SYNTHESIS OF CARBOCYCLIC NUCLEOSIDES AS POTENTIAL ANTIVIRAL AGENTS

by

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(Under the Direction of Chung K. Chu)

ABSTRACT

This dissertation describes the synthesis, biological evaluation and molecular modeling studies of carbocyclic nucleosides as potential antiviral agents.

Chapter 1 is a comprehensive review which covers the synthesis and biological activities of important carbocyclic nucleosides. This review provides not only the basic information of carbocyclic nucleosides but also provides most recent advances in this field.

Chapter 2 details the synthesis, anti-HIV-1 activity and mechanism of drug resistance of D- and L-2',3'-didehydro-2',3'-dideoxy-2'-fluoro-carbocyclic nucleosides. The L-form adenosine analog (L-2'F-C-d4A) exhibited the most potent anti-HIV-1 activity (EC_{50} 0.77 μM) without cytotoxicity, while it is cross-resistant to the lamivudine-resistant variant (HIV-1_{M184V}). Molecular modeling studies suggested that the steric hindrance between the sugar moiety of L-2'F-C-d4A and the side chain of Val184 might destabilize the RT-nucleoside triphosphate complex, which causes the inactivity of L-2'F-C-d4A against M184V mutant.

Chapter 3 deals with D- and L-2',3'-didehydro-2',3'-dideoxy-3'-fluoro-carbocyclic nucleosides, which are positional isomers of those described in Chapter 2. New schemes were developed to synthesize the 3'-F isomers, and D-3'F-C-d4G was found to be a very potent anti-HIV-1 compound (EC_{50} 0.4 μM, EC_{90} 2.8 μM), although it was inactive against M184V mutant.

According to the molecular modeling studies, cross-resistance of D-3'-F-C-d4G to M184V mutant may be caused by the realignment of the primer and template in the HIV-RT_{M184V} interaction, which destabilizes the RT-inhibitor triphosphate complex, resulting in a significant reduction in anti-HIV activity of the D-guanine derivative.

Chapter 4 describes the anti-HCV drug discovery program and consists of four parts: (1) a mini-review of current status of anti-HCV nucleosides; (2) asymmetric synthesis of 2'-fluorine(s) substituted-2'-hydroxyl entecavir analogs as anti-HCV agents; (3) attempts at the synthesis of 2'-β-*C*-methyl-2'-hydroxyl entecavir analogs; (4) synthesis of 2'-*O*-methyl-2'-hydroxyl entecavir analogs as potential anti-HCV agents.

Finally, a short summary of this dissertation is given in the chapter 5.

INDEX WORDS: Antiviral activity, carbocyclic nucleosides, cross-resistance, human immunodeficiency virus (HIV), hepatitis C virus (HCV), entecavir analog

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

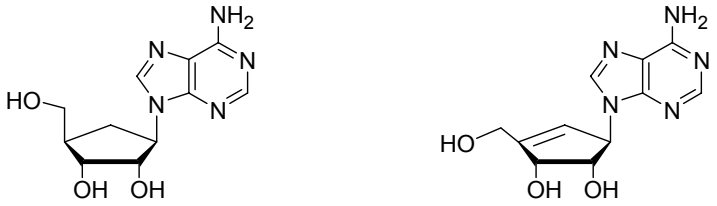
As fundamental building blocks of nucleic acids, nucleosides are essential to the process of conservation, replication and transcription of genetic information in living organisms.[1] Therefore, nucleoside analogs are able to interfere with the replication of pathogenic agents or with the proliferation of cancer cells by competing with their natural counterparts, and this conception has attracted considerable attention in the field of chemotherapy. Indeed, the past decades have witnessed the emergence of numerous therapeutically important nucleosides. In antiviral chemotherapy, eight nucleosides/nucleotides are currently licensed for the treatment of human immunodeficiency virus (HIV) infection; and five nucleosides/nucleotides have been approved for anti-hepatitis B virus (HBV) therapy. A number of other nucleoside analogs are widely used against herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), influenza virus, respiratory syncytial virus (RSV) and hepatitis C virus (HCV), etc.[2] In cancer chemotherapy, several nucleoside analogs have also demonstrated their clinical application.[3]

Carbocyclic nucleosides are analogs of natural nucleosides in which a methylene group replaces the oxygen atom in the sugar ring. This modification results in the loss of the labile glycosidic bond and thus increases their metabolic stability towards phosphorylase and/or hydrolase.[4] Although carbocyclic nucleosides were first conceived and synthesized by medicinal chemists,[5] aristeromycin **1** and neplanocin A **2,** two naturally occurring antibiotic and anticancer agents,[6-8] prompted extensive investigations in carbocyclic nucleosides. Synthesis of carbocyclic nucleosides mainly focuses on the five-membered ring system, three-, four- and six-membered carbocyclic nucleosides have been synthesized as well. Thus, a large number of novel carbocyclic nucleosides have been prepared and many of these compounds are endowed with

interesting biological activities. Particularly, this strategy has successfully led to the discoveries of abacavir (a pro-drug of carbovir) (**89)** and entecavir (**154**) as potent anti-HIV and anti-HBV agents, respectively. Despite the substantial progress that has been achieved,[9-14] the effort to discover novel chemotherapeutic agents with enhanced biological activity and reduced toxicity continues, in order to treat emerging infectious organisms. This article covers the most recent advances in carbocylic nucleosides.

FIVE-MEMBERED CARBOCYCLIC NUCLEOSIDES

Aristeromycin and Neplanocin



Aristeromycin **1** Neplanocin A **2**

Figure 1.1. Aristeromycin and neplanocin A

Although the majority of carbocyclic nucleosides are of synthetic origin, nature has provided two of the most interesting compounds, aristeromycin (**1**) and neplanocin A (**2**) (Figure 1.1). The D-(-)-aristeromycin was first isolated from *Streptomyces citricolor* in 1968,[7] while the racemic form was chemically synthesized in 1966.[5] The discovery of neplanocin A, another five-membered carbocylic nucleoside, was achieved thirteen years later in 1981.[6, 8] These two carbocyclic furanose nucleosides exhibit significant anti-tumor as well as anti-viral activity. In

particular, the broad-spectrum antiviral activity of these agents has been correlated with potent inhibitory effect of *S*-adenosyl-*L*-homocysteine (SAH) hydrolase.[15-21]

It is well known that SAH hydrolase is one of the key enzymes in regulating the methylation reactions which are essential to a number of important biological processes.[22-25] For instance, methylation of mRNA (i.e., 5'-capping) is required for mRNA maturation in many viruses. As shown in Figure 1.2, after the methyl transfer reaction, *S*-adenosyl-*L*-methionine (SAM) is converted to *S*-adenosyl-*L*-homocysteine (SAH), which is a powerful feedback inhibitor of this cycle. SAH hydrolase efficiently removes SAH by cleaving it to adenosine and homocysteine and maintains the balance of SAM and SAH. Inhibition of SAH hydrolase (tight binding) results in increased level of SAH and consequent inhibition of viral mRNA methylation.[12] A number of species of viruses, encompassing poxviruses, paramyxoviruses, herpesviruses, reoviruses, arenavirus, rhabdoviruses and retroviruses, have been shown to be susceptible to the SAH inhibitors.[26]

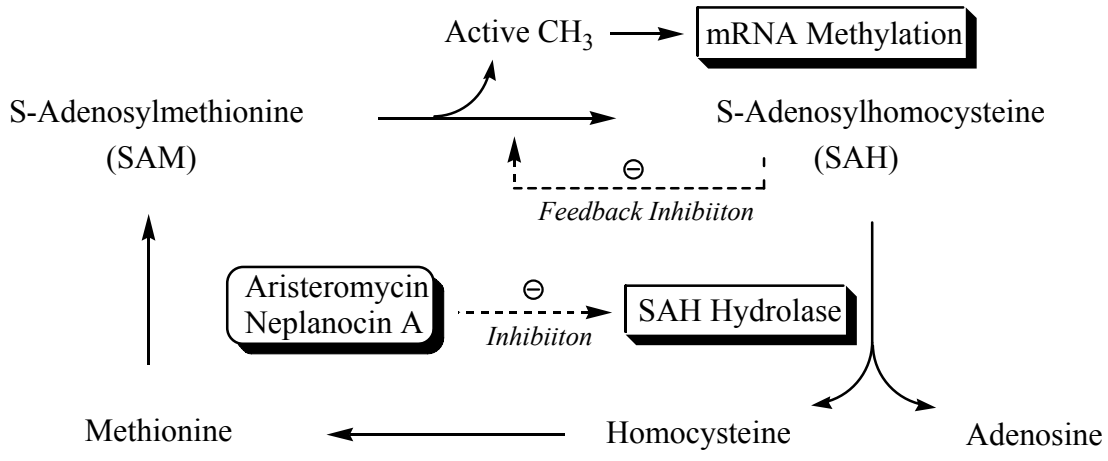


Figure 1.2. Inhibition of SAH hydrolase by aristeromycin and neplanocin A[12]

There are two types of mechanisms by which SAH hydrolase acts. The first one was elucidated by Palmer and Abeles.[23] The reaction proceeds with oxidation, deprotonation, elimination, Michael-type addition and reduction with all steps reversible. Methionine-bound aristeromycin and neplanocin A appear to act as analogs of SAH and are oxidized by SAH hydrolase at 3'-position. This process leads to a depletion of enzyme-bound NAD, and SAH hydrolase can no longer initiate the catalysis cycle.[20, 27-29] Such inhibition of SAH hydrolase can be reversed after incubation with NAD^+ or dialysis. On the other hand, the second type of inhibition is caused not only by the NAD^+ depletion, but also the covalently binding of the inhibitor to SAH hydrolase, which can't be rescued by the addition of NAD^+.[30-35] In view of the interesting mechanism of action as well as the significant biological activity, syntheses of SAH hydrolase inhibitors, particularly aristeromycin and neplanocin A as well as their analogs, have been the subject of a number of investigations.

*Aristeromycin (**1**) (Figure 1.1).* There have been a number of approaches to synthesize aristeromycin since Shealy's original work.[5] Several published methods took advantage of the aminotriol **3** as an important intermediate for building up the target nucleosides, while others focused on utilizing alcohol **4** (or appropriate derivatives therefrom) as a pseudo-sugar source to couple with the base moiety (Figure 1.3)

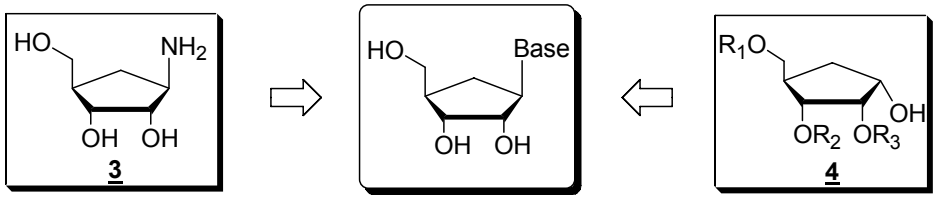
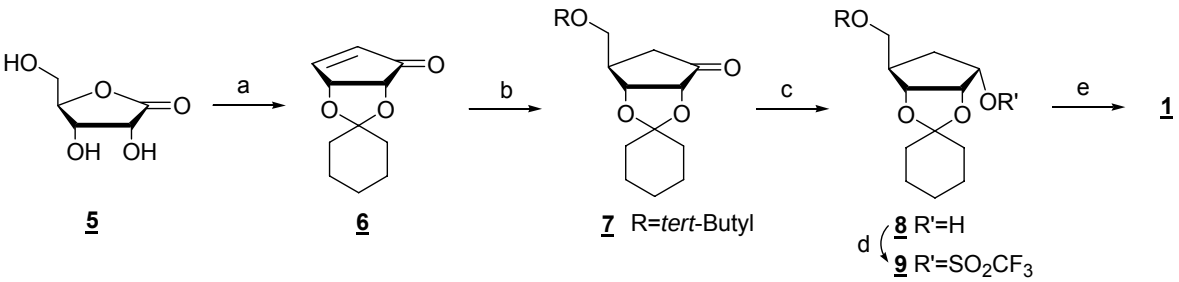


Figure 1.3. General approaches of the synthesis of aristeromycin

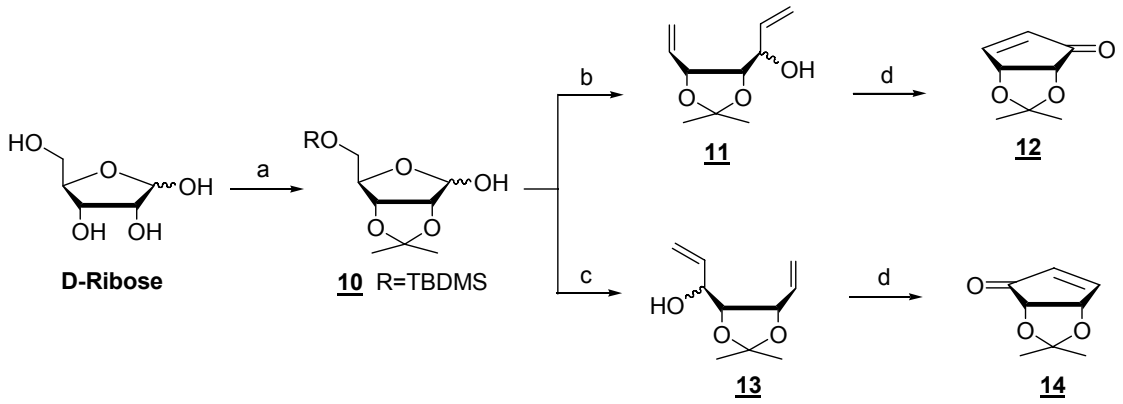
In general, the aminotriol routes always started from achiral materials and generated racemic compounds.[5, 36-41] Although in other instances, the chirality can be resolved by using enzymatic resolutions,[42] asymmetric cycloaddition;[43-46] or palladium-catalyzed reactions,[47-49] the synthesis of optically pure **1** is unsatisfactory due to the long sequences, low yields as well as scale-up difficulties. On the contrary, the pathway *via* alcohol **4** has been more fruitful. Borchardt and co-workers[50, 51] developed a more direct method starting from D-ribonic acid γ-lactone **5**, *via* an enone **6** intermediate, which was treated with lithium di-(*tert*-butoxymethylene)cuprate followed by DIBAL-H to provide the desired key intermediate **8**. Condensation of triflate **9** with adenine salt followed by deprotection afforded target aristeromycin (Scheme 1.1).



Reagents and conditions: (a) i) cyclohexanoe, $FeCl_3$; $NaIO_4$, NaOH, ii) 2-propanol, PPTS, iii) $CH_3PO(OMe)_2$, nBuLi, THF; (b) $(^tBuOCH_2)_2CuLi$; c) DIBAL-H; d)Tf_2O/Py; e) i) adenine, NaH, ii) TFA/H_2O

Scheme 1.1. Synthesis of aristeromycin by Borchardt and co-workers[50]

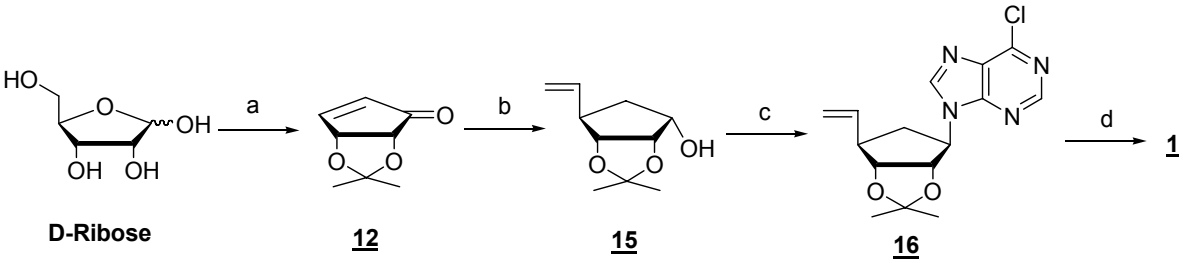
Chu and co-workers made modifications of this scheme in which D-ribose was converted to the key intermediate **12** as well as its enantiomer **14** in eight steps in large scale (> 40 g) in good yield (D-series >54 %; L-series >45 %, Scheme 1.2).[52, 53] A series of L-aristeromycin analogs have been prepared using **14** as a key intermediate.[54, 55]



Reagents and conditions: (a) i) 2,2-dimethoxypropane, *p*-TSA, ii) TBDMSCl, Im; (b) i) vinylmagnesium bromide, ii) TBAF, iii) $NaIO_4$; iv) NaH, DMSO, Ph_3PMeBr; (c) i) NaH, DMSO, Ph_3PMeBr, ii) DCC, DMSO, Py, TFA, iii) vinylmagnesium bromide, iv) TBAF, v) $NaIO_4$, vi) $NaBH_4$, $CeCl_3 \cdot 7H_2O$; (d) i) Grubbs' catalyst, ii) PDC, 4Å MS, AcOH

Scheme 1.2. Modified sequences of the synthesis of aristeromycin by Chu and co-workers[52, 53]

Recently, Schneller and co-workers also described a hybrid sequence of the previous reported methods to produce aristeromycin.[56] The key step of this scheme is the 1,4-addition step using an anion of vinyl instead of *tert*-butoxymethylene. By this modification, the deprotection step of *tert*-butyl group is avoided, which requires very harsh conditions, resulting in incompatibility of some protecting groups.



Reagents and conditions: (a) i) $(MeO)_2CMe_2$, MeOH, ii) Ph_3P, I_2, Im, iii) Zn, iv) vinylmagnesium bromide, v) Grubbs catalyst, vi) PCC; (b) i) vinylmagnesium bromide, TMSCl, HMPA, $CuBr \cdot Me_2S$, ii) $LiAlH_4$; (c) Ph_3P, DIAD, 6-chloropurine; (d) i) $NaIO_4$, OsO_4, ii) $NaBH_4$, iii) NH_3/MeOH, iv) HCl/MeOH

Scheme 1.3. Synthesis of aristeromycin by Schneller and co-workers[56]

*Neplanocin (**2**) (Figure 1.1).* An endocyclic double bond distinguishes neplanocin A from aristeromycin. Therefore, the allylic alcohol **17**, which is more reactive than the saturated alcohol **4**, has been employed frequently as a precursor for the synthesis of neplanocin A (Figure 1.4).

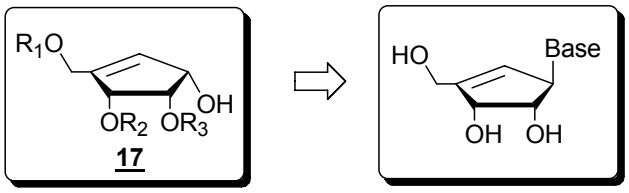
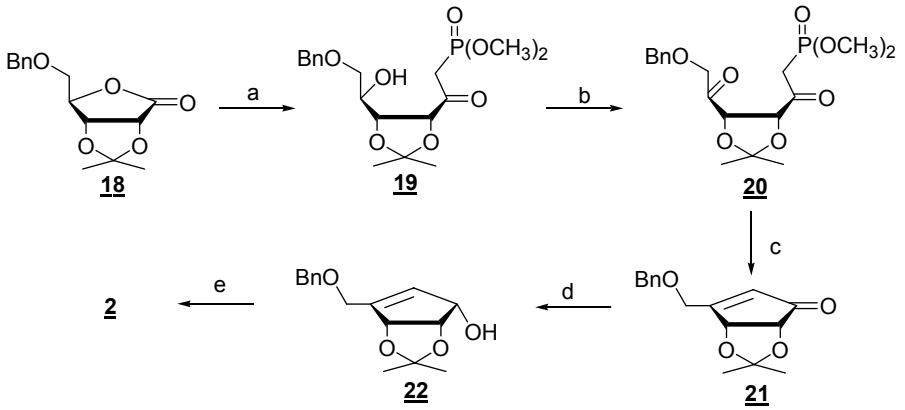


Figure 1.4. General synthesis approach *via* allylic alcohol **17**

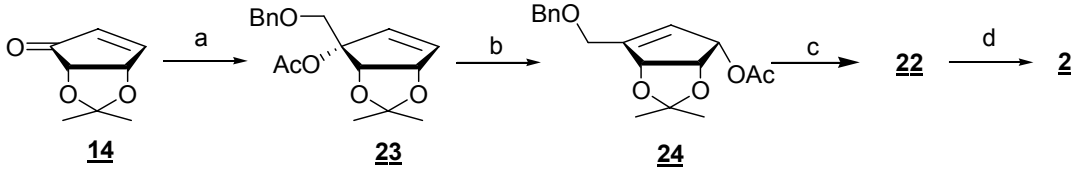
The representative sequence was developed by Lim and Marquez (Scheme 1.4.)[57] in which neplanocin A was synthesized from ribonolactone **18**, which, in turn, was available in two steps from D-ribonic acid γ-lactone **5**.[58] Treatment of lactone **18** with lithium dimethyl methylphosphonate followed by sodium methoxide in methanol afforded keto phosphonate **19**. Oxidation of **19** with modified Collins reagent produced ketone **20**, which underwent intramolecular cyclization under basic condition to generate key intermediate **21**. The allylic alcohol **22** was eventually converted to the neplanocin A.



Reagents and conditions: (a) i) $LiCH_2P(O)(OCH_3)_2$, ii) NaOMe; (b) CrO_3, Py; c) K_2CO_3, 18-crown-6; (d) $NaBH_4$, $CeCl_3$; (e) i) *p*-CH_3PhSO_2Cl, ii) 6-chloropurine, NaH, iii) NH_3/MeOH, iv) BCl_3

Scheme 1.4. Synthesis of neplanocin A by Lim and Marquez[57]

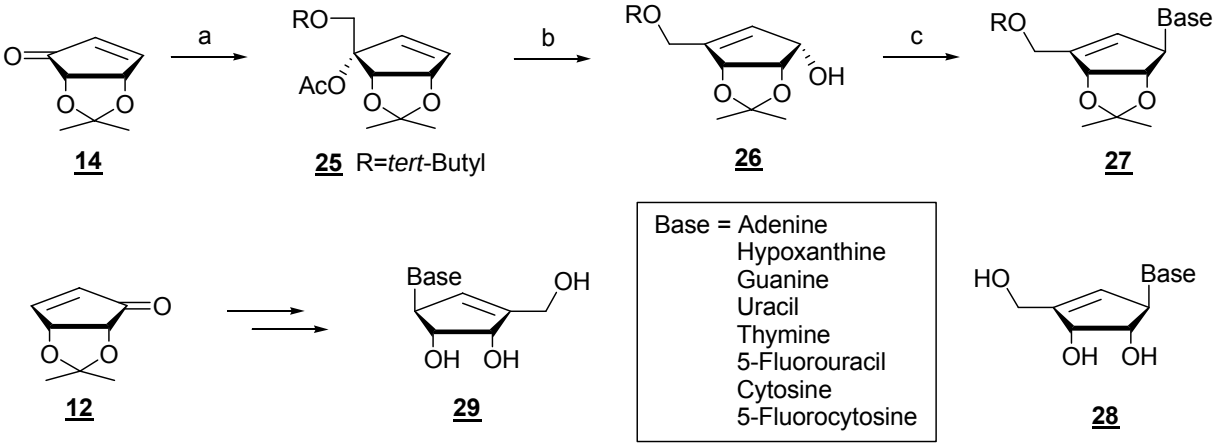
Optimization of this sequence was carried out by Johnson and co-workers[59] who utilized the enone **14** as a precursor which was converted to acetate **24** *via* a sequence of 1,2-addition/acetylation/ 1,3-σ rearrangement. Deprotection of **24** provided known compound **22** which was converted to the target nucleoside **2** by the known chemistry.



Reagents and conditions: (a) i) *n*-Bu_3SnCH_2OBn, *n*-BuLi, ii) Ac_2O, Et_3N, DMAP; (b) $PdCl_2(CH_3CN)_2$, benzoquinone, c) i) K_2CO_3, ii) MsCl, Et_3N, iii) adenine, K_2CO_3, 18-crown-6, iv) $Pd(OH)_2$, cyclohexene, v) HCl/MeOH

Scheme 1.5. Synthesis of neplanocin A by Johnson and co-workers[59]

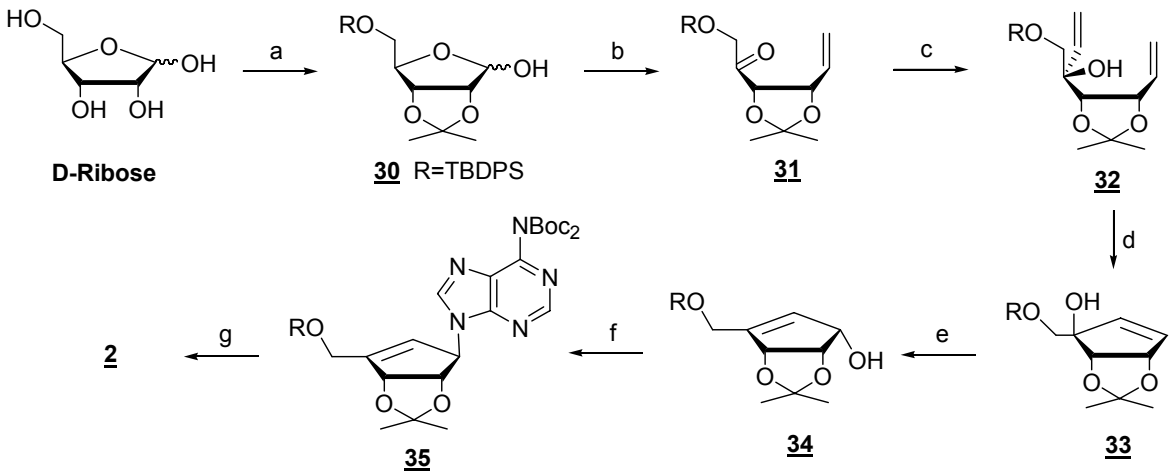
In view of the interesting biological activity as well as the unique structure of neplanocin A, Chu and co-workers conducted the SAR study of D- and L-neplanocin analogs. Noteworthily, Mitsunobu reaction was performed to construct the nucleosides instead of classic SN_2 coupling reaction (Scheme 1.6).[60]



Reagents and conditions: (a) i) $(CH_3)_3COCH_3$, ^{t}BuOK, *sec*-BuLi, ii) Ac_2O, Et_3N, DMAP; (b) i) $PdCl_2(CH_3CN)_2$, benzoquinone, ii) K_2CO_3; (c) Mitsunobu conditions, proper base moieties.

Scheme 1.6. Synthesis of D- & L- neplanocin analogs by Chu and co-workers[60]

More recently, Strazewski and Michel reported a short pathway to synthesis neplanocin A with the highest published overall yield (Scheme 1.7).[61] This approach used allylic alcohol **34** as a key intermediate which was prepared from D-ribose in eight steps. Mitsunobu reaction coupled the sugar moiety with *di*-Boc protected adenine moiety to give the desired nucleoside **35**, which was deblocked to provide target compound **2**.



Reagents and conditions: (a) i) acetone, H^+, ii) TBDPSCl, Et_3N, DMAP; (b) i) Ph_3PMeBr, ^tBuOK, ii) $(COCl)_2$, DMSO, Et_3N; (c) vinylmagnesium bromide; (d) Neolyst dichloride; e) i) PDC, 4Å MS, ii) $NaBH_4$, $CeCl_3$; (f) i) PPh_3, DIAD, base, ii) TBAF; (g) TFA

Scheme 1.7. Synthesis of neplanocin A by Strazewski and Michel[61]

Aristeromycin and Neplanocin A Analogs

Although aristeromycin and neplanocin A are potent SAH inhibitors, their therapeutic utility has been limited due to their significant toxicity, which was shown to be mediated through phosphorylation by adenosine kinase and subsequent conversion to the corresponding cytotoxic nucleotides.[62-65] Therefore, modifications based on the prototypes of these natural products have

generated a number of carbocyclic analogs that retain the inhibitory activity toward SAH hydrolase but are devoid of toxicity.

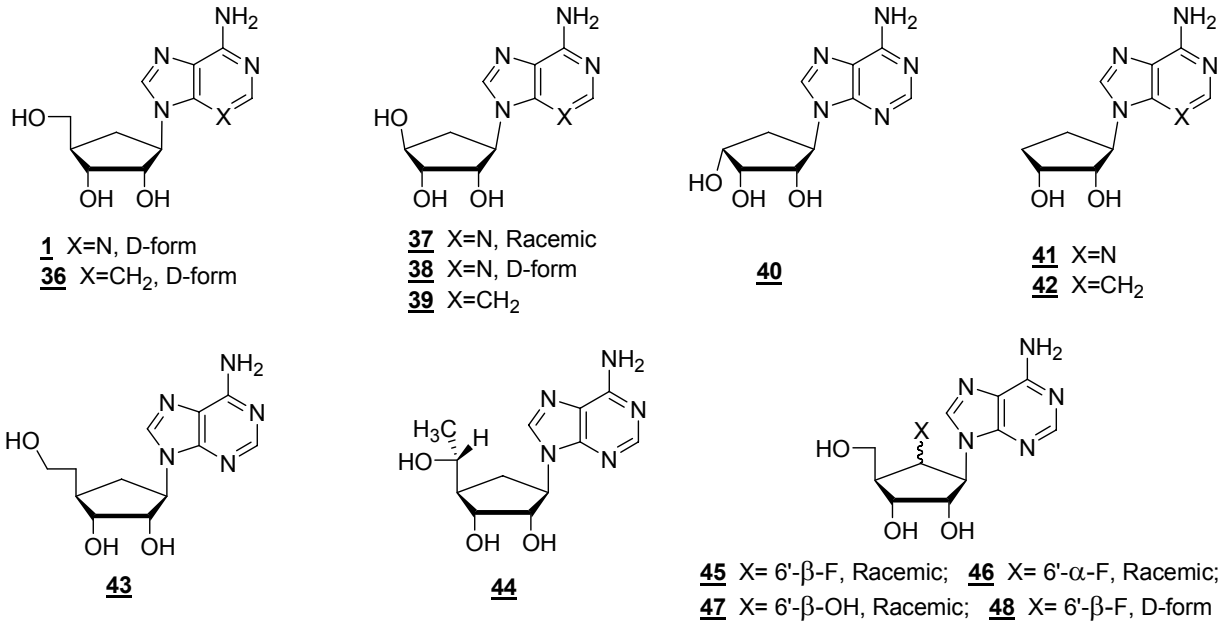


Figure 1.5. Biologically active aristeromycin analogs

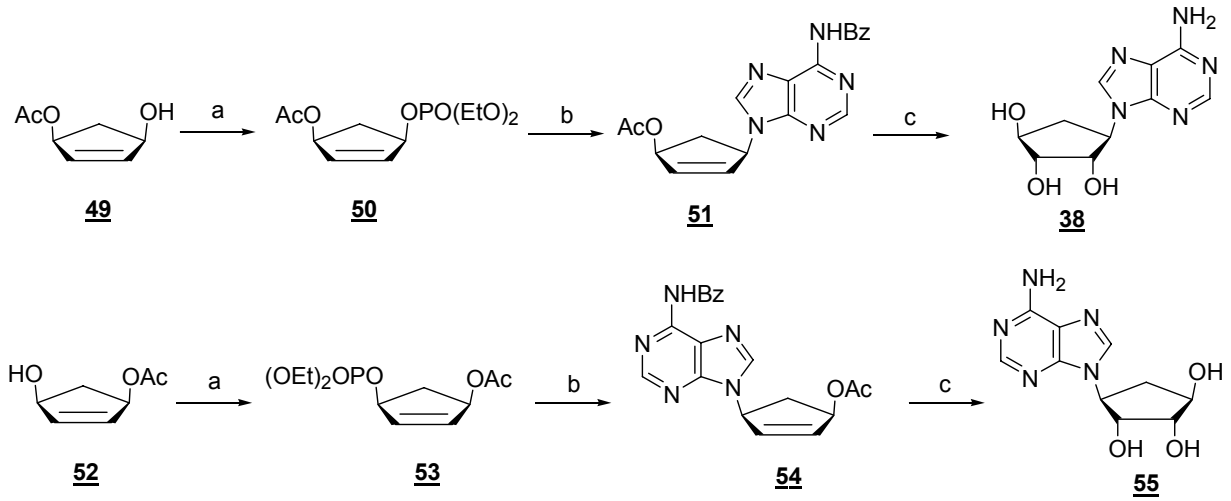
Aristeromycin Analogs (Figure 1.5). Given the fact that the cytotoxicity of aristeromycin is attributed to the metabolism to its 5'-phosphates, Schneller and co-workers addressed this situation by preparing (±) 5'-noraristeromycin **37** to avoid the phosphorylation step by displacing the 5'-phosphate-accepting hydroxyl group from its original place.[66] Surprisingly, nucleoside **37** was found to be non-toxic to host cells but still active against a variety of viruses (Table 1.1).

Table 1.1. Antiviral Activity of (-) & (+) 5'-Noraristeromycin

Virus	Cell	EC_{50} (μg/mL)			
		Compd 37 (racemic)	Compd 38 (D-form)	Compd 55 (L-form)	neplanocin A
Vaccinia virus	E_6SM	0.3	0.04	0.7	0.2[a] / 0.2[b]
Vesicular stomatitis virus	E_6SM	0.07	0.1	2.0	0.2[a] / 2.0[b]
Parainfluenza-3	HeLa	0.4	0.07	0.2	0.4[a] / 0.2[b]
Reovirus-1	Vero	0.07	0.7	7.0	0.4[a] / 0.7[b]
Cytomegalovirus	HEL	0.4	0.01-0.05	5-20	0.4[a] / 0.2-0.5[b]
Measles	Vero	0.4	/	/	0.4[a]
Respiratory syncytial virus	Hela	2.0	/	/	0.2[a]
Tacaribe	Vero	1.0	8	50	0.4[a] / 0.4[b]

[a]Positive control for compound **37**; [b]Positive control for compounds **38** and **55**

Subsequently, the enantiomerically pure D-5'-noraristeromycin and its L-isomer were synthesized using chiral precursors **49** and **52** as starting materials. The Pd catalyzed reaction was conducted to couple the base and sugar to provide the target nucleosides (Scheme 1.8).[67]



Reagents and conditions: (a) $(EtO)_2P(=O)Cl$, Py; (b) i) NH_3/MeOH, ii) $Pd(PPh_3)_4$, Ph_3P, Base; (c) i) OsO_4, NMO, ii) NH_4OH/MeOH

Scheme 1.8. Synthesis of D & L-noraristeromycin by Siddiqi and co-workers[67]

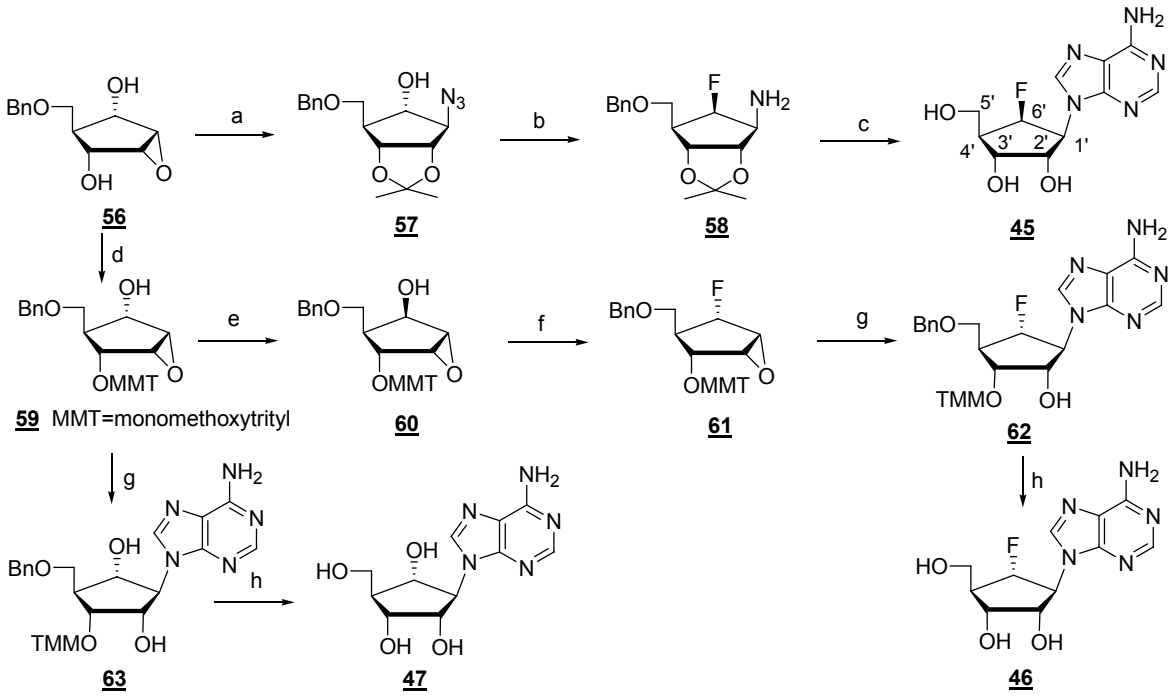
As shown in Table 1.1, D-form **38** was, on the average, 10-fold more potent than its L-enantiomer **55** in inhibiting virus replication. The 4'-epimer of 5'-norariste romycin **40**[68] and 5'-homoaristeromycin **43**[69] were prepared as well (Figure 1.5). Compound **40** inhibited the replication of various DNA and RNA viruses at concentrations similar to those for neplanocin A, but was significantly less cytotoxic. Interestingly, an extension of 5'-hydroxylmethyl chain (**43**) also retained antiviral activity against vaccinia (EC_{50} 1.2 μg/mL), cowpox (EC_{50} 0.12 μg/mL) and moneypox (EC_{50} 0.12 μg/mL) viruses without cytotoxicity up to 100 μg/mL.

Borchardt and co-workers also described several other aristeromycin analogs without a 5'-OH.[70, 71] Among these compounds, nucleoside **41** (Figure 1.5) was one of the most potent SAH hydrolase inhibitors. A number of other aristeromycin analogs with modifications at the 5'-position have been reported.[72-76] However, no significant antiviral activity was observed for those molecules with one exception of nucleoside **44** (Figure 1.5) which showed potent antiviral activity against yellow fever (EC_{50} 0.32 μg/mL).

Modification of aristeromycin was carried out on its 6'-position as well. Nucleoside **45** (Figure 1.5) is a racemic compound with a fluorine atom on the 6'-β face.[77] The synthesis of **45** was accomplished, starting from the epoxide precursor **56**, which was subjected to nucleophilic attack by an azide anion to open up the epoxide ring. Fluorination reaction and azide reduction provided the amine intermediate **58**, which was used to construct the target nucleoside **45** (Scheme 1.9).

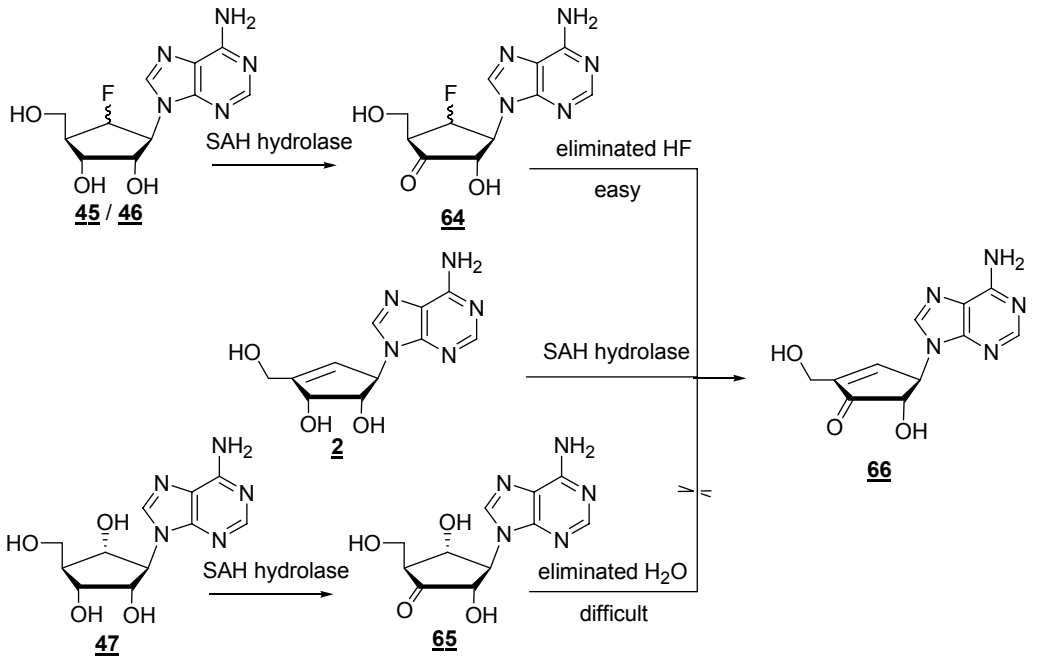
While **45** and its α-epimer **46** are good SAH hydrolase inhibitors (IC_{50} 8 and 80 nM for β and α epimers, respectively), its analog **47** is not active at all (IC_{50} 28000 nM). As shown in Scheme 1.10, the mechanism of this type of SAH hydrolase inhibitors has been proposed. The intermediate **66**, generated from 6'-fluoroaristeromycin, is the same one produced by the action

of SAH hydrolase on neplanocin A, and can irreversibly bind with enzyme and consequently inhibits the SAH hydrolase. Therefore, the poor inhibition of **47** may be explained by the fact that the 6'-hydroxyl group would be more difficult to eliminate than 6'-fluorine to generate active intermediate **66**. On the basis of this information, the D-enantiomer of 6'-β-fluoroaristeromycin (**48**) was synthesized.[78] However, no antiviral data have been reported. Installing exo-cyclic double bond on the 6'-position of aristeromycin produced novel nucleoside **187** (Figure 1.11) which will be discussed in detail in the next section (*vide infra*).



Reagents and conditions: (a) i) NaN_3, ii) 2,2-dimethoxypropane, H^+; (b) i) Tf_2O, Py, ii) TASF, iii) H_2/Lindlar catalyst; (c) i) 5-amino-4,6-dichloropyrimidine, Et_3N, ii) diethoxymethyl acetate, iii) NH_3/MeOH, iv) cyclohexene/$Pd(OH)_2$/C, v) HCl; (d) *p*-anisylchlorodiphenylmethane, Py, (e) Tf_2O, 2,6-di-*tert*-butyl-4-methylpyridine, iii) lithium benzonate, iv) NH_3/MeOH; (f) i) Tf_2O, 2,6-di-*tert*-butyl-4-methylpyridine, ii) TBAF; (g) adenine, K_2CO_3; (h) i) formic acid, ii) cyclohexene/$Pd(OH)_2$/C

Scheme 1.9. Synthesis of 6'-modified aristeromycin analogs[77]



Scheme 1.10. Proposed mechanism of 6'-F-neplanocin analogs as SAH hydrolase inhibitors[77]

One important discovery of selective SAH hydrolase inhibitors is the replacement of adenine moiety with 3-deazaadenine resulting in the reduced metabolic susceptibility of the nucleoside to the adenosine deaminase as well as adenosine kinase and consequently reduced cytotoxicity.[79] For instance, compounds **36**[80], **39**[81] and **42**[71] are of interest due to their potent antiviral activity and reduced cytotoxicity.

Neplanocin A Analogs (Figure 1.6). Modifications of neplanocin A are specifically aimed at improving the selectivity of this nucleoside. 3-Deazaneplanocin A (**67**) is a base-modified analog exhibiting potent inhibitory effect towards SAH hydrolase without toxicity after 24-hr exposure up to 100 μM.[81]

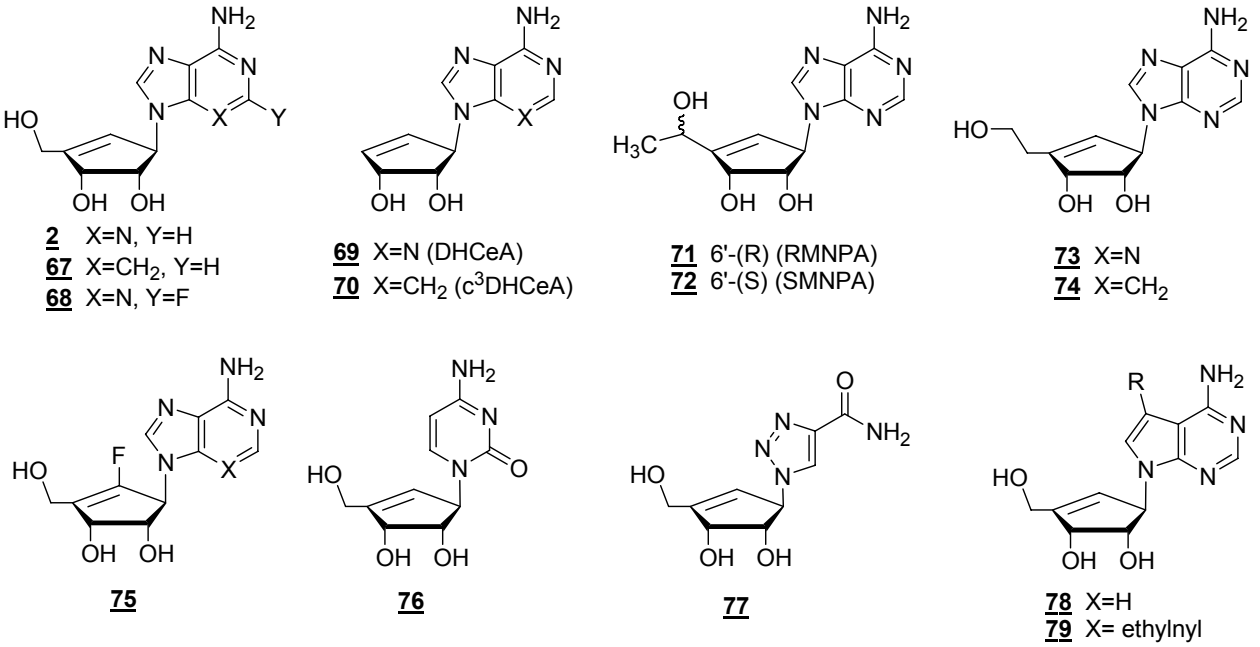
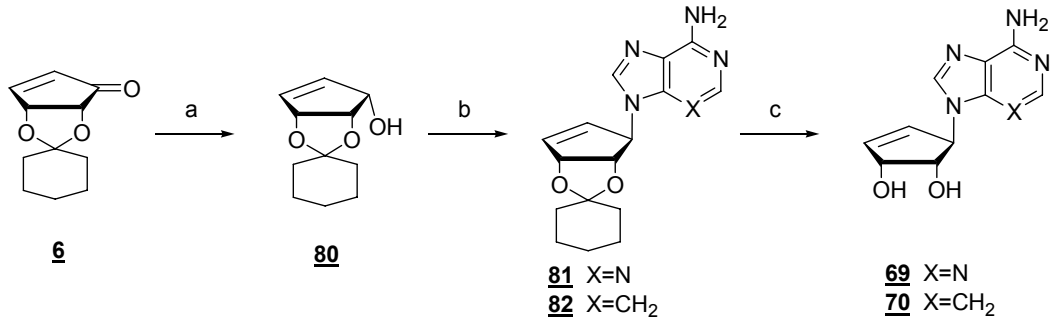


Figure 1.6. Biologically active neplanocin A analogs

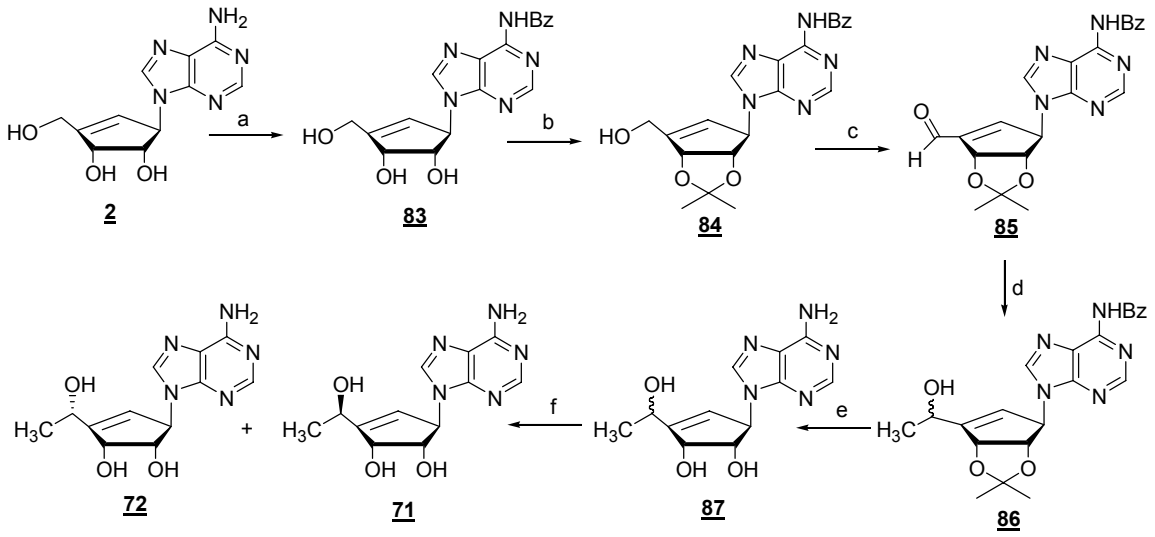
The 5'-nor derivative **69** or **70**,[50] prepared *via* enone **6** through a convergent approach (Scheme 1.11), have been demonstrated to be approximately 10-fold better than the parent compound **2** in terms of antiviral activity (CC_{50} / IC_{50}).[82] A comparative study analyzed the antiviral activity and toxicity of neplanocin A (**2**), 3-deazaneplanocin A (**67**), 5'-norneplanocin A (**69**) and 5'-nor-3-deazaneplanocin A (**70**) against a wide range of DNA and RNA viruses,[83] in which **67**, **69** and **70** showed greater selectivity than neplanocin A against vesicular stomatitis virus and rotavirus. Especially, 3-deazaneplanocin A in newborn mice showed marked protective effect against a lethal infection with vesicular stomatitis virus at a dose of 0.5 mg/kg/day.



Reagents and conditions: (a) $NaBH_4$, $CeCl_3$; (b) i) TsCl, Et_3N, ii) base, NaH; (c) HCl

Scheme 1.11. Synthesis of 5'-norneplanocin analogs[50]

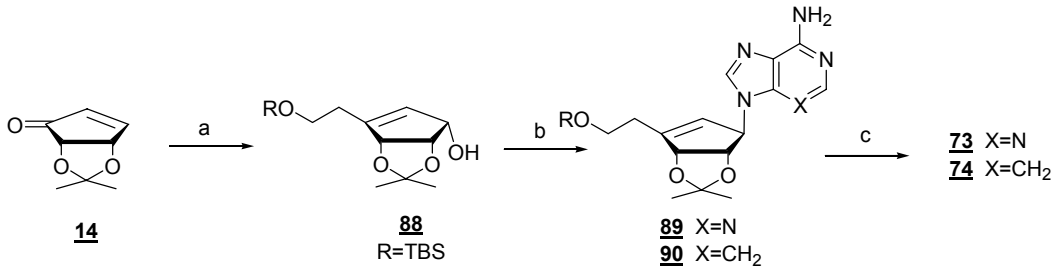
In addition to these analogs, a series of 6'-position modified neplanocin A analogs were prepared.[84-86] Among the synthesized compounds, (6'*R*)-6'-methylneplanocin A (**71**, RMNPA) demonstrated excellent antiviral potency and selectivity superior to that of the neplanocin A. Interestingly, its diastereomer, (6'*S*)-6'-methylneplanocin A (**72**, SMNPA)**,** was completely biologically inactive. The synthesis of RMNPA **71** was accomplished starting from neplanocin A (Scheme 1.12). Protection of 2'- and 3'-positions as well as the amino group on the base moiety left a free 6'-OH, which was oxidized, alkylated and then separated *via* HPLC to provide optically pure **71** and **72**. Matsuda and co-workers have described 2-F-neplanocin A (**68**), one of the 2-halo derivatives of neplanocin A.[87] The compound (**68**) was as active as neplanocin A against viruses which are susceptible to SAH hydrolase inhibitors, but is completely resistant to adenosine deaminase.[88]



Reagents and conditions: (a) i) TMSCl, Py, ii) BzCl, iii) NH_4OH; (b) $HClO_4$, acetone; (c) $BaMnO_4$; (d) Ph_3PCH_3Br, *n*-BuLi; (e) Me_3Al; (f) HPLC (C_{18}) separation

Scheme 1.12. Synthesis of RMNPA[86]

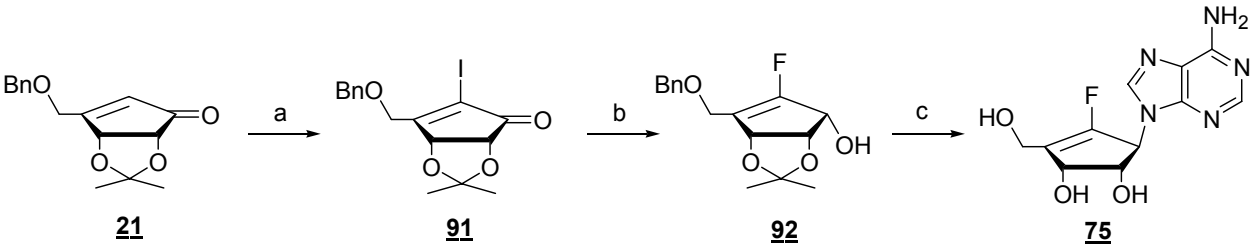
Another interesting compound is 6'-homoneplanocin A (**73**), which displayed particular activity against human cytomegavirus (EC_{50} 0.15-0.5 μg/mL), vaccinia virus (EC_{50} 0.1 μg/mL) and vescular stomatitis virus (EC_{50} 1.0 μg/mL).[89] Starting from enone **14**, the key intermediate **88** was obtained *via* an addition/reduction/rearrangement sequence. Treating Mesylated alcohol **88** with adenine or 3-deazaadenine salt, followed by deprotection afforded desired 6'-homoneplanocin analogs (Scheme 1.13).



Reagents and conditions: (a) i) $(TMS)_2NH$, BuLi, EtOAc, ii) $LiBH_4$, iii) TBSCl, Im, iv) Ac_2O, DMAP, Et_3N, v) $PdCl_2(MeCN)_2$, *p*-benzoquinone, vi) K_2CO_3; (b) i) MsCl, DMAP, ii) base, NaH, 15-crown-5; (d)HCl

Scheme 1.13. Synthesis of 6'-homoneplanocin A[89]

Jeong and co-workers designed and synthesized a fluoroneplanocin A **75** (Scheme 1.14), which was believed to inhibit SAH hydrolase based on the type II mechanism (*vide supra*).[30] The synthesis of **75** was straight forward as depicted in Scheme 1.14. The fluorosugar was prepared starting from eneone **21**, which was converted to iodo derivative **91** by treating with I_2/CCl_4/pyridine. Compound **91** was then reduced, protected, fluorinated and deprotected to provide desired key intermediate **92**. Once compound **92** in hand, the preparation of target nucleoside **75** was smoothly accomplished *via* SN_2 type coupling reaction and deprotection steps.

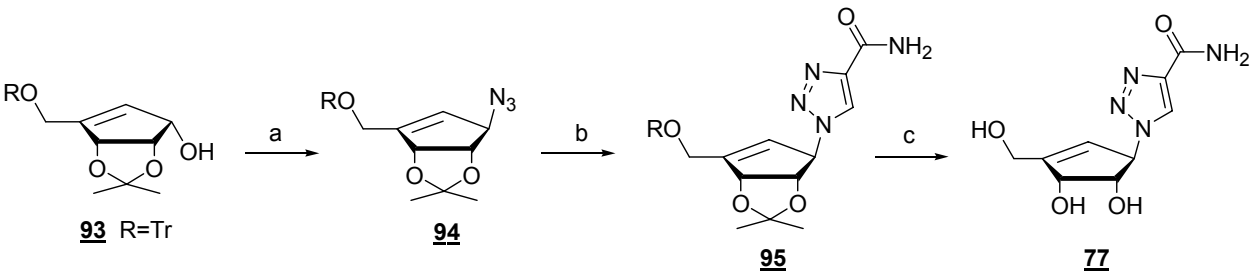


21 **91** **92** **75**

Reagents and conditions: (a) I_2, CCl_4, Py; (b) i) $NaBH_4$, $CeCl_3$, ii) TBDPSCl, Im, iii) *n*-BuLi, *N*-fluorobenzenesulfonimide, v) TBAF; (c) i) MsCl, Py, ii) adenine, K_2CO_3, 18-crown-6, iii) BBr_3, iv) Ac_2O, Py, v) NH_3/MeOH

Scheme 1.14. Synthesis of fluoroneplanocin A[30]

In a comprehensive structure activity relationship study of D- and L-neplanocin analogs (*vide supra*, Scheme 1.6), Chu and co-workers found that cytosine analog **76** (D-form) exhibited potent anti-HIV, anti-orthopoxvirus as well as anti-WNV (West Nile virus) activities.[60, 90] Recently, the same group also reported that the triazole analog **77** (Scheme 1.15) was a potent antiviral agent against vaccinia virus with an EC_{50} of 0.4 μM while the positive control cidofovir had an EC_{50} of 6 μM.[91]



93 R=Tr **94** **95** **77**

Reagents and conditions: (a) i) MsCl, Et_3N, ii) NaN_3; (b) i) methyl propiolate, CuI, Et_3N, ii) NH_3 / MeOH; (c) HCl

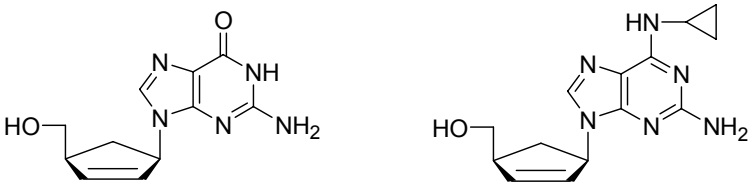
Scheme 1.15. Synthesis of triazol analog[91]

Another base modified analog 7-deazanepalnocin A (**78**) was also synthesized.[92] It was active against cowpox and vaccinia with EC_{50}s of 1.2 and 3.4 μM, respectively; it also exhibited significant anti-HCV and anti-HBV activity. On the basis of this preliminary data, a number of 7-substituted 7-deazanepalnocin A analogs were synthesized and screened for antiviral activity. Among target nucleosides, the 7-ethylnyl substituted compound **79** exhibited interesting anti-HBV activity against wild type as well as several HBV mutants including rtL180M, rtM204I, rtM204V and rtN236T.[93]

Carbovir, Abacavir and Their Analogs

Carbovir and Abacavir (Figure 1.7). (±)-Carbovir was first reported by Vince et al. with potent anti-HIV activity and low cytotoxicity.[94] Although the (-)-D form is approximately 75-fold more potent than its enantiomer, triphosphates of (-)-D and (+)-L- carbovir are equally active against HIV reverse transcriptase.[94, 95] Therefore, the reduced activity seen with (+)-L-carbovir *in vitro* could, in part, be attributed to low level of conversion to its phosphate. Cytosolic 5'-nucleotidase

converts (-)-D-carbovir to its triphosphate, which can incorporate into viral DNA and disturb viral replication but have no interaction with host cell DNA polymerase α, β and γ. Unfortunately, the low aqueous solubility, poor oral bioavailability as well as inefficient central nervous system penetration prevented it from further developing as anti-HIV agents.[96-98] To improve its preclinical profile, a number of prodrug of carbovir were prepared and a 6-cyclopropylamino substituted analog, which was later known as the clinically useful drug, Abacavir (Figure 1.7). [97]



96 Carbovir **97** Abacavir

Figure 1.7. Carbovir and abacavir

Abacavir also exhibits significant anti-HIV effect with low cytotoxicity,[97] and more importantly, it has excellent pharmacokinetic as well as toxicological profiles. The unique activation process of abacavir to its triphosphate is described in Figure 1.8: (1) adenosine phosphortransferases are responsible for the monophosphorylation; (2) deamination to carbovir monophosphate is performed by cytosolic deaminase; (3) cytosolic enzymes are responsible for the conversion of carbovir monophosphate to triphosphate (Figure 1.8).[99]

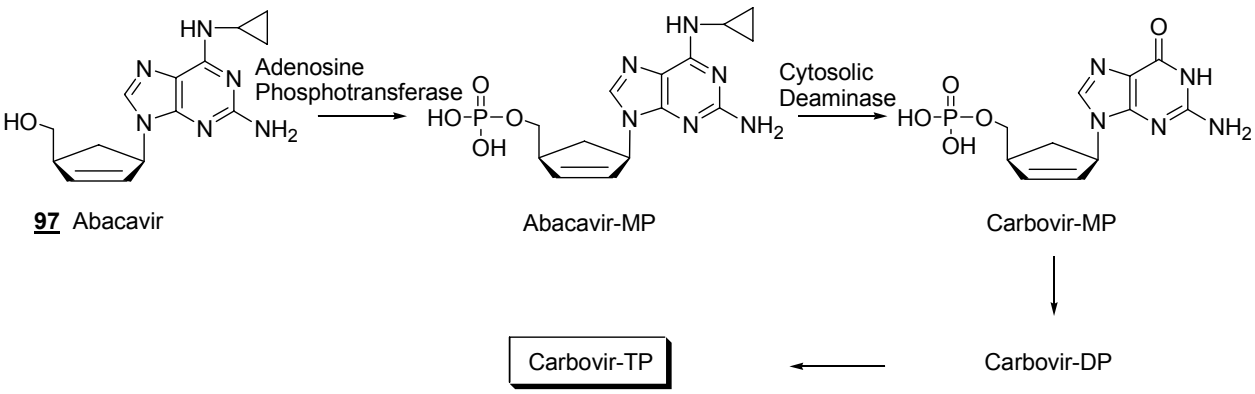
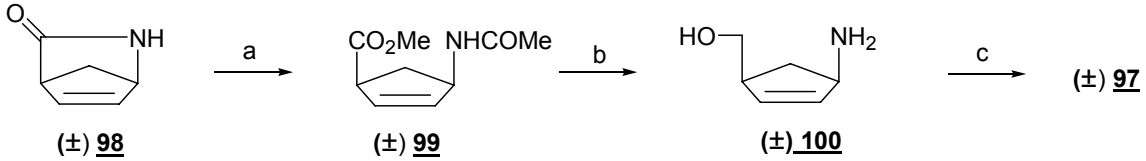


Figure 1.8. Activation pathway of abacavir[99]

In Dec. 1998, abcarvir was approved by FDA for the treatment of HIV infection under the trade name of Ziagen™. It has been used in combinations with AZT and 3TC (Trizivir™) and later with 3TC (Epizcom™).

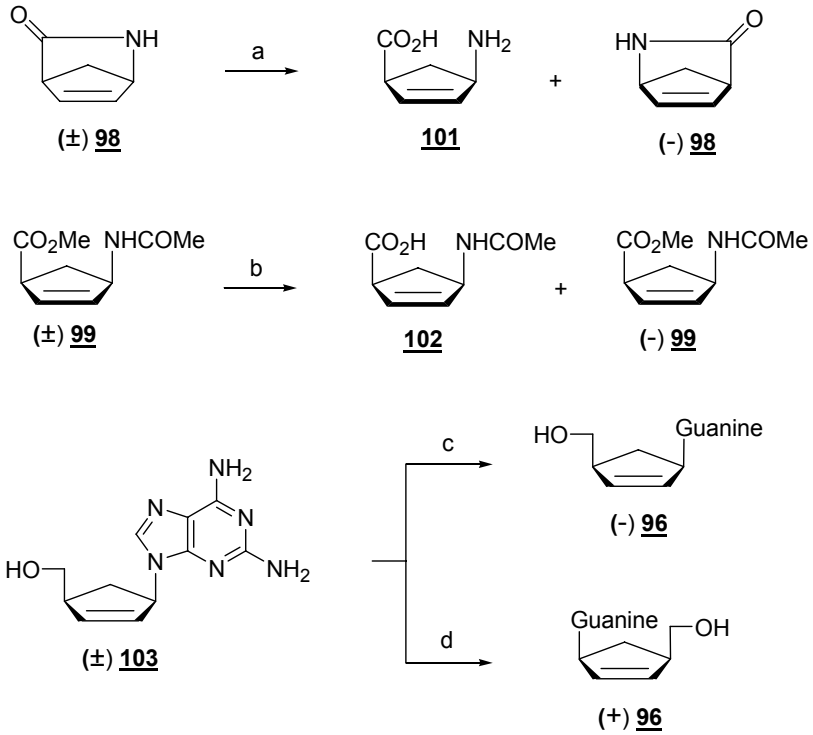
Vince's procedure[94, 100] (Scheme 1.16) utilized the racemic compound **98** as the starting material, which underwent a sequence of hydrolyzation/esterification/reduction/deprotection to generate key intermediate **100**. Compound **100** was converted to the target nucleoside (±)-**97** as well as other analogs by reported methodology.[101]



Reagents and conditions: (a) i) H^+, ii) MeOH, H^+, iii) esterification; (b) i) $LiBH_4$, ii) H^+; (c) Base construction

Scheme 1.16. Synthesis of racemic carbovir[94, 100]

Later, it was found that bicycle compound (±) **98** can be resolved by using *Psedomomonas solanacearum*;[102, 103] pig liver esterase (PLE) distinguishes the two enantiomers of (±) **99**;[104] and adenosine deaminase recognizes either (-) or (+) **103** at different temperature (Scheme 1.17).[100] By using these methods, optically pure (-) **96** could be prepared.

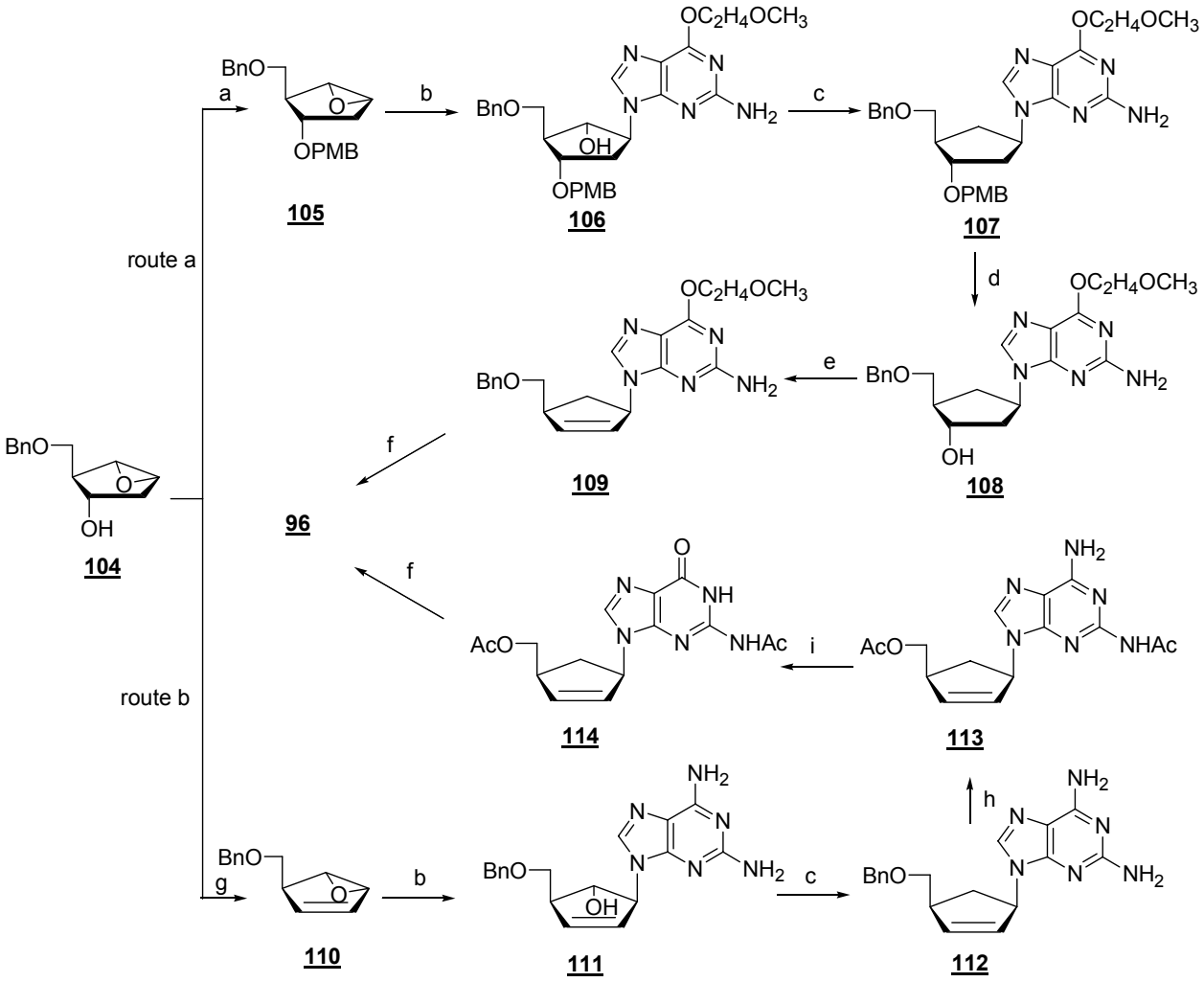


Reagents and conditions: (a) *Psedomomonas solanacearum*; (b) PLE; (c) Adenosine deaminase

Scheme 1.17. Enzymatic resolution reactions in the synthesis of carbovir[100, 102-104]

Enantioselective synthesis of (-) carbovir was reported by Jones and co-workers from Glaxo.[105] Starting from the same chiral epoxide **104**, two different routes were developed, in which the first one (route a) generated a double bond at the nucleoside level, while the second one (route b) produced a vinyl epoxide **110** at the very beginning of the sequence. However, both

routes needed an extra step to remove the 6'-hydroxyl group by Barton-McCombie radical reaction (Scheme 1.18).

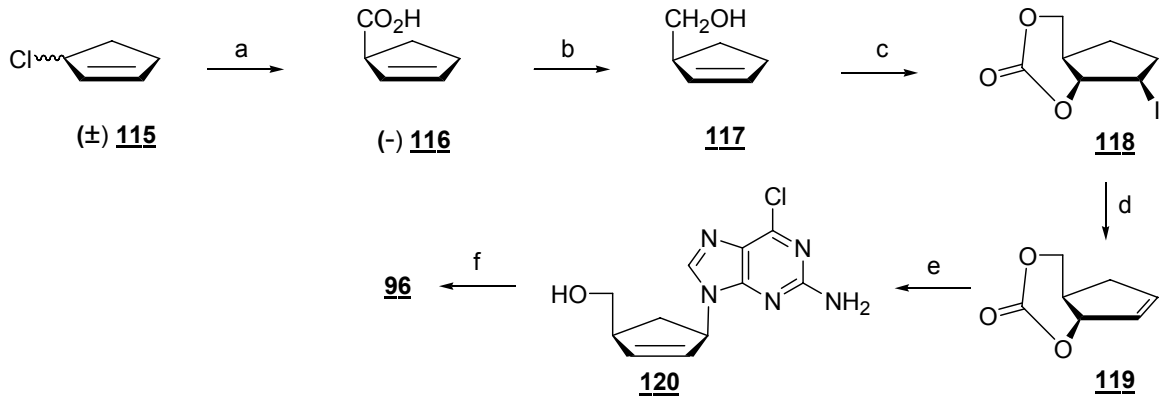


Reagents and conditions: (a) PMBCl, NaH; (b)Ph_3P, DIAD, base; (c) i) PhOCSCl, DMAP, ii) BuSnH, AIBN; (d) DDQ; (e) i) MsCl, DMAP, ii) $NaOCH_2CH_2OMe$; (f) Base derivation and deprotection; (g) i) MsCl, DMAP, ii) TBAF; (h) $BF_3{\cdot}Et_2O$, Ac_2O; (i) $NaNO_2$, AcOH

Scheme 1.18. Synthesis of carbovir from epoxide **104**[105]

Crimmins's approach[106] to prepare (-)-carbovir relies on the Trost's palladium-catalyzed nucleophilic coupling reaction.[48] The racemic homoallylic chloride **115** was converted to optically active endocyclic vinyl compound **119** in four steps by using a chemical resolution

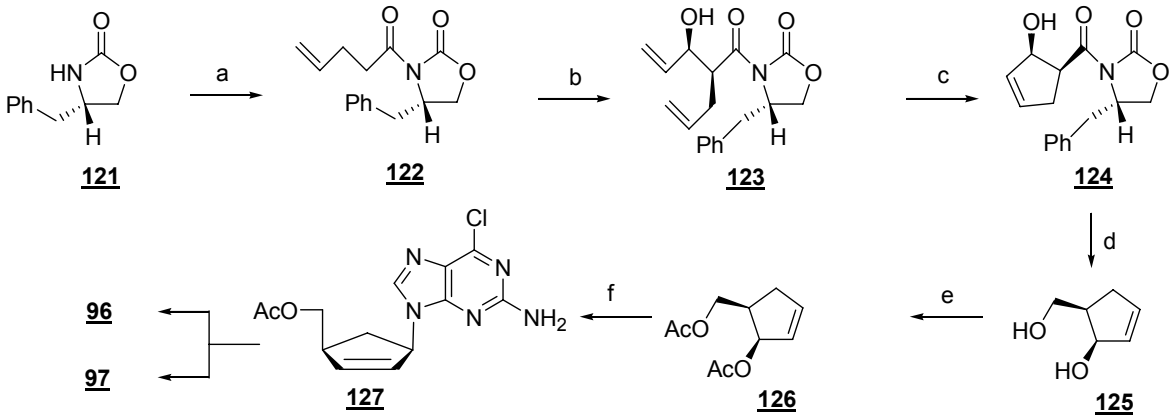
method. Compound **119** was then coupled with base moiety under Trost's conditions (allylpalladium dichloride dimer/Ph_3P) to give nucleoside **120** which was further hydrolyzed to afford the target compound **96** (Scheme 1.19).



Reagents and conditions: (a) Mg, CO_2, recrystallization as (-)-(α-phenylethyl)amine salt; (b) LAH; (c) BuLi, CO_2, I_2; (d) DBU; (e) 2-amino-6-chloropurine, allylpalladium chloride dimmer, PPh_3; f) NaOH

Scheme 1.19. Synthesis of carbovir from chloride compound **115**[106]

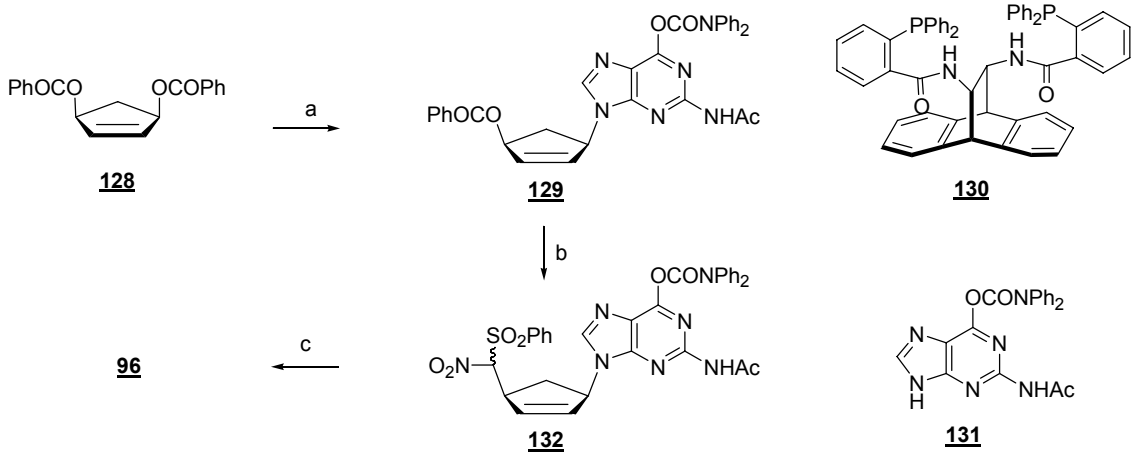
A chiral auxiliary assisted asymmetric synthesis of carbovir and abacavir was accomplished by the same group (Scheme 1.20).[106] Pentenoic pivalic mixed anhydride was coupled with **121** to obtain **122,** which was subjected to the *syn* aldol condensation to provide the diene **123**. A metathesis/reduction/esterification sequence afforded **126,** which underwent Pd(0) catalyzed coupling reaction and base derivation to smoothly generate abacavir or carbovir. More recently, Crimmins and co-workers optimized the selectivity of the coupling reaction by a solid phase synthesis methodology.[107]



Reagents and conditions: (a) n-BuLi, pentenoic pivalic mixed anhydride; (b)Bu_2BOTf, Et_3N, CH_2=CHCHO; (c)Grubbs catalyst; (d) $LiBH_4$; (e) Ac_2O, Et_3N, DMAP; (f) 2-amino-6-chloropurine, $Pd(PPh_3)_4$

Scheme 1.20. Chiral auxiliary assisted asymmetric synthesis of carbovir[106]

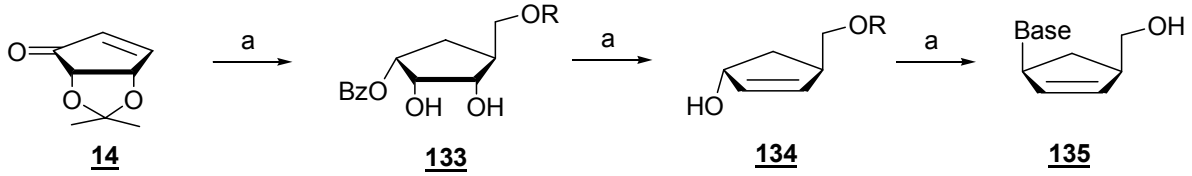
Trost and co-workers reported improvements in the asymmetric desymmertrization reaction in 1996 (Scheme 1.21).[108] A unique ligand **130**, a tertiary amine base (pempidine) and a modified guanine equivalent **131**, were employed in the coupling step. By this method, both enantioselectivity and regioselectivity were significantly improved.



Reagents and conditions: (a) $(C_3H_5PdCl)_2$, pempidine, **130**, **131**, DMSO/THF; (b) $[Pd_2(dba)_3]$-$CHCl_3$, Ph_3P; (c) i) tetramethylgunidine, ii) tetrabutylammonium oxone, Na_2CO_3, iii) $Ca(BH_4)_2$, iv) NH_4OH

Scheme 1.21. Improved asymmetric synthesis of carbovir[108]

Chu and co-workers developed another sequence to prepare L-carbovir analogs as shown in Scheme 1.22.[109] Starting from L-enone **14**, which could be synthesized from D-ribose in several steps, the diol **133** was obtained *via* a four-step sequence in high yield. Treating diol **133** under pyrolytic elimination condition successful provided the allylic alcohol **134**. Condensation of allylic alcohol **134** with proper base moieties, such as 6-chloropurine, under Mitsunobu conditions provided desired nucleosides which was subjected to base derivation and deprotection steps to furnish the target L-carvovir analogs (**135**). This scheme is very straightforward and suitable for scale-up.



14 **133** **134** **135**

Reagents and conditions: (a) i) *tert*-butyl methyl ether, *sec*-BuLi, ^{t}BuOK, CuBr·Me_2S, ii) $NaBH_4$, $CeCl_3$·7H $_2O$, iii) BzCl, iv) HCl; (b) i) $CH(OMe)_3$/ Py/ *p*-TSA, ii) Ac_2O, 120ºC-130ºC; iii) NaOH; (c) Mitsunobu couplings & base derivation

Scheme 1.22. Synthesis of L-carbovir analogs[109]

Carbovir and Abacavir Analogs (Figure 1.9). In light of the fact that noraristeromycin and norneplanocin increased selectivity, racemic 5'-norcarbovir **136** and 5'-norabacavir **137** were prepared by Huang et al.[110] However, it was found that only norabacavir **137** showed moderate anti-HIV-1 activity with an EC_{50} of 5.0 μg/mL, but it was toxic to host cells. The synthesis of both compounds started from an epoxide **144**, which underwent the Trost type coupling reaction to give nucleoside **145**. Further base derivation afforded desired **136** and **137** (Scheme 1.23).

