

Inhibition of Hepatitis C Virus Replication by Octadecyloxyethyl- (S)-HPMPA in Genotype 1b and 2a Replicons

**Karl Y. Hostetler, David L. Wyles, Kelly A.
Kaihara, James R. Beadle and Robert T. Schooley**

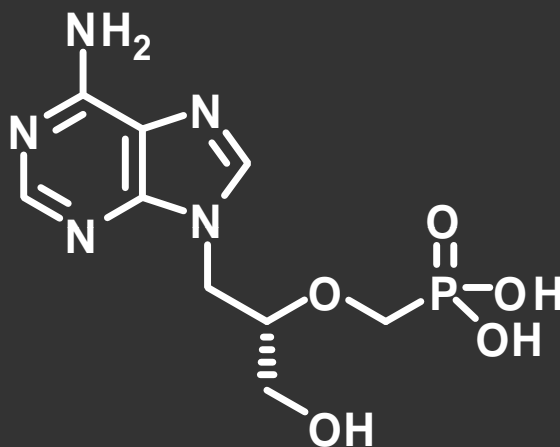
Dept. of Medicine, Div. Infectious Disease,
University of California, San Diego, La Jolla, CA
92093

Disclosure: Dr. Hostetler is a consultant to Chimerix Inc. and this relationship has been reviewed and approved by the UCSD Conflict of Interest Office in keeping with University Policies.

Background

- As part of NIAID biodefense initiatives, our group was asked to synthesize an orally active version of cidofovir (CDV) for defense against pox viruses
- We synthesized the hexadecyloxypropyl ester analog of CDV (HDP-CDV)
- HDP-CDV was orally active in various poxvirus animal models
- HDP-CDV was >10 fold more active than CDV against poxviruses and >100 fold against HCMV
- This approach is applicable to all nucleoside phosphates and phosphonates...

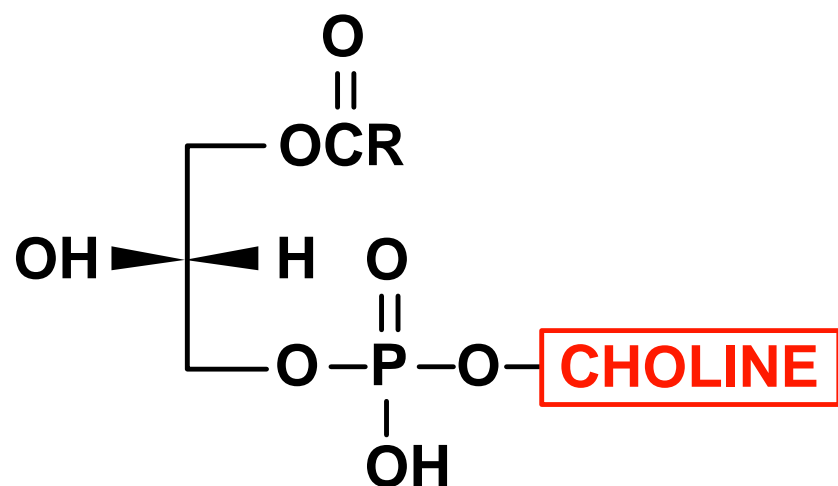
(S)-9-(3-Hydroxy-2-phosphonomethoxypropyl)adenine



