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Cyclic monophosphate prodrugs of base-modified 2′-*C*-methyl ribonucleosides as potent inhibitors of hepatitis C virus RNA replication

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Abstract—A new series of heterobase-modified 2′-*C*-methyl ribonucleosides was synthesized and tested as inhibitors of hepatitis C virus (HCV) RNA replication. The nucleosides showed a weak inhibitory activity in a HCV replicon system (EC_{50} = 92 μM) and did not exhibit any cytotoxicity (CC_{50} > 300 μM). Cyclic monophosphate (cMP) prodrugs of the same nucleosides were synthesized and also tested in the HCV replicon system. Prodrugs exhibited strong potency (EC_{50} = 0.008 μM) without significant cytotoxicity (CC_{50} > 50 μM).

The hepatitis C virus (HCV) was identified in the late 1980s as the etiological agent of non-A, non-B hepatitis.[1] HCV is a positive-stranded RNA virus in the family of *Flaviviridae*. It is estimated that more than 170 million people are infected by HCV worldwide. In the majority of cases (70–80%), the immune system is not capable of clearing the infection which results in a chronic infection. Such persistent infection can lead to end-stage liver diseases such as cirrhosis and hepatocellular carcinoma in a proportion of patients (up to 20%).[2] The FDA-approved HCV therapies include interferon (IFN) monotherapies and combinations of interferon-α, usually pegylated IFN-α, with ribavirin. However, efficacy is less than ideal as only about 52–54% of patients achieve a sustained virological response. Treatment is also associated with severe side effects, so there exists an urgent need for better antiviral agents to fight chronic HCV infection.

Historically, nucleoside derivatives have been successfully employed as antiviral agents, especially in the treatment of HIV, HBV, and HCV. Nucleoside inhibitors are usually phosphorylated to their triphosphate derivatives, and act as competitive substrate analogs, which can cause premature chain termination during replication of the viral genome. In the last few years, several novel nucleoside analogs have been described in the literature[3] (compounds **1**–**4**) which exhibited respectful cell-based activities against HCV, EC_{50} values in the range of 0.2–13.0 μM, without any significant cytotoxicity (Fig. 1).

It was recently reported that compound **3** (MK-0608, Merck)[4] dosed in chimpanzees infected with hepatitis C virus demonstrated robust suppression of viral replication.[5] NM283 (Idenix), a prodrug of compound **4** (currently in phase II clinical development for the treatment of chronic hepatitis C), has demonstrated dose-related viral load reduction alone and in combination with pegylated interferon.[6]

To explore this class of 2′-methyl ribonucleosides which has exhibited a relatively good safety profile, two novel nucleosides[7] were synthesized. These nucleosides showed very low (**5**, EC_{50} = 300 μM) to weak anti-HCV replicon activity (**6**, EC_{50} = 92 μM), but no cytotoxicity was observed (CC_{50} > 300 μM). In contrast to compounds **1**–**4**, compounds **5** and **6** were not substrates for adenosine kinase, which might indicate that intracellular formation of the monophosphate (MP), and ultimately the triphosphate, may be inefficient. To obtain MPs **7** and **8,** the corresponding nucleosides (**5** and **6**)

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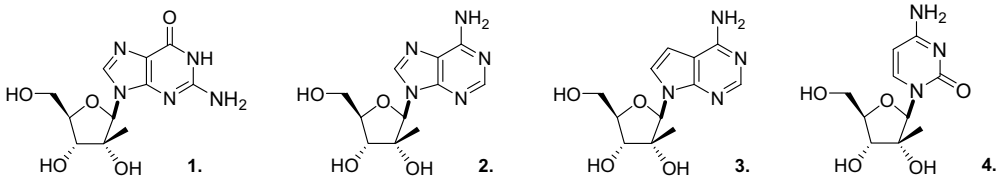


Figure 1. The most advanced nucleoside inhibitors of hepatitis C virus RNA replication: **1**, 2′-*C*-methylguanosine; **2**, 2′-*C*-methyladenosine; **3**, 7-deaza-2′-*C*-methyladenosine; **4**, 2′-*C*-methylcytosine.

were reacted with $POCl_3$ in the presence of $PO(OMe)_3$ (Scheme 1). The cMPs **9** and **10** were synthesized by ring closure with DCC in pyridine. The MPs **7** and **8** were tested against the HCV replicon, and the activities were better than those of the nucleosides (EC_{50} = 100 and 23.8 μM, respectively) despite diminishing cell penetration capabilities. By synthesizing cyclic monophosphates (cMP, **9** and **10**), the polarity of the compounds was reduced and the potency against the HCV replicon was further enhanced (EC_{50} = 68.5 and 1.7 μM, respectively). These data suggest that intracellular formation of MP from nucleosides **5** and **6** is limited.

