SOLOMON T. GARNER, JR
The Synthesis of 1-[3-C-cyano-2, 3-Dideoxy-β-L-*glycero*-pent-2-enofuranosyl]thymine; 1-[2-C-cyano-2, 3-Dideoxy-β-L-*glycero*-pent-2-enofuranosyl]thymine and 1-(3-C-Cyano-3-deoxy-β-L-*arabino*-pentofuranosyl]thymine and Computational Studies of 1-[2-C-cyano-2-deoxy-1-β-D-arabinofuranosyl]cytosine.
(Under the Direction of C. K. CHU)

The attempt herein was to give a concise overview of the results and general milestones that have led to towards the synthesis of 1-[3-C-cyano-2, 3-Dideoxy-β-L-*glycero*-pent-2-enofuranosyl]thymine; 1-[2-C-cyano-2, 3-Dideoxy-β-L-*glycero*-pent-2-nofuranosyl]-thymine and 1-(3-C-Cyano-3-deoxy-β-L-*arabino*-pentofuranosyl]thymine, nucleoside analogues. Also included is a computational evaluation, whereas a density functional theory (DFT) method has been employed to study the epimerization of 2'-C-Cyano-2'-deoxy-1-β-D-*arabino*-pentofuranosylcytosine (CNDAC) to 2'-C-Cyano-2'-deoxy-1-α-D-*ribo*-pentofuranosylcytosine (CNDC) as well as their degradation to 1,4-anhydro-2-C-cyano-2-deoxy-D-*erytho*-pent-1-enitol (glycal) and cytosine by way of glycosyl bond cleavage.

INDEX WORDS: Nucleoside, 1-[3-C-cyano-2, 3-Dideoxy-β-L-*glycero*-pent-2-enofuranosyl]thymine, 1-[2-C-cyano-2, 3-Dideoxy-β-L-*glycero*-pent-2-enofuranosyl]thymine, (3-C-Cyano-3-deoxy-β-L-*arabino*-pentofuranosyl]thymine, 1-[2-C-cyano-2-deoxy-1-β-D-arabinofuranosyl]cytosine (CNDAC)/2'-C-Cyano-2'-deoxy-1-β-D-*arabino*-pentofuranosylcytosine (CNDAC), Density functional theory (DFT), Conformer, Eperimerization

THE SYNTHESIS OF 1-[3-C-CYANO-2, 3-DIDEOXY-β-L-*GLYCERO*-PENT-2-ENOFURANOSYL]THYMINE; 1-[2-C-CYANO-2, 3-DIDEOXY-β-L-*GLYCERO*-PENT-2-ENOFURANOSYL]THYMINE AND 1-(3-C-CYANO-3-DEOXY-β-L-*ARABINO*-PENTOFURANOSYL]THYMINE AND COMPUTATIONAL STUDIES OF 1-[2-C-CYANO-2-DEOXY-1-β-D-ARABINOFURANOSYL]CYTOSINE

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B.S., Jackson State University, 1999

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2001

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December 2001

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CHAPTER 1

THE SYNOPSIS: BRIEF INTRODUCTION OF L-NUCLEOSIDES AND 2', 3'-UNSATURATED NUCLEOSIDES

Introduction of L-Nucleosides

L-Nucleoside research began with the pioneering work of Smejkal and Sorm's[1] synthesis of L-thymidine as well as Holy's[2] *et* al. synthesis of the enantiomers of the natural D-nucleoside's uridine, cytidine, adenosine and guanosine. More importantly, L-nucleoside anti-metabolites are known to inhibit the synthesis of DNA or RNA after conversion triphosphate analogues and exhibit evidence of antiviral/anti-neoplastic activities.[3,4] Figure 1.1 illustrates a nucleoside analogue's general mode of action, given that these processes are extremely important for the biochemical activation of therapeutic nucleosides.[5] Nucleoside monophosphate kinases (NMPKs) and nucleoside diphosphates kinases (NDPKs) catalyze the reversible phosphotransferase reactions of a nucleoside to its corresponding 5'-nucleotidases via pathways to their corresponding triphosphates.[5]

The anti-human immunodeficiency virus type I (HIV-1) activity of L-(-)-3TC (3TC, Lamivudine) **1** was the first L-nucleoside approved by the Food and Drug Administration (FDA) to be used as therapy against HIV and chronic Hepatitis B viral infections [Figure 1.2]. Its cytotoxicity was observed to be less than its enatiomer (+)-BCH-189 **2.** The D- nucleosides were previously thought to be the only biologically active enatiomers.[6, 7, 8]

Correspondingly, L-thymidine **3** was reported to be a substrate of thymidine

kinase that is coded by herpes viruses and to has weak anti-herpes simplex virus type 1 (HSV-1) activity.[7] The idea arose that L-nucleosides are not recognized by normal mammalian enzymes and by virus-encoded or bacterial enzymes, thus giving rise to minimal host toxicity while maintaining good antiviral/antibacterial activity.[9] On the other hand, it has been found that L-nucleosides are recognized by normal mammalian enzymes.[9]

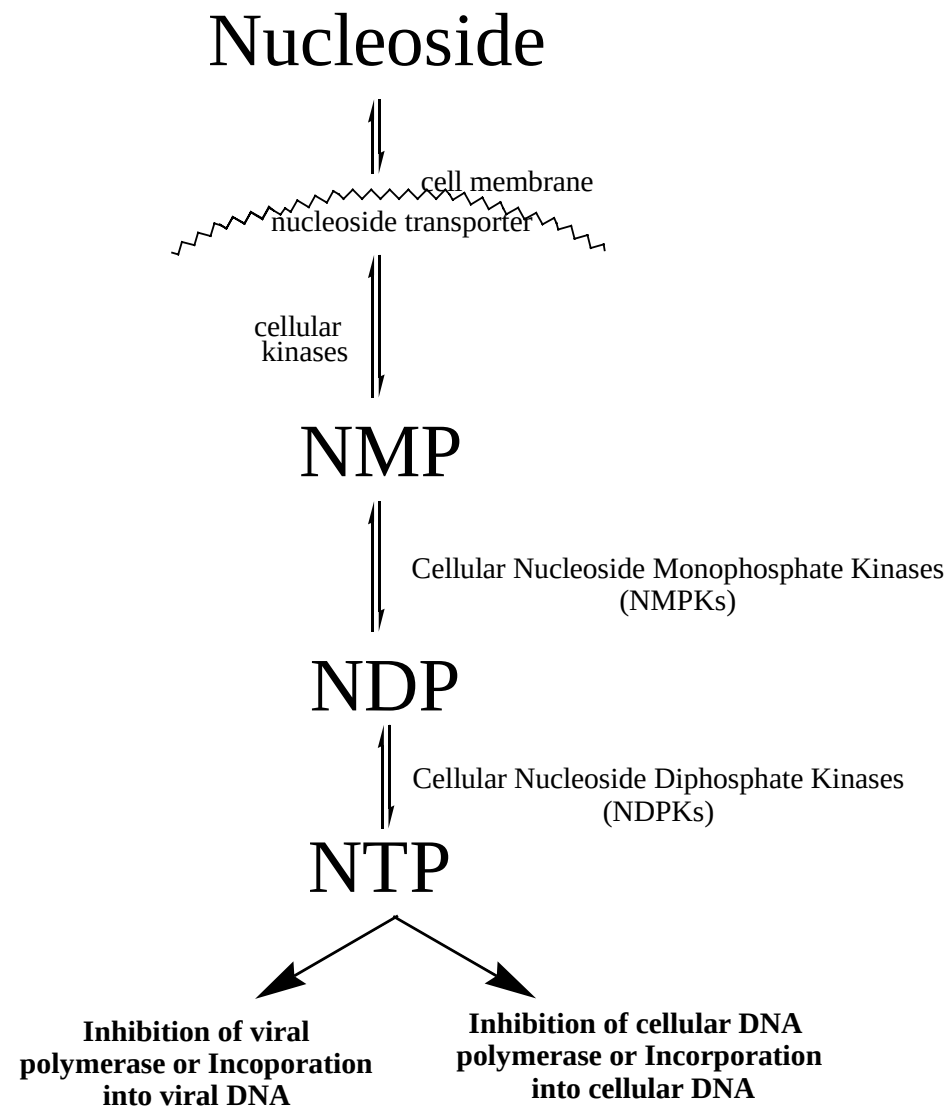


Figure 1.1 General Nucleoside Mode of Action**:** Nucleoside monophosphate (NMP), Nucleoside diphosphate (NDP), Nucleoside triphosphate (NTP)

Because of these findings, L-nucleosides such as L-FMAU[11] **4** and FTC[13] **5** have been cited as potential selective antiviral agents. L-FMAU, as reviewed by Wang et *al*[12] exhibited potent anti-HBV and anti-EBV activities[11]. Triangle Pharmaceuticals, Inc. is currently undertaking L-FMAU in Phase I/II dose-range finding study in patients with HBV infection.[13] FTC[14] is a 5-fluorinated analog of 3TC which has been demonstrated as a potent inhibitor of HIV-1 and is currently undergoing clinical trials.[15] As an example, it has been shown that FTC is phosphorylated in a manner similar to that of the natural D-nucleosides.[16,17] [Figure 1.2]

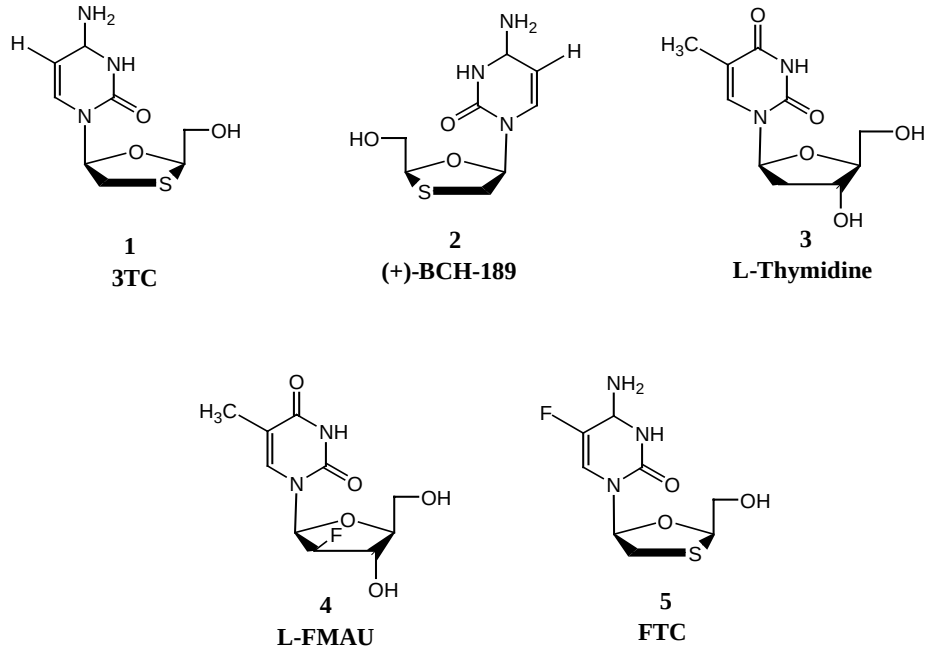
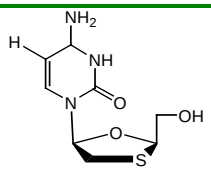
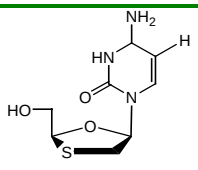
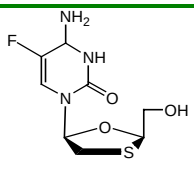
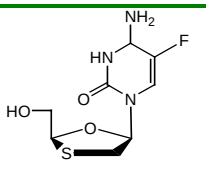


Figure 1.2 Structures of Biologically Active L-Nucleosides

For example, the activity/toxicity of the previously mentioned compounds 3TC, FTC and L-FMAU is compared to that of their D-enantiomers. 3TC and its enatiomer (+)-BCH-189 both showed potent anti-HIV activity but cytotoxicity is primarily

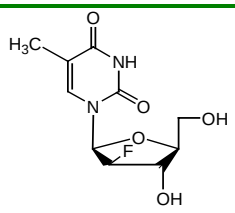
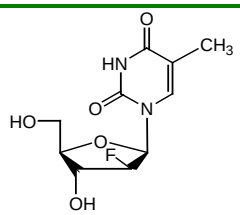
associated with the D- enantiomer. This comparison (Table 1.1) comes from *in vitro* data reported by Chu and co-workers, which shows that 3TC (EC_{50} 0.0018 µM in PBM cells) was more potent than (+)-BCH-189 (EC_{50} 0.002-0.06 µM) against HIV-1 PBM cells[7, 18]. 3TC was also shown to be more effective against HBV than (+)-BCH-189, where each compound had EC_{50} values of 0.01 µM and 0.5 µM respectively.[19] The 5-fluorinated analogues of these compounds FTC and D-FTC showed EC_{50} values of 0.0008 µM and 0.84 µM *in vitro*, respectively against HIV-1 in PBM cells, whereas FTC had the greater potency.[20] Similarly, these compounds exhibited the same trends of anti-HBV activity in potency against HBV hepatoma cell lines (2.2.15)[21] as listed in Table 1.1.