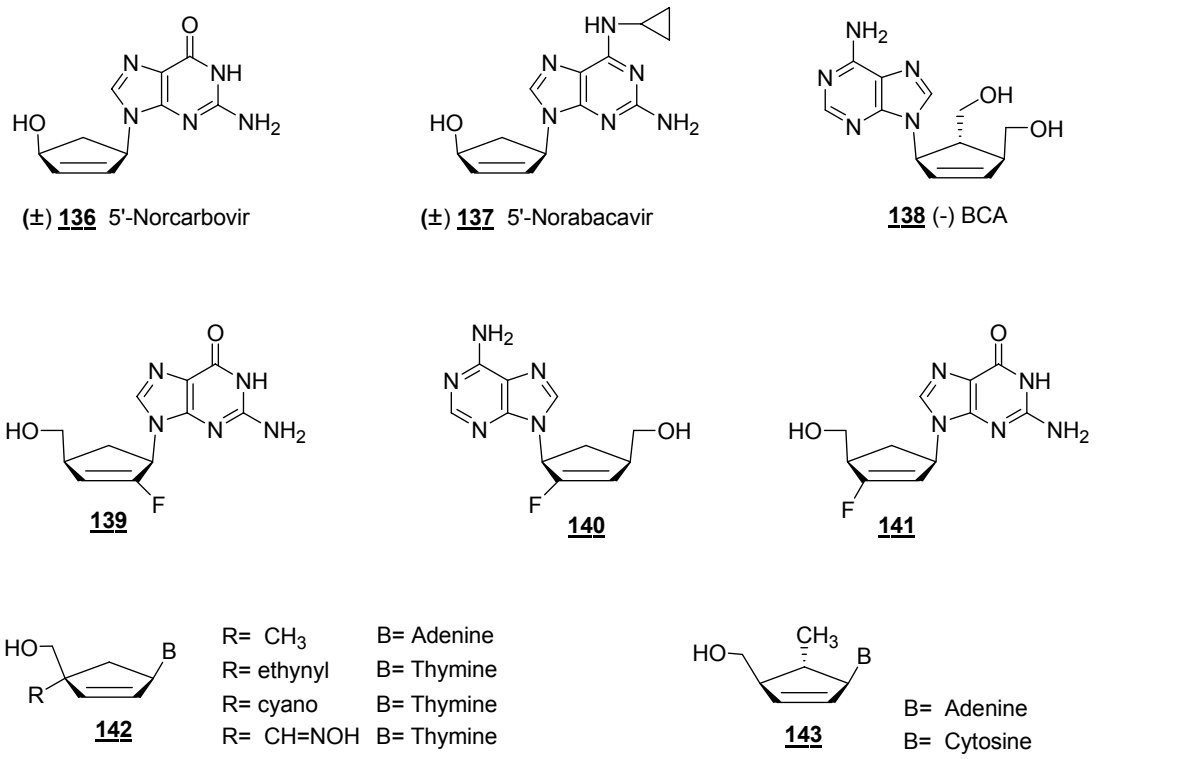
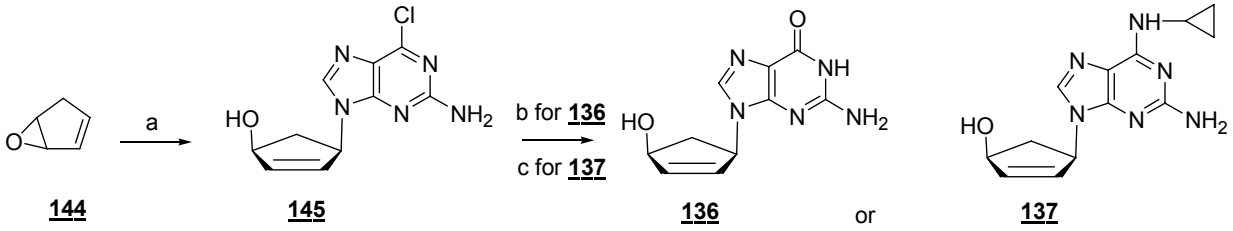


Figure 1.9. Carbovir and abacavir analogs

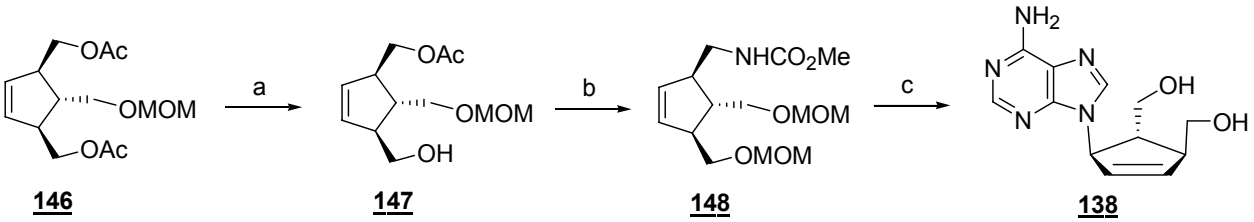


Reagents and conditions: (a) $Pd(OAc)_2$, TPP, base (b) NaOH; (c) EtOH, cyclopropylamine

Scheme 1.23. Synthesis of norcarbovir and norabacavir[110]

Katagiri and Kaneko reported (-) BCA **138**, an unnatural L-carbocylic nucleoside as a potent anti-HIV-1 agent with an EC_{50} of 0.71 μM in MT-4 cells.[111-113] However, no updated

information of this compound is available. The enzymatic resolution by *Rhizopus delemar* lipase (RDL) was applied in the synthesis of optically pure intermediate **146**, which was then oxidized and underwent Curtius rearrangement to give carbamate **148**. A deprotection step followed by a well known base construction procedure afforded the target nucleoside **138** (Scheme 1.24).



146 **147** **148** **138**

Reagents and conditions: (a) *Rhizopus delemar* lipase; (b) i) MOMCl, ii) K_2CO_3/MeOH; iii) PCC and then $NaClO_2$, iv) DPPA; (c) i) KOH, ii) Base construction, iii) NH_3

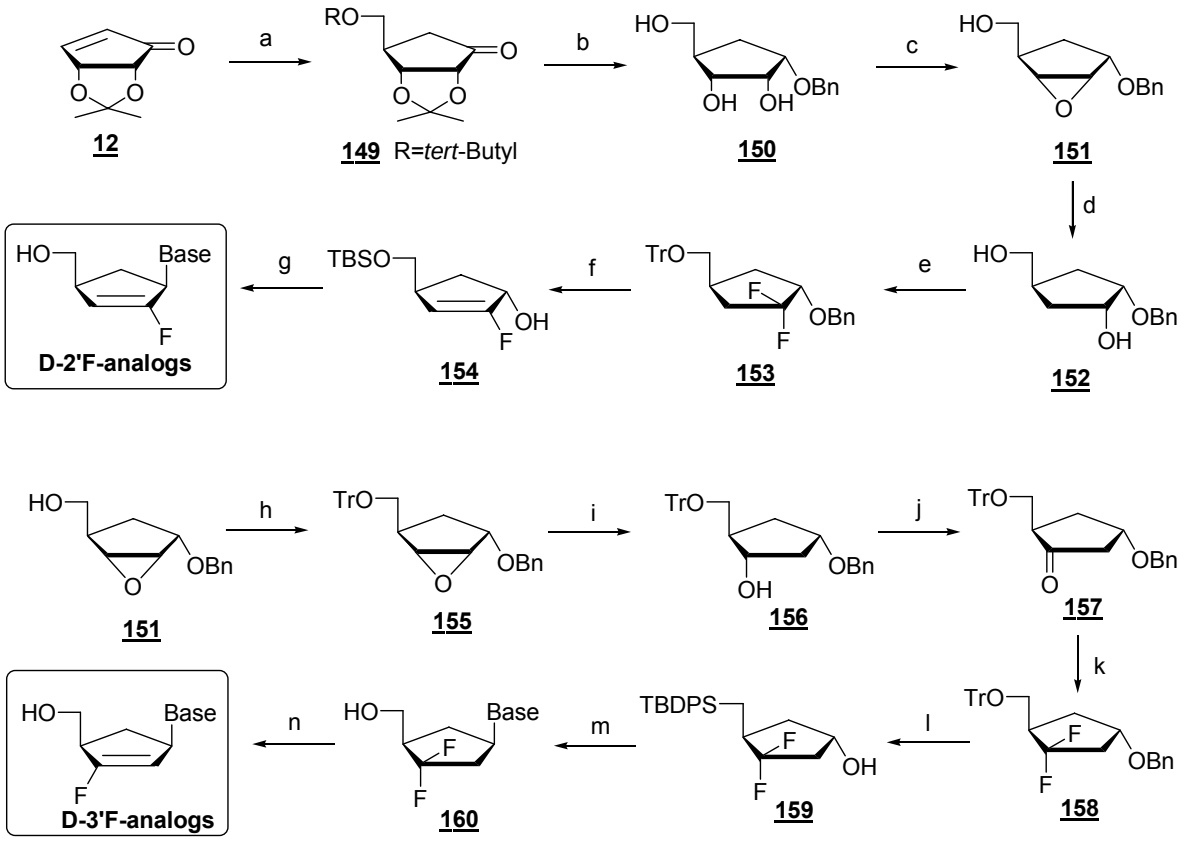
Scheme 1.24. Synthesis of norcarbovir and norabacavir[113]

Toyota and co-workers reported the synthesis of 3'-fluorine substituted carbovir analog **139** in 1998, however no biological data were provided.[114] Since then, Chu and co-workers have accomplished the synthesis, and antiviral screening of both 2'- and 3'-fluorine substituted analogs including D- and L-nucleosides were reported (Table 1.2).[115, 116]

Table 1.2. Antiviral activity of compounds **140** and **141** against HIV-wild type (xxBRU) and M184V mutant

Compds	**xxBRU**		**M184V**		**FI**[a]
	EC_{50} (μM)	**EC_{90} (μM)**	**EC_{50} (μM)**	**EC_{90} (μM)**	
140	0.77	8.34	75.3	>100	98
3TC[b]	0.027	0.25	>100	>100	>100
141	0.098	0.58	3.8	14.9	38.8
Carbovir[c]	0.087	0.27	0.20	1.1	2.3

[a]FI is the fold increase (EC_{50} HIV-1_{M184V} / EC_{50} HIV-1_{xxBRU}; [b]positive control for compound **140**; [c]Positive control for compound **141**.



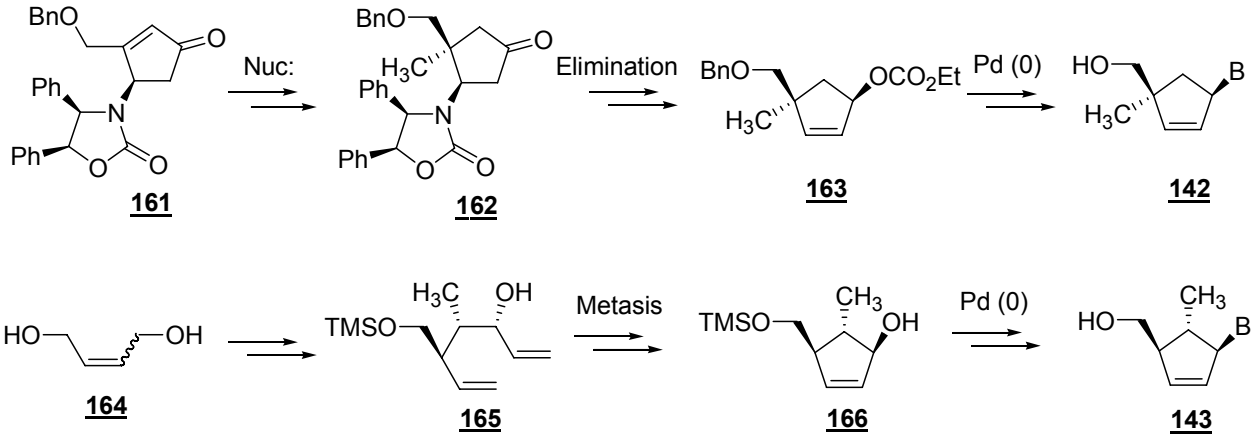
Reagents and conditions: (a) i) *tert*-butyl methyl ether, *sec*-BuLi, ^{t}BuOK, CuBr·Me_2S; (b) i) $NaBH_4$, $CeCl_3$·7H_2O, ii) BnBr, iii) HCl; (c) AIBBr, K_2CO_3; (d) LAH; (e) i) TrCl, Py, ii) PDC, iii) DAST; (f) i) ^{t}BuOK, THF, ii) HCl, iii) TBSCl, Im, iv) Na/NH_3 (liq.); (g) Mitsunobu conditions and base derivations; (h) TrCl, DMAP; (i) Super-hydride; (j) PDC; (k) neat DAST; (l) i) TMSI, ii) TBDPSCl, Im; (m) i) Mitsunobu conditions and base derivations, ii) deprotection; (n) microwave assisted elimination, ^{t}BuOK, DMF

Scheme 1.25. Synthesis of 2'-F & 3'-F d4 carbocyclic nucleosides[115, 116]

Among 2'-F substituted nucleosides, L-adenine derivative **140** was the most potent anti-HIV agent with an EC_{50} of 0.77 μM without toxicity at a concentration up to 100 μM. In the other series, D-3'-fluorinated guanosine analog **141** exhibited potent anti-HIV activity (EC_{50} 0.41 μM) with marginal toxicity. Interestingly, this compound (**141**) showed significant cross-resistance to the HIV M184V mutant which was believed to be the result of template/primer realignment as indicated in the molecular modeling studies.

To synthesize 2'-fluorinated compounds, 2'-F-allylic alcohol **154** was prepared from a common enone intermediate **12** in thirteen steps. Compound **12** was then condensed with bases under Mitsunobu conditions and subjected to base derivations to afford target nucleosides. However, the 3'-F analog of **154** was difficult to prepare due to its instability. Therefore, in the case of 3'-fluorinated analogs, the double bond was generated in the final stage of the whole sequence by microwave assisted reactions (Scheme 1.25). The L-series compounds were prepared in the same manner.

Modifications of 4'- and 6'-position of carbocyclic ring have also generated compounds **142**[117, 118] and **143**[119] (Scheme 1.26). However, neither of them showed significant biological activity.

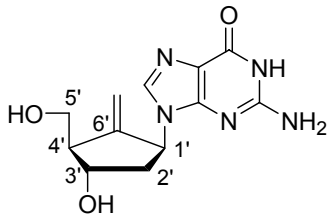


Scheme 1.26. General schemes for the synthesis of compounds **142** and **143**[117, 119]

Entecarvir and Analogs

Entecavir (Figure 1.10). Entecavir (**167**) is a 2'-deoxy carbocyclic guanosine analog with an exo-cyclic double bond on the 6'-position. This compound undergoes rapid intracellular phosphorylation to the active triphosphate form,[120] which inhibits HBV replication by acting as a

non-obligate chain terminator in priming, at the RNA dependent DNA synthesis and DNA dependent DNA synthesis stages.[121] *In vitro* studies demonstrated that entecavir was most potent inhibitor of HBV replication in comparison to other anti-HBV agents (EC_{50} 3.75 nM in HepG 2.2.15 cell assay).[122-125] Resistance studies indicated that 3TC/FTC-double mutant (rtM204V/I and rtL180M) reduced the viral susceptibility to entecavir by 20 to 30-fold while adefovir-resistant mutant (rtN236T) retained full susceptibility to this compound.[126] In the clinical trial, a small proportion (6 %) of patients developed entecavir-associated mutation after a long-term administration. However, most of them did not experience confirmed virological rebounds.[127, 128] The main resistance mutations of entecavir are rtT184G, rtS202I and rtM250V on a background of lamivudine-resistant mutations.[129, 130]



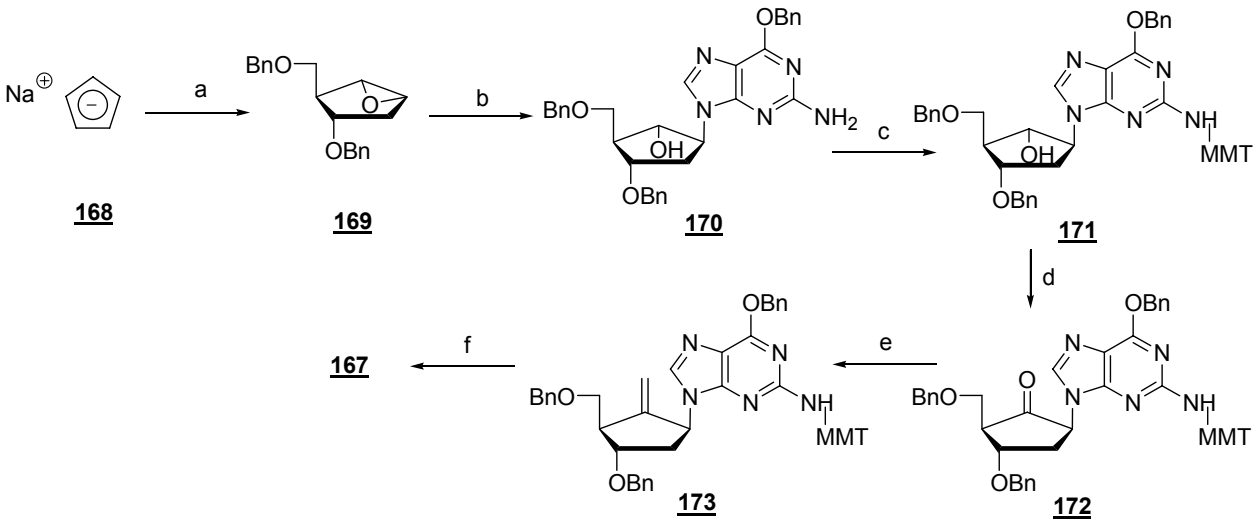
167 Entecavir

Figure 1.10. Structure of entecavir

Phase II trials showed that entecavir administration at dose of 0.5 or 1.0 mg/day for 4 weeks produced significant reduction of serum HBV DNA in both nucleoside-naïve and lamivudine-experienced patients, and the viral load rebound was slower than lamivudine treatment after cessation of the therapy.[131-134] In three randomized, multi-center phase III trials, nucleoside-naïve or -experienced patients (HBeAg positive or negative) were included. After 2 years of administration, 81 % of entecavir recipients (0.5 mg/day) had a viral load below 300 copies/mL versus only 39 % in the lamivudine recipients (100 mg/day), while ALT

normalization ratio and clearance of HBsAg ratio were 79 % versus 68 % and 5 % versus 3 %, respectively. In addition, entecavir administration at 1.0 mg/day produced significant viral load reduction in lamivudine refractory patients in comparison to the control group.[132] Based on these impressive results from the clinical trials, the US FDA has approved 0.5 & 1.0 mg dose of entecavir as an oral, once-daily drug (Baraclude®) for the treatment of chronic hepatitis B infection.

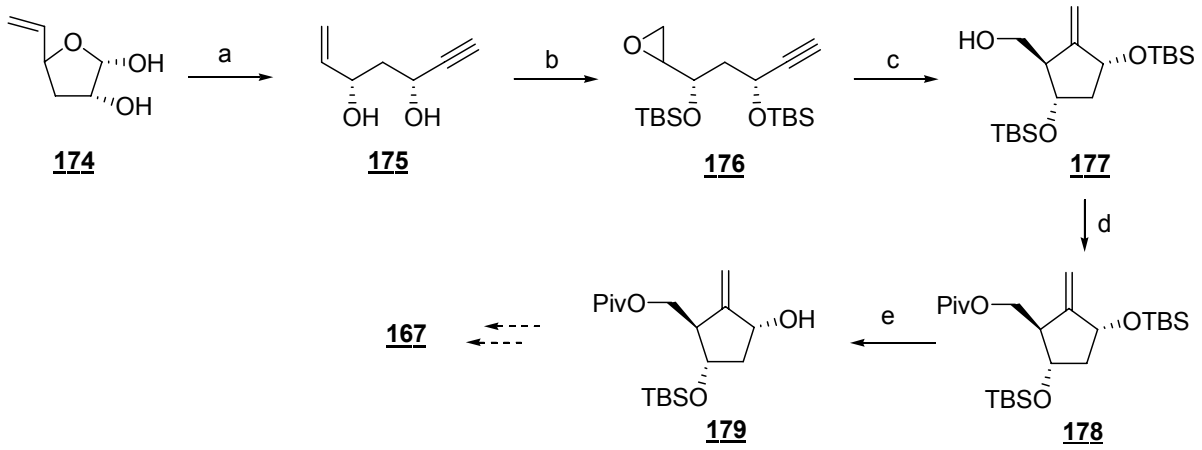
The first synthesis of entecavir was accomplished by Bisacchi et al.,[122] in which chiral epoxide **169** was prepared from sodium cyclopentadienide **168** by an asymmetric hydroboration/epoxidation/protection sequence. Treatment of epoxide **156** with a purine salt provided nucleoside **170** in 60 % yield with desired regioselectivity. After the protection of the amino group of **170**, Dess-Martin oxidation and Nysted methylenation afforded the exo-cyclic double bond compound 1**73**, which was converted to entecavir **167** after base derivation and deblocking steps (Scheme 1.27)



Reagents and conditions: (a) i) $BnOCH_2Cl$, ii) diisopinylcampheylborane, iii) NaOH, H_2O_2, iv) $VO(acac)_2$, *t*-BuOOH, v) BnBr, NaH; (b) NaH, Base; (c) MMTrCl; (d) Dess-Martin reagent; (e) Nysted reagent, $TiCl_4$; (f) Base derivation and deprotection

Scheme 1.27. Synthesis of entecavir[122]

Ziegler and Sarpong studied the radical cyclization of the important intermediate **179** toward the synthesis of entecavir (Scheme 1.28).[135] Compound **174** was prepared from D-diacetone by a known procedure.[136] Ohira's protocol[137] was applied to convert unsaturated **174** into terminal acetylene **175**, which was protected with a TBS group and treated with *m*CPBA to provide epoxide **176**. Compound **176** underwent intramolecular radical cyclization in the presence of Cp_2TiCl. Desired carbocyclic intermediate **179** was obtained after standard protection group manipulations.



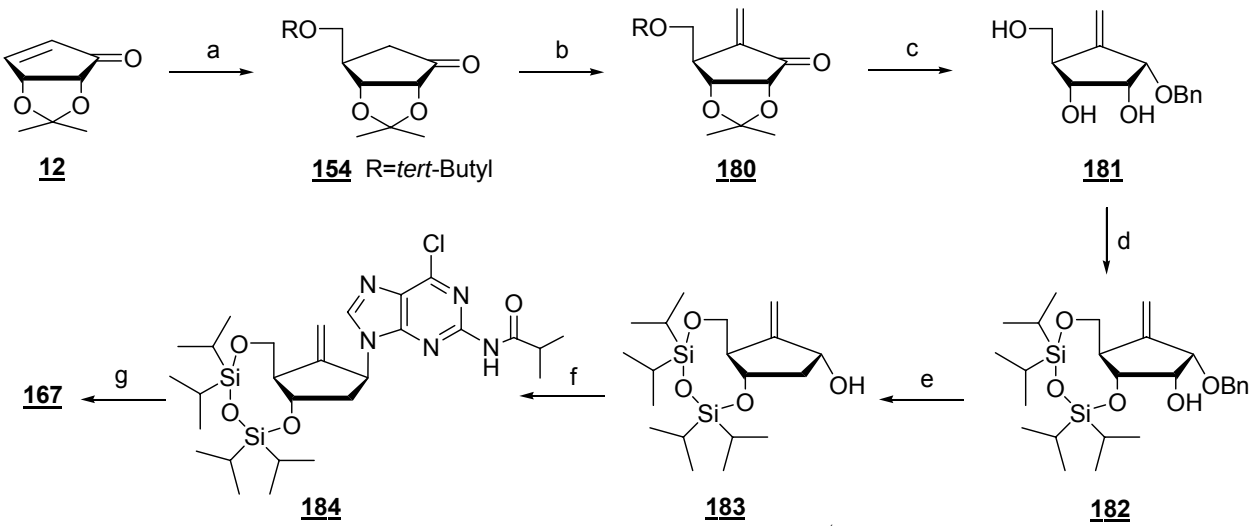
174 **175** **176** **177** **178** **179**

Reagents and conditions: (a) i) $(MeO)_2POCN_2COMe$, K_2CO_3; (b) i) TBSOTf, 2,6-lutidine, ii) *m*CPBA; (c) Cp_2TiCl; (d) PivCl, DMAP; (e) HOAc

Scheme 1.28. Synthesis of carbocyclic core **179** as an intermediate for preparing entecavir[135]

Alternative route to prepare entecavir was developed by Chu and co-workers starting from enone **12,** which was transformed to **154** using 1,4-addition method.[54] The exocyclic double bond was constructed under standard Mannich reaction/Hoffman elimination protocol which led to α, β-unsaturated ketone **180**. Compound **80** was subjected to reduction, protection and deprotection steps afforded triol **181**. The protection of the 3' and 5' hydroxyl groups was followed by Barton-McCombie deoxygenation and Birch reduction to yield key intermediate **183**.

Standard Mitsunobu coupling, base derivation and protecting-group manipulations furnished entecavir (**167**) (Scheme 1.29).[93]



Reagents and conditions: (a) *tert*-butyl methyl ether, *sec*-BuLi, ^{t}BuOK, CuBr·Me_2S; (b) i) LDA, Eschenmoser's salt, ii) MeI, $NaHCO_3$; (c) i) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, ii) BnBr, NaH, iii) HCl; (d) $TIPDSCl_2$/Imidazole; (e) i). NaH, CS_2, MeI; ii) Bu_3SnH, AIBN, f) Na/Liq NH_3; (f) DIAD, TPP, Base; (d) Base derivation and deprotection

Scheme 1.29. New scheme towards the synthesis of entecavir by Chu and co-workers[93]

Entecavir analogs (Figure 1.11). During the course of the development of entecaivr, its regio-isomers, **185** and **186**, were synthesized and screened against HBV. Unfortunately, both compounds proved to be inactive against HBV.[138]

The antiviral activity of (±)-**187**, a carbocyclic ribo-type adenine analog with exo-cyclic double bond, was first described in 1988.[77] It was found that this compound was active against vaccinia virus. Recently, Chu and co-workers have accomplished the asymmetrical synthesis of the whole series of D-form compounds for a complete SAR study.[93] However, no interesting biological activity of the target nucleosides were observed except marked cytotoxicity effect of (-)-**187**. The synthesis of **187** in Chu's protocol utilized the allylic alcohol **189**, which was obtained

by reducing α, β-unsaturated ketone **170** depicted in Scheme 1.30. Mitsunobu coupling of alcohol **189** with proper base equivalents, followed by base derivations and deprotection steps yielded the desired nucleosides.

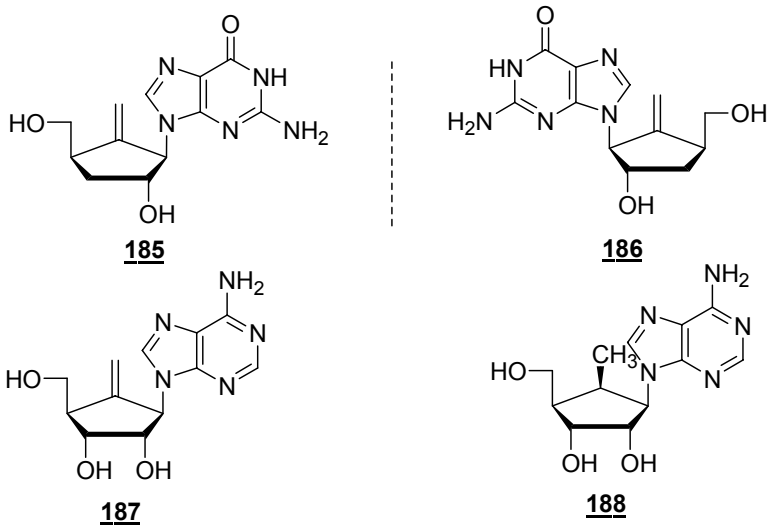
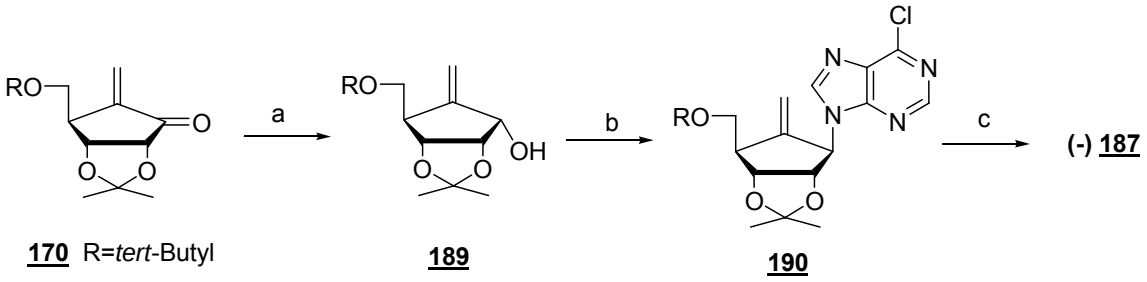


Figure 1.11. Entecavir analogs



Reagents and conditions: (a) $NaBH_4$, $CeCl_3 \cdot 7H_2O$; (b) DIAD, Ph_3P, Base; (c) Base derivation and deprotection

Scheme 1.30. Synthesis of (-) **187**[93]

Carbocyclic Arabino- and Xylo-Nucleosides

Cyclaradine **191**, a carbocyclic analog of ara-A, was discovered by Vince and co-workers.[139] It was resistant against adenosine deaminase and exhibited antiviral activity against HSV-1 (EC_{50} 2.8-9.0 μM) and vaccinia virus (EC_{50} 9.0 μM).[139] Carbocyclic xylo-nucleosides **192**, was reported to exhibit potent antitumor activity with EC_{50} of 0.38 μM. Its guanine analog **193** was active against HSV-1 (EC_{50} 1.8-3.0 μM).

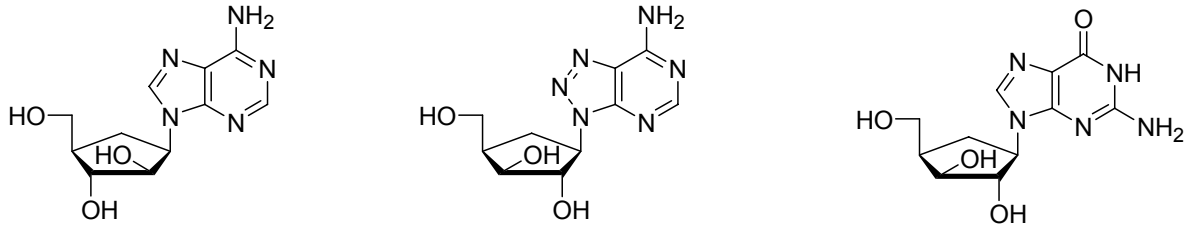
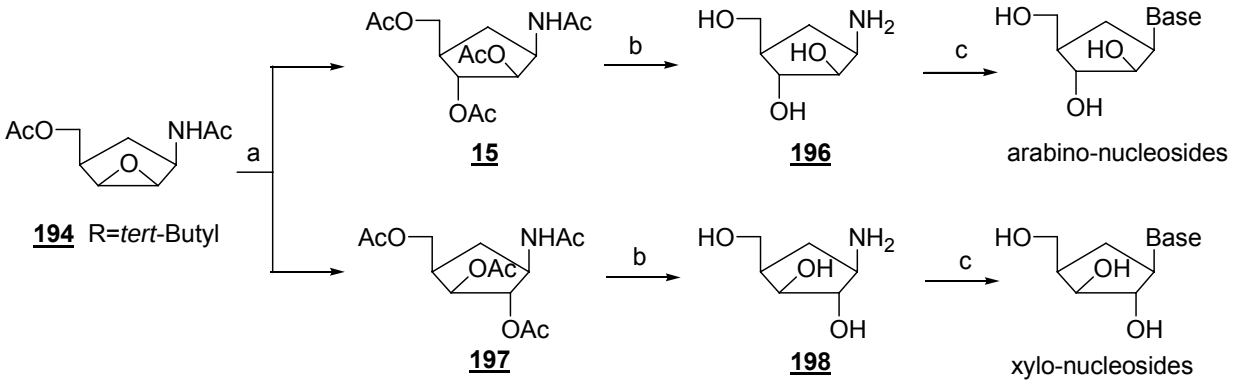


Figure 1.12. Biological active carbocyclic arabino- and xylo-nucleosides

The synthesis of both classes of compounds started from the same epoxide **194** which was hydrolyzed to yield two products: arabino-type **195** and xylo-type **197** (Scheme 1.31). Two intermediates were deprotected and further converted to the desired arabino- and xylo-nucleosides by known chemistry.

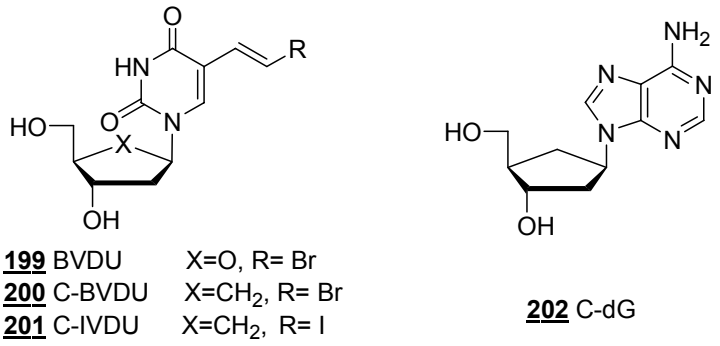


Reagents and conditions: (a) i) H_2SO_4, ii) Ac_2O; (b) i) HCl, ii) OH^-; (c) Bases construction

Scheme 1.31. Synthesis of carbocyclic arabino- and xylo-nucleosides[139]

Carbocyclic 2'-Deoxy-Nucleosides and Related Nucleosides

Carbocyclic 2'-deoxy nucleosides (Figure 1.13) (E)-5-(2-Bromovinyl)-2'-deoxyuridine (BVDU) is a highly active anti-herpes agent.[140] However, the fast degradation to (E)-5-(2-Bromovinyl)-2'-deoxyuracil (BVU) catalyzed by pyrimidine nucleoside phosphorylases limits the therapeutic usage of BVDU.[141] The carbocyclic counterpart, *C*-BVDU, however, is no longer a substrate for phosphorylases while it maintains the antiviral potency.[142] Its analog, (E)-5-(2-iodovinyl)-2'-deoxyuridine (*C*-IVDU), exhibits similar selectivity as well as antiviral activity.

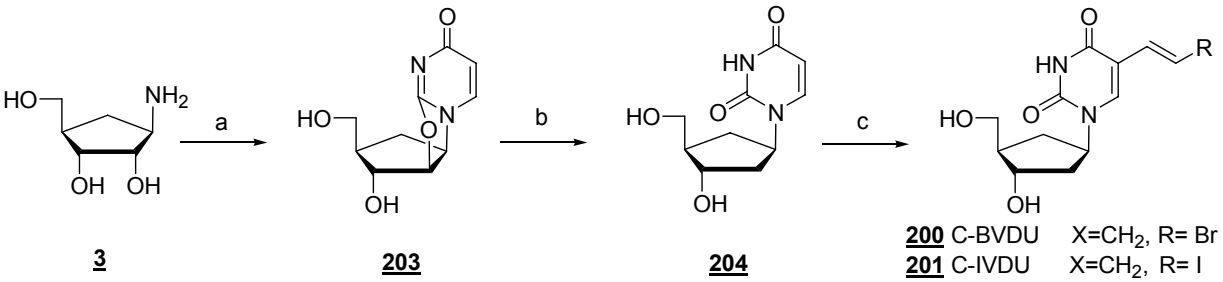


199 BVDU X=O, R= Br
200 C-BVDU X=CH_2, R= Br
201 C-IVDU X=CH_2, R= I

202 C-dG

Figure 1.13. Biological active carbocyclic 2'-deoxy nucleosides

The synthesis of *C*-BVDU and *C*-IVDU started from aminotriol **3**, which was converted to anhydrouridine **203** in three steps. Treatment of **203** with acetyl bromide followed by dehalogenation and deprotection provided carbocyclic 2'-deoxyuridine **204**. Introduction of 5-vinylbromide was accomplished *via* an iodination/coupling reaction/hydrolyzation/bromination sequence (Scheme 1.32). *C*-IVDU was prepared in a similar manner.[142]

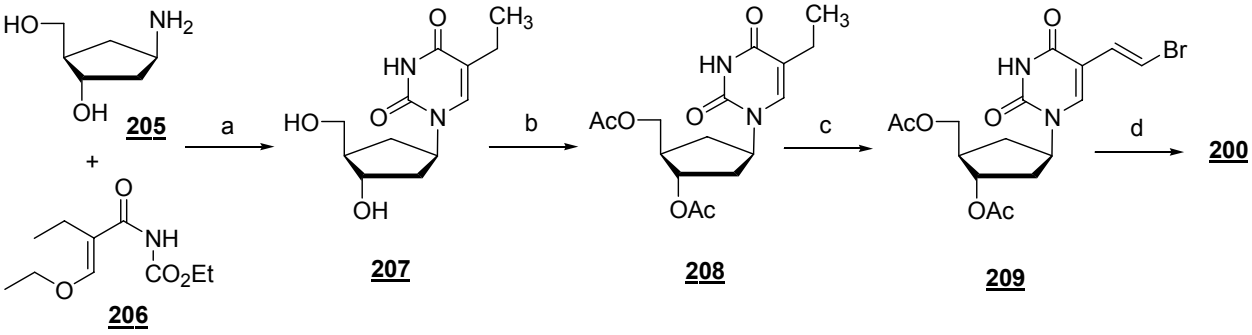


3 **203** **204**

Reagents and conditions: (a) i) silver cyanate, β-methoxyacryloyl chloride, ii) NH_4OH, iii) diphenyl carbonate; (b) i) acetyl bromide, ii) Bu_3SnH, AIBN; (c) i) I_2, nitric acid, ii) methyl acrylae, $Pd(OAc)_2$, Ph_3P, Et_3N, iii) KOH, iv) NBS for **200** or I_2, iodic acid, K_2CO_3 for **201**

Scheme 1.32. Synthesis of *C*-BVDU and *C*-IVDU[142]

Another approach to the synthesis of *C*-BVDU was described by Wyatt et al. as outlined in Scheme 1.33, which is comparably concise with the original one.[143]



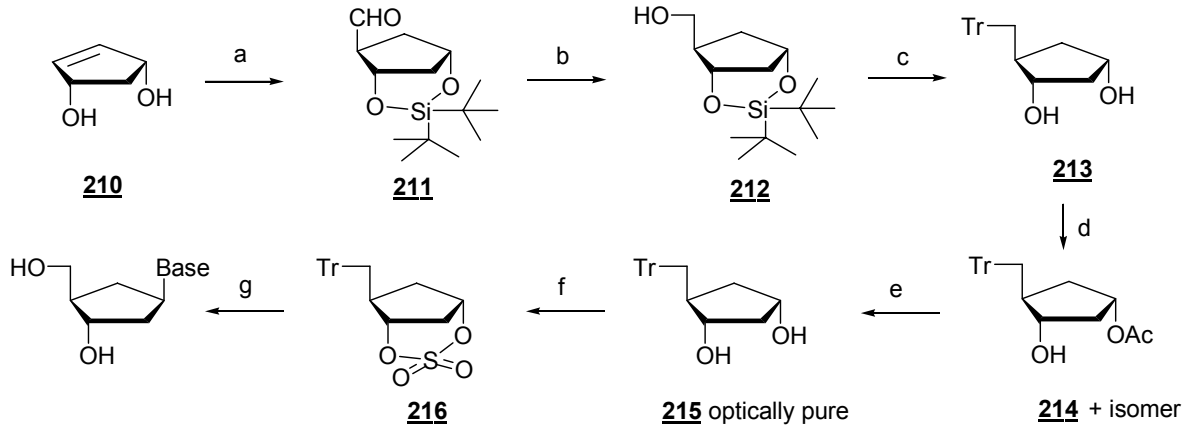
Reagents and conditions: (a) i) Et_3N, ii) HCl; (b) Ac_2O, DMAP; (c) NBS or Br_2; (d) NaOH

Scheme 1.33. Alternative route of the synthesis of *C*-BVDU[143]

Another interesting compound in this series is carbocyclic 2'-deoxyguanosine (**202**, *C*-dG), which demonstrated potent antiviral activity against herpes simplex virus (HSV-1 & 2),[144] human cytomegalovirus (HCMV)[145] and HBV.[146] *C*-dG apparently is activated by virus-encoded kinase to exhibit anti-herpes activity while it is a poor substrate for cellular phosphorylating

enzymes.[147] Parker et al. showed that *C*-dG can incorporate into viral DNA and functions as a competitive inhibitor.

Racemic *C*-dG was synthesized by a linear method.[144] It is noteworthy that the enantiomeric synthesis of *C*-dG was accomplished by Liang and Moser *via* an enzymatic approach (Scheme 1.34).[148] Protection and hydroformylation of a racemic vinyl diol **210** led to an aldehyde **211**, which was reduced to an alcohol **212**. A standard protecting-group manipulation provided compound **213**. Enzymatic resolution of **213** with *Pseudomonas fluorescens* lipase (PFL) in the presence of vinyl acetate followed by the deprotection of the acetate group furnished optically pure compound **215**. Cyclic sulfate **215** was then prepared and condensed with base moieties to provide the target carbocyclic nucleosides.

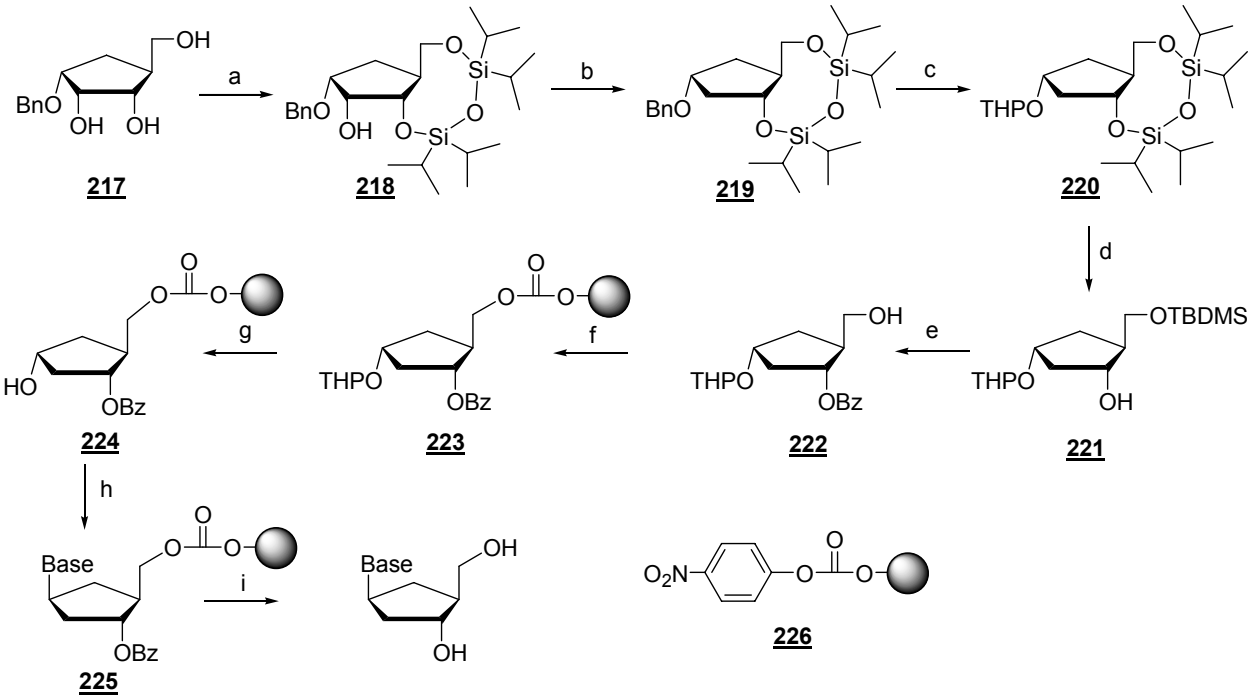


Reagents and conditions: (a) i) $(t\text{-Bu})_2SiOTf_2$, 2,3-lutidine, ii) $[RhCl(PPh_3)_3]$, H_2/CO, 80 bar; (b) $NaBH_4$; (c) i) TrCl, DMAP, Et_3N, ii) TBAF; (d) PFL, vinyl acetate; (e) ethylenediamine; (f) i) $SOCl_2$, Et_3N, ii) $RuCl_3$, $NaIO_4$; (g) i) NaH, base, ii) deprotection

Scheme 1.34 Synthesis of carbocyclic 2'-deoxynucleoside[144]

Borthwick et al. described another enzymatic resolution method soon after Liang and Moser's report.[149] Chu and co-workers developed a solid phase synthesis of L-*C*-dG as well as

its analogs (Scheme 1.35).[150] Standard protecting-group manipulation and radical deoxygenation led to compound **222**, which was coupled with *p*-nitrophenyl carbonate resin **225** to yield compound **223**. Fully protected **223** was subjected to the acidic hydrolysis to remove the THP group to provide alcohol **224** ready for Mitsunobu coupling reaction. In the coupling reaction, it was found that both regioselectivity and yield were generally improved under the solid phase condition in comparison with solution phase synthesis.[150]

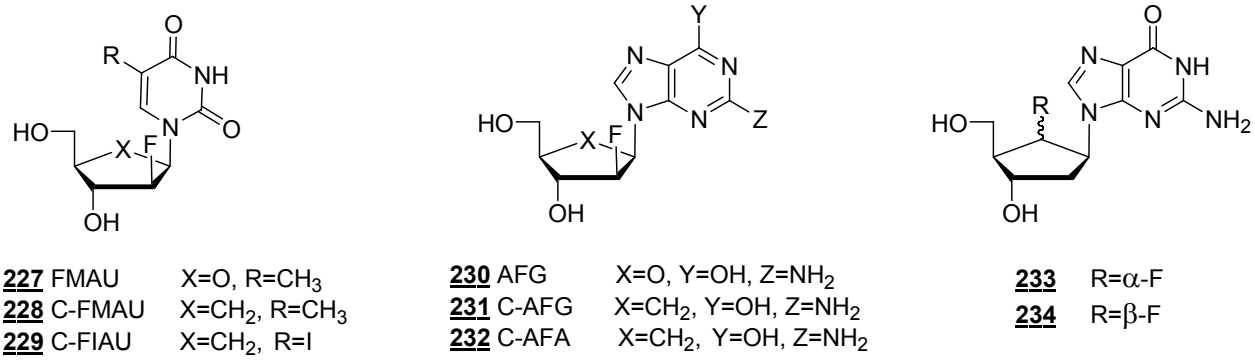


Reagents and conditions: (a)$TIPDSCl_2$, Py; (b) i) NaH, CS_2, MeI; ii) Bu_3SnH, AIBN; (c) i) Pb(OH), H_2, ii) DHP, PPTS; (d) i) TBAF, ii) TBDMSCl, Im; (e) i) BzCl, Py, ii) TBAF; (f) **213**, DMAP, DIPEA; (g) PPTS, 1-butanol/1,2-dichloroethane; (h) DIAD, Ph_3P, Bases; (i) K_2CO_3

Scheme 1.35. Solid phase synthesis of L-carbocyclic 2'-deoxynucleosides[150]

Analogs of carbocyclic 2'-deoxynucleosides (Figure 1.14) The most interesting analogs of carbocyclic 2'-deoxynucleosides are a series of 2'-fluoro substituted compounds. Among these analogs, the adenine derivative **232** was approximately 10-fold more active than cyclaridine (**191**)

against herpes viruses (HSV-1 & 2).[151] Guanine derivative **231** initially displayed significant antiviral activity against HSV-1 & 2 with EC_{50}s of 0.006 & 0.05 μg/mL, respectively, and did not show any toxicity up to 300 μg/mL. [152] Unfortunately, compound **231** was found to be toxic in later studies. Soon after this discovery, the same group in Glaxo described the synthesis and antiviral activity of fluorinated pyrimidine analogs.[153] However, only *C*-FMAU (**228**) and *C*-FIAU (**229**) exhibited moderate anti-HSV-1 activities which were both significantly lower than that of the parent compound FMAU (**227**). 6'-Fluoro substituted guanosine analogs were also prepared. The β-F analog **221** (EC_{50} 0.16 and 0.77 μg/mL for HSV-1 & 2, respectively) was approximately 50 to 100-fold more potent than α-F isomer **233**.



227 FMAU X=O, R=CH_3
228 C-FMAU X=CH_2, R=CH_3
229 C-FIAU X=CH_2, R=I

230 AFG X=O, Y=OH, Z=NH_2
231 C-AFG X=CH_2, Y=OH, Z=NH_2
232 C-AFA X=CH_2, Y=OH, Z=NH_2

233 R=α-F
234 R=β-F

Figure 1.14. Analogs of carbocyclic 2'-deoxynucleosides

The synthesis of these fluorinated carbocyclic nucleosides all followed a linear methodology *via* **235**, **236** or **237** as key intermediates that can be prepared from compound **3** by standard methods (Figure 1.15).[154]

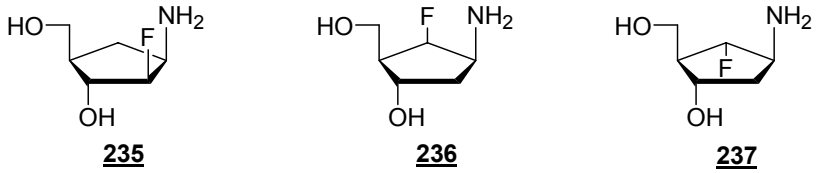


Figure 1.15. Key intermediates for the preparation of fluorinated nucleosides

Conformationally locked carbocyclic nucleosides

Neplanocin C (**238**), a naturally occurring carbocyclic nucleoside isolated from *Ampullariela regularis* in the early 1980's, provided a prototype of conformationally locked nucleoside with a cyclopropane ring fused on the 4', 5' position of a cyclopentane ring (Figure 1.16).[8, 155]

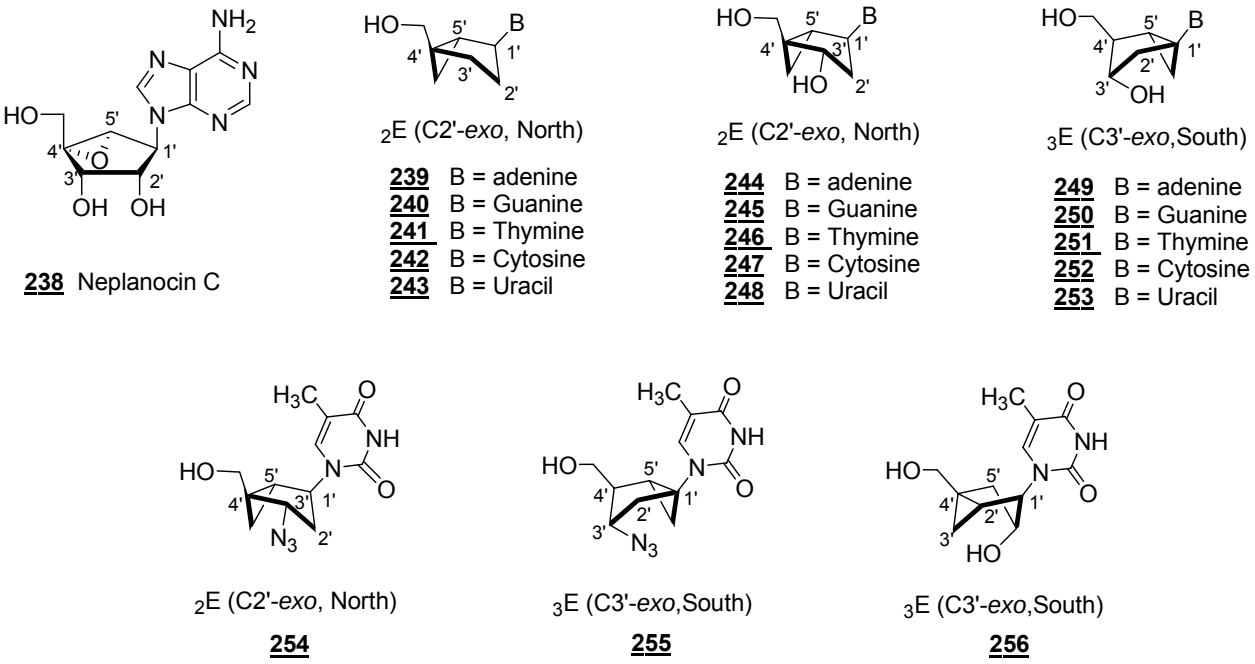
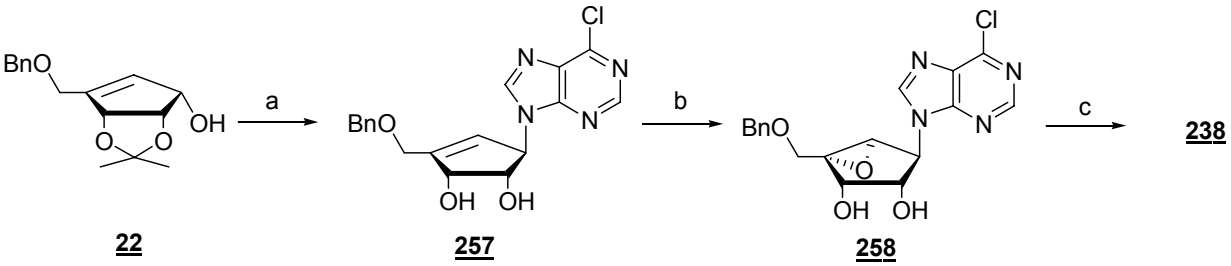


Figure 1.16. Conformationally locked carbocyclic nucleosides

The [3,1,0]-bicyclic system adopted a predominant Northern conformation as indicated in the X-ray analysis.[156] The synthesis of the neplanocin C started from a known cyclopentenol

22.[157] The cyclopentenol **22** was condensed with 6-chloropurine and deblocked the acetonide group to provide nucleoside **257**, which was further subjected to the epoxidation, amination followed by debenzylation to furnish the target neplanocin C (Scheme 1.36).

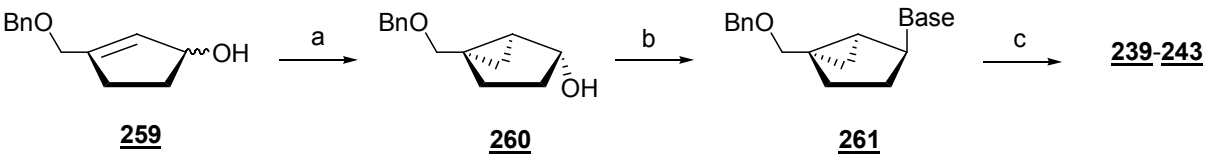


Reagents and conditions: (a) i) DIAD, Ph_3P, 6-chloropurine, ii) AcOH; (b) *m*-CPBA; (c) NH_3/MeOH, ii) H_2, Pd/C

Scheme 1.36. Synthesis of neplanocin C[157]

Inspired by the novel structure of neplanocin C, a number of carbocyclic analogs have been prepared. Among these compounds, the most systematically and extensively studied are those 1',5'-methano (**249-253**) as well as the 4',5'-methano carbocyclic nucleosides (**244-248**) as shown in Figure 1.16.

D-form adenine derivative **239** was found to adopt a typical Northern conformation and exhibited moderate anti-HIV activity while its enantiomer was devoid of the antiviral activity. The synthesis of the target nucleosides was accomplished *via* a cyclopropane fused cyclopentanal **260**, which was prepared by treating compound **259** with chloroiodomethane in the presence of samarium (+2) at -78 °C. Condensation of various base moieties with **260** followed by base derivation provided the desired nucleosides **239-243** (Scheme 1.37).[158, 159]

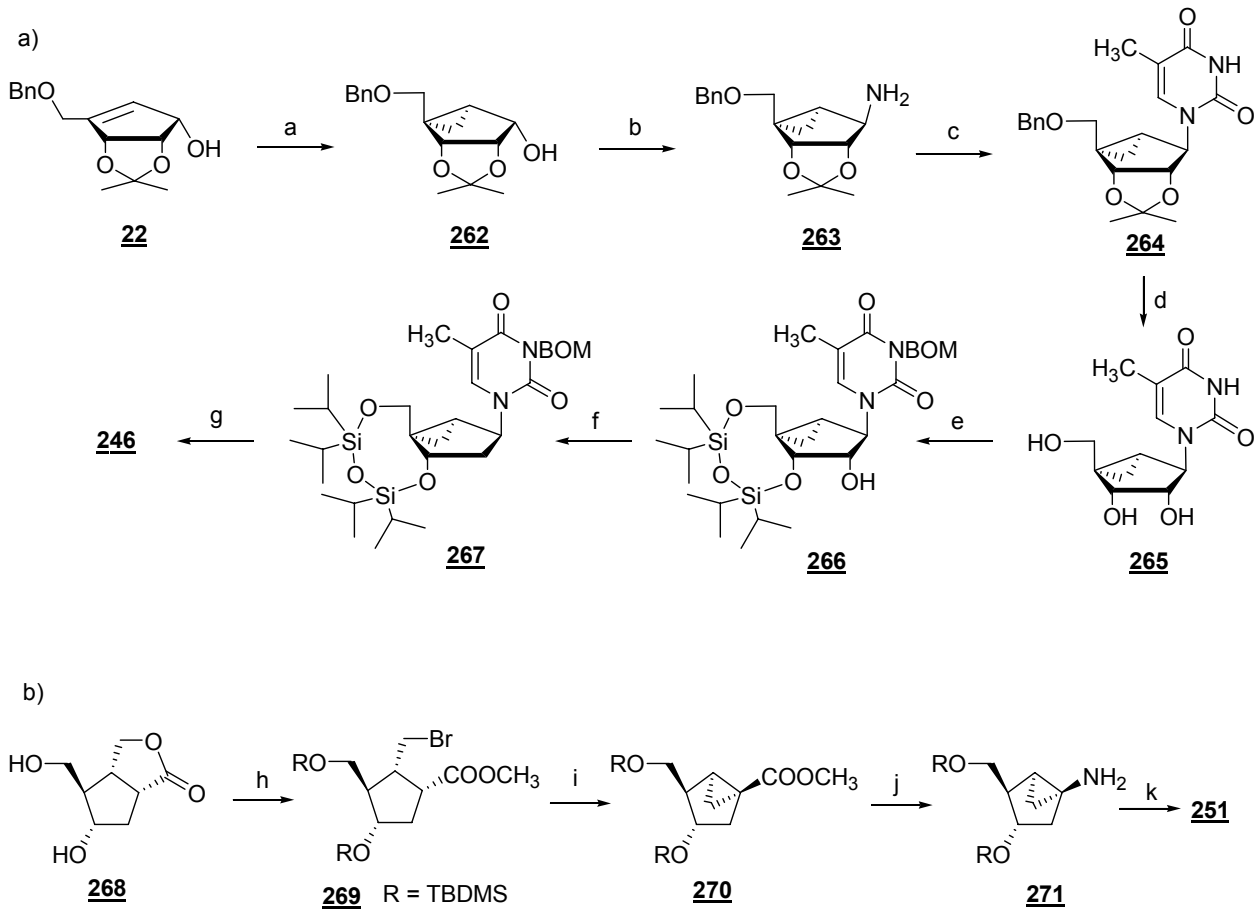


259 **260** **261**

Reagents and conditions: (a) CH_2ICl, Sm, $HgCl_2$; (b) DIAD, Ph_3P, Bases; (c) Base derivation and deprotection

Scheme 1.37. Synthesis of compounds **239-243**[158, 159]

Soon after the report of conformationally locked carbocyclic dideoxynucleosides as described above, Altmann et al. also accomplished the synthesis of both 4',5'- and 1',5'-methano-2'-deoxy carbocyclic thymidine (**246** and **251**, Scheme 1.38).[160] The synthesis of **246** was accomplished starting from allylic alcohol **22**, which was subjected to the Simmons-Smith cyclopropanation to give ring fused compound **262** with the desired stereochemistry due to the directing effect of the allylic hydroxyl group.[161] After the alcohol **262** was converted into the amine **263**, the heterocyclic moiety was constructed under standard conditions to give nucleoside **265**, which was protected and subjected to the Barton McCombie deoxygenation to afford target compound **246**. On the other hand, the synthesis of 1',4'-methano-2'-deoxy carbocyclic thymidine utilized the bicyclic lactone **268** as a key intermediate, which was treated with TMSBr followed by *N*-TBDMS to provide compound **269**. Formation of the three-membered ring went smoothly under basic condition. Subsequent deprotection, Curtius rearrangement, deprotection and base construction furnished target nucleoside **251**. The X-ray analysis indicated that 4',5'-methano-2'-deoxy carbocyclic thymidine **246** preferred boat-like Northern conformation while the isomer **251** existed predominately as boat-like Southern conformation.



Reagents and conditions: (a) Zn/Cu, CH_2I_2; (b) i) TsCl, Et_3N, DMAP, ii) NaN_3, iii) H_2, Lindar's catalyst; (c) Base construction; (d) i) HCl, ii) H_2, Pd/C; (e) i) $TIPDSCl_2$, Im, ii) BOM-Cl, DBU; (f) i) $CH_3C_6H_4OC(S)Cl$, DMAP, Et_3N, ii) Bu_3SnH, AIBN; (g) i) TBAF, ii) H_2, Pd/C, ii) NaOMe; (h) i) TMSBr, $ZnBr_2$, ii) *N*-methyl acetamide; (i) ^{t}BuOK, *t*-BuOH; (j) i) KOH, ii) DPPA, Et_3N, iii) H_2, Pd/C; (k) base construction and deprotection

Scheme 1.38. Synthesis of **246** and **251**[160]

In the series of Northern 2'-deoxy nucleosides, the adenine analog **243** was prepared by Siddiqui and Marquez et al. and showed good antiviral activity against HCMV and EBV.[162] To explore the full potential of this class of molecules, a series of nucleoside bases (adenine, uracil, cytosine and guanine) built on the Northern bicyclo[3,1,0]hexane pseudo-sugar ring were synthesized by using a convergent approach.[163] Nucleoside **246** displayed excellent anti-herpes activity with EC_{50} of 0.03 and 0.09 μg/mL against HSV-1 and HSV-2, respectively. It was non-

toxic to host cells at concentration up to 100 μg/mL. Interestingly, the isomer of compound **251**, Southern bicyclo[3,1,0]hexane thymidine, was devoid of antiviral activity. Conformational analysis revealed that not only the ring pucker of these two conformationally rigid nucleosides were quite different (**246**: Northern, **251**: Southern), but also the base rotation angle (χ) of compound **251** was more stiff in comparison to **246**. All of these disparities together might explain, to some extent, the difference in the antiviral activity of these two compounds. Based on these findings, Marquez and co-workers performed a systematic SAR study of a number of conformationally locked carbocyclic nucleosides (**254-256**).[164-168] Their data showed that herpes thymidine kinase had a strong preference to the Southern conformation and anti-base disposition in the monophosphorylation step but insensitive to the presence or absence of 3'-OH. However, in the diphosphorylation step, the 3'-OH was extremely important to this enzyme and Sourthern conformation was still preferred.[165-167] Cellular DNA polymerase and HIV reverse transcriptase favored exclusively the triphosphate of the Northern conformers.[164, 166, 168]

THREE-MEMBERED CARBOCYCLIC NUCLEOSIDES

In general, three-membered carbocyclic nucleosides can be divided in two classes. The first ones have the base moiety directly attached to the ring, while the other ones have a spacer between the base and the ring (Figure 1.17).

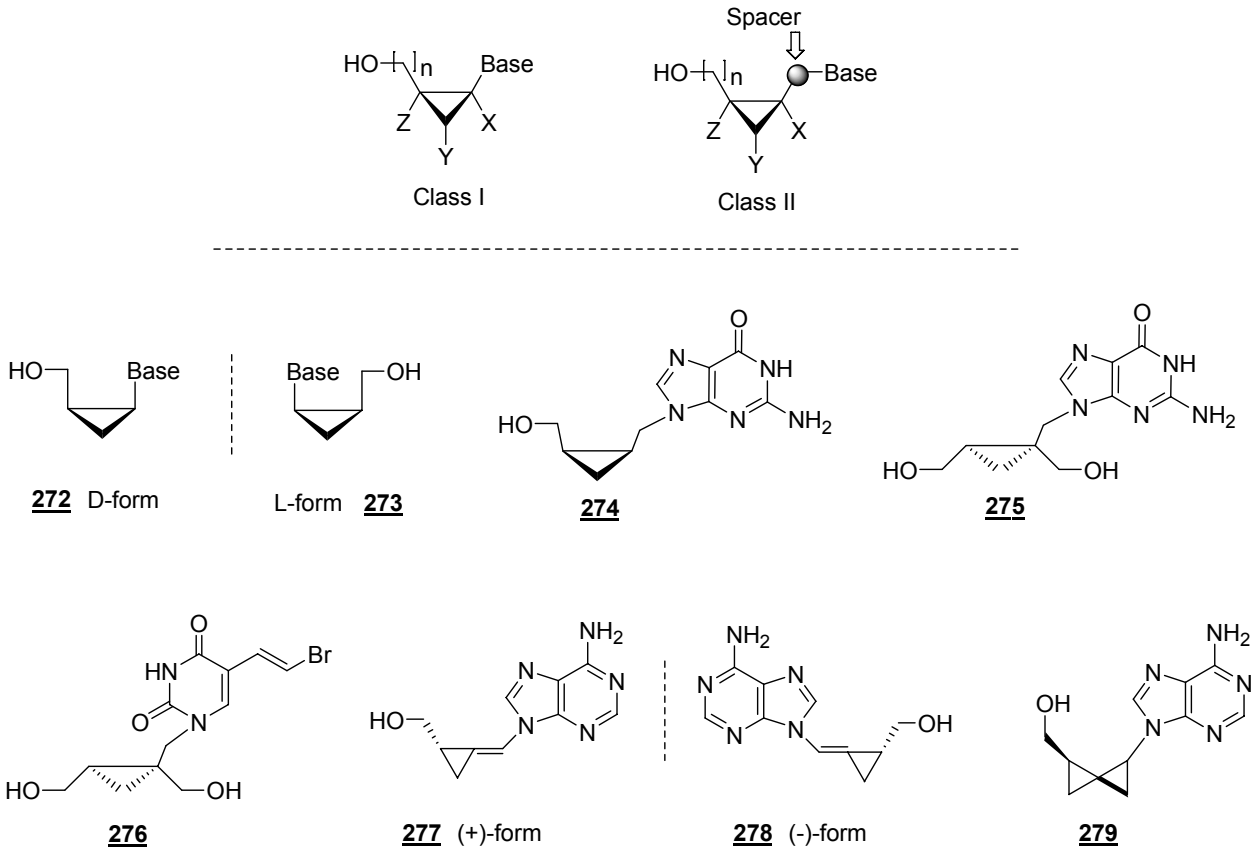
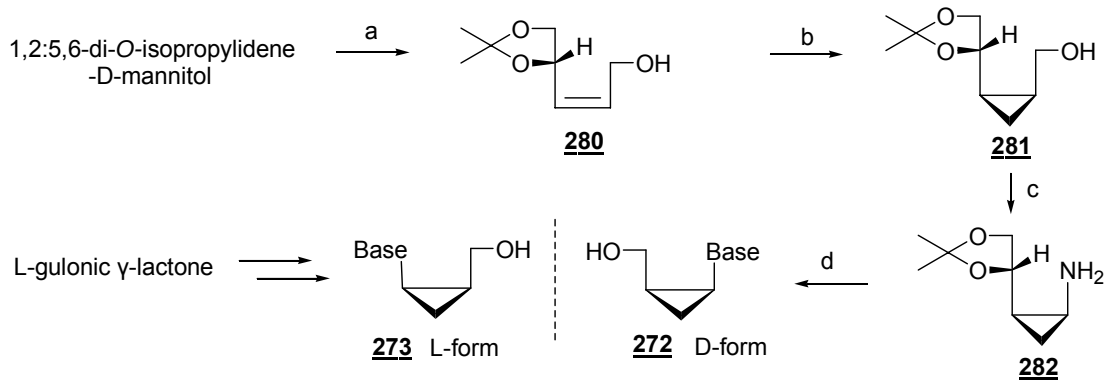


Figure 1.17. General structures of three-membered ring carbocyclic nucleosides and some representative molecules

Chu and co-workers accomplished the first asymmetric synthesis of D- & L-cyclopropyl nucleosides which belong to the first category (Scheme 1.39).[169] Protected D-mannitol was converted to the vinyl alcohol **280** by standard oxidation/wittig reaction/reduction sequence. The requisite cyclopropyl ring was installed by Simmons-Smith cyclopropanation following oxidation, Curtius rearrangement and deprotection protocol to yield cyclopropyl amine **282**. The target D-nucleosides **272** were obtained by a linear methodology. L-Cylcopropyl nucleosides **273** were synthesized in similar fashion using L-gulonic γ-lactone as chiral starting material. Unfortunately, no significant biological activity was exhibited by synthesized nucleosides.

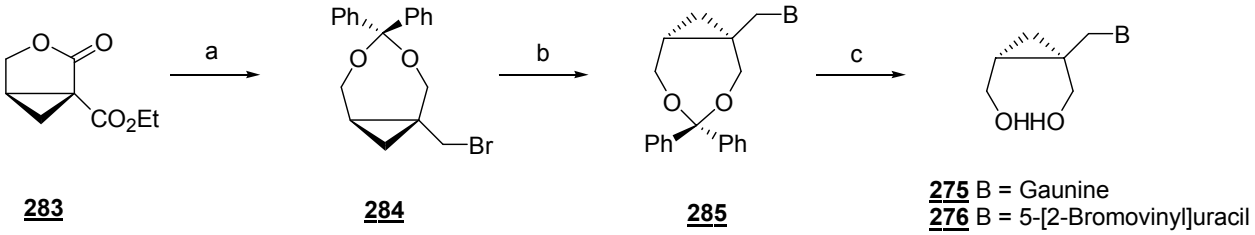


Reagents and conditions: (a) i) $Pb(OAc)_4$, ii) Ph_3P=CHCOOMe, iii) DIBAL-H; (b) $ZnEt_2$, CH_2I_2; (c) i) $RuO_2/NaIO_4$, ii) $ClCO_2Et$, triethylamine, iii) NaN_3, iv) BnOH, heat, v) H_2, Pd/C; (d) Base construction, deprotection

Scheme 1.39. Synthesis of cyclopropyl nucleosides **272** and **273**[169]

On the other hand, in the second category, several biologically interesting nucleosides were discovered. Ashton et al. reported that the conformationally constrained acyclovir analog **274** showed similar anti-HSV-1 & 2 activities to the parent nucleoside.[170, 171] Tsuji and co-workers explored extensive SAR of carbocyclic nucleosides bearing a methylene spacer between the base and pseudo-sugar ring.[171, 172] The guanine derivative **275** was active against HSV-1 and HSV-2 with EC_{50}s of 0.0093-0.035 and 0.12-0.24 μg/mL, respectively, in comparison to 0.27-1.0 and 0.25-1.3 μg/mL for acyclovir and 0.54-2.0 and 1.2-2.7 μg/mL for penciclovir. Furthermore, this nucleoside was 8 to 20-fold more potent than acyclovir and penciclovir against VZV, and the selectivity index of nucleoside **275** was also high. Studies demonstrated that **275** can be phosphorylated by HSV-1 thymidine kinase (TK) very efficiently. As an extension of the research, a series of 5-substituted uracil derivatives were prepared and some of target nucleosides exhibited potent anti-VZV activity.[173] Particularly, 5-bromovinyl nucleoside **276** was about 40-fold more potent than acyclovir and had good oral bioavailability in rats (68.5%). The

enantiomeric syntheses of compounds **275** and **276** were accomplished using chiral cyclopropane lactone **283** as starting material. The key intermediate was condensed with base moiety *via* classic SN_2 reaction followed by deprotection/derivation to afford target nucleosides (Scheme 1.40).[173]

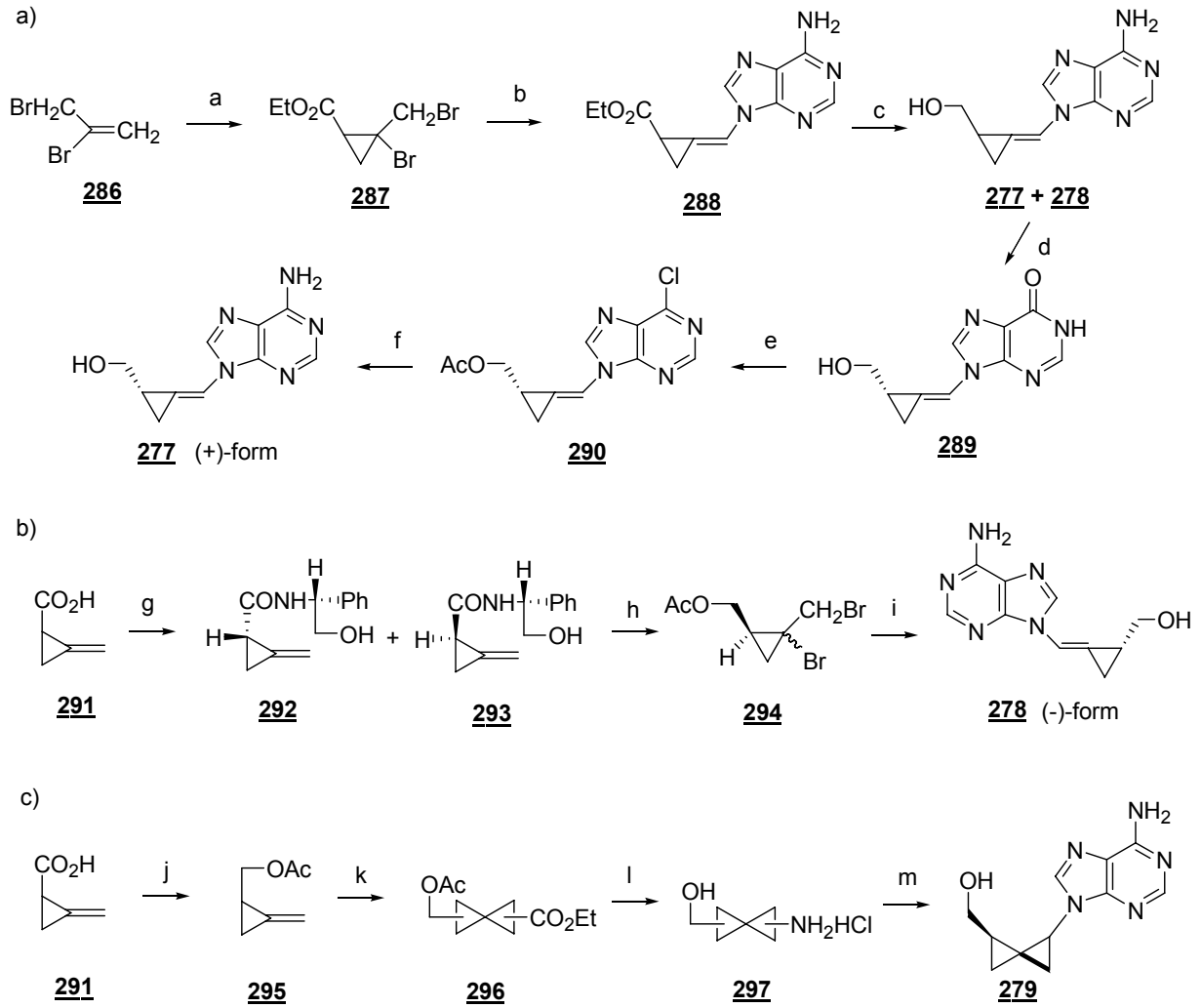


Reagents and conditions: (a) i) $NaBH_4$, ii) Ph_2CN_2, DDQ, iii) $LiBH_4$, iv) CBr, Ph_3P, Et_3N; (b) BVU, K_2CO_3; (c) HCl

Scheme 1.40. Synthesis of cyclopropyl nucleosides **275** and **276**[173]

Zemlicka and co-workers described another type of interesting nucleosides, in which the spacer between the base and the ring is an unsaturated double bond.[174, 175] Compounds, such as **277** and **278** (Figure 1.17), displayed broad-spectrum antiviral activity. The pair of enantiomers (**277** and **278**) were synthesized through enzymatic as well as chemical resolutions (Scheme 1.41). It was interesting to find that nucleosides **277** and **278** exhibited equipotent anti-HCMV activity (EC_{50} 2.9 and 2.4 μM, respectively). However, compound **277** was somehow more potent than **278** against HSV-1 & 2 with EC_{50}s of 8.8 vs. 38 μM and 35 vs. >50 μM, respectively. But compound **277** was less effective (EBV) or devoid of activity (HIV-1) in comparison to **278**. Further modifications of the spacer generated spiropentane nucleoside **279**. Although no antiviral activity was found at the nucleoside level, phosphoralaninate nucleotide of **279** showed

significant antiviral activity against HCMV, HSV-1&2, VZV, EBV HIV-1 as well as HBV, which indicated the inefficient phosphorylation of this class of nucleosides *in vitro*.[176]



Reagents and conditions: (a) N_2CHCO_2Et, $Rh(OAc)_4$; (b) K_2CO_3; (c) K_2CO_3, heat; (d) Adenosine deaminase, pH 7.5; (e) i) Ac_2O, Py, ii) $[Me_2N{=}CHCl]^+Cl^-$; (f) NH_3/MeOH; (g) *i*-BuOCOCl, Et_3N, ii) *(R)*-2-phenylglycinol, separation; (h) i) H_2SO_4, ii) HCl, EtOH, iii) Br_2, iv) DIBAL-H, v) Ac_2O, Py; (i) i) K_2CO_3, adenine, ii) NH_3/MeOH; (j) i) LAH, ii) Ac_2O, Py; (k) N_2CHCO_2Et, $Rh(OAc)_4$; (l) i) NaOH, separation, ii) Ac_2O, Py, iii) $(PhO)_2P(O)N_3$, Et_3N, *t*BuOH, iv) K_2CO_3, aq. MeOH, v) separation, vi) HCl, MeOH; (m) Base construction and deprotection

Scheme 1.41. Synthesis of cyclopropyl nucleosides **277**, **278** and **279**[174, 175]

A natural nucleoside, oxetanocin A **298** (OXT-A, Figure 1.18) is a four-member-ring nucleoside produced by *Bacillus megaterium*.[177, 178] The broad spectrum antiviral activity of the compound has prompted considerable attention to this class of nucleosides.[179]

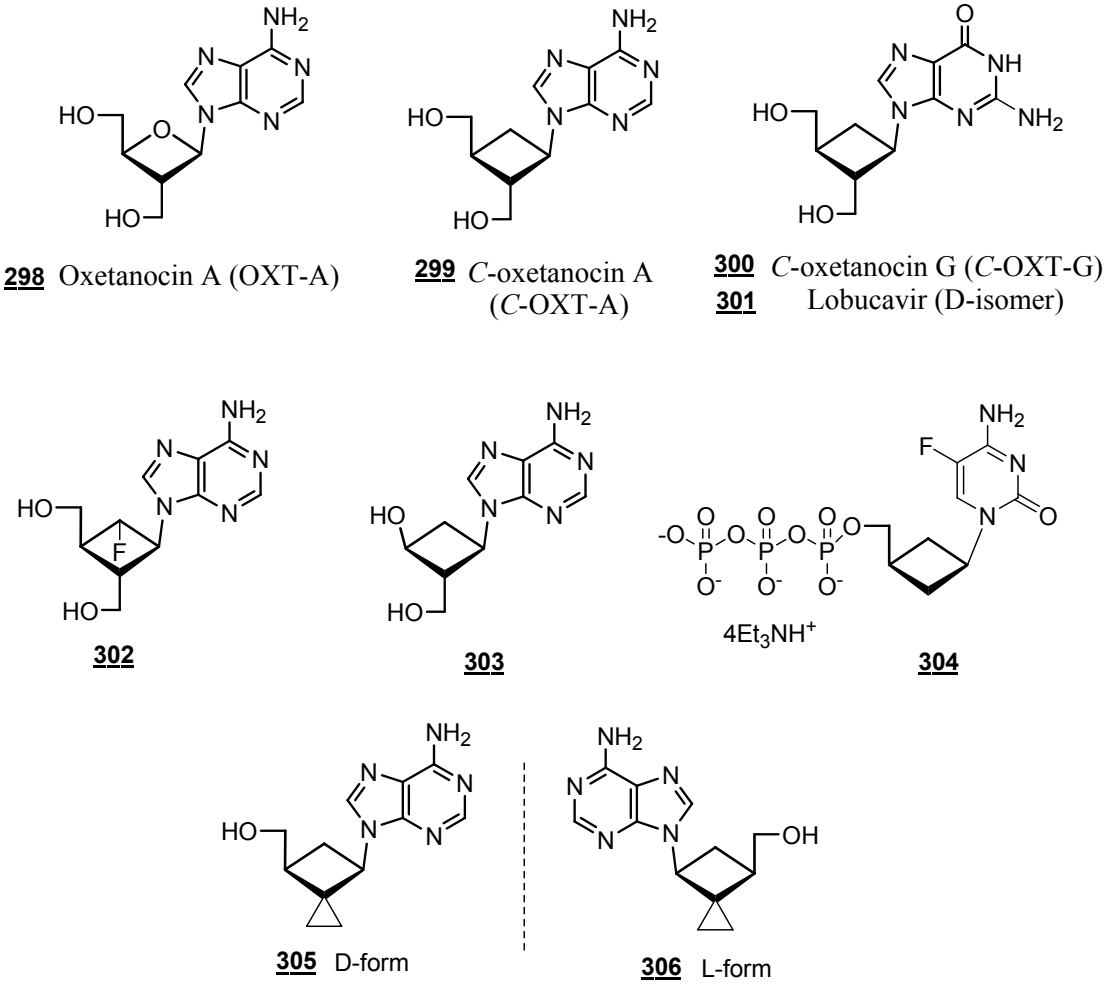
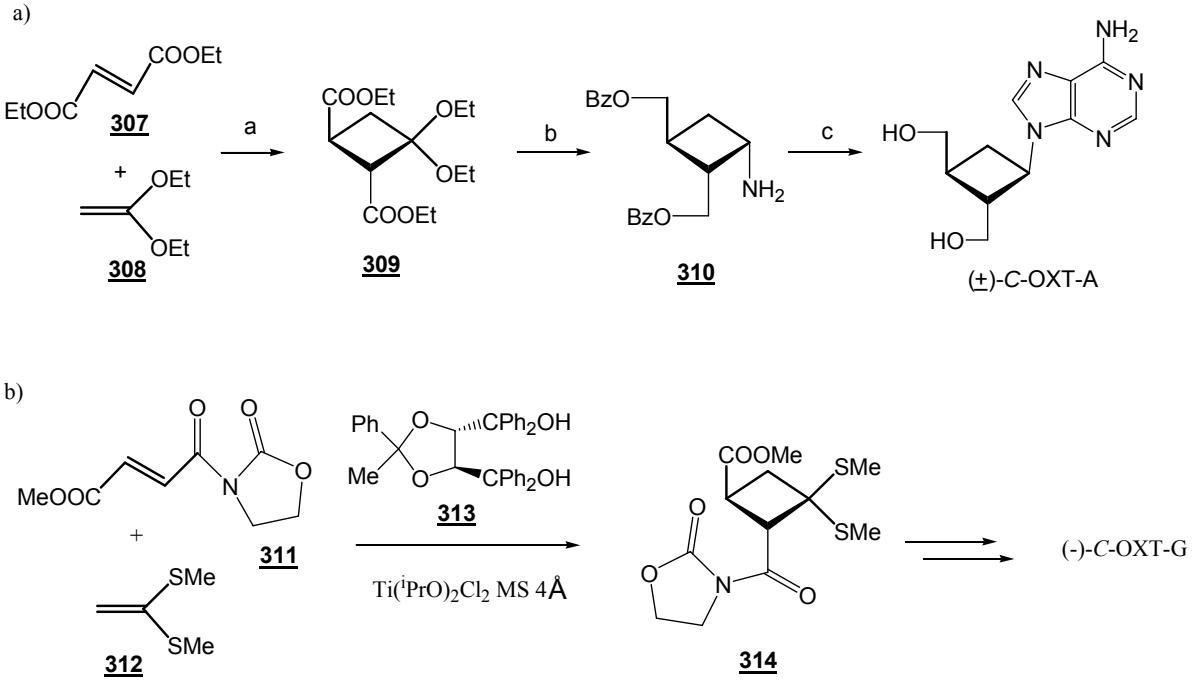


Figure 1.18. Four-membered carbocyclic nucleosides

Preparation of carbocyclic analogs of the natural counterparts was first reported by Honjo.[180] A [2+2] formation provided cyclobutane intermediate **309** which underwent a series of

manipulations to afford cyclobutylamines **310**. The racemic *C*-OXT-A was constructed through a linear approach (Scheme 1.42.a.). In the same year, the synthesis of optically pure *C*-OXT-G was accomplished by Narasaka and co-workers by using an asymmetric [2+2] addition as a key step (Scheme 1.42. b.).[181]



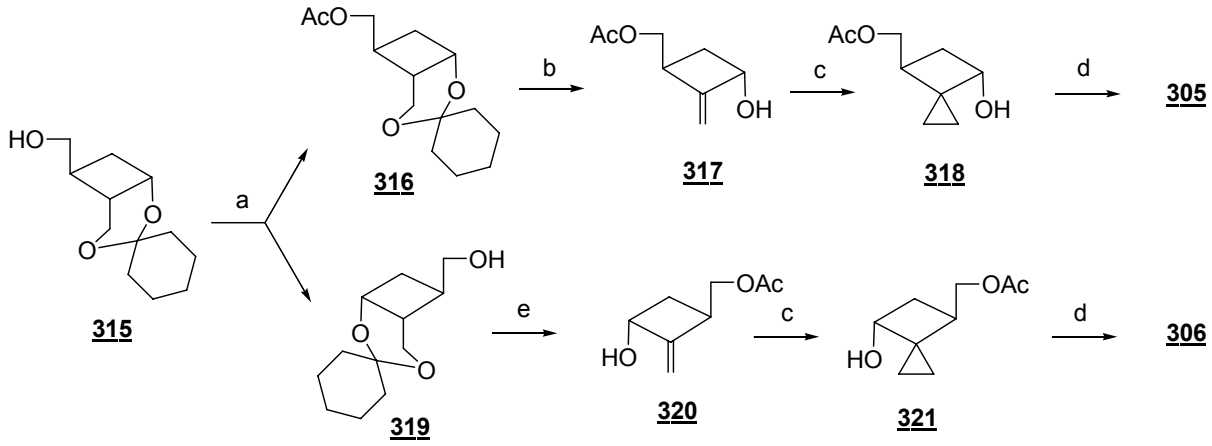
Reagents and conditions: (a) CH_3CN, heat; (b) i) LAH, ii) BzCl, Py, iii) *p*-TsOH, acetone, iv) NH_2OH, v) H_2, PtO_2; (c) Base construction and deprotection

Scheme 1.42. Synthesis of racemic and optically pure *C*-OXT-A and *C*-OXT-G[180, 181]

Among synthesized nucleosides, the guanine (**300**, *C*-OXT-G) and adenine (**299**, *C*-OXT-A) derivatives were active against HIV in ATH18 cells (EC_{50} 1-2 μM).[182] In addition, the D-enantiomer of *C*-OXT-G (**301**, lobucavir, LBV) could be phosphorylated to its triphosphate by viral TK as well as protein kinase[183] and exhibited broad-spectrum antiviral activity against HBV and herpes viruses.[184, 185] Lobucavir was advanced to clinical trials as an anti-HBV agent by the

Bristol-Myers Squibb Phamaceuticals. However, the clinical studies were suspended due to oncogenicity in rodents.[186]

Further modifications based on the structure of *C*-OXTs generated series of interesting four-membered carbocyclic nucleosides. Monofluoro nucleoside (-) **302** showed significant antiviral activity against HSV-1&2 (EC_{50} 0.7-1.8 μM), VZV (EC_{50} 1.8-3.5 μM) and HCMV (EC_{50} 3.5-35 μM), however it was toxic to cells.[187, 188] Removal of the 4'-methylene group of *C*-OXTs (**303**) resulted in a considerable decrease of anti-HSV and anti-VZV activity in comparison to the parent compounds.[189] Interestingly, the triphosphate of nucleoside **304**, which did not have a 2'-hydroxylmethyl group, was reported to be active against wild type HIV-RT as well as M184V mutant.[190] Novel spiro-carbocyclic nucleosides **305** and **306** have been prepared by Chu and co-workers *via* enzymatic resolution (Scheme 1.43). Both D- and L- nucleosides exhibited some anti-HIV activity with EC_{50} values of 22.4 and 48.6 μM, respectively, while L-enantiomer was less toxic than its D-counterpart.[191]



Reagents and conditions: (a) *P. cepacia* lipase, $AcOCH{=}CH_2$; (b) i) Amberlite IR-120, ii) TrCl, Py, iii) TBDPSCl, Im, iv) $BF_3{\cdot}OEt_2$, v) $Me_3P(OPh_3)I$, vi) DBU; (c) Et_2Zn, CH_2I_2; (d) Mitsunobu coupling and base derivations and deptrotection; (e) Ac_2O, Py, then followed the procedure for compound **317**

Scheme 1.43. Synthesis of optically pure spiro-carbocyclic nucleosides[191]

SIX-MEMBERED CARBOCYCLIC NUCLEOSIDES

Herdewijin and co-workers have prepared a number of cyclohexenyl and cyclohexanyl analogs (Figure 1.19), for instance, nucleosides **322**, **323**, **324**, **325** and **326**.[192-196] However, no biological activity was noticed with the exceptions of guanine derivatives of C3-hydroxyl cyclohexenyl **325** and **326** which were shown to be potent and highly selective antiviral agents against herpes virus (HSV-1 & 2 and VZV) with EC_{50}s comparable to acyclovir and ganciclovir.[195] The NMR conformational studies suggested that the nucleosides antiviral activity was correlated with their predominant conformation.[195]

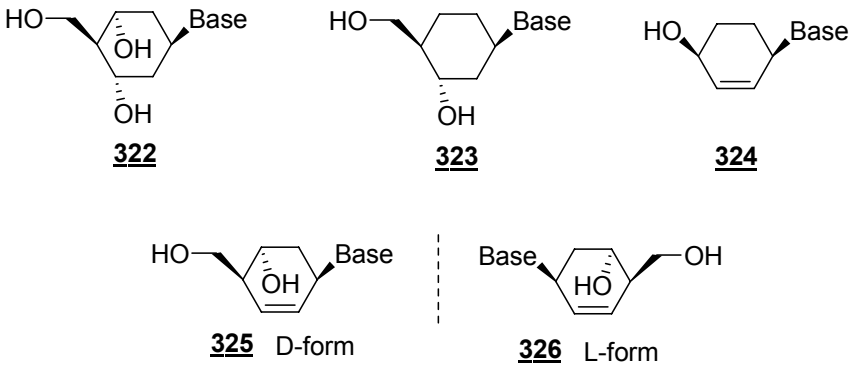


Figure 1.19. Six-membered carbocyclic nucleosides

CONCLUSION

Carbocyclic nucleosides have been a subject of great interest in the medicinal chemistry for the past decades. Particularly, the discovery of abacavir and entecavir as clinical effective antiviral agents prompted the studies of various carbocyclic nucleosides. Although the synthesis of

carbocyclic nucleosides has advanced dramatically, more efficient and practical methods are still in demand for the preparation of biologically active compounds as well as chiral key intermediates. In the future, novel structures will continue to be discovered and the vital structure-activity relationships will be used to improve existing chemotherapeutic agents.

