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The Stereoselective Synthesis of a Novel Aromatic Peptide Nucleic Acid (APNA) Oligomer

Jacqueline F. Lunetta

A Thesis

in

The Department

of

Chemistry and Biochemistry

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Science at
Concordia University
Montreal, Quebec, Canada

January 1997

ABSTRACT

The Stereoselective Synthesis of a Novel Aromatic Peptide Nucleic Acid (APNA) Oligomer

Jacqueline F. Lunetta

The synthesis of the thymine derivative (S)-**50**, was achieved in high enantiomeric purity (98% ee). The (R) enhanced enantiomer was also synthesized with moderate enantiomeric purity (46% ee). This acyclic pyrimidine analog ((S)-**50**) is a useful building block for the synthesis of a novel class of oligomers, the aromatic peptide nucleic acids (APNA). The APNA tetramer **70** was prepared from the amino acid monomer (S)-**50** using classical peptide synthesis. Chemical shift differences between the ^{1}H NMR of monomer (S)-**57** and tetramer **70** were observed which suggested that base stacking interactions in the oligomers may be favorable.

Acknowledgments

First, I would like to thank my supervisor, Dr. Tsantrizos, for her constant support, encouragement and much valued advice. You have shown me that anything is possible, given the will. I am grateful to Dr. Boismenu (McGill Biomedical Mass Spectrometry Unit) for assistance with the ESMS analysis and Dr. F. Sauriol (Chemistry Department of McGill University) for recording the COSY, HMQC, and HMBC NMR spectra of tetramer **70.** A personal thanks to Dr. L. Colebrook for the recording of the HMQC and HETCOR NMR spectra of dimer **67** and for being there with a patient smile on the occasion when the NMR spectrometer misbehaved. I would also like to acknowledge Dr. Joyce and Dr. Tee for the final corrections of this manuscript (otherwise the 'its' would have gone unnoticed). Special thanks to Mike Boyd, Marie-Claire Wilson and Lee Fader for their assistance in generating some of the monomer intermediates and for being one of the contributors for making the summer of '96 so memorable. Chris Wilds, you are not forgotten; your advice and sincere honesty was very much appreciated.

to my family and friends,the ones who put life in perfect balance

to Don

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List of Schemes

ABBREVIATIONS

A	adenine
AIDS	Acquired Immunodefficiency Syndrome
APNA	Aromatic Peptide Nucleic Acid
AZT	3'-azido-3'-deoxy-thymidine
BOC	tert-butyl carbamate
Bn	benzyloxy
BSLDH	bacillus stearothermophilus lactate dehydrogenase
C	cytidine
Cbz	benzyloxycarbonyl
DCC	N,N'-dicyclohexylcarbodiimide
DCU	N,N'-dicyclohexylurea
DhbtOH	3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazene
DIPEA	diisopropylethylamine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
ds DNA	double stranded deoxyribonucleic acid
Fmoc	fluorenylmethylcarbamate
HATU	O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HBTU	O-benzyltriazol-1-yl-1,1,3,3-tetramethyluronium hexafluorophosphate
HOBt	1-hydroxybenzotriazole
HPLC	high pressure liquid chromatography
G	guanine

HIV	human immunodeficiency virus
μM	micromolar
MOM	methoxymethyl
mRNA	messenger ribonucleic acid
PNA	peptide nucleic acid
PyBrop	bromotris(pyrrolidino)phosphonium hexafluorophosphate
RNA	ribonucleic acid
RNase H	ribonuclease H
rRNA	ribosomal ribonucleic acid
ss DNA	single stranded deoxyribonucleic acid
T	thymine
3TC	2',3'-dideoxy-3'-thiacytidine
THF	tetrahydrofuran
Tm	melting temperature
tRNA	transfer ribonucleic acid
X-mer	an oligomer of X nucleotide units long

1.0 INTRODUCTION

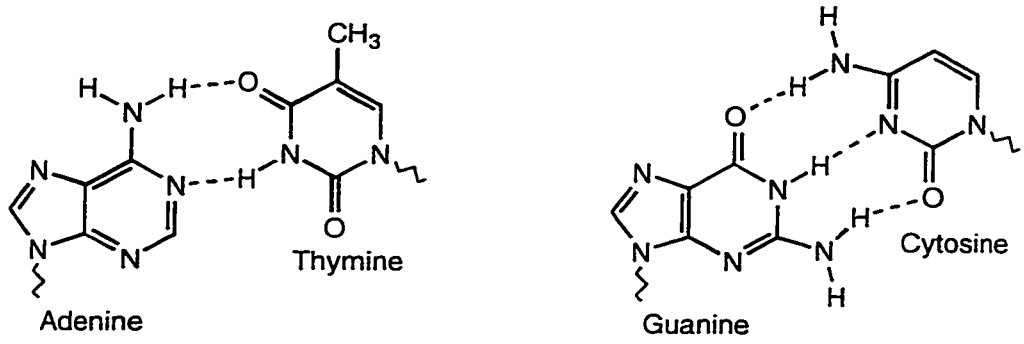
1.1 DNA: the Molecule of Life

Thanks to the pioneering work of James Watson and Francis Crick, the 3-dimensional structure of the 'gene carrying' molecule, deoxyribonucleic acid (DNA), was discovered in 1953.[1] DNA is undoubtedly one of the most celebrated and vital molecules of life involved in the storage, replication and translation of genetic information.

DNA is a polymer of deoxyribonucleotides. Each deoxyribonucleotide unit consists of: 1) a purine or pyrimidine nitrogenous base, 2) a deoxyribose sugar and, 3) a phosphate group. The purine bases are adenine (A) and guanine (G) whereas the pyrimidine bases are thymine (T) and cytosine (C). The bases are the determinants of the genetic information, whereas, the sugar and phosphate moieties serve mainly a structural role.

DNA exists as a set of two helical polynucleotide chains which are coiled around a common axis and whose chains run in opposite directions. The bases are positioned on the inside of the helix, perpendicular to the axis of the helix and are held together via hydrogen bonding (Watson-Crick bonding) of the complementary base pairs. Adenine forms two hydrogen bonds with thymine, whereas guanine forms three hydrogen bonds with cytosine (Figure 1). The phosphate group and deoxyribose sugar are positioned on the outside of the helix, perpendicular to the plane of the bases.

Figure 1. The Complementary Base Pairs



1.2. From Genes to Proteins

Genes are what specify the kinds of proteins to be made by cells. However, DNA on its own is not a direct template for protein synthesis. DNA first transcribes or encodes its information to RNA (ribonucleic acid) and then RNA, in turn, translates its genetic information and synthesizes the specified protein.

RNA is a single stranded polynucleotide whose sugars are riboses instead of deoxyriboses (found in DNA). Unlike DNA, RNA contains uracil bases instead of thymines which form base pairs with adenine. There are several classes of RNAs, namely messenger RNAs (mRNAs) which are the information carrying intermediates in protein synthesis, transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs) which are part of the actual protein synthesizing machinery.

Therefore, DNA transcribes its genetic code to mRNA. The genetic code is divided into codons consisting of a sequence of three bases which specify particular amino acids. The tRNAs then sequentially read the codons on mRNA and finally protein synthesis takes place on ribosomes which are complex

assemblies of rRNA and proteins. Once the protein is synthesized it contains signals which enable it to be targeted to specific destinations.[2]

1.3. Biologically Active Nucleoside Analogs

Most traditional drugs work by inhibiting a disease-causing enzyme or protein. Most cancer therapy involves killing cells at a rate proportional to their growth. Unfortunately, these treatments are associated with unpleasant side effects due to limited selectivity of the drugs for the target cells.

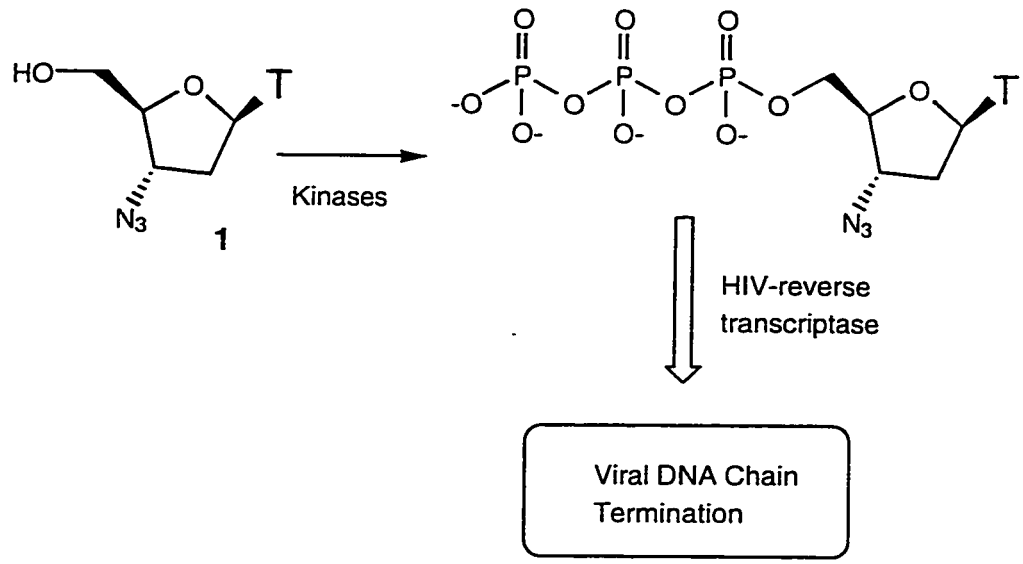
Interestingly, nucleoside analogs, both synthetic[3] and natural,[4] have been shown to be important models for drug discovery and therapeutic intervention in human diseases including cancer, fungal and viral infections. A classic example of a synthetic nucleoside analog, AZT (**1**, 3'-azido-3'-deoxy-thymidine), is used for the treatment of the acquired immune deficiency syndrome (AIDS).

Human immunodeficiency virus is a retrovirus which transcribes its genetic information in the reverse or 'retro' direction: from RNA to DNA. The virus contains a coat of glycoproteins which binds and fuses to the membrane of a host cell, releasing its viral RNA, along with its reverse transcriptase, into the cytoplasm of the host cell. The reverse transcriptase then synthesizes DNA from the viral RNA. The DNA inserts itself into the host's chromosomes to give proviral DNA. Later, this proviral DNA can be transcribed back to RNA and then translated to make the viral proteins. These proteins then assemble into virus particles, which emerge from the host cell and infect new cells.[3]

The mechanism of action of AZT (**1**) initially involves its activation upon phosphorylation of the 5'-hydroxyl to the corresponding 5'-triphosphate by

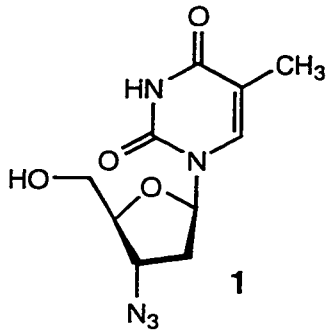
cellular kinases (Figure 2). The HIV reverse transcriptase then incorporates the AZT-5'-triphosphate into the replicating viral DNA. At this point DNA chain elongation is terminated since the terminal 'unnatural' nucleoside does not contain a 3'-hydroxyl necessary for further elongation of the chain.

Figure 2. Mechanism of Action of AZT



Unfortunately, AZT (**1**) along with its mono and di-phosphate derivatives are not specific for their particular polymerase targets. They have affinity for other cellular enzymes, resulting in undesired side effects, such as the bone marrow toxicity and low platelet and white blood cell counts, observed in AZT-treated patients.

The more recent drug, 2'3'-dideoxy-3'-thiacytidine (**2**, 3TC) has shown to be active against HIV. Since it lacks the 3' hydroxyl, its mode of action is similar to that of AZT. Interestingly, the 3' carbon on the sugar has been replaced by a sulfur atom. This alteration results in the drug being more stable in cells than AZT.[5]



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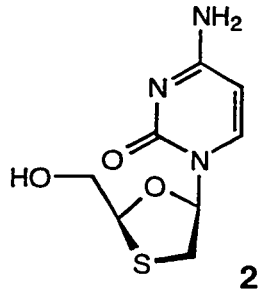

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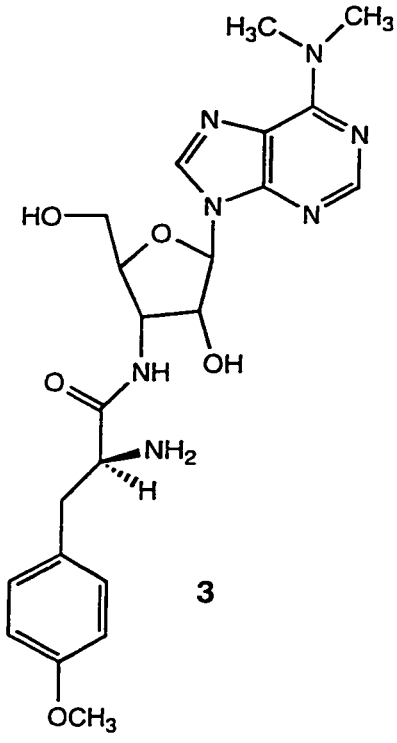
Among the naturally occurring nucleosides shown to exhibit potent antiviral and antitumor activity are the *Streptomyces* metabolites, Puromycin (**3**)[6] and Nikkomycin B (**4**).[7] Both metabolites contain an aromatic ring and a peptidic moiety, in addition to alterations of the nucleoside portion of the molecule. Both their peptidic and aromatic nature may contribute to their antiviral/antitumor activity. Their peptidic nature may allow them to behave as peptide mimics and their aromatic nature may contribute to their potency by increasing lipophilicity, thereby facilitating passage through membranes. Unfortunately, the ability of these compounds to discriminate between the deleterious cells and the healthy cells is limited.



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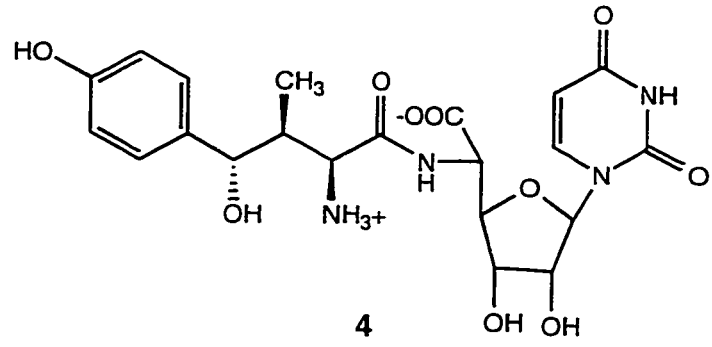

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1.4. The Antigene/Antisense Strategy

The antisense strategy uses the principle of Watson-Crick base pairing between a synthetic oligonucleotide and a target RNA sequence (the sense strand). This base pairing interaction leads to the selective inhibition of gene expression. If the target is a cancer-specific oncogene, then the cancer cells will be selectively destroyed. If the target is a viral gene, then the oligonucleotide will act as a selective antiviral drug. It is important to note that selectivity is a critical factor and must be maintained in an effective therapeutic drug.

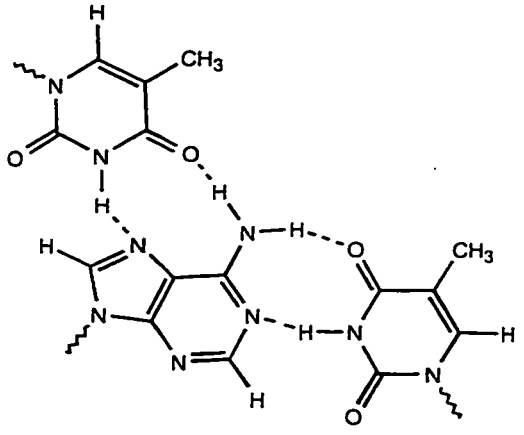
The antisense oligomer may inhibit the expression of a gene by preventing access of the RNA to the translation machinery such as spliceosomes, ribosomes and polymerases. Interestingly, Kim and Wold have found evidence that an antisense oligomer can prevent mRNA from being

exported from the cell nucleus to the cytoplasm and thus never having access to the translation machinery.[8]

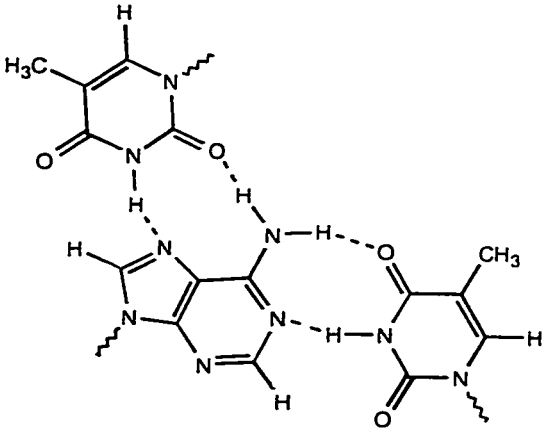
Inhibition of gene expression can also be mediated by the activation of Ribonuclease H (RNase H), which digests the RNA component of an RNA-DNA duplex. This mechanism has the advantage that one antisense molecule can inactivate many target RNA molecules; thus, the oligonucleotide's function is catalytic.

In the antigene model, the antisense oligomer will hybridize *via* Hoogsteen base pairing with double stranded DNA to form a triple helix, thereby inhibiting transcription by steric effects as described previously (i.e. inhibition of DNA binding proteins such as restriction endonucleases, methylases, transcriptases etc). Hoogsteen base pairing arises from the formation of base triplets thymine-adenine-thymine (T-A-T) and protonated cytosine-guanine-cytosine (C^+-G-C), where the oligomer strand binds in the major groove of DNA, parallel to the purine strand (Figure 3).[9] The oligomer can also hybridize to duplex DNA *via* reverse Hoogsteen base pairing. In this case the thymine and protonated cytosine bases are reversed and the oligomer will bind in an antiparallel fashion to the purine strand.

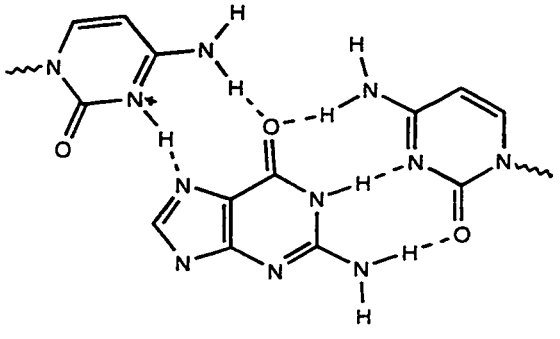
Figure 3. Hoogsteen and Reverse Hoogsteen Base Pairing



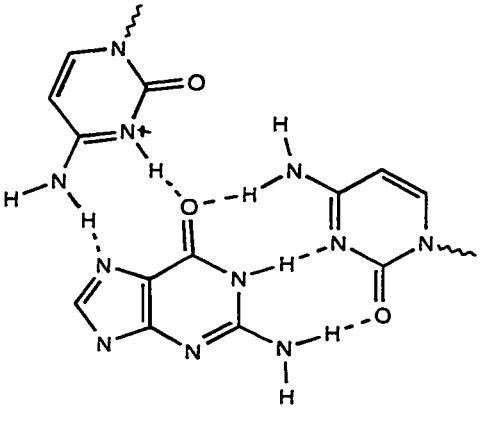
TAT
Hoogsteen



TAT
Reverse Hoogsteen



C+GC
Hoogsteen



C+GC
Reverse Hoogsteen

The oligonucleotide's specificity is derived from both its sequence and its length. A specific sequence of 16 nucleotide units (16-mer) is unlikely to occur at random in the human genome. Although, shorter oligomers are more likely to cross the cell membrane with relative ease, they may not be sufficiently specific to their targets and thereby may create adverse side effects. The use of very lengthy oligomers poses the problems of limited solubility and cell membrane

permeability. Furthermore, long oligomers may also bind to regions of partial complementarity which may lead to side effects due to reduced specificity.

In order for an oligomer to be an effective antisense or antigene drug four key requirements must be met: the oligonucleotide must (a) be stable to both exonucleases and endonucleases, (b) be able to penetrate into the cell in order to reach its target, (c) bind to its target with high specificity, and (d) be able to hybridize with sufficient affinity in order to inhibit gene expression.

Since natural oligonucleotides are not stable to intra and inter-cellular enzymes and have low membrane permeability, researchers have had to design chemically modified oligonucleotide analogs that will circumvent these limitations and still allow for good selectivity and affinity.

1.5 Melting Temperatures (Tm)

The binding affinity between complementary oligonucleotide strands is characterized by the melting temperature (Tm) of the duplex they form. The Tm is the temperature at which 50% of the duplexes have dissociated into two single strands. It is measured by observing the absorbance of the duplex as a function of increasing temperature and is dependent on the concentration of the oligomers and on the solvent. Tm can also apply to triple helices, which would exhibit two Tm values; one where a single strand melts leaving the duplex and a second Tm value representing the complete dissociation of the double helix to finally give three single strands.

An estimate of the Tm for natural oligonucleotide of 12-20 nucleotides long can be found by employing "Wallace's Rule" which holds true for double stranded DNA.[10]

$$Tm = n(2\ ^{o}C) + m(4\ ^{o}C)$$

n= number of A-T base pairs

m= number of G-C base pairs

The stability of the duplex increases with the proportion of G-C base pairs as a result of the three hydrogen bonds formed by this pair. Furthermore, under physiological conditions, 37 °C and low salt concentration, at least 12 base pairs are required to achieve reasonably stable duplexes.

1.6 Antigene/Antisense Oligonucleotides

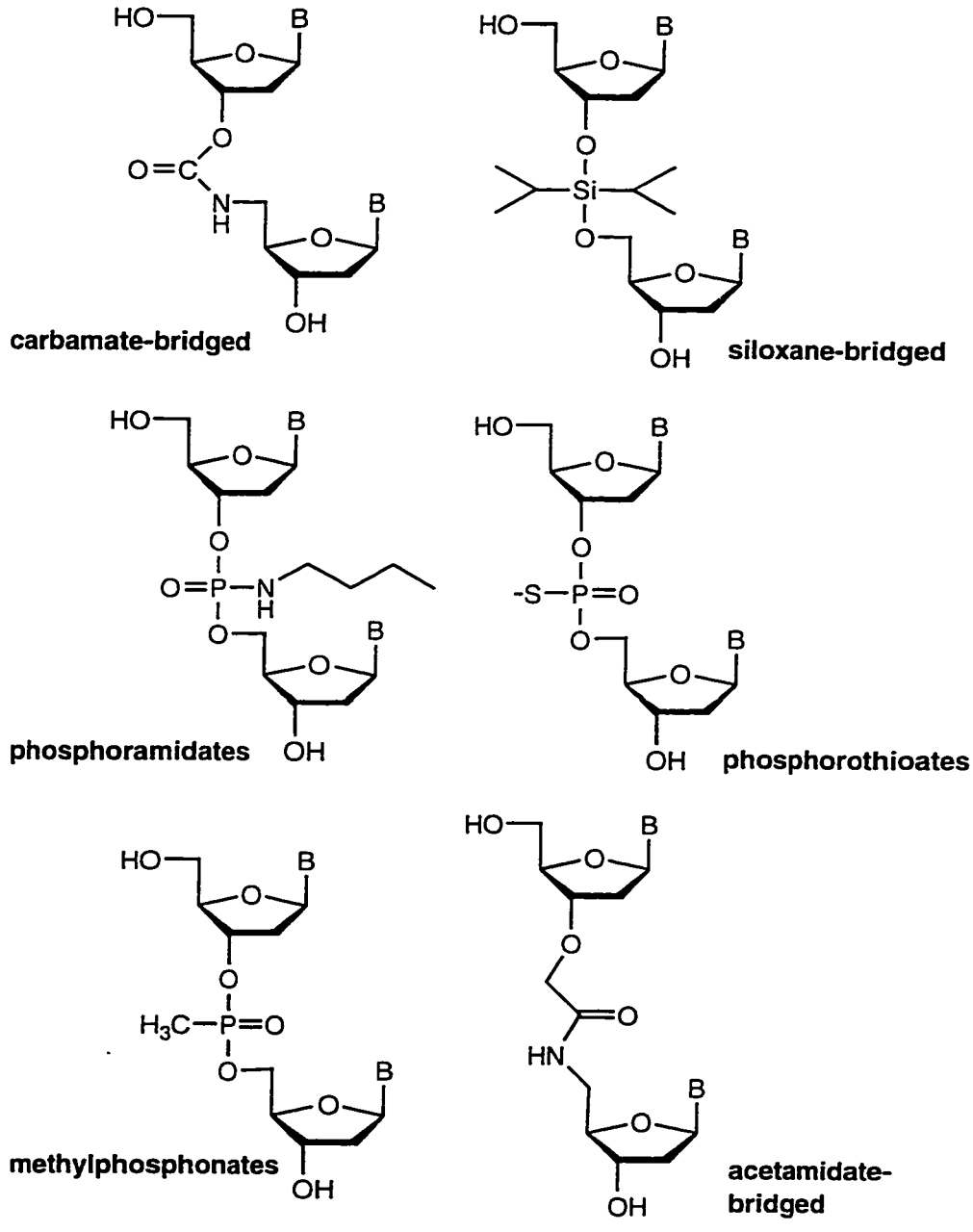
The idea of using antisense oligodeoxynucleotides as sequence specific inhibitors of gene expression was proposed in 1967.[11] But, at the time it was very laborious and expensive to produce sufficient amounts of oligomers. In 1978, Zamecnik and Stephenson were the first to propose the use of synthetic antisense oligonucleotides for therapeutic purposes.[12] They designed a 13-mer com-plementary to the RNA of the Rous sarcoma virus and used it to inhibit the virus's growth in cell culture.

Then in 1981, the development of the phosphoroamidite method of DNA synthesis by Beaucage and Caruthers,[13] along with the development of the gene synthesizer by Ogilvie *et. al.*[14] led to an explosion in the field of antisense research.

Over the last 20 years hundreds of DNA analogs have been synthesized and a sampling of them is shown in Figure 4. Modification of the backbone such that it is no longer a substrate for cellular nucleases is the most straightforward solution to the problem of biological stability. First generation oligomers

involved subtle modifications to the phosphodiester backbone such as the methylphosphonates, phosphorothioates and phosphoramidates. The next generation of nucleic acid analogs were those involving the replacement of the phosphodiester moiety with siloxane, acetamide and carbamate bridges.

