

# Preclinical Characterization of a Series of Highly Potent Achiral Phosphorodiamidate Nucleotide Analogue Inhibitors of Hepatitis C Polymerase

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J. Vernachio<sup>1</sup>, B. Bleiman<sup>1</sup>, K. D. Bryant<sup>1</sup>, S. Chamberlain<sup>1</sup>, B. Ganguly<sup>1</sup>, E. Gorovits<sup>1</sup>, A. Hall<sup>1</sup>, G. Henson<sup>1</sup>, D. Hunley<sup>1</sup>, J. Hutchins<sup>1</sup>, A. Kolykhalov<sup>1</sup>,

J. Muhammad<sup>1</sup>, A. Obikhod<sup>1</sup>, N. Raja<sup>1</sup>, C. R. Walters<sup>1</sup>, J. Wang<sup>1</sup>, K. Williams<sup>1</sup>, H. Zhao<sup>1</sup>, C. McGuigan<sup>2</sup>, K. Madela<sup>2</sup>, M. Aljarah<sup>2</sup>, C. Bourdin<sup>2</sup>, S. Jones<sup>2</sup> and J. Patti<sup>1</sup>

<sup>1</sup>Inhibitex, Inc., Alpharetta, Georgia, USA, <sup>2</sup>Welsh School of Pharmacy, Cardiff University, Cardiff, UK

## Introduction

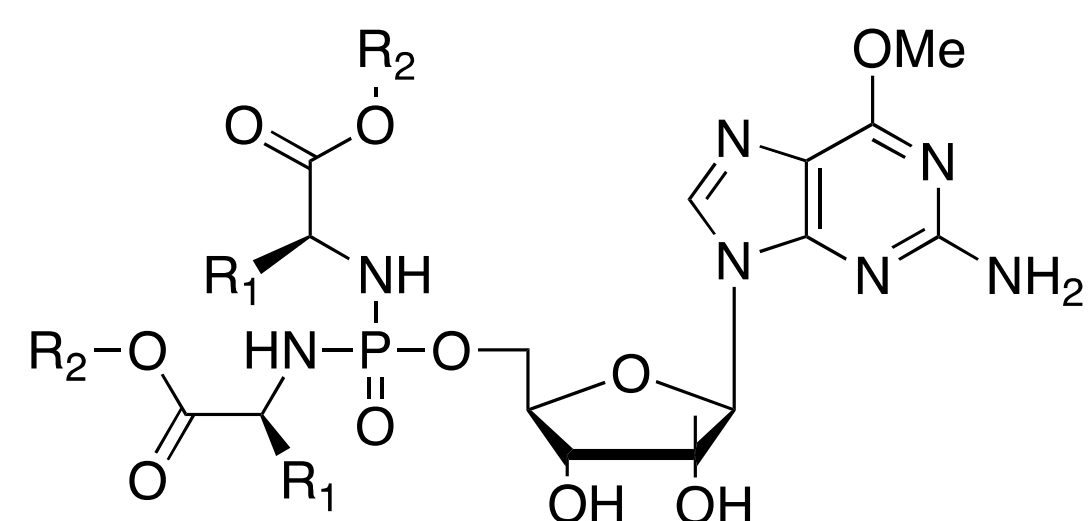
The advantages of using a phosphoramidate prodrug strategy for the delivery of monophosphate forms of nucleoside analog inhibitors of HCV have been clearly demonstrated in clinical trials.

Achiral phosphorodiamidates represent a new approach for this prodrug strategy and provide the advantages of a single diastereomer around phosphorus with the potential of improved physical properties while building on the positive attributes associated with other phosphoramidates. Results from the preclinical characterization of a series of novel phosphorodiamidate prodrugs of 6-O-methyl-2'-C-methyl guanosine are presented.

