

## Synthesis and Pharmacokinetics of Valopicitabine (NM283), an Efficient Prodrug of the Potent Anti-HCV Agent 2'-C-Methylcytidine

Claire Pierra,<sup>†</sup> Agnès Amador,<sup>†</sup> Samira Benzaria,<sup>†</sup> Erika Cretton-Scott,<sup>‡</sup> Marc D'Amours,<sup>‡</sup> John Mao,<sup>‡</sup> Steven Mathieu,<sup>‡</sup> Adel Moussa,<sup>‡</sup> Edward G. Bridges,<sup>‡</sup> David N. Standring,<sup>‡</sup> Jean-Pierre Sommadossi,<sup>‡</sup> Richard Storer,<sup>§,∇</sup> and Gilles Gosselin<sup>\*,†</sup>

Laboratoire Coopératif Idenix–CNRS–Université Montpellier II, Case Courrier 008, Université Montpellier II, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France, and Idenix Pharmaceuticals Inc., 60 Hampshire Street, Cambridge, Massachusetts 02139, and Laboratoires Idenix SARL, Laboratoire de Chimie Médicinale, Cap Gamma, 1682 Rue de la Valsière, BP 50001, 34189 Montpellier Cedex 4, France

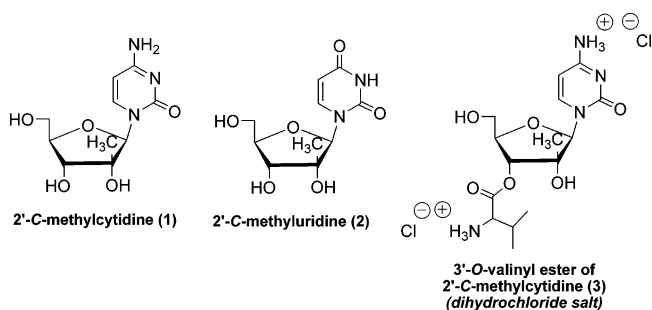
Received March 28, 2006

In our search for new therapeutic agents against chronic hepatitis C, a ribonucleoside analogue, 2'-C-methylcytidine, was discovered to be a potent and selective inhibitor in cell culture of a number of RNA viruses, including the pestivirus bovine viral diarrhea virus, a surrogate model for hepatitis C virus (HCV), and three flaviviruses, namely, yellow fever virus, West Nile virus, and dengue-2 virus. However, pharmacokinetic studies revealed that 2'-C-methylcytidine suffers from a low oral bioavailability. To overcome this limitation, we have synthesized the 3'-O-L-valinyl ester derivative (dihydrochloride form, valopicitabine, NM283) of 2'-C-methylcytidine. We detail herein for the first time the chemical synthesis and physicochemical characteristics of this anti-HCV prodrug candidate, as well as a comparative study of its pharmacokinetic parameters with those of its parent nucleoside analogue, 2'-C-methylcytidine.

### Introduction

Hepatitis C virus (HCV) has infected an estimated 170 million individuals, 3% of the world's population.<sup>1</sup> The virus establishes a persistent infection in the majority of cases, leading to chronic hepatitis that often develops into cirrhosis and, in many cases, causes hepatocellular carcinoma.<sup>1</sup> There is no vaccine available against HCV, and current therapies, namely, pegylated or nonpegylated interferon- $\alpha$  (IFN- $\alpha$ ) monotherapy and combination of IFN- $\alpha$  with oral ribavirin, are expensive, often poorly tolerated, and effective only in half of the patient population.<sup>2,3</sup> Therefore, there is an urgent need to develop new and more effective therapies in response to this important unmet medical need.<sup>4</sup>

In the course of our HCV program, we recently discovered that 2'-C-methylcytidine (**1**, Figure 1) is a potent and selective inhibitor of *Flaviviridae* virus replication in cell culture.<sup>5,6</sup> More particularly, **1** inhibited the replication of the bovine viral diarrhea virus (BVDV, a pestivirus surrogate model for HCV),<sup>7</sup> eliminated persistent BVDV infection at nontoxic concentrations, and was synergistic in combination with interferon- $\alpha_{2b}$  but not with ribavirin.<sup>8</sup> Compound **1** has no activity against human immunodeficiency virus (HIV) or against DNA viruses. In contrast to ribavirin, which is not effective alone in reducing viral RNA levels but stimulates the immune boosting capacity of interferon- $\alpha$  when used in combination, **1** is the first example of an anti-RNA virus agent that is active via a nucleoside analogue mode of action. Thus, in primary human hepatocyte cultures, in a human hepatoma cell line (HepG2), and in a bovine kidney cell line (MDBK), **1** is converted into its major metabolite, 2'-C-methylcytidine-5'-triphosphate, along with smaller amounts of 2'-C-methyluridine-5'-triphosphate, resulting from deamination. The active metabolite 2'-C-methylcytidine



**Figure 1.** Structures of compounds **1**–**3**.

triphosphate is a competitive inhibitor of purified BVDV RNA polymerase in vitro ( $K_i = 160$  nM).<sup>9</sup>

Preliminary pharmacokinetic studies in animals revealed that further development of **1** would be hampered by its low oral bioavailability. To overcome this limitation, we devoted our efforts to the design of 2'-C-methylcytidine prodrugs with more favorable oral absorption profiles. From a literature survey, it was found that a broad variety of amino acid ester derivatives have been studied and successfully employed as nucleoside prodrug forms. More particularly, efficacy of such derivatives has been proved in the case of valacyclovir, the L-valinyl ester of acyclovir (Figure 2).<sup>10,11</sup> After active absorption via peptide transport mechanism, valacyclovir is rapidly and almost completely converted into acyclovir by enzymatic hydrolysis, increasing considerably the oral bioavailability and cellular uptake of the parent drug.<sup>10,11</sup> The efficacy of L-valinyl derivatives has been also demonstrated in the case of ganciclovir (GCV), since valganciclovir (L-valinyl ester of GCV, Figure 2) has an oral bioavailability 10-fold higher than the parent nucleoside.<sup>12,13</sup> Recently, as part of our hepatitis B program, we have synthesized and studied several L-valinyl ester prodrugs of 2'-deoxy- $\beta$ -L-cytidine (L-dC) in order to improve the oral bioavailability of L-dC. Among them, the 3'-O-L-valinyl ester of L-dC (Val-L-dC, valtorcitabine, Figure 2) emerged as the most attractive L-dC prodrug<sup>14</sup> and is currently in phase II clinical studies.<sup>15</sup>

On the basis of these considerations, we decided to synthesize and study the 3'-O-L-valinyl ester derivative (NM283, valop-

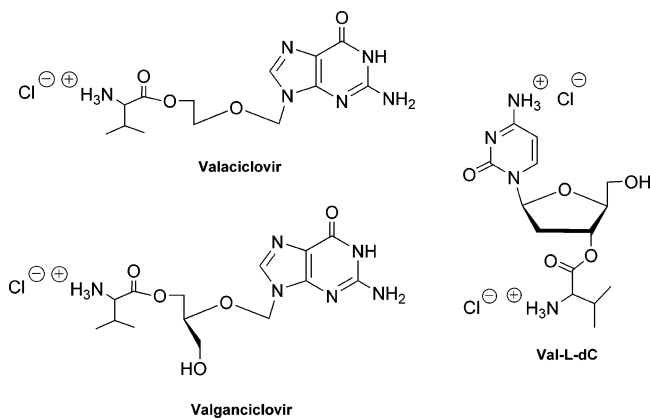
\* Corresponding author. Tel: +33-4-67143855. Fax: +33-4-67549610. E-mail: gosselin@univ-montp2.fr.

