

Discovery of LdT (Telbivudine) as a New Potent Nucleoside Analogue for the Treatment of Chronic Hepatitis B

- ❖ GENERAL CONSIDERATIONS
- ❖ LdT REVISITED
- ❖ CURRENT STATUS of LdT and FUTURE PROSPECTS
- ❖ CONCLUSION

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Université Montpellier II (UM II)

Bridging the Sciences
HIV, HBV, HCV and Emerging Viruses
Paris, France
May 31 – June 2, 2006

GENERAL CONSIDERATIONS

- ❖ **Hepatitis B Virus (HBV) Infection :**
 - ★ World-wide significance
 - ★ A blood-borne disease
 - ★ Major therapeutic treatments
- ❖ **Currently approved anti-HBV nucleos_T^Side analogues**
 - ★ Lamivudine
 - ★ Adefovir dipivoxil
 - ★ Entecavir

and their mode of action
- ❖ **The Montpellier**
«Laboratoire de Chimie Organique Biomoléculaire de Synthèse»

HEPATITIS B VIRUS (HBV) INFECTION

- **World-wide significance :**

- Over 2 billion people infected worldwide
- Around 350 million are chronic carriers
- At least 1 million chronically infected individuals die each year

- **A typically blood-borne disease :**

- Viral loads as high as 10^6 to 10^{11} virions/ml of blood in chronic carriers
- Minimum required volume of blood to transmit HBV infection = 0.00004 ml
- Risk of infection following needlestick injury with positive HBV patient = 7-30%

- **Prevention of HBV infection :**

Safe and effective anti-HBV vaccines are available

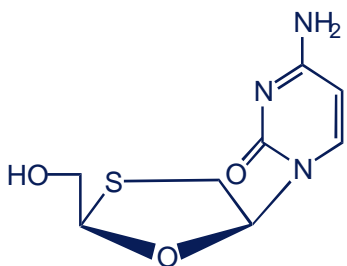
However

Several million people are still being newly infected annually

- **Current major therapies :**

**Only Interferon α -2b,
two nucleoside analogues (Lamivudine and Entecavir)
and one nucleotide analogue (Adefovir Dipivoxil)
have been approved by the FDA
for the treatment of chronic HBV infection**

CURRENTLY APPROVED ANTI-HBV NUCLEO^S_TIDE ANALOGUES



LAMIVUDINE

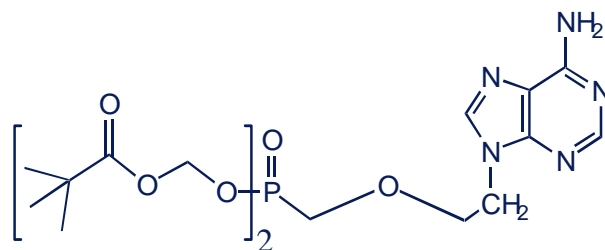
[EpiVir, 3TC]

(Glaxo Wellcome PLC and
Biochem Pharma Inc.)

First Approvals : 1995 HIV; 1998 HBV



**L-enantiomer
dideoxycytidine analogue**



ADEFOVIR DIPIVOXYL

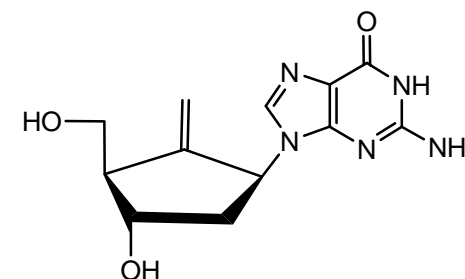
[Hepsera, Bis(POM) PMEA]

(Gilead)

First Approval : September 2002 HBV



**Acyclic phosphonate
dideoxyadenosine analogue
(Prodrug)**



ENTECAVIR

[Baraclude]

(Bristol-Myers Squibb)

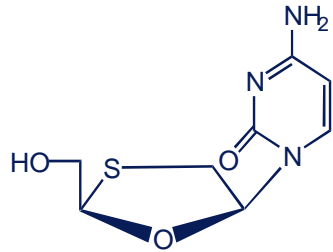
First Approval : March 2005 HBV



**D-enantiomer carbocyclic
2'-deoxyguanosine
analogue**

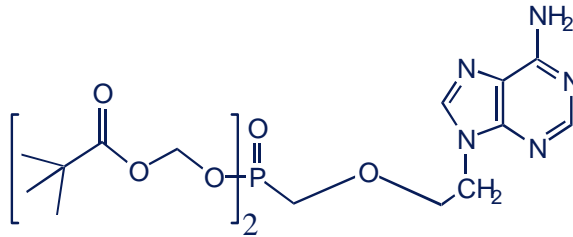
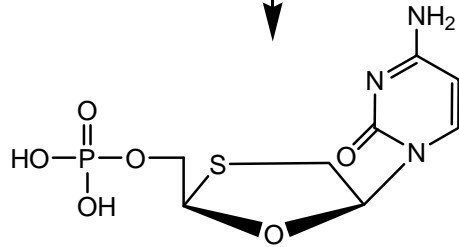
**All are orally-bioavailable drugs, which are converted
intracellularly into their active triphosphate form**

MODE OF ACTION OF ANTI-HBV NUCLEO^S_TIDE ANALOGUES



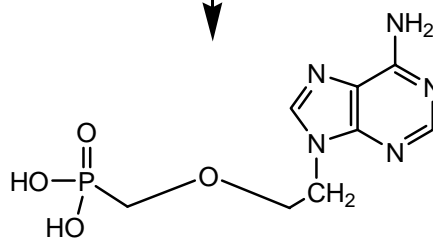
LAMIVUDINE

*Kinase intracellular
monophosphorylation*

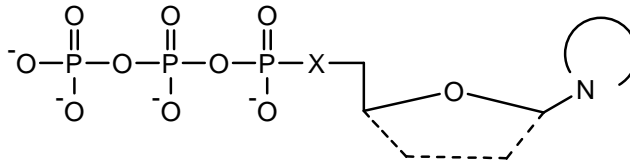


ADEFOVIR DIPIVOXYL

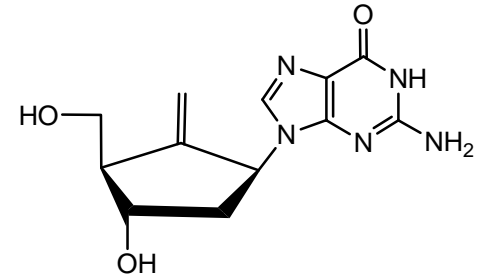
Esterases



Intracellular di- and tri-phosphorylations

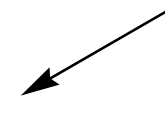
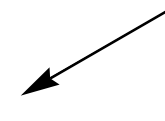
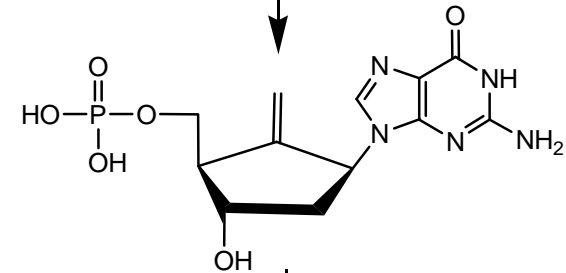


NUCLEOSIDE ANALOGUE TRIPHOSPHATE
[active form]

