AN ABSTRACT OF THE THESIS OF

Sung-Ben Huang for the degree of Doctor of Philosophy in Chemistry presented on May 29, 1990

Title: Synthesis and Oligomerization of Delta,4-diamino-2-oxo-1(2H)-pyrimidinehexanoic Acid

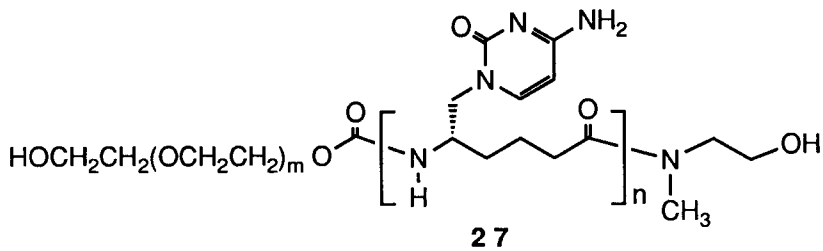
Redacted for Privacy

Abstract approved: _____
Dwight D. Weller

The title compound, with suitably protected functional groups, was prepared in optically pure form from (S)-(+)-6-hydroxymethyl-2-piperidone **30b** to serve as cytosine-containing building blocks for the construction of an uncharged stereoregular oligonucleotide analogue. The key intermediate, alcohol **30b**, was previously unknown. Several methods to prepare optically pure alcohol **30b** were investigated. The best route is the lithium borohydride reduction of the (S)-(+)-6-oxo-piperidine-2-carboxylates **33a,b**, prepared from L-α-aminoadipic acid by a slightly modification of the literature procedure used for the homologue (S)-(+)-5-hydroxymethyl-2-pyrrolidinone **30a**.

Upon stepwise oligomerization of the monomeric subunit (protected title compound) to hexamer, and subsequent appended a polyethylene glycol tail on the N-terminus then removal of protecting groups of the hexamer provide a cytosine-containing, uncharged stereoregular oligonucleotide analogue **27** (n = 6). All of the intermediate oligomers along the synthesis were satisfactorily characterized by ^{1}H-NMR, homonuclear ^{1}H-COSY, and negative ion fast atom bombardment mass

spectroscopy. Association of hexamer **27** (n = 6) with $p(dG)_6$ was evidenced by a hypochromic effect following annealing. A thermal denaturation experiment of the complex was also performed. However, the T_m value of this thermal denaturation cannot be obtained, as a non-ideal sigmoidal curve was derived; it was estimated to be 48°C.



27

Synthesis and Oligomerization of Delta,4-diamino-2-oxo-1(2H)-pyrimidinehexanoic Acid

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Redacted for Privacy

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Redacted for Privacy

Dean of Graduate School

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Typed by Sung-Ben Huang

To My Parents

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SCHEMES

SYNTHESIS AND OLIGOMERIZATION OF DELTA,4-DIAMINO-2-OXO-1(2H)-PYRIMIDINEHEXANOIC ACID

INTRODUCTION

The utilization of naturally occurring or chemically synthesized oligonucleotides and oligonucleotide analogues to achieve control of genetic expression at the level of transcription or translation is normally referred to as the antisense approach.[1-7] It is based upon the sequence specificity of nucleic acid binding. Thus, the transfer of genetic information can be blocked by duplex formation of a complementary antisense agent with a target segment of mRNA via Watson-Crick base pairing interactions. Naturally occurring antisense RNAs have been found in both prokaryotes [8-32] and eukaryotes.[33-46] Inhibition of gene expression of viral and cellular mRNAs by antisense RNA and DNA has been observed by several research groups.[47-69] These results reveal that the antisense approach may provide a tool in the area of gene research, and suggest that antisense oligomers may serve as potential therapeutic agents for viral diseases and cancers. In fact, several types of antisense oligomers have been shown to cause inhibition of a number of RNA and DNA viruses, including Rous sarcoma virus (RSV),[70-72] human immunodeficiency virus (HIV),[73-79] vesicular stomatitis virus (VSV),[80-82] herpes simplex virus type I (HSV-1) [82,83] and influenza virus.[3]

