National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

The synthesis of amide-linked 2',3'-cyclopropanated dinucleosides and the effect of their incorporation into DNA-strands on duplexation

by

Wen-Qiang Zhou

A thesis submitted to the Faculty of Graduate Studies and Research of McGill University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Department of Chemistry
McGill University
Montreal, Quebec, Canada
December 1995

National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

ISBN 0-612-12523-8

Canada

To My Parents and Sisters

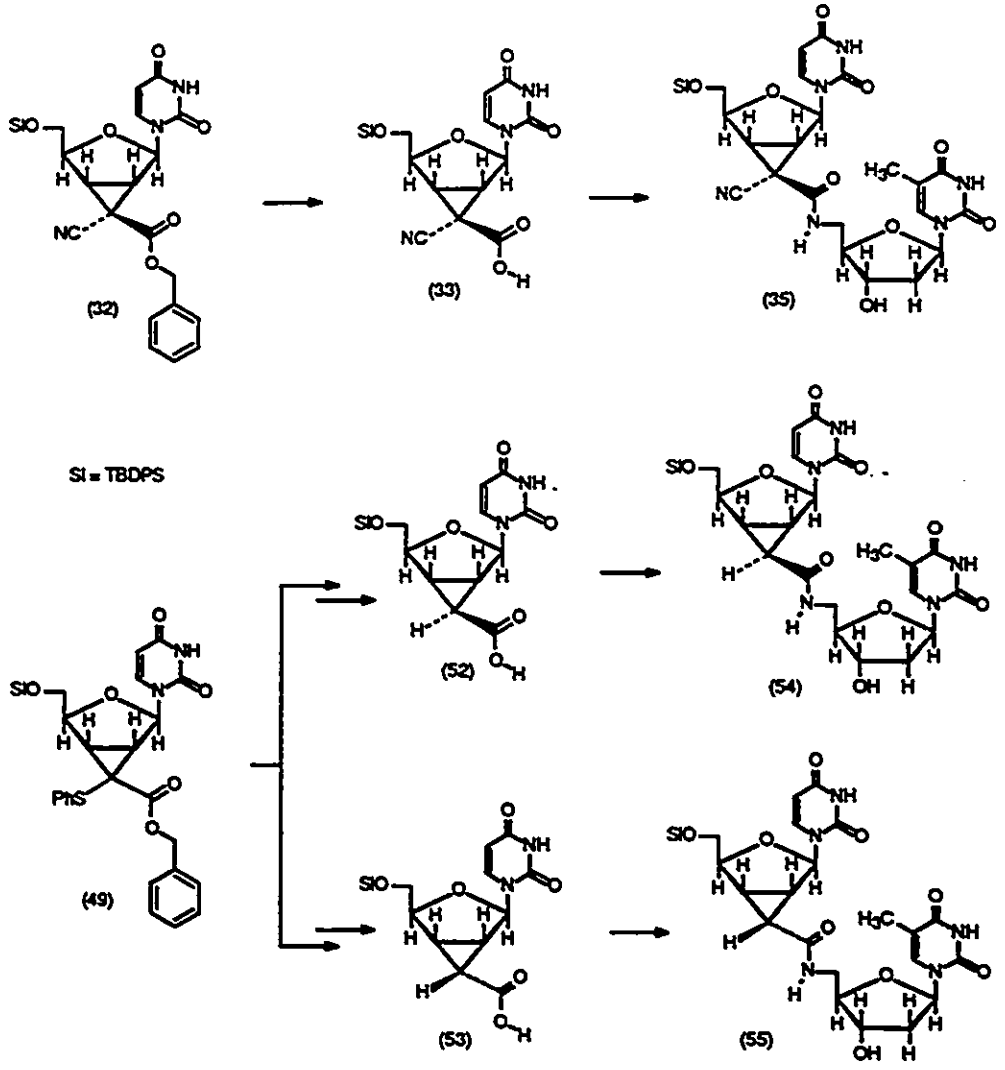
To My Loving Wife,

Jing

ABSTRACT

In order to establish if binding affinity could be improved by conformationally restricting the amide backbone, *exo*-amide-linked and *endo*-amide-linked 2',3'-cyclopropanated dinucleoside analogs (e.g. dimers **35**, **54**, and **55**) have been studied. The dimers were synthesized by coupling the corresponding cyclopropyl acids (5'-end building unit) with aminothymidines (3'-end building unit) by standard peptide synthesis methodology. After proper functionalizations, the dimers were incorporated into DNA sequences, and the effects of their incorporation into DNA-strands on binding to complementary DNA and RNA were evaluated.

Different strategies were explored to prepare the carboxylic acid-functionalized 2',3'-cyclopropanated nucleoside analogs as the 5'-end building block. It was found that reaction of the α,β-unsaturated selenonyl uridine with the anions of 2-substituted acetates could efficiently yield the ester-functionalized cyclopropanes (e.g. **32** and **49**) in a stereoselective manner, through a Michael-type cyclopropanation mechanism. Proper transformations of the ester precursor successfully provided the desired acid derivatives (e.g. **33**, **52** and **53**).


SiO
NC
(32)
(33)
(35)
Si = TBDPS
PhS
(49)
(52)
(53)
(54)
(55)
OH

Résumé

Nous avons étudié des analogues de dinucléotides comportant une fonction 2',3'-cyclopropane, reliée à une fonction amide (dimères **35**, **54** et **55**) ayant l'orientation exo-ou endo-, avec pour objectif de déterminer si cette restriction conformationnelle pouvait augmenter l'affinité de la liaison amide lors de l'appariement de ces analogues. Les dimères ont été synthétisés par couplage des acides cyclopropylecarboxyliques correspondants (unité monomérique 5') avec une aminothymidine (unité monomérique 3') dans des conditions habituelles de synthèse peptidique. Après fonctionnalisation, ces dimères ont été incorporés dans une séquence d'ADN, et nous avons étudié l'effet de leur incorporation dans un brin d'ADN sur l'affinité avec un brin d'ADN ou d'ARN complémentaire.

Nous avons exploré différentes stratégies pour préparer ces analogues comportant une fonction 2',3'-cyclopropane carboxylique. Nous avons trouvé que la réaction de la sélénonyluridine α,β-insaturée avec des anions d'acétates substitués sur la position 2 pouvait produire des cyclopropanes (**32**, et **49**) de façon stéréosélective, via un mécanisme de cyclopropanation de type Michael. Une transformation appropriée des esters nous mena aux acides correspondants (**33**, **52** et **53**).

ACKNOWLEDGMENTS

I would like to express my sincere thanks and appreciation to Professor George Just for his guidance and support throughout the course of my studies. Without his enthusiasm and encouragement, this project would not be possible.

I also wish to express my appreciation to Dr. Yogesh S. Sanghvi at ISIS Pharmaceuticals for his interest in this project, and his help in the DNA synthesis and the binding studies.

I wish to thank Costa Yannopoulos and Peter Nower for their collaboration and contribution to the synthesis of the vinyl selenone **17b**.

I would also like to thank:

Irene Idziak, Dr. Bing Meng, Dr. Daniela Montesarchio, Dr. Kristina Kutterer, and Eric Marsault as well as all my co-worker for their friendships and helpful discussion.

Dr. Françoise Sauriol for her instruction and assistance in NMR experiments.

Dr. Orval Mamer for recording mass spectra and advice in this area.

Ms. Renée Charron for her help with administrative matters.

Glossary of Abbreviations

A	adenine
bp	base pair
b.p.	boiling point
br	broad (in NMR)
^{13}C-NMR	carbon NMR
COSY	correlated spectroscopy
Bn	benzyl group
BOP	benzotriazol-1ylcxy-tris(dimethyl-amino)phosphcnium hexafluorophosphate
C	cytosine
δ	chemical shift
d	doublet (in NMR)
dd	doublet of doublets
DCC	1,3-dicyclohexylcarbodiimide
DMF	*N,N*-dimethylformamide
DMAP	4-dimethylaminopyridine
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
eq.	equivalent
EtOAc	ethyl acetate
FAB	fast atom bombardment
g	gram(s)
G	guanine
HMQC	2D-Heteronuclear Multiple Quantum Coherence spectroscopy
^{1}H-NMR	proton NMR

HPLC	high performance liquid chromatography
hrs.	hours
Hz	hertz
HRMS	high resolution mass spectroscopy
LRMS	low resolution mass spectroscopy
m	multiplet (in NMR)
MCPBA	*m*-chloroperbenzoic acid
m /e	mass to charge ratio
mRNA	messenger RNA
MS	mass spectroscopy
MsCl	methanesulfonyl chloride
mins	minutes
ml	milliliter
mmol	1×10^{-3} mole
m.p.	melting point
N	normality
NMR	nuclear magnetic resonance
NOESY	Nuclear Overhauser Effect SpectroscopY
NOE	Nuclear Overhauser Effect
Ph	phenyl group
ppm	parts per million
Pyr.	pyridine
q	quartet (in NMR)
R_f	retention factor
RNA	ribonucleic acid
RNase H	Ribonuclease H
RT	room temperature

ss	single strand
t	triplet (in NMR)
T	thymine
tert	tertiary
TBAF	tetrabutylammonium fluoride
TBDMSCl	*tert*-butyldimethylsilyl chloride
TBDMS	*tert*-butyldimethylsilyl
TBDPSCl	*tert*-butyl diphenylsilyl chloride
TBDPS	*tert*-butyldiphenylsilyl
THF	tetrahydrofuran
TLC	thin layer chromatography
T_m	melting temperature
TsCl	toluenesulfonyl chloride
U	uracil

TABLE OF CONTENTS

Chapter 1. Introduction

1.1. What Is Antisense

Synthetic oligonucleotides that form stable complexes with natural DNA and RNA according to the Watson-Crick base-pairing rules[1](Figure 1) recently attracted enormous interest as specific inhibitors of protein biosynthesis. The use of oligonucleotides in suppressing specific gene expression based on sequence specificity has been termed the antisense approach.

The sequence of bases along a messenger RNA (mRNA) that carries genetic information for protein synthesis in cell , is said to be the sense sequence. The so-called "antisense" sequences refer to the strands complementary to the "sense" strands.

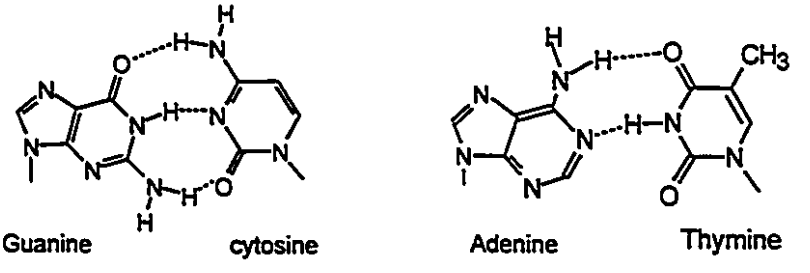


Figure 1. Watson-Crick base pairing

Practically, antisense oligonucleotides are defined as short sequences of single-stranded DNA or RNA, usually less than 30 nucleoside units in length, which are co.nplementary to a specific intracellular target, normally messenger RNA (mRNA)[2].

---

[1]Watson, J.D.; Crick, F.H.C., *Nature*, 333, 866 (1953)
[2]Murray, J.A.H.; Crockett, N., In *Antisense RNA and DNA*, Murray J.A.H., Eds., Wiley-Liss, New York, 8 (1992)

1.2. mRNA in Eukaryotes Gene Expression

During gene expression, only the 3'-5' DNA is transcribed into a pre-mRNA. The mRNA then translocates from nucleus into cytoplasm, and functions as a template for peptide synthesis (Figure 2).

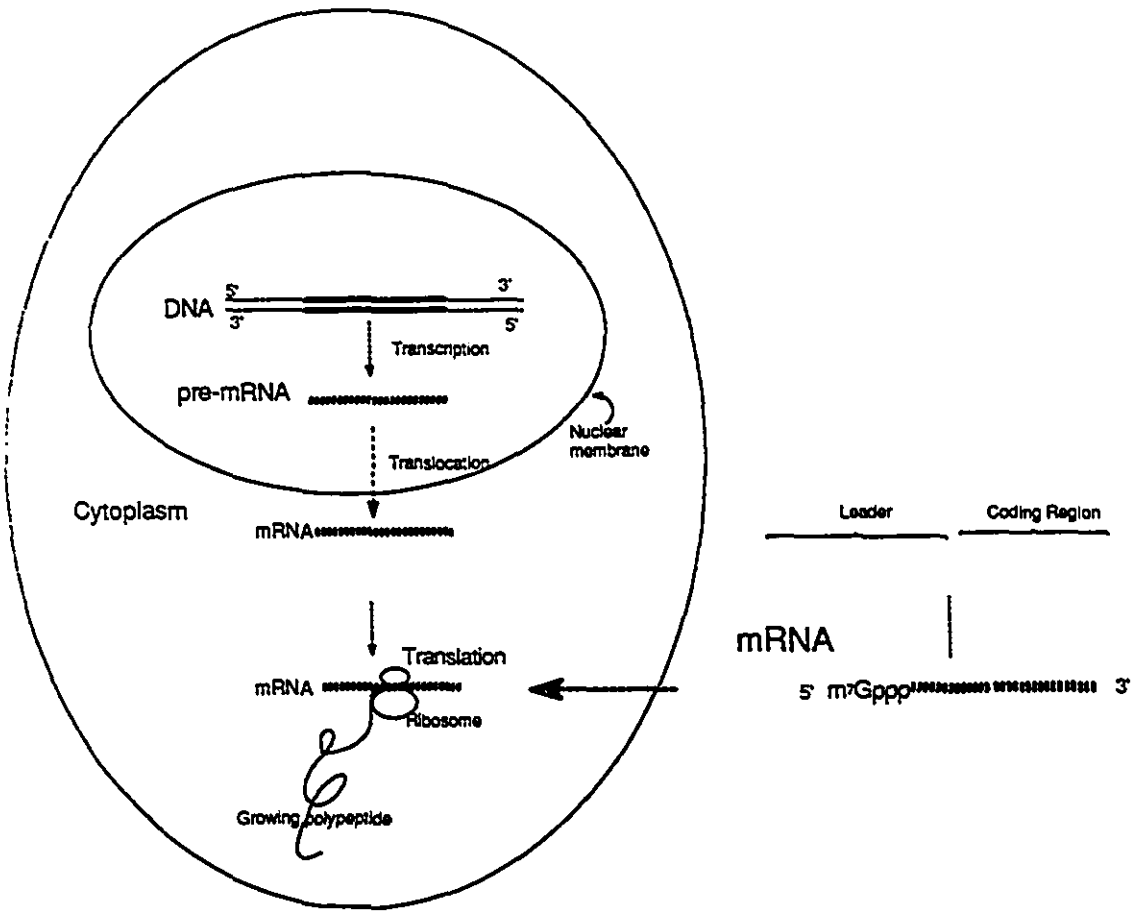


Figure 2. Simplified model of gene expression in eukaryotes: the transcription of a gene to the complementary mRNA and its subsequent translation to the protein products.

The initiation of translation involves the recognition of the mRNA sequence by the ribosome and subsequent formation of the ribosome-mRNA complex. Once complexed, the ribosome aligns with the starting codon coding. The ribosome then moves along the

mRNA's coding region in a 5' to 3' direction, joining amino acids brought to the ribosome-mRNA complex by transfer RNA (tRNA).

In the whole process, mRNA functions as a message carrier and bears genetic information from the gene in the nucleus.

1.3. Naturally Occurring Antisense RNA Regulation

Genetic control by naturally occurring antisense RNAs has been reported since the early 1980s in a number of cases[3].The biological activities controlled are diverse, including transposition[4], phage development[5,6,7], chromosomal gene expression[8], and plasmid replication, compatibility and conjugation[9,10,11]. Although most of the observations were made in prokaryotes, a large body of evidence strongly suggested that such a control could also happen in eukaryotic systems.

In 1983, Simons and Kleckner detected in procaryotes RNA(RNA-OUT) that was complementary to the 5'-end of the transpose mRNA (RNA-IN) at the ribosome-binding site and suppressed its expression by binding to it in a complementary, antisense manner[4].

One year later, micF RNA (mRNA-interfering complementary RNA) was discovered[8] to function as a antisense RNA to control the expression of a bacterial gene (ompF) in E. coli. This is the first example of antisense control. MicF RNA is a 174-base s/s RNA which shows much sequence homology to the leader region (including the Shine-Delgarno sequences) and 5'-end of the coding region of ompF mRNA which codes for the outer membrane protein OmpF. When expressed from a multicopy plasmid, micF RNA severely decreases the level of ompF message and protein. Consistent with a role in controlling ompF, micF RNA is expressed under conditions in which ompF expression is inhibited, thus maintaining a constant level of total membrane protein.

After the discovery of MicF RNA, it became very apparent that the translational regulation could be achieved through the use of RNA complementary to the mRNA of the

---

[3]Simons, R.W.; Kleckner, N., *Annu. Rev. Genet.*, 22, 567 (1988)
[4]Simons, R.W.; Kleckner, N., *Cell*, 34, 683 (1983)
[5]Wu, T.-H.; Liao, S.-M.; Mclure, W.R.; Susskind, M.M., *Genes Dev.*, 1, 204 (1987)
[6]Hoopes, B.C.; McClure, W.R., *Pro. Natl. Acad. Sci. USA*, 82, 3134 (1985)
[7]Krinke, L.; Wulff, D.L., *Genes Dev.*, 1, 1005 (1988)
[8]Mizuno, T.; Chou, M-Y.; Inouye, M., *Proc. Natl. Acad. Sci. USA*, 81, 1966 (1984)
[9]Light, J.; Molin, S., *EMBO J.*, 2, 93 (1983)
[10]Dempsey, W.B., *Mol. Gen. Gent.*, 209, 533 (1987)
[11]Finlay, B.B.; Frost, L.S.; Paranchych, W.; Willetts, N.S., *J. Bacteriol*, 167, 754 (1986)

targeted polypeptide.

Although the exact mechanisms of antisense RNA regulation are not fully understood, the control was observed to be exerted at many different levels, by both direct and long-range effects[12]. In direct effects of antisense binding, when an antisense RNA is complementary to the translation initiation site on the target RNA, it is likely that control occurs by inhibition of ribosome binding[4,5,8]. When an antisense RNA is complementary to some other region of the target RNA, cleavage may play an essential role[7]. On the other hand, when action is at a distance, the antisense binding may induce a conformational change in the target RNA that prevents its function[9].

[12]Simons, R.W., *Gene*, 72 (1988)

1.4. Artificial Regulation of Gene Expression by Antisense Oligomers

The discovery of natural antisense RNA in prokaryotes and in eukaryotes has led to a blossoming of studies in the field. Antisense control has received considerable attention in recent years, not only because of a general interest in the mechanism involved and using it to investigate normal and pathological gene functions, but also with the eye toward the design of efficient, therapeutic antisense agents.

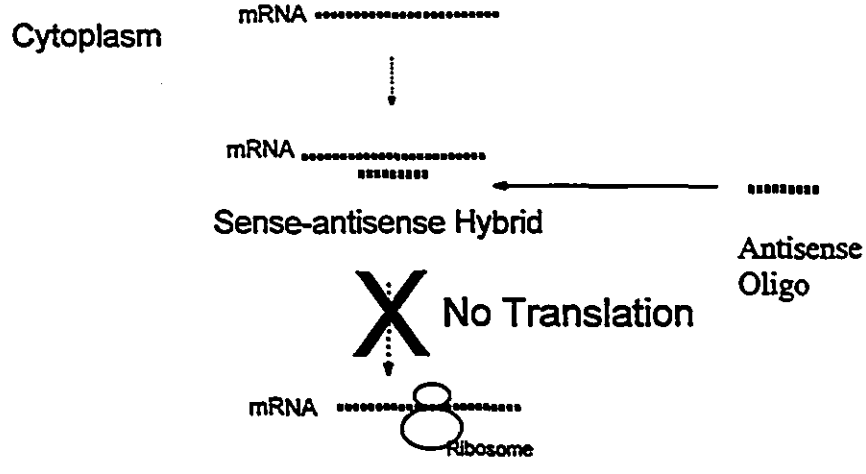


Figure 3. Schematic of translation arrest with antisense oligomer

In theory, the formation of complementary sense-antisense duplexes could result in inhibition of mRNA's translation into the encoded protein (Figure 3). If the protein coded by the messenger is vital to cellular or viral growth and/or reproduction, then inhibition of this process could lead to a significant diminution in cellular or viral viability. Thus the antisense oligonucleotides targeted to mRNA represents an attempt at the sequence-specific, genetically-based therapy[13,14,15].

[13]Stein, C.A.; Cheng, Y.-C., *Science*, 261, 1004 (1993)
[14]Calabretta, B.; Skorski, T.; Szczylik, C.; Zon, G., *Cancer Treat. Rev.*, 19, 169 (1993)

1.5. Design and Chemical Synthesis of Antisense Oligonucleotides

Practically, antisense sequences can be constructed either genetically[16] or chemically (antisense oligoside)[17]. Even if conceptually identical, these two approaches have different requirements and uses. Because my research is mainly on synthetic antisense chemistry, this thesis will only focus on reviewing the synthetic oligonucleotides (oligosides).

To enable the therapeutic uses of antisense oligonucleotide, several requirements must be fulfilled: (1) an antisense oligomer must hybridize with the target mRNA with sufficient affinity and specificity to inhibit the translation; (2) the antisense compounds should be resistant towards nucleases existing in serum and in cells; (3) since the antisense oligomers will be applied externally, they should be able to penetrate through the cell membrane to maintain a high, effective concentration in cells; and finally the antisense compounds should be obtained at a reasonable price.

The most important advantage of chemical synthesis of antisense oligosides is that the full power of synthetic chemistry can be used to design oligomers with particular modified sites or terminal groups, thereby improving the antisense properties. It is on such modified oligonucleotides that most hope is pinned for developing antisense therapeutics for human antiviral or anticancer treatment.

---

[15]Castanotto, D.; Rossi, J.J., *Adv. Pharmcol.*, 25, 289 (1994)
[16]For review see :Takayama, K.M.; Inouye, M., *Crit. Rev. Biochem. Mol. Biol.*, 25, 155 (1990); and Inouye, M., *Gene*, 72, 25 (1988)
[17]For review see: Uhlmann, E.; Peyman, A., *Chem. Rev.*, 90, 543 (1990)

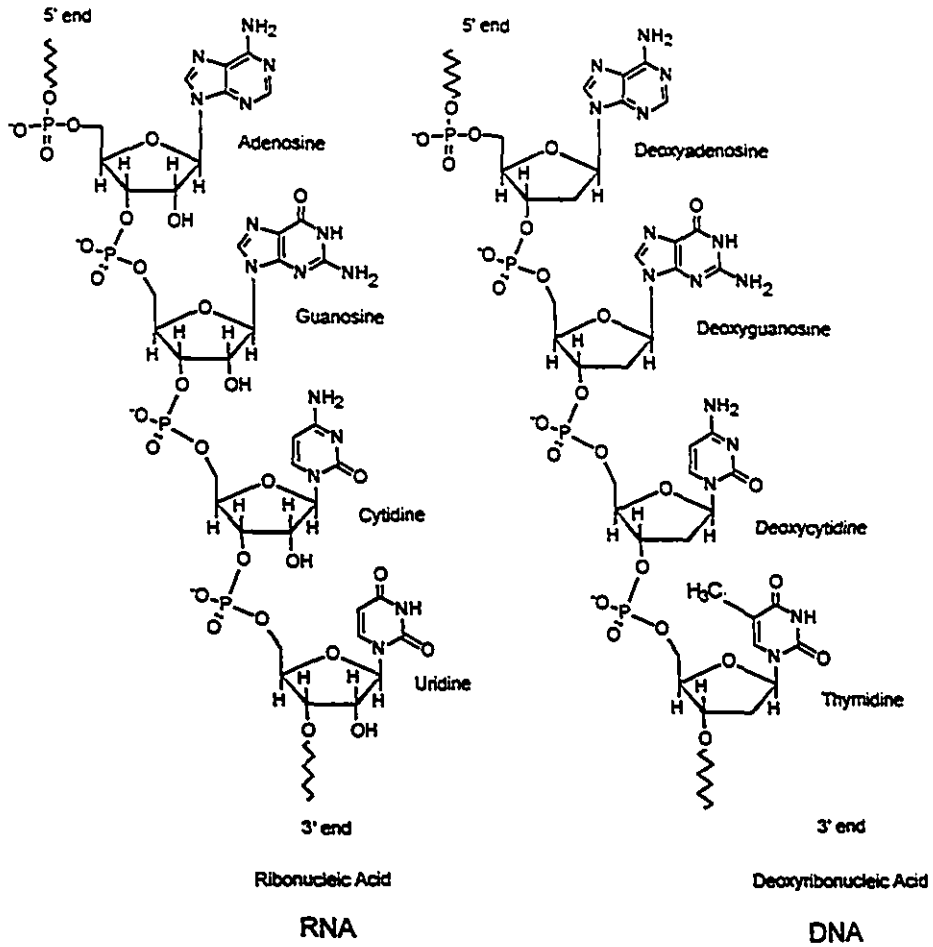


Figure 4. General structure of the RNA and DNA oligonucleotide strand with natural phosphodiester linkers

1.5.1. Unmodified Phosphodiester Oligodeoxynucleotides

It is interesting that even before the discovery of the naturally-occurring antisense-mediated gene regulations, synthetic antisense DNAs (Figure 4) were designed to inhibit gene expression. In 1978, Zamecnik and Stephenson[18] synthesized a 13-mer strand with phosphodiester internucleoside linkers, protected at the ends as phenyl isocyanate complementary to the 21 bp sequence repeated at the ends of Rous Sarcoma Virus (RSV) 35S RNA. When added to the culture medium, the oligomer demonstrated direct inhibition of the Rous Sarcoma Virus in cultured cell lines through an antisense

[18]Zamecnik, P.C.; Stephenson, M.L., *Proc. Natl. Acad. Sci. USA*, 75, 280 (1978)

mechanism. Subsequent experiments[19,20,21,22] have shown that inhibition of various viruses and the expression of genes can be achieved, clearly indicating that the synthetic oligosides are internalized by cells and can interact with intracellular targets[23].

Two serious problems were encountered for the utilization of the oligonucleotides with the phosphodiester linkers as antisense agents: (1) the phosphodiester linkers are rapidly attacked and degraded easily by nucleases present in the serum of culture medium; (2) also, efficiency of membrane penetration for this highly charged oligonucleotide is low. Only 1%-2% of added oligonucleotides were internalized by cells after 4 hours[24,25].

1.5.2. Oligonucleotide Analogs with Backbone Modifications

In an attempt to enhance the nuclease resistance of the antisense molecule as well as cellular uptake, modification of virtually all possible sites of oligosides have been explored during the past 15 years (figure 5) [17, 26]. Due to the fact that the hydrolytic cleavage of the phosphodiester backbone is the main cause for the rapid degradation of natural oligonucleotide by nucleases, replacement of the phosphodiester internucleoside linker with other moieties (backbone modification) was of the highest interest for the investigators in antisense research. It was shown from pioneering investigations[27,28] that the backbone modifications could significantly improve the stability of the oligosides

---

[19]Zamecnik, P.C.; Goodchild,J.; Yagushi, Y.; Sarin, P., *Proc. Natl. Acad. Sci. USA*, 83, 4143 (1986)
[20]Szcylik, C.; Skorski, T.; Nicolaides, N.C.; Manzella, L.; Malaguarnera, L.; Venturelli, D.; Gerwitz, A.M.; Calabretta, B., *Science*, 253, 562 (1991)
[21]Blum, H.E.; Galun, E.; Weizacker, F.V.; Wands, J.R., *Lancet*, 337, 1230 (1992)
[22]Colige, A.; Sokolov, B.P.; Nugent, P.; Baserga, R.; Prockop, D.J., *Biochemistry*, 32, 7 (1992)
[23]For a review see: To, R.Y., In *Antisense RNA and DNA*, Murray, J.A.H., Eds., Wiley-Liss, New York, 267 (1992)
[24]Wickstrom, E., *J.Biochem. Biophys. Methods*, 13, 97 (1986)
[25]Wickstrom, E.L.; Bacon, T.A.; Gonzalez, A.; Freeman, D.L.; Lyman, G.H.; Wickstrom, E., *Proc. Natl. Acad. Sci. USA*, 85, 1028 (1988)
[26]For a recent review see: Cohen, J.S.; Hogan, M.E., *Scentific American*, 12, 82 (1994)
27Milligan, J.F.; Matteucci, M.D.; Martin, J.C., *J. Med. Chem.*, 36, 1923 (1993)
28Sanghvi, Y.S.; Cook, P.D., In *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*; Chu, C.K., Baker, D.C., Eds.; Plenum press: New York, 312 (1993)

towards nucleases, as well as influence other biophysical properties of the oligosides such as binding affinity or cellular uptake. The backbone modifications will be briefly reviewed in this Section.

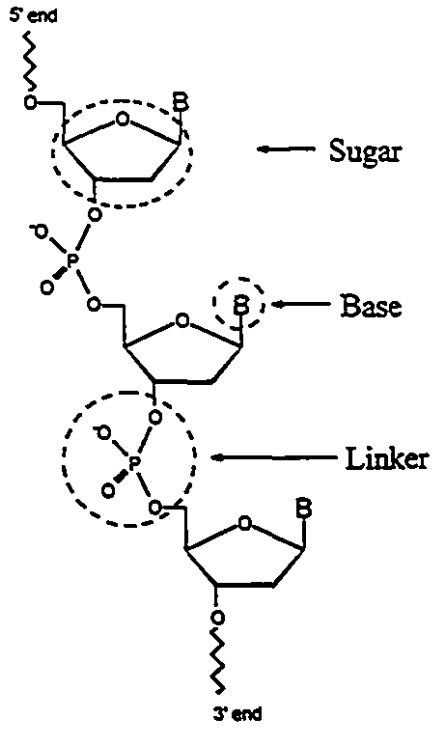


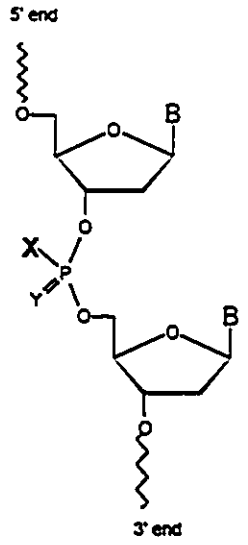
Figure 5. Possible Modification Sites on the Natural DNA

Phosphodiester Analogs

The dual aims of improving both cellular uptake and resistance to nucleases were first tackled by synthesizing derivatives with substitutions of one or two of the non-binding oxygens of the phosphate group. Some of the well-known substitutions for the phosphodiester are depicted in Figure 6. The main concern for these relatively conservative modifications was to ensure retention of hybridization with the target. Methylphosphonates[29] and phosphorothioates[30] were two of the most successful examples for antisense application, both in cell-free and cellular systems, and are usually

[29] a) Miller, P.S.; Yano, J.;Yano, E; Carroll, C.; Jayaraman, K.; Ts'o, P.O.P., *Biochemistry*, 18, 5134 (1979): b) Miller, P.S.; McParland, K.B.; Jayaraman, K.; Ts'o, P.O.P., *Biochemistry*, 20, 1874 (1981)
[30] Eckstein, F., *Angew. Chem.*, 6, 431 (1983)

referred as the first generation of antisense compounds.



X = CH_3, Y = O Methylphosphonate

X = S-, Y = O Phosphorothioate

X = NHR, Y = O Phosphoramidate

X = OR, Y = O Phosphotriester

X = S-, Y = S Phosphorodithioate

Figure 6. Oligosides with substitutions of one or two non-binding phosphate atoms.

The uncharged methylphosphonate DNA analog was chronologically the first one developed to explore the value of backbone-modified natural DNA. This analog was originally designed without charge to improve cellular uptake as well as nuclease resistance[31]. Studies involving short oligomers (~ 15 mers) of this modification appeared to indicate that these uncharged analogs bound more strongly to DNA (or RNA) than their natural counterparts. This was rationalized as being due to the absence of electrostatic repulsion between the phosphate groups of opposing strand. The methylphosphonate-linked oligonucleotides were found to be resistant towards endonucleases[32].

Phosphorothioates, in which a nonbridging oxygen is substituted by a sulfur atom, are the most widely used analogs of phosphodiester as antisense agents. The melting temperature of the 15-mer phosphorothioate-DNA duplex was found to be close to the

[31]Miller, P.S.; Ts'o, P.O.P., *Anti Cancer Drug Des.*, 2, 117 (1987)
[32]Ts'o, P.O.P., *Antisense Research and Development*, 1, 273 (1991)

natural DNA-DNA hybrid, with only a 0.6 °C decrease / modification[33]. Also the water-soluble phosphorothioate oligomer can cross cell membranes by receptor-mediated endocytosis like natural DNA, and is distributed evenly to most tissues. No acute toxicity was seen with therapeutic doses of this DNA analog given to animals[34]. Importantly, this modification of the phosphodiester resulted in stability towards endonucleases. Phase II clinical trials of phosphorothioate DNA analogs as anti-viral drugs are currently conducted by pharmaceutical companies[35].

However, both methylphosphonate and phosphorothioate analogs have introduced the problem of chirality, because the synthesis by the convenient, currently used automatic methods are not stereospecific[36,37]. Isotopic or elemental replacement of any of two nonbridging oxygen atoms attached to the internucleoside phosphorus atom creates new centers of chirality and results in formation of diastereoisomers. This leads, on the basis of the preparation of roughly equal amounts of both diastereoisomers during synthesis to a large number of diastereoisomeric compounds exceeding half-million individual compounds for a 20-mer. These diastereoisomers do not have the same biophysical properties. Both in phosphorothioate and methylphosphonate, single strand oligosides with bulky substitutions (S^- or CH_3) orienting outward have less stable binding to their complementary sequences than their diastereoisomeric oligosides with the bulky substitutions (S^- or CH_3) pointing inward. It has been postulated that decreased binding affinity of the former diastereomers is mainly due to unfavorable steric interaction between the outward-bulky groups and flanking residues in the major grooves.

---

[33]Stein, C.A.; Subasinghe, C.; Shinozuka, K.; Cohen, J.S., *Nucleic Acids Res.*, 16, 3209 (1988)
[34]Crooke, S.T., 1994 Experimental Biology Meeting, American Society for Pharmacology and Experimental Therapeutics, Anaheim, CA (1994)
[35]Crooke, S.T., *Antisense Res. Dev.*, 4, 145 (1994)
[36]Connonlly, B.A.; Potter, B.V.L.; Eckstein, F.; Pingoud, A.; Grotjahn, L., *Biochemistry*, 23, 3443 (1984)
[37]Miller, P.S.; Agris, C.H.; Aurelian, L.; Blake, K.R.; Murakami, A.; Reddy, M.P.; Spitz, S.A.; Ts'o, P.O.P., *Biochimie*, 67, 769 (1985)

Backbone-Modified Analogs Containing No Phosphorus Atoms

Compounds have been synthesized in which the whole phosphodiester linkage has been replaced by various linear groups[38], especially those achiral and neutral linkers with four atoms in length. Some of the well-known linkers (e.g. formacetal[39], thioformacetal[40], sulfide[41], alkyl derivative, aminoalkyl derivatives, carbamates, ureas and amides) are depicted in Figure 7.

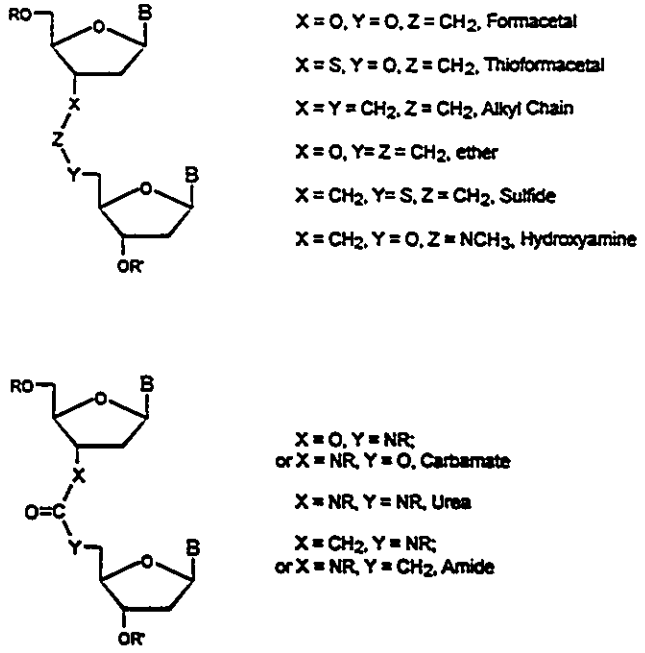


Figure 7. Summary of backbone-modified oligonucleotide analogs obtained by replacement of the natural phosphodiester backbone with nonphosphorus linkers.

The most remarkable improvement of these nonphosphorus-containing linkers was their total resistance towards nucleases. This was expected when considering the difference between these linkers and the natural phosphodiester. In addition, due to their

---

38 For a recent review see: De Mesmaeker, A.; Haner, R.; Martin, P.; Moser, H.E., *Acc. Chem. Res.*, 28, 366 (1995), and references therein.
[39]Jones, R. J.; Lin, K.-Y.; Milligan, J.F.; Wadwani, S.; Matteucci, M. D., *J. Org. Chem.*, *58*, 2983 (1993).
[40]Cao,X.; Matteucci, M.D., *Bioorg. Med. Chem. Lett.*, 4, 807 (1994).
[41]Kawai, S.H.; Wang, D.; Giannaris, P.A.; Damha, M.J.; Just, G., *Nucl. Acids Res.*, 21, 1473 (1993).

neutral nature, the oligosides bearing such linkers should more readily cross various cellular membranes. Furthermore, as these linkers are achiral, stereochemical problems associated with the synthesis and biological activity should be minimized. Finally, all the linkers as depicted in Figure 7 are readily accessible by well-known synthetic methodologies, and the properly functionalized dinucleosides connected by these linkers are compatible with the solid-phase oligonucleotide synthesis. Due to their nuclease resistance and synthetic simplicity, the nonphosphorus-containing linkers are very attractive as antisense agents. The growing attention on these non-phosphorus modifications is evidenced by the number of publication in this area[28,38,42,43].

Theoretically, incorporation of these uncharged mimics of natural phosphodiester into the DNA should eliminate or minimize the electrostatic repulsion upon hybridization of the DNA with the complementary target, resulting in stronger association and more stable duplex formation with the target nucleic acid. However, most of these modifications have an adverse effect on the binding affinity to the DNA and RNA complements compared with their natural DNA counterparts[39], although the interatomic distances in the synthetic oligosides should be essentially preserved because the modified linkers are isosteric with the natural phosphodiester backbone. *N*-Methylhydroxylamine[44] and amide[45,46] are two of the exceptions, which displayed similar binding to their DNA and RNA complements. The main problem resulting from the introduction of above backbone substitutions is the need to ensure that the bases on the oligomers are capable of hybridizing with the target complements, which requires proper geometry of the bases as well as the interatomic distances in the synthetic oligonucleotide. The improvement of

---

[42]Agrawal, S.; Iyer, R.P., *Current Opinion in Biotechnology*, 6, 12 (1995).
[43]Varma, R.S., *Synlett*, 621 (1993).
[44](a)Vasseur, J.-J.; Debart, F.; Sanghvi, Y.S.; Cook, P.D., *J. Am. Chem. Soc.*, 114, 4006 (1992).
(b)De Mesmaeker,A; Waldner, A.; Sanghvi, Y.S.; Lebreton, J., *Bioorg Med Chem Lett*, 4, 807 (1994).
[45](a)Idziak, I.; Just, G.; Damha, M.; Giannaris, P.A., *Tetrahedron Lett.*, 34, 5417 (1993)
(b)Idziak, I., Ph. D. Thesis, McGill University, Canada (1996)
[46](a)Lebreton, J.; Waldner, A.; Lesueur, C.; De Mesmaeker, A., *Synlett*, 137 (1994);
(b)De Mesmaeker, A.; Waldner, A.; Lebreton, J.; Hoffmann, P.; Fritsch, V.; Wolf, R.M.; Freier, S.M., *Angew. Chem., Int. Ed. Engl.*, 33, 226 (1994)

binding affinity of the uncharged nonphosphorus modifications is the focus of the research in this area[17,28,38].

1.6. Conformationally Restricted Oligosides

Optimization of the binding affinity of an oligonucleotide to complementary DNA or RNA can be achieved by decreasing the entropy of duplexation as well as increasing the enthalpy. The latter strategy has been widely adopted with the uncharged modifications since the appearance of the methylphosphonate analog by Miller and Ts'o as the first prototype of these replacements, and has been briefly discussed in the previous section. Decreasing the entropy of hybridization, on the other hand, can be accomplished by restricting the rotation of the backbone of the oligomer to resemble its binding conformation. Conceptually, one can expect a higher degree of pre-organization of single strand oligomers to lead to less entropy change upon duplexation with natural complements, thereby diminishing the entropy component of hybridization and increasing the binding affinity. Attempts to improve the binding affinity through rigid modification have already been demonstrated in a few literature examples, which will be briefly described.

"Homo DNA"

The enhancement of duplexation through conformational restriction was first demonstrated in Eschenmoser's study of oligo-2',3'-dideoxy-β-D-glucopyranonucleotides ("homo DNA") [47, 48, 49](Figure 8). In a comparable sequence (dA_5T_5), the homo-DNA was able to form anti-parallel duplexes to its complementary

[47]Eschenmoser, A.; Loewenthal, E., *Chem. Soc. Rev.*, 21, 1 (1992)
[48]Eschenmoser, A.; Dobler, M., *Helv. Chim. Acta*, 75, 218 (1992)
[49]Hunziker, J.; Roth, H.J.; Bohringer, M.; Giger, A.; Diederichsen, U.; Gobel, M.; Krishnan, R.; Jaun, B.; Leumann, C.; Eschenmoser, A., *Helv. Chim. Acta*, 76, 259 (1993)

homo-DNA and have a Tm significantly higher (~2 °C/base pair) than that observed in natural DNA. Thermodynamic calculations, as summarized in Table 1, indicated that homo-DNA showed a slightly smaller (less negative) enthalpy change (Δ H) and a distinctly smaller (less negative) entropy change (Δ S) upon duplexation with respect to natural DNA. This in general leads to a more negative free enthalpy of pairing (Δ G) in homo-DNA and, eventually, to thermodynamically more stable duplexes (higher Tm). Structurally, the entropy of duplex stabilization can be due to homo-DNA's more rigid pyranose chair conformation as compared to the flexible furanose ring in the natural DNA.

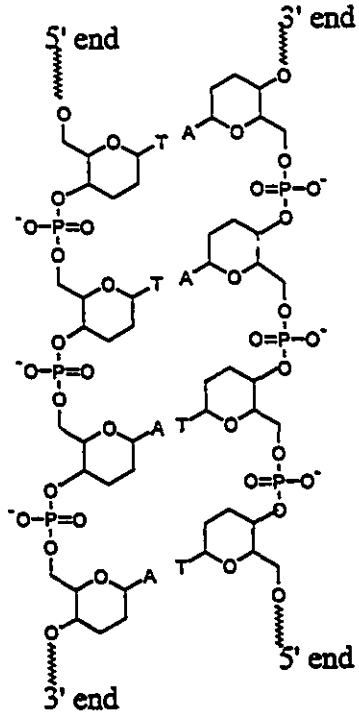


Figure 8. Homo-DNA duplex

Table 1. Thermodynamic parameters for natural DNA duplex (dA5T5) and comparable homo-DNA duplex.

	ΔH	$T\cdot\Delta S$	ΔG	T_m
Natural DNA	-61.3	-54.2	-7.1	33
Homo-DNA	-44.0	-33.5	-10.5	55

Riboacetal

This concept of entropy duplex stabilization was later extended by Jones et al..[50] to design conformationally restricted oligonucleotide analogs to pair to complementary DNA and RNA for 'antisense' applications. This type of rigid modifications has been introduced both on sugar moieties and the internucleoside linkages (Figure 9). By replacing the phosphodiester internucleoside connection with a conformationally restricted 'riboacetal' linkage, ΔTm / insert was observed to be decreased by 1.07 °C with a DNA 15 mer (containing seven riboacetal linkers alternating with seven phosphodiester linkers) binding to the complementary RNA. Interestingly, with the same modified DNA, Δ Tm / insert was observed to be between 3.5 °C and 3.7 °C higher in triple helix formation. Formacetal (-OCH_2O-), which is a linear analog, did not show such enhanced binding when tested under the same condition in the same sequences[41]. It was clearly demonstrated by this riboacetal modification that the binding affinity of the modified DNA with flexible backbone could be significantly improved through the covalent conformational restriction of the internucleoside linkage along with the perturbation of the ribose sugar pucker.

[50]Jones, R. J.; Swaminathan, S.; Milligan, J.F.; Wadwani, S.; Froehler, B.C.; Matteucci, M.D., *J. Am. Chem. Soc.*, 115, 9817 (1993)

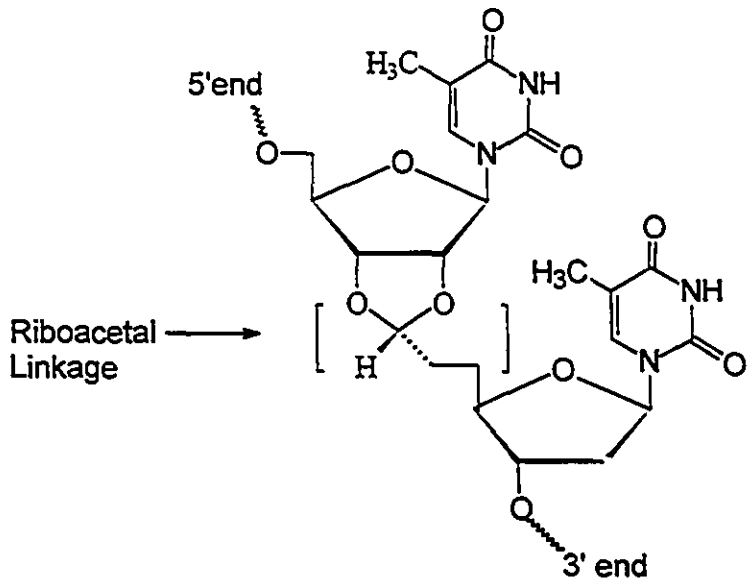


Figure 9. Oligonucleotide containing riboacetal internucleoside linkage

Bicyclodeoxynucleosides

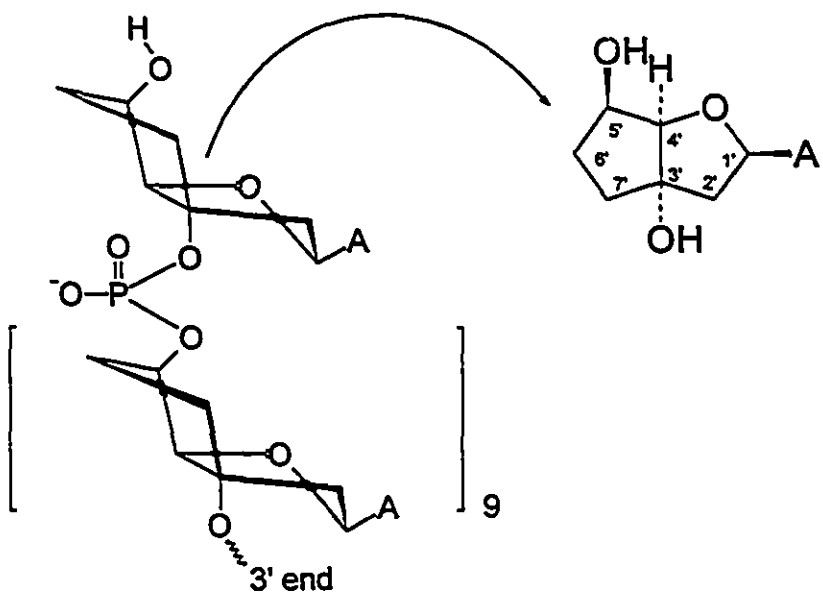


Figure 10. Nucleic-acid analog with constrained conformational flexibility in sugar-phosphate backbone ('bicyclo-DNA')

In Tarköy and Leumann's effort[51,52], natural 2'-deoxyribonucleosides were restricted by adding an ethylene bridge between the centers C-3' and C-5'˙ to form constrained 'bicyclodeoxynucleosides' (BCD) (Figure 10). The configuration at these centers (3'S, 5'R) was chosen to match the geometry of a repeating nucleoside unit in duplex DNA as closely as possible, thus, leading to a higher degree of pre-organization of a single strand.

Although the Tm evaluation under comparable conditions showed that the duplexes of deca(bicyclodeoxynucleotide) $(BCD\text{-}T)_{10}$ (thymidine derivative) with natural poly(A) and $d(A)_{10}$ complements were less stable than its natural counterparts (4 °C and 13 °C decreased respectively), the $(BCD\text{-}A)_{10}$ showed enhanced binding to its natural RNA target (13 °C more stable) as well as the natural DNA target (25 °C vs 23 °C). Thermodynamic calculations clearly indicated that the more stable $(BCD\text{-}A)_{10} \bullet d(T)_{10}$ hybrid was due to the numeric reduction of its pairing entropies term (Δ S), which could overcome the unfavorable reduction of pairing enthalpy (Δ H) and still lead to an overall reduction of free enthalpy Δ G of $(BCD\text{-}A)_{10} \bullet d(T)_{10}$ duplex compared to its natural counterpart. The significant reduction of the entropy term of the $(BCD\text{-}A)_{10} \bullet d(T)_{10}$ duplex was attributed to the reduced degree of rotational freedom in the sugar-phosphate backbone of the $(BCD\text{-}A)_{10}$, although other contributions, especially from differences in duplex and single strand solvation, might also play an important role.

It was clearly demonstrated in this example that the binding affinity of a DNA strand could be enhanced through proper conformational restriction of the internucleoside backbone, which was aimed to reduce the pairing entropies term (Δ S) of the duplex.

---

[51]Tarköy, M.; Bolli, M.; Schweizer, B.; Leumann, C., *Helv. Chim. Acta.*, 76, 481 (1993)
[52]Tarköy, M.; Leumann, C., *Angew. Chem. Int. Ed. Engl.*, 32, 1432 (1993)

Bicyclo[3.1.0] hexane thymidine analogs

The most recent example of conformationally constrained oligonucleotides was reported by Altmann and co-workers[53, 54](Figure 11).

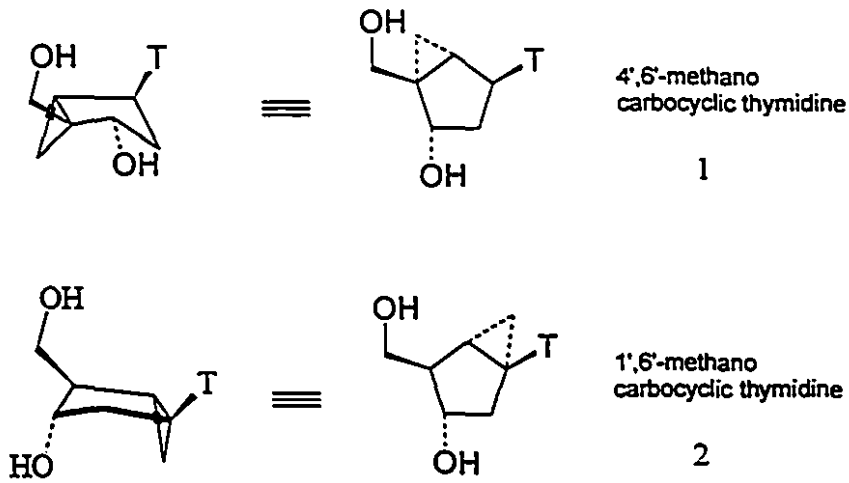


Figure 11. Bicyclo [3.1.0] hexane derivatives: 4',6'-methano carbocyclic thymidine (1) and 1',6'-methano carbocyclic thymidine (2)

X-ray crystallography revealed the bicyclic skeleton of type 1 molecule adopted a boat-like ("2'-exo") conformation. The hybridization data indicated that the substitution of type 1 molecule for natural thymidine in DNA/RNA heteroduplexes increased their thermodynamic stability. The bicyclic skeleton of type 2 molecule adopted a boat-like (3'-exo) conformation as revealed by X-ray crystallography. Oligodeoxyribonucleotides incorporating up to 10 building blocks of type 2 molecule in place of natural thymidine were still capable of binding to complementary DNA or RNA, albeit with lower affinity than the unmodified parent compounds.

[53]Altmann, K-H.; Kesselring, R.; Francotte, E.; Rihs, G., *Tetrahedron Lett.*, 35, 2331 (1994)
[54]Altmann, K-H.; Imwinkelried, R.; Kesselring, R.; Rihs, G., *Tetrahedron Lett.*, 35, 7625 (1994)

Conclusion

Although other studies still have to be brought forward, there is substantial evidence above to suggest that conformational restriction of the DNA strand through rigid backbone modification may lead to an improvement of its binding to the complementary target, thereby opening novel routes to improve the binding properties of the backbone-modified oligosides.

1.7. Research Plan and Rationale of the Project

In our effort to search for novel antisense constructs, we were interested in exploring the uncharged nonphosphorus analogs for the natural phosphodiester backbone. As we have previously discussed in Section 1.5.2., such modified linkers have the potential advantages as being nuclease-resistant and readily crossing various cellular membranes, which make them very attractive in antisense applications.

The previous results from our group[45] and others[46] have shown that substituting the phosphodiester backbone of a DNA with an amide linker (see **A**, Figure 12) had little effect on DNA binding towards both DNA and RNA complements. The preservation of the binding affinity of DNA with amide modification was thought to be a result of the relative rigidity of the amide bond[38]. In order to establish if the binding affinity could be further improved by conformationally restricting this amide link, we decided to prepare analogs of this amide in which the methylene group of the amide link was covalently attached to the 2'-position of the ribose ring. The general structure of the amide-linked 2',3'-cyclopropanated dinucleosides (see **B**) is depicted in Figure 12.

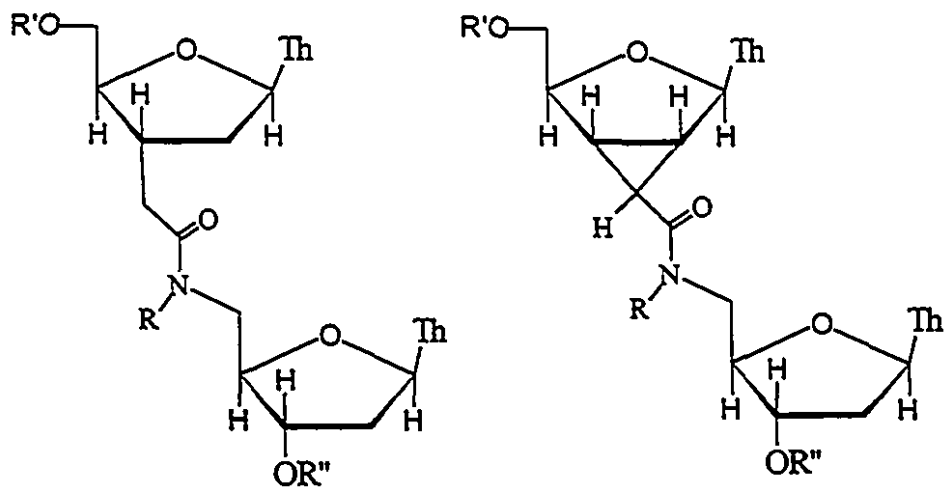


Figure 12. (A) amide-linked dinucleoside; (B) amide-linked 2',3'-cyclopropanated dinucleosides

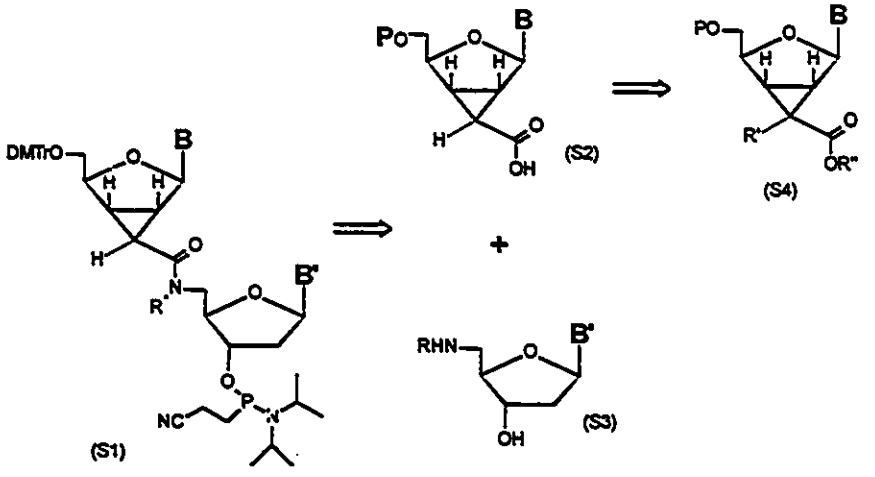
Because an additional degree of rigidity is introduced by the highly constrained [3.1.0]-bicyclic structure in the cyclopropanated amide modification, the number of the energetically favored conformations of the modified DNA strand should be significantly reduced in this way. Theoretically, such preorganization could provide an entropy advantage for the Watson-Crick base-pairing process, thereby leading to an enhanced binding.

2. Results and Discussion

2.1. Synthetic Strategy for Dinucleoside Analogs Synthesis

In principle, the dinucleoside analog of type **S1** with a cyclopropyl amide linker can be prepared[45] by coupling of acid **S2** (5'-end building unit) with amine **S3** (3'-end building unit) (Scheme 1). Transforming the 5'-end protecting group of the dimer into a dimethoxytrityl group, followed by phosphitylation at the 3'-end will provide a properly functionalized dimer that may be incorporated into a DNA sequence by the standard automated DNA synthesis methodology[55].

Scheme 1



The 5'-amino-5'-deoxynucleoside analog **S3**, which will serve as the 3'-end building unit in the coupling, can be obtained by a reported procedure[56]. Since the chemistry of transforming ester to acid is also well-established, the uncertain step in this strategy is the formation of the cyclopropyl carboxylate **S4**.

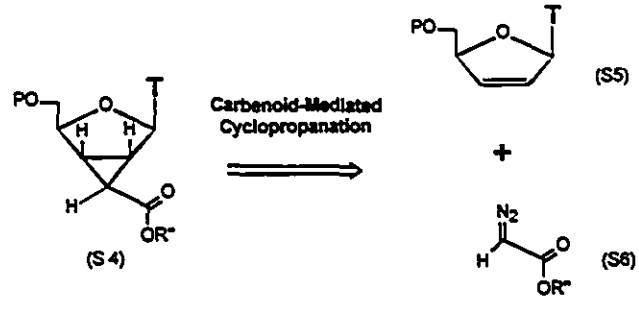
---

[55]Brown, T.; Brown, D.J.S., In *Oligonucleotides and Analogues: a Practical Approach*, Eckstein, F., Eds., Oxford University Press, New York, 1 (1991).
[56]Horwitz, J.P.; Tomson, J.A.; Chua, J., *J. Org. Chem.*, 27, 3045 (1962)

2.2. Carbenoid-Mediated Cyclopropanation Attempts

One obvious way to prepare the cyclopropyl carboxylate S4 was to add a carbenoid species derived from ethyl diazoacetate to the olefinic thymidine derivative S5 (Scheme 2)

Scheme 2



The reactions of an olefin and ethyl diazoacetate, proceeding with nitrogen elimination and generation of carbenes or of their complexes with transition metals, have been widely used for the production of cyclopropanes[57,58] since the discovery of ethyl diazoacetate (N_2CHCO_2Et). A systematic screening of common transition metal complexes revealed that Rh(II) carboxylates were the mildest and most efficient catalysts for the cyclopropanation[59,60,61]. The Rh (II) mediated reaction of α-diazo carbonyl compounds has been reported to add to different alkenes to produce cyclopropyl derivatives in an *inter*- or *intra*-molecular manner[61]. Therefore the olefinic thymidine S5 was prepared, and the carbenoid-mediated cyclopropanation was attempted.

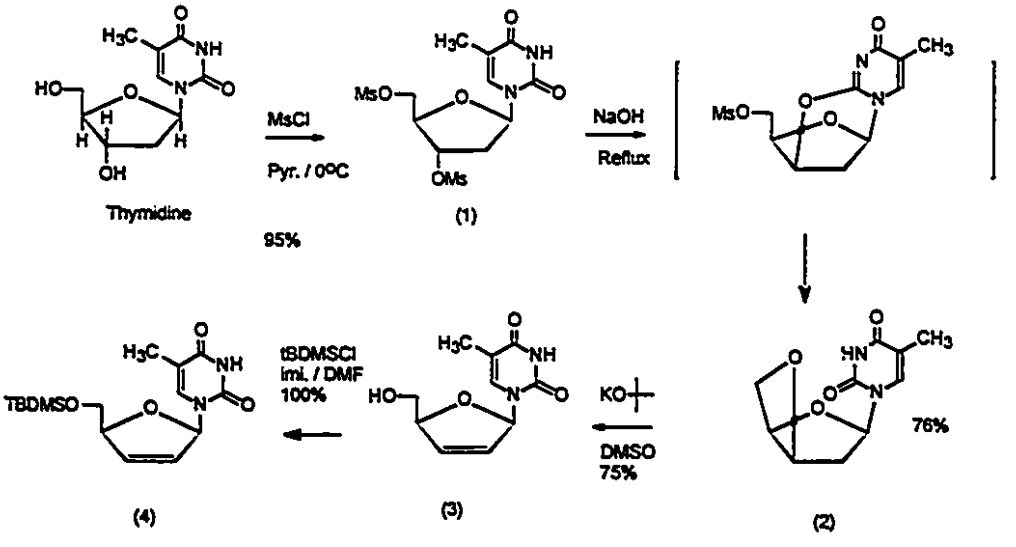
---

[57]Nefedov, O.M., Shapiro, E.A., Dyatkin, A.B., In *Supplement B: The Chemistry of Acid Derivatives*, Patai, S., Eds, John Wiley & Sons, Vol. 2, 1477 (1992)
[58]Adams, J.; Spero, D.M., *Tetrahedron*, 47, 1765 (1991)
[59]Anciaux, A.J.; Hubert, A.J.; Noels, A.F.; Petiniot, N.; Teyssie, P.; *J. Org. Chem.*, 45, 695 (1980)
[60]Doyle, M.P.; Van Leusen, D.; Tamblyn, W.H.; *Synthesis*, 778 (1981)
[61]Doyle, M.P.; *Chem. Rev.*, 86, 919 (1986)

2.2.1. Synthesis of Silylated Olefinic Thymidine 4

The 2',3'-olefinic thymidine analog 3 was prepared from the commercially available thymidine in three steps as outlined in Scheme 3, following a published procedure[62,63] .

