Published in final edited form as:
Bioorg Med Chem Lett. 2013 June 1; 23(11): 3385–3388. doi:10.1016/j.bmcl.2013.03.077.

Synthesis and evaluation of Janus type nucleosides as potential HCV NS5B polymerase inhibitors

Longhu Zhou[a], **Franck Amblard**[a], **Hongwang Zhang**[a], **Tamara R. McBrayer**[b], **Mervi A. Detorio**[a], **Tony Whitaker**[b], **Steven J. Coats**[b], and **Raymond F. Schinazi**[a]

[a]Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, and Veterans Affairs Medical Center, Decatur, GA 30033, USA

[b]RFSPharma, LLC, 1860 Montreal Road, Tucker, GA 30084, USA

Abstract

The synthesis of new ribo and 2'-β-*C*-methyl ribo Janus type nucleosides J-AA, J-AG and J-AU is reported along with their ability to block HCV and HIV replication. Their toxicity was also assessed in Huh7, human lymphocytes, CEM and Vero cells

Keywords

HCV; Antiviral; prodrug; Janus

Hepatitis C virus (HCV), an enveloped single-stranded positive sense enveloped RNA virus discovered in 1989,[1] is a leading cause of long term liver cirrhosis, resulting in liver transplantation, liver failure and hepatocellular carcinoma. [2] Globally, there are an estimated 170 million persons infected with the virus and 3 to 4 million persons are newly infected each year. Despite the existence of treatments involving pegylated interferon-α (IFN) and ribavirin (RBV), with or without protease inhibitors (PI) boceprevir (Victrelis) and telaprevir (Incivek),[3] the limited efficacy and side effects of current therapies emphasize the need for additional improved therapeutic agents. Nucleoside inhibitors that target HCV NS5B polymerase have demonstrated clinical advantages of broader activity against various HCV genotypes and a higher barrier to the development of resistant viruses when compared to all other classes of HCV inhibitors.[4] To date a number of 2'-modified nucleosides have shown potent activity against HCV (Figure 1).[5,6] IDX-184 **1**, RO-5024048/RG-7128 **2**, and PSI-7977 (GS-7977) **3** are in advanced clinical trials as effective anti-HCV agents. Interestingly, highly base-modified tricyclic derivatives such as **4**[7] and **5**[8] have also shown potent anti-HCV activity.

Based on these compounds and inspired by Townsend's work on linear tricyclic nucleosides, [9] we prepared a series of new nucleosides with potential anti-HCV activity that may be viewed as possessing the feature of two completely different bases simultaneously (Figure 2). These dual bases or Janus type [10] nucleosides, such as J-GA (Figure 2), presenting one face with a Watson-Crick donor/acceptor array of a guanine and the other

Correspondence to: Raymond F. Schinazi.

face with an array of an adenine, could in principle, by rotation of the glycosidic bond, pair with either a cytosine or a uracil. Thus, we report herein the synthesis and the biological evaluation of ribo and new 2'-β-*C*-methyl ribo Janus type nucleosides J-AA, J-AG and J-AU (Figure 2; R = H or Me).

The Janus type nucleosides **12a, 14a** and **19a** were previously reported by Townsend,[9] we have included our resynthesis of these nucleosides for comparison to the synthesis of the 2'-β-*C*-methyl ribo series and also to evaluate and compare both series for antiviral and cytotoxic activity. The synthesis of Janus type nucleosides **12a-b** (J-AU) is summarized in Scheme 1. The Vorbr ggen coupling reaction between 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine **6**[11] and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **7a** or 2'-β-*C*-methyl-1,2-di-*O*-benzoyl-3,5-di-*O*-toluyl-β-D-ribofuranose **7b**[12] using *N,O*-bis(trimethylsilyl)acetamide (BSA) as the silylation agent and TMSOTf as the Lewis acid provided compounds **8a** and **8b** in 71% and 65% respectively. [13] Compounds **8a-b** were then deprotected in saturated methanolic ammonia to give **9a-b**. Subsequent halogen displacement with liquid ammonia in a steel bomb was followed by treatment with hydroxylamine[14] in ethanol which afforded compounds **11a-b** in good yields. Finally, J-AU derivatives **12a-b**[15] were obtained in 51% and 46% yield, respectively, by treatment of amides **11a-b** with diethyl carbonate in presence of sodium ethoxide. It is noteworthy that 1H NMR spectral data for Janus base nucleosides denote the presence of only one isomer (For compound **12b**, 2'Me signal appears as a singlet), clearly indicating free rotation of the base.

J-AA compounds **14a-b**[16] were easily prepared without isolation of intermediates by reaction of **10a-b** with diethoxymethyl acetate followed by treatment with saturated methanolic ammonia solution followed by 25% aqueous acetic acid (Scheme 2).