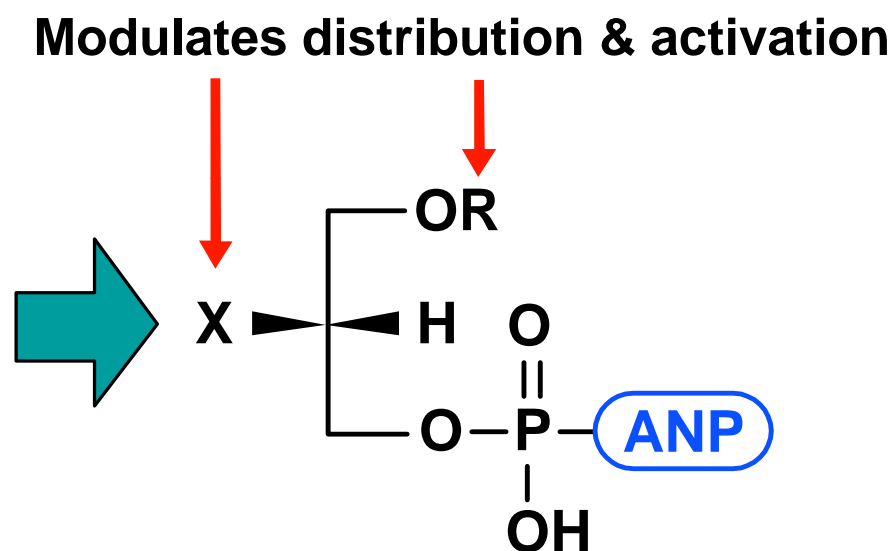
(S)-HPMPA

- (S)-HPMPA was the 1st antiviral acyclic nucleoside phosphonate
- Described by De Clerq, Holý et al. *Nature*, 323: 464-467, 1986
- Adenine analog of cidofovir (HPMPC)
- Broad spectrum antiviral against double stranded DNA viruses
- More potent against orthopoxviruses than CDV

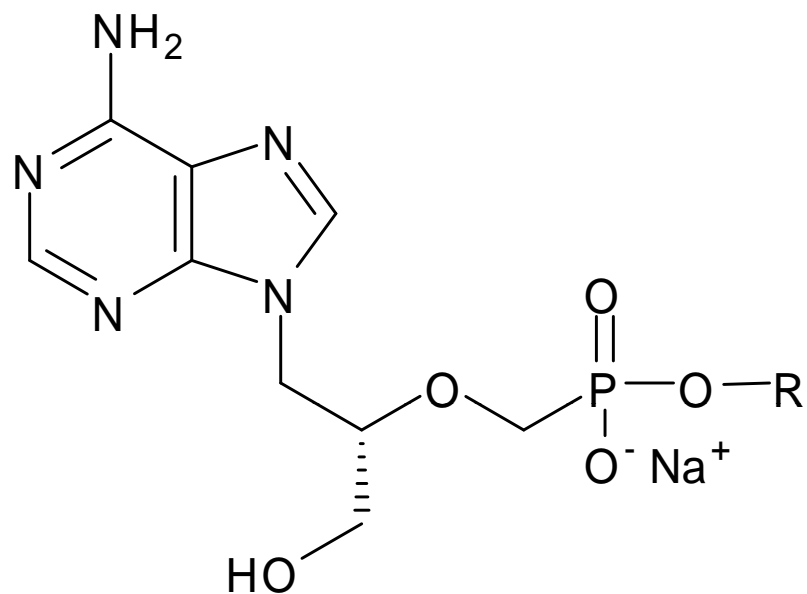
Drug Design Paradigm: Acyclic Nucleoside Phosphonates



Lysophosphatidylcholine
(a natural lipid metabolite)



Phospholipase C cleavage
inside cells releases ANP



HDP-(S)-HPMPA R = (CH₂)₃O(CH₂)₁₅CH₃
ODE-(S)-HPMPA R = (CH₂)₂O(CH₂)₁₇CH₃

Figure 1. Structure of (S)-HPMPA Analogs

Synthesis described in Beadle et al, J. Med. Chem., 49:2010-2015, 2006.