In their pioneering work, Zamecnik and Stephenson demonstrated that viral replication and viral RNA translation of Rous sarcoma virus can be inhibited by a sequence-specific oligodeoxynucleotide 13-mer.[71,72] Studies by Inouye and coworkers on prokaryotes [84,85] and by Izant and Weintraub on eukaryotes [57,58] demonstrated that exogenous antisense sequences could be used for physiological regulation of gene

expression in intact cells. More recent investigations by Zamecnik and coworkers further showed that the inhibition of replication and expression of human T-cell lymphotropic virus type III (HIV) can be achieved by synthetic oligodeoxyribonucleotides complementary to the viral RNA.[79]

Oligodeoxyribonucleotides **1** are quite effective as antisense agents. They apparently can form DNA-RNA hybrids that are susceptible to cleavage by RNAase-H, and can thus act in a stoichiometric fashion to destroy the target mRNA.[86-91] They are also routinely available by use of the automatic DNA synthesizer. However, unprotected oligodeoxyribonucleotides are readily destroyed by nuclease degradation.[92-94] Furthermore, the uptake of such oligomers by cells is difficult because polyanionic species may not easily penetrate the cell membrane. Therefore, the biological effectiveness of oligodeoxynucleotide is expected to be diminished in practical applications due to the combination of these factors. Efforts have been made to overcome the problems associated with the use of oligodeoxyribonucleotides by structural modifications of the normal oligonucleotides, so that the new agents would retain the capability of sequence-specific binding, be nuclease insenstive, and be able to freely pass through the cell membrane.

From a structural point of view, the modifications performed on oligodeoxyribonucleotides can be classified into two categories. The first strategy (Figure I) involves replacement of the internucleoside phosphodiester groups with other linkages that are not recognized by nucleases. The sugar moieties are retained with little or no modification. Ionic analogues derived from this approach include α-anomeric DNA **2**, [54,95-108] which is epimeric with natural oligodeoxyribonucleotides at the C1' carbon, and phosphorothioates **3**.[75,78,109-118] Both are less readily cleaved by nucleases than are oligonucleotides. To foster membrane permeability, uncharged linkers, which yield non-ionic backbones, have been proposed. The lack of backbone

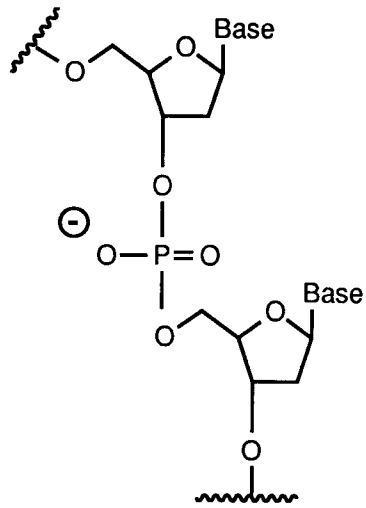
charges is beneficial to the binding ability of the oligomers with their complementary mRNA target, due to the lack of charge-charge repulsions between the strands. Uncharged analogues include methanephosphonates **4**,[74,110,113,119-148] phosphotriesters **5**,[109,114,149-158] phosphoramidates **6**,[75,159-163] acetate-linked **7**,[164-168] acetamido-linked **8**,[169,170] carbonate-linked **9**,[171-173] carbamate-linked **10**[174-177] and morphorlinocarbamate **11**[178] analogues. Among these, the phosphorothioates, methanephosphonates and phosphotriesters have been the most extensively studied. In terms of classification, the strategy employed to conceptually derive these types of analogues is referred to as the "cyclic strategy" because the cyclic structure of the "sugar" is retained.

Several types of antisense analogues derived from the cyclic approach have been found to be effective gene regulators and potent antiviral agents. For example, the inhibition of gene expression by α-anomeric DNA,[54,95-108] phosphorothioate,[75,78,109-118] methanephosphonate,[74,110,113,119-148] and phosphoramidate[75,159-163] analogues have been reported by many research groups. Furthermore, recent discoveries by Zamenick and coworkers also demonstrated that phosphorothioate, methanephosphonate, and phosphoramidate analogues inhibit human immunodeficiency virus (HIV) replication in cell culture.[74,75,78,110] Phosphorothioate analogues are currently undergoing pharmacokinetic and pre-clinical studies.[179]

