Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5290–5293
Bioorganic & Medicinal Chemistry Letters

Cytostatic and antiviral 6-arylpurine ribonucleosides. Part 7: Synthesis and evaluation of 6-substituted purine L-ribonucleosides☆

Michal Hocek,[a,*] Peter Šilhár,[a] I-hung Shih,[b] Eric Mabery[b] and Richard Mackman[b]

[a]*Gilead Sciences & IOCB Research Center, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nam. 2, CZ-16610 Prague 6, Czech Republic*
[b]*Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, CA 94404, USA*

Received 15 July 2006; revised 28 July 2006; accepted 31 July 2006
Available online 14 August 2006

Abstract—A series of purine L-ribonucleosides **2a**–**2i** bearing diverse C-substituents (alkyl, aryl, hetaryl or hydroxymethyl) in the position 6 were prepared by Pd-catalyzed cross-coupling reactions of 6-chloro-9-(2,3,5-tri-*O*-acetyl-β-L-ribofuranosyl)purine with the corresponding organometallics followed by deprotection. Unlike their D-ribonucleoside enantiomers that possess strong cytostatic and anti-HCV activity, the L-ribonucleosides were inactive except for 6-benzylpurine nucleoside **2h** showing moderate anti-HCV effect in replicon assay. A triphosphate of **2h** did not inhibit HCV RNA polymerase.

Purine nucleosides **1** bearing C-substituents in position 6 are an important class of biologically active compounds. 6-Methyl- and 6-ethylpurine nucleosides are cytotoxic.[1] 6-(hydroxymethyl)-,[2] 6-(fluoromethyl)-,[3] 6-(difluoromethyl)-[4], and 6-(trifluoromethyl)purine[5] ribonucleosides are all strongly cytostatic. 6-Aryl-, 6-hetaryl-, and 6-benzylpurine ribonucleosides were also found[6] to possess significant cytostatic effects. A subclass of them, purine ribonucleosides bearing 5-membered heterocycles in position 6, have recently been reported to exert[7] strong anti-HCV activities. Though the mechanism of action of the latter class of compounds is not yet fully understood, they all inhibit RNA synthesis. In order to achieve selective inhibition of HCV RNA polymerase, some additional sugar modifications may be pursued. From the previous studies it is known that 2′- and 5′-deoxyribonucleosides,[8] as well as 2′-C-methyl-ribonucleosides[9] of the 6-aryl- or 6-hetarylpurine series are inactive, while some carbocyclic homonucleosides still exert[10] cytostatic effects.

L-Nucleosides, enantiomers of the natural nucleosides, were first reported[11] in the 1960s. Much later some L-nucleosides were found[12] to possess strong antiviral activity, lower toxicity and higher metabolic stability compared to their D-counterparts. Several of them are clinically used antiviral drugs and some other ones are in final stages of clinical trials.[13] These findings prompted extensive research of nucleobase- as well as sugar-modified L-nucleosides in recent years.[14]

Combining the structural features of the two above-mentioned classes of biologically active compounds, we report here on the synthesis and biological activity of L-ribonucleosides **2** (Fig. 1) derived from purines bearing diverse C-substituents in position 6. The selection of substituents was based both on diversity and on analogy to the most active compounds of the D-ribo

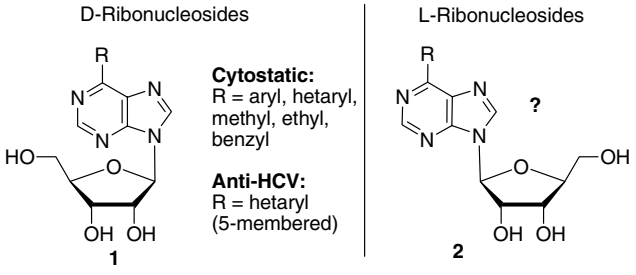


Figure 1. Structures of biologically active purine D-ribonucleosides **1** and the title L-nucleosides **2**.