<sup>†</sup> Université Montpellier II.

<sup>‡</sup> Idenix Pharmaceuticals Inc.

<sup>§</sup> Laboratoires Idenix SARL.

<sup>∇</sup> Present address: VASTox plc, 91 Milton Park, Abingdon, Oxfordshire, OX14 4RY, U.K.



**Figure 2.** Structures of valaciclovir, valganciclovir, and val-L-dC.

icitabine, **3**; Figure 1) of **1** in order to improve the oral bioavailability of the parent nucleoside.

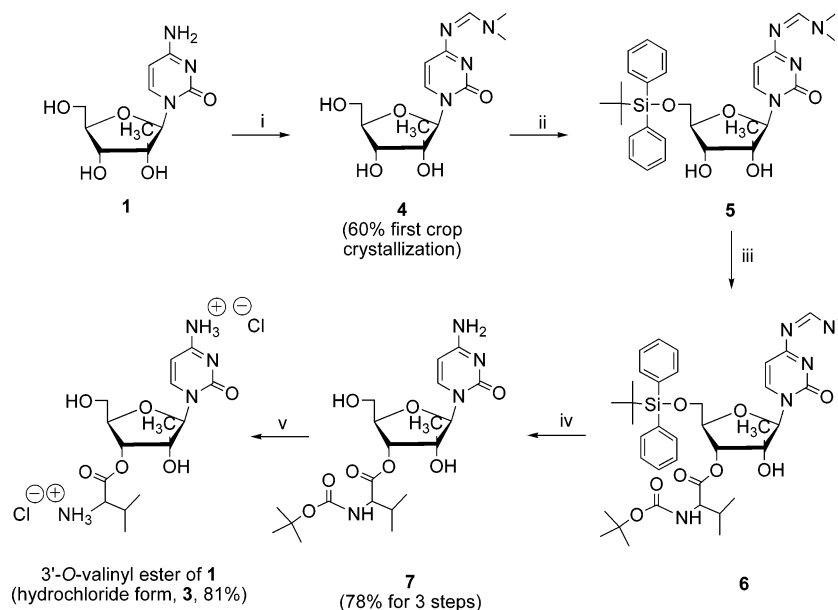
## Results and Discussion

**Synthesis.** The 3'-*O*-valinyl ester prodrug of 2'-*C*-methylcytidine (**3**) was first prepared by the route described in Scheme 1. Our multistep sequence involved successive protections of the exocyclic amino function and of the 5'-hydroxyl group of **1**, followed by condensation with *N*-*tert*-butyloxycarbonyl-L-valine (L-Boc-valine) and, finally, total deprotection. *N,N*-Dialkylformamidines and especially *N,N*-dimethylformamide have been widely used as protective groups for exocyclic amino function of nucleosides.<sup>16,17</sup> In the present work, the synthesis of *N*<sup>4</sup>-[(dimethylamino)methylene]-2'-*C*-methylcytidine (**4**) was carried out following a procedure described by Kerr et al for the protection of **1** using *N,N*-dimethylformamide dimethylacetal.<sup>18</sup> Selective silylation of the 5' primary hydroxyl group using a reported method<sup>19</sup> led to the protected key intermediate **5**. Condensation of **5** with L-Boc-valine using the coupling agent *N*'-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC),<sup>20</sup> with 4-(dimethylamino)pyridine (DMAP) as a catalyst,<sup>11</sup> provided the *N*-Boc-protected ester derivative **6**. Finally, desilylation of **6** with excess ammonium fluoride (NH<sub>4</sub>F) in methanol,<sup>21</sup> an economical alternative to tetrabutylammonium

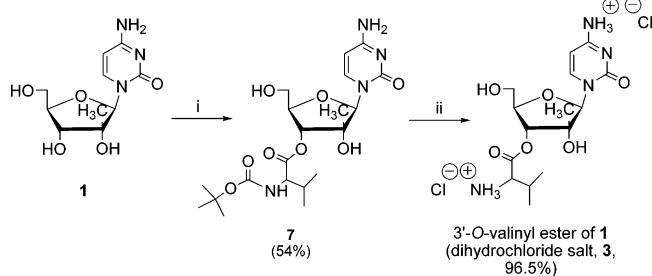
fluoride in tetrahydrofuran (TBAF/THF), followed by acidic hydrolysis using a saturated solution of hydrogen chloride in ethyl acetate,<sup>22</sup> led to the target prodrug **3** as a dihydrochloride salt.

Although this conventional synthetic route afforded the desired prodrug in a satisfactory overall yield of 52%, several chemical difficulties (instability of the formamidine intermediate **4** and partial racemization of the L-valine during the coupling step), as well as cost issues (use of expensive *tert*-butyldiphenylsilyl group), made scale-up using this initial route unattractive. In our search for a more direct and scalable synthetic process, we then developed a new synthetic strategy based on the direct condensation of **1** 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 (Scheme 2). We decided to explore this direct coupling approach after examining a report by McCormick et al, who described the room-temperature reaction of unprotected guanosine with Boc-anhydride in the presence of triethylamine, DMAP, and DMSO. This reaction selectively gave 3'-Boc-guanosine in 55% yield.<sup>23</sup> In our first attempt, we reacted compound **1** in DMSO with carbonyldiimidazole (CDI)-activated L-Boc-valine in the presence of triethylamine and DMAP. The reaction did not progress at all when kept at room temperature. The reaction produced the desired product and several byproducts when heated at 50 °C using 4 equiv of CDI-activated L-Boc-valine. Optimum selectivity was achieved by using 1.1 equiv of CDI-activated L-Boc-valine and running the reaction for only 1 h at 80 °C. We used only 0.1 equiv of DMAP in order to avoid the racemization of the L-valine. Other coupling reagents such as EDC and *N,N*-dicyclohexylcarbodiimide (DCC) did not produce desired product, and DMF was difficult to change due to the insolubility of compound **1** in most organic solvents. HPLC analysis showed 68% of the desired compound, 11% of starting nucleoside **1**, and the two 3',5'/3',*N*<sup>4</sup>-divaliny ester byproducts. Pure product **3** was obtained in 54% yield and 99% purity by using simple acid–base extraction, eliminating the extensive chromatographic step. Thus, this time- and labor-saving alternative process could be accomplished without involvement of chromatographic

### Scheme 1. A Conventional Route for the Synthesis of **3**<sup>a</sup>



<sup>a</sup> Reagents and conditions: (i) Me<sub>2</sub>NCH(OMe)<sub>2</sub>, DMF, rt, 1.5 h; (ii) TBDPSCI, imidazole, pyridine, rt, 6 h; (iii) *N*-Boc-L-valine, EDC, DMAP, CH<sub>3</sub>CN/DMF, rt, 2 d; (iv) NH<sub>4</sub>F, MeOH, reflux, 3 h; (v) HCl/EtOAc, EtOAc, rt.