Scheme 3



Mesylation of thymidine with methanesulfonyl chloride (MsCl) in dry pyridine readily gave the dimesylate 1 in 95% yield after crystallization from methanol. The resulting dimesylate 1 was then refluxed in 1N aqueous sodium hydroxide for 2 hours, followed by neutralization to yield the oxetane 2. After crystallization from ethanol, oxetane 2 was obtained as yellow prisms in 76% yield. Subsequent transformation of oxetane 2 to the desired dideoxythymidine 3 was accomplished by treating 2 with two equivalents of potassium *tert*-butoxide in dimethylsulfoxide. The reaction gave the olefin

---

[62]Horwitz, J.; Chua, J., In *synthetic procedures in Nucleic Acid Chemistry*, Zorbach, W.W., Tipson, R.S., Eds.; Interscience, New York, 1, 344 (1968)

[63]Mansuri, M.M.; Scarrecett, J. E.; Ghazzouli, I.; Hitchcock, M.J.M.; Sterzycki, R.Z.; Bankovan, V.; Lin, T.S.; August, E.M.; Prusoff, W.H.; Sommadossi, J.P.; Martin, J.C., *J. Med. Chem.*, 32, 461 (1989)

3 in 75% yield after crystallization. The resulting olefin 3 was then silylated with *tert*-butyldimethylsilyl chloride in dimethylformamide in the presence of imidazole to afford the 5'-silylated olefin 4. The ^{1}H-NMR spectrum of the silylated olefin 4 (Figure 13) is quite similar to that of 3 as reported except for the additional peaks at 0.88 ppm and 0.07 ppm due to the *tert*-butyldimethylsilyl group. The 5' silylated olefin 4 was going to serve as the olefin substrate in latter attempts of the carbenoid-mediated cyclopropanation.

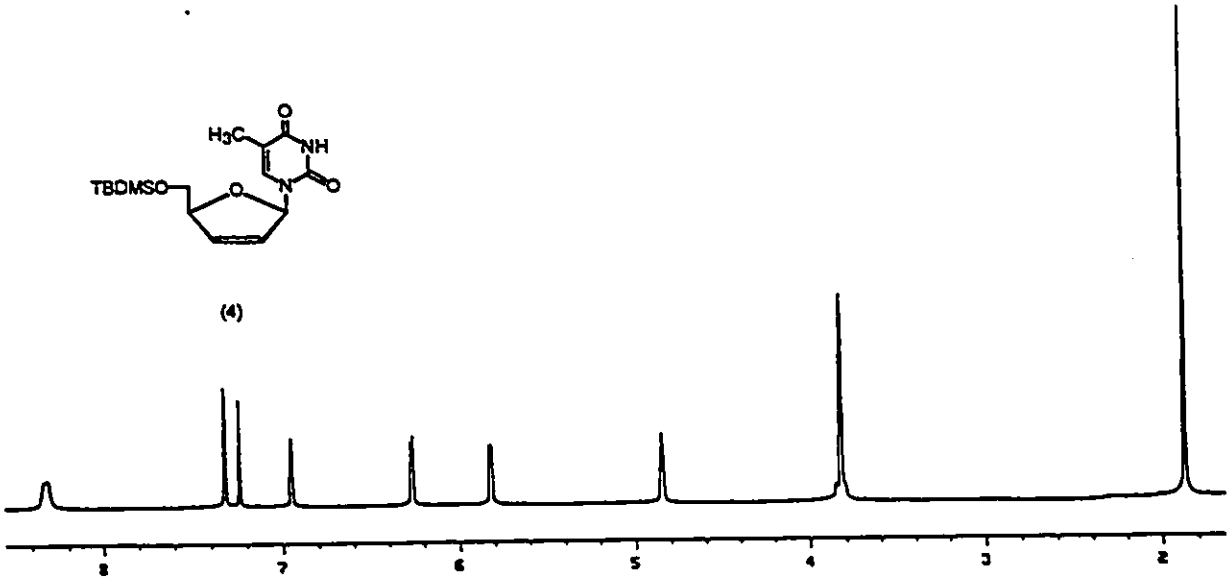


Figure 13. ^{1}H-NMR spectrum of the 5'-*tert*-butyldimethylsilyl-2',3'-olefinic thymidine analog 4 (500 MHz, $CDCl_3$).

2.2.2. Initial Cyclopropanation Attempts with Dideoxythymidine 4

One possible competing reaction accompanying the desired cyclopropanation is known to be the dimerization of the reactive carbenoid intermediate derived from ethyl diazoacetate[58]. Three strategies are commonly employed to minimize such dimerization, namely using a large excess of olefin substrate, and a minimum amount of $Rh_2(OAc)_4$ (usually 2-3% by weight), as well as maintaining a minimum level of diazo substrate in the reaction solution by adding the diazo compound slowly[58]. In our application, the olefinic substrate (2',3'-olefinic thymidine analog 4) had to be prepared in a four-steps transformation from thymidine, so its supply was limited. Therefore, minimization of the competing dimerization could only be addressed by using a minimum amount of catalyst, and controlling the addition of the ethyl diazoacetate.

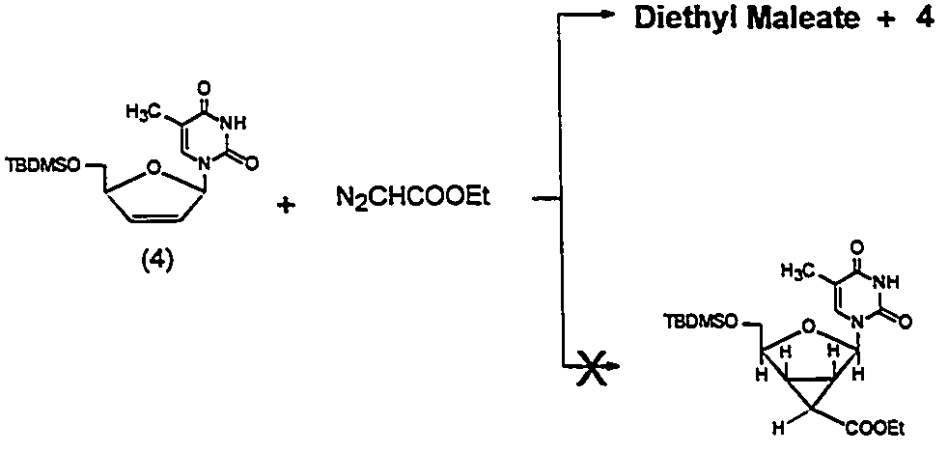
In the initial attempt, ethyl diazoacetate (~ 2 mmol, 200 µl) was added as slow as possible into a stirred mixture of olefin 4 (2 mmol, 676 mg) and $Rh_2(OAc)_4$ catalyst (3 % by weight, ~ 10 mg) in fresh-distilled dichloromethane (0.5 ml) under argon . Evolution of nitrogen was observed with the addition of ethyl diazoacetate. However the reaction resulted only in recovered starting material 4, and there was no evidence of the desired cyclopropane product. The only new species in the product mixture was diethyl maleate, which was produced from the dimerization of the carbenoid species.

To make sure that the dominant dimerization of the carbenoid species was not caused by the relatively fast addition of the ethyl diazoacetate, the ethyl diazoacetate (2 mmol, 200 µl) was then diluted in 10 ml of freshly distilled dichloromethane at 0°C, and added to the reaction mixture over 2 hours using a syringe pump. Disappointingly, the same result was obtained as before, and the starting material 4 was again fully recovered. The reaction was subsequently repeated at different reaction temperatures, and using copper-based catalysts like Cu and $Cu(OTf)_2$[64]. Unfortunately, no cyclopropane product

[64]Doyle, M.P.; Griffin, J.H.; Bagheri, V.; Dorow, R.L., *Organometallics*, 3, 53 (1984)

was detected in all these attempts (Scheme 4).

Scheme 4

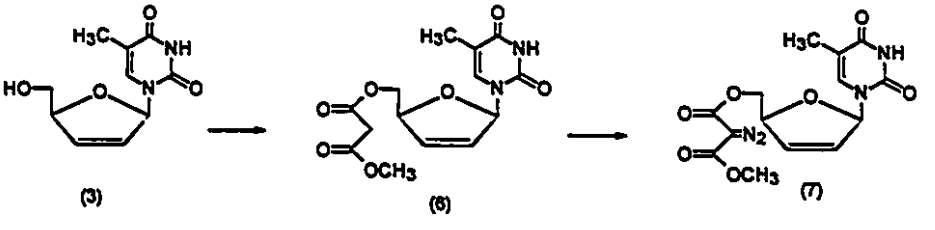


2.2.3. Cyclopropanation Attempt in an Intramolecular Approach

The dilemma that was encountered in the previous attempts was due to the relative ease of the carbenoid dimerization over the cyclopropanation. To optimize the desired carbenoid addition to olefin for the cyclopropanation, an intramolecular approach was then explored.

In principle, the intramolecular reaction provides an entropic advantage, thus occurs with higher efficiency than its intermolecular counterpart. It was hoped that this desired carbene addition could be significantly promoted by the intramolecular approach, thereby suppressing the intermolecular dimerization of the carbenoid species. The intramolecular strategy in carbenoid-mediated cyclopropanations has been adopted in the literature in many cases[65,66], and the power of this chemistry has been elegantly demonstrated in the synthesis of some unusually strained ring systems[67,68,69].

Scheme 5



To allow a quick look at this possibility, the 5'-diazomalonated 2',3'-olefinic thymidine analog 7 was prepared. Esterification of the olefinic thymidine 3 with methyl malonyl chloride, followed by a diazo-transfer reaction with 4-

65Adams, J.; Belley, M.; *Tetrahedron Lett.*, 27, 2075 (1986)
66Adams, J.; Frenette, R.; Belley, M.; Chibante, F.; Springer, J.P.; *J. Am. Chem. Soc.*, 109, 5432 (1987)
67Martin, S.F.; Spaller, M.R.; Liras, S.; Hartmann, B., *J. Am. Chem. Soc.*, 16, 4493 (1994)
68Reingold, I.D.; Drake, J., *Tetradedron Lett.*, 30, 1921 (1989)
69Ceccherelli, P.; Curini, M.; Marcotullio, M.C.; Wenkert, E.; *J. Org. Chem.*, 51, 738 (1986)

carboxybenzenesulfonazide[70] in the presence of triethyl amine in acetonitrile successfully yielded the diazo functionalized olefin 7 (Scheme 5). The structure of diazomalonate 7 was confirmed by NMR analysis as well as mass spectrometry (FAB), in which the molecular ion $[M + 1]^+$ was observed at 351.30. A strong absorption at 2132.1 cm^{-1} due to the diazo group was also observed in the IR spectrum of compound 7, as an indication for the formation of the diazomalonate type of structure. The ^{1}H-NMR spectrum of diazomalonate 7 is showed in Figure 14.

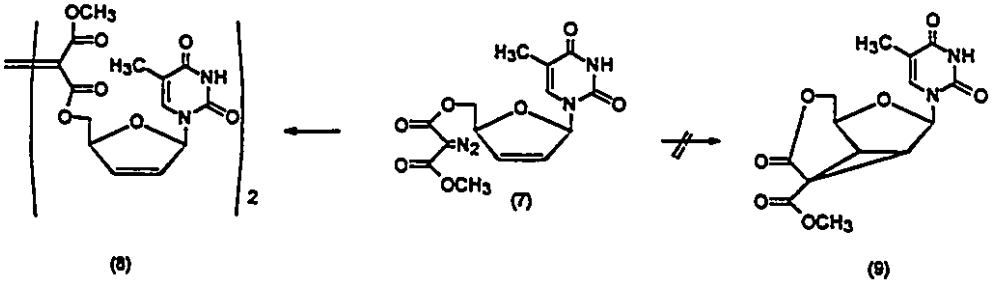
The 5'-diazomalonated olefinic thymidine analog 7 was then dissolved in dry dichloromethane and stirred with a catalytic amount (2% by weight) of $Rh_2(OAc)_4$ at room temperature for 30 minutes. Bubbles were observed to evolve from the solution. The reaction mixture was then filtered through celite to remove the rhodium catalyst. After evaporating dichloromethane from the filtrate, the residue was directly subjected to NMR analysis. Since the ^{1}H-NMR spectrum of the residue was different from the starting material 7, it was believed that the starting material 7 was consumed. However, no pair of AB doublets were observed between δ 2-3 ppm, which were expected for the cyclopropanated H-2' and H-3' protons if the desired tricyclic structure 9 had been generated from the reaction. The occurring of the dimerization of the carbenoid intermediate was strongly suggested by the similarity of the NMR spectra of the starting material and the product, and was also supported by mass spectrometry analysis of the residue (FAB), in which a peak at 645 was observed to match the molecular ion $[M+1]^+$ of the dimer 8. It was not known whether the E or the Z isomer of 8 was formed. Unfortunately, dimerization of the carbenoid was still dominant in this approach.

Since the cyclopropanation was unable to compete with the carbenoid dimerization in an *inter-* or *intra-* molecular reaction, it appeared that the 2',3'-olefinic function in the dideoxythymidine was too electron-deficient to react with the carbenoid species. Therefore, the carbenoid-mediated cyclopropanation approach was given up. The

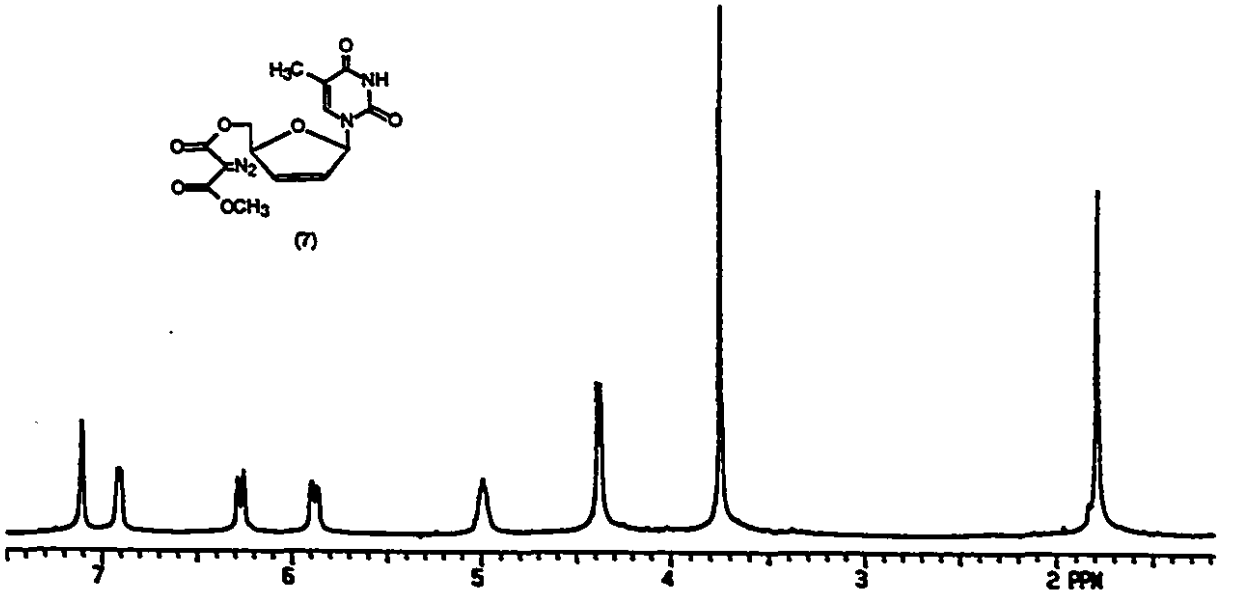
[70]Hendrickson, J.B.; Wolf, W.A., *J. Org. Chem.*, 33, 3610 (1968)

failure of Simmons-Smith and related methods for cyclopropanation of a similar substrate had been already reported[71], and they were therefore not attempted. A different methodology had to be used to introduce the functionalized cyclopropane into the nucleosides.

Scheme 6



(8) (7) (9)



(7)

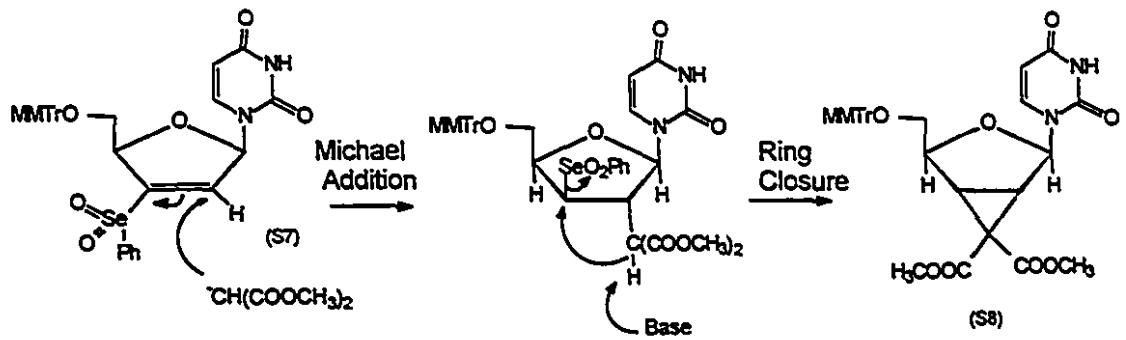
Figure 14. ^{1}H-NMR spectrum of 5'-diazomalonated olefin 7 (200 MHz, $CDCl_3$).

[71]Samano, V.; Robins, M., *J.Am. Chem. Soc.*, 87, 1353 (1992)

2.3. New Strategy to Synthesize the 5'-End Building Block

Due to our lack of success in preparing the cyclopropyl carboxylate of type S4 by the carbenoid-mediated cyclopropanation, we next explored the possibility of decarboxylating malonate of type S8, which has been recently described by Wu and Chattopadhyaya[72]. The important, malonate-functionalized cyclopropane forming steps are the Michael addition of malonate anion to the vinyl selenone S7, followed by the S_N2 type nucleophilic ring closure reaction of reionized malonate (Scheme 7).

Scheme 7



To generate the cyclopropyl acid of type S2, it was planned (Scheme 8) to convert malonate S8 to the diacid S9 first, then decarboxylate the resulting diacid. Malonic acids are reported to be more readily decarboxylated under mild condition[73,74] via a six-center mechanism[75]. It was hoped that the thermolysis of diacid S10 could result in cleavage of the bond connecting the carboxyl carbon, loss of carbon dioxide, and formation of the enol S10, which should be easily tautomerized to afford the carboxylic acid S2.

---

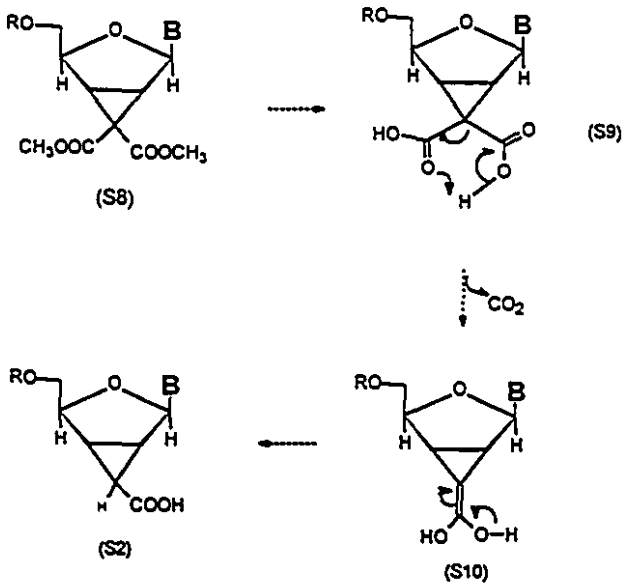
[72]Wu, J.C., Chattopadhyaya, J., *Tetrahedron*, 45, 4507 (1989)

[73]Choi, W.-B.; Churchill, H. R.-O.; Lynch, J.E.; Thompson, A.S.; Humphrey, G.R.; Volante, R.P.; Reider, P.J.; Shinkai, I., *Tetrahedron Lett.*, 35, 2275 (1994).

[74]Jones, D.K.; Liotta, D.C.; Choi, W.-B.; Volante, Reider, P.J.;R.P.; Shinkai, I; Churchill, H. R.-O.; Lynch, J.E., *J. Org. Chem.*, 59, 3749 (1994).

[75]Westheimer, F.H., Jones, W.A., *J. Am. Chem. Soc.*, 63, 3283 (1941)

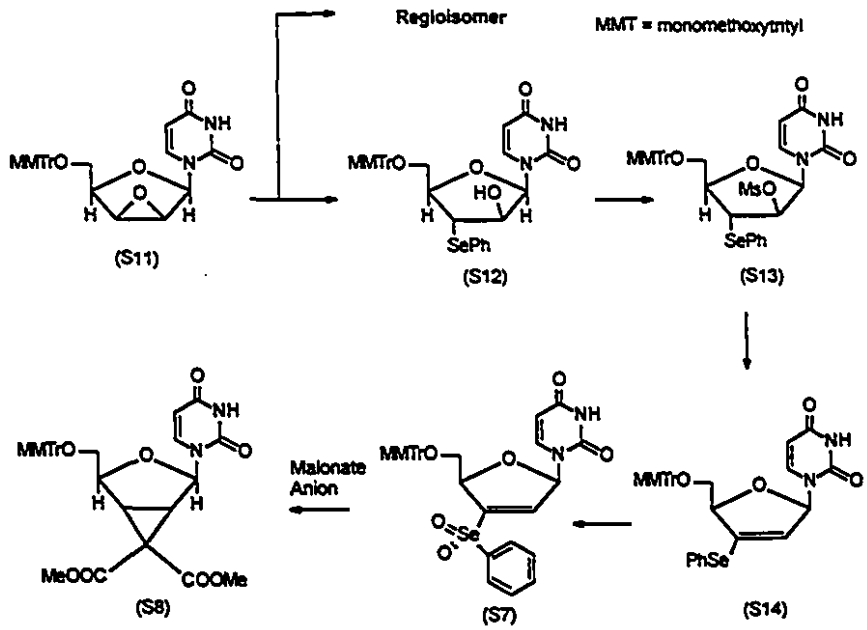
Scheme 8



2.4. A Modified Synthesis of the Vinyl Selenone Substrates

To carry out this decarboxylation strategy, our first task was to prepare the vinyl selenone substrate of type S7 for the Michael-type of cyclopropanation. The Wu and Chattopadhyaya approach[72] to synthesize S7 is summarized in Scheme 9.

Scheme 9



Since we planned to carry out the decarboxylation via a malonic acid intermediate, the acid-labile 5'-monomethoxytrityl protecting group used by Wu *et al* was replaced by a more acid-resistant group in the selenone synthesis. Silyl (*tert*-butyldimethylsilyl and especially *tert*-butyldiphenylsilyl) ethers are known for their stability under both basic and mildly acidic conditions[76], and the chemistry of silyl protecting groups in nucleoside synthesis are well established. In addition, the *tert*-butyldimethylsilyl group only shows as two singlets around 0 ppm and 1 ppm in the ^{1}H-NMR spectrum, which are away from most nucleoside signals. Therefore, the using of the

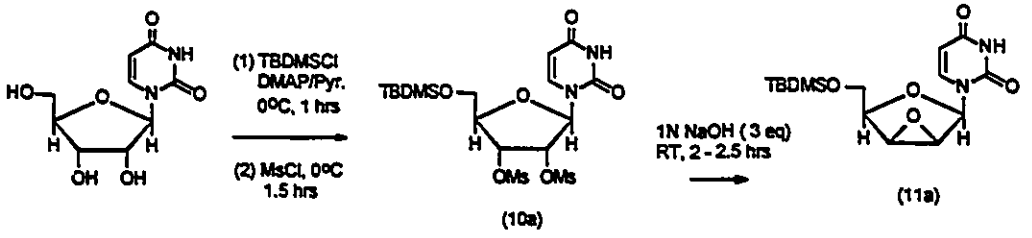
[76]Greene, T.W.; Wuts, P.G.M., *Protective Groups in Organic Synthesis*, John Wiley & Sons Inc., New York (1991)

5'-*tert*-butyldimethylsilyl protecting group in the preliminary investigation would benefit the structure elucidation of the nucleoside analogs by ^{1}H-NMR.

2.4.1. Synthesis of Epoxide 11a

To have a good supply of epoxide **11a** as the starting material and to carry out the similar synthetic sequence as depicted in Scheme 9, Codington's methodology[77,78] for epoxide formation was modified for our application (Scheme 10).

Scheme 10



The silylation and dimesylation of uridine was found to readily proceed in an one pot reaction in pyridine in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP) at 0°C. Because the silylation of the most reactive 5'-hydroxyl group of uridine was inevitably accompanied by a relatively slow silylation of the 2'- and 3'-hydroxyl groups, the silylation was carefully controlled by maintaining the reaction temperature at 0°C and adding *tert*-butyldimethylsilyl chloride (1.2 equivalents) portionwise. Once the silylation of the most reactive 5'-hydroxyl group was almost completed and silylation of the less reactive 2'- and 3'-hydroxyl groups became obvious (about 1 hour), 6 equivalents of methanesulfonyl chloride were added to the reaction mixture to quickly mesylate the

[77]Codington, J.F.; Fecher, R.; Fox, J.J., *J. Am. Chem. Soc.*, 82, 2794 (1960)
[78]Codington, J.F.; Fecher, R.; Fox, J.J., *J. Org. Chem.*, 27, 163 (1962)

2'- and 3'-hydroxyl groups, and suppress the ongoing of the 2'-O and 3'-O silylation. After the mesylation of the 2'- and 3'-hydroxyl groups (2 hours), water was then added, and the mixture was stirred at 0°C for another hour to destroy the remaining methanesulfonyl chloride or *t*-butyldimethylsilyl chloride in the solution. The mixture was then slowly poured into a large amount of ice-water with vigorous stirring. In this way, the crude dimesylate **10a** was obtained as a precipitate, and easily separated from other by-products. The crude dimesylate **10a** was then filtered off, and crystallized from methanol to remove the small amount of disilylated side-product. Pure dimesylate **10a** was obtained as fine white crystals in ~ 90% yield.

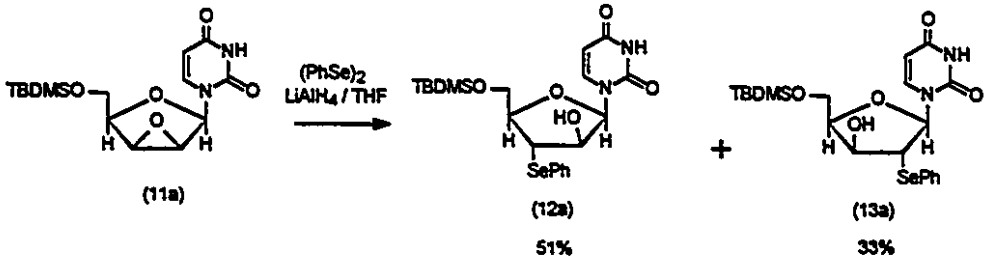
The dimesylate **10a** obtained above was subsequently added to aqueous sodium hydroxide (1 N, 3 equivalents) solution at room temperature with vigorous stirring. After 2 hours, the resulting thick solution was diluted with large amount of ice-water, and neutralized to pH 8 with 0.1 N HCl at 0°C to provide crude epoxide **11a** as a yellow crystals. The crude epoxide was then recrystallized from 95% ethanol to give the pure epoxide **11a** as white crystals. The overall yield from uridine to epoxide **11a** was about 75%.

The above method can be easily applied to a 20 mmol scale preparation, and no chromatography was needed in the whole procedure.

2.4.2. Reaction of Epoxide 11a with Phenylselenium Anion

With the epoxide **11a** in hand, we then proceeded with the epoxide opening reaction with phenylselenium anion. The phenylselenium anion was generated in *situ* by treating diphenyldiselenide (slightly in excess) with lithium aluminum hydride in dry tetrahydrofuran. Similar to Wu and Chattopadhyaya's observation[72], the reaction of epoxide **11a** with the nucleophilic phenylselenium anion yielded a mixture of regioisomeric alcohols **12a** and **13a** (Scheme 11) in a ratio of about 5:3, due to lack of regioselectivity of the reaction. Following Wu *et al*'s protocol, the regioisomeric mixture was then carefully separated by chromatography to provide the 3'-selenyl uridine analog **12a** as the major regioisomer (51% yield). The minor isomer **13a** was isolated in 33% yield after chromatography.

Scheme 11

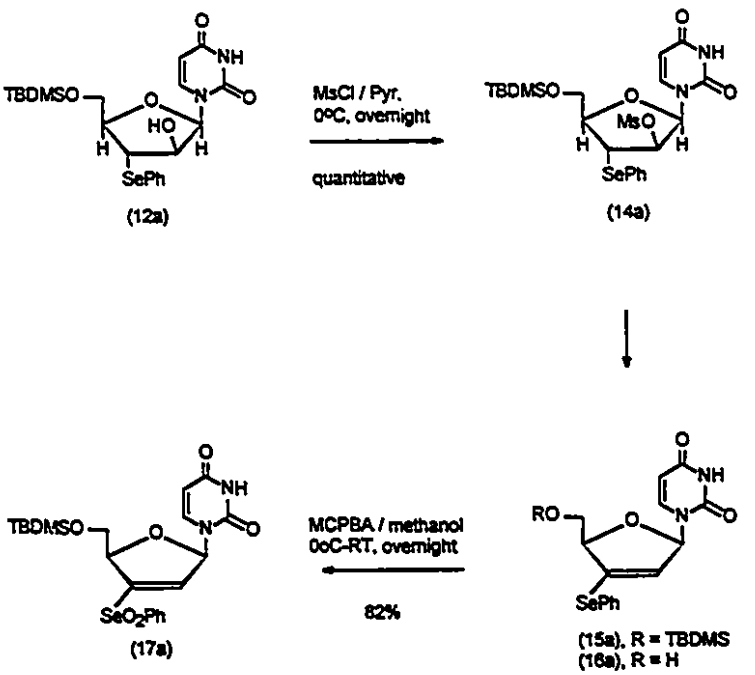


2.4.3. Preparation of 3'-Selenonyl Uridine 17a

The major isomer **12a** was then treated with methanesulfonyl chloride in dry pyridine at 0 °C overnight. The mesylation of the 2'-β-hydroxyl group proceeded smoothly, and yielded the 2'-β-O-mesylate **14a** in a clean reaction. After workup, mesylate **14a** was dried *in vacuo*, followed by the treatment with potassium *t*-butoxide (2.4 equivalents) in *N,N*-dimethylformamide at room temperature for 4 hours. Besides the

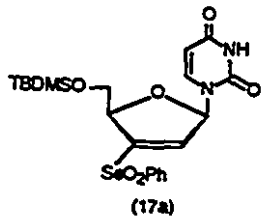
desired elimination, an unexpective desilylation also occurred under this reaction condition, and the product isolated was the 5'-hydroxyl olefin **16a** instead of the 5'-silylated olefin **15a**. No mechanistic study was carried out at this stage, and the problem was simply circumvented by resilylating **16a** to give back 5'-silylated vinyl selenide **15a**. Oxidation of 3'-selenyl olefin **15a** with *m*-chloroperbenzoic acid (MCPBA) in methanol gave the vinyl selenone **17a** in 82% yield after chromatography.

Scheme 12



The ^{1}H-NMR (Figure 15) signals of selenone **17a** were similar to those as reported by Wu *et al* for a similar structure[72] except for the peaks due to 5'-protecting group and the H-5 proton. The H-5 of **17a** appeared as a doublet ($J_{5,6}$ = 8.1 Hz) at δ 5.67 ppm, which was significantly downshifted as compared to its counterpart (δ 4.56) in Wu and Chattopadhyaya's report. The upshift of H-5 proton in Wu's compound was presumably due to the influence of the neighboring benzene rings of the 5'-monomethoxytrityl group. A *tert*-butyldimethylsilyl group was used as the 5'-protecting

group in **17a**, therefore such influence on H-5 proton did not exist, and the chemical shift of H-5 in **17a** remained at the usual position around 5.5 ppm as the H-5 in uridine. The synthetic sequence from 3'-selenyl uridine **12a** to vinyl selenone **17a** is outlined in Scheme 12.



(17a)

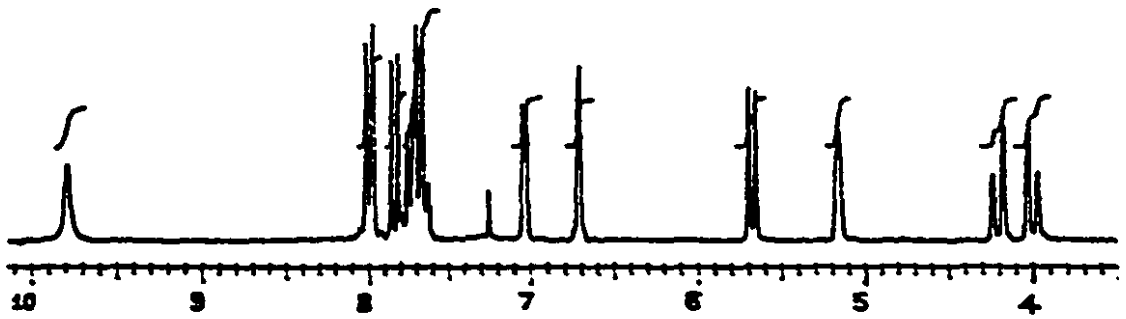


Figure 15. ^{1}H-NMR spectrum of 5'-*tert*-butyldimethylsilyl 3'-selenonyl uridine analog **17a** (200 MHz, $CDCl_3$).

2.4.3. Synthesis of 2'-Selenonyl Uridine 20a from 2'-Selenide 13a

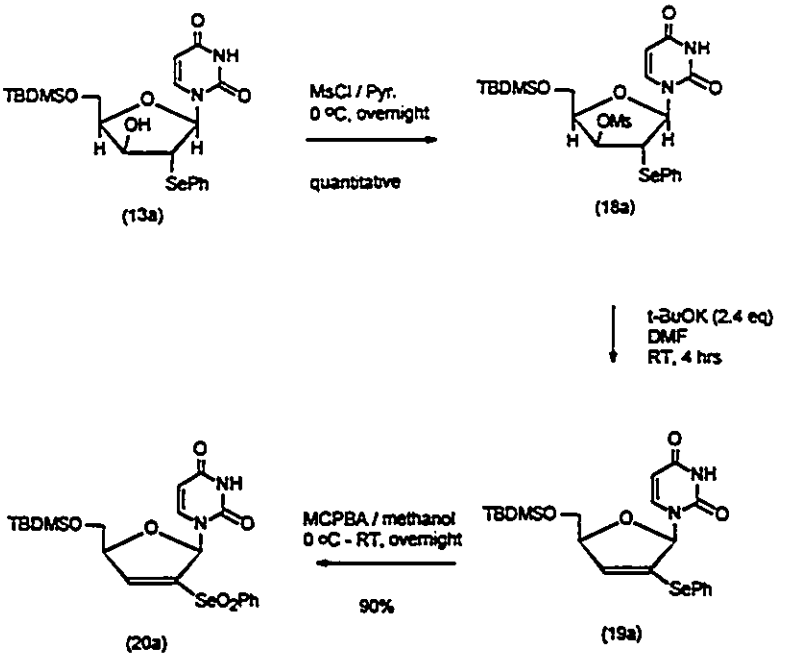
A serious problem was encountered in applying Wu and Chattopadhyaya's strategy to a large scale preparation of the selenone substrate **17a**: the R_f difference between the regioisomeric alcohols **12a** and **13a** obtained from the nonregioselective epoxide opening was measured to be only 0.1 (MeOH / CH_2Cl_2), which made the grams-

scale separation of these regioisomeric mixture by silica gel chromatography very tedious. In addition, in Wu and Chattopadhyaya's report[72], only the 3'-selenide of type **12a** (the major regioisomer) was converted to the 3'-selenonyl uridine of type **17a**, which was later used in the Michael-type cyclopropanation. However, the other regioisomeric 2'-selenide **13a** contributed to almost 40% of all selenide product after the epoxide opening reaction, and should not be ignored in a scale-up synthesis.

To address these problems, we decided to investigate the chemistry of the 2'-selenide **13a** to see if 2'-selenone **20a** can be prepared from 2'-selenide **13a** in a method compatible with the synthesis of its regioisomer **17a**. If the answer was positive, and the **20a** could be proved to function in the Michael-type of cyclopropanation in a similar manner as **17a**, the selenide **12a** and **13a** might be used as a mixture without separation.

Similarly to the reaction with 3'-selenide **12a**, the regioisomeric 2'-selenyl uridine **13a** was treated with methanesulfonyl chloride in dry pyridine at 0°C overnight. As we expected, the reaction afforded the 3'-O-mesylate **18a** in excellent yield. After workup, mesylate **18a** was dried *in vacuo*, and treated with 2.4 equivalents of potassium *tert*-butoxide in *N,N*-dimethylformamide at room temperature for 4 hours to generate the vinyl selenide. Interestingly, the desilylation which had occurred in the previous elimination of mesylate **14a** did not happen in this case under the same reaction condition, and the 5'-silyl protected vinyl selenide **19a** was obtained in quantitative yield. The vinyl selenide **19a** was then subsequently treated with *m*-chloroperbenzoic acid (2.5 equivalents) in dry methanol at room temperature overnight, and the reaction gave corresponding 2'-selenone **20a** in 90% yield after chromatography. The ^{1}H-NMR spectrum of 2'-selenone **20a** is shown in Figure 16, and the transformation sequence from 2'-selenide **13a** to 3'-selenone **20a** is summarized in Scheme 13.

Scheme 13



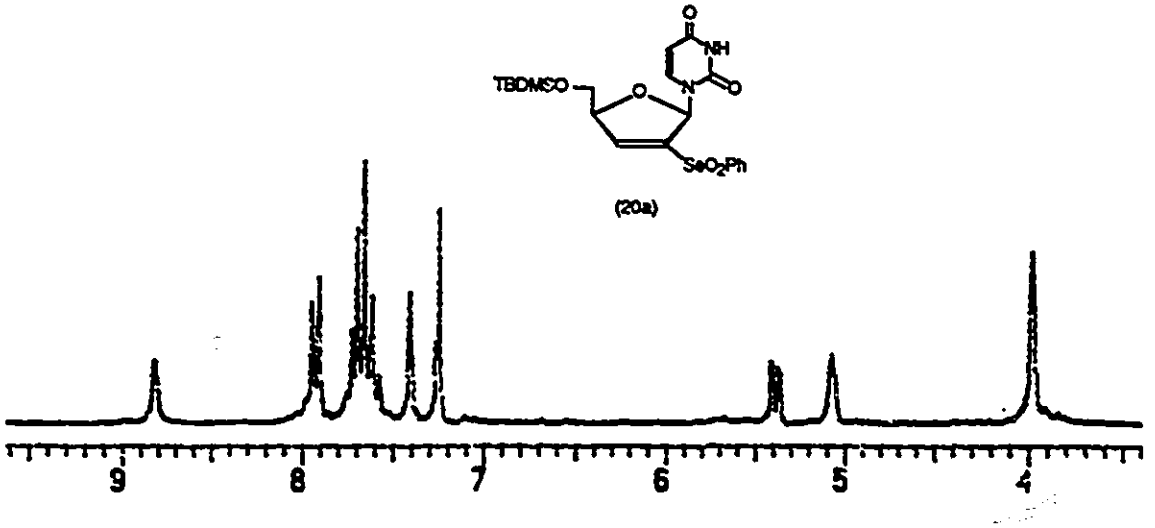


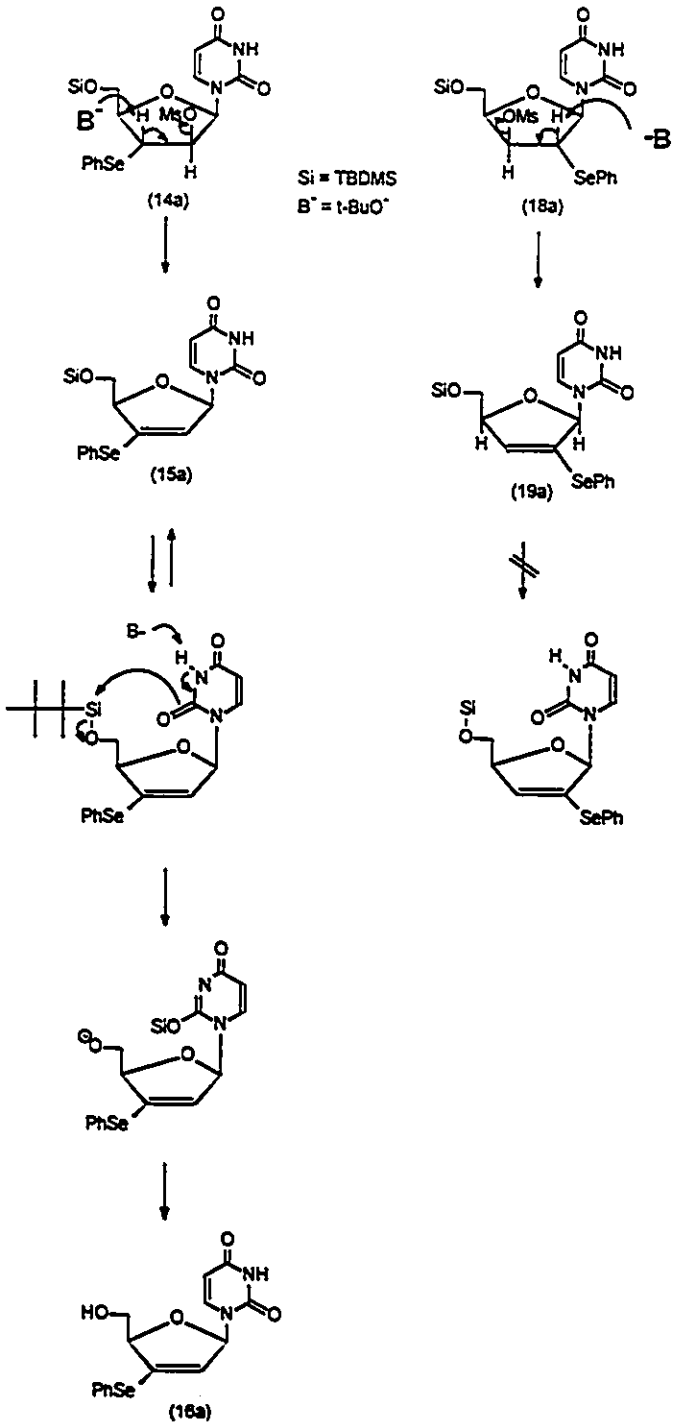
Figure 16. ^{1}H-NMR spectrum of 2'-selenonyl uridine **20a** (200 MHz, $CDCl_3$).

2.4.4. Different Stability of the 5'-Silyl Groups in the Eliminations

Since the regioisomeric mesylates **14a** and **18a** were treated under the same reaction condition, the desilylation occurring during the elimination of mesylate **14a** must result from an intramolecular mechanism. It was speculated that the desilylation of the 3'-selenyl isomer occurred by the participation of the 2-carbonyl oxygen of the uracil after the vinyl selenide **15a** was formed, where the formation of the 2',3'-double bond resulted in a shorter distance between H-1' and H-4'. The molecular mechanics studies[84] showed that uracil of **15a** can rotate freely around the C1'-N bond at room temperature with energy barriers lower than 5 Kcal/mol, thereby allowing an *endo*-2-carbonyl conformation. Such a free rotation of the uracil is prevented in case of olefin **19a** due to the Van der Waals interaction between the uracil and the neighboring 2'-phenyl selenyl group. Molecular mechanics studies of 2'-selenide **19a** using the same method showed a much higher energy barrier (about 15 Kcal/mol) in the interconversion process of the uracil from the energetically favored *exo*-2-carbonyl conformation to the less favored *endo*-2-carbonyl conformation, thereby preventing the 2-carbonyl oxygen from attacking the 5'-silyl group. The mechanism proposed is outlined in Scheme 14.

[84]The molecular mechanics studies were carried out on a Power Macintosh workstation by Costa Yannopoulos in our group, using Oxford's molecular CACHe Scientific software (version 3.7).

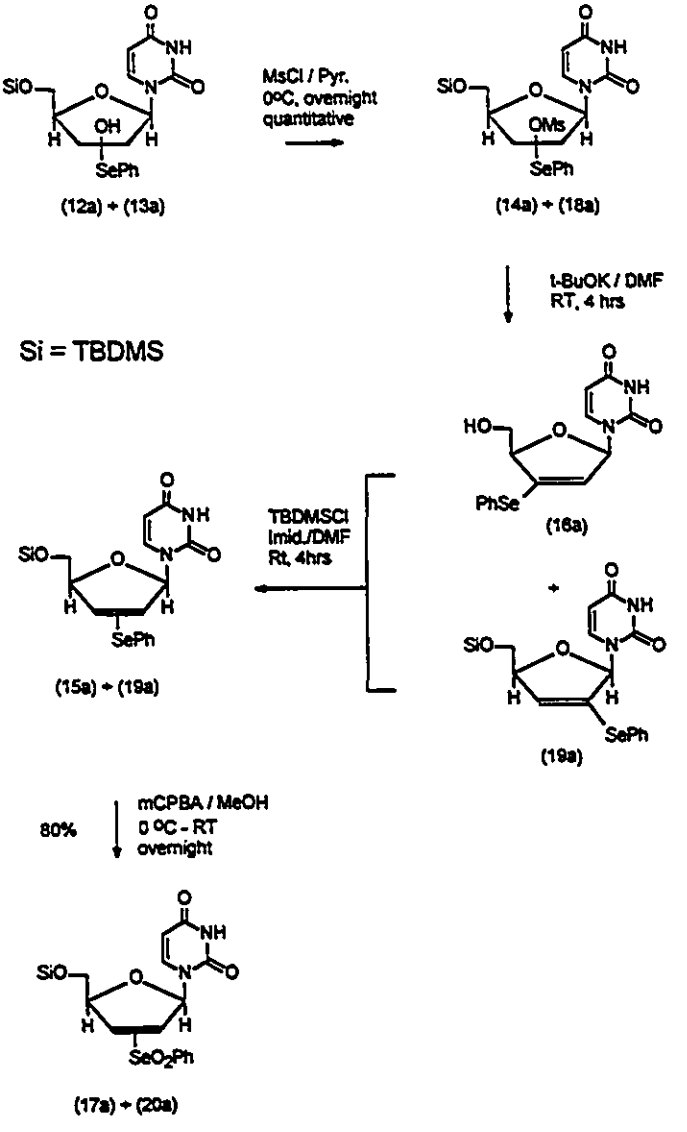
Scheme 14



2.4.5. Preparation of 17a and 20a from a Mixture of 12a and 13a

After our success in preparing both regioisomeric selenones **17a** and **20a** from their corresponding selenides **12a** and **13a**, the preparation of selenones **17a** and **20a** from the mixture of **12a** and **13a** was then attempted. The transformation sequence is outlined in Scheme 15, which is similar to Scheme 12.

Scheme 15

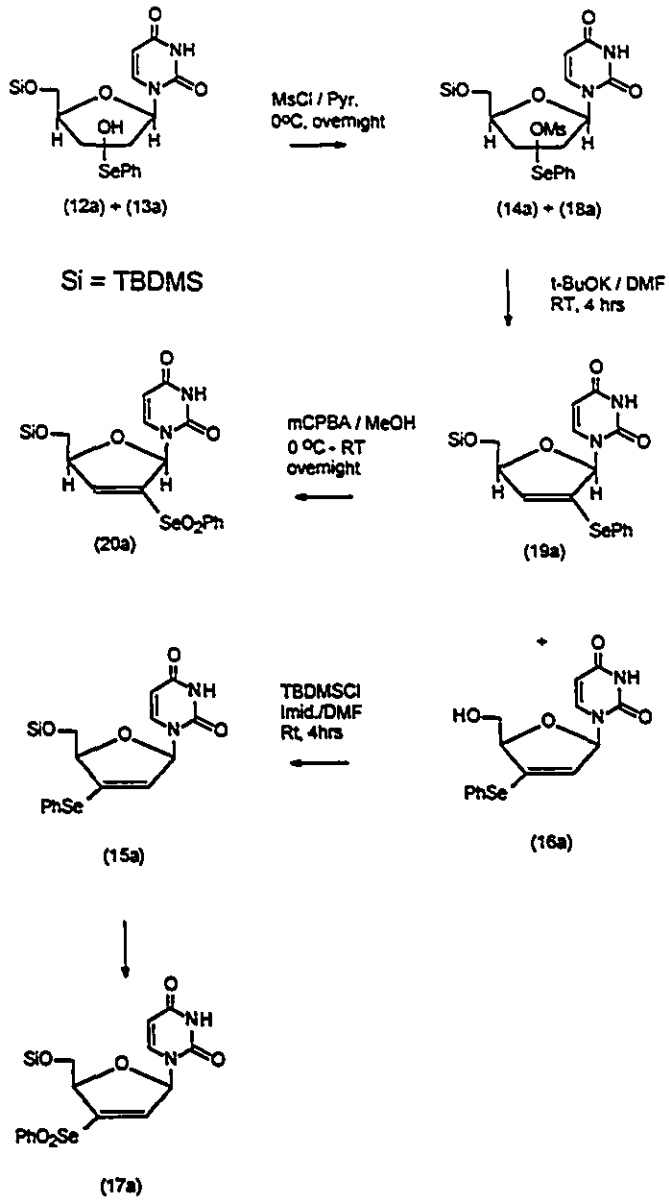


The mixture of **12a** and **13a** obtained from the epoxide opening reaction, was treated with methanesulfonyl chloride in pyridine at 0 °C overnight. The reaction gave a mixture of mesylates **14a** and **18a** with a R_f difference less than 0.1. After workup, the mixture was dissolved in *N,N*-dimethylformamide, and treated with 2.4 equivalents of potassium *t*-butoxide at room temperature for 4 hours. The product mixture was then poured into saturated ammonium chloride, and extracted with ethyl acetate. The extract, which contained a mixture of 5'-hydroxyl-3'-selenide **16a** and 5'-silyl-2'-selenide **19a**, was dried and redissolved in *N,N*-dimethylformamide, followed by the treatment with *tert*-butyldimethylsilyl chloride in the presence of imidazole to provide the 5'-silylated regioisomeric mixture of **15a** and **19a**. The mixture of **15a** and **19a** were then oxidized by *m*-chloroperbenzoic acid to give a mixture of regioisomeric selenones **17a** and **20a**.

It later occurred to us that the problematic separation of the regioisomers **12a** and **13a** could also handled by taking advantage of the different stability of the silyl groups between olefins **16a** and **19a**. The strategy is depicted in Scheme 16.

Instead of separating the selenyl regioisomers **12a** and **13a** after the epoxide opening reaction, the regioisomeric selenides were mesylated as a mixture. It was known that treatment of the mesylates mixture with potassium *t*-butoxide at room temperature for 4 hours resulted in a mixture of 5'-hydroxyl-vinyl-3'-selenide **16a** and 5'-silyl-vinyl-2'-selenide **19a**, which are nonregioisomeric. The R_f difference between **16a** and **19a** is about 0.43, which allowed for an easy separation of these two compounds by chromatography. After separation, 3'-selenide **16a** was then resilylated, followed by oxidation with *m*-chloroperbenzoic acid in methanol to give 3'-selenone **17a**. In another experiment, similar oxidation of 2'-selenide **19a** gave the corresponding 2'-selenone **20a**.

Scheme 16



2.4.6. Preparation of Vinyl Selenone 17b

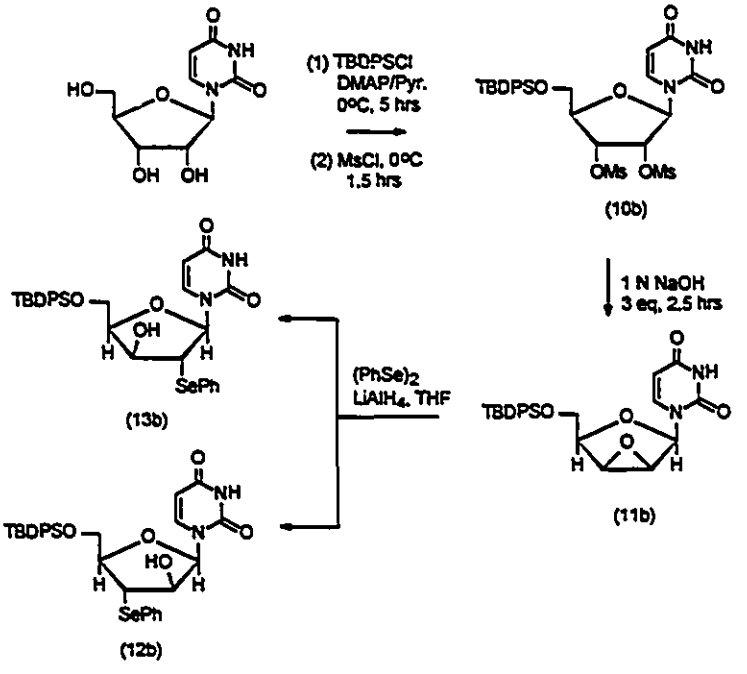
A similar synthetic sequence was also carried out to prepare the vinyl selenone substrate, where a 5'- *tert*-butyldiphenylsilyl protecting group was used. The *tert*-butyldiphenylsilyl protecting group is more acid-resistant[76], and should be more favorable

in our later handling of the acid-funtionalized analogs.

Preparation of Selenides 12b and 13b

The synthesis of the 5'-*tert*-butyldiphenylsilyl protected epoxide **11b** from uridine was similar to the synthesis of 5'-*tert*-butyldimethylsilyl protected analog **11a** as previously described. A longer silylation time (4-5 hours) was needed with *tert*-butyldiphenylsilyl chloride than with *tert*-butyldimethylsilyl chloride, due to the less reactive nature of the former. Epoxide **11b** was then treated with phenylselenium anion, from which a mixture of regioisomeric alcohols **12b** and **13b** were produced. These regioisomers were separated by chromatography to give the major isomer **12b** in 57% yield and the minor isomer **13b** in 30% yield. The reaction sequence is summarized in Scheme 17.

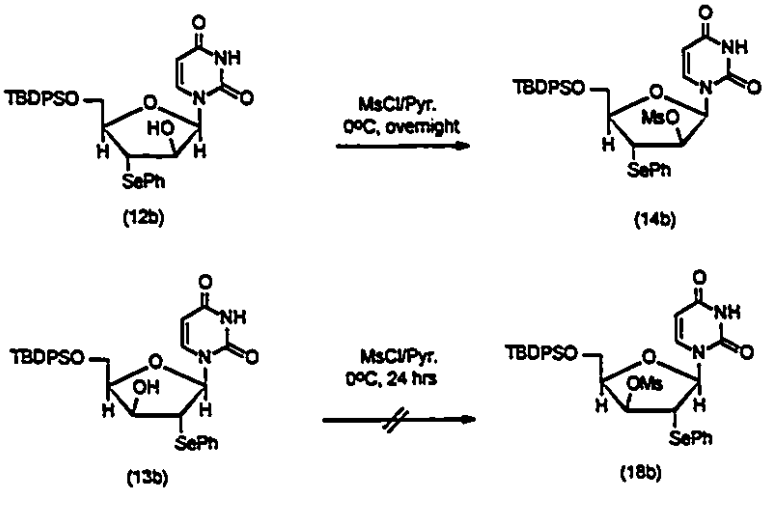
Scheme 17



Mesylation of 12b and 13b

In two parallel experiments, the regioisomeric alcohols **12b** and **13b** were treated with methanesulfonyl chloride in pyridine at 0 °C. The mesylation of the 2'-β-hydroxyl group of **12b** proceeded smoothly, and gave mesylate **14b** in a clean reaction overnight. However, a similar mesylation of the 3'-β-hydroxyl of **13b** proved unsuccessful, and no mesylate **18b** was obtained under these reaction conditions even after 24 hours (Scheme 18). The starting material **13b** was fully recovered after the treatment. A modeling study showed that the 3'-β-hydroxyl group in **13b** was highly shielded by the neighboring 5'-*tert*-butyldiphenylsilyl group, and might be too hindered to be mesylated. The mesylation of the 3'-β-hydroxyl group of 2'-selenyl uridine was not described in Wu and Chattopadhyaya's report where a 5'-monomethoxytrityl protecting group was used.

Scheme 18

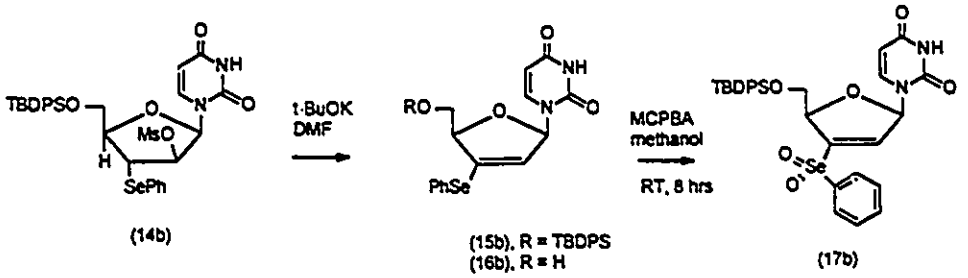


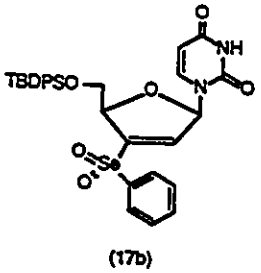
Converting Mesylate 14b to Vinyl Selenone 17b

Mesylate **14b** from the previous step was then treated with potassium *t*-butoxide (2.4 equivalents) at room temperature for four hours. The 5'-*tert*-butyldiphenylsilyl group was still not base-resistant enough to withstand these reaction conditions, and the elimination reaction gave a mixture of 5'-silylated vinyl selenide **15b** and the 5'-desilylated vinyl selenide **16b** in a ratio of about 1:1. Since the desilylation was thought to be caused by the participation of the 2-carbonyl oxygen of the uracil in an intramolecular manner, the *N,N*-dimethylformamide solvent was reduced to a minimum level to increase the concentration of the potassium *tert*-butoxide, thereby promoting the intermolecular elimination. It was found[80] that by lowering the reaction temperature to 0°C, the elimination reaction could be completed in 7 minutes, while no significant amount of desilylation product was observed. The product mixture was then poured into the saturated ammonium chloride solution. After workup and purification by chromatography, the 5'-silylated vinyl selenide **15b** was obtained in about 90% yield. Subsequent oxidation of selenide **15b** with *m*-chloroperbenzoic acid in methanol (8 hours, room temperature) gave 3'-selenonyl uridine **17b** in 85% yield (Scheme 19). The ^{1}H-NMR data of **17b** (spectrum see Figure 17) were quite similar to those reported by Wu *et al* for the similar 3'-selenonyl uridine except for the peaks due to the 5' protecting group. The peak for H-5 of **17b** appeared at δ 4.62 ppm as a doublet with $J_{5,6}$ = 8.1 Hz, which was significantly upshifted as compared to its counterpart in uridine or the *tert*-butyldimethylsilyl protected analog **17a** (δ 5.67 ppm). The upshift of H-5 signal was probably due to the influence of the neighboring benzene ring of the 5'-protecting group, and such an upshift was also observed in Wu and Chattopadhyaya's compound, where a 5'-monomethoxytrityl protecting group was used.

[80]These reaction conditions were first worked out by Costa Yannopoulus in our group, who shared the 3'-selenonyl uridine **17b**.

Scheme 19





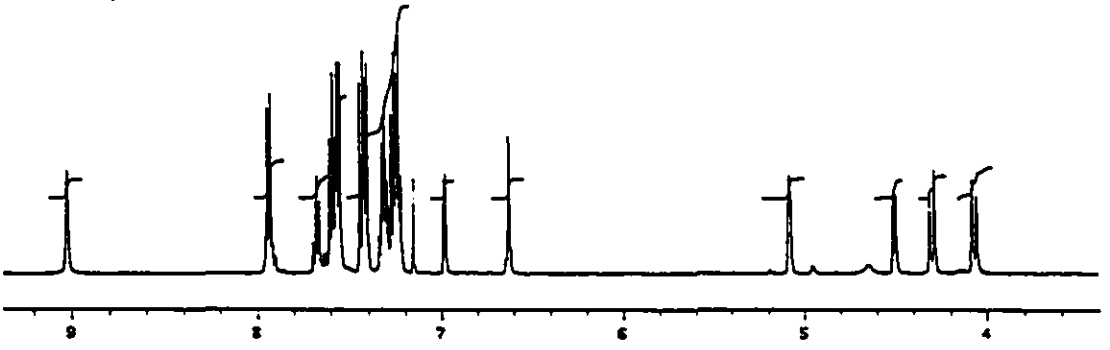


Figure 17. ^{1}H-NMR spectrum of 5'-*tert*-butyldiphenylsilyl 3'-selenonyl uridine analog **17b** (500 MHz, $CDCl_3$).

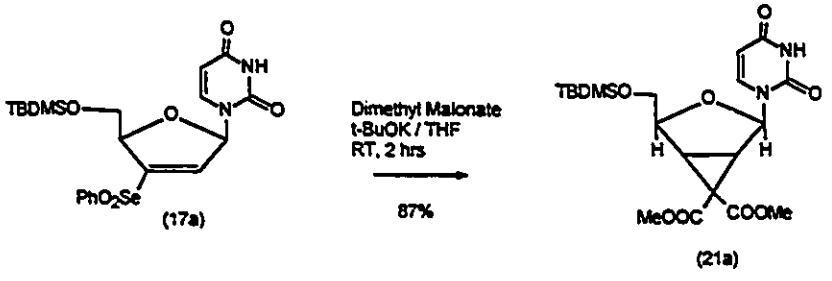
2.5. Preparation of Cyclopropyl Diacid 24 and Decarboxylation

2.5.1. Cyclopropanation of 3'-Selenone 17a with Dimethyl Malonate

To allow a comparison of our experimental results to those reported by Wu and Chattopadhyaya in their initial investigation[72], 3'-selenonyl uridine **17a** was first used as the vinyl selenone substrate to carry out the Michael-type cyclopropanation with dimethyl malonate.

The Wu and Chattopadhyaya's procedure[72] was modified in our application by using only 4 equivalents of dimethyl malonate as the reactant, and by using tetrahydrofuran as the solvent. In this experiment, the anion of malonate was readily generated in a homogenous solution by treating dimethyl malonate (4 equivalents) in dry tetrahydrofuran with potassium *tert*-butoxide (4 equivalents) at room temperature for 30 minutes. The vinyl selenone **17a** was then added to the tetrahydrofuran solution to react with the malonate anion. The cyclopropanation was completed in two hours, and cyclopropane **21a** was obtained in 87 % yield as a white foam after chromatography (Scheme 20).

Scheme 20



In the ^{1}H-NMR spectrum of cyclopropane **21a**, the cyclopropane-fused H-2' and

H-3' protons were present as an AB system at δ 2.82 ppm and 2.56 ppm. The anomeric proton (H-1') of **21a** appeared at δ 6.06 ppm as a sharp singlet, and no coupling between H-1' and H-2' was observed. This signal pattern of H-1' suggested a 90° dihedral angle between H-1' and H-2', which was significantly different from its counterparts in other uridine analogs without the bicyclic-[3.1.0.] structure. The NMR data of **21a** are virtually identical to the data as described for a similar structure by Wu *et al*[72] except for the peaks due to the 5'-protecting group. The ^{1}H NMR spectrum of cyclopropane **21a** is shown in Figure 18.