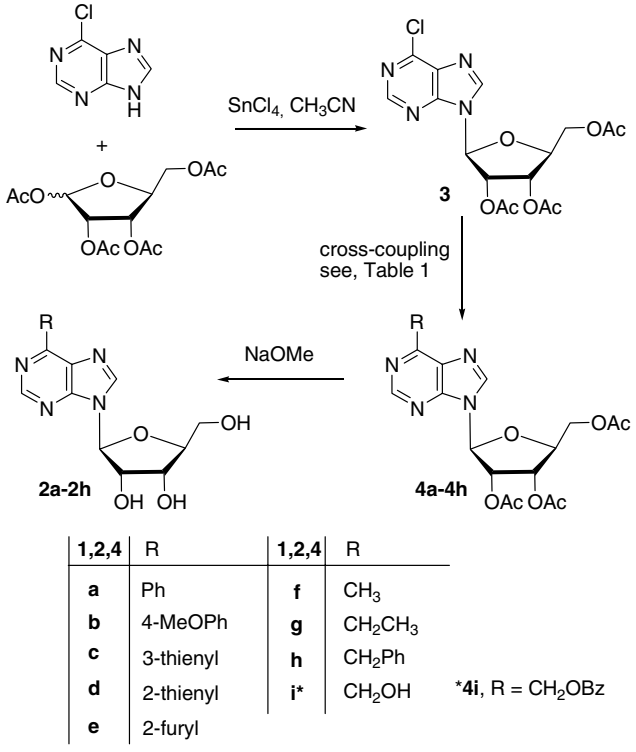
Keywords: Purines; Nucleosides; Antivirals; HCV.
☆ Part 6, see Ref. 7.
* Corresponding author. Tel.: +420 220183324; fax: +420 220183559; e-mail: hocek@uochb.cas.cz

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.07.092

series: two examples of phenyl groups, three hetaryl, three alkyl groups and one functionalized, hydroxymethyl group.

All the title L-ribonucleosides **2** were prepared by cross-coupling reactions[15] of the corresponding protected 6-chloropurine nucleoside **3**. This intermediate was prepared[16] in 61% yield by glycosidation of 6-chloropurine by commercially available 1,2,3,5-tetra-*O*-acetyl-β-L-ribose in presence of $SnCl_4$.

The 6-chloropurine nucleoside **3** was then subjected to a series of cross-coupling reactions with organometallics (Scheme 1, Table 1). The Suzuki–Miyaura coupling with phenyl-, 4-methoxyphenyl-, and 3-thienylboronic acid in toluene catalyzed by $Pd(PPh_3)_4$ gave 6-phenyl-, 6-(4-methoxyphenyl)-, and 6-(3-thienyl)purine nucleosides **4a**–**4c** (analogy to Ref. 6a,7). The Stille reactions with 2-thienyl- and 2-furyl(tributyl)stannanes in DMF catalyzed by $PdCl_2(PPh_3)_2$ gave 6-(2-thienyl)- and 6-(2-furyl)purine nucleosides **4d** and **4e** (analogy to Ref. 6b). Cross-coupling reactions with trimethylaluminum, triethylaluminum (analogy to Ref. 17), and benzylzinc chloride (analogy to Ref. 6b) in the presence of $Pd(PPh_3)_4$ in THF at 70 °C gave the corresponding 6-methyl-, 6-ethyl-, and 6-benzylpurines **4f**–**4h**. Cross-coupling with (benzoyloxymethyl)zinc iodide in presence of $Pd(PPh_3)_4$ in THF at rt gave 6-(benzoyloxymethyl)purine **4i** (analogy to Ref. 2). All the acylated nucleosides **4** were deprotected using standard treatment with NaOMe in methanol to give the series of L-ribonucleosides **2a**–**2i** in good yields.[18]



1,2,4	R	1,2,4	R
a	Ph	f	CH_3
b	4-MeOPh	g	CH_2CH_3
c	3-thienyl	h	CH_2Ph
d	2-thienyl	i*	CH_2OH
e	2-furyl		

Scheme 1. Synthesis of the title L-nucleosides **2**.

Title L-nucleosides **2a**–**2i** 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). None of these compounds showed any significant cytostatic effect at 10 μM concentration.

Antiviral activity of L-nucleosides **2a**–**2i** was evaluated in an HCV subgenomic replicon assay[19] and the results are presented in Table 2. Analogs, **2d** and **2h**, displayed antiviral activity with no detectable cytotoxicity in Huh-7 or MT-4 cells. Close structural analogs of **2d**, for example 3-thienyl (**2c**) and 2-furyl (**2e**), were inactive

Table 2. HCV antiviral activity, cytotoxicity, and HCV NS5B inhibition

Compound	HCV replicon[a] EC_{50} (μM)	Huh7[b] CC_{50} (μM)	MT-4[c] CC_{50} (μM)	NS5B[d] IC_{50} (μM)
2a	>100	>50	—	—
2b	>50	>100	—	—
2c	>50	>50	—	—
2d	56	>100	>100	—
2e	>100	>100	—	—
2f	>500	>500	—	—
2g	>500	>500	—	—
2h	41	>500	>500	>100
2i	>500	>500	—	—

[a] Antiviral activity in HCV con1 replicon ($N = 2$).
[b] Cellular toxicity in Huh-7 cells harboring con-1 replicon ($N = 2$).
[c] Cellular toxicity in MT-4 cells ($N = 2$).
[d] In vitro inhibition toward purified HCV NS5B ($N = 2$).