Finally, nucleosides J-AG **19a-b** were prepared in 5 steps from intermediate **11a-b** (Scheme 3). Thus, treatment of compounds **11a-b** with carbon disulfide under basic conditions afforded, in quantitative manner, tricyclic sodium salts **15a-b**, which were converted to their ammonium salt counterparts **17a-b** after acidification and treatment with NH_4OH. **17a-b** were then oxidized using hydrogen peroxide and the resulting ammonium sulfonate intermediates **18a-b** were subsequently reacted with ammonia in a steal vessel to give the desired J-AG nucleosides **19a-b**.[17]

Overall the presence of the 2'-*C*-Me group had little effect on the yield of the various reactions, however, in most cases there was a slight reduction in isolated yield. All synthesized tricyclic dual-base nucleosides **12a-b, 14a-b, 19a-b** along with intermediates **9a-b, 10a-b, 11a-b** were evaluated for inhibition of HCV RNA replication in Huh7 cells using a subgenomic HCV replicon system. [18] Cytotoxicity in Huh7 cells was determined simultaneously with anti-HCV activity by extraction and amplification of both HCV RNA and cellular ribosomal RNA (rRNA). [19] To determine the spectrum of activity of the compounds, anti-HIV activity was evaluated against HIV-1_{LAI} in primary human peripheral blood mononuclear (PBM) cells and 3'-azido-3'-deoxythymidine (AZT) was used as a positive control. Cytotoxicity was determined in PBM, human lymphoblastoid CEM, and African Green monkey Vero cells (Table 1).[20]

From among the prepared compounds, targeted Janus nucleosides compounds, **12a** (J-AU) and **19a-b** (J-GA) along with intermediates **10a-b** and **11a** showed HCV activities that were not differentiable from the toxicity observed in the replicon Huh7 cell line. The remaining three Janus compounds **12b** (J-AU) and **14a-b** (J-AA) and intermediates **9a-b, 11b**, and **16a-b** did not display any anti-HCV activity in our Huh7 based replicon system. A similar result was seen in the HIV assay with all compounds that displayed anti-HIV activity were also

found to have cytotoxicity toward PBM cells (compound **10a** was the most toxic with a CC_{50} in PBM cells of 0.1 μM).

Since the lack of antiviral activity or cytotoxicity observed for **12b** and **14b** may be due to inefficient uptake and/or their inability to be intracellularly metabolized to the corresponding nucleoside triphosphates, a J-AA McGuigan type phosphoramidate prodrug **24** was prepared (Scheme 4). We chose **24** for prodrug synthesis as the adenine groups are presented in a manner consistent with adenosine while the J-AU nucleoside **12b** presents the uracil group in a manner inconsistent with uridine.

Attempts to synthesize prodrug **24** by direct coupling of nucleoside **14b** with phenyl(ethoxy-L-alaninyl)phosphorochloridate in presence of either *t*-BuMgCl or NMI afforded only trace amount of the desired product. These difficulties lead us to envisage a temporarily protection of the exocyclic amino groups before coupling with the chlorophosphoramidate. Thus, compound **14b** was reacted with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine to form 3',5'-protected intermediate **20**. Subsequently, the amino groups were protected with *tert*-butoxycarbonyl anhydride ($(Boc)_2O$) to give the corresponding tri-*N*-Boc protected derivative **21**. Interestingly, despite the use of a large excess of $(Boc)_2O$, we were unable to introduce four Boc groups on compound **20**. Treatment of **21** with $Et_3N.3HF$ provided *N*-Boc-protected nucleoside **22**, which was then reacted with phenyl(ethoxy-L-alaninyl)phosphorochloridate in presence of *t*-BuMgCl to give phosphoramidate **23**. Deprotection with 80% aqueous TFA at room temperature provided target monophosphate prodrug **24** in 33% yield over 2 steps. Unfortunately, monophosphate prodrug **24** did not display any inhibition of HCV RNA replication in the replicon system.

In conclusion, as a part of our drug discovery program, we have synthesized some ribo and novel 2'-*C*-Me tricyclic dual base nucleosides J-AU, J-AG and J-AA and a McGuigan type phosphoramidate prodrug of J-AA. Many of these Janus type nucleosides were found to be cytotoxic in multiple cell lines with only 2'-*C*-Me J-AA **12b** and 2'-*C*-Me J-AG **14b** being devoid of cytotoxicity in the four cell lines tested. Our studies did not reveal any anti-HCV or -HIV activity that was free from cytotoxicity indicating that our changes to the nucleoside base might have been too drastic for recognition by phosphorylation kinases and/or HCV NS5B polymerase.

Acknowledgments

This work was supported in part by NIH grant 5P30-AI-50409 (CFAR), 1R01-MH-100999 and by the Department of Veterans Affairs. Dr. Schinazi is the founder and a major shareholder of RFS Pharma, LLC. Emory received no funding from RFS Pharma, LLC to perform this work and *vice versa*.