Table 1.1 Comparison of 3TC's and FTC's Enantiomers

Nucleosides	 **3TC**	 **(+)-BCH-189**	 **L-FTC**	 **D-FTC**
Anti-HIV-1 (EC_{50}, µM)				
PBM	0.002	0.2	0.008	0.84
CEM	0.07	0.1	0.009	1.4
Anti-HBV-(EC_{50}, µM)				
2.2.15	0.01	0.05	0.01	0.96
Cytotoxicity (IC_{50}, µM)				
PBM	>100	2.7	>100	>100
CEM	>100	>100	>100	>100

L-FMAU was found to have potent anti-HBV (EC_{50} 0.1 μM in 2.2.15 cells) and anti-Epstein Barr (EC_{50} 5.0 μM in H1 cells) viral activities. In comparison with D-FMAU , L-FMAU exhibited lower cytotoxicity and was a more potent anti-HBV agent.[11] [Table 1.2]

Table 1.2 Comparisons of D- and L-FMAU

Nucleoside	 **L-FMAU**	 **D-FMAU**
Anti-HBV-(EC_{50}, μM)		
2.2.15	0.1	0.2
Anti-HBV-(EC_{50}, μM)		
H1	5.0	0.1
Cytotoxicity-(IC_{50}, μM)		
MT2	100	8.0

2', 3'-Unsaturated Nucleosides

2', 3'-Unsaturated-nucleosides have become an important class of potent anti-viral agents in light of the FDA's approval[23] of L-2', 3'-didehydro-2', 3'-dideoxythymidine (d4T**,** Stavudine, Zerit) as an anti-HIV drug during the mid-1990s.[24] The corresponding cytosine analogue (d4C, 2', 3'-didehydro-2', 3'-dideoxycytidine) has also been found to be a strong inhibitor of HIV replication[25, 24]. In effect, many of these types of nucleosides have been synthesized and identified as anti-HIV agents such as

abacavir,[26, 27] L-2', 3'-didehydro-2', 3'-dideoxycytidine (β-L-d4C, L-d4C) and its 5-flouro analogue (β-L-Fd4C, L-Fd4C).[28, 29] [Figure 1.3]

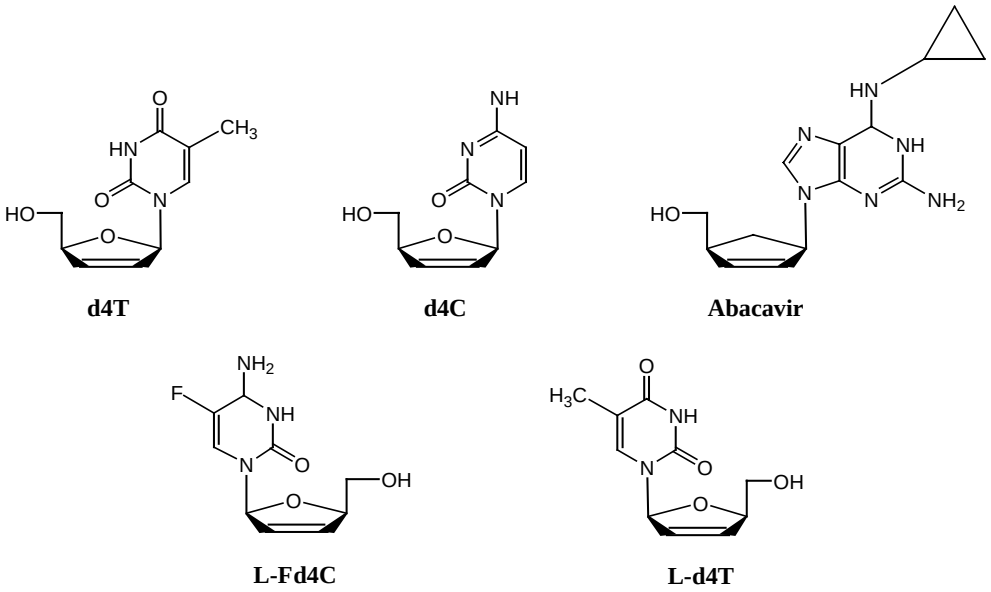
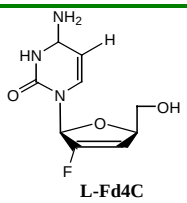
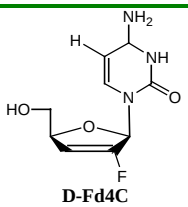
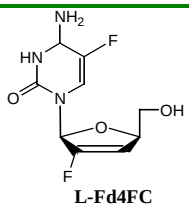
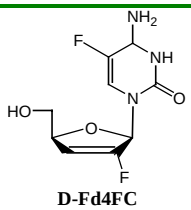


Figure 1.3 Structures of Biologically Active 2', 3'-Unsaturated-nucleosides

In view of interesting biological activities of this class of compounds, our research group has reported the synthesis and anti-HIV/HBV activities of 2'-flouro-2',3'-unsaturated-L-nucleosides such as L-Fd4C, L-Fd4FC, which were found to be more potent and have lower cytotoxicities than their D-enantiomers(Table 1.3).[12, 30, 31] L-Fd4C listed in Table 1.3 is currently undergoing pre-clinical studies.[12] As an electron withdrawing group, the 2'-flouro substituent stabilizes the glycosyl bond by providing acid stability, thus making a more stable 2',3'-unsaturated nucleoside.[30, 32] It has also been found that the 2'-flouro as an electron withdrawing group substituents confers more interesting biological activities on 2', 3'-unsaturated nucleosides.[30, 32]

Table 1.3 Comparison Fd4C's and Fd4FC's Enantiomers

Nucleosides	 L-Fd4C	 D-Fd4C	 L-Fd4FC	 D-Fd4FC
Anti-HIV-1 ($EC_{50,}$ µM)				
PBM	0.51	3-10	0.17	0.35
Anti-HBV-($EC_{50,}$ µM)				
2.2.15	2.0	2.0	4.0	Not reported
Cytotoxicity ($IC_{50,}$ µM)				
PBM cells	>100	2.6	>100	>100
CEM cells	>100	>29.9	>100	>100
Vero cells	>100	>100	>100	>100

References

1. Smejkal, J; Sorm, F. Nucleic acid Components and Their Analogues. Preparation of 1, 2-deoxy-β-L-ribofuranosythymine, 'L-thymine', *Collect. Czech. Chem. Commun.* 1964, 29, 2809-13.
2. Holy, A.; Sorm, F. Preparation of some β- L—ribonucleosides, their 2', (3')-phosphates and 2', 3'-cyclic phosphates. *Collect. Czech. Chem. Commun.* 1969. 34, 3383-3391.

3. Satoh, H.; Yoshimura, Y.; Sakata, Shinji, S.; Miura, S.; Machida, H.; Matsuda, A. Synthesis of 4'-Thioarabinofuranosyl Pyrimidine Nucleosides. *Bioorg. Med. Chem. Lett.* 1998, 8, 989-992.

4. Ono, T.; Fujii, A.; Yamagami, K.; Hosoya, M.; Okumoto, T.; Sakata, S.; Matsuda, A.; Sasaki, T. Cell kill kinetics of an antineoplastic nucleoside, 1-(2-deoxy-2-methylene-β-D-erythro-pentofuranosyl)cytosine. *Biochem. Pharmacol.* 1996, 32, 1279-1285.

5. Van Rompay, An R.; Johansson, M.; Karlsson. A. Phosphorylation of nucleoside and nucleoside analogs by mammalian nucleoside monophosphate kinases. *Pharmacology &Therapeutics.* 2000, 87, 189-198.

6. Coates, J. A. V.; Cammeck, N.; Jenkinson, H. J.; Mutton, I. M.; Pearson, B. A. ; Cameron, J. M.; Penn, C. R. The Separated Enantiomers of 2'-Deocy-3'-Thiacytidine (BCH-189) Both Inhibit Human-Immunodeficiency-Virus Replication In *vitro.* *Antimicrob. Agents Chemother.* 1992, 36, 202-205

7. Schinazi, R. F.; Chu, C. K.; Peck, A.; McMillan, A.; Mathis, R.; Cannon, D.; Jeong, L.-S.; Beach, J. W.; Choi, W.-B.; Yeola. S.; Liotta, D. Activities of the 4 Optical Isomers of 2'-Deoxy-3'-Thiacytidine (BCH-189) Against Human-Immunodeficiency-Virus Type-1 in Human-Lymphocytes. *Antimicrob. Agents Chemother*. 1992, 36, 672-676.

8. Coates, J. A. V.; Cammack, N. Jenkinson, H. J.; Jowett, A. J.; Jowett, M. I.; Pearson, B. A.; Penn, C. R.; Rouse, P. L.; Viner, K. C.; Cameron, J. M**. (-)-**2'-Deoxy-3'-Thiacytidine is a Potent, Highly Selective Inhibitor of Human-

Immunodeficiency-Virus Type-1 and Type-2 Replication In Vitro. *Antimicrob. Agents Chemother.* **1992**, 36, 733-739.

9. Spadari, S.; Maga, G.; Focher, F.; Biarrochi, G.; Manservigi, R.; Arcamone, F.; Capoblanco, M.; Carcuro, A.; Colonna, F.; Iotti, S.; Garbesi, A. *J. Med. Chem.* L-Thymidine is Phosphorylated by Herpes-Simplex Virus Type-1 Thymidine Kinase and Inhibits Viral Growth. **1992**, 35, 4214-4220.

10. Kotra, L. P.; Xiang, Y.; Newton, M. G.; Schinazi, R. F.; Cheng, Y.-C.; Chu, C. K. Structure-activity relationships of 2'-deoxy-2',2'-difluoro-L-erythro-pentofuranosyl nucleosides. *J. Med. Chem.* **1997**, 40, 3635-3644.

11. Chu, C. K.; Ma, T.; Shanmuganathan, K; Wang, C.; Xiang, Y.; Pai, S. B.; Yao, G. Q.; Sommadossi, J. P.; Cheng, Y. –C. Use of 2'-fluoro-5-methyl-β-L-arabinofuranosyluracil as a novel antiviral agent for hepatitis B virus and Epstein-Barr virus. *Antimicrob. Agents Chemother.* **1995**, 39, 979-981.

12. a) Wang, P.; Hong, J. H.; Cooperwood, J. S.; Chu, C. K. Recent advances in L-nucleosides: chemistry and biology. **1998**, 40, 19-44. b) Gumina, G.; Song, G. –Y.; Chu, C. K. L-Nucleosides as chemotherapeutic agents. *FEMS Microbiology Letters.* **2001**, 202, 9-15.

13. Triangle Pharmaceuticals. L-FMAU: A potent antiviral pyrimidine nucleoside. September **2001,** www.tripharm.com/lfmau.html.

14. Van Draanen, N. A.; Tisdale, M.; Parry, N. R.; Jansen, R.; Dornsife, R. E.; Tuttle, J. V.; Averett, D. R.; Kosazalka, G. W. Influence of Stereochemistry on Antiviral Activities and Resistance Profiles of Dideoxycytidine Nucleosides. *Antimicrob. Agents Chemother.* **1994**, 38, 868.

15. Triangle Pharmaceuticals. Study Name: FTC-301. September **2001**, www.tripharm.com/trialsmain.html

16. Schinazi, R.F.; Lloyd, R. M.; Nguyen, M.-N.; Cannon, D .L.; Mcmillian, A.; Ilksoy, N.; Chu, C.K.; Liotta, D.C.; Bazmi, H.Z.; Mellors, J.W., Characterization of human immunodeficiency virus resistant to oxathiolane-cytosine nucleosides. *Antimicrob. Agents Chemother.* **1993**, 37, 875-881.

17. Shewach, D. S.; Liotta, D. C.; Schinazi, R. F., Affinity of the antiviral enantiomers of oxathiolane cytosine nucleosides for human 2'-dioxycytidine kinase. *Biochem. Pharmacol*. **1994**, 45, 1540-1543.

18. Chu, C. K.; Beach, J. W.; Jeong, L-. S. ; Choi, B. G.; Comer, F. I.; Alves, A. J.; Schinazi, R. F.; Enatiomeric Synthesis of (+)-BCH-189 [(+)-(*2S, 5R*)-1-[2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]cytosine] from D-manose and its Anti-HIV Activity. *J. Org. Chem.* **1991**, 56, 6503-6505.

19. Jeong, L-. S. ; Schinazi, R. F.; Beach, J. W.; Kim, H. O.; Shanmuganathan, K.; Alves, A. J.; McMillian, A.; Chu, C. K.; Mathis, R. Asymmetric Synthesis and Biological Evaluation of β-L- (*2R, 5S*) and α-L-(*2R, 5S*)-2, 3-oxathiolane-Pyrimidine and Purine Nucleosides as Potential Anti-HIV Agents. *J. Med. Chem.* **1993**, 36, 2627-2638

20. Schinazi, R. F.; McMillian, A.; Cannon, D.; Mathis, R.; Lloyd, R. M.; Peck, A.; Sommadossi, J. –P.; St. Clair, M. ; Wilson, J.; Furman, P.A.; Painter, G.; Choi, W. B.; Liotta, D. C. Selective Inhibition of Human Immunodeficiency Viruses by the Enantiomers of Cis-5-fluoro-1-[2-hydroxymethyl-1, 3-oxathiolanyl]cytosine. *Antimicro. Agents Chemother.* **1992**, 36, 2423-2431

21. Furman, P. A.; Davis, M.; Liotta, D. C.; Paff, M.; Frick, L. W.; Nelson, D. J.; Dornsife, R. E.; Wurst, J. A.; Wilson, L. J.; Fyfe, J. A.; Tuttle, J. V.; Miller, W. H.; Condreay, L.; Averette, D. R.; Schinazi, R. F.; Painter, G. R. The Anti-hepatitis B Virus Activities, Cytotoxicities ad Anabolic Profiles of the (-) and (+) Enantiomers of Cis-5-fluoro-1-[2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]cytosine. *Antimicro. Agents Chemother.* **1992**, 36, 2686-2692.

22. Chu, C. K.; Ma, T. W.; Shanmuganathan, K.; Wang, C., –G.; Xiang, Y,-J.; Pai, S. B.; Yao, G.- Q.; Sommadossi, J.-P.; Cheng, Y, -C. Use of 2'-Fluoro-5 Methyl-β-L-arabinofuranosyluracil as a Novel Antiviral Agent for Hepatitis B Virus and Epstein Barr Virus. *Antimicrob. Agents Chemother.* **1995**, 39, 979-981.

23. Barrish, J. C.; Zahler, R. Antiviral Agents. *Annu. Rep. Med. Chem.* **1993**, 28, 131-140.

24. Winter, H.; Maeda, Y.; Uchida, H.; Mitsuya, H; Zemlicka, J. Phosphodiester Amidates of Unsaturated Nucleoside Analogues: Synthesis and Anti-HIV Activity. *J. Med. Chem.* **1997**, 40, 2191-2195.

