

3',5'-DI-O-ACYLATED NUCLEOSIDES FOR HCV TREATMENT

The present invention provides 3',5'-di-O-acylated nucleosides which are prodrugs of an inhibitor of Hepatitis C Virus (HCV) RNA-dependent RNA viral polymerase. These compounds when administered orally are readily absorbed from the GI tract and efficiently revert to the parent nucleoside in vivo. These prodrugs inhibit RNA-
5 dependent RNA viral replication and are useful as inhibitors of HCV NS5B polymerase, as inhibitors of HCV replication, and for the treatment of hepatitis C infection in mammals.

In particular, the invention is concerned with the use of acylated pyrimidine nucleosides which provide improved drug absorption when the nucleoside is
10 administered orally and which are superior to previously disclosed prodrugs.

Hepatitis C virus is a major health problem and the leading cause of chronic liver disease throughout the world. (Boyer, N. et al. J. Hepatol. 2000 32:98-112). Patients infected with HCV are at risk of developing cirrhosis of the liver and subsequent hepatocellular carcinoma and, hence, HCV is the major indication for liver
15 transplantation.

According to the World Health Organization, there are more than 200 million infected individuals worldwide, with at least 3 to 4 million people being infected each year. Once infected, about 20% of people clear the virus, but the rest can harbor HCV the rest of their lives. Ten to twenty percent of chronically infected individuals eventually
20 develop liver-destroying cirrhosis or cancer. The viral disease is transmitted parenterally by contaminated blood and blood products, contaminated needles, or sexually and is transmitted vertically from infected mothers or carrier mothers to their offspring. Current treatments for HCV infection, which are restricted to immunotherapy with recombinant interferon- α alone or in combination with the nucleoside analog ribavirin,
25 are of limited clinical benefit as resistance develops rapidly. There is an urgent need for improved therapeutic agents that effectively combat chronic HCV infection.

Currently there are a limited number of approved therapies available for the treatment of HCV infection. New and existing therapeutic approaches to treating HCV and inhibition of HCV NS5B polymerase have been reviewed: R. G. Gish, *Sem. Liver. Dis.*,
30 1999 19:5; Di Besceglie, A. M. and Bacon, B. R., *Scientific American*, October: 1999 80-85; G. Lake-Bakaar, "Current and Future Therapy for Chronic Hepatitis C Virus Liver
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Disease", *Curr. Drug Targ. Infect Dis.* **2003** 3(3):247-253; P. Hoffmann *et al.*, "Recent patents on experimental therapy for hepatitis C virus infection (1999-2002)", *Exp. Opin. Ther. Patents* **2003** 13(11):1707-1723; F. F. Poordad *et al.* "Developments in Hepatitis C therapy during 2000-2002", *Exp. Opin. Emerging Drugs* **2003** 8(1):9-25; M. P. Walker *et al.*, "Promising Candidates for the treatment of chronic hepatitis C", *Exp. Opin. Investig. Drugs* **2003** 12(8):1269-1280; S.-L. Tan *et al.*, "Hepatitis C Therapeutics: Current Status and Emerging Strategies", *Nature Rev. Drug Discov.* **2002** 1:867-881; R. De Francesco *et al.* "Approaching a new era for hepatitis C virus therapy: inhibitors of the NS3-4A serine protease and the NS5B RNA-dependent RNA polymerase", *Antiviral Res.* **2003** 58:1-16; Q. M. Wang *et al.* "Hepatitis C virus encoded proteins: targets for antiviral therapy", *Drugs of the Future* **2000** 25(9):933-8-944; J. A. Wu and Z. Hong, "Targeting NS5B-Dependent RNA Polymerase for Anti-HCV Chemotherapy", *Cur. Drug Targ.-Inf. Dis.* **2003** 3:207-219. The reviews cite compounds presently in various stages of the development process. Combination therapy with two or three anti-viral agents directed to the same or different targets has become standard anti-viral therapy to avoid or slow the development of resistant strains of a virus and the compounds disclosed in the above reviews could be used in combination therapy with compounds of the present invention and these reviews are hereby incorporated by reference in their entirety.

Ribavirin (1-((2R,3R,4S,5R)-3,4-Dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-1H-[1,2,4]triazole-3-carboxylic acid amide; VIRAZOLE[®]) is a synthetic, non-interferon-inducing, broad spectrum antiviral nucleoside analog. Ribavirin has *in vitro* activity against several DNA and RNA viruses including *Flaviviridae* (G. L. Davis, *Gastroenterology* **2000** 118:S104-S114). In monotherapy ribavirin reduces serum amino transferase levels to normal in 40% of patients, but it does not lower serum levels of HCV-RNA. Ribavirin also exhibits significant toxicity and is known to induce anemia. Ribovirin is not approved in monotherapy against HCV but the compound is approved in combination therapy with interferon α -2a and interferon α -2b. Viramidine is a prodrug converted to ribavirin in hepatocytes.

Interferons (IFNs) have been available for the treatment of chronic hepatitis for nearly a decade. IFNs are glycoproteins produced by immune cells in response to viral infection. Two distinct types of interferon are recognized: Type 1 includes several interferon alphas and one interferon β , type 2 includes interferon γ . Type 1 interferon is produced mainly by infected cells and protects neighboring cells from *de novo* infection. IFNs inhibit viral replication of many viruses, including HCV, and when used as the sole treatment for hepatitis C infection, IFN suppresses serum HCV-RNA to undetectable levels. Additionally, IFN normalizes serum amino transferase levels. Unfortunately, the

effects of IFN are temporary. Cessation of therapy results in a 70% relapse rate and only 10-15% exhibit a sustained virological response with normal serum alanine transferase levels. (L.-B. Davis, *supra*)

One limitation of early IFN therapy was rapid clearance of the protein from the blood. Chemical derivatization of IFN with polyethyleneglycol (PEG) has resulted in proteins with substantially improved pharmacokinetic properties. PEGASYS® is a conjugate of interferon α -2a and a 40 kD branched mono-methoxy PEG and PEG-INTRON® is a conjugate of interferon α -2b and a 12 kD mono-methoxy PEG. (B. A. Luxon *et al.*, *Clin. Therap.* **2002** 24(9):1363-1383; A. Kozlowski and J. M. Harris, *J. Control. Release*, **2001** 72:217-224).

Interferon α -2a and interferon α -2b are currently approved as monotherapy for the treatment of HCV. ROFERON-A® (Roche) is the recombinant form of interferon α -2a. PEGASYS® (Roche) is the pegylated (i.e. polyethylene glycol modified) form of interferon α -2a. INTRON-A® (Schering Corporation) is the recombinant form of interferon α -2b, and PEG-INTRON® (Schering Corporation) is the pegylated form of interferon α -2b.

Other forms of interferon α , as well as interferon β , γ , τ and ω are currently in clinical development for the treatment of HCV. For example, INFERGEN® (interferon alfacon-1) by InterMune, OMNIFERON® (natural interferon) by Viragen, ALBUFERON® by Human Genome Sciences, REBIF® (interferon β -1a) by Ares-Serono, Omega Interferon by BioMedicine, Oral Interferon Alpha by Amarillo Biosciences, and interferon γ , interferon τ , and interferon γ -1b by InterMune are in development.

Combination therapy of HCV with ribavirin and interferon- α currently represent the optimal therapy. Combining ribavirin and PEG-IFN (*infra*) results in a sustained viral response (SVR) in 54-56% of patients. The SVR approaches 80% for type 2 and 3 HCV. (Walker, *supra*) Unfortunately, the combination also produces side effects which pose clinical challenges. Depression, flu-like symptoms and skin reactions are associated with subcutaneous IFN- α and hemolytic anemia is associated with sustained treatment with ribavirin.

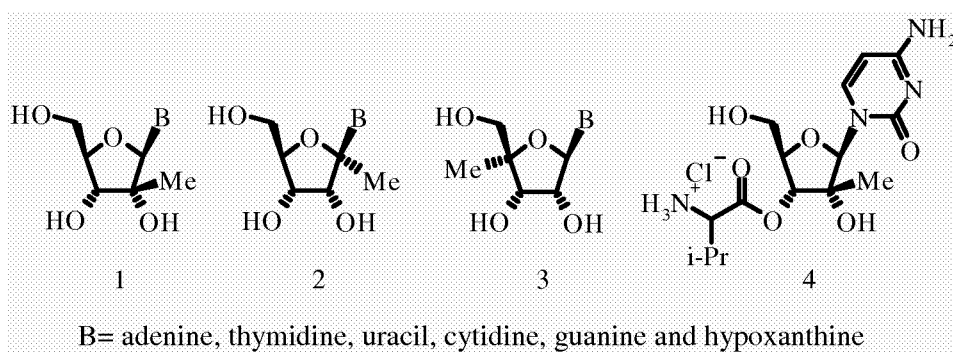
Other macromolecular compounds currently in preclinical or clinical development for treatment of hepatitis C virus infection include: Interleukin-10 by Schering-Plough, IP-SO1 by Intemuron, Merimebodib (VX-497) by Vertex, HEPTAZYME® by RPI, IDN-6556 by Idun Pharma., XTL-002 by XTL., HCV/MFS9 by Chiron, CIVACIR® (hepatitis C Immune Globulin) by NABI, ZADAXIN® (thymosin α -1) by SciClone, thymosin plus pegylated interferon by SciClone, CEPLENE®; a therapeutic vaccine directed to E2 by

Innogenetics, therapeutic vaccine by Intercell, therapeutic vaccine by Epimmune/Genencor, a therapeutic vaccine by Merix, a therapeutic vaccine, Chron-VacC, by Tripep.

