(12) STANDARD PATENT (11) Application No. **AU 2002243791 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Nucleoside derivatives as inhibitors of RNA-dependent RNA viral polymerase

(51) International Patent Classification(s)
C07H 19/00 (2006.01) ***A61P 31/14*** (2006.01)
A61K 31/7064 (2006.01) ***C07H 19/14*** (2006.01)

(21) Application No: **2002243791** (22) Date of Filing: **2002.01.18**

(87) WIPO No: **WO02/057287**

(30) Priority Data

(31) Number	(32) Date	(33) Country
60/263,313	**2001.01.22**	**US**
60/299,320	**2001.06.19**	**US**
60/282,069	**2001.04.06**	**US**
60/344,528	**2001.10.25**	**US**

(43) Publication Date: **2002.07.30**
(43) Publication Journal Date: **2003.02.13**
(44) Accepted Journal Date: **2006.06.29**

(71) Applicant(s)
Merck & Co., Inc.;Isis Pharmaceuticals, Inc.

(72) Inventor(s)
Prhavc, Marija;Cook, Phillip Dan;Olsen, David, B.;Song, Quanlai;Bhat, Neelima;Maccoss, Malcolm;Prakash, Thazha, P.;Carroll, Steven, S.;Eldrup, Anne, B.;Bhat, Balkrishen

(74) Agent / Attorney
Spruson & Ferguson, Level 35 St Martins Tower 31 Market Street, Sydney, NSW, 2000

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 July 2002 (25.07.2002)

PCT

(10) International Publication Number
WO 02/057287 A3

(51) International Patent Classification[7]: C07H 19/14, A61K 31/7064, A61P 31/14

(21) International Application Number: PCT/US02/03086

(22) International Filing Date: 18 January 2002 (18.01.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/263,313	22 January 2001 (22.01.2001)	US
60/282,069	6 April 2001 (06.04.2001)	US
60/299,320	19 June 2001 (19.06.2001)	US
60/344,528	25 October 2001 (25.10.2001)	US

(71) Applicants *(for all designated States except US)*: **MERCK & CO., INC.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **ISIS PHARMACEUTICALS, INC.** [US/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CARROLL, Steven, S.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **MACCOSS, Malcolm** [GB/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **OLSEN, David, B.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **BHAT, Balkrishen** [IN/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US). **BHAT, Neelima** [IN/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US). **COOK, Phillip Dan** [US/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US). **ELDRUP, Anne, B.** [DK/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US). **PRAKASH, Thazha, P.** [IN/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US). **PRHAVC, Marija** [SI/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US). **SONG, Quanlai** [CN/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US).

(74) Common Representative: **DURETTE, Philippe, L.**; Merck & Co., Inc., 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
10 October 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/057287 A3

(54) Title: NUCLEOSIDE DERIVATIVES AS INHIBITORS OF RNA-DEPENDENT RNA VIRAL POLYMERASE

(57) Abstract: The present invention provides nucleoside compounds and certain derivatives thereof which are inhibitors of RNA-dependent RNA viral polymerase. These compounds are inhibitors of RNA-dependent RNA viral replication and are useful for the treatment of RNA-dependent RNA viral infection. They are particularly useful as inhibitors of hepatitis C virus (HCV) NS5B polymerase, as inhibitors of HCV replication, and/or for the treatment of hepatitis C infection. The invention also describes pharmaceutical compositions containing such nucleoside compounds alone or in combination with other agents active against RNA-dependent RNA viral infection, in particular HCV infection. Also disclosed are methods of inhibiting RNA-dependent RNA polymerase, inhibiting RNA-dependent RNA viral replication, and/or treating RNA-dependent RNA viral infection with the nucleoside compounds of the present invention.

NUCLEOSIDE DERIVATIVES AS INHIBITORS OF RNA-DEPENDENT RNA VIRAL POLYMERASE

FIELD OF THE INVENTION

The present invention is concerned with nucleoside compounds and certain derivatives thereof, their synthesis, and their use as inhibitors of RNA-dependent RNA viral polymerase. The compounds of the present invention are inhibitors of RNA-dependent RNA viral replication and are useful for the treatment of RNA-dependent RNA viral infection. They are particularly useful as inhibitors of hepatitis C virus (HCV) NS5B polymerase, as inhibitors of HCV replication, and for the treatment of hepatitis C infection.

BACKGROUND OF THE INVENTION

Hepatitis C virus (HCV) infection is a major health problem that leads to chronic liver disease, such as cirrhosis and hepatocellular carcinoma, in a substantial number of infected individuals, estimated to be 2-15% of the world's population. There are an estimated 4.5 million infected people in the United States alone, according to the U.S. Center for Disease Control. According to the World Health Organization, there are more than 200 million infected individuals worldwide, with at least 3 to 4 million people being infected each year. Once infected, about 20% of people clear the virus, but the rest harbor HCV the rest of their lives. Ten to twenty percent of chronically infected individuals eventually develop liver-destroying cirrhosis or cancer. The viral disease is transmitted parenterally by contaminated blood and blood products, contaminated needles, or sexually and vertically from infected mothers or carrier mothers to their off-spring. Current treatments for HCV infection, which are restricted to immunotherapy with recombinant interferon-α alone or in combination with the nucleoside analog ribavirin, are of limited clinical benefit. Moreover, there is no established vaccine for HCV. Consequently, there is an urgent need for improved therapeutic agents that effectively combat chronic HCV infection. The state of the art in the treatment of HCV infection has been reviewed, and reference is made to the following publications: B. Dymock, et al., "Novel approaches to the treatment of hepatitis C virus infection," Antiviral Chemistry & Chemotherapy, 11: 79-96 (2000); H. Rosen, et al., "Hepatitis C virus: current understanding and prospects for future therapies," Molecular Medicine Today, 5: 393-

399 (1999); D. Moradpour, et al., "Current and evolving therapies for hepatitis C," European J. Gastroenterol. Hepatol., 11: 1189-1202 (1999); R. Bartenschlager, "Candidate Targets for Hepatitis C Virus-Specific Antiviral Therapy," Intervirology, 40: 378-393 (1997); G.M. Lauer and B.D. Walker, "Hepatitis C Virus Infection," N. Engl. J. Med., 345: 41-52 (2001); B.W. Dymock, "Emerging therapies for hepatitis C virus infection," Emerging Drugs, 6: 13-42 (2001); and C. Crabb, "Hard-Won Advances Spark Excitement about Hepatitis C," Science: 506-507 (2001); the contents of all of which are incorporated by reference herein in their entirety.

Different approaches to HCV therapy have been taken, which include the inhibition of viral serine proteinase (NS3 protease), helicase, and RNA-dependent RNA polymerase (NS5B), and the development of a vaccine.

The HCV virion is an enveloped positive-strand RNA virus with a single oligoribonucleotide genomic sequence of about 9600 bases which encodes a polyprotein of about 3,010 amino acids. The protein products of the HCV gene consist of the structural proteins C, E1, and E2, and the non-structural proteins NS2, NS3, NS4A and NS4B, and NS5A and NS5B. The nonstructural (NS) proteins are believed to provide the catalytic machinery for viral replication. The NS3 protease releases NS5B, the RNA-dependent RNA polymerase from the polyprotein chain. HCV NS5B polymerase is required for the synthesis of a double-stranded RNA from a single-stranded viral RNA that serves as a template in the replication cycle of HCV. NS5B polymerase is therefore considered to be an essential component in the HCV replication complex [see K. Ishi, et al., "Expression of Hepatitis C Virus NS5B Protein: Characterization of Its RNA Polymerase Activity and RNA Binding," Hepatology, 29: 1227-1235 (1999) and V. Lohmann, et al., "Biochemical and Kinetic Analyses of NS5B RNA-Dependent RNA Polymerase of the Hepatitis C Virus," Virology, 249: 108-118 (1998)]. Inhibition of HCV NS5B polymerase prevents formation of the double-stranded HCV RNA and therefore constitutes an attractive approach to the development of HCV-specific antiviral therapies.

It has now been found that nucleoside compounds of the present invention and certain derivatives thereof are potent inhibitors of RNA-dependent RNA viral replication and in particular HCV replication. The 5'-triphosphate derivatives of these nucleoside compounds are inhibitors of RNA-dependent RNA viral polymerase and in particular HCV NS5B polymerase. The instant nucleoside compounds and derivatives thereof are useful to treat RNA-dependent RNA viral infection and in particular HCV infection.

It is therefore an object of the present invention to provide nucleoside compounds and certain derivatives thereof which are useful as inhibitors of RNA-dependent RNA viral polymerase and in particular as inhibitors of HCV NS5B polymerase.

It is another object of the present invention to provide nucleoside compounds and certain derivatives thereof which are useful as inhibitors of the replication of an RNA-dependent RNA virus and in particular as inhibitors of the replication of hepatitis C virus.

It is another object of the present invention to provide nucleoside compounds and certain derivatives thereof which are useful in the treatment of RNA-dependent RNA viral infection and in particular in the treatment of HCV infection.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside compounds of the present invention in association with a pharmaceutically acceptable carrier.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside compounds and derivatives thereof of the present invention for use as inhibitors of RNA-dependent RNA viral polymerase and in particular as inhibitors of HCV NS5B polymerase.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside compounds and derivatives thereof of the present invention for use as inhibitors of RNA-dependent RNA viral replication and in particular as inhibitors of HCV replication.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside compounds and derivatives thereof of the present invention for use in the treatment of RNA-dependent RNA viral infection and in particular in the treatment of HCV infection.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside compounds and derivatives thereof of the present invention in combination with other agents active against an RNA-dependent RNA virus and in particular against HCV.

It is another object of the present invention to provide methods for the inhibition of RNA-dependent RNA viral polymerase and in particular for the inhibition of HCV NS5B polymerase.

2002243791 01 Jun 2006

It is another object of the present invention to provide methods for the inhibition of RNA-dependent RNA viral replication and in particular for the inhibition of HCV replication.

It is another object of the present invention to provide methods for the treatment of RNA-dependent RNA viral infection and in particular for the treatment of HCV infection.

It is another object of the present invention to provide methods for the treatment of RNA-dependent RNA viral infection in combination with other agents active against RNA-dependent RNA virus and in particular for the treatment of HCV infection in combination with other agents active against HCV.

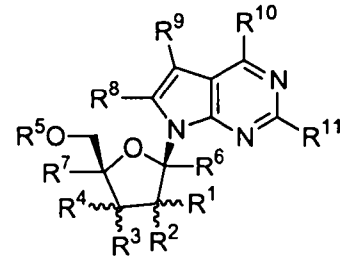
It is another object of the present invention to provide nucleoside compounds and certain derivatives thereof and their pharmaceutical compositions for use as a medicament for the inhibition of RNA-dependent RNA viral replication and/or the treatment of RNA-dependent RNA viral infection and in particular for the inhibition of HCV replication and/or the treatment of HCV infection.

It is another object of the present invention to provide for the use of the nucleoside compounds and certain derivatives thereof of the present invention and their pharmaceutical compositions for the manufacture of a medicament for the inhibition of RNA-dependent RNA viral replication and/or the treatment of RNA-dependent RNA viral infection and in particular for the inhibition of HCV replication and/or the treatment of HCV infection.

These and other objects will become readily apparent from the detailed description which follows.

Summary of the Invention

The present invention relates to a compound of structural formula I:



(I)

or a pharmaceutically acceptable salt thereof;

wherein R^1 is C_{2-4} alkenyl, C_{2-4} alkynyl, or C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

2002243791 01 Jun 2006

R^2 is hydrogen, fluorine, hydroxy, mercapto, C_{1-4} alkoxy, or C_{1-4} alkyl; or R^1 and R^2 together with the carbon atom to which they are attached form a 3- to 6-membered saturated monocyclic ring system optionally containing a heteroatom selected from O, S, and NC_{0-4} alkyl;

R^3 and R^4 are each independently selected from the group consisting of hydrogen, cyano, azido, halogen, hydroxy, mercapto, amino, C_{1-4} alkoxy, C_{2-4} alkenyl, C_{2-4} alkynyl, and C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^5 is hydrogen, C_{1-10} alkylcarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or $P(O)R^{13}R^{14}$;

R^6 and R^7 are each independently hydrogen, methyl, hydroxymethyl, or fluoromethyl;

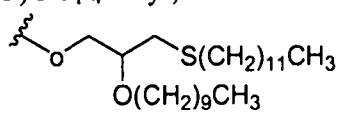
R^8 is hydrogen, C_{1-4} alkyl, C_{2-4} alkynyl, halogen, cyano, carboxy, C_{1-4} alkyloxycarbonyl, azido, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, hydroxy, C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfonyl, (C_{1-4} alkyl)$_{0-2}$ aminomethyl, or C_{4-6} cycloheteroalkyl, unsubstituted or substituted with one to two groups independently selected from halogen, hydroxy, amino, C_{1-4} alkyl, and C_{1-4} alkoxy;

R^9 is hydrogen, cyano, nitro, C_{1-3} alkyl, $NHCONH_2$, $CONR^{12}R^{12}$, $CSNR^{12}R^{12}$, $COOR^{12}$, $C(=NH)NH_2$, hydroxy, C_{1-3} alkoxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, halogen, (1,3-oxazol-2-yl), (1,3-thiazol-2-yl), or (imidazol-2-yl); wherein alkyl is unsubstituted or substituted with one to three groups independently selected from halogen, amino, hydroxy, carboxy, and C_{1-3} alkoxy;

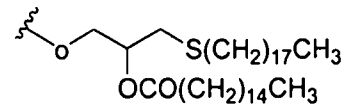
R^{10} and R^{11} are each independently hydrogen, hydroxy, halogen, C_{1-4} alkoxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, C_{3-6} cycloalkylamino, di(C_{3-6} cycloalkyl)amino, or C_{4-6} cycloheteroalkyl, unsubstituted or substituted with one to two groups independently selected from halogen, hydroxy, amino, C_{1-4} alkyl, and C_{1-4} alkoxy;

each R^{12} is independently hydrogen or C_{1-6} alkyl; and

R^{13} and R^{14} are each independently hydroxy, $OCH_2CH_2SC(=O)C_{1-4}$ alkyl, $OCH_2O(C=O)OC_{1-4}$ alkyl, $NHCHMeCO_2Me$, $OCH(C_{1-4}$ alkyl$)O(C=O)C_{1-4}$ alkyl,



or



;

with the proviso that when R^1 is β-methyl, R^4 is hydrogen, R^2 and R^3 are α-hydroxy, R^{10} is amino, and R^5, R^6, R^7, R^8, and R^{11} are hydrogen, then R^9 is not cyano or $CONH_2$.

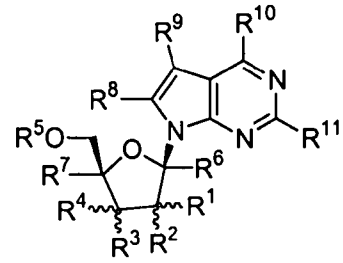
2002243791 01 Jun 2006

The compounds of formula I are useful as inhibitors of RNA-dependent RNA viral polymerase and in particular of HCV NS5B polymerase. They are also inhibitors of RNA-dependent RNA viral replication and in particular of HCV replication and are useful for the treatment of RNA-dependent RNA viral infection and in particular for the treatment of HCV infection.

Also encompassed within the present invention are pharmaceutical compositions containing the compounds alone or in combination with other agents active against RNA-dependent RNA virus and in particular against HCV as well as methods for the inhibition of RNA-dependent RNA viral replication and for the treatment of RNA-dependent RNA viral infection.

Detailed Description of the Invention

The present invention relates to a compound of structural formula I:



(I)

or a pharmaceutically acceptable salt thereof;

wherein R^1 is C_{2-4} alkenyl, C_{2-4} alkynyl, or C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^2 is hydrogen, fluorine, hydroxy, mercapto, C_{1-4} alkoxy, or C_{1-4} alkyl; or R^1 and R^2 together with the carbon atom to which they are attached form a 3- to 6-membered saturated monocyclic ring system optionally containing a heteroatom selected from O, S, and NC_{0-4} alkyl;

R^3 and R^4 are each independently selected from the group consisting of hydrogen, cyano, azido, halogen, hydroxy, mercapto, amino, C_{1-4} alkoxy, C_{2-4} alkenyl, C_{2-4} alkynyl, and C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^5 is hydrogen, C_{1-10} alkylcarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or $P(O)R^{13}R^{14}$;

R^6 and R^7 are each independently hydrogen, methyl, hydroxymethyl, or fluoromethyl;

R^8 is hydrogen, C_{1-4} alkyl, C_{2-4} alkynyl, halogen, cyano, carboxy, C_{1-4} alkyloxycarbonyl, azido, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, hydroxy, C_{1-6}

2002243791 01 Jun 2006

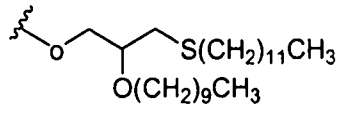
alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfonyl, $(C_{1-4}$ alkyl$)_{0-2}$ aminomethyl, or C_{4-6} cycloheteroalkyl, unsubstituted or substituted with one to two groups independently selected from halogen, hydroxy, amino, C_{1-4} alkyl, and C_{1-4} alkoxy;

R^9 is hydrogen, cyano, nitro, C_{1-3} alkyl, $NHCONH_2$, $CONR^{12}R^{12}$, $CSNR^{12}R^{12}$, $COOR^{12}$, $C(=NH)NH_2$, hydroxy, C_{1-3} alkoxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, halogen, (1,3-oxazol-2-yl), (1,3-thiazol-2-yl), or (imidazol-2-yl); wherein alkyl is unsubstituted or substituted with one to three groups independently selected from halogen, amino, hydroxy, carboxy, and C_{1-3} alkoxy;

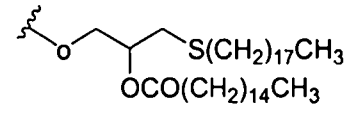
R^{10} and R^{11} are each independently hydrogen, hydroxy, halogen, C_{1-4} alkoxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, C_{3-6} cycloalkylamino, di(C_{3-6} cycloalkyl)amino, or C_{4-6} cycloheteroalkyl, unsubstituted or substituted with one to two groups independently selected from halogen, hydroxy, amino, C_{1-4} alkyl, and C_{1-4} alkoxy;

each R^{12} is independently hydrogen or C_{1-6} alkyl; and

R^{13} and R^{14} are each independently hydroxy, $OCH_2CH_2SC(=O)C_{1-4}$ alkyl, $OCH_2O(C=O)OC_{1-4}$ alkyl, $NHCHMeCO_2Me$, $OCH(C_{1-4}$ alkyl$)O(C=O)C_{1-4}$ alkyl,



or

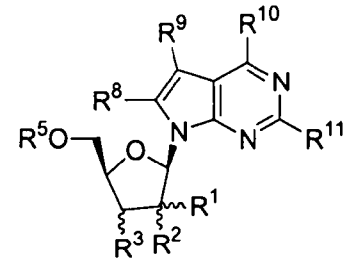


;

with the proviso that when R^1 is β-methyl, R^4 is hydrogen, R^2 and R^3 are α-hydroxy, R^{10} is amino, and R^5, R^6, R^7, R^8, and R^{11} are hydrogen, then R^9 is not cyano or $CONH_2$.

The compounds of formula I are useful as inhibitors of RNA-dependent RNA viral polymerase. They are also inhibitors of RNA-dependent RNA viral replication and are useful for the treatment of RNA-dependent RNA viral infection.

In one embodiment of the compounds of structural formula I are the compounds of structural formula II:



(II)

or a pharmaceutically acceptable salt thereof;

wherein

2002243791 01 Jun 2006

R^1 is C_{1-3} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-3} alkoxy, C_{1-3} alkylthio, or one to three fluorine atoms;

R^2 is hydroxy, fluoro, or C_{1-3} alkoxy;

R^3 is hydrogen, halogen, hydroxy, amino, or C_{1-3} alkoxy;

R^5 is hydrogen, $P_3O_9H_4$, $P_2O_6H_3$, or PO_3H_2;

R^8 is hydrogen, amino, or C_{1-4} alkylamino;

R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and

R^{10} and R^{11} are each independently hydrogen, halogen, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, or C_{3-6} cycloalkylamino;

with the proviso that when R^1 is β-methyl, R^2 and R^3 are α-hydroxy, R^{10} is amino, and R^5, R^8, and R^{11} are hydrogen, then R^9 is not cyano or $CONH_2$.

In a second embodiment of the compounds of structural formula I are the compounds of structural formula II wherein:

R^1 is methyl, fluoromethyl, hydroxymethyl, difluoromethyl, trifluoromethyl, or aminomethyl;

R^2 is hydroxy, fluoro, or methoxy;

R^3 is hydrogen, fluoro, hydroxy, amino, or methoxy;

R^5 is hydrogen or $P_3O_9H_4$;

R^8 is hydrogen or amino;

R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and

R^{10} and R^{11} are each independently hydrogen, fluoro, hydroxy, or amino;

with the proviso that when R^1 is β-methyl, R^2 and R^3 are α-hydroxy, R^{10} is amino, and R^5, R^8, and R^{11} are hydrogen, then R^9 is not cyano or $CONH_2$.

Illustrative but nonlimiting examples of compounds of the present invention of structural formula I which are useful as inhibitors of RNA-dependent RNA viral polymerase are the following:

4-amino-7-(2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-methylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-dimethylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-7-(2-*C*-vinyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-7-(2-*C*-hydroxymethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid,

4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2,4-diamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
4-amino-7-(2-*C*-ethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
2-amino-5-methyl-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
4-amino-7-(3-deoxy-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine,
4-amino-7-(3-deoxy-2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine,
4-amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(3-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(3-*C*-methyl-β-D-xylofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2,4-di-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine, and
4-amino-7-(3-deoxy-3-fluoro-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;
and the corresponding 5'-triphosphates;
or a pharmaceutically acceptable salt thereof.