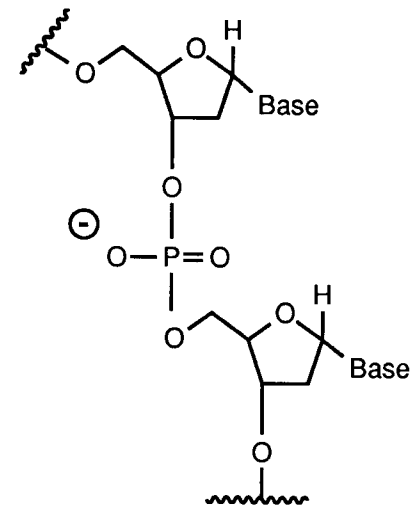
The potency of antisense agents may vary with the type of backbone. For instance, comparative studies on the inhibition of chloramphenicol acetyltransferase gene expression at 30 μM concentration of analogues (15-mers) showed that the phosphorothioate analogue was about twice as potent as the methanephosphonate analogue, which in turn was approximately two times more potent than the normal oligodeoxynucleotide.[113] However, studies on anti-HIV activities of oligonucleotide analogues by Zamecnik and coworkers showed that phosphorothioates, as well as

certain phophoramidates and methanephosphonates, all give 100% inhibition at a concentration of 20 μg/mL (~ 3μM).[74,75]

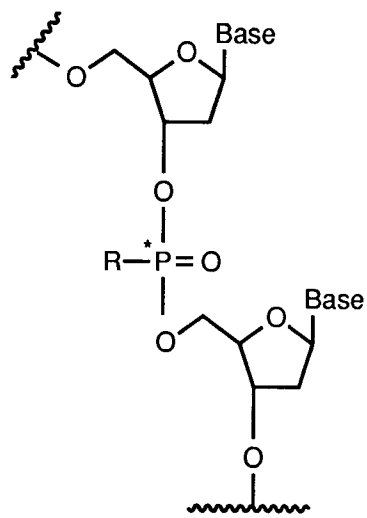
Figure Ia. Analogues Derived From "Cyclic" Strategy.



1
Normal
Oligodeoxyribonucleotide



2
α-Anomer of
Oligodeoxyribonucleotide



3 Phosphorothioates R = $S^{\ominus}$

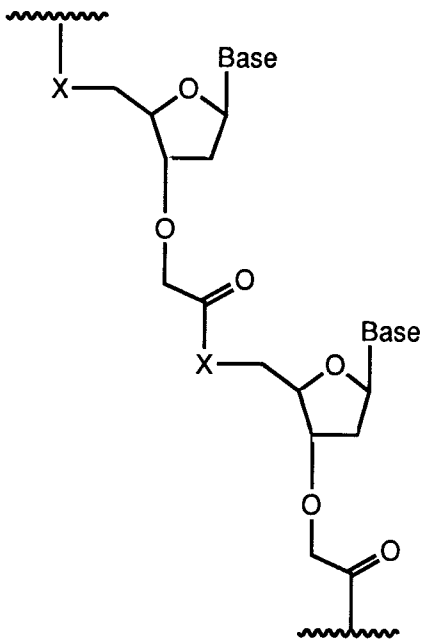
4 Methanephosphonates R = CH_3

5 Phosphotriesters R = O-Alkyl

6 Phosphoroamidates R = NR'R"
R', R" = H or Alkyl groups

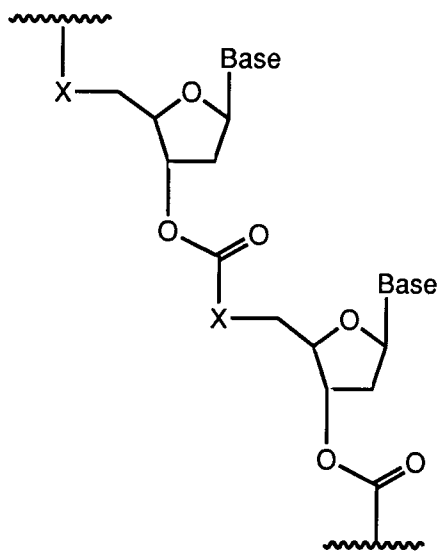
Base = A, T, C, G

Figure Ib. Analogues Derived From "Cyclic" Strategy (continued)