## Methods

A luciferase-reporter genotype 1b subgenomic replicon cell line, was obtained from Apath, LLC, Brooklyn, NY. HCV replication was measured using the Renilla luciferase reporter assay (Promega, Madison, WI). Cell cytotoxicity was measured using the CellTiter-Glo<sup>®</sup> luminescent assay. Intrinsic clearance of compounds (10 μM) by human, rat and primate liver and intestinal microsomes in the presence of NADPH was measured by LC-MS/MS. The levels of intracellular 2'-C-MeGTP produced in primary, plated human hepatocytes after incubation with compounds at 10 μM was measured using LC-MS/MS. The pharmacokinetics of selected compounds was evaluated in rats at a dose of 10 mg/kg and in cynomolgus monkeys at 25 mg/kg following a single oral administration. The levels of intracellular 2'-C-methyl guanosine triphosphate in liver samples and plasma concentrations of 2'-C-methyl guanosine were measured using LC-MS/MS. Pharmacokinetic analysis was performed with WinNonLin Software (Pharsight<sup>®</sup>, St. Louis, MO).

## Phosphorodiamidate Structure



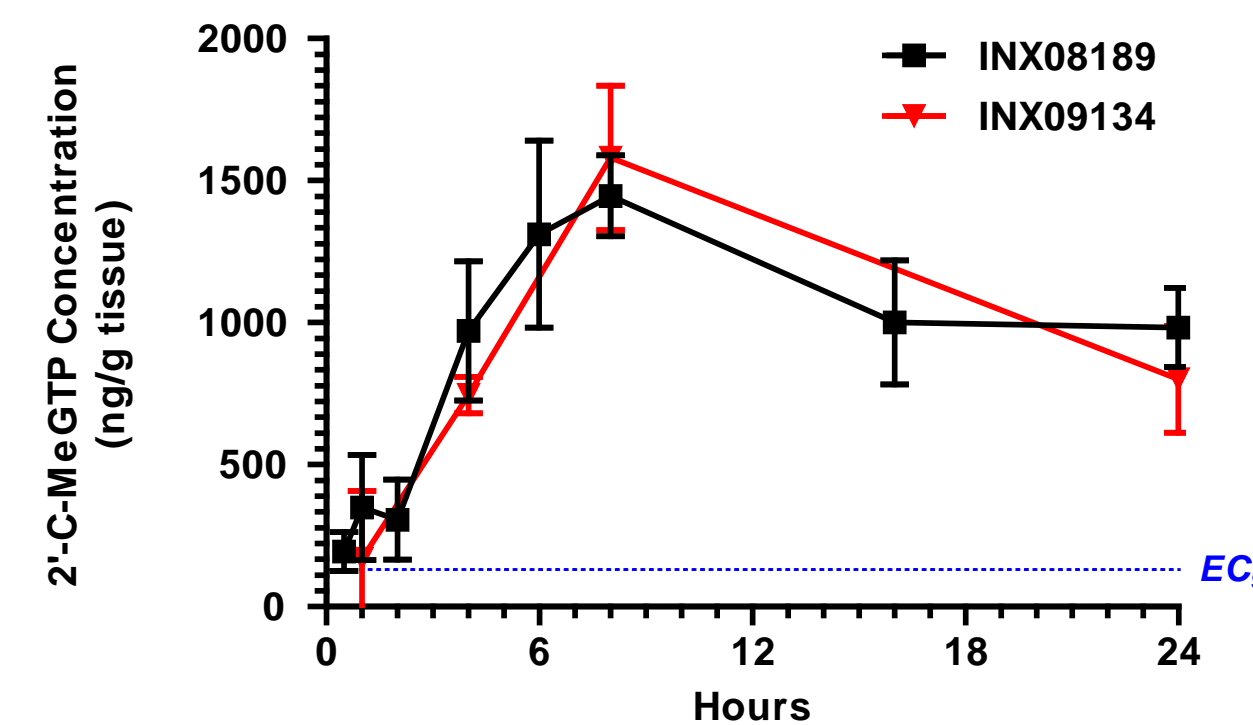
## Potency in HCV GT1b Replicon

AA (R1) ester (R2)	EC <sub>50</sub> (μM)		CC <sub>50</sub> (μM)
	Avg	SD	
INX08189	0.01	0.01	7
2'-C-MeG Nucleoside	2.20	1.40	>100
6OMe2'-C-MeG Nucleoside	4.40	2.20	>100
L-Ala OMe	5.90	0.76	>100
L-Ala OEt	1.20	0.08	>100
L-Ala OnPr	0.28	0.05	>100
L-Ala OButyl	0.07	0.01	>100
L-Ala OPentyl	0.03	0.01	65
L-Ala OiPr	0.49	0.18	>100
L-Ala O-(R,S)-2Bu	0.15	0.00	>100
L-Ala O-3,3-dimethylbutyl	0.02	0.01	71