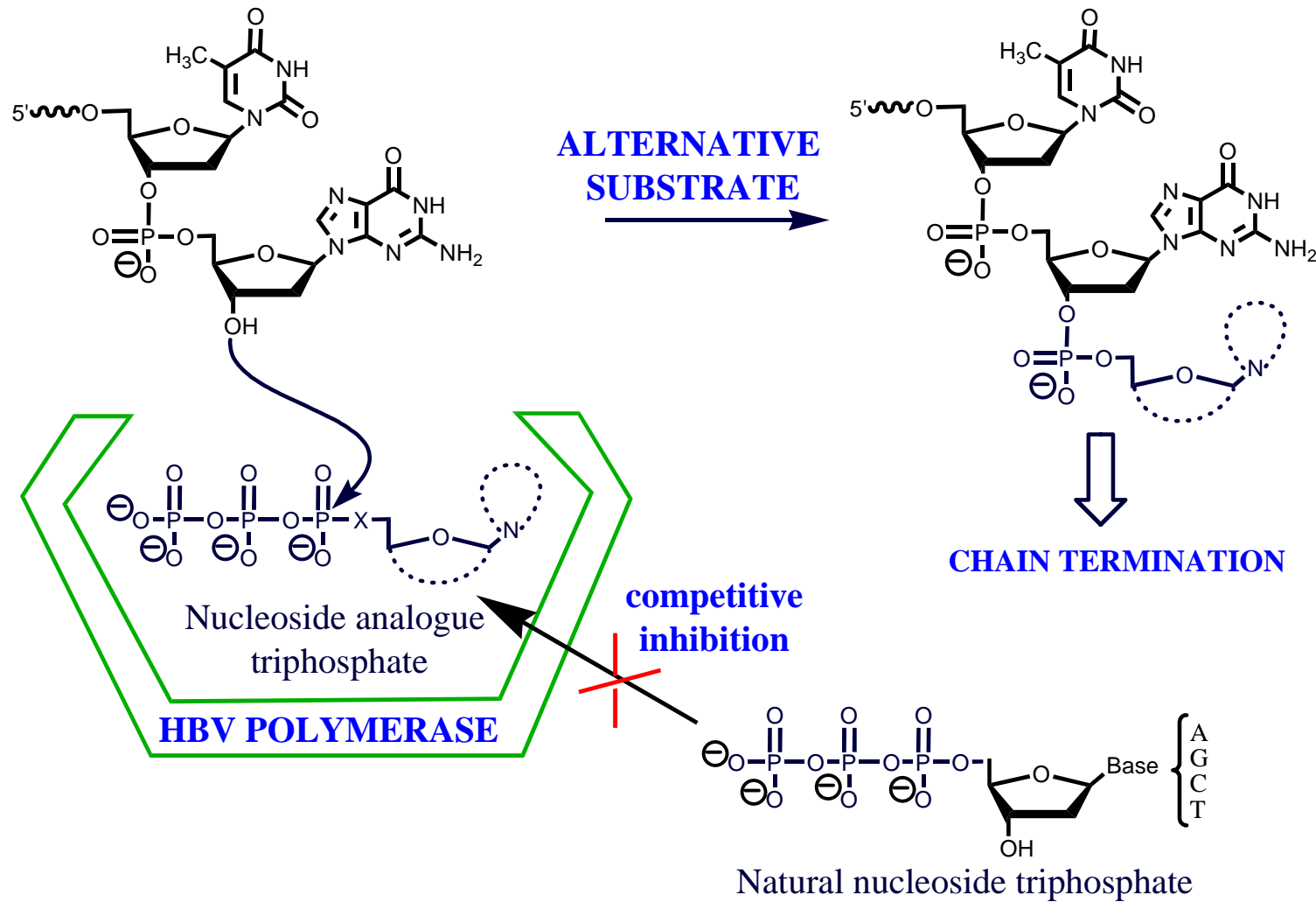


ENTECAVIR

*Kinase intracellular
monophosphorylation*



MODE OF ACTION OF ANTI-HBV NUCLEO^S_TIDE ANALOGUES



LABORATOIRE DE CHIMIE ORGANIQUE BIOMOLECULAIRE DE SYNTHÈSE



CNRS - Unité Mixte de Recherche 5625 - Université Montpellier II

Site web : <http://lcobs.univ-montp2.fr>

Director: Dr. Gilles GOSSELIN

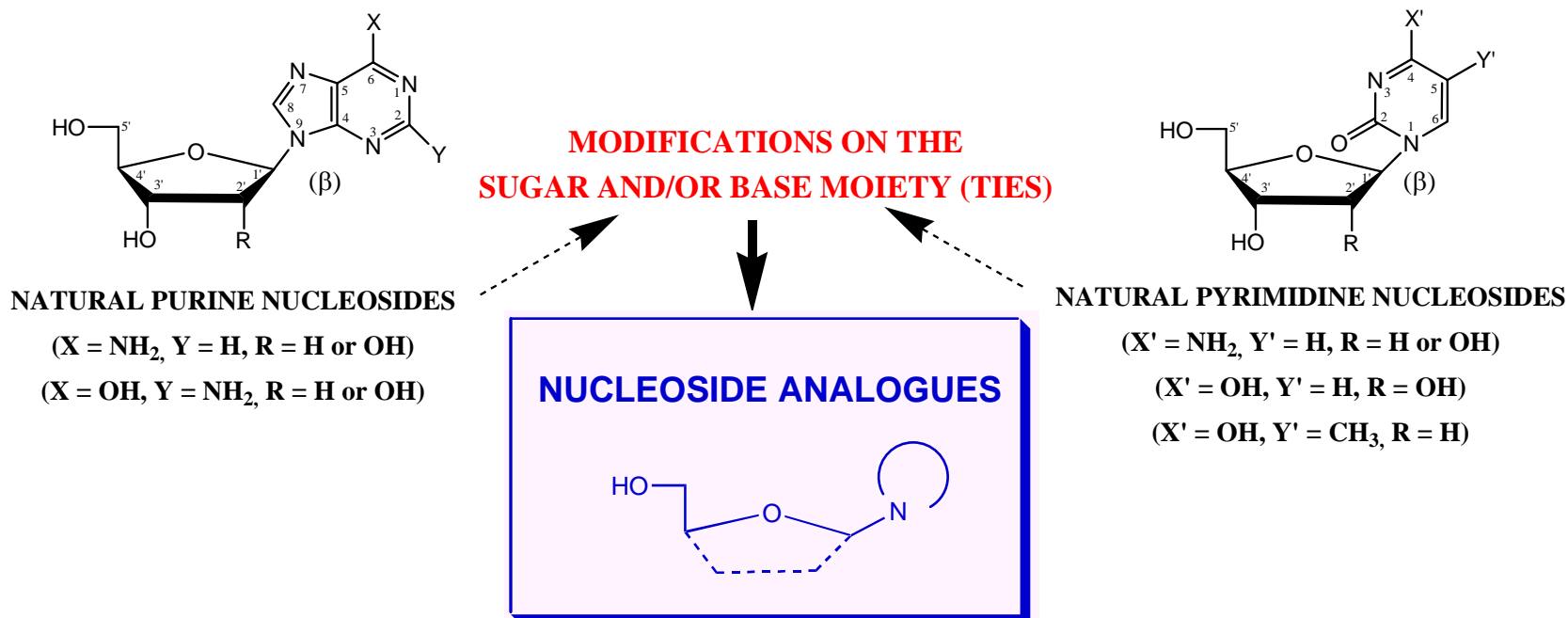


since its establishment

(in 1971 by Professor Jean-Louis Imbach)