Table 1. Cross-coupling reactions of **3** with organometallics

Entry	Reagent	Catalyst	Product (yield)	Deprotection (yield)
1	$PhB(OH)_2$	$Pd(PPh_3)_4$	**4a** (88%)	**2a** (75%)
2	4-MeOPh$B(OH)_2$	$Pd(PPh_3)_4$	**4b** (85%)	**2b** (87%)
3	3-Thienyl$B(OH)_2$	$Pd(PPh_3)_4$	**4c** (90%)	**2c** (79%)
4	2-Thienyl$SnBu_3$	$PdCl_2(PPh_3)_2$	**4d** (93%)	**2d** (72%)
5	2-Furyl$SnBu_3$	$PdCl_2(PPh_3)_2$	**4e** (92%)	**2e** (76%)
6	Me_3Al	$Pd(PPh_3)_4$	**4f** (66%)	**2f** (88%)
7	Et_3Al	$Pd(PPh_3)_4$	**4g** (68%)	**2g** (73%)
8	BnZnCl	$Pd(PPh_3)_4$	**4h** (62%)	**2h** (62%)
9	$BzOCH_2ZnI$	$Pd(PPh_3)_4$	**4i** (88%)	**2i** (80%)

indicating that the antiviral structure–activity relationships for 6-modified purine L-ribonucleosides are quite narrow.

To verify the antiviral mode of action, the triphosphate of **2h** was prepared[20] and the in vitro inhibition of HCV NS5B polymerase evaluated.[21] Inhibition was determined using recombinant NS5B derived from HCV con1 strain (1b), with a C-terminal hexahistidine tag and the last 21 amino acids deleted, and a heteropolymeric RNA template.[22] No inhibition of the NS5B polymerase activity was observed up to 100 μM concentration of inhibitor (Table 2). In addition, the ability of **2h** triphosphate to serve as a NS5B polymerase substrate in a single-nucleotide incorporation assay was determined.[23] The incorporation assay was performed using recombinant NS5B from HCV con1 strain (1b), with a C-terminal hexahistidine tag and the last 55 amino acids deleted, a dinucleotide primer (GpC), and a RNA oligonucleotide template (5′-CAAAAAAAUGC-3′).[24] No incorporation of **2h** triphosphate by NS5B was observed up to concentrations of 1 mM. Furthermore, the triphosphate of L-adenosine was also not incorporated by NS5B polymerase under the same conditions. Taken together, these results suggest that purine L-ribonucleosides are not substrates of the viral polymerase and that the antiviral activity of **2h** was not due to incorporation by NS5B polymerase into the viral RNA and subsequent termination of further synthesis, but most likely inhibition of other cellular or viral targets.

Acknowledgments

This work is a part of the research project Z4 055 0506. It was supported by the 'Centre for New Antivirals and Antineoplastics' (1M0508), by the Programme of Targeted Projects of Academy of Sciences of the Czech Republic (1QS400550501), and by Gilead Sciences, Inc. (Foster City, CA, USA).

