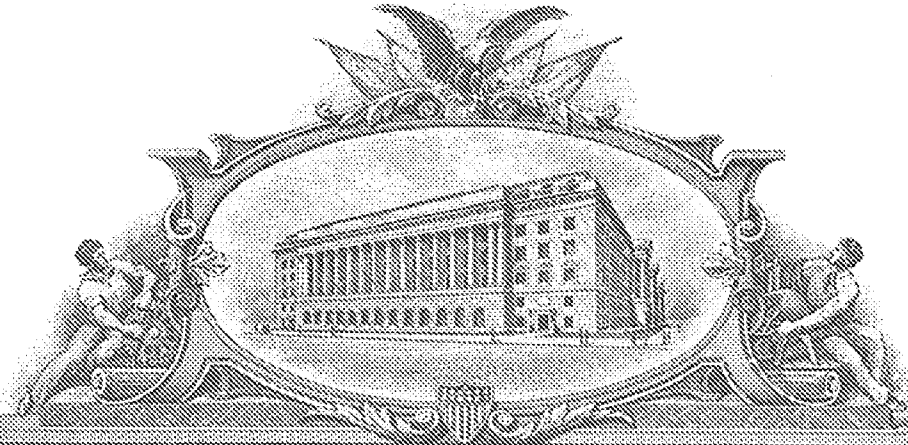


DOCUMENT MADE AVAILABLE UNDER THE PATENT COOPERATION TREATY (PCT)

International application number:	PCT/US2012/055621
International filing date:	14 September 2012 (14.09.2012)
Document type:	Certified copy of priority document
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489641



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United States Patent and Trademark Office

September 22, 2012

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APPLICATION NUMBER: 61/535,885

FILING DATE: *September 16, 2011*

RELATED PCT APPLICATION NUMBER: *PCT/US12/55621*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US61/535,885*



Certified by

David J. Kappas

Under Secretary of Commerce
for Intellectual Property
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET – Page 1 of 2

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)		
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William E.	Delaney	Foster City, California
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David W.	Oldach	Chapel Hill, North Carolina
Adrian S.	Ray	Redwood City, California
Additional inventors are being named on the <u>1</u> separately numbered sheets attached hereto.		
TITLE OF THE INVENTION (500 characters max):		
METHODS FOR TREATING HCV		
<div style="display: flex; justify-content: space-between;"> Direct all correspondence to: CORRESPONDENCE ADDRESS </div> <div style="display: flex; align-items: flex-start;"> <div style="flex: 1;"> <input checked="" type="checkbox"/> The address corresponding to Customer Number: </div> <div style="border: 1px solid black; width: 150px; height: 30px; text-align: center; margin-left: 10px;">25000</div> </div> <div style="margin-top: 5px;"> <input type="checkbox"/> OR <input type="checkbox"/> Firm or Individual Name </div>		
Address		
City	State	Zip
Country	Telephone	Email
ENCLOSED APPLICATION PARTS (check all that apply)		
<input checked="" type="checkbox"/> Application Data Sheet. See 37 CFR 1.76 <input type="checkbox"/> CD(s), Number of CDs _____		
<input type="checkbox"/> Drawing(s) Number of Sheets _____ <input type="checkbox"/> Other (specify) _____		
<input checked="" type="checkbox"/> Specification (e.g. description of the invention) Number of Pages <u>172</u>		
<small>Fees Due: Filing Fee of \$220 (\$110 for small entity). If the specification and drawings exceed 100 sheets of paper, an application size fee is also due, which is \$270 (\$135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).</small>		
METHOD OF PAYMENT OF THE FILING FEE AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT		
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. <div style="float: right; border: 1px solid black; padding: 5px; margin-top: 10px;">490.00</div>		
<input type="checkbox"/> A check or money order made payable to the <i>Director of the United States Patent and Trademark Office</i> is enclosed to cover the filing fee and application size fee (if applicable). <div style="text-align: right;">TOTAL FEE AMOUNT (\$)</div>		
<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.		
<input checked="" type="checkbox"/> The Director is hereby authorized to charge the filing fee and application size fee (if applicable) or credit any overpayment to Deposit <div style="margin-top: 5px;">Account Number: <u>07-1250</u></div>		

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: 9/16/2014

Signature: Donna Macedo

(Donna Macedo)

PROVISIONAL APPLICATION COVER SHEET
Page 2 of 2

PTO/SB/16 (12-08)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.



No.



Yes, the name of the U.S. Government agency and the Government contract number are:

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SIGNATURE



Date

September 16, 2011

TYPED or PRINTED NAME

Eric K. Voelk

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(if appropriate)

45,185

TELEPHONE

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Docket Number:

851.P3

**SUPPLEMENTAL SHEET TO
PROVISIONAL APPLICATION COVER SHEET (PTO/SB/16)
(Page 1 of 1)**

First Named Inventor	William E. Delaney, IV	Docket Number	851.P3
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Application Data Sheet

Application Information

Application Type::	Provisional
Subject Matter::	Utility
Suggested Group Art Unit::	N/A
CD-ROM or CD-R?::	None
Sequence submission?::	None
Computer Readable Form (CRF)?::	No
Title::	METHODS FOR TREATING HCV
Attorney Docket Number::	851.P3
Request for Early Publication?::	No
Request for Non-Publication?::	No
Small Entity?::	No
Petition included?::	No
Secrecy Order in Parent Appl.?::	No

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Correspondence Customer Number:: 25000

Representative Information

Representative Customer Number:: 25000

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Foreign Priority Information

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METHODS FOR TREATING HCV

FIELD OF THE INVENTION

5 This invention relates to combinations of therapeutic molecules useful for treating hepatitis C virus infection. The present invention relates to methods, uses, dosing regimens, and compositions.

BACKGROUND OF THE INVENTION

10 Hepatitis is a disease occurring throughout the world. Hepatitis is generally of viral nature, although, if considered a state of chronic inflammation of the liver, there are other known, non-infectious causes. Viral hepatitis is by far the most common form of hepatitis. The U.S. Centers for Disease Control has estimated that at least 1.8% of the U.S. population has serologic evidence of HCV infection, in the majority of
15 cases associated with chronic active infection. HCV is a positive-stranded RNA virus belonging to the Flaviviridae family and has closest relationship to the pestiviruses that include hog cholera virus and bovine viral diarrhea virus.

 The HCV genome is a single-stranded, positive-sense RNA of about 9,600 bp coding for a polyprotein of 3009-3030 amino acids, which is cleaved co- and post-
20 translationally by cellular and two viral proteinases into mature viral proteins (core, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B). The structural proteins, E1 and E2, are believed to be embedded into a viral lipid envelope and form stable heterodimers. The structural core protein is believed to interact with the viral RNA genome to form the nucleocapsid. The nonstructural proteins designated NS2 to NS5 include proteins
25 with enzymatic functions involved in virus replication and protein processing including a polymerase, protease, and helicase. HCV replicates through the production of a complementary negative-strand RNA template.

 HCV is a genetically diverse virus. Within a single infected patient, many variant viruses can be identified, leading to the description 'viral swarm', or viral
30 quasispecies. Within the global human population, HCV is also genetically diverse, with at least 6 major 'genotypes' identified (Genotypes 1-6), and numerous subtypes (i.e., HCV Genotype 1a and 1b). HCV genotypes are defined by genomic phylogenetic analysis, and diagnosed (in a given patient) by HCV RNA sequence-based diagnostic assays.

35 The main route of infection with HCV is blood exposure. The magnitude of the HCV infection as a health problem is illustrated by the prevalence among high-risk

groups. For example, in some surveys, 60% to 90% of hemophiliacs and more than 80% of intravenous drug abusers in western countries had chronic HCV infection. For intravenous drug abusers, the prevalence varies from about 28% to 80% depending on the population studied. The proportion of new HCV infections associated with blood or blood product transfusion has been markedly reduced due to pharmaceutical advances and widespread use of sensitive serologic and RNA detection assays used to screen blood donors, however, a large cohort of aging, chronically infected persons is already established.

One available treatment for HCV infection is pegylated interferon- α (PEG-IFN α 1a or PEG-IFN α 1b), which is, under current treatment guidelines, administered weekly by subcutaneous injection for 24 to 48 weeks, dependent upon the HCV viral genotype being treated. Although greater than 50% of patients with Genotype 1 HCV infection may be expected to have suppression of HCV viremia at the completion of 48 weeks therapy, a significant proportion of these patients will have viral relapse. Accordingly, a Sustained Virologic Response (SVR, defined as HCV RNA negativity 24 weeks post treatment cessation, and considered tantamount to 'cure') is only achieved in 30-40% of Genotype 1 HCV infections treated with PEG-IFN alone. In addition, treatment with PEG-IFN + RBV is not well tolerated, with an adverse event profile that includes flu-like symptoms, thrombocytopenia, anemia, and serious psychiatric side effects. While treatment with the current standard of care is suboptimal, many patients are precluded from ever starting therapy due to comorbidities common in HCV-infected populations, including psychiatric disorders, advanced liver disease, and substance abuse.

Ribavirin is a nucleoside analog antiviral drug. Ribavirin is typically taken orally (by mouth) twice a day. The exact mechanism for ribavirin is unknown. However, it is believed that when ribavirin enters a cell it is phosphorylated; it then acts as an inhibitor of inosine 5'-monophosphate dehydrogenase (IMPDH). IMPDH inhibitors such as ribavirin reduce the intracellular synthesis and storage of guanine, a nucleotide "building block" necessary for DNA and RNA production, thus inhibiting viral replication. IMPDH inhibitors also interfere with the reproduction of rapidly proliferating cells and cells with a high rate of protein turnover. Treatment with ribavirin monotherapy has little effect on HCV RNA levels, but is associated with a decline in serum alanine transferase (ALT). This observation suggests that ribavirin may not be acting as an antiviral agent, but rather as a modulator of immune system function. Ribavirin is only approved for use, for HCV infection, in combination with IFN.

Treatment with the combination of PEG-IFN plus ribavirin improves SVR rates over those observed with PEG-IFN alone, in large part due to reduction in the frequency of viral relapse at the cessation of therapy. Large clinical trial SVR rates for PEG-IFN/ribavirin treated patients with HCV Genotype 1 infection have ranged from 40-55%. At the present time, PEG-IFN/ribavirin therapy is considered the 'standard-of-care' treatment for chronic HCV infection. The standard of care is, however, expected to change rapidly in the near future with approval of direct acting antiviral agents which will, initially, be used in combination with PEG-IFN/ribavirin.

Unfortunately, different genotypes of HCV respond differently to PEG-IFN/ribavirin therapy; for example, HCV genotype 1 is more resistant to therapy than types 2 and 3. Additionally, many current treatments for HCV produce unwanted side effects. Thus, there is currently a need for new anti-viral therapies. In particular there is a need for new antiviral therapies that produce fewer unwanted side-effects, that are more effective against a range of HCV genotypes, or that have less complicated dosing schedules, i.e. that require administration of agents fewer times during a day.

SUMMARY OF THE INVENTION

The present invention provides compositions and therapeutic methods that are useful for treating viral infections (e.g. HCV). Certain compositions and methods of the invention produce fewer unwanted side-effects, are more effective against a range of HCV genotypes, reduce the potential for viral rebound due to resistance selection and have shortened less complicated dosing schedules than currently available therapies.

Accordingly, in one embodiment the invention provides a composition comprising two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11 and pharmaceutically acceptable salts thereof.

In another embodiment the invention provides a method of treating an HCV infection in a human, comprising administering two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11 and pharmaceutically acceptable salts thereof to the human.

In another embodiment the invention provides a method for ameliorating one or more symptoms of an HCV infection in a human, comprising administering two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4,

Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11 and pharmaceutically acceptable salts thereof to the human.

5 In another embodiment the invention provides a method for reducing viral load in a human with HCV, comprising administering two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11 and pharmaceutically acceptable salts thereof to the human.

10 In another embodiment the invention provides a method for reducing emergence of HCV quasiespecies with resistance to coadministered oral antiviral agents in a human, comprising administering two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. and pharmaceutically acceptable salts thereof to the human.

15 In another embodiment the invention provides the use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. and pharmaceutically acceptable salts thereof in medical therapy.

20 In another embodiment the invention provides the use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. and pharmaceutically acceptable salts thereof for the prophylactic or therapeutic treatment of a viral (e.g. HCV) infection.

In another embodiment the invention provides the use of a composition of the invention for the prophylactic or therapeutic treatment of a viral (e.g. HCV) infection.

25 In another embodiment the invention provides the use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. and pharmaceutically acceptable salts thereof to prepare a medicament for treating a viral (e.g. HCV) infection in a human.

30 In another embodiment the invention provides the use of a composition of the invention to prepare a medicament for treating a viral (e.g. HCV) infection in a human.

35 In another embodiment the invention provides the use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. and pharmaceutically acceptable salts thereof to prepare a

medicament for ameliorating one or more symptoms of a viral (e.g. HCV) infection in a human.

In another embodiment the invention provides the use of a composition of the invention to prepare a medicament for ameliorating one or more symptoms of a viral
5 (HCV) infection in a human.

In another embodiment the invention provides the use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. and pharmaceutically acceptable salts thereof to prepare a
10 medicament for reducing viral load in a human.

In another embodiment the invention provides the use of a composition of the invention to prepare a medicament for reducing viral load in a human.

In another embodiment the invention provides the use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4,
15 Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. and pharmaceutically acceptable salts thereof to prepare a medicament for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents in a human.

In another embodiment the invention provides the use of a composition of the invention to prepare a medicament for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents in a human.
20

The compositions and methods of the invention may provide "synergy" and "synergistic effects", i.e. the effect achieved when the active ingredients (including two or more Combination Compounds) are used together is greater than the sum of the
25 effects that results from using the compounds separately.

The compositions and methods of the invention are beneficial because they provide treatments for a wide range of HCV genotypes and because they cause fewer or less serious side effects than current HCV therapies (e.g. treatments that include the administration of interferon).
30

DETAILED DESCRIPTION OF THE INVENTION

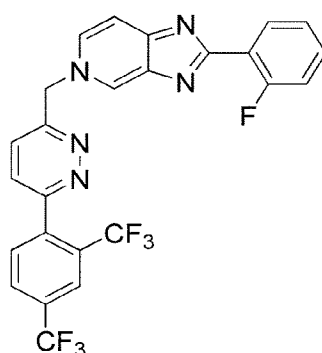
Definitions

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings. The fact that a particular term or phrase is
35 not specifically defined should not be correlated to indefiniteness or lacking clarity, but

rather terms herein are used within their ordinary meaning. When trade names are used herein, applicants intend to independently include the trade name product and the active pharmaceutical ingredient(s) of the trade name product.

As used herein the term "Combination Compounds" refers to Compound 1,
 5 Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7,
 Compound 9, Compound 10 and Compound 11.

As used herein, Compound 1 is:

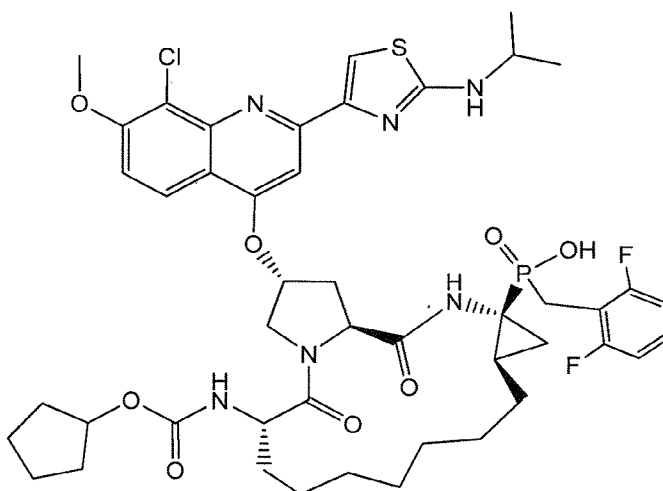


10

Compound 1 may also be referred to as 5-((6-(2,4-bis(trifluoromethyl)phenyl)pyridazin-3-yl)methyl)-2-(2-fluorophenyl)-5H-imidazo[4,5-c]pyridine or 5H-imidazo[4,5-c]pyridine, 5-[[6-[2,4-bis(trifluoromethyl)phenyl]pyridazin-3-yl]methyl]-2-(2-fluorophenyl).

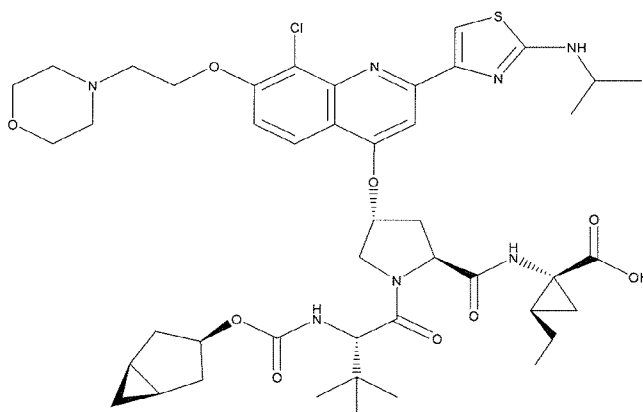
As used herein, Compound 2 is:

15

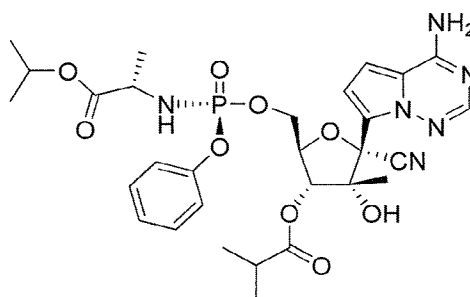


Compound 2 may also be referred to as (2*R*,6*S*,13*aR*,14*aS*,16*aS*)-2-(8-chloro-2-(2-(isopropylamino)thiazol-4-yl)-7-methoxyquinolin-4-yloxy)-6-(cyclopentyloxycarbonylamino)-5,16-dioxooctadecahydrocyclopropa[*e*]pyrrolo[1,2-*a*][1,4]diazacyclopentadecin-14*a*-yl(2,6-difluorobenzyl)phosphinic acid.

5 As used herein, Compound 3 is:

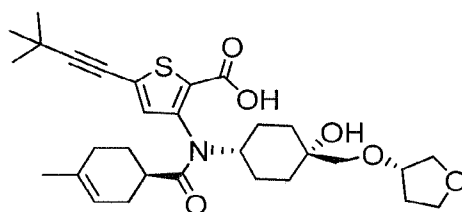


As used herein, Compound 4 is:



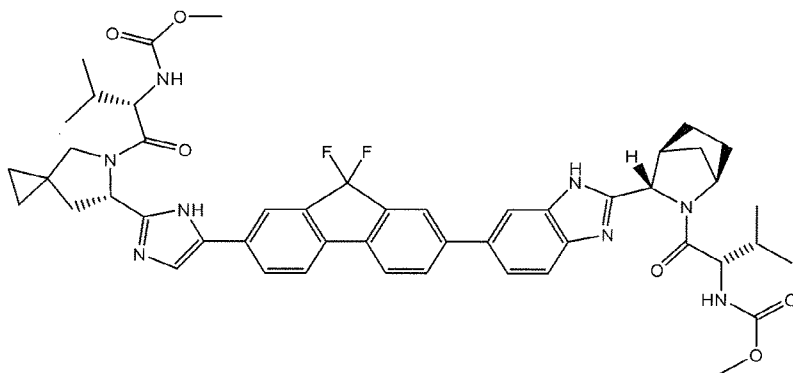
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As used herein, Compound 5 is:



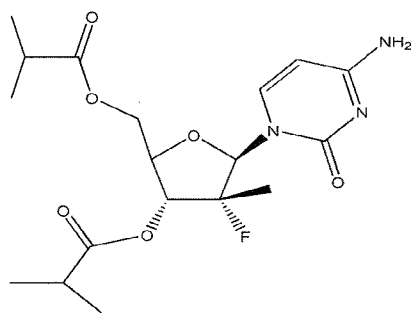
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As used herein, Compound 6 is:

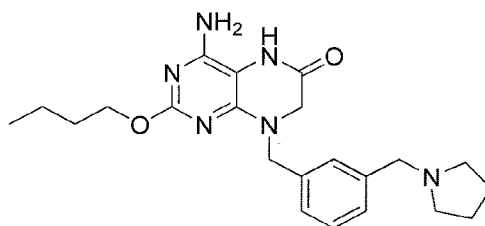


As used herein, Compound 7 is:

5

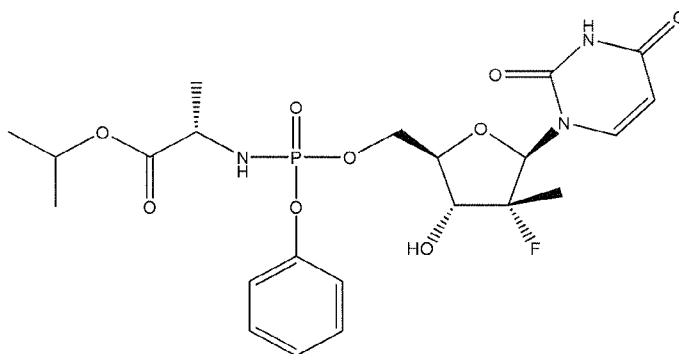


As used herein, Compound 8 is:



10

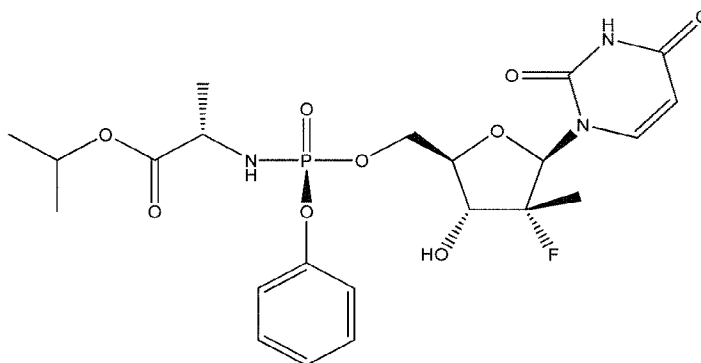
As used herein Compound 9 (diastereomer at P) is:



9

5 With regard to Compound 9, reference is made to US 7,964,580 and US 2010/0298257, (both of which are incorporated by reference) with regard to manufacture and purification Compound 9.

As used herein, Compound 10 (S-isomer of Compound 9) is:

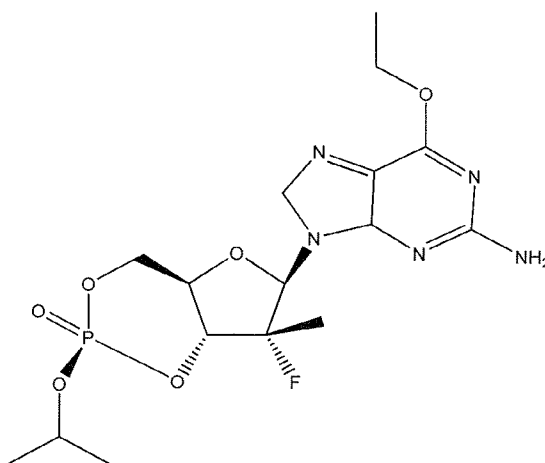


10

10

With regard to Compound 10, reference is made to US 7,964,580 and US 2010/0298257, (both of which are incorporated by reference) with regard to manufacture and purification of Compound 10.

15 As used herein, Compound 11 is:

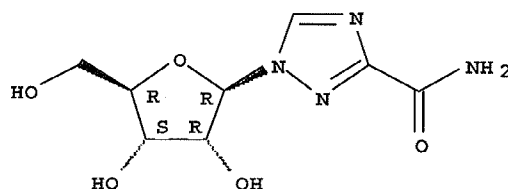


11

With regard to Compound 11, reference is made to US 2010/0081628, (both of which are incorporated by reference) with regard to manufacture and purification

5 Compound 11.

With regard to ribavirin, reference is made to EP 0 093 401 B1, herein incorporated by reference with regard to a process for manufacture as well as to nomenclature concerning ribavirin. As used herein, ribavirin refers to:



10

Ribavirin is also referred to as 1-β-D-ribofuranosyl-1H-1,2,4-Triazole-3-carboxamide, 1-β-D-ribofuranosyl-1,2,4-triazol-3-carboxamide; 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide; COPEGUS (Roche); DRG-0028; HSDB 6513; ICN 1229; MegaRibavirin (e.g. in formulations of 100 mg of ribavirin/mL); NSC 163039;

15 RAVANEX (BioPartners); REBETOL (Schering-Plough; AescA; Bayer Schering Pharma; Essex; Pfizer; Trading Pharma; Zuellig Pharma); Ribamide; RIBAMIDIL (Biopharma, Russia); RIBASPHERE (Three Rivers Pharmaceuticals); Ribavarin; Ribavirina; Tribavirin; VILONA (Valeant Pharmaceuticals; ICN Pharmaceuticals); VIRAMID (ICN Pharmaceuticals ; Alfa Wassermann); VIRAZOLE (Valeant

20 Pharmaceuticals); and VIRIZADOLE (Uci-farma, Sao Bernardo do Campo, Sao Paulo,

Brazil). In addition, as used herein ribavirin includes analogs of ribavirin, including taribavirin (VIRAMIDINE, ICN 3142).

The term "interferon" includes 1) interferons, *e.g.*, pegylated rIFN-alpha 2b (PEG-Intron, Merck & Co., Inc.), pegylated rIFN-alpha 2a (PEGASYN, Hoffmann-La Roche Inc.), rIFN-alpha 2b (INTRON® A, Merck & Co., Inc.), rIFN-alpha 2a (Roferon® A, Hoffmann-La Roche Inc.), interferon alpha (MULTIFERON® Viranative AB Corporation, OPC-18, Alfaferone, Alfaferone, subalin), interferon alfacon-1 (Valeant), interferon alpha-n1 (Wellferon™, Glaxo Wellcome), interferon alpha-n3 (ALFERON® Hemispherx Biopharma, Inc.), interferon-beta-1a (AVONEX® Biogen Idec, DL-8234 Daiichi Pharmaceutical Co. Ltd), interferon-omega (omega DUROS®, Alza Corporation, Intarcia Therapeutics, Inc.; Biomed 510, Intarcia Therapeutics, Inc.), albinterferon alpha-2b (ALBUFERON®, Human Genome Sciences, INC.), IFN alpha-2b XL, BLX-883 (LOCTERON®, Biolex Therapeutics, INC.), DA-3021, glycosylated interferon alpha-2b (AVI-005), PEG-INFERGEN®, Amgen, Inc., Pegylated interferon lambda-1(type III) (PEGylated IL-29), and BELEROFON®, Nautilus Biotech.

The term "combination therapy" means compositions or methods or uses or the like that incorporate two or more of the Combination Compounds. Combination therapy may also incorporate other active ingredients in addition to the two or more of the Combination Compounds including, but not limited to: ribavirin, an interferon, an alpha-glucosidase 1 inhibitor, a hepatoprotectant, a Toll-like receptor (TLR)-7 agonist, a cyclophilin inhibitor, an HCV viral entry inhibitor, an HCV maturation inhibitor, and an HCV IRES inhibitor.

The term "active ingredient" means a component of a combination therapy that a exerts or is capable of exerting a pharmaceutical effect including any of the Combination Compounds, ribavirin, an interferon, an alpha-glucosidase 1 inhibitor, a hepatoprotectant, a TLR-7 agonist, a cyclophilin inhibitor, an HCV viral entry inhibitor, an HCV maturation inhibitor, and an HCV IRES inhibitor.

The term "treating" and grammatical equivalents thereof, when used in the context of treating a disease, means slowing or stopping the progression of a disease, or ameliorating at least one symptom of a disease, more preferably ameliorating more than one symptom of a disease. For example, an HCV patient may experience an improvement in one or all of the following symptoms that can be associated with HCV infection: increase in alanine aminotransferase (ALT) levels, fever, headache, muscle aches, jaundice, fatigue, loss of appetite, nausea, vomiting and diarrhea. Treatment of

a hepatitis C virus infection can include reducing the HCV viral load in an HCV infected human being.

Certain of the compounds described herein contain one or more chiral centers, or may otherwise be capable of existing as multiple stereoisomers. The scope of the present invention includes mixtures of stereoisomers as well as purified enantiomers or enantiomerically/diastereomerically enriched mixtures. Also included within the scope of the invention are the individual isomers of the compounds represented by the formulae shown herein, as well as any wholly or partially equilibrated mixtures thereof. The present invention also includes the individual isomers of the compounds represented by the formula shown herein as mixtures with isomers thereof in which one or more chiral centers are inverted. Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., McGraw-Hill Dictionary of Chemical Terms (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., Stereochemistry of Organic Compounds (1994) John Wiley & Sons, Inc., New York, herein incorporated by reference in its entirety.

Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory.

A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which may occur where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

Combinations

The present invention encompasses combinations of two or more of the Combination Compounds. Table I showing possible two-way (Combinations 1-21), three-way (Combinations 22-56), four-way (Combinations 57-92) and five-way (Combinations 93-113) combinations of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10

and Compound 11 of the invention is provided below. Compound 4, Compound 9, Compound 10 and Compound 11 are nucleoside inhibitors of HCV NS5b polymerase and in certain embodiments, combinations of Combination Compounds can include only one of Compound 4, Compound 9, Compound 10 and Compound 11 (for
5 example, see Column 6 of Table I). In other embodiments, combinations of Combination Compounds can include one or two or three of Compound 4, Compound 9, Compound 10 and Compound 11.

TABLE I

10

Combo #	Compound 1	Compound 2	Compound 3	Compound 4 Or Compound 9 Or Compound 10 Or Compound 11	Compound 5	Compound 6	Compound 7
1	X	X					
2	X		X				
3	X			X			
4	X				X		
5	X					X	
6	X						X
7		X	X				
8		X		X			
9		X			X		
10		X				X	
11		X					X
12			X	X			
13			X		X		
14			X			X	
15			X				X
16				X	X		
17				X		X	
18				X			X
19					X	X	
20					X		X
21						X	X
22	X	X	X				
23	X	X		X			
24	X	X			X		
25	X	X				X	
26	X	X					X
27	X		X	X			
28	X		X		X		
29	X		X			X	

30	X		X				X
31	X			X	X		
32	X			X		X	
33	X			X			X
34	X				X	X	
35	X				X		X
36	X					X	X
37		X	X	X			
38		X	X		X		
39		X	X			X	
40		X	X				X
41		X		X	X		
42		X		X		X	
43		X		X			X
44		X			X	X	
45		X			X		X
46		X				X	X
47			X	X	X		
48			X	X		X	
49			X	X			X
50			X		X	X	
51			X		X		X
52			X			X	X
53				X	X	X	
54				X	X		X
55				X		X	X
56					X	X	X
57	X	X	X	X			
58	X	X	X		X		
59	X	X	X			X	
60	X	X	X				X
61	X	X		X	X		
62	X	X		X		X	
63	X	X		X			X
64	X	X			X	X	
65	X	X			X		X
66	X	X				X	X
67	X		X	X	X		
68	X		X	X		X	
69	X		X	X			X
70	X		X		X	X	
71	X		X		X		X
72	X		X			X	X
73	X			X	X	X	
74	X			X	X		X
75	X			X		X	X
76	X				X	X	X
77		X	X	X	X		
78		X	X	X		X	

79		X	X	X			X
80		X	X		X	X	
81		X	X		X		X
82		X	X			X	X
83		X		X	X	X	
84		X		X	X		X
85		X		X		X	X
86		X			X	X	X
87			X	X	X	X	
88			X	X	X		X
89			X	X		X	X
90			X		X	X	X
91				X	X	X	X
92							
93	X	X	X	X	X		
94	X	X	X	X		X	
95	X	X	X	X			X
96	X	X	X		X	X	
97	X	X	X		X		X
98	X	X	X			X	X
99	X	X		X	X	X	
100	X	X		X	X		X
101	X	X		X		X	X
102	X	X			X	X	X
103	X		X	X	X	X	
104	X		X	X	X		X
105	X		X	X		X	X
106	X		X		X	X	X
107	X			X	X	X	X
108		X	X	X	X	X	
109		X	X	X	X		X
110		X	X	X		X	X
111		X	X		X	X	X
112		X		X	X	X	X
113			X	X	X	X	X

Compositions

One aspect of the present invention includes a composition, e.g. a
5 pharmaceutical composition, the composition comprising Compound 1 and further
comprising a second compound selected from the group consisting of Compound 2,
Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9,
Compound 10 and Compound 11. In one specific embodiment of the invention, the
second compound may be Compound 2, Compound 3, Compound 4, Compound 5,
10 Compound 6, Compound 7, Compound 9, Compound 10, or Compound 11. In

another embodiment, the second compound may be Compound 2, Compound 3, Compound 4, Compound 5 or Compound 6.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 2 and further comprising a second compound selected from the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. In one specific embodiment of the invention, the second compound may be Compound 4. In one specific embodiment of the invention, the second compound may be Compound 3. In one specific embodiment of the invention, the second compound may be Compound 5.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 3 and further comprising a second compound selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. In one specific embodiment of the invention, the second compound may be Compound 1. In one specific embodiment of the invention, the second compound may be Compound 4. In one specific embodiment of the invention, the second compound may be Compound 5. In one specific embodiment of the invention, the second compound may be Compound 6. In one specific embodiment of the invention, the second compound may be Compound 7. In one specific embodiment of the invention, the second compound may be Compound 9. In one specific embodiment of the invention, the second compound may be Compound 10. In one specific embodiment of the invention, the second compound may be Compound 11.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising a first compound that is Compound 4 and further comprising a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7. In another aspect, the first compound selected from the group consisting of Compound 4, Compound 7, Compound 9, Compound 10 and Compound 11 and further comprising a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, and Compound 6. In one specific embodiment of the invention, the second compound may be Compound 1 or Compound 2 or Compound 3 or Compound 6. In one specific embodiment of the invention, the second compound may be Compound 1. In one specific embodiment of

the invention, the second compound may be Compound 2. In one specific embodiment of the invention, the second compound may be Compound 3. In one specific embodiment of the invention, the second compound may be Compound 5. In one specific embodiment of the invention, the second compound may be Compound 6.
 5 In one specific embodiment of the invention, the second compound may be Compound 7. In one specific embodiment of the invention, the second compound may be Compound 9. In one specific embodiment of the invention, the second compound may be Compound 10. In one specific embodiment of the invention, the second compound may be Compound 11.

10 Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 5 and further comprising a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. In one specific embodiment of the invention, the
 15 second compound may be Compound 1. In one specific embodiment of the invention, the second compound may be Compound 6.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 6 and further comprising a second compound selected from the group consisting of Compound 1,
 20 Compound 2, Compound 3, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. In one specific embodiment of the invention, the second compound may be Compound 1. In one specific embodiment of the invention, the second compound may be Compound 2. In one specific embodiment of the invention, the second compound may be Compound 3. In one specific embodiment of
 25 the invention, the second compound may be Compound 4. In one specific embodiment of the invention, the second compound may be Compound 7. In one specific embodiment of the invention, the second compound may be Compound 9. In one specific embodiment of the invention, the second compound may be Compound 10. In one specific embodiment of the invention, the second compound may be
 30 Compound 11.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 7 and further comprising a second compound selected from the group consisting of Compound 1,
 35 Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 9, Compound 10 and Compound 11.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising a first compound selected from the group consisting of Compound 9, Compound 10 and Compound 11 and further comprising a second compound selected from the group consisting of
 5 Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 1 and further comprising a second compound and a third compound each selected from the group
 10 consisting of Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 3, or Compound 4, or Compound 5 or Compound 6. The second compound may be Compound 2 and the third compound may be selected from the group consisting of Compound 4, Compound 7, Compound 9, Compound 10 and
 15 Compound 11. The second compound may be Compound 2 and the third compound may be selected from the group consisting of Compound 4. The second compound may be Compound 2 and the third compound may be selected from the group consisting of Compound 4, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 2 and the third compound may be
 20 Compound 4. The second compound may be Compound 3 and the third compound may be selected from the group consisting of Compound 4, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 3 and the third compound may be Compound 4. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be
 25 Compound 3 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 3. The second compound may be Compound 2 and the third compound may be Compound 5. The second compound may be Compound 3 and the third compound may be
 30 Compound 5.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 2 and further comprising a second compound and a third compound each selected from the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6,
 35 Compound 7, Compound 9, Compound 10 and Compound 11. The second compound

may be Compound 4. The second compound may be Compound 1 and the third compound may be selected from the group consisting of Compound 4, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1 and the third compound may be Compound 4. The second compound
 5 may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be selected from the group consisting of Compound 4, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 3 and the third compound
 10 may be Compound 4. The second compound may be Compound 3 and the third compound may be Compound 5. The second compound may be Compound 3 and the third compound may be Compound 6.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 3 and further
 15 comprising a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1 or Compound 6. The second compound may be Compound 1 and the third compound may be selected from the group consisting of Compound 4,
 20 Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1 and the third compound may be Compound 4. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be selected from the group consisting of Compound 4, Compound 7, Compound 9, Compound 10 and Compound 11 and the third compound
 25 may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6. The second compound may be selected from the group consisting of Compound 4, Compound 7, Compound 9, Compound 10 and Compound 11 and the third compound may be Compound 5. The second compound may be Compound 4 and the third compound may be Compound 5.

30 Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising a first compound selected from the group consisting of selected from the group consisting of Compound 4, Compound 7, Compound 9, Compound 10 and Compound 11 and further comprising a second compound and a third compound each selected from the group consisting of
 35 Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound

7. The second compound may be Compound 1, Compound 2, Compound 3 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6. The second compound may be Compound 1 and the third compound may be Compound 5. The second compound may be Compound 2 and the third compound may be Compound 5.

10 Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising a first compound selected from the group consisting of Compound 4 and further comprising a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7. The second compound may be Compound 1, Compound 2, Compound 3 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6. The second compound may be Compound 1 and the third compound may be Compound 5. The second compound may be Compound 2 and the third compound may be Compound 5.

25 Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 5 and further comprising a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1.

30 Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 6 and further comprising a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1, Compound 2, Compound 3 or Compound 4. The second

compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 4 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound
5 may be selected from the group consisting of Compound 4, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 2 and the third compound may be Compound 4. The second compound may be Compound 3 and the third compound may be selected from the group consisting of Compound 4, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound
10 may be Compound 3 and the third compound may be Compound 4. The second compound may be Compound 1 and the third compound may be Compound 5. The second compound may be Compound 2 and the third compound may be Compound 5. The second compound may be Compound 3 and the third compound may be Compound 5.

15 Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 7 and further comprising a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 9, Compound 10 and Compound 11.

20 Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising a first compound selected from the group consisting of Compound 9, Compound 10 and Compound 11 and further comprising a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, and
25 Compound 6.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising a first compound selected from the group consisting of Compound 9 and further comprising a second compound and a third compound each selected from the group consisting of Compound 1,
30 Compound 2, Compound 3, Compound 5, and Compound 6. The second compound may be Compound 1, Compound 2, Compound 3 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 1 and the third compound may be
35 Compound 6. The second compound may be Compound 2 and the third compound

may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6. The second compound may be Compound 1 and the third compound may be Compound 5. The second compound may be Compound 2 and the third compound may be Compound 5.

5 Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising a first compound selected from the group consisting of Compound 10 and further comprising a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, and Compound 6. The second compound
10 may be Compound 1, Compound 2, Compound 3 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound
15 may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6. The second compound may be Compound 1 and the third compound may be Compound 5. The second compound may be Compound 2 and the third compound may be Compound 5.

 Another aspect of the present invention includes a composition, e.g. a
20 pharmaceutical composition, the composition comprising a first compound selected from the group consisting of Compound 11 and further comprising a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, and Compound 6. The second compound may be Compound 1, Compound 2, Compound 3 or Compound 6. The second
25 compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third
30 compound may be Compound 6. The second compound may be Compound 1 and the third compound may be Compound 5. The second compound may be Compound 2 and the third compound may be Compound 5.

 Another aspect of the present invention includes a composition, e.g. a
35 pharmaceutical composition, the composition comprising Compound 1 and further comprising a second compound, a third compound and a fourth compound each

selected from the group consisting of Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 3, Compound 4, Compound 5, or Compound 6. The second compound may be Compound 2 and the
5 third compound may be Compound 4. The second compound may be Compound 3 and the third compound may be Compound 4. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6. The
10 second compound may be Compound 2, the third compound may be Compound 4, and the fourth compound may be Compound 6. The second compound may be Compound 3, the third compound may be Compound 4, and the fourth compound may be Compound 6.

Another aspect of the present invention includes a composition, e.g. a
15 pharmaceutical composition, the composition comprising Compound 2 and further comprising a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 4. The second compound
20 may be Compound 1 and the third compound may be Compound 4. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes a composition, e.g. a
25 pharmaceutical composition, the composition comprising Compound 3 and further comprising a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 4.
30 The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6. The second compound may be Compound 4, the third compound may be Compound 5, and the fourth compound may be Compound 6.

Another aspect of the present invention includes a composition, e.g. a
35 pharmaceutical composition, the composition comprising a first compound selected

from the group consisting of Compound 4, Compound 7, Compound 9, Compound 10 and Compound 11 and further comprising a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, and Compound 6. The second compound
5 may be Compound 1, Compound 2, Compound 3, or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound
10 may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising a first compound selected from the group consisting of Compound 4 and further comprising a second compound,
15 a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7. The second compound may be Compound 1, Compound 2, Compound 3, or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third
20 compound may be Compound 3. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising a first compound selected from the group consisting of Compound 4 and further comprising a second compound,
25 a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, and Compound 6. The second compound may be Compound 1, Compound 2, Compound 3, or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third
30 compound may be Compound 3. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6.
35 and the third compound may be Compound 6.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 5 and further comprising a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3,
5 Compound 4, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 6 and further comprising a second compound, a third compound and a fourth compound each
10 selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1, Compound 2, Compound 3, or Compound 4. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1
15 and the third compound may be Compound 3. The second compound may be Compound 4 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 4. The second compound may be Compound 3 and the third compound may be Compound 4.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 7 and further comprising a second compound, a third compound and a fourth compound each
20 selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 9, Compound 10 and Compound 11.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising a first compound selected from the group consisting of Compound 9, Compound 10 and Compound 11 and further comprising a second compound, a third compound and a fourth compound
25 each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 1 and further comprising a second compound, a third compound, a fourth compound and a fifth
30 compound each selected from the group consisting of Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10

and Compound 11. The second compound may be Compound 3, Compound 4, Compound 5 or Compound 6. The second compound may be Compound 2 and the third compound may be Compound 4. The second compound may be Compound 3 and the third compound may be Compound 4. The second compound may be
5 Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 2 and further
10 comprising a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 4. The second compound may be Compound 1 and the third compound may be Compound 4. The
15 second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 3 and further
20 comprising a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1 or Compound 6. The second compound may be Compound 1 and the third compound may be
25 Compound 4. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising a first compound selected
30 from the group consisting of Compound 4, Compound 7, Compound 9, Compound 10 and Compound 11 and further comprising a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, and Compound 6. The second compound may be Compound 1, Compound 2, Compound 3 or Compound 6.
35 The second compound may be Compound 1 and the third compound may be

Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3
5 and the third compound may be Compound 6.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising a first compound selected from the group consisting of Compound 4 and further comprising a second compound, a third compound, a fourth compound and a fifth compound each selected from the
10 group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7. The second compound may be Compound 1, Compound 2, Compound 3 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second
15 compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising a first compound selected from the group consisting of Compound 4 and further comprising a second compound, a third compound, a fourth compound and a fifth compound each selected from the
20 group consisting of Compound 1, Compound 2, Compound 3, Compound 5, and Compound 6. The second compound may be Compound 1, Compound 2, Compound 3 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 1
25 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 5 and further comprising a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2,

Compound 3, Compound 4, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11.