The sharp singlet of the anomeric proton around δ 6 ppm and the AB system for the cyclopropanated H-2' and H-3' protons located between δ 2 ppm to 3 ppm in ^{1}H-NMR spectrum are very characteristic for the 2',3'-cyclopropane-fused nucleoside analogs, and proved to be convenient characterization tools for the formation of such a bicyclic-[3.1.0] structure as well as the diastereomeric purity of the product.

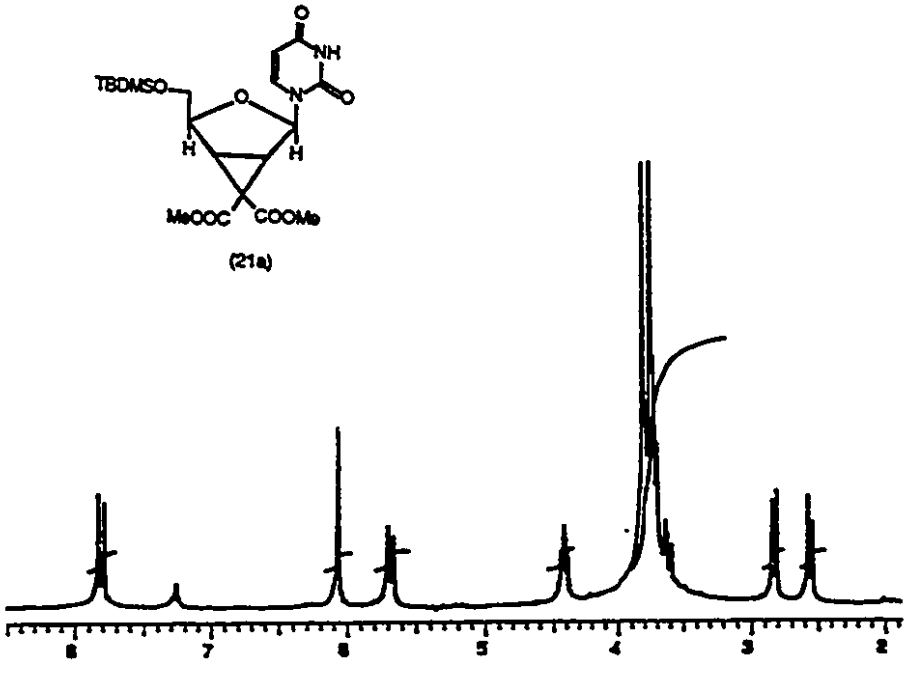
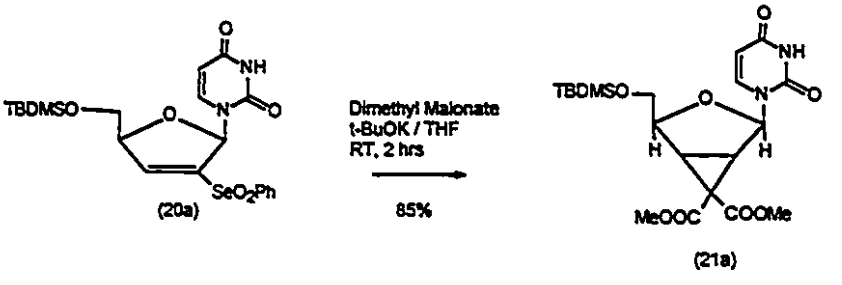


Figure 18. ^{1}H-NMR spectrum of malonated cyclopropane **21a** (200 MHz, $CDCl_3$).

2.5.2. Cyclopropanation of 2'-Selenone 20a with Dimethyl Malonate

The reaction of 2'-selenonyl uridine **20a** with dimethyl malonate was then investigated. Using a similar protocol, 2'-selenone **20a** was treated with dimethyl malonate anion in dry tetrahydrofuran. As expected, all 2'-selenone **20a** was consumed in 2 hours, and the reaction gave the same cyclopropane **21a** in about 85% yield (Scheme 21). This result indicated that both regioisomeric selenones **17a** and **20a** functioned in a similar manner in the Michael-type of cyclopropanation with dimethyl malonate .

Scheme 21



2.5.3. Ester Cleavage Attempts

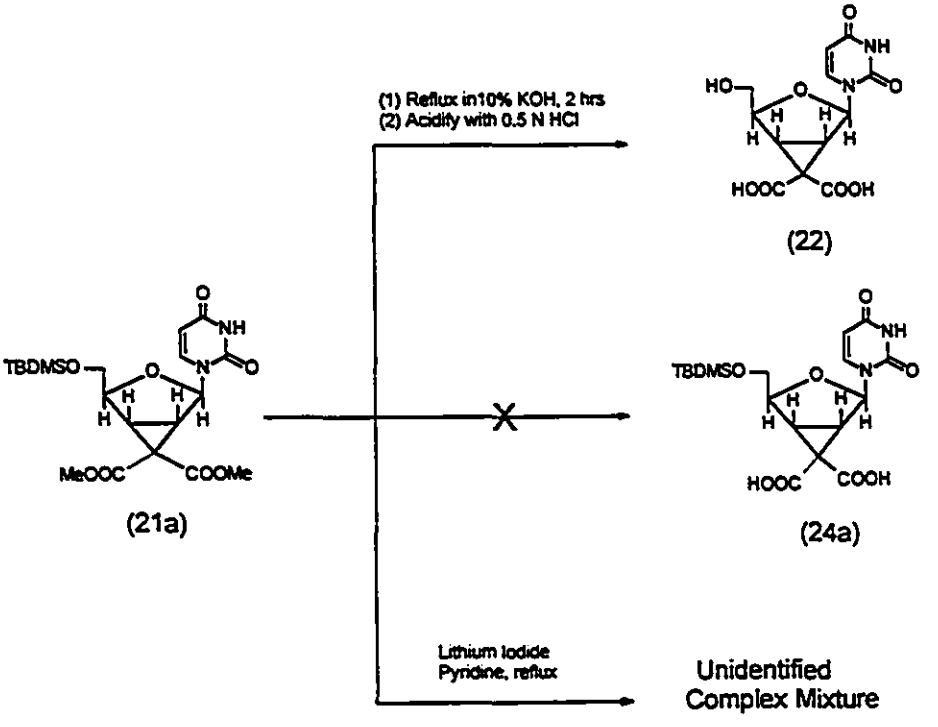
With the cyclopropanated malonate **21a** in hand, we then attempted the cleavage of the methyl ester to prepare the diacid for the decarboxylation. Using standard saponification conditions, **21a** was refluxed in 10% aqueous potassium hydroxide solution for 2 hours, followed by acidification of the solution to pH 2. Although the saponification of the malonate ester was successful, the 5'-silyl protecting group was also removed under this treatment, and the product was found to be the desilylated diacid **22** instead of the desired diacid **24a**. Because the silane odor was detected during the reflux with potassium hydroxide, it was believed that the 5'-silyl protecting group was removed by the hydroxyl anion, albeit with the possibility that the desilylation also occurred

during the acidification. The *t*-butyldimethylsilyl ethers are only stable in solution with a pH above 4[76]. A selective reprotection of the 5'-hydroxyl group in a molecule like diacid 22 turned out to be difficult, therefore the saponification approach was abandoned.

In another experiment, cyclopropane **21a** was treated with anhydrous lithium iodide in refluxing pyridine[81]. A complex mixture was observed after a few hours of refluxing. All efforts to separate the product mixture for characterization proved unsuccessful, and no clean acid derivatives were obtained. This approach was also considered impractical.

Since no other better methods occurred us to selectively cleave the methyl ester and allow for an easy isolation of the acid derivatives, we felt that it might be easier to look for other malonate substrates for the cyclopropanation, where the ester could be selectively cleaved by a mild and convenient method without touching the silyl ether.

Scheme 22



[81]Elsinger, F.; Schreiber, J.; Eschenmoser, A., *Helv. Chimi. Acta.*, 43, 113 (1960)

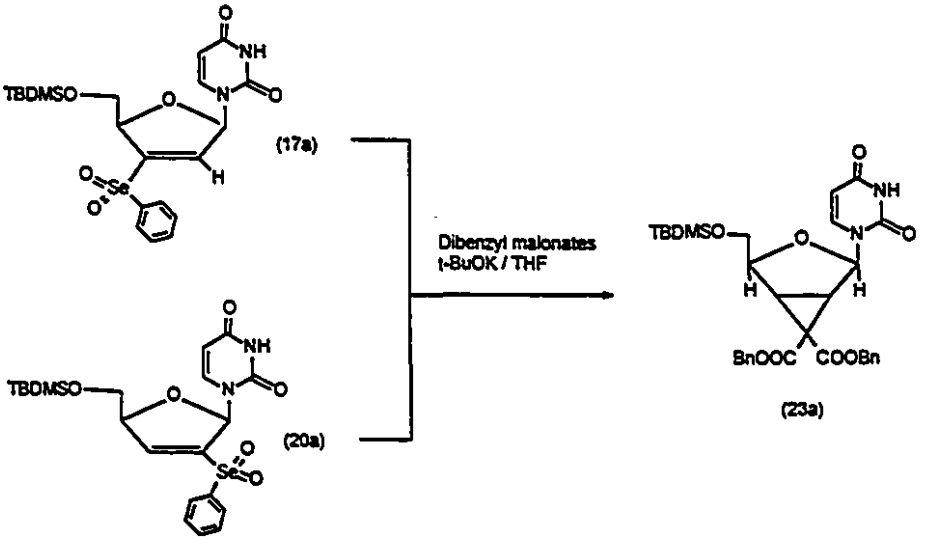
2.5.4. Synthesis of Cyclopropanated Diacids 24a

Silyl ethers are generally resistant to the conditions of hydrogenation over 10% palladium on carbon[76], which is normally used to cleave benzyl esters[76]. We therefore carried out the addition of dibenzyl malonate to the selenone **17a**, and then investigated the hydrogenation of the cyclopropane **23a**.

Preparation of Cyclopropane 23a

Following a procedure similar to the previous synthesis of methyl analog **21a**, cyclopropane **23a** was successfully prepared by reaction of 3'-selenone **17a** with the dibenzyl malonate anion in dry tetrahydrofuran in 85% yield after chromatography. The same cyclopropane **23a** was also obtained by reaction of the other regioisomeric selenone **20a** with the dibenzyl malonate anion in a similar yield (Scheme 23). The ^{1}H-NMR spectrum of **23a** was quite similar to that of **21a** except for the peaks due to the benzyl ester. The anomeric proton of H-1' appeared as a sharp singlet at δ 6.15 ppm, while the H-2' and H-3' protons appeared as a pair of doublets (AB, J = 6.8 Hz) at δ 2.83 ppm and δ 2.61 ppm.

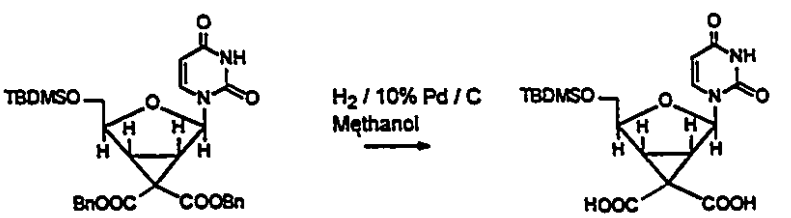
Scheme 23



Hydrogenation of 23a

The cyclopropanated dibenzyl malonate **23a** obtained above was then hydrogenated in dry methanol over 10% palladium on carbon. TLC analysis indicated that all starting material **23a** was converted to one product with high polarity in 4 hours. After filtering off the palladium/Carbon catalyst, the methanol filtrate was evaporated to dryness to give a white solid as diacid **24a** with reasonable purity. In the ^{1}H-NMR spectrum of diacid **24a**, the complete cleavage of the benzyl ester was clearly evidenced by the absence of the peaks due to the benzyl ester, and the *tert*-butyldimethylsilyl protecting group remained in the molecule. The anomeric proton of **24a** appeared as a sharp singlet at δ 6.42 ppm, while the H-2' and H-3' protons appeared as an AB quartet centered at δ 2.67. The formation of diacid **24a** was also confirmed by mass spectrometry (FAB, NBA), in which the pseudomolecular ions $[M + Na]^+$ and $[M -1 + 2Na]^+$ of **24a** were observed at 449 and 471 respectively.

Scheme 24

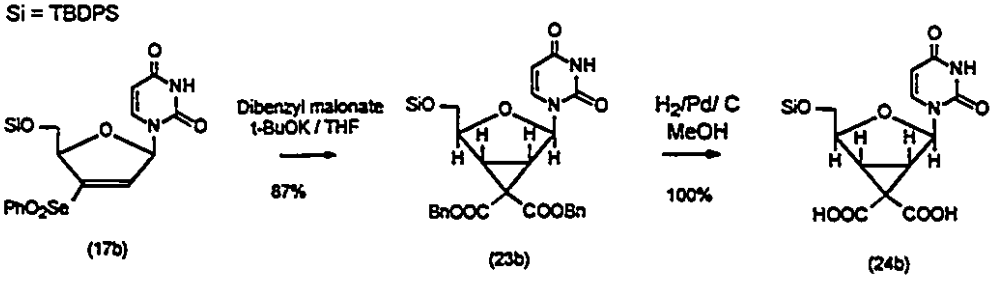


2.5.5. Preparation of Diacid 24b

With the supply of the 5'-*tert*-butyldiphenylsilyl protected 3'-selenone **17b**, cyclopropane **23b** was prepared as described for 23a. The resulting cyclopropanated dibenzyl malonate **23b** was then hydrogenated over 10% palladium on carbon in methanol to yield the cyclopropyl diacid derivative **24b** in a clean reaction. In the ^{1}H-

NMR spectrum of diacid **24b** (Figure 19), the anomeric proton appeared at δ 6.25 ppm as a sharp singlet. The H-2' and H-3' protons appeared as an AB quartet centered at δ 2.69 ppm. The complete removal of the benzyl ester was clearly evidenced by the absence of the peaks due to benzyl ester in the spectrum of **24b**. The structure of diacid **24b** was also confirmed by ^{13}C-NMR as well as mass spectrometry (FAB), in which the pseudomolecular ion $[M+1]^+$and $[M+Na^+]$ were observed at 551 and 573, respectively.

Scheme 25



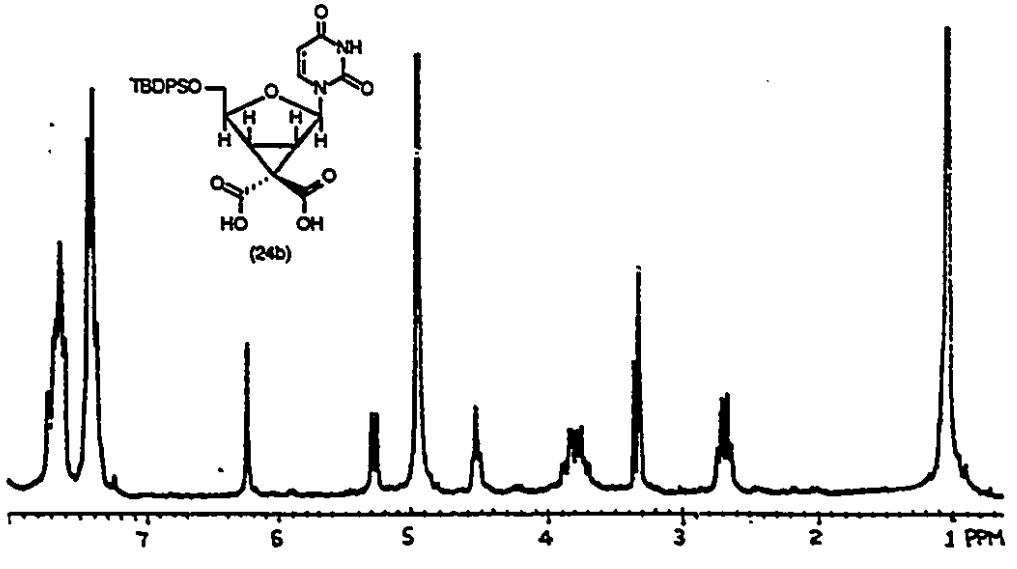


Figure 19. ^{1}H-NMR spectrum of diacid **24b** (200 MHz, CD_3OD).

2.5.6. Decarboxylation Attempts

The cyclopropyl diacids **24a** and **24b** were first refluxed in alkanols (e.g. ethanol, n-butanol [82]) for more than 24 hours. However, under these conditions no decarboxylation took place, and the cyclopropyl diacids **24a** and **24b** were fully recovered.

Because the 5'-*tert*-butyldimethylsilyl protecting group of **24a** was unstable in solution under pH 2[76], the acid-catalyzed thermolysis was then carried out with the 5'-*tert*-butyldiphenylsilyl protected **24b**. After refluxing cyclopropyl diacid **24b** in ethyl acetate in the presence of formic acid[73, 74] or acetic acid (5 - 10 equivalents) for 24 hours, all volatile solvents were removed. Although the ^{1}H-NMR spectrum of the residue was slightly different from the spectrum of the starting material, methylating the residue with diazomethane gave cyclopropyl malonate **21b**, which was the same as that obtained from the reaction of selenone **17b** with dimethyl malonate. No decarboxylated derivative was detected (Scheme 26). Diacid **24b** was then refluxed in glacial acetic acid at 117°C under much more forcing condition. Unfortunately, the molecule slowly decomposed into a mixture of sugar moieties and uracil, and was totally consumed in 20 hours. Methylation of the residue did not give any cyclopropanated nucleoside analogs.

Base-catalyzed thermolysis[82] was also attempted. Disappointingly, no decarboxylation took place after refluxing diacid **24b** in pyridine or heating **24b** in quinoline. In case of heating diacid **24b** in quinoline above 150°C, the diacid **24b** was slowly degraded into a complex mixture in a few hours.

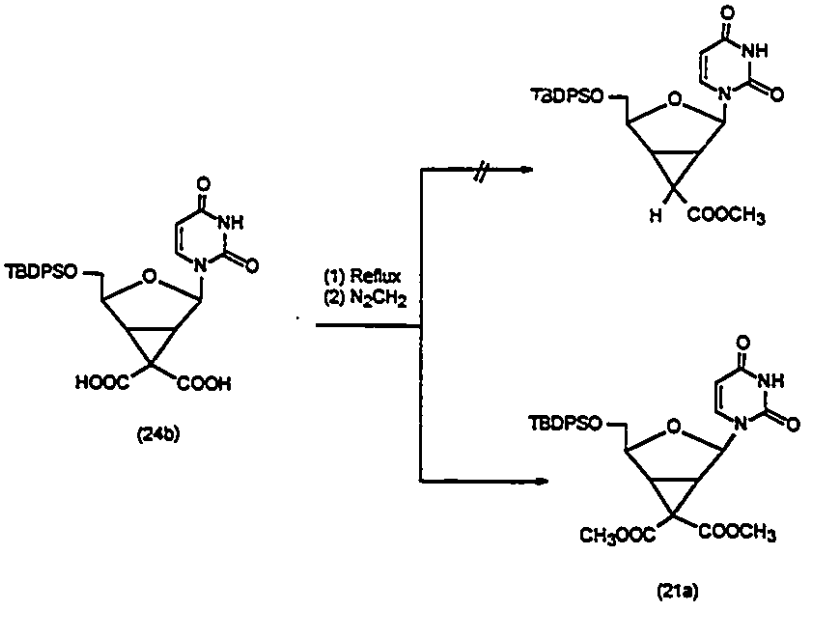
In conclusion, various efforts to decarboxylate the cyclopropyl diacids **24a** and **24b** proved unsuccessful. Compared to the easy decarboxylation of other malonic acids, the difficulty of decarboxylating the cyclopropanated malonic acid was presumably due

---

[82]Clark, L.W., In *Chem. Carboxylic Acids Esters*, Patai, S., Eds., Intersci. Publ., New York, N.Y., 589 (1967)

to the high energy needed to convert the cyclopropanated diacid to its highly strained cyclopropanated enol or enolate intermediate. The reluctance to form such cyclopropanated enols was also suggested by comparing the transformations of the cyclopropanated Meldrum's acid[83] with that of the monocyclic Meldrum's acid. Therefore the decarboxylation approach had to be abandoned.

Scheme 26

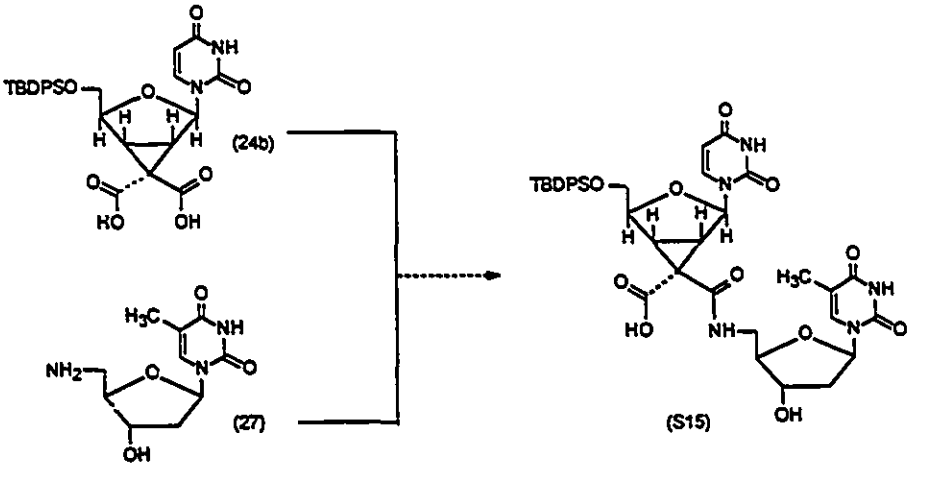


[83]Izquierdo, M.L., Arenal, I., Bernabe, M., Alvarez, E.F., *Tetrahedron*, 41, 215 (1985)

2.6. Coupling of Diacid 24b and Amine 27

After our lack of success in decarboxylating diacid **24b**, a direct coupling of **24b** with the 3'-end building block **27** was then attempted before giving up the diacid approach. It was hoped that the difference in steric hindrance between the *exo*- and *endo*-carboxylic acid groups of **24b** might allow for a selective or at least dominant coupling of the *exo*-carboxylic acid with amine **27** to give an acid-functionalized dimer of type **S15** with the desired cyclopropyl amide linker (Scheme 27). Dimer **S15** could be used as an analog to our designed dimer for the preliminary investigations.

Scheme 27



2.6.1. Preparation of Amines 27 and 28

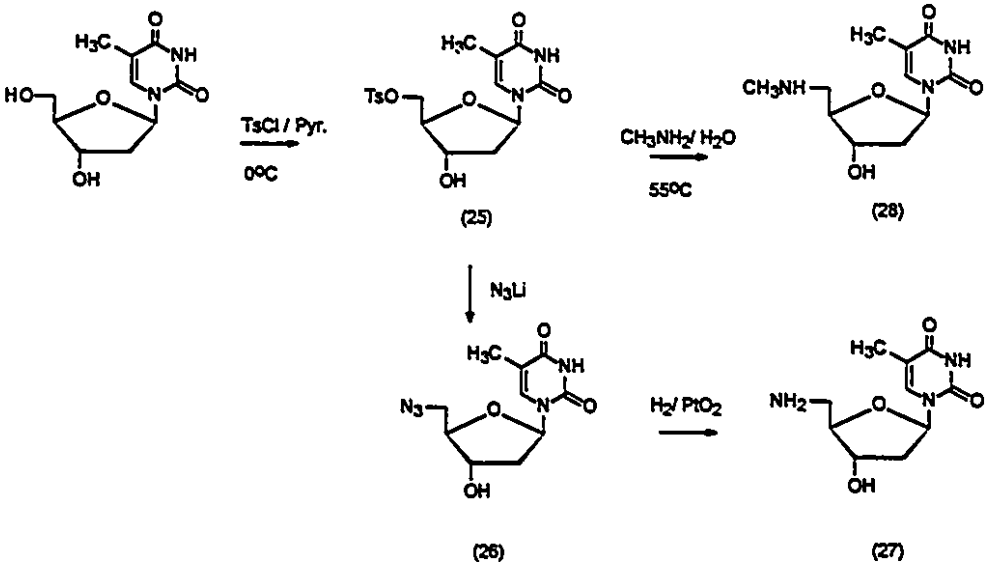
The amines **27** and **28**, which would serve as 3'-end building blocks in the dinucleoside synthesis, were prepared as outlined in Scheme 28.

Following a literature procedure[56], reaction of thymidine with *p*-toluenesulfonyl chloride in pyridine at room temperature readily gave crystalline tosylate **25**. After

treating **25** with lithium azide in dimethylformamide at 100°C for 2 hours, the 5'-tosyl group of **25** was replaced by an azido group to yield azide **26** quantitatively. Hydrogenolysis of the resulting azide **26** over platinum dioxide in ethanol then afforded the 5'-aminothymidine **27** in a clean reaction.

In another experiment, tosylate **25** was heated in aqueous methylamine at 55°C for 2 hours to give the methylamine **28** in quantitative yield, following a protocol developed by Idziak in our group.

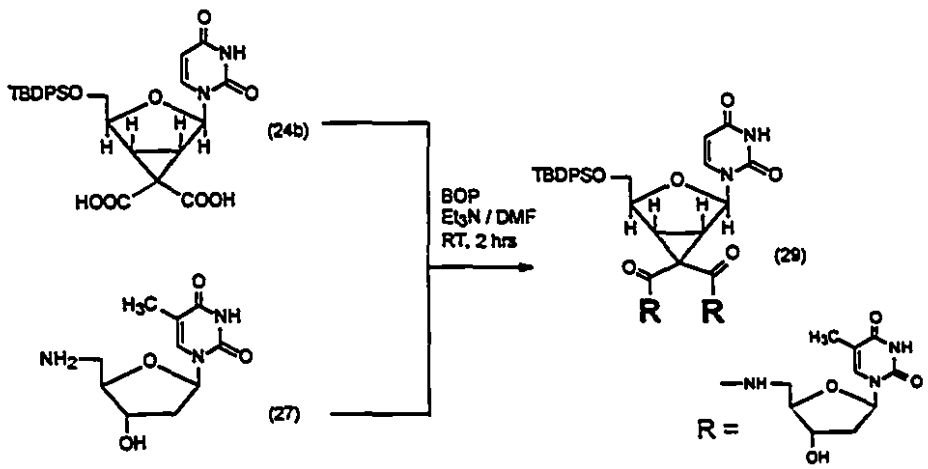
Scheme 28



2.6.2. Reaction of Diacid 24b with Amine 27

Using a standard peptide coupling methodology[45], cyclopropyl diacid **24b** was dissolved in *N,N*-dimethylformamide, and treated with triethyl amine (3 equivalents). BOP (1 equivalent)[84] was then added as the coupling reagent. After stirring the mixture for 15 minutes, amine **27** (1 equivalent) was added slowly. All amine **27** was consumed in 2 hours, and the reaction gave a dominant product which, disappointingly, was found to be the branched trinucleoside derivative **29** (Scheme 29). No desired dinucleoside analog of type **S16** was detected. The structure of the trimer **29** was confirmed by detailed analysis of ^{1}H-NMR as well as mass spectrometry (FAB), in which the pseudomolecular ion of **29** ($[M + Na]^+$) was observed at 1019. It was suggested from the above reaction that the reactivity of the *exo*-acid group and the *endo*-acid group in diacid **24b** were very similar in the amide formation under the above reaction conditions.

Scheme 29



[84]Castro, B., Dormoy, J.R., Evin, R., Selve, C., *Tertahedron Lett.*, 1219 (1975)

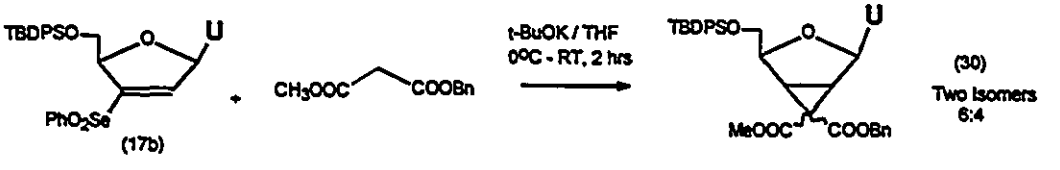
2.7. Preparation of Substituted Cyclopropyl Acid

Due to the lack of success in the cyclopropyl diacid approach, other suitable nucleophilic substrates for the Michael-type cyclopropanation were then investigated. To simplify our effort in the initial attempt, we first looked for molecules like XCH_2COOR, where **X** was a carbanion stabilizing group. The **X** substituent was expected to be carried through the synthesis of the dimer, so we did not need to worry about the removal of **X**. The dinucleoside analog containing a **X** substituted amide linker would then serve as an analog to our targeted dimer **S1** for a preliminary binding study.

2.7.1. Cyclopropanation of Selenone 17b with Benzyl Methyl Malonate

Based on the fact that the benzyl group in benzyl methyl malonate can be selectively removed by hydrogenation, cyclopropanation with the commercially available benzylmethyl malonate was first investigated.

Scheme 30



Using the same protocol as previously described for the Michael-type cyclopropanation, reaction of selenone **17b** with benzyl methyl malonate anion proceeded smoothly, and yielded cyclopropane **30** as one spot by TLC. However, ^{1}H-NMR analysis of the cyclopropane **30** indicated that it was present as a mixture of diasterecmers (Scheme 30). The ^{1}H-NMR spectrum of cyclopropane **30** clearly showed two sharp

singlets for H-1' proton at δ 6.08 ppm and 6.09 ppm, respectively, in a ratio of 6: 4. The cyclopropane-fused H-2' and H-3' protons were also observed as two sets of overlapping AB quartets centered at δ 2.75 ppm and 2.59 ppm. Unfortunately all efforts to separate the diastereomers proved difficult. Therefore the malonate approach was abandoned.

2.7.2. Synthesis of Cyano Substituted Cyclopropane 32

Next, we investigated the reaction of alkyl cyanoacetates with selenone **17b**. The pK_a of cyanoacetate is similar to that of malonate, and an anion of cyanoacetate is also a good soft nucleophile. It was expected that the cyanoacetate anion would react with vinyl selenone **17b** in a manner similar to the malonate anion. Although it would have been difficult to replace the cyano group with a hydrogen atom in the resulting cyclopropane, we felt that the reasonably inert nature of the cyano substitute might enable it to withstand the later transformation conditions for the dimer synthesis.

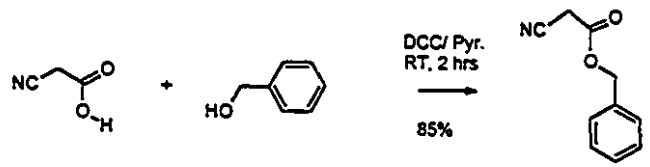
Synthesis of Benzyl Cyanoacetate 31

The selection of benzyl 2-cyanoacetate **31** as the nucleophilic substrate for the Michael-type cyclopropanation was based on our previous experience of obtaining the cyclopropyl acid from its benzyl ester precursor through a mild and convenient hydrogenation. The cyano group should be stable towards the hydrogenation conditions over 10% Pd / C. In addition, use of the benzyl substitution could maximize the difference of the steric bulk between cyano group and the ester group, and might result in a diastereoselective reaction.

Benzyl cyanoacetate **31** was prepared by coupling of benzyl alcohol (2 equivalents) and cyanoacetic acid (1 equivalents) in pyridine, using 1,3-dicyclohexylcarbodiimimide (DCC) as the coupling reagent (Scheme 31). Excess benzyl alcohol was used in the reaction to make sure that all the cyanoacetic acid was consumed.

Due to the high boiling point of benzyl cyanoacetate, it was difficult to purify it by distillation even under reduced pressure. After workup, the crude **31** was therefore flashed through a short column of silica gel to provide pure benzyl 2-cyanoacetate as a colorless liquid.

Scheme 31



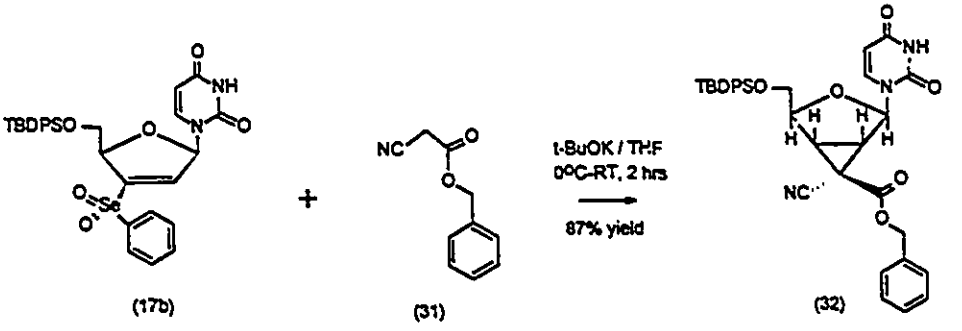
(31)

Cyclopropanation

Using the same protocol as previously described for the cyclopropanation with malonates, benzyl 2-cyanoacetate **31** (5 equivalents) was dissolved in dry tetrahydrofuran at room temperature, and treated with potassium *tert*-butoxide (4 equivalents) to generate the anion *in situ*. Reaction with selenone **17b** was completed after two hours, and only one UV active product was found in the mixture with the polarity matching the expected cyclopropane product. The mixture was chromatographed on silica gel to afford cyclopropane **32** in 84% yield (Scheme 32). Interestingly, the ^{1}H-NMR analysis of this product showed that cyclopropane **32** was present as a diastereomerically pure compound. In the ^{1}H-NMR spectrum (Figure 20) of the cyclopropane **32**, only one sharp singlet for one proton was observed at δ 6.04 ppm for the anomeric proton (H-1') , and the H-2' and H-3' protons appeared as a pair of doublets (AB character) at δ 3.04 ppm and 2.79 ppm, respectively, with an AB coupling constant of 7.0 Hz. Careful examination of all other components from the chromatography revealed no sign of the other diastereoisomer of **32**. Therefore it was concluded that the cyclopropanation of vinyl selenone **17b** with benzyl cyanoacetate **32** was stereoselective. The significant difference in steric hindrance between the cyano group and the benzyl carboxylate group was

thought to be the controlling factor in the cyclopropanation (general mechanism see Scheme 7). Because the *exo*-face of the nucleoside was considered to be less hindered, the more bulky benzyl carboxylate should be oriented in an *exo*-configuration in the cyclopropane product to minimize the steric repulsion. As expected, the configuration of cyclopropane **32** was later established as having an *exo*-carboxylate.

Scheme 32



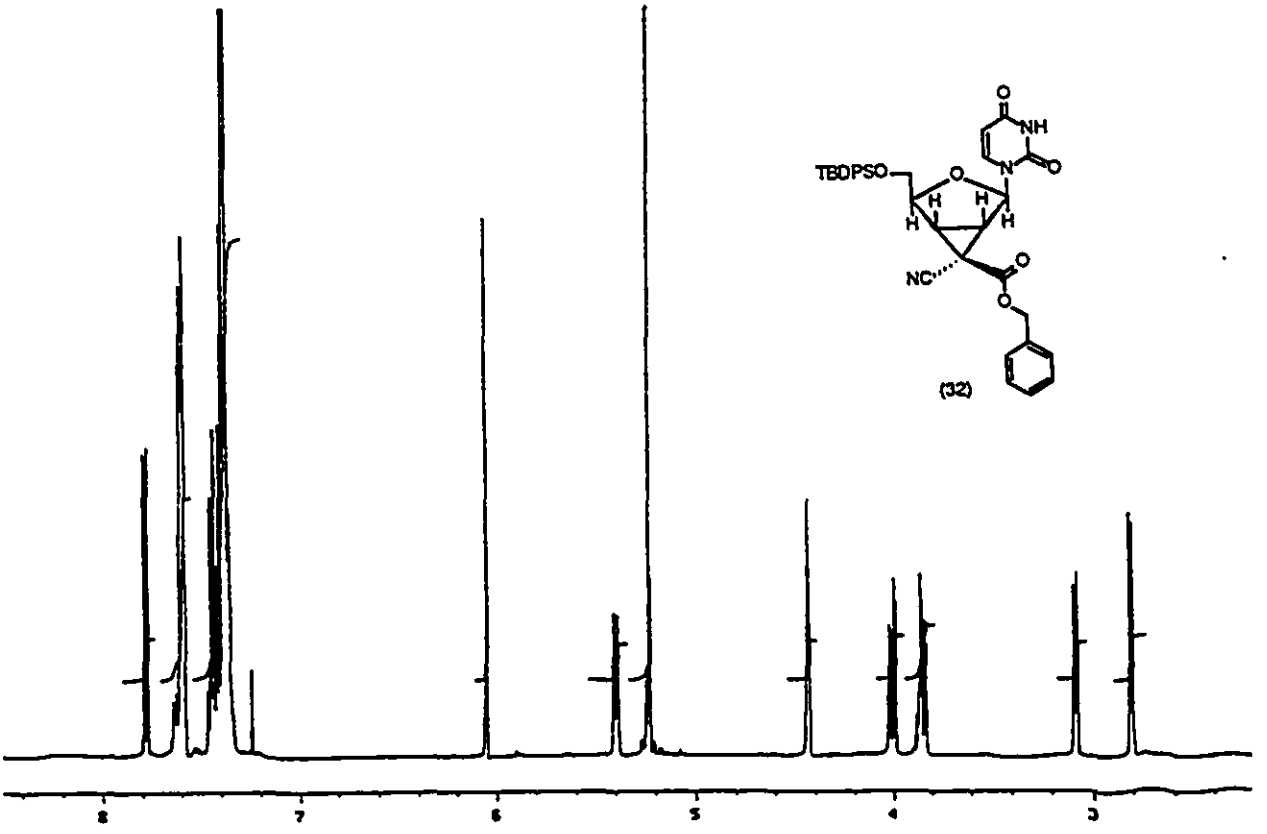
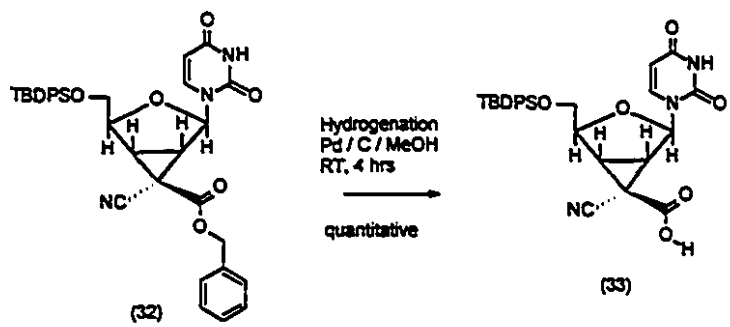


Figure 20. ^{1}H-NMR spectrum of cyano substituted cyclopropane **32** (500 MHz, $CDCl_3$).

2.7.3. Synthesis of Cyclopropyl Acid 33 as the 5'-End Building Unit

Benzyl ester **32** obtained above was subsequently hydrogenated over 10% palladium on carbon in dry methanol for 4 hours to yield acid **33** in a clean reaction (Scheme 33). After filtering off the catalyst, all the volatile materials in the methanol filtrate were removed to give acid **33** as a pure compound. In the ^{1}H-NMR spectrum of acid **33**, the sharp singlet of the anomeric proton appeared at δ 6.07 ppm, and the peaks for the H-2' and H-3' protons were observed as an AB quartet centered at δ 2.99 ppm. The structure of acid **33** was also confirmed by detailed analysis of its ^{13}C-NMR spectrum as well as by mass spectrometry, in which the pseudomolecular ion [M + H^{+}] was observed at 532 (FAB, LRMS) and the pseudomolecular ion [M + Na^{+}] was observed at 554.17217 (FAB, HRMS).

Scheme 33



2.7.4. Determination of the Configuration of Acid 33

In order to determine the configuration of the carboxyl group (*exo* or *endo*) in acid **33** by proton *N*uclear *O*verhauser *E*ffect (NOE) NMR, it was necessary to transform acid **33** to its alcohol derivative **34**. This allowed the replacement of the carbonyl oxygen in acid **33** with two hydrogens H_a and H_b in the resulting alcohol derivative **34**. If the

carboxyl group in acid **33** is in an *exo* configuration, the resulting alcohol group in **28** must be in an *exo* configuration also as shown in type **A** (Figure 21). In this case, the distances between the two newly generated protons (H_a and H_b) and H-1' or H-4' would be too far to show any detectable NOE between each other. Therefore H_a and H_b should only show detectable NOEs with H-2' and H-3'. On the other hand, if the carboxyl group in the acid **33** was in an *endo* configuration, the resulting alcohol group in **34** would be in an *endo* configuration also as shown in **B** (Figure 21). The newly generated protons, H_a and H_b, of alcohol **34** were then expected to have strong NOEs with H-1' and H-4' as well as with H-2' and H-3' according to a modeling study of alcohol **B**. In this way, the configuration of the carboxyl group in acid **33** could be assigned by analyzing the NOESY spectrum of alcohol **34**.

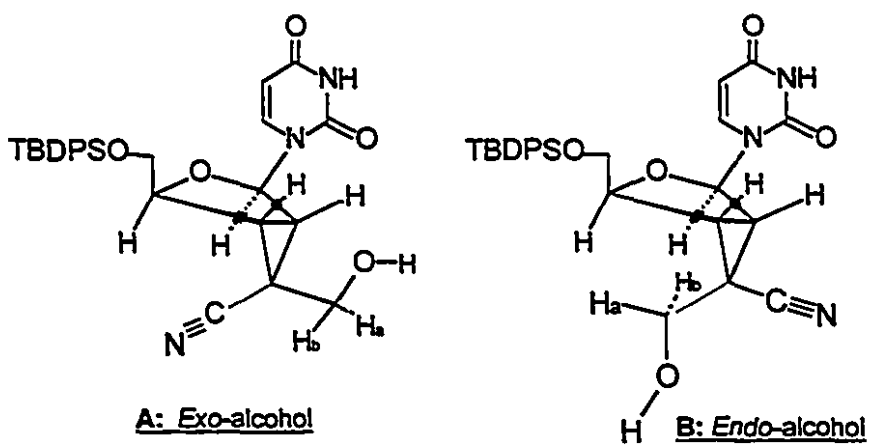


Figure 21. Configurations of *exo*-alcohol **A** and *endo*-alcohol **B**.

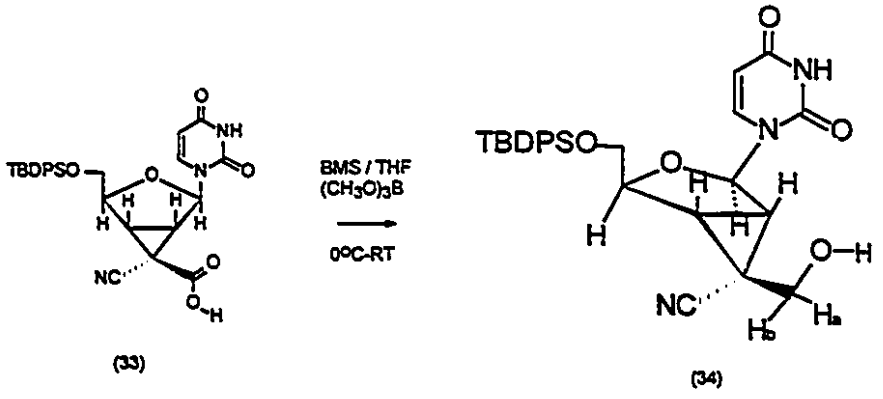
Borane reductions of carboxylic acids with borane-methyl sulfide complex and trimethyl borate are known to proceed much faster than the reduction of a cyano group under the same reaction condition[85]. By this mild method, it was possible to selectively

[85](a)Brown, H.C., *Boranes in Organic Chemistry*, Cornell Univ. Press, Ithaca, NY (1972)
(b)Brown, H.C., Heim, P., Yoon, N.M., *J. Am. Chem. Soc.*, 92, 1637 (1970);
(c)Braun, L. M., Braun, R. A., Crissman, H.R., Opperman, M., Adams, R.M., *J. Org. Chem.*, 36, 2388 (1971);

reduce the carboxylic acid to the alcohol without touching the cyano group in the same molecule.

Following a Hanessian procedure[86], cyclopropyl acid 33 was treated with borane-methyl sulfide complex and trimethyl borate in dry tetrahydrofuran at room temperature. After 2 hours, the product mixture was chromatographed to give alcohol 34 as a white solid in 58% yield. The ^{1}H-NMR data of alcohol 34 (spectrum see Appendix II. 1.) are listed in Table 2. The newly generated protons H_a and H_b were observed at δ 3.79 and 3.61 ppm representing an AB system. The structure of alcohol 34 was also supported by mass spectrometry, in which the molecular ion $[M+1]^+$ was observed at 518.

Scheme 34



(33) (34)

In the NOESY spectrum of cyclopropyl alcohol 34 (Appendix II. 1.), both H_a (δ 3.79 ppm) and H_b (δ 3.61 ppm) were observed to have considerable NOEs with H-2' and H-3', but had no detectable NOEs with H-1' (δ 5.93 ppm) or H-4' (δ 4.42 ppm). According to our previous rationalization, these NOE observations suggested an *exo* configuration of the CH_2 group in the cyclopropyl alcohol 34. Because the configuration of the CH_2OH in 34 is the same as that of the carboxyl group in either acid 33 or carboxylate 32, the configurations of both cyclopropyl acid 33 and cyclopropyl

(d)Yoon, N.M., Pak, C.S., Brown, H.C., Kirshamurthy, S., Stocky, T.P., *J. Org. Chem.*, 38, 2786 (1973)

[86]Hanessian, S.; Ugolini, A.; Dube, D.; Glamyan A., *Can. J. Chem.*, 62, 2146 (1984)

carboxylate **32** were therefore established as shown.

Table 2. NMR data of alcohol **34**

	δ (ppm)	J (Hz)	NOE*
NH	9.04 (br)		
H-6	7.82 (d)	8.1	H-5(+++), H-2'(++)
Arom.	7.60-7.43 (m)		
H-1'	5.93 (s)		H-2'(+++), H-3'(+), H-4'(+)
H-5	5.40 (d)	8.1	H-6(+++)
H-4'	4.42 (dd, ABX)	3.4, 3.9	H-5'(+++), H-3'(++), H-1'(+)
H-5'a	3.96 (dd, ABX)	11.7, 3.4	H-4'(+++), H-5'b(+++)
H-5'b	3.81 (dd, ABX)	11.7, 3.9	H-4'(+++), H-5'a(+++)
H_a	3.79 (d, AB)	11.7	H_b(+++), H-3'(++), H-2'(+)
H_b	3.61 (d, AB)	11.7	H_a(+++), H-2'(+++), H-3'(++)
H-2'	2.63 (d, AB)	6.4	H-1'(+++), H_b(+++), H_a(+), H-6 (++), H-3' (+++)
H-3'	2.16 (d, AB)	6.4	H-2'(+++), H_a(++), H_b(++)
OH	1.66 (br)		
t-Butyl	1.06 (s)		

* In column of NOE, +++, ++ and + mean strong, modest and weak respectively.

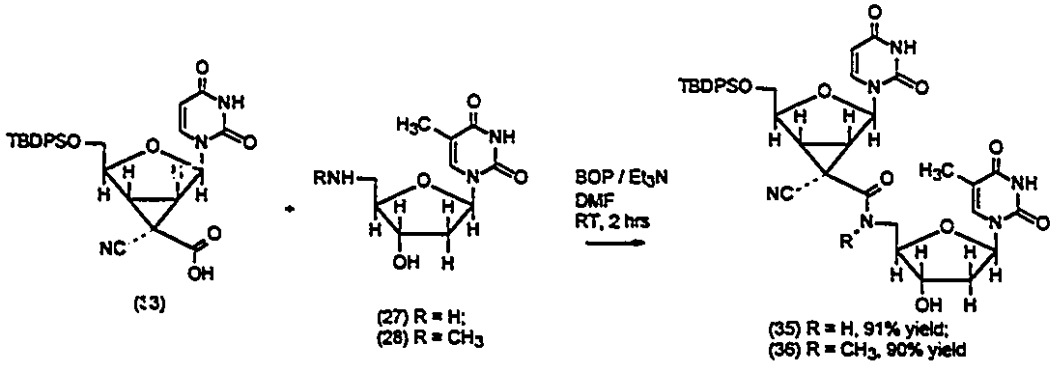
In summary, an *endo*-cyano substituted cyclopropyl acid **33** was successfully synthesized in this section by hydrogenolysis of its benzyl ester precursor **32**. The cyclopropyl carboxylate **32** was prepared by the Michael-type cyclopropanation of selenonyl nucleoside **17b** with benzyl cyanoacetate **31** in a stereoselective manner. The

cyclopropyl acid 33 was established as having an *exo*-carboxyl configuration through a NOE study of alcohol 34, which was derived from reduction of 33 with borane.

2.8. Synthesis of Dinucleoside Analogs 35 and 36 Containing a Cyano Substituted Cyclopropyl *exo*-Amide Linker

2.8.1. Coupling of Cyclopropyl Acid 33 with Amines 27 and 28

Scheme 35



The *endo*-cyano substituted cyclopropyl *exo*-acid 33 obtained from the previous reaction was then used as the 5'-end building block to react with amine 27 (3'-end building block) to synthesize dinucleoside 35 with a cyclopropyl *exo*-amide linker (Scheme 35). Using BOP (1 equivalent) as the coupling reagent[84], coupling of cyclopropyl acid 33 and amine 27 was performed in DMF at room temperature in the presence of 3 equivalents of triethylamine[45]. The reaction was completed in 2 hours, and gave dimer 35 in excellent yield. The formation of the dinucleoside was clearly indicated by ^{1}H-NMR analysis. In the ^{1}H-NMR spectrum of 35 (Figure 22), two anomeric protons were observed at δ 5.90 ppm (singlet, 1H) and 5.62 ppm (doublet of doublets, ABX, 1H) respectively. The sharp singlet was found to be the H-1' proton of the 5'-end uridine unit, while the ABX doublet of doublets was due to the H-1' proton of the 3'-end thymidine

unit. The cyclopropane-fused H-2' and H-3' of the 5'-end unit were observed as a pair of doublets (AB) at δ 3.39 ppm and 2.82 ppm, respectively, with a coupling constant of 6.4 Hz. The two H-2' protons of the 3'-end thymidine unit were observed at δ 2.34 ppm as a multiplet. The formation of dinucleoside 35 was also confirmed by detailed analysis of the ^{13}C-NMR spectrum and by mass spectrometry (FAB, HRMS), in which the molecular ion $[M+1]^+$ was observed at 755.2859.

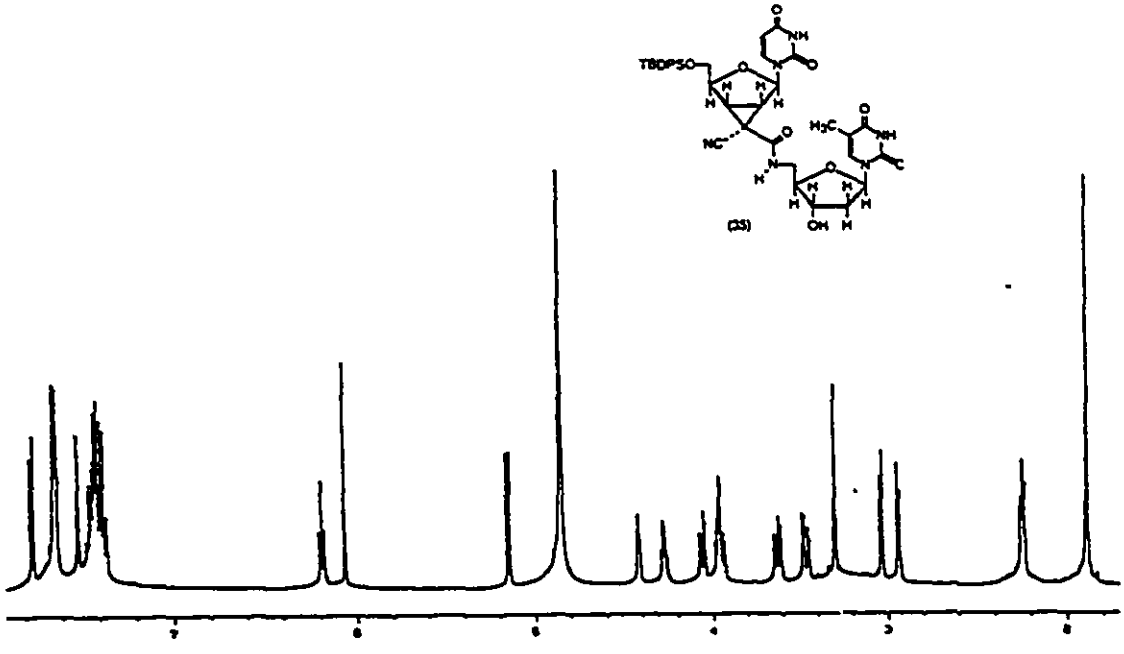


Figure 22. ^{1}H-NMR spectrum of dimer 35 with a cyano substituted cyclopropyl H-*exo*-amide linker (500 MHz, CD_3OD).

Using the same methodology, coupling of cyclopropyl acid 33 and the methylaminothymidine 28 readily provided dimer 36 in 90% yield after chromatography (Scheme 35). Although it was one spot by chromatography, dimer 36 with a methylamide linker showed two sets of ^{1}H-NMR signals in a ratio of 3:1, indicating that it was present as a pair of rotamers (Figure 23). Such a phenomenon was not observed in dimer 35, which has a H-amide linker.

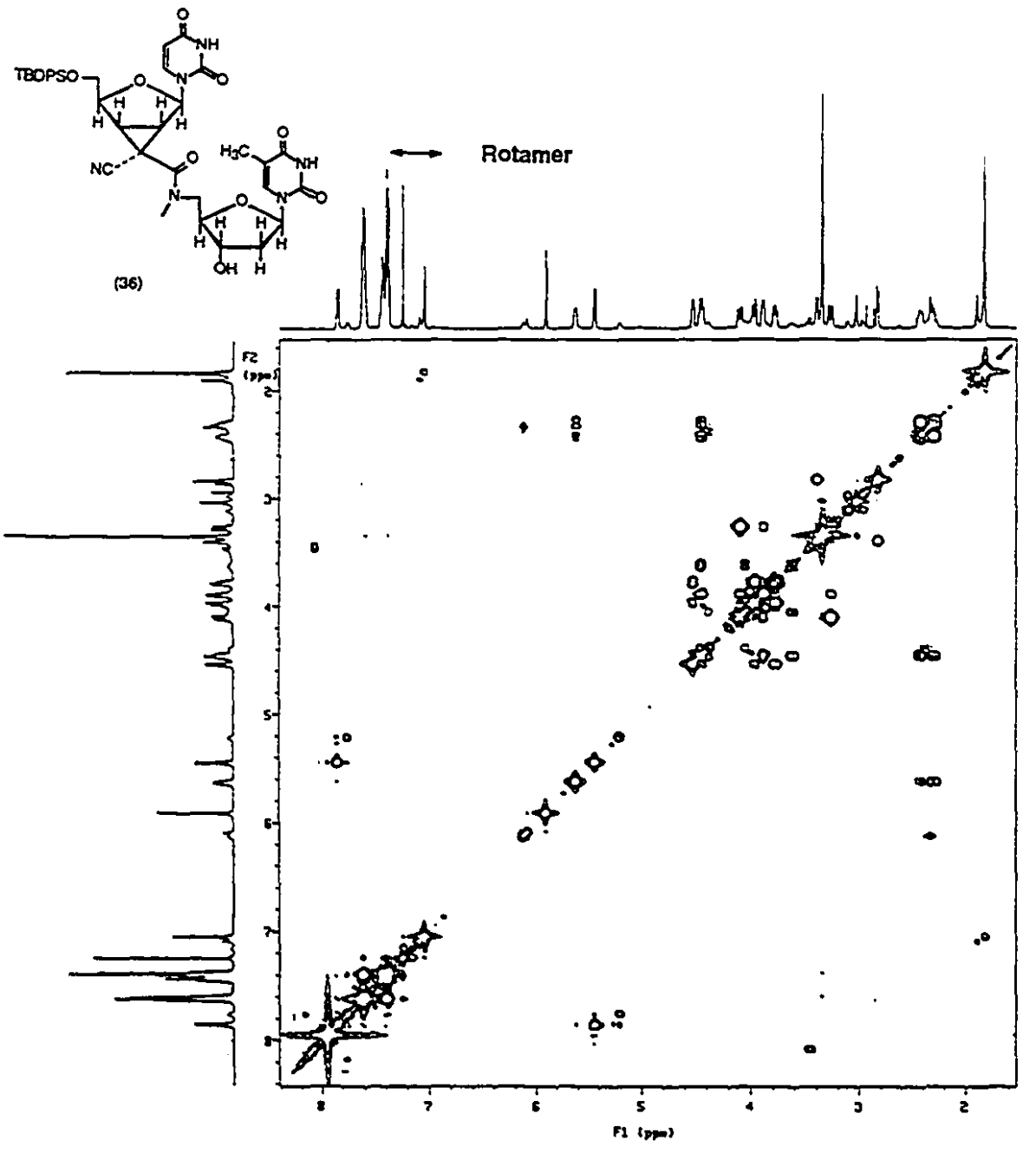
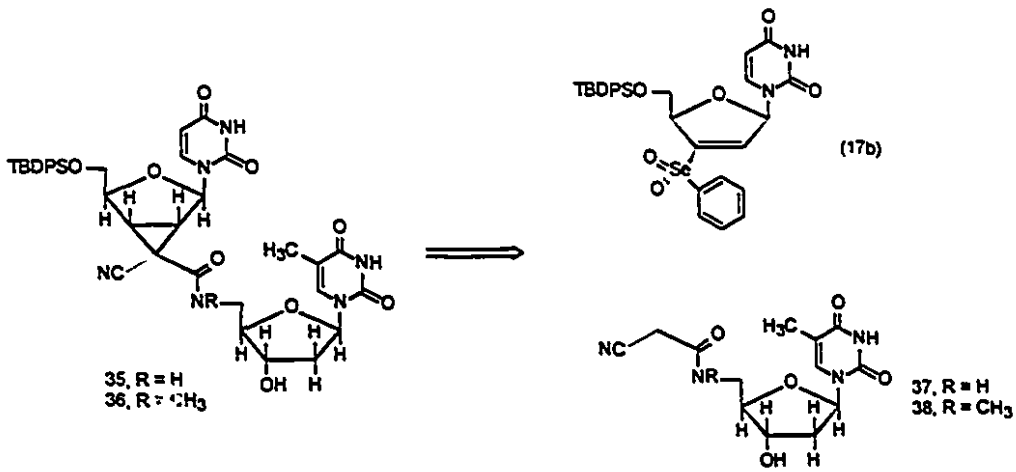


Figure 23. COSY spectrum of dimer 36 with a cyclopropyl methyl-*exo*-amide linker (500 MHz, CD_3OD). The set of small signals is due to the minor rotamer of 36.

2.8.2. Cyclopropanation of 3'-Selenone 17b with Amides 37 and 38 – Alternative Strategy for Dimer Synthesis

In another approach, the synthesis of dimer **35** and **36** was also attempted by direct cyclopropanation of selenone **17b** with amides **37** and **38** respectively, as outlined in Scheme 36. This strategy was based on our previous observation of the stereoselective cyclopropanation of selenone **17b** with benzyl cyanoacetate. In addition, a preliminary test also showed that amides **37** and **38** were able to withstand the treatment with potassium *tert*-butoxide in DMF at room temperature.

Scheme 36

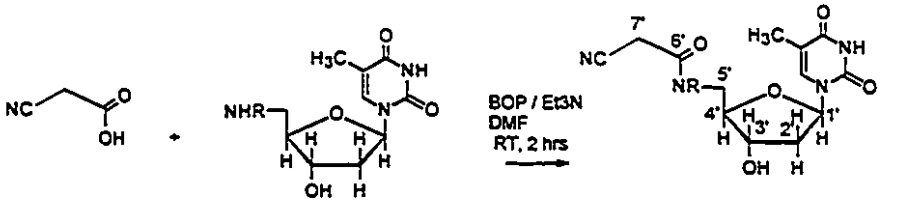


Preparation of Amides 37 and 38

Amide **37** was prepared by coupling of cyanoacetic acid (1.1 equivalent) and aminothymidine **27** (1 equivalent) in DMF in the presence of BOP (1 equivalent) as the coupling reagent. The reaction proceeded smoothly, and yielded amide **37** in good yield after chromatography (Scheme 37). In a parallel reaction, methylamide **38** was prepared similarly by coupling of methylaminothymidine **28** and cyanoacetic acid in a clean reaction (Scheme 37). The methyl amide **38** was also present as a pair of rotamers in a ratio of 2:1 at room temperature (COSY spectrum of **38** is shown in Appendix III. 3., and

COSY spectrum of 37 is shown in Appendix III. 2.).

Scheme 37



27, R = H
28, R = CH_3

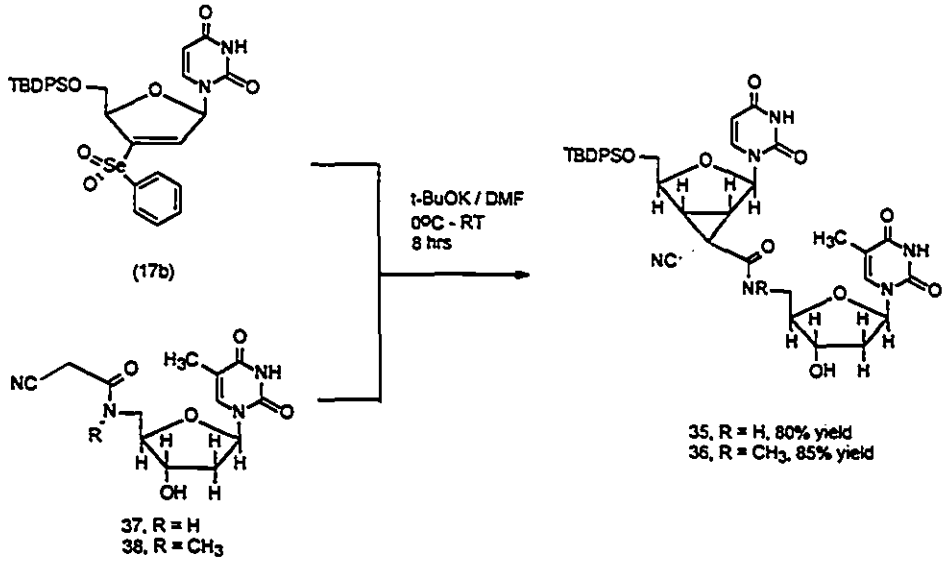
37, R = H, 94% yield
38, R = CH_3, 90% yield

Cyclopropanation

Because potassium *tert*-butoxide could deprotonate the protons at 3'-OH, 3-NH (uracil), CONH as well as the 7'-CH_2 in a molecule like amide **37**, 4 equivalents of potassium *tert*-butoxide were employed to make sure the desired 7'-CH anion was generated. Reaction of selenone **17b** with the anion of amide **37** proceeded smoothly, and yielded the desired dimer **35** in a clean reaction. After all selenone **17b** was consumed, the reaction mixture was added to a saturated ammonium chloride solution with vigorous stirring to give dimer **35** as a fine solid with reasonable purity in about 80% yield (Scheme 38). The dimer **35** obtained in this way was identical to the product previously prepared by coupling of acid **33** and amine **27**. The cyclopropanation of selenone **17b** and amide **37** was stereoselective.