Scheme 2. Alternative Route for the Synthesis of 3<sup>a</sup>

<sup>a</sup> Reagents and conditions: (i) *N*-Boc-L-valine, CDI, DMAP, TEA, DMF/THF; (ii) HCl/EtOH.

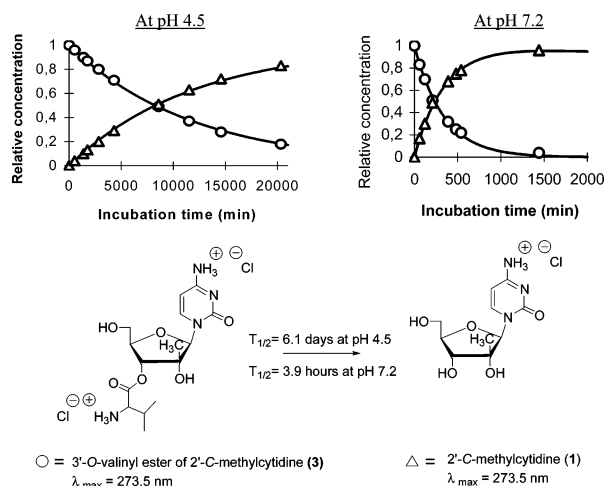


Figure 3. Kinetic curves of hydrolysis of prodrug 3 at pH 4.5 and 7.2.

purifications, and compound 3 was obtained in high purity using simple crystallization.

**Aqueous Solubility, Lipophilicity, and Chemical Stability of Prodrug 3.** To be considered as a suitable prodrug for oral administration, the 3'-*O*-valinyl ester of 2'-*C*-methylcytidine (3) should possess adequate solubility in aqueous media in order to dissolve in the small intestine, thus being available for absorption. The aqueous solubility of 3 has been determined in comparison with the solubility of the parent nucleoside 1. The concentration of saturated solutions of 1 and 3 in water were 32 and 423 g/L, respectively. Both compounds were found to be highly soluble in aqueous media, which has been corroborated by their low distribution coefficient (log *P*) values: -0.965 and -1.34 for 1 and 3, respectively.

The main aim of stability studies was to determine whether the prodrug of 2'-*C*-methylcytidine would be sufficiently chemically stable in the gastrointestinal tract before its absorption. Prodrug 3 appeared to be fully stable at pH 1.2 but was hydrolyzed at pH 4.5 and 7.2 into the parent drug 1 following first-order kinetics. The half-lives of 3 at pH 4.5 and 7.2 were 6.1 days and 3.9 h, respectively (Figure 3). It is noteworthy that 1 was fully stable at all the acidic and neutral studied pHs.

**Stability in Human Blood, Plasma, and Liver Cytosol.** Prodrug 3 was rapidly converted into 1 in both human plasma and whole blood, exhibiting *in vitro* half-life values of 130 and 40 min, respectively. This rapid conversion is primarily due to the presence of esterases in blood and plasma. Conversion of 3 into 1 was also observed in human liver cytosol and S9 fractions, with approximately 30% of 3 converted into 1 within 1 h. Differences in half-life for prodrug 3 in plasma, whole blood,

Table 1. Pharmacokinetic Parameters of Compounds 1 and 3, Following a Single Oral Administration of 3 to Sprague–Dawley Rats<sup>a</sup>

prodrug 3 dose (mg/kg)	compd 1 equiv dose (mg/kg)	drug	AUC (μg h/mL)	T <sub>1/2</sub> (h)	C <sub>max</sub> (μg/mL)	T <sub>max</sub> (h)	F (%)
100	72	3	8.95	0.64	3.62	1.0	—
		1	30.0	7.10	6.12	2.0	33.6

<sup>a</sup> AUC<sub>0–t</sub>: area under the plasma concentration–time curve from time 0 to *t*. T<sub>1/2</sub>: terminal elimination half-life. C<sub>max</sub>: maximum plasma concentration. T<sub>max</sub>: time to maximum plasma concentration. F: apparent oral bioavailability calculated on the basis of a dose-normalized AUC value of 1.24 (μg h/mL) from a single iv dose of 1 to the rat.

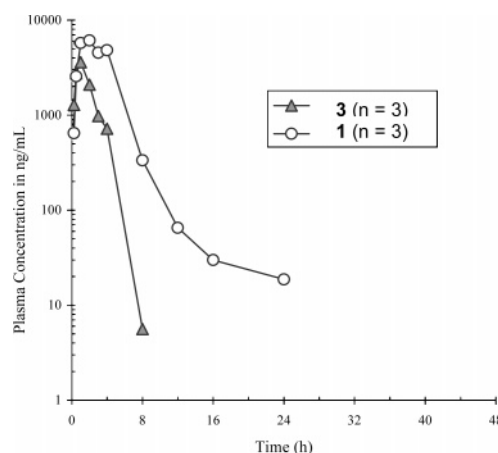


Figure 4. Mean plasma concentration of compounds 1 and 3, following a single oral administration of 3 to Sprague–Dawley rats.

human liver cytosol, and S9 fractions are not surprising and depend on the activity/quantity of enzyme(s) present in these samples. In all cases, no further metabolism of 1, such as deamination to its corresponding uridine metabolite 2, has been observed.

**Protein Binding of Compounds 1 and 3 to Rat Plasma.**

The *in vitro* binding of compounds 1 and 3 to rat plasma proteins was investigated by the ultrafiltration method using radiolabeled test articles ([<sup>3</sup>H]-2'-*C*-methylcytidine and [<sup>3</sup>H]-3'-*O*-valinyl ester of 2'-*C*-methylcytidine) at a concentration of 20 μM for each compound. As expected for nucleosides, the protein binding for the two compounds was low, with 7% and 5% bound for 3 and 1, respectively.

**Oral Bioavailability and Pharmacokinetic Studies of Compounds 1 and 3.** The pharmacokinetics (PK) of prodrug 3 were evaluated in Sprague–Dawley rats in a mass balance using radiolabeled 3. Both intact and bile duct cannulated (BDC) rats were used in this study. PK parameters of 1 and its 3'-*O*-valinyl ester 3 are presented in Table 1. Plasma concentration–time profiles of 1 and 3 are shown in Figure 4.

Following a single oral dose of [<sup>14</sup>C]-3'-*O*-valinyl ester of 2'-*C*-methylcytidine (3) at 100 mg/kg (as free base), urine, feces, and cage rinses were collected for a period of 72–168 h postdose to evaluate the excretion of radioactivity and the overall mass balance. Plasma samples were collected over a 48-h period postdose to study the PK of 1 and 3. In intact rats, fecal excretion was also the predominant route of elimination of total radioactivity, accounting for 63.5% of the administered dose. Excretion of radioactivity occurred largely within the first 24 h (averaging 60% of the dose). Urinary excretion accounted for 31.9% of the administered dose. The mean total recovery of radioactivity, in urine, feces, and cage rinses, was 96.6%. Biliary excretion of total radioactivity in BDC rats accounted for a mean