Effect of Alkoxyalkyl Esters of (S)-HPMPA on Poxvirus Replication

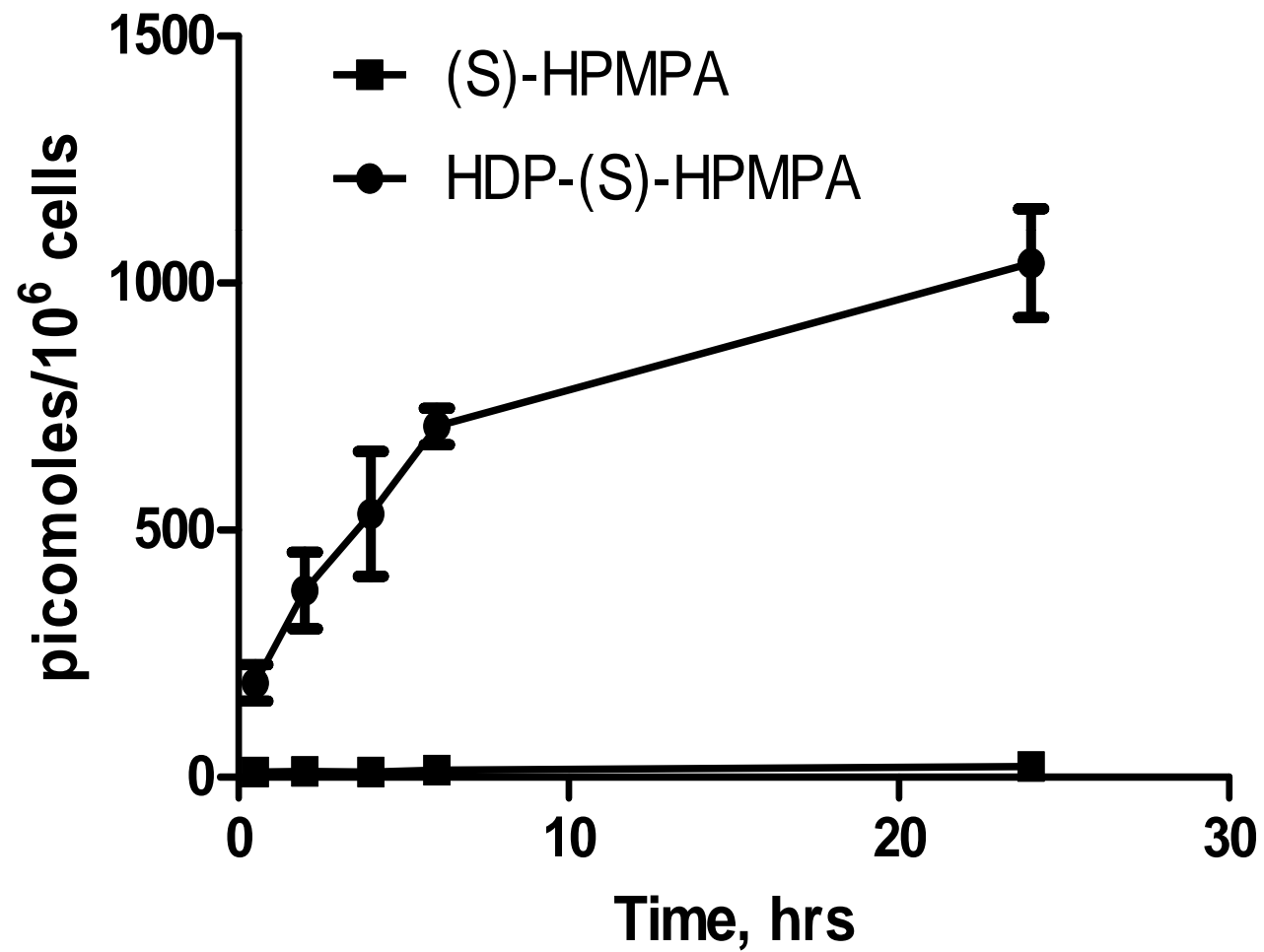
Compound	Vaccinia EC ₅₀ μM	Cowpox EC ₅₀ μM	Variola EC ₅₀ μM	Selectivity Range
(S)-HPMPA	2.7	4.0	7.9	>70
HDP-HPMPA	0.010 (270x)	0.020 (200x)	<0.05 (>118)	480-970
15M-HDP-HPMPA	0.008	0.012	0.046	28-530
ODBG-HPMPA	0.010	0.020	<0.05	75-150
CDV	31	42	27	>12
HDP-CDV	0.85 (36x)	1.20 (35x)	0.10 (270x)	50-110

