Novel Chemical Strategies for Making Orally Active Antiviral Drugs

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Optimizing Antiviral “Hits”

- Poor or inadequate oral bioavailability is a recurring issue in the design and development of drugs
- The literature abounds with promising leads that failed because of poor oral activity in man
- We have developed a general approach for converting poorly bioavailable drugs to orally active compounds

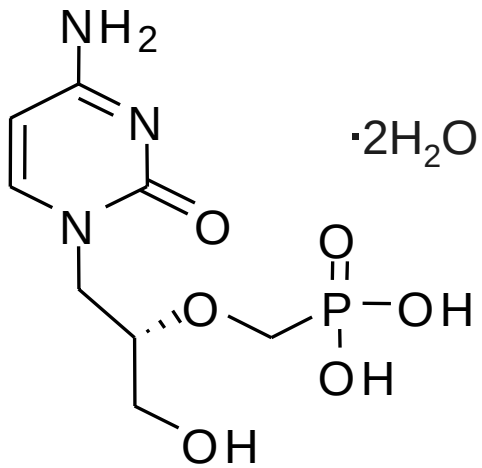
Why Drugs for Smallpox?

- Vaccination is the standard way to prevent death from smallpox but many persons are not good candidates for the live vaccine
  - AIDS, immunosuppression
  - Skin diseases, eczema, atopic dermatitis
  - Cancer chemotherapy
  - Pregnancy
- IL-4 modified poxviruses bypass established immunity

Converting an IV Drug for Smallpox to an Oral Form

- Cidofovir (CDV) is active IV in lethal poxvirus infections in various animal models but too toxic to develop for Bioshield Repository
- Asked by NIAID/Army to synthesize an oral version of CDV based on our prior success with foscarnet and acyclovir/ganciclovir monophosphates

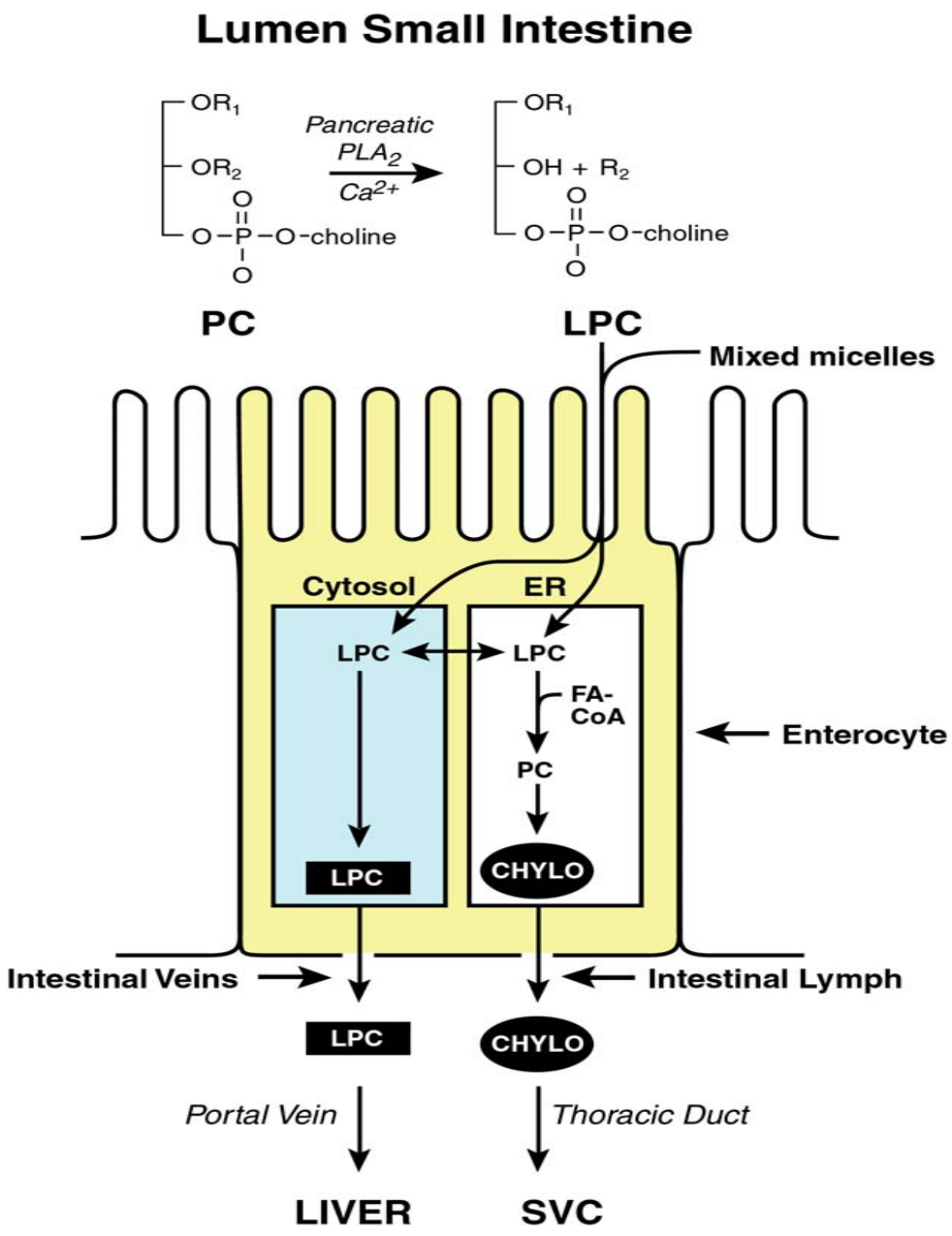
Cidofovir (Vistide®)



- Nucleotide analog discovered and evaluated at the Institute of Organic Chemistry and Biochemistry in Prague and the Rega Institute in Belgium.
- Developed by Gilead and FDA approved for intravenous treatment of cytomegalovirus retinitis in patients with AIDS
- Requires intravenous infusion. Causes kidney damage due to concentration in the proximal tubule by an organic anion transporter, limiting the human dose to 5 mg/kg iv once a week.