Further illustrative of the present invention are the compounds selected from the group consisting of:
4-amino-7-(2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
and
4-amino-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
and the corresponding 5'-triphosphates;

or a pharmaceutically acceptable salt thereof.

In one embodiment of the present invention, the nucleoside compounds of the present invention are useful as inhibitors of positive-sense single-stranded RNA-dependent RNA viral polymerase, inhibitors of positive-sense single-stranded RNA-dependent RNA viral replication, and/or for the treatment of positive-sense single-stranded RNA-dependent RNA viral infection. In a class of this embodiment, the positive-sense single-stranded RNA-dependent RNA virus is a *Flaviviridae* virus or a *Picornaviridae* virus. In a subclass of this class, the *Picornaviridae* virus is a rhinovirus, a poliovirus, or a hepatitis A virus. In a second subclass of this class, the *Flaviviridae* virus is selected from the group consisting of hepatitis C virus, yellow fever virus, dengue virus, West Nile virus, Japanese encephalitis virus, Banzi virus, and bovine viral diarrhea virus (BVDV). In a subclass of this subclass, the *Flaviviridae* virus is hepatitis C virus.

Another aspect of the present invention is concerned with a method for inhibiting RNA-dependent RNA viral polymerase, a method for inhibiting RNA-dependent RNA viral replication, and/or a method for treating RNA-dependent RNA viral infection in a mammal in need thereof comprising administering to the mammal a therapeutically effective amount of a compound of structural formula I.

In one embodiment of this aspect of the present invention, the RNA-dependent RNA viral polymerase is a positive-sense single-stranded RNA-dependent RNA viral polymerase. In a class of this embodiment, the positive-sense single-stranded RNA-dependent RNA viral polymerase is a *Flaviviridae* viral polymerase or a *Picornaviridae* viral polymerase. In a subclass of this class, the *Picornaviridae* viral polymerase is rhinovirus polymerase, poliovirus polymerase, or hepatitis A virus polymerase. In a second subclass of this class, the *Flaviviridae* viral polymerase is selected from the group consisting of hepatitis C virus polymerase, yellow fever virus polymerase, dengue virus polymerase, West Nile virus polymerase, Japanese encephalitis virus polymerase, Banzi virus polymerase, and bovine viral diarrhea virus (BVDV) polymerase. In a subclass of this subclass, the *Flaviviridae* viral polymerase is hepatitis C virus polymerase.

In a second embodiment of this aspect of the present invention, the RNA-dependent RNA viral replication is a positive-sense single-stranded RNA-dependent RNA viral replication. In a class of this embodiment, the positive-sense single-stranded RNA-dependent RNA viral replication is *Flaviviridae* viral replication or *Picornaviridae* viral replication. In a subclass of this class, the *Picornaviridae*

viral replication is rhinovirus replication, poliovirus replication, or hepatitis A virus replication. In a second subclass of this class, the *Flaviviridae* viral replication is selected from the group consisting of hepatitis C virus replication, yellow fever virus replication, dengue virus replication, West Nile virus replication, Japanese encephalitis virus replication, Banzi virus replication, and bovine viral diarrhea virus replication. In a subclass of this subclass, the *Flaviviridae* viral replication is hepatitis C virus replication.

In a third embodiment of this aspect of the present invention, the RNA-dependent RNA viral infection is a positive-sense single-stranded RNA-dependent viral infection. In a class of this embodiment, the positive-sense single-stranded RNA-dependent RNA viral infection is *Flaviviridae* viral infection or *Picornaviridae* viral infection. In a subclass of this class, the *Picornaviridae* viral infection is rhinovirus infection, poliovirus infection, or hepatitis A virus infection. In a second subclass of this class, the *Flaviviridae* viral infection is selected from the group consisting of hepatitis C virus infection, yellow fever virus infection, dengue virus infection, West Nile virus infection, Japanese encephalitis virus infection, Banzi virus infection, and bovine viral diarrhea virus infection. In a subclass of this subclass, the *Flaviviridae* viral infection is hepatitis C virus infection.

Throughout the instant application, the following terms have the indicated meanings:

The alkyl groups specified above are intended to include those alkyl groups of the designated length in either a straight or branched configuration. Exemplary of such alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tertiary butyl, pentyl, isopentyl, hexyl, isohexyl, and the like.

The term "alkenyl" shall mean straight or branched chain alkenes of two to six total carbon atoms, or any number within this range (e.g., ethenyl, propenyl, butenyl, pentenyl, etc.).

The term "alkynyl" shall mean straight or branched chain alkynes of two to six total carbon atoms, or any number within this range (e.g., ethynyl, propynyl, butynyl, pentynyl, etc.).

The term "cycloalkyl" shall mean cyclic rings of alkanes of three to eight total carbon atoms, or any number within this range (i.e., cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, or cyclooctyl).

The term "cycloheteroalkyl" is intended to include non-aromatic heterocycles containing one or two heteroatoms selected from nitrogen, oxygen and

sulfur. Examples of 4-6-membered cycloheteroalkyl include azetidinyl, pyrrolidinyl, piperidinyl, morpholinyl, thiamorpholinyl, imidazolidinyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydrothiophenyl, piperazinyl, and the like.

The term "alkoxy" refers to straight or branched chain alkoxides of the number of carbon atoms specified (e.g., C_{1-4} alkoxy), or any number within this range [i.e., methoxy (MeO-), ethoxy, isopropoxy, etc.].

The term "alkylthio" refers to straight or branched chain alkylsulfides of the number of carbon atoms specified (e.g., C_{1-4} alkylthio), or any number within this range [i.e., methylthio (MeS-), ethylthio, isopropylthio, etc.].

The term "alkylamino" refers to straight or branched alkylamines of the number of carbon atoms specified (e.g., C_{1-4} alkylamino), or any number within this range [i.e., methylamino, ethylamino, isopropylamino, t-butylamino, etc.].

The term "alkylsulfonyl" refers to straight or branched chain alkylsulfones of the number of carbon atoms specified (e.g., C_{1-6} alkylsulfonyl), or any number within this range [i.e., methylsulfonyl ($MeSO_2$-), ethylsulfonyl, isopropylsulfonyl, etc.].

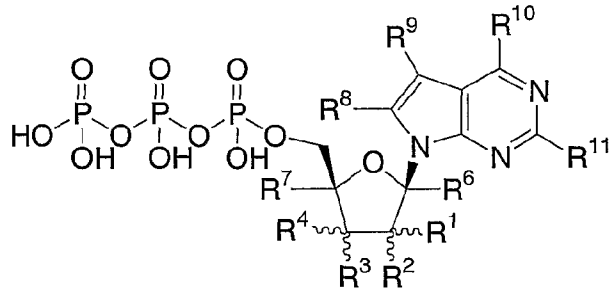
The term "alkyloxycarbonyl" refers to straight or branched chain esters of a carboxylic acid derivative of the present invention of the number of carbon atoms specified (e.g., C_{1-4} alkyloxycarbonyl), or any number within this range [i.e., methyloxycarbonyl (MeOCO-), ethyloxycarbonyl, or butyloxycarbonyl].

The term "aryl" includes both phenyl, naphthyl, and pyridyl. The aryl group is optionally substituted with one to three groups independently selected from C_{1-4} alkyl, halogen, cyano, nitro, trifluoromethyl, C_{1-4} alkoxy, and C_{1-4} alkylthio.

The term "halogen" is intended to include the halogen atoms fluorine, chlorine, bromine and iodine.

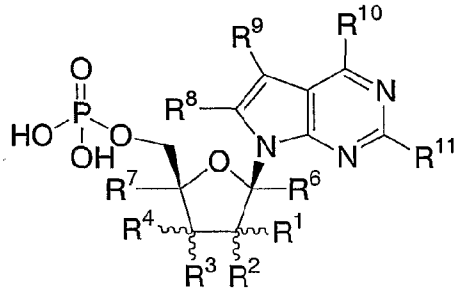
The term "substituted" shall be deemed to include multiple degrees of substitution by a named substituent. Where multiple substituent moieties are disclosed or claimed, the substituted compound can be independently substituted by one or more of the disclosed or claimed substituent moieties, singly or plurally.

The term "5'-triphosphate" refers to a triphosphoric acid ester derivative of the 5'-hydroxyl group of a nucleoside compound of the present invention having the following general structural formula III:

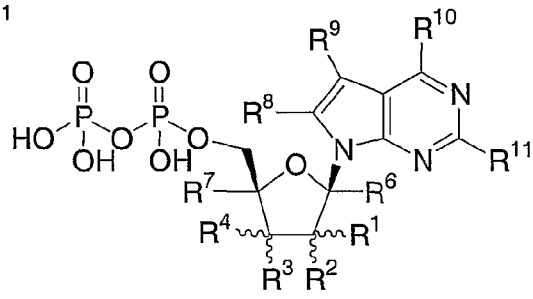


(III)

wherein R^1-R^{11} are as defined above. The compounds of the present invention are also intended to include pharmaceutically acceptable salts of the triphosphate ester as well as pharmaceutically acceptable salts of 5'-monophosphate and 5'-diphosphate ester derivatives of the structural formulae IV and V, respectively,

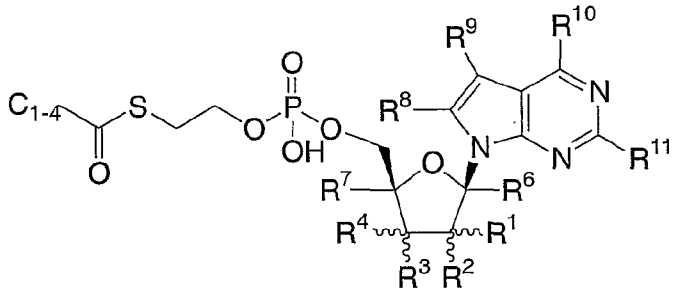


(IV)

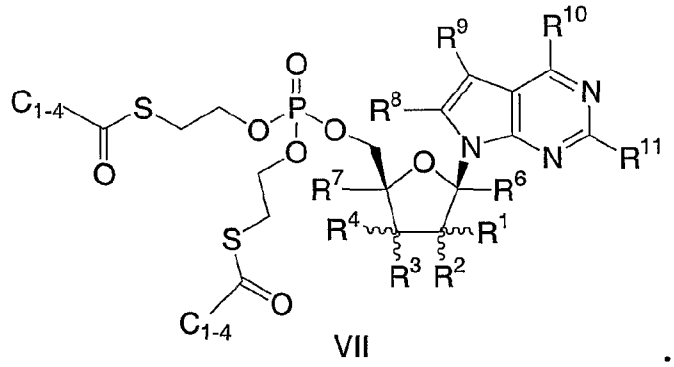


(V)

The term "5'-(S-acyl-2-thioethyl)phosphate" or "SATE" refers to a mono- or di-ester derivative of a 5'-monophosphate nucleoside derivative of the present invention of structural formulae VI and VII, respectively, as well as pharmaceutically acceptable salts of the mono-ester,



VI



VII

The term "composition", as in "pharmaceutical composition," is intended to encompass a product comprising the active ingredient(s) and the inert ingredient(s) that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing a compound of the present invention and a pharmaceutically acceptable carrier.

The terms "administration of" and "administering a" compound should be understood to mean providing a compound of the invention or a prodrug of a compound of the invention to the individual in need.

Another aspect of the present invention is concerned with a method of inhibiting HCV NS5B polymerase, inhibiting HCV replication, or treating HCV infection with a compound of the present invention in combination with one or more agents useful for treating HCV infection. Such agents active against HCV include, but are not limited to, ribavirin, levovirin, viramidine, thymosin alpha-1, interferon-α, pegylated interferon-α (peginterferon-α), a combination of interferon-α and ribavirin,

a combination of peginterferon-α and ribavirin, a combination of interferon-α and levovirin, and a combination of peginterferon-α and levovirin. Interferon-α includes, but is not limited to, recombinant interferon-α2a (such as Roferon interferon available from Hoffmann-LaRoche, Nutley, NJ), pegylated interferon-α2a (Pegasys™), interferon-α2b (such as Intron-A interferon available from Schering Corp., Kenilworth, NJ), pegylated interferon-α2b (PegIntron™), a recombinant consensus interferon (such as interferon alphacon-1), and a purified interferon-α product. Amgen's recombinant consensus interferon has the brand name Infergen®. Levovirin is the L-enantiomer of ribavirin which has shown immunomodulatory activity similar to ribavirin. Viramidine represents an analog of ribavirin disclosed in WO 01/60379 (assigned to ICN Pharmaceuticals). In accordance with this method of the present invention, the individual components of the combination can be administered separately at different times during the course of therapy or concurrently in divided or single combination forms. The instant invention is therefore to be understood as embracing all such regimes of simultaneous or alternating treatment, and the term "administering" is to be interpreted accordingly. It will be understood that the scope of combinations of the compounds of this invention with other agents useful for treating HCV infection includes in principle any combination with any pharmaceutical composition for treating HCV infection. When a compound of the present invention or a pharmaceutically acceptable salt thereof is used in combination with a second therapeutic agent active against HCV, the dose of each compound may be either the same as or different from the dose when the compound is used alone.

For the treatment of HCV infection, the compounds of the present invention may also be administered in combination with an agent that is an inhibitor of HCV NS3 serine protease. HCV NS3 serine protease is an essential viral enzyme and has been described to be an excellent target for inhibition of HCV replication. Both substrate and non-substrate based inhibitors of HCV NS3 protease inhibitors are disclosed in WO 98/22496, WO 98/46630, WO 99/07733, WO 99/07734, WO 99/38888, WO 99/50230, WO 99/64442, WO 00/09543, WO 00/59929, and GB-2337262. HCV NS3 protease as a target for the development of inhibitors of HCV replication and for the treatment of HCV infection is discussed in B.W. Dymock, "Emerging therapies for hepatitis C virus infection," Emerging Drugs, 6: 13-42 (2001).

Ribavirin, levovirin, and viramidine may exert their anti-HCV effects by modulating intracellular pools of guanine nucleotides via inhibition of the

intracellular enzyme inosine monophosphate dehydrogenase (IMPDH). IMPDH is the rate-limiting enzyme on the biosynthetic route in *de novo* guanine nucleotide biosynthesis. Ribavirin is readily phosphorylated intracellularly and the monophosphate derivative is an inhibitor of IMPDH. Thus, inhibition of IMPDH represents another useful target for the discovery of inhibitors of HCV replication. Therefore, the compounds of the present invention may also be administered in combination with an inhibitor of IMPDH, such as VX-497, which is disclosed in WO 97/41211 and WO 01/00622 (assigned to Vertex); another IMPDH inhibitor, such as that disclosed in WO 00/25780 (assigned to Bristol-Myers Squibb); or mycophenolate mofetil [see A.C. Allison and E.M. Eugui, Agents Action, 44 (Suppl.): 165 (1993)].

For the treatment of HCV infection, the compounds of the present invention may also be administered in combination with the antiviral agent amantadine (1-aminoadamantane) [for a comprehensive description of this agent, see J. Kirschbaum, Anal. Profiles Drug Subs. 12: 1-36 (1983)].

By "pharmaceutically acceptable" is meant that the carrier, diluent, or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Also included within the present invention are pharmaceutical compositions comprising the nucleoside compounds and derivatives thereof of the present invention in association with a pharmaceutically acceptable carrier. Another example of the invention is a pharmaceutical composition made by combining any of the compounds described above and a pharmaceutically acceptable carrier. Another illustration of the invention is a process for making a pharmaceutical composition comprising combining any of the compounds described above and a pharmaceutically acceptable carrier.

Also included within the present invention are pharmaceutical compositions useful for inhibiting RNA-dependent RNA viral polymerase in particular HCV NS5B polymerase comprising an effective amount of a compound of the present invention and a pharmaceutically acceptable carrier. Pharmaceutical compositions useful for treating RNA-dependent RNA viral infection in particular HCV infection are also encompassed by the present invention as well as a method of inhibiting RNA-dependent RNA viral polymerase in particular HCV NS5B polymerase and a method of treating RNA-dependent viral replication and in particular HCV replication. Additionally, the present invention is directed to a pharmaceutical composition comprising a therapeutically effective amount of a

compound of the present invention in combination with a therapeutically effective amount of another agent active against RNA-dependent RNA virus and in particular against HCV. Agents active against HCV include, but are not limited to, ribavirin, levovirin, viramidine, thymosin alpha-1, an inhibitor of HCV NS3 serine protease, interferon-α, pegylated interferon-α (peginterferon-α), a combination of interferon-α and ribavirin, a combination of peginterferon-α and ribavirin, a combination of interferon-α and levovirin, and a combination of peginterferon-α and levovirin. Interferon-α includes, but is not limited to, recombinant interferon-α2a (such as Roferon interferon available from Hoffmann-LaRoche, Nutley, NJ), interferon-α2b (such as Intron-A interferon available from Schering Corp., Kenilworth, NJ), a consensus interferon, and a purified interferon-α product. For a discussion of ribavirin and its activity against HCV, see J.O. Saunders and S.A. Raybuck, "Inosine Monophosphate Dehydrogenase: Consideration of Structure, Kinetics, and Therapeutic Potential," Ann. Rep. Med. Chem., 35: 201-210 (2000).

Another aspect of the present invention provides for the use of the nucleoside compounds and derivatives thereof and their pharmaceutical compositions for the manufacture of a medicament for the inhibition of RNA-dependent RNA viral replication, in particular HCV replication, and/or the treatment of RNA-dependent RNA viral infection, in particular HCV infection. Yet a further aspect of the present invention provides for the nucleoside compounds and derivatives thereof and their pharmaceutical compositions for use as a medicament for the inhibition of RNA-dependent RNA viral replication, in particular HCV replication, and/or for the treatment of RNA-dependent RNA viral infection, in particular HCV infection.

The pharmaceutical compositions of the present invention comprise a compound of structural formula I as an active ingredient or a pharmaceutically acceptable salt thereof, and may also contain a pharmaceutically acceptable carrier and optionally other therapeutic ingredients.

The compositions include compositions suitable for oral, rectal, topical, parenteral (including subcutaneous, intramuscular, and intravenous), ocular (ophthalmic), pulmonary (nasal or buccal inhalation), or nasal administration, although the most suitable route in any given case will depend on the nature and severity of the conditions being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well-known in the art of pharmacy.

In practical use, the compounds of structural formula I can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral (including intravenous). In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations such as, for example, powders, hard and soft capsules and tablets, with the solid oral preparations being preferred over the liquid preparations.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques. Such compositions and preparations should contain at least 0.1 percent of active compound. The percentage of active compound in these compositions may, of course, be varied and may conveniently be between about 2 percent to about 60 percent of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that an effective dosage will be obtained. The active compounds can also be administered intranasally as, for example, liquid drops or spray.

The tablets, pills, capsules, and the like may also contain a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin. When a dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier such as a fatty oil.

Various other materials may be present as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain, in addition to the active ingredient, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and a flavoring such as cherry or orange flavor.

Compounds of structural formula I may also be administered parenterally. Solutions or suspensions of these active compounds can be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

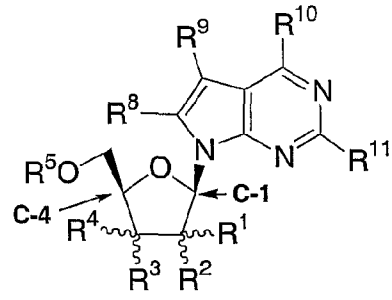
The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g. glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

Any suitable route of administration may be employed for providing a mammal, especially a human with an effective dosage of a compound of the present invention. For example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, and the like may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, and the like. Preferably compounds of structural formula I are administered orally.

For oral administration to humans, the dosage range is 0.01 to 1000 mg/kg body weight in divided doses. In one embodiment the dosage range is 0.1 to 100 mg/kg body weight in divided doses. In another embodiment the dosage range is 0.5 to 20 mg/kg body weight in divided doses. For oral administration, the compositions are preferably provided in the form of tablets or capsules containing 1.0 to 1000 milligrams of the active ingredient, particularly, 1, 5, 10, 15, 20, 25, 50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 750, 800, 900, and 1000 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated.

The effective dosage of active ingredient employed may vary depending on the particular compound employed, the mode of administration, the condition being treated and the severity of the condition being treated. Such dosage may be ascertained readily by a person skilled in the art. This dosage regimen may be adjusted to provide the optimal therapeutic response.

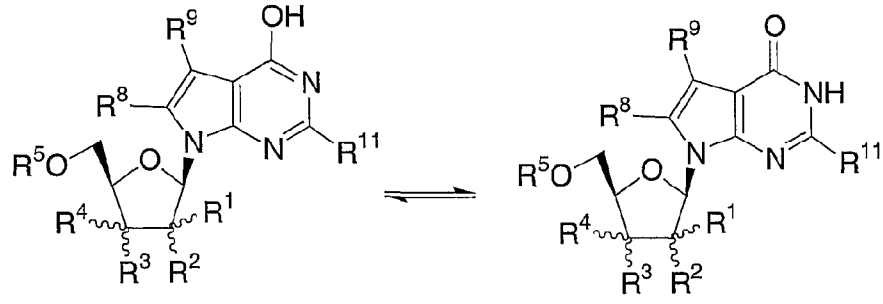
The compounds of the present invention contain one or more asymmetric centers and can thus occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereomers. The present invention is meant to comprehend nucleoside compounds having the β-D stereochemical configuration for the five-membered furanose ring as depicted in the structural formula below, that is, nucleoside compounds in which the substituents at C-1 and C-4 of the five-membered furanose ring have the β-stereochemical configuration ("up" orientation as denoted by a bold line).



