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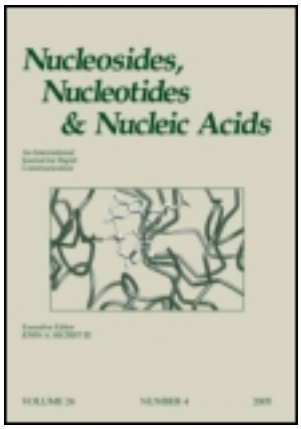


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# SYNTHESIS OF $N^3$,5′-CYCLO-4-(β-D-RIBOFURANOSYL)-VIC-TRIAZOLO[4,5-b]PYRIDIN-5-ONE AND ITS 3′-DEOXYSUGAR ANALOGUE AS POTENTIAL ANTI-HEPATITIS C VIRUS AGENTS

Peiyuan Wang [a], Laurent Hollecker [a], Krzysztof W. Pankiewicz [a], Steven E. Patterson [a], Tony Whitaker [b], Tamara R. McBrayer [b], Phillip M. Tharnish [b], Lieven J. Stuyver [b], Raymond F. Schinazi [c], Michael J. Otto [b] & Kyoichi A. Watanabe [a]

[a] Department of Chemistry, Pharmasset, Inc., Tucker, Georgia, USA

[b] Department of Biology, Pharmasset, Inc., Tucker, Georgia, USA

[c] Department of Pediatrics, Emory University, School of Medicine/Veterans Affairs Medical Center, Decatur, Georgia, USA

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# SYNTHESIS OF $N^3$,5′-CYCLO-4-(β-D-RIBOFURANOSYL)-*vic*-TRIAZOLO[4,5-*b*]PYRIDIN-5-ONE AND ITS 3′-DEOXYSUGAR ANALOGUE AS POTENTIAL ANTI-HEPATITIS C VIRUS AGENTS

**Peiyuan Wang, Laurent Hollecker, Krzysztof W. Pankiewicz, and Steven E. Patterson** ▫ *Department of Chemistry, Pharmasset, Inc., Tucker, Georgia, USA*

**Tony Whitaker, Tamara R. McBrayer, Phillip M. Tharnish, and Lieven J. Stuyver** ▫ *Department of Biology, Pharmasset, Inc., Tucker, Georgia, USA*

**Raymond F. Schinazi** ▫ *Department of Pediatrics, Emory University, School of Medicine/ Veterans Affairs Medical Center, Decatur, Georgia, USA*

**Michael J. Otto** ▫ *Department of Biology, Pharmasset, Inc., Tucker, Georgia, USA*

**Kyoichi A. Watanabe** ▫ *Department of Chemistry, Pharmasset, Inc., Tucker, Georgia, USA*

▫ *We recently discovered a novel compound, identified as $N^3$,5′-cyclo-4-(β-D-ribofuranosyl)-vic-triazolo[4,5-b]pyridinin-5-one, with anti-hepatitis C virus (HCV) activity in vitro. The structure was confirmed by chemical synthesis from 2-hydroxy-5-nitropyridine. It showed anti-HCV activity with $EC_{50}$=19.7 μM in replicon cells. Its 3′-deoxy sugar analogue was also synthesized, but was inactive against HCV in vitro.*

## INTRODUCTION

Since hepatitis C virus (HCV) is responsible for the second most common cause of viral hepatitis, much attention has been given toward the discovery of clinically useful anti-HCV agents. HCV is an RNA virus that replicates without the

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Address correspondence to Peiyuan Wang, Department of Chemistry, Pharmasset, Inc., Tucker, GA 30084, USA.



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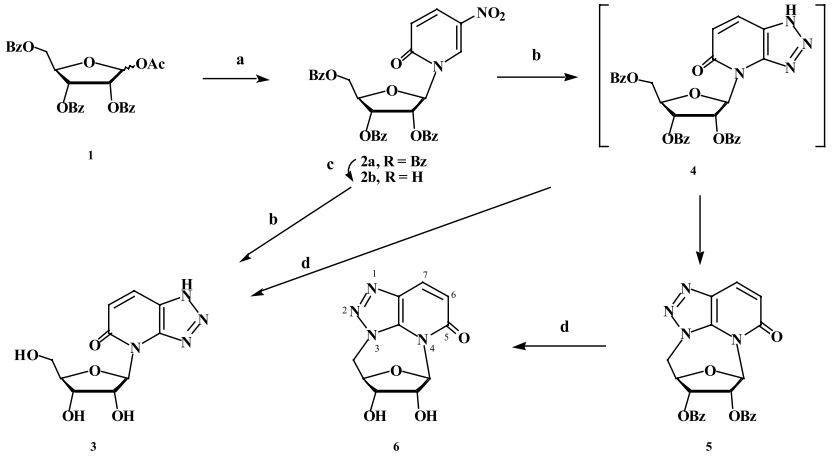
involvement of DNA. It is one of the most important Flaviridae infections in humans and an estimated 170 million people worldwide are HCV carriers.[1] Currently, there is no effective cure for this disease and the only medicines available are alpha interferon, either alone or in combination with ribavirin.[2] However, the therapeutic value of these treatments has been compromised largely due to adverse effects,[2,3] which highlights the need for the development of additional options for treatment.