Strategy for Making Cidofovir Orally Active

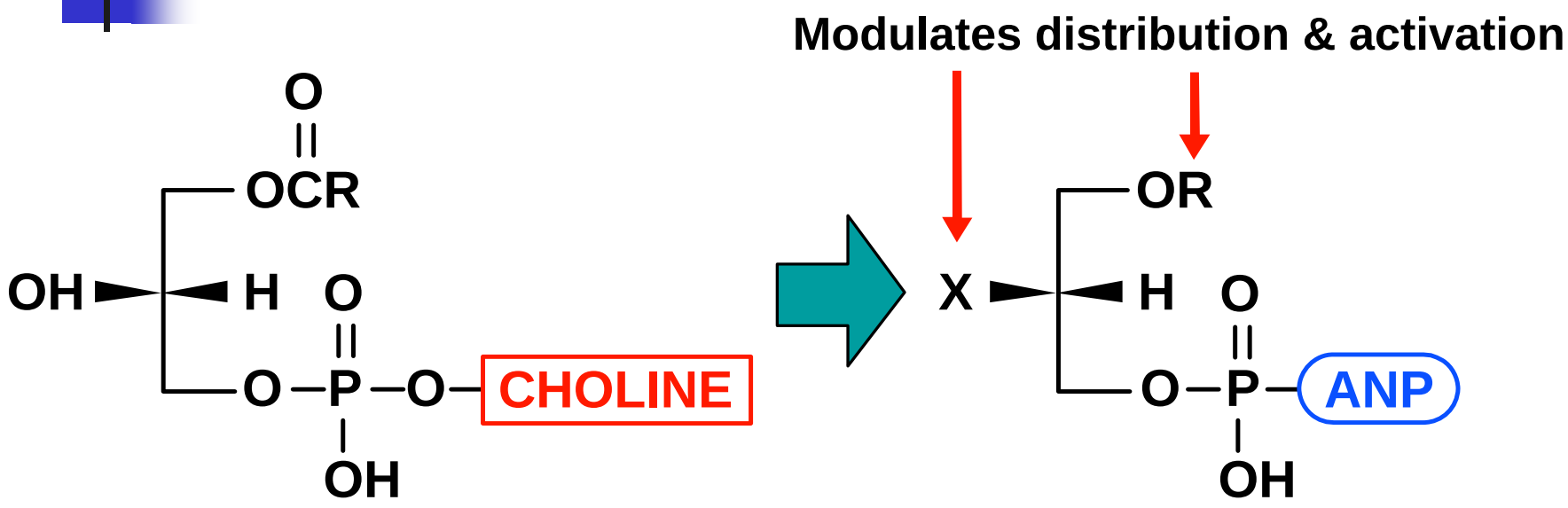
- Two negative charges on cidofovir's phosphonate impair its absorption from GI tract
- Oral bioavailability <6%.
- Lysophosphatidylcholine (LPC), is known to be highly orally absorbed intact (Scow et al and Borgstrom & coworkers publications in 1967-68)


Lumen Small Intestine
OR1
OR2
O
O-P-O-choline
O
Pancreatic
PLA2
Ca2+
OR1
OH + R2
O
O-P-O-choline
O
PC
LPC
Mixed micelles
Cytosol
ER
LPC
LPC
FA-
CoA
PC
LPC
CHYLO
Enterocyte
Intestinal Veins
Intestinal Lymph
LPC
CHYLO
Portal Vein
Thoracic Duct
LIVER
SVC

Strategy for Making Cidofovir Orally Active

- Our approach was to disguise CDV as LPC to take advantage of natural pathways of small intestine lipid absorption.
- The choline residue was replaced with CDV (or the drug of interest)
- Hexadecyloxypropyl-CDV survives absorption and circulates intact, loosely bound to albumin; since it has only one negative charge HDP-CDV is not taken up avidly by the kidney OAT

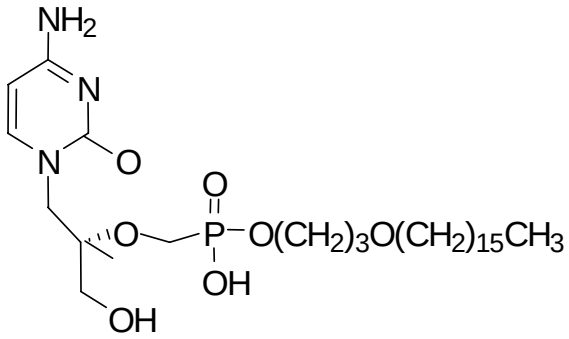
Drug Design Paradigm:
Acyclic Nucleoside Phosphonates



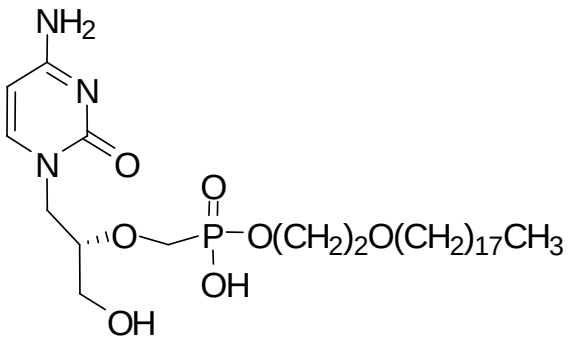
Lysophosphatidylcholin e
(a natural lipid metabolite)

Phospholipase C cleavage
inside cells releases ANP

Lead Compounds for Poxvirus Infection



Hexadecyloxypropyl-CDV



Octadecyloxyethyl-CDV

Activity of HDP-CDV Against Poxviruses and Herpes Viruses *

Virus	EC_{50} Value (μM) [a] HDP-CDV	CDV	Fold Change
Cowpox-Brighton	0.60	44.7	75
Vaccinia-Copenhagen	0.80	46.2	58
Vaccinia-WR	1.10	45.8	42
Vaccinia-Elstree	1.20	41.6	35
HCMV-AD169	0.0009	0.38	422
HCMV-Towne	0.0009	0.40	444
HSV-1	0.06	15.2	253
HSV-2	0.08	10.5	131

[a] EC_{50} values were determined by plaque reduction assays in HFF cells.

