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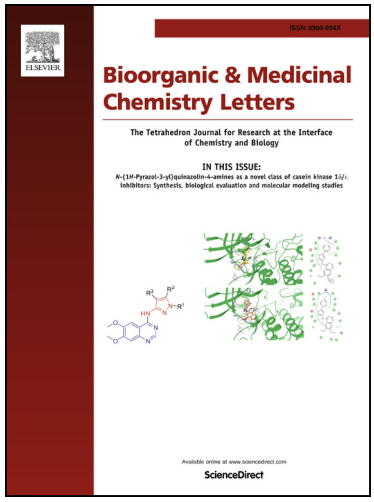

The discovery of IDX21437: design, synthesis and antiviral evaluation of 2'-α-chloro-2'-β-*C*-methyl branched uridine pronucleotides as potent liver-targeted HCV polymerase inhibitors

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Graphical Abstract

The discovery of IDX21437: Design, synthesis and antiviral evaluation of 2'-α-chloro-2'-β-*C*-methyl branched uridine pronucleotides as potent liver-targeted HCV polymerase inhibitors

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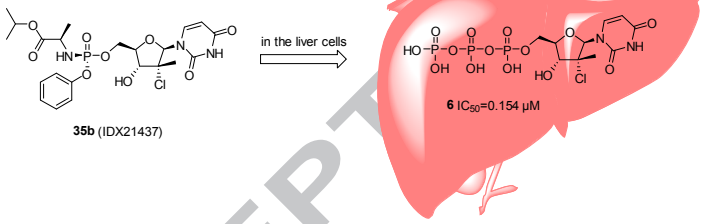
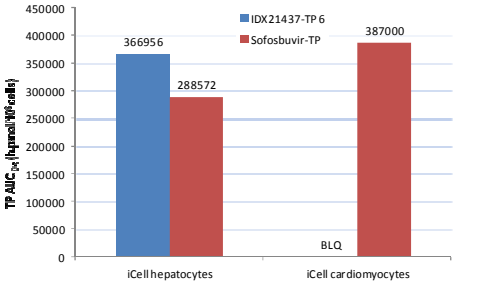
François-René Alexandre[a,*], Eric Badaroux[a,†], John P. Bilello[b,c], Stéphanie Bot[a], Tony Bouisset[a,†], Guillaume Brandt[a], Sylvie Cappelle[a], Christopher Chapron[c,†], Dominique Chaves[a,†], Thierry Convard[a], Clément Counor[a,†], Daniel Da Costa[a], David Dukhan[a], Marion Gay[a,†], Gilles Gosselin[d], Jean-François Griffon[a], Kusum Gupta[c,†], Brenda Hernandez-Santiago[c,†], Massimiliano LaColla[c,†], Marie-Pierre Lioure[a,†], Julien Milhau[a], Jean-Laurent Paparin[a], Jérôme Peyronnet[a,†], Christophe Parsy[a,†], Claire Pierra Rouvière[a], Houcine Rahali[a], Rachid Rahali[a], Aurélien Salanson[a], Maria Seifer[c,†], Ilaria Serra[c,†], David Standring[c,†], Dominique Surleraux[a,†] and Cyril B. Dousson[a]

[a]IDENIX an MSD Company, Cap Gamma, 1682 rue de la Valsière, 34189 Montpellier Cedex 4, France
[b]Merck & Co., Inc., PO Box 4, 770 Sumneytown Pike, West Point, PA 19486, USA
[c]Formerly at IDENIX Pharmaceuticals, 320 Bent Street - 4th Floor, Cambridge, MA 02139, USA
[d]Former employee of the Institut des Biomolécules Max Mousseron (IBMM), UMR 5247 CNRS-Université Montpellier-ENSCM, case courrier 1705, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France
[†]Former Idenix employees





Cells were exposed to 100 µM of IDX21437 and 10 µM of sofosbuvir

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ABSTRACT

Herein we describe the discovery of IDX21437 **35b**, a novel R_P D-aminoacid-based phosphoramidate prodrug of 2'-α-chloro-2'-β-*C*-methyluridine monophosphate. Its corresponding triphosphate **6** is a potent inhibitor of the HCV NS5B RNA-dependent RNA polymerase (RdRp). Despite showing very weak activity in the *in vitro* Huh-7 cell based HCV replicon assay, **35b** demonstrated high levels of active triphosphate **6** in mouse liver and human hepatocytes. A biochemical study revealed that the metabolism of **35b** was mainly attributed to carboxyesterase 1 (CES1), an enzyme which is underexpressed in HCV Huh-7-derived replicon cells. Furthermore, due to its metabolic activation, **35b** was efficiently processed in liver cells compared to other cell types, including human cardiomyocytes. The selected R_P diastereoisomeric configuration of **35b** was assigned by X-ray structural determination. **35b** is currently in Phase II clinical trials for the treatment of HCV infection.

* Corresponding author. Tel.: +33(0)499522252; fax: +33-(0)499522250; francois-rene.alexandre@merck.com

Hepatitis C Virus (HCV) is a single-stranded positive sense RNA virus belonging to the Flaviviridae family. This virus is the causative agent of chronic liver diseases affecting around 3% of the world's population.[1] Initially asymptomatic, this infection can ultimately lead to liver cirrhosis, fibrosis and hepatocellular carcinoma. In developed countries, HCV is the primary cause for liver transplants. Introduced in 2001, the initial standard of care therapy (SOC) combined a subcutaneous injection of a modified interferon-α (IFN-α) with an oral administration of ribavirin. However this treatment was inconvenient, very poorly tolerated and induced a sustained viral response (SVR) in only 42-82% of patients depending of the virus genotype.[2] In this context development of orally administered, effective and well-tolerated therapies became a major stake for industrial researchers. In recent years, progress in the understanding of the HCV morphology, virus life cycle, as well as the development of HCV replicon (RPL) cell-based assays has resulted in a large number of research groups taking on the rational design of so-called direct acting antivirals (DAA) as specific inhibitors of the virus life cycle.[3] In 2011, two new DAAs, both HCV NS3/4A protease inhibitors, boceprevir and telaprevir, became available to reinforce the existing antiviral arsenal. Albeit bringing clear improvement over the previous therapies, they possess several drawbacks that included low efficacy rates in some patient populations, high pill burdens and serious side effects.[3,4] However, the major advance toward an oral, interferon-free regimen, came in 2014 with the approved combination of sofosbuvir (NS5B polymerase nucleoside inhibitor (NI)) and ledipasvir (NS5A replication inhibitor). This combination demonstrated an improved cure rate up to 99% in certain patient populations, but remains suboptimal in others.[5] To this end, one critical focus for future HCV therapy is the identification of a pan-genotypic DAA combination therapy that could be effective across all HCV patient populations.

