Supporting information for

The Design, Synthesis and Anti-viral Activity of Monofluoro and Difluoro Analogues of 4'-Azidocytidine Against Hepatitis C Virus Replication: The Discovery of 4'-Azido-2'-deoxy-2'-fluoroarabinocytidine and 4'-Azido-2'-dideoxy-2',2'-difluorocytidine.

David B. Smith,[†] Genadiy Kalayanov,[§] Christian Sund,[§] Anna Winqvist,[§] Tatiana Maltseva,[§] Vincent J-P. Leveque,[†] Sonal Rajyaguru,[†] Sophie Le Pogam,[†] Isabel Najera,[†] Kurt Benkestock,[§] Xiao-Xiong Zhou,[§] Ann C. Kaiser,[†] Hans Maag,[†] Nick Cammack ,[†] Joseph A. Martin,[†] Steven Swallow,[†] Nils Gunnar Johansson,[§] Klaus Klumpp,[†] and Mark Smith[†]*

[†]Roche Palo Alto LLC, 3431 Hillview Avenue, Palo Alto, CA 94304, USA.

[§]Medivir AB, Department of Medicinal Chemistry, Lunastigen 7, SE-141, 44 Huddinge, Sweden.

*Corresponding author. Tel.: +1 650 855 5076; fax: +1 650 852 1132; e-mail Mark.Smith@Roche.com

Contents:

Biological methods

The HCV replicon assay was performed in the stable replicon cell line 2209-23 derived from Huh-7 cells stably transfected with a bicistronic HCV replicon (genotype 1b) expressing the Renilla luciferase reporter gene, as described.[1,2]

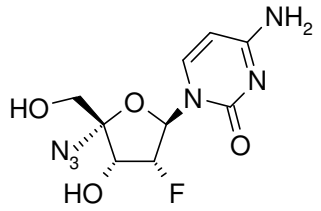
Analytical methods.

The analytical RP-HPLC system consisted of Waters 2695 Alliance separation module, Waters 996 photodiode array detector, and Micromass ZQ2000 mass detector (operated in +ESI). The columns used were an Atlantis dC18, 3 × 150 mm, 3 µm, 100A from Waters and Hypercarb, 50 × 3, 3 µm, from Thermo Electron Corporation. The mobile phases were based on water/acetonitrile containing 5 mM ammonium acetate. LC-MS accurate mass measurements were performed using a HDMS Synapt instrument from Waters (UK) equipped with a lockspray interface, connected to a Waters Aquity system. The acquisition range was *m/z* 100 to 1000 with an acquisition time of 0.15 s (+ESI). Leucine enkephalin was used as lock mass. The reversed phase column was an YMC-UltraHT Pro C_{18}, 2.1 × 50 mm, 2µm, 120A from YMC (U.S.A) and the mobile phases were based on water/acetonitrile containing 0.2% formic acid. ^{1}H - and ^{13}C - NMR experiments were carried out on Varian spectrometer (UNITY INOVA) at magnetic field strength of 11.7 T operating at 499.84 MHz for ^{1}H and 125.67 MHz for ^{13}C, unless otherwise stated. The spectrometer was equipped with ^{1}H, ^{13}C, ^{15}N 5 mm Indirect detected Cryo Probe. ^{1}H and ^{13}C pulses were applied with 36.8 kHz and 15.7 kHz field, respectively. ^{13}C decoupling was performed using GARP with 8.8 kHz field strength. To avoid the spinning artifacts, all spectra were measured on non-spinning samples. All experiments have been done at temperature 25 °C. The assignment of the ^{1}H - and ^{13}C resonances have been based on homonuclear 2D COSY and NOESY experiments as well as on inverse heteronuclear experiments, gHSQC and gHMBC. For COSY and NOESY experiments, the data sets were recorded as 2 K × 256 real matrix with 4 (and 16) scans for each t_1 value and a spectral width of 14 ppm. For gHSQC and gHMBC, the

data sets were recorded as 2 K × 256 real matrix with 4 scans for each t_1 value and a spectral width of 14 ppm in F2 and 230 ppm in F1 with the carrier 6 and 125 ppm, respectively. In all cases the recycle delay used was 2.0 s.