**Kern et al., Antimicrob. Agents Chemother. 46: 991-995 (2002) & Beadle et al., Antimicrob. Agents Chemother. 46: 2381-2386 (2002)*

Effect of Lipid Analogs of Cidofovir on Poxvirus Replication

	Variola*		Monkeypox*		Cowpox	
Drug_	V76	MK2	V76	MK2	V76	MK2
CDV	27.3	10.2	4.6	4.3	36.5	7.0
HDP-CDV	0.10	0.04	0.07	0.013	0.10	0.008
ODE-CDV	0.03	0.01	0.006	0.006	0.04	0.009

Data are EC_{50}, μM, in Vero 76 and MK2 cells

** - John Huggins et al, Antiviral Research, 53:A66(104), 2002.*

Antiviral Activity of CDV Analogs vs WT and IL-4 + Ectromelia

	EC_{50}, (μM)	
Compound	ECTV wt	ECTV IL-4 7.5E
CDV	12.0	12.0
HDP-CDV	0.5	0.2
ODE-CDV	0.2	0.2

Plaque reduction assay in CV-1 cells; ECTV, ectromelia virus

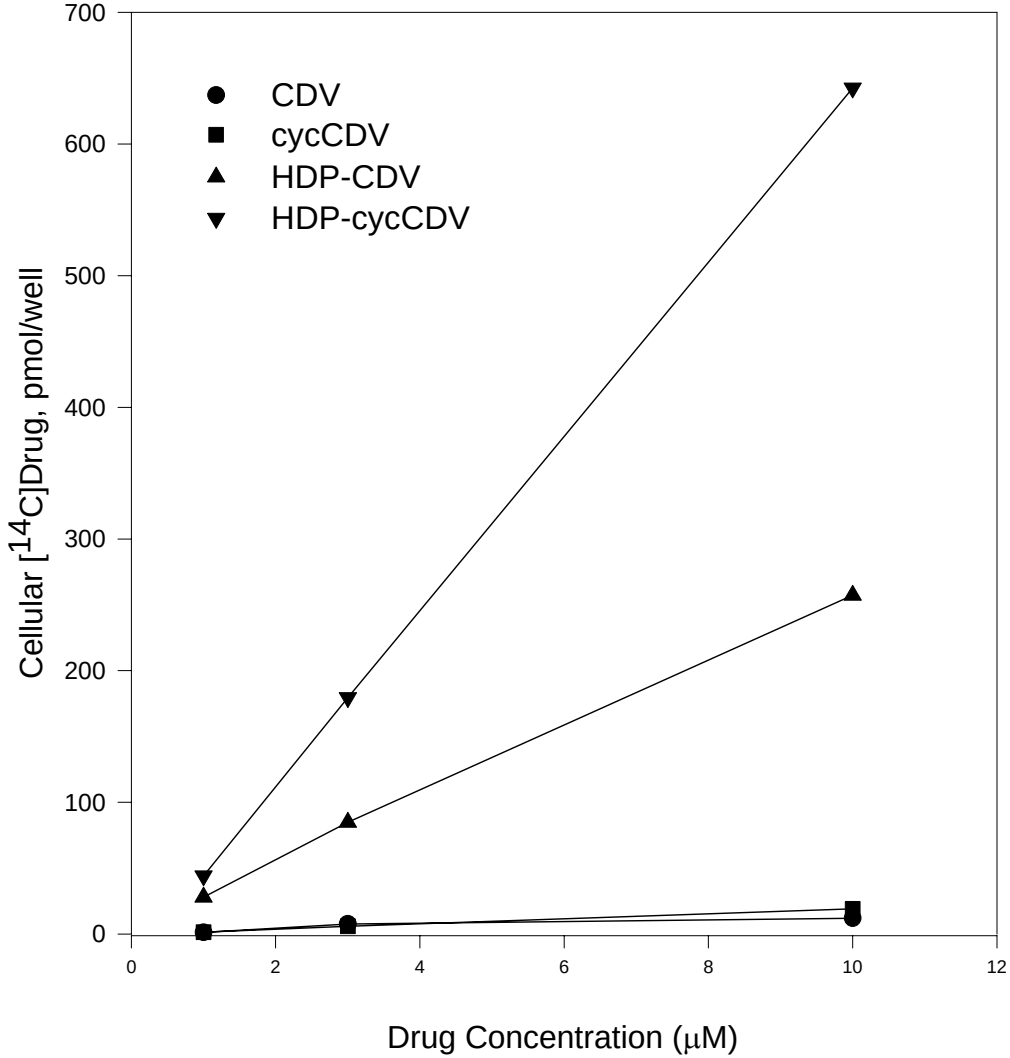
**Buller et al, Virology, 318:474-81, 2004.*

Why is the Antiviral Activity of HDP-CDV 100x greater?

