Improving Metabolic Stability with Deuterium: The Discovery of BMT-052, a Pan-genotypic HCV NS5B Polymerase Inhibitor

Kyle Parcella*, Kyle Eastman, Kap-Sun Yeung , Katharine A. Grant-Young, Juliang Zhu, Tao Wang, Zhongxing Zhang, Zhiwei Yin, Dawn Parker, Kathy Mosure, Hua Fang, Ying-Kai Wang, Julie Lemm, Xiaoliang Zhuo, Umesh Hanumegowda, Mengping Liu, Karen Rigat, Maria Donoso, Maria Tuttle, Tatyana Zvyaga, Zuzana Haarhoff, Nicholas A. Meanwell, Matthew G. Soars, Susan B. Roberts, and John F. Kadow

ACS Med. Chem. Lett. **2017**, *8*, 771-774.

Wipf Group Current Literature

Chaemin Lim

08/05/2017

Hepatitis C Virus (HCV) prevalence

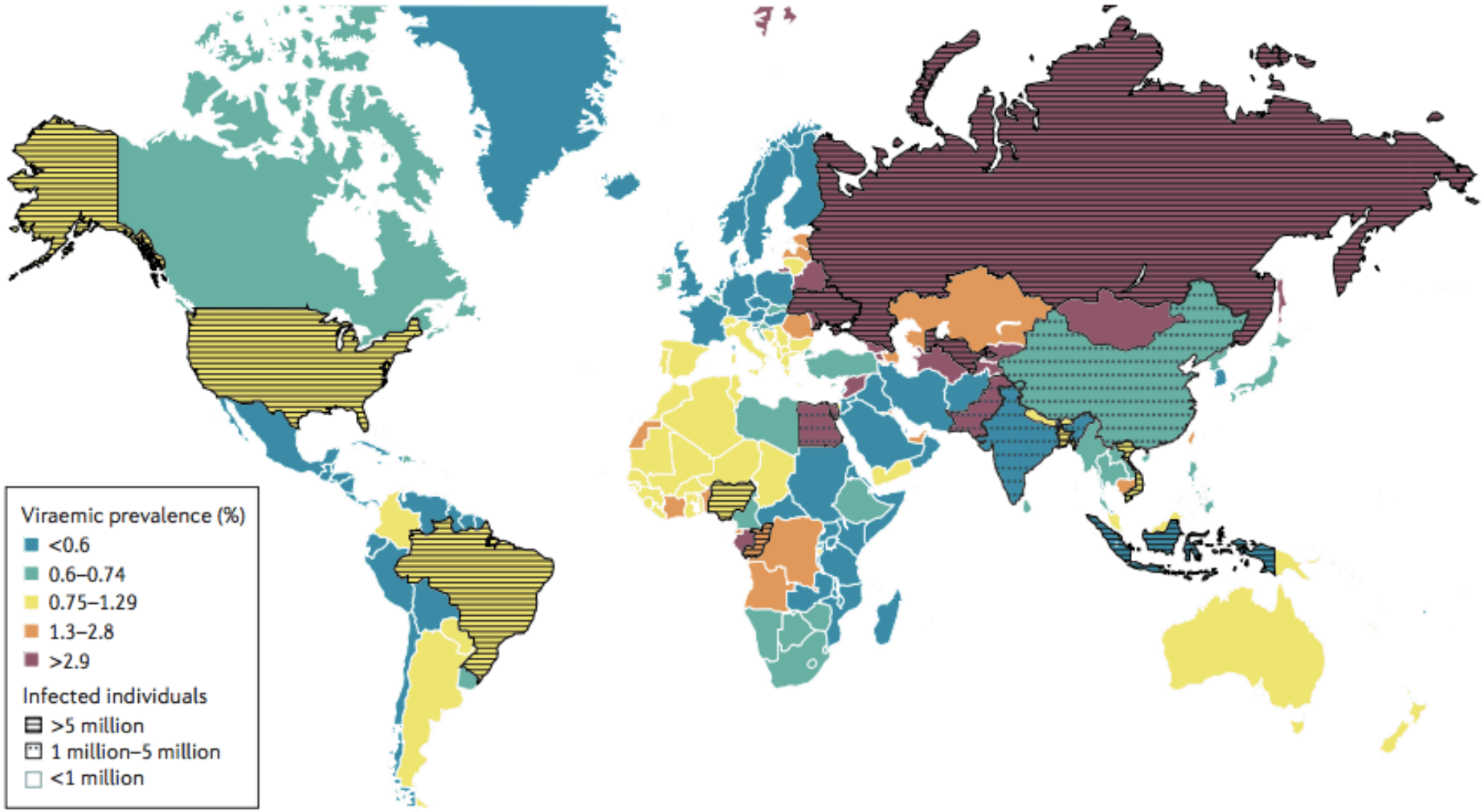
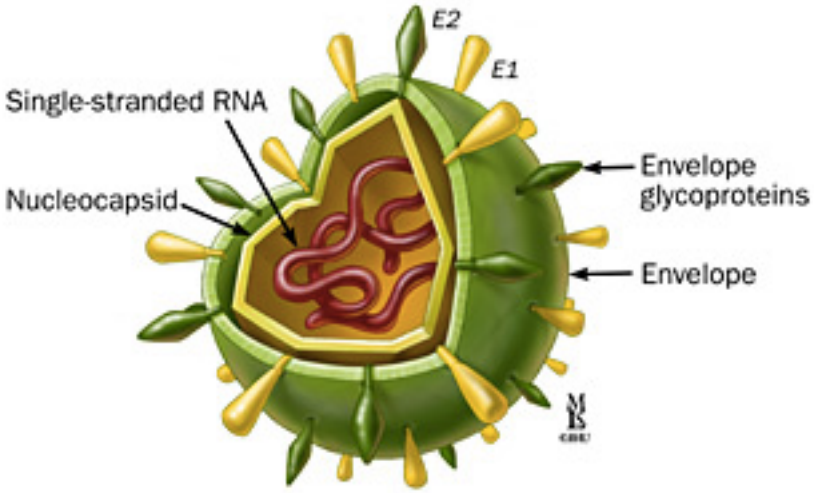


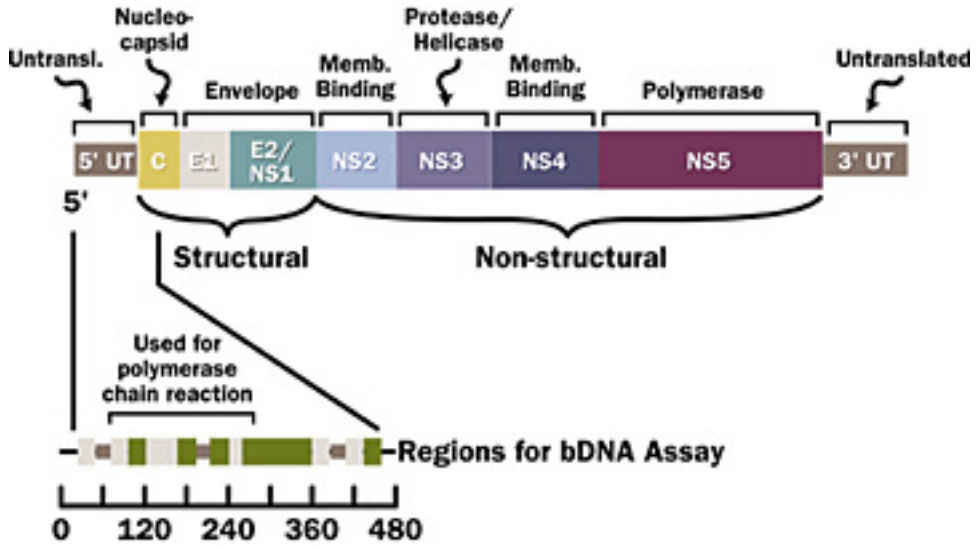
Figure 2 | **HCV prevalence.** Schematic representation of the actual viraemic hepatitis C virus (HCV) prevalence and the extrapolated total HCV infections per country. Figure based on data obtained from REF. 15.