Ribozymes have been targeted at HCV RNA. Ribozymes are short naturally occurring molecules with endonuclease activity that catalyze the sequence-specific cleavage of RNA. An alternate approach is the use of antisense oligonucleotides bind to RNA and stimulate RNaseH mediated cleavage.

A number of potential molecular targets for drug development as anti-HCV therapeutics have now been identified including, but not limited to, the NS2-NS3 autoprotease, the N3 protease, the N3 helicase and the NS5B polymerase. The RNA-dependent RNA polymerase is absolutely essential for replication of the single-stranded, positive sense, RNA genome and this essential enzyme has elicited significant interest among medicinal chemists.

Nucleoside inhibitors of NS5B polymerase can act either as a non-natural substrate incapable of chain elongation or as a competitive inhibitor which competes with natural nucleotides for binding to the polymerase. To function as a chain terminator the nucleoside analog must be taken up by the cell and converted *in vivo* to a triphosphate to compete for the polymerase nucleotide binding site. Conversion to the triphosphate is commonly mediated by cellular kinases which impart additional structural requirements on a potential nucleoside polymerase inhibitor. Unfortunately, this limits the direct evaluation of nucleosides as inhibitors of HCV replication to cell-based assays capable of *in situ* phosphorylation.

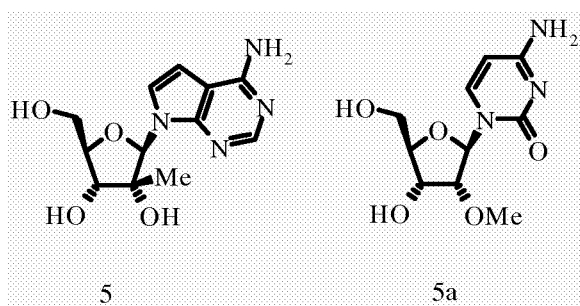


The 2β-methyl nucleoside 1 was first described by E. Walton *et al.* (*J. Med. Chem.* 1969 12(2):306-309). Esters of substituted nucleosides have been used as protected carbohydrate intermediates in synthetic sequences (E. Walton, GB 12909654 published October 21, 1970) When the base is cytidine, acylated derivatives are commonly di- and tri-esters of 2 in which the amino substituent is acylated (E. Walton, *supra*, J. L. Clark *et*

al. J. Med Chem. 2005 48(17):5504-5508; R. E. Harry-O'kuru *et al. J. Org. Chem.* 1997 62(6):1754-1759; J.-P. Sommadossi *et al.* WO 2004002422). Ester intermediates are typically benzoate or phenylacetate esters which are more like to afford crystalline compounds. Antiviral activity of 4-Amino-1-((2R,3R,4R,5R)-3,4-dihydroxy-5-hydroxymethyl-3-methyl-tetrahydro-furan-2-yl)-1H-pyrimidin-2-one derivatives has been reported (E. Walton, *J. Med Chem.*, *supra*).

In WO 01 90121 published November 29, 2001, J.-P. Sommadossi and P. Lacolla disclose and exemplify the anti-HCV polymerase activity of 1'-alkyl- and 2'-alkyl nucleosides of formulae 1 and 2. In WO 01/92282, published December 6, 2001, J.-P. Sommadossi and P. Lacolla disclose and exemplify treating *Flaviviruses* and *Pestiviruses* with 1'-alkyl- and 2'-alkyl nucleosides of formulae 1 and 2. In WO 03/026675 published April 3, 2003, G. Gosselin discloses 4'-alkyl nucleosides 3 for treating *Flaviviruses* and *Pestiviruses*. In WO2004/046331 published June 3, 2004, J.-P. Sommadossi *et al.* disclose 2'-branched nucleosides and *Flaviviridae* mutations. In WO03/026589 published April 3, 2003 G. Gosselin *et al.* disclose methods of treating hepatitis C virus using 4'-modified nucleosides. In WO2005009418 published February 3, 2005, R. Storer *et al.* disclose purine nucleoside analogues for treatment of diseases caused by *Flaviviridae* including HCV.

Other patent applications disclose the use of certain nucleoside analogs to treat hepatitis C virus infection. In WO 01/32153 published May 10, 2001, R. Storer discloses nucleoside derivatives for treating viral diseases. In WO 01/60315 published August 23, 2001, H. Ismaili *et al.*, disclose methods of treatment or prevention of Flavivirus infections with nucleoside compounds. In WO 02/18404 published March 7, 2002, R. Devos *et al.* disclose 4'-substituted nucleotides for treating HCV virus. In WO 01/79246 published October 25, 2001, K. A. Watanabe disclose 2'- or 3'-hydroxymethyl nucleoside compounds for the treatment of viral diseases. In WO 02/32920 published April 25, 2002 and in WO 02/48 165 published June 20, 2002 L. Stuyver *et al.* disclose nucleoside compounds for the treatment of viral diseases.



In WO 03/105770 published December 24, 2003, B. Bhat *et al.* disclose a series of carbocyclic nucleoside derivatives that are useful for the treatment of HCV infections. In WO 2004/007512 published January 22, 2003, B. Bhat *et al.* disclose nucleoside compounds that inhibit of RNA-dependent RNA viral polymerase. The nucleosides
5 disclosed in this publication are primarily 2'-methyl-2'-hydroxy substituted nucleosides. In WO 2002/057425 published July 25, 2002, S. S. Carroll *et al.* disclose nucleoside derivatives which inhibit RNA-dependent viral polymerase and methods of treating HCV infection. In WO02/057287 published July 25, 2002, S. S. Carroll *et al.* disclose related 2 α -methyl and 2 β -methylribose derivatives wherein the base is an optionally substituted
10 7H-pyrrolo[2,3-d]pyrimidine radical 5. The same application discloses one example of a 3 β -methyl nucleoside. S.S. Carroll *et al.* (*J. Biol. Chem.* **2003** 278(14):11979-11984) disclose inhibition of HCV polymerase by 4-amino-1-((2R,3R,4R,5R)-4-hydroxy-5-hydroxymethyl-3-methoxy-tetrahydro-furan-2-yl)-1H-pyrimidin-2-one (5a). In WO 2004/009020 published January 29, 2004, D. B. Olsen *et al.* disclose a series of
15 thionucleoside derivatives as inhibitors of RNA dependent RNA viral polymerase.

PCT Publication No. WO 99/43691 to Emory University, entitled "2'-Fluoronucleosides" discloses the use of certain 2'-fluoronucleosides to treat HCV. U.S. Patent No. 6,348,587 to Emory University entitled "2'-fluoronucleosides" discloses a family of 2'-fluoronucleosides useful for the treatment of hepatitis B, HCV, HIV and
20 abnormal cellular proliferation. Both configurations of the 2' fluoro substituent are disclosed.

Eldrup *et al.* (Oral Session V, Hepatitis C Virus, Flaviviridae; 16th International Conference on Antiviral Research (Apr. 27, 2003, Savannah, Ga.)) described the structure activity relationship of 2'-modified nucleosides for inhibition of HCV.

25 Bhat *et al.* (Oral Session V, Hepatitis C Virus, Flaviviridae; 16th International Conference on Antiviral Research (Apr. 27, 2003, Savannah, Ga.); p A75) describe the synthesis and pharmacokinetic properties of nucleoside analogues as possible inhibitors of HCV RNA replication. The authors report that 2'-modified nucleosides demonstrate potent inhibitory activity in cell-based replicon assays.

30 Olsen *et al.* (Oral Session V, Hepatitis C Virus, Flaviviridae; 16th International Conference on Antiviral Research (Apr. 27, 2003, Savannah, Ga.) p A76) also described the effects of the 2'-modified nucleosides on HCV RNA replication.

Non-nucleoside allosteric inhibitors of HIV reverse transcriptase have proven effective therapeutics alone and in combination with nucleoside inhibitors and with

protease inhibitors. Several classes of non-nucleoside HCV NS5B inhibitors have been described and are currently at various stages of development including: benzimidazoles, (H. Hashimoto *et al.* WO 01/47833, H. Hashimoto *et al.* WO 03/000254, P. L. Beaulieu *et al.* WO 03/020240 A2; P. L. Beaulieu *et al.* US 6,448,281 B1; P. L. Beaulieu *et al.* WO 03/007945 A1); indoles, (P. L. Beaulieu *et al.* WO 03/0010141 A2); benzothiadiazines (D. Dhanak *et al.* WO 01/85172 A1; D. Dhanak *et al.* WO 03/037262 A2; K. J. Duffy *et al.* WO03/099801 A1, D.Chai *et al.* WO 2004052312, D.Chai *et al.* WO2004052313, D.Chai *et al.* WO02/098424, J. K. Pratt *et al.* WO 2004/041818 A1; J. K. Pratt *et al.* WO 2004/087577 A1), thiophenes, (C. K. Chan *et al.* WO 02/100851); benzothiophenes (D. C. Young and T. R. Bailey WO 00/18231); β -ketopyruvates (S. Attamura *et al.* US 6,492,423 B1, A. Attamura *et al.* WO 00/06529); pyrimidines (C. Gardelli *et al.* WO 02/06246 A1); pyrimidinediones (T. R. Bailey and D. C. Young WO 00/13708); triazines (K.-H. Chung *et al.* WO 02/079187 A1); rhodanine derivatives (T. R. Bailey and D. C. Young WO 00/10573, J. C. Jean *et al.* WO 01/77091 A2); 2,4-dioxopyrans (R. A. Love *et al.* EP 256628 A2); phenylalanine derivatives (M. Wang *et al.* *J. Biol. Chem.* **2003** 278:2489-2495).