The second compound may be Compound 1.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 6 and further comprising a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1, Compound 2, Compound 3, and Compound 4. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 4 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 4. The second compound may be Compound 3 and the third compound may be Compound 4.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising Compound 7 and further comprising a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 9, Compound 10 and Compound 11.

Another aspect of the present invention includes a composition, e.g. a pharmaceutical composition, the composition comprising a first compound selected from the group consisting of Compound 9, Compound 10 and Compound 11 and further comprising a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

Salts

The Combination Compounds and other active ingredients can be in the form of a salt. Typically, but not absolutely, the salts of the Combination Compounds and other active ingredients are pharmaceutically acceptable salts. Salts encompassed within the term "pharmaceutically acceptable salts" refer to non-toxic salts of the Combination Compounds and/or other active ingredients. Examples of suitable pharmaceutically acceptable salts include inorganic acid addition salts such as

chloride, bromide, sulfate, phosphate, and nitrate; organic acid addition salts such as acetate, galactarate, propionate, succinate, lactate, glycolate, malate, tartrate, citrate, maleate, fumarate, methanesulfonate, p-toluenesulfonate, and ascorbate; salts with acidic amino acid such as aspartate and glutamate; alkali metal salts such as sodium salt and potassium salt; alkaline earth metal salts such as magnesium salt and calcium salt; ammonium salt; organic basic salts such as trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, dicyclohexylamine salt, and N,N'-dibenzylethylenediamine salt; and salts with basic amino acid such as lysine salt and arginine salt. The salts may be in some cases hydrates or ethanol solvates.

Pharmaceutical Formulations

The Combination Compounds and/or other active ingredients can be formulated with conventional carriers or excipients, which can be selected in accord with ordinary practice. Tablets typically contain excipients, glidants, fillers, binders and the like. Aqueous formulations can be prepared in sterile form, and when intended for delivery by other than oral administration generally will be isotonic. All formulations will optionally contain excipients such as those set forth in the Handbook of Pharmaceutical Excipients (1986), herein incorporated by reference in its entirety. Excipients include ascorbic acid and other antioxidants, chelating agents such as EDTA, carbohydrates such as dextrin, hydroxyalkylcellulose, hydroxyalkylmethylcellulose, stearic acid and the like.

The pH of the formulations ranges from about 3 to about 11, but is ordinarily about 7 to 10.

While it is possible for an active ingredient to be administered alone it may be preferable to present one or more active ingredients as pharmaceutical formulations. The formulations of the invention, both for veterinary and for human use, comprise at least one active ingredient, together with one or more acceptable carriers and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and physiologically innocuous to the recipient thereof.

The formulations include those suitable for the administration routes set forth below. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Techniques and formulations generally can be found in Remington's Pharmaceutical Sciences (Mack Publishing Co., Easton, Pa.), herein incorporated by reference in its entirety.

Such methods include the step of bringing into association an active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations can be prepared by uniformly and intimately bringing into association one or more active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of an active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. An active ingredient may also be administered as a bolus, electuary or paste.

A tablet can be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine an active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredient moistened with an inert liquid diluent. The tablets may optionally be coated or scored and optionally can be formulated so as to provide slow or controlled release of an active ingredient.

For administration to the eye or other external tissues e.g., mouth and skin, the formulations can be preferably applied as a topical ointment or cream containing an active ingredient(s) in an amount of, for example, 0.075 to 20% w/w (including active ingredient(s) in a range between 0.1% and 20% in increments of 0.1% w/w such as 0.6% w/w, 0.7% w/w, etc.), preferably 0.2 to 15% w/w and most preferably 0.5 to 10% w/w. When formulated in an ointment, an active ingredient may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, an active ingredient may be formulated in a cream with an oil-in-water cream base.

If desired, the aqueous phase of the cream base may include, for example, at least 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane 1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol (including PEG 400) and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of an active ingredient through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethyl sulphoxide and related analogs.

The oily phase of the emulsions of Combination Compounds and/or other active ingredients may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Emulgents and emulsion stabilizers suitable for use in the formulation of the invention include Tween® 60 (ICI Americas Inc.), Span 80, cetostearyl alcohol, benzyl alcohol, myristyl alcohol, glyceryl mono-stearate and sodium lauryl sulfate.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties. The cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Pharmaceutical formulations according to the present invention comprise one or more active together with one or more pharmaceutically acceptable carriers or excipients and optionally other therapeutic agents. Pharmaceutical formulations containing active ingredients may be in any form suitable for the intended method of administration. When used for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation. Tablets containing an active ingredient in admixture with non-toxic pharmaceutically acceptable excipient which are suitable for

manufacture of tablets are acceptable. These excipients may be, for example, inert diluents, such as calcium or sodium carbonate, lactose, lactose monohydrate, croscarmellose sodium, povidone, calcium or sodium phosphate; granulating and disintegrating agents, such as maize starch, or alginic acid; binding agents, such as cellulose, microcrystalline cellulose, starch, gelatin or acacia; and lubricating agents, such as magnesium stearate, stearic acid or talc. Tablets may be uncoated or may be coated by known techniques including microencapsulation to delay disintegration and adsorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

Formulations for oral use may be also presented as hard gelatin capsules where an active ingredient(s) is mixed with an inert solid diluent, for example calcium phosphate or kaolin, or as soft gelatin capsules wherein an active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions of the invention contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxy-benzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose or saccharin.

Oil suspensions may be formulated by suspending an active ingredient in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oral suspensions may contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth herein, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

Dispersible powders and granules of the invention suitable for preparation of an aqueous suspension by the addition of water provide an active ingredient in admixture with a dispersing or wetting agent, a suspending agent, and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those disclosed above. Additional excipients, for example sweetening, 5 flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable 10 emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan monooleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan monooleate. The emulsion may also contain 15 sweetening and flavoring agents. Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

The pharmaceutical compositions of the invention may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous 20 suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned herein. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butane-diol or prepared as a lyophilized powder. Among the 25 acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

30 The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain approximately 1 to 1000 mg of active material compounded with an appropriate and convenient amount of carrier material 35 which may vary from about 5 to about 95% of the total compositions (weight:weight).

The pharmaceutical composition can be prepared to provide easily measurable amounts for administration. For example, an aqueous solution intended for intravenous infusion may contain from about 3 to 500 µg of an active ingredient per milliliter of solution in order that infusion of a suitable volume at a rate of about 30 mL/hr can occur.

Formulations suitable for administration to the eye include eye drops wherein an active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for an active ingredient. An active ingredient is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% particularly about 1.5% w/w.

Formulations suitable for topical administration in the mouth include lozenges comprising an active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising an active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising an active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulations suitable for intrapulmonary or nasal administration have a particle size for example in the range of 0.1 to 500 µm (including particle sizes in a range between 0.1 and 500 µm in increments such as 0.5 µm, 1 µm, 30 µm, 35 µm, etc.), which is administered by rapid inhalation through the nasal passage or by inhalation through the mouth so as to reach the alveolar sacs. Suitable formulations include aqueous or oily solutions of an active ingredient. Formulations suitable for aerosol or dry powder administration may be prepared according to conventional methods and may be delivered with other therapeutic agents such as compounds heretofore used in the treatment or prophylaxis of infections as described herein.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to an active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents.

The formulations can be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules and tablets of the kind previously described. Preferred unit dosage formulations can be those containing a daily dose or unit daily sub-dose, as herein above recited, or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of Combination Compounds and/or other active ingredients may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents.

Combination Compounds and other active ingredients can also be formulated to provide controlled release of an active ingredient to allow less frequent dosing or to improve the pharmacokinetic or toxicity profile of an active ingredient. Accordingly, the invention also provides compositions comprising two or more of the Combination Compounds formulated for sustained or controlled release.

Dosages

The effective dose of an active ingredient depends at least on the nature of the condition being treated, toxicity, whether the compound is being used prophylactically (lower doses) or against an active disease or condition, the method of delivery, and the pharmaceutical formulation, and can be determined by the clinician using conventional dose escalation studies.

By way of example, compositions of the invention (e.g. tablets) can be formulated to provide effective doses. For example, with respect to Compound 1, or a pharmaceutically acceptable salt thereof, the composition may comprise from 1.0 mg to 100 mg, from 5 mg to 40 mg, from 30 mg to 50 mg, or 20 mg or 40 mg and can be adapted to be administered one or more times daily to a human being in need thereof in combination with any one or more of Compound 2, Compound 3, Compound 6, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. With respect to Compound 2 or a pharmaceutically acceptable salt thereof, the composition may comprise from 25 mg to 800 mg, from 50 mg to 400 mg, or from 60 mg to 300 mg or from 70 mg to 200 mg or may be 150 mg and can be

adapted to be administered one or more times daily to a human being in need thereof in combination with any one or more of Compound 1, Compound 3, Compound 6, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. With respect to Compound 3, or a pharmaceutically acceptable salt thereof, the composition may comprise from 10 mg to 1000 mg, or 50 to 400 mg, or 100mg to 400mg or 200 mg to 400 mg and can be adapted to be administered one or more times daily to a human being in need thereof in combination with any one or more of Compound 1, Compound 2, Compound 6, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. With respect to Compound 4, or a pharmaceutically acceptable salt thereof, the composition may comprise from 25mg to 400mg or from 25mg to 200mg can be adapted to be administered one or more times daily to a human being in need thereof in combination with any one or more of Compound 1, Compound 2, Compound 3, Compound 6, Compound 5 and Compound 7. With respect to Compound 5, or a pharmaceutically acceptable salt thereof, the composition may comprise from 50mg to 1000mg or 100mg to 750mg can be adapted to be administered one or more times daily to a human being in need thereof in combination with any one or more of Compound 1, Compound 2, Compound 3, Compound 6, Compound 4, Compound 7, Compound 9, Compound 10 and Compound 11. With respect to Compound 6, or a pharmaceutically acceptable salt thereof, the composition may comprise from 1mg to 500mg or from 3 mg to 300 mg or from 3 mg to 200mg or from 3 mg to 100 mg or from 10 mg to 90 mg or from 30 mg to 90 mg can be adapted to be administered one or more times daily to a human being in need thereof in combination with any one or more of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. With respect to Compound 7, or a pharmaceutically acceptable salt thereof, the composition may comprise from 100 micrograms up to 3000mg, from 25mg up to 2000mg, or from 50mg up to 1000mg and can be adapted to be administered one or more times daily (e.g. four times daily) to a human being in need thereof in combination with any one or more of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 9, Compound 10 and Compound 11. With respect to Compounds 9 and 10, or pharmaceutically acceptable salts thereof, the composition may comprise 10 mg to 1000mg per day (according to US 2010/0298257). With respect to Compound 11, or pharmaceutically acceptable salts thereof, the composition may comprise 1mg to 1000mg per day (according to US 2010/0081628). Dosages for Compounds 1-7 that are co-

administered may need to be adjusted to account for potential drug-drug interactions. For example, although it does not appear that Compound 1 affects drug metabolizing systems, Compound 2 appears to have the effect of increasing the exposure of Compound 1 approximately 2-3X. Therefore, a dose reduction (e.g. 2x-3x) of Compound 1 would be anticipated when Compound 1 is combined with Compound 2. In combination with Compound 16, Compound 2 appears to have the effect of increasing the exposure of Compound 6 approximately 5x, so dose reduction (e.g. 3x-5x) of Compound 16 would be anticipated when Compound 16 is dosed with Compound 2. Therefore, a 10 mg dose of Compound 6 when coadministered with Compound 2 approximate to a 30 mg dose.

The two or more Combination Compounds may be administered in conjunction with Ribavirin in amounts of about 800mg, 1000mg or 1200mg per day in single or multiple dosages (e.g. about 400mg, 500mg or 600mg twice daily).

Use of Combinations of the Invention

In practice of this aspect of the invention, Combination Compounds may be used in the dosages set forth above.

One aspect of the present invention includes Compound 1 for use in a method of treating HCV infections, wherein compound 1 is used in combination with a second compound selected from the group consisting of Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 3, Compound 4, Compound 5 or Compound 6.

Another aspect of the present invention includes Compound 2 for use in a method of treating HCV infections, wherein compound 2 is used in combination with a second compound selected from the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 4.

Another aspect of the present invention includes Compound 3 for use in a method of treating HCV infections, wherein compound 3 is used in combination with a second compound selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1 or Compound 6.

Another aspect of the present invention includes Compound 4 for use in a method of treating HCV infections, wherein Compound 4 is used in combination with a

second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7. The second compound may be Compound 1 or Compound 2 or Compound 3 or Compound 6.

5 Another aspect of the present invention includes Compound 5 for use in a method of treating HCV infections, wherein Compound 5 is used in combination with a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1.

10 Another aspect of the present invention includes Compound 6 for use in a method of treating HCV infections, wherein Compound 6 is used in combination with a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1, Compound 2, Compound 3 or Compound 4.

15 Another aspect of the present invention includes Compound 7 for use in a method of treating HCV infections, wherein Compound 7 is used in combination with a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 9, Compound 10 and Compound 11.

20 Another aspect of the present invention includes Compound 9 for use in a method of treating HCV infections, wherein Compound 9 is used in combination with a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6, and Compound 7.

25 Another aspect of the present invention includes Compound 10 for use in a method of treating HCV infections, wherein Compound 10 is used in combination with a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6, and Compound 7.

30 Another aspect of the present invention includes Compound 11 for use in a method of treating HCV infections, wherein Compound 11 is used in combination with a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6, and Compound 7.

35 Another aspect of the present invention includes Compound 1 for use in a method of treating HCV infections, wherein compound 1 is used in combination with a second compound and a third compound each selected from the group consisting of Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7,

Compound 9, Compound 10 and Compound 11. The second compound may be Compound 3, or Compound 4, or Compound 5 or Compound 6. The second compound may be Compound 2 and the third compound may be Compound 4. The second compound may be Compound 3 and the third compound may be Compound 4.

5 The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

10 Another aspect of the present invention includes Compound 2 for use in a method of treating HCV infections, wherein compound 2 is used in combination with a second compound and a third compound each selected from the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 4. The second compound may be Compound 1 and the third compound
15 may be Compound 4. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

20 Another aspect of the present invention includes Compound 3 for use in a method of treating HCV infections, wherein compound 3 is used in combination with a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 4. The second compound may be Compound 1
25 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

30 Another aspect of the present invention includes Compound 4 for use in a method of treating HCV infections, wherein Compound 4 is used in combination with a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7. The second compound may be Compound 1, Compound 2, Compound 3 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 1 and the
35 third compound may be Compound 6. The second compound may be Compound 2

and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6.

Another aspect of the present invention includes Compound 5 for use in a method of treating HCV infections, wherein Compound 5 is used in combination with a
5 second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1.

Another aspect of the present invention includes Compound 6 for use in a
10 method of treating HCV infections, wherein Compound 6 is used in combination with a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1, Compound 2, Compound 3 or Compound 4. The second compound
15 may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 4 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 4. The second compound may be Compound 3 and the third compound
20 may be Compound 4.

Another aspect of the present invention includes Compound 7 for use in a method of treating HCV infections, wherein Compound 7 is used in combination with a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6,
25 Compound 9, Compound 10 and Compound 11.

Another aspect of the present invention includes Compound 9 for use in a method of treating HCV infections, wherein Compound 9 is used in combination with a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound
30 7.

Another aspect of the present invention includes Compound 10 for use in a method of treating HCV infections, wherein Compound 10 is used in combination with a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound
35 7.

Another aspect of the present invention includes Compound 11 for use in a method of treating HCV infections, wherein Compound 11 is used in combination with a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

Another aspect of the present invention includes Compound 1 for use in a method of treating HCV infections, wherein compound 1 is used in combination with a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 3, Compound 4, Compound 5, or Compound 6. The second compound may be Compound 2 and the third compound may be Compound 4. The second compound may be Compound 3 and the third compound may be Compound 4. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes Compound 2 for use in a method of treating HCV infections, wherein compound 2 is used in combination with a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 4. The second compound may be Compound 1 and the third compound may be Compound 4. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes Compound 3 for use in a method of treating HCV infections, wherein compound 3 is used in combination with a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 4. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes Compound 4 for use in a method of treating HCV infections, wherein Compound 4 is used in combination with a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5,
 5 Compound 6 and Compound 7. The second compound may be Compound 1, Compound 2, Compound 3, or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 1 and the third compound may be Compound 6. The
 10 second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6.

Another aspect of the present invention includes Compound 5 for use in a method of treating HCV infections, wherein Compound 5 is used in combination with a
 15 second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1.

Another aspect of the present invention includes Compound 6 for use in a
 20 method of treating HCV infections, wherein Compound 6 is used in combination with a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1, Compound 2, Compound 3, or Compound 4.
 25 The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 4 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 4. The second compound may be Compound 3
 30 and the third compound may be Compound 4.

Another aspect of the present invention includes Compound 7 for use in a method of treating HCV infections, wherein Compound 7 is used in combination with a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4,
 35 Compound 5, Compound 6, Compound 9, Compound 10 and Compound 11.

Another aspect of the present invention includes Compound 9 for use in a method of treating HCV infections, wherein Compound 9 is used in combination with a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5,
 5 Compound 6 and Compound 7.

Another aspect of the present invention includes Compound 10 for use in a method of treating HCV infections, wherein Compound 10 is used in combination with a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5,
 10 Compound 6 and Compound 7.

Another aspect of the present invention includes Compound 11 for use in a method of treating HCV infections, wherein Compound 11 is used in combination with a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5,
 15 Compound 6 and Compound 7.

Another aspect of the present invention includes Compound 1 for use in a method of treating HCV infections, wherein compound 1 is used in combination with a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 2, Compound 3, Compound 4,
 20 Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 3, Compound 4, Compound 5 or Compound 6. The second compound may be Compound 2 and the third compound may be Compound 4. The second compound may be Compound 3 and the third compound may be Compound 4. The second compound may be
 25 Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes Compound 2 for use in a method of treating HCV infections, wherein compound 2 is used in combination with a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 3, Compound 4,
 30 Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 4. The second compound may be Compound 1 and the third compound may be Compound 4. The second

compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes Compound 3 for use in a method of treating HCV infections, wherein compound 3 is used in combination with a
 5 second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 4.
 10 The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes Compound 4 for use in a method of treating HCV infections, wherein Compound 4 is used in combination with a
 15 second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7. The second compound may be Compound 1, Compound 2, Compound 3 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second
 20 compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6.

Another aspect of the present invention includes Compound 5 for use in a method of treating HCV infections, wherein Compound 5 is used in combination with a
 25 second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 6, Compound 7, Compound 9, Compound 10 and
 30 Compound 11. The second compound may be Compound 1.

Another aspect of the present invention includes Compound 6 for use in a method of treating HCV infections, wherein Compound 6 is used in combination with a
 second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3,
 35 Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and

Compound 11. The second compound may be Compound 1, Compound 2, Compound 3, or Compound 4. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be
5 Compound 4 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 4. The second compound may be Compound 3 and the third compound may be Compound 4.

Another aspect of the present invention includes Compound 7 for use in a method of treating HCV infections, wherein Compound 7 is used in combination with a
10 second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 9, Compound 10 and Compound 11.

Another aspect of the present invention includes Compound 9 for use in a method of treating HCV infections, wherein Compound 9 is used in combination with a
15 second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

Another aspect of the present invention includes Compound 10 for use in a method of treating HCV infections, wherein Compound 10 is used in combination with
20 a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

Another aspect of the present invention includes Compound 11 for use in a method of treating HCV infections, wherein Compound 11 is used in combination with
25 a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

One aspect of the present invention includes a method for ameliorating one or
30 more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 1 and further comprising administering a second compound selected from
35 the group consisting of comprising Compound 2, Compound 3, Compound 4,

Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 3, Compound 4, Compound 5 or Compound 6.

Another aspect of the present invention includes a method for ameliorating one
5 or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 2 and further comprising administering a second compound selected from
10 the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 4.

Another aspect of the present invention includes a method for ameliorating one
or more symptom of HCV infection in a human, a method for reducing viral load in a
15 human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 3 and further comprising administering a second compound selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5,
20 Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1 or Compound 6.

Another aspect of the present invention includes a method for ameliorating one
or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a
25 method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 4 and further comprising administering a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7. The second compound may be Compound 1 or
30 Compound 2 or Compound 3 or Compound 6.

Another aspect of the present invention includes a method for ameliorating one
or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a
method for reducing emergence of HCV quasispecies with resistance to
35 coadministered oral antiviral agents, each method comprising administering

Compound 5 and further comprising administering a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1.

5 Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering
10 Compound 6 and further comprising administering a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1, Compound 2, Compound 3 or Compound 4.

 Another aspect of the present invention includes a method for ameliorating one
15 or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering
20 Compound 7 and further comprising administering a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 9, Compound 10 and Compound 11.

 Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a
25 method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 9 and further comprising administering a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

30 Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering
35 Compound 10 and further comprising administering a second compound selected from

the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 11 and further comprising administering a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 1 and further comprising administering a second compound and a third compound each selected from the group consisting of Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 3, or Compound 4, or Compound 5 or Compound 6. The second compound may be Compound 2 and the third compound may be Compound 4. The second compound may be Compound 3 and the third compound may be Compound 4. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 2 and further comprising administering a second compound and a third compound each selected from the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 4. The second compound may be Compound 1 and the third compound may be Compound 4. The

second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes a method for ameliorating one
5 or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to
coadministered oral antiviral agents, each method comprising administering
Compound 3 and further comprising administering a second compound and a third
10 compound each selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 4. The second compound may be Compound 1 and the third compound
15 may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes a method for ameliorating one
or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a
20 method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 4 and further comprising administering a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7. The second compound
25 may be Compound 1, Compound 2, Compound 3 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound
30 may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6.

Another aspect of the present invention includes a method for ameliorating one
or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a
35 method for reducing emergence of HCV quasispecies with resistance to

coadministered oral antiviral agents, each method comprising administering Compound 5 and further comprising administering a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 6 and further comprising administering a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1, Compound 2, Compound 3 or Compound 4. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 4 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 4. The second compound may be Compound 3 and the third compound may be Compound 4.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 7 and further comprising administering a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 9, Compound 10 and Compound 11.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 9 and further comprising administering a second compound and a third

compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 10 and further comprising administering a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 11 and further comprising administering a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 1 and further comprising administering a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 3, Compound 4, Compound 5, or Compound 6. The second compound may be Compound 2 and the third compound may be Compound 4. The second compound may be Compound 3 and the third compound may be Compound 4. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to

5 coadministered oral antiviral agents, each method comprising administering Compound 2 and further comprising administering a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be

10 Compound 4. The second compound may be Compound 1 and the third compound may be Compound 4. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes a method for ameliorating one

15 or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 3 and further comprising administering a second compound, a third

20 compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 4. The second compound may be Compound 1

25 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a

30 method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 4 and further comprising administering a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound

35 7. The second compound may be Compound 1, Compound 2, Compound 3, or

Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 5 and further comprising administering a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 6 and further comprising administering a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1, Compound 2, Compound 3, or Compound 4. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 4 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 4. The second compound may be Compound 3 and the third compound may be Compound 4.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a

method for reducing emergence of HCV quasispecies with resistance to
coadministered oral antiviral agents, each method comprising administering
Compound 7 and further comprising administering a second compound, a third
compound and a fourth compound each selected from the group consisting of
5 Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6,
Compound 9, Compound 10 and Compound 11.

Another aspect of the present invention includes a method for ameliorating one
or more symptom of HCV infection in a human, a method for reducing viral load in a
human diagnosed with HCV, a method of treating HCV in a human subject, and a
10 method for reducing emergence of HCV quasispecies with resistance to
coadministered oral antiviral agents, each method comprising administering
Compound 9 and further comprising administering a second compound, a third
compound and a fourth compound each selected from the group consisting of
Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound
15 7.

Another aspect of the present invention includes a method for ameliorating one
or more symptom of HCV infection in a human, a method for reducing viral load in a
human diagnosed with HCV, a method of treating HCV in a human subject, and a
method for reducing emergence of HCV quasispecies with resistance to
20 coadministered oral antiviral agents, each method comprising administering
Compound 10 and further comprising administering a second compound, a third
compound and a fourth compound each selected from the group consisting of
Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound
7.

Another aspect of the present invention includes a method for ameliorating one
or more symptom of HCV infection in a human, a method for reducing viral load in a
human diagnosed with HCV, a method of treating HCV in a human subject, and a
method for reducing emergence of HCV quasispecies with resistance to
coadministered oral antiviral agents, each method comprising administering
25 Compound 11 and further comprising administering a second compound, a third
compound and a fourth compound each selected from the group consisting of
Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound
30 7.

Another aspect of the present invention includes a method for ameliorating one
35 or more symptom of HCV infection in a human, a method for reducing viral load in a

human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 1 and further comprising administering a second compound, a third
5 compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 3, Compound 4, Compound 5 or Compound 6. The second compound may be Compound 2 and the third compound may be Compound 4. The
10 second compound may be Compound 3 and the third compound may be Compound 4. The second compound may be Compound 2 and the third compound may be Compound 6. The second compound may be Compound 3 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

15 Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering
20 Compound 2 and further comprising administering a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 4. The second compound may be Compound 1 and the third
25 compound may be Compound 4. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a
30 human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 3 and further comprising administering a second compound, a third compound, a fourth compound and a fifth compound each selected from the group
35 consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6,

Compound 7, Compound 9, Compound 10 and Compound 11. The second compound may be Compound 1 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 4. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound
5 may be Compound 4 and the third compound may be Compound 6.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to
10 coadministered oral antiviral agents, each method comprising administering Compound 4 and further comprising administering a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7. The second compound may be Compound 1, Compound 2,
15 Compound 3 or Compound 6. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 1 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound may be Compound 6. The second
20 compound may be Compound 3 and the third compound may be Compound 6.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to
25 coadministered oral antiviral agents, each method comprising administering Compound 5 and further comprising administering a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound
30 may be Compound 1.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to
35 coadministered oral antiviral agents, each method comprising administering

Compound 6 and further comprising administering a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 7, Compound 9, Compound 10 and Compound 11. The second compound
5 may be Compound 1, Compound 2, Compound 3, and Compound 4. The second compound may be Compound 1 and the third compound may be Compound 2. The second compound may be Compound 1 and the third compound may be Compound 3. The second compound may be Compound 4 and the third compound may be Compound 6. The second compound may be Compound 2 and the third compound
10 may be Compound 4. The second compound may be Compound 3 and the third compound may be Compound 4.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a
15 method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 7 and further comprising administering a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5,
20 Compound 6, Compound 9, Compound 10 and Compound 11.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to
25 coadministered oral antiviral agents, each method comprising administering Compound 9 and further comprising administering a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to
30 coadministered oral antiviral agents, each method comprising administering Compound 10 and further comprising administering a second compound, a third
35

compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

Another aspect of the present invention includes a method for ameliorating one or more symptom of HCV infection in a human, a method for reducing viral load in a human diagnosed with HCV, a method of treating HCV in a human subject, and a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents, each method comprising administering Compound 11 and further comprising administering a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

Routes and Modes of Administration

Two or more of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. and any other components of a combination therapy can be adapted to be administered by any route appropriate to the condition to be treated. Suitable routes include oral, rectal, nasal, topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) and the like. It will be appreciated that the preferred route may vary with, for example, the condition of the recipient.

A synergistic effect may be attained when the active ingredients are: (1) co-formulated (e.g. in a unitary dosage form) and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g., in separate tablets, pills or capsules, or by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

Co-administration of a Combination Compound with one or more Combination Compounds generally refers to simultaneous or sequential administration of one or more Combination Compounds, such that therapeutically effective amounts of two or

more Combination Compounds are present in the body of the patient. In some cases, Combination Compounds (e.g. two, three or four Combinations Compounds) will be co-formulated to allow administration at the same time. In some cases, co-formulated Combination Compounds may be co-administered with one or more additional

5 Combination Compounds.

Co-administration also includes administration of unit dosages of the Combination Compounds before or after administration of unit dosages of one or more other active ingredients, for example, administration of two or more Combination Compounds within seconds, minutes, or hours of the administration of one or more

10 other active ingredients. For example, a unit dose of a Combination Compound can be administered first, followed within seconds or minutes by administration of a unit dose of a second Combination Compound, followed within seconds or minutes by administration of a unit dose of one or more other active ingredients. Alternatively, a unit dose of one or more other active ingredients can be administered first, followed

15 within seconds or minutes by administration of a unit dose of a Combination Compound, followed within seconds or minutes by administration of a unit dose of a second Combination Compound. In some cases, it may be desirable to administer a unit dose of a Combination Compound first, followed, after a period of hours (e.g., 1-12 hours), by administration of a unit dose of a second Combination Compound, followed,

20 after a period of hours (e.g., 1-12 hours), by administration of a unit dose of one or more other active ingredients. In other cases, it may be desirable to administer a unit dose of one or more other active ingredients first, followed, after a period of hours (e.g., 1-12 hours), by administration of a unit dose of a Combination Compound, followed, after a period of hours (e.g., 1-12 hours), by administration of a unit dose of a

25 second Combination Compound. Where three or more Combinations Compounds are administered with one or more additional active ingredients, the Combination Compounds may be administered one after another within seconds, minutes, or hours (e.g. 1-12 hours) of each other and the one or more additional active ingredients may be administered before, during or after the administration of the Combination

30 Compounds. Where Combination Compounds are co-formulated, they can be administered simultaneously, or before or after the administration of one or more additional active ingredients.

Unless otherwise specified, the combination therapy may be administered as separate dosage forms with each active ingredient, administered together or

separately, sequentially or concurrently, and close in time or remote in time to each other.

The course of treatment can extend, for example, from about 12 weeks to about 48 weeks, or longer, for example, from about 12 weeks to about 24 weeks.

5 The present invention includes a combination of therapeutically effective components to ameliorate at least one symptom of HCV infection in a human being including, but not limited to, nausea, vomiting, loss of appetite, fatigue, jaundice, vomiting, diarrhea, dehydration, abdominal pain, cirrhosis of the liver. In addition, in some HCV infected individuals the use of combination therapy is effective to reduce
10 the viral load of HCV viral particles present in the body of the infected person by a statistically significant amount. Viral load can be measured, for example, by measuring plasma HCV RNA levels using, for example, the COBAS TaqMan HCV assay (Roche Molecular Systems). Typically, an HCV infected person who is treated with the Combination Compounds in accordance with the present invention
15 experiences an improvement in one or all of the symptoms associated with the HCV infection.

Combinations of Two or more of the Combination Compounds with Ribavirin but not Interferon

20 As discussed above, some current HCV treatments include the administration of interferon, but this treatment typically produces unwanted side effects. Therefore it would be desirable to find effective HCV treatments that do not require the administration interferon.

25 One aspect of the present invention provides for compositions, methods, uses and the like for the treatment of HCV comprising administering two or more of the Combination Compounds or pharmaceutically acceptable salts thereof and ribavirin, without administering one or more interferons. This aspect of the invention may be particularly useful because it allows for the effective treatment of HCV without the side effects associated with the administration of one or more interferon.

30 In one embodiment of the present invention, the combined amount of ribavirin and Combination Compounds or pharmaceutically acceptable salts thereof, optionally with one or more additional agents, is effective to treat HCV infection.

35 Another aspect of the present invention includes a method for ameliorating one or more symptoms of HCV infection in a human comprising: administering two or more of the Combination Compounds or pharmaceutically acceptable salts thereof and

ribavirin, without concurrent administration of one or more interferon. In this regard, the present invention does not foreclose the potential for dosing one or more interferon. Rather, the present invention may be used in conjunction with another therapy that, in fact, includes one or more interferon. An aspect of the present invention includes efficacious treatment of HCV with ribavirin without the need for one or more interferon.

Another aspect of the present invention includes a method for reducing viral load in a human diagnosed with HCV comprising: administering two or more of the Combination Compounds or pharmaceutically acceptable salts thereof and ribavirin, but not one or more interferon.

Another aspect of the present invention includes a method for treating HCV in a human subject consisting essentially of administration of ribavirin in conjunction with two or more of the Combination Compounds or pharmaceutically acceptable salts thereof.

Another aspect of the present invention includes a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents comprising: administering two or more of the Combination Compounds or pharmaceutically acceptable salts thereof and ribavirin, without concurrent administration of one or more interferon.

Similarly, another aspect of the present invention includes a composition, e.g. a pharmaceutical composition for ameliorating one or more symptom of HCV infection in a human comprising two or more of the Combination Compounds or pharmaceutically acceptable salts thereof and ribavirin, without one or more interferon. Another aspect of the present invention includes a composition for reducing viral load in a human diagnosed with HCV comprising two or more of the Combination Compounds or pharmaceutically acceptable salts thereof and ribavirin, but not one or more interferon. Another aspect of the present invention includes a composition for treating HCV in a human subject consisting essentially of ribavirin in conjunction with two or more of the Combination Compounds or pharmaceutically acceptable salts thereof. Another aspect of the present invention includes a composition for ribavirin-based HCV therapy comprising two or more of the Combination Compounds or pharmaceutically acceptable salts thereof, with the proviso that said composition does not include one or more interferon. Another aspect of the present invention includes a composition for reducing emergence of HCV quasispecies with resistance to coadministered oral

antiviral agents comprising two or more of the Combination Compounds or pharmaceutically acceptable salts thereof and ribavirin, without one or more interferon.

Similarly, another aspect of the present invention includes use of: two or more of the Combination Compounds or pharmaceutically acceptable salts thereof and ribavirin, without one or more interferon, in the manufacture of a medicament for ameliorating one or more symptoms of HCV infection in a human; as well as use of: two or more of the Combination Compounds or pharmaceutically acceptable salts thereof and ribavirin, but not one or more interferon, in the manufacture of medicament for reducing viral load in a human diagnosed with HCV; as well as use of ribavirin in conjunction with two or more of the Combination Compounds or pharmaceutically acceptable salts thereof in the manufacture of a medicament for treating HCV in a human subject, wherein said use does not include use of one or more interferon; as well as use of two or more of the Combination Compounds or pharmaceutically acceptable salts thereof, in the manufacture of a medicament for ribavirin-based HCV therapy, wherein said use avoids administration of one or more interferon; as well as use of two or more of the Combination Compounds or pharmaceutically acceptable salts thereof and ribavirin, without one or more interferon in the manufacture of a medicament for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents.

Another aspect of the present invention includes a combination comprising ribavirin and two or more of the Combination Compounds or pharmaceutically acceptable salts thereof, which combination is substantially free of one or more interferon. In one embodiment, the combination may occur as separate dosage forms with each active ingredient, administered together or separate, sequentially or concurrently, and close in time or remote in time to each other.

Another aspect of the present invention includes a kit comprising: ribavirin, two or more of the Combination Compounds and instruction regarding a treatment regimen to treat, reduce viral load, or delay onset or progression of HCV wherein the treatment regimen includes administration of the two or more of the Combination Compounds and ribavirin without administration of one or more interferon. In one embodiment, such a kit may also include packaging, such as a blister pack. Alternatively, such a kit may provide for individual prescription and dosing of each component as separately packaged pharmaceuticals, but when combined with the instruction regarding a treatment regimen to treat, reduce viral load, or delay onset or progression of HCV, such is intended to be within the scope of the present invention.

Another aspect of the present invention includes a pharmaceutical composition comprising: ribavirin; two or more of the Combination Compounds or pharmaceutically acceptable salts thereof and one or more pharmaceutically acceptable carriers. In one embodiment, the pharmaceutical composition may be a unitary dosage form.

5 Unless otherwise specified, the combination therapy with Ribavirin may be administered as separate dosage forms with each active ingredient administered (including the Combination Compounds), may be administered together (e.g., in the form of a unit dosage, such as a tablet) or separately, sequentially or concurrently, and close in time or remote in time to each other. If administered separately, each
10 compound may be administered with the other(s) at the same time, or either before or after such administration of the other(s). The active ingredients can be administered daily. In one embodiment, a daily dosage of the active ingredients is administered in separate sub-doses, such as one, two, three or four times per day. Advantageously, the daily dosage of Combination Compounds or pharmaceutically acceptable salts
15 thereof and ribavirin may be administered once per day.

Although the present invention includes compositions, methods, uses and the like for the treatment of HCV comprising administering two or more Combination Compounds or a pharmaceutically acceptable salt thereof; and ribavirin, but not one or more interferon, the present invention does not foreclose the potential for dosing one
20 or more interferon to the human. Rather, the present invention may be used in conjunction with another therapy for another indication that, in fact, includes one or more interferon.

25 Combinations of Two or more of the Combination Compounds with Ribavirin and Interferon

Another aspect of the present invention provides for compositions, methods, uses and the like comprising administering two or more of the Combination Compounds or pharmaceutically acceptable salts thereof and ribavirin, and one or more interferon for treatment of HCV. The administration of more interferon may be in
30 temporal relation to the administration of the Combination Compounds and ribavirin.

Another aspect of the present invention includes a method for ameliorating one or more symptoms of HCV infection in a human comprising administering two or more of the Combination Compounds or pharmaceutically acceptable salts thereof, ribavirin, and one or more interferons. Another aspect of the present invention includes a
35 method for reducing viral load in a human diagnosed with HCV comprising:

administering two or more of the Combination Compounds or pharmaceutically acceptable salts thereof along with ribavirin and one or more interferons.

Another aspect of the present invention includes a method of ribavirin-based HCV therapy comprising administering two or more of the Combination Compounds or
5 pharmaceutically acceptable salts thereof along with ribavirin, and one or more interferons.

Another aspect of the present invention includes a method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents comprising: administering two or more of the Combination Compounds or
10 pharmaceutically acceptable salts thereof along with ribavirin and one or more interferons.

Another aspect of the present invention includes use of two or more of the Combination Compounds or pharmaceutically acceptable salts thereof ribavirin, and one or more interferons, in the manufacture of a medicament for ameliorating one or
15 more symptoms of HCV infection in a human. Another aspect of the present invention includes use of two or more of the Combination Compounds or pharmaceutically acceptable salts thereof along with ribavirin and one or more interferons, in the manufacture of medicament for reducing viral load in a human diagnosed with HCV. Another aspect of the present invention includes use of ribavirin in conjunction with two
20 or more of the Combination Compounds or pharmaceutically acceptable salts thereof in the manufacture of a medicament for treating HCV in a human subject, wherein said use includes use of one or more interferons. Another aspect of the present invention includes use of two or more of the Combination Compounds or pharmaceutically acceptable salts thereof, in the manufacture of a medicament for ribavirin-based HCV
25 therapy, wherein said use includes administration of one or more interferon. Another aspect of the present invention includes use of two or more of the Combination Compounds or pharmaceutically acceptable salts thereof, ribavirin, and one or more interferons in the manufacture of a medicament for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents.

30 Another aspect of the present invention includes a combination comprising ribavirin and two or more of the Combination Compounds or pharmaceutically acceptable salts thereof, which combination includes one or more interferons.

Another aspect of the present invention includes a kit comprising: ribavirin, two or more of the Combination Compounds and one or more interferon; and instructions
35 regarding a treatment regimen to treat, reduce viral load, or delay onset or progression

of HCV wherein the treatment regimen includes administration of the two or more of the Combination Compounds and ribavirin and administration of one or more interferon. In one embodiment, such a kit may also include packaging, such as a blister pack. Alternatively, such a kit may provide for individual prescription and dosing of each component as separately packaged pharmaceuticals, but when combined with the instruction regarding a treatment regimen to treat, reduce viral load, or delay onset or progression of HCV, such is intended to be within the scope of the present invention.

Another aspect of the present invention includes a pharmaceutical composition comprising: two or more of the Combination Compounds or pharmaceutically acceptable salts thereof, ribavirin, and one or more interferon; and one or more pharmaceutically acceptable carriers. In one embodiment, the pharmaceutical composition may be a unitary dosage form.

Unless otherwise specified, the combination therapy with Ribavirin and one or more interferons may be administered as separate dosage forms with the one or more interferons administered to the patient and each of the remaining active ingredients to be employed in the combination therapy (including the Combination Compounds) are administered together (e.g., in the form of a unit dosage, such as a tablet) or separately, sequentially or concurrently, and close in time or remote in time to each other. If administered separately, each active ingredient may be administered with the other(s) at the same time, or either before or after such administration of the other(s). The active ingredients can be administered daily. In one embodiment, a daily dosage is administered in separate sub-doses, such as one, two, three or four times per day.