of 0.3% of the dose over the 72-h collection period. In this group, fecal excretion was the predominant route of elimination, accounting for a mean 65.9% of the dose. A mean 29.8% of the dose was recovered in urine. Excretion of radioactivity was largely completed by 24 h in BDC rats. On the basis of the combined mean recovery of radioactivity in bile and urine, it was apparent that at least 30.0% of the [ $^{14}\text{C}$ ]-3'-*O*-valinyl ester of 2'-*C*-methylcytidine (**3**) oral dose was absorbed. The mean recovery of total radioactivity in this group was 97.0% of the administered dose. In the group following a single dose of  $^{14}\text{C}$ -**3** at 100 mg/kg (as free base), maximum plasma concentrations of total radioactivity (averaging 11.9  $\mu\text{g}$  equiv/mL) were achieved 1–2 h after the oral dose. Plasma concentrations of **3**, determined by LC/MS/MS, were greatest 0.5–1 h after dosing, and  $C_{\text{max}}$  averaged 3.62  $\mu\text{g}/\text{mL}$ . Plasma **3** concentrations decayed with a half-life of 0.64 h, and no prodrug **3** was detected in plasma by 12 h. Plasma concentrations of **1**, determined by LC/MS/MS, were greatest 1–2 h after dosing, and  $C_{\text{max}}$  averaged 6.12  $\mu\text{g}/\text{mL}$ . Plasma concentrations of **1** decayed with a half-life of 7.1 h. The AUC of **1** was more than 3-fold greater than the AUC of **3**. 2'-*C*-Methyluridine (**2**) was not detected in rat plasma (<0.1  $\mu\text{g}/\text{mL}$ ).

The pharmacokinetics of **1** were also studied in Sprague–Dawley rats in a toxicokinetic (TK) study in which compound **1** was administered intravenously daily for 15 days. There were three dose groups (60, 120, and 300 mg/kg/day) in the study, and TK sampling was conducted on day 1 following the first dose and on day 15 after the last dose. For the scope of this paper, only the TK parameters of the 60 mg/kg/day dose group on day 1 are presented. Following a single iv dose of compound **1** to rats at 60 mg/kg, the mean plasma  $C_{\text{max}}$  of **1** was 75.6  $\mu\text{g}/\text{mL}$  and the mean AUC was 74.4  $\mu\text{g h}/\text{mL}$ . The mean AUC value of **1** from this dose group was compared to the AUC value of **1** from the prodrug **3** rat study above, and an apparent oral bioavailability was calculated for **1**. Thus, the apparent oral bioavailability of **1** following oral administration of the valinyl ester derivative **3** at 100 mg/kg (as free base) was calculated to be 33.6%.

## Conclusion

3'-*O*-Valinyl ester of 2'-*C*-methylcytidine (dihydrochloride salt, NM283, valopicitabine, **3**) has been synthesized in order to improve the oral bioavailability of the parent compound 2'-*C*-methylcytidine (**1**). For that purpose, two different strategies have been developed, both starting from **1**. The first one is a conventional route that involves successive protection steps, and the second one is more appropriate for large-scale synthesis and is based on selective 3'-*O*-esterification. Physicochemical, pharmacokinetic, and toxicokinetic studies have shown that compound **3** is an acid-stable prodrug of **1** with excellent pharmacokinetic and toxicokinetic profiles. Prodrug **3** is rapidly converted into compound **1** in both human plasma and whole blood, probably due to the presence of esterases. The apparent oral bioavailability of the parent drug **1** following oral administration of the prodrug **3** is 34%.

Thus, on the basis of its ease of synthesis, its physicochemical properties, and pharmacokinetic profile, 3'-*O*-valinyl ester **3** has emerged as a promising prodrug of 2'-*C*-methylcytidine (**1**). The 3'-*O*-valinyl ester of 2'-*C*-methylcytidine (**3**, NM283, valopicitabine) is currently undergoing phase IIb clinical trials for the treatment of chronic HCV infection.<sup>24</sup>

## Experimental Section

**General Methods for Chemistry.**  $^1\text{H}$  NMR spectra were recorded at ambient temperature on a Bruker AC 200 MHz, 250,

300, or 400 MHz spectrometer.  $^1\text{H}$  NMR chemical shifts ( $\delta$ ) are quoted in parts per million (ppm) referenced to the residual solvent peak [dimethyl sulfoxide ( $\text{DMSO}-d_6$ )] set at 2.49 ppm. The accepted abbreviations are as follows: s, singlet; d, doublet; t, triplet; m, multiplet. FAB mass spectra were recorded in the positive-ion or negative-ion mode on a JEOL DX 300 mass spectrometer operating with a JMA-DA 5000 mass data system and using a mixture of glycerol and thioglycerol (1/1, v/v, G–T) as the matrix. Melting points were determined in open capillary tubes with a Büchi B-545 apparatus and are uncorrected. UV spectra were recorded on an Uvikon XS spectrophotometer. Elemental analyses were carried out by the Service de Microanalyses du CNRS, Division de Vernaison (France). Thin-layer chromatography (TLC) was performed on precoated aluminum sheets of silica gel 60 F<sub>254</sub> (Merck, Art. 5554), visualization of products being accomplished by UV absorbance and by charring with 10% ethanolic sulfuric acid with heating or with a 0.2% ethanolic ninhydrin solution for compounds bearing an amide function.

Column chromatography was carried out on silica gel 60 (Merck, Art. 9385). Evaporation of solvents was carried out in a rotary evaporator under reduced pressure. All moisture-sensitive reactions were carried out under rigorous anhydrous conditions under an argon atmosphere using oven-dried glassware. Solvents were dried and distilled prior to use and solids were dried over  $\text{P}_2\text{O}_5$  under reduced pressure. Analytical high-performance liquid chromatography (HPLC) studies were carried out on a Waters Associates unit (multisolute delivery system, 717 autosampler injector, 996 photodiode array detector and a Millennium data workstation) using a reverse-phase analytical column (Nova-Pak Silica 60 Å, 4  $\mu\text{m}$ , C<sub>18</sub>, 150  $\times$  3.9 mm). The compound to be analyzed was eluted using a linear gradient of 0–50% acetonitrile in 20 mM triethylammonium acetate buffer (TEAC, pH 7) programmed over a 30-min period with a flow rate of 1 mL/min.

**General Procedure for Aqueous Solubility Studies.** Aqueous solubilities were determined in distilled water at room temperature.<sup>25</sup> An excess of studied compounds was added to aqueous solution, and the suspension was shaken and centrifugated. Samples of supernatant were analyzed by HPLC (same conditions as described in General Methods for Chemistry), and the concentration of saturated solution was determined according to a calibration curve. The experiment was repeated three times.

**General Procedure for Distribution Coefficient Studies.** Distribution coefficients between 1-octanol and an aqueous phase (phosphate buffer solution 0.02 M) were determined at room temperature using a shake-flask procedure.<sup>25</sup> An aliquot of a  $10^{-2}$  M aqueous solution of studied compounds was diluted to 1 mL with the aqueous phase previously saturated with octanol. An equal volume of octanol, previously saturated with the aqueous phase, was added to give a total volume of 2 mL. The mixture was shaken vigorously. The two phases were centrifugated and separated. Samples of each phase were collected and analyzed by HPLC (same conditions as described in General Methods for Chemistry). UV absorbances for both phases were measured at the respective maximum wavelength. The distribution coefficient was calculated from the ratio of the area of the signal detected in the octanol and aqueous phases. Each experiment was repeated twice.