The NS3 protease has emerged as a major target for discovery of new anti-HCV therapy. In WO 98/22496 published May 28, 1998, M. R. Attwood *et al.* have disclosed mechanism-base active site inhibitors of the protease (M.R. Attwood *et al.*, *Antiviral Chemistry and Chemotherapy* **1999** 10:259-273; M.R. Attwood *et al.*, Preparation and use of amino acid derivatives as anti-viral agents, German Patent Pub. DE 19914474). In WO98/17679 published April 30, 1998 R. D. Tung *et al.* disclosed mechanism-based peptide inhibitors on the NS3 protease.

In WO99/07734 published February 18, 1999 and in WO00/09543 published August 9, 1999, M Llinas-Brunet *et al.* disclose peptide inhibitors of the HCV protease. In WO00/59929 published October 12, 2000, Y. S. Tsantrizos *et al.* disclose macrocyclic tripeptides which are potent inhibitors of the HCV NS3 protease. A series of related patents from Boehringer-Ingelheim disclose related protease inhibitors and have lead to the identification of tripeptide derivative BILN 2061 (M. Llinas-Brunet *et al.* *Biorg. Med. Chem. Lett.* **2000** 10(20):2267-70; *J. Med Chem.* **2004** 47(26):6584-94; *J. Med. Chem.* **2004** 47(7):1605-1608; *Angew. Chem. Int. Ed. Eng.* **2003** 42(12):1356-60).

Other tripeptide inhibitors identified by Bristol-Myers Squibb have been disclosed, *inter alia*, in WO03/099274 published December 4, 2003, in WO2004/032827 published April 22, 2004, in WO03/053349 published July 3, 2003, in WO2005/046712 published May 26, 2005 and in WO2005/051410 published June 9, 2005. In WO2004/072234 published August 26, 2004 and in WO2004/093798 published November 4, 2004 further

tripeptide protease inhibitors were disclosed by Enanta Pharmaceuticals. In WO2005/037214 published April 28, 2005 L. M. Blatt *et al.* disclose still other tripeptide derivatives that inhibit HCV NS3 protease. In WO2005/030796 published April 7, 2005, S. Venkatraman *et al.* disclose macrocyclic inhibitors of HCV NS3 serine protease. In
5 WO 2005/058821 published June 30, 2005, F. Velazquez *et al.* disclose inhibitors of HCV NS3/NS4a serine protease. In WO02/48172 published June 20, 2002, Z. Zhu disclose diaryl peptides as inhibitors of NS3 protease. In WO02/08187 and in WO02/08256 both published January 31, 2002, A. Saksena *et al.* disclose peptide inhibitors of HCV NS3 protease. In WO02/08251 published January 31, 2002 M. Lim-Wilby *et al.* disclose
10 peptide inhibitors of the NS3 protease. In US 6,004,933 published December 21, 1999, L. W. Spruce *et al.* disclose heterocyclic peptide derivatives which inhibit cysteine proteases including HCV endopeptidase.

Non-substrate-based NS3 protease inhibitors such as 2,4,6-trihydroxy-3-nitro-benzamide derivatives (Sudo K. *et al.*, *BBRC* 1997 238:643-647; Sudo K. *et al.* *Antiviral*
15 *Chemistry and Chemotherapy* 1998 9:186), including RD3-4082 and RD3-4078, the former substituted on the amide with a 14 carbon chain and the latter processing a *para*-phenoxyphenyl group are also being investigated.

SCH 68631, a phenanthrenequinone, is an HCV protease inhibitor (Chu M. *et al.* *Tetrahedron Lett.* 1996 37:7229-7232). In another example by the same authors, SCH
20 351633, isolated from the fungus *Penicillium griseofulvum*, was identified as a protease inhibitor (Chu M. *et al.*, *Bioorg. Med. Chem. Lett.* 1999 9:1949-1952). Nanomolar potency against the HCV NS3 protease enzyme has been achieved by the design of selective inhibitors based on the macromolecule eglin c. Eglin c, isolated from leech, is a potent inhibitor of several serine proteases such as *S. griseus* proteases A and B, α -
25 chymotrypsin, chymase and subtilisin (Qasim M. A. *et al.*, *Biochemistry* 1997 36:1598-1607).

Thiazolidine derivatives which show relevant inhibition in a reverse-phase HPLC assay with an NS3/4A fusion protein and NS5A/5B substrate (Sudo K. *et al.* *Antiviral Research* 1996 32:9-18), especially compound RD-1-6250, possessing a fused cinnamoyl
30 moiety substituted with a long alkyl chain, RD4 6205 and RD4 6193. Thiazolidines and benzanilides were identified by N. Kakiuchi *et al.* in *FEBS Lett.* 1998 421:217-220 and N. Takeshita *et al.* *Anal. Biochem.* 1997 247:242-246.

Imidazolidinones as NS3 serine protease inhibitors of HCV are disclosed in WO 02/08198 to Schering Corporation published January 31, 2002 and in WO 02/48157 to

Bristol Myers Squibb published June 20, 2002. In WO02/48116 published June 20, 2002 P. Glunz *et al.* disclose pyrimidinone inhibitors of NS3 protease.

Other enzymatic targets for anti-HCV include the HCV IRES site (Internal Ribosomal Entry Site) and HCV helicase. IRES inhibitors have been reported by
5 Immusol, Rigel Pharmaceuticals (R803) and by Anadys (ANA 245 and ANA 246). Vertex has disclosed an HCV helicase inhibitor.

Combination therapy which can suppress resistant mutant strains, has become well established approach to anti-viral chemotherapy. The nucleoside inhibitors disclosed herein can be combined with other nucleoside HCV polymerase inhibitors, non-
10 nucleoside HCV polymerase inhibitors, and HCV protease inhibitors. As other classes of HCV drugs, e.g. viral entry inhibitors, helicase inhibitors, IRES inhibitors, ribozymes and antisense oligonucleotides emerge and are developed they will also be excellent candidates for combination therapy. Interferon derivatives have already been successfully combined with ribavirin and interferons and chemically modified interferons will be useful in
15 combination with the nucleosides herein disclosed.

The object of the present invention is to provide new compounds, methods and compositions for the treatment of a host infected with hepatitis C virus.

Nucleoside derivatives often are potent anti-viral (*e.g.*, HIV, HCV, *Herpes simplex*, CMV) and anti-cancer chemotherapeutic agents. Unfortunately their practical utility is
20 often limited by two factors. Firstly, poor pharmacokinetic properties frequently limit the absorption of the nucleoside from the gut and the intracellular concentration of the nucleoside derivatives and, secondly, suboptimal physical properties restrict formulation options which could be employed to enhance delivery of the active ingredient.

Albert introduced the term prodrug to describe a compound which lacks intrinsic
25 biological activity but which is capable of metabolic transformation to an active drug substance (A. Albert, *Selective Toxicity*, Chapman and Hall, London, 1951). Prodrugs have been recently reviewed (P. Ettmayer *et al.*, *J. Med Chem.* 2004 47(10):2393-2404; K. Beaumont *et al.*, *Curr. Drug Metab.* 2003 4:461-485; H. Bundgaard, *Design of Prodrugs: Bioreversible derivatives for various functional groups and chemical entities* in *Design of*
30 *Prodrugs*, H. Bundgaard (ed) Elsevier Science Publishers, Amsterdam 1985; G. M. Pauletti *et al. Adv. Drug Deliv. Rev.* 1997 27:235-256; R. J. Jones and N. Bischofberger, *Antiviral Res.* 1995 27; 1-15 and C. R. Wagner *et al., Med. Res. Rev.* 2000 20:417-45) and these references are hereby incorporated by reference in their entirety. While the

transformations can catalyzed by specific enzymes, often hydrolases, the active compound can also be regenerated by non-specific chemical processes.

Pharmaceutically acceptable prodrugs refer to a compound that is metabolized, for example hydrolyzed or oxidized, in the host to form the compound of the present invention without forming fragments with toxicological liabilities. Typical examples of prodrugs include compounds that have biologically labile protecting groups linked to a functional moiety of the active compound. Alkylation, acylation or other lipophilic modification of the hydroxy group(s) on the sugar moiety have been utilized in the design of pronucleotides. These pronucleotides can be hydrolyzed or dealkylated *in vivo* to generate the active compound.

Factors limiting oral bioavailability frequently are absorption from the gastrointestinal tract and first-pass excretion by the gut wall and the liver. Optimization of trans-cellular absorption through the GI tract requires a $D_{(7.4)}$ greater than zero. Optimization of the distribution coefficient does not, however, insure success. The prodrug may have to avoid active efflux transporters in the enterocyte. Intracellular metabolism in the enterocyte can result in passive transport or active transport of the metabolite by efflux pumps back into the gut lumen. The prodrug must be designed to avoid undesired biotransformations in the blood before reaching the target cells or receptors.