L-Ala OcBu	0.32	0.05	>100
L-Ala OcPentyl	0.06	0.01	>100
L-Ala OcHx	0.05	0.02	>100
L-Ala OBn	0.49	-	>100
L-Ala OPhEt	0.49	-	88
L-Ala O-2,4-diFBn	0.26	0.21	54
L-Ala OTHP	13.33	13.02	>100
L-Ala OIndanol	0.58	0.39	70
L-Ala OCH2tBu	0.06	0.04	>100
L-Ala OCH2iPr	0.07	0.01	>100
L-Ala OCH2cPropyl	0.21	0.00	>100
D-Ala OCH2tBu	0.11	0.03	>100
L-Asp OMe	10.18	3.17	>100
L-Asp OBn	0.61	0.08	>100
L-Gly OBn	0.60	0.13	>100
L-Gly OCH2tBu	0.13	0.02	>100
L-Leu OcHx	0.45	0.52	20
L-Leu OBn	0.38	0.02	39
L-Leu OCH2tBu	0.47	0.15	27
L-Ile OMe	5.17	1.94	>100
L-Ile OcHx	4.00	0.47	14
L-Ile OBn	0.40	0.11	24
L-Ile OCH2tBu	2.90	0.33	15
L-Met OcHx	0.60	0.17	51
L-Met OBn	0.25	0.04	>100
L-Met OCH2tBu	2.22	-	>100
L-Phe OcHx	0.50	0.59	25
L-Phe OBn	0.32	0.14	67
L-Phe OCH2tBu	0.05	0.02	24
L-Pro OBn	0.52	0.21	>100
L-Pro OCH2tBu	0.81	0.05	56
L-Val OcHx	2.50	0.35	20
L-Val OBn	0.12	0.04	49
L-Val OCH2tBu	0.72	-	32
L-Tyr (tBu) OMe	0.11	0.04	89
L-PhG OcHx	0.32	0.00	18
L-PhG OCH2tBu	0.27	0.03	24
L-Val-L-Ala OCH2tBu	0.54	0.26	>100

## Cell Cytotoxicity in a Panel of Human Cell Lines

Compound #	AA ester	Cell Line CC <sub>50</sub> (μM)							
		HepG2 Liver	MT4 Lymphoid	CEM Lymphoid	293 Embryonic Kidney	A204 Skeletal Muscle	HEL Lung Fibroblast	CaCo2 Colon Epithelium	HeLa Epithelium
INX08189		11	1	12	3	18	23	34	36
INX09134	L-Ala OCH2tBu	>100	85	93	>100	>100	>100	>100	>100
INX100045	L-Ala OcPentyl	>100	98	96	>100	95	94	>100	>100
INX100059	L-Ala OButyl	93	8	11	76	28	59	92	60
INX100060	L-Ala OPentyl	47	3	5	34	11	26	95	23
INX100063	D-Ala OCH2tBu	>100	>100	>100	>100	>100	>100	>100	>100