25. Declercq, E. HIV Inhibitors Targeted at the Reverse Transcriptase. *AIDS Res. Hum. Retroviruses.* **1992**, 8, 119-134.

26. Daluge, S. M.; Good, S. S.; Faletto, M. B.; Miller, W. H.; St. Clair, M. H.; Boone, L. R.; Tisdale, M.; Parry, N. R.; Reardon, J. E.; Dornsife, R. E; Averet, D. R.; Krenitsky, T. A. 1592U89, A Novel Carbocyclic Nucleoside Analogue Potent, Selective Anti-Human Immunodeficiency Virus Activity. *Antimicro. Agents Chemother.* **1997**, 41, 1082-1093.

27. Tisdale, M.; Alnadar, T.; Cousens, D. Combination of Mutations in Human Immunodeficiency Virus Type I Reverse Transcriptase Required for Resistance to the Carbocyclic Nucleoside 1592U89. *Antimicro. Agents Chemother.* **1997**, 41, 1094-1098.

28. Lin, T. S.; Luo, M. Z.; Liu, M. C.; Zhu, Y. L.; Gullen, E.; Dutchman, G. E.; Cheng, Y.-C.; Design and Synthesis of Dideoxy-2', 3'-Didehydro-β-L-Cytidine (β-L-d4C) and of 2', 3'-Dideoxy-2', 3'-Didehydro-β-L-5-Fluorocytidine (β-L-d4C), Two Exceptionally Potent Inhibitors of Hepatitis B Virus (HBV) and Potent Inhibitors of Human Immunodeficiency Virus (HIV). *J. of Med. Chem.* **1996**, 39, 1757-1759

29. Gosselin, G.; Schinazi, R. F.; Sommadossi, J. –P; Mathe, C.; Bergogone, M. C.; Aubertin, A. –M.; Kirn, A.; Imbach, J.-L. Anti-Human Immunodeficiency Viruses Activities of the β-L-Enantiomer of 2', 3'-Dideoxycytidine and its 5-Fluoro Derivative *in vitro. Antimicrob. Agents Chemother.* **1994**, 38, 1292-1297.

30. Lee, K.; Choi, Y.; Gullen, E.; Schlueter-Wurtz, S.; Schinazi, R.; Cheng, Y. –C.; Chu, C. K. Synthesis and Anti-HIV and Anti-HBV Activities of 2'-Fluoro-2', 3'-unsaturated L-Nucleosides. *J. Med. Chem.* **1999**, 42, 1320-1328.

31. Pai, S. B.; Chu, C. K.; Liotta, D. C.; Schinazi, R. F. 2'-Fluoro-2'; 3'-unsaturated nucleosides as selective antivirals against hepatitis B virus. *Antivi. Ther.* **2000**, 5 (Suppl. 1), B66.

32. Ma, T. W.; Pai, S. B.; Zhu, Y. L.; Lin, J. S.; Shamuganatha, K.; Du, J. F.; Wang, C.; Kim, H. B.; Newton, M. G.; Cheng, Y. –C.; Chu, C. K. Structure-

Activity Relationships of 1-(2-Deoxy-2-Fluoro-β-L-Arabinofuranosyl) Pyrimidine Nucleosides as Anti-Hepatitis B Virus Agents. *J. Med. Chem.* **1996**, 39, 2835-2843.

CHAPTER 2

SYNTHESIS OF 1-[3-C-CYANO-2, 3-DIDEOXY-β-L-*GLYCERO*-PENT-2-ENOFURANOSYL]THYMINE; 1-[2-C-CYANO-2, 3-DIDEOXY-β-L-*GLYCERO*-PENT-2-ENOFURANOSYL]THYMINE AND 1-(3-C-CYANO-3-DEOXY-β-L-*ARABINO*-PENTOFURANOSYL]THYMINE

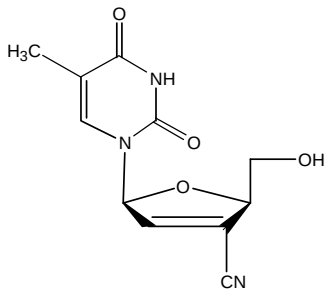
Introduction

Our approach has been to synthesize the key carbohydrate intermediate. Once the key carbohydrate moiety is synthesized, many nucleosides may be prepared. This convergent approach for the synthesis of nucleoside analogues is a very desirable and flexible process, since it provides the basis for the preparation of numerous pyrimidine and purine analogues from the same intermediate. Thus, a series of nucleosides can be tested for biological activities as potential medicinal agents.

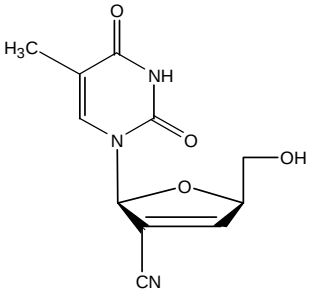
In perspective, as found, completing the sugar moiety before making the nucleoside, however, is not always the best approach when chemical properties limit the route toward the final nucleoside product. Correspondingly, two groups Matsuda and co-workers [1-3] as well as Velázquez and Camarasa [4] successfully synthesized 2'-C-Cyano-β-D-arabinofuranosyl pyrimidine nucleoside analogues, the enantiomers of our targeted compounds. They synthesized the analogues by divergent methods of completing a 2'-CN addition into a nucleoside to obtain the final product.

Therefore, to add to the knowledge of the nucleoside chemistry, it was our objective to synthesize these types of pyrimidine nucleoside analogues in the β-**L**-configuration by convergent methods. Due to the unsuccessful reaction as shown in Scheme 2.1, a divergent method was tried to obtain 2'-CN and 3'-CN substituted β-L- 5-methyluridine nucleoside analogues, as well as their 2', 3'-unsaturated analogues. From this approach, 1-[3-C-Cyano-2, 3-dideoxy-β-L-*glycero*-pent-2-enofuran-osyl]thymine; 1-[2-C-Cyano-2, 3-dideoxy-β-L-*glycero*-pent-2-enofuranosyl]thymine; and 1-(3'-C-Cyano-3'-deoxy-β-L-*arabino*-pentofuranosyl]thymine were synthesized. (Figure 2.1).

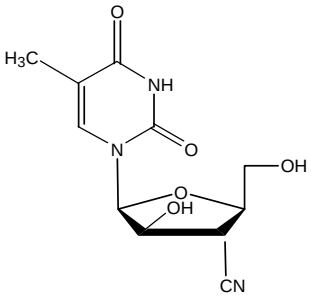
Matsuda *et* al evaluated the inhibition of cytopathogenicity of HIV by 3'-C-cyano-3'-deoxy-2', 3'-didehydro-β-D-5-methyluridine **I** and 1-(3-C-Cyano-3-deoxy-β-D-*arabino*-pentofuranosyl)-thymine **II**, the enantiomers of 1-[3-C-Cyano-2, 3-dideoxy-β-L-*glycero*-pent-2-enofuranosyl]thymine and 1-(3-C-Cyano-3-deoxy-β-L-*arabino*-pentofuranosyl]thymine respectively.[5] Neither compound exhibited inhibitory activity up to 500 μg/ml concentration when tested by using HTLV-1 in MT-4 cells.[5] (Figure 2.2) These results conflict with a report [6] that compound **1** is more active than AZT. Others report the inhibitory activity of compounds **I** and **II** as >500 μg/ml and >250 μg/ml concentration, respectively, when tested by using HTLV-1 in MT-4.[7] Only one group Azuma *et* al[2] synthesized and reported the biological activity of 2'-C-Cyano-2', 3'-didehydro-2', 3'-dideoxy-5-methyluridine **III**; the enatiomer of 1-[2-C-Cyano-2, 3-dideoxy-β-L-*glycero*-pent-2-enofuranosyl]thymine; as its *in vitro* cell growth inhibitory activity against murine leukemia L1210 and human oral epidermoid carcinoma KB cells; IC_{50} values were 12.4 and 97.8 respectively.[1] [Figure 2.2]



1-[3-C-cyano-2, 3-Dideoxy-ß-L-glycero-pent-2-enofuranosyl]thymine

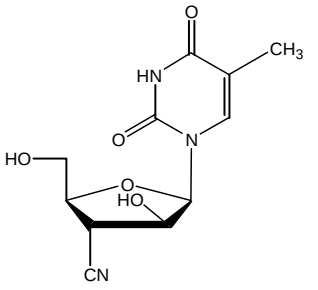


1-[2-C-cyano-2,3-Dideoxy-ß-L-glycero-pent-2-enofuranosyl]thymine

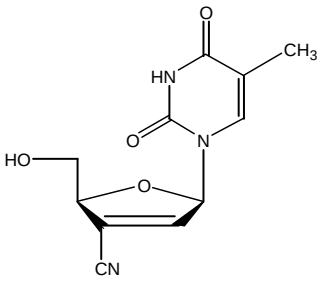


1-(3'-C-Cyano-3'-deoxy-ß-L-arabino-pentofuranosyl]thymine

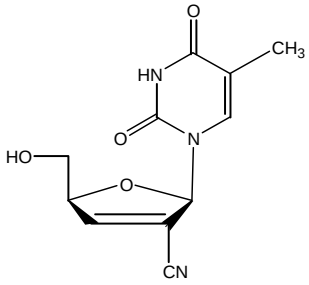
Figure 2.1 Synthesized Compounds



I



II



III

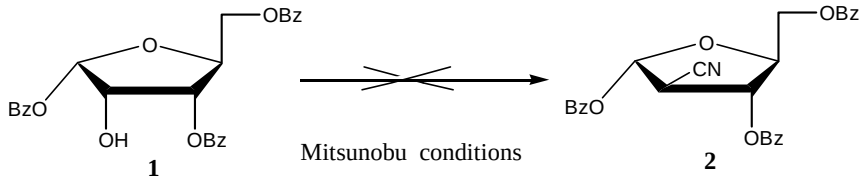
Figure 2.2 Corresponding D-enatiomers

Discussion and Results

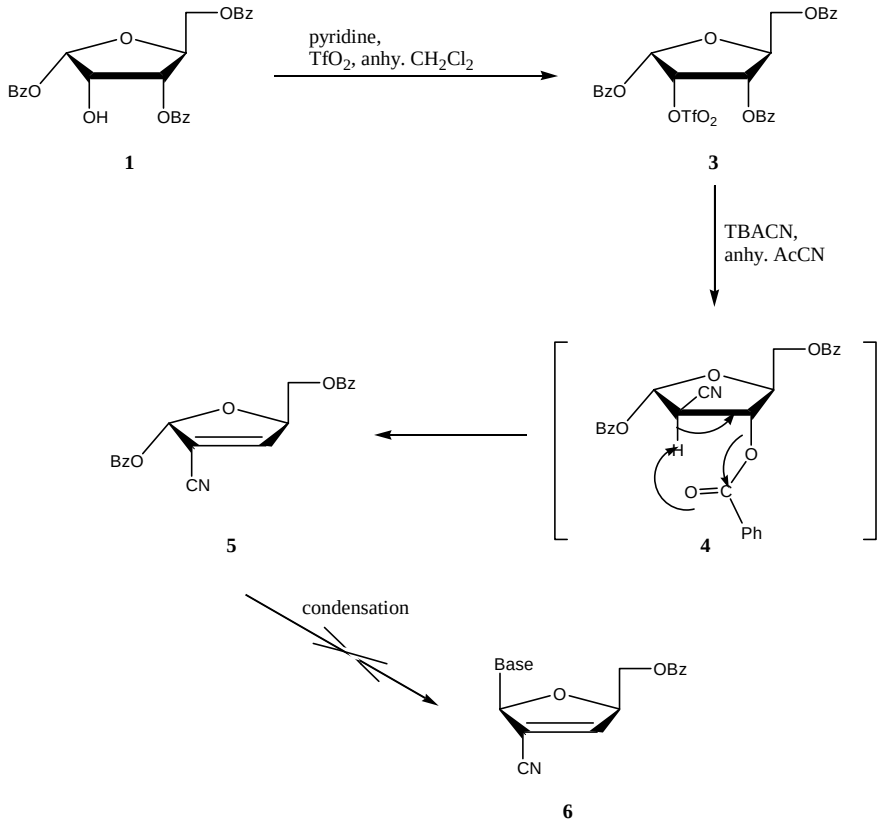
Strategy A (Scheme 2.1)**.** Mitsunobu reaction conditions [8-12] in were employed as the preliminary method, since good yields from use of this SN2 type substitution reactions were observed by Chu and Co-workers towards the synthesis of 2'-substituted-flouro-L-ribofuranosyl carbohydrate moieties by use of 1,3,5-tri-O-benzoyl-ribofuranosyl **1** as the starting material.[13-15] However, the Mitsunobu reaction conditions failed to provide key intermediate **2,** because the primary reagent sodium cyanide proved to be too weak.

Strategy B (Scheme 2.2). Methods were explored that would provide a good leaving group as well as non-acidic conditions, which would not cause 1,3,5-tri-O-benzoyl-ribofuranosyl to decompose. Further exploration led to Fleet *et* al's method of using tetrabutylammoniumcyanide (TBACN) in the SN2 substitution of the cyano group into the 3'-position of the pentose-carbohydrate moiety.[16] To explore this method, a triflic anhydride intermediate **3** was synthesized. The method failed to provide compound **2**, but provided a 2'-C-Cyano-2'3'-dideoxy-2'3'-didehyro-L-ribo-pento carbohydrate moiety **5** via the leaving of 3'-*O*-benzoyl induced by an acidic 2'-proton. The condensation of compound **5** by common methodologies for carbohydrate moiety condensations involved the use of a Lewis acid and an electrophilic base in conjunction with a 1-*O*-alkyl/acyl sugar moiety, which undergoes direct nucleophilic displacement or oxonium ion formation.[17] These methods of condensation, however, failed towards the synthesis of 2'-C-Cyano-2'3'-dideoxy-2'3'-didehydro-L-nucleoside analogues **6**, due to unsuccessful condensation reactions under various conditions.