**General Procedure for Chemical Stability Studies.** Chemical hydrolysis rates were determined in KCl–HCl buffer 0.135 M solution (pH 1.2, 37 °C), acetate buffer 0.02 M solution (pH 4.5, 37 °C), and phosphate buffer 0.02 M solution (pH 7.2, 37 °C).<sup>26</sup> Solutions ( $10^{-4}$  M) of **3** have been incubated at 37 °C in buffer solutions. Aliquot samples were collected at different time intervals and analyzed by HPLC (the same conditions as described in General Methods for Chemistry). Rates of decomposition have been easily determined using a method developed in our laboratory and based on pseudo-first-order kinetic models.<sup>27</sup>

**General Procedure for in Vitro Blood and Plasma Stability Studies.** A stock solution of **3** was aliquoted (1 mg/mL stored in MeOH at –20 °C), dried down, and resuspended to 20  $\mu\text{g}/\text{mL}$  with either human plasma or whole blood (Bioreclamation Inc., Hicksville, NY) preheated to 37 °C. Aliquot samples were collected at

different time intervals 0, 2, 5, 10, 20, 40, and 60 min for blood studies and 0, 30, 70, 100, and 140 min for plasma studies. Samples were quenched in 5 volumes of ice-cold solvent consisting of 50% acetonitrile/50% methanol and centrifuged at 16 000 rcf for 3 min. Supernatant was removed, dried down in a centrifugal concentrator, resuspended in mobile phase (5% MeOH–95% potassium phosphate, monobasic, pH 5.0), filtered (0.2  $\mu$ m nylon Spin X tubes), and analyzed by reverse-phase HPLC using an Agilent model 1100 instrument with automatic injection and a diode-array spectrophotometric detector. The mobile phase consisted of buffer A (25 mM potassium phosphate, pH 5) and buffer B (methanol). A linear gradient from 5% to 100% buffer B was run over 15 min. The HPLC system used a C<sub>18</sub> Columbus column (5  $\mu$ m, 250  $\times$  4.6 mm, Phenomenex, Torrance, CA). Authentic standards were used to identify the peaks in HPLC. This assay was done one time (blood and plasma from one donor) in duplicate.

**General Procedure for Metabolic Stability Studies.** The ability of derivative **3** to function as substrate for relevant metabolic enzymes was examined using human liver subcellular fractions obtained from BD Gentest (Woburn, MA). The prodrug was incubated for 1 h at 50  $\mu$ g/mL with 1 mg/mL liver S9 or liver cytosol. Prior to HPLC analysis, samples were quenched in 5 volumes of ice-cold solvent (50% acetonitrile/50% methanol) and centrifuged (16 000 rcf for 3 min). Supernatant was removed, dried down in a centrifugal concentrator, resuspended in mobile phase (5% MeOH–95% potassium phosphate, monobasic, pH 5.0), filtered (0.2  $\mu$ m nylon Spin X tubes), and analyzed via HPLC (same conditions as described in General Procedure for in vitro Blood and Plasma Stability Studies).

**General Procedure for Protein Binding Studies.** The protein binding of prodrug **3** and parent nucleoside **1** in rat plasma was investigated using the ultrafiltration centrifugation method at a concentration of 20  $\mu$ M for each. The studies used both nonradiolabeled and radiolabeled test articles. The [<sup>3</sup>H]-3'-*O*-valinyl ester of 2'-*C*-methylcytidine and [<sup>3</sup>H]-2'-*C*-methylcytidine, provided by Moravak Biochemicals, Inc. (Brea, CA), were prepared by tritium exchange (catalyst and labile tritium were removed during purification). To achieve the desired concentrations, concentrated stock solutions for each test article containing the appropriate amounts of the radiolabeled and nonradiolabeled articles were prepared in HPLC grade water. Rat plasma (1 mL) was then added to each tube and vortexed. The sample tubes were incubated at 37 °C for 1 h.

Centrifuge micropartition units (1-mL capacity, Amicon Inc.) with a molecular weight cutoff of 30 000 Da were used to separate the unbound from the bound test article by membrane filtration. Fortified plasma (0.6 mL) was added to the sample reservoir portion of the ultrafiltration device. Plasma samples were centrifuge for 10 min at 37 °C and 1800g so that the amount of sample filtered ranged between 20 and 50% of the total volume. After centrifugation, the ultrafiltrate of each sample was collected, and 10  $\mu$ L aliquots were analyzed for radioactivity using liquid scintillation counting (LSC) on a Beckman Coulter LS6500. Protein binding determinations were done in duplicate for each test article.

**General Procedure for Pharmacokinetic Studies.** The pharmacokinetic and mass balance study of the [<sup>14</sup>C]-3'-*O*-valinyl ester of 2'-*C*-methylcytidine (<sup>14</sup>C label at C-2, Moravak Biochemicals, Inc.) was conducted in three groups of male Sprague–Dawley rats. A single oral dose of the [<sup>14</sup>C]-3'-*O*-valinyl ester of **1** was administered as a solution in 0.01 N HCl at a target dose of 100 mg/kg (as free base) to the three groups of male rats after fasting overnight. Group 1 was used to characterize the excretion of the radioactivity derived from the [<sup>14</sup>C]-3'-*O*-valinyl ester of 2'-*C*-methylcytidine in urine and feces. Urine and feces were collected up to 168 h postdose from group 1 rats. Group 2 comprised bile duct cannulated (BDC) male rats and was used to characterize the excretion of the radioactivity derived from the [<sup>14</sup>C]-3'-*O*-valinyl ester of 2'-*C*-methylcytidine in bile, urine, and feces. Urine, bile, and feces were collected up to 72 h postdose in group 2 rats. Group 3 consisted of jugular vein cannulated (JVC) male rats in which the pharmacokinetics of plasma radioactivity derived from the [<sup>14</sup>C]-

3'-*O*-valinyl ester of 2'-*C*-methylcytidine, and **1** and **3** were evaluated. Blood samples were collected from group 3 male rats predose and 0.25, 0.5, 1, 2, 3, 4, 8, 12, 16, 24, and 48 h postdose (3 rats/time point), and the separated plasma was analyzed by LSC and by LC/MS/MS. The LC/MS/MS method was validated according to ICH guidelines for bioanalytical method validation prior to sample analysis. A daily cage rinse, a cage wash, and a cage wipe at termination were also performed for groups 1 and 2. All postdose samples of bile, urine, feces, plasma, and cage residues (cage rinse, cage wash, and cage wipe) were analyzed for radioactivity by LSC. Mean plasma concentrations of **1** and its 3'-*O*-valinyl ester were used to calculate descriptive pharmacokinetic parameters by noncompartmental analysis using WinNonlin 4.1 (Pharsight).

The 15-day toxicokinetic study of nucleoside analogue **1** was conducted to evaluate the pharmacokinetics of **1** following iv administration to Sprague–Dawley rats. Compound **1** was administered once daily to rats intravenously for 15 days at dose levels of 60, 120, and 300 mg/kg/day. Each dose group consisted of eight animals/gender. On days 1 and 15, blood samples were collected from the first set of four rats/sex/group at predose and approximately 20 min and 2 h postdose and from the second set of four rats/sex/group at approximately 5 min and 1 and 4 h postdose. Plasma samples were analyzed for 2'-*C*-methylcytidine and 2'-*C*-methyluridine by a validated bioanalytical method (LC/MS/MS), and composite PK parameters were calculated by noncompartmental analysis using WinNonlin 4.1 (Pharsight).