CHAPTER 2

SYNTHESIS, ANTIVIRAL ACTIVITY, AND MECHANSIM OF DRUG RESISTANCE OF D- AND L-2',3'-DIDEHYDRO-2',3'-DIDEOXY-2'-FLUORO-CARBOCYCLIC NUCLEOSIDES

Jianing Wang, Yunho Jin, Kimberly L. Rapp, Matthew Bennett, Raymond F. Schinazi, and Chung K. Chu. 2005, *Journal of Medicinal Chemistry* 48:3736-3748.

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ABSTRACT

Carbocyclic nucleosides have received much attention due to their interesting biological activity and metabolic stability. Among nucleoside analogs, a fluorine substitution on the carbohydrate moiety or the introduction of a 2',3'-unsaturated structure motif has been proven to be successful in producing effective antiviral agents. By combining these structural features, both D- and L-2',3'-dideoxy-2',3'-didehydro-2'-fluoro-carbocyclic nucleosides (D- and L-2'F-C-d4Ns) were synthesized as potential anti-HIV agents. The target D- and L-carbocyclic nucleosides were both stereospecifically synthesized from D-ribose. The structure activity relationships of synthesized compounds against HIV-1 in activated human peripheral blood mononuclear (PBM) cells were studied, from which we found L-2',3'-dideoxy-2',3'-didehydro-2'-fluoro adenosine analog (L-2'F-C-d4A) **46** showed potent anti-HIV activity (EC_{50} 0.77 μM), although it is cross-resistant to the lamivudine-resistant variant (HIV-1_{M184V}). Modeling studies demonstrated a good correlation between calculated relative binding energies and activity/resistance data. The modeling study also indicated that an additional hydrogen bond and a favorable van der Waals interaction contribute to the higher antiviral activity of L-2'F-C-d4A in comparison to its D- counterpart. Also, like other L-nucleosides, the unfavorable steric hindrance of the sugar moiety of L-2'F-C-d4A and the side chain of Val184 could explain the cross-resistance of L-2'F-C-d4A with the M184V mutant. The significant difference of antiviral activity between carbovir and its analog D-2'F-C-d4G **25** may be due to distortion of the phenyl ring of Tyr115 in L-2'F-C-d4G-TP/HIV-RT complex which resulted in a poor π-π interaction.

INTRODUCTION

During the past two decades, extensive work has been conducted in the field of nucleosides in search of novel anti-HIV agents. As a result, eight clinically useful nucleosides/nucleotide have been approved by the US FDA for the treatment of HIV infection which are being used as part of the highly active antiretroviral treatment (HAART).[2] However, the efficacy of nucleoside reverse transcriptase inhibitors (NRTIs), as well as other classes of anti-HIV agents, has been compromised by the emergence of drug-resistant variants[197-199] and toxicity.[200-203] Consequently, new classes of NRTIs with less toxic and less cross-resistant with existed regimes are highly desirable.

A fluorine substitution on the carbohydrate moiety has been proven to be successful in producing effective antiviral agents[204-206] and the 2',3'-unsaturated analogs have also demonstrated promising antiviral activity.[207, 208] Based on this information, D- and L-2', 3'-didehydro-2',3'-dideoxy-2'-fluoro-nucleosides (D- and L-2'F-d4Ns) and their 4'-thio count parts (D- and L-2'F-4'-Sd4Ns) have been synthesized in order to access the structure-activity relationships (Figure 2.1).[14, 209-213] Among them, L-2',3'-didehydro-2',3'-dideoxy-2'-fluorocytosine, 5-fluorocytosine and adenine derivatives exhibited potent anti-HIV activity (EC_{50} 0.51, 0.17, and 1.5 μM, respectively) as well as anti-HBV activity (EC_{50} 0.8, 0.22, and 1.7 μM, respectively).[209, 212] D-2',3'-Didehydro-2',3'-dideoxy-2'-fluoro-5-fluorocytosine and adenine were also active against HIV in PBM cells without significant cytotoxicity (EC_{50} 0.82 and 4.4 μM, respectively).[213] In the case of 4'-thio series, D-2',3'-didehydro-2',3'-dideoxy-2'-fluoro-4' thiocytosine, L-2',3'-didehydro-2',3'-dideoxy-2'-fluoro-4'thiocytosine and 5-fluorocytosine demonstrated significant anti-HIV activity (EC_{50} 1.3, 0.12 and 0.15 μM, respectively).[210, 211]

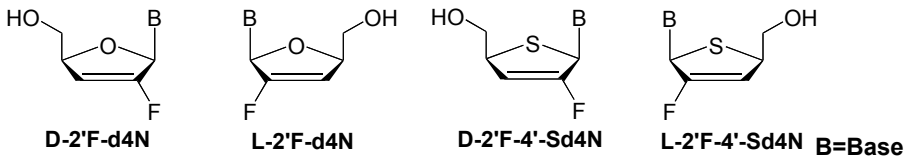


Figure 2.1. D- and L-2',3'-didehydro-2',3'-dideoxy-2'-fluoro(-4'-thio)nucleosides

Carbocyclic nucleosides are analogs of natural nucleoside in which the methylene group replaces the oxygen atom of a furanose ring. As a consequence, the glycosidic bond is resistant to nucleoside phosphorylases and hydrolases which makes the carbocyclic nucleosides more stable toward metabolic degradation.[10] Interestingly, in some cases the substitution of the sugar ring to a carbocyclic ring does not affect enzyme recognition (especially kinases and target enzymes).[10] Due to these features, carbocyclic nucleosides have received much attention as potential chemotherapeutic agents,[9, 10, 14, 214, 215] such as abacavir and entecavir (Figure 2.2).[12] Recently, carbocyclic nucleosides have also been reported to exhibit antiviral activity against smallpox, monkeypox as well as West Nile virus.[53, 60, 90]

Previously, optically active neplanocin A, D- and L-aristeromycin (Figure 2.2) have been synthesized in our laboratory.[60, 109, 216] More recently, the efficient and practical synthetic methodology of the key intermediates, D- and L- cyclopent-2-enone, have been also accomplished by our group.[53, 90] Therefore, efficient synthesis of carbocyclic nucleosides with 2'-fluoro-2',3'-unsaturated structure motif has become feasible. Herein, we report the full accounts of the synthesis, antiviral activity and molecular modeling studies of the both D- and L- form of the title nucleosides.

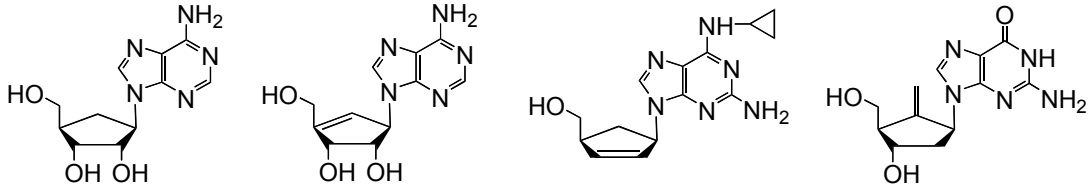


Figure 2.2. Important carbocyclic nucleosides

RESULTS AND DISSCUSION

Chemistry

Several different synthetic approaches have been explored for the synthesis of carbocyclic nucleosides. Basically, there are two synthetic methods to construct carbocyclic nucleosides: (1) the convergent approach attaching a heterocyclic base to an appropriate carbocyclic ring by a substitution reaction; (2) the linear approach constructing a base moiety from an amino-subsistent on a carbocyclic moiety.[9, 10, 14, 214, 215]

Fully protected D- and L- enantiomeically pure cyclopentane **1** and **26** were synthesized from D-ribose *via* 12 and 13 steps, respectively by the reported method.[53] Further modifications of **1** and **26** gave the key intermediates **12** and **37** using the procedure shown in Scheme 2.1 and Scheme 2.3, respectively. Due to the similarity between the syntheses of D- and L- isomers, the following discussion is based on Scheme 2.1 unless otherwise described. Compound **1** was deprotected under the acidic condition to give a triol **2** in 74% yield which was treated with 1-

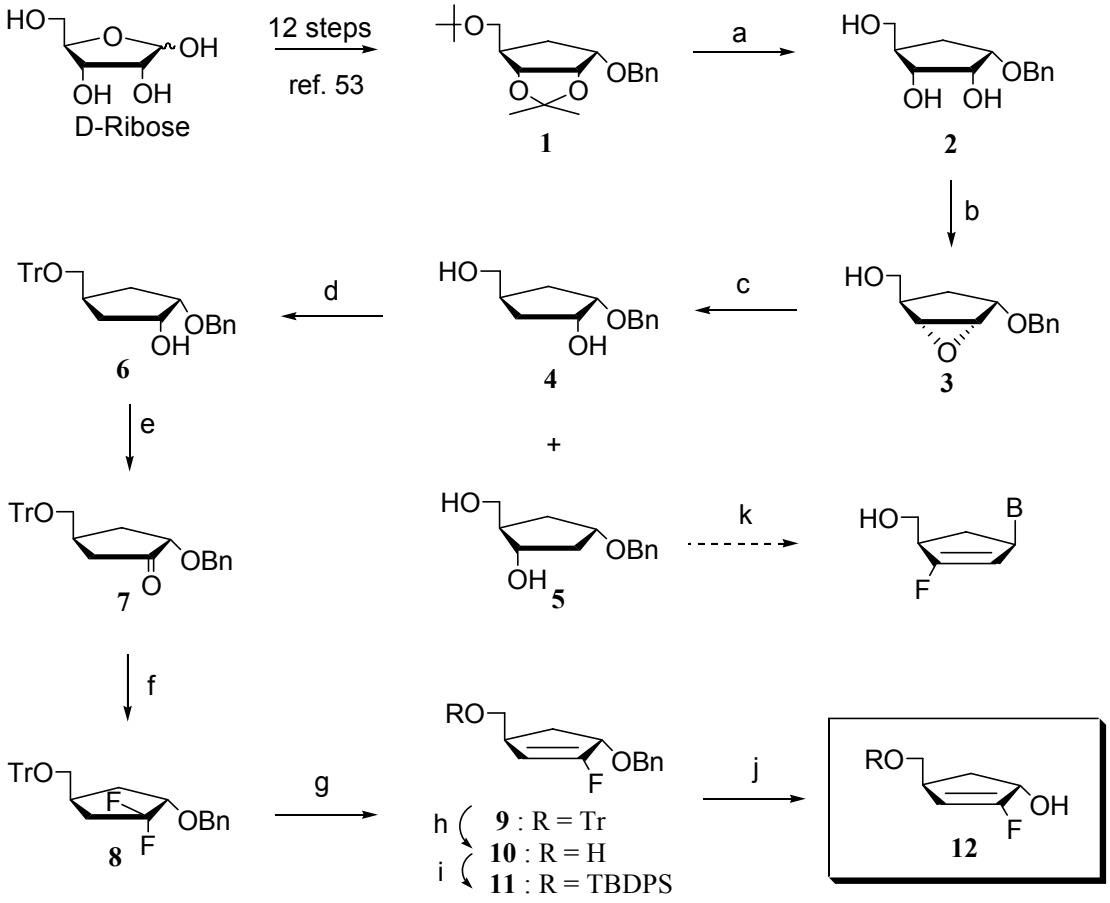
bromocarbonyl-1-methylethyl acetate at -30 °C followed by the addition of potassium carbonate at room temperature to give an epoxide **3,** which in turn was ready for the ring-opening reaction. In the case of D-isomers, lithium aluminum hydride (LAH) was selected as the most efficient reducing reagent which gave the 2- and 3- hydroxyl compound in a ratio of 4.3:1. These conditions were also confirmed in the L-series, which provided a ratio of 6:1. Furthermore, the 3-hydroxyl compound **5** could also be used as the intermediate for the synthesis of 3-fluorine substituted isomers for which synthesis is in progress and will be reported in the future. The primary hydroxyl group of **4** was selectively protected with trityl group, followed by the oxidation of 2-hydroxy group to give the ketone **7,** which was further treated with diethylaminosulfur trifluoride (DAST) to give a difluorinated compound **8** in 69% yield in 3 steps. Treatment of **8** with potassium *tert*-butoxide in THF at 50 °C gave an allyic alcohol **9** in 97% yield. After removing the trityl group under acidic conditions, *tert*-butyl diphenyl silyl group (TBDPS) was used to protect the primary alcohol which was further subjected to a debenzylation reaction using sodium metal in liquid ammonia with a small amount of 1,4-dioxane as the solvent at -78 °C to afford the key intermediate alcohol **12** in 64% yield in 3 steps. However, the phenyl groups of TBDPS sometimes were also reduced by using the sodium/liquid ammonia. Additionally, 1,4-dioxane was solidified under -78 °C which made the reaction take place in heterogeneous conditions. Hence, very careful control of the debenzylation reaction conditions was needed to obtain good yield. Due to these difficulities as described in Scheme 2.3, *tert*-butyl dimethyl silyl (TBDMS) group was used instead of the TBDPS group as the protecting group, and THF was used instead of 1,4-dioxane in L-series. These modifications made the reaction easier to control and the reaction was completed within 5 minutes with improved yield from 70% to 90% in comparison to the D-series. Corresponding

D- and L- carbocyclic nucleosides were synthesized as shown in Scheme 2.2 and Scheme 2.4, respectively. The following description is based on Scheme 2.2 unless otherwise indicated.

To synthesize the uridine and thymidine analogs, Mitsunobu reaction was used to condense the key intermediate **12** with protected uracil and thymine to give the corresponding nucleosides as crude products, which were contaminated with reduced diisopropyl azodicarboxylate. These crude compounds were directly treated with methanolic ammonia to give debenzoylated compounds **13** and **17** in 41% and 54% yield, respectively in two steps. To synthesize the cytidine analog, the uridine analog **13** was further subjected to the ammonolysis using 2,4,6-triisopropylbenzenesulfonyl chloride, 4-dimethylaminopyridine and triethylamine in acetonitrile to give **15** in 65% yield. The uridine, cytidine and thymidine analogs **14**, **16** and **18** were obtained by the deprotection of the silyl group using 3 N HCl in 88% - 92% yield.

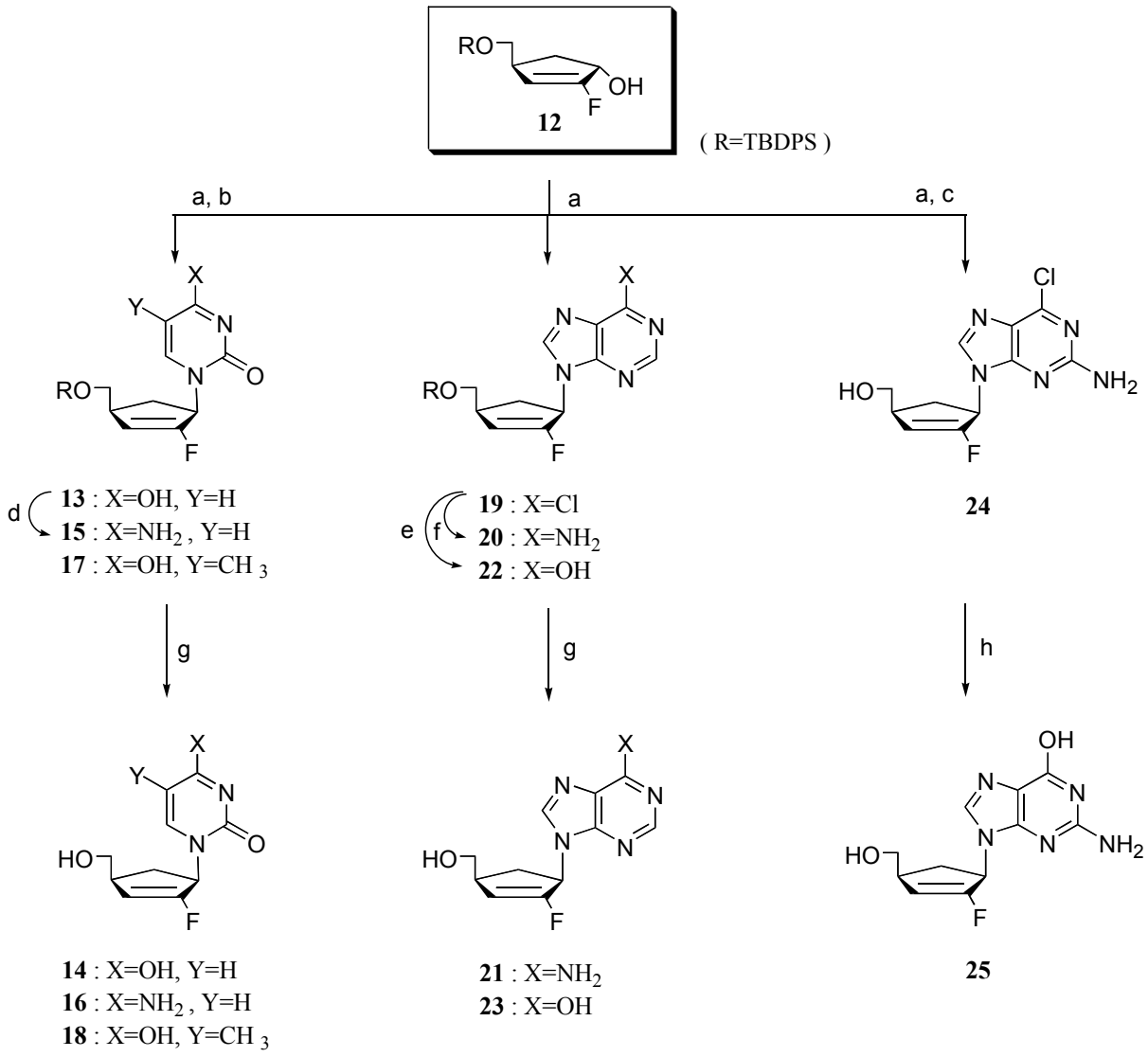
The synthesis of purine analogs followed the similar procedure. The key intermediate **12** was condensed with 6-chloropurine to give the corresponding nucleosides **19** which was further treated with methanolic ammonia in a steel bomb at 110 °C to obtain an adenine derivative **20** in 36% yield in two steps, and the deprotection of silyl group gave the target compound **21** in 93% yield. Compound **19** was also treated with 2-mercaptoethanol and sodium methoxide in refluxing methanol, and the silyl group was then removed under acidic conditions to give an inosine analog **23** in 64% yield in two steps. Condensation of 6-chloro-2-ammino purine with **12** needed careful control of the solvent and reaction temperature. Although treatment of **12** with 2-amino-6-chloropurine in the presence of triphenylphosphine and diisopropyl azodicarboxylate in THF or DMF at 0 °C failed to provide the corresponding 2-amino-6-chlorpurine nucleoside, the desired compound was obtained in a mixture THF and 1,4-dioxane (7:1) at –78 °C. The removal of the silyl group using tetrabutylammonium fluoride

(TBAF) gave **24** in 40% yield in 2 steps. The guanosine analog **25** was obtained by treatment of **24** with formic acid at 80 °C followed by 28% ammonium hydroxide solution in 84% yield.



Reagents and conditions: (a) 6 N HCl, H_2O; (b) α-AIBBr, CH_3CN and then K_2CO_3; (C) LAH, anhydrous THF ; (d) TrCl, pyridine; (e) PDC, AcOH, CH_2Cl_2; (f) DAST, anhydrous CH_2Cl_2; (g) ^{t}BuOK, THF; (h) 3 N HCl, MeOH; (i) TBDPSCl, imidazole, CH_2Cl_2; (j) Na/ liq. NH_3; (k) Synthesis of 3'-fluoro isomers

Scheme 2.1 Synthesis of D-form key intermediate **12**

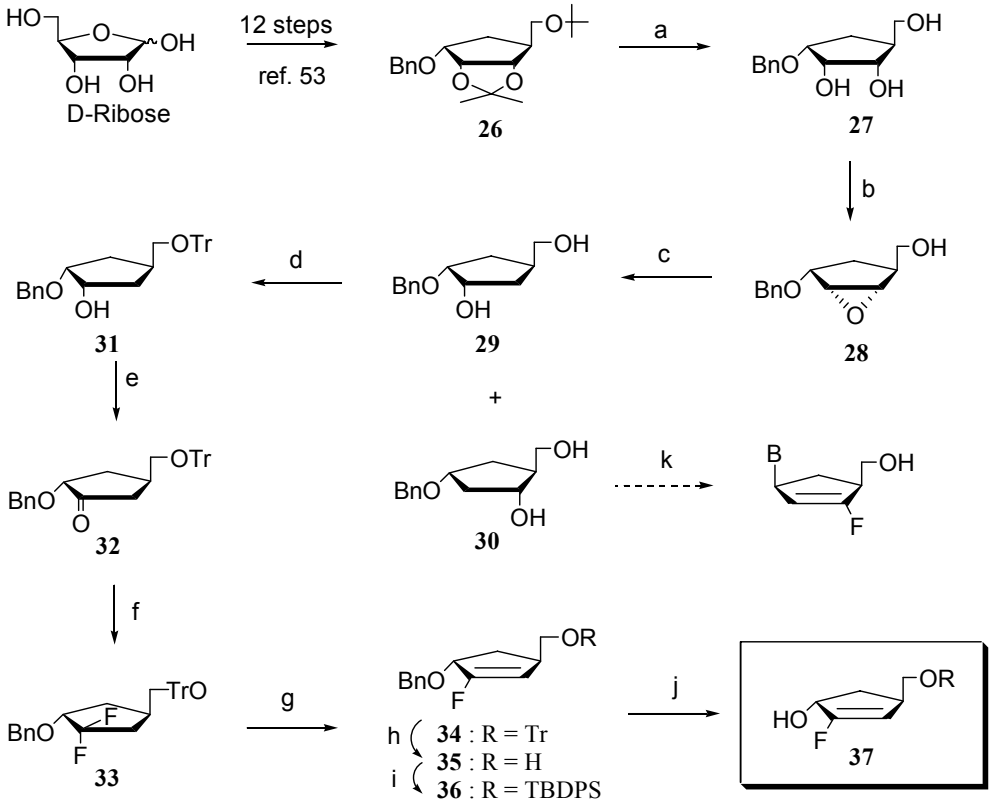


Reagents and conditions: (a) DIAD, Ph_3P, pyrimidines, THF or THF/1,4dioxane; (b) NH_3/MeOH, room temp.; (c) TBAF, THF, room temp.; (d) (i) 2,4,6-triisopropylbenzenesulfonyl chloride , DMAP, Et_3N, CH_3CN, room temp. (ii) NH_4OH; MeOH, room temp; (e) NH_3, MeOH, steal bomb, 110 °C; (f) $HOCH_2CH_2SH$, NaOMe, 70 °C; (g) 3 N HCl, MeOH; (h) (1) HCOOH, 80 °C, (2) NH_4OH, MeOH, room temp.

Scheme 2.2. Synthesis of D-form target nucleosides

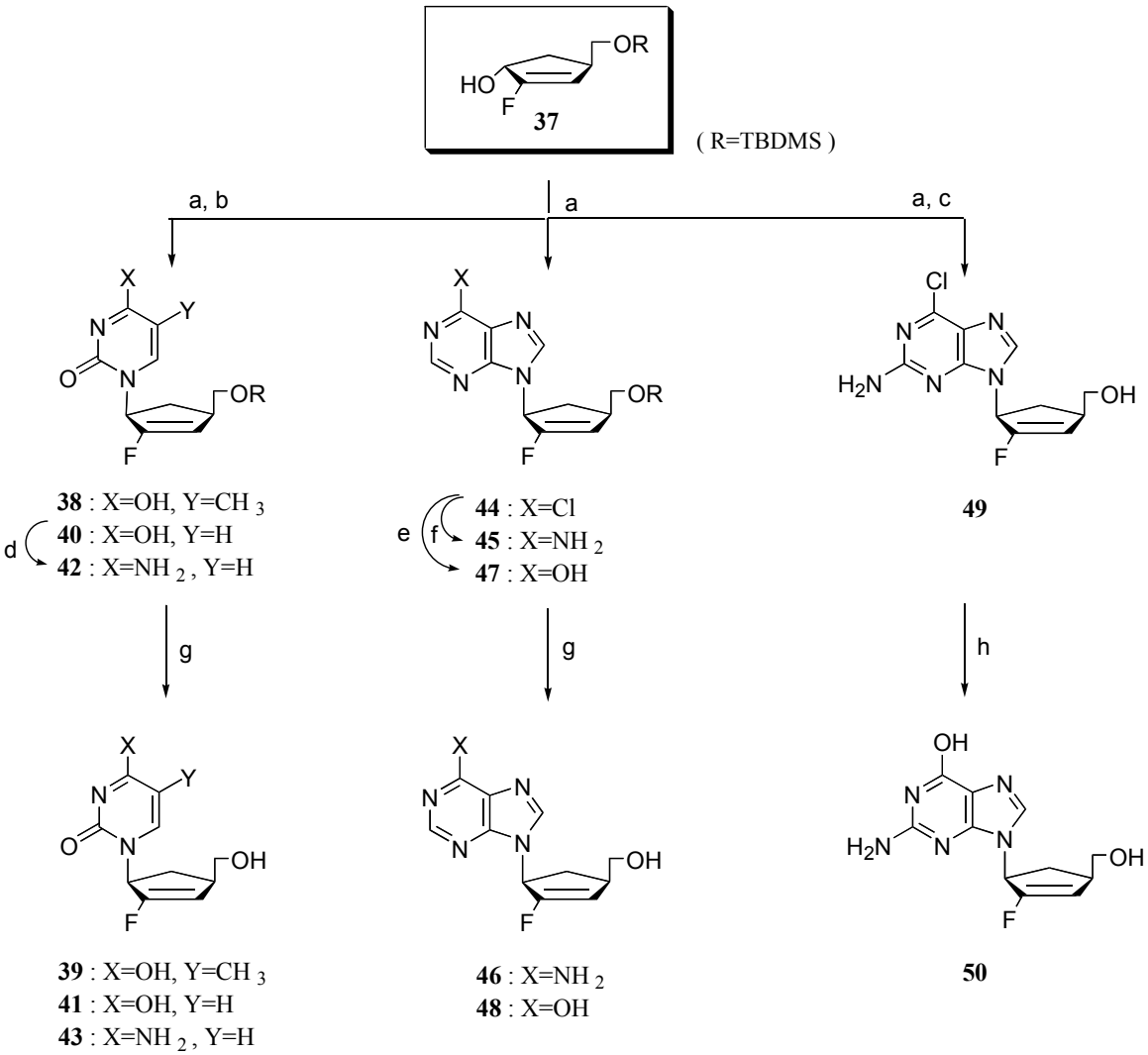
For the L-series (as shown in Scheme 2.4), syntheses were quite similar to the D-isomers except the condensation conditions applied in the Mitsunobu reaction: Triphenylphosphine and

diisopropyl azodicarboxylate were first mixed in THF at 0 °C to form the yellowish suspension. The key intermediate **37** and the corresponding protected base moiety were then added and allowed the reaction gradually warmed up to room temperature. The conditions could be applied for both purines and pyrimidines to provide the desired products. The synthetic variations applied between the D- and L- series may be due to the different 5'-protecting groups. Assignment of the structures of newly synthesized nucleosides was based on NMR, mass spectroscopy, elemental analysis and UV spectroscopy.



Reagents and conditions: (a) 6 N HCl, H_2O; (b) α-AIBBr, CH_3CN and then K_2CO_3; (C) LAH, anhydrous THF ; (d) TrCl, pyridine; (e) PDC, AcOH, CH_2Cl_2; (f) DAST, anhydrous CH_2Cl_2; (g) ^tBuOK, THF; (h) 3 N HCl, MeOH; (i) TBDMSCl, imidazole, CH_2Cl_2; (j) Na/ liq. NH_3; (k) Synthesis of 3'-fluoro isomers

Scheme 2.3. Synthesis of L-form key intermediate **37**



Reagents and conditions: (a) DIAD, Ph_3P, pyrimidines, THF; (b) NH_3/MeOH, room temp.; (c) TBAF, THF, room temp.; (d) (i) 2,4,6-triisopropyl-benzenesulfonyl chloride , DMAP, Et_3N, CH_3CN, room temp. (ii) NH_4OH; 3 N HCl, MeOH, room temp; (e) NH_3, MeOH, steal bomb, 110 °C; (f) $HOCH_2CH_2SH$, NaOMe, 70 °C; (g) 3 N HCl, MeOH; (h) (1) HCOOH, 80 °C, (2) NH_4OH, MeOH, room temp.

Scheme 2.4. Synthesis of L-form target nucleosides

Table 2.1. *In vitro* anti-HIV-1 activity and toxicity of D- and L- 2'-fluoro-2',3'-didehydro-carbocyclic nucleosides

14 - 25 **39 - 50**

B	Configuration	Antiviral activity (μM)	Cytotoxicity (μM)		
		HIV-1 EC_{50}	PBM	CEM	Vero
Uracil **14**	D	>100	>100	>100	>100
Cytosine **16**	D	>100	>100	>100	>100
Thymine **18**	D	>100	>100	>100	>100
Adenine **21**	D	72.8	>100	>100	>100
Hypoxanthine **23**	D	>100	>100	>100	>100
Guanine **25**	D	37.8	>100	>100	>100
Thymine **39**	L	>100	>100	>100	>100
Uracil **41**	L	>100	>100	>100	>100
Cytosine **43**	L	37.7	>100	>100	>100
Adenine **46**	L	0.77	>100	>100	>100
Hypoxanthine **48**	L	>100	>100	91.3	>100
Guanine **50**	L	>100	>100	>100	>100
AZT	D	0.0018	>100	14.3	28.0

Anti-HIV Activity

The newly synthesized carbocyclic nucleosides were tested for anti-HIV activity as well as cytotoxicity using AZT as the positive control, and the results are summarized in Table 2.1. Anti-HIV activity was performed in human peripheral blood mononuclear (PBM) cells infected with HIV-1. Cytotoxicity was tested in three cell lines (PBM, CEM, and Vero). Among the target nucleosides, L-adenosine analog 46 showed the most potent activity against wild type HIV-1(EC_{50} 0.77 μM), although its D-counterpart was inactive. In the D-series, only cytidine 21 (EC_{50} 72.8 μM) and guanosine 25 (EC_{50} 37.8 μM) analogs showed weak anti-HIV activity. It is interesting to note that, although compound 25 has the similar structure with carbovir, it wasn't as potent as carbovir. The lower antiviral potency may be due to the decreased level of phosphorylation by the initial nucleoside kinase and/or by reduced binding of the triphosphate to the HIV reverse transcriptase at the catalytic site (*vide infra* for molecular modeling studies).

Antiviral Activity against Lamivudine-Resistant (HIV-1_{M184V}) Mutant Strain

Since its introduction in 1996, lamivudine (3TC) has been widely used in HIV treatment. However, the rapidly emerged lamivudine-resistant mutant strain compromised its efficacy. The single point mutation at codon 184 (M184V) in YMDD motif increases the 50% inhibitory concentration at least 1,000-fold.[217, 218] Consequently, discovering effective novel NRTIs against these resistant variants is of great interest. Thus, the most potent compound 46 was studied with the M184V mutant using 3TC as control (Table 2.2). From this study, it was found that there was significantly reduced antiviral activity against HIV-1_{M184V} for compound **46**. Thus, its mechanism was also investigated by molecular modeling as below.

Table 2.2. Activity of selected nucleosides against lamivudine-resistant virus (HIV-1_{M184V}) in human PBM cells

Compounds	xxBRU		M184V		FI[a]
	EC_{50} (μM)	EC_{90} (μM)	EC_{50} (μM)	EC_{90} (μM)	
L-2'F-CdA	0.77	8.34	75.3	>100	98
3TC	0.027	0.25	>100	>100	>100

[a]FI is the fold increase (EC_{50} HIV-1_{M184V} / EC_{50} HIV-1_{xxBRU}).

Molecular Modeling

Considering the anti-HIV activities of D- and L-2',3'-dideoxy-2',3'-didehydro-2'-fluoro-carbocyclic nucleosides (D- and L-2'F-C-d4Ns), there are several interesting points that need to be understood: (a) L-2'F-C-d4A is significantly more active than its D-counterpart; (b) it is cross-resistant to HIV-$RT_{(M184V)}$; (c) D-2'F-C-d4G is significantly less potent compared to that of carbovir although these two compounds share similar structural features.

To understand the molecular basis of different anti-HIV potency as described above, molecular modeling studies to analyze the binding energy as well as the interaction between HIV-RT and NRTI triphosphates (Table 2.3 and 2.4). In our previous studies, the relative binding energy is proved to be qualitatively correlated with anti-HIV activity.[211-213, 219, 220] In this study, we found that L-2'F-C-d4A had the most favorable relative binding energy (-39.5 kcal/mol) among the synthesized carbocyclic nucleosides, which is in agreement with its antiviral activity. The relative binding energy of carbovir is significantly higher than that of D-2'F-C-d4G by nearly 60 kcal/mol, which is also in accordance with the difference of their antiviral activity. Analogously, in L-2'F-C-d4A-TP/HIV-$RT_{(M184V)}$ complex, a less favorable relative binding energy indicated a nearly 100-fold decrease of anti-HIV activity. As expected, our model provided a qualitative, but not a quantitative correlation with the experimental data.

Table 2.3. *In vitro* anti-HIV activity of selected 2'F-C-d4Ns against HIV wild type (WT) virus and correlation with calculated energy of complex (2'F-C-d4N-TPs) / HIV-RT

Compound	EC_{50} (μM)[a]	E_{rel} (Kcal/mol)[b]
L-2'F-CdA	0.77	–39.5
D-2'F-CdA	72.8	–22.7
D-2'F-CdG	37.8	–5.2
Carbovir	4.6[c]	–65.2
	0.55[d]	
3TC	0.027	–51.4
AZT	0.0018	–101.8

a. EC_{50} in PBM cells unless otherwise indicated.
b. E_{rel} = (Binding energy of inhibitor-TP) – (Binding energy of natural 2'-dNTP)
c. IC_{50} in MT4 cells, Ref. **15.**
d. EC_{50} in PBM cells, Communicated biological data.

Table 2.4. *In vitro* anti-HIV activity of selected 2'F-C-d4Ns against wild type (WT) and M184V virus in human PBM cells and correlation with calculated energy of complex (2'F-C-d4N-TPs) / HIV-RT

Compound	xxBRU (WT)		M184V		FI[a]	ΔE_{rel}[c]
	EC_{50} (μM)	E_{rel}[b] (Kcal/mol)	EC_{50} (μM)	E_{rel}[b] (Kcal/mol)		
L-2'F-CdA	0.77	–39.5	75.3	–26.3	98	–13.2
3TC	0.027	–51.4	>100	–18.3	>3700	–33.1

a. FI is the Fold Increase (EC_{50} HIV-1_{M184V} / EC_{50} HIV-1_{xxBRU}).
b. E_{rel} = (Binding energy of inhibitor-TP) – (Binding energy of natural 2'-dNTP)
c. $\Delta E_{rel} = E_{rel}$ (WT) – E_{rel} (M184V)

The energy minimized structures of L- and D-2'F-C-d4A-TP were bound to the HIV-RT catalytic site, in which Arg72, Lys65, Ala 114 and Asp 113 stabilize the triphosphate moiety by multiple hydrogen bonds (Figure 2.3-2.5).

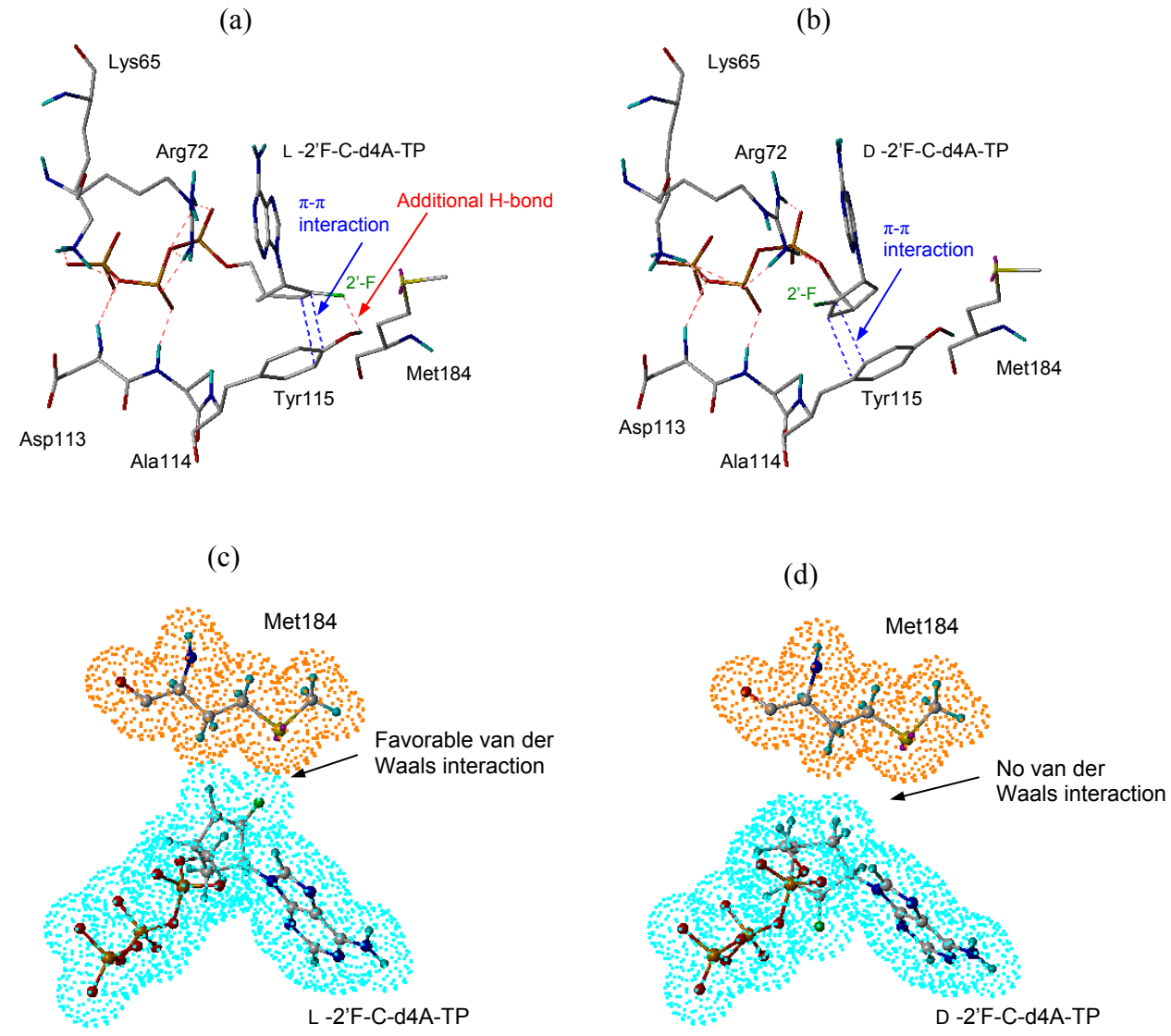


Figure 2.3. (a) Binding mode of L-2'F-C-d4A-TP in the catalytic site of HIV-RT. Blue dot lines indicate the π-π interaction. The 2'-fluorine involves in the hydrogen bonding with –OH on the phenyl ring of Tyr115. (b) Binding mode of L-2'F-C-d4A-TP in the catalytic site of HIV-RT. 2'-Fluorine of D-2'F-C-d4A-TP doesn't have the hydrogen bond. (c) A favorable van der Waals interaction between the sugar ring of L-2'F-C-d4A-TP and side chain of Met184 which increases its binding affinity to HIV-RT. (d) Side chain of Met184 is far away from the D-2'F-C-d4A-TP.

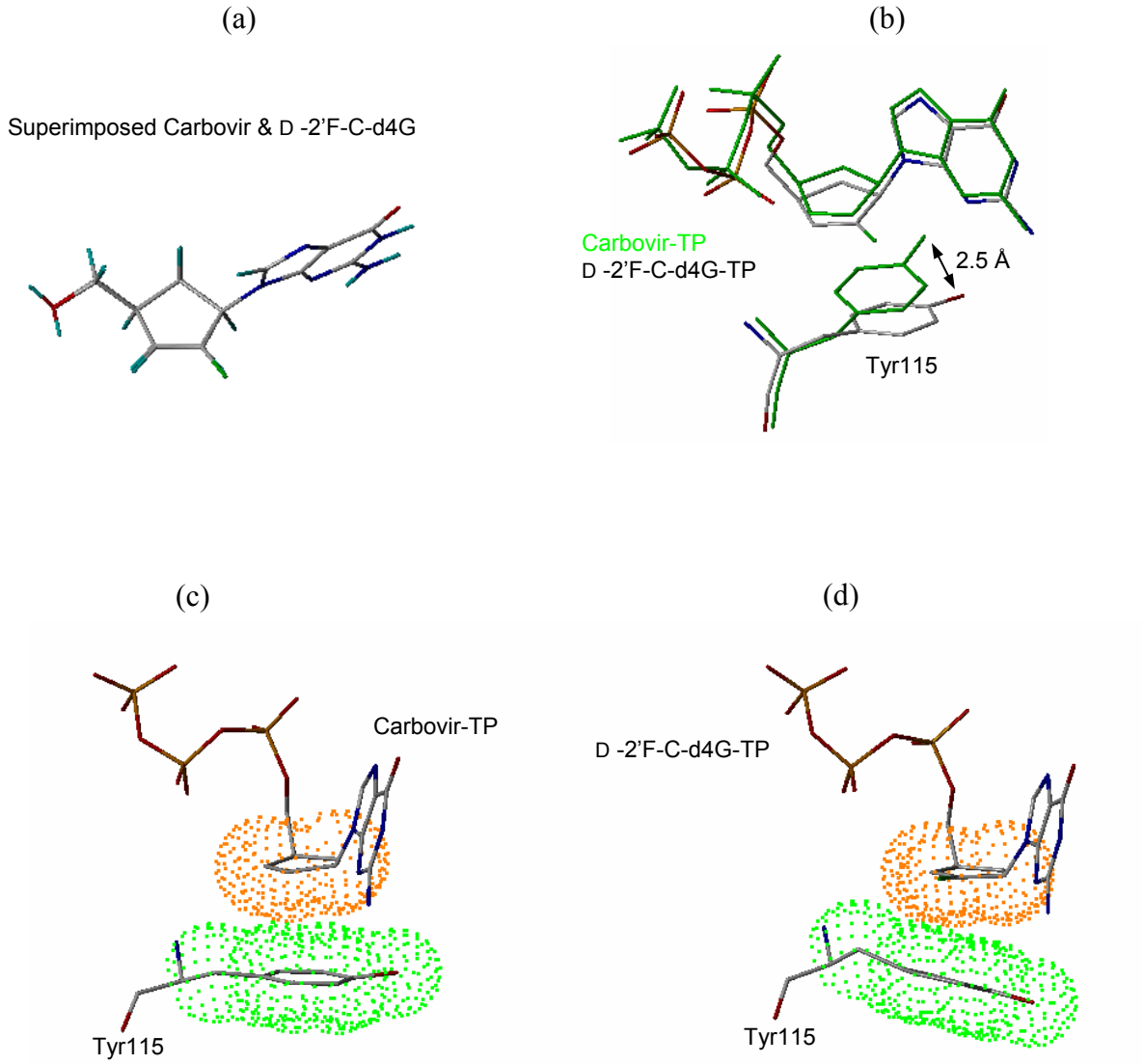


Figure 2.4. (a) Carbovir and D-2'F-C-d4G superimposed very well on each other indicated the similar affinity to nucleoside kinases. (b) Green one is the binding mode of carbovir-TP and corresponding Tyr115 which has the good π-π interaction between phenyl ring and 2',3'-double bond. D-2'F-C-d4G-TP and corresponding Tyr115 are indicated in CPK mode in which Tyr115 moves away from the bottom of D-2'F-C-d4G-TP and decrease the π-π interaction. (c) Side view of carbovir-TP and Tyr115. As can be seen in the figure, carbovir-TP has a favorable π-π interaction with Tyr115. (d) Side view of D -2'F-C-d4G-TP and Tyr115. Trp115 is distorted and moves away from the bottom of the sugar ring which indicated an unfavorable π-π interaction.

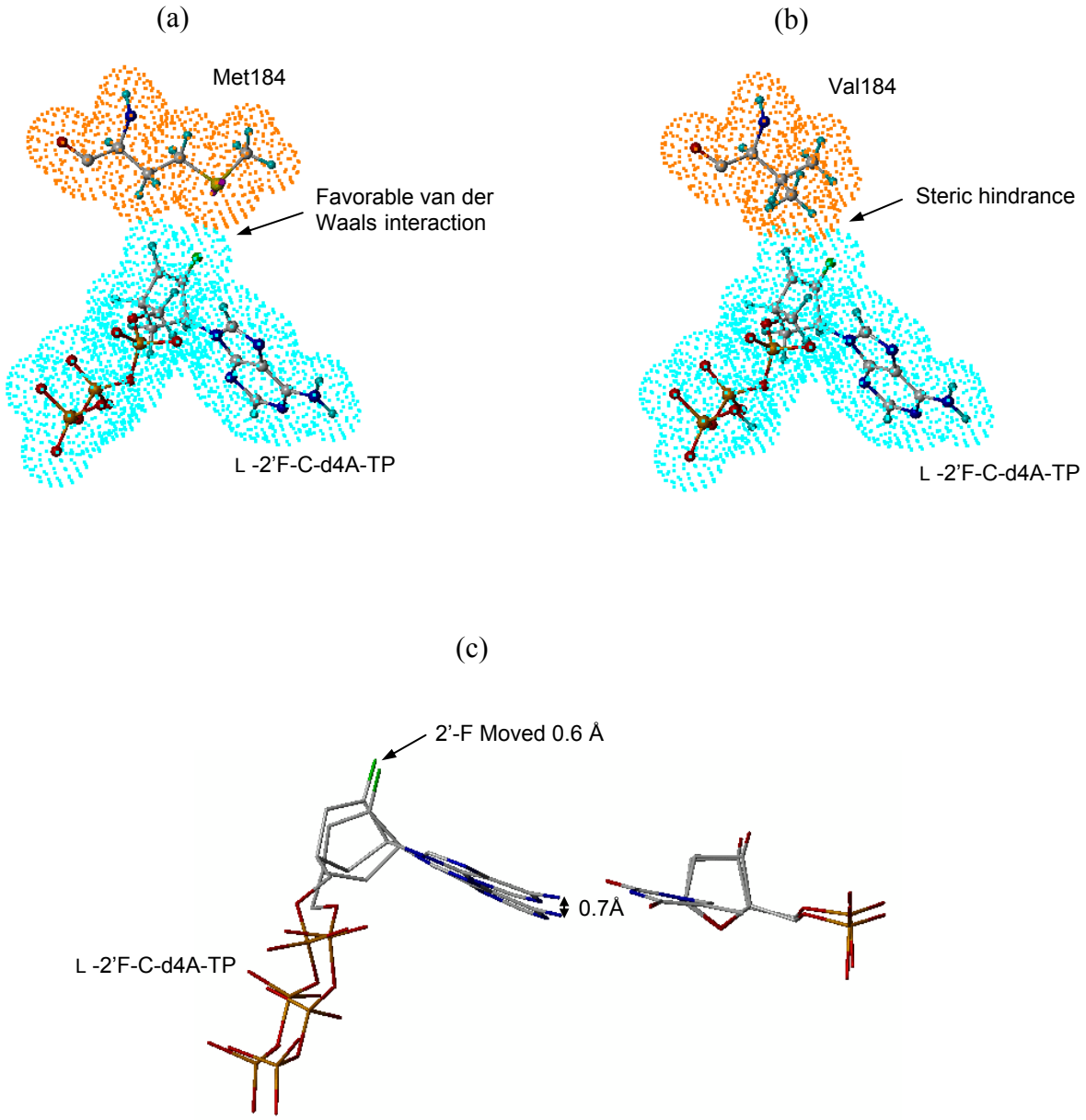


Figure 2.5. (a) In L-2'F-C-d4A-TP/HIV-RT$_{(WT)}$ complex, no unfavorable steric hindrance between Met184 and sugar ring. (b) In L-2'F-C-d4A-TP/HIV-RT$_{(M184V)}$ complex, the side chain of Val184 and the sugar ring of inhibitor experience the steric hindrance. (c) Comparing with L-2'F-C-d4A-TP/HIV-RT$_{(WT)}$ complex, the minimized structure of L-2'F-C-d4A-TP/HIV-RT$_{(M184V)}$ complex shows the movement of 2'-fluorine which cause losing of hydrogen bonding with Tyr115, and a disrupted base-pairing with the complementary base in the template strand.

A π-π interaction between the 2',3'-double bond and the aromatic ring of Tyr115 may also contribute to a positive binding (Figure 2.3a). In the case of L-2'F-C-d4A-TP, the 2'-fluorine is stabilized by a hydrogen bonding to OH in the phenyl ring of Tyr115 which is not observed in the D-counterpart (Figure 2.3b). Additionally, the carbocyclic ring of L-2'F-C-d4A-TP shows an additional favorable van der Waals interaction between the side chain of Met184 (Figure 2.3c) which is absent in the D-counterpart (Figure 2.3d). The increased favorable van der Waals interaction as well as the additional hydrogen bond, may result in the L-form to bind more tightly to HIV-RT, reflecting the higher relative binding energy and consequently higher level of anti-HIV activity, although the initial kinase might have also played a significant role in determining the observed anti-HIV potency.

In view of the fact that carbovir is a potent anti-HIV agent with an EC_{50} value of 4.6 μM, it is interesting to find out the significantly lower binding energy of D-2'F-C-d4G on HIV-RT. The two optimized-structures of D-2'F-C-d4G and carbovir superimposed nicely on each other (Figure 2.4a). As indicated by the modeling studies, D-2'F-C-d4G might be also a good substrate for nucleoside kinase like carbovir.[221, 222] Studies showed that carbovir can be stereoselectively phosphorylated by 5'-nucleotidase and GMP kinase to its triphosphate.[95] Hence, D-2'F-C-d4G might also be converted to its triphosphate by these kinases and then interacts with HIV-RT. Analysis of the binding mode of D-2'F-C-d4G-TP with HIV-RT, we found that Tyr115, which is involved in the π-π interaction with the 2',3'-double bond of D-2'F-C-d4G, is distorted and moves away from the bottom of the sugar ring (Figure 2.4b), in contrast to the favorable π-π interaction in carbovir-TP/HIV-RT complex (Figure 2.4c). Decrease of the favorable π-π interaction (Figure 2.4d) implies the loss of binding affinity resulting in lower anti-HIV activity.

Similar to other L-nucleosides,[219] in the L-2'F-C-d4A-TP/HIV-$RT_{(M184V)}$ complex, the binding pocket of sugar ring points toward the side chain of Val184 and tends to provide the steric hindrance when L-2'F-C-d4A-TP binds to M184V RT (Figure 2.5b). To circumvent this unfavorable binding, the L-2'F-C-d4A-TP/HIV-$RT_{(M184V)}$ must undergo significant conformational change, resulting in a loss of the hydrogen bond between 2'-fluorine and OH of Tyr115 and a disrupted base-pairing with the complementary base in the template strand, which in turn, results in a decrease in the relative binding energy (Figure 2.5c).[223]

EXPERIMENTAL SECTION

Melting points were determined on a Mel-temp II apparatus and were uncorrected. Nuclear magnetic resonance spectra were recorded on a Bruker AMX 400 spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR or Varian Inova 500 spectrometer at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR with tetramethylsilane as the internal standard. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or bs (broad singlet). UV spectra were recorded on a Beckman DU-650 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. High resolution mass spectra were recorded on a Micromass Autospec high-resolution mass spectrometer. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Column chromatography was performed using either silica gel-60 (220-440 mesh) for flash chromatography or silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

(Compounds 1-25 belong to D-series)

(+)-(1*S*,2*R*,3*R*,4*S*)-1-*O*-Benzyloxy-2,3-dihydroxyl-4-hydroxymethyl-cyclopentane (2) A solution of fully protected cyclopentane **1** (29.9 g, 89.4 mmol)[53] in MeOH (150 mL) was treated with 3N HCl (150 mL). After refluxed for 4 h, the resulting brown mixture was co-evaporated *in vacuo* with EtOH (100 mL x 2) and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:20) to afford **2** (15.7 g, 73.7%) as an off-white solid. mp : 115.7-116.9 °C $[\alpha]^{22}_D$ +11.37° (*c* 0.71, MeOH); ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.38–7.24 (m, 5H), 4.55 (dd, *J* = 11.9 and 35.1 Hz, 1H), 4.00 (t, *J* = 3.9 Hz, 1H), 3.85 (dt, *J* = 3.7 and 7.4 Hz, 1H), 3.72 (dd, *J* = 4.2 and 7.6 Hz, 1H), 3.60 (dd, *J* = 4.6 and 10.7 Hz, 2H), 3.50 (dd, *J* = 6.1 and 10.7 Hz, 2H), 2.23 (m, 1H), 2.00 (m, 1H), 1.72 (m, 1H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 137.8, 128.8, 128.3, 128.0, 78.7, 75.8, 72.9 (d, *J* = 11.8 Hz), 72.1, 65.1 (d, *J* = 11.4 Hz), 45.9, 30.1, 0.2. Anal. Calcd. for ($C_{12}H_{18}O_4$) C, H.

(-)-(1*S*,2*R*,3*R*,4*S*)-1-*O*-Benzyloxy-2,3-anhydro-4-hydroxymethyl-cyclopentane (3) A solution of triol **2** (15.7 g, 65.9 mmol) in anhydrous acetonitrile (200 mL) was cooled to –30 °C and then treated with 1-bromocarbonyl-methylethylacetate (24.2 mL, 164.7 mmol). After stirring at room temperature for 1 h, H_2O (150 mL) and EtOAc (150 mL) were added to the resulting mixture and the organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* and the residue was dissolved in MeOH (200 mL). Potassium carbonate (36.4 g, 263.6 mmol) was added and reacted for 12 h at room temperature. The reaction mixture was filtered over Celite pad (~5 cm) and the filtrate was concentrated under reduced pressure. The residue was dissolved in EtOAc (200 mL), washed with H_2O (100 mL), dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:3 to 1:1) to give **3** (11.5 g, 79.3%) as a colorless oil. $[\alpha]^{22}_D$ –54.06° (*c* 0.11,

$CHCl_3$); ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.39–7.27 (m, 5H), 4.63 (s, 2H), 4.14 (t, *J* = 8.0 Hz, 1H), 3.61 (dd, *J* = 5.3 and 10.6 Hz, 1H), 3.52 (m, 2H), 3.45 (d, *J* = 2.6 Hz, 1H), 2.52 (q, *J* = 6.7 Hz, 1H), 1.76 (dd, *J* = 8.1 and 13.2 Hz, 1H), 2.00 (m, 1H), 1.60 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.5, 128.7, 128.0, 127.9, 79.2, 71.9, 64.0, 57.7, 56.7, 41.1, 28.5. Anal. Calcd. for ($C_{12}H_{16}O_3$) C, H.

(-)-(1*S*,2*R*,4*S*)-1-*O*-Benzyloxy-2-hydroxyl-4-hydroxymethyl-cyclopentane (4) and (+)-(1*S*,3*S*,4*S*)-1-*O*-Benzyloxy-3-hydroxyl-4-hydroxymethyl-cyclopentane (5) To a solution of epoxide **3** (10.0 g, 45.4 mmol) in anhydrous THF (150 mL) at –78 °C, lithium aluminum hydride (LAH, 5.4 g, 136.2 mmol) was slowly added. After addition of LAH, the reaction mixture was warmed up to room temperature and stirred for 4 h. Celite (20 g) was added to the reaction mixture and iced H_2O (150 mL) was added slowly to quench the reaction. The slurry was filtered over Celite pad (~10 cm) and the filtrate was extracted with EtOAc (100 mL x 4). The combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:2) to give **4** (6.9 g, 68.6%) as a colorless oil and **5** (1.5 g, 14.8%) as a colorless oil. Compound **4** : $[\alpha]^{23}_D$ – 6.08° (*c* 0.38, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.29 (m, 5H), 4.56 (dd, *J* = 11.7 and 29.3 Hz, 2H), 4.17 (bs, 1H), 3.90 (m, 1H), 3.48 (m, 2H), 2.60 (bs, 1H), 2.49 (m, 1H), 1.97-1.87 (m, 2H), 1.66 (m, 1H), 1.48 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.0, 128.5, 127.8, 127.7, 81.0, 72.3, 71.6, 66.9, 36.8, 34.3, 31.1. Anal. Calcd. for ($C_{12}H_{18}O_3$) C, H. Compound **5** : $[\alpha]^{22}_D$ +6.00° (*c* 0.15, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.27 (m, 5H), 4.48 (s, 2H), 4.03 (m, 2H), 3.71 (dd, *J* = 5.5 and 10.4 Hz, 1H), 3.53 (dd, *J* = 8.0 and 10.4 Hz, 1H), 2.34 (m, 1H), 2.09 (m, 2H), 1.92 (m, 1H), 1.40 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2, 128.4, 127.6, 78.9, 76.1, 70.6, 65.4, 48.7, 40.8, 33.4. Anal. Calcd. for ($C_{12}H_{18}O_3$) C, H.

(-)-(1*S*,2*R*,4*S*)-1-*O*-Benzyloxy-2-hydroxyl-4-(*O*-trityloxymethyl)-cyclopentane (6) A mixture of diol **4** (7.27 g, 32.7 mmol) and trityl chloride (10.0 g, 32.7 mmol) in pyridine (100 mL) was refluxed for 4h. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in EtOAc (200 mL), washed with brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:30) to give **6** (15.1 g, 99.4%) as a colorless oil. $[\alpha]^{24}_D$ –11.18° (*c* 0.30, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.12 (m, 20H), 4.47 (dd, *J* = 11.8 and 29.0 Hz, 2H), 4.07 (bs, 1H), 3.81 (dd, *J* = 6.6 and 10.7 Hz, 1H), 2.86 (m, 2H), 2.54 (m, 1H), 2.45 (d, *J* = 3.6 Hz, 1H), 1.86 (m, 2H), 1.55 (m, 1H), 1.43 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.3, 128.7, 128.5, 127.8, 127.7, 126.9, 81.0, 72.4, 71.6, 67.2, 35.1 (d, *J* = 19.8 Hz), 31.8. Anal. Calcd. for ($C_{32}H_{32}O_3$) C, H.

(-)-(2*S*,4*S*)-2-*O*-Benzyloxy-4-(*O*-trityloxymethyl)-cyclopentan-1-one (7) To a solution of alcohol **6** (15.1 g, 32.5 mmol) in anhydrous CH_2Cl_2 (250 mL), 4 Å molecular sieve (15 g), pyridinium dichromate (24.5 g, 65.0 mmol) and acetic acid (0.7 mL, 0.05 mol%) were added respectively. After being stirred at room temperature for 12 h, the resulting brown slurry mixture was filtered over a silica gel pad (~15 cm) with EtOAc. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:50 to 1:20) to give ketone **7** (12.2 g, 81.3%) as a white solid. mp: 100-102 °C $[\alpha]^{23}_D$ –27.05° (*c* 0.14, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.24 (m, 20H), 4.82 (d, *J* = 11.9 Hz, 1H), 4.35 (d, *J* = 11.9 Hz, 1H), 3.91 (t, *J* = 6.6, 1H), 3.13 (m, 2H), 2.70 (m, 1H), 2.53 (dd, *J* = 8.8 and 19.0 Hz, 1H), 2.17 (dd, *J* = 6.0 and 18.6 Hz, 1H), 2.09-1.99 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.0, 143.9, 86.7, 78.3, 71.9, 66.4, 39.6, 33.6, 32.6. Anal. Calcd. for ($C_{32}H_{30}O_3$) C, H.

(-)-(2*S*,4*S*)-1-Difluoro-2-*O*-benzyloxy-4-(*O*-trityloxymethyl)-cyclopentane (8) To a solution of ketone **7** (11.2 g, 24.2 mmol) in anhydrous CH_2Cl_2 (100 mL), diethyl aminosulfur trifluoride (DAST, 14.8 mL, 121.5 mmol) was added at room temperature. After being stirred at room temperature for 4 h, the reaction mixture was poured into a saturated $NaHCO_3$ (150 mL) solution and extracted with CH_2Cl_2 (100 mL x 2). The combined organic layer was washed with brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:100) to give difluorinated compound **8** (10.0 g, 85.5%) as a colorless oil. $[\alpha]^{22}_D$ –12.95° (*c* 0.16, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.27 (m, 20H), 4.84 (d, *J* = 11.9 Hz, 1H), 4.62 (d, *J* = 11.9 Hz, 1H), 3.94 (m, 1H), 3.05 (m, 2H), 2.64 (m, 1H), 2.40 (m, 1H), 2.04-1.94 (m, 2H), 1.78 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.5, 144.1, 138.3, 128.9, 128.7, 128.0, 128.0, 127.8, 127.3, 86.8, 74.9, 70.8, 62.3, 46.7, 45.8, 33.1. Anal. Calcd. for ($C_{32}H_{30}F_2O_2$) C, H.

(-)-(3*S*,5*S*)-1-Fluoro-3-(*O*-trityloxymethyl)-5-*O*-benzyloxy-cyclopent-1-ene (9) To a solution of compound **8** (10.9 g, 22.5 mmol) in anhydrous THF (150 mL), potassium *tert*-butoxide (13.3 g, 112.5 mmol) was added at room temperature. After being stirred at 50 °C for 28 h, (the completion of reaction was monitored by ^{1}H NMR.) H_2O (150 mL) was added to the resulting dark brown mixture and extracted with EtOAc (150 mL x 2). The combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:30) to give vinyl compound **9** (9.1 g, 87.5%) as a colorless oil. $[\alpha]^{23}_D$ –74.99° (*c* 0.95, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.24 (m, 20H), 5.40 (s, 1H), 4.62 (dd, *J* = 11.7 and 20.7 Hz, 2H), 4.54 (d, *J* = 5.67 Hz, 1H), 3.09 (m, 2H), 2.97 (t, *J* = 7.33 Hz, 1H), 2.08 (dd, *J* = 8.1 and 13.9 Hz, 1H), 1.97 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.5, 129.1, 128.8, 128.2, 128.0, 127.4, 110.2 (d, *J* =

8.9 Hz), 78.6 (d, *J* = 20.9 Hz), 71.5, 67.7, 38.9 (d, *J* = 7.7 Hz), 33.1 (d, *J* = 7.8 Hz). Anal. Calcd. for ($C_{32}H_{30}FO_2$) C, H.

(-)-(3*S*,5*S*)-1-Fluoro-3-hydroxymethyl-5-*O*-benzyloxy-cyclopent-1-ene (10) To a solution of vinyl **9** (1.0 g, 2.15 mmol) in THF (15 mL), 6 N HCl (4 mL) solution and MeOH (4 mL) were added at room temperature. After being heated at 50 °C for 5 h, the resulting mixture was cooled to 0 °C and neutralized by 1 N NaOH solution. After evaporation of half volume, the residue was extracted with EtOAc (50 mL x 2). The combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10 to 1:5) to give alcohol **10** (429 mg, 89.7%) as a colorless oil. $[\alpha]^{23}_D$ –154.82° (C 0.76, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.28 (m, 5H), 5.28 (s, 1H), 4.61 (dd, *J* = 13.0 and 24.7 Hz, 2H), 4.57 (bs, 1H), 3.58 (dd, *J* = 5.5 and 10.5 Hz, 1H), 3.51 (dd, *J* = 5.8 and 10.5 Hz, 1H), 2.97 (m, 1H), 2.04 (m, 2H), 1.37 (t, *J* = 5.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.3, 128.7, 128.0, 128.0, 108.8 (d, *J* = 7.3 Hz), 78.4 (d, *J* = 16.8 Hz), 71.5, 66.6 (d, *J* = 2.7 Hz), 40.6 (d, *J* = 5.7 Hz), 32.4 (d, *J* = 5.7 Hz). Anal. Calcd for ($C_{13}H_{15}FO_2 \cdot 0.3CH_2Cl_2$) C, H.

(-)-(3*S*,5*S*)-1-Fluoro-3-(*O*-*tert*-butyldiphenylsilyloxymethyl)-5-*O*-benzyloxcyclopent-1-ene (11) To a solution of vinyl alcohol **10** (3.0 g, 13.5 mmol) in CH_2Cl_2 (50 mL), *tert*-butyldiphenylsilane chloride (3.9 mL, 14.8 mmol) and imidazole (1.38 g, 20.2 mmol) were added at room temperature. After being stirred at room temperature for 1 h, H_2O (150 mL) was added to the resulting white suspension mixture and the organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:100) to give **11** (6.2 g, 99%) as a colorless oil. $[\alpha]^{24}_D$ –74.99° (C 0.79, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64-7.61 (m, 4H), 7.40-7.34

(m, 11H), 5.29 (s, 1H), 4.61 (dd, *J* = 131.7 and 22.1 Hz, 2H), 4.55 (bs, 1H), 3.52 (m, 2H), 2.97 (bs, 1H), 1.99 (m, 2H), 1.03 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.2, 160.9, 138.2, 135.6 (d, *J* = 8.4 Hz), 129.7 (d, *J* = 2.2 Hz), 128.4, 127.8, 127.8, 127.7, 127.7, 109.5 (d, *J* = 6.8 Hz), 78.3 (d, *J* = 16.4 Hz), 71.1, 67.5 (d, *J* = 2.7 Hz), 40.4 (d, *J* = 5.7 Hz), 32.2 (d, *J* = 5.7 Hz), 26.8, 19.2. Anal. Calcd. for ($C_{29}H_{33}FO_2Si$) C, H.

(-)-(3*S*,5*S*)-1-Fluoro-3-(*O*-*tert*-butyldiphenylsilyloxymethyl)-5-hydroxyl-cyclopent-1-ene (12) Liquid ammonia (20 mL) was trapped in three neck round bottom flask (50 mL) at –78 °C and then sodium (825 mg, 35.8 mmol) was added slowly. To a resulting dark blue solution, a solution of **10** (660 mg, 1.43 mmol) in 1,4-dioxane (3 mL) was added slowly at –78 °C for 5 min. After being stirred at the same temperature for 10 min, CH_2Cl_2 (50 mL) was added slowly to the reaction mixture. The resulting brown mixture was carefully poured into iced H_2O (50 mL). (Caution: Remaining sodium causes flames.) The mixture was extracted with CH_2Cl_2 (50 mL x 2). The combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:5) to give vinyl alcohol **12** (380 mg, 71.7%) as a colorless oil. $[\alpha]^{25}_D$ – 90.52° (*c* 0.47, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (m, 4H), 7.42-7.36 (m, 6H), 5.23 (s, 1H), 4.79 (bs, 1H), 3.55 (m, 2H), 2.96 (bs, 1H), 2.12 (m, 2H), 1.91 (m, 1H), 1.04 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 161.4, 155.2, 135.8 (d, *J* = 4.5 Hz), 133.8 (d, *J* = 9.1 Hz), 130.0 (d, *J* = 2.3 Hz), 127.9, 108.5 (d, *J* = 8.4 Hz), 72.1 (d, *J* = 22.9 Hz), 71.9, 67.6 (d, *J* = 3.0 Hz), 40.4 (d, *J* = 7.6 Hz), 34.9 (d, *J* = 6.8 Hz), 27.0, 19.5. Anal. Calcd. for ($C_{22}H_{27}FO_2Si$) C, H.

(-)-(1'*R*,4'*S*)-1-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6-(*O*-*tert*-butyldiphenylsilyloxymethyl)-cyclopent-2-enyl]uracil (13) A solution of vinyl alcohol **12** (1.0 g, 2.70 mmol), triphenyl phosphine (2.83 g, 10.80 mmol) and N^3-benzoyluracil (1.17 g, 5.40

mmol) in anhydrous THF (10 mL) was cooled to 0 °C and then diisopropyl azodicarboxylate (2.17 g, 10.80 mmol) was added slowly. The reaction mixture was slowly allowed to warm to room temperature and stirred for 12 h. The yellowish resulting mixture was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10) to the corresponding nucleoside as crude product, which was used for the next reaction without further purification. The crude product (700 mg) was treated with methanolic ammonia and stirred at room temperature for 12 h. After the reaction mixture was concentrated *in vacuo*, the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:5) to give **13** (530 mg, 41% from **12**) as a white foam. $[\alpha]^{24}_{D}$ –14.76° (*c* 0.41, $CHCl_3$); UV (MeOH) λ_{max} 263.5 nm; Anal. Calcd. for ($C_{26}H_{29}FN_2O_3Si \cdot 0.7H_2O$) C, H, N.

(-)-(1'*R*,4'*S*)-1-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-cyclopent-2-enyl]uracil (14) To a solution of **13** (130 mg, 0.27 mmol) in MeOH (5 mL), 3 N HCl (5 mL) was added at room temperature. After being stirred at room temperature for 1 h, the resulting mixture was co-evaporated with EtOH and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:15) to give **14** (45 mg, 68%) as a white foam. $[\alpha]^{23}_{D}$ –20.97° (*c* 0.56, MeOH); UV (H_2O) λ_{max} 264.5 nm (ε 11145, pH 2), 264.5 nm (ε 8932, pH 7), 264.5 nm (ε 13131, pH 11); MS: *m/z* 227(M+1); Anal. Calcd. for ($C_{10}H_{11}FN_2O_3 \cdot 0.2H_2O$) C, H, N.

(-)-(1'*R*,4'*S*)-1-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6-(*O-tert*-butyldiphenylsilyloxymethyl)-cyclopent-2-enyl]cytosine (15) To a solution of uracil derivative **13** (120 mg, 0.26 mmol) in anhydrous acetonitrile (5 mL), 2,4,6-triisopropyl benzene-sulfonyl chloride (156 mg, 0.52 mmol), 4-(dimethylamino)pyridine (32 mg, 0.26 mmol) and triethylamine (0.15 mL, 1.04 mmol) were added respectively at 0 °C. After being stirred at room temperature for 12 h, 28% solution of ammonium hydroxide (5 mL) was added to the resulting brown

mixture solution and stirred at room temperature for another 12 h. The reaction mixture was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:30) to give **15** (100 mg, 83%) as a colorless oil. $[\alpha]^{24}_D$ –45.68° (*c* 0.76, $CHCl_3$); UV (MeOH) λ_{max} 272.5 nm; Anal. Calcd. for ($C_{26}H_{30}FN_2O_2Si$) C, H, N.

(-)-(1'*R*,4'*S*)-2-*O*-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-cyclopent-2-enyl]cytosine (16) Compound **15** (100 mg, 0.22 mmol) was converted to the cytosine derivative **16** (45 mg, 92%) as a white solid using same procedure as described for **14**. mp: 185-187 °C (dec.) $[\alpha]^{23}_D$ –36.92° (*c* 0.64, MeOH); UV (H_2O) λ_{max} 281.5 nm (ε 9661, pH 2), 272.5 nm (ε 13881, pH 7), 272.0 nm (ε 16461, pH 11); MS: *m/z* 227 (M+2); Anal. Calcd. for ($C_{10}H_{12}FN_3O_2$) C, H, N.

(-)-(1'*R*,4'*S*)-1-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6-(*O-tert*-butyldiphenylsilyloxymethyl)-cyclopent-2-enyl]thymine (17) Compound **12** (400 mg, 1.08 mmol) was converted to **17** (243 mg, 46% from **12**) as a white foam using same procedure as described for **13**. $[\alpha]^{22}_D$ –24.72° (*c* 0.61, $CHCl_3$); UV (MeOH) λ_{max} 267.5 nm; Anal. Calcd. for ($C_{27}H_{31}FN_2O_4Si$) C, H, N.

(+)-(1'*R*,4'*S*)-1-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-cyclopent-2-enyl]thymine (18) Compound **17** (225 mg, 0.46 mmol) was converted to the thymine derivative **18** (104 mg, 88%) as a white foam using same procedure as described for **14**. $[\alpha]^{23}_D$ +28.19° (*c* 0.76, $CHCl_3$); UV (H_2O) λ_{max} 269.0 nm (ε 5678, pH 2), 271.0 nm (ε 6971, pH 7), 279.0 nm (ε 6911, pH 11); MS: *m/z* 241 (M+1); Anal. Calcd. for ($C_{11}H_{13}FN_2O_3$) C, H, N.

(+)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6-(*O-tert*-butyldiphenylsilyloxymethyl)-cyclopent-2-enyl]adenine (20) A solution of vinyl alcohol **12** (700 mg, 1.89 mmol), tripheylphosphine (990 mg, 3.78 mmol) and 6-chloropurine (613 mg, 3.96 mmol) in anhydrous THF (10 mL) was cooled to 0 °C and then diisopropyl

azodicarboxylate (760 mg, 3.78 mmol) was added slowly. The reaction mixture was slowly allowed to warm to room temperature and stirred for 12 h. The yellowish resulting mixture was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10) to give the corresponding nucleoside **19** as crude product (570 mg) , which was used for the next reaction without further purification. The crude product (520 mg) was treated with methanolic ammonia and heated in a steel bomb at 100 °C for 24 h. After the reaction mixture was concentrated *in vacuo*, the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:20) to give **20** (335 mg, 36% from **12**) as a colorless oil. $[\alpha]^{24}_D$ +2.18° (*c* 0.40, $CHCl_3$); UV (MeOH) λ_{max} 260.5 nm; Anal. Calcd. for ($C_{27}H_{30}N_5FOSi \cdot 0.7H_2O$) C, H, N.

(+)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6'-hydroxymethylcyclopent -2-enyl]adenine (21) Compound **20** (180 mg, 0.37 mmol) was converted to adenine derivative **21** (85 mg, 93%) as a white solid using same procedure as described for **14**. $[\alpha]^{23}_D$ +79.60° (*c* 0.35, MeOH); mp = 218-220 °C; UV (H_2O) λ_{max} 258.0 nm (ε 11106, pH 2), 260.5 nm (ε 8708, pH 7), 260.5 nm (ε 10234, pH 11); MS: *m/z* 250 (M+1); Anal. Calcd. for ($C_{11}H_{12}FN_5O \cdot HCl$) C, H, N.

(+)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6-(*O-tert*-butyldiphenylsilyloxymethyl)-cyclopent-2-enyl]inosine (22) To a solution of 6-chloropurine analog **19** (240 mg, 0.47 mmol) in MeOH (10 mL), 2-mercaptoethanol (130 mg, 1.65 mmol) and sodium methoxide (94 mg, 1.65 mmol) were added at room temperature. After being refluxed for 12 h, the reaction mixture was neutralized with acetic acid and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:50) to give **22** (180 mg, 78%) as a colorless oil. $[\alpha]^{22}_D$ +22.28° (*c* 1.65, $CHCl_3$); UV (MeOH) λ_{max} 220.5 and 249.5 nm; Anal. Calcd. for ($C_{27}H_{39}FN_4O_2Si \cdot 0.6H_2O$) C, H, N.