Similarly, reaction of selenone **17b** with the anion of methylamide **38** also yielded dimer **36** with a cyclopropyl methylamide linker in a clean reaction (Scheme 38). After the same workup as previously described, dimer **36** was obtained as a single product, which was identical to the dimer obtained by coupling of acid **33** and methylamine **28**. No chromatography was needed in the above preparation of dimers **35** and **36**.

Scheme 38



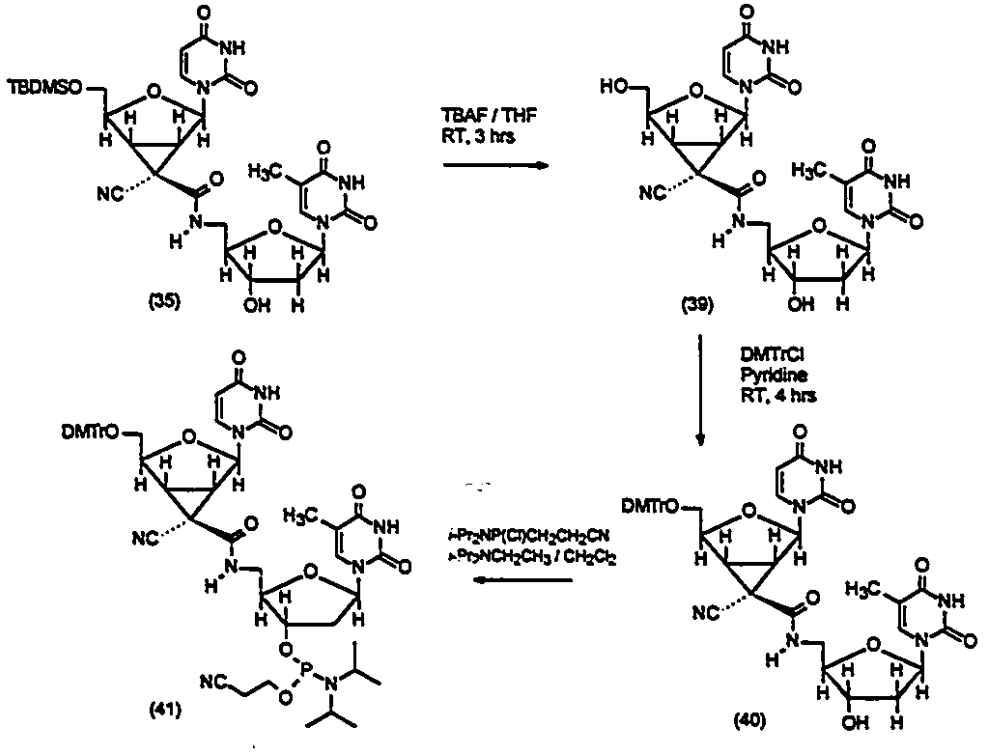
As compared to the method of coupling acid **33** and amines **27** and **28**, the merit of this dimer preparation approach was obvious in term of using selenone **17b** economically: only one step was needed from selenone **17b** to dimers in Scheme 38, while three steps were needed in the previous approach of coupling acid and amine. The selenone **17b** had to be prepared from the commercially available uridine in 6 steps, and its supply was limited. The nonchromatographic purification also made this approach desirable. The other potential application of this approach was that it might allow using the acid-labile 4',4'-dimethoxytrityl (DMTr) group as the 5'-protecting group for the selenone substrate, thereby generating the dinucleosides with the 5'-DMTr group, which was required for the later automated DNA synthesis.

2.9. Binding Properties of DNA Strands Bearing the Cyano Substituted Cyclopropyl *exo*-Amide Linker

2.9.1. Functionalization of Dimer 35

Appropriate functionalizations (protection and activation) of the dinucleoside 35 were required to make the dinucleoside 35 compatible with the automated phosphoramidite DNA synthesis methodology[55]. The transformations included replacing the 5'-silyl protecting group of the dinucleoside 35 by a 4,4'-dimethoxytrityl group, and activating the 3'-OH of the dimer with a (2-cyanoethyl) *N,N*-diisopropylphosphoramidite. The transformation sequence is outlined in Scheme 39.

Scheme 39



The 5'-*tert*-butyldiphenylsilyl group of dimer **35** was easily removed by *tetra*-n-butylammonium fluoride (TBAF) in tetrahydrofuran at room temperature in a clean reaction. However the attempt to purify the resulting diol **39** by chromatography (Et_3N/MeOH/CH_2Cl_2, 1/20/79) proved very difficult, and less than 20% of diol **39** was recovered after chromatography. Alternatively, after removal of the tetrahydrofuran solution from the product mixture, the residue was triturated with ether, followed by removal of the supernatant and repeated washing with ether to afford the crude diol **39** as a fine white solid. The ^{1}H-NMR of this crude diol **39** clearly showed the presence of about one equivalent of tetra-*n*-butylammonium salt as an impurity, even after repeated washing with ether. However, attempted tritylation of this crude diol on a small scale in dry pyridine with 3 equivalents of 4,4'-dimethoxytrityl chloride at room temperature was quite successful, and no diol **39** was left in 3 hours. It seemed that the presence of the remaining tetra-*n*-butyl ammonium species would not affect the tritylation of the 5'-hydroxyl group of diol **39**, as long as the sample was dry enough. Therefore it was decided to carry out the tritylation with this crude diol **39**. As expected, the tritylation proceeded smoothly, and gave the 5'-dimethoxytritylated dimer **40** in good yield after chromatography on silica gel (Et_3N / MeOH / CH_2Cl_2, 1/10/89). The remaining tetra-*n*-butylammonium salt from the previous step was easily removed by the same chromatography. Less than 5% of the ditritylated side-product was generated as a side-product under these reaction conditions.

The incorporation studies of the functionalized dimer **41** into DNA sequences for T_m evaluation were carried out in the ISIS laboratory at Carlsbad, California by Dr. Y. Sanghvi's group. Because of the concerns about the instability of the phosphoramidite derivative, the phosphitylation of dimer **40** was carried out by Dr. Y. Sanghvi's group in ISIS before using the dimer **41** in the automated DNA synthesis.

2.9.2. Stability of the Cyano Substituted Cyclopropyl Amide Linker

To make sure that our modified dimer was compatible with the automated DNA synthesis, the stability of the cyano substituted cyclopropyl amide linker under the standard reaction conditions in the automated DNA synthesis[55] was tested. The tests were carried out with diol **39**, and the results are summarized in Table 3. It shows that diol **39** with a cyano substituted cyclopropyl amide linker is able to stand the standard conditions used in the DNA synthesis. Therefore, the properly functionalized dinucleoside **41** should be compatible with the automated phosphoramidite DNA synthesis methodology.

Table 3. Results of the stability tests with diol **39**

	Conditions used in DNA synthesis		**Stability tests with diol 39**		
Solutions used	**Temperature**	**Duration**	**Temperature**	**Duration**	**Stability**
Detritylation solution [a]	RT [b]	50 sec. [b]	RT	24 hrs.	stable
Coupling solution [a]	RT [b]	40 sec. [b]	RT	24 hrs.	stable
Oxidation solution [a]	RT [b]	50 sec. [b]	RT	24 hrs.	stable
Conc. $NH_3.H_2O$ [c]	55° C [c]	12 hrs. [c]	55° C	12 hrs.	stable

[a] Detritylation solution: 3% trichloroacetic acid in dichloromethane; coupling solution: 0.5 M tetrazole in acetonitrile; oxidation solution: 0.1 M iodine in water / pyridine / THF 2/20/80; [b] conditions used in standard automatic DNA synthesis per coupling cycle; [c] conditions used for the removal of the protecting groups of the bases and the cleavage of the oligonucleotide from the polymer support after the DNA synthesis.

2.9.3. Binding Properties of the Modified DNA

Using the standard phosphoramidate DNA synthesis methodology, the dinucleoside amidite **41** with the *endo*-cyano substituted cyclopropyl *exo*-amide linker was then incorporated into then DNA sequences (Isis 10419 and Isis 10446, Figure 24) in the ISIS laboratory by Dr. Y. Sanghvi's group to evaluate the influence of the incorporation of the cyclopropyl amide-linked dinucleoside into the DNA strands on the modified DNA's hybridization property towards its complementary RNA. Because our modified dimer had a U-T base sequence, a 2'-deoxyuridine (dU) was used as the counterpart of our 2',3'-cyclopropanated uridine analog in the control sequence. The sequences used in the binding experiment are depicted in Figure 24, and the hybridization results are summarized in Table 4.

Isis 10419 Modified DNA strand with 5 incorporations of the amide-linked 2',3'-cyclopropanated dinucleoside:

5' - GCG UcaT UcaT UcaT UcaT UcaT GCG -3'

Isis 10794 Control sequence for sequence ISIS 10419:

5' - GCG dU T dU T dU T dU T dU T GCG -3'

Isis 10446 Modified DNA strand with 2 incorporations of the amide-linked 2',3'-cyclopropanated dinucleoside:

5' - CTC GTA C UcaT UcaT C CGG TCC - 3'

Isis 10793 Control sequence for sequence ISIS 10446:

5' - CTC GTA C dU T dU T C CGG TCC - 3'

Figure 24. Sequences synthesized for hybridization evaluation. The nucleosides are linked with phosphodiester unless specified. UcaT = cyclopropyl-amide-linked dinucleoside; dU = 2'-deoxyuridine.

Table 4. Effects of the cyano substituted cyclopropyl *exo*-amide modification on DNA's affinity to RNA and DNA

Test No.	Isis #	No. of modifications	Tm	ΔTm[a] / mod.[b] (vs RNA)	ΔTm / mod.[b] (vs DNA)
1	10794	5	46.6	- 0.44	- 0.62
2	10419	5		no hybridization	no hybridization
3	10793	2	60.9	- 0.30	not tested
4	10446	2	50.1	- 5.41	not tested

[a] ΔT_m: Difference in melting temperature (T_m) between the modified DNA/RNA or DNA/DNA duplex and the unmodified wide type (WT) duplex per modification ($\Delta T_m = T_m - T_m(WT)$); [b] modification or mod. = replacement of the phosphodiester-linked TT dinucleoside unit with other dinucleoside analogs.

With two incorporations of the *exo*-amide-linked 2',3'-cyclopropanated UT dimer into a 5'-CTCGTACUcaTUcaTCCGGTCC-3' sequence (Isis 10446, Figure 24), a drop of 10.8 °C in Tm was found in the modified DNA's binding to its complementary RNA sequence, as compared to the corresponding Tm of the phosphodiester-linked DNA/RNA duplex. With five incorporations of our cyclopropyl *exo*-amide-linked dimers into a 5'-GCGUcaTUcaTUcaTUcaTUcaTGCG-3' sequence (Isis 10419, Figure 24), no hybridization of the modified DNA (Isis 10419) with its complementary DNA or RNA strand was observed. The above hybridization data (Tm) suggested that the replacement of the natural phosphodiester linker with the cyano substituted cyclopropyl *exo*-amide linker decreased the modified DNA's binding to its complementary RNA target.

2.10. Synthesis of the Cyclopropyl Carboxylates

In our previous efforts, we have prepared an *endo*-cyano substituted cyclopropyl *exo*-carboxylate **32**, which was then hydrogenated to cyano substituted acid **33**. Acid **33** was then used as the 5'-end building unit to react with amine **27** to provide dimer **35** with a 2',3'-cyclopropanated *endo*-cyano-*exo*-amide linker. Preliminary T_m evaluation of a DNA strand bearing such a dimer unit showed that the cyclopropyl *endo*-cyano-*exo*-amide modification decreased the modified DNA's binding to its natural RNA complement.

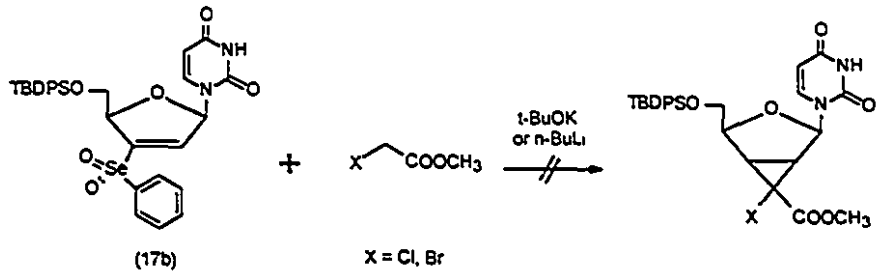
Thinking that the cyano substituent might be structurally unfavourable factor to cost the modified DNA's binding affinity, we next attempted to prepare our targeted cyclopropyl amide-linked dinucleoside analog **S1** without the cyano substituent. To obtain the key cyclopropyl carboxylate, a program was then pursued to search for the suitable **X** substituted acetates (XCH_2COOR) for the cyclopropanation, where the **X** was removable.

2.10.1 Reactions of Selenone 17b with 2-Haloacetates

Reactions of selenone **17b** with 2-haloacetates were first attempted. In two parallel experiments, neat methyl 2-chloroacetate and methyl 2-bromoacetate were treated with 10 equivalents of potassium t-butoxide at room temperature for 30 minutes, followed by the addition of selenone **17b**. The reaction mixtures were stirred at room temperature for a few hours before being poured into a saturated ammonium chloride solution. However, no desired cyclopropanation occurred in both attempts, and the selenone **17b** was fully recovered after the reactions. The reactions were then repeated with a stronger base as *n*-Butyl lithium, and no cyclopropanation took place either (Scheme 40). It was later realized that the 2'-haloacetyl anion could easily react with 2'-

haloacetate either by Claisen condensation or by substitution of the halo group, thus had no chance to react with selenone **17b** for the cyclopropanation.

Scheme 40



2.10.2. Reaction of 17b with Ethyl-2-nitroacetate

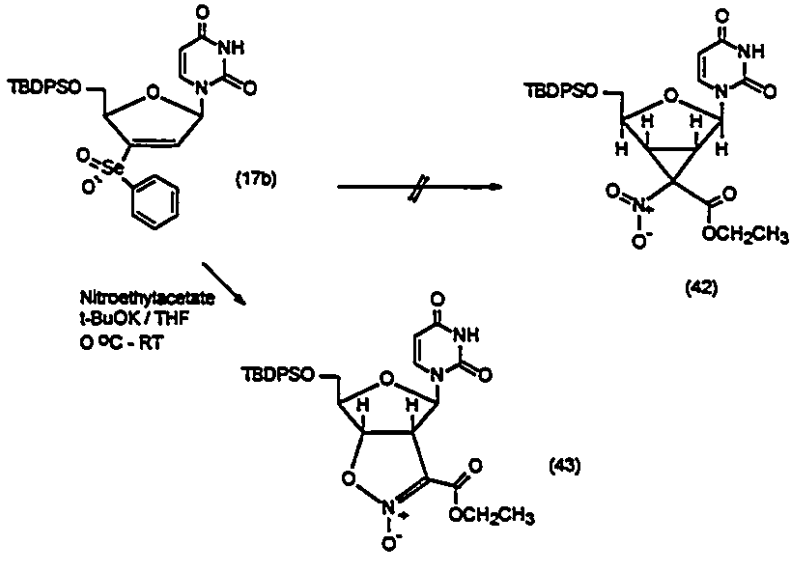
It is known that secondary and tertiary nitro groups can be directly replaced by hydrogen with tributyltin hydride[87]. In addition, the anions of the alkyl 2-nitroacetates are good soft nucleophiles. If the cyclopropanations of selenone **17b** with alkyl 2-nitroacetates were to succeed, the nitro group of the resulting cyclopropane could be easily removed to give the desired cyclopropyl carboxylate.

Using the same procedure as previously adopted for the Michael-type cyclopropanation, a solution of ethyl 2-nitroacetate (10 equivalents) in dry tetrahydrofuran was treated with 5 equivalents of potassium *t*-butoxide to generate the anion in *situ*, followed by the addition of selenone **17b**. All selenone **17b** was consumed after stirring the reaction mixture at room temperature overnight, and converted to a single product. Disappointingly, the product was found to be a [3.3.0]-bicyclic nucleoside analog **43** instead of the desired [3.1.0]-bicyclic analog **42** (Scheme 41). The ^{1}H-NMR spectrum of **43** (Figure 25) showed the anomeric proton (H-1') as a doublet (δ 6.04 ppm)

[87](a)Ono, N.; Miyake, H.; Tamura, R.; Kaji, A., *Tetrahedron Lett.*, 22, 1705 (1981)
(b)Ono, N.; Kamimura, A.; Miyake, H.; Hamamoto, I.; Kaji, A., *J. Org. Chem.*, 50, 3692 (1985)

with a coupling constant of 4.4 Hz. This signal pattern of H-1' was significantly different from the characteristic singlet of the anomeric proton observed for the [3.1.0]- bicyclic analogs. Also, the H-3' proton appeared as a doublet of doublets at δ 5.33 ppm (ABX, J = 10.3 Hz, J = 9.8 Hz) instead of an AB doublet around δ 2 - 3 ppm, as usually observed in the cyclopropyl analogs. The H-2' proton appeared at δ 4.35 ppm as a multiplet overlapping with the H-4' and the OCH_2- protons. These NMR data of **43** were quite similar to the published data of a similar [3.3.0]-bicyclic uridine analog[72], which was obtained from the reaction of selenonyl uridine with methyl acetoacetate. Mass spectrometry (FAB) analysis agreed with our structure assignment, in which the molecular ion $[M+1]^+$ was observed at 580.

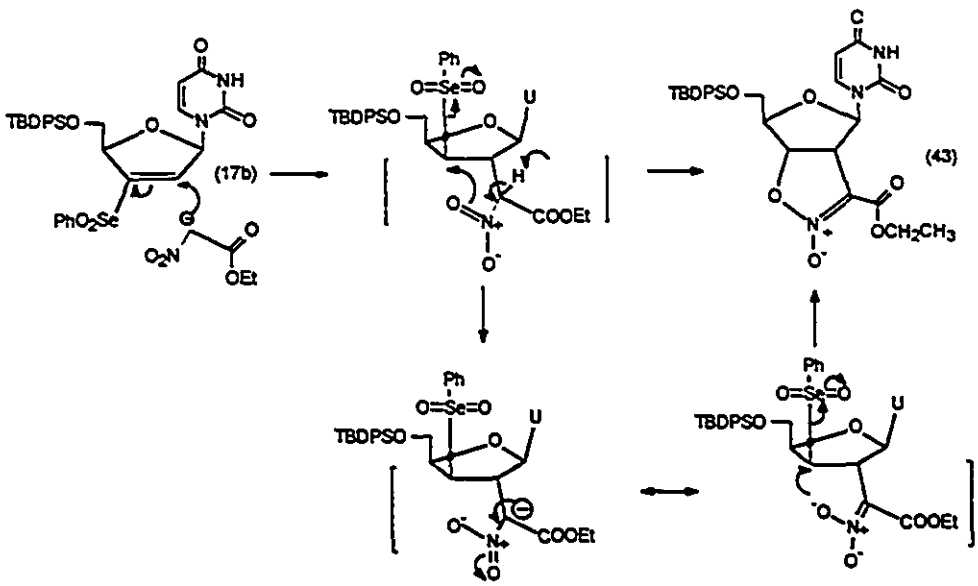
Scheme 41

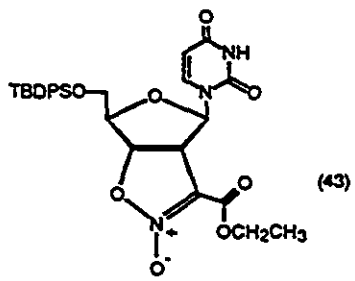


The formation of the [3.3.0]-bicyclic nucleoside **43** proceeds probably through a similar mechanism as the reaction of selenonyl uridine with methyl acetoacetate[72]. The first Michael-type addition of the ethyl 2-nitroacetate anion to vinyl selenone **17b** was considered the same as that of the malonate anion. However, the ring closure step proceeded through the oxygen atom of the nitro group rather than the carbon atom of the

acetate, thereby forming a five-member ring instead of a cyclopropane structure (Scheme 42). As a conclusion, the ethyl nitroacetate anion did react with vinyl selenone **17b**, but in a different manner. Therefore this approach was abandoned.

Scheme 42





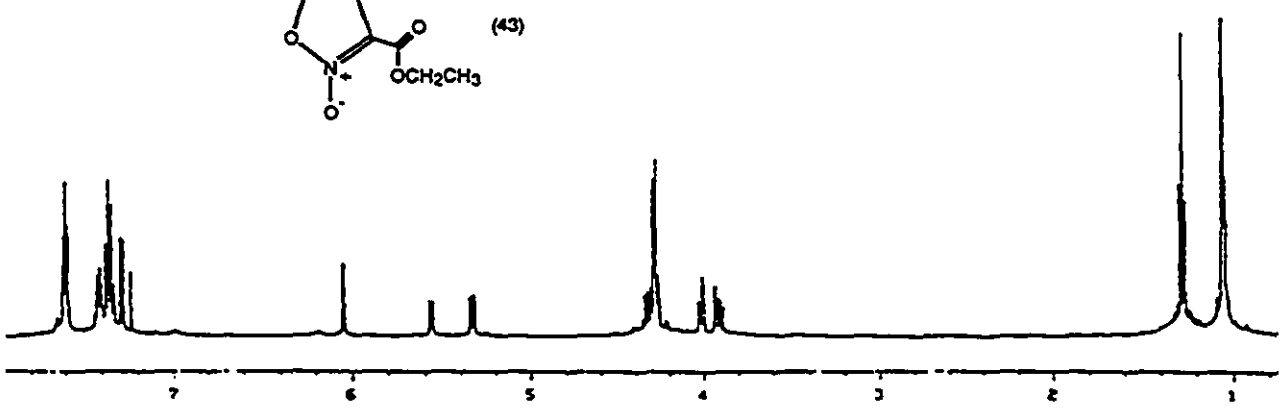
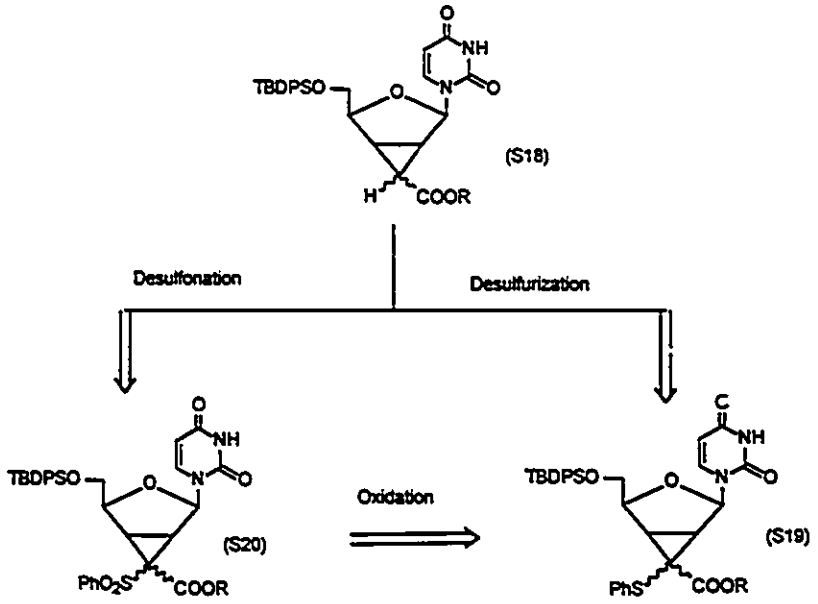


Figure 25. ^{1}H-NMR spectrum of [3.3.0]-bicyclic nucleoside analog 43 (500 MHz, $CDCl_3$)

2.10.3. Reaction of Selenone 17b with Methyl 2-Thiophenoxyacetate

Ethyl 2-thiophenoxyacetate has been reported by Kuwajima[88] to react with vinyl selenones in a Michael-type cyclopropanation. If the cyclopropanation of selenone **17b** with alkyl 2-thiophenoxyacetates were to succeed, the thiophenoxyl group in the resulting cyclopropane product might be removed by direct desulfurization[89] or through desulfonation[90](Scheme 43).

Scheme 43



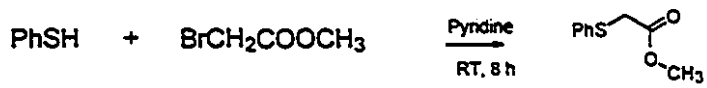
Preparation of Methyl 2-Thiophenoxyacetate 44

The preliminary investigation was carried out with methyl 2-thiophenoxyacetate **44**, which could be easily prepared by reaction of thiophenol and methyl 2-bromoacetate in pyridine. In order to minimize the notorious odor of thiophenol, 1.5 equivalents of methyl-2-bromoacetate were employed in the reaction to make sure that all the thiophenol

88Kuwajima, I.; Ando, R.; Sugawara, T., *Tetrahedron Lett.*, 24, 4429 (1983)
89Hylton, T.; Boekelheide, V., *J. Am. Chem. Soc.*, 90, 6887 (1968)
90Brown, A.C.; Carpino, L.A., *J. Org. Chem.*, 50, 1749 (1985)

used in the reaction was consumed. After stirring the mixture of thiophenol and methyl 2-bromoacetate in pyridine at room temperature for 8 hours, no thiophenol was detected in the reaction mixture, and acetate **44** was generated in a clean reaction (Scheme 44). After workup, the crude acetate **44** was purified by distillation at reduced pressure to give the pure methyl 2-thiophenoxyacetate **44** as a colorless liquid.

Scheme 44



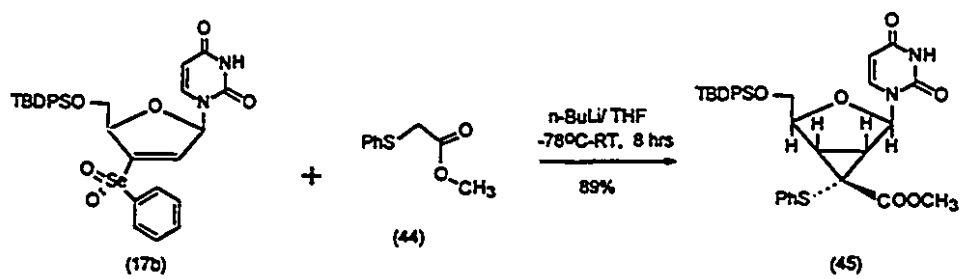
(44)

Cyclopropanation of Selenone 17b with Acetate 44

Our initial attempt to form a cyclopropane **45** using our standard reaction procedure (potassium *tert*-butoxide, THF) failed, presumably due to the high pKa of the methyl thiophenoxyacetate **44**. The cyclopropane product was barely detected by TLC, and most of the selenone **17b** starting material was recovered from the reaction mixture. A stronger base, *n*- butyl lithium, was then employed to treat methyl 2-thiophenoxyacetate **44** in tetrahydrofuran at - 78 °C to generate the anion of **44**. In this case, the cyclopropanation proceeded smoothly, and yielded the diastereomerically pure **45** in 89% yield after chromatography. In the ^{1}H-NMR spectrum of **45** (Figure 26), only one sharp singlet for one proton was observed at δ 5.94 ppm for the anomeric proton (H-1'). The cyclopropanated H-2' and H-3' protons appeared as a pair of doublets at δ 3.03 and 2.95 ppm (AB, J = 7.8 Hz). The ^{1}H-NMR spectrum of **45** is showed in Figure 26. Interestingly, the above ^{1}H-NMR data suggested that cyclopropane **45** obtained from the above reaction was present as only one isomer. Cyclopropane **45** was speculated to have

an *exo*-carboxylate (in other words, *endo*-thiophenoxyl) configuration. This speculation was based on an analysis of the chemical shifts of the H-1' and H-4' protons in cyclopropane 45. As later summarized in Section 2.10.4. (Table 7), the chemical shifts of the H-1' and H-4' protons in the [3.1.0]-bicyclic systems were usually downshifted by the *endo*-carbonyl group if present, compared to the cyclopropyl carboxylates containing no endo-carbonyl groups.

Scheme 45



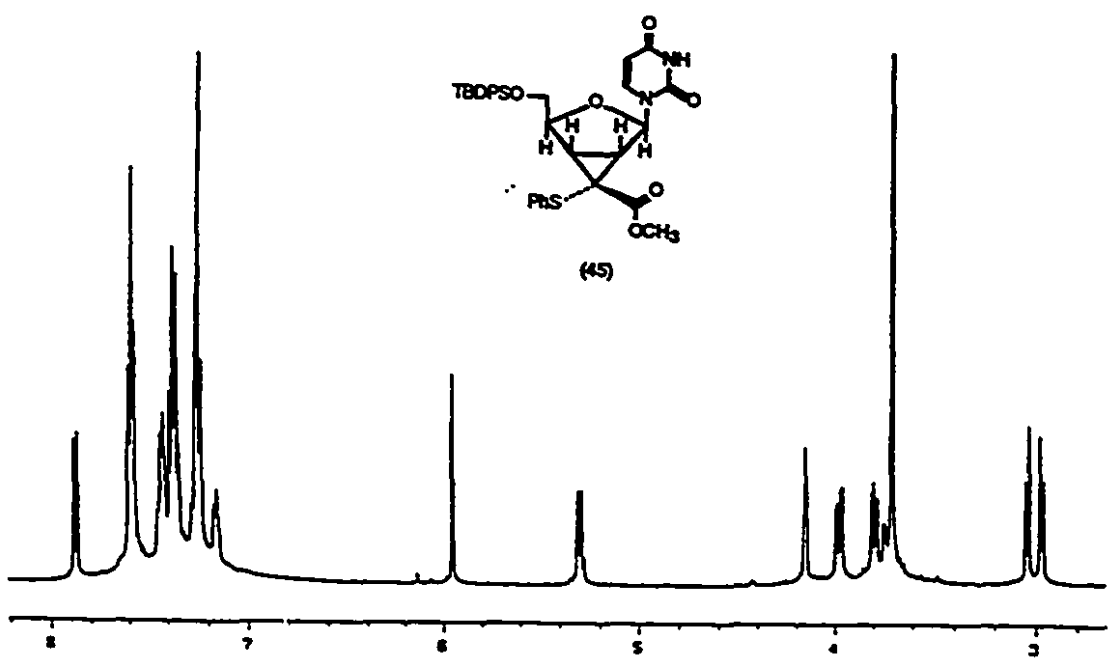


Figure 26. ^{1}H-NMR spectrum of cyclopropane 45 (500 MHz, $CDCl_3$).

2.10.4. Desulfurization of Thiophenoxylate 45

With Raney Nickel

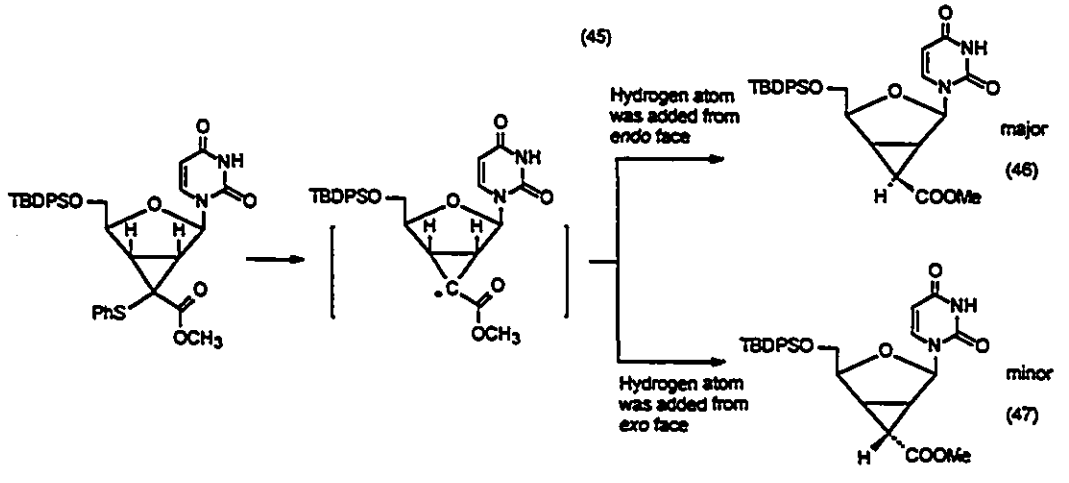
With the thiophenoxyl substituted cyclopropyl carboxylate 45 in hand, we then proceeded with the desulfurization. The preliminary desulfurization of 45 was attempted on a small scale by refluxing 45 with Raney nickel in ethanol. However, neither starting material 45 nor any other UV activated derivatives were present in the ethanol solution after a few hours of reflux. It seemed that the nucleoside derivatives were absorbed by the Raney nickel. Washing the Raney nickel with a large amount of methanol did not bring any detectable nucleoside analogs into the methanol solution. Because the typical odor of thiophenol had not been detected during the reaction, it was concluded that no desulfurization occurred in the attempt. Being aware of the possible inactivity of the "old" Raney nickel, fresh Raney nickel (from Aldrich) was then employed to repeat the reaction, but no desulfurization was observed.

With Tributyltinhydride

A radical-mediated desulfurization was then attempted. Thiophenoxylate 45 was refluxed with tributyltinhydride and azobisisobutyronitrile (AIBN) in dry benzene. As expected, all starting material 46 was consumed overnight, and two new products were generated with polarities slightly higher than that of the starting material 45. The two UV active products were distinguishable in TLC, with a 0.08 difference in R_f value (EtOAc/Hexane, 1/1). The typical odor of thiophenol was also detected during the reaction indicating the occurring of the desulfurization. The two products were then carefully separated by preparative TLC to give diastereomeric cyclopropanes 46 and 47 in a ratio of 5:1 (Scheme 46). The ^{1}H-NMR spectrum of the major isomer 46 (Figure 27) showed a sharp singlet at δ 6.00 ppm for the anomeric proton (H-1'). The newly

generated cyclopropyl proton (H-6')[91] was observed as an triplet at δ 1.71 ppm with $J_{2',6'} = J_{3',6'} = 2.0$ Hz. The cyclopropanated H-2' and H-3' protons were observed as two sets of ABX doublet of doublets centered at δ 2.47 ppm and δ 2.44 ppm respectively, as the results of coupling with each other ($J_{2',3'} = 6.3$ Hz) and with the H-6' proton. In the ^{1}H-NMR of the minor isomer **47** (Figure 28), the anomeric proton (H-1') was observed as a sharp singlet at δ 6.27 ppm, which was significantly downshifted compared to its counterpart in isomer **46** (δ 6.00 ppm). A similar downshift was also observed for signal of H-4' in carboxylate **47** compared to its counterpart in isomer **46**. The newly generated cyclopropyl proton H-6' of **47** was observed as a triplet at δ 2.05 ppm with coupling constants ($J_{2',6'} = J_{3',6'} = 8.3$ Hz) significantly higher than their counterparts (2.0 Hz) in isomer **46**. The H-2' and H-3' protons in **47** were observed as two sets of ABX doublet of doublets centered at δ 2.33 ppm and 2.40 ppm respectively. The ^{1}H-NMR data of carboxylates **46** and **47** are listed in the Table 5. The structures of carboxylates **46** and **47** were also confirmed by detailed analysis of the ^{13}C-NMR spectra, as well as the mass spectra (FAB, NBA), in which the molecular ions were observed.

Scheme 46



[91]H-6' is used in this thesis to represent the cyclopropyl proton adjacent to the carboxyl group in all the cyclopropyl carboxylates.

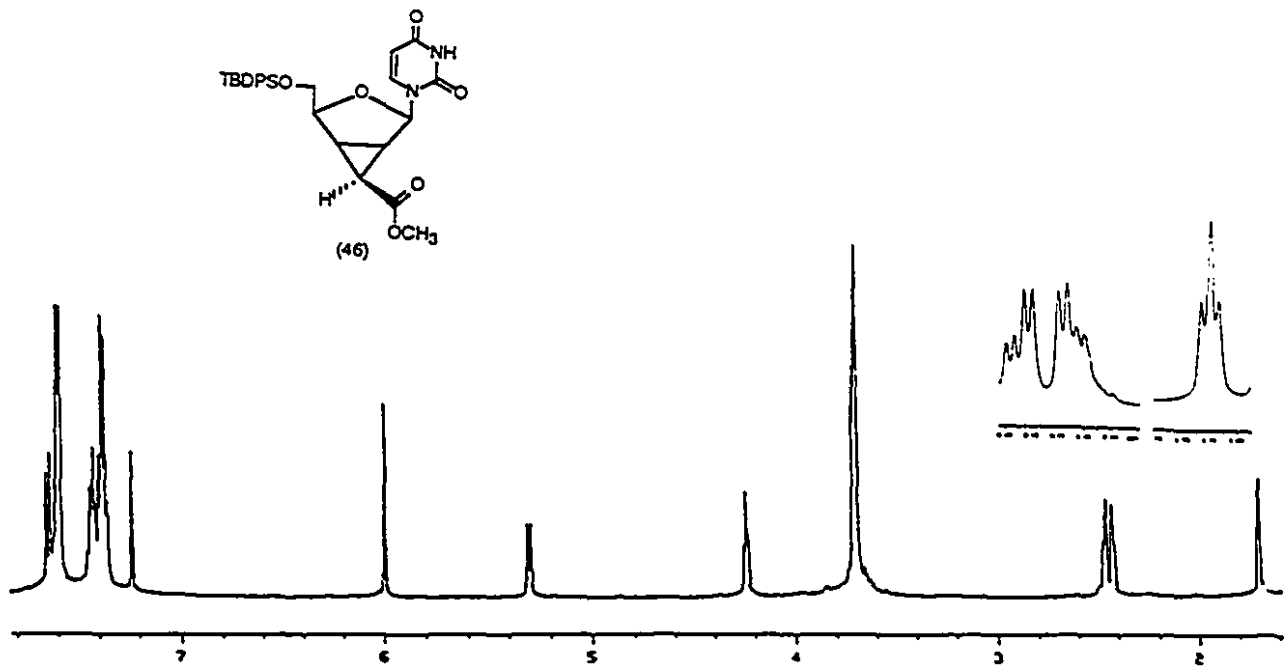


Figure 27. ^{1}H-NMR spectrum of cyclopropyl carboxylate 46 (500 MHz, $CDCl_3$).

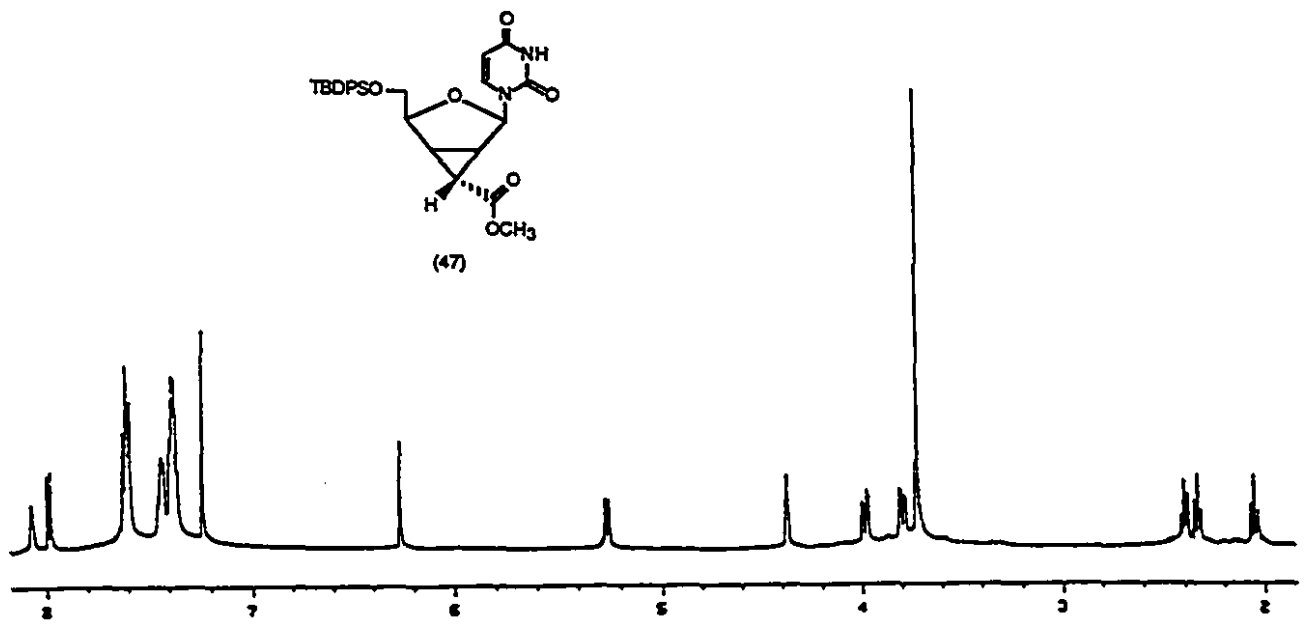


Figure 28. ^{1}H-NMR spectrum of cyclopropyl carboxylate 47 (500 MHz, $CDCl_3$).

Table 5. ^{1}H-NMR data of carboxylates **46** and **47**

	Carboxylate 46		Carboxylate 47	
	δ (ppm)*	J (Hz)	δ (ppm)*	J (Hz)
NH	8.34 (br)		8.08 (br)	
H-6	7.65 (d, AX)	8.3	7.99 (d, AX)	8.3
Arom.	7.61-7.36 (m)		7.63-7.36 (m)	
H-1'	6.00 (s)		6.27 (s)	
H-5	5.30 (d, AX)	8.3	5.27 (d, AX)	8.3
H-4'	4.24 (dd, ABX)	5.4, 3.5	4.38 (dd, ABX)	2.9, 3.5
H-5'a	3.72		3.99 (dd, ABX)	11.7, 2.9
H-5'b	3.71		3.80 (dd, ABX)	11.7, 3.5
OCH_3	3.70 (s)		3.73 (s)	
H-2'	2.47 (dd, ABX)	6.3, 2.0	2.40 (dd, ABX)	7.3, 8.3
H-3'	2.44 (dd, ABX)	6.3, 2.0	2.33 (dd, ABX)	7.3, 8.3
H-6'	1.71 (t)	2.0, 2.0	2.05 (t)	8.3, 8.3
t-Butyl	1.06 (s)		1.07 (s)	

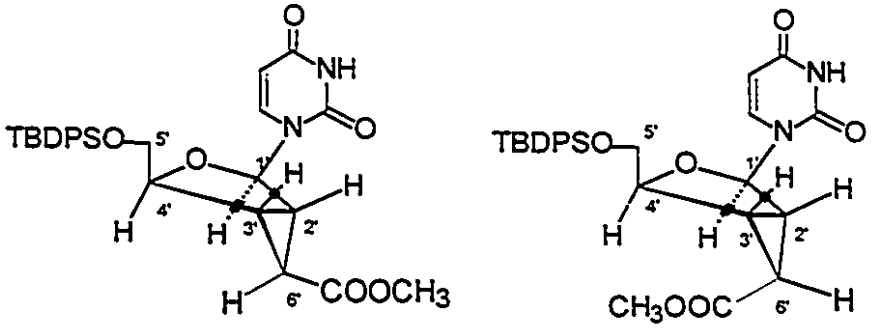
* s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet.

Configurational Assignments of 46 and 47

According to the modeling studies[92], the *endo* H-6' proton in the *exo*-carboxylate of type A (Figure 29) should have detectable NOEs with H-1', H-2', H-3' and H-4'. On the other hand, the *exo* H-6' in the *endo*-carboxylate (**B**, Figure 29) should only have strong NOEs with H-2' and H-3'. The distance between the *exo* H-6' and H-1' or between the *exo*

[92]All MM calculations were performed on IBM-PC using HyperChem modeling program from Autodesk (Sausalito, California, 1993) unless specified.

H-6' and H-4' is too far to have any detectable NOE.



A: exo-carboxylate B: *endo*-carboxylate

Figure 29. General structure of *exo*-carboxylate (A) and *endo*-carboxylate (B).

In our NOE studies of the cyclopropane **46**, the H-6' proton showed strong NOEs with H-2' and H-3', as well as with H-2' and H-3'. showed no detectable NOEs with H-1' and H-4' (NOESY spectrum of **46** see Appendix II. 2.). In the NOESY spectrum of cyclopropane **47** (Appendix II. 3), the H-6' proton was observed to only have strong NOEs with H-2' and H-3', but no detectable NOEs with H-1' and H-4'. The NOE observations of carboxylates **46** and **47** are summarized in Table 6. Therefore the major isomer **46** was established as with the carboxylate in an *exo*-configuration (the H-6' proton was in an *endo*-configuration), and the minor isomer **47** was established as with the carboxylate in an *endo*-configuration (the H-6' proton was in an *exo*-configuration).

The configurations of **46** and **47** established by NOE studies could be used to explain the difference between the ^{1}H-NMR data of **46** and those of **47** (Table 5). Compared to the chemical shifts of the H-1' and H-4' protons in **46** (δ 6.00 and 4.24 ppm, respectively), the significant downshifts of their counterparts (δ 6.27 and 4.38 ppm, respectively) in **47** could be explained as due to the influence of the neighboring *endo*-carbonyl group in **47**, where the *endo*-carbonyl group in **47** is close to the H-1' and H-4'. In *exo*-carboxylate **46**, the *exo*-carbonyl is too far to have any influence on the chemical

shifts of the H-1' and H-4' protons. Such an influence of the *endo*-carbonyl group on the chemical shifts of the H-1' and H-4' protons in the [3.1.0]-bicyclic system could also be observed in other similar cyclopropanes, as shown in Table 7.

Table 6. NOE observations of carboxylates **46** and **47**.

	Carboxylate 46		**Carboxylate 47**	
	δ (ppm)	**NOE**	δ (ppm)	**NOE**
H-6	7.65	H-5	7.99	H-5
H-1'	6.00	H-2', H-6'	6.27	H-2'
H-5	5.30	H-6	5.27	H-6
H-4'	4.24	H-3', H-5', H-6'	4.38	H-3', H-5'
H-5'	3.72	H-4'	3.99, 3.80	H-4'
H-2'	2.47	H-1', H-3', H-6'	2.33	H-1', H-3', H-6'
H-3'	2.44	H-2', H-4', H-6'	2.40	H-2', H-4', H-6'
H-6'	1.71	H-1', H-2', H-3', H-4'	2.05	H-2', H-3'

The above configurational assignments of **46** and **47** established by NOE observations were also supported by the coupling constant ($J_{2',6'}$ and $J_{3',6'}$) analysis of cyclopropanes **46** and **47**. The $J_{2',6'}$ and $J_{3',6'}$ of isomer **46** were measured to be 2.0 Hz. The $J_{2',6'}$ and $J_{3',6'}$ of isomer **47** were measured to be 8.3 Hz (Table 6), which are significantly larger than their counterparts of **46**. According to the molecular mechanics studies of the *exo*-carboxylate **A** (Figure 29), the dihedral angles between H-6' and H-2', and between H-6' and H-3' were calculated to be 138.4° and 138.1°, respectively. In case of the *endo*-carboxylate **B** (Figure 29), the dihedral angles between H-6' and H-2', and between H-6' and H-3' were calculated to be 3.1° and 1.7°, respectively. By correlating

the dihedral angles with the coupling constants, isomer **46** with $J_{2',6'} = J_{3',6'} = 2.0$ Hz should be the *exo*-carboxylate of type **A**, and its stereoisomer **47** with $J_{2',6'} = J_{3',6'} = 8.3$ Hz should be the *endo*-carboxylate of type **B**. The coupling constants and the corresponding dihedral angles are listed in Table 8. The above configuration assignments of **46** and **47** by coupling constants analysis agreed well with those established by NOESY analysis

Table 7. the chemical shifts of the H-1' and H-4' protons
in all the 5'-TBDPS protected cyclopropanes prepared in the study.

Cyclopropane	*endo*-carbonyl	δ H-1' (ppm)	δ H-4' (ppm)
21b	yes	6.07	4.46
23b	yes	6.16	4.52
33	no	6.04	4.42
35	no	5.90	4.53
45	no *	5.94	4.13
46	no	6.00	4.24
47	yes	6.27 #	4.38 #
49 Δ	no *	6.01	4.18
50 Δ	no	6.01	4.24
51 Δ	yes	6.31 #	4.39 #
54 Δ	no	6.00	4.26
55 Δ	yes	6.32 #	4.47 #

Δ Compounds were prepared in the later sections; * stereochemistry of which was speculated; # the chemical shift is significantly downshifted compared to its counterpart in the diastereoisomer with an *exo*-carbonyl group.

Table 8. Correlation of the $J_{6',2'}$ and $J_{6',3'}$ with the dihedral angles obtained from the molecular mechanics studies of the carboxylates.

***exo*-Carboxylate 46**		***endo*-Carboxylate 47**	
J (Hz)	Dihedral angle (°)	J(Hz)	Dihedral angle (°)
$J_{H6',H2'} = 2.0$	138.4	$J_{H6',H2'} = 8.3$	3.1
$J_{H6',H3'} = 2.0$	138.1	$J_{H6',H3'} = 8.3$	1.7

In summary, the reduction of the thiophenoxyl substituted cyclopropane **45** by refluxing it with tributyltinhydride and azobisisobutyronitrile in benzene successfully gave two stereoisomers (*exo*-carboxylate **46** and *endo*-carboxylate **47**) in a clean reaction. Although the R_f values of the *exo*-carboxylate **46** and the *endo*-carboxylate **47** were very close to each other (with a 0.08 difference in EtOAc/Hexane, 1/1), these diastereomers were still separable by chromatography on silica gel. Configurations of these two conformers were assigned by NOESY or coupling constants analysis.

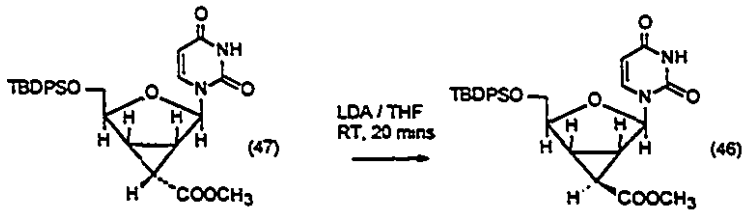
2.10.4. Equilibration between *exo*-Carboxylate 46 and *endo*-Carboxylate 47

Initial attempt to epimerize the mixture of carboxylates **46** and **47** (1/1) with sodium methoxide in methanol for up to 24 hours resulted in no epimerization taking place, and only desilylation of the starting materials was observed.

Being aware of the fact that the methoxyl anion might not be basic enough to deprotonate the cyclopropyl proton, a stronger base like lithium diisopropylamide (LDA) was then employed for the epimerization. To allow for easy monitoring of the reaction, pure carboxylates **46** and **47** were used in two parallel experiments. It was found that *endo*-carboxylate **47** was partly converted to *exo*-carboxylate **46** in 20 minutes (Scheme

47). In contrast, *exo*-carboxylate **46** virtually remained intact under these conditions.

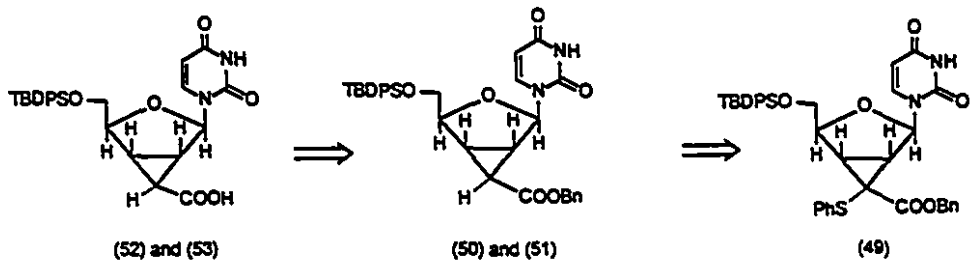
Scheme 47



2.11. Synthesis of Cyclopropyl Carboxylic Acids

After our success in generating the cyclopropyl carboxylate through the thiophenoxyacetate approach, we then carried out the synthesis of the cyclopropyl acids **52** and **53** as the 5'-end building units for later dinucleosides synthesis. Similarly to our previous strategy to prepare the cyano substituted acid **33** (Section 2.8.3.), it was decided to generate the acids from their benzyl ester precursors **50** and **51**, which should be obtained by the radical-mediated desulfurization of the cyclopropanated thiophenoxylate **49** (Scheme 48).

Scheme 48

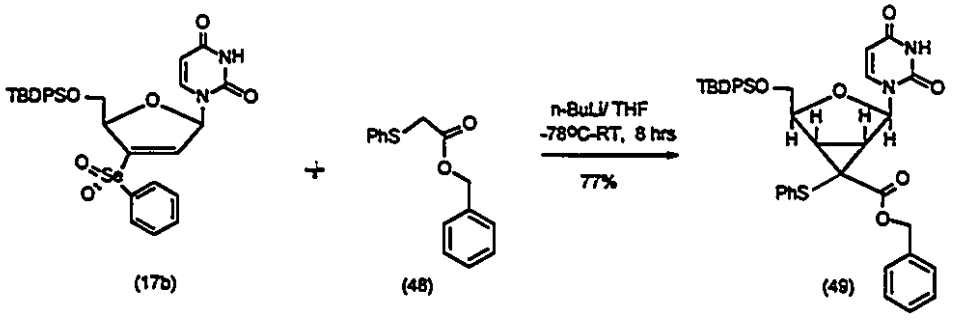


2.11.1. Synthesis of Cyclopropane 49

Similarly to the previous synthesis of cyclopropane **45**, reaction of selenone **17b**

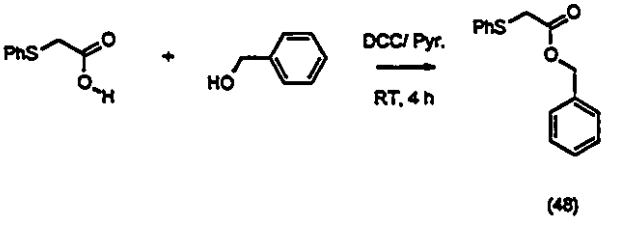
with the anion of benzyl 2-thiophenoxyacetate **48** in dry tetrahydrofuran gave diastereomerically pure cyclopropane **49** as a single product in 77% yield after chromatography (Scheme 49). Cyclopropane **49** was speculated to have an *exo*-carboxylate configuration based on the analysis of the chemical shifts of the H-1' and H-4' protons, which had been previously discussed in Section 2.10.4. and summarized in Table 7. The ^{1}H- and ^{13}C-NMR spectra of cyclopropane **49** were quite similar to those of **46** except for the peaks due to benzyl group. The anomeric proton of **49** was observed at δ 6.01 ppm as a sharp singlet in the ^{1}H-NMR spectrum.

Scheme 49



Benzyl 2-thiophenoxyacetate **48** used in the above cyclopropanation was prepared by coupling of thiophenoxyacetic acid (1 equivalent) and benzyl alcohol (1.1 equivalent) in pyridine, using 1,3-dicyclohexylcarbodiimide (DCC, 1 equivalent) as the coupling reagent (Scheme 50).

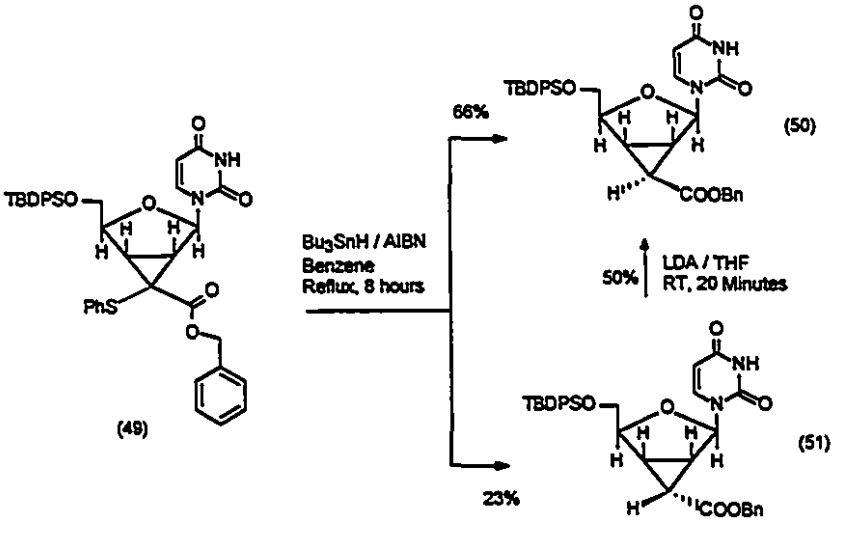
Scheme 50



2.11.2. Desulfurization of Cyclopropane 49

Cyclopropane **49** was then refluxed with tributyltin hydride and AIBN in dry benzene for desulfurization. The starting material **49** was totally consumed in 8 hours, and converted to two new products **50** and **51** with higher polarity. The two products, which had a R_f difference of about 0.15 (EtOAc/Hexanes, 1/1), were then carefully separated by column chromatography to give *exo*-carboxylate **50** and *endo*-carboxylate **51** in a ratio of about 3:1 (Scheme 51, configurational assignments will be discussed later in the same Section). Similar to its methyl analog **47**, the *endo*-carboxylate **51** could be epimerized to its stereoisomer **50** by treating it with lithium diisopropylamide in dry tetrahydrofuran.

Scheme 51



The ^{1}H-NMR spectrum of the major isomer **50** was quite similar to that of the *exo*-carboxylate **46** obtained in the previous Section. The anomeric proton (H-1') was observed as a sharp singlet at δ 6.01 ppm. The newly-generated cyclopropyl proton (H-6') was observed as triplet at δ 1.75 ppm with $J_{2',6'} = 3.4$ Hz and $J_{3',6'} = 3.4$ Hz. The H-2' and H-3' protons of **50** were observed as two sets of doublet of doublets (ABX, $J_{2',3'} =$

6.8 Hz) centered at δ 2.50 ppm and δ 2.46 ppm, respectively.

The ^{1}H-NMR spectrum of the minor isomer **51** was similar to the *endo*-carboxylate **47** obtained in the previous section. The anomeric proton (H-1') in **51** was observed as a sharp singlet at δ 6.31 ppm, and the H-4' was observed as an ABX doublet of doublets centered at 4.39 ppm. Both chemical shifts of the H-1' and H-4' protons were significantly downshifted as compared to their counterparts (δ 6.01 ppm and 4.24 ppm, respectively) in the major stereoisomer **50**. The newly-generated cyclopropyl proton (H-6') was observed as an ABX quartet centered at δ 2.05 ppm, with $J_{2',6'}$ = 7.8 Hz and $J_{3',6'}$ = 8.4 Hz. The H-2' and H-3' protons of isomer **51** appeared as an ABX multiplet centered at δ 2.38 ppm. The ^{1}H-NMR data of carboxylates **50** and **51** are listed in Table 9.

A comparison of the NMR data listed in Table 9 and Table 5 suggested that carboxylate **50** was the analog of *exo*-carboxylate **46** with an *exo*-carboxyl configuration, and carboxylate **51** was, on the other hand, the analog of *endo*-carboxylate **47** with an *endo*-carboxyl configuration. Compared to the chemical shifts of the H-1' and H-4' protons in **50**, the significant downshifts of their counterparts in **51** were observed, which presumably due to the influence of the neighboring *endo*-carboxyl group in **51**. The coupling constants of the H-6' proton in **51** were measured to be 7.8 Hz ($J_{2',6'}$) and 8.4 Hz ($J_{3',6'}$), which were significantly larger than their counterparts in **50** ($J_{2',6'} = J_{3',6} = 3.4$ Hz). According to the molecular mechanics studies, the dihedral angle between H-6' and H-2' in the [3.1.0] bicyclic system was calculated to be ~138° when the H-6' proton was in an *endo* configuration (in other words, H-6' was *trans* to H-2' and H-3'), and was calculated to be ~2° when H-6' was in an *exo*-configuration (in other words, H-6' was *cis* to H-2' and H-3'). Similarly, the dihedral angle between H-6' and H-3' in the [3.1.0] bicyclic system was calculated to be ~138° when H-6' was in an *endo* configuration, and was calculated to be ~3° when H-6' was in an *exo*-configuration. The dihedral angles obtained from the molecular mechanics calculation agreed with the corresponding coupling constants.

Table 9. ^{1}H-NMR assignments of carboxylates **50** and **51**

	Carboxylate 50		Carboxylate 51	
	δ (ppm)*	J (Hz)	δ (ppm)*	J (Hz)
NH	8.71(br)		8.33 (br)	
H-6	7.67 (d, AX)	7.3	7.98 (d, AX)	7.8
Arom.	7.65-7.33 (m)		7.65-7.31 (m)	
H-1'	6.01 (s)		6.31 (s)	
H-5	5.30 (d, AX)	7.3	5.27 (d, AX)	7.8
OCH_2Ph	5.12 (2 x s)		5.18 (2 x s)	
H-4'	4.24 (dd, ABX)	5.4, 5.3	4.39 (dd, ABX)	2.9, 4.4
H-5'a	3.72		3.99 (dd, ABX)	11.2, 2.9
H-5'b	3.71		3.79 (dd, ABX)	11.2, 4.4
H-2'	2.50 (dd, ABX)	6.8, 3.4	2.33 (m, ABX)	7.8, 8.3
H-3'	2.46 (dd, ABX)	6.8, 3.4	2.40 (m, ABX)	7.8, 8.3
H-6'	1.75 (t)	3.4, 3.4	2.05 (t)	8.3, 8.3
t-Butyl	1.06 (s)		1.07 (s)	

* s = singlet, d = doublet, dd = doublet of doublets, m = multiplet, t = triplet.

The configurations of carboxylates **50** and **51** could also be clearly established by NOE studies using a method similar to that discussed in Section 2.12.3. The NOE observations are summarized in Table 10. In the NOESY spectrum of the major isomer **50** (Appendix II. 4.), the newly generated cyclopropane proton H-6' was observed to have strong NOEs with the H-1' and H-4' protons, as well as with the H-2' and H-3' protons. On the other hand, the newly generated cyclopropane proton H-6' of the minor isomer **51** only showed strong NOEs with the H-2' and H-3' protons, but did not showed any

detectable NOEs with the H-1' and H-4' protons in the NOESY spectrum of carboxylate **51** (Appendix II. 5.). Therefore, the major isomer **50** was established as with the benzyl ester in an *exo*-configuration, and the minor isomer **51** was established as with the benzyl ester in an *endo* configuration.