Replication of HCV occurs predominantly in hepatocytes, where the genome is translated into a polyprotein that is subsequently cleaved to produce structural and non-structural proteins constitutive of the virus. Among the most studied viral targets, including NS3/4A protease, the NS5A protein and RdRp NS5B polymerase, the latter is among the most attractive within the HCV life cycle due to its high pan-genotypic conservation. Nucleoside inhibitors (NIs) which have long been demonstrated to inhibit the NS5B polymerase possess a high barrier to drug resistance and a high genotypic coverage compared to other DAAs, indicating that they are likely to remain key components in future treatment regimens.

Our laboratory is engaged in the research of chemotherapeutics against HCV, with the discovery of the antiviral properties of the 2'-*C*-methyl-β-D-ribonucleoside analogues over 15 years ago. This effort led to the discovery of NM283, the first nucleoside to enter Phase II clinical trials for the treatment of chronic hepatitis C (Chart 1).[6] NM283, similarly to other NIs, is metabolized in cells in a three-step process to its nucleoside monophosphate, diphosphate and triphosphate consecutively. This nucleoside triphosphate (NTP) is the active anabolite that binds to the active site of the NS5B polymerase as a substrate, is incorporated to the growing chain of viral RNA and acts as a nonobligate chain terminator by blocking the further production of the viral RNA. Despite the efficacy shown in clinical trials, development of NM283 was halted due to gastrointestinal (GI) adverse events.[7]

In order to improve the overall therapeutic index and potency of the parent nucleoside, a prodrug approach was investigated and applied to pyrimidine and purine 2'-*C*-methyl-β-D-ribonucleoside. The aim of this approach was to target the liver and to bypass the initial monophosphorylation step, which is known to be rate-limiting in numerous cases.[8] From our preclinical studies and based initially on replicon activity and *in vivo* data, we selected IDX184 a phosphoramidate pronucleotide of 2'-*C*-methyl-guanosine as a clinical candidate (Chart 1), possessing S-acyl-2-thioethyl (SATE) and benzylamine moieties.[9] This compound was the first pronucleotide evaluated in the clinic through Phase II trials to treat HCV. In combination with IFN-α and ribavirin in genotype 1 treatment-naïve patient in Phase IIa clinical trials, a 4-log HCV RNA reduction by day 14 was observed at a daily dose of 100 mg.[10] Despite those encouraging results, and although no evidence of cardiac effects in all IDX184 clinical studies was found, IDX184 was placed on clinical hold by the FDA due to the cardiac adverse events observed with BMS-986094 (INX-189), which shares the same 2'-MeGTP anabolite as IDX184. As a consequence, the program was re-oriented to other pronucleotides bearing nucleosides other than 2'Me-G.

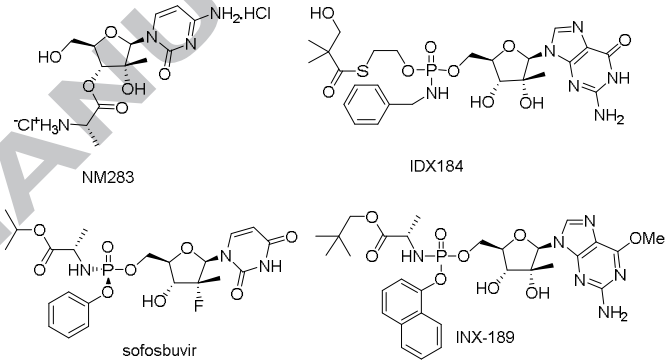
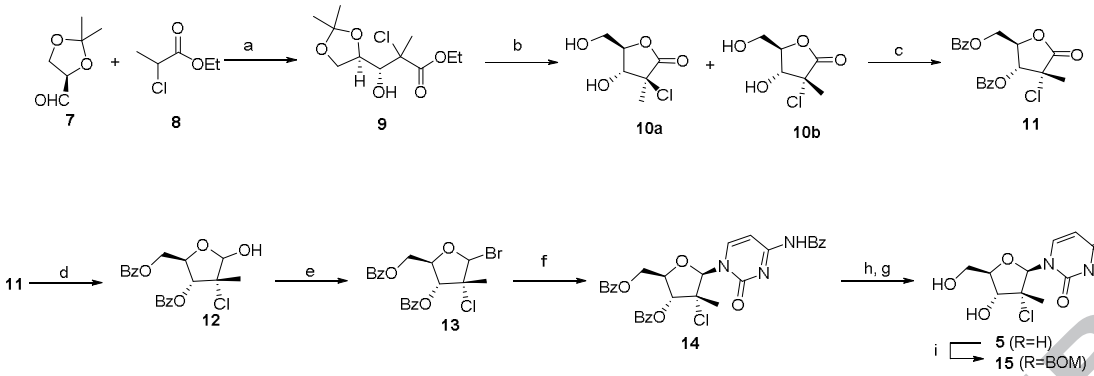


Chart 1. Sofosbuvir, INX-189 (stopped for cardiac toxicity) and Idenix pioneered anti HCV clinical molecules: nucleoside NM283 and pronucleotide IDX184.

Uridine derivatives are generally known to be poorly recognized by the deoxycytidine kinase (dCK), the enzyme responsible for the first phosphorylation step en route to NTP.[11] This lack of recognition when associated with a good activity against the HCV polymerase of the corresponding NTP, made such nucleosides ideal candidates for a monophosphate prodrug approach. We first re-evaluated the 2'-C-methyluridine derivative **1** (NM106), and its triphosphate **2** in the cell-based HCV replicon assay and in the NS5B polymerase biochemical assay respectively (Table 1).[12] While **1** displayed poor activity of 30 μM in the replicon assay (Table 1, Entry 1), the corresponding triphosphate **2** was very potent at 0.081 μM against the wild type (WT) NS5B polymerase (Table 1, Entry 4), validating our interest in a prodrug approach. However, against the HCV S282T mutant polymerase, the mutant selected by the 2'-methyl nucleosides class, 2'Me-UTP **2** showed a 102-fold loss in activity compared to the WT (Table 1, Entry 4).

With the aim of improving the mutation profile of such inhibitors, we investigated the 2'-α-chloro-2'-β-*C*-methyl substitution. The synthesis of 2'-α-chloro-2'-β-*C*-methylcytidine and 2'-α-chloro-2'-β-*C*-methyluridine is depicted in Scheme 1.[13]



Scheme 1. Reagents and conditions: (a) LDA, Toluene, −78 °C, 5 min; (b) AcOH, 90°C, 17% (2 steps); (c) (1) BzCl, DMAP, CH_3CN, (2) Et_3N, 84% ; (d) Red-Al, THF, −35 °C to 0 °C, (α/β 45/55) 96% ; (e) CBr_4, PPh_3, −20 °C, 66%; (f) N-4-benzoylcytosine, HMDS, $SnCl_4$, 4-chlorobenzene, 70 °C, 89%; (g) 7 N NH_3 in MeOH, RT, 79%; (h) AcOH, H_2O, 110 °C, quant. ; (i) benzyloxymethylchloride (BOMCl), DBU, DMF, −5 °C to 5 °C, 80%.