Compound	Mw (Da)	Purity (%)
10	286.1	98.9
17	286.1	99.6
20	286.1	94.2
28	286.1	97.6
34	305.1	99.8

1-(4’-Azido-2'-deoxy-2'-fluoro-β-D-ribofuranosyl)cytosine (10).



10

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Data File C:\EZXDATA\GENADIY\11-08\201108-MSV12111-03374.D
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    Acq. Instrument : MIKI                              Location : Vial 5
    Injection Date  : 2008-11-20 12:47:35                  Inj :   1
                                                       Inj Volume : 5 µl
    Acq. Method     : C:\Chem32\1\METHODS\ROCHE5.M
    Last changed    : 2008-11-20 12:47:10  by Genadiy Kalayanov
                      (modified after loading)
    Analysis Method : C:\Chem32\1\METHODS\PN0599A3.M
    Last changed    : 2008-11-18 11:26:26  by Christian Sund
                      (modified after loading)
    Method Info     : STANDARD METHOD (pos and neg ionizaion); 5 to 99 % ACN in 3 min
                      Flow: 0.8 ml/min. UV=210-400 nm, ACE C8 3 * 50 mm
                      Mobile phase A: 10 mM NH4Ac in 90% H2O, B: 10 mM NH4Ac in 90% ACN

    Sample Info     : a
                      Agilent Easy-Access Method: 'Roche 5 min'
                      *** No target masses specified ***
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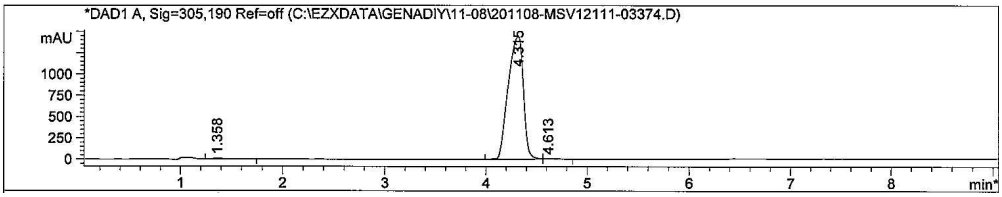


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 Sample Name: MSV121

    ================================================================================
    ================================================================================
                              Area Percent Report
    ================================================================================

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    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=305,190 Ref=off
     Signal has been modified after loading from rawdata file!

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]       [min]    [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   1.358 BB    0.0903   67.67107   11.22322   0.4451
       2   4.315 BV    0.1754 1.50299e4  1444.23279  98.8579
       3   4.613 VB    0.1209  105.97323   11.56340   0.6970

