Supporting information for

The design, synthesis and antiviral activity of 4'-azidocytidine analogues against hepatitis C virus replication: The discovery of 4'-azidoarabinocytidine.

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Contents:

Biology

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]

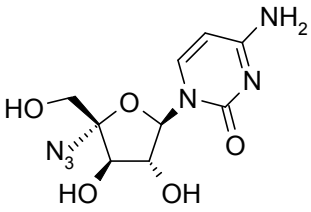
Analytical methods

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. 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 (%)
9	284.1	94.8
13	284.1	96.6
21	298.1	96.3
24	268.2	89.7
25	298.2	100

1-(4’-Azido-β-D-xylofuranosyl)cytosine (9).



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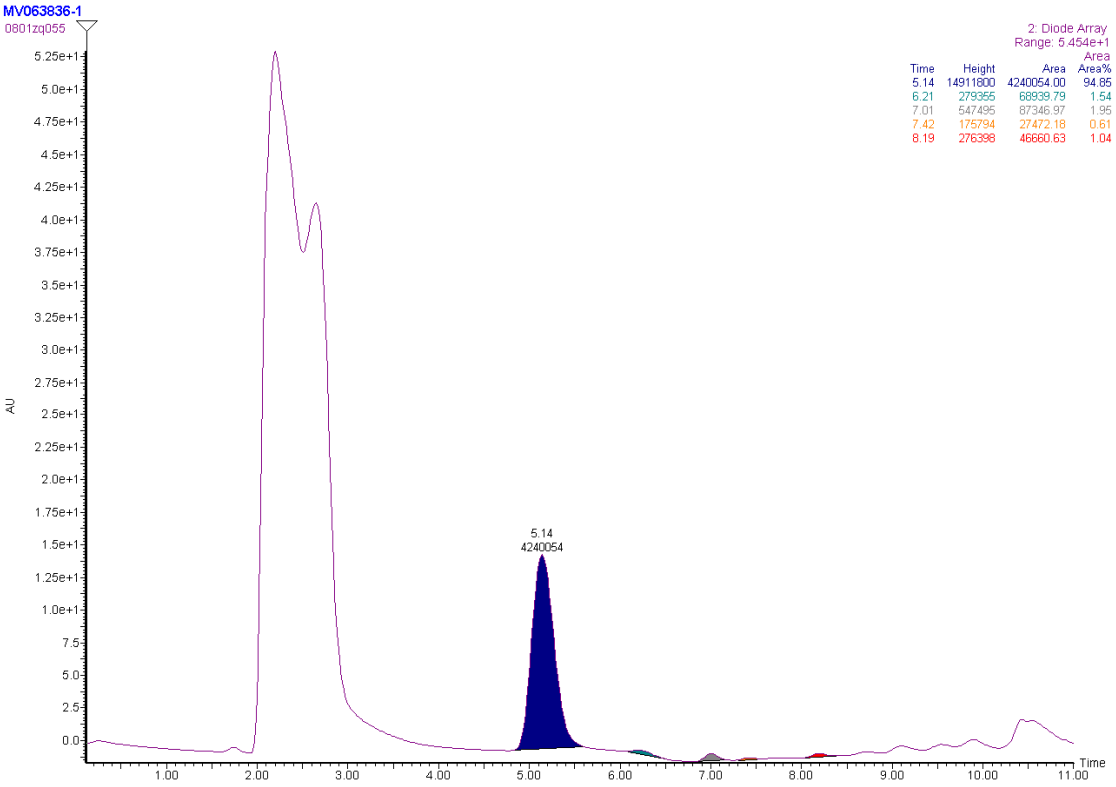
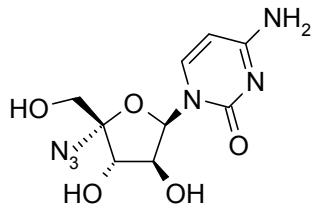


1-(4’-Azido-β-D-arabinofuranosyl)cytosine (13).