For LC/MS/MS analysis, compound **1**, its 3'-*O*-valinyl ester, and added internal standards were extracted from plasma (50  $\mu$ L) using protein precipitation with acetonitrile (0.5 mL). The supernatant was dried, reconstituted, and analyzed by LC/MS/MS using HPLC coupled to a PE Sciex API3000 tandem mass spectrometer with a Turbo IonSpray interface. The HPLC system used an Alltech Platinum C<sub>18</sub> column (3  $\mu$ m, 53  $\times$  7 mm, Alltech Associates, Deerfield, IL). HPLC elution was carried out using a gradient of ammonium acetate (10 mM, pH 4) and acetonitrile. The flow rate was 2.5 mL/min with a split of 0.5 mL/min into the mass spectrometer. Each analyte was detected by multiple reaction monitoring (MRM) under the positive ion mode using the precursor to product ion pair of *m/z* 357 to 112 (collision energy 27 eV) and *m/z* 258 to 112 (collision energy 20 eV), for **3** and **1**, respectively. Quantitation was achieved by constructing a calibration curve by weighted linear regression of the ratio of the analyte peak area to that of the added internal standard. Calibration standards were in blank rat plasma. The calibration range was 0.01–5  $\mu$ g/mL in plasma for **3** and 0.05–25  $\mu$ g/mL in plasma for the parent nucleoside **1**.

**N<sup>4</sup>-[(Dimethylamino)methylene]-2'-*C*-methyl- $\beta$ -D-cytidine (**4**).** A solution of **1**<sup>5,6,28</sup> (1.65 g, 6.43 mmol) in *N,N*-dimethylformamide (DMF, 32 mL) was treated with dimethylformamide dimethylacetal (8.2 mL, 61.73 mmol) and stirred for 1.5 h at room temperature.<sup>18</sup> The solution was evaporated under reduced pressure and coevaporated with ethanol. Crystallization from ethanol/ether yielded the hitherto unknown title compound **4** (first crop = 1.21 g, 60%; second crop slightly impure on TLC = 0.46 g, 23%) as crystals. All the following physicochemical characteristics have been determined on the crystals issued from the first crop crystallization: mp 169–172 °C; <sup>1</sup>H NMR  $\delta$  8.62 (s, 1H, N=CH), 8.17 (d, 1H, H-6, *J*<sub>5-6</sub> = 7.3 Hz), 5.91 (m, 2H, H-1', H-5), 5.16 (m, 1H, OH-5', D<sub>2</sub>O exchangeable), 5.06 (s, 1H, OH-2', D<sub>2</sub>O exchangeable), 3.8–3.5 (m, 4H, H-3', H-4', H-5', and H-5''), 3.15 and 3.02 (2s, 6H, N(CH<sub>3</sub>)<sub>2</sub>), 0.92 (s, 3H, CH<sub>3</sub>); FAB > 0 *m/z* 625 (2M + H)<sup>+</sup>, 313 (M + H)<sup>+</sup>, 167 (B + 2H)<sup>+</sup>; FAB < 0 *m/z* 419 (M + T - H)<sup>-</sup>, 403 (M + G - H)<sup>-</sup>, 311 (M - H)<sup>-</sup>, 165 (B)<sup>-</sup>; HPLC *t*<sub>R</sub> = 5.96 min;  $\lambda$ <sub>max</sub> = 316.1 nm; UV (H<sub>2</sub>O):  $\lambda$ <sub>max</sub> = 313 nm ( $\epsilon$  30 200),  $\lambda$ <sub>min</sub> = 242 nm ( $\epsilon$  3600); [ $\alpha$ ]<sub>D</sub><sup>20</sup> = +134.7 (*c* = 0.95, H<sub>2</sub>O). Anal. (C<sub>13</sub>H<sub>20</sub>N<sub>4</sub>O<sub>5</sub>·0.1H<sub>2</sub>O) C, H, N.

**3'-*O*-[*N*-(*tert*-Butoxycarbonyl)-L-valinyl]-2'-*C*-methyl- $\beta$ -D-cytidine (**7**).** Procedure A Starting from Protected Intermediate **4**. To a solution of compound **4** (1.17 g, 3.73 mmol) in dry pyridine (15 mL) were added successively imidazole (0.76 g, 11.19 mmol)

and *tert*-butyldiphenylchlorosilane (TBDPSCI, 0.66 mL, 2.53 mmol). The solution was stirred at room temperature. After 4 h, the reaction mixture was recharged with TBDPSCI (0.40 mL, 2.28 mmol) and stirred at room temperature for 2 h. After sodium bicarbonate treatment, the organic layer was washed with water, dried over sodium sulfate, and evaporated under reduced pressure. The crude compound **5** was taken up in a mixture of dry acetonitrile (CH<sub>3</sub>CN, 30 mL) and dry DMF (15 mL). To that solution were successively added *N*-(*tert*-butoxycarbonyl)-L-valine (Boc-Val-OH, 0.40 g, 1.87 mmol), *N'*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC, 0.71 g, 3.73 mmol), and 4-(dimethylamino)pyridine (DMAP, 0.07 g, 0.56 mmol), and the solution was stirred at room temperature.<sup>11</sup> The reaction profile was followed by HPLC and the reaction mixture was recharged three times with Boc-Val-OH (0.40 g × 3), EDC (0.71 g × 3) and DMAP (0.07 g × 3) and finally one time with Boc-Val-OH (0.20 g), EDC (0.36 g) and DMAP (0.03 g). After 2 days the starting material was totally consumed, and the DMF was removed under reduced pressure. The residue **6** was taken up in dry methanol (70 mL). To that solution was added ammonium fluoride (1.38 g, 37.30 mmol), the reaction mixture was refluxed for 3 h, the mixture was filtered, and the solvent was removed from the filtrate under reduced pressure. The residue was taken up in ethyl acetate (EtOAc) and extracted several times with water. The organic phase was evaporated under vacuum and purified by silica gel column chromatography [eluant: MeOH (20%) in EtOAc (80%)]. The desired compound **7** was isolated (1.37 g, 78% for the three steps) as a white foam: <sup>1</sup>H NMR δ 7.99 (d, 1H, *H*-6, *J*<sub>6-5</sub> = 7.4 Hz), 7.3–7.1 (m, 3H, *CH* and *NH*<sub>2</sub>, D<sub>2</sub>O exchangeable), 5.9 (s, 1H, *H*-1'), 5.75 (d, 1H, *H*-5, *J*<sub>6-5</sub> = 7.4 Hz), 5.43 (s, 1H, *OH*-2', D<sub>2</sub>O exchangeable), 5.24 (m, 1H, *OH*-5'), 5.04 (d, 1H, *H*-3', *J*<sub>3'-4'</sub> = 9.1 Hz), 4.1–4.0 (m, 2H, *H*-4', *CH*), 3.8–3.4 (2 m, 2H, *H*-5', *H*-5''), 2.2–2.0 (m, 1H, *CH*), 1.40 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>C), 1.0 (s, 3H, CH<sub>3</sub>), 0.9–0.8 (m, 6H, (CH<sub>3</sub>)<sub>2</sub>CH); FAB <0 *m/z* 911 (2M – H)<sup>-</sup>, 455 (M – H)<sup>-</sup>, 256 (M – BocVal)<sup>-</sup>, 216 (BocValO)<sup>-</sup>, 110 (B)<sup>-</sup>; FAB >0 *m/z* 913 (2M + H)<sup>+</sup>, 457 (M + H)<sup>+</sup>, 112 (B + 2H)<sup>+</sup>, 57 (CH<sub>3</sub>)<sub>3</sub>C<sup>+</sup>.