Uptake and Metabolism of

HDP-[2-^{14}C]CDV

Cellular uptake of drugs in MRC-5 human lung fibroblasts with an incubation time of four hours



Intracellular Metabolites After Exposure to HDP-[2-^{14}C]CDV

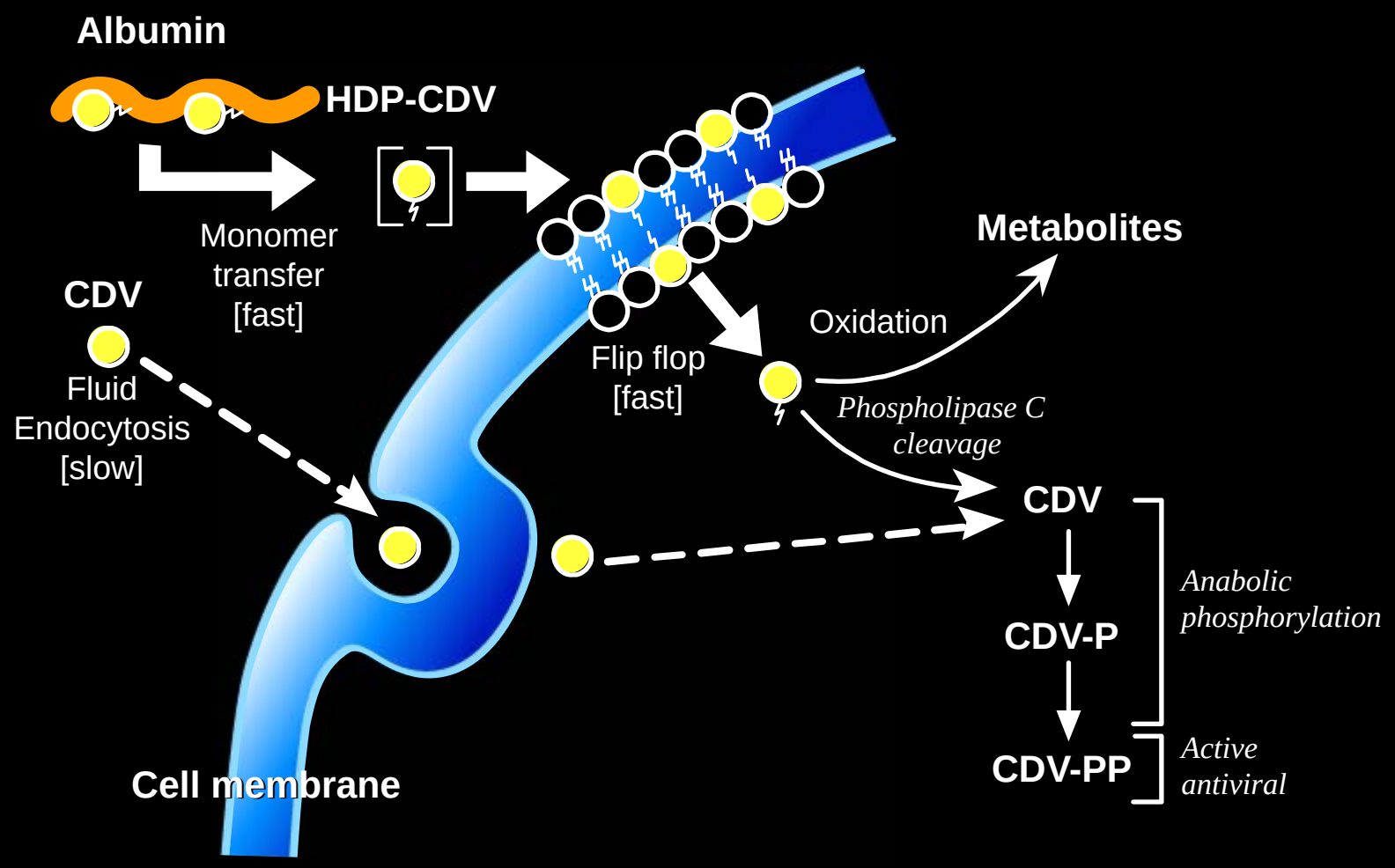
Metabolite	CDV*	HDP-CDV*	
CDV	129	702	
CDVp	1.2	71	
CDVpp	1.8	184	*100x*

*- Picomoles per well in MRC-5 human lung fibroblasts after 48 hrs exposure to ^{14}C-HDP-CDV