References and notes

1. Montgomery, J. A.; Hewson, K. *J. Med. Chem.* **1968**, *11*, 48.
2. (a) Šilhár, P.; Pohl, R.; Votruba, I.; Hocek, M. *Org. Lett.* **2004**, *6*, 3225; (b) Šilhár, P.; Pohl, R.; Votruba, I.; Hocek, M. *Collect. Czech. Chem. Commun.* **2005**, *70*, 1669.
3. Šilhár, P.; Pohl, R.; Votruba, I.; Hocek, M. *Org. Biomol. Chem.* **2005**, *3*, 3001.
4. Šilhár, P.; Pohl, R.; Votruba, I.; Hocek, M. *Synthesis* **2006**, 1848.
5. Hocková, D.; Hocek, M.; Dvořáková, H.; Votruba, I. *Tetrahedron* **1999**, *55*, 11109.
6. (a) Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *J. Med. Chem.* **2000**, *43*, 1817; (b) Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *Collect. Czech. Chem. Commun.* **2001**, *66*, 483.
7. Hocek, M.; Nauš, P.; Pohl, R.; Votruba, I.; Furman, P. A.; Tharnish, P. M.; Otto, M. J. . *J. Med. Chem.* **2005**, *48*, 5869.
8. Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *Collect. Czech. Chem. Commun.* **2000**, *65*, 1683.
9. Ding, Y.; Girardet, J.-L.; Hong, Z.; Lai, V. C. H.; An, H.; Koh, Y.-h.; Shaw, S. Z.; Zhong, W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 709.
10. Fernandez, F.; Garcia-Mera, X.; Morales, M.; Rodriguez-Borges, J.; De Clercq, E. *Synthesis* **2002**, 1084.
11. (a) Šmejkal, J.; Šorm, F. *Collect. Czech. Chem. Commun.* **1964**, *29*, 2809; (b) Acton, E. M.; Ryan, K. J.; Goodman, L. *J. Am. Chem. Soc.* **1964**, *86*, 5352; (c) Holý, A.; Šorm, F. *Collect. Czech. Chem. Commun.* **1969**, *34*, 3383; (d) Holý, A. *Collect. Czech. Chem. Commun.* **1972**, *37*, 4072.
12. Reviews: (a) Wang, P. Y.; Hong, J. H.; Cooperwood, J. S.; Chu, C. K. *Antiviral Res.* **1998**, *40*, 19; (b) Gumina, G.; Song, G.-Y.; Chu, C. K. *FEMS Microbiol. Lett.* **2001**, *202*, 9; (c) Gumina, G.; Chong, Y.; Choo, H.; Song, G.-Y.; Chu, C. K. *Curr. Top. Med. Chem.* **2002**, *2*, 1065.
13. Bryant, M. L.; Bridges, E. G.; Placidi, L.; Faraj, A.; Loi, A. G.; Pierra, C.; Dukhan, D.; Gosselin, G.; Imbach, J. L.; Hernandez, B.; Juodawlkis, A.; Tennant, B.; Korba, B.; Cote, P.; Marion, P.; Cretton-Scott, E.; Schinazi, R. F.; Sommadossi, J. P. *Antimicrob. Agents Chemother.* **2001**, *45*, 229.
14. Recent examples: (a) Mugnaini, C.; Botta, M.; Coletta, M.; Corelli, F.; Focher, F.; Marini, S.; Renzulli, M. L.; Verri, A. *Bioorg. Med. Chem.* **2003**, *11*, 357; (b) Zhu, W.; Chong, Y. H.; Choo, H.; Mathews, J.; Schinazi, R. F.; Chu, C. K. *J. Med. Chem.* **2004**, *47*, 1631; (c) Seela, F.; Lin, W.; Kazimierczuk, Z.; Rosemeyer, H.; Glacon, V.; Peng, X.; He, Y.; Ming, X.; Andrzejewska, M.; Gorska, A.; Zhang, X.; La Colla, P. *Nucleosides Nucleotides Nucleic Acids* **2005**, *24*, 859; (d) Clinch, K.; Evans, G. B.; Fleet, G. W. J.; Furneaux, R. H.; Johnson, S. W.; Lenz, D. H.; Mee, S. P. H.; Rands, P. R.; Schramm, V. L.; Taylor Ringia, E. A.; Tyler, P. C. *Org. Biomol. Chem.* **2006**, *4*, 1131; (e) Seela, F.; Peng, Q. *Collect. Czech. Chem. Commun.* **2006**, *71*, 956.
15. Reviews: (a) Hocek, M. *Eur. J. Org. Chem.* **2003**, 245; (b) Agrofoglio, L. A.; Gillaizeau, I.; Saito, Y. *Chem. Rev.* **2003**, *103*, 1875.
16. Compound **3**: a mixture of chloropurine (3.8 g, 20 mmol) and 1,2,3,5-tetra-*O*-acetyl-β-L-ribose (6.4 g, 20 mmol) in acetonitrile (100 ml) was stirred at rt and $SnCl_4$ (5 ml) was added dropwise. The stirring was continued for 10 h and the solvent was evaporated. The residue was dissolved in ethyl acetate (250 ml) and washed with satd aq $NaHCO_3$ (2× 250 ml) and H_2O (2× 250 ml). The organic phase was dried, evaporated, and the residue chromatographed on silica gel (ethyl acetate) to give **3** as yellowish foam (5 g, 61%), $[\alpha]_D$ +15.9 (*c* 0.35, $CHCl_3$). Spectral data were in accord with those of D-enantiomer: Buck, I.M.; Reese, C.B. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2937–2942.
17. (a) Hirota, K.; Kitade, Y.; Kanbe, Y.; Maki, Y. *J. Org. Chem.* **1992**, *57*, 5268; (b) Česnek, M.; Hocek, M.; Holý, A. *Collect. Czech. Chem. Commun.* **2000**, *65*, 1357; (c) Šilhár, P.; Pohl, R.; Votruba, I.; Klepetářová, B.; Hocek, M. *Collect. Czech. Chem. Commun.* **2006**, *71*, 788.
18. Spectral data of compounds **2** and **4** were in accord with those of the corresponding D-ribonucleosides. Comparison of mp and $[\alpha]_D$ values of L-nucleosides **2** with those of D-nucleosides **1**: **2a**: mp 229–231 °C, $[\alpha]_D$ +60.4 (*c* 0.34, DMF) [lit.[6a] for **1a**: mp 228–230 °C, $[\alpha]_D$ −56.1 (*c* 0.5, DMF)]. Compound **2b**: mp 172–176 °C, $[\alpha]_D$ +55.9 (*c* 0.15, DMF) [lit.[6a] for **1b**: mp 173–175 °C, $[\alpha]_D$ −61.5 (*c* 0.5, DMF)]. Compound **2c**: mp 198–200 °C, $[\alpha]_D$ +60.8 (*c* 0.39, DMF) [lit.[7] for **1c**: mp 196–199 °C, $[\alpha]_D$ −51.8 (*c* 0.5, DMF)]. Compound **2d**: mp 225–229 °C, $[\alpha]_D$ +68.2 (*c* 0.33, DMF) [lit.[6b] for **1d**. H_2O: mp 126–129 °C, $[\alpha]_D$ −55.3 (*c* 0.2, DMF)]. Compound **2e**: mp 179–181 °C, $[\alpha]_D$ +62.5 (*c*