Figure 4. DNA Analogs

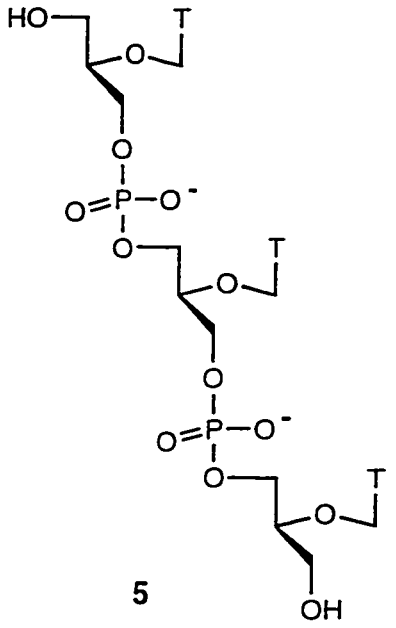


Among the best of the non-natural oligonucleotides are the methylphosphonates and the phosphorothioates. In the case of the phosphorothioates, an oxygen atom on the phosphate moiety was replaced with a sulfur atom. This substitution conserves the original charge and retains its water solubility. Since the phosphothioates possess a chiral center, it was important to devise a stereoselective synthesis so as to avoid any erroneous hybridization effects and to avoid the potential problem of biological side effects. Both the (R) and (S) enantiomers were synthesized and their biological properties studied.[15] These oligomers exhibit properties that are different from the natural phosphodiester nucleotides. The phosphorothioates are nuclease resistant[16] and are the only oligomers to this date, that elicit RNase H activity. In addition, they have shown promising results both *in vitro* and *in vivo*.[17]

In the case of the methylphosphonates, an oxygen atom of the phosphodiester backbone has been replaced by a methyl group. The methylphosphonates are nuclease resistant and are also chiral at the phosphorus, but since they now lack an ionizable group, they tend to have reduced solubility in aqueous media. Furthermore, due to the lack of a negative charge, there is little repulsion upon hybridization between the methyphosphonate strand with its complementary DNA/RNA strand and thereby have good hybridization stability. Although they do not elicit RNase H activity, several examples of antisense gene inhibition have been reported both *in vitro* and *in vivo*.[18]

In 1990, Benner and Schneider synthesized the acyclic glyceronucleosides (**5**).[19] It was found that the incorporation of a single glyceronucleoside unit in a natural 9-mer oligonucleotide depressed the Tm by 15 °C and that a homo- thymidine 12-mer glyceronucleoside showed no hybridization at all with its complementary DNA strand. Although it was anticipated that the incorporation of flexible nucleoside analogs would decrease

the stability of the duplexes formed, they were quite surprised that the decrease in stability was so great.



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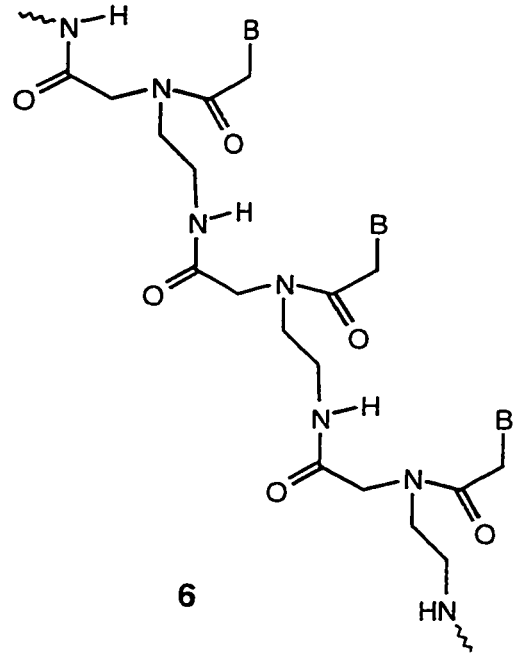
1.7 The Peptide Nucleic Acids (PNAs)

Nielson et. al. were the first to drastically alter the DNA backbone in hopes of meeting the requirements for producing effective antigene/antisense agents.[20] They showed that the complete replacement of the natural deoxyribose phosphate backbone of DNA by repeating N-(2-aminoethyl)glycine units with the nucleobases attached through methylene carbonyl linkages gave a DNA analog with unique properties. The nucleotides are linked together via peptide linkages and hence the name, Peptide Nucleic Acids (**6**, PNAs).



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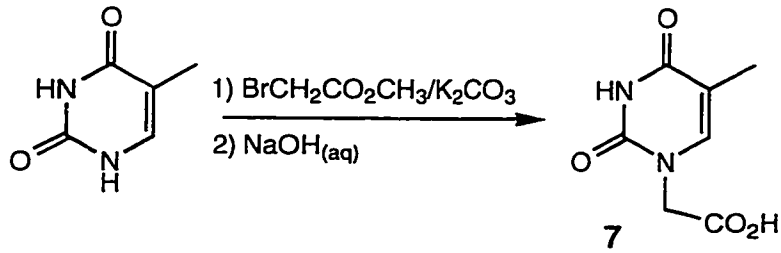
The unique structure of PNAs proved to exhibit several advantages over previous DNA analogs. PNAs are linked together *via* neutral peptide linkages which do not contribute to any electrostatic repulsion upon hybridization with a complementary DNA strand. Hence they are advantageous to the natural negatively charged DNA oligomers. Furthermore, these peptide-like oligomers can be easily assembled via automated peptide synthesis (Merrifield Peptide Synthesis) to give oligomers in milligram to gram quantities. Since they are structurally dramatically different from natural oligodeoxynucleotides and proteins, they are not recognized by nucleases or proteases.[21] Finally, PNAs are achiral, thus avoiding the problems of designing stereoselective synthetic strategies.

1.8 Synthesis of The PNAs

The synthesis of PNAs first involves the derivatization of the four nucleobases.[22] For example, thymine can be alkylated with methylbromoacetate, followed by the hydrolysis of the resulting ester to give compound **7**.

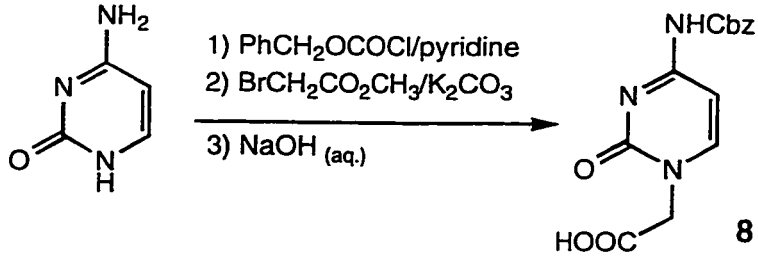
Scheme 1. Derivitization of Thymine



7

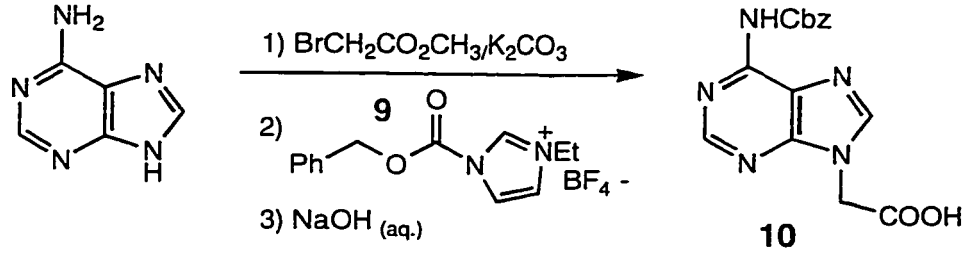
In the case of cytosine, the exocyclic amino group must be protected in order to prevent N-alkylation at this position or acetylation during the capping procedure used in the oligomerization process. The exocyclic amino group was protected with a carbonyl benzyloxy (cbz) group prior to the alkylation with methylbromoacetate (Scheme 2). The use of the cbz group also served the purpose of increasing lipophilicity, thereby increasing the solubility of cytosine in organic solvents.

Scheme 2. Derivitization of Cytosine



Adenine was alkylated with methylbromoacetate and alkylation was found only at the 9-position. This was followed by the protection of the 6-amino moiety with a cbz group which was introduced using N-benzyloxy-carbonyl-N'-ethyl-imidazolium tetrafluoroborate (**9**, Rapoport's reagent), instead of the traditional benzyloxy carbonyl chloride (Scheme 3).[23] In the case of the benzyloxy carbonyl chloride reagent, the chloride ion was not a sufficiently good leaving group to allow acylation of the weakly nucleophilic center of adenine, where as the N-ethylimidazole was found to be a far better leaving group.[24]

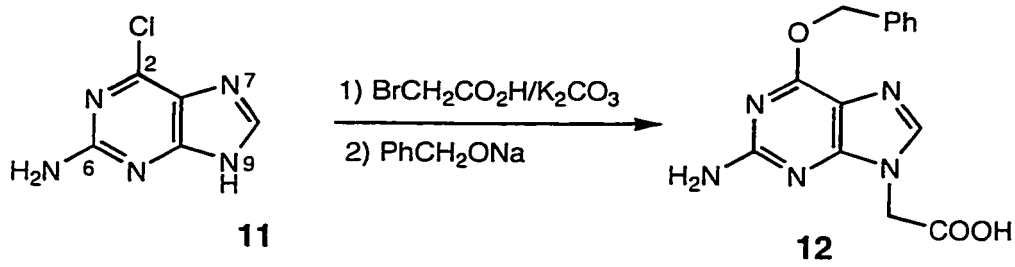
Scheme 3 . Derivitization of Adenine



Since guanine cannot be alkylated exclusively at the 9-position, 2-aminochloropurine (**11**) has been used as the starting material (Scheme 4). Reaction of 2-aminochloropurine with bromoacetic acid also produces 9 and 7-alkylated compounds; the 9-isomer (**12**) is the predominant product which was easily separated from the 7-isomer by column chromatography. The chlorine

group was then exchanged for a benzyloxy group (Bn) due to poor solubility. The protection of guanine's exocyclic amine was attempted using both benzyloxycarbonyl chloride and Rapoport's reagent, but neither resulted in any reaction. Therefore, the amino group of the guanine monomer would get acylated during the capping procedure in the oligomerization via Merrifield peptide synthesis (Note: use acetic anhydride for the capping procedure). The problem was circumvented by omitting the capping step after introduction of the first guanine residue.

Scheme 4 . Derivatization of Guanine



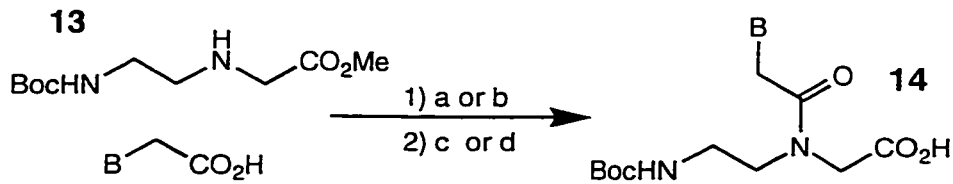
11 **12**

Activation using 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazene (DhbtOH) and N, N'-dicyclohexylcarbodiimide (DCC) in combination with the ethyl ester backbone was found to work best for the introduction of the derivatized thymine **7**, cytosine **8** and adenine **10** nucleobases. The resulting monomers were then saponified with base (Scheme 5).

The derivatized guanine nucleobase **12** was attached to the backbone via condensation using bromotris(pyrrolidino)phophonium hexafluorophosphate (PyBrop) giving the guanine monomer ester which was then saponified in the presence of base.

The oligomerization of the monomers has been done via standard solid phase peptide synthesis using a 4-(methylbenzylhydryl)amine polystyrene resin as the solid support. Thymine and cytosine monomers were oligomerized using DCC in quantitative yields. The adenine and guanine residues could be incorporated with DCC, however, N,N'-diisopropylcarbodiimide was found to be a more effective coupling reagent for these monomers. Similar results were obtained with O-benzyltriazol-1-yl-1,1,3,3,-tetramethyluronium hexafluorophosphate as the coupling reagent (HBTU).

Scheme 5. Synthesis of the PNA Monomers



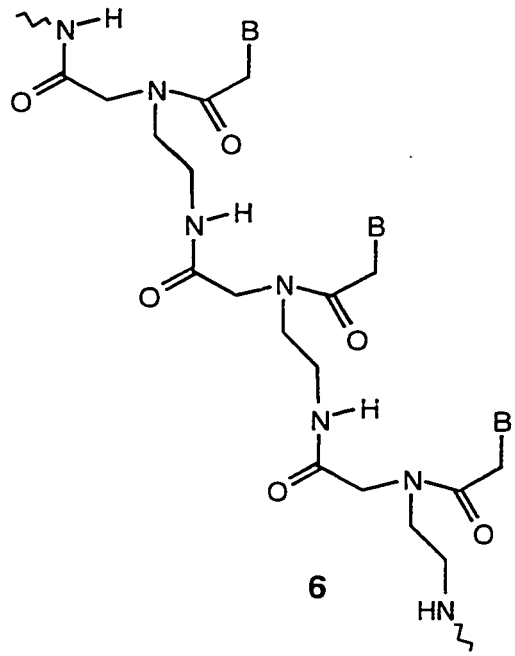
(a) DCC, DhbtOH (when B=thymine, Cbz-cytosine or Cbz-adenine)
(b) PyBrop, DIEA (when B=Bn-guanine)
(c) LiOH THF/H_2O (when B=thymine, Cbz-cytosine or Cbz-adenine)
(d) NaOH, EtOH/H_2O (when B=Bn-guanine).

1.9 PNA Hybridization Properties

The PNA-DNA hybridization studies have been conducted using a pentadecamer PNA with a mixed base sequence which was shown to bind with single stranded DNA or RNA to form a duplex.[25] It was shown that the PNA-DNA hybrids melted at higher temperatures than normal DNA-DNA hydrids, 69.5

°C and 53.3 °C, respectively. The PNA-RNA melted at 72.3 °C. Furthermore, single mismatches in the PNA sequence decreases the Tm's by 8-20 °C.

PNA's ability to form such stable hybrids was rather surprising, since, as in the case of Benner's glyceronucleosides, they appear to be quite flexible. Molecular modelling calculations suggest that the carbonyl oxygen of the tertiary amide in PNA and the inter-residue backbone NH group participate in hydrogen bonding thereby stabilizing the single PNA strand on its own.[26] In fact, when the corresponding PNA analog **15** was modelled, which lacked the critical carbonyl moeity, mathematical calculations showed a decrease in conformational stability of the single strand on its own and further docking calculations showed that **15** does not interact with its complemetary DNA strand.[27]



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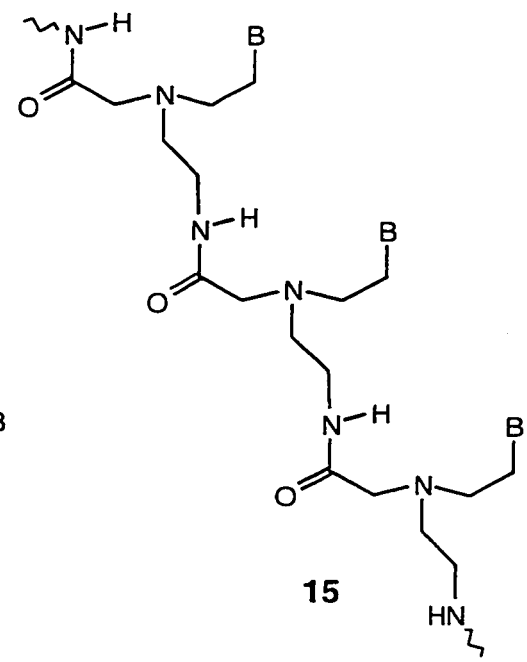

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An interesting phenomenon occurs when homopyrimidine PNA oligomers bind to their complementary single stranded DNA (ss DNA) oligomers. One would expect simple duplex formation, but, instead what actually forms are

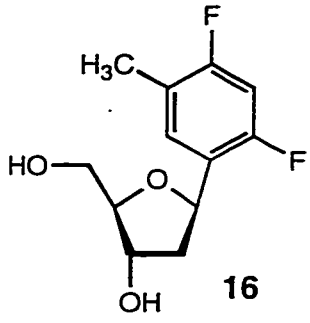
triplexes involving two PNA strands to a single DNA strand involving Hoogsteen and Watson-Crick base pairing. The Watson-Crick base pairing PNA strand is in the anti-parallel mode relative to the DNA strand and the Hoogsteen strand is in a parallel orientation relative to the DNA strand (i.e. the two PNA strands are anti-parallel relative to each other).[28]

In the case of homopyrimidine PNAs binding to their complementary double stranded DNA, strand displacement occurs. The PNAs rich in homo-pyrimidine displace the pyrimidine strand of the complementary double stranded DNA (ds DNA) target and the PNA hybridizes with the homopurine strand of DNA via Watson-Crick base pairing. This displacement renders the displaced strand sensitive to digestion by single strand-specific nuclease, S1.[20] *In vitro* studies, using microinjection, have shown that PNAs do exhibit both antigene and antisense gene inhibition at low concentrations (1μM).[29] They have been shown to function selectively and to be biologically stable. Their only drawback is poor cellular uptake.

1.10 Effects of π-Stacking Interactions

Various groups have been studying the effects or role of π-stacking interactions on the stability of the double helix.[30] Most recently, Kool and co-workers have shown that hydrogen bonding is responsible for the fidelity of base pairing but is not necessary for duplex stability.[31] By replacing the natural bases in DNA with hydrophobic isosteres, Kool showed that duplex formation still occurred (Figure 5). Since there is no hydrogen bonding to attract the bases to each other, it was hypothesized that the pairs may rely on base stacking interactions with the nearest neighbors for stabilization.

Figure 5. Hydrophobic Isosteres of Nucleobases



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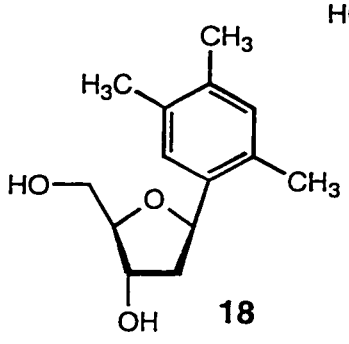

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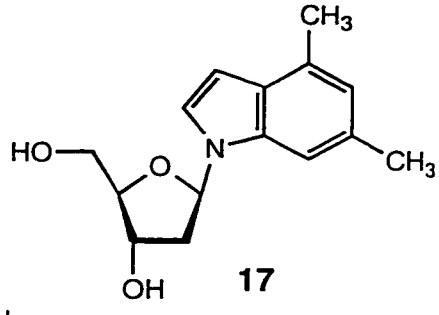

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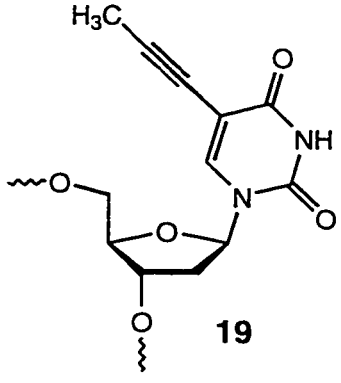
Other researchers have been working on the design and synthesis of various bases which have the ability to participate in additional base stacking interactions. For example, C-5 propyne analogs of uridine **19** and cytidine **20** phosphorothioates,[32] were shown to inhibit gene expression in cell assays using microinjection.[33] These analogs show enhanced affinity for complementary RNA, and maintain their nuclease stability as well as high selectivity.



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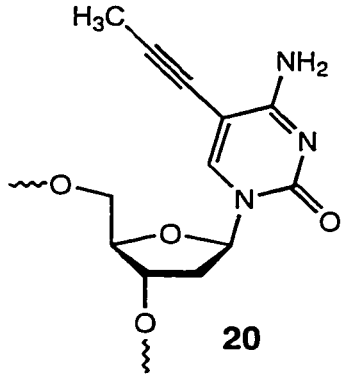

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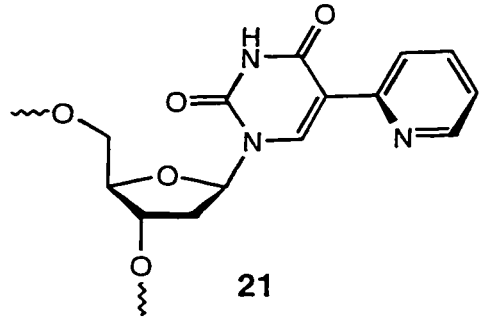
A year later, the same group designed a series of C-5 hetroaryl deoxy-uridines **21** and have shown that they exhibit higher Tm's than the natural

control.[34] They used a 15-mer where the last five terminal bases were the modified nucleotides and found that for every unnatural nucleotide incorporated, there was an increases in Tm between 0.3 to 1.7 °C. The C-5 propynyl deoxy-uridine was also tested and the Tm was increased by 1.6 °C per substitution.



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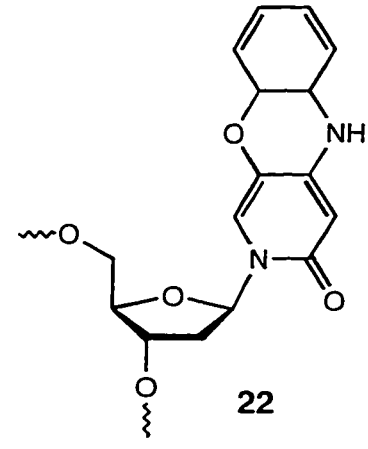

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Other examples include the tricyclic heterocyclic deoxycytosine analog **22** which was shown to specifically hybridize with a complementary guanosine. Incorporation of three tricyclic deoxyribosides in the middle of a mixed sequence 15-mer showed an increased Tm of 15 °C (corresponds to a 5 °C increase in Tm per substitution).

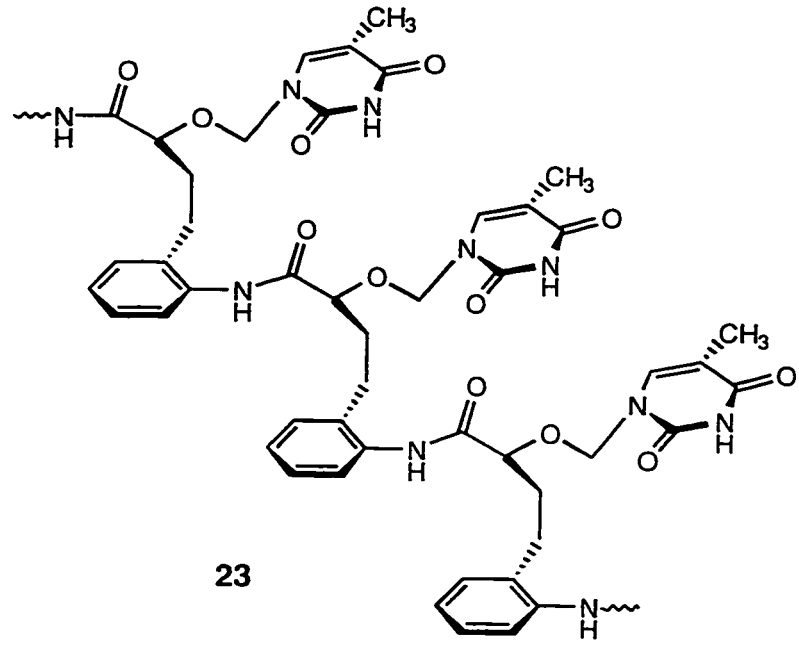
2.0 Results and Discussion

2.1 Objective

One of the important lessons learned from past analogs is that alterations of the phosphodiester linkage are responsible for nuclease resistance. The drastic replacement of the phosphodiester linkage by an amide linkage, as in the PNAs, render them nuclease resistant. As an additional advantage, the PNAs are also resistant to proteases, improving their bioavailability.

Furthermore, with PNAs, it has been demonstrated that not only can the phosphodiester linkage be completely redesigned, but, the ribose sugar moiety can also be replaced. The critical replacement for the ribose sugar is that the oligomer must maintain some conformational stability as a single strand. If the oligomer is too flexible, as in Benner's glyceronucleosides, it is not likely to hybridize with the target. In addition, the oligomers which have been made so far have the limitation of poor cellular uptake, which obliges one to add lipofectins or lipophilic moieties on the terminal ends of the oligomer.

Our contribution to this field are the Aromatic Peptide Nucleic Acids (**23,** APNAs). The backbone of this oligomer is composed of a system of aromatic rings which will render the molecule more lipophilic than previous oligomers, thereby making it more likely to penetrate the outer cell membrane.



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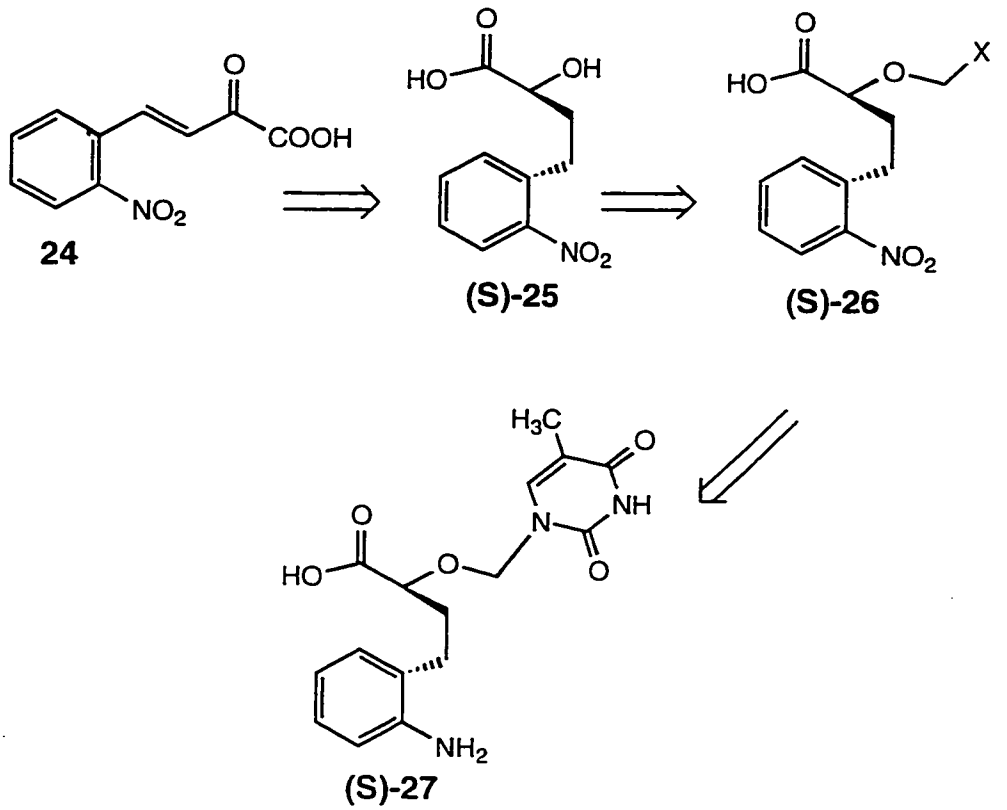
The aromatic ring system may also contribute to the conformational stability of the oligomer as a single strand. Polyaromatic systems have been shown to participate in π-stacking interactions such as the nucleobase stacking found in DNA/RNA[35] and other self-assembling polyaromatic systems.[36] In addition, there may be potential inter-residue hydrogen bonding between the face of the aromatic ring and the amide proton which has been shown to contribute to the stabilization of the tertiary structures of proteins involving phenylalanine and tyrosine residues.[37]

2.2 Synthetic Plan

The overall synthetic strategy involves the stereoselective reduction of the unsaturated keto acid **24** to the (S)-α hydroxy acid **25**. Subsequently, the hydroxyl moiety will be derivatized to the methylene derivative (S)-**26** which is in

turn attached to a good leaving group **X** for easy substitution with a nucleobase to give the (S)-monomer **27** (Figure 6).

