INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA

UMI®
800-521-0600

Synthesis of Aromatic Peptide Nucleic Acids

Michael J. Boyd

A Thesis

in

The Department

of

Chemistry and Biochemistry

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

July 1999

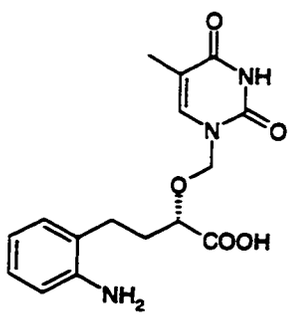
ABSTRACT

Synthesis of Aromatic Peptide Nucleic Acids

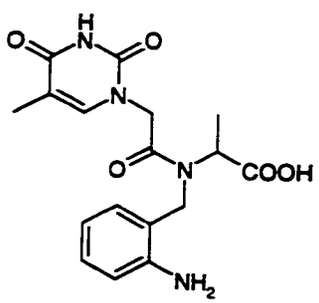
Michael J. Boyd

The synthesis of two analogues of Aromatic Peptide Nucleic Acids (APNAs) monomers was successfully achieved. These Peptide Nucleic Acid analogues could be used as building blocks for the synthesis of oligomers with the DNA and RNA recognition properties of PNAs in addition to a more hydrophobic backbone which may improve cell permeability. Such analogues may be useful as antisense/antigene therapeutic agents.

A first generation APNA hexamer, composed of monomers of general structure **A** was synthesized in solution. Thermal denaturation studies failed to indicate if these compounds hybridize to DNA or RNA. The second generation APNA monomers (**B**) were incorporated into PNA hexamers by solid phase peptide synthesis. The resulting PNA/APNA chimeras hybridize to both DNA and RNA by triple helix formation, however these complexes were found to be less stable than the unmodified PNAs.



A



B

ACKNOWLEDGMENTS

☺ My supervisor, Dr. Youla S. Tsantrizos: For all your advice, support and encouragement. You have, at countless times, gone beyond the call of duty to ensure a fulfilled graduate education. I appreciate it very much.

☺ Dr. Oswald S. Tee: The experience working for you as an undergraduate was both very fulfilling and enjoyable. You were a big contribution to my education. Thank you for all I have learnt from you and also for the use of a certain fumehood that I needed from time to time. I would also like to thank you for agreeing to serve on my graduate committee.

☺ Lee Fader: For technical advice and assistance, namely the hybridization studies, the occasional encouragement, amusing conversations and all those so memorable insults, which were all greatly appreciated.

☺ Dr. Lawrence Colebrook: For advice and assistance on the NMR instrument.

☺ Alexei Fedorchenko: For company during the night shifts.

☺ Sylvain Bordeleau and Serge Valois (Bio-Mega): For running mass spectra.

☺ Dr. Masad Damha: For advice and for agreeing to serve on my graduate committee.

☺ And in no particular order: Dirk Bandilla, Isabelle Turner, Paul Loncke, Delna Ghadiali, Angela Salinaro and Jennifer Yazbeck for friendship and for making the Concordia University Chemistry Department an enjoyable place to work.

to Cécile Sarrazin, who sparked the scientist in me.

and

to my parents for all their support and encouragement.

"Nothing great was ever achieved without enthusiasm."

Ralph Waldo Emerson.

"...humanity also needs dreamers, for whom the disinterested development of an enterprise is so captivating that it becomes impossible for them to devote their care to their own material profit."

Marie Curie.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES ix

LIST OF SCHEMES xi

ABBREVIATIONS xii

1. INTRODUCTION 1

1.1 DNA 1

1.2 RNA 5

1.3 Triple Helices 6

1.4 Genes 8

1.5 Transcription 9

1.6 Splicing 11

1.7 Translation 13

1.8 Antisense / Antigene Oligonucleotides 16

1.8.1 Choosing a Site 18

1.8.2 Hurdles and DNA Analogues 19

1.8.3 Ribonuclease H 22

1.9 The Melting Temperature 22

1.10 Peptide Nucleic Acids (PNAs) 23

1.10.1 Binding Affinity of PNAs 25

1.10.2 Structure of PNA/DNA and PNA/RNA Complexes 26

1.10.3 Antisense / Antigene potential of PNAs 27

1.11 Solid Phase Peptide Synthesis 28

1.11.1 Solid Supports and Linkers 30

1.11.2 Amino Protecting Groups 31

1.11.3 Carboxyl Activation used in SPPS 32

1.11.4 Monitoring Reactions on Solid Support 36

1.12 Research Project 38

2. RESULTS AND DISCUSSION 43

2.1 First Generation Aromatic Peptide Nucleic Acids 43

2.1.1 Synthesis of APNA Thymine Monomer 43

2.1.2 Synthesis and Properties of APNA Oligomers 50

2.2 Second Generation APNAs 54

2.2.1 Synthesis of the L- and D- Alanine Thymine Monomers 54

2.2.2 Synthesis of APNA Chimera Units 58

2.2.3 Synthesis of Boc Protected PNA Backbone 60

2.2.4 Synthesis of Fmoc Protected PNA Backbone 62

2.2.5 SPPS of APNA/PNA Chimeras 64

2.2.6 Preliminary Binding Studies of APNA/PNA Chimeras 67

3. CONCLUSIONS 71

4. EXPERIMENTAL 72

APPENDICES 105

REFERENCES 119

LIST OF FIGURES

FIGURE 1. DNA 1

FIGURE 2. Watson-Crick Base Pairing 2

FIGURE 3. DNA Conformations 4

FIGURE 4. a) Uracil b) RNA Secondary Stucture 6

FIGURE 5. Triple Helix Formation 7

FIGURE 6. The Central Dogma of Molecular Genetics 9

FIGURE 7. Transcription 10

FIGURE 8. Splicing 1 12

FIGURE 9. Splicing 2 13

FIGURE 10. tRNA and Codons 14

FIGURE 11. Translation 15

FIGURE 12. Antisense and Antigene Inhibition 17

FIGURE 13. Base Analogues 19

FIGURE 14. DNA Analogues 20

FIGURE 15. Base Modifications 21

FIGURE 16. The Melting Temperature 23

FIGURE 17. Peptide Nucleic Acids 23

FIGURE 18. Glyceroside Analogues 25

FIGURE 19. Solid Phase Peptide Synthesis 29

FIGURE 20. Solid Supports and Linkers 30

FIGURE 21. Amino Protecting Groups 31

FIGURE 22. DCC Coupling and Racemization 33

FIGURE 23. Pfp and HOBt Esters 34

FIGURE 24. Stand Alone Coupling Reagebts 35

FIGURE 25. Tests for Amines 37

FIGURE 26. Aromatic Peptide Nucleic Acids (APNAs) 39

FIGURE 27. Aromatic Ring in the Backbone 40

FIGURE 28. Second Generation APNAs 41

FIGURE 29. Bacillus Stearothermopholis Lactate Dehydrogenase 45

FIGURE 30. Mechanism of BSLDH 46

FIGURE 31. Ester Interference 48

FIGURE 32. Protons Near a Conjugated System 52

FIGURE 33. Intramolecular Acyl Transfer 63

FIGURE 34. PNA / APNA Hexamer Synthesis 65

FIGURE 35. Melting Curves for the APNA / PNA Hexamers 70

TABLE 1. 53

LIST OF SCHEMES

SCHEME 1.	First Generation APNA Synthesis	44
SCHEME 2.	Methoxy Substitution	47
SCHEME 3.	First Generation APNA Synthesis Continued	49
SCHEME 4.	First Generation APNA Oligomer Synthesis	51
SCHEME 5.	Second Generation APNA Synthesis	55
SCHEME 6.	Reductive Amination	56
SCHEME 7.	Synthesis of PNA / APNA Chimeras	58
SCHEME 8.	Synthesis of PNA / APNA Chimeras Continued	59
SCHEME 9.	PNA Synthesis	60
SCHEME 10.	Synthesis of Boc Protected PNA Backbone 1	61
SCHEME 11.	Synthesis of Boc Protected PNA Backbone 2	62
SCHEME 12.	Synthesis of Fmoc Protected PNA Backbone 1	63
SCHEME 13.	Synthesis of Fmoc Protected PNA Backbone 2	64

ABBREVIATIONS

δ	Chemical shift
2-Cl-Z	2-Chlorobenzyloxycarbonyl
A	Absorbance
A	Adenine
AA	Amino acid
Abs	Absorbance
Ala	Alanine
APNA	Aromatic peptide nucleic acid
Ar	Aromatic
Arg	Arginine
atm	Atmosphere
B	Base
Boc	tert-butoxycarbonyl
BroP	(Bromo tris(dimethylamino) Phosphonium hexafluorophosphate
BSLDH	Bacillus Stearothermophols Lactatedehydrogenase
C	Cytosine
C^+	Protonated cytosine
d	Doublet
D	Dextrorotatory

d^2A/dT^2	Second derivative of function A with respect to T
DCC	Dicylclohexylcarboiimide
DCM	Dichloromethane
dd	doublet of doublets
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic Acids
eq	equivalent
Fmoc	9-Fluorenylmethoxycarbonyl
g	gram
G	Guanine
h	Hour
HATU	(O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
His	Histidine
HOBt	Hydroxybenztriazole
HPLC	High pressure liquid chromatography
hv	Radiation
J	Coupling constant
K	Kelvin

L	Leveorotatory
LDH	Lactate Dehydrogenase
Lys	Lysine
m	multiplet
M	Molar
MBHA	Methylbenzhydrylamine
mg	milligram
MHz	Megahertz
min	Minutes
ml	millilitre
mmol	millimole
MP	Melting point
mRNA	Messenger RNA
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide hydride
Nle	Norleucine
nm	Nanometer
NMP	N-methylpyrrolidine
NMR	Nuclear magnetic resonance
Nuc	Nucleophile
°C	degrees Celcius
Pfp	Pentafluorophenoxy

PG	Protecting group
Phe	Phenylalanine
PNA	Peptide Nucleic Acid
ppm	Parts per million
PS	Polystyrene
q	Quartet
RaNi	Raney Nickel
R_f	Rentention fraction
RNA	Ribonucleic Acids
RT	Room temperature
s	Singlet
Ser	Serine
snRNP	Small nuclear ribonucleoprotein
SPPS	Solid phase peptide synthesis
t	triplet
T	Temperature
T	Thymine
TEA	Triethylamine
TFA	Trifluoroacetic acid
TFMSA	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography

T_m	Melting temperature
TMS	Trimethylsilane
tRNA	Transfer RNA
Tyr	Tyrosine
U	Uracil
UV	Ultra-violet
$[\alpha]_D$	Optical rotation
Å	Angstrom

1. INTRODUCTION

1.1 DNA

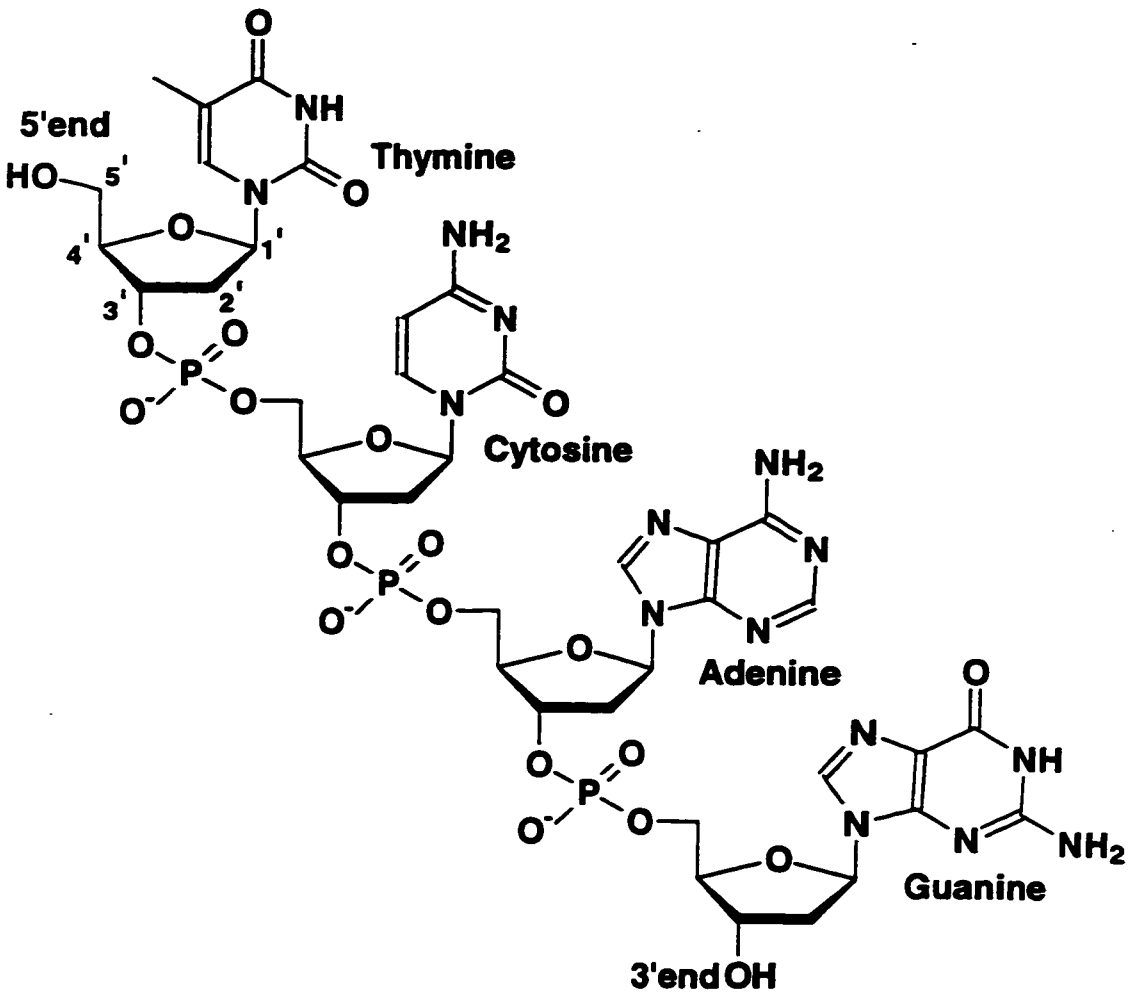


FIGURE 1: DNA

Deoxyribonucleic Acid (DNA) is the "blueprint" for the function and structure of each cell in every biological system. DNA is an oligomer made up of nucleosides interconnected by phosphodiester linkages (Figure 1), each nucleoside contains a sugar (2'-deoxy-D-ribose) and a base of which there are 4 kinds, two pyrimidine derivatives [Thymine (T) and Cytosine (C)] and two purine derivatives [Adenine (A)

and Guanine (G)]. The nucleosides are interconnected *via* a phosphodiester linkage through the 3' hydroxyl of one nucleoside to the 5' hydroxyl of the other. It is the sequence of nucleotide bases that is the code or script for biological systems.

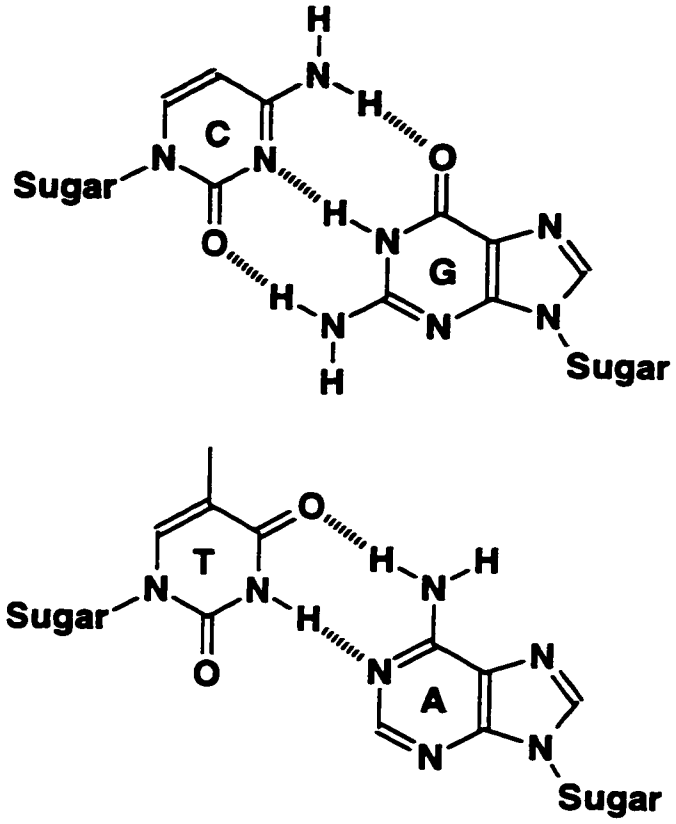


FIGURE 2: Watson-Crick Base Pairing

In most biological systems, these oligomers exist as dimers, by the formation of hydrogen bonds between the 4 bases as shown in Figure 2, where the two strands run in opposite directions (one strand runs from its 3'OH terminal to its 5'OH terminal and the other from its 5'OH terminal to its 3'OH terminal). Thymine shows

selective binding with Adenine *via* two hydrogen bonds and Cytosine shows selective binding with Guanine *via* 3 hydrogen bonds, this greater number of H bonds in the latter case is responsible for the stronger duplex formation in GC rich strands relative to AT rich strands. And the selectivity in both cases is known as Watson-Crick base pairing. The driving force for DNA complexation is associated with the formation of the H-bonds involved in Watson-Crick base pairing and interstrand π stacking interactions of the bases.

The main form of the DNA duplex, known as the B-form, is a right handed helix where the hydrophobic bases are stacked inside of the helix (avoiding water) nearly perpendicular to the helix axis (around 1° inclination relative to the helix axis) and offset to the right about 36° relative to each other [1, 2], this results in a complete right turn per 10 bases (34 Å) (Figure 3). The hydrophilic sugars and ionized phosphates point outside the helix (exposing them to water) perpendicular to the bases. Moreover the sugars are in a C2'-endo conformation and the N-sugar bond angle is in the anti configuration (Figure 3). The complete helix forms two distinct grooves of equal depth but of different width, the larger is known as the major groove and the smaller as the minor groove (Figure 3).

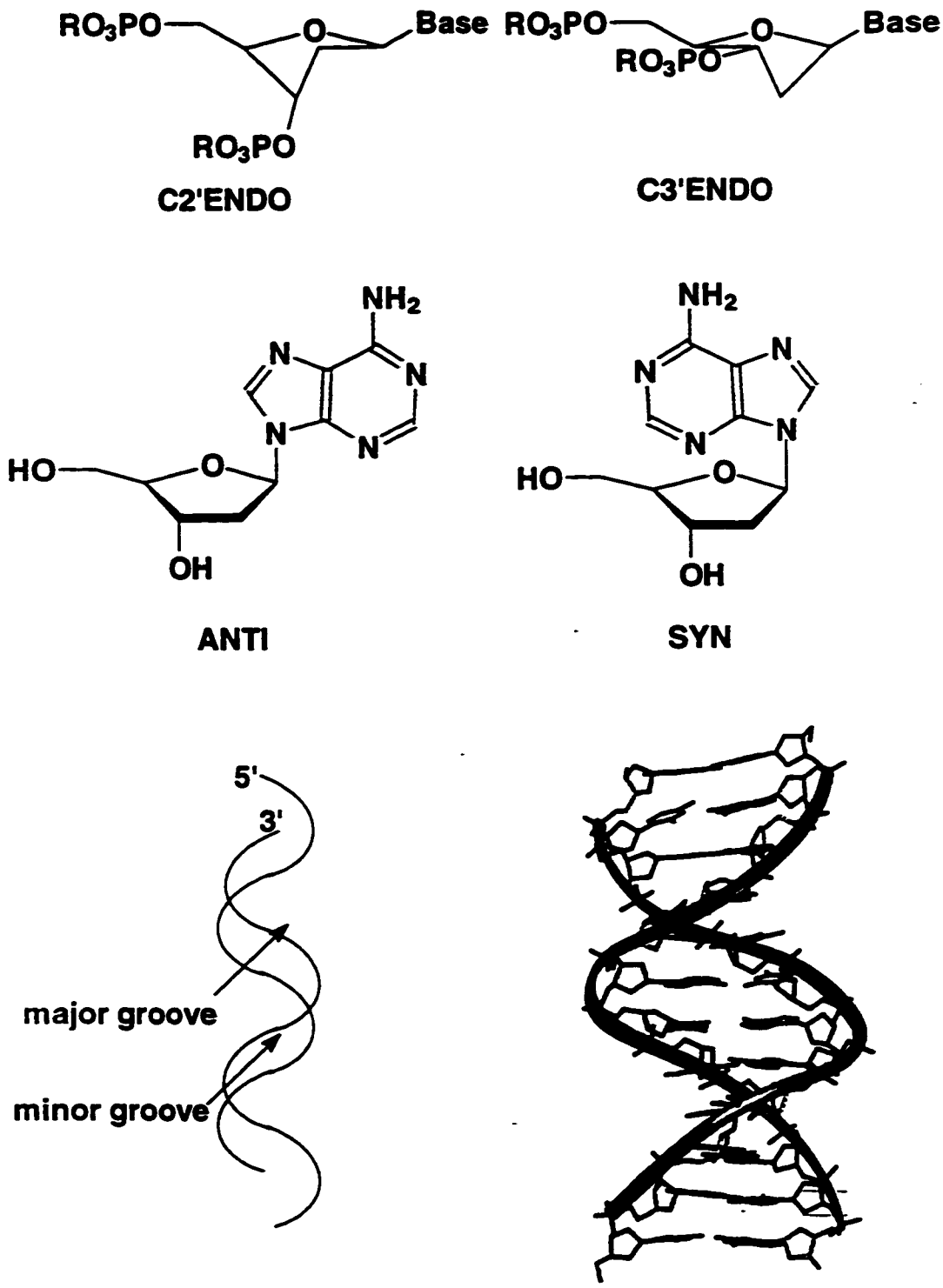


FIGURE 3: DNA Conformations

The B-form is the major form of DNA under physiological conditions, but, under different conditions DNA can exist in two other well characterized forms: the A and Z forms [1, 3]. The A-form exists in dryer systems (low humidity crystal structures), its main characteristics are that it adopts a 3'-endo sugar configuration rather than a 2'-endo as in the B-form and its major groove is narrow and deep while its minor groove is wide and shallow. The Z-form exists predominantly at very high ionic strength (3-4 M) with alternating purine-pyrimidine base sequences. Moreover, there is diversity in the structure of these duplexes depending on the base sequence [5], each nucleoside behaves a little differently depending on what other bases are above and below it, creating slight differences in the form of the helix in each particular environment.

1.2 RNA

There are three major differences between DNA and RNA: a) in RNA all thymine bases are replaced with uracil (Figure 4), b) the sugar is D-ribose (there is a OH on the 2'C unlike DNA) and c) RNA does not usually exist as a duplex although it has a secondary structure resulting in the formation of intra strand double helices with complementary portions of the single strand (Figure 4). In addition, RNA has tertiary structure dictated by non-Watson-Crick base pairing interactions [6, 7]. RNA-RNA duplexes form A-form helices with the sugar in the 3'-endo sugar configuration [9]. Secondary and tertiary structure determination of large strands of RNAs (particularly mRNA) is difficult due to their increased vulnerability to hydrolytic

cleavage relative to DNA and also the amount of material necessary for detailed studies [11].

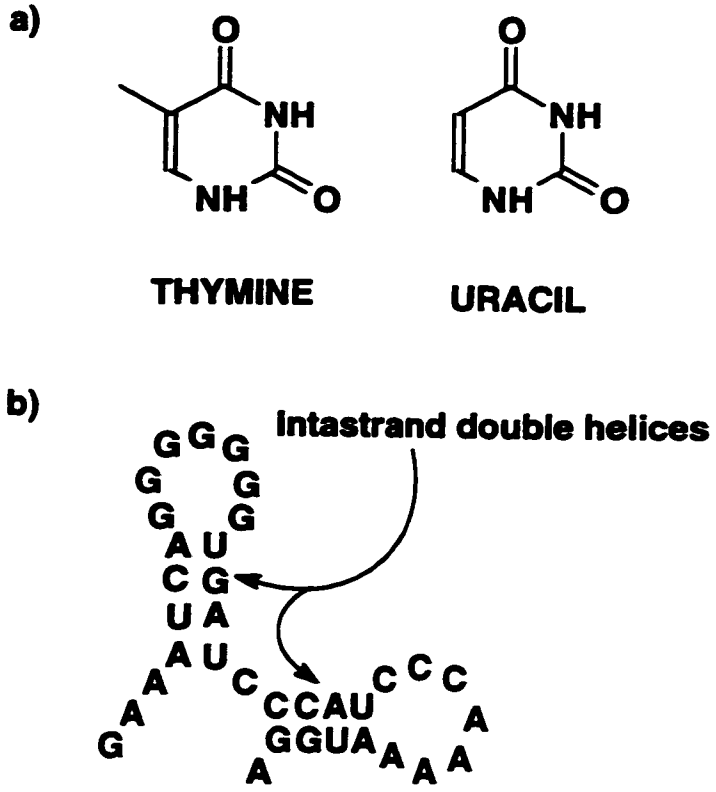
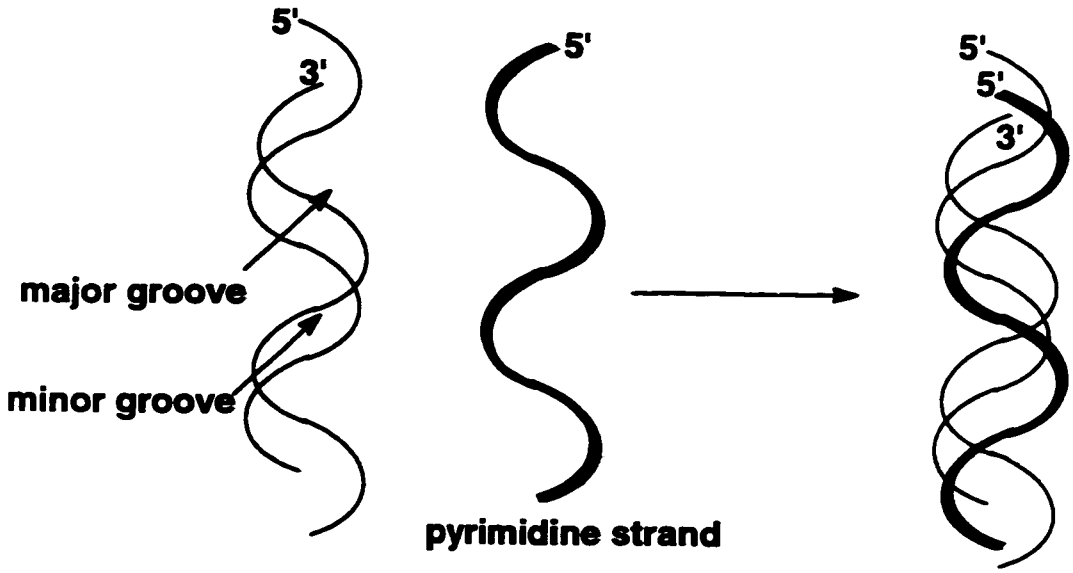


FIGURE 4: a) Uracil b) RNA Secondary Structure

1.3 Triple Helices

DNA and RNA strands can also form triple helices, where a third strand binds in the major grove of a double helix (Figure 5) [15]. These structures are formed in the presence of a purine rich stand and two pyrimidine rich stands, where the purine strand and a pyrimidine strand form a double helix *via* Watson-Crick base pairing, the third pyrimidine strand binds in the major groove antiparallel to the other



1 purine and 1 purimidine strand

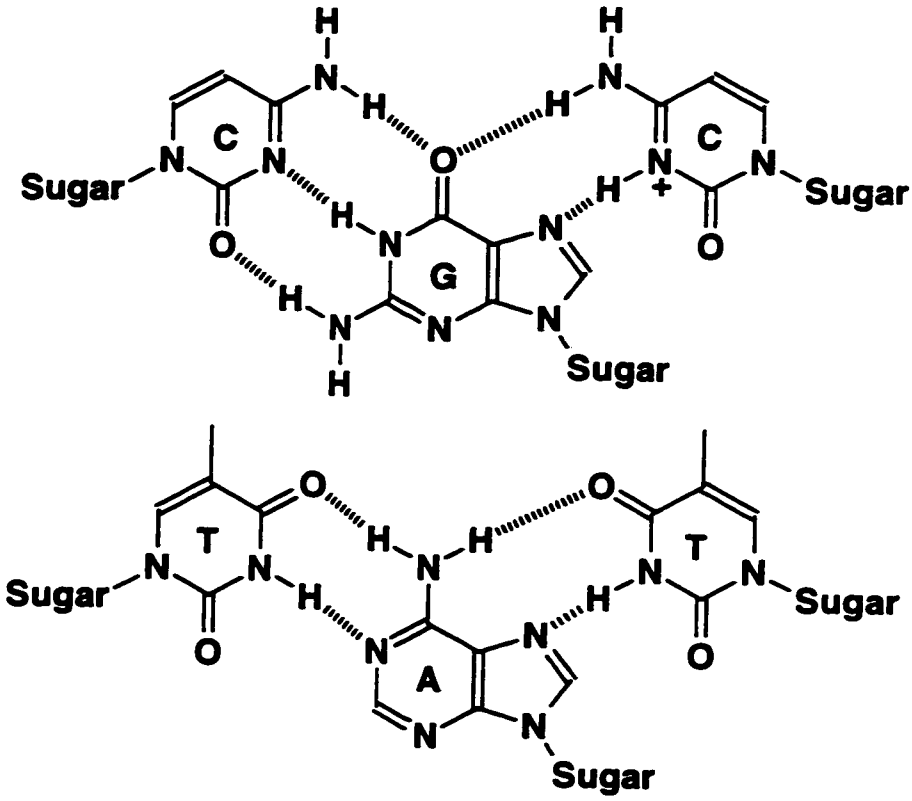


FIGURE 5: Triple Helix Formation

pyrimidine strand *via* a novel base pairing model, known as Hoogsteen base pairing (Figure 5) [16,17,18] where ATA and C^+GC triplets are formed. The C^+ indicates that the C must be protonated for binding to the double helix. Triple helices can also be composed of different combinations of RNA and DNA strands [12,21]. Most combinations of DNA and RNA can form triple helices, however some combinations are not observed [12,21].

1.4 Genes

Genes are long segments of DNA that contain the information or code for the formation of a biological product, such as proteins. The mechanism of the formation of proteins from the gene is known as the central dogma of molecular genetics (Figure 6). The gene is not directly responsible for the formation of the protein; the DNA is first expressed as mRNA through transcription. In eukaryotes the mRNA strand is known as the primary transcript and it contains the code for the formation of proteins but only in scattered segments. In the mRNA strands these segments are called introns and exons. Only the exons are relevant to protein biosynthesis. Through a process known as splicing these strands of mRNA are cleaved and the exons fused together to obtain a mature mRNA strand. This mRNA strand is subsequently used in translation of the genetic material into proteins. Therefore, in eukaryotes the information in genes is not continuous, since the genetic codes are interrupted through the expression of introns.

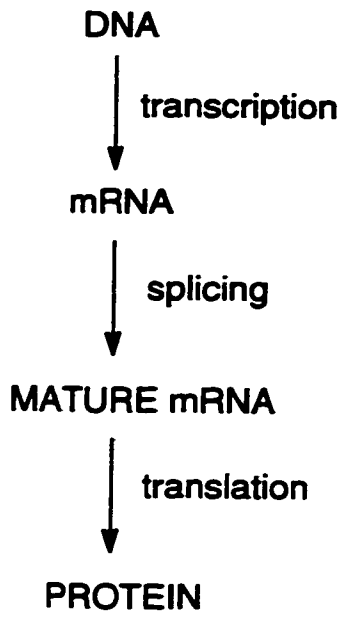


FIGURE 6: The Central Dogma of Molecular Genetics

1.5 Transcription

In transcription an enzyme system transforms the information stored in the double stranded DNA into mRNA (Figure 7) which is synthesized from its 5' end to its 3' end. An enzyme called RNA polymerase unwinds the DNA to form what is known as a transcription bubble which is about 17 base pairs at any given time. The 5'–3' DNA strand is called the non template strand and it has no direct function in transcription. The 3'–5' strand or the template strand is read by the polymerase and the complementary RNA strand is synthesized while forming a RNA-DNA double helix. As the enzyme winds the DNA the mRNA strand is extruded from the system and double stranded DNA is reformed.