The key protected ribonolactone **11** was synthesized in three steps from the *R*-glyceraldehyde acetonide **7**. The first step was an aldol condensation between **7** and **8** to provide **9** as a diastereomeric mixture. **9** was engaged crude in a cyclization reaction to give **10a** and **10b** in a 1:9 ratio, the latter being the major product, which was isolated in 17% yield by recrystallization from a toluene-butanone mixture. The hydroxyl groups of **10b** were then protected as benzoates to obtain the ribonolactone **11** in 84% yield. The lactone was reduced with Red-Al in THF to afford lactol **12** as a diastereomeric mixture at the 1'-position, and the resulting hydroxyl group of **12** was substituted to a bromo group with carbontetrabromide and triphenylphosphine to afford **13** as 44/56 mixture of α/β anomers. These anomers were readily separable by chromatography on silica gel, however it was unnecessary to proceed with this separation as the anomeric mixture reacted with sylilated N-4-benzoylcytosine and tin tetrachloride in 4-chlorobenzene affording only the β nucleoside **14** in 89% yield. Subsequent hydrolysis/deprotection of **14** led to uridine derivative **5** in good yields.

As expected and similarly to 2'-α-fluoro-2'-β-*C*-methyluridine **3**, the uridine derivative **5** showed very modest activity in the RPL cell-based assay (Table 1, Entry 3). The corresponding triphosphates **6** was synthesized according known procedures and its activity is shown in Table 1.[14] Triphosphate **6** was found to be a potent inhibitor of the HCV NS5B polymerase. Against the S282T mutant polymerase, **6** showed a 6-fold decrease in activity compared to the wild type, while a 102-fold decrease was measured for NM106-TP **2**. **6** showed similar activity across all genotypes against HCV polymerases (Table 2, Entries 1–6) and proved to be selective for HCV polymerases *vs* human polymerases (Table 2, Entries 7–11). In addition, the half-life of compound **6** was determined to be over 18h in human hepatocytes which supported the potential for once daily (QD) dosing. These promising results prompted us to synthesize and evaluate a series of prodrugs based on nucleoside **5**.

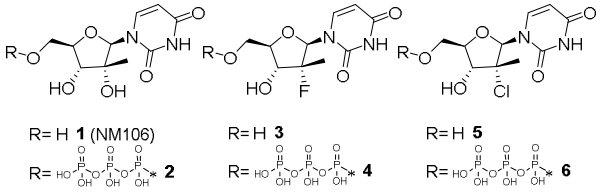


Chart 2. Evaluated nucleosides and corresponding NTPs

Table 1. Activities of Nucleosides **1**, **3**, **5** in the HCV replicon gen1b assay and of their corresponding nucleoside 5'-triphosphates **2**, **4**, **6** against the HCV con 1b NS5B polymerase

Entry	Cpd	EC_{50}[a] (μM)	CC_{50}[b] (μM)	Entry	Cpd	IC_{50}[c] WT (μM)	IC_{50}[d] S282T (μM)	FC[e]
1	**1**	30	>100	4	**2**	0.081	8.24	102
2	**3**	50	>100	5	**4**	0.079	0.606	7.7
3	**5**	57	>100	6	**6**	0.154	0.944	6.1

[a] Inhibition of HCV replication Huh-7 cells containing HCV Con1 subgenomic replicon (GS4.1 cells) with a luciferase read-out. EC_{50} values were determined from the 50% inhibition versus concentration data. [b] CC_{50}'s were calculated as the concentration that caused a 50% of cells death versus concentration data. [c] Biochemical assay for inhibition capacity on the 1b HCV NS5B polymerase; IC_{50} values were determined from the percent inhibition versus concentration data. [d] Biochemical assay for inhibition capacity on the S282T HCV NS5B polymerase; IC_{50} values were determined from the percent inhibition versus concentration data. [e] FC= fold change = IC_{50} S282T/IC_{50} WT.

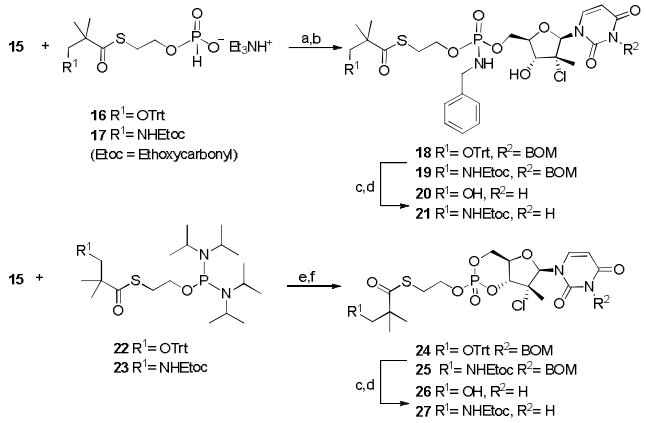
Table 2. *In vitro* inhibition of wild type HCV and cellular polymerases by nucleoside 5'triphosphate **6**.[13]

Entry	Species	Enzyme	IC_{50} (μM)[a]
1	**HCV polymerases**	Genotype 1a	0.14
2		Genotype 2a	0.25
3		Genotype 3a	0.16
4		Genotype 4a	0.15
5		Genotype 5a	0.23
6		Genotype 6a	0.10
7	**Human polymerases**	DNA pol alpha	>500
8		DNA pol beta	>500
9		DNA pol gamma	95
10		RNA pol II	>500
11		Mitochondrial RNA pol	>500

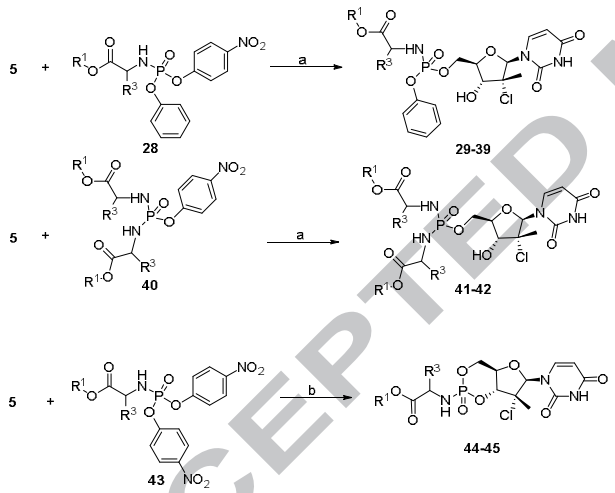
[a] Biochemical assay for inhibition capacity on various polymerases; IC_{50} values were determined from the percent inhibition versus concentration data.