Manns, M. P., et al. Hepatitis C virus infection. *Nature reviews. Disease primers*, **2017**, *3*, 17006.

Hepatitis C Virus (HCV)

- The hepatitis C virus (HCV) is a single-stranded RNA virus of the Hepacivirus genus in the Flaviviridae family.
- A major cause of hepatitis (acute and chronic) and cirrhosis - 21% of all acute viral hepatitis in the United States may be attributed to hepatitis C viral infection. Infection with hepatitis C almost always results in chronic infection. There are approximately 30,000 new cases of acute hepatitis C diagnosed each year in the United States.
- Hepatitis C virus (HCV) is a very heterogeneous virus; seven genotypes have been detected thus far. Genotype distribution differs between countries according to the World Bank income categories.
- The genomic organization of the hepatitis C virus shows highly conserved 5' and 3' nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B).





https://www.halstedsurgery.org/Upload/200710261000_13974_000.jpg
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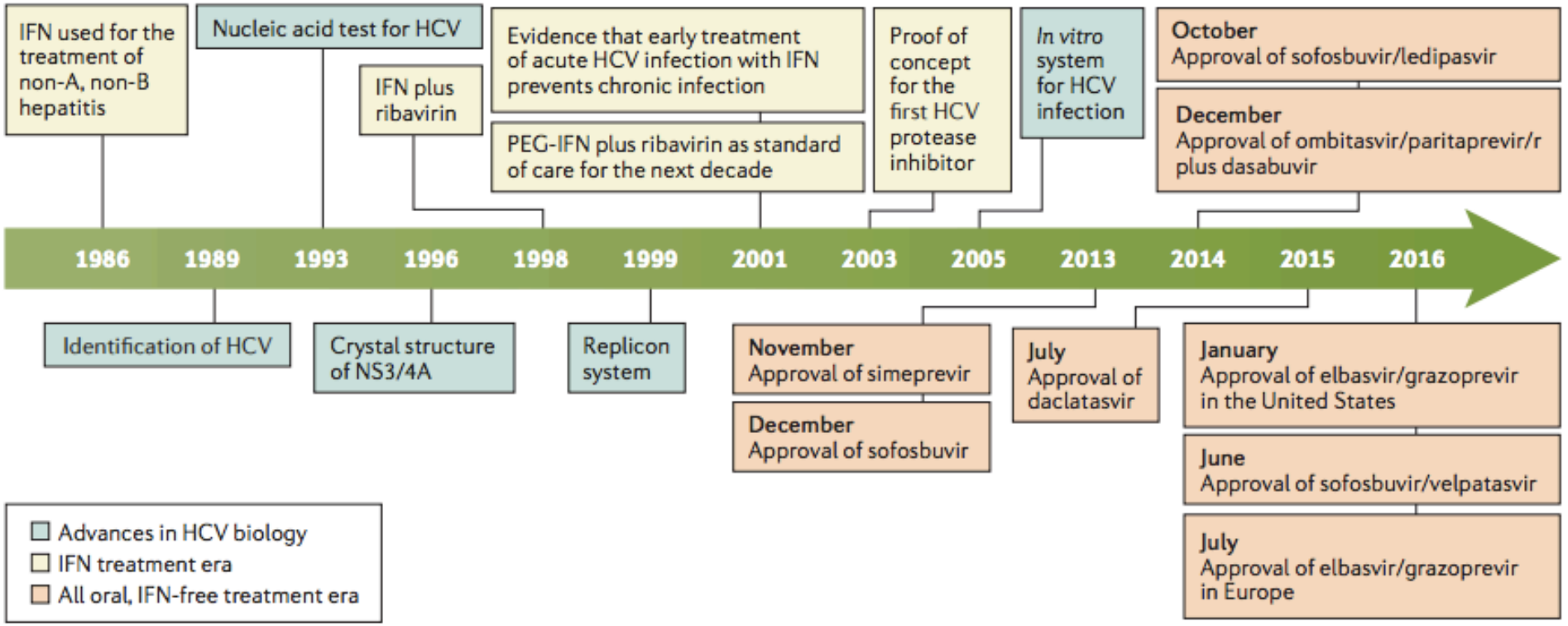
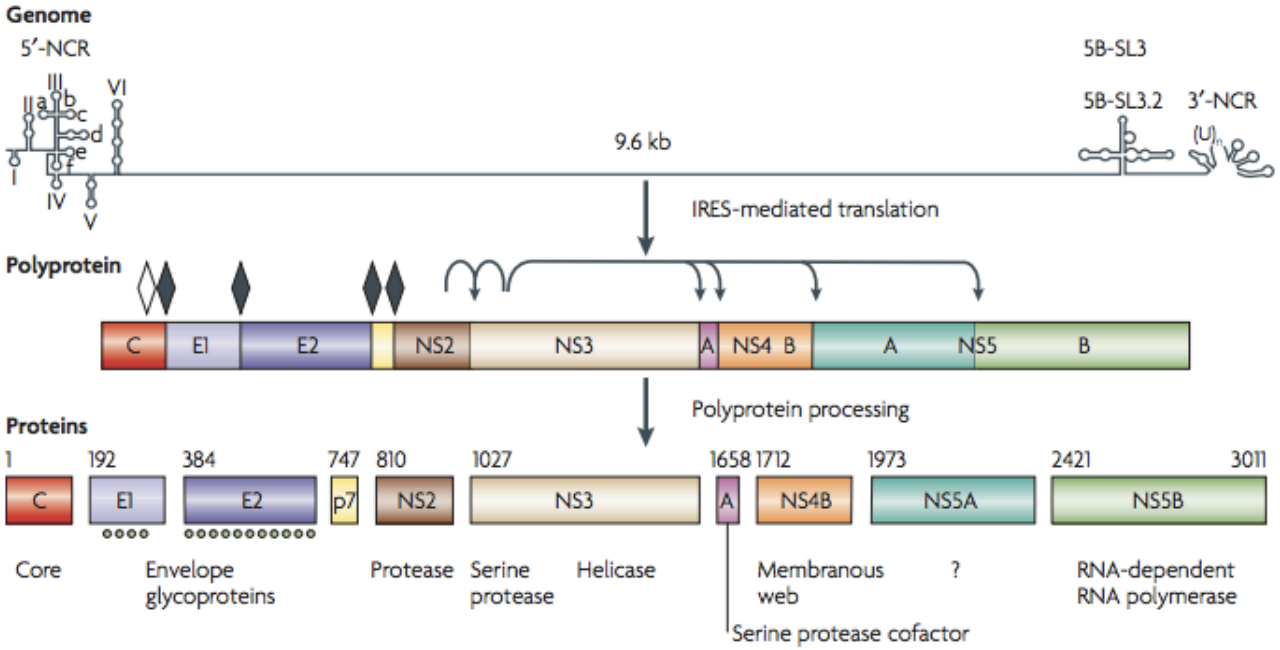
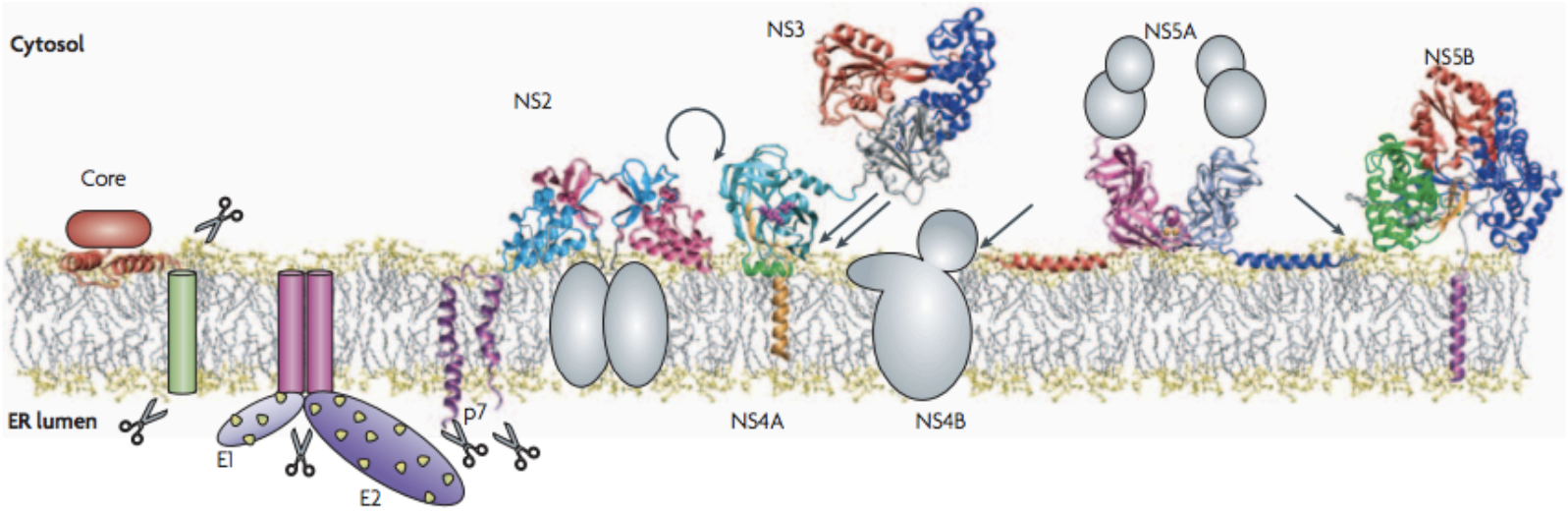
Milestones in HCV Research and Management