β-D-

Some of the compounds described herein contain olefinic double bonds, and unless specified otherwise, are meant to include both E and Z geometric isomers.

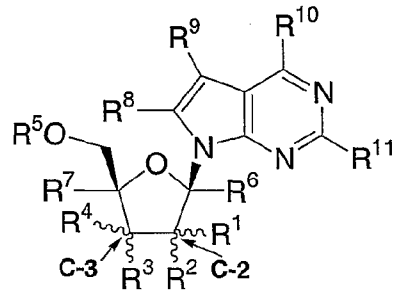
Some of the compounds described herein may exist as tautomers such as keto-enol tautomers. The individual tautomers as well as mixtures thereof are encompassed with compounds of structural formula I. An example of keto-enol tautomers which are intended to be encompassed within the compounds of the present invention is illustrated below:



Compounds of structural formula I may be separated into their individual diastereoisomers by, for example, fractional crystallization from a suitable solvent, for example methanol or ethyl acetate or a mixture thereof, or via chiral chromatography using an optically active stationary phase.

Alternatively, any stereoisomer of a compound of the structural formula I may be obtained by stereospecific synthesis using optically pure starting materials or reagents of known configuration.

The stereochemistry of the substituents at the C-2 and C-3 positions of the furanose ring of the compounds of the present invention of structural formula I is denoted by squiggly lines which signifies that substituents R^1, R^2, R^3 and R^4 can have either the α (substituent "down") or β (substituent "up") configuration independently of one another. Notation of stereochemistry by a bold line as at C-1 and C-4 of the furanose ring signifies that the substituent has the β-configuration (substituent "up").



(I)

The compounds of the present invention may be administered in the form of a pharmaceutically acceptable salt. The term "pharmaceutically acceptable salt" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and inorganic or organic acids. Salts of basic compounds encompassed within the term "pharmaceutically acceptable salt" refer to non-toxic salts of the compounds of this invention which are generally prepared by reacting the free base with a suitable organic or inorganic acid. Representative salts of basic compounds of the present invention include, but are not limited to, the following: acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate,

glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, N-methylglucamine ammonium salt, oleate, oxalate, pamoate (embonate), palmitate, pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, sulfate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide and valerate. Furthermore, where the compounds of the invention carry an acidic moiety, suitable pharmaceutically acceptable salts thereof include, but are not limited to, salts derived from inorganic bases including aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic, mangamous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, cyclic amines, and basic ion-exchange resins, such as arginine, betaine, caffeine, choline, N,N-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.

Also, in the case of a carboxylic acid (-COOH) or alcohol group being present in the compounds of the present invention, pharmaceutically acceptable esters of carboxylic acid derivatives, such as methyl, ethyl, or pivaloyloxymethyl, or acyl derivatives of alcohols, such as acetate or maleate, can be employed. Included are those esters and acyl groups known in the art for modifying the solubility or hydrolysis characteristics for use as sustained-release or prodrug formulations.

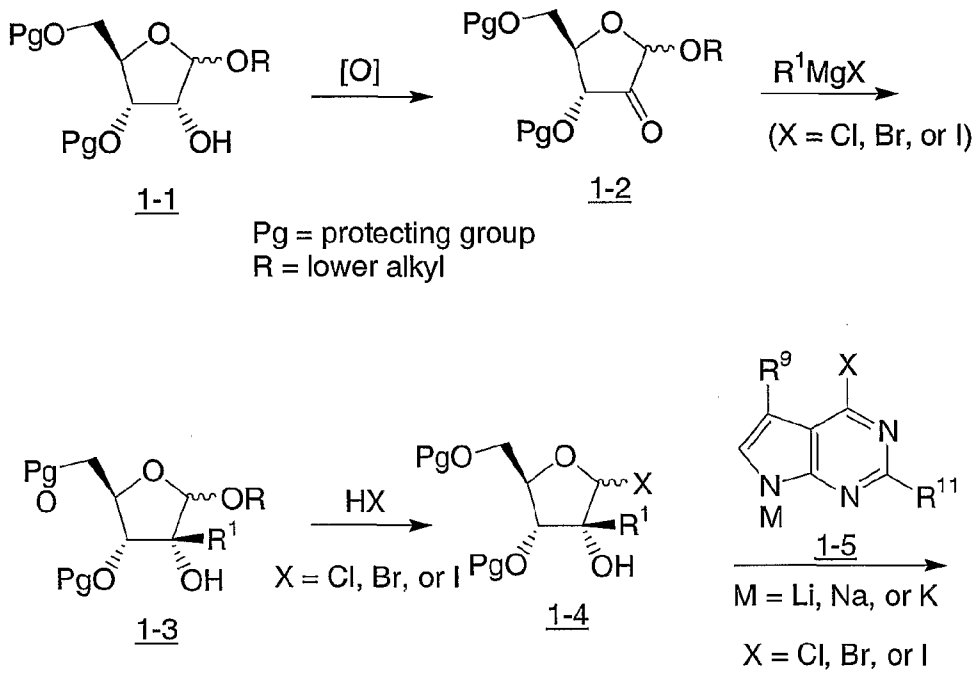
Preparation of the Nucleoside Compounds and Derivatives of the Invention

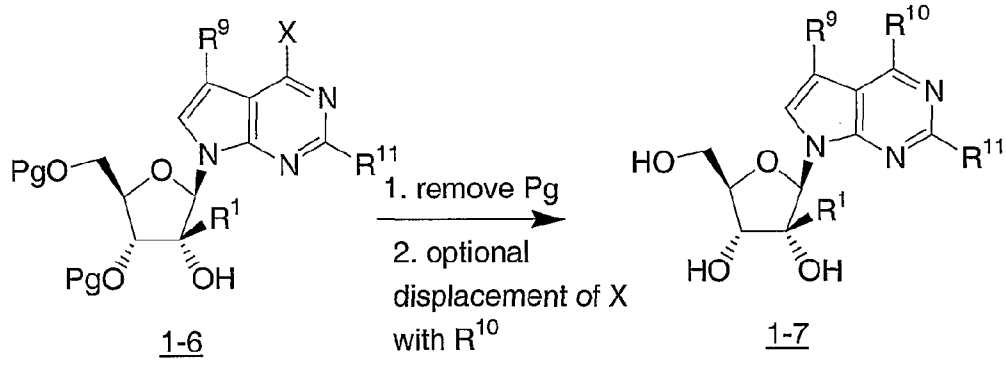
The nucleoside compounds and derivatives thereof of the present invention can be prepared following synthetic methodologies well-established in the practice of nucleoside and nucleotide chemistry. Reference is made to the following text for a description of synthetic methods used in the preparation of the compounds of the present invention: "Chemistry of Nucleosides and Nucleotides," L.B. Townsend, ed., Vols. 1-3, Plenum Press, 1988, which is incorporated by reference herein in its entirety.

A representative general method for the preparation of compounds of the present invention is outlined in Scheme 1 below. This scheme illustrates the synthesis of compounds of the present invention of structural formula 1-7 wherein the furanose ring has the β-D-ribo configuration. The starting material is a 3,5-bis-O-protected alkyl furanoside, such as methyl furanoside, of structural formula 1-1. The C-2 hydroxyl group is then oxidized with a suitable oxidizing agent, such as a chromium trioxide or chromate reagent, Dess-Martin periodinane, or by Swern oxidation, to afford a C-2 ketone of structural formula 1-2. Addition of a Grignard reagent, such as an alkyl, alkenyl, or alkynyl magnesium halide (for example, MeMgBr, EtMgBr, vinylMgBr, allylMgBr, and ethynylMgBr) or an alkyl, alkenyl, or alkynyl lithium, such as MeLi, across the carbonyl double bond of 1-2 in a suitable organic solvent, such as tetrahydrofuran, diethyl ether, and the like, affords the C-2 tertiary alcohol of structural formula 1-3. A good leaving group (such as Cl, Br, and I) is next introduced at the C-1 (anomeric) position of the furanose sugar derivative by treatment of the furanoside of formula 1-3 with a hydrogen halide in a suitable organic solvent, such as hydrogen bromide in acetic acid, to afford the intermediate furanosyl halide 1-4. A C-1 sulfonate, such methanesulfonate ($MeSO_2O$-), trifluoromethane-sulfonate (CF_3SO_2O-), or p-toluenesulfonate (-OTs), may also serve as a useful leaving group in the subsequent reaction to generate the glycosidic (nucleosidic) linkage. The nucleosidic linkage is constructed by treatment of the intermediate of structural formula 1-4 with the metal salt (such as lithium, sodium, or potassium) of an appropriately substituted 1*H*-pyrrolo[2,3-d]pyrimidine 1-5, such as an appropriately substituted 4-halo-1*H*-pyrrolo[2,3-d]pyrimidine, which can be generated *in situ* by treatment with an alkali hydride (such as sodium hydride), an alkali hydroxide (such as potassium hydroxide), an alkali carbonate (such as potassium carbonate), or an alkali hexamethyldisilazide (such as NaHMDS) in a suitable anhydrous organic solvent, such as acetonitrile, tetrahydrofuran, 1-methyl-2-pyrrolidinone, or N,N-dimethylformamide (DMF). The displacement reaction can be catalyzed by using a phase-transfer catalyst, such as TDA-1 or triethylbenzyl-ammonium chloride, in a two-phase system (solid-liquid or liquid-liquid). The optional protecting groups in the protected nucleoside of structural formula 1-6 are then cleaved following established deprotection methodologies, such as those described in T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," 3rd ed., John Wiley & Sons, 1999. Optional introduction of an amino group at the 4-position of the pyrrolo[2,3-d]pyrimidine nucleus is effected by

treatment of the 4-halo intermediate 1-6 with the appropriate amine, such as alcoholic ammonia or liquid ammonia, to generate a primary amine at the C-4 position (-NH_2), an alkylamine to generate a secondary amine (-NHR), or a dialkylamine to generate a tertiary amine (-NRR'). A 7H-pyrrolo[2,3-d]pyrimidin-4(3H)one compound may be derived by hydrolysis of 1-6 with aqueous base, such as aqueous sodium hydroxide. Alcoholysis (such as methanolysis) of 1-6 affords a C-4 alkoxide (-OR), whereas treatment with an alkyl mercaptide affords a C-4 alkylthio (-SR) derivative. Subsequent chemical manipulations well-known to practitioners of ordinary skill in the art of organic/medicinal chemistry may be required to attain the desired compounds of the present invention.

Scheme 1

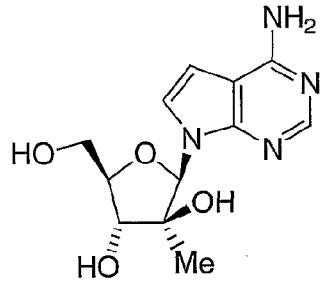




The examples below provide citations to literature publications, which contain details for the preparation of final compounds or intermediates employed in the preparation of final compounds of the present invention. The nucleoside compounds of the present invention were prepared according to procedures detailed in the following examples. The examples are not intended to be limitations on the scope of the instant invention in any way, and they should not be so construed. Those skilled in the art of nucleoside and nucleotide synthesis will readily appreciate that known variations of the conditions and processes of the following preparative procedures can be used to prepare these and other compounds of the present invention. All temperatures are degrees Celsius unless otherwise noted.

EXAMPLE 1

4-Amino-7-(2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



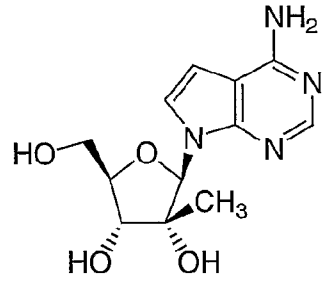
To chromium trioxide (1.57 g, 1.57 mmol) in dichloromethane (DCM) (10 mL) at 0°C was added acetic anhydride (145 mg, 1.41 mmol) and then pyridine (245 mg, 3.10 mmol). The mixture was stirred for 15 min, then a solution of 7-[3,5-*O*-[1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl]-β-D-ribofuranosyl]-7*H*-

pyrrolo[2,3-*d*]pyrimidin-4-amine [for preparation, see J. Am. Chem. Soc. 105: 4059 (1983)] (508 mg, 1.00 mmol) in DCM (3 mL) was added. The resulting solution was stirred for 2 h and then poured into ethyl acetate (10 mL), and subsequently filtered through silica gel using ethyl acetate as the eluent. The combined filtrates were evaporated *in vacuo*, taken up in diethyl ether/THF (1:1) (20 mL), cooled to -78°C and methylmagnesium bromide (3M, in THF) (3.30 mL, 10 mmol) was added dropwise. The mixture was stirred at -78°C for 10 min, then allowed to come to room temperature (rt) and quenched by addition of saturated aqueous ammonium chloride (10 mL) and extracted with DCM (20 mL). The organic phase was evaporated *in vacuo* and the crude product purified on silica gel using 5% methanol in dichloromethane as eluent. Fractions containing the product were pooled and evaporated *in vacuo*. The resulting oil was taken up in THF (5 mL) and tetrabutylammonium fluoride (TBAF) on silica (1.1 mmol/g on silica) (156 mg) was added. The mixture was stirred at rt for 30 min, filtered, and evaporated *in vacuo*. The crude product was purified on silica gel using 10% methanol in dichloromethane as eluent. Fractions containing the product were pooled and evaporated *in vacuo* to give the desired compound (49 mg) as a colorless solid.

^{1}H NMR (DMSO-d_6): δ 1.08 (s, 3H), 3.67 (m, 2H), 3.74 (m, 1H), 3.83 (m, 1H), 5.19 (m, 1H), 5.23 (m, 1H), 5.48 (m, 1H), 6.08 (1H, s), 6.50 (m, 1H), 6.93 (bs, 2H), 7.33 (m, 1H), 8.02 (s, 1H).

EXAMPLE 2

4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-ribofuranose

A mixture of 2-*O*-acetyl-3,5-bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-ribofuranose [for preparation, see: Helv. Chim. Acta 78: 486 (1995)]

(52.4 g, 0.10 mol) in methanolic K_2CO_3 (500 mL, saturated at rt) was stirred at room temperature for 45 min. and then concentrated under reduced pressure. The oily residue was suspended in CH_2Cl_2 (500 mL), washed with water (300 mL + 5 × 200 mL) and brine (200 mL), dried (Na_2SO_4), filtered, and concentrated to give the title compound (49.0 g) as colorless oil, which was used without further purification in Step B below.

^1H NMR (DMSO-d_6): δ 3.28 (s, 3H, OCH_3), 3.53 (d, 2H, $J_{5,4}$ = 4.5 Hz, H-5a, H-5b), 3.72 (dd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{3,2}$ = 6.6 Hz, H-3), 3.99 (ddd, 1H, $J_{2,1}$ = 4.5 Hz, $J_{2,OH-2}$ = 9.6 Hz, H-2), 4.07 (m, 1H, H-4), 4.50 (s, 2H, CH_2Ph), 4.52, 4.60 (2d, 2H, J_{gem} = 13.6 Hz, CH_2Ph), 4.54 (d, 1H, OH-2), 4.75 (d, 1H, H-1), 7.32-7.45, 7.52-7.57 (2m, 10H, 2Ph).

^{13}C NMR (DMSO-d_6): δ 55.40, 69.05, 69.74, 71.29, 72.02, 78.41, 81.45, 103.44, 127.83, 127.95, 129.05, 129.28, 131.27, 131.30, 133.22, 133.26, 133.55, 133.67, 135.45, 135.92.

Step B: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-*erythro*-pentofuranos-2-ulose

To an ice-cold suspension of Dess-Martin periodinane (50.0 g, 118 mmol) in anhydrous CH_2Cl_2 (350 mL) under argon (Ar) was added a solution of the compound from Step A (36.2 g, 75 mmol) in anhydrous CH_2Cl_2 (200 mL) dropwise over 0.5 h. The reaction mixture was stirred at 0°C for 0.5 h and then at room temperature for 3 days. The mixture was diluted with anhydrous Et_2O (600 mL) and poured into an ice-cold mixture of $Na_2S_2O_3.5H_2O$ (180 g) in saturated aqueous $NaHCO_3$ (1400 mL). The layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ (600 mL), water (800 mL) and brine (600 mL), dried ($MgSO_4$), filtered and evaporated to give the title compound (34.2 g) as a colorless oil, which was used without further purification in Step C below.

^1H NMR ($CDCl_3$): δ 3.50 (s, 3H, OCH_3), 3.79 (dd, 1H, $J_{5a,5b}$ = 11.3 Hz, $J_{5a,4}$ = 3.5 Hz, H-5a), 3.94 (dd, 1H, $J_{5b,4}$ = 2.3 Hz, H-5b), 4.20 (dd, 1H, $J_{3,1}$ = 1.3 Hz, $J_{3,4}$ = 8.4 Hz, H-3), 4.37 (ddd, 1H, H-4), 4.58, 4.69 (2d, 2H, J_{gem} = 13.0 Hz, CH_2Ph), 4.87 (d, 1H, H-1), 4.78, 5.03 (2d, 2H, J_{gem} = 12.5 Hz, CH_2Ph), 7.19-7.26, 7.31-7.42 (2m, 10H, 2Ph).

^{13}C NMR (DMSO-d_6): δ 55.72, 69.41, 69.81, 69.98, 77.49, 78.00, 98.54, 127.99, 128.06, 129.33, 129.38, 131.36, 131.72, 133.61, 133.63, 133.85, 133.97, 134.72, 135.32, 208.21.

Step C: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose

To a solution of MeMgBr in anhydrous Et_2O (0.48 M, 300 mL) at –55 °C was added dropwise a solution of the compound from Step B (17.40 g, 36.2 mmol) in anhydrous Et_2O (125 mL). The reaction mixture was allowed to warm to –30°C and stirred for 7 h at –30°C to –15°C, then poured into ice-cold water (500 mL) and the mixture vigorously stirred at room temperature for 0.5 h. The mixture was filtered through a Celite pad (10 × 5 cm) which was thoroughly washed with Et_2O. The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in hexanes (~30 mL), applied onto a silica gel column (10 × 7 cm, prepacked in hexanes) and eluted with hexanes and hexanes/EtOAc (9/1) to give the title compound (16.7 g) as a colorless syrup.

^{1}H NMR ($CDCl_3$): δ 1.36 (d, 3H, $J_{Me,OH}$ = 0.9 Hz, 2C-Me), 3.33 (q, 1H, OH), 3.41 (d, 1H, $J_{3,4}$ = 3.3 Hz), 3.46 (s, 3H, OCH_3), 3.66 (d, 2H, $J_{5,4}$ = 3.7 Hz, H-5a, H-5b), 4.18 (apparent q, 1H, H-4), 4.52 (s, 1H, H-1), 4.60 (s, 2H, C*H*$_2$Ph), 4.63, 4.81 (2d, 2H, J_{gem} = 13.2 Hz, C*H*$_2$Ph), 7.19-7.26, 7.34-7.43 (2m, 10H, 2Ph).

^{13}C NMR ($CDCl_3$): δ 24.88, 55.45, 69.95, 70.24, 70.88, 77.06, 82.18, 83.01, 107.63, 127.32, 129.36, 130.01, 130.32, 133.68, 133.78, 134.13, 134.18, 134.45, 134.58.

Step D: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step C (9.42 g, 19 mmol) in anhydrous dichloromethane (285 mL) at 0°C was added HBr (5.7 M in acetic acid, 20 mL, 114 mmol) dropwise. The resulting solution was stirred at 0°C for 1 h and then at rt for 3 h, evaporated *in vacuo* and co-evaporated with anhydrous toluene (3 × 40 mL). The oily residue was dissolved in anhydrous acetonitrile (50 mL) and added to a solution of the sodium salt of 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine in acetonitrile [generated *in situ* from 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine [for preparation, see: J. Chem. Soc.: 131 (1960)] (8.76 g, 57 mmol) in anhydrous acetonitrile (1000 mL), and NaH (60% in mineral oil, 2.28 g, 57 mmol), after 4 h of vigorous stirring at rt]. The combined mixture was stirred at rt for 24 h, and then evaporated to dryness. The residue was suspended in water (250 mL) and extracted with EtOAc (2 × 500 mL). The combined extracts were washed with brine (300 mL, dried over Na_2SO_4, filtered and evaporated. The crude product was purified on a silica gel column (10 cm × 10

cm) using ethyl acetate/hexane (1:3 and 1:2) as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product (5.05 g) as a colorless foam.

^{1}H NMR ($CDCl_3$): δ 0.93 (s, 3H, CH_3), 3.09 (s, 1H, OH), 3.78 (dd, 1H, $J_{5',5''}$ = 10.9 Hz, $J_{5',4}$ = 2.5 Hz, H-5'), 3.99 (dd, 1H, $J_{5'',4}$ = 2.2 Hz, H-5''), 4.23-4.34 (m, 2H, H-3', H-4'), 4.63, 4.70 (2d, 2H, J_{gem} = 12.7 Hz, CH_2Ph), 4.71, 4.80 (2d, 2H, J_{gem} = 12.1 Hz,CH_2Ph), 6.54 (d, 1H, , $J_{5,6}$ = 3.8 Hz, H-5), 7.23-7.44 (m, 10H, 2Ph).

^{13}C NMR ($CDCl_3$): δ 21.31, 69.10, 70.41, 70.77, 79.56, 80.41, 81.05, 91.11, 100.57, 118.21, 127.04, 127.46, 127.57, 129.73, 129.77, 130.57, 130.99, 133.51, 133.99, 134.33, 134.38, 134.74, 135.21, 151.07, 151.15 152.47.