Combination Therapy, Including Additional Therapeutics

In another embodiment, non-limiting examples of suitable combinations include the combinations of two or more of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11. with one or more additional active ingredients including HCV NS3 protease inhibitors, alpha-glucosidase 1 inhibitors, hepatoprotectants, nucleoside or nucleotide inhibitors of HCV NS5B polymerase, non-nucleoside inhibitors of HCV NS5B polymerase, HCV NS5A inhibitors, TLR-7 agonists, cyclophilin inhibitors, HCV IRES inhibitors, HCV entry inhibitors, HCV maturation inhibitors, HCV assembly inhibitors, HCV infectivity inhibitors and pharmacokinetic enhancers, as well as other drugs for treating HCV. More specifically, one or more compounds of the present

invention may be combined with one or more compounds selected from the group consisting of:

- 5 (i) HCV NS3 protease inhibitors, e.g., boceprevir (SCH-503034, SCH-7), telaprevir (VX-950), TMC-435 (IUPAC N-
[(2R,3aR,10Z,11aS,12aR,14aR)-2-[2-(4-Isopropylthiazol-2-yl)-7-methoxy-8-methylquinolin-4-yloxy]-5-methyl-4,14-dioxo-
1,2,3,3a,4,5,6,7,8,9,11a,12,12a,13,14,14a-
hexadecahydrocyclopenta[c]cyclopropa[g][1,6]diazacyclotetradecin-
12a-ylcarbonyl]cyclopropanesulfonamide], ABT-450, ACH-1625,
10 ACH-2684, BI-201335, BI-1230, MK-5172, MK-7009, SCH-900518, VBY-376, VX-500, GS-9256, GS-9451, BMS-605339, PHX-1766, AS-101, YH-5258, YH-5530, YH-5531, and ITMN-191 (R-7227);
- (ii) alpha-glucosidase 1 inhibitors, e.g., celgosivir (MX-3253), UT-231B, Miglitol;
- 15 (iii) hepatoprotectants, e.g., emericasan (IDN-6556), ME-3738, silibinin, and MitoQ;
- (iv) nucleoside or nucleotide inhibitors of HCV NS5B polymerase, e.g., .., R1626, R7128 (R4048), IDX184, IDX-102, PSI-661, PSI-938, PSI-7851, PSI-7977, BCX-4678, valopicitabine (NM-283), MK-0608 and
20 TMC649128;
- (v) non-nucleoside inhibitors of HCV NS5B polymerase, e.g., filibuvir (PF-868554), ABT-333, ABT-072, BI-207127, VCH-759, VCH-916, JTK-652, MK-3281, VBY-708, VCH-222, A848837, ANA-598, GL60667, GL59728, A-63890, A-48773, A-48547, BC-2329, VCH-
25 796 (nesbuvir), GSK625433, BILN-1941, and XTL-2125;
- (vi) HCV NS5A inhibitors, e.g., ACH-2928, AZD-2836 (A-831), AZD-7295 (A-689), BMS-766, BMS-790052, BMS-824393, and PPI-461;
- (vii) TLR-7 agonists, e.g., imiquimod, 852A, ANA-773, ANA-975, AZD-8848 (DSP-3025), PF-04878691, and SM-360320 and Compound 8;
- 30 (viii) cyclophilin inhibitors, e.g., DEBIO-025, SCY-635, and NIM811;
- (ix) HCV IRES inhibitors, e.g., MCI-067;
- (x) pharmacokinetic enhancers, e.g. roxythromycin, BAS-100, SPI-452, PF-4194477, TMC-41629;
- (xi) HCV entry inhibitors
- 35 (xii) HCV assembly inhibitors;

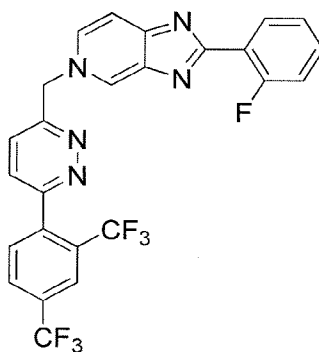
- (xiii) HCV maturation inhibitors;
 (xiv) HCV infectivity inhibitors; and
 (xv) other drugs for treating HCV, e.g., thymosin alpha 1 (Zadaxin), nitazoxanide (Alinea, NTZ), BIVN-401 (virostat), PYN-17 (altirex), KPE02003002, actilon (CPG-10101), KRN-7000, civacir, GI-5005, XTL-6865, BIT225, PTX-111, ITX2865, TT-033i, ANA 971, NOV-205, tarvacin, EHC-18, VGX-410C, EMZ-702, AVI 4065, BMS-650032, BMS-791325, Bavituximab, MDX-1106 (ONO-4538), Oglufanide, FK-788, and VX-497 (merimepodib)..

SYNTHETIC EXAMPLES

Synthetic protocols for the preparation of Compounds 1, 2, 3, 6, 7, and 8 are known in the literature. Additionally, a synthetic protocol for preparing each of the Combination Compounds is provided in the Examples below.

Compound **1** can be prepared using synthetic methods and intermediates like those described in US 7,754,720. Compound **1** can also be prepared as described in the following Example.

Example 1: 5-({6-[2,4-bis(trifluoromethyl)phenyl]pyridazin-3-yl}methyl)-2-(2-fluorophenyl)-5H-imidazo[4,5-c]pyridine **1**.



1

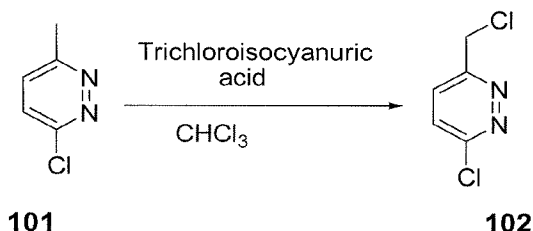
Compound	MW	Amount	Moles	Equivalents
104	453.79	95mg	0.209	1
DME	500μL			
2N aq. Na ₂ CO ₃		313μL	0.626	3
105	257.93	80.9mg	0.313	1.5
Pd(PPh ₃) ₄	1155	12mg	0.0104	0.05

Compound **103** was dissolved in dimethoxyethane (DME). To this solution was added 2,4-bis(trifluoromethyl)phenylboronic acid **105** and a 2N aq. Na₂CO₃ solution. To the resulting biphasic mixture was added Pd(PPh₃)₄ and the reaction was then heated at 80°C for 72 hrs. The reaction was cooled to room temperature and filtered through
 5 Celite and the Celite washed with EtOAc. The filtrate was concentrated *in vacuo*. The residue was purified on 6g SiO₂ using MeOH/CH₂Cl₂ to elute compound. The compound thus obtained was contaminated with PPh₃(O). The product was repurified on a 1 mm Chromatotron plate with 0 to 5% MeOH/CH₂Cl₂ in 1% steps. The pure fractions were combined and concentrated *in vacuo*, then dried on high vacuum for 12
 10 hrs. 11.8 mg of the free base of compound 1 was obtained with no PPh₃ contamination. ¹H NMR (300MHz, CD₃OD) δ 6.20 (s, 2), 7.32 (m, 3), 7.52 (m, 1), 7.78 (d, 1), 7.89 (d, 1), 7.95 (s, 2), 8.15 (m, 3), 8.35 (d, 1), 9.12 (s, 1); LC/MS M+H = 518.

The intermediate compound **104** was prepared as follows.

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a. Preparation of Compound **102**.



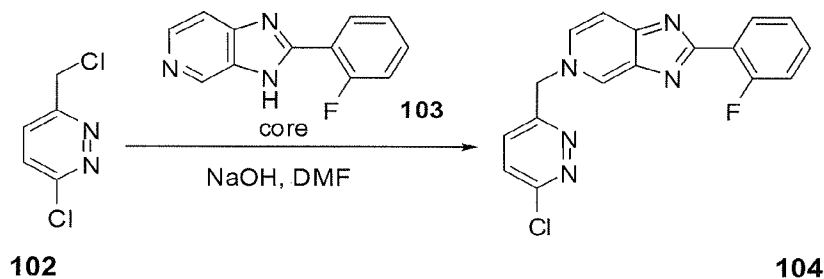
Compound	MW	Amount	mmoles	Equivalents
101	128.56	5 g	38.9	1
TCCA	232.41	3.62 g	15.6	0.4
CHCl ₃		130 mL		

20

To a solution of the commercially available starting material **101** in CHCl₃, trichloroisocyanuric acid (TCCA) was added at 60°C. Then the solution was stirred for 1.5 hrs, cooled, and filtered with HiFlo-Celite. The filtrate was concentrated and dried with vacuum. The yield was 5.037 g of compound **102**.

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b. Preparation of Compound **104**.



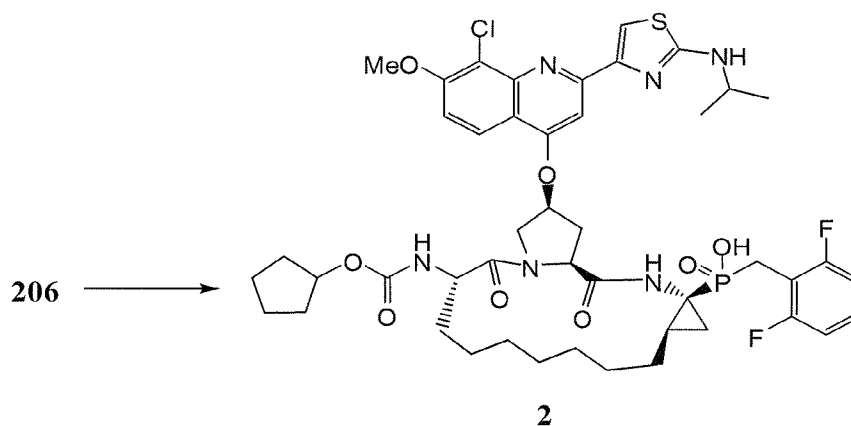
Compound	MW	Amount	mmoles	Equivalents
102	163	5.073 g	31.12	1
103	213.2	6.635 g	31.12	1
NaOH (10%)	40	1.245 g	31.12	1
DMF		320 mL		

5 To a solution of compound **103** in DMF (dimethylformamide), NaOH was added. Compound **102** was dissolved in DMF (20 mL) and added to the solution slowly. The reaction was stirred for 3 hrs, was diluted with water and extracted with EtOAc. The organic layer was dried with Na₂SO₄. The solvent was removed and the product recrystallized with dichloromethane. The yield was 5.7 g of compound **103**.

10

Compound **2** can be prepared using synthetic methods and intermediates like those described in USSN 12/202319 (US 20100051763 A1). Compound **2** can also be prepared as described in the following Example.

15 Example 2: Preparation of Compound 2.



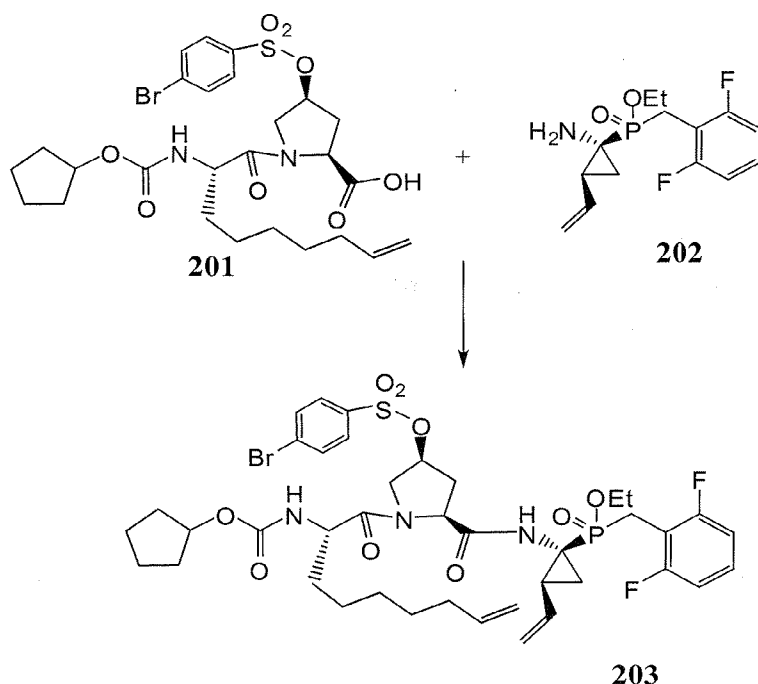
Phosphinate ester **206** (23.7 g, 24.05 mmol) was dissolved in CH₃CN (240 mL) and cooled to 0°C. Iodotrimethylsilane (17.4 mL, 122.3 mmol) was added at a fast drop-wise pace followed by, after 10 min, 2,6-lutidine (17.0 mL, 146.4 mmol). The

reaction mixture was slowly warmed to room temperature and stirred for 1 h then cooled back down to 0°C and 2,6-lutidine (11.1 mL, 95.6 mmol) followed by MeOH (24 mL) were added. The solution was concentrated *in vacuo* and the crude residue was purified by HPLC to afford 12.68 g of Compound **2** in 55% yield. ¹H NMR (300 MHz, CDCl₃) δ 8.35 (d, J = 9.3 Hz, 1H), 8.28 (s, 1H), 7.85 (s, 1H), 7.64 (d, J = 9.6 Hz, 1H), 7.35-7.22 (m, 1H), 7.02-6.89 (m, 2H), 5.85 (bs, 1H), 4.82-4.71 (m, 2H), 4.33 (bs, 1H), 4.28-3.99 (m, 3H), 4.16 (s, 3H), 3.57-3.28 (m, 2H), 2.90-2.78m, 1H), 2.63-2.50 (m, 1H), 2.08-1.91 (m, 1H), 1.91-1.70 (m, 2H), 1.70-1.13 (m, 22H), 1.37 (d, J = 6.9 Hz, 6H); ³¹P NMR (121.4 MHz, CD₃OD) δ 42.4; LCMS (M+1): 957.35. g.

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Intermediate compound **206** was prepared as follows.

a. Preparation of Compound **203**.



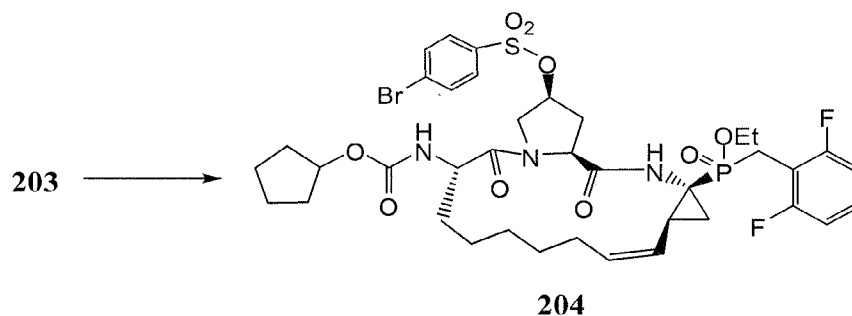
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Compound **201** (17.42 g, 28.30 mmol) was dissolved in THF (136 mL) and cooled to 0°C. To the solution was added N-methylmorpholine (4.7 mL, 42.7 mmol). After 10 min at 0°C, *i*-butylchloroformate (4.05 mL, 30.96 mmol) was added dropwise. After an additional 1 h, (1-amino-2-vinyl-cyclopropyl)-(2,6-difluorobenzyl)-phosphinic acid ethyl ester **202** (8.94 g, 29.70 mmol) was slowly added as a solution in THF (20

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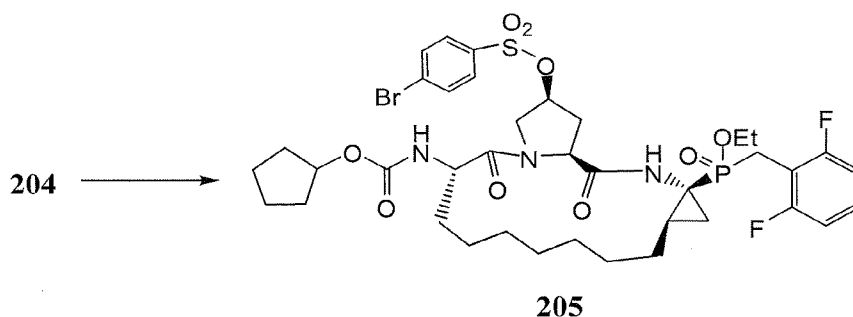
mL). The suspension was warmed to room temperature and after 2 h it was partitioned between H₂O (400 mL) and ethylacetate (200 mL). The aqueous layer was extracted with ethylacetate (200 mL x 2) and the combined organic layers were washed with HCl (1N, 225 mL) and H₂O (200 mL). The acid wash and aqueous wash
 5 were combined and back-extracted with ethylacetate (175 mL x 2, 100 mL x 2). The combined organic layers were washed with brine (400 mL), dried over Na₂SO₄, and concentrated *in vacuo* providing 25.06 g of diene **203** in 98.5% crude yield. LCMS (M + 1): 898.06.

10 b. Preparation of Compound **204**.



Compound **203** (12.91 g, 14.36 mmol) was dissolved in CH₂Cl₂ (1440 mL) and the solution was degassed for 30 minutes. The solution was heated to 40°C and
 15 Grubb's G1 catalyst (2.95 g, 3.59 mmol) was added. The reaction was refluxed for 17 h whereupon tris-hydroxymethylphosphine (22.3 g, 18.0 mmol), TEA (50 mL, 35.9 mmol), and H₂O (400 mL) were added and the reaction mixture was heated to reflux for an additional 16 hours. The reaction mixture was cooled to room temperature and
 20 the two layers were separated. The organic layer was washed with H₂O (400 mL) and brine (300 mL), dried over MgSO₄, and concentrated. The crude residue was purified by silica-gel chromatography to afford 8.30 g of macrocyclic olefin **204** in 66% yield. LCMS (M + 1): 870.09.

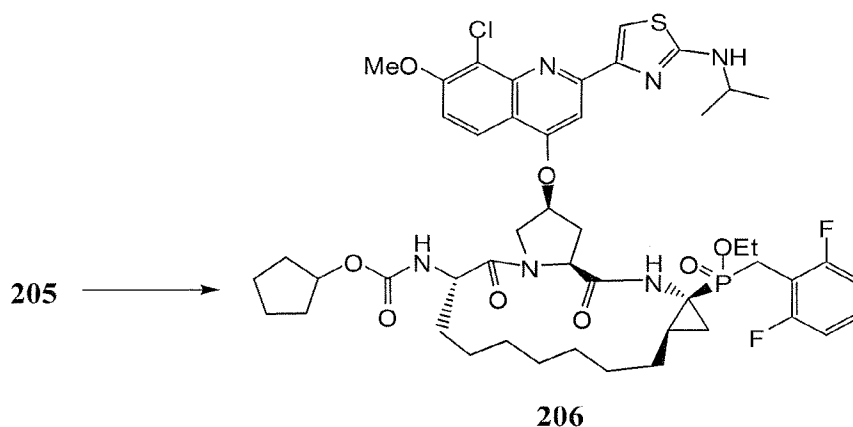
25 c. Preparation of Compound **205**.



The macrocyclic olefin **204** (7.34 g, 8.42 mmol) was dissolved in ethylacetate (105 mL) and rhodium on alumina (5% wt, 2.945 g, 0.40 wt %) was added. The system was evacuated and flushed with H₂ (1 atm, 3x). To the system, after 3 h, was added more rhodium on alumina (5% wt, 842 mg, 0.10 wt %) and evacuated and flushed with H₂ (1 atm, 3x). After an additional 1 h the suspension was filtered and concentrated *in vacuo* providing 6.49 g of reduced macrocycle **205** in 88% crude yield. LCMS (M + 1): 872.04.

10

d. Preparation of Compound **206**.



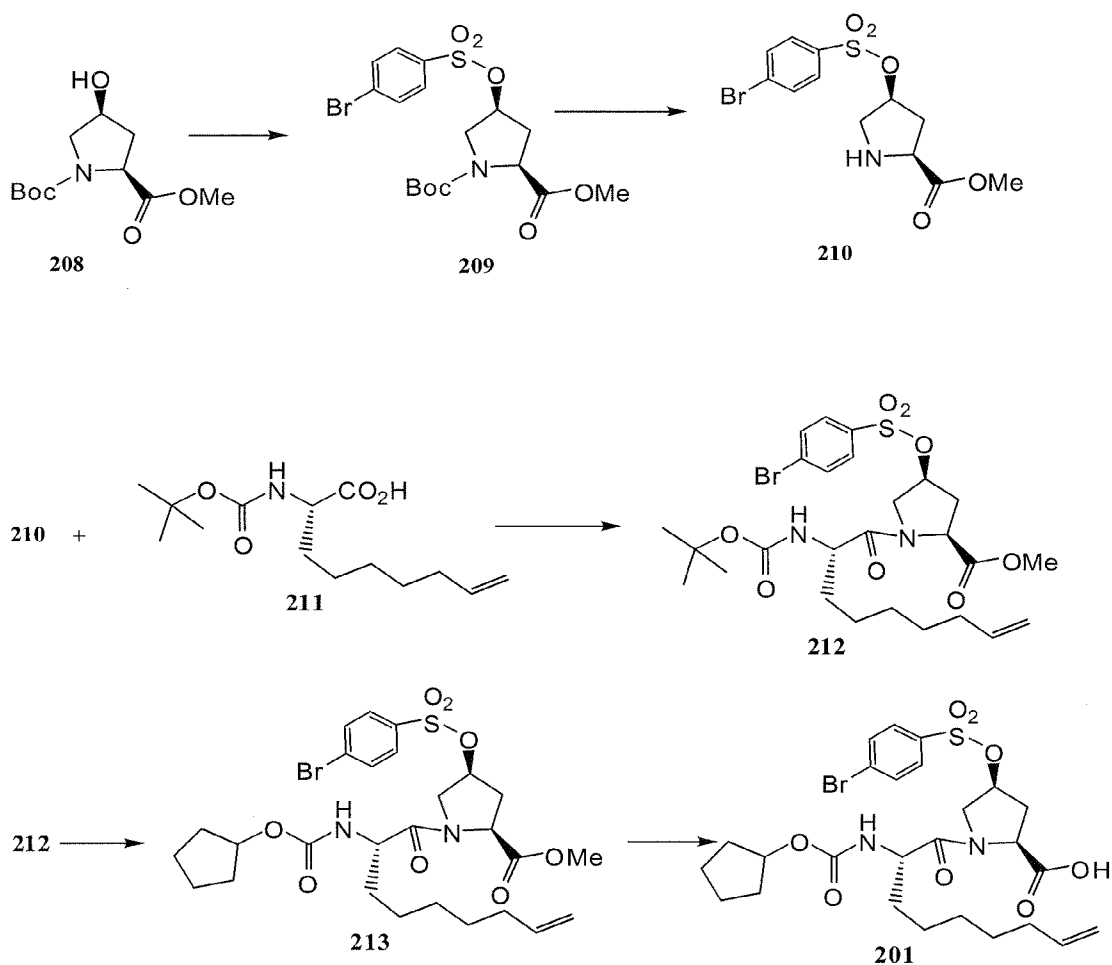
The brosylate macrocycle **205** (6.49 g, 7.67 mmol) was dissolved in N-methylpyrrolidinone (25.0 mL) and 8-chloro-2-(2-isopropylamino-thiazol-4-yl)-7-methoxy-quinolin-4-ol **207** (2.564 g, 7.33 mmol) followed by Cs₂CO₃ (4.40 g, 13.50 mmol) were added. The mixture was heated to 65°C for 6 h then diluted with ethylacetate (200 mL) and washed with LiCl (5%, 250 mL). The aqueous layer was extracted with ethylacetate (100 mL x 2) and the combined organic layers were washed with brine (150 mL), dried over Na₂SO₄/MgSO₄, and concentrated *in vacuo*.

20

The crude residue was purified via silica-gel chromatography (ethylacetate-methanol) affording 4.39 g of aminothiazole **206** in 58% yield. LCMS (M + 1): 985.28.

Intermediate Compound **201** can be prepared as follows.

5



10

e. Preparation of compound **209**.

Compound **208** (7.00 g, 28.55 mmol) and DABCO (5.13 g, 45.94 mmol) were dissolved in toluene (30 mL). A toluene (11 mL) solution of brosylchloride (10.22 g, 40.01 mmol) was added. The reaction mixture was stirred at room temperature overnight. The reaction was diluted with EtOAc (210 mL) and 0.5N HCl (200 mL) was added. The two layers were separated and the aqueous layer was extracted with EtOAc (2 x 200 mL). The combined organic layers were washed with brine (200 mL),

15

dried with Na₂SO₄, filtered, and concentrated. The crude product was purified by combi-flash to give 12.23 g of compound **209** in 92% yield.

f. Preparation of compounds **210** and **212**.

5 Compound **209** (12.2 g, 26.3 mmol) was treated with 4 N HCl / 1,4-dioxane (60 mL) and stirred for 1 hour. The reaction mixture was concentrated and dried under vacuum for 20 minutes. The crude amine HCl salt of compound **210** was dissolved in DMF (150 mL) and acid **211** (14.2 g, 52.6 mmol) was added. HATU (20.0 g, 52.6 mmol) and NMM (13.5 g, 131.5 mmol) were added. The reaction mixture was stirred
10 at room temperature overnight. The reaction was diluted with EtOAc (300 mL), washed with 1 N HCl (200 mL), saturated NaHCO₃, brine, dried with Na₂SO₄, and concentrated. The crude product was purified by combi-flash to give 15.1 g of compound **212** in 93% yield.

15 g. Preparation of compound **213**.

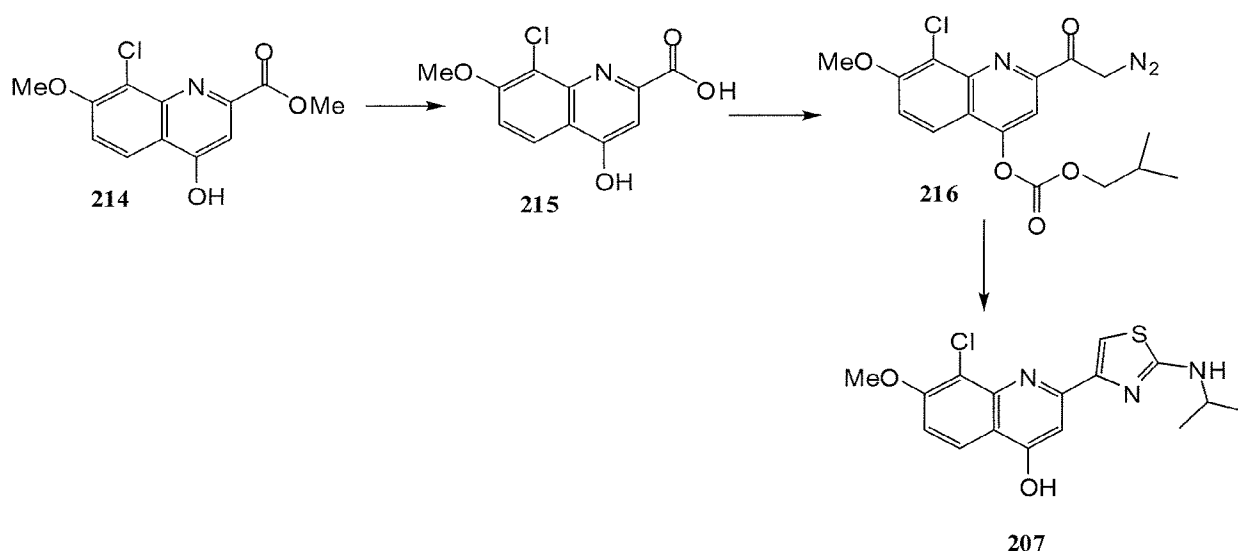
To a solution of **212** (12.8 g, 20.7 mmol) in CH₂Cl₂ (50 mL) was added 4 N HCl in 1,4- dioxane (50 mL, 200 mmol). The reaction mixture was stirred at room temperature for 2 hours, concentrated, dried under vacuum for 20 minutes, and then dissolved in CH₃CN (50 mL). Saturated NaHCO₃ in H₂O (50 mL) was added and stirred
20 for 5 minutes. Freshly prepared cyclopentylchloroformate in THF (50mL) was added. The reaction was complete within 1 h. The solvent was removed under reduced pressure and the residue was diluted with EtOAc. The mixture was brought to pH = 2 with 1 N HCl and the two layers were separated. The organic layers were washed with brine, dried with Na₂SO₄, filtered, and concentrated to give crude compound **213** (3.18 g).
25 g).

h. Preparation of compound **201**.

The crude ester **213** (3.18 g, 5.07 mmol) was dissolved in THF (25 mL), H₂O (25 mL), and then MeOH (6 mL) and LiOH (660 mg, 25.4 mmol) was added. The
30 reaction mixture was stirred at room temperature for 1h and diluted with EtOAc. The reaction mixture was acidified to pH 2 with 1 N HCl and the two layers were separated. The aqueous layer was extracted with EtOAc (2 x). The combined organic layers were washed with brine, dried with Na₂SO₄ concentrated and dried under vacuum to give 3.09 g of acid **201**.

35

Intermediate 8-chloro-2-(2-isopropylamino-thiazol-4-yl)-7-methoxy-quinolin-4-ol **207** can be prepared as follows.



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i. Preparation of 8-chloro-4-hydroxy-7-methoxyquinoline-2-carboxylic acid **215**.

To a solution of methyl 8-chloro-4-hydroxy-7-methoxyquinoline-2-carboxylate **214** (36.5g, 0.145 mol) in a mixture of 1:1 of MeOH: THF (160 mL total) was added a solution of LiOH (30.5 g, 0.725 mol) in H₂O (80 mL). The mixture was stirred at room temperature for an hour when LCMS analysis showed complete conversion to the carboxylic acid. The reaction was worked up by removal of the volatiles and adjusting the pH of the solution to 6 using aqueous 6N HCl. The resulted gummy residue was filtered and dried on the lyophilizer for 2 days to provide 34.4 g (99.6 %) of compound **215** as a white solid. EI MS (*m/z*) 253.9 [M+H].

15

j. Preparation of 2-(2-diazo-1-oxo)-8-chloro-7-methoxyquinolin-4-yl isobutyl carbonate **216**.

To a solution of 8-chloro-4-hydroxy-7-methoxyquinoline-2-carboxylic acid **215** (10.2 g, 0.04 mol) in THF (400 mL) was added triethyl amine (12.3 mL, 0.088 mol) and *i*-Butylchloroformate (11.6 mL, 0.088 mol) at 0°C under an argon atmosphere. The mixture was stirred at 0°C for 1 hour when LCMS analysis demonstrated completion of the reaction to provide the desired mixed anhydride. EI MS (*m/z*) 454.0 [M+H]. To the reaction mixture of the anhydride was added a 1M solution of diazomethane (121 mL, 0.121 mol) in diethyl ether via a plastic funnel at 0°C. This mixture was allowed to stir while warming up to room temperature for additional 2 hours. Analysis of the mixture

25

by LCMS demonstrated completion of the reaction. The septum was removed and the reaction was stirred for additional 20 minutes before removal of the solvent. The residue was dried further under high vacuum to provide compound **216**, which was carried on to the next step. EI MS (*m/z*) 377.9 [M+H].

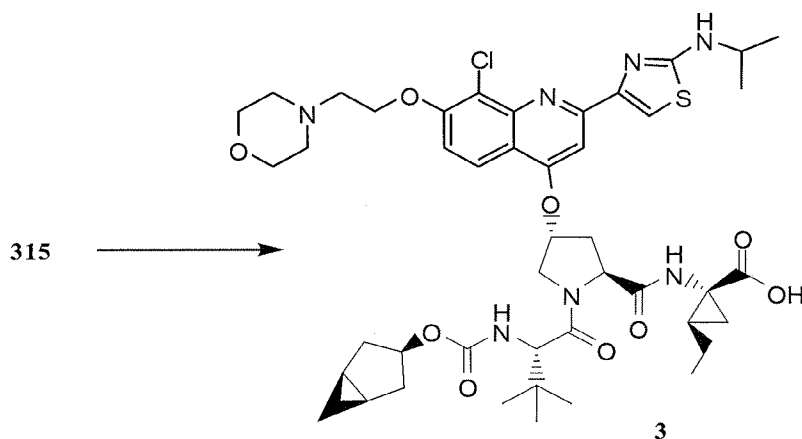
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k. Preparation of 8-chloro-2-(2-(isopropylamino)thiazol-4-yl)-7-methoxyquinolin-4-ol **207**.

To a cooled solution of 2-(2-diazo-1-oxo)-8-chloro-7-methoxyquinolin-4-yl isobutyl carbonate **216** (15.2 g, 0.040 mol) at 0°C in THF (268 mL) was added 48% HBr (23 mL, 0.201 mol) slowly over 15 minutes. The solution was stirred at 0°C for an additional 40 minutes when LCMS analysis demonstrated complete reaction. The reaction was worked up by addition of aqueous 1N NaOH (180 mL) at 0°C to adjust the pH of the aqueous layer to 9. The layers were separated and the aqueous layer was washed with EtOAc (2 x 200 mL). Combined organic extracts were washed with brine and dried over MgSO₄. The solvent was removed *in vacuo* to provide 17.7 g of a yellow solid. EI MS (*m/z*) 4³¹.9 [M+H].

The solution of the bromoketone obtained from the previous reaction was suspended in i-propanol (270 mL) and isopropylisourea (9.4 g, 0.080 mol). The reaction mixture was heated at 72 °C for 32 hours. LCMS analysis of the reaction demonstrated complete conversion to the desired product. The reaction was allowed to cool to room temperature to allow for the product to precipitate out of the solution. The reaction was further cooled to 0°C for 12 hours before filtration. The filtrate was washed with ether and dried on lyophilizer to provide 8.03 g of compound **207** as an orange solid. ¹H NMR (500 MHz, CDCl₃): δ 8.21 (d, *J*= 9 Hz, 1H), 7.74 (s, 1H), 7.44 (d, *J*= 10Hz), 1H), 7.07 (s, 1H), 4.05 (s, 3H), 3.92 (pentet, *J*=6 Hz, 1H), 1.25 (d, *J*= 7 Hz, 6H); EI MS (*m/z*) 350.0 [M+H].

Compound **3** can be prepared using synthetic methods and intermediates like those described in USSN 12/215,605 (US 20090257978 A1). Compound **3** can also be prepared as described in the following Example.

Example 3: Preparation of Compound 3.

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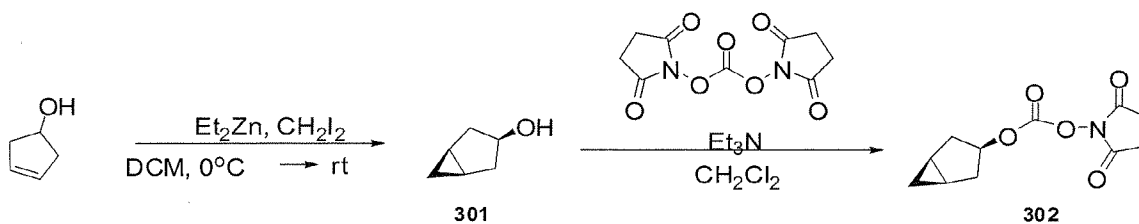
Compound **315** (12 g, 13 mmol) was dissolved in THF (200 ml), LiOH (11g, 260 mmol) in H₂O (200 ml) was added, followed by MeOH (200 ml). The mixture was kept stirring at room temperature for 20 hours. Upon completion of the reaction, 4 N HCl in H₂O was added to adjust pH to 7 at 0 °C. The mixture was extracted with EtOAc (2 x 400 ml). The combined organic layer was washed with brine, dried (Na₂SO₄) and concentrated *in vacuo* to give compound **3** as a yellow solid (11 g, 93%).

LC/MS = 911.52(M⁺+1). ¹H NMR (300MHz, CD₃OD) δ 7.95 (d, 1H), 7.90 (s, 1H), 7.48 (s, 1H), 7.31 (d, 1H), 5.42 (s, 1H), 4.37 (dd, 1H), 4.20 (m, 2H), 3.83-3.56 (m, 7H), 3.50 (m, 2H), 3.39 (m, 2H), 2.45 (m, 1H), 2.27(m, 1H), 1.62 (m, 2H), 1.50 (m, 1H), 1.33 (m, 2H), 1.18 (m, 1H), 1.05 (m, 8H), 0.90 (m, 3H), 0.76 (m, 11H), 0.14-0.04 (m, 2H)

15

The intermediate compound **315** was prepared as follows.

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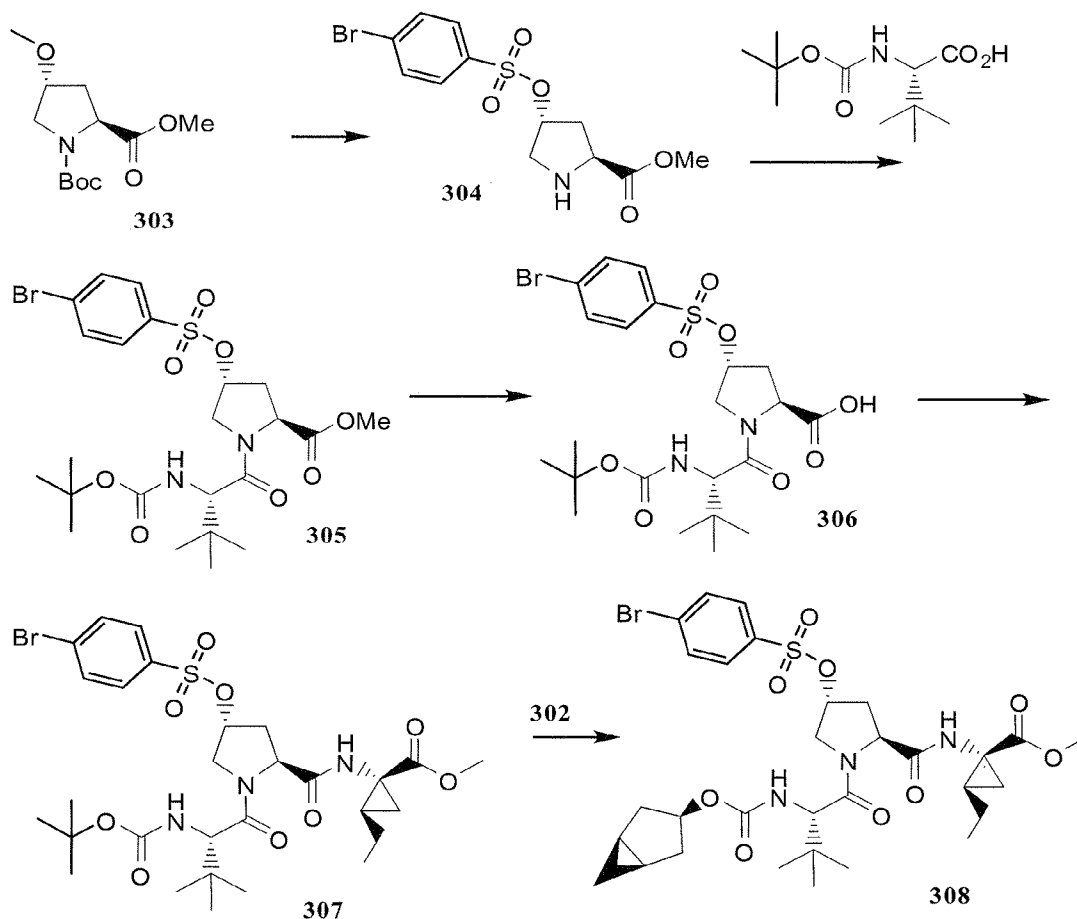


a. Preparation of compound **301**.

To a dry, argon purged three-neck round bottom flask (1000 mL) were added anhydrous dichloromethane (100 mL) and Et₂Zn (28 mL, 273 mmol) at 0 °C. (CAUTION: Source of argon can not be from needle. Use appropriate glass adapter only. A second bubbler can also be attached to the flask to prevent excessive pressure build up.) Cyclopenten-3-ol (10.0 mL, 119 mmol) was then added dropwise (large quantity of ethane gas was produced) to the flask and the reaction mixture was allowed to stir until the evolution of gas had ceased. Diiodomethane (22 mL, 242 mmol) was then added dropwise over a period of 30 minutes. The reaction was allowed to warm to room temperature and continued to stir overnight under a positive flow of argon, at which point TLC analysis had indicated complete disappearance of the starting alcohol. The reaction was then diluted with CH₂Cl₂ and quenched with 2M HCl (white precipitate should be completely dissolved). The biphasic mixture was poured into a separatory funnel and the organic layer was collected. The solvent was removed under reduced pressure until 100 mL of material containing compound **301** remained.

b. Preparation of compound **302**.

Anhydrous dichloromethane (525 mL) was added to the flask followed by the dropwise addition of triethylamine (34 mL, 245 mmol). The reaction continued to stir at room temperature under a positive flow of nitrogen at which point, disuccinimidylcarbonate (40.7 g, 159 mmol) was added to the flask portion wise. The reaction was allowed to stir until TLC analysis indicated complete disappearance of the starting material (2-3 days). Upon completion, the reaction mixture was quenched with 1M HCl (200 mL x 2) and washed with H₂O (200 mL x 2). The desired material was extracted using CH₂Cl₂ and the combined organic layers were dried using anhydrous MgSO₄ and passed through a silica plug. The solvent was removed under reduced pressure and the crude material was purified using flash chromatography (R_f = 0.33, 1:1 Hex/EtOAc) to provide compound **302** (22 g, 75%): ¹H NMR (300 MHz, CDCl₃): δ 5.24 (t, 1H), 3.82 (s, 4H), 2.24 (m, 2H), 2.03 (d, 2H), 1.38 (m, 2H), 0.48 (m, 1H), 0.40 (m, 1H).



5

c. Preparation of compound **304**.

N-*t*-Boc-cis-4-Hydroxy-L-Proline methyl ester **303** (100.0 g, 407.7 mmol) and DABCO (1.5eq, 68.6g, 611.6 mmol) were dissolved in anhydrous toluene (200 mL) in a 2 L three necked round bottom flask with a mechanical stirrer and an addition funnel. After cooling the solution to 0 °C under N₂, A solution of 4-Bromo-benzenesulfonyl chloride (1.3eq, 135.6g, 530.0 mmol) in 300 mL of toluene was added through addition funnel over 60 minutes. The reaction mixture was stirred and warmed to room temperature overnight (16 hours). The mixture was slowly poured into 2L 1M Na₂CO₃ (aq.), and the product was extracted with EtOAc (2L). After the organic phase was washed by 0.5 N HCl (2L), H₂O (1L), and brine (1L), it was dried (MgSO₄), concentrated to give 195.45 g of a yellow oily brosylate product.

To a solution of the above brosylate (407.7 mmol) in dichloromethane (300 mL) was slowly added 4.0 M HCl in dioxane (500 mL, 5eq) and the resulting solution was

allowed to stir at room temperature for 2 hours. After ether (500mL) was added to the reaction mixture, the mixture was stirred for 15 minutes and the white precipitate was collected by filtration. The solid was washed with ether and hexane and then dried under vacuum overnight to obtain 153.0 g of the HCl amine salt of compound **304**,
5 381.8 mmol, in 94% yield for two steps.

d. Preparation of compound **305**.

To a solution of Boc-*tert*-butyl-glycine (97.0g, 420.0 mmol) in DMF (200mL) and methylene chloride (200mL) were added HATU (217.76g, 572.7 mmol) and
10 Hunig's base (126 mL, 1145.4 mmol) at room temperature. After the mixture was stirred for 20 minutes at room temperature, a solution of the previous HCl salt (153.0 g, 381.8 mmol) and Hunig's base (126 mL, 1145.4 mmol) in DMF (200mL) and dichloromethane (200mL) was added to the above acid mixture in one portion. The reaction mixture was stirred at room temperature for 3h, with monitoring by LCMS.
15 The reaction mixture was concentrated to remove dichloromethane under reduced pressure and the white solid that formed was filtered off. The remaining DMF solution was diluted with ethyl acetate (1L), washed successively with 3% LiCl (aq) (3x650mL), sat'd NH₄Cl (2x500mL), 0.5N HCl (aq) (2x600mL), brine (500mL), sat'd NaHCO₃ (3x500mL), and brine (500mL). The resulting organic fraction was dried (MgSO₄) and
20 concentrated to afford compound **305** (111g).

e. Preparation of compound **306**.