Manns, M. P., et al. Hepatitis C virus infection. *Nature reviews. Disease primers*, **2017**, *3*, 17006.

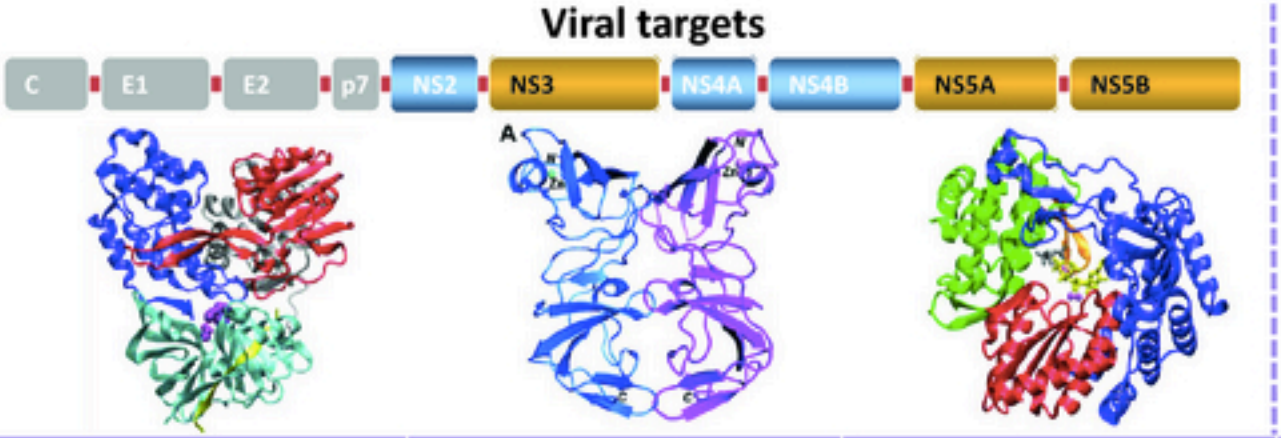
HCV: Genetic Organization and Protein Association





Nature Reviews. Microbiology, **2007**, *5*, 453.

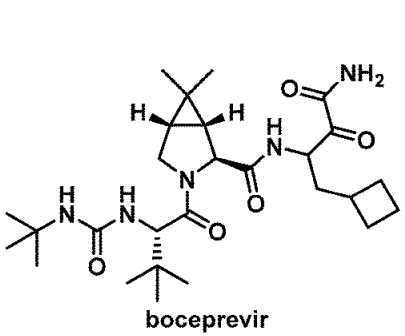
Different Classes of Direct-Acting Antiviral Drugs



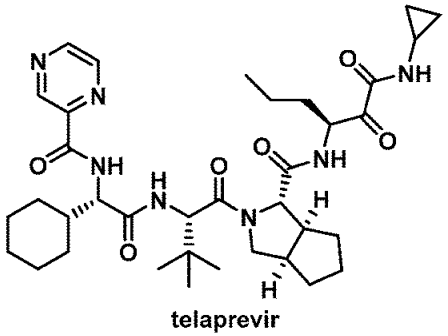
NS3	NS5A	NS5B	Cyclophilin A
The NS3/4A serine protease	Multifunctional phosphoprotein, component of the HCV-RNA replication complex	RNA-dependent RNA polymerase	Host protein interacting with NS5A and the NS5B
Boceprevir **Telaprevir** ABT-450/r, ACH-1625 Asunaprevir, TMC-435 (Simeprevir), BI-201335 Danoprevir/r, GS-9451 MK-5172	Daclatasvir GS-5885 ABT-267 PPI-668 MK	Nucleos(t)ide analogue GS-7977 (Sofosbuvir), Mericitabine, IDX-184 Non-nucleoside analogue BI-207127, ABT-333 ABT-072, BMS-791325 Tegobuvir, Setrobuvir VX-222, Filibuvir	Alisporivir SCY-635