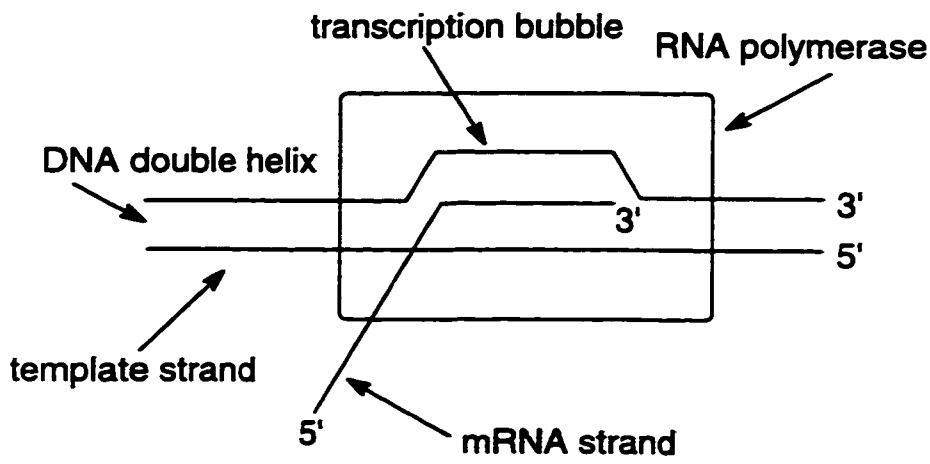


FIGURE 7: Transcription

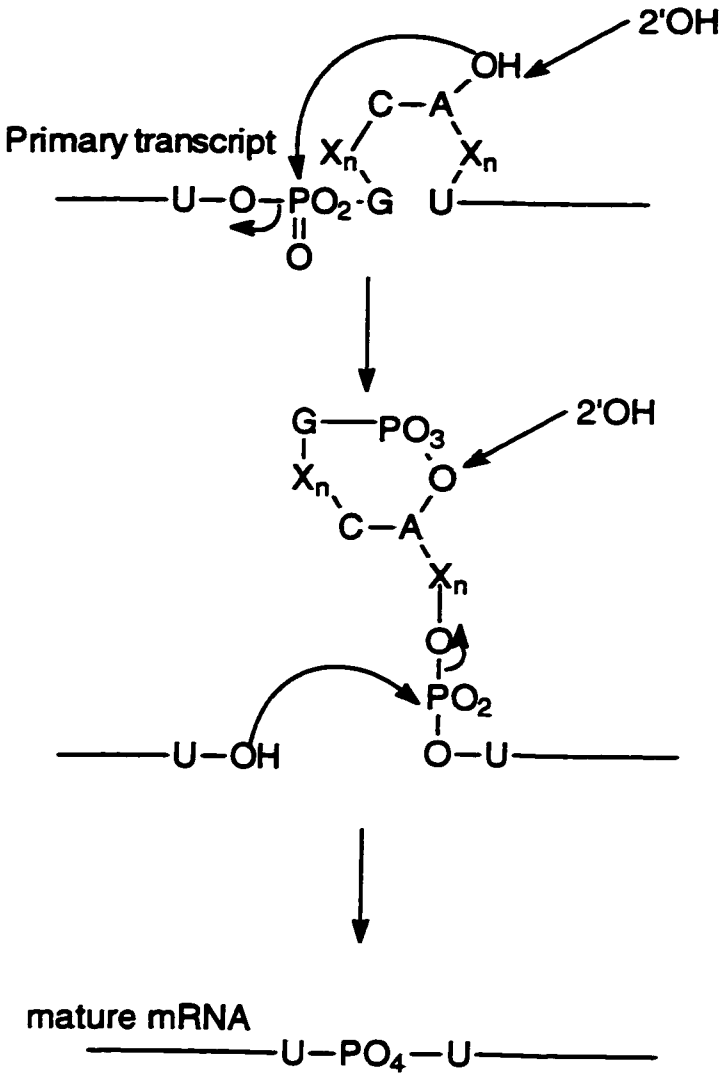
Transcription in bacteria is initiated by promoter small regions (6 base pairs long) on the template strand located at 10 and 35 base pairs away from where transcription begins; these regions are known as the -10 and -35 regions. The enzyme migrates to the -35 region and clamps on the DNA, it then moves to the -10 region and unwinds the DNA strand and exposes the template strand after which RNA synthesis begins. After a few base pairs have been made the enzyme loses a subunit known as the σ subunit, this unit is only needed for the recognition of the promoter site and not required for RNA synthesis.

In eukaryotic cells mRNA synthesis is more complex. mRNAs are made from RNA polymerase II. Many of the promoter sites have common features such as a binding site for proteins known a transcription factors. These proteins recognize regions (7 to 10 base pairs long) on DNA and guide binding of the polymerases to the promoter sites.

1.6 Splicing

As mentioned earlier splicing is the conversion of the primary transcript RNA into mature RNA by removal of unneeded introns. In the case of mRNA, two different mechanisms exist for the conversion of primary transcripts into mature mRNA. The first mechanism does not involve any enzymes or co-factors, the introns involved are known as self-splicing introns (Figure 8). In this mechanism an adenosine 2' OH attacks the phosphate in a transesterfication reaction releasing the exon 3' OH and forming a branched intron. The free 3' OH on the exon reacts with the phosphate between the intron and the other exon in another transesterfication reaction to obtain the "spliced RNA".

The other more common mechanism requires RNA-protein complexes called small nuclear ribonucleoproteins (snRNPs) (Figure 9). The mechanism is essentially the same as the self-slicing mechanism except that a snRNP binds to the primary transcript and defines the splice site. Another snRNP which has a complementary region minus a uracil to a another site on the primary transcript binds and forms a bulged adenosine which is now activated and attacks the splice site.



X_n= chain of no particular sequence or length

FIGURE 8: Splicing 1

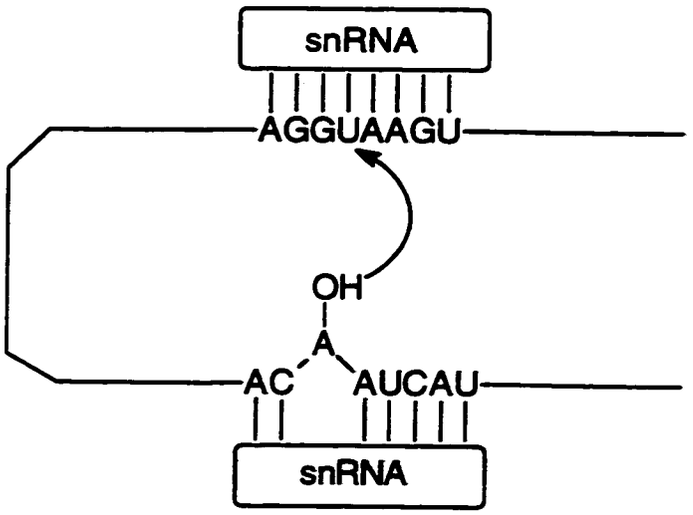


FIGURE 9: Splicing 2

1.7 Translation

Translation is the use of the genetic information expressed in mRNA to form proteins. This process involves 3 stages: Initiation, Elongation and Termination. The whole cycle is controlled by ribosomes and tRNA. In eukaryotic cells the ribosomes consist of 2 subunits called S60 and S40, in bacteria they are known as 50S and 30S. tRNAs are the carriers of the amino acids, there is one tRNA for every one of the 20 amino acids. Each of the tRNAs have 3 specific bases which form an anticodon which is complementary to a site on the mRNA known as the codon (Figure 10). For example the codon for phenylalanine is UUU, UCU for serine and UAU for tyrosine. Thus the mRNA codes for the protein using 3 bases on the mRNA for each amino acid in the sequence. These amino acids are

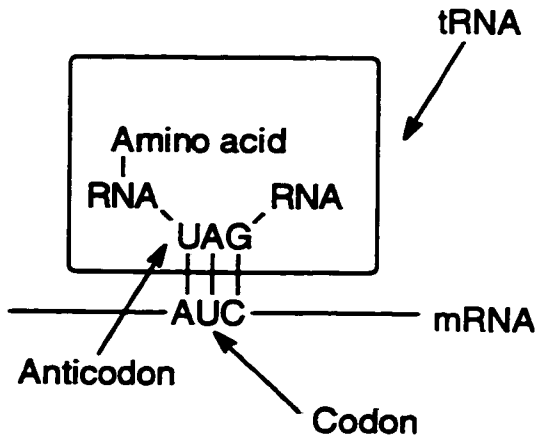


FIGURE 10: tRNA and Codons

connected from carboxyl terminal to amino terminal by reading of the mRNA by the ribosome and recognition of the codons by the amino acid carrying tRNAs. For example an mRNA portion containing UUUUCUUAU would code a protein containing phe-ser-tyr.

The translation process in bacteria is shown in Figure 11. First the 30S subunit is guided to an initiation codon (AUG) by recognition of a particular sequence 8 to 13 bases away from the translation site. The first tRNA then binds to the initiation codon and the 50S unit grips on to the chain, then the next tRNA binds and the amino acid is transferred from the first tRNA to the second, the process continues until the whole protein is made. The end of the synthesis is possible by the binding of a release factor which binds to the mRNA strand at a termination codon and the peptide is released.

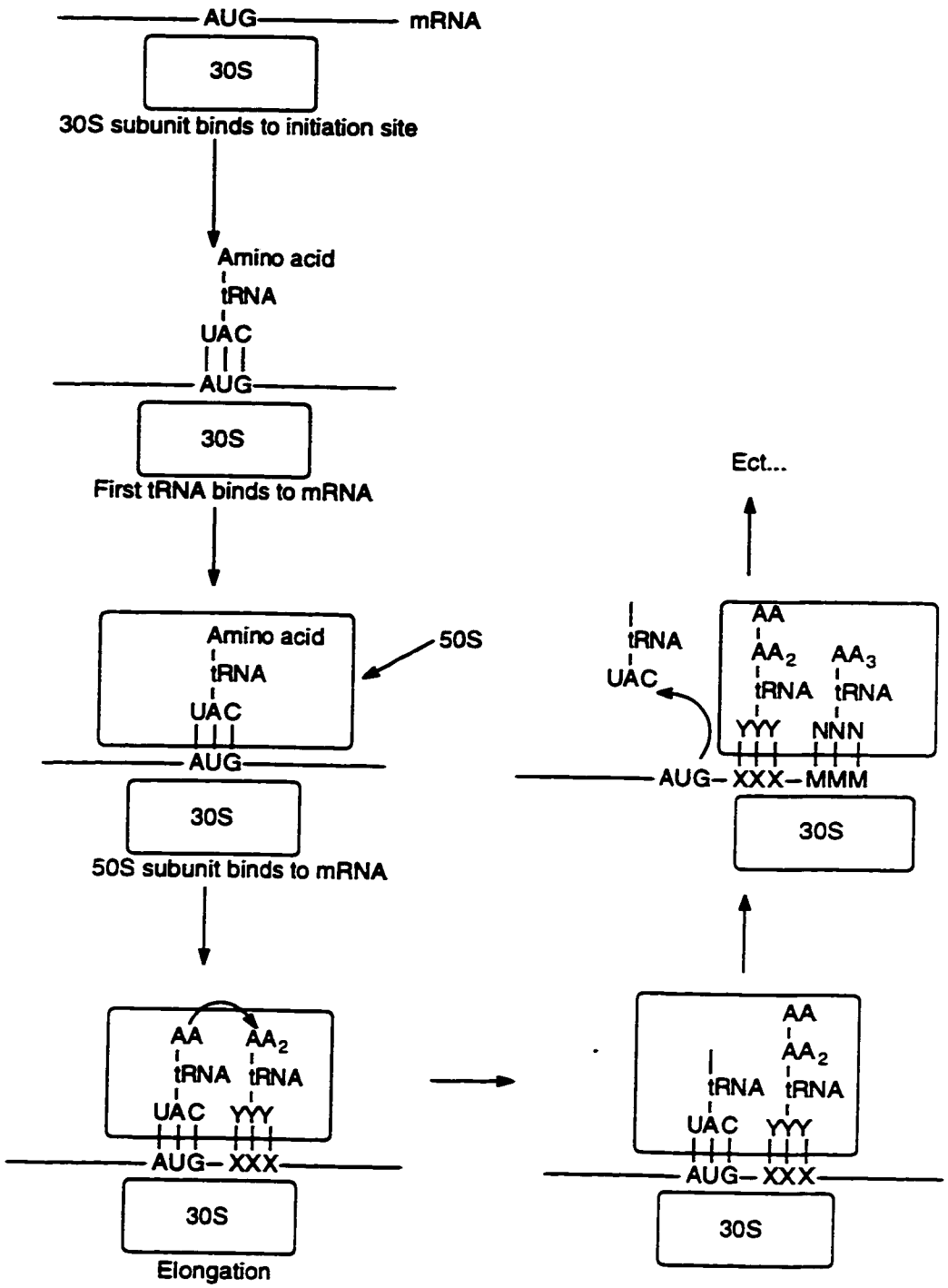


FIGURE 11: Translation

1.8 Antisense / Antigene Oligonucleotides

Modern drug development often requires the synthesis and testing of thousands of compounds before a lead compound is found. In many cases these compounds are targeted against proteins, in order to inhibit their biological role. However, intervention at the genetic level could be more effective by blocking the expression of the gene responsible for the biosynthesis of the protein. There are many drugs on the market today that do intervene at the genetic level, however they do so in a non specific manner. This often leads to serious side effects. A compound that could block the expression of any particular gene would be invaluable. One could selectively block the expression of any desired gene including oncogenes and viral genes. Compounds known as antisense and antigene agents could potentially be used for this purpose.

In 1978 Zamecnik and Stephenson [22] proposed the use of synthetic oligonucleotides as therapeutic agents. Instead of targeting gene products (enzymes or proteins) for inhibition, one can target the source of the gene product at the genetic level by inhibiting the expression of the gene. Theoretically, this can be done by inhibiting translation, transcription or splicing using oligonucleotides that can form complexes with DNA or RNA using the well understood rules of Watson-Crick or Hoosteen base pairing (Figure 12). When complexed these molecules can stericly block the biological machineries from performing their role. At the transcriptional level this can be accomplished by blocking RNA polymerase or transcription factors from binding to DNA by the formation of a triple helix with a

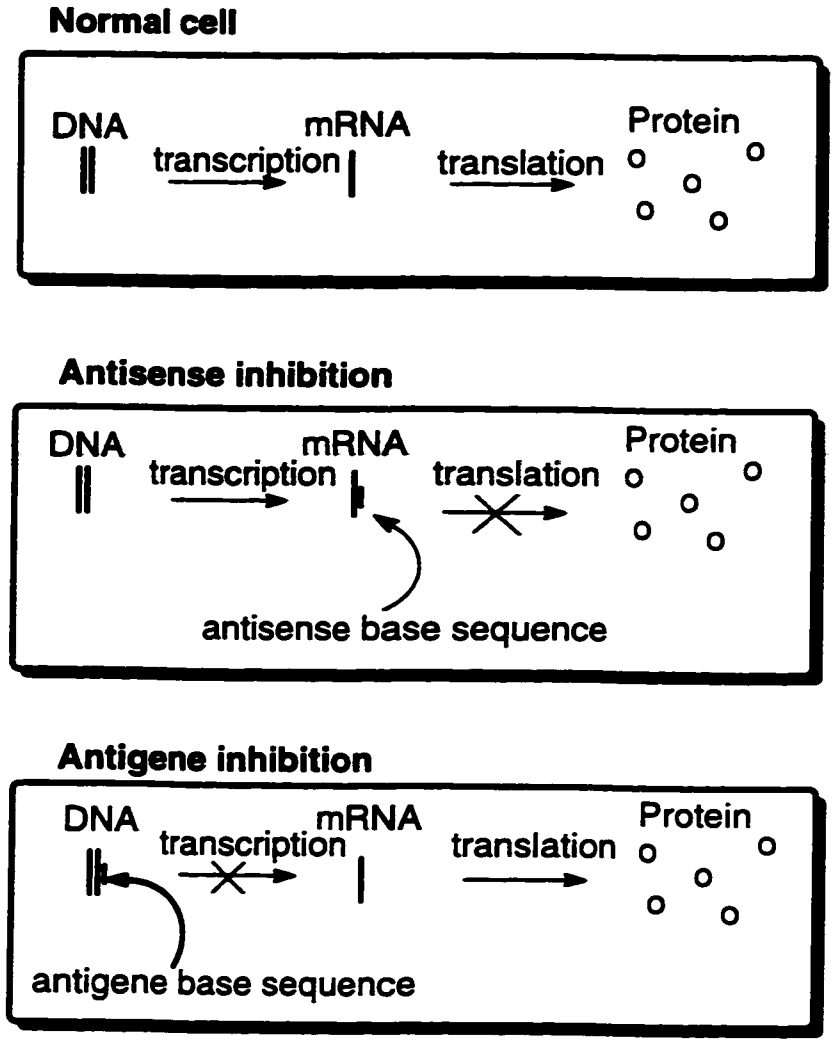


FIGURE 12: Antisense and Antigene Inhibition

synthetic oligonucleotide. This strategy is known as the antigene strategy. The other option is to block translation by formation of a double helix with a synthetic oligonucleotide and RNA. In this case, the ribosomal subunits are blocked from binding to the RNA initiation sites. This strategy is known as antisense.

The main advantage of these strategies is that in principle one can treat a

disease by simply targeting the genes responsible for the expression of the disease. Moreover, this can be done selectively by taking advantage of base pairing rules.

1.8.1 Choosing a Site

In blocking translation one can block initiation sites or sites in between initiation and termination. However, the most effective sites are the initiation sites [23,24] . Unfortunately mRNAs can form secondary structures making some sites inaccessible and it has been shown that this could have profound effects on the ability of the oligo to bind to its target [25]. Moreover the prediction of secondary structure in mRNA is difficult , making choice of a rational site challenging [27]. However, it has been shown that there is some correlation between binding strength of the oligo and inhibition of translation, indicating that the oligo may be able to compete to a certain extent with the secondary structure of mRNA [28,30]. Splicing can also be blocked by blockage of snRNA binding sites [31-33]. It has also been shown *in vitro* that transcription can be blocked by oligos that bind to the RNA polymerase initiation sites on DNA [34-36]. One possible problem in the antigene strategy is that a homopurine site must be chosen; in this case pyrimidine interruptions in the target would weaken binding. In order to overcome this problem, base derivatives have been made that can deal with these interruptions (Figure 13) [37-40]. Finally, it has also been shown that antigene and antisense oligos can be effective in vivo [41-43].

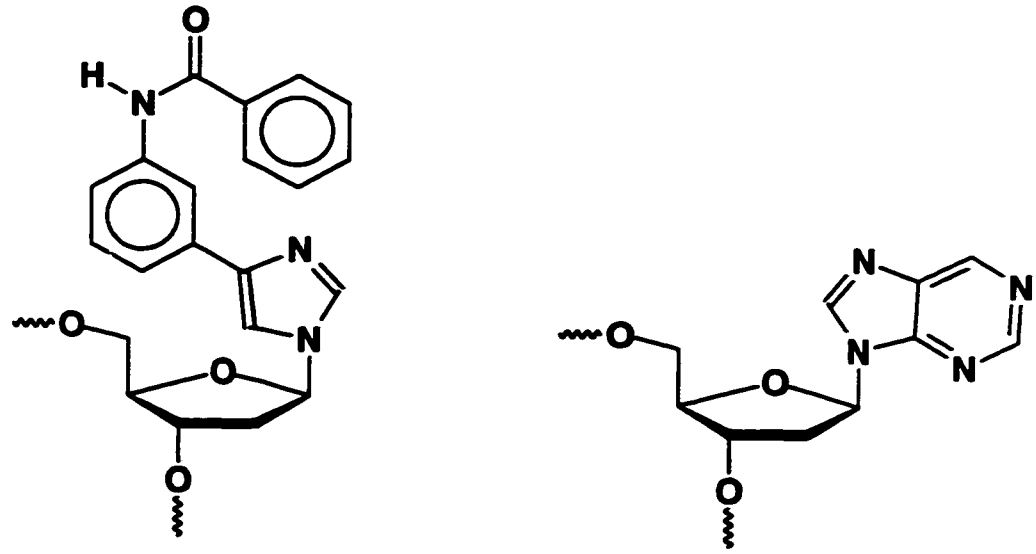


FIGURE 13: Base Analogues

1.8.2 Hurdles and DNA Analogues

There are several hurdles that must be overcome in order to achieve a practical therapeutic application of an oligunucleotide [44]. These compounds must be stable *in vivo* conditions and they must penetrate the cell membrane. They must also bind strongly but selectively to the target RNA or DNA. Moreover, some studies must be done in the determination of mRNA structure to facilitate site selection. Finally, these oligos must be technically feasible (easy to prepare). The latter has been facilitated by modern methodologies in DNA synthesis and the development of the gene machine [45,46].

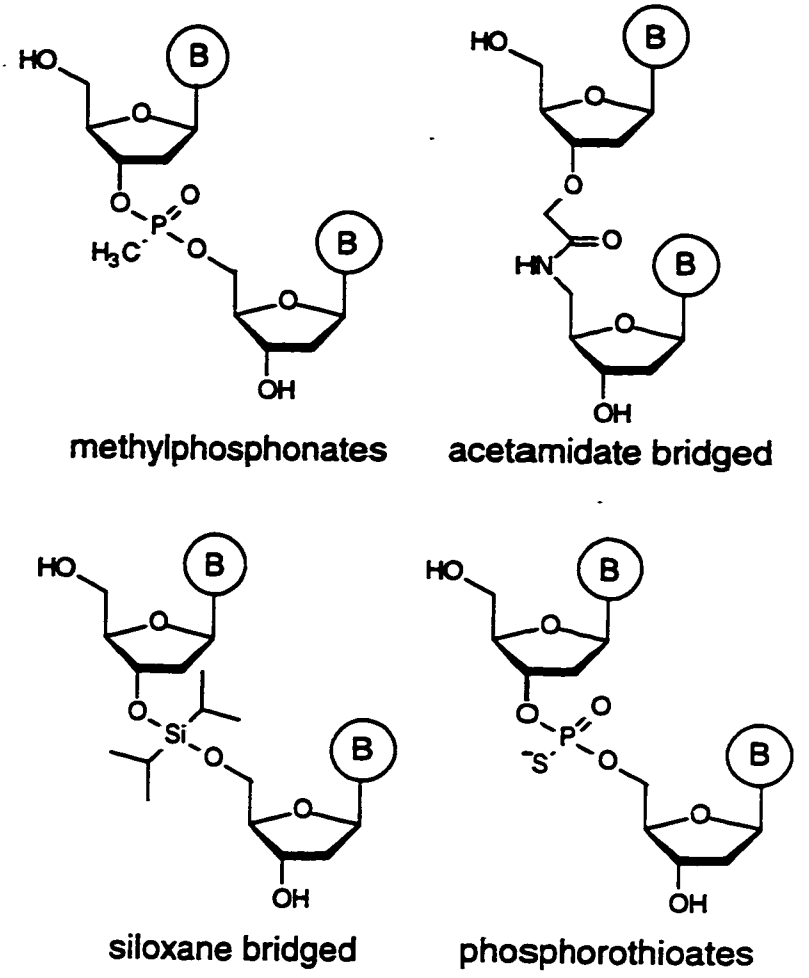


FIGURE 14: DNA Analogues

For the above requirements to be satisfied, DNA must be structurally modified since in its natural form it is vulnerable to enzymatic cleavage by nucleases. The synthesis of these DNA derivatives and their potential use has been reviewed [47]. Most of these derivatives involve modification of the phosphate backbone in order to improve nuclease resistance, a few examples are shown in Figure 14. Base modifications are less common since base modifications could easily disrupt the Watson-Crick base pairing required for specificity. Most of the modifications that

have been done involve the addition of π systems to the bases (Figure 15) [44]. This increases the ability of the bases to π-stack, which in turn helps preorganize the single stand in a favorable conformation for binding with its target. This results in higher affinity by lowering the entropic barrier for binding. These increased interactions are also increased in the duplex which also contributes to binding affinity.

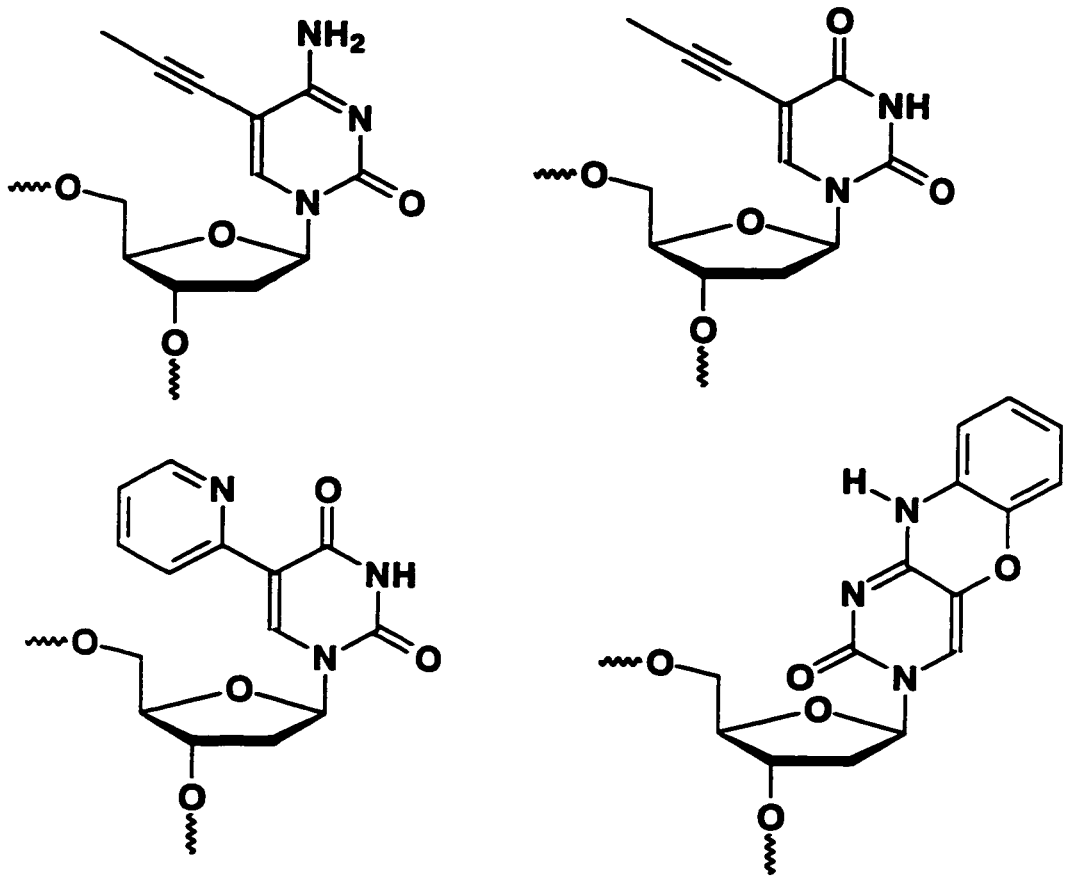


FIGURE 15: Base Analogues

1.8.3 Ribonuclease H

In eukaryotic and bacterial cells, another more efficient mechanism for translation arrest has been observed, which involves the enzyme Ribonuclease H. This enzyme recognizes double stranded RNA and selectively cleaves the RNA portion of the double strand. It has been found that this mechanism is limited to natural oligos [48] and a few others including the phosphothioate analogues [26,49], since the enzyme is very sensitive to structure deviations.

1.9 The Melting Temperature

The simplest way of investigating duplex and triplex formation in DNA, RNA and their analogues is by determining their "melting temperature"; that is the temperature where half of the molecules are in the complexed form and the other half in the non complexed form. This is done by taking advantage of the hypochromicity observed when the bases π stack in the complexed form. In other words, the total absorbance associated with the purine and pyrimidine bases decreases when they are involved in π-stacking interactions (and π-stacking interactions are greater in the complexed form relative to the single stand). The UV absorbance can be measured at varying temperature to obtain a curve as in Figure 16. The melting temperature is the inflection point between both plateaus (T when, $dA^2/dT=0$). This temperature is a direct measure of the strength of complexation between the two species of interest. When a triple helix is formed two transitions occur, with one transition due to a triplex to duplex and the other due to a duplex to single strand.

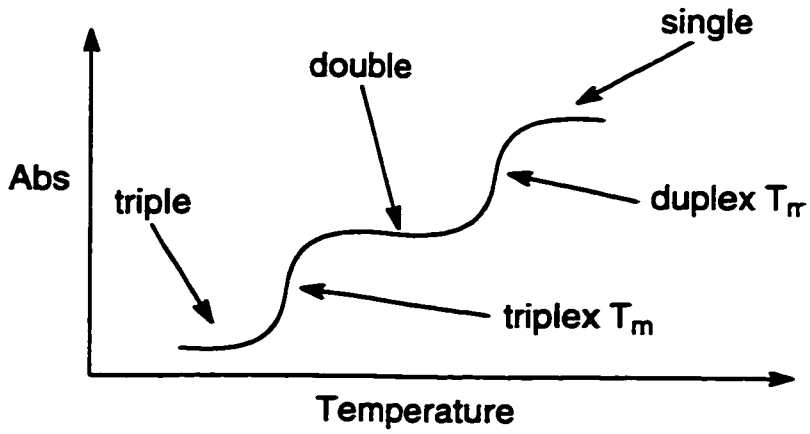


FIGURE 16: The Melting Temperature

1.10 Peptide Nucleic Acids (PNAs)

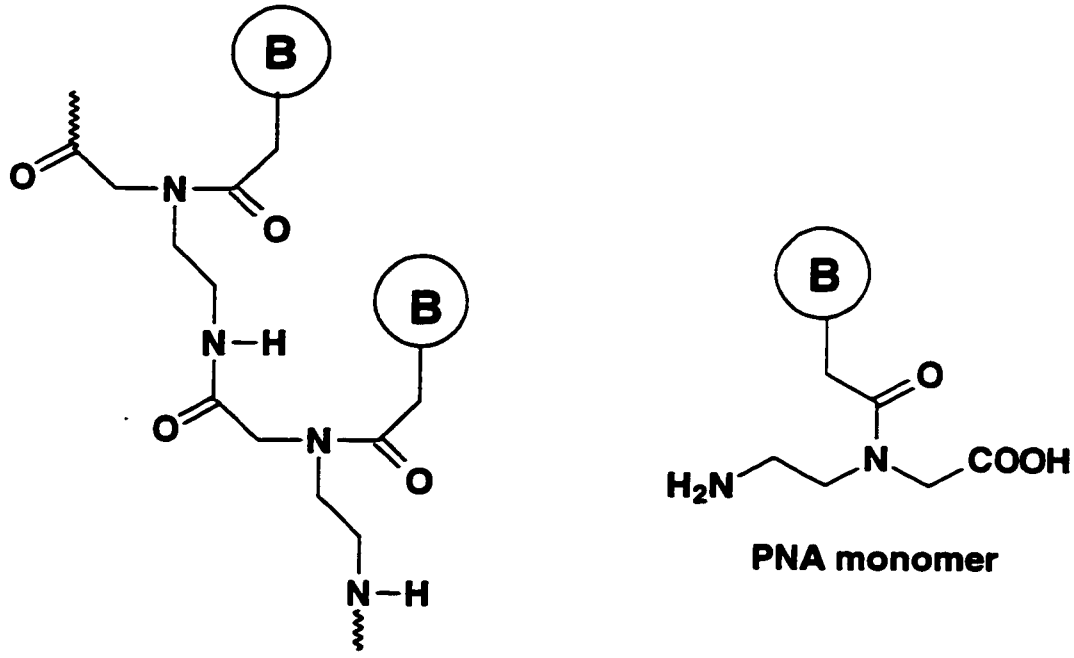


FIGURE 17: Peptide Nucleic Acids

One of the most unique DNA analogue is Peptide Nucleic Acids (PNAs) where the phosphate backbone and the sugar have been completely replaced by a pseudo peptide backbone (Figure 17) [50-53]. These compounds bind to nucleic acids obeying Watson-Crick base pairing, with higher affinity than their natural counterparts [54], mainly because they are uncharged eliminating the electrostatic repulsion present in DNA, due to the charged phosphate groups. Furthermore, the NH and carbonyls of PNAs could be involved in hydrogen bonding and the rotational restrictions provided by the amide bonds in the backbone could lead to preorginization in the PNA single strand lowering the entropic barrier for binding (for a review on the importance of preorganization in DNA see [55]). This analogue in contrast to other acyclic analogues of DNA such as the glyceroside analogs made by Olgilvie and Benner [56] (Figure 18), where the 2'C is removed, providing increased flexibility, decreases binding drastically presumably because of the loss in preorganization in the single strand increasing the entropic barrier for complexation. Moreover it has been shown that DNA analogues which are more rigid than DNA can increase affinity [55]. But PNAs show that the rigid sugar is not a requirement for DNA recognition as long as some conformational restrictions are provided for good preorganization.

Many different approaches have been used for the synthesis of the PNA monomers [57], and a few of them will be discussed in Section 2. The PNA oligomers, are composed of PNA monomers interconnected by peptide linkages using standard solid phase peptide synthesis [57-59].

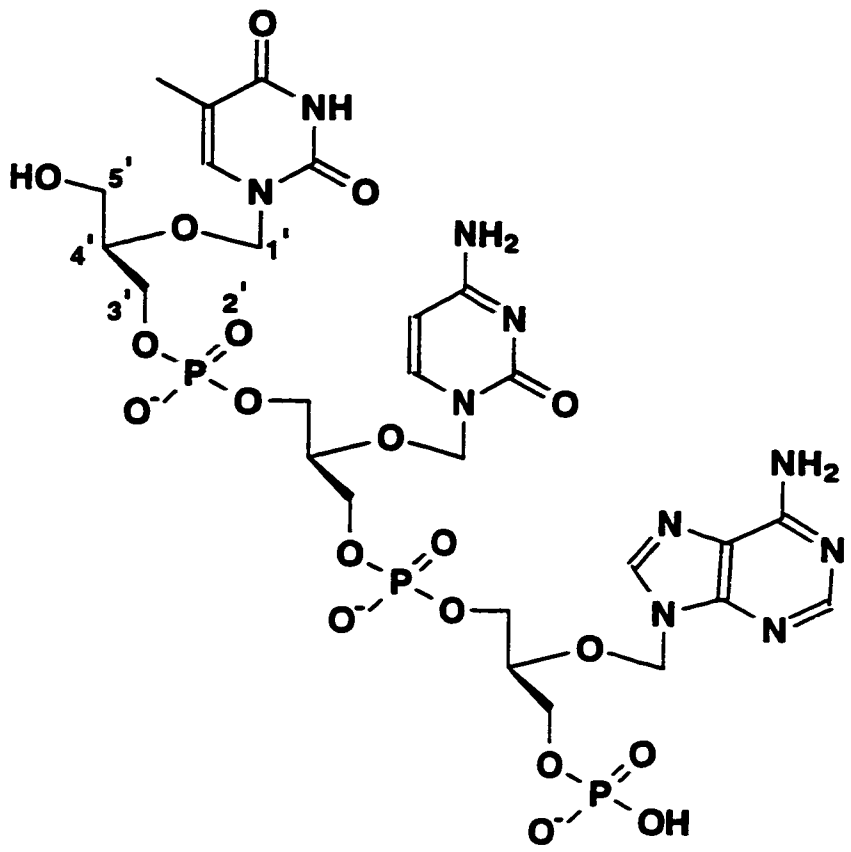


FIGURE 18: Glyceroside Analogues

1.10.1 Binding Affinity of PNAs

PNAs form strong duplexes with DNA and RNA obeying Watson-Crick base pairing rules. These compounds do so in two possible orientations parallel and antiparallel [54], where the C terminus corresponds to the 3'end and the N terminus corresponds to the 5'end, however the antiparallel hybridization is the most stable. The duplexes formed are about 1.5 °C/base more stable than their natural counterparts with DNA and 1.5 °C/base with RNA at 100 mmol NaCl [54].

Moreover, it seems that these compounds would be more selective than their natural counterparts since a single mismatch results in a 8-20 K decrease in stability which corresponds to double of that in DNA duplexes [54].