(+)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6'-hydroxymethylcyclopent-2-enyl]inosine (23) Compound **22** (180 mg, 0.37 mmol) was converted to inosine derivative **23** (85 mg, 93%) as a white solid using same procedure as for **14**. $[\alpha]^{23}_{D}$ +81.45° (*c* 0.22, MeOH); mp = 195-197 °C; UV (H_2O) λ_{max} 249.0 nm (ε 11354, pH 2), 248.5 nm (ε 15927, pH 7), 254.0 nm (ε 12152, pH 11); MS: *m/z* 251 (M^+); Anal. Calcd. for ($C_{11}H_{12}FN_4O_2 \cdot 0.6H_2O$) C, H, N.

(+)-(1'*R*,4'*S*)-2-Amino-6-chloro-9-[2',3'-dideoxy-2',3'-didehydro-2'-fluoro-6-hydroxymethyl-cyclopent-2-enyl]purine (24) A solution of tripheylphosphine (1.25 g, 4.75 mmol) in anhydrous THF (10 mL) was cooled to 0 °C and then treated with a solution of diisopropyl azodicarboxylate (956 mg, 4.75 mmol) in anhydrous 1,4-dioxane (2 mL) slowly. The resulting suspension was stirred at 0 °C for 30 min and then cooled to –78 °C. A solution of vinyl alcohol **12** (440 mg, 1.19 mmol) in anhydrous THF (5 mL) was added slowly, followed by the addition of 2-amino-6-chloropurine (806 mg, 4.75 mmol). The reaction mixture was slowly allowed to warm to room temperature and stirred for 6 h. The precipitate was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:4) to corresponding nucleoside (970 mg) as a crude product, which was used for the next reaction without further purification. The crude product (970 mg) was dissolved in THF (10 mL) and then treated with a 1.0 M solution of tetrabutylammonium fluoride in THF. After being stirred at room temperature for 3 h, the reaction mixture was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:40) to give **24** (135 mg, 40% from **12**) as a white foam. $[\alpha]^{23}_{D}$ +9.76° (*c* 1.09, MeOH); UV (MeOH) λ_{max} 247.0 and 309.0 nm; Anal. Calcd. for ($C_{11}H_{11}ClFN_5O \cdot 0.3MeOH$) C, H, N .

(+)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6'-hydroxymethylcyclopent-2-enyl]guanine (25) A mixture of **24** (95 mg, 0.33 mmol) and formic acid (4 mL) was heated at 90 °C for 2 h and then concentrated *in vacuo*. The residue was dissolved in methanol (4 mL) and treated with a 28% solution of ammonium hydroxide (1 mL). After being stirred at room temperature for 2 h, the reaction mixture was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:30) to give **25** (15.5 g, 85%) as a white solid. $[\alpha]^{24}_D$ +23.13° (*c* 0.45, MeOH); mp = 252 °C (dec), ; UV (H_2O) λ_{max} 253.5 nm (ε 1259.2, pH 2), 252.5 nm (ε 12600, pH 7), 252.0 nm (ε 12779, pH 11); MS: *m/z* 266 (M^+); Anal. Calcd. for ($C_{11}H_{12}FN_5O_2 \cdot 1.6H_2O$) C, H, N.

(Compounds 26-50 belong to L-series)

(-)-(1*R*,2*S*,3*S*,4*R*)-1-*O*-Benzyloxy-2,3-dihydroxyl-4-hydroxymethylcyclopentane (27) was prepared from the fully protected cyclopentane **26** [10c] on a 18.9 mmol scale in 83% yield by a similar procedure as described for **2**. mp: 118.5-119.5 °C; $[\alpha]^{26}_D$ –13.62° (*c* 1.03, MeOH) ^{1}H NMR (MeOH-d_4, 500 MHz) δ 7.38-7.26 (m, 5H), 4.60 (d, *J* = 11.5 Hz, 1H)). 4.52 (d, *J* = 12.0 Hz, 1H), 4.01(t, *J* = 5.0 Hz, 1H), 3.86 (td, *J*= 4.0 and 7.5 Hz, 1H), 3.72 (dd, *J* = 4.0 and 7.5 Hz, 1H), 3.61 (dd, *J* = 5.0 and 11 Hz, 1H), 3.51 (dd, *J* = 6.0 and 10.5 Hz, 1H), 3.31 (t, *J* = 1.5 Hz, 1H), 2.25-22 (m, 1H), 2.04-1.98 (m, 1H), 1.76-1.71 (m, 1H) ^{13}C NMR (MeOH-d_4, 125 MHz) δ 138.63,128.15,127.78, 127.46, 78.50, 73.52,72.83, 71.18, 63.24, 45.01, 30.12 Anal. Calcd. for ($C_{13}H_{18}O_4$) C, H.

(+)-(1*R*,2*S*,3*S*,4*R*)-1-*O*-Benzyloxy-2,3-anhydro-4-hydroxymethyl-cyclopentane (28) was prepared from **27** on a 110 mmol scale in 80% yield by a similar procedure as described for **3**. $[\alpha]^{26}_D$ +76.91° (*c* 0.71, $CHCl_3$) ^{1}H NMR (MeOH-d_4, 500 MHz) δ 7.39-7.26 (m, 5H), 4.62 (s, 2H), 4.19-4.16 (m, 1H), 3.63-3.60(m, 1H), 3.55-3.52 (m, 2H), 3.45 (d, *J* = 3 Hz, 1H), 2.52 (dd,

J = 6.5 and 13 Hz, 1H), 1.76 (dd, *J* = 8 and 13 Hz, 1H), 1.67-1.54 (m, 2H); ^{13}C NMR (MeOH-d_4, 125 MHz) δ 138.46, 128.68, 127.99,127.97, 79.16, 71.93, 63.98, 57.69, 56.69, 41.15, 28.53 Anal. Calcd. for ($C_{13}H_{16}O_3 \cdot 0.24H_2O$) C, H.

(+)-(1*R*,2*S*,4*R*)-*O*-Benzyloxy-2-hydroxyl-4-hydroxymethyl-cyclopentane (29) and (-)-(1*R*,3*R*,4*R*)-*O*-Benzylox-3-hydroxyl-4-hydroxymethyl-cyclopentane (30) were prepared from **28** on a 78 mmol scale in 72% and 14.5% yield respectively, by similar procedures as described for **4** and **5**. **29**: $[\alpha]^{26}_D$ +12.69° (*c* 0.72, MeOH) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38-7.27 (m, 5H), 4.60 (d, *J* = 11.5 Hz, 2H), 4.53 (d, *J* = 11.5 Hz, 1H), 4.19-4.16 (m, 1H), 3.90 (td, *J* = 4 and 6.5 Hz, 1H), 3.53-3.45 (m, 2H), 2.58 (d, *J* = 3.5 Hz, 1H), 2.53- 2.46 (m, 1H), 1.99-1.89 (m, 2H), 1.69-1.64 (m, 2H), 1.52-1.46 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.03, 128.56, 127.92, 127.75, 81.06, 72.38, 71.64, 67.01, 36.90, 34.36, 31.18 Anal. Calcd. for ($C_{13}H_{18}O_3 \cdot 0.18H_2O$) C, H. **30**: $[\alpha]^{25}_D$ –36.43° (*c* 0.85, MeOH) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36-7.26 (m, 5H), 4.48 (s, 1H), 4.06-4.00 (m, 2H), 3.72-3.68 (m, 1H), 3.54-3.49 (m, 1H), 2.86 (d, *J* = 7 Hz, 1H), 2.35-2.30 (m, 1H), 2.13-2.05 (m, 2H), 1.95-1.90 (m, 1H), 1.43-1.37 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.24, 128.48, 127.69, 79.05, 76.34, 70.70, 65.58, 48.76, 40.89, 33.41 Anal. Calcd. for ($C_{13}H_{18}O_3 \cdot 0.5H_2O$) C, H.

(+)-(1*R*,2*S*,4*R*)-*O*-Benzyloxy-2-hydroxyl-4-(*O*-trityl-oxymethyl)-cyclopentane (31) was prepared from **29** on a 54 mmol scale in 83% yield by a similar procedure as described for **6**. $[\alpha]^{24}_D$ +12.00° (*c* 0.65, MeOH) ^{1}H NMR (MeOH-d_4, 500 MHz) δ 7.42-7.20 (m, 20H), 4.78 (d, *J* = 11.5 Hz, 1H), 4.51 (d, *J* = 12 Hz, 1H), 4.15 (s, 1H), 3.88-3.86 (m, 1H), 2.98-2.92 (m, 2H), 2.61 (m, 1H), 2.56 (d, *J* = 3.5 Hz, 1H), 1.96-1.63 (m, 2H), 1.67-1.63 (m, 1H), 1.51 (m, 1H); ^{13}C NMR (MeOH-d_4, 125 MHz) δ 144.36, 138.16, 128.78, 128.57, 127.90, 127.80, 127.78, 126.96, 86.24, 81.13, 72.44, 71.63, 67.31, 35.18, 34.98, 31.85 Anal. Calcd. for ($C_{32}H_{32}O_3$) C, H.

(+)-(2*R*,4*R*)-2-*O*-Benzyloxy-4-(*O*-trityl-oxymethyl)-cyclopentan-1-one (32) was prepared from **31** on a 43 mmol scale in 72% yield by a similar procedure as described for **7**. (14.3 g, 72%). mp: 102-103 °C $[\alpha]^{26}_{D}$ +37.29° (*c* 0.32, MeOH) ^{1}H NMR (MeOH-d_4, 500 MHz) δ 7.37-7.22 (m, 20H) 4.79 (d, *J* = 12 Hz, 1H), 4.60 (d, *J* = 11.5 Hz, 1H), 3.88 (t, *J* = 7 Hz, 1H), 3.13-3.06 (m, 2H), 2.68-2.65 (m, 1H), 2.52-2.47 (dd, *J* = 8.5 and 19 Hz, 1H), 2.14-2.08 (dd, *J* = 6 and 19 Hz, 1H), 2.08-2.04 (m, 1H), 1.98-1.93 (m, 1H); ^{13}C NMR (MeOH-d_4, 125 MHz) δ 216.11, 143.90, 137.71, 128.65, 128.45, 128.08, 127.87, 127.84, 127.08, 86.68, 78.30, 71.90, 66.42, 39.66, 33.63, 32.64 Anal. Calcd. for ($C_{32}H_{30}O_3$) C, H.

(+)-(2*R*,4*R*)-1-Difluoro-2-*O*-benzyloxy-4-(*O*-trityloxymethyl)-cyclopentane (33) was prepared from **32** on a 30 mmol scale in 85% yield by a similar procedure as described for **8**. $[\alpha]^{28}_{D}$ +12.29° (*c* 0.24, MeOH) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.44-7.22 (m, 20H), 4.77 (d, *J* = 11.5 Hz, 1H), 4.57 (d, *J* = 11.5 Hz, 1H), 3.89 (t, *J* = 5.5 Hz, 1H), 3.03-2.97 (m, 2H), 2.62-2.59 (m, 1H), 2.39-2.34 (m, 1H), 1.99-1.88 (m, 2H), 1.75-1.71 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 144.07, 137.91, 131.98, 129.98, 128.69, 128.48, 127.85, 127.05, 86.41, 79.58 (dd, *J* = 15.2 and 23.6 Hz), 72.39, 66.41, 35.88 (t, *J* = 18.3 Hz), 33.14, 32.59 Anal. Calcd. for ($C_{32}H_{30}F_2O_2$) C, H.

(+)-(3*R*,5*R*)-1-Fluoro-3-(*O*-trityl-oxymethyl)-5-*O*-benzyloxy-cyclopent-1-ene (34) was prepared from **33** on a 24.8 mmol scale in 95% yield by a similar procedure as described for **9**. $[\alpha]^{28}_{D}$ +93.52° (*c* 0.61, $CHCl_3$) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.42-7.21 (m, 20H), 5.37 (s, 1H), 4.62-4.56 (dd, *J* = 11.5 and 24.5 Hz, 2H), 4.52-4.50 (m, 1H), 3.05-2.92 (m, 2H), 1.88-1.85 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.05 (d, *J* = 225.1 Hz) 144.14, 138.29, 128.94, 128.85, 128.72, 128.60, 128.53, 128.51, 128.47, 128.00, 127.97, 127.85, 127.83, 127.77, 127.71, 127.67, 127.05, 109.81 (d, *J* = 7.3 Hz), 86.42, 78.11 (d, *J* = 16.8 Hz), 71.17, 67.38 (d, *J* = 2.7

Hz), 38.48 (d, *J* = 6.1 Hz), 32.72 (d, *J* = 5.4 Hz) Anal. Calcd. for ($C_{32}H_{29}FO_2$) C, H.

(+)-(3*R*,5*R*)-1-Fluoro-3-hydroxymethyl-5-*O*-benzyloxy-cyclopent-1-ene (35) was prepared from **34** on a 24 mmol scale in 89% yield by a similar procedure as described for **10**. $[\alpha]^{27}_D$ +166.85° (*c* 1.0, MeOH) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.39-7.26 (m, 5H), 5.27 (d, *J* = 1.5Hz), 4.65-4.57 (m, 2H), 3.59-3.48 (m, 2H), 2.98-2.95 (m, 2H), 2.09-1.97 (m, 2H), 1.43 (t, *J* = 5.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.58 (dd, *J* = 8 and 225.9 Hz) 138.15 (d, *J* = 8 Hz), 128.57, 128.49, 128.46, 128.08, 128.00, 127.95, 127.87, 127.83, 127.82, 127.79, 108.79 (t, *J* = 7.6 Hz), 78.20 (dd, *J* = 8 and 16.8 Hz), 71.35 (d, *J* = 6.9 Hz), 66.34 (dd, *J* = 2.3 and 8 Hz), 40.44 (dd, *J* = 6.1 and 8.1 Hz), 32.21 (dd, *J* = 5.3 and 8 Hz) Anal. Calcd. for ($C_{13}H_{15}FO_2$) C, H.

(+)-(3*R*,5*R*)-1-Fluoro-3-(*O*-*tert*-butyldimethylsilyloxymethyl)-5-*O*-benzyloxycyclopent-1-ene (36) was prepared from **35** on a 5 mmol scale in 83% yield by a similar procedure as described for **11**. $[\alpha]^{25}_D$ +120.19° (*c* 0.73, $CHCl_3$) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37-7.26 (m, 5H), 5.27 (s, 1H), 4.61 (dd, *J* = 12 and 24.5 Hz, 2H), 4.57-4.54 (m, 1H), 3.52-3.42 (m, 2H), 2.93-2.89 (m, 1H), 2.04-1.98 (m, 1H), 1.94-1.89 (m, 1H), 0.87 (t, *J* = 3 Hz, 9H), 0.025 (d, *J* = 1 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ168.44 (d, *J* = 225.1 Hz), 143.66, 133.79, 133.17, 133.02, 114.83 (d, *J* = 7.3 Hz), 83.57 (d, *J* = 16.8 Hz), 76.48, 72.32 (d, *J* = 2.6 Hz), 45.90 (d, *J* = 5.7 Hz), 37.52 (d, *J* = 5.4 Hz), 31.28 (d, *J* = 5.3 Hz), 23.66, 0.01 (d, *J* = 2.3 Hz) Anal. Calcd. for ($C_{19}H_{29}FO_2Si$) C, H.

(+)-(3*R*,5*R*)-1-Fluoro-3-(*O*-*tert*-butyldimethylsilyloxymethyl)-5-*O*-hydroxylcyclopent-1-ene (37) Liquid ammonia (30 mL) was trapped in three neck round bottle flask at -78 °C and then sodium (220 mg, 9.6 mmol) was added. To the resulting dark blue solution was added a solution of **36** (500 mg, 1.5 mmol) in anhydrous THF (10 mL) fast. After keeping the reaction mixture shaken very well for 4.5 min at same temperature, a saturated NH_4Cl solution was added to

quench and the mixture was extracted with EtOAc. The organic layer was combined and dried over $MgSO_4$, concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:30) to give **37** as colorless oil (330 mg, 90%). $[\alpha]^{25}_D$ +128.07° (*c* 0.92, $CHCl_3$) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.22 (d, *J* = 2 Hz, 1H), 4.78-4.76 (m, 1H), 3.53-3.43 (m, 2H), 2.91-2.88 (m, 1H), 2.08-2.03 (m, 1H), 1.93-1.85(m, 1H), 0.88 (t, *J* = 3 Hz, 9H), 0.034 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.52 (d, *J* = 223.5 Hz), 108.14 (d, *J* = 7.3 Hz), 71.51 (d, *J* = 18.3 Hz), 66.82 (d, *J* = 2.6 Hz), 40.12 (d, *J* = 5.7 Hz), 34.47 (d, *J* = 5 Hz), 25.80, 18.20, -5.46 (d, *J* = 1.9 Hz) Anal. Calcd. for ($C_{12}H_{23}FO_2Si$) C, H.

(+)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6'-(*O-tert*-butyldimethylsilyloxymethyl)-cyclopent-2-enyl]thymine (38) Triphenyl phosphine (320 mg, 1.2 mmol) and diisopropyl azodicarboxylate (790 mg, 3.0 mmol) were dissolved in anhydrous THF and cooled to 0 °C. After forming a yellowish suspension, the mixture was further cooled to -78 °C. N^3-benzyluracil (700 mg, 3.0 mmol) and a solution of alcohol **12** (250 mg, 1.0 mmol) in THF were added successively. The resulting mixture was kept at -78 °C for 0.5 h and then stirred at room temperature for 1 h. MeOH was added to quench the reaction and the mixture was evaporated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10) to give corresponding nucleoside as crude product, which was treated directly with methanolic ammonia at room temperature for 24 h. After evaporation *in vacuo*, the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:6) to give **38** (192 mg, 54% from **37**) as a white solid. mp: 86.5-88.5 °C $[\alpha]^{23}_D$ +39.56° (*c* 0.30, $CHCl_3$); UV (H_2O) λ_{max} 271.0 nm (ε 12158, pH 2), 271.0 nm (ε 11977, pH 7), 270.0 nm (ε 9799, pH 11). Anal. Calcd. for ($C_{17}H_{27}FN_2O_3Si$) C, H, N.

(-)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6'-hydroxymethyl-cyclopent-2-enyl]thymine (39) Compound **38** was treated with 3 N HCl in MeOH at room temperature for 45 min. $NaHCO_3$ was added to neutralize the solution. The mixture was evaporated under reduced pressure and the resulting mixture was dissolved in H_2O and extracted with *i*-PrOH:$CHCl_3$ = 1:4. The organic layer was dried over $MgSO_4$, evaporated *in vacuo*. The residue was purified by column chromatography on a silica gel (MeOH: CH_2Cl_2 = 1: 20) to give **39** as white foam (65 mg, 82%). $[\alpha]^{24}_D$ –25.76° (*c* 0.42, $CHCl_3$); UV (H_2O) λ_{max} 271.0 nm (ε 14280, pH 2), 271.0 nm (ε 14676, pH 7), 269.0 nm (ε 12382, pH 11). MS: *m/z* 241 (M+1); Anal. Calcd. for ($C_{11}H_{13}FN_2O_3 \cdot 0.1H_2O$) C, H, N.

(+)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6'-hydroxymethylcyclopent-2-enyl]uracil (41) was prepared from **37** on a 0.41 mmol scale in 34% yield by a similar procedure as described for **39**. $[\alpha]^{23}_D$ +19.08° (*c* 0.48, MeOH); UV (H_2O) λ_{max} 265.0 nm (ε 11713, pH 2), 265.0 nm (ε 11887, pH 7), 264.0 nm (ε 9153, pH 11). MS: *m/z* 227 (M+1); Anal. Calcd. for ($C_{10}H_{11}FN_2O_3 \cdot 0.2H_2O$) C, H, N.

(+)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6'-(*O*-*tert*-butyldimethylsilyloxymethyl)-cyclopent-2-enyl]cytosine (42) was prepared from **37** on a 0.6 mmol scale in 34% yield by a similar procedure as described for **15**. mp: 99-102 °C $[\alpha]^{22}_D$ +42.65° (*c* 0.30, MeOH); UV (H_2O) λ_{max} 282.0 nm (ε 20683, pH 2), 273.0 nm (ε 14192, pH 7), 274.0 nm (ε 13886, pH 11). Anal. Calcd. for ($C_{16}H_{26}FN_3O_2Si$) C, H, N.

(+)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6'-hydroxymethylcyclopent-2-enyl]cytosine (43) was prepared from **42** on a 0.05 mmol scale in 85% yield by a similar procedure as described for **39**. mp: 182 °C (dec.) $[\alpha]^{24}_D$ +34.01° (*c* 0.32, MeOH); UV (H_2O) λ_{max}

282.0 nm (ε 22250, pH 2), 273.0 nm (ε 15123, pH 7), 273.0 nm (ε 14938, pH 11). MS: *m/z* 226 (M+1); Anal. Calcd. for ($C_{10}H_{12}FN_3O_2 \cdot 0.38H_2O$) C, H, N.

(-)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6'-(*O-tert*-butyldimethylsilyloxymethyl)-cyclopent-2-enyl]adenine (45) was prepared from **37** on a 0.93 mmol scale in 55% yield by a similar procedure as described for **20**. mp: 167-168 °C $[\alpha]^{23}_D$ – 47.35° (*c* 0.49, MeOH); UV (H_2O) λ_{max} 258.5 nm (ε 18037, pH 2), 260.0 nm (ε 19612, pH 7), 260.0 nm (ε 17301, pH 11). Anal. Calcd. for ($C_{17}H_{26}FN_2OSi$) C, H, N.

(-)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6'-hydroxymethylcyclopent-2-enyl]adenine (46) was prepared from **45** on a 0.3 mmol scale in 82% yield by a similar procedure as described for **39**. mp: 224 °C (dec.). $[\alpha]^{24}_D$ –83.80° (*c* 0.32, MeOH); UV (H_2O) λ_{max} 259.0 nm (ε 13344, pH 2), 260.0 nm (ε 13894, pH 7), 260.0 nm (ε 13144, pH 11). MS: *m/z* 250 (M+1); Anal. Calcd. for ($C_{11}H_{12}FN_5O$) C, H, N.

(-)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6'-hydroxymethyl-cyclopent-2-enyl]inosine (48) was prepared from **37** on a 1.0 mmol scale in 33% yield by a similar procedure as described for **23**. mp: 226-228 °C (dec.) $[\alpha]^{24}_D$ –83.20° (*c* 0.34, MeOH); UV (H_2O) λ_{max} 249.0 nm (ε 15157, pH 2), 252.0 nm (ε 24290, pH 7), 255.0 nm (ε 16164, pH 11). MS: *m/z* 251 (M+1); Anal. Calcd. for ($C_{11}H_{11}FN_4O_2$) C, H, N.

(-)-(1'*S*,4'*R*)-2-amino-6-chloro-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6'-hydroxymethyl-cyclopent-2-enyl]purine (49) Triphenyl phosphine (630 mg, 2.4 mmol) and diisopropyl azodicarboxylate (479 mg, 2.4 mmol)) were dissolved in anhydrous THF and cooled to 0 °C. After forming a yellowish suspension, the mixture was further cooled to –78 °C. 2-amino-6-chloropurine (407 mg, 2.4 mmol) and a solution of alcohol **12** (150 mg, 0.61 mmol) in THF were added successively. The resulting mixture was kept at –78 °C for 0.5 h and then

stirred at room temperature for 4 h. MeOH was added to quench the reaction and the mixture was evaporated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10) to give **18** as crude product, which was treated with tetrabutylammonium fluoride (1.0 M in THF, 0.6 mL, 0.6 mmol) for 1hr. After evaporation *in vacuo*, the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:20) to give **49** as white foam (105 mg, 61%). $[\alpha]^{23}_D$ –12.65° (*c* 0.42, MeOH); UV (H_2O) λ_{max} 308.0 nm (ε 9044, pH 2), 308.0 nm (ε 9349, pH 7), 308.0 nm (ε 9201, pH 11). Anal. Calcd. for ($C_{11}H_{11}ClFN_5O \cdot 0.33H_2O$) C, H, N.

(-)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',3'-didehydro-2'-fluoro-6'-hydroxymethyl-cyclopent-2-enyl]guanosine (50) was prepared from **49** on a 0.3 mmol scale in 81% yield by a similar procedure as described for **25**. mp: 256 °C (dec.) $[\alpha]^{23}_D$ –23.80° (*c* 0.35, MeOH); UV (H_2O) λ_{max} 254.0 nm (ε 14452, pH 2), 252.0 nm (ε 15511, pH 7), 257.0 nm (ε 12473, pH 11). MS: *m/z* 266 (M+1); Anal. Calcd. for ($C_{11}H_{12}FN_5O_2 \cdot 1.7H_2O$) C, H, N.

Table 2.5. Elemental analysis data

Cmpd No.	Formula	Calc for C	Calc for H	Calc for N	Found for C	Found for H	Found for N
2	$C_{13}H_{18}O_4$	65.53	7.61	-	65.42	7.54	-
3	$C_{13}H_{16}O_3$	70.89	7.32	-	70.54	7.29	-
4	$C_{13}H_{18}O_3$	70.24	8.16	-	69.98	8.24	-
5	$C_{13}H_{18}O_3$	70.24	8.16	-	70.58	8.33	-
6	$C_{32}H_{32}O_3$	82.73	6.94	-	82.86	7.22	-
7	$C_{32}H_{30}O_3$	83.09	6.54	-	82.84	6.61	-
8	$C_{32}H_{30}F_2O_2$	79.32	6.24	-	79.36	6.34	-
9	$C_{32}H_{30}FO_2$	82.73	6.29	-	82.89	6.44	-
10	$C_{13}H_{15}FO_2 \cdot 0.3CH_2Cl_2$	69.96	6.87	-	69.83	6.87	-
11	$C_{29}H_{33}FO_2Si$	75.61	7.22	-	75.58	7.22	-
12	$C_{22}H_{27}FO_2Si$	71.31	7.34	-	71.32	7.44	-
13	$C_{26}H_{29}FN_2O_3Si \cdot 0.7H_2O$	65.44	6.42	5.87	65.39	6.41	5.88
14	$C_{10}H_{11}FN_2O_3 \cdot 0.2H_2O$	52.26	5.00	12.19	52.24	5.09	11.96
15	$C_{26}H_{30}FN_2O_2Si$	67.36	6.52	9.06	67.27	6.57	8.97
16	$C_{10}H_{12}FN_3O_2$	45.90	5.01	16.06	45.86	4.97	16.00
17	$C_{27}H_{31}FN_2O_4Si$	67.75	6.53	5.85	67.92	6.64	5.80
18	$C_{11}H_{13}FN_2O_3$	55.00	5.45	11.66	54.71	5.40	11.39
20	$C_{27}H_{30}FN_5OSi \cdot 0.7H_2O$	64.83	6.33	14.00	64.95	6.25	13.98
21	$C_{11}H_{12}FN_5O \cdot HCl$	46.24	4.59	24.51	46.35	4.59	24.44
22	$C_{27}H_{39}FN_4O_2Si \cdot 0.6H_2O$	64.93	6.09	11.22	65.02	6.07	10.94
23	$C_{11}H_{11}FN_4O_2 \cdot 0.6H_2O$	50.61	4.71	21.46	50.29	4.74	21.45
24	$C_{11}H_{11}ClFN_5O \cdot 0.3MeOH$	46.31	4.16	23.96	46.35	4.14	23.65
25	$C_{11}H_{12}FN_5O_2 \cdot 1.6H_2O$	44.93	4.62	23.14	45.01	4.68	23.11
27	$C_{13}H_{18}O_4$	65.53	7.61	-	65.59	7.67	-
28	$C_{13}H_{16}O_3 \cdot 0.24H_2O$	69.52	7.40	-	69.57	7.45	-
29	$C_{13}H_{18}O_3 \cdot 0.18H_2O$	69.23	8.21	-	69.21	8.27	-
30	$C_{13}H_{18}O_3 \cdot 0.5H_2O$	67.51	8.28	-	67.78	8.30	-
31	$C_{32}H_{32}O_3$	82.73	6.94	-	82.75	7.03	-
32	$C_{32}H_{30}O$	83.09	6.54	-	82.80	6.59	-
33	$C_{32}H_{30}F_2O_2$	79.32	6.24	-	79.36	6.22	-
34	$C_{32}H_{29}FO_2$	82.73	6.29	-	82.75	6.39	-
35	$C_{13}H_{15}FO_2$	70.25	6.80	-	69.98	6.81	-
36	$C_{19}H_{29}FO_2Si$	67.81	8.69	-	68.03	8.81	-
37	$C_{12}H_{23}FO_2Si$	58.50	9.41	-	58.78	9.40	-
38	$C_{17}H_{27}FN_2O_3Si$	57.60	7.68	7.90	57.85	7.74	8.07
39	$C_{11}H_{13}FN_2O_3 \cdot 0.1H_2O$	54.59	5.50	11.57	54.21	5.50	11.33
41	$C_{10}H_{11}FN_2O_3 \cdot 0.2H_2O$	52.26	5.00	12.19	52.12	4.69	11.88
42	$C_{16}H_{26}FN_3O_2Si$	56.61	7.72	12.38	56.58	7.73	12.13
43	$C_{10}H_{12}FN_3O_2 \cdot 0.38H_2O$	51.76	5.54	18.11	51.43	5.15	17.73
45	$C_{17}H_{26}FN_2OSi$	56.17	7.21	19.27	55.87	7.18	19.21
46	$C_{11}H_{12}FN_5O$	53.01	4.85	28.10	53.07	4.97	28.14
48	$C_{11}H_{11}FN_4O_2$	52.80	4.43	22.39	52.82	4.52	22.20
49	$C_{11}H_{11}ClFN_5O \cdot 0.33H_2O$	45.62	4.06	24.18	45.99	3.96	23.79
50	$C_{11}H_{12}FN_5O_2 \cdot 1.7H_2O$	44.65	5.24	23.67	44.65	4.91	23.49

Table 2.6. ^{1}H NMR data-1

Cmpd	H-1'	H-3'	H-4'	H-5'	H-6'	Other protons
13[a]	5.70 (bs, 1H)	5.46 (s, 1H)	2.86 (bs, 1H)	2.68 (m, 1H), 1.61 (m, 1H)	3.73 (dd, *J* = 4.4 and 10.0 Hz, 1H), 3.60 (dd, *J* = 3.6 and 9.2 Hz, 1H)	8.66 (s, 1H), 7.62 (d, *J* = 6.8 Hz, 4H), 7.48-7.37 (m, 6H), 7.29-7.26 (m, 2H)
14[a]	5.66 (m ,1H)	5.48 (t, *J* = 2.0 Hz, 1H)	2.89 (m, 1H)	2.74 (dt, *J* = 9.0 and 14.5 Hz, 1H), 1.72 (dt, *J* = 5.0 and 14.0 Hz, 1H)	3.77 (m, 1H), 3.62 (m, 1H)	8.85 (bs, 1H), 7.58 (dd, *J* = 1.5 and 8.0 Hz, 1H), 5.74 (d, *J* = 7.5 Hz, 1H), 1.96 (t, *J* = 4.5 Hz, 1H)
15[a]	5.80 (bs, 1H)	5.42 (s, 1H)	2.78 (m, 1H)	2.65 (m, 1H), 1.45 (m, 1H)	3.65 (dd, J = 5.0 and 10.0 Hz, 1H), 3.50 (dd, J = 5.5 and 11.0 Hz, 1H)	7.61-7.23 (m, 10H), 6.96, (s, 1H), 5.57 (d, *J* = 6.5 Hz, 1H), 1.05 (s, 9H)
16[a]	5.71 (bs, 1H)	5.54 (s, 1H)	2.84 (bs, 1H)	2.72 (dt, *J* = 9.5 and 14.0 Hz, 1H), 1.61 (dt, *J* = 4.5 and 14.5 Hz, 1H)	3.62 (dd, *J* = 4.5 and 11.5 Hz, 1H), 3.50 (ddd, *J* = 1.5, 4.5 and 11.5 Hz, 1H)	7.80 (d, *J* = 7.0 Hz, 1H), 6.00 (d, *J* = 6.5 Hz, 1H),
17[a]	5.69 (bs, 1H)	5.47 (s, 1H)	2.85 (bs, 1H)	2.64 (m, 1H), 1.26 (s, 1H)	3.68 (dd, *J* = 4.4 Hz, 1H), 3.60 (dd, *J* = 4.8 and 10.4 Hz, 1H)	8.30 (s, 1H), 7.63-7.26 (m, 10H), 6.99 (s, 1H), 1.77 (s, 3H), 1.07 (s, 9H)
18[a]	5.66 (bs, 1H)	5.48 (s, 1H)	2.88 (bs, 1H)	2.73 (m, 1H), 1.75 (m, 1H)	3.77 (dd, *J* = 4.0 and 10.4 Hz, 1H), 3.64 (dd, *J* = 2.8 and 10.4 Hz, 1H)	9.15 (bs, 1H), 7.36 (s, 1H), 1.91 (s, 3H)
20[a]	5.68 (bs, 1H)	5.66 (s, 1H)	2.97 (bs, 1H)	2.84 (dt, *J* = 9.0 and 14.5 Hz, 1H), 1.81 (dt, *J* = 8.0 and 14.0 Hz, 1H)	3.66 (dd, *J* = 2.5 and 6.0 Hz, 2H)	8.35 (s, 1H), 7.77 (s, 1H), 7.63-7.35 (m, 10H), 5.55 (t, *J* = 2.0 Hz, 2H), 1.06 (s, 9H)
21[b]	5.85 (bs, 1H)	5.60 (s, 1H)	2.96 (bs, 1H)	2.92 (d, *J* = 12.5 Hz, 1H), 2.00 (d, *J* = 12.5 Hz, 1H)	3.69 (dd, *J* = 3.5 and 11.0Hz, 1H), 3.60 (dd, *J* = 7.5 and 14.0 Hz, 1H)	8.53 (s, 1H), 8.42 (s, 1H)
22[a]	5.65 (m, 1H)	5.45 (m, 1H)	2.98 (d, *J* = 5.5 Hz, 1H)	2.87 (m, 1H), 1.82 (dt, *J* = 6.0 and 14.0 Hz, 1H)	3.68 (d, *J* = 6.5 Hz, 2H)	13.16 (bs, 1H), 7.77 (s, 1H),7.66-7.37 (m, 10H), 1.07 (s, 9H)
23[b]	5.78 (m, 1H)	5.67 (s, 1H)	2.92(m, 1H)	2.88 (m, 1H), 1.97 (dt, *J* = 5.90 and 13.5 Hz, 1H)	3.67 (dd, *J* = 4.5 and 10.5, 1H), 3.60 (ddd, *J* = 1.5, 5.0 and 11.0 Hz, 1H)	8.15 (s, 1H), 8.07 (s, 1H)
24[b]	5.68 (m, 1H)	5.53 (t, *J* = 2.0 Hz, 1H)	2.96 (m, 1H)	2.87 (dt, *J* = 11.5and 17.5 Hz, 1H), 1.99 (ddt, *J* = 1.5, 6.8 and 16.5 Hz, 1H)	3.72 (m, 1H), 3.63 (ddd, *J* = 2.0, 6.0 and 14.0 Hz, 1H)	8.26 (d, *J* = 1.0 Hz, 1H)
25[c]	5.53 (s, 1H)	5.39 (bs, 1H)	2.76 (d, *J* = 4.0 Hz, 1H)	2.67 (dt, *J* = 9.5 and 14.0 Hz, 1H), 1.74 (dt, *J* = 4.5 and 14.0 Hz, 1H)	3.45 (m, 2H)	7.77 (s, 1H), 6.50 (bs, 2H), 4.83 (bs, 1H)

[a] $CDCl_3$, [b] CD_3OD, [c] DMSO

Table 2.6. continue

Cmpd	H-1'	H-3'	H-4'	H-5'	H-6'	Other protons
38[a]	5.74-5.72 (m, 1H)	5.44 (t, *J* = 2 Hz, 1H)	2.83-2.81 (m, 1H)	2.67-2.62 (dt, *J* = 3.5, 14.5Hz, 1H), 1.64-1.60 (m, 1H)	3.71-3.68 (dd, *J* = 4.5, 10.5 Hz, 1H), 3.56-3.52 (ddd, *J* = 1.5, 4.5, 10 Hz, 1H)	8.72 (s, 1H), 1.94 (d, *J* = 1Hz,3H), 0.90 (t, *J* = 6 Hz, 9H), 0.063 (m, 6H)
39[a]	5.68-5.66 (m, 1H)	5.46 (d, *J* = 1.5 Hz, 1H)	2.89 (m, 1H)	2.76-2.72 (dt, *J* = 9.5, 14.5 Hz, 1H), 1.71-1.68 (m, 1H)	3.80-3.78 (dd. *J* = 3.5, 10.5 Hz, 1H), 3.64-3.63 (m, 1H)	8.15 (s,1H), 7.34 (t, *J* = 1.5 Hz, 1H), 1.92 (d, *J* = 1.5Hz, 3H)
41[b]	5.55-5.52 (m, 1H)	5.42 (s, 1H)	2.74-2.70 (m, 1H)	2.62-2.56 (dt, *J* = 9.0, 14.5 Hz, 1H), 1.55-1.50 (m, 1H)	3.53-3.50 (dd, *J* = 5.0, 11.5 Hz, 1H), 3.42-3.38 (ddd, *J* = 1.5, 5.0, 11.5 Hz, 1H)	7.62 (dd, *J* = 1.5 and 8 Hz, 1H), 5.61 (d, *J* = 8.5 Hz, 1H)
42[b]	5.70 (m, 1H)	5.48 (s, 1H)	2.84 (m, 1H)	2.73-2.67 (dt, *J* = 9.5, 14.0 Hz, 1H), 1.60-1.57 (m, 1H)	3.73-3.70 (dd, *J* = 4.5, 10.0 Hz, 1H), 3.61-3.59 (dd, *J* = 3.0, 10.5 Hz, 1H)	7.64 (dd, *J* = 1.5 and 7 Hz, 1H), 5.90 (d, *J* = 7.5 Hz, 1H), 0.91 (s, 9H), 0.08 (d, *J* = 2.0 Hz, 6H)
43[b]	5.68 (m 1H)	5.50 (s, 1H)	2.82-2.81 (m, 1H)	2.74-2.68 (dt, *J* = 9.0, 14.0 Hz, 1H), 1.59-1.55 (dt, *J* = 5.5, 13.5 Hz, 1H)	3.61-3.58 (dd, *J* = 5.0, 11.5 Hz, 1H), 3.50-3.47 (dd, *J* = 4.5, 10.5 Hz, 1H)	7.80 (dd, *J* = 2 and 7.5 Hz, 1H), 5.91 (d, *J* = 7 Hz, 1H)
45[b]	5.64-5.47 (m,1H)	5.48 (t, *J* = 2.0 Hz, 1H)	2.89-2.86 (m, 1H)	2.82-2.76 (dt, *J* = 9.0, 14.5 Hz 1H), 1.88-1.84 (m, 1H)	3.70-3.67 (dd, *J* = 4.5, 10 Hz, 1H), 3.65-3.61 (ddd, *J* = 1.5, 5, 10 Hz, 1H)	8.13 (s, 1H), 8.12 (s, 1H), 0.81 (m, 9H), 0.002 (m, 6H)
46[b]	5.78-5.75 (m, 1H)	5.61 (s, 1H)	3.00-2.97 (m, 1H)	2.96-2.90 (dt, *J* = 9.5, 13.5 Hz, 1H), 2.04-1.99 (m, 1H)	3.75-3.71 (dd, *J* = 5, 8.5 Hz, 1H), 3.66-3.62 (ddd, *J* = 1.5, 5, 11 Hz, 1H)	8.31 (s, 1H), 8.24 (s, 1H)
48[b]	5.77-5.74 (m, 1H)	5.56 (s, 1H)	2.94-2.92 (m, 1H)	2.91-2.84 (dt, *J* = 9, 13.5 Hz, 1H), 1.98-1.94 (m, 1H)	3.69-3.66 (dd, *J* = 5, 11.5 Hz, 1H), 3.61-3.57 (m, 1H)	8.23 (s, 1H), 8.05 (s, 1H)
49[b]	5.70-5.68 (m, 1H)	5.59 (s, 1H)	2.97-2.96 (m, 1H)	2.90-2.84 (m, 1H), 2.02-1.97 (m, 1H)	3.74-3.71 (dd, *J* = 5.0, 11.0 Hz, 1H), 3.66-3.63 (m, 1H)	8.27 (s, 1H)
50[c]	5.44-5.42 (m, 1H)	5.57 (s, 1H)	2.80-2.79 (m, 1H)	2.74-2.67 (dt, *J* = 9.5, 14 Hz, 1H), 1.81-1.76 (dt, *J* = 4.5, 13.5 Hz, 1H)	3.50-3.46 (m, 2H)	7.80 (s, 1H), 6.60 (s, 2H), 4.88 (t, *J* = 5.5 Hz, 1H)

Table 2.7. ^{13}C NMR data

Cmpd No.	^{13}C Chemical shift ([a] $CDCl_3$, [b] CD_3OD, [c] DMSO)
13[a]	163.0, 157.8, 155.0, 151.1, 140.5, 135.8, 133.4 (d, *J* = 12.2 Hz), 130.2 (d, *J*= 3.0 Hz), 128.1 (d, *J* = 3.0 Hz), 112.5 (d, *J* = 7.6 Hz), 103.3, 66.3, 56.8 (d, *J* = 21.3 Hz), 53.7, 39.8 (d, *J* = 6.1 Hz), 31.4, 27.2, 19.6
14[a]	163.4 (d, *J*= 10.4 Hz), 151.2 (d, *J* = 5.6 Hz), 142.1, 140.7, 112.7, 111.4, 103.7, 102.2, 78.3, 64.6, 57.7
15[b]	165.1, 158.4, 156.1, 142.1, 135.6, 133.3 (d, *J* = 22.9 Hz), 129.9, 127.8 (d, *J* = 5.3 Hz), 112.0 (d, *J* = 7.6 Hz), 94.4, 66.4, 57.4 (d, *J* = 20.0 Hz), 39.6 (d, *J* = 6.8 Hz), 32.2, 27.0 (d, *J*= 6.5 Hz), 22.0, 19.3, 0.0
16[b]	165.1, 158.2, 155.4, 142.8, 112.3 (d, *J* = 9.5 Hz), 64.0, 58.0 (d, *J* = 27.6 Hz), 53.6, 39.9 (d, , *J* = 6.6 Hz), 31.7
17[b]	231.7, 185.3, 163.6, 159.4, 135.8, 134.6, 133.4, 130.3, 128.1, 112.0, 98.2, 83.4, 41.5, 27.1, 19.6, 12.7, 11.5
18[b]	164.1, 151.4, 137.1, 111.8 (d, *J* = 8.4 Hz), 91.4, 64.8, 57.0 (d, *J* = 21.4 Hz), 39.7 (d, *J* = 6.1 Hz), 31.1, 12.8
20[b]	157.9, 155.7 (d, *J* = 14.8 Hz), 153.3, 150.3, 138.6, 135.8, 133.4 (d, *J* = 4.4 Hz), 130.1, 128.0, 111.5 (d, *J* = 8.1 Hz), 67.1, 55.3 (d, *J* = 21.4 Hz), 40.4 (d, *J* = 6.3 Hz), 33.3 (d, *J* = 4.8 Hz), 27.1, 19.5, 0.2
21[b]	157.7, 154.9, 150.5, 149.02, 144.1 (d, *J* = 3.9 Hz), 143.0, 118.8, 111.5 (d, *J* = 10.5 Hz), 64.1, 56.5 (d, *J* = 26.6 Hz), 40.5 (d, *J* = 7.2 Hz), 32.3 (d, *J* = 6.8 Hz), 17.2
22[b]	159.6, 155.4, 149.3, 145.1, 138.4, 135.8, 133.4, 130,2, 128.1, 124.9, 111.5, 67.1, 55.8 (d, *J* = 21.3 Hz), 40.4, 33.4, 27.1, 19.5
23[b]	157.7, 154.9, 150.5, 149.0, 144.1 (d, *J* = 3.9 Hz), 143.0, 118.8, 111.5 (d, *J* = 10.5 Hz), 64.1, 56.5 (d, *J* = 26.6 Hz), 40.5 (d, *J* = 7.6 Hz), 32.3 (d, *J* = 5.8 Hz), 17.2
24[b]	160.2, 158.1, 155.3, 153.7, 150.2, 141.5, 123.6, 110.6 (d, *J* = 10.5 Hz), 64.0 (d, *J* = 2.9 Hz), 55.4 (d, *J* = 26.8 Hz), 40.2 (d, *J* = 8.6 Hz), 31.6 (d, *J* = 6.8 Hz)
25[c]	158.5, 157.2, 155.7, 154.1, 151.6, 135.6, 117.0, 111.1 (d, *J* = 9.5 Hz), 64.4, 54.4 (d, *J* = 27.6 Hz), 32.6
38[a]	163.79, 156.37 (d, *J*= 223.9 Hz), 135.80, 111.91 (d, *J*= 6.1 Hz), 111.62, 65.12, 56.40 (d, 16.5 Hz), 39.51 (d, *J*= 5.4 Hz), 31.00 (d, *J*= 3.8 Hz), 25.89, 18.40, 12.53, -5.37 (d, *J* = 4.6 Hz)
39[a]	163.47, 156.94 (d, *J*= 222.4 Hz), 150.96, 136.58, 111.37, 111.17 (d, *J*= 6.5 Hz), 64.74 (d, *J*= 1.9 Hz), 56.54 (d, *J*= 16.8 Hz), 39.33 (d, *J*= 5.3 Hz), 30.89 (d, *J*= 4.2 Hz), 12.51
41[b]	166.17, 157.87 (d, *J*= 222.0 Hz), 152.82, 143.21, 113.08 (d, *J*= 6.5 Hz), 103.06, 65.14 (d, *J*= 1.9 Hz), 58.09 (d, *J*= 16.8 Hz), 41.06 (d, *J*= 5.3 Hz), 32.08 (d, *J*= 4.2 Hz)
42[b]	167.46, 158.91, 158.49 (d, *J*= 222.0 Hz), 143.25, 112.88 (d, *J*= 6.9 Hz), 96.68, 66.71 (d, *J*= 1.5 Hz), 58.79 (d, *J*= 16.8 Hz), 41.05 (d, *J*= 5.7 Hz), 32.65 (d, *J*= 4.2 Hz), 26.46, 19.344, -5.23
43[b]	166.12, 157.55, 157.16 (d, *J*= 222.0 Hz), 142.13, 111.37 (d, *J*= 6.5 Hz), 95.10, 64.00, 57.64 (d, *J*= 16.8 Hz), 39.71 (d, *J*= 5.7 Hz), 31.50 (d, *J*= 4.2 Hz)
45[b]	158.18 (d, *J*= 222.4 Hz), 157.47, 153.97, 150.72, 140.77, 120.24, 112.24 (d, *J*= 6.8 Hz), 67.01 (d, *J*=1.5 Hz), 56.72 (d, *J*=17.6Hz) , 41.70 (d, *J*= 5.8Hz), 33.57 (d, *J*= 3.8 Hz), 26.57, 19.46, -5.12 (d, *J*= 8Hz)
46[b]	156.74 (d, *J*= 222.5 Hz), 156.00, 149.18, 139.64, 118.77, 110.58 (d, *J*= 6.4 Hz), 64.02 (d. *J*= 1.9 Hz), 55.40 (d, *J*= 17.6 Hz), 40.14 (d, *J*= 5.7 Hz), 32.00 (d, *J*= 4.2 Hz)
48[b]	158.03 (d, *J*= 222.4 Hz), 150.17, 146.73, 140.54, 125.29, 112.01 (d, *J*= 6.5 Hz), 65.34 (d, *J*= 1.9 Hz), 57.06 (d, *J*= 17.2 Hz), 41.54 (d, *J*= 5.8 Hz), 33.50 (d, *J*= 3.8 Hz)
49[b]	160.21 (d, *J* = 3.8 Hz), 156.63 (d, *J* = 222.4 Hz), 153.69, 150.25, 141.45, 123.58, 110.62 (d, *J*= 6.5 Hz), 63.92 (d, *J*= 1.5 Hz), 55.22 (d, *J*= 17.6 Hz), 40.12 (d, *J*= 5.7 Hz), 31.62 (d, *J*= 3.8 Hz)
50[c]	157.19, 157.11 (d, *J*= 221.7 Hz), 154.19, 151.56, 135.56, 116.96, 111.08 (d, *J*= 4.8 Hz), 64.41, 54.26 (d, *J*= 17.2 Hz), 32.51 (d, *J*= 4.2 Hz)

Antiviral Assay

Human peripheral blood mononuclear (PBM) cells (obtained from the Atlanta Red Cross) were isolated by Ficoll-Hypaque discontinuous gradient centrifugation from healthy seronegative donors. Cells were stimulated with phytohemagglutinin A (Difco, Sparks, MD) for 2-3 days prior to use. HIV-1_{LAI} obtained from the Centers for Disease Control and Prevention (Atlanta, GA) was used as the standard reference virus for the antiviral assays. The molecular infectious clones HIV-1_{xxBru} and HIV-$1_{M184Vpitt}$ were obtained from Dr. John Mellors (University of Pittsburgh). Infections were done in bulk for 1 h, either with 100 $TCID_{50}$ /1 x 10^7 cells for a flask (T25) assay or with 200 $TCID_{50}$ / 6 x 10^5 cells/well for a 24 well plate assay. Cells were added to a plate or flask containing a ten-fold serial dilution of the test compound. Assay medium was RPMI-1640 supplemented with heat inactivated 16% fetal bovine serum, 1.6 mM L-glutamine, 80 IU/mL penicillin, 80 µg/mL streptomycin, 0.0008% DEAE-Dextran, 0.045% sodium bicarbonate, and 26 IU/mL recombinant interleukin-2 (Chiron Corp, Emeryville, CA). AZT was used as a positive control for the assay. Untreated and uninfected PBM cells were grown in parallel at equivalent cell concentrations as controls. The cell cultures were maintained in a humidified 5% CO_2-air at 37 °C for 5 days and supernatants were collected for reverse transcriptase (RT) activity.

Supernatants were centrifuged at 12,000 rpm for 2 h to pellet the virus. The pellet was solubilized with vortexing in 100 µL virus solubilization buffer (VSB) containing 0.5% Triton X-100, 0.8 M NaCl, 0.5 mM phenylmethylsulfonyl fluoride, 20% glycerol, and 0.05 M Tris, pH 7.8. Ten µL of each sample was added to the 75 µL RT reaction mixture (0.06 M Tris, pH 7.8, 0.012 M $MgCl_2$, 0.006 M dithiothreitol, 0.006 mg/mL poly $(rA)_n$ oligo $(dT)_{12-18}$, 96 µg/mL dATP, and 1 µM of 0.08 mCi/mL ^{3}H-thymidine triphosphate (Moravek Biochemicals, Brea, CA)

and incubated at 37 °C for 2 h. The reaction was stopped by the addition of 100 μL 10% trichloroacetic acid containing 0.05% sodium pyrophosphate. The acid insoluble product was harvested onto filter paper using a Packard Harvester (Meriden, CT), and the RT activity was read on a Packard Direct Beta Counter (Meriden, CT). The RT results were expressed in counts per minute (CPM) per milliliter. The antiviral 50% effective concentration (EC_{50}) and 90% effective concentration (EC_{90}) were determined from the concentration-response curve using the median effect method.[224]

Cytotoxicity assays

The compounds were evaluated for their potential toxic effects on uninfected PHA-stimulated human PBM cells, in CEM (T-lymphoblastoid cell line obtained from American Type Culture Collection, Rockville, MD.) and Vero (African green monkey kidney) cells. PBM cells were obtained from whole blood of healthy seronegative donors (HIV-1 and hepatitis B virus) by single-step Ficoll-Hypaque discontinuous gradient centrifugation. Log phase Vero, CEM and PHA-stimulated human PBM cells were seeded at a density of 5×10^3, 2.5×10^3 and 5×10^4 cells/well respectively. All of the cells were plated in 96-well cell culture plates containing ten-fold serial dilutions of the test drug. The cultures were incubated for 3, 4, and 5 days for Vero, CEM, and PBM cells, respectively in a humidified 5% CO_2-air at 37 °C. At the end of incubation, MTT tetrazolium dye solution (Cell titer 96®, Promega, Madison, WI) was added to each well and incubated overnight. The reaction was stopped with stop solubilization solution (Promega, Madison, WI). The plates were incubated for 5 h to ensure that the formazan crystals were dissolved. The plates were read at a wavelength of 570 nm using an ELISA plate reader (Bio-tek instruments, Inc., Winooski, VT, Model # EL 312e). The 50% inhibition concentration

(IC_{50}) was determined from the concentration-response curve using the median effect method as previous described.[224]

Molecular Modeling Study (a) Conformational analysis: The initial conformation of D-2'F-C-d4A 21, D-2'F-C-d4G 25, L-2'F-C-d4A 46 were constructed by builder module in MACROMODEL®, version 8.5 (Schrodinger, Inc.) based on the crystal structure of carbovir. The Monte Carlo conformational search was performed in 5,000-step, in the presence of GB/SA water model using MMFFs force field in MACROMODEL. (b) Binding affinity study to HIV-1 reverse transcriptase: All molecular modeling studies of the enzyme-substrate complexes were performed using Sybyl® 6.91 (Tripos Associates, St. Louis, MO) on a Silicon Graphics Tezro® workstation. The enzyme site of the enzyme-ligand complex was built based on the X-ray structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer-template duplex (PDB entry 1rtd).[225] A model of the NRTI binding site was built, which consisted of residues between Lys1 and Pro243 in the p66 subunit, and a 7:4 (template-primer) duplex. The conformationally optimized structure of 2'-fluoro-2',3'-dideoxy-2',3'-didehydro carbocyclic nucleosides were used to define the initial Cartesian coordinates. The heterocyclic moiety of the $n+1^{th}$ nucleotide in the template overhang was modified to the base complementary to the incoming NRTIs if needed, i.e. the adenine moiety which was in the original X-ray structure (1rtd)[225] was modified to guanine. The inhibitor triphosphates were manually docked to the active site of the enzyme by adjusting the torsional angles to those found in the X-ray structure.[225] Gästeiger-Hückel charges were given to the enzyme-ligand complex with formal charges (+2) to the two Mg atoms in the active site. Then, Kollman-All-Atom charges were loaded to the enzyme site using the biopolymer module in Sybyl. Fluorine parameters were

obtained from literature[186, 226, 227] and MM2 parameters were entered into the parameter files. In order to eliminate local strains resulting from merging inhibitors and/or point mutations, residues inside 6 Å from the merged inhibitors and mutated residues were annealed until energy change from one iteration to the next was less than 0.05 Kcal/mol. The annealed enzyme-inhibitor complexes were minimized by using Kollman-All-Atom Force Field until iteration number reached 5,000.

Acknowledgement

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CHAPTER 3

D- AND L-2',3'-DIDEHYDRO-2',3'-DIDEOXY-3'-FLUORO-CARBOCYCLIC NUCLEOSIDES: SYNTHESIS, ANTI-HIV ACTIVITY AND MECHANISM OF RESISTANCE

Jianing Wang, Yunho Jin, Kimberly L. Rapp, Matthew Bennett, Raymond F. Schinazi, and Chung K. Chu. 2007, *Journal of Medicinal Chemistry* 50:1828-1839.

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ABSTRACT

Introducing 2'-fluoro substitution on the 2',3'-double bond in carbocyclic nucleosides has provided biologically interesting compounds with potent anti-HIV activity. As an extension of our previous works in the discovery of anti-HIV agents, D- and L-2',3'-unsaturated 3'-fluoro carbocyclic nucleosides were synthesized and evaluated against HIV-1 in human peripheral blood mononuclear (PBM) cells. Among the synthesized L-series nucleosides, compounds **18**, **19**, **26**, **28** exhibited moderate antiviral activity (EC_{50} 7.1 μM, 6.4 μM, 10.3 μM and 20.7 μM, respectively), while among the D-series, the guanosine analogue (**35**, D-3'-F-C-d4G) exhibited the most potent anti-HIV activity (EC_{50} 0.4 μM, EC_{90} 2.8 μM). However, the guanosine analogue **35** was cross-resistant to the lamivudine-resistant variants (HIV-1_{M184V}). Molecular modeling studies suggest that hydrophobic interaction as well as hydrogen bonding stabilize the binding of compound **35** in the active site of wild type HIV reverse transcriptase (HIV-RT). In the case of L-nucleosides, these two effects are opposite which results in a loss of binding affinity. According to the molecular modeling studies, cross-resistance of D-3'-F-C-d4G (**35**) to M184V mutant may be caused by the realignment of the primer and template in the HIV-RT_{M184V} interaction, which destabilizes the RT-inhibitor triphosphate complex, resulting in a significant reduction in anti-HIV activity of the D-guanine derivative **35**.

INTRODUCTION

Nucleoside reverse transcriptase inhibitors (NRTI) have played important role in the treatment of HIV infections.[228] However, major drawbacks of NRTI include the emergence of drug resistant variants and toxicity.[200-203] Therefore, conservative efforts have been made to improve the antiviral efficacy as well as to reduce the toxicity by modifying the structure. The structures of several potent NRTIs, such as stavudine (d4T), abacavir, reverset (D-d4FC) and elvucitabine (L-d4FC) highlight the important role of a 2',3'-double bond to enhance the antiviral activity (Figure 3.1).[228] Additionally, carbocyclic nucleosides such as abacavir, have attracted considerable attention due to their potent antiviral activity as well as the stability toward metabolic degradation.[10]

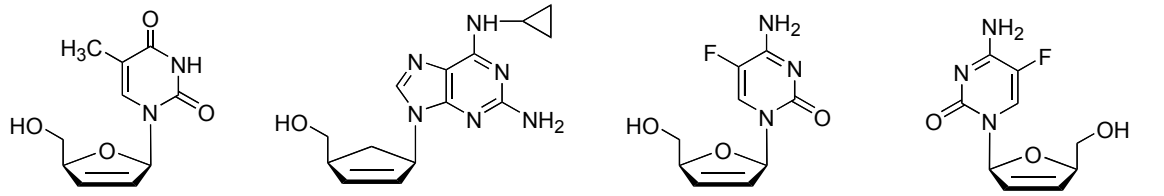


Figure 3.1. Several potent NRTIs with 2',3'-double bond

In view of these facts, it was of interest to incorporate these structural features into nucleoside analogues. Several interesting compounds with potent anti-HIV activity have been discovered as the result of this concept.[209-213, 220] In connection to these efforts, our laboratory recently reported the stereo-selective synthesis and anti-HIV activity of D- & L-2',3'-didehyhydro-2',3'-dideoxy-2'-fluoro-carbocyclic nucleosides.[115] Among the series, the adenosine analogue with L-configuration showed the most potent anti-HIV activity (EC_{50} 0.77 μM). Based

on molecular modeling studies, it was found that both the double bond on the carbocyclic ring as well as the 2'-fluoro substitution contribute to the favorable binding affinity between the inhibitor and the HIV-RT. Hence, further exploring the antiviral activity of 3'-fluorine congeners was of interesting to expand our knowledge on the structure activity relationships of the same class of nucleosides.

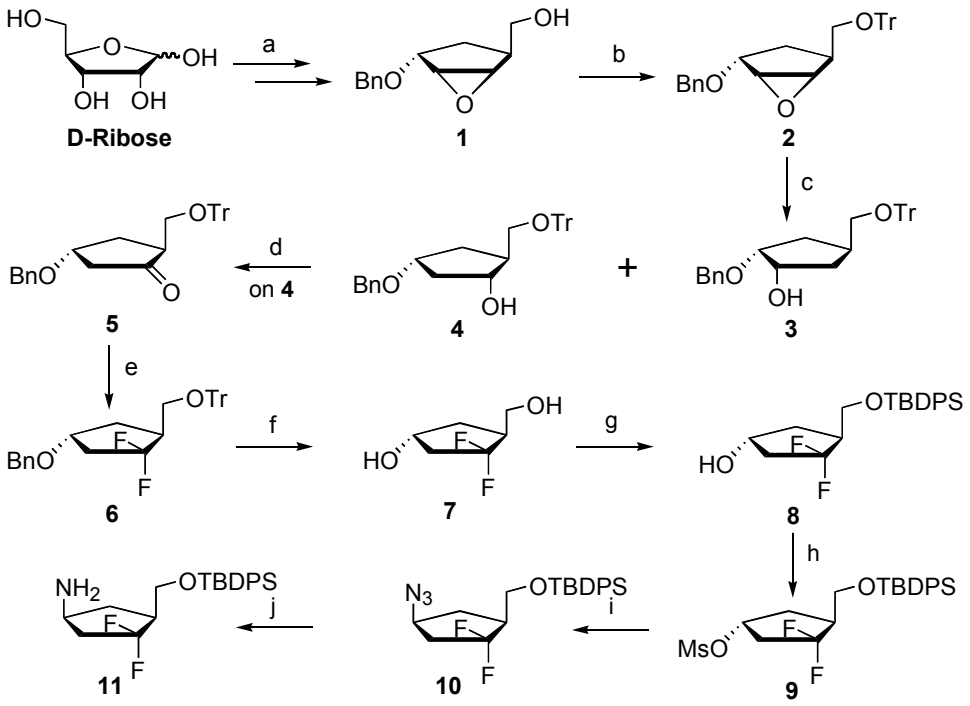
For the synthesis of 2'-fluoro carbocyclic nucleosides, the fluorine substituted unsaturated carbocyclic ring was first constructed followed by coupling with various heterocyclic bases. However, this method was found to be unsuccessful in the synthesis for the 3'-fluoro congeners due to the instability of the final products under the same conditions used. Therefore, condensation of a gem-3',3'-difluoro sugar **8** or **29** with base moieties followed by an elimination reaction in the last step was successful to obtain the target purine nucleosides. Pyrimidine nucleosides were, however, synthesized *via* the linear method using the intermediate **11** or **30**. Herein, synthesis, anti-HIV activity and molecular modeling studies of D- & L-2',3'-didehyhydro-2',3'-dideoxy-3'-fluoro-carbocyclic nucleosides are reported.

RESULTS AND DISCUSSION

Chemistry

Both D- & L-nucleosides were synthesized, however, the following descriptions are mainly based on the L-series according to the Scheme 3.1 - 3.3, unless otherwise indicated. The

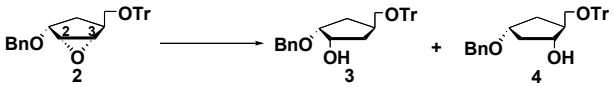
epoxide **1** was synthesized from D-ribose by the known method in our laboratory.[115] The desired regio-isomer was obtained by reductive ring opening of the epoxide with judicious selection of reducing reagents (Scheme 3.1). The preliminary study suggested that compound **4** was the major product when treated the epoxide **2** with LAH. The selective opening of the epoxide by the hydride may be due to the steric hindrance of the bulky trityl group adjacent to the 3'-position. Based on this result, several other reducing reagents were investigated (Table 3.1). Among them, Super-Hydride® gave predominantly compound **4** (compound **4** : compound **3** = 15.3 : 1) in excellent yield (98 %).



Reagents and conditions: (a) i) Ref. **14**, ii) α-AIBBr, CH_3CN iii) K_2CO_3, MeOH; (b) TrCl, DMAP, Et_3N, CH_2Cl_2; (C) Super-Hydride® (1.0 M in THF), 0 °C to room temp.; (d) PDC, AcOH, 4 Å molecular sieve, CH_2Cl_2; (e) Neat DAST, 40 °C; (f) TMSI, CH_2Cl_2; (g) TBDPSCl, imidazole, CH_2Cl_2; (h) MsCl, Et_3N, CH_2Cl_2, room temp.; (i) NaN_3, DMF, 130 °C; (j) H_2/Pd/C, 30 psi, room temp.

Scheme 3.1. Synthesis of L-form key intermediates **8** and **11**

Table 3.1. Ring-opening reaction of epoxide **2**



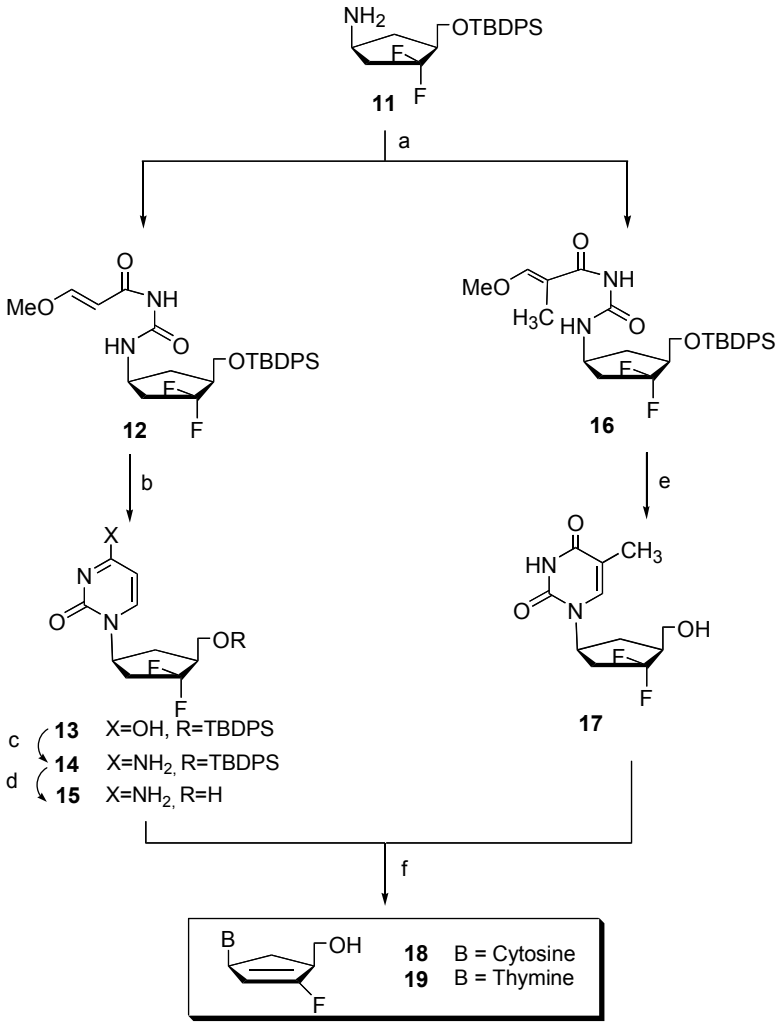
Entry	Reducing agent	4:3	yield
1	Super-Hydride®	15.3 : 1	98 %
2	LAH	5.1:1	88 %
3	DIBAL-H	-	0 %
4	Red-Al	-	0 %
5	LiAl(*t*-butoxy)$_3$	-	0 %

Oxidation of the alcohol **4** with PDC gave the ketone **5** as an unstable compound. It was interesting to note that introducing a di-fluorine group to **5** was quite difficult in comparison to the 2'-fluoro isomer.[115] A harsh condition of neat diethylaminosulfur trifluoride (DAST) with reaction temperature at 40 °C for 36 h was needed to convert the ketone **5** to difluoro compound **6** in 68 % yield. The following elimination reaction also gave significant problems. Treating compound **6** with potassium *tert*-butoxide (^{t}BuOK) in THF at 50 °C did not produce the desired 3'-fluorovinyl moiety and only recovered the starting material. Hence, a modified synthetic sequence was adopted as illustrated in Scheme 3.1 (**6** to **11**). Both trityl and benzyl groups were removed using iodotrimethylsilane (TMSI) in 70 % yield. The resulting diol **7** was selectively protected by *tert*-butyldiphenylsilyl chloride (TBDPSCl) to give the key intermediate **8** which was converted to amine **11** in three steps.

For the pyrimidine nucleosides, the linear synthetic methodology reported by Shealy et al.,[229, 230] was used, as a direct coupling reaction using the alcohol **8** under Mitsunobu condition resulted in the decomposition of the starting material. The amine **11** was coupled with

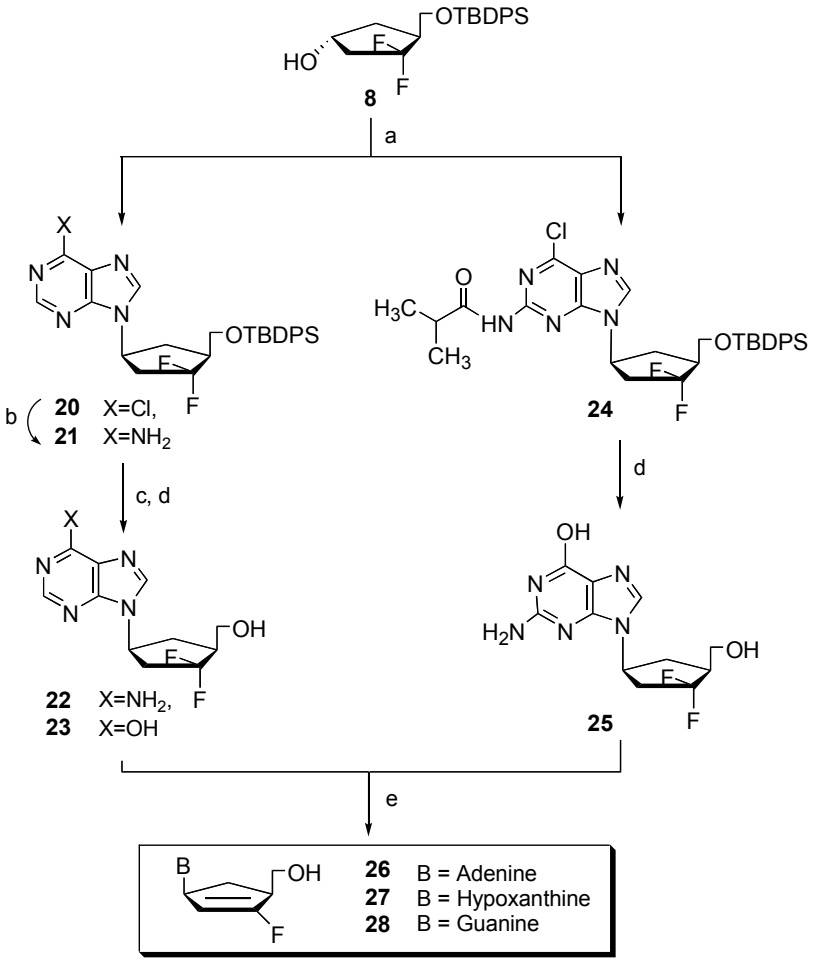
substituted isocynate to give the corresponding urea **12** or **16** (Scheme 3.2). Reaction of **12** with conc. ammonium hydroxide/ethanol/1,4-dioxane in a steel bomb gave the uridine analogue **13** in 41 % yield. Amination of **13** followed by deprotection afforded the cytidine analogue **15**. For the thymidine analogue **17**, ring closure and deprotection were accomplished in one step under acidic condition from compound **16.** Lastly, the 2',3'-double bond was obtained under basic elimination conditions to afford the target cytidine **18** and thymidine **19** analogues in 35 % and 46 % yield, respectively. To synthesize adenosine analogues, triphenylphosphine (TPP) and diisopropyl azodicarboxylate (DIAD) were first mixed in the THF:1,4-dioxane co-solvent at 0 °C, and then further cooled to -78 °C. The key intermediate **8** and the 6-chloropurine were added sequentially and the reaction was allowed to gradually warm up to room temperature until all the starting material was consumed (Scheme 3.3). The crude product **20**, which was contaminated with reduced DIAD species, was directly treated with methanolic ammonia in a steel bomb at 100 °C to give the adenosine analogue **21** in 37 % yield in two steps. After removing the silyl group under acidic condition, compound **22** was treated with ^{t}BuOK in THF at 90 °C to furnish the adenine derivative **26** in 50 % yield. The compound **21** could also be converted to inosine analogue **23** by treating with formic acid followed by ammonium hydroxide in 39 % yield in two steps. After the elimination reaction similar to the method described for the adenosine analogue, the final inosine analogue **27** was obtained in 49 % yield. Condensation of alcohol **8** with 2-amino-6-chloropurine or 6-chloro-N^2-isobutyrylpurine, under the Mitsunobu condition as described above, failed to give the corresponding nucleoside. However, when a mixture of TPP, 6-chloro-N^2-isobutyrylpurine and alcohol **8** in dry THF was treated with DIAD at 0 °C, the desired product **24** was able to be isolated. The compound **24** was converted to the guanosine analogue **25** using formic acid followed by ammonium

hydroxide in 18 % yield from **8** in two steps. The nucleoside **25** was further subjected to an elimination reaction using ^{t}BuOK in DMF at 70 °C to give the guanosine analogue **28** in 45 % yield.



Reagents and conditions: (a) (i) β-methoxyacryloyl isocyanate, THF, -30 °C to room temp. (for **12**) or β-methoxy-α-methacryloyl isocyanate, THF, -30 °C to room temp.(for **16**); (b) NH_4OH, 1,4-dioxane/EtOH, steel bomb, 90-100 °C; (c) (i) 2,4,6-triisopropylbenzenesulfonyl chloride, DMAP, Et_3N, CH_3CN, room temp. (ii) NH_4OH or NH_3/MeOH, room temp.; (d) HCl/ MeOH, room temp.; (e) 3N HCl/1,4-dioxane, reflux 3h; (f) ^{t}BuOK, THF/1,4-dioxane, 90 °C.

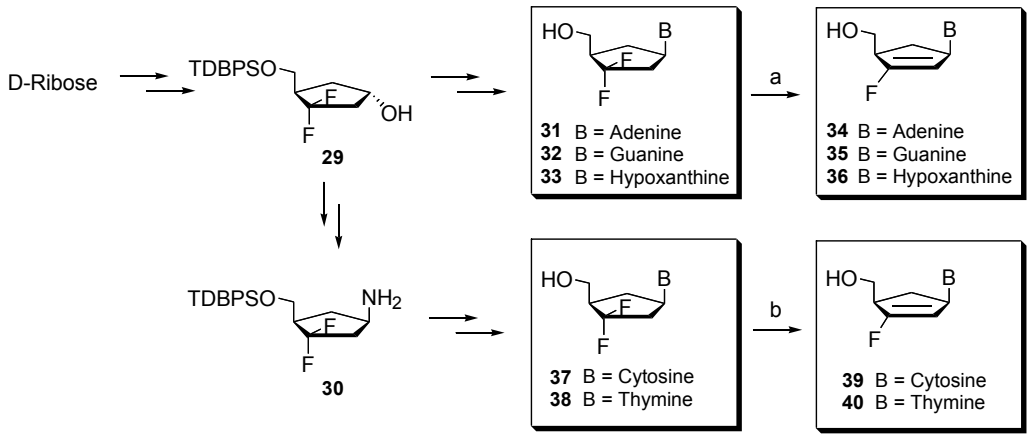
Scheme 3.2. Synthesis of target L-form pyrimidine analogs



Reagents and conditions: (a) DIAD, Ph_3P, purines, THF or THF/1,4-dioxane; (b) NH_3/MeOH, steel bomb, 110 °C; (c) 3 N HCl, MeOH; (d) (i) Formic acid, (ii) NH_4OH or NH_3/MeOH, room temp.; (e) ^{t}BuOK, THF/1,4-dioxane, 90 °C or ^{t}BuOK, DMF, 70 °C.

Scheme 3.3. Synthesis of target L-form purine analogs

Similar procedures were conducted to synthesize the D-series (Scheme 3.4). As the difluoro-substituted nucleosides on the carbohydrate moiety exhibit interesting activity,[231] the difluoro-nucleosides **31**-**33**, **37** and **38** in the D-series were also evaluated against HIV-1.



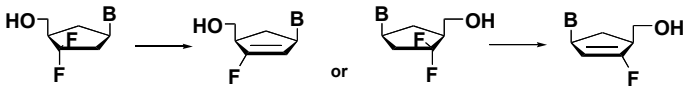
Reagents and conditions: (a) ^{t}BuOK, THF/1,4-dioxane, 90 °C, conventional oil bath heating for **34**, **36**; ^{t}BuOK, DMF, 70 °C, microwave-assisted for **35**; (b) ^{t}BuOK, THF/1,4-dioxane, 60 °C, traditional oil bath heating for **40**; ^{t}BuOK, DMF, 70 °C, microwave-assisted for **39**.

Scheme 3.4. Synthesis of D-form target pyrimidine and purine analogs

It is noteworthy that the elimination reaction in the last step proceeded with significant difficulties. First of all, the starting material and product have almost identical R_f values on a silica gel plate as well as on a column, which makes it extremely difficult to identify and separate. Secondly, the difluoro compounds are inert to the conventional methods of elimination using ^{t}BuOK in aprotic solvent, and therefore, the reaction mixture has to be heated for long periods of time to convert all the starting material to the product (Table 3.3, entries 1 and 3). Unfortunately, under these conditions, the newly formed target nucleosides decomposed, which resulted in low yields. Fortunately, in the search for better conditions for the elimination step during the synthesis of the D-compounds, it was found that the microwave-assisted method had several advantages over the traditional method. Upon irradiation of the difluoro-nucleosides in the microwave synthesizer with maximum output power of 300 W, the elimination reaction was completed within 5-10 min at 70 °C and gave 70-80 % yield, while a lower yield were obtained after a longer time in the traditional thermally-assisted conditions

(Table 3.2). This methodology may provide an efficient way for preparing molecules which have a fluorovinyl moiety. Assignment of the structures of newly synthesized nucleosides was accomplished by NMR, elemental analysis, mass, UV and IR spectroscopy.

Table 3.2. Elimination reactions using traditional or microwave (MW)-assisted methods



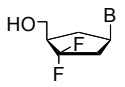
Entry	Substrate	Temperature	Reaction time	Isolated yield (%)	Methods
1	L-cytidine analogue	90 °C	9 h	35	traditional oil bath heating
2	D-cytidine analogue	70 °C	6 min	84	MW-assisted
3	L-guanosine analogue	70 °C	24 h	45	traditional oil bath heating
4	D-guanosine analogue	70 °C	10 min	72	MW-assisted

Anti-HIV Activity

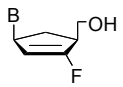
All the synthesized pyrimidine (**18**, **19** and **37**-**40**) and purine (**26**-**28**, **31**-**36**) nucleosides were evaluated against HIV-1 in human PBM cells. The EC_{50}, EC_{90} and toxicity data are listed in Table 3.3. In the D-3',3'-difluoro series, none of the compounds showed any antiviral activity nor cytotoxicity up to 100 μM. Among the 3'-fluoro-2',3'-unsaturated nucleosides, some of them exhibited moderate to potent anti-HIV activity. The cytidine **18**, thymidine **19**, adenosine **26** and guanosine **28** analogues in the L-series inhibited HIV-1 in PBM cells with EC_{50} ranged from 6.4 μM to 20.7 μM. The D-guanosine analogue **35** is the most active compound among synthesized nucleosides (EC_{50} 0.41 μM, EC_{90} 2.8 μM), although it exhibited moderate cytotoxicity (IC_{50} 21.1

μM, PBM cells). The antiviral activities of 3'-fluoro-2',3'-unsaturated carbocyclic nucleosides in the current studies were generally maintained or enhanced, in comparison to that of 2'-fluoro congeners.[115] Thus, the role of the fluorine substitution may have significant effects (*vide infra*).

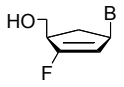
Table 3.3. *In vitro* anti-HIV-1 activity and toxicity of D-3',3'-difluoro-2',3'-dideoxy-carbocyclic nucleosides and D- & L- 3'-fluoro-2',3'-didehydro-carbocyclic nucleosides



31, 32, 33, 37, 38



18, 19, 26, 27, 28



34, 35, 36, 39, 40

B	Config.	Anti-HIV-1 activity (μM)[a]		Cytotoxicity (μM)		
		EC_{50}	EC_{90}	PBM	CEM	Vero
Adenine **31**	D	>100	>100	95.1	>100	>100
Guanosine **32**	D	>100	>100	>100	>100	>100
Hypoxanthine **33**	D	>100	>100	>100	>100	>100
Cytosine **37**	D	>100	>100	>100	>100	>100
Thymine **38**	D	>100	>100	>100	>100	>100
Cytosine **18**	L	7.1	72.0	>100	>100	>100
Thymine **19**	L	6.4	>100	>100	>100	>100
Adenine **26**	L	10.3	33.5	>100	>100	>100
Hypoxanthine **27**	L	>100	>100	>100	>100	>100
Guanine **28**	L	20.7	>100	>100	>100	>100
Adenine **34**	D	14.8	40.6	>100	>100	>100
Guanine **35**	D	0.41	2.8	21.1	>100	>100
Hypoxanthine **36**	D	>100	>100	>100	>100	>100
Cytosine **39**	D	>100	>100	>100	>100	>100
Thymine **40**	D	68.8	>100	>100	>100	>100
Carbovir	D	0.087[b]	0.27[b]	N/A	N/A	N/A

[a]Anti-HIV activity evaluated in PBM cells against HIV-1_{LAI} unless otherwise indicated.
[b]Anti-HIV activity evaluated in PBM cells against HIV-1_{xxBRU}.

Antiviral Activity against Lamivudine-Resistant (HIV-1_{M184V}) Mutant Strain

One of the drawbacks of the NRTI is the emergence of drug-resistant mutant strains during the extended treatment period, which may significantly compromise the clinical efficacy. Lamivudine, an important component of the highly active antiretroviral therapy (HAART), confers a single mutation at residue 184 (M184V), which caused at least a 1,000-fold decrease in its antiviral activity.[217, 218] Discovery of novel NRTI agents against lamivudine-resistant mutant strain is of great interest. Unfortunately, all the potent NRTI with L-configuration against wild type HIV-1, are always cross-resistant to the lamivudine resistant mutant (M184V), which may be due to the steric hindrance between the bulky side chain of Val184 and the adjacent NRTIs' sugar ring.[232, 233] The situation is generally better in D-nucleosides, as their sugar rings project far away from the residue 184. However, the M184V mutant has also been isolated after using the abacavir, a prodrug of carbovir, which is a D-nucleoside.[94, 97, 234-237] In view of the structural similarity between the compound **35** and carbovir, it was of interest to understand its resistance profile. Hence, we further evaluated the compound **35** against HIV_{M184V} using carbovir (Table 3.4) as well as 3TC/AZT (data not shown) as control. Based on this study, the compound **35** appears to confer resistance to HIV-1_{M184V} (Table 3.4). Molecular modeling was thus performed to understand the potential mechanism of the cross-resistance (*vide infra*).