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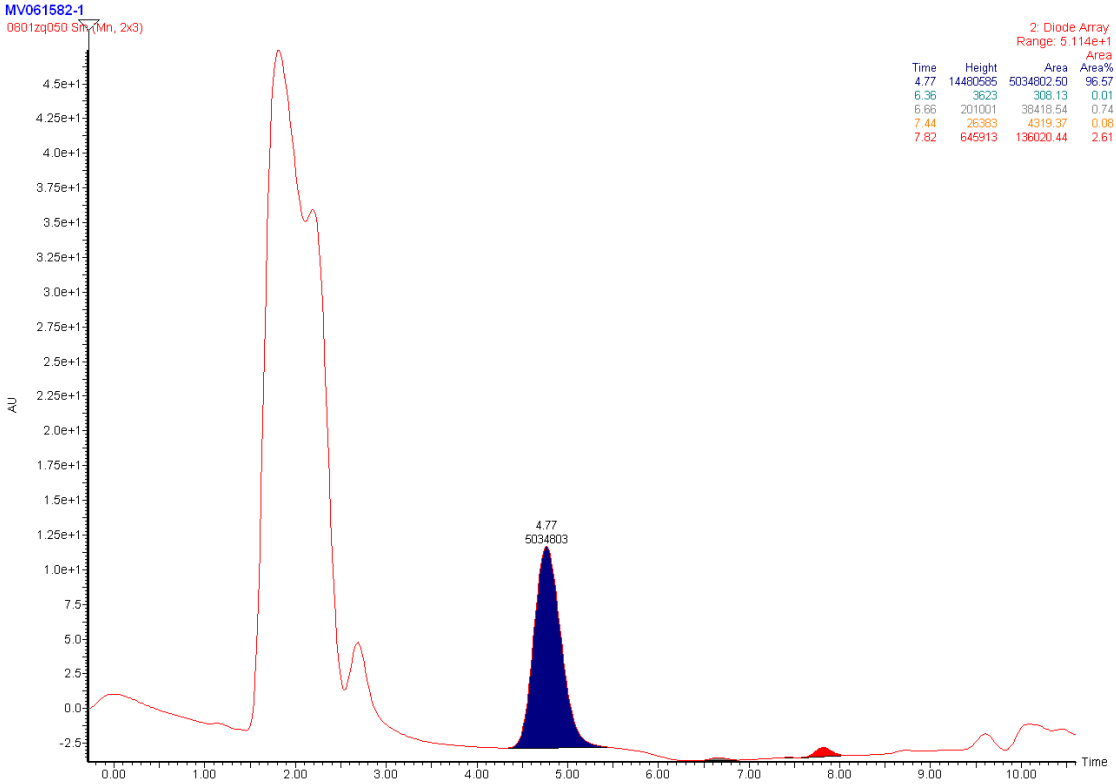
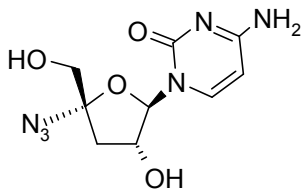


1-(4’-Azido-3’-deoxy-β-D-ribofuranosyl)cytosine (21).



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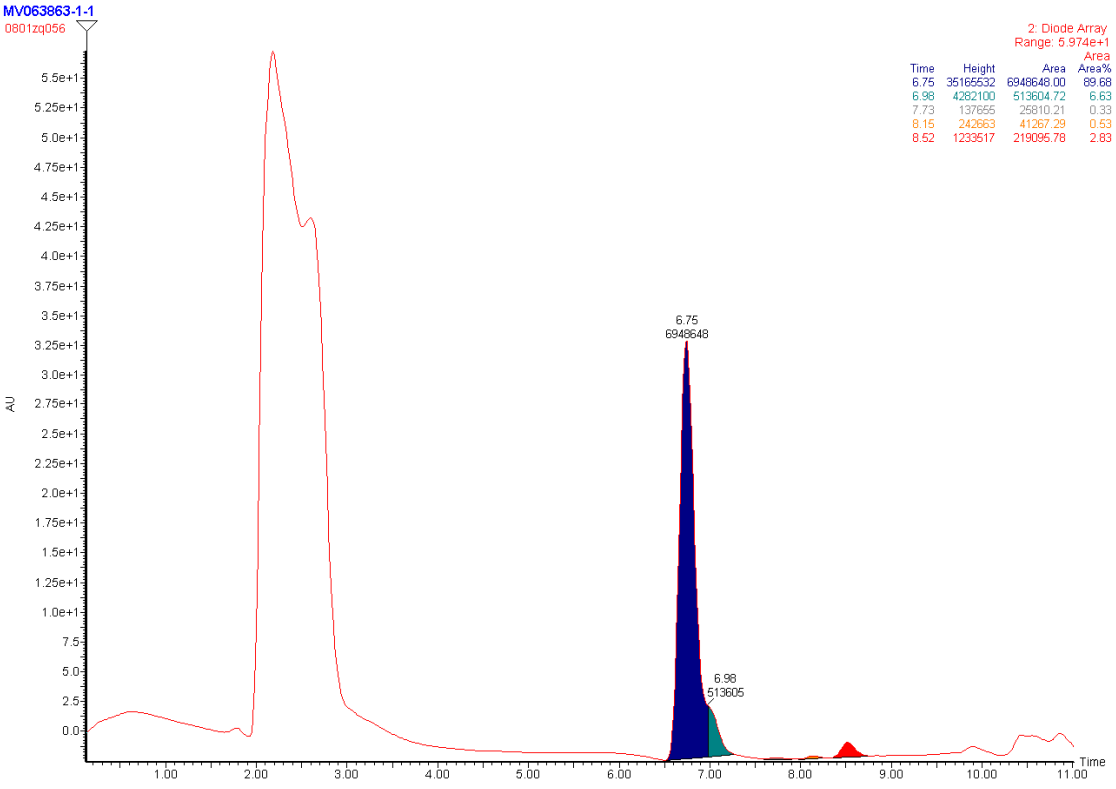
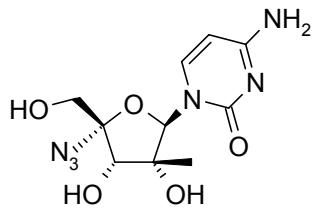