Liver International **2014**, DOI:10.1111/liv.12405

Currently Marketed HCV Drugs



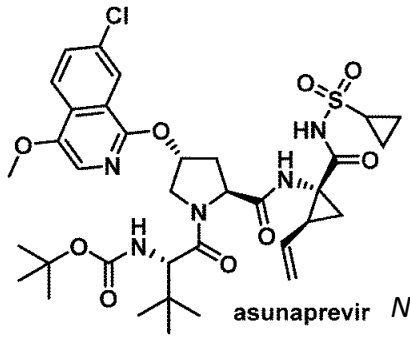
boceprevir



telaprevir

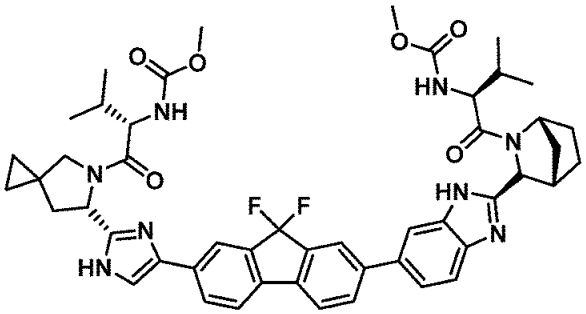
daclatasvir

NS5A inhibitor



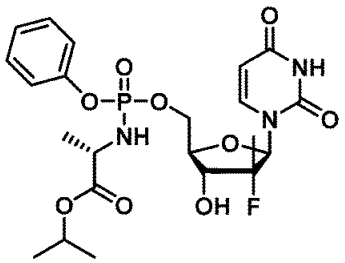
asunaprevir

NS3 protease inhibitor

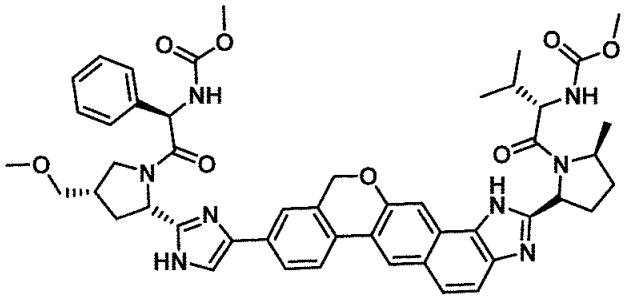


NS5A inhibitor

ledipasvir



sofosbuvir



velpatasvir

Harvoni®

Epclusa®

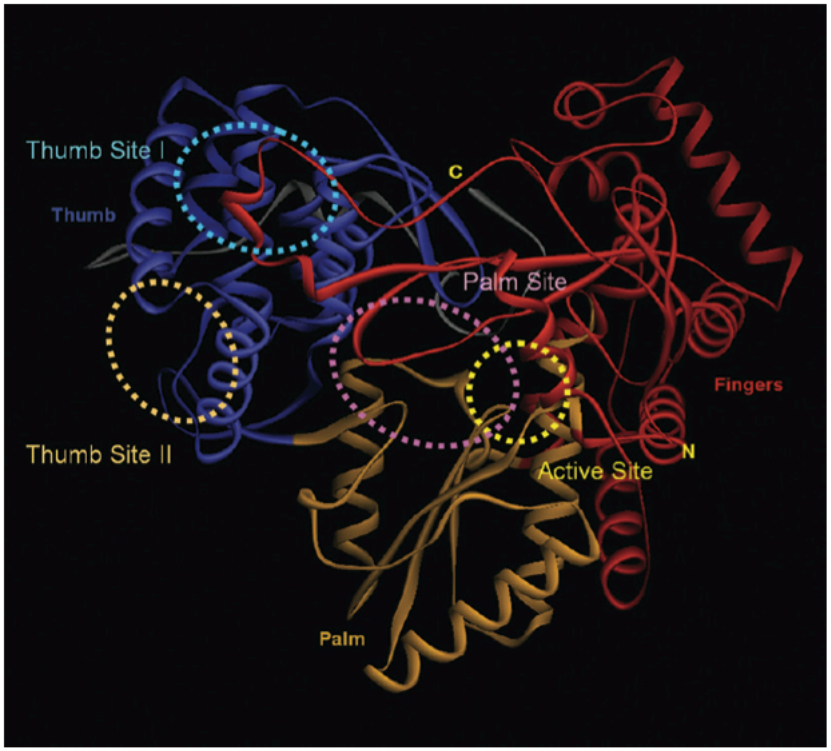
Treatment for GT-2 and GT-3 infected patients

Treatment for all HCV GTs

Med. Chem. Commun. **2017**, *8*, 796-806.

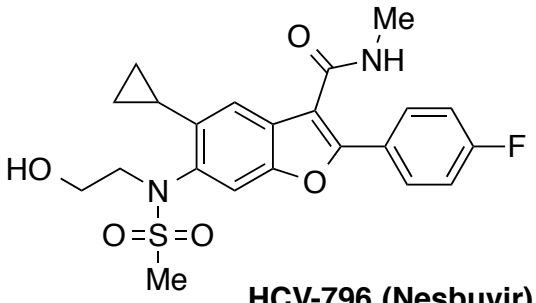
NS5B RNA Dependent RNA Polymerase

- 66 kDa protein of ~590 amino acids found at the C-terminus of the virally encoded HCV polyprotein of ~3000 amino acids.
- Essential for viral replication
- Contains three main domains (thumb, palm, and fingers) and four allosteric sites (thumb-I, thumb-II, palm-I, and palm-II).

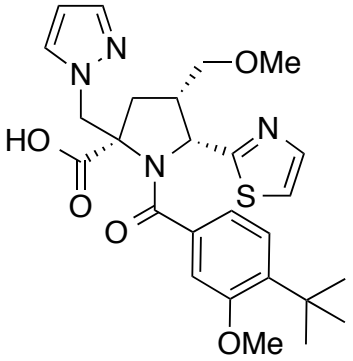


Ribbon diagram of the NS5b RNA dependent RNA polymerase (using 1C2P data) oriented to show the NTP entrance to the active site in the palm subdomain (gold ribbon). The archway of the entrance is formed by the fingerloops with the finger domain to the right in red. The thumb domain is in blue on the left. It hosts two allosteric binding sites alone and provides key interactions on the interior to the palm allosteric binding site. The C-terminal loop connecting the thumb domain to the transmembrane anchoring helix (truncated) is in gray. These domain ribbon colors are maintained throughout the molecular graphics.

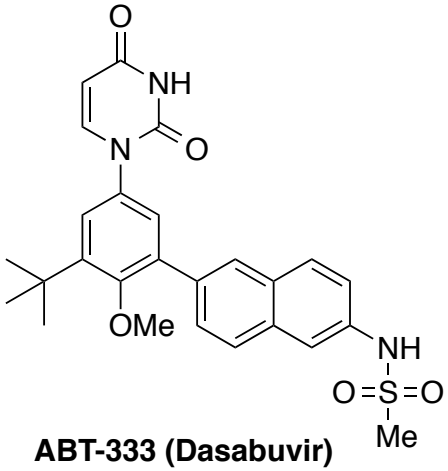
1st gen. Palm Inhibitors of HCV NS5B polymerase



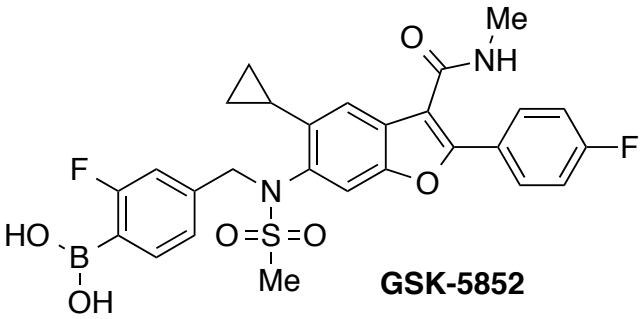
HCV-796 (Nesbuvir)



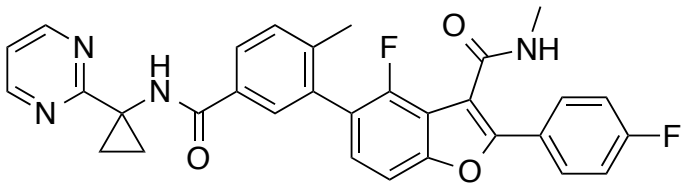
GSK625433



ABT-333 (Dasabuvir)

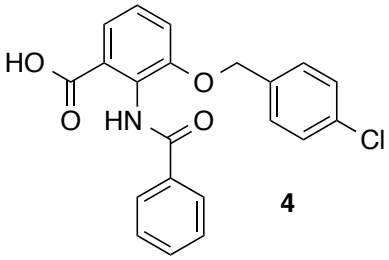


GSK-5852



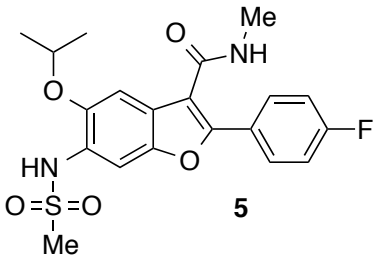
BMS-929075 (4)

Discovery of HCV gt-1b NS5B enzyme inhibitors



4

IC_{50} 1b = 8.4 μM
EC_{50} = inactive



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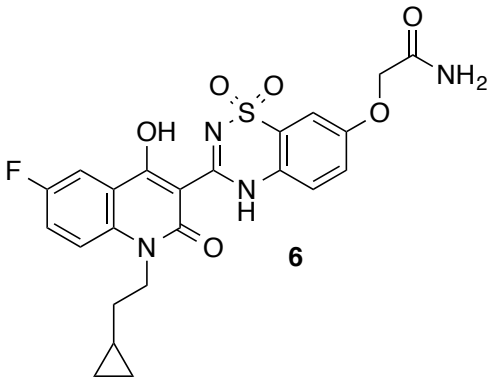