Table 10. NOE observations of carboxylates **50** and **51**

	Carboxylate 50		**Carboxylate 51**	
	δ (ppm)	**NOE**	δ (ppm)	**NOE**
H-1'	6.01	H-2', H-6'	6.31	H-2'
H-4'	4.24	H-3', H-5', H-6'	4.39	H-3', H-5'
H-5'	3.72	H-4'	3.99, 3.79	H-4'
H-2'	2.50	H-1', H-3', H-6'	2.33	H-1', H-3', H-6'
H-3'	2.46	H-2', H-4', H-6'	2.40	H-2', H-4', H-6'
H-6'	1.75	H-1', H-2', H-3', H-4'	2.05	H-2', H-3'

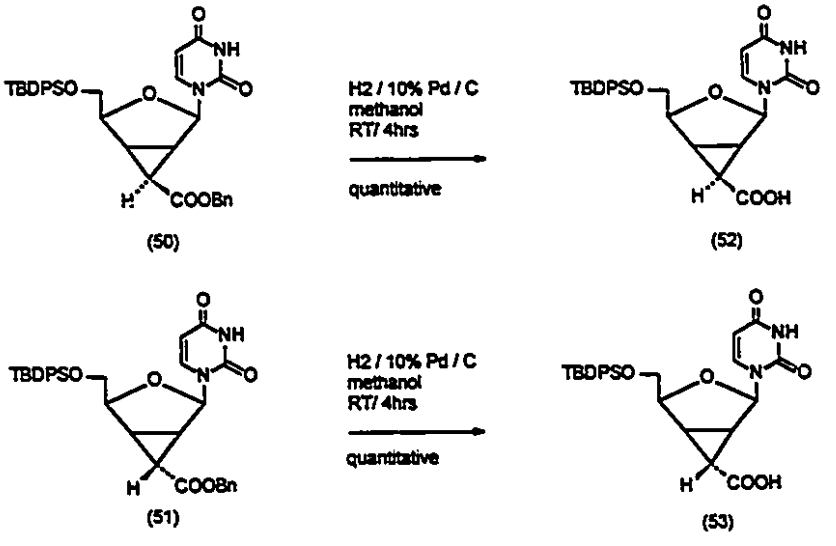
2.11.4. Synthesis of *exo*-Acid 52 and *endo*-Acid 53

Because the R_f difference between carboxylates **50** and **51** was 0.15 (EtOAc / Hexanes, 1 / 1), it was practical to separate these two isomers on an one-gram scale by column chromatography. Besides our initial plan to prepare the *exo*-acid **52** to synthesize the *exo*-amide-linked dimer of type **S1**, we decided to isolate *endo*-carboxylate **51** to prepare another similar dinucleoside analog with a cyclopropanated *endo*-amide linker. Our interest in this type of *endo*-conformer was influenced by the work of Jones et al.[50], who had shown that the DNA strand bearing the *endo* "riboacetal" linker enhanced its

binding to the complementary double stranded DNA. It had been shown in our previous coupling of diacid **24b** with amine **27** (Section 2.7.2.) that the reactivity of the *exo*- and *endo*- acids were very similar in the amide formation when using BOP peptide coupling reagent.

The *exo*-benzyl carboxylate **50** obtained in the previous section was hydrogenated over 10% palladium on carbon in dry methanol for 4 hours to give the corresponding *exo*-acid **52** in a quantitative yield. Similarly, hydrogenation of the *endo*-benzyl ester **51** also proceeded smoothly and yielded the corresponding *endo*-acid **53** quantitatively (Scheme 52). The structures of *exo*-acid **52** and *endo*-acid **53** were confirmed by detailed analysis of ^{1}H- and ^{13}C-NMR spectra as well as mass spectrometry (HRMS, FAB), in which the pseudomolecular ions [M + Na^{+}] of both **52** and **53** were observed at 529.17728.

Scheme 52

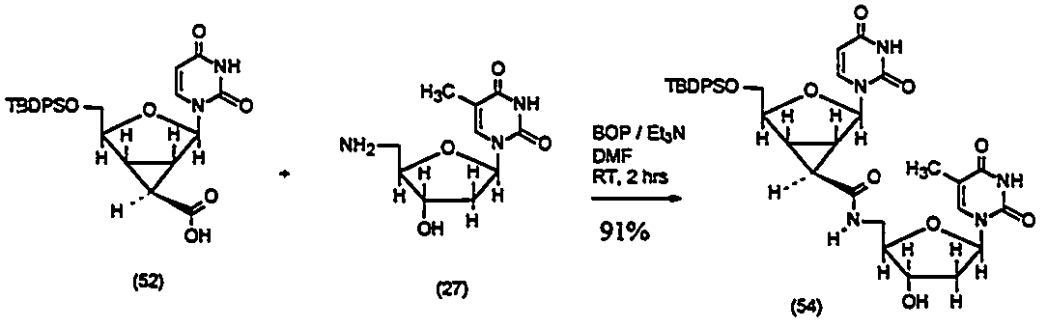


2.12. Synthesis of Dimers Containing the Cyclopropyl Amide Linkers

With the *exo*-acid **52** in hand, we then carried out the synthesis of dinucleoside analog **54** with cyclopropyl *exo*-amide linker. By the same peptide coupling methodology

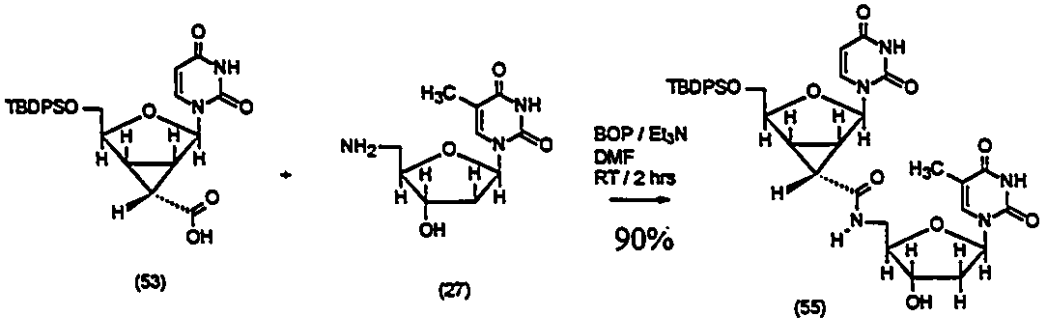
as employed in the previous synthesis of dimer **35** (Section 2.8.), coupling of *exo*-acid **52** (5'-end building unit) and aminothymidine **27** (3'-end building unit) was performed in *N,N*-dimethylformamide in the presence of triethylamine (3 equivalents), using BOP as the coupling reagent. The reaction gave dimer **54** in 91% yield after chromatography (Scheme 53).

Scheme 53



Similarly, dimer **55** with a cyclopropyl *endo*-amide linker was successfully prepared by coupling of *endo*-acid **51** and amine **27** in excellent yield (Scheme 54).

Scheme 54



The ^{1}H-NMR spectra of **54** and **55** (Figure 30 and 31, respectively) clearly indicated the formation of the cyclopropyl amide-linked dinucleosides, in which the

signals corresponding to the upper nucleoside units, lower nucleoside units and the internucleoside linkers were unequivocally assigned (detailed assignments see Table 11). The structures of dimers **54** and **55** were also confirmed by detailed analysis of the ^{13}C-NMR spectra as well as mass spectrometry (FAB, HRMS), in which the pseudomolecular ions [M + Na^+] were observed.

The chemical shifts of the ^{3}H-1' protons (anomeric proton of the lower sugar moiety) are virtually the same (δ 6.18 ppm, doublet of doublets) in the ^{1}H-NMR spectra of both dimers **54** and **55**. The ^{5}H-1' proton (anomeric proton of the upper sugar moiety) of the *exo*-amide-linked dimer **54** is located at δ 6.00 ppm as a sharp singlet, which is relatively upshifted compared to the peak of the ^{3}H-1' proton in the same molecule (Figure 30). Interestingly, the sequence of the chemical shifts of the ^{5}H-1' and ^{3}H-1' protons is just opposite in the ^{1}H-NMR spectrum of *endo*-amide-linked dimer **55** (Figure 31): the ^{5}H-1' proton is located at δ 6.32 ppm, which is relatively downshifted compared to the signal of the ^{3}H-1' proton in the same molecule. From the different sequences of the ^{5}H-1' and ^{3}H-1' signals in the ^{1}H-NMR spectra of dimers, the *exo*-amide-linked and *endo*-amide-linked stereoisomers were easily distinguished.

The downshift of the ^{5}H-1' signal in the ^{1}H-NMR spectrum of the *endo*-amide-linked isomer is presumably due to the influence of the *endo*-carbonyl in **55**, as observed in its carboxylate precursor.

Table 11. ^{1}H-NMR assignments of dimers 54 and 55

	Dimer 54 with Cyclopropyl *exo*-Amide Linker		Dimer 55 with Cyclopropyl *endo*-Amide Linker	
	δ (ppm)*	J (Hz)	δ (ppm)*	J (Hz)
Arom.	7.65-7.37 (m)		7.63-7.33 (m)	
^{5}H-5	5.18 (d)	8.3	5.19 (d)	7.8
^{5}H-6	7.73 (d)	8.3	7.79 (d)	7.8
^{5}H-1'	6.00 (s)		6.32 (s)	
^{5}H-2'	2.44 (ABX)	6.8, 3.4	2.27 (m)	7.8
^{5}H-3'	2.40 (ABX)	6.8, 2.9	2.27 (m)	8.3
^{5}H-4'	4.24 (dd)	4.4, 5.9	4.47 (dd)	5.1, 3.9
^{5}H-5'a	3.80 (dd)	11.7, 4.4	3.82 (dd)	11.3, 5.1
^{5}H-5'b	3.72 (dd)	11.7, 5.9	3.70 (dd)	11.3, 3.9
^{5}H-6'	1.72 (ABX)	2.9, 3.4	2.14 (ABX)	7.8, 8.3
^{3}H-6	7.48 (s)		7.47 (s)	
^{3}H-1'	6.18 (dd)	6.8, 6.8	6.18 (dd)	6.8, 7.2
^{3}H-2'	2.25 (m)		2.27 (m)	
^{3}H-3'	4.27 (m)		4.31 (m)	
^{3}H-4'	3.91 (dd)	3.9, 3.9	3.96 (dd)	
^{3}H-5'	3.50 (d)	3.9	3.52(m)	
^{3}T-CH$_3$	1.90 (s)		1.87 (s)	
t-Butyl	1.04 (s)		1.00(s)	

* s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet.

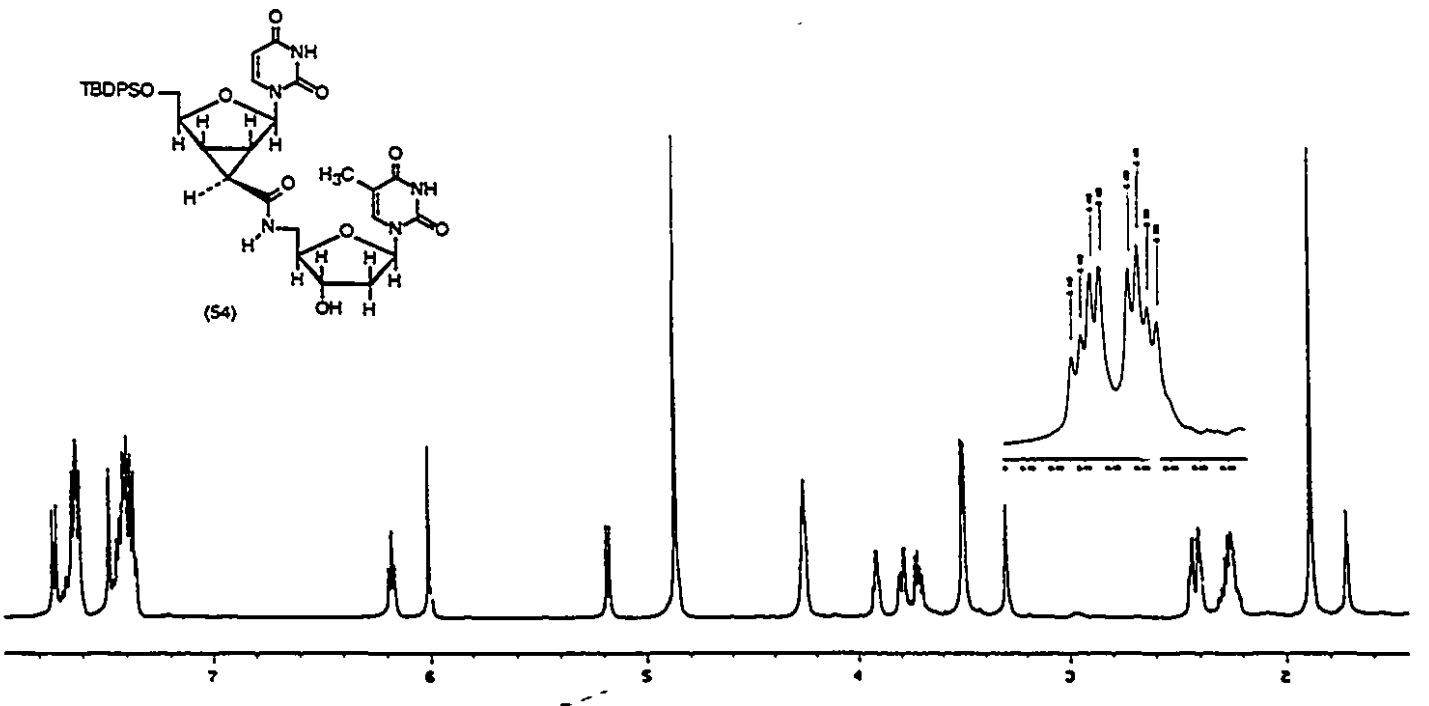


Figure 30. ^{1}H-NMR spectrum of dimer 54 with a cyclopropyl *exo*-amide linker (500 MHz, CD_3OD).

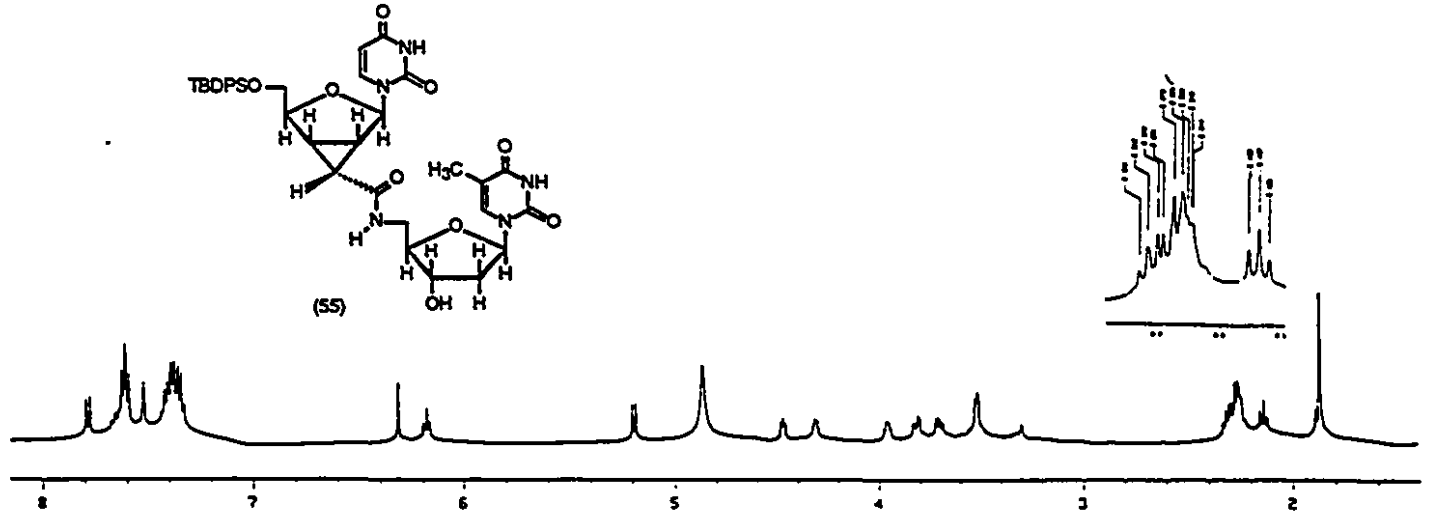


Figure 31. ^{1}H-NMR spectrum of dimer 55 with a cyclopropyl *exo*-amide linker (500 MHz, CD_3OD).

2.13. Binding Properties of DNA Strands Bearing the Cyclopropyl Amide Linkers

2.13.1. Functionalization of Dimers 54 and 55

To allow the incorporation of the dinucleoside analogs into DNA sequences by automated phosphoramidate methodology, appropriate functionalizations of dimers **54** and **55** were required, namely the 5'-end dimethoxytritylation and 3'-end phosphitylation of the dimer. The transformation sequences are outlined in Scheme 55.

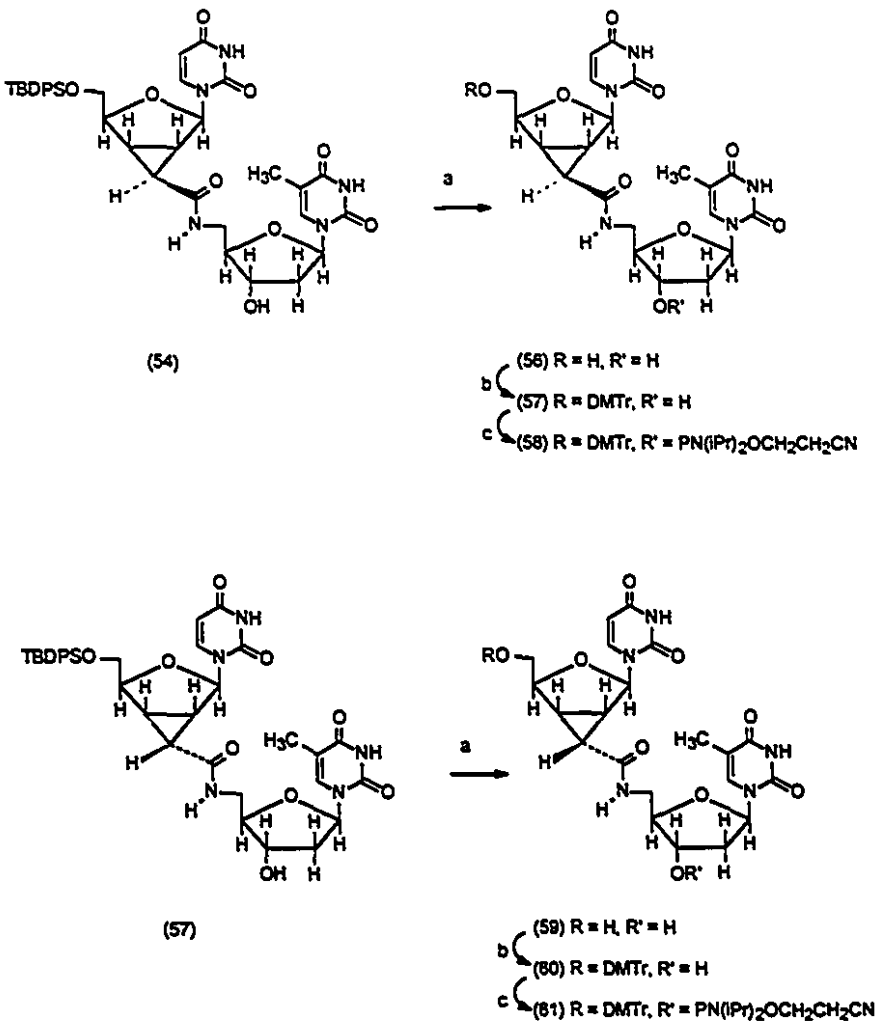
The desilylation of dimer **54** with tetra-*n*-butyl-ammonium fluoride (Bu_4NF) in tetrahydrofuran proceeded smoothly at room temperature, and resulted in diol **56** as a gummy yellow solid. As encountered in the previous desilylation of dimer **35**, attempted purification of diol **56** by chromatography resulted in very poor recovery. Alternatively, trituration of the resulting crude solid with ether followed by removal of the supernatant and repeated washing afforded a fine white solid. The ^{1}H-NMR of this material clearly showed the presence of about one equivalent of a tetra-*n*-butyl ammonium group as an impurity with the crude diol **56** even after repeated washing with ether. As shown in the previous tritylation of diol **39**, the presence of the tetra-*n*-butyl ammonium species does not affect the tritylation of the diol. The crude diol **56** obtained was directly submitted to the reaction with 4,4'-dimethoxytrityl chloride in pyridine at room temperature. The 5'-tritylated dimer **56** was obtained as the main product in excellent yield. The mixture was then separated by chromatography on silica gel to give dimer **57** as a white foam.

Similarly, dimer **55**, which has a cyclopropyl *endo*-amide linker, was first desilylated, followed by tritylation to yield the 5'-tritylated derivative **61** as a white foam in good yield.

Due to the instability of the phosphoramidite structure, the final phosphitylation of the dimers (**57** and **60**) were performed at ISIS Pharmaceuticals by Dr. Y. Sanghvi's

group before the solid-phase DNA synthesis.

Scheme 55



a: 3 eq. TBAF/THF, RT/ 3 hrs, 94% for **56**, 91% for **59**; b: 4 eq. DMTrCl/pyr., RT/ 4 hrs, 84% for **57**, 84% for **60**.

2.13.2. Stability of the Cyclopropyl Amide Linkers

To make sure that the functionalized dimers **58** (cyclopropyl *exo*-amide-linked) and **62** (cyclopropyl *endo*-amide-linked) were fully compatible with the automated DNA

synthesis methodology, the stability of the cyclopropyl amide linkers under standard conditions[55] used in solid-phase automatic DNA synthesis was tested prior to the DNA synthesis. The tests were carried out with diols **56** (cyclopropyl *exo*-amide-linked) and **59** (cyclopropyl *endo*-amide-linked). Diols **56** and **59** were subsequently incubated in the detritylation solution (3% trichloroacetic acid in dichloromethane), coupling solution (0.5 M tetrazole in acetonitrile), oxidation solution (0.1 M iodine in water / pyridine / THF 2/20/80) and the concentrated aqueous ammonia solution under the temperature and duration as listed in Table 12. Both diols **56** and **59** were found to be stable in all tests.

Table 12. Results of the stability tests of the cyclopropyl amide linkers

Solutions used in the tests	Temperature	Duration	**Diol 56**	**Diol 59**
Detritylation solution	RT	24 hrs	Stable	Stable
Coupling solution	RT	24 hrs	Stable	Stable
Oxidation Solution	RT	24 hrs	Stable	Stable
Conc. $NH_3.H_2O$	55°C	12 hrs	Stable	Stable

2.13.3. Effects of the Cyclopropyl-Amide Modifications on DNA's Affinity to RNA and DNA Targets

Using standard automated solid phase DNA synthesis methodology, the functionalized dimers **58** and **62** were then incorporated separately into DNA sequences of type 5'-GCGTTTTTTTTTTGCG-3', where one or three of phosphodiester-linked T-T dimer units of the DNA strand were substituted by the cyclopropyl amide-linked UT dimers. The binding affinity of the modified sequences to the complementary RNA and

DNA targets were then evaluated by thermal denaturation methodology, and the results are summarized in Table 13:

Table 13. Effect of the Cyclopropyl-amide Modifications on DNA's Binding to RNA and DNA Targets with a 5'-GCGTTTTTTTTTGCG-3' Type of Sequence

Type of the Modifications	Mod.[c] No.	vs RNA target			vs DNA target		
		Tm	ΔTm[d]	ΔTm/mod.	Tm	ΔTm[d]	ΔTm/mod.
exo-Amide[a]	1	41.5	-6.05	-6.05	46.9	-5.86	-5.86
exo-Amide[a]	3	19.4	-27.2	-9.07	33.0	-18.4	-6.16
endo-Amide[b]	1	41.6	-7.05	-7.05	45.8	-6.86	-6.86

[a] *exo*-Amide = UT dinucleoside with the cyclopropyl *exo*-amide linker; [b] *endo*-Amide = UT dinucleoside with the cyclopropyl *endo*-amide linker; [c] Mod. = substituting the phosphodiester-linked TT dinucleoside unit in the 5'-GCGTTTTTTTTTGCG-3' sequence with the cyclopropyl amide-linked UT dinucleoside; [d] difference in melting temperature (Tm) between the modified DNA/RNA or DNA/DNA duplex and the unmodified wide type (WT) duplex per modification (ΔTm = Tm - Tm(WT)). The numbers are corrected by taking into account that each phosphodiester-linked dU-T dimer decreases a duplex stability by -0.45°/dimer vs RNA target and -0.64°/dimer vs DNA target.

Substituting one or three of the phosphodiester-linked TT dinucleoside units in sequence 5'-GCGTTTTTTTTTGCG-3' with one or three cyclopropyl-*exo*-amide-linked UT dinucleoside, a drop of 6.05° C or a drop of 27.2° C in Tm was observed, respectively, in the modified DNA's binding towards the RNA complement. Under the same conditions, the modified DNA strands demonstrated a slightly preferred binding to their DNA complement with a drop of 5.84° C and a drop of 18.4° C, respectively. Similarly, a drop of -7.05° C and a drop of -6.86° C in Tm value was observed in the cyclopropyl-*endo*-amide-modified DNA's binding towards its RNA and DNA complements. The above thermal denaturation studies suggested that replacing the

phosphodiester-linked T-T dimer unit in a GCGTTTTTTTTTTGCG DNA strand with the *exo*-amide-linked 2',3'-cyclopropanated U-T dimer disturbed the DNA's binding to its natural DNA and RNA complements in a similar degree. A similar effect was also observed in the hybridization with the *endo*-amide modification.

2.14. Summary

In order to investigate the influence of conformational restriction on DNA's binding affinity, a new type of amide-linked 2',3'-cyclopropanated dinucleoside analog has been synthesized. The rigid modification was meant to reduce the number of the energetically favored conformations of the DNA, thereby preorganizing the DNA strand to its binding conformation. Theoretically, such modification can introduce an entropy advantage for the Watson-Crick base-pairing process.

Initial attempts to add a carbenoid species derived from ethyl diazoacetate to 2',3'-olefinic thymidine analogs proved unsuccessful, presumably due to the electron-deficient nature of the olefin. As an alternative approach, cyclopropanation of vinyl selenone **17b** with benzyl 2-thiophenoxyacetate efficiently generated the 6'-ester-functionalized 2',3'-cyclopropanated uridine analog **49**. Subsequent desulfurization of **49**, followed by hydrogenation gave the cyclopropyl *exo*- and *endo*-acids **52** and **53** as the key 5'-end building units. By standard peptide methodology, acids (**52** and **53**) and amine **27** were coupled to yield the desired *exo*-amide-linked 2',3'-cyclopropanated dinucleoside analog **55** and its *endo*-stereoisomer **56** respectively.

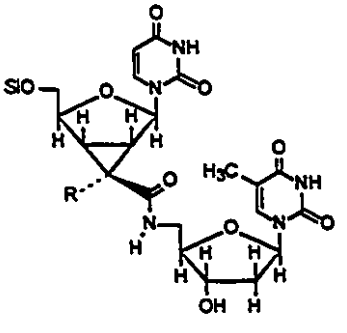
After proper functionalizations, the modified dinucleosides were successfully incorporated into DNA by standard automated methodology. The preliminary thermal denaturation studies showed that replacement of a phosphodiester-linked T-T dimer in a GCGTTTTTTTTTTGCG DNA strand with an *exo*-amide-linked 2',3'-cyclopropanated U-T dimer decreased the DNA's binding affinity to its complementary DNA and RNA targets. Similar effects were observed with the *endo*-amide modification.

3. Contributions to Knowledge

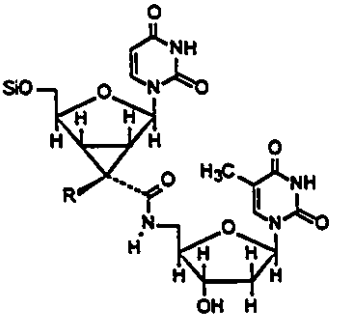
1. A modified synthesis of the α,β-unsaturated selenonyl uridine was developed, which was useful in preparing the functionalized 2',3'-cyclopropanated uridine analogs by the Michael-type cyclopropanation. It was found that the cyclopropanation of the α,β-unsaturated selenonyl uridine with 2-substituted acetates was stereoselective.

2. A number of novel bicyclic uridine analogs were efficiently synthesized, which might be interesting candidates as antiviral agents.

3. A method was developed to prepare the *exo*-acid-functionalized or *endo*-acid-functionalized 2',3'-cyclopropanated uridine analogs, which were used as the 5'-end building blocks to synthesize a novel type of conformationally constrained dinucleoside analogs (e.g. dimers **35**, **54** and **55**) bearing the cyclopropyl amide linkages.



(35) R = CN;
(54) R = H



(55) R = H

4. Experimental

4.1. General Methods

Low resolution chemical ionization (CI), electron ionization (EI) and part of the fast atom bombardment (FAB) mass spectra (MS) were obtained on an KRATOS MS 25RFA and a ZAB 2F HS spectrometers in the direct-inlet mode. All High resolution FAB mass spectra of the key compounds were obtained on a ZAB 2F HS spectrometer in the direct inlet mode (Biomedical Spectrometry Unit) with a resolving power of 10,000.

Thin Layer Chromatography (TLC) was performed using Kieselgel 60 F_{254} aluminum backed plates (0.2 mm thickness). Spot(s) were visualized by UV and / or dipping in solution A followed by heating [Solution A: Ammonium molybdate (2.5 g) and ceric sulfate (1.0 g) in 10% v/v aqueous sulfuric acid (100 ml)]. Kieselgel 60 (230-400 mesh) silica gel was employed for column chromatography. The ratio of silica gel to substance to be purified is approximately 20:1.

Melting points (m. p.) were determined on a Gallenkamp block and are uncorrected.

^{1}H-NMR spectra were recorded on Varian XL-200, Gemini 200 and UNITY 500 spectrometers at 200, 200 and 500 MHz respectively. Peak assignments were made with homonuclear spin (H-H) decoupling experiments and 2D-COrrelation SpectroscopY (COSY, Appendix III). The residual proton signals of chloroform, methanol and pyridine (assigned values of 7.24, 3.30 and 7.17 ppm respectively) were used as reference in these solvents. The multiplicities are recorded using the following abbreviations: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet; br, broad.

^{13}C-NMR spectra were recorded on Varian XL-200, Gemini 200 and Varian UNITY 500 spectrometers at 55.5, 50 and 125.7 MHz, respectively. The ^{13}C signals of $CDCl_3$, CD_3OD and pyridine-d_6 (assigned values of δ 77.00, 49.00 and 123.3 ppm,

respectively) were used as reference in these solvents. Peak assignments were made with 2D-Heteronuclear Multiple Quantum Coherence (HMQC) spectroscopies (Appendix III).

All air sensitive reactions were carried out under N_2 flow with freshly distilled solvents (BDH grade). Pyridine was refluxed for 4 hours over BaO. Triethyl amine (Et_3N) was distilled over CaH_2. Methanol was distilled over magnesium. Anhydrous N,N-dimethylformamide (DMF) was purchased from Aldrich. Tetrahydrofuran (THF) was distilled over sodium benzophenone ketyl.

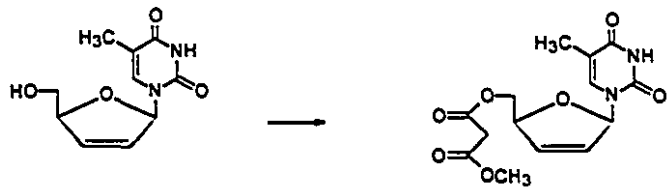
4.2. Experimental

1-[5'-O-*t*-Butyldimethylsilyl-2',3'-ene-β-D-ribofuranosyl] thymine (4)

(3) (4)

A solution of dideoxythymidine 3 (448 mg) and imidazole (136 mg) in 5 ml of DMF was stirred at RT for 15 minutes. The solution was then cooled in an ice bath, followed by addition of *tert*-butyldimethylsilyl chloride (450 mg, 3 mmol) with strong stirring. After 2 hours, the reaction mixture was poured into 50 ml of ice-cold aqueous $NaHCO_3$ (5%), which was extracted with EtOAc (3 x 20 ml). The combined extracts were dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel (EtOAc / hexanes 1:1) to provide 4 as a white foam (635 mg, 94% yield). ^{1}H-NMR ($CDCl_3$) δ: 8.33 (br, 1H) NH; 7.32 (s, 1H) H-6; 6.95 (d, J = 1.5 Hz, 1H) H-1'; 6.27 (d, $J_{2',3'}$ = 5.9 Hz, 1H) H-3'; 5.82 (dd, 1H) H-2'; 4.85 (s, 1H) H-4'; 3.83 (2 x s, 2H) H-5'a, H-5'b; 1.88 (s, 3H) T-CH_3; 0.88 (s, 9H) t-Butyl; 0.06 (s, 6H) $Si(CH_3)_2$; ^{13}C-NMR ($CDCl_3$) δ:136.0, C-6; 134.6, C-2'; 126.3, C-3'; 110.8, C-5; 89.8, C-1'; 87.0, C-4'; 64.6, C-5'; 25.9, t-Butyl; 12.6, T-CH_3; LRMS (FAB, NBA) m/e: 339 ([M + H^+] 1.7), 310 (1.3).

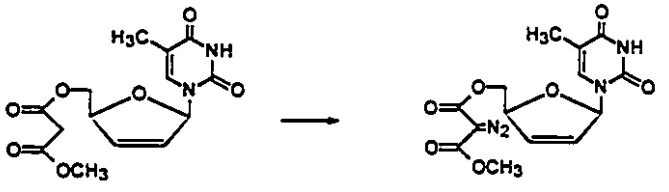
1-[5'-O-Malonate-2',3'-ene-β-D-ribofuranosyl] thymine (6)



(3) (6)

A solution of methyl malonyl chloride (160 µl, 1.5 mmol) and DMAP (11.2 mg, 0.1 mmol) in 5 ml of dry pyridine was stirred at 0°C for 15 minutes, followed by addition of olefin 3 (224 mg, 1 mmol). The reaction temperature was raised to RT, and the stirring was continued for 20 hours. The solution was then cooled with an ice-bath, and water (1 ml) was added. After stirring for another half hour, the product mixture was evaporated to dryness. The residue was then chromatographed on silica gel to give malonate **6** as a white foam (195 mg, 60% yield). ^{1}H-NMR ($CDCl_3$) δ: 9.71 (br, 1H) NH; 7.12 (d, J =1.3 Hz, 1H) H-6; 6.94 (m, 1H) H-1'; 6.25 (dd, $J_{2',3'}$=6.0 Hz, $J_{3',4'}$=1.6 Hz, 1H) H-3'; 5.87 (dd, $J_{2',1'}$=1.4 Hz, 1H) H-2'; 5.00 (m, 1H) H-4'; 4.40 (dd, $J_{5'a,5'b}$=12.3 Hz, $J_{5'a,4'}$=4.4 Hz, 1H) H-5'a; 4.24 (dd, $J_{5'b,4'}$=3.3 Hz, 1H) H-5'b; 3.69 (s, 3H) OCH_3; 3.36 (s, 2H) $CH_2(CO)_2$; 1.85 (s, 3H) T-CH_3; ^{13}C-NMR ($CDCl_3$) δ: 166.4, COOR; 166.0, COOR'; 164.0, C-4; 150.9, C-2; 135.3, C-6; 132.9, C-2'; 127.2, C-3'; 111.1, C-5; 89.7, C-1'; 83.7, C-4'; 65.5, C-5'; 52.6, OCH_3; 40.9, CH_2(COOR)COOR'; 12.4, T-CH3; LRMS (FAB, glycerol) m/e: 347 ([M + Na^+], 9), 325 ([M + H^+], 80), 185 (100).

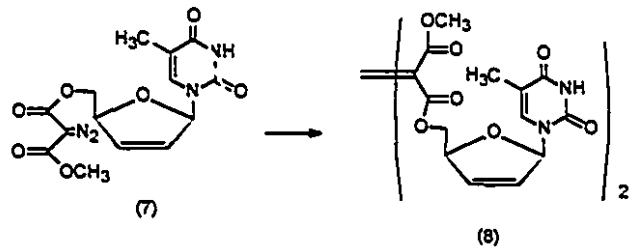
1-[5'-O-Diazomalonate-2',3'-ene-β-D-ribofuranosyl] thymine (6)



(6) (7)

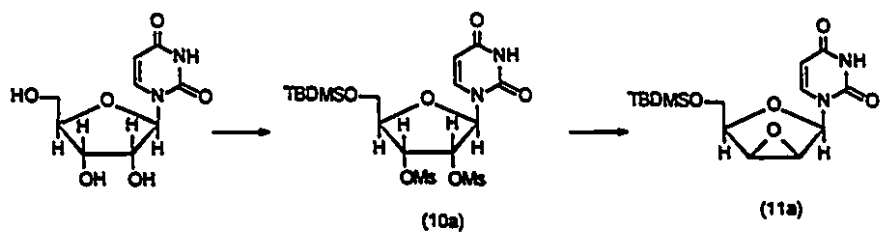
p-Carboxylbenzenesulfonazide (114 mg, 0.5 mmol) was suspended in a stirred solution of malonate **6** (129 mg, 0.4 mmol) in 2 ml of acetonitrile. The mixture was then cooled in an ice bath, followed by addition of 207 µl of triethylamine (1.5 mmol), upon which the azide went into solution. The ice bath was removed and the stirring was continued for 1 hour. The reaction mixture was then poured into 20 ml of EtOAc, and washed with saturated $NaHCO_3$ solution (3 x 10 ml). After drying over $MgSO_4$, the EtOAc layer was evaporated, and the residue was chromatographed on silica gel to give diazomalonate **7** as a white foam (132 mg, 94%). ^{1}H-NMR ($CDCl_3$) δ: 7.11 (br, 1H) NH; 6.91 (dd, $J_{2',1'}$=1.9 Hz, 1H) H-1'; 6.27 (dd, $J_{2',3'}$=6.0 Hz, $J_{3',4'}$=1.4 Hz, 1H) H-3'; 5.88 (dd, 1H) H-2'; 4.99 (m, 1H) H-4'; 4.40, 4.38 (2 x s, 2H) H-5'a, H-5'b; 3.75 (s, 3H) OCH_3; 1.79 (s, 3H) T-CH_3; ^{13}C-NMR ($CDCl_3$) δ: 164.0, C-4; 161.0, 160.5, 2 x CO; 150.9, C-2; 135.2, C-6; 132.8, C-2'; 127.1, C-3'; 110.0, C-5; 89.6, C-1'; 83.5, C-4'; 65.6, C-5'; 52.3, OCH_3; 12.0, T-CH_3; LRMS(FAB, NBA) m/e: 351 ([M + H$^+$] 14), 289 (20.6), 225 (3.8); IR: 2132.1 cm^{-1}, diazo group.

Dimerized adduct (8)



(7) (8)

A solution of 105 mg of diazomalonate 7 in 10 ml of dry CH_2Cl_2 was added 3 mg of $[Rh(OAc)_2]_2$ at RT. After stirring for 20 hours, the reaction mixture was filtered through celite to remove the $[Rh(OAc)_2]_2$. The CH_2Cl_2 was then removed to give dimer 8 as a glass (90 mg, 93% yield). ^{1}H-NMR ($CDCl_3$) δ: 7.29, 7.20 (2 x s, 2H) H-6(a), H-6(b); 7.04 (m, 2H) H-1'(a), H-1'(b); 6.25 (m, 2H) H-3'(a), H-3'(b); 5.95 (m, 2H) H-2'(a), H-2'(b); 5.07 (m, 2H) H-4'(a), H-4'(b); 4.72-4.40 (m, 4H) H-5'$_a$, H-5'$_b$ (a), H-5'$_a$, H-5'$_b$(b); 3.81, 3.79 (2 x s, 2 x 3H) 2 x OCH_3; 2.02, 1.96 (2 x s, 2 x 3H) 2 x CH_3; LRMS (FAB, NBA) m/e: 645 ([M + H$^+$] 0.7).



(10a) (11a)

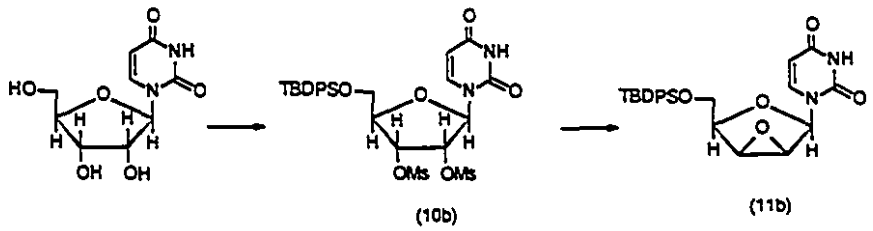
1-[5'-O-(tert-Butyldimethylsilyl)-2',3'-di-O-mesyl-β-D-ribofuranosyl] uracil (10a)

To a solution of uridine (4.88 g) and DMAP (0.244 g) in dry pyridine (20 ml) at 0°C was added *tert*-butyldimethylsilyl chloride (3.30g, 22 mmol) in small portions. After 1 hour, MsCl (4.63 ml, 60 mmol) was added, and the reaction mixture was stirred at 0 °C for another 2 hours. Water (5 ml) was then added. After stirring at 0 °C for 1 hour, the cold solution was poured slowly into a 600 ml ice water with vigorous stirring. The

resulting precipitate was then filtered, and washed with large amount of cold water until it was free of pyridine. The precipitate was then crystallized from methanol to provide dimesylate **10a** as white crystals. (8.6 g, 84% yield). ^{1}H-NMR ($CDCl_3$) δ: 8.82 (br, 1H) NH; 7.88 (d, $J_{5,6}$=8.1 Hz, 1H) H-6; 6.21 (d, $J_{1',2'}$= 3.8 Hz) H-1'; 5.76 (d, 1H) H-5 ; 5.22 (m, 2H) H-2',H-3'; 4.42 (m, 1H) H-4'; 4.03 (dd, $J_{5',5''}$= 12.2 Hz, 1H) H-5'; 4.03 (dd, 1H) H-5''; 3.22, 3.19 (2 x s, 2 x 3H) 2 x CH_3SO_2OR; 0.94 (s, 9H) t-Butyl; 0.16 (s, 6H) $Si(CH_3)_2$; LRMS (FAB, NBA) m/e: 515 ([M + H$^+$] 0.9), 401 (13.4), 154; m.p. (dec.): 158 °C.

1-[5'-O-(tert-Butyldimethylsilyl)-2',3'-epoxy-β-D-ribofuranosyl] uracil (11a)

The dimesylate **10a** (8.2 g) was added to 48 ml of aqueous NaOH (1N). After stirring at RT for 2 hours, the mixture was diluted with 500 ml of ice-water, and neutralized with 0.1 N HCl to pH 8-9 gave a crystalline solid. The solid was filtered, washed with water, and then crystallized from 95% ethanol to give epoxide **11a** as white crystals (4.24 g, 78%). ^{1}H-NMR ($CDCl_3$) δ: 7.60 (d, $J_{5,6}$=8.2 Hz, 1H) H-6; 6.18 (s, 1H) H-1'; 5.75 (d, 1H) H-5; 4.12 (dd, 1H) H-4'; 4.10-3.80 (m, 4H) H-2', H-3', H-5_a', H-5_b'; 0.92 (s, 9H) t-Butyl; 0.10 (s, 6H) $Si(CH_3)_2$; LRMS (FAB, NBA) m/e: 341 ([M + H$^+$] 100); m.p.: 153- 155 °C.



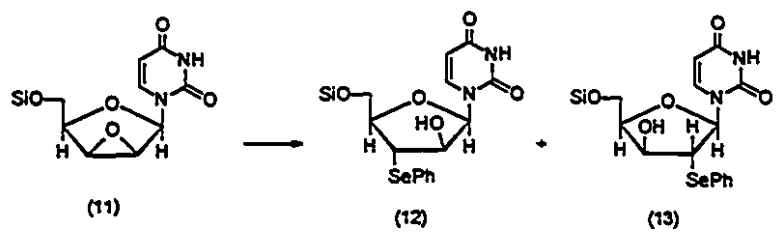
1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-di-O-mesyl-β-D-ribofuranosyl] uracil (10b)

To a solution of uridine (4.88 g) and DMAP (0.244 g) in dry pyridine (20 ml) at 0 °C was added *t*-butydiphenylsilyl chloride (3.30 g) in small portions. The ice bath was removed, and the mixture was stirred at room temperature for 5 hours. The solution was then cooled in an ice bath, followed by the addition of methanesulfonyl chloride (4.63 ml). After stirring for another 2 hours, water (5 ml) was added. The mixture was continued at 0 °C for another hour. The cold solution was then poured slowly into a 600 ml of ice water with vigorous stirring. The resulting precipitate was collected, and washed with large amount of cold water until it was free of pyridine. The precipitate was then crystallized from methanol to provide dimesylate **10b** as white crystals (10.5 g, 82% yield). ^{1}H-NMR ($CDCl_3$) δ: 9.31 (br, 1H) NH; 7.34-7.70 (m, 11H) H-6 + Arom.; 6.09 (d, $J_{1',2'}$= 3.9 Hz) H-1'; 5.22-5.33 (m, 3H) H-5, H-2', H-3'; 4.33 (m, $J_{3',4'}$= 5.2 Hz, $J_{4',5'}$= 1.8 Hz, $J_{4',5''}$= 1.9 Hz) H-4'; 4.10 (dd, $J_{5',5''}$ = 12.4 Hz, 1H) H-5'; 3.93 (dd, 1H) H-5''; 3.18 , 3.11 (2 x s, 2 x 3H) 2 x CH_3SO_2OR; 1.02 (s, 9H) *t*-Butyl; ^{13}C-NMR ($CDCl_3$) δ: 161.8, C-4; 149.6, C-2; 138.5, C-6; 127.5-135.2, Arom.; 103.1, C-5; 87.3, C-1'; 82.5, C-4'; 78.1, C-2'; 73.6, C-3'; 62.1, C-5'; 39.4, 39.3, 2 x CH_3SO_2OR; 27.7, CH_3; 20.1, CMe_3; LRMS (FAB, NBA) m/e: 639 ([M + H$^+$] 8.8), 581 (16.8), 561 (24.5); m.p.(dec.): 152 °C.

1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-epoxy-β-D-ribofuranosyl] uracil (11b)

The dimesylate **10b** (9.60 g) was added to 45 ml of aqueous NaOH (1N). After stirring at RT for 2 hours, the mixture was diluted with 500 ml of ice-water, and neutralized with 0.1 N HCl to pH 8-9 gave a crystalline solid as the crude **11b**. The solid

was collected, washed with water, and then crystallized from 95% ethanol to provide epoxide **11b** as white crystals (6.2 g, 89% yield). ^{1}H-NMR ($CDCl_3$) δ: 8.71 (br, 1H) NH; 7.33-7.70 (m, 11H) H-6 + Arom.; 6.11 (s, 1H) H-1';5.58 (d, $J_{5,6}$= 7.62 Hz, 1H) H-5; 3.80-4.09 (m, 5H) H-2', H-3', H-4', H-5', H-5'';1.02 (s, 9H) *t*-Butyl; ^{13}C-NMR ($CDCl_3$) δ: 162.7, C-4; 150.3, C-2; 140.7, C-6; 135.8-127.6, Arom.; 102.6, C-5; 81.7, C-1'; 77.5, C-4'; 61.8, C-5'; 56.1, C-2'; 55.9, C-3'; 26.7, CH_3; 19.1, $SiCMe_3$; LRMS (FAB, NBA) m/e: 487 ([M + Na^+] 100), 465 ([M + H^+] 19.3); m.p.: 164 - 169 °C.



1-[5'-O-(tert-Butyldimethylsilyl)-3'-deoxy-3'-phenylseleno-β-D-arabinofuranosyl] uracil (12a) and 1-[5'-O-(tert-Butyldimethylsilyl)-2'-deoxy-2'-phenylseleno-β-D-xylofuranosyl] uracil (13a) (from epoxide **11a**, Si = TBDMS)

To a solution of diphenyldiselenide (4.99g, 16 mmol) in dry THF (60 ml) was added lithium aluminum hydride (0.46 g, 12 mmol) in portions under nitrogen at 0 °C. The ice bath was removed, and the mixture was allowed to stir at RT for half hour. A solution of epoxide **11a** (3.41 g, 10 mmol) in dry THF (35 ml) was then added using a syringe pump, and the stirring was continued until no epoxide **11a** was left (ca. 3 hours). The reaction mixture was poured slowly into a saturated NH_4Cl (50 ml) with stirring, and was extracted with EtOAc (3 x 50 ml). The combined extracts were evaporated to dryness to give a mixture of regioisomers **12a** and **13a**, which were then carefully separated by chromatography to yield 3'-selenyl uridine **12a** (2.5g , 51% yield) as a major isomer, and

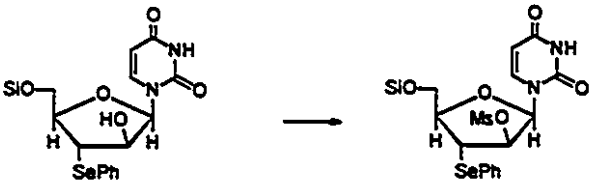
2'-selenyl uridine **13a** (1.6g , 33% yield) as a minor isomer. **Selenide 12a:** ^{1}H-NMR ($CDCl_3$) δ: 8.00 (d, $J_{5,6}$= 8.1 Hz, 1H) H-6; 7.66-7.28 (m, 5H) Arom.; 6.10 (d, $J_{1',2'}$=4.8 Hz, 1H) H-1'; 5.65 (d, 1H) H-5; 4.38 (m, 1H) H-3'; 4.18 (br, 1H) OH; 4.06-3.84 (m, 3H) H-4', H-5'a, H-5'b; 3.54 (m, 1H) H-2'; 0.90 (s, 9H) t-Butyl; 0.10 (s, 6H) $Si(CH_3)_2$; ^{13}C-NMR ($CDCl_3$) δ: 141.3, C-6; 135.8-129.0, Arom.; 101.9, C-5; 86.2, C-1'; 83.8, C-4'; 76.8, C-3'; 63.0, C-5'; 44.2, C-2'; 27.4, t-Butyl, $Si(CH_3)_2$; 20.0, CMe_3; LRMS (FAB, NBA) m/e: 499 ([M + H$^+$] 51.5), 387 (25.8), 195 (100). **Selenide 13a:** ^{1}H-NMR ($CDCl_3$) δ: 8.84 (br, 1H) NH; 7.75 (d, $J_{5,6}$= 8.1 Hz, 1H) H-6; 7.62-7.28 (m, 5H) Arom.; 6.09 (d, $J_{1',2'}$ = 4.1 Hz, 1H) H-1'; 5.63 (d, 1H) H-5; 4.37 (dd, 1H) H-2'; 4.27-4.07 (m, 3H) H-4', H-5'a, H-5'b; 3.66(dd, $J_{2',3'}$ = 3.1 Hz, 1H) H-3'; 0.90 (s, 9H) t-Butyl; 0.10 (s, 6H) $Si(CH_3)_2$; ^{13}C-NMR ($CDCl_3$) δ: 163.0, C-4; 150.0, C-2; 140.7, C-6; 135.0-128.7, Arom; 102.4, C-5; 89.2, C-1'; 80.0, C-4'; 77.3, C-2'; 62.6, C-5'; 51.5, C-3'; 25.7, CH_3 ; 18.2, CMe_3; LRMS (CI) m/e: 499 ([M + H$^+$], 7.6), 237 (100).

1-[5'-O-(tert-Butyldiphenylsilyl)-3'-deoxy-3'-phenylseleno-β-D-arabinofuranosyl] uracil (12b) and 1-[5'-O-(tert-Butyldiphenylsilyl)-2'-deoxy-2'-phenylseleno-β-D-xylofuranosyl] uracil (13b) (from epoxide **11b**, Si = TBDPS)

Similarly, reaction of epoxide **11b** (4.64 g, 10 mmol) with phenyl selenium anion in dry THF also yielded a mixture of regioisomeric selenides **12b** and **13b**, which was then separated on silica gel to give the major isomer **12b** (3.5g, 57% yield), and minor isomer **13b** (1.8 g, 30%). **Selenide 12b:** ^{1}H-NMR ($CDCl_3$) δ: 7.99 (d, $J_{5,6}$= 8.1 Hz, 1H) H-6; 7.26-7.64 (m, 15H) Arom.; 6.09 (d, $J_{1',2'}$= 6.1 Hz, 1H) H-1'; 5.32 (d, 1H) H-5; 4.50 (br, 1H) OH; 4.45 (m, 1H) H-3'; 3.87-4.04 (m, 3H) H-4', H-5', H-5" ; 3.88 (dd, 1H) H-2'; 1.02 (S, 9H) CMe_3; ^{13}C-NMR ($CDCl_3$) δ: 162.9, C-4; 150.3, C-2; 140.6, C-6; 125.9-134.9, Arom.; 101.2, C-5; 84.9, C-1'; 82.3, C-4'; 76.2, C-3'; 62.2, C-5'; 43.4, C-2'; 27.5, CH_3; 20.0, CMe_3; LRMS (FAB, NBA) m/e: 623 ([M + H$^+$], 31.4), 545 (8.8), 433 (5.0), 255 (100). **Selenide 13b:** ^{1}H-NMR ($CDCl_3$) δ: 8.58 (br, 1H) NH; 7.70-7.30 (m, 16H)

Arom. + H-6; 6.09 (d, $J_{1',2'}$= 3.7 Hz, 1H) H-1'; 5.47 (d, $J_{5,6}$ = 8.1 Hz, 1H) H-5; 4.39 (dd, J = 2.7 Hz, J = 3.4 Hz, 1H) H-2'; 3.87-4.04 (m, 3H) H-4', H-5', H-5''; 3.71 (dd, J = 3.3 Hz, J = 3.0 Hz, 1H) H-3'; 1.02 (S, 9H) CMe_3; ^{13}C-NMR ($CDCl_3$) δ: 161.8, C-4; 149.2, C-2; 139.9, C-6; 134.8-127.3, Arom.; 102.0, C-5; 89.4, C-1'; 80.4, C-4'; 77.3, C-2'; 63.1, C-5'; 51.6, C-3'; 27.4, CH_3; 19.8, CMe_3; LRMS (FAB, NBA) m/e: 623 ([M + H$^+$], 27.8), 545 (12.1), 433 (8.4), 255 (100).

1-[5'-O-(tert-Butyldimethylsilyl)-3'-deoxy-3'-phenylseleno-2'-O-mesyl-β-D-arabinofuranosyl] uracil (14a, Si = TBDMS), and 1-[5'-O-(tert-Butyldiphenylsilyl)-3'-deoxy-3'-phenylseleno-2'-O-mesyl-β-D-arabinofuranosyl] uracil (14b, Si= TBDPS)



From 2'-β-hydroxyl-3'-selenide 12a (Si = TBDMS):

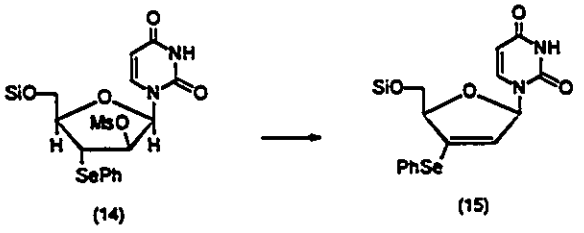
To a solution of 2'-β-hydroxyl-3'-selenyl uridine **12a** (2.0 g) in dry pyridine (50 ml) was added methanesulfonyl chloride (0.78 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 24 hours. Water (1 ml) was then added, and the mixture was stirred at 0 °C for another hour. The solvent was then removed. The residue was redissoved in 100 ml of EtOAc, and washed with 2 x 50 ml of saturated $NaHCO_3$ solution. The EtOAc layer was dried ($MgSO_4$), and evaporated to dryness to give 2'-O-mesylate **14a** as a white foam (2.4 g, 96% yield). ^{1}H-NMR ($CDCl_3$) δ: 7.80 (d, $J_{5,6}$=8.2 Hz, 1H) H-6; 7.67-7.30 (m, 5H) Arom.; 6.17 (d, $J_{1',2'}$=5.6 Hz, 1H) H-1'; 5.68 (d, 1H) H-5; 5.24 (t, $J_{2',3'}$=1.4 Hz, 1H) H-2'; 3.92-3.68 (m, 4H) H-3', H-4', H-5'a, H-5'b; 2.95 (s, 3H) CH_3SO_2OR; 0.88 (s,

9H) t-Butyl; 0.06 (s, 6H) $Si(CH_3)_2$; ^{13}C-NMR ($CDCl_3$) δ: 162.0, C-4; 149.3, C-2; 139.5, C-6; 135.6-124.5, Arom.; 101.8, C-5; 82.1, C-1'; 81.8, C-4'; 81.0, C-2'; 60.8, C-5'; 41.6, C-3'; 39.0, CH_3SO_2OR; 26.4, t-butyl, $Si(CH_3)_2$; 20.0, CMe_3.

From 2'-β-hydroxyl-3'-selenide 12b (Si = TBDPS):

Similarly, 2'-β-hydroxyl-3'-selenyl uridine **12b** (3.10 g) was treated with methanesulfonyl chloride (12.5 ml) to give 2'-O-mesylate **14b** in a clean reaction. After a similar workup as described for **14a**, the residue was dried *in vacuo* to afford mesylate **14b** as a white foam (3.12 g, 90% yield). ^{1}H-NMR ($CDCl_3$) δ: 9.30 (br, 1H) NH; 7.28-7.67 (m, 16H) H-6 + Arom.; 6.18 (d, $J_{1',2'}$= 5.1 Hz, 1H) H-1'; 5.42 (d, $J_{5,6}$= 8.1 Hz, 1H) H-5; 5.27 (t, $J_{2',3'}$= 5.5 Hz, 1H) H-2'; 3.85-4.02 (m, 4H) H-3', H-4', H-5', H-5''; 2.84 (s, 3H) CH_3SO_2OR; 1.08 (s, 9H) *t*-butyl; ^{13}C-NMR ($CDCl_3$) δ: 161.9, C-4; 149.3, C-2; 139.5, C-6; 124.8-135.6, Arom.; 101.8, C-5; 82.5, C-1'; 82.4, C-4'; 81.8, C-2'; 61.9, C-5'; 42.4, C-3'; 38.8, CH_3SO_2OR; 27.5, CH_3; 20.0, CMe_3; MS (FAB-NBA) m/e: 701 [M + H^+].

1-[5'-O-(tert-Butyldimethylsilyl)-2',3'-dideoxy-3'-phenylseleno-β-D-enofuranosyl] uracil (15a, Si = TBDMS), and 1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-dideoxy-3'-phenylseleno-β-D-enofuranosyl] uracil (15b, Si = TBDPS)



From 2'-β-mesylate 14a (Si = TBDMS):

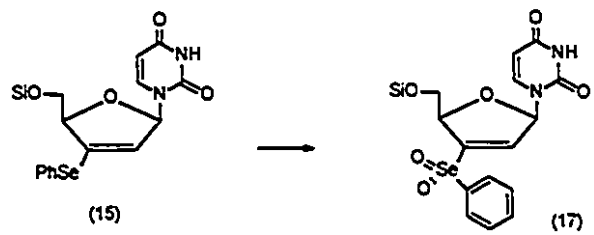
Potassium *tert*-butoxide (1.12 g) was added to a solution of mesylate **14a** (2.00 g)

in 20 ml dry DMF at RT. After 4 hours, the mixture was poured into a saturated NH_4Cl solution (20 ml), and extracted with EtOAc (3 x 10 ml). The combined extracts were dried ($MgSO_4$), and evaporated to dryness. The residue was then redissolved in dry DMF (5 ml), and treated with imidazole (270 mg), and *t*-butyldimethylsilyl chloride (0.63 ml) at 0 °C for 2 hours. The DMF was removed by coevaporating with toluene, and the residue was chromatographed on silica ($MeOH/CH_2Cl_2$, 5/95) to give vinyl selenide **15a** as a white foam (1.3g, 68 % yield). ^{1}H-NMR ($CDCl_3$) δ: 8.00 (br, 1H) NH; 7.78 (d, $J_{5,6}$ = 8.1 Hz, 1H) H-6; 7.62-7.28 (m, 5H) Arom.; 6.88 (m, 1H) H-2'; 5.60 (d, 1H) H-5; 5.36 (d, $J_{1',2'}$=1.7 Hz, 1H) H-1'; 4.80 (m, 1H) H-4'; 3.81-3.67 (m, 2H) H-5'a, H-5'b; 0.86 (s, 9H) t-Butyl; 0.04 $Si(CH_3)_2$; ^{13}C-NMR ($CDCl_3$) δ: 162.6, C-4; 149.9, C-2; 140.0, C-6; 139.0-122.5, Arom., C-2', C-3'; 101.8, C-5; 89.3, C-1'; 88.6, C-4'; 62.3, C-5'; 27.4, CH_3; 20.1, CMe_3.

From 2'-β-mesylate 14b (Si = TBDPS):

Potassium *tert*-butoxide (1.23 g) was added to a solution of mesylate **14b** (3.00 g) in 5 ml dry DMF at 0 °C. After 7 minutes, the mixture was poured into a saturated NH_4Cl solution (20 ml), and extracted with EtOAc (3 x 10 ml). The combined extracts were dried ($MgSO_4$), and evaporated to dryness. The residue was then chromatographed on silica ($MeOH/CH_2Cl_2$, 5/95) to give vinyl selenide **15b** as a white foam (2.27 g, 88% yield).^{1}H-NMR ($CDCl_3$) δ: 9.24 (br, 1H) NH; 7.68 (d, $J_{5,6}$ = 8.1 Hz, 1H) H-6; 7.37-7.67 (m, 15H) Arom.; 6.93 (dd, $J_{2',1'}$= 1.5 Hz, $J_{4',1'}$=0.7 Hz, 1H) H-1'; 5.35 (dd, $J_{4',2'}$=2.0 Hz, 1H) H-2'; 4.95 (d, 1H) H-5; 4.84 (dd, $J_{5',4'}$=3.5 Hz, 1H) H-4'; 4.04-3.91 (m, 2H) H-5'a, H-5'b; 1.02 (s, 9H) t-Butyl; ^{13}C-NMR ($CDCl_3$) δ: 162.2, C-4; 149.8, C-2; 140.0, C-6; 139.4-122.5, Arom., C-2', C-3'; 102.1, C-5; 89.0, C-1'; 88.3, C-4'; 64.3, C-5'; 27.7, CH_3; 20.2, CMe_3; LRMS (FAB, NBA) m/e: 627 [M + Na^+].