References and notes

1. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Science. 1989; 244:359–362. [PubMed: 2523562]
2. Kanwal F, Hoang T, Kramer JR, Asch SM, Goetz MB, Zeringue A, Richardson P, El-Serag HB. BMC Gastroenterol. 2011; 140:1182.
3. Sheridan C. Nature Biotech. 2011; 29:553.
4. McCown MF, Rajyaguru S, Le Pogam S, Ali S, Jiang WR, Kang H, Symons J, Cammack N, Najera I. Antimicrob Agents Chemother. 2008; 52:1604. [PubMed: 18285474]
5. (a) Eldrup AB, Prhavc M, Brooks J, Bhat B, Prakash TP, Song Q, Bera S, Bhat N, Dande P, Cook PD, Bennett CF, Carroll SS, Ball RG, Bosserman M, Burlein C, Colwell LF, Fay JF, Flores OA, Getty K, LaFemina RL, Leone J, MacCoss M, McMasters DR, Tomassini JE, Von Langen D, Wolanski B, Olsen DB. J Med Chem. 2004; 47:5284. [PubMed: 15456273] (b) Clark JL, Hollecker L, Mason JC, Stuyver LJ, Tharnish PM, Lostia S, McBrayer TR, Schinazi RF, Watanabe KA, Otto

MJ, Furman PA, Stec WJ, Patterson SE, Pankiewicz KW. J Med Chem. 2005; 48:5504. [PubMed: 16107149]

6. For a recent review of NS5B anti-HCV agents, see: Sofia MJ, Chang W, Furman PA, Mosley RT, Ross BS. J Med Chem. 2012; 55:2481. [PubMed: 22185586]
7. Keicher, JD.; Roberts, CD.; Liehr, SJR.; Zheng, X.; Prhavc, M.; Rajwanshi, VK.; Griffttth, RC.; Kim, CU. PCT. WO2006093987.
8. Seley-Radtke KL, Zhang Z, Wauchope OR, Zimmermann S, Ivanov A, Korba BE. Nucleic Acids Symp. 2008; 52:635–636.
9. Chung F-L, Schram KH, Panzica RP, Earl RA, Wotring LL, Townsend LB. J Med Chem. 1980; 23:1158. [PubMed: 7452664]
10. Inspired by Janus, the Roman god of gates and doors, beginnings and endings, and hence represented with a double-faced head, each looking in opposite directions.
11. Tolman RL, Robins RK, Townsend LB. J Am Chem Soc. 1969; 91:2102. [PubMed: 5805382]
12. Franchetti P, Cappellacci L, Marchetti S, Trincavelli L, Martini C, Mazzoni MR, Lucacchini A, Grfantini M. J Med Chem. 1998; 41:1708. [PubMed: 9572897]
13. (a) Wang G, Tam RC, Gunic E, Du J, Bard J, Pai B. J Med Chem. 2000; 43:2566. [PubMed: 10891116] (b) Ding Y, An H, Hong Z, Girardet J-L. Bioorg Med Chem Lett. 2005; 15:725. [PubMed: 15664845]
14. Hurd CD. Inorg Synth. 1939; 1:87.
15. *Selected spectral data for compound* ***12b***: ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.74 (s, 3H), 3.16 (d, 2H, J = 5.2 Hz), 3.71-3.90 (m, 3H), 4.09-4.13 (m, 1H), 5.19 (br s, 1H), 5.43 (br s, 1H), 6.33 (s, 1H), 7.43 (br s, 1H), 7.51 (br s, 1H), 8.15 (s, 1H), 11.29 (br s, 1H), 12.03 (br s, 1H). LC/MS (*m/z*), calcd for $C_{14}H_{16}N_6O_6$ (M^++H), 364.1; found, 365.2.
16. *Selected spectral data for compound* ***14b***: ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.82 (s, 3H, CH_3), 3.76-3.78 (m, 2H, H_5'), 3.90-3.93 (m, 1H, H_4'), 4.61-4.65 (m, 1H, H_3'), 4.99 (br s, 2H, 2× OH), 5.20 (d, 1H, J = 7.2 Hz, OH), 6.55 (s, 1H, H_1'), 6.88 (br s, 4H, 2× NH_2), 8.32 (s, 2H, ArH). LC/MS (*m/z*), calcd for $C_{14}H_{17}N_7O_4$ (M^++H), 348.1; found, 348.2.
17. *Selected spectral data for compound* ***19b***: ^{1}H-NMR (400 MHz, CD_3OD) δ: 0.98 (s, 3H), 3.99-4.08 (m, 3H), 4.61 (d, 1H, J = 8.8 Hz), 6.47 (s, 1H), 8.19 (s, 1H). LC/MS (*m/z*), calcd for $C_{14}H_{17}N_7O_5$ (M^++H), 363.1; found, 364.1.
18. Rondla R, Coats SJ, McBrayer TR, Grier J, Johns M, Tharnish PM, Whitaker T, Zhou L-H, Schinazi RF. Antivir Chem Chemother. 2009; 20:99. [PubMed: 19843980]
19. Stuyver LJ, Whitaker T, McBrayer TR, Hernandez-Santiago BI, Lostia S, Tharnish PM, Ramesh M, Chu CK, Jordan R, Shi J, Rachakonda S, Watanabe KA, Otto MJ, Schinazi RF. Antimicrob Agents Chemother. 2003; 47:244. [PubMed: 12499198]
20. (a) Schinazi RF, Sommadossi JP, Saalmann V, Cannon DL, Xie M-W, Hart GC, Smith GA, Hahn EF. Antimicrob Agents Chemother. 1990; 34:1061. [PubMed: 2393266] (b) Stuyver LJ, Lostia S, Adams M, Mathew J, Pai BS, Grier J, Tharnish P, Choi Y, Chong Y, Choo H, Chu CK, Otto MJ, Schinazi RF. Antimicrob Agents Chemother. 2002; 46:3854. [PubMed: 12435688]