Abbreviations: CDVp, CDV monophosphate; CDVpp, CDV diphosphate

Aldern et al, Mol. Pharmacol. 63:678-681, 2003

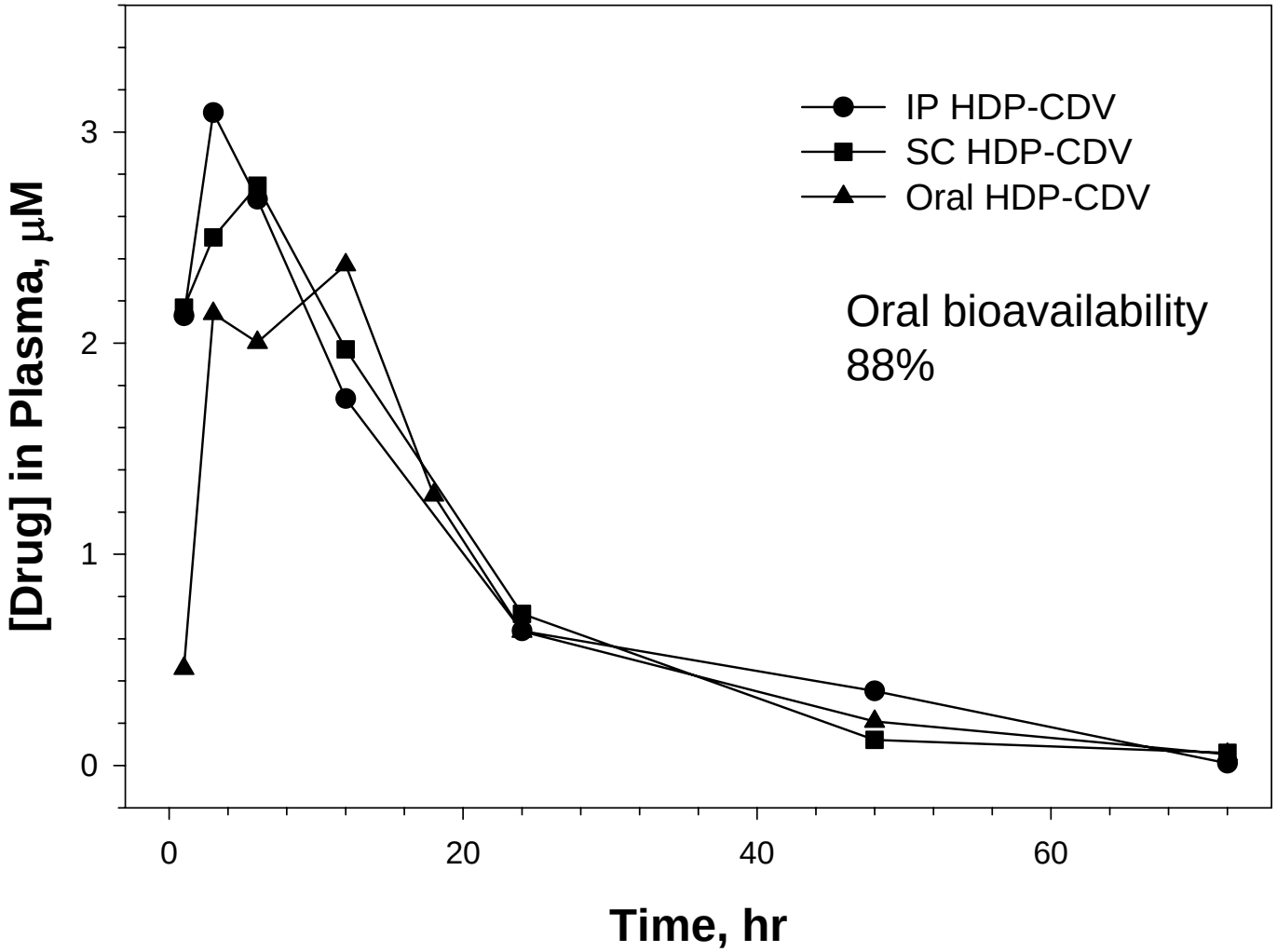
Mechanism of Cellular Uptake and Activation of 1-0-Hexadecyloxypropyl-Cidofovir and Cidofovir



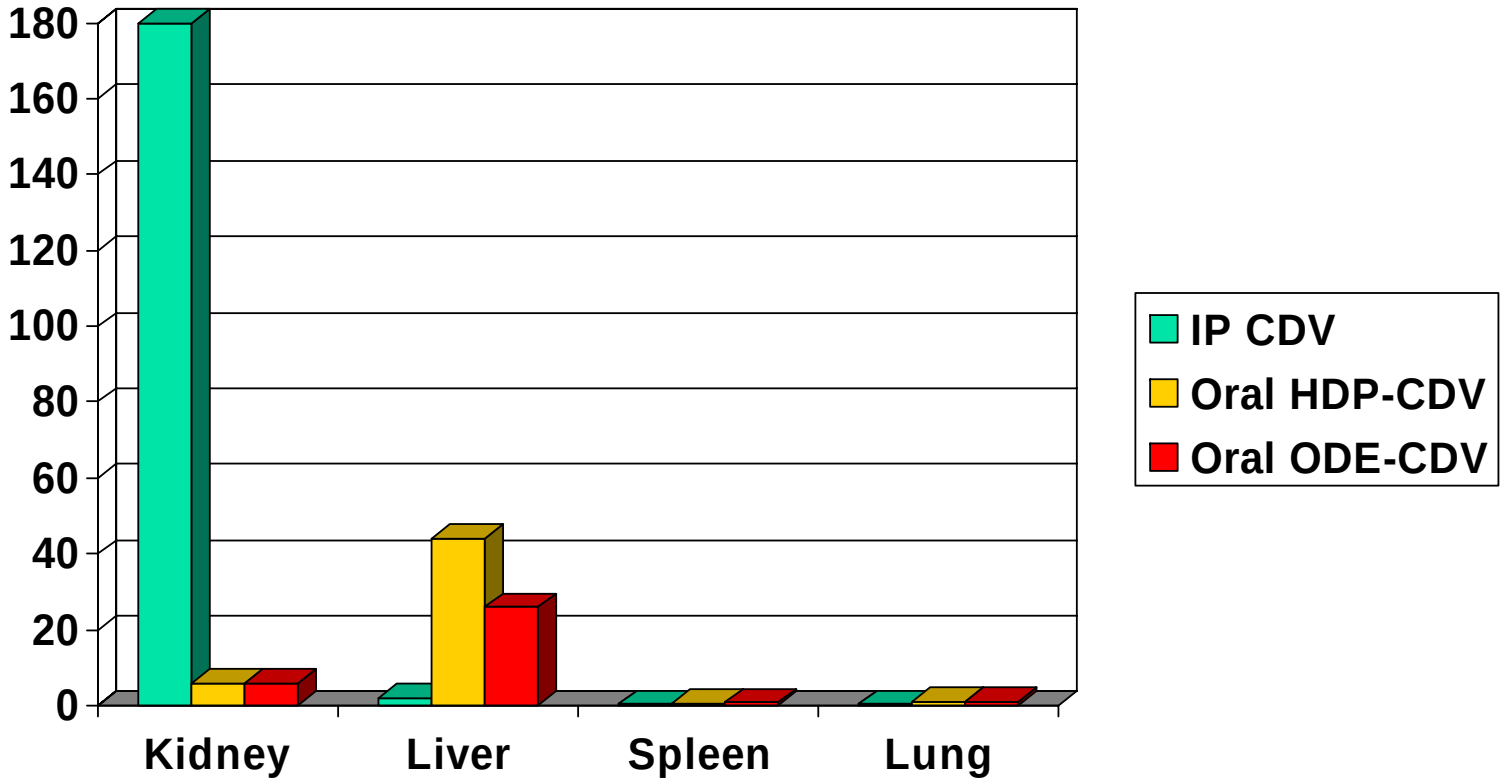
Oral Pharmacokinetics of HDP-[2-^{14}C]CDV in Mice