To a solution of the methyl ester **305** (120 g, 207.8 mmol) in THF (300 mL), MeOH (75 mL) was added a solution of LiOH (26.18 g, 623.4 mmol) in H₂O (150 mL).
25 The solution was allowed to stir at room temperature for 4 hours. The mixture was cooled in an ice-bath while acidifying with 3N HCl to pH about 5.5, stirred for 10minutes, and the resulting white solids were collected by filtration. The solids were washed with more water, ether and hexane. The solids were dried under vacuum at 40°C overnight to give 95.78g (82%) of the acid **306**.
30

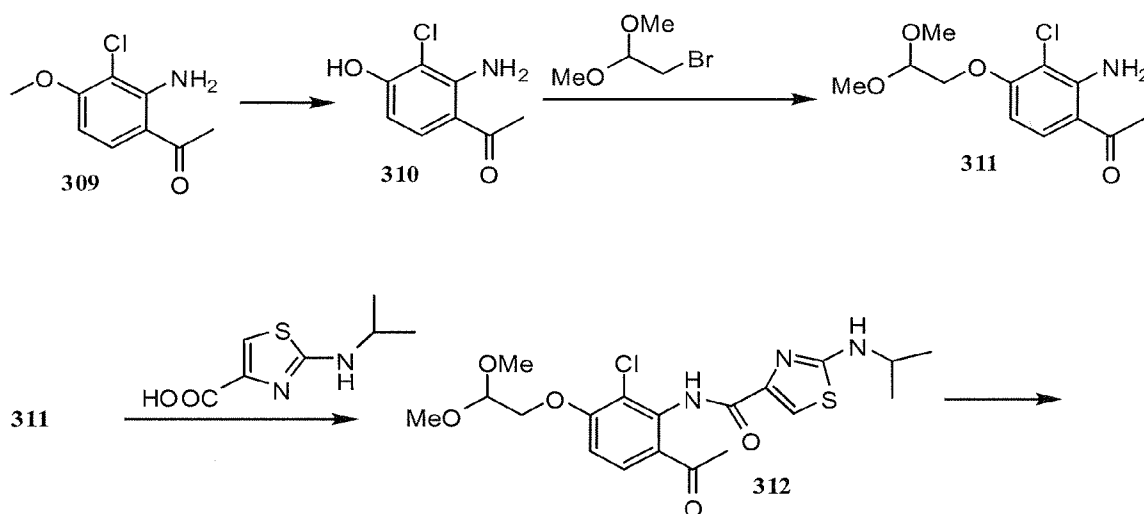
f. Preparation of compound **307**.

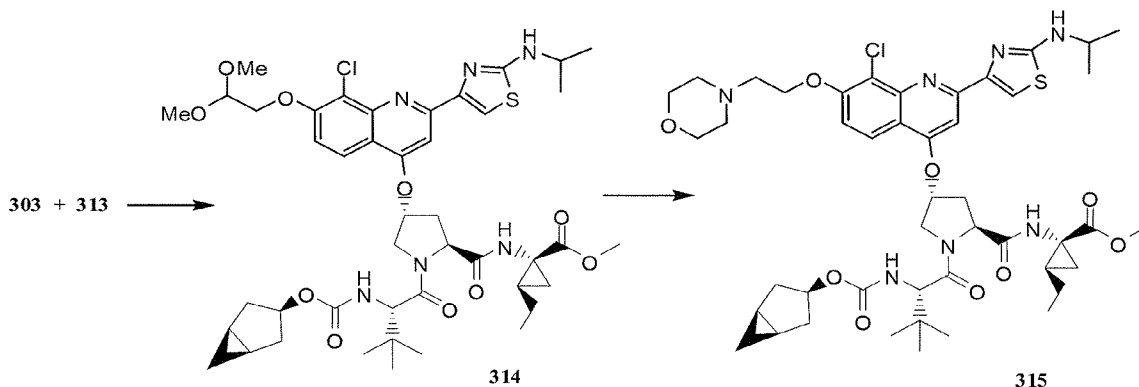
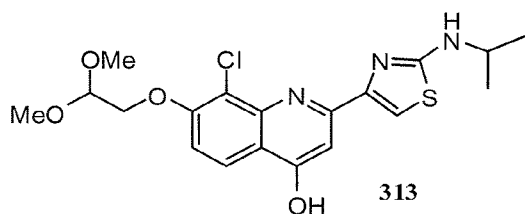
To a solution of the carboxylic acid **306** (81.4 g, 144.27 mmol) in DMF (200mL) and dichloromethane (200mL) was added HATU (82.3g, 216.4 mmol) and Hunig's base (47.5 mL, 432.8 mmol) at room temperature. After the mixture was stirred for
35 20 minutes at room temperature, a solution of amine (158.7 mmol) and Hunig's base

(47.5 mL, 1145.4 mmol) in DMF (200mL) and dichloromethane (200mL) was added to the above acid mixture in one portion. The reaction mixture was stirred at room temperature for 3 hours and monitored by LCMS. After the mixture was concentrated under reduced pressure to remove dichloromethane, the white solids that formed were filtered off. The remaining DMF solution was diluted with ethyl acetate (600mL) and successively washed with 3% LiCl (aq) (2x550mL), sat'd NH₄Cl (500mL), 1N HCl (aq) (500mL), sat'd NaHCO₃ (500mL), and brine (300mL). The resulting organic fraction was dried (Na₂SO₄) and concentrated to afford compound **307** (111g).

g. Preparation of compound **308**.

Compound **307** was dissolved in 4N HCl in dioxane (300 mL) at room temperature and stirred for 2 hours. It was then concentrated under vacuum, and co-evaporated with dichloromethane (2 x 200mL) to dryness. The residue was dissolved in EtOAc (600mL) and sat'd aq. NaHCO₃ (1L). It was stirred vigorously. After 10 minutes, carbonic acid bicyclo[3.1.0]hex-3-yl ester 2,5-dioxo-pyrrolidin-1-yl ester **302** (41.4 g, 173.1 mmol) was added in one portion. After the resulting mixture was stirred for another 30 minutes, the organic layer was collected and washed with brine (500mL), dried (Na₂SO₄), and concentrated. The crude product was purified by flash chromatography on silica gel with ethyl acetate/hexane to afford 94.44 g (92%) of compound **308**.





5

h. Preparation of compound **310**.

1-(2-Amino-3-chloro-4-hydroxy-phenyl)-ethanone **309** (70.7 g, 354 mmol) was stirred in 48% aq. HBr (500 mL) at 110 °C for 72 hours. After the mixture was cooled to 0 °C with stirring, the solids were filtered and washed with water. The resulting solids were triturated with a saturated NaHCO₃ solution (~350 mL), filtered, washed with water, and dried under vacuum to give ~ 40 g (61%) of crude **310** as a dark brown solid. LC/MS = 186 (M⁺+1).

15 i. Preparation of compound **311**.

1-(2-Amino-3-chloro-4-hydroxy-phenyl)-ethanone **310** (40 g, 215 mmol) was dissolved in DMF (360 ml). Cesium carbonate (140 g, 430 mmol) was added, followed by bromoacetaldehyde dimethyl acetal (54.5 g, 323 mmol). The mixture was then vigorously stirred at 65 °C for 24 hours. Upon cooling to room temperature, EtOAc (1 L) and H₂O (1 L) were added to the mixture. The organic layer was extracted with EtOAc (1 x 400 ml). The combined organic layer was washed with aqueous 3% LiCl solution (2 x 1L), brine, dried (Na₂SO₄) and concentrated *in vacuo*. The residue was purified by silica gel chromatography to give compound **311** as a white solid (39 g, 67%).

j. Preparation of compound **312**.

To a mixture of 1-[2-Amino-3-chloro-4-(2,2-dimethoxy-ethoxy)-phenyl]-ethanone **311** (13 g, 47.5 mmol) and isopropylaminothiazole-4-carboxylic acid hydrobromide (12.64 g, 47.5 mmol) in pyridine (150 ml) was slowly added phosphorus oxychloride (9.47 g, 61.8 mmol) at -40 °C. The mixture was then stirred at 0 °C for 4 hours. Upon completion of the reaction, H₂O (30 ml) was added dropwise to the mixture. The mixture was then stirred at 0 °C for another 15 minutes. The mixture was concentrated *in vacuo*. The residue was diluted with EtOAc, washed with a sat. NaHCO₃ aqueous solution. The organic layer was dried (Na₂SO₄) and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂, hexanes were added slowly to the solution, and a yellow solid started to crash out. More hexanes were added until not much product was left in the mother liquid to provide compound **312** (18 g, 85%).

k. Preparation of compound **313**.

2-Isopropylamino-thiazole-4-carboxylic acid [6-acetyl-2-chloro-3-(2,2-dimethoxy-ethoxy)-phenyl]-amide **312** (18 g, 40.7 mmol) was suspended in toluene (400 ml). NaH (2.4 g, 61 mmol) was added to the vigorously stirred mixture while monitoring H₂ evolution. The mixture became a clear solution during heating to reflux. The reaction was complete after refluxing for 3 hours. The mixture was cooled to room temperature. A solution of AcOH (69.2 mmol) in H₂O (3 vol) was added to the mixture. After vigorous agitation for 1 hour at 0 °C, the solids were collected by filtration, rinsed forward with H₂O. The wet cake was dried under high vacuum to a constant weight to provide compound **313** (15 g, 86%).

25

l. Preparation of compound **314**.

To a mixture of brosylate intermediate **303** (15 g, 35 mmol) and compound **313** (27.5 g, 38.5 mmol) in NMP (200 ml) was added cesium carbonate (25.1 g, 77 mmol). The mixture was stirred at 65 °C for 5 hours. The reaction was cooled to room temperature and EtOAc (600 ml) and an aqueous solution of 3% LiCl (600 ml) were added to the mixture. The organic layer was washed with aqueous 3% LiCl (1 x 600 ml), brine, dried (Na₂SO₄) and concentrated *in vacuo*. The residue was purified by silica gel chromatography to give the desired methyl ester as a yellow solid (23.6 g, 75%). LC/MS = 900.13(M⁺+1).

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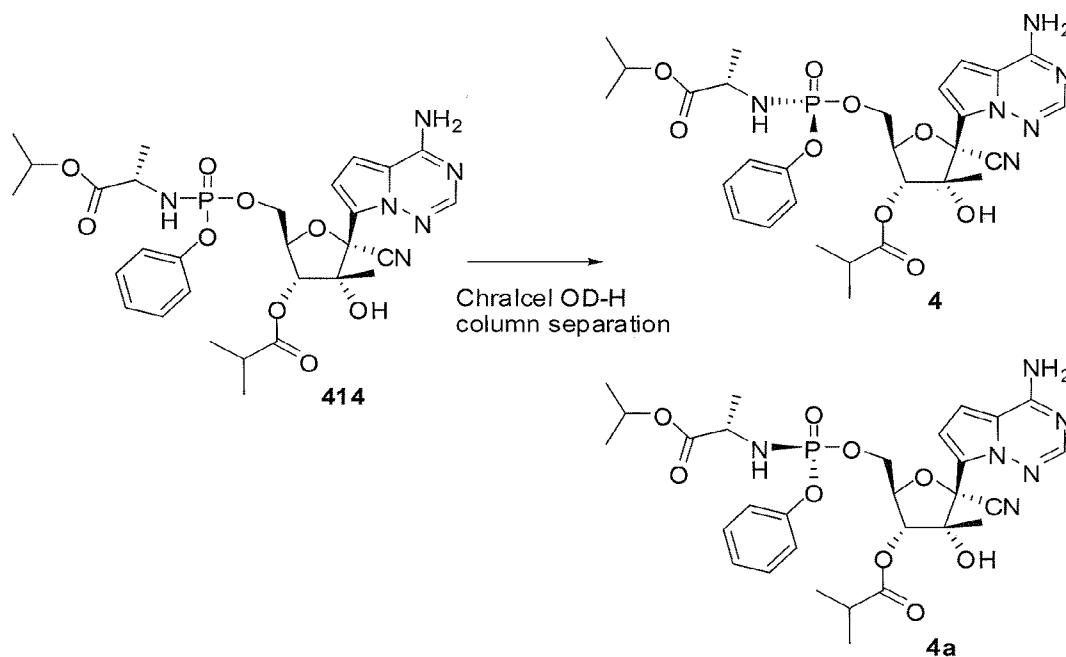
m. Preparation of compound **315**.

Methyl ester **314** (23.6 g, 26 mmol) was dissolved in glacial acetic acid (200 ml), 1.4 N HCl in H₂O (75 ml) was added to the solution. The mixture was stirred at 60 °C for 1 hour. Upon completion of the reaction, the mixture was concentrated to remove the solvents, coevaporated with toluene (x 2) to remove residual acetic acid. The residue was then dissolved in EtOAc (500 ml) and sat. NaHCO₃ aqueous solution (enough to neutralize the mixture) while monitoring CO₂ evolution. The organic layer was washed with brine, dried (Na₂SO₄) and concentrated *in vacuo*. The residue was further dried under high vacuum for 1 h and used as is for the next step. The crude

was dissolved in CH₂Cl₂ (360 ml), morpholine (3.4 g, 39 mmol) and sodium triacetoxyborohydride (7.2 g, 34 mmol) were added to the mixture at 0 °C. Then glacial acetic acid (0.47 g, 7.8 mmol) was added dropwise to the mixture. The reaction was complete in 10 minutes at 0 °C. Sat. NaHCO₃ aqueous solution was added to quench the reaction. After stirring for another 20 minutes, the organic layer was washed with brine, dried (Na₂SO₄) and concentrated *in vacuo*. The residue was purified by silica gel chromatography to give the desired amine product **315** as a yellow solid (12 g, 50%). LC/MS = 924.63(M⁺+1).

Compound **4** can be prepared as described in the following Example.

Example 4: Preparation of Compound 4.



Diastereomeric mixture **414** was dissolved in heptane and isopropanol (70%:30%, 230 mg in 4.5 mL of the mixed solvents) and subjected to chiral column separation under the following conditions:

Column: Chiralcel OD-H, 2 x 25 cm

5 Solvent system: 70% heptane and 30% isopropanol

Flow rate: 6 mL/min.

Loading volume per run: 2.5 mL

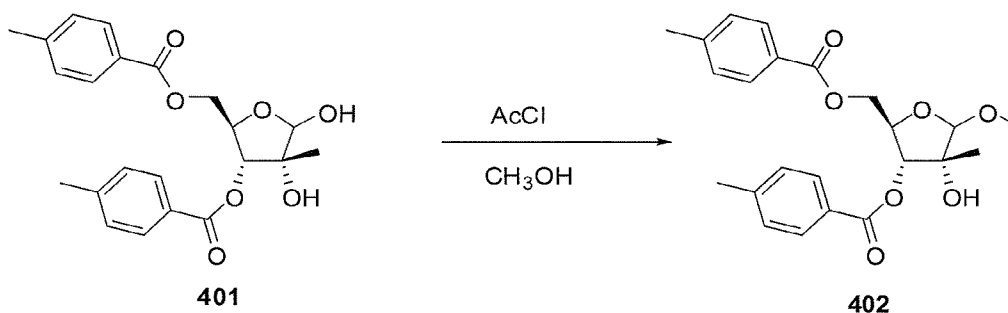
Compound **4** had a retention time of 20 minutes. ^1H NMR (300 MHz, CDCl_3): δ 8.00 (s, 1H), 7.1-7.3 (m, 5H), 6.83 (d, 1H), 6.71 (d, 1H), 6.09 (brs, 2H), 5.95 (s, 1H),
 10 5.04 (m, 2H), 4.67 (q, 1H), 4.35-4.52 (m, 2H), 4.00 (m, 2H), 2.74 (m, 1H), 1.40 (d, 3H), 1.2-1.3 (12H), 0.98 (s, 3H). ^{31}P NMR (121.4 MHz, CDCl_3): δ 2.72 (s). Compound **4** was subsequently recrystallized from MTBE for x-ray quality crystals.

Compound **4a** had a retention time 50 min. ^1H NMR (300 MHz, CDCl_3): δ 7.98 (s, 1H), 7.1-7.3 (m, 5H), 6.83 (d, 1H), 6.73 (d, 1H), 6.02 (brs, 2H), 5.95 (s, 1H), 5.08 (d, 1H),
 15 5.00 (m, 1H), 4.68 (q, 1H), 4.38-4.56 (m, 2H), 3.98 (m, 2H), 2.74 (m, 1H), 1.40 (d, 3H), 1.2-1.3 (12H), 0.99 (s, 3H). ^{31}P NMR (121.4 MHz, CDCl_3): δ 2.61 (s).

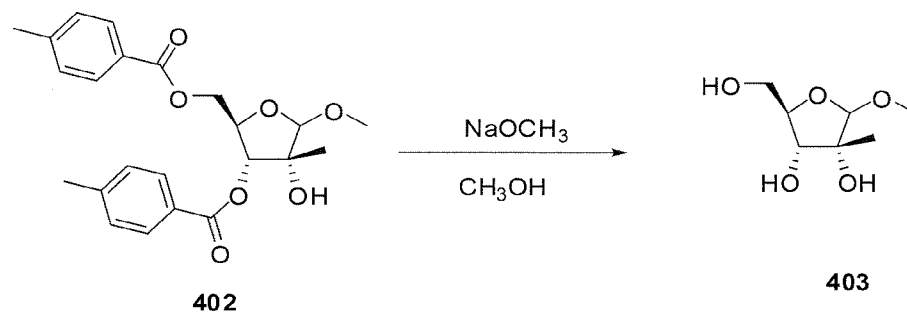
The intermediate diastereomeric mixture **414** was prepared as follows.

a. Preparation of Compound **402**.

20



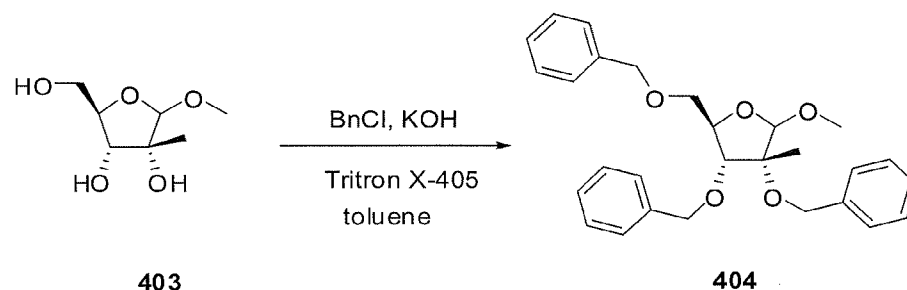
To a solution of compound **401** (22.0 g, 54.9 mmol, prepared according to the procedures described in *J.O.C.*, 2004, 6257) in methanol (300 mL) was dropwise
 25 added acetyl chloride (22 mL) at 0 °C using a dropping funnel over a period of 30 minutes and then stirred at room temperature for 16 hours. The mixture was concentrated, re-dissolved in ethyl acetate (400 mL), washed with ice-cold 2 N NaOH, and concentrated to dryness, affording the crude methyl ether **402** as an oil. MS = 437.2 (M + Na⁺).

b. Preparation of Compound **403**.

5

To a solution of compound **402** in methanol (300 mL) was added 0.5 M sodium methoxide solution in methanol (20 mL, 10 mmol), and stirred for 16 hours at room temperature. The reaction was quenched with 4.0 N HCl solution in dioxane (2.5 mL, 10 mmol). The mixture was then concentrated, affording the crude compound **403**. MS = 201.0 ($\text{M} + \text{Na}^+$).

10

c. Preparation of Compound **404**.

15

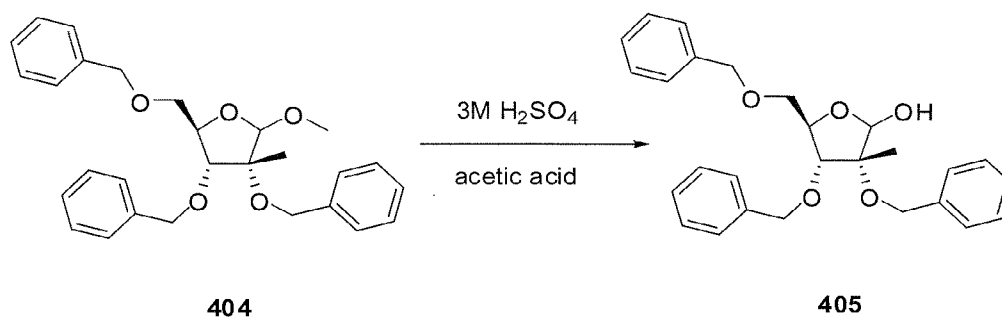
A mixture of compound **403**, Triton X-405 (70% in water, 6.0 g), 50% KOH (in water, 85 g) in toluene (500 mL) was heated to reflux with a Dean-Stark trap attached. After 1 hour collecting 25 mL of water, benzyl chloride (33 g, 260 mmol) was added and continued to reflux with stirring for 16 hours. The mixture was then cooled and partitioned between ethyl acetate (400 mL) and water (300 mL). The organic layer was washed with water (300 mL), and concentrated. The residue was purified by silica gel column chromatography (20% EtOAc / hexanes), affording the methyl ether **404** as an

20

oil (22.0 g, 89% in three steps). ^1H NMR (300 MHz, CDCl_3): δ 7.3 (m, 15H), 4.5 - 4.9 (m, 7H), 4.37 (m, 1H), 3.87 (d, 1H), 3.56 (m, 2H), 3.52 (s, 3H), 1.40 (s, 3H).

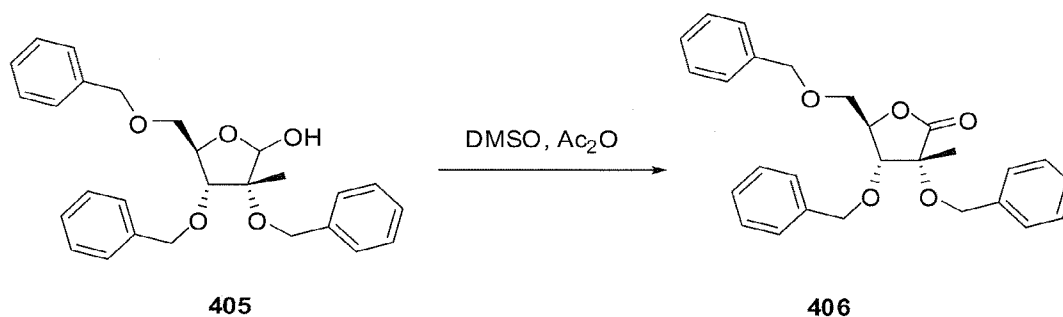
d. Preparation of Compound **405**.

5



To a solution of **404** (22.0 g, 49.0 mmol) in acetic acid (110 mL) was added 3 M
 10 sulfuric acid (prepared by mixing 4.8 g of concentrated sulfuric acid with 24 mL of
 water) and stirred at 70 °C for 8 hours. The mixture was concentrated to a volume of
 20 mL, and partitioned between ethyl acetate and ice-cold 2N NaOH. The ethyl
 acetate layer was concentrated, and purified by silica gel column chromatography
 (~35% EtOAc / hexanes), affording compound **405** as an oil (17.0 g, 80%). MS =
 15 457.2 (M + Na⁺).

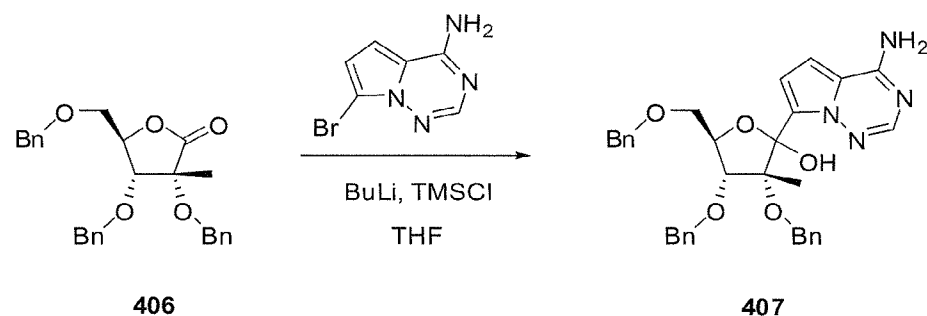
e. Preparation of Compound **406**.



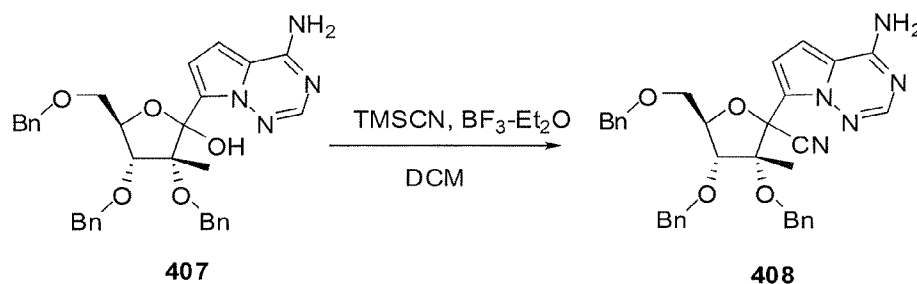
20

To a solution of compound **405** (45 g, 104 mmol) in DMSO (135 mL) was
 dropwise added acetic anhydride (90 mL, 815 mmol) at room temperature under

10



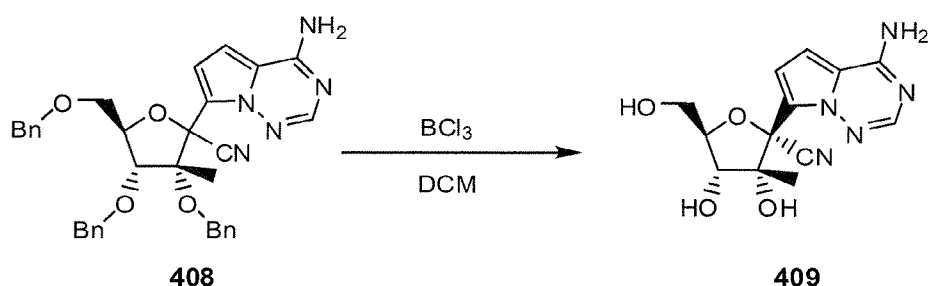
To a dry, argon purged round bottom flask (100 mL) were added 7-bromo-
pyrrolo[2,1-f][1,2,4]triazin-4-ylamine (234 mg, 1.10 mmol) (prepared according to
WO2007056170) and anhydrous THF (1.5 mL). TMSCl (276 μ L, 2.2 mmol) was then
added and the reaction mixture stirred for 2 hours. The flask was placed into a dry
ice/acetone bath (-78 $^{\circ}$ C) and BuLi (2.5 mL, 4.0 mmol, 1.6M in hexanes) was added
dropwise. After 1 hour, a solution of compound 406 (432.5 mg, 1.0 mmol) in THF was
cooled to 0 $^{\circ}$ C and then added to the reaction flask dropwise. After 1 hour of stirring at
-78 $^{\circ}$ C, the flask was warmed to 0 $^{\circ}$ C and sat. NH_4Cl (5 mL) was added to quench the
reaction. The organics were extracted using EtOAc (3 x 10 mL) and the combined
organic layers were dried using MgSO_4 . The solvent was removed under reduced
pressure and the crude material was purified using flash chromatography (hexanes /
EtOAc). 560 mg (90 %) of compound **407** was isolated as a mixture of two anomers.
LC/MS = 567.2 ($\text{M} + \text{H}^+$). ^1H NMR (300 MHz, CDCl_3): δ 7.85 (m, 1H), 7.27 (m, 15H),
7.01 (m, 1H), 6.51 (m, 1H), 4.66 (m, 8H), 4.40 (m, 2H), 3.79 (m, 3H), 1.62 (s, 2'- CH_3
from the one anomer), 1.18 (s, 2'- CH_3 from the other anomer).

g. Preparation of Compound **408**.

5

To a solution of Compound **407** (1 g, 1.77 mmol) in CH_2Cl_2 (20 mL) at 0°C was added TMS-CN (1.4 mL, 10.5 mmol) and $\text{BF}_3\cdot\text{Et}_2\text{O}$ (1 mL, 8.1 mmol). The reaction mixture was stirred at 0°C for 0.5 hours, then at room temperature for additional 0.5 hour. The reaction was quenched with NaHCO_3 at 0°C , and diluted with $\text{CH}_3\text{CO}_2\text{Et}$.

10 The organic phase was separated, washed with brine, dried over Na_2SO_4 , filtered and concentrated. The residue was purified by chromatography on silica gel, eluted with $\text{CH}_3\text{CO}_2\text{Et}$ -hexanes (1:1 to 2:1), to give compound **408** (620 mg, 61%) as an isomeric mixture. MS = 576.1 ($\text{M} + \text{H}^+$).

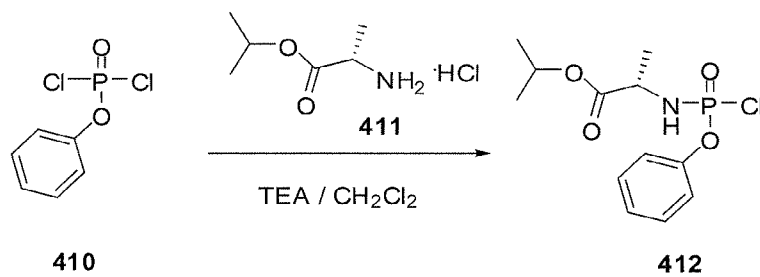
15 h. Preparation of Compound **409**.

20 To a solution of compound **408** (150 mg, 0.26 mmol) in CH_2Cl_2 (4 mL) at -78°C was added BCl_3 (2 mL, 1M in CH_2Cl_2). The reaction mixture was stirred at -78°C for 1 hour. The reaction was quenched at -78°C by dropwise addition of TEA (2 mL) and MeOH (5 mL). The mixture was allowed to warm up to room temperature, evaporated, and co-evaporated with MeOH several times. The residue was treated with NaHCO_3 (1 g in 10 mL H_2O), concentrated and purified by HPLC to give the desired product

compound **409** (48 mg, 60%). ^1H NMR (300 MHz, D_2O): δ 7.74 (s 1H), 6.76 (d, J = 5 Hz, 1H), 6.73 (d, J = 5 Hz, 1H), 4.1 (m, 1H), 3.9 (m, 1H), 3.8 (m, 2H), 0.84 (s, 3H). MS = 305.9 ($\text{M} + \text{H}^+$). The other alpha-anomer was also obtained (9 mg, 11%): ^1H NMR (300 MHz, D_2O): δ 7.70 (s 1H), 6.8 (d, J = 5 Hz, 1H), 6.7 (d, J = 5 Hz, 1H), 4.25 (d, J = 9 Hz, 1H), 4.07 (m, 1H), 3.85 (m, 1H), 3.7 (m, 1H), 1.6 (s, 3H). MS = 306.1 ($\text{M} + \text{H}^+$).

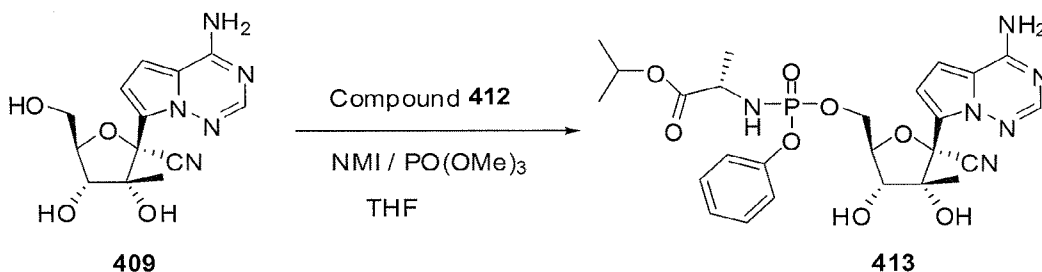
i. Preparation of Compound **412**.

10



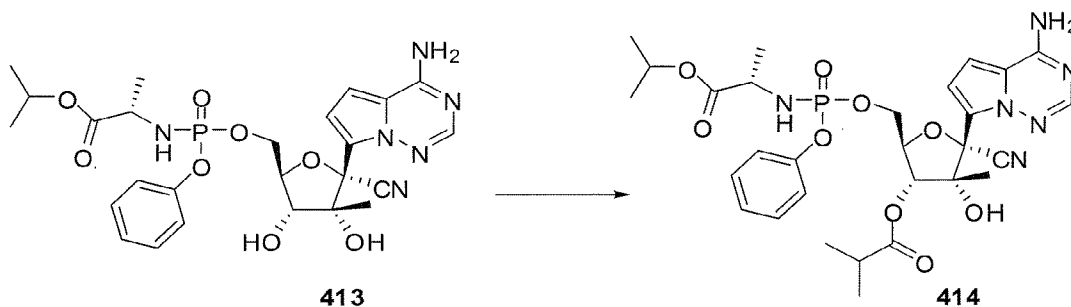
Compound **410** (commercially available, 4.99 g, 23.8 mmol) was dissolved in dichloromethane (100 mL) and alanine isopropyl ester hydrochloride **411** (3.98 g, 23.8 mmol) was added. The resulting clear solution was cooled -78°C for 30 min. Triethylamine (6.63 mL, 47.5 mmol) was added dropwise over 15 minutes. The mixture was then allowed to warm to room temperature. After 16 hours, the solvent was removed by argon stream. The residue was re-dissolved in MTBE (25 mL) and the insoluble was removed by filtration under argon. The filtrate was condensed by argon stream and the crude product **412** was used for the next reaction without further purification. ^1H NMR (300 MHz, CDCl_3): 7.1-7.4 (m, 5H), 5.1 (m, 1H), 4.35 (m, 1H), 4.15 (m, 1H), 1.5 (d, 3H), 1.2 (m, 6H). ^{31}P NMR (121.4 MHz, CDCl_3): δ 7.8 and 8.4 (2s).

25 j. Preparation of Compound **413**.



To a solution of compound **409** (1.03 g, 3.37 mmol) in trimethyl phosphate (2.0 mL) and THF (20 mL) was added *N*-methyl imidazole (1.5 g, 18.3 mmol) at 0 °C. A solution of compound **412** (2.5 g, 8.18 mmol) in THF (3 mL) was dropwise added. The resulting mixture was allowed to warm to room temperature over 1.5 hours. The mixture was partitioned between ethyl acetate and water. The ethyl acetate layer was concentrated and the residue was purified by silica gel chromatography (ethyl acetate to 10% ethanol / ethyl acetate), affording 1.15 g (59%) of compound **413** as 1:1 diastereomeric mixture at phosphorous. ¹H NMR (300 MHz, CDCl₃): δ 8.02 (s, 1H), 7.1-7.4 (m, 5H), 6.8 (2d, 1H), 6.7 (2d, 1H), 6.08 (brs, 2H), 5.03 (m, 1H), 4.6 (m, 1H), 4.4 (m, 2H), 3.9-4.1 (m, 3H), 1.31 (d, 3H), 1.2 (m, 6H), 0.83 (s, 3H). ³¹P NMR (121.4 MHz, CDCl₃): δ 2.78 (s). MS = 575.1 (M + H⁺).

k. Preparation of Compound **414**.

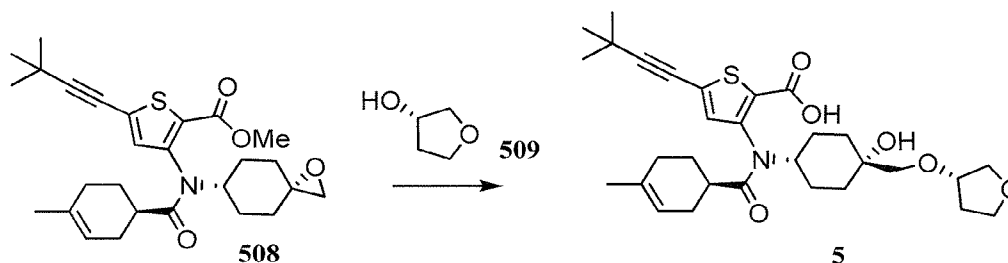


To a solution of compound **413** (175 mg, 0.305 mmol) in acetonitrile (2 mL) was added *N,N*-dimethylformamide dimethyl acetal (41 μL, 0.34 mmol, 1.1 eq.) and stirred at room temperature for 1 hour. The reaction was complete (by LCMS). The mixture was then concentrated to dryness. To the residue were added DCC (250 mg, 1.21 mmol, 4 eq.), acetonitrile (5 mL) and isobutyric acid (55 mg, 58 μL, 2 eq.). The mixture was stirred at room temperature for 48 hours. Water (0.2 mL) and trifluoroacetic acid (0.1 mL) were added at 0 °C and stirred at room temperature for 64

hours. Sodium bicarbonate (500 mg) was added at 0 °C. The mixture was stirred at room temperature for 0.5 hour and filtered. The filtrate was concentrated and the residue was purified by silica gel column chromatography (5% methanol / dichloromethane), affording 144 mg (73%) of compound **414** as 1:1 diastereomeric mixture at phosphorus. ¹H NMR (300 MHz, CDCl₃): δ 8.00 (s, 1H), 7.1-7.4 (m, 5H), 6.83 (d, 1H), 6.71 (2d, 1H), 5.97 (brs, 2H), 5.94 (d, 1H), 5.07 (2d, 1H), 5.01 (m, 1H), 4.68 (m, 1H), 4.4 (m, 2H), 4.0 (m, 2H), 2.74 (m, 1H), 1.4 (2d, 3H), 1.2-1.3 (12H), 0.98 and 0.99 (2s, 3H). ³¹P NMR (121.4 MHz, CDCl₃): δ 2.56 and 2.65 (2s). MS = 645.1 (M + H⁺).

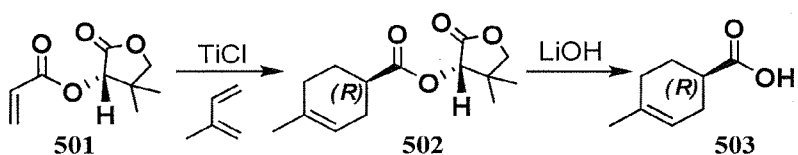
Compound **5** can be prepared as described in the following Example.

Example 5: Preparation of 5: 5-(3,3-dimethylbut-1-yn-1-yl)-3-[(*cis*-4-hydroxy-4-[(3*S*)-tetrahydrofuran-3-yloxy]methyl)cyclohexyl]{[(1*R*)-4-methylcyclohex-3-en-1-yl]carbonyl}amino]thiophene-2-carboxylic acid
5.



5-(3,3-dimethyl-but-1-ynyl)-3-[[[(1*R*)-4-methyl-cyclohex-3-enecarbonyl]-(1-oxa-spiro[2.5]oct-6-yl)-amino]-thiophene-2-carboxylic acid methyl ester **508** (132 mg, 0.28 mmol) and (*S*)-tetrahydro-furan-3-ol **509** (247 mg, 2.8 mmol) in 1-methyl-pyrrolidin-2-one (3 mL) were treated with potassium *tert*-butoxide (251 mg, 2.24 mmol), sealed and heated to 40 °C for 16 hours. After cooling the mixture was treated with 2 M HCl until pH 3, partitioned between ethyl acetate and water and separated. The organic layer was washed with 5% lithium chloride solution, water, brine, and dried over sodium sulfate. After filtration and concentration the residue was purified by HPLC with CH₃CN (0.1% TFA)/H₂O(0.1% TFA) to afford 107 mg (70% yield) of compound **5** as a white powder: MS (m/z): 544.0 [M+H]⁺; HPLC retention time 4.22 min (2-98% acetonitrile: water with 0.05% trifluoroacetic acid).

The intermediate compound **508** was prepared as follows.



5

a. Preparation of Compound **502**.

(S)-3-hydroxy-4,4-dimethyldihydrofuran-2(3H)-one (2.60 g, 20 mmol) and diisopropylethylamine (5.2 mL, 30 mmol) in dichloromethane (25 mL) was cooled to -10 °C and treated dropwise with acryloyl chloride (2.03 mL, 25 mmol) and stirred for 2 h. 1M HCl (20 mL) was added and the organic layer was washed with sodium bicarbonate and water. The organic layer was dried over sodium sulfate, filtered and concentrated. Flash chromatography (10-40% EtOAc, hexanes) afforded 2.09 g (57% yield) of the desired (S)-4,4-dimethyl-2-oxotetrahydrofuran-3-yl acrylate **501** as a clear oil.

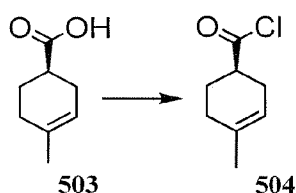
(S)-4,4-dimethyl-2-oxotetrahydrofuran-3-yl acrylate **501** (2.05 g, 11.1 mmol) in dichloromethane (17.5 mL) and hexanes (2.5 mL) was cooled to -10 °C and treated with titanium tetrachloride (2.2 mL, 1 M in dichloromethane, 2.2 mmol). The yellow solution was stirred for 15 minutes and treated with isoprene (1.67 mL, 16.7 mmol) dropwise over 5 minutes. After stirring for 2 hours, an additional portion of isoprene (1.67 mL, 16.7 mmol) was added and the reaction mixture was stirred at -10 to 0 °C for 3.5 hours. The reaction mixture was quenched with ammonium chloride (sat. aq.). Water and ethyl acetate: hexanes (1:1) were added. The organic layer was separated and the aqueous layer was extracted again with ethyl acetate:hexanes (1:1). The combined organic layers were dried over sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography (10-50% EtOAc:Hex, 80 g column) to afford 1.30 g (46% yield) of (R)-((S)-4,4-dimethyl-2-oxotetrahydrofuran-3-yl) 4-methylcyclohex-3-enecarboxylate **502** as a clear oil.

b. Preparation of Compound **503**.