Hostetler, Huggins, Kern, Prichard et al, unpublished, 2007

Activity of HDP- and ODE-(S)-HPMPA in HCV genotype 1b and 2a replicons

Compound	EC ₅₀	CC ₅₀	Selectivity
<u>Replicon BM4-5, Genotype 1b</u>			
(S)-HPMPA	>100	>100	-
HDP-(S)-HPMPA	2.0±0.95	63.8±6.3	31.6
ODE-(S)-HPMPA	1.3±0.66	27.2±4.3	20.7
<u>Replicon JFH-1, Genotype 2a</u>			
(S)-HPMPA	>100	>100	-
HDP-(S)-HPMPA	8.5±12.5	63.8±6.3	7.5
ODE-(S)-HPMPA	0.68±0.70	27.2±4.2	40
Data μM; Wyles, Schooley, Hostetler et al, unpublished, 2007			

Figure 2. Drug Uptake in Hep G2 Cells

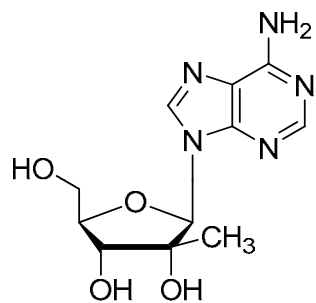


Conversion of HDP-[8-¹⁴C]-(S)-HPMPA to Nucleotides in HepG2 cells

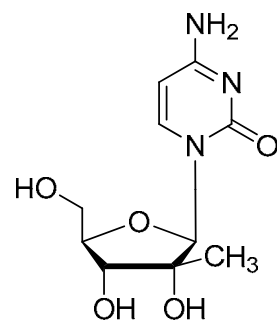
Compound	24 hours	48 hours
(S)-HPMPA	260	460
(S)-HPMPAp	1200	1070
(S)-HPMPApp	160	650