Step E: 4-Chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step D (5.42 g, 8.8 mmol) in dichloromethane (175 mL) at -78°C was added boron trichloride (1M in dichloromethane, 88 mL, 88 mmol) dropwise. The mixture was stirred at –78°C for 2.5 h, then at –30°C to –20°C for 3h. The reaction was quenched by addition of methanol/dichloromethane (1:1) (90 mL) and the resulting mixture stirred at –15°C for 30 min., then neutralized with aqueous ammonia at 0°C and stirred at rt for 15 min. The solid was filtered and washed with CH_2Cl_2/MeOH (1/1, 250 mL). The combined filtrate was evaporated, and the residue was purified by flash chromatography over silica gel using CH_2Cl_2 and CH_2Cl_2:MeOH (99:1, 98:2, 95:5 and 90:10) gradient as the eluent to furnish desired compound (1.73 g) as a colorless foam, which turned into an amorphous solid after treatment with MeCN.

^{1}H NMR (DMSO-d_6): δ 0.64 (s, 3H, CH_3), 3.61-3.71 (m, 1H, H-5'), 3.79-3.88 (m, 1H, H-5''), 3.89-4.01 (m, 2H, H-3', H-4'), 5.15-5.23 (m, 3H, 2'-OH, 3'-OH, 5'-OH), 6.24 (s, 1H, H-1'), 6.72 (d, 1H, $J_{5,6}$ = 3.8 Hz, H-5), 8.13 (d, 1H, H-6), 8.65 (s, 1H, H-2).

^{13}C NMR (DMSO-d_6): δ 20.20, 59.95, 72.29, 79.37, 83.16, 91.53, 100.17, 117.63, 128.86, 151.13, 151.19, 151.45.

Step F: 4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To the compound from Step E (1.54 g, 5.1 mmol) was added methanolic ammonia (saturated at 0°C; 150 mL). The mixture was heated in a

stainless steel autoclave at 85°C for 14 h, then cooled and evaporated *in vacuo*. The crude mixture was purified on a silica gel column with CH_2Cl_2/MeOH (9/1) as eluent to give the title compound as a colorless foam (0.8 g), which separated as an amorphous solid after treatment with MeCN. The amorphous solid was recrystallized from methanol/acetonitrile; m.p. 222°C.

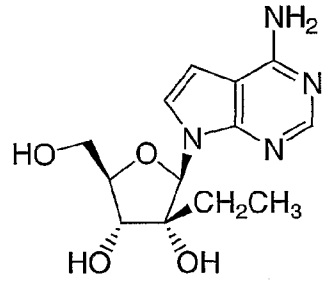
^{1}H NMR (DMSO-d_6) δ 0.62 (s, 3H, CH_3), 3.57-3.67 (m, 1H, H-5'), 3.75-3.97 (m, 3H, H-5", H-4', H-3'), 5.00 (s, 1H, 2'-OH), 5.04 (d, 1H, $J_{3'OH,3'}$ = 6.8 Hz, 3'-OH), 5.06 (t, 1H, $J_{5'OH,5',5"}$ = 5.1 Hz, 5'-OH), 6.11 (s, 1H, H-1'), 6.54 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5), 6.97 (br s, 2H, NH_2), 7.44 (d, 1H, H-6), 8.02 (s, 1H, H-2).

^{13}C NMR (DMSO-d_6) δ 20.26, 60.42, 72.72, 79.30, 82.75, 91.20, 100.13, 103.08, 121.96, 150.37, 152.33, 158.15.

LC-MS: Found: 279.10 ($M-H^+$); calc. for $C_{12}H_{16}N_4O_4+H^+$: 279.11.

EXAMPLE 3

4-Amino-7-(2-*C*-ethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-ethyl-1-*O*-methyl-α-D-ribofuranose

To diethyl ether (300 mL) at –78°C was slowly added EtMgBr (3.0 M, 16.6 mL) and then dropwise the compound from Step B of Example 2 (4.80 g, 10.0 mmol) in anhydrous Et_2O (100 mL). The reaction mixture was stirred at –78 °C for 15 min, allowed to warm to –15°C and stirred for another 2 h, and then poured into a stirred mixture of water (300 mL) and Et_2O (600 mL). The organic phase was separated, dried ($MgSO_4$), and evaporated *in vacuo*. The crude product was purified on silica gel using ethyl acetate/hexane (1:2) as eluent. Fractions containing the product were pooled and evaporated *in vacuo* to give the desired product (3.87 g) as a colorless oil.

Step B: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-ethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step A (1.02 mg, 2.0 mmol) in dichloromethane (40 mL) was added HBr (5.7 M in acetic acid) (1.75 mL, 10.0 mmol) dropwise at 0°C. The resulting solution was stirred at room temperature for 2 h, evaporated *in vacuo* and co-evaporated twice from toluene (10 mL). The oily residue was dissolved in acetonitrile (10 mL) and added to a vigorously stirred mixture of 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine (307 mg, 2.0 mmol), potassium hydroxide (337 mg, 6.0 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (130 mg, 0.4 mmol) in acetonitrile (10 mL). The resulting mixture was stirred at rt overnight, and then poured into a stirred mixture of saturated ammonium chloride (100 mL) and ethyl acetate (100 mL). The organic layer was separated, washed with brine (100 mL), dried over $MgSO_4$, filtered and evaporated *in vacuo*. The crude product was purified on silica gel using ethyl acetate/hexane (1:2) as eluent to give the desired product (307 mg) as a colorless foam.

Step C: 4-Chloro-7-(2-*C*-ethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step B (307 mg, 0.45 mmol) in dichloromethane (8 mL) was added boron trichloride (1M in dichloromethane) (4.50 mL, 4.50 mmol) at -78°C. The mixture was stirred at -78°C for 1h, then at -10°C for 3h. The reaction was quenched by addition of methanol/dichloromethane (1:1) (10 mL), stirred at -15°C for 30 min, and neutralized by addition of aqueous ammonium hydroxide. The mixture was evaporated under diminished pressure and the resulting oil purified on silica gel using methanol/dichloromethane (1:9) as eluent. Fractions containing the product were pooled and evaporated *in vacuo* to give the desired product (112 mg) as a colorless foam.

Step D: 4-Amino-7-(2-*C*-ethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To the compound from Step C (50 mg, 0.16 mmol) was added saturated ammonia in methanol (4 mL). The mixture was stirred at 75°C for 72 h in a closed container, cooled and evaporated *in vacuo*. The crude mixture was purified on silica gel using methanol/dichloromethane (1:9) as eluent. Fractions containing the

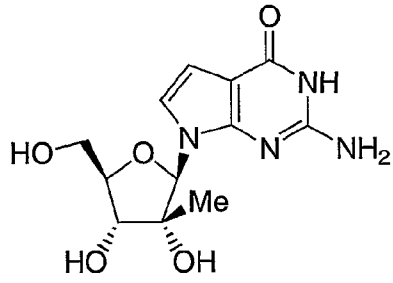
product were pooled and evaporated *in vacuo* to give the desired product (29 mg) as a colorless powder.

^{1}HNMR (200 MHz, DMSO-d_6): δ 0.52 (t, 3H), 1.02 (m, 2H), 4.01-3.24 (m, 6H), 5.06 (m, 1H), 6.01 (s, 1H), 6.51 (d, 1H), 6.95 (s br, 2H), 6.70 (d, 1H), 7.99 (s, 1H).

LC-MS: Found: 295.2 ($M+H^+$); calc. for $C_{13}H_{18}N_4O_4+H^+$: 295.14.

EXAMPLE 4

2-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one



Step A: 2-Amino-4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cold solution of the product from Step C of Example 2 (1.27 g, 2.57 mmol) in CH_2Cl_2 (30 mL) was added HBr (5.7 M in acetic acid; 3 mL) dropwise. The reaction mixture was stirred at room temperature for 2 h, concentrated under diminished pressure and coevaporated with toluene (2 × 15 mL). The resulting oil was dissolved in acetonitrile (MeCN) (15 mL) and added dropwise into a well-stirred mixture of 2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine [for preparation, see Heterocycles 35: 825 (1993)] (433 mg, 2.57 mmol), KOH (85%, powdered) (0.51 g, 7.7 mmol), tris-[2-(2-methoxyethoxy)ethyl]amine (165 μL, 0.51 mmol) in acetonitrile (30 mL). The resulting mixture was stirred at rt for 1h, filtered and evaporated. The residue was purified on a silica gel column using hexanes/EtOAc, 5/1, 3/1 and 2/1, as eluent to give the title compound as a colorless foam (0.65 g).

Step B: 2-Amino-4-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the product from Step A (630 mg, 1.0 mmol) in CH_2Cl_2 (20 mL) at -78°C was added boron trichloride (1M in CH_2Cl_2) (10 mL, 10

mmol). The mixture was stirred at -78°C for 2 h, then at -20°C for 2.5 h. The reaction was quenched with CH_2Cl_2/MeOH (1:1) (10 mL), stirred at -20°C for 0.5 h, and neutralized at 0°C with aqueous ammonia. The solid was filtered, washed with CH_2Cl_2/MeOH (1:1) and the combined filtrate evaporated *in vacuo*. The residue was purified on a silica gel column with CH_2Cl_2/MeOH, 50/1 and 20/1, as eluent to give the title compound as a colorless foam (250 mg).

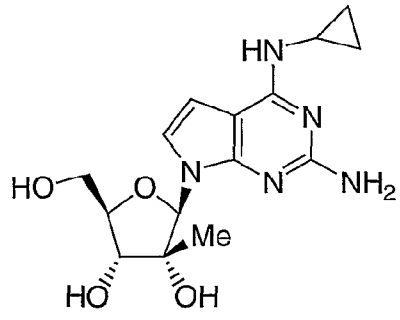
Step C: 2-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one

A mixture of the product from Step B (90 mg, 0.3 mmol) in aqueous NaOH (2N, 9 mL) was heated at reflux temperature for 5 h, then neutralized at 0°C with 2 N aqueous HCl and evaporated to dryness. Purification on a silica gel column with CH_2Cl_2/MeOH, 5/1, as eluent afforded the title compound as a white solid (70 mg).

^{1}H NMR (200 MHz, CD_3OD): δ 0.86 (s, 3H), 3.79 (m 1H), 3.90-4.05 (m, 3H), 6.06 (s, 1H), 6.42 (d, J = 3.7 Hz, 1H), 7.05 (d, J = 3.7 Hz, 1H).

EXAMPLE 5

2-Amino-4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

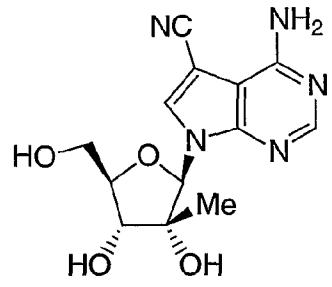


A solution of 2-amino-4-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (Example 4, Step B) (21 mg, 0.07 mmol) in cyclopropylamine (0.5 mL) was heated at 70°C for two days, then evaporated to an oily residue and purified on a silica gel column with CH_2Cl_2/MeOH, 20/1, as eluent to give the title compound as a white solid (17 mg).

^{1}H NMR (200 MHz, CD_3CN): δ 0.61 (m, 2H), 0.81 (m, 2H), 0.85 (s, 3H), 2.83 (m, 1H), 3.74-3.86 (m, 1H), 3.93-4.03 (m, 2H), 4.11 (d, J = 8.9 Hz, 1H), 6.02 (s, 1H), 6.49 (d, J = 3.7 Hz, 1H), 7.00 (d, J = 3.7 Hz, 1H).

EXAMPLE 6

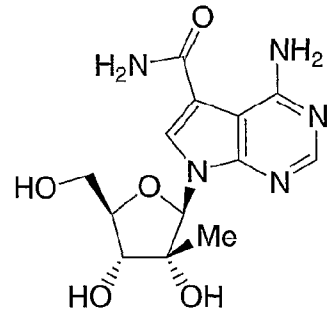
4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile



This compound was prepared following procedures described by Y. Murai et al. in Heterocycles 33: 391-404 (1992).

EXAMPLE 7

4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide



This compound was prepared following procedures described by Y. Murai et al. in Heterocycles 33: 391-404 (1992).

EXAMPLE 8

General process to SATE prodrug moiety

S-Acyl-2-thioethyl (SATE) pronucleotides are discussed in C.R. Wagner, V.V. Iyer, and E.J. McIntee, "Pronucleotides: Toward the In Vivo Delivery of Antiviral and Anticancer Nucleotides," Med. Res. Rev., 20: 1-35 (2000), which is incorporated by reference herein in its entirety. SATE derivatives of nucleosides are also disclosed U.S. Patent Nos. 5,770,725; 5,849,905; and 6,020,482, the contents of each of which are incorporated by reference herein in their entirety.

Bis(*S*-acetyl-2-thioethyl)-*N*,*N*-diisopropylphosphoramidite

2-Mercaptoethanol (5 g, 64 mmol) was dissolved in CH_2Cl_2 (50 mL). To this solution was added triethylamine (7.67 mL, 57.6 mmol), and the reaction mixture was cooled in an ice bath to 0 °C. Acetic anhydride (4.54 mL, 48 mmol) was added dropwise in 10 min., and the reaction mixture was stirred for 1 h at 0 °C. The reaction mixture was then allowed to come to room temperature over a period of 2 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with water (75 mL), 5% aqueous $NaHCO_3$ (75 mL) and brine (75 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oil. The oil was then dissolved in anhydrous THF (40 mL) and anhydrous triethylamine (7.76 mL) was added. To this mixture was added activated molecular sieves (4Å) and was kept at room temperature for 10 min. The reaction mixture was cooled in an ice bath to 0°C and diisopropylphosphoramidous dichloride (6.47 g, 32.03 mmol) was added. The reaction mixture was stirred at 0 °C for 2 h under inert atmosphere. Hexane (40 mL) was added to the reaction mixture and the precipitate formed was filtered. The filtrate was concentrated to one fourth of the volume, purified by loaded silica gel column chromatography and eluted with hexane containing 3 % triethylamine and incremental amount of ethyl acetate (0 to 7 %) to give the title compound as an oil (2.36 g). ^{1}H NMR ($CDCl_3$): δ 1.17 (s, 6H), 1.21 (s, 6H), 2.36 (s, 6H), 3.14 (t, J = 6.44 Hz), 3.51-3.84 (m, 6H); ^{13}C NMR ($CDCl_3$): δ 24.47, 24.61, 30.48, 42.85, 43.1, 61.88, 62.23, 195.26; ^{13}P NMR ($CDCl_3$): δ 146.96.

EXAMPLE 9

5'-Triphosphate Derivatives

The nucleoside 5'-triphosphates of the present invention were prepared following general procedures described in *Chem. Rev.* 100: 2047 (2000).

EXAMPLE 10

Purification and Purity Analysis of 5'-Triphosphate Derivatives

The triphosphate derivatives were purified by anion exchange (AX) chromatography using a 30 x 100 mm Mono Q column (Pharmacia) with a buffer system of 50 mM Tris, pH 8. Elution gradients were typically from 40 mM NaCl to 0.8 M NaCl in two column volumes at 6.5 mL/min. Appropriate fractions from anion exchange chromatography were collected and desalted by reverse-phase (RP) chromatography using a Luna C18 250 × 21 mm column (Phenomenex) with a flow rate of 10 mL/min. Elution gradients were generally from 1% to 95% methanol in 14 min at a constant concentration of 5 mM triethylammonium acetate (TEAA).

Mass spectra of the purified triphosphates were determined using on-line HPLC mass spectrometry on a Hewlett-Packard (Palo Alto, CA) MSD 1100. A Phenomenex Luna (C18(2)), 150 × 2 mm, plus 30 x 2 mm guard column, 3-μm particle size was used for RP HPLC. A 0 to 50% linear gradient (15 min) of acetonitrile in 20 mM TEAA (triethylammonium acetate) pH 7 was performed in series with mass spectral detection in the negative ionization mode. Nitrogen gas and a pneumatic nebulizer were used to generate the electrospray. The mass range of 150-900 was sampled. Molecular masses were determined using the HP Chemstation analysis package.

The purity of the purified triphosphates was determined by analytical RP and AX HPLC. RP HPLC with a Phenomonex Luna or Jupiter column (250 × 4.6 mm), 5-μm particle size was typically run with a 2-70% acetonitrile gradient in 15 min in 100 mM TEAA, pH 7. AX HPLC was performed on a 1.6 × 5 mm Mono Q column (Pharmacia). Triphosphates were eluted with a gradient of 0 to 0.4 M NaCl at constant concentration of 50 mM Tris, pH 8. The purity of the triphosphates was generally >80%.

EXAMPLE 11

5'-Monophosphate Derivatives

The nucleoside 5'-monophosphates of the present invention were prepared following the general procedures described in *Tetrahedron Lett.* 50: 5065 (1967).

EXAMPLE 12

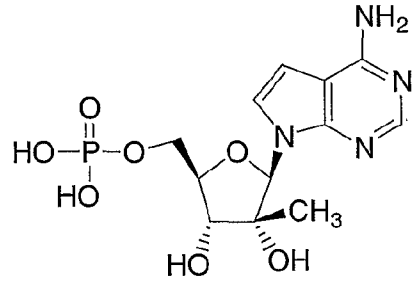
Mass Spectral Characterization of 5'-Triphosphate Derivatives

Mass spectra of 5'-triphosphates of the compounds of the present invention were determined as described in Example 10. Listed in the following table are the calculated and experimental masses for representative 5'-triphosphates prepared according to the procedures of Example 9. The example numbers correspond to the parent compound of the 5'-triphosphate.

Example	Calculated	Found
1	520.0	519.9
2	520.0	520.0
3	534.0	534.0
4	536.0	536.0

EXAMPLE 13

[4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine]-5'-monophosphate



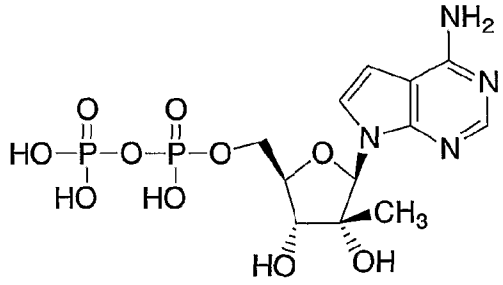
To the compound from Step F of Example 2 (14 mg, 0.05 mmol) (dried by coevaporation with pyridine and several times with toluene) was added trimethyl phosphate (0.5 mL). The mixture was stirred overnight in a sealed container. It was then cooled to 0°C and phosphorous oxychloride (0.0070 mL, 0.075

mmol) was added via a syringe. The mixture was stirred for 3 h at 0°C, then the reaction was quenched by addition of tetraethylammonium bicarbonate (TEAB) (1M) (0.5 mL) and water (5 mL). The reaction mixture was purified and analyzed according to the procedure described in Example 10.

Electron spray mass spectrum (ES-MS): Found: 359.2 ($M-H^+$), calc. for $C_{12}H_{17}N_4O_7P - H^+$: 359.1.

EXAMPLE 14

[4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine]-5'-diphosphate

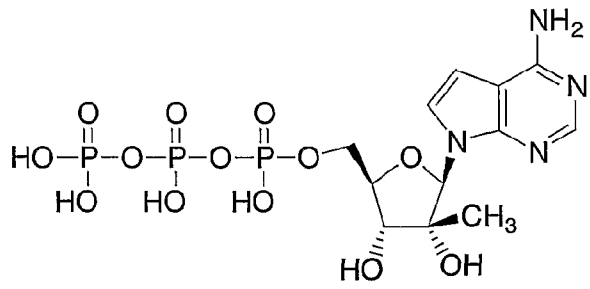


To the compound from Step F of Example 2 (56 mg, 0.20 mmol) (dried by coevaporation with pyridine and several times with toluene) was added trimethyl phosphate (stored over sieves) (1.0 mL). The mixture was stirred overnight in a sealed container. It was then cooled to 0°C and phosphorous oxychloride (0.023 mL, 0.25 mmol) was added via a syringe. The mixture was stirred for 2 h at 0°C, then tributylamine (0.238 mL, 1.00 mmol) and tributylammonium phosphate (generated from phosphoric acid and tributylamine in pyridine, followed by repeated azeotropic evaporation with pyridine and acetonitrile) (1.0 mmol in 3.30 mL acetonitrile) was added. The mixture was stirred for an additional 30 min at 0°C, the sealed vial was then opened and the reaction quenched by addition of TEAB (1M) (1.0 mL) and water (5 mL). The reaction mixture was purified and analyzed according to the procedure described in Example 10.

ES-MS: Found: 439.0 ($M-H^+$), calc. for $C_{12}H_{18}N_4O_{10}P_2 - H^+$: 439.04.

EXAMPLE 15

[4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine]-5'-triphosphate

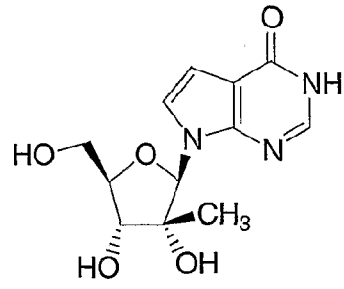


To the compound from Step F of Example 2 (20 mg, 0.07 mmol) (dried by coevaporation with pyridine and several times with toluene) was added trimethyl phosphate (stored over sieves) (0.4 mL). The mixture was stirred overnight in a sealed container. It was then cooled to 0°C and phosphorous oxychloride (0.0070 mL, 0.075 mmol) was added via syringe. The mixture was stirred for 3 h at 0°C, then tributylamine (0.083 mL, 0.35 mmol), tributylammonium pyrophosphate (127 mg, 0.35 mmol) and acetonitrile (stored over sieves) (0.25 mL) were added. The mixture was stirred for an additional 30 min at 0°C, the sealed vial was then opened and the reaction quenched by addition of TEAB (1M) (0.5 mL) and water (5 mL). The reaction mixture was purified and analyzed according to the procedure described in Example 10.