Synthesis and Study of Nucleoside Analogues as Potential Antiviral and/or Antitumor Agents



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CNRS - Unité Mixte de Recherche 5625 - Université Montpellier II

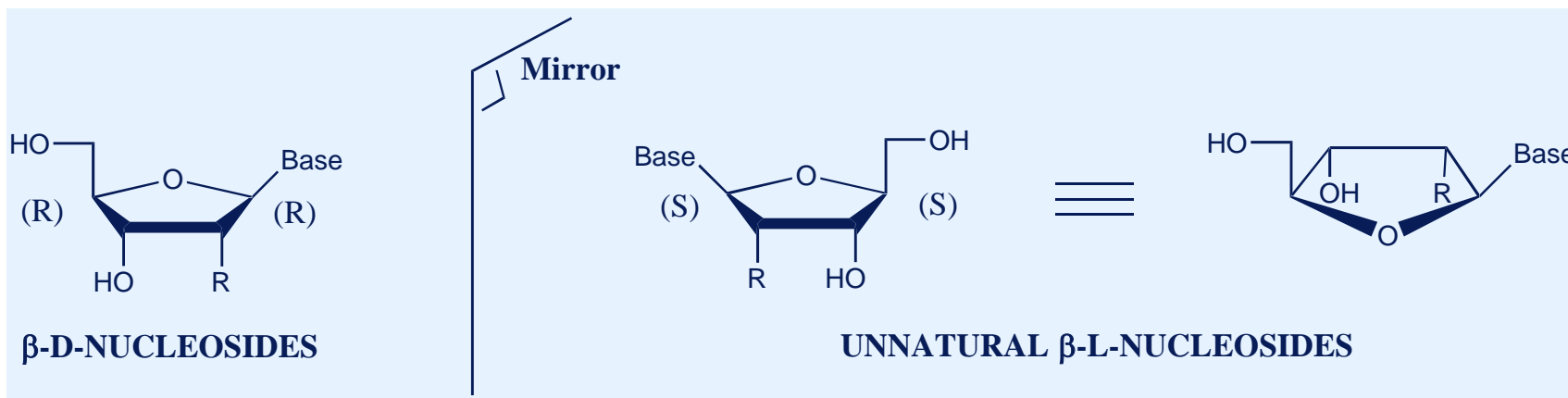
Site web : <http://lcobs.univ-montp2.fr>



starting in the 1990's



Synthesis and Biological Evaluation of Numerous New Nucleoside Analogues Endowed with the β -L-configuration



LABORATOIRE DE CHIMIE ORGANIQUE BIOMOLECULAIRE DE SYNTHÈSE

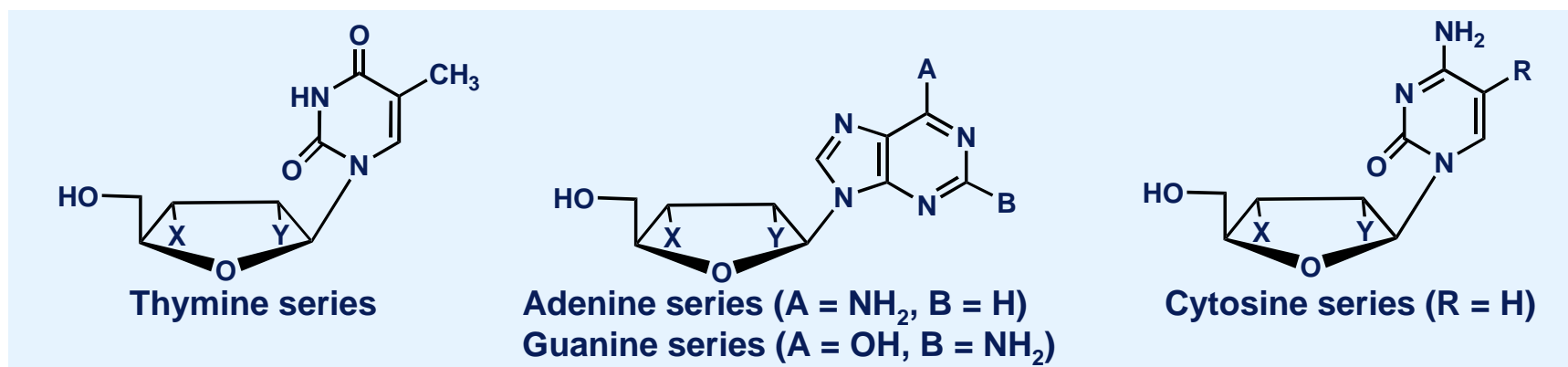


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Examples of β -L-Nucleoside Analogues (X, Y = H, OH, N₃, F, double bond, ...):



Structure-Activity Relationships



Discovery that nucleosides from the β -L-2'-deoxyribofuranosyl series (X = OH; Y = H) are endowed with a unique specificity for anti-HBV activity

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**Discovery that nucleosides
from the β -L-2'-deoxyribofuranosyl series
are endowed with a unique specificity for anti-HBV activity**



- **File on August 10, 1998 a provisional patent application (US 60/096, 110) entitled « β -L-2'-Deoxy-Nucleosides for the Treatment of Hepatitis B Virus » which was issued as WO 00/009531 on Feb 24, 2000 with G. Gosselin, J.-L. Imbach and M.L. Bryant as inventors, and assigned to CNRS (France) and Novirio (currently Idenix).**
- **Establishment in January 1999 of the *Laboratoire Coopératif Idenix (formerly Novirio) – CNRS – Université Montpellier II* within the *Laboratoire de Chimie Organique Biomoléculaire de Synthèse*.**

GENERAL CONSIDERATIONS

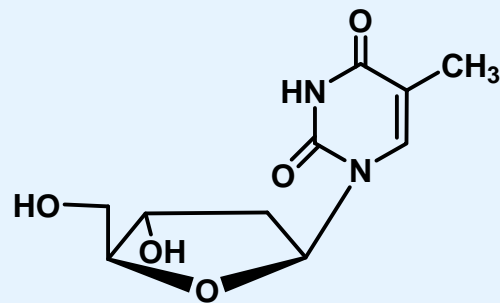
SUMMARY

more than 8 years ago :

Discovery that nucleosides from the β -L-2'-deoxyribofuranosyl series are endowed with a unique specificity for anti-HBV activity