Figure 6. Synthetic Strategy

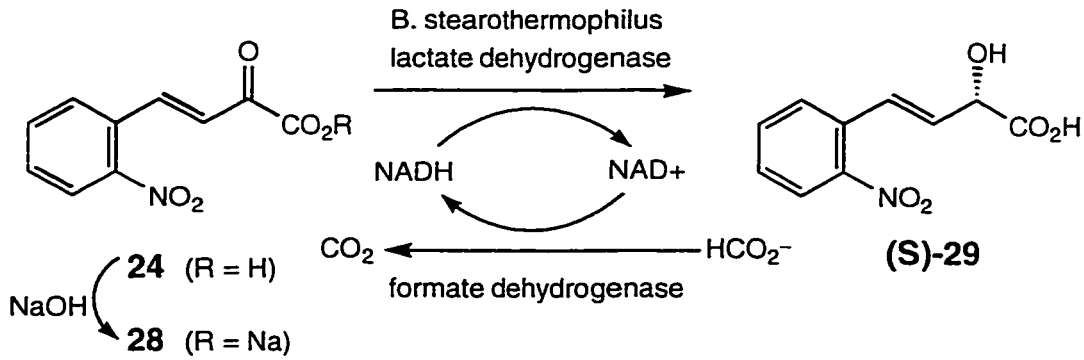


It was decided to begin with the synthesis of the (S) enantiomer, since this is the chirality found in natural oligodeoxynucleotides. Although, this chirality will not necessarily ensure better hybridization, synthesis of the (R) enantiomer was also carried out and will be discussed later.

2.3 Stereoselective Reduction of Compound 24

The unsaturated α keto acid **24** was stereoselectively reduced to the (S) unsaturated α hydroxy acid **29** using a lactate dehydrogenase enzyme that was isolated from *Bacillus stearothermophilus* (BSLDH, Scheme 6).[38] Since the reaction is conducted in aqueous buffer, compound **24** was rendered more soluble by converting it to the sodium salt **28**.

Scheme 6. Enzymatic Reduction of α Keto Acid **24**



The natural role of BSLDH is in the conversion of pyruvate to L-lactate. It is the only enzyme to date that will accept, as a substrate, rigid conjugated molecules such as **24**. The BSLDH enzyme is very well suited for use as a synthetic catalyst because of its high specific activity, good thermostability and a relatively broad substrate specificity, relative to most other enzymes of this type. Reactions can be successfully carried out in scales as large as 10 g in 2 litres of buffer solution. Formate dehydrogenase, along with its substrate, sodium formate, was used to recycle NADH in situ. The reaction can be carried out rather effortlessly, however, close attention must be paid to the maintenance of the pH in the range of 6.0 to 6.2. The enzymatic reaction gave quantitative

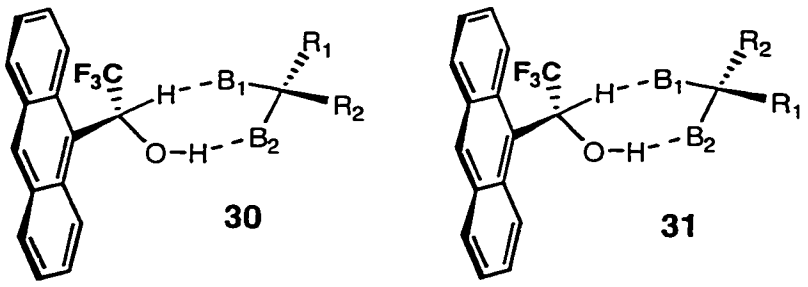
yields as well as an enantiomeric purity of greater than 98% (discussed in Section 2.4).

2.4 Determination Of the Enantiomeric Purity of Compound (S)-29

The assignment of the absolute configuration was determined by ^1H NMR analysis in the presence of Pirkle's chiral shift reagent (R)-(-)-2,2,2-trifluoro-1,(9-anthryl)ethanol.[39] Although enantiomers exhibit identical physical properties in an achiral environment, they can be easily distinguished by ^1H NMR in the presence of a chiral reagent. Pirkle's reagent is able to form a diastereomeric complex via hydrogen bonding interactions with the compound to be assigned. In order for this to occur, two basic sites on the substrate are required, B1 and B2, which are able to participate in hydrogen bonding (Figure 7). The electron withdrawing effects of the trifluoro, hydroxyl, and anthryl substituents of Pirkle's reagent render its proton very acidic and thereby able to participate in a dipole-dipole interaction with the less basic group, B1, on the compound to be analyzed. In addition, the hydroxyl group of the Pirkle reagent will also participate in hydrogen bonding with the more basic moiety, B2, of the substrate.

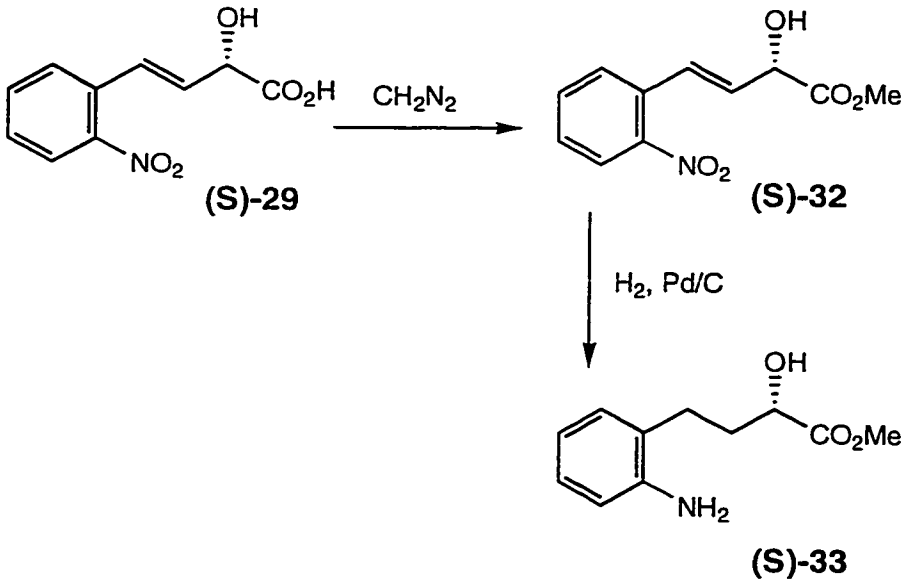
Depending on where the substituents of the substrate, R1 and R2, are located relative to the anthryl group of Pirkle's reagent, one will observe shifts in the ^1H NMR spectrum between the two enantiomers. In complex **30**, R2 is located over the region of the diamagnetic field of the anthryl system and is therefore shielded. The result of shielding is an upfield shift in the ^1H NMR signal of R2. In the case of the other diastereomeric complex, **31**, R1 will be shifted upfield.

Figure 7. Diastereomeric Chelates formed with (R)-Pirkle's Reagent and its Substrate



In order to prevent the interference of the carboxylic acid proton of (S)-**29** from hydrogen bonding with Pirkle's reagent, it was first esterified to the methyl ester (S)-**32** with diazomethane (Scheme 7).

Scheme 7. Reduction of Compound (S)-**29**

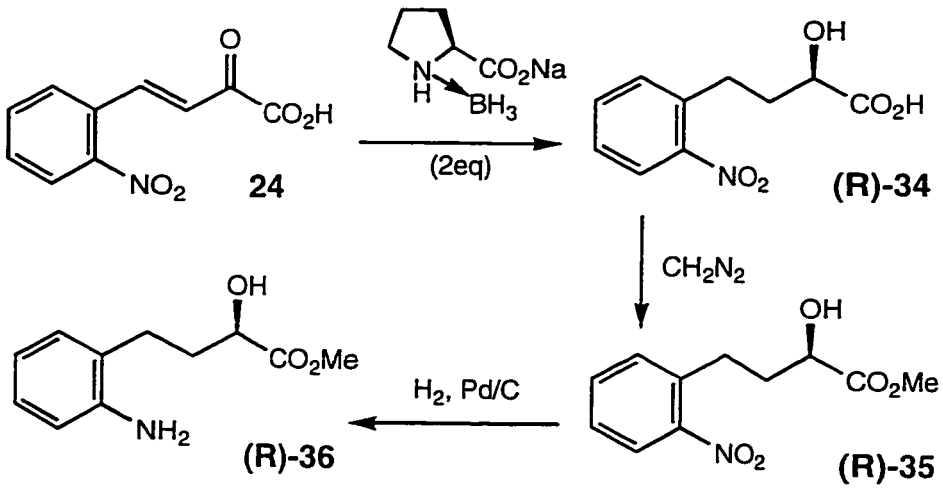


The (R) enhanced analog was also synthesized for comparison. Since, no enzyme was found that would turnover **24** to the (R) analog, it was stereo-selectively reduced via a synthetic route using an L-proline sodium borohydride

complex (Scheme 8). This reduction not only reduced the carbonyl moiety to the alcohol, but it also reduced the double bond giving compound (R)-**34**. Like, the (S) analog it was also esterified to the methyl ester (R)-**35** with diazomethane.

In order to compare the (R) and (S) analogs it was important for both compounds to be structurally identical. Therefore it was necessary to reduce the double bond in (S)-**32**. Attempts at selectively reducing the double bond, while not affecting the nitro moiety, by hydrogenation with a ruthenium catalyst did not prove successful. Therefore, the alternative was to reduce both the double bond and the nitro to give (S)-**33** via catalytic hydrogenation with palladium on activated charcoal. By analogy (R)-**35** was also reduced to the amine (R)-**36**.

Scheme 8. Synthesis of the Amino Hydroxy Ester (R)-**36**

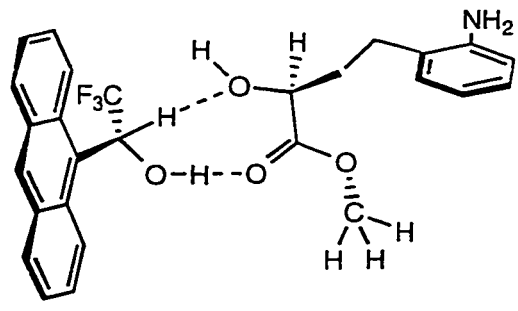


In the case of the α hydroxy ester (R)-**36**, the α proton is oriented above the ring current of the anthryl moiety of (R)-Pirkle's reagent as shown in figure 8, thereby exhibiting an upfield shift in the signal corresponding to the doublet of doublets (at approximately δ 4.17-4.27) shown in figure 9. Consequently, the corresponding enantiomer, (S)-**33**, is the more downfield signal. Interestingly,

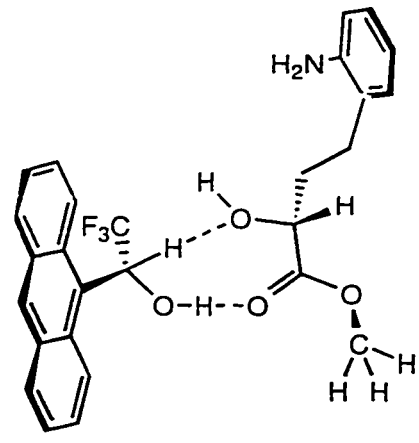
the methyl ester singlet (δ 3.74) is also shifted upfield in the (R) enantiomer **36**, indicating that this moiety is also located above the ring current. This proves to be advantageous since this singlet is much better resolved than the doublet of doublets of the α proton and thereby its integral is more reliable.

The relative integrations of the singlets were used to determine the enantiomeric excess of the compound. In the case of (R)-**36**, the enantiomeric purity was found to be 50%. On the other hand, the enzymatic product, (S)-**33**, showed no evidence of any (R) enantiomer, thus its enantiomeric purity was estimated to be greater than 98%. In order to confirm that the enzymatic product was actually the (S) and not the (R) enantiomer, the sample of enzymatic origin was spiked with (R)-**36** and the corresponding downfield signal became apparent.

Figure 8. Chelate Complex of (R)-Pirkle's Reagent with (S)-**33** and (R)-**36**

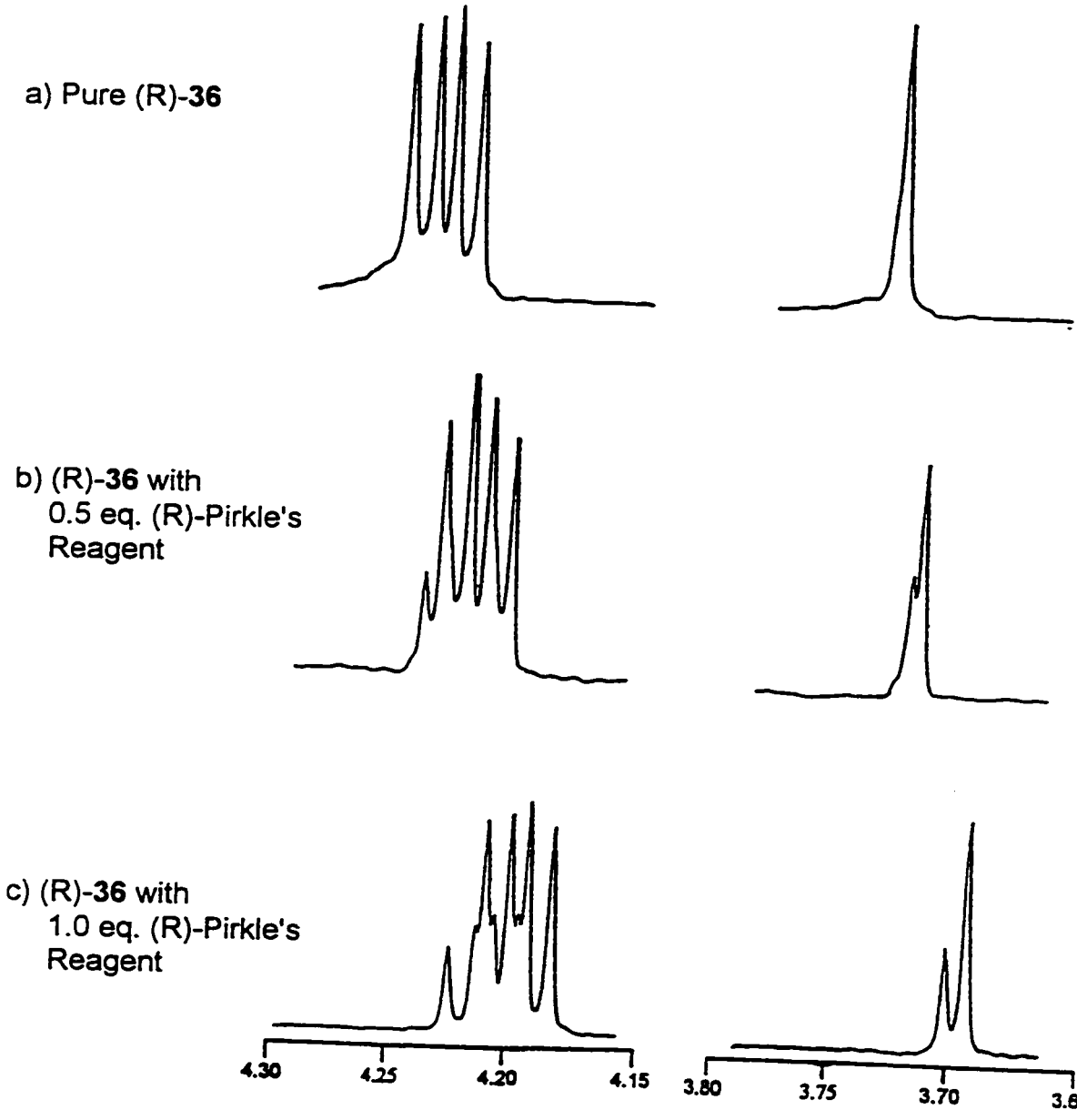


(S)-33



(R)-36

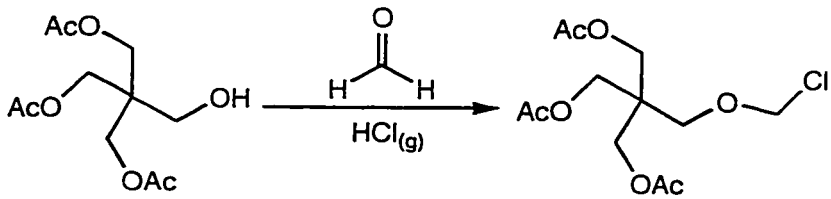
Figure 9. ^1H NMR Spectra (270MHz, $CDCl_3$) of the α Proton and Methyl Ester Region of (R)-**36** in the Presence of (R)-Pirkle's Reagent



2.4 Methylene Extension of Compound (S)-33

The next step of the synthetic strategy is the derivatization of the hydroxyl in (S)-**33** to the methylene derivative (Figure 6). Ogilvie and his coworkers converted a primary hydroxyl to the chloromethyl ether using paraformaldehyde and HCl gas (Scheme 9).[40] Unfortunately, performing the analogous reaction on compound (S)-**33** gave back the starting material.

Scheme 9. Conversion to the Chloromethyl ether

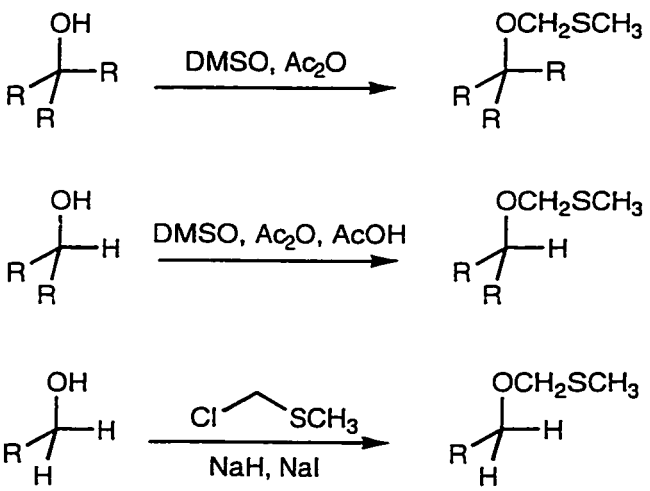


An alternative approach is the conversion of the hydroxyl to a methylthiomethyl ether. Methylthiomethyl ethers are well known side products in the Moffat oxidation.[41] Under the reaction conditions of the Moffat oxidation which involve dimethyl sulfoxide (DMSO) and acetic anhydride (Ac_2O), the methylthiomethyl ether is the sole product in reactions involving tertiary alcohols (Scheme 10).[42] Similarly, primary alcohols can be easily converted to their methylthiomethyl ethers by reaction with sodium hydride and iodomethyl methyl sulfide, formed in situ from commercially available chloromethyl methyl sulfide and sodium iodide.[43] Unfortunately, the conversion of secondary hydroxyls is less effective. However, later it was found that secondary hydroxyls could be easily converted to their methylthiomethyl ethers by the simple addition of acetic acid to the Moffat oxidative system.[44] The presence of the acid shifts the

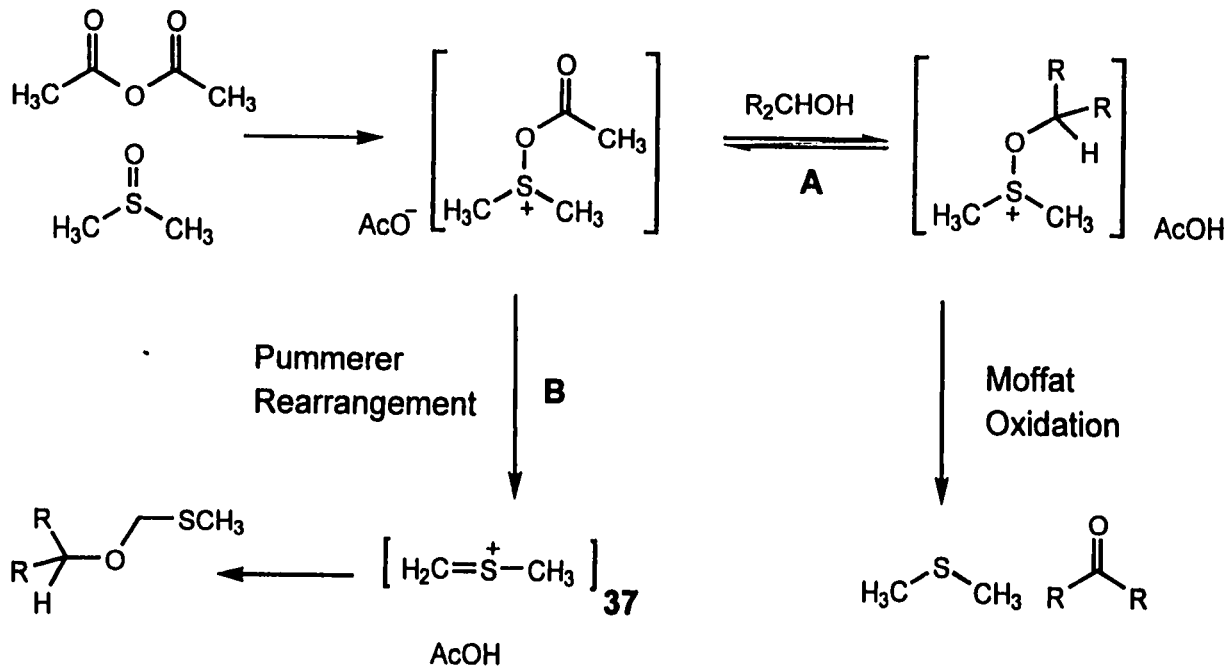
equilibrium to favor the formation of the methylthiomethyl ether, as shown in scheme 11.

Acetic anhydride reacts with DMSO to give a sulfonium ion which can then react via two alternate pathways; pathway A favors the Moffat oxidation to give the ketone product and pathway B favors the Pummerer rearrangement to give the ether product. If one assumes that path A is reversible, the addition of acetic acid will shift the equilibrium to favor the Pummerer rearrangement. Acetic acid is also generated in pathway B indicating that the unsaturated sulfonium ylid **37** reacts very rapidly with the alcohol, thereby rendering pathway B virtually irreversible.[40]

Scheme 10. Conversion of Primary, Secondary and Tertiary Hydroxyls to Methylthiomethyl Ethers

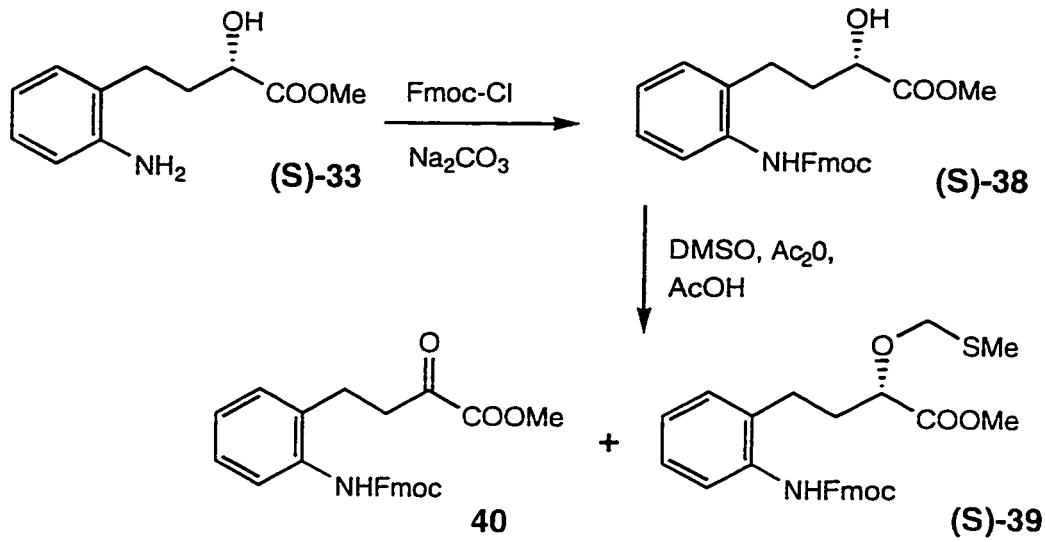


Scheme 11. Mechanism for the Formation of the Methylthiomethyl Ether



Prior to converting (S)-**33** to the corresponding methylthiomethyl ether, the amino group was first protected as the fluorenylmethylcarbamate (Fmoc) (S)-**38** in order to abolish its nucleophilicity and so it would not interfere with the reaction. This was subsequently followed by treatment with DMSO, Ac_2O and acetic acid to give 15-20% yield of the methylthiomethyl ether (S)-**39** and 75% yield of the oxidized keto product **40** obtained by the competing Moffat oxidation reaction (Scheme 12). Various attempts at improving the yields by either increasing the amount of acid as well as using a stronger acid such as trifluoroacetic acid, did not result in any improvement.

Scheme 12. Synthesis of (S)-**39** *via* the Pummerer Rearrangement

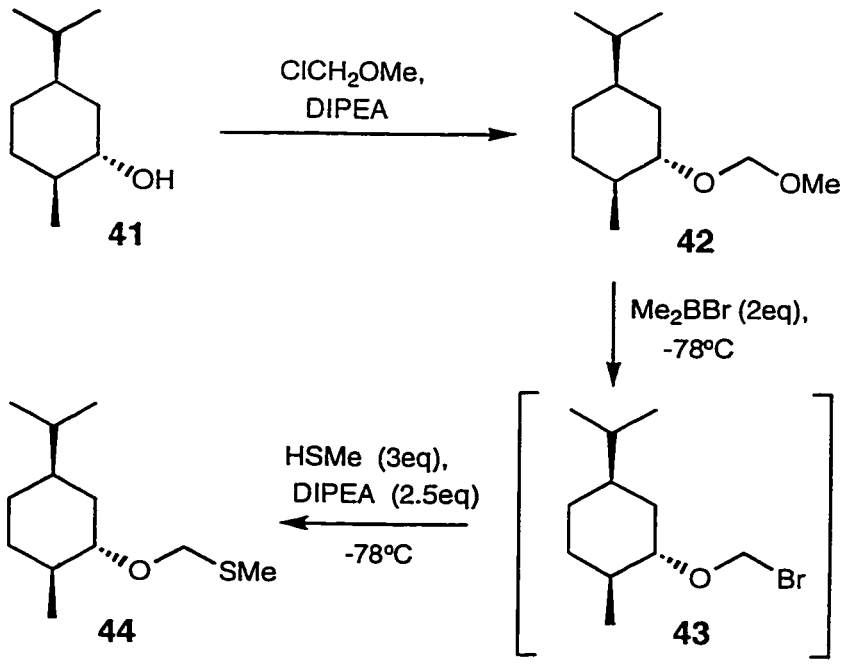


Another variation of this reaction was attempted which involved reaction of the alcohol with dimethyl sulfide (8 eq) and benzoyl peroxide (4 eq).[45] The excess dimethyl sulfide served to prevent the oxidation of the methylthiomethyl ether. Unfortunately, this did not lead to a significant improvement in the yields (20-25%) resulting, as previously, with compound **40** as the main product.

2.6 Indirect Synthesis of the Thioether Intermediate

Guindon and his coworkers were successful in converting secondary hydroxyls to their methylthiomethyl ethers via an indirect approach involving a methylene bromide intermediate (Scheme 13).[46] The hydroxyl group of menthol **41** was first converted to the methoxymethyl ether (MOM) **42** and subsequently treated with dimethylboron bromide at -78°C to give the unstable methylene bromide intermediate **43**. Intermediate **43** was then quenched with methanethiol to give the corresponding methylthiomethyl ether **44** in 80-95% yields. Interestingly, it was found that quenching the methylene bromide with MeOH, gave back the MOM protected product and quenching with water gave the starting hydroxyl compound.