(R)-((S)-4,4-dimethyl-2-oxotetrahydrofuran-3-yl) 4-methylcyclohex-3-enecarboxylate **502** (1.30 g, 5.15 mmol) in THF (10 mL), water (1 mL) and methanol (1 mL) was treated with lithium hydroxide monohydrate (2.16 g, 51.5 mmol) and warmed to 50 °C with stirring. After 1 hour, the reaction mixture treated with 1M HCl. The

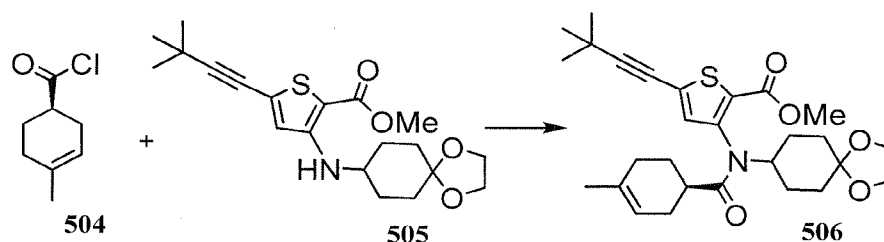
mixture was extracted with hexanes:THF (10:1), dried over sodium sulfate, filtered and concentrated to 0.738 g (quantitative yield) of (R)-4-methylcyclohex-3-enecarboxylic acid **503** as a white powder.

5 c. Preparation of Compound **504**.



(R)-4-methylcyclohex-3-enecarboxylic acid **503** (371 mg, 2.65 mmol), azeotropically dried by evaporation from toluene, was treated with potassium phosphate tribasic (1.13 g, 7.94 mmol), suspended in dichloromethane (7.6 mL) and treated with dimethylformamide (4 drops). The reaction mixture was cooled to 0 °C and treated dropwise with oxalyl chloride (0.75 mL, 7.9 mmol). The reaction mixture was allowed to warm to ambient temperature while stirring for 2 hours. After filtering the solids, the solution was concentrated, treated with hexanes and concentrated again to afford (R)-4-methylcyclohex-3-enecarbonyl chloride **504** as a light yellow oil which was used immediately in the next step.

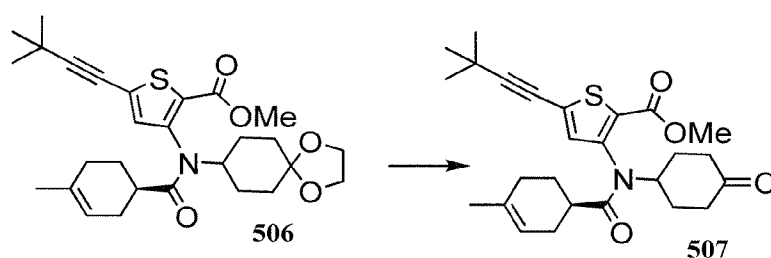
d. Preparation of Compound **506**.



(R)-4-methylcyclohex-3-enecarbonyl chloride **504** (2.65 mmol), 5-(3,3-dimethylbut-1-ynyl)-3-(1,4-dioxaspiro[4.5]dec-8-ylamino)-thiophene-2-carboxylic acid methyl ester **505** (250 mg, 0.66 mmol) and potassium phosphate tribasic (562 mg, 2.65 mmol) were suspended in dichloroethane (1.7 mL), sealed with a cap and heated to 90 °C. After 16 hours, the reaction mixture was cooled and partitioned between ethyl acetate and water. The organic layer was separated and the aqueous extracted again with

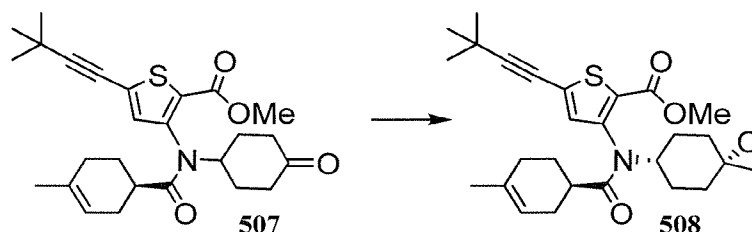
ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and concentrated. Flash chromatography (10-40% EtOAc:Hexanes) afforded 220 mg (67% yield) of the desired 5-(3,3-dimethyl-but-1-ynyl)-3-[(1,4-dioxo-spiro[4.5]dec-8-yl)-((1*R*)-4-methyl-cyclohex-3-enecarbonyl)-amino]-thiophene-2-carboxylic acid methyl ester **506** as a beige foam.

e. Preparation of Compound **507**.



5-(3,3-Dimethyl-but-1-ynyl)-3-[(1,4-dioxo-spiro[4.5]dec-8-yl)-((1*R*)-4-methyl-cyclohex-3-enecarbonyl)-amino]-thiophene-2-carboxylic acid methyl ester **506** (219 mg, 0.438 mmol) was dissolved in THF (3.5 mL) and treated with 4M HCl (1.75 mL, 7.01 mmol). The reaction mixture was heated to 45 °C and stirred 2 h. Ethyl acetate was added and the organic layer was separated then washed with water, sodium bicarbonate (sat aq), water, and brine. The organic layer was dried over sodium sulfate, filtered and concentrated to 0.190 g (95% yield) of the desired 5-(3,3-dimethyl-but-1-ynyl)-3-[(1*R*)-4-methyl-cyclohex-3-enecarbonyl)-(4-oxo-cyclohexyl)-amino]-thiophene-2-carboxylic acid methyl ester **507** as a white foam.

f. Preparation of Compound **508**.

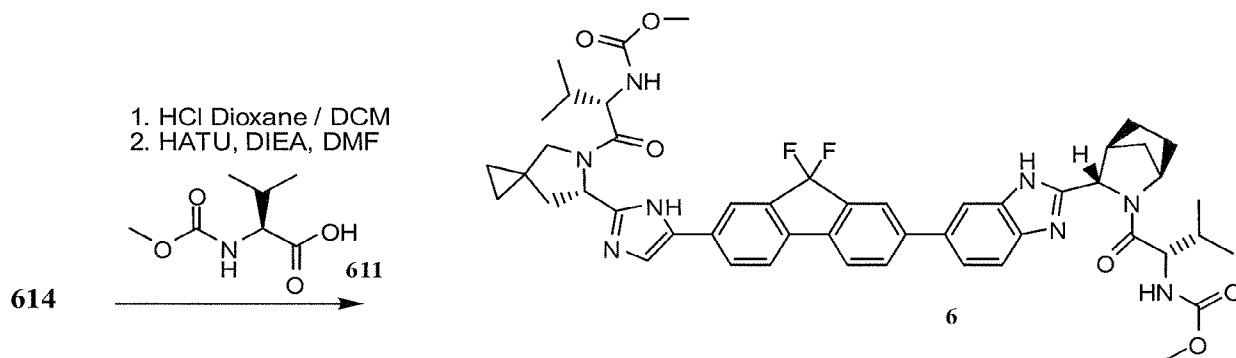


Trimethylsulfoxonium chloride (79 mg, 0.62 mmol) in DMSO (1.5 mL) was treated with sodium hydride (21 mg, 60% oil dispersion, 0.53 mmol) and stirred at ambient temperature for 10 min. 5-(3,3-Dimethyl-but-1-ynyl)-3-[(1*R*)-4-methyl-cyclohex-3-enecarbonyl)-(4-oxo-cyclohexyl)-amino]-thiophene-2-carboxylic acid methyl ester **507** in THF (1 mL + 0.5 mL) was added dropwise and the reaction mixture was

stirred for 45 min. The orange solution was treated with 5% citric acid until pH 3 and partitioned between water and ethyl acetate. The organic layer was separated and the aqueous was extracted again with ethyl acetate. The combined organics were washed with 5% LiCl, water and brine, and dried over sodium sulfate. After filtration and
 5 concentration, the residue was purified by flash chromatography (20-75% EtOAc:hexanes) to afford 0.134 g (70% yield) of 5-(3,3-dimethyl-but-1-ynyl)-3-(((1*R*)-4-methyl-cyclohex-3-enecarbonyl)-(1-oxa-spiro[2.5]oct-6-yl)-amino]-thiophene-2-carboxylic acid methyl ester **508** as a white powder.

10 Compound **6** can be prepared using synthetic methods and intermediates like those described in USSN 12/779,023 (US 20100310512 A1). Compound **6** can also be prepared as described in the following Example.

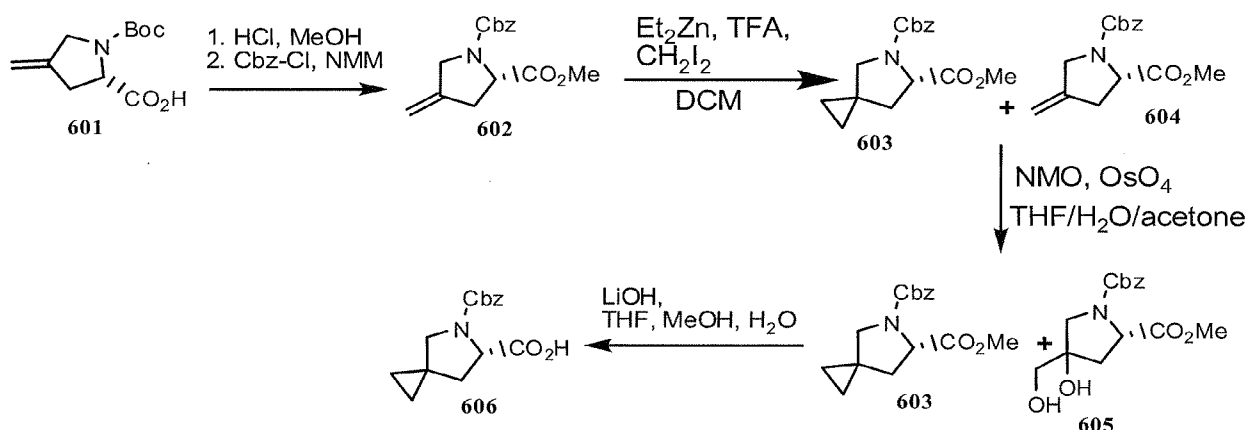
15 Example 6: Preparation of (1-{3-[6-(9,9-Difluoro-7-{2-[5-(2-methoxycarbonylamino-3-methyl-butyl)-5-aza-spiro[2.4]hept-6-yl]-3H-imidazol-4-yl]-9H-fluoren-2-yl]-1H-benzoimidazol-2-yl]-2-aza-bicyclo[2.2.1]heptane-2-carbonyl]-2-methyl-propyl)-carbamic acid methyl ester **6**.



20 3-[6-(9,9-Difluoro-7-{2-[5-(2-methoxycarbonylamino-3-methyl-butyl)-5-aza-spiro[2.4]hept-6-yl]-3H-imidazol-4-yl]-9H-fluoren-2-yl]-1H-benzoimidazol-2-yl]-2-aza-bicyclo[2.2.1]heptane-2-carboxylic acid tert-butyl ester **614** (115 mg, 0.138 mmol) was dissolved in methylene chloride (2 mL) and HCl in dioxane (4M, 2 mL) was added and stirring at room temperature was continued. After 20 minutes, all volatiles were
 25 removed *in vacuo*. The crude material was used in the next step without further purification. The crude material was dissolved in DMF (1.5 mL) and DIEA (53.4 mg, 0.414 mmol) was added. A solution of 2- (L) Methoxycarbonylamino-3-methyl-butyric acid **611** (24.2 mg, 0.138 mmol), HATU (52.4 mg, 0.138 mmol) and DIEA (17.8 mg, 0.138 mmol) in DMF (1 mL) was added. The reaction was stirred at room temperature.

After 20 minutes, the reaction was diluted with EtOAc and was washed with aqueous bicarbonate solution, aqueous LiCl solution (5%), brine, and was dried over sodium sulfate. Filtration and removal of solvents *in vacuo* gave the crude material, which was purified by RP-HPLC (eluent: water / MeCN w/ 0.1% TFA) to yield compound **6** (76 mg). LCMS-ESI⁺: calc'd for C₄₉H₅₄F₂N₈O₆: 888.9 (M⁺); Found: 890.0 (M+H⁺). ¹H-NMR: 300 MHz, (dmso-d₆) δ: 8.20-7.99 (m, 8H), 7.73 (s, 2H), 7.37 – 7.27 (m, 2H), 5.25 (dd, J = 7.2 Hz, 1H), 4.78 (s, 1H) 4.54 (s, 1H), 4.16 (m, 1H), 4.02 (m, 1H), 3.87 (m, 1H), 3.74 (m, 1H), 3.55 (s, 3H), 3.53 (s, 3H), 2.75 (m, 1H), 2.25 (m, 2H), 2.09 – 2.04 (m, 2H), 1.88 – 1.79 (m, 2H), 1.54 (m, 1H), 0.94 – 0.77 (m, 15H) 0.63 (m, 4H) ppm. ¹⁹F-NMR: 282 MHz, (dmso-d₆) δ: -109.1 ppm [-74.8 ppm TFA].

The intermediate compound **614** was prepared as follows.



15

a. Preparation of compound 4-Methylene-pyrrolidine-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester **602**.

4-Methylene-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester **601** (10.0 g, 44 mmol) was dissolved in MeOH (75 mL) at room temperature and HCl (4M in dioxane, 75 mL) was added. Stirring at room temperature was continued for 4 hours. All volatiles were removed *in vacuo* and a beige solid was obtained. The crude material was suspended in methylene chloride (100 mL) and N-Methyl morpholine (13.3 g, 132 mmol) was added. The mixture was cooled to 0 °C and benzyl chloroformate (8.26 g, 48.4 mmol) was added while stirring. After 30 minutes, the reaction was warmed to room temperature and the solution was washed with water and aqueous HCl (1M). The solution was dried over sodium sulfate. Filtration and evaporation of solvents gave

crude product, which was purified by silica gel chromatography (eluent: EtOAc / hexanes) to yield compound **602** (10.2 g). LCMS-ESI⁺: calc'd for C₁₅H₁₇NO₄: 275.3 (M⁺); Found: 276.4 (M+H⁺).

5 b. Preparation of a mixture of Compounds **603** and **604**.

An oven-dried 3-neck round bottom flask was equipped with a nitrogen inlet adaptor and a 250 mL addition funnel. The third neck was sealed with a septum. The flask was charged with a stir bar, dichloromethane (120 mL) and diethyl zinc (1.0 M in hexane, 118 mL, 118 mmol) then cooled to 0 °C in an ice bath. The addition funnelled
10 was charged with dichloromethane (40 mL) and trifluoroacetic acid (9.1 mL, 118 mmol). After the diethyl zinc solution had cooled to 0 °C (about 25 minutes), the trifluoroacetic acid solution was added dropwise over 20 min to the stirred reaction mixture. After stirring for another 20 min at 0 °C, diiodomethane (9.5 mL, 118 mmol) was added slowly over 4 minutes. After another 20 min, 4-methylene-pyrrolidine-1,2-
15 dicarboxylic acid 1-benzyl ester 2-methyl ester **602** (8.10 g, 29.4 mmol) was added in 30 mL dichloromethane by cannula. The flask containing 4-methylene-pyrrolidine-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester was then rinsed with another 10 mL dichloromethane and this solution was also transferred to the reaction mixture by cannula. The reaction mixture was allowed to warm to RT and stirred for 110 h (about
20 5 days) after which the reagents were quenched with saturated aqueous ammonium chloride (~150 mL). The contents of the flask were slowly poured into a 2 L sep funnel containing saturated aqueous sodium bicarbonate (800 mL). The aqueous phase was extracted three times with 300 mL ethyl acetate. The combined organics were dried over magnesium sulfate and concentrated to provide a mixture of Compounds **603** and
25 **604**.

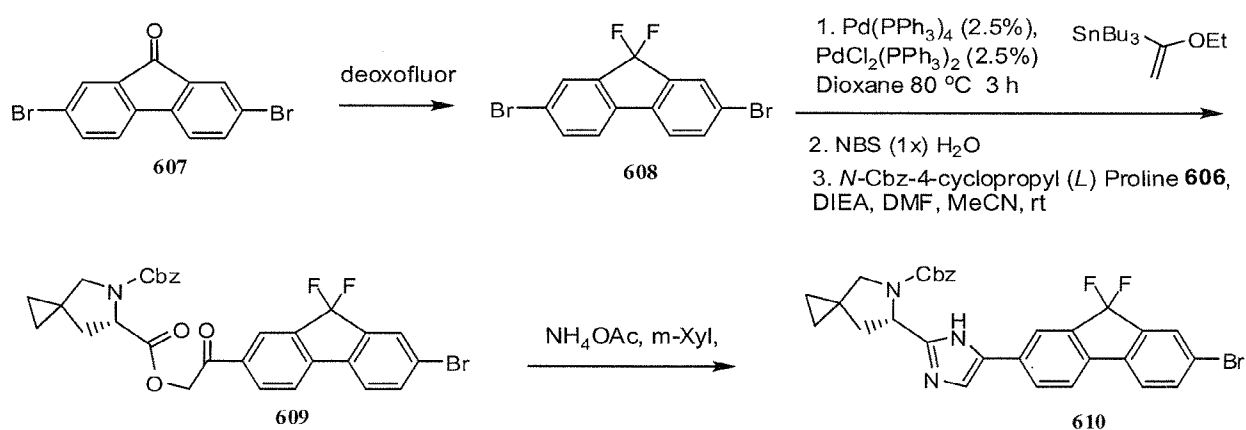
c. Preparation of a Compound **603**.

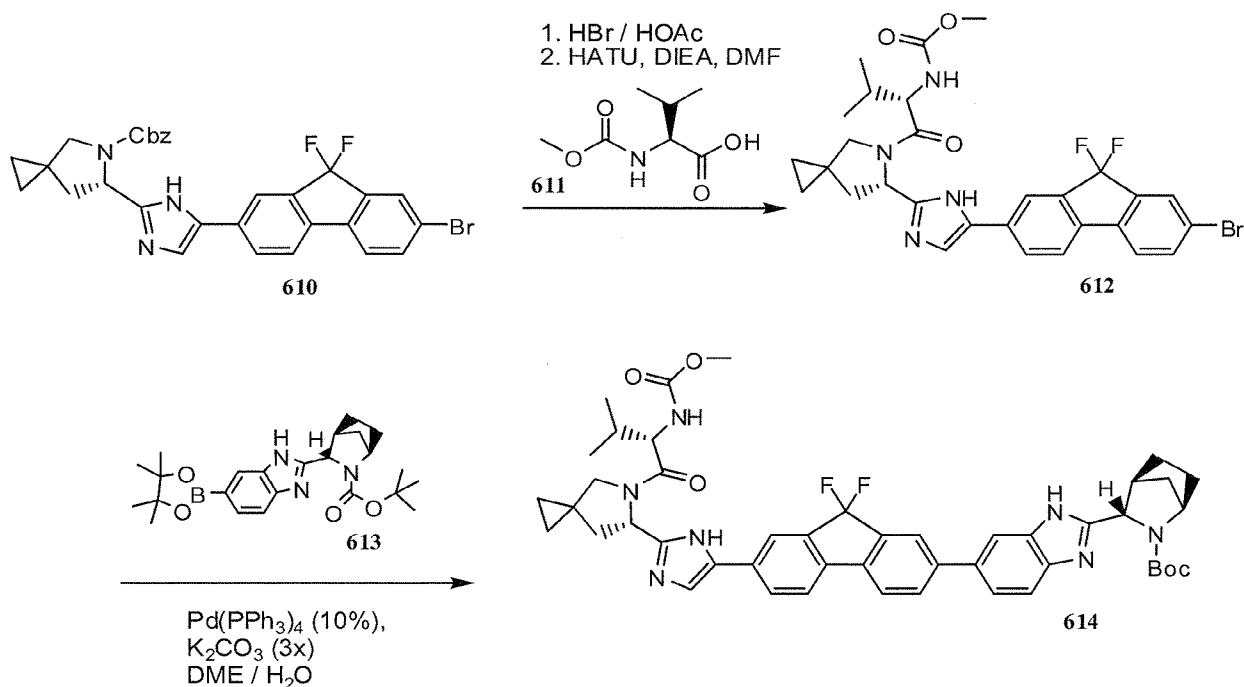
The crude material from sub-part b was dissolved in 3:1:1 THF/water/acetone (165 mL) then treated with *N*-methyldimorpholine-*N*-oxide (3.45 g, 29.4 mmol) and
30 osmium tetroxide (4 wt% in water, 5 mL, 0.818 mmol). After stirring at RT for 7 h, the reagents were quenched with 1 M aqueous sodium thiosulfate (~100 mL). The contents of the flask were then poured into a 1 L sep funnel containing water (~300 mL). The aqueous phase was extracted three times with 300 mL dichloromethane. The combined organics were dried over magnesium sulfate and concentrated. The
35 crude residue was purified by silica column chromatography (5% to 45%

EtOAc/hexane) to provide 5-aza-spiro[2.4]heptane-5,6-dicarboxylic acid 5-benzyl ester 6-methyl ester **603** as a clear oil (5.54g, 19.15 mmol, 65%) as a clear oil. ¹H NMR (CDCl₃) δ 7.36-7.29 (m, 5H), 5.21-5.04 (m, 2H), 4.56-4.47 (m, 1H), 3.75 (s, 1.5H), 3.60 (m, 1.5H), 0.51-0.37 (m, 2H), 2.32-2.25 (m, 1H), 1.87-1.80 (m, 1H), 0.64-0.51 (m, 4H).

d. Preparation of 5-Aza-spiro[2.4]heptane-5,6-dicarboxylic acid 5-benzyl ester **606**.

5-Aza-spiro[2.4]heptane-5,6-dicarboxylic acid 5-benzyl ester 6-methyl ester **603** (244 mg, 0.840 mmol) was dissolved in THF (2.0 mL) / MeOH (1.5 mL). An aqueous solution of LiOH (35.5 mg, 0.84 mmol) was added and stirring at room temperature was continued. After 3 hours, the reaction was neutralized with aqueous HCl (1M) and the organic solvents were removed *in vacuo*. The crude mixture was diluted with water and EtOAc and the organic layer was collected. All volatiles were removed *in vacuo* and the crude acid **606** was used without further purification. LCMS-ESI⁺: calc'd for C₁₅H₁₇NO₄: 275.3 (M⁺); Found: 276.3 (M+H⁺).





5 e. Preparation of a 2,7-Dibromo-9,9-difluoro-9H-fluorene **608**.

2,7-Dibromo-fluorene-9-one **607** (4.0 g, 11.8 mmol) was suspended in deoxofluor (12 mL) at room temperature and EtOH (4 drops) was added. The stirred suspension was heated at T = 90° C for 24 hours (CAUTION: Use of deoxofluor at elevated temperatures, as described above, is cautioned as rapid and violent exotherms may occur). The reaction was cooled to room temperature and poured onto ice containing sodium bicarbonate. A solid formed and was collected via filtration. The crude material was taken into EtOAc and was washed with aqueous HCl (1M) and brine. The solution was dried over sodium sulfate. Filtration and evaporation of solvents gave crude product, which was purified by silica gel chromatography (eluent: EtOAc / hexanes) to yield **608** (3.2 g). ¹⁹F-NMR: 282 MHz, (dms_o-d₆) δ: -111.6 ppm. Before using the material in the next step, it was exposed as a solution in EtOAc to charcoal.

20 f. Preparation of 5-Aza-spiro[2.4]heptane-5,6-dicarboxylic acid 5-benzyl ester 6-[2-(7-bromo-9,9-difluoro-9H-fluorene-2-yl)-2-oxo-ethyl] ester **609**.

2,7-Dibromo-9,9-difluoro-9H-fluorene **608** (372 mg, 1.04 mmol), Pd(PPh₃)₄ (30.0 mg, 0.026 mmol), PdCl₂(PPh₃)₂ (18.2 mg, 0.026 mmol), As(PPh₃)₃ (5.0 mg)

were dissolved in dioxane (10 mL) under an argon atmosphere. Ethoxyvinyl-tributyl tin (376.4 mg, 1.04 mmol) was added. The mixture was heated for 140 minutes at 85 °C (oil bath). The reaction was cooled to room temperature. *N*-bromo succinimide (177 mg, 1.0 mmol) was added followed by water (2 mL). The reaction was stirred at room temperature for 3 hours, after which the majority of the dioxane was removed *in vacuo*. The crude reaction mixture was diluted with EtOAc and was washed with water. All volatiles were removed *in vacuo*. Toluene was added and all volatiles were removed *in vacuo* for a second time. The crude material was dissolved in DMF / MeCN (2 mL, 1:1) at room temperature. A solution of *N*-Cbz-4-cyclopropyl (*L*) proline **606** (0.84 mmol) and DIEA (268 mg, 2.08 mmol) in MeCN (2 mL) was added and stirring at room temperature was continued. After 14 hours, most of the MeCN was removed *in vacuo* and the crude reaction mixture was diluted with EtOAc. The mixture was washed with aqueous HCl (1M), aqueous LiCl solution (5%), brine, and was dried over sodium sulfate. Filtration and evaporation of solvents gave the crude reaction product, which was purified via silica gel chromatography (eluent: EtOAc / hexanes) to yield compound **609** (176 mg). LCMS-ESI⁺: calc'd for C₃₀H₂₄BrF₂NO₅: 596.4 (M⁺); Found: 595.2 / 597.2 (M+H⁺).

g. Preparation of 6-[5-(7-Bromo-9,9-difluoro-9H-fluoren-2-yl)-1H-imidazol-2-yl]-5-aza-spiro[2.4]heptane-5-carboxylic acid benzyl ester **610**.

5-Aza-spiro[2.4]heptane-5,6-dicarboxylic acid 5-benzyl ester 6-[2-(7-bromo-9,9-difluoro-9H-fluoren-2-yl)-2-oxo-ethyl] ester **609** (172 mg, 0.293 mmol) was dissolved in *m*-xylenes (6.0 mL). Ammonium acetate (226 mg, 2.93 mmol) was added and the reaction was stirred at 140 °C for 60 minutes under microwave conditions. The reaction was cooled to room temperature and all volatiles were removed *in vacuo*. The crude material was purified via silica gel chromatography (eluent: EtOAc / hexanes) to yield compound **610** (80.3 mg). LCMS-ESI⁺: calc'd for C₃₀H₂₄BrF₂N₃O₂: 576.4 (M⁺); Found: 575.2 / 577.2 (M+H⁺).

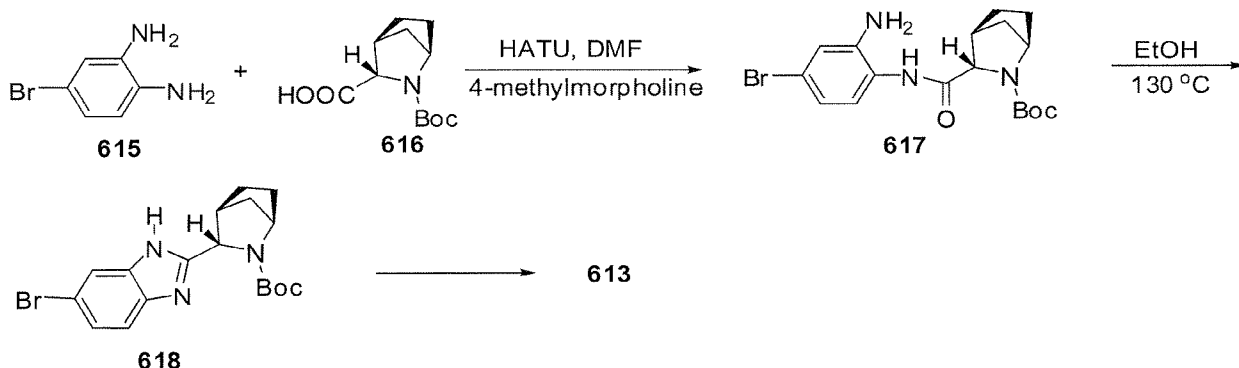
h. Preparation of (1-{6-[5-(7-Bromo-9,9-difluoro-9H-fluoren-2-yl)-1H-imidazol-2-yl]-5-aza-spiro[2.4]heptane-5-carbonyl}-2-methyl-propyl)-carbamic acid methyl ester **612**.

6-[5-(7-Bromo-9,9-difluoro-9H-fluoren-2-yl)-1H-imidazol-2-yl]-5-aza-spiro[2.4]heptane-5-carboxylic acid benzyl ester **610** (800 mg, 1.38 mmol) was dissolved in methylene chloride (15 mL) and HBr in AcOH (37%, 2 mL) was added and stirring at room temperature was continued. After 180 minutes, the suspension was

diluted with hexanes and the solid was collected via filtration and was washed with hexanes and subjected to vacuum. The crude material was used in the next step without further purification. The crude material was dissolved in DMF (4.0 mL) and DIEA (356 mg, 2.76 mmol) was added. A solution of 2-(*L*)-Methoxycarbonylamino-3-methyl-butyric acid **611** (242 mg, 1.38 mmol), HATU (524 mg, 1.38 mmol) and DIEA (178 mg, 1.38 mmol) in DMF (1 mL) was added. The reaction was stirred at room temperature. After 50 minutes, the reaction was diluted with EtOAc and was washed with aqueous bicarbonate solution, aqueous LiCl solution (5%), brine, and was dried over sodium sulfate. Filtration and removal of solvents *in vacuo* gave the crude material, which was purified by silica gel chromatography (eluent: EtOAc / hexanes) to yield the slightly impure compound **612** (878 mg). LCMS-ESI⁺: calc'd for C₂₉H₂₉BrF₂N₄O₃: 599.5 (M⁺); Found: 598.5 / 600.5 (M+H⁺).

i. Preparation of 3-[6-(9,9-Difluoro-7-{2-[5-(2-methoxycarbonylamino-3-methylbutyryl)-5-aza-spiro[2.4]hept-6-yl]-3H-imidazol-4-yl]-9H-fluoren-2-yl)-1H-benzoimidazol-2-yl]-2-aza-bicyclo[2.2.1]heptane-2-carboxylic acid tert-butyl ester **614**.
(1-{6-[5-(7-Bromo-9,9-difluoro-9H-fluoren-2-yl)-1H-imidazol-2-yl]-5-aza-spiro[2.4]heptane-5-carbonyl}-2-methyl-propyl)-carbamic acid methyl ester **612** (840 mg, 1.4 mmol), 3-[6-(4,4,5,5-Tetramethyl-[1,3,2]dioxaborolan-2-yl)-1H-benzoimidazol-2-yl]-2-aza-bicyclo[2.2.1]heptane-2-carboxylic acid tert-butyl ester **613** (615 mg, 1.4 mmol), Pd(PPh₃)₄ (161 mg, 0.14 mmol), K₂CO₃ (579 mg, 4.2 mmol), were dissolved in DME (15 mL) / water (3 mL) under an argon atmosphere. The mixture was heated for 120 minutes at 85 – 90 °C (oil bath). After 120 minutes additional boronate ester (61 mg, 0.14 mmol) was added and heating was continued. After 3 hours, the reaction was cooled to room temperature. Most of the DME was removed *in vacuo* and the crude reaction mixture was diluted with EtOAc. The mixture was washed with brine and was dried over sodium sulfate. Filtration and evaporation of solvents gave the crude reaction product, which was purified via silica gel chromatography (eluent: EtOAc / hexanes) to yield compound **614** (878 mg). LCMS-ESI⁺: calc'd for C₄₇H₅₁F₂N₇O₅: 831.9 (M⁺); Found: 832.7 (M+H⁺).

The intermediate compound **613** can be prepared as follows



j. Preparation of 3-(2-Amino-4-bromo-phenylcarbamoyl)-2-aza-bicyclo[2.2.1]heptane-2-carboxylic acid *tert*-butyl ester **617**.

5 To a solution of 2-Aza-bicyclo[2.2.1]heptane-2,3-dicarboxylic acid 2-*tert*-butyl ester **616** (0.327 g, 1.36 mmol, 1 eq.), 4-Bromo-benzene-1,2-diamine **615** (0.507 g, 2.71 mmol, 2 eq.) and 4-methylmorpholine (0.299 mL, 2 eq.) in 10 mL DMF was added HATU (0.543g, 1.05 eq.). The reaction mixture was stirred at room temperature for 1 hour then concentrated. The reaction mixture was diluted with ethyl acetate and
 10 washed with diluted NaHCO₃ aqueous solution and brine. The organic layer was concentrated down and purified by flash column chromatography (silica gel, 20 to 80% ethyl acetate/hexane) to give a mixture of regioisomer 3-(2-Amino-4-bromo-phenylcarbamoyl)-2-aza-bicyclo[2.2.1]heptane-2-carboxylic acid *tert*-butyl ester **617**.

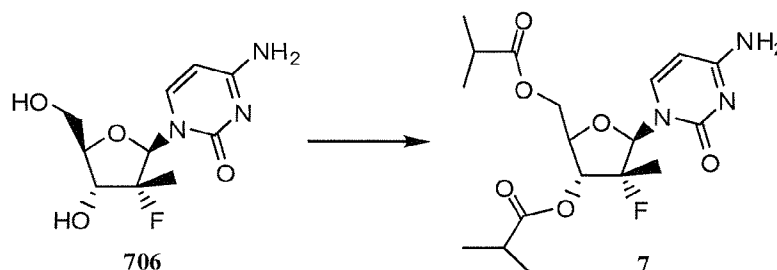
15 k. Preparation of 3-(6-Bromo-1H-benzimidazol-2-yl)-2-aza-bicyclo[2.2.1]heptane-2-carboxylic acid *tert*-butyl ester **618**.

The above mixture of regioisomer 3-(2-Amino-4-bromo-phenylcarbamoyl)-2-aza-bicyclo[2.2.1]heptane-2-carboxylic acid *tert*-butyl ester **617** was dissolved in ethanol and heated to 130°C in sealed tube overnight and continue heating at 170°C
 20 for 3 days. LC-MS showed desired product and Boc cleaved product (about 1:1 ratio). The mixture was concentrated down and dissolved HCL. Di-*tert*-butyl dicarbonate (0.6 eq.) was added and reaction was stirred overnight at room temperature. The reaction mixture was concentrated down and purified by flash column chromatography (silica gel, 20 to 80% ethyl acetate/hexane) to give 3-(6-Bromo-1H-benzimidazol-2-yl)-2-aza-bicyclo[2.2.1]heptane-2-carboxylic acid *tert*-butyl ester **618** (0.383 g, 72%) as an
 25 orange foam.

I. Preparation of Compound **613**.

A mixture of 3-(6-Bromo-1H-benzimidazol-2-yl)-2-aza-bicyclo[2.2.1]heptane-2-carboxylic acid *tert*-butyl ester **618** (264 mg, 0.673 mmol), benzene-1,4-diboronic acid dipinacol ester (5 eq., 3.36 g, 6.95 mmol), tetrakis(triphenylphosphine)palladium (5%, 39 mg) and 2M potassium carbonate aqueous solution (3 eq., 1.01 mL) in 5 mL DME was heated to 90°C under Ar for 4 hours. The reaction mixture was cooled and diluted in ethyl acetate and washed with saturated sodium bicarbonate solution. The organic layer dried (MgSO₄), concentrated and purified by flash column chromatography (silica gel, 20 to 60% ethyl acetate/hexane) to give 3-{6-[4-(4,4,5,5-Tetramethyl-
[1,3,2]dioxaborolan-2-yl)-phenyl]-1H-benzimidazol-2-yl}-2-aza-bicyclo[2.2.1]heptane-2-carboxylic acid *tert*-butyl ester **613** (295 mg, yield 85%). LCMS-ESI: calc'd for C₃₀H₃₈BN₃O₄: 515.45; Found: 516.1 (M+H⁺).

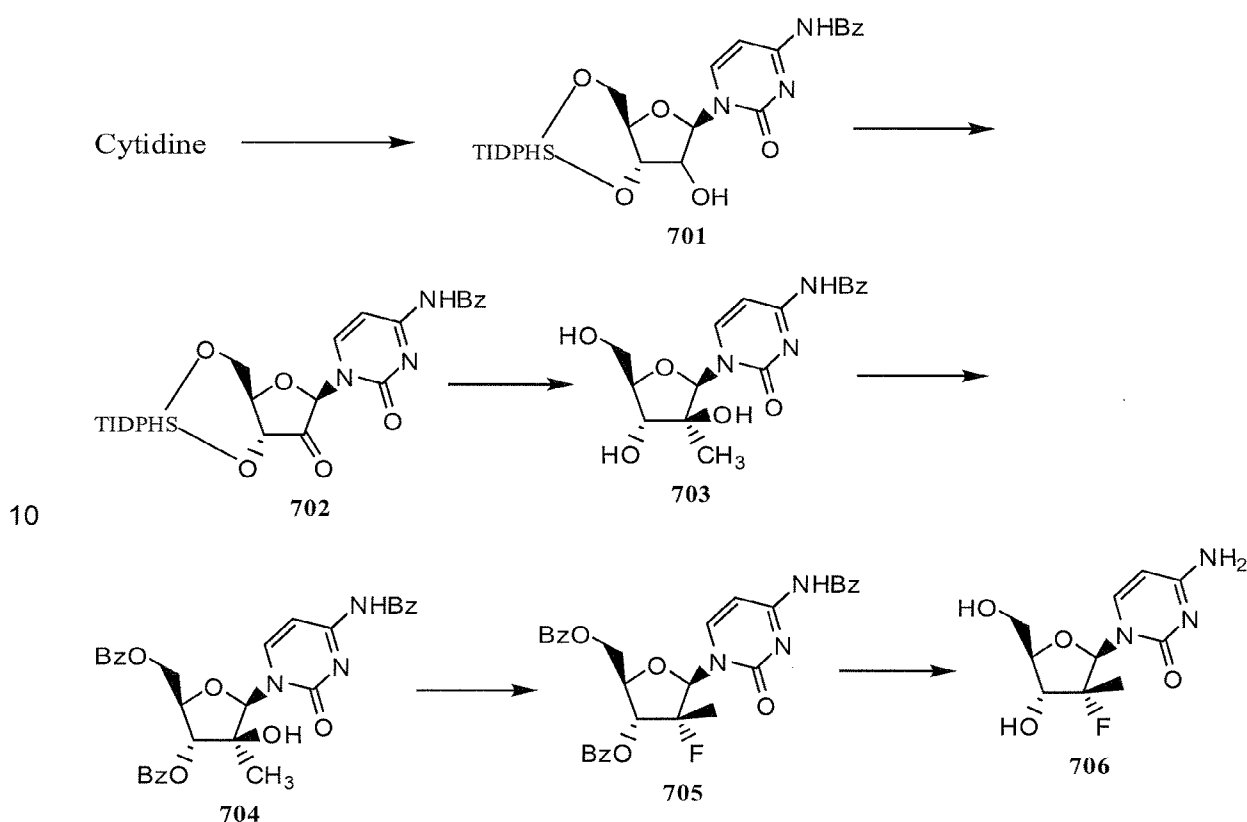
Compound **7** can be prepared using synthetic methods and intermediates like those described in US 7,429,572. Compound **7** can also be prepared as described in the following Example.

Example 7: Preparation of Compound **7**.

To an ice-cold suspension of compound 701 (970 g, 3.74 mol) and DMAP (50 g, 0.412 mol) in THF (10 L) is added TEA (2.3 kg, 16.5 mol) and water (7 L) which produces a clear solution. Isobutyryl chloride (3 equivalents) is added slowly to the stirred mixture while maintaining the temperature at about 0° C. An additional 1.2 then 0.7 equivalents of isobutyl chloride is added until the HPLC indicates the reaction had proceeded essentially to completion (a total of about 1.95 kg). The reaction mixture is acidified with concentrated HCl to a pH of about 6.4 and the organic phase is washed with EtOAc (2 x 10 L). The combined extracts are washed with water (1 x 15 L). The organic phase is filtered and concentrated *in vacuo*. The residue is dissolved in IPA (ca. 20 kg) and heptane (14.2 kg) is added. The solution is heated to about 74-75°C to produce a clear solution, then about 5L is removed by distillation. The resulting

solution is cooled slowly to RT. A precipitate is formed at about 42-43° C. Cooling is continued slowly to 5°C then stirred overnight. The resulting solid is filtered and the filtrate is washed with IPA/heptane (1:8) mixture (13.4 kg), and dried under vacuum at about 60- 70°C to afford 1.295 kg (86.65%) of compound **7** which is 99.45% pure by HPLC.

The intermediate compound **706** can be prepared as follows.



a. Preparation of compound **701**.

To a suspension of cytidine (100 g, 0.411 mol) in DMF (2.06 L) is added benzoic anhydride (102.4 g, 0.452 mol). The mixture was stirred at room temperature for 20 hours. The DMF was removed *in vacuo* and the residue was triturated with diethyl ether. The resulting solid was collected by suction filtration and washed with diethyl ether (2 x 200 mL). Further drying *in vacuo* at room temperature gave the *N*⁴ benzamide (140.6 g, 98.3%). A portion of this material (139.3 g, 0.401 mol) was dissolved in anhydrous pyridine (1.2 L) and was treated with 1,3-dichloro-1,1,3,3-

tetraisopropyl-disiloxane (141.4 mL, 0.441 mol) at room temperature. The solution was stirred at room temperature overnight. The mixture was concentrated to near dryness *in vacuo* and coevaporated with toluene (3 x 200 mL). The residue was treated with EtOAc (1.8 L) and washed with HCl (2 x 200 mL, 0.05 N), NaHCO₃ (5 %, 2 x 400 mL). The organic layer was washed dried (Na₂SO₄), filtered, and evaporated to dryness. Compound **701** (256.5 g, >100%) was isolated as a white foam and used without further purification.

b. Preparation of compound **702**.

Compound **701** (236.5 g, 0.40 mol) was dissolved in dry THF (1.22 L). Anhydrous DMSO (180.8 mL, 2.1 mol) was added and the resulting solution was cooled to between -20°C and -15°C. Trifluoroacetic anhydride (90.6 mL, 0.64 mol) was added dropwise over 45 minutes and the solution was stirred between -20°C and -15 °C for 2 hrs after which anhydrous triethylamine (223.5 mL, 1.6 mol) was added over 20 minutes. The crude reaction containing ketone **702** was dissolved in EtOAc (500 mL), and the resulting solution was washed with H₂O (3 x 400 mL), dried (Na₂SO₄) and the solvents were removed *in vacuo* to give a yellow solid that was purified on a silica gel column eluting with a stepwise gradient of Et₂O (0-60%) in hexanes followed by a stepwise gradient of EtOAc (50-100%) in hexanes. The crude ketone so-obtained (~192 g) was crystallized from petroleum ether to give ketone **702** (138.91 g, 57.5% from cytidine) as a white solid and 22 g of unreacted starting material, **701**, as a yellow solid.

c. Preparation of compound **703**.