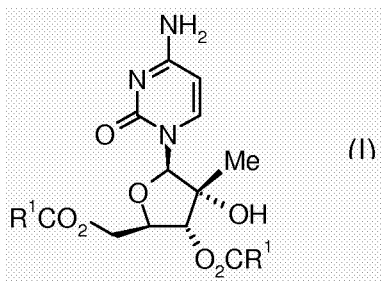
Peptide transporters (PEPT proteins) are proton-coupled active transport systems localized in the brush-border membranes of intestinal and renal epithelial cells which play an important role in protein and amino acid absorption. The discovery that these transport systems could mediate uptake of drugs linked to amino acids has provided a powerful tool for drug delivery (T. Terada and K. Inui, *Curr. Drug Metab.* **2004** 5(1):85-94; E. De Clercq and H. J. Field, *Brit. J. Pharmacol.* **2006** 147(1):1-11). The anti-herpes nucleosides acyclovir and ganciclovir when linked to L-amino acid esters were found to be efficiently transported into cells by PEPT proteins (H. Han *et al.*, *Pharm. Res.* **1998** 15(8):1154-1159; C. U. Nielsen and B. Brodin, *Curr. Drug Targets* **2003** 4(5):373-388). The L-valinyl ester of acyclovir and valacyclovir were found to be substrates for PEPT uptake (G. M. Friedrichsen *et al.* *Eur. J. Pharm. Sci.* **2002** 16(1-2):1-13; A. Guo *et al.* *J. Pharm. Exp. Ther.* **1999** 289(32):29509-29514; M. E. Ganapathy *et al.*, *Biochem. Biophys. Res. Commun.* **1998** 246(2):470-475).

In WO2004/003000 published January 8, 2004, J.-P. Sommadossi *et al.* disclose 2'- and 3' prodrugs of 1'-, 2'-, 3'- and 4'-substituted β -D and β -L nucleosides. In WO 2004/002422 published January 8, 2004, 2'-C-methyl-3'-O-valine ester ribofuransyl

cytidine for the treatment of *Flaviviridae* infections. Idenix has reported clinical trials for a related compound NM283 which is believed to be the valine ester 4 of 1 (B = cytosine). In WO 2004/002999 published Jan. 8, 2004, J.-P. Sommadossi *et al.* disclose a series of 2' or 3' prodrugs of 1', 2', 3', or 4' branched nucleosides for the treatment of flavivirus
 5 infections including HCV infections. In WO 2004/052899 published June 24, 2004, R. Storer *et al.* disclose a process for the preparation of 3'-O-valinyl derivatives of 2 β -methyl-cytosine. In WO2004/058792 published July 15, 2004, R. Storer *et al.* disclose a one step process for the preparation of 3'-O-valinyl derivatives.

In US 6,846,810 issued January 25, 2005, J. A. Martin *et al.* disclose acyl derivatives
 10 of 4'-azido-cytosine as prodrugs useful for treating patients with an HCV infection. In U.S. Ser. No. 11/635898, K. Sarma *et al.* disclose diacyl derivatives of 2'-deoxy-2'- α -fluoro-2'- β -methyl-cytidine which are useful for treating patients with an HCV infection. In U.S. Publication No. 2006/0040890 published February 23, 2006, J. A. Martin *et al.* disclose 4'-azido-cytarabine and prodrugs thereof which are useful for treating patients
 15 with HCV infection.

The present invention is directed toward novel di-acyl derivatives of 4-amino-1-((2R,3R,4R,5R)-3,4-dihydroxy-5-hydroxymethyl-3-methyl-tetrahydro-furan-2-yl)-1H-pyrimidin-2-one (I, also referred to as (2R)-2 β -methyl-cytidine):



20 wherein R¹ is independently selected from the group consisting of C₂₋₅ unbranched or branched alkyl, C₂₋₅ unbranched or branched alkenyl, C₃₋₅ cycloalkyl and C₂₋₅ haloalkyl; and,
 hydrates, solvates and acid addition salts thereof.

Compounds of the present invention are useful for treating a disorder mediated by
 25 HCV the invention further comprises a method for treating a patient infected with HCV with compounds of the present invention in mono- or combination therapy and pharmaceutical compositions containing said compounds. The invention further relates to a process to prepare the diacyl derivatives.

The HCV polymerase inhibitory activity of 4-amino-1-((2R,3R,4R,5R)-3,4-dihydroxy-5-hydroxymethyl-3-methyl-tetrahydro-furan-2-yl)-1H-pyrimidin-2-one I-4 has been disclosed (J.-P. Sommadossi *et al.*, *supra*). In clinical practice, it would be desirable to provide high blood levels of I-4 to quickly inhibit HCV polymerase and thereby lower viral levels under conditions that minimize the opportunity for the virus to mutate and to select for resistant strains. Sufficiently high levels may be difficult to achieve with the parent nucleoside. Prodrugs could improve the pharmacokinetic and physical properties of a compound and thereby optimize the bioavailability. The valine ester I-5 has been investigated as a prodrug of I-4.

While prodrug candidates are deceptively simple to envision, the identification of compounds with the appropriate physiochemical and pharmacodynamic properties, *in vivo* transformations and safety profile is a complex multi-disciplinary undertaking that requires significant experimentation. Hurdles to identification of prodrugs for oral delivery include maintaining sufficient aqueous solubility, lipophilicity and chemical stability while enabling rapid and efficient release of the active moiety post administration. Non-esterase metabolism and transporter mediated clearance of the prodrug must be minimized (K. Beaumont *et al. Curr. Drug Metab.* 2003 4(6):461-485). Also inhibition or induction of cytochrome P₄₅₀ enzymes can produce undesirable drug-drug interactions which are undesirable.

It has now been found that diesters of I-4 are superior prodrugs and provide substantially higher blood levels of I-4.

In one embodiment of the present invention there is provided a compound according to formula I wherein R¹ is as defined herein above.

In another embodiment of the present invention there is provided a compound according to formula I wherein R¹ is ethyl, *n*-propyl, *iso*-propyl, *n*-butyl or *iso*-butyl.

In still another embodiment of the present invention there is provided a compound according to formula I wherein R¹ is ethyl or *iso*-propyl.

In yet another embodiment of the present invention there is provided a compound according to formula I wherein R¹ is *iso*-propyl and the compound is a hydrochloride, mesylate, hemisulfate or sulfate salt.

Another embodiment of the present invention is the use of compounds according to formula I as defined above or a hydrates, solvates or acid addition salts thereof as antiviral agent for the treatment of a disease mediated by the HCV.

Another embodiment of the present invention is the use of compounds according to formula I as defined above or a hydrates, solvates or acid addition salts thereof as antiviral agent in combination with at least one immune system modulator and/or at least one antiviral agent that inhibits replication of HCV for the treatment of a disease

5 mediated by the HCV.

Another embodiment of the present invention is the use of compounds according to formula I as defined above or a hydrates, solvates or acid addition salts thereof for the preparation of a medicament for treating a disease mediated by the HCV.

Another embodiment of the present invention is the use of compounds according to formula I as defined above or a hydrates, solvates or acid addition salts thereof in combination with at least one immune system modulator and/or at least one antiviral agent that inhibits replication of HCV for the preparation of a medicament for treating a disease mediated by the HCV.

In still another embodiment of the present invention there is provided a method for treating a disease mediated by the HCV comprising administering to a patient in need thereof a therapeutically effective dose of a compound according to formula I wherein R^1 is selected from the group consisting of C_{2-5} unbranched or branched alkyl, C_{2-5} unbranched or branched alkenyl, C_{2-5} haloalkyl and C_{3-5} cycloalkyl, or a hydrates, solvates or acid addition salts thereof.

In still another embodiment of the present invention there is provided a method for treating a disease mediated by the HCV comprising administering to a patient in need thereof dose of 0.1 to 10 g per day of a compound according to formula I wherein R^1 is selected from the group consisting of C_{2-5} unbranched or branched alkyl, C_{2-5} unbranched or branched alkenyl, C_{2-5} haloalkyl and C_{3-5} cycloalkyl, or a hydrates, solvates or acid addition salts thereof. In yet another embodiment the dose is between 0.5 and 7 g per day and in a further embodiment the dose is between 1.0 and 6.0 g per day.

In another embodiment of the present invention there is provided a method for treating a disease mediated by the HCV comprising co-administering to a patient in need thereof a therapeutically effective dose of a compound according to formula I wherein R^1 is selected from the group consisting of C_{2-5} unbranched or branched alkyl, C_{2-5} unbranched or branched alkenyl, C_{2-5} haloalkyl and C_{3-5} cycloalkyl, or a hydrates, solvates or acid addition salts thereof and a therapeutically effective amount of at least one immune system modulator and/or at least one antiviral agent that inhibits replication of HCV.

In another embodiment of the present invention there is provided a method for treating a disease mediated by the HCV comprising co-administering to a patient in need thereof a therapeutically effective dose of a compound according to formula I wherein R¹ is selected from the group consisting of C₂₋₅ unbranched or branched alkyl, C₂₋₅

5 unbranched or branched alkenyl, C₂₋₅ haloalkyl and C₃₋₅ cycloalkyl, or a hydrates, solvates or acid addition salts thereof and a therapeutically effective amount of at least one immune system modulator which immune system modulator is an interferon, interleukin, tumor necrosis factor or colony stimulating factor.

In another embodiment of the present invention there is provided a method for
10 treating a disease mediated by the HCV comprising co-administering to a patient in need thereof a therapeutically effective dose of a compound according to formula I wherein R¹ is selected from the group consisting of C₂₋₅ unbranched or branched alkyl, C₂₋₅ unbranched or branched alkenyl, C₂₋₅ haloalkyl and C₃₋₅ cycloalkyl, or a hydrates, solvates or acid addition salts thereof, and a therapeutically effective amount of at least
15 one immune system modulator which immune system modulator is an interferon or a chemically derivatized interferon.

In another embodiment of the present invention there is provided a method for treating a disease mediated by the HCV comprising co-administering to a patient in need thereof a therapeutically effective dose of a compound according to formula I wherein R¹
20 is selected from the group consisting of C₂₋₅ unbranched or branched alkyl, C₂₋₅ unbranched or branched alkenyl, C₂₋₅ haloalkyl and C₃₋₅ cycloalkyl, or a hydrates, solvates or acid addition salts thereof, and a therapeutically effective amount of at least one other antiviral compound.