Table 3.4. Activity of D-3'-F-C-d4G against lamivudine-resistant virus (HIV-1_{M184V}) in human PBM Cells using carbovir as control

Compounds	xxBRU		M184V		FI[a]
	EC_{50} (μM)	EC_{90} (μM)	EC_{50} (μM)	EC_{90} (μM)	
D-3'-F-C-d4G **35**	0.098	0.58	3.8	14.9	38.8
Carbovir[b]	0.087	0.27	0.20	1.1	2.3

[a]FI is the fold increase (EC_{50} HIV-1_{M184V} / EC_{50} HIV-1_{xxBRU}).
[b]We also performed the experiment using AZT and 3TC as control, in which AZT is not but 3TC is highly cross-resistant to M184V mutant, and compound **35** is cross-resistant to M184V mutant (data not shown).

Molecular Modeling Studies

Among all the synthesized nucleosides, D-3'-F-C-d4G is the most active compound, while its L-form exhibited only marginal activity against HIV-1. From the studies of drug resistant mutant, it was found that the anti-HIV activity of D-3'-F-C-d4G to HIV-RT_{M184V} significantly decreased in comparison to the wild type virus. To understand the molecular basis of antiviral activity as well as the drug-resistance, molecular modeling studies were conducted on the interactions between the NRTI and HIV-RT. Our previous modeling studies have qualitatively demonstrated the relationship between the binding affinity and the antiviral activity.[116, 211, 220, 223] From present studies, the most active compound D-3'-F-C-d4G **35** has the most favorable relative binding energy (-24.4 kcal/mol, Table 3.5), which is significantly higher than that of less activity L-counterpart **28** (+18.8 kcal/mol, Table 3.5).

The minimized structure showed that D-3'-F-C-d4G was bound tightly in the well defined binding pocket inside the wild type HIV-RT (Figure 3.2a). The triphosphate moiety is stabilized

by the extensive hydrogen bonds with amino acids Arg65, Lys70, Lys72, Asp113 and Ala114. The carbocyclic ring stacks right over the phenyl ring of Tyr115 forming a favorable hydrophobic π-π interaction, which has been observed in our previous reports.[220, 223] Also, the 3'-fluorine is strongly interacting with the backbone amide of Tyr115 (Figure 3.2a). Stabilized by the combined effects of hydrophobic interaction and hydrogen bonding with Tyr115, D-3'-F-C-d4G is thus bound tightly with HIV-RT, reflecting a higher level of anti-HIV activity, although the initial kinase might have also played a significant role in determining the observed anti-HIV potency. In the case of L-3'-F-C-d4G, the 3'-fluorine is at a reasonable distance (2.0 Å) to interact in a hydrogen bond with backbone amide of Asp185 in lieu of Tyr115. However, we noticed that this interaction pulls the carbocyclic ring away from the Tyr115, which decreases the hydrophobic π-π interaction (Figure 3.2b), leads to a lower binding affinity (Table 3.5).

It has been well understood that the M184V mutation causes serious problem in positioning the L-nucleoside triphosphate at the active site by interfering the sugar ring with the bulky side chain of Val184. However, reports for the D-nucleosides, which confer significant cross-resistant to M184V, are rare. The antiviral activity of D-3'-F-C-d4G in the current report showed a marked decrease in the HIV-1_{M184V} in comparison to the HIV-1_{WT} (Table 3.4, 3.6). To understand the underlying mechanism, we further conducted the molecular dynamics studies of D-3'-F-C-d4G-TP, carbovir-TP and dGTP binding with the wild type HIV-RT as well as M184V mutant. According to our model, it is unlikely that the resistance of M184V mutant is caused by the steric hindrance as we observed for the L-nucleosides. However, a steric clash was noticed between the Val184 side chain and the sugar ring of the final residue of the primer. In the D-3'-F-C-d4G/HIV-RT_{M184V} complex, Val184 pushes the last residue of primer away and changes the conformation of adjacent Asp185. The conformational change of Asp185 propagates to

magnesium atoms, Asp110 and triphosphate moiety of D-3'-F-C-d4G, through the strong electrostatic interactions between two magnesium atoms and nearby negative charged residues such as Arg72 (Figure 3.3a). Although the D-3'-F-C-d4G still maintains the hydrogen bond between the 3'-fluorine and backbone amide of Tyr115, inhibitor's sugar ring was lifted away from the surface of the Ty115 aromatic ring, resulting in the loss of hydrophobic stacking interaction which may decrease the relative binding energy (Figure 3.3b right, Table 3.6). Furthermore, the primer/template reposition results in the significant increase of the catalytic distance (3.9 Å in the wild type vs. 5.1 Å in the M184V mutant) between the 3'-OH (last residue of the primer) and α-phosphate (D-3'-F-C-d4G-TP) (Figure 3.3b). Consequently, the incorporation of D-3'-F-C-d4G-MP into viral DNA chain in HIV-1_{M184V} would be expected to be more difficult than in the HIV_{WT}, reflecting its decreased antiviral activity against the mutant. In the case of carbovir-TP, the relative binding energy to the M184V mutation also decreased, but to a less extent than the D-3'-F-C-d4G-TP, which is in accordance with the biological data (Table 3.6). Binding mode analysis of carbovir-TP/HIV-RT_{M184V} complex revealed that the hydrophobic interaction was almost maintained and catalytic distance experienced only a small increase (3.2 Å in wild type vs. 3.8 Å in M184V mutant, Figure 3.3c). These changes may not significantly affect the incorporation of carbovir-MP into viral DNA.

In summary, molecular modeling studies illustrated the important roles of π-π interaction and the additional hydrogen bond in the binding affinity of D- and L-nucleosides in the HIV-RT activity site. The cross-resistance of compound **35** to HIV-RT_{M184V} may be partially due to the primer/template repositioning and resultant increased catalytic distance and the loss of hydrophobic interaction.

Table 3.5. *In vitro* anti-HIV activity of selected 3'-F-C-d4Ns and carbovir against HIV wild type virus and correlation with calculated energy of complex (Inhibitor-TP)/HIV-RT

Compound	EC_{50} (μM)[a]	Erel (Kcal/mol)[c]
D-3'-F-C-d4G **35**	0.41	–24.4
L-3'-F-C-d4G **28**	20.7	+18.8
Carbovir	0.087[b]	-10.8

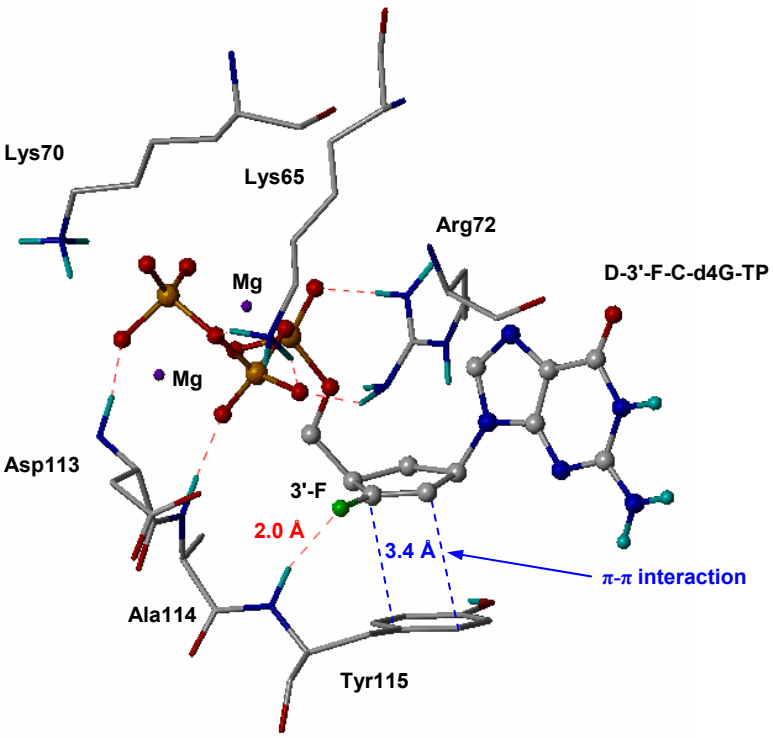
[a]EC_{50} in PBM cells against HIV-1_{LAI} unless otherwise indicated.
[b]EC_{50} in PBM cells against HIV-1_{xxBRU}.
[c]E_{rel} = (Binding energy of inhibitor-TP) – (Binding energy of natural 2'-dNTP).

Table 3.6. *In vitro* anti-HIV-1 activity of D-3'-F-C-d4G against wild type (WT) and M184V virus in human PBM cells using carbovir as positive control and correlation with calculated energy of complex (Inhibitor-TP) / HIV-RT after molecular dynamics simulations.

Compound	xxBRU (WT)		M184V		FI[b]	ΔE_{rel}[c]
	EC_{50} (μM)	E_{rel}[a] (Kcal/mol)	EC_{50} (μM)	E_{rel}[a] (Kcal/mol)		
D-3'-F-C-d4G **35**	0.098	-270.2[d]	3.8	84.9[d]	38.8	-355.1[d]
Carbovir	0.087	-310.0[d]	0.20	-63.4[d]	2.3	-246.6[d]

[a]E_{rel} = (Binding energy of inhibitor-TP) – (Binding energy of natural 2'-dNTP)
[b]FI is the Fold Increase (EC_{50} HIV-1_{M184V} / EC_{50} HIV-1_{xxBRU}).
[c]$\Delta E_{rel} = E_{rel}$ (WT) – E_{rel} (M184V).
[d]These values were calculated based on the molecular dynamics results (refer to experimental section)

(a)



(b)

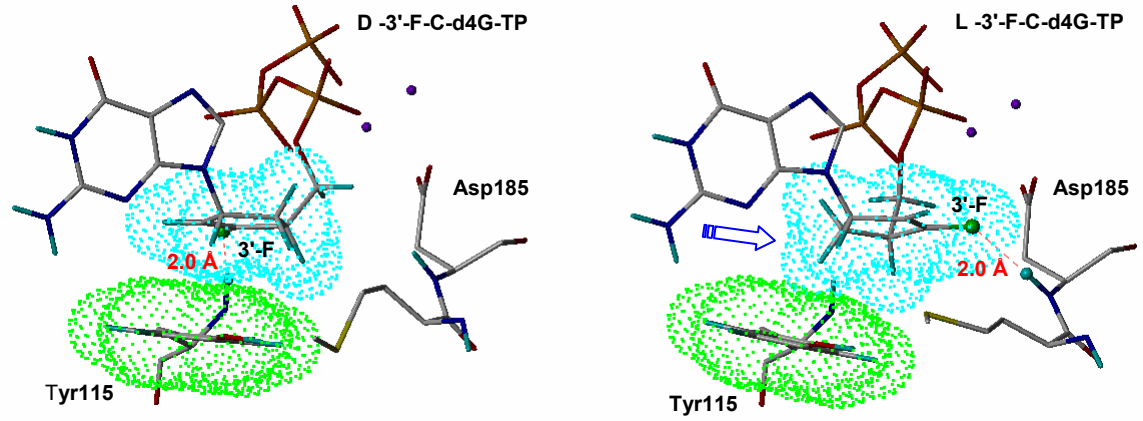
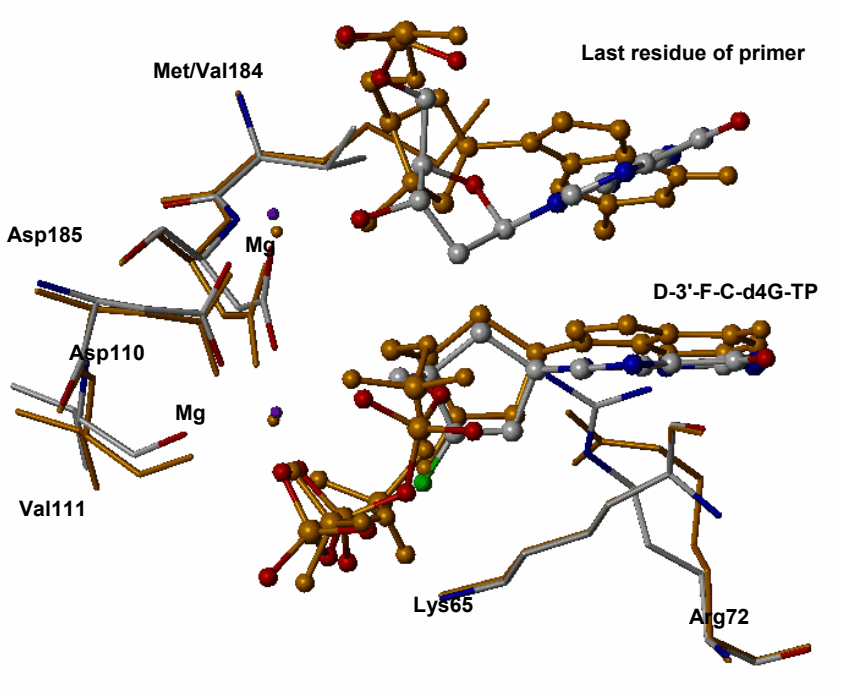
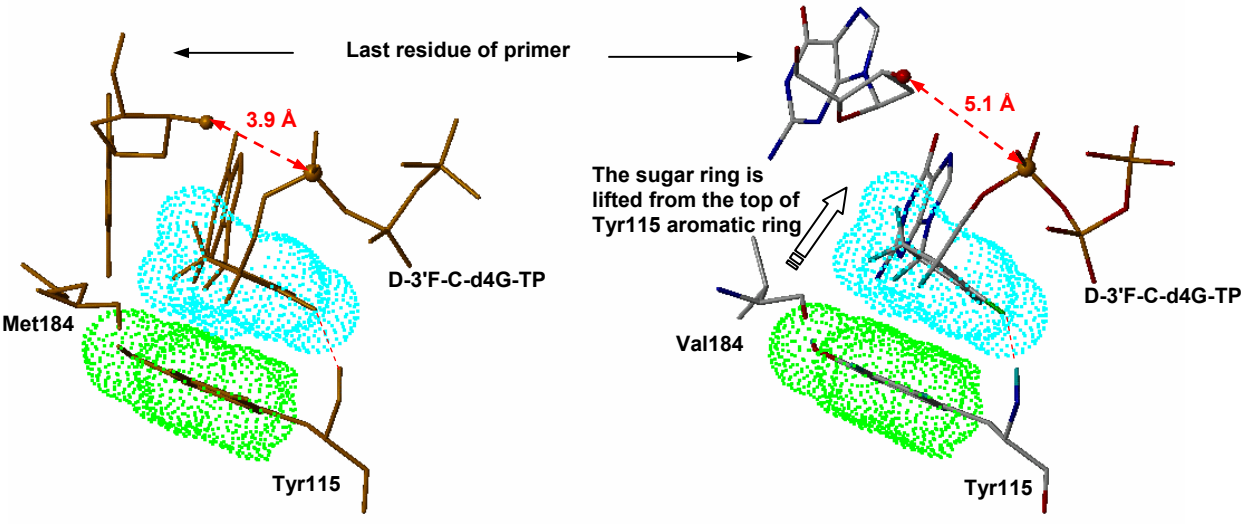


Figure 3.2. (a) Binding mode of D-3'-F-C-d4G-TP/HIV-RT_{WT} complex. The triphosphate moiety is stabilized by hydrogen bonding with residues Lys65, Arg72, Lys70, Asp113 and Ala114. The other strong hydrogen bond is detected between the 3'-fluoro and backbone amide of Tyr115. Also, the sugar ring is located right over the phenyl ring of Tyr115 forming a favorable hydrophobic interaction. (b) Comparing the binding mode of D- and L- 3'-F-C-d4G-TP, a decreased hydrophobic interaction is observed for the latter due to the hydrogen bond of its 3'-fluoro with backbone amide of Asp185 pulls the sugar ring away from the top of Tyr115 (indicated by the blue arrow).

(a)


Yellow: D-3'F-C-d4G-TP/HIV-RT$_{WT}$
Atom type: D-3'-F-C-d4G-TP/HIV-RT$_{M184V}$
Met/Val184
Last residue of primer
Asp185
Mg
D-3'-F-C-d4G-TP
Asp110
Mg
Val111
Lys65
Arg72

(b)


Last residue of primer
3.9 Å
D-3'F-C-d4G-TP
Met184
Tyr115
5.1 Å
The sugar ring is
lifted from the top of
Tyr115 aromatic ring
D-3'F-C-d4G-TP
Val184
Tyr115

(c)

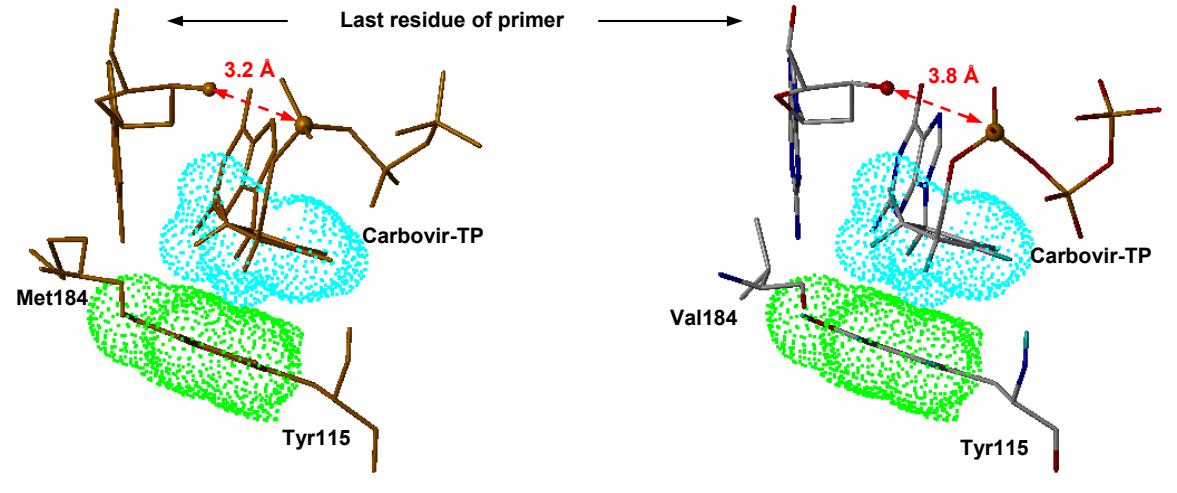


Figure 3.3. (a) Mutation of methionine to valine at the position 184 induces conformational changes of the key residues inside the active site, such as Asp185, Asp110, Val111 and Arg72. (b) The comparison of the bind modes of D-3'-F-d4G-TP with HIV-RT_{WT} (yellow color, left) and HIV-RT_{M184V} (atom type, right). The propagated effected from the mutation on the codon 184 lifted the D-3'-F-d4G-TP from the surface of Tyr115 causes a decrease of the hydrophobic interaction, resulting in a loss of binding affinity. Further more, the catalytic distance lengthened significantly (3.9 Å to 5.1 Å). (c) The comparison of the bind modes of carbovir-TP with HIV-RT_{WT} (yellow color, left) and HIV-RT_{M184V} (atom type, right). The binding mode is almost maintained in the mutant enzyme compared with the wild type enzyme.

EXPERIMENTAL SECTION

General Methods. Melting points were determined on a Mel-temp II apparatus and were uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian Mercury 400 spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR or Varian Inova 500 spectrometer at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR with tetramethylsilane as the

internal standard. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or bs (broad singlet). UV spectra were recorded on a Beckman DU-650 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. High resolution mass spectra were recorded on a Micromass Autospec high-resolution mass spectrometer. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Column chromatography was performed using either silica gel-60 (220-440 mesh) for flash chromatography or silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

Microwave-Assisted Synthesis. Reactions were run in the Discover™ reactor module (CEM Corporation) of focused microwaves with a magnetron operating at a frequency at 2.45 GHz and a maximum power output of 300 W. The thick-wall tube was heated in a closed cavity located inside the instrument with continuous stirring. The temperature was measured by an IR pyrometer inside the reactor.

(+)-(1*R*,2*S*,3*S*,4*R*)-2,3-Anhydro-1-*O*-benzyloxy-4-(*O*-triphenylmethyloxymethyl)-cyclopentane (2) To a suspension of epoxide **1**[115] (16.0 g, 72.6 mmol) in anhydrous CH_2Cl_2, DMAP (4.4 g, 36.3 mmol), triethylamine (15.1 ml, 109 mmol) and trityl chloride (30.4 g, 109 mmol) were added at room temperature. The reaction mixture was stirred for 24 h at room temperature and concentrated *in vacuo*. EtOAc/H_2O was added to the residue and the organic layer was collected, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:40 to 1:20) to give **2** as a white solid (30.6 g, 91 %). mp 102-103 °C $[\alpha]^{24}_D$ +46.71º (*c* 1.47, $CHCl_3$) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.39-7.24 (m, 20H), 4.59 (d, *J* = 2.5 Hz, 2H), 4.17 (t, *J* = 7.5 Hz, 1H), 4.19-4.16 (m, 1H), 3.54

(s, 1H), 3.45 (s, 1H), 3.14-3.15 (m, 1H), 2.96-2.99 (m, 1H), 2.57 (d, J = 7.0 Hz, 1H), 1.66-1.55 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 143.9, 138.4, 128.7, 128.4, 127.9, 127.8, 127.7, 127.1, 86.8, 79.1, 71.7, 64.2, 57.9, 56.7, 39.2, 28.7. Anal. Calcd for ($C_{32}H_{30}O_3$): C, H.

(+)-(1*R*,2*S*,4*R*)-1-*O*-Benzyloxy-2-hydroxyl-4-(*O*-triphenylmethyloxymethyl)-cyclopentane (3) and (+)-(1*R*,3*S*,4*R*)-1-*O*-Benzyloxy-3-hydroxyl-4-(*O*-triphenylmethyloxymethyl)-cyclopentane (4) Epoxide **2** (26.0 g, 56.2 mmol) was dissolved in a 1.0 M THF solution of Super-Hydride® (180 mL, 180 mmol) at 0 °C. The suspension was allowed to warm up to room temperature and stirred for 30 min and EtOAc/H_2O was added to quench the reaction. The organic layer was collected and the aqueous layer was extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:8) to give **3** (1.5 g, 6 %) as a colorless oil and **4** (24.0 g, 92 %) as a colorless oil. Compound **3**: ^{1}H NMR data is identical to the literature; [14] Compound **4**: $[\alpha]^{25}_D$ +18.98° (*c* 0.93, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43–7.22 (m, 20H), 4.48 (s, 2H), 4.01-3.96 (m, 2H), 3.25-3.22 (m, 1H), 2.96 (t, J = 8.5 Hz, 1H), 2.82 (d, J = 6.0 Hz, 1H), 2.49-2.44 (m, 1H), 2.17-2.04 (m, 2H), 1.94-1.89 (m, 1H), 1.44-1.25 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.1, 138.4, 128.7, 128.5, 127.9, 127.7, 127.6, 127.1, 86.8, 79.1, 76.6, 70.7, 66.2, 46.9, 40.7, 34.0. Anal. Calcd. for ($C_{32}H_{32}O_3$) C, H.

(-)-(2*R*,4*R*)-4-*O*-Benzyloxy-2-(*O*-triphenylmethyloxymethyl)-cyclopentan-1-one (5) To a solution of alcohol **4** (23.5 g, 50.6 mmol) in anhydrous CH_2Cl_2 (300 mL), 4 Å molecular sieve (40.5 g), pyridinium dichromate (37.6 g, 101.2 mmol) and acetic acid (4.4 mL, 76.0 mmol) were added. After stirred at room temperature for 3 h, Celite was added and stirred for another 30 min. The resulting brown slurry mixture was filtered over a Celite pad. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel

(EtOAc:Hexanes = 1:50 to 1:20) to give ketone **5** (19.0 g, 81 %) as a white solid. mp: 106-108 °C; $[\alpha]^{25}_{D}$ –59.32° (*c* 0.62, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.20 (m, 20H), 4.52 (s, 2H), 4.35 (t, *J* = 5.0 Hz, 1H), 3.48-3.45 (m, 1H), 3.21-3.19 (m, 1H), 2.67-2.62 (m, 1H), 2.57-2.38 (m, 3H), 2.17-2.10 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 184.7, 143.9, 138.1, 128.7, 128.5, 127.8, 127.7, 127.6, 127.0, 86.6, 74.8, 70.6, 62.1, 46.6, 45.6, 33.0. Anal. Calcd. for ($C_{32}H_{30}O_3$) C, H.

(+)-(2*R*,4*R*)-4-*O*-Benzyloxy-1,1-difluoro-2-(*O*-triphenylmethyloxymethyl)-cyclopentane (6) Ketone **5** (19.0 g, 41.1 mmol) was dissolved in neat diethyl aminosulfur trifluoride (DAST, 86.0 mL, 656.2 mmol) at room temperature. After stirred at 40 °C for 36 h, the reaction mixture was diluted with 300 mL CH_2Cl_2 and then slowly added into saturated $NaHCO_3$ (600 mL) solution. The organic layer was collected and the aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:100) to give **6** (13.5 g, 68 %) as a pale yellow syrup. $[\alpha]^{27}_{D}$ +3.00° (*c* 0.90, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48-7.21 (m, 20H), 4.47 (s, 2H), 4.05 (s, 1H), 3.37-3.34 (m, 1H), 3.11-3.08 (m, 1H), 2.83-2.78 (m, 1H), 2.44-2.32 (m, 2H), 2.22-2.18 (m, 1H), 1.75-1.69 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.0, 138.1, 128.9, 130.7 (t, *J* = 252.5 Hz), 128.8, 128.5, 127.9, 127.8, 127.7, 127.6, 127.0, 86.8, 75.0, 70.8, 61.0 (d, *J* = 7.6 Hz), 44.7 (t, *J* = 21.5 Hz), 43.0 (t, *J* = 11.2 Hz), 34.3 (d, *J* = 6.1 Hz). Anal. Calcd. for ($C_{32}H_{30}F_2O_2$) C, H.

(-)-(2*R*,4*R*)-1,1-Difluoro-4-hydroxy-2-hydroxymethyl-cyclopentane (7) To a solution of **6** (12.7 g, 26.2 mmol) in anhydrous CH_2Cl_2 (300 mL), iodotrimethylsilane (11.2 mL, 78.6 mmol) was added at -20 °C. The reaction mixture was allowed to warm up to room temperature and stirred for 6 h. Additional portion of iodotrimethylsilane (5.6 mL, 39.3 mmol) was added and

stirred for another 8 h. The reaction was quenched with MeOH at -20 °C and carefully neutralized with solid $NaHCO_3$. The resulting brown mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:100 to 1:10) to give diol **7** (2.8 g, 70 %) as a pale brown oil. $[\alpha]^{25}_D$ –21.53° (*c* 0.90, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.46-4.43 (m, 1H), 3.83-3.76 (m, 2H), 2.82-2.70 (m, 1H), 2.48-2.38 (m, 1H), 2.28-2.19 (m, 1H), 2.02-1.90 (m, 2H), 1.77 (bs, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 131.8 (dd, *J* = 252.4 and 248.0 Hz) 68.6 (t, *J* = 5.2 Hz), 60.5 (d, *J* = 8.6 Hz), 46.2 (t, *J* = 21.5 Hz), 45.7 (t, *J* = 23.9 Hz), 36.3 (d, *J* = 5.8 Hz). Anal. Calcd for ($C_6H_{10}F_2O_2$) C, H.

(-)-(2*R*,4*R*)-2-(*O*-*tert*-Butyldiphenylsilyloxymethyl)-1,1-difluoro-4-hydroxy-cyclopentane (8)

To a solution of diol **7** (2.6 g, 17.1 mmol) and imidazole (1.9 g, 27.4 mmol) in anhydrous CH_2Cl_2 (100 mL) *tert*-butyldiphenylsilane chloride (4.8 mL, 18.8 mmol) was slowly added at 0 °C during 1 h. The resulting mixture was stirred at 0 °C for another 45 min and quenched with MeOH. The mixture was concentrated *in vacuo* and the residue was dissolved in EtOAc/H_2O. The organic layer was collected and the aqueous layer was extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:20 to 1:4) to give **8** (4.9 g, 74 %) as a colorless oil. $[\alpha]^{27}_D$ –14.62° (*c* 0.90, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67-7.37 (m, 10H), 4.44 (s, 1H), 3.76 (ddd, *J* = 450.0, 10.5 and 5.5Hz, 2H), 2.81-2.69 (m, 1H), 2.50-2.40 (m, 1H), 2.24-2.16 (m, 1H), 2.04-1.95 (m, 2H), 1.05 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.6, 133.4, 133.4, 131.1, 129.7, 127.7, 68.7 (d, *J* = 6.7 Hz), 61.5 (d, *J* = 8.1 Hz), 46.4 (t, *J* = 22.4 Hz), 45.8 (t, *J* = 23.9 Hz), 37.1 (d, *J* = 5.2 Hz), 26.8, 19.2. Anal. Calcd. for ($C_{22}H_{28}F_2O_2Si$) C, H.

(-)-(2*R*,4*R*)-2-(*O*-*tert*-Butyldiphenylsilyloxymethyl)-1,1-difluoro-4-[(methylsulfonyl)oxy]cyclopentane (9)

Methanesulfonyl chloride (0.4 mL, 5.12 mmol) in 10 mL anhydrous CH_2Cl_2 (10 mL) was slowly added to a solution of alcohol **8** (1.00 g, 2.56 mmol) and triethylamine (1.4 mL, 10.2 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and kept for 4 h. After removing the solvent *in vacuo*, the residue was dissolved in EtOAc and washed with water. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:20 to 1:10) to give **9** (1.17 g, 98 %) as a colorless oil. $[\alpha]^{25}_D$ –19.66° (*c* 0.32, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67-7.38 (m, 10H), 5.26 (m, 1H), 3.76 (d, *J* = 5.5Hz, 2H), 3.03 (s, 3H), 2.74-2.50 (m, 3H), 2.36-2.32 (m, 1H), 2.18-2.12 (m, 1H), 1.05 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.6, 133.0 (d, *J* = 2.0 Hz), 129.9, 129.8 (t, *J* = 250.9 Hz), 127.8, 77.8 (dd, *J* = 3.75 and 7.6 Hz), 61.0 (dd, *J* = 2.9 and 4.8 Hz), 46.4 (dd, *J* = 21.5 and 23.9 Hz), 43.7 (t, *J* = 26.8 Hz), 34.8, 34.7 (t, *J* = 4.8 Hz), 26.8, 19.2. HR-MS Calcd. for $(C_{23}H_{30}NF_2O_4SSi+H)^+$ 469.1680, found 469.1662.

(+)-(2*R*,4*S*)-4-Azido-2-(*O*-*tert*-butyldiphenylsilyloxymethyl)-1,1-difluoro-cyclopentane (10)

Compound **9** (1.17 g, 2.5 mmol) was dissolved in anhydrous DMF (45 mL) and heated at 130 °C for 1.5 h. After removing the solvent *in vacuo*, the residue was dissolved in EtOAc and washed with water. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:200 to 1:100) to give **10** (0.97 g, 93 %) as a colorless oil. $[\alpha]^{26}_D$ +4.2° (*c* 0.51, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67-7.38 (m, 10H), 3.96 (m, 1H), 3.78 (ddd, *J* = 38.0, 11.0 and 5.5Hz, 2H), 2.56-2.34 (m, 1H), 2.21-2.10 (m, 1H), 1.82-1.75 (m, 1H), , 2.04-1.95 (m, 2H), 1.06 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ

135.6, 133.2 (d, *J* = 3.4 Hz), 129.8, 128.9 (dd, *J* = 248.5 and 254.2 Hz), 127.8, 61.2 (dd, *J* = 7.1 and 2.9 Hz), 56.4 (dd, *J* = 6.1 and 3.8 Hz), 47.2 (dd, *J* = 23.4 and 21.0 Hz), 41.9 (t, *J* = 24.8 Hz), 32.9 (t, *J* = 1.5 Hz), 26.8, 19.2. HR-MS Calcd. for $(C_{22}H_{27}F_2N_3OSi+H)^+$ 416.1970, found 416.2022.

(-)-(2*R*,4*S*)-2-(*O*-*tert*-Butyldiphenylsilyloxymethyl)-1,1-difluoro-cyclopentanamine (11)

A suspension of azido compound **10** (0.97 g, 2.33 mmol) and 10 % Pd/C (330 mg) in absolute EtOH was shaken under 30 psi of H_2 at room temperature for 2.0 h. Celite was added into the solution and the slurry was filtered through a Celite pad. The volatile was removed *in vacuo* and the residue was purified by column chromatography on a silica gel ($MeOH:CH_2Cl_2$ = 1:50 to 1:10) to give **11** (0.86 g, 95 %) as a colorless oil. $[\alpha]^{26}_D$ –9.98° (*c* 0.79, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68-7.36 (m, 10H), 3.78 (ddd, *J* = 55.0, 10.0 and 5.0 Hz, 2H), 3.46-3.39 (m, 1H), 2.51-2.28 (m, 3H), 1.93-1.82 (m, 1H), 1.45-1.39 (m, 3H), 1.05 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.6 (d, , *J* = 2.4 Hz), 133.4 (d, , *J* = 1.2 Hz), 130.0 (dd, , *J* = 252.8 and 3.2 Hz), 129.7, 127.8, 61.6 (dd, *J* = 7.6 and 1.9 Hz), 48.0 (dd, *J* = 23.4 and 20.2 Hz), 47.9 (dd, *J* = 6.1 and 4.2 Hz), 45.8 (t, *J* = 22.4 Hz), 37.3 (d, *J* = 4.8 Hz) 26.8, 19.3. HR-MS Calcd. for $(C_{22}H_{29}F_2NOSi+H)^+$ 390.2065, found 390.2030. Anal. Calcd. for ($C_{22}H_{29}F_2NOSi$) C, H, N.

(+)-(1'*S*,4'*R*)-9-[6'-(*O*-*tert*-Butyldiphenylsilyloxymethyl)-2',3'-dideoxy-3',3'-difluoro-cyclopentanyl]uracil (13) To a suspension of silver cyanate (810 mg, 5.4 mmol) in anhydrous benzene (20 mL), β–methoxyacryloyl chloride (650 mg, 5.4 mmol) was added. The mixture was heated under reflux for 30 min and cooled to room temperature. The supernatant solution was added into the solution of amine **11** (700 mg, 1.8 mmol) in anhydrous THF (30 mL) at –30 °C during 15 min. The mixture was allowed to gradually warmed up to room temperature and kept overnight. After removing the solvent *in vacuo*, the residue was purified by column

chromatography on a silica gel (EtOAc:hexanes = 1:3 to 1:1) to give crude **12** (600 mg) as a yellow syrup which was directly used for the next step. Crude compound **12** (600 mg) was dissolved in 1,4-dioxane/ethanol (20 mL/20 mL) and treated with 28 % solution of ammonium hydroxide (20 mL) in a steel bomb at 90 -100 °C for 17 h. After removing the solvent *in vacuo*, the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:200 to 1:100) to give **13** (360 mg, 41 %) as a pale yellow syrup. $[\alpha]^{26}_D$ +13.12° (*c* 0.31, $CHCl_3$); UV (H_2O) λ_{max} 266.0 (MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.22 (s, 1H), 7.67-7.38 (m, 10H), 7.22 (d, *J* = 8.5 Hz, 1H), 5.68 (d, *J* = 8.0 Hz, 1H), 5.26-5.19 (m, 1H), 3.88 (dtd, *J* = 11.0, 10.0 and 5.0 Hz, 2H), 2.70-2.60 (m, 1H), 2.2.52-2.44 (m, 2H), 2.27-2.16 (m, 1H), 1.79-1.70 (m, 1H), 1.07 (s, 9H); ^{13}C NMR (125 MHz, CD_3OD) δ 162.8, 150.9, 140.0 (d, *J* = 2.0 Hz), 135.6, 133.1, 133.0, 129.9, 128.3 (t, *J* = 250.9 Hz), 127.9, 103.6, 61.0 (d, *J* = 5.2 Hz), 49.6, 47.0 (t, *J* = 21.9 Hz), 40.7 (t, *J* = 24.9 Hz), 32.3 (d, *J* = 4.2 Hz), 26.8, 19.3. HR-MS Calcd. for $(C_{26}H_{30}F_2N_2O_3Si+H)^+$ 485.2072, found 485.2169 Anal. Calcd. for ($C_{26}H_{30}F_2N_2O_3Si$) C, H, N.

(+)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-3',3'-difluoro-6'-(*O*-*tert*-butyldiphenylsilyloxymethyl)-cyclopentanyl]cytosine (14) To a solution of uracil derivative **13** (360 mg, 0.74 mmol) in anhydrous acetonitrile (25 mL), 2,4,6-triisopropyl benzenesulfonyl chloride (450 mg, 1.48 mmol), 4-(dimethylamino)pyridine (90.4 mg, 0.74 mmol) and triethylamine (0.42 mL, 3.0 mmol) were added at 0 °C. After stirred at room temperature for 12 h, 28 % solution of ammonium hydroxide (15 mL) was added to the brown mixture and stirred at room temperature for another 12 h. The reaction mixture was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:30) to give **14** (300 mg, 84 %) as a white solid. mp 250-252 °C $[\alpha]^{24}_D$ +15.86° (*c* 0.31, MeOH); UV (MeOH) λ_{max} 272.0 nm; ^{1}H NMR (500 MHz, CD_3OD) δ 7.59-7.30 (m, 10H), 7.44 (d, *J* = 7.0 Hz, 1H), 5.75 (d, *J* = 7.5 Hz, 1H),

5.00-4.92 (m, 1H), 3.88 (ddd, *J* = 56.0, 10.5 and 6.0 Hz, 2H), 2.59-2.45 (m, 2H), 2.30-2.19 (m, 2H), 1.78-1.71 (m, 1H), 0.96 (s, 9H); ^{13}C NMR (125 MHz, CD_3OD) δ 165.8, 157.4, 142.0, 135.4, 133.1, 133.0, 129.7, 128.8 (t, *J* = 250.9 Hz), 127.5, 95.2, 61.1 (d, *J* = 8.1 Hz), 52.0 (d, *J* = 5.8 Hz), 40.2 (t, *J* = 24.8 Hz), 32.0 (d, *J* = 4.2 Hz), 25.9, 18.6. HR-MS Calcd. for $(C_{26}H_{31}F_2N_3O_2Si+H)^+$ 484.2232, found 484.2212 Anal. Calcd. for ($C_{26}H_{31}F_2N_3O_2Si$) C, H, N.

(+)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-3',3'-difluoro-6'-hydroxymethylcyclopentanyl]cytosine (15) To a solution of **14** (300 mg, 0.62 mmol) in MeOH (2 mL), 3 N HCl (2 mL) was added. After stirred at room temperature for 17 h, the resulting mixture was co-evaporated with EtOH and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:15) to give **15** (110 mg, 72%) as a white solid. mp 134-136 °C; $[\alpha]^{23}_D$ +4.07° (*c* 0.22, MeOH); UV (H_2O) λ_{max} 275.0 nm (MeOH), ^{1}H NMR (400 MHz, CD_3OD) δ 7.72 (d, *J* = 8.0 Hz, 1H), 5.95 (d, *J* = 7.0 Hz, 1H), 5.14-5.07 (m, 1H), 3.88 (ddd, *J* = 47.0, 11.5 and 5.5 Hz, 2H), 2.70-2.35 (m, 4H), 1.97-1.90 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.8, 157.4, 142.0, 128.8 (t, *J* = 249.2 Hz), 95.2, 59.0 (d, *J* = 7.6 Hz), 52.0 (t, *J* = 7.6 Hz), 40.1 (t, *J* = 25.1 Hz), 32.0 (d, *J* = 3.8 Hz). HR-MS Calcd. for $(C_{10}H_{13}F_2N_3O_2+H)^+$ 246.1054, found 246.0975 Anal. Calcd. for ($C_{10}H_{13}F_2N_3O_2 \cdot 0.65H_2O$) C, H, N.

(+)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',3'-didehydro-3',3'-fluoro-6'-hydroxymethylcyclopent-2-enyl]cytosine (18) To a suspension of **15** (110 mg, 0.45 mmol) in anhydrous THF:1,4-dioxane (10 mL:10 mL) co-solvent, potassium *tert*-butoxide (121 mg, 1.0 mmol) was added. The reaction mixture was stirred at 90 °C for 9 h. The yellow suspension was filtered through a short silica gel pad and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:30 to 1:15) to give **18** (35 mg, 35 %) as a white solid. mp 244-248 °C; $[\alpha]^{25}_D$ +127.49° (*c* 0.36, MeOH); UV (H_2O) λ_{max} 284.0 nm (ε

17115, pH 2), 274.0 nm (ε 11975, pH 7), 274.0 nm (ε 11680, pH 11); ^{1}H NMR (400 MHz, CD_3OD) δ 7.83 (d, *J* = 7.5 Hz, 1H), 5.90 (d, *J* = 7.5 Hz, 1H), 5.62-5.61 (m, 1H), 5.19 (s, 1H), 3.70 (ddd, *J* = 132.5, 11.5 and 3.5 Hz, 2H), 2.95-2.93 (m, 1H), 2.86-2.79 (m, 1H), 1.72-1.69 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 166.1, 166.0 (d, *J* = 282.8 Hz), 157.6, 142.5, 103.3 (d, *J* = 13.0 Hz), 94.3, 59.8, 55.9 (d, *J* = 12.2 Hz), 42.9 (d, *J* = 19.0 Hz), 31.7 (d, *J* = 6.1 Hz). Anal. Calcd. for ($C_{10}H_{12}F_1N_3O_2$) C, H, N.

(+)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-3',3'-difluoro-6'-hydroxymethylcyclopentanyl]thymine (17)

To a suspension of silver cyanate (400 mg, 2.7 mmol) in anhydrous benzene (8 mL), β-methoxy-α-methacryloyl chloride (360 mg, 2.7 mmol) was added. The mixture was heated under reflux for 30 min and cooled to room temperature. The supernatant solution was added into the solution of amine **11** (350 mg, 0.89 mmol) in anhydrous THF (8 mL) at –30 °C during 15 min. The mixture was allowed to gradually warmed up to room temperature and kept overnight. After removing the solvent *in vacuo*, the residue was purified by column chromatography on a silica gel (EtOAc:hexanes = 1:3 to 1:1) to give crude **16** (360 mg) as a yellow syrup which was directly used for the next step. Crude compound **16** (360 mg) was dissolved in 1,4-dioxane (60 mL) and treated with 3 N HCl (15 mL) at the refluxed temperature for 2.5 h. After removing the solvent *in vacuo*, the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:50 to 1:20) to give **17** (145 mg, 63 %) as a white solid. mp 140-142 °C; $[\alpha]^{23}_{D}$ +3.9° (*c* 0.15, MeOH); UV (H_2O) λ_{max} 270.0 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 7.52 (s, 1H), 5.07-4.98 (m, 1H), 3.76 (ddd, *J* = 33.2, 10.8 and 5.2 Hz, 2H), 2.63-2.32 (m, 4H), 1.98-1.89 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ 164.8, 151.4, 137.6, 128.7 (dd, *J* = 250.8 and 248.5 Hz), 110.6, 58.9 (dd, *J* = 7.6 and 2.3 Hz), 50.8 (t, *J* = 6.8 Hz), 39.5 (t, *J* = 25.1 Hz), 31.3 (d, *J* = 3.8 Hz), 11.0. Anal. Calcd. for ($C_{11}H_{14}F_2N_2O_3$) C, H, N.

(+)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',3'-didehydro-3'-fluoro-6'-hydroxymethylcyclopent-2-enyl]thymine (19)

Compound **17** (140 mg, 0.54 mmol) was converted to thymine derivative **19** (60 mg, 46 %) as a white solid using the same procedure as for **18**. mp 182-184 °C (dec.); $[\alpha]^{26}_{D}$ +23.99° (*c* 0.26, MeOH); UV (H_2O) λ_{max} 272.0 nm (ε 14258, pH 2), 272.0 nm (ε 14240, pH 7), 271.0 nm (ε 11651, pH 11); ^{1}H NMR (400 MHz, CD_3OD) δ 7.69 (s, 1H), 5.58-5.53 (m, 1H), 5.15 (s, 1H), 3.69 (ddd, *J* = 121.6, 11.6 and 3.2 Hz, 2H), 2.91-2.90 (m, 1H), 2.78-2.70 (m, 1H), 1.76-1.70 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.2, 166.0 (d, *J* = 282.7), 151.6, 138.2, 109.7, 103.4 (d, *J* = 13.7 Hz), 59.6 (d, *J* = 1.5 Hz), 54.7 (d, *J* = 12.2 Hz), 42.7 (d, *J* = 18.3 Hz), 30.9 (d, *J* = 6.1 Hz), 11.0. Anal. Calcd. for ($C_{11}H_{13}FN_2O_3$•0.1H_2O) C, H, N.

(-)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-3',3'-difluoro-6'-(*O-tert*-butyldiphenylsilyloxymethyl)-cyclopentanyl]adenine (21) Triphenylphosphine (2.12 g, 8.1 mmol) and diisopropyl azodicarboxylate (1.59 mL, 8.1 mmol)) were dissolved in anhydrous THF:1,4-dioxane (14 mL:7 mL) co-solvent and cooled to 0 °C. The resulting yellowish suspension was further cooled to –78 °C. 6-Chloropurine (1.25 g, 8.1 mmol) and a solution of alcohol **8** (630 mg, 1.61 mmol) in THF (14 mL) were added successively. The resulting mixture was kept at –78 °C for 0.5 h and then stirred at room temperature for 24 h. MeOH was added to quench the reaction and the mixture was evaporated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10) to give **20** as a crude product, which was treated with methanolic ammonia in a steel bomb at 100 °C for 24 h. After evaporation *in vacuo*, the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:50 to 1:20) to give **21** as a colorless syrup (305 mg, 37 % from **8**). $[\alpha]^{25}_{D}$ –10.89° (*c* 0.45, $CHCl_3$); UV (MeOH) λ_{max} 260.0; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.84 (s, 1H), 7.68-7.38 (m, 10H), 5.07 (m, 1H), 3.92

(ddd, *J* = 31.0, 10.5 and 5.0 Hz, 2H), 2.91-2.81 (m, 1H), 2.73-2.56 (m, 3H), 2.28-2.22 (m, 1H), 2.06 (bs, 1H), 1.08 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.6, 153.0, 150.2, 138.2, 135.6, 135.6, 133.2, 133.1, 129.9, 128.4 (dd, *J* = 252.4 and 250.0 Hz), 127.8, 120.0, 61.1 (d, *J* = 5.8 Hz), 49.8 (t, *J* = 4.8 Hz), 47.5 (t, *J* = 21.5 Hz), 33.7 (d, *J* = 17.0 Hz), 26.8, 19.3. Anal. Calcd. for ($C_{27}H_{31}F_2N_5OSi$) C, H, N.

(-)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-3',3'-difluoro-6'-hydroxymethylcyclopentanyl]adenine (22) Compound **21** (300 mg, 0.59 mmol) was dissolved in MeOH (5 mL) and treated with 3N HCl (10 mL) at room temperature for 16 h. After neutralizing with solid $NaHCO_3$, the resulting suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:50 to 1:20) to give **22** (140 mg, 88 %) as a white solid. mp 156-158 °C; $[\alpha]^{25}_D$ –9.86° (*c* 0.56, MeOH); UV (H_2O) λ_{max} 260.0 (MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.28 (s, 1H), 8.24 (s, 1H), 5.13 (m, 1H), 3.86 (ddd, *J* = 50.0, 11.5 and 6.0 Hz, 2H), 2.89-2.77 (m, 2H), 2.71-2.57 (m, 2H), 2.36-2.30 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 156.0, 152.3, 149.4, 139.5, 128.9 (dd, *J* = 251.8 and 248.0 Hz), 119.0, 59.1 (dd, *J* = 8.1 and 1.9 Hz), 50.4 (dd, *J* = 7.6 and 4.4 Hz), 41.1 (t, *J* = 25.8 Hz), 33.0 (d, *J* = 3.9 Hz). Anal. Calcd. for ($C_{11}H_{13}F_2N_5O$) C, H, N.

(-)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',3'-didehydro-3'-fluoro-6'-hydroxymethylcyclopent-2-enyl]adenine (26) Compound **22** (60 mg, 0.22 mmol) was converted to adenine derivative **26** (27 mg, 49 %) as a white solid using the same procedure as for **18**. mp 225 °C (dec.); $[\alpha]^{29}_D$ – 43.80° (*c* 0.18, DMSO); UV (MeOH) λ_{max} 261.0 nm (ε 17931, pH 2), 261.0 nm (ε 17780, pH 7.4), 261.0 nm (ε 18556, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 8.31 (s, 1H), 8.24 (s, 1H), 5.69-5.64 (m, 1H), 5.44 (s, 1H), 3.75 (ddd, *J* = 126.5, 11.0 and 4.0 Hz, 2H), 3.09-3.04 (m, 1H), 3.02-3.00 (m, 1H), 2.05-2.00 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 166.3 (d, *J* = 283.2 Hz),

156.0, 152.2, 148.8, 139.8, 118.9, 103.2 (d, J = 13.8 Hz), 60.1, 54.0 (d, J = 12.4 Hz), 43.2 (d, J = 18.6 Hz), 32.2 (d, J = 6.2 Hz). Anal. Calcd. for ($C_{11}H_{12}FN_5O$) C, H, N.

(-)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',2'-difluoro-6-hydroxymethylcyclopentanyl] hypoxanthine (23) A crude 6-chloropurine analogue **20** (850 mg) was treated with 85 % formic acid (40 mL) at 90 °C for 3 h. After completely removing the volatile *in vacuo*, the residue was dissolved in concentrated ammonium hydroxide (35%) and stirred at room temperature overnight. The solution was evaporated *in vacuo* and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:30 to 1:15) to give **23** (93 mg, 39 % from **8**) as a white solid. mp 244-246 °C; $[\alpha]^{24}_D$ –10.08° (*c* 0.23, MeOH); UV (MeOH) λ_{max} 248.0 nm; ^{1}H NMR (500 MHz, CD_3OD) δ 8.22 (s, 1H), 8.09 (s, 1H), 5.18-5.10 (m, 1H), 3.84 (ddd, J = 53.5, 11.5 and 6.0 Hz, 2H), 2.93-2.76 (m, 2H), 2.70-2.58 (m, 2H), 2.36-2.29 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.6, 148.8, 145.0, 139.0, 128.8 (dd, J = 251.9 and 248.0 Hz), 124.3, 59.1 (d, J = 6.2 Hz), 50.7 (dd, J = 7.1 and 4.2 Hz), 41.3 (t, J = 25.8 Hz), 33.1 (d, J = 4.2 Hz). Anal. Calcd. for ($C_{11}H_{12}F_2N_4O_2$) C, H, N.

(-)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',3'-didehydro-3'-fluoro-6'-hydroxymethylcyclopent-2-enyl]hypoxanthine (27) Compound **23** (22 mg, 0.081 mmol) was converted to inosine derivative **27** (10 mg, 49 %) as a white solid using the same procedure as for **18**. mp 226-230 °C; $[\alpha]^{27}_D$ –33.05° (*c* 0.20 MeOH); UV (H_2O) λ_{max} 249.0 nm (ε 12220, pH 2), 249.0 nm (ε 12820, pH 7), 255.0 nm (ε 13552, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 8.26 (s, 1H), 8.08 (s, 1H), 5.66-5.72 (m, 1H), 5.43 (s, 1H), 3.75 (ddd, J = 124.5, 11.5 and 4.0 Hz, 2H), 3.03-3.09 (m, 1H), 2.97 (dt, J = 14.0 and 9.5 Hz, 1H), 2.05-2.00 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 166.4 (d, J = 283.9 Hz), 157.6, 148.4, 145.1, 139.2, 123.9, 103.1 (d, J = 14.8 Hz), 60.0, 54.2 (d, J = 12.9 Hz), 43.3 (d, J = 18.6 Hz), 32.4 (d, J = 5.8 Hz). Anal. Calcd. for ($C_{11}H_{11}FN_4O_2 \cdot 0.1H_2O$) C, H, N.

(-)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-3',3'-difluoro-6'-hydroxymethylcyclopentanyl]guanine (25) A solution of alcohol **8** (600 mg, 1.54 mmol), tripheylphosphine (1.61 g, 6.1 mmol) and 6-chloro-N^2-isobutyrylpurine (1.47 g, 6.1 mmol) in anhydrous THF (20 mL) was cooled to 0 °C and then diisopropyl azodicarboxylate (1.2 mL, 6.1 mmol) was slowly added during 2 h. The reaction mixture was allowed to warm up to room temperature and stirred for 4 h. The clear yellowish solution was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10 to 1:1) to give the corresponding nucleoside **24** as a crude product, which was treated with 85% formic acid (30 mL) at 90 °C for 4 h. After completely removing the volatile, the residue was further treated with methanolic ammonia at room temperature for 24 h. After the concentration *in vacuo*, the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:20 to 1:10) to give **25** (80 mg, 18 % from **8**) as a white solid. mp: 253 °C (dec.); $[\alpha]^{27}_D$ –9.30° (*c* 0.25, MeOH); UV (MeOH) λ_{max} 253.0 nm; ^{1}H NMR (500 MHz, CD_3OD) δ 7.86 (s, 1H), 3.83 (ddd, *J* = 50.5, 11.5 and 5.5 Hz, 2H), 2.81-2.72 (m, 2H), 2.61-2.51 (m, 2H), 2.30-2.22 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 158.0, 153.7, 151.8, 136.4, 128.9 (dd, *J* = 252.2 and 248.0 Hz), 116.5, 59.1 (dd, *J* = 8.1 and 2.4 Hz), 50.0 (dd, *J* = 7.1 and 3.4 Hz), 41.1 (t, *J* = 25.8 Hz), 32.8 (d, *J* = 3.4 Hz). HR-MS Calcd. for $(C_{11}H_{13}N_5F_2O_2+H)^+$ 286.1116, found 286.1137.

(+)-(1'*S*,4'*R*)-9-[2',3'-Dideoxy-2',3'-didehydro-3'-fluoro-6'-hydroxymethylcyclopent-2-enyl]guanine (28) To a suspension of **25** (70 mg, 0.25 mmol) in anhydrous DMF (8 mL), potassium *tert*-butoxide (120 mg, 1.0 mmol) was added. The reaction mixture was stirred at 70 °C for 24 h. The yellow suspension was filtered through a short silica gel pad and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:20 to 1:10) to give **28** (30 mg, 45 %) as a white solid. mp 224-227 °C (dec.);

$[\alpha]^{27}_D$ +40.13° (*c* 0.11, MeOH); UV (H_2O) λ_{max} 253.0 nm (ε 13343, pH 2), 251.0 nm (ε 13635, pH 7), 260.0 nm and 268.0 nm (ε 11470 and 11893, respectively, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 7.92 (s, 1H), 5.52-5.46 (m, 1H), 5.37 (s, 1H), 3.74 (ddd, *J* = 114.0, 11.0 and 4.0 Hz, 2H), 3.05-3.00 (m, 1H), 2.90 (dt, *J* = 14.0 and 9.0 Hz, 1H), 2.01-1.96 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 165.9 (d, *J* = 282.9 Hz), 158.0, 153.8, 151.2, 136.6, 116.2, 103.1 (d, *J* = 13.9 Hz), 60.1, 53.2 (d, *J* = 12.9 Hz), 43.2 (d, *J* = 19.1 Hz), 32.2 (d, *J* = 5.6 Hz). Anal. Calcd. for ($C_{11}H_{12}FN_5O_2 \cdot 1.1H_2O$) C, H, N.

(+)-(2*S*,4*S*)-2-(*O*-*tert*-Butyldiphenylsilyloxymethyl)-1,1-difluoro-4-hydroxy-cyclopentane (29)

$[\alpha]^{24}_D$ +14.97° (*c* 0.83, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD) δ 7.67-7.66 (m, 4H), 74.5-7.37 (m, 11H), 4.44 (bs, 1H), 3.80 (dd, *J* = 5.0 and 10.5 Hz, 1H), 3.72 (dd, *J* = 6.0 and 10.5 Hz, 1H), 2.75 (m, 1H), 2.45 (m, 1H), 2.20 (q, *J* = 14.0 Hz, 1H), 2.02-1.98 (m, 2H), 1.05 (s, 9H); ^{13}C NMR (125 MHz, CD_3OD) δ 135.6, 133.3 (d, *J* = 3.9 Hz), 133.2, 131.2, 129.8 (d, *J* = 1.4 Hz), 129.1, 127.8 (d, *J* = 1.4 Hz), 68.8, 61.5, 46.4 (t, *J* = 21.4 Hz), 46.0 (t, *J* = 28.1 Hz), 37.1 (d, *J* = 5.3 Hz), 26.8, 19.3. Anal. Calcd. for ($C_{22}H_{28}F_2O_2Si$) C, H, N.

(+)-(2*S*,4*R*)-2-(*O*-*tert*-Butyldiphenylsilyloxymethyl)-1,1-difluoro-cyclopentanamine (30)

$[\alpha]^{26}_D$ +10.02° (*c* 0.68, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68-7.37 (m, 10H), 3.86-3.70 (m, 2H), 3.49-3.39 (m, 1H), 2.52-2.28 (m, 3H), 1.94-1.79 (m, 1H), 1.45-1.39 (m, 3H), 1.05 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.6 (d, , *J* = 2.4 Hz), 133.4, 130.0 (dd, , *J* = 253.1 and 249.2 Hz), 129.7, 127.7, 61.6 (dd, *J* = 7.7 and 2.3 Hz), 48.1 (m), 48.0 (dd, *J* = 22.9 and 21.3 Hz), 46.1 (t, *J* = 22.9 Hz), 37.3 (d, *J* = 4.6 Hz) 26.8, 19.2. Anal. Calcd. for ($C_{22}H_{29}F_2NOSi \cdot 0.2H_2O$) C, H, N.

(+)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-3',3'-difluoro-6'-hydroxymethylcyclopentanyl]adenine (31)

mp 158-160 °C; $[\alpha]^{25}_D$ +9.50° (*c* 0.33, MeOH); UV (H_2O) λ_{max} 260.0 (MeOH); ^{1}H NMR (500

MHz, CD_3OD) δ 8.24 (s, 1H), 5.12 (m, 1H), 3.92 (ddd, *J* = 50.0, 11.5 and 5.5 Hz, 2H), 2.85 (m, 2H), 2.65 (m, 2H), 2.32 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 155.9, 152.3, 149.3, 139.5, 128.7 (dd, *J* = 251.6 and 248.0 Hz), 119.0, 59.2 (t, *J* = 5.8 Hz), 50.3, 41.1 (t, *J* = 25.8 Hz), 32.9. Anal. Calcd. for ($C_{11}H_{13}F_2N_5O$) C, H, N.

(+)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-3'-difluoro-6'-hydroxymethylcyclopentanyl]guanine (32) mp 258 °C (dec); $[\alpha]^{28}_D$ +9.29° (*c* 0.15, MeOH); UV (H_2O) λ_{max} 254.0 nm (ε 10631, pH 2), 252.0 nm (ε 11008, pH 7), 256.0 nm (ε 9234, pH 11); mp >250 °C; ^1H NMR (500 MHz, CD_3OD) δ 7.83 (s, 1H), 3.79 (ddd, *J* = 51.0, 14.5 and 6.5 Hz, 2H), 2.77-2.70 (m, 2H), 2.57-2.54 (m, 2H), 2.32-2.21 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 158.0, 153.7, 151.8, 136.4, 128.9 (dd, *J* = 251.5 and 246.9 Hz), 116.5, 59.1 (d, *J* = 8.4 Hz), 49.9 (d, *J* = 3.8 Hz), 41.0 (t, *J* = 25.9 Hz), 32.8 (d, *J* = 3.8 Hz). Anal. Calcd. for ($C_{11}H_{13}F_2N_5O_2 \bullet 0.3H_2O$) C, H, N.

(+)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-3',3'-difluoro-6-hydroxymethylcyclopentanyl]hypoxanthine (33) mp 227 °C; $[\alpha]^{23}_D$ +9.97° (*c* 0.45, MeOH); UV (MeOH) λ_{max} 247.0 nm; ^1H NMR (500 MHz, CD_3OD) δ 8.25 (s, 1H), 8.09 (s, 1H), 5.14 (m, 1H), 3.84 (ddd, *J* = 55.0, 11.5 and 6.0 Hz, 2H), 2.82 (m, 2H), 2.65 (m, 2H), 2.32 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.6, 148.8, 145.0, 139.0, 128.7 (dd, *J* = 251.5 and 247.5 Hz), 124.3, 59.0 (dd, *J* = 8.0 and 2.4 Hz), 50.7, 41.3 (t, *J* = 25.9 Hz), 33.1 (d, *J* = 4.8 Hz). Anal. Calcd. for ($C_{11}H_{12}F_2N_4O_2$) C, H, N.

(+)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-2',3'-didehydro-3'-fluoro-6'-hydroxymethylcyclopent-2-enyl]adenine (34) mp 218-220 °C (dec.); $[\alpha]^{22}_D$ +41.00° (*c* 0.13, DMSO); UV (MeOH) λ_{max} 260.0 nm (ε 10925, pH 2), 261.0 nm (ε 10807, pH 7), 261.0 nm (ε 11780, pH 11); ^1H NMR (500 MHz, CD_3OD) δ 8.31 (s, 1H), 8.24 (s, 1H), 5.66 (br, 1H), 5.44 (s, 1H), 3.75 (ddd, *J* = 130.0, 11.0 and 3.5 Hz, 2H), 3.05 (br, 1H), 2.98 (m, 1H), 2.00 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ

166.2 (d, *J* = 287.5 Hz), 155.9, 152.1, 148.8, 139.8, 118.8, 103.2 (d, *J* = 14.3 Hz), 60.0, 53.9 (t *J* = 12.9 Hz), 43.2 (d, *J* = 18.6 Hz), 32.2 (d, *J* = 5.8 Hz); MS: *m/z* 250 (M+1); Anal. Calcd. for ($C_{11}H_{12}FN_5O$) C, H, N.

(-)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-2',3'-didehydro-3'-fluoro-6'-hydroxymethylcyclopent-2-enyl]guanine (35) To a suspension of **33** (60 mg, 0.21 mmol) in anhydrous DMF (5 mL), potassium *tert*-butoxide (82 mg, 0.69 mmol) was added. The reaction mixture in the thick-walled tube was placed in a microwave synthesizer and irradiated at maximum output power of 300 W with air-cooling at 70 °C for 10 min. The brown suspension was filtered through a short silica gel pad and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:20 to 1:10) to give **35** (40 mg, 72 %) as a white solid. mp 220 °C (dec.); $[\alpha]^{25}_D$ –44.12° (*c* 0.11, MeOH); UV (H_2O) λ_{max} 253.0 nm (ε 13553, pH 2), 252.0 nm (ε 14393, pH 7), 256.0 nm and 268.0 (ε 11186 and 11829, respectively, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 7.92 (s, 1H), 5.49 (m, 1H), 5.37 (s, 1H), 3.74 (ddd, *J* = 113.5, 11.5 and 4.0 Hz, 2H), 3.02 (m, 1H), 2.90 (dt, *J* = 14.0 and 9.0 Hz, 1H), 2.00-1.97 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 165.9 (d, *J* = 282.0 Hz), 158.0, 153.8, 151.2, 136.6, 116.2, 103.1 (d, *J* = 13.7 Hz), 60.0, 53.2 (d, *J* = 12.9 Hz), 43.2 (d, *J* = 18.3 Hz), 32.2 (d, *J* = 5.4 Hz). Anal. Calcd. for ($C_{11}H_{12}FN_5O_2$•0.9H_2O) C, H, N.

(+)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-2',3'-didehydro-3'-fluoro-6'-hydroxymethylcyclopent-2-enyl]hypoxanthine (36) mp 254-256 °C; $[\alpha]^{27}_D$ +34.01° (*c* 1.51, MeOH); UV (H_2O) λ_{max} 249.0 nm (ε 10354, pH 2), 248.5 nm (ε 13925, pH 7), 254.0 nm (ε 10142, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 8.15 (s, 1H), 8.08 (s, 1H), 5.68 (m, 1H), 5.44 (s, 1H), 3.86 (dd, *J* = 10.5 and 4.5 Hz, 1H), 3.60 (m, 1H), 2.96-2.85 (m, 2H), 1.97 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 166.4 (d, *J*

= 283.9 Hz), 157.6, 148.4, 145.1, 139.2, 123.9, 103.1 (d, *J* = 14.8 Hz), 60.0, 54.2 (d, *J* = 12.9 Hz), 43.3 (d, *J* = 18.6 Hz), 32.4 (d, *J* = 5.8 Hz). Anal. Calcd. for ($C_{11}H_{11}FN_4O_2$) C, H, N.

(-)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-3',3'-difluoro-6'-hydroxymethylcyclopentanyl]cytosine (37) mp 132-134 °C; $[\alpha]^{26}_D$ –4.62° (*c* 0.3, MeOH); UV (H_2O) λ_{max} 282.0 nm (ε 16381, pH 2), 274.0 nm (ε 10363, pH 7), 273.0 nm (ε 11099, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 7.72 (d, *J* = 7.5 Hz, 1H), 5.95 (d, *J* = 7.0 Hz, 1H), 5.14-5.07 (m, 1H), 3.81 (ddd, *J* = 47.0, 11.5 and 5.5 Hz, 2H), 2.70-2.35 (m, 4H), 1.97-1.90 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 165.8, 157.5, 142.2, 128.9 (dd, *J* = 250.9 and 249.0 Hz), 95.2, 59.0 (t, *J* = 5.6 Hz), 52.0 (t, *J* = 6.2 Hz), 40.1 (t, *J* = 25.2 Hz), 32.0 (d, *J* = 4.4 Hz). Anal. Calcd. for ($C_{10}H_{13}F_2N_3O_2$•0.6H_2O) C, H, N.

(-)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-3',3'-difluoro-6'-hydroxymethylcyclopentanyl]thymine (38) $[\alpha]^{26}_D$ –3.2° (*c* 0.25, MeOH); mp 142-144 °C; UV (H_2O) λ_{max} 271.0 nm (ε 8478, pH 2), 271.0 nm (ε 8509, pH 7), 270.0 nm (ε 7587, pH 11); ^{1}H NMR (400 MHz, CD_3OD) δ 7.56 (s, 1H), 5.08-5.02 (m, 1H), 3.80 (ddd, *J* = 33.2, 9.2 and 4.8 Hz, 2H), 2.65-2.37 (m, 4H), 2.01-1.92 (m, 4H); ^{13}C NMR (125 MHz, CD_3OD) δ 164.9, 151.4, 137.7, 128.8 (dd, *J* = 250.9 and 248.0 Hz), 110.7, 58.9 (dd, *J* = 8.0 and 2.4 Hz), 50.8 (t, *J* = 4.2 Hz), 39.5 (t, *J* = 23.6 Hz), 31.4 (d, *J* = 3.8 Hz), 11.0. Anal. Calcd. for ($C_{11}H_{14}F_2N_2O_3$) C, H, N.

(-)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-2',3'-didehydro-3'-fluoro-6'-hydroxymethylcyclopent-2-enyl]cytosine (39) Compound **37** (60 mg, 0.24 mmol) was converted to cytosine derivative **39** (46 mg, 84 %) as a white solid using the same procedure as for **35**. mp 220-230 °C; $[\alpha]^{25}_D$ – 124.63° (*c* 0.33, MeOH); UV (H_2O) λ_{max} 283.0 nm (ε 15638, pH 2), 274.0 nm (ε 10805, pH 7), 274.0 nm (ε 10512, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 7.83 (d, *J* = 7.5 Hz, 1H), 5.90 (d, *J* = 7.5 Hz, 1H), 5.63-5.59 (m, 1H), 5.19 (s, 1H), 3.70 (ddd, *J* = 132.5, 10.5 and 3.5 Hz, 2H), 2.95-2.93 (m, 1H), 2.82 (td, *J* = 14.0 and 9.0 Hz, 1H), 1.73-1.68 (m, 1H); ^{13}C NMR (125 MHz,

CD_3OD) δ 166.1, 166.0 (d, *J* = 283.2 Hz), 157.5, 142.6, 103.3 (d, *J* = 13.4 Hz), 94.3, 59.8 (d, *J* = 1.9 Hz), 55.9 (d, *J* = 8.1 Hz), 42.9 (d, *J* = 18.6 Hz), 31.7 (d, *J* = 6.1 Hz). Anal. Calcd. for ($C_{10}H_{12}FN_3O_2$) C, H, N.

(-)-(1'*R*,4'*S*)-9-[2',3'-Dideoxy-2',3'-didehydro-3'-fluoro-6'-hydroxymethylcyclopent-2-enyl]thymine (40) mp 184-186 °C (dec.); $[\alpha]^{25}_D$ –24.17° (*c* 0.15, MeOH); UV (H_2O) λ_{max} 272.0 nm (ε 15633, pH 2), 273.0 nm (ε 15750, pH 7), 271.0 nm (ε 12548, pH 11); ^{1}H NMR (400 MHz, CD_3OD) δ 7.69 (d, *J* = 1.2 Hz, 1H), 5.57-5.54 (m, 1H), 5.15 (s, 1H), 3.69 (ddd, *J* = 121.6, 11.6 and 3.2 Hz, 2H), 2.93-2.90 (m, 1H), 2.78-2.70 (td, *J* = 14.4 and 9.2 Hz, 1H), 1.76-1.70 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.2, 166.0 (d, *J* = 282.7), 151.6, 138.2, 109.7, 103.4 (d, *J* = 13.7 Hz), 59.6 (d, *J* = 2.3 Hz), 54.7 (d, *J* = 12.2 Hz), 42.7 (d, *J* = 18.3 Hz), 30.9 (d, *J* = 6.1 Hz), 11.0. Anal. Calcd. for ($C_{11}H_{13}FN_2O_3$) C, H, N.

Table 3.7. Elemental analysis data

Cmpd No.	Formula	Calc for C	Calc for H	Calc for N	Found for C	Found for H	Found for N
2	$C_{32}H_{30}O_3$	83.09	6.54	-	82.89	6.57	-
4	$C_{32}H_{32}O_3$	82.73	6.94	-	82.54	6.81	-
5	$C_{32}H_{30}O_3$	83.09	6.54	-	82.80	6.69	-
6	$C_{32}H_{30}F_2O_2$	79.32	6.24	-	79.42	6.32	-
7	$C_6H_{10}F_2O_2$	47.37	6.63	-	47.63	6.78	-
8	$C_{22}H_{28}F_2O_2Si$	67.66	7.23	-	67.67	7.27	-
11	$C_{22}H_{29}F_2NOSi$	67.83	7.50	3.60	67.64	7.62	3.61
13	$C_{26}H_{30}F_2N_2O_3Si$	64.44	6.24	5.78	64.63	6.28	5.71
14	$C_{26}H_{31}F_2N_3O_2Si$	64.57	6.46	8.69	64.39	6.31	8.51
15	$C_{10}H_{13}F_2N_3O_2 \cdot 0.65H_2O$	46.75	5.61	16.35	47.03	5.73	16.02
17	$C_{11}H_{14}F_2N_2O_3$	50.77	5.42	10.76	50.54	5.68	10.65
18	$C_{10}H_{12}FN_3O_2$	53.33	5.37	18.66	53.02	5.22	18.34
19	$C_{11}H_{13}FN_2O_3 \cdot 0.1H_2O$	54.59	5.50	11.57	54.59	5.41	11.52
21	$C_{27}H_{31}F_2N_5OSi$	63.88	6.16	13.80	63.73	6.21	13.70
22	$C_{11}H_{13}F_2N_5O$	49.07	4.87	26.01	49.14	4.87	25.89
23	$C_{11}H_{12}F_2N_4O_2$	48.89	4.48	20.73	48.83	4.38	20.51
26	$C_{11}H_{12}FN_5O$	53.01	4.85	28.10	52.90	4.84	28.18
27	$C_{11}H_{11}FN_4O_2 \cdot 0.1H_2O$	52.42	4.48	22.23	52.30	4.41	22.23
28	$C_{11}H_{12}FN_5O_2 \cdot 1.1H_2O$	46.35	5.02	24.57	46.65	4.99	24.29
29	$C_{22}H_{28}F_2O_2Si$	67.66	7.23	-	67.62	7.47	-
30	$C_{22}H_{29}F_2NOSi \cdot 0.2H_2O$	67.21	7.54	3.56	67.26	7.62	3.56
31	$C_{11}H_{13}F_2N_5O$	49.07	4.87	26.01	49.15	4.77	26.29
32	$C_{11}H_{13}F_2N_5O_2 \cdot 0.3H_2O$	45.46	4.72	24.09	45.26	4.65	23.88
33	$C_{11}H_{12}F_2N_4O_2$	48.89	4.48	20.73	49.10	4.51	20.99
34	$C_{11}H_{12}FN_5O$	53.01	4.85	28.10	53.31	4.59	28.44
35	$C_{11}H_{12}FN_5O \cdot 0.9H_2O$	46.94	4.94	24.88	46.85	4.63	24.73
36	$C_{11}H_{11}FN_4O_2$	52.80	4.43	22.39	52.99	4.74	22.45
37	$C_{10}H_{13}F_2N_3O_2 \cdot 0.6H_2O$	46.91	5.59	16.41	47.27	5.71	16.02
38	$C_{11}H_{14}F_2N_2O_3$	50.77	5.42	10.76	50.56	5.40	10.69
39	$C_{10}H_{12}FN_3O_2$	53.33	5.37	18.66	53.44	5.35	18.36
40	$C_{11}H_{13}FN_2O_3$	55.00	5.45	11.66	54.80	5.46	11.51

Antiviral and Cytotoxicity Assay. HIV drug susceptibility assays were performed as previously described.[238] Cytotoxicity assays in PBM, CEM and Vero cells were conducted as previously described.[239]

Molecular Modeling Study. (a) Conformational analysis: The initial conformations of inhibitors were constructed by builder module in MACROMODEL®, version 8.5 (Schrodinger, Inc.) based on the crystal structure of carbovir. The Monte Carlo conformational search was performed in 5,000-step, in the presence of GB/SA water model using MMFFs force field in MACROMODEL. (b) Binding affinity study to HIV-1 reverse transcriptase: All molecular modeling studies of the enzyme-substrate complexes were performed using Sybyl® 7.0 (Tripos Associates, St. Louis, MO) on a Silicon Graphics Tezro® workstation or a SGI Origin 300 workstation. The enzyme site of the enzyme-ligand complex was built based on the X-ray structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer-template duplex (PDB entry 1rtd).[225] A model of the NRTI binding site was built, which consisted of residues between Lys1 and Pro243 in the p66 subunit, and a 7:4 (template-primer) duplex. The conformationally optimized structures of carbocyclic nucleosides were used to define the initial Cartesian coordinates. The heterocyclic moiety of the n+1th nucleotide in the template overhang was modified to the base complementary to the incoming NRTIs if needed, i.e. the adenine moiety which was in the original X-ray structure (1rtd)[225] was modified to guanine. The inhibitor triphosphates were manually docked to the active site of the enzyme by adjusting the torsional angles to those found in the X-ray structure.[225] Gästeiger-Hückel charges were then given to the nucleoside triphosphate with formal charges (+2) to the two Mg atoms in the active site and Kollman-All-Atom charges were loaded to the enzyme site using the biopolymer module in Sybyl. Fluorine parameters were obtained from literature[186, 226, 227] and MM2 parameters were entered into the parameter files. In order to eliminate local strains resulting from merging inhibitors and/or point mutations, residues inside 6 Å from the merged inhibitors and mutated residues were annealed until energy change from one iteration to the next was less than 0.05

Kcal/mol. The annealed enzyme-inhibitor complexes were minimized by using Kollman-All-Atom force field until iteration number reached 5,000.

The structures (D-3'-F-C-d4G-TP/HIV-RT_{WT}, D-3'-F-C-d4G-TP/HIV-RT_{M184V}, carbovir-TP/HIV-RT_{WT}, carbovir-TP/HIV-RT_{M184V}, GTP/HIV-RT_{WT} and GTP/HIV-RT_{M184V}) were further confirmed by the molecular dynamics studies using MACROMODEL®, version 9.1 (Schrodinger, Inc.). The complex was minimized until there was no significant movement in atomic coordinates using MMFF94s force field in the presence of GB/SA continuum water model before performing molecular dynamics simulations. A conjugate gradient, Polak-Ribiere 1st derivative method was used for energy minimization. Molecular dynamics simulations on nucleoside-TP/RT complex was performed with MMFF94s in the presence of GB/SA continuum water model on a SGI Origin 300 workstation running the IRIX 6.5 operating system by heating from 0 to 300K over 5 ps and equilibrating at 300K for an additional 10 ps. Production dynamics simulations were carried out for 500 ps with a step size of 1.5 fs at 300 K. A shake algorithm was used to constrain covalent bonds to hydrogen atoms. A distance constrain was used to constrained the two magnesium atoms with Asp110, Val111, Asp185, α- and β-phosphate of nucleotide. For simulation of the nucleoside-TP/RT complex, the residues further away than 15 Å from the active site were not considered and the residues from 6 to 15 Å were constrained by harmonic constraints. Only residues inside 6 Å sphere from the bound nucleoside-TP were allowed to move freely.

Acknowledgement This research was supported by the U.S. Public Health Service Grants (AI25899, AI32351 & AI41980) from the National Institute of Allergy and Infectious Diseases Emory's CFAR Grand 5-P30-AI-50409 and the Department of Veterans Affairs.

CHAPTER 4

ASYMMETRIC SYNTHESIS OF NOVEL 2'-FLUORINE(S) SUBSTITUTED, 2'-β-*C*-METHYL-2'-HYDROXYL AND 2'-*O*-METHYL-2'-HYDROXYL ENTECAVIR ANALOGS AS POTENTIAL ANTI-HCV AGENTS

PART I: INTRODUCTION - BIOLOGICALLY ACTIVE

ANTI-HCV NUCLEOSIDES

Approximately 170 million people worldwide are infected by hepatitis C virus (HCV). Once infected, around 80 % of patients become chronic carriers and have a substantial risk of developing liver cirrhosis as well as hepatocellular carcinoma.[240, 241] According to the World Health Organization, more than 280,000 deaths were attributable to HCV infection in the year 2002.[186] HCV has been recognized as one of the leading causes of liver impairment.

HCV is classified in the genus *Hepacivirus*, *Flaviviridaeis*, containing a single-strand, positive-sense RNA genome of approximately 9,600 nucleosides in length.[242] The viral genome contains a 5'-noncoding region (5'-NCR), a long open reading frame encoding a polyprotein precursor and a 3'- noncoding region (3'-NCR). The polyprotein precursor is cleaved by cellular and viral proteases to yield the mature structural and non-structural proteins. Among the non-structural (NS) proteins, NS3 contains both a serine protease and RNA helicase; NS5B possesses a RNA-dependent RNA polymerase (Figure 4.1).[243] Based on genomic differences, there are at least six known HCV genotypes worldwide, with genotype 1 (~75%), genotype 2 (~10%) and genotype 3 (~10%) being the most common in the United States.[244]

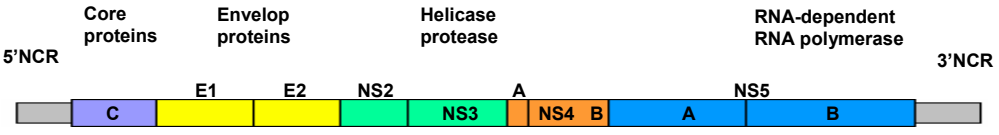
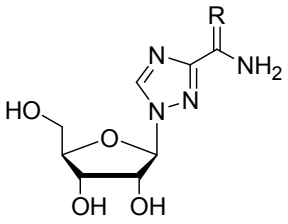


Figure 4. 1. Schematic drawing of HCV genomic map

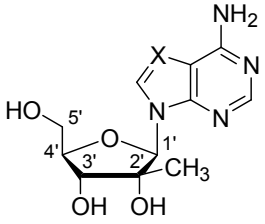
HCV replicates primarily within hepatocytes.[245, 246] Like other flaviviruses, HCV replication follows: (a) host cell attachment, entry and uncoating; (b) translation of HCV genome into viral proteins; (c) cleavage and processing of viral proteins; (d) replication of HCV genome; (e) assembly of new virions and release from the host cell. Theoretically, all of these stages could be potential targets for antiviral therapy. However, the current recommended therapy for HCV infection is limited to a combination of ribavirin with α-interferon (IFN-α) or pegylated-IFN-α, which has been shown to be more effective.[247-250] Ribavirin and interferon are non-specific anti-HCV agents, and sustained virological response (SVR) rates to this treatment in genotype 1 patients are only 40%-50%.[251, 252] Furthermore, side effects such as fatigue, flu-like symptoms and ribavirin-related hemolytic anemia can result in need of dose reduction or even discontinuation of treatment. Therefore, more effective and HCV-specific antiviral agents are urgently needed.

Considerable efforts have been conducted in developing novel and HCV-specific antiviral agents, for instance, protease inhibitors, polymerase inhibitors, ribozyme, antisense oligodeoxynucleotide and small interfering RNA have been evaluated.[248] Among these, RNA-dependent RNA polymerase (RdRp) inhibitors have been the focus for drug discovery over the past several years. HCV RdRp is a key enzyme strictly required in the HCV replication cycle.[253] Close homologs of HCV RdRp do not exist within the uninfected host cell. Thus, HCV RdRp is an ideal target for developing specific antiviral agents.[254] Application of nucleoside analogs as antiviral agents targeting viral polymerase has been demonstrated to be a very efficient and successful approach in viral chemotherapy, especially in the treatment of HIV, HBV and herpes virus infections. Actually, a number of specific anti-HCV nucleosides have been identified and some of them are undergoing clinical or preclinical trials (Figure 4.2): (1) ribavirin and its

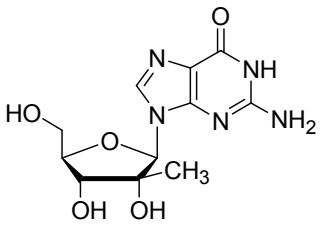
analogs (**1** & **2**); (2) 2'-modified nucleosides (**3-9**); (3) 4'-azido nucleosides (**10** & **11**); (4) carbocyclic nucleosides (**12** & **13**). A briefly discussion of these nucleosides is given in the Part 1 of this chapter.