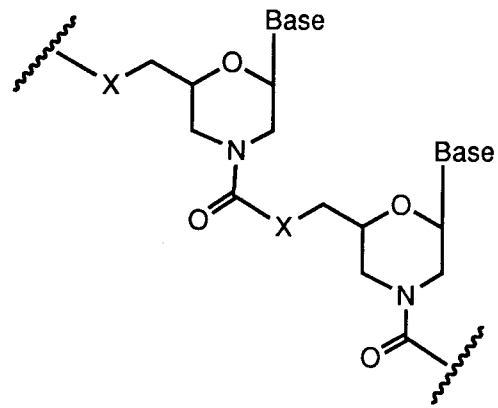
7 when X = O
Acetate-linked analogue

8 when X = NH
Acetamido-linked analogue



9 when X = O
Carbonate-linked analogue

10 when X = NH
Carbamate-linked analogue



11 when X = NH
Morpholinocarbamate

Base = A, T, G, C

It is important to notice that the structural modifications of the internucleoside phosphodiester linkages with substitution of various types of functional groups may create a stereogenic phosphorus atom. Therefore, an analogue may exist in 2^n stereoisomeric forms when there are n units of modified internucleoside linkages. Each of the diastereomeric oligomers may have a different binding affinity for the target RNA/DNA as can be measured by the T_m values for the complex thus formed. If the stereoisomeric oligomers have relatively similar duplex stability, this multiplicity of stereoisomerism might be acceptable in a chemotherapeutic application. Unfortunately, the available evidence indicates that the stereochemical configuration at phosphorus plays a large role in the stability of complexes of the oligomer.

Miller and coworkers illustrated that the T_m values for the two stereoisomers of bis(adenosine) monomethanephosphonate complexed to polyuridylic acid were different, with a T_m value of 15.4°C for one isomer and 19.8°C for the other isomer.[146] Similar results are also reported for the decathymidylate analogues having a stereoregular, alternating methanephosphonate and phosphodiester backbone.[145] These analogues were paired with poly(dA). The T_m values of the resulting compexes are 33.5°C for one isomer and 2°C for the other isomer. Recent studies by Zon and coworkers provide further evidence that the complex stability is related to (1) the charge of the oligomer, (2) electronic and other substituent effects and (3) steric interactions.[125] They prepared a series of self-complementary oligomers containing a stereochemically defined methanephosphonate linkage. Analogues containing an S_p configuration at the methanephosphonate linkage gave lower T_m values than the R_p containing analogues. This is attributed to the orientation of the P-CH_3 bond of the S_p isomer, which is pointed toward the major groove of the duplex, thereby destabilizing the complex.

Similar observations and explanations were reported for alkyl phosphotriesters[149,150] and phosphorothioate[115] analogues. In these cases, the S_p

configuration of phosphotriesters and the R_p configuration of phosphorothioates causes these oligomers to possess lower T_m values. This is explained by the orientations of the substituents on the phosphorus atoms in these oligomers, which are based on the results of 2D-NOE experiments. Furthermore, the observed low binding affinities for atactic methanephosphonates relative to normal oligodeoxynucleotides (275-fold less) in the hybrid arrest studies on human dihydrofolate reductase (DHFR) mRNA by Dolnick and coworkers was attributed to the diastereoisomerism of the oligomers.[120]

Although it is generally believed that the chirality of the backbones does play an important role in the stability of the duplex, uncertainties still persist. Letsinger and coworkers prepared several N-alkyl phosphoramidate analogues and tested the effect of various alkyl substitution at phosphorus on binding properties.[161] Dimeric and trimeric phosphoramidates of deoxyadenylic acid were found to bind with poly(U) and poly(dT), even when the analogues possessed large lipophilic side chains. The complexes with poly(U) are more stable than the poly(dU)·poly(dA) complex. Binding to poly(dT) was comparable or in some cases stronger relative to the parent oligodeoxynucleotide duplex. They also found that stereochemistry at phosphorus influenced the stability of the complexes, but the effect was not a major one.

Recent studies by Froehler and coworkers demonstrated similar results.[160] They synthesized a series of oligodeoxynucleoside 15-mers containing 12 units of N-alkylphosphoramidate linkages and 2 phosphodiester linkages. All of these analogues were found to bind with a complementary strand of DNA, but the duplexes formed were less stable than the normal phosphodiester duplex. The chirality at phosphorus of these analogues was thought not critical for stability of the duplexes, as relatively sharp melting curves were measured. The cause of the lowered T_m values for these oligomers is still not clear, however. It was rationalized that a spine of hydration about the phosphorus species is dramatically disturbed by the neutral backbones.