Based on our previous experience in the IDX184 program, we selected several classes of phosphorylated moieties from our prodrug collection for evaluation.[9] We initially synthesized SATE derivative phosphoramidate prodrugs **20** and **21** (Scheme 2). BOM protected **15**, obtained from **5** in 80% yield (Scheme 1), reacted with **16** and **17** (synthesized according known methods)[9,15] to afford intermediate phosphonic acid derivatives, which underwent Atherton–Todd oxidative amination with benzylamine in carbon tetrachloride to give **18** and **19**. Trityl (for **18**) and BOM protecting groups were removed by reaction with BBr_3 at low temperature followed by treatment with formic acid in acetonitrile and water to remove

residual hydroxymethyl resulting from incomplete deprotection. This sequence furnished **20** and **21** as mixtures of diastereomers, which were separated into their S_P and R_P isomers by preparative HPLC. Cyclic phosphate triesters (CPO) **26** and **27**, derived from SATE prodrugs, were obtained from the reaction of protected uridine **15** and phosphorodiamidite **22** and **23**, followed by oxidation and deprotection (Scheme 2). As previously, the diastereomers were separated by preparative HPLC.



Scheme 2. Synthesis of SATE prodrug derivatives.[13] Reagents and conditions: (a) Pivaloyl chloride, pyridine, RT; (b) $BnNH_2$, CCl_4, DCM, RT, 46-63% (2 steps); (c) BBr_3, DCM −80 °C; (d) HCO_2H, ACN, H_2O, 50°C, 15-26% (2 steps); (e) Pyridine, tetrazole (0.45 M in ACN), −5°C to RT; (f) *tert*-butylhydroperoxide, 0 °C to RT, 32-46% (2 steps).



Scheme 3. Synthesis of phosphoramidate, phosphorodiamidate and cyclic phosphoramidate prodrug derivatives **29-45**.[13] Reagents and conditions: (a) *tert*-butyl magnesium chloride 1M in THF, THF, RT, 8-56% ; (b) DBU, ACN, THF, RT, 11-67%.

A series of linear amino acid phosphoramidate (PON) prodrugs was also prepared by reaction of **5** with variety of phosphoramidate reagents **28** in the presence of *tert*-butyl magnesium chloride in THF (Scheme 3).[16] Again, the diastereomers were separated by preparative HPLC or by column chromatography on silica gel. Two related phosphorodiamidates (PDN), **41** and **42**, were synthesized from **5** and **40** using the same protocol. Finally, cyclic phosphoramidates (CPN) **44** and **45** were prepared from phosphoramidate reagent **43** using a known protocol.[17] In the case of **35b**, **45a** and **45b**, single crystals were isolated and X-ray structure determination was performed allowing us to assign compound **35b** as the R_P isomer (Figure 1). **45a** was determined to be the R_P isomer and **45b** as the S_P isomer. The phosphorus stereochemistry of the other prodrugs was assigned by analogy to the ^{31}P NMR chemical shifts with those of **35b**, **45a**, **45b** and similar structures reported in the literature.[17,18]

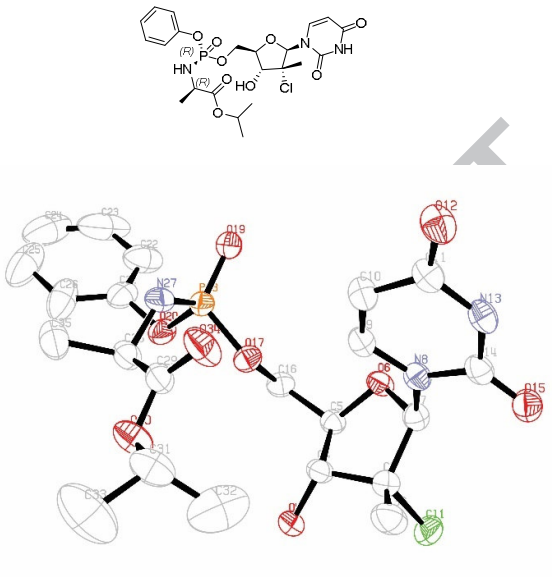


Figure 1. Chemical structure and ORTEP drawing of **35b** showing R_P configuration at phosphorous (hydrogens were removed for clarity).

An initial set of SATE derivatives **20–21** and L-aminoacid phosphoramidates **29–35** was evaluated in the HCV replicon assay and results are reported in Table 3. SATE derivatives **20a–b** and **21a–b** displayed modest activities in the micromolar range (Entries 1–4) with no significant difference between the diastereomers as observed previously for this class of prodrug.[15,19] L-aminoacid phosphoramidate protides SAR is well documented and our results were in accordance with reported data in other nucleoside series.[20] A brief study of the amino acid side chain of phosphoramidate protides showed that compounds displayed excellent to good potencies when R^3 =Me (**29a–b**, Table 2, Entries 5–6) and R^1= CH_2CHMe_2 (**30a–b**, Table 2, Entries 7–8) and are completely inactive in the case of R^1= $CHMe_2$ (**31a–b**, Table 2, Entries 9–10). Among the diastereomers, the S_P isomers **29a**, **30a**, **32a**, **33a** and **34a** demonstrated activities 2.6- to 11-fold more active than their R_P analogues, and **29a**, which bears the same phosphoramidate moiety as sofosbuvir, was 1.8-fold more active that the latter. Terminal carboxylic acid ester variations in the case of alanine side chains indicated that the most lipophilic esters were the most potent with Bn > iPr > Et (Table 2, Entries 5–6 and 13–16). For leucine, ethyl esters **32a-b** were more active than isopropyl analogues **30a-b**. Finally, D-amino acid phosphoramidates **35a-b** showed poor activities with high EC_{50} values (Table 2, Entries 17–18), in line with what has been reported previously in other series.[20]

In our initial screening cascade, the replicon activity was the first filter, and was intended to be indicative of the ability of prodrugs to deliver high triphosphate levels in the target cells.[21] Thus, to verify this assumption and to further select the compounds of interest based on their pharmacokinetics properties, an *in vivo* evaluation of liver levels of triphosphate **6** and plasma levels of parent nucleoside **5** in mice at a single oral dose of 10mg/kg was performed on an initial set of compounds (Table 2). The highest liver exposures were obtained with R_P phosphoramidates prodrugs **29b**, **33b** and

34b which delivered 1.4- to 2.1-fold more NTP than their S_P counterparts **29a**, **33a**, and **34a**, and 2.7- to 3.9-fold more than sofosbuvir (Entry 37). This difference between diastereomers is significant compared to the case of sofosbuvir (S_P) and its R_P analogue PSI-7976. Indeed despite having a 7.3-fold difference in EC_{50}, both showed the same levels of NTP in the liver (Table 2, Entries 37–38). Interestingly, the weakest R_P phosphoramidate compounds in terms of replicon activities provided the highest levels of liver NTP demonstrating that antiviral activity observed in the replicon system did not correlate with amounts of NTP produced in the mouse liver. This observation may indicate a different metabolic pathway or differentiated kinetics of cleavage between the diastereomers as it was observed in the 2'β-*C*-Me-2'-α-C-F-uridine series.[22] In the case of leucine **32**, liver TP levels were lower than with alanine derivative **34**; however **32b**, which displayed only 2.5μM activity, gave same levels of liver NTP as **32a** (Table 2, Entry 12). Similarly prodrugs such as SATE **20a** and **20b** were also evaluated *in vivo*, despite their poor EC_{50}, in order to provide a comparison to IDX184. In these cases, significant levels of liver NTP were observed (Table 2, Entries 1–2 and 39) within 1.8 to 2.6-fold less compared to IDX184. These unexpected results prompted us to further evaluate compounds *in vivo* that exhibited modest to poor EC_{50} to make sure we would base our selection on the liver delivery propensity of the prodrug rather than its *in vitro* replicon activity. Compounds **35a** and **35b** were therefore evaluated *in vivo* and very high NTP levels of 3750 and 6200 pmol•h/g respectively (Table 2, Entries 17–18), were observed, significantly above the levels resulting from sofosbuvir administration.