PNAs can also form triplexes with DNA and RNA [51-53,60]. Homopyrimidine PNAs form very strong PNA_2DNA triplexes, where the Watson-Crick PNA strand binds in an antiparallel fashion and the Hoogsteen strand a parallel fashion to the DNA purine strand [60]. A homothymine 10 mer binds to DNA with an affinity of 73 °C while its natural counterpart binds with only 23 °C [53]. Each TAT triplet increases stability by about 10 °C [51] and mismatches cause a drop of 14-25 °C in T_m. The hybridization of PNA_2-DNA is so strong that homopyrimidine PNA strands can displace DNA strand in the middle of a DNA duplex to form a PNA_2-DNA triple helix. This mechanism is known as strand displacement [53].

1.10.2 Structure of PNA/DNA and PNA/RNA Complexes

It has been shown by NMR experiments [62,63] that Watson-Crick base pairing does occur in PNA complexes and that DNA-PNA complexes show characteristics similar to the B-form DNA. The DNA portion shows the anti conformation of the base with the sugar in the C2'-endo form like the B-form, the bases also have base inclination of the B-form (1°). However there are 13 base pairs per turn compared to 10 in the B-form of DNA, the major groove is wider and the minor groove shallower and narrower. The tertiary amide bonds are all in the trans conformation and point towards the carboxyl terminus. No evidence of hydrogen bonds has

been conclusively observed by NMR, however, computational studies have shown that intra residue H bonds are possible in the backbone [64,65].

NMR studies on PNA-RNA duplexes show that PNAs complex *via* Watson-Crick base pairing and that the sugars in the RNA are in the 3'endo conformation (A-like) with the bases in the anti conformation [66]. Unlike the PNA in the PNA-DNA duplex the PNA tertiary amides are in the *cis* conformation and the carbonyl group is exposed to solvent.

Information on the structure of PNA_2DNA triple helices is available by X-ray crystal structure analysis [67] which confirms Hoogsteen base pairing of the third strand. The study shows a structure different from that of the A- or B-form DNA. The PNA amide protons are hydrogen bonded to the phosphate groups and 16 base pairs per turn are observed. The sugars are in a 3'endo conformation (A-type) but the bases are inclined like the B-form.

1.10.3 Antisense / Antigene potential of PNAs

There are three major advantages of PNAs over other DNA analogues. Firstly, PNAs can be easily made by automated solid phase peptide synthesis on a larger scale. Secondly, PNAs show very strong affinity and better selectivity relative to natural DNA and RNA and finally, PNAs are stable in biological systems [68]. Unfortunately, they show very poor cellular uptake [69,70]. This problem can be overcome by the attachment of carriers [71-72] or by the synthesis of DNA.PNA

chimeras (strands which contain an DNA portion and a PNA portion) [73] which increase cellular uptake. Although these chimeras show less affinity for their targets than PNAs they penetrate the cell better and they have been shown to activate Rnase H [57] at the DNA portions of the chimeras.

Not surprisingly, PNAs show efficient and specific translation and transcription inhibition properties *in vitro* [61,74] as well as *in vivo* after microinjection into cells [75].

1.11 Solid Phase Peptide Synthesis

Solid phase peptide synthesis (SPPS), developed by Merrifield in 1963 [76], is the sequential coupling of amino acids on a solid support. Normally, this is done by the addition of an amino acid having a protected amino and free acid moiety on to the free amino group of the amino acid attached to the solid support. The SPPS procedure is summarized in Figure 19.

The solid support is composed of a polymer and a linker. The first amino protected amino acid is covalently attached to the linker *via* its carboxyl group. The protecting group is then removed and the next protected amino acid is added through formation of a peptide bond, the deprotection and peptide coupling cycle is repeated until the desired sequence is obtained. Finally, the peptide is removed by cleavage of the linker peptide bond.

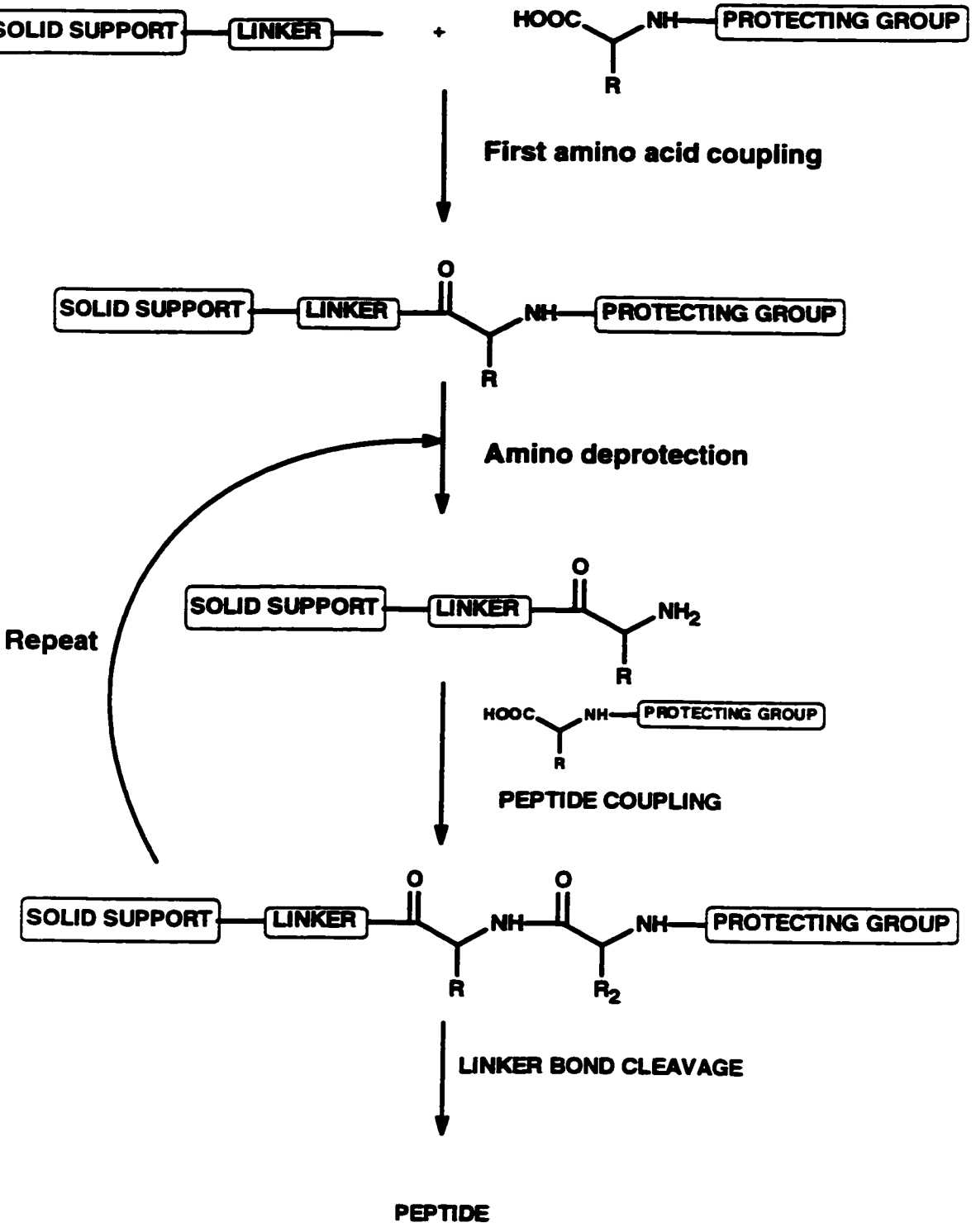


FIGURE 19: Solid Phase Peptide Synthesis

1.11.1 Solid Supports and Linkers

The most common solid support used in SPPS is polystyrene (PS) cross linked with 1% divinylbenzene (Figure 20). The support is cross linked to decrease

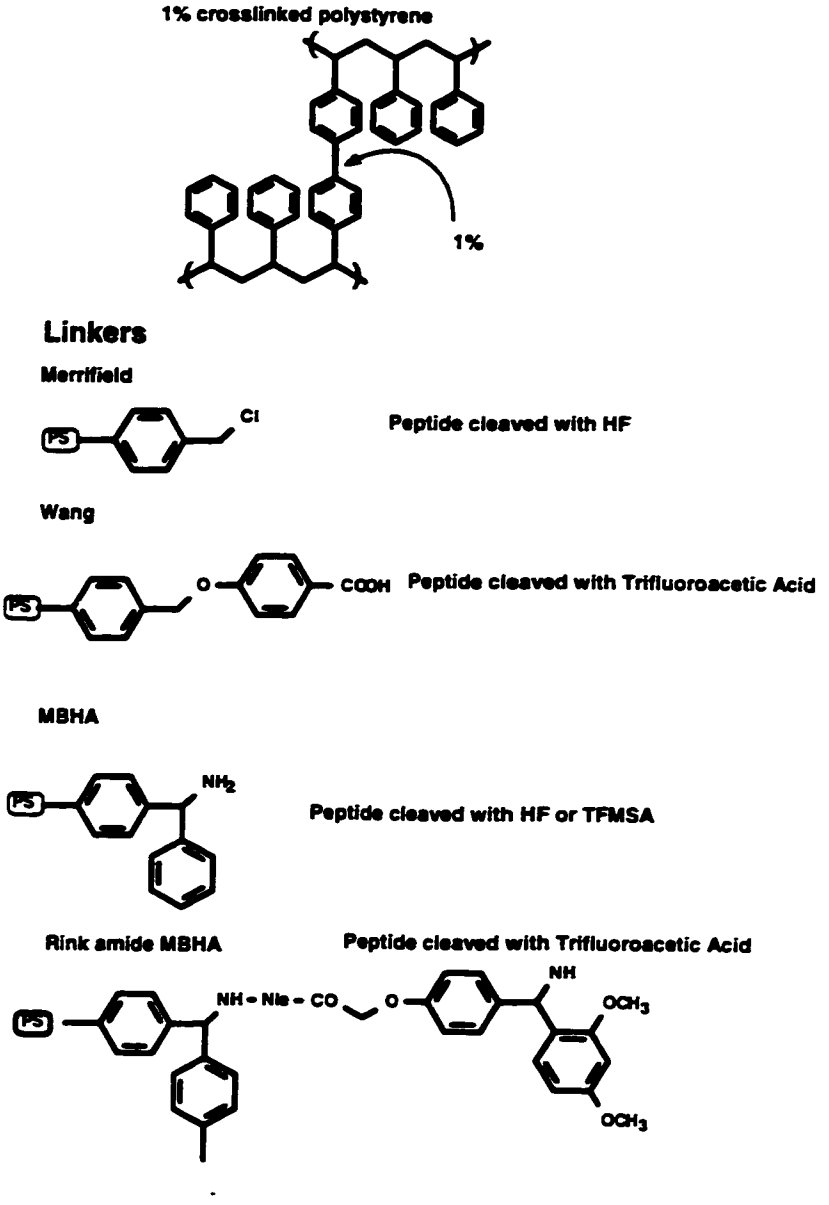
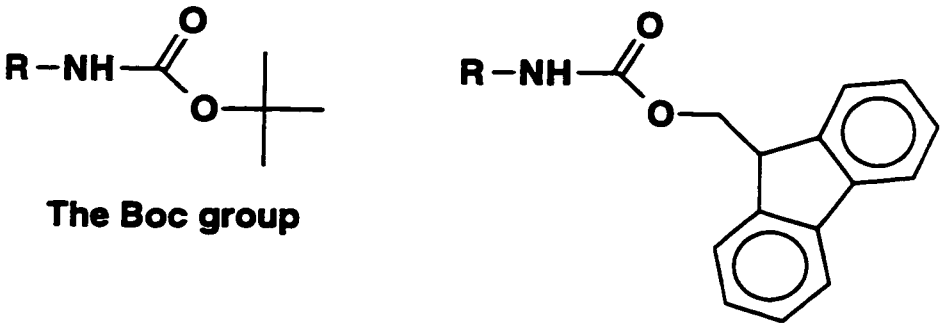


FIGURE 20: Solid Supports and Linkers

solubility of the support in organic solvents. Many different linkers are available [77], a few examples are shown in Figure 19 [76,78-80]. In almost all cases, the bond between the linker and the peptide is cleaved under acidic conditions (HF, trifluoroacetic acid (TFA) or trifluormethanesulfonic acid (TFMSA)) but there are also photosensitive linkers [81]. Base sensitive linkers are usually avoided because of the possibility of racemization of the amino acids during cleavage, although these are commonly used for non-peptidic organic synthesis on solid support [82].

1.11.2 Amino Protecting Groups used in SPPS



The Boc group

The Fmoc group

FIGURE 21: Amino Protecting Groups

There are primarily two amino protecting groups used in SPPS; these are the acid labile Boc (tert-butoxycarbonyl) group [83] and the base labile Fmoc [85] group (Figure 21). The Boc method is cheaper but requires TFA for deprotection, a chemical that many laboratories try to avoid since it is extremely toxic and corrosive. Moreover, Boc compatible linkers require very harsh conditions for cleavage (HF or

TFMSA). The Fmoc method is more expensive, however, Fmoc cleavage is milder (20% pipiridine) and linkers which are compatible with Fmoc chemistry usually require milder conditions for cleavage.

1.11.3 Carboxyl Activation used in SPPS

To form a peptide bond the carboxyl group must be activated to a more reactive intermediate (in most cases an ester) followed by aminolysis. Originally, dicyclohexylcarboiimide (DCC) in the presence of a tertiary amine, was used for this purpose [86] (Figure 22). Unfortunately DCC couplings are often plagued with racemization. This occurs because of the removal of the α-proton of the amino acid in the active intermediate which could happen by removal of the proton by the tertiary amine or by a intramolecular deprotonation as shown in Figure 22 [87]. Moreover DCC mediated reactions are also known to form side products. This can be suppressed by the addition of pentafluorophenol (Pfp) to form pentafluorophenyl esters [88] or by hydroxybenztriazole (HOBt) to form HOBt esters [89] which are reactive intermediates not prone to racemization (Figure 23). Recently, more efficient coupling reagents have been designed to increase reactivity and suppress racemization. These reagents are known as stand alone reagents since they do not require DCC for formation of the activated ester. Two of the most active reagents are shown in Figure 24 [90,91].

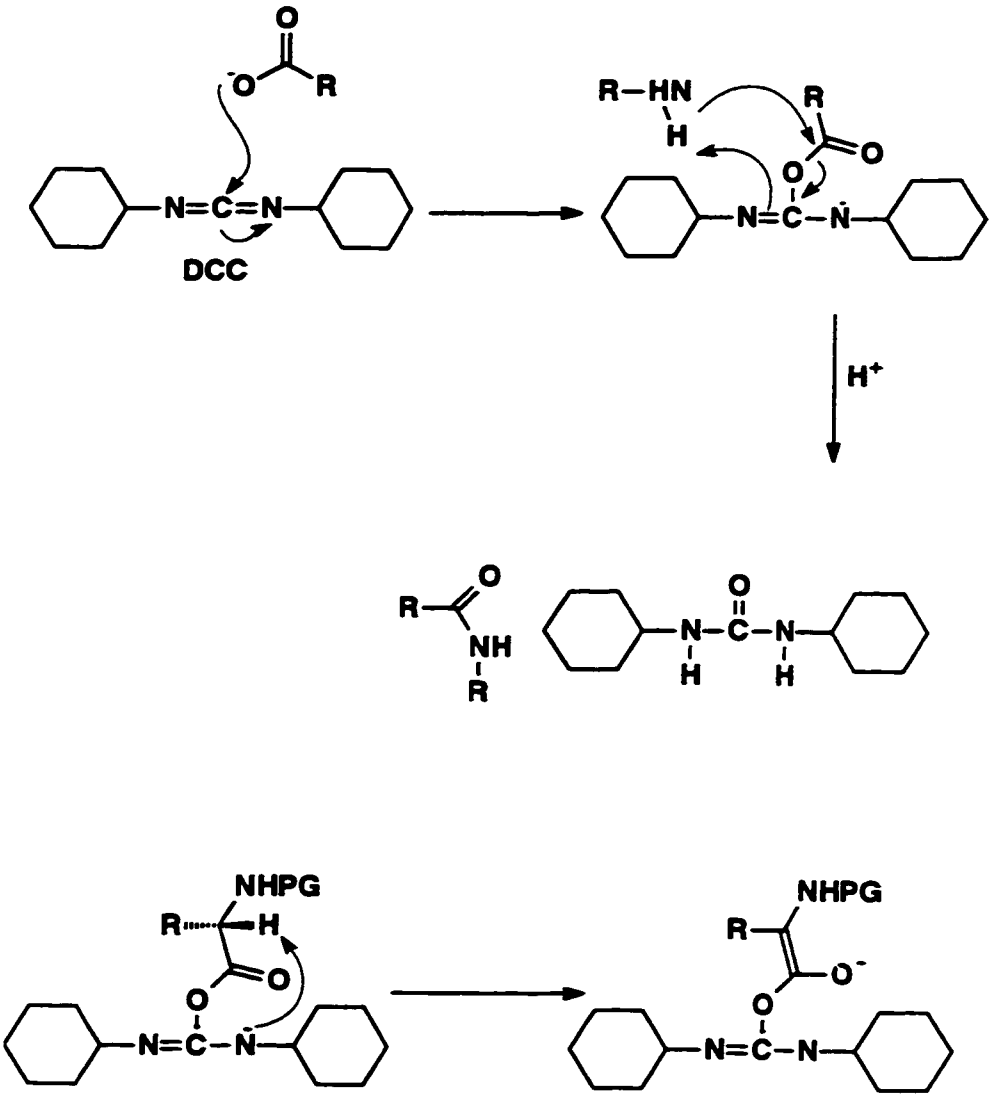


FIGURE 22: DCC Coupling and Racemization

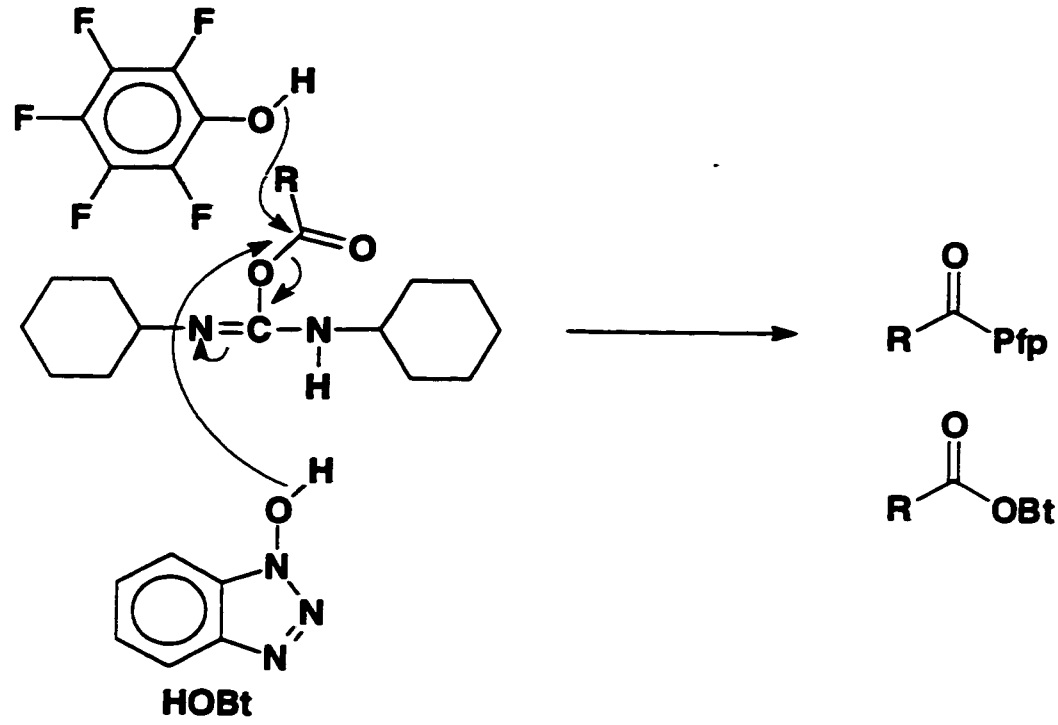
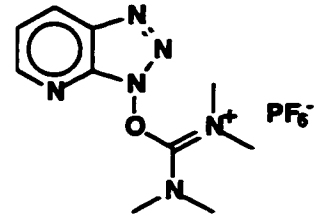
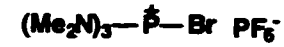


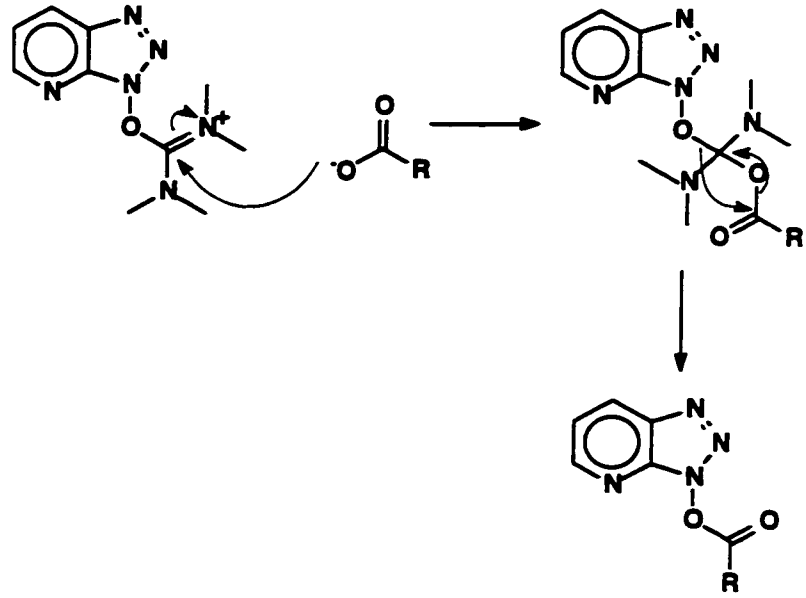
FIGURE 23: Pfp and HOBt Esters



HATU
(O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)



BroP
(Bromo tris(dimethylamino) Phosphonium hexafluorophosphate)



Activated ester

FIGURE 24: Stand Alone Coupling Reagents

1.11.4 Monitoring Reactions on Solid Support

Solid phase synthesis offers the advantage of eliminating all purification between all steps in a synthesis because all excess reagents are simply washed away while the compound remains bound to the support. This is unlike solution synthesis where excess reagents must be removed by crystallization or chromatography. Unfortunately, unlike solution synthesis where reactions can be monitored by thin layer, high pressure or gas chromatography, the polymer bound product cannot be characterized and reaction progression is difficult to monitor. This problem can be overcome by using a large excess of reagents and/or prolonged reaction times. However, this strategy does not insure reaction completion and the evaluation of the synthesis is usually done when the product is removed from the support at the end of the synthesis. However, progress has been done with some NMR techniques that can be used on polymer bound compounds [8,10].

Monitoring reactions on solid support involves the detection of the amino groups by the use of specialized reagents. The presence of amino groups confirms that deprotection steps are working and the lack of free amino groups indicates completion of the coupling reactions. Two tests are commonly used for this purpose. The most common is the Kaiser test which uses ninhydrin [92,10] to monitor amino groups as shown in Figure 25a. The product known as Ruhemann's purple gives a distinct blue color on the beads in the presence of free amino groups. This qualitative test can be sensitive up to 99% in certain cases and only requires

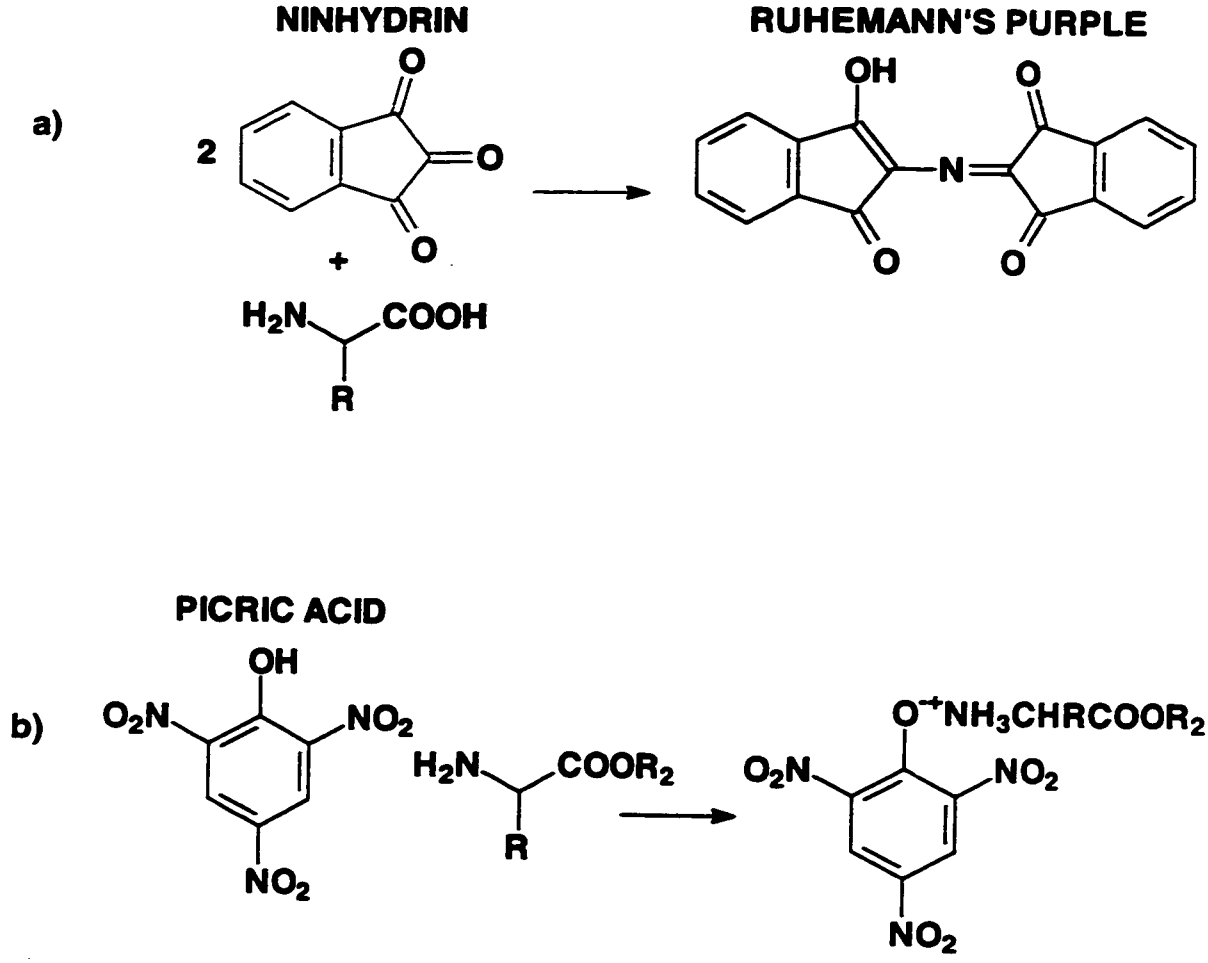


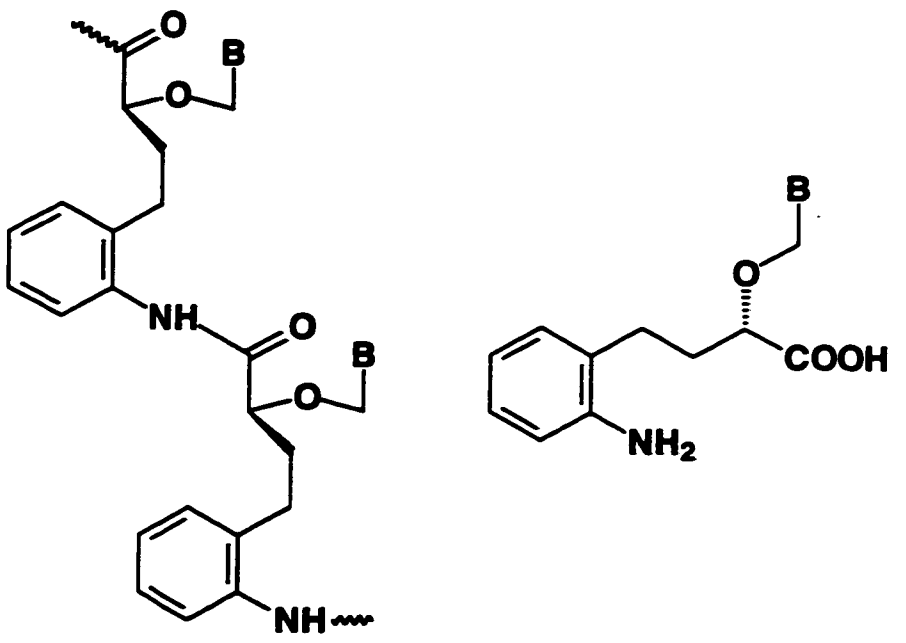
FIGURE 25: Tests for Amines

a few beads of resin (the term resin is used for the solid support and linker). However, sensitivity depends on sequence, and can decrease dramatically with unusual or hindered amino acids (secondary amino acids such as proline or anilines) or with sequences which can aggregate on the solid support, situations which are difficult to predict [93]. There is also a quantitative version of the Kaiser test which involves photometric determination of Ruhemann's purple at 570 nm [94]. Another commonly used test is the picric acid test [95] (Figure 25b). In this case the

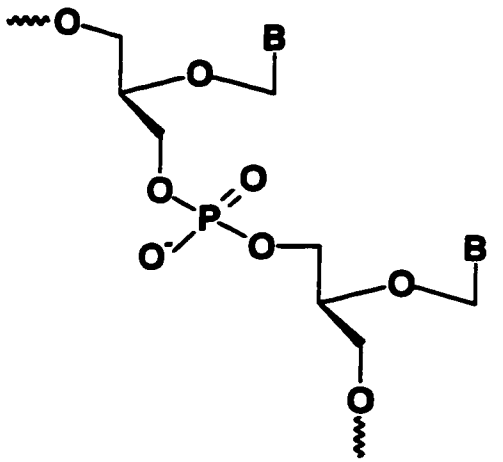
resin is treated with picric acid and to form a picric acid salt with the amino groups. The picric acid is subsequently displaced with a base and can be determined photometrically at 358 nm. Sensitivity is excellent for natural amino acids (except proline) but has the same limitations as the Kaiser test.

1.12 Research Project

The main drawback of PNAs oligomers is their poor cellular uptake which is most likely due to low hydrophobicity. The goal of this project was to develop a new class of PNAs called Aromatic Peptide Nucleic Acids (APNAs) (Figure 26). As shown in Figure 26, these APNAs are analogous to the glycerocide analogues. The aromatic rings in the backbone should make these compounds more lipophilic, thus increase cellular uptake. Moreover, conformational restrictions along the backbone may be provided by π-staking interactions between the aromatic rings, or hydrogen bond formation between the rings and the amide protons, a phenomenon often observed in protein folding [96]. Similar to the PNAs, the amide bond rotational restrictions may provide additional stability. This idea of inserting hydrophobic moieties in the backbone has been examined in other DNA analogs (Figure 27) [97], it was shown that these groups have little effect on duplex stability. However, their ability to increase cellular uptake remains unknown. The goal of this part of the project was to synthesize an APNA hexamer in solution and examine its binding properties.



APNA and its monomer



Glyceroside analogues

FIGURE 26: Aromatic Peptide Nucleic Acids (APNAs)

The hexamer was made using the APNA monomer shown in Figure 26, using procedures initially developed by J. Lunetta (MSc. Concordia University 1997). This

monomer was condensed into a dimer by formation of a peptide bond between amino and carboxyl terminals and subsequently used to prepare a hexamer following the same synthetic procedures.