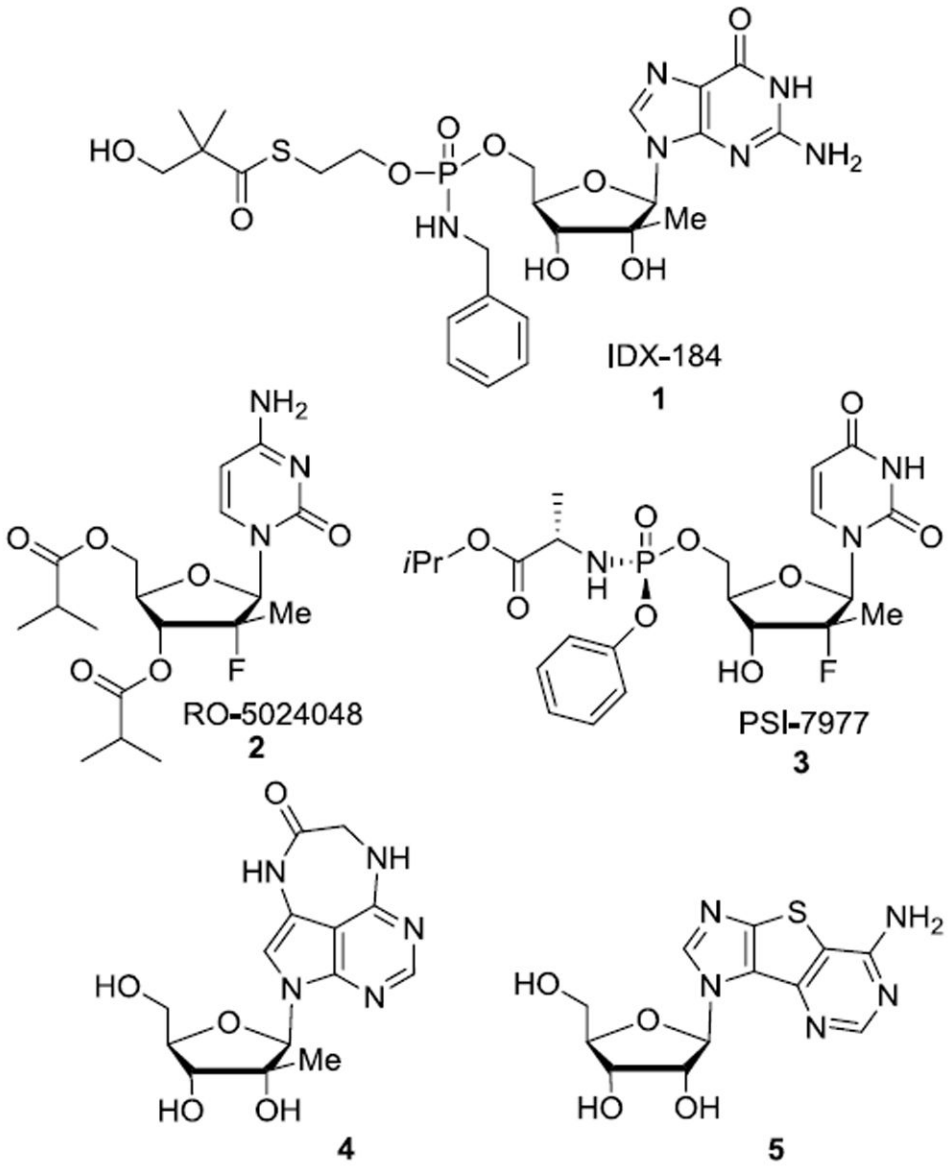


Figure 1.
Nucleoside analogs exhibiting anti-HCV properties.