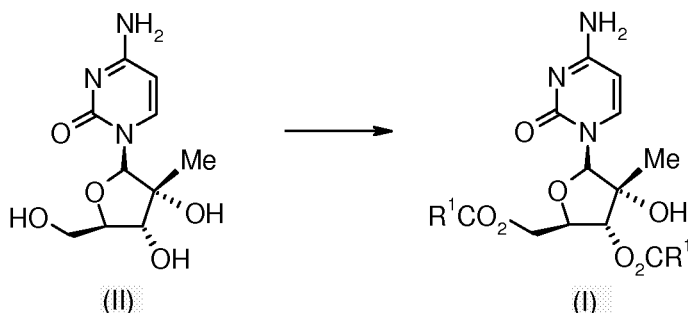
In another embodiment of the present invention there is provided a method for
25 treating a disease mediated by the HCV comprising co-administering to a patient in need thereof a therapeutically effective dose of a compound according to formula I wherein R¹ is selected from the group consisting of C₂₋₅ unbranched or branched alkyl, C₂₋₅ unbranched or branched alkenyl, C₂₋₅ haloalkyl and C₃₋₅ cycloalkyl, or a hydrates, solvates or acid addition salts thereof, and a therapeutically effective amount of at least
30 one other antiviral compound which compound is a HCV protease inhibitor, another nucleoside HCV polymerase inhibitor, a non-nucleoside HCV polymerase inhibitor, an HCV helicase inhibitor, an HCV primase inhibitor or a HCV fusion inhibitor.

In another embodiment of the present invention there is provided a pharmaceutical composition of comprising a compound according to formula I wherein R¹ is selected
35 from the group consisting of C₂₋₅ unbranched or branched alkyl, C₂₋₅ unbranched or

branched alkenyl, C₂₋₅ haloalkyl and C₃₋₅ cycloalkyl, or a hydrates, solvates or acid addition salts thereof admixed with at least one pharmaceutically acceptable carrier, diluent or excipient.

In another embodiment of the present invention there is provided a process for the preparation of a compound according to formula I wherein R¹ is selected from the group consisting of C₂₋₅ unbranched or branched alkyl, C₂₋₅ unbranched or branched alkenyl, C₂₋₅ haloalkyl and C₃₋₅ cycloalkyl, or a hydrates, solvates or acid addition salts thereof which process comprises steps (i)-(v) enumerated below and exemplified in the examples. The process comprises treating I in a basic aqueous organic media which can be homogenous or biphasic with an acylating agent as defined herein in the presence of DMAP and sufficient base to maintain the solution at a pH of at least about 7.5. The present process allows the acylation without concomitant reaction with the heterocyclic base.

A further embodiment of the present invention is a process for the selective O-acylation of a nucleoside I to afford an O-acyl nucleoside II under basic reaction conditions



wherein R¹ is selected from the group consisting of C₂₋₅ unbranched or branched alkyl, C₂₋₅ unbranched or branched alkenyl, C₃₋₅ cycloalkyl, C₂₋₅ haloalkyl,

and optionally substituted phenyl which process comprises the steps of:

(i) dissolving II and DMAP in an heterogeneous aqueous solvent mixture and adding aqueous base to adjust the pH from about 7.5 to about 12;

(ii) optionally adding sufficient saturated aqueous NaCl to produce a biphasic reaction mixture;

(iii) adding an acylating agent and additional base sufficient to maintain the pH from about 7.5 to about 12;

(iv) monitoring the reaction and discontinuing addition of said acylating agent and said base when the conversion reaches a satisfactory level;

(v) optionally contacting the O-acyl nucleoside with a pharmaceutically acceptable acid to form an acid additional salt of the O-acyl nucleoside.

5 The phrase "a" or "an" entity as used herein refers to one or more of that entity; for example, a compound refers to one or more compounds or at least one compound. As such, the terms "a" (or "an"), "one or more", and "at least one" can be used interchangeably herein.

10 The phrase "as defined herein above" refers to the first definition provided in the specification.

15 The term "independently" is used herein to indicate that a variable is applied in any one instance without regard to the presence or absence of a variable having that same or a different definition within the same compound. Thus, in a compound in which R¹ appears twice and is defined as "independently selected from the group consisting of C₂₋₅ unbranched or branched alkyl, C₂₋₅ unbranched or branched alkenyl, C₃₋₅ cycloalkyl and C₂₋₅ haloalkyl", both R¹'s can be identical or the R¹'s can have different meanings.

20 The terms "optional" or "optionally" as used herein means that a subsequently described event or circumstance may but need not occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not.

25 The term "independently" is used herein to indicate that a variable is applied in any one instance without regard to the presence or absence of a variable having that same or a different definition within the same compound. Thus, in a compound in which R¹ appears twice and is defined as "independently selected from the group consisting of C₂₋₅ unbranched or branched alkyl, C₂₋₅ unbranched or branched alkenyl, C₃₋₅ cycloalkyl and C₂₋₅ haloalkyl" both R¹'s can be identical or different from each other.

30 The term "alkenyl" as used herein denotes an unsubstituted hydrocarbon chain radical having from 2 to 10 carbon atoms having one or two olefinic double bonds [preferably one olefinic double bond]. C₂₋₅ alkenyl" as used herein refers to an alkenyl composed of 2 to 5 carbons. Examples are vinyl, 1-propenyl, 2-propenyl (allyl) or 2-butenyl (crotyl).

The term "alkyl" as used herein denotes an unbranched or branched chain, saturated, monovalent hydrocarbon residue containing 1 to 10 carbon atoms. "C₂₋₅ alkyl" as used herein refers to an alkyl composed of 2 to 5 carbons. Examples of alkyl groups include, but are not limited to ethyl, propyl, *i*-propyl, *n*-butyl, *i*-butyl, *t*-butyl or pentyl, isopentyl, neopentyl.

The term "cycloalkyl" as used herein denotes a saturated carbocyclic ring containing 3 to 8 carbon atoms, i.e. cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or cyclooctyl. "C₃₋₅ cycloalkyl" as used herein refers to a cycloalkyl composed of 3 to 5 carbons in the carbocyclic ring.

The term "haloalkyl" as used herein denotes an unbranched or branched chain alkyl group as defined above wherein 1, 2, 3 or more hydrogen atoms are substituted by a halogen. "C₂₋₅ haloalkyl" as used herein refers to a haloalkyl composed of 2 to 5 carbons and 1-11 halogen substituents. Examples are 1-fluoromethyl, 1-chloromethyl, 1-bromomethyl, 1-iodomethyl, trifluoromethyl, trichloromethyl, tribromomethyl, triiodomethyl, 1-fluoroethyl, 1-chloroethyl, 1-bromoethyl, 1-iodoethyl, 2-fluoroethyl, 2-chloroethyl, 2-bromoethyl, 2-iodoethyl, 2,2-dichloroethyl, 3-bromopropyl or 2,2,2-trifluoroethyl.

The term "di-acyl" derivative as used herein refers to a derivatized nucleoside compound as described herein wherein the 3'- and 5'-hydroxy are an ester -OC(=O)R¹ wherein R¹ is as defined in claim 1.

The term "acylating agent" as used herein refers to either an anhydride or an acid halide. The term "anhydride" as used herein refers to compounds of the general structure R¹C(O)-O-C(O)R¹ wherein R¹ is as defined in claim 1. The term "acid halide" as used herein refers to compounds of the general structure R¹C(O)X wherein X is a halogen. The term "acyl imidazole" refers to a compound of general structure R¹C(O)X wherein X is N-imidazolyl.

The term "activated derivative" of a compound as used herein refers to a transient reactive form of the original compound which renders the compound active in a desired chemical reaction, in which the original compound is only moderately reactive or non-reactive. Activation is achieved by formation of a derivative or a chemical grouping within the molecule with a higher free energy content than that of the original compound, which renders the activated form more susceptible to react with another reagent. In the context of the present invention activation of the carboxy group is of particular importance and corresponding activating agents or groupings which activate

the carboxy group are described in more detail below. Of particular interest for the present invention is carboxylic acid anhydrides and carboxylic acid chlorides.

The phrase "heterogeneous aqueous solvent mixture" as used here refers to a mixture of water and an organic co-solvent which produces a two-phase or
5 heterogeneous mixture. This heterogeneous aqueous solvent mixture may result from a co-solvent with limited aqueous solubility or the ionic strength of the aqueous component can be adjusted to limit the solubility of the co-solvent in the aqueous phase.

The term "alkali metal hydroxide" refers to a compound of formula MOH wherein M is lithium sodium, potassium or cesium, "alkali metal bicarbonate" refers to a group
10 MHCO_3 wherein M is sodium or potassium and "alkali metal carbonate" refers to a group M_2CO_3 where M is sodium or potassium. One skilled in the art will appreciate that other bases can be used to maintain the pH with desired range and other bases are within the scope of the invention.