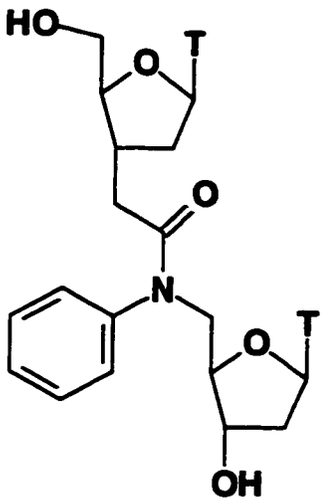
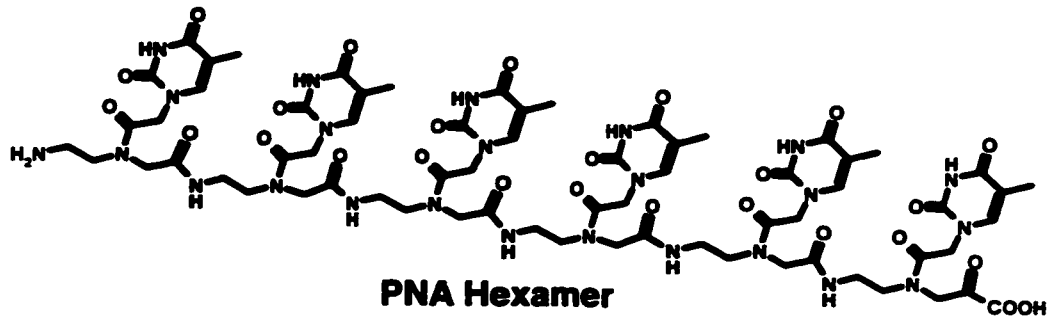
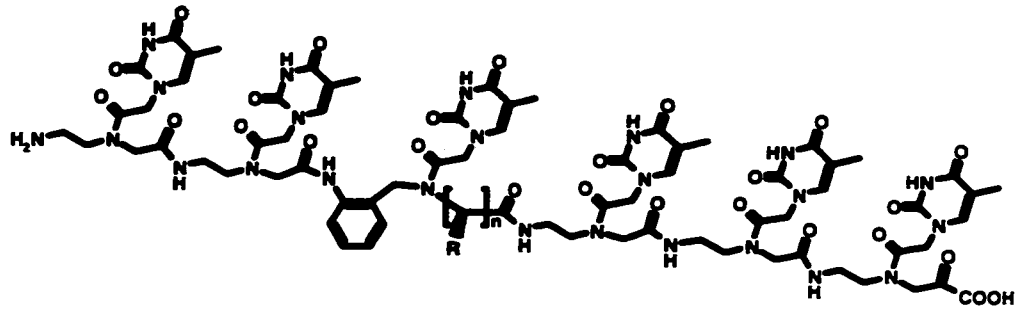


FIGURE 27: Aromatic Ring in Backbone

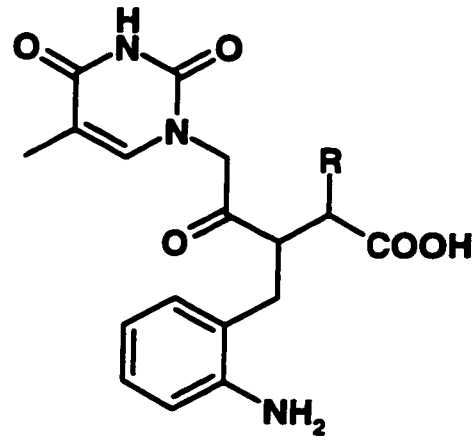
The second aspect of this project involved the synthesis of PNA hexamers containing a "mutation" with an novel APNA unit as shown in Figure 28. The latter APNA unit is structurally more similar to the original PNAs. The goal was to make PNAs more hydrophobic by the addition of aromatic rings. These aromatic rings could also be involved in π-staking interactions or aromatic H-bonding which could provide some conformational stability. Another novelty of these APNAs is the presence of the side chain (R). This side chain could easily be modified using the same synthetic procedures, with different amino acids as starting materials. This



PNA Hexamer



PNA Hexamer containing APNA unit



APNA Monomer

FIGURE 28: Second Generation APNAs

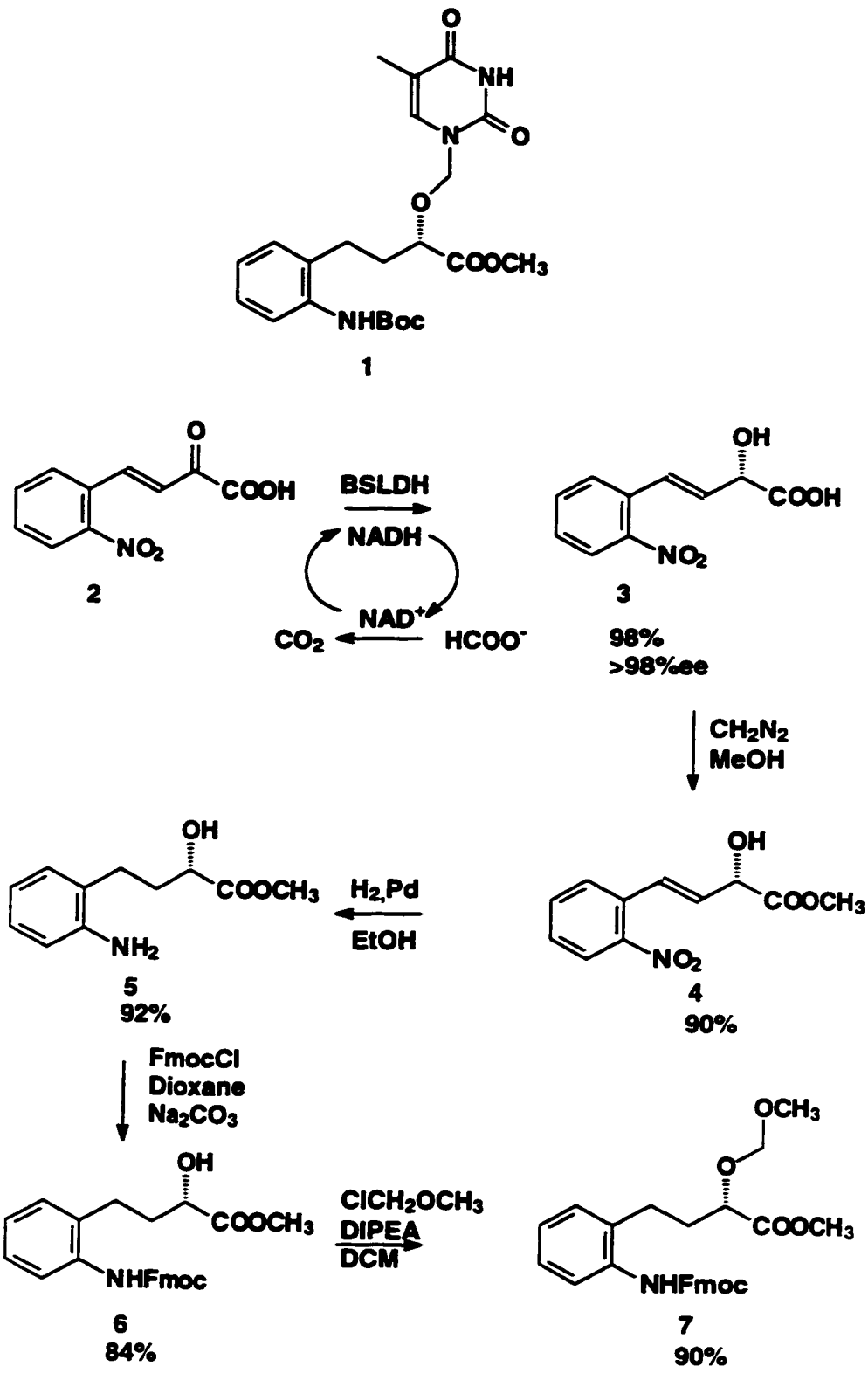
was done in order to examine the effect of substitution on the binding affinity of the hexamers. The synthesis of APNA units, where R = CH_3 (both stereoisomers), were synthesized and inserted into PNA hexamers. These oligomers were assembled on solid support and all units contained thymine as the base in order the simplify the synthesis.

2. RESULTS AND DISCUSSION

2.1 First Generation Aromatic Peptide Nucleic Acids

2.1.1 Synthesis of APNA Thymine Monomer

Our strategy was to synthesize the APNA monomer **1**, with the orthogonal Boc (removed in acid) and methyl ester (removed in base) protecting groups for the preparation of oligomers, from the α-keto acid **2** [98]. The keto ester **2** was enzymatically reduced with NADH in the presence of the enzyme *Bacillus Stearothermopholis Lactate Dehydrogenase* (BSLDH), using *formate dehydrogenase* (FDH) and sodium formate as a recycling system for NADH (Scheme 1) [99]. The natural substrate of this enzyme is pyruvate (Figure 29a), however, broad substitutions at the methyl position are well accepted giving good yields and enantiomeric excess, even though catalytic efficiency can drop as far as 0.1% with bulkier substituents [100]. To our advantage, this enzyme works especially well with α-β unsaturated ketones [99]. It has been proposed that the double bond results in a flattened and conformationally restricted structure which eases substrate binding [99]; the substrate binding in the active site and the proposed reaction mechanism are shown in Figures 29b and 30 respectively. An arginine amino acid stabilizes the carboxyl terminal and another forms a hydrogen bond with the keto group which helps polarize the bond, favoring hydride transfer



SCHEME 1: First Generation APNA Synthesis

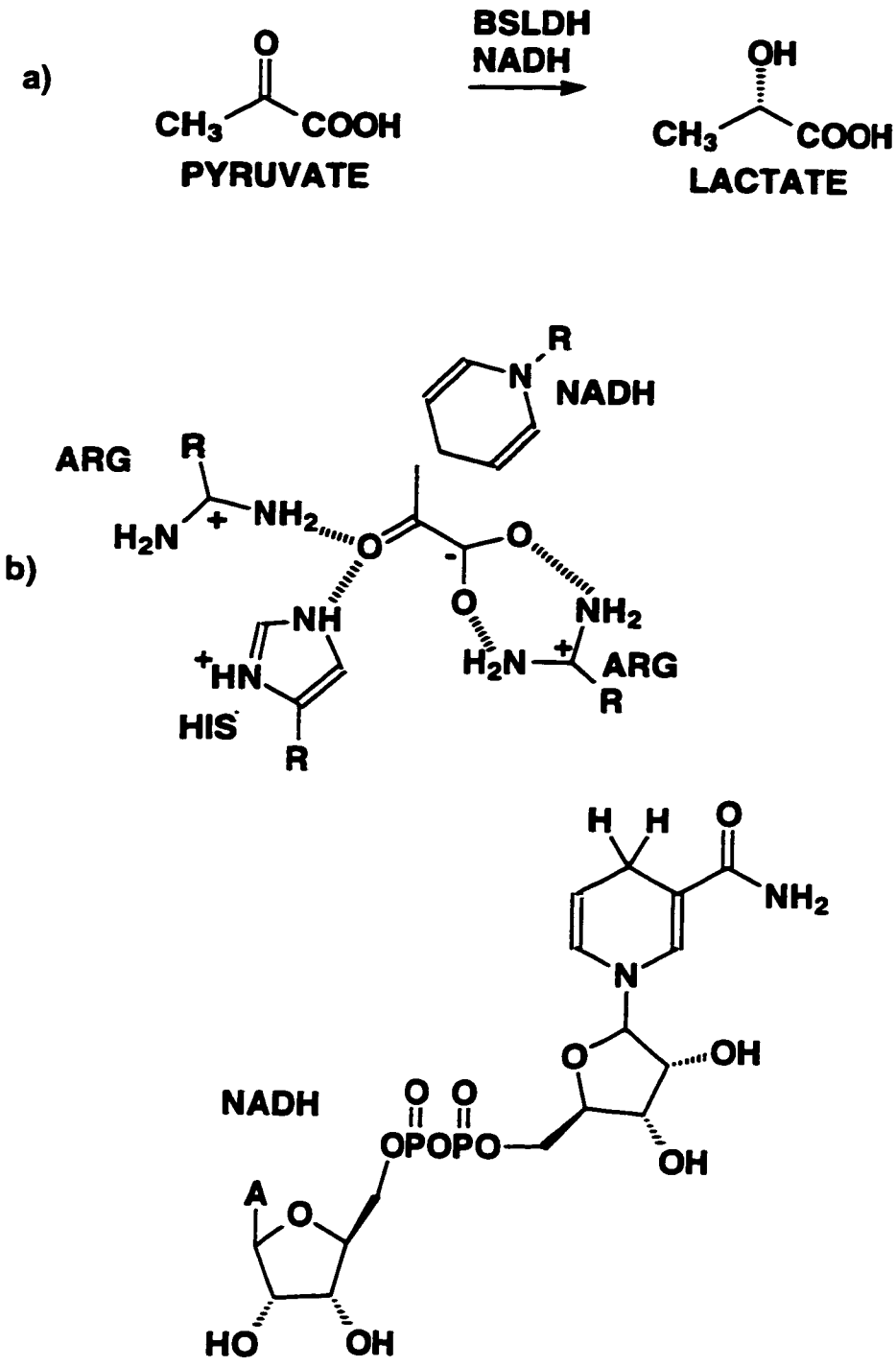


FIGURE 29: Bacillus Stearothermopholis Lactate Dehydrogenase

[101]. Moreover, a histidine (pK_a=5.6-7.0) residue is the proton source of the reaction. Therefore the pH of the reaction is kept at 6.0-6.2 to keep the His in its protonated form [102]. The (S)-alcohol (3) was obtained in 98% chemical yield and greater than 98% enantiomeric excess from the α-keto acid **2**.

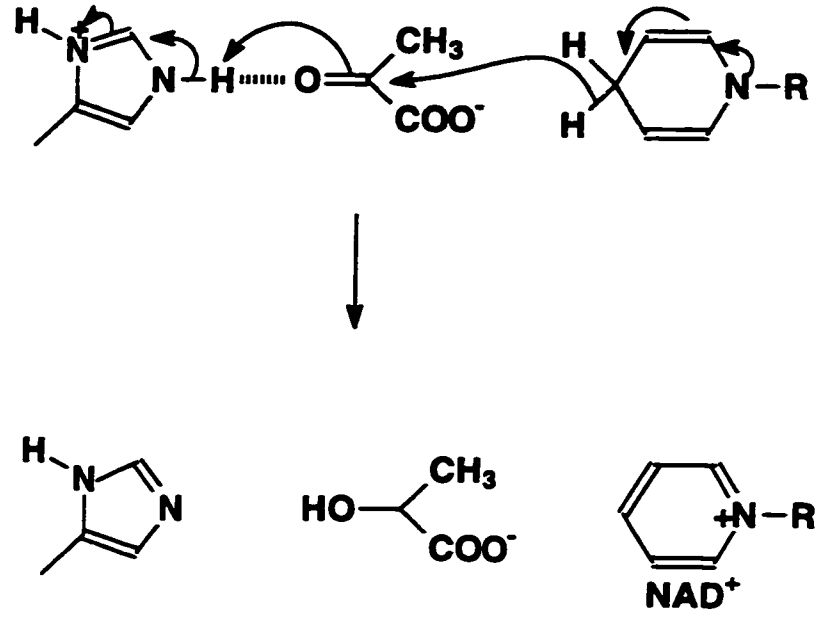
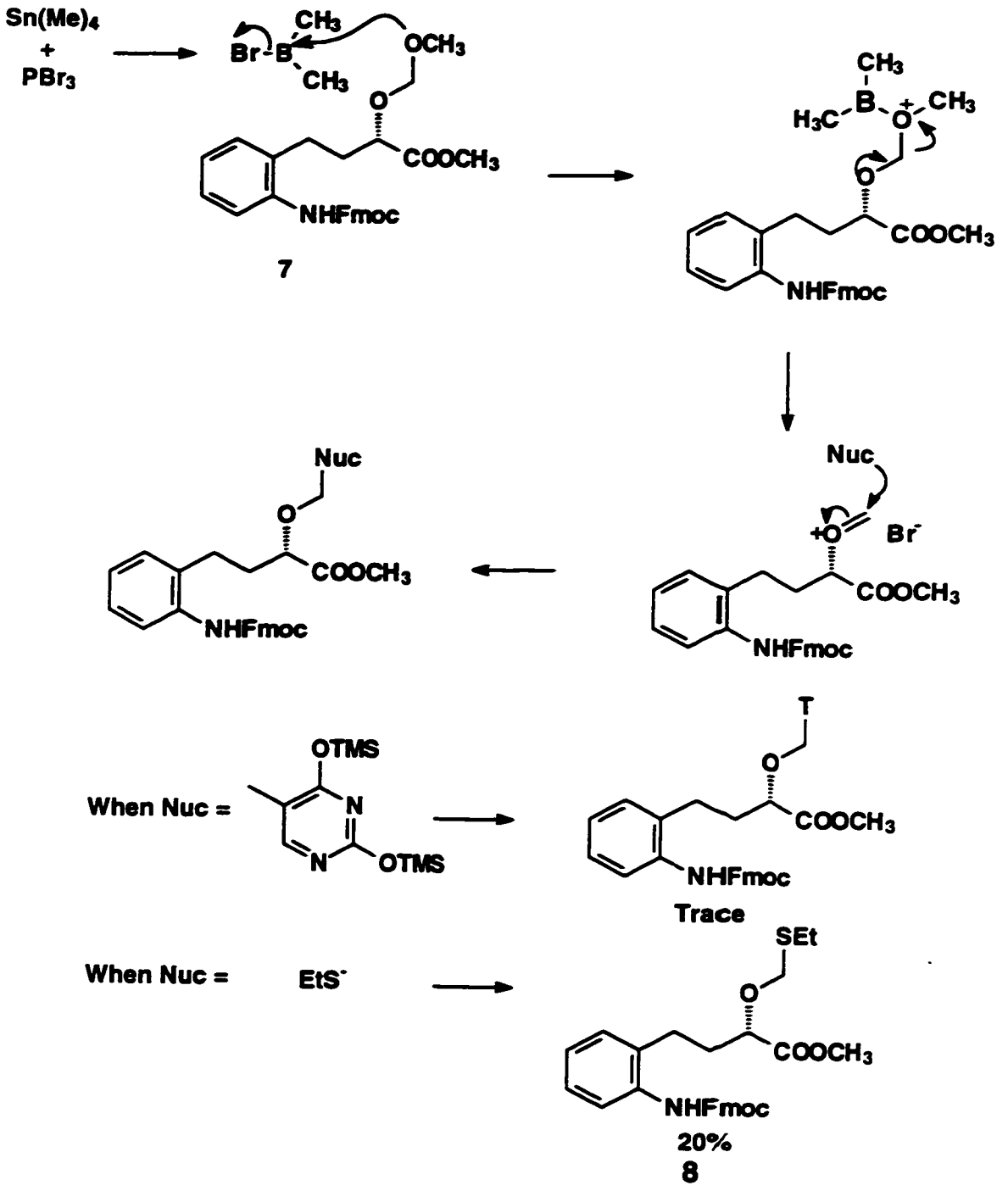


FIGURE 30: Mechanism of BSLDH

The acid group was then protected as its methyl ester **4** with the use of diazomethane and the double bond and nitro group reduced with palladium catalyst under hydrogen to obtain **5**. The aniline was then protected with the Fmoc group (the aniline could not be directly Boc protected due to the Lewis acid used in a later step) and compound **6** was obtained in good yields.



SCHEME 2: Methoxy Substitution

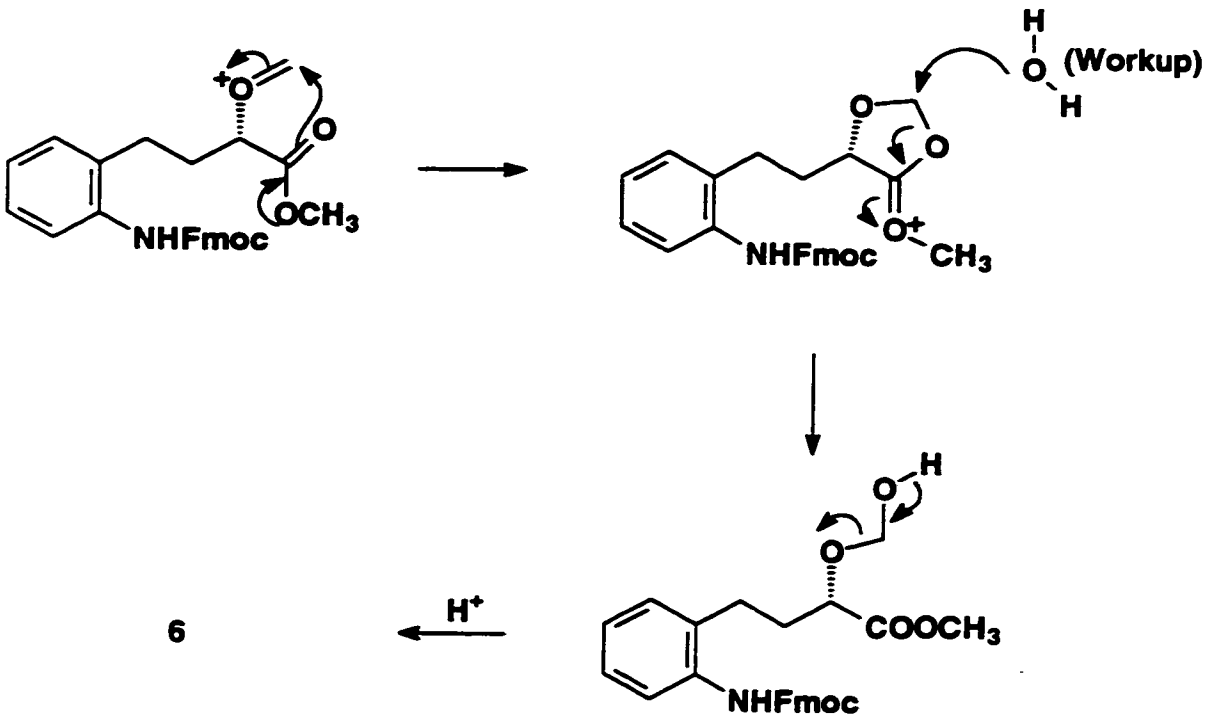
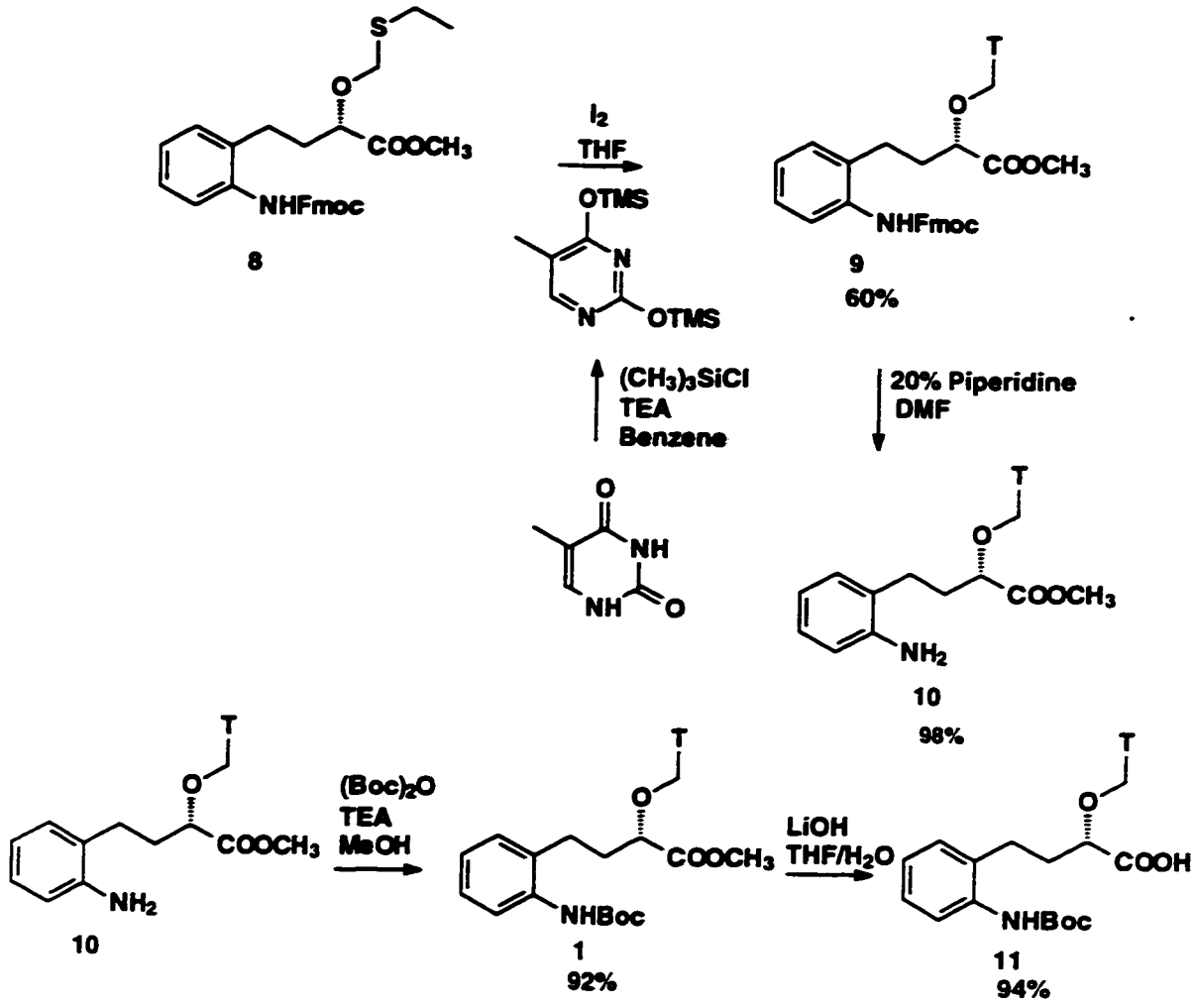


FIGURE 31: Ester Interference

The methylene extension of compound **6** was performed by the formation of the methoxy methyl ether with chloromethyl methyl ether (Scheme 1). It has been shown that the methoxy group can be substituted with nucleophiles with the use of dimethylboron bromide (synthesized from phosphorus tribromide and tetramethyl tin), by the formation of a bromo intermediate followed by substitution in excellent yields (>70%) [103,104] (Scheme 2). Unfortunately, attempts to use bis-trimethylsilyl thymine as the nucleophile resulted in only traces of the desired product **9** (Scheme 2) with recovery of the alcohol **6**. However, slightly better yields (~20%) were obtained with ethanethiol as the nucleophilic species to obtain the



SCHEME 3: First Generation APNA Synthesis continued

thioether **8** along with recovered alcohol **6**. The poor yields may be a consequence of the interference of the ester functionality as shown in Figure 31. Nevertheless, the thymine moiety can be added by iodine assisted sulfur displacement with bis-trimethylsilyl thymine (prepared from thymine and trimethylsilylchloride [105]) to obtain **9** in 60% yield (Scheme 3). The silylated derivative was used since it is much more soluble than thymine in organic solvents. Finally, the Fmoc group was

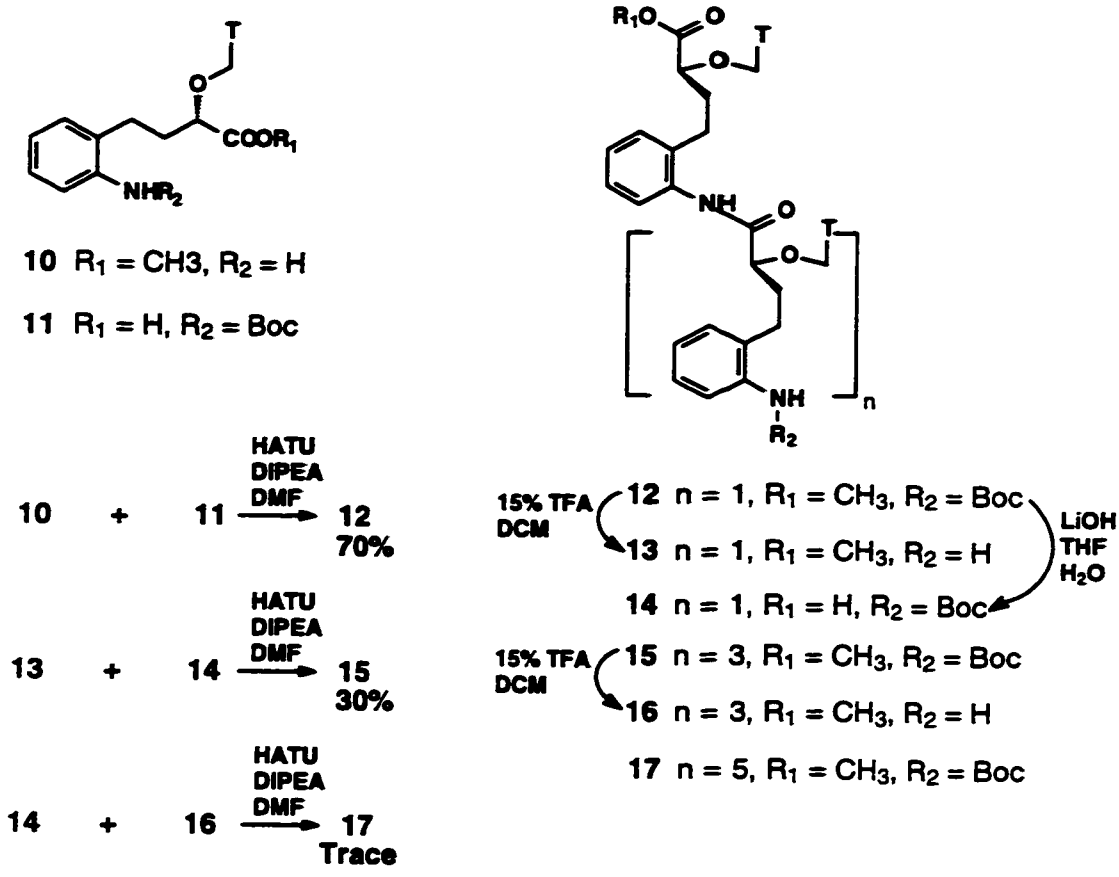
removed with piperidine to obtain the free aniline monomer **10**. Subsequently some of the aniline was Boc protected to obtain compound **1** and saponified with LiOH to obtain the free acid **11**. It should be noted that the chiral integrity of the monomer was confirmed by chiral HPLC using compound **8**, and confirmed again after the saponification since deuterium exchange was not observed when the reaction was performed in D_2O.

2.1.2 Synthesis and Properties of APNA Oligomers

The APNA dimer **12** was made *via* the formation of peptide coupling between the aniline and carboxyl groups using compounds **10** and **11**, with HATU as the coupling reagent as shown in Scheme 4. The dimer was then appropriately deprotected and the resulting compounds were subsequently coupled to obtain tetramer **15**, which was then deprotected (**16**) and condensed with dimer to obtain hexamer **17**.

The synthesis of an APNA dimer on solid support was also attempted using the Wang resin, using Fmoc free acid **18** (made from completely deprotected monomer). Unfortunately, the cleaved crude mixture showed mostly recovered monomer.

The fully deprotected monomer **19**, dimer **20** and tetramer **21** were also prepared in order to carry out some conformational studies in an aqueous medium



SCHEME 4: First Generation APNA Oligomer Synthesis

which is of biological relevance for DNA molecular recognition. We investigated the ability of the thymine bases to π stack , which is important in the preorganization of single strands in natural oligonucleotides. The nature of these interactions is not fully understood only that attraction is in part driven by Coulombic interactions between partial charges on the bases and a non classical hydrophobic effect, it seems that the exclusion of high energy water from the hydrophobic surfaces is unimportant [106,107].

π-stacking interactions can easily be detected by two methods. The first is by ^{1}H NMR spectroscopy. When bases are involved in π stacking the protons near the conjugated system show an upfield shift relative to the unstacked bases as shown in Figure 32 [106,108]. This phenomenon is due to the magnetic field induced by the current produced by the mobile electrons in the conjugated system, which is parallel to the applied magnetic field in the region of the protons. This effect gives rise to a lower energy transition and hence an upfield shift. As shown in Table 1, the methyl and vinyl protons on the thymines show a downfield shift rather than an upfield shift from the monomer to the dimer, however, the expected upfield shift from the dimer to the tetramer is observed.

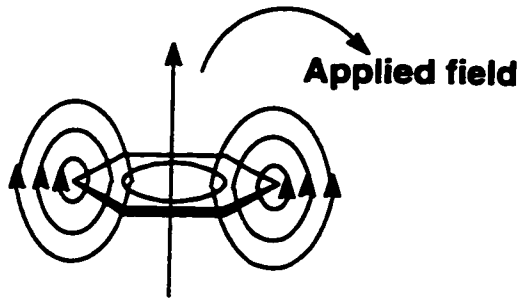


FIGURE 32: Protons near a conjugated system

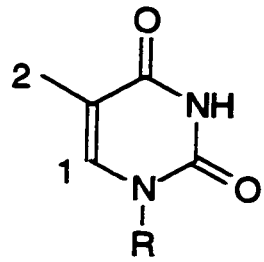
TABLE 1

UV-SPECTROSCOPY

264nm

	EXTINCTION COEFFICIENT/THYMINES ($cm^{-1}M^{-1}$)
Monomer	6110
Dimer	8945
Tetrmer	7090

^{1}H NMR SPECTROSCOPY



Chemical shifts in D_2O

	1	2
Monomer	7.34	1.80
Dimer	7.54,7.41	1.87,1.83
Tetramer	7.45,7.41,7.38,7.30	1.81,1.80,1.80,1.76

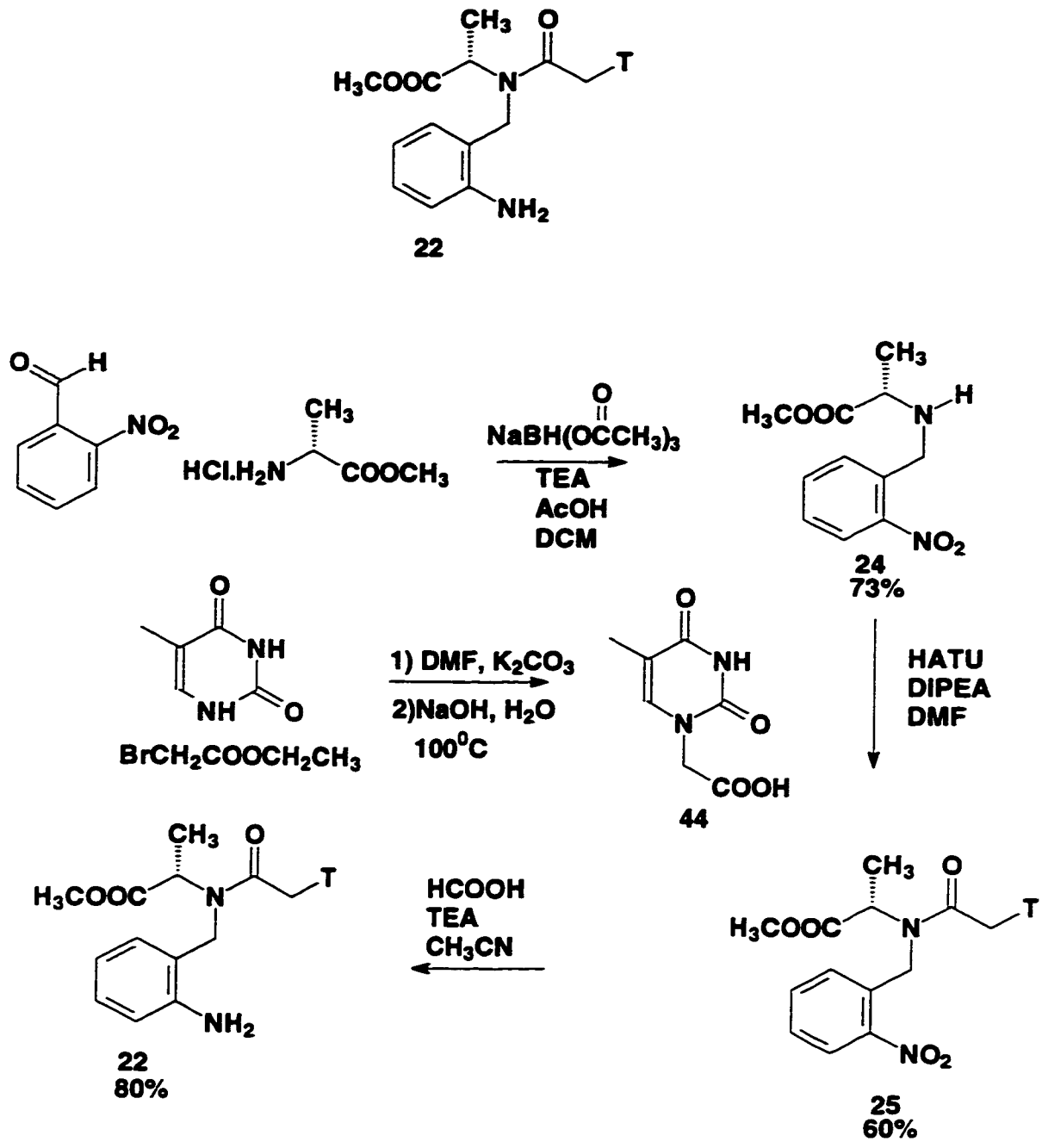
Another method which is commonly used to detect these interactions is UV-visible spectroscopy. When the bases stack they exhibit a decrease in absorbance relative to the unstacked forms, this phenomenon is known as hypochromicity. This can be explained by the fact that the magnitude of absorbance is proportional to the dipole induced by the radiation. When a base is involved in stacking the dipole induced is smaller because the opposing dipoles of the neighboring bases [109]. The absorbance of the monomer, dimer and tetramer is shown in Table 1. An increase in absorbance was observed from the monomer to the dimer and the expected decrease was observed from the dimer to the tetramer.