1-[5'-O-(tert-Butyldimethylsilyl)-2',3'-dideoxy-3'-phenylselenonyl-β-D-enofuranosyl] uracil (17a, Si =TBDMS), and 1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-dideoxy-3'-phenylselenonyl-β-D-enofuranosyl] uracil (17b, Si = TBDPS)



From vinyl-3'-selenide 15a (Si = TBDMS):

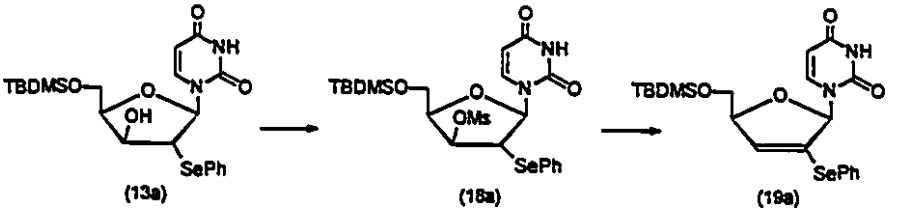
To a solution of 3'-selenyl uridine **15a** (720 mg) in dry methanol (5 ml) at 0°C was added MCPBA (647 mg). After stirring at RT overnight, the reaction mixture was poured into a saturated $NaHCO_3$ solution (100 ml, ice-cold), and extracted with EtOAc (3 x 50 ml). The combined extracts were then dried over $MgSO_4$, evaporated, and chromatographed on silica gel to give selenone **17a** as a white foam (630 mg, 82% yield). ^{1}H-NMR ($CDCl_3$) δ: 9.80 (br, 1H) NH; 7.83 (d, $J_{5,6}$=8.1 Hz, 1H) H-6; 8.02-7.67 (m, 5H) Arom.; 7.03 (d, $J_{2',1'}$=4.1 Hz, 1H) H-1'; 6.71 (d, 1H) H-2'; 5.67 (d, 1H) H-5; 5.15 (dd, $J_{5'a,4'}$=1.6 Hz, $J_{5'b,4'}$=1.2 Hz, 1H) H-4'; 4.18 (d, $J_{5'a,5'b}$=12.1 Hz, 1H) H-5'a; 4.00 (d, 1H) H-5'b; 0.86 (s, 9H) t-Butyl; 0.05 (s, 6H) $Si(CH_3)_2$; ^{13}C-NMR ($CDCl_3$) δ: 162.2, C-4; 149.5, C-2; 139.5, C-6; 147.1-126.2, Arom., C-2', C-3'; 102.9 C-5; 87.4, C-1'; 85.9, C-4'; 63.1, C-5'; 27.6, CH_3; 20.6, CMe_3; LRMS (FAB, NBA) m/e: 513 ([M + H^+], 47.6); 402 (5.2).

From vinyl-3'-selenide 15b (Si = TBDPS):

Similarly, 3'-selenyl uridine **15b** (2.00 g) was oxidized with MCPBA (1.71 g) in methanol to yield the 3'-selenonyl uridine **17b** as a white foam after chromatography (1.77 g, 84%). ^{1}H-NMR ($CDCl_3$) δ: 9.98 (br, 1H) NH; 8.01 (d, $J_{5,6}$ = 6.8 Hz, 1H) H-6;

7.71-7.26 (m, 15H) Arom.; 7.07 (dd, $J_{1',4'}$ = 4.5 Hz, $J_{1',2'}$ = 1.5 Hz, 1H) H-1'; 6.79 (d, 1H) H-2'; 5.15 (dd, $J_{5',4'}$ = 1 Hz, 1H) H-4'; 4.34 (d, $J_{5'',5'}$ = 11.8 Hz, 1H) H-5'a; 4.12 (d, 1H) H-5'b; 1.08 (s, 9H) t-Butyl; ^{13}C-NMR ($CDCl_3$) δ: 163.2, C-4; 150.5, C-2; 140.2, C-6; 138.1-127.0, Arom., C-2', C-3'; 102.6, C-5; 87.3, C-1'; 85.5, C-4'; 63.7, C-5'; 27.6, CH_3; 20.2, CMe_3; LRMS (FAB, NBA) m/e: 637 ([M + H$^+$] 7.4), 579 (23.0), 559 (44.8), 448 (14.1), 447 (47.8), 307 (100).

1-[5'-O-(tert-Butyldimethylsilyl)-2'-deoxy-3'-O-mesyl-2'-phenylseleno-β-D-xylofuranosyl] uracil (18a), and 1-[5'-O-(tert-Butyldimethylsilyl)-2',3'-dideoxy-2'-phenylseleno-β-D-enofuranosyl] uracil (19a)

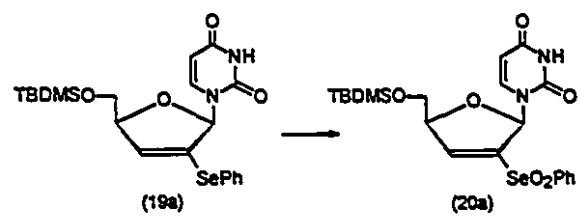


To a solution of 3'-β-hydroxyl-2'-selenyl uridine **13a** (0.995 g, 2.0 mmol) in dry pyridine (25 ml) at 0°C was added MsCl (0.4 ml, 5.0 mmol), and the reaction mixture was stirred at 0°C for 24 hours. Water (1 ml) was then added, and the mixture was stirred at 0°C for another hour. The solvent was then removed. The residue was redissoved in 20 ml of EtOAc, and washed with 2 x 10 ml of saturated $NaHCO_3$ solution. The organic layer was dried ($MgSO_4$), and evaporated to dryness to give the crude mesylate **18a** as a slightly yellow solid.

The mesylate **18a** obtained was then dissolved in 5 ml of dry DMF, and treated with potassium *tert*-butoxide (0.56 g) at RT for 4 hours. The mixture was poured into a saturated NH_4Cl solution (15 ml), and extracted with EtOAc (3 x 10 ml). The combined extracts were dried ($MgSO_4$), and evaporated to dryness. The residue was then

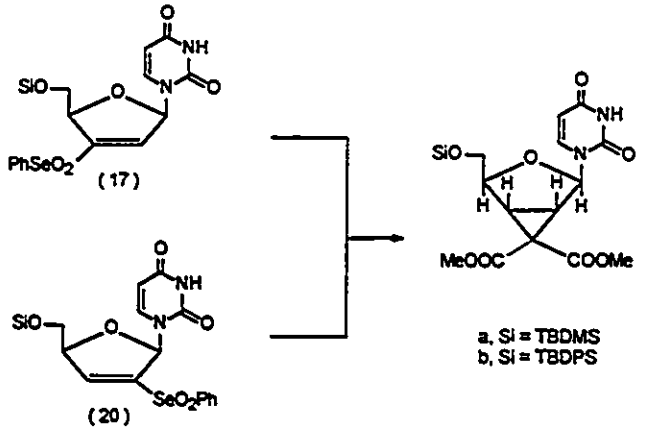
chromatographed on silica ($MeOH/CH_2Cl_2$, 5/95) to give vinyl selenide **19a** as a white foam (0.90 g, 95% yield from **13a**). ^{1}H-NMR ($CDCl_3$) δ: 7.74 (d, $J_{5,6}$= 8.1 Hz, 1H) H-6; 7.56-7.28 (m, 5H) Arom.; 6.92 (dd, J = 1.4 Hz, J = 3.4 Hz, 1H) H-1'; 6.07 (dd, J =1.6 Hz, J = 1.6 Hz, 1H) H-3'; 5.51 (d, 1H) H-5; 4.86 (m, 1H) H-4'; 3.88 (dd, $J_{5'a,5'b}$=11.8 Hz, $J_{5'a,4'}$=2.5 Hz, 1H) H-5'a; 3.78 (dd, $J_{5'b,4'}$ = 2.5 Hz, 1H) H-5'b; 0.89 (s, 9H) t-Butyl; 0.06 $Si(CH_3)_2$; ^{13}C-NMR ($CDCl_3$) δ: 139.9, C-6; 134.8-128.5, Arom., C-2', C-3'; 102.1, C-5; 90.9, C-1'; 87.0, C-4'; 64.2, C-5'; 26.4, CH_3; 19.2, $C(Me)_3$; MS (FAB, NBA) m/e: 481 ([M + H$^+$], 5.2), 237 (100).

1-[5'-O-(tert-Butyldimethylsilyl)-2',3'-dideoxy-2'-phenylselenonyl-β-D-enofuranosyl] uracil (20a)



To a solution of 3'-selenyl uridine **19a** (720 mg, 1.5 mmol) in dry methanol (5 ml) was added MCPBA (647 mg, 3.75 mmol) at 0°C. After stirring at RT overnight, the reaction mixture was poured into a saturated $NaHCO_3$ (100 ml, ice-cold), and extracted with EtOAc (3 x 50 ml). The combined extracts were dried over $MgSO_4$, evaporated, and chromatographed on silica gel to give selenone **20a** as a white foam (690 mg, 90% yield). ^{1}H-NMR ($CDCl_3$) δ: 8.81(br, 1H) NH; 7.95-7.59 (m, 6H) Arom., H-6; 7.42 (br, 1H) H-1'; 7.25 (d, $J_{4',3'}$ = 3.8 Hz, 1H) H-3'; 5.39 (d, 1H) H-5; 5.08 (m, 1H) H-4'; 4.00 (s, 2H) H-5'; 0.88 (s, 9H) *t*-Butyl; 0.07(s, 6H) $Si(CH_3)_2$; ^{13}C-NMR ($CDCl_3$) δ: 162.7, C-4; 150.0, C-2; 141.1, C-6; 146.0-126.9, Arom., C-2', C-3'; 103.3, C-5; 86.5, C-1'; 86.4, C-4'; 63.6, C-5'; 25.9, CH_3; 18.7, $C(Me)_3$; LRMS (FAB, NBA) m/e: 513 ([M + H$^+$], 5).

1-[5'-O-(tert-Butyldimethylsilyl)-2',3'-dideoxy-2',3'-(1,1-dimethoxycarbonyl)cyclopropane-β-D-ribofuranosyl] uracil (21a, Si = TBDMS), and 1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-dideoxy-2',3'-(1,1-dimethoxycarbonyl)cyclopropane-β-D-ribofuranosyl] uracil (21b, Si = TBDPS)



From vinyl 3'-selenonyl uridine 17a (Si = TBDMS):

Dimethyl malonate (106 mg) in dry THF (5 ml) was treated with potassium *t*-butoxide (90 mg) at RT for 30 mins. The solution was then cooled in in an ice bath. After the addition of 3'-selenonyl uridine **17a** (102 mg), the ice bath was removed, and the stirring was continued at RT for 2 hours. The solution was then diluted with saturated NH_4Cl (5 ml), and extracted with EtOAc (3 x 5 ml). The combined extracts were dried ($MgSO_4$), evaporated, and chromatographed on silica gel (CH_2Cl_2 first, then with CH_2Cl_2 / MeOH: 97/3) to give the cyclopropane **21a** as a white foam (79 mg, 87%).

From vinyl 2'-selenonyl uridine 20a (Si = TBDMS):

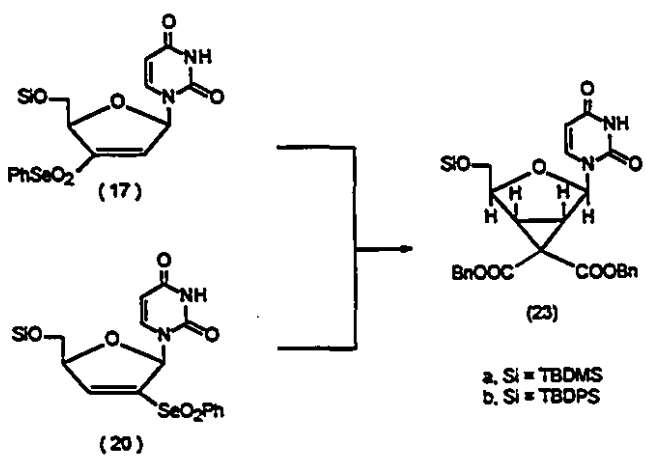
Similarly, reaction of 2'-selenonyl uridine **20a** (51 mg) with dimethyl malonate (53 mg) in dry THF (2 ml) gave the same cyclopropane **21a** as a white foam after chromatography (38 mg, 85%). ^{1}H-NMR ($CDCl_3$) δ: 9.52 (br, 1H) NH; 7.80 (d, $J_{5,6}$ = 8.2 Hz, 1H) H-6; 6.06(s, 1H) H-1'; 5.68 (d, 1H) H-5; 4.41 (m, 1H) H-4'; 3.80, 3.74(2 x s,

2 x 3H) OCH_3; 3.93-3.62 (m, 2H) H-5',H-5''; 2.82 (d, $J_{2',3'}$= 6.8 Hz, 1H) H-2'; 2.56(d, 1H) H-3'; 0.87(s, 9H) *t*-Butyl; 0.05 (s, 6H) *t*-Butyl; LRMS (FAB, NBA) m/e: 455.24 ([M + H^+], 9.3).

From vinyl 3'-selenonyl uridine 17b (Si = TBDPS):

Similarly, reaction of 3'-selenonyl uridine **17b** (64 mg) with dimethyl malonate (53 mg) in dry THF (2 ml) gave cyclopropane **21b** as a white foam after chromatography (46 mg, 80% yield). ^{1}H-NMR ($CDCl_3$) δ: 7.63-7.35 (m, 10H) Arom.; 7.51 (d, $J_{5,6}$= 7.8 Hz, 1H) H-6; 6.07 (s, 1H) H-1'; 5.43 (d, 1H) H-5; 4.46 (t, $J_{4',5'}$= $J_{4',5''}$ = 5.8 Hz, 1H) H-4'; 3.81, 3.67 (2 x s, 2 x 3H) OCH_3; 3.80-3.67 (m, 2H) H-5', H-5''; 2.73 (d, $J_{2',3'}$= 6.3 Hz, 1H) H-2'; 2.55 (d, 1H) H-3'; 1.04 (s, 9H) t-Butyl.

1-[5'-O-(tert-Butyldimethylsilyl)-2',3'-dideoxy-2',3'-(1,1-dibenzyloxycarbonyl) cyclopropane-β-D-ribofuranosyl] uracil (23a, Si = TBDMS), and 1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-dideoxy-2',3'-(1,1-dibenzyloxycarbonyl)cyclopropane-β-D-ribofuranosyl] uracil (23b, Si = TBDPS)



From vinyl 3'-selenonyl uridine 17a (Si = TBDMS):

A solution of dibenzyl malonate (1.24 g, 4 mmol) in 10 ml of dry THF (10 ml) was treated with potassium *tert*-butoxide (448 mg, 4 mmol) at RT for 30 minutes, and then cooled in an ice bath. 3'-selenonyl uridine **17a** (511 mg, 1 mmol) was then added with stirring, and the ice bath was removed. After stirring at RT for 2 hours, the solution was poured into saturated NH_4Cl (50 ml), and extracted with EtOAc (3 x 50 ml). The combined extracts were dried ($MgSO_4$), evaporated, and chromatographed on silica gel (CH_2Cl_2 first, then with CH_2Cl_2 / MeOH: 97/3) to give cyclopropane **23a** as a white foam (516 mg , 85% yield).

From vinyl 2'-selenonyl uridine 20a (Si = TBDMS):

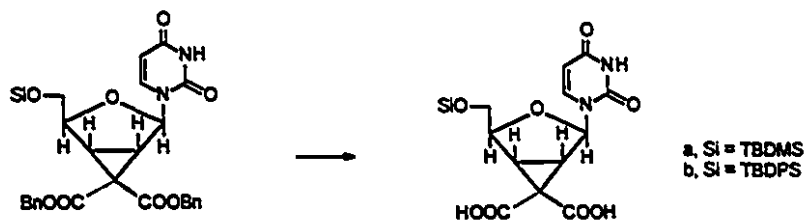
Similarly, reaction of 2'-selenonyl uridine **20a** (51 mg, 0.1 mmol) with dibenzyl malonate (114 mg, 0.4 mmol) in dry THF (2 ml) gave the same cyclopropane **23a** as a white foam after chromatography (49 mg, 80% yield). ^{1}H-NMR ($CDCl_3$) δ: 8.45 (br, 1H) NH; 7.82 (d, $J_{5,6}$=8.2 Hz, 1H) H-6; 7.45-7.25 (m, 10H) Arom; 6.15 (s, 1H) H-1'; 5.68 (d, 1H) H-5; 5.21, 5.14 (2 x s, 2 x 2H) 2x OCH_2Ph; 4.43 (m, 1H) H-4'; 3.73 (2 x d, 2H) H-5',H-5''; 2.82 (d, $J_{2',3'}$ = 6.8 Hz, 1H) H-2'; 2.61 (d, 1H) H-3'; 0.87 (s, 9H) t-Butyl; 0.04 (s, 6H) $Si(CH_3)$; ^{13}C-NMR ($CDCl_3$) δ: 167.8, 164.9, 162.5, 2 x COOBn, C-4; 149.9, C-2; 140.3, C-6; 134.4-127.8, Arom.; 101.8, C-5; 86.2, C-1'; 82.5, C-4'; 68.1, 68.0, 2 x OCH_2Ph; 64.5, C-5'; 35.8, C-2'; 33.7, C-3'; 26.0, CH_3; 18.7 CMe_3.

From vinyl 3'-selenonyl uridine 17b (Si = TBDPS):

Similarly, reaction of 5'-*t*-butyldiphenylsilyl-3'-selenonyl uridine **17b** (1.27 g, 2 mmol) and dibenzyl malonate (1.24 g) in dry THF gave cyclopropane **23b** as a white foam after chromatography (1.26 g, 87% yield). ^{1}H-NMR ($CDCl_3$) δ: 9.35 (br, 1H) NH; 7.63-7.20 (m, 21H) Arom. + H-6; 6.16 (d, $J_{2',1'}$=1.2 Hz, 1H) H-1'; 5.35 (d, $J_{5,6}$=8.0 Hz, 1H) H-5; 5.23, 5.16 (2 x s, 2 x 2H) 2x OCH_2Ph; 4.52 (t, 1H) H-4'; 3.73, 3.70 (2 x s, 2H) H-5',H-5''; 2.80 (dd, $J_{2',3'}$=6.6 Hz, 1H) H-2'; 2.64 (d, 1H) H-3'; 1.08 (s, 9H) t-Butyl;

^{13}C-NMR ($CDCl_3$) δ: 166.4, 162.9, 162.1, 2 x COOBn, C-4; 150.4, C-2; 139.2, C-6; 134.7-126.3, Arom.; 101.6, C-5; 85.9, C-1'; 81.5, C-4'; 68.1, 68.0, 2 x OCH_2Bn; 65.4, C-5'; 36.3, 35.7, C-2', C-3'; 27.4, CH_3; 19.9, CMe_3; LRMS (FAB, NBA) m/e: 753 ([M + Na^+], 1.9); 731 ([M + H^+], 3.3); HRMS (FAB, glycerol), m/e calcd. for $C_{42}H_{43}N_2O_8Si$ [M + H^+] 731.27887, found 731.27893.

1-[5'-O-(tert-Butyldimethylsilyl)-2',3'-dideoxy-2',3'-(1,1-dicarboxylic acid)cyclopropane-β-D-ribofuranosyl] uracil (24a, Si = TBDMS), and 1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-dideoxy-2',3'-(1,1-dicarboxylic acid)cyclopropane-β-D-ribofuranosyl] uracil (24b, Si = TBDPS)



(23) (24)

From cyclopropane 23a (Si = TBDMS):

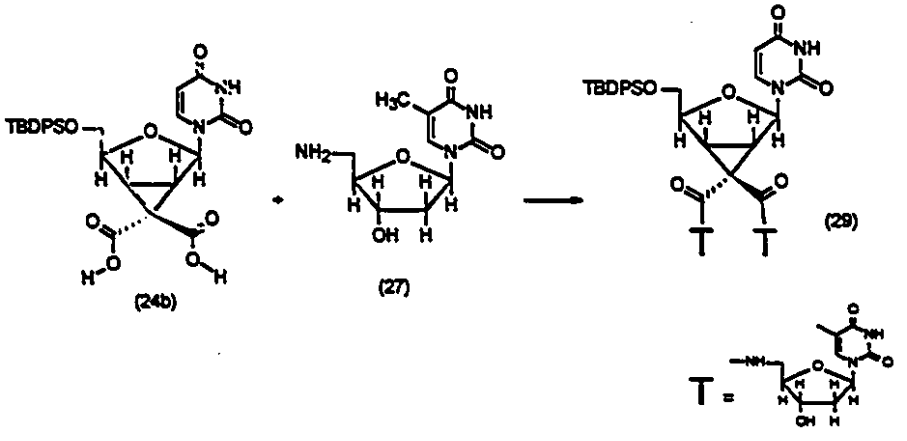
Cyclopropane **23a** (485 mg, 0.8 mmol) in dry methanol (15 ml) was hydrogenated over 100 mg palladium / carbon (10%) at an initial pressure of 40 p.s.i. for 4 hours. The reaction mixture was then filtered through celite, and the filtrate was evaporated to dryness to give diacid **24a** as a white foam in quantitative yield. ^{1}H-NMR (CD_3OD) δ: 8.24 (d, $J_{5,6}$= 8.1 Hz, 1H) H-6; 6.40 (s, 1H) H-1'; 5.68 (d, 1H) H-5; 4.42 (m, 1H) H-4'; 3.91-3.82 (m, 2 H) H-5'a, H-5'b; 2.67 (AB quartet, 2H) H-2', H-3'; 0.93 (s, 9H) t-Butyl; 0.17 (s, 6H) $Si(CH_3)_2$; ^{13}C-NMR (CD_3OD) δ: 175.6, 173.0, 2 x COOH; 166.1, C-4; 152.2, C-2; 143.4, C-6; 102.5, C-5,; 87.1, C-1'; 83.0, C-4'; 66.6, C-5'; 39.6, 37.9, C-2', C-3'; 26.4, $SiCH_3$; 19.3, $SiCMe_3$; LRMS (FAB, NBA) m/e: 471 ([M - 1 + 2Na^+], 15),

449 ([M + Na^+], 28), 157(100).

From cyclopropane 23b (Si = TBDPS):

Similarly, cyclopropane **23b** (731 mg , 1 mmol) in dry methanol (30 ml) was hydrogenated over 150 mg palladium / carbon (10%) at an initial pressure of 40 p.s.i. for 4 hours. The reaction mixture was filtered through celite, and the filtrate was evaporated to dryness to give **24b** as a white foam quantitatively. ^{1}H-NMR (CD_3OD) δ: 7.73-7.35 (m, 11H) Arom. + H-6; 6.25 (s, 1H) H-1'; 5.28 (d, $J_{5,6}$= 8.0 Hz, 1H) H-5; 4.54 (dd, $J_{4',5'}$=2.9 Hz, $J_{4',5''}$=4.5 Hz, 1H) H-4'; 2.73 (d, $J_{2',3'}$=6.5 Hz, 1H) H-2'; 2.65 (d, 1H) H-3'; 1.05 (s, 9H) t-Butyl; ^{13}C-NMR (CD_3OD) δ: 171.6, 168.7, 2 x COOH; 165.9, C-4; 152.4, C-2; 142.6, C-6; 136.6 - 129.0, Arom.; 102.6, C-5; 87.2, C-1'; 83.1, C-4'; 67.2, C-5'; 35.9, 35.0, C-2', C-3'; 27.4, $SiCH_3$; 20.1, $SiCMe_3$; LRMS (FAB, NBA) m/e: 595 ([M - 1 + 2Na^+], 1.8), 573 ([M + Na^+], 14.6), 551 ([M + H^+], 3.0).

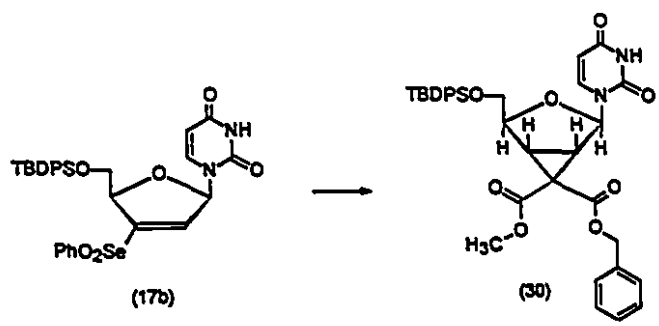
Branched Trinucleoside 29



To a solution of diacid **24b** (55 mg, 0.1 mmol) and amine **27** (24 mg, 0.1 mmol) in dry DMF (5 ml) was added triethylamine (42 µl, 0.3 mmol), and then BOP (52 mg, 0.1

mmol). After stirring the mixture at RT for 2 hours, the DMF was evaporated, and the residue was chromatographed on silica gel (MeOH / CH_2Cl_2, 5/95) to give **29** as a white foam (78 mg, 78 % yield). ^{1}H-NMR (CD_3OD) δ: 7.72 (d, $J_{5,6}$ = 8.1 Hz, 1H) ^{5}H-6; 7.55, 7.50 (2 x s, 2 x 1H) ^{3}H-6; 7.67-7.37 (m, 10H) Arom.; 6.22, 6.18 (2 x dd, 2 x 1H) ^{3}H-1'; 6.16 (s, 1H) ^{5}H-1'; 5.25 (d, 1H) ^{5}H-5; 4.58 - 4.23 (m, 3H) ^{3}H-3', ^{5}H-4', ^{3}H-3'; 4.02 (m, 1H) ^{3}H-4'; 3.88-3.35 (m, 7H) ^{3}H-4', ^{5}H-5', ^{3}H-5'; 2.65, 2.60 (2 x d, $J_{2',3'}$=6.7 Hz) ^{5}H-2', ^{5}H-3'; 2.37-2.19 (m, 4H) ^{3}H-2'; 1.92, 1.90 (2 x s, 2 x 3H) ^{3}T-CH_3; 1.02 (s, 9H) T-Butyl; LRMS (FAB, NBA) m/e: 1019 ([M + Na^+], 2.4), 907 (2.0).

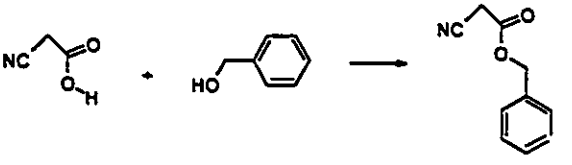
1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-dideoxy-2',3'-(1-methoxycarbonyl-1-benzyloxycarbonyl)cyclopropane-β-D-ribofuranosyl] uracil (30)



A solution of benzylmethyl malonate(0.2 ml) in 1 ml of dry THF was treated with potassium *t*-butoxide (28 mg , 0.25 mmol) at RT for 30 minutes. The solution was cooled in an ice bath, and selenone (32 mg, 0.05 mmol) was added. After stirring at RT for 4 hours, the solution was diluted with saturated NH_4Cl (10 ml) and extracted with EtOAc (3 x 5 ml). The combined extracts were dried ($MgSO_4$), evaporated, and chromatographed on silica gel (CH_2Cl_2 first, then CH_2Cl_2 / MeOH: 97/3) to give cyclopropane **30** as a white foam (27 mg, 83% yield). ^{1}H-NMR ($CDCl_3$) δ: 8.31, 8.00 (2 x br) NH; 7.60-7.29 (m) Arom.+ H-6; 6.09, 6.08 (2 x s) H-1'; 5.32, 5.31 (2 x d, J = 8.0 Hz, J = 8.1 Hz) H-5;

5.24 - 5.16 (4 x s) OCH_2Ph; 4.47 (m) H-4'; 3.73-3.51 (m) OCH_3, H-5', H-5''; 2.75 (2 x d, J = 6.7 Hz, J = 5.5 Hz) H-2'; 2.59 (2 x d, J = 6.7 Hz, J = 5.5 Hz) H-3'; 1.05 (s) t-Butyl; ^{13}C-NMR ($CDCl_3$) δ: 167.1, 166.5, 163.0, 162.1, 2 x COOBn, 2 x COOMe; 161.5, C-4; 149.3, 149.2, C-2; 139.2, C-6; 135.1-127.3, Arom.; 101.6, C-5; 86.4, 86.3, C-1'; 81.7, 81.6, C-4'; 68.2, 68.1, C-5'; 65.4, OCH_2Ph; 53.6, 53.4, OCH_3; 35.8, 35.7, C-2'; 33.8, 33.7, C-3'; 27.5 CH_3; 19.9 CMe_3.

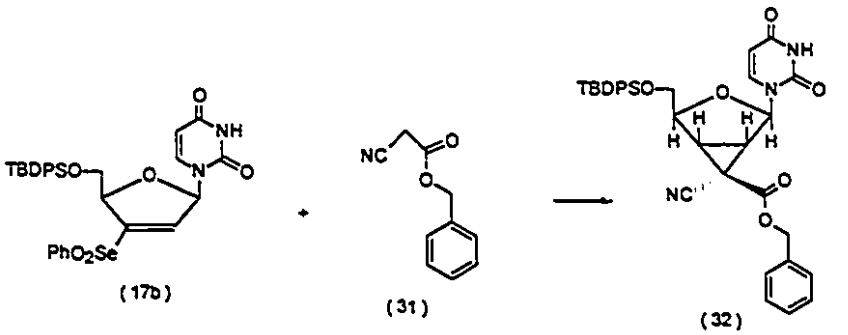
Benzyl cyanoacetate (31)



(31)

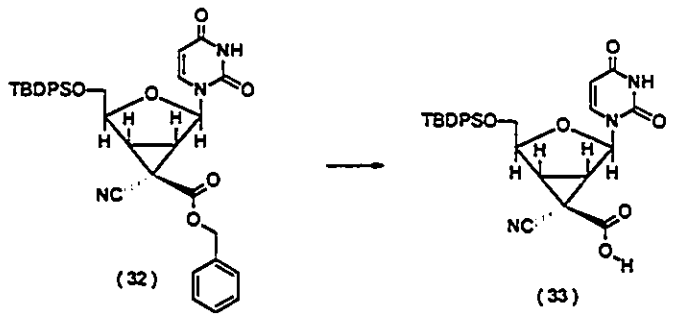
To a solution of cyanoacetic acid (3.4 g, 40 mmol) and benzyl alcohol (8.4 ml, 80 mmol) in 200 ml of pyridine was added DCC (8.24g, 40 mmol) portionwise at RT with strong stirring. After 4 hours, the pyridine was evaporated, and hexanes (200 ml) was then added to the residue. The organic salts were filtered off. The filtrate was dried ($MgSO_4$), evaporated, and chromatographed on silica gel (EtOAc/hexanes, 1/4) to provide benzyl cyanoacetate **31** as a colorless oil (5.8 g, 83%). ^{1}H-NMR ($CDCl_3$) δ: 7.37 (s, 5 H) Arom.; 5.18 (s, 2 H) CH_2Ph; 3.42 (s, 2 H) $NCCH_2COOR$; LRMS (CI) m/e: 176 ([M + H^+], 3.7), 91 (100).

1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-dideoxy-2',3'-(1-*exo*-benzyloxycarbonyl-1-*endo*-cyano)cyclopropane-β-D-ribofuranosyl] uracil (32)



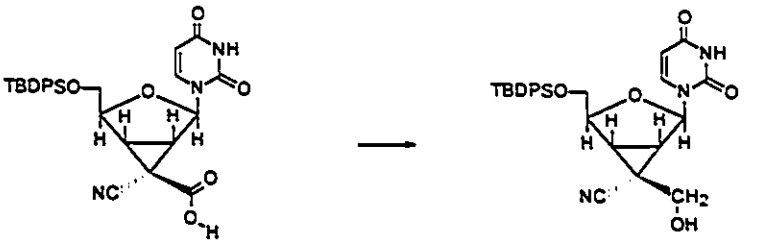
A solution of benzyl cyanoacetate **31** (1.4 g, 8.0 mmol) in 10 ml of dry THF was treated with potassium *t*-butoxide (0.90 g, 8.0 mmol) at RT for 30 minutes. The solution was then cooled in an ice bath, and selenone **17b** (1.27 g, 2 mmol) was added. After stirring at RT for 4 hours, the solution was poured into saturated NH_4Cl (50 ml), and extracted with EtOAc (3 x 50 ml). The combined extracts were dried ($MgSO_4$), evaporated, and chromatographed on silica gel (CH_2Cl_2 first, then CH_2Cl_2 / MeOH: 97/3) to give cyclopropane **32** as a white foam (1.05 g, 84% yield).^{1}H-NMR ($CDCl_3$) δ: 8.44 (br, 1H) NH; 7.78 (d, $J_{5,6}$=8.1 Hz, 1H) H-6; 7.33-7.60 (m, 15H) Arom.; 6.04 (s, 1H) H-1'; 5.34 (d, 1H) H-5; 5.23 (s, 2H) OCH_2Ph; 4.42 (dd, $J_{4',5'}$= 4.2 Hz, $J_{4',5''}$= 3.5 Hz, 1H) H-4'; 4.01 (dd, $J_{5',5''}$= 11.9 Hz, 1H) H-5'; 3.83 (dd, 1H) H-5''; 3.04, (d, $J_{2',3'}$= 7.0 Hz, 1H) H-2'; 2.79(d, 1H) H-3'; 1.05 (s, 9H) t-Butyl; ^{13}C-NMR ($CDCl_3$) δ:164.7, COOBn; 162.5, C-4; 160.3, C-2; 139.9, C-6; 149.9-127.9, Arom.; 112.3, CN; 102.5, C-5; 87.4, C-1'; 83.4, C-4'; 69.1, C-5'; 65.0, OCH_2Bn; 39.5, C-2'; 36.7, C-3'; 26.9, CH_3; 26.8, C(CN)COOBn; 19.2, $SiCMe_3$; LRMS (FAB, NBA) m/e: 644 ([M + Na^+], 3.1), 622 ([M + H^+], 5.7).

1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-dideoxy-2',3'-(1-*exo*-carboxylic acid-1-*endo*-cyano)cyclopropane-β-D-ribofuranosyl] uracil (33)



Benzyl ester **32** (931 mg, 1.5 mmol) was dissolved in 20 ml of dry methanol, and hydrogenated over 150 mg palladium / carbon (10%) at an initial pressure of 40 p.s.i. for 5 hours. The reaction mixture was then filtered through celite. The filtrate was evaporated to dryness to give acid **33** quantitatively as a glass. ^{1}H-NMR (CD_3OD) δ: 7.77 (d, $J_{5,6}$= 7.8 Hz, 1H) H-6; 7.69-7.36 (m, 10H) Arom.; 6.07 (s, 1H) H-1'; 5.16 (d, 1H) H-5; 4.41 (dd, $J_{5',4'}$= 3.5 Hz, $J_{5'',4'}$= 4.9 Hz, 1H) H-4'; 4.06 (dd, $J_{5',5''}$= 11.7 Hz, 1H) H-5'; 3.95 (dd, 1H) H-5''; 3.05 (d, $J_{3',2'}$= 6.8 Hz, 1H, H-2'; 2.92 (d, 1H) H-3'; 1.06 (s, 9H) t-Butyl; ^{13}C-NMR (CD_3OD) δ: 168.0, COOH; 165.8, C-4; 152.1, C-2; 142.8, C-6; 136.8-129.0, Arom.; 115.0, CN; 102.9, C-5; 88.7, C-1'; 84.8, C-4'; 66.9, C-5'; 39.2, C-2'; 38.9, C-3'; 27.5, CH_3; 27.4, C(CN)COOH; 20.1, $SiCMe_3$; LRMS (FAB, NBA) m/e: 576 ([M - 1 + $2Na^+$], 1.8), 554 ([M + Na^+], 7.8), 532 ([M + H^+], 2.5)223 (100); LRMS (FAB, glycerol) m/e: 576 ([M - 1 + $2Na^+$], 8.5), 554 ([M + Na^+], 61.7), 532 ([M + H^+], 25); HRMS (FAB, glycerol), m/e calcd for $C_{28}H_{29}N_3O_6Si$ + Na^+ [M + Na^+] 554.17233, found 554.17217.

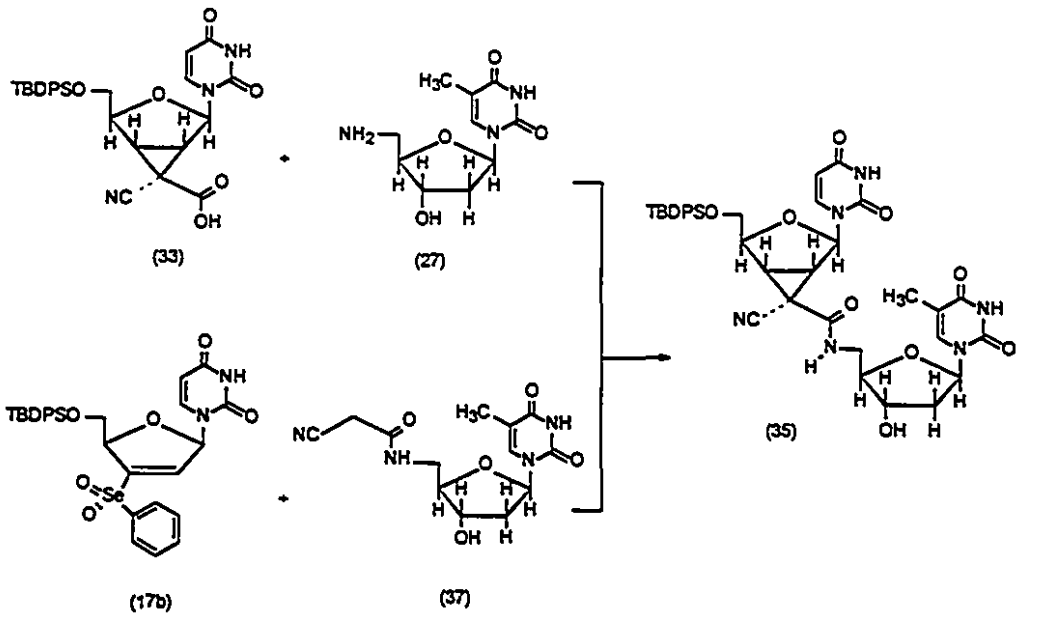
Alcohol 34



Cyclopropyl acid **33** (11 mg, 0.02 mmol) was added to a stirred solution of borane-methyl sulfide complex in THF (2M, 40 µl, 0.08 mmol) and trimethylborate (0.5 ml) at 0°C. The mixture was stirred at room temperature for 2 hours, and methanol (1 ml) was then added. After removing the solvent, the residue was chromatographed on silica gel to provide alcohol **34** as a colorless glass (6 mg, 58 % yield). ^{1}H-NMR ($CDCl_3$) δ: 9.04 (br, 1H) NH; 7.82 (d, $J_{5,6}$=8.1 Hz, 1H) H-6; 7.60-7.43 (m, 10H) Arom.; 5.93 (s, 1H) H-1'; 5.40 (d, 1H) H-5; 4.42 (dd, $J_{4',5'}$=3.4 Hz, $J_{4',5''}$=3.9 Hz, 1H) H-4'; 3.96 (dd, $J_{5',5''}$=11.7 Hz, 1H) H-5'; 3.85-3.78 (m, 2H) H-5'', CH_aOH; 3.62(d, $J_{a,b}$=11.7 Hz, 1H) CH_bOH; 2.63 (d, $J_{2',3'}$= 6.4 Hz, 1H) H-2'; 2.17 (d, 1H) H-3'; 1.66 (br, 1H) OH; 1.06 (s, 9H) t-Butyl; ^{13}C-NMR ($CDCl_3$) δ: 164.9, C-4; 150.3, C-2; 139.9, C-6; 135.4-127.9, Arom.; 115.9, CN; 102.1, C-5; 87.8, C-1'; 83.3, C-4'; 65.16, C-5'; 62.9, CH_2OH; 29.9, C-2'; 29.5, C-3'; 26.8, CH_3; 23.7, $C(CN)CH_2OH$; 19.1, $SiCMe_3$; LRMS(FAB, NBA) m/e: 540 ([M + Na^+], 5.9), 518 ([M + H^+], 2.6).

Dinucleoside analog containing an *endo*-cyano substituted cyclopropyl *exo*-amide

linker (35)



From reaction of cyclopropyl carboxylic acid 33 and 5'-amino-5'-deoxythymidine 27:

To a solution of acid **33** (638 mg, 1.2 mmol) and amine **27** (318 mg, 1.32 mmol) in dry DMF (5 ml) was added triethylamine (484 µl, 3.6 mmol) and then BOP (584 mg, 1.32 mmol). After stirring the mixture at RT for 2 hours, the DMF was evaporated, and the residue was chromatographed on silica gel (MeOH / CH_2Cl_2, 5/95) to give dimer **35** as a white foam (820 mg, 91% yield).

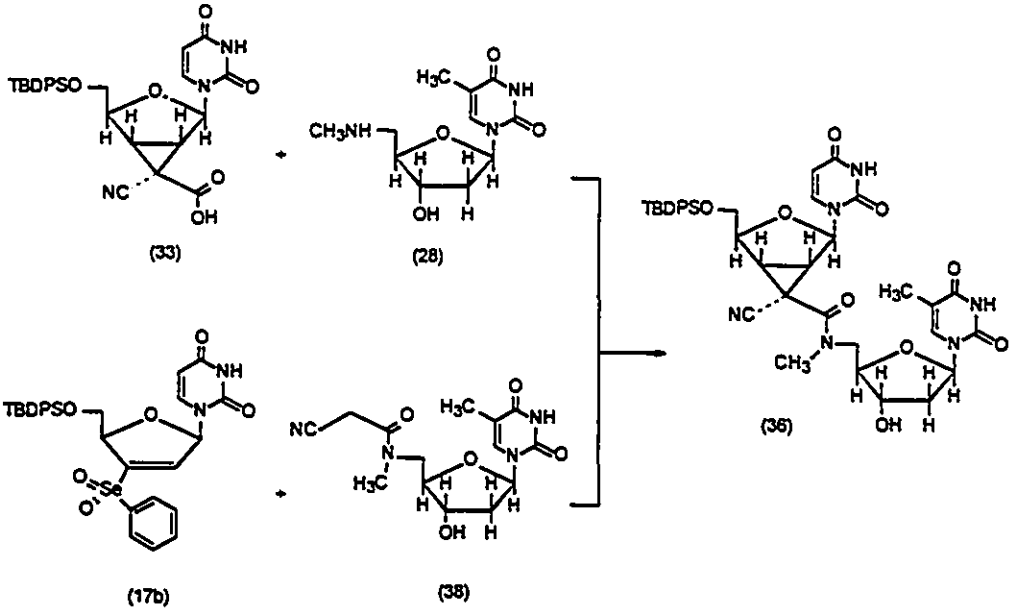
From reaction of vinyl selenone 17b and amide 37:

A solution of amide **37** (65 mg, 0.21 mmol) in 1 ml of dry DMF was treated with potassium *t*-butoxide (95 mg, 0.84 mmol) at RT for 30 minutes, and then cooled in an ice bath. Selenone **17b** (127 mg, 0.2 mmol) was added, and the reaction mixture was stirred at RT for 4 hours. The product mixture was then slowly poured into 10 ml of saturated NH_4Cl with vigorous stirring to provide the crude dimer **35** as a white precipitate. The

precipitate was collected, washed with water, and then dried in *vacuo* to give dimer **35** as a white solid (119 mg, 80%). ^{1}H-NMR (CD_3OD) δ : 7.77 (d, $J_{5,6}$= 7.8 Hz, 1H) ^{5}H-6; 7.51 (d, 1H) ^{3}H-6; 7.36-7.67 (m, 10H) Arom.; 6.21 (dd, $J_{1',2'}$= 6.8 Hz, $J_{1',2''}$= 6.8 Hz, 1H) ^{3}H-1'; 6.07 (s, 1H) ^{5}H-1'; 5.15 (d, 1H) ^{5}H-5; 4.42 (dd, $J_{4',5'}$= 3.4 Hz, $J_{4',5''}$= 4.9 Hz, 1H) ^{5}H-4'; 4.28 (dd, $J_{4',2'}$= 8.8 Hz, $J_{4',2''}$= 4.4 Hz, 1H) ^{3}H-4'; 4.06 (dd, $J_{5',5''}$= 11.7 Hz, 1H) ^{5}H-5'; 3.95 (dd, $J_{3',5'}$= 7.1 Hz, $J_{3',5''}$= 4.9 Hz, 1H) ^{3}H-3'; 3.95 (dd, 1H) ^{5}H-5''; 3.63 (dd, $J_{5',5''}$= 14.16 Hz, 1H) ^{3}H-5'; 3.46 (dd, 1H) ^{3}H-5''; 3.04, 2.94 (2 x d, $J_{2',3'}$= 6.8 Hz, 2H) ^{5}H-2', ^{5}H-3'; 2.25 (dd, 2H) ^{3}H-2', ^{3}H-2''; 1.89 (d, 1H) ^{3}T-CH_3; 1.06 (s, 9H) t-Butyl; ^{13}C-NMR (CD_3OD) δ: 164.9, 164.4, 163.9, ^{5}C-4, ^{3}C-4, C-amide; 151.0, 150.9, ^{5}C-2, ^{3}C-2; 141.6, ^{5}C-6; 137.2, ^{3}C-6; 136.4-128.1, Arom.; 114.3, CN; 111.1, ^{3}C-5; 102.2, ^{5}C-5; 88.2, ^{5}C-1'; 86.4, ^{3}C-1'; 85.6, ^{3}C-3'; 84.4, ^{5}C-4'; 72.7, ^{3}C-4'; 66.6, ^{5}C-5'; 43.3, ^{3}C-5'; 40.1, ^{3}C-2'; 38.8, ^{5}C-2'; 37.1, ^{5}C-3'; 27.4, CH_3; 26.9, C(CN)CONHR; 20.5, $SiCMe_3$); 12.9, ^{3}T-CH_3; LRMS (FAB, NBA) m/e: 777 ([M + Na^+], 57), 755 ([M + H^+], 31), 307 (90); HRMS (FAB-glycerol), m/e calcd for $C_{38}H_{42}N_6O_9Si$ + H^+ [M + H^+] 755.28608, found 755.28590.

Dinucleoside analog containing an *endo*-cyano substituted cyclopropyl *exo*-

methylamide linker (36)



From reaction of acid 33 and 5'-methylamino-5'-deoxythymidine 28:

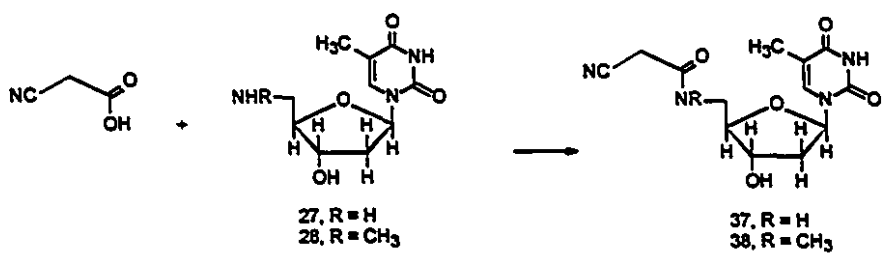
To a solution of acid **33** (53 mg, 0.10 mmol) and amine **28** (28 mg, 0.11 mmol) in dry DMF (1 ml) was added triethylamine (72 µl, 0.30 mmol), and then BOP (46 mg, 0.105 mmol). After stirring the mixture at RT for 2 hours, the DMF was evaporated. The residue was chromatographed on silica gel to give dimer **36** as a white foam (77 mg, 90% yield).

From reaction of selenone 17b and amide 38:

A solution of amide **38** (34 mg, 0.105 mmol) in 1 ml of DMF was treated with potassium *t*-butoxide (48 mg, 0.42 mmol) at RT for 30 minutes, and then cooled in an ice bath. Selenone **17b** (64 mg, 0.1 mmol) was added, and the reaction mixture was stirred at room temperature for 4 hours. The product mixture was then slowly poured into 10 ml of saturated NH_4Cl with vigorous stirring to provide the crude dimer **36** as a white precipitate. The precipitate was collected, washed with water, and then dried *in vacuo* to

give dimer **36** as a white solid (65 mg, 85%). ^{1}H-NMR ($CDCl_3$) δ: 7.85 (d, $J_{5,6}$=8.3 Hz, 1H) ^{5}H-6; 7.62-7.36 (m, 10H) Arom.; 7.04 (s, 1H) ^{3}H-6; 5.90 (s, 1H) ^{5}H-1'; 5.62 (m, 1H) ^{3}H-1'; 4.53 (m, 1H) ^{5}H-4'; 4.45 (m, 1H) ^{3}H-3'; 4.10 (dd, J = 3.9 Hz, J = 14.7 Hz, 1H) ^{3}H-5'; 3.28 (d, J = 14.7 Hz, 1H) ^{3}H-5''; 3.96 (dd, J = 3.4 Hz, J = 11.7 Hz, 1H) ^{5}H-5'; 3.89 (dd, J = 3.4 Hz, J = 2.5 Hz, 1H) ^{3}H-4'; 3.77 (m, 1H) ^{5}H-5''; 3.39 (d, J = 6.4 Hz, 1H) ^{5}H-2'; 2.82 (d, J = 6.4 Hz, 1H) ^{5}H-3'; 2.34 (m, 2H) ^{3}H-2'; 1.90 (s, 3H) ^{3}T-CH_3; 1.06 (s, 9H) t-Butyl; ^{13}C-NMR ($CDCl_3$) δ: 164.8, 163.3, 163.0, ^{5}C-4, ^{3}C-4, C-Amide; 151.3, 150.0, ^{5}C-2, ^{3}C-2; 139.3-128.0, ^{5}C-6, ^{3}C-6, Arom.; 113.3, CN; 110.5, ^{3}C-5; 102.3, ^{5}C-5; 89.1, ^{5}C-1'; 88.7, ^{3}C-1'; 84.6-37.8, ^{5}C-1', ^{3}C-1', ^{5}C-4', ^{3}C-4', ^{3}C-3', ^{5}C-5', ^{3}C-5', ^{3}C-2'; 36.6, ^{5}C-2'; 32.2, ^{5}C-3'; 26.8, t-Butyl; 24.3, C(CN)CONR; 19.2, t-Butyl; 12.3, ^{3}T-CH_3; LRMS (FAB, NBA) m/e: 769 ([M + H$^+$] 1.1), 711 (0.4), 643 (1.1), 522 (1.3).

Amide 37 (R = H), and Amide 38 (R = CH_3)



From 5'-Amino-5'-deoxythymidine 27:

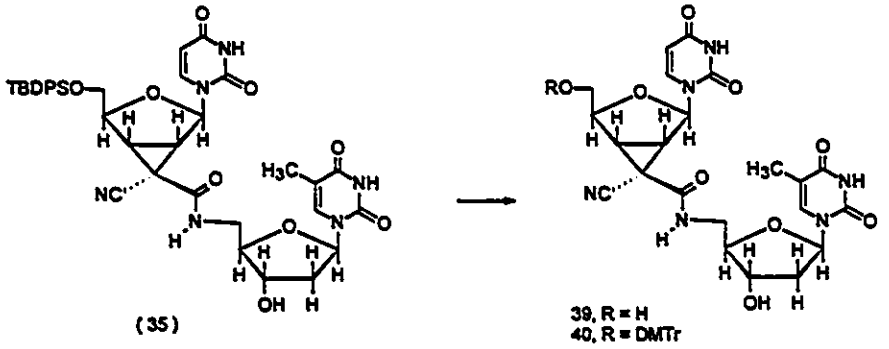
To a solution of cyanoacetic acid (89 mg, 1.05 mmol) and amine **27** (241 mg, 1.0 mmol) in dry DMF (5 ml) was added triethylamine (417 µl, 3.1 mmol) and then BOP (442 mg, 1.0 mmol). After stirring the mixture at RT for 2 hours, the DMF was removed by co-evaporating with toluene. The residue was chromatographed on silica gel (Et_3N / MeOH / CH_2Cl_2, 1/5/94) to give amide **37** as a white foam (290 mg, 94% yield). ^{1}H-NMR ($CDCl_3$) δ: 7.47 (d, J=1.5 Hz, 1H) H-6; 6.17 (dd, J = 6.4 Hz, J = 7.3 Hz, 1H) H-1';

4.26 (m, 1H) H-3'; 3.90 (m, 1H) H-2'; 3.57, 3.56 (2 x s, 2H) $NCCH_2CO$; 3.51, 3.50 (2 x s, 2H) H-5', H-5''; 2.25 (m, 2H) H-2'; 1.90 (d, 1H) $T\text{-}CH_3$; LRMS (FAB, glycerol) m/e: 309 ([M + H^+], 0.7), 185 (100).

From 5'-Methylamino-5'-deoxythymidine 28:

To a solution of cyanoacetic acid (89 mg, 1.05 mmol) and methylamine **28** (241 mg, 1.0 mmol) in dry DMF (5 ml) was added triethylamine (417 µl, 3.1 mmol) and then BOP (442 mg, 1.0 mmol). After stirring the mixture at RT for 2 hours, the DMF was removed by co-evaporating with toluene, and the residue was chromatographed on silica gel (Et_3N / MeOH / CH_2Cl_2, 1/5/94) to give amide **38** as a white foam (288 mg, 90% yield). ^{1}H-NMR ($CDCl_3$) δ (main rotamer): 7.55 (d, J=1.0 Hz, 1H) H-6; 6.24 (t, J=6.3 Hz, 1H) H-1'; 4.24 (m, 1H) H-3'; 4.05 (m, 1H) H-4'; 3.80-3.56 (m) H-5'a, H-5'b; 3.31 (s, 2H) $CNCH_2$; 3.06 (s, 3H) $N\text{-}CH_3$; 2.22 (m) H-2'; 1.92 (s, 3H) $T\text{-}CH_3$; δ (minor rotamer): 7.37 (d, J=1 Hz) H-6; 6.09 (dd, J=5.8 Hz, J=5.4 Hz) H-1'; 4.30 (m) H-3'; 3.91 (m) H-4'; 3.80-3.56 (m) H-5'a, H-5'b; 3.31 (s) $CNCH_2$; 3.01 (s) $N\text{-}CH_3$; 2.22 (m) H-2'; 1.89 (s) $T\text{-}CH_3$; LRMS(CI) m/e: 323 ([M + H^+], 5.2).

Diol (39), and 5'-DMTr protected dinucleoside analog (40)



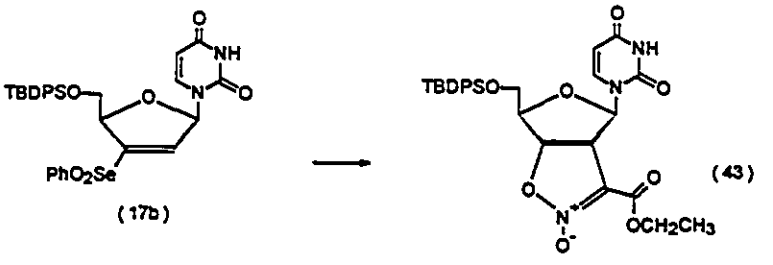
A solution dimer **35** (754 mg, 1.0 mmol) in dry THF (5 ml) was treated with

TBAF / THF (1 M, 3 ml, 3.0 mmol) at RT for 3 hours. The THF was then evaporated, and the resulting solid was triturated with ether three times to afford diol **39** as a white solid (489 mg, 95%). ^{1}H-NMR (CD_3OD) δ: 8.04 (d, $J_{5,6}$=8.2 Hz, 1H) ^{5}H-6; 7.46 (d, $J_{6,T\text{-}CH_3}$=1.1 Hz, 1H) ^{3}H-6; 6.14 (m, $J_{1',2'}$ = 6.6 Hz, 1H) ^{3}H-1'; 6.10 (s, 1H) ^{5}H-1'; 5.65 (d, 1H) ^{5}H-5; 4.25 (m, 1H) ^{3}H-3'; 3.91 (m, 1H) ^{3}H-4'; 3.74 (m, 1H) ^{5}H-4'; 3.59-3.34 (m, 4H) ^{5}H-5', ^{5}H-5", ^{3}H-5', ^{3}H-5"; 2.88, 2.63 (2 x s, 2H) ^{5}H-2', ^{5}H-3'; 2.2 (m, 2H) ^{3}H-2'; 1.86 (s, 3H) ^{3}T-CH_3; ^{13}C-NMR (CD_3OD) δ: 165.2, 165.0, 164.0, C-amide, ^{5}C-4, ^{3}C-4; 151.5, 151.1, ^{5}C-2, ^{3}C-2; 142.1, ^{5}C-6; 137.2, ^{3}C-6; 114.5, CN; 111.0, ^{3}C-5; 102.1, ^{5}C-5; 87.7, ^{5}C-1'; 86.3, ^{3}C-1'; 85.6, ^{5}C-4'; 84.6, ^{3}C-4'; 72.7, ^{3}C-3'; 64.0, ^{5}C-5'; 43.4, ^{3}C-5'; 40.0, ^{3}C-2'; 38.9, 37.4, ^{5}C-2', ^{5}C-3'; 27.1, C-cyclo; 12.9, ^{3}T-CH_3; LRMS (FAB, NBA) m/e: 517 ([M + H$^+$] 0.5), 242 (100).

Diol **39** (412 mg, 0.80 mmol) was co-evaporated with dry pyridine (3 x 3 ml) three times until it turned into a white foam. Diol **39** was then redissolved in 5 ml of dry pyridine, and treated with 4,4'-dimethyoxytrityl chloride (675 mg, 2 mmol) at RT for 4 hours. Water (2 ml) was then added. After removing the solvent, the resulting syrup was chromatographed on silica gel (Et_3N / MeOH / CH_2Cl_2, 1/5/94, v/v) to afford dinucleoside **40** as a white foam (536 mg, 82%). ^{1}H-NMR (CD_3 OD) δ: 7.77 (d, $J_{5,6}$=8.0 Hz, 1H) ^{5}H-6; 7.52 (s, 1H) ^{3}H-6; 7.49-6.79 (m, 13H) Arom.; 6.19 (dd, J=2.3 Hz, J=4.7 Hz, 1H) ^{3}H-1'; 6.12 (s, 1H) ^{5}H-1'; 5.10 (d, 1H) ^{5}H-5; 4.46 (m, 1H) ^{5}H-4'; 4.29 (m, 1H) ^{3}H-3'; 3.95 (m, 1H) ^{3}H-4'; 3.77 (s, 6H) 2 x OCH_3; 3.61-3.35 (m, 4H) ^{5}H-5', ^{5}H-5'', ^{3}H-5', ^{3}H-5''; 3.06 (d, J 2',3'=6.9 Hz, 1H) ^{5}H-2'; 2.97 (d, 1H) ^{5}H-3'; 1.90 (s, 3H) ^{3}T-CH_3; ^{13}C-NMR (CD_3 OD) δ: 165.0, 164.4, 164.0, C-Amide, ^{5}C-4, ^{3}C-4; 151.0, 150.9, ^{5}C-2, ^{3}C-2; 141.9, 137.2, ^{5}C-6, ^{3}C-6; 135.7-120.4, Arom.; 114.3, CN; 111.1, ^{3}C-5; 102.2, ^{5}C-5; 87.8, ^{5}C-1'; 86.4, ^{3}C-1'; 85.5, ^{5}C-4'; 83.0, ^{3}C-4'; 72.7, ^{3}C-3'; 66.1, ^{5}C-5'; 55.7, OCH_3; 43.4, ^{3}C-5'; 40.3, ^{3}C-2'; 38.6, 37.2, ^{5}C-2', ^{5}C-3'; 27.1, C-cyclo; 12.9, ^{3}T-CH_3; LRMS (FAB, NBA) m/e: 819 ([M + H$^+$], 0.3); HRMS (FAB, glycerol), m/e calcd. for

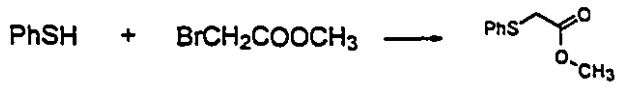
$C_{43}H_{42}N_6O_{11}$ + H^+ [M + H^+] 819.29898, found 819.29918.

[3.3.0]-Bicyclic uridine (43)



A solution of ethyl 2-nitroacetate (0.5 ml) in 2 ml of THF was treated with potassium *tert*-butoxide (28 mg , 0.25 mmol) at RT for 30 minutes, then cooled in an ice bath. Selenone **17b** (32 mg, 0.05 mmol) was added with stirring, and the ice bath was removed. After stirring at RT for 4 hours, the mixture was added to saturated NH_4Cl (5 ml), and extracted with EtOAc (3 x 5 ml). The combined extracts were dried ($MgSO_4$), evaporated, and chromatographed on silica gel (CH_2Cl_2 first, then CH_2Cl_2 / MeOH: 97/3) to give compound **43** as a white foam (26 mg, 90% yield). ^{1}H-NMR ($CDCl_3$) δ: 9.25 (br, 1H) NH; 7.29 (d, $J_{5,6}$=7.8 Hz, 1H) H-6; 7.65-7.33 (m, 10H) Arom.; 6.04 (d, $J_{2',1'}$=4.4 Hz, 1H) H-1'; 5.56 (dd, J=1.9 Hz, 1H) H-5; 5.33 (dd, J=10.3 Hz, J=9.8 Hz, 1H) H-3'; 4.35-4.22 (m, 4H) H-2', H-4', OCH_2; 4.01 (dd, $J_{4',5'}$=3.9 Hz, $J_{5',5''}$=11.3 Hz, 1H) H-5'; 3.92 (dd, $J_{4',5''}$=3.9 Hz, 1H) H-5''; 1.28 (t, J=8.3 Hz, 3H) CH_3 ; 1.05 (S, 9H) t-Butyl; ^{13}C-NMR ($CDCl_3$) δ: 162.5, 158.4, COOEt, C-4; 149.3, C-2; 140.9, C-6; 135.5 - 127.9, Arom.; 103.2, C-5; 91.4, C-1'; 86.0, C-4'; 78.1, C-3'; 63.1, 62.6 C-5', C-2'; 54.0, CO_2CH_2R; 26.9, CH_3(t-Butyl); 19.3, $SiCMe_3$; 14.1, CH_3; LRMS (FAB, NBA) m/e: 580 ([M + H^+], 4.5).

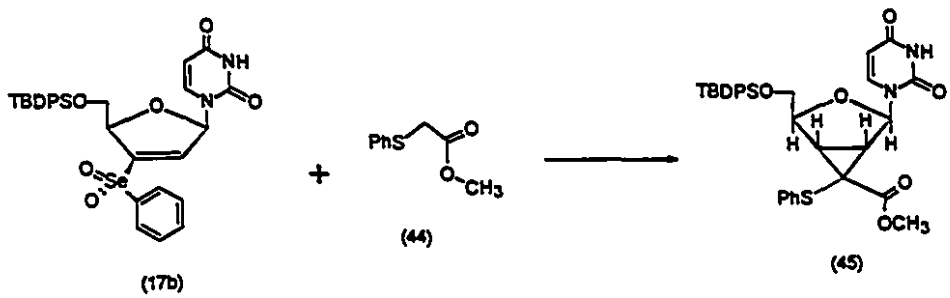
Methyl thiophenoxyacetate (44)



(44)

To a solution of thiophenol (5 ml, 49 mmol) in pyridine (100 ml) at 0°C was added methyl 2-bromoacetate (7 ml, 75 mmol) slowly with vigorous stirring. The ice-bath was removed, and the reaction mixture was stirred at RT overnight. The pyridine was then evaporated, and hexanes (200 ml) was added to the residue. The crystalline organic salts were filtered off. The filtrate was washed with saturated Na_2CO_3 (3 x 100 ml), then dried over $MgSO_4$. The solvent was evaporated to give a thick liquid, which was distilled to provide 44 as a colorless liquid. b.p. 122 °C (0.2 mm Hg); ^{1}H-NMR ($CDCl_3$) δ: 7.43-7.26 (m, 5H) Arom.; 3.71(s, 3H) OCH_3; 3.66n (s, 1H) CH_2COOMe; ^{13}C-NMR ($CDCl_3$) δ: 170.0 COOMe, 134.8-126.9 Arom., 52.4 OCH_3, 36.4 CH_2(SPh)COOMe.