We therefore changed our screening cascade by the placing *in vivo* mouse study as the primary filter for our prodrug selection as it was apparent that the *in vitro* cell assay and *in vivo* cell displayed differentiate metabolic activity. To this end, a new series of prodrugs consisting of D-amino acid phosphoramidates **36**–**39**, phosphorodiamidates **41**–**42**, CPOs **26**–**27** and CPN **45** were synthesized and evaluated *in vitro* and *in vivo* (Table 2). As mentioned previously, increasing lipophilicity by incorporation of the carboxylic ester with *n*-butyl **36** and benzyl **37** improved EC_{50} values and provided slightly higher liver NTP levels. Based on the potential liabilities found by others in the literature,[20c, 23] n-butyl ester derivative **36b** was abandoned and **35b**, namely IDX21437, was selected for further preclinical studies.

While having selected **35b** as a preclinical candidate, our efforts to evaluate other prodrugs continued. Phosphorodiamidates bearing two L-alanine units **41** or two D-alanine units **42** displayed 12 μM and >100 μM EC_{50}, respectively. As the D-alanine moiety provided high liver TP levels in the phosphoramidate prodrug series, and although **42** is inactive in the HCV replicon system, it was evaluated *in vivo* and good levels of NTP were detected in the liver. This additional result demonstrated clearly that it is very likely that a different metabolism, specific to the D-amino acid moeity, occurred in the liver compared to the HCV replicon cells system.

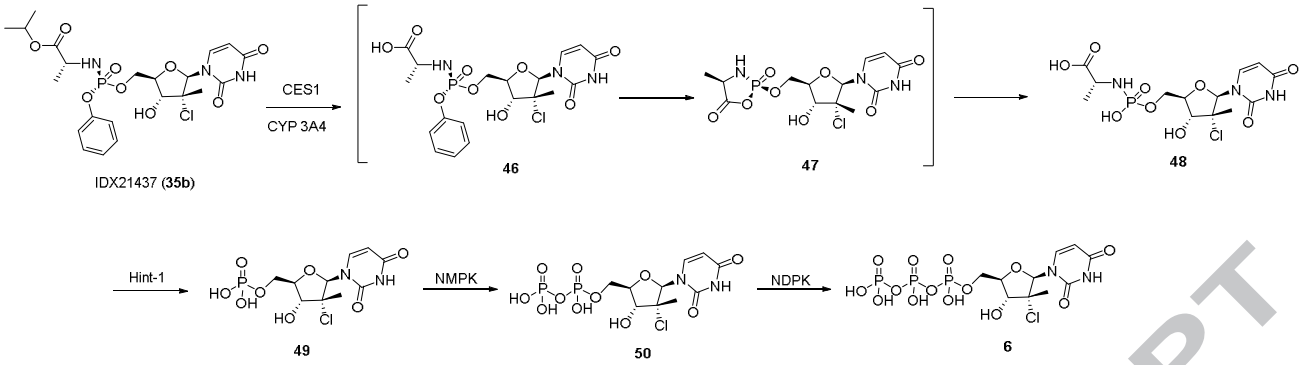
Continuing our SAR of D-amino-acid derived prodrugs, CPN bearing L-alanine **44** and D-alanine **45** were studied. Similarly to the linear phosphoramidates, the D-alanine derivatives **45a** and **45b** produced high levels of liver NTP whereas L-alanine derivatives **44a** and **44b** showed lower levels with a dramatic difference of 11-fold between the diastereomers. On the basis of the CPN results, we investigated cyclic SATE prodrugs **26**–**27**. Dramatic improvements in EC_{50} and in liver NTP levels were observed with **26** compared to **20**. However concerns around potential toxic metabolites such as episulfide led us to discontinue further evaluation of **26** and **27**, despite the lack of conclusive evidence of its formation *in vivo* in the case of IDX184. Despite **35b** having already being selected for further preclinical evaluation, **45a** and **45b** were also selected as preclinical candidates and their biological profiles will be reported in due course.

Table 2. HCV replicon activity, selectivity index and pharmacokinetic parameters for pronucleotides **20**–**45**[13]

Entry	Cpd	Series	AA series[a]	R^1	R^3	P conf.[b]	EC_{50} (μM)[c]	SI[d]	2'-*C*-MeUTP Liver $DNAUC_{0\text{-}24h}$ (pmol•h/g)[e]	2'-*C*-MeU Plasma $DNAUC_{0\text{-}24h}$ (pmol•h/mL)[e]
1	**20a**	SATE	/	OH	/	S_P	3.61	>28	96	590
2	**20b**	"	/	OH	/	R_P	3.99	>25	150	930
3	**21a**	"	/	NHEtoc	/	S_P	1.269	>79	ND	ND
4	**21b**	"	/	NHEtoc	/	R_P	1.159	>86	ND	ND
5	**29a**	PON	*L*	iPr	Me	S_P	0.158	>633	560	1300
6	**29b**	"	*L*	iPr	Me	R_P	0.953	>105	840	970
7	**30a**	"	*L*	iPr	CH_2CHMe_2	S_P	1.108	>90	ND	ND
8	**30b**	"	*L*	iPr	CH_2CHMe_2	R_P	12.596	>8	ND	ND
9	**31a**	"	*L*	iPr	$CHMe_2$	S_P	>100	>1	ND	ND
10	**31b**	"	*L*	iPr	$CHMe_2$	R_P	>100	>1	ND	ND
11	**32a**	"	*L*	Et	CH_2CHMe_2	S_P	0.291	>344	270	2100
12	**32b**	"	*L*	Et	CH_2CHMe_2	R_P	2.486	>40	220	940