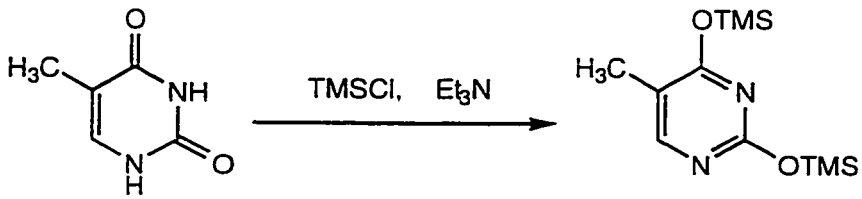
Scheme 13. Indirect Conversion to the Methylthiomethyl Ether



2.7 Model Study

A model reaction was performed on menthol, to see if it would be feasible to attach the thymine nucleobase directly to methylene bromide **43** rather than go through the methylthiomethyl ether intermediate. Thymine was first converted to the bis(trimethylsilyl) derivative **45** (Scheme 14). Silylation of thymine transforms it from a rather insoluble, non-volatile compound into a very soluble and volatile silyl compound, which can be easily purified by distillation and can allow for coupling reactions to be carried out in homogeneous solutions. Thymine was silylated with chlorotrimethylsilane in the presence of triethylamine.[47] The mixture was then filtered under an anhydrous atmosphere in order to remove the bulk of the salt and it proved advantageous to distill the bis(trimethylsilyl)thymine from the reaction mixture since minor impurities would greatly depress the subsequent coupling of the nucleobase to the backbone.

Scheme 14. Silylation of Thymine



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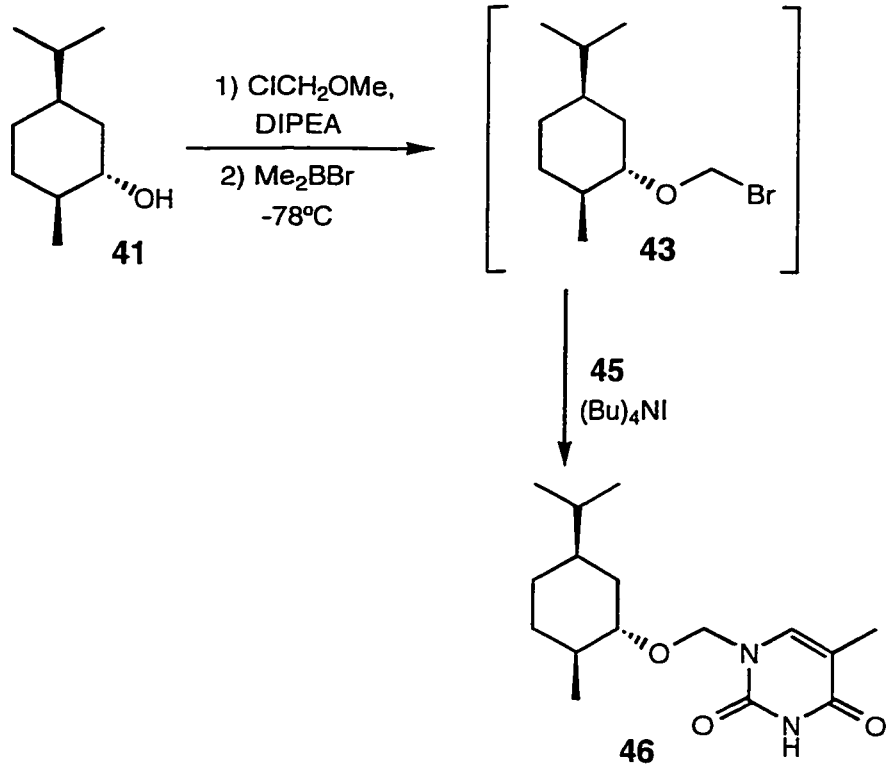
Quenching of the menthol methylene bromide derivative **43** with bis-(trimethylsilyl)thymine **45** in the presence of tetrabutylammonium iodide (which served to deprotect the thymine and render it more nucleophilic) resulted in 80% of the desired thymine coupled menthol product **46** (Scheme 15). Unfortunately,

carrying out the same reaction with compound (S)-**38**, resulted in traces if any at all of the desired product and recovery of the starting material .

The ^{1}H and ^{13}C NMR of compound **46** are depicted in figures 10 and 11 respectively. In the ^{1}H NMR, the singlet at δ 1.95 (3H), corresonds to methyl H5' of the thymine base. The AB system at δ 5.17 (2H), represents the OCH2Th protons, confirming that the thymine base is indeed linked onto the menthol. Thymine H6 is evident as a singlet in the aromatic region at δ 7.16 and the broad singlet at δ 8.42 corresponds to the NH signal. The remaining unassigned signals are the menthol protons.

The demonstrative signals in the ^{13}C NMR are the most upfield signal at δ 12.49 corresponding to methyl C5', the signal at δ 78.27 is the OCH2Th carbon and the remaining signals in the aromatic region, δ 111.62, 139.36, 151.10, and 163.99 correspond to C5, C6, C2 and C4 respectively. The remaining un-assigned signals in the hydrocarbon region correspond to the carbons on menthol.

Scheme 15. Model Reaction towards Direct Thymine Coupling



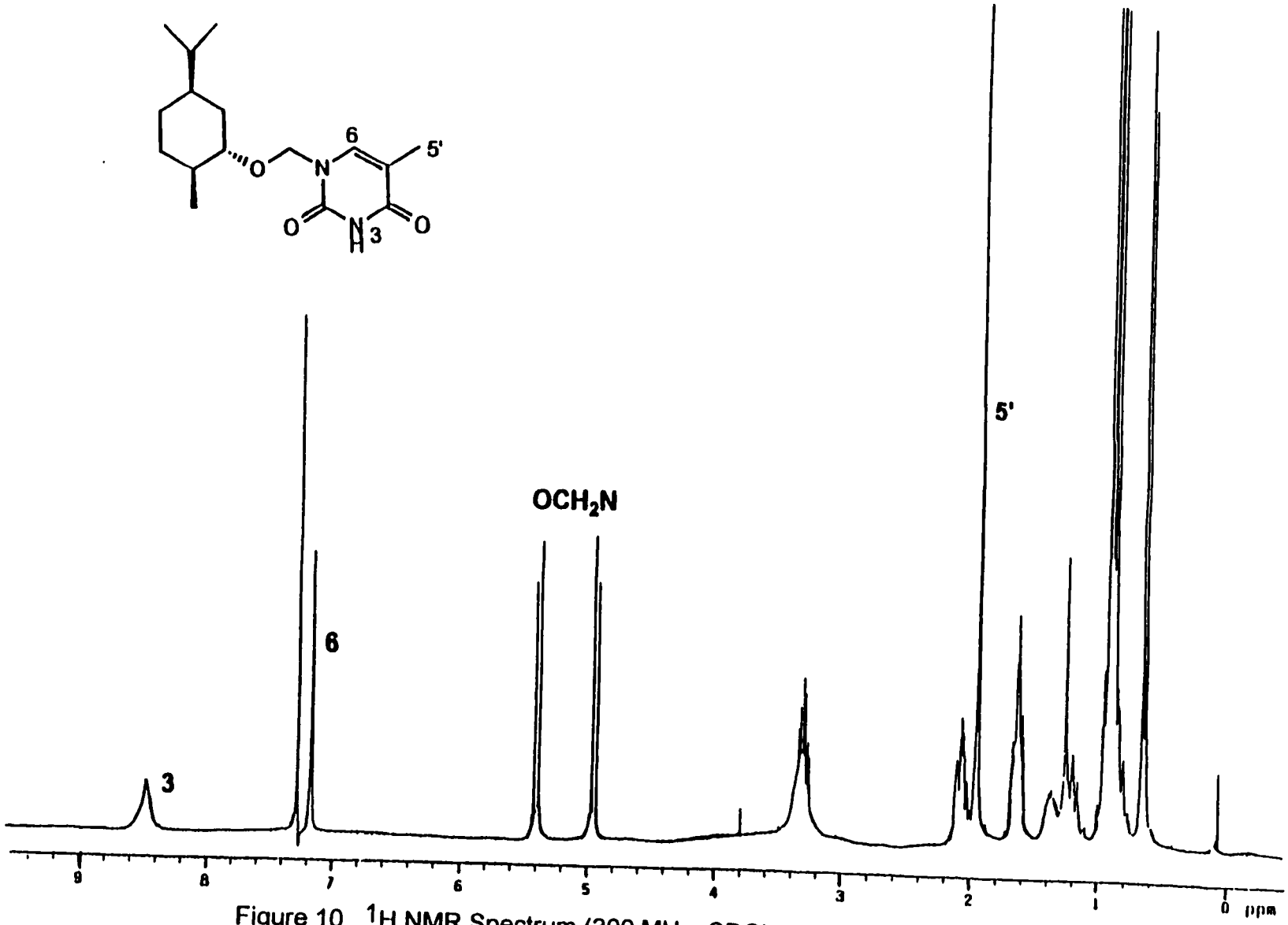


Figure 10. ^{1}H NMR Spectrum (300 MHz, $CDCl_3$) of Compound **46**

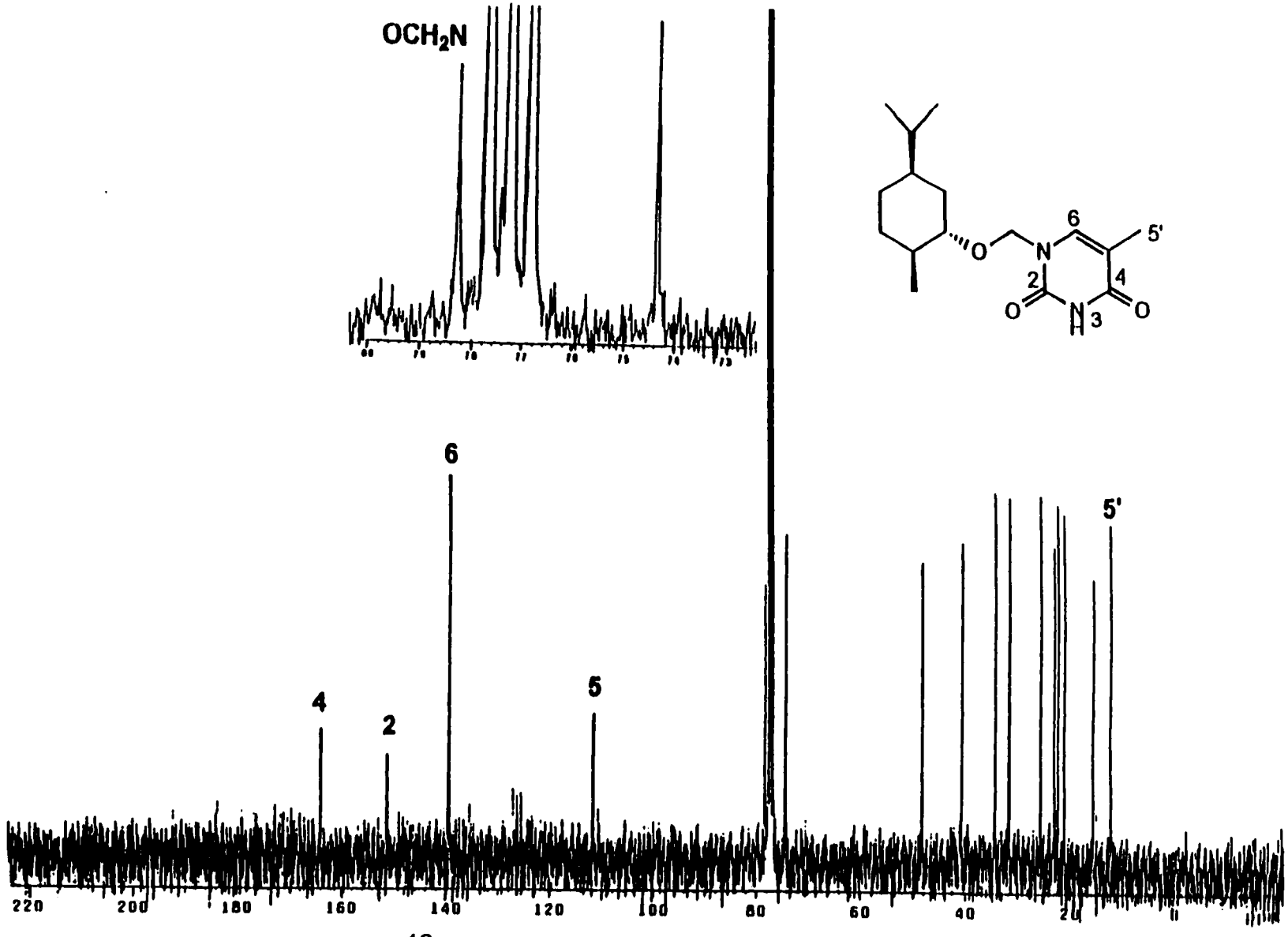
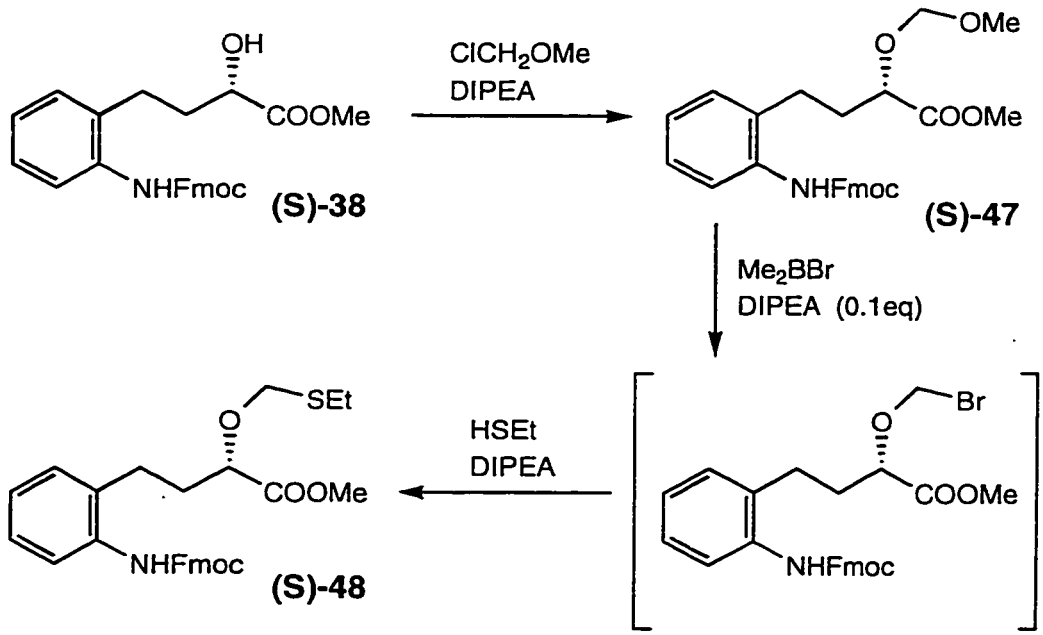


Figure 11. ^{13}C NMR Spectrum (75 MHz, $CDCl_3$) of Compound **46**

2.8 Synthesis of the Monomer (S)-50

Compound (S)-**38** was converted to the methoxy methyl ether, (S)-**47**, in 95% yield using chloromethylmethyl ether and diisopropylethylamine (DIPEA). Subsequent treatment with dimethylboron bromide followed by quenching with ethanethiol and DIPEA gave 30% yield of the desired methylthioethyl ether (S)-**48** and 65% of the hydroxy starting compound (S)-**38** (scheme 16). A trace amount (0.1eq) of DIPEA was added along with the dimethylboron bromide to act as an acid scavenger since the boron reagent may decompose over time to release HBr. Ethanethiol proved to be more convenient than methanethiol since the latter is a gas. The thiomethane sodium salt is commercially available but proved to be disadvantageous since salts are seldom completely free of water and the above reaction is extremely sensitive to moisture.

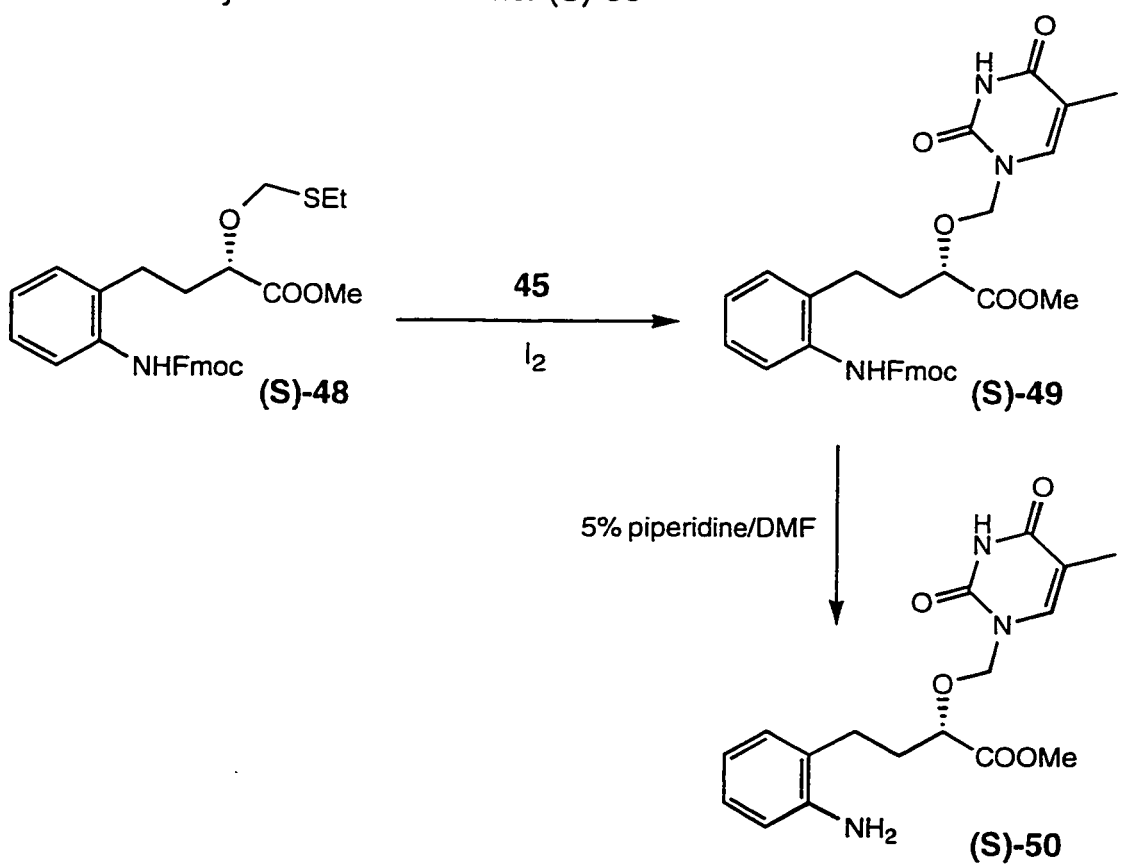
Scheme 16. Synthesis of Methylthiomethyl Ether (S)-**48**



Although the yield is only improved by approximately 10% relative to previous methods, in this case the side product is immediately recyclable with no loss in chirality (optical rotations of the initial hydroxy compound (S)-**38** and the recovered hydroxy compound after the reaction were identical).

The subsequent reaction of the methylthioethyl ether (S)-**48**, with bis(trimethylsilyl)thymine (**45**) in the presence of iodine yielded 60-70% of the thymine coupled product (S)-**49** (Scheme 17). The purpose of the iodine was to activate the methylthioethyl ether as well as to deprotect and increase the nucleo-philicity of the silylated thymine. Finally, treatment of (S)-**49** with 5% piperidine in DMF gave the amino monomer (S)-**50**.

Scheme 17. Synthesis of Monomer (S)-**50**



The ^{1}H and ^{13}C NMR spectra of the monomer (S)-**50** are shown in figures 12 and 13. The most upfield singlet in the ^{1}H NMR at δ 1.95 corresponds to methyl H5'. The underlying set of multiplets at δ 1.82-2.19 and the triplet at δ 2.60 corresponds to methylenes H3' and H4'. The methoxy protons are represented as a singlet at δ 3.67 and the single proton H2' is evident as a doublet of doublets at δ 4.18. The characteristic AB system at δ 5.20 is that of the OCH2Th protons and the lone singlet at δ 7.42 is that of H6. The aromatic protons are represented by the multiplets in the region between δ 6.55 and 6.98.

The most upfield signal in the ^{13}C NMR spectra is that of the methyl C5' at δ12.26, followed by the two methylenes C3' and C4' at δ 27.55 and 32.96. The methoxy carbon is evident at δ 52.73 and the OCH2Th and C2' signals are overlapping at δ 77.86 and 77.80. The thymine signals C5 and C6 are found at δ 112.05 and 142.31ppm, and their carbonyls C2 and C4 are at δ 153.38 and 166.85 respectively. The carbonyl ester C1' is the most downfield signal at δ 174.47.

