

# CHARACTERIZATION OF A SERIES OF HIGHLY POTENT PHOSPHORAMIDATE NUCLEOSIDE ANALOGUE INHIBITORS OF HEPATITIS C POLYMERASE

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## Introduction

Phosphoramidate nucleoside analogues, or pronucleotides (ProTides), possess several pharmacological advantages over their parent nucleoside that include a significant increase in antiviral activity, higher concentrations of the triphosphate in liver, and potentially less toxicity due to reduced systemic exposure. The aims of this study were to characterize the *in vitro* and *in vivo* properties of a series of novel aminoacyl ProTides of 2'-C-methyl guanosine (2'-C-MeG).

## Methods

The antiviral activity of the ProTides alone and in combination with ribavirin and IFN-2α was determined using the genotype 1b subgenomic replicon assay. Cell cytotoxicity was measured using an XTT based ELISA and mitochondrial toxicity was quantitated by Q-PCR. The levels of intracellular 2'-C-MeGTP produced in primary human hepatocytes was measured using LC-MS/MS. Plasma concentrations of the ProTides and parent nucleoside in the peripheral circulation and portal vein of cannulated cynomolgus monkeys were measured using LC-MS/MS following a single oral dose (25mg/kg).

## Results

ProTides exhibited potent anti-HCV activity with EC<sub>50</sub>s ranging from 60-230 nM and combined with ribavirin or interferon-2α, the anti-HCV effects were demonstrated to be synergistic. Cytotoxicity was not observed in Huh-7 cells (CC<sub>50</sub>>100μM). In an MT-4 cell line, the cytotoxicity of the series produced CC<sub>50</sub> values ranging from 20 to >100 μM. ProTides evaluated for mitochondrial toxicity did not reduce the mitochondrial copy number after continuous treatment for 3 days at 100 μM or for 13 days at 5 μM. Conversion of ProTides in hepatocytes to the triphosphate demonstrated C<sub>max</sub> levels of 78 pmol/10<sup>6</sup> cells, representing approximately a 20-fold increase over the EC<sub>90</sub>. PK studies in primates suggested efficient extraction by the liver as demonstrated by low systemic levels of the ProTides. Analysis of liver biopsies indicated that levels of the triphosphate exceeding the EC<sub>90</sub> were achieved following oral dosing.