**Procedure B Starting from 1.** A solution of *N*-(*tert*-butoxycarbonyl)-L-valine (46.50 g, 214 mmol), carbonyldiimidazole (CDI, 34.70 g, 214 mmol), and anhydrous tetrahydrofuran (THF, 1000 mL) was stirred at 25 °C under argon for 1.5 h and then at 40–50 °C for 20 min. This mixture was slowly added to a clear solution of **1** (50.0 g, 195 mmol), DMAP (2.38 g, 19 mmol), and triethylamine (TEA, 500 mL) in anhydrous DMF (1000 mL). The temperature was maintained up to 80 °C during the addition process and the reaction was stirred at this temperature for 1 h. The reaction mixture was cooled to room temperature, and TEA and THF were removed under vacuum. The remaining DMF solution was then neutralized with acetic acid (pH 7.69), and DMF was evaporated under reduced pressure. The residue was taken up in EtOAc (500 mL) and successively washed with water, an aqueous saturated brine solution, and an aqueous solution of malonic acid. The acidic aqueous extracts were combined, cooled in an ice bath, and neutralized with TEA (pH 7.40). The desired compound **7** (99.09% pure according to HPLC) which precipitated was collected by vacuum filtration (48.44 g, 54%).

**3'-O-(L-valinyl)-2'-C-methyl-β-D-cytidine (Dihydrochloride Salt, 3).** **Procedure A.** A solution of **7** (1.32 g, 2.9 mmol) in dry EtOAc (75 mL) was treated with a 20% HCl/EtOAc solution (75 mL).<sup>22</sup> The reaction mixture was stirred at room temperature for 2 h. The title compound **3**, which precipitated in the reaction mixture, was filtered and washed with ethyl ether (1.01 g, 81%): mp 210 °C (brown), 234–241 °C (melted); <sup>1</sup>H NMR δ 10.0 (s, 1H, 1/2NH<sub>2</sub>, D<sub>2</sub>O exchangeable), 8.9–8.6 (2 br s, 4H, 1/2NH<sub>2</sub>, NH<sub>3</sub>, D<sub>2</sub>O exchangeable), 8.42 (d, 1H, *H*-6, *J*<sub>5-6</sub> = 7.9 Hz), 6.24 (d, 1H, *H*-5, *J*<sub>5-6</sub> = 7.9 Hz), 5.84 (s, 1H, *H*-1'), 5.12 (d, 1H, *H*-3', *J*<sub>3'-4'</sub> = 8.8 Hz), 4.22 (d, 1H, *H*-4, *J*<sub>3'-4'</sub> = 8.7 Hz), 4.0–3.9 (m, 1H, *CH*), 3.8–3.5 (m, 2H, *H*-5', *H*-5''), 2.3–2.1 (m, 1H, *CH*), 1.16 (s, 3H, CH<sub>3</sub>), 1.0 (m, 6H, (CH<sub>3</sub>)<sub>2</sub>CH); FAB >0 *m/z* 713 (2M + H)<sup>+</sup>, 449 (M + G + H)<sup>+</sup>, 357 (M + H)<sup>+</sup>, 246 (S)<sup>+</sup>, 112 (B + 2H)<sup>+</sup>; FAB <0 *m/z* 747 (2M + Cl)<sup>-</sup>, 483 (M + G + Cl)<sup>-</sup>, 391 (M + Cl)<sup>-</sup>, 355 (M – H)<sup>-</sup>, 116 (Val)<sup>-</sup>, 110 (B)<sup>-</sup>, 35 (Cl)<sup>-</sup>; HPLC *t*<sub>R</sub> = 7.26 min, λ<sub>max</sub> =

273.5 nm; UV (H<sub>2</sub>O): λ<sub>max</sub> = 271 nm (ε 7500), λ<sub>min</sub> = 249 nm (ε 5200), λ<sub>s</sub> = 234 nm (ε 6200); [α]<sub>D</sub><sup>20</sup> = +75.2 (c = 1.01, H<sub>2</sub>O). Anal. (C<sub>15</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>6</sub> · 0.2H<sub>2</sub>O) C, H, N, Cl.

**Procedure B.** Hydrogen chloride gas (22.00 g) was bubbled into a clear solution of compound **7** (21.00 g, 46 mmol) in ethanol (EtOH, 168 mL) over a period of 1 h. The reaction temperature was kept below 30 °C using an ice–water bath for 4 h. Solids were collected by filtration and washed with EtOH and ethyl ether to give 19.06 g (96.5%) of the desired compound **3** (97.3% pure according to HPLC).