Relative oral bioavailability and levels of drug & metabolites in key tissues over time

Plasma Drug Levels following Intraperitoneal (IP), Subcutaneous (SC) and Oral Administration of ^{14}C HDP-CDV to Mice

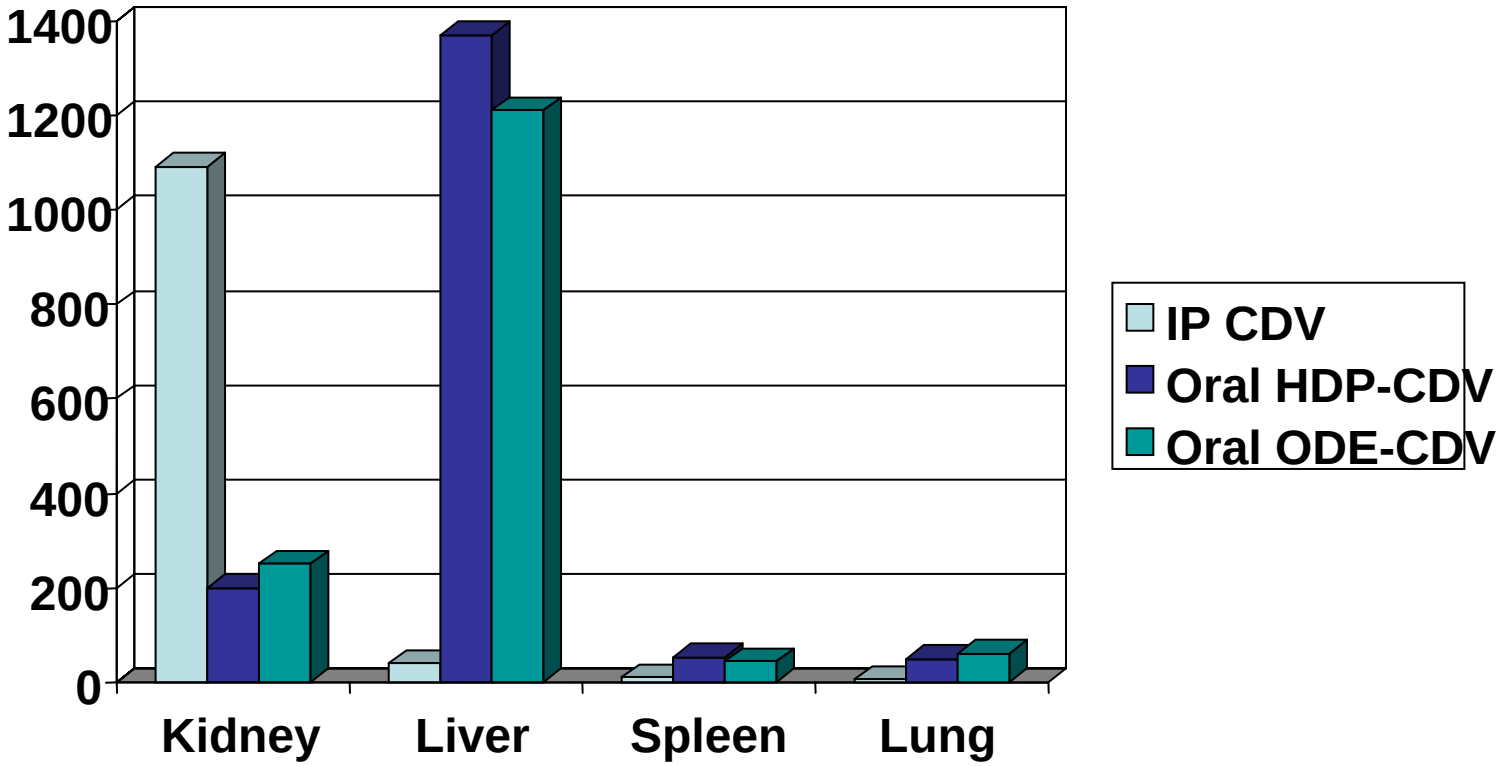


Tissue C_{max}: Intraperitoneal CDV vs Oral HDP-CDV and ODE-CDV in Mice



Data in nmol/gm

Tissue AUC: Intraperitoneal CDV versus Oral HDP-CDV and ODE-CDV

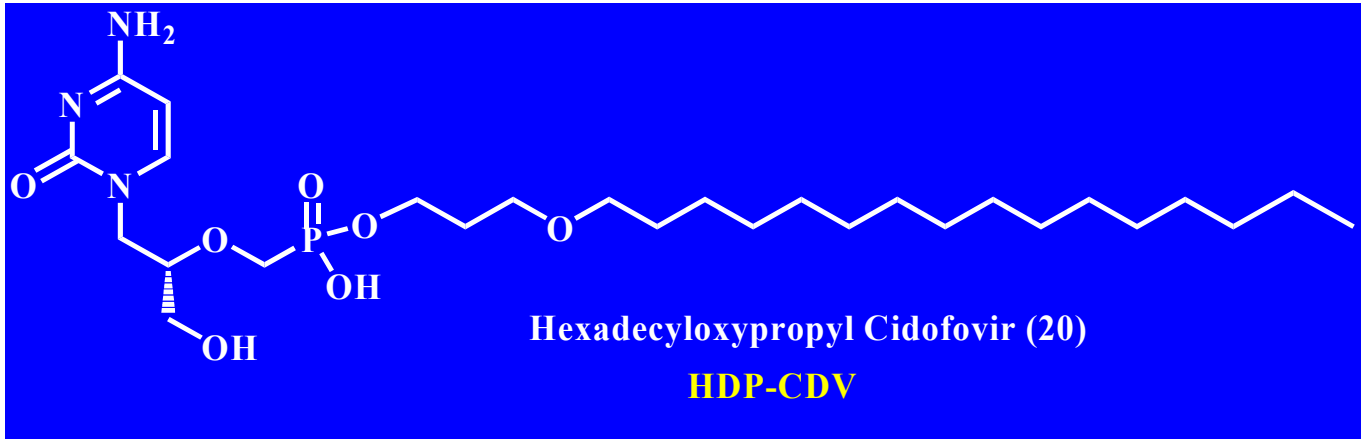


Data in nmol.hrs/gm

Structure-Activity

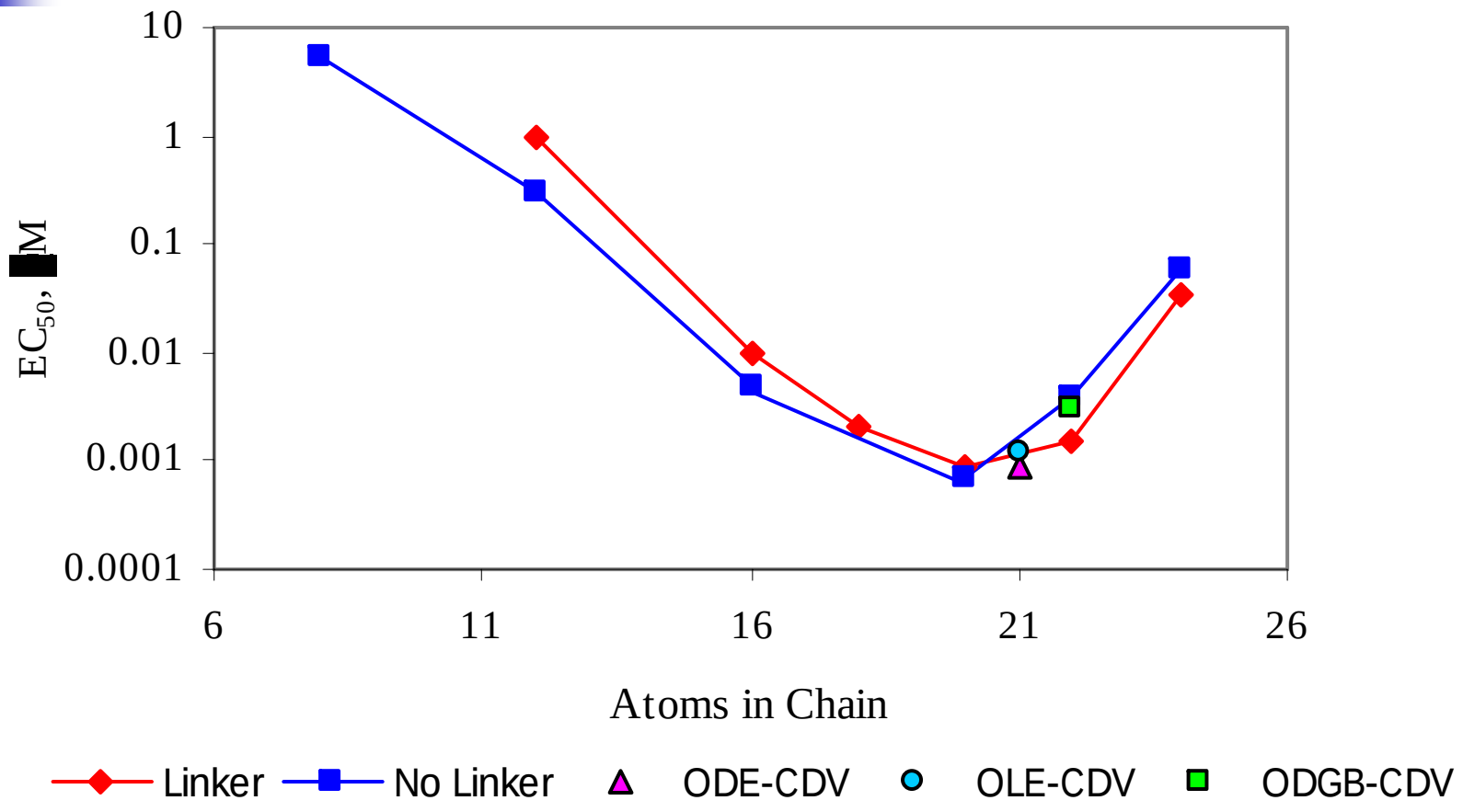
Varying the Linker and the Number of Atoms
and Double bonds in the Lipid Ester

Effect of Chain Length and Structure on Antiviral Activity



- Overall chain length
- Unsaturation in the alkyl chain
- With and without oxygen heteroatom
- Length of Linker (ethyl vs propyl)
- Substitution with glycerol linker

Effect of Chain Length and Linker on anti-HCMV activity, in vitro*



Wan et al, AAC, 49:656,2005/similar results vs pox, Keith et al, AAC, 48:1869-71, 2004.

In Vivo Antiviral Activity of HDP-CDV and ODE-CDV

Activity in Lethal Animal Models of Poxvirus Infection

Effect of Oral HDP-CDV on Lethal Cowpox Infection in Mice*

Drug	Dose	Survival	MDD
HDP-CDV	10 mg/kg po	10/10	-
HDP-CDV	5 mg/kg po	10/10	-
HDP-CDV	2.5 mg/kg po	7/10	10.5
Oral CDV	20 mg/kg po	1/10	10.1
Oral CDV	10 mg/kg po	1/10	11.2
IP CDV	100 mg/kg ip	10/10	-
Placebo	-	1/10	8.2

Treatment started 2 hrs after infection with 5 x 10^7 pfu of intranasal cowpox virus; MDD, mean day of death

