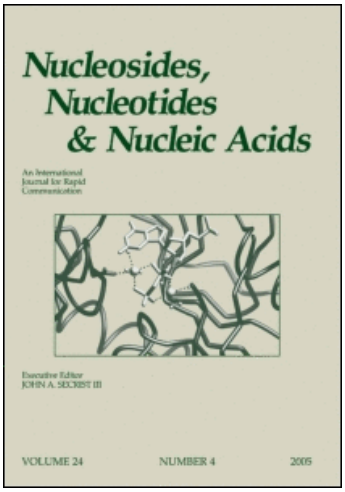

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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

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To cite this Article Wang, Peiyuan , Hollecker, Laurent , Pankiewicz, Krzysztof W. , Patterson, Steven E. , Whitaker, Tony , McBrayer, Tamara R. , Tharnish, Phillip M. , Stuyver, Lieven J. , Schinazi, Raymond F. , Otto, Michael J. and Watanabe, Kyoichi A.(2005) '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', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 957 — 960
To link to this Article: DOI: 10.1081/NCN-200059314
URL: http://dx.doi.org/10.1081/NCN-200059314

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):957–960, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200059314

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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

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▫ *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

Supported in parts by NIH grants 1R43 AI-52868 (biology) and 1 R43 AI-056720 (chemistry). We would like to acknowledge the contribution of D. L. Barnard, J. D. Morrey, and D. F. Smee at the Institute for Antiviral Research at Utah State University for their testing of RNA viruses under NIH Contract No. N01-A1-85348. Dr. Schinazi is a founder and consultant of Pharmasset, Inc. His laboratory did not receive any funding from Pharmasset, Inc.

Parts of this work were presented at the International Society for Nucleosides, Nucleotides & Nucleic Acids, XVI International Roundtable, Minneapolis, Minnesota, USA, September 12–16, 2004.

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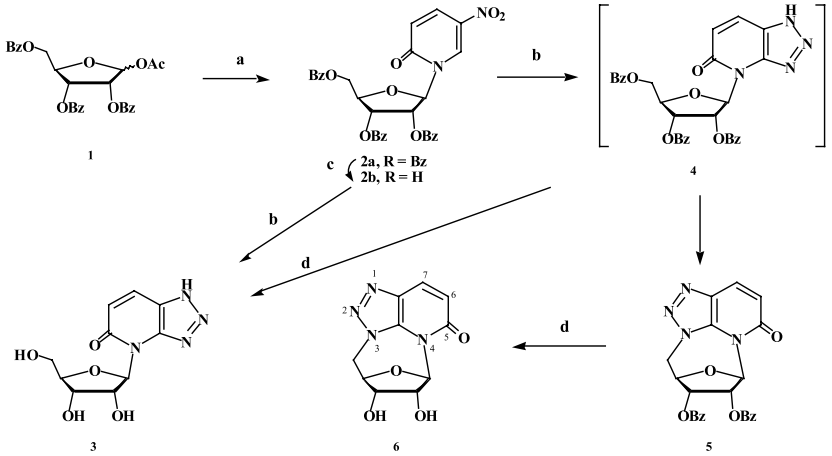
involvement of DNA. It is one of the most important Flaviridae infections in humans and an estimated 170 million people worldwide are HCV carriers.[1] Currently, there is no effective cure for this disease and the only medicines available are alpha interferon, either alone or in combination with ribavirin.[2] 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.

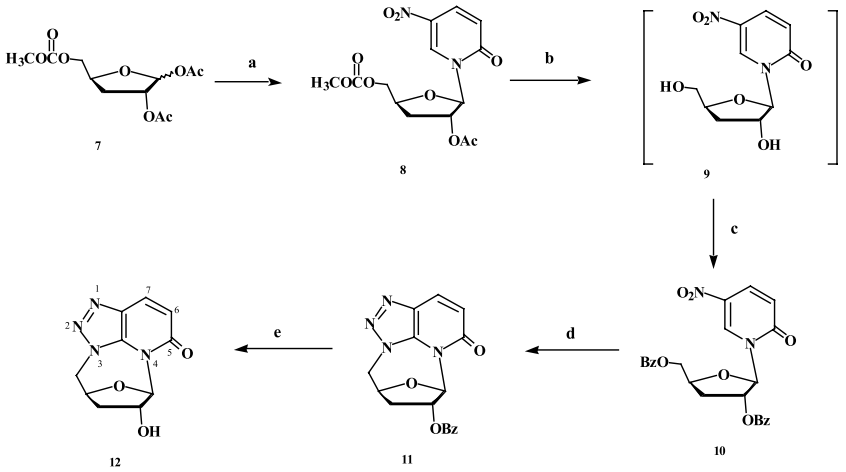
RESULTS AND DISCUSSION

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.



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.

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.

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