ES-MS: Found: 519.0 ($M-H^+$), calc. for $C_{12}H_{19}N_4O_{13}P_3$- H^+: 519.01.

EXAMPLE 16

7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one

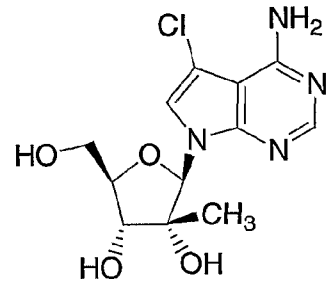


To the compound from Step E of Example 2 (59 mg, 0.18 mmol) was added aqueous sodium hydroxide (1M). The mixture was heated to reflux for 1hr, cooled, neutralized with aqueous HCl (2M) and evaporated in vacuo. The residue was purified on silica gel using dichloromethane/methanol (4:1) as eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (53 mg) as a colorless oil.

^{1}H NMR (CD_3CN): δ 0.70 (s, 3H), 3.34-4.15 (overlapping m, 7H), 6.16 (s, 1H), 6.57 (d, 3.6 Hz, 1H), 7.37 (d, 3.6 Hz, 1H), 8.83 (s, 1H).

EXAMPLE 17

4-Amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



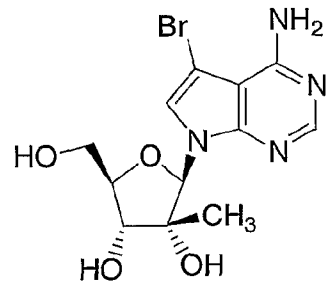
To a pre-cooled solution (0°C) of the compound from Step F of Example 2 (140 mg, 0.50 mmol) in DMF (2.5 mL) was added *N*-chlorosuccinimide (0.075 g, 0.55 mmol) in DMF (0.5 mL) dropwise. The solution was stirred at rt for 1h and the reaction quenched by addition of methanol (4 mL) and evaporated in vacuo. The crude product was purified on silica gel using methanol/dichloromethane (1:9) as eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (55 mg) as a colorless solid.

^{1}H NMR (CD_3CN): δ 0.80 (s, 3H), 3.65-4.14 (overlapping m, 7H), 5.97 (s br, 2H), 6.17 (s, 1H), 7.51 (s, 1H), 8.16 (s, 1H).

ES-MS: Found: 315.0 ($M+H^+$), calc.for $C_{12}H_{15}ClN_4O_4 + H^+$: 315.09.

EXAMPLE 18

4-Amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



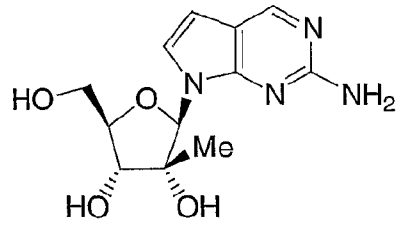
To a pre-cooled solution (0°C) of the compound from Step F of Example 2 (28 mg, 0.10 mmol) in DMF (0.5 mL) was added *N*-bromosuccinimide (0.018 g, 0.10 mmol) in DMF (0.5 mL) dropwise. The solution was stirred at 0°C for 20 min, then at rt for 10 min. The reaction was quenched by addition of methanol (4 mL) and evaporated in vacuo. The crude product was purified on silica gel using methanol/dichloromethane (1:9) as eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (13.0 mg) as a colorless solid.

^{1}H NMR (CD_3CN): δ 0.69 (s, 3H), 3.46-4.00 (overlapping m, 7H), 5.83 (s br, 2H), 6.06 (s, 1H), 7.45 (s, 1H), 8.05 (s, 1H).

ES-MS: Found: 359.1 ($M+H^+$), calc.for $C_{12}H_{15}BrN_4O_4 + H^+$: 359.04.

EXAMPLE 19

2-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



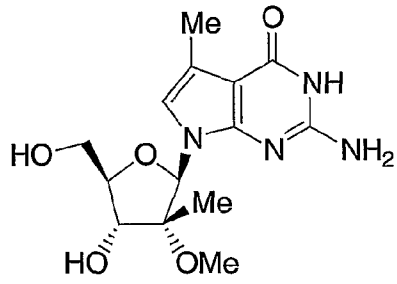
A mixture of 2-amino-4-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (Example 4, Step B) (20 mg, 0.07 mmol) in EtOH (1.0 mL), pyridine (0.1 mL) and 10% Pd/C (6 mg) under H_2 (atmospheric pressure) was stirred overnight at room temperature. The mixture was filtered through a Celite pad which was thorougly washed with EtOH. The combined filtrate was evaporated and purified

on a silica gel column with CH_2Cl_2/MeOH, 20/1 and 10/1, as eluent to give the title compound as a white solid (16 mg).

^{1}H NMR (200 MHz, CD_3OD): δ 0.86 (s, 3H, 2'C-Me), 3.82 (dd, $J_{5',4'}$= 3.6 Hz, $J_{5',5''}$ = 12.7 Hz, 1H, H-5'), 3.94-4.03 (m, 2H, H-5', H-4'), 4.10 (d, $J_{3',4'}$ = 8.8 Hz, 1H, H-3'), 6.02 (s, 1H, H-1'), 6.41 (d, $J_{5,6}$ = 3.8 Hz, 1H, H-5), 7.39 (d, 1H, H-6), 8.43 (s, 1H, H-4). ES MS: 281.4 (MH^+).

EXAMPLE 20

2-Amino-5-methyl-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one



Step A: 2-Amino-4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-β-D-ribofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cold solution of the product from Step C of Example 2 (1.57 g, 3.16 mmol) in CH_2Cl_2 (50 mL) was added HBr (5.7 M in acetic acid; 3.3 mL) dropwise. The reaction mixture was stirred at 0°C for 1 h and then at room temperature for 2 h, concentrated in vacuo and co-evaporated with toluene (2 × 20 mL). The resulting oil was dissolved in MeCN (20 mL) and added dropwise to a solution of the sodium salt of 2-amino-4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine in acetonitrile [generated *in situ* from 2-amino-4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine [for preparation, see Liebigs Ann. Chem. 1984: 708-721] (1.13 g, 6.2 mmol) in anhydrous acetonitrile (150 mL), and NaH (60% in mineral oil, 248 mg, 6.2 mmol), after 2 h of vigorous stirring at rt]. The combined mixture was stirred at rt for 24 h and then evaporated to dryness. The residue was suspended in water (100 mL) and extracted with EtOAc (300 + 150 mL). The combined extracts were washed with brine (100 mL), dried over Na_2SO_4, filtered and evaporated. The crude product was purified on a silica gel column (5 × 7 cm) using ethyl

acetate/hexane (0 to 30% EtOAc in 5% step gradient) as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product (0.96 g) as a colorless foam.

Step B: 2-Amino-4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cold mixture of the product from Step A (475 mg, 0.7 mmol) in THF (7 mL) was added NaH (60% in mineral oil, 29 mg) and stirred at 0 °C for 0.5 h. Then MeI (48 μL) was added and reaction mixture stirred at rt for 24 h. The reaction was quenched with MeOH and the mixture evaporated. The crude product was purified on a silica gel column (5 × 3.5 cm) using hexane/ethyl acetate (9/1, 7/1, 5/1 and 3/1) as eluent. Fractions containing the product were combined and evaporated to give the desired compound (200 mg) as a colorless foam.

Step C: 2-Amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-4(3*H*)-one

A mixture of the product from Step B (200 mg, 0.3 mmol) in 1,4-dioxane (15 mL) and aqueous NaOH (2N, 15 mL) in a pressure bottle was heated overnight at 135 °C. The mixture was then cooled to 0 °C, neutralized with 2N aqueous HCl and evaporated to dryness. The crude product was suspended in MeOH, filtered, and the solid thoroughly washed with MeOH. The combined filtrate was concentrated, and the residue purified on a silica gel column (5 × 5 cm) using CH_2Cl_2/MeOH (40/1, 30/1 and 20/1) as eluent to give the desired compound (150 mg) as a colorless foam.

Step D: 2-Amino-5-methyl-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one

A mixture of the product from Step C (64 mg, 0.1 mmol) in MeOH (5 mL) and Et_3N (0.2 mL) and 10% Pd/C (24 mg) was hydrogenated on a Parr hydrogenator at 50 psi at r.t. for 1.5 days, then filtered through a Celite pad which was thoroughly washed with MeOH. The combined filtrate was evaporated and the residue purified on a silica gel column (3 × 4 cm) with CH_2Cl_2/MeOH (30/1, 20/1) as eluent to yield 2-amino-5-methyl-7-(5-*O*-benzyl-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one. The compound (37 mg) was

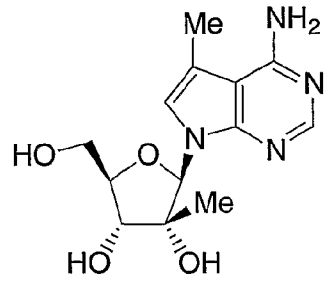
further hydrogenated in EtOH (2 mL) with 10% Pd/C and under atmospheric pressure of hydrogen. After stirring 2 days at r.t., the reaction mixture was filtered through Celite, the filtrate evaporated and the crude product purified on a silica gel column (1 × 7 cm) with CH_2Cl_2/MeOH (30/1, 20/1 and 10/1) as eluent to yield the title compound (12 mg) after freeze-drying.

^{1}H NMR (200 MHz, CD_3OD): δ 0.81 (s, 3H, 2'C-Me), 2.16 (d, $J_{H\text{-}6,C5\text{-}Me}$ = 1.3 Hz, 3H, C5-Me), 3.41 (s, 3H, 2'-OMe), 3.67 (dd, $J_{5',4'}$= 3.4 Hz, $J_{5',5''}$ = 12.6 Hz, 1H, H-5'), 3.81-3.91 (m, 3H, H-5", H-4', H-3'), 6.10 (s, 1H, H-1'), 6.66 (d, 1H, H-6).

ES MS: 323.3 $(M-H)^+$.

EXAMPLE 21

4-Amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-β-D-ribofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cold solution of the product from Step C of Example 2 (1.06 g, 2.1 mmol) in CH_2Cl_2 (30 mL) was added HBr (5.7 M in acetic acid; 2.2 mL) dropwise. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 2 h, concentrated in vacuo and co-evaporated with toluene (2 × 15 mL). The resulting oil was dissolved in MeCN (10 mL) and added dropwise into a solution of the sodium salt of 4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine in acetonitrile [generated *in situ* from 4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine [for preparation, see J. Med. Chem. 33: 1984 (1990)] (0.62 g, 3.7 mmol) in anhydrous acetonitrile (70 mL), and NaH (60% in mineral oil, 148 mg, 3.7 mmol), after 2 h of vigorous stirring at rt]. The combined mixture was stirred at rt for 24 h and then evaporated to dryness. The residue was suspended in water (100 mL) and extracted with EtOAc (250 + 100 mL). The combined extracts were washed with brine (50

mL), dried over Na_2SO_4, filtered and evaporated. The crude product was purified on a silica gel column (5 × 5 cm) using hexane/ethyl acetate (9/1, 5/1, 3/1) gradient as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product (0.87 g) as a colorless foam.

Step B: 4-Chloro-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

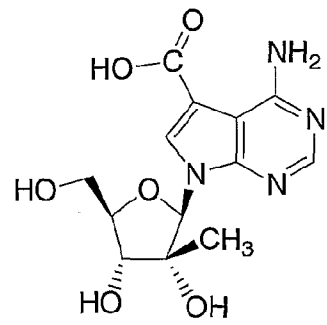
To a solution of the compound from Step A (0.87 g, 0.9 mmol) in dichloromethane (30 mL) at -78°C was added boron trichloride (1M in dichloromethane, 9.0 mL, 9.0 mmol) dropwise. The mixture was stirred at –78°C for 2.5 h, then at –30°C to –20°C for 3 h. The reaction was quenched by addition of methanol/dichloromethane (1:1) (9 mL) and the resulting mixture stirred at –15°C for 30 min., then neutralized with aqueous ammonia at 0°C and stirred at rt for 15 min. The solid was filtered and washed with CH_2Cl_2/MeOH (1/1, 50 mL). The combined filtrate was evaporated, and the residue was purified on a silica gel column (5 × 5 cm) using CH_2Cl_2 and CH_2Cl_2/MeOH (40/1 and 30/1) gradient as the eluent to furnish the desired compound (0.22 g) as a colorless foam.

Step C: 4-Amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To the compound from Step B (0.2 g, 0.64 mmol) was added methanolic ammonia (saturated at 0°C; 40 mL). The mixture was heated in a stainless steel autoclave at 100°C for 14 h, then cooled and evaporated *in vacuo*. The crude mixture was purified on a silica gel column (5 × 5 cm) with CH_2Cl_2/MeOH (50/1, 30/1, 20/1) gradient as eluent to give the title compound as a white solid (0.12 g). ^{1}H NMR (DMSO-d_6): δ 0.60 (s, 3H, 2'C-Me), 2.26 (s, 3H, 5C-Me), 3.52-3.61 (m, 1H, H-5'), 3.70-3.88 (m, 3H, H-5", H-4', H-3'), 5.00 (s, 1H, 2'-OH), 4.91-4.99 (m, 3H, 2'-OH, 3'-OH, 5'-OH), 6.04 (s, 1H, H-1'), 6.48 (br s, 2H, NH_2), 7.12 (s, 1H, H-6), 7.94 (s, 1H, H-2). ES MS: 295.2 (MH^+).

EXAMPLE 22

4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid

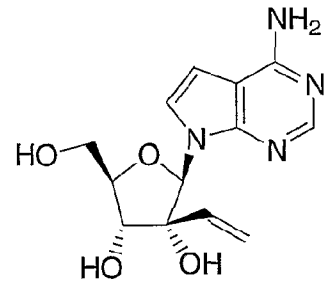


The compound of Example 6 (0.035 g, 0.11 mmol) was dissolved in a mixture of aqueous ammonia (4 mL, 30 wt %) and saturated methanolic ammonia (2 mL), and a solution of H_2O_2 in water (2 mL, 35 wt %) was added. The reaction mixture was stirred at room temperature for 18 h. Solvent was removed under reduced pressure, and the residue obtained was purified by HPLC on a reverse phase column (Altech Altima C-18, 10x 299 mm, A = water, B = acetonitrile, 10 to 60 % B in 50 min, flow 2 mL/min) to yield the title compound (0.015 g, 41 %) as a white solid.

^{1}H NMR (CD_3OD): δ 0.85 (s, 3H, Me), 3.61 (m, 1H), 3.82 (m, 1H) 3.99-4.86 (m, 2H), 6.26 (s, 1H), 8.10 (s, 2H) 8.22(s, 1H); ^{13}C NMR (CD_3OD): 20.13, 61.37, 73.79, 80.42, 84.01, 93.00, 102.66, 112.07, 130.07, 151.40, 152.74, 159.12, 169.30. HRMS (FAB) Calcd for $C_{13}H_{17}N_4O_6^+$ 325.1148, found 325.1143.

EXAMPLE 23

4-Amino-7-(2-*C*-vinyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-vinyl-1-*O*-methyl-α-D-ribofuranose

Cerium chloride heptahydrate (50 g, 134.2 mmol) was finely crushed in a pre-heated mortar and transferred to a round-bottom flask equipped with a mechanical stirrer. The flask was heated under high vacuum overnight at 160°C. The vacuum was released under argon and the flask was cooled to room temperature. Anhydrous THF (300 mL) was cannulated into the flask. The resulting suspension was stirred at room temperature for 4 h and then cooled to –78 °C. Vinylmagnesium bromide (1M in THF, 120 mL, 120 mmol) was added and stirring continued at –78 °C for 2 h. To this suspension was added a solution of 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-erythro-pentofuranose-2-ulose (14 g, 30 mmol) [from Example 2, Step B] in anhydrous THF (100 mL), dropwise with constant stirring. The reaction was stirred at –78 °C for 4 h. The reaction was quenched with saturated ammonium chloride solution and allowed to come to room temperature. The mixture was filtered through a celite pad and the residue washed with Et_2O (2 × 500 mL). The organic layer was separated and the aqueous layer extracted with Et_2O (2 × 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to a viscous yellow oil. The oil was purified by flash chromatography (SiO_2, 10% EtOAc in hexanes). The title compound (6.7 g, 13.2 mmol) was obtained as a pale yellow oil.

Step B: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-vinyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step A (6.4 g, 12.6 mmol) in anhydrous dichloromethane (150 mL) at –20 °C was added HBr (30% solution in AcOH, 20 mL, 75.6 mmol) dropwise. The resulting solution was stirred between –10°C and 0°C for 4 h, evaporated in vacuo and co-evaporated with anhydrous toluene (3 × 40 mL). The oily residue was dissolved in anhydrous acetonitrile (100 mL) and added to a solution of the sodium salt of 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine (5.8 g, 37.8 mmol) in acetonitrile (generated in situ as described in Example 2) at –20 °C. The resulting mixture was allowed to come to room temperature and stirred at room temperature for 24 h. The mixture was then evaporated to dryness, taken up in water and extracted with EtOAc (2 × 300 mL). The combined extracts were dried over Na_2SO_4, filtered and evaporated. The crude mixture was purified by flash chromatography (SiO_2, 10% EtOAc in hexanes) and the title compound (1.75 g) isolated as a white foam.

Step C: 4-Amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-vinyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

The compound from Step B (80, mg) was dissolved in the minimum amount of 1,4-dioxane and placed in a stainless steel bomb. The bomb was cooled to –78°C and liquid ammonia was added. The bomb was sealed and heated at 90°C for 24 h. The ammonia was allowed to evaporate and the residue concentrated to a white solid which was used in the next step without further purification.

Step D: 4-Amino-7-(2-*C*-vinyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

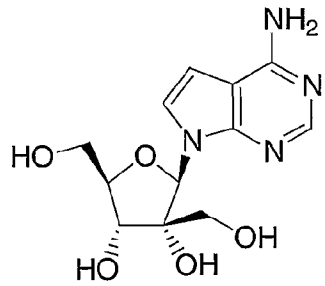
To a solution of the compound from Step C (60 mg) in dichloromethane at –78 °C was added boron trichloride (1M in dichloromethane) dropwise. The mixture was stirred at –78 °C for 2.5 h, then at –30 °C to –20 °C for 3h. The reaction was quenched by addition of methanol/dichloromethane (1:1) and the resulting mixture stirred at –15 °C for 0.5 h, then neutralized with aqueous ammonia at 0°C and stirred at room temperature for 15 min. The solid was filtered and washed with methanol/dichloromethane (1:1). The combined filtrate was evaporated and the residue purified by flash chromatography (SiO_2, 10% methanol in EtOAc containing 0.1% triethylamine). The fractions containing the product were evaporated to give the title compound as a white solid (10 mg).

^{1}H NMR (DMSO-d_6): δ 3.6 (m, 1H, H-5'), 3.8 (m, 1H, H-5"), 3.9 (m d, 1-H, H-4'), 4.3 (t, 1H, H-3'), 4.8-5.3(m, 6H, CH=CH_2, 2'-OH, 3'-OH, 5'-OH) 6.12 (s, 1H, H-1'), 6.59 (d, 1H, H-5), 7.1 (br s, 1H, NH2), 7.43 (d, 1H, H-6), 8.01 (s, 1H, H-2).

ES-MS: Found: 291.1 ($M-H^-$); calc. for $C_{13}H_{16}N_4O_4$ - H^-: 291.2.

EXAMPLE 24

4-Amino-7-(2-*C*-hydroxymethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-hydroxymethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Example 23, Step B (300 mg, 0.48 mmol) in 1,4-dioxane (5 mL) were added *N*-methylmorpholine-*N*-oxide (300 mg, 2.56 mmol) and osmium tetroxide (4% solution in water, 0.3 mL). The mixture was stirred in the dark for 14 h. The precipitate was removed by filtration through a celite plug, diluted with water (3 ×), and extracted with EtOAc. The EtOAc layer was dried over Na_2SO_4 and concentrated in vacuo. The oily residue was taken up in dichloromethane (5 mL) and stirred over $NaIO_4$ on silica gel (3 g, 10% $NaIO_4$) for 12 h. The silica gel was removed by filtration and the residue was evaporated and taken up in absolute ethanol (5 mL). The solution was cooled in an ice bath and sodium borohydride (300 mg, 8 mmol) was added in small portions. The resulting mixture was stirred at room temperature for 4 h and then diluted with EtOAc. The organic layer was washed with water (2 × 20 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was evaporated and the residue purified by flash chromatography (SiO_2, 2:1 hexanes/EtOAc) to give the title compound (160 mg, 0.25 mmol) as white flakes.

Step B: 4-Amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-hydroxymethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

The compound from Step A (150 mg, 0.23 mmol) was dissolved in the minimum amount of 1,4-dioxane (10 mL) and placed in a stainless steel bomb. The bomb was cooled to –78 °C and liquid ammonia was added. The bomb was sealed and heated at 90°C for 24 h. The ammonia was allowed to evaporate and the residue concentrated to a white solid which was used in the next step without further purification.

Step C: 4-Amino-7-(2-*C*-hydroxymethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

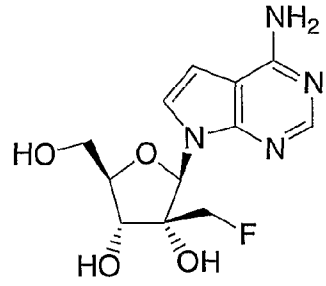
The compound from Step B (120 mg, 0.2 mmol) was dissolved in 1:1 methanol/dichloromethane, 10% Pd-C was added, and the suspension stirred under an H_2 atmosphere for 12 h. The catalyst was removed by filtration through a celite pad and washed with copious amounts of methanol. The combined filtrate was evaporated in vacuo and the residue was purified by flash chromatography (SiO_2, 10% methanol in EtOAc containing 0.1% triethylamine) to give the title compound (50 mg) as a white powder.