Strategy C (Scheme 2.3). This method explored method of completing a 2'-CN addition into a nucleoside to create nucleoside analogue 13b as well as its 3'-C-cyano isomer 13a and its analogue 14. We focused on first synthesizing 5-methyl-L-uridine[18] (**9, scheme II**), which could be potentially more active than uridine as well as to avoid the problems associated with the reactive N^4 position of cytidine towards the key intermediate, epoxide 11. We proceeded from commercially available L-ribose via an



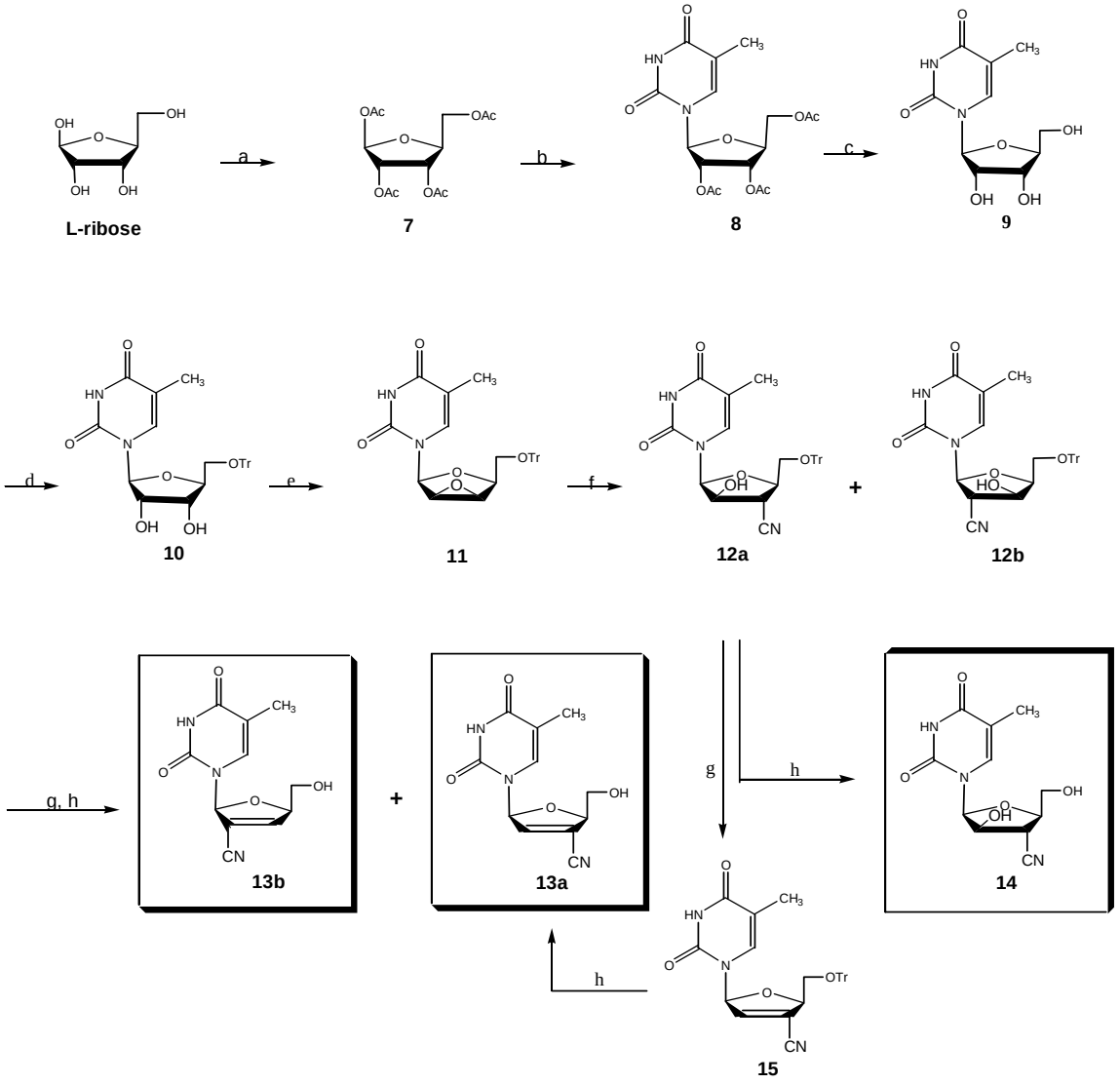
Scheme 2.1 Strategy A



Scheme 2.2 Strategy B acetylation method[19] to produce 1, 2, 3, 5-tri-*O*-acetyl-β-L-ribofuranose **7** in 74.5 % yield. The introduction of the β-N-glycoside bond was completed by the method reported by by Mayroud and Strazewski[20] or Vorbrüggen *et* al, [21, 22] to produce 2, 3, 5-tri-*O*-acetyl-β-L-5-methyl-uridine **8** in 92.1% yield. Compound **8** was treated with NH_3-CH_3OH to give 5-Methyl-L-uridine **9** in 98.0% yield, and then the 5'-OH was protected by use tritylation methodology[23-25] to provide compound **10** in high yield. Subsequently, key epoxide intermediate **11** was also prepared by methods previously reported.[23-25] Faul *et* al.[24] used two procedures towards the epoxide ring opening reaction with varying conditions by the use of LiCN or Et_2AlCN. Using Et_2AlCN as the reaction reagent, we obtained a mixture of both 2'- and 3'-C-cyano isomers in a ratio of 5:1 of compounds **12a** and **12b** in 82% overall yield. The 2'-isomer was found to be relatively unstable and inseparable.

In spite of this, relatively pure fractions of compound **12a** were obtained. Hence, we were able to synthesize 1-[3-C-Cyano-2, 3-Dideoxy-β-L-*glycero*-pent-2-enofuran-osyl]thymine **13a** in 86% yield via 2'-deoxygenation[25, 26] of intermediate **12a** and subsequent detritylation of compound **15** by use of BF_3-OEt_2. Uncontaminated fractions of **12a** were also deprotected to provide the 2'-OH congener of **13a**, 1-(3'-C-Cyano-3'-deoxy-β-L-*arabino*-pentofuranosyl]thymine **14** in 89% yield. A partially separated mixture containing the 2'-C-cyano nucleoside **12b** and **12a** was detritylated and found to also be inseparable. Consequently, we were not able to obtain the 3'-OH congener of **13b.** However, this mixture was deoxygenated by conditions described for related nucleosides[25, 26] to obtain a mixture of nucleosides **13a** and **13b** from which 1-[2-C-

Cyano-2, 3-Dideoxy-β-L-*glycero*-pent-2-enofuranosyl]thymine **13b** was successfully separated and acquired in 13% yield.



Reagents: **(a)** 1.) H_2SO_4, CH_3OH , r.t 2.) pyridine, Ac_2O r.t. 3. Acetic acid, Ac_2O, r.t. **(b)** BSA, Thymine, CH_3CN, TMSOTf, 60-65 ^oC **(c)** sat. NH_3/CH_3OH **(d)** pyridine,TrCl **(e)** 1.) MsCl, Et_3N, THF, -5-5 ^oC 2.) 5 *N* NaOH, CH_2Cl_2, 40 ^oC **(f)** Et_2AlCN, THF, 80 ^oC **(g)** MsCl, DMAP, CH_2Cl_2, reflux **(h)** BF_3-OEt_2/CH_3OH, CH_2Cl_2, r.t.

Scheme 2.3 Strategy C: The Synthesis of 1-[3-C-cyano-2, 3-Dideoxy-β-L-*glycero*-pent-2-enofuranosyl]thymine **13a**; 1-[2-C-cyano-2, 3-Dideoxy-β-L-*glycero*-pent-2-enofuranosyl]thymine **13b** and 1-(3-C-Cyano-3-deoxy-β-L-*arabino*-pentofuranosyl]thymine **14**

Summary

Attempts made to synthesize a series 2'-C-cyano-L-nucleosides and 2'-C-cyano-2', 3' unsaturated- L-nucleoside analogues were not successful by use of Schemes 2.1 and 2.2. Scheme 2.3 proceeded with difficulties in the separation of intermediates **12a** and **12b** as well as with the subsequent purification of their corresponding detritylated products**.** Thus, we were not able to obtain 2'-C-cyano-5-methyl-L-uridine, **13b's** congener. Overall, **13a**; **13b** and **14** were prepared in fair yields using a tritylated epoxide (**11)** derived from L-5-methyl-uridine. Data obtained in these syntheses is presented and is relative to the referenced D-enantiomers. On the other hand, in vitro biological activity data for nucleosides **13a**, **13b** and **14** currently has not been returned to our group.

Experimental Section

General. The reactions were performed under a nitrogen atmosphere and all reagents were used as received unless noted otherwise. TLC was performed on Analtech Co. Uniplates (silica gel). Column chromatography was performed using silica gel 60 (220-440 mesh) or silica gel G (TLC grad>440 mesh) for vacuum column chromatography. Melting points were determined on a Mel-temp II and are uncorrected. ^{1}H NMR spectra at 400 MHz and ^{13}C NMR spectra at 100 MHz were obtained by use of a Brüker 400 AMX spectrometer. NMR chemical shifts (δ) are reported in units parts per million (ppm) referenced to TMS or residual proton signals in the deuterated solvents as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or nm (narrow multiplet). Mass spectra were either recorded under fast atom bombardment

(FAB), on a Micromass Autospect high-resolution mass spectrometer unless noted. Electron spray ionization (ESI) high-resolution mass spectrometry obtained from the University of Georgia Chemistry Department's Mass Spectrometer Laboratory. Optical rotations calculation were performed on a Jasco DIP-370 Digital Polarimeter. UV spectra were obtain on a Beckman DU-650 spectrophotometer. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

2-Trifluoromethanesulfonyl-1, 3, 5-tri-O-benzoyl-L-ribofuranose (3). Compound **1** (Scheme 2.2) (1 g, 2.17 mmol) was dissolved into anhydrous methylene chloride (15 ml) and cooled to -50° C under a nitrogen atmosphere. Upon cooling, the solution was treated with pyridine (2 ml) and subsequently with triflouromethanesulfonic anhydride (0.91 g, 3.24 mmol). The reaction mixture was allowed to stir for 40 min from –50°-0° C, then the mixture washed with of water (20 ml), extracted with ethyl acetate (3 x 50 ml) and concentrated by rotary evaportion. The residual material was purified on silica gel column using (EtOAc: hexanes, 0.3:0.7). Compound **3** was obtained as a pale yellow oil 1.29 g (77.5%). ^{1}H NMR ($CDCl_3$) δ (ppm) 7.29-8.17 (15 H, m, Ar-H), 6.90 (1 H, s, H-1), 5.82 (1H, s, H-2), 5.59 (1 H, s, H-3), 5.90 (1 H, s, H-4), 4.80-4.64 (2 H, m, H-5'. H-5''). . Anal. Calcd. for $C_{27}H_{21}F_3O_{10}S{\cdot}H_2O$: C, 54.65; H, 3.56; found C, 53.90; H, 3.69

2-C-Cyano-2, 3-deoxy-2, 3- didehydro-1, 5, di-O-benzoyl-L-ribofuranose (5). Compound **5** (580 mg, 0.976 mmol) was dissolved into anhydrous acetonitrile (25 ml) at r.t. To this, mixture tertiarybutylammoniumcyanide [TBACN] (1.57 g, 5.92 mmol) was added. The solution was allowed to stir for 6 hrs at r.t. Upon which the solvent was removed and the concentrate mixed in a saturated $NaCO_3$ (25 ml) solution and brine (25 ml). This mixture was washed with ether (3 x 50 ml). The organic phase was then

concentrated and purified by silica gel on a vacuum column (EtOAc: hexanes, 1:9) upon to obtain a orange oil (150 mg, 34% yield) was obtained. $[\alpha]^{25}_{D}$ –40.8. Spectral data: ($CDCl_3$) δ (ppm) 7.96-7.38 (10 H, m, Ar-H), 6.07 (1 H, t, H-3, $J_{3',4'}$=1.8 Hz), 6.02(d, J=2.96, 1H, H-1), 6.02 (t, J=3.3, J=6.3 Hz, 1 H, H-3), 4.97 (m, 1 H, H-4), 4.57 (m, 2 H, H-5', H5''): FABMS *m/z* Calcd. 350.1028 $(M+H)^+$; found 350. 1011 $(M+H)^+$: IR (Nujol) –CN 2240 cm^{-1}: Anal. Calcd. for $C_{20}H_{15}NO_5 \cdot 0.1$ hexanes: C, 69.12.; H, 4.62; N, 3.91; found C, 69.31; H, 4.58; N, 3.91.

1,2,3,5-Tri-*O*-acetyl-β-L-ribofuranose (7). **L-ribose** (32.0 g, 0.21 mol) in anhydrous methanol (500 ml) and sulfuric acid (7.5 ml) was stirred at –10˚ to 5˚ C for 13 hrs. Anhydrous pyridine (100 ml) was added to neutralize the solution and subsequently removed via reduced pressure rotary evaporation providing a stiff syrup. The syrup was dissolved into anhydrous pyridine (100 ml) and cooled to 0˚ C. Acetic anhydride (200 ml) was slowly poured into this solution via syringe and the mixture was allowed to stir at room temperature for 60 hrs. The pyridine was removed via reduced pressure rotary evaporation providing pyridine free syrup. This syrup was dissolved into acetic acid (300 ml), treated with acetic anhydride (70 ml) and sulfuric acid (15 ml) at 0˚ C and stirred at room temperature for 24 hrs. This reaction mixture was subsequently neutralized with sodium hydrogen carbonate until the evolution of carbon dioxide ceased while maintaining the temperature below 30˚ C. The product was extracted with CH_2Cl_2 (5 x 100 ml). The organic layer was washed with brine (2 x 100 ml) and condensed by reduced pressure rotary evaporation to form syrupy solid, which was seeded with product crystals and kept in a refrigerator for 24 hours. The resulting solid was washed with ice-cold ethanol to remove the impurities to give a white crystalline solid (35.0 g, 74.5%

yield): Mp 83-84˚ C: $[\alpha]^{26}_{D}$ 11.9˚ (c 1.0, MeOH). Spectral Data: ^{1}H NMR ($CDCl_3$) δ (ppm) 6.15(s, 1H, H-1'), 5.32 (m, 2H, H-2' and H-3'), 4.38-4.30 (m, 2H, H-5', and H-4'), 4.13 (m, 1H, H-5''), 2.12 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.06 (s, 3H, CH_3): ^{13}C NMR δ ($CDCl_3$) 20.8 (2C, CH_3)), 21.0 (CH_3), 21.3 (CH_3), 63.9 (C-5'), 70.8 (C-3'), 74.4 (C-2'), 79.6 (C-4'), 98.5 (C-1') 169.3 (C=O), 169.7 (1C, C=O), 170.0 (C=O), 170.7 (C=O): FABMS *m/z* calc. 259.0818 $[M+H-AcOH]^+$; found 259.0821 $[M+H-AcOH]^+$: Anal Calcd for $C_{13}H_{18}O_9$: C, 49.06; H, 5.70. Found: C, 49.04; H, 5.71.