In both cases it appears that the monomer does not follow the expected trend. We have speculated that the monomer stacks on its own with the base and the aromatic ring, since no other aromatic system is present. However, the data from the dimer to the tetramer does support stacking interactions in the bases necessary for preorganization. Unfortunately no hybridization between the hexamer **17** and could be observed by melting temperature measurements supporting the absence of DNA/RNA recognition.

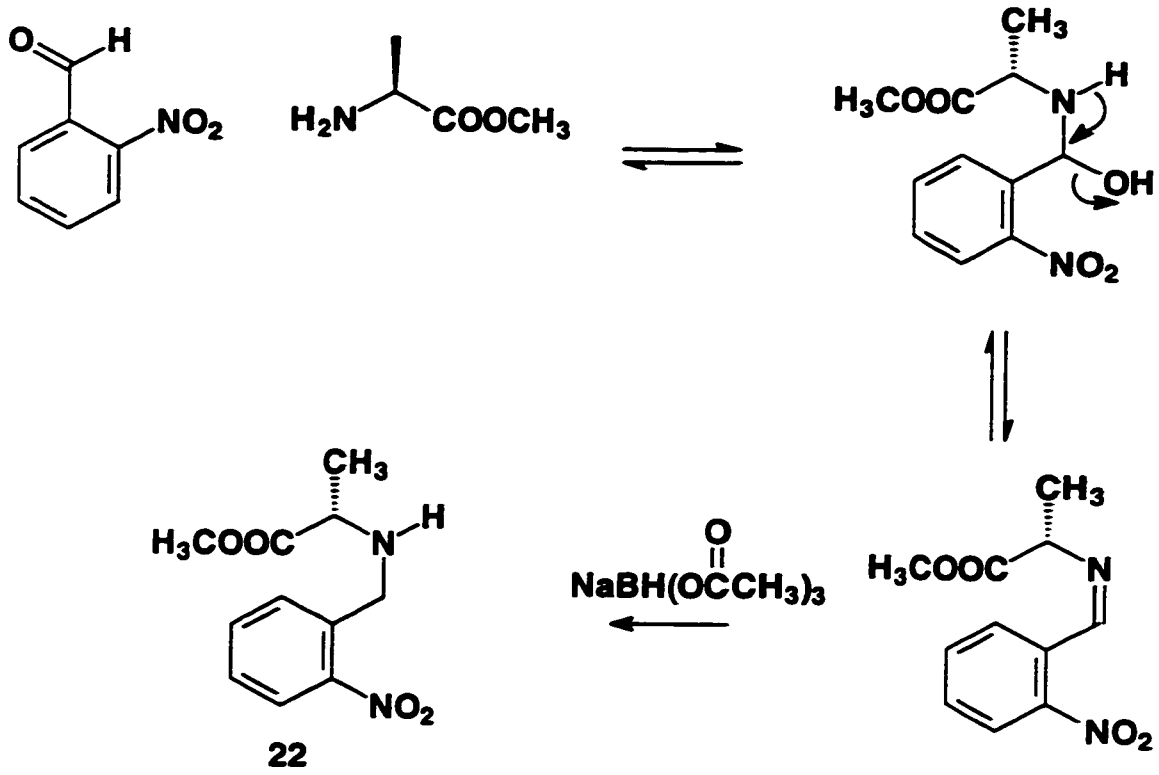
2.2 Second Generation APNAs

2.2.1 Synthesis of the L- and D- Alanine Thymine Monomers

Starting from L- (or D-) alanine methyl ester and *o*-nitrobenzaldehyde (Scheme



SCHEME 5: Second Generation APNA Synthesis

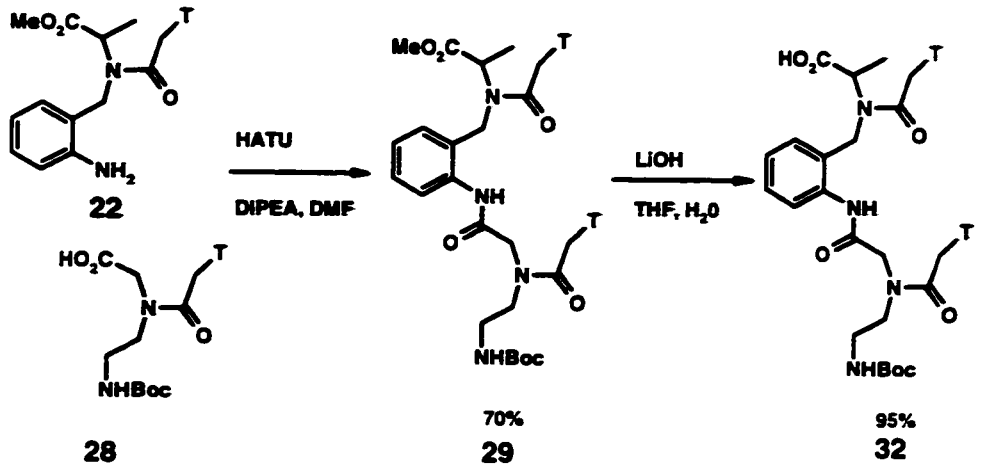


SCHEME 6: Reductive Amination

5), the APNA backbone **24** was made by reductive amination [110,111], which consists of the formation of a Schiff base followed by *in situ* reduction of the double bond with triacetoxy sodium borohydride (Scheme 6). The latter is a mild reducing agent due to the stabilization of the charged boron by the acetoxy groups, which selectively reduces Schiff bases in the presence of aldehydes. A potential problem with this reaction is the possibility of dialkylation which can be diminished by the use of excess amine. Unfortunately, this led to complex reaction mixtures which consequently led to poor yields (~40 %) after chromatography. Optimum conditions were achieved when a slight excess of aldehyde was used (10%) and 5 equivalents of acetic acid catalyst were added (72% yield). This led to a clean reaction mixture

composed only of product and nitrobenzyl alcohol. The product could then be separated by formation of its hydrochloride salt in ether, with no chromatography. Subsequently, the base was attached to the backbone by peptide bond formation between the backbone secondary amine **24** and the thymine derivative **44** as shown in Scheme 5 (the thymine derivative is made by alkylation of thymine with bromoethyl acetate, followed by ester hydrolysis [58]). All attempts to attach the thymine derivative by generation of its pentafluoro ester, proved futile (Figure 22) even though this method works well with structurally similar analogues, such as the glycine derivative. It appears that the bulk of the methyl group hinders peptide bond formation. However, compound **25** could be obtained if the more effective HATU reagent was used as the coupling reagent. Finally, the nitro group was reduced by catalytic transfer hydrogenation [112], using formic acid as the source of hydrogen and palladium as the catalyst to obtain compound **22**. The ^{1}H NMR spectra of all these compounds containing the thymine base clearly show that at room temperature they exist as rotamers. This is due to the double bond character of the nitrogen of the tertiary amide. To facilitate interpretation, the NMRs of these compounds were acquired at 120-140°C, where the interconversion between rotamers is very fast, giving one set of ^{1}H resonances. The effect of temperature on the equilibrium between the two rotamers is shown in Appendix A. Based on the integrals it appears that these compounds exist in a 1:3 ratio at room temperature.

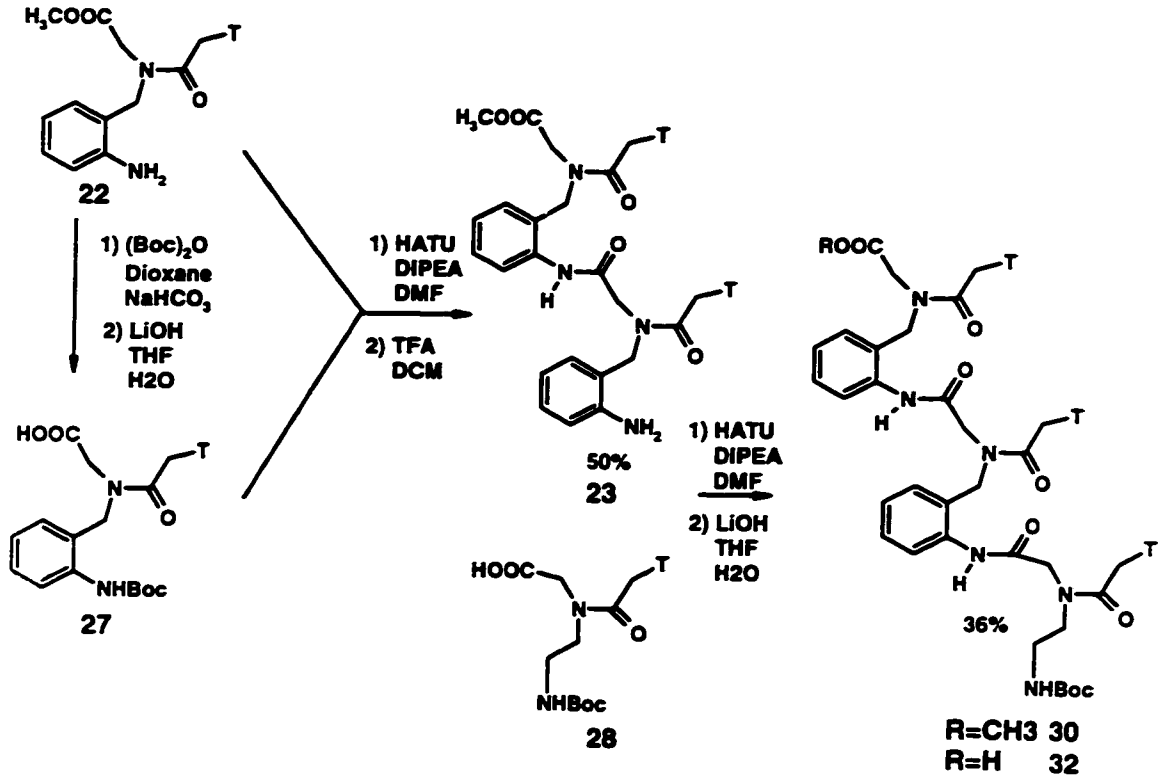
2.2.2 Synthesis of APNA Chimera Units



SCHEME 7: Synthesis of APNA / PNA Chimeras

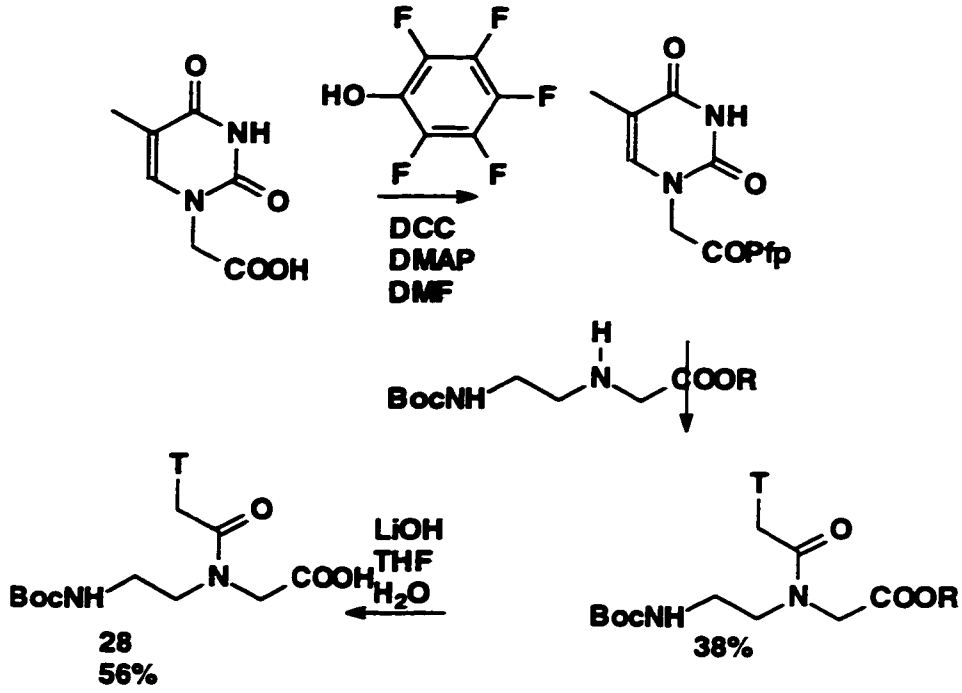
Before incorporation of the APNAs into the PNA hexamers, the APNA unit was first condensed with a PNA unit (to form an APNA/PNA chimera) as shown in Scheme 7. This was done in order to avoid exposure of the aniline group during SPPS, since this group is difficult to monitor using conventional tests for amines (see section 1.11.4). The chimera units were made by peptide couplings of **22** with free acid PNA **28** (see next section for synthesis of PNAs) using HATU in DMF to obtain **29** (Scheme 7). The chimera was then saponified with LiOH and **31** was obtained. The APNA is now ready for SPPS. (Identical procedures were followed for both the D- and L- alanine APNAs).

It was also desirable to study the effect of two APNA mutations in a PNA hexamer (in particular the L-alanine APNA) . This was acheived by first condensing an APNA dimer **23** with a PNA unit **28,** followed by saponification to obtain a PNA/APNA chimera containing two APNA units **32** (Scheme 8). The dimer was obtained by Boc protection of the aniline followed by ester hydrolysis, the free acid APNA **27** was subsequently condensed with **22** and the resulting dimer was deprotected with TFA to obtain **23.**



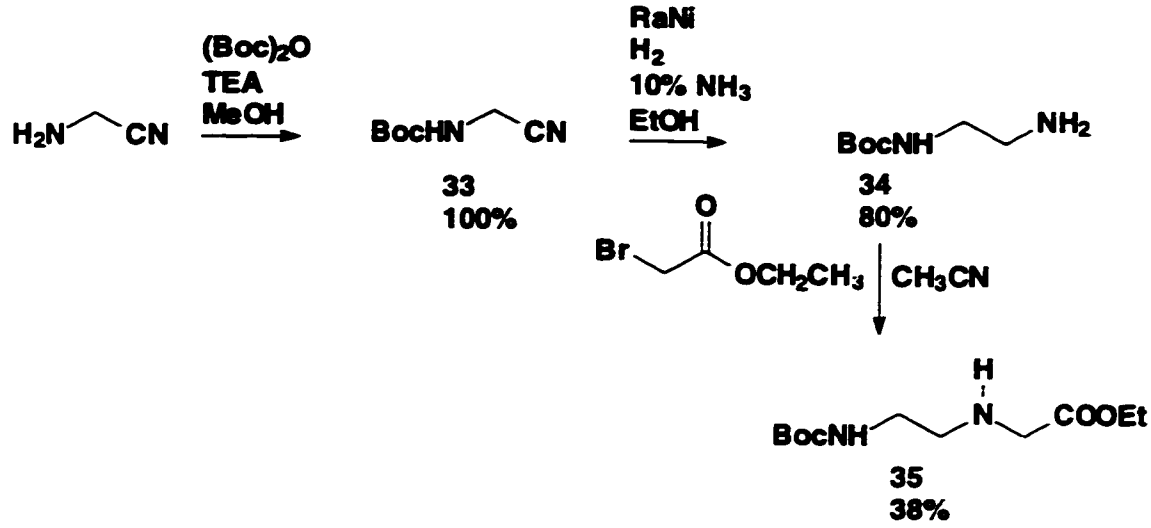
SCHEME 8: Synthesis of APNA/PNA Chimeras continued

2.2.3 Synthesis of Boc Protected PNA Backbone



SCHEME 9: PNA Synthesis

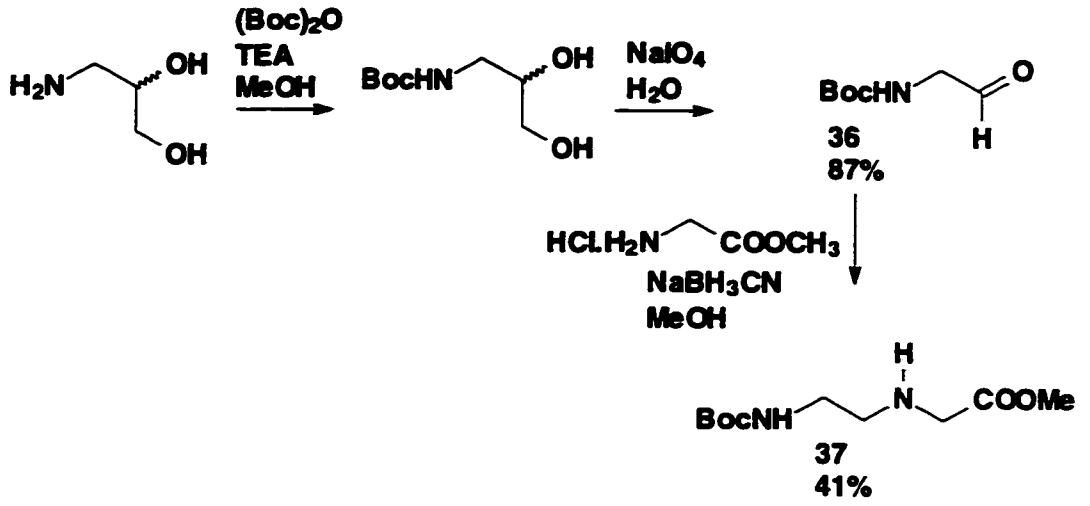
PNAs thymine monomer **28** was prepared using a similar synthetic strategy, where the same thymine derivative is coupled on to the PNA backbone as shown in Scheme 9. In this case the pentflurophenol ester method works well. The free acid PNA monomer is then obtained often saponification with LiOH.



SCHEME 10: Synthesis of Boc Protected PNA Backbone 1

Two different strategies were used to build the Boc protected PNA backbone based on literature procedures [113,114]. The first method (Scheme 10) involved the use of aminoacetonitrile which was Boc protected with Boc anhydride to obtain **33.** The cyano group reduced under hydrogen with Raney Nickel catalyst to obtain **34** and the PNA backbone **35** was obtained after alkylation with bromoethyl acetate.

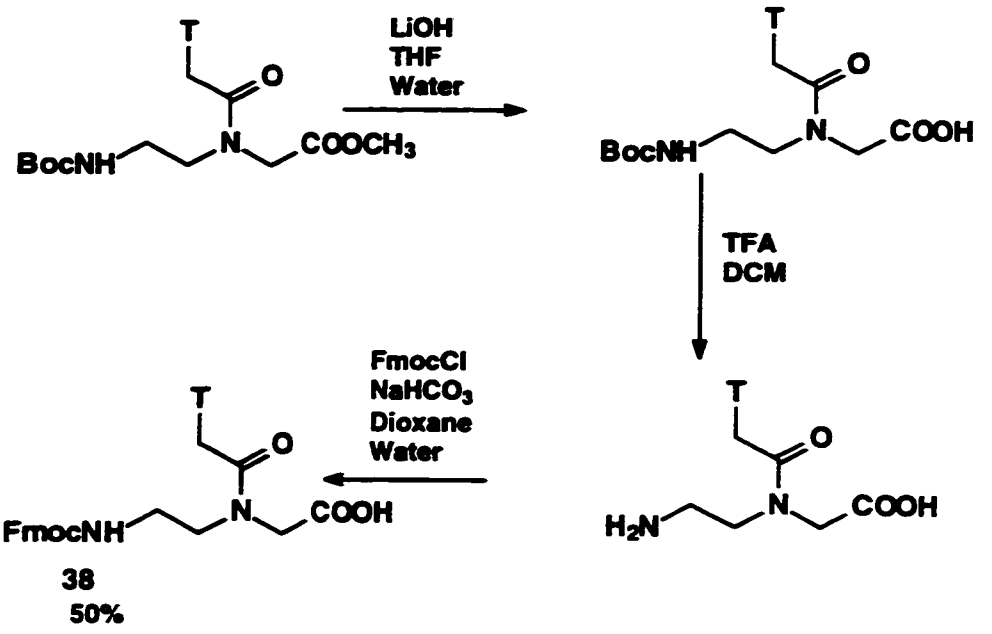
Alternatively a second method was used, aminopropanediol was Boc protected to obtain **35** and oxidatively cleaved with $NaIO_4$ to obtain Boc protected aldehyde **36** (Scheme 11). The backbone **37** was obtained after reductive amination of **36** with glycine methyl ester, with *in situ* Schiff base reduction with $NaBH_3CN$.



SCHEME 11: Synthesis of Boc Protected PNA Backbone 2

2.2.4 Synthesis of Fmoc Protected PNA Backbone

Initially, we believed that we could easily replace the Boc group of compound **28** for the Fmoc group to obtain Fmoc protected PNA **38** (Scheme 12). Unfortunately, the yields for the conversion were poor, presumably due to an intramolecular acyl transfer reaction that has been observed for free amine PNAs under neutral and basic conditions [59] (Figure 33). However, Fmoc PNAs were prepared using other literature procedures [115] (Scheme 13). Alkylation of ethylenediamine with tert-butyl bromoacetate, followed by selective Fmoc protection of the primary amine with Fmoc succinimide produced the Fmoc backbone in good yields. This compound must be stored as the HCl salt to avoid self deprotection by the secondary amine.



SCHEME 12: Synthesis of Fmoc Protected PNA Backbone 1

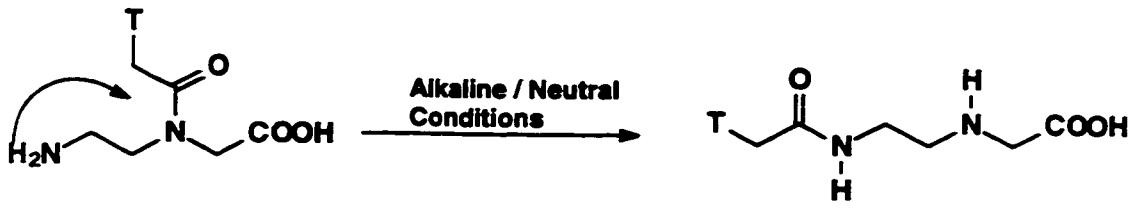
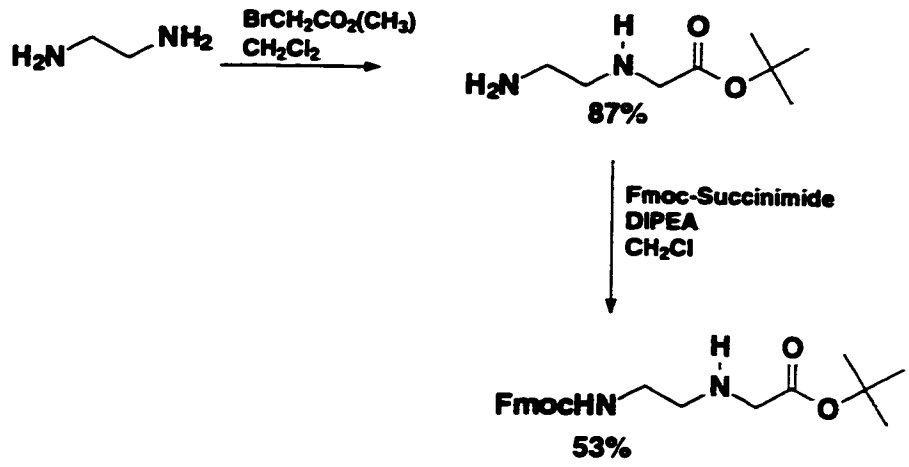


FIGURE 33: Intramolecular Acyl Transfer



SCHEME 13: Synthesis of Fmoc Protected PNA backbone 2

2.2.5 SPPS of APNA/PNA Hexamers

Initially, we decided to attempt the synthesis of the hexamers on the Wang resin, (Figure 20) since this approach is one of the most successful and commonly used methods. Moreover peptide cleavage conditions (TFA) are milder relative to Boc compatible resins which require very harsh conditions. Unfortunately, after many attempts this strategy was abandoned due to the sluggish deprotections and/or couplings observed as the chain was extended to 3-5 units. We believe that this is due to the aggregation of the chains on the solid support. Synthesis on the Rink amide MBHA resin was also attempted (Figure 20). In this case the Fmoc protected PNA hexamer control **39** was obtained (Figure 34). However only small amounts could be obtained, presumably due to aggregation.

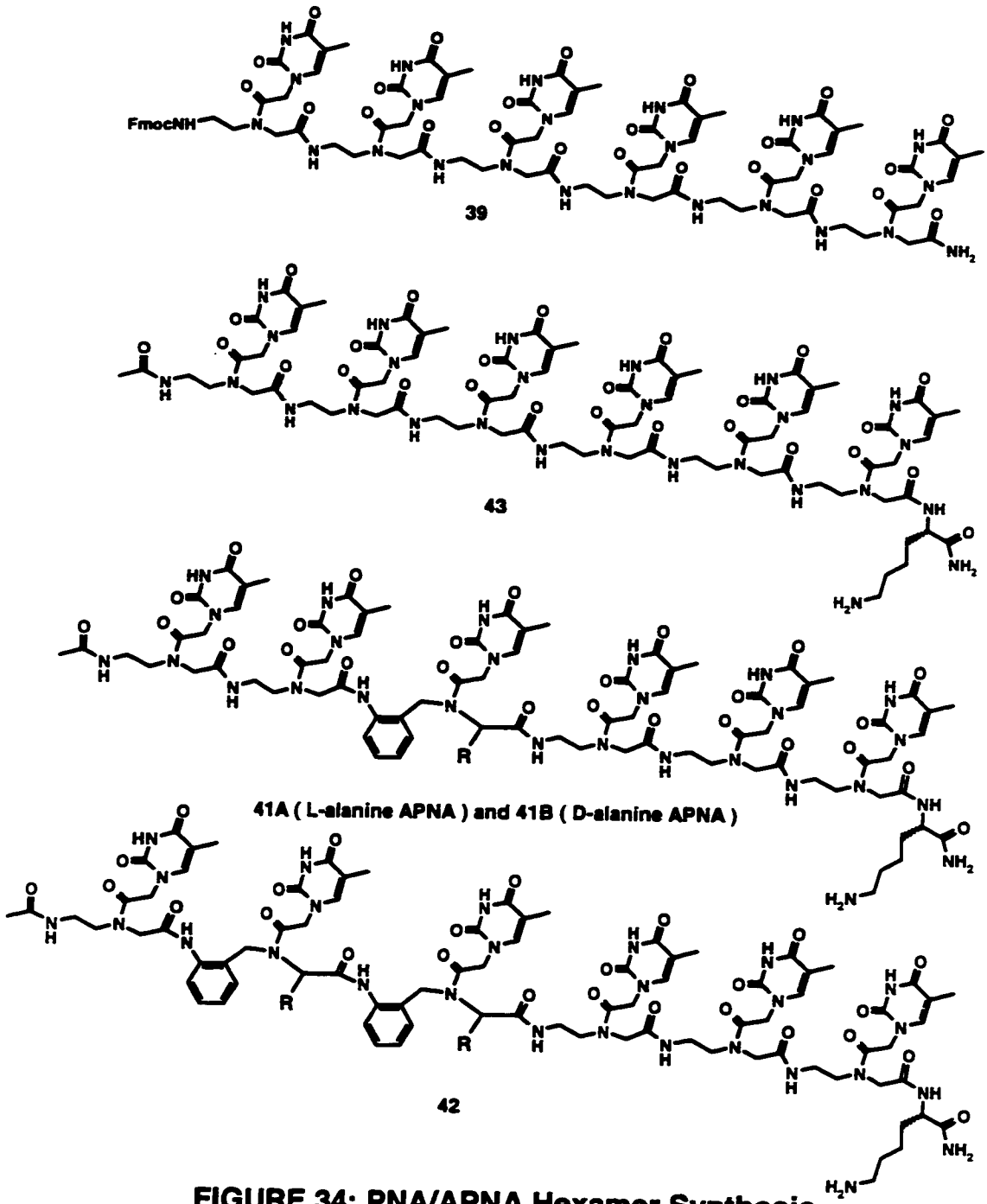
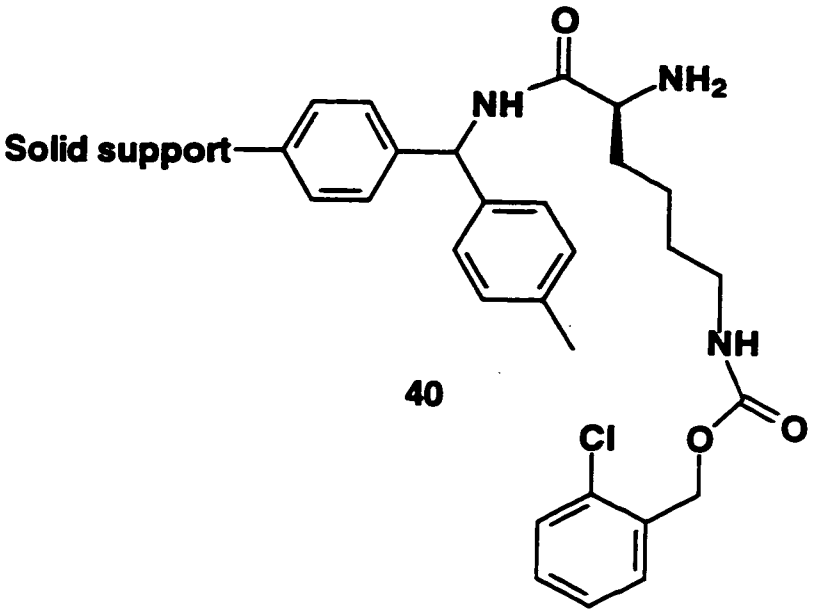


FIGURE 34: PNA/APNA Hexamer Synthesis

Finally, best results were obtained when the peptide was synthesized on the MBHA resin (Figure 20) with a 2-Cl-Z protected lysine attached as the first residue **40** (the

lysine must be protected since its side chain amino group will interfere with the synthesis). This single lysine residue helps reduce the tendency of these compounds to aggregate on the solid support, however the reasons for this effect are unclear [58]. One possible explanation could be that the protected lysine increases solvation of the growing chain making it more accessible to the medium.



40

Moreover, the compounds are acetylated at the end of the synthesis to avoid the intramolecular acyl transfer reaction. Three hexamers **41A** (with one APNA L-alanine mutation), **41B** (with one D-alanine APNA mutation), **42** (with two APNA L-alanine mutations) and the hexamer control **43** (a PNA hexamer) were successfully synthesized on this resin (Figure 14), cleaved from the solid support with TFMSA and were purified by preparative HPLC. The synthesis of **41** (**A** and **B**) worked very

well and resulted in a relatively pure crude mixture with the desired product as the main component. However, the synthesis of **42** resulted in a mixture, with a pentamer containing only one APNA unit. We have speculated that some of the APNA-APNA bond mat have been cleaved during the ester cleavage of **30**, resulting in coupling of an APNA-PNA unit during SPPS rather than $(APNA)_2$-(PNA). Nevertheless, the desired compound was obtained as a minor product.

2.2.6 Preliminary Binding Studies of APNA/PNA Chimeras

The hybridization of compounds **41A, 41B** and **42** with DNA and RNA were examined by measuring their melting temperature with polydeoxyribo Adenine and polyribo Adenine. The results obtained were compared to those a PNA hexamer control **43** (Conditions: 2:1 Hexamer / PolyA, 150mmol, 10 mM NaH2PO4, 0.1 EDTA, pH=7.02). The goal of this particular experiment was to examine the effect of backbone substitution on triple helix formation. It was expected that these compounds would form triple helices with both RNA and DNA since homothymine PNA oligomers are known to form triple helices with DNA and RNA [52]. The resulting melting curves for compounds **41** (A and B) are shown in Figure 35, along with the respective melting curves for the PNA hexamer control **43**.

As shown in Figure 35, we observed a T_m at about 55°C for the PNA control with DNA and 65°C with RNA, whereas the T_m values of compound **41A** were 25°C and 30°C with DNA and RNA respectively. Thus, insertion of a single alanine APNA unit

into the PNA hexamer led to destabilization of the triple helix in both cases. The T_ms for compound **41B** (containing D-alanine APNA) were 30°C and 35°C with DNA and RNA respectively, indicating that substituents with the R configuration are better tolerated, but severe destablization is still observed. Based on these results it is not surprising that hybridization with compound **42** (containing two alanine APNAs) could not be detected. However , as shown in Figure 35, analogues containing a glycine moiety instead of an alanine shows significantly less destablization of the triplex (unpublished results), indicating that the substitutions have a negative effect on binding

These studies indicate that triple helix formation is destabilized by the substitution of one PNA unit for an APNA unit in a PNA hexamer. It is possible that the presence of the aromatic ring in the backbone increases the distance between the bases at the point of mutation, making binding difficult. It is also possible that the mutation locks the compound in an inappropriate conformation for binding. Another possible explanation is that the aromatic ring and the methyl substitution provide extra bulk that is simply not well tolerated in the complex. Even though stability is lost, it is expected that these compounds should penetrate the cell better than PNAs. In such a case, loss of stability could be compensated by increased cell permeability. Longer chains can easily be made using the developed protocol with different substitution patterns. Moreover, the APNA effect in the formation of double

helices has not yet been examined.