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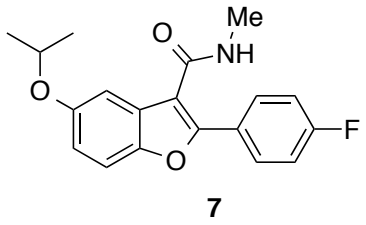
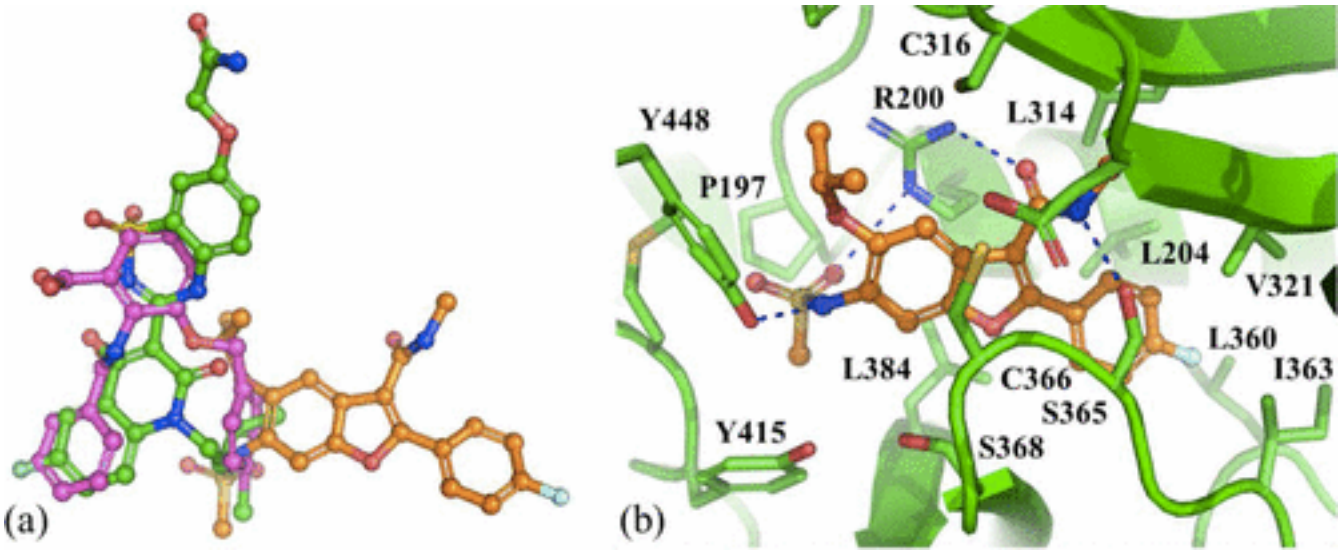


7

IC_{50} 1b = 21 μM
EC_{50} 1b / 1a = 14 / >11 μM

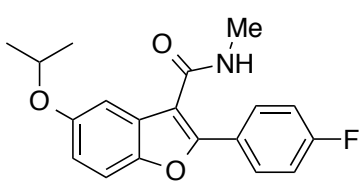
LE = 0.27; MW = 327
HAC = 24; cLogP = 4.18



(a) Overlay of the bound structures of anthranilic acid **4** (magenta), benzofuran **5** (orange), and benzothiadiazine **6** (green) in the palm site of the wild-type (WT) gt-1b NS5B protein. (b) X-ray structure of **5** bound gt-1b NS5B WT protein.

J. Med. Chem. **2017**, *60*, 4369.

Discovery of BMS-929075: 1st gen. Clinical Candidate



7

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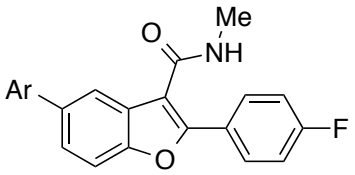
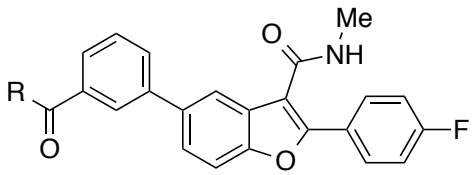
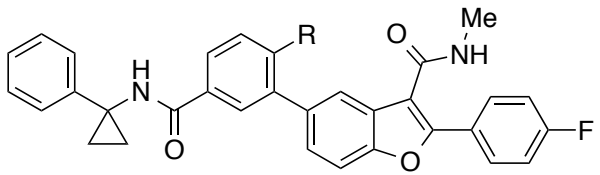


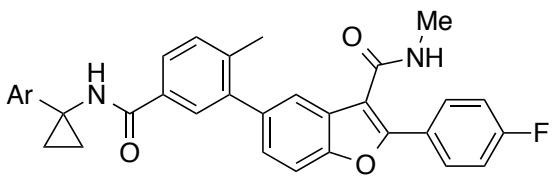
Benzofuran C5 aryl library
- meta-benzoic acid
gt-1b NS5B enzyme IC_{50} = 1.1 μM
- para-benzoic acid
gt-1b NS5B enzyme IC_{50} = 0.65 μM

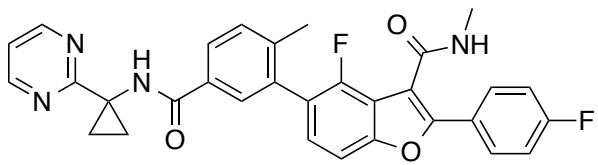




R = H
gt-1b NS5B enzyme IC_{50} = 0.006 μM
Replicon EC_{50} = 3.6 nM (gt-1b), 36 nM (gt-1a)

R = Me
Replicon EC_{50} = 1.3 nM (gt-1b), 2.1 nM (gt-1a)





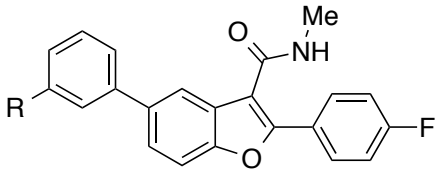
BMS-929075

Replicon EC_{50} = 4 nM (gt-1b), 9 nM (gt-1a), 18 nM (gt-1bC316N)
Good in vitro profiling data, PK parameters in rats, etc.

*fragment growing approach starting from **7** ultimately resulted in the identification of clinical candidate BMS-929075.

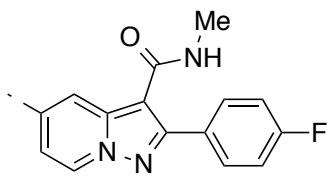
J. Med. Chem. **2017**, *60*, 4369.