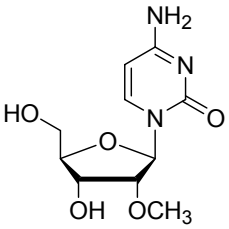
1 R=O, Ribavirin, Virazole®
2 R=NH, Viramidine



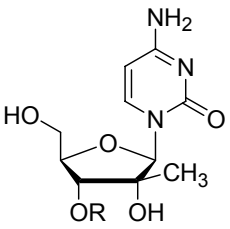
3 X= N, 2'-*C*-Me-Ade
4 X=CH, 7-deaza-2'-*C*-Me-Ade, MK-0608



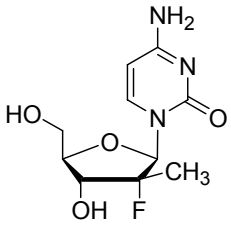
5 2'-*C*-Me-Gua



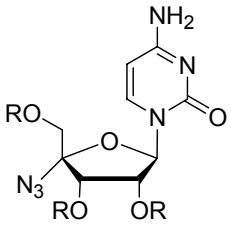
6 2'-O-Me-Cyt



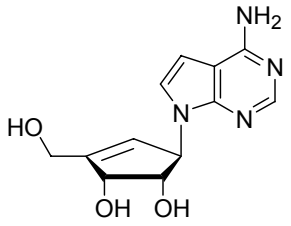
7 R=valine, Valopicitabine, NM-283
8 R=H, 2'-*C*-Me-Cyt, NM-107



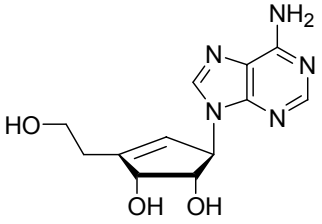
9 2'-Deoxy-2'-F-*C*-Me-Cyt, R1656
PSI-6130, R7128 (prodrug of R1656)



10 R=*iso*-Butyric ester, R1626
11 R=H, 4'-azidocytidine, R1479



12 7-Deazaneplanocin A



13 5'-Homoaneplanocin A

Figure 4.2. Anti-HCV nucleosides in clinical or pre-clinical studies

Ribavirin and its analogs

Ribavirin, Virazole® (Figure 4.2). Currently, ribavirin is the only nucleoside in clinical use for the treatment of HCV infections.[255-258] This compound is a guanosine analog with broad antiviral

spectrum. Several mechanisms of action of ribavirin have been proposed to explain its antiviral activity.[259] Ribavirin is converted to its mono-, di- and tri-phosphates in the cells. Ribavirin monophosphate (ribavirin-MP) is a direct competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH) and thus decreases the intracellular guanosine triphosphate (GTP) level. Because GTP is essential for the viral replication, ribavirin-MP can therefore inhibit viral replication by depleting intracellular GTP pools.[260] Ribavirin triphosphate (ribavirin-TP) also functions as a viral polymerase inhibitor.[261] Furthermore, incorporation of ribavirin-MPs into the viral genome can increase the viral replication error and eventually induces the replication error catastrophe.[262-264] In addition to these direct mechanisms, ribavirin is also recognized as an immune modulator by shifting the immune response towards Th1 cells and their associated cytokines which may inhibit the production of HCV virion.[265] However, the complete mechanisms by which ribavirin function as anti-HCV agent, remain unknown.[266] The efficacy of ribavirin monotherapy has been demonstrated to be modest and transient by several clinical trials.[267-270] The addition of IFN-α to ribavirin treatment, significantly improved the virological outcomes by reducing relapse rates. Patients in clinical trials were randomized to receive Intron® A of 3 million international units (MIU) three times per week and either oral ribavirin 1,000-1,200 mg/day or a placebo for 24 weeks of treatment. At a follow-up time of 6 months after therapy, 79 patients (45.7%) who received the combination therapy had undetectable viral load versus 8 patients (4.7%) in placebo group.[259] The major adverse effect was ribavirin-related anemia which resulted in a dose reduction of ribavirin in 22% of patients.[259]

Viramidine (Figure 4.2). Viramidine is a liver-targeting prodrug of ribavirin. It is converted to ribavirin by hepatic adenosine deaminase and thus specifically targets liver (Figure 4.3).[271]

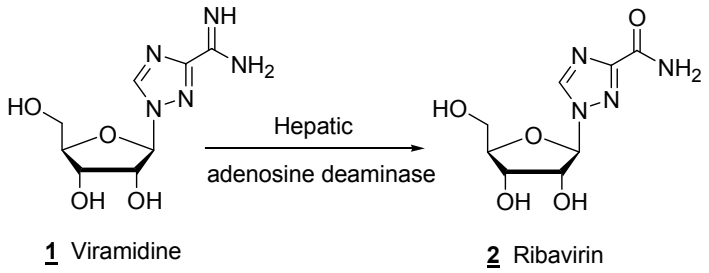


Figure 4.3. Activation process of Viramidine in liver

In monkeys, multiple oral dosing (10 mg/kg) of viramidine achieved three times the concentration of ribavirin in the liver and only half the concentration in red blood cells in comparison to ribavirin (10 mg/kg). Furthermore, viramidine had a much better safety profile than ribavirin in a 28-day toxicity study in monkeys.[272] In healthy volunteers, a single dose of viramidine, ranging from 200 to 1200 mg, did not produce serious adverse effects.[273] A randomized, active-controlled phase II study compared the efficacy and tolerability of viramidine or ribavirin with pegylated INF-α in 180 treatment-naïve patients. According to the report, the proportion of the subjects that had undetectable HCV RNA level were similar in both groups. However, significantly fewer patients developed anemia in the viramidine/INF-α group than in ribavirin/INF-α group.[259] Two phase III trials of viramidine plus pegylated INF-α 2a or 2b (so called VIramidine's Safety and Efficacy vs. Ribavirin 1 & 2, VISER1 & 2) have been recently completed.[186] Based on the data from VISER1 & 2, viramidine (600 mg/bid) was not better than standard ribavirin (weight-based 1,000 or 1,200 mg/daily) in terms of efficacy (Table 4.1). However, Valeant Pharmaceuticals emphasized the more favorable safety profile of viramidine (less likely to cause anemia) than ribavirin (Table 4.1), which may lead the company to reevaluate viramidine at a higher dose in the near future.

Table 4.1. Viramidine versus ribavirin in two phase III trials: Efficacy and safety

Study	Sustained Virologic Response		Anemia (Hemoglobin < 10 g/dL)	
	Viramidine	Ribavirin	Viramidine	Ribavirin
VISER 1	38%	52%	5%	24%
VISER 1	40%	55%	6%	22%

2'-modified nucleosides

2'-C-methyl (2'-O-methyl) ribonucleosides (Figure 4.2). Various modifications on the sugar or base moiety of nucleosides have been studied to search for novel anti-HCV agents. One of the most promising candidates is 2'-*C*-methyl / 2'-*O*-methyl nucleoside. Merck Research Laboratory described 2'-*C*-methyladenosine (2'-*C*-Me-Ade) and 2'-*O*-methylcytidine (2'-*O*-Me-Cyt) which specifically inhibited HCV RNA replication without apparent cytotoxicity.[274] Triphosphates of both nucleosides inhibited the catalytic activity of HCV RNA polymerase with comparable IC_{50} values (1.9 μM and 3.8 μM for adenosine and cytidine analogs, respectively), but the adenosine analog was more potent than the cytidine analog in the cell based HCV replicon assay (EC_{50} 0.3 μM and 21 μM for adenosine and cytidine analogs, respectively). This likely reflected the combination effects of the different metabolic properties and the fact that the analogs act as chain terminators. Actually, a lower amount of intracellular 2'-*O*-Me-Cyt triphosphate was detected than that of 2'-*C*-Me-Ade.[274] In another *in vitro* study using different cell lines, it was found that 2'-*O*-Me-Cyt had difficulty penetrating into the replicon cells and yielded much less triphosphates with extensive metabolism to UTP and CTP, and consequently exhibited an EC_{50} more than 100 μM.[275] Based on these preliminary results, a comprehensive SAR study of 2'- or

3'-modified nucleosides has been conducted.[276, 277] Interestingly, the study demonstrated a fairly stringent SAR which indicated that a methyl substitution on the β-face on the 2'-position of ribonucleosides was necessary for maintaining potent anti-HCV activity. Molecular modeling studies suggested that the active nucleoside (2'-*C*-Me-Ade) preferred the *C*3'-endo conformation while the inactive nucleoside (3'-*C*-Me-Ade) adopted the *C*2'-endo conformation, which may explain, at least in part, their different anti-HCV activities. Resistant mutants against 2'-*C*-Me-Ade have been selected and characterized. The single mutation on the codon 282 (Ser282Thr) reduced the HCV susceptibility to 2'-*C*-Me-Ade by 40-fold.[278]

Pharmacokinetic studies of 2'-*C*-Me-Ade and 2'-*C*-Me-Gua were investigated in rats.[278] Oral dosing of 2'-*C*-Me-Ade didn't produced detectable drug levels in plasma, suggesting little or no bioavailability and/or rapid degradation of this compound. In contrast, 2'-*C*-Me-Gua had a much better pharmacokinetic profile with oral bioavailability of 82% and plasma half-life around 2 h. However, further development of 2'-*C*-Me-Gua is in doubt due to its poor phosphorylation. Although 2'-*C*-Me-Ade and 2'-*C*-Me-Gua may not be promising anti-HCV drug candidates due to their poor metabolic properties, they did provide important prototypes, upon which further modifications have been made and led to the discovery of more interesting molecules, such as valopicitabine, 2'-*C*-methyl-7-deazaadenosine and 2'-deoxy-2'-fluoro-2'-*C*-methylcytidine.

Valopicitabine (NM-283, prodrug of 2'-C-methyl-cytidine NM-107) (Figure 4.2). Valopicitabine (NM283) is a valine prodrug of 2'-*C*-methyl-cytidine (2'-*C*-Me-Cyt, NM-107) and had been undergoing phase II clinical trial by Idenix Pharmaceuticals Inc and Novartis AG. However, it was discontinued due to the gastrointestinal (GI) side effects. *In vitro*, NM-107 is a potent and selective inhibitor of flavivirus.[279, 280] In bovine viral diarrhea virus (BVDV, a pestivirus

surrogate model for HCV), NM-283 suppressed viral replication with an EC_{50} of 0.67 μM. Further evaluation of this compound revealed its antiviral activity against yellow fever virus, dengue virus and West Nile virus.[281] Another *in vitro* study evaluated the efficacy of the combination of ribavirin with valopicitabine.[282] In the HCV replicon assay, EC_{50}s of ribavirin and valopicitabine were 87 and 0.27 μM, respectively. However, ribavirin antagonized the antiviral activity of valopicitabine when they were used in combination. These data suggest avoiding the combination therapy of ribavirin and valopicitabine. An *in vivo* study of valopicitabine was conducted using HCV genotype 1-infected chimpanzees.[283] After 7-day treatment, the mean reduction of vial load was 0.83 $\log_{10}$ at dose of 8.3 mg/kg, and 1.05 $\log_{10}$ at dose of 16.6 mg/kg, while there was no change in the placebo group.

Long term treatment of BVDV using valopicitabine selected a Ser405Thr mutation, which was equivalent to a Ser282Thr mutation in HCV polymerase. The Ser405Thr mutation reduced the susceptibility of BVDV to valopicitabine by 50-fold but it resulted in a 38-fold in the increased susceptibility to INF-α2b, which suggested benefits of a combination of valopicitabine with INF- α2b.[284]

Although 2'-*C*-methyl-cytidine had low bioavailability in animals, its prodrug, valopicitabine demonstrated much better pharmacokinetic profiles.[285] After a single dose oral administration of valopicitabine dihydrochloride 100 mg/kg in rats, at least 30 % of the oral dose was absorbed and the half-life of the drug was 0.64 h. In healthy individuals, the mean oral bioavailability was 68.3 % with a terminal phase half-life of approximately 4.5 h.

In a dose-escalation, double blinded, randomized phase I/II study, 12 patients who were treatment-naïve or had failed IFN plus ribavirin treatment, received valopicitabine treatment for 14 days. The reduction of HCV RNA level ranged from 0.2 $\log_{10}$ at 50 mg/day to 1.2 $\log_{10}$ at 800

mg/day. The efficacy of combination of valopicitabine plus pegylated-IFN-α2b was demonstrated in phase IIa trials in which 11 (91.6%) patients had significant reductions of HCV RNA level ranging from -1.7 to -6.2 $\log_{10}$ after 10 weeks treatment.[286] Two phase IIb studies also confirmed the efficacy of the combination of valopicitabine and pegylated-INF-α2a.[287, 288] However, serious gastrointestinal (GI) intolerability was found frequently in the valopicitabine 800 mg/day group. Based on these data, the study was amended.[289, 290] Patients receiving 800 mg/day plus pegylated-INF-α2a randomly assigned to 200 mg or 400 mg valopicitabine plus pegylated-INF-α2a. Interestingly, more than 50 % of patients receiving a 200 mg dose of valopicitabine plus pegylated-INF-α2 had undetectable HCV RNA level at week 48 (Figure 4.4). However, GI-related adverse effects were still observed at this dose and were occasionally severe.[289, 290] Therefore, the suspension of NM283 clinical trial was announced in July, 2007 due to its GI toxicity.[291]

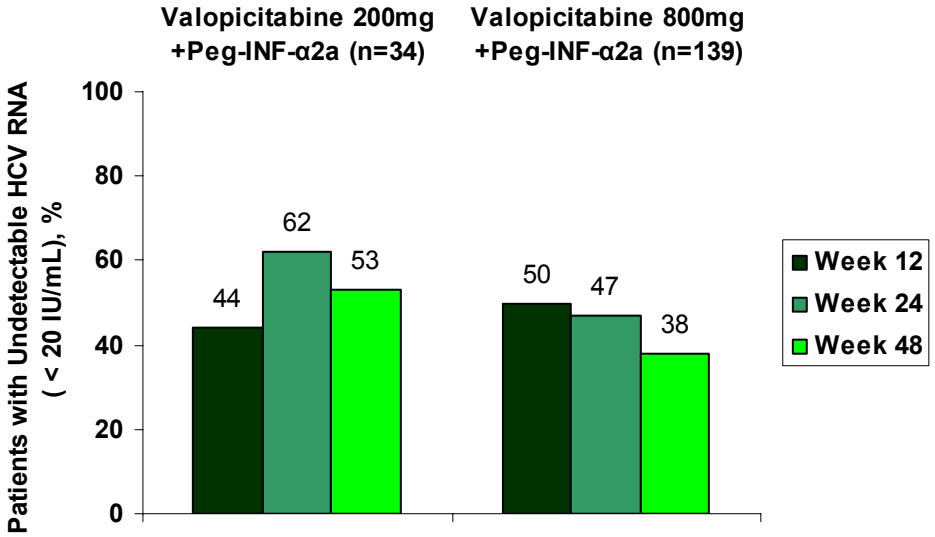


Figure 4.4. Percentage of patients with undetectable HCV RNA level at week 12, 24 and 48

2'-C-methyl-7-deazaadenosine (MK-0608) (Figure 4.2). Introduction of a 7-deaza modification to 2'-*C*-methyl-adenosine generates another interesting nucleoside, 2'-*C*-methyl-7-deazaadenosine (2'-*C*-Me-7-deazaade).[277, 292] In enzyme assay, 2'-*C*-Me-7-deazaade triphosphate exhibited more potent anti-HCV activity (IC_{50} 0.11 μM) in comparison to adenosine counterpart (IC_{50} 1.8 μM). Notably, the modification on the 7-position of the base moiety significantly improved the pharmacokinetic properties of the parent compound. The oral bioavailability of 2'-*C*-Me-7-deazaade was 51%, 51% and 98% in monkeys, rats and dog, respectively and the plasma half-life was 9.0, 1.6 and14 h. In contrast to 7-deazaadenosine, known as tubercidin, 2'-*C*-Me-7-deazaade exhibited marginal cytotoxicity in cells and in animals. However, *in vitro* resistance studies found HCV polymerase containing S282T mutation reduced the susceptibility to 2'-*C*-Me-7-deazaade by approximately 34-fold in comparison to the wild type.[292]

In vivo efficacy and tolerability studies of 2'-*C*-Me-7-deazaade were conducted in chronically HCV-infected chimpanzees. After 7 days intravenously administration, viral loads decreased by >5 $\log_{10}$ in two animals. However, viral loads rebounded after dosing stopped and the S282T mutation was detected. Orally administration of 2'-*C*-Me-7-deazaade 1 mg/kg/day for 37 days also achieved substantial viral load reductions in two chimpanzees. Moreover, when 2 mg/kg/day nucleoside was administrated for 37 days, the viral load decline was very steep and remained undetectable for at least 6 days after the treatment.[293] Currently, 2'-*C*-methyl-7-deazaadenosine (MK-0608) is being developed in phase I study by Merck.[186]

2'-Deoxy-2'-fluoro-2'-C-methylcytidine (R1656, PSI-6130, prodrug/R7128) (Figure 4.2). 2'-Deoxy-2'-fluoro-2'-*C*-methylcytidine (R1656, previous PSI-6130) is a cytidine analog containing a 2'-α-fluorine substitution. It is a selective anti-HCV compound and approximately 4-fold more

potent than valopicitabine, with an EC_{90} of 4.6 μM in HCV replicon assay.[294, 295] Unlike previous reported 2'-*C*-methyl-ribonucleosides, this compound shows only weak or no activity against other flavivirus, such as BVDV, dengue and West Nile viruses, indicating its specificity to HCV.[295] R1656 is also active against S282T mutant, which is known to confer resistance to 2'-*C*-Me-Ade.[295] *In vitro* studies did not observe apparent cytotoxicity and mitochondrial toxicity in a diverse set of cell lines. [294, 295]

Metabolic profiles of R1656 have been reported recently.[296] In the study, R1656 was converted to its 5'-triphosphate (R1656-TP) and also deaminated by deoxycytidine deaminase to the uridine analog (RO2433) which was further converted to the corresponding triphosphate (RO2433-TP, Figure 4.5). Interestingly, the RO2433-TP was also found to be a potent anti-HCV agent with comparable activity to R1656-TP. Furthermore, the intracellular half-life of RO2433-TP was around 38 h suggesting the possibility of once daily dosing of R1656 for HCV therapy.

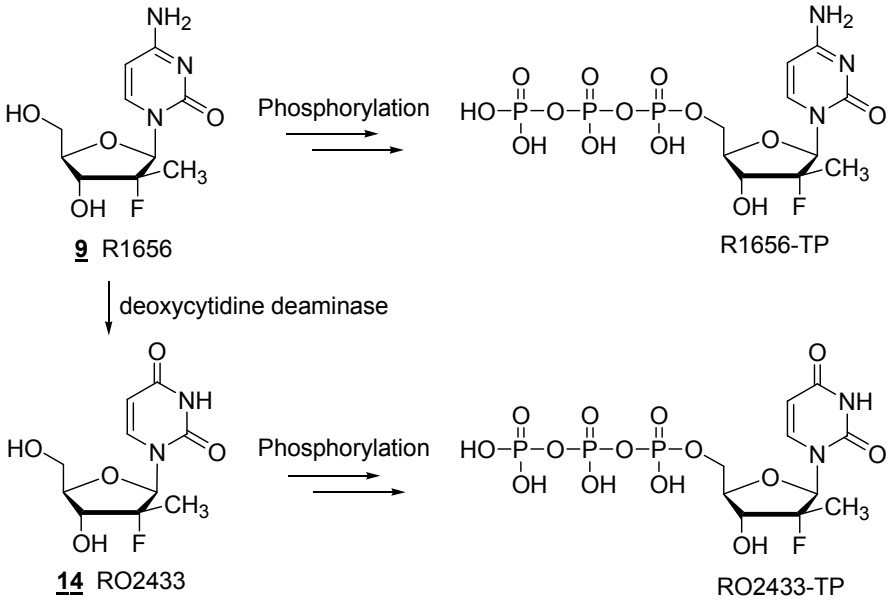


Figure 4.5. Metabolic profile of R1656

In a completed single dose-escalation phase I study, R1656 was well tolerated at all doses without serious adverse effects up to 3,000 mg / day. Currently, Roche and Pharmasset are collaborating to develop a prodrug (R7128) of R1656. In another multi-center, observer-blinded, randomized and placebo controlled phase I trial, R7128 was generally well tolerated at all doses in single-dose oral administration and no significant abnormalities were evident so far.[186]

4'-Azido nucleosides

4'-Azidocytidine, R1479, prodrug/R1626 (Figure 4.2). Ribonucleosides with 4'-subtitutions have been explored by scientists at Roche. 4'-Azidocytidine (R1479) was found to be a potent inhibitor of HCV polymerase with an IC_{50} of 0.32-1.28 μM in HCV replicon assay without cytotoxicity with concentration up to 2 mM.[297, 298] Biochemical studies of this compound showed that R1479 functioned as a CTP competitive inhibitor with a Ki value of 40 nM. It was incorporated into viral RNA by HCV polymerase and inhibited further RNA chain elongation. Interestingly, the S282T mutation did not confer any cross-resistance to R1479.[297] In another preclinical study, R1479 exerted moderate synergistic effects when combined with either INF-α2a or ribavirin.[299] Because R1479 was identified with suboptimal oral bioavailability due to its absorption problem, a number of prodrugs have been synthesized. Among them, tri-isobutyric ester (R1626) demonstrated much improved pharmacokinetic profiles than R1479.[300]

In a phase I dose-ascending study, 47 patients, who were HCV genotype 1 infected and treatment-naïve, were randomized and orally treated with R1626 twice daily for 14 days and followed up for another 14 days. After oral administration, R1626 was converted to its active form R1479 efficiently. The mean reduction of HCV RNA level was 1.2, 2.6 and 3.7 $\log_{10}$ at dose of 1500, 3000 and 4500 mg, respectively. All doses were generally well tolerated and no

severe adverse effect was observed. These positive data warranted the future studies of R1626 in combination therapy for anti-HCV treatment.[301] The ongoing phase II study is evaluating the efficacy of R1626 in combination with pegylated-IFN-α2a or pegylated-IFN-α2a plus ribavirin. Recently, two novel 4'-azido-2'-deoxy nucleotides (RO-0622-TP and RO-9187-TP) have been reported with sub-micromole anti-HCV activities (IC_{50} 0.17 and 0.024 μM, respectively) without cytotoxicity at concentrations of 1 mM. [302] *In vivo*, oral administration of RO-9187 10 mg/kg could achieve effective plasma concentration above 150-fold of the IC_{50} in HCV replicon assay.[302]

Carbocyclic nucleosides

7-deaza-7-substituted neplanocin A analogs (Figure 4.2). Neplanocin A is a fermentation-derived carbocyclic nucleoside bearing a double bond in its carbocyclic ring. It exhibits potent and broad-spectrum antiviral activity. Chu and co-workers have recently described one analog, 7-deazaneplanocin A (7-DNPA), as a potent agent against orthopoxviruses (vaccinia and cowpox virus) with a very good selectivity index.[92] Further screening of this compound revealed its potent anti-HCV activity with low cytotoxicity (EC_{50} 2.5 μM, IC_{50} > 100 μM). A variety of 7-substituted 7-DNPA were therefore synthesized and evaluated against HCV. However, none of them was superior to the parent compound 7-DNPA in terms of potency and cytotoxicty. Interestingly, some of the 7-DNPA derivatives were also active against wild type as well as drug-resistant HBV variants.[303]

5'-homoneplanocin A (Figure 4.2). 5'-homoneplanocin A is another interesting nucleoside which is active against both HCV and HBV *in vitro*.[69] In HCV replicon assay in AVA5 cell cultures,

this compound demonstrated anti-HCV activity with an EC_{50} and EC_{90} of 7.3 and 31 μM, respectively.

PART II: ASYMMETRIC SYNTHESIS OF NOVEL 2'-FLUORINE(S) SUBSTITUTED ENTECAVIR ANALOGS AS POTENTIAL ANTI-HCV AGENTS

Introduction

Hepatitis C virus (HCV) infection has been recognized as a threat to the public health worldwide.[240, 241] The standard combination peginterferon/ribavirin treatment has several limitations including nonresponse, poor tolerability and high cost. [247-250] Therefore, new protocols for HCV treatment are needed.

During the past several years, a number of nucleosides analogs have been identified with potent antiviral activity (refer to Part 1, Figure 4.2). Particularly, a group of 2'-modified (numbering system indicated in Figure 4.5) furanosyl nucleosides including NM283 (**7**)[281], MK-0608 (**4**)[277, 292] and R7182 (**9**)[294, 295] (Figure 4.2) etc., have drawn considerable attention. On the basis of this information, a recent report described the synthesis of a series of 2'-β-*C*-Me cyclopentyl nucleosides (compound **15** and related guanine, cytosine and uracil derivatives, Figure 4.6) as anti-HCV agents.[304, 305] Unfortunately, none of synthesized carbocyclic compounds exhibited significant antiviral activity, despite their similar structure to the furanosyl counterparts. We performed conformational analysis of purine derivative **15** and found that the base moiety of **15** predominately took an unfavorable *syn* disposition (*vide infra*). Given the fact that antiviral activities of nucleosides are closely correlated with their specific

conformations,[166,167,306,307] the unfavorable base-disposition may explain, at least in part, the inactivity of this compound. On the other hand, we found that in the presence of an exo-cyclic double bond at the 6'-position of cyclopentyl ring (entecavir analogs **16**), the sugar part of nucleoside **16** preferred *C*3'-endo Northern conformation and importantly, its base moiety could adjust from *syn* to *anti* disposition with a very low energy barrier (*vide infra*). Furthermore, 2',2'-difluoro entecavir analog (**17**) was able to adopt a very similar conformation to that of 2'-β-*C*-Me-adenosine (**3**) (*vide infra*). Hence, modifications focusing on the 2'-postion of entecavir have directed our synthesis efforts. In Part 2 of this chapter, efforts towards the attempted synthesis of 2',2'-difluoro entecavir analog **17** are summrized. To obtain a full picture of SAR of 2'-fluorine(s) substituted entecavir analogs, the preparations of 2'-α-fluoro **18** and 2'-β-fluoro **19** are also reported here. The attempted synthesis of 2'-β-*C*-metyl entecavir analog **16** is discussed in Part 3 (*vide infra*).

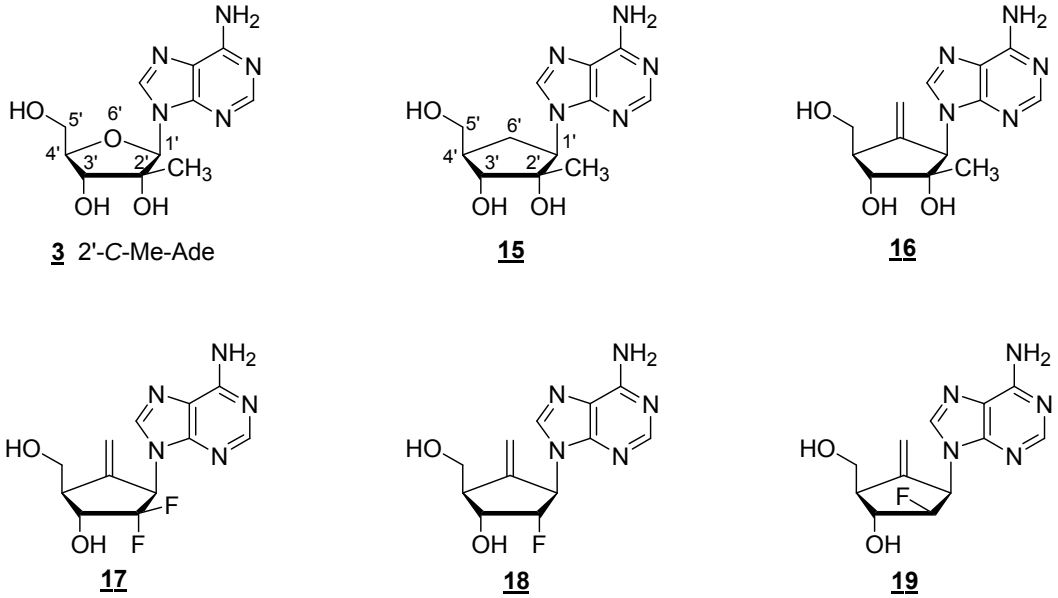


Figure 4.6. Several nucleosides with 2'-modifications

Preliminary Conformational Studies

It has been well documented that nucleoside conformation plays a crucial role in determining their antiviral activities.[166, 167, 307] In the SAR studies of purine ribonucleoside analogs as HCV NS5B inhibitors, Eldrup et al. have demonstrated that a 2'-β-*C*-methyl modification on the furanosyl sugar ring generated nucleosides with the preferred *C*3'-endo Northern sugar conformation with *anti* base disposition. This conformation was required for purine derivatives to exhibit potent anti-HCV activity.

Recently, Gosselin and Meillon et al. described the synthesis as well as the antiviral activity of a series of ribo-type carbocyclic nucleosides with the same 2'-β-*C*-mehyl modification (such as compound **15**).[304, 305] However, none of the synthesized compounds exhibited any antiviral activity against bovine viral diarrhea virus (BVDV, a pestivirus surrogate model for HCV). To understand the possible mechanism, we performed the conformational study of compound **15** as well as the active anti-HCV nucleoside 2'-*C*-Me-Ade (**3**) (Table 4.2). To our surprise, although the sugar portion of compound **15** adopted a desired *C*3'-endo Northern conformation (Figure 4.7), the base moiety of **15** strictly took an unusual *syn* disposition (χ -60.34° and 179.93° for compound **15** and **3**, respectively, Table 4.2, Figure 4.6), which may affect the phosphorylation step of the nucleoside[166, 167, 306, 307] and the further interaction with HCV polymerase.

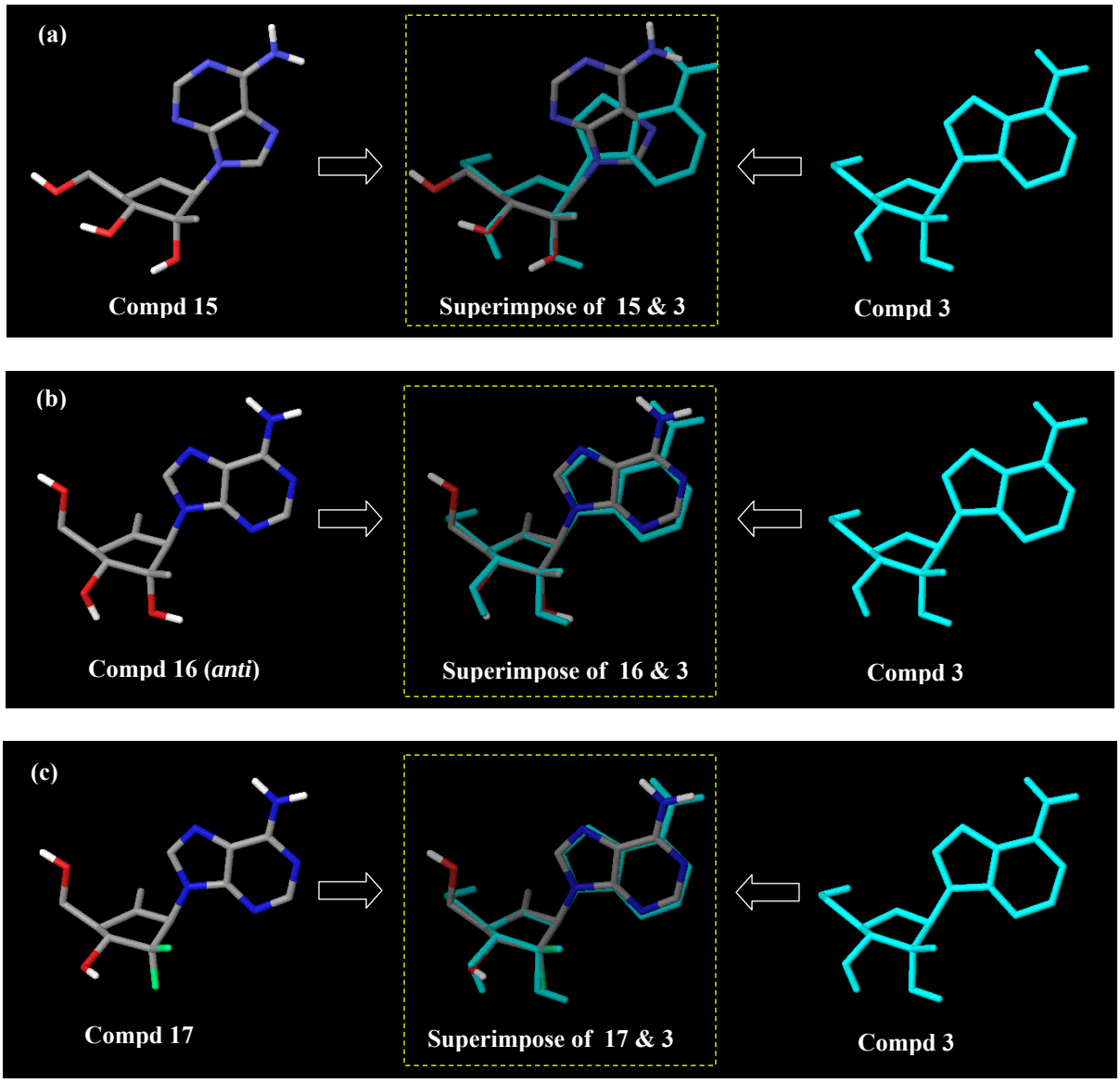


Figure 4.7. (a) Low-energy conformer of compound **15** took different base disposition with that of positive control, compound **3**; (b) Compound **16** (*anti*) had similar conformation with compound **3** and *anti* conformer of compound **16** can convert to *syn* conformer with only ~0.5 KJ/mol energy barrier; (c) Difluorine substituted compound **17** overlapped very well with compound **3**.

Table 4.2. Conformational parameters of compounds **3**, **15**, **16**, and **17**

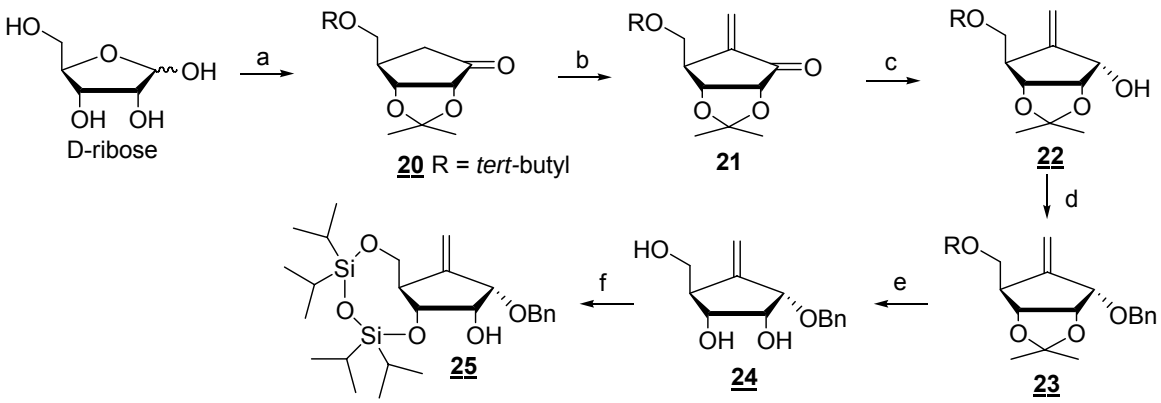
	P	**υ**	**X**	**γ**	Type
Compd **3**	18.85	39.03	-146.76	179.93	*C*3'-endo
Compd **15**	20.91	40.69	54.44	-60.34	*C*3'-endo
Compd **16** (anti)	3.66	40.06	-143.13	58.19	*C*3'-endo
Compd **17**	5.88	40.25	-146.71	58.04	*C*3'-endo

Interestingly, we noticed that 2'-β-*C*-Me modification on the entecavir scaffold (**16**) gave a better result: the sugar was still in the Northern conformation while the base can rotate almost freely from *syn* to *anti* disposition with very little energy expense (~0.5 kJ/mol) (Figure 4.7). Another modification using difluoro substitution on the 2'-position on the entecavir scaffold provided compound **17** which also adopted a very similar conformation to our positive control 2'-*C*-Me-Ade (**3**) (Figure 4.7). In view of fact that introducing fluorine substitutions on the carbohydrate moiety has been proven to be successful in producing effective antiviral agents,[115, 116, 204-206] we decided to synthesize **17** as well as its analogs, 2'-α-monofluoro **18** and 2'-β-monofluoro **19** (Figure 4.6), for the purpose of exploring the SAR of 2'-fluorine(s) substituted entecavir analogs.

Chemistry

*Synthesis of 2',2'-difloro entecavir analog **17*** Starting from D-ribose, the ketone **20** was synthesized *via* nine steps according to the known procedure developed in our group.[52, 116] The synthesis of enone **21** was accomplished by the Mannich reaction-Hofmann degradation protocol. Steric-selective reduction of enone **21** using sodium borohydride/cerium chloride heptahydrate complex ($NaBH_4/CeCl_3 \cdot 7H_2O$) gave exclusively α-hydroxyl compound **22**. After protecting the

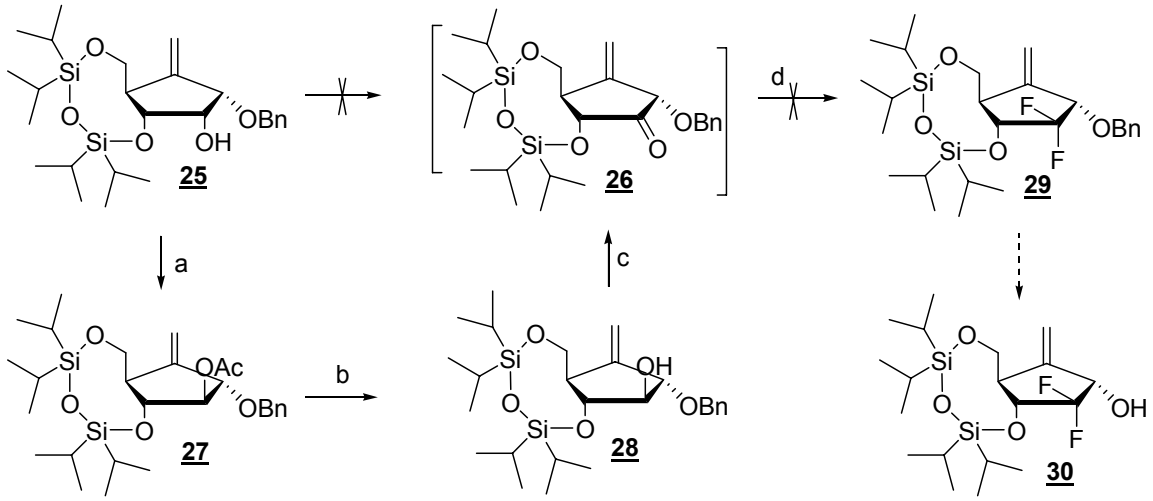
allylic hydroxyl with benzyl group, compound **23** was treated with HCl/MeOH at refluxing temperature to give triol **24** in 80% yield in two steps. The triol **24** was then protected with a tetraisopropyldisiloxane (TIPDS) group to yield compound **25** (Scheme 4.1).



Reagents and conditions: a) published method[53, 115, 116]; b) (i) LDA, Echenmoser's salt, THF, -78 °C (ii) MeI, rt (iii) sat. $NaHCO_3$ solution, rt; c) $NaBH_4/CeCl_3 \cdot 7H_2O$, THF, -78 °C; d) NaH, BnBr, THF, rt; e) 6N HCl, MeOH, 90 °C; f) TIPDSCl, Py, -30 °C to rt.

Scheme 4.1. Synthesis of intermediate **25**

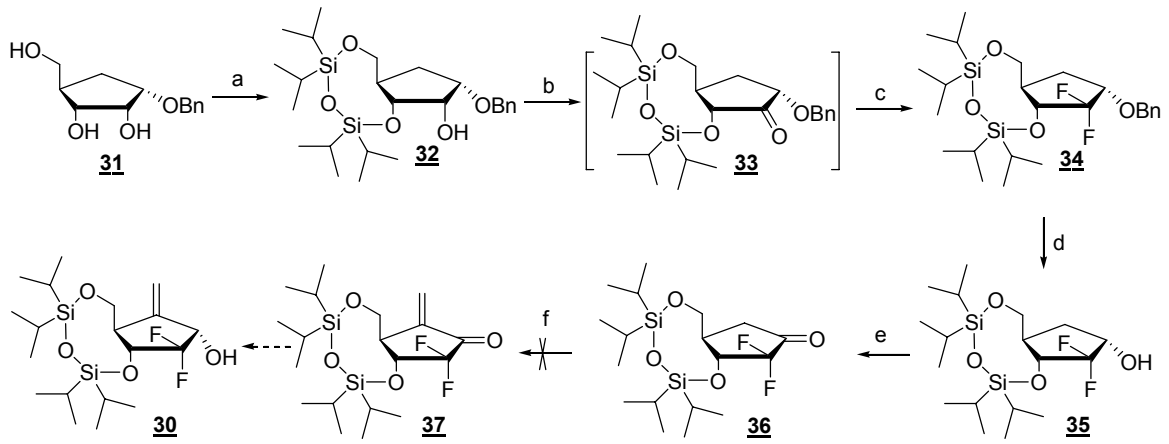
Various conditions, such as Swern, Dess-Martin and Moffart oxidation, were tried to oxidize the 2-hydroxyl group of **25**. However, all of these conditions resulted in very complex inseparable mixtures. Interestingly, when compound **28**, a 2-β-hydroxyl isomer of **25** was treated with Dess-Martin periodinane, an unstable white solid was obtained which was identified by ^{1}H-, ^{13}C-NMR and IR as the desired ketone **26**. With ketone **26** in hand, we planned to perform the difluorination reaction on the 2-position followed by debenzylation to reach the key intermediate **30,** which can be coupled with proper base moiety to give the desired nucleosides. Unfortunately, when the crude ketone **26** was treated with diethylaminosulfur trifluoride (DAST) from -78 ºC to room temperature, a very complex TLC pattern was obtained and no major spot could be isolated (Scheme 4.2).



Reagents and conditions: a) (i) Tf_2O, Py, -30 °C to rt, (ii) CeOAc, benzene, 50 °C; b) NaOMe, MeOH, rt; c) Dess-Martin reagent, CH_2Cl_2, rt; d) DAST, CH_2Cl_2 or DAST, toluene or neat DAST.

Scheme 4.2. Attempted synthesis of key intermediate **31**

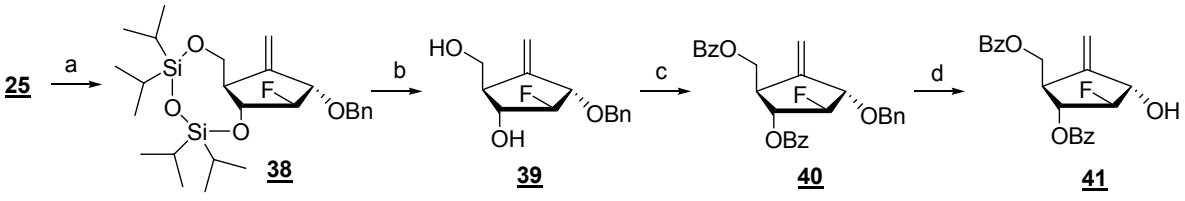
Therefore, we modified our sequence according to Scheme 4.3. Compound **32** was synthesized starting from a known triol **31**. Oxidation of **32** went smoothly under the Swern oxidation condition. The crude ketone **33** was then treated with DAST in toluene and heated in a microwave synthesizer at 80 ºC to provide difluoro compound **34**. Debenzylation of **34** using ammonium formate at refluxed temperature in MeOH provided alcohol **35** which was converted to ketone **36** using Dess-Martin periodinane. However, we were unable to introduce a methylene group on the 6'-position of compound **36** and again failed to obtain the desired key intermediate **30** (Scheme 4.3). At this point, the synthesis of 2'-difluoro entecavir analogs was abandond.



Reagents and conditions: a) TIPDSCl, Py, rt; b) Swern oxidation condition, -78 °C to rt; c) DAST, toluene, microwave assisted, 80 °C; d) ammonium formate, MeOH, reflux; e) Dess-Martin, CH_2Cl_2, rt; f) (i) LDA, Echenmoser's salt, THF, -78 °C (ii) MeI, rt (iii) sat. $NaHCO_3$ solution, rt.

Scheme 4.3. Alternative synthesis of key intermediate **30**

Synthesis of 2'-α-fluoro entecarvir analogs Transformation of the 2-β-hydroxyl group to 2-α-fluorine was accomplished by treating the alcohol **25** with DAST (Shceme 4.4). However, debenzylation of **38** was unsuccessful under Birch reduction condition or lewis acids condition (BCl_3). Therefore, the silyl group of the fluorinated compound **38** was changed to benzoyl groups to provide **40**. Compound **40** was then treated with BCl_3 at -78 °C to obtain key intermediate **41** in 95% yield (Scheme 4.4).

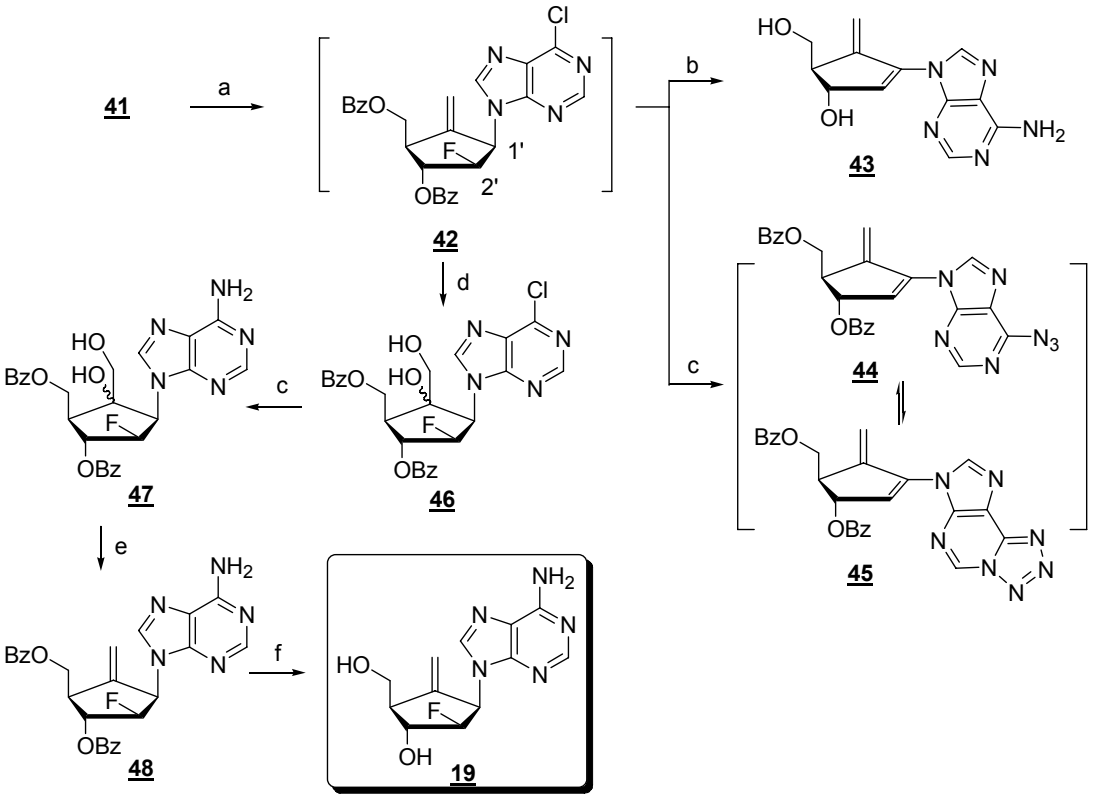


Reagents and conditions: a) DAST, CH_2Cl_2, rt; b) TBAF, THF, rt; c) B_ZCl, Py, rt; d) BCl_3, DCM, -78 °C.

Scheme 4.4. Synthesis of key intermediate **41**

Key intermediate **41** was condensed with 6-chloropurine under the standard Mitsunobu condition to yield a crude product **42** which was contaminated with reduced diisopropyl azodicarboxylate (DIAD) species and directly used for the next step without further purification (Scheme 4.5). However, amination of the 6-chloropurine derivative to the corresponding adenine derivative by methanolic ammonia at ~100 °C was unsuccessful. Only a byproduct **43** was isolated which was formed by the loss of a HF under the basic condition. To our surprise, the elimination reaction dominated even under sodium azide condition. It was speculated that the stability of the elimination product, a conjugated diene (such as **43**, **44** or **45**), is the driving force to promote the side reaction. Therefore, transient protection of the exo-cyclic double bond of compound **42** was required. Compound **42** was hence treated with osmium tetraoxide/NMO to provide a diol **46**. As we anticipated, conversion of **46** to the adenine derivative **47** went smmothly by reacting with sodium azide followed by H_2 reduction. Several conditions were studied to regenerate the olefin from diol. The Corey's olefin synthesis by the desulfurization of 1,3-dioxolane-2-thiones with 1,3-dimethyl-2-phenyl-1,3,2-diazaphospholidine is well known due to its mildness and effectiveness.[308] However, when we applied this condition to compound **47**, only complex reaction mixture was obtained. Another general method by heating 2-methoxy-1,3-dioxolane derivatives in acetic anhydride was also unsuccessful in the present case due to the high reaction temperature.[309] Finally, we adopted the reductive elimination protocol, which was widely used in the synthesis of 2', 3'-dideoxy-2', 3'-dihydro nucleosides or 2',3'-dideoxy nucleosides.[310-312] Diol **47** was treated with 1-bromocarbonyl-1-methylethyl acetate followed by activated Zn in DMF in the presence of catalytic amount of HOAc at room temperature for 8 hours to furnish desired nucleoside **48** in 68% yield in two steps. Eventually, the target adenosine

analog **19** was obtained in 76 % yield, by the treatment of protected **48** with diisobutylaluminium hydride (DIBAL-H) in CH_2Cl_2 at -78 °C.



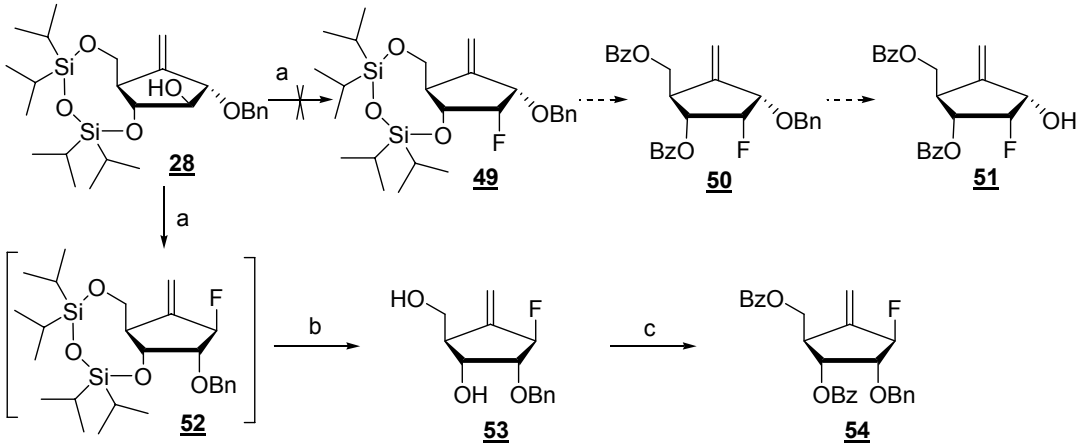
Reagents and conditions: a) DIAD, Ph_3P, 6-chloropurine, THF, rt; b) NH_3, MeOH, 100 °C; c) (i) NaN_3, DMF, 140 °C, (ii) H_2/Pd/C, EtOH, rt; d) OsO_4/NMO, Acetone/H_2O, rt; e) (i) 1-bromocarbonyl-1-methylethyl acetate, acetonitrile, rt; (ii) Zn/HOAc, DMF; f) DIBAL-H, CH_2Cl_2, -78 °C

Scheme 4.5. Synthesis of target adenosine analog **19**

*Synthesis of 2'-β-fluoro entecavir analog **18*** The first attempt of the synthesis of 2'-β-fluoro isomer started from the intermediate **25**. Based on our experience with the 2'-α-fluoro nucleosides, compound **51** would be a good intermediate for coupling with base moieties to provide 2'-β-fluoro nucleosides. Unfortunately, treating intermediate **28** with DAST in CH_2Cl_2 at room temperature gave only benzyl migrated compound **52** instead of desired compound **49**

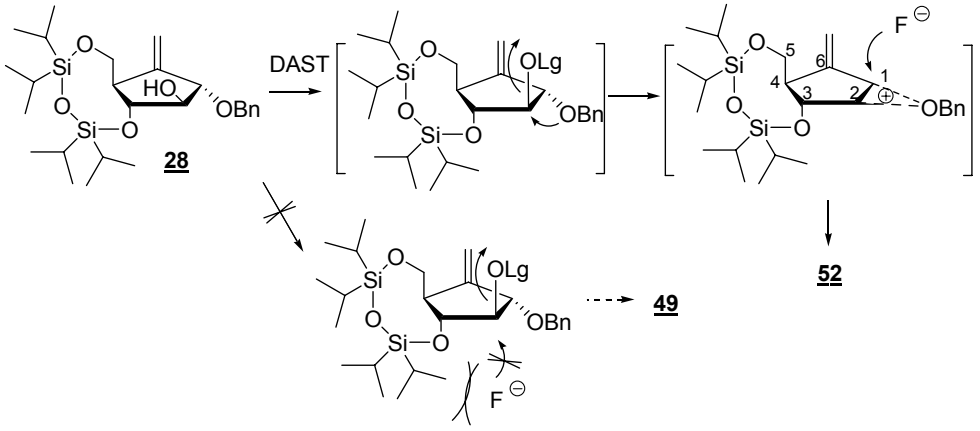
(Scheme 4.6.a). After the silyl groups of **52** were converted to benzoyl groups, the structure of compound **54** was fully identified by ^{1}H-NMR, ^{13}C-NMR, HRMS, DEPT, COSY, NOE as well as HMBC spectroscopy. The mechanism of this abnormal reaction was believed to be occurred *via* a three-member ring intermediate, which was then attacked by the fluoride ion from the less hindrance α-face on the allylic position (Scheme 4.6.b). Actually, benzyl migration have been also observed in similar cases in carbohydrate chemistry.[313-315]

(a)



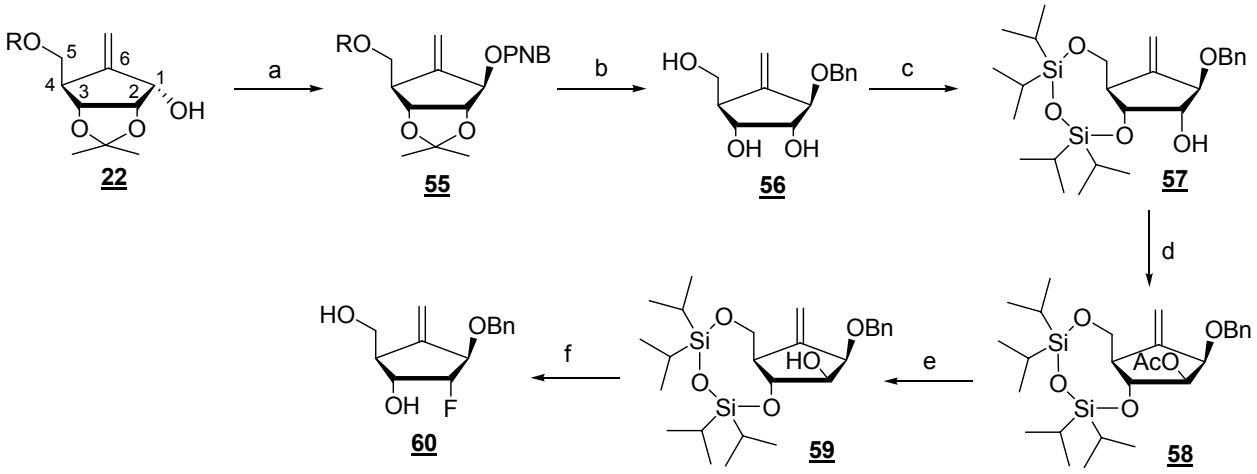
Reagents and conditions: a) DAST, CH_2Cl_2, rt; b) TBAF/HOAc, THF, rt; c) B_ZCl, Py, rt

(b)



Scheme 4.6. (a) Attempted synthesis of key intermediate **51**; (b) Proposed mechanism of benzyl migration during fluorination reaction of compound **28**

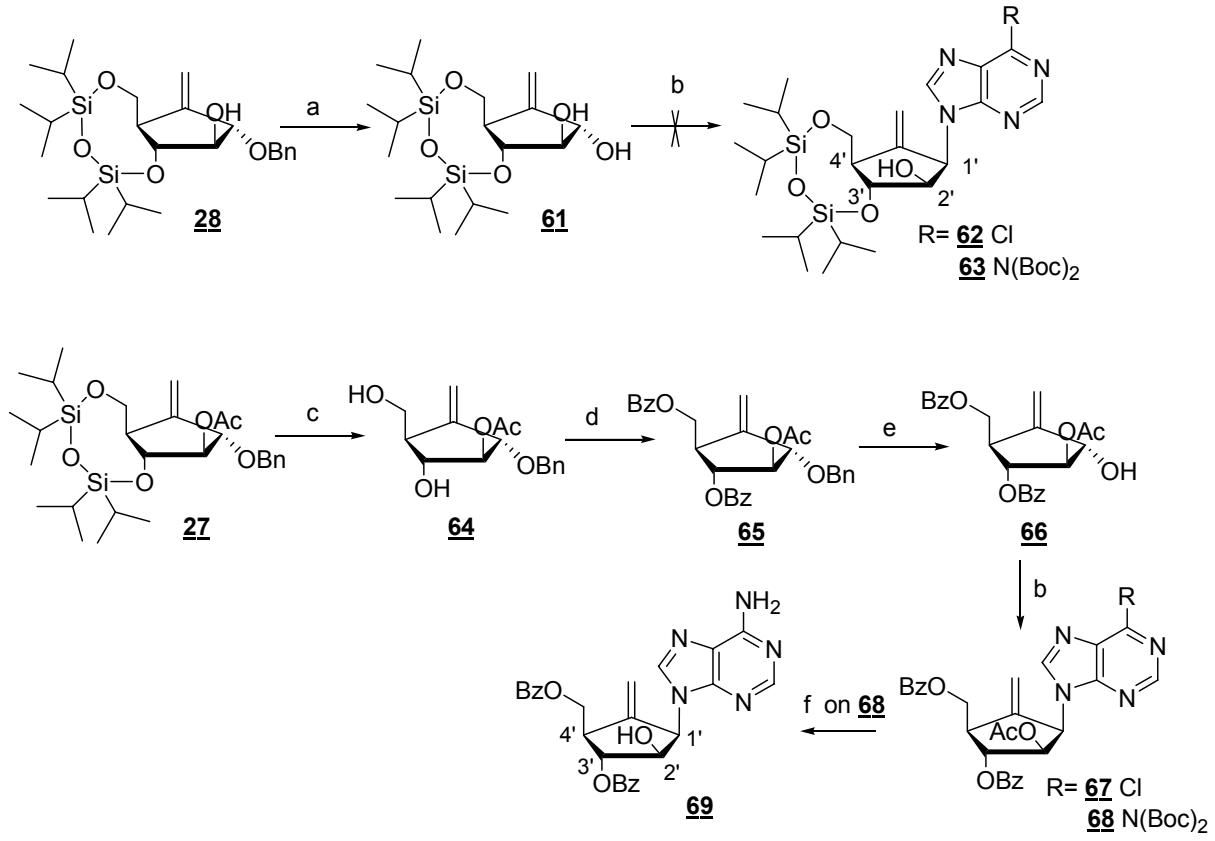
In view of the fact that the steric hindrance near the α-face of 2-position may block the approach path of the incoming fluoride ion from the rearside of the activated alcohol, it was of interest to check the fluorination reaction after reversing the configuration of the 1-hydroxyl group (Scheme 4.7). The Mitsunobu protocol was conducted to convert the 1-hydroxyl group from α to β face to yield the desired *p*-nitrobenzonate **55**. Protecting group manipulations led to triol **56** in 64 % yield from **55**. The 3- and 5-hydroxyl groups in triol **56** were selectively blocked with TIPDS group to afford compound **57** in good yield. Triflation/SN_2 reaction/deprotection sequence was applied to reverse the configuration of 2-hydroxyl of **57** to generate alcohol **59**. With **59** in hand, we were able to try the fluorination reaction at this stage. As we expected, desired compound **60** was isolated after the deprotection step, but in a very low yield (6%). Using **60** as a key intermediate to synthesize the target nucleoside is not feasible due to the low yield, but we realized that reducing the steric hindrance near the α-face of 2-position was one of the keys for the successful fluorination reaction.



Reagents and conditions: a) DIAD, Ph_3P, *p*-nitrobenzoic acid, THF, rt; b) (i) NaOMe/MeOH, rt, (ii) NaH, BnBr, rt, (iii) 3N HCl, MeOH, refluxing; c) $TIPDSCl_2$, Py, 0 °C to rt; d) (i) Tf_2O, Py, -30 °C to rt, (ii) CeOAc, benzene, 50 °C; e) NaOMe, MeOH, rt; f) (i) DAST, CH_2Cl_2, rt, (ii) TBAF, THF, rt.

Scheme 4.7. Synthesis of intermediate **60**

Apparently, nucleosides such as **62**, **63** and **69** bearing arabino-configuration have little steric hindrance on the α-face of 2'-position, which may lead **62**, **63** and **69** as good substrates for introducing α-fluorine by DAST reaction. Furthermore, if fluorination reaction was successful, the target nucleosides could be easily obtained by one simple deprotection step. Therefore, we attempted to synthesize compounds **62**, **63** and **69** for the purpose of testing DAST reaction on these substrates (Scheme 4.8).

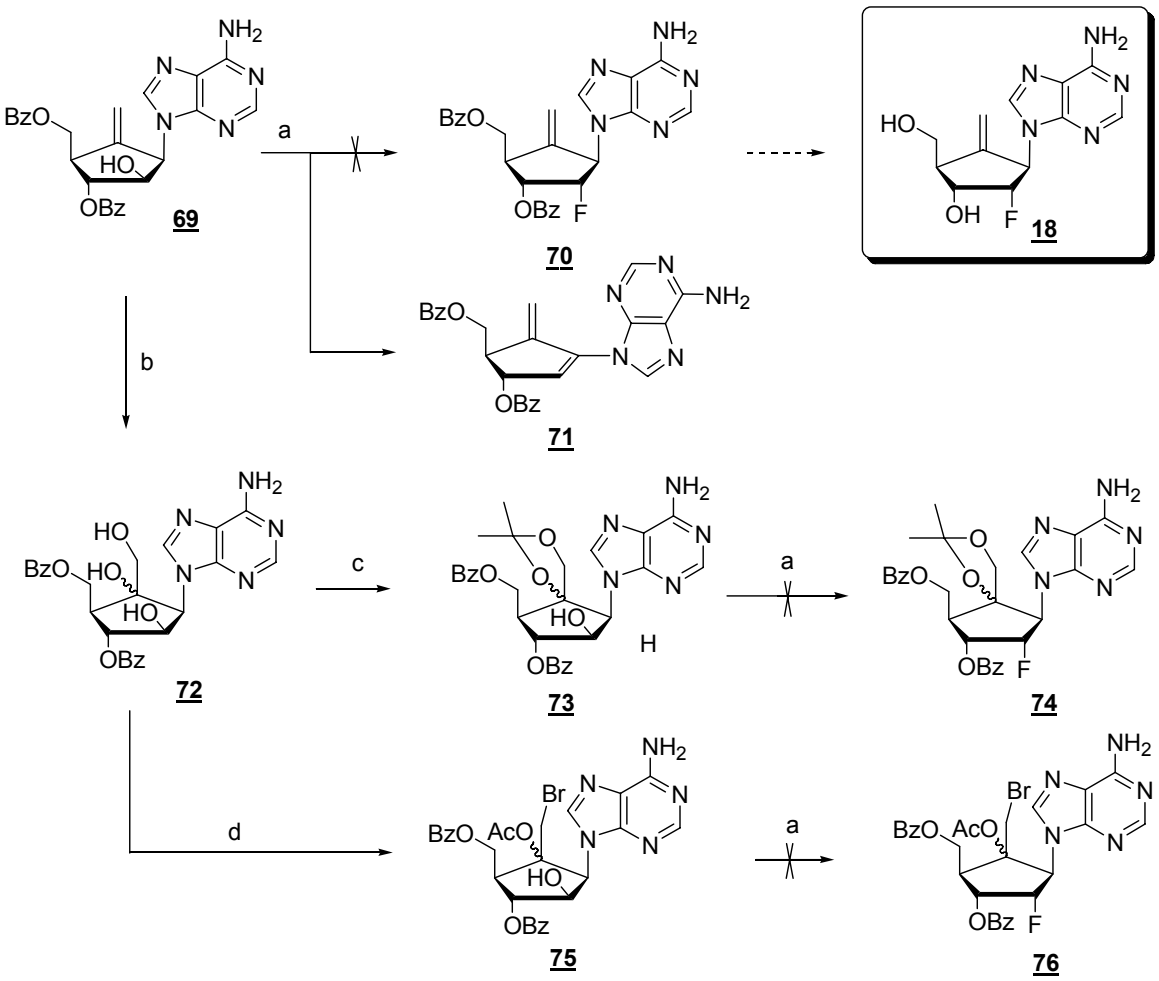


Reagents and conditions: a) Na/NH_3(liq), -78 °C b) DIAD, Ph_3P, base, THF, rt; c) TBAF/HOAc,THF, rt; d) B_ZCl, Py, rt; e) BCl_3, -78 °C; f) (i) TFA/CH_2Cl_2, rt, (ii) HCl/MeOH, rt.

Scheme 4.8. Synthesis of adenosine intermediate **69**

To reach the proper nucleoside intermediate with only a 2-hydroxyl free, the 3', 5'-TIPDS functionality was employed. Compound **61**, which was prepared from **28** by the Birch reduction, was unable to condense with base moieties under Mitsunobu condition. Presumably the 2-hydroxyl group may cause problem during coupling reaction. A modified sequence was employed as shown in Scheme 4.8. Compound **27** was deprotected under TBAF/HOAc condition and then re-protected with benzoyl groups to give compound **48**, which was treated with BCl_3 at -78 °C to smoothly provide intermediate **66** in high yield. Compound **66** was able to coupled with either 6-chloropurine or di-*tert*-butyl carbamate (Boc) protected adenine to give the corresponding nucleosides **67** or **68**. Nucleoside **68** was further deprotected under acidic condition to give 2'-β-hydroxyl adenine derivative **69** in 52 % yield from **66** (Scheme 4.8).

With adenine derivative **69** in hand, several conditions were explored to introduce 2'F from the α-face (Scheme 4.9). However, when compound **69** was treated with DAST in CH_2Cl_2 in the presence of excess of pyridine, only elimination product **71** was obtained instead of the desired fluorinated compound **70**. Since we had a similar experience in the case of synthesis of α-2'-F compound, we decided to temporarily protect the exo-cyclic double bond before conducting fluorination reaction. Treatment of nucleoside **69** with OsO_4/NMO smoothly generated the triol **72** which was further protected with acetonide group to yield compound **73**. Unfortunately, the fluorination reaction of **73** only gave a complex mixture. Similarly, the treatment of compound **75** with DAST resulted in an unseparable high-polarity mixture. Once again, introduction of a fluorine atom to the α-face on 2'-position at the nucleosides level were unfruitful.

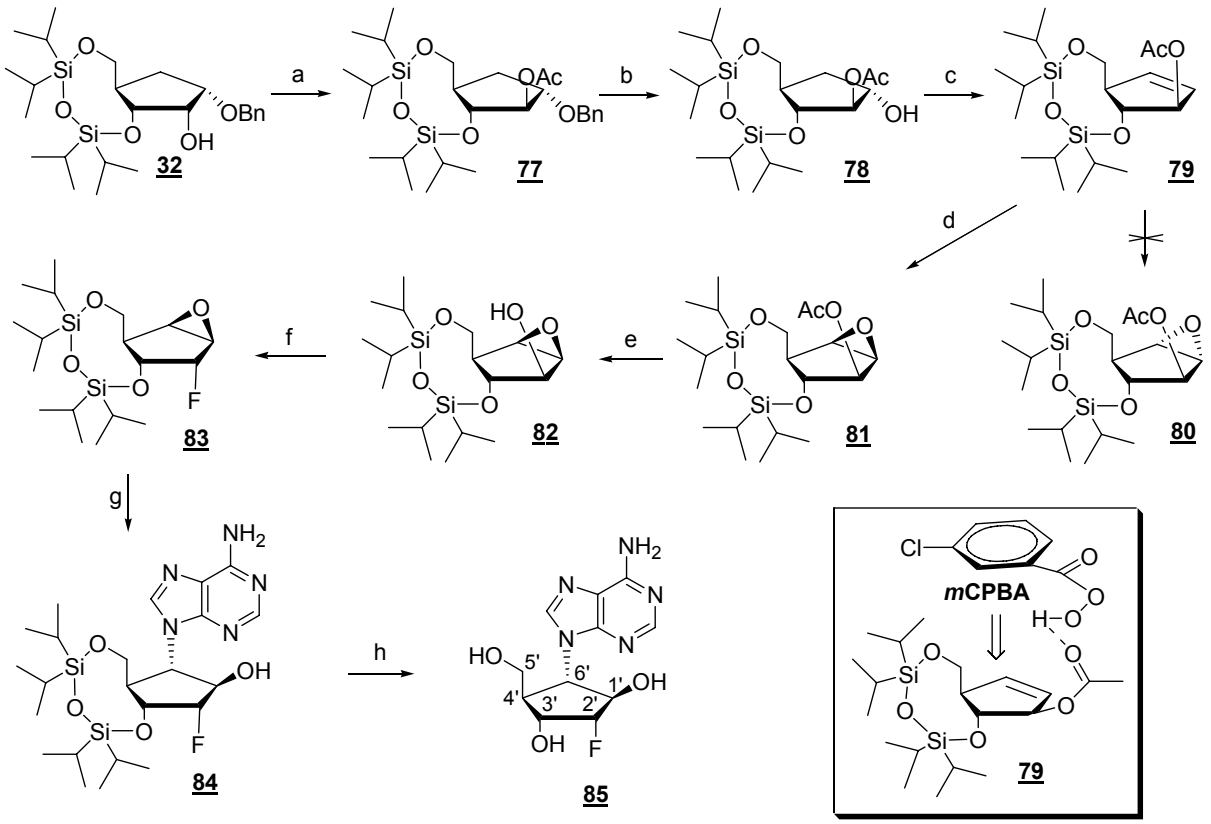


Reagents and conditions: a) DAST/Py, CH_2Cl_2, rt; b) OsO_4/NMO, acetone, H_2O; c) 2,2-dimethoxypropane/CSA, acetone, rt; d) α-AIBBr, acetonitrile/H_2O, 0 °C-rt;

Scheme 4.9. Attempted synthesis of target compound **18** at nucleoside level

The information obtained from previous unsuccessful experiences led us to reconsider the whole synthetic plan. Our new synthetic route (scheme 4.10) relies on an epoxide opening reaction *via* similar method described in the entecavir synthesis.[122] The initial scheme commenced from the alcohol **32**, readily obtained *via* protecting-group manipulation from triol **31** (*vide supra*) in high yield. Standard two-step protocol gave the 2-acetate compound **77** which was deprotected using transfer hydrogenation to provide alcohol **78** in quantative yield. The

alcohol **78** was subjected to Mitsunobu-type elimination reaction to give, although in a low yield, olefin **79**. Epoxidation of the endo-cyclic double bond was carried out using *m*-chloroperoxybenzoic acid (*m*CPBA). From the oxidation reaction, an α-epoxide was expected to be obtained due to the steric crowding of 2-acetate group on the β-face. However, it was difficult to identify the sterochemistry at this stage because of overlapping signals in the ^{1}H-NMR as well as NOE spectrum, Therefore, epoxide **81** was deprotected, fluorinated and condensed with base moiety to provide nucleoside **84** which was further desilylated to obtained free nucleoside **85,** contaminated with tetrabutylammonium salt. The ^{1}H NMR of **85**, assigned with the aid of a 2D-COSY analysis, showed that H-1' instead of H-6' had a correlation with the free hydroxyl group, which indicated that the base moiety was attached at the 6-position of the epoxide **67** with the free hydroxyl on the 1'-position. Furthermore, in NOE experiments, irradiation of H-4' gave rise to a significant enhancement of the integral associate with the proton on the base moiety indicating the short distance between the H-4' and base. Therefore ^{1}H NMR, 2D-COSY and NOE spectrums concomitantly corroborated the structure of **85** as an undesired product as shown in scheme 4.10. Apparently, the β-epoxide **81,** instead of desired compound **80,** was the actual product obtained during the epoxidation reaction. Presumably, *m*CPBA approached predominantly from the β-face of the olefin **79**. No explanation is available on precisely why this selectivity can be achieved, although it is clear that the selectivity of *m*CPBA reaction depends on not only the steric but also the electronic environment around the reaction site in case there is no allylic or homo-allylic hydroxyl, which can form a hydrogen bond with *m*CPBA and control the steric chemistry (lower right panel in Scheme 4.10).[316]

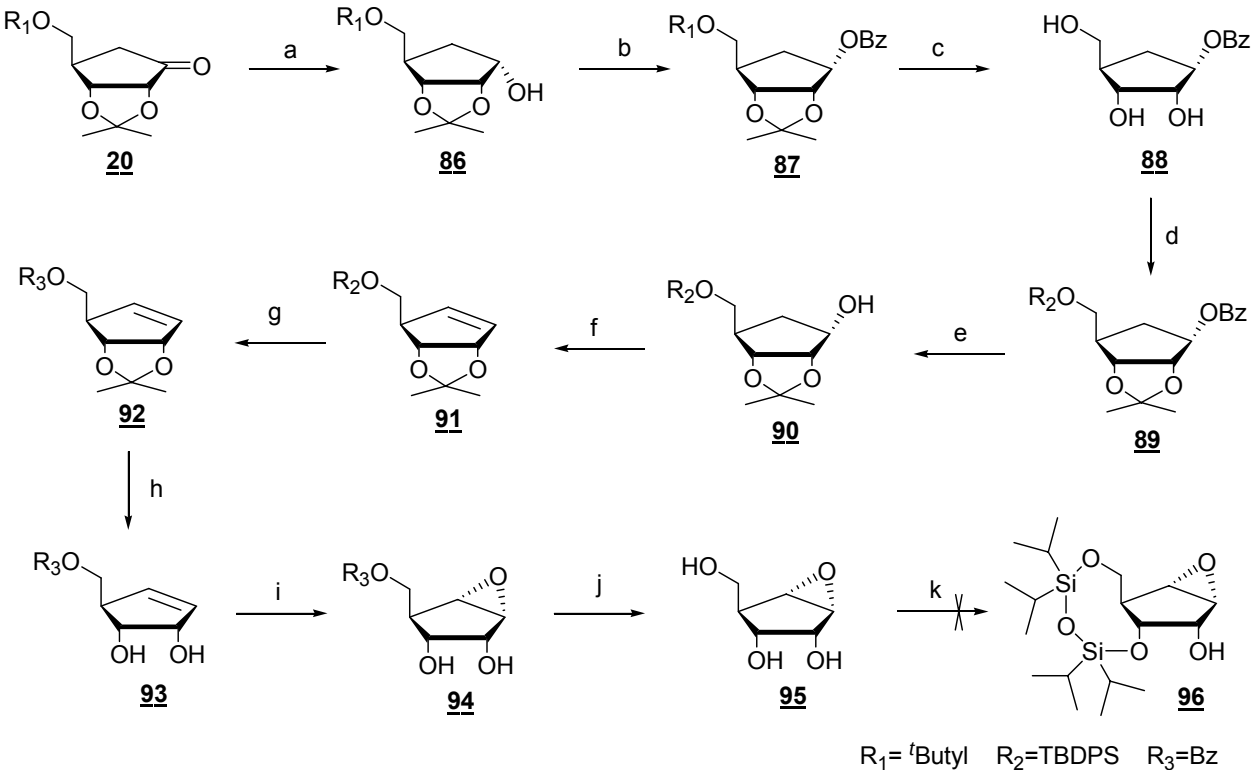


Reagents and conditions: a) (i) Tf_2O, Py, -30 °C to rt, (ii) CeOAc, benzene, 50 °C; b) Ammonium formate, Pd/C, MeOH, refluxing; c) TPP, DIAD, toluene, 85 °C; d) mCPBA, CH_2Cl_2; e) NaOMe, MeOH; f) DAST, CH_2Cl_2; g) Adenine, NaH, 15-C-5, DMF, 130 °C; h) TBAF, THF, rt.

Scheme 4.10. Synthesis of undesired nucleoside **85**

The unexpected epoxidation reaction in scheme 4.10 prompted us to introduce the epoxide in such a way that sterochemistry can be controlled unequivocally. For such chemistry to be applicable in the present case, an olefin with 2-α-hydroxyl group would be required to direct the approach of *m*CPBA.[316] The new sequence (Scheme 4.11) was conducted starting from ketone **20** which was reduced, followed by Mitsunobu inversion of the hydroxyl group to give the compound **87**. Standard protecting-group manipulation provided alcohol **90** in 63% yield. Mitsunobu-type elimination reaction was optimized to afford unsaturated carbocycle **91** using microwave conditions which increased the yield reduced the reaction time (Table 4.3). However,

the selective deprotection of acetonide group of **91** was not successful. Consequently, a TBDPS group was replaced by a benzoyl group on the 5-position follpwed by the acidic hydrolysis to give the desired intermediate **93** in 83% yield. Although epoxidation and debenzoylation went smoothly in the next two steps to obtain triol **95**, selective protection of 3,5-hydroxyl groups by TIPDS group was unsuccessful in spite of several attempts.



Reagents and conditions: a) $NaBH_4/CeCl_3 \cdot 7H_2O$, THF, -78 °C; b) TPP, DIAD, benzoic acid, THF, 50 °C; c) $TiCl_4$, CH_2Cl_2, 0 °C to rt; d) (i) TBDPSCl, Im, CH_2Cl_2, (ii) 2,2-dimethoxypropane, PTSA, acetone, rt; e) NaOMe, MeOH, rt; f) TPP, DIAD, toluene, MW 90 °C; g) (i) TBAF, THF, rt, (ii) benzoyl chloride, Py. Rt; h) 3N HCl / MeOH, rt; i) mCPBA, CH_2Cl_2, rt; j) NH_3 / MeOH, rt; k) $TIPDSCl_2$, Py. rt to 80 °C.

Scheme 4.11. Attemplted synthesis of intermediate **96**

Therefore, a modified procedure was adopted as shown in scheme 4.12. Compound **87** was debenzoylated and subjected to Mitsunobu-type elimination as described in scheme 4.10 to provide olefin **98**. Again, microwave condition was superior to the conventional method in terms of reaction time and yield (Table 4.3).

Table 4.3. Elimination reactions using traditional or microwave (MW)-assisted methods

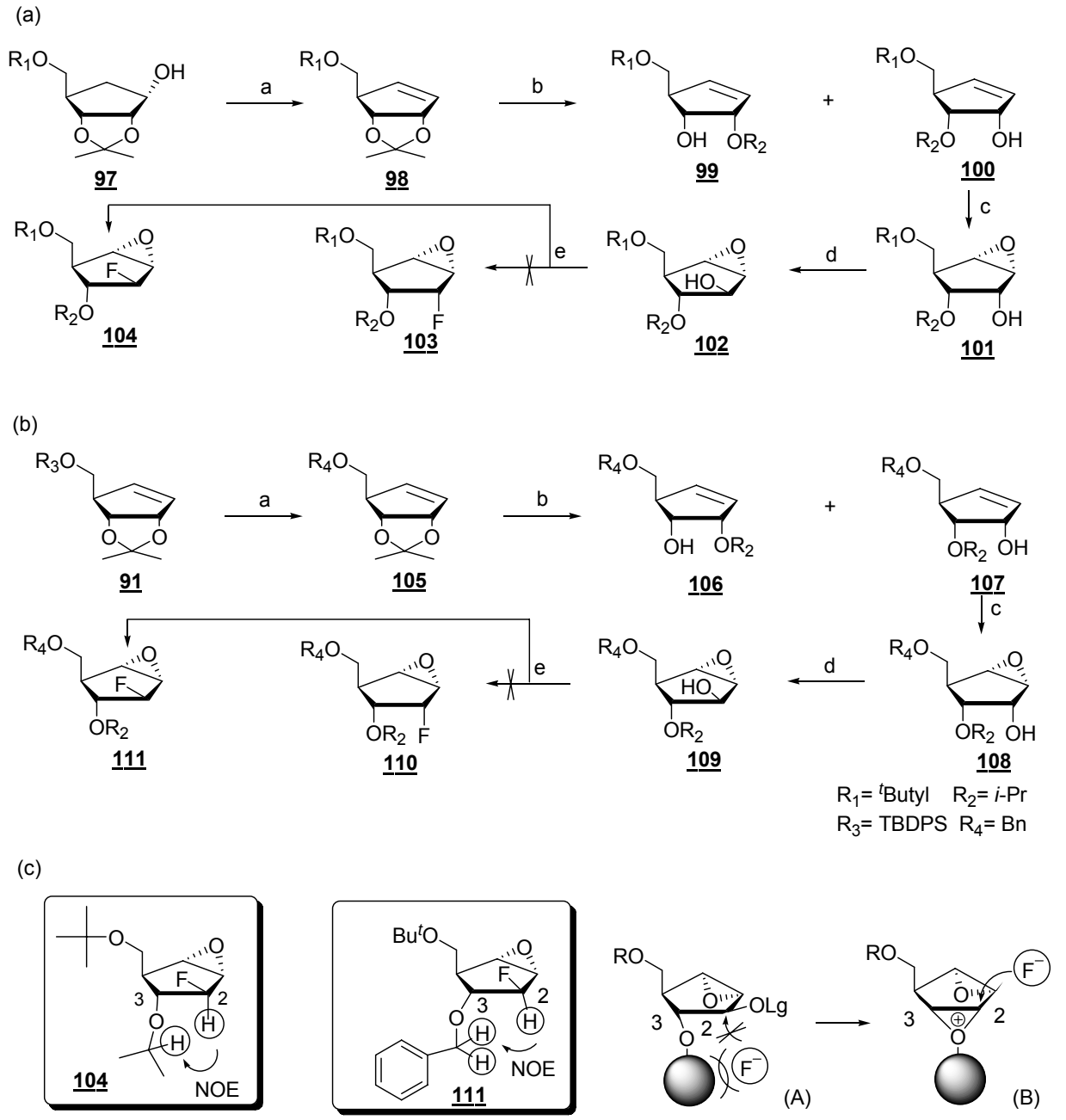
Entry	Substrate	Temperature	Reaction time	Isolated yield (%)	Methods
1	Compd. **90**	90 °C	2.5 h	60	traditional oil bath heating
2	Compd. **90**	90 °C	10 min	89	MW-assisted, open vessel
3	Compd. **97**	80 °C	1.5 h	81	traditional oil bath heating
4	Compd. **97**	80 °C	10 min	94	MW-assisted, open vessel

The olefin **98** underwent the reductive acetonide deprotection using DIBAL-H in CH_2Cl_2 to give the desired 3-isopropyl protected alcohol **100**, along with the isomer **99** with a ratio of 2.5 to 1 as indicated by ^{1}H-NMR of a crude product. Epoxidation of **100** by *m*CPBA gave desired compound **101** which was directly used for the next step without further purification. Following a standard two-step Mitsunobu hydroxyl inversion protocol, the alcohol **102** was able to be prepared in 90 % yield. Unfortunately, treatment of alcohol **102** with DAST provided the β-fluorine compound **104** instead of desired α-fluorine **103**, which was evidenced by NOE studies.