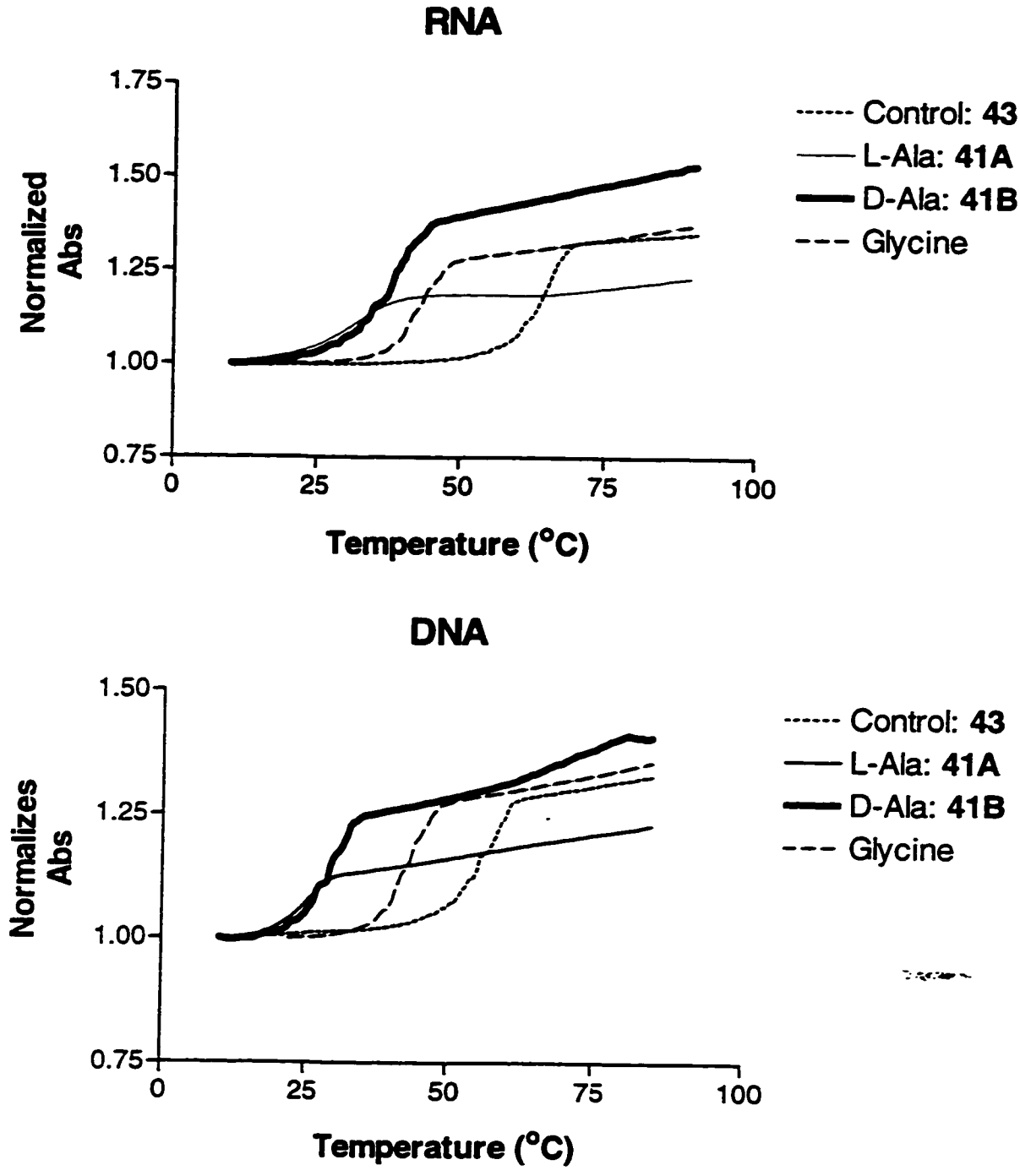


Figure 35: Melting Curves for PNA / APNA Hexamers

3. CONCLUSIONS

An APNA thymine hexamer, composed of monomer **1**, was successfully synthesized in solution. However, thermal denaturation studies failed to indicate if the compound hybridizes to DNA or RNA.

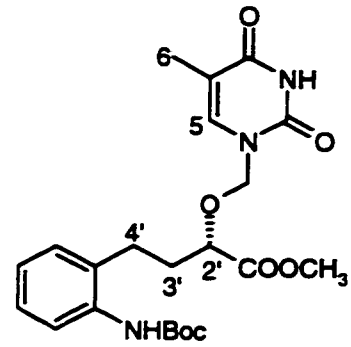
APNA (D and L) alanine monomers **22** were successfully synthesized and incorporated into PNA hexamers by solid phase peptide synthesis. Thermal denaturation studies indicate that DNA and RNA recognition does occur. However, the triple helices are destabilized relative to a PNA hexamer control.

4. EXPERIMENTAL

^{1}H NMRs of some key compounds **(17,18,22,28,29 and 30)** are shown in the appendices

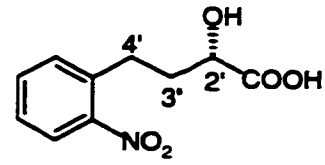
Instrumentation and General Methods. NMR spectra were obtained at RT unless stated otherwise. ^{1}H and ^{13}C-NMR chemical shifts are quoted in ppm and are referenced to the internal deuterated solvent unless otherwise indicated. Mass spectral data were obtained at McGill University, Biomedical Mass Spectrometry Unit and Bio-Mega (Laval, PQ). All reactions were run under a nitrogen atmosphere using oven-dried syringes and glassware when appropriate. THF was distilled from Na/benzophenone, DCM was distilled from P_2O_5, MeOH from Mg turnings, DMF was distilled from CaO. Reagents and solvents were purchased from Aldrich Chemical Co. and VWR Scientific of Canada respectively. Resins were purchased from Novabiochem. The enzymes BSLDH and FDH were purchased from Genzyme (Cambridge, MA) and Boeringer Mannheim (Montreal, Que) respectively. Reversed and normal phase flash column chromatography was carried out on silica gel, following previously reported procedures [116,117]. More detailed caracterization of the compounds involved in the synthesis of **1** and its oligomers has been published [118].

Synthesis of compound 1



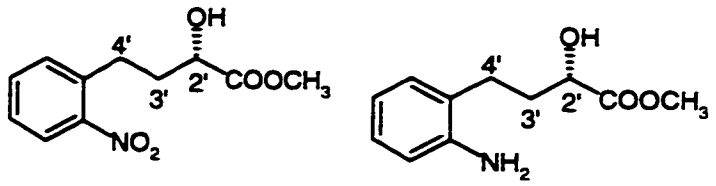
Compound **10** (52 mg, 1 eq, 0.15 mmol) was dissolved in 10% triethylamine in MeOH (1mL) and mixed with di-tert-butyldicarbonate (66 mg, 2 eq, 0.30 mmol). The mixture was stirred at RT for 17 h. The solvent was then evaporated and the residue was partitioned between H_2O (10 mL) and EtOAc (20 mL). The 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 **1** as a light yellow foam with a 92% yield and 98-99% ee. $[\alpha]_D$ -24 (c 0.40, MeOH). TLC (3:1 EtOAc/Hex): Rf= 0.30. ^{1}H 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, $0CH_2$Th), 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.

Synthesis of compound 3



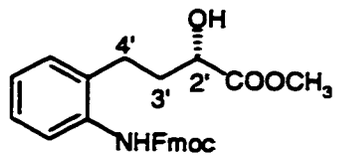
An aqueous suspension of compound **2** was titrated to pH 7 with 1N NaOH and freeze-dried to obtain the sodium salt as a yellow powder. The salt (2.42 g, 1 eq, 10 mmol) was dissolved in TRIS.HCl buffer (5 mM, pH 6, 500 mL) containing sodium formate (1.56 g, 2.3 eq, 23 mmol), NADH (150 mg, 0.02 eq, 0.2 mmol) and dithiothrietol (7.8 mg, 0.005 eq, 0.05 mmol), and the solution was degassed under 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 at 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; 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'), 7.53-7.93 (4ArH). ^{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 compounds 4 and 5



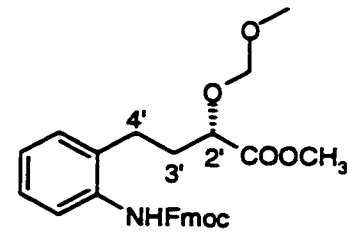
The α-hydroxy acid **3** was dissolved in methanol and reacted with excess diazomethane at RT until the evolution of gas had ceased. Pure methyl ester **4** was obtained after flash column chromatography in 90% yield. $[\alpha]_D$ +55 (c 0.40, $CHCl_3$). TLC (3:2 hex/EtOAc): Rf = 0.23. ^1H NMR (270 MHz, $CDCl_3$) 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, ArH), 7.37-7.59 (m, 2ArH), 7.93 (d, J=7.9 Hz, ArH). 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 EtOH 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. $[\alpha]_D$ +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.

Synthesis of compound 6



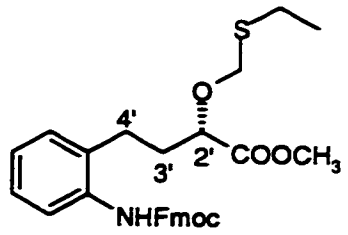
Compound **5** (3.06 g, 1 eq, 14.66 mmol) was dissolved in a mixture of 10% aqueous Na_2CO_3 (17.6 mL, 1 eq) and dioxane (10 mL) and cooled to 0 °C. Fluoroenylmethylchloroformate (3.79 g, 1 eq, 14.66 mmol) was dissolved in dioxane (20 mL) and added dropwise via a syringe, giving a white milky reaction mixture. The mixture was stirred at 0 °C for 1.5 h and allowed to warm up to RT for an additional 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 **6** as a white solid in 84% yield. TLC (1:1 Hex/EtOAc): Rf=0.35. M.p.: 119-120 °C. ^{1}H 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, $NHCOOCH_2C\underline{H}$), 4.48 (d, J=6.9 Hz, $NHCOOC\underline{H}_2CH$)), 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 compound 7



Compound **6** (1.1 g, 1 eq, 2.3 mmol) was dissolved in freshly distilled THF (20 mL) at 0 °C under N_2. Chloromethylmethyl ether (4 ml, 20 eq., 53.2 mmol,) was added, followed by the dropwise addition of dry diisopropylethlamine (1.4 ml, 3 eq., 0.8.0 mmol). 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 (50 mL), followed by extraction with EtOAc (3x50 mL); the organic layer was dried with anhydrous $MgSO_4$ and concentrated to give a light amber oil. Purification by flash column chromatography using 2:1 Hex/EtOAc as the solvent system gave product **7** 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. ^{1}H 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.

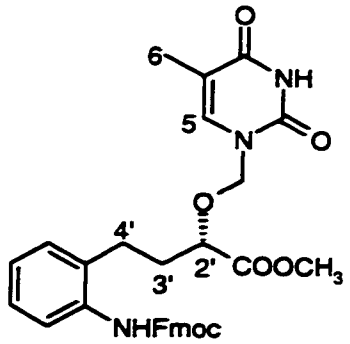
Synthesis of compound 8



Compound **7** (1.52 g, 1 eq, 3.2 mmol) was dissolved in freshly distilled CH_2Cl_2 (40 mL) and cooled to -78 °C under N_2. A solution of dimethylboron bromide in CH_2Cl_2 (1.5 M, 7.6 mL, 11.2 mmol) was added along with diisopropylethylamine (0.04 ml, 0.1 eq., 0.32 mmol). After 20 min, more diisopropylethylamine (1.2 mL, 6.4 mmol) was added followed by ethanethiol (0.8 mL, 9.6 mmol), and the resulting mixture was stirred for 2 h at -78 °C. The reaction was quenched with saturated aqueous $NaHCO_3$ (50 mL) and the mixture was allowed to warm up to R.T. The aqueous layer was extracted with EtOAc (3x100 mL) and the combined organic layers were washed with saturated NaCl (150 mL). The organic layer was then dried with anhydrous $MgSO_4$ and concentrated to dryness. Pure compound **8** 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 **6** . $[\alpha]_D$ -54 (c 1.80, $CHCl_3$). TLC (4:1 hex/EtOAc): Rf=0.19. ^{1}H 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.

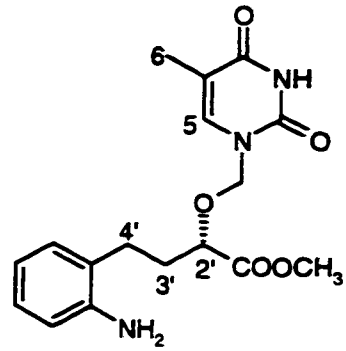
Synthesis of compound 9



Methoxymethylthioethyl ether **8** (218 mg, 1 eq, 0.43 mmol) was dissolved in dry THF (1 mL) in the presence of activated molecular sieves (3Å). A solution of bis(trimethylsilyl)thymine (1.4 mL, 1.5 M in dry THF) was added, followed by the addition of I_2 (109 mg, 1 eq, 0.43 mmol), 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 (10 mL), extracted with EtOAc (3x15 mL) and the organic layer was washed with H_2O (45 mL) and saturated NaCl (45 mL). The organic layer was then dried with anhydrous $MgSO_4$ and concentrated. Purification by flash column chromatography, using 1:2 hex/EtOAc as the eluting solvent, led to the isolation of the desired product in 58% yield (98% based on recovery of starting material). TLC (1:2 hex/EtOAc): Rf= 0.25. ^1H NMR (270 MHz, $CDCl_3$) δ: 1.84 (s, H5), 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, -CHC<u>H</u>$_2$- 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, $CDCl_3$) d: 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.

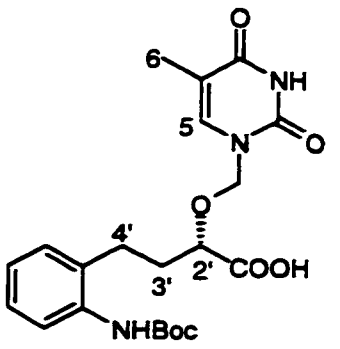
Synthesis of compound 10



Compound **9** (460 mg, 1 eq, 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 **10** 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, H5), 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.

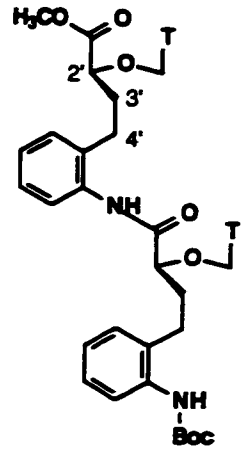
Synthesis of compond 11



To a solution of compound **1** (105 mg, 1 eq, 0.234 mmol) in 3:1 THF/MeOH (5 mL), aqueous LiOH (250 µL, 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 **11** in 94% yield. $[\alpha]_D$ -15 (c 0.37, MeOH). TLC on C_{18}-silica (2:1 MeOH/H_2O): Rf = 0.53. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.50 (s, $C(CH_3)_3$), 1.88 (d, J=1.2 Hz, H5), 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.

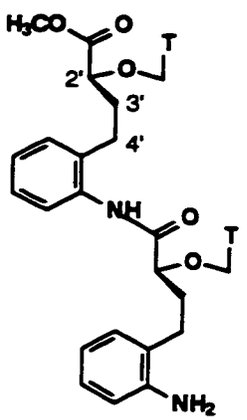
Synthesis of compound 12



Free acid monomer **11** (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 µL, 0.44mmol) was added and the reaction was allowed to stir for 10 min. The free aniline monomer **10** (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, OCH_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, Th vinyH), 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.

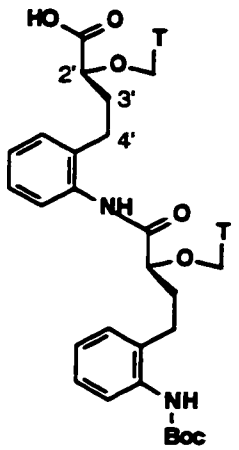
Synthesis of compound 13



Boc-methylester dimer **12** (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 **13** eluted from the column with 50-60% aqueous MeOH; 40% yield of pure **14** 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, Th vinyl H). ^{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.

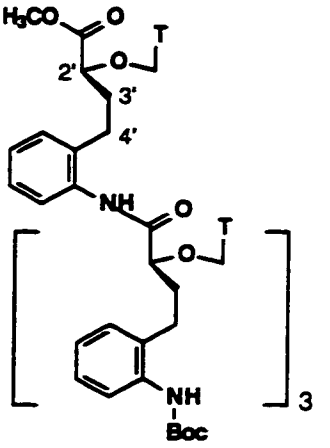
Synthesis of compound 14



Hydrolysis of methyl ester dimer **12** to the corresponding free acid (~99% yield) was carried out using the same reaction conditions as in the preparation of the free acid analog **11**. $[\alpha]_D$ -25 (c 0.25, MeOH). TLC on C_{18}-silica (2:1 MeOH/H_2O): Rf =

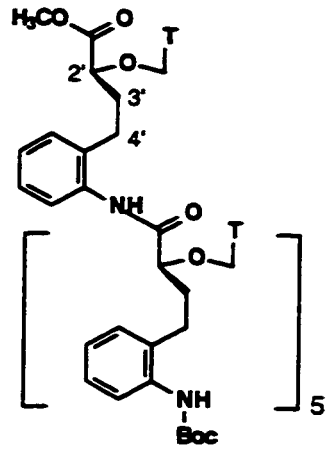
0.38. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.47 (s, $C(CH_3)_3$), 1.84 (bs, Th-CH_3), 1.88 (bs, Th-CH_3), 2.00-2.15 (m, 4H3'), 2.60-2.85 (m, 4H4'), 4.16-4.25 (m, 2H2'), 5.08 (d, A part of AB, J=11.1 Hz, 1H, OCH_2Th), 5.20 (d, B part of AB, J=11.1 Hz, 1H, OCH_2Th), 5.26 (d, A part of AB, J=10.2 Hz, 1H, OCH_2Th), 5.33 (d, B part of AB, J=10.2 Hz, 1H, OCH_2Th), 7.05-7.35 (8H, m, Ar), 7.38 (s, Th vinyl H). 7.55 (s, Th vinyl H), 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.

Synthesis of compound 15



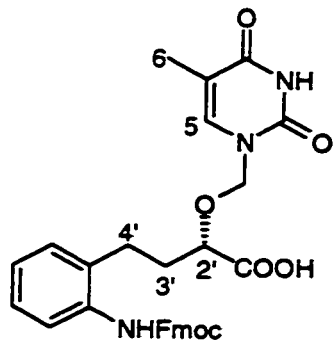
Refer to synthesis of **21**.

Synthesis of compound 17



17 was made from dimer **14** and tetramer **16** using the same procedure used for the synthesis of **12**. Purified on C_{18} column, 13.2 min on Vydac C_{18} 5-100% acetonotrile in water, 0.06% TFA in 15min, 1.5ml/min ^{1}H NMR (300 MHz, CD3OD) δ: 1.48 (s, C(CH3)3), 1.79-1.84 (m, Th-CH3), 2.04 (broad, H3'), 2.7 (m, H4'), 3.55 (s, OCH3), 4.1-4.2 (m, H2'), 5.04-5.24 (m, OCH_2Th), 7.0-7.52 (m, ArH,Th vinyl H), 9.48, 9.34,9.31 (NHs) (See Appendix B).

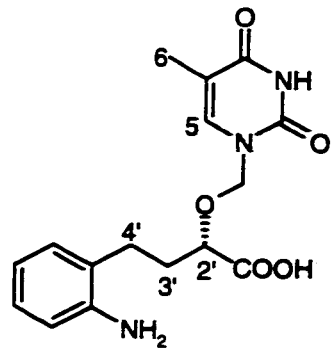
Synthesis of compound 18



19 was protected using the same procedure as for the protection of **6**. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.76 (s, H5), 1.86-2.10 (m, 2H3'), 2.6-2.8 (m, 2H4'), 4.20 (dd,

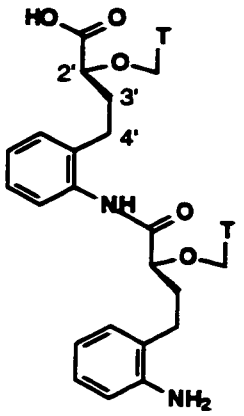
J= 5.2 Hz, H2'), 4.23 (broad, -C<u>H</u>CH_2- of Fmoc), 4.3-4.45 (d, -CHC<u>H</u> $_2$ of Fmoc), 5.02 (d, J=12 Hz, A part of AB, 1H, OCH_2Th), 5.17 (d, J=12 Hz, B part of AB, 1H, OCH_2Th), 7.06-7.84 (14H, Ar, NH, H6) (see Appendix C).

Synthesis of compound 19



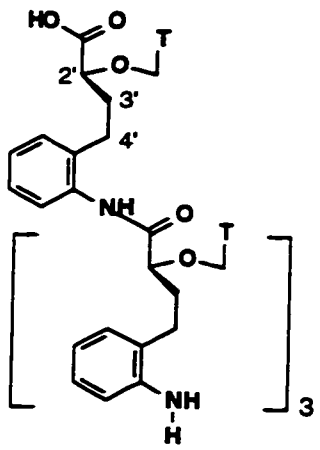
Boc-protected monomer **11** (~10 mg) was dissolved in an anhydrous solution of 15 % trifluoroacetic acid in CH_2Cl_2 (2 mL) and stirred at R.T. under N_2 for 15 min. The fully deprotected monomer **19** was obtained in high purity after evaporation of the reaction mixture to dryness. C_{18}-TLC (3:1 MeOH/H_2O): Rf = 0.77. ^{1}H NMR (300 MHz, D_2O) δ: 1.80 (d, J=1.2 Hz, H5), 1.77-1.93 and 2.05-2.15 (2m, 2H3'), 2.57 (t, J = 7 Hz, 2H4'), 3.85-3.89 (dd, J = 9.3, 3.6, H2'), 5.01 (d, A part of AB, J=11 Hz, 1H, OCH_2Th), 5.22 (d, B part of AB, J=11 Hz, 1H, OCH_2Th), 6.72-7.10 (m, 4H, ArH), 7.34 (d, J=1.2 Hz, H6).

Synthesis of compound 20



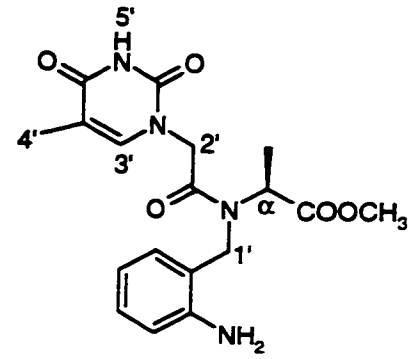
Boc-protected dimer **14** (~15 mg) was dissolved in an anhydrous solution of 15 % trifluoroacetic acid in CH_2Cl_2 (2 mL) and stirred at R.T. under N_2 for 15 min. The fully deprotected dimer **20** was obtained after evaporation of the reaction mixture to dryness. C_{18}-TLC (2:1 MeOH/H_2O): Rf = 0.71. ^1H NMR (300 MHz, D_2O) δ: 1.83 (bs, Th-CH_3), 1.87 (bs, Th-CH_3), 1.70-2.33 (4m, 4H3'), 2.63-2.71 and 2.84-2.91 (2m, 4H4'), 4.00-4.04 and 4.23-4.27 (2m, 2H2'), 5.08 (s, 2H OCH_2Th), 5.26 (d, A part of AB, J=11.1 Hz, 1H, OCH_2Th), 5.32 (d, B part of AB, J=11.1 Hz, 1H, OCH_2Th), 7.05-7.43 (8H, m, Ar), 7.41 (Th vinyl H). 7.54 (bs, Th vinyl H).

Synthesis of compound 21



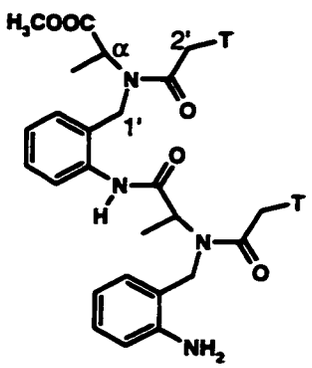
The peptide coupling reaction between dimers **13** and **14** was achieved using the same reaction procedure as in the coupling of their respective monomers. The crude product was purified by C_{18} reversed phase flash column chromatography using a gradient from 100% H_2O to 100% MeOH. The desired APNA tetramer **15** 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. ES MS m/z (assignment): 1392.6 (M^+), 1393.6 (MH^+). Hydrolysis of the methyl ester, followed by removal of the Boc group (using standard conditions in both cases) led to the isolation of tetramer **21** which was purified by preparative C_{18}-TLC (3:1 MeOH/H_2O): Rf = 0.50. ^{1}H NMR (500 MHz, D_2O) showed resonances in the expected chemical shifts regions, based on the ^{1}H NMR spectra of compound **15** . However, due to the great extend of overlapping resonances, the exact chemical shifts of all resonances were not assigned; only those resonances associated with the methyls and H6 protons of the thymine moieties were assigned from the 1D COSY NMR spectra. (See Table 1)

Synthesis of compound 22



To a mixture of **25** (3.49 g, 1 eq; 8.6 mmol) and 10% Pd/C (1.75g) in 100ml acetonitrile was added formic acid (0.87 ml, 2.7 eq; 23 mmol) and TEA (3.5 ml, 2.9 eq; 25 mmol). The mixture was stirred at RT for 18 hours. Reaction was filtered, rinsed with 20 ml 10% TEA in acetonitrile and solvent was removed. Pure **22** (80%) was obtained after flash chromatography (10% DMF in DCM). TLC: Rf= 0.40 (EtOAc). H^1NMR (300MHz, DMSO, 120°C): δ 1.34 (d, J=6.9Hz, alanine CH_3), 1.80 (s, H4'), 3.60 (s, $COOCH_3$), 4.41-4.64 (5H, alanine αH, 2H1', 2H2'), 6.62 (t, J=7.2Hz, 1ArH), 6.72 (d, J=7.8Hz, 1ArH), 7.01 (t, J=7.5Hz, 1ArH), 7.12 (d, 7.5Hz, 1ArH), 7.25 (s, H3'), 10.70 (s, H5'). (see Appendix D)

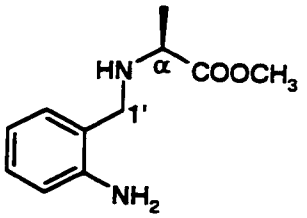
Synthesis of compound 23



HATU (1.05 g, 1.1 eq, 3.1 mmol) and free acid APNA **27** (1.15 g, 1 eq, 3.1 mmol) were dissolved in 20 ml DMF and DIPEA (0.94 ml, 5 eq, 5.5 mmol) was added. After 10 min free amine APNA **22** (1.15 g, 1.2 eq, 3.1 mmol) was added in 30 ml DMF. After 24 hours the solvent was removed and the product was extracted with 3x100 ml EtOAc from 50 ml water. The organic layers were combined and dried with $MgSO_4$. Solvent was removed and the compound is purified by flash

chromatography (5% MeOH, EtOAc), 1g (50%) of dimer was obtained. TLC: Rf= 0.27 (5% MeOH, EtOAc). An analytically pure sample of the dimer could only be obtained in the free acid form after the following procedure. The dimer (1 eq, 0.64 mmol) was dissolved in 100 ml THF and LiOH (4 eq, 2.5 mmol) was added in 10 ml water. After 2 hours the solution was neutralized with 1 M HCl and the solvent was removed. 50 ml EtOAc and 50 ml pH 3 water (0°C) were added and the product was extracted with 3x100 ml EtOAc, maintaining the pH of the water at 3. The organic layers were combined and dried with $MgSO_4$. Solvent was removed to obtain 450 mg (88%) free acid. ^1H NMR (300MHZ, DMSO, 140°C): δ 1.32 (d, J=7.5Hz, alanine CH_3), 1.41 (d, J=6.9 Hz, alanine CH_3), 1.47 (s, BocCH_3), 1.79 (s, 2H3'), 4.47-4.93 (10H, 2αH, 4H1',4H2'), 7.22-7.38 (10H, 8ArH, 2 vinyl H), 8.23 (s, BocNH), 9.14 (s, APNA-NH-APNA), 10.63 (s, 2H5'). MS ES: 803 (M+).

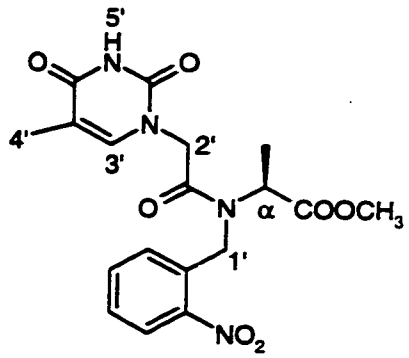
Synthesis of compound 24



To a mixture of L-alanine methyl ester hydrochloride (309 mg, 1.1 eq; 2.2 mmol), 2-nitro benzaldehyde (302 mg, 1.0 eq; 2 mmol), TEA (0.31 ml, 1.1 eq; 2.2 mmol) and acetic acid (240 mg, 2 eq; 4mmol) in 60 ml DCM was added triacetoxysodium borohydride (633 mg, 1.5 eq; 3 mmol) in 10 ml DCM. The mixture was stirred under

nitrogen for 1 hour and 1 eq triacetoxysodium borohydride was added. After 30 min 15 ml of 5% $NaHCO_3$ was added and the product was extracted with DCM (3x50 ml). The organic layers were combined and dried with $MgSO_4$. Pure **24** (73%) was obtained after precipitation o the HCl salt from ether with 3 eq HCl (from a 4M HCl solution in dioxane). TLC: Rf= 0.16 (4:1 hexanes/EtOAc). ^1H NMR (300MHz, DMSO): δ 1.17 (d, J=6.9Hz, alanine CH_3), 2.73 (s,NH), 3.24 (q, J=6.9Hz, alanine αH), 3.59 (s, $COOCH_3$), 3.85 (d, J=15Hz, Part A of AB 1H1'), 4.00 (d, J=15Hz, Part B of AB 1H1'), 7.47-7.53 (m, 1ArH), 7.67-7.69 (m, 2ArH), 7.91 (d, J=7.5Hz, 2 ArH).

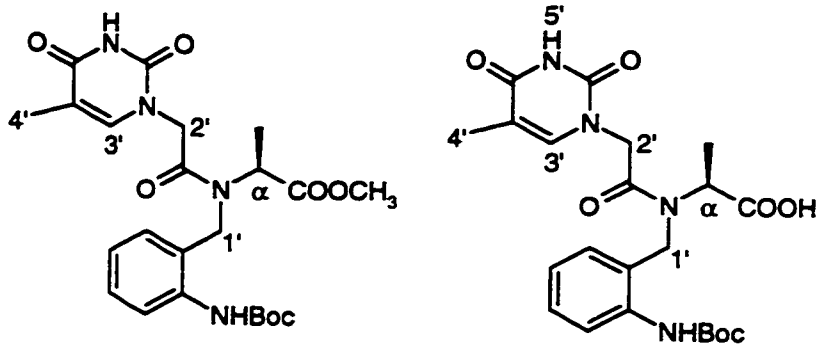
Synthesis of compound 25



To a mixture of HATU (4.76 g, 1.1eq; 12.5 mmol) and thymine acetic acid (2.65 g, 1.1 eq; 12.5 mmol) in 50 ml DMF was added DIPEA (4.9 ml, 2.3 eq; 29 mmol). The mixture was stirred at 0°C for 10 min. **24** (3.14 g, 1 eq; 13.2 mmol) was then added in 75 ml DMF. The mixture was stirred at RT for 24 hours. Solvent was removed, 100 ml water was added and the product was extracted with EtOAc (3x100 ml). The organic layers were combined and dried with $MgSO_4$ and solvent was removed.

Pure **25** (60%) was obtained after trituration with 100ml EtOAc. TLC: Rf= 0.35 (EtOAc). ^{1}H NMR (300MHz, DMSO, 140°C): δ 1.37 (d, J=7.2Hz, alanine CH_3), 1.79 (d, J=1.2Hz, H4'), 3.64 (s, $COOCH_3$), 4.48-5.08 (5H, alanine αH, 2H1', 2H2'), 7.80 (d, J=1.2Hz, H3'), 7.53-7.59 (m, 1ArH), 7.73 (d (broad), 2ArH), 8.06 (d, J=8.1Hz, 1ArH), 10.76 (1s, H5'). αH confirmed by COSY. MS ES: 404 (M+)

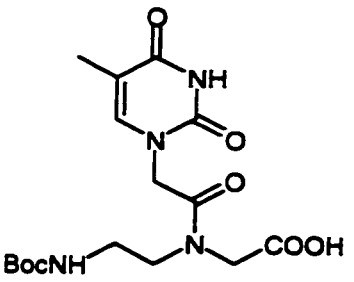
Synthesis of compound 26 and 27



To a mixture of **22** (1.15 G, 1 eq; 3.1 mmol) and sodium bicarbonate (287 mg, 1.1eq; 3.4 mmol) in 125 ml 1,4-dioxane at reflux was added $(Boc)_2O$ (4.66 g, 7 eq; 21.4 mmol). The mixture was stirred at reflux for 3 hours. Solvent was removed and 100 ml water was added and the product was extracted with EtOAc (3x100 ml). The organic layers were combined and dried with $MgSO_4$. Pure **26** (100%) was obtained after flash chromatography (4:1EtOAc). TLC: Rf= 0.36 (4:1 hexanes/EtOAc). ^{1}H NMR (300MHz, DMSO, 140°C): δ 1.31 (d, J=6.9 Hz, alanine CH_3), 1.48 (s, BocCH_3), 1.79 (d, J=0.9Hz, H4'), 3.61 (s, $COOCH_3$), 4.43-4.71 (5H, alanine αH, 2H1', 2H2'), 7.17-7.36 (5H, ArH, H3'), 8.32 (s, BocNH), 10.75 (s, H5'). The ester (1.5 g, 1 eq,

3.2 mmol) was dissolved in 50 ml THF and LiOH (4eq, 12.8 mmol) was added in 15 ml water. After 2 hours the solution was neutralized with 1 M HCl and the solvent was removed. 100 ml EtOAc and 50 ml pH 3 water (0°C) were added and the product was extracted with 3x100 ml EtOAc, maintaining the pH of the water at 3. The organic layers were combined and dried with $MgSO_4$. Solvent was removed to obtain 1.15 g (87%) free acid **27**. ^1H NMR (300MHz, DMSO, 140°C): δ 1.31 (d, J=6.9 Hz, alanine CH_3), 1.48 (s, BocCH_3), 1.79 (d, J=0.9Hz, H4'), 3.61 (s, $COOCH_3$), 4.43-4.71 (5H, alanine αH, 2H1', 2H2'), 7.17-7.36 (5H, ArH, H3'), 8.32 (s, BocNH), 10.75 (s, H5').