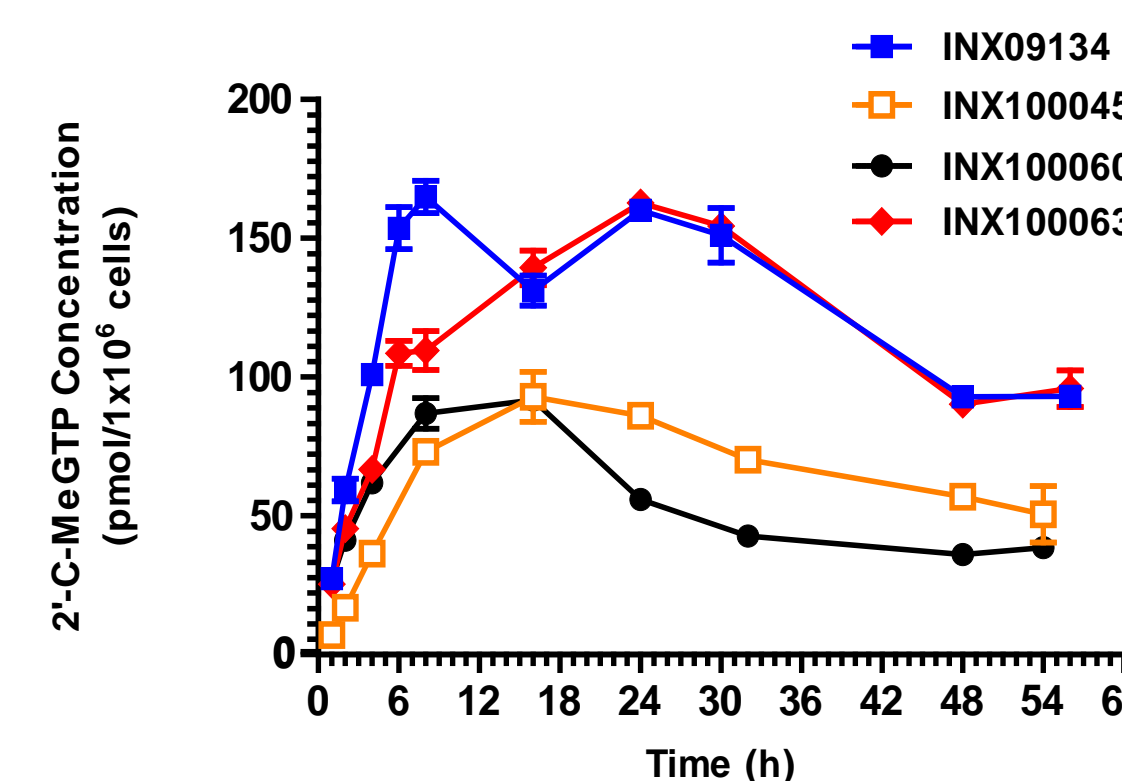
## Pharmacokinetics of Active Triphosphate in Rat Liver



Liver 2'-C-MeGTP Pharmacokinetic Comparison

Compound	AA ester	C <sub>max</sub> (ng/g)	C <sub>last</sub> (ng/g)	T <sub>max</sub> (h)	AUC <sub>0-t</sub> (ng-h/g)
INX08189		1447	983	8	24,557
INX09134	L-Ala OCH <sub>2</sub> tBu	1580	800	8	25,147
INX100045	L-Ala O-cycloPentyl	1523	610	8	24,546
INX100059	L-Ala O-n-Butyl	1553	720	8	27,671
INX100060	L-Ala O-n-Pentyl	1042	506	8	18,600
INX100063	D-Ala OCH <sub>2</sub> tBu	598	442	8	11,608