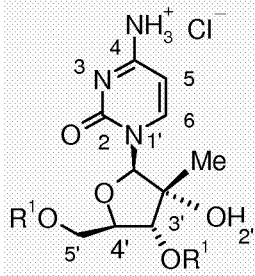
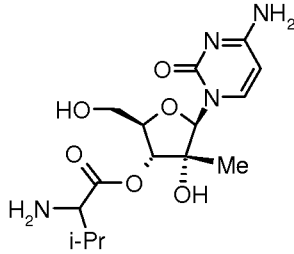
Abbreviations used in this application include: acetyl (Ac), atmospheres (Atm),
15 high pressure liquid chromatography (HPLC), methyl (Me), acetonitrile (MeCN), butyl (Bu), methanol (MeOH), melting point (mp), MeSO_2^- (mesyl or Ms), mass spectrum (ms), methyl *t*-butyl ether (MTBE), dichloromethane (DCM), propyl (Pr), pounds per square inch (psi), pyridine (pyr), room temperature, rt or RT, N,N-dimethyl acetamide (DMA), 4-N,N-dimethylaminopyridine (DMAP), triethylamine (Et_3N or TEA), N,N-
20 dimethylformamide (DMF), dimethyl sulfoxide (DMSO), thin layer chromatography (TLC), ethyl acetate (EtOAc), tetrahydrofuran (THF), diethyl ether (Et_2O), ethyl (Et), 4-Me- $\text{C}_6\text{H}_4\text{SO}_2^-$ or tosyl (Ts), *iso*-propyl (*i*-Pr), ethanol (EtOH). Conventional nomenclature including the prefixes *normal* (*n*), *iso* (*i*-), *secondary* (*sec*-), *tertiary* (*tert*-) and *neo* have their customary meaning when used with an alkyl moiety. (J. Rigaudy and
25 D. P. Klesney, *Nomenclature in Organic Chemistry*, IUPAC 1979 Pergamon Press, Oxford.).

Examples of representative compounds encompassed by the present invention and within the scope of the invention are provided in TABLE I. These examples and preparations which follow are provided to enable those skilled in the art to more clearly
30 understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

In general, the nomenclature used in this Application is based on AUTONOMTM v.4.0, a Beilstein Institute computerized system for the generation of IUPAC systematic

- nomenclature. If there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, by bold or dashed lines, the structure or portion of the structure is to be
- 5 interpreted as encompassing all stereoisomers of it. The numbering system for these ring systems is shown in TABLE 1.

TABLE 1

Cpd. No.		mp	ms	Caco2 (7 day)	Rat
				cm/sec x 10 ⁻⁶	AUC ¹ μg·h/mL
	R ¹				
I-1	C(=O)-C ₂ H ₅ HCl salt	164.5-167.0	369	0.11	2.4
I-2	C(=O)- <i>i</i> -C ₃ H ₇ HCl salt	167.0-167.8	397	1.06	5.6 ± 0.3
I-3	C(=O)- <i>n</i> -C ₄ H ₉ HCl salt	158.2-159.2	425	4.21	5.5 ± 1.8
I-4 ²	H			0.76	0.4 ± 0.08
I-5				0.12	1.1 ± 0.3

¹. AUC(cyt) is the area-under-the curve for parent cytidine nucleoside I-5

². parent nucleoside

To further evaluate potential behavior in human subjects, the transport through Caco-2 cells was evaluated for the putative prodrugs. Caco-2 cells are commonly used to evaluate the potential absorption/permeability of molecules (G. Gaviraghi *et al.* in

5 *Pharmacokinetic optimization in drug research. Biological, Pharmacokinetic and*

Computational Strategies. B. Testa *et al.* eds. Wiley Interscience VCH, Zurich 2001 pp 3-14). Caco-2 permeability was found to be acceptable for C2-4 alkyl diesters of I-4.

The compounds of the present invention are conveniently prepared in one step by acylation of I-4 in an aqueous organic solvent. The solvent can either be a homogenous
5 aqueous solution or a two-phase solution. The pH of the aqueous organic solvent is maintained above 7.5 by addition of base to neutralize acid produced by the acylation. The base can be either an alkali or alkaline metal hydroxide or a tertiary amine. The reaction is carried out in the presence of DMAP which is known in the art to be a catalyst for acylation. An advantage of the present process is the desired product can be obtained
10 without acylation of the heterocyclic base. No protecting group is required which eliminates the need for a protection/deprotection step. The process is described in the accompanying examples.

The compounds of the present invention may be formulated in a wide variety of oral administration dosage forms and carriers. Oral administration can be in the form of
15 tablets, coated tablets, hard and soft gelatine capsules, solutions, emulsions, syrups, or suspensions. Compounds of the present invention are efficacious when administered by suppository administration, among other routes of administration. The most convenient manner of administration is generally oral using a convenient daily dosing regimen which can be adjusted according to the severity of the disease and the patient's response to the
20 antiviral medication.

A compound or compounds of the present invention, as well as their pharmaceutically useable salts, together with one or more conventional excipients, carriers, or diluents, may be placed into the form of pharmaceutical compositions and unit dosages. The pharmaceutical compositions and unit dosage forms may be
25 comprised of conventional ingredients in conventional proportions, with or without additional active compounds and the unit dosage forms may contain any suitable effective amount of the active ingredient commensurate with the intended daily dosage range to be employed. The pharmaceutical compositions may be employed as solids, such as tablets or filled capsules, semisolids, powders, sustained release formulations, or
30 liquids such as suspensions, emulsions, or filled capsules for oral use; or in the form of suppositories for rectal or vaginal administration. A typical preparation will contain from about 5% to about 95% active compound or compounds (w/w). The term "preparation" or "dosage form" is intended to include both solid and liquid formulations of the active compound and one skilled in the art will appreciate that an active ingredient

can exist in different preparations depending on the desired dose and pharmacokinetic parameters.

The term "excipient" as used herein refers to a compound that is used to prepare a pharmaceutical composition, and is generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes excipients that are acceptable for veterinary use as well as human pharmaceutical use. The compounds of this invention can be administered alone but will generally be administered in admixture with one or more suitable pharmaceutical excipients, diluents or carriers selected with regard to the intended route of administration and standard pharmaceutical practice.

A "pharmaceutically acceptable salt" form of an active ingredient may also initially confer a desirable pharmacokinetic property on the active ingredient which were absent in the non-salt form, and may even positively affect the pharmacodynamics of the active ingredient with respect to its therapeutic activity in the body. The phrase "pharmaceutically acceptable salt" of a compound as used herein means a salt that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound. Such salts include: (1) acid addition salts, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as glycolic acid, pyruvic acid, lactic acid, malonic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, salicylic acid, muconic acid, and the like. It should be understood that all references to pharmaceutically acceptable salts include solvent addition forms (solvates) or crystal forms (polymorphs) as defined herein, of the same acid addition salt.

Solid form preparations include powders, tablets, pills, capsules, suppositories, and dispersible granules. A solid carrier may be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. In powders, the carrier generally is a finely divided solid which is a mixture with the finely divided active component. In tablets, the active component generally is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired. Suitable carriers include but are not limited to magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth,

methycellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. Solid form preparations may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

5 Liquid formulations also are suitable for oral administration include liquid formulation including emulsions, syrups, elixirs and aqueous suspensions. These include solid form preparations which are intended to be converted to liquid form preparations shortly before use. Emulsions may be prepared in solutions, for example, in aqueous propylene glycol solutions or may contain emulsifying agents such as lecithin, sorbitan
10 monooleate, or acacia. Aqueous suspensions can be prepared by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well known suspending agents.

 The compounds of the present invention may be formulated for administration as
15 suppositories. A low melting wax, such as a mixture of fatty acid glycerides or cocoa butter is first melted and the active component is dispersed homogeneously, for example, by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and to solidify.

 The compounds of the present invention may be formulated for vaginal
20 administration. Pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

 Suitable formulations along with pharmaceutical carriers, diluents and excipients are described in *Remington: The Science and Practice of Pharmacy 1995*, edited by E. W. Martin, Mack Publishing Company, 19th edition, Easton, Pennsylvania. A skilled
25 formulation scientist may modify the formulations within the teachings of the specification to provide numerous formulations for a particular route of administration without rendering the compositions of the present invention unstable or compromising their therapeutic activity.

 The modification of the present compounds to render them more soluble in water
30 or other vehicle, for example, may be easily accomplished by minor modifications (e.g., salt formulation), which are well within the ordinary skill in the art. It is also well within the ordinary skill of the art to modify the route of administration and dosage regimen of a particular compound in order to manage the pharmacokinetics of the present compounds for maximum beneficial effect in patients.

The term "therapeutically effective amount" as used herein means an amount required to reduce symptoms of the disease in an individual. The dose will be adjusted to the individual requirements in each particular case. That dosage can vary within wide limits depending upon numerous factors such as the severity of the disease to be treated, the age and general health condition of the patient, other medicaments with which the patient is being treated, the route and form of administration and the preferences and experience of the medical practitioner involved. For oral administration, a daily dosage of between about 0.1 and about 10 g per day should be appropriate in monotherapy and/or in combination therapy. A preferred daily dosage is between about 0.5 and about 7.5 g per day, more preferred 1.5 and about 6.0 g per day. Generally, treatment is initiated with a large initial "loading dose" to rapidly reduce or eliminate the virus following by a decreasing the dose to a level sufficient to prevent resurgence of the infection. One of ordinary skill in treating diseases described herein will be able, without undue experimentation and in reliance on personal knowledge, experience and the disclosures of this application, to ascertain a therapeutically effective amount of the compounds of the present invention for a given disease and patient.

Therapeutic efficacy can be ascertained from tests of liver function including, but not limited to protein levels such as serum proteins (e.g., albumin, clotting factors, alkaline phosphatase, aminotransferases (e.g., alanine transaminase, aspartate transaminase), 5'-nucleosidase, γ -glutamyltranspeptidase, etc.), synthesis of bilirubin, synthesis of cholesterol, and synthesis of bile acids; a liver metabolic function, including, but not limited to, carbohydrate metabolism, amino acid and ammonia metabolism. Alternatively the therapeutic effectiveness may be monitored by measuring HCV-RNA. The results of these tests will allow the dose to be optimized.