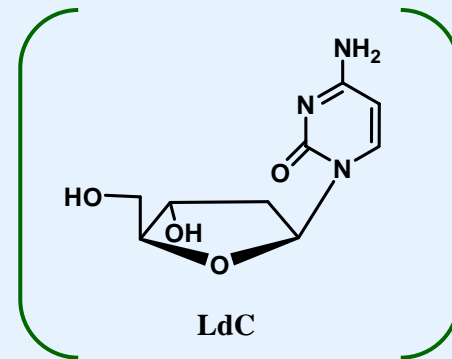
FURTHERMORE

among these β -L-2'-deoxy ribofuranonucleosides



LdT

and



LdC

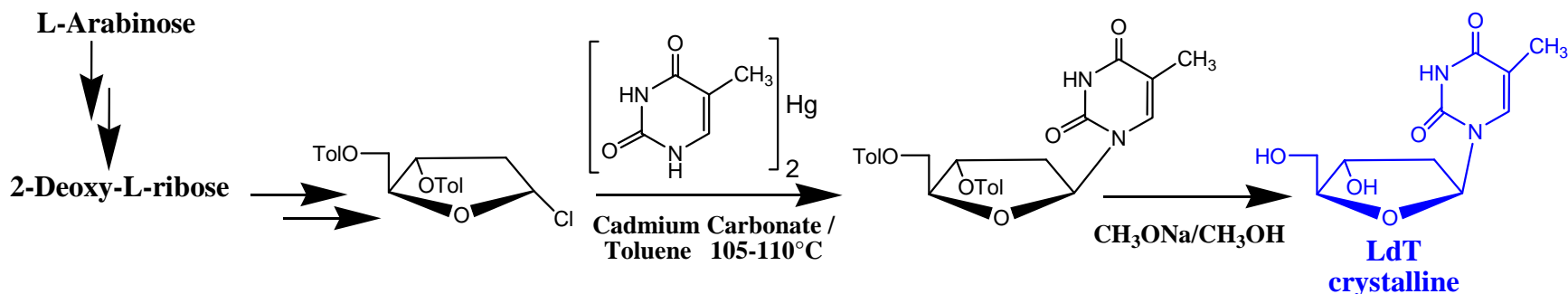
have the most potent, selective and specific activity against HBV replication

LdT (telbivudine) REVISITED

- ❖ **LdT : previously reported data**
- ❖ **First Montpellier implemented synthetic approach**
- ❖ **Subsequent semi-large scale synthesis**
- ❖ **Assessment of specific anti-HBV activity and safety profiles**
- ❖ **Intracellular activation (metabolism and pharmacology)**
- ❖ **Anti-HBV activity in animal models (woodchuck)**
- ❖ **Pharmacological studies (cynomologus monkeys)**

FIRST SYNTHESIS OF LdT :

[J. Smejkal and F. Sorm, *Collect. Czech. Chem. Commun.* 29, 2809 (1964)]



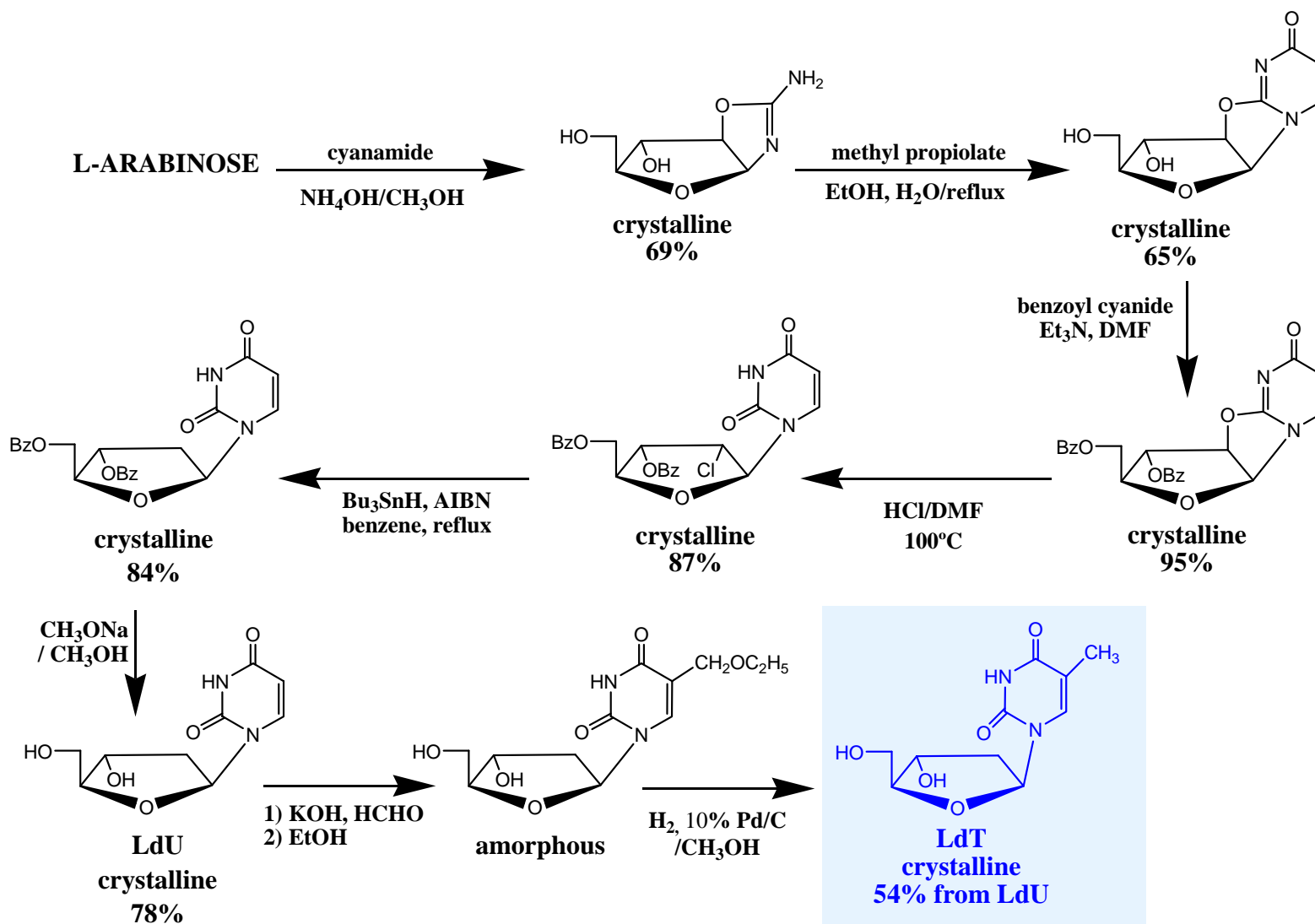
Other literature data on LdT

- **Synthesis** : A. Holy, *Collect. Czech. Chem. Commun.* 37, 4072 (1972); C.B. Reese and Y.S. Sanghvi, *J. Chem. Soc. Chem. Commun.* 877 (1983); S. Fujimori et al., *Nucleosides & Nucleotides* 11, 341 (1992)
- **Biochemical studies** : M. Jurovcik and A. Holy, *Nucleic Acids Res.* 3, 2143 (1976); A. Holy et al., *Biol. Chem. Hoppe-Seyler*, 366, 355 (1985); S. Spadari et al., *J. Med. Chem.* 35, 4214 (1992); G. Maga et al., *Biochem. J.* 294, 381 (1993) and 302, 279 (1994); A. Verri et al. *Biochem. J.* 328, 317 (1997); J. Wang et al., *Nucleosides & Nucleotides* 18, 807 (1999) and *Biochemistry* 51, 16993 (1999)
- **LdT 5'-triphosphate and polymerases** : T. Yamaguchi et al., *Nucleic Acids Symposium Series* 9, 135 (1993); T. Yamaguchi et al., *Biochem. Bioph. Res. Commun.* 200, 1023 (1994); F. Focher et al., *Nucleic Acids Res.* 23, 2840 (1995); D.G. Semizarov et al., *J. Biol. Chem.* 272, 9556 (1997); M. van Janta-Lipinski et al., *J. Med. Chem.* 41, 2040 (1998); T. Yamaguchi et al., *Biochem. Bioph. Res. Commun.* 279, 475 (2000)