## RESULTS AND DISCUSSION

### Chemistry

Recently, a novel HCV agent was identified by our group as $N^3$,5′-cyclo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one[4] from Pharmasset's compound library (**6**, Scheme 1). Compound **6** was synthesized from the known 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5-nitropyridin-2-one (**2a**),[5] which was obtained in 70% yield by condensation of the trimethylsilylated pyridine with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**1**) under Vorbrüggen's conditions. The $^1$H NMR spectrum of the product was consistent with those previously reported.[5] The benzoyl protecting groups were removed and 1-(β-D-ribofuranosyl)-5-nitropyridin-2-one (**2b**) was obtained in 81% yield. Treatment of **2b** with sodium azide in DMF at 110–120°C for 12 hr afforded the *vic*-triazolopyridine nucleoside **3** in 60% yield. Reaction of **2a** with $NaN_3$ in DMF at 80–95°C afforded only 4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (**4**). However, **5** was produced when the reaction temperature was increased to 110°C or higher with the concomitant




**SCHEME 1** Reagent: (a) silylated 5-nitropyrimidine-2-one/$SnCl_4$/DCE; (b) $NaN_3$, DMF, (at 110–120°C, 12 h, from **2b** to **3**; at 80–95°C, 24 h $NaN_3$, DMF, from **2a** to **4**; at 110–120°C, 48 h, from **2a** to **5**); (c) $NH_3$/MeOH, rt; (d) 0.5 M NaOMe in MeOH, rt.

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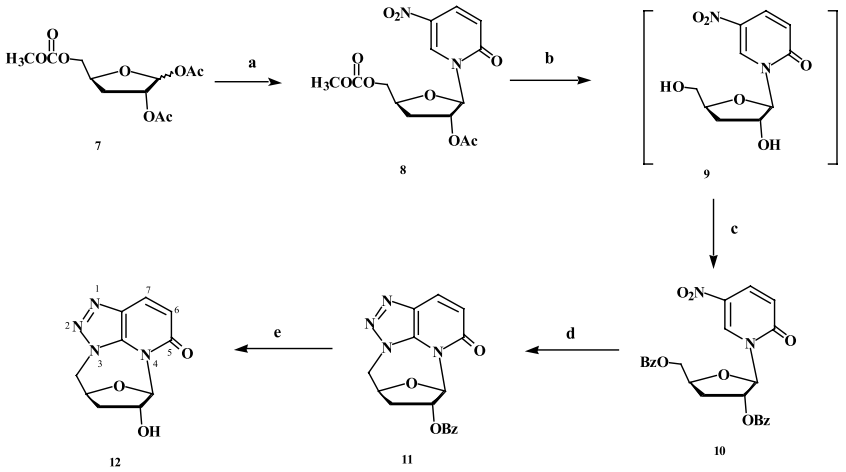

**SCHEME 2** Reagent: (a) silylated 5-nitropyrimidine-2-one/$SnCl_4$; (b) NaOMe/MeOH; (c) BzCl/Py; (d) $NaN_3$, DMF, 115°C, 4 d; (e) NaOMe/MeOH.

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decrease of **4**. Compound **5** was obtained in 44% yield after prolonged heating. Saponification of **5** with MeONa/MeOH furnished **6** in 62% yield.

The 3′-deoxy analogue **12** was synthesized by the condensation of 1,2-*O*-acetyl-5-*O*-methoxycarbonyl-3-deoxy-D-glyceropentofuranose (**7**, Scheme 2) with 5-nitropyridine-2-one to afford **8** in 79.5% yield as the first step. The methoxycarbonyloxy group at C-5′ was not a good leaving group, and underwent saponification upon treatment with two equivalent of NaOMe/MeOH to produce the free nucleoside **9**. Without purification, **9** was benzoylated to **10** in 97% yield from **8**. Treatment of **10** with 1.5 equivalent of $NaN_3$ in DMF at 115°C for 4 days gave **11**. Saponification of **11** with 1.5 equivalent of NaOMe/MeOH at room temperature for 1 hr afforded **12** in 62% yield as colorless solid after silica gel chromatographic purification with a stepwise gradient of MeOH (0 to 4%) in $CH_2Cl_2$.