2, 3, 5-tri-*O*-acetyl-β-L-5-methyluridine (8). N, O-Bis(trimethylsilyl)acetamide [BSA] (43.45 ml, 0.18 mol) was added at room temperature to a mixture of compound **7** (18.65 g, 0.059 mol) and thymine (22.13 g, 0.33mol) in anhydrous CH_3CN (120 ml) and stirred under argon for 6 hrs at 65-70^{o} C under an argon atmosphere. TMSOTf (19.50 g, 0.088 mol) was slowly added to the reaction mixture, which was allowed stir for 16 hrs at 65-70^{o} C under an argon atmosphere. The reaction mixture was cooled to room temperature and subsequently quenched with saturated $NaHCO_3$ and stirred until the evolution of carbon dioxide ceased. The resulting mixture was diluted CH_2Cl_2 (250 ml). The mixture was filtered and the solid washed with CH_2Cl_2 (2 x 100 ml). The filtrate was washed with brine (2 x 100 ml). Subsequently, the organic layer was dried with $MgSO_4$. The organic solvent was removed via reduced pressure rotary evaporation and the resulting product was purified by silica gel column chromatography (CH_2Cl_2/MeOH 9:1). Compound **8** was afforded as a hygroscopic white crystalline foam (23.22 g, 91.2%). Mp 60˚ C: $[\alpha]^{27}_{D}$ -56.8˚ (c 1.0, CH_2Cl_2): Spectral Data: ^{1}H NMR ($CDCl_3$) δ (ppm) 9.03 (s, NH), 7.32 ((s, H-6), 6.14(d, J=3.7,1H, H-1'), 5.40-5.35 (m, 2H, H-2' and H-3'), 4.43-

4.38 (m, 3H, H-4', H-5', and H-5''), 2.19 (s, 3H, CH_3), 2.15(s, 3H, CH_3), 2.09 (s, 3H, CH_3), 1.99 (s, 3H, 5-CH_3): ^{13}C NMR ($CDCl_3$) δ (ppm) 12.64 (5-CH_3), 20.3 (CH_3)), 20.5 (CH_3), 20.7 (CH_3), 63.2 (C-5'), 70.2 (C-3'), 72.5 (C-2'), 79.7 (C-4'), 86.9 (C-1'), 110.5 (C-5), 111.92 (C-6), 134.9 (C-4), 150.4 (C-2), 163.4 (C=O), 169.6 (C=O): UV λ_{max} 263 nm (CH_2Cl_2): FABMS *m/z C*alcd. 385.1247 $[M+H]^+$; found 385.1265$[M+H]^+$: Anal Calcd for $C_{16}H_{12}N_2O_9$: C, 49.06; H, 5.70. Found C, 49.04; H, 5.71.

5-Methyl-L-uridine (9). Compound **8** (17g, 6.58 mmol) was stirred in saturated NH_3/CH_3OH until the TLC indicated that the reaction had completed. Upon completion, the solvent removed via reduced pressure rotary evaporation. The resulting solid/syrup was purified via a silica gel column (1:9, MeOH/CH_2Cl_2) to give 11.3g (98.0%) of a white solid. mp 182-184 C (Lit.[18] mp 183-184 ˚ C): $[\alpha]^{27}_D$ -13.2˚ (c 1.0, MeOH): Spectral Data: ^{1}H NMR (D_2O) δ (ppm) 7.8 (d, J= 7.3, H-6), 7.7 (br. s, NH), 5.9 (d, J=3.2, 1H, H-1'), 4.34 (t, 1H, J=4.8, H-3'), 4.23 (t, 1H, J=5.2, H-2'), 4.11 (m, 1H, H-4'), 3.91 (dd, 1H, $J_{5'b,5'a}$=12.5, $J_{5'b,4'}$=2.8, H-5'b), 3.81 (dd, 1H, $J_{5'a,5'b}$=12.7, $J_{5'a,5'b}$=2.5, H- 5'a), 2.91 (t, 1H, J=8.0, 0H-5'), 1.76 (m, 1H, OH-2'), 0.62 (t, 1H, J=8.4, OH-3'): ^{13}C NMR (D_2O) δ (ppm) 14.3 (5-CH_3), 57.1 (C-5'), 63.4 (C-3'), 72.1 (C-2'), 86.8 (C-4'), 91.7 (C-1'), 114.0 (C-5), 140.0 (C-6), 154.0 (C-2), 169.1 (C-4): UV λ_{max} 266 nm (MeOH): FABMS *m/z C*alcd. 259.0930 $[M+H]^+$; found 259.0929 $[M+H]^+$.

5'-*O*-(Triphenylmethyl)-5-methyl-L-uridine (10). A mixture of triphenylmethyl chloride (11.2 g, .40.7 mmol) and compound **9** (8.4g, 32.5 mmol) and pyridine (60 ml) was heated at 75˚-80˚ C for 2.0 hrs and cooled r.t. After completion of the reaction, the pyridine was removed via reduced pressure rotary evaporation. The residual material was diluted with CH_2Cl_2 (100 ml) and cooled to 0˚ C. The acidity of the mixture was

adjusted to pH 4 with use of 6 N HCl while maintaining the temperature below 10˚ C. The organic layer was washed with saturated aqueous $NaHCO_3$ (50 ml) and brine (100 ml) and dried with $MgSO_4$. The solvent was removed by rotary evaporation to give 11.3g (89.0%) of a pale yellow solid. Mp 182-184˚ C (lit**.** D-*enatiomer* 181-184˚ C): $[\alpha]^{25}_D$ 18.2˚ (c 1.0, CH_2OH). Spectral Data: ^{1}H NMR (CD_3OD) δ (ppm) 10.32 (br s, 1H), 7.21 (d, J=8.0 Hz, 1H), 6.63-6.47, m, 15H), 5.08 (br s, 1H), 4.57 (br d, 1H), 4.25 (br d, 1H), 3.42 (br s, 3H), 3.23 (br s, 2H), 1.77 (s, 3H); ^{13}C NMR(CD_3OD) δ (ppm) 12.0, 64.5, 71.9, 75.6, 85.0, 88.7, 90.0, 111.7, 128.5 (3C) 129.0 (6C), 130.0 (6C), 137.5, 137.7, 144.9 (3C), 163.0; UV λ_{max} (MeOH) 263 nm; FABMS *m/z C*alcd 501.2026 $[M+H]^+$; found 501.2360 $[M+H]^+$; Anal Calcd for $C_{29}H_{28}N_2O_6 \cdot 0.25\ CH_2Cl_2$:. C, 67.33; H, 5.51; N, 5.30. Found: C, 67.38; H, 5.84; N, 5.16.

1-[2, 3-Anhydro-2, 3-dideoxy -5'-*O*-trityl-(*β*-L-lyxofuranosyl)]thymine (11).

Compound **10** (11.5 g, 22. 9 mmol) was stirred in THF (70 ml). This mixture was cooled to –30˚ C upon which Et_3N (12.0 g, 0.19 mol) was added to the solution. The reaction mixture was allowed to warm to from -10˚ to 0˚ C and subsequently MsCl (37 g, 0.32 mol) was added drop wise to the reaction mixture over .75 hr. The reaction mixture was stirred for an additional 2 hrs. CH_2Cl_2 (100 ml) was stirred into the reaction mixture for 5 min, the organic was separated and condensed by rotary evaporation. Then EtOAc (100) along with a 5 *N* solution of NaOH (20 ml) solution was added and the resultant two-phase reaction mixture was allowed to stir at 40˚ C for 13 hrs. The pH of the aqueous phase was adjusted to 0.8 with 3.0 *N* HCl. The phases were separated and the organic fraction was washed with brine, concentrated to an oil by rotary evaporation and diluted with EtOAc (50 ml). The resulting suspension was stirred for 30 min at 50˚ C and the

precipitated product was filtered, rinsed with EtOAc and dried *in vacuo* to give 9 g (81%) of compound **11.** m.p. 132-136 (lit.[23] **D**-enatiomer 130-134˚ C$[\alpha]^{29}_{D}$ -17.13 (c 0.5, CH_3OH): ^{1}H-NMR (CD_3OD) δ (ppm) 7.75 (s, H-6), 7.09 (dd, 1H, $J_{1',2'}$=1.4 Hz, $J_{2',4'}$=3.9 Hz, H-2'), 6.90 (t, 1H, $J_{1',4'}$=1.6 Hz, H-1'), 4.85 (m, 2H, 5'-OH, NH, D_2O exchange·CD_3OD), 5.00 (m, 1H, H-4'), 3.88 (s, 2H, H-5', H-5''), 1.84 (s, 3H, 5-CH_3); ^{13}C-NMR δ (ppm) (CD_3OD) 166.378 (C-2), 152.48 (C-4), 142.57 (C-6), 138.61(d, J-=19.3 Hz, C-3'), 120.26 (3'-CN), 113.27 (C-5), 111.83 (C1'), 90.71 (d, J=13.4 Hz, C-2'), 88.29 (d, J=17.0 Hz, C-4'), 62.04 (C-5'), 12.40 (5-CH_3); IR(CH_3OH film) 2260 (CN); UV (H_2O) λ_{max} 263.0 nm (ε 13400) (pH 7), λ_{max} 263.0 nm (ε 14400) (pH 2), λ_{max} 266.5 nm (ε 2800) (pH 11); ESI-MS m/z 250 (M + H) [calcd 250 (M + H)]. .

1-[3-C-Cyano-3-deoxy-5-*O*-triphenylmethyl-(β-L-arabinofuranosyl)]thymine (12a) and its 2'-isomer; 1-[2-C-Cyano-2-deoxy-5-*O*-triphenylmethyl-(β-L-arabinofuranosyl)]thymine (12b). Diethylaluminumcyanide (1.0 **M** in toluene**,** 48.3 mmol) was added dropwise to over 30 min. to a slurry of compound **11** (9.3 g, 19.3 mmol) in THF (60 ml) while maintaining the temperature be 10º C. The mixture was heated at reflux an 75º C for 3 hrs and cooled to 0º C. To the solution ice cold 2 **N** HCl (100 ml, 85.1 mmol) and EtOAc (100 ml) were slowly pour into the solution. The mixture was allowed to reach r.t. and stirred for 30 min. The aqueous layer was separated and the organic layer was washed with 1 **N** HCl (2 x 100 ml) and brine (2x100 ml). The organic layer was dried with $MgSO_4$. Removal of the solvent provided **12a** and **12b** 7.8 g in 82% overall yield as a orange syrup in approximately a 5:1 ratio respectively from which 2'-isomer **12b** was inseparable except for a minuet amount obtained from preparative TLC (EtOAc/Hexane 0.1~1:1.0) used for ^{1}H NMR

characterization. Whereas, a larger amount of an **12a** (orange solid) was obtained by silica gel column chromatography (EtOAc/Hexane 1:1) whereas the 2'-isomer decomposed. Data for **12a**: m.p. 146-148 $[\alpha]^{27}_{D}$ –64.0 (c 1.0, CH_2Cl_2) (lit.[5] **D**-*enatiomer* physical data not reported). Spectral Data: ^{1}H NMR ($CDCl_3$) δ (ppm) 10.82 (br s, 1H, NH), 7.57-7.28 (m, 16H, Tr, H-6), 6.17 (d, J=5.0 Hz, 1H, H-1'), 5.09 (d, J=3.3 Hz, 1H, H-3'), 4.15 (dt, J-8.8 Hz, J=3.3 Hz, 1H, H-4'), 3.50 (dd, J=8.0 Hz, J=3.2 Hz, 1H, 2'-OH), 3.41-3.34(m, 3H, H-3', H-5', H-5''), 1.69 (s, 3H, 5-CH_3); ^{13}C NMR ($CDCl_3$) δ 12.0, 37.5, 62.0, 74.4, 77.7, 85.5, 87.7, 108.9, 117.6 (CN), 127.5 (3C), 128.8 (6C), 128.5 (6C), 138.7, 143.2 (3C),150.8, 165.3: UV λ_{max} (MeOH) 264 nm; FABMS *m/z C*alcd 501.2026 $[M+H]^+$; found 501.2360: Anal Calcd for $C_{30}H_{27}N_2O_5 \cdot 0.3\ CH_2Cl_2$:. C, 67.90; H, 5.19; N, 7.83. Found: C, 67.90; H, 5.27; N, 7.83. ^{1}H NMR ($CDCl_3$) data for the 2'-isomer **12b**: δ (ppm) δ 8.04 (br s, 1H, NH), 7.417-7.19 (m, 16H, Tr and H-6), 6.11 (s, 1H, H-1'), 4.09 (t, J=5.7 Hz, 1H, H-2'), 3.84 (dd, J=11.9 Hz, J=2.8 Hz, 2H, H-4'), 3.41 (n m, 3H, H-3',H-5'a, 3'-OH), 3.30(m, 2H, H-5'b), 1.69 (s, 3H, 5-CH_3).