FIRST MONTPELLIER IMPLEMENTED SYNTHETIC APPROACH

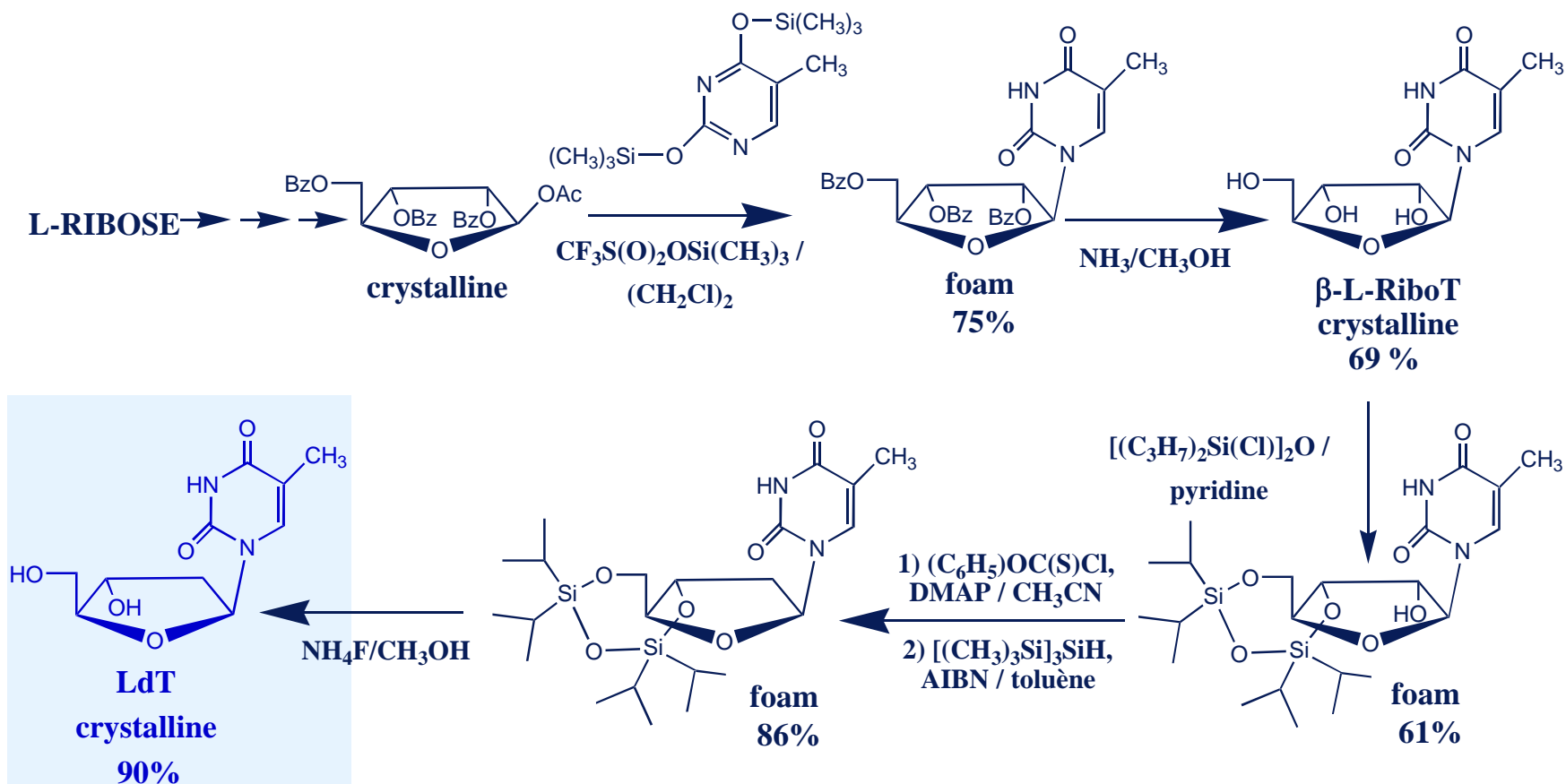
F. Morvan, ..., G. Gosselin and J.-L. Imbach, *Biochem. Res. Commun.* **172**, 537 (1990)

[similar procedure as that already reported by A. Holy, *Collect. Czech. Chem. Commun.* **37**, 4072 (1972)]



LdT : SEMI-LARGE SCALE (20g) CHEMICAL SYNTHESIS

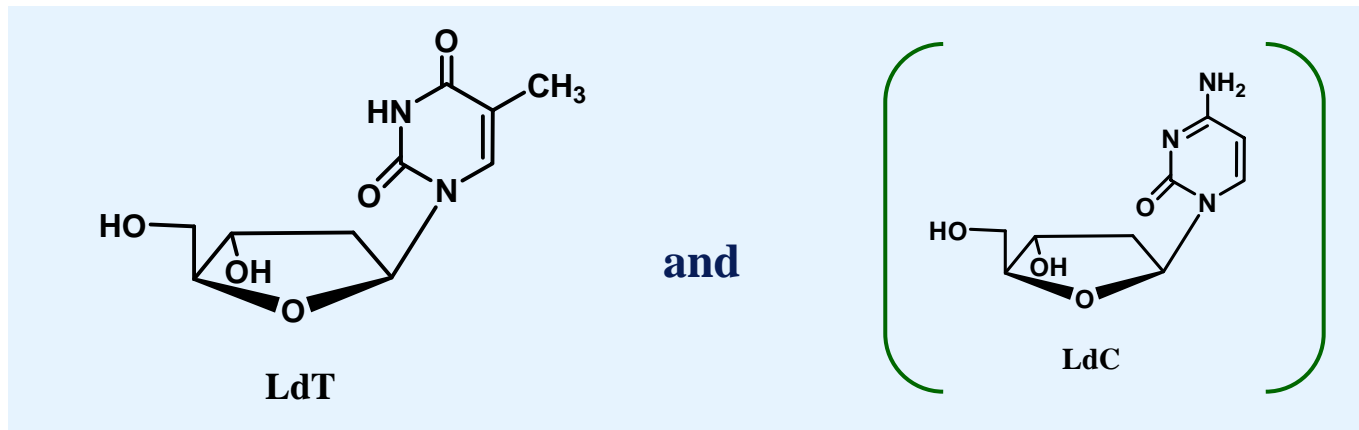
C. Pierra, ... , J.-P. Sommadossi and G. Gosselin, *Antiviral Res.* **46** (1), A62 (2000)



ASSESSMENT OF THE SPECIFIC ANTI-HBV ACTIVITY OF LdT

M. L. Bryant, ... G. Gosselin, ... J.-P. Sommadossi,
Nucleosides, Nucleotides & Nucleic Acids. 20, 597 (2001)

When screened against 15 different RNA and DNA viruses
in cell culture experiments,



- Potent inhibition of human and duck HBV replication (**EC₅₀ < 0.2 μM**)
- No activity (**EC₅₀ > 100 μM**) against other viruses (**HIV-1, HSV-1, HSV-2, VZV, EBV, HCMV, Adenovirus type-1, Influenza A, Influenza B, Measles, Parainfluenza type-3, Rhinovirus type-5, RSV type A**)

ASSESSMENT OF THE SAFETY PROFILE OF LdT

M. L. Bryant, ... G. Gosselin, ... J.-P. Sommadossi, *Antimicrobial Agents Chemother.* 45, 229 (2001) and *Nucleosides, Nucleotides & Nucleic Acids*, 20, 597 (2001)