^{1}H NMR (CD_3OD): δ 3.12 (d, 1H, CH_2'), 3.33 (d, 1H, CH_2''), 3.82 (m, 1H, H-5'), 3.99-4.1(m, 2H, H-4', H-5''), 4.3 (d, 1H, H-3'), 6.2 (s, 1H, H-1'), 6.58 (d, 1H, H-5), 7.45 (d, 1H, H-6), 8.05 (s, 1H, H-2).

LC-MS: Found: 297.2 ($M+H^+$); calc. for $C_{12}H_{16}N_4O_5 + H^+$: 297.3.

EXAMPLE 25

4-Amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-fluoromethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Example 24, Step A (63 mg, 0.1 mmol) in anhydrous dichloromethane (5 mL) under argon, were added 4-dimethylaminopyridine (DMAP) (2 mg, 0.015 mmol) and triethylamine (62 μL, 0.45 mmol). The solution was cooled in an ice bath and p-toluenesulfonyl chloride (30 mg, 0.15 mmol) was added. The reaction was stirred at room temperature overnight, washed with $NaHCO_3$ (2 × 10 mL), water (10 mL), brine (10 mL), dried over Na_2SO_4 and concentrated to a pink solid in vacuo. The solid was dissolved in anhydrous THF (5 mL) and cooled in an icebath. Tetrabutylammonium fluoride (1M solution in THF,

1 mL, 1 mmol) was added and the mixture stirred at room temperature for 4 h. The solvent was removed in vacuo, the residue taken up in dichloromethane, and washed with $NaHCO_3$ (2 × 10 mL), water (10 mL) and brine (10 mL). The dichloromethane layer was dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by flash chromatography (SiO_2, 2:1 hexanes/EtOAc) to afford the title compound (20 mg) as a white solid.

Step B: 4-Amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-fluoromethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

The compound from Step A (18 mg, 0.03 mmol) was dissolved in the minimum amount of 1,4-dioxane and placed in a stainless steel bomb. The bomb was cooled to –78 °C and liquid ammonia was added. The bomb was sealed and heated at 90 °C for 24 h. The ammonia was allowed to evaporate and the residue concentrated to a white solid which was used in the next step without further purification.

Step C: 4-Amino-7-(2-C-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

The compound from Step B (16 mg) was dissolved in 1:1 methanol/dichloromethane, 10% Pd-C was added, and the suspension stirred under an H_2 atmosphere for 12 h. The catalyst was removed by filtration through a celite pad and washed with copious amounts of methanol. The combined filtrate was evaporated in vacuo and the residue was purified by flash chromatography (SiO_2, 10% methanol in EtOAc containing 0.1% triethylamine) to give the title compound (8 mg) as a white powder.

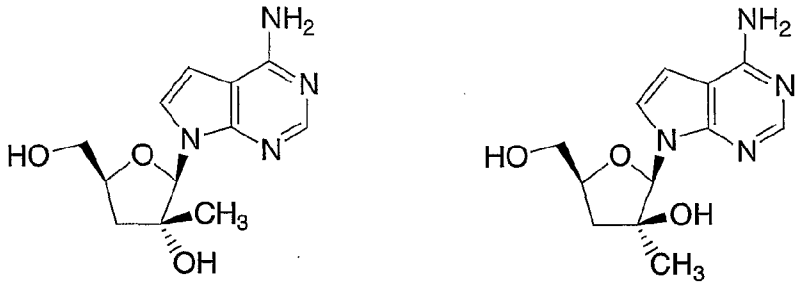
^1H NMR (DMSO-d_6): δ 3.6-3.7 (m, 1H, H-5'), 3.8 – 4.3 (m, 5H, H-5'', H-4', H-3', CH_2) 5.12 (t, 1H, 5'-OH), 5.35 (d, 1H, 3'-OH), 5.48 (s, 1H, 2'-OH), 6.21 (s, 1H, H-1'), 6.52 (d, 1H, H-5), 6.98 (br s, 2H, NH2), 7.44 (d, 1 H, H-6), 8.02 (s, 1H, H-2).

^{19}F NMR (DMSO-d_6): δ -230.2 (t).

ES-MS: Found: 299.1 ($M+H^+$), calc.for $C_{12}H_{15}FN_4O_4 + H^+$: 299.27.

EXAMPLES 26 and 27

4-Amino-7-(3-deoxy-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine and 4-amino-7-(3-deoxy-2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine



Step A: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine and 7-[3,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*] pyrimidine

To a stirred solution of tubercidin (5.0 g, 18.7 mmol) in a mixture of pyridine (7.5 mL) and DMF (18.5 mL) was added silver nitrate (6.36 g, 38.8 mmol). This mixture was stirred at room temperature for 2 h. It was cooled in an ice bath and THF (37.4 mL) and *tert*-butyldimethylsilyl chloride (5.6 g, 37 mmol) was added and the mixture was stirred at room temperature for 2 h. The mixture was then filtered through a pad of celite and washed with THF. The filtrate and washings were diluted with ether containing a small amount of chloroform. The organic layer was washed successively with sodium bicarbonate and water (3 × 50 mL), dried over anhydrous sodium sulfate and concentrated. The pyridine was removed by coevaporation with toluene and the residue was purified by flash chromatography on silica gel using 5-7% MeOH in CH_2Cl_2 as the eluent; yield 3.0 g.

Step B: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl)]-4-[di-(4-methoxyphenyl)phenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine and 7-[3,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-[di-(4-methoxyphenyl)phenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of mixture of the compounds from Step A (3.0 g, 6.0 mmol) in anhydrous pyridine (30 mL) was added 4,4'-dimethoxytrityl chloride (2.8 g, 8.2 mmol) and the reaction mixture was stirred at room temperature overnight. The

mixture was then triturated with aqueous pyridine and extracted with ether. The organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated to a yellow foam (5.6 g). The residue was purified by flash chromatography over silica gel using 20-25% EtOAc in hexanes as the eluent. The appropriate fractions were collected and concentrated to furnish 2',5'-bis-O-(*tert*-butyldimethylsilyl)- and 3',5'-bis-O-(*tert*-butyldimethylsilyl) protected nucleosides as colorless foams (2.2 g and 1.0 g, respectively).

Step C: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-*O*-tosyl-β-D-ribofuranosyl)]-4-[di-(4-methoxyphenyl)phenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cooled solution of 2',5'-bis-O-(*tert*-butyldimethylsilyl)-protected nucleoside from Step B (2.0 g, 2.5 mmol) in pyridine (22 mL) was added p-toluenesulfonyl chloride (1.9 g, 9.8 mmol). The reaction mixture was stirred at room temperature for four days. It was then triturated with aqueous pyridine (50%, 10 mL) and extracted with ether (3 × 50 mL) containing a small amount of CH_2Cl_2 (10 mL). The organic layer was washed with sodium bicarbonate and water (3 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated. Pyridine was removed by co-evaporation with toluene (3 × 25 mL). The residual oil was filtered through a pad of silica gel using hexane:ethyl acetate (70:30) as eluent; yield 1.4 g.

Step D: 4-[di-(4-methoxyphenyl)phenylmethyl]amino-7-[3-*O*-tosyl-β-D-ribofuranosyl-7H-pyrrolo[2,3-*d*]pyrimidine

A solution of the compound from Step C (1.0 g, 1.1 mmol) and THF (10 mL) was stirred with tetrabutylammonium fluoride (1M solution in THF, 2.5 mL) for 0.5h. The mixture was cooled and diluted with ether (50 mL). The solution was washed with water (3 × 50 mL), dried over anhydrous Na_2SO_4, and concentrated to an oil. The residue was purified by passing through a pad of silica gel using hexane: ethyl acetate (1:1) as eluent; yield 780 mg.

Step E: 4-Amino-7-(3-deoxy-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]- pyrimidine and 4-amino-7-(3-deoxy-2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidine

A solution of CH_3MgI (3.0 M solution in ether, 3.0 mL) in anhydrous toluene (3.75 mL) was cooled in an ice bath. To this was added a solution of the

compound from Step D (500 mg, 0.8 mmol) in anhydrous toluene (3.7 mL). The resulting mixture was stirred at room temperature for 3.5 h. It was cooled and treated with aqueous NH_4Cl solution and extracted with ether (50 mL containing 10 mL of CH_2Cl_2). The organic layer was separated and washed with brine (2 × 30 mL) and water (2 × 25 mL), dried over anhydrous Na_2SO_4 and concentrated to an oil which was purified by flash chromatography on silica gel using 4% MeOH in CH_2Cl_2 to furnish the 2-C-α-methyl compound (149 mg) and the 2-C-β-methyl compound (34 mg). These derivatives were separately treated with 80% acetic acid and the reaction mixture stirred at room temperature for 2.5 h. The acetic acid was removed by repeated co-evaporation with ethanol and toluene. The residue was partitioned between chloroform and water. The aqueous layer was washed with chloroform and concentrated. The evaporated residue was purified on silica gel using 5-10% MeOH in CH_2Cl_2 as the eluent to furnish the desired compounds as white solids.

4-Amino-7-(3-deoxy-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (9.0 mg):

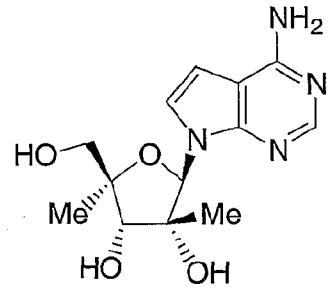
^{1}H NMR (DMSO-d_6): δ 0.74 (s, 3H, CH_3), 1.77 (dd, 1H, H-3'), 2.08 (t, 1H, H-3"), 3.59 (m, 1H, H-5'), 3.73 (m, 1H, H-5"), 4.15 (m, 1H, H-4'), 5.02 (t, 1H, OH-5'), 5.33 (s, 1H, OH-2'), 6.00 (s, 1H, H-1'), 6.54 (d, 1H, H-7), 6.95 (br s, 2H, NH_2), 7.47 (d, 1H, H-8), 8.00 (s, 1H, H-2); ES-MS: 263.1 [M-H].

4-Amino-7-(3-deoxy-2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (15 mg):

^{1}H NMR (DMSO-d_6): δ 1.23 (s, 3H, CH_3), 2.08 (ddd, 2H, H-3'and 3"), 3.57 (m, 2H, H-5'and 5"), 4.06 (m, 1H, H-4), 5.10 (s, 1H, OH-2'), 5.24 (t, 1H, OH-5'), 6.01 (s, 1H, H-1'), 6.49 (d, 1H, H-7),6.89 (br s, 2H, NH_2), 7.35 (d, 1H, H-8), 8.01 (s,1H,H-2). ES-MS: 265.2[M+H].

EXAMPLE 28

4-Amino-7-(2,4-*C*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 5-Deoxy-1,2-*O*-isopropylidene-D-xylofuranose

1,2-*O*-Isopropylidene-D-xylofuranose (38.4 g, 0.2 mol), 4-dimethylaminopyridine (5 g), triethylamine (55.7 mL, 0.4 mol) were dissolved in dichloromethane (300 mL). p-Toluenesulfonyl chloride (38.13 g, 0.2 mol) was added and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was then poured into saturated aqueous sodium bicarbonate (500 mL) and the two layers were separated. The organic layer was washed with aqueous citric acid solution (20%, 200 mL), dried (Na_2SO_4) and evaporated to give a solid (70.0 g). The solid was dissolved in dry THF (300 mL) and $LiAlH_4$ (16.0 g, 0.42 mol) was added in portions over 30 min. The mixture was stirred at room temperature for 15. Ethyl acetate (100 mL) was added dropwise over 30 min and the mixture was filtered through a silica gel bed. The filtrate was concentrated and the resulting oil was chromatographed on silica gel (EtOAc/hexane 1/4) to afford the product as a solid (32.5 g).

Step B: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-4-methyl-α-D-ribofuranose

Chromium oxide (50 g, 0.5 mol), acetic anhydride (50 mL, 0.53 mol) and pyridine (100 mL, 1.24 mol) were added to dichloromethane (1 L) in an ice-water bath and the mixture was stirred for 15 min. 5-Deoxy-1,2-*O*-isopropylidene-D-xylofuranose (32 g, 0.18 mol) in dichloromethane (200 mL) was added, and the mixture was stirred at the same temperature for 30 min. The reaction solution was diluted with ethyl acetate (1 L) and filtered through a silica gel bed. The filtrate was concentrated to give a yellow oil. The oil was dissolved in 1,4-dioxane (1 L) and formaldehyde (37%, 200 mL). The solution was cooled to 0°C and solid KOH (50 g) was added. The mixture was stirred at room temperature overnight and was then extracted with ethyl acetate (6 × 200 mL). After concentration, the residue was

chromatographed on silica gel (EtOAc) to afford the product as an oil (1.5 g). The oil was dissolved in 1-methyl-2-pyrrolidinone (20 mL) and 2,4-dichlorophenylmethyl chloride (4 g, 20.5 mmol) and NaH (60%, 0.8 g) were added. The mixture was stirred overnight and diluted with toluene (100 mL). The mixture was then washed with saturated aqueous sodium bicarbonate (3 × 50 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in methanol (50 mL) and HCl in dioxane (4 M, 2 mL) was added. The solution was stirred overnight and evaporated. The residue was chromatographed on silica gel (EtOAc/hexane: 1/4) to afford the desired product as an oil (2.01 g).

Step C: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-2,4-di-*C*-methyl-1-*O*-methyl-α-D-ribofuranose

The product (2.0 g, 4.0 mmol) from Step B and Dess-Martin periodinane (2.0 g) in dichloromethane (30 mL) were stirred overnight at room temperature and then concentrated under reduced pressure. The residue was triturated with ether ether (50 mL) and filtered. The filtrate was washed with a solution of $Na_2S_2O_3.5H_2O$ (2.5 g) in saturated aqueous sodium bicarbonate solution (50 mL), dried ($MgSO_4$), filtered and evaporated. The residue was dissolved in anhydrous Et_2O (20 mL) and was added dropwise to a solution of MeMgBr in Et_2O (3 M, 10 mL) at –78 °C. The reaction mixture was allowed to warm to –30°C and stirred at –30°C to –15°C for 5 h, then poured into saturated aqueous ammonium chloride (50 mL). The two layers were separated and the organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was chromatographed on silica gel (EtOAc/hexane: 1/9) to afford the title compound as a syrup (1.40 g).

Step D: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2,4-di-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To the compound from Step C (0.70 g, 1.3 mmol) was added HBr (5.7 M in acetic acid, 2 mL). The resulting solution was stirred at room temperature for 1 h, evaporated in vacuo and co-evaporated with anhydrous toluene (3 × 10 mL). 4-Chloro-*1H*-pyrrolo[2,3-*d*]pyrimidine (0.5 g, 3.3 mmol) and powdered KOH (85%, 150 mg, 2.3 mmol) were stirred in 1-methyl-2-pyrrolidinone (5 mL) for 30 min and the mixture was co-evaporated with toluene (10 mL). The resulting solution was poured into the above bromo sugar residue and the mixture was stirred overnight.

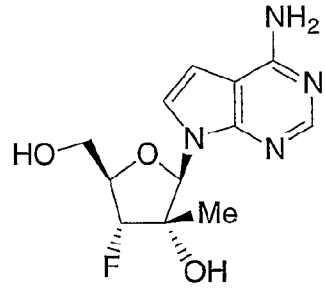
The mixture was diluted with toluene (50 mL), washed with water (3 × 50 mL) and concentrated under reduced pressure. The residue was chromatographed on silica gel eluting with EtOAc/ Hexane (15/85) to afford a solid (270 mg).

Step E: 4-Amino-7-(2,4-*C*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

The compound from Step D (270 mg) was dissolved in dioxane (2 mL) and liquid ammonia (20 g) was added in a stainless steel autoclave. The mixture was heated at 100°C for 15, then cooled and evaporated. The residue was chromatographed on silica gel (EtOAc) to afford a solid (200 mg). The solid (150 mg) and Pd/C (10% 150 mg) in methanol (20 mL) were shaken under H_2 (30 psi) for 3 h, filtered and evaporated. The residue was chromatographed on silica gel ($MeOH/CH_2Cl_2$: 1/9) to afford the desired product as a solid (35 mg).
^1H NMR (DMSO-d_6): δ 0.65 (s, 3H), 1.18 (s, 3H), 3.43 (m, 2H), 4.06 (d, 1H, *J* 6.3 Hz), 4.87 (s, 1H), 5.26 (br, 1H), 5.08 (d, 1H, *J* 6.3 Hz), 5.25 (t, 1H, *J* 3.0 Hz), 6.17 (s, 1H), 6.54 (d, 1H, *J* 3.5 Hz), 6.97 (s, br, 2H), 7.54 (d, 1H, *J* 3.4 Hz), 8.02 (s, 1H).
^{13}C NMR (DMSO-d_6): δ 18.19, 21.32, 65.38, 73.00, 79.33, 84.80, 90.66, 99.09, 102.41, 121.90, 149.58, 151.48, 157.38.
LC-MS: Found: 295.1 ($M+H^+$); calculated for $C_{13}H_{18}N_4O_4+H^+$: 295.1.

EXAMPLE 29

4-Amino-7-(3-deoxy-3-fluoro-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 3-Deoxy-3-fluoro-1-*O*-methyl-5-*O*-toluoyl-α-D-ribofuranose

1,2-*O*-Isopropylidene-D-xylofuranose (9.0 g, 50 mmol) and *p*-toluoyl chloride (7.0 mL, 50 mmol) in pyridine (50 mL) were stirred for 30 min. Water (10 mL) was added and the mixture was concentrated under reduced pressure. The residue was dissolved in toluene (500 mL) and the solution was washed with water (200 mL) and saturated aqueous sodium bicarbonate (200 mL). The two layers were separated and the organic layer was evaporated. The residue was dissolved in methanol (100 mL) and HCl in dioxane (4 M, 10 mL) was added. The mixture was stirred at room temperature overnight and was then evaporated under reduced pressure. The resulting oil was chromatographed on silica gel (EtOAc/hexane: 1/1) to afford an oil (10.1 g). The oil was dissolved in dichloromethane (100 mL) and diethylaminosulfur trifluoride (DAST) (5.7 mL) was added. The mixture was stirred overnight and was then poured into saturated aqueous sodium bicarbonate solution (100 mL). The mixture was extracted with toluene (2 × 50 mL) and the combined organic layers were concentrated. The residue was chromatographed on silica gel (EtOAc/hexane: 15/85) to afford the title compound as an oil (1.50 g).

Step B: 3-Deoxy-3-fluoro-2-*C*-methyl-1-*O*-methyl-5-*O*-toluoyl-α-D-ribofuranose

The product from Step A (1.0 g, 3.5 mmol) and Dess-Martin periodinane (2.5 g) in dichloromethane (20 mL) were stirred overnight at room temperature and was then concentrated under reduced pressure. The residue was triturated with diethyl ether (50 mL) and filtered. The filtrate was washed with a solution of $Na_2S_2O_3.5H_2O$ (12.5 g) in saturated aqueous sodium bicarbonate (100 mL), dried ($MgSO_4$), filtered and evaporated. The residue was dissolved in anhydrous THF (50 mL). $TiCl_4$ (3 mL) and methyl magnesium bromide in ethyl ether (3 M, 10 mL) were added at –78°C and the mixture was stirred at –50 to –30°C for 2 h. The mixture was poured into saturated aqueous sodium bicarbonate solution (100 mL) and filtered through Celite. The filtrate was extracted with toluene (100 mL) and evaporated. The residue was chromatographed on silica gel (EtOAc/hexane: 15/85) to afford the title compound as an oil (150 mg).

Step C: 4-Amino-7-(3-deoxy-3-fluoro-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

The product from Step B (150 mg, 0.5 mmol) was dissolved in HBr (30%) in acetic acid (2 mL). After one hour, the mixture was evaporated under reduced pressure and co-evaporated with toluene (10 mL). 4-Chloro-*1H*-pyrrolo[2,3-*d*]pyrimidine (0.5 g, 3.3 mmol) and powdered KOH (85%, 150 mg, 2.3 mmol) were stirred in DMF (3 mL) for 30 min and the mixture was co-evaporated with toluene (2 mL). The resulting solution was poured into the above bromo sugar and the mixture was stirred overnight. The mixture was diluted with toluene (50 mL), washed with water (3 × 50 mL) and concentrated under reduced pressure. The residue was chromatographed on silica gel (EtOAc/hexane: 15/85) to afford an oil (60 mg). The oil was dissolved in dioxane (2 mL) and liquid ammonia (20 g) was added in a stainless steel autoclave. The mixture was heated at 85°C for 18 h, then cooled and evaporated. The residue was chromatographed on silica gel (methanol/dichloromethane: 1/9) to afford the title compound as a solid (29 mg).

^{1}H NMR (DMSO-d_6): δ 0.81 (s, 3H), 3.75 (m, 2H), 4.16 (m, 1H), 5.09 (dd, 1H, *J* 53.2, 7.8 Hz), 5.26 (br, 1H), 5.77 (s, 1H), 6.15 (d, 1H, *J* 2.9 Hz), 6.59 (d, 1H, *J* 3.4 Hz), 7.02 (s br, 2H), 7.39 (d, 1H, *J* 3.4 Hz), 8.06 (s, 1H).