Discovery of BMS-986139: 2nd gen. pan-genotypic inhibitor

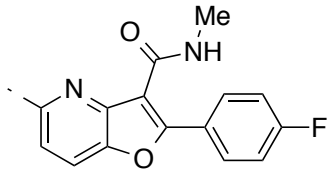


Benzofuran-based inhibitors
-> lack of inhibition of GT-2a virus

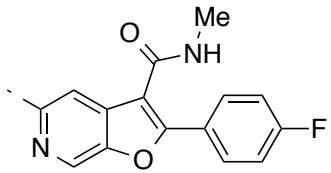
Alternative cores



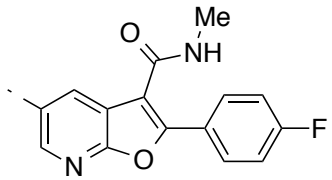
pyrazolopyridine



4-azabenzofuran

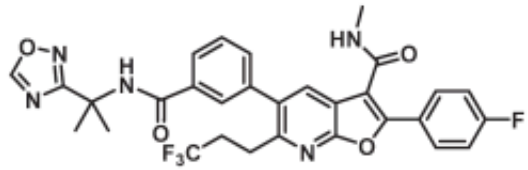


6-azabenzofuran



7-azabenzofuran

Table 9 Full GT activity data and three species PK for **43**



43, BMS-986139

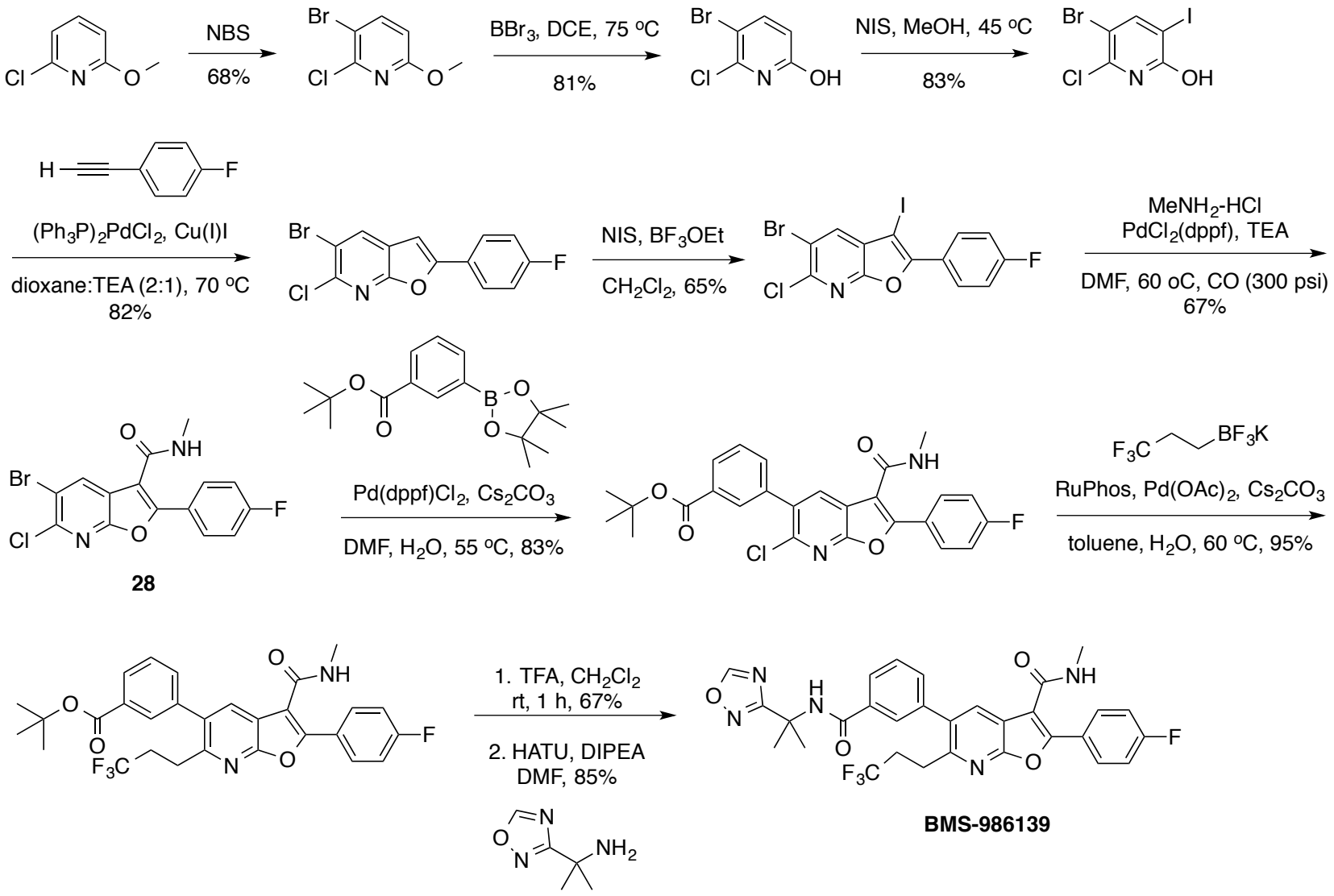
	EC_{50} (nM)										EC_{50} (nM)	
Cmpd	GT: 1a	1b	2a	2b	3a	4a	5a	6a	1b C316N[a]	CC_{50}[b]	1a (FBS)	1a (HS)
43	9	3	4	3	3	2	0.3	6	6	>100 μM	11	52

Species	IV clearance (mL min^{-1} kg^{-1})	Vss (L kg^{-1})	$t_{1/2}$ (h) IV/PO	PO AUC (μM h)	% oral F
Rat[c]	5.7	5.6	12/6.7	13	58
Dog[d]	1.2	3.3	34/35	25	85
Cyno[d]	5.8	2.8	6.1/6.4	8.3	61

[a] Enzyme data (polyC:pGpG). [b] GT-1b LUC CC_{50}: see ESI for experimental details. [c] IV dose = 2 mg kg^{-1}; PO dose 6 mg kg^{-1}. [d] IV dose = 1 mg kg^{-1}; PO dose 3 mg kg^{-1}.

Med. Chem. Commun. **2017**, *8*, 796-806.

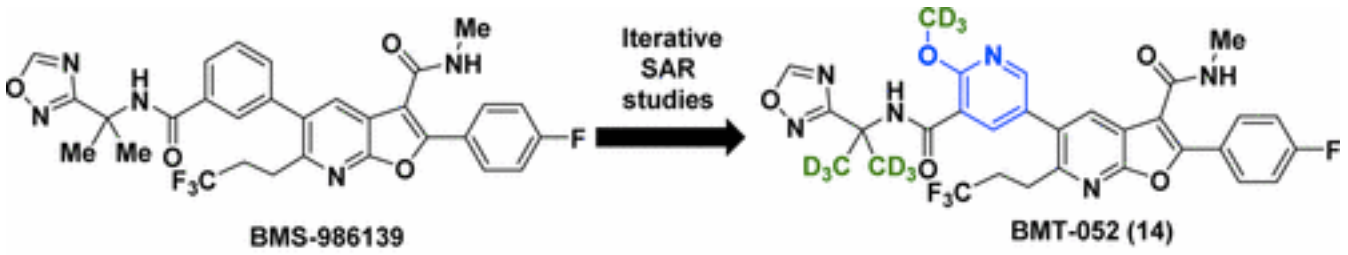
Synthesis of BMS-986139



Med. Chem. Commun. **2017**, *8*, 796-806.

In this Study

- Identification of the second generation pan-genotypic HCV NS5B polymerase primer grip inhibitor BMT-052.



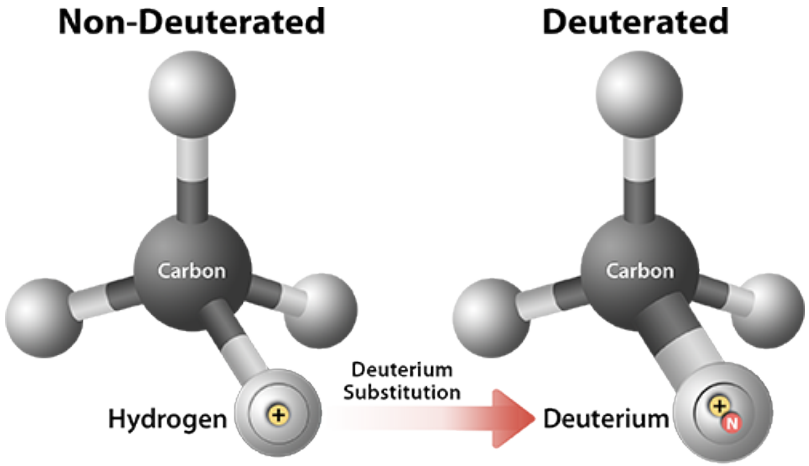
Highly unexpected microcrystallization in multiple tissues at elevated doses in both rats and dogs

- Iterative structure–activity analyses in a class of highly functionalized furo[2,3-b] pyridines led to the identi- fication of the second generation pan-genotypic hepatitis C virus NS5B polymerase primer grip inhibitor BMT-052 (14), a potential clinical candidate. The key challenge of poor metabolic stability was overcome by strategic incorporation of deuterium at potential metabolic soft spots. The preclinical profile and status of BMT-052 (14) is described.