    Totals :                  1.52035e4  1467.01941
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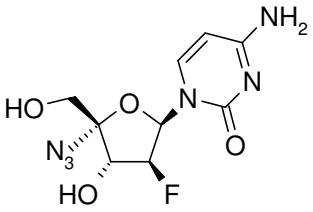
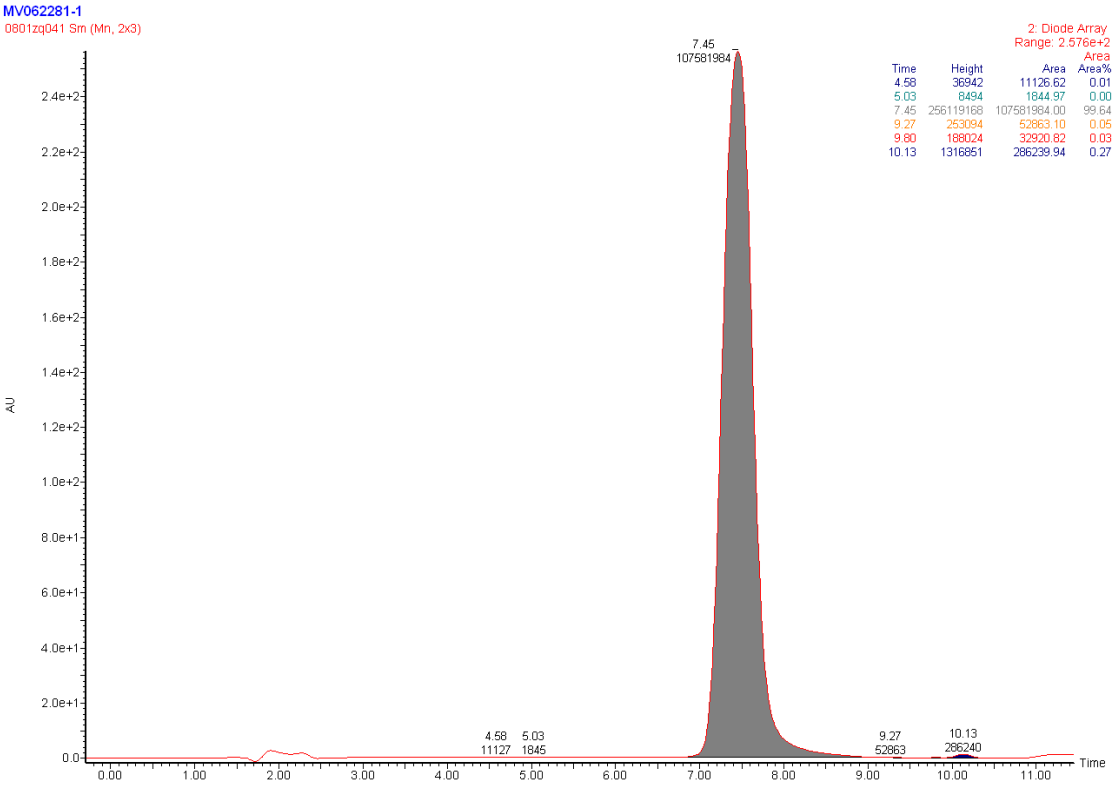
1-(4’-Azido-2’-deoxy-2’-fluoro-β-D-arabinofuranosyl)cytosine (17).



17



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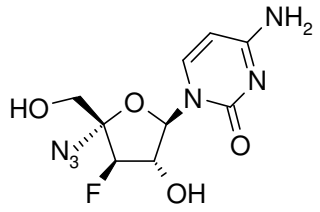

20

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    Chiralpak AS-H 85/15 Hexanes/EtOH @ 1.2 ml/min

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    Injection Date  : 10/27/2008 5:18:57 PM
    Sample Name     : PA35102-147                    Location : Vial 33
    Acq. Operator   : Marquis Cummings
    Acq. Instrument : 1100HPLC                    Inj Volume : 5 µl
    Acq. Method     : C:\CHEM32\1\METHODS\25 AS AN.M
    Last changed    : 10/27/2008 4:48:25 PM by Marquis Cummings
                      (modified after loading)
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    Last changed    : 10/28/2008 2:56:04 PM by Marquis Cummings
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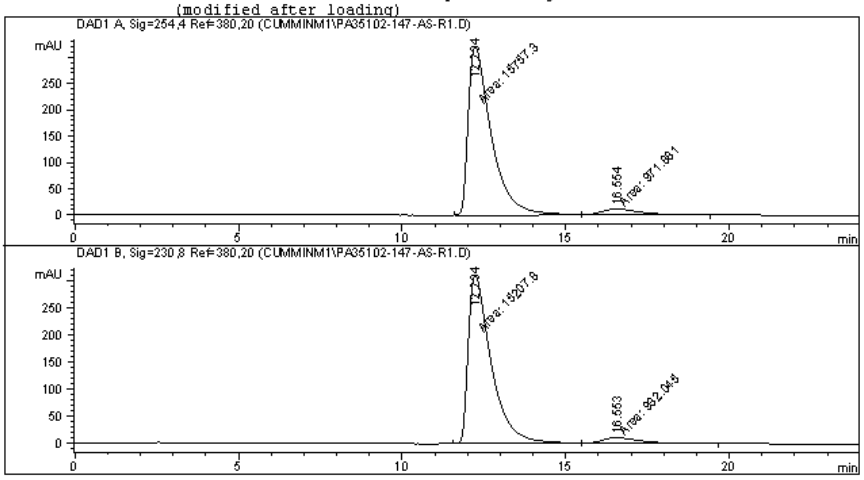


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                              Area Percent Report
    ===================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,4 Ref=380,20