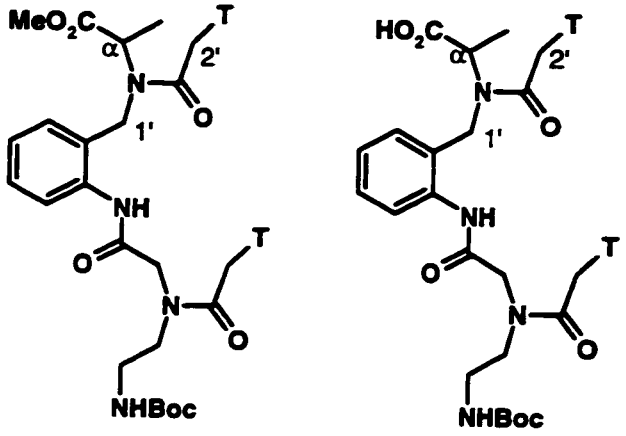
Synthesis of compound 28



Thymine acetic acid (9.1 g, 1 eq, 39 mmol), DCC (8.0 g, 2 eq, 39 mmol) and DMAP (939 mg, 0.2eq, 7.7 mmol) were dissolved in 500 ml DMF at 0°C. The mixture was left stirring for 10 min and a solution of pentafluorophenol (14.2 g, 2 eq, 77 mmol) in 400 ml DMF was added. After 3 hours **37** (16.3 g, 2 eq, 77 mmol) was added in 100 ml DMF and the mixture was left stirring overnight. The mixture was filtered, solvent removed and 100 ml water was added and product was extracted with

3x200ml DCM. The organic layers were combined and dried with $MgSO_4$. Pure PNA (38%) was obtained after flash chromatography (EtOAc).TLC: Rf= 0.15 (EtOAc). ^{1}H NMR (300 MHZ, DMSO, 140°C): δ 1.42 (s, $BocCH_3$), 1.80 (d, J=1.2Hz, $ThCH_3$), 3.20 (q, J=6.6Hz, J=5.7Hz, CH_2), 6.46 (t, J=6.3Hz, CH_2), 3.70 (s, $COOCH_3$), 4.15 (s, CH_2), 4.58 (s, CH_2), 6.29 (s, BocNH), 7.20 (s, vinyl H), 10.66 (s, ThNH). For ester cleavage refer to procedure for the synthesis of **27** from **26** 75% yield. ^{1}H NMR (300 MHZ, DMSO, 140°C): δ 1.42 (s, $BocCH_3$), 1.80 (d, J=1.2Hz, $ThCH_3$), 3.20 (q, J=6.0Hz, J=6.0Hz, CH_2), 6.46 (t, J=6.0Hz, CH_2), 4.07 (s, CH_2), 4.57 (s, CH_2), 6.28 (s, BocNH), 7.20 (d, 0.9, vinyl H), 10.64 (s, ThNH) see Appendix E.

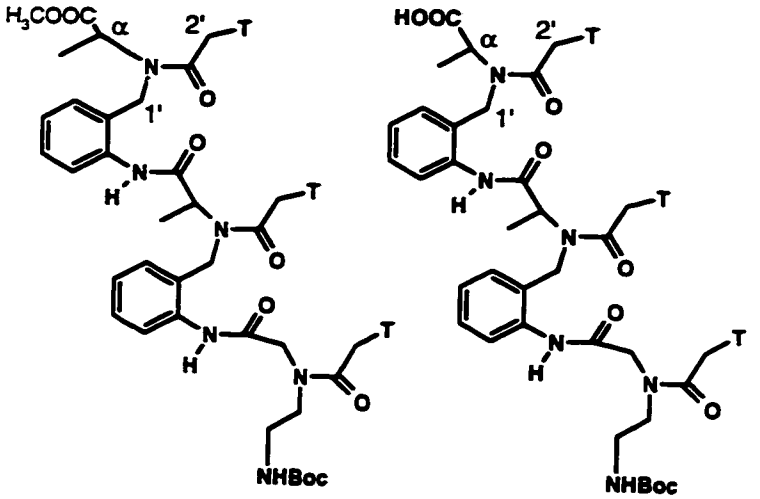
Synthesis of compounds 29 and 31



HATU (457 mg, 1.5 eq, 1.2 mmol) and free acid PNA **28** (461 mg, 1.2 eq, 1.2 mmol) were dissolved in 10 ml DMF and DIPEA (0.41 ml, 2.4 eq, 2.4 mmol) was added. After 10 min free amine APNA **22** (299 mg, 1 eq, 0.8 mmol) is added in 15 ml DMF. After 24 hours the solvent was removed and the product was extracted with 3x50

ml EtOAc from 25 ml water. The organic layers were combined and dried with $MgSO_4$. Solvent was removed and the compound was purified by flash chromatography (10% MeOH, EtOAc), 420 mg (70%) of dimer **29** was obtained. TLC: Rf= 0.20 (5% MeOH, EtOAc). ^1H NMR (300MHZ, DMSO, 140°C): δ 1.34 (d, J=7.2Hz, alanine CH_3), 1.42 (s, BocCH_3), 1.80 (d, 2ThCH_3), 3.25 (q, J=6.3Hz, 6.3Hz, PNA CH_2), 3.29-3.50 (PNA CH_2), 3.60 (s, $COOCH_3$), 4.22 (s, PNA CH_2), 4.44-4.68 (7H, PNA CH_2, αH, 2H1', 2H2'), 6.32 (s, BocNH), 7.23-7.31, 7.39-7.46 (6H, 4ArH, 2 vinyl H), 9.26 (s, PNA-NH-APNA), 10.63 (s, 2ThNHs) See Appendix F, MS ES: 764 $(M+Na)^+$. The chimera (385 mg, 1 eq, 0.52 mmol) was dissolved in 15 ml THF and LiOH (4 eq, 2.1 mmol) was added in 5 ml water. After 2 hours the solution 30 ml water was added and the pH set to 3 at 0°C. The product was extracted with 5x50 ml EtOAc. The organic layers were combined and dried with $MgSO_4$. Solvent was removed to obtain 96% yield free acid.

Synthesis of compounds 30 and 32



Refer to the procedures used for the synthesis of **29** and **31**. Yield: 36% ^{1}H NMR (300MHz, DMSO, 140°C): δ 1.30-1.44 (m, alanine CH_3, BocCH_3), 1.76, 1.79 (2s, ThCH_3), 3.22 (PNA, CH_2), 3.50 (PNA CH_2), 3.58 (s, COOCH_3), 4.23 (s, PNA CH_2), 4.43-4.88 (PNA CH_2, αCH3,2 H1', 2H2'), 6.30 (s, BocNH), 7.20-7.43 (ArH, vinyl H), 9.15, 9.33 (2s, PNA-NH-APNA, APNA-NH-APNA), 10.63 (s, ThNH) (See Appendix G) MS ES: 1105 $(M+Na)^+$

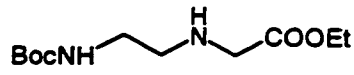
Synthesis of compound 33

$(NCCH_2NH_4)_2SO_4$ (15.4 g, 1 eq, 100 mmol) and $(Boc)_2O$ (24 g, 1.1 eq, 110 mmol) were dissolved in 300 ml DCM at 0°C followed by dropwise addition of TEA (39 ml, 2.8 eq, 280 mmol). Reaction was left stirring overnight, solvent was removed and the residue was dissolved in 300 ml ether. The ether layer was washed with 100 ml water and brine and was dried with $MgSO_4$. The solvent was removed and 11.7g (75%) of **33** was obtained. TLC: Rf= 0.55 (1:1 EtOAc\Hexanes). ^{1}H NMR (300MHZ, CD_3OD): δ 1.49 (s,9H), 3.05-3.25 (m, 2H), 3.45-3.56 (m, 2H), 3.63-3.70 (m, 2H)

Synthesis of compound 34

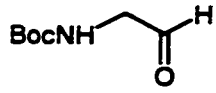
6g of **33** was dissolved in a 10% NH_3/EtOH (100 ml) suspension of Raney Nickel (~1 tablespoon) and left under 50 psi H_2 for 20 hours. The mixture was filtered through Celite and solvent was removed. Obtained 4.5 g of **34**.TLC: Rf= 0.20 (10% MeOH/ EtOAc).

Synthesis of compound 35



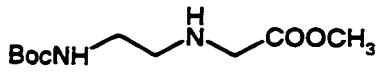
Ethyl bromoacetate (2.18 ml, 0.7 eq, 19 mmol) was added to a solution of **34** (4.5 g, 1 eq; 28mmol) in 95 ml acetonitrile at 60°C. After 15 hours the reaction was cooled, filtered and the solvent was removed. The crude mixture was purified by flash chromatography. (EtOAc). 1.8 g obtained (38%). ^{1}H NMR (300 MHZ, $CDCl_3$): δ 1.28 (t, J=6.3Hz, $COOCH_2\mathit{CH_3}$), 1.44 (s, $BocCH_3$), 1.66 (s, NH), 2.74 (t, J=6Hz, CH_2), 3.21 (q, J= 6Hz, 5.4Hz, CH_2), 3.39 (s, CH_2), 4.19 (q, J=6.3Hz, 7.5Hz, $COOCH_2$), 5.02 (s, BocNH).

Synthesis of compound 36



3-aminopropane-1,2-diol (10 g, 1 eq, 0.11 mol) and $(Boc)_2O$ (25 g, 1.1 eq, 0.12 mmol) were dissolved in 100ml 20% TEA/MeOH. The mixture was refluxed for 30 min and the solvent was removed. Yield 100%. TLC: Rf= 0.11 (1:1 DCM/ EtOAc). H^1 NMR (300MHZ, $CDCl_3$): δ 1.47 (s, $BocCH_3$), 4.07 (d, J=5.4Hz, CH_2), 4.98 (s, BocNH). The Boc protected diol (20.7 g, 1 eq, 0.11 mol) is dissolved in water and $NaIO_4$ (27.8 g, 1.2 eq, 27.8 g) is added. The mixture is stirred for 3 hours, filtered and extracted with 5x400ml DCM. The organic layers were combined and dried with $MgSO_4$. 15.3 g (87%) was obtained after removal of solvent. TLC: Rf= 0.41 (1:1 DCM/ EtOAc).

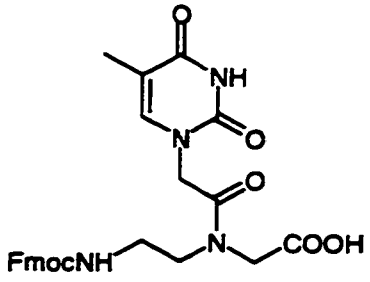
Synthesis of compound 37



$NaBH_3CN$ (5.7 g, 1 eq, 81 mmol) was added to a solution of glycine methyl ester hydochloride (25.8 g, 2.5 eq, 204 mmol) and **36** (12.9 g, 1 eq, 81 mmol). After 24 hours of stirring at room temperature, the solvent was removed 200 ml was water added, the pH set to 8 (1M HCl) and the product extracted with 5x200 ml DCM. The organic layers were combined and dried with $MgSO_4$. Pure **37** (41%) was

obtained after flash column chromatography (EtOAc). TLC: Rf= 0.15 (EtOAc). ^{1}H NMR (300 MHZ, $CDCl_3$): δ 1.44 (s, $BocCH_3$), 1.73 (s, NH), 2.74 (t, J= 5.4Hz, $BocNHCH_2\mathit{CH_2}NH$), 3.21 (q, J= 5.4Hz, 6Hz, $BocNH\mathit{CH_2}CH_2NH$), 3.42 (s, $NH\mathit{CH_2}COOCH_3$), 3.73 (s, $COOCH_3$), 5.02 (s, BocNH).

Synthesis of compound 38



28 was treated with 15% TFA in DCM and solvent was removed. The crude mixture was Fmoc protected as **6**. ^{1}H NMR (300 MHZ, DMSO): δ 1.72 (d, $ThCH_3$), 3.14-3.39 (m, $NH\mathit{CH_2}CH_2$, $NHCH_2\mathit{CH_2}$), 3.98-4.65 (m, $2CH_2$, $FmocCH_2$, FmocCH), 7.26-7.90 (Ar, vinyl H), 11.31,11.29 (2s, ThNH).

OLIGOMERIZATION ON SOLID SUPPORT

Synthesis of $H_2NCO(PNA)_6NHFmoc$ on Rink Amide resin (100 mg, 0.54 mmol/g loading). 39

DEPROTECTION:

2 ml 20% piperidine in 20% DMSO/NMP (3x5min). Wash with 3x20% DMSO/NMP. Do Kaiser test

COUPLING:

Add 1ml 20% DMSO/NMP, HATU (2 eq, 0.108 mmol), free acid Fmoc PNA (2 eq, 0.108 mmol) and DIPEA (4 eq, 0.216 mmol), shake for 1hr (first coupling 4 hours). Do Kaiser test.

CAPPING:

Wash with 3x2 ml 20% DMSO/NMP. Add 2 ml 0.5M acetic anhydride, 0.5M DIPEA in NMP for 2x10 min. Wash 3x2 ml 20% DMSO/NMP. (No capping after last coupling)

After last coupling wash with 3x2 ml 20% DMSO/NMP, 3x2 ml DCM and dry overnight under vaccum. Add 5 ml 95% TFA (5% water) and stir for 3 hours. Filter and remove solvent. Purified on C_{18} column, 14.1min on Vydac C_{18} 5-100% acetonotrile in water, 0.06% TFA in 15min, 1.5ml/min . MS EI: 1837 (M+)

Synthesis of 41A, 41B, 42 and 43 on Lys(2-Cl-Z) preloaded MBHA resin (100 mg, 0.43 mmol/g loading)).

Resin is preswollen in DCM overnight.

DEPROTECTION:

With 2 ml 1:1 TFA/DCM 1x2min, 1x30min. Wash with 2ml DCM, 4x20s, DMF, 2x20s, DCM, 4x20s.

NEUTRALIZATION:

With 2 ml DIPEA/DCM (1:19) 2x3min. Wash with 2 ml DCM, 4x20s.

COUPLING:

Add 1ml DMF, HATU (2 eq, 0.086 mmol), free acid Boc PNA (2 eq, 0.086 mmol) and DIPEA/DCM (1:19) (4 eq, 0.17 mmol), shake for 1hr (first coupling 2 hours). Do Kaiser test. Wash with 2 ml DMF, 2x20s, 1x2min, DCM, 4x1min.

CAPPING:

Add 10ml 2:1:1 DCM/$(Ac)_2O$/pyridine for 5min (Except end cap 2x10min). Wash with DCM 6x1min.

After last coupling wash with 3x2 ml DCM and dry overnight under vaccum. Add

2ml TFA and 0.3ml thioanisole at 0°C, stir for 10 min, add 0.2ml TFMSA and stir for 3 hours.

Workup A (neutralization method)

Filter, rinse with 4x2ml TFA, add 10ml water and neutralize with conc. ammonium hydroxide. Remove solvent. Purification by reverse phase chromatography to remove salts (water to acetonitrile), followed by preparative HPLC.

Workup B (precipitation method)

Filter, rinse with 4x2ml TFA, add 10ml ether. The precipitate is centriuged and washed with ether . The crude mixture is then purified by HPLC. Retentions times on Purified on C_{18} column, 13.2 min on Vydac C_{18} 5-100% acetonotrile in water, 0.06% TFA in 15min, 1.5ml/min .

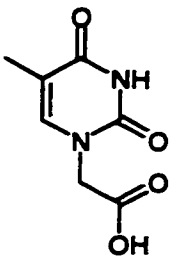
41L: 9.5 min, MS ES: 1861

41D: 9.5 min, MS ES: 1861

42: 10.7 min, MS ES: 1937

43: 8.2 min, MS ES: 1785

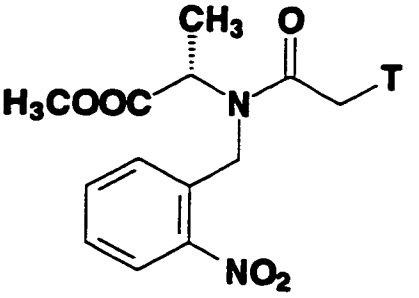
Synthesis of thymine acetic acid 44



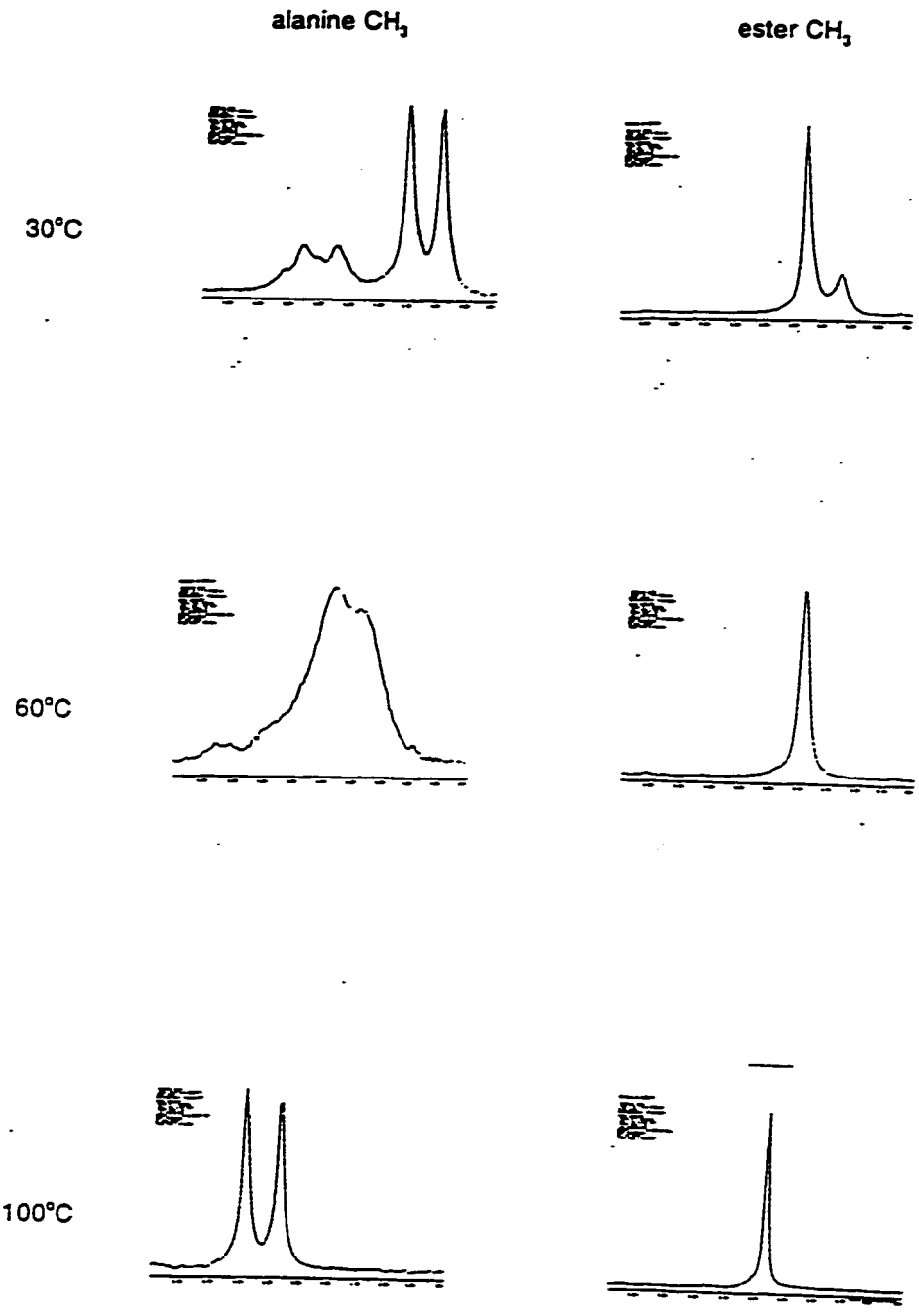
To a suspension of thymine (1 eq, 4 mmol) and K_2CO_3 (1eq, 4mmol) in dry DMF (12 ml) was added $BrCH_2COOEt$ (1 eq, 4 mmol), the mixture was stirred under nitrogen for 12 hours. The resulting solution was filtered and evaporated to dryness. The solid residue was cooled to 0°C, treated with 4 ml water and 0.15 ml 4M HCl then stirred for 30 min. The solid was collected by filtration and washed with 2x2 ml water. The compound was purified by flash column chromatography (2% MeOH, $CHCl_3$). TLC: Rf= 0.15 (2% MeOH, $CHCl_3$). ^1H NMR (300MHz, DMSO): δ 1.26 (t, J=6.9Hz, $COOCH_2\mathit{CH_3}$), 1.86 (d, J=1.5Hz, vinyl H), 4.21 (q, J=7.2 Hz, $COO\mathit{CH_2}CH_3$), 4.47 (s, CH_2), 7.37 (d, J=0.9Hz, CH_3). The solid was treated with 4ml water, 2ml 2M NaOH and boiled for 10 min. The mixture was cooled to 0°C and treated with 3 ml 4 M HCl for 30 min. The solution was filtered and the solid washed with 3x2ml water. Pure compound (36%) was obtained after drying overnight over P_2O_5. ^1H NMR (300MHZ, DMSO): δ 1.74 (s, CH_3), 4.36 (s, CH_2), 7.50 (s, vinyl H), 11.36 (s, NH).

APPENDIX A

Temperature dependance of methyl resonances of :

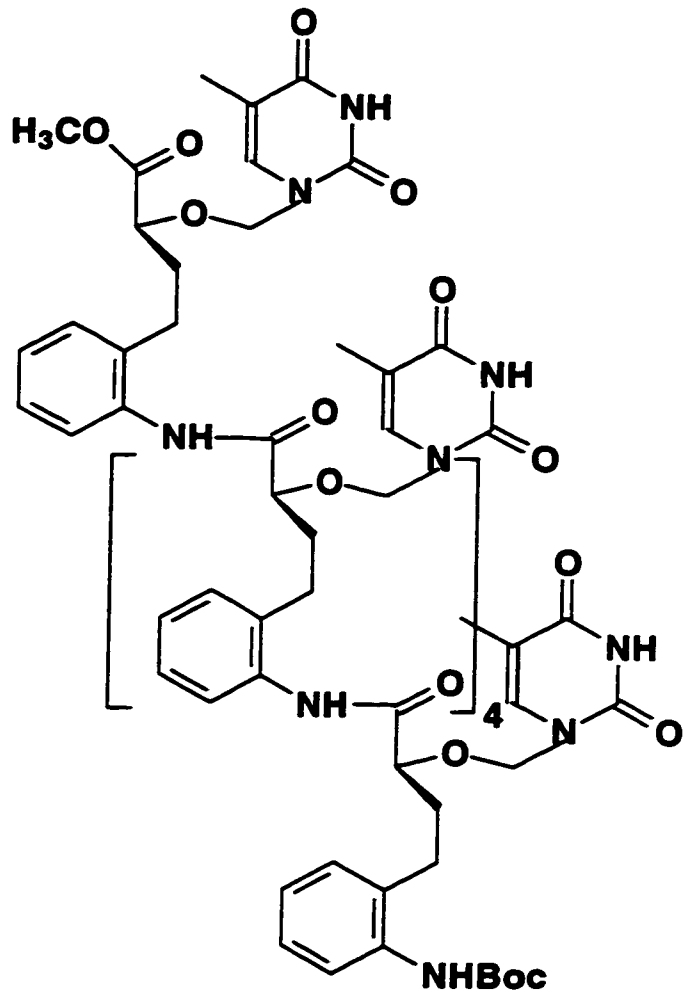


(22)

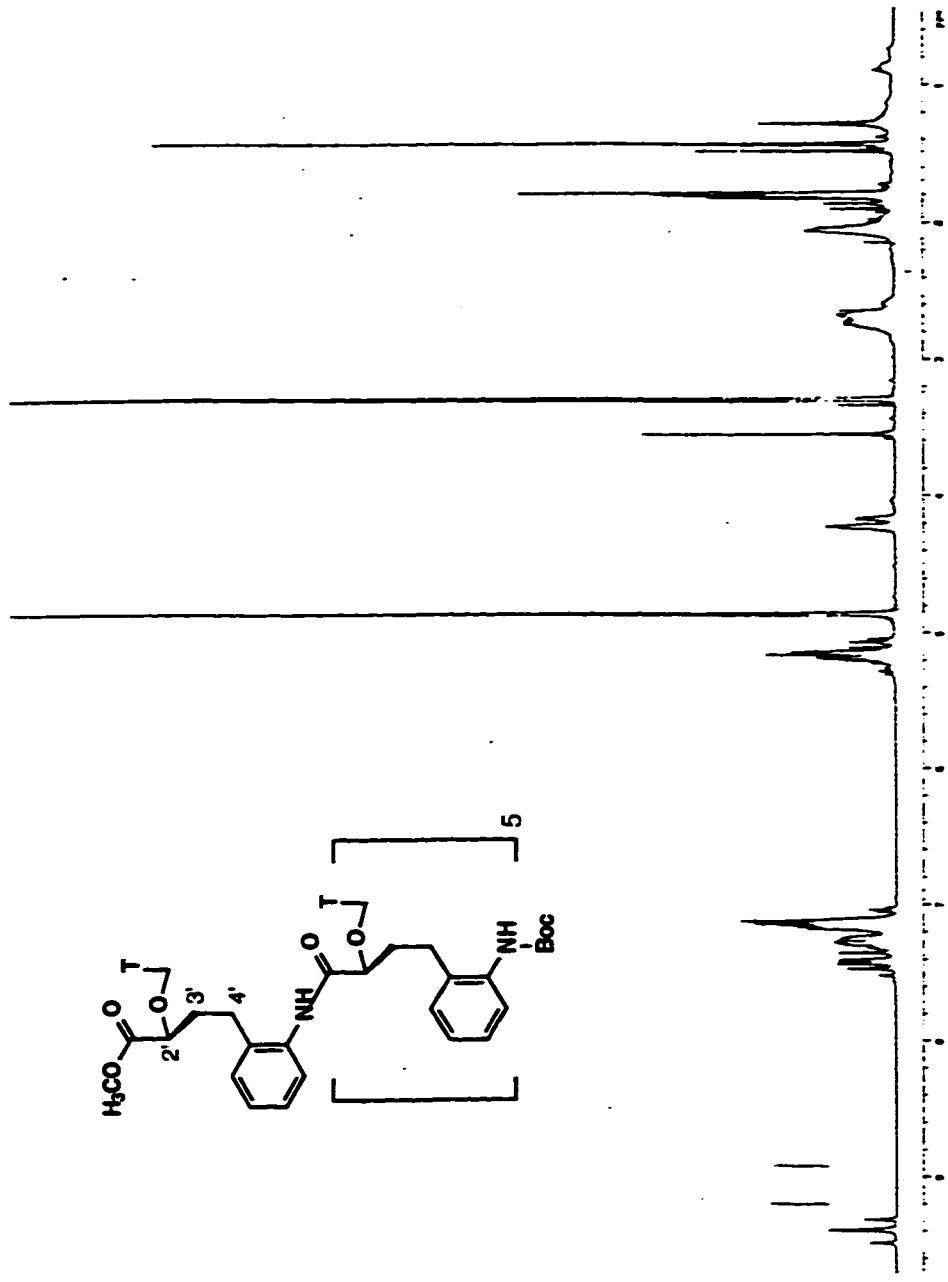


Appendix A: Temperature Dependance of the Methyl ^1H Resonances of 22 in DMSO at 300MHz

APPENDIX B

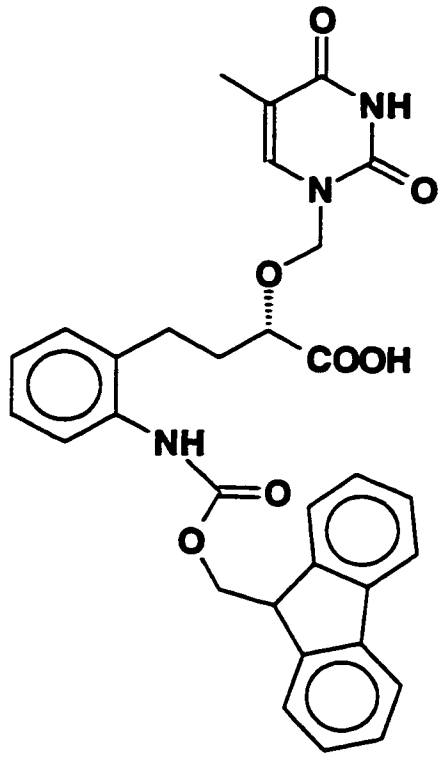


(17)

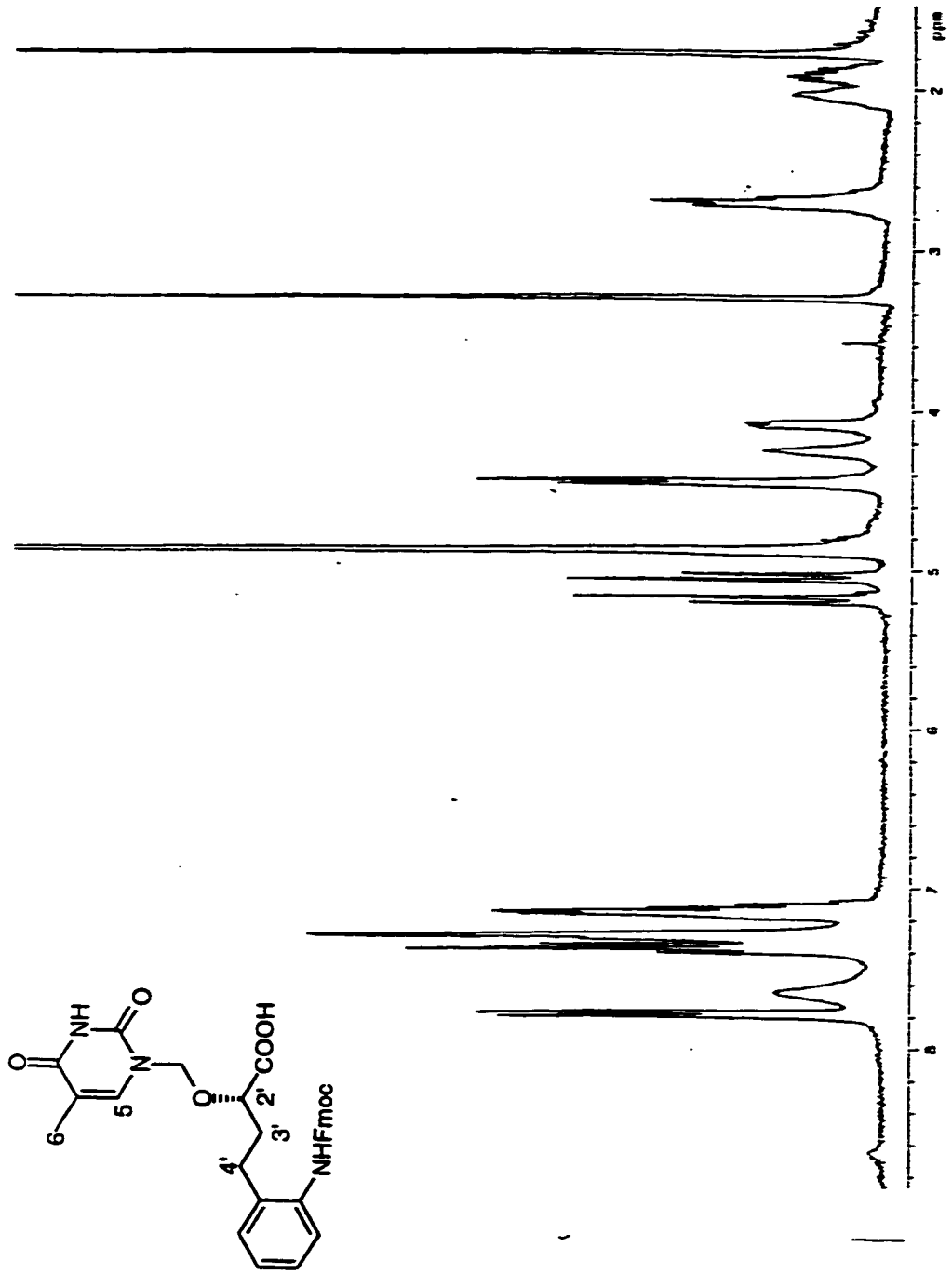


Appendix B: ^{1}H NMR Spectrum of 17 (500 MHz, CD_3OD)

APPENDIX C

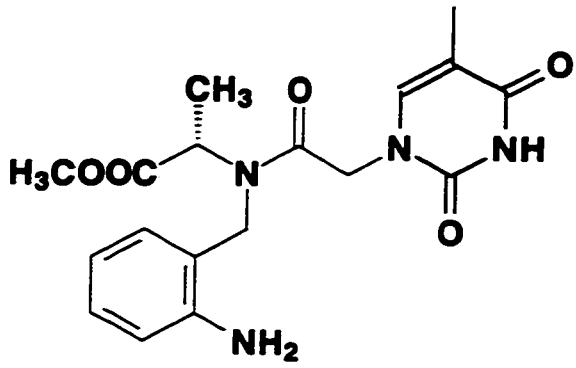


(18)

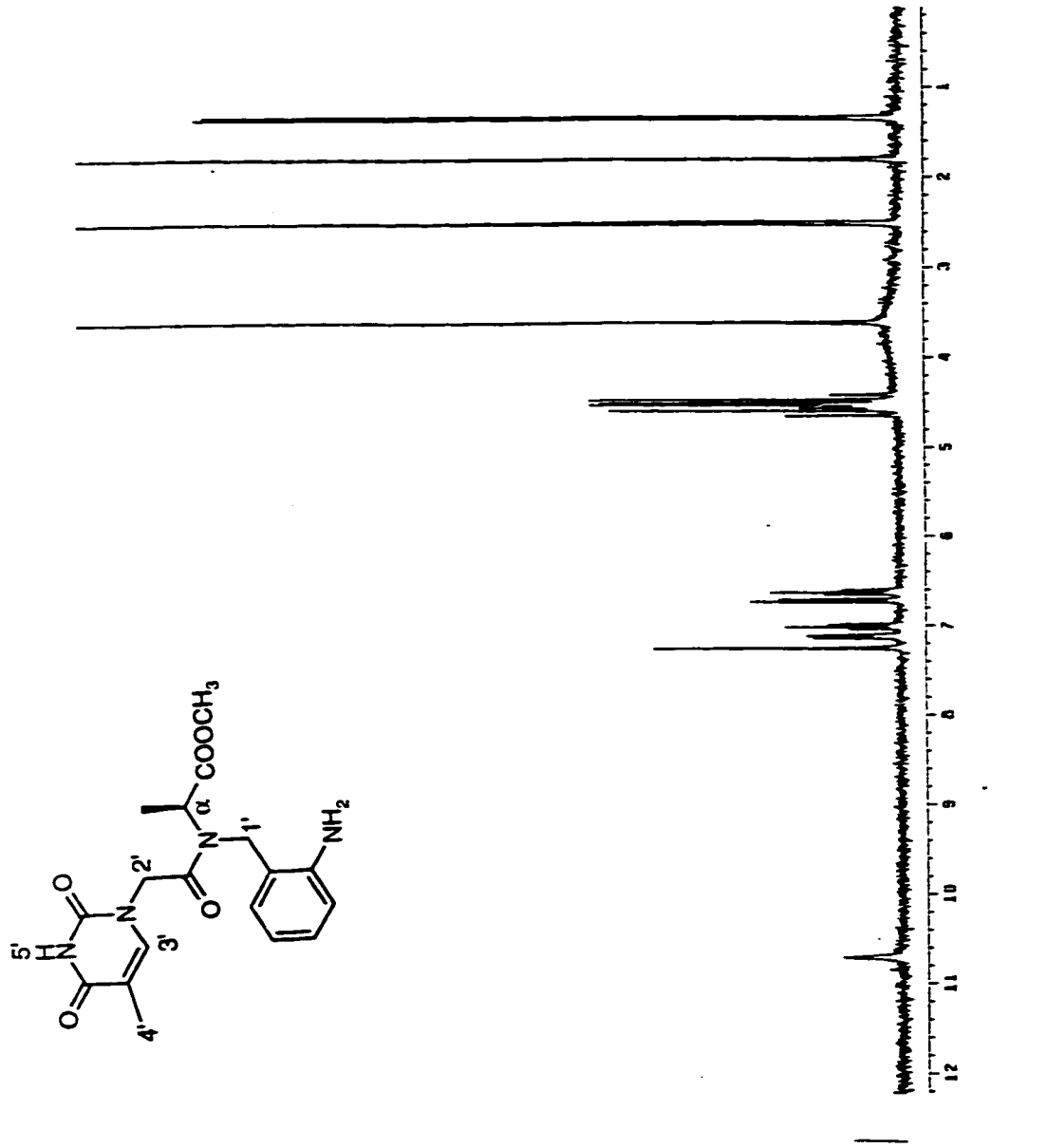


Appendix C: ^{1}H NMR Spectrum of 18 (300 MHz, CD_3OD)