The H-2 showed NOE effect with 3-isopropyl group indicating a β-fluorine configuration of **104**, as shown in Scheme 4.12.

The unexpected fluorination reaction was observed even after changing the protection group (scheme 4.12 b). An undesired fluorinated compound **111** was obtained upon the treatment of **109** with DAST. This was also confirmed by NOE studies of compound **111**. Actually, the difficulties of fluorination reaction presented herein have been observed in our previous synthesis (*vide supra* scheme 4.6). Therefore, neighboring group participations were always observed when the approach path of the incoming fluoride ion to the activated alcohol was relatively hindered as indicated in the right panel of scheme 4.12 c.

In summary, various conditions and approaches have been studied towards the synthesis of 2'-fluorine(s) substituted entecavir analogs **17**, **18** and **19**. One of the target molecules, 2'-α-fluoro entecavir analog **19** has been successfully prepared *via* a 24-step sequence starting from D-ribose. However, all attempts to synthesize the other two compounds **17** and **18**, met with failure. Actually, with an exo-cyclic double bond on the 6'-position of the cyclopentyl ring, the protons on the 1' & 4'-position have been activated. Therefore, any reaction, such as the fluorination reaction, involved in the possible formation of carbocation on the nearby atom may cause rearrangement of the exo-double bond or elimination of H-1' and/or H-4' that will significantly complicate the desired process. The limitations associated with the development of 2'-fluorine(s) substituted entecavir analogs have now been defined. Nevertheless, the enlightment gained in the current study provides important information for designing alternative entecavir analogs as potent antiviral agents.



Reagents and conditions:a) For **98**: (i) NaOMe, MeOH, rt, (ii) Ph_3P/DIAD, toluene, MW 80 °C; For **105**: (i) TBAF, THF, rt, (ii) BnBr/TBAI/NaH, THF, rt; b) DIBAL-H, CH_2Cl_2, rt; c) *m*CPBA, CH_2Cl_2, rt; d) (i) Ph_3P/DIAD/Benzoic acid, THF, rt, (ii) NaOMe, MeOH, rt; e) DAST, CH_2Cl_2, -78 °C-rt;

Scheme 4.12. (a) & (b) Modified schemes based on the epoxide-opening route; (c) Proposed mechanism of unexpected fluorination reaction

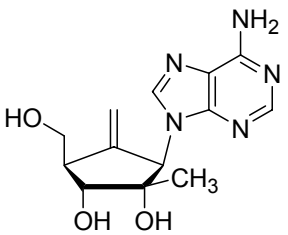
Antiviral Activities

Compound **19** was evaluated against a variety of viruses, including Rhinovirus, parainfluenza virus, respiratory syncytial virus-A, Adeno, Tacaribe, SARS, Flu A (H5N1), Flu B, Yellow fever, Rife Valley Fever and Dengue viruses. However, no significant antiviral activity or cytotoxicity was found. The anti-HCV screening of compound **19** is still in progress.

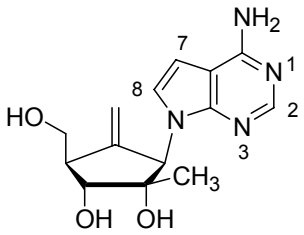
PART III: ASYMMETRIC SYNTHESIS OF NOVEL 2'-β-*C*-METHYL-2'-HYDROXYL ENTECAVIR ANALOGS AS POTENTIAL ANTI-HCV AGENTS

Introduction

Based on our initial molecular modeling studies (*vide supra*), we realized that adenine derivative **16** can adopt a favorable conformation with 3'-endo sugar ring and *anti* base disposition. However, 2'-β-*C*-methyl adenine derivatives could be deaminated to hypoxanthine by adenosine deaminase (ADA)[276, 277] and the glycosidic bond hydrolyzed *via* purine nucleoside phosphorylase (PNP).[277] On the contrary, a 7-deaza modification on the adenine moiety has been shown to be stable to both enzymes.[277] Furthermore, our modeling studies indicated a perfect overlap between the low-energy conformations of adenine **16** and 7-deazaadeninederivative **112** (Figure 4.8).



16



112

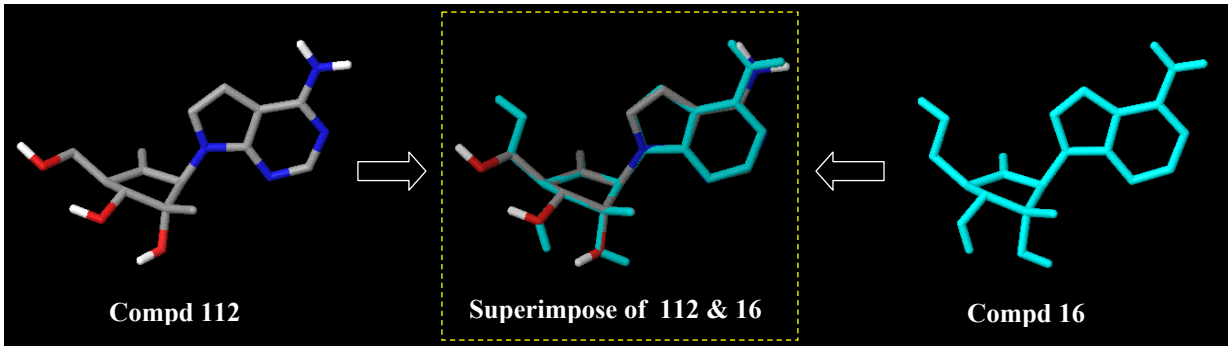


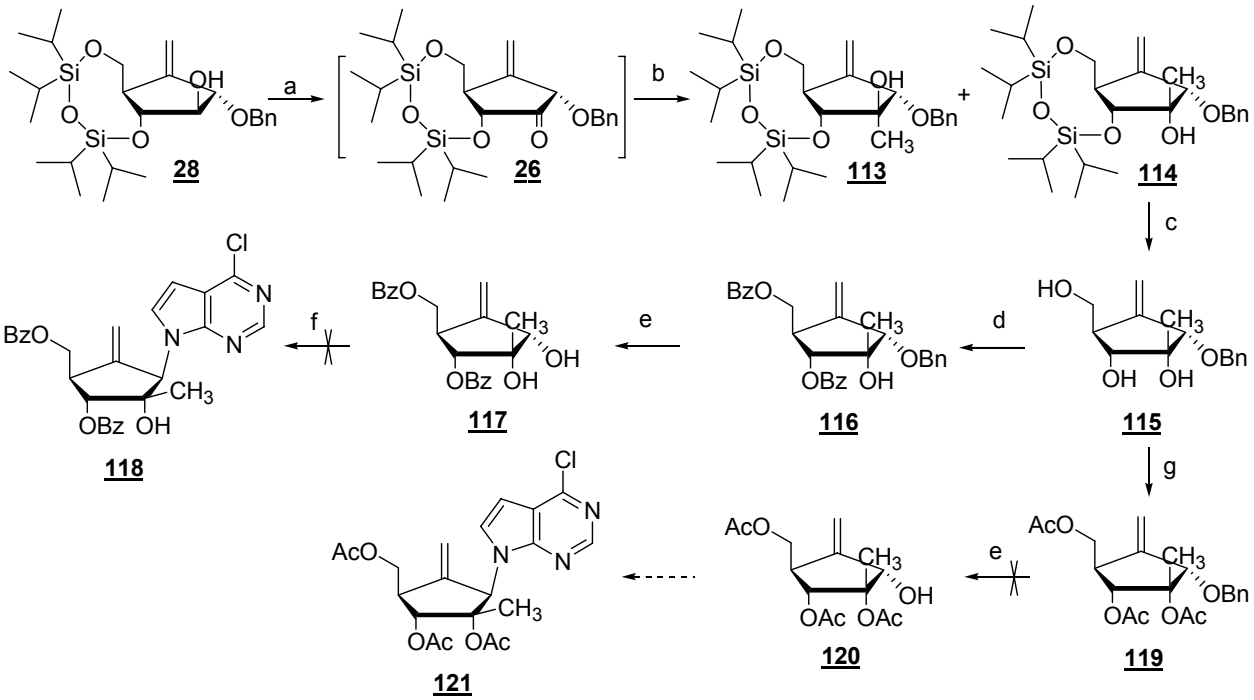
Figure 4.8. Low-energy conformers of compound **16** and its 7-deaza analog **112** overlapped very good

Actually, 2'-β-*C*-methyl-2'-hydroxyl entecavir analogs were briefly described in a patent published in 2003.[317] However, only limited experimental details were provided and most importantly, no biological data were disclosed. Therefore, it is of great interest to prepare compound **112**, which can serve as a parent compound for further modifications on its 7-position to generate a series of 7-functionalized analogs *via* similar strategy employed by Eldrup et al.[277] as well as our group.[303] Herein, we reported the efforts towards the synthesis of compound **112**.

Chmeistry

The synthesis of compound **112** commenced from the intermediate **28** based on Scheme 4.13. Oxidation of **28** using Dess-Martin periodiane provided an unstable ketone **26**, which was directly subjected to the Grignard addition to yield β-methyl compound **114** (61% yield) as well as α-isomer **113** (22% yield). The desired compound **114** was then deprotected using TBAF to afford triol **115**. However, treatment of **115** with benzoyl chloride in the presence of DMAP in pyridine at 40 ºC overnight only gave a partially protected compound **116**, leaving tertiary 2-hydroxyl free. Debenzylation of compound **116** went smoothly using BCl_3 at -78 ºC. Unfortunately, Mitsunobu coupling of diol **117** with base moiety was unsuccessful and gave a

very complex TLC pattern. Presumably, the 2-hydroxyl was the cause of the problem during coupling reaction. Therefore, all three hydroxyl groups were protected with acetates under more harsh conditions to afford fully protected intermediate **119**. However, difficulty in deblocking the 1-benzyl group of compound **119** prompted us to pursue a new scheme.

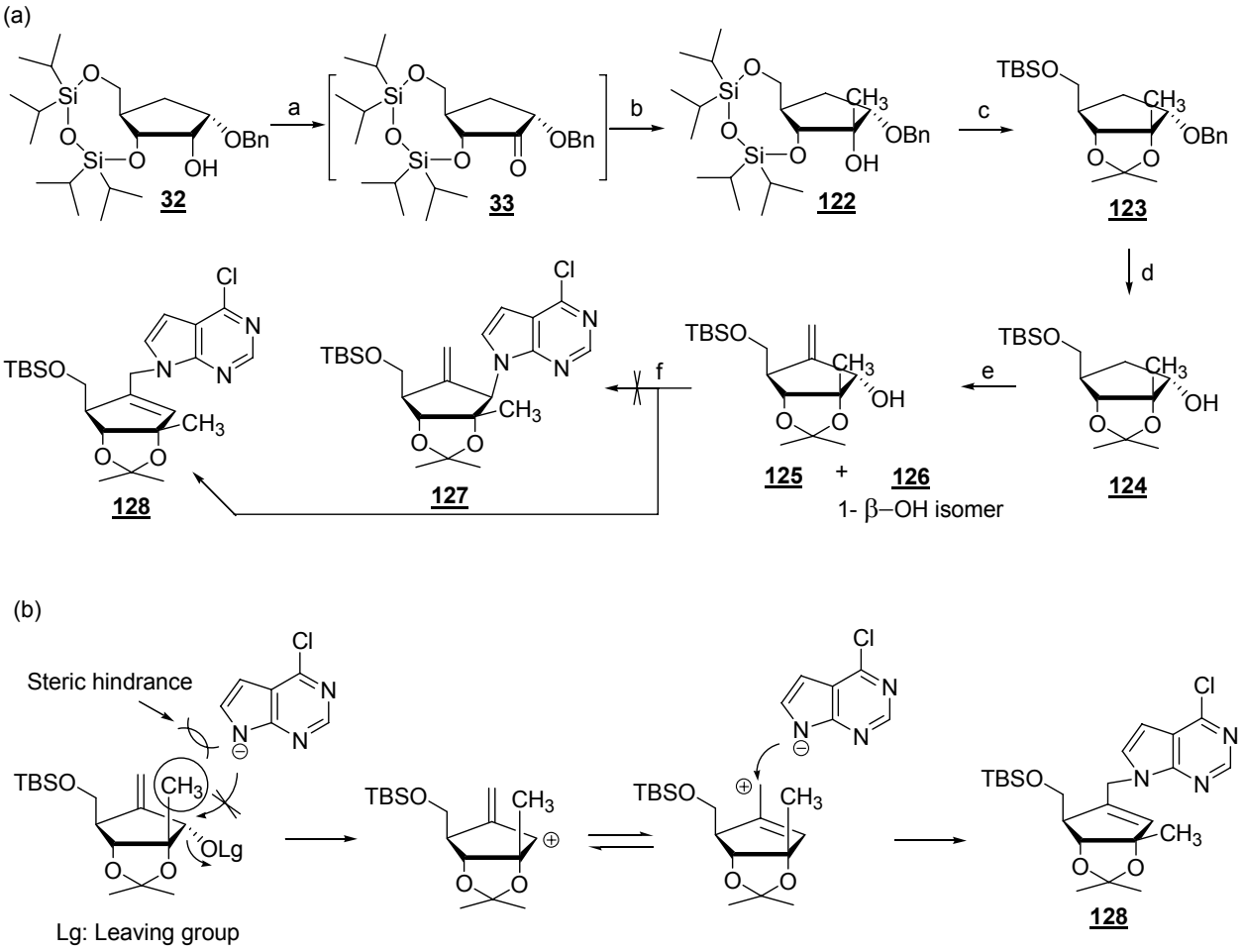


Reagents and conditions: a) Dess-Martin periodiane, CH_2Cl_2, rt; b) CH_3MgBr, Et_2O, -78 °C - 30 °C; c) TBAF, THF, rt; d) BzCl, DMAP, Py, 40 °C; e) BCl_3, CH_2Cl_2, -78 °C; f) Ph_3P, DIAD, 6-chloro-7-deazapurine.

Scheme 4.13. Attempted syntheses of intermediate **118** and **121**

The new scheme 4.14 focused on the synthesis of key intermediate **125**. Oxidation of **32** followed by Grignard addition and protecting group manipulations provided compound **123**. Deprotection of benzyl group using transfer hydrogenation gave alcohol **124** in 93 % yield. Installation of the exo-cyclic double bond took advantage of the known method (refer to scheme 4.1) to give conjugated enone which was reduced to afford compound **125** and its epimer **126** in

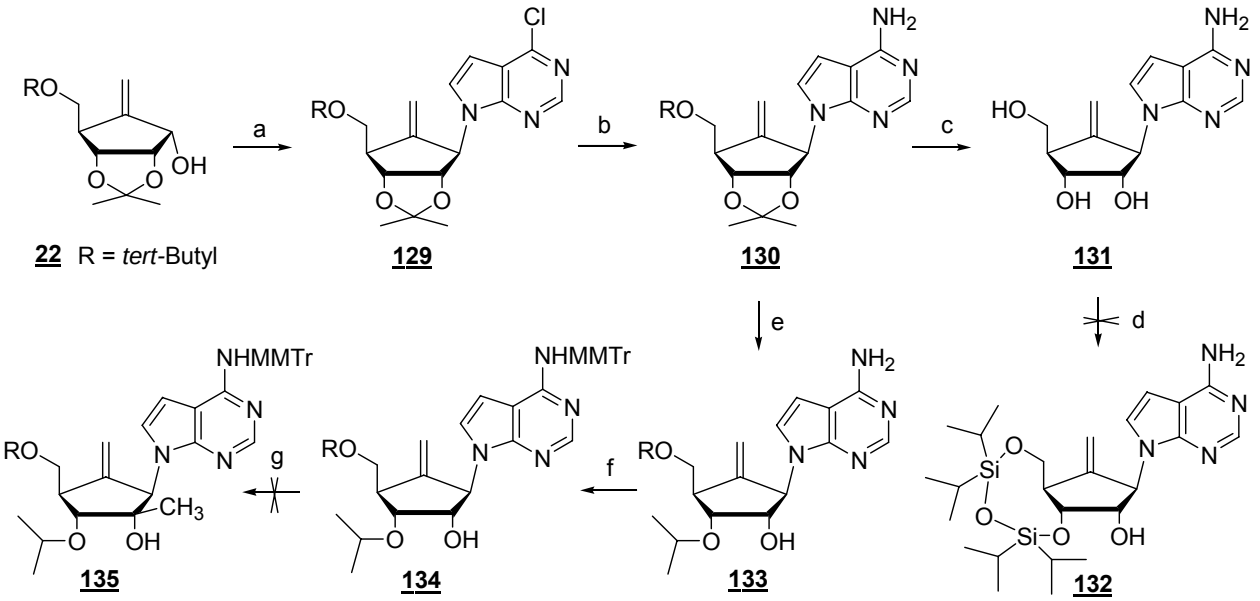
56 % and 12 % yield, respectively. To our surprise, Mitsunobu coupling generated an undesired nucleoside **128**. Presumably, this is caused by the steric hindrance of the 2-β-methyl group and the subsequent 1,3-migration of the carbon cation (Scheme 4.14 panel b).



Reagents and conditions: a) DMSO, oxalyl chloride, Et_3N, CH_2Cl_2, -78 °C - rt; b) CH_3MgBe, Et_2O, -78 °C; c) (i) TBAF, THF, rt, (ii) TBDMSCl, Im, CH_2Cl_2, (iii) 2,2-dimethoxypropane, *p*-TSA, acetone; d) ammonium formate, Pd/C, MeOH, 90 °C; e) (i) Dess-Martin periodiane, CH_2Cl_2, (ii) LDA, Eschenmoser's salt, THF, (iii) MeI, (iv) $NaHCO_3$ solution; f) Ph_3P, DIAD, 6-chloro-7-deazapurine.

Scheme 4.14. (a) Scheme of the synthesis of undesired compound **128**; (b) Proposed mechanism of the formation of compound **128**

Since the target compound **112** could not be prepared *via* a coupling reaction of 2-β-methyl-6-methylene carbocycles and base moiety, introduction of a 2'-β-methyl group was attempted at the nucleoside level. The detailed steps were outlined in Scheme 4.15. Without the 2-β-methyl group, an alcohol **22** was able to be smoothly coupled with proper base moiety, such as 7-deaza-6-chloropurine, to provide the nucleoside **129** in 85 % yield. Amination followed by deprotection afforded free nucleoside **131**. However, selective protection of 3'- and 5'-hydroxyls by TIPDS group was unsuccessful under various conditions. Based on our previous experience (refer to Scheme 4.12), we decided to perform reductive acetonide deprotection by treating compound **130** with ten equivalents of DIBAL-H at -78 °C. Compound **133** was obtained in 86 % yield. Since the protection of the amino group on the purine is necessary for the subsequent oxidation step,[122] monomethoxytrytyl (MMTr) was used to protect the amino group to yield nucleoside **131** in 60 % yield. Unfortunately, the conversion of compound **134** to **135** was again found to be difficult. Oxidation of **134** by Dess-Martin periodiane or tetrapropylammonium perruthenate (TPAP)/NMO led only the recovery of the starting material. While using Swern oxidation followed by the treatment of CH_3MgBr, gave two unknown compounds which were not stable enough to be fully identified. Based on the ^{1}H-NMR data, however, it was clear that there was no vinyl proton in either compound which indicated the migration of the exocyclic double bond.



Reagents and conditions: a) Ph_3P, DIAD, 6-chloro-7-deazapurine, THF, 40 °C; b) NH_3/MeOH, 90 °C; c) TFA/H_2O, rt; d) $TIPDSCl_2$, Py, DMAP, rt; e) DIBAL-H, CH_2Cl_2, -78 °C; f) (i) TMSCl, Py, (ii) MMTrCl, DMAP, Py, (iii) NH_4OH/H_2O; g) oxidation conditions followed by CH_3MgBr, Et_2O, -78 °C.

Scheme 4.15. Tentative synthesis of compound **135**

PART IV: ASYMMETRIC SYNTHESIS OF NOVEL 2'-*O*-METHYL-2'-HYDROXYL ENTECAVIR ANALOGS AS POTENTIAL ANTI-HCV AGENTS

Introduction

In view of the synthesis difficulties in both 2'-fluoro and 2'-*C*-methyl entecavir analogs, we turned our attention to another class of nucleosides with 2'-*O*-methyl modification, such as compound **136**, which was believed to be easier to access. In fact, a 2'-*O*-methyl motif is also an attractive functional group as the ribo-type nucleoside **6** is a potent anti-HCV agent (Figure 4.9).[274] Furthermore, a conformational search also revealed the possibility that adenine derivative

137 may adapt a 3'-endo Northern conformation with an *anti* base disposition. Therefore, we conducted the synthesis of 2'-*O*-methyl-2'-hydroxyl entecavir analogs.

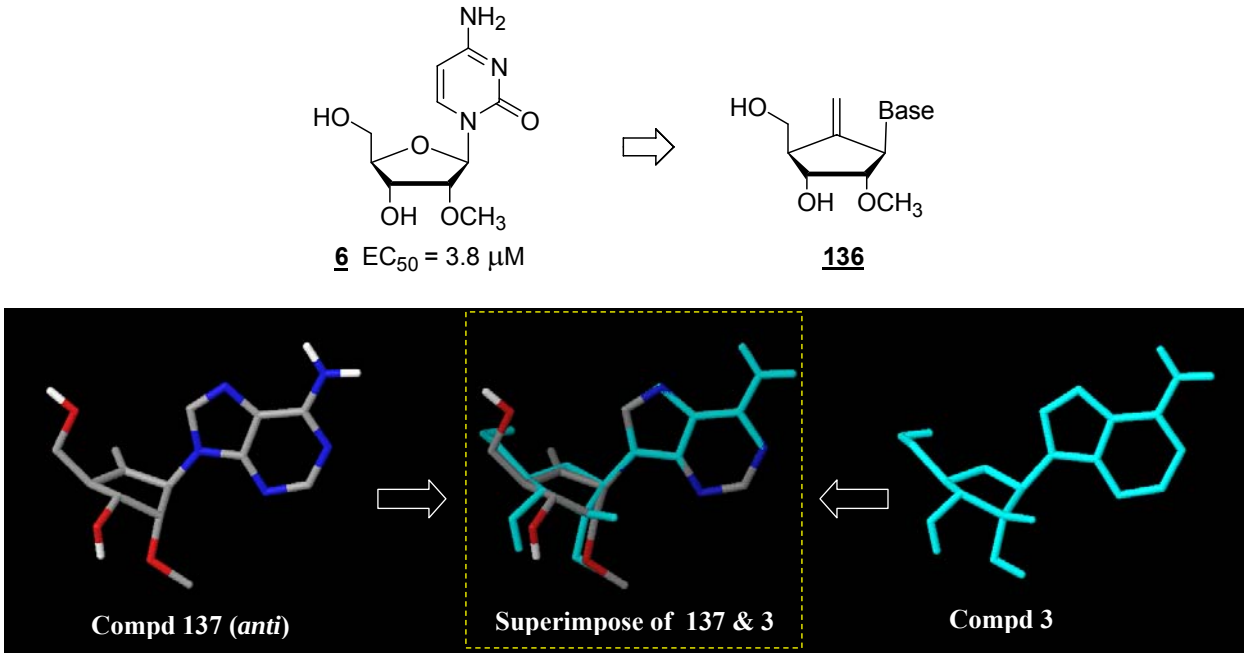
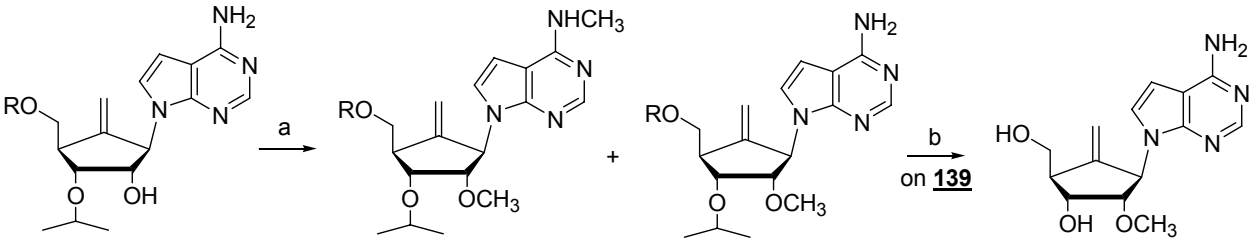


Figure 4.9. Two low-energy conformers of compound **137** (conformer b as *Northern* and conformer a as *Southern*) can convert to each other with only ~5.1 KJ/mol energy barrier; Compound **137** (conformer b) had similar conformation with compound **3**.

Chemistry

Since we have an interesting intermediate **133** in hand (*vide supra*), we first performed the synthesis of 7-deaza analog **140** (Scheme 4.16). Methylation reaction was conducted by the treatment of compound **133** with NaH followed by methyl iodide and two products were isolated: dimethylated compound **138** (26 %) as well as desired monomethylated compound **139** (50 %). Selective deprotection of *tert*-butyl and *iso*-propyl group in compound **139** was accomplished

under a Lewis acid condition (BCl_3) at -30 ºC. The target 7-deazaadenine derivative **140** was isolated in 87 % yield.



133 R=*tert*-Butyl **138** **139** **140**

Reagents and conditions: a) NaH, MeI, DMF, 0 °C; b) BCl_3, CH_2Cl_2, -30°C.

Scheme 4.16. Synthesis of target compound **140**

Antiviral activity

The antiviral screening of compound **140** is still in progress.

EXPERIMENTAL SECTION

General Methods. Melting points were determined on a Mel-temp II apparatus and were uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian Mercury 400 spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR or Varian Inova 500 spectrometer at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR with tetramethylsilane as the

internal standard. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or bs (broad singlet). UV spectra were recorded on a Beckman DU-650 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. High resolution mass spectra were recorded on a Micromass Autospec high-resolution mass spectrometer. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Column chromatography was performed using either silica gel-60 (220-440 mesh) for flash chromatography or silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

Microwave-Assisted Synthesis. Reactions were run in the Discover™ reactor module (CEM Corporation) of focused microwaves with a magnetron operating at a frequency at 2.45 GHz and a maximum power output of 300 W. The thick-wall tube was heated in a closed cavity located inside the instrument with continuous stirring. The temperature was measured by an IR pyrometer inside the reactor.

(-)-(3a*R*,4*S*,6*R*,6a*R*)-4-(benzyloxy)-6-(tert-butoxymethyl)-2,2-dimethyl-5-methylenetetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole (23) To a mixture of enone **20**[53, 115, 116] (8.4 g, 34.6 mmol) in THF solution lithium diisopropylamine (2.0 M solution, 19.1 mL, 38.1 mmol) was added slowly at -78 ^{o}C. After stirring at the same temperature for 3 h, Eshenmoser's salt (25.9 g, 138.4 mmol) was added in one portion. The mixture was stirred for additional 3 h at the same temperature and overnight at room temperature. Then iodomethane (108.8 mL, 1.73 mol) was added and stirred for another 4 h at room temperature before quenching with 10% aqueous $NaHCO_3$ (100 mL). The mixture was stirred for 1 h and extracted with diethyl ether (2 X 400 mL). The combined ether extracts were washed with 10% aqueous $NaHCO_3$ followed by

brine and dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by vacuum silica gel column chromatography (EtOAc:Hexanes = 1:30 to 1:10) to give an oil (4.6 g) which was dissolved in MeOH and treated with $CeCl_3 \cdot 7H_2O$ (7.5 g, 19.6 mmol) for 10 min at room temperature. After cooling down to -78 ^oC, $NaBH_4$ (0.75 g, 20.0 mmol) was added slowly. The reaction was kept at the same temperature for 20 min and quenched with HOAc. Solvent was removed *in vacuo* and the residue was dissolved in EtOAc and washed with H_2O and brine, dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by vacuum silica gel column chromatography (EtOAc:Hexanes = 1:30 to 1:10) to give white solid (4.0 g) which was used directly for next step. White solid (8.0 g, 31.2 mmol) was dissolved in THF and treated with NaH (60 %, 1.62 g, 40.5 mmol) for 15 min at room temperature. Benzyl bromide (4.81 mL, 40.5 mmol) and tetrabutylammonium iodide (TBAI) were added subsequently and the mixture was stirred for 3.5 h at 40 ^oC. After quenching with ice/water, the mixture was taken into Et_2O and washed with H_2O and brine, dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by vacuum silica gel column chromatography (EtOAc:Hexanes = 1:30 to 1:20) to give desired compound **23** (9.7 g, 43 % from **20**). $[\alpha]^{24}_D$ -121.09° (c 0.83, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.43-7.26 (m, 5H), 5.28 (d, *J* = 1.0 Hz, 1 H), 5.07 (t, *J* = 1.0 Hz, 1 H), 4.83 (d, *J* = 12.0 Hz, 1H), 4.68 (d, *J* = 13.0 Hz, 1H), 4.56 (t, *J* = 5.5 Hz, 1H), 4.44 (t, *J* = 1.0 Hz , 1H), 4.32-4.30 (m, 1 H), 3.42 (dd, *J* = 4.0 and 8.5 Hz, 1 H), 3.21 (dd, *J* = 5.0 and 8.5 Hz, 1 H), 2.59-2.57 (m, 1H), 1.46 (s, 3H), 1.34 (s, 3H), 1.02 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 150.6, 138.6, 128.3, 127.8, 127.6, 110.8, 108.9, 81.3, 79.7, 78.5, 72.6, 71.8, 64.5, 49.9, 27.3, 26.9, 25.3; HR-MS Calcd. for $(C_{21}H_{30}O_4+H)^+$ 347.2222, found 347.2225.

(-)-(1*S*,2*S*,3*S*,5*R*)-3-(benzyloxy)-5-(hydroxymethyl)-4-methylenecyclopentane-1,2-diol (24) Fully protected compound **23** (450 mg, 1.3 mmol) was dissolved in MeOH and treated with 3 N HCl at refluxed temperature for 3.5 h. After removing the solvent, the residue was purified by vacuum silica gel column chromatography (MeOH:CH_2Cl_2 = 1:30 to 1:10) to give triol **24** (280 mg, 85 %) as a white solid. mp 122-124 ^oC; $[\alpha]^{24}_D$ -123.05° (c 0.37, MeOH); ^1H NMR (500 MHz, CD_3OD) δ 7.46-7.30 (m, 5 H), 5.34 (dd, *J* = 1.0 and 3.0 Hz , 1H), 5.21 (s, 1H), 4.77 (d, *J* = 12.0 Hz , 1H), 4.62 (d, *J* = 12.5 Hz , 1H), 4.17-4.14 (m, 2 H), 3.95-3.93 (m, 1H), 3.82-3.73 (m, 2H), 2.69-2.66 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 148.9, 138.3, 128.0, 127.6, 127.3, 109.1, 80.8, 71.7, 71.0, 70.8, 61.8, 49.6; HR-MS Calcd. for $(C_{14}H_{18}O_4+H)^+$ 251.1283, found 251.1281.

(-)-*(6a*R*,8*S*,9*R*,9a*R*)*-8-(benzyloxy)-2,2,4,4-tetraisopropyl-7-methyleneperhydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-ol (25) 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (5.5 mL, 16.8 mmol) was added dropwise to a solution of triol **24** (4.0 g, 16.0 mmol) in anhydrous pyridine at -30 ºC. The reaction mixture was allowed to warm up to room temperature gradually and kept at the same temperature for 2 h. After removing the pyridine *in vacuo,* the residue was dissolved in EtOAc and washed with H_2O and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:30 to 1:5) to yield the alcohol **25** (6.5 g, 82 %). $[\alpha]^{24}_D$ -105.94° (*c* 0.58, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.41-7.26 (m, 5H), 5.36 (t, *J* = 2.5 Hz, 1H), 5.11 (t, *J* = 2.5 Hz, 1H), 4.77 (d, *J* = 12.0 Hz, 1H), 4.62 (d, *J* = 12.5 Hz, 1H), 4.18-4.14 (m, 2H), 4.05 (dd, *J* = 4.5 and 12.0 Hz, 1H), 3.78 (dd, *J* = 8.0 and 12.0 Hz, 1H), 2.90-2.88 (m, 1H), 1.08-0.97 (m, 27H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.3, 138.1, 128.4, 127.6,

127.5, 111.1, 80.2, 74.2, 71.2, 71.1, 64.9, 50.1, 17.6, 17.5, 17.4, 17.3, 17.2, 17.1, 17.0. Anal. Calcd. for $C_{26}H_{44}O_5Si_2$: C, 63.37; H, 9.00. Found: C, 63.64; H, 9.05.

(-)-*(6aR,8S,9R,9aR)*-8-(benzyloxy)-2,2,4,4-tetraisopropyl-7-methyleneperhydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-ol (28) A solution of compound **25** (2.1 g, 4.3 mmol) and anhydrous pyridine (1.05 mL, 12.6 mmol) in anhydrous CH_2Cl_2 (20 mL) was treated with trifluoromethanesulfonic anhydride (0.94 mL, 5.6 mmol) at -78 ºC. The reaction mixture was allowed to warm up to room temperature gradually and kept at the same temperature for 20 min. After removing the solvent *in vacuo,* the residue was dissolved in EtOAc and washed with H_2O and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was dissolved in anhydrous benzene (40 mL), and 18-crown-6 (2.25 g, 8.6 mmol) and cesium acetate (2.47 g, 12.6 mmol) were added. The suspension was heated at 50 ºC for 30 min and cooled to room temperature. After removing the solvent, the residue was used directly for the next step without further purification. The analytic sample **27** was obtained by the purification using column chromatography on a silica gel (EtOAc:Hexanes = 1:30 to 1:5). Compound **27** was dissolved in the MeOH and treated with sodium methoxide at room temperature for 3 h and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10 to 1:3) to give **28** (1.7 g, 81 % from **7**). Compound **27** $[\alpha]^{25}_D$ -84.09° (*c* 0.30, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41-7.26 (m, 5H), 5.44 (dd, *J* = 7.5 Hz and 9.5 Hz, 1H), 5.31 (t, *J* = 3.0 Hz, 1H), 5.22 (t, *J* = 3.0 Hz, 1H), 4.60 (s, 2H), 4.17 (m, 1H), 4.10 (dd, *J* = 4.5 Hz and 11.5 Hz, 1H), 4.00 (m, 2H), 2.67 (m, 1H), 1.09-1.02 (m, 27H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 143.3, 138.1, 128.4, 127.9, 127.7, 112.0, 81.4, 81.2, 73.6, 70.3, 60.7, 49.6, 21.2, 17.5, 17.4, 17.3, 17.2, 17.1, 17.0, 13.6, 13.4, 12.8, 12.5. Anal. Calcd. for $C_{28}H_{46}O_6Si_2$: C, 62.88; H, 8.67. Found: C, 63.20; H, 8.79. Compound **28** $[\alpha]^{24}_D$ -

76.47° (*c* 0.82, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41-7.26 (m, 5H), 5.34 (t, *J* = 2.5 Hz, 1H), 5.16 (t, *J* = 2.0 Hz, 1H), 4.80 (q, *J* = 12.0 Hz, 2H), 4.12-3.89 (m, 5H), 2.60 (m, 1H), 1.09-0.94 (m, 27H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.4, 138.6, 128.5, 127.7, 127.6, 111.5, 82.4, 82.3, 77.3, 77.0, 76.8, 76.2, 71.8, 62.7, 49.4, 17.6, 17.5, 17.4, 17.3, 17.2, 17.1, 17.0, 13.6, 13.4, 12.8, 12.6. HR-MS Calcd. for $(C_{26}H_{44}O_5Si_2+H)^+$ 493.2806, found 493.2736.

(-)-(6a*R*,8*S*,9a*R*)-8-(benzyloxy)-2,2,4,4-tetraisopropyl-7-methylenetetrahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9(*9aH*)-one (26) Alcohol **28** (33 mg, 0.067 mmol) in anhydrous CH_2Cl_2 was treated with Dess-Martin reagent at room temperature for 30 min. The reaction was quenched by adding saturated sodium thiosulfate solution. The organic layer was collected and thoroughly washed again with saturated sodium thiosulfate solution and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo* to give the title ketone **26** (30 mg, 91 %). However, the compound **26** decomposed over one night. IR: 1701 (ketone peak); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.26 (m, 5H), 5.349-5.46 (m, 2H), 4.87 (d, *J* = 12.0 Hz, 1H), 4.72 (d, *J* = 11.0 Hz, 1H), 4.8 (dd, *J* = 2.0 and 10.5 Hz, 1H), 4.38-4.36 (m, 1H), 4.18 (dd, *J* = 3.5 and 12.0 Hz, 1H), 4.08 (dd, *J* = 4.0 and 12.0 Hz, 1H), 2.92-2.89 (m, 1H), 1.10-0.97 (m, 27H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.0, 140.0, 137.5, 128.4, 128.1, 127.9, 115.1, 76.8, 74.4, 71.2, 59.6, 49.9, 17.5, 17.4, 17.3, 17.0, 16.9, 16.8, 13.6, 13.2, 12.7, 12.4.

(-)-(6a*R*,8*S*,9a*R*)-8-(benzyloxy)-9,9-difluoro-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocine (34) 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (4.4 mL, 13.8 mmol) was added dropwise to a solution of triol **31** (3.04 g, 12.8 mmol) in anhydrous pyridine at -30 ºC. The reaction mixture was allowed to warm up to room temperature gradually and kept at the same temperature for 1 h. After removing the

pyridine *in vacuo,* the residue was dissolved in EtOAc and washed with H_2O and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:30 to 1:5) to yield the alcohol **32** (5.4 g, 88 %) which is directly used for the next step. Oxalyl chloride (1.96 mL, 22.4 mmol) was added dropwise into the solution of DMSO (3.18 mL, 44.8 mmol) in anhydrous CH_2Cl_2 (80 mL) at - 78 ºC and kept at the same temperature for 30 min. Compound **32** (5.4 g, 11.2 mmol) in anhydrous CH_2Cl_2 (80 mL) was then added into the mixture and stirred at - 78 ºC for 50 min. Triethylamine (12.5 mL, 89.6 mmol) was added and the reaction was allowed to warmed up to room temperature. The reaction was quenched by adding H_2O and extracted with EtOAc and washed with H_2O and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:30 to 1:15) to yield the ketone **33** (4.67 g, 87 %) which is directly used for the next step. Ketone **33** (0.82 g, 1.7 mmol) was dissolved in the anhydrous toluene and treated with (diethylamino)sulfur trifluoride (DAST) (0.57 mL, 3.4 mmol). The reaction mixture in 100 mL flask was placed in a microwave synthesizer and irradiated at maximum output power of 300 W with air-cooling at 80 $^{\circ}$C for 30 min. The remainder DAST (0.57 mL, 3.4 mmol) was added and kept under same condition for another 30 min. The reaction mixture was poured into ice-cold saturated $NaHCO_3$ solution and extracted with EtOAc and washed with H_2O and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:30 to 1:20) to give **34** (600 mg, 70 %). Compound **33** IR: 1762 (ketone peak); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37-7.26 (m, 5H), 4.93 (d, J = 11.5 Hz, 1H), 4.68 (d, J = 12.0 Hz, 1H), 4.22 (dd, J = 1.5 and 12.0 Hz, 1H), 4.02 (dd, J = 3.5 and 11.5 Hz, 1H), 3.87 (d, J = 7.5 Hz, 1H), 3.80 (dd, J = 1.5 and 12.0 Hz, , 1H), 2.39-2.36 (m, 1H), 2.07-2.00 (m,

1H), 1.92-1.88 (m, 1H), 1.11-0.96 (m, 27H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.2, 137.7, 128.4, 128.2, 127.8, 75.6, 74.1, 72.1, 59.7, 43.0, 27.6, 17.5, 17.4, 17.3, 17.1, 17.0, 16.9, 13.6, 13.2, 12.8, 12.4; $(C_{25}H_{42}O_5Si_2+NH_4)^+$ 496.2914, found 496.2850; Compound **34** ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.26 (m, 5H), 4.81 (d, *J* = 11.5 Hz, 1H), 4.53 (d, *J* = 11.5 Hz, 1H), 4.03-3.91 (m, 2H), 3.68-3.65 (m, 1H), 2.26-2.24 (m, 1H), 1.98-1.91 (m, 1H), 1.73-1.69 (m, 1H), 1.08-0.96 (m, 27H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.6, 128.5, 128.4, 128.0, 127.9, 127.8, 125.0 (dd, *J* = 255.6 and 260.9 Hz), 70.5, 76.4, 76.3, 76.1, 72.4, 72.3, 72.2, 72.0, 71.8, 42.7, 27.3, 17.6, 17.4, 17.3, 17.1, 17.0, 16.9, 16.8, 14.0, 13.9, 13.5, 13.2, 12.7, 12.5. ^{19}F NMR (376.5 MHz, $CDCl_3$) δ -129.6 (dd, *J* = 9.0 and 16.6 Hz), -130.2 (dd, *J* = 9.0 and 16.6 Hz); HR-MS Calcd. for $(C_{25}H_{42}F_2O_4Si_2+NH_4)^+$ 518.2933, found 518.2907.

(-)-(6a*R*,9a*R*)-9,9-difluoro-2,2,4,4-tetraisopropyltetrahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-8(6*H*)-one (36) A suspension of compound **34** (100 mg, 0.2 mmol), ammonium formate (60 mg, 1.3 mmol) and Pd/C (100 mg) in MeOH (4 mL) was refluxed for 20 min. After filtration, the filtrate was evaporated *in vacuo* to give a oil which was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10 to 1:4) to yield the alcohol **35** (66 mg, 81 %) which is directly used for the next step. Alcohol **35** (40 mg, 0.1 mmol) was dissolved in anhydrous CH_2Cl_2 and treated with Dess-Martin reagent (64 mg, 0.15 mmol) at room temperature for 1 h. After quenched with a mixed solution of saturated $NaHCO_3$ and $Na_2S_2O_3$, the organic layer was collected and washed with washed with $NaHCO_3$ solution and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo* to give the title ketone **36** (30 mg, 74 %). Compound **35**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.20-4.17 (m, 2H), 3.96-3.93 (m, 1H), 3.70-3.66 (m, 1H), 2.32-2.30 (m ,1 H), 2.14-2.05 (m, 1H), 1.88-1.82 (m, 1H), 1.73-1.67 (m, 1H), 1.08-0.98 (m., 27 H); ^{19}F NMR (376.5 MHz, $CDCl_3$) δ -111.10 (td, *J* = 9.4

and 186.4 Hz), -129.55 (d, J = 183.3 Hz); Compound **36**: IR: 1782 (ketone peak); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.24-4.16 (m, 1H), 4.11 (dd, J = 3.0 and 11.5 Hz, 1H), 3.80 (d, J = 12.5 Hz, 1H), 2.56-2.51 (m ,1 H), 2.43-2.36 (m, 1H), 2.29-2.26 (m, 1H), 1.14-0.97 (m, 27H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 220.1 (t, J = 22.1 Hz), 113.1 (dd, J = 256.8 and 262.9 Hz), 70.7, 70.4, 70.3, 56.6, 37.6, 37.5, 37.3, 34.2, 28.7, 16.4, 16.3, 16.2, 16.1, 16.0, 15.9, 15.8, 13.1, 12.4, 12.2, 11.7, 11.4; ^{19}F NMR (376.5 MHz, $CDCl_3$) δ -125.34 - -126.77 (m); HR-MS Calcd. for $(C_{18}H_{34}F_2O_4Si_2+H)^+$ 409.2042, found 409.2047.

(-)-(6a*R*,8*S*,9*R*,9a*R*)-8-(benzyloxy)-9-fluoro-2,2,4,4-tetraisopropyl-7-methylenehexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocine (38) To a solution of alcohol **25** (6.5 g, 13.2 mmol) in anhydrous CH_2Cl_2, (diethylamino)sulfur trifluoride (DAST) was added slowly at room temperature. The reaction mixture was quenched with iced H_2O after 20 min. The organic layer was collected and the aqueous phase was extracted with dichloromethane. The organic layer was then combined, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was used immediately for the next deprotection step. The analytic sample was obtained by the purification using column chromatography on a silica gel (EtOAc:Hexanes = 1:100 to 1:20). $[\alpha]^{24}_{D}$ -104.08° (*c* 0.51, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39-7.26 (m, 5H), 5.36 (t, J = 2.5 Hz, 1H), 5.20 (dd, J = 2.5 and 5.0 Hz, 1H), 4.92 (ddd, J = 6.0, 7.5 and 55.0 Hz, 1H), 4.78 (d, J = 11.5 Hz, 1H), 4.65 (d, J = 11.5 Hz, 1H), 4.31-4.26 (m, 1H), 4.23-4.16 (m, 1H), 4.01-3.92(m, 1H), 1.08-0.94 (m, 27H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.6 (d, J = 9.2 Hz), 137.9, 128.4, 127.8, 127.7, 112.7, 103.4 (d, J = 189.0 Hz), 80.4 (d, J = 21.3 Hz), 73.8 (d, J = 19.8 Hz), 71.3, 61.6, 48.8 (d, J = 5.3 Hz), 17.5, 17.4, 17.1, 17.0, 16.9, 16.8, 13.4, 13.3, 12.7, 12.5. HR-MS Calcd. for $(C_{26}H_{43}FO_4Si_2+H)^+$ 495.2762, found 495.2769.

(-)-(1*R*,2*S*,3*S*,5*R*)-3-(benzyloxy)-2-fluoro-5-(hydroxymethyl)-4-methylenecyclopentanol (39) The crude fluorinated compound **38** was dissolved in THF and treated with acetic acid (3.2 mL, 53.0 mmol) followed by tetrabutylammonium fluoride (TBAF) (40 mL, 40.0 mmol) at room temperature for 1 h. After removing the solvent *in vacuo*, the residue was dissolved in isopropyl alcohol/chloroform (4:1) co-solvent and washed with H_2O. The organic layer was collected, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:4 to 1:1) to give **39** (2.1 g, 61 % from **25**). $[\alpha]^{24}_D$ -101.07° (*c* 0.74, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39-7.26 (m, 5H), 5.40 (t, *J* = 3.5 Hz, 1H), 5.17 (dd, *J* = 3.0 and 6.0 Hz, 1H), 4.86 (td, *J* = 8.5 and 66.5 Hz, 1H), 4.78 (d, *J* = 14.5 Hz, 1H), 4.67 (d, *J* = 14.5 Hz, 1H), 4.34-4.28 (m, 1H), 4.21-4.13 (m, 1H), 3.94-3.90 (m, 1H), 3.79-3.75 (m, 1H), 2.70 (br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.2 (d, *J* = 9.1 Hz), 137.7, 128.5, 128.0, 127.9, 113.2, 102.4 (d, *J* = 188.0 Hz), 80.8 (d, *J* = 21.5 Hz), 75.0 (d, *J* = 25.0 Hz), 71.6, 63.3, 48.2 (d, *J* = 5.3 Hz). HR-MS Calcd. for $(C_{14}H_{17}FO_3+H)^+$ 253.1240, found 253.1240.

(-)-[(1*R*,2*Ri*3*R*,4*R*)-2-(benzoyloxy)-4-(benzyloxy)-3-fluoro-5-methylenecyclopentyl]methyl benzoate (40) Benzoyl chloride (1.88 mL, 16.0 mmol) was added into the solution of diol **39** (1.0 g, 4.0 mmol) in anhydrous pyridine at room temperature. Pyridine was removed in vacuo after 4 h and the residue was dissolved in EtOAc. The solution was washed with H_2O and brine, dried over magnesium sulfate, filtered and concentrated under *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:20 to 1:3) to give **40** (1.8 g, 99 %). $[\alpha]^{24}_D$ -52.71° (*c* 0.55, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03-7.26 (m, 15H), 5.68 - 5.61 (m, 1H), 5.49 (t, *J* = 2.5 Hz, 1H), 5.34 (dd, *J* = 2.5 and 4.5 Hz, 1H), 5.20 (td, *J* = 6.0 and 53.0 Hz, 1H), 4.82 (d, *J* = 11.5 Hz, 1H), 4.73 (d, *J* = 11.5 Hz, 1H), 4.62 (dd, *J* = 5.0 and 10.5 Hz,

1H), 4.55-4.50 (m, 2H), 3.24-3.23 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.3, 165.7, 142.8 (d, *J* = 7.6 Hz), 137.5, 133.4, 133.0, 129.8, 129.6, 129.5, 129.3, 128.5, 128.4, 128.3, 128.0, 127.9, 114.3, 99.9 (d, *J* = 189.9 Hz), 81.2 (d, *J* = 22.0 Hz), 76.2 (d, *J* = 23.8 Hz), 71.7, 64.9, 45.0 (d, *J* = 4.5 Hz). HR-MS Calcd. for $(C_{28}H_{25}FO_5+H)^+$ 461.1764, found 461.1756.

(-)-[(1*R*,2*R*,3*R*,4*R*)-2-(benzoyloxy)-3-fluoro-4-hydroxy-5-methylenecyclopentyl]methyl benzoate (41) A solution of compound **40** (1.4 g, 3.0 mmol) in anhydrous CH_2Cl_2 was treated with boron trichloride (9.1 mL of 1M solution in CH_2Cl_2, 9.1 mmol) at -78 ºC during 15 min. After stirred at the same temperature for another 15 min, additional portion of boron trichloride (6.1 mL of 1M solution in CH_2Cl_2, 6.1 mmol) was added. The reaction was quenched with MeOH at -78 ºC after 15 min and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10 to 1:3) to give **41** (1.0 g, 89 %). $[\alpha]^{26}_D$ -53.55° (*c* 0.25, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03-7.32 (m, 10H), 5.66 (td, *J* = 6.8 and 16.4 Hz, 1H), 5.49 (t, *J* = 2.0 Hz, 1H), 5.32 (dd, *J* = 2.0 and 4.4 Hz, 1H), 4.96 (td, *J* = 6.8 and 54.4 Hz, 1H), 4.80 (m, 1H), 4.64-4.52 (m, 2H), 3.21 (m, 1H), 2.66 (d, *J* = 7.0 Hz, D_2O exchangeable, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.3, 165.8, 144.4 (d, *J* = 8.4 Hz), 133.4, 133.1, 129.8, 129.6, 129.2, 128.4, 128.3, 113.1, 99.9 (d, *J* = 191.3 Hz), 75.3, 75.2, 75.1, 75.0, 65.4, 44.8 (d, *J* = 3.8 Hz). Anal. Calcd. for $C_{21}H_{19}FO_5$: C, 68.10; H, 5.17. Found: C, 67.78; H, 5.27.

(1*R*,5*S*)-3-(6-amino-9*H*-9-purinyl)-5-(hydroxymethyl)-4-methylene-2-cyclopenten-1-ol (43) To a solution of compound **41** (1.07 g, 2.89 mmol), triphenylphosphine (TPP, 1.13 g, 4.33 mmol) and 6-chloropurine (0.67 g, 4.33 mmol) in anhydrous THF (20 mL), diisopropyl azodicarboxylate (DIAD, 0.89 mL, 4.33 mmol) was added at 0 ºC during 5 min. The reaction was allowed to warmed up to room temperature and kept for 1 h. The reaction was quenched by

adding MeOH (1mL) and evaporated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:4 to 1:2) to give a coupling product **42** as a mixture which was contaminated with the reduced DIAD species. The crude product **42** was treated with saturated methanolic ammonia at 100 ºC for 18 h in a steel bomb. After removing the solvent, the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:30 to1:10) to give compound **43**. UV (MeOH) λ_{max} 248.0 nm; ^{1}H NMR (500 MHz, CD_3OD) δ 8.24 (s, 1H), 8.18 (s , 1H), 7.40 (br, 2 H), 6.45 (s, 1H), 5.36 (d, *J* = 7.2 Hz, 1H), 5.13 (s, 1H), 4.89 (d, *J* = 4.0 Hz, 1H), 4.80 (t, *J* = 5.5 Hz, 1H), 4.73 (m, 1H), 3.71-3.65 (m, 2H), 2.72 (s, 1H).

[(1*R*,3*R*,4*R*,5*R*)-5-(benzoyloxy)-3-(6-chloro-9*H*-9-purinyl)-4-fluoro-2-hydroxy-2-(hydroxymethyl)cyclopentyl]methyl benzoate (46) The crude compound **42** (660 mg) was dissolved actone/H_2O (15 mL/2.5 mL) and directly treated with osmium tetroxide (1.3 mL 5 % H_2O solution)/NMO (480 mg) for 24 h. The reaction mixture was quenched with saturated sodium thiosulfate aqueous solution. The organic solution was removed *in vacuo* and the aqueous phase was extracted with isopropyl alcohol/chloroform (4:1) co-solvent. The organic layer was colleted and dried over Na_2SO_4 and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:60 to1:40) to give compound **46** as a diastereomers (640 mg, 41 % from **41**). Major isomer: ^{1}H NMR (500 MHz, CD_3OD) δ 8.80 (d, *J* = 5.0 Hz, 1H), 8.78 (s , 1H), 7.95-7.11 (m, 10 H), 6.10 (ddd, *J* = 3.0, 12.5 and 17.5 Hz, 1H), 5.80 (dd, *J* = 10.0 and 35.0 Hz, 1H), 5.38 (ddd, *J* = 3.0, 10.5 and 67.5 Hz, 1H), 4.80 (m, 2H), 3.73 (d, *J* = 14.5 Hz, 1H), 3.40 (d, *J* = 14.5 Hz, 1H), 3.00 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 165.6, 153.1, 151.8, 151.7, 149.9, 148.2, 148.1, 129.4, 129.1, 128.2, 127.9, 93.1 (d, *J* = 193.1 Hz), 80.8, 79.3 (d, *J* = 26.2 Hz), 62.8, 61.9 (d, *J* = 5.0 Hz), 60.2 (d, *J* =

13.4 Hz), 48.6 (d, *J* = 5.6 Hz). HR-MS Calcd. for $(C_{26}H_{22}ClFN_4O_6+H)^+$ 541.1290, found 541.1290.

[(1*R*,3*R*,4*R*,5*R*)-3-(6-amino-9*H*-9-purinyl)-5-(benzoyloxy)-4-fluoro-2-hydroxy-2-(hydroxymethyl)cyclopentyl]methyl benzoate (47) Nucleoside **46** (620 mg, 1.15 mmol) in anhydrous DMF was treated with sodium azide (750 mg, 11.5 mmol) at 70-80 ºC for 1.5 h. The volatile was removed *in vacuo* and the residue was dissolved in isopropyl alcohol/chloroform (4:1) co-solvent and washed with H_2O, dried over Na_2SO_4 and evaporated to dryness. The resulting crude azide compound was dissolved in EtOH and treated with Pd/C (200 mg) under H_2 atmosphere at 40 ºC for 3 h. After removing the solid, the filtrate was evaporated and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:40 to1:20) to give desired adenosine analogue **47** (370 mg, 62 %) as a diastereomers. Major isomer: UV (MeOH) λ_{max} 259.0 nm; ^{1}H NMR (500 MHz, CD_3OD) δ 8.43 (d, *J* = 4.0 Hz, 1H), 8.29 (s , 1H), 7.99-7.16 (m, 10 H), 6.11 (ddd, *J* = 2.5, 9.5 and 14.5 Hz, 1H), 5.59 (dd, *J* = 8.0 and 29.0 Hz, 1H), 5.35 (ddd, *J* = 2.5, 8.5 and 43.5 Hz, 1H), 4.89 (m, 2H), 3.72 (d, *J* = 11.0 Hz, 1H), 3.50 (d, *J* = 11.0 Hz, 1H), 3.00 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 165.6, 156.0, 152.4, 150.5, 142.8, 142.7, 133.1, 132.7, 129.4, 129.1, 128.2, 127.8, 117.7, 93.3 (d, *J* = 193.1 Hz), 80.8, 79.4 (d, *J* = 26.2 Hz), 63.0, 61.9 (d, *J* = 17.6 Hz), 60.3, 48.9 (d, *J* = 5.2 Hz). HR-MS Calcd. for $(C_{26}H_{25}FN_5O_6+H)^+$ 522.1789, found 522.1774.

(+)-[(1*R*,3*R*,4*R*,5*R*)-3-(6-amino-9*H*-9-purinyl)-5-(benzoyloxy)-4-fluoro-2-methylenecyclopentyl]methyl benzoate (48) A mixture of **47** (260 mg, 0.50 mmol) with its diastereomer was dissolved in moist acetonitrile (9 μL H_2O was added into 10 mL anhydrous acetonitrile) and cooled to -30 ºC. Excess 1-bromocarbonyl-methylethylacetate (0.54 mL, 3.68 mmol) was added dropwise into the mixture and allowed to warm up to room temperature. After

stirring at room temperature for 1 h, the reaction mixture was again cooled to -30 ºC and additional 1-bromocarbonyl-methylethylacetate (0.2 mL, 1.47 mmol) was added. Crushed ice was added to quenched the reaction and neutralized with saturated $NaHCO_3$ (20 mL) solution and extracted with EtOAc (100 mL x 2). The combined organic layer was washed with brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated *in vacuo* and the residue was dissolved in anhydrous DMF and treated with activated zinc (c.a. 2.0 g) and HOAc (0.2 mL) and stirred at room temperature for 8 h. The volatile was removed *in vacuo* and the residue was dissolved in isopropyl alcohol/chloroform (4:1) co-solvent and washed with saturated $NaHCO_3$ (15 mL) solution, H_2O and brine. The organic layer was colleted and dried over Na_2SO_4 and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 2:1 to 4:1) to give exo-cyclic double bond compound **48** (165.0 mg, 68 %) as a white solid. mp: 195-198 °C (dec.) $[\alpha]^{25}_{D}$ +77.66° (*c* 0.27, $CHCl_3$); UV (MeOH) λ_{max} 231.0, 259.0 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.12-8.06 (m ,2H), 7.94 (d, *J* = 3.6 Hz, 1H), 7.65-7.44 (m, 3H), 6.0 (dd, *J* = 2.4 and 33.2 Hz, 1H), 5.86 (br, 2H, D_2O exchangeable), 5.75 (d, (d, *J* = 14.8 Hz, 1H), 5.50 (s, 1H), 5.21 (dd, *J* = 4.0 and 50.8 Hz, 1H), 4.98 (d, *J* = 1.2 Hz, 1H), 4.82-4.64 (m, 1H), 4.66-4.61 (m, 1H), 3.42 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 165.0, 155.5, 153.2, 150.5, 144.4, 140.9, 140.8, 133.8, 133.3, 130.0, 129.7, 129.6, 128.7, 128.6, 128.5, 118.8, 113.2, 93.6 (d, *J* = 184.4 Hz), 75.8 (d, *J* = 29.0 Hz), 64.4 (d, *J* = 3.1 Hz), 58.3 (d, *J* = 17.5 Hz), 46.5. HR-MS Calcd. for $(C_{26}H_{22}FN_5O_4+H)^+$ 488.1734, found 488.1731.

(+)-(1*R*,2*R*,3*R*,5*R*)-3-(6-amino-9*H*-9-purinyl)-2-fluoro-5-(hydroxymethyl)-4-methylenecyclopentan-1-ol (19) Diisobutylaluminum hydride (DIBAL-H, 1.6 mL, 1.0 M in toluene) was added slowly into the solution of compound **48** (160.0 mg, 0.33 mmol) in

anhydrous CH_2Cl_2 at -78 °C. After 30 min at the same temperature, the reaction was diluted with isopropyl alcohol/chloroform (4:1) co-solvent (30 mL) and saturated potassium sodium tartrate solution (10 mL) was added. The mixture was stirred at room temperature for 2 h and the organic layer was colleted. The aqueous layer was extracted with isopropyl alcohol/chloroform (4:1) co-solvent (3 X 10 mL) and organic layer were combined, dried over Na_2SO_4 and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:20 to1:10) to give adenosine analogue **19** (70.0 mg, 76 %) as a white solid. mp: 215-218 °C (dec.) $[\alpha]^{25}_D$ +151.80° (*c* 0.23, $CHCl_3$) UV (H_2O) λ_{max} 259.0 nm (ε 13998, pH 2), 260.0 nm (ε 15590, pH 7), 260.0 nm (ε 15579, pH 11); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 8.06 (d, *J* = 2.4 Hz, 1H), 5.86 (dd, *J* = 2.4 and 25.6 Hz, 1H), 5.42 (t, *J* = 2.4 Hz, 1H), 4.93 (td, *J* = 3.2 and 52.4 Hz, 1H), 4.92 (s, 1H, partially buried inside the H_2O peak), 4.40 (td, *J* = 3.2 and 10.8 Hz, 1H), 3.88-3.76 (m, 2H), 2.78 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 152.5, 149.9, 146.1 (d, *J* = 1.0 Hz), 141.1 (d, *J* = 5.2 Hz), 117.9, 111.8, 95.9 (d, *J* = 186.0 Hz), 72.9 (d, *J* = 22.9 Hz), 61.8 (d, *J* = 3.4 Hz), 57.6 (d, *J* = 17.2 Hz), 51.1. Anal. Calcd. for $C_{12}H_{14}FN_5O_2$: C, 51.61; H, 5.05; N, 25.08. Found: C, 51.74; H, 5.09; N, 24.92.

(-)-(1*R*,2*R*,3*R*,5*R*)-2-(benzyloxy)-3-fluoro-5-(hydroxymethyl)-4-methylenecyclopentanol (53)

A solution of compound **25** (300 mg, 0.61 mmol) in wet CH_2Cl_2 (6 mL, 0.18 mmol H_2O) was treated with (diethylamino)sulfur trifluoride (DAST) (0.38 mL, 1.83 mmol) at -78 ºC. The reaction mixture was allowed to warm up to room temperature gradually and kept at the same temperature for 20 min. After quenching the reaction with saturated $NaHCO_3$ solution, the organic layer was collected and washed with H_2O, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was dissolved in anhydrous THF (5 mL), and HOAc (0.22 ml, 3.6 mmol) and tetrabutylammonium fluoride (TBAF) (2.4 mL, 2.4 mmol) at 40 ºC for 2 h. After

removing the solvent *in vacuo*, the residue was dissolved in isopropyl alcohol/chloroform (4:1) co-solvent and washed with 0.5 N NaOH solution, 0.5 N HCl solution and brine. The organic layer was collected, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:100 to1:20) to give **53** (100 mg, 65 % from **25**). $[\alpha]^{25}_D$ -8.07° (*c* 0.24, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34-7.26 (m, 5H), 5.51 (m, 1H), 5.30 (m, 1H), 5.29 (m, *J* = 65.0 Hz, 1H), 4.79 (d, *J* = 14.5 Hz, 1H), 4.67 (d, *J* = 14.5 Hz, 1H), 4.16 (m, 1H), 4.00 (td, *J* = 6.5 Hz and 11.0 Hz, 1H), 3.80 (m, 1H), 2.72 (m, 1H), 2.60 (d, *J* = 1.0 Hz, 1H), 1.66 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.8, 144.6, 137.2, 128.6, 128.2, 127.9, 114.8, 114.7, 95.6 (d, *J* = 183.7 Hz), 82.2, (d, *J* = 21.4 Hz), 77.2, 72.7 (d, *J* = 6.1 Hz), 72.4, 63.7, 49.6 (d, *J* = 8.5 Hz). HR-MS Calcd. for $(C_{14}H_{17}FO_3+H)^+$ 253.1240, found 253.1362.

(+)-((1*R*,2*R*,3*R*,4*R*)-2-(benzoyloxy)-3-(benzyloxy)-4-fluoro-5-methylenecyclopentyl)methyl benzoate (54) Diol **53** (100 mg, 0.39 mmol) was dissolved in anhydrous pyridine (2 mL) and treated benzoyl chloride (0.19 mL, 1.6 mmol) at room temperature for 15 min. Pyridine was removed in vacuo and the residue was dissolved in EtOAc. The solution was washed with H_2O and brine, dried over magnesium sulfate, filtered and concentrated under *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:20 to 1:3) to give **54** (170 mg, 95 %). $[\alpha]^{24}_D$ +48.20° (*c* 0.54, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04-7.20 (m, 15H), 5.61 -5.60 (m, 1H), 5.57-5.55 (m, 1H), 5.47-5.46 (m, 1H), 5.37 (m, *J* = 54.0 Hz, 1H), 4.67 (d, *J* = 12.5 Hz, 1H), 4.63 (d, *J* = 12.0 Hz, 1H), 4.60-4.47 (m, 2H), 4.30 (td, *J* = 5.0 Hz and 13.5 Hz, 1H), 3.34-3.30 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.4, 165.8, 142.9, 142.8, 137.4, 133.3, 133.1, 129.8, 129.7, 129.6, 128.5, 128.4, 127.9, 127.7, 116.2, 116.1, 95.6 (d, *J* = 184.5

Hz), 80.3 (d, *J* = 21.5 Hz), 73.9 (d, *J* = 5.2 Hz), 72.6, 64.9 (d, *J* = 1.4 Hz), 44.7. ^{19}F NMR (376.5 MHz, $CDCl_3$) δ -180.7 (m), HR-MS Calcd. for $(C_{28}H_{25}FO_5+H)^+$ 461.1764, found 461.1760.

(+)-(1*R*,2*R*,3*R*,5*R*)-3-(benzyloxy)-5-(hydroxymethyl)-4-methylenecyclopentane-1,2-diol (56)

A mixture of alcohol **22** (1.26 g, 5.0 mmol), triphenylphosine (5.1 g, 19.0 mmol) and *p*-nitrobenzoic acid (3.36 g, 19.0 mmol) in anhydrous THF (30 mL) was treated with diisopropyl azodicarboxylate (3.86 mL, 19.0 mmol) at 0 ºC and kept at room temperature for 1 h. After quenching with MeOH, the volatile was removed *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:15 to 1:3) to give compound **55** as a pale yellow oil. The analytic sample **55** was obtained by second time chromatography on a silica gel. The fully protected **55** was directly deprotected by treating with NaOMe in MeOH. After removing the solvent, the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10 to 1:2) to give deprotected alcohol which was directly protected with benzyl group under NaH / BnBr system. The benzyl protected compound was then treated with 3N HCl / MeOH at refluxing teperature for 4 h. After removing the solvent, the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:30 to 1:10) to yield compound **56** (0.8 g, 64 % from **22**) as a syrup. Compound **55** $[\alpha]^{25}_D$ -10.04° (*c* 0.71, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.32-9.19 (m, 4H), 5.69 (s, 1H), 5.84 (s, 1H), 5.37 (s, 1H), 4.66 (s, 2H), 3.51-3.43 (m, 2H), 2.91 (t, *J* = 9.0 Hz, 1H), 3.61-3.49 (m, 1H), 1.51 (s, 3H), 1.35 (s, 3H), 1.18 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.8, 150.6, 147.3, 135.4, 130.7, 123.6, 116.6, 111.4, 84.4, 82.1, 73.0, 63.1, 51.0, 27.5, 27.2, 25.0. HR-MS Calcd. for $(C_{21}H_{27}ON_7+H)^+$ 406.1866, found 406.1843; $(C_{21}H_{27}ON_7+Na)^+$ 428.1685, found 428.1664. Compound **56**: $[\alpha]^{26}_D$ +11.8° (*c* 0.23, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47-7.24 (m, 5H), 5.37 (m, 1H), 5.20 (m, 1H), 4.68 (d, *J* = 12.5 Hz, 1H), 4.61 (d, *J* = 11.5 Hz, 1H), 4.25 (m, 1H), 4.17 (t, *J* = 2.0 Hz, 1H),

4.10 (m, 1H), 3.94 (m, 1H), 3.78 (m, 1H), 3.49 (d, J = 5.0 Hz, 1H), 2.72 (m, 1H), 2.61 (d, J = 3.5 Hz, 1H), 2.948 (d, J = 3.5 Hz, 1H), 1.77 (s, br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.8, 138.0, 128.5, 127.9, 127.8, 113.3, 84.2, 75.4, 75.0, 71.3, 64.3, 49.1; HR-MS Calcd. for $(C_{14}H_{18}O_4+H)^+$ 251.1283, found 251.1356.

(-)-(6a*R*,8*R*,9*S*,9a*R*)-8-(benzyloxy)-2,2,4,4-tetraisopropyl-7-methylenehexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-ol (57) 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (1.15 mL, 3.5 mmol) was added dropwise to a solution of triol **56** (0.8 g, 3.2 mmol) in anhydrous pyridine (30 mL) at -30 ºC. The reaction mixture was allowed to warm up to room temperature gradually and kept at the same temperature overnight. After removing the pyridine *in vacuo,* the residue was dissolved in EtOAc and washed with H_2O and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:30 to 1:5) to yield the alcohol **57** (1.5 g, 95 %). $[\alpha]^{27}_D$ -25.7° (*c* 0.62, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.30 (m, 5H), 5.34 (t, J = 1.5 Hz, 1H), 5.25 (s, 1H), 4.64 (d, J = 12.0 Hz, 1H), 4.52-4.50 (m, 2H), 4.12-4.07 (m, 3H), 3.90 (dd, J = 10.0 and 12.0 Hz, 1H), 2.85 (d, J = 1.5 Hz, D_2O exchangeable, 1H), 2.77-2.75 (m, 1H), 1.12-1.02 (m, 28 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.1, 138.1, 128.4, 127.7, 127.6, 113.7, 84.0, 76.6, 75.6, 70.1, 66.7, 50.5, 17.7, 17.5, 17.4, 17.3, 17.2, 17.1, 17.0, 13.4, 13.3, 13.2, 12.9, 12.7, 12.6; HR-MS Calcd. for $(C_{26}H_{44}O_5Si_2+H)^+$ 493.2806, found 493.2815.

(-)-(6a*R*,8*R*,9*R*,9a*R*)-8-(benzyloxy)-2,2,4,4-tetraisopropyl-7-methylenehexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-yl acetate (58) A solution of compound **57** (1.5 g, 3.0 mmol) and anhydrous pyridine (0.5 mL, 0.6 mmol) in anhydrous CH_2Cl_2 (20 mL) was treated with trifluoromethanesulfonic anhydride (0.60 mL, 3.6 mmol) at -78 ºC. The reaction mixture was allowed to warm up to room temperature gradually and kept at the

same temperature for 20 min. After removing the solvent *in vacuo,* the residue was dissolved in EtOAc and washed with H_2O and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was dissolved in anhydrous benzene (20 mL), and 18-crown-6 (1.57 g, 6.0 mmol) and cesium acetate (1.76 g, 9.0 mmol) were added. The suspension was heated at 50 ºC for 30 min and cooled to room temperature. After removing the solvent, the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10 to 1:3) to give **58** (1.3 g, 80 % from **57**). $[\alpha]^{25}_{D}$ -100.74° (*c* 0.41, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.29 (m, 5H), 5.29 (d, *J* = 2.0 Hz, 1H), 5.24 (d, *J* = 1.5 Hz, 1H), 4.84 (dd, *J* = 4.5 and 8.0 Hz, 1H), 4.65 (dd, *J* = 5.5 and 7.5 Hz, 1H), 4.58 (d, *J* = 12.5 Hz, 1H), 4.37 (d, *J* = 12.0 Hz, 3H), 4.29 (d, *J* = 4.5 Hz, 1H), 4.00 (dd, *J* = 5.0 and 12.0 Hz, 1H), 3.91 (dd, *J* = 9.5 and 11.5 Hz, 1H), 2.66-2.63 (m, 1H), 2.15 (s, 3H), 1.13-1.00 (m, 28 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 144.5, 138.1, 128.3, 127.6, 127.6, 115.4, 79.9, 78.2, 77.8, 69.5, 67.8, 50.9, 20.1, 17.7, 17.6, 17.5, 17.4, 17.1, 17.0, 13.5, 13.4, 12.8, 12.4; HR-MS Calcd. for $(C_{28}H_{46}O_6Si_2+H)^+$ 535.2912, found 535.2893.

(-)-(6a*R*,8*R*,9*R*,9a*R*)-8-(benzyloxy)-2,2,4,4-tetraisopropyl-7-methylenehexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-ol (59) Compound **58** (100 mg, 0.19 mmol) in MeOH (3 mL) was treated with NaOMe (21 mg, 0.38 mmol) at room temperature for 3h. After reomving volatile, the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10 to 1:3) to give compound **59** (75 mg, 81 %). $[\alpha]^{27}_{D}$ -56.74° (*c* 0.13, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37-7.26 (m, 5H), 5.27 (d, *J* = 2.5 Hz, 1H), 5.18 (1H, *J* = 1.5 Hz, 1H), 4.63 (d, *J* = 12.0 Hz, 1H), 4.38 (d, *J* = 24.0 Hz, 1H), 4.27 (t, *J* = 6.0 Hz, 1H), 4.05 (d, *J* = 5.0 Hz, 1H), 3.98 (dd, *J* = 4.5 and 11.0 Hz, 1H), 3.95-3.91 (m, 1H), 3.84 (dd, *J* = 9.5 and 11.5 Hz, 1H), 2.60-2.56 (m, 1H), 2.51 (d, *J* = 9.0 Hz, 1H), 1.09-0.92 (m, 28 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.1, 137.6, 128.5, 127.9, 127.8, 114.7, 81.1, 80.5, 78.6, 77.2, 69.7, 67.5, 51.6,

17.7, 17.6, 17.5, 17.1, 13.5, 13.4, 12.8, 12.5; HR-MS Calcd. for $(C_{26}H_{44}O_5Si_2+H)^+$ 493.2906, found 493.2800.

(+)-(1*R*,2*R*,3*R*,5*R*)-3-(benzyloxy)-2-fluoro-5-(hydroxymethyl)-4-methylenecyclopentanol (60) Compound **59** (60 mg, 0.12 mmol) was dissolved in CH_2Cl_2 (1 mL) and treated with (diethylamino)sulfur trifluoride (DAST, 0.016 mL, 0.24 mmol) at rt for 15 min. After removing the solvent the residue was redissolved in THF and treated with tetrabutylammonium fluoride (TBAF, 0.5 mL, 0.5 mmol) and trace amount of HOAc at rt for 1 h. Silic gel was added into the mixture and evaperated to dryness. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10 to 1:2) to give **60** (2 mg, 6 %) as a pale yellow liquid $[\alpha]^{25}_D$ +20.60° (*c* 0.10, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.40-7.30 (m, 5H), 5.38 (d, *J* = 11.0 Hz, 1H), 4.81 (dt, *J* = 52.5 and 4.0 Hz, 1H), 4.71-4.65 (m, 2H), 4.41 (dt, *J* = 13.5 and 2.0 Hz, 1H), 4.18-4.13 (m, 1H), 3.74-3.72 (m, 1H), 2.63-2.62 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 146.2, 138.1, 128.0, 127.6, 127.4, 113.0, 95.3 (d, *J* = 183.6 Hz), 81.7 (d, *J* = 23.8 Hz), 71.9 (d, *J* = 16.2 Hz), 70.8, 62.5, 49.3; HR-MS Calcd. for $(C_{14}H_{18}FO_3+H)^+$ 253.1240, found 253.1271.

(-)-(6a*R*,8*S*,9*R*,9a*R*)-2,2,4,4-tetraisopropyl-7-methylenehexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocine-8,9-diol (61) Liquid ammonium was collected in a 100 mL flask at -78 ºC. Sodium (160 mg, 7.1 mmol) was dissolved in the liquid ammonium and formed a dark blue solution. Compound **28** (350 mg, 0.71 mmol) in anhydrous THF (2 mL) was then added into the mixture and stirred at -78 ºC for 10 min. The reaction was quenched with saturated NH_4Cl solution and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over magnesium sulfate, filtered and concentrated under *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:20 to 1:3) to give **61** (200 mg, 70 %) as well as starting material **28** (90 mg,

26 %). $[\alpha]^{24}_D$ -81.54° (*c* 0.95, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.31 (t, *J* = 3.0 Hz, 1H), 5.12 (t, *J* = 2.0 Hz, 1H), 4.23 (m, 1H), 4.00-3.96 (m, 2H), 3.86-3.79 (m, 2H), 2.65-2.61 (m, 1H), 2.53 (br, 1H, D_2O exchangeable), 2.28 (br, 1H, D_2O exchangeable), 1.10- 0.93 (m, 27 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.7, 109.2, 81.9, 75.1, 74.9, 63.6, 48.6, 16.5, 16.4, 16.3, 16.2, 16.1, 16.0, 12.5, 12.4, 12.3, 11.7, 11.6. HR-MS Calcd. for $(C_{19}H_{39}O_5Si_2+H)^+$ 403.2336, found 403.2396.

(-)-(1*S*,2*S*,4*R*,5*R*)-2-(benzyloxy)-5-hydroxy-4-(hydroxymethyl)-3-methylenecyclopentyl acetate (64) Fully protected compound **27** (1.28 g, 2.39 mmol) and acetic acid (0.66 mL) in anhydrous THF (20 mL) was treated with TBAF (6.6 mL, 6.6 mmol) at room temperature for 3 h. After removing the solvent *in vacuo*, the residue was dissolved in isopropyl alcohol/chloroform (4:1) co-solvent and washed with H_2O. The organic layer was collected, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:4 to 1:1) to give **64** (0.64 g, 91 %). $[\alpha]^{26}_D$ -75.44° (*c* 0.37, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37-7.30 (m, 5H), 5.34 (t, *J* = 3.0 Hz, 1H), 5.29 (t, *J* = 2.5 Hz, 1H), 4.68 (d, *J* = 11.5 Hz, 1H), 4.63 (d, *J* = 11.5 Hz, 1H), 4.28-4.26 (m, 1H), 3.94 (t, *J* = 7.5 Hz, 1H), 3.84-3.74 (m, 2H), 2.62-2.60 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5, 145.6, 138.2, 128.0, 127.6, 127.4, 110.5, 82.3, 81.9, 72.8, 70.9, 61.2, 49.7, 19.7. Anal. Calcd. For $C_{16}H_{20}O_5 \cdot 0.05\, H_2O$: C, 65.54; H, 6.91. Found C, 65.23; H, 7.01.

(-)-((1*R*,2*R*,3*S*,4*S*)-3-acetoxy-2-(benzoyloxy)-4-(benzyloxy)-5-methylenecyclopentyl)methyl benzoate (65) Diol **64** (640 mg, 2.2 mmol) was dissolved in anhydrous pyridine (10 mL) and treated with benzoyl chloride (1.0 mL, 8.8 mmol) at room temperature for 30 min. After removing the solvent *in vacuo*, the residue was dissolved in EtOAc and washed with H_2O. The organic layer was collected, dried over magnesium sulfate, filtered and concentrated *in vacuo*.

The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:4 to 1:1) to give **65** (1.0 g, 95 %). $[\alpha]^{26}_{D}$ -19.01° (*c* 0.87, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00-7.28 (m, 10H), 5.53-5.44 (m, 2H), 5.34 (t, *J* = 2.5 Hz, 1H), 4.70 (d, *J* = 11.5 Hz, 1H), 4.66 (d, *J* = 12.0 Hz, 1H), 4.61 (dd, *J* = 5.0 and 11.0 Hz, 1H), 4.53 (dd, *J* = 6.5 and 11.0 Hz, 1H), 4.40-4.39 (m, 1H), 3.35 (m, 1H), 2.02 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.0, 166.3, 166.0, 150.0, 144.1, 137.8, 133.2, 133.0, 129.9, 129.7, 129.6, 129.5., 129.4, 129.3, 127.8, 113.8, 82.1, 80.0, 77.2, 71.2, 64.7, 45.7, 21.0. HR-MS Calcd. for $(C_{30}H_{29}O_7+H)^+$ 501.1913, found 501.1962.

(-)-((1*R*,2*R*,3*R*,4*S*)-3-acetoxy-2-(benzoyloxy)-4-hydroxy-5-methylenecyclopentyl)methyl benzoate (66) Compound **65** (220 mg, 0.45 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL) and treated with boron trichloride (2.2 mL, 2.2 mmol) at -78 ºC for15 min. After quenching with MeOH and neutralized with solid $NaHCO_3$, the solvent was removed *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:15 to 1:3) to give **66** (155 mg, 86 %). $[\alpha]^{27}_{D}$ -39.51° (*c* 0.68, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.0-7.29 (m, 10H), 5.60 (t, *J* = 7.5 Hz, 1H), 5.49 (t, *J* = 2.5 Hz, 1H), 5.33 (t, *J* = 2.5 Hz, 1H), 5.20 (t, *J* = 8.0 Hz, 1H), 4.62-4.58 (m, 2H), 4.52 (dd, *J* = 6.5 and 11.5 Hz, 1H), 3.32-3.28 (m, 1H), 3.18 (d, *J* = 5.0 Hz, 1H), 2.12 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.2, 166.3, 165.8, 145.1, 133.4, 133.0, 129.8, 129.6, 129.5, 129.3, 128.4, 128.3, 112.9, 83.4, 75.6, 75.2, 65.1, 45.3, 21.0. HR-MS Calcd. for $(C_{23}H_{23}O_7+H)^+$ 411.1444, found 411.1438.