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]    [mAU*s]    [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1  12.234 MF    0.8174 1.57573e4   321.27060  94.1905
       2  16.554 FM    1.4087  971.88129   11.49824   5.8095

    Totals :                  1.67292e4   332.76884
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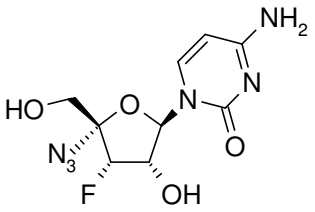
1-(4’-Azido-3'-deoxy-3'-fluoro-β-D-ribofuranosyl)cytosine (28)



28

```
Print of window 38: Current Chromatogram(s)
Bpure12
Agilent Easy-Access Method: 'Roche Method'
*** No target masses specified ***

=====================================================================
Injection Date  : 2004-07-01 13:06:39 PM
Sample Name     : 39944Bpure12                    Location : Vial 3
Acq. Operator   : Christian Sund
Acq. Instrument : INSTRUMENT1                  Inj Volume : 5 µl
Acq. Method     : C:\HPCHEM\1\METHODS\ROCHE_GR.M
Last changed    : 2004-07-01 13:04:25 PM by Christian Sund
                  (modified after loading)
Analysis Method : C:\HPCHEM\1\METHODS\ROCHE_GR.M
Last changed    : 2004-07-01 14:32:20 PM by Genadiy Kalayanov
                  (modified after loading)
Gradient ACN/H2O: 10/90 --> 90/10 in 10 min
Flow: 1.0 ml/min, 266 nm
HyperCArb 4.6 * 100 mm
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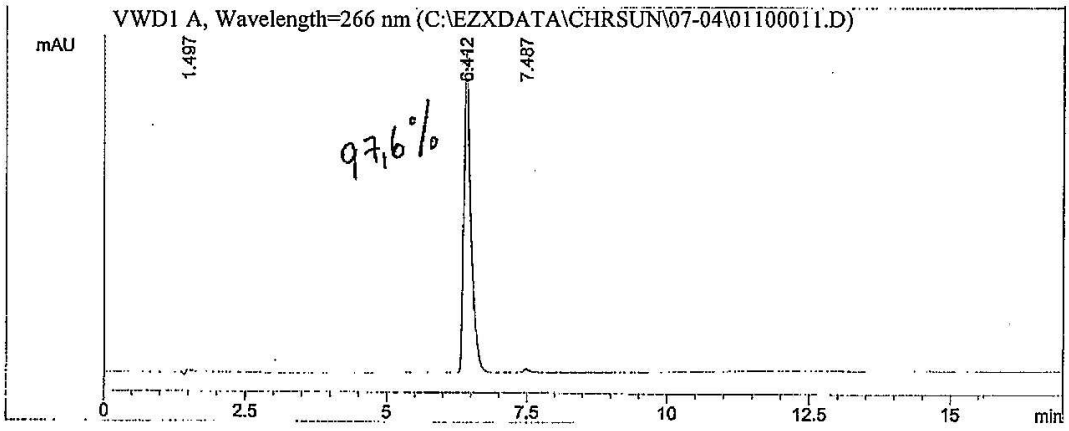


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Data File C:\EZXDATA\CHRSUN\07-04\01100011.D

=====================================================================
                      Area Percent Report
=====================================================================

Signal 1: VWD1 A, Wavelength=266 nm

Peak    Time    Type      Area        Height      Area
  #     [min]           [mAU*s]       [mAU]        %
----|--------|----|-----------|-----------|--------|
   1     1.497 PB       27.44311     2.81430   1.4286
   2     6.412 BB     1874.70911   212.52504  97.5971
   3     7.487 VB       18.71299     2.19273   0.9742
```
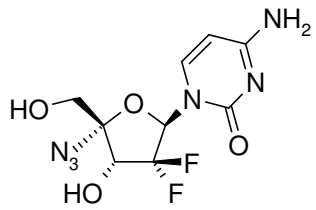
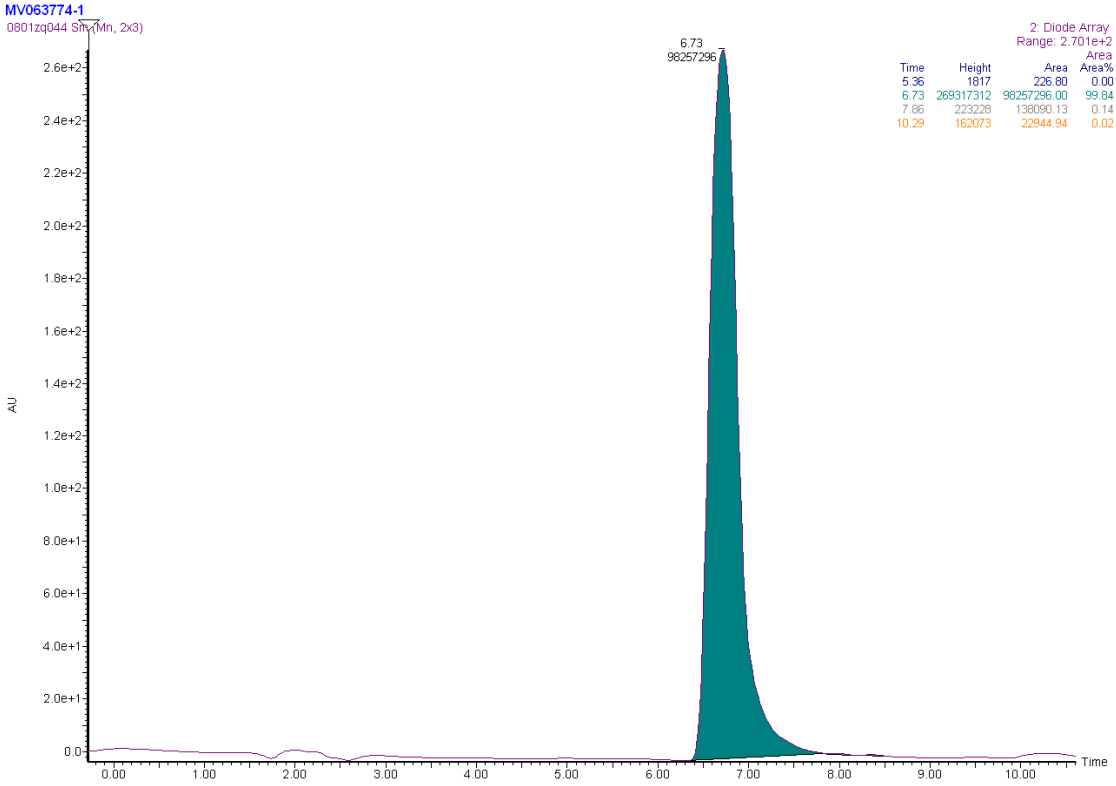