1-(4’-Azido-2’-β-methyl-β-D-ribofuranosyl)cytosine (24).



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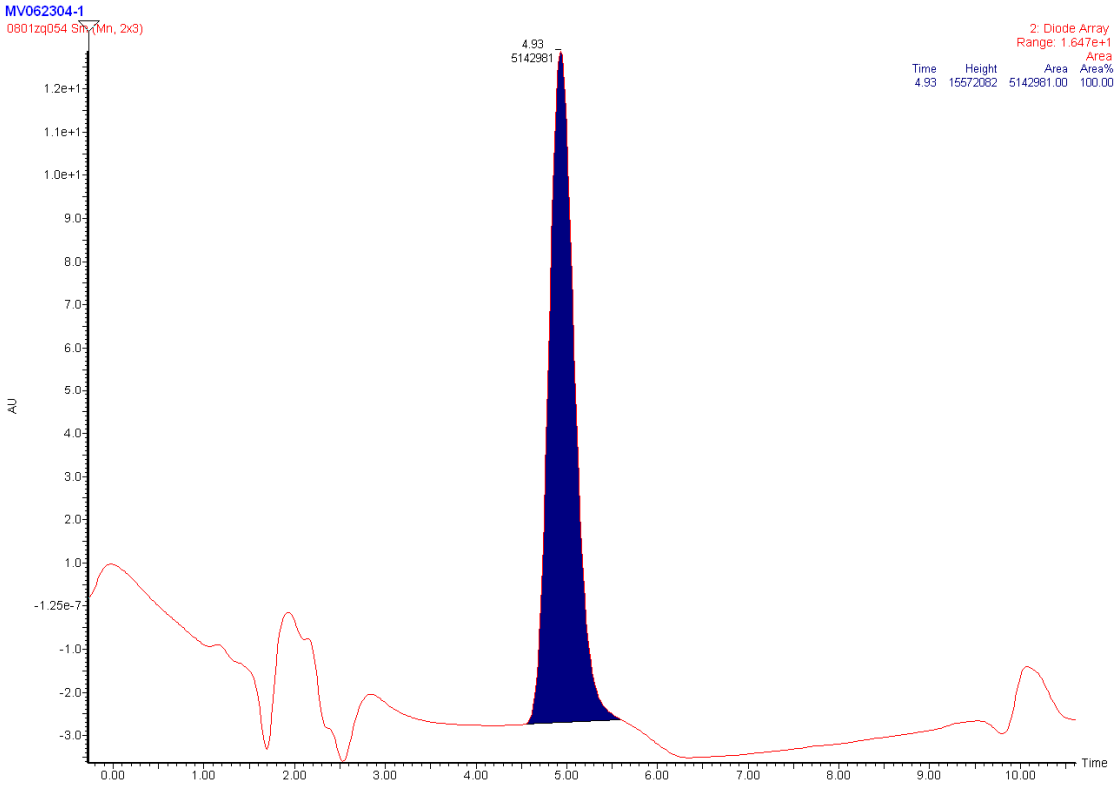
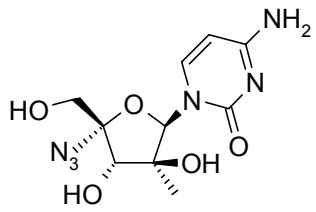


1-(4’-Azido-2’-α-methyl-β-D-ribofuranosyl)cytosine (25).