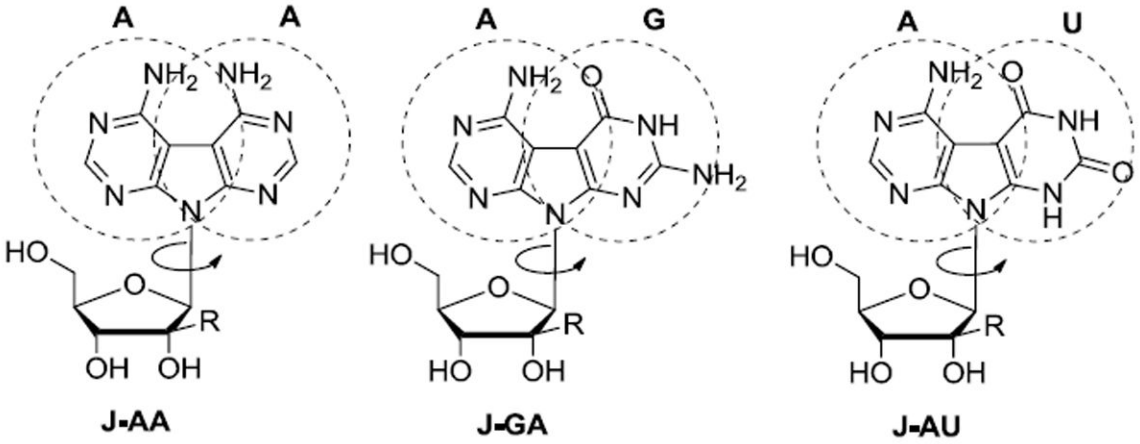
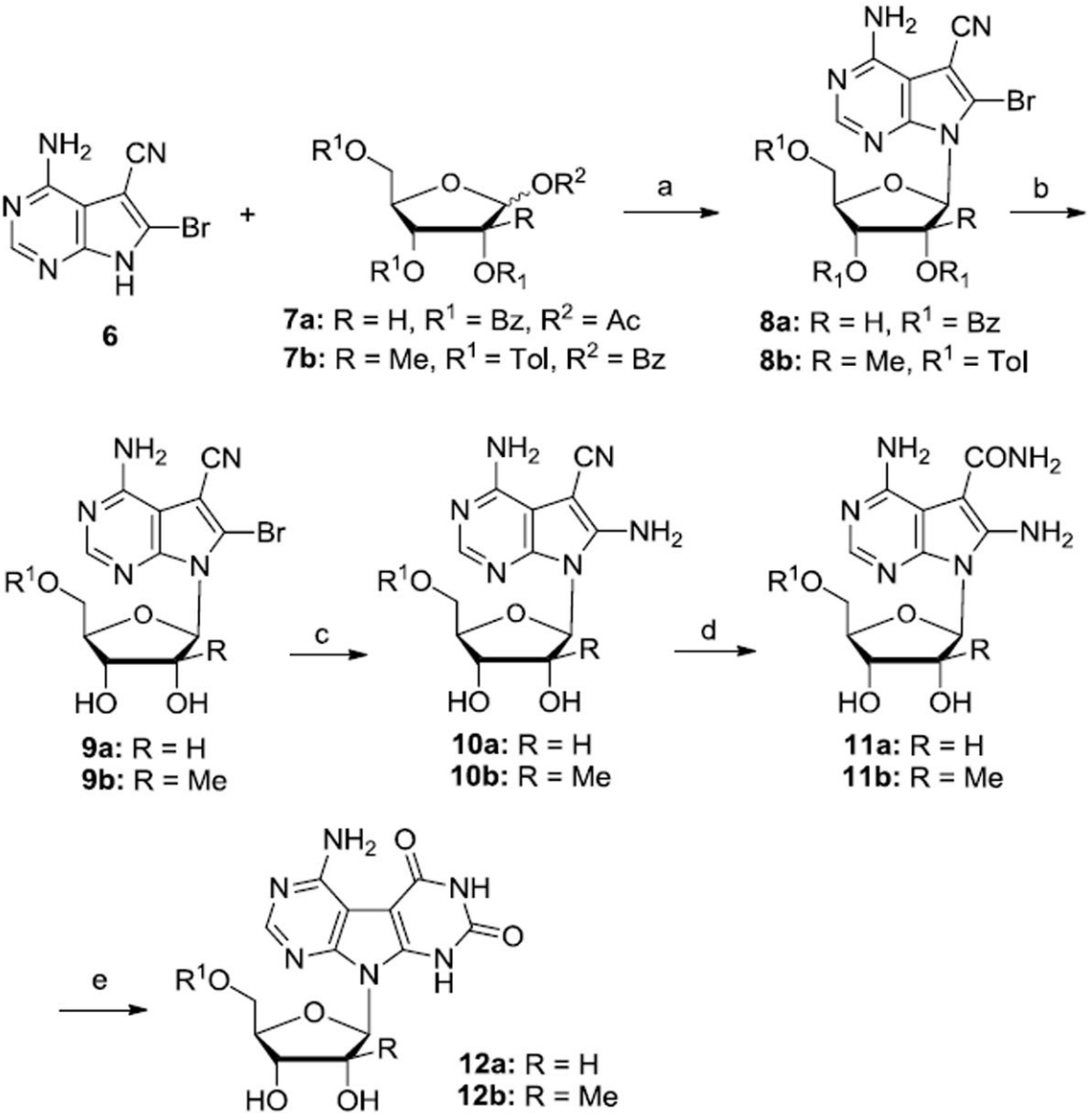
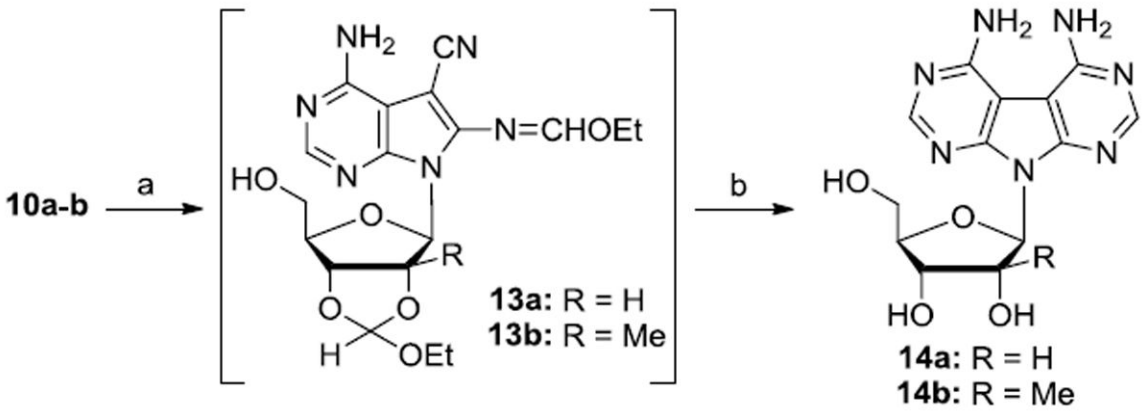