1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-dideoxy-2',3'-(1-methoxycarbonyl-1-phenylthio) cyclopropane-β-D-ribofuranosyl] uracil (45)

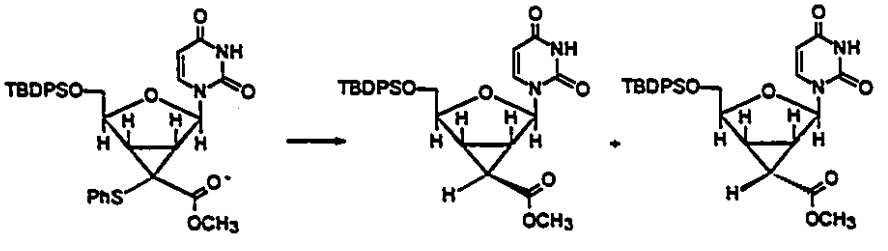


(17b) (44) (45)

A solution of methyl thiophenoxyacetate (273 mg, 1.5 mmol) in 2 ml of dry THF was treated with *n*-butyl lithium / hexane (1.6 M, 0.5 ml, 0.8 mmol) at -78°C for 30

minutes, followed by addition of selenone **17b** (95 mg, 0.15 mmol). After stirring at RT for 8 hours, the reaction mixture was added to saturated NH_4Cl (10 ml), and extracted with EtOAc (3 x 10 ml). The combined extracts were dried ($MgSO_4$), evaporated, and chromatographed on silica gel (EtOAc/Hexane, 1/3) to give cyclopropane **45** as a white foam (86 mg , 89% yield). ^{1}H-NMR ($CDCl_3$) δ: 8.83 (br, 1H) NH; 7.87 (d, $J_{5,6}$=8.3 Hz, 1H) H-6; 7.59-7.13 (m, 15H) Arom.; 5.94 (s, 1H) H-1'; 5.29 (d, 1H) H-5; 4.13 (dd, $J_{4',5'}$= 3.4 Hz, $J_{4',5''}$= 3.9 Hz, 1H) H-4'; 3.96 (dd, $J_{5',5''}$ = 11.2 Hz, 1H) H-5'; 3.78 (dd, 1H) H-5''; 3.68 (s, 3H) $COOCH_3$; 3.03, 2.95 (2 x d, $J_{2',3'}$= 7.8 Hz, 2H) H-2', H-3'; 1.06 (s, 9H) t-Butyl; ^{13}C-NMR ($CDCl_3$) δ: 169.1, COOR; 162.1, C-4; 149.2, C-2; 139.6, C-6; 134.8 - 125.5, Arom.; 102.0, C-5; 86.9, C-1'; 82.8, C-4'; 65.7, C-5'; 54.0, OCH_3; 41.5, 38.2, C-2', C-3'; 36.3, $COOCH_3$; 27.6, CH_3; 20.0, $SiCMe_3$; LRMS (FAB, NBA) m/e: 651 ([M + Na^+], 1.5), 629 ([M + H^+] 6.1).

1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-dideoxy-2',3'-(1-*exo*-methoxycarbonyl)cyclopropane-β-D-ribofuranosyl] uracil (46), and 1-[5'-O-(tert-butyldiphenylsilyl)-2',3'-dideoxy-2',3'-(1-*endo*-methoxycarbonyl)cyclopropane-β-D-ribofuranosyl] uracil (47)



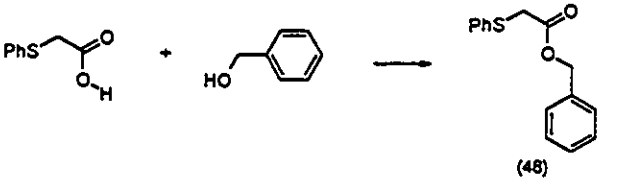
A solution of cyclopropane **45** (80 mg, 0.13 mmol), tributyltin hydride (107 μl, 0.4 mmol) and AIBN (5 mg) in 2 ml of dry benzene was refluxed for 20 hours. The

product mixture was then separated by preparative TLC (silica) (EtOAc / Hexanes: 6/4) to give **46** (53 mg, 78 % yield) and **47** (12 mg, 18% yield) as a pair of diastereoisomers. **Major isomer 46**: ^{1}H-NMR ($CDCl_3$) δ: 8.34 (br, 1H) NH; 7.65 (d, $J_{5,6}$=8.3 Hz, 1H) H-6; 7.61-7.36 (m, 10H) Arom.; 6.00 (s, 1H) H-1'; 5.30 (d, 1H) H-5; 4.24 (dd, $J_{4',5'}$= 5.4 Hz, $J_{4',5''}$= 3.5 Hz, 1H) H-4'; 3.72 (d, 1H) H-5'; 3.71 (d, 1H) H-5''; 3.70 (s, 3H) OCH_3; 2.47 (dd, $J_{2',3'}$= 6.3 Hz, $J_{2',6'}$=2.0 Hz, 1H) H-2'; 2.44 (dd, $J_{3',6'}$=2.0 Hz, 1H) H-3'; 1.71 (t, 1H) H-6'; 1.06 (s, 9H) t-Butyl; ^{13}C-NMR ($CDCl_3$) δ: 171.4, COOMe; 162.7, C-4; 150.4, C-2; 139.8, C-6; 135.5-128.0, Arom.; 102.1, C-5; 87.3, C-1'; 84.1, C-4'; 66.4, C-5'; 52.3, OCH_3; 31.5, C-2'; 29.2, C-3'; 26.9, CH_3; 24.9, C-6'; 19.3, $SiCMe_3$; LRMS (FAB NBA) m/e: 521 ([M + H$^+$], 5.0), 463 (8.2), 411 (2.2), 409 (10.2), 291 (29.1), 235 (42.5). **Minor isomer 47**: ^{1}H-NMR ($CDCl_3$) δ: 8.08 (br, 1H) NH; 7.99 (d, $J_{5,6}$=8.3 Hz, 1H) H-6; 7.63-7.36 (m, 10H) Arom.; 6.27 (s, 1H) H-1'; 5.27 (d, 1H) H-5; 4.379 (t, $J_{4',5'}$= 2.9 Hz, $J_{4',5''}$= 3.5 Hz, 1H) H-4'; 3.99 (dd, $J_{5',5''}$=11.7 Hz, 1H) H-5'; 3.80 (dd, 1H) H-5''; 3.73 (s, 3H) O-CH_3; 2.40 (dd, $J_{2',3'}$= 7.3 Hz, $J_{2',6'}$ =8.3 Hz, 1H) H-2'; 2.33 (dd, $J_{3',6'}$ =8.3 Hz ,1H) H-3'; 2.05 (t, 1H) H-6'; 1.07 (s, 9H) t-Butyl; ^{13}C-NMR ($CDCl_3$) δ: 167.9, COOMe; 161.7, C-4; 149.2, C-2; 140.0, C-6; 134.8-127.2, Arom.; 101.9, C-5; 85.5, C-1'; 80.9, C-4'; 65.9, C-5'; 52.4, OCH_3; 32.0, C-2'; 28.3, C-3'; 27.6, CH_3; 23.6, C-6'; 20.1, $SiCMe_3$; LRMS (FAB-NBA) m/e: 521 [M + H$^+$] (4.4), 463 (9.3), 410 (4.5), 409 (12.4), 291 (17.3), 235 (21.7).

Equilibration of 47:

endo-Carboxylate **47** (10 mg, 0.02 mmol), previously coevaporated with dry THF, was dissolved in 1 ml of dry THF, followed by addition of a few drops of lithium diisopropylamide in heptane/THF/ethylbenzene (2.0 M) at room temperature with stirring. After 20 minutes, the mixture was cooled in an ice-bath and quenched with drops of glacial acetic acid. The mixture was then examined by TLC, with carboxylates **46** and **47** as the references. About half of the starting material **47** was converted to **46**.

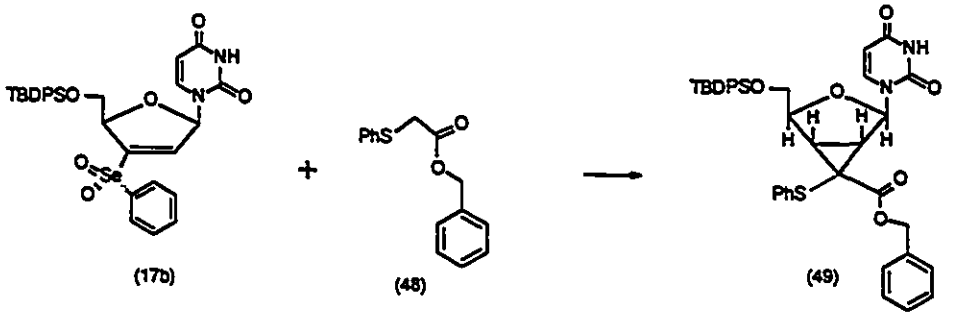
Benzyl thiophenoxyacetate 48



(48)

To a solution of thiophenoxyacetic acid (6.72g, 40 mmol) and benzyl alcohol (4.6 ml, 44 mmol) in pyridine (150 ml) was added DCC (8.24g, 40 mmol) portionwise at RT with strong stirring. After 4 hours, the pyridine was evaporated. Hexanes (200 ml) were then added. After filtering the crystalline organic salts, the filtrate was evaporated to give a thick oil, which was chromatographed (EtOAc / hexanes, 1/4) to provide benzyl thiophenoxyacetate **48** as a colorless oil (5.42g, 78% yield). ^{1}H-NMR ($CDCl_3$) δ: 7.37-7.21 (m, 5H) Arom.; 5.12 (s, 2H) $PhSCH_2COOBn$; 3.66 (s, 2H) OCH_2Ph; ^{13}C-NMR ($CDCl_3$) δ: 169.5, COOBn; 135.3-126.9, Arom.; 67.2, OCH_2Ph; 36.7, $PhSCH_2COOBn$.

1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-dideoxy-2',3'-(1-benzyloxycarbonyl-1-phenylthio)cyclopropane-β-D-ribofuranosyl] uracil (49)

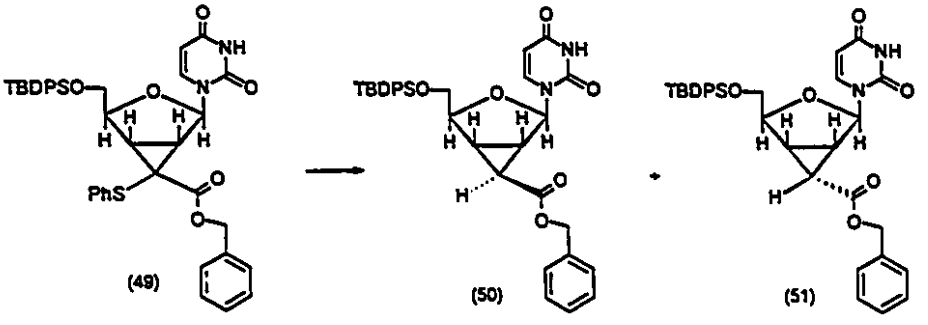


(17b) (48) (49)

A solution of benzyl thiophenoxyacetate **48** (258 mg, 10 mmol) in 10 ml of dry THF was treated with *n*-butyl lithium / hexanes (1.6 M, 5 ml) at -78°C for 30 minutes. Selenone **17b** (950 mg, 1.5 mmol) was then added. After stirring at RT for 8 hours, the mixture was poured into saturated NH_4Cl (100 ml), and extracted with EtOAc (3 x 50

ml). The combined extracts were dried ($MgSO_4$), evaporated, and chromatographed on silica gel (EtOAc/hexanes, 1/3) to give cyclopropane **49** as a white foam (810 mg , 77% yield). ^{1}H-NMR ($CDCl_3$) δ: 8.63 (br, 1H) NH; 7.92 (d, $J_{5,6}$=8.1 Hz, 1H) H-6; 7.71-7.11 (m, 15H) Arom.; 6.01 (s, 1H) H-1'; 5.32 (d, 1H) H-5; 5.15, 5.12 (2 x s, 2H) OCH_2Ph; 4.18 (dd, $J_{4',5'}$=3.9 Hz, $J_{4',5''}$=2.8 Hz, 1H) H-4'; 3.99 (dd, $J_{5',5''}$=7.3 Hz, 1H) H-5'; 3.82 (dd, 1H) H-5''; 3.09 (d, $J_{2',3'}$=8.0 Hz, 1H) H-2'; 3.00(d, 1H) H-3'; 1.07 (s, 9H) t-Butyl; ^{13}C-NMR ($CDCl_3$) δ: 169.5, COOBn; 162.9, C-4; 149.9, C-2; 140.4, C-6; 135.5-126.0, Arom.; 102.3, C-5; 87.0, C-1'; 82.9, C-4'; 68.3, OCH_2Ph; 66.5, C-5'; 41.0, C-2'; 37.7, C-3'; 29.2, C(SPh)COOBn; 27.0, CH_3; 19.3 $SiCMe_3$; LRMS (FAB, NBA) m/e: 727 ([M + Na^+], 2.0), 705 ([M + H^+] 1.6), 597 (1.0), 595 (0.9).

1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-dideoxy-2',3'-(1-*exo*-benzyloxycarbonyl)cyclopropane-β-D-ribofuranosyl] uracil (50), and 1-[5'-O-(tert-butyldiphenylsilyl)-2',3'-dideoxy-2',3'-(1-*endo*-benzyloxycarbonyl)cyclopropane-β-D-ribofuranosyl] uracil (51)



A solution of thiophenoxylate **49** (704 mg, 1.00 mmol), tributyltin hydride (0.54 ml, 2.00 mmol) and AIBN (10 mg) in 20 ml of dry benzene was refluxed for 20 hours. The mixture was chromatographed on silica gel (EtOAc / hexanes: 6/4) to provide the diastereomeric *exo*-carboxylate **50** (395 mg, 66% yield), and *endo*-carboxylate **51** (139

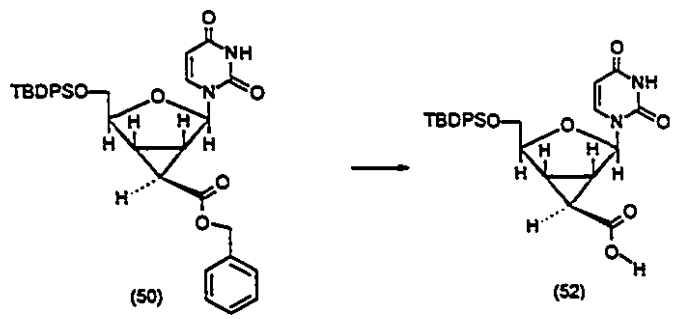
mg, 23%). *exo*-Carboxylate **50**: ^{1}H-NMR ($CDCl_3$) δ: 8.71 (br, 1H) NH; 7.67 (d, $J_{5,6}$ = 7.3 Hz, 1H) H-6; 7.65-7.33 (m, 15H) Arom.; 6.01 (s, 1H) H-1'; 5.30 (d, 1H) H-5; 5.12, 5.11 (2 x s, 2H) OCH_2Ph; 4.24 (dd, $J_{4',5'}$ = 5.4 Hz, $J_{4',5''}$ = 5.3 Hz, 1H) H-4'; 3.72 (dd, 2H) H-5', H-5''; 2.50 (dd, $J_{2',3'}$ = 6.8 Hz, $J_{6',2'}$ = 3.4 Hz, 1H) H-2'; 2.46 (dd, $J_{6',3'}$ = 3.4 Hz, 1H) H-3'; 1.75 (t, 1H) H-6'; 1.06 (s, 9H) t-Butyl; ^{13}C-NMR ($CDCl_3$) δ: 169.3, COOBn; 161.7, C-4; 149.5, C-2; 139.1, C-6; 134.8-127.3, Arom.; 101.8, C-5; 87.2, C-1'; 84.0, C-4'; 67.2, OCH_2 Ph; 65.7, C-5'; 32.1, C-2'; 30.0, C-3'; 27.5, CH_3; 25.7, CHCOOBn; 20.0, $SiCMe_3$; LRMS (FAB, NBA) m/e: 619 ([M + Na$^+$]), 597 ([M + H$^+$] 2.6), 539 (5.3), 505 (3.6), 485 (4.3), 451 (5.6); HRMS (FAB-glycerol) m/e: calcd for $C_{27}H_{30}N_2O_6Si$+H$^+$ [M + H$^+$] 597.242091, found 597.24207. *endo*-carboxylate **51**: ^{1}H-NMR ($CDCl_3$) δ: 8.33 (br, 1H) NH; 7.98 (d, $J_{5,6}$=7.8 Hz, 1H) H-6; 7.65-7.31 (m, 15H) Arom.; 6.31 (s, 1H) H-1'; 5.27 (d, 1H) H-5; 5.19, 5.17 (2 x s, 2 x 1H) OCH_2Ph; 4.39 (dd, $J_{4',5'}$=2.9 Hz, $J_{4',5''}$=4.4 Hz, 1H) H-4'; 3.99 (dd, $J_{5',5''}$=11.2 Hz, 1H) H-5'; 3.79 (dd, 1H) H-5''; 2.40 (dd, $J_{2',3'}$ = 8.3 Hz, $J_{6',2'}$ = 8.4 Hz 1H) H-3'; 2.35 (dd, $J_{6',3'}$ = 7.80 Hz, 1H) H-2'; 2.08 (dd, 1H) H-6'; 1.07 (s, 9H) t-Butyl; ^{13}C-NMR ($CDCl_3$) δ: 167.3, COOBn; 161.7, C-4; 149.2, C-2; 139.9, C-6; 134.8-127.3, Arom.; 101.8, C-5; 85.6, C-1'; 80.9, C-4'; 67.1, OCH_2Ph; 65.9, C-5'; 32.0, 28.4, C-2', C-3'; 27.6, CH_3; 23.8, CHCOOBn; 20.1, $SiC(Me)_3$; LRMS (FAB, NBA) m/e: 619 ([M + Na$^+$], 1.0), 597 ([M + H$^+$] 1.7), 539 (2.4), 505 (1.3), 485 (4.3), 451 (3.3); HRMS (FAB-glycerol) m/e: calcd for $C_{27}H_{30}N_2O_6Si$+H$^+$ [M + H$^+$] 597.242091, found 597.24207.

Epimerization of 51:

endo-Carboxylate **51** (12 mg, 0.02 mmol), previously co-evaporated with dry THF, was dissolved in 1 ml of dry THF, followed by addition of a few drops of lithium diisopropylamide in heptane/THF/ethylbenzene (2.0 M) at room temperature with stirring. After 20 minutes, the mixture was cooled in an ice-bath and quenched with glacial acetic acid. The mixture was then examined by TLC, with carboxylates **50** and **51**

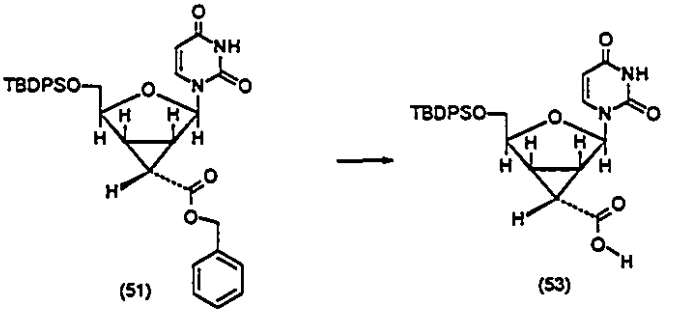
as the references. About half of the starting material **51** was converted to **50**.

1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-dideoxy-2',3'-(1-*exo*-carboxylic acid)cyclopropane-β-D-ribofuranosyl] uracil (52)



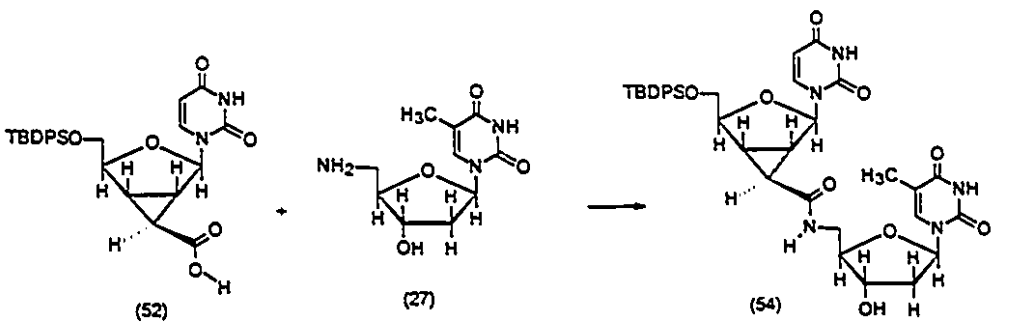
Benzyl *exo*-carboxylate **50** (370 mg, 0.62 mmol) was dissolved in 15 ml of dry methanol and hydrogenated over 100 mg of palladium / carbon (10%) at an initial pressure of 40 p.s.i. for 4 hours. The mixture was filtered through celite, and the filtrate was evaporated to dryness to give cyclopropyl *exo*-acid **52** as a colorless glass (302 mg, 92% yield). ^{1}H-NMR (CD_3 OD) δ: 7.72 (d, $J_{5,6}$=8.3 Hz, 1H) H-6; 7.64-7.35 (m, 10H) Arom.; 6.01 (s, 1H) H-1'; 5.21 (d, 1H) H-5; 4.26 (dd, $J_{4',5'}$=4.9 Hz, $J_{4',5''}$=5.3 Hz, 1H) H-4'; 3.79 (dd, $J_{5',5''}$=11.2 Hz, 1H) H-5'; 3.70 (dd, 1H) H-5''; 2.44 (m, 2H) H-2', H-3'; 1.65 (m, 1H) H-6'; 1.03 (s, 9H) t-Butyl; ^{13}C-NMR (CD_3 OD) δ: 175.2, COOH; 165.9, C-4; 152.4, C-2; 142.4, C-6; 136.7-128.9, Arom.; 102.4, C-5; 88.4, C-1'; 85.4, C-4'; 67.3, C-5'; 31.5, C-2'; 30.1, C-3'; 27.4, CH_3; 27.3 C-6', 20.0 $SiC(Me)_3$; LRMS (FAB, NBA) m/e: 573 ([M - 2 + 3Na$^+$] 16), 551 ([M - 1 + 2Na$^+$] 19.9), 529 ([M + Na$^+$] 23.3), 411 (1.3), 291 (1.5); HRMS (FAB-glycerol), m/e calcd. for $C_{27}H_{30}N_2O_6Si$ + Na$^+$ [M+Na$^+$] 529.177085, found 529.17728.

1-[5'-O-(tert-Butyldiphenylsilyl)-2',3'-dideoxy-2',3'-(1-*endo*-carboxylic acid)cyclopropane-β-D-ribofuranosyl] uracil (53)



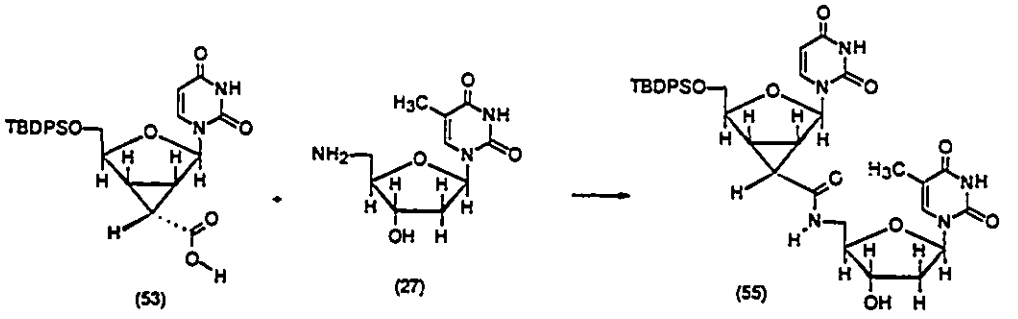
Benzyl carboxylate **51** (150 mg, 0.25 mmol) was dissolved in 10 ml of dry methanol and hydrogenated over 80 mg palladium / carbon (10%) at an initial pressure of 40 p.s.i. for 4 hours. The mixture was then filtered through celite, and the filtrate was evaporated to dryness to give the cyclopropyl *endo*-acid **53** as a glass (125 mg, 95%). ^{1}H-NMR (CD_3 OD) δ: 7.86 (d, $J_{5,6}$=7.8 Hz, 1H) H-6; 7.65-7.36 (m, 10H) Arom.; 6.35 (s, 1H) H-1'; 5.11 (d, 1H) H-5; 4.40 (dd, $J_{4',5'}$=3.3 Hz, $J_{4',5''}$=5.4 Hz, 1H) H-4'; 3.90 (dd, $J_{5',5''}$=11.2 Hz, 1H) H-5'; 3.79 (dd, 1H) H-5''; 2.40 (dd, $J_{2',3'}$=7.6 Hz, $J_{2',6'}$ =8.8 Hz, 1H) H-2'; 2.13 (dd, $J_{3',6'}$ =8.5 Hz, 1H) H-3'; 1.05 (s, 9H) *t*-Butyl; ^{13}C-NMR (CD_3 OD) δ: 173.0, COOH; 166.0, C-4; 152.3, C-2; 142.9, C-6; 136.6-129.0, Arom.; 102.6, C-5; 86.4, C-1'; 82.2, C-4'; 67.6, C-5'; 29.8, C-2'; 27.9, C-3'; 27.5, CH_3; 27.4, C-6'; 20.1, $SiCMe_3$; LRMS (FAB, NBA) m/e: 573 ([M - 2 + 3Na$^+$] 3.7), 551 ([M - 1 + 2Na$^+$] 10.9), 529 ([M + Na$^+$] 42.4), 291 (1.5); HRMS (FAB-glycerol), m/e calcd. for $C_{27}H_{30}N_2O_6Si$ + Na$^+$ [M+Na$^+$] 529.177085, found 529.17728.

Dinucleoside analog containing a cyclopropyl *exo*-amide linker (54)



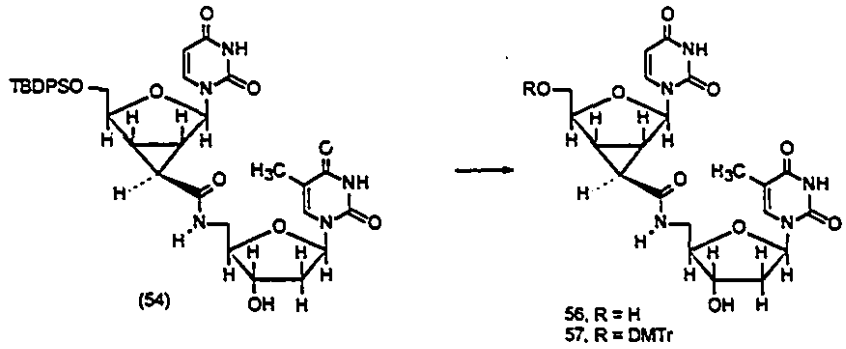
To a solution of *exo*-acid **52** (270 mg, 0.53 mmol) and 5'-amino-5'-deoxythymidine **27** (141 mg, 0.59 mmol) in dry DMF (5 ml) was added triethylamine (221 µl, 1.6 mmol), and then BOP (292 mg, 0.66 mmol). After stirring the mixture at RT for 2 hours, the DMF was evaporated. The residue was chromatographed on silica gel (MeOH / CH_2Cl_2, 5/95) to give dimer **54** as a white foam (351 mg, 91% yield). ^{1}H-NMR (CD_3 OD) δ: 7.73 (d, $J_{5,6}$=8.3 Hz, 1H) ^{5}H-6; 7.48 (s, 1H) ^{3}H-6; 7.65-7.37 (m, 10H) Arom.; 6.18 (t, $J_{2',1'}$=6.8 Hz, 1H) ^{3}H-1'; 6.00 (s, 1H) ^{5}H-1'; 5.18 (d, 1H) ^{5}H-5; 4.27-4.25 (m, 2H) ^{3}H-3', ^{5}H-4'; 3.91 (t, $J_{4',5'}$ = 3.9 Hz, 1H) ^{3}H-4'; 3.80 (dd, $J_{5'a,5'b}$ = 11.7 Hz, $J_{5'a,4'}$=4.4 Hz, 1H) ^{5}H-5'a; 3.72 (dd, $J_{4',5'b}$=5.9 Hz, 1H) ^{5}H-5'b; 3.50 (d, 2H) ^{3}H-5'a, ^{3}H-5'b; 2.44 (dd, $J_{2',3'}$=6.8 Hz, $J_{2',6}$=3.4 Hz, 1H) ^{5}H-2'; 2.40 (dd, $J_{3',6'}$ =2.9 Hz, 1H) ^{5}H-3'; 2.25 (m, 2H) ^{3}H-2'a, ^{3}H-2'b; 1.90 (s, 3H) ^{3}T-CH_3; 1.72 (dd, 1H) ^{5}H-6'; 1.04 (s, 9H) t-Butyl; ^{13}C-NMR (CD_3 OD) δ: 172.4, C-amide; 166.3, 165.9, ^{5}C-4, ^{3}C-4; 152.5, 152.2, ^{5}C-2, ^{3}C-2; 142.3, ^{5}C-6; 138.4, ^{3}C-6; 136.6-129.0, Arom.; 111.8, ^{3}C-5; 102.5, ^{5}C-5; 88.4, ^{5}C-1'; 87.1, ^{3}C-1'; 86.3, ^{3}C-4'; 85.5, ^{5}C-4'; 72.9, ^{3}C-3'; 67.4, ^{5}C-5'; 42.6, ^{3}C-5'; 39.9, ^{3}C-2'; 30.8, 29.2, ^{5}C-2', ^{5}C-3'; 27.4, CH_3; 27.3, 27.2, ^{5}C-6', $SiCMe_3$; 12.5 ^{3}T-CH_3; LRMS (FAB, NBA) m/e: 752 ([M + Na^+] 10), 730 ([M + H^+] 1.6), 492 (2.5); HRMS (FAB-glycerol), m/e calcd. for $C_{27}H_{30}N_2O_6Si$ + Na^+ [M + Na^+] 752.272777, found 752.27294.

Dinucleoside analog containing a cyclopropyl *endo*-amide linker (55)



To a solution of *endo*-acid **53** (125 mg, 0.25 mmol) and 5'-amino-5'-deoxythymidine **27** (72 mg, 0.30 mmol) in dry DMF (4 ml) was added triethylamine (105 µl, 0.75 mmol), and then BOP (292 mg, 0.66 mmol). After stirring the mixture at RT for 2 hours, the DMF was evaporated. The residue was chromatographed on silica gel (MeOH / CH_2Cl_2, 5/95) to give dimer **54** as a white foam (164 mg, 90% yield). ^{1}H-NMR (CD_3 OD) δ: 7.79 (d, $J_{5,6}$= 8.3 Hz, 1H) ^{5}H-6; 7.52 (d, $J_{T\text{-}CH_3,6}$ = 1.0 Hz, 1H) ^{3}H-6; 7.63-7.33 (m, 10H) Arom.; 6.32 (s, 1H) ^{5}H-1'; 6.18 (t, $J_{2',1'}$=6.8 Hz, $J_{2'',1'}$=7.2 Hz, 1H) ^{3}H-1'; 5.19 (d, 1H) ^{5}H-5; 4.47 (dd, $J_{4',5'}$=5.1 Hz, $J_{4',5''}$=3.9 Hz, 1H) ^{5}H-4'; 4.31 (m, 1H) ^{3}H-3'; 3.96 (m, 1H) ^{3}H-5'; 3.82 (dd, 1H) ^{5}H-5'; 3.70 (dd, 1H) ^{5}H-5''; 3.52 (m, 1H) ^{3}H-5', ^{3}H-5''; 2.27 (m, 4H) ^{5}H-2', ^{5}H-3', ^{3}H-2'', ^{3}H-2''; 2.14 (dd, $J_{2',6'}$ = 7.8 Hz, $J_{3',6'}$=8.3 Hz, 1H) ^{5}H-6'; 1.87 (s, 3H) ^{3}T-CH_3; 1.00 (s, 9H) t-Butyl; ^{13}C-NMR (CD_3 OD) δ: 170.0, COONHR; 166.3, 165.9, ^{5}C-4, ^{3}C-4; 152.4, 152.3, ^{5}C-2, ^{3}C-2; 142.4, ^{5}C-6; 138.4, ^{3}C-6; 136.8-128.8, Arom.; 111.8, ^{3}C-5; 102.5, ^{5}C-5; 87.1, ^{3}C-1'; 86.6, ^{3}C-4'; 86.5, ^{5}C-1'; 82.2, ^{5}C-4'; 72.9, ^{3}C-3'; 67.7, ^{5}C-5'; 42.4, ^{3}C-5'; 40.1, ^{3}C-2'; 28.6, ^{5}C-2'; 27.4, CH_3; 27.0, C-6'; 24.7, ^{5}C-3'; 20.1, $SiCMe_3$; 12.6 T-CH_3; LRMS (FAB, NBA) m/e: 752 ([M + Na^+] 17.6), 492 (4.5); HRMS (FAB-glycerol), m/e calcd. for $C_{27}H_{30}N_2O_6Si$ + Na^+ [M + Na^+] 752.272777, found 752.27294.

Cyclopropyl *exo*-amide-linked diol (56), and 5'-DMTr protected dinucleoside analog (57)

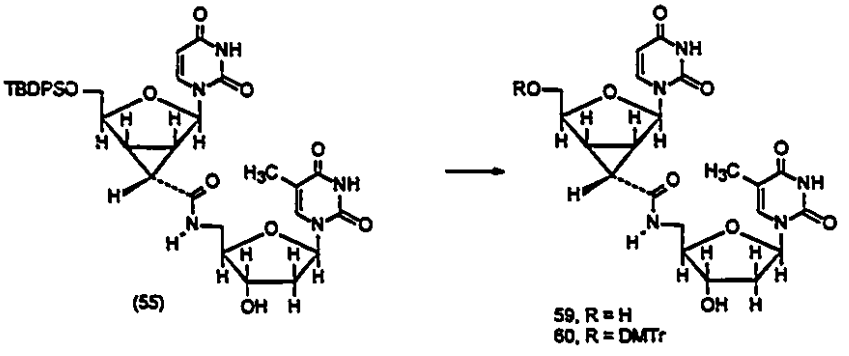


To a solution of silylated dimer **54** (379 mg, 0.52 mmol) in 5 ml THF was added TBAF / THF (1 M, 1.56 ml) at room temperature. After 3 hours, the THF was decanted. The remaining gummy residue was triturated with ether until it turned into white fine solid. The solid was dried *in vacuo* to provide diol **56** (240 mg, 94% yield). ^{1}H-NMR (d_5-Pyridine) δ: 9.76 (s, 2H) ^{3}NH, ^{5}NH; 8.21 (d, $J_{5,6}$=7.8 Hz, 1H) ^{5}H-6; 7.66 (s, 1H) ^{3}H-6; 6.83 (dd, $J_{1',2'}$=6.8 Hz, $J_{1',2''}$=6.3 Hz, 1H) ^{3}H-1'; 6.52 (s, 1H) ^{5}H-1'; 5.80 (d, 1H) ^{5}H-5; 5.04 (br) OH; 4.88 (dd, $J_{2',3'}$=1.5 Hz, $J_{2'',3'}$=1.0 Hz, 1H) ^{3}H-3'; 4.55 (d, 1H) ^{3}H-4'; 4.40 (dd, 1H) ^{5}H-4'; 4.02 (d, 2H) ^{3}H-5'a, ^{3}H-5'b; 3.96-3.88 (m, 2H) ^{5}H-5'a, ^{5}H-5'b; 2.89, 2.74 (2 x m, 2H) ^{5}H-2', ^{5}H-3'; 2.62 (m, 2H) ^{3}H-2'; 2.18 (m, 1H) ^{5}H-6'; 1.96 (s, 3H) ^{3}T-CH_3; ^{13}C-NMR (d_5-Pyridine) δ: 110.9, ^{3}C-5; 102.0, ^{5}C-5; 87.1, 86.4, 86.3, 85.9, ^{5}C-1', ^{3}C-1', ^{3}C-4', ^{5}C-4'; 72.5, ^{5}C-3'; 64.6, ^{5}C-5'; 42.5, ^{3}C-5'; 39.9, ^{3}C-2'; 30.5, 29.4, ^{5}C-2', ^{5}C-3'; 27.3, ^{5}C-6'; 12.7, ^{3}T-CH_3; LRMS (FAR, NBA) m/e: 492 ([M + H$^+$] 0.9).

Diol **56** (200 mg) obtained was coevaporated with dry pyridine (3 x 2 ml) to give diol **56** in a white foam. Diol **56** was then redissolved in 5 ml of dry pyridine, and treated with 4,4'-dimethyoxytrityl chloride (270 mg, 0.80 mmol) at RT for 4 hours. Water (2 ml) was then added. After removing the volatile solvent, the resulting syrup was chromatographed on silica gel (Et_3N / MeOH / CH_2Cl_2, 1/5/94, v/v) to afford dinucleoside **57** as a white foam (264 mg, 84%). ^{1}H-NMR (CD_3 OD) δ: 7.67 (d, $J_{5,6}$=7.8

Hz, 1H) ^{5}H-6; 7.47 (s, 1H) ^{3}H-6; 7.38-6.81 (m, 13H) Arom.; 6.18 (t, $J_{2',1'}$=6.8 Hz, 1H) ^{3}H-1'; 6.02 (s, 1H) ^{5}H-1'; 5.12 (d, 1H) ^{5}H-5; 4.33 (dd, $J_{4',5'}$=5.9 Hz, $J_{4',5''}$=3.9 Hz, 1H) ^{5}H-4'; 4.27 (dd, $J_{2',3'}$=2.9 Hz, $J_{2'',3'}$=2.0 Hz, 1H) ^{3}H-3'; 3.91 (dd, $J_{5',4'}$ =4.4 Hz, $J_{5'',4'}$=6.8 Hz, 1H) ^{3}H-4'; 3.76 (s, 6H) OCH_3; 3.51, 3.50 (dd, 2H) ^{3}H-5'a, ^{3}H-5'b; 3.24 (dd, $J_{5',5''}$=9.8 Hz, 1H) ^{5}H-5'a; 3.08 (dd, 1H) ^{5}H-5'b; 2.43 (dd, $J_{2',3'}$=6.3 Hz, $J_{2',6'}$ =3.5 Hz, 1H) ^{5}H-2'; 2.40 (dd, $J_{3',6'}$ =2.9 Hz, 1H) ^{5}H-3'; 2.25 (m, 2H) ^{3}H-2'; 1.88 (s, 3H) ^{3}T-CH_3; 1.73 (dd, 1H) ^{5}H-6'; ^{13}C-NMR (CD_3 OD) δ: 172.5 COONHR; 166.3, 165.9, ^{5}C-4, ^{3}C-4; 152.6, 152.2, ^{5}C-2, ^{3}C-2; 146.0, C-amide; 142.5, ^{5}C-6; 138.4, ^{3}C-6; 131.6-114.1, Arom.; 111.8, ^{3}C-5; 102.4, ^{5}C-5; 88.2, ^{5}C-1'; 87.0, ^{3}C-1'; 86.3, ^{3}C-4'; 84.1, ^{5}C-4'; 73.0, ^{3}C-3'; 67.0, ^{5}C-5'; 55.7, OCH_3; 42.6, ^{3}C-5'; 39.9, ^{3}C-2'; 30.7, 29.4, ^{5}C-2', ^{5}C-3'; 27.3, ^{5}C-6'; 12.5, ^{3}T-CH_3; LRMS (FAB, NBA) m/e: 816 ([M + Na^+] 0.2), 794 ([M + H^+] 0.4), 519 (25), 242 (100).

Cyclopropyl *endo*-amide-linked diol (59), and 5'-DMTr protected dinucleoside analog (60)



To a solution of silylated dimer 55 (150 mg, 0.30 mmol) in 5 ml THF was added TBAF / THF (1 M, 0.9 ml) at RT. After 3 hours, the THF solution was decanted. The remaining gummy residue was triturated with ether until it turned into white fine solid. The solid was dried *in vacuo* to give diol 59 (134 mg, 91%). ^{1}H-NMR (d_5-Pyridine) δ: 10.46 (br, 2H) ^{3}NH, ^{5}NH; 8.42 (d, 1H) ^{5}H-6; 7.16 (s, 1H) ^{5}H-1'; 6.90 (dd, $J_{1',2'}$=5.9 Hz, J

$J_{1',2''}$=6.3 Hz, 1H) ^{3}H-1'; 5.90 (d, $J_{5,6}$=6.8 Hz, 1H) ^{5}H-5; 5.07 (m, 1H) ^{3}H-3'; 4.92 (m, 1H) ^{3}H-4'; 4.50 (m, 1H) ^{5}H-4'; 4.06, 3.74 (m, 4H) ^{3}H-5'a, ^{3}H-5'b; ^{5}H-5'a, ^{5}H-5'; 2.89, 2.57 (2 x dd, 2 x 1H) ^{5}H-2', ^{5}H-3'; 2.50, 2.39 (2 x m, 2x 1H) ^{3}H-2'a, ^{3}H-2'b; 2.24 (dd, 1H) H-cyclo; 2.05 (s, 3H) ^{3}T-CH_3; ^{13}C-NMR (d_5-Pyridine) δ: 110.9, ^{3}C-5; 103.1, ^{5}C-5; 86.3, ^{3}C-1'; 85.6, ^{3}C-4'; 85.2, ^{5}C-1'; 81.7, ^{5}C-4'; 72.8, ^{3}C-3'; 65.0, ^{5}C-5'; 42.4, ^{3}C-5'; 39.8, ^{3}C-2'; 30.4, 28.4, ^{5}C-2', ^{5}C-3'; 24.2, ^{5}C-6'; 12.6 ^{3}T-CH_3; LRMS (FAB, NBA) m/e: 514 ([M + Na^+] 0.4), 492 ([M + H^+] 0.6), 242 (86).

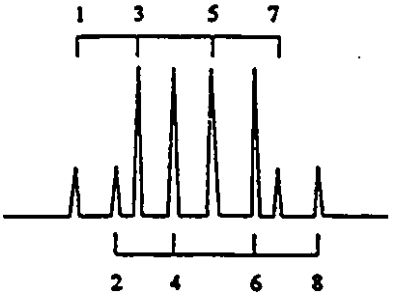
Diol **59** (120 mg) obtained was co-evaporated with dry pyridine (3 x 2 ml) until it turned into a white foam. Diol **59** was then redissolved in 5 ml of dry pyridine, and treated with 4,4'-dimethoxytrityl chloride (124 mg) at RT for 4 hours. Water (2 ml) was then added. After removing the volatile solvent, the resulting syrup was chromatographed on silica gel (Et_3N / MeOH / CH_2Cl_2, 1/5/94, v/v) to afford dinucleoside **60** as a white foam (162 mg, 84% yield). ^{1}H-NMR (CD_3 OD) δ: 7.67 (d, $J_{5,6}$=7.8 Hz, 1H) ^{5}H-6; 7.49 (s, 1H) ^{3}H-6; 7.32-6.75 (m, 13H) Arom.; 6.31 (s, 1H) ^{5}H-1'; 6.16 (t, $J_{2',1'}$=6.8 Hz, 1H) ^{3}H-1'; 5.11 (d, 1H) ^{5}H-5; 4.50 (m, 1H) ^{5}H-4'; 4.30 (m, 1H) ^{3}H-3'; 3.94 (m, 1H) ^{3}H-4'; 3.70 (s, 6H) 2 x OCH_3; 3.52 (dd, $J_{5',5''}$=14.2 Hz, $J_{4',5'}$=6.8 Hz, 1H) ^{5}H-5'a; 3.45 (dd, $J_{4',5''}$=4.4 Hz, 1H) ^{5}H-5'b; 3.11-3.02 (dd, 2H) ^{3}H-5'a, ^{3}H-5'b; 2.23 (m, 4H) ^{3}H-2'a, ^{3}H-2'b, ^{5}H-2', ^{5}H-3'; 2.10 (dd, $J_{3',6'}$ =8.8 Hz, $J_{2',6}$=8.3 Hz, 1H) ^{5}H-6'; 1.80 (s, 3H) ^{3}T-CH_3; ^{13}C-NMR (CD_3 OD) δ: 169.9, COONHR; 166.3, 165.8, ^{5}C-4, ^{3}C-4; 152.5, 152.2, ^{5}C-2(5), ^{3}C-2; 142.8 ^{5}C-6; 138.4 ^{3}C-6; 131.4-114.1 Arom.; 111.8 ^{3}C-5; 102.5 ^{5}C-5; 87.1 ^{3}C-1'; 86.5 ^{3}C-4'; 86.2 ^{5}C-1'; 80.8 ^{5}C-4'; 72.9 ^{3}C-3'; 67.4 ^{5}C-5; 55.8 OCH_3; 42.3 ^{3}C-5'; 40.3 ^{3}C-2'; 28.2, 27.1 ^{5}C-2', ^{5}C-3'; 24.7 ^{5}C-6'; 12.6 ^{3}T-CH_3; LRMS (FAB, NBA) m/e: 816 ([M + Na^+] 0.2), 794 ([M + H^+] 0.5), 519 (17), 242 (100).

5. APPENDIXES

Appendix I. Analysis of ABX systems in ^{1}H-NMR spectra.

The Chemical shifts and coupling constants of second order AB portions of ABX systems were calculated by method shown below[93].
The ABX spectrum is divided into two AB system.

AB #1



AB #2

$$J_{AB} = (8 - 6) = (7 - 5) = (4 - 2) = (3 - 1)$$

AB #1

$$\vartheta_1 = (1 + 3 + 5 + 7)/4$$
$$(\Delta\vartheta_1)/2 = [(1 - 7)(3 - 5)]^{1/2}/2$$
$$\Delta 1^+ = \vartheta_1 + (\Delta\vartheta_1)/2$$
$$\Delta 1^- = \vartheta_1 - (\Delta\vartheta_1)/2$$

$$\vartheta_A = (\Delta 1^+ + \Delta 2^+)/2$$
$$J_{AX} = \Delta 1^+ - \Delta 2^+$$
or
$$\vartheta_A = (\Delta 1^+ + \Delta 2^-)/2$$
$$J_{AX} = \Delta 1^+ - \Delta 2^-$$

AB #2

$$\vartheta_2 = (2 + 4 + 6 + 8)/4$$
$$(\Delta\vartheta_2)/2 = [(2 - 8)(4 - 6)]^{1/2}/2$$
$$\Delta 2^+ = \vartheta_2 + (\Delta\vartheta_2)/2$$
$$\Delta 2^- = \vartheta_2 - (\Delta\vartheta_2)/2$$

$$\vartheta_B = (\Delta 1^- + \Delta 2^-)/2$$
$$J_{BX} = \Delta 1^- - \Delta 2^-$$
or
$$\vartheta_B = (\Delta 1^- + \Delta 2^+)/2$$
$$J_{BX} = \Delta 1^- - \Delta 2^+$$

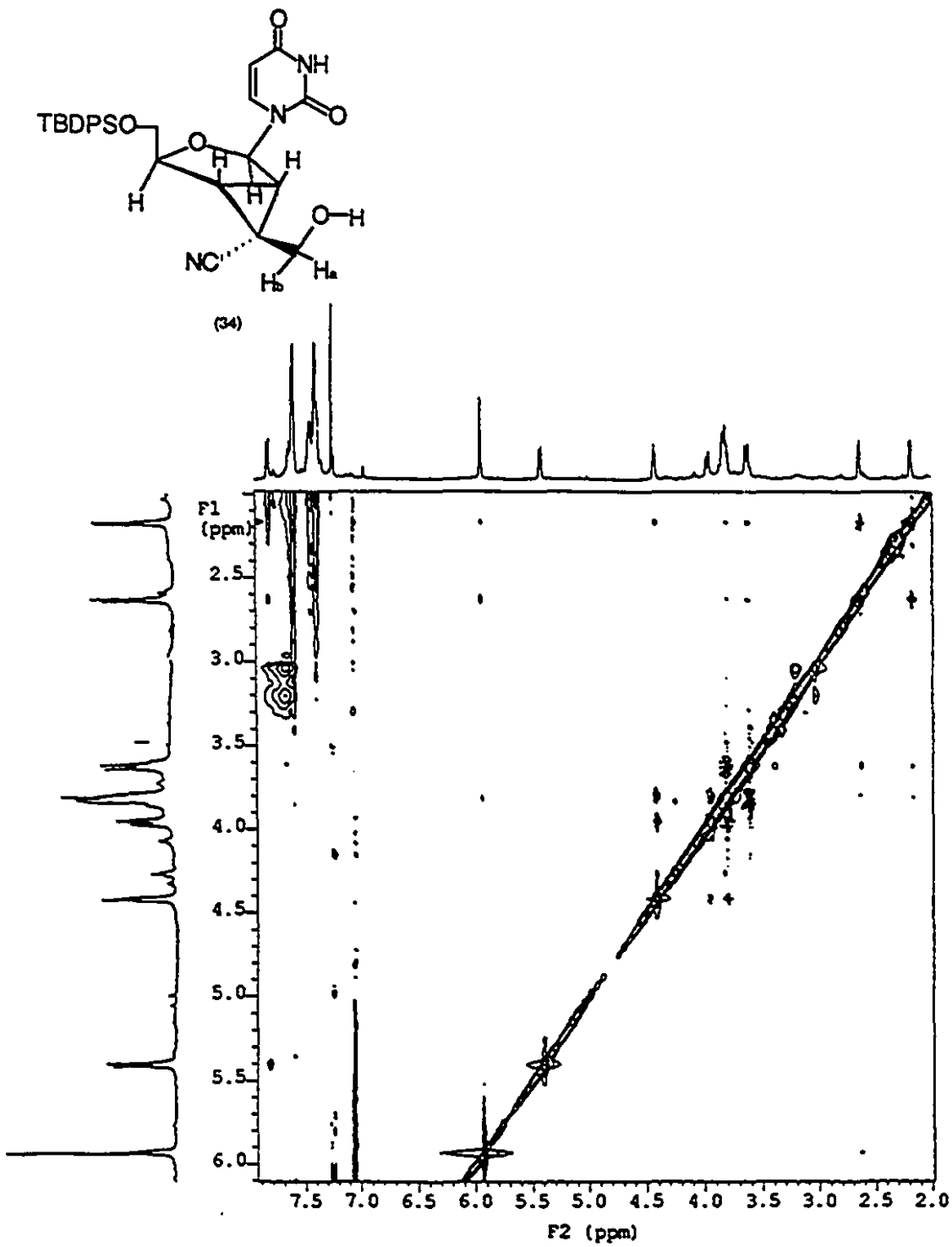
Two possible sets of values are generated, but one gives unrealistic coupling constants.

---

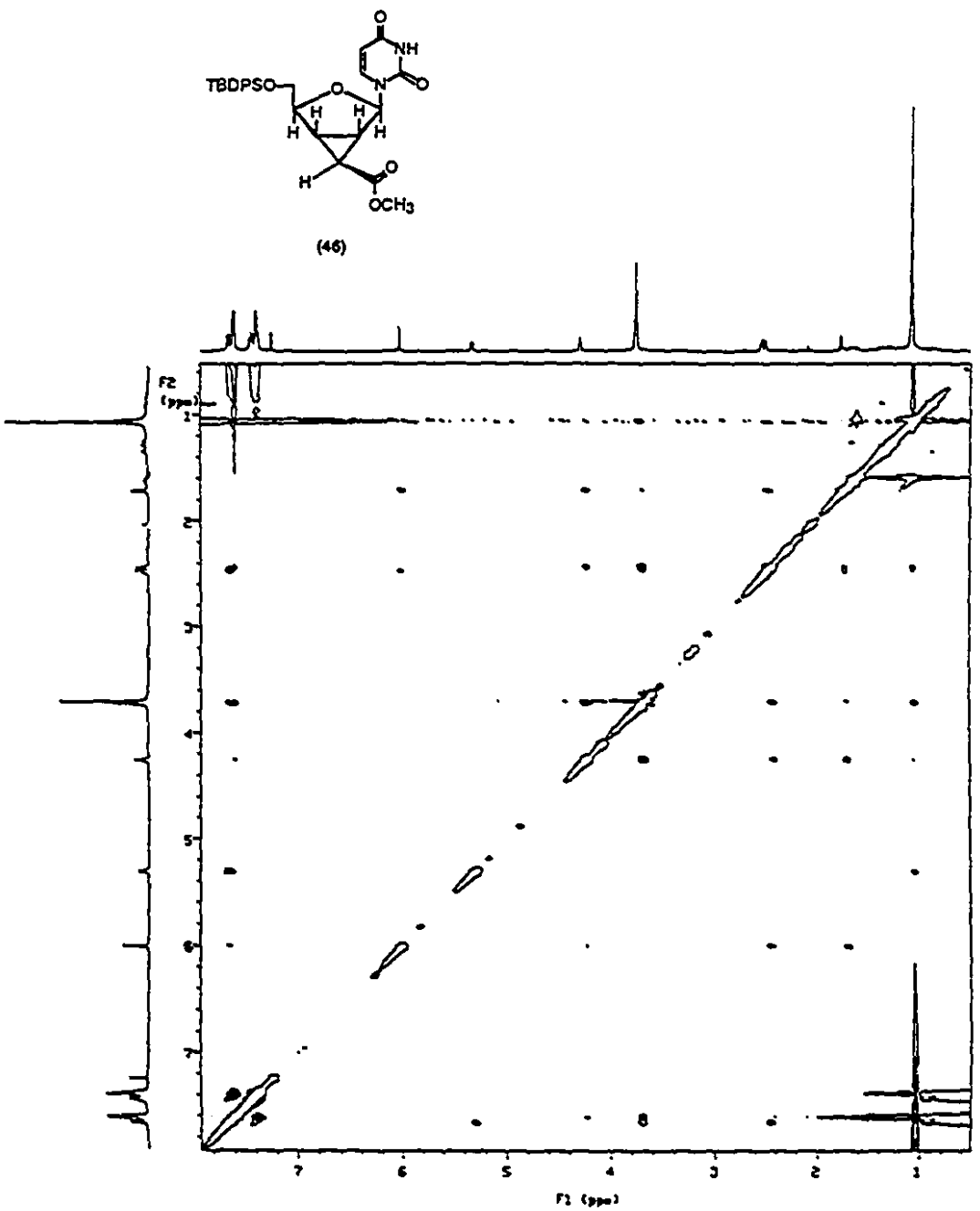
[93]Becker, E.D., (ed.), High Resolution NMR-Theory and chemical applications, Academic Press, Inc., London, (1980), chap. 7.

Appendix II. NOESY spectra of compound 34, 46, 47, 50 and 51 (500 MHz).

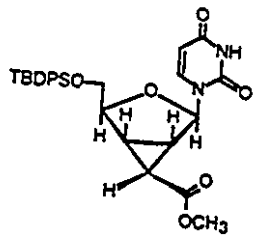
1. NOESY spectrum of alcohol **34** (Section 2.7.4.)



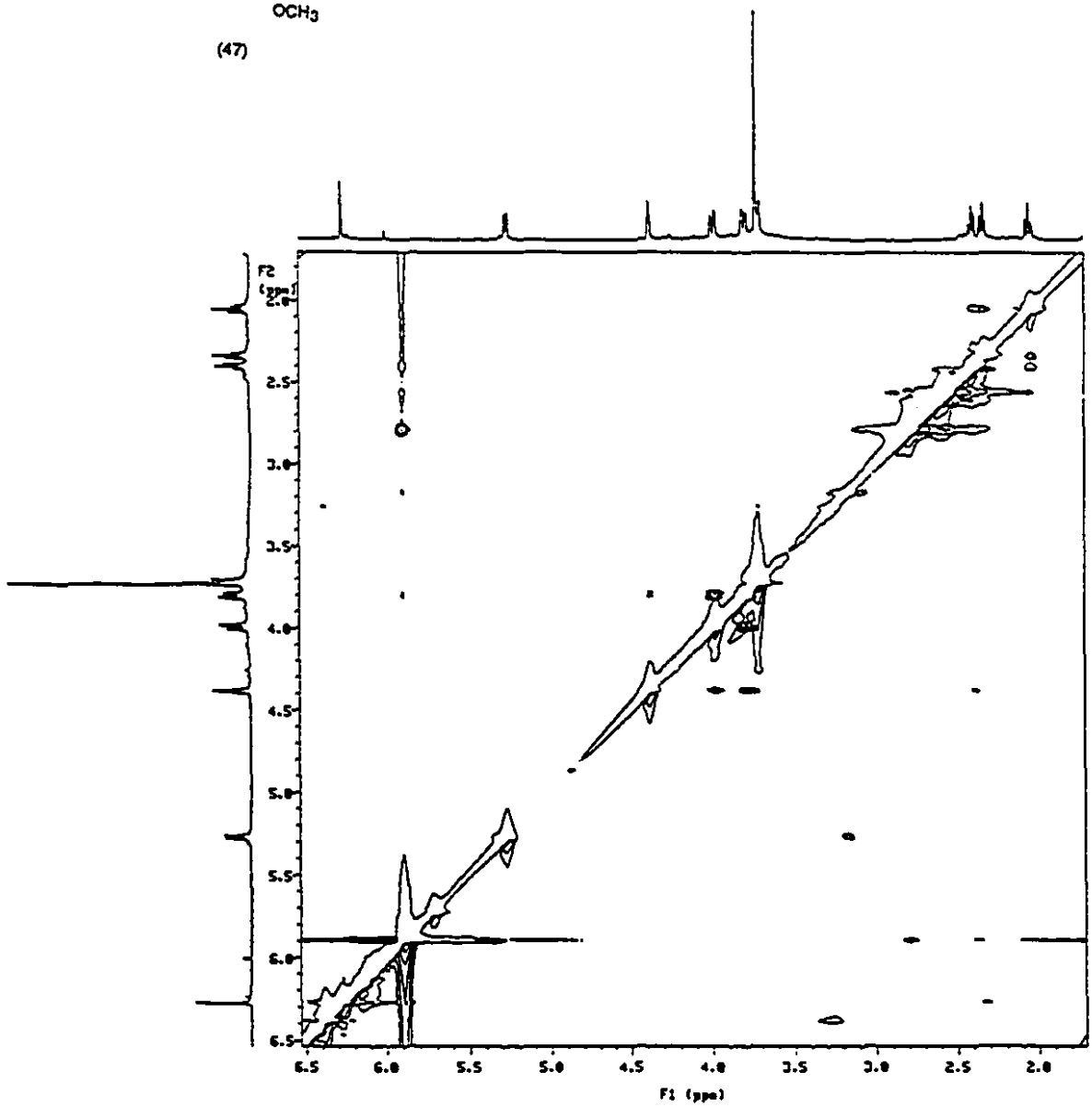
2. NOESY spectrum of methyl *exo*-carboxylate **46** (Section 2.10.3).



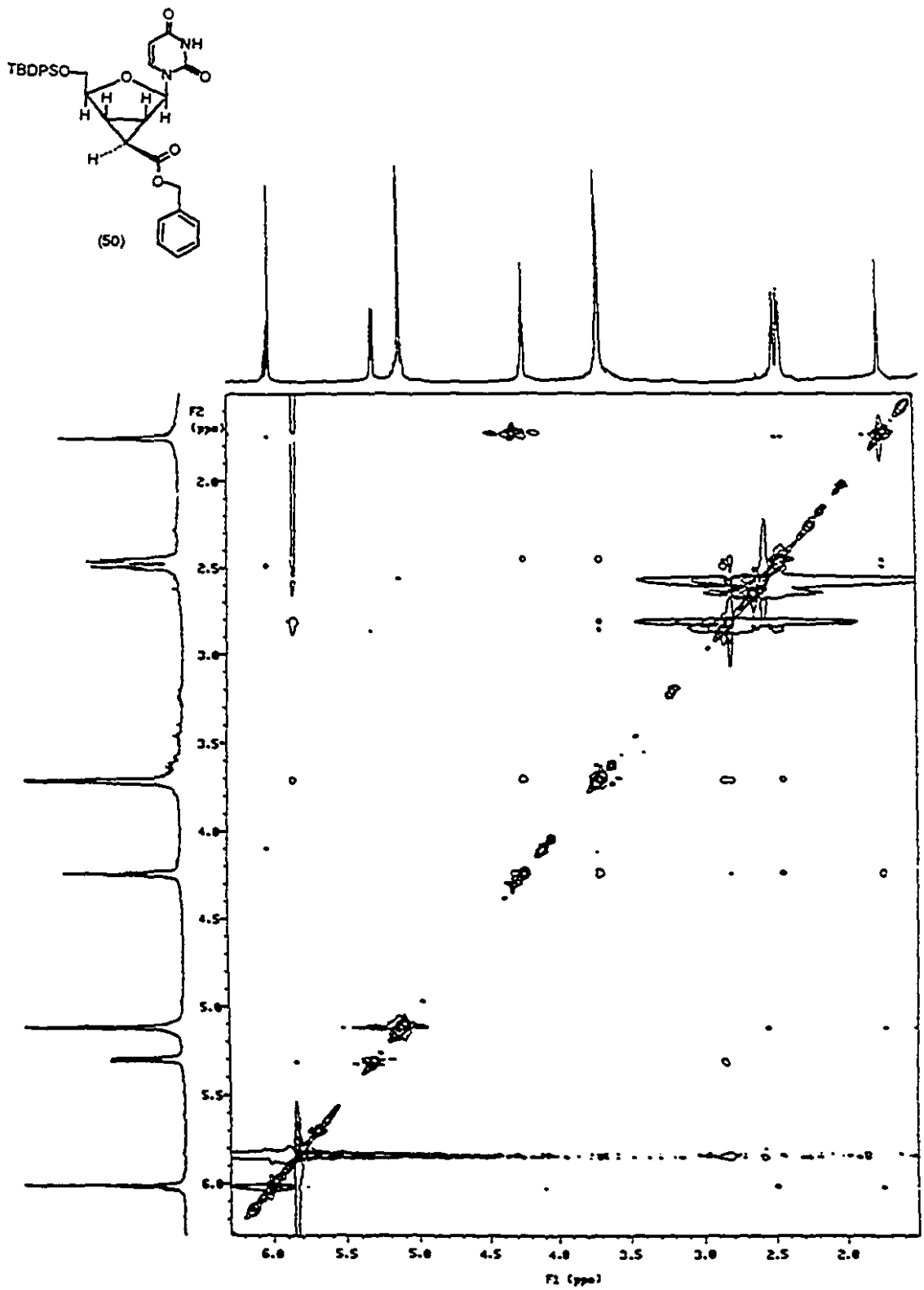
3. NOESY spectrum of methyl *endo*-carboxylate **47** (Section 2.10.3).