• picomoles/T-75 flask
• results for [8-¹⁴C]-(S)-HPMPA are pending

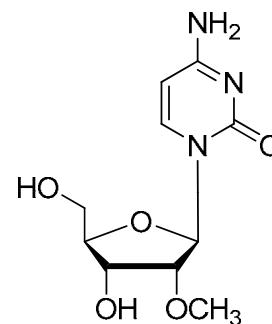
(S)-HPMPA vs Other HCV Leads



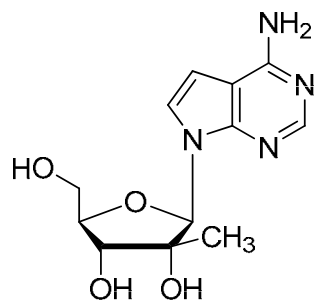
2'-C-methyladenosine



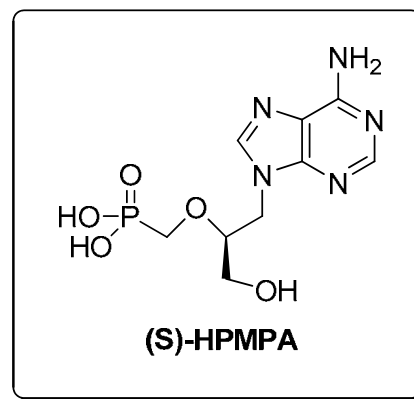
2'-C-methylcytidine



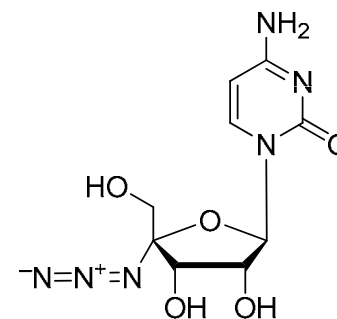
2'-O-methylcytidine



7-deaza-2'-C-methyladenosine



(S)-HPMPA

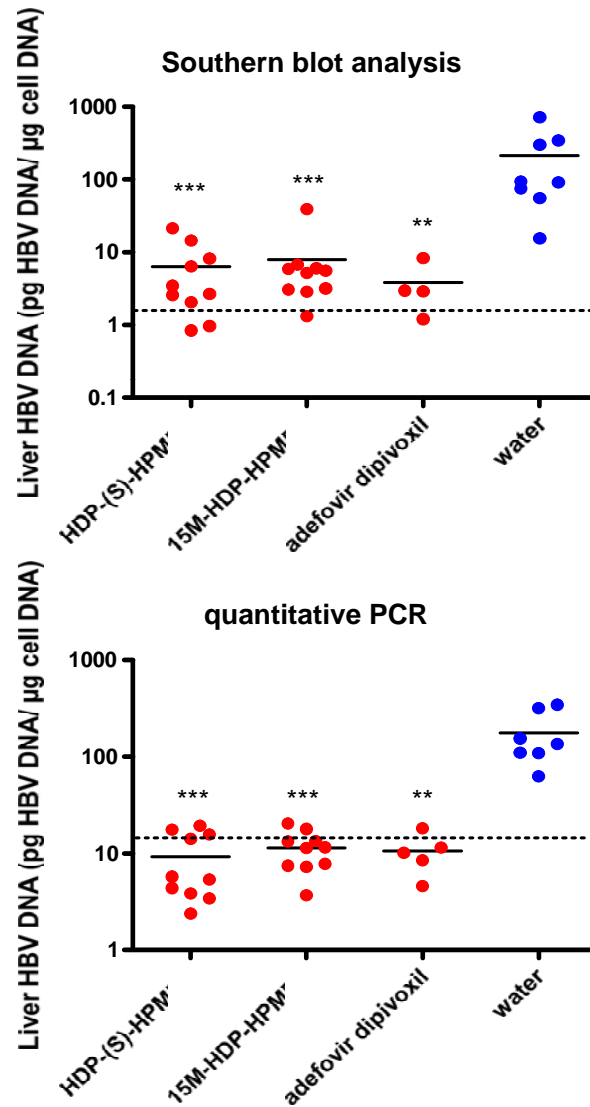


4'-azidocytidine

(S)-HPMPA structure compared with other leads

- Phosphonate vs ribonucleosides & may bypass initial phosphorylation
- Other leads have substitutions at 2' or 4' carbon or the 2' hydroxyl of ribose
- (S)-HPMPA lacks the ribofuranose moiety and has an acyclic chain retaining only a small part of the ribofuranose motif
- Mechanism of action of (S)-HPMPA vs HCV is not known
- Studies with HPMPApp and NS5B polymerase are in process

Figure 1. Expt. NHA-75. Effect of oral 15M-HDP-HPMPA and HDP-(S)-HPMPA on liver HBV DNA in transgenic mice ---- 2 SD above normal. All doses were 4 mg/kg, per os, qd X 14 days. (**P < 0.01, ***P < 0.001 compared to water using Mann-Whitney non-parametric test)



ODE- and HDP-(S)-HPMPA: Spectrum of Antiviral Activities

Virus	EC ₅₀ , μM
HBV	0.11–0.25
HCV	0.68–2.0
HIV-1	0.007
HCMV	0.003
HSV-1	<0.01
Adenovirus	0.04–1.1
Cowpox	0.02
Ectromelia	0.06
Vaccinia	0.01
Variola	<0.05
Orf virus	0.00002
BK virus	0.015

Conclusions

- ODE-(S)-HPMPA and related compounds are active and selective against HCV in both type 1b and 2a replicons, in vitro
- Esterification of (S)-HPMPA with alkoxyalkyl groups greatly increases cellular uptake and antiviral activity against HCV.
- (S)-HPMPA is inactive in vitro because its uptake in liver cells and conversion to HPMPA diphosphate is minimal compared with that of the alkoxyalkyl esters of HPMPA
- HDP-(S)-HPMPA given orally is effective in lowering hepatitis B virus titers in liver in the transgenic mouse model
- ODE-(S)-HPMPA and HDP-(S)-HPMPA have broad spectrum activity against HCV, HBV, HIV, HSV, HCMV, adenovirus, orthopoxviruses, BK virus and ORF virus and are worthy of further evaluation for possible use in human viral diseases

Acknowledgements

- ▶ **NIH/NIAID Grants**

- AI-066499
- AI-064615
- AI-071803

- ▶ **Univ. Cal., San Diego**

- Nadejda Valiaeva
- Kathy Aldern
- Julissa Trahan

- ▶ **Utah State Univ.**

- John Morrey
- Don Smee

- ▶ **Georgetown Univ.**

- Brent Korba

- ▶ **St. Louis University**

- R. Mark Buller

- ▶ **Univ. Alabama**

- Mark Prichard
- Earl R. Kern
- Debra Quenelle