0.46, DMF) [lit.[6b] for **1e**. 1/2 H_2O: mp 165–168 °C, $[\alpha]_D$ −54.1 (*c* 0.2, DMF)]. Compound **2f**: mp 206–209 °C, $[\alpha]_D$ +50.6 (*c* 0.23, DMF) [lit.[1] for **1f**: mp 209 °C, $[\alpha]_D$ −52.1 (MeOH)]. Compound **2g**: mp 192–195 °C, $[\alpha]_D$ +48.5 (*c* 0.20, DMF) [lit.[1] for **1g**. H_2O: mp 105 °C, $[\alpha]_D$ −50 (MeOH)]. Compound **2h**: mp 103–106 °C, $[\alpha]_D$ +58.6 (*c* 0.13, DMF) [lit.[6b] for **1h**: mp 97–100 °C, $[\alpha]_D$ −47.7 (*c* 0.2, DMF)]. Compound **2i**: mp 75–78 °C, $[\alpha]_D$ +27.6 (*c* 0.3, H_2O) [lit.[2] for **1i**. H_2O: mp 65–69 °C, re-measured $[\alpha]_D$ −27.1 (*c* 0.3, H_2O)].

19. Lohmann, V.; Korner, F.; Koch, J.; Herian, U.; Theilmann, L.; Bartenschlager, R. *Science* **1999**, *285*, 110.
20. Triphosphates were prepared from the corresponding nucleosides by Chemcyte Inc., San Diego, USA.
21. IC_{50} determination: The reaction was assembled in 50 mM Tris/HCl, pH 7.5, 10 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA, 40 ng/μl RNA template, 75 nM NS5B-delta21 enzyme, 0.5 μCi of [α-^{33}P] adenosine triphosphate, 3 μM ATP, and 500 μM remaining NTP's. Purified enzyme was preincubated with compounds for 20 min at 34 °C, after which time the reaction was started by the addition of RNA template and NTP substrates. The reaction was allowed to proceed for 90 min and then transferred onto 96-well DE81 filter membranes, washed three times with 100 mM Na_2HPO_4, once with ethanol, and dried. Scintillation fluid was added to the wells and counts per minute (cpm) were measured in a TopCount. Radioactivity of each reaction was plotted against the drug concentration. IC_{50} was calculated by nonlinear regression analysis using GraphPad software package.
22. Hung, M.; Gibbs, C. S.; Tsiang, M. *Antiviral Res.* **2002**, *56*, 99.
23. Single-nucleotide incorporation assay: the reaction was carried out in 50 mM HEPES (pH 7.3), 5 mM $MgCl_2$, 10 mM DTT, 20 μM ^{33}P-labeled-GpC primer, 20 μM oligonucleotide, 1 mM nucleotide triphosphate, and 2 μM of NS5B-delta55 enzyme, at 34 °C for 60 min. The reaction mixture was resolved on 20% denaturing acrylamide gel and quantified by PhosphorImager (Amersham).
24. Cramer, J.; Jaeger, J.; Restle, T. *Biochemistry* **2006**, *45*, 3610.