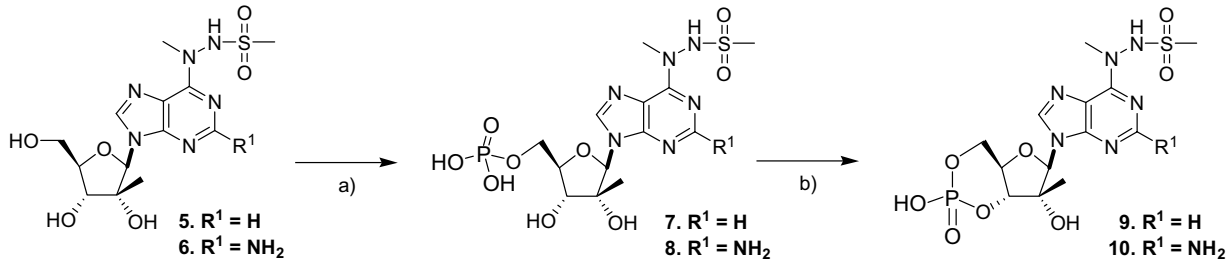
To enhance the cell permeability characteristics of this series, a prodrug strategy was initiated. The *S*-acetyl-2-thioethyl (SATE) MP prodrugs were synthesized and tested for anti-HCV activity, with encouraging results.[7] In this article, the design and synthesis of cMP-prodrugs is described (Table 1). Compounds **11** and **15** were obtained by coupling cMP (**9** or **10**) with chloromethyl pivalate in the presence of DIEA in DMF, while **12** and **16** were generated by coupling the cMP (**9** or **10**) with chloromethyl isopropyl carbonate, also in the presence of DIEA in DMF. For compounds **13**, **14**, **17**, and **18**, the cMP (**9** or **10**) was coupled with *S*-2-hydroxyethyl 2,2-dimethylpropanethioate or *S*-2-hydroxyethyl 2,2-dimethyl-3-propoxypropanethioate (Scheme 2) in the presence of MSNT in pyridine.

The data in Table 1 show that cMP prodrugs of nucleosides **5** and **6** displayed remarkable improvement in HCV replicon inhibition. Enhancement in potency was more than 7000-fold for compound **14** (R^1 = H) versus compound **5** and more than 11,000-fold for compound **18** (R^1=NH_2) vs. compound **6**. The activities observed with SATE MP prodrugs[7] were similar to those of SATE-cMP prodrugs (**13**, **14**, **17**, and **18**). On the other hand, pivaloyloxymethyl (POM)-cMP prodrugs (**12** and **16**) and carbonate-cMP prodrugs (**11** and **15**) had comparable HCV replicon activities. In addition to the extraordinary improvement in HCV replicon activities from the parent nucleosides (**5** and **6**), compounds **12**–**18** showed consistent antiviral activity when tested against BVDV, a surrogate replicon model for HCV. Compounds **12**–**18** were analyzed for cytotoxicity, and no significant cytotoxicity was observed.

Compounds **12**–**18** represent three classes of cMP nucleoside prodrugs with SATE, POM, and carbonate residues which mask the negative charge of cMPs **9** and **10.** All three classes of prodrugs were stable for two hours in simulated intestinal fluid (SIF) and simulated gastric fluid (SGF) stability studies. This good stability in SIF contrasts with the stability observed with 5′-phosphoramidate prodrugs attached to the same nucleosides.[8]

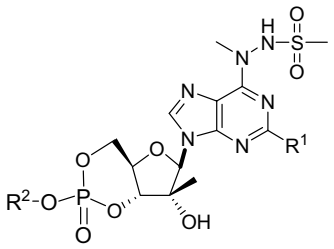
The ultimate goal of this prodrug strategy was to deliver the cMP or MP species to the hepatocyte where it will be trapped due to its charge. To determine the stability of these compounds in plasma, compound **18** was selected for further biological evaluation. After 1 h incubation in rat plasma, only 4% of compound **18** remain intact. The major metabolites were cMP (**10**, 59%), MP (**8**, 19%), and nucleoside (**6**, 18%). In contrast, compound **18** was stable in monkey and human plasma for one hour. These data suggest that compound **18** may reach the hepatocyte intact after intravenous administration to monkeys or humans.

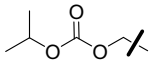
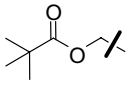
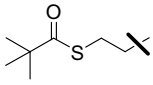
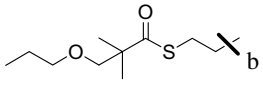
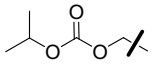
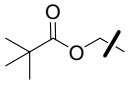
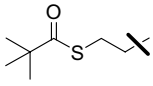
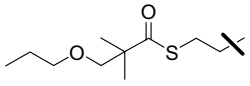
The structure–activity relationship (SAR) analysis of these prodrugs demonstrates that potency is in direct correlation with lipophilicity of the prodrug residue, probably due to better cell penetration capabilities. Additional SAR studies for cMP prodrugs by monitoring cell penetration and/or release of monophosphates in hepatocytes may be warranted. Through our work, a cMP-prodrug approach can potentially become a valuable technology platform for any nucleoside which needs to be delivered intracellularly as a MP.