ACS Med. Chem. Lett. **2017**, *8*, 771-774.

Deuterium: An Attractive Hydrogen Bioisostere

- The deuterium-carbon bond is typically six to nine times more stable than the hydrogen-carbon bond. This has important implications for drug development because drug metabolism often involves the breaking of hydrogen-carbon bonds.

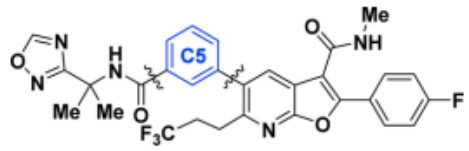


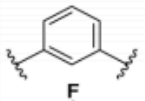
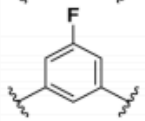
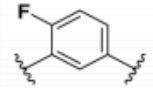
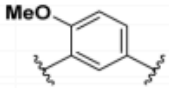
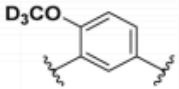
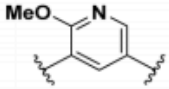
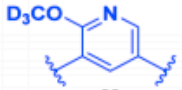
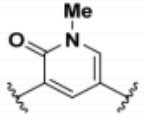
- Because deuterium forms more stable bonds with carbon, deuterium substitution can in some cases alter drug metabolism including, through improved metabolic stability, reducing the formation of toxic metabolites, increasing the formation of desired active metabolites, or a combination of these effects.

15

http://www.concertpharma.com/technology-overview/

C5 Modifications of BMS-986139 (5)



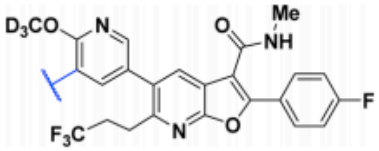
cmpd	C5	EC50 (nM)[12] 1a	EC50 (nM)[12] 1aHS[b]	CC50 (μM)	t1/2 (min)[a] Human	Rat	Dog	Cyno
5		9	52	51	>120	>120	>120	87
6		8	108	57	48	>120	111	70
7		9	79	>100	17	95	>120	8
8		3	31	>100	6	70	>120	2
9		3	36	>100	15	>120	>120	9
10		3	33	>100	14	>120	>120	5
11		5	34	76	24	>120	>120	14
12		7	24	72	>120	>120	81	12

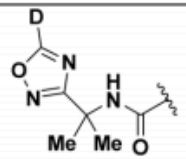
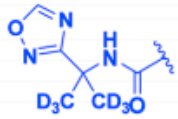
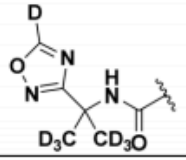
[a]Half-lives determined using liver microsomes in the presence of NADPH. [b]GT 1a in the presence of 40% human serum.

- SAR studies began with modification to the C5 phenyl ring of 5.
- Screening: measuring the replicon inhibitory activity against genotype (GT) 1a, 1b, 2a, and the 1b existing mutant C316N.
- It was determined that transposing the methyl group from the oxygen to the nitrogen (10 to 12) maintained 1aHS activity and improved stability in HLM significantly, as represented by compound 12. Unfortunately, 12 was shown to be a potent competitive inhibitor of CYP3A4 (IC50 = 0.44 μM) and was not pursued further.

Additional Deuterium Incorporation

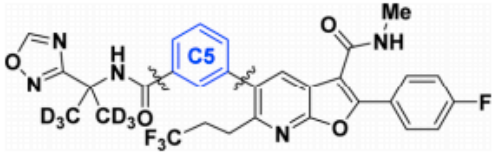
Table 2. Deuterium Incorporation into the Oxadiazole Amide

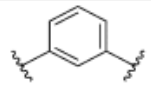
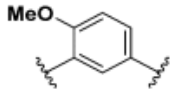
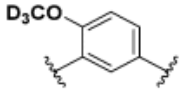
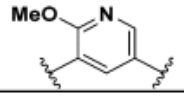


cmpd	amide	EC_{50} (nM)[12] 1a	EC_{50} (nM)[12] 1aHS[b]	CC_{50} (μM)	$t_{1/2}$ (min)[a] Human	Rat	Dog	Cyno
13		3	23	>100	26	>120	>120	16
BMT-052 14		4	33	>100	59	>120	>120	48
15		3	31	>100	67	>120	>120	48

[a]Half-lives determined using liver microsomes in the presence of NADPH. [b]GT 1a in the presence of 40% human serum.

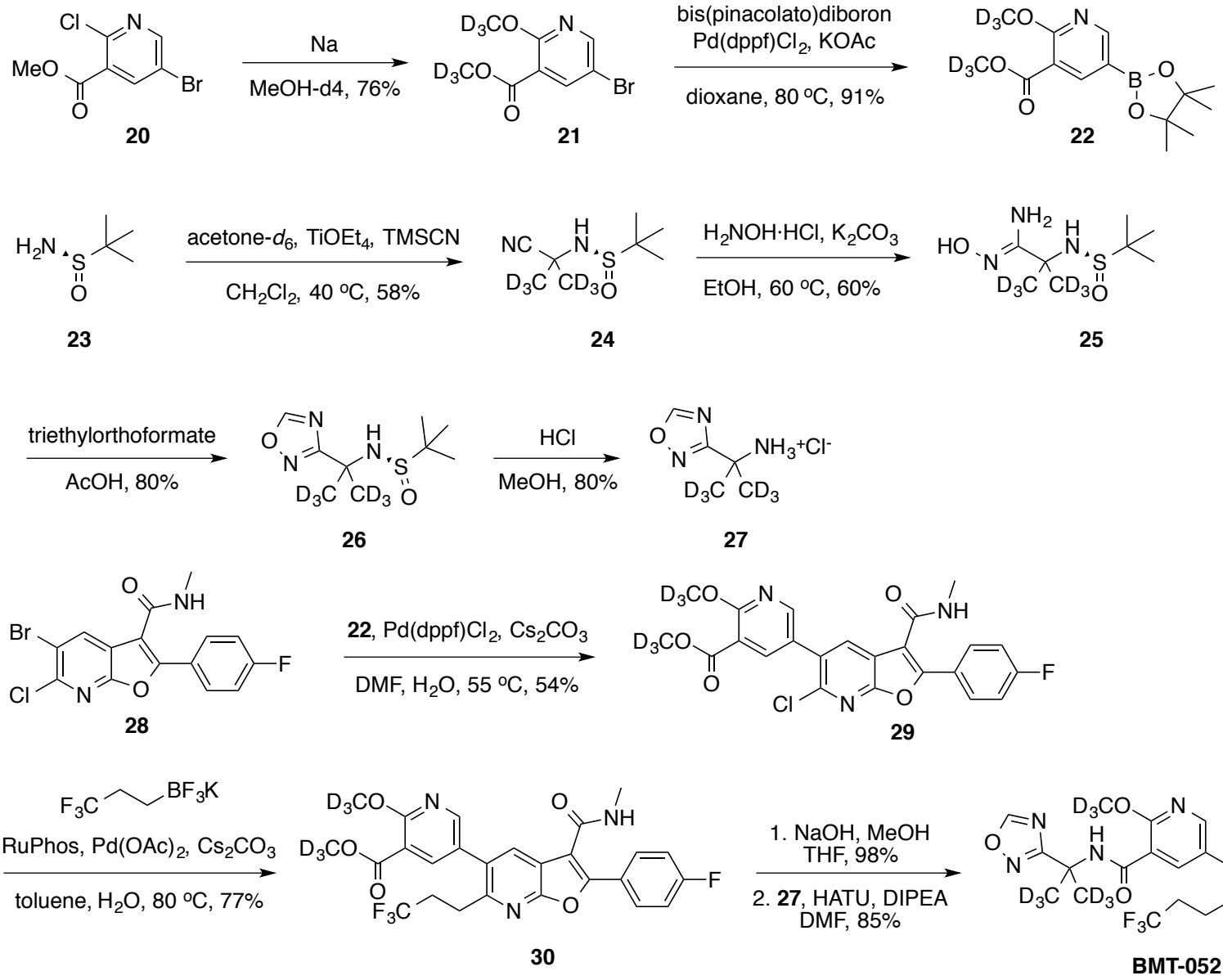
Table 3. C5 Modifications of BMT-052 (14)



