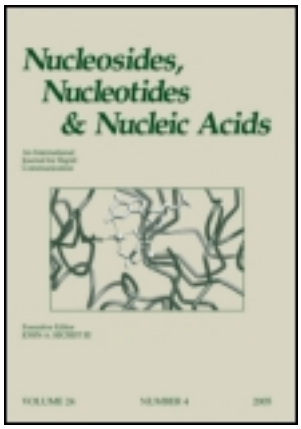

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NM 283, AN EFFICIENT PRODRUG OF THE POTENT ANTI-HCV AGENT 2′-C-METHYLCYTIDINE

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NM 283, AN EFFICIENT PRODRUG OF THE POTENT ANTI-HCV AGENT 2′-*C*-METHYLCYTIDINE

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▫ *In order to improve the oral bioavailability of 2′-C-methylcytidine, a potent anti-HCV agent, the corresponding 3′-O-L-valinyl ester derivative (NM 283) has been synthesized. Based on its ease of synthesis and its physicochemical properties, NM 283 has emerged as a promising antiviral drug for treatment of chronic HCV infection.*

Keywords Hepatitis C Virus, 2′-*C*-Methyl Branched Nucleosides, Prodrugs

INTRODUCTION

Hepatitis C virus (HCV) has infected an estimated 170 million individuals, 3% of the world's population. There is no vaccine available against HCV, and current therapies, subcutaneous interferon-α (IFN-α) monotherapy and IFN-α with oral ribavirin combination, are expensive, often poorly tolerated, and effective only in half of the patients population.

In our search for improved therapeutic agents against chronic hepatitis C, a ribonucleoside analog, 2′-*C*-methylcytidine **4**, was discovered to be a potent and

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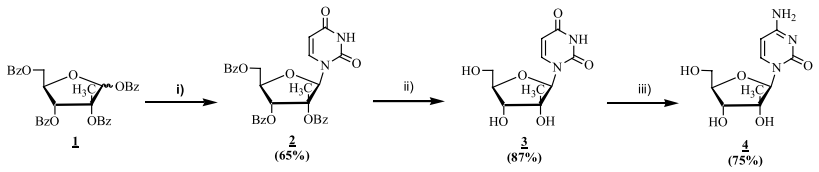


(i) bis(trimethylsilyl)acetamide, uracil, $SnCl_4$, CH_3CN, reflux; (ii) CH_3ONa, CH_3OH, room temperature (rt); (iii) a) trimethylsilyl chloride, *N*-methylpyrrolidine, CH_3CN, rt; b) trifluoroacetic anhydride, 0ºC; c) 4-nitrophenol, 0ºC; d) NH_4OH, dioxane, 50ºC

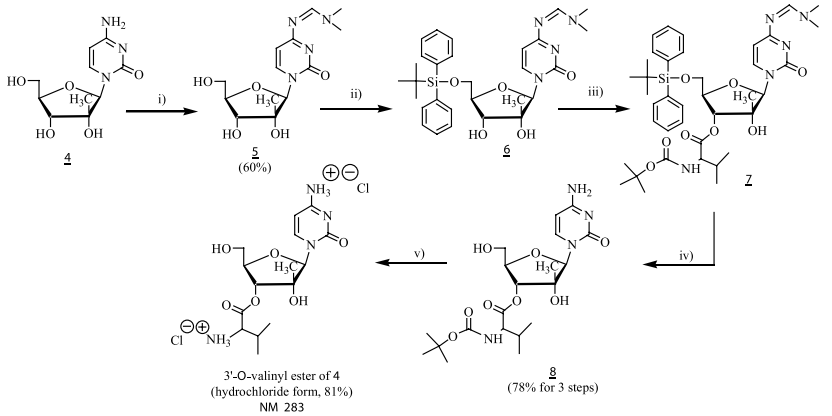
SCHEME 1 Synthesis of 2′-*C*-methylcytidine.

selective inhibitor in cell culture of a number of related RNA viruses, including bovine viral diarrhea virus (BVDV), yellow fever virus (YFV), and West Nile virus (WNV).[1,2] In order to improve the oral bioavailability of 2′-*C*-methylcytidine, its 3′-*O*-L-valinyl ester derivative (NM 283) has been synthesized.