13	**33a**	"	*L*	Bn	Me	S_P	0.071	>1408	850	2500
14	**33b**	"	*L*	Bn	Me	R_P	0.185	>541	1200	1900
15	**34a**	"	*L*	Et	Me	S_P	0.257	>389	400	1100
16	**34b**	"	*L*	Et	Me	R_P	0.845	>118	840	1100
17	**35a**	"	*D*	iPr	Me	S_P	9.6	>10	3750	1050
18	**35b**	"	*D*	iPr	Me	R_P*	56.8	>2	6200	1400
19	**36a**	"	*D*	nBu	Me	S_P	0.528	>189	7200	1700
20	**36b**	"	*D*	nBu	Me	R_P	2.18	>46	8400	1700
21	**37a**	"	*D*	Bn	Me	S_P	0.819	>122	6900	1700
22	**37b**	"	*D*	Bn	Me	R_P	4.283	>23	5600	1400
23	**38a**	"	*D*	Et	Me	S_P	2.548	>39	ND	ND
24	**38b**	"	*D*	Et	Me	R_P	8.136	>12	ND	ND
25	**39a**	"	*D*	Et	CH_2CHMe_2	S_P	>100	>1	ND	ND
26	**39b**	"	*D*	Et	CH_2CHMe_2	R_P	37.4	>3	ND	ND
27	**41**	PDN	*L*	iPr	Me	NA	12.03	>8	ND	ND
28	**42**	"	*D*	iPr	Me	NA	>100	>1	490	210
29	**44a**	CPN	*L*	iPr	Me	R_P	0.508	>197	21	570
30	**44b**	"	*L*	iPr	Me	S_P	4.022	>25	520	1100
31	**45a**	"	*D*	iPr	Me	R_P*	31.834	>3	2400	770
32	**45b**	"	*D*	iPr	Me	S_P*	42.299	>2	7500	910
33	**26a**	CPO SATE	/	OH	/	S_P	0.248	>403	5000	1300
34	**26b**	"	/	OH	/	R_P	0.417	>240	3600	710
35	**27a**	"	/	NHEtoc	/	S_P	0.351	>285	ND	ND
36	**27b**	"	/	NHEtoc	/	R_P	0.073	>1370	3300	1600
37	Sofosbuvir	PON	*L*	iPr	Me	S_P*	0.284	>352	310	330
38	PSI-7976	"	*L*	iPr	Me	R_P	2.08	>48	310	320
39	IDX184	SATE					0.203	>370	275	45

[a] AA = aminoacids; [b] P conf. = phosphorus configuration assumed and assigned by ^{31}P NMR chemical shift comparison with compounds marked with * indicating that X-ray structure determination was performed; [c] Inhibition of HCV replication Huh-7 cells containing HCV Con1 subgenomic replicon (GS4.1 cells) with a luciferase read-out. EC_{50} values were determined from the 50% inhibition *versus* concentration data; [d] SI= selectivity index = CC_{50}/EC_{50}, CC_{50}'s were calculated as the concentration that caused a 50% of cells death *versus* concentration data, all compounds had CC_{50} >100 µM; [e] DNAUC: Dose Normalized AUC= AUC/mg/kg



Scheme 4: Proposed metabolic pathway for **35b** (IDX21437) in liver cells.

It has been reported that preferred phosphoramidates of the 2'β-*C*-methyl-2'-α-C-F-uridine series contain L-amino acid moieties. L-Alanine phosphoramidates, such as sofosbuvir and its diastereomer PSI-7976, are metabolized to their intermediate monophosphate through the action of cathepsin A (CatA), and carboxylesterase 1 (CES1), that are responsible of the ester cleavage. The second metabolic step, which is the cleavage of the P-N bond, is promoted by the histidine triad nucleotide binding protein 1 (Hint-1).[21] It was also reported that CES1 is poorly expressed in Huh-7 cells containing the HCV con1 genomic replicon which explains the difference in activity between the sofosbuvir (S_P) and PSI-7976 (R_P), the former preferentially cleaved by CatA and the latter by CES1, despite both being substrates of the two enzymes. Unlike L-amino acid phosphoramidates, little has been reported about the metabolic activation of D-amino acid-containing phosphoramidate pronucleotides. As such, the metabolic activation of **35b** was investigated. Our initial hypothesis was that D-amino-acid phosphoramidates would be largely cleaved by CES1 which would rationalize the lack of activity in the HCV replicon assay. Furthermore, as CES1 is predominantly expressed in the liver and intestine,[24] while CatA is abundantly present in many organs e.g. in human heart tissue,[25] a prodrug mainly activated by CES1 would be able to deliver high level of active NTP in the liver as it is the case for the D-alanine phosphoramidates.

To this end, a transient HCV replicon assay overexpressing CES1 was set up. In this assay, **35b** had an EC_{50} of 0.323µM while it had an EC_{50} of 56.8 µM in the wild-type cell assay (Table 2, Entry 18), indicating that CES1 plays an important role in the metabolic activation of **35b**. Sofosbuvir, being efficiently hydrolyzed by CatA, remained largely unaffected by transfection with CES1, with its activity slightly decreasing in the transient HCV replicon assay (EC_{50}=0.552 µM) *versus* the wild type assay (EC_{50}= 0.284 µM).

Following these preliminary results, an *in vitro* metabolism study of D-Alanine phosphoramidate pronucleotides **35a–b** and sofosbuvir by purified CES1 and CatA enzymes was undertaken, and the results are reported in Table 3. CatA did not hydrolyse the D-alanine phosphoramidates **35a** and **35b** (IDX21437) even after 18 hours of incubation, while sofosbuvir was completely metabolized under the same conditions. CES1 displayed a clear substrate preference for **35b** (R_P) with 94% of the compound hydrolyzed after 21 hours and only 4.5% of the S_P diastereomer **35a** hydrolyzed during the same time. Further, sofosbuvir hydrolysis by this enzyme did not progress in a time-dependent manner (Entry 3, Table 3) with 12% processed at 3 hours and 15% at 21 hours, in accordance with a previous report.[23] These results revealed a clear difference between the metabolic pathways of sofosbuvir and IDX21437 (**35b**) and confirmed that the weak *in vitro* anti-HCV activity of IDX21437 in the commonly used Huh-7-derived replicon cells was the result of under-expression of CES1.

Additional experiments (data not shown) indicated that cytochromes 3A4/5 (CYP 3A4/5) were also able to process **35b** to **46** (Scheme 4), such CYP-mediated activation *via* isopropyl oxidation of esters was observed by other groups in CPO series.[26] It demonstrated that the metabolism of **35b** is more complex than original hypothesis, and that the formation of cytidine metabolites, as shown elsewhere,[27] was not studied in this article but was further observed and will be discuss in a separate report.

Table 3. *In vitro* processing of **35a–b** and sofosbuvir by CES1 and CatA purified enzymes at indicated times.

Entry	Cpd	PD type	% metabolized		
			CES1 3h	CES1 21h	CatA 18h
1	**35a**	D-S_P	ND	4.5	0
2	**35b**	D-R_P	23	94	0
3	**SOF**	L-S_P	12	15	100

ND= not determined.