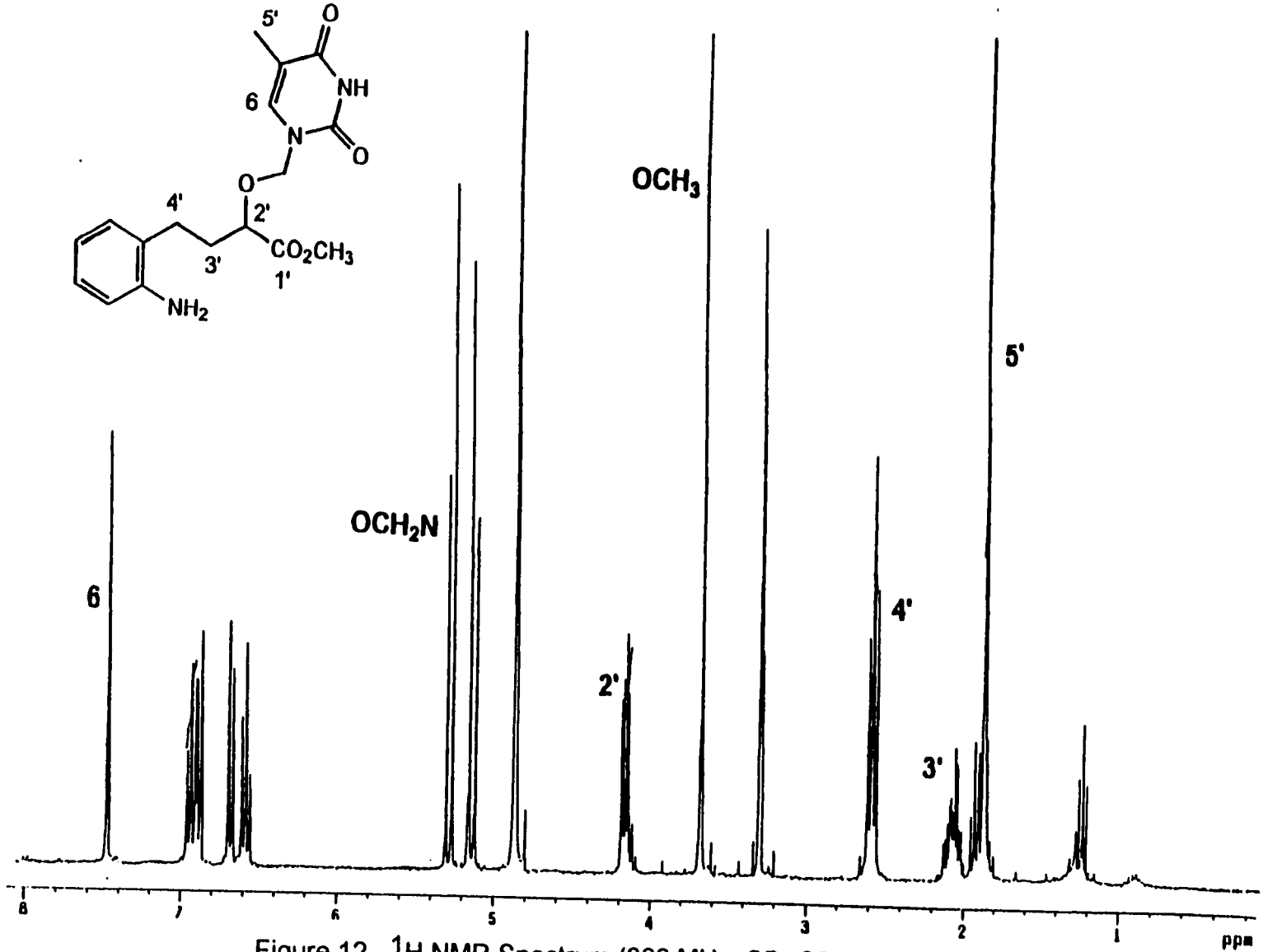


Figure 12. ^{1}H NMR Spectrum (300 MHz, CD_3OD) of Monomer (S)- **50**

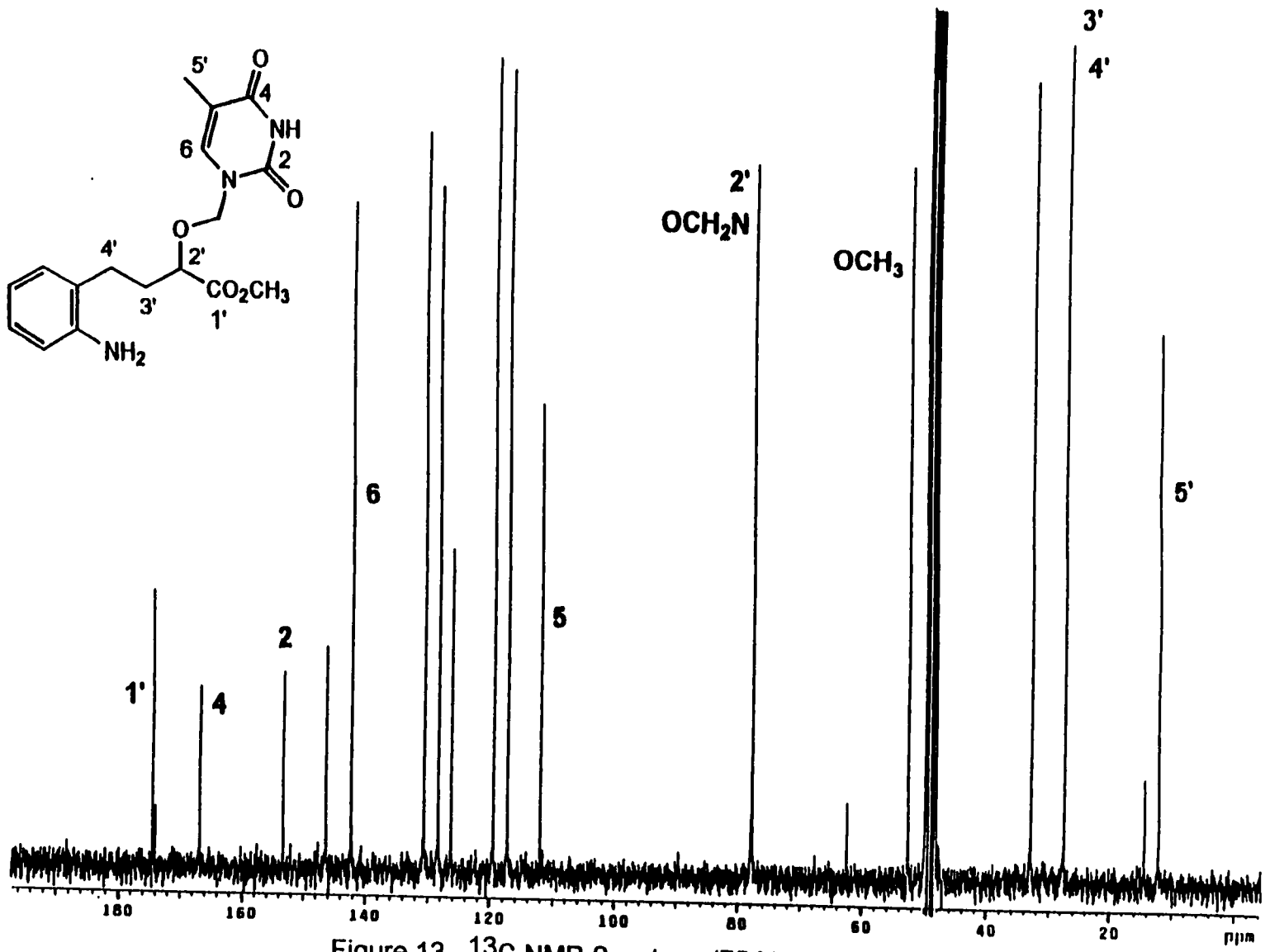


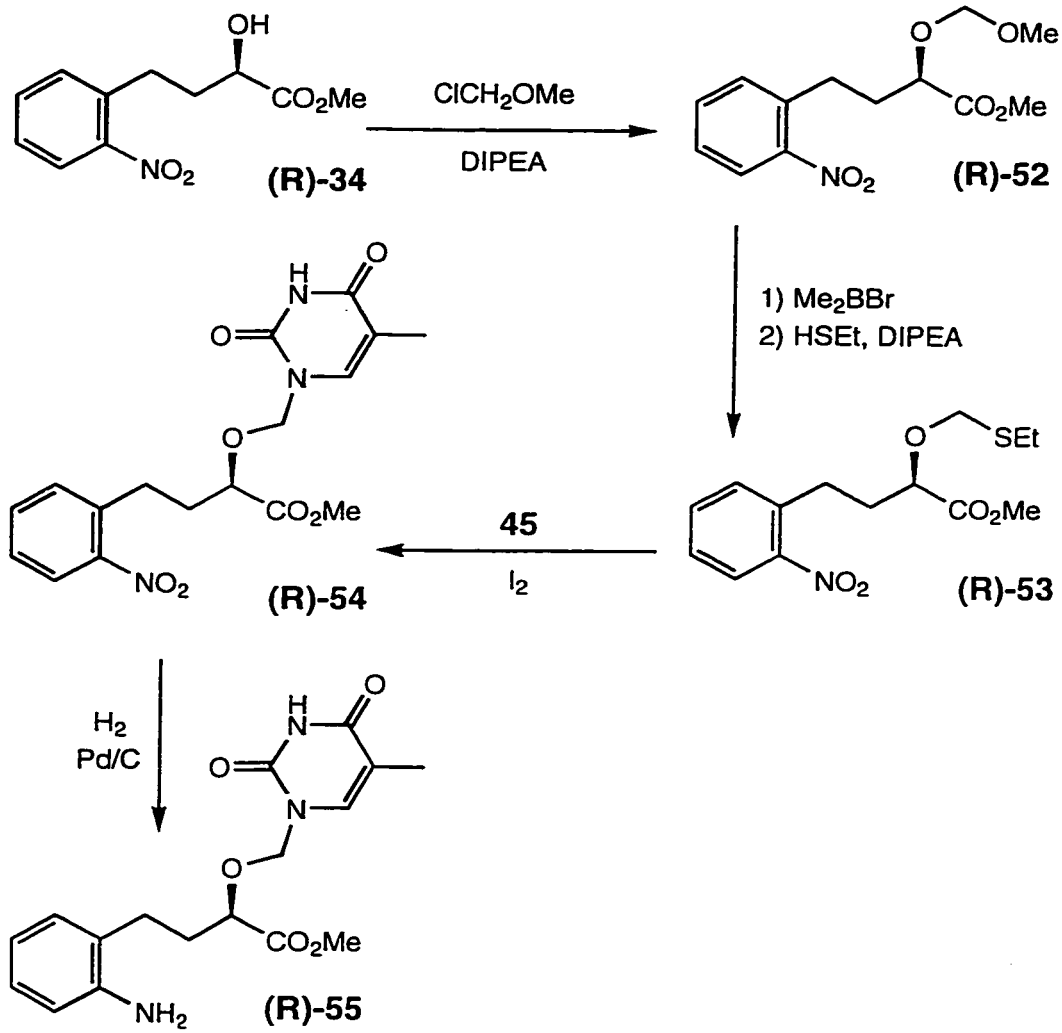
Figure 13. ^{13}C NMR Spectrum (75 MHz, CD_3OD) Monomer (S)-**50**

2.9 Synthesis of Monomer (R)-55

The analogous (R) enriched monomer was synthesized for various purposes. It was important in the determination of the assignment of the actual chirality and enantiomeric purity as shown previously. Furthermore, in the future, it will be interesting to compare the hybridization affinity of the (R) oligomer with the (S) oligomer.

The synthesis of the (R) monomer was similar to that of the (S) analog, but with some minor differences such as in the introduction of the chiral center and in the sequence of the reactions (Scheme 18). Since, no enzyme was found that would convert **24** to the (R)-**29** analog, it was converted to (R)-34 via a chemical chiral auxiliary, L-proline sodium borohydride (outlined in Scheme 8). Because this reaction reduces both the ketone, as well as the double bond, the hydro-genation at this step was not necessary. This proved advantageous since the nitro moiety did not require protection. The synthesis was carried out as in the (S) monomer and the nitro group was then reduced at the very final step via hydrogenation to give (R)-**55**.

Scheme 18. Synthesis of Monomer (R)-**55**



2.10 Determination of the Enantiomeric Purity of the (R) and (S) Monomers

Unfortunately, attempts at determining the enantiomeric purity of the monomers (S)-**50** and (R)-**55** using Pirkle's shift reagent proved unsuccessful. The signals which were expected to show different chemical shifts did not behave as expected. As Pirkle's reagent was added (0.5 eq. at a time) the

signals simply broadened without any resolution. Although this was unfortunate, it was not unexpected since (R)-**55** and (S)-**50** are not α amino acids. These monomers exhibit a larger distance between the amino and carbonyl moiety, in addition to, possible interference caused by the thymine base.

An alternative approach is the resolution of the monomers via chiral high pressure liquid chromatography (HPLC). One of the problems with this method is that the columns are very expensive and finding the correct type of column for a novel compound can sometimes be a challenge. On the other hand, once the correct column and solvent system is found the results are quick and very accurate.

Both the Fmoc protected and free amine (R) and (S) monomers were eluted through a cellulose chiralcel^{T} column using various solvent systems, with no resolution. Later, it was found that eluting the tert-butyl carbamate (BOC) analog (R) **56** and (S) **57** through the same column using 10% ethanol in hexane resulted in the resolution of the two enantiomers (Figure 14). The BOC analogs were synthesized by reacting the free amino monomers (R)-**55** and (S)-**50** with ditert-butyldicarbonate in the presence of triethylamine (Scheme 19).

As shown by the HPLC chromatograms, (S)-**50** exhibited a retention time of 23.9 minutes whereas (R)-**56** eluted later at 27.7 minutes (Figure14a and b). (S)-**50** showed no evidence of the (R)-**56** enantiomer except for a suspected impurity that eluted at 32.7 minutes (Figure 14a). In order to confirm that the (S) enantiomer was the sole enantiomer and to rule out the possibility that the peak at 32.7 minutes is not the (R) enantiomer, (S)-**50** was spiked with a small amount of (R)-**56** and it was found that the peak corresponding to (S)-**50** is enhanced and the smaller peak due to the (R) enantiomer becomes evident (Figure 14c). After comparison of the areas under each signal, it was determined that the enantiomeric purity of (S)-**50** was greater than 99% and that of (R)-**56** was 46%.

Scheme 19. Synthesis of the BOC protected Monomers

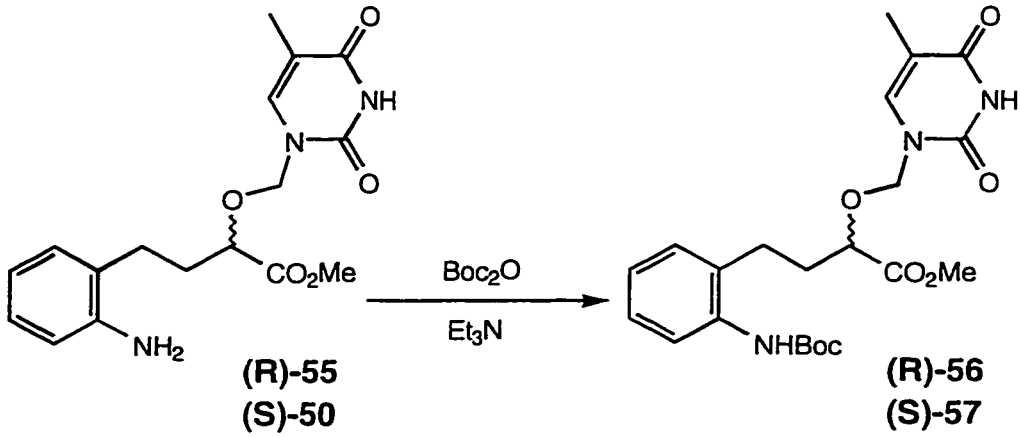
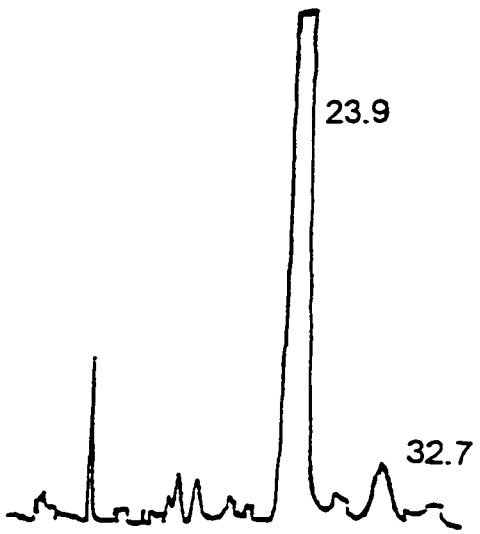
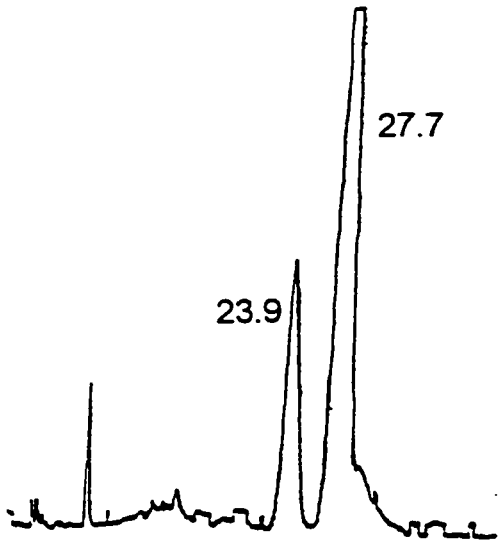


Figure 14. Determination of the Enantiomeric Purity of the Monomers via Chiral HPLC

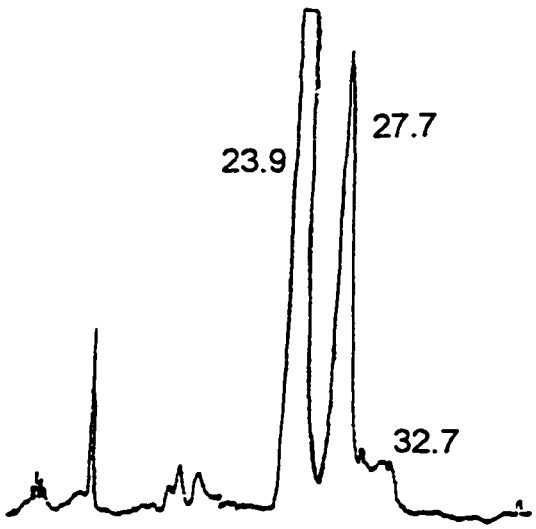
a) (S)-**50**



b) (R)-**56**



c) (S)-**50** + (R)-**56**

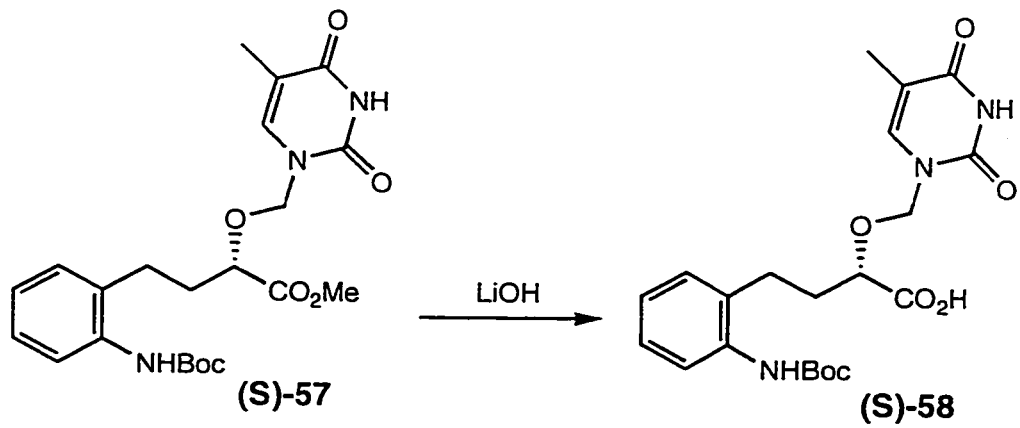


2.11 Synthesis of the Dimer 67

Coupling of monomer units to make oligomers was done via conventional peptide synthesis. In order to efficiently synthesize the dimer and prevent the formation of intramolecular peptide bonds, one monomer was reacted as the free amine ester and the other unit as the BOC protected free carboxylic acid.

Unfortunately, the hydrolysis of the Fmoc protected monomer (S)-**49** with LiOH not only generated the free carboxylic acid but, also resulted in some deprotection of the amine. It was therefore necessary to exchange the Fmoc group of (S)-**49** for the base resistant BOC protecting group (S)-**57**. The subsequent hydrolysis of (S)-**57** with LiOH (3eq) resulted in a clean reaction to give the free acid (S)-**58** while the BOC goup remained intact (Scheme 20).

Scheme 20. Ester Hydrolysis of Monomer

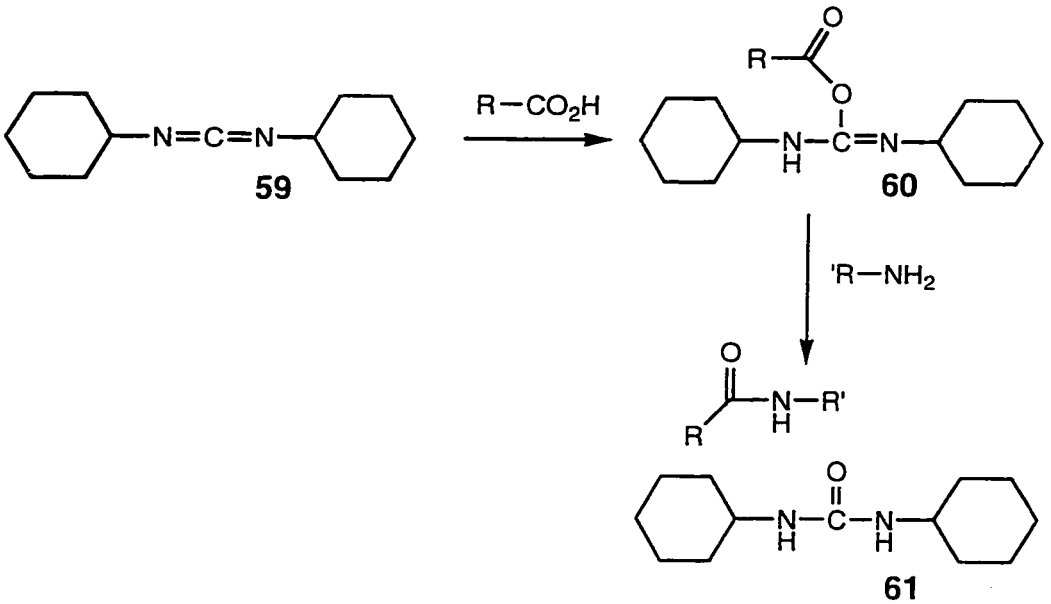


(S)-57 **(S)-58**

The introduction of carbodiimides, such as DCC (**59**), as reagents for the formation of peptide bonds was a revolutionary contribution to peptide synthesis.[48] While carboxylic acids react with carbodiimides very rapidly, the rate of reaction with amines is negligible. The reaction of the carboxylic acid

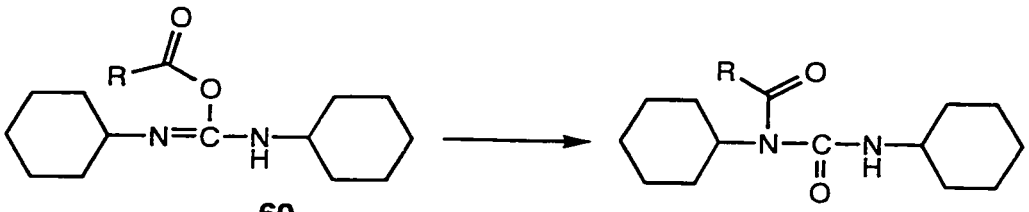
with DCC (**59**) yields an O-acyl-isourea reactive intermediate **60**, which results in the activation of the carboxylic acid leading to the coupling with the amine (Scheme 21). The simplicity of the reaction, where the activation and coupling are done in a single step, and the insoluble byproduct, N,N'-dicyclohexylurea (**61**, DCU) is removed with filtration, is what has contributed to the popularity of this method.

Scheme 21. DCC Coupling Reaction



Not, surprisingly, there is a price to be paid for such high reactivity. The over-reactivity of the O-acylisourea intermediate **60** results in some loss of chirality when the peptide segments are coupled. In addition, the nucleophilic nitrogen centre of **60** competes with the amine compound for the acyl residue leading to the formation of the unreactive N-acylurea **62**, resulting in deadlock (Scheme 22).

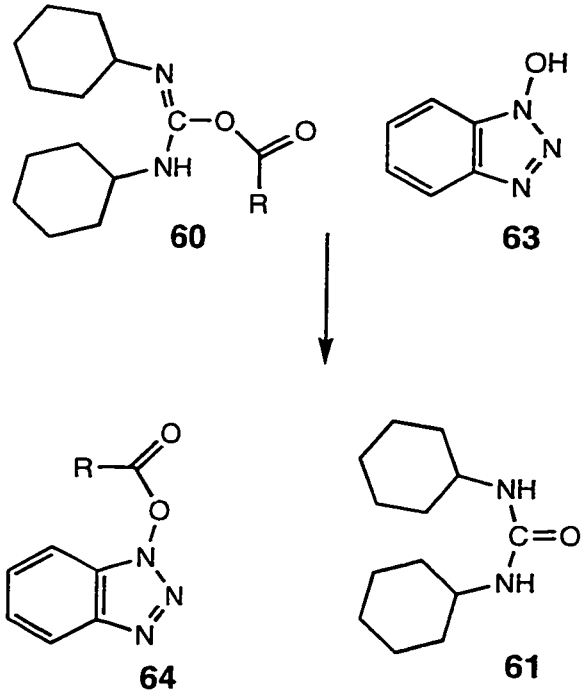
Scheme 22. N-acylurea Deadlock



60 62

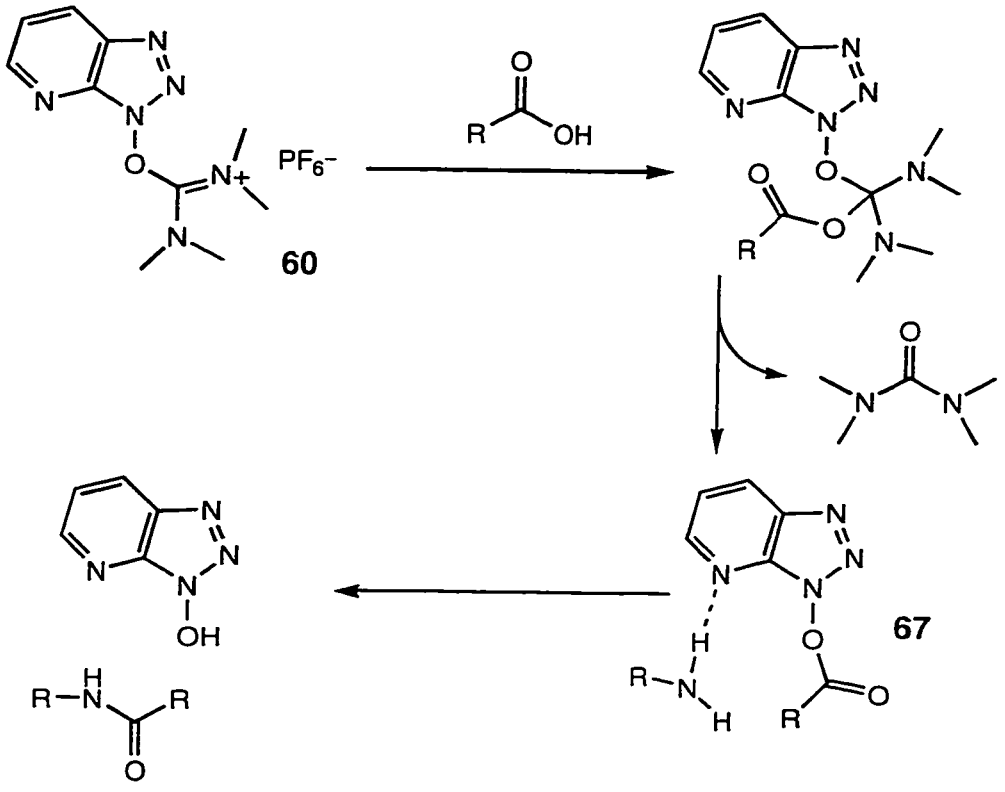
The simple addition of an auxiliary nucleophile, such as 1-hydroxy-benzotriazole (**63**, HOBt), can serve to suppress both racemization and N-acylurea formation.[49] HOBt attacks the O-acylisourea intermediate to give an O-acyl-1-hydroxybenzotriazole **64** which is a powerful acylating agent (Scheme 24). The resulting intermediate **64** serves to reduce the concentration of the O-acylisourea **60** and thereby reduces racemization. In addition, the weakly acidic nature of HOBt itself prevents proton abstraction from the chiral carbon and contributes to the suppression of racemization. Furthermore, the availability of HOBt serves to shorten the lifetime of the over-reactive O-acylisourea **60** and thereby, reduces the extent of oxygen to nitrogen acyl migration, obliterating the deadlock N-acylurea side-product **62**.[50]

Scheme 23. Addition of the HOBt Auxiliary

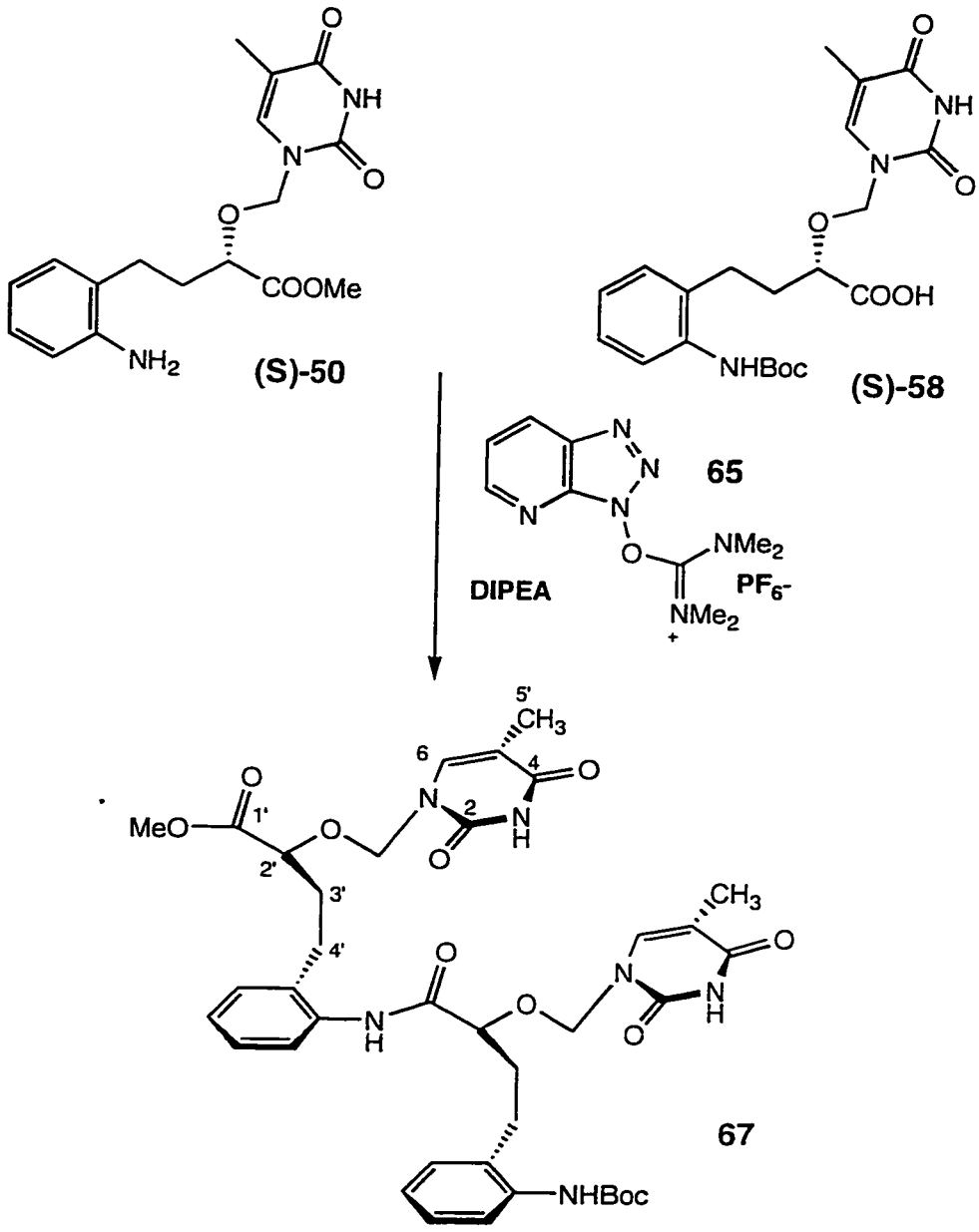


The advantage of the HATU coupling reagent (**65,** O-(7-azabenzo-triazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate coupling reagent) is that it is both a peptide coupler and auxiliary, all in one. Furthermore, it contains an additional aromatic nitrogen which serves to coordinate with the amine component (as shown in intermediate **66**), thereby bringing the two segments to be condensed in close proximity to each other, allowing for a more efficient reaction (Scheme 24). Finally, dimer **67** was synthesized from the coupling of monomers (S)-**58** and (S)-**50**, in the presence of the HATU coupling reagent (**65,** Scheme 25).[51]

Scheme 24. Mechanism of HATU Coupling Reaction



Scheme 25. Synthesis of Dimer **67**



2.12 NMR Assignment of Dimer 67

The structural identity and chemical shift assignments of dimer **67** were determined *via* ^{1}H, ^{13}C, COSY, HETCOR and HMBC NMR experiments (Figures 15-18). The upfield set of singlets at δ 1.84 and 1.88 in the ^{1}H NMR (Figure 15) correspond to the methyls H5' of the two thymine bases. The HETCOR (Figure 17) indicates that the corresponding C5' signals are nearly overlapping at δ 12.37 and 12.43. Through the COSY NMR (Figure 18), H5' shows coupling with aromatic signals at δ 7.38 and 7.57 corresponding to H6 of the two bases.