## Antiviral Assay

Compound **6** was evaluated in the HCV subgenomic RNA provided by Apath LLC (St. Louis, MO).[6] Compound **6** had an anti-HCV effect with an $EC_{50}$=19.7 μM and $EC_{90}$=79.8 μM. In addition to the antiviral effect, ribosomal RNA was also reduced. Huh7 replicon cells were kept in culture for 7 days, either in presence (100 μM) or in absence of the compound. These experiments showed that **6** caused a cytostatic effect at high concentrations ($CC_{50}$ = 30.6 μM). Concomitantly with the slower cell proliferation, a significant decrease in intracellular HCV RNA was observed. Compound **6** does not inhibit purified HCV RNA-dependent RNA polymerase (NS5B) in vitro when tested up to 100 μM.[7] In addition, **6** was tested against a range of other RNA viruses including Influenza viruses A and B, respiratory syncytial virus, rhinovirus, parainfluenza virus, Pinchinde virus, Venezuelian equine encephalitis virus, yellow fever, West Nile,

adenovirus type 1, Punta Toro A, hepatitis B virus, and bovine viral diarrhea virus. The compound was inactive and generally nontoxic,[8–10] with the exception of weak activity against influenza B virus with an $EC_{50}$ of 28 μM. Compound **12** showed no activity in the HCV replicon system. As expected, **6** was inactive against DNA viruses, such as HIV-1 in primary human lymphocytes and HSV type 1 in Vero cells.[9]

## CONCLUSIONS

A novel, potential anti-HCV agent was discovered from the Pharmasset compound library. The structure was identified as $N^3$,5′-cyclo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (**6**) and confirmed by chemical synthesis. Compound **6** inhibited production of HCV-RNA, in the HCV-subgenomic replicon cell line (Huh7 cells) with $EC_{50} = 19.7$ μM and $CC_{50} = 30.6$ μM. Compound **6** did not inhibit HCV-RNA polymerase in vitro, suggesting that this nucleoside does not interact with this viral enzyme. The 3′-deoxy analogue **12** was synthesized and it showed no antiviral activity in the replicon system, suggesting that the presence of a 3′-OH group was important for anti-HCV activity.

## REFERENCES


1. Alter, M.J.; Kruszon-Moran, D.; Nainan, O.V.; McQuillan, G.M.; Gao, F.; Moyer, L.A.; Kaslow, R.A.; Margolis, H.S. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. N. Engl. J. Med. **1999**, *341*, 556–562.
2. Collier, J.; Chapman, R. Combination therapy with interferon-alpha and ribavirin for hepatitis C: practical treatment issues. BioDrugs **2001**, *15*, 225–238.
3. Di Bisceglie, A.M.; McHutchison, M.J.; Rice, C.M. New therapeutic strategies for hepatitis C. Hepatology **2002**, *285*, 224–231.
4. Wang, P.Y.; Hollecker, L.; Pankiewicz, K.P.; Patterson, S.E.; Whitaker, T.; McBrayer, T.R.; Tharnish, P.M.; Stuyver, L.J.; Otto, M.J.; Schinazi, R.F.; Watanabe, K.A. Synthesis of $N^3$,5′-Cyclo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one, a novel compound with potential anti-hepatitis C virus activity. J. Med. Chem. **2004**, *in press*.
5. Lynch, B.M.; Sharma, S.C. Synthesis, structural and conformational assignments, and conversions of pyridine and triazopyridine nucleosides. Can. J. Chem. **1976**, *54*, 1029–1038.
6. Stuyver, L.J.; McBrayer, T.R.; Tharnish, P.M.; Hassan, A.E.A.; Chu, C.K.; Pankiewicz, K.W.; Watanabe, K.A.; Schinazi, R.F.; Otto, M.J. Dynamics of subgenomic hepatitis C virus replicon RNA levels in Huh-7 cells after exposure to nucleoside antimetabolites. J. Virol. **2003**, *77*, 10689–10694.
7. Stuyver, L.J.; Whitaker, T.; McBrayer, T.R.; Hernandez, B.I.; Lostia, S.; Tharnish, P.M.; Ramesh, M.; Chu, C.K.; Shi, J.; Jordan, R.; Watanabe, K.A.; Rachakonda, S.; Otto, M.J.; Schinazi, R.F. Ribonucleoside analogue that blocks replication of bovine viral diarrhea and hepatitis C virus in culture. Antimicrob. Agents Chemother. **2003**, *47*, 244–254.
8. Barnard, D.L.; Hubbard, V.D.; Burton, J.; Smee, D.F.; Morrey, J.D.; Otto, M.J.; Sidwell, R.W. Inhibition of severe acute respiratory syndrome-associated coronavirus (SARSCoV) by calpain inhibitors and β-D-N4-hydroxycytidine. Antivir. Chem. Chemother. **2004**, *15*, 15–22.
9. Schinazi, R.F.; Sommadossi, J.P.; Saalmann, V.; Cannon, D.L.; Xie, M.-W.; Hart, G.C.; Smith, G.A.; Hahn, E.F. Activity of 3′-azido-3′-deoxythymidine nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1. Antimicrob. Agents Chemother. **1990**, *34*, 1061–1067.
10. Stuyver, L.J.; Lostia, S.; Adams, M.; Mathew, J.; Pai, B.S.; Grier, J.; Tharnish, P.; Choi, Y.; Chong, Y.; Choo, H.; Chu, C.K.; Otto, M.J.; Schinazi, R.F. Antiviral activities and cellular toxicities of modified 2′,3′-dideoxy-2′,3′-didehydrocytidine analogues. Antimicrob. Agents Chemother. **2002**, *46*, 3854–3860.


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