D. N. Standing, ... G. Gosselin, ... J.-P. Sommadossi, *Antiviral Chem. Chemother.* 12 (Supp.1), 119 (2001)

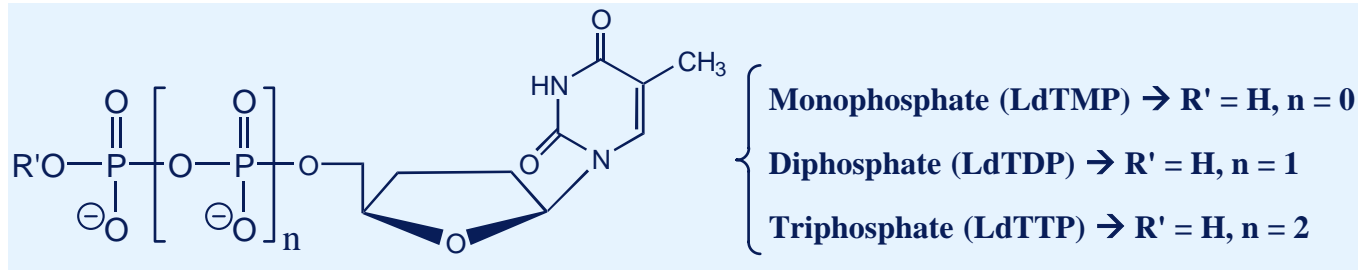
- LdT had no cytotoxic effect (**CC₅₀ values > 100 μM**) on human hepatoma cell line 2.2.15, on primary human peripheral blood mononuclear cells (**PBMC**), on human foreskin fibroblast (**HFF**) or on other cell types of mammalian and avian origins (**PDH, Daudi, A549, MDCK, CV-1, MA-104, KB**).
- LdT would not induce hematotoxicity, since human bone marrow stem cells in primary culture [**Granulocyte-macrophage (CFU - GM) and erythroid precursors (BFU - E)**] exposed to LdT in clonogenic assays at concentrations up to 10 μM were not affected.
- LdT would not show clinically delayed toxicities such as peripheral neuropathy, myopathy and pancreatitis, since exposure of Hep-G2 cells to 10 μM of LdT had no effect on lactic acid production, mitochondrial DNA content, function and morphology.

PHARMACOLOGY OF LdT (1)

Intracellular concentrations of metabolites detected in Hep-G2 cells and hepatocyte cells after 24 hr of incubation with 10 μM of [^3H]LdT

B. Hernandez-Santiago, ... G. Gosselin, ... J.-P. Sommadossi,
Antimicrob. Agents Chemother. 46, 1728 (2002)

Structure of the detected metabolites :

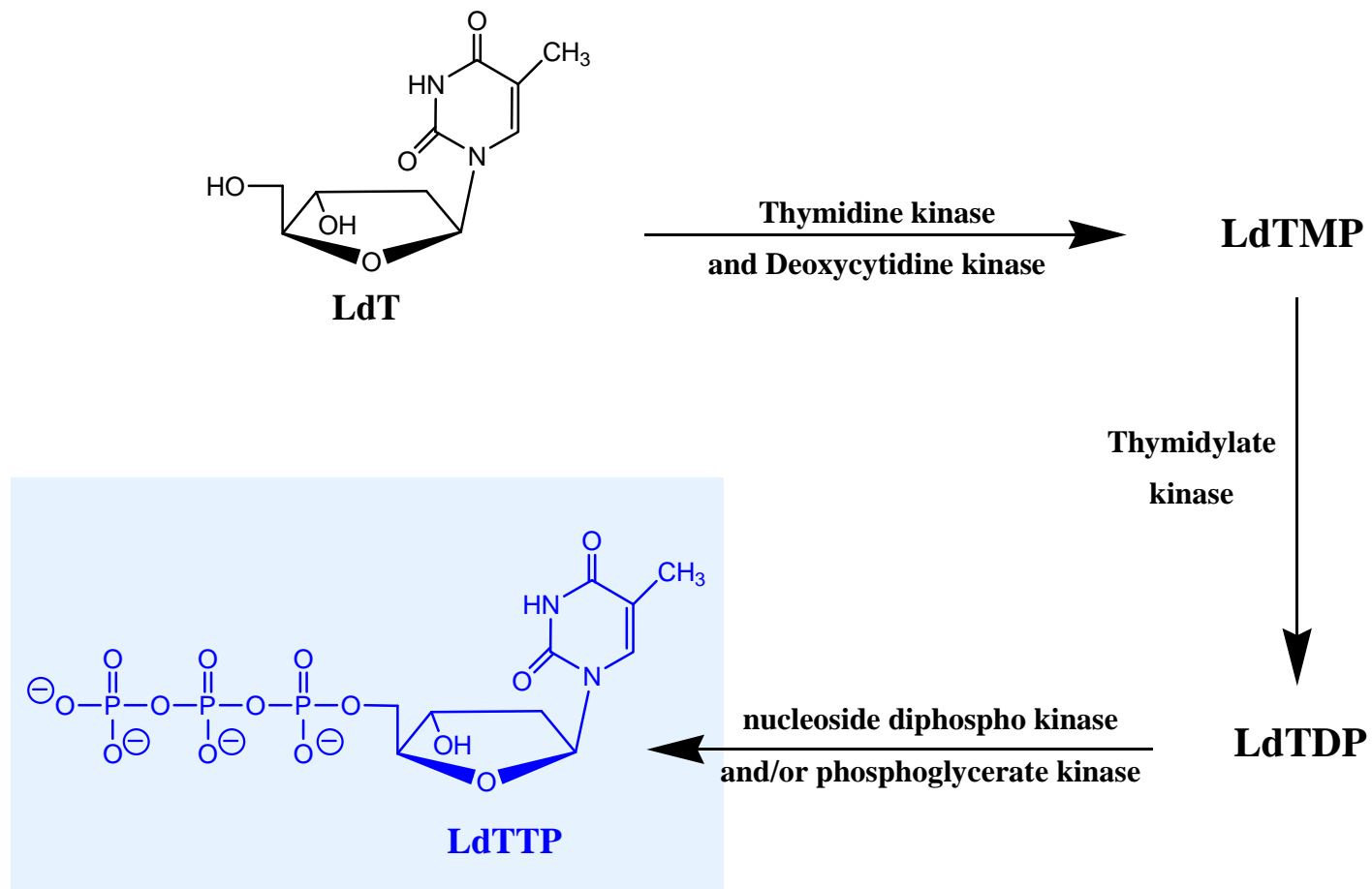


	Intracellular concentration (pmol / 10 ⁶ cells)		
	LdTMP	LdTDP	LdTTP
Hep-G2 cells	8.1	2.9	27.7
Primary hepatocytes (isolated from human liver)	15.2	2.5	16.5

LdT is rapidly and extensively converted into its 5'-triphosphate derivative

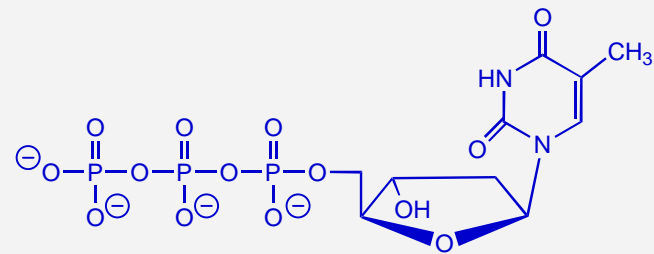
PROPOSED INTRACELLULAR METABOLIC PATHWAY FOR LdT