Long range HMBC experiments were helpful is assigning the thymine carbon signals. The HMBC NMR showed ^{2}J and ^{3}J coupling of H5' with olefinic C6 at δ 137.52 and 137.37, C5 at δ 111.95 and 112.17 and carbonyls C4 at δ 166.78 (it should be mentioned that two sets of signals for all carbons were not always apparent, some signals are overlapping). H6 correlates with OCH2Th carbons at δ 78.04 and 78.18 and the HETCOR and ^{1}H NMR show that the corresponding protons are exhibited as AB quartets at δ 5.13 and 5.30.

The methoxy protons appear as a singlet on the ^{1}H NMR at δ 3.60 and the corresponding carbon is found at δ 52.81. HMBC experiment shows correlation with the carbonyl ester C1' at δ 171.16 and C2' at δ 78.82. The corresponding H2' signals are obseved between δ 4.18 and 4.25.

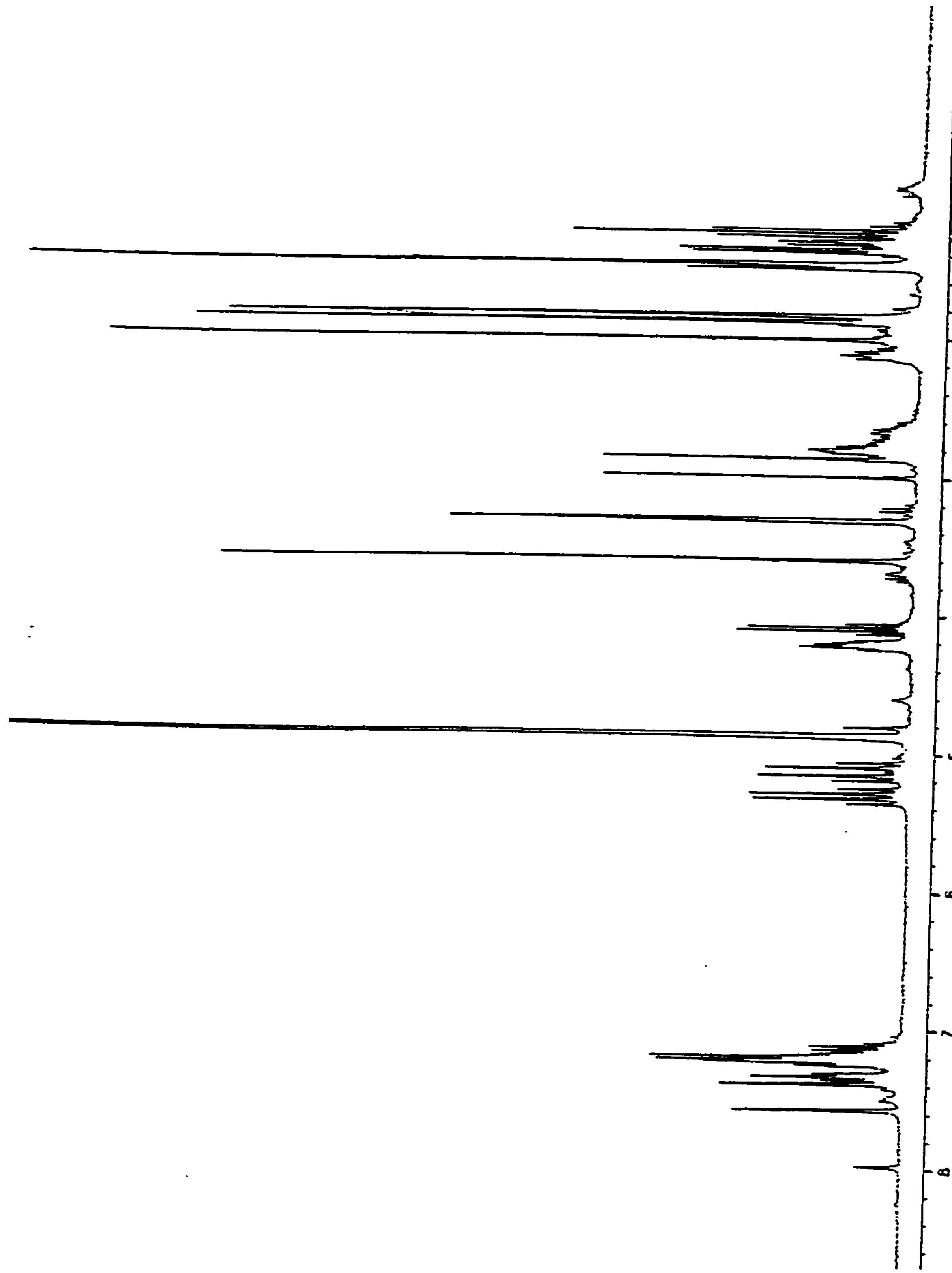
Of the two methylene signals H3' and H4', the more upfield multiplet between δ 1.90 and 2.18 in the ^{1}H NMR corresponds H3' and the more downfeild multiplet between δ 2.60 and 2.84 is that of H4'. Through the HETCOR experiment, H3' shows that it is connected to C3' at δ 34.38 and 34.81 and H4' is connected to C4' at δ 28.17 and 28.26. The COSY experiment shows correlation of H3' to both H2' and H4'. In turn, H4' shows coupling with only H3'.

The *tert*-butyl protons were shown as a singlet at δ 1.47 whose corresponding carbon through the HETCOR was seen at δ 28.90. HMBC showed ^2J correlation of the *tert*-butyl protons with the quaternary carbon at δ 81.80 and the carbamate carbonyl at δ 156.89.

The remaining carbonyl C2 on thymine, and the amide carbonyl are found at δ 153.32 and 173.52 respectively. The amide and imino protons were not observed due to the exchange with CD_3OD. A complete summary of the NMR data is outlined in Table 1.

Table 1: ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR Data of Dimer **67** in CD_3OD.

assignment	^{13}C (δ)	^{1}H (δ)	HMBC	COSY	int, mult
1' (C=O)	174.16				
(OCH3)	52.81	52.81	C1', C2'		3H, s
2'	79.70 78.30	4.18-4.25	C1', C3', OCH2Th	H3'	2H, m
3'	34.38 34.81	1.90-2.18	C1', C2'	H2', H4'	4H, m
4'	28.17 28.26	2.60-2.84	C3', ArC	H3'	4H, m
OCH_2Th	78.04 78.18	5.07-5.19 5.25-5.36			2H, AB q. 2H, AB q.
Ph-NH-CO	173.52				
$CO_2C(CH_3)_3$	156.89				
$CO_2C(CH_3)_3$	81.18				
$CO_2C(CH_3)_3$	28.90	1.47	$CO_2C(CH_3)_3$		9H, s
2	153.32				
4	166.78				
5	111.95 112.17				
5'	12.37 12.43	1.84 1.88	C4, C6, C5	H6	3H, s 3H, s
6	137.37 137.52	7.38 7.57	C4, OCH_2Th, C5'	H5'	1H, d 1H, d


8
7
6
5
4

60
80
100
120
140
160
180

Figure 17. COSY NMR Spectrum (300 MHz, CD_3OD) of Dimer **67**

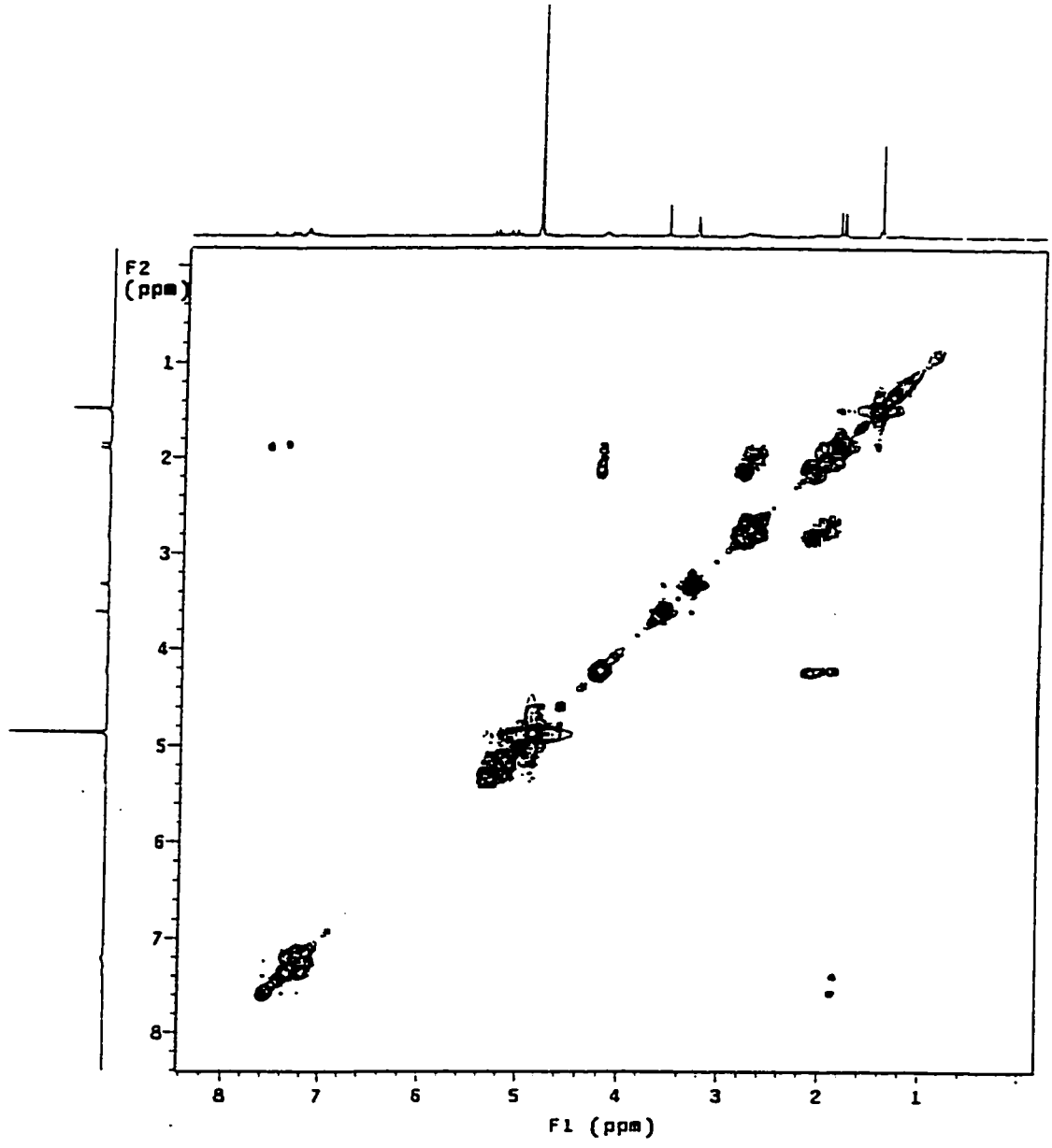
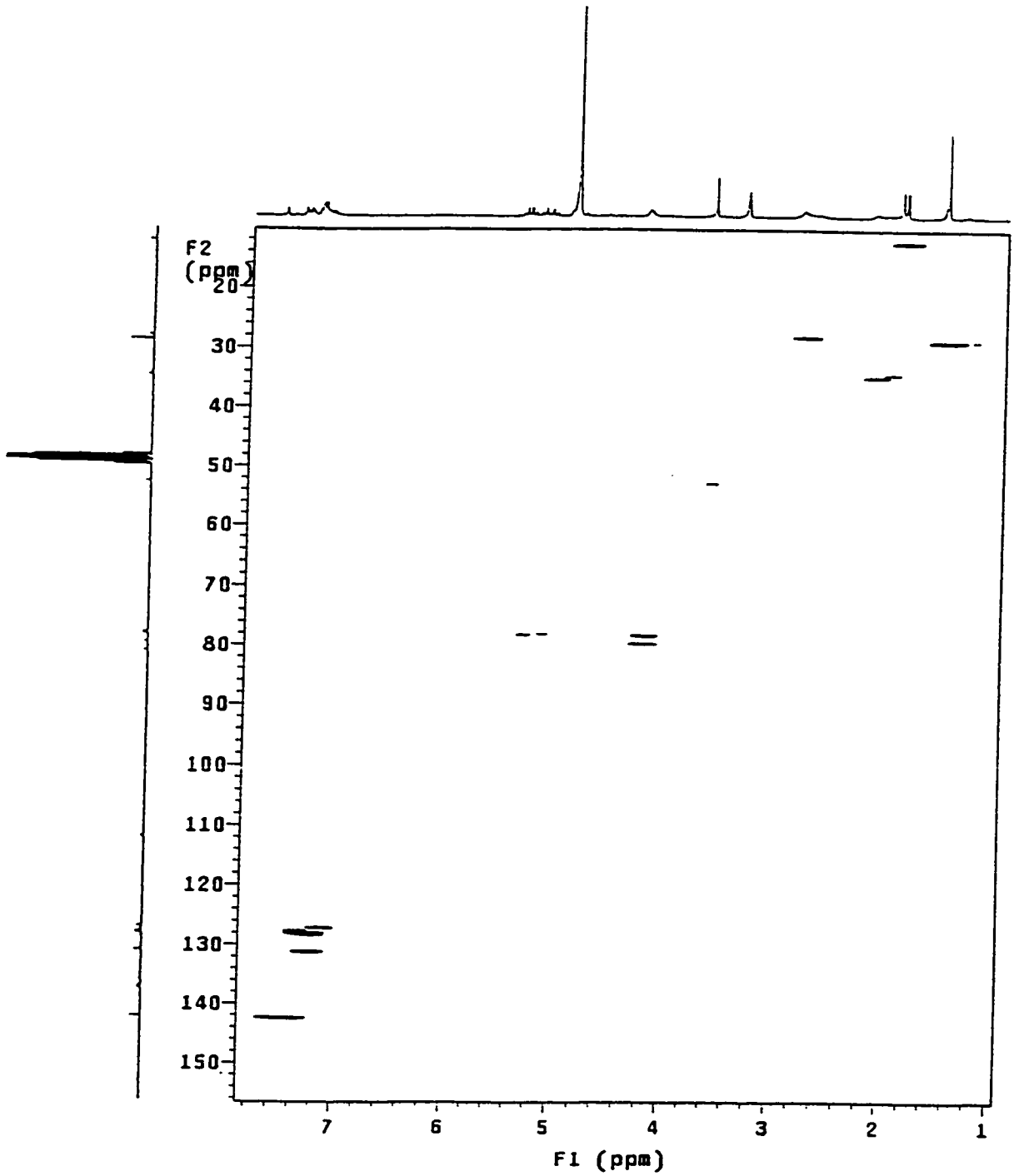


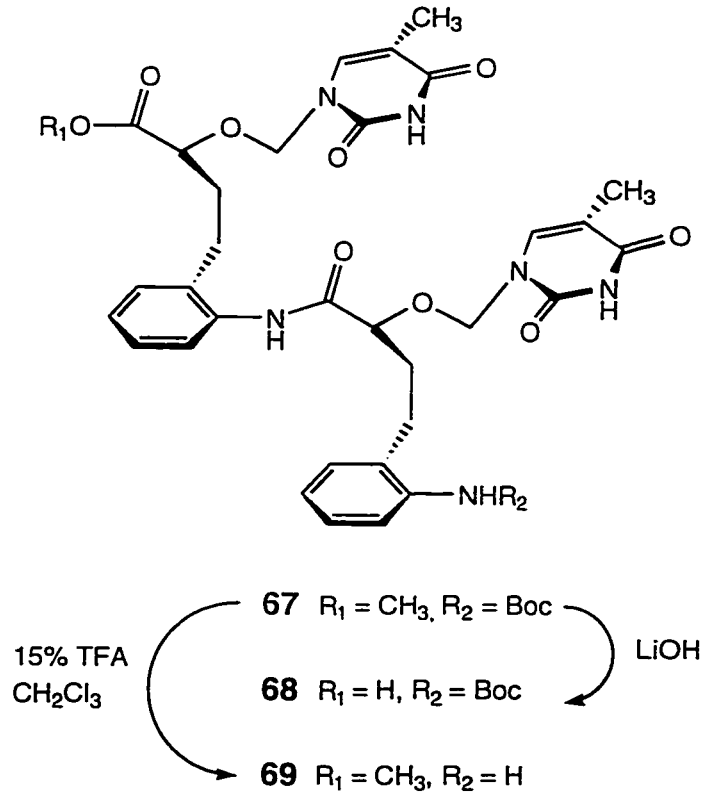
Figure 18. HETCOR NMR Spectrum (300 MHz, CD_3OD) of Dimer **67**



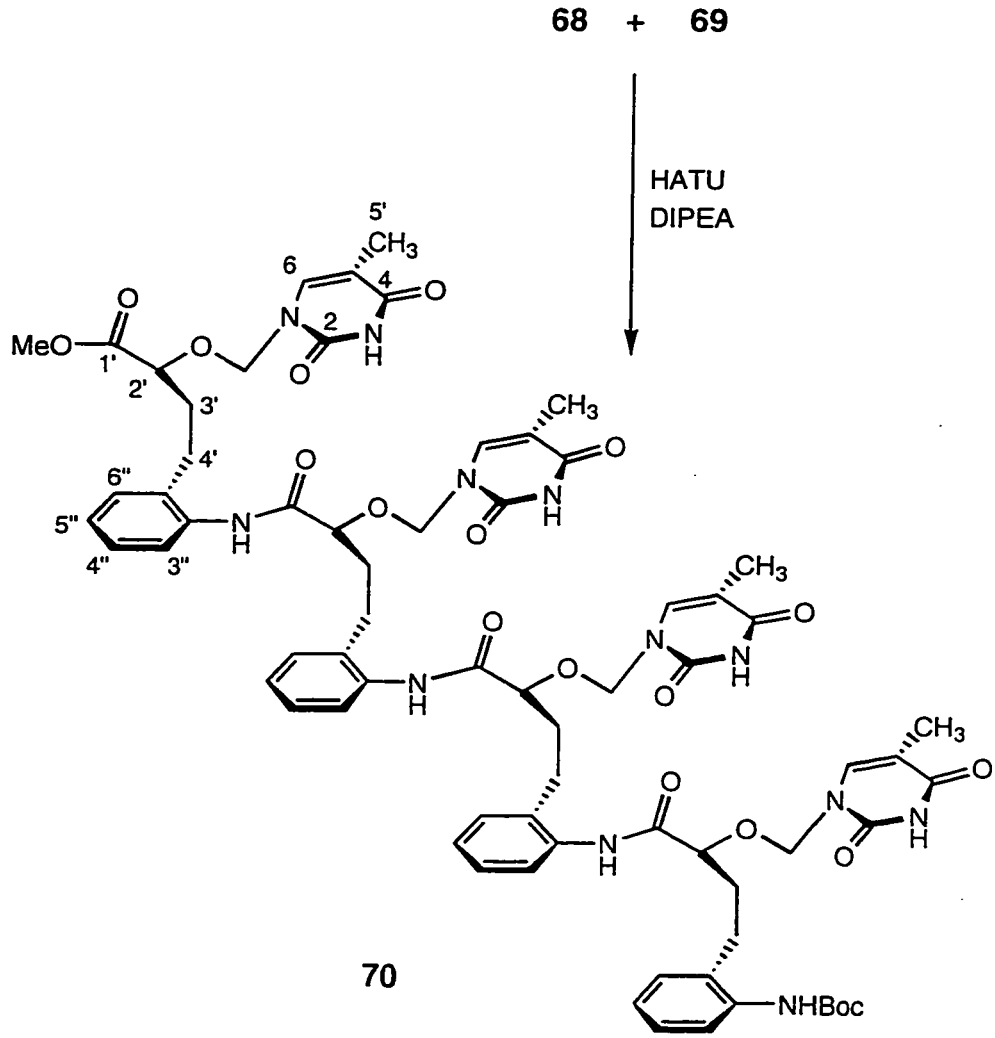
2.13 Synthesis of the Tetramer

Tetramer **70** was synthesized through another cycle of peptide coupling with the dimer. Dimer **67** was hydrolysed to the free acid **68** with LiOH as discussed previously (Scheme 26). The free amine dimer **69** was generated through the Boc deprotection of **67** with 15% TFA in anhydrous CH_2Cl_2. Coupling of the two dimer units **68** and **69** resulted in a 65% yield of the tetramer **70** (Scheme 27).

Scheme 26. Deprotection of the Dimer Coupling Units



Scheme 27. Synthesis of Tetramer **70**



70

2.14 NMR Assignment of Tetramer

The ^{1}H NMR of the monomer served as a starting point for the assignment of the tetramer. All the characteristic signals in the monomer were apparent in the tetramer, the only difference being in the complexity of the signals. The chemical shift assignments of the tetramer were based on the combined ^{1}H, ^{13}C, HMBC, HMQC and COSY NMR data (Table 2 and Figures 19 to 22). The two ends of the molecule were easily identified from the characteristic chemical shifts of the methyl ester and the BOC group, as well as the correlations observed in the HMBC spectrum from these resonances to the adjacent moieties.

In the ^{1}H NMR spectrum, the methyl ester appeared as a singlet at δ 3.64 which was coupled to a carbon at δ 52.90 in the HMQC spectrum and to a carbonyl at δ 172.51 in the HMBC spectrum. The HMBC spectrum also showed ^{2}J and ^{3}J coupling between the H3' (δ 1.90-2.24) and the carbons at δ 77.94 (C2') and δ 172.51 (C1', CO_2CH_3) respectively. Coupling of the H2', H3' and H4' protons was observed in the COSY spectrum, as well as coupling of these protons to C2', C3' and C4' in the HMQC spectrum; the expected correlations between these signals were also observed in the HMBC spectrum. Thus, the combined NMR data allowed both ^{1}H and ^{13}C chemical shift assignments to be made for the butanyl fragments of the backbone.

Two sets of amide protons, at δ ~8.6-8.8 and d 9.9-10.3, were observed in the ^{1}H NMR of the tetramer $CDCl_3$. The most downfield signals were assigned to the thymine imino resonances, whereas those at δ ~8.6-8.8 were assigned to the peptide backbone. The latter assignments were consistent with correlations observed in the HMBC spectrum, showing ^{2}J coupling of these protons with the backbone carbonyls [δ 151.94 (Boc carbonyl), 170.33, 170.72 and 170.80], in

addition to ^2J and ^3J coupling to the expected carbons in the phenyl ring (C2" and C1", C3" respectively). A summary of all the chemical shift assignments of the backbone is shown in Table 2.

Assignment of all of the thymine resonances of the tetramer was also achieved from analysis of the 2D NMR data. The methyl resonances appeared at δ 1.86 and 1.88 (9H and 3H, respectively) in the ^{1}H NMR spectrum and they were attached to overlapping carbon resonances at δ 12.50 in the HMQC spectrum. In the HMBC spectrum, ^2J and ^3J couplings from these methyls to the four C5 (δ ~112) and C6 (δ ~139-140) carbons of thymine were also observed. Finally, the chemical shifts of the four H6 protons (δ ~7.05-7.18) were confirmed from both their coupling to the methyls in the COSY spectrum and their coupling to the C6 resonances (δ ~139-140) in the HMQC spectrum.

In conclusion, the structural characterization of the tetramer is complete from the NMR data and its integrations and further confirmed by the ES mass spectrum giving a molecular ion of 1392.6 (M+).

Table 2. ^{1}H (500 MHz) and ^{13}C (125MHz) NMR Data of Tetramer **70** in $CDCl_3$

assignment	^{13}C (δ)	^{1}H (δ)	HMBC	COSY	intensity
1'	172.51				
2'	77.94 78.22 78.76 79.88	4.17 - 4.23[b]	C1',C3',C4', O<u>C</u>H$_2$Th, NH<u>C</u>O	H3'	4H, m
3'	32.46 33.07 33.43 33.75	1.90 - 2.24[b]	C1",C2',C1', NH<u>C</u>O	H2', H3'	8H, m
4'	26.65 26.78 26.90 27.11	2.62 - 2.75[b]	C1",C2",C6" C2',C3'	H3'	8H, m
O<u>C</u>H$_2$Th	76.40[a] 76.71[a] 77.03[a]	5.06[b] 5.12[b] 5.18[b] 5.26[b]	C2,C6, C2'		8H, m
N<u>H</u>CO		8.62 8.72 8.84	NH<u>C</u>O, C3', NH<u>C</u>OO, C3",C2"		3H, bs
NH<u>C</u>O	170.33 170.72 170.80				
N<u>H</u>COO		8.71	NH<u>C</u>OO		1H, bs
NH<u>C</u>OO	151.94				
$CO_2\underline{C}(CH_3)_3$	80.76				
$CO_2C(\underline{CH_3})_3$	28.57	1.47	$CO_2\underline{C}(\underline{C}H_3)_3$		9H, s
OCH_3	52.90	3.64	C1'		3H, s

	151.94				
2	152.00				
	152.16				
	152.23				
		9.95			
3		10.02			
		10.16			
		10.27			
	164.04				
4	164.22				
	164.43				
	164.73				
	112.03				
5	112.17[c]				
	112.24				
	139.64	7.10-7.17			
6	139.81				
	140.02				
5'	12.50[c]	1.88		H6	12H
		1.86			
1"	131.63				
	131.64				
	132.97				
	133.43				
2"	134.66				
	134.83				
	135.03				
	136.34				
3"	123.42	7.66	C1", C4", C2"	H4"	1H, d
	124.50	7.74			1H, d
	125.55	7.61			1H, bs
	124.87	7.54			1H, d
4"	127.24	7.15 - 7.20[b]	C2",C3",	H3",	4H, m
	127.32		NHCOO,	H5"	
	127.33[c]		NHCO		

5"	125.11 126.09 126.27 126.48	6.98 - 7.01[b]	C1", C6"	H6", H4"	4H, m
6"	129.35 129.61 129.78 129.87	7.09 - 7.19[b]	C1", C2", C5", C4", C4'	H5"	4H, m

[a] Due to overlapping ^{13}C resonances these chemical shifts were based on the HMQC and HMBC NMR data.
[b] Due to overlapping resonances in the ^{1}H NMR spectrum, the exact chemical shifts and coupling constants could not be determined; the values given were obtained from the HMQC and HMBC data.
[c] The strong intensity of the signal suggest overlapping ^{13}C resonances.

Finally,in hopes of gaining some insight as to the possibility of π-stacking interactions of the tetramer, the ^{1}H NMR resonances of the monomer (S)-**57**, dimer **67** and tetramer **70** were compared. Small upfield shifts of both the H6 and H5' resonances in the tetramer, as compared to the dimer and the monomer, were observed (Table 3.). This phenomenon is generally attributed to the ring-current magnetic anisotropy effect of the purine and pyrimidine bases and is usually observed in systems involving base stacking interactions. Interestingly, a similar upfield shift was observed for the backbone amide protons.