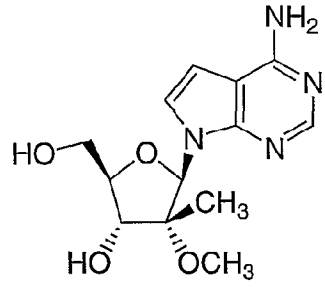
^{13}C NMR (DMSO-d_6): 19.40, 59.56, 77.24, 79.29, 90.15, 91.92, 99.88, 102.39, 121.17, 149.80, 151.77, 157.47.

^{19}F NMR (DMSO-d_6): δ 14.66 (m).

ES-MS: Found: 283.1 ($M+H^+$); calculated for $C_{12}H_{15}FN_4O_3+H^+$: 283.1.

EXAMPLE 30

4-Amino-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a pre-cooled (0°C) solution of the compound from Example 2, Step D (618 mg, 1.0 mmol) in THF (8 mL) was added methyl iodide (709 mg, 5.0 mmol) and NaH (60% in mineral oil) (44 mg, 1.1 mmol). The resulting mixture was stirred overnight at rt and then poured into a stirred mixture of saturated aqueous ammonium chloride (50 mL) and dichloromethane (50 mL). The organic layer was washed with water (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The resulting crude product was purified on silica gel using ethyl acetate/hexane as the eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (735 mg) as a colorless foam.

Step B: 4-amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To the compound from Step A (735 mg, 1.16 mmol) was added methanolic ammonia (saturated at 0°C) (20 mL). The mixture was heated in a stainless steel autoclave at 80°C overnight, then cooled and the content evaporated in vacuo. The crude mixture was purified on silica gel using ethyl acetate/hexane as the eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (504 mg) as colorless foam.

Step C: 4-amino-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

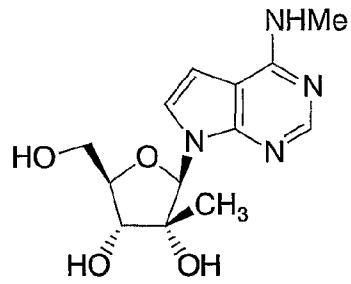
A mixture of the product from Step C (64 mg, 0.1 mmol), MeOH (5 mL), Et_3N (0.2 mL) and 10% Pd/C (61 mg) was hydrogenated on a Parr hydrogenator at 50 psi at room temperature overnight. The mixture was filtered throught celite, evaporated in vacuo and filtered through a pad of silica gel using 2% methanol in dichloromethane as eluent. The desired product was collected and evaporated in vacuo. The compound was redissolved in methanol (10 mL) and 10% Pd/C (61 mg) was added. The mixture was hydrogenated on a Parr hydrogenator at 55 psi at room temperature for two weeks. The mixture was filtered through celite, evaporated in vacuo and purified on silica gel using 10% methanol in dichloromethane as eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (110 mg) as a colorless foam.

^{1}H NMR (DMSO-d_6): δ 0.68 (s, 3H,), 3.40 (s, 3H), 3.52-3.99 (overlapping m, 4H), 4.92 (d, 1H), 5.07 (t, 1H), 6.26 (s, 1H), 6.55 (d, 1H), 7.00s br, 2H), 7.46 (d, 1H), 8.05 (s, 1H).

LC-MS: Found: 293.1 (M-H$^+$); calc. for $C_{12}H_{16}N_4O_4$-H$^+$: 293.12.

EXAMPLE 31

4-Methylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



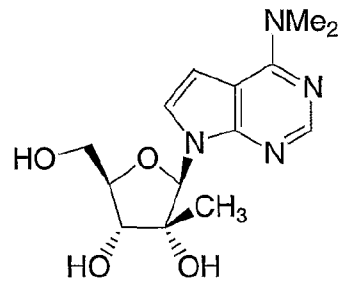
The compound from Step E of Example 2 (200 mg, 0.67 mmol) was added to methylamine (5 mL condensed in a small stainless steel autoclave) and warmed at 85°C for 48 h, then cooled and evaporated in vacuo. The crude mixture was purified on a silica gel with ethanol as the eluent to give the title compound which separated as an amorphous solid after treatment with MeCN. The amorphous solid was dissolved in water and lyophilized to give a colorless powder (144 mg).

^{1}H NMR (DMSO-d_6): δ 0.63 (s, 3H, CH_3), 3.32 (s, 3H, N CH_3), 3.58-3.67 (m, 1H, H-5'), 3.79-3.39 (m, 3H, H-5", H-4', H-3'), 5.03 (s, 1H, 2'-OH), 5.04-5.11 (1H,3'-OH, 1H, 5'-OH), 6.14 (s, 1H, H-1'), 6.58 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5), 7.46 (d, 1H, H-6), 7.70 (br s, 1H, NH), 8.14 (s, 1H, H-2).

LC-MS: Found: 295.1 (M-H$^+$); calc. for $C_{13}H_{18}N_4O_4$+H$^+$: 294.3.

EXAMPLE 32

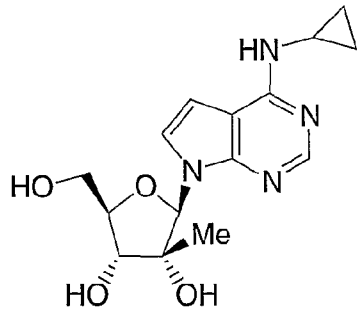
4-Dimethylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



The compound from Step E of Example 2 (200 mg, 0.67 mmol) was added to dimethylamine (5 mL condensed in a small stainless steel autoclave) and warmed at 85°C for 48 h, then cooled and evaporated in vacuo. The crude mixture was purified on a silica gel with ethanol as the eluent to give the title compound which separated as an amorphous solid after treatment with MeCN. The amorphous solid was dissolved in water and lyophilized to give a colorless powder (164 mg). ^{1}H NMR (DMSO-d_6): δ 0.64 (s, 3H, CH_3), 3.29 (s, 3H, N CH_3), 3.32 (s, 3H, N CH_3), 3.60-3.66 (m, 1H, H-5'), 3.77-3.97 (m, 3H, H-5", H-4', H-3'), 5.04 (s, 1H, 2'-OH), 5.06-5.11 (1H, 3'-OH, 1H, 5'-OH), 6.21 (s, 1H, H-1'), 6.69 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5), 7.55 (d, 1H, H-6), 8.13 (s, 1H, H-2).
LC-MS: Found: 309.3 ($M-H^+$); calc. for $C_{14}H_{20}N_4O_4+H^+$: 308.33.

EXAMPLE 33

4-Cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

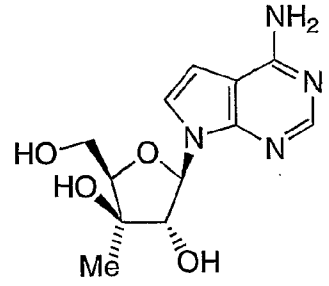


The compound from Step E of Example 2 (200 mg, 0.67 mmol) was added to cyclopropylamine (5 mL condensed in a small stainless steel autoclave) and warmed at 85°C for 48 h, then cooled and evaporated in vacuo. The crude mixture was purified on a silica gel with ethanol as the eluent to give the title compound

which separated as an amorphous solid after treatment with MeCN. The amorphous solid was dissolved in water and lyophilized to give a colorless powder (148 mg). ^{1}H NMR (DMSO-d_6): δ 0.51- 0.58 (m, 2H), 0.64 (s, 3H, CH_3), 0.74- 0.076 (m, 2H), 3.62-3.67 (m, 1H, H-5'), 3.79-3.82 (m, 3H, H-5"), 3.92-3.96 (m, H-4', H-3'), 5.03 (s, 1H, 2'-OH), 5.05-5.10 (1H, 3'-OH, 1H, 5'-OH), 6.15 (s, 1H, H-1'), 7.48 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5), 7.59 (d, 1H, H-6), 8.13 (s, 1H, H-2).
LC-MS: Found: 321.1 ($M-H^+$); calc. for $C_{15}H_{20}N_4O_4+H^+$: 320.3.

EXAMPLE 34

4-Amino-7-(3-*C*-methyl-β-D-xylofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl)]-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine and 7-[3,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of mixture of the compounds from Step A of Examples 26 and 27 (0.32 g, 0.65 mmol) in anhydrous pyridine (6 mL) was added monomethoxytrityl chloride (0.30 g, 0.98 mmol) and the reaction mixture was stirred at room temperature overnight. The mixture was then concentrated and the residue was partitioned between CH_2Cl_2 (70 mL) and water (20 mL). The organic layer was washed with water and brine, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel column using 5-13% EtOAc in hexanes as the eluent. The appropriate fractions were collected and concentrated to furnish 2',5'-bis-O-(*tert*-butyldimethylsilyl)- and 3',5'-bis-O-(*tert*-butyldimethylsilyl) protected nucleosides as colorless foams (343 mg and 84 mg, respectively).

Step B: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-*erythro*-pentofuranos-3-ulosyl]-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a well-stirred suspension of chromium trioxide (91 mg, 0.91 mmol) in CH_2Cl_2 (4 mL) at 0°C were added pyridine (147 μL, 1.82 mmol) and then acetic anhydride (86 μL, 0.91 mmol). The mixture was stirred at room temperature for 0.5 h. Then the 2',5'-bis-O-(*tert*-butyldimethylsilyl) protected nucleoside from step A (343 mg 0.45 mmol) in CH_2Cl_2 (2.5 mL) was added and the mixture stirred at room temperature 2 h. The mixture was then poured into ice-cold EtOAc (10 mL) and filtered through a short silica gel column using EtOAc as the eluent. The filtrate was evaporated and the residue purified on a silica gel column with hexanes and hexanes/EtOAc (7/1) as the eluent to give the title compound (180 mg).

Step C: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-*C*-methyl-β-D-ribofuranosyl)-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine and 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-*C*-methyl-β-D-xylofuranosyl)-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a mixture of MeMgBr (3.0 M solution in ether; 0.17 mL, 0.5 mmol) in anhydrous hexanes (1.5 mL) at room temperature was added dropwise a solution of the compound from Step B (78 mg, 0.1 mmol) in anhydrous hexanes (0.5 mL). After 2 h stirring at room temperature, the reaction mixture was poured into ice-cold water (10 mL) and diluted with EtOAc (20 mL), then filtered through Celite which was then thoroughly washed with EtOAc. The layers were separated and the organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified on a silica gel column using 8 to 25% EtOAc in hexanes as eluent to give the 3-C-methyl xylo- (60 mg) and the 3-C-methyl ribo-isomer (20 mg).

Step D: 4-Amino-7-(3-*C*-methyl-β-D-xylofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

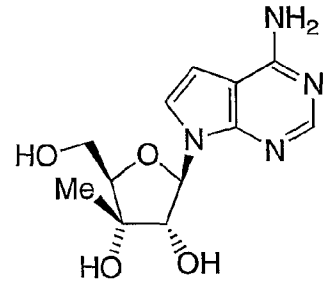
To an ice-cold solution of 3-C-methyl-xylo isomer from Step C (60 mg, 0.08 mmol) in THF (2 mL) was added TBAF (1 M in THF; 0.32 mL, 0.32 mmol). The reaction mixture was stirred at room temperature for 5 h, then diluted with CH_2Cl_2 (50 mL), washed with water (3 × 15 mL), dried,and evaporated. The residue was dissolved in dioxane (0.3 mL) and 80% acetic acid (3 mL) was added. The

reaction mixture was stirred at room temperature for 1 d and then evaporated. The residue was co-evaporated with dioxane, taken up in water (50 mL) and washed with CH_2Cl_2 (2 × 10 mL). The aqueous layer was concentrated and then freeze-dried. The residue was purified on silica gel column with CH_2Cl_2/MeOH (20/1 and 10/1) as the eluent to give the title compound as a white fluffy compound after freeze drying (10 mg).

^{1}H NMR (CD_3CN): δ 1.28 (s, 3H, CH_3), 3.56 (br s, 1H, OH), 3.78 (m, 3H, H-4', H-5', H-5"), 4.10 (br s, 1H, OH), 4.44 (d, 1H, $J_{2',1'}$ = 3.9 Hz, H-2'), 5.58 (d, 1H, H-1'), 5.85 (br s, 2H, NH_2), 6.15 (br s, 1H, OH), 6.48 (d, 1H, $J_{5,6}$ = 3.7 Hz, H-5), 7.23 (d, 1H, H-6), 8.11 (s, 1H, H-2). ES-MS: 281 $[MH]^+$.

EXAMPLE 35

4-Amino-7-(3-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

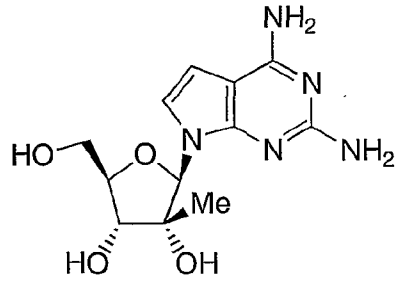


The ribo-isomer (20 mg) from Step C of Example 32 was deprotected using the procedure described in Step D of Example 32 to yield the title compound (4 mg).

^{1}H NMR (CD_3CN): δ 1.43 (s, 3H, CH_3), 3.28 (br s, 1H, OH), 3.58 (m, 2H, H-5', H-5"), 3.99 (m, 1H, H-4'), 4.10 (br s, 1H, OH), 4.62 (d, 1H, $J_{2',1'}$ = 8.1 Hz, H-2'), 5.69 (d, 1H, H-1'), 5.88 (br s, 3H, OH, NH_2), 6.45 (br s, 1H, OH), 6.51 (d, 1H, $J_{5,6}$ = 3.7 Hz, H-5), 7.19 (d, 1H, H-6), 8.12 (s, 1H, H-2). ES-MS: 281 $[MH]^+$.

EXAMPLE 36

2,4-Diamino-7-(2-C-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



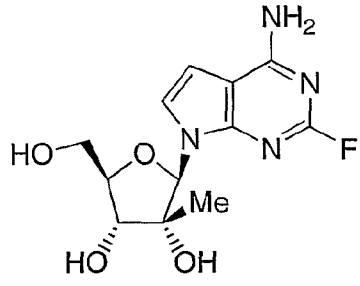
A mixture of the product from Step B of Example 4 (24 mg) in aqueous ammonia (30%, 10 mL) was heated in a stainless steel autoclave at 100 °C overnight, then cooled and evaporated. The residue was purified on a silica gel column with CH_2Cl_2/MeOH (10/1 and 5/1) as the eluent to afford the title compound (15 mg).

^{1}H NMR (DMSO-d_6): δ 0.68 (s, 3H, CH_3), 3.48-3.58 (m 1H, H-5'), 3.68-3.73 (m, 2H, H-5", H-4'), 3.84 (m, 1H, H-3'), 4.72 (s, 1H, 2'-OH), 4.97-5.03 (m, 2H, 3'-OH, 5'-OH), 5.45 (br s, 2H, NH_2), 6.00 (s, 1H, H-1'), 6.28 (d, 1H, J = 3.7 Hz, H-5), 6.44 (br s, 2H, NH_2) 6.92 (d, 1H J = 3.7 Hz, H-6).

ES MS: 294.1 ($M-H^+$).

EXAMPLE 37

4-Amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

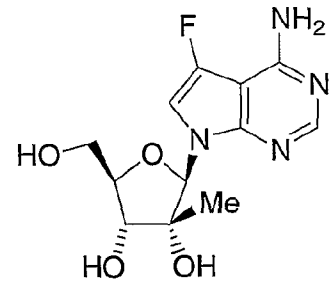


To a solution of HF/pyridine (70%, 2 mL) diluted with pyridine (1 mL) at –30 °C is added the compound of Example 36 (60 mg, 0.2 mmol) in 0.5 mL pyridine followed by *tert*-butyl nitrite (36 μL, 0.3 mmol). Stirring is continued for 5 min –25 °C. Then the solution is poured into ice-water (5 mL), neutralized with 2 N aqueous NaOH, and evaporated to dryness. The residue is purified on a silica gel

column with CH_2Cl_2/MeOH (20/1 and 10/1) as the eluent to afford the title compound.

EXAMPLE 38

4-Amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 4-Acetylamino-7-(2,3,5-tri-*O*-acetyl-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from step F of Example 2 (280 mg, 1.00 mmol) in pyridine is added acetic anhydride (613 mg, 6.0 mmol). The resulting solution is stirred overnight at ambient temperature evaporated in vacuo and the resulting crude mixture is purified on silica gel using ethyl acetate/hexane as the eluent. Fractions containing the desired product are pooled and evaporated in vacuo to give the desired product.

Step B: 4-Acetylamino-5-bromo-7-(2,3,5-tri-*O*-acetyl-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a pre-cooled (0°C) solution of the compound from Step A (460 mg, 1.00 mmol) in DMF is added *N*-bromosuccinimide (178 mg, 1.0 mmol) in DMF. The resulting solution is stirred at 0°C for 30 min then at room temperature for another 30 min. The reaction is quenched by addition of methanol and evaporated in vacuo. The resulting crude mixture is purified on silica gel using ethyl acetate/hexane as the eluent. Fractions containing the desired product are pooled and evaporated in vacuo to give the desired product.

Step C: 4-Amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a pre-cooled (-78°C) solution of the compound from Step B (529 mg, 1.00 mmol) in THF is added butyl lithium (2M in hexanes) (0.5 mL, 1.00 mmol). The resulting solution is stirred at -78°C for 30 min and then quenched with *N*-fluorobenzensulfonimide (315 mg, 1.00 mmol) in THF. The resulting solution is very slowly allowed to come to ambient temperature and then poured into a stirred mixture of saturated aqueous ammonium chloride and dichloromethane. The organic phase is evaporated in vacuo and treated with ammonium hydroxide at 55°C in a closed container overnight. The resulting crude mixture is purified on silica gel using dichloromethane/methanol as the eluent. Fractions containing the desired product are pooled and evaporated in vacuo to give the desired product.

BIOLOGICAL ASSAYS

The assays employed to measure the inhibition of HCV NS5B polymerase and HCV replication are described below.

The effectiveness of the compounds of the present invention as inhibitors of HCV NS5B RNA-dependent RNA polymerase (RdRp) was measured in the following assay.

A. Assay for Inhibition of HCV NS5B Polymerase:

This assay was used to measure the ability of the nucleoside derivatives of the present invention to inhibit the enzymatic activity of the RNA-dependent RNA polymerase (NS5B) of the hepatitis C virus (HCV) on a heteromeric RNA template.

Procedure:

Assay Buffer Conditions: (50 µL -total/reaction)

20 mM Tris, pH 7.5

50 µM EDTA

5 mM DTT

2 mM $MgCl_2$

80 mM KCl

0.4 U/µL RNAsin (Promega, stock is 40 units/µL)

0.75 µg t500 (a 500-nt RNA made using T7 runoff transcription with a sequence from the NS2/3 region of the hepatitis C genome)

1.6 μg purified hepatitis C NS5B (form with 21 amino acids C-terminally truncated)
1 μM A,C,U,GTP (Nucleoside triphosphate mix)
[alpha-^{32}P]-GTP or [alpha-^{33}P]-GTP

The compounds were tested at various concentrations up to 100 μM final concentration.

An appropriate volume of reaction buffer was made including enzyme and template t500. Nucleoside derivatives of the present invention were pipetted into the wells of a 96-well plate. A mixture of nucleoside triphosphates (NTP's), including the radiolabeled GTP, was made and pipetted into the wells of a 96-well plate. The reaction was initiated by addition of the enzyme-template reaction solution and allowed to proceed at room temperature for 1-2 h.

The reaction was quenched by addition of 20 μL 0.5M EDTA, pH 8.0. Blank reactions in which the quench solution was added to the NTPs prior to the addition of the reaction buffer were included.

50 μL of the quenched reaction were spotted onto DE81 filter disks (Whatman) and allowed to dry for 30 min. The filters were washed with 0.3 M ammonium formate, pH 8 (150 mL/wash until the cpm in 1 mL wash is less than 100, usually 6 washes). The filters were counted in 5-mL scintillation fluid in a scintillation counter.

The percentage of inhibition was calculated according to the following equation: %Inhibition = [1-(cpm in test reaction - cpm in blank) / (cpm in control reaction - cpm in blank)] x 100.

Representative compounds tested in the HCV NS5B polymerase assay exhibited IC_{50}'s less than 100 micromolar.

B. Assay for Inhibition of HCV RNA Replication:

The compounds of the present invention were also evaluated for their ability to affect the replication of Hepatitis C Virus RNA in cultured hepatoma (HuH-7) cells containing a subgenomic HCV Replicon. The details of the assay are described below. This Replicon assay is a modification of that described in V. Lohmann, F. Korner, J-O. Koch, U. Herian, L. Theilmann, and R. Bartenschlager, "Replication of a Sub-genomic Hepatitis C Virus RNAs in a Hepatoma Cell Line," Science 285:110 (1999).

Protocol:

The assay was an *in situ* Ribonuclease protection, Scintillation Proximity based-plate assay (SPA). 10,000 - 40,000 cells were plated in 100-200 μL of media containing 0.8mg/mL G418 in 96-well cytostar plates (Amersham). Compounds were added to cells at various concentrations up to 100 μM in 1% DMSO at time 0 to 18 h and then cultured for 24-96 h. Cells were fixed (20 min, 10% formalin), permeabilized (20 min, 0.25% Triton X-100/PBS) and hybridized (overnight, 50°C) with a single-stranded ^{33}P RNA probe complementary to the (+) strand NS5B (or other genes) contained in the RNA viral genome. Cells were washed, treated with RNAse, washed, heated to 65°C and counted in a Top-Count. Inhibition of replication was read as a decrease in counts per minute (cpm).

Human HuH-7 hepatoma cells, which were selected to contain a subgenomic replicon, carry a cytoplasmic RNA consisting of an HCV 5' non-translated region (NTR), a neomycin selectable marker, an EMCV IRES (internal ribosome entry site), and HCV non-structural proteins NS3 through NS5B, followed by the 3' NTR.