APPENDIX D

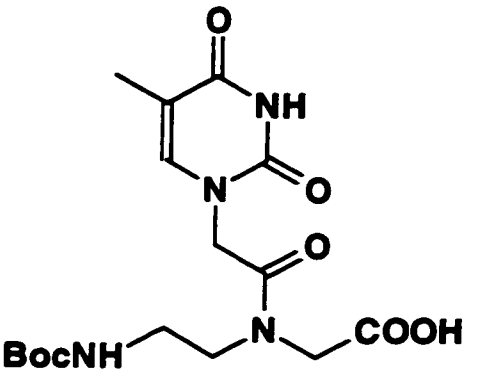


(22)

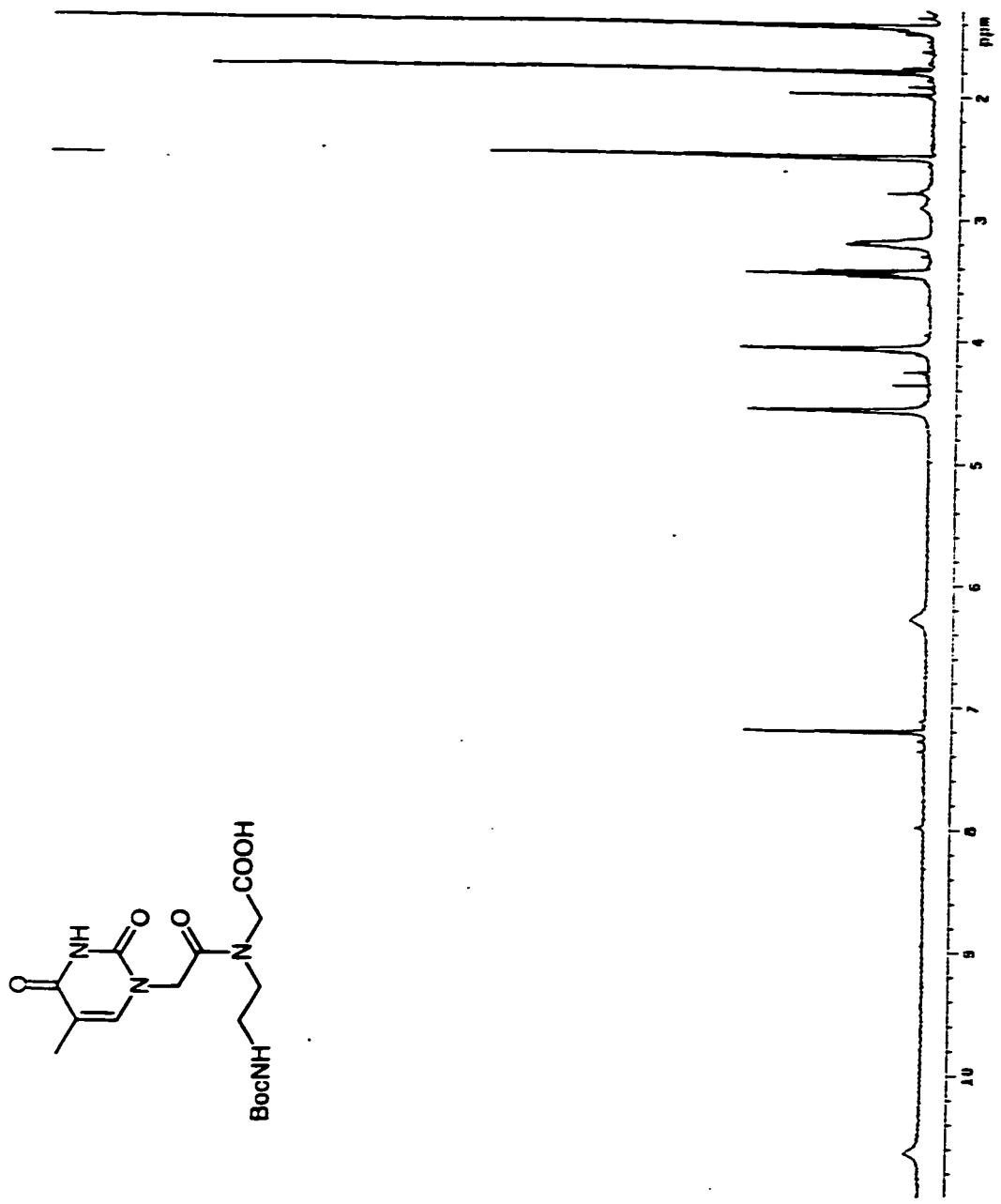


Appendix D: ^{1}H NMR Spectrum of 22 (300 MHz, DMSO at 120 °C)

APPENDIX E

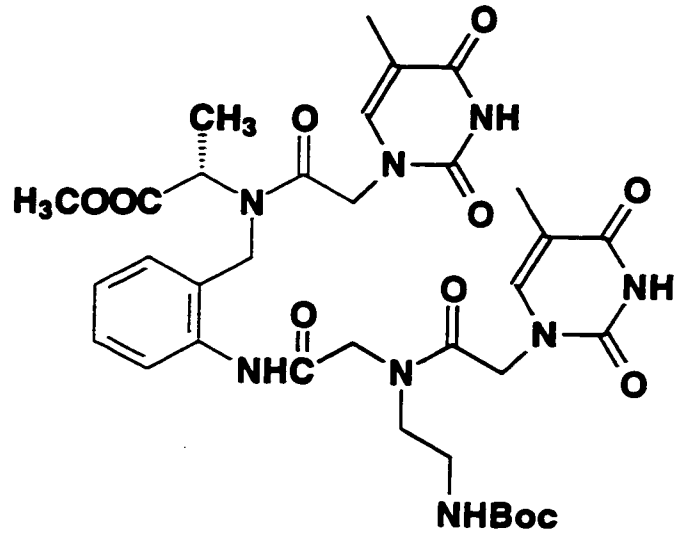


(28)

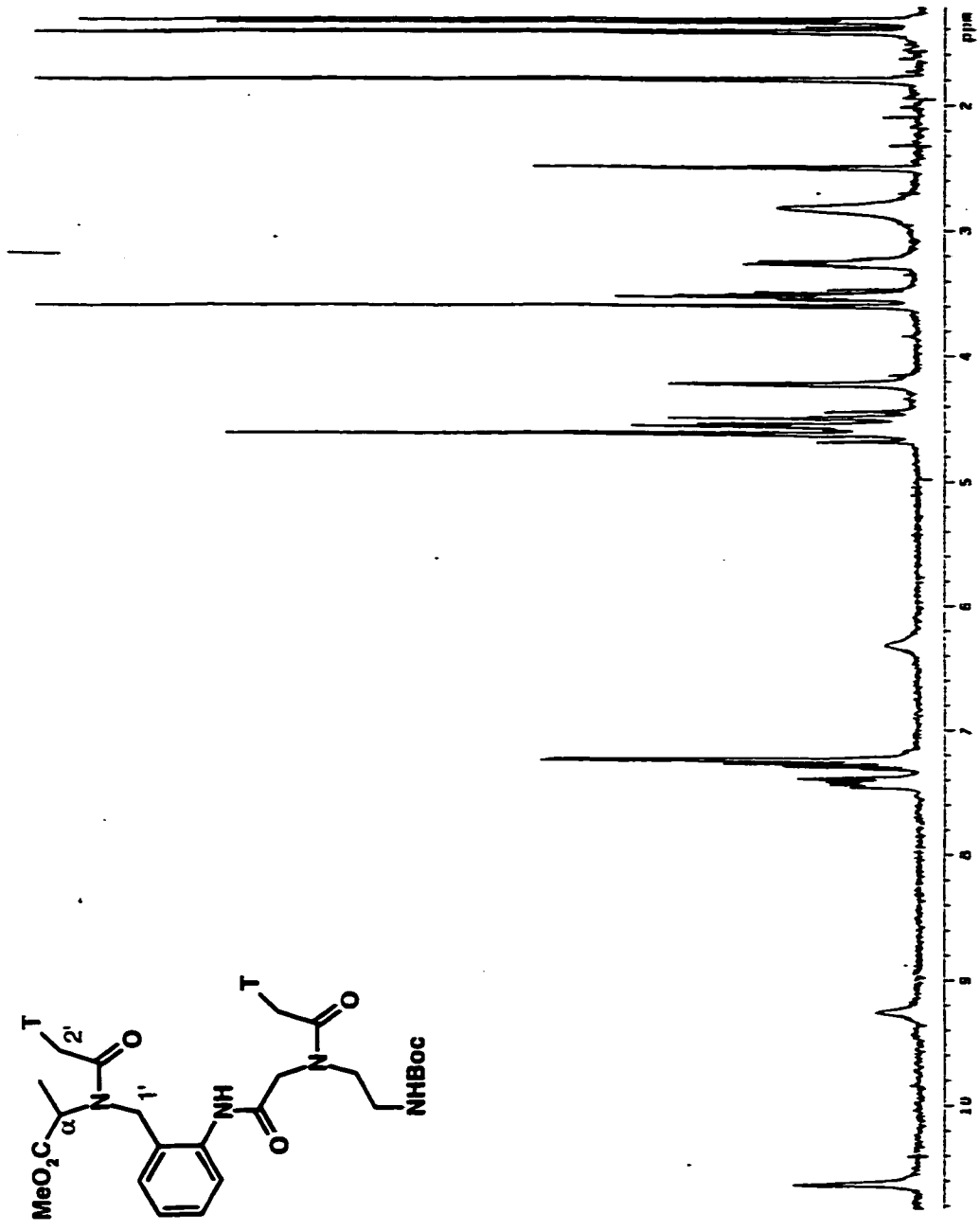


Appendix E: ^{1}H NMR Spectrum of 28 (300 MHz, DMSO, 140°C)

APPENDIX F

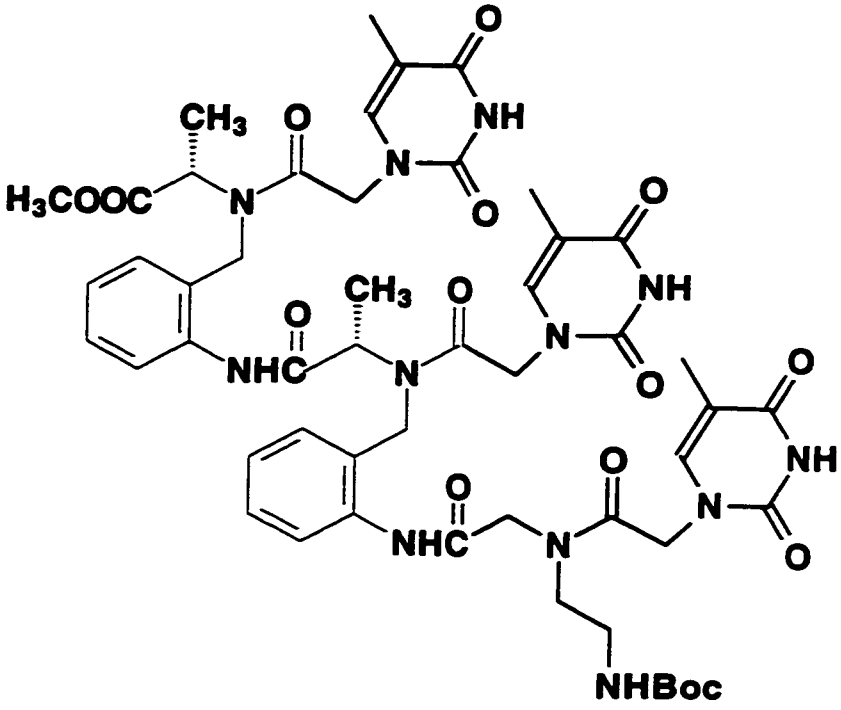


(29)

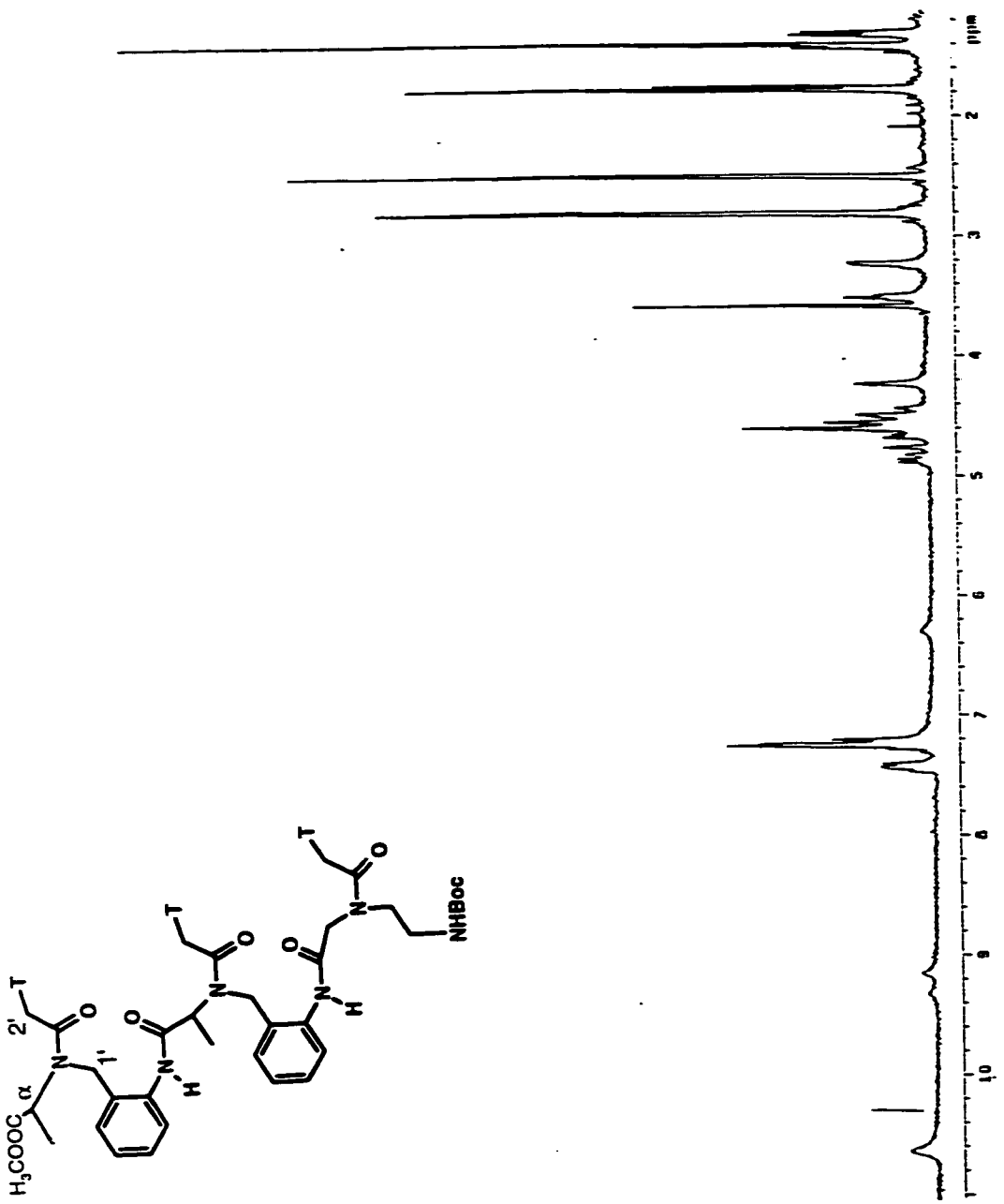


Appendix F: ^{1}H NMR Spectrum of 29 (300 MHz, DMSO, 140°C)

APPENDIX G



(30)



Appendix G: ^{1}H NMR Spectrum of 30 (300 MHz, DMSO, 140°C)

REFERENCES

[1]. Dickerson R. E., Drew H. R., Conner B. N., Wing R. M., Fratini A. V., Kopka M. L., *Science*, **216**, 475 (1982).

[2]. Wing R., Drew H., Takano T., Broka C., Tanaka S., Itakura K., Dickerson R. E., *Nature*, **287**, 755 (1980).

[3]. Calladine C. R., *J. Mol. Biol.*, **161**, 343 (1982).

[4]. Benevides J. M., Wang H.-J., Rich A., Kyogoku Y., van der Marel G.A., van Boom J. H., Thomas G.J., *Biochemistry*, **25**, 41(1986).

[5]. Drew H. R., Travers A. A., *Cell*, **37**, 491 (1984).

[6]. Rich A., *Acc. Chem. Res.*, **10**, 388 (1977).

[7]. Westhof E., Dumas P., Moras D., *J. Mol. Biol.*, **184**, 119 (1985).

[8]. Sarkar K. S., Garigipati R. S., Adams J. L., Keifer P. A., *J. Am. Chem. Soc.*, 118, 2305 (1996).

[9]. Arnott S., Hukins D. W. L., Dover S. D., Fuller W., Hodgson A. R., *J. Mol. Biol.*, **81**, 107 (1973).

[10]. Keifer P. A., *J. Org. Chem.*, **61**, 1558 (1996).

[11]. Hecht S. M., *Bioorganic Chemistry: Nucleic Acids*, Oxford University Press, New York, (1996).

[12]. Roberts R. W., Crothers D. M., *Science*, **258**, 146 (1992).

[13]. Friedman M., Sigel C. W., *Biochemistry*, 5, 478 (1966).

[14]. Chou S.-H., Flynn P., Reid B., *Biochemistry*, **28**, 2435 (1989).

[15]. Felsenfeld G., Davies D. R., Rich A., *J. Am. Chem. Soc.*, **79**, 2023 (1957).

[16]. de los Santos C., Rosen M., Patel D., *Biochemistry*, **28**, 7282 (1989).

[17]. Rajagopal P., Feigon J., *Biochemistry*, **28**, 7859 (1989).

[18]. Rajagopal P., Feigon J., *Nature*, **339**, 637 (1989).

[19]. Macaya R., Wang E., Schultze P., Sklenar V., Feigon J., *J. Mol. Biol.*, **225**, 755 (1992).

[20]. Macaya R., Wang E., Schultze P., Feigon J., *J. Am. Chem. Soc.*, **114**, 781 (1992).

[21]. Han H., Dervan P. B., *Proc. Natl. Acad. Sci. USA*, **90**, 3806 (1993).

[22]. Stephenson M. L., Zamecnik P. C., *Proc. Natl. Acad. Sci. USA*, **75**, 285 (1978).

[23]. Liebhaber S. A., Cash F. E., Shakin S. H., *J. Biol. Chem.*, **259**, 15597 (1984).

[24]. Goodchild J., Agrawal S., Civeira M. P., Sarin P. S., Sun D., Zamecnik P. C., *Proc. Natl. Acad. Sci. USA*, **85**, 5507 (1988).

[25]. Lima W. F., Monia B. P., Ecker D. J., Freier S. M., *Biochemistry*, **31**, 12055, (1992).

[26]. Damha M. J., Wilds C. J., Noronha A., Brukner I., Borkow G., Arion D., Parniak M. A., *J. Am. Chem. Soc.*, **120**, 12976 (1998).

[27]. Jaeger J. A., Turner D. H., Zuker M., *Proc. Natl. Acad. Sci. USA*, **86**, 7706 (1989).

[28]. Stull R. A., Taylor L. A., Szoka F. C., *Nucleic Acids Res.*, **20**, 3501 (1992).

[29]. Monia B. P., Johnston J. F., Ecker D. J., Zounes M. A., Lima W. F., Freier S. M., *J. Biol. Chem.*, **267**, 19954 (1992).

[30]. Wagner R. W., Matteucci M.D., Lewis J. G., Gutierrez A. J., Moulds C., Froehler B. C., *Science*, **260**, 1510 (1993).

[31]. Smith C. C., Aurelian L., Reddy M. P., Miller P. S., Ts'o P. O. P., *Proc. Natl. Acad. Sci. USA*, **83**, 2787 (1985).

[32]. Kulka M., Smith C. C., Aurelian L., Fishelevich R., Meade K., Miller P., Ts'o P. O. P., *Proc. Natl. Acad. Sci. USA*, **86**, 6868 (1989).

[33]. Morrison R. S., *J. Biol. Chem.*, **266**, 728 (1991).

[34]. Cooney M., Czernuszewicz G., Postel E. H., Flint S. J., Hogan M. E., *Science*, *241*, 456 (1988).

[35]. Maher III J. L., Dervan P. B., Wold B., *Biochemistry*, **31**, 70 (1992).

[36]. Skoog J. U., Maher III L. J., *Nucleic Acids Res.*, **21**, 2131 (1993).

[37]. Kiessling L. L., Griffin L. C., Dervan P. B., *Biochemistry*, **31**, 2829 (1992).

[38]. Griffin L. C., Kiessling L. L., Beal P. A., Gillespie P., Dervan P. B., *J. Am. Chem. Soc.*, **114**, 7976 (1992).

[39]. Koshlap K. M., Gillespie P., Dervan P. B., Feigon J., *J. Am. Chem. Soc.*, **115**, 7908 (1993).

[40]. Stilz H. U., Dervan P. B., *Biochemistry*, **32**, 2177 (1993).

[41]. Akhtar S., Agrawal S., *TiPS*, **18**, 12 (1997).

[42]. Chang E. H., Miller P. S., Cushman C., Devadar K., Pirollo K. F., Ts'o P. O. P., Yu Z. P., *Biochemistry*, **30**, 8283 (1991).

[43]. Grigoriev M., Praseuth D., Guieysse A. L., Robin P., Thuong N. T., Helene C., Harel-Bellan A., *Proc. Natl. Acad. Sci. USA*, **90**, 3501 (1993).

[44]. De Mesmaeker A., Haner R., Martin P., Moser H. E., *Acc. Chem. Res.*, **28**, 366 (1995).

[45]. Beaucage S. L., Caruthers M. H., *Tet. Lett.*, **22**, 1859 (1981).

[46]. McBride L. J., Caruthers M. H., *Tet. Lett.*, **24**, 245 (1983).

[47]. Uhlmann E., Peyman A., *Chem Rev.*, **90**, 544 (1990).

[48]. Cazenave C., Chevrier M., Thuong N. T., Helene C., *Nucleic Acids Res.*, **15**, 10507 (1987).

[49]. Stein C. A., Subasinghe C., Shinozuka K., Cohen J. S., *Nucleic Acids Res.*, **16**, 3209 (1988).

[50]. Egholm M., Behrens C., Christensen L., Berg R. H., Nielsen P. E., Buchardt O., *J. Chem. Soc. Chem. Commun.*, 800 (1993).

[51]. Egholm M., Buchardt O., Nielson P. E., Berg R. H., *J Am. Chem. Soc.*, **114**,

1895 (1992).

[52]. Egholm M., Nielsen P. E., Buchardt O., Berg R. H., *J. Am. Chem. Soc.*, **114**, 9677 (1992).

[53]. Nielsen P. E., Egholm M., Berg R. H., Buchardt O., *Science*, **254**, 1497 (1991).

[54]. Egholm M., Buchardt O., Christensen L., Behrens C., Freier S. M., Driver D. A., Berg R. H., Kim S. K., Norden B., Nielsen P. E., *Nature*, **365**, 566 (1993).

[55]. Kool E. T., *Chem. Rev.*, **97**, 1473 (1997).

[56]. Benner S. A., Schneider K. S., J. *Am. Chem. Soc.*, **112**, 453 (1990).

[57]. Uhlmann E., Peyman A., Breipohl G., Will D. W., *Angew. Chem Int. Ed.*, **37**, 2796 (1998).

[58]. Dueholm K. L., Egholm M., Behrens C., Christensen L., Hansen H. F., Vulpius T., Petersen K. H., Berg R. H., Nielsen P. E., Buchardt O., *J. Org. Chem.*, **59**, 5767 (1994).

[59]. Christensen L., Fitzpatrick R., Gildea B., Petersen K. H., Hansen H. F., Koch T., Egholm M., Buchardt O., Nielsen P. E., Coull J., Berg R. H., *J. Pept. Sci.*, **3**, 175 (1995).

[60]. Egholm M., Christensen L., Dueholm K. L., Buchardt O., Coull J., Nielsen P. E., *Nucleic Acids Res.*, **23**, 217 (1995).

[61]. Knudsen H., Nielsen P. E., *Nucleic Acids Res.*, **24**, 494 (1996).

[62]. Leijon M., Graslund A., Nielsen P. E., Buchardt O., Norden B., Kristensen S. M., Eriksson M., *Biochemistry*, **33**, 9820 (1994).

[63]. Magdalena E., Nielsen P. E., *Nature Struct. Biol.*, **3**, 410 (1996).

[64]. Almarsson O., Bruice T. C., *Proc. Natl. Acad. Sci. USA*, **90**, 9542 (1993).

[65]. Almarsson O., Bruice T. C., Kerr J., Zuckermann R. N., *Proc. Natl. Acad. Sci. USA*, **90**, 7518 (1993).

[66]. Brown S. C., Thomson S. A., Veal J. M., Davis D. G., *Science*, **265**, 777 (1994).

[67]. Betts L., Josey J. A., Veal J. M., Jordan S. R., *Science*, **270**, 1838 (1995).

[68]. Demidov V. V., Potaman V. N., Frank-Kamenetskii M. D., Egholm M., Buchard O., Sonnichsen S. H., Nielsen P. E., *Biochem. Pharmacol.*, **48**, 1310 (1994).

[69]. Bonham M. A., Brown S., Boyd A. L., Brown P. H., Bruckenstein D. A., Hanvey J. C., Thomson S. A., Pipe A., Hassman F., Bisi J. E., Froehler B. C., Matteucci M. D., Wagner R. W., Noble S. A., Babiss L. E., *Nucleic Acids Res.*, **23**, 1197 (1995).

[70]. Hyrup B., Nielsen P. E., *Bioorg. Med. Chem.*, **4**, 5 (1996).

[71]. Aldrian-Herrada G., Desarmenien M. G., Orcel H., Boissin-Agasse L., Mery J., Brugidou J., Rabie A., *Nucleic Acids Res.*, **26**, 4910 (1998).

[72]. Brugidou J., Legrand Ch., Mery J., Rabie A., *Biochem. Biophys. Res. Comm.*, **214**, 685 (1995).

[73]. Ulmann E., Will D. W., Breipohl G., Langner D., Ryte A., *Angew. Chem. Int. Ed. Engl.*, **35**, 2632 (1996).

[74]. Gambacorti-Passerini C., Mologni L., Bertazzoli C., le Coutre P., Marchesi E., Grignani F., Nielsen P. E., *Blood*, **88**, 1411 (1996).

[75]. Hanvey J. C., Peffer N. J., Bisi J. E., Thomson S. A., Cadilla R., Josey J. A., Ricca D. J., Hassman C. F., Bonham M. A., Au K. G., Carter S. G., Bruckerstein D. A., Boyd A. L., Noble S. A., Babiss L. E., *Science*, **258**, 1481 (1992).

[76]. Merrifield R. D., *J. Am. Chem. Soc.*, **85**, 2149 (1963).

[77]. *Novabiochem Catalog & Peptide Synthesis Hanbook* (1999).

[78]. Wang S.-S., *J. Am. Chem Soc.*, **95**, 1328 (1973).

[79]. Rink H., *Tet. Lett.*, **28**, 3787 (1987).

[80]. Matsueda *G. R.*, Stewart J. M.,*Peptides*, **2**, 45 (1981).

[81]. Holmes C. P., Jones D. G., *J. Org. Chem.*, **60**, 2318 (1995).

[82]. Fruchtel J. S., Jung G., *Angew. Chem. Int. Ed. Engl.*, **35**, 17 (1996).

[83]. Ponnusamy E., Fotadar U., Fiat D., *Synthesis*, 48 (1996).

[84]. Sabatier J. M., Tessier-Rochat M., Granier C., Van Rietscoten J., Pedroso E., Grandas A., Albericio F., Giralt E., *Tetrahedron*, **43**, 5973 (1987).

[85]. Carpino L. A., Han G. Y., *J. Am. Chem. Soc., J. Org. Chem.*, **37**, 3404 (1972).

[86]. Sheehan J. C., Hess G. P., *J. Am. Chem. Soc.*, **77**, 1067 (1955).

[87]. Bodanszky M., *Peptide Chemistry: A Practical Textbook*, Springer-Verlag, Berlin Heidelberg New-York (1988).

[88]. Kisfaludy L., Roberts J. E., Johnson R. H., Mayers G. L., Kovacs J., *J. Org. Chem.*, **35**, 3563 (1970).

[89]. *J. Pept. Prot. Res.*, **7**, 495 (1975).

[90]. Carpino L. A., *J. Am. Chem. Soc.*, **115**, 4397 (1993).

[91]. Coste J., Dufour M-N., Pantaloni A., Castro B., *Tet. Lett.*, **31**, 669 (1990).

[92]. Kaiser E., Colescott R. L., Bossinger C. D., Cook P. I., *Anal. Biochem.*, **34**, 595 (1970).

[93]. Chu S. S., Reich S. H., *Bioorg. Med. Chem. Lett.*, **5**, 1053 (1995).

[94]. Sarin V. K., Kent S. B. H., Tam J. P., Merrifield R. B., *Anal. Biochem.* **117**, 147 (1981).

[95]. Gisin B. F., *Anal. Chim. Acta.*, **58**, 248 (1972).

[96]. Adams H., Harris K. D. M., Hembury G. A., Hunter C. A., Livingstone D., McCabe J. M., *Chem. Commun.*, 2531 (1996).

[97]. Waldner A., De Mesmaeker A., Wendeborn S., *Bioorg. Med. Chem. Lett.*, **6**, 2363 (1996).

[98]. Stecher E. D., Gelblum E., *J. Org. Chem*, **26**, 2693 (1961).

[99]. Casy G., Lee T. V., Lovell H., *Tet. Lett.*, **33**, 817 (1992).

[100]. Kim M. J., Whitesides G. M., *J. Am. Chem. Soc.*, **110**, 2959 (1988).

[101]. Hart K. W., Clarke A. R., Wigley D. B., Waldman A. D. B., Chia W. N.,

Barstow D. A., Atkinson T., Jones J. B., Holbrook J. J., *Biochim. Biophys. Acta.*, **914**, 294 (1987).

[102]. Clarke A. R., Wilks H. M., , Chia W. N., Barstow D. A., Atkinson T., Holbrook J. J., *Biochemistry*, **27**, 1617 (1988).

[103]. Morton H. E., Guindon Y., *J. Org. Chem.*, **50**, 5379 (1985).

[104]. Morton H. E., Guindon Y., Yoakim C., *J. Org. Chem.*, **49**, 3912 (1984).

[105]. Nishimura T., Iwai I.,*Chem. Pharm. Bull.*, **12**, 352 (1964).

[106]. Newcomb L. F., Gellman S. H., *J. Am. Chem. Soc.*, **116**, 4993 (1994).

[107]. Guckian K. M., Schweitzer B. A., Ren R. X. F., Sheils C. J., Paris P. L., Tahmassebi D. C., Kool E. T., *J. Am. Chem. Soc.*, **118**, 8182 (1996).

[108]. Ts'O P., *Basic Principles in Nucleic Acid Chemistry*, Academic Press, New York, 1974, p.548.

[109]. Bloomfield V. A., Crothers D. M., Tinoco I., *Physical Chemistry of Nucleic Acids*, Harper and Row, New York 1974, p.132.

[110]. Abdel-Magid A. F., Carson K. G., Harris B. D., Maryanoff C. A., Shah R. D., *J. Org. Chem.*, **61**, 3849 (1996).

[111]. Sim M. M., *J. Org. Chem.*, **62**, 3230 (1997).

[112]. Parker K. A., Dermatakis A., *J. Org. Chem.*, **64**, 4164 (1997).

[113]. Meltzer P. C., Liang A. Y., Matsudaira P., *J. Org. Chem.*, **60**, 4305 (1195).

[114]. Finn P. J., Gibson N. J., Fallon R., Hamilton A., Brown T., *Nucleic Acids Res.*, **24**, 3357 (1996).

[115]. Thomson S. A., Josey J. A., Cadilla R., Gaul M. D., Hassman C. F., Luzzio M. J., Pipe A. J., Rees K. L., Ricca D. J., Wiethe R. W., Noble S. A., *Tetrahedron*, **21**, 6179 (1995).

[116]. Still W. C., Kahn M., Mitra A., *J. Org. Chem.*, **43**, 64 (1978).

[117]. Blunt J. W., Calder V. L., Fenwick G. D., Lake R. J., McCombs J. D., Munro M. H. G., Perry N. B., *J. Nat. Prod.*, **50**, 290 (1987).

[118]. Tsantrizos Y. S., Lunetta J. F., Boyd M., Fader L. D., Wilson M. C., *J. Org. Chem.*, **62**, 5451 (1997).