Similar results of chirality irrelevance were also reported for the "phosphate-methylated" DNA[149] (phosphotriester **5**; alkyl group = CH_3) by a Dutch group.[180-184] This neutral analogue, derived from DNA by esterification, hybridizes strongly and specifically with complementary natural DNA and RNA. The stability of the duplexes is independent of the ionic strength, which is consistent with the absence of interstrand phosphate-phosphate charge repulsions. The relatively narrow melting transitions observed (within 10°C) for the duplexes points out the insensitivity of the binding to the absolute configuration of the stereogenic phosphorus atoms of the backbone. Both antisense and sense phosphate-methylated DNA were found to effectively inhibit the human immunodeficiency virus type-1 (HIV-1) at very low concetration (0.15 to 3.0 μM) as compared to other types of modified DNAs (e.g. phosphorothioates, phosphoramidates, or methanephosphonates; 3 to 15 μM). The inhibitory effect of antisense phosphate-methylated DNA is strongly influenced by the secondary structure of the target region in the viral RNA. It was also suggested that the target selection should focus on hairpin loop structures in the viral RNA.

The second general approach to oligonucleotide analogues involves the complete replacement of the sugar phosphate backbones of the natural nucleotides with acyclic backbones, and is termed the "acyclic strategy". This approach has been explored to a much lesser extent. The acyclic nucleotide analogues (Figure II) reported to date include polyvinyl **12**,[185-199] polyacrylate **13**,[200-211] polyacrylamide **14**,[212,213] polyvinylamine **15**,[214-217] poly(vinyl alcohol) **16**,[218-221] polyethylenimines **17a,b**,[222-231] polytrimethylenimine **18**,[232,233] polyphosphate **19**[234] and polypeptide **20-23**[235-239] analogues.

Figure IIa. Nucleic Acid Analogues Derived From "Acyclic" Strategy.