** - John Huggins et al, USAMRIID w/ permission*

Effect of Oral CDV-Analogs on Aerosol Ectromelia Virus Infection *

Drug	Survival	MTD	PFU in Liver	PFU in Lung
CDV *(10 mg/kg/d x5)*	2/11	9.3d	3×10^5	1×10^4
HDP-CDV *(10 mg/kg/d x5)*	11/11	-	$< 10^2$	4×10^3
ODE-CDV *(5 mg/kg/d x5)*	11/11	-	$< 10^2$	2×10^3
Control	0/11	7.3d	2×10^7	1×10^6

**Buller et al, Virology, 318:474-81, 2004.*

Single-Dose Oral Treatment of Cowpox Infected Mice*

Treatment	Mortality			
12.5 mg/kg	Number	%	P-Value	MDD
Vehicle	16/30	53	---	12.1
Day -5 HDP-CDV	3/15	20	<0.05	10.3
Day -3 HDP-CDV	0/15	0	<0.001	---
Day -1 HDP-CDV	0/15	0	<0.001	---
Day +1 HDP-CDV	0/15	0	<0.001	---
Day +3 HDP-CDV	2/15	13	<0.05	11.5

**Quenelle et al, Antimicrobial Agents & Chemotherapy, 48:404-412, 2004*

Approach is Generally Applicable

HIV

HBV

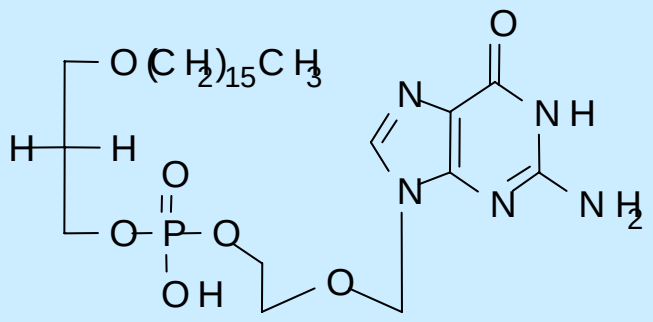
HCV

Anti-HIV Compounds*

Compound	EC_{50} (μM)	Fold Δ	CC_{50} (μM)	S.I.
(S)-HPMPA	77.5	-	>100	>1.29
HDP-(S)-HPMPA	0.007	11,000	1.0	143
PMEA	1.1	-	157	142
HDP-PMEA	1.5×10^{-5}	73,000	0.06	4,000
Tenofovir	5.9	-	>300*	>51
HDP-Tenofovir	$<1 \times 10^{-5}$	>590,000	54	>5.4Mil
PMEDAP	0.08	-	5.3	66.3
HDP-PMEDAP	1.6×10^{-5}	5,000	0.02	1250
PME-N^6-cPr-DAP	0.07		4.2	60
HDP-N^6-cPr-DAP	$<1 \times 10^{-5}$	>7,000	0.12	>12,000
PME-5FC	>100	-	>100	-
HDP-PME-5FC	0.14	>710	24	174

*Valiaeva et al, Antiviral Res. 72:10-9, 2006

Modification of ACV-Phosphate for Hepatitis B Virus Infection

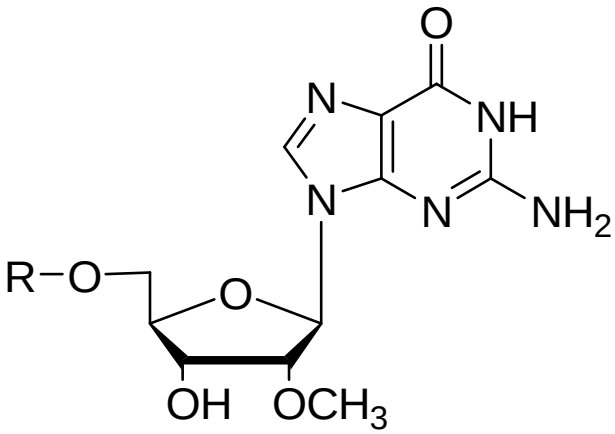


HDP-P-ACV

- Good anti-Hepatitis B activity in cell culture
- Orally bioavailable (>90%)
- Favorable distribution to the liver
- Good oral activity in woodchuck model of HBV infection
- ACV inactive at max tolerated dose

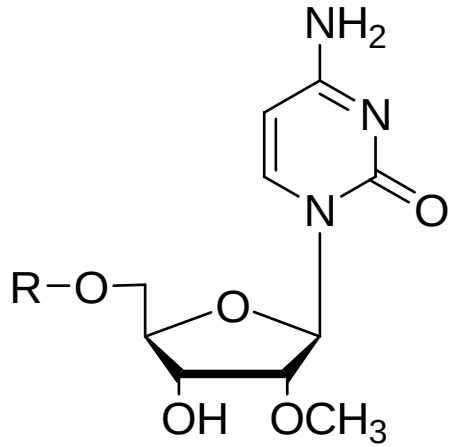
Hostetler, Korba et al, Antimicrob Agents Chemother, 44:1964-1969, 2000.

Anti-HCV activity of hexadecyloxypropyl-5´-phospho-2´-O-methyl-guanosine



R =	EC_{50} (μM)
-H	29.0
$-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{\|}}{P}}-O-(CH_2)_3O(CH_2)_{15}CH_3$	2.5

Anti-HCV Activity of 2´-O-methyl-cytosine and its 5´-hexadecyloxy-propyl-phosphate ester



R =	EC_{50} (μM)
-H	19.0
$-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{\|}}{P}}-O-(CH_2)_3O(CH_2)_{15}CH_3$	1.8

Requirements: Making Drugs Orally Active

- Applicable to nucleosides, nucleotides, nucleoside phosphonates and other small molecules having M.W. <600-1,000 (?)
- Should be hydrophilic in nature
- Have an –OH, -SH, -COOH or -NH_2 linker
- Linked to alkoxyalkyl (phosphate) ester having 20-22 atoms in side chain
- Approaches are covered by U.S. Patents #5,411,947 and #6,716,825 various pending applications

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  - Nadejda Valiaeva
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  - Julissa Trahan
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  - Kathy Keith
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