In embodiments of the invention, the active compound or a salt can be administered in combination with another antiviral agent such as ribavirin, another nucleoside HCV polymerase inhibitor, a HCV non-nucleoside polymerase inhibitor, a HCV protease inhibitor, a HCV helicase inhibitor or a HCV fusion inhibitor. When the active compound or its derivative or salt are administered in combination with another antiviral agent the activity may be increased over the parent compound. When the treatment is combination therapy, such administration may be concurrent or sequential with respect to that of the nucleoside derivatives. "Concurrent administration" as used herein thus includes administration of the agents at the same time or at different times. Administration of two or more agents at the same time can be achieved by a single formulation containing two or more active ingredients or by substantially simultaneous administration of two or more dosage forms with a single active agent.

It will be understood that references herein to treatment extend to prophylaxis as well as to the treatment of existing conditions. Furthermore, the term "treatment" of a HCV infection, as used herein, also includes treatment or prophylaxis of a disease or a condition associated with or mediated by HCV infection, or the clinical symptoms
5 thereof.

Example 1

Propionic acid (2R,3R,4R,5R)-5-(4-amino-2-oxo-2H-pyrimidin-1-yl)-4-hydroxy-4-methyl-3-propionyloxy-tetrahydro-furan-2-ylmethyl ester; hydrochloride salt 9 (I-1)

5 To a solution of I-4 (1.0 g, 3.89 mmol) in THF (15 mL) and H₂O (7 mL) was added TEA (4.72 g, 33.87 mol) and the reaction mixture was cooled to 5° C. Propionyl chloride (1.44 g, 15.45 mmol) was added slowly while maintaining the temperature of the reaction mixture below 0° C. The reaction was monitored by hplc and after the initial addition was complete monopropionate ester was still present and additional propionyl chloride
10 (0.72 g, 7.77 mmol) was added and the reaction stirred overnight at RT. The reaction mixture was cooled to 0° C and the pH was adjusted to *ca.* 6.5 with concentrated HCl. The mixture was partitioned between EtOAc (30 mL) and H₂O (15 mL) and the phases separated. The aqueous phase was extracted with EtOAc (2 x 25 mL) and the combined organic extracts were washed with dilute brine (2 x 20 mL), dried (Na₂SO₄), filtered and
15 concentrated *in vacuo* to afford the free base as an oil. The oil was dissolved in IPA (5 mL) and treated with HCl in IPA (1 mL of *ca.* 4 N HCl) and concentrated *in vacuo*. The residue was recrystallized from IPA/MTBE/*iso*-propyl acetate (1:3:15) and the resulting crystals filtered and washed with *iso*-propyl acetate and dried *in vacuo* at 80° C to afford 0.95 g (60.1%) of I-1.

20 Example 2

Iso-butyric acid (2R,3R,4R,5R)-5-(4-amino-2-oxo-2H-pyrimidin-1-yl)-4-hydroxy-3-isobutyryloxy-4-methyl-tetrahydro-furan-2-ylmethyl ester; hydrochloride salt (I-2)

To a solution of I-4 (0.50 g, 1.94 mmol), DMAP (0.026 g, 0.21 mmol), THF (8 mL) and H₂O (4 mL) was added TEA (2.36 g, 16.93 mmol) and the reaction mixture was
25 cooled to *ca.* -5° C. Iso-butyryl chloride (0.93 mL, 8.75 mmol) was added dropwise at a rate which allowed the reaction temperature to be maintained below 0° C. The reaction was monitored by HPLC-MS. Additional aliquots of 0.41 g and 0.21 g of *iso*-butyryl chloride (total of 1.55 g, 14.58 mmol) were added to eliminate monoacylated byproduct (determined by hplc). The reaction was stirred overnight at RT, cooled to 0° C and the
30 pH was adjusted to pH 6.5 with *con* HCl. The mixture was partitioned between EtOAc (30 mL) and H₂O (15 mL) and the phases separated. The aqueous phase was extracted with EtOAc (2 x 15 mL) and the combined organic extracts were washed sequentially with dilute brine (20 mL), dilute NaHCO₃ (20 mL), dilute brine (15 mL) dried (Na₂SO₄), filtered and concentrated *in vacuo* to afford the free base as an oil. The oil was dissolved
35 in IPA (5 mL) and treated with HCl in IPA (1 mL of *ca.* 4 N HCl) and concentrated *in*

vacuo. The residue was recrystallized from IPA/MTBE (1:10) and the resulting crystals filtered and washed with MTBE and dried *in vacuo* at 80° C to afford 0.61 g (72.6%) of I-2.

Example 3

5 **Pentanoic acid (2R,3R,4R,5R)-5-(4-amino-2-oxo-2H-pyrimidin-1-yl)-4-hydroxy-4-methyl-3-pentanoyloxy-tetrahydro-furan-2-ylmethyl ester; hydrochloride salt (I-3)**

To a solution of I-4 (0.490 g, 1.90 mmol), DMAP (0.026 g, 0.21 mmol), THF (7 mL) and H₂O (3 mL) was added TEA (2.31 g, 16.59 mmol) and the reaction mixture was cooled to *ca.* 0° C. Valeric anhydride (1.6 g, 2.57 mmol) was added dropwise at a rate
10 which allowed the reaction temperature to be maintained below 5° C. The reaction was monitored by HPLC-MS. Two 0.32 g aliquots of valeric anhydride (2.24 g, 11.99 mmol total) were added to eliminate monoacylated byproduct. The reaction mixture was cooled to 0° C and the pH was adjusted to pH 6.8 with concentrated HCl. The mixture was partitioned between EtOAc (30 mL) and H₂O (15 mL) and the phases separated. The
15 aqueous phase was extracted with EtOAc (2 x 15 mL) and the combined organic extracts were washed sequentially with dilute NaHCO₃ (2 x 20 mL) and water (1 x 20 mL), dried (Na₂SO₄), filtered and concentrated *in vacuo* to afford the free base as an oil. The oil was dissolved in IPA (5 mL) and treated with HCl in IPA (0.8 mL of *ca.* 4 N HCl) and concentrated *in vacuo*. The residue was recrystallized from IPA/MTBE/heptane (1:0.5:10;
20 15 mL) and the resulting crystals filtered and washed with heptane and dried *in vacuo* at 80° C under N₂ to afford 0.88 g (55.7%) of I-3.

Example 4

Determination of pharmacokinetic parameters in rats

Intact male IGS Wistar Han Rats Crl:WI(GLx/BRL/Han)IGS BR (Hanover-Wistar)
25 rats weighing 200-250 g were used. Groups of three rats were used for each dose level of an experimental compound. Animals were allowed normal access to chow and water throughout the experiment. The test substance was formulated as an aqueous suspension containing Captex 355EP, Capmul MCM, EtOH, and propylene glycol (30:20:20:30) at a dose equivalent to 10 mg/kg of the I-5 and was administered orally by gavage. A blood
30 sample (0.3 mL) was collected from the treated rats at, 0.25, 0.5, 1, 3, 5, and 8 h from a jugular cannula and at 24 h by cardiac puncture. Potassium oxalate/NaF were added to the samples which were stored on ice during sampling procedure. The samples were spun in a refrigerated centrifuge at -4° C as soon as possible and the plasma samples were stored in a -80° C freezer until analysis. Aliquots of plasma (0.05 mL) were mixed with
35 0.1 mL of acetonitrile. Internal standard (0.05 mL in water) and 0.02 mL blank solvent

were added. A set of calibration standards was prepared by mixing 0.05 mL aliquots of plasma from untreated rats with 0.1 mL acetonitrile, 0.02 mL aliquots of standard solution in methanol:water (1:1) and 0.05 mL aliquots of the internal standard in water. Each plasma sample and calibration standard was vortexed thoroughly and then

5 centrifuged at 3000 rpm for 5 min to precipitate the protein. Supernatant (100 μ L each) from centrifugation was transferred into a 96-well plate containing 200 μ L of aqueous mobile phase for LC/MS/MS analysis.

Sample Analysis – Prodrugs were analyzed using high-performance liquid chromatography with tandem mass-spectrometry (HPLC/MS/MS). An Thermo Aquasil

10 C18 4.6 $\times$ 50 mm column (5 μ M) was used for separation. Electrospray Ionization (ESI) was used for the ionization process. The mobile phase A contained 5 mM ammonium acetate in water with 0.1% formic acid and mobile phase B contained MeOH with 0.1% Formic Acid. Elution was performed with a gradient at a flow rate of 1 mL/min.

Example 5

15 Caco2 Protocol

Materials

Powders of Krebs-Henseleit buffer, calcium chloride dihydrate, and sodium bicarbonate were purchased from Sigma (St. Louis, MO). Caco-2 cells (Passage ~100) were obtained from Roche Basel. Dulbecco's Modified Eagle's Medium (DMEM)/high

20 medium, 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid (HEPES), and bovine serum were obtained from JRH Bioscience (Lenexa, KS). MEM nonessential amino acids, L-glutamine, penicillin, and streptomycin were obtained from GIBCO Labs, Life Tech. LLC (Grand Island, NY). Snapwell cell culture inserts (6.5 mm diameter, 1.12 cm², 0.4 μ m pore size) were obtained from Costar (Cambridge, MA).

25 Cell Cultures

Cells were grown in 75-cm² flasks and maintained at 37°C in an atmosphere of 5% CO₂ and 95% air. Culture media consisted of DMEM/high medium supplemented with 5% bovine serum, 25 mM HEPES, 1% MEM non-essential amino acids, 1% L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin. Cultures were passaged every week at

30 a split ratio of 1-3. For the permeability studies, Caco-2 cells at passage number 110-120 were plated at a density of 400,000 cells/cm² on Transwell polycarbonate filters in Snapwell inserts and allowed to culture for 7 days prior to use.