Table 3. 1H Chemical Shifts of Monomer (S)-**57**, Dimer **67** and Tetramer **70** in $CDCl_3$

assignment	**(S)-57** (δ)	**67** (δ)	**70** (δ)
H6	7.22	7.08, 7.12	7.05-7.18
H5'	1.95	1.91	1.86, 1.88
NH (amide)	9.31	8.44, 8.59	8.62, 8.71, 8.71, 8.84

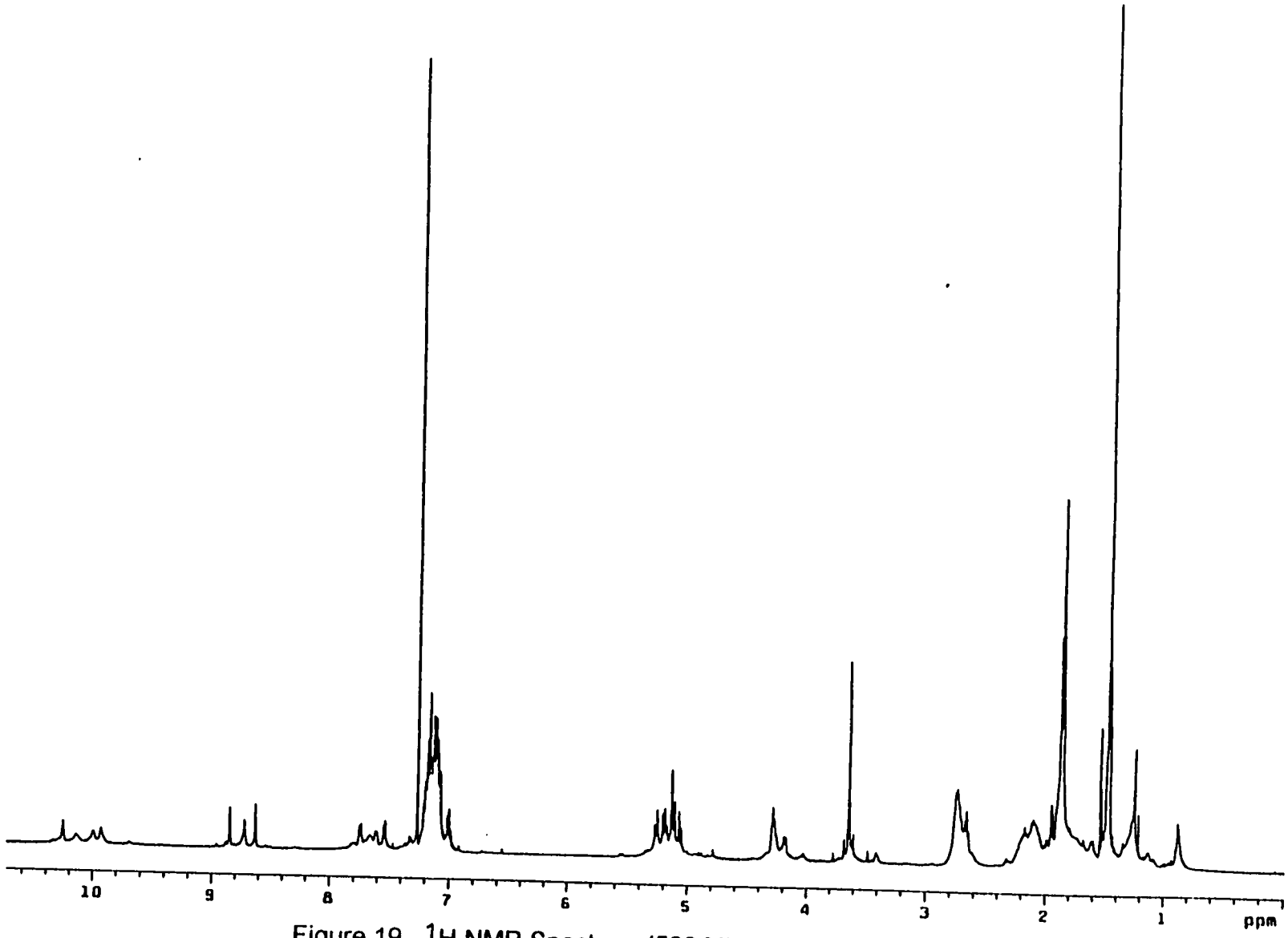


Figure 19. ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of T

Figure 20. COSY NMR Spectrum (500 MHz, $CDCl_3$) of Tetramer **70**

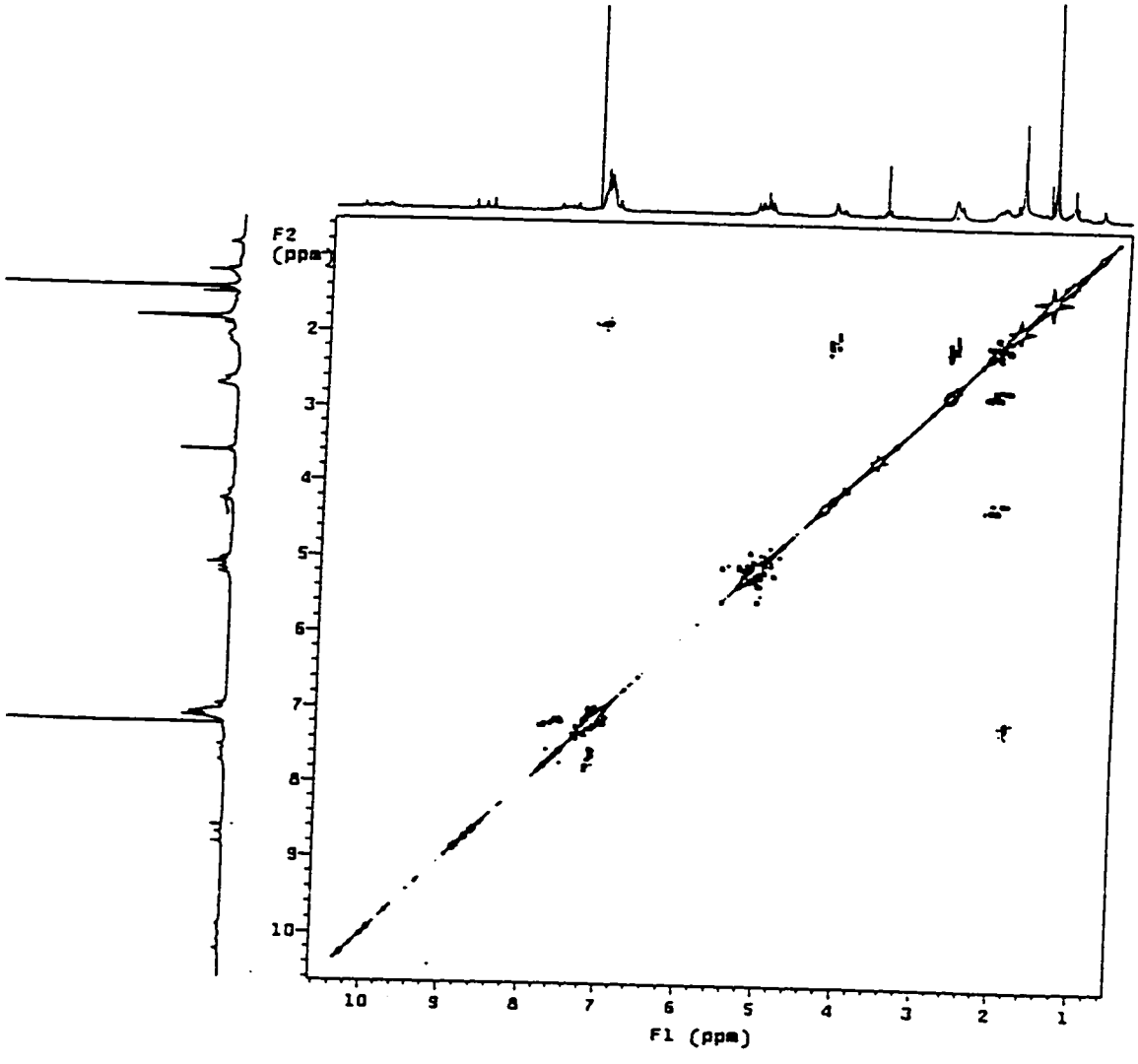


Figure 21. COSY NMR Spectrum (500 MHz, $CDCl_3$) of the Aromatic Region o
Tetramer **70**

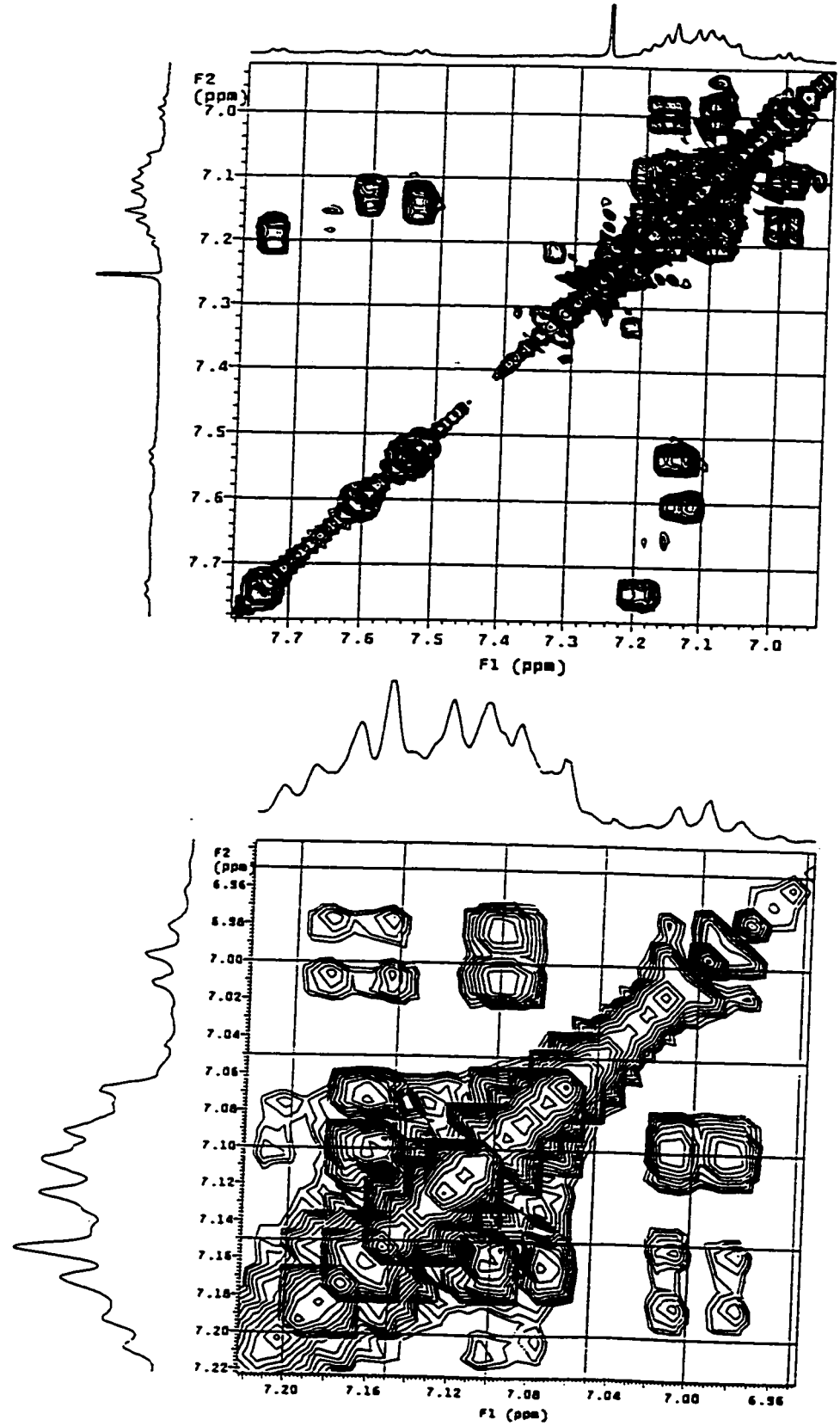
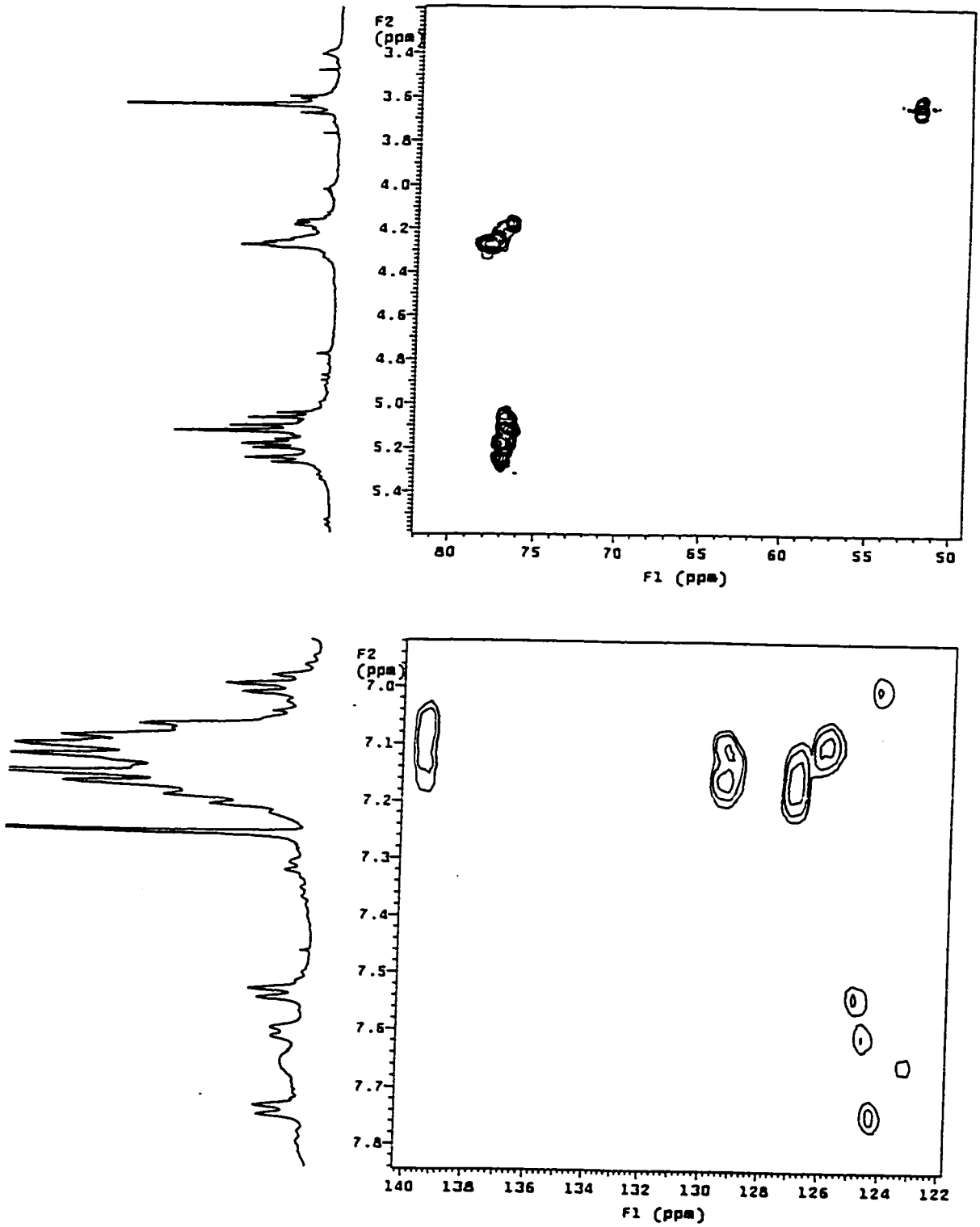


Figure 22. HMQC NMR Spectrum (500 MHz, $CDCl_3$) of Tetramer **70**



2.15 Conclusion

Both the (R) and (S) thymidine monomers ((S)-**50** & (R)-**55**) have been synthesized and their enantiomeric purities have been determined (>98% ee. and 46% ee. respectively) by chiral HPLC. Monomer (S)-**50** has been oligomerized to the tetramer **70** in moderate yields and its structure has been completely characterized by NMR. It is expected that the APNA analogs will be stable to nucleases as well as proteases and the lipophilic character of their novel aromatic backbone may have a beneficial effect on the cell membrane permeability of these compounds. It was encouraging to find out that the Boc deprotected tetramer was fairly soluble in aqueous medium. It is expected that the completely deprotected tetramer will be completely water soluble.

3.0 EXPERIMENTAL

Instrumentation and General Methods. NMR spectra were obtained at 20-22° both at Concordia and McGill University. ^{1}H and ^{13}C-NMR chemical shifts ar quoted in ppm and are referenced to the internal deuterated solvent. Mass spectra data were obtained at McGill University, Biomedical Mass Spectrometry Unit an the Department of Chemistry. All reactions were run under a nitrogen atmospher using oven-dried syringes and glassware when appropriate. THF was distilled fro Na/benzophenone, CH_2Cl_2 was distilled from P_2O_5, MeOH from Mg turnings, DM was distilled from CaO. Reagents and solvents were purchased from Aldric Chemical Co. and VWR Scientific of Canada respectively. The enzymes BSLDH and FDH were purchased from Genzyme (Cambridge, MA) and Boeringe Mannheim (Montreal, Que), respectively. Reversed phase flash colum chromatography was carried out on silica gel reacted with *n* octadecyltrichlorosilane, following previously reported procedures.[52]

Synthesis of α-hydroxy acid (S)-29

An aqueous suspension of compound **24** was titrated to pH 7 with 1N NaOH an freeze-dried to obtain the sodium salt **28** as a yellow powder. Compound **28** (2.42 g, 10 mmol) was dissolved in TRIS.HCl buffer (5 mM, pH 6, 500 mL) containing sodium formate (2.3 eq, 23 mmol, 1.56 g), NADH (0.02 eq, 0.2 mmol, 150 mg) an dithiothrietol (0.005 eq, 0.05 mmol, 7.8 mg), and the solution was degassed unde vacuum for at least 30 min. Lyophilized powders of the two enzymes, BSLDH (600 units) and FDH (50 units), were added and the mixture was gently stirred at RT under N_2 for 24 h with a periodic addition of acid (0.2 N HCl) to maintain the pH a

6.0-6.2. The solution was then acidified to pH 3 with 1N HCl and extracted with EtOAc (4x300 mL). The organic layer was concentrated to give the desired compound as a light brown solid which was found to be fairly pure by ^{1}H NMR (98% yield). MP: 123-125 °C. $[\alpha]_D$ +43.2 (c 0.10, MeOH), >98% ee; the enantiomers of **29** could not be separated by chiral HPLC, however, the enantiomeric purity was estimated from the %ee of compound **33**. TLC on normal silica gel (1:1 MeOH/EtOAc): Rf=0.56; on C18 reversed phase silica gel (1:1 MeOH/H_2O): Rf=0.32. ^{1}H NMR (270 MHz, acetone-d_6) δ: 4.94 (dd, J=2.0, 4.9 Hz, H2'), 6.53 (dd, J=4.9, 15.8 Hz, H3'), 7.21 (dd, J=2.0, 15.8 Hz, H4'), 7.53 (dt, J=7.9, 1.5, H4"), 7.70-7.73 (tm, J=8.0 Hz, H5") 7.81 (dd, J=1.5, 8.0 Hz, H6"), 7.93 (dd, J=1.0, 8.0 , H3"). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ: 72.24, 125.30, 127.25, 129.62, 129.65, 132.84, 133.36, 134.18, 149.61, 175.00.

Synthesis of aniline methyl ester (S)-33

The α-hydroxy acid (S)-**29** was dissolved in methanol and reacted with excess diazomethane at RT until the evolution of gas had ceased. Pure methyl ester (S)-**32** was obtained after flash column chromatography in 90% yield. $[\alpha]_D$ +55 (c 0.40, $CHCl_3$). TLC (3:2 hex/EtOAc): Rf = 0.23. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 3.85 (s, OCH_3), 4.89 (dd, J=1.7, 5.2 Hz, H2'), 6.22 (dd, J=5.2, 15.8 Hz, H3'), 7.29 (dd, J=1.7, 15.8 Hz, H4"), 7.37-7.45 and 7.55-7.59 (2m, 3H, Ar), 7.93 (d, J=7.9 Hz, H3"). Hydrogenation of both the nitro group and the double bond was achieved by reacting the methyl ester (3.3 g) with H_2 gas (3 atm) in the presence of 10% Pd/C (0.33g) in MeOH for 15 h. The solution was filtered through celite and concentrated to give a 92% yield of the desired product as a light yellow oil. The enantiomeric purity of the (S)-enantiomer was measured to be >98% ee from its ^{1}H NMR observed in the presence of 1 equivalence of (R)-(-)-2,2,2-trifluoro-1-(9-anthryl)

ethanol; (R)-**36** having ~50% ee was also synthesized and used for reference in the NMR analysis of the optical purity. $[\alpha]_D$ +28 (c 0.40, $CHCl_3$). TLC (1:2 hex/EtOAc): Rf = 0.25. ^1H NMR (270 MHz, $CDCl_3$) δ: 1.85-2.14 (2m, 2H3'), 2.55-2.75 (m, 2H4'), 3.72 (s, OCH_3), 3.78 (bs, OH), 4.21 (dd, J=8.2, 3.7 Hz, H2'), 6.65-6.78 (m, 2H, Ar), 7.00-7.05 (m, 2H, Ar). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ: 25.99, 33.54, 52.34, 69.48, 115.87, 118.83, 125.34, 127.17, 129.70, 144.14, 175.30. MS $(NH_3)CI$ *m/z* (assignment): 210 (base, MH^+), 209 (M^+), 178 ($M-OCH_3^+$), 177 ($M-CH_3OH^+$), 149 (177-CO).

Synthesis of aniline methyl ester (R)-36

Sodium borohydride (200 mg, 5.29 mmol) and L-proline (610 mg, 5.29 mmol) were dissolved in THF (10ml) and refluxed for 5 h under N_2. The mixture was then cooled to 0 °C and to it was added compound **24** (560 mg, 2.65 mmol) as a solid. The resulting jelly-like mixture was warmed to RT and stirred for an additional 18 h. The reaction mixture was quenched with 10% HCL (10ml) and the THF was evaporated. The resulting mixture was extracted with EtOAc (4x10 ml) , washed with saturated NaCl (40ml), dried over anhydrous MgSO4 and evaporated to dryness to give a crude product (R)-**34** as a reddish brown oil (~90% yield). $[\alpha]_D$ -10 (c 0.12, $CHCl_3$). TLC (1:1 MeOH, EtOAc): Rf=0.51. ^1H NMR (270 MHz, $CDCl_3$) δ: 1.95-2.23 (m, H3'), 3.12 (m, H4'), 4.12 (dd, J=4.0, 7.2 Hz, H2'), 6.40 (bs, OH), 7.37-8.00 (m, 4H, Ar).

The α hydroxy acid (R)-**34** was dissolved in methanol and reacted with excess diazomethane at RT until the evolution of gas had ceased. The mixture was then concentrated to give 95% yield of the desired compound (R)-**35**. $[\alpha]_D$ -22 (c 0.13 $CHCl_3$). TLC (2:1 hex/EtOAc): Rf=0.27. ^1H NMR (270 MHz, $CDCl_3$) δ: 1.98-2.15 (m, H3'), 3.00 (m, H4'), 3.74 (s, OCH_3), 4.22 (m, H1'), 7.30-8.00 (m, 4H, Ar).

Hydrogenation of the nitro group was achieved by reacting the methyl ester (R)-3 (4.1 g) with H_2 gas (2 atm) in the presence of 10% Pd/C (0.42 g) in MeOH for 15 h The solution was filtered through celite and concentrated to give 94% yeild of th desired product as a light amber oil. The enatiomeric purity was determined from it 1H NMR in the presence of the chiral solvating agent (R)-(-)-2,2,2-trifluoro-1-(9 anthryl)-ethanol. $[\alpha]_D$ -13 (c 0.15, $CHCl_3$), ~50% ee. NMR data as in (S)-33.

Synthesis of Fmoc-protected (S)-38

Compound (S)-**33** (3.06 g, 14.66 mmol) was dissolved in a mixture of 10% aqueous Na_2CO_3 (17.6 mL, 1 eq) and dioxane (10 mL) and cooled to 0 °C. Fluoroenyl methylchloroformate (3.79 g, 1 eq, 14.66 mmol) was dissolved in dioxane (20 mL and added dropwise *via* a syringe, giving a white milky reaction mixture. The mixture was stirred at 0 °C for 1.5 h and allowed to warm up to RT for an additiona hour. The reaction was then quenched with H_2O (30 mL), acidified to pH 3 and extracted with EtOAc (4x30 mL); the combined organic layers were washed with saturated NaCl (120 mL) and dried over anhydrous $MgSO_4$. Flash column chromatography using 3:2 hex/EtOAc as the solvent system led to the isolation of the desired product (S)-**38** as a white solid in 84% yield. TLC (1:1 Hex/EtOAc): Rf=0.35. M.p.: 119-120 °C. ^1H NMR (270 MHz, $CDCl_3$) δ: 1.89-2.15 (m, 2H3'), 2.65-2.89 (m, 2H4'), 3.09 (bs, OH), 3.70 (s, OCH_3), 4.06 (dd, J=8.6, 3.7 Hz, H2'), 4.27, (t, J=6.9 Hz, NHCOOCH$_2$C<u>H</u>), 4.48 (d, J=6.9 Hz, NHCOOC<u>H$_2$</u>CH)), 7.04-7.78 (12H, Ar and Fmoc-Ar). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ: 25.87, 34.26, 47.09, 52.57, 66.85, 68.75, 119.88, 124.51, 125.05, 127.00, 127.14, 127.63, 129.82, 135.88, 141.24, 143.81, 154.27, 175.04.

Synthesis of methylthiomethyl ether (S)-39 *via* Pummerer Rearrangement

To a solution of the Fmoc derivative (S)-**38** (1.6 g, 3.8 mmol) in acetonitrile (40 ml) at 0 °C, was added methyl sulfide (1.8 g, 30.2 mmol) and benzoyl peroxide (3.6 g, 15.2 mmol) over a 20 min period. After stirring at 0 °C for 1.5 h, the reaction mixture was diluted with ether (100 ml) and washed with 1N NaOH (100 ml) and saturated NaCl (100 ml). The organic layer was then dried with $MgSO_4$ and concentrated to give the crude product as a yellow oil. Purification via flash column chromatography using 3:1 hex/EtOAc as the eluting solvent, gave pure compound (S)-**39** (20% yield) and the keto compound **40** (75% yield). $[\alpha]_D$ -52 (c 0.20, $CHCl_3$), TLC (1:1 hex/EtOAc): Rf=0.56. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.05-2.15 (m, H3'), 2.18 (s, SCH_3), 2.98 (m, H4'), 3.75 (s, OCH_3), 4.34 (dd, J= 2.0, 4.8 Hz, H2'), 4.64 (d, J=12 Hz, A part of AB, 1H, OCH_2S), 4.82 (d, J=12 Hz, 1H, A part of AB, 1H, OCH_2S), 7.30-7.92 (m, 4H, Ar).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.33, 28.93, 33.40, 52.15, 74.51, 74.91, 124.91, 127.41, 132.07, 133.12, 136.31, 149.40, 172.43. MS $(NH_3)Cl$ *m/z* (assignment): 317 ($M+NH3^+$), 299 (M^+), 238 ($M-CH2SCH3^+$)

General Procedure for the Preparation of the methoxymethyl ethers

The alcohol derivative (1 eq.) was dissolved in freshly distilled THF (9 mL/mmol alcohol)) at 0 °C under N_2. Chloromethylmethyl ether (20 eq.), was added, followed by the dropwise addition of dry DIPEA (3 eq.) The reaction mixture was allowed to warm up to R.T. and to stir for 18 h. The reaction was quenched with the addition of water, followed by extraction with EtOAc; the organic layer was dried with anhydrous $MgSO_4$ and concentrated to give a light amber oil.