Representative compounds tested in the replication assay exhibited EC_{50}'s less than 100 micromolar.

The nucleoside derivatives of the present invention were also evaluated for cellular toxicity and anti-viral specificity in the counterscreens described below.

C. COUNTERSCREENS:

The ability of the nucleoside derivatives of the present invention to inhibit human DNA polymerases was measured in the following assays.

a. Inhibition of Human DNA Polymerases alpha and beta:

Reaction Conditions:

50 μL reaction volume

Reaction buffer components:

20 mM Tris-HCl, pH 7.5

200 μg/mL bovine serum albumin

100 mM KCl

2 mM β-mercaptoethanol
10 mM $MgCl_2$
1.6 μM dA, dG, dC, dTTP
α-^{33}P-dATP

Enzyme and template:
0.05 mg/mL gapped fish sperm DNA template
0.01 U/μL DNA polymerase α or β

Preparation of gapped fish sperm DNA template:
Add 5 μL 1M $MgCl_2$ to 500 μL activated fish sperm DNA (USB 70076);
Warm to 37°C and add 30 μL of 65 U/μL of exonuclease III (GibcoBRL 18013-011);
Incubate 5 min at 37°C;
Terminate reaction by heating to 65 °C for 10 min;
Load 50-100 μL aliquots onto Bio-spin 6 chromatography columns (Bio-Rad 732-6002) equilibrated with 20 mM Tris-HCl, pH 7.5;
Elute by centrifugation at 1,000Xg for 4 min;
Pool eluate and measure absorbance at 260 nm to determine concentration.

The DNA template was diluted into an appropriate volume of 20 mM Tris-HCl, pH 7.5 and the enzyme was diluted into an appropriate volume of 20 mM Tris-HCl, containing 2 mM β-mercaptoethanol, and 100 mM KCl. Template and enzyme were pipetted into microcentrifuge tubes or a 96 well plate. Blank reactions excluding enzyme and control reactions excluding test compound were also prepared using enzyme dilution buffer and test compound solvent, respectively. The reaction was initiated with reaction buffer with components as listed above. The reaction was incubated for 1 hour at 37°C. The reaction was quenched by the addition of 20 μL 0.5M EDTA. 50 μL of the quenched reaction was spotted onto Whatman DE81 filter disks and air dried. The filter disks were repeatedly washed with 150 mL 0.3M ammonium formate, pH 8 until 1 mL of wash is < 100 cpm. The disks were washed twice with 150 mL absolute ethanol and once with 150 mL anhydrous ether, dried and counted in 5 mL scintillation fluid.

The percentage of inhibition was calculated according to the following equation: % inhibition = [1-(cpm in test reaction - cpm in blank)/(cpm in control reaction - cpm in blank)] x 100.

b. Inhibition of Human DNA Polymerase gamma :

The potential for inhibition of human DNA polymerase gamma was measured in reactions that included 0.5 ng/ μL enzyme; 10 μM dATP, dGTP, dCTP, and TTP; 2 μCi/reaction [α-^{33}P]-dATP, and 0.4 μg/μL activated fish sperm DNA (purchased from US Biochemical) in a buffer containing 20 mM Tris pH8, 2 mM β-mercaptoethanol, 50 mM KCl, 10 mM $MgCl_2$, and 0.1 μg/μL BSA. Reactions were allowed to proceed for 1 h at 37°C and were quenched by addition of 0.5 M EDTA to a final concentration of 142 mM. Product formation was quantified by anion exchange filter binding and scintillation counting. Compounds were tested at up to 50 μM.

The percentage of inhibition was calculated according to the following equation: % inhibition = [1-(cpm in test reaction - cpm in blank)/(cpm in control reaction - cpm in blank)] x 100.

The ability of the nucleoside derivatives of the present invention to inhibit HIV infectivity and HIV spread was measured in the following assays.

c. HIV Infectivity Assay

Assays were performed with a variant of HeLa Magi cells expressing both CXCR4 and CCR5 selected for low background β-galactosidase (β-gal) expression. Cells were infected for 48 h, and β-gal production from the integrated HIV-1 LTR promoter was quantified with a chemiluminescent substrate (Galactolight Plus, Tropix, Bedford, MA). Inhibitors were titrated (in duplicate) in twofold serial dilutions starting at 100 μM; percent inhibition at each concentration was calculated in relation to the control infection.

d. Inhibition of HIV Spread

The ability of the compounds of the present invention to inhibit the spread of the human immunedeficiency virus (HIV) was measured by the method described in U.S. Patent No. 5,413,999 (May 9, 1995), and J.P.Vacca, et al., Proc.

Natl. Acad. Sci., 91: 4096-4100 (1994), which are incorporated by reference herein in their entirety.

The nucleoside derivatives of the present invention were also screened for cytotoxicity against cultured hepatoma (HuH-7) cells containing a subgenomic HCV Replicon in an MTS cell-based assay as described in the assay below. The HuH-7 cell line is described in H. Nakabayashi, et al., Cancer Res., 42: 3858 (1982).

e. Cytotoxicity assay:

Cell cultures were prepared in appropriate media at concentrations of approximately 1.5 x 10^5 cells/mL for suspension cultures in 3 day incubations and 5.0 x 10^4 cells/mL for adherent cultures in 3 day incubations. 99 µL of cell culture was transferred to wells of a 96-well tissue culture treated plate, and 1 µL of 100-times final concentration of the test compound in DMSO was added. The plates were incubated at 37°C and 5% CO_2 for a specified period of time. After the incubation period, 20 µL of CellTiter 96 Aqueous One Solution Cell Proliferation Assay reagent (MTS) (Promega) was added to each well and the plates were incubated at 37°C and 5% CO_2 for an additional period of time up to 3 h. The plates were agitated to mix well and absorbance at 490 nm was read using a plate reader. A standard curve of suspension culture cells was prepared with known cell numbers just prior to the addition of MTS reagent. Metabolically active cells reduce MTS to formazan. Formazan absorbs at 490 nm. The absorbance at 490 nm in the presence of compound was compared to absorbance in cells without any compound added. Reference: Cory, A. H. *et al.*, "Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture," Cancer Commun. 3: 207 (1991).

The following assays were employed to measure the activity of the compounds of the present invention against other RNA-dependent RNA viruses:

a. Determination of In Vitro Antiviral Activity of Compounds Against Rhinovirus (Cytopathic Effect Inhibition Assay):

Assay conditions are described in the article by Sidwell and Huffman, "Use of disposable microtissue culture plates for antiviral and interferon induction studies," Appl. Microbiol. 22: 797-801 (1971).

Viruses:

Rhinovirus type 2 (RV-2), strain HGP, was used with KB cells and media (0.1% $NaHCO_3$, no antibiotics) as stated in the Sidwell and Huffman reference. The virus, obtained from the ATCC, was from a throat swab of an adult male with a mild acute febrile upper respiratory illness.

Rhinovirus type 9 (RV-9), strain 211, and rhinovirus type 14 (RV-14), strain Tow, were also obtained from the American Type Culture Collection (ATCC) in Rockville, MD. RV-9 was from human throat washings and RV-14 was from a throat swab of a young adult with upper respiratory illness. Both of these viruses were used with HeLa Ohio-1 cells (Dr. Fred Hayden, Univ. of VA) which were human cervical epitheloid carcinoma cells. MEM (Eagle's minimum essential medium) with 5% Fetal Bovine serum (FBS) and 0.1% $NaHCO_3$ was used as the growth medium.

Antiviral test medium for all three virus types was MEM with 5% FBS, 0.1% $NaHCO_3$, 50 μg gentamicin/mL, and 10 mM $MgCl_2$.

2000 μg/mL was the highest concentration used to assay the compounds of the present invention. Virus was added to the assay plate approximately 5 min after the test compound. Proper controls were also run. Assay plates were incubated with humidified air and 5% CO_2 at 37°C. Cytotoxicity was monitored in the control cells microscopically for morphologic changes. Regression analysis of the virus CPE data and the toxicity control data gave the ED50 (50% effective dose) and CC50 (50% cytotoxic concentration). The selectivity index (SI) was calculated by the formula: SI = CC50 ÷ ED50.

b. Determination of In Vitro Antiviral Activity of Compounds Against Dengue, Banzi, and Yellow Fever (CPE Inhibition Assay)

Assay details are provided in the Sidwell and Huffman reference above.

Viruses:

Dengue virus type 2, New Guinea strain, was obtained from the Center for Disease Control. Two lines of African green monkey kidney cells were used to culture the virus (Vero) and to perform antiviral testing (MA-104). Both Yellow fever virus, 17D strain, prepared from infected mouse brain, and Banzi virus, II 336 strain, isolated from the serum of a febrile boy in South Africa, were obtained from ATCC. Vero cells were used with both of these viruses and for assay.

Cells and Media:

MA-104 cells (BioWhittaker, Inc., Walkersville, MD) and Vero cells (ATCC) were used in Medium 199 with 5% FBS and 0.1% $NaHCO_3$ and without antibiotics.

Assay medium for dengue, yellow fever, and Banzi viruses was MEM, 2% FBS, 0.18% $NaHCO_3$ and 50 μg gentamicin/mL.

Antiviral testing of the compounds of the present invention was performed according to the Sidwell and Huffman reference and similar to the above rhinovirus antiviral testing. Adequate cytopathic effect (CPE) readings were achieved after 5-6 days for each of these viruses.

c. Determination of In Vitro Antiviral Activity of Compounds Against West Nile Virus (CPE Inhibition Assay)

Assay details are provided in the Sidwell and Huffman reference cited above. West Nile virus, New York isolate derived from crow brain, was obtained from the Center for Disease Control. Vero cells were grown and used as described above. Test medium was MEM, 1% FBS, 0.1% $NaHCO_3$ and 50 μg gentamicin/mL.

Antiviral testing of the compounds of the present invention was performed following the methods of Sidwell and Huffman which are similar to those used to assay for rhinovirus activity. Adequate cytopathic effect (CPE) readings were achieved after 5-6 days.

d. Determination of In Vitro Antiviral Activity of Compounds Against rhino, yellow fever, dengue, Banzi, and West Nile Viruses (Neutral Red Uptake Assay)

After performing the CPE inhibition assays above, an additional cytopathic detection method was used which is described in "Microtiter Assay for Interferon: Microspectrophotometric Quantitation of Cytopathic Effect," Appl. Environ. Microbiol. 31: 35-38 (1976). A Model EL309 microplate reader (Bio-Tek Instruments Inc.) was used to read the assay plate. ED50's and CD50's were calculated as above.

EXAMPLE OF A PHARMACEUTICAL FORMULATION

As a specific embodiment of an oral composition of a compound of the present invention, 50 mg of the compound of Example 1 or Example 2 is formulated

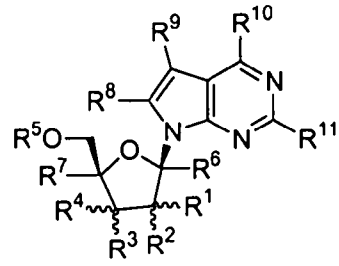
with sufficient finely divided lactose to provide a total amount of 580 to 590 mg to fill a size O hard gelatin capsule.

While the invention has been described and illustrated in reference to specific embodiments thereof, those skilled in the art will appreciate that various changes, modifications, and substitutions can be made therein without departing from the spirit and scope of the invention. For example, effective dosages other than the preferred doses as set forth heeinabove may be applicable as a consequence of variations in the responsiveness of the human being treated for severity of the HCV infection. Likewise, the pharmacologic response observed may vary according to and depending upon the particular active compound selected or whether there are present pharmaceutical carriers, as well as the type of formulation and mode of administration employed, and such expected variations or differences in the results are contemplated in accordance with the objects and practices of the present invention. It is intended therefore that the invention be limited only by the scope of the claims which follow and that such claims be interpreted as broadly as is reasonable.

2002243791 01 Jun 2006

The claims defining the invention are as follows:

1. A compound of the structural formula I:



(I)

or a pharmaceutically acceptable salt thereof;

wherein R^1 is C_{2-4} alkenyl, C_{2-4} alkynyl, or C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^2 is hydrogen, fluorine, hydroxy, mercapto, C_{1-4} alkoxy, or C_{1-4} alkyl; or R^1 and R^2 together with the carbon atom to which they are attached form a 3- to 6-membered saturated monocyclic ring system optionally containing a heteroatom selected from O, S, and NC_{0-4} alkyl;

R^3 and R^4 are each independently selected from the group consisting of hydrogen, cyano, azido, halogen, hydroxy, mercapto, amino, C_{1-4} alkoxy, C_{2-4} alkenyl, C_{2-4} alkynyl, and C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^5 is hydrogen, C_{1-10} alkylcarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or $P(O)R^{13}R^{14}$;

R^6 and R^7 are each independently hydrogen, methyl, hydroxymethyl, or fluoromethyl;

R^8 is hydrogen, C_{1-4} alkyl, C_{2-4} alkynyl, halogen, cyano, carboxy, C_{1-4} alkyloxycarbonyl, azido, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, hydroxy, C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfonyl, (C_{1-4} alkyl)$_{0-2}$ aminomethyl, or C_{4-6} cycloheteroalkyl, unsubstituted or substituted with one to two groups independently selected from halogen, hydroxy, amino, C_{1-4} alkyl, and C_{1-4} alkoxy;

R^9 is hydrogen, cyano, nitro, C_{1-3} alkyl, $NHCONH_2$, $CONR^{12}R^{12}$, $CSNR^{12}R^{12}$, $COOR^{12}$, $C(=NH)NH_2$, hydroxy, C_{1-3} alkoxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, halogen, (1,3-oxazol-2-yl), (1,3-thiazol-2-yl), or (imidazol-2-yl); wherein alkyl is unsubstituted or substituted with one to three groups independently selected from halogen, amino, hydroxy, carboxy, and C_{1-3} alkoxy;

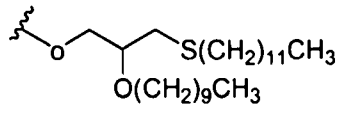
R^{10} and R^{11} are each independently hydrogen, hydroxy, halogen, C_{1-4} alkoxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, C_{3-6} cycloalkylamino, di(C_{3-6}

2002243791 01 Jun 2006

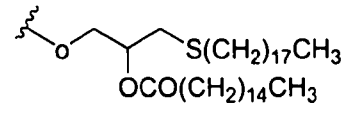
cycloalkyl)amino, or C_{4-6} cycloheteroalkyl, unsubstituted or substituted with one to two groups independently selected from halogen, hydroxy, amino, C_{1-4} alkyl, and C_{1-4} alkoxy;

each R^{12} is independently hydrogen or C_{1-6} alkyl; and

R^{13} and R^{14} are each independently hydroxy, $OCH_2CH_2SC(=O)C_{1-4}$ alkyl, $OCH_2O(C=O)OC_{1-4}$ alkyl, $NHCHMeCO_2Me$, $OCH(C_{1-4}$ alkyl$)O(C=O)C_{1-4}$ alkyl,



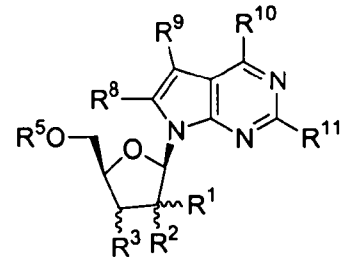
or



;

with the proviso that when R^1 is β-methyl, R^4 is hydrogen, R^2 and R^3 are α-hydroxy, R^{10} is amino, and R^5, R^6, R^7, R^8, and R^{11} are hydrogen, then R^9 is not cyano or $CONH_2$.

2. The compound of Claim 1 of the structural formula II:



(II)

or a pharmaceutically acceptable salt thereof;

wherein

R^1 is C_{1-3} alkyl, wherein alkyl is optionally substituted with hydroxy, amino, C_{1-3} alkoxy, C_{1-3} alkylthio, or one to three fluorine atoms;

R^2 is hydroxy, fluoro, or C_{1-4} alkoxy;

R^3 is hydrogen, halogen, hydroxy, amino, or C_{1-4} alkoxy;

R^5 is hydrogen, $P_3O_9H_4$, $P_2O_6H_3$, or PO_3H_2;

R^8 is hydrogen, amino, or C_{1-4} alkylamino;

R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and

R^{10} and R^{11} are each independently hydrogen, halogen, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, or C_{3-6} cycloalkylamino;

with the proviso that when R^1 is β-methyl, R^2 and R^3 are α-hydroxy, R^{10} is amino, and R^5, R^8, and R^{11} are hydrogen, then R^9 is not cyano or $CONH_2$.

3. The compound of Claim 2 wherein

R^1 is methyl, fluoromethyl, hydroxymethyl, difluoromethyl, trifluoromethyl, or aminomethyl;

R^2 is hydroxy, fluoro, or methoxy;

2002243791 01 Jun 2006

R^3 is hydrogen, fluoro, hydroxy, amino, or methoxy;

R^5 is hydrogen or $P_3O_9H_4$;

R^8 is hydrogen or amino;

R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and

R^{10} and R^{11} are each independently hydrogen, fluoro, hydroxy, or amino;

with the proviso that when R^1 is β-methyl, R^2 and R^3 are α-hydroxy, R^{10} is amino, and R^5, R^8, and R^{11} are hydrogen, then R^9 is not cyano or $CONH_2$.

4. The compound of Claim 1 selected from the group consisting of:

4-amino-7-(2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-methylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-dimethylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-7-(2-*C*-vinyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-7-(2-*C*-hydroxymethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid,

4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

2,4-diamino-7-(2-*C*-methyl-β-D-ribofuransoyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

2-amino-4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

2002243791 01 Jun 2006

2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,

4-amino-7-(2-*C*-ethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimdine,

4-amino-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,

2-amino-5-methyl-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3*d*]pyrimidin-4(3*H*)-one,

4-amino-7-(3-deoxy-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-7-(3-deoxy-2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine,

4-amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-7-(2,4-di-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine, and

4-amino-7-(3-deoxy-3-fluoro-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;

and the corresponding 5'-triphosphates;

or a pharmaceutically acceptable salt thereof.

5. The compound of Claim 4 selected from the group consisting of:

4-amino-7-(2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine, and

4-amino-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

and the corresponding 5'-triphosphates;
or a pharmaceutically acceptable salt thereof.

6. The compound of Claim 5 which is
4-amino-7-(2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

7. The compound of Claim 5 which is
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

8. The compound of Claim 5 which is 4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

9. The compound of Claim 5 which is 4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

10. The compound of Claim 5 which is 4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

11. A pharmaceutical composition comprising a compound of Claim 1 and a pharmaceutically acceptable carrier.

12. The pharmaceutical composition of Claim 11 useful for inhibiting RNA-dependent RNA viral polymerase, inhibiting RNA-dependent RNA replication, and/or treating RNA-dependent RNA viral infection.

13. The pharmaceutical composition of Claim 12 wherein said RNA-dependent RNA viral polymerase is HCV NS5B polymerase, said RNA-dependent RNA viral replication is HCV replication, and said RNA-dependent RNA viral infection is HCV infection.

14. A method of inhibiting RNA-dependent RNA viral polymerase and/or inhibiting RNA-dependent RNA viral replication comprising administering to a mammal in need of such inhibition an effective amount of a compound according to Claim 1.

15. The method of Claim 14 wherein said RNA-dependent RNA viral polymerase is HCV NS5B polymerase and said RNA-dependent RNA viral replication is HCV viral replication.

16. A method of treating RNA-dependent RNA viral infection comprising administering to a mammal in need of such treatment an effective amount of a compound according to Claim 1.

17. The method of Claim 16 wherein said RNA-dependent RNA viral infection is HCV infection.

18. The method of Claim 17 in combination with a therapeutically effective amount of another agent active against HCV.

19. The method of Claim 18 wherein said agent active against HCV is ribavirin; levovirin; thymosin alpha-1; an inhibitor of NS3 serine protease; an inhibitor of inosine monophosphate dehydrogenase; interferon-α or pegylated interferon-α, alone or in combination with ribavirin or levovirin.

20. The method of Claim 19 wherein said agent active against HCV is interferon-α or pegylated interferon-α, alone or in combination with ribavirin.

21. Use of a compound of Claim 1 for the inhibition of RNA-dependent RNA viral polymerase or inhibition of RNA-dependent RNA viral replication in a mammal.

22. Use of a compound of Claim 1 for treatment of RNA-dependent RNA viral infection in a mammal.

2002243791 01 Jun 2006

23. The use of Claim 22 wherein said RNA-dependent RNA viral infection is hepatitis C infection.

24. Use of a compound of Claim 1 in the manufacture of a medicament for the inhibition of RNA-dependent RNA viral polymerase or the inhibition of RNA-dependent RNA viral replication in a mammal.

25. Use of a compound of Claim 1 in the manufacture of a medicament for treatment of RNA-dependent RNA viral infection in a mammal.

26. The use of Claim 25 wherein said RNA-dependent RNA viral infection is hepatitis C infection.

27. The compound of Claim 5 which is 4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;

or a pharmaceutically acceptable salt thereof.

28. A compound of formula (I) as defined in claim 1 or a pharmaceutically acceptable salt thereof and substantially as herein described with reference to any one of Examples 1 to 38.

29. A pharmaceutical composition comprising a compound as defined in claim 28 or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier.

30. A process of making a compound of formula (I) as defined in claim 1 which process is substantially as herein described with reference to any one of Examples 1 to 38.

31. Use of a compound of claim 28 in the manufacture of a medicament for the inhibition of RNA-dependent RNA viral polymerase or inhibition of RNA-dependent RNA viral replication in a mammal.

Dated 29 May, 2006

Merck & Co., Inc.

Patent Attorneys for the Applicant/Nominated Person

SPRUSON & FERGUSON