**Supporting Information Available:** Elemental analysis results for compounds **3** and **4**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) Marcellin, P.; Boyer, N. Chronic viral hepatitis. *Best Pract. Res. Clin. Gastroenterol.* **2003**, *17*, 259–275.
- (2) Wu, J. W.; Yao, N.; Walker, M.; Hong, Z. Recent advances in discovery and development of promising therapeutics against hepatitis C virus NS5B RNA-dependent RNA polymerase. *Mini-Rev. Med. Chem.* **2005**, *5*, 1103–1112.
- (3) McHutchison, J.; Patel, K. Future therapy of hepatitis C. *Hepatology* **2002**, *S245–S252*.
- (4) De Francesco, R.; Migliaccio, G. Challenges and successes in developing new therapies for hepatitis C. *Nature* **2005**, *436*, 953–960.
- (5) Sommadossi, J.-P.; La Colla, P. Preparation of antiviral nucleosides and methods for treating hepatitis C virus. *Novirio Pharmaceuticals Limited, Universita Degli Studi Di Cagliari*; WO 2001090121, CAN 136:6296, 2001; 296 pp.
- (6) Sommadossi, J.-P.; La Colla, P. Methods and compositions using modified nucleosides for treating flaviviruses and pestiviruses. *Novirio Pharmaceuticals Limited, Universita Degli Studi Di Cagliari*; WO 2001092282, CAN 136:590, 2001; 302 pp.
- (7) Buckwold, V. E.; Beer, B. E.; Donis, R. O. Bovine viral diarrhea as a surrogate model of hepatitis C virus for the evaluation of antiviral agents. *Antiviral Res.* **2003**, *60*, 1–15.
- (8) Bichko, V.; Tausek, M.; Qu, L.; La Colla, M.; Bergelson, S.; Pierra, C.; Benzaria, S.; Storer, D.; Gosselin, G.; Sommadossi, J.-P.; Standing, D. Enhanced antiviral activity of NM 107, alone or in combination with interferon α. *J. Hepatol.* **2005**, *42* (Suppl. 2), 154.
- (9) Standing, D. N.; Lanford, R.; Wright, T.; Chung, R. T.; Bichko, V.; Cretton-Scott, E.; Pan-Zou, X.; Bergelson, S.; Qu, L.; Tausek, M.; Bridges, E.; Moussa, A.; Storer, R.; Pierra, C.; Benzaria, S.; Gosselin, G.; La Colla, P.; Sommadossi, J.-P. NM 283 has potent antiviral activity against genotype 1 chronic hepatitis C virus (HCV-1) infection in the chimpanzee. *J. Hepatol.* **2003**, *38* (Suppl. 2), 3.
- (10) Weller, S.; Blum, M. R.; Doucette, M.; Burnette, T.; Cederberg, D. M.; De Miranda, P.; Smiley, M. L. Pharmacokinetics of the acyclovir prodrug valaciclovir after escalating single and multiple dose administration to normal volunteers. *Clin. Pharmacol. Ther.* **1993**, *54*, 595–605.
- (11) Beauchamp, L. M.; Orr, G. F.; De Miranda, P.; Burnette, T.; Krenitsky, T. A. Amino acid ester prodrugs of acyclovir. *Antiviral Chem. Chemother.* **1992**, *3*, 157–164.
- (12) Pescovitz, M. D.; Rabkin, J.; Merion, R. M.; Paya, C. V.; Pirsch, J.; Freeman, R. B.; O'Grady, J.; Robinson, C.; To, Z.; Wren, K.; Banken, L.; Buhles, W.; Brown, F. Valganciclovir results in oral absorption of ganciclovir in liver transplant recipients. *Antimicrob. Agents Chemother.* **2000**, *44*, 2811–2815.
- (13) Oral ganciclovir prodrug, valganciclovir, approved for cytomegalovirus retinitis. *Antiviral Agents Bulletin. Antiviral Drug and Vaccine Development Information*; Biotechnology Information Institute: Rockville, MD, 2001; pp 65–66.
- (14) Gosselin, G.; Pierra, C.; Benzaria, S.; Dukhan, D.; Imbach, J.-L.; Loi, A.-G.; La Colla, P.; Cretton-Scott, E.; Bridges, E. G.; Standing, D. N.; Sommadossi, J.-P. β-L-2'-deoxythymidine (L-dT) and β-L-2'-deoxycytidine (L-dC): How simple structures can be potent, selective and specific anti-HBV drugs. *Frontiers in Nucleosides and Nucleic Acids*, IHL Press: Arlington, MA, 2004; pp 309–317.
- (15) Lim, S. G.; Lai, C. L.; Myers, M.; Yuen, M. F.; Wai, C. T.; Lloyd, D.; Pietropaolo, K.; Zhou, X. J.; Cho, G.; Brown, N. A. Final results of a phase I/II dose escalation trial of valtorcitabine in patients with chronic hepatitis B. *J. Hepatol.* **2005**, *42* (Suppl. 2), 16.
- (16) Vincent, S.; Mioskowski, C.; Lebeau, L. Hydrolysis and hydrolysis of formamides: *N,N*-Dimethyl and *N,N*-dibenzyl formamides as protective groups for primary amines. *J. Org. Chem.* **1999**, *64*, 991–997.

- (17) Pierra, C.; Benzaria, S.; Dukhan, D.; Loi, A.-G.; La Colla, P.; Bridges, E.; Mao, J.; Standing, D.; Sommadossi, J.-P.; Gosselin, G. Synthesis, physicochemical and pharmacokinetic studies of potential prodrugs of  $\beta$ -L-2'-deoxycytidine, a selective and specific anti-HBV agent. *Antiviral Chem. Chemother.* **2004**, *15*, 269–279.
- (18) Kerr, S. G.; Kalman, T. I.  $N^4$ -(Dialkylamino)methylene derivatives of 2'-deoxycytidine and arabinocytidine: Physicochemical studies for potential prodrug applications. *J. Pharm. Sci.* **1994**, *83*, 582–586.
- (19) Hanessian, S.; Lavallee, P. Preparation and synthetic utility of *tert*-butyldiphenylsilyl ethers. *Can. J. Chem.* **1975**, *53*, 2975–2977.
- (20) Dhaon, M. K.; Olsen, R. K.; Ramasamy, K. Esterification of *N*-protected  $\alpha$ -amino acids with alcohol/carbodiimide/4-(dimethylamino)-pyridine. Racemisation of aspartic and glutamic acid derivatives. *J. Org. Chem.* **1982**, *47*, 1962–1965.
- (21) Zhang, W.; Robins, M. J. Removal of silyl protecting groups from hydroxyl functions with ammonium fluoride in methanol. *Tetrahedron Lett.* **1992**, *33*, 1177–1180.
- (22) Cavelier, F.; Enjalbal, C. Studies of selective Boc Removal in the presence of silyl ethers. *Tetrahedron Lett.* **1996**, *37*, 5131–5134.
- (23) McCormick, J.; Li, Y.; McCormick, K.; Duynstee, H. I.; Van Engen, A. K.; Van der Marel, G.; Ganem, B.; Van Boom, J. H.; Meinwald, J. Structure and total synthesis of HF-7, a neuroactive glyconucleoside disulfate from the funnel-web spider *Hololena curta*. *J. Am. Chem. Soc.* **1999**, *121*, 5661–5665.
- (24) (a) Afdhal, N.; Rodriguez-Torres, M.; Lawitz, E.; Godofsky, E.; Chao, G.; Fielman, B.; Knox, S.; Brown, N. Enhanced antiviral efficacy for valopicitabine (NM283) plus PEG-Interferon in hepatitis C patients with HCV genotype-1 infection: Results of a phase IIa multicenter trial. *J. Hepatol.* **2005**, *42* (Suppl. 2), 39–40. (b) Zhou, X. J.; Afdhal, N.; Godofsky, E.; Dienstag, J.; Rustgi, V.; Schick, L.; McNery, D.; Fielman, B. A.; Brown, N. Pharmacokinetics and pharmacodynamics of valopicitabine (NM283), a new nucleoside HCV polymerase inhibitor: Results of a phase I/II dose-escalation trial in patients with HCV-1 infection. *J. Hepatol.* **2005**, *42* (Suppl. 2), 229.
- (25) Valette, G. Biotransformation et détermination du caractère lipophile de pronucléotides à visée antivirale: Étude par chromatographie liquide hautes performances (CLHP) et couplage CLHP/spectrométrie de masse). Ph.D. Thesis, Chimie des Biomolécules: Synthèse, Structure et Réactivité, Université de Montpellier II—Sciences et Techniques du Languedoc; January 21, 1998.
- (26) Bates, R. G.; Paabo, M. Measurement of pH. In *Handbook of Biochemistry*, 2nd ed.; Sober, H. A. Ed.; CRC Press: Cleveland, Ohio, 1970; pp J227–J237.
- (27) Lefebvre, I.; Perigaud, C.; Pompon, A.; Aubertin, A.-M.; Girardet, J.-L.; Kirm, A.; Gosselin, G.; Imbach, J.-L. Mononucleoside phosphotriester derivatives with *S*-acyl-2-thioethyl bioreversible phosphate-protecting groups: Intracellular delivery of 3'-azido-2',3'-dideoxythymidine 5'-monophosphate. *J. Med. Chem.* **1995**, *38*, 3941–3950.
- (28) Pierra, C.; Benzaria, S.; Amador, A.; Moussa, A.; Mathieu, M.; Storer, D.; Gosselin, G. NM 283, an efficient prodrug of the potent anti-HCV agent 2'-C-methylcytidine. *Nucleosides Nucleotides Nucleic Acids* **2005**, *24*, 767–770.

JM0603623