1-[3-C-Cyano-2, 3-dideoxy-β-L-*glycero*-pent-2-enofuranosyl]thymine (13a).

Compound **15** (800 mg, 1.62 mmol) dissolved in CH_2Cl_2 (10 ml) and diluted with MeOH (25 ml) was cooled to 0° C. BF_3-OEt_2 (2 ml) was subsequently added to the cool solution, which was stirred at r.t. (2 hr) until the TLC indicated a completed reaction. Saturated $NaHCO_2$ was added to the solution and organic phase was dried with Na_2SO_4. The solvent was removed and the residual material was purified by silica gel chromatography (CH_2Cl_2/MeOH 100:1~50:1) to give compound **13a** (300 mg, 86%) as a white solid. mp 200-202° C (lit.[26] mp 200-201° C, **D**-*enantiomer*); $[\alpha]^{29}_{D}$ -17.13 (c 0.5, CH_3OH): ^{1}H-NMR (CD_3OD) δ (ppm) 7.75 (s, H-6), 7.09 (dd, 1H, $J_{1',2'}$=1.4 Hz, $J_{2',4'}$=3.9

Hz, H-2'), 6.90 (t, 1H, $J_{1',4'}$=1.6 Hz, H-1'), 4.85 (m, 2H, 5'-OH, NH, D_2O exchange·CD_3OD), 5.00 (m, 1H, H-4'), 3.88 (s, 2H, H-5', H-5''), 1.84 (s, 3H, 5-CH_3); ^{13}C-NMR (CD_3OD) δ (ppm) 166.378 (C-2), 152.48 (C-4), 142.57 (C-6), 138.61(d, J-=19.3 Hz, C-3'), 120.26 (3'-CN), 113.27 (C-5), 111.83 (C1'), 90.71 (d, J=13.4 Hz, C-2'), 88.29 (d, J=17.0 Hz, C-4'), 62.04 (C-5'), 12.40 (5-CH_3); IR(CH_3OH film) 2260 (CN); UV (H_2O) λ_{max} 263.0 nm (ε 13400) (pH 7), λ_{max} 263.0 nm (ε 14400) (pH 2), λ_{max} 266.5 nm (ε 2800) (pH 11); ESI-MS m/z 250 (M + H); calcd 250 (M + H). Anal.Calcd. for $C_{11}H_{11}N_3O_4$·C, 53.01: H, 4.45: N, 16.86. Found C, 52.97: H, 4.51: N, 16.53.

1-[2-C-Cyano-2, 3-dideoxy-β-L-*glycero*-pent-2-enofuranosyl]thymine (13b). MsCl (2 eq., 460 µl) was added to a cooled (0º C) solution of compounds **12a** and **12b** (1.62 g, 3.2 mmol, approximately a 5:1 mixture, respectively) along with DMAP (1g, 8.2 mmol) in CH_2Cl_2 (30 ml) and stirred for 1 hr. The reaction mixture was refluxed for 2 hr and the solution cooled to r.t. Water (50 ml) was added to the solution and the phases were separated. The aqueous phase was washed with CH_2Cl_2 (2x30 ml). The organic phases were combined, dried with Na_2SO_4 and the solvents were removed via rotary evaporation. Due to the inseparability, the reduced products were dissolved in CH_2Cl_2 (10 ml) and MeOH (50 ml) was added. The solution was cooled to 0º C and BF_3-OEt_2 (2 ml) was subsequently added. The solution was stirred at r.t. (2 hr) until the TLC indicated a completed reaction. Saturated $NaHCO_2$ was added to the solution and organic phase was separated and dried with Na_2SO_4. The solvent was removed and the residual material was purified via silica gel chromatography (CH_2Cl_2/MeOH 100:1~50:1~30:1~20:1) to remove **13a** (330 mg, 42%) and give compound **13b** (100 mg, 13%, crystallized CH_3OH -Hexane) as a white solid: mp 190-192° C (lit.[3] mp 191-192, **D**-*enantiomer*); $[\alpha]^{29}_D$ -

25.53 (c 0.5, CH_3OH); ^{1}H-NMR (DMSO-d_6) δ (ppm) 11.32 (s, 1H, NH), 7.59 (d, 1H, $J_{6,Me}$=1.1 Hz, H-6), 7.42 (t, 1H, $J_{1',3'}$=1.7 Hz, H-1'), 6.81 (dd, 1H, $J_{3',1'}$=1.7 Hz, $J_{3',4}$=3.8 Hz, H-3'), 5.10 (t, 1H, J=4.7 Hz, 5'-OH), 4.85 (m, 1H, H-4'), 3.50 (ddd, 1H, $J_{5'a,4'}$=2.7 Hz, $J_{5'a,OH}$=4.2 Hz, $J_{a,b}$=13.5 Hz), 3.45 (ddd, 1H, $J_{5'b,4'}$=2.7 Hz, $J_{5'b,OH}$=4.2 Hz, $J_{b,a}$=13.5 Hz) 3.66 (m, 2H, H-5', H-5''), 1.54 (d, $J_{6,Me}$=1.1 Hz, 3H, 5-CH_3); ^{13}C-NMR (CD_3OD) δ (ppm) 169.06 (C-6), 155.49 (C-4), 154.61 (d, J=30, C-6), 140.4 (d, J=47, C-2'), 116.12 (CN), 115.78 (C-5), 115.51 (C-1'), 92.86 (C-3'), 92.58 (C-4'), 85.88 (C-5'), 15.37 (5-CH_3); IR(CH_3OH-film) 2230 cm^{-1} (CN); UV (H_2O) λ_{max} 263.0 nm(ε 9200) (pH 7), λ_{max} 263.0 nm (ε 8900) (pH 2), λ_{max} 262.5 nm (ε 6800) (pH 11); ESI-MS m/z 250 (M + H); calcd 250 (M + H). Anal.Calcd. for $C_{11}H_{11}N_3O_4$; C, 53.01: H, 4.45: N, 16.86. Found C, 53.07: H, 4.50: N, 16.73.

1-(3-C-Cyano-3-deoxy-β-L-*arabino*-pentofuranosyl]thymine (14). Compound **12a** (530 mg, 1.04 mmol) dissolved in CH_2Cl_2 (10 ml) and diluted with MeOH (25 ml). The solution was cooled to 0º C. BF_3-OEt_2 (2 ml) was subsequently added to the cool solution, which was stirred at r.t. (2 hr) until the TLC indicated a completed reaction. Then saturated $NaHCO_2$ was added to the solution and organic phase was separated and dried with Na_2SO_4. The solvent was removed and the residual material was purified via silica gel chromatography (CH_2Cl_2/MeOH 100:1~50:1) to give compound **14** (261 mg, 94%)as a white solid. mp 241-242º C (lit.[7] 243º C, **D**-*enantiomer*); $[\alpha]^{26}_D$ -15.98 (c 0.98, DMSO), $[\alpha]^{26}_D$ -39.187 (c 0.57, CH_3OH),, [lit[27]. $[\alpha]^{20}_D$ 12.5 (c 0.92, DMSO), **D**-*enantiomer*); IR(CH_3OH-film) 2230 cm^{-1} (CN); ^{1}H-NMR (DMSO-d_6) δ (ppm) 11.17 (s, NH). 76.60 (s, 1H, H-6), 6.26 (d, 1H, J=6.5 Hz, H-1'), 6.13 (d, 1H, OH-2'), 5.36 (t, 1H, OH-5'), 4.80 (q, 1H, J=6.8 Hz, H-2'), 4.11 (d, 1H, J=13.8 Hz, H-4'), 3.77 (d, 1H, J=13,

H-5'), 3.65 (d, 1H, J=13, H-5'), 3.22-3.36 (m, 1H, H-3'), 1.77 (s, 3H, 5-CH_3): ^{13}C-NMR (DMSO-d_6) δ (ppm) 167.09 (C-4), 153.85 (C-2), 140.88 (C-6), 122.07 (3'-CN), 111.37 (C-5), 86.70 (C-1'), 81.30 (C-2'), 76.72 (C-4'), 62.98 (C-5'), 39.49 (C-3'), 15.55 (5-CH_3); UV (H_2O) λ_{max} 263.5 nm (ε 11 300) (pH 7), λ_{max} 263.5 nm(ε 6 200) (pH 2), λ_{max} 266.0 nm(ε 8 100) (pH 11); ESI-MS *m/z* 268 (M+H); calcd 268 (M+H). Anal.Calcd for $C_{11}H_{13}N_3O_5$: C, 49.44: H, 4.90: N, 15.72. Found C, 49.34: H, 4.90: N, 15.63.

1-[3-C-Cyano-2, 3-didehydro-2, 3-dideoxy- 5'-*O*-triphenylmethyl-(β-L-pentofuranosyl)]thymine (15). MsCl (2 mol. eq., 230 µl) was added to a cooled solution (0º C) of compound **12a** (820 mg, 1.61 mmol) along with DMAP (480 mg, 3.9 mmol) in CH_2Cl_2 (30 ml). The solution was stirred for 1 hr while maintaining 0º C. Following this stage of the reaction, the solution was refluxed for 2 hr and allowed to cool to r.t. Water (50 ml) was added to the solution and the phases were separated. The aqueous phase was wash with CH_2Cl_2 (2x30 ml). The organic phases were combined, dried with $NaSO_4$ and the solvents were removed via rotary evaporation. The residual material was via silica gel chromatography (CH_2Cl_2/MeOH 20:1) to give compound **15** (690 mg, 89%) as a yellow solid. m.p. 154-156 (lit.[5] no physical data given on **D-*enatiomer***): $[\alpha]^{25}_D$ -51.3 (c 1.0, CH_3OH): ^{1}H-NMR ($CDCl_3$) δ (ppm) 10.17 (s, 1H, NH), 7.47-7.18 (m, 16H, Tr, H-6), 6.78 (s, 1H, H-1'), 5.23 (s, 1H, 4'), 4.97 (m, 1H, H-2'), 3.57-3.51 (m, 2H, H-5', H-5''): ^{13}C-NMR ($CDCl_3$) δ (ppm) 163.4 (C-2), 152.5 (C-4), 142.5 3(C), 141.0 (C-2'), 135.4 (C-6), 129.6 [C(6)], 128.1 [C(6)], 127.6 [C(3)], 119.3 (CN), 112.4 (C-3'), 111.9 (C-5), 88.8 (C-1'), 87.5 [C(1)], 85.1 (C-4'), 62.7 (C-5'); UV (CH_2Cl_2) λ_{max} 263.0 nm; ESI-MS m/z 491 (M + H), calcd 491 (M + H): Anal.Calcd for $C_{30}H_{25}N_3O_4$: C, 73.30: H, 5.13: N, 8.55. Found C, 73.41: H, 5.39: N, 8.33.

.

References

1. Matsuda, A.; Nakajima, Y.; Azuma, A.;. Tanaka, M.; Sasaki, T. Nucleosides and Nucleotides, 141: 2'-C-Cyano-2'deoxy-1-β-D-arabinofuranosylcystosine (CNDAC) – Design of a Potential Mechanism-Based DNA-Strand-Breaking Antineoplastic Nucleoside. *J. Med. Chem*. **1991**, 34, 2919-22.
2. Matsuda, A.; Nakajima, Y.; Azuma, A.;. Tanaka, M.; Sasaki, T. Nucleosides and Nucleotides, 141: 2'-C-Cyano-2'deoxy-1-β-D-arabinofuranosylcystosine (CNDAC) – Design of a Potential Mechanism-Based DNA-Strand-Breaking Antineoplastic Nucleoside. *J. Med. Chem*. **1991**, 34, 2919-22.
3. Azuma, A.; Nakajima, Y.; Nishizono, N.; Minakawa, M.; Suzuki, M.; Hanaoka, K.; Kobayashi, T.; Tanaka, M.; Sasaki, T.; Matsuda, A. Nucleosides and Nucleotides 122: 2'-C-Cyano-2'deoxy-1-β-D-arabinofuranosylcystosine and its Derivatives. A. New Class of Nucleoside with a Broad Antitumor Spectrum. *J. Med. Chem*. **1993**, 36, 4183-4189
4. Velázquez, S.; Camarasa, M. –J. Synthesis of 2'-C-cyano-2', 3'-dideoxy-β-D-arabinofuranosyl Nucleoside. *Tetrahedron.* **1992,** 48, 1683-1694.
5. Matsuda, A.; Satoh, M.; Nakashima, H.; Yamamoto, N.; Ueda, T. Synthesis and Anti-HIV Activity of 3'-Cyano-2', 3'-dideoxythymidine and 3'-Cyano-2', 3'-didehydro-2', 3'-dideoxythymidine. *Heterocycles.* **1988,** 27, 2545-2548.
6. Race for Patents of relative of AZT. *New Scientist*. 23 July **1987,** p. 22

7. Herdewijin, P.; Balazarini, J, Babe, M.; Pauwels, R.; Aerschot, A. V.; Janssen, G.; De Clercq, E. Synthesis and Anti-HIV Activity of Different Sugar-Modified Pyrimidine and Purine Nucleosides. *J. Med Chem.* **1988**, 31, 2040-2048.

8. Mitsunobu, O. Review: The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products. *Synthesis.* **1981**, 1-28.

9. Csuk, R.; Thiede, G. Preparation of novel difluorocyclopropane nucleosides *Tetrahedron*. **1999,** 55, 739-750.

10. Chien Tun-Cheng; Chen Chien-Shu; Yeh Jun-Yen; Wang Kuang-Chao; Chern Ji-Wang. Nucleosides VII: Synthesis of N-Triphenylphosphoranylidene Nucleosides by Mitsunobu Reaction. A Novel Protecting Group for Primary Amines of Nucleosides *Tetrahedron Letters.* **1995,** 36, 7881-7884.

11. Harusawa, S.; Murai, Y.; Moriyama H.; Yoneda, H. O. R.; Kurihara, T. Stereoselective Synthesis of the C-4 Linked Imidazole Nucleosides Using Modified Mitsunobu Reaction. *Tetrahedron Letters*. **1995,** 36, 18, 3165-3168.