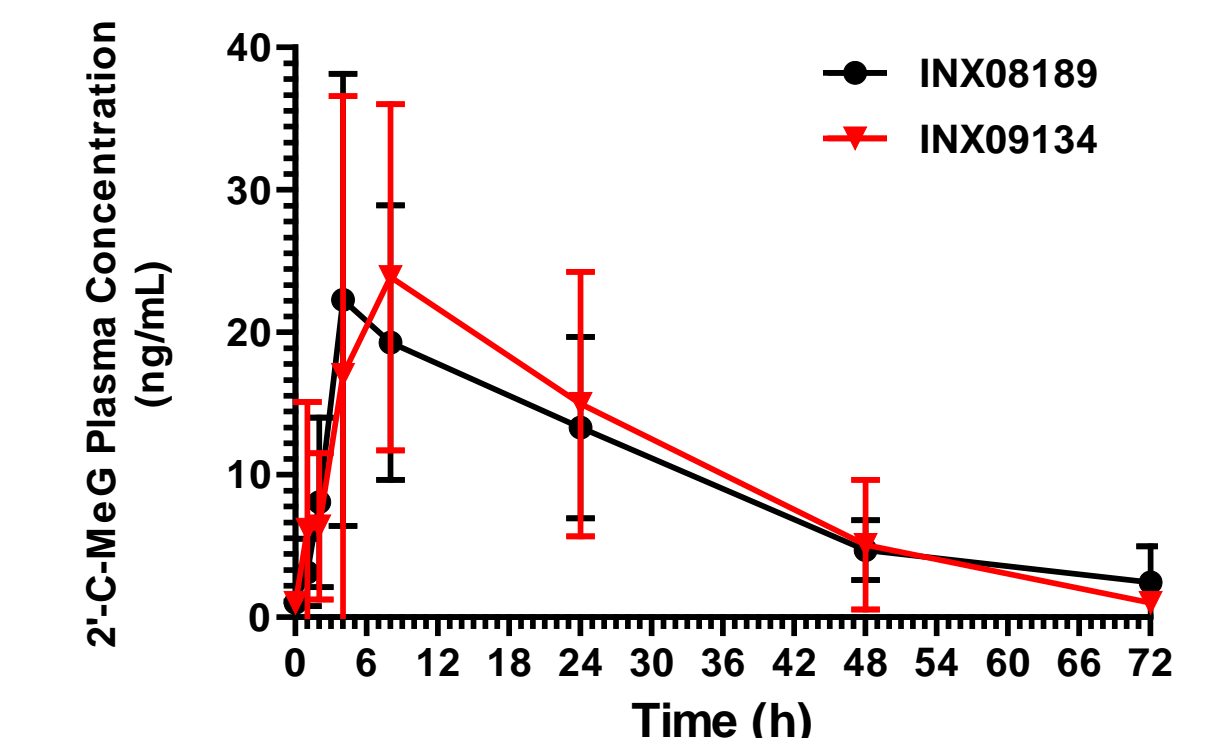
## Intracellular 2'-C-MeGTP in 1<sup>0</sup> Human Hepatocyte Cultures



## Ratio of Liver to Intestinal Clearance

Compound #	AA ester	Microsome Intrinsic Clearance Ratio (Cl <sub>liv</sub> :Cl <sub>int</sub> ) (mL/min/g)		
		Human	Rat	Primate
INX08189		4.4	4.4	7.3
INX09134	L-Ala OCH2tBu	5.0	3.1	4.8
INX100045	L-Ala OcPentyl	8.4	6.9	7.8
INX100059	L-Ala OButyl	5.2	5.7	11.1
INX100060	L-Ala OPentyl	7.7	9.4	6.7
INX100063	D-Ala OCH2tBu	6.7	1.4	6.7

## Pharmacokinetics of Nucleoside Metabolite in Primate Plasma



2'-C-MeG Nucleoside Metabolite Exposure in Primate Plasma

Compound	AA ester	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	AUC <sub>0-24</sub> (ng-h/mL)
INX08189		27	8	349
INX09134	L-Ala OCH2tBu	27	4	424
INX100045	L-Ala OcPentyl	46	14	506
INX100059	L-Ala OButyl	118	4	1081
INX100060	L-Ala OPentyl	68	14	707
INX100063	D-Ala OCH2tBu	28	14	393

## Conclusions

Phosphorodiamidate prodrugs of 6-O-methyl-2'-C-methyl guanosine have a high potential for clinical utility in the treatment of chronic HCV with the following characteristics:

- Lack of chirality at the phosphorus eliminates mixed diastereomers
- Highly potent inhibitors of HCV NS5B with good selectivity
- Favorable ratio of liver to intestinal clearance
- Excellent pharmacokinetic and pharmacodynamic properties in preclinical studies
- Rapid and efficient conversion to active triphosphate in human hepatocytes

**Disclosures:** Inhibitex authors are employees of Inhibitex, Inc. C.M. is a director of Inhibitex, Inc. K.M and M.A. have received stipends from Inhibitex, Inc.