M. L. Bryant, ... G. Gosselin, ... J.-P. Sommadossi,
In *Frontiers in Viral Hepatitis*, R.F. Schinazi et al. Eds, Elsevier, pp 245 -261 (2003)



PHARMACOLOGY OF LdT (2)

B. Hernandez-Santiago, ... G. Gosselin, ... J.-P. Sommadossi,
Antimicrob. Agents Chemother. 46, 1728 (2002)



LdTTP

EC₅₀ (μM) cellular DNA polymerases (α, β, γ)	> 100
IC₅₀ (μM) woodchuck HBV DNA polymerase	0.24
Intracellular Half-life	≥ 15h*
Concentration : 24h after removal of the parent drug LdT from the cell	7.4 pmol/10⁶ cells**

* This long half-life and ** intracellular persistence indicate that LdTTP concentration remains above the EC₅₀ for HBV in 2.2.15 cells for 24 h (**Predictive of once daily dosing**)

IN VIVO ANTI-HBV ACTIVITY AND SAFETY OF LdT (chronic HBV infection in woodchucks)

M. L. Bryant, ... G. Gosselin, ... J.-P. Sommadossi,
Nucleosides, Nucleotides & Nucleic Acids, 20, 597 (2001)

**When LdT was given orally
at one daily dose of 10 mg/kg/day for 4 weeks :**



**Serum woodchuck hepatitis virus DNA levels (*HBV viremia*)
decreased up to 8 logs in the treated animals**

**[At the same dose, lamivudine used for comparison reduced the
woodchuck hepatitis virus DNA level in serum by 0.5 logs]**

- LdT was well tolerated and caused no drug-related toxicity through 12 weeks of treatment and 4 weeks of follow-up**

LdT : PRECLINICAL PHARMACOLOGICAL STUDIES

D. N. Standring, ... G. Gosselin, ... J.-P. Sommadossi,
Antiviral Chem. Chemother. 12 (Supp.1), 119 (2001)





Absolute oral bioavailability (%F) =

 **in woodchucks : 38%**  **in cynomologous monkeys : 69%**

(Renal clearance appeared to be the major pathway of LdT elimination)

FURTHERMORE

[Bridges, 41st Annual Meeting of the European Association for the Study of the Liver (EASL), April 26-30, 2006, Vienna, Austria]

-  **No effect of LdT on physiology of rats or cynomolgus monkeys at doses up to 1000 mg/kg/day, treated for 6 or 9 months respectively**
-  **No evidence of carcinogenicity in rats or transgenic mice at LdT doses up to 2000 mg/kg/day, treated for 2 years or 6 months respectively**
-  **No effect on reproductive fertility in rats at doses up to 2000 mg/kg/day**
-  **No effect of LdT on fetal or postnatal development in rats or rabbits at doses up to 1000 mg/kg/day**

LdT (telbivudine) REVISITED

SUMMARY :

G. Gosselin, C. Pierra, ... and J.-P. Sommadossi,
in Frontiers in Nucleosides and Nucleic Acids,
R.F. Schinazi & D.C. Liotta eds, IHL Press (Tucker, USA), pp 309-318 (2004)

Owing to its antiviral potency and to its specific and selective profile, LdT appeared as a highly attractive clinical development candidate for the treatment of chronic HBV infection.

CURRENT STATUS OF LdT AND FUTURE PROSPECTS

❖ CURRENT STATUS :

- ★ Clinical Phase IIb : Completed
- ★ Clinical Phase III : Ongoing, year 1 data reported Nov 2005
- ★ NDA submitted to FDA in December 2005
- ★ Other regulatory filings completed in Europe (EMA), Australia and Asia (including China)

❖ FUTURE PROSPECTS

CURRENT STATUS OF LdT :

CLINICAL PHASE IIb (*Completed*)

- Once daily oral dose at 400 or 600 mg LdT, vs. lamivudine (3TC) 100 mg
- Endpoints : serum HBV DNA reduction, ALT normalization, HBeAg seroconversion



Results after 1 year:

- 😊 Significantly greater reduction of HBV in the blood with LdT vs lamivudine:
HBV DNA undetectable in 61% of patients, vs. 32% with lamivudine
- 😊 Serum ALT, a marker of HBV-related liver inflammation, normalized in 86% of LdT-treated patients, vs. 63% with lamivudine

Data support antiviral superiority of LdT over Lamivudine (3TC)

For a review, see :

Telbivudine : A Novel Nucleoside Analog for Chronic Hepatitis B;
J.W. Kim, S.H. Park and S.G. Louie. *The Annals of Pharmacotherapy*, 40, 472-478 (2006)

CURRENT STATUS OF LdT

International phase III clinical trial (the GLOBE study):

- Enrolled 1,367 patients from approximately 135 clinical centers
 - Both HBeAg positive and HBeAg negative patients enrolled
 - Patients receive LdT 600 mg/day or lamivudine (3TC) 100 mg/day

[Lai *et al.*, Meeting of American Association for the Study of Liver Diseases (AASLD), Nov. 11-15, 2005, San Francisco, USA]

- Efficacy and safety of LdT after one year:
 - ☺ High degree of efficacy on all virologic and clinical endpoints
 - ☺ Significantly greater reduction of serum HBV DNA vs. lamivudine
 - ☺ More LdT patients with HBV DNA non-detectable by PCR assay
 - ☺ Greater improvement of liver histology with LdT in HBeAg+ve patients
 - ☺ Significantly less resistance with LdT
 - ☺ Excellent safety and tolerability profile
- Final (two-year) results available late 2006

CURRENT STATUS OF LdT

International clinical trial comparing LdT and adefovir dipivoxyl:

[Chan *et al.*, 41st Annual Meeting of the European Association for the Study of the Liver (EASL), April 26-30, 2006, Vienna]

- **Enrolled 135 HBeAg positive patients**
 - **Patients receive LdT 600 mg/day or adefovir dipivoxyl 10 mg/day**
- **Results after 24 weeks:**
 - ☺ **Significantly greater and more rapid viral suppression with LdT, apparent by week 2**
 - ☺ **Significantly more LdT patients with HBV DNA non-detectable by PCR assay at Week 24: 39%, vs. 12% for adefovir**
 - ☺ **Significantly less treatment failure with LdT: only 5% of LdT patients failed to achieve viral load below 5 log₁₀ copies/mL, vs. 42% for adefovir**
 - ☺ **Both treatments well tolerated**
- **One-year results available late 2006**

LdT (and val-LdC) : FUTURE PROSPECTS

Come Back to the Beginning :

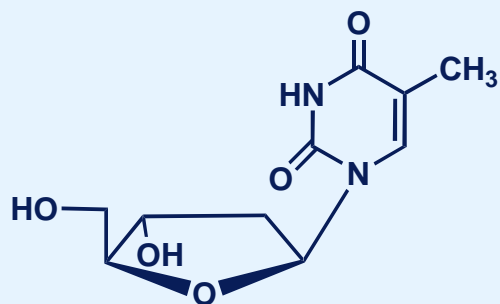
LABORATOIRE DE CHIMIE ORGANIQUE BIOMOLECULAIRE DE SYNTHÈSE

more than 8 years ago :

Discovery that nucleosides from the β -L-2'-deoxyribofuranosyl series are endowed with a unique specificity for anti-HBV activity