Figure 2.
Janus type dual-base nucleosides



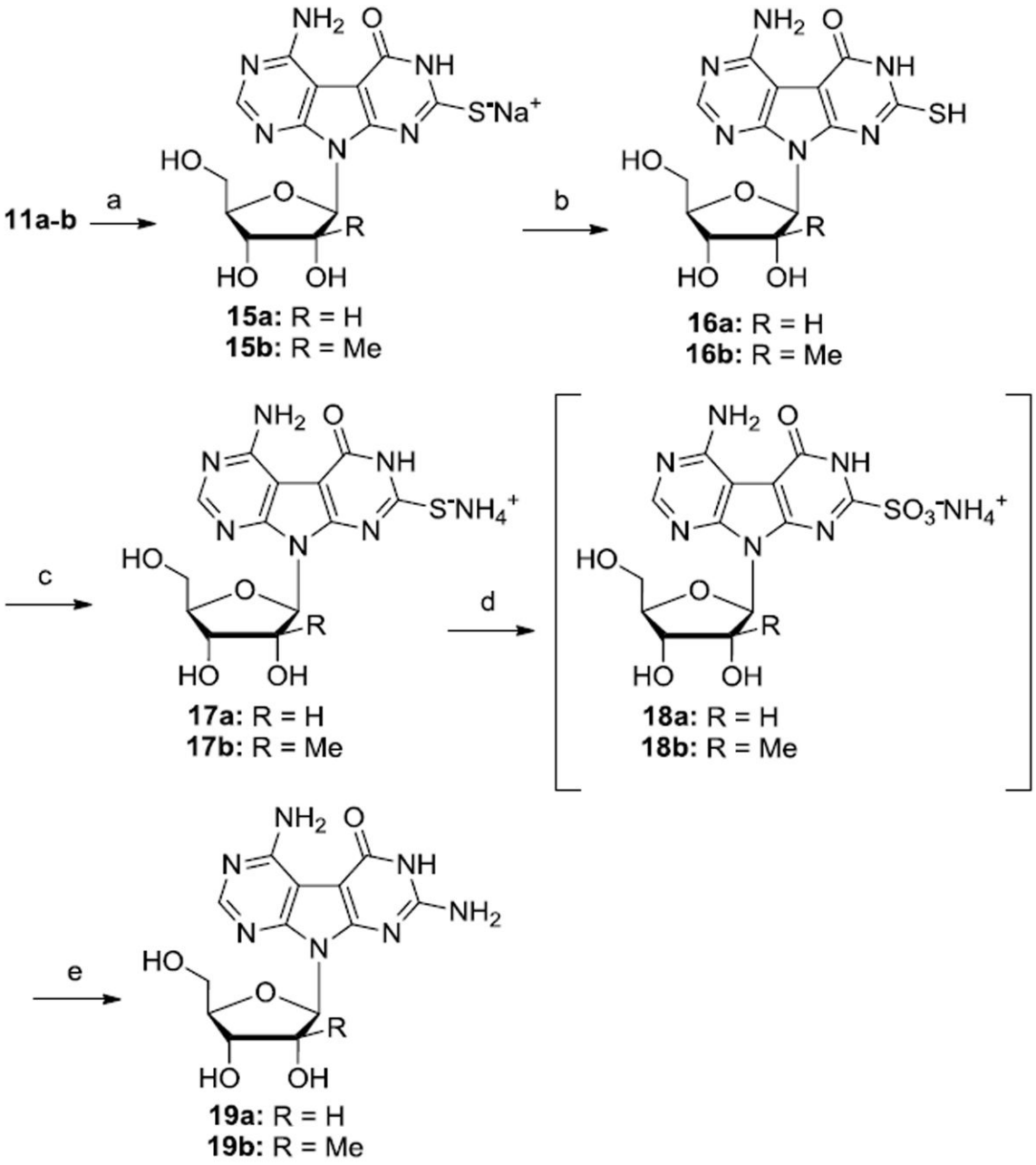
Scheme 1.