Compound **702** (48.57 g, 8.26 mmol) was dissolved in anhydrous toluene (~400 mL) and the solvent was removed *in vacuo* with exclusion of moisture. The residue was then further dried *in vacuo* (oil pump) for another 2 hours. With strict exclusion of moisture, the residual foam was dissolved in anhydrous diethyl ether (1.03 L) under argon. The resulting solution was cooled to -78°C under argon and MeLi (1.6 M, 258.0 mL, 0.413 mol) was added dropwise *via* additional funnel. After the addition was complete, the mixture was stirred for 2 hours at -78°C. Aqueous 1M NH₄Cl (500 mL) was added slowly. After warming to room temperature, the mixture was washed with H₂O (2 x 500 mL), dried (Na₂SO₄), and then concentrated to dryness to give a brown foam (~60 g, >100%).

The reaction was performed two more times using 37.62 g and 56.4 g of compound **702**. The combined crude products (128.0 g, 0.212 mol) were dissolved in THF (1.28 L) and treated with concd HOAc (23 mL, 0.402 mol). To the solution was added TBAF (384.0 mL, 1 M in THF). The solution was stirred at room temperature for 5 0.75 hours and the mixture was treated with silica gel (750 g) and concentrated to dryness. The powder was placed on a silica gel column packed in CH₂Cl₂. Elution with 1:7 EtOH-CH₂Cl₂ afforded a dark waxy solid that was pre-adsorbed on silica gel (300 g) and chromatographed as before. Compound **703** (46.4 g, 53.0 % from **702**) was isolated as an off-white solid. ¹H NMR (DMSO-d₆): δ 1.20 (s, 3H, CH₃), 3.62-3.69 (m, 10 2H,), 3.73-3.78 (m, 2H,), 5.19 (t, 1H, *J* = 5.4 Hz, OH-5'), 5.25 (s, 1H, OH-2'), 5.52 (d, 1H, *J* = 5.0 Hz, OH-3'), 5.99 (s, 1H, H-1'), 7.32 (d, 1H, *J* = 5.8 Hz), 7.50 (Ψt, 2H, *J* = 7.7 Hz), 7.62 (Ψ, 1H, *J* = 7.3 Hz), 8.00 (d, 2H, *J* = 7.3 Hz), 8.14 (d, 1H, *J* = 6.9 Hz), 11.22 (s, 1H, NH). Anal. Calcd for C₁₇H₁₉N₃O₆ • 0.5 H₂O: C, 55.13; H, 5.44; N, 11.35. Found: C, 55.21; H, 5.47; N, 11.33.

15

d. Preparation of compound **704**.

Compound **703** (46.0 g, 0.13 mol) was dissolved in anhydrous pyridine and concentrated to dryness *in vacuo*. The resulting syrup was dissolved in anhydrous pyridine under argon and cooled to 0°C with stirring. The brown solution was treated 20 with benzoyl chloride (30mL, 0.250 mol) dropwise over 10 minutes. The ice bath was removed and stirring continued for 1.5 hours whereby TLC showed no remaining starting material. The mixture was quenched by the addition of water (5 mL) and concentrated to dryness. The residue was dissolved in a minimal amount of CH₂Cl₂ and washed with satd NaHCO₃ (1 x 500 mL) and H₂O (1 x 500 mL). The organic 25 phase was dried (Na₂SO₄) and filtered, concentrated to dryness and chromatographed on silica gel eluting with a stepwise gradient of EtOAc-hexanes (25-60%) to provide compound **704** as yellow foam (48.5 g, 67%). ¹H NMR (CDCl₃): δ 1.64 (s, 3H, CH₃), 4.50 (m, 1H, H-4), 4.78-4.85 (m, 2H, H-5',5a'), 5.50 (d, 1H, *J* = 3.4 Hz, H-3'), 6.42 (s, 1H, H-1'), 7.44-7.54 (m, 7H, Ar), 7.57-7.66 (m, 3H, Ar), 7.94 (d, 2H, *J* = 7.8 Hz), 8.05- 30 8.09 (m, 4H, Ar), 8.21 (d, 1H, *J* = 7.3 Hz). Anal. Calcd for C₃₁H₂₇NO₈: C, 65.37; H, 4.78; N, 7.38. Found: C, 65.59; H, 4.79; N, 7.16.

e. Preparation of compound **705**.

Compound **704** (7.50 g, 0.013 mol) was dissolved in anhydrous toluene (150 35 mL) under argon and cooled to -20°C. DAST (2.5 mL, 18.9 mmol) was added slowly

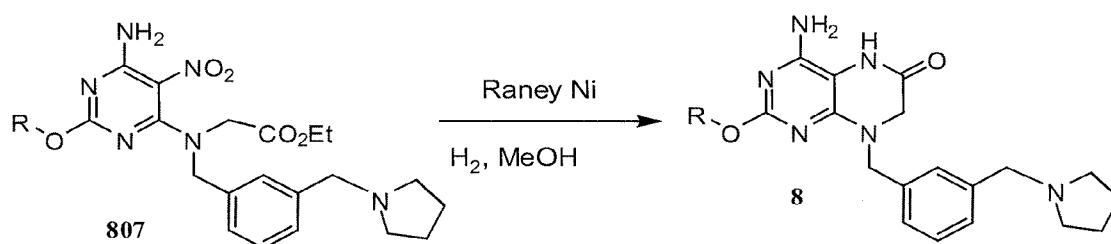
and the cooling bath was removed after the addition was complete. Stirring was continued for 1 hours and the mixture was poured into satd NaHCO_3 (100 mL) and washed until gas evolution ceased. The organic phase was dried (Na_2SO_4), concentrated, and purified by silica gel chromatography eluting with 1:1 EtOAc-hexanes. Yield was 1.22 g (16.3%) of pure **705** as a white solid. mp 241°C (CH_2Cl_2 -hexanes); ^1H NMR (CDCl_3): δ 1.49 (d, 3H, $J=22.4$ Hz, CH_3), 4.64 (dd, 1H, $J=3.44$, 12.9 Hz, H-5'), 4.73 (d, 1H, $J=9.5$ Hz, H-4'), 4.90 (dd, 1H, $J=2.4$, 12.7 Hz, H-5a'), 5.56 (dd, 1H, $J=8.6$, 20.7 Hz, H-3'), 6.52 (d, 1H, $J=18.0$ Hz, H-1'), 7.47-7.57 (m, 7H, Ar), 7.62-7.71 (m, 3H, Ar), 7.89 (d, 2H, $J=6.9$ Hz), 8.07-8.11 (m, 5H, Ar), 8.67 (bs, 1H, NH). ^{19}F NMR (CDCl_3): δ 3.3 (m). Anal. Calcd for $\text{C}_{31}\text{H}_{26}\text{FN}_3\text{O}_7 \cdot 0.7 \text{H}_2\text{O}$: C, 63.74; H, 4.72; N, 7.20. Found: C, 63.71; H, 4.54; N, 7.20.

f. Preparation of compound **706**.

Compound **705** (6.30 g, 0.011 mol) was suspended in methanolic ammonia (ca 7 N, 150 mL) and stirred at room temperature overnight. The solvent was removed *in vacuo*, co-evaporated with methanol (1 x 20 mL), and pre-adsorbed onto silica gel. The white powder was placed onto a silica gel column (packed in CHCl_3) and the column was eluted with 9% EtOH in CHCl_3 , then 17% EtOH and finally 25% EtOH in CHCl_3 . Concentration of the fractions containing the product, filtration through a 0.4 μm disk, and lyophilization from water afforded compound **706**, 2.18 g (76%). ^1H NMR ($\text{DMSO}-d_6$): δ 1.17 (d, 3H, $J=22.3$ Hz, CH_3), 3.63 (dd, 1H, $J=2.7$, 13.7 Hz, H-5'), 3.70-3.84 (m, 3H, H-3', H-4', H-5a'), 5.24 (app s, 1H, OH-3'), 5.60 (d, 1H, $J=5.4$ Hz, H-5'), 5.74 (d, 1H, $J=7.71$ Hz, H-5), 6.07 (d, 1H, $J=18.9$ Hz, H-1'), 7.31 (s, 1H, NH₂), 7.42 (s, 1H, NH₂), 7.90 (d, 1H, $J=7.3$ Hz, H-6). ^{19}F NMR ($\text{DMSO}-d_6$): δ 2.60 (m). Anal. Calcd for $\text{C}_{10}\text{H}_{14}\text{FN}_3\text{O}_4 \cdot 1.4 \text{H}_2\text{O}$: C, 44.22; H, 5.95; N, 14.77. Found: C, 42.24; H, 5.63; N, 14.54. Compound **706** (0.10 g, 0.386 mmol) was converted to the hydrochloride salt by dissolving in water (2 mL) and adjusting the pH to approximately 3.0 with 1 M HCl. The water was removed *in vacuo* and the residue was crystallized from aqueous EtOH to give Compound **706** as the hydrochloride salt (71.0 mg). mp 243°C (dec); ^1H NMR ($\text{DMSO}-d_6$): δ 1.29 (d, 3H, $J=22.6$ Hz, CH_3), 3.65 (dd, 1H, $J=2.3$, 12.7 Hz, H-5'), 3.76-3.90 (m, 3H, H-3', H-4', H-5a'), 5.96 (d, 1H, $J=17.3$ Hz, H-1'), 6.15 (d, 1H, $J=7.9$ Hz, H-5), 8.33 (d, 1H, $J=7.9$ Hz, H-6), 8.69 (s, 1.5H, NH), 9.78 (s, 1.5H, NH). ^{19}F NMR ($\text{DMSO}-d_6$): δ 1.69 (m). Anal. Calcd for $\text{C}_{10}\text{H}_{14}\text{FN}_3\text{O}_4 \cdot \text{HCl}$: C, 40.62; H, 5.11; N, 14.21. Found: C, 40.80; H, 5.09; N, 14.23.

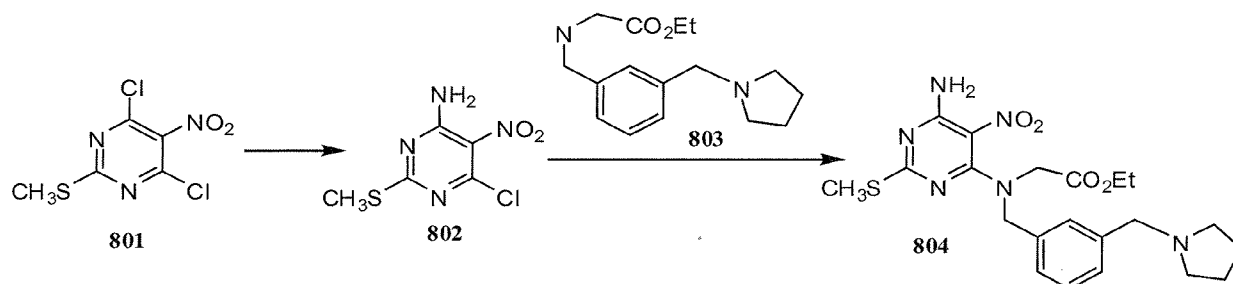
Compound **8** can be prepared using synthetic methods and intermediates like those described in USSN 12/632,194. Compound **8** can also be prepared as described in the following Example.

- 5 **Example 8:** Preparation of 4-amino-2-*n*-butoxy-8-[3'-(pyrrolidin-1"-ylmethyl)-benzyl]-5,6,7,8-tetrahydropteridin-6-one **8**. (R = *n*-butyl)



- 10 To a solution of nitro compound **807** (730 mg, 1.5 mmol) in MeOH (10 mL) was added a Raney Nickel (~200 μ L, slurry in H₂O). The reaction vessel was flushed with H₂ and then stirred under an H₂ atmosphere for 1.5 hours. The mixture was filtered through celite with CH₂Cl₂ and MeOH (1:1). The filtrate was concentrated under vacuum and left on lyophilizer overnight. The free base of compound **8** was obtained
- 15 as a white solid. To obtain the HCl salt of **8**, a sample of the filtrate above was spiked with 1.0 M HCl to pH = 1-2 and lyophilized. ¹H NMR (CD₃OD, 300 MHz): δ 7.65 (s, 1H), 7.50 (m, 3H), 4.96 (s, 2H), 4.44 (t, *J* = 7 Hz, 2H), 4.40 (s, 2H), 4.16 (s, 2H), 3.48 (m, 2H), 3.19 (m, 2H), 2.02-2.17 (m, 4H), 1.74 (m, 2H), 1.45 (m, 2H), 0.94 (t, *J* = 7 Hz, 3H) - [HCl salt]. LCMS-ESI⁺: calc'd for C₂₂H₃₁N₆O₂: 411.5 (M+H⁺); Found: 411.3
- 20 (M+H⁺).

The intermediate compound **807** was prepared as follows.

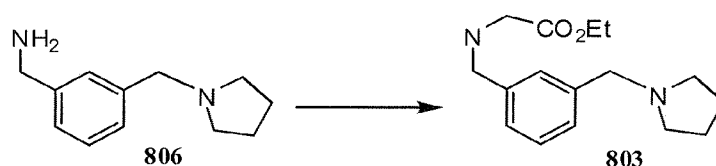


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- a. Preparation of compound **802**.

To a solution of compound **801** (2.46 g, 10.2 mmol) in THF (34 mL) at $-20\text{ }^{\circ}\text{C}$ was added Et_3N (3.14 mL, 22.5 mmol) followed by a solution of NH_3 (2.0 M in MeOH, 5.4 mL, 11 mmol). The mixture was stirred while warming to $0\text{ }^{\circ}\text{C}$ for 1.5 h (LC/MS indicated consumption of starting materials). The reaction mixture containing
 5 compound **802** was taken forward without work-up.

b. Preparation of compound **803**.



10 To a solution of 3-((1-pyrrolidin-1-yl)methyl)phenylmethanamine **806** (1.95 g, 10.2 mmol) in THF (34 mL) at $0\text{ }^{\circ}\text{C}$ was added Et_3N (3.14 mmol, 22.5 mmol) followed by methyl bromoacetate (1.04 mL, 22.3 mmol) dropwise. The reaction mixture was stirred until LC/MS indicated consumption of starting materials, approximately 2 hours. The mixture containing compound **803** was taken forward without work up.

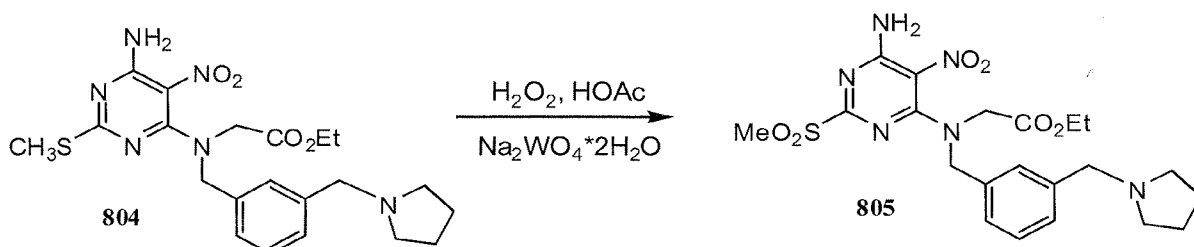
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c. Preparation of compound **804**.

The reaction mixture containing compound **803** was added to the reaction mixture containing compound **802** at $0\text{ }^{\circ}\text{C}$. The reaction mixture was stirred until LC/MS indicated the consumption of compound **802**, approximately 45 minutes. A
 20 saturated solution of NH_4Cl (50 mL) was added. The layers were separated, and the aqueous layer was extracted with EtOAc (2 x 30 mL). The combined organic layers were dried over MgSO_4 , filtered, and concentrated under vacuum. Purification by silica gel chromatography provided 2.11 g of compound **804**. ^1H NMR (CD_3OD , 300 MHz): δ (ppm) 7.32-7.16 (m, 4H), 4.69 (s, 2H), 4.19 (q, $J = 7\text{ Hz}$, 2H), 4.07 (s, 2H), 3.60 (s,
 25 2H), 2.49 (m, 4H), 2.40 (s, 3H), 1.78 (m, 4H), 1.23 (t, 3H, $J = 7\text{ Hz}$). LCMS-ESI $^+$: calc'd for $\text{C}_{21}\text{H}_{29}\text{N}_6\text{O}_4\text{S}$: 461.2 ($\text{M}+\text{H}^+$); Found: 461.0 ($\text{M}+\text{H}^+$).

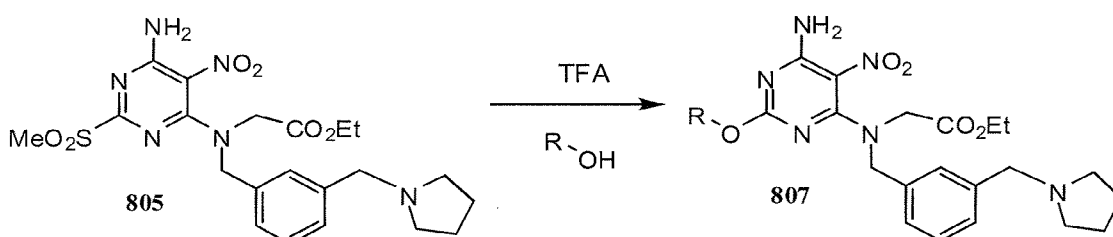
d. Preparation of Ethyl- N_α -[4-amino-2-methanesulfonyl-5-nitropyrimidin-6-yl], N_α -[3'-(pyrrolidin-1'-ylmethyl)-benzyl]-glycinate **805**.

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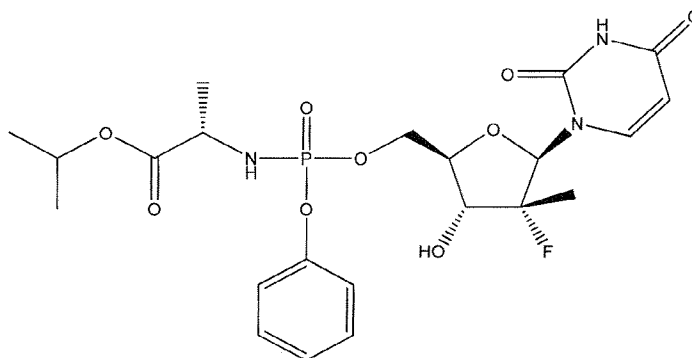
To a solution a suspension of the sulfide **804** (3.68 g, 8.00 mmol) in EtOH (40 mL) at 0 °C was added sodium tungstate dihydrate (792 mg, 2.40 mmol), acetic acid (4.6 mL, 80 mmol), and hydrogen peroxide (3.4 mL, ~40 mmol, 35% w/w in H₂O) sequentially. After 3 hours, additional acetic acid (4.6 mL) and hydrogen peroxide (3.4 mL) were added. The reaction was maintained at 0 °C for 16 hours. A saturated solution of Na₂SO₃ (50 mL) was added carefully while at 0 °C followed by CH₂Cl₂ (75 mL). The layers were separated, and the aqueous layer was extracted with CH₂Cl₂ (4 x 50 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated under vacuum to provide a material containing compound **805** that was used without further purification.

e. Preparation of Compound **807**. (R = n-butyl)



To a solution of sulfone **805** (1.0 g, 2.0 mmol) in n-butanol (10 mL) was added TFA (470 μL, 6.1 mmol). The reaction was stirred at 100 °C for 1 hour. The reaction mixture was poured onto a saturated solution of NaHCO₃ (20 mL) and CH₂Cl₂ (30 mL). The layers were separated, and the aqueous layer was extracted with CH₂Cl₂ (30 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated under vacuum. Purification was conducted by silica gel chromatography (1 g substrate/10 g SiO₂) (2-15% MeOH/CH₂Cl₂) to provide compound **807**.

Preparation of Compound 9 (from US2010/0298257)



5

9

Preparation of (S)-2-[[[(1R,4R,5R)-5-(2,4-Dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-4-(R)-fluoro-3-hydroxy-4-methyl-tetrahydro-furan-2-yl-methoxy]-phenoxyphosphorylamino]-propionic acid isopropyl ester (from US2010/0298257 Example 2). Synonym: 5'-O-(Isopropyl-L-alanate, phenyl phosphoramidyl)-2'-deoxy-2'-fluoro-2'-C-methyl-uridine diastereomeric mixture.

A 5 L 3-necked flask was fitted with a mechanical stirrer, brine ice bath, internal thermometer, and a nitrogen atmosphere. The flask was charged with L-alanine isopropyl ester hydrochloride (82.0 g, 0.490 moles) and anhydrous dichloromethane (0.80 L). While this was stirring, phenyl dichlorophosphate (85.0 g, 0.40 moles) was added in one lot and stirred. While maintaining the internal temperature between -5 to 5 °C., a solution of N-methylimidazole (NMI, 250 g, 3.07 moles) in dichloromethane (250 mL) was added over a period of a half hour. The solution was allowed to stir for 1 h in this temperature range. 2'-Deoxy-2'-fluoro-2'-C-methyl-uridine (3,80.0 g, 0.307 moles) was added at 0° C. in one portion and then the reaction flask was allowed to warm up slowly in the brine bath. At 1 h, the internal temperature was up to -2° C. TLC (5% Methanol in HCL) at 1 h showed that more than 50% of nucleoside was consumed. The bath was removed and the reaction flask reached ambient temperature over 1 h more. TLC after 3 h and at 5 h total showed 95% of the starting nucleoside was consumed. The reaction mixture was quenched by adding methanol (100 mL) and stirring the reaction for 5 minutes.

The reaction mixture was washed with 1N HCl (2x500 mL) followed by saturated sodium bicarbonate solution (2x500 mL). The separated organic layer was

dried over anhydrous sodium sulfate (50 g) and filtered. The solution was evaporated under reduced pressure and then under high vacuum to dryness to give the crude product as a viscous oil (170 g). NMRs of the crude product (^{31}P and ^1H) were taken. The ^{31}P -NMR indicated about 1% of the total phosphorus integration was due to the presence of the 3' isomer 5.

To the crude product was added anhydrous pyridine (1700 mL). The solvent was evaporated under reduced pressure and then under high vacuum in order to reduce the water content of the crude mixture through co-evaporation. The resulting oil was re-dissolved in anhydrous pyridine (500 ml) and then was added excess t-butyltrimethylsilyl chloride (9.0 g, 60 mM). The reaction was stirred at ambient temperature. Reaction progress was monitored by UPLC/MS. After 3 hours, the 3' impurity 5 could no longer be detected and the reaction was quenched by the addition of methanol (50 mL).

The reaction was evaporated under reduced pressure to an oil. The residue was dissolved in ethyl acetate (1.5 L) and washed with 1N HCl (2x500 mL), followed by saturated sodium bicarbonate solution (2x500 mL). The organic layer was dried over anhydrous sodium sulfate (50 g), filtered and evaporated under reduced pressure to give the crude product as a pale yellow oil.

The crude oil was diluted with the same volume of dichloromethane and loaded onto a 2.5 Kg silica gel cartridge in a radial compression module at 100 psi of air pressure. Using a gradient pump at 60 psi and a flow rate of 400 ml/min, the cartridge was washed with methylene chloride (4L) followed by a gradient 1-4% methanol in methylene chloride (48 L). Most of the major impurities (di-(isopropylalanyl) phenyl phosphate, 3',5'-bis phosphoramidate, 3'-phosphoramidate-5'-TBDMS adduct (7)) eluted with ~3% gradient. The desired product eluted between 3 and 4% methanol. The product containing fractions were sorted into two lots. The first contained small amounts of upper impurities and the latter was pure product. The first set of fractions contained small amounts of less polar impurities (upper impurities) such as the 3',5'-bis phosphoramidate and the di-alanylphenyl phosphate and a mostly the *Rp* diastereomer and required a second column purification. (The relative terminology, upper vs. lower refers to the elution on normal phase silica-gel chromatography, where the "upper isomer" means the first eluting isomer.) The second set of fractions did not have a significant amount of impurities-just the remaining *Rp* and mostly the *Sp* diastereomers. It was later recombined with the twice-columned fractions. The solvent was evaporated under reduced pressure and the resulting white foam was

further dried (0.20mmHg) for 1 h to give 42 g of the impure lot (4:1 upper vs lower isomer based on ^{31}P -NMR) and 38 g of the pure lot (1:3 upper vs lower isomer). The impure lot was recolumned in a similar manner to give 3.8 g of 97% pure upper isomer (fraction set aside) and 36 g of pure product in a 4: 1 ratio. The two main lots were
 5 dissolved in HCL, combined, evaporated under reduced pressure and dried (50° C., 0.2 mmHg, 24 h) to get 74 g (45.7%) of pure product (Compound 9) with a diastereomeric ratio of 48:51, as a white foam, mp about 75-85° C.

In order to produce an amorphous solid of the diastereomeric mixture, 74 g of the white foam was stirred in with t-butyl methyl ether (750 mL) resulting in a partial
 10 solution and a gummy solid residue. While stirring, heptanes (750 mL) was added slowly and the suspension was mechanically stirred for 1 hour until most of the gum was converted to a white solid. The solid was scraped up with a spatula and the resulting slurry was filtered. The solid was washed with heptanes (4x50mL) and dried under vacuum (50° C., 0.2mmHg, 24 h) to give a white, amorphous powder (64 g) with
 15 a broad melting range of ca 70-80° C. ^1H and ^{31}P NMR conformed to structure and HPLC showed a purity of 99.8% with a diastereomeric ratio of 46:54 (also confirmed by ^{31}P NMR).

Alternative method to make a solid mixture of Compound 9. After chromatography, the residue was co-evaporated with dichloromethane twice (5 mL/g) and dried for 24 h at 35-40° C. at 35-45 mTorr. The foam residue was sieved through a
 20 250 micron screen and further dried under the same conditions until the residual dichloromethane fell below 400 ppm as measured by headspace GC. The resulting fine off-white to white amorphous powder has a glass transition temperature range of 53.7 to 63.5° C.

25 Characterization of Compound 9 (mixture of isomers):

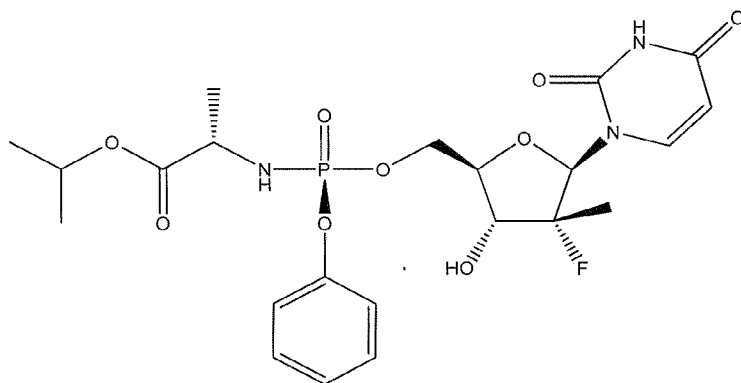
^1H -NMR (CDCl_3) 010.05 (brs, 1H, NH, *Sp*), 10.00 (brs, 1H, NH, *Rp*), 7.49 (d, 1H, C6-H, *Sp*), 7.36 (m, 5H, C6-H, *Rp*, aromatic), 7.23-7.14 (m, 6H, *Rp/Sp*, aromatic), 6.18 (br d, 2H, Cl'-H, *Rp/Sp*), 5.63 (d, 1H, C5-H, *Sp*), 5.58 (d, 1H, C5-H, *Rp*), 5.01 (m, 2H, CH-(CH₃)₂ *Rp/Sp*), 4.46-4.33 (m, 8H, C-5'-H₂, ala-NH, C3'-OH, *Rp/Sp*), 4.12 (m, 2H, ala-
 30 CHCH₃, *Rp/Sp*), 4.01-3.85 (m, 4H, C3'-H, C4'-H, *Rp/Sp*), 1.391.22 (m, 12H, all CH₃, *Rp/Sp*).

^{31}P -NMR (CDCl_3) 03.60 (*Rp*), 3.20 *Sp* relative to triphenylphosphate at -17.80 ppm. ES-MS M+1 530.2. Elemental Analysis: Calculated % (including 0.29% water as found by Karl Fisher analysis) C, 49.75; H, 5.54; N, 7.90; F, 3.58, P, 5.84. Found %: C,
 35 49.50; H, 5.44; N, 7.85; F, 3.62; P, 6.05.

Preparation of 2'-deoxy-2'-fluoro-2'-C-methyluridine (from US2010/0298257 Example 1)

In a 10 L flask, was added 3', 5'-O-dibenzozy-2'-deoxy-2'-fluoro-2'-C-methyl-N4-benzoylcytidine (500 g, 0.874 mol) and 70% aqueous acetic acid (7.5 L). The solution was heated to reflux (110° C.) for 20 h. TLC indicated a complete reaction (Rf 0.6 in 5% methanol in dichloromethane (HCL)). The mixture was cooled to ambient temperature and diluted with water (2 L). After stirring for 2 h, the resulting precipitate was collected by filtration and the solid was rinsed with water (5 L) and dried in the atmosphere at ambient temperature for 12 h to afford 360 g (88%). This dibenzoyluridine intermediate was used directly in the next step by adding it all to freshly prepared methanolic ammonia (5.4 L, ca 25%) at 0° C. This temperature was maintained for 3 h and then allowed to warm to 15° C. for 24 h. TLC indicated a complete reaction (Rf 0.4 in 10% methanol in HCL). The reaction mixture was filtered through a Celite bed and concentrated under reduced pressure to give the crude product (216 g). The crude product was stirred with ethyl acetate (325 mL) for 3 h at ambient temperature. The resulting solid was collected by filtration and washed with ethyl acetate (216 mL). The solid was dried under vacuum at ambient temperature for 4 h to afford 160 g (78%) of the desired product in 98.7% HPLC purity. ¹H-NMR (DMSO-d₆) 011.44 (br s, 1H, NH), 7.95 (d, 1H, C-6H), 5.97 (d, 1H, C-1'H), 5.64 (d, 1H, C-5H), 3.84-3.77 (m, 3H, C-5'-Ha, C-3'H, C-4'H), 3.63-3.60 (m, 1H, C5'-Hb), 1.23 (d, 3H, C-2'-CH₃). ES-MS M-I 259.

Preparation of Compound 10 (from US2010/0298257)



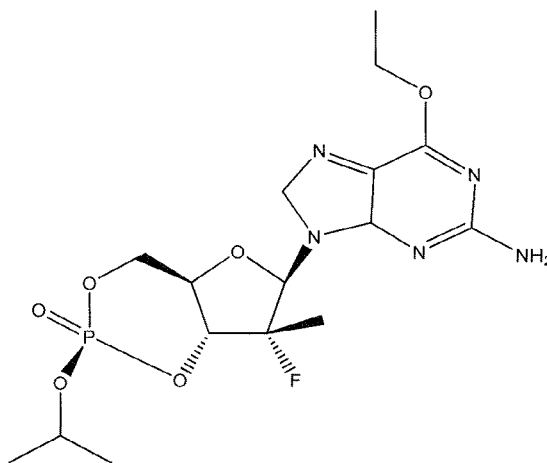
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Direct precipitation of Compound 10 (from US2010/0298257 Example 4): To a stirred solution of L-alanine isopropyl ester hydrochloride (10.5 g, 61.5 mmol, azeotropically dried, two times, with 50 mL of toluene each time) in dichloromethane (100 mL) was added phenyldichlorophosphate (7.5 mL, 50 mmol) at room temperature. The mixture was cooled to -10° C. and then was added a solution of N-Methylimidazole (30.5 mL, 384.3 mmol) in 30 mL of dichloromethane over a period of 30 min. After completion of the addition, the mixture was stirred between -10 and -15° C. for 1 h. To the above mixture was added 2'-deoxy-2'-fluoro-2'- C-methyluridine (10 g, 38.4 mmol) (see US2010/0298257 Example 1) in one lot and the mixture was stirred below -10° C. for 3 h and then slowly allowed to warm to 20° C. (6 h). The mixture was stirred at this temperature overnight (15 h) and then quenched with 10 mL of methanol. The solvent was evaporated and the residue was re-dissolved in EtOAc (200 mL). The EtOAc layer was washed with water (100 mL), 1N HCl (3x75 mL), 2% aqueous NaHCO₃ solution (50 mL) and brine (50 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated. The residue was dried under high vacuum for 2 h to give white foam (22 g).

The above foam was dissolved in 33 mL of HCl and then was added 65 mL of isopropyl ether to give a saturated solution. The solution was filtered through a small pad of Celite and the filtrate was stirred with seeds of Compound 10 for 72 h at ambient temperature (about 22° C.-note that cooling the suspension to 0° C. led to oiling out the crude product). The white solid was filtered, washed with isopropyl ether (20 mL) and dried to give 4.58 g (~85:15 mixture of Compound 10: R isomer at P respectively as determined by ³¹P NMR) of a white powder. The above solid was suspended in 23 mL of HCL and then refluxed for 3 h. The mixture was cooled to room temperature and stirred for 15 h. The white solid was filtered, washed with 4.5 mL of cold HCl and dried under high vacuum at 45° C. to give pure Compound 10, mp 93.9-104.7° C HPLC purity 99.74% (3.11 g, 15.2% from the uridine nucleoside).

Compound 10: ¹H-NMR (CDCl₃) δ 8.63 (br s, 1H, NH), 7.47 (d, 1H, C6-H), 7.30 (m, 2H, o-aromatic), 7.26-7.18 (m, 3H, m,p-aromatic), 6.18 (br d, 1H, C1'-H), 5.70 (d, 1H, C5-H), 5.02 (sept, CH-(CH₃)₂), 4.53 (m, 2H, C-5'-H₂), 4.11 (d, 1H, C3'-H), 3.97 (m, 3H, C3'-OH, C4'-H, ala-CH-CH₃), 3.77 (br s, 1H, ala-NH), 1.39 (d, 3H, C2'-CH₃), 1.37 (d, 3H, ala-CH₃) 1.24 (d, 6H, CH-(CH₃)₂).

Preparation of Compound 11 (from US 2010/0081628)



11

Synthesis of 6-Ethoxy-9-((4aR,6R,7R,7aR)-7-fluoro-2-isopropoxy-7-methyl-2-oxo-
 5 tetrahydro-2,5-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl)-9H-purin-2-yl-amine (Compound
11) (Compound 19, US 2010/0081628)

(2R,3R,4R,5R)-5-(2-Amino-6-ethoxy-purin-9-yl)-4-fluoro-2-hydroxymethyl-4-methyl-tetrahydro-furan-3-ol (150 mg, 0.46 mmol) was dissolved in anhydrous pyridine (2 ml) at 0° C. A solution of 0.45 M 1H-tetrazole in acetonitrile (2.55 mL) was added
 10 followed by bis (N,N-diisopropylamino) isopropylphosphoramidite (0.16 mL, 0.55 mmol, 1.2 eq). The mixture was allowed to slowly warm to ambient temperature over 3 h. TLC indicated a complete reaction. The reaction was quenched upon the addition of water (0.1 mL). The reaction solution was concentrated under reduced pressure and then the residue was triturated with ethyl acetate (5 mL). The resulting white
 15 precipitate was removed by filtration and the filtrate was concentrated under reduced pressure.

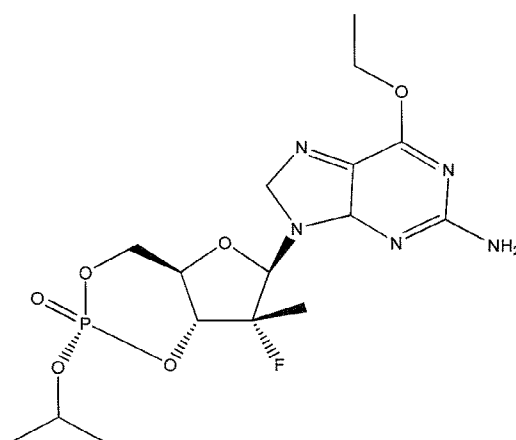
The resulting intermediate cyclic phosphite residue was dissolved in acetonitrile (2 mL) and then treated with t-butyl hydroperoxide (70% in water, 0.19 mL) for 5 h at ambient temperature. TLC indicated a complete reaction. The reaction solution was
 20 concentrated under reduced pressure and the residue was purified by column chromatography (Analogix using a gradient of 0 to 5% IPA in HCL). The two diastereomers (Compound 11 and R-isomer at P) were separable. Fractions containing each diastereomer were separately combined and concentrated under reduced pressure to white solids to give 20 mg of each diastereomer (combined yield
 25 20%).

Compound 11 (S-isomer at P): ^{31}P -NMR (162 MHz, DMSO): δ -6.49;

^1H -NMR (400 MHz, DMSO): δ = 8.17 (s, 1H), 6.47 (bs, 2H), 6.27 (d, J =21.2 Hz, 1H), 4.73-4.62 (m, 4H), 4.45 (q, J =7.0 Hz, 2H), 4.27-4.21 (m, 1H), 1.39-1.34 (m, 9H), 1.20 (d, J =22.8 Hz, 3H).

5 MS (ESI): m/z 432.4 $[\text{M}+\text{H}]^+$

R-isomer at P:



10

^{31}P -NMR (162 MHz, DMSO): δ = -4.68;

^1H -NMR (400 MHz, DMSO): δ = 8.15 (s, 1H), 6.63 (s, 2H), 6.27 (d, J =21.2 Hz, 1H), 4.74-4.58 (m, 4H), 4.45 (q, J =6.4 Hz, 2H), 4.42-4.37 (m, 1H), 1.36 (t, J =7.2 Hz, 3H), 1.32 (d, J =3.6 Hz, 3H), 1.30 (d, J =3.6 Hz, 3H), 1.22 (d, J =22.8 Hz, 3H).

15 MS (ESI): m/z 432.4 $[\text{M}+\text{H}]^+$

Synthesis of (2R,3R,4R,5R)-5-(2-amino-6-ethoxy-9H-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydro-furan-3-ol (Compound 16, US 2010/0081628)

To a 500 mL of dry round-bottomed flask was loaded (2R,3R,4R,5R)-5-(2-amino-6-chloro-9H-purin-9-yl)-2-(benzoyloxymethyl)-4-fluoro-4-methyltetrahydrofuran-3-yl benzoate (11 g, 20.92 mmol). Anhydrous absolute ethanol (210 mL) was added and followed by anhydrous K_2CO_3 (28.91 g, 209.2 mmol). The suspension was stirred and heated at 75° C under nitrogen for 5.5 h. All the starting material was consumed at that time by TLC test. The mixture was cooled to room temperature and solid was filtered out. The filtrate was neutralized by addition of glacial acetic acid (2.52 g) to pH~7 and concentrated under reduced pressure. The residue was dissolved in methanol and mixed with silica gel (15 g). The dried mixture of crude product and silica

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gel was transferred to an empty cartridge and separated through column chromatography (Analogix 220 g, gradient of 0 to 15% MeOH in DCM) to afford product (5% MeOH in DCM) as a white foam solid (3.73 g, 54.5%). A second white solid was isolated from column (10% MeOH in DCM, 1.44 g) and it is a mixture of two dimers of nucleoside. A more polar, third white solid was collected from column (15% MeOH in DCM, 0.47 g) and it is a mixture of trimers of nucleoside. HPLC purity of product 99.94%. ¹H-NMR (DMSO-d₆): δ 8.16 (s, 1H, 8-H), 6.55 (s, 2H, NH₂), 6.04 (d, 1H, C1'-H), 5.66 (d, 1H, 3'-OH), 5.24 (m, 1H, 5'-OH), 4.44 (q, 2H, 6-OCH₂), 4.23-4.08 (m, 1H, C3'-H), 3.91-3.82 (m, 2H, C4'-H and C5'-H_a), 3.71-3.66 (m, 1H, C5'-H_b), 1.36 (t, 3H, CH₃ of ethyl), 1.06 (d, 3H, C2'-CH₃).

Synthesis of (2R,3R,4R,5R)-5-(2-amino-6-chloro-9H-purin-9-yl)-2-(benzoyloxymethyl)-4-fluoro-4-methyltetrahydrofuran-3-yl benzoate (Compound 12, US 2010/0081628)

To a 12 L of three-neck round-bottomed flask was charged 6-chloro-2-aminopurine (225.4 g, 1.329 mol). Anhydrous tert-BuOH (4.5 L) was added and the solution was stirred with a mechanical stirrer at ambient temperature. Potassium tert-butoxide (solid, 151.6 g, 1.35 mol) was added portion-wise under a flow of nitrogen gas while stirring. The mixture was stirred at RT for an additional 30 min. To a 5 L round-bottomed flask was loaded the α-bromide (10, 197 g, 0.451 mol) and 3 L of anhydrous acetonitrile at ambient temperature. The bromide solution was added to the purine base suspension over 1 min at ambient temperature. The 5 L flask was rinsed with acetonitrile (2x1 L) to transfer bromide completely to the reaction mixture. The mixture was heated gradually to 50° C. over 2 h with a heating mantle and controller, and stirred for 20 h. The reaction was almost complete as shown by TLC beta (R_f 0.28, 30% EtOAc in hexanes). The reaction was quenched by the addition of sat. NH₄Cl (200 mL) to form a suspension. The suspended solid was removed by filtration through a 3 cm pad of Celite in a 2.5 L porcelain Buchner funnel. The solid was washed with toluene (3x100 mL). The combined filtrate was neutralized by adding 6 N HCl solution until pH 7 (approx 220 mL). The mixture was concentrated under reduced pressure. When the volume of mixture was reduced to about one-third volume, additional precipitated solid was removed by filtration in a similar manner. The filtrate was further concentrated to a volume of about 800 mL. The residue was loaded onto a plug column (1.6 kg flash grade silica gel in a 6 L sintered glass Buchner funnel) and eluted (via suction) with a gradient of 10% ethyl acetate in hexanes (6 L) to remove non-polar impurities, 30% ethyl acetate in hexanes to afford a small amount of lactol (6 L), and

then 40%-45% ethyl acetate in hexanes (4 L) to elute the main amount of product. The product containing fractions were combined, concentrated under reduced pressure and dried under vacuum (0.2 mmHg, 24 h, ambient temp.) to a white foam solid (150.7 g, $\beta/\alpha=14:1$ by NMR). $^1\text{H-NMR}$. (CDCl_3) β : $\delta=1.33$ (d, 22.4 Hz, 2'-C-CH₃), α :

5 1.55 (d, 22 Hz, 2'-C—CH₃).

The product mixture foam was dissolved in methanol (700 mL) at ambient temperature. Upon standing, a solid slowly formed over 2 h. The suspension was cooled in a freezer to -5° C. for 17 h. The resulting white solid was collected by filtration and washed with cold MeOH (-5° C 3x60 mL) and ethyl ether (3x100 mL). The solid was dried under vacuum (0.2 mmHg, 24 h, ambient temp.) to afford 110.5 g of β -
 10 product with excellent de (β/α 99.8:1 by HPLC). The filtrate was partially concentrated (ca. 400 mL) and then diluted with more MeOH (400 mL) while heating to 60° C. The solution was cooled down to ambient temperature, seeded and the cooled to -5° C. The second crop was collected, washed and dried in a similar manner to give more
 15 product as a white solid (12.26 g) with similar diastereomeric purity. The mother liquor was concentrated to dryness under reduced pressure (ca. 25 g). The residue was a mixture of β and α -isomers. It was subjected to automated silica gel column chromatography (Analogix, 240 g cartridge, 40% to 50% ethyl acetate in hexanes) to afford 14.52 g of product foam which was recrystallized from MeOH, washed and dried
 20 in a similar manner to afford an additional 8.46 g of product in high purity.