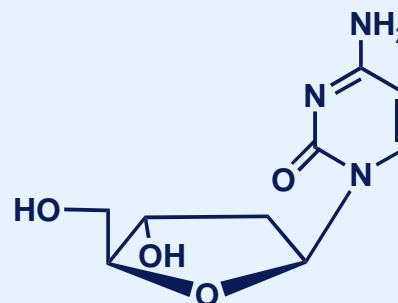
FURTHERMORE

among these β -L-2'-deoxyribofuranonucleosides



not only LdT(telbivudine)

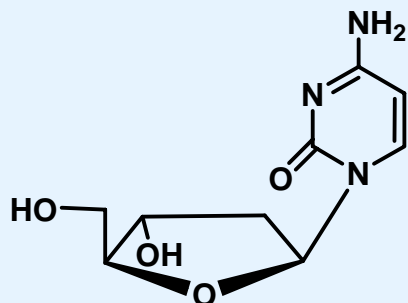
but also



LdC(torcitabine)

have potent, selective and specific activity against HBV replication

LdT (and val-LdC) : FUTURE PROSPECTS



LdC (torcitabine)

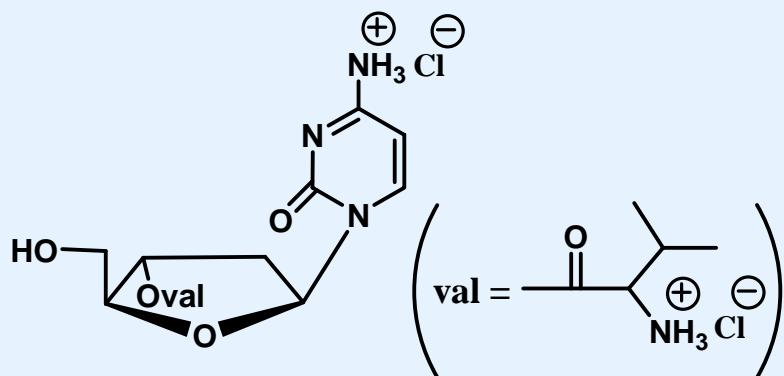
😊 potent, specific and selective inhibitor of HBV replication
but

☹️ %F = 16% in cynomologous monkeys

LABORATOIRE COOPERATIF IDENIX – CNRS – UNIVERSITE MONTPELLIER II
synthesis and comparative study of various ester derivatives of the LdC

S. Benzaria, ... D. Standring, and G. Gosselin, *Nucleosides, Nucleotides & Nucleic Acids*, 22, 1003 (2003)

C. Pierra, ... J.-P. Sommadossi, and G. Gosselin, *Antiv. Chem. & Chemother.*, 15, 269 (2004)



3'-O-L-valinyl ester (val-LdC) : valtorcitabine

😊 %F = 84% oral bioavailability
increased more than fivefold

LdT (and val-LdC) : FUTURE PROSPECTS

[LdT + val-LdC] as a candidate HBV combination therapy

INDEED :

- Neither LdT nor LdC interferes with the level of phosphorylation of the other compound in Hep-G2 cells [B. Hernandez-Santiago *et al*, *Antimicrob. Agents Chemother.* 46, 1728 (2002)]
- LdT preferentially inhibits 2nd-strand HBV DNA synthesis, while LdC inhibits both 1st- and 2nd-strand synthesis [M. Seifer *et al*, HEP DART 2001, *Maui-Hawaiï* = Abstract 080]
- In vitro studies on the combination of LdT and LdC as well as combination studies in the woodchuck model of chronic HBV infection suggest that the two drugs have potent antiviral synergy [A. Juodawlkis *et al*, *Antiviral. Res.* 50, A43 (2001)]

PLANS :

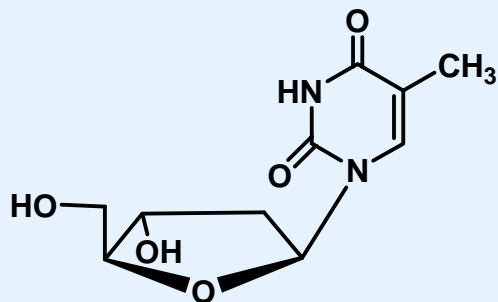
Development of valtorcitabine as a fixed dose combination with telbivudine for patients for whom treatment with a single agent may not be adequate

CURRENT STATUS :

Phase IIb clinical program initiated in late 2004 and currently underway

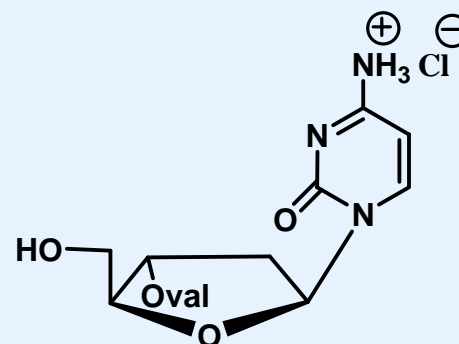
Discovery of LdT (telbivudine) as a New Potent Nucleoside Analogue for the Treatment of Chronic Hepatitis B

CONCLUSION



Not only **LdT (telbivudine)**

but also



val-LdC (valtorcitabine)

are two examples of a fruitful collaboration between an academic team (**Laboratoire de Chimie Organique Biomoléculaire de Synthèse, UMR 5625 CNRS – Université Montpellier II**) and a Company (**Idenix**).

Idenix is developing telbivudine and valtorcitabine in collaboration with Novartis Pharma AG under a development and commercialization agreement established in May 2003

Discovery of LdT (telbivudine) as a New Potent Nucleoside Analogue for the Treatment of Chronic Hepatitis B

Concepts and Chemical Syntheses

Laboratoire de Chimie Organique Biomoléculaire de Synthèse
Laboratoire Coopératif Idenix - CNRS – Université Montpellier II (France)

S. Benzaria (*Ph. D.*)

D. Dukhan (*Ph. D.*)

C. Pierra (*Ph. D.*)

G. Gosselin (*Ph. D., Research Director*)

J.-L. Imbach (*Ph. D., Emeritus Prof.*)

Pharmacology, Pharmacokinetics and Clinical Phase Studies

Idenix Pharmaceuticals, Cambridge (USA)

J.-P. Sommadossi (*Ph. D., Chair & CEO*)

N. Brown (*M. D., Sr V.P.*)

M. Bryant[†] (*M.D., Ph. D., Exec.V.P.*)

E. Cretton-Scott (*Ph. D.*)

A. Juodawlkis (*Sr. Manager*)

L. Placidi (*Ph. D.*)

M. Seifer (*Ph. D.*)

D. Standring (*Ph. D., V.P.*)

X.-J. Zhou (*Ph. D.*)

[†] *Deceased on March 04, 2002*

OTHER COLLABORATIONS

Dipartimento di Biologia Sperimentale (Pr P. La Colla), Università di Cagliari (Italy)
University Department of Medicine (Pr C.-L. Lai), University of Hong Kong (China), etc



International Society for Nucleosides, Nucleotides and Nucleic Acids (IS3NA)

Founders : P. D. Cook, G. Gosselin, P. Herdewijn, A. Matsuda, J. A. Secrist

- * Made Official on January 1st, 2001, Currently more than 350 registered members**
- * Aims : Focused on research related to nucleic acid components and analogues, to encourage international collaborations on research and application among academic, industrial, governemental and private institutional organizations.**

Preliminary IS3NA membership benefits :

- sponsoring the XVII International RoundTable (IRT) to be held in Bern, Switzerland on September 03-07, 2006.**
- offering *Nucleosides, Nucleotides & Nucleic Acids* Journal to members at reduced rates**

More information : <http://www.is3na.org>

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