Krebs-Henseleit Buffer

Krebs-Henseleit bicarbonate buffer containing 10 mM glucose and 2.5 mM CaCl_2 adjusted to pH 6.5 and 7.4 were prepared per packet instructions. Powder salts were quantitatively dissolved in about 90% of required volume with Millipore water. Calcium chloride dihydrate and sodium bicarbonate were added before pH adjustment with 1N HCl or 1N NaOH. Additional Millipore water was added to bring the solution to final volume. The solution was sterilized by filtration using membrane with a porosity of 0.22 microns and stored in the refrigerator ($\sim 20^\circ\text{C}$) until use.

Cell Preparation

Differentiated cells were obtained from Cell Culture Core Facility and allowed to equilibrate at 37°C in an atmosphere of 5% CO_2 and 95% air. Snapwells inserts, containing caco-2 monolayers were rinsed in 37°C equilibrated Krebs-Henseleit pH 7.4 buffer.

Method

The cell insert was utilized as the diffusion chamber. The pH of the Krebs-Henseleit buffer in the apical and basolateral chambers was 6.5 and 7.4, respectively, and the initial concentration of substrate in apical side was $100\ \mu\text{M}$. The cells with test compound in apical chamber were pre-incubated for approximately 30 min at 37°C under an atmosphere of 5% CO_2 and 95% air. The experiments were initiated when the cell inserts with $100\ \mu\text{M}$ compounds in pH 6.5 Krebs-Henseleit buffer were transferred into a new plate with pre-equilibrated buffer in basolateral chamber. Samples from donor side at 0 min, and both donor and receiver sides at 30 min were collected for analysis.

Post-Experiment Control

Lucifer Yellow was used to assess the performance of the diffusion system. Following the last sampling for test compounds, Lucifer Yellow was added to the apical chamber to give an initial concentration of $100\ \mu\text{M}$. After 60 min of incubation, $250\ \mu\text{L}$ was removed from the basal chamber and assayed.

Calculation of Permeability Coefficient (P_{app})

The P_{app} was calculated using the following formula:

$$P_{app} = \frac{V \times dC}{A \times C_o \times dt} = (\text{cm/sec})$$

Where V is the volume (cm³) of the receiver solution, A is the surface area (cm²) of the Snapwell insert, C_o is the initial concentration (nM), and dC/dt is the change in concentration in the receiver chamber over time, i.e., the slope (nM/min) of the concentration in the receiver chamber vs. time. Concentrations at each sampling time point were corrected to account for the aliquots removed or transferring of donor inserts to new plates depending on the experiment.

Example 6

Representative pharmaceutical compositions for the subject compounds

10 Composition for wet granular formulation for oral administration (A)

Ingredient	% wt./wt.
Intragranular	
Active ingredient (I-2)	50.0
Mannitol (Partech M200) USP	39.0
Povidone K30 USP	5.0
Croscarmellose sodium NF	4.0
Purified water USP	for granulation
Extragranular	
Croscarmellose USP	2.0

The ingredients are mixed, granulated and dispensed into hard gelatin capsules containing about 500 mg of active compound

Composition for Oral Administration (B)

Ingredient	% wt./wt.
Active ingredient (I-2)	50.0
Mannitol (Partech M200)USP	30.0
Povidone K30 USP	5.0
Croscarmellose sodium NF	9.5
Microcrystalline cellulose	5.0
Magnesium stearate	0.5

The ingredients are combined and granulated using a solvent such as water. The formulation is then dried and formed into tablets containing about 500 mg of active compound with an appropriate tablet machine.

Composition for Oral Administration (C)

Ingredient	% wt./wt.
Active compound	6 g
Carboxymethylcellulose	1 g
Methyl paraben	0.15 g
Propyl paraben	0.05 g
Flavoring	0.035 mL
Colorings	0.5 mg
Distilled water	q.s. to 100 mL
Hydrochloric Acid	q.s. to adjust ca. pH 4

- 5 The ingredients are mixed to form a suspension for oral administration.

Suppository Formulation (D)

Ingredient	% wt./wt.
Active ingredient	1.0%
Polyethylene glycol 1000	74.5%
Polyethylene glycol 4000	24.5%

The ingredients are melted together and mixed on a steam bath, and poured into molds containing 2.5 g total weight.

- 10 The features disclosed in the foregoing description, or the following claims, expressed in their specific forms or in terms of a means for performing the disclosed function, or a method or process for attaining the disclosed result, as appropriate, may, separately, or in any combination of such features, be utilized for realizing the invention in diverse forms thereof.

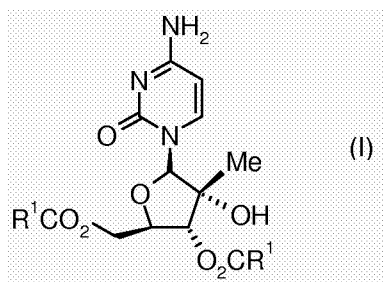
- 15 The foregoing invention has been described in some detail by way of illustration and example, for purposes of clarity and understanding. It will be obvious to one of skill in the art that changes and modifications may be practiced within the scope of the appended claims. Therefore, it is to be understood that the above description is intended to be illustrative and not restrictive. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be

determined with reference to the following appended claims, along with the full scope of equivalents to which such claims are entitled.

All patents, patent applications and publications cited in this application are hereby incorporated by reference in their entirety for all purposes to the same extent as if each
5 individual patent, patent application or publication were so individually denoted.

Claims

1. A compound of formula I



wherein R^1 is selected independently from the group consisting of C_{2-5} unbranched or branched alkyl, C_{2-5} unbranched or branched alkenyl, C_{3-5} cycloalkyl and C_{2-5} haloalkyl; and,

hydrates, solvates and acid addition salts thereof.

2. A compound according to claim 1 wherein R^1 is ethyl, *n*-propyl, *iso*-propyl, *n*-butyl or *iso*-butyl.

3. A compound according to claim 2 wherein R^1 is ethyl or *iso*-propyl.

4. A compound according to claim 3 wherein R^1 is *iso*-propyl and the compound is the hydrochloride, mesylate, hemisulfate or sulfate salt.

5. The use of compounds according to formula I as defined in anyone of claims 1 to 4, or a hydrates, solvates or acid addition salts thereof as antiviral agent for the treatment of a disease mediated by the HCV.

6. The use of compounds according to formula I as defined in anyone of claims 1 to 4, or a hydrates, solvates or acid addition salts thereof as antiviral agent in combination with at least one immune system modulator and/or at least one antiviral agent that inhibits replication of HCV for the treatment of a disease mediated by the HCV.

7. The use of compounds according to formula I as defined in anyone of claims 1 to 4, or a hydrates, solvates or acid addition salts thereof for the preparation of a medicament for treating a disease mediated by the HCV.

8. The use of compounds according to formula I as defined in anyone of claims 1 to 4, or a hydrates, solvates or acid addition salts thereof in combination with at least one immune system modulator and/or at least one antiviral agent that inhibits replication of HCV for the preparation of a medicament for treating a disease mediated by the HCV.

9. A method for treating a disease mediated by the Hepatitis C Virus (HCV) virus comprising administering to a mammal in need thereof, a therapeutically effective quantity of a compound according to claim 1.

10. The method of Claim 9 wherein the compound a dose of between 0.1 and 10 g per day is administered to the patient.

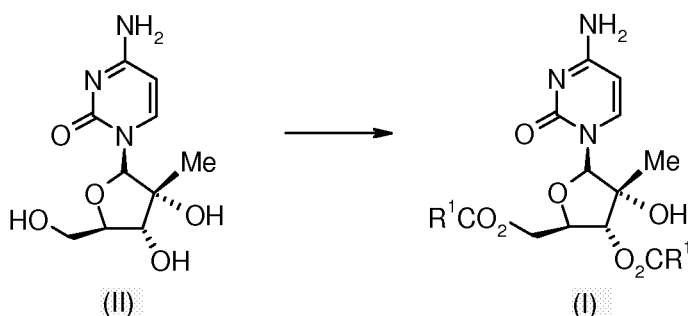
11. A method according to claim 10 wherein a dose of between 0.5 and 7.5 g per day is administered to the patient.

12. A method according to claim 11 wherein a dose of between 1.0 and 6.0 g per day is administered to the patient.

13. The method of Claim 9 further comprising administering at least one immune system modulator and/or at least one antiviral agent that inhibits replication of HCV.

14. A pharmaceutical composition comprising a therapeutically effective quantity of a compound according to claim 1 admixed with at least one pharmaceutically acceptable carrier, diluent or excipient.

15. A process for the selective O-acylation of a nucleoside **I** to afford an O-acyl nucleoside **II** under basic reaction conditions



wherein R^1 is selected from the group consisting of C_{2-4} unbranched or branched alkyl, C_{2-4} unbranched or branched alkenyl, C_{3-6} cycloalkyl, C_{2-5} haloalkyl,

and optionally substituted phenyl which process comprises the steps of:

(i) dissolving **II** and DMAP in an heterogeneous aqueous solvent mixture and adding aqueous base to adjust the pH from about 7.5 to about 12;

(ii) optionally adding sufficient saturated aqueous NaCl to produce a biphasic reaction mixture;

- (iii) adding an acylating agent and additional base sufficient to maintain the pH from about 7.5 to about 12;
- (iv) monitoring the reaction and discontinuing addition of said acylating agent and said base when the conversion reaches a satisfactory level;
- 5 (v) optionally contacting the O-acyl nucleoside with a pharmaceutically acceptable acid to form an acid additional salt of the O-acyl nucleoside.

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