12. Saady, M.; L. Lebeau, L.; Mioskowski, C. Convenient "One-Pot" Synthesis of Chlorophosphonates, Chlorophosphates and Chlorophosphoramides from the Corresponding Benzyl Esters. *Tetrahedron Letters.* **1995,** 36, 13, 2239-242.

13. Ma, T.; Lin, J. –S; Newton, M. G.; Cheng, Y. -C.; Chu, C. K. *J. Med. Chem.* **1997**, 40, 2750-2754

14. Kotra L.P.; Xiang, Y; Newton, M. G; Schinazi R. F.; Cheng Y.-C.;. Chu, C. K. *J. Med. Chem.* **1997**, 40, 3635-3644

15. Ma, T.; Pai, S. B.; Zhu, Y, L.; Lin, J. S.; Shanmuganathan, K.; Du, J.; Wang, C.; Kim, H.; Newton, M. G.; Cheng, Y. –C.; Chu, C. K.; *J. Med. Chem.* **1996**, 39, 2835-2843.

16. Fleet, G. W. J.; Son, J. C.; Derome, A. E. Methyl 5-O-Tert-butyldiphenylsilyl-2-deoxy-αβ-D-Threo-Pentofuranoside as a Divergent Intermediated for the Synthes of 3'-Substistued-2', 3'-dideoxynucleosides: Synthesis of 3'-Azido-3'-deoxythymidine, 3'-Deoxy-3'-fluorothymidine and 3'-Cyano-3'-deoxythymidine. *Tetrahedron.* 1988, 44, 625-636

17. Wilson, L. J.; Hager, M. W.; Y. A.; Liotta, D. C. Nitrogen Glycosylation Reactions Involving Pyrimidine and Purine Nucleoside Bases with Furanoside Sugars. *Synthesis* **1995**, 1465-79.

18. Moyroud, E.; Biala, E.; Strazewski, P. Synthesis and Enzymatic Digestion of an RNA Nonamer in Both Enatiomeric Forms. *Tetrahedron.* **2000**, 56, 1475-1484

19. Guthrie, R. D.; Smith, S. C. 1, 2, 3, 5-*0*-acetyl-β-D-ribofuransose. *Biochemical Preparations.* **1971**, 1-3.

20. Moyroud, E.; Strazewski, P. L-Ribonucleosides from L-Xylose. *Tetrahedron.* **1999,** 55, 1277-1284.

21. Vorbrüggen, H.; Hofle, G. Nucleoside Syntheses: 23. On the Mechanism of Nucleoside Synthesis. *Chem Ber-Recl.* **1981,** 114, 1256-1268.

22. Vorbrüggen, H. Some recent rends and progress in nucleoside synthesis. *Acta. Biochim. Pol.* **1996**, 43, 25-36.

23. Huang, J. –T.; Chen, L. –C.; Wang, L.; Kim, M. –H.; Warshaw, J. A.; Armstrong, D.; Zhu, Q. -Y.; Chou, T. –C.; Wantanabe, K. A.; Matulic-Adamic, J.; Su, T. –S.;

Fox, J. J.; Polsky, P.; Baron, P. A.; Gold, J. W. M.; Hardy, W. D., Zuckerman, E. Fluorinated Sugar Analogues of Potential Anti-HIV-1 Nucleosides. *J. Med. Chem.* **1991,** 34, 1640-1646.

24. Faul, M. M.; Huff, B. E.; Dunlap, S. E.; Frank, S. A.; Fritz, J. E.; Kaldor, S. W.; LeTourneau, M. E.; Staszak, M. A.; Ward, J. A.; Wener, J. A.; Winneroski, L. L. Synthesis of 2', 3'-Dideoxy-3'-hydroxymethylcytidine; Aunique Antiviral Nucleoside. *Tetrahedron.* **1997**, 53, 8085-8104.

25. Yung, C. C.; Fox, J. J. Nucleosides 15. Synthesis of 1-β-D-Lyxofuranosylcytosine via Thiation of an Anhydronucleoside. *J. Org. Chem.* **1962**, 27, 1477.

26. Calvo-Meteo, A.; Camarasa, M. –J.; Díaz-Ortíz, A.; De las Heraś, R. G.; Alemany, A. Synthesis of 3'-C-Cyano-3'-deoxy-pentofuranosylthymine nucleosides. *Tetrahedron.* **1988,** 44, 4895-4903.

27. Barth, W.; Häbich, D. Synthesis of 3'-Cyano-3'-deoxy-β-D-*arabino*-nucleosides. *Synthesis.* **1988,** 943-947.

CHAPTER 3

COMPUTATIONAL STUDIES OF CNDAC: THE DFT ENERGY CALCULATIONS OF THE TWO CONFORMERS OF 2'-C-CYANO-2'-DEOXY-1- β -D-*ARABINO*-PENTOFURANOSYLCYTOSINE (CNDAC) AND 2'-C-CYANO-2'-DEOXY-1-β-D-*RIBO*-PENTOFURANOSYLCYTOSINE (CNDC) AND THEIR PRODUCTS FORMED BY GLYCOSYL BOND CLEAVAGE

Introduction

A density functional theory (DFT) method has been employed to study the epimerization of 2'-C-Cyano-2'-deoxy-1-β-D-*arabino*-pentofuranosylcytosine (CNDAC), also stated as 1-[2-C-Cyano-2-deoxy-β-D-arabinofuranosyl]cytosine to 2'-C-Cyano-2'-deoxy-1-α-D-*ribo*-pentofuranosylcytosine (CNDC) as well as their degradation to 1,4-anhydro-2-C-cyano-2-deoxy- D-*erytho*-pent-1-enitol (glycal) and cytosine by way of glycosyl bond cleavage. CNDAC and CNDC geometries were optimized to the C_1 point group while cytosine and glycal were optimized to C_s point group. The initial optimizations were performed at the AM1 semi-empirical theory. Cartesian coordinates of the AM1 optimized structures into GAUSSIAN 94 at the B3LYP level of theory using a 6-31G(d) basis set. The calculated energies and dipole moments were recorded. CNDAC 's calculated energy was found to be 2.50 kcal/mol greater than that of CNDC. CNDC's dipole moment was approximately 2.95 Debye greater than that of CNDAC. As products of glycosyl bond cleavage, cytosine and glycal energy values were observed as

-1.688356×10^5 and -2.025143×10^5 kcal/mol respectively. Their dipole moments were observed to be 6.3803 and 2.6372 Debye.

Frequently, nucleoside analogs are developed which have antiviral activity against various cell lines. Nevertheless, those particular compounds are later found to be inactive in vivo within the human body due to the slightly alkaline blood. As a precursor to future research, it may be advantageous to study nucleoside mechanisms quantum mechanically in determination of which nucleoside analogs will most likely undergo this cleavage rendering those compounds inactive. In retrospect CNDAC presents an interesting mechanism to be proved or disproved quantum mechanically.

Background

CNDAC was designed and synthesized by Matsuda *et* al. and hypothesized to be a potential DNA-strand-breaking nucleoside after showing quality anti-tumor activity.[1, 2, 3] Matsuda and Azuma were able to further demonstrate the hypothesis in a cell-free model system from which CNDAC was theoretically presented to exhibit a DNA-self-strand-breaking mechanism.[1] The cell-free model consisted of alkaline medium, from which they found that CNDAC readily eperimized to CNDC at ratio of 3:5 respectively and slowly decomposed to glycal and cytosine.[1] Deuterium incorporation experiments with CNDAC in D_2O buffer were performed. This experiment suggested that β-elimination occurs via an E1cB type reaction mechanism initiated through the presence of a highly acidic 2'-α-proton. Correspondingly, Azuma and co-workers observed CNDAC's epimerization and the conformers' subsequent degradation reactions in neutral media (pH 7.5) as well. [1]

As a carbohydrate nucleoside, CNDAC could be strongly affected by the anomeric effect and its other substituents which are a nucleophilic base (cytosine) bonded to the anomeric carbon and an electron withdrawing group (cyano) bonded to the 2'-position carbon within the ribose sugar moiety. One assumption is that the presence of cyano as a strong Lewis base at the 2'-position further stimulates CNDAC's epimerization and degradation. To evaluate the route of epimerization and degradation, the total energies of CNDAC, CNDC and products formed through glycosyl bond cleavage as observed by Matsuda and coworkers, were determined.

CNDAC and CNDC consist of thirty atoms each, which is considered an extremely large and difficult system to calculate due to the molecules size. Due to limited time, choosing a method proved to be somewhat difficult in relationship the molecules size. In perspective, Holmén and Broo[4] found that Hartree-Fock ab initio and semi-empirical methods used for DNA base geometry calculations differ significantly from those obtained experimentally. Full geometry optimizations using more expensive and time consuming experiments by implementation of Møller-Plesset pertubation theory (MP2) [6] with medium sized basis sets like 6-31G(d, p) [5] were employed. In respect to this information, Broo and Holmén compared their MP2 results with experimental data finding reasonable agreement for most DNA bases with that of Stewart *et* al.'s work.[7] Therefore, our first thought was to try MP2 and MP4 with various basis sets to obtain quality data.

After contemplating the amount of time it would take to complete these types of calculations for molecules **1**, **2**, **6** and **7** in Figure 3.1, we decided to explore other areas that led us to density functional theories (DFT). In doing so, we viewed Broo and

Holmén's work which compared a few DNA base calculations taken from the use DFT(B3LYP) with various basis sets and MP2/6-31G(d).[8] Both methods yielded relatively accurate geometry approximations in respect to 9-methyladenine's experimentally found geometry by neutron diffraction Overall, Broo and Holmén showed that both the MP2/6-31G(d) and the DFT(B3LYP)/6-31G(d) calculations give accurate geometries for a series of purines (Fig. 1)**.** Several studies other than Broo and Holmén's also relate the use of B3LYP functional. The B3LYP system consists of Becke's three parameter (B3) functional for the exchange[9] and the Lee, Yang and Parr[10] for the correlation part, and MP2 with the same basis sets yield similar geometries for medium size molecules.[11, 12] B3LYP functional has also been applied in the study of hydrogen bonding where it was shown it provides practical approximations of hydrogen bond lengths and bonding energies.[11,13] In relation, Sponer *et al.* [14, 15] reported that when DFT(B3LYP)/6-31G(d, p) theory was applied to nucleic acid base pairs, the hydrogen bond energies relatively agreed with MP2 calculations but found that DFT did not do well in describing base stacking interactions. Since these studies provided relative data in relation to energies and angles, the assumption taken is that B3LYP along with medium sized basis should provide relatively accurate data until crystal structure data are provided to compare the calculated angles.

Computational Methods

DFT(B3LYP) was evaluated as a useful tool for the investigation of large molecules. We employed its use in obtaining geometry optimizations, total energies and dipole moments of molecules **1**, **2**, **6** and **7** (Fig. 3.1)**.** The initial geometris optimizations

were performed at the AM1 semi-empirical level of theory within Spartan 5.l.[14] The cartesian coordinates obtained were further optimized in GAUSSIAN 94[15]

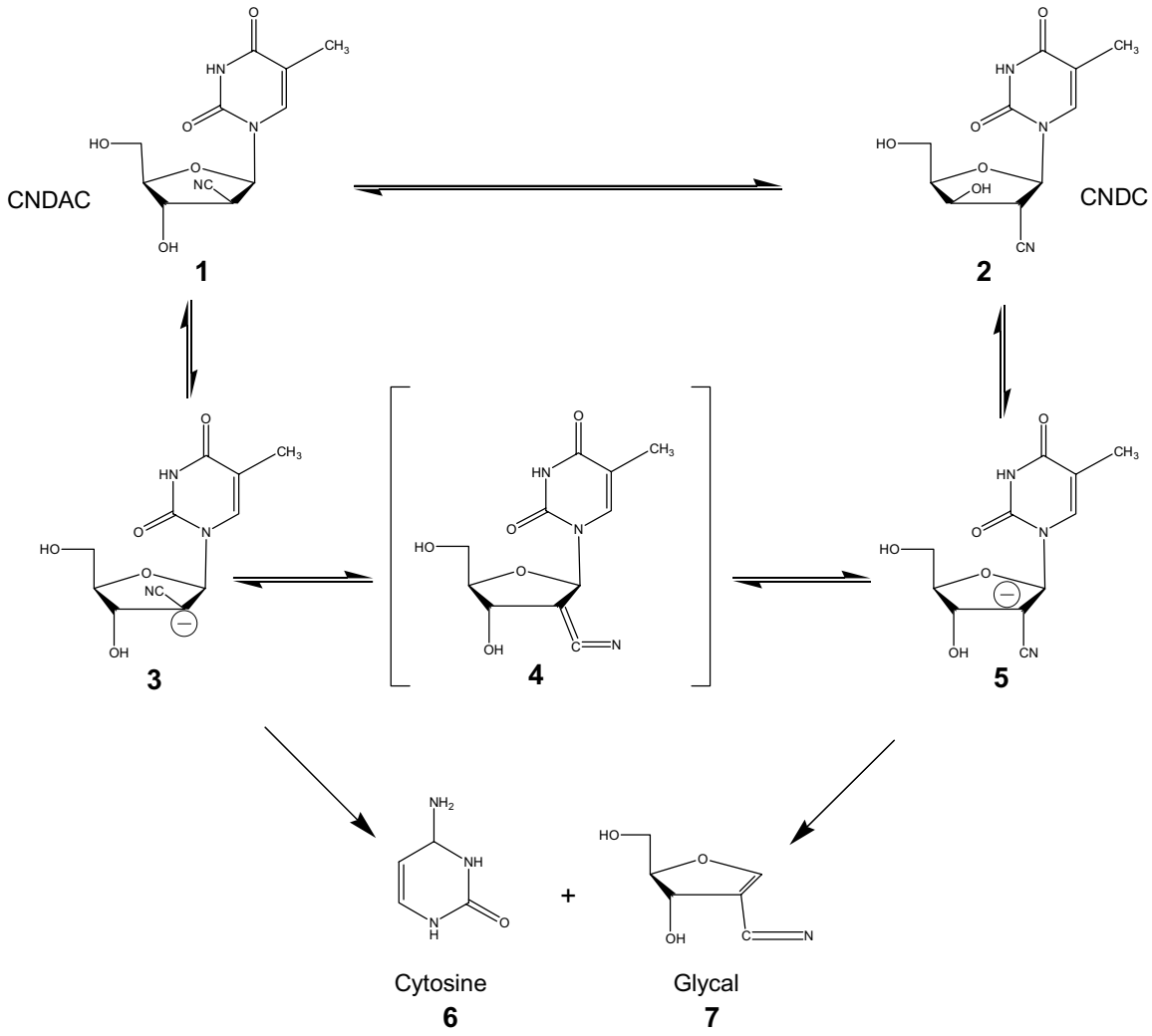


Figure 3.1 Azuma et *al*.'s proposed pathways for the equilibrium/epimerization and degradation of CNDAC and CNDC

using DFT to perform final geometry optimizations of CNDAC, CNDC, glycal and cytosine. Becke's three parameter (B3) functional for the exchange[9] and the functional Lee, Yang, and Parr [10] for the correlation part in combination with the 6-31G(d) basis set,

a split valence double zeta standard basis set augmented with a d-type polarization function, were utilized.[16] The 6-31G(d) basis set was used as the standard since the C_1 point group of nucleosides prohibits the use of large basis sets. GAUSSIAN94's default atomic radii values were used. Negative eigenvalues were not found within the Hessian's (force constant matrix) of the conformers, cytosine or glycal. Geometry optimizations and transition state calculations were performed on **3**,**4** and **5** (Fig. 3.1) using AM1 theory within Spartan 5.l.[14]