Reagents and conditions: (a) (i) **6**, BSA, CH_3CN, rt, 20 min; (ii) **7**, TMSOTf, 80-85°C, 3 h, **8a**: 71%, **8b**: 65%; (b) NH_3/MeOH, overnight, **9a**:90%, **9b**:92%; (c) NH_3(l), 110 °C, **10a**: 64%; **10b**: 66%; (d) NH_2OH, EtOH, reflux, 17 h, **11a**: 68%; **11b**: 81%; (e) $(EtO)_2CO$, NaOEt, reflux, 30 h, **12a**: 51%; **12b**: 46%.



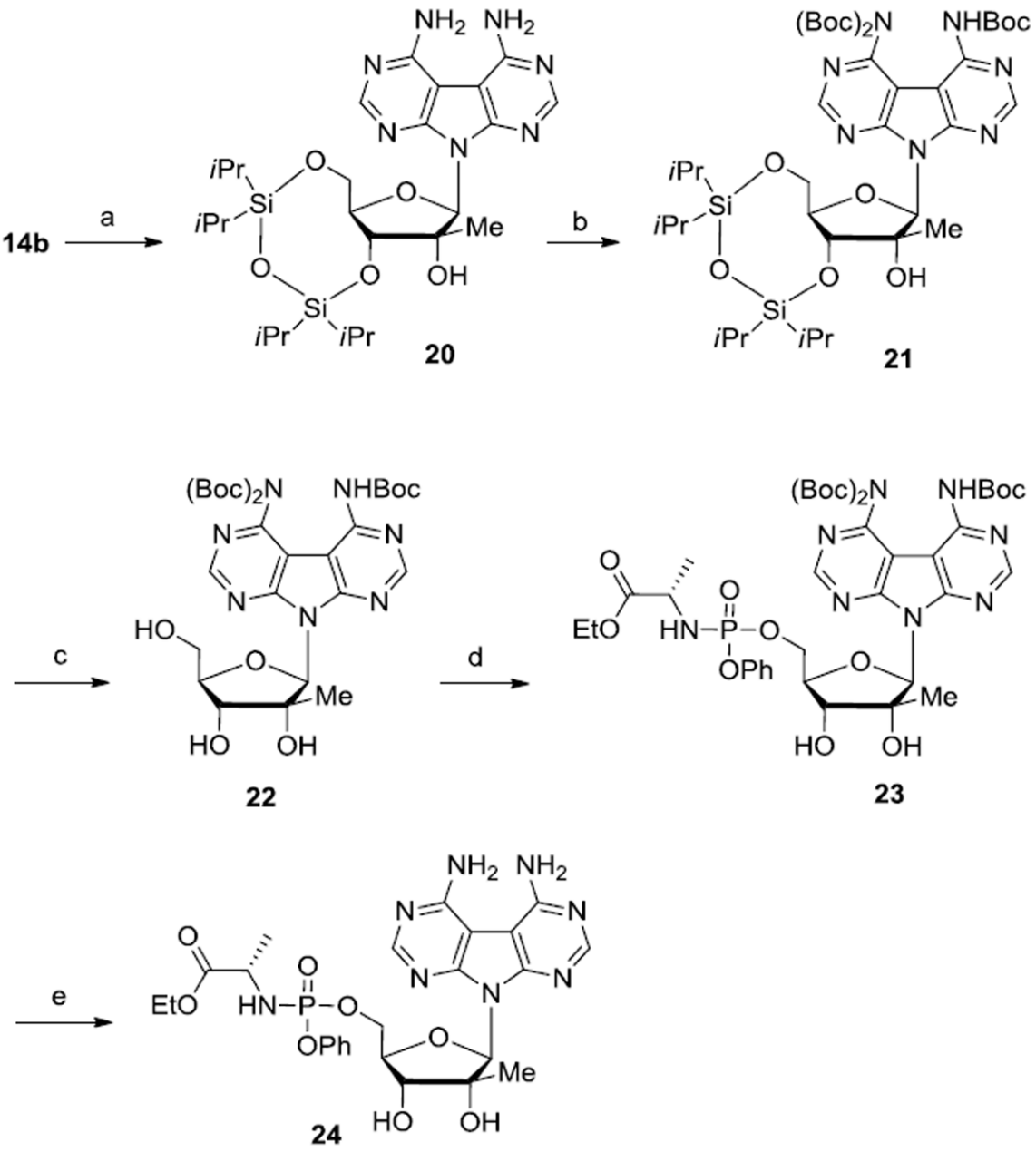
Scheme 2.