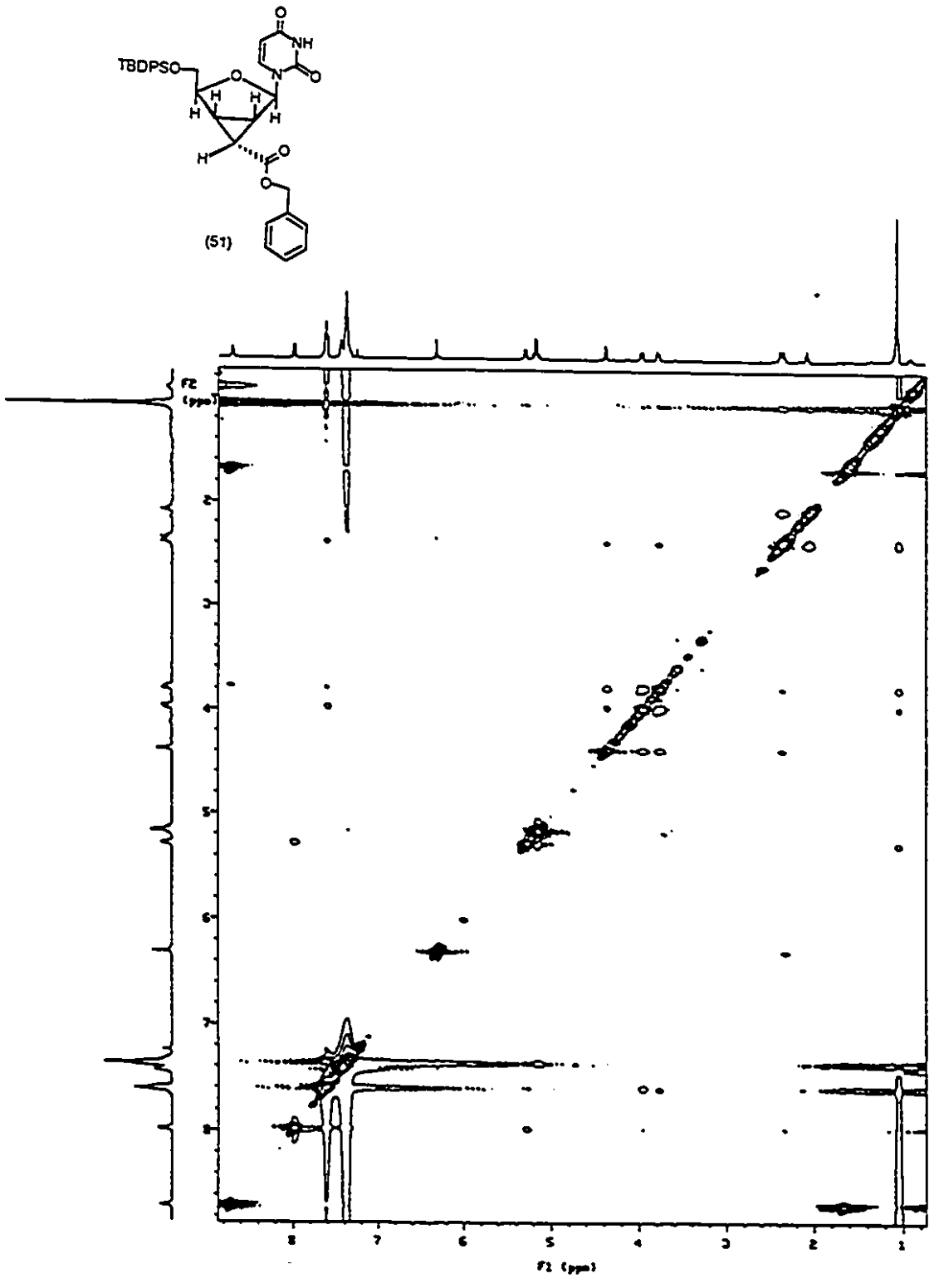
(47)



4. NOESY spectrum of benzyl *exo*-carboxylate 50 (Section 2.11.2).

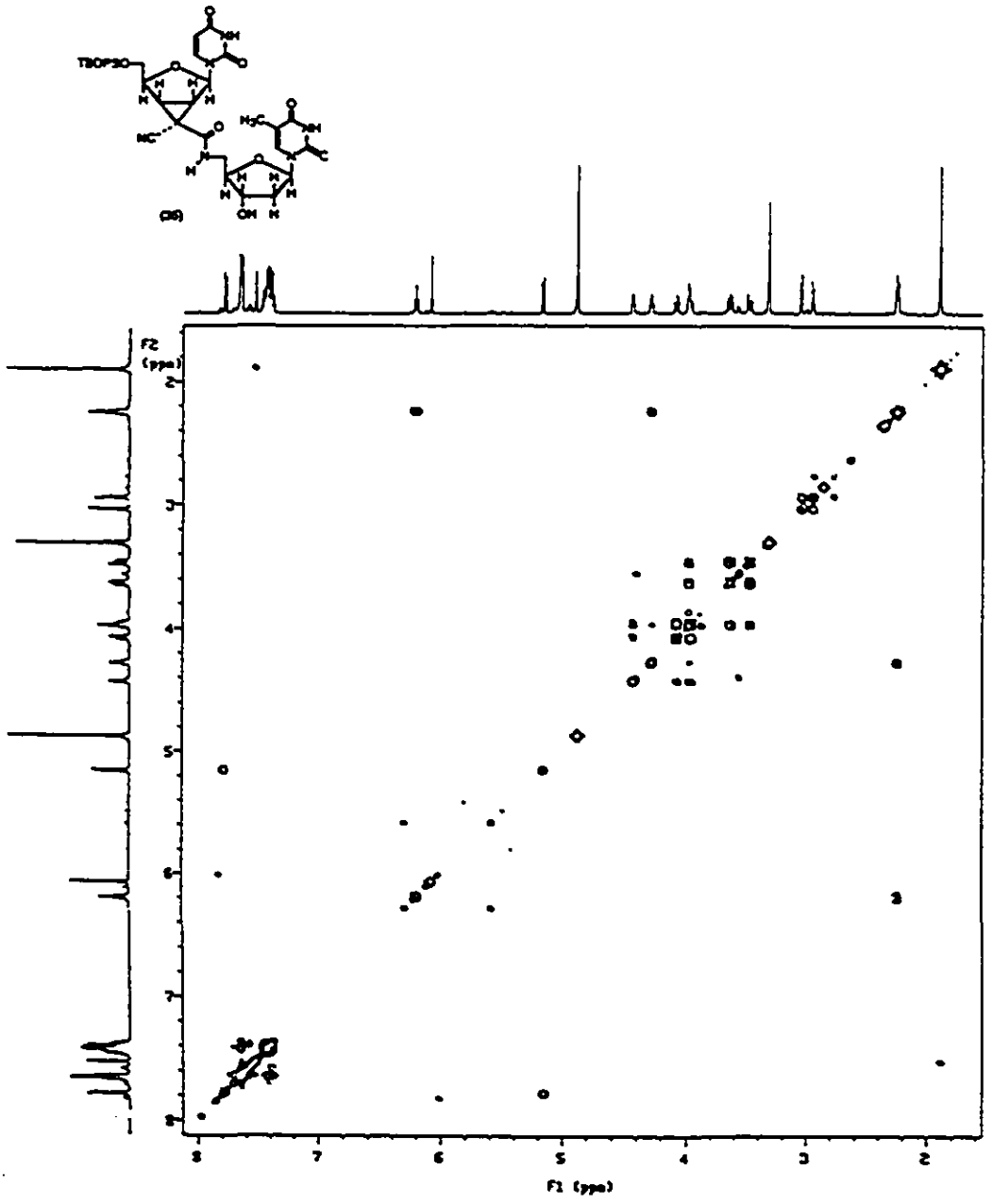


5. NOESY spectrum of benzyl *endo*-carboxylate **51** (Section 2.11.2).

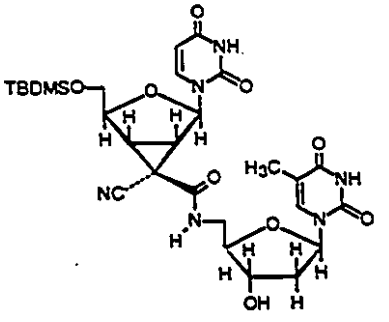


Appendix III. COSY and HMQC spectra of the key compounds (500 MHz).

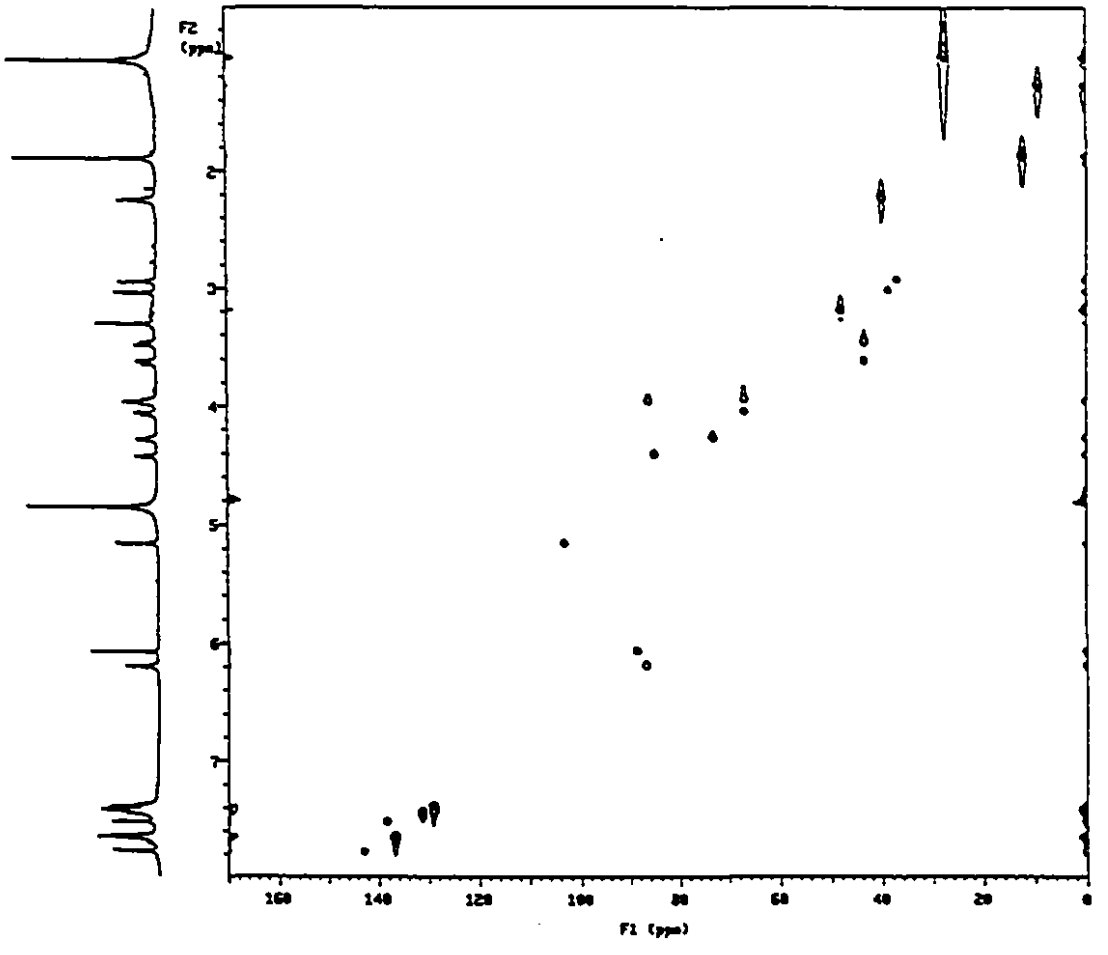
1. COSY spectrum of dimer 35 containing a cyclopropyl *endo*-cyano-*exo*-amide linker (Section 2.8.1.)



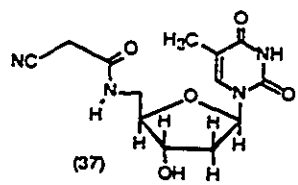
2. HMQC spectrum of dimer 35 containing a cyclopropyl *endo*-cyano-*exo*-amide linker (Section 2.8.1.)



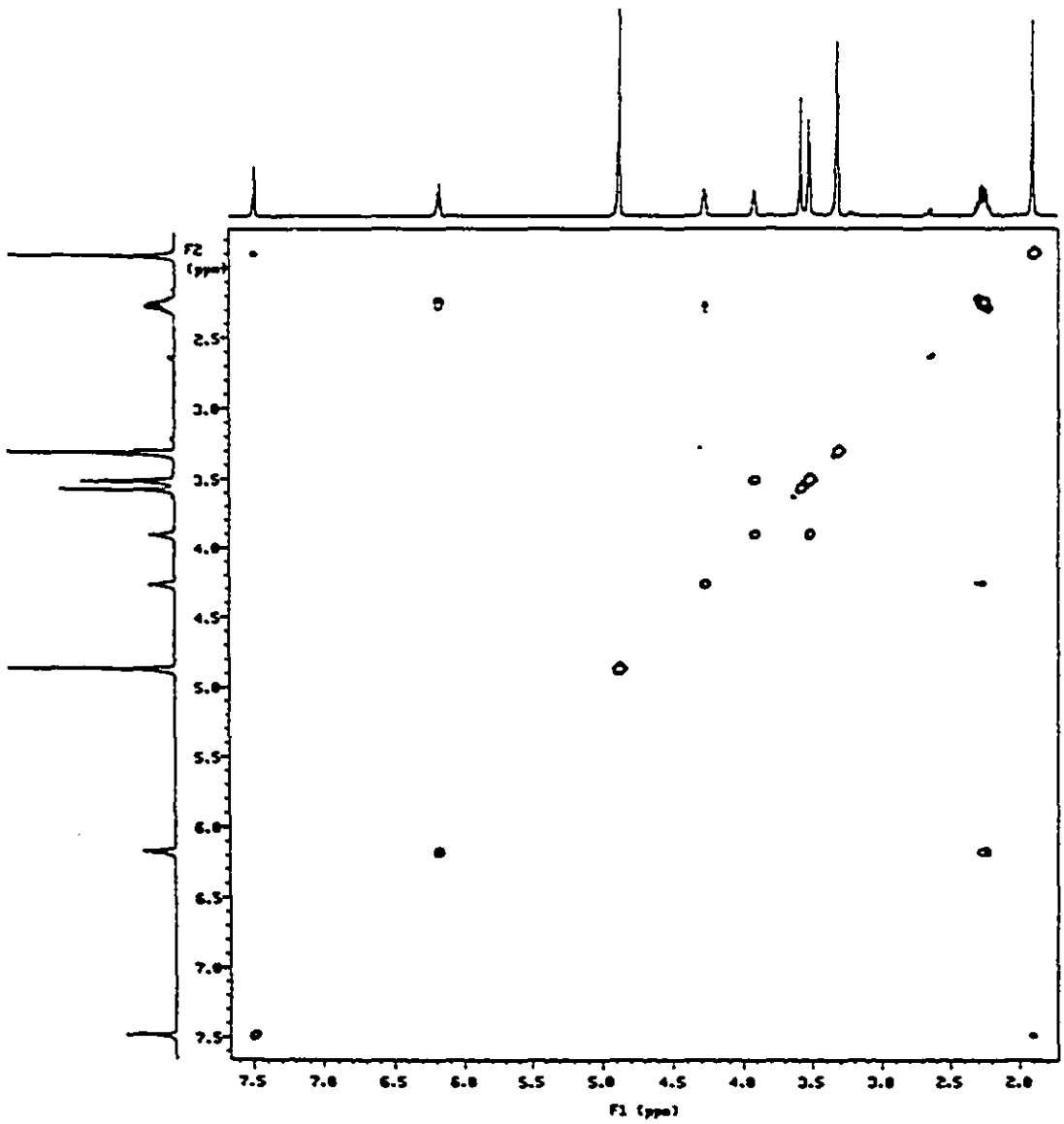
(35)



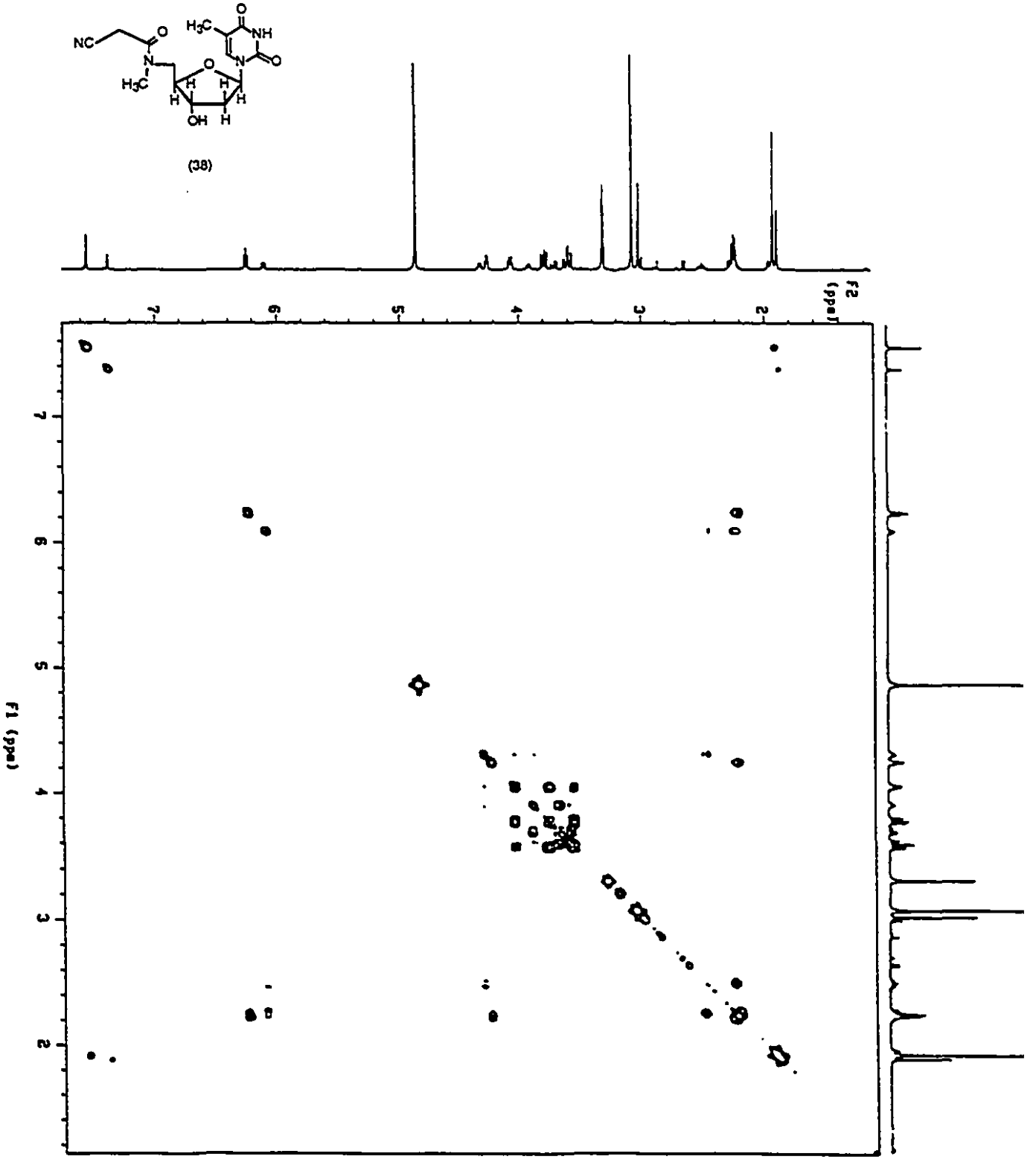
3. COSY spectrum of amide 37 (Section 2.8.2).



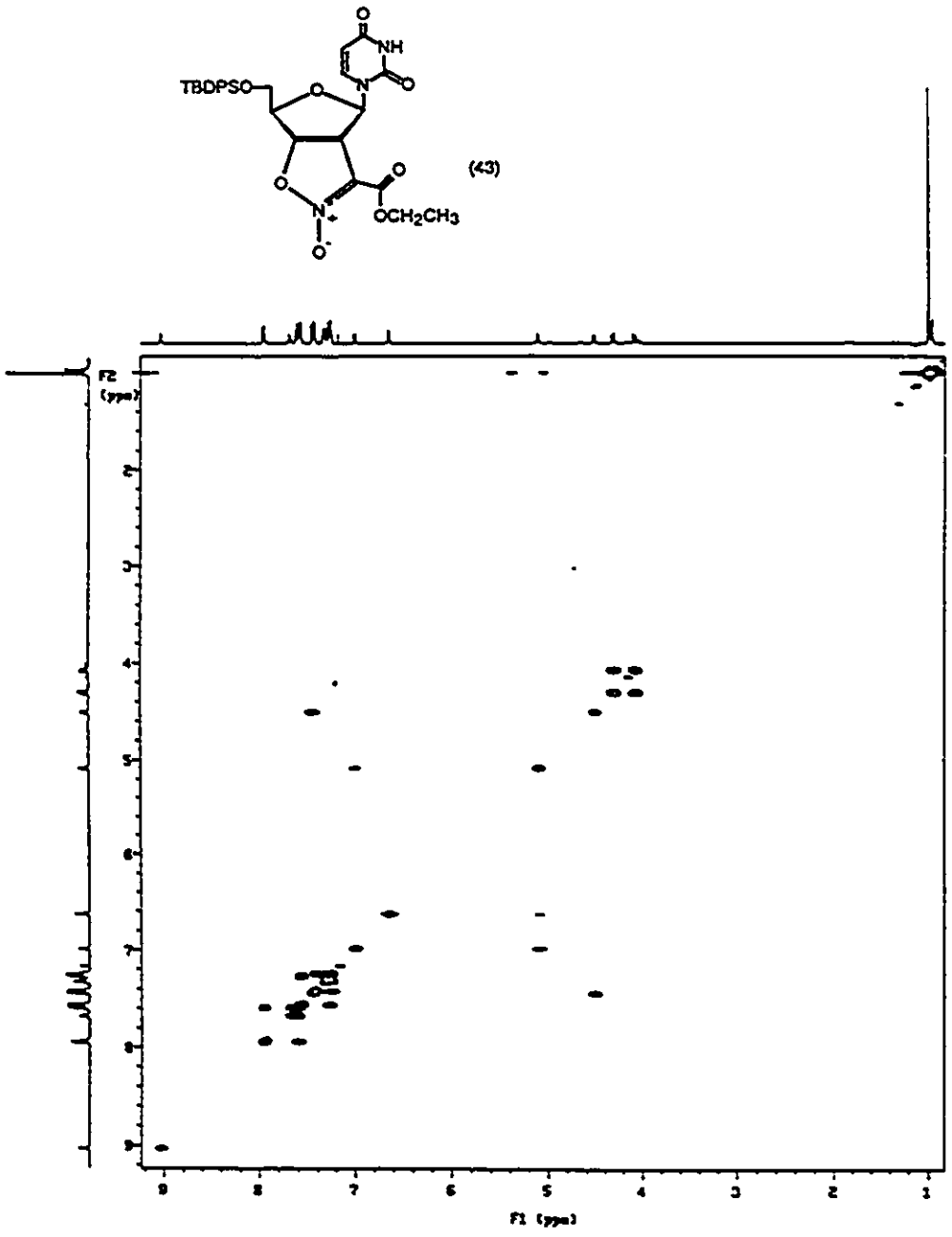
(37)



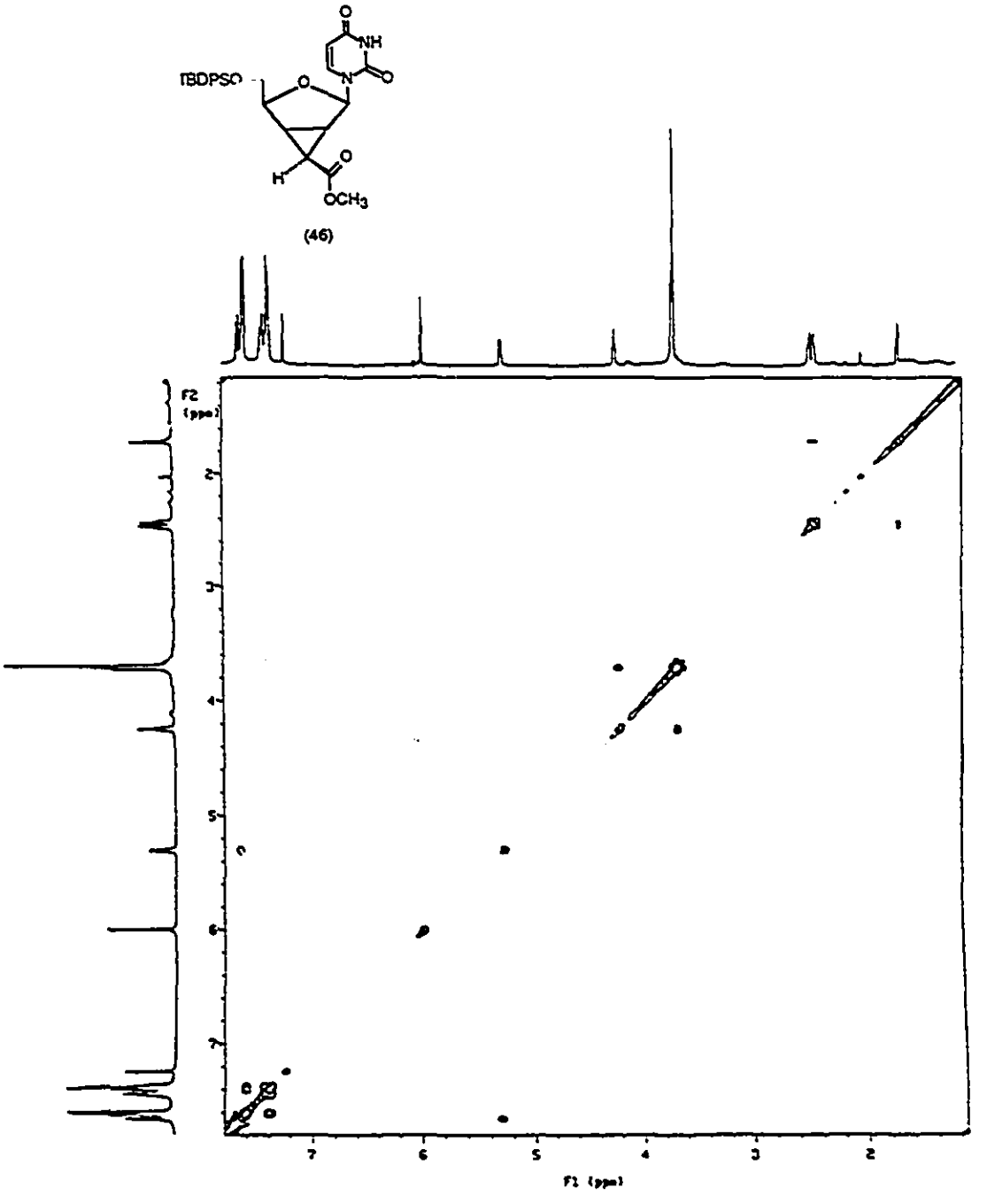
4. COSY spectrum of methyl amide **38** (Section 2.8.2).



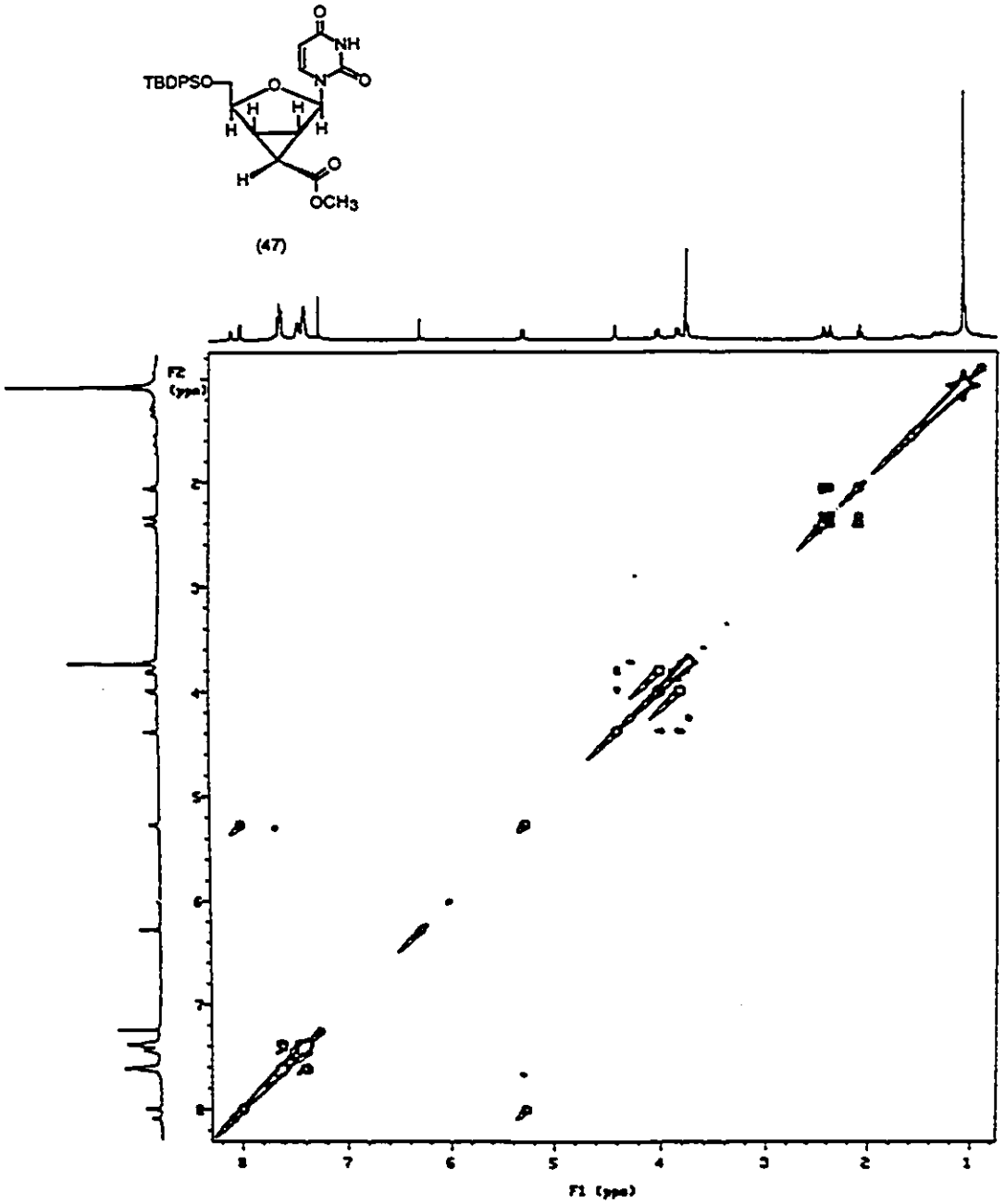
5. COSY spectrum of [3.3.1]-bicyclic nucleoside analog **43** (Section 2.10.2).



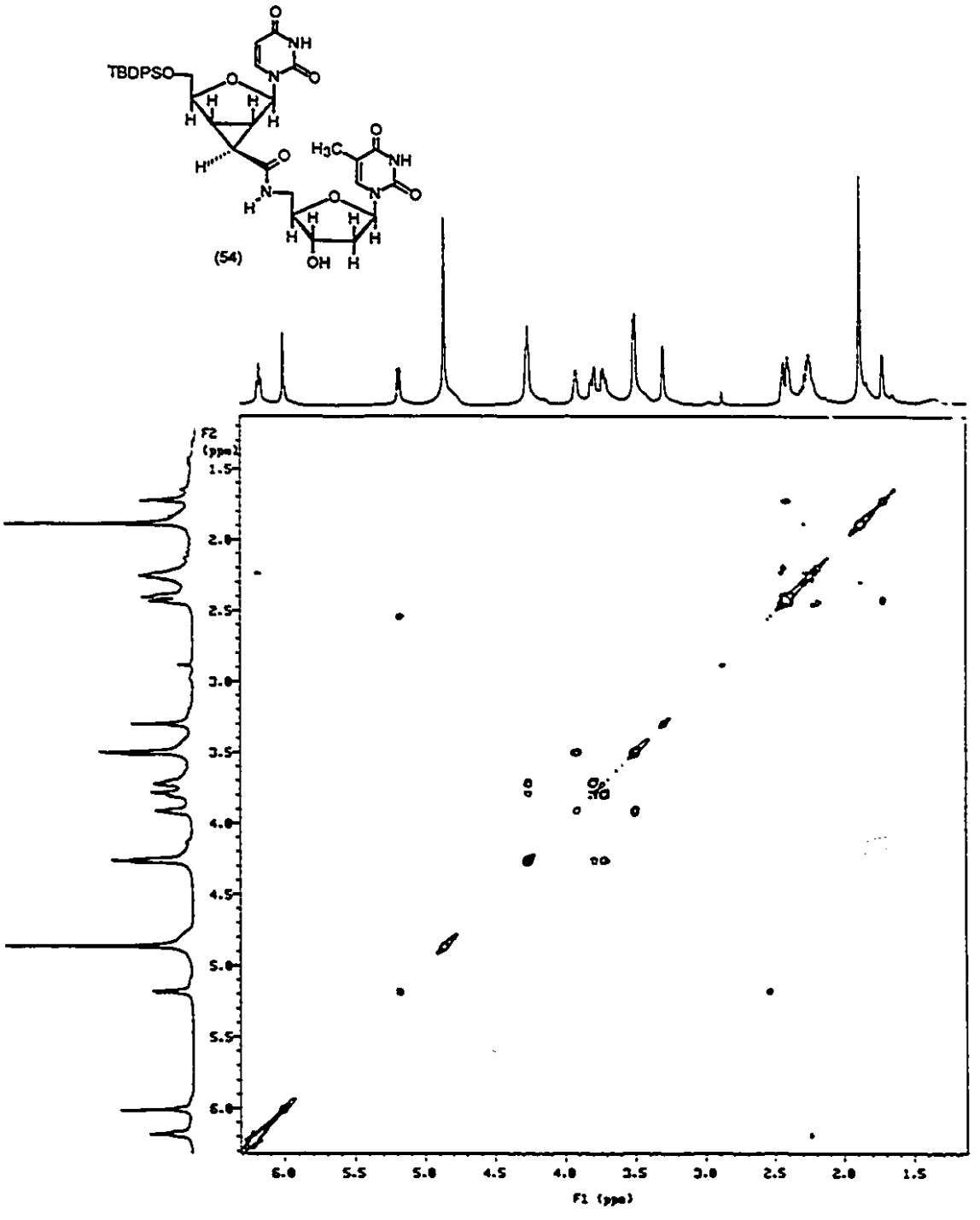
6. COSY spectrum of methyl *exo*-carboxylate **46** (Section 2.10.3).



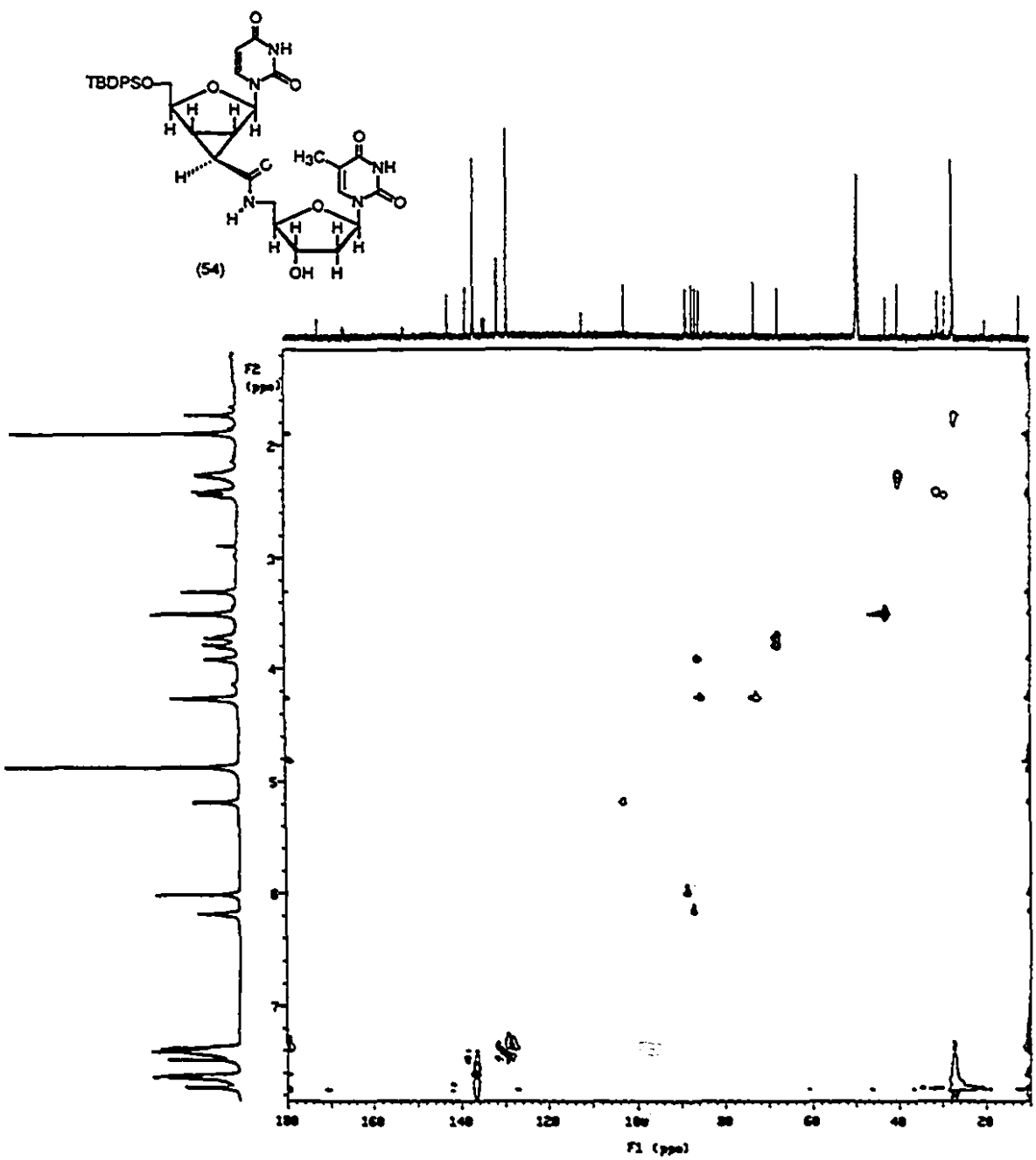
7. COSY spectrum of methyl *endo*-carboxylate **47** (Section 2.10.3).



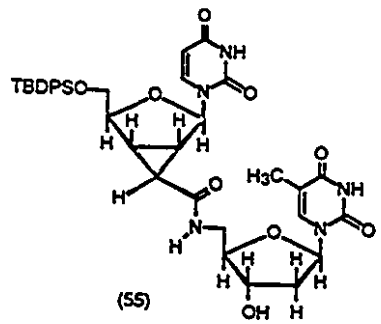
8. COSY spectrum of 5'-t-butyldiphenylsilyl protected dimer **54** containing a cyclopropyl *exo*-amide linker (Section 2.12.).



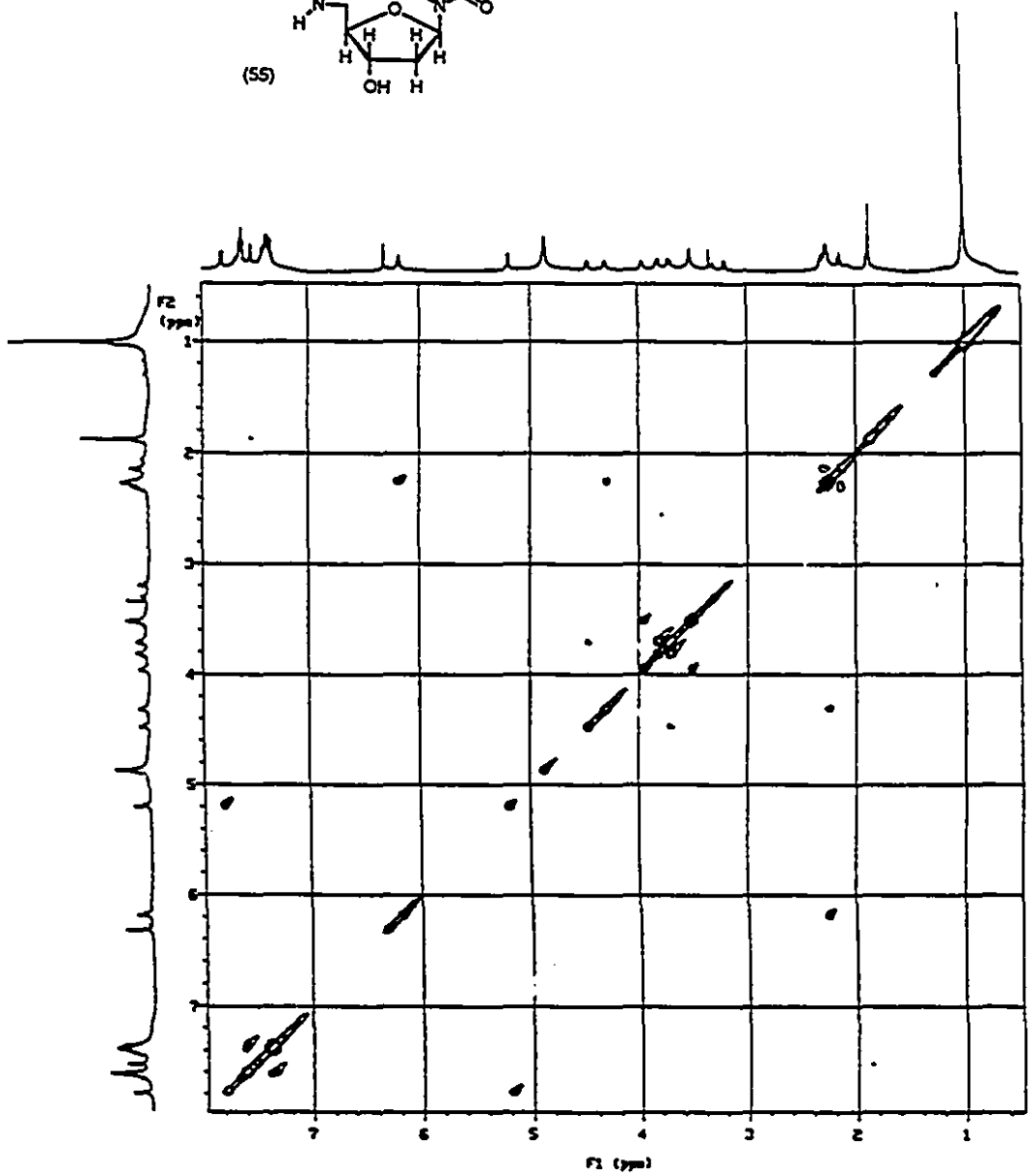
9. HMQC spectrum of 5'-t-butyldiphenylsilyl protected dimer **54** containing a cyclopropyl *exo*-amide linker (Section 2.12.).



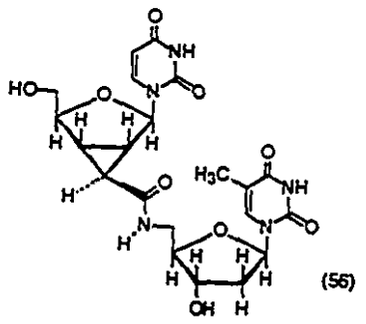
10. COSY spectrum of 5'-t-butyldiphenylsilyl protected dimer **55** containing a cyclopropyl *endo*-amide linker (Section 2.12.).



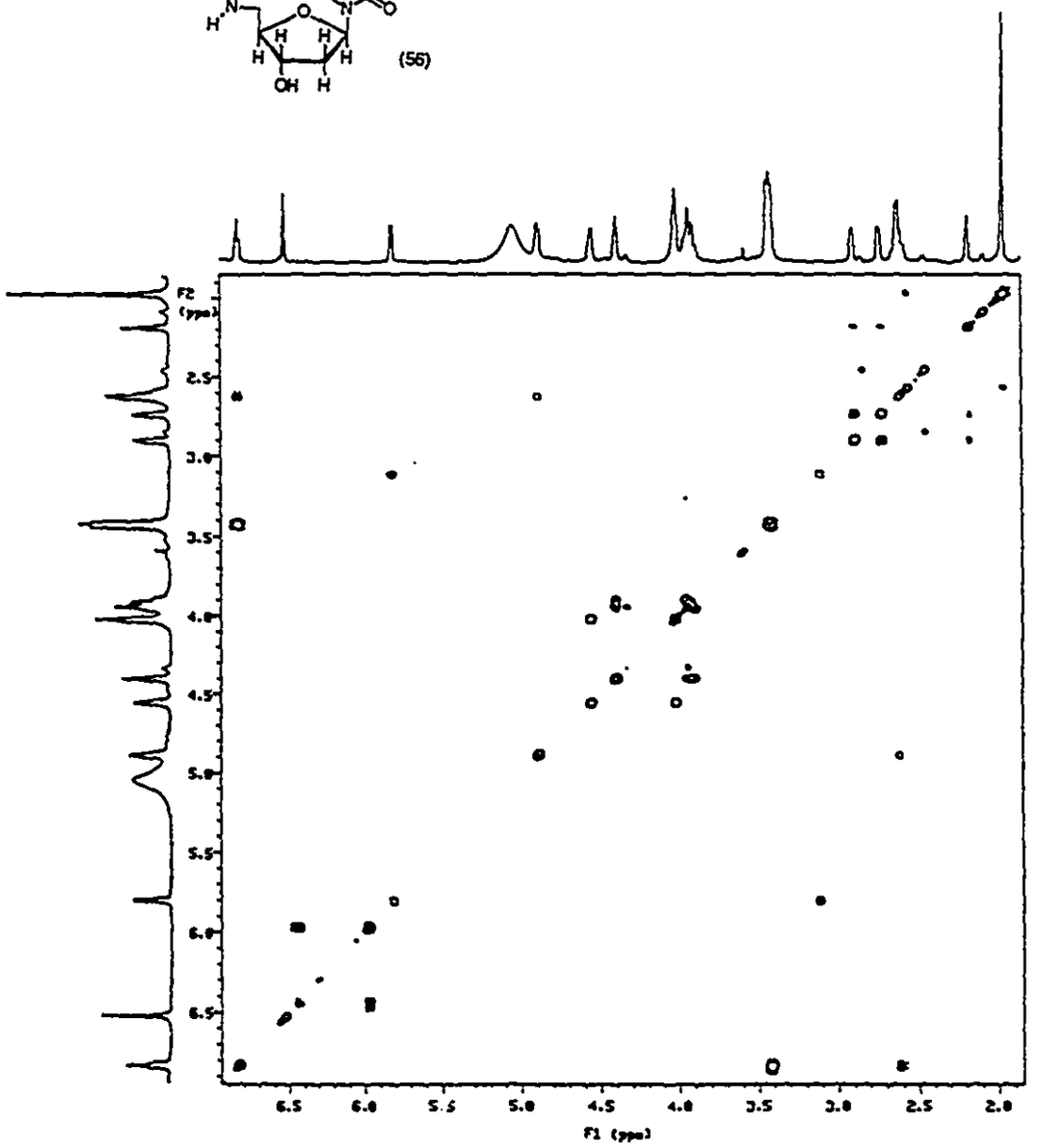
(55)



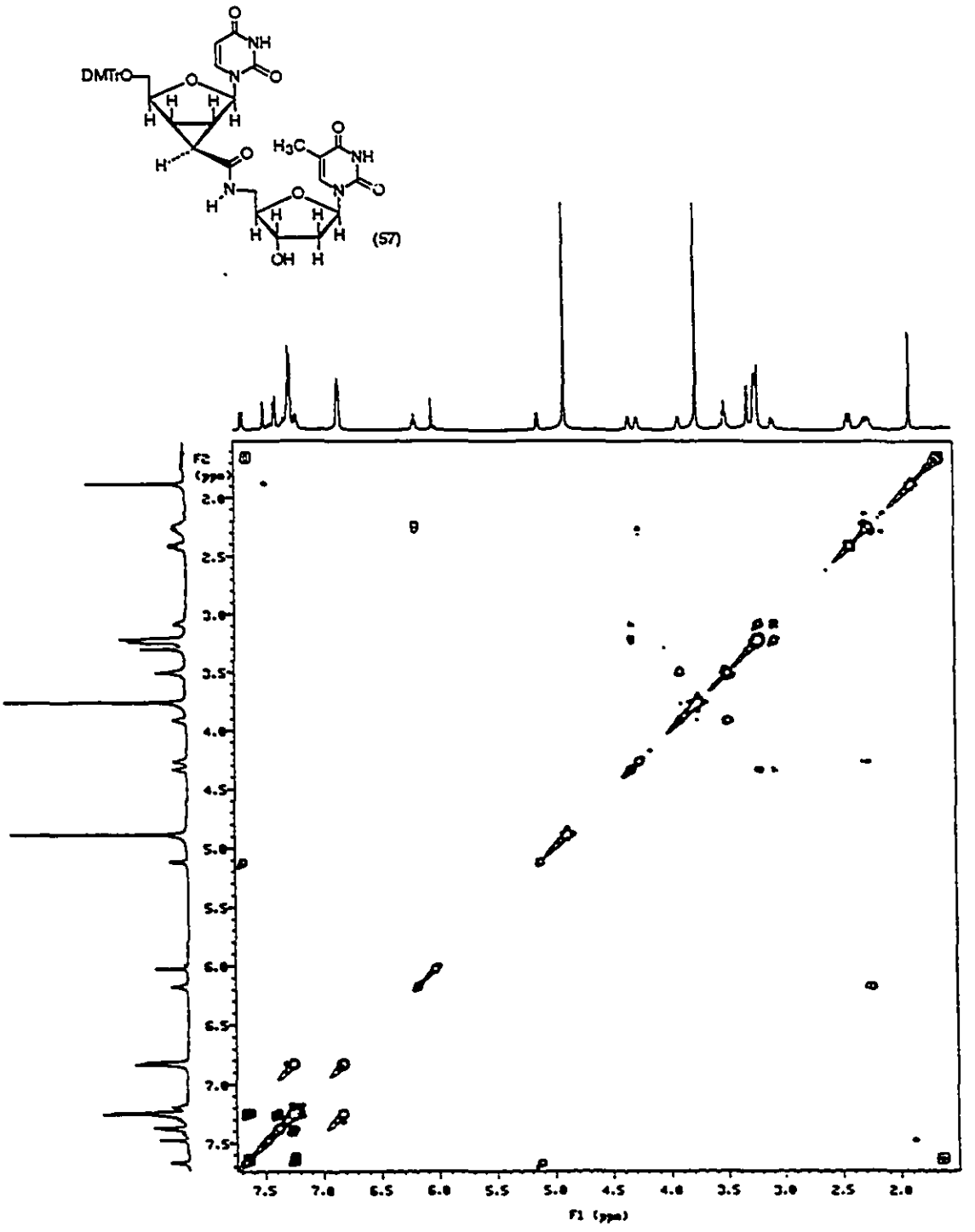
11. COSY spectrum of diol **56** containing a cyclopropyl *exo*-amide linker (Section 2.13.).



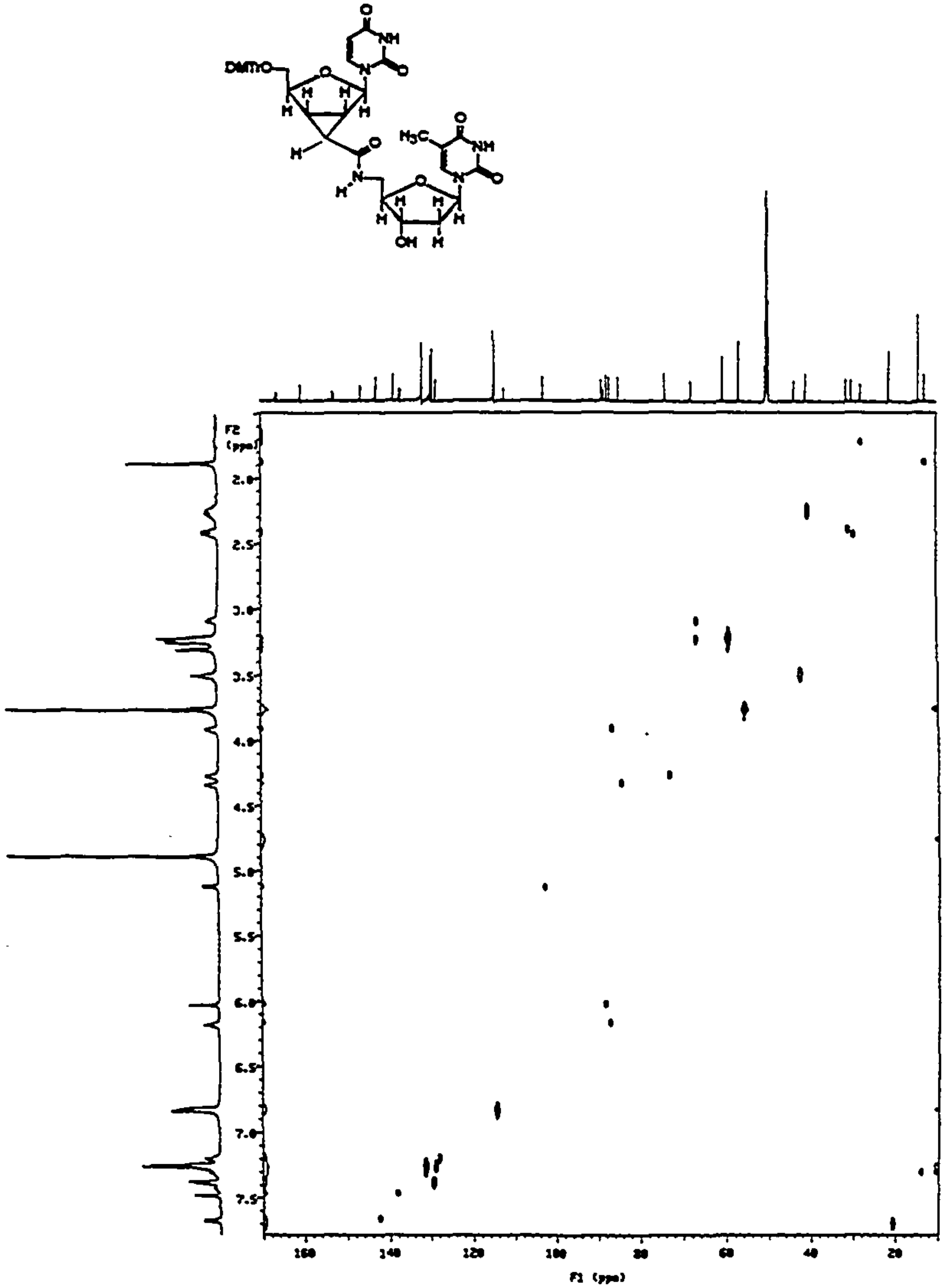
(56)



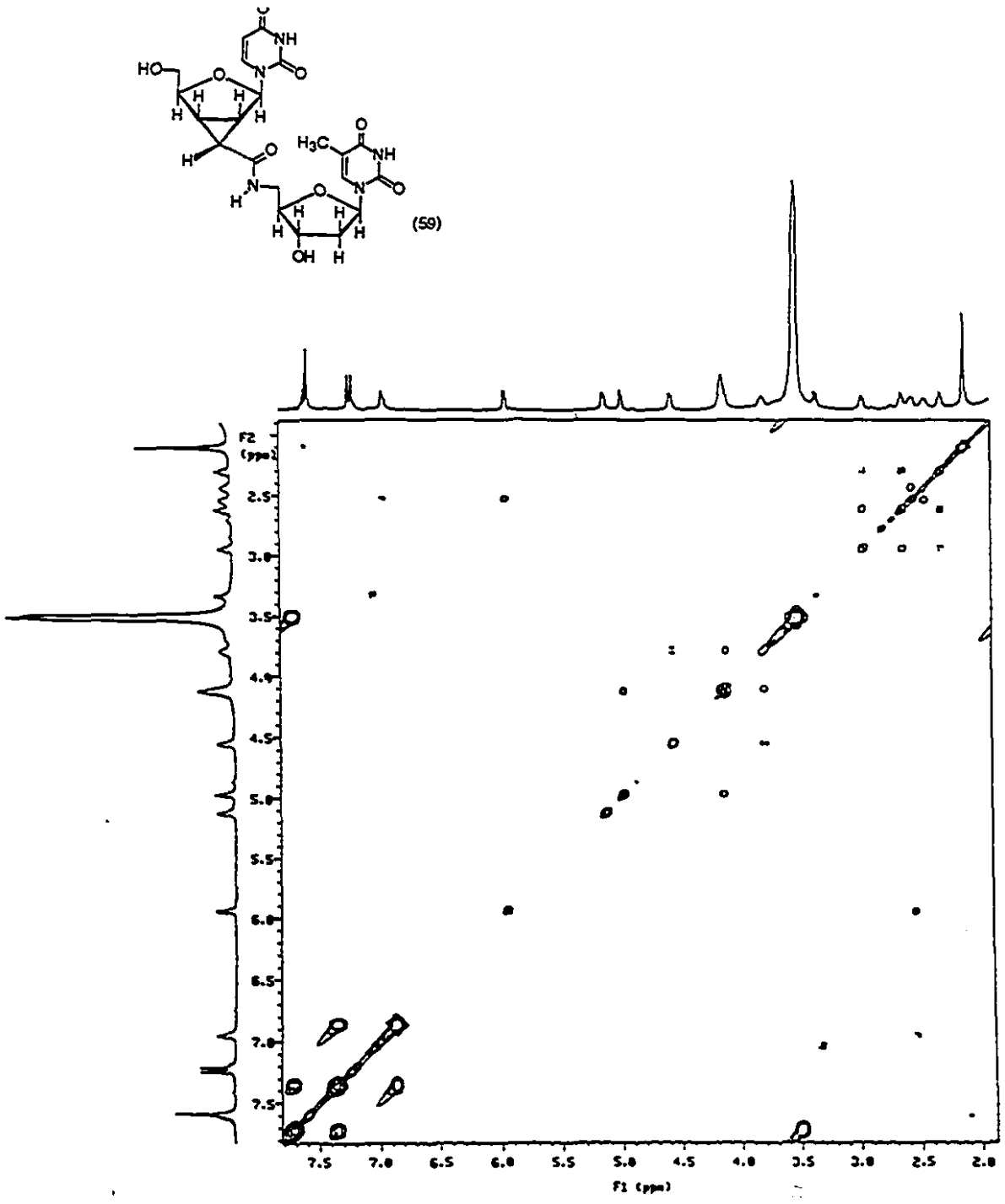
12. COSY spectrum of 5'-DMTr protected dimer 57 containing a cyclopropyl *exo*-amide linker (Section 2.13.).



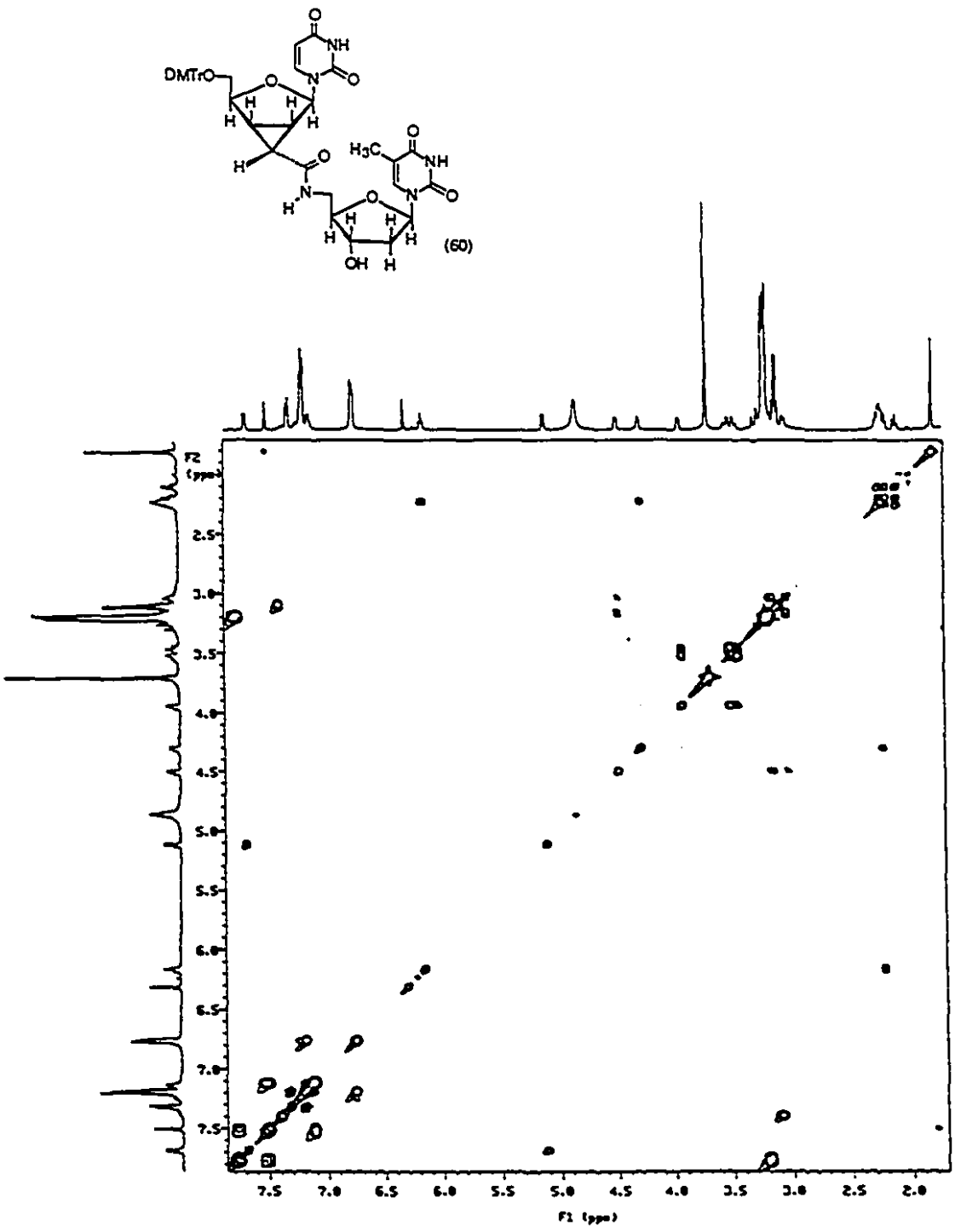
13. HMQC spectrum of 5'-DMTr protected dimer 57 containing a cyclopropyl *exo*-amide linker (Section 2.13.).



14. COSY spectrum of diol **59** containing a cyclopropyl *endo*-amide linker (Section 2.13.).



15. COSY spectrum of 5'-DMTr protected dimer **60** containing a cyclopropyl *endo*-amide linker (Section 2.13.).



16. HMQC spectrum of 5'-DMTr protected dimer **60** containing a cyclopropyl *endo*-amide linker (Section 2.13.).