In order to be an efficient inhibitor of HCV replication, **35b** must be metabolized not only transiently to its monophosphate **49**, but also to its corresponding active triphosphate anabolite **6**. In order to measure the effective *in vitro* formation of NTP **6**, a study in cultured human cells was performed using multiple different types of cells, including: hepatoma cell line Huh-7, which are commonly used in the HCV replicon model system, fresh iCell hepatocytes (human stem cells derived), and iCell cardiomyocytes to assess metabolic specificity of IDX21437. These cells were exposed to 100 µM IDX21437 and then analyzed for the formation of NTP **6**. The cells were also exposed to 10 µM sofosbuvir for comparison and analyzed for sofosbuvir-TP formation. As shown on Figure 2, IDX21437 and sofosbuvir were both readily metabolized to the corresponding triphosphates in hepatocytes. As expected IDX21437 was inefficiently processed in Huh-7 cells to NTP **6** and yielded an AUC of 437 pmol•h/10^6cells, confirming that the lack of CES1 expression in Huh-7 cells explains its weak *in vitro* anti-HCV activity in the Huh-7-derived replicon cells. In the same assay, and at a 10-fold lower dose than IDX21437, sofosbuvir was efficiently processed and an AUC of 35748 pmol•h/10^6cells was measured (Figure 2). More notably, only

traces of NTP **6** were found in human cardiomyocytes after exposure for 72 h and 96 h to IDX21437, whereas sofosbuvir was efficiently processed to its triphosphate analogue, again at a 10 times lower concentration. These data and further study[28] suggested that, contrary to sofosbuvir, the metabolism of pronucleotide **35b** occurs efficiently in hepatocytes with little uptake and/or metabolism in cardiac cells such as cardiomyocytes or CES1 deficient cells such as Huh-7.

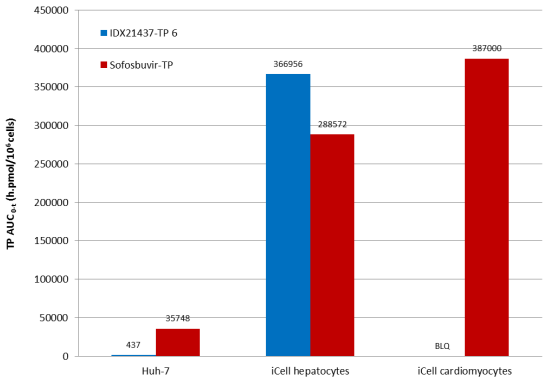


Figure 2: Measured AUC of NTP **6** and sofosbuvir-TP after incubation of cells with 100µM of **35b** (IDX21437) or 10µM of sofosbuvir for 24 h to 72 h in Huh-7 cells, 48 h to 96 h in hepatocytes, or 24 h to 168 h in cardiomyocytes.

Based on its overall favorable profile and its high level of NTP delivered in the liver, **35b** (IDX21437) was selected as clinical development candidate and is currently in phase II clinical trials as MK-3682 (uprifosbuvir).[29]

In conclusion, we have described the discovery of **35b** (IDX21437), a novel R_P D-amino acid-based phosphoramidate monophosphate prodrug of 2'-α-chloro-2'-β-C-methyluridine. Despite showing very weak activity in the *in vitro* replicon cell assay, **35b** demonstrated high levels of active triphosphate **6** in mouse liver and in human hepatocytes. Furthermore, due to its important CES-1 metabolic activation, **35b** displayed high level of NTP in the liver cells compared to other cell types such as CES1-deficient Huh-7 cells and human cardiomyocytes. **35b** is currently in phase II clinical development for the treatment of HCV infection.