Results and Discussion

Implementation of DFT(B3LYP) functional for the calculations was due to the size and the nucleosides symmetry factor. The properties within the systems could be derived from the knowledge of the molecules electronic density as related to the size of CNDAC and CNDC. Particularly, the systems studied were large molecules and we were not concerned with the systems total energy property. The 6-31G(d) basis set was chosen due to its addition polarisation functions on heavy non-hydrogen atoms as well as the fact that the nucleosides concerned had no symmetry.

Table 3.1 Ground state calculations of geometry optimized molecules

Method	B3LYP/6-31G(d)	B3LYP/6-31G(d)	B3LYP/6-31G(d)	B3LYP/6-31G(d)
Molecule	CNDAC	CNDC	Cytosine	Glycal
Energy (au)	-908.147759	-908.1517456·	-394.927856·	-475.7065476·
μ, (D)	6.8875	9.8335	6.3803	2.6372

CNDAC ‘s calculated energy has been established to be 2.5 kcal/mol greater than that of CNDC. Since CNDC has a slightly lower heat of formation. In perspective, we find that CNDAC and CNDC are conformers due to their differences in energy being close in proximity. Correspondingly CNDC **2** is assumed quantum mechanically to the most stable of the conformers due to its lower energy, which corresponds to Azuma *et al.’s* kinetic study[3], which found CNDC **2** in greater concentration. The dipole moment of CNDC **2** was observed to be approximately 2.95 Debye greater than CNDAC’s **1**, indicating that CNDAC **1** has a smaller separation of charges. As products of glycosyl bond cleavage, cytosine and glycal energy values were observed as -1.69×10^5 and -2.03×10^5 kcal/mol respectively. Their dipole moments were observed at 6.38 and 2.64 Debye, respectively. Data related to any of these or similar molecules are not available presently. Therefore, we were not able to compare the calculated total energies and dipole moments of molecules **1**, **2**, **6** or **7** (Fig.3.1). Calculations were neglected for **3** and **5** (Fig. 1). Both structures are essentially resonance forms as compared to **4** (Fig. 3.1). AMI transition state calculations were performed on **4,** (Fig. 3.1) as a relative resonance form of **3** and **5** (Fig. 1)**.** The expectation was to find one of the resonant forms as a transition state molecule. Neither of the resonant forms was found to be a transition state molecule.

Our observation showed an indication that compounds **1** and **2** inter-converts by inversion, in the same manner as alkyl carbanions, or tertiary amines, thus giving rise to conformers **1** and **2**. On the other hand, OH_2 elimination does not occur because the cyano group is a highly polar substituent molecule as well as a strong Lewis base which, lends toward the idea that it much would rather “pull” the corresponding proton to

stabilize the system. Thus the –CN group in this case seems to be acting as versatile character in the exchange of charges by means of classical electrostatic effects. In other terms, theoretically the R-group (nucleoside moiety) can be either an σ-acceptor or π-donor whereas –CN acts as an σ-acceptor/π-donor. This brings up the question of, how/why do one-way and two-way charge transfers interact in CNDAC glycosyl bond cleavage? Qualitatively, the data correlates with the experimental data expressed by Azuma and co-workers. Thus, we find that the proposed mechanism does not accurately describe the decomposition of conformers' CNDAC (**1**) and CNDC **2** cytosine **6** and glycal **7** via the proposed intermediates **3** and **5** (fig. 3.1). Therefore we conclude that the mechanism involves only the equilibrium of conformers' CNDAC **1** and CNDC **2** which go through subsequent glycosyl bond cleavage to provide cytosine **6** and glycal **7**. [Figure 3.2]

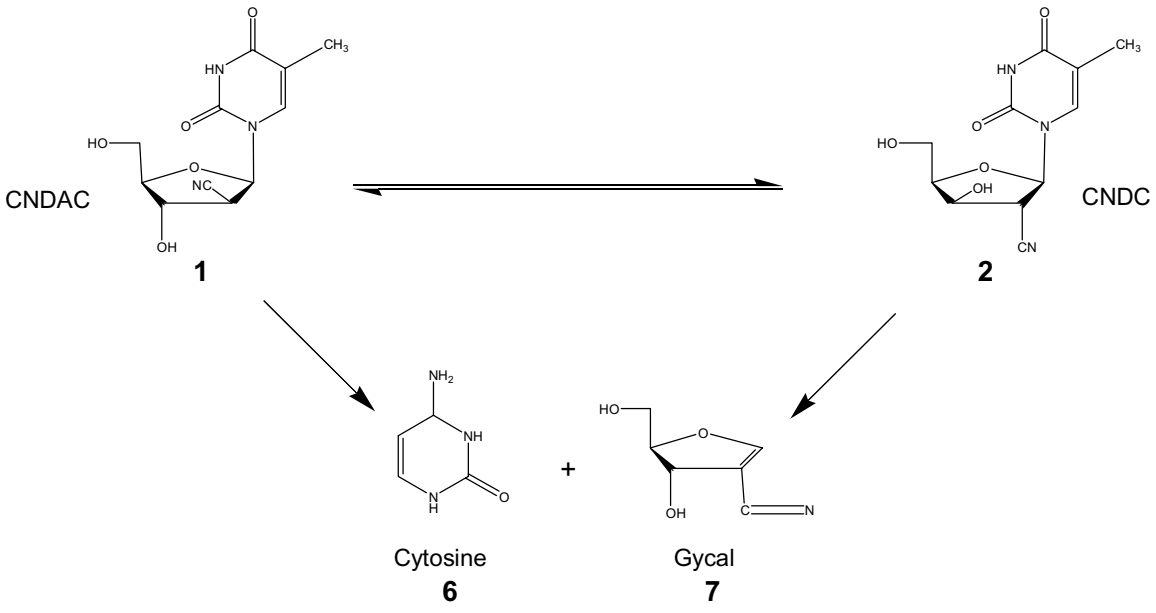


Figure 3.2 Proposed pathway for the equilibrium of conformers' CNDAC and CNDC towards their subsequent glycosyl bond cleavage

References

1. Azuma, A.; Hanaoka, K.; Kurihara, A.; Kobayashi, T.; Miyauchi, S.; Kamo, N. Tanaka, M.; Sasaki, T.; Matsuda, A. Nucleosides and Nucleotides, 141: Chemical Stability of a New Antitumor Nucleoside, 2'-C-Cyano-2'deoxy-1-β-D-arabinopentofuranosylcystosine in Alkaline Medium: Formation of 2'-C-Cyano-2'deoxy-1-β-D-ribo-pentofuranosylcystosine and its Antitumor Activity. *J. Med. Chem*. **1995**, 38, 3391-97.
2. Matsuda, A.; Nakajima, Y.; Azuma, A.;. Tanaka, M.; Sasaki, T. Nucleosides and Nucleotides, 141: 2'-C-Cyano-2'deoxy-1-β-D-arabinofuranosylcystosine (CNDAC) – Design of a Potential Mechanism-Based DNA-Strand-Breaking Antineoplastic Nucleoside. *J. Med. Chem*. **1991**, 34, 2919-22.
3. Azuma, A.; Nakajima, Y.; Nishizono, N.; Minakawa, M.; Suzuki, M.; Hanaoka, K.; Kobayashi, T.; Tanaka, M.; Sasaki, T.; Matsuda, A. Nucleosides and Nucleotides 122: 2'-C-Cyano-2'deoxy-1-β-D-arabinofuranosylcystosine and its Derivatives. A. New Class of Nucleoside with a Broad Antitumor Spectrum. *J. Med. Chem*. **1993**, 36, 4183-4189
4. A. Holmén and A. Broo. A Theoretical Investigation of the Solution N(7)H[-]N(9)H Tautomerism of Adenine. *Int. J. Quantum Chem*. **1995**, 22 113-122.
5. (a) Lezczynski, J.. Are the Amino-Groups in the Nucleic-Acid Bases Coplanar with the Molecular Ring-Ab *Initio* HF/6-31G* and MP2/6-31G* Studies. *Int. J. Quantum Chem*. **1992,** 19, 43-55. (b) Stewart; E. L.; Foley, G. K.; Allinger, N. L.; Bowen, J. P.; *J. Am. Chem. Soc*. **1994**, 116, 7282-7286. (c) Sponer, J.; Lezczynski, J.; Hobza, P. Structures and Energies of Hydrogen-bonded DNA

Base Pairs. A non-empirical study with inclusion of electron correlation. *J. Phys. Chem.* **1996**,100, 1965-. (d) Sponer, J. and Hobza, P. Nonplanar Geometries of DNA Bases- Ab initio 2nd-Order Møller- Plesset Study. *J. Phys. Chem.* **1994,** 98, 3161-3164. (e) Sponer, J. and Hobza, P. DNA Base Amino Groups and Their Role in Molecular Interactions: Ab initio and Preliminary Density Functional Theory Calculations. *Int. J. Quantum Chem*. **1996,** 57, 959-970. (g) Gould, I. R.; Burton, N. A.; Hall, R. J.; Hillier, I. H. *J. Mol. Struct. THEOCHEM.* **1995,** 331, 147-. (h) Holmén, A. and Broo, A. Ab initio MP2 and DFT Calculations of Geometry and Solution Tautomerism of Purine and some Purine Derivatives. *Chem. Phy*. **1996**, 211, 147-161.

6. (a) Møller and M. S. Plesset, Phys. Rev 46 (1934) 618. (b) Pople, J. A.; Brinkley, J. S.; Seeger, R. Theoretical Models incorporating Electron Correlation. *Int. J. Quantum Chem*. **1976,** Suppl. Y-10, 1-19.
7. Stewart, E. L.; Foley, G. K.; Allinger, N. L.; Bowen, J. P.; Ab-Initio Calculations with Electronic Correlation (MP2) on the Nucleic-acid Bases and Their Metheyl-Derivatives. *J. Am. Chem. Soc.* **1994**, 116, 7282-7286.
8. Holmén, A. and Broo, A. Ab initio MP2 and DFT Calculations of Geometry and Solution Tautomerism of Purine and some Purine Derivatives. *Chem. Phy*. **1996**, 211, 147-161
9. Becke, A. D. A New Mixing of Hartree-Fock and Local Density –Functional Theories. *J. Chem. Phys*. **1993,** 98; 1372-1377.

10. Lee, W.; Yang, R. G.; Parr, R. G. Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron-Density. *Phys. Rev. B.* **1988**, 37, 785-789.

11. (a) Novoa, J. J. and Sosa, C. Evaluation of the Density-Funtional Approximation on the Computation of Hydrogen-bond Interactions. *J. Phys. Chem*. **1995,** 99, 15837-15845.

12. Stephens, P. J.; Devlin, F. J.; Chabalowski, C. F.; Frisch, M. J. Ab-Initio Calculation of Vibrational Absorption and Circular-Dichroism Spectra using Density-Functional Force-Fields. *J. Phys. Chem*. **1994,** 98, 11623. Finley, J.; Stephens, P. J.; Frisch, M. J.; *J. Phys. Chem*. **1995,** 98, 16883.

13. Hobza, P; Sponer, J.; Reschel, T. J. Density-Funtional Theory and Molecular Clusters. *Comput. Chem*. **1995,** 16, 1315.

14. Spartan 5.1, Wavefunction, Inc.,18401 Von Karman Ave., Ste. 370,Irvine, CA 92612 U.S.A

15. *Gaussian 94* (Revision D.3), Frisch, M. J., Trucks, G. W., Schlegel, H. B., Gill, P. M. W., Johnson, B. G., Robb, M. A., Cheeseman, J. R., Keith, T. A., Petersson, G. A., Montgomery, J. A., Raghavachari, K., Al-Laham, M. A., Zakrzewski, V. G., Ortiz, J. V., Foresman, J. B., Peng, , C. Y., Ayala, P. A., Wong, M. W., Andres, J. L., Replogle, E. S., Gomperts, R., Martin, R. L., Fox, D. J., Binkley, J. S., Defrees, D. J., Baker, J., Stewart, J. P., Head-Gordon, M., Gonzalez, C., and Pople, J. A., Gaussian, Inc., Pittsburgh PA, 1995.

16. Harihara, P. C and Pople, J. A. Influence of Polarization Functions on Molecular Orbital Hydrogenation Energies. *Theor. Chim. Acta.* **1973,** 28, 973) 213-222.