cmpd	C5	EC_{50} (nM)[12] 1a	EC_{50} (nM)[12] 1aHS[b]	CC_{50} (μM)	$t_{1/2}$ (min)[a] Human	Rat	Dog	Cyno
16		13	146	69	>120	>120	>120	118
17		2	57	>100	15	111	>120	2
18		3	27	>100	36	>120	>120	21
19		2	27	>100	25	>120	>120	7

[a]Half-lives determined using liver microsomes in the presence of NADPH. [b]GT 1a in the presence of 40% human serum.

Synthesis of BT-052 (14)



PK Properties of BMT-052 (14)

Table 4. Genotype Coverage of BMT-052 (14)

EC_{50} (nM)[12]								
1b	1a	2a	2b	3a	4a	5a[a]	6a	C316N
4	4	6	3	3	1	2	4	7

[a]IC_{50}.

Table 5. Preclinical PK Properties of BMT-052 (14)

	dose (mg/kg)				$t_{1/2}$ (h)			
species	IV[a]	PO[b]	Cl (mL/min/kg)	Vss (L/kg)	IV	PO	PO AUC (μM h)	F %
rat	2	6	1.6	11	79	76	95	100
dog	1	3	1	2.1	18	>48	125	90
cyno	1	3	4.7	2.3	6.3	10	16	85

[a]Dose formulations: PEG 400/ethanol (90:10). [b]Dose formulations: PEG 400/ethanol/vitamin E TPGS (90:5:5).

- The pharmacokinetic (PK) properties of **14** were evaluated in three preclinical species: rat, dog, and cyno.
- Compound **14** exhibited low clearance (Cl), a moderate volume of distribution (Vss), and good oral bioavailability (F%) across the species. The overall profile of **14** supported a low projected human dose consistent with once daily (QD) dosing similar to that of 5 (35 mg QD).

Solubility of BMT-052 (14) vs BMS-986139 (5)

- Compound **14** has both a lower measured log D at pH 6.5 (3.73) and a lower melting point (177 °C) when compared to **5** (log D = 4.43, mp = 241 °C, respectively).
- The solubility of **5** and **14** were evaluated in a range of media. While there was little difference observed between the two compounds in phosphate buffer and fasted state simulated intestinal fluid (FaSSIF), **14** had an improved solubility in fed state simulated intestinal fluid (FeSSIF) at both pH 6.5 and 5 (Figure 2).

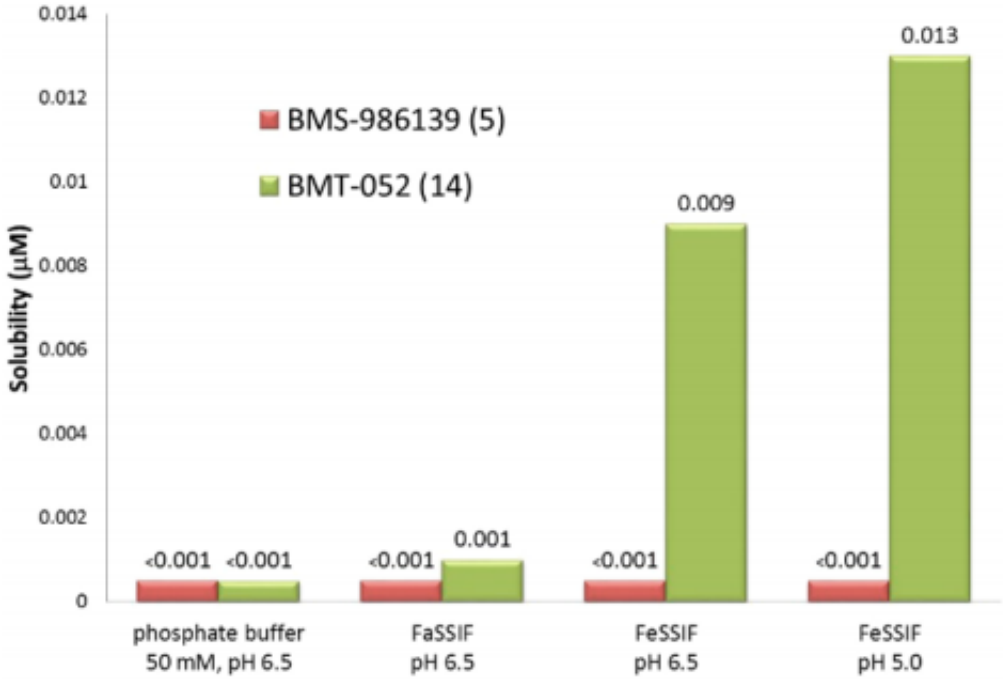
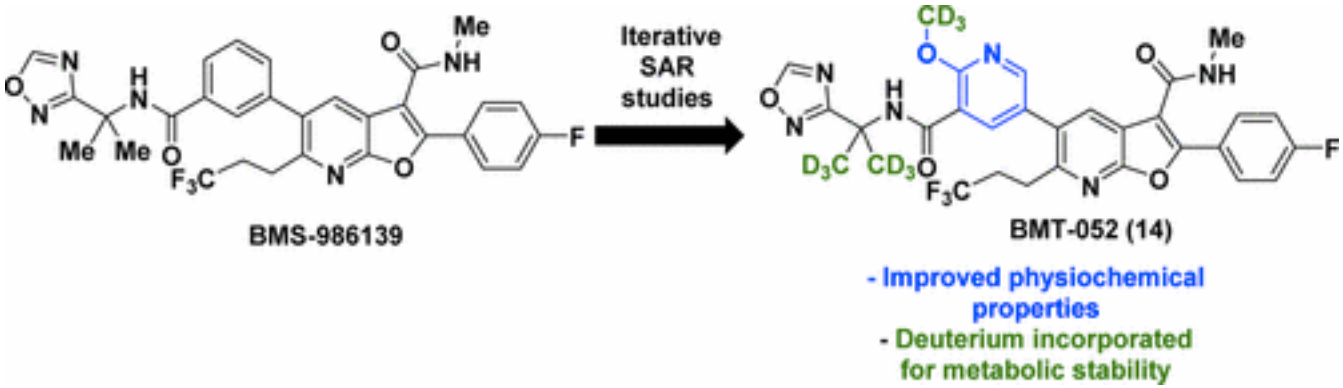


Figure 2. Solubility of **14** vs **5**.

Summary



- Iterative SAR studies and systematically incorporating deuterium into both the C5 and amide substituents, the promising preclinical compound 14 was identified.
- Compound **14** expressed potent, pan-genotype HCV inhibition, a PK profile predictive of QD dosing in humans and improved physiochemical properties compared to **5**.
- Empirically, in this context we have shown the ability of deuterium to reduced metabolic rates in LMs as evident by the longer recorded half-lives.