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References and notes

1. Hepatitis C. Fact sheet #164; WHO, updated April **2017**; retrieved from: http://www.who.int/mediacentre/factsheets/fs164/en/
2. Ghany, M. G.; Strader, D. B.; Thomas, D. L.; Seeff, L. B. *Hepatology* **2009**, *49*, 1335.
3. Kwong, A. D. *ACS Med. Chem. Lett.* **2014**, *5*, 214.
4. (a) Lim, T. R.; Tan, B. H.; Mutimer, D. J. *Int. J. Antimicrobial Agents* **2014**, *43*, 17. (b) Hayes, C. N.; Chayama, K. *J. Formosan Medical Association* **2015**, *114*, 204.
5. Afdhal, N.; Reddy, K. R.; Nelson, D. R.; Lawitz, E.; Gordon, S. C.; Schiff, E.; Nahass, R.; Ghalib, R.; Gitlin, N.; Herring, R.; Lalezari, J.; Younes, Z. H.; Pockros, P. J.; Di Bisceglie, A. M.; Arora, S.; Subramanian, G. M.; Zhu, Y.; Dvory-Sobol, H.; Yang, J. C.; Pang, P. S.; Symonds, W. T.; McHutchison, J. G.; Muir, A. J.; Sulkowski, M.; Kwo, P. *N. Engl. J. Med.* **2014**, *370*, 1483.
6. Pierra, C.; Amador, A.; Benzaria, S.; Cretton-Scott, E.; D'Amours, M.; Mao, J.; Mathieu, S.; Moussa, A.; Bridges, E. G.; Standring, D. N.; Sommadossi, J.-P.; Storer, R.; Gosselin, G. *J. Med. Chem.* **2006**, *49*, 6614.
7. Coats, S. J.; Garnier-Amblard, E. C.; Amblard, F.; Ehteshami, M.; Amiralaei, S.; Zhang, H.; Zhou, L.; Boucle, S. R. L.; Lu, X.; Bondada, L.; Shelton, J. R.; Li, H.; Liu, P.; Li, C.; Cho, J. H.; Chavre, S. N.; Zhou, S.; Mathew, J.; Schinazi, R. F. *Antiviral Res.* **2014**, *102*, 119.
8. Golitsina, N. L.; Danehy, F. T. J.; Fellows, R.; Cretton-Scott, E.; Standring, D. N. *Antiviral Res.* **2010**, *85*, 470.
9. Sizun, G.; Pierra, C.; Peyronnet, J.; Badaroux, E.; Rabeson, C.; Benzaria-Prad, S.; Surleraux, D.; Loi, A. G.; Musiu, C.; Liuzzi, M.; Seifer, M.; Standring, D.; Sommadossi, J.-P.; Gosselin, G. *Future Med. Chem.* **2015**, *7*, 1675.
10. Sofia, M. J. *Antiviral Chem. Chemother.* **2011**, *22*, 23.
11. Murakami, E.; Niu, C.; Bao, H.; Micolochick Steuer, H. M.; Whitaker, T.; Nachman, T.; Sofia, M. J.; Wang, P.; Otto, M. J.; Furman, P. A. *Antimicrob. Agents Chemother.* **2008**, 458.
12. Idenix unpublished results but activities of NM106 **1** and **2** were reported by: Kirschberg, T. A.; Mish, M. R.; Zhang, L.; Squires, N. H.; Wang, K.-Y.; Cho, A.; Feng,J. Y.; Fenaux, M.; Babusis, D.; Park, Y.; Ray, A. S.; Kim, C. U. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1040.
13. Gosselin, G.; C. Parsy, C.; Alexandre, F.-R.; Rahali, H.; Griffon, J.-F.; Milhau, J.; Surleraux, D.; Dousson, C.; Pierra, C.; Moussa, A.; Mayes, B.WO2014058801A1, 2014.
14. Gillerman, I.; Fischer, B. *Nucleosides Nucleotides Nucleic Acids* **2010**, *29*, 245.
15. Mayes, B. A.; Wang, J.; Arumugasamy, J.; Arunachalam, K.; Baloglu, E.; Bauer, D.; Becker, A.; Chaudhuri, N.; Glynn, R.; Latham, G. M.; Li, J.; Lim, J.; Liu, J.; Mathieu, S.; McGarry, F. P.; Rosinovsky, E.; Soret, A. F.; Stewart, A.; Moussa, A. *Org. Process Res. Dev.* **2015**, *19*, 520.
16. (a) Ross, B. S.; Ganapati Reddy, P.; Zhang, H.-R.; Rachakonda, S.; Sofia, M. J. *J. Org. Chem.* **2011**, *76*, 8311. (b) Pradere, U.; Garnier-Amblard, E. C.; Coats, S. J.; Amblard, F.; Schinazi, R. F. *Chem. Rev.* **2014**, *114*, 9154.
17. Jain, H. V.; Kalman, T. I. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4497.
18. Meppen, M.; Pacini, B.; Bazzo, R.; Koch, U.; Leone, J. F.; Koeplinger, K. A.; Rowley, M.; Altamura, S.; Di Marco, A.; Fiore, F.; Giuliano, C.; Gonzalez-Paz, O.; Laufer, R.; Pucci, V.; Narjes, F.; Gardelli, C. *Eur. J. Med. Chem.* **2009**, *44*, 3765.
19. Cretton-Scott, E.; Hernandez-Santiago, B.; Larsson, M.; Gupta, K. US2010003217A1 (2010).
20. (a) McGuigan, C.; Perrone, P.; Madela, K.; Neyts, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4316 (b) Gardelli, C.; Attenni, B.; Donghi, M.; Meppen, M.; Pacini, B.; Harper, S.; Di Marco, A.; Fiore, F.; Giuliano, C.; Pucci, V.; Laufer, R.; Gennari, N.; Marcucci, I.; Leone, J. F.; Olsen, D. B.; MacCoss, M.; Rowley, M.; Narjes, F. *J. Med. Chem.* **2009**, *52*, 5394. (c) Sofia, M. J.; Bao, D.; Chang, W.; Du, J.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Wang, P.; Zhang, H.-R.; Bansal, S.; Espiritu, C.; Keilman, M.; Lam, A. M.; Micolochick Steuer, H. M.; Niu, C.; Otto, M. J.; Furman, P. A. *J. Med. Chem.*, **2010**, *53*, 7202.
21. (a) Ma, H.; Jiang, W.-R.; Robledo, N.; Leveque, V.; Ali, S.; Lara-Jaime, T.; Masjedizadeh, M.; Smith, D. B.; Cammack, N.; Klumpp, K.; Symons, J. *J. Biol. Chem.* **2007,** *282,* 29812. (b) Chang, W.; Bao, D.; Chun, B.-K.; Naduthambi, D.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Zhang, H.-R.; Bansal, S.; Espiritu, C. L.; Keilman, M.; Lam, A. M.; Niu, C.; Steuer, H. M.; Furman, P. A.; Otto, M. J.; Sofia, M. J. *ACS Med. Chem. Lett.* **2011**, *2*, 130.
22. Murakami, E.; Tolstykh, T.; Bao, H.; Niu, C.; Micolochick Steuer, H. M.; Bao, D.; Chang, W.; Espiritu, C.; Bansal, S.; Lam, A. M.; Otto, M. J.; Sofia, M. J.; Furman, P. A. *J. Biol. Chem.* **2010**, *285*, 34337.
23. Cho, A.; Zhang, L.; Xu, J.; Lee, R.; Butler, T.; Metobo, S.; Aktoudianakis, V.; Lew, W.; Ye, H.; Clarke, M.; Doerffler, E.; Byun, D.; Wang, T.; Babusis, D.; Carey, A. C.; German, P.;

Sauer, D.; Zhong, W.; Rossi, S.; Fenaux, M.; McHutchison, J. G.; Perry, J.; Feng, J.; Ray, A. S.; Kim, C. U. *J. Med. Chem.* **2014**, *57*, 1812.

24. Zhu, H. J. ; Brinda, B. ; Froehlich, T. E.; Markowitz, J. S. *Pharmacogenetics Genomics* **2012**, *22*, 215.
25. Jackman, H. L.; Massad, M. G.; Sekosan, M.; Tan, F.; Brovkovych, V.; Marcic, B. M.; Erdös, E. G. *Hypertension* **2002**, *39*, 976.
26. (a) Niu, C.; Tolstykh, T.; Bao, H.; Park, Y.; Babusis, D.; Lam, A. M.; Bansal, S.; Du, J.; Chang, W.; Reddy, P. G.; Zhang, H.-R.; Woolley, J.; Wang, L.-Q.; Chao, P. B.; Ray, A. S.; Otto, M. J.; Sofia, M. J.; Furman, P. A.; Murakami, E. *Antimicrob. Agents Chemother.* **2012**, *56*, 3767 (b) Jonckers, T. H. M.; Tahri, A.; Vijgen, L.; Berke, J. M.; Lachau-Durand, S.; Stoops, B.; Snoeys, J.; Leclercq, L.; Tambuyzer, L.; Lin, T.-I.; Simmen, K.; Raboisson, P. *J. Med. Chem.* **2016**, *59*, 5790.
27. Pinho, P.; Kalayanov, G.; Westerlind, H.; Rosenquist, Å.; Wähling, H.; Sund, C.; Almeida, M.; Ayesa, S.; Tejbrant, J.; Targett-Adams, P.; Eneroth, A.; Lindqvist, A. *Bioorg. Med. Chem. Lett.* **2017**, *27,* 3468.
28. Lagrutta, A.; Regan, C. P.; Zeng, H.; Imredy, J. P.; Koeplinger, K.; Morissette, P.; Liu, L.; Wollenberg, G.; Brynczka, C.; Lebrón, J.; DeGeorge, J.; Sannajust, F. *Scientific Reports* **2017**, *7*, 44820.
29. Press Release 22 April 2017 http://investors.merck.com/news/press-release-details/2017/Merck-Announces-New-Phase-2-Data-on-Investigational-Triple-Combination-Therapy-MK-3682B-for-Chronic-Hepatitis-C/default.aspx