Figure 1: Antiviral Activity

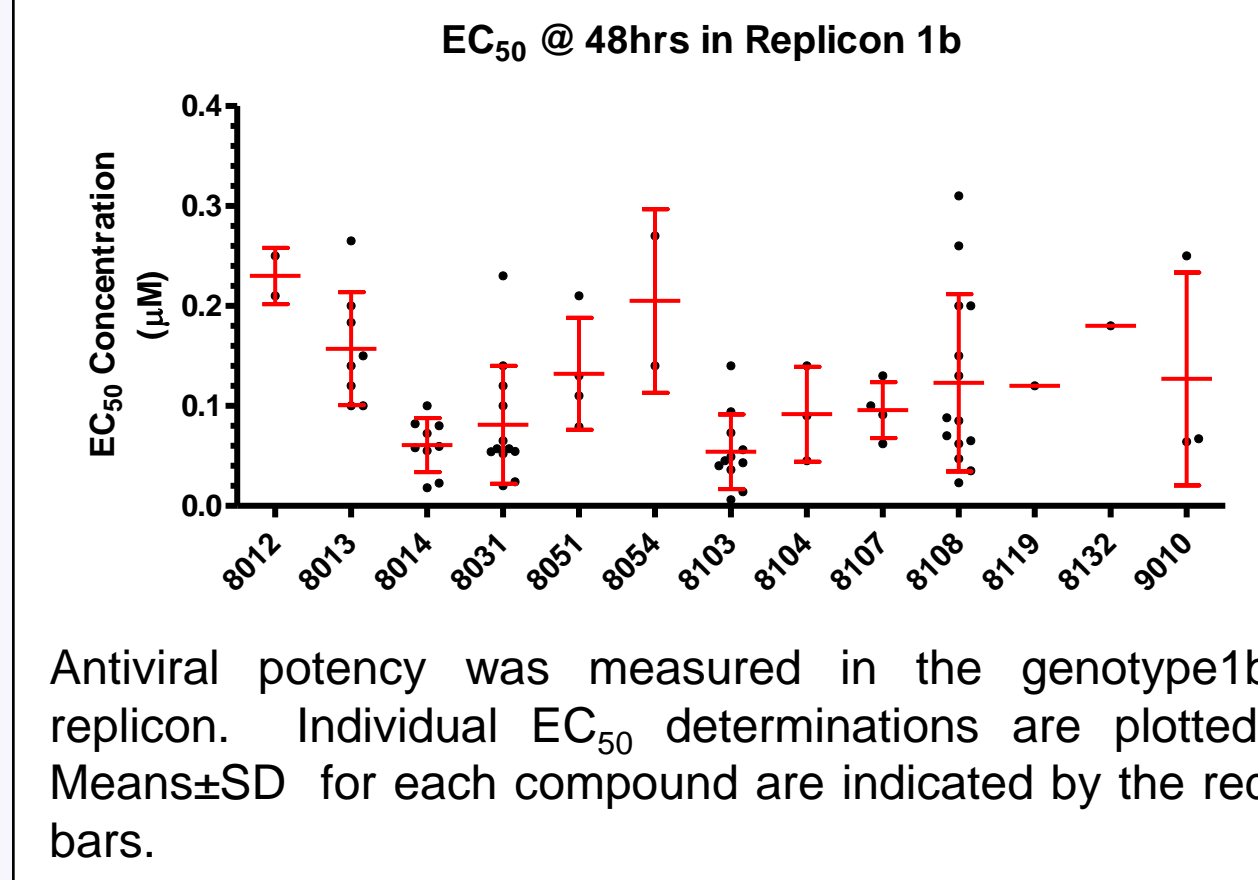


Table 1: Therapeutic Indices

Compound (INXO-)	Potency Measurements in Genotype 1b Replicon (µM±SD)						CC <sub>50</sub>	TI
	48hr		72hr		EC <sub>90</sub>	TI		
	EC <sub>50</sub>	EC <sub>90</sub>	EC <sub>50</sub>	EC <sub>90</sub>				
Ribavirin	28.445 ± 9.135	153.332 ± 17.471	33.693 ± 1.500	172.143 ± 15.800	>100	88		
2'-C-MeG	0.992 ± 0.280	3.608 ± 0.525	1.135 ± 0.310	3.408 ± 0.788	>100	88		
8012	0.220 ± 0.028	0.775 ± 0.158	0.247 ± 0.091	0.734 ± 0.119	>100	404		
8013	0.177 ± 0.060	0.769 ± 0.221	0.171 ± 0.060	0.552 ± 0.107	>100	585		
8014	0.056 ± 0.028	0.257 ± 0.119	0.080 ± 0.043	0.258 ± 0.158	>100	1247		
8031	0.076 ± 0.054	0.321 ± 0.238	0.062 ± 0.034	0.252 ± 0.145	>100	1612		
8051	0.130 ± 0.048	0.513 ± 0.153	0.117 ± 0.047	0.436 ± 0.075	>100	852		
8054	0.173 ± 0.063	0.541 ± 0.165	0.270 ± 0.040	0.690 ± 0.109	>50	185		
8103	0.054 ± 0.036	0.254 ± 0.143	0.053 ± 0.028	0.231 ± 0.116	>100	1874		
8104	0.091 ± 0.045	0.398 ± 0.114	0.120 ± 0.063	0.342 ± 0.102	>100	834		
8107	0.095 ± 0.027	0.474 ± 0.091	0.116 ± 0.047	0.367 ± 0.070	>100	860		
8108	0.119 ± 0.086	0.592 ± 0.518	0.085 ± 0.044	0.298 ± 0.169	>100	1173		
8119	0.125 ± 0.008	0.483 ± 0.070	0.116 ± 0.021	0.340 ± 0.003	>100	863		
8132	0.184 ± 0.001	0.735 ± 0.009	0.194 ± 0.009	0.692 ± 0.058	>100	515		
9010	0.127 ± 0.107	0.573 ± 0.543	0.096 ± 0.073	0.340 ± 0.277	>100	1042		

Table 2: Toxicity

Compound (INXO-)	Cytotoxicity (CC <sub>50</sub> µM)					Mitochondrial Toxicity (CC <sub>50</sub> µM)	
	LIVER		LYMPHOID (T cell)		Kidney Vero	CEM Exposure Time	
	Huh7b	HepG2	MT-4	CEM		3 Days	13 Days
Ribavirin	>100	>100	>100	>100	>100	>100	>5
2'-C-MeG	>100	>100	>100	>100	>100	>100	>5
8012	>100	70	30	>100	>100	>100	>5
8013	>100	>100	>100	>100	>100	>100	>5
8014	>100	>100	100	>100	>100	>100	>5
8031	>100	>100	>100	>100	>100	>100	>5
8051	>100	>100	>100	>100	>100	>100	>5
8054	>50	75	36	>100	>100	>100	>5
8103	>100	>100	57	>100	>100	>100	>5
8104	>100	>100	65	>100	>100	>100	>5
8107	>100	>100	57	>100	>100	>100	>5
8108	>100	>100	>100	>100	>100	>100	>5
8119	>100	>100	64	>100	>100	>100	>5
8132	>100	>100	>100	>100	>100	>100	>5
9010	>100	>100	>100	>100	>100	>100	>5