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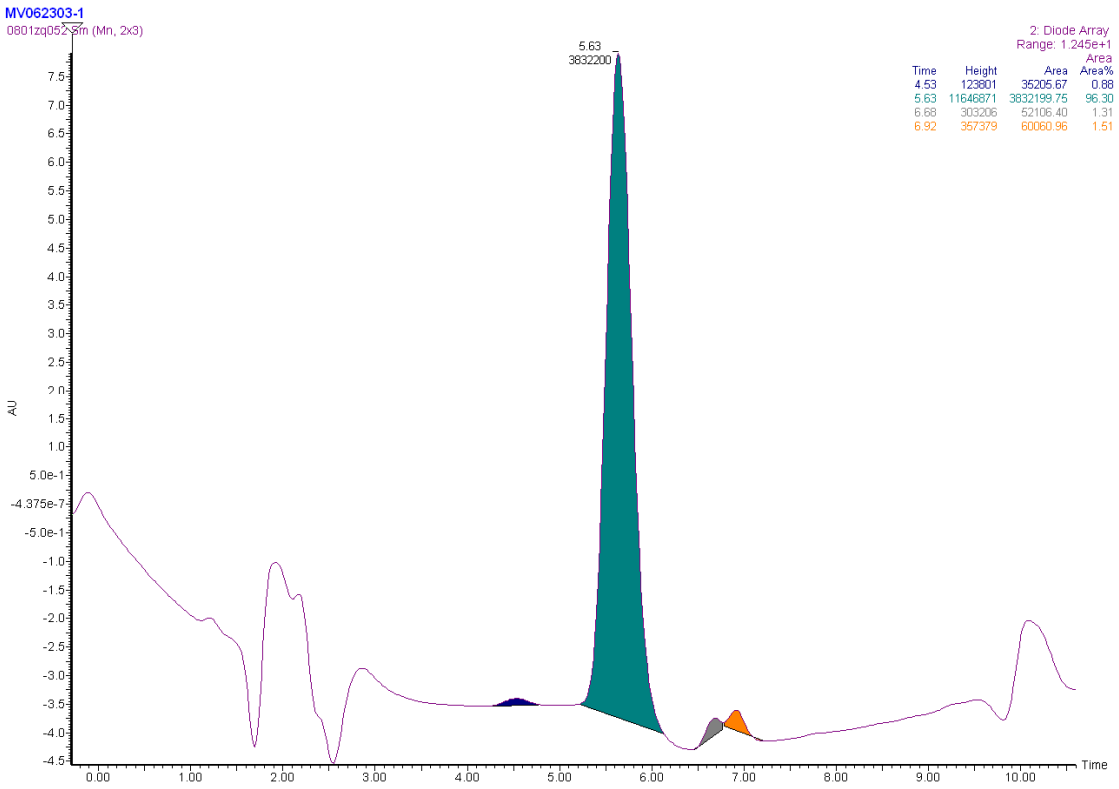

1-(4'-Azido-β-D-ribofuranosyl)cytosine (3). ^{1}H NMR (DMSO-d_6): δ 7.72 (d, *J* = 7.5 Hz, 1H), 7.26 (br. d, 2H), 6.10 (d, *J* = 5.2 Hz, 1H), 5.75 (d, *J* = 7.5 Hz, 1H), 5.61–5.48 (m, 2H), 5.49 (d, *J* = 6.2 Hz, 1H), 4.21–4.14 (m, 2H), 3.50 (dd, *J* = 5.8 and 11.8 Hz, 1H), 3.41 (dd, *J* = 5.8 and 11.8 Hz, 1H).

1-(4'-Azido-β-D-ribofuranosyl)uracil (10). ^{1}H NMR (DMSO-d_6): δ 11.39 (s, 1H), 7.80 (d, *J* = 8 Hz, 1H), 6.07 (d, *J* = 6.4 Hz, 1H), 5.76 (d, *J* = 5.7 Hz, 1H), 5.70 (d, *J* = 8 Hz, 1H), 5.62–5.56 (m, 2H), 4.25 (dd, *J* = 6.5 and 11.8 Hz, 1H), 4.17 (t, *J* = 5.5 Hz, 1H), 3.52–3.41 (m, 2H).

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.