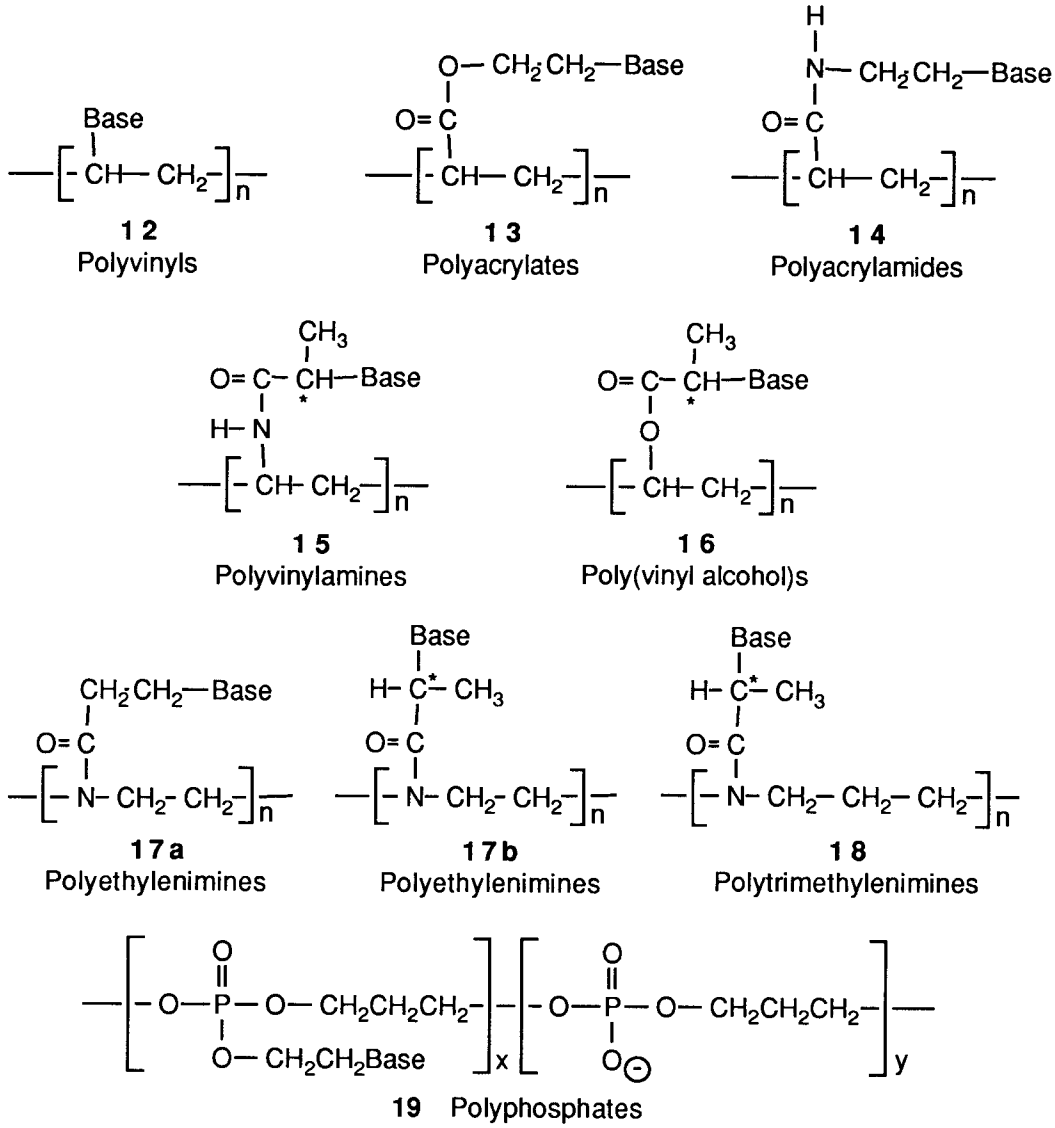
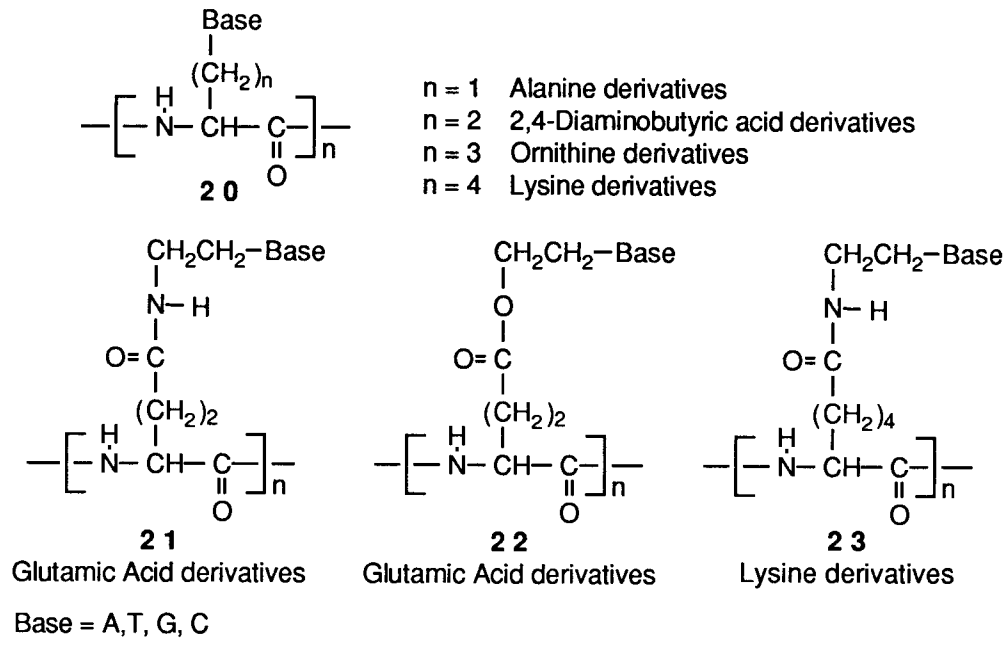


Figure IIb. Nucleic Acid Analogues Derived From "Acyclic" Strategy (continued).



The pioneering work on polyvinyl derivatives has been conducted by Pitha and coworkers.[185-199] They prepared a series of purine- and pyrimidine-containing vinyl polymers via radical polymerization of the corresponding monomers. The interaction of these poly(vinyl) analogues with normal oligoribonucleotides was studied. The results showed that poly(vinyluracil) forms a complex with poly(A) which is less stable than the poly(A)·poly(U) complex.[198] Poly(vinylcytosine) forms highly stable complexes with poly(G) and poly(I). It does not form an ordered hydrogen bonded secondary structure at acid pH as does poly(C).[197] Poly(vinyladenine) forms a complex with poly(U) in 1:1 base ratio at neutral condition.[240] Poly(vinylhypoxanthine) does not form a complex with poly(C), but a complex forms with poly(vinylcytosine) after heating and then cooling of the mixed polymer solution.[195]