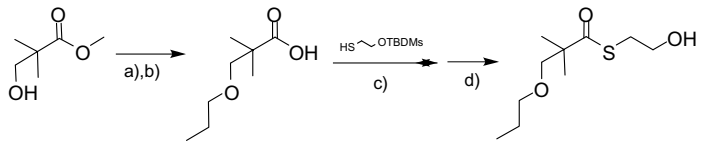
Scheme 1. Reagents and conditions: (a) $POCl_3$, $PO(OMe)_3$, 0 °C, 5 h, 86% for R^1 = H and 72% for R^1 = NH_2; (b) DCC, pyridine, reflux, 6 h, 30% for R^1 = H and 28% for R^1 = NH_2.

Table 1.



Compound	R^1	R^2	HCV EC_{50} (μM)	BVDV EC_{50} (μM)	CC_{50} (μM)
11[a]	H		0.463	0.420	>50
12[b]	H		0.414	0.962	>50
13[c]	H		0.145	0.245	>50
14[c,d]	H		0.039	0.110	>50
15[a]	NH_2		0.163	0.216	>50
16[b]	NH_2		0.092	0.223	>50
17[c]	NH_2		0.022	0.033	>50
18[c,d]	NH_2		0.008	0.016	>50

[a] **9** or **10**, chloromethyl isopropyl carbonate, DIEA, DMF, 60 °C, 29–40%.
[b] **9** or **10**, chloromethyl pivalate, DIEA, DMF, 80 °C, 8 h, 38–41%.
[c] **9** or **10**, *S*-2-hydroxyethyl 2,2-dimethylpropanethioate or *S*-2-hydroxyethyl 2,2-dimethyl-3-propoxypropanethioate, MSNT, pyridine, rt, 48 h, 42–46%.
[d] See Scheme 2 for synthesis of R_2-OH.



Scheme 2. Reagents and conditions: (a) 1-iodopropane, NaH, THF, rt, 12 h, 68%; (b) NaOH, THF/MeOH/H_2O, rt, 12 h, 82%; (c) CDI, DCM, rt, 18 h, 88%; (d) TBAF, THF, rt, 2 h, 94%.

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References and notes

1. Choo, Q.-L.; Kuo, G.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. *Science* **1989**, *244*, 359.
2. Wasley, A.; Alter, M. J. *Semin. Liver Dis.* **2000**, *20*, 1.
3. (a) Eldrup, A. B.; Prhavc, M.; Brooks, J.; Bhat, B.; Prakash, T. P.; Song, Q.; Bera, S.; Bhat, N.; Dande, P.; Cook, P. D.; Bennett, C. F.; Carroll, S. S.; Ball, R. G.; Bosserman, M.; Burlein, C.; Colwell, L. F.; Fay, J. F.; Flores, O. A.; Getty, K.; LaFemina, R. L.; Leone, J.; MacCoss, M.; McMasters, D. R.; Tomassini, J. E.; Von Langen, D.; Wolanski, B.; Olsen, D. B. *J. Med. Chem.* **2004**, *47*, 5284; (b) Pierra, C.; Amador, A.; Benzaria, S.; Cretton-Scott, E.; D'Amours, M.; Mao, J.; Mathieu, S.; Moussa, A.; Bridges, E. G.; Standring, D. N.; Sommadossi, J.-P.; Storer, R.; Gosselin, G. *J. Med. Chem.* **2006**, *49*, 6614.

4. Carroll, S. S.; Tomassini, J. E.; Bosserman, M.; Getty, K.; Stahlhut, M. W.; Eldrup, A. B.; Bhat, B.; Hall, D.; Simcoe, A. L.; LaFemina, R.; Rutkowski, C. A.; Wolanski, B.; Yang, Z.; Migliaccio, G.; De Francesco, R.; Kuo, L. C.; MacCoss, M.; Olsen, D. B. *J. Biol. Chem.* **2003**, *278*, 11979.
5. Carroll, S. S.; Davies, M.; Handt, L.; Koeplinger, L. K.; Zhang, R.; Ludmerer, S.; MacCoss, M.; Hazuda, D.; Olsen, D. *Hepatology* **2006**, *44*(suppl.1), Abs. 935.
6. (a) Pockros, P.; O'Brien, C.; Godofsky, E.; Rodriguez-Torres, M.; Afdhal, N.; Pappas, S.; Lawitz, E.; Bzowej, N.; Rustgi, V.; Sulkowski, M.; Sherman, K.; Jacobson, I.; Chao, G.; Knox, S.; Pietropaolo, K.; Brown, N. *Gastroenterology* **2006**, *130*(suppl 2), Abs. 4; (b) Dieterich, D.; Lawitz, E.; Nguyen, T.; Younes, Z.; Santoro, J.; Gitlin, N.; McEniry, D.; Chasen, R.; Goff, J.; Knox, S.; Kleber, K.; Belanger, B.; Brown, N. A. *Hepatology* **2006**, *44*(suppl.1), Abs. 93.
7. Gunic, E.; Rong F. Patent WO 2006/122207.
8. Gunic, E.; Chow, S.; Rong, F.; Ramasamy, K.; Raney, A.; Li, D.; Huang, J.; Hamatake, K. R.; Hong, Z.; Girardet, J.-L. *Bioorg. Med. Chem. Lett.* **2007**. doi:10.1016/j.bmcl.2007.02.029.