The three solids were judged to be of similar purity and they were combined to give 131.2 g of white crystalline product, (55% from bromosugar, 49% from lactol). Mp 160.5-162.0° C. HPLC purity 99.5% including 0.20% α . $^1\text{H-NMR}$ (pure β -anomer, CDCl_3): $\delta=8.03$ (m, 2H, arom.), 7.93 (m, 2H, arom.), 7.88 (s, 1H, C8-H), 7.60 (m, 1H, arom.), 7.50 (m, 1H, arom.), 7.44 (m, 2H, arom.), 7.33 (m, 2H, arom.), 6.44 (dd, 1H, CII'-H), 6.12 (d, 1H, C3'-H), 5.35 (s, 2H, NH₂), 5.00 (dd, 1H, C5'-Ha), 4.76 (m, 1H, C4'-H), 4.59 (dd, 1H, C5'-Hb), 1.33 (d, 3H, CH₃).

BIOLOGICAL EXAMPLES

30 Assay Protocol

High throughput replicon assay (HTBS)

Replicon cells harboring H77 (genotype 1a) or Con1 (genotype 1b) HCV RNA and Renilla luciferase reporter were seeded in 384-well black plates at a density of 1.6×10^3 cells per well in 90 μL of DMEM culture medium, excluding G-418.
 35 Compounds were serially diluted in 100% DMSO and added to cells at a 1:225 dilution, achieving a final concentration of 0.44% DMSO in a total volume of

90 µL with a Biotek µFlow Workstation. Cell plates were incubated at 37°C with 5% CO₂ for 3 days, after which culture media were removed and cells were assayed for luciferase activity as a marker for replication level. Luciferase expression was measured using Dual-Glo luciferase assay reagents (Promega, Madison, WI). Briefly, 20 µL of Dual-Glo luciferase buffer was added to lyse the cells for 10 min and subsequently 20 µL of a diluted Dual-Glo Stop & Glo substrate (1:100) was added to each well. Luminescence signal was measured on a Perkin Elmer Envision Plate Reader after incubation for 10 minute. Luciferase levels were converted into percentages relative to the untreated controls (defined as 100%) and data were fit to the logistic dose response equation $y = a/(1+(x/b)^c)$ using XLFit4 software (IDBS, Emeryville, CA). EC₅₀ values were calculated from the resulting equations. Alternatively, antiviral activity may be analyzed by HCV NS3 Protease IC₅₀ Determination.

HCV NS3 protease activity was monitored using a fluorescence resonance energy transfer (FRET) depsipeptide substrate (RET S1, Anaspec, San Jose, CA) based on the method of Taliani, Taliani M, Bianchi E, Narjes F, Fossatelli M, Urbani A, Steinkuhler C, et al. A continuous assay of hepatitis C virus protease based on resonance energy transfer depsipeptide substrates. *Anal Biochem* 1996; 240 (1):60-7, herein incorporated by reference with regard to performing such assay.

Briefly, 2–10 nM of purified NS3 protease domains were pre-incubated at 37°C for 10 minutes with 20 µM isogenic NS4A peptide cofactors (Sigma, St. Louis, MO), in 40% glycerol buffer with 50 mM HEPES pH 7.5 and 10 mM DTT. Compounds were diluted serially 1:3 in DMSO, incubated with the enzyme/cofactor mixture for 10 minutes and reactions were started by the addition of 2 µM RET S1 substrate (final concentration). Fluorescence increase was measured continuously over one hour using a Victor3 V fluorescence plate reader (Perkin Elmer, Waltham, MA). Initial velocities were calculated for each inhibitor concentration using Workout 1.5 software (DAZDAQ, East Sussex, UK) with the maximal slope algorithm. Velocity data were converted into percentages relative to the untreated control (defined as 100%) and non-linear regression was performed to calculate 50% inhibitory concentrations (IC₅₀ values).

NS3 Enzymatic Potency: Purified NS3 protease is complexed with NS4A peptide and then incubated with serial dilutions of the compounds (DMSO used as solvent). Reactions are started by addition of dual-labeled peptide substrate and the resulting kinetic increase in fluorescence is measured. Non-linear regression of velocity data is

performed to calculate IC_{50} s. Activity is initially tested against genotype 1b protease. Depending on the potency obtained against genotype 1b, additional genotypes (1a, 2a, 3) and or protease inhibitor resistant enzymes (D168Y, D168V, or A156T mutants) may be tested. BILN-2061 is used as a control during all assays. Compounds of the

5 Examples were evaluated in this assay and were found to have IC_{50} values of less than about 1 μ M.

Replicon Potency and Cytotoxicity: Huh-luc cells (stably replicating Bartenschlager's I389luc-ubi-neo/NS3-3'/ET genotype 1b replicon) are treated with serial dilutions of compound (DMSO is used as solvent) for 72 hours. Replicon copy number is

10 measured by bioluminescence and non-linear regression is performed to calculate EC_{50} s. Parallel plates treated with the same drug dilutions are assayed for cytotoxicity using the Promega CellTiter-Glo cell viability assay. Depending on the potency achieved against the 1b replicon, compounds may be tested against a genotype 1a replicon and/or inhibitor resistant replicons encoding D168Y or A156T mutations.

15 BILN-2061 is used as a control during all assays. Compounds of the Examples were evaluated in this assay and were found to have EC_{50} values of less than about 5 μ M.

Effect of serum proteins on replicon potency

Replicon assays are conducted in normal cell culture medium (DMEM + 10%FBS) supplemented with physiologic concentrations of human serum albumin (40 mg/mL) or

20 α -acid glycoprotein (1 mg/mL). EC_{50} s in the presence of human serum proteins are compared to the EC_{50} in normal medium to determine the fold shift in potency.

Enzymatic Selectivity: The inhibition of mammalian proteases including Porcine Pancreatic Elastase, Human Leukocyte Elastase, Protease 3, and Cathepsin D are measured at K_m for the respective substrates for each enzyme. IC_{50} for each enzyme

25 is compared to the IC_{50} obtained with NS3 1b protease to calculate selectivity.

MT-4 Cell Cytotoxicity: MT4 cells are treated with serial dilutions of compounds for a five day period. Cell viability is measured at the end of the treatment period using the Promega CellTiter-Glo assay and non-linear regression is performed to calculate CC_{50} .

Compound Concentration Associated with Cells at EC_{50} : Huh-luc cultures are

30 incubated with compound at concentrations equal to EC_{50} . At multiple time points (0 – 72 hours), cells are washed 2X with cold medium and extracted with 85% acetonitrile; a sample of the media at each time-point is also extracted. Cell and media extracts are analyzed by LC/MS/MS to determine the molar concentration of compounds in each fraction

Solubility and Stability: Solubility is determined by taking an aliquot of 10 mM DMSO stock solution and preparing the compound at a final concentration of 100 μ M in the test media solutions (PBS, pH 7.4 and 0.1 N HCl, pH 1.5) with a total DMSO concentration of 1%. The test media solutions are incubated at room temperature with shaking for 1 hr. The solutions are then centrifuged and the recovered supernatants are assayed on the HPLC/UV. Solubility can be calculated by comparing the amount of compound detected in the defined test solution compared to the amount detected in DMSO at the same concentration. The stability of compounds after 1 hour incubation in the test media at 37°C is also determined.

Stability in Cryo-preserved Human, Dog, and Rat Hepatocytes: Each compound is incubated for up to 1 hour in hepatocyte suspensions (100 μ L, 80,000 cells per well) at 37°C. Cryopreserved hepatocytes are reconstituted in the serum-free incubation medium. The suspension is transferred into 96-well plates (50 μ L/well). The compounds are diluted to 2 μ M in incubation medium and then are added to hepatocyte suspensions to start the incubation. Samples are taken at 0, 10, 30 and 60 minutes after the start of incubation and reaction can be quenched with a mixture consisting of 0.3% formic acid in 90% acetonitrile/10% water. The concentration of the compound in each sample is analyzed using LC/MS/MS. The disappearance half-life of the compound in hepatocyte suspension is determined by fitting the concentration-time data with a monophasic exponential equation. The data is also scaled up to represent intrinsic hepatic clearance and/or total hepatic clearance.

Stability in Hepatic S9 Fraction from Human, Dog, and Rat: Each compound is incubated for up to 1 hour in S9 suspension (500 μ L, 3 mg protein/mL) at 37°C (n = 3). The compounds are added to the S9 suspension to start the incubation. Samples are taken at 0, 10, 30, and 60 minutes after the start of incubation. The concentration of the compound in each sample is analyzed using LC/MS/MS. The disappearance half-life of the compound in S9 suspension is determined by fitting the concentration-time data with a monophasic exponential equation.

Caco-2 Permeability: Both forward (A-to-B) and reverse (B-to-A) permeability is measured. Caco-2 monolayers are grown to confluence on collagen-coated, microporous, polycarbonate membranes in 12-well Costar Transwell® plates. The compounds are dosed on the apical side for forward permeability (A-to-B), and are dosed on the basolateral side for reverse permeability (B-to-A). The cells are incubated at 37°C with 5% CO₂ in a humidified incubator. At the beginning of incubation, at 1 hr and 2 hr after incubation, a 200- μ L aliquot is taken from the receiver

chamber and replaced with fresh assay buffer. The concentration of the compound in each sample is determined with LC/MS/MS. The apparent permeability, P_{app}, is calculated.

Plasma Protein Binding: Plasma protein binding is measured by equilibrium dialysis.

- 5 Each compound is spiked into blank plasma at a final concentration of 2 µM. The spiked plasma and phosphate buffer is placed into opposite sides of the assembled dialysis cells, which is then rotated slowly in a 37°C water bath. At the end of the incubation, the concentration of the compound in plasma and phosphate buffer is determined. The percent unbound is calculated using the following equation:

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$$\% \text{ Unbound} = 100 \cdot \left(\frac{C_f}{C_b + C_f} \right)$$

Where C_f and C_b are free and bound concentrations determined as the post-dialysis buffer and plasma concentrations, respectively.

- CYP450 Profiling: Each compound is incubated with each of 5 recombinant human CYP450 enzymes, including CYP1A2, CYP2C9, CYP3A4, CYP2D6 and CYP2C19 in
15 the presence and absence of NADPH. Serial samples can be taken from the incubation mixture at the beginning of the incubation and at 5, 15, 30, 45 and 60 min after the start of the incubation. The concentration of the compound in the incubation mixture is determined by LC/MS/MS. The percentage of the compound remaining after incubation at each time point is calculated by comparing with the sampling at the
20 start of incubation.

- Stability in Rat, Dog, Monkey and Human Plasma: Compounds are incubated for up to 2 hour in plasma (rat, dog, monkey, or human) at 37°C. Compounds are added to the plasma at final concentrations of 1 and 10 µg/mL. Aliquots are taken at 0, 5, 15, 30, 60, and 120 min after adding the compound. Concentration of compounds and major
25 metabolites at each timepoint are measured by LC/MS/MS. Biological data (antiviral potency [EC₅₀] is determined using a *Renilla* luciferase (RLuc)-based HCV replicon reporter assay – HCV 1b RLuc).

- Biological Example 1: Anti-HCV Activity of the Combination of Compound 1 and
30 Compound 2
Cell Lines

HCV genotype 1b replicon cells (Huh-luc) were obtained from Reblikon (Mainz, Germany). The replicon in these cells is designated I389luc-ubi-neo/NS3-3'/ET and

encodes a selectable resistance marker (neomycin phosphotransferase) as well as the firefly luciferase reporter gene. Huh-luc cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; GIBCO, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT) and 0.5 mg/mL of G-418 (GIBCO). Cells were
5 passaged twice a week and maintained at subconfluent levels.

EC₅₀ Determinations

Replicon cells were seeded in 96-well plates at a density of 5×10^3 cells per well in 100 μ L of DMEM culture medium, excluding G-418. Compounds 1 and 2 were serially diluted 1:3 in 100% DMSO (Sigma). These serial dilutions were added to the
10 cells at a 1:200 dilution to achieve a final concentration of 0.5% DMSO in a total volume of 200 μ L. Plates were incubated at 37°C for 3 days, after which culture media were removed and cells were lysed and assayed for luciferase activity using a commercial luciferase assay (Promega, Madison, WI). HCV replication levels in drug-treated samples were expressed as a percentage of those in untreated controls
15 (defined as 100%), and data were fit to the logistic dose response equation $y=a/(1+(x/b)^c)$ using XLFit4 software (IDBS, Emeryville, CA). EC₅₀ values were calculated from the resulting equations as described previously (Delaney, W.E., et al., Antimicrobial Agents Chemotherapy, 45(6):1705-1713 (2001)).

20 Antiviral Combination Studies

Replicon cells were seeded in 96-well plates at a density of 5×10^3 cells per well in 100 μ L of culture medium. Compounds 1 and 2 were serially diluted in 100% DMSO as described above and added in a matrix format to 96-well plates, achieving a defined set of different drug concentrations and ratios in a final volume of 200 μ L and a
25 final DMSO concentration of 0.5%. For each individual drug, the EC₅₀ value was selected as the midpoint for the concentration range tested. Cells were incubated for three days and analyzed for luciferase expression as indicated above. For the combination study, two independent experiments were performed in triplicate.

30 Combination Data Analysis

Data were analyzed using the MacSynergy II program developed by Prichard and Shipman (Prichard MN, Aseltine KR, Shipman C, Jr., MacSynergyTM II, Version 1.0. University of Michigan, Ann Arbor, Michigan, 1993; Prichard M.N., Shipman C.,

Jr., *Antiviral Res* 14 (4-5):181-205 (1990); Prichard M.N., Shipman C, Jr., *Antivir Ther* 1 (1):9-20 (1996); Prichard M.N., et al., *Antimicrob Agents Chemother* 37 (3):540-5 (1993). The software calculates theoretical inhibition assuming an additive interaction between drugs (based on the Bliss Independence model) and quantifies statistically significant differences between the theoretical and observed inhibition values. Plotting these differences in three dimensions results in a surface where elevations in the Z-plane represent antiviral synergy and depressions represent antiviral antagonism between compounds. The calculated volumes of surface deviations are expressed in $\text{nM}^2\%$. Per Prichard and Shipman, combination effects are defined as:

- Highly synergistic if volumes $> 100 \text{ nM}^2$.
- Slightly synergistic if volumes are > 50 and $\leq 100 \text{ nM}^2$.
- Additive if volumes are $> -50 \text{ nM}^2$ and $\leq 50 \text{ nM}^2$.
- Slightly antagonistic if volumes are $> -100 \text{ nM}^2$ and $\leq -50 \text{ nM}^2$.
- Antagonistic if volumes are $\leq -100 \text{ nM}^2$.

Prior to initiating combination experiments, EC_{50} values in Huh-luc replicon cells were determined for Compound 1 and Compound 2 and results are shown in Table II. Both compounds had an antiviral effect.

Table II

Individual EC_{50} s for Anti-HCV Compounds 1 and 2 in Huh-luc Replicon Cells

Compound	EC_{50} (nM) ^a
Compound 1	3 ± 2
Compound 2	11 ± 3

^a EC_{50} indicates average \pm standard deviation for two or more independent experiments.

The antiviral effect of the combination of Compound 1 and Compound 2 was measured, and the resulting data were analyzed using MacSynergy II, which provides surface plots displaying significant deviations from additivity. Quantification of

statistically significant deviations from additivity indicated that the combination of Compounds 1 and 2 had synergy/antagonism volumes between -50 nM^2 and 50 nM^2 indicating additive antiviral effects as shown in Table III.

Table III

5 Quantification of Antiviral Synergy and Antagonism and Drug Interactions for
Combination of Compound 1 and Compound 2

Drug(s) Used in Combination with Compound 2	Synergy Volume (nM^2) ^a	Antagonism Volume (nM^2) ^a	Interaction
Compound 1	13.5 ± 10.5	0.07 ± 0.07	Additive

^a Values represent the mean \pm standard deviation of two independent experiments performed in triplicate

10 The results of the *in vitro* experiments set forth in Table III indicate that
Compound 2 has additive antiviral activity when combined with Compound 1.

Biological Example 2: Combinations with Compound 3

Cell Lines

15 HCV genotype 1b replicon cells (Huh-luc) were obtained from Reblikon (Mainz,
Germany). The replicon in these cells is designated I389luc-ubi-neo/NS3-3'/ET and
encodes a selectable resistance marker (neomycin phosphotransferase) as well as the
firefly luciferase reporter gene. Huh-luc cells were maintained in Dulbecco's Modified
Eagle Medium (D-MEM) with GlutaMAX™ (Invitrogen, Carlsbad, CA) supplemented
20 with 10% fetal bovine serum (FBS, Hyclone, Logan, UT) and 0.5 mg/mL of G-418
(Invitrogen). Cells were passaged twice a week and maintained at subconfluent
levels.

EC₅₀ Determinations

25 Replicon cells were seeded in 96-well plates at a density of 5×10^3 cells per well in
100 μL of DMEM plus 10% FBS culture medium, excluding G-418. Compounds were
serially diluted 1:3 in 100% DMSO (Sigma). These serial dilutions were added to the
cells at a 1:200 dilution to achieve a final concentration of 0.5% DMSO in a total

volume of 200 μ L. Plates were incubated at 37°C for 3 days, after which culture media were removed and cells were lysed and assayed for luciferase activity using a commercial luciferase assay (Promega, Madison, WI). HCV replication levels in drug-treated samples were expressed as a percentage of those in untreated controls (defined as 100%), and data were fit to the logistic dose response equation $y=a/(1+(x/b)^c)$ using XLFit4 software (IDBS, Emeryville, CA). EC₅₀ values were calculated from the resulting equations as described previously.

Antiviral Combination Studies

Replicon cells were seeded in 96-well plates at a density of 5×10^3 cells per well in 100 μ L of culture medium, excluding G-418. Compound 3 and other compounds were serially diluted in 100% DMSO as described above and added in a matrix format to 96-well plates, achieving a defined set of different drug concentrations and ratios in a final volume of 200 μ L and a final DMSO concentration of 0.5%. For each individual drug (with the exception of Ribavirin), the EC₅₀ value was selected as the midpoint for the concentration range tested. For Ribavirin, which did not have a selective antiviral effect, a top dose of 6.2 μ M was selected since this was approximately 3-fold below the concentration at which cytotoxicity started to be observed. Cells were incubated with drugs for three days and analyzed for luciferase expression as indicated above. For each combination study, two independent experiments were performed in triplicate.

Combination Data Analysis

Data were analyzed using the MacSynergy II program developed by Prichard and Shipman. The software calculates theoretical inhibition assuming an additive interaction between drugs (based on the Bliss Independence model) and quantifies statistically significant differences between the theoretical and observed inhibition values. Plotting these differences in three dimensions results in a surface where elevations in the Z-plane represent antiviral synergy and depressions represent antiviral antagonism between compounds. The calculated volumes of surface deviations are expressed in nM²%. Per Prichard and Shipman, combination effects are defined as follows:

- Strong synergy if volumes > 100 nM²; this amount of synergy is probably important *in vivo*

- Moderate synergy if volumes are > 50 and ≤ 100 nM^2 ; this amount of synergy may be important *in vivo*
- Minor synergy if volumes are > 25 and < 50 nM^2
- Additivity if volumes are > -25 nM^2 and ≤ 25 nM^2
- 5 • Minor antagonism if volumes are < -25 and > -50 nM^2
- Moderate antagonism if volumes are > -100 nM^2 and ≤ -50 nM^2 ; this amount of antagonism may be important *in vivo*
- Strong antagonism if volumes are ≤ -100 nM^2 ; this amount of antagonism is probably important *in vivo*

10

EC₅₀ Values for Individual Compounds in Huh-luc Replicon Cells.

Prior to initiating combination experiments, EC₅₀ values in Huh-luc replicon cells were determined for each compound as shown in Table IV. All compounds had an antiviral effect with the exception of Ribavirin, which had no antiviral activity up to

15 concentrations which were beginning to show cytotoxicity.

Table IV

Individual EC₅₀s for Anti-HCV Compounds in Huh-luc Replicon Cells

Compound	EC ₅₀ (nM) ^a
Compound 3	2.3 ± 2.6
IFN-α	0.105 ± .003 (U/mL) ^b
Ribavirin	> 12,500
Compound 1	0.4 ± 0.14

^a EC₅₀ indicates average ± standard deviation for two or more independent experiments.

20 ^b INF-α EC₅₀ is expressed in Units (U) per milliliter (mL) instead of a nanomolar concentration.

Combination Antiviral Effects and Drug Interactions

The antiviral effects of Compound 3 when combined with IFN- α , Ribavirin, and Compound 1 were assayed. The resulting data were analyzed using MacSynergy II, which provides surface plots displaying significant deviations from additivity.

- 5 Quantification of statistically significant deviations from additivity indicated that combinations of Compound 3 with IFN- α resulted in minor synergy (synergy volumes of 32 and 36.5 nM², respectively; Table V). The combination of Compound 3 with the non-nucleoside NS5B inhibitor Compound 1 yielded an synergy volume of 14.5 nM² which indicates an additive antiviral interaction. None of the compounds yielded
- 10 antiviral antagonism volumes outside of the additive range (> -25 nM²) when combined with Compound 3 as shown in Table V.

Table V

Quantification of Antiviral Synergy and Antagonism and Drug Interactions for Drug Combinations with Compound 3

Drug(s) Used in Combination with Compound 3	Synergy Volume (nM ²) ^a	Antagonism Volume (nM ²) ^a	Interaction
IFN- α	32 \pm 4.2	0.15 \pm 0.2	Minor synergy
Ribavirin	54 \pm 14.1	1.6 \pm 2.3	Moderate synergy
Compound 1	14.5 \pm 0.7	4.22 \pm 5.0	Additive

- 15 ^a Values represent the mean \pm standard deviation of two independent experiments performed in triplicate

- 20 These *in vitro* antiviral combination experiments indicate that the novel HCV NS3 protease inhibitor Compound 3 has minor synergy when combined with IFN- α and moderate synergy when combined with Ribavirin. These results suggest that Compound 3 could potentially be used in combination with the current standard of care (PEG-IFN- α plus ribavirin) in HCV patients to achieve enhanced viral load suppression without reducing the efficacy of any of the individual drugs. Combinations of Compound 3 with non-nucleoside (Compound 1) NS5B polymerase inhibitors resulted

in additivity. These results indicate that Compound 3 may also be suitable for exploring drug combinations comprised of multiple classes of specific HCV inhibitors in patients.

Biological Example 3: Compound 4 Combinations

Cell Line and Cell Culture

- 5 The HCV genotype 1a replicon cell line used in this study was described previously. The cells were grown in cell culture medium containing Dulbecco's Modified Eagle Medium (DMEM) with GlutaMAX (Gibco, Carlsbad, CA, Cat# 10569-044), supplemented with 10% FBS (HyClone, Logan, UT, Cat#SH30071.03), 100 Units/mL Penicillin, 100 µg/mL Streptomycin (Gibco, Carlsbad, CA, Cat# 15140-122), and 0.1
- 10 mM non-essential amino acids (Gibco, Carlsbad, CA, Cat#11140-050). Replicon cells were maintained in 0.5 mg/mL Geneticin (Invitrogen, Carlsbad, CA, Cat# 10131-035) to prevent the loss of HCV replicon. The cells were passaged every 3-4 days before reaching confluency.

HCV Replicon Assay for EC₅₀, CC₅₀ Determinations and Combination Studies

- 15 All compounds were supplied in 100% DMSO except for IFN-α, which was supplied in buffer specified by the manufacture (Sigma, St. Louis, MO, Cat#I4276). Compound serial dilutions were performed in 100% DMSO except for IFN-α, which was serially diluted in cell culture medium described in section 3.2. All serial dilutions were performed in 384-well polypropylene plates (Thermo Scientific, Hudson, NH,
- 20 Cat#4341) using a Biomek FX Workstation. For EC₅₀ and CC₅₀ determinations, test compounds were serially diluted in ten steps of 1:3 dilutions in columns 3-20 of the 384-well plates. For combinational studies, one compound was serially diluted in nine steps of 1:2 dilutions toward the horizontal direction with the other compound serially diluted in seven steps of 1:2 dilutions toward the vertical direction. This achieved a
- 25 defined set of different drug concentrations and ratios. For each individual drug, the EC₅₀ value was selected as the midpoint for the concentration range tested. All serial dilutions were performed in four replicates per compound within the same 384-well plate. 100% DMSO was added into column 1-2 of each serial dilution 384-well plate. A HCV protease inhibitor ITMN-191 at 100 µM was added into column 23 as a control of
- 30 100% inhibition of HCV replication while puromycin at 10 mM was added into column 24 as a control of 100% cytotoxicity.

To each well of a black polystyrene 384-well plate (Greiner Bio-one, Monroe, NC, Cat#781086, cell culture treated), 90 μ L of cell culture medium (without geneticin) containing 2000 suspended HCV replicon cells was added with a Biotek μ Flow Workstation. For compound transfer into cell culture plates, 0.4 μ L of compound solution from the compound serial dilution plate was transferred to the cell culture plate on a Biomek FX Workstation. The DMSO concentration in the final assay wells was 0.44%. The plates were incubated for 3 days at 37 °C with 5% CO₂ and 85% humidity.

The HCV replicon assay was a multiplex assay which can assess both cytotoxicity and anti-replicon activity from the same well. The CC₅₀ assay was performed first. The media in the 384-well cell culture plate was aspirated and the wells were washed four times with 100 μ L 1 X PBS each, using a Biotek ELX405 plate washer. A volume of 50 μ L of a solution containing 400 nM calcein AM (Anaspec, Fremont, CA, Cat#25200-056) in 1 X PBS was added to each well of the plate with a Biotek μ Flow Workstation. The plate was incubated for 30 minutes at room temperature before the fluorescence signal (excitation 490 nm, emission 520 nm) was measured with a Perkin Elmer Envision Plate Reader.

EC₅₀ assay was performed in the same wells as CC₅₀ assay. The calcein-PBS solution in the 384-well cell culture plate was aspirated with a Biotek ELX405 plate washer. A volume of 20 μ L of Dual-Glo luciferase buffer (Promega, Madison, WI, Cat#E298B) was added to each well of the plate with a Biotek μ Flow Workstation. The plate was incubated for 10 minutes at room temperature. A volume of 20 μ L of a solution containing 1:100 mixture of Dual-Glo Stop & Glo substrate (Promega, Madison, WI, Cat#E313B) and Dual-Glo Stop & Glo buffer (Promega, Madison, WI, Cat#E314B) was then added to each well of the plate with a Biotek μ Flow Workstation. The plate was then incubated at room temperature for 10 minutes before the luminescence signal was measured with a Perkin Elmer Envision Plate Reader.

Data Analysis

The cytotoxicity effect was determined by calcein AM conversion to fluorescent product. The percent cytotoxicity was calculated by equation 1:

$$\% \text{ cytotoxicity or \% inhibition} = 100 \times \left(1 - \frac{X_C - M_B}{M_D - M_B} \right) \quad (1)$$

where X_C is the fluorescence signal from the compound-treated well; M_B is the average fluorescence signal from puromycin-treated wells; M_D is the average fluorescence signal from DMSO-treated wells. The anti-HCV replication activity was determined by the luminescence signal generated from the reporter renilla luciferase of the HCV replicon. The percent inhibition on HCV replicon was calculated using equation 1, where X_C is the luminescence signal from compound-treated well; M_B is average luminescence signal from the ITMN-191-treated wells; M_D is the average luminescence signal from DMSO-treated wells.

The CC_{50} values were determined as the testing compound concentration that caused a 50% decrease of cell viability. The EC_{50} values were determined as the testing compound concentration that caused a 50% decrease in HCV replication. Both CC_{50} and EC_{50} values were obtained using Pipeline Pilot 5.0 software package (Accelrys, San Diego, CA) by nonlinear regression fitting of experimental data to equation 2:

$$y = d + \frac{a - d}{[1 + (\frac{x}{c})^b]}$$

(2)

where y is the observed % inhibition of HCV replicon at x concentration of compound; d is estimated response at zero compound concentration; a is estimated response at infinite compound concentration; c is the mid-range concentration (CC_{50} or EC_{50}); b is the Hill slope factor.

The combination study experimental data were analyzed using the MacSynergy II program developed by Prichard and Shipman. The software (MacSynergy™ II, University of Michigan, MI) calculates theoretical inhibition assuming an additive interaction between drugs (based on the Bliss Independence model) and quantifies statistically significant differences between the theoretical and observed inhibition values. Plotting these differences in three dimensions results in a surface where elevations in the Z-plane represent antiviral synergy and depressions represent antiviral antagonism between compounds. The calculated volumes of surface deviations are expressed in $nM^2\%$. Per Prichard and Shipman, combination effects are defined as:

- Strong synergy: $> 100 nM^2\%$

- Moderate synergy: > 50 and ≤ 100 nM²%
- Minor synergy: > 25 and ≤ 50 nM²%
- Additivity: ≤ 25 and > -25 nM²%
- Minor antagonism: ≤ -25 and > -50 nM²%
- 5 • Moderate antagonism: ≤ -50 and > -100 nM²%
- Strong antagonism: ≤ -100 nM²%

For each combination study, three independent experiments were performed with four replicates in each experiment.

Results

10 Antiviral Activity and Cytotoxicity of Individual Compounds in HCV Genotype 1a Replicon Assay.

The anti-HCV activity and cytotoxicity of Compound 4 and other compounds were tested in Huh-7 cells carrying a HCV genotype 1a replicon. The EC₅₀ and CC₅₀ values are listed in Table VI. There is no significant cytotoxicity observed for all compounds up to the highest concentrations tested.

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Table VI

EC₅₀ and CC₅₀ of Compounds used in this Study against HCV Genotype 1a Replicon

Compounds	EC ₅₀ ^a (nM)	CC ₅₀ ^a (nM)
Compound 1	19 ± 8	>44400
Compound 2	496 ± 135	>22200
Compound 3	49 ± 18	>22200
Compound 4	201 ± 74	>44400
Compound 5	15 ± 2.4	>44400
Compound 6	0.033 ± 0.011	>44400
IFN-α	1.4 ± 0.3 ^b	>50 ^b
Ribavirin	36482 ± 17507	>88800

- ^a Values are average \pm standard deviation for three or more independent experiments
- ^b IFN- α values are expressed in Units (U) per milliliter (mL) instead of a nanomolar concentration

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Antiviral Activity and Cytotoxicity of Compound 4 in Combination with Other Classes of Anti-HCV Agents.

The antiviral effects of Compound 4 in combination with other anti-HCV compounds were evaluated using the HCV genotype 1a replicon. The results were analyzed using MacSynergy II, which provides surface plots displaying significant deviations from additivity. Synergy and antagonism volumes ($\text{nM}^2\%$) calculated from deviations from additive surface are summarized in Table VII. At 95% confidence interval, the mean synergy and antagonism volumes are between 25 and $-25 \text{ nM}^2\%$ when Compound 4 was combined with IFN- α , Compound 2 and Compound 6, indicative of additive interaction with those compounds. Furthermore, Compound 4 shows synergy volumes in the range of 25 to $50 \text{ nM}^2\%$ when combined with Compound 1, Compound 5 or Compound 3, suggesting minor synergistic interaction.

Table VII

Quantification of Antiviral Synergy and Antagonism and Drug Interactions for Drug Combinations with Compound 4

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Drug(s) Used in Combination with Compound 4	Synergy Volume ($\text{nM}^2\%$) ^a	Antagonism Volume ($\text{nM}^2\%$) ^a	Interaction
Compound 1	34 ± 26	-1 ± 2	Minor synergy
Compound 2	22 ± 14	-2 ± 3	Additivity
Compound 3	26 ± 6	-3 ± 2	Minor synergy
Compound 5	26 ± 28	-1 ± 3	Minor synergy
Compound 6	19 ± 17	-7 ± 7	Additivity
IFN- α	12 ± 6	0 ± 0	Additivity
Ribavirin	1 ± 1	-43 ± 20	Minor antagonism

Values represent the mean \pm standard deviation of three independent experiments performed in four replicates

In all combination studies, the cell viability is higher than 85% at all concentration ratios and all drug combinations show additive effects on the cytotoxicity as shown in Table VIII.

Table VIII

Quantification of Cytotoxicity Synergy and Antagonism and Drug Interactions for Drug Combinations with Compound 4

Drug(s) Used in Combination with Compound 4	Synergy Volume (nM ² %) ^a	Antagonism Volume (nM ² %) ^a	Interaction
Compound 1	13 \pm 11	0 \pm 1	Additivity
Compound 2	17 \pm 14	0 \pm 0	Additivity
Compound 3	3 \pm 5	0 \pm 0	Additivity
Compound 5	15 \pm 8	-10 \pm 7	Additivity
Compound 6	8 \pm 4	0 \pm 0	Additivity
IFN- α	8 \pm 12	-7 \pm 13	Additivity
Ribavirin	4 \pm 3	-1 \pm 2	Additivity

^a Values represent the mean \pm standard deviation of three independent experiments performed in four replicates

However, Compound 4 shows an antagonism volume of -43 nM²% when combined with Ribavirin, suggesting a minor antagonistic interaction.

Table IX

Quantification of Cytotoxicity Synergy and Antagonism and Drug Interactions for Drug Combinations with Ribavirin

Drug Used in Combination with Ribavirin	Synergy Volume ($\mu\text{M}^{2\%}$) ^a	Antagonism Volume ($\mu\text{M}^{2\%}$) ^a	Interaction
Compound 4	4 ± 3	-1 ± 2	Additivity

^a Values represent the mean \pm standard deviation of three independent experiments performed in four replicates

The Ribavirin concentration that shows the highest antagonism with Compound 4 is around 0.5 to 1 μM , which is about 10-fold lower than the steady-state plasma concentration of Ribavirin (6-11 μM) observed in human at a dose of 800 mg/day. At this physiological concentration of Ribavirin (6-11 μM), the antagonistic interaction between Ribavirin and Compound 4 is minimal across a wide range of Compound 4 concentrations (0-0.44 μM). Therefore, the observed minor antagonism between Ribavirin and Compound 4 in the *in vitro* replicon system is unlikely to have clinical significance.

The antiviral activity of Compound 4 (in a diastereomeric mixture) was tested in combination with the current standard of care (IFN- α /Ribavirin), as well as Gilead Sciences' internal developmental candidates Compound 1 and Compound 5 (non-nucleoside NS5B inhibitors), Compound 2 and Compound 3 (NS3 protease inhibitors), and Compound 6 (NS5A inhibitor). As summarized in Table VIII, Compound 4 showed additive antiviral activity in combination with IFN- α , Compound 2 and Compound 6 and minor synergy with Compound 1, Compound 5 and Compound 3.

The combination of Compound 4 with Ribavirin resulted in a minor antagonism at Ribavirin concentrations between 0.5 to 1 μM , which is approximately 10-fold lower than its steady-state physiological concentration (6-11 μM) in human plasma. At the clinically relevant Ribavirin concentration, the antagonistic interaction between the two compounds became negligible.

Biological Example 4: Compound 5 Combinations

The antiviral activity of Compound 5 was tested in GT-1b Huh-lunet cells (using substantially the same methods as in the assays for Compound 4) in combination with the internal developmental compounds Compound 1, Compound 2 and Compound 3 (NS3 protease inhibitors), Compound 6 (NS5A inhibitor), Compound 4 (C-nuc NS5B inhibitor) and also the approved HCV therapeutics PEG-IFN- α and Ribavirin. Combination data were analyzed based on the Bliss Independence model using MacSynergy II software. Results of the combination assays were expressed as mean synergy and antagonism volumes (nM^2) calculated at 95% confidence from two independent experiments performed in triplicate. Combination effects are defined as:

- Strong synergy if volumes $> 100 \text{ nM}^2$; this amount of synergy is probably important *in vivo*
- Moderate synergy if volumes are > 50 and $\leq 100 \text{ nM}^2$; this amount of synergy may be important *in vivo*
- Minor synergy if volumes are > 25 and $< 50 \text{ nM}^2$
- Additivity if volumes are > -25 and $\leq 25 \text{ nM}^2$
- Minor antagonism if volumes are < -25 and $> -50 \text{ nM}^2$
- Moderate antagonism if volumes are $> -100 \text{ nM}^2$ and $\leq -50 \text{ nM}^2$; this amount of antagonism may be important *in vivo*
- Strong antagonism if volumes are $\leq -100 \text{ nM}^2$; this amount of antagonism is probably important *in vivo*.

The combination of the allosteric NS5B inhibitors Compound 1 and Compound 5 resulted in moderate synergy in the replicon assay. Studies with other HCV inhibitors, including PEG-IFN- α and Ribavirin, revealed additive to minor synergistic interactions.

Table X

Antiviral effects of Compound 5 in combination with other anti-HCV drugs in 1b Huh-luc replicon cells

Drug used in combination with Compound 5	Synergy Volume (nM ²) ^a	Antagonism Volume (nM ²) ^a	Interaction
Compound 1	70 ± 26	0 ± 0	Moderate synergy
Compound 2	22 ± 12	-7 ± 7	Additive
Compound 3	19 ± 13	-2 ± 2	Additive
Compound 4	26 ± 28	-1 ± 3	Minor synergy
Compound 6	34 ± 19	0 ± 0	Minor synergy
PEG-IFN-α	31 ± 23	-2 ± 4	Minor synergy
Ribavirin	12 ± 8	-12 ± 9	Additive

^aValues represent the mean ± standard deviation of two independent experiments performed in triplicate

Biological Example 5: Compound 6 Combinations

5 Cell Lines

HCV genotype 1b replicon cells (Huh-luc) were obtained from Reblikon (Mainz, Germany). The replicon in these cells is designated I389luc-ubi-neo/NS3-3'/ET and encodes a selectable resistance marker (neomycin phosphotransferase) as well as the firefly luciferase reporter gene. Huh-luc cells were maintained in Dulbecco's Modified Eagle's Medium GlutaMax (DMEM; Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT), 1X penicillin/streptomycin, 1X nonessential amino acids and 0.5 mg/mL of G-418 (all from Invitrogen, Carlsbad, CA). Cells were passaged twice a week and maintained at subconfluent levels.

Assays

15 Antiviral Activity Assay in HCV Huh-luc Replicon Cells

Replicon cells were seeded in 96-well plates at a density of 7×10^3 cells per well in 100 µL of DMEM culture medium, excluding G-418. Compounds were serially diluted 1:2 in 100% DMSO. Serial dilutions were added to the cells at a 1:200 dilution to achieve a final concentration of 0.5% DMSO in a total volume of 200 µL. Plates were incubated at 37°C for 3 days, after which culture media were removed and cells were lysed and assayed for luciferase activity using a commercial luciferase assay (Promega, Madison, WI).

Antiviral Combination Studies

Replicon cells were seeded in 96-well plates at a density of 7×10^3 cells per well in 100 μ L culture medium, excluding G-418. Compound 6 and other compounds were serially diluted 1:2 in 100% DMSO and added in a matrix format to 96-well plates, achieving a defined set of different drug concentrations and ratios in a final volume of 200 μ L and a final DMSO concentration of 0.5%. For each individual drug, the EC₅₀ value was selected as the midpoint for the concentration range tested. Cells were incubated for 3 days and analyzed for luciferase expression using a commercial luciferase assay (Promega). For each combination study, two independent experiments were performed in triplicate.

Cellular Cytotoxicity Determination

Replicon cells were seeded and treated with drugs as described for the antiviral combination studies above. After three day incubation at 37°C, the culture media was removed and cells were lysed and assayed for cytotoxicity using a CellTiter-Glo Luminescent Cell Viability Assay (Promega) according to the manufacturer's instructions. Relative Light Units were converted into percentages relative to the untreated controls (defined as 100%).

Data Analysis

EC₅₀ Calculations

Following EC₅₀ assays, luciferase levels in drug-treated samples were expressed as a percentage of those in untreated controls (defined as 100%). EC₅₀ values were calculated by nonlinear regression analysis of replicate data sets using XLfit 4 software (IDBS, Emeryville, CA).

Calculation of Antiviral Synergy and Antagonism

Following combination assays, luciferase levels in drug-treated samples were expressed as a percentage of those in untreated controls (defined as 100%). Replicate data sets were then analyzed using the MacSynergy II program developed by Prichard and Shipman. The software (MacSynergy™ II, University of Michigan, MI) calculates theoretical inhibition assuming an additive interaction between drugs (based on the Bliss Independence model) and quantifies statistically significant differences between the theoretical and observed inhibition values. Plotting these differences in three dimensions results in a surface where elevations in the Z-plane represent

antiviral synergy and depressions represent antiviral antagonism between compounds. The calculated volumes of surface deviations are expressed in $\text{nM}^2\%$. Per Prichard and Shipman, combination effects are defined as:

- 5 • Strong synergy if volumes $> 100 \text{ nM}^2$; this amount of synergy is probably important *in vivo*
- Moderate synergy if volumes are > 50 and $\leq 100 \text{ nM}^2$; this amount of synergy may be important *in vivo*
- Minor synergy if volumes are > 25 and $< 50 \text{ nM}^2$
- Additivity if volumes are $> -25 \text{ nM}^2$ and $\leq 25 \text{ nM}^2$
- 10 • Minor antagonism if volumes are < -25 and $> -50 \text{ nM}^2$
- Moderate antagonism if volumes are $> -100 \text{ nM}^2$ and $\leq -50 \text{ nM}^2$; this amount of antagonism may be important *in vivo*
- Strong antagonism if volumes are $\leq -100 \text{ nM}^2$; this amount of antagonism is probably important *in vivo*.

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Results

Antiviral Activity of Individual Compounds in Huh-luc Replicon Cells.

Prior to initiating combination experiments, the antiviral activity of individual compounds was determined in Huh-luc replicon cells. EC_{50} values consistent with

20 historical results were observed with all seven compounds.

Table XI

Individual EC_{50} Values for Anti-HCV Compounds in Huh-luc Replicon Cells

Compound	EC ₅₀ (nM) ^a
IFN- α ^b	0.05 U/ml \pm 0.04
Ribavirin	> 12 \pm 2.4
Compound 1	0.96 \pm 0.39
Compound 2	5.0 \pm 0.0
Compound 3	3.0 \pm 1.2
Compound 6	0.0018 \pm 0.0007
Compound 7	1245 \pm 341

^a EC₅₀ indicates arithmetic mean \pm standard deviation for three or more independent experiments.