Reagents and conditions: (a) $AcOCH(OEt)_2$, reflux, 2 h; (b) (i) NH_3/MeOH, rt, 24 h; (ii) 25% HOAc, rt, 30 h for **13a**, 72 h for **13b**; (iii) 1N NaOH, **14a**:33%; **14b**:36%.



Scheme 3.

Reagents and conditions: (a) Carbon disulfide, NaOH, MeOH, 160-180 °C, 3 h, **15a**: 100%; **15b**: 100%; (b) 1N HCl, **16a**: 62%; **16b**: 51%; (c) NH_4OH, rt; (d) H_2O_2, 0 °C;(e) NH_3(l), 0 °C, then 120 °C, 3 h, over three steps, **19a**: 40%; **19b**: 35%.



Scheme 4.

Reagents and conditions: (a) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, pyridine, rt, 72%; (b) $(Boc)_2O$, DMAP, THF, rt, 30 h, 51%; (c) $Et_3N.3HF$, THF, rt, 15 h, 55%; (d) phenyl(ethoxy-L-alaninyl)phosphorochloridate, *t*-BuMgCl, THF, rt, overnight; (e) 80% aq. TFA, rt, 2 h, 33% over two steps.

Table 1

In vitro antiviral activity and cytotoxicity of compounds **12a-b, 14a-b, 19a-b, 9a-b, 10a-b, 11a-b** and phosphoramidate prodrug **24**.[a]

Cmpd	Janus Type	Anti-HCV activity (μM)		rRNA (μM)	Anti-HIV-1 activity (μM)		Cytotoxicity, CC_{50} (μM)		
		EC_{50}	EC_{90}	CC_{50}[b]	EC_{50}	EC_{90}	PBM	CEM	Vero
12a	**J-AU**	5.7	9.4	5.7	6.8	16.5	51.8	7.9	13.4
12b	**J-AU**	> 10	> 10	> 10	> 100	> 100	> 100	>100	> 100
14a	**J-AA**	> 10	> 10	> 10	> 100	> 100	53.8	29.5	82.5
14b	**J-AA**	> 10	> 10	> 10	≥100	≥100	> 100	>100	> 100
19a	**J-GA**	3	22.5	< 10	9.4	24.0	3.1	20.2	63.1
19b	**J-GA**	~ 3	17.9	~ 3	3.3	> 100	92.1	36.5	> 100
24	**NA**	> 10	>10	>10	ND	ND	ND	ND	ND
9a	**NA**	> 10	> 10	> 10	2.7	16.2	10.5	65.1	87.3
9b	**NA**	> 10	> 10	> 10	>100	> 100	> 100	> 100	> 100
10a	**NA**	0.2	0.3	0.15	0.38	1.1	0.10	ND	0.96
10b	**NA**	6.8	21.8	~ 10	24.7	56.0	4.9	10.0	> 100
11a	**NA**	0.5	2.4	~ 10	0.49	1.5	3.1	<1.0	7.6
11b	**NA**	> 10	> 10	> 10	> 100	> 100	> 100	> 100	> 100
16a	**NA**	> 10	> 10	> 10	9.6	30.2	2.9	8.2	43.1
16b	**NA**	> 10	> 10	> 10	> 100	> 100	> 100	> 100	> 100
AZT	**NA**	> 10	> 10	> 10	0.0040	0.028	> 100	14.3	53.0

[a] All values are based on mean of replicate assays (n ≥ 2)

[b] CC_{50}: cytotoxic concentration that reduced the rRNA levels by 50% at 120 h.

ND: not determined.

Bioorg Med Chem Lett. Author manuscript; available in PMC 2014 June 01.