Synthesis of methoxymethyl ether (S)-47

(S)-**38** (1.1 g, 2.3 mmol), chloromethylmethyl ether (4 ml, 53.2 mmol) and DIPEA (1.4 mL, 7.0 mmol) were reacted as described in the general procedure. The crude product was purified by flash column chromatography using 2:1 Hex/EtOAc as the solvent system to give product (S)-**47** as a light yellow oil in 90% yield. $[\alpha]_D$ -19.6 (c 0.30, $CHCl_3$). TLC (2:1 hex/EtOAc): Rf= 0.24. ^1H NMR (300 MHz, $CDCl_3$) δ 2.06-2.13 (m, 2H3'), 2.73 (t, J=7.2 Hz, 2H4'), 3.35 (s, $CH_2OC\underline{H}_3$), 3.68 (s $COOCH_3$), 4.19 (t, J=6 Hz, H2'), 4.29 (t, J=6.9 Hz, Fmoc-C$\underline{H}CH_2$), 4.48-4.60 (m Fmoc-CHC$\underline{H}_2$), 4.64 (d, J=6.9 Hz, A part of AB, 1H, OCH_2O), 4.71 (d, J=6.9 Hz, B part of AB, 1H, OCH_2O), 6.90-7.64 (13H, Ar, Fmoc-Ar, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 26.37, 33.13, 47.41, 52.24, 56.30, 66.99, 74.40, 96.34, 120.15, 122.87, 124.72, 125.25, 127.26, 127.39, 127.90, 129.76, 131.70, 135.79, 141.49, 143.97, 144,03, 154.38, 172.67. EIMS *m/z* (assignment): 476 $(MH)^+$, 475 (M^+), 297 [(M-Fmoc+COOH)$^+$], 279 [(297-H2O)$^+$].

Synthesis of methoxymethyl ether (R)-52

(R)-**34** (1.2 g, 5.0 mmol), chloromethylmethyl ether (20 ml, 100.0 mmol) and DIPEA (3 mL, 15.0 mmol) were reacted as described in the general procedure. The crude product was purified by flash column chromatography using 2:1 Hex/EtOAc as the solvent system to give product (R)-**52** as a light yellow oil in 94% yield. TLC (2:1 hex/EtOAc): Rf= 0.25. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.02-2.18 (m, H3'), 2.92-3.01 (m, H4'), 3.38 (s, $CH_2OC\underline{H}_3$), 3.72 (s, $COOCH_3$), 4.19 (t, J=6 Hz, H2'), 4.66 (d, J=6 Hz, A part of AB, 1H, OCH_2O), 4.70 (d, J=6 Hz, B part of AB, 1H, OCH_2O), 7.30-7.89 (m, 4H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.85, 33.56, 52.11, 56.23, 77.94, 96.40, 124.92, 127.46, 132.10, 133.18, 136.30, 149.42, 172.62.

General Procedure for the preparation of the methylthioethyl ethers

Synthesis of methylthioethyl ether intermediate (S)-48

The methoxymethyl ether compound (1 eq.) was dissolved in freshly distille CH_2Cl_2 (12 mL/mmol methoxymethyl ether) and cooled to -78 °C under N_2. A solution of dimethylboron bromide in CH_2Cl_2 (3.5 eq.) was added along with DIPEA (0.1 eq.). After 20 min, more DIPEA (2 eq.) was added followed by ethanethiol (3 eq.), and the resulting mixture was stirred for 2 h at -78 °C. The reaction was quenched with saturated aqueous $NaHCO_3$ and the mixture was allowed to warm up to R.T. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with saturated NaCl. The organic layer was then dried with anhydrous $MgSO_4$ and concentrated to dryness.

Synthesis of methylthioethyl ether (S)-48

Compound (S)-**47** (1.52, 3.2 mmol), dimethylboron bromide (1.5 M, 7.6 mL, 11.2 mmol) and DIPEA (0.04 mL, 0.32 mmol) were first added, followed by more DIPEA (1.2 mL, 6.4 mmol) and ethanethiol (0.8 mL, 9.6 mmol) as described in the general procedure. Pure compound (S)-**48** was isolated as a light yellow oil (450 mg) after flash column chromatography using 4:1 Hex/EtOAc as the solvent system. The average yield of this reaction was 55-60%, based on the amount of recovered alcohol (S)-**38**. $[\alpha]_D$ -54 (c 1.80, $CHCl_3$). TLC (4:1 hex/EtOAc): Rf=0.19. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.24 (t, J=7.5 Hz, $SCH_2C\underline{H}_3$), 2.07-2.14 (m, 2H3'), 2.60 (q, J=7.4 Hz, $SC\underline{H}_2CH3$), 2.73 (t, J=6.6 Hz, 2H4'), 3.67 (s, OCH_3), 4.31 (t, $OCH_2C\underline{H}$-FMoc), 4.40 (t, J=5.5 Hz, H2'), 4.45-4.61 (m, $OC\underline{H}_2CH$-Fmoc), 4.67 (d, J=11 Hz, A part of AB, 1H, OCH_2S), 4.87 (d, J=11 Hz, B part of AB, 1H, OCH_2S), 7.06-7.81 (13H, ArH, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 15.01, 25.29, 26.55, 32.99, 47.48, 52.37, 67.19, 73.04, 73.90, 120.26, 124.78, 125.36, 127.36, 127.51, 128.00, 129.85,

135.87, 141.58, 144.04, 144.12, 154.43, 172.64. FAB MS m/z (assignment): 506 (MH^+), 505 (M^+), 444 [$(M\text{-}SEt)^+$].

Synthesis of methylthioethyl ether (R)-53

Compound (R)-**52** (1.20 g, 4.2 mmol), dimethylboron bromide (1.5 M, 8.9 mL, 14.7 mmol) and DIPEA (0.05 mL, 0.42 mmol) were first added, followed by more DIPEA (1.6 mL, 8.4 mmol) and ethanethiol (1.1 mL, 12.6 mmol) as described in the genera procedure. Pure compound (R)-**53** was isolated as a light yellow oil after flash column chromatography using 3:1Hex/EtOAc as the solvent system. The average yield of this reaction was 55-60%, based on the amount of recovered alcohol (R)-**34**. TLC (5:1 hex/EtOAc): Rf=0.28. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.30 (t, J=7.5 Hz, $SCH_2C\underline{H_3}$), 2.05-2.18 (m, H3'), 2.52-2.54 (q, J=7.5 Hz, $SC\underline{H_2}CH_3$), 3.01 (t, J=8.1 Hz, H4'), 3.76 (s, OCH_3), 4.37 (dd, J=3.2, 7.5 Hz, H2'), 4.66 (d, J=11.7 Hz, A part of AB, 1H, OCH_2S), 4.92 (d, J=11.7 Hz, B part of AB, 1H, OCH_2S), 7.32-7.58 (m, 4H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.99, 25.18,29.05, 33.56, 52.30, 72.84, 74.65, 125.06, 127.57, 132.27, 133.28, 136.51, 149.55, 172.60. FAB MS m/z (assignment): 336 ($M+Na^+$), 320 ($M+Li^+$), 314 ($M+H^+$).

General procedure for the preparation of the thymine coupled compound

The methoxymethylthioethyl ether compound (1 eq.) was dissolved in dry THF (2 mL/mmol methoxythioethyl ether) in the presence of activated molecular sieves (3Å). A solution of bis-(trimethylsilyl)thymine (5 eq.) was added, followed by the addition of I_2 (1 eq.), and the reaction mixture was stirred at RT under N_2 for 48 h. The mixture was then poured into a 5% aqueous sodium sulfite solution, extracted with EtOAc and the organic layer was washed with H_2O and saturated NaCl. The organic layer was then dried with anhydrous $MgSO_4$ and concentrated.

Synthesis of thymine analog (S)-49

(S)-**48** (218 mg, 0.43 mmol), bis(trimethylsilyl)thymine (1.4 mL/1.5 M in dry THF and I2 (109 mg, 0.43 mmol) were reacted as described in the general procedure Purification by flash column chromatography, using 1:2 hex/EtOAc as the elutin solvent, led to the isolation of the desired product in 58% yield (98% based o recovery of starting material). TLC (1:2 hex/EtOAc): Rf= 0.25. ^{1}H NMR (270 MHz $CDCl_3$) δ: 1.84 (s, CH_3), 2.00-2.18 (m, 2H3'), 2.61-2.74 (m, 2H4'), 3.65 (s, OCH_3) 4.20 (dd, J= 5.2 Hz, H2'), 4.29 (t, J=9 Hz, -C<u>H</u>CH_2- of Fmoc), 4.58-4.68 (m, $CHCH_2$- of Fmoc), 4.90 (d, J=12 Hz, A part of AB, 1H, OCH_2Th), 5.13 (d, J=12 Hz B part of AB, 1H, OCH_2Th), 6.9-8.3 (14H, Ar, NH, H6). ^{13}C NMR (75 MHz, $CDCl_3$ δ: 12.46, 26.31, 32.81, 47.50, 52.55, 66.83, 76.38, 77.42, 111.87, 120.26, 125.21 125.23, 127.31, 127.39, 127.57, 128.03, 129.81, 135.66, 139.58, 141.56, 143.89 144.03. MALDI MS m/z (assignment): 592 [$(M+Na)^+$].

Synthesis of thymine analog (R)-54

(R)-**53** (400 mg, 1.26 mmol), bis(trimethylsilyl)thymine (4.2 mL, 1.5 M in dry THF and I_2 (319 mg, 1.26 mmol) were reacted as described in the general procedure Purification by flash column chromatography, using 1:5 hex/EtOAc as the eluting solvent, led to the isolation of the desired product in 60% yield (94% based or recovery of starting material). TLC (1:5 hex/EtOAc): Rf= 0.26. ^{1}H NMR (270 MHz $CDCl_3$) δ: 1.92 (s, CH_3), 2.01-2.27 (m, H3'), 2.93 (t, J=7.4 Hz, H4'), 3.71 (s, OCH_3) 4.22 (m, H2'), 5.07 (d, J=11 Hz, A part of AB, 1H, OCH_2Th), 5.31 (d, J=11 Hz, B part of AB, 1H, OCH_2Th), 7.17-7.89 (m, 5H, H6, Ar). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.34, 28.57, 33.19, 52.39, 76.29, 76.79, 111.86, 124.92, 127.52, 132.13, 133.17, 135.81, 139.50, 149.36, 151.40, 164.22, 171.93. MS EI m/z (assignment): 347 (M^+), 348 ($M+H^+$).

Synthesis of aniline monomer (R)-55

(R)-**54** (1.0 g) was dissolved in MeOH (100 mL) and and the nitro group wa reduced by hydrogenation with H_2 gas (3 atm) in the presence of 10% Pd/C (100mg) in MeOH for 15 h. The solution was filtered through celite and concentrated to give 90% yield of the desired product as a light yellow oil. $[\alpha]_D$ +18 (c 0.2, MeOH). NMR data as compound (S)-**33**.

Preparation of aniline analog (S)-50

Compound (S)-**49** (460 mg, 0.81mmol) was treated with 5% piperidine in DMF (13 mL) at R.T. for 20 min. The DMF was then removed by evaporation, and the crude mixture was partitioned between H_20 (10 mL) and EtOAc (20 mL). The aqueous layer was extracted with more EtOAc (3x20 mL), the combined organic layers were dried with anhydrous $MgSO_4$ and concentrated to give a light yellow oil. Purification by flash column chromatography using 5:1 EtOAc/Hex afforded compound (S)-**50** in 98% yeild. $[\alpha]_D$ -37 (c 0.2, MeOH). TLC (5:1 hex/EtOAc): Rf= 0.20. ^1H NMR (300 MHz, CD_3OD) δ: 1.87 (d, J=1.2 Hz, Th-CH_3), 1.83-2.13 (m, 2H3'), 2.59 (t, J=7.5 Hz, 2H4'), 3.68 (s, OCH_3), 4.17 (dd, J=3.6, 8.4 Hz, H2'), 5.14 (d, A part of AB, J=11.1 Hz, OCH_2Th), 5.29 (d, B part of AB, J=11.1 Hz, 1H, OCH_2Th), 6.59 (dt, J=7.5, 1.2 1H, Ar), 6.68 (dd, J=8.1, 1.2 Hz, 1H, Ar), 6.89 (dd, J=7.2, 1.2 Hz, 1H, Ar), 6.94 (dt, J=8.1, 1.2 Hz, 1H, Ar), 7.48 (q, J=1.2 H6). ^{13}C NMR (75 MHz, CD_3OD) δ: 12.36, 27.55, 32.96, 52.73, 77.80, 77.86, 112.05, 117.29, 119.51, 126.27, 128.36, 130.63, 142.31, 146.40, 153.38, 166.85, 174.48. MS EI *m/z* (assignment): 347 (M^+), 348 (MH^+).

Preparation of Boc protected analog (S)-57

Compound (S)-**50** (52 mg, 0.15 mmol) was dissolved in 10% triethylamine in MeOH (1mL) and mixed with ditertbutyldicarbonate (66 mg, 0.30 mmol). The mixture was stirred at RT for 17 h. The solvent was then evaporated and the residue was partitioned between H_2O (10 mL) and EtOAc (20 mL). The aqueous layer was extracted with more EtOAc (3x20 mL), the combined organic layers were dried with anhydrous $MgSO_4$ and concentrated to give the Boc-protected analog as a light yellow foam with a 92% yield and 98-99% ee. The enantiomeric purity of (S)-**57** was determined by HPLC using a chiralcel OD column with 10% ethanol in hexane as the eluting solvent and a flow rate of 1ml/min. Under these conditions, base-line separation of the two enantiomers was observed with a reference sample containing both enantiomers; the retention time for the S- and R-enantiomer is 23.8 and 27.7 min respectively. $[\alpha]_D$ -24 (c 0.40, MeOH). TLC (3:1 EtOAc/Hex): Rf= 0.30. ^1H NMR (300 MHz, CD_3OD) δ: 1.58 (9H, tBoc), 1.95 (Th-CH_3), 1.94-2.16 (m, 2H3'), 2.67-2.74 (m, 2H4'), 3.75 (s, OCH_3), 4.30 (dd, J=6 Hz, H2'), 5.18 (d, A part of AB, J=10.5 Hz, 1H, $0CH_2Th$), 5.39 (d, B part of AB, J=10.5 Hz, 1H, OCH_2Th), 7.04-7.27 (m, 5H, ArH, H6), 9.35 (s, NHBoc). ^{13}C NMR (75 MHz, CD_3OD) δ: 12.51, 26.44, 28.59, 32.66, 52.52, 76.44, 77.44, 80.68, 112.02, 122.97, 124.41, 127.38, 129.59, 131.09, 136.14, 139.52, 151.52, 153.69, 164.20, 172.12.

Preparation of the free acid analog (S)-58

To a solution of compound **(S)-57** (105 mg, 0.234 mmol) in 3:1 THF/MeOH (5 mL), aqueous LiOH (250 mL, 1.4 M) was added and the reaction mixture was stirred at R.T. for 3 h. The mixture was subsequently evaporated to dryness and the resulting residue was dissolved in H_2O (10 mL) at pH 3 and extracted with EtOAc (3x20 mL). The organic layer was dried with anhydrous $MgSO_4$ and concentrated to give a

fairly pure sample of the free acid analog (S)-**58** in 94% yield. $[\alpha]_D$ -15 (c 0.37 MeOH). TLC on C_{18}-silica (2:1 MeOH/H_2O): Rf = 0.53. ^1H NMR (300 MHz CD_3OD) δ: 1.50 (s, $C(CH_3)_3$), 1.88 (d, J=1.2 Hz, Th-CH_3), 1.86-2.14 (m, 2H3') 2.62-2.81 (m, 2H4'), 4.10-4.15 (m, H2'), 5.14 (d, A part of AB, J=10.5 Hz, 1H OCH_2Th), 5.39 (d, B part of AB, J=10.5 Hz, 1H, OCH_2Th), 7.05-7.32 (m, 4H, ArH) 7.48 (d, J=1.2 Hz, H6), 8.21 (s, NH). ^{13}C NMR (75 MHz, CD_3OD) δ: 12.40, 28.28 28.87, 34.11, 77.69, 77.86, 81.11, 111.93, 126.94, 127.49, 127.96, 130.91, 136.92 137.30, 142.33, 153.29, 156.83, 166.84, 175.60.

Synthesis of dimer 67

Free acid monomer (S)-**58** (96 mg in 2 mL of dry DMF, 0.22mmol) was added to a solution of the HATU coupling reagent (101 mg in dry DMF, 0.27 mmol) at 0 °C under N_2. Diisopropylethylamine (80 mL, 0.44mmol) was added and the reaction was allowed to stir for 10 min. The free aniline monomer (S)-**50** (100 mg in 1 mL dry DMF, 0.29 mmol) was then added via a syringe and the resulting solution was stirred at RT for 24 h. The reaction was quenched by diluting with H_2O (10 mL) and the product was extracted with EtOAc (3x20 mL). Purification *via* C_{18} reversed phase chromatography using a solvent gradient from 100% H_2O to 100% MeOH led to the isolation of the desired product in 70% yield as a pale yellow solid (eluted from column in ~55% aqueous MeOH). $[\alpha]_D$ -24 (c 0.40, MeOH). TLC on C_{18}-silica (1:1 MeOH/H_2O): Rf = 0.35. ^1H NMR (300 MHz, CD_3OD) δ: 1.47 (s, $C(CH_3)_3$), 1.84 (d, J=1.5 Hz, Th-CH_3), 1.88 (d, J=1.5 Hz, Th-CH_3), 1.88-2.18 (2m, 4H3'), 2.6-2.8 (2m, 4H4'), 3.60 (s, OCH_3), 4.16-4.28 (m, 2H2'), 5.08 (d, A part of AB, J=10.5 Hz, 1H, $0CH_2Th$), 5.17 (d, B part of AB, J=10.5 Hz, 1H, OCH_2Th), 5.27 (d, A part of AB, J=10.2 Hz, 1H, OCH_2Th), 5.35 (d, B part of AB, J=10.2 Hz, 1H, OCH_2Th), 7.0-7.35 (8H, m, ArH), 7.38 (q, J=1.5 Hz, H6), 7.57 (q, J=1.5 Hz, H6), 7.9

(s, NH). ^{13}C NMR (300 MHz, CD_3OD) δ: 12.37, 12.24, 28.17, 28.26, 28.90, 34.38 34.81, 52.81, 78.04, 78.18, 78.30, 79.71, 81.18, 111.95, 112.17, 127.06, 127.54 127.93, 128.03, 128.14, 128.18, 131.03, 131.08, 142.31, 153.32, 156.89, 166.78 173.52, 174.16, MALDI MS m/z (assignment): 786 [$(M+Na)^+$].

Preparation of free acid dimer 68

Hydrolysis of methylester dimer **67** to the corresponding free acid **68** (~99% yield) was carried out using the same reaction conditions as in the preparation of the free acid analog (S)-**58**. $[\alpha]_D$ -25 (c 0.25, MeOH). TLC on C_{18}-silica (2:1 MeOH/H_2O): Rf = 0.38. ^1H NMR (300 MHz, CD_3OD) δ: 1.47 (s, $C(CH_3)_3$), 1.84 (bs, Th-CH_3), 1.88 (bs, Th-CH_3), 2.00-2.15 (m, 4H3'), 2.60-2.85 (m, 4H4'), 4.16-4.25 (m, 2H2'), 5.08 (d, A part of AB, J=11.1 Hz, 1H, OCH_2Th), 5.20 (d, B part of AB, J=11.1 Hz, 1H, OCH_2Th), 5.26 (d, A part of AB, J=10.2 Hz, 1H, OCH_2Th), 5.33 (d, B part of AB, J=10.2 Hz, 1H, OCH_2Th), 7.05-7.35 (8H, m, Ar), 7.38 (bs, H6). 7.55 (bs, H6), 8.25 (s, NH). ^{13}C NMR (75 MHz, CD_3OD) δ: 12.42, 12.45, 24.37, 28.10, 28.49, 28.90, 30.83, 34.54, 34.74, 77.92, 78.17, 79.66, 81.18, 111.86, 112.16, 127.06, 127.52, 127.85, 128.00, 128.08, 128.17, 131.00, 131.20, 136.00, 137.09, 137.33, 137.70, 142.37, 153.29, 156.90, 166.70, 173.54, 175.73, 210.21. FAB MS m/z (assignment): 749 (M^+).

Preparation of free aniline dimer 69

Boc-methylester dimer **67** (53 mg, 0.07 mmol) was dissolved in an anhydrous solution of 15 % trifluoroacetic acid in CH_2Cl_2 (2 mL) and stirred at R.T. under N_2 for 15 min. The reaction mixture was subsequently evaporated to dryness and partitioned by chromatography on a dianion HP20 column using a solvent gradient from

100% H_2O to 100% MeOH. The aniline dimer **69** eluted from the column with 50-60% aqueous MeOH; 40% yield of pure **69** was isolated, however, some unreacted starting material was detected by TLC. $[\alpha]_D$ -43 (c 0.17, MeOH). TLC on C_{18}-silica (2:1 MeOH/H_2O): Rf = 0.30. ^1H NMR (300 MHz, CD_3OD) δ: 1.82 (d, J= 1.2 Hz, Th-CH_3), 1.87 (d, J=1.2 Hz, Th-CH_3), 1.9-2.21 (m, 4H3'), 2.56-2.80 (m, 4H4'), 3.58 (s, OCH_3), 4.20 (dd, J=3.9, 8.1 Hz, H2'), 4.24 (dd, J=3.9, 8.1 Hz, H2'), 5.07 (d, A part of AB, J=10.8 Hz, 1H, OCH_2Th), 5.17 (d, B part of AB, J=10.8 Hz, 1H, OCH_2Th), 5.27 (d, A part of AB, J=10.2 Hz, 1H, OCH_2Th), 5.34 (d, B part of AB, J=10.2 Hz, 1H, OCH_2Th), 6.62 (dt, J=7.5, 1.2 Hz, 1H, Ar), 6.70 (dd, J=7.2, 1.2 Hz, 1H, Ar), 6.91-7.00 (m, 2H, Ar), 7.17-7.23 (m, 3H, Ar), 7.29-7.30 (m, 1H, Ar), 7.32 (q, J= 1.2 Hz, H6), 7.50 (q, J=1.2 Hz, H6). ^{13}C NMR (75 MHz, CD_3OD) δ: 12.38, 12.42, 27.92, 28.34, 33.77, 34.44, 52.81,78.04, 78.27, 78.38, 79.80, 111.96, 112.19, 117.29, 119.60, 126.67, 128.05, 128.17, 128.26, 128.42, 130.75, 131.10, 136.01, 137.67, 142.31, 146.49, 153.32, 153.35, 166.81, 173.78, 174.15. FAB MS m/z (assignment): 663 (M^+).

Sythesis of Tetramer 70

The peptide coupling reaction between dimers **68** and **69** was achieved using the same reaction procedure as in the coupling of their respective monomers. The crude product was purified by C_{18} reversed phase flash column chromatography using a gradient from 100% H_2O to 100% MeOH. The desired APNA tetramer **70** eluted with 70-80% aqueous MeOH. $[\alpha]_D$ -31 (c 0.08, MeOH). TLC on C_{18}-silica (2:1 MeOH/H_2O): Rf = 0.13. NMR data in Table 2. ES MS m/z (assignment): 1392.6 (M^+), 1393.6 (MH^+)

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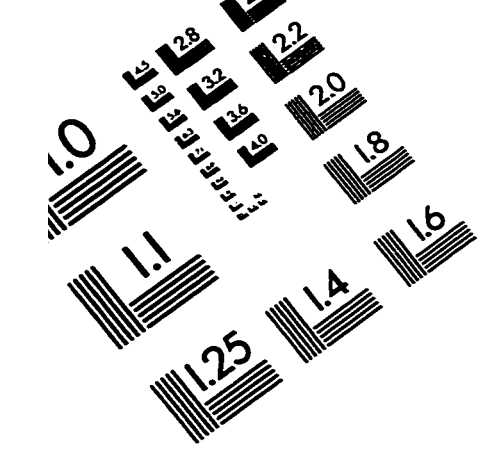

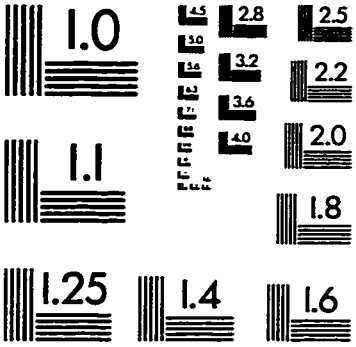

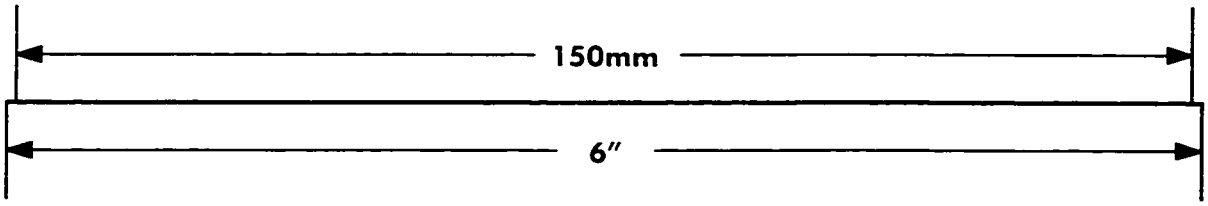

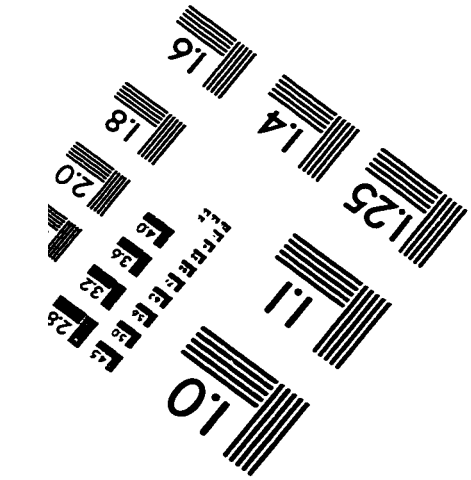

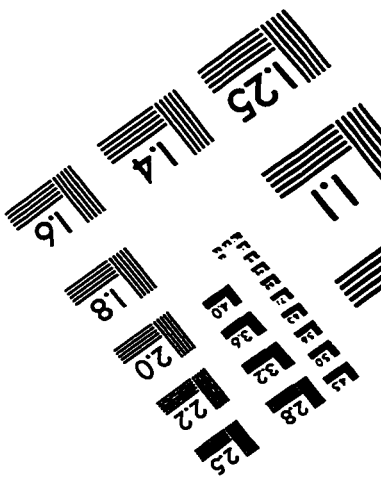

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