^b IFN- α EC₅₀ is expressed in Units (U) per milliliter (mL) instead of a nanomolar concentration.

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Combination Antiviral Effects and Drug Interactions

The antiviral effects of Compound 6 in combination with other HCV inhibitors were evaluated using the HCV 1b replicon system. The resulting data were analyzed using MacSynergy II, which provides surface plots displaying significant deviations from additivity. Quantification of statistically significant deviations from additivity from two independent experiments is summarized in Table XII. Combinations of Compound 6 with IFN- α or Compound 1 resulted in synergy volumes of 32 and 34 nM², respectively, indicating minor synergy. Ribavirin, Compound 2 and Compound 7 yielded synergy volumes of 61, 52 and 51 when combined with Compound 6, respectively, indicating a moderate synergistic interaction between Compound 6 and these three HCV inhibitors. The combination of Compound 6 with Compound 3 resulted in a synergy volume of 132 nM² % signifying a strongly synergistic antiviral interaction. None of the compounds yielded antiviral antagonism volumes outside of the additive range (> -25 nM) when combined with Compound 6.

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Table XII

Quantification of Antiviral Synergy and Antagonism and Drug Interactions for Drug Combinations with Compound 6

Drug(s) Used in Combination with Compound 6	Synergy Volume (nM ²) ^a	Antagonism Volume (nM ²) ^a	Interaction
IFN- α	32 \pm 1.4	0.0 \pm 0.0	Minor Synergy
Ribavirin	61 \pm 0.5	-0.5 \pm 0.1	Moderate Synergy
Compound 1	34 \pm 9.9	-17 \pm 0.7	Minor Synergy
Compound 2	52 \pm 5.1	-0.7 \pm 0.7	Moderate Synergy
Compound 3	132 \pm 44	-0.1 \pm 0.2	Strong Synergy
Compound 7	51 \pm 7.8	-0.2 \pm 0.1	Moderate Synergy

^a Values represent the arithmetic mean \pm standard deviation of two independent experiments performed in triplicate.

Cell Viability Percentages for Compound 6 in Combination with Other HCV Inhibitors

- 5 To ensure that antiviral combination results were not confounded by combination cytotoxicity, the cytotoxicity was investigated in parallel using the same compound concentrations tested in the antiviral assays (Table XIII). For all compounds, cell viability was at least 98% of untreated controls for combinations at the highest concentrations tested. Therefore, no significant *in vitro* cytotoxicity was observed while
- 10 testing Compound 6 alone, or in combination with these agents.

Table XIII

Cell Viability Percentages for Compound 6 Combinations in Huh-luc Replicon Cells

Compounds	Concentration(s) (nM)	Cell Viability % ^a
Compound 6	0.014	99 ± 1
Compound 6 + IFN- α ^b	0.014 + 0.8	102 ± 3
Compound 6 + Ribavirin	0.014 + 8000	105 ± 4
Compound 6 + Compound 1	0.014 + 4.0	99 ± 3
Compound 6 + Compound 2	0.014 + 24.0	103 ± 3
Compound 6 + Compound 3	0.014 + 12.8	104 ± 4
Compound 6 + Compound 7	0.014 + 8800	103 ± 3

^a Cell viability % indicates arithmetic mean \pm standard deviation for at least two independent experiments performed in triplicate.

^b IFN- α is expressed in Units (U) per milliliter (mL) instead of a nanomolar concentration.

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Results of these *in vitro* experiments indicate that Compound 6 has minor antiviral synergy when combined with IFN- α or the non-nucleoside NS5B polymerase inhibitor Compound 1. Combinations of Compound 6 with Ribavirin, the NS3 protease inhibitor Compound 2 or the nucleoside NS5B polymerase inhibitor Compound 7 resulted in moderate antiviral synergy. Strong antiviral synergy was observed between Compound 6 and the NS3 protease inhibitor Compound 3. No significant *in vitro* cytotoxicity was identified while testing these drug combinations. These results suggest that Compound 6 could rationally be combined with the current standard of care.

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Biological example 6:

Cell Lines

HCV genotype 1b replicon cells (Huh-luc) were obtained from Reblikon (Mainz, Germany). The replicon in these cells is designated I389luc-ubi-neo/NS3-3'/ET and

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encodes a selectable resistance marker (neomycin phosphotransferase) as well as the firefly luciferase reporter gene. Huh-luc cells were maintained in Dulbecco's Modified Eagle's Medium GlutaMax (DMEM; Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT), 1X penicillin/streptomycin, 1X
5 nonessential amino acids and 0.5 mg/mL of G-418 (all from Invitrogen, Carlsbad, CA). Cells were passaged twice a week and maintained at subconfluent levels.

Assays

Determination of compound concentration required to suppress replicon RNA by 1-1.5
10 log over 6 days of treatment

Genotype 1b replicon cells were seeded in T-75 flasks at a cell density of 2.5×10^5 cells/ flask, excluding G418. Compounds were individually added to the cells at variable concentrations: Compound 6 was added at concentrations of either 1pM,
15 2pM, 4pM, 6pM, 8pM, or 12pM, Compound 4 was added at 125nM, 250nM, 375nM, 500nM or 1000nM, Compound 1 was added at 1.25nM, 2.5nM, 5nM, 2.75nM or 10nM, and Compound 3 was added at concentrations of 3.75nM, 7.5nM, 11.25nM, 15nM, 30nM or 60nM. Flasks were incubated at 37°C, media and compounds were refreshed every two days. After 6 days of incubation the replicon cells were collected for RNA
20 extraction and replicon RNA QRT-PCR analysis.

Compound combination replicon cure assay

Genotype 1b replicon cells were seeded in T-75 flasks at a density of 2.5×10^5
25 cells/flask. Compounds were added to the T-75 flasks at the following concentrations: Compound 6 at 4pM, Compound 4 at 1000nM, Compound 1 at 10nM, and Compound 3 at 26.25nM. Flasks were incubated at 37°C and compounds and media were refreshed every two days. All experiments were performed in duplicate and will be noted in as flask 1 and flask 2. On day 6 all cells were collected from flask 1 for RNA
30 extraction followed by HCV replicon specific QRT-PCR analysis and the cells from flask 2 were replated on a 10cm tissue culture dishes in the presence of G418 for 14 days to record colony formation of uncured replicon cells.

QRT-PCR assay

Total RNA was extracted with the RiboPure kit (AM1924 Life Technologies Corporation Carlsbad, CA) following the manufacturer's protocol. Extracted RNA samples were stored at -80°C until use. For the Quantitative RT-PCR assay the Qiagen One-step QRT-PCR kit was used according to manufacturer's protocol (Qiagen, Valencia CA). The genotype 1b HCV NS3 gene specific primers, forward primer NS3_180FL 5'-CGGCGGACTGTCTATCATGGTGC[FAM]G-'3 and reverse NS3_180 5'-GGTCCTGGTCCACATTGGTGT-'3 and 18S rRNA LUX™ [FAM] endogenous control primer set (115HM-01) were produced by Invitrogen corporation (Carlsbad, CA). For the reverse transcriptase step, the reactions were incubated at 44°C for 30 min, the reverse transcriptase enzyme was then degraded by heating the sample to 94°C for 10 min. The Q-PCR step included 38 cycles at 94°C for 15 s and 58°C for 30 s.

Prior to initiating combination replicon cure experiments the compound concentration required to suppress genotype 1b replicon RNA by 1-1.5 log was determined for Compound 6, Compound 4, Compound 1, and Compound 3. The replicon RNA log drop is relative to the RNA levels in DMSO control treated replicon cells maintained for 6 days.

Table XIV

Individual compound dose able to induce replicon RNA 1-1.5 log drop in a 6 day assay

Compound	Replicon RNA log drop	Compound concentration (nM)
Compound 1	-1.0	10
Compound 3	-0.9	26.25
Compound 4	-1.2	1000
Compound 6	-1.4	0.004

Combination genotype 1b replicon cure assay

The replicon RNA suppression by compounds Compound 6, Compound 4, Compound 1 and Compound 3 was determined in a 6 day assay as individual compounds and in various double, triple, and quadruple combinations. The replicon RNA log drop is relative to the RNA levels in DMSO control treated replicon cells maintained for 6 days alongside the treatment flasks. The ability of the various compound combinations to

cure the cells from the HCV replicon was determined by colony formation. Colony formation occurred after compound treatment was removed and G418 pressure was returned for 14 days. If a compound combination completely cures the cell population from the HCV replicon no colonies will develop since the cells lack resistance to G418.

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Table XV

Quantification of compound combination in the replicon cure assay

Compounds	Replicon RNA log drop	Uncured colony number
Compound 6	-1.4	634
Compound 4	-1.2	1054
Compound 1	-1.0	657
Compound 3	-0.9	989
Compound 4+Compound 6	-2.67	15
Compound 1+Compound 4	-2.022	14
Compound 3+Compound 4	-2.26	23
Compound 1+Compound 6	-2.3	148
Compound 3+Compound 6	-2.62	13
Compound 1+Compound 3	-1.8	113
Compound 1+Compound 4+Compound 6	-2.66	0
Compound 3+Compound 4+Compound 6	-2.71	0
Compound 1+Compound 3+Compound 4	-2.69	0
Compound 1+Compound 3+Compound 6	-2.69	0
Compound 1+Compound 3+Compound 4+Compound 6	-2.71	0
DMSO (0.2% to match Quadruple combination)	0	6330

- 10 Results of these *in vitro* experiments indicate that combination of two compounds increases the viral RNA log drop over 6 day treatment and increases the rate of cured

replicon cells. The dual combinations of Compound 6 with Compound 4 or Compound 3 results in larger replicon RNA log suppression and lowest number of uncured colonies compared to all other dual compound combinations. The combination of three or four compounds cures all replicon cells and the combination treatments suppress the replicon RNA levels to the assay limit of detection.

Clinical Example 1: Clinical Testing of Anti-HCV Activity of the Combination of Compound 1 and Compound 2

This Clinical Example shows that the combination of Compound 1 and Compound 2 plus ribavirin is more effective at reducing HCV viral load, and suppressing HCV viral rebound, than the combination of Compound 1 plus Compound 2 without ribavirin.

Clinical Trial Design:

A Phase 2, randomized, open-label trial of Compound 2 plus Compound 1 alone and in combination with ribavirin for 28 days in treatment-naïve subjects with chronic genotype 1 HCV infection. Subjects in Arm 1 received Compound 2 at 75 mg + Compound 1 at 40 mg, both administered twice daily (BID) (double regimen) and subjects in Arm 2 received Compound 2 at 75 mg + Compound 1 at 40 mg, both administered BID, and plus ribavirin, also administered BID (triple regimen) for 28 days.

On Day 28, all subjects were to initiate PEG/Ribavirin. Additionally, the protocol called for subjects with an insufficient virologic response ($< 2 \log_{10}$ IU/mL reduction from baseline HCV RNA by Day 5) or virologic rebound (HCV RNA increase of $> 0.5 \log_{10}$ IU/mL from nadir confirmed over two time points occurring after Day 5 with an absolute value > 1000 IU/mL) to initiate PEG/RIBA prior to Day 28.

For subjects with insufficient virologic response, the combination of pegylated interferon (PEG) and ribavirin (RIBA) was initiated prior to Day 28 with or without continuation Compound 2 + Compound 1. As a result, by Day 28 of the study, subjects were receiving one of four treatments:

- (i) Compound 2 + Compound 1,
- (ii) Compound 2 + Compound 1 + RIBA,
- (iii) Compound 2 + Compound 1 + PEG/RIBA, or
- (iv) PEG/RIBA.

A total of 31 subjects were enrolled and started dosing (16 subjects received the double regimen in Arm 1 and 15 subjects received the triple regimen in Arm 2).

Preliminary subject demographics and baseline characteristics (Table XVI) were generally comparable between Arms 1 and 2, aside from a greater number of subjects with genotype 1b in Arm 2. Four subjects were identified as HCV genotype 1b at screening (one subject on the dual regimen and three subjects on the triple regimen), but have not been confirmed as genotype 1a or 1b upon further analysis, with further assessment ongoing.

No subjects have experienced serious adverse events. Study medications have been generally well-tolerated, with all adverse events being Grade 1-2 in severity, except for a single Grade 3 fatigue, which was the only treatment emergent adverse event leading to study drug discontinuation. Prior to the initiation of PEG/Ribavirin, the most common treatment-emergent adverse events occurring in more than one subject were headache (n=5), and diarrhea or nausea (n=3 each) in Arm 1 and headache (n=7), diarrhea or fatigue (n=3 each), nausea, asthenia, pruritis or insomnia (n=2 each) in Arm 2. When Compound 2 + Compound 1 were given in combination with PEG/RIBA, the only adverse events occurring in more than one subject were influenza-like illness (n=5) and myalgia (n=3), both common adverse events with PEG/RIBA therapy. With regard to laboratory abnormalities, there were no Grade 4 events during the 28-day treatment period. Among subjects receiving the study drugs, there were two treatment-emergent Grade 3 elevations in total bilirubin in the ribavirin containing Arm 2 (occurring at Day 7, but resolving with continued dosing of study drug). There were also 2 Grade-1 elevations and a single Grade-2 elevation in total bilirubin among other subjects in this dosing Arm (with ribavirin). Among subjects in Arm-1 (no ribavirin), there were four Grade-1 total bilirubin elevations. ALT values were reduced approximately 40 U/L from baseline in both arms by Day 14. Median QTcF was not significantly changed from baseline in either study arm and no subjects discontinued study drugs due to QTc abnormalities. Preliminary safety data are summarized in Table XVIII.

Plasma HCV RNA was monitored approximately twice weekly to gauge virologic response in relation to the protocol-specified criteria for early initiation of PEG/RIBA. From preliminary analysis of the HCV RNA values, the median maximal decline in HCV RNA was 3.9 log₁₀ IU/mL for the dual regimen and 5.0 log₁₀ IU/mL for the triple regimen. The median time to maximal decline in HCV RNA was 7 days for the dual regimen and 14 days for the triple regimen, with the difference attributed to delayed incidence and onset of viral breakthrough in the ribavirin containing arm.

Three of 15 (20%) subjects receiving the dual regimen and 10 of 13 (77%) subjects

receiving the triple regimen had nadir HCV RNA values ≤ 30 IU/mL (excluding non-GT1 subjects). 13/16 (81%) subjects receiving Compound 2/Compound 1 and 6/15 (40%) subjects receiving Compound 2/Compound 1/Ribavirin initiated PEG or PEG/Ribavirin prior to the scheduled start on Day 28 of the study. Additional details of virologic outcomes are provided in

Compound 2 + Compound 1 alone and in combination with RIBA were well-tolerated for up to 28 days by HCV subjects in this study, both before and following the addition of PEG or PEG/Ribavirin. Both regimens yielded potent suppression of HCV RNA, with greater and more sustained activity in the three drug regimen.

Table XVI Preliminary Subject Demographics and Baseline Characteristics

	Arm #1: Compound 2 at 75 mg BID + Compound 1 at 40 mg BID (n=16)	Arm #2: Compound 2 at 75 mg BID + Compound 1 at 40 mg BID + RIBA (n=15)
Age in years - Median (range)	47 (30,66)	55 (27, 63)
Sex	14 male 2 female	11 male 4 female
Ethnicity	16 Non-Hispanic/Latino	15 Non-Hispanic/Latino
Race	13 White 2 Black 1 Asian	13 White 2 Black 0 Asian
Baseline Weight in kg – Median (range)	86.1 (57.8, 110.5)	79.0 (51, 127.5)
Baseline BMI in kg/M ² – Median (range)	27.1 (21.5, 34.1)	24.7 (19.9, 37.6)
Baseline Log ₁₀ HCV RNA (IU/mL) from Central lab– Median (range) Central lab	6.17 (5.25, 7.26)	6.34 (5.41, 7.19)
Baseline HCV Genotype	8 1a 8 1b	3 1a 12 1b

Table XVII Preliminary Safety Results

	Arm 1: Compound 2 at 75 mg BID + Compound 1 at 40 mg BID (n=16)	Arm 2: Compound 2 at 75 mg BID + Compound 1 at 40 mg BID + RIBA (n=15)
Grade 3 Adverse Events (AEs): Fatigue	1	0
Grade 1/Grade 2 (AEs): Headache	5 (31%)	7 (47%)
Diarrhea	3 (19%)	3 (20%)
Nausea	3 (19%)	2 (13%)
Fatigue	0	3 (20%)
Asthenia	0	2 (13%)
Pruritis	1 (6%)	2 (13%)
Insomnia	0	2 (13%)
Grade 3 Laboratory Abnormalities: Bilirubin	0	2
Grade 1/Grade 2 Laboratory Abnormalities: Bilirubin	4	3
Hemoglobin	0	2
Glucose (nonfasting)	8	5

Table XVIII Preliminary Virologic Outcomes

	Arm 1: Compound 2 at 75 mg BID + Compound 1 at 40 mg BID (n=16)	Arm 1: Compound 2 at 75 mg BID + Compound 1 at 40 mg BID <i>Unconfirmed GT1 Subjects Excluded (n=15)*</i>	Arm 2: Compound 2 at 75 mg BID + Compound 1 at 40 mg BID + Ribavirin (n=15)	Arm 2: Compound 2 at 75 mg BID + Compound 1 at 40 mg BID + Ribavirin <i>Unconfirmed GT1 Subjects Excluded (n=13)</i>
Median maximal HCV RNA decline	-3.9 log ₁₀ IU/mL	-4.0 log ₁₀ IU/mL	-5.0 log ₁₀ IU/mL	-5.0 log ₁₀ IU/mL
Mean maximal HCV RNA decline	-3.4 log ₁₀ IU/mL	-3.6 log ₁₀ IU/mL	-4.5 log ₁₀ IU/mL	-4.9 log ₁₀ IU/mL
Mean time to Breakthrough	16 days	16 days	23 days	23 days
Subjects with HCV RNA nadir <50 IU/mL	3/16 (19%)	3/15 (20%)	10/15 (63%)	10/13 (77%)
Subjects with Breakthrough**	12 (75%)	12/15 (80%)	6/15 (40%)	6/13 (46%)

	Arm 1: Compound 2 at 75 mg BID + Compound 1 at 40 mg BID (n=16)	Arm 1: Compound 2 at 75 mg BID + Compound 1 at 40 mg BID <i>Unconfirmed GT1 Subjects Excluded (n=15)*</i>	Arm 2: Compound 2 at 75 mg BID + Compound 1 at 40 mg BID + Ribavirin (n=15)	Arm 2: Compound 2 at 75 mg BID + Compound 1 at 40 mg BID + Ribavirin <i>Unconfirmed GT1 Subjects Excluded (n=13)</i>
Day 28 Response: RVR at < 25 IU/mL RVR at < 50 IU/mL	1/16 (6%) 1/16 (6%)	1/15 (7%) 1/15 (7%)	5/15 (33%) 6/15 (40%)	5/13 (38%) 6/13 (46%)

* GT1 is an abbreviation for HCV Genotype 1.

Subjects 1011, 1012, and 1043 at one French study center were excluded;

Subject 1004 was not excluded

- 5 ** Breakthrough defined as > 1 log increase in HCV RNA above nadir value or HCV RNA > 25 IU/mL following a nadir of < 25 IU/mL

The data presented in Table XVIII show that there was an approximately 10 fold greater decline in both the median maximal HCV RNA level and the mean maximal HCV RNA level in response to the combination of Compound 2 + Compound 1 in the presence of ribavirin compared to the absence of ribavirin. Also, the number of study subjects having an HCV RNA nadir below 50 IU/mL is greater in the presence of ribavirin than in the absence of ribavirin. These results show that ribavirin, in the absence of interferon, significantly potentiates the antiviral activity of the combination of Compound 1 and Compound 2.

Additionally, the mean time to HCV breakthrough, which is a measure of the eventual increase in HCV viral load as the virus mutates and becomes less susceptible to the antiviral drugs, is greater in the presence of ribavirin than in the absence of ribavirin. Further, the number of subjects showing viral breakthrough is substantially less in the presence of ribavirin than in the absence of ribavirin. These results show that the HCV virus is less able to develop resistance to the combination of Compound 1 and Compound 2 in the presence of ribavirin.

Further, the data presented in Table XVIII shows that the number of patients achieving a Rapid Virologic Response (RVR) in the presence of ribavirin is significantly greater than in the absence of ribavirin. Achievement of RVR positively correlates with cure of HCV infection.

Taken together the data presented in Table XVIII show that the combination of Compound 1, Compound 2, and ribavirin causes a rapid and clinically significant reduction in HCV viral load, with a reduced viral rebound, even in the absence of administration of interferon.

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Although specific embodiments of the present invention are herein illustrated and described in detail, the invention is not limited thereto. The above detailed descriptions are provided as exemplary of the present invention and should not be construed as constituting any limitation of the invention. Modifications will be obvious to those skilled in the art, and all modifications that do not depart from the spirit of the invention are intended to be included with the scope of the appended claims.

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CLAIMS

What is claimed is:

1. A composition comprising two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof.
2. The composition of claim 1 which comprises Compound 1 and further comprises a second compound selected from the group consisting of Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7.
3. The composition of claim 2, wherein the second compound is Compound 3.
4. The composition of claim 2, wherein the second compound is Compound 4.
5. The composition of claim 2, wherein the second compound is Compound 5.
6. The composition of claim 2, wherein the second compound is Compound 6.
7. The composition of claim 1 which comprises Compound 2 and further comprises a second compound selected from the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7.
8. The composition of claim 7, wherein the second compound is Compound 4.
9. The composition of claim 1 which comprises Compound 3 and further comprises a second compound selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6 and Compound 7.
10. The composition of claim 9, wherein the second compound is Compound 2.
11. The composition of claim 9, wherein the second compound is Compound 5.
12. The composition of claim 1 which comprises Compound 4 and further comprises a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.
13. The composition of claim 12, wherein the second compound is Compound 1.
14. The composition of claim 12, wherein the second compound is Compound 2.
15. The composition of claim 12, wherein the second compound is Compound 3.
16. The composition of claim 12, wherein the second compound is Compound 6.

17. The composition of claim 1 which comprises Compound 5 and further comprises a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 6 and Compound 7.
18. The composition of claim 17, wherein the second compound is Compound 2.
19. The composition of claim 1 which comprises Compound 6 and further comprises a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5 and Compound 7.
20. The composition of claim 19, wherein the second compound is Compound 1.
21. The composition of claim 19, wherein the second compound is Compound 2.
22. The composition of claim 19, wherein the second compound is Compound 3.
23. The composition of claim 19, wherein the second compound is Compound 5.
24. The composition of claim 1 which comprises Compound 7 and further comprises a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5 and Compound 6.
25. The composition of claim 1 which comprises Compound 1 and further comprises a second compound and a third compound each selected from the group consisting of Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7.
26. The composition of claim 25, wherein the second compound is Compound 3.
27. The composition of claim 25, wherein the second compound is Compound 4.
28. The composition of claim 25, wherein the second compound is Compound 5.
29. The composition of claim 25, wherein the second compound is Compound 6.
30. The composition of claim 25, wherein the second compound is Compound 3 and the third compound is Compound 5.
31. The composition of claim 25, wherein the second compound is Compound 3 and the third compound is Compound 4.
32. The composition of claim 25, wherein the second compound is Compound 2 and the third compound is Compound 3.
33. The composition of claim 25, wherein the second compound is Compound 3 and the third compound is Compound 6.

34. The composition of claim 25, wherein the second compound is Compound 4 and the third compound is Compound 6.
35. The composition of claim 1 which comprises Compound 2 and further comprises a second compound and a third compound each selected from the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7.
36. The composition of claim 35, wherein the second compound is Compound 4.
37. The composition of claim 35, wherein the second compound is Compound 1 and the third compound is Compound 5.
38. The composition of claim 35, wherein the second compound is Compound 3 and the third compound is Compound 4.
39. The composition of claim 35, wherein the second compound is Compound 4 and the third compound is Compound 6.
40. The composition of claim 1 which comprises Compound 3 and further comprises a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6 and Compound 7.
41. The composition of claim 40, wherein the second compound is Compound 1.
42. The composition of claim 40, wherein the second compound is Compound 6.
43. The composition of claim 40, wherein the second compound is Compound 2 and the third compound is Compound 5.
44. The composition of claim 38, wherein the second compound is Compound 5 and the third compound is Compound 6.
45. The composition of claim 40, wherein the second compound is Compound 4 and the third compound is Compound 6.
46. The composition of claim 1 which comprises Compound 4 and further comprises a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.
47. The composition of claim 46, wherein the second compound is Compound 1.
48. The composition of claim 46, wherein the second compound is Compound 2.

49. The composition of claim 46, wherein the second compound is Compound 3.
50. The composition of claim 46, wherein the second compound is Compound 6.
51. The composition of claim 46, wherein the second compound is Compound 1 and the third compound is Compound 2.
52. The composition of claim 46, wherein the second compound is Compound 1 and the third compound is Compound 3.
53. The composition of claim 46, wherein the second compound is Compound 2 and the third compound is Compound 5.
54. The composition of claim 46, wherein the second compound is Compound 1 and the third compound is Compound 5.
55. The composition of claim 46, wherein the second compound is Compound 3 and the third compound is Compound 5.
56. The composition of claim 1 which comprises Compound 5 and further comprises a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 6 and Compound 7.
57. The composition of claim 56, wherein the second compound is Compound 1.
58. The composition of claim 1 which comprises Compound 6 and further comprises a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5 and Compound 7.
59. The composition of claim 58, wherein the second compound is Compound 1.
60. The composition of claim 58, wherein the second compound is Compound 2.
61. The composition of claim 58, wherein the second compound is Compound 3.
62. The composition of claim 58, wherein the second compound is Compound 4.
63. The composition of claim 58, wherein the second compound is Compound 5.
64. The composition of claim 58, wherein the second compound is Compound 1 and the third compound is Compound 2.
65. The composition of claim 58, wherein the second compound is Compound 1 and the third compound is Compound 5.

66. The composition of claim 58, wherein the second compound is Compound 4 and the third compound is Compound 5.
67. The composition of claim 58, wherein the second compound is Compound 2 and the third compound is Compound 5.
68. The composition of claim 58, wherein the second compound is Compound 2 and the third compound is Compound 3.
69. The composition of claim 1 which comprises Compound 7 and further comprises a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5 and Compound 6.
70. The composition of claim 1 which comprises Compound 1 and further comprises a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7.
71. The composition of claim 70, wherein the second compound is Compound 3.
72. The composition of claim 70, wherein the second compound is Compound 4.
73. The composition of claim 70, wherein the second compound is Compound 5.
74. The composition of claim 70, wherein the second compound is Compound 6.
75. The composition of claim 70, wherein the second compound is Compound 2 and the third compound is Compound 4 or Compound 6.
76. The composition of claim 70, wherein the second compound is Compound 3 and the third compound is Compound 4 or Compound 6.
77. The composition of claim 70, wherein the second compound is Compound 4 and the third compound is Compound 6.
78. The composition of claim 70, wherein the second compound is Compound 2, the third compound is Compound 4, and the fourth compound is Compound 6.
79. The composition of claim 70, wherein the second compound is Compound 3, the third compound is Compound 4, and the fourth compound is Compound 6.
80. The composition of claim 1 which comprises Compound 2 and further comprises a second compound, a third compound and a fourth compound each

selected from the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7.

81. The composition of claim 80, wherein the second compound is Compound 4.

82. The composition of claim 80, wherein the second compound is Compound 1 and the third compound is Compound 4.

83. The composition of claim 80, wherein the second compound is Compound 1 and the third compound is Compound 6.

84. The composition of claim 80, wherein the second compound is Compound 4 and the third compound is Compound 6.

85. The composition of claim 1 which comprises Compound 3 and further comprises a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6 and Compound 7.

86. The composition of claim 85, wherein the second compound is Compound 1.

87. The composition of claim 85, wherein the second compound is Compound 6.

88. The composition of claim 85, wherein the second compound is Compound 1 and the third compound is Compound 4.

89. The composition of claim 85, wherein the second compound is Compound 1 or Compound 4 and the third compound is Compound 6.

90. The composition of claim 85, wherein the second compound is Compound 4, the third compound is Compound 5 and the fourth compound is Compound 6.

91. The composition of claim 1 which comprises Compound 4 and further comprises a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

92. The composition of claim 91, wherein the second compound is Compound 1.

93. The composition of claim 91, wherein the second compound is Compound 2.

94. The composition of claim 91, wherein the second compound is Compound 3.

95. The composition of claim 91, wherein the second compound is Compound 6.

96. The composition of claim 91, wherein the second compound is Compound 1 and the third compound is Compound 2.
97. The composition of claim 91, wherein the second compound is Compound 1 and the third compound is Compound 3.
98. The composition of claim 91, wherein the second compound is Compound 1 and the third compound is Compound 6.
99. The composition of claim 91, wherein the second compound is Compound 2 and the third compound is Compound 6.
100. The composition of claim 91, wherein the second compound is Compound 3 and the third compound is Compound 6.
101. The composition of claim 1 which comprises Compound 5 and further comprises a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 6 and Compound 7.
102. The composition of claim 101, wherein the second compound is Compound 1.
103. The composition of claim 1 which comprises Compound 6 and further comprises a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4 and Compound 5.
104. The composition of claim 103, wherein the second compound is Compound 1.
105. The composition of claim 103, wherein the second compound is Compound 2.
106. The composition of claim 103, wherein the second compound is Compound 3.
107. The composition of claim 103, wherein the second compound is Compound 4.
108. The composition of claim 103, wherein the second compound is Compound 1 and the third compound is Compound 2.
109. The composition of claim 103, wherein the second compound is Compound 1 and the third compound is Compound 3.
110. The composition of claim 103, wherein the second compound is Compound 4 and the third compound is Compound 6.
111. The composition of claim 103, wherein the second compound is Compound 2 and the third compound is Compound 4.

112. The composition of claim 103, wherein the second compound is Compound 3 and the third compound is Compound 4.

113. The composition of claim 1 which comprises Compound 7 and further comprises a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5 and Compound 6.

114. The composition of claim 1 which comprises Compound 1 and further comprises a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7.

115. The composition of claim 114, wherein the second compound is Compound 3.

116. The composition of claim 114, wherein the second compound is Compound 4.

117. The composition of claim 114, wherein the second compound is Compound 5.

118. The composition of claim 114, wherein the second compound is Compound 6.

119. The composition of claim 114, wherein the second compound is Compound 2 and the third compound is Compound 4.

120. The composition of claim 114, wherein the second compound is Compound 3 and the third compound is Compound 4.

121. The composition of claim 114, wherein the second compound is Compound 2 and the third compound is Compound 6.

122. The composition of claim 114, wherein the second compound is Compound 3 and the third compound is Compound 6.

123. The composition of claim 114, wherein the second compound is Compound 4 and the third compound is Compound 6.

124. The composition of claim 1 which comprises Compound 2 and further comprises a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7.

125. The composition of claim 124, wherein the second compound is Compound 4.

126. The composition of claim 124, wherein the second compound is Compound 1 and the third compound is Compound 4.

127. The composition of claim 124, wherein the second compound is Compound 1 and the third compound is Compound 6.
128. The composition of claim 124, wherein the second compound is Compound 4 and the third compound is Compound 6.
129. The composition of claim 1 which comprises Compound 3 and further comprises a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 4, Compound 5, Compound 6 and Compound 7.
130. The composition of claim 129, wherein the second compound is Compound 1.
131. The composition of claim 129, wherein the second compound is Compound 6.
132. The composition of claim 129, wherein the second compound is Compound 1 and the third compound is Compound 4.
133. The composition of claim 129, wherein the second compound is Compound 1 and the third compound is Compound 6.
134. The composition of claim 129, wherein the second compound is Compound 4 and the third compound is Compound 6.
135. The composition of claim 1 which comprises Compound 4 and further comprises a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.
136. The composition of claim 135, wherein the second compound is Compound 1.
137. The composition of claim 135, wherein the second compound is Compound 2.
138. The composition of claim 135, wherein the second compound is Compound 3.
139. The composition of claim 135, wherein the second compound is Compound 6.
140. The composition of claim 135, wherein the second compound is Compound 1 and the third compound is Compound 2.
141. The composition of claim 135, wherein the second compound is Compound 1 and the third compound is Compound 3.
142. The composition of claim 135, wherein the second compound is Compound 1 and the third compound is Compound 6.

143. The composition of claim 135, wherein the second compound is Compound 2 and the third compound is Compound 6.
144. The composition of claim 135, wherein the second compound is Compound 3 and the third compound is Compound 6.
145. The composition of claim 1 which comprises Compound 5 and further comprises a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 6 and Compound 7.
146. The method of claim 145, wherein the second compound is Compound 1.
147. The composition of claim 1 which comprises Compound 6 and further comprising a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5 and Compound 7.
148. The composition of claim 147, wherein the second compound is Compound 1.
149. The composition of claim 147, wherein the second compound is Compound 2.
150. The composition of claim 147, wherein the second compound is Compound 3.
151. The composition of claim 147, wherein the second compound is Compound 4.
152. The composition of claim 147, wherein the second compound is Compound 1 and the third compound is Compound 2.
153. The composition of claim 147, wherein the second compound is Compound 1 and the third compound is Compound 3.
154. The composition of claim 147, wherein the second compound is Compound 4 and the third compound is Compound 6.
155. The composition of claim 147, wherein the second compound is Compound 2 and the third compound is Compound 4.
156. The composition of claim 147, wherein the second compound is Compound 3 and the third compound is Compound 4.
157. The composition of claim 1 which comprises Compound 7 and further comprises a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 4, Compound 5 and Compound 6.

158. The composition of any one of claims 1-157 which further comprises one or more pharmaceutically acceptable diluents or carriers.
159. The composition of any one of claims 1-158 which is formulated as a unit dosage form for once daily administration.
160. The composition of any one of claims 1-159 which is formulated for oral administration.
161. The composition of any one of claims 1-160 which is formulated as a tablet.
162. A method of treating an HCV infection in a human, comprising administering two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to the human.
163. The method of claim 162 wherein a composition as described in any one of claims 1-160 is administered to the human.
164. A method for ameliorating one or more symptoms of an HCV infection in a human, comprising administering two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to the human.
165. The method of claim 164 wherein a composition as described in any one of claims 1-160 is administered to the human.
166. A method for reducing viral load in a human with HCV, comprising administering two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to the human.
167. The method of claim 166 wherein a composition as described in any one of claims 1-160 is administered to the human.
168. A method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents in a human, comprising administering two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to the human.
169. The method of claim 168 wherein a composition as described in any one of claims 1-160 is administered to the human.

170. The method of any one of claims 162-169 wherein an interferon is not administered to the human.
171. The method of any one of claims 162-169 further comprising administering ribavirin to the human.
172. The method of any one of claims 162-169 further comprising administering one or more additional agents selected from ribavirin, an interferon, alpha-glucosidase 1 inhibitors, hepatoprotectants, TLR-7 agonists, cyclophilin inhibitors, HCV viral entry inhibitors, HCV maturation inhibitors, and HCV IRES inhibitors to the human.
173. The use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof in medical therapy.
174. The use of a composition as described in any one of claims 1-160 in medical therapy.
175. The use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof for the prophylactic or therapeutic treatment of an HCV infection.
176. The use of a composition as described in any one of claims 1-157 for the prophylactic or therapeutic treatment of an HCV infection.
177. The use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to prepare a medicament for treating an HCV infection in a human.
178. The use of a composition as described in any one of claims 1-157 to prepare a medicament for treating an HCV infection in a human.
179. The use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to prepare a medicament for ameliorating one or more symptoms of an HCV infection in a human.
180. The use of a composition as described in any one of claims 1-157 to prepare a medicament for ameliorating one or more symptoms of an HCV infection in a human.

181. The use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to prepare a medicament for reducing viral load in a human.
182. The use of a composition as described in any one of claims 1-157 to prepare a medicament for reducing viral load in a human.
183. The use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to prepare a medicament for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents in a human.
184. The use of a composition as described in any one of claims 1-157 to prepare a medicament for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents in a human.
185. A composition comprising two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, Compound 7, Compound 9, Compound 10 and Compound 11 and pharmaceutically acceptable salts thereof.
186. The composition of claim 185 which comprises a first compound selected from the group consisting of Compound 4, Compound 9, Compound 10 and Compound 11 and further comprises a second compound selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.
187. The composition of claim 186, wherein the first compound is Compound 9.
188. The composition of claim 186, wherein the first compound is Compound 10.
189. The composition of claim 186, wherein the first compound is Compound 11.
190. The composition of claim 186, wherein the second compound is Compound 1.
191. The composition of claim 186, wherein the second compound is Compound 2.
192. The composition of claim 186, wherein the second compound is Compound 3.
193. The composition of claim 186, wherein the second compound is Compound 6.

194. The composition of claim 185 which comprises a first compound selected from the group consisting of Compound 4, Compound 9, Compound 10 and Compound 11 and further comprises a second compound and a third compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.
195. The composition of claim 194, wherein the first compound is Compound 9.
196. The composition of claim 194, wherein the first compound is Compound 10.
197. The composition of claim 194, wherein the first compound is Compound 11.
198. The composition of claim 194, wherein the second compound is Compound 1.
199. The composition of claim 194, wherein the second compound is Compound 2.
200. The composition of claim 194, wherein the second compound is Compound 3.
201. The composition of claim 194, wherein the second compound is Compound 6.
202. The composition of claim 194, wherein the second compound is Compound 1 and the third compound is Compound 2.
203. The composition of claim 194, wherein the second compound is Compound 1 and the third compound is Compound 3.
204. The composition of claim 194, wherein the second compound is Compound 2 and the third compound is Compound 5.
205. The composition of claim 194, wherein the second compound is Compound 1 and the third compound is Compound 5.
206. The composition of claim 194, wherein the second compound is Compound 3 and the third compound is Compound 5.
207. The composition of claim 185 which comprises a first compound selected from the group consisting of Compound 4, Compound 9, Compound 10 and Compound 11 and further comprises a second compound, a third compound and a fourth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.
208. The composition of claim 207, wherein the second compound is Compound 1.
209. The composition of claim 207, wherein the second compound is Compound 2.
210. The composition of claim 207, wherein the second compound is Compound 3.

211. The composition of claim 207, wherein the second compound is Compound 6.

212. The composition of claim 207, wherein the second compound is Compound 1 and the third compound is Compound 2.

213. The composition of claim 207, wherein the second compound is Compound 1 and the third compound is Compound 3.

214. The composition of claim 207, wherein the second compound is Compound 1 and the third compound is Compound 6.

215. The composition of claim 207, wherein the second compound is Compound 2 and the third compound is Compound 6.

216. The composition of claim 207, wherein the second compound is Compound 3 and the third compound is Compound 6.

217. The composition of claim 185 which comprises a first compound selected from the group consisting of Compound 4, Compound 9, Compound 10 and Compound 11 and further comprises a second compound, a third compound, a fourth compound and a fifth compound each selected from the group consisting of Compound 1, Compound 2, Compound 3, Compound 5, Compound 6 and Compound 7.

218. The composition of claim 217, wherein the second compound is Compound 1.

219. The composition of claim 217, wherein the second compound is Compound 2.

220. The composition of claim 217, wherein the second compound is Compound 3.

221. The composition of claim 217, wherein the second compound is Compound 6.

222. The composition of claim 217, wherein the second compound is Compound 1 and the third compound is Compound 2.

223. The composition of claim 217, wherein the second compound is Compound 1 and the third compound is Compound 3.

224. The composition of claim 217, wherein the second compound is Compound 1 and the third compound is Compound 6.

225. The composition of claim 217, wherein the second compound is Compound 2 and the third compound is Compound 6.

226. The composition of claim 217, wherein the second compound is Compound 3 and the third compound is Compound 6.

227. The composition of any one of claims 185-226 which further comprises one or more pharmaceutically acceptable diluents or carriers.
228. The composition of any one of claims 185-227 which is formulated as a unit dosage form for once daily administration.
229. The composition of any one of claims 185-228 which is formulated for oral administration.
230. The composition of any one of claims 185-229 which is formulated as a tablet.
231. A method of treating an HCV infection in a human, comprising administering two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to the human.
232. The method of claim 231 wherein a composition as described in any one of claims 185-230 is administered to the human.
233. A method for ameliorating one or more symptoms of an HCV infection in a human, comprising administering two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to the human.
234. The method of claim 233 wherein a composition as described in any one of claims 185-230 is administered to the human.
235. A method for reducing viral load in a human with HCV, comprising administering two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to the human.
236. The method of claim 235 wherein a composition as described in any one of claims 185-230 is administered to the human.
237. A method for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents in a human, comprising administering two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to the human.
238. The method of claim 237 wherein a composition as described in any one of claims 185-230 is administered to the human.

239. The method of any one of claims 231-238 wherein an interferon is not administered to the human.
240. The method of any one of claims 231-238 further comprising administering ribavirin to the human.
241. The method of any one of claims 231-238 further comprising administering one or more additional agents selected from ribavirin, an interferon, alpha-glucosidase 1 inhibitors, hepatoprotectants, TLR-7 agonists, cyclophilin inhibitors, HCV viral entry inhibitors, HCV maturation inhibitors, and HCV IRES inhibitors to the human.
242. The use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof in medical therapy.
243. The use of a composition as described in any one of claims 185-230 in medical therapy.
244. The use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof for the prophylactic or therapeutic treatment of an HCV infection.
245. The use of a composition as described in any one of claims 185-226 for the prophylactic or therapeutic treatment of an HCV infection.
246. The use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to prepare a medicament for treating an HCV infection in a human.
247. The use of a composition as described in any one of claims 185-226 to prepare a medicament for treating an HCV infection in a human.
248. The use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to prepare a medicament for ameliorating one or more symptoms of an HCV infection in a human.
249. The use of a composition as described in any one of claims 185-226 to prepare a medicament for ameliorating one or more symptoms of an HCV infection in a human.

250. The use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to prepare a medicament for reducing viral load in a human.

251. The use of a composition as described in any one of claims 185-226 to prepare a medicament for reducing viral load in a human.

252. The use of two or more compounds selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6 and Compound 7 and pharmaceutically acceptable salts thereof to prepare a medicament for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents in a human.

253. The use of a composition as described in any one of claims 185-226 to prepare a medicament for reducing emergence of HCV quasispecies with resistance to coadministered oral antiviral agents in a human.

ABSTRACT OF THE INVENTION

This invention relates to combinations of therapeutic molecules useful for treating hepatitis C virus infection. The present invention relates to methods, uses, dosing regimens, and compositions.

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	Document Description	Start	End
	Specification	1	153
	Claims	154	171
	Abstract	172	172

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