We report here the chemical syntheses of both 2′-*C*-methylcytidine **4** and NM 283, as well as some physicochemical characteristics of NM 283.

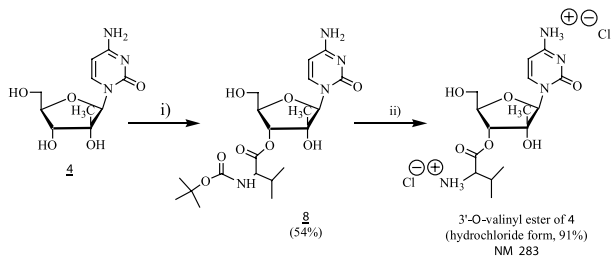
CHEMISTRY

2′-*C*-methylcytidine **4** was synthesized using a strategy related to that reported by Harry-O'kuru et al.[3] The precursor sugar **1** was easily accessible from commercially available material. Condensation of this peracylated sugar **1** with uracil, followed by removal of benzoyl groups under basic conditions, led to 2′-*C*-methyluridine **3**. Conversion of **3** *via* transient generation of its 4-nitrophenoxy



(i) $(CH_3)_2NCH(OCH_3)_2$, dimethylformamide (DMF), rt, 1h30; (ii) tert-butyldiphenylchlorosilane, imidazole, pyridine, rt, 6h; (iii) L-Boc-valine, DEC, DMAP, tetrahydrofuran (THF)/DMF, rt, 2 days; (iv) NH_4F, CH_3OH, reflux, 3h; (v) HCl/ethyl acetate, rt

SCHEME 2 Synthesis of NM 283.



(i) L-Boc-valine, carbonyldiimidazole, DMAP, triethylamine, DMF/THF;
(ii) HCl/ethanol

SCHEME 3 Alternative route to the synthesis of NM 283.

derivative,[4] followed by substitution with aqueous ammonia, led to the desired 2′-*C*-methylcytidine **4** (Scheme 1).

A multi-step sequence involving dimethylaminomethylene protection[5] of the exocyclic amino function, silylation[6] of the 5′-hydroxyl group, condensation with *N*-tert-butyloxycarbonyl-L-valine (L-Boc-valine) using the coupling agent *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (DEC) in the presence of 4-dimethylaminopyridine (DMAP),[7] and total deprotection,[8] led to NM 283 as its dihydrochloride salt (Scheme 2).

In our search for a more direct and scalable synthetic process, we then developed a new synthetic strategy for the preparation of NM 283, based on the direct condensation of **4** with L-Boc-valine, followed by removal of the *N*-tert-butyloxycarbonyl group, thus reducing the number of the synthetic steps from 5 to 2. This time and labor saving process could be accomplished without involvement of chromatographic purifications and NM 283 was obtained in high purity following simple crystallization (Scheme 3).

PHYSICOCHEMICAL PROPERTIES

To be considered as a suitable prodrug for oral administration, NM 283 should possess adequate solubility in aqueous media in order to dissolve in the small intestine, and thus be available for absorption. Aqueous solubility of NM 283 has been determined in comparison with the solubility of the parent nucleoside **4**. Both compounds are highly soluble in aqueous media (32 g/L for **4** and 423 g/L for NM 283), commensurate with their low distribution coefficient (log P) values: −0.965 and −1.34 for **4** and NM 283, respectively.

Stability studies of NM 283 were also carried out in order to determine whether it would be sufficiently chemically stable in biological fluids and in the gastrointestinal tract before its absorption. NM 283 appeared to be fully stable at pH 1.2, but is hydrolyzed at pH 4.5 and 7.2 into the parent drug **4** following first-order kinetics. The half-life of NM 283 at pH 4.5 and 7.2 is 6.1 days and 3.9 h, respectively.

CONCLUSION

NM 283, the 3′-*O*-valinyl ester derivative of 2′-*C*-methylcytidine, has been synthesized in order to improve the oral bioavailability of the parent drug 2′-*C*-methylcytidine. Based on its ease of synthesis and its physicochemical properties, NM 283 has emerged as a promising antiviral agent for chronic HCV infection. Further studies have revealed a good pharmacokinetic profile in monkey, and promising results in human clinical trials.[9,10]

Expanded testing of NM 283, alone and in combination with interferon, is anticipated.

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