(-)-((1*R*,3*R*,4*R*,5*R*)-3-(6-amino-9*H*-purin-9-yl)-5-(benzoyloxy)-4-hydroxy-2-methylenecyclopentyl)methyl benzoate (69) DIAD (0.39 mL, 2.0 mmol) was added into a solution of alcohol **66** (360 mg, 0.90 mmol), TPP (440mg, 2.0 mmol) and N^6,N^6-di-Boc protected adenine (600 mg, 2.0 mmol) in THF at 0 ºC. The reaction mixture was kept at room temperature overnight. After purification by column chromatography on a silica gel (EtOAc:Hexanes = 1:15

to 1:3), the white solid was dissolved in CH_2Cl_2 (20 mL) and treated with TFA (4 mL) for 8 h. The volatile was removed and the residue was treated with HCl/MeOH (0.8 ml acetyl chloride in 15 mL) at 40-50 ºC for 4 h and neutralized with solid $NaHCO_3$. The solvent was removed *in vacuo* and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:60 to1:40) to give target nucleoside **69** as a white foam (230 mg, 53% from **66**). $[\alpha]^{26}_D$ 48.36° (*c* 0.35, MeOH); UV (H_2O) λ_{max} 259.0 nm; ^{1}H NMR (500 MHz, CD_3OD) δ 8.28 (s, 1H), 8.23 (s, 1H), 8.19-7.49 (m, 10H), 5.96 (d, *J* = 2.5 Hz, 1H), 5.61 (m, 1H), 5.54 (m, 1H), 5.01 (t, *J* = 2.5 Hz, 1H), 4.87-4.83 (m, 1H), 4.75-4.72 (m, 1H), 4.43 (d, *J* = 5.5 Hz, 1H), 3.42 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.6, 165.5, 156.0, 152.3, 149.9, 147.1, 142.2, 133.2, 132.9, 129.9, 129.6, 129.5, 129.4, 128.3, 128.2, 117.9, 111.8, 78.7, 73.6, 65.5, 59.4, 46.7. HR-MS Calcd. for $(C_{26}H_{24}O_5N_5+H)^+$ 486.1778, found 486.1764.

((1*R*,5*S*)-3-(6-amino-9*H*-purin-9-yl)-5-(benzoyloxy)-2-methylenecyclopent-3-enyl)methyl benzoate (71) Adenosine analog **69** (20 mg, 0.04 mmol) and pyridine (32 μL, 0.4 mmol) were dissolved in anhydrous CH_2Cl_2 and treated with DAST (16 μL, 0.12 mmol) at 0 ºC and then warmed up to room temperature for 30 min. The mixture was quenched with MeOH and purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:30) to give compound **71** as pale yellow syrup (10 mg, 50 %). UV (H_2O) λ_{max} 231.0 nm, 260 nm (buried); ^{1}H NMR (500 MHz, CD_3OD) δ 8.43 (s, 1H), 8.07-7.34 (m, 11H), 6.74 (s, 1H), 6.20 (t, *J* = 2.5 Hz, 1H), 5.87 (br, 2H, D_2O exchangeable), 5.37 (d, *J* = 1.5 Hz, 1H), 5.27 (s, 1H), 4.80 (dd, *J* = 5.5 and 11.5 Hz, 1H), 4.80 (dd, *J* = 6.5 and 11.5 Hz, 1H), 3.61-3.49 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.4, 166.2, 155.7, 153.9, 150.2, 144.4, 139.6, 139.4, 133.4, 133.2, 129.8, 129.7, 129.6, 129.5, 128.8, 128.4, 128.4, 119.7, 108.8, 77.8, 65.1, 47.6. HR-MS Calcd. for $(C_{26}H_{24}O_5N_5+H)^+$ 469.1750, found 469.2127.

((1*R*,5*S*)-3-(6-amino-9*H*-purin-9-yl)-5-(benzoyloxy)-2-methylenecyclopent-3-enyl)methyl benzoate (72) Adenosine analog **66** (30 mg, 0.06 mmol) and NMO (23 mg, 0.19 mmol) was dissolved in a mixture of acetone/H_2O (2 mL/0.3 mL) and treated with OsO_4 (62 μL 5 % H_2O solution). The mixture was kept at room temperature for 24 h and quenched with $Na_2S_2O_3$ solution. The organic solution was removed *in vacuo* and the aqueous phase was extracted with isopropyl alcohol/chloroform (4:1) co-solvent. The organic layer was colleted and dried over Na_2SO_4 and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:30) to give compound **72** as a diastereomers (24 mg, 75 %). Major isomer: ^{1}H NMR (500 MHz, CD_3OD) δ 8.51 (s, 1H), 8.27, (s, 1H), 8.02-7.17 (m, 10H), 5.74 (dd, *J* = 3.0 and 8.0 Hz, 1H), 5.46 (d, *J* = 8.0 Hz, 1H), 4.87-4.79 (m, 2H), 4.48 (dd, *J* = 2.5 and 8.0 Hz, 1H), 3.73 (d, *J* = 11.5 Hz, 1H), 3.62 (d, *J* = 11.5 Hz, 1H), 3.55-3.34 (m, 2H), 3.00-2.95 (m, 1H). HR-MS Calcd. for $(C_{26}H_{25}O_7N_5+H)^+$ 520.1832, found 520.1880.

(*6R*,*7R*,*8R*,*9S*)-9-(6-amino-9H-purin-9-yl)-6-(benzoyloxymethyl)-8-hydroxy-2,2-dimethyl-1,3-dioxaspiro[4.4]nonan-7-yl benzoate (73) Diol **72** (30 mg, 0.058 mmol) and catalytic amount of 10-camphorsulfonic acid (CSA) was dissolved in DMF (2 mL) and treated with 2,2-dimethoxypropane (1.5 mL) at 50 ºC for 1 h then microwave-assisted 80 ºC for 10 min. After quenching with solid $NaHCO_3$, the volatile was removed *in vacuo* and the aqueous phase was extracted with isopropyl alcohol/chloroform (4:1) co-solvent. The organic layer was colleted and dried over Na_2SO_4 and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:30) to give compound **73** as pale yellow oil (22 mg, 68 %). ^{1}H NMR (500 MHz, CD_3OD) δ 8.33 (s, 1H), 8.11 (s, 1H), 8.03-7.32 (m, 10H), 5.41 (d, *J* = 3.0 Hz, 1H), 5.15 (d, *J* = 3.5 Hz, 1H), 5.02 (dd, *J* = 4.5 and 11.0

Hz, 1H), 4.60-4.56 (m, 2H), 4.38 (d, *J* = 10.5 Hz, 1H), 3.50 (d, *J* = 10.5 Hz, 1H), 2.88-2.84 (m, 1H), 1.50 (s, 3H), 0.88 (s, 3H). HR-MS Calcd. for $(C_{29}H_{29}O_7N_5+H)^+$ 560.2145, found 560.2220.

((1*R*,3*S*,4*R*,5*R*)-2-acetoxy-3-(6-amino-9*H*-purin-9-yl)-5-(benzoyloxy)-2-(bromomethyl)-4-hydroxycyclopentyl)methyl benzoate (75) Compound **72** (20 mg, 0.038 mmol) was dissolved in moist CH_3CN (2 mL) and treated with excess 1-bromocarbonyl-methylethylacetate at room temperature until the solution became clear. Crushed ice was added to quench the reaction and neutralized with saturated $NaHCO_3$ solution and extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:30) to give compound **75** (22 mg, 92 %) as diastereomers. Major isomer: ^{1}H NMR (500 MHz, CD_3OD) δ 8.60 (s, 1H), 8.30, (s, 1H), 8.09-7.38 (m, 10H), 5.83 (m, 1H), 5.68 (d, *J* = 5.5 Hz, 1H), 5.08 (dd, *J* = 5.5 and 11.0 Hz, 1H), 4.95 (buried in the solvent peak, 1H), 4.65 (dd, *J* = 2.0 and 4.5 Hz, 1H), 4.54 (d, *J* = 12.0 Hz, 1H), 4.22 (d, *J* = 12.0 Hz, 1H), 3.37 (m, 1H), 2.11 (s, 3H). HR-MS Calcd. for $(C_{28}H_{26}BrO_7N_5+H)^+$ 624.1094, found 624.1079.

(-)-(6a*R*,8*S*,9*R*,9a*R*)-8-(benzyloxy)-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-yl acetate (77) A solution of compound **32** (1.1 g, 2.3 mmol) and anhydrous pyridine (0.36 mL, 4.6 mmol) in anhydrous CH_2Cl_2 (20 mL) was treated with trifluoromethanesulfonic anhydride (0.44 mL, 2.6 mmol) at -78 ºC. The reaction mixture was allowed to warm up to room temperature gradually and kept at the same temperature for 20 min. After removing the solvent *in vacuo,* the residue was dissolved in EtOAc and washed with H_2O and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was dissolved in anhydrous benzene (30 mL), and 18-crown-6 (600 mg, 2.3 mmol) and cesium acetate (666 mg, 3.4 mmol) were added. The suspension was heated at 50

ºC for 30 min and cooled to room temperature. The mixture was dissolved in EtOAc and washed with H_2O and brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:40 to 1:10) to give compound **77** (1.1 g, 92 %) as a syrup. $[\alpha]^{25}_D$ -2.3° (*c* 0.21, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33-7.25 (m, 5H), 5.32-5.30 (m, 1H), 4.61 (d, *J* = 12.0 Hz, 1H), 4.48 (d, *J* = 12.0 Hz, 1H), 4.03 (dd, *J* = 7.5 and 10.5 Hz, 1H), 3.95 (dd, *J* = 3.0 and 11.5 Hz, 1H), 3.74-3.71 (m, 1H), 3.68 (dd, *J* = 2.0 and 12.0 Hz, 1H), 2.21-2.15 (m, 1H), 2.08 (s, 3H), 1.94-1.88 (m, 1H), 1.75-1.71 (m, 1H), 1.07-0.92 (m, 27H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 138.3, 128.3, 127.7, 127.5, 84.0, 79.8, 74.9, 70.7, 59.9, 43.6, 29.8, 21.2, 17.5, 17.4,17.3, 17.2, 17.1, 17.0, 16.9, 13.6, 13.3, 12.8, 12.5; HR-MS Calcd. for $(C_{27}H_{46}O_6Si_2+H)^+$ 523.2911, found 523.2900.

(-)-(6a*R*,8*S*,9*R*,9a*R*)-8-hydroxy-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-yl acetate (78) Compound **77** (100 mg, 0.19 mmol) in MeOH (5 mL) was treated with ammonium formate (88 mg, 1.14 mmol) and Pd/C (100 mg) at refluxing temperature for 30 min before filtration and concentration *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:20 to 1:10) to give compound **78** (90 mg, quantitative yield) as a syrup. $[\alpha]^{26}_D$ -32.54° (*c* 0.22, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.56 (dd, *J* = 3.5 and 8.5 Hz, 1H), 4.04 (dd, *J* = 9.0 and 10.5 Hz, 1H), 3.98-3.93 (m, 2H), 3.69 (dd, *J* = 1.5 and 11.5 Hz, 1H), 3.41 (s, br, 1H), 2.17-2.12 (m, 4H), 2.03-1.96 (m, 1H), 1.68-1.64 (m, 1H), 1.10- 0.96 (m, 27H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3, 89.9, 73.2, 72.8, 59.2, 42.8, 30.0, 20.8, 17.4, 17.3, 17.2, 17.1, 17.0, 16.9, 13.5, 12.3, 12.7, 12.4; HR-MS Calcd. for $(C_{20}H_{40}O_6Si_2+H)^+$ 433.2442, found 433.2441.

(-)-(6a*S*,7*R*,8*R*,9*R*,9a*R*)-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocine-7,8-oxiran-9-triol (82)

Alcohol **78** (290 mg, 0.67 mmol) and TPP (527 mg, 2.0 mmol) were dissolved in anhydrous toluene (5 mL) and treated with DIAD (0.4 mL, 2.0 mmol) at rt. The mixture was heated at 80 ºC overnight. After removing the solvent, the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:200 to 1:20) to give a syrup which was directly treated with *m*-chloroperoxybenzoic acid (*m*CPBA) in CH_2Cl_2 at rt overnight before quenched with Na_2CO_3 solution. The mixture was further washed with Na_2CO_3 solution and brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated *in vacuo* and the residue was dissolved in MeOH and treated with NaOMe for 40 min. After removing the solvent, the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:20 to 1:10) to give compound **82** (45 mg, 17 %) as a syrup. $[\alpha]^{25}_{D}$ -13.66° (*c* 0.61, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.18 (dd, *J* = 3.5 and 12.5 Hz, 1H), 4.12 (t, *J* = 6.5 Hz, 1H), 4.06 (dd, *J* = 8.5 and 11.5 Hz, 1H), 3.77 (dd, *J* = 5.0 and 6.0 Hz, 1H), 3.48 (dd, *J* = 1.5 and 3.5 Hz, 1H), 3.37 (dd, *J* = 1.5 and 3.0 Hz, 1H), 2.18-2.14 (m, 1H), 1.99 (d, *J* = 8.0 Hz, 1H), 1.11-0.88 (m ,27H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 80.9, 79.0, 64.4, 55.6, 55.4, 48., 17.6, 17.5, 17.4, 17.3, 17.2, 17.1, 13.3, 13.,2, 12.7, 12.6; HR-MS Calcd. for $(C_{18}H_{36}O_5Si_2+H)^+$ 389.2180, found 389.2180.

(+)-(6a*S*,7*R*,8*R*,9*S*,9a*R*)-9-fluoro-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocine-7,8-oxirane (83) Alcohol **82** (37 mg, 0.095 mmol) in CH_2Cl_2 was treated with DAST (0.013 mL, 0.28 mmol) at rt for 30 min. After removing the solvent, the residue was was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:50 to 1:20) to give compound **83** (32 mg, 86 %) as a syrup. $[\alpha]^{23}_{D}$ +33.66° (*c* 0.29, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.91 (dd, *J* = 4.5 and 53.5 Hz, 1H), 4.22 (dd, *J* = 3.0 and 11.5 Hz, 1H), 4.12-4.05 (m, 2H), 3.56 (d, *J* = 3.0 Hz, 1H), 3.46 (d, *J* = 2.0 Hz, 1H), 2.36-2.32 (m, 1H), 1.19-0.88 (m ,27H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 89.4 (d, *J* = 181.2

Hz), 72.0 (d, *J* = 15.2 Hz), 61.9, 57.2, 51.2 (d, *J* = 34.4 Hz), 45.6, 16.5, 16.4, 16.3, 16.2, 16.1, 16.0, 12.2, 12.1, 11.8, 11.6; ^{19}F NMR (376.5 MHz, $CDCl_3$) δ -209.5 (dd, d, *J* = 20.0 and 53.5 Hz); HR-MS Calcd. for $(C_{18}H_{35}FO_4Si_2+H)^+$ 391.2736, found 391.2829.

(-)-(1*R*,2*R*,3*R*,4*S*,5*R*)-4-(6-amino-9*H*-purin-9-yl)-2-fluoro-5-(hydroxymethyl)cyclopentane-1,3-diol (85) To a flask charged with adenine (26 mg, 0.19 mmol) in anhydrous DMF (1 mL), sodium hydride (60 %, 6.8 mg, 0.17 mmol) was added and heated to 120 ºC for 1 h before cooled to the rt. Epoxide **83** (25 mg, 0.064 mmol) in anhydrous DMF (1 mL) was then added into the suspension and re-heated 15 min at 50 ºC, followed by 120 ºC 4 h. After removing the solvent *in vacuo*, the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:30 to 1:20) to give a syrup which was directly dissolved in THF and treated with TBAF at rt for 3 h. After purification by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:20 to 1:10), a pale yellow solid **85** was obtained, however, it was contaminated with tetrabutylammonium salt. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.91 (dd, *J* = 4.5 and 53.5 Hz, 1H), 4.22 (dd, *J* = 3.0 and 11.5 Hz, 1H), 4.12-4.05 (m, 2H), 3.56 (d, *J* = 3.0 Hz, 1H), 3.46 (d, *J* = 2.0 Hz, 1H), 2.36-2.32 (m, 1H), 1.19-0.88 (m ,27H); HR-MS Calcd. for $(C_{11}H_{14}FN_5O_3+H)^+$ 284.1159, found 284.1157.

(-)-(3a*S*,4*R*,6*R*,6a*R*)-6-(*tert*-butoxymethyl)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl benzoate (87) Ketone **20** (2.4 g, 10.0 mmol) was dissolved in the MeOH (20 mL) and treated with cesium chloride hepatahydrate (5.4 g, 14.9 mmol) at rt for 10 min and then cooled to -78 ºC. Sodium borohydride (450 mg, 12.0 mmol) was added to the pre-cooled solution slowly and the reaction was kept at the same temperature for 30 min before quenched with HOAc. After removing the solvent *in vacuo*, the residue was dissolved in EtOAc and washed with H_2O, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The

residue, TPP (5.2 g, 20.0 mmol) and benzoic acid (2.4 g, 20.0 mmol) were dissolved in anhydrous THF in 100 mL round-bottom flask and were treated with DIAD (4.0 mL, 20.0 mmol) at 0 ºC.The reaction was kept at 40-50 ºC overnight. The dark red solution was concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:50 to 1:10) to give compound **87** (2.4 g, 70 % from **20**) as a sticky syrup. $[\alpha]^{23}_{D}$ -19.90° (*c* 0.48, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03-7.44 (m, 5H), 5.31-5.29 (m, 1H), 4.67-4.61 (m, 2H), 3.46-3.32 (m, 2H), 2.50-2.38 (m, 2H), 1.83-1.78 (m, 1H), 1.50 (s, 3H), 1.32 (s, 3H), 1.16 (s, 9H); ^{13}C NMR (125 MHz, CD_3OD) δ 165.6, 133.1,130.1, 129.5, 128.4, 111.0, 85.1, 82.6, 80.4, 72.7, 62.6, 45.7, 32.2, 27.5, 26.8, 24.4; HR-MS Calcd. for $(C_{21}H_{28}O_5+H)^+$ 349.2015, found 349.2029.

(-)-(1*R*,2*R*,3*R*,4*R*)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl benzoate (88) To a solution of compound **87** (2.4 g, 6.9 mmol) in CH_2Cl_2 (50 mL), titanium tetrachloide (2.3 mL, 20.9 mmol) was added slowly at 0 ºC then the reaction was allowed to warm up to rt for 30 min. The reaction mixture was re-cooled to 0 ºC, and additional amount of titanium tetrachloide (1.2 mL, 10.9 mmol) was added. After kept at rt for another 15 min, the reaction was slowly quenched with MeOH at 0 ºC. After removing the solvent, the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:20 to 1:10) to give compound **88** contaminated with a higher polar inpurity. The mixture was then dissolved in $CHCl_3$/*i*-propanol (4:1) co-solvent and washed with diluted HCl soltion, H_2O and brine, dried over Na_2SO_4 and concentrated *in vacuo* to give desired compound **88** (1.5 g, 86 %) as a pure product. $[\alpha]^{25}_{D}$ -29.05° (*c* 0.40, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06-7.50 (m, 5H), 5.23-5.20 (m, 1H), 4.09 (t, *J* = 4.5 Hz, 1H), 3.99 (t, *J* = 4.5 Hz, 1H), 3.71 (dd, *J* = 5.0 and 10.5 Hz, 1H), 3.63 (dd, *J* = 6.0 and 11.0 Hz, 1H), 2.55-2.49 (m, 1H), 2.26-2.18 (m, 1H), 1.55-1.50 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 166.2, 132.9, 130.1,

129.1, 128.2, 78.8, 75.9, 72.9, 63.2, 44.6, 29.8; HR-MS Calcd. for $(C_{13}H_{16}O_5+H)^+$ 253.1076, found 253.1078.

(-)-(3a*S*,4*R*,6*R*,6a*R*)-6-((*tert*-butyldiphenylsilyloxy)methyl)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-ol (90) To a solution of triol **88** (1.5 g, 6.0 mmol) and imidazole (800 mg, 12.0 mmol) in anhydrous CH_2Cl_2, *tert*-butyldiphenylchlorosilane (1.62 mL, 6.3 mmol) was added slowly. The reaction mixture was kept at room temperature for 1 h and then quenched with MeOH. After removing the solvent, the residue was dissolved in EtOAc and washed with H_2O and brine, dried over magnesium sulfate. The volatile was removed *in vacuo* and the residue was dissolved in acetone and treated with *p*-tolunenesulfonic acid (25 mol %) and excess 2,2-dimethoxypropane at room temperature for 20 min. The reaction mixture was neutrulized with solid NaOMe and evaperated to dry *in vacuo*. The residue was then dissolved in MeOH and treated with NaOMe (650 mg, 12.0 mmol) at room temperature overnight. After removing the solvent, the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:20 to 1:10) to give alcohol **90** (1.9 g, 73 % from **88**). $[\alpha]^{24}_D$ -5.38° (*c* 0.65, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69-7.26 (m, 5H), 4.58 (d, *J* = 6.0 Hz, 1H), 4.47 (d, *J* = 6.0 Hz, 1H), 4.17-4.14 (m, 1H), 3.78-3.72 (m, 2H), 3.66 (dd, *J* = 4.0 and 10.0 Hz, 1H), 2.46-2.40 (m, 1H), 2.30-2.28 (m, 1H), 1.67 (d, *J* = 15.0 Hz, 1H), 1.42 (s, 3H), 1.29 (s, 3H), 1.07 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.8, 135.6, 132.4, 130.1, 130.0, 128.0, 127.9, 110.1, 88.0, 83.4, 76.7, 66.3, 47.5, 35.5, 26.9, 26.8, 24.2, 19.2; HR-MS Calcd. for $(C_{25}H_{34}O_4Si+H)^+$ 427.2305, found 427.2338.

(+)-*tert*-butyl(((3a*R*,4*R*,6a*S*)-2,2-dimethyl-4,6a-dihydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl)methoxy)diphenylsilane (91) To a solution of **90** (1.4 g, 3.2 mmol) in anhydrous toluene (35 mL), triphenylphosphine (2.5 g, 9.6 mmol) and diisopropyl azodicarboxylate (1.93 mL, 9.6 mmol) were added. The reaction mixture in the 100 mL round bottom flask equipped with a

condenser, was placed in a microwave synthesizer and irradiated at maximum output power of 300 W with air-cooling at 90 ºC for 10 min. The dark red solution was concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:200 to 1:20) to give **91** (1.2 g, 89 %) as a syrup. $[\alpha]^{24}_{D}$ +85.02° (*c* 0.26, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65-7.36 (m, 5H), 5.88 (d, *J* = 5.5 Hz, 1H), 5.76 (d, *J* = 3.5 Hz, 1H), 5.15 (d, *J* = 5.5 Hz, 1H), 4.58 (d, *J* = 6.0 Hz, 1H), 3.74 (dd, *J* = 4.5 and 9.0 Hz, 1H), 3.60 (dd, *J* = 5.0 and 10.0 Hz, 1H), 2.98 (s, br, 1H), 1.42 (s, 3H), 1.35 (s, 3H), 1.03 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.7, 135.6, 134.1, 133.5, 133.4, 132.5, 129.8 127.8, 127.7, 109.8, 85.3, 81.2, 64.7, 54.4, 27.5, 26.8, 25.7, 19.3; HR-MS Calcd. for $(C_{25}H_{32}O_3Si+H)^+$ 409.2199, found 409.2199.

(+)-((3a*R*,4*R*,6a*S*)-2,2-dimethyl-4,6a-dihydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl)methyl benzoate (93) Compound **91** (1.4 g, 3.3 mmol) was treated with tetrabutylammonium fluoride (1.0 M solution in THF, 5.0 mL, 5.0 mmol) in THF for 40 min. After removing the solvent *in vacuo*, the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:50 to 1:10) to give a syrup which was directly dissolved in anhydrous pyridine and treated with benzoyl chloride (0.58 mL, 5.0 mmol). The reaction mixture was kept at room temperature for 3 h and quenched with MeOH. After removing the volatile, the residue was dissolved in EtOAc and throughly washed with diluted HCl solution and H_2O, dried over magnisum sulfate, filtered and concentrated *in vacuo*. The residue was dissolved in MeOH (10 mL) and treated with 3 N HCl solution (10 mL) for 1 h. After neutrulized with solid $NaHCO_3$, the solvent was removed and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:4 to 1:1) to give **93** (660 mg, 85 %) as a syrup. $[\alpha]^{24}_{D}$ +193.56° (*c* 0.25, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01-7.42 (m, 5H), 5.97-5.93 (m,, 2H), 4.67 (d, *J* = 5.5 Hz, 1H), 4.46 (dd, *J* = 6.0 and 11.5 Hz, 1H), 4.36 (dd, *J* = 5.5 and 11.0 Hz, 1H), 4.16 (dd, *J* = 4.0 and 5.0 Hz, 1H), 3.12-3.08 (m,

1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.6, 134.8, 133.2, 133.0, 129.6, 129.9, 128.5, 75.1, 73.8, 65.0, 51.5; HR-MS Calcd. for $(C_{13}H_{14}O_4+NH_4)^+$ 252.1236, found 252.1238.

(+)-((1*S*,2*R*,3*R*,4*R*,5*R*)-3,4-dihydroxy-6-oxabicyclo[3.1.0]hexan-2-yl)methyl benzoate (94) To a solution of diol **93** (600 mg, 2.56 mmol) in CH_2Cl_2 (25 mL), 3-chloroperoxy benzoic acid (77 % max., 2.3 g, 10.2 mmol) was added at 0 ºC. The reaction mixture was allowed to warmed up to room temperature and kept overnight. After diluted with isopropyl alcohol/chloroform (4:1) co-solvent (40 mL), a aquous solutoin of sodium carbonate (25 mL) was added and stirred for 15 min. Collecting the organic phase and washed again with sodium carbonate solution twice and H_2O twice, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:50 to 1:20) to yield the compound **94** (450 mg, 70 %) as a wax. $[\alpha]^{24}_D$ +39.52° (*c* 1.83, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00-7.45 (m, 5H), 4.44 (dd, *J* = 5.0 and 11.0 Hz, 1H), 4.36-4.33 (m, 2H), 3.91 (s, br, 1H), 3.72 (s, br, 1H), 3.67 (s, br, 1H), 3.05 (s, br, 1H), 2.84 (t, *J* = 5.5 Hz, 1H), 2.44 (s, br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2, 133.5, 120.6, 129.3, 128.7, 72.9, 71.6, 62.7, 60.0, 56.8, 47.6; HR-MS Calcd. for $(C_{13}H_{14}O_5+H)^+$ 251.0920, found 251.0919.

(+)-(1*R*,2*R*,3*R*,4*R*,5*S*)-4-(hydroxymethyl)-6-oxabicyclo[3.1.0]hexane-2,3-diol (95) Compound **94** (440 mg, 1.76 mmol) was treated with saturated methanolic ammonia at room temperature over night. After removing the solvent *in vacuo*, the residue was purified twice by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:40 to 1:10) to obtain the compound **95** (200 mg, 78 %) as a pale yellow syrup. $[\alpha]^{26}_D$ +45.10° (*c* 36, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.27 (d, *J* = 7.0 Hz, 1H), 3.78 (d, (m, *J* = 7.0 Hz, 1H), 3.63-3.61 (m, 2H), 3.56 (s, br, 1H), 3.51 (s, br, 1H), 2.42 (t, *J* = 5.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 72.9, 70.8, 60.2, 59.2, 56.4, 50.8; HR-MS Calcd. for $(C_6H_{10}O_4+H)^+$ 147.0657, found 147.0655.

(+)-(3a*R*,4*R*,6a*S*)-4-(*tert*-butoxymethyl)-2,2-dimethyl-4,6a-dihydro-3a*H*-cyclopenta[*d*][1,3]dioxole (98) Ketone **20** (2.0 g, 8.3 mmol) was dissolved in the MeOH (20 mL) and treated with cesium chloride hepatahydrate (4.5 g, 12.4 mmol) at rt for 10 min and then cooled to -78 ºC. Sodium borohydride (376 mg, 10 mmol) was added to the pre-cooled solution slowly and the reaction was kept at the same temperature for 30 min before quenched with HOAc. After removing the solvent *in vacuo*, the residue was dissolved in EtOAc and washed with H_2O, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue, TPP (6.5 g, 24.9 mmol) and benzoic acid (3.0 g, 24.9 mmol) were dissolved in anhydrous THF in 100 mL round-bottom flask and were treated with DIAD (5.0 mL, 24.9 mmol) at 0 ºC. The reaction flask equipped with a condenser, was placed in a microwave synthesizer and irradiated at maximum output power of 300 W with air-cooling at 90 ºC for 10 min. The dark red solution was concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:50 to 1:10) to give a syrup which was treated directly with NaOMe in MeOH at rt overnight. After concentration *in vacuo*, the residue was then purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:30 to 1:10) to give a coloress syrup. The reaction flask which was charged with above syrup, TPP (3.22 g, 12.3 mmol) and DIAD (2.5 mL, 12.3 mmol) and equipped with a condenser, was placed in a microwave synthesizer and irradiated at maximum output power of 300 W with air-cooling at 80 ºC for 10 min. After concentration, the residue was The syrup was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:200 to 1:20) to give olefin **98** (1.3 g, 69 % from **20**) as a pink syrup. $[\alpha]^{25}_{D}$ +130.67° (*c* 0.79, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.84-5.78 (m, 2H), 5.12 (d, *J* = 5.5 Hz, 1H), 4.49 (d, *J* = 6.0 Hz, 1H), 3.37 (dd, *J* = 6.0 and 9.0 Hz, 1H), 3.24 (dd, *J* = 6.5 and 9.0 Hz, 1H), 2.95 (s, br, 1H), 1.41 (s, 3H), 1.36 (s, 3H), 1.16 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ

134.5, 132.0, 109.8, 85.1, 81.5, 72.7, 62.8, 53.0, 27.5, 25.8; HR-MS Calcd. for $(C_{13}H_{22}O_3+H)^+$ 244.1913, found 244.1934.

(+)-(1*R*,2*R*,5*S*)-2-(*tert*-butoxymethyl)-5-isopropoxycyclopent-3-enol (99) and (1*S*,4*R*,5*R*)-4-(*tert*-butoxymethyl)-5-isopropoxycyclopent-2-enol (100) Olefin **98** (40 mg, 0.18 mmol) was dissoleved in CH_2Cl_2 and treated with DIBAL-H (1.0 M in CH_2Cl_2, 1.8 mL, 1.8 mmol) at 0 ºC which was allowed to warmed up to room temperature for 30 min before quenched with CH_3OH. The mixture was poured into a solution of potassium sodium tartrate solution and stirred for 30 min and extracted with CH_2Cl_2, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:20 to 1:5) to yield the desired compound **100** (27 mg, 66 %) as well as its isomer **99** (10 mg, 24 %). Compound **99**: $[\alpha]^{27}_D$ +115.82° (*c* 0.39, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.92-5.78 (m, 2H), 4.39-4.36 (m, 1H), 4.00- 3.98 (m, 1H), 3.80-3.74 (m, 1H), 3.45 (dd, *J* = 7.0 and 10.5 Hz, 1H), 3.33 (dd, *J* = 8.0 and 11.0 Hz, 1H), 3.02 (s, br, 1H), 2.81-2.76 (m, 1H), 1.22 (t, *J* = 7.0 Hz, 1H), 1.16 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.2, 120.5, 80.8, 73.1, 72.6, 72.0, 62.5, 53.4, 27.5, 22.8, 22.7; HR-MS Calcd. for $(C_{13}H_{24}O_3+H)^+$ 229.1804, found 229.1814. Compound **100**: $[\alpha]^{27}_D$ +116.74° (*c* 1.21, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.88-5.80 (m, 2H), 4.61 (s, br, 1H), 3.88- 3.82 (m, 1H), 3.81-3.75 (m, 1H), 3.36 (dd, *J* = 7.0 and 11.0 Hz, 1H), 3.23 (dd, *J* = 9.0 and 11.0 Hz, 1H), 3.05 (d, *J* = 8.0 H,z 1H), 2.84-2.81 (m, 1H), 1.22 (d, *J* = 8.0 Hz 1H), 1.18 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.0, 133.6, 78.4, 74.2, 72.6, 71.9, 62.1, 51.8, 27.5, 22.5, 22.4; HR-MS Calcd. for $(C_{13}H_{24}O_3+Na)^+$ 251.1623, found 251.1645.

(+)-(1*R*,2*R*,3*R*,4*R*,5*S*)-4-(*ter*t-butoxymethyl)-3-isopropoxy-6-oxabicyclo[3.1.0]hexan-2-ol (101) To a solution of **100** (55 mg, 0.24 mmol) in CH_2Cl_2 (1.5 mL), mCPBA (216 mg, 77 % max) was added at 0 ºC. The mixture was allowed to warm up to room temperature and kept for 8 h

before Na_2CO_3 aquous solution was added. The water phase was extracted with EtOAc, and dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:20 to 1:5) to give compound **101** as a pale yellow liquid. $[\alpha]^{25}_{D}$ +55.03° (*c* 0.31, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.29 (t, *J* = 8.5 Hz, 1H), 3.64-3.60 (m, 2H), 3.52 (s, br, 1H), 3.41 (dd, *J* = 5.0 and 9.5 Hz, 1H), 3.36-3.33 (m, 2H), 3.10 (d, *J* = 10.5 Hz, 1H), 2.46 (d, *J* = 4.5 Hz, 1H), 1.18 (d, *J* = 6.5.0 Hz, 1H), 1.16 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 77.3, 73.7, 72.8, 60.4, 59.8, 48.0, 27.3, 22.5, 22.3; HR-MS Calcd. for $(C_{13}H_{24}O_4+H)^+$ 245.1753, found 245.1741.

(+)-(1*R*,2*S*,3*R*,4*R*,5*S*)-4-(*tert*-butoxymethyl)-3-isopropoxy-6-oxabicyclo[3.1.0]hexan-2-ol (102) Alcohol **101** (440 mg, 1.8 mmol), TPP (1.4 g, 5.4 mmol) and benzoic acid (650 mg, 5.4 mmol) were dissolved in anhydrous THF in 100 mL round-bottom flask and were treated with DIAD (1.1 mL, 5.4 mmol) at 0 ºC. The reaction flask equipped with a condenser, was placed in a microwave synthesizer and irradiated at maximum output power of 300 W with air-cooling at 70 ºC for 15 min. The dark red solution was concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:50 to 1:10) to give a syrup which was treated directly with NaOMe in MeOH at rt overnight. After concentration *in vacuo*, the residue was then purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:30 to 1:10) to give **102** as coloress syrup (400 mg, 90 % from **101**). $[\alpha]^{25}_{D}$ +52.73° (*c* 0.13, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.99 (d, *J* = 12.0 Hz, 1H), 3.92 (d, *J* = 12.5 Hz, 1H), 3.67-3.54 (m, 4H), 3.50 (s, 1H), 3.37 (d, *J* = 2.0 Hz, 1H), 2.37 (s, 1H), 1.27-1.13 (m, 15 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 88.0, 75.0, 74.5, 71.3, 61.1, 59.5, 59.2, 47.0, 27.2, 22.8, 22.2; HR-MS Calcd. for $(C_{13}H_{24}O_4+H)^+$ 245.1753, found 245.1757.

(+)-(1*S*,2*S*,3*R*,4*S*,5*S*)-2-(*tert*-butoxymethyl)-4-fluoro-3-isopropoxy-6-oxabicyclo[3.1.0]hexane (104) To a solution of alchohol **102** (50 mg, 0.20 mmol) in CH_2Cl_2, DAST (0.08 mL, 0.60 mmol) was added dropwise at -78 ºC. The reaction mixture was allow to warm up to rt and kept for 15 min before quenched with MeOH/$NaHCO_3$ (soild). After purifiation by column chromatography on a silica gel (EtOAc:Hexanes = 1:40 to 1:20) to give **104** (24 mg, 48 %) as a pale yellow liquid. $[\alpha]^{25}_D$ +33.28° (*c* 0.70, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.90 (d, *J* = 50.0 Hz, 1H), 3.85 (d, *J* = 26.0 Hz, 1H), 3.68-3.66 (m, 1H), 3.61 (ds, br, 1H), 3.52 (s, br, 1H), 3.40 (d, *J* = 7.5 Hz, 1H), 3.33 (d, *J* = 8.0 Hz, 1H), 2.37 (t, *J* = 7.0 Hz, 1H), 1.20 (s, 9 H), 1.16 (t, *J* = 5.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 96.8 (d, *J* = 176.5 Hz), 84.6 (d, *J* = 22.9 Hz), 73.0, 71.4, 69.3, 59.5, 57.0 (d, *J* = 40.5 Hz), 47.9 (d, *J* = 2.9 Hz), 27.4, 22.2, 22.1; HR-MS Calcd. for $(C_{13}H_{23}FO_3+H)^+$ 247.1710, found 247.1714.

(+)-(3a*R*,4*R*,6a*S*)-4-(benzyloxymethyl)-2,2-dimethyl-4,6a-dihydro-3a*H*-cyclopenta[*d*][1,3]dioxole (105) To a solution of olefin **91** (200 mg, 0.47 mmol) in THF, TBAF (1.0 M in THF, 0.71 mL, 0.71 mmol) was added and kept at rt for 35 min. The reaction mixture was concentrated and purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:10 to 1:3) to give a syrup which was directly dissolved in THF (5 mL) and treated with NaH (25 mg, 0.63 mmol) at rt for 20 min. TBAI and BnBr were added to this slurry and kept at rt for another 4 h before quenching with ice-water. The mixture was extracted with EtOAc and dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:40 to 1:10) to give compound **105** as a pale yellow liquid (105 mg, 85 % from **91**). $[\alpha]^{25}_D$ +113.52° (*c* 0.51, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.04-7.32 (m, 5H), 5.92-5.82 (m, 2H), 5.19 (d, *J* = 5.0 Hz, 1H), 4.60 (d, *J* = 6.0 Hz, 1H), 4.57 (s, 2H), 3.54 (dd, *J* = 5.5 and 9.5 Hz, 1H), 3.42 (dd, *J* = 6.5 and 9.5 Hz, 1H), 3.10 (s,

br, 1H), 1.47 (s, 3 H), 1.39 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.3, 134.0, 132.3, 128.4, 127.6, 127.5, 110.0, 85.1, 81.5, 73.1, 71.2, 52.6, 27.4, 25.6; HR-MS Calcd. for $(C_{16}H_{20}O_3+NH_4)^+$ 278.1756, found 278.1762.

(+)-(1*R*,2*R*,5*S*)-2-(benzyloxymethyl)-5-isopropoxycyclopent-3-enol (106) and (+)-(1*S*,4*R*,5*R*)-4-(benzyloxymethyl)-5-isopropoxycyclopent-2-enol (107) Olefin **105** (95 mg, 0.36 mmol) was dissoleved in CH_2Cl_2 and treated with DIBAL-H (1.0 M in CH_2Cl_2, 1.8 mL, 1.8 mmol) at 0 ºC which was allowed to warmed up to room temperature for 30 min before quenched with CH_3OH. The mixture was poured into a solution of potassium sodium tartrate solution and stirred for 30 min and extracted with CH_2Cl_2, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:20 to 1:5) to yield the desired compound **107** (40 mg, 42 %) as well as its isomer **106** (50 mg, 52 %). Compound **107**: $[\alpha]^{25}_D$ +132.66° (*c* 0.37, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36-7.26 (m, 5H), 5.92-5.85 (m, 2H), 4.59 (s, br, 1H), 4.53 (d, *J* = 2.5 Hz, 1H), 3.90 (dd, *J* = 3.5 and 5.5 Hz, 1H), 3.75-3.70 (m, 1H), 3.50-3.43 (m, 2H), 2.96 (d, *J* = 5.5 Hz, 1H), 2.93-2.92 (m, 1H), 1.20 (d, *J* = 6.5 Hz, 1H), 1.17 (d, *J* = 6.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.3, 134.0, 133.7, 128.4, 127.6, 127.5, 78.4, 74.0, 73.2, 72.1, 70.4, 51.1, 22.6, 22.4; HR-MS Calcd. for $(C_{16}H_{22}O_3+NH_4)^+$ 280.1913, found 280.1936. compound **106**: $[\alpha]^{23}_D$ +171.56° (*c* 0.61, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.26 (m, 5H), 5.92-5.82 (m, 2H), 4.53 (s, 2H), 4.38 (d, *J* = 6.0 Hz, 1H), 4.06-4.03 (m, 1H), 3.79-3.74 (m, 1H), 3.56 (dd, *J* = 5.0 and 9.0 Hz, 1H), 3.49 (dd, *J* = 6.5 and 9.0 Hz, 1H), 3.03 (d, *J* = 7.0 Hz, 1H), 2.90-2.88 (m, 1H), 1.21 (t, *J* = 6.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 135.7, 131.0, 128.4, 127.6, 80.7, 73.1, 73.0, 72.2, 70.9, 53.1, 22.8, 22.7; HR-MS Calcd. for $(C_{16}H_{22}O_3+NH_4)^+$ 280.1913, found 280.1917.

(+)-(1*R*,2*R*,3*R*,4*R*,5*S*)-4-(benzyloxymethyl)-3-isopropoxy-6-oxabicyclo[3.1.0]hexan-2-ol (108)

To a solution of olefin **107** (36 mg, 0.14 mmol) in CH_2Cl_2 (1 mL), mCPBA (125 mg, 0.56 mmol) was added at 0 ºC which was allowed to warmed up to room temperature for overnight before quenched with Na_2CO_3 solution. The mixture was extracted with CH_2Cl_2 and washed with Na_2CO_3 solution and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:20 to 1:3) to yield compound **108** (27 mg, 71 %). $[\alpha]^{24}_D$ +37.04 (*c* 0.26, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.26 (m, 5H), 4.51 (s, 2H), 4.32 (t, *J* = 8.0 Hz, 1H), 3.68-3.46 (m, 6H), 3.37 (d, *J* = 2.8 Hz, 1H), 3.07 (d, *J* = 10 Hz, 1H), 2.53 (t, *J* = 4.8 Hz, 1H), 1.16 (t, *J* = 5.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.8,128.5, 127.8, 127.4, 77.1, 73.8, 73.4, 72.5, 68.9, 59.8, 57.1, 47.9, 22.4, 22.3; HR-MS Calcd. for $(C_{16}H_{22}O_4+NH_4)^+$ 296.1862, found 296.1875.

(+)-(1*R*,2*S*,3*R*,4*R*,5*S*)-4-(benzyloxymethyl)-3-isopropoxy-6-oxabicyclo[3.1.0]hexan-2-ol (109)

DIAD (0.13 mL, 0.65 mmoL) was added dropwise to a solution of epoxide **108** (60 mg, 0.22 mmoL), TPP (170 mg, 0.65 mmol) and benzoic acid (80 mg, 0.65 mmoL) in THF (2 mL) at 0 ºC. The reaction mixture was kept at rt for overnight and evaporated *in vacuo* to give a yellowish residue which was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:30 to 1:10) to give a syrup which was directly dissolved in MeOH (3 mL) and treated with NaOMe (23 mg, 0.43 mmol) at rt overnight. The mixture was concentrated *in vacuo*, extracted with EtOAc and dried over magnesium sulfate, filtered and concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:20 to 1:10) to give compound **109** as a liquid (49 mg, 82 % from **108**). $[\alpha]^{24}_D$ +49.12 (*c* 0.17, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.27 (m, 5H), 4.56 (s, 2H), 4.00 (t, *J* = 12.4 Hz, 1H), 3.70-3.42 (m, 7H), 2.37 (s, 1H), 1.14 (dd, *J* = 6.4 and 16.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.6,

128.7, 128.4, 128.0, 88.0, 75.3, 73.9, 71.3, 69.5, 59.4, 59.0, 47.3, 22.7, 22.1; HR-MS Calcd. for $(C_{16}H_{22}O_4+NH_4)^+$ 296.1862, found 296.1874.

(+)-(1*S*,2*S*,3*R*,4*S*,5*S*)-2-(benzyloxymethyl)-4-fluoro-3-isopropoxy-6-oxabicyclo[3.1.0]hexane (111) Alcohol **109** (40 mg, 0.14 mmol) in CH_2Cl_2 (1mL) was treated with DAST (0.055 mL, 0.42 mmoL) at -78 ºC. The reaction mixture was allowed to warm up to rt for 15 min. After quenching with MeOH, the mixture was concentrated *in vacuo* and was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:40 to 1:20) to give compound **111** as a liquid (20 mg, 51 %). $[\alpha]^{25}_D$ +40.10 (*c* 0.18, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.26 (m, 5H), 4.85 (d, *J* = 50.4 Hz, 2H), 4.55 (s, 2H), 3.87 (d, *J* = 25.6 Hz, 1H), 3.68-3.50 (m, 5H), 2.48 (m, 1H), 1.14 (dd, *J* = 6.0 and 10.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.9, 128.4, 127.8, 127.6, 96.5 (d, *J* = 176.8 Hz), 84.7 (d, *J* = 23.7 Hz), 73.2, 71.6, 68.7, 59.3, 56.9 (d, *J* = 39.6 Hz), 47.5, (d, *J* = 3.0 Hz), 22.2 (d, *J* = 30.0 Hz); HR-MS Calcd. for $(C_{16}H_{21}FO_3+NH_4)^+$ 298.1818, found 298.1805.

(-)-(6a*R*,8*S*,9*S*,9a*R*)-8-(benzyloxy)-2,2,4,4-tetraisopropyl-9-methyl-7-methylenehexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-ol (113) and (-)-(6a*R*,8*S*,9*R*,9a*R*)-8-(benzyloxy)-2,2,4,4-tetraisopropyl-9-methyl-7-methylenehexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-ol (114) Alcohol **25** (770 mg, 1.56 mmol) in CH_2Cl_2 (10 mL) was treated with Dess-Martin periodiane (1.0 g, 2.34 mmol) at -0 ºC to rt for 1h before quenching with cold $Na_2S_2O_3$ / $NaHCO_3$ solution. The reaction mixture was then extracted with CH_2Cl_2 and washed with $NaHCO_3$ solution, H_2O and brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* and the residue was directly used for the next step. The above residue was dissolved in Et_2O (10 mL) and added slowly to a solution of CH_3MgBr in Et_2O (0.35 M, 20 mL) at -78 ºC. The reaction was kept at -78 ºC for 30 min and

then warmed up to -30 ºC for another 30 min before quenching with NH_4Cl solution. The reaction mixture was extracted with Et_2O and washed with NH_4Cl, brine and dried over magnesium sulfate, filtered and concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:40 to 1:15) to give compound **113** (175 mg, 22 % from **25**) as a liquid and compound **114** (485 mg, 61 %) as a liquid. Compound **113**: $[\alpha]^{25}_D$ -89.87 (*c* 0.46, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47-7.30 (m, 5H), 5.38 (t, *J* = 2.5 Hz, 1H), 5.09 (t, *J* = 2.5 Hz, 1H), 4.94 (d, *J* = 11.5 Hz, 1H), 4.81 (d, *J* = 12.0 Hz, 1H), 4.20 (d, *J* = 2.5 Hz, 1H), 4.10 (d, *J* = 8.0 Hz, 1H), 4.05-3.89 (m, 2H), 2.55 (s, br, 1H), 1.72 (s, 1H, D_2O exchangable), 1.27 (s, 3H), 1.16-1.00 (m, 27H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.9, 128.4, 127.7, 127.6, 110.4, 84.2, 82.0, 79.1, 66.0, 49.5, 17.6, 17.5, 17.4, 17.3, 17.2, 15.7, 13.6, 13.4, 12.9, 12.8; HR-MS Calcd. for $(C_{27}H_{46}O_5Si_2+H)^+$ 507.2962, found 507.2910.Compound **114**: $[\alpha]^{26}_D$ -43.25 (*c* 0.54, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43-7.30 (m, 5H), 5.29 (s, 1H), 5.09 (s, 1H), 4.81 (d, *J* = 12.5 Hz, 1H), 4.68 (d, *J* = 12.0 Hz, 1H), 4.17 (dd, *J* = 4.5 and 9.0 Hz, 1H), 3.95 (d, *J* = 5.5 Hz, 1H), 3.84 (d, *J* = 1.0 Hz, 1H), 3.78 (dd, *J* = 9.5 and 12.0 Hz, 1H), 3.06 (s, br, 1H, D2O exchangable), 2.93 (s, br, 1H), 1.30 (s, 3H), 1.19-0.90 (m, 27 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.6, 138.4, 128.3, 127.8, 127.6, 110.8, 85.8, 78.8, 76.3, 72.1, 65.2, 51.5, 24.1, 17.7, 17.6, 17.5, 17.4, 17.3, 17.2, 17.0, 13.7, 13.5, 13.1, 12.6; HR-MS Calcd. for $(C_{27}H_{46}O_5Si_2+H)^+$ 507.2962, found 507.2967.

(+)-((1*R*,2*R*,3*R*,4*S*)-2-(benzoyloxy)-4-(benzyloxy)-3-hydroxy-3-methyl-5-methylenecyclopentyl)methyl benzoate (116) Compound **114** (450 mg, 0.89 mmol) was dissolved in THF (10 mL) and treated with TBAF (3.5 mL, 3.5 mmol) for 1 h. After removing the solvent, the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:40 to 1:10) to give compound **115** which was dissolved in pyridine (10 mL) and treated with

benzoyl chloride (0.53 mL, 4.5 mmol) and DMAP (100 mg, 0.89 mmol) at 40 ºC overnight. After removing the volatile, the residue was dissolved in EtOAc and washed with H_2O, brine and dried over Na_2SO_4, filtered and concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:40 to 1:15) to give compound **116** (360 mg, 86 % from **114**) as a liquid. $[\alpha]^{26}_D$ +30.40 (*c* 0.58, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02-7.28 (m, 15H), 5.38 (d, *J* = 2.0 Hz, 1H), 5.34 (d, *J* = 1.0 Hz, 1H), 5.26 (d, *J* = 6.0 Hz, 1H), 4.78 (d, *J* = 11.5 Hz, 1H), 4.67-4.15 (m, 3H), 3.91 (s, 1H), 3.41-3.40 (m, 1H), 3.30 (s, 1H), 1.37 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.5, 166.0, 145.5, 137.7, 133.1, 133.0, 129.9, 129.8, 128.7, 128.4, 128.3, 128.0, 113.7, 86.2, 79.6, 76.2, 71.6, 64.7, 46.0, 23.7; HR-MS Calcd. for $(C_{29}H_{28}O_6+NH_4)^+$ 490.2229, found 490.2220.

(-)-((1*R*,2*R*,3*S*,4*S*)-2-(benzoyloxy)-3,4-dihydroxy-3-methyl-5-methylenecyclopentyl)methyl benzoate (117) Compound **116** (25 mg, 0.053 mmol) in CH_2Cl_2 (1 mL) was treated with BCl_3 (1.0 M in CH_2Cl_2, 0.53 mL, 0.53mmol) at -78 ºC for 15 min before quenching with MeOH. After removing the solvent the residue was purified by preparative TLC to obtain compound **117** (18 mg, 89 %) a yellow oil which was solidifed on standing at rt. mp: 92-94 ºC; $[\alpha]^{25}_D$ -2.09 (*c* 0.37, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03–7.18 (m, 10H), 5.46 (d, *J* = 2.5 Hz, 1H), 5.38 (d, *J* = 8.0 Hz, 1H), 5.28 (s, 1H), 4.55 (dd, *J* = 5.0 and 11.5 Hz, 1H), 4.41 (dd, *J* = 7.0 and 11.0 Hz, 1H), 4.40 (s, 1H), 3.41 (d, *J* = 2.5 Hz, 1H), 2.83 (s, br, 1H), 2.74 (s, br, 1H), 1.37 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.5, 166.4, 149.1, 133.4, 132.9, 129.9, 129.6, 129.5, 129.4, 128.5, 128.2, 111.3, 78.2, 77.7, 77.3, 65.7, 44.3, 22.1; HR-MS Calcd. for $(C_{22}H_{22}O_6+NH_4)^+$ 400.1760, found 400.1729.

(+)-(1*R*,2*R*,3*R*,5*S*)-3-(acetoxymethyl)-5-(benzyloxy)-1-methyl-4-methylenecyclopentane-1,2-diyl diacetate (119) Compound **115** (50 mg, 0.19 mmol), DMAP (22 mg, 0.20 mmol) and

acetic anhydride (0.19 mL, 2.0 mmol) were dissolved in pyridine (2 mL) and heated at 80-100 ºC at conventinal oil bath overnight and Microwave assisted oven 15 min. After removing the volatile, the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:40 to 1:20) to give compound **119** (65 mg, 95 %). $[\alpha]^{26}_{D}$ +26.25 (*c* 0.71, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.26 (m, 5H), 5.31 (d, *J* = 3.0 Hz, 1H), 5.18 (d, *J* = 2.0 Hz, 1H), 5.00 (d, *J* = 5.0 Hz, 1H), 4.62 (d, *J* = 12.0 Hz, 1H), 4.42 (d, *J* = 12.0 Hz, 1H), 4.31-4.26 (m, 2H), 3.12-3.11 (m, 1H), 2.11 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.50 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.5, 169.7, 150.0, 144.8, 138.4, 128.2, 127.4, 127.3, 113.4, 84.7, 82.1, 79.1, 70.3, 63.2, 45.0, 21.7, 20.9, 20.8; HR-MS Calcd. for $(C_{21}H_{26}O_7+NH_4)^+$ 408.2022, found 408.2018.

(+)-(6a*R*,8*S*,9*S*,9a*R*)-8-(benzyloxy)-2,2,4,4-tetraisopropyl-9-methylhexhydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-ol (122) To a solution of DMSO (0.27 mL, 3.76 mmol) in CH_2Cl_2 (10 mL), oxalyl chloride (0.16 mL, 1.88 mmol) was added slowly at -78 ºC and reaction mixture was kept at the same temperature for 30 min. Alcohol **32** (450 mg, 0.94 mmol) in CH_2Cl_2 (10 mL) was added into this mixture and kept at -78 ºC for 1 h before Et_3N (1.0 mL, 7.52mmol) was added and allowed to warm up to rt for another 40 min. The reaction micture was partitioned in CH_2Cl_2 and H_2O. The organic layer was collected and washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:40 to 1:20) to give compound **33** (400 mg, 89 %) directly used for the next step. Compound **33** (50 mg, 0.1 mmol) was dissolved in Et_2O (2 mL) and added slowly to a solution of CH_3MgBr in Et_2O (0.35 M, 12 mL) at -78 ºC. The reaction was kept at -78 ºC for 20 min and then quenched with NH_4Cl solution. The reaction mixture was extracted with Et_2O and washed with NH_4Cl,

brine and dried over magnesium sulfate, filtered and concentrated *in vacuo* and the residue purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:30 to 1:10) to give compound **122** (36 mg, 70 %). $[\alpha]^{28}_{D}$ +12.68 (*c* 0.28, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47-7.30 (m, 5H), 4.69 (d, *J* = 12.5 Hz, 1H), 4.60 (d, *J* = 12.5 Hz, 1H), 3.98-3.95 (m, 1H), 3.76 (d, *J* = 8.0 Hz, 1H), 3.64-3.61 (m, 1H), 3.55 (t, *J* = 6.5 Hz, 1H), 2.96 (s, 1H), 2.43 (s, br, 1H), 1.97-1.91 (m, 1H), 1.62-1.56 (m,1 H), 1.31 (s, 3H), 1.10-0.96 (m, 27H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4, 128.3, 127.6, 127.5, 81.4, 78.1, 77.7, 71.7, 63.9, 45.4, 29.6, 24.5, 17.6, 17.5, 17.4, 17.3, 17.2, 17.1, 13.8, 13.4, 12.9, 12.5; HR-MS Calcd. for $(C_{26}H_{46}O_5Si_2+H)^+$ 495.2962, found 495.2934.

(+)-(((3a*R*,4*R*,6*S*,6a*S*)-6-(benzyloxy)-2,2,6a-trimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl)methoxy)(*tert*-butyl)dimethylsilane (123) Compound **122** (280 mg, 0.57 mmol) was dissolved in THF (5 mL) and treated with TBAF (2.3 mL, 2.3 mmol) at rt for 1h. After removing the volatile, the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:40 to 1:10) to provide a syrup. The syrup was dissolved in CH_2Cl_2 (5 mL) and treated with TBDMSCl (130 mg, 0.86 mmol) and imidazole (77 mg, 1.14 mol) for 2 h. The reaction was quenched with H_2O and extracted with CH_2Cl_2 and washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* and the residue was directly dissolved in acetone and treated with 2,2-dimethoxypropane and catalytic amount *p*-TSA at rt for 1h. After quenching with solid $NaHCO_3$, the solvent was removed *in vacuo* and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:30 to 1:10) to yield compound **123** (210 mg, 91 %). $[\alpha]^{25}_{D}$ +11.22 (*c* 0.20, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39-7.28 (m, 5H), 4.67 (t, *J* = 6.5 Hz, 1H), 4.14 (s, 1H), 3.63-3.59 (m, 1H), 3.51-3.48 (m, 1H), 2.23-2.21 (m, 1H), 2.06-2.03 (m, 1H), 1.85- 1.83 (m, 1H), 1.52 (s, 3H), 1.45 (3, 3H),

1.44 (s, 3H), 0.86 (s, 9H), 0.02 (s, 3H), 0.00 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 149.9, 138.8, 128.2, 127.6, 127.3, 111.7, 86.7, 88.6, 88.2, 83.4, 72.0, 64.6, 44.4, 32.6, 27.8, 27.6, 25.8, 24.3, 18.1, -5.6, -5.7; HR-MS Calcd. for $(C_{24}H_{38}O_4Si+NH_4)^+$ 424.2883, found 424.2829.

(+)-(3a*S*,4*SiR*,6a*R*)-6-((*tert*-butyldimethylsilyloxy)methyl)-2,2,3a-trimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-ol (124) A suspension of compound **123** (40 mg, 0.1 mol), ammnoiun formate (63 mg, 1.0 mmol) and Pd/C (20 mg) in MeOH (3 mL) was heated at 80 ºC for 1 h. The solvent was removed and the residue was purified by column chromatography on a silica gel (EtOAc:Hexanes = 1:30 to 1:10) to provide compound **124** (29 mg, 93 %) as a pale yellow oil. $[\alpha]^{25}_D$ +12.41 (*c* 0.24, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.17 (s, 1H), 3.71-3.69 (m, 1H), 3.60 (dd, *J* = 5.0 and 10.5 Hz, 1H), 3.45 (dd, *J* = 5.0 and 10.0 Hz, 1H), 2.46 (d, *J* = 7.0 Hz, 1H), 2.22-2.20 (m, 1H), 1.88-1.80 (m, 2H), 1.43 (s, 3H), 1.39 (s, 3H), 1.37 (s, 3H), 0.84 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 111.7, 88.4, 88.0, 64.1, 44.6, 35.8, 27.4, 26.7, 26.0, 24.1, 18.5, -5.3, -5.4; HR-MS Calcd. for $(C_{16}H_{32}O_4Si+H)^+$ 317.2148, found 317.2134.

(+)-(3a*S*,4*S*,6*R*,6a*R*)-6-((*tert*-butyldimethylsilyloxy)methyl)-2,2,3a-trimethyl-5-methylenetetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-ol (126) and (3a*S*,4*R*,6*R*,6a*R*)-6-((*tert*-butyldimethylsilyloxy)methyl)-2,2,3a-trimethyl-5-methylenetetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-ol (126) To a solution of compound **124** (150 mg, 0.37 mmol) in CH_2Cl_2 (5 mL), Dess-Martin periodiane (313 mg, 7.4 mmol) was added at 0 ^{o}C. The reaction was kept at rt for 1 h and then quenched with $Na_2S_2O_3$ / $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 and washed with $NaHCO_3$ solution followed by brine and dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was directly used for the next step. To a solution of above mixture in THF, lithium diisopropylamine (2.0 M solution, 0.37 mL, 0.74 mmol) was added slowly at -78 ^{o}C. After stirring at the same temperature for 3 h, Eshenmoser's

salt (410 mg, 2.2 mmol) was added in one portion. The mixture was stirred for additional 3 h at the same temperature and overnight at room temperature. Then iodomethane (0.28 mL, 4.4 mmol) was added and stirred for another 4 h at room temperature before quenching with 10% aqueous $NaHCO_3$ (5 mL). The mixture was stirred for 1 h and extracted with diethyl ether. The combined ether extracts were washed with 10% aqueous $NaHCO_3$ followed by brine and dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by vacuum silica gel column chromatography (EtOAc:Hexanes = 1:30 to 1:10) to give an oil (90 mg, 0.22 mmol) which was dissolved in MeOH and treated with $CeCl_3 \cdot 7H_2O$ (100 mg, 0.26 mmol) for 10 min at room temperature. After cooling down to -78 ^oC, $NaBH_4$ (8 mg, 0.22 mmol) was added slowly. The reaction was kept at the same temperature for 20 min and quenched with HOAc. Solvent was removed *in vacuo* and the residue was dissolved in EtOAc and washed with H_2O and brine, dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:30 to 1:10) to give compound **125** (70 mg, 56 % from **124**) and compound **126** (15 mg, 12 % from **124**). Compound **125**: $[\alpha]^{24}_D$ -54.81 (*c* 0.20, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.24 (s, 1H), 5.04 (s, 1H), 4.27 (s, 1H), 4.06-4.03 (m, 1H), 3.72-3.70 (m, 1H), 3.56-3.53 (m, 1H), 2.55 (s, br, 1H), 2.24 (d, *J* = 10.5 Hz, 1H), 1.47 (s, 3H), 1.38 (s, 3H), 1.31 (s, 3H), 0.85 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.8, 111.0, 109.9, 87.6, 87.2, 79.5, 66.0, 52.2, 28.2, 27.8, 26.1, 23.8, 18.5, -5.3, -5.4; HR-MS Calcd. for $(C_{17}H_{32}O_4Si+Na)^+$ 351.1968, found 351.1934.Compound **126**: $[\alpha]^{26}_D$ -10.94 (*c* 0.18, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.24 (s, 1H), 5.02 (s, 1H), 4.16 (d, *J* = 1.0 Hz, 1H), 4.12 (d, *J* = 9.0 Hz, 1H), 3.80 (dd, *J* = 3.5 and 10.0 Hz, 1H), 3.62 (dd, *J* = 4.5 and 9.5 Hz, 1H), 3.15 (d, *J* = 9.0 Hz, 1H), 2.62 (s, br 1H), 1.31 (s, 6H), 1.27 (s, 3H), 0.81 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.7, 111.8, 111.3, 90.7,

88.1, 80.8, 65.7, 51.3, 28.7, 28.1, 26.0, 19.2, 18.5, -5.4, -5.5; HR-MS Calcd. for $(C_{17}H_{32}O_4Si+H)^+$ 329.2148, found 329.2133.

(+)-7-(((3*aR*,4*R*,6*aS*)-4-((*tert*-butyldimethylsilyloxy)methyl)-2,2,6a-trimethyl-4,6a-dihydro-3*aH*-cyclopenta[*d*][1,3]dioxol-5-yl)methyl)-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (128) To a mixture of compound **125** (10 mg, 0.024 mmol), Ph_3P (13 mg, 0.048 mmol) and 6-chloro-7-deaza-purine (8 mg, 0.048 mmol) in THF (15 mL), DIAD (0.01 mL, 0.048 mmol) was added at 0 ºC. The reaction mixture was kept at 40 ºC overnight and purified by preparative TLC to obtain nucleoside **128** (10 mg, 75 %) as s syrup. UV (MeOH) λ_{max} 271.0 nm; $[\alpha]^{26}_D$ +63.25 (*c* 0.30, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (s, 1H), 7.17 (d, *J* = 4.0 Hz, 1H), 6.57 (d, *J* = 3.5 Hz, 1H), 5.37 (s, 1H), 4.93 (d, *J* = 16.0 Hz, 1H), 4.80 (d, *J* = 16.5 Hz, 1H), 4.12 (s, 1H), 3.71-3.62 (m, 2H), 2.56 (t, *J* = 4.5 Hz, 1H), 1.37 (s, 6H), 1.29 (s, 3H), 1.26 (s, 3H), 0.83 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.3, 152.1, 140.8, 135.5, 130.1, 118.4, 110.9, 101.1, 92.8, 88.8, 84.0, 55.7, 44.7, 29.1, 28.0, 27.0, 25.6, 19.5, -4.3, -4.4; HR-MS Calcd. for $(C_{23}H_{34}ClO_3N_3Si+H)^+$ 464.2136, found 464.2116.

(-)-7-((3*aS*,4*R*,6*R*,6*aR*)-6-(*tert*-butoxymethyl)-2,2-dimethyl-5-methylenetetrahydro-3*aH*-cyclopenta[*d*][1,3]dioxol-4-yl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (129) To a solution of alcohol **22** (1.0 g, 3.9 mmol), Ph_3P (2.0 g, 7.8 mmol) and 6-chloro-7-deazapurine (1.19 g, 7.8 mmol) in THF, DIAD (1.57 mL, 7.8 mmol) was added slowly. The reaction mixture was kept at 40 ºC overnight and the solvent was removed. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:30 to 1:10) to give compound **129** (1.3 g, 85 %) as a pale yellow syrup. UV (MeOH) λ_{max} 272.0 nm; $[\alpha]^{25}_D$ -48.91 (*c* 0.35, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (s, 1H), 7.47 (d, *J* = 4.0 Hz, 1H), 6.63 (d, *J* = 3.5 Hz, 1H), 5.81 (t, *J* = 4.0 Hz, 1H), 5.22 (t, *J* = 4.0 Hz, 1H), 4.72-4.70 (m, 1H), 4.64-4.62 (m, 1H), 3.75-3.66 (m, 2H), 3.08 (s, 1H),

1.65 (s, 3H), 1.36 (s, 3H), 1.24 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 151.9, 151.8, 150.8, 150.1, 127.9, 117.4, 112.1, 111.9, 99.9, 85.3, 82.2, 73.2, 64.7, 63.7, 47.9, 28.0, 27.5, 25.1; HR-MS Calcd. for $(C_{20}H_{26}ClN_3O_3+H)^+$ 392.1741, found 392.1704.

(-)-7-((3*aS*,4*R*,6*R*,6*aR*)-6-(*tert*-butoxymethyl)-2,2-dimethyl-5-methylenetetrahydro-3*aH*-cyclopenta[*d*][1,3]dioxol-4-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (130) A solution of compound **129** (1.3 g, 3.32 mmol) in MeOH was saturated with ammonia at -30 ºC. The resulting methnolic solution was heated at 90 ºC in a steel bomb overnight. After removing the volatile, the residue was purified by silica gel column chromatography (MeOH:CH_2Cl_2 = 1:40 to 1:10) to give nucleoside **130** (1.0 g, 85 %) as a pale yellow foam. UV (MeOH) λ_{max} 274.0 nm; $[\alpha]^{25}_D$ -46.57 (*c* 0.20, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD) δ 8.12 (s, 1H), 7.24 (d, *J* = 3.5 Hz, 1H), 6.67 (d, *J* = 3.5 Hz, 1H), 5.66-5.65 (m, 1H), 5.20-5.19 (m, 1H), 4.81 (t, *J* = 6.0 Hz, 1H), 4.69-4.67 (m, 1H), 4.56 (s, 1H), 3.79-3.69 (m, 1H), 3.35-3.34 (m ,1H), 3.03 (s, 1H), 1.62 (s, 3H), 1.37 (s, 3H), 1.28 (s, 9H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.5, 150.7, 150.6, 150.0, 122.7, 112.0, 109.7, 99.4, 84.3, 81.9, 72.9, 64.3, 63.0, 26.7, 26.4, 24.4; HR-MS Calcd. for $(C_{20}H_{28}N_4O_3+H)^+$ 373.2240, found 373.2206.

(-)-(1*R*,2*S*,3*R*,5*R*)-3-(4-amino-7H-pyrrolo[2,3-*d*]pyrimidin-7-yl)-5-(hydroxymethyl)-4-methylenecyclopentane-1,2-diol (131) Nucleoside **130** (100 mg, 0.27 mmol) was dissolved in the a mixture of trifluoroacetic acid / H_2O (2 mL / 0.7 mL) and heated at 50-60 ºC for 4 h. After completely removing the volatile, the residue was co-evaperated with ethanol (10 mL X 2). The residue was dissolved in MeOH and passed through a pre-washed IRA-400 resin column (10 mL). The fractions were collected and evaperated *in vacuo* to provide a pale yellow syrup which became a while foam as compound **131** (70 mg, 94 %) when co-evaperateded with Et_2O. UV (MeOH) λ_{max} 273.0 nm; $[\alpha]^{25}_D$ -12.98 (*c* 0.25, MeOH); ^{1}H NMR (500 MHz, DMSO-d6) δ 8.04

(s, 1H), 7.13 (d, *J* = 3.5 Hz, 1H), 7.04 (s, b, 2H), 6.60 (d, *J* = 3.5 Hz, 1H), 5.43 (d, *J* = 10.5 Hz, 1H), 5.01-4.98 (m, 2H), 4.80 (d, *J* = 3.0 Hz, 1H), 4.35 (s, 1H), 4.01 (s, 1H), 3.59-3.57 (m, H), 2.61 (s, br, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.6, 150.3, 149.6, 147.6, 123.8, 110.3, 103.4, 98.8, 75.8, 72.9, 63.8, 63.6, 51.3.

(-)-(1*S*,2*R*,4*R*,5*R*)-2-(4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-4-(*tert*-butoxymethyl)-5-isopropoxy-3-methylenecyclopentanol (133) To a solution of compound **130** (110 mg, 0.30 mmol) in CH_2Cl_2, DIBAL-H (1.0 M solution in CH_2Cl_2, 3.0 mL, 3.0 mmol) was added slowly at -78 ºC. After 15 min at the same temperature, the reaction was quenched with *iso*-PrOH / $CHCl_3$ (1 / 4). Potasium sodium tartrate solution was then added and stiired at rt for 1 h. The organic layer was collected and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (MeOH:CH_2Cl_2 = 1:40 to 1:10) to give compound **133** (95 mg, 86 %) UV (MeOH) λ_{max} 270.0 nm; $[\alpha]^{24}_D$ -22.94 (*c* 1.75, $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d6) δ 8.04 (s, 1H), 7.13 (d, *J* = 3.5 Hz, 1H), 6.59 (s, *J* = 3.5 Hz, 1H), 2H), 5.40 (d, *J* = 9.5 Hz, 1H), 5.05 (s, 1H), 4.36-4.31 (m, 2H), 3.80-3.74 (m, 2H), 3.50-3.43 (m, 2H), 2.66 (s, br, 1H), 1.18 (s, 9 H), 1.52 (s, 3HU0 ,1.50 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 156.5, 151.5, 151.4, 147.4, 123.0, 111.0, 103.1, 98.1, 77.9, 76.0, 73.0, 70.8, 63.5, 63.1, 47.3, 27.5, 23.3, 22.0; Anal. Calcd. For $C_{20}H_{30}N_4O_3$: C, 64.15; H, 8.07; N, 14.96 Found C, 63.85; H, 8.14; N, 14.81.

(-)-(1*S*,2*R*,3*R*,5*R*)-3-(*tert*-butoxymethyl)-2-isopropoxy-5-(4-((4-methoxyphenyl)diphenylmethylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-4-methylenecyclopentanol (134) Chloro trimethylsilane (0.29 mL, 1.33 mmol) was added to a solution of compound **133** (50 mg, 0.13 mmol) in pyridine (2 mL). The reaction was kept at 40 ºC for 2 h and 4-methoxytrytyl chloride (MMTrCl, 120 mg, 0.39 mmol) and DMAP (16 mg,

0.13 mmol) were added. After kept at the same temperature for 2 h, conc. NH_4OH / H_2O (2 mL / 0.7 mL) were added and stirred for another 1 h. After removing the volatile, the residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:5 to 1:1) to give compound **134** (60 mg, 60 %) . UV (MeOH) λ_{max} 275.0 nm; $[\alpha]^{23}_{D}$ -23.86 (*c* 0.22, $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d6) δ 8.08 (s, 1H), 7.35-7.20 (m, 13H), 7.00 (s, 1H), 6.80 (s, 1H), 6.78 (s, 1H), 6.22 (s, 1H), 6.13 (s, 1H), 5.55 (d, *J* = 9.5 Hz, 1H), 5.10 (s, 1H), 4.32-4.29 (m, 1H), 3.92 (d, *J* = 5.5 Hz, 1H), 3.81-3.76 (m, 4H), 3.59-3.56 (m, 1H), 3.45 (t, *J* = 7.0 Hz, 1H), 3.08-2.97 (br, 1H), 2.85 (s, 1H), 1.21-1.19 (m, 15 H); ^{13}C NMR (125 MHz, CD_3OD) δ 158.3, 155.7, 151.1, 147.5, 145.7, 137.7, 130.3, 129.0, 127.9, 126.8, 122.2, 113.2, 111.1, 104.2, 77.9, 76.0, 72.9, 71.0, 70.9, 63.3, 63.1, 55.2, 47.4, 27.5, 23.3, 22.0.

(-)-7-((1*R*,3*R*,4*R*,5*S*)-3-(*tert*-butoxymethyl)-4-isopropoxy-5-methoxy-2-methylenecyclopentyl)-*N*-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin)-4-amine (138) & (-)-7-((1*R*,3*R*,4*R*,5*S*)-3-(*tert*-butoxymethyl)-4-isopropoxy-5-methoxy-2-methylenecyclopentyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin)-4-amine (139) A sloution of compound **133** (50 mg, 0.13 mmol) in anhydrous DMF (1.5 mL) was treated with sodium hydride (60 % in mineral oil, 7.8 mg, 0.2 mmol) at 0 ºC for 45 min before methyl iodide (10 μL, 0.17 mmol) was added. The brown solution was stirred at 0 ºC for another 2 h. After removing the volatile, the residue was purified by preparative TLC to obatin a dimethylated compound **138** (14 mg, 26 %) as well as desired prodcut **139** (26 mg, 50 %). For compound **138**: UV (MeOH) λ_{max} 272.0 nm; $[\alpha]^{27}_{D}$ -47.14 (*c* 0.23, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.13 (d, *J* = 3.5 Hz, 1H), 6.35 (d, *J* = 3.5 Hz, 1H), 5.67-5.66 (m, 1H), 5.17 (t, *J* = 2.5 Hz, 1H), 5.10 (br, 1H), 4.81 (t, *J* = 2.5 Hz, 1H), 4.07-4.02 (m, 2H), 3.73-3.69 (m, 1H), 3.62-3.60 (m, 2H), 3.38 (s, 3H), 3.19 (d, *J* = 4.5 Hz, 1H), 2.85-2.84 (m, 1H), 1.25 (s, 9H), 1.20-1.18 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.1,

151.7, 150.5, 146.8, 123.0, 111.7, 103.1, 97.6, 83.8, 75.6, 72.8, 70.4, 61.9, 60.8, 57.6, 47.3, 27.5, 22.9, 22.2; Anal. Calcd. For $C_{22}H_{34}N_4O_3$: C, 65.64; H, 8.51; N, 13.92 Found C, 65.41; H, 8.60; N, 13.65. For compound **139**: UV (MeOH) λ_{max} 270.0 nm; $[\alpha]^{27}_D$ -14.29 (*c* 0.27, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.19 (d, *J* = 4.0 Hz, 1H), 6.36 (d, *J* = 3.5 Hz, 1H), 5.67-5.66 (m, 1H), 5.58 (br, 2H), 5.18 (m, *J* = 2.5 Hz, 1H), 4.81 (t, *J* = 2.5 Hz, 1H), 4.07-4.03 (m, 2H), 3.72-3.70 (m, 1H), 3.61-3.60 (m, 2H), 3.38 (s, 3H), 2.85-2.84 (m, 1H), 1.25 (s, 9H), 1.20-1.18 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 156.4, 151.0, 150.9, 146.7, 124.0, 111.8, 103.2, 97.6, 83.8, 75.5, 72.9, 70.4, 62.0, 61.0, 57.6, 47.3, 27.5, 22.9, 22.1; Anal. Calcd. For $C_{21}H_{32}N_4O_3 \cdot 0.2\ H_2O$: C, 64.33; H, 8.33; N, 14.29 Found C, 64.09; H, 8.15; N, 13.97.

(-)-(1*R*,2*S*,3*R*,5*R*)-3-(4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-5-(hydroxymethyl)-2-methoxy-4-methylenecyclopentanol (140) Compound **139** (20 mg, 0.052 mmol) was dissolved in anhydrous CH_2Cl_2 (1.0 mL) and was treated with BCl_3 (1.0 M in CH_2Cl_2, 0.5 mL, 0.5 mmol) at -78 ºC for 30 min before warmed up to -30 ºC for another 30 min. After quenching with MeOH and neutralizing with $NaHCO_3$, the solvent was removed and the residue was purified by preparative TLC to gave target nucleoside **140** (13 mg, 87 %) as off-white solid. mp: 236-238 °C (dec.) $[\alpha]^{28}_D$ -11.94° (*c* 0.22, MeOH) UV (H_2O) λ_{max} 274.0 nm (ε 10027, pH 2), 271.0 nm (ε 10843, pH 7), 272.0 nm (ε 10796, pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 8.07 (s, 1H), 7.20 (d, *J* = 3.5 Hz, 1H), 6.65 (d, *J* = 3.5 Hz, 1H), 5.58 (d, *J* = 10.0 Hz, 1H), 5.22 (s, 1H), 4.68 (s, 1H), 4.40 (s, d, *J* = 4.0 Hz, 1H), 4.28-4.25 (m, 1H), 3.86-3.85 (m, 2H), 3.31 (s, 3H), 2.83 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.6, 150.2, 149.5, 147.4, 123.8, 110.5, 103.3, 99.0, 84.8, 70.2, 63.8, 61.9, 56.6, 51.4. Anal. Calcd. For $C_{14}H_{18}N_4O_3 \cdot 0.3\ H_2O$: C, 56.86; H, 6.34; N, 18.95 Found C, 56.91; H, 6.20; N, 18.70.

Molecular Modeling Study. Conformational analysis: The initial conformations of compound **3**, **15**, **16**, **17**, **18** and **19** were constructed by builder module in MACROMODEL®, version 8.5 (Schrodinger, Inc.). The Monte Carlo conformational search was performed in 5,000-step, in the presence of GB/SA water model using MMFFs force field in MACROMODEL®. Conformational parameters of compounds **3**, **15**, **16** and **17** were obtained *via* online calculation by running program PROSIT.[186, 318]

CHAPTER 5

CONCLUSION

Extensive research has been carried out in the field of nucleosides / nucleotides for the purpose of mining biologically active compounds. One significant finding has been the discovery of carbocyclic nucleosides in which a methylene group replaces the oxygen of the natural nucleosides. This modification results in molecules with improved metabolic stability as well as enhanced biological activity, represented by two antiviral nucleosides, abacavir and entecavir. However, because of drug toxicity, and drug-resistant issues, there is a continued need for novel antiviral nucleosides. We have tried to address this problem by developing novel carbocyclic nucleosides as potential antiviral agents.

Chapter 1 covers the recent advances in the field of carbocyclic nucleosides. This review provides detailed information regarding the synthesis as well as biological activity of important carbocyclic nucleosides.

In Chapter 2, we described the synthesis and molecular modeling studies of D- and L-2',3'-dideoxy-2',3'-didehydro-2'-fluoro-carbocyclic nucleosides (D- and L-2'F-C-d4Ns) as potential anti-HIV agents. The target D- and L-carbocyclic nucleosides were both stereospecifically synthesized from D-ribose. The structure activity relationships of synthesized compounds against HIV-1 in activated human peripheral blood mononuclear (PBM) cells were studied, from which we found that L-2',3'-dideoxy-2',3'-didehydro-2'-fluoro adenosine analog (L-2'F-C-d4A) showed potent anti-HIV activity (EC_{50} 0.77 μM), although it is cross-resistant to the lamivudine-resistant variant (HIV-1_{M184V}). Modeling studies demonstrated a good correlation between calculated relative binding energies and activity/resistance data. The modeling study also indicated that an additional hydrogen bond and favorable van der Waals interactions contribute to the higher antiviral activity of L-2'F-C-d4A in comparison to its D-counterpart. Also, like other L-nucleosides, the unfavorable steric hindrance of the sugar moiety of L-2'F-C-

d4A and the side chain of Val184 could explain the cross-resistance of L-2'F-C-d4A with the M184V mutant. The significant difference of antiviral activity between carbovir and its analog D-2'F-C-d4G may be due to distortion of the phenyl ring of Tyr115 in L-2'F-C-d4G-TP/HIV-RT complex which resulted in a poor π-π interaction.

Introducing 2'-fluoro substitution on the 2',3'-double bond in carbocyclic nucleosides has provided biologically interesting compounds with potent anti-HIV activity. Therefore, as an extension of our previous works in the discovery of anti-HIV agents, it was interesting to conduct the SAR of the 3'-fluoro isomers. Chapter 3 details the synthesis, anti-HIV-1 activity and mechanism of drug resistance of D- and L-2',3'-didehydro-2',3'-dideoxy-3'-fluoro-carbocyclic nucleosides. Among the synthesized L-series nucleosides, cytidine, thymidine, adenosine and guanosine derivatives exhibited moderate antiviral activity (EC_{50} 7.1 μM, 6.4 μM, 10.3 μM and 20.7 μM, respectively), while among the D-series, the guanosine analogue (D-3'-F-C-d4G) exhibited the most potent anti-HIV activity (EC_{50} 0.4 μM, EC_{90} 2.8 μM). However, the guanosine analogue was cross-resistant to the lamivudine-resistant variants (HIV-1_{M184V}). Molecular modeling studies suggest that hydrophobic interaction as well as hydrogen bonding stabilize the binding of guanosine analog D-3'-F-C-d4G in the active site of wild type HIV reverse transcriptase (HIV-RT). In the case of L-nucleosides, these two effects are opposite which results in a loss of binding affinity. According to the molecular modeling studies, cross-resistance of D-3'-F-C-d4G to M184V mutant may be caused by the realignment of the primer and template in the HIV-RT_{M184V} interaction, which destabilizes the RT-inhibitor triphosphate complex, resulting in a significant reduction in anti-HIV activity of the D-guanine derivative.

HCV has been recognized as one of the leading causes of liver impairment and a number of nucleosides have been discovered as potential anti-HCV agents. A short review of potential

anti-HCV nucleosides is provided in the Part I of Chapter 4. Chapter 4 (in Part II, III and IV) also describes our efforts to synthesize novel carbocyclic nucleosides as potential anti-HCV agents. Although the synthesis of 2,2-difluoro, 2'-α-fluoro and 2'-*C*-methyl entecavir analogs was not successful, the synthesis of 2'-β-fluoro and 2'-*O*-methyl-entecavir analogs has been accomplished. The anti-HCV screening of the synthesized compounds is still in progress.

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