1-(4’-Azido-2’-dideoxy-2’,2’-difluoro-β-D-ribofuranosyl)cytosine (34).



34



References.

(1) Klumpp, K.; Leveque, V.; Le Pogam, S.; Ma, H.; Jiang, W-R.; Kang, H.; Granycome, C.; Singer, M.; Laxton, C.; Hang, J. Q.; Sarma, K.; Smith, D. B.; Heindl, D.; Hobbs, C. J.; Merrett, J. H.; Symons, J.; Cammack, N.; Martin, J. A.; Devos, R. and Najera, I. The Novel Nucleoside

Analog R1479 (4'-Azidocytidine) Is a Potent Inhibitor of NS5B-dependent RNA Synthesis and Hepatitis C Virus Replication in Cell Culture. *J. Biol. Chem*. **2006**, *281*, 3793–3799.

(2) Klumpp, K.; Kalayanov, G.; Ma, H.; Le Pogam, S.; Leveque, V.; Jiang, W-R.; Inocencio, N.; De Witte.; Rajyaguru, S.; Tai, E.; Chanda, S.; Irwin, M. R.; Sund, C.; Winquist, A.; Maltseva, T.; Eriksson, S.; Usova, E.; Smith, M.; Alker, A.; Najera, I.; Cammack, N.; Martin, J. A.; Johansson, N. G. and Smith, D. B. 2'-deoxy-4'-azido nucleoside analogs are highly potent inhibitors of HCV replication despite the lack of 2'-alpha hydroxyl groups. *J. Biol. Chem*. **2008**, *283*, 2167–2175.