Table 3: Drug Combination Studies

Compound (INXO-)	Synergy Volume* (µM <sup>2</sup> %)		Combination Index**	
	IFNα-2a	Ribavirin	IFNα-2a	Ribavirin
8014	5	62	ED90: 0.37 ED75: 0.34 ED50: 0.37	ED90: 0.49 ED75: 0.47 ED50: 0.46
8108	27		ED90: 0.50 ED75: 0.47 ED50: 0.46	

\* Synergy Volume: Analysis performed with MacSynergyII (Synergy: >25; Antagonism: <-25)  
 \*\* Combination Index: Isobologram analysis performed with CalcuSyn (Synergy: <0.5; Additivity: 0.5-<0.5; Antagonism: >1)

Figure 2: 2'-C-MeGTP in 1<sup>o</sup> Human Hepatocytes

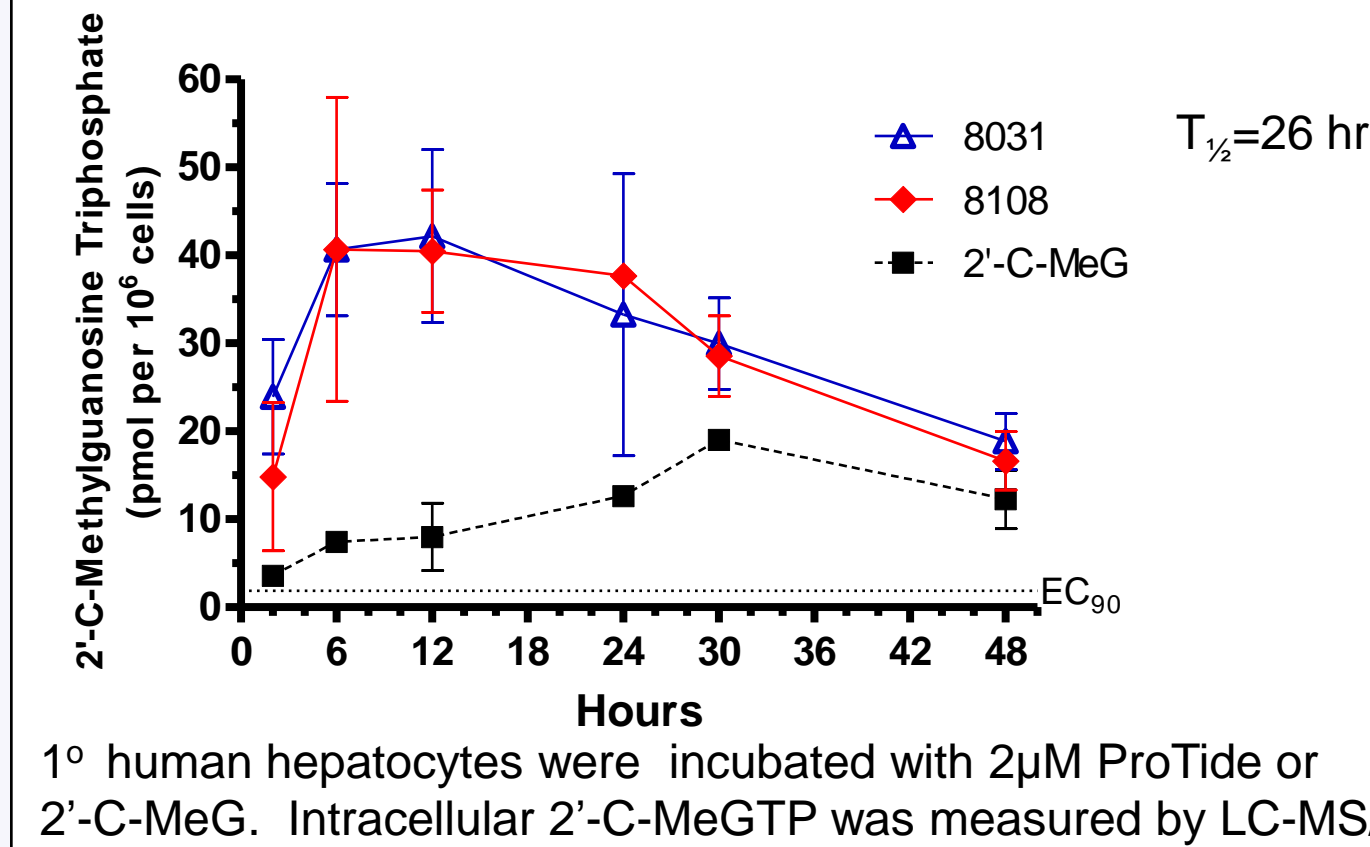


Figure 3: 2'-C-MeGTP in Rodent Liver Tissue

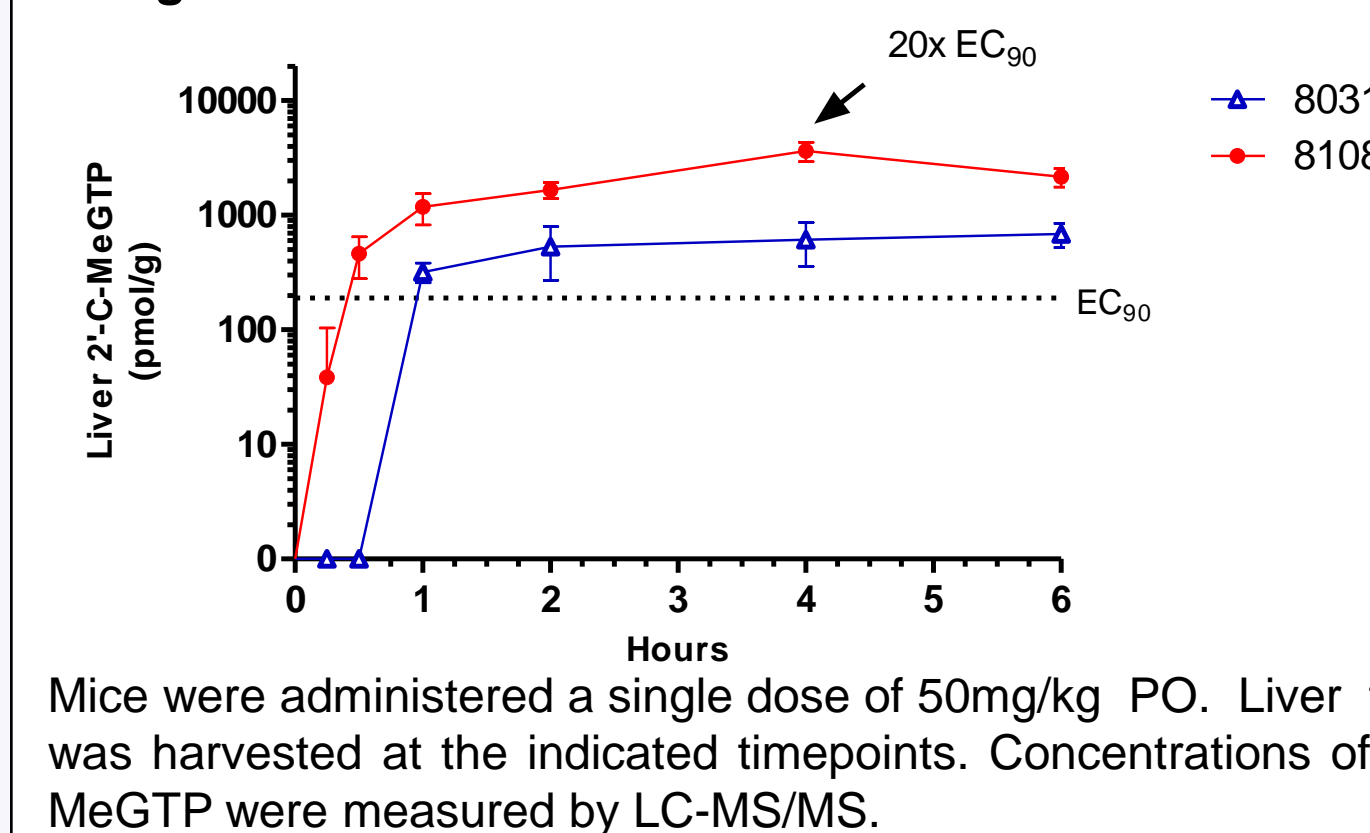
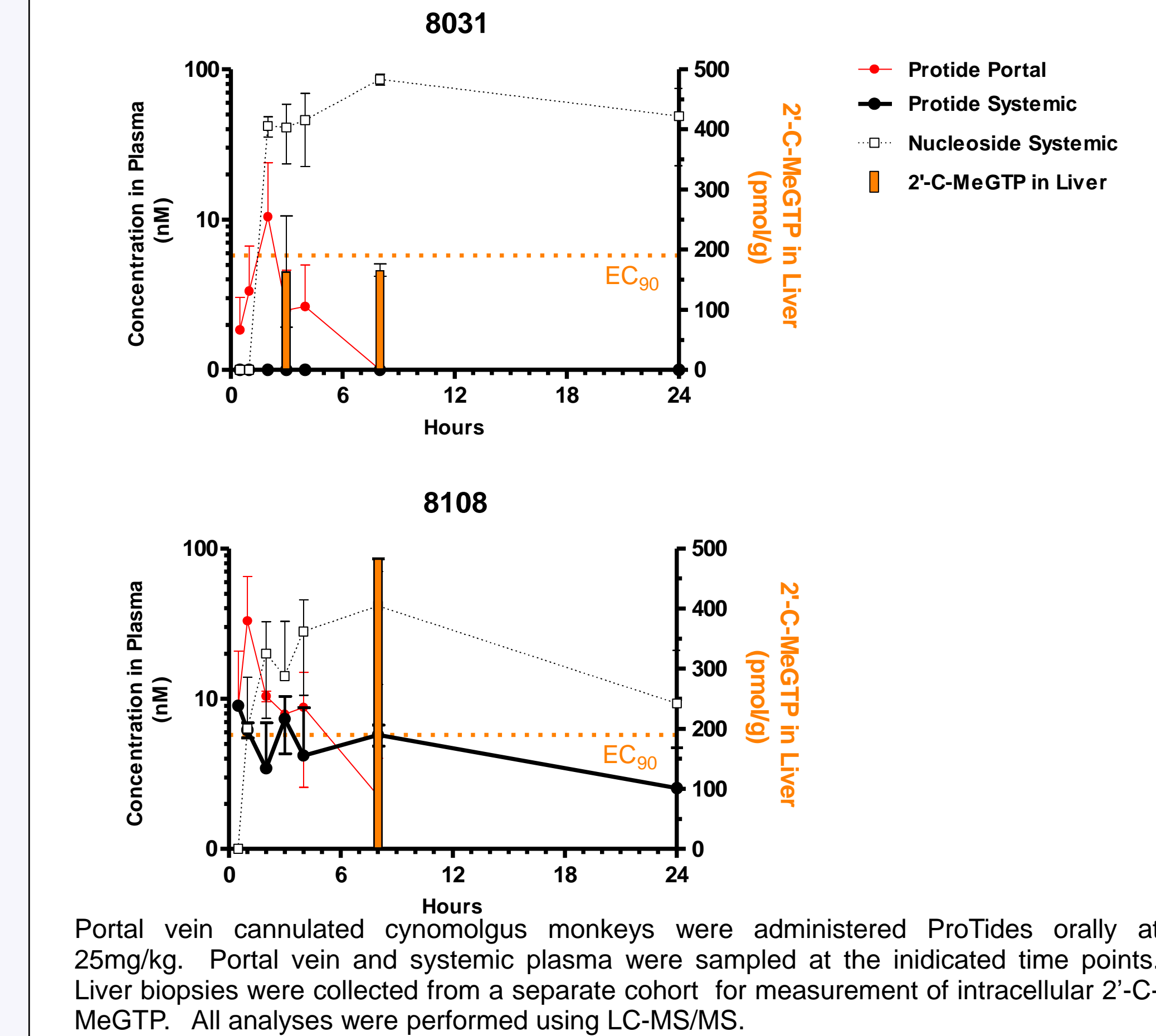


Figure 4: Oral Pharmacokinetics in Primates



Portal vein cannulated cynomolgus monkeys were administered ProTides orally at 25mg/kg. Portal vein and systemic plasma were sampled at the indicated time points. Liver biopsies were collected from a separate cohort for measurement of intracellular 2'-C-MeGTP. All analyses were performed using LC-MS/MS.

## Conclusions

ProTides of 2'-C-MeG exhibit excellent therapeutic indices and conversion to 2'-C-MeGTP in primary human hepatocytes exceed the EC<sub>90</sub> in the genotype 1b replicon. Primate PK studies support delivery of the ProTides to the liver and subsequent conversion to the triphosphate after oral administration. These data suggest that ProTides of 2'-C-MeG represent a promising class of compounds for the treatment of HCV infections.