Biological and biochemical properties of these uncharged polyvinyl analogues were also investigated.[185] It was found that the complex of poly(vinylcytosine) with

poly(I) has potent antiviral activity due to its interferon-inducing ability.[194] It was suggested that the high activity is related to the low charge/mass ratio and to the aggregation of the complex. These two factors are generally thought to enhance the uptake of compounds by cells.[120,148,241] In cell-free systems, poly(vinyladenine) and poly(vinyluracil) have been found to inhibit poly(U)-directed incorporation of phenylalanine and poly(A) directed incorporation of lysine into polypeptides, respectively.[193,242]

In addition, acute murine leukemia virus infection in mouse embryo cells can be blocked by introduction of poly(vinyladenine) and poly(vinyluracil); however, these agents do not significantly inhibit the replication of Sindbis and vesicular stomatitis virus.[192] These agents also inhibit murine tumor and avian myeloblastosis viral RNA-dependent DNA polymerase (reverse transcriptase), and competitively inhibit *E. coli* RNA polymerase when single-stranded templates complementary to the vinyl polymers are used, but not with non-complementary or with double-stranded templates.[186,191,243] Studies on the mechanism of inhibition of reverse transcriptase showed that the enzyme activity was inhibited in all cases where base pairing between the vinyl polymer and the template occurred.[190] Poly(vinyladenine) also inhibits tumor development and death induced by Moloney sarcoma virus-leukemia virus complex in newborn mice, and interferes directly with the replication of Friend leukemia virus in mice.[244] Furthermore, administration of this polymer did not affect the host immune reponse, or induce interferon, or affect the induction of interferon by other agents.[187]

Although some of the polyvinyl analogues are biologically active, they have several distinct differences from naturally occurring oligonucleotides: (1) they are uncharged macromolecules, (2) they possess a stereo-irregular backbone, (3) the steric constraints restrict complete base pairing interactions between strands, and (4) the base-containing region is more hydrophilic than the backbone. Because of the incomplete

base pairing of a single strand, this could result in weaker binding between strands relative to the natural occurring polynucleotides. Among other types of acyclic nucleic acid analogues mentioned above, nearly all of them possess backbones which are more hydrophilic than the polyvinyl backbone. Proper spacing between bases is also taken into consideration by adjustment of backbone length so that base pairing interactions between synthetic polymers and polynucleotides are enhanced.

As was the case for the analogues derived from cyclic strategy, the stereoregularity of the polymer may play an important role in the complex stability of acyclic analogues with nucleic acids. In fact, different complex stabilities have been observed for the optically active polyvinylamine analogues by Overberger and co-workers.[217] In this case, optically pure 2-(thymine-1-yl)propionic acid was attached on to achiral polyvinylamine backbones (PVA-T). It was found that PVA-(-)T interacts with poly(A) as evidenced by the observed hypochromicity, while PVA-(+)T and PVA-(+/-)T showed no significant hypochromicity or hyperchromicity. The utilization of achiral or stereoregular backbones with chiral pendant side chains has been employed for polyvinylamines, polytrimethylenimines and poly-L-lysine analogues.[214-217,232,233,235,236]

General strategies for the construction of these acyclic analogues include polymerization of the corresponding monomers[238,239,245-252], or grafting the nucleic acid base containing side chains onto the backbone polymers.[214-233] All of the attempts to prepare optically active acyclic nucleic acid analogues have concentrated on the preparation of optically pure monomers,[238] stereoregular polymeric backbones[203,204,208,209] and optically pure pendant side chains containing nucleic acid bases.[253] Subsequent polymerization of the optically pure monomers, or attachment of the nucleic acid base onto stereoregular polymeric backbones, or grafting the optically pure side chains onto backbone polymers will result in an optically active analogue. For

instance, optically pure propanoic acids, substituted at the 2-position with nucleic acid bases, have been prepared and grafted onto polyvinylamine, poly(vinyl alcohol), and polytrimethylenimine backbones by active ester techniques.[218,232] Stereoregular polymers were also prepared from β-bromoethyl methacrylate via anionic polymerization. Subsequent alkylation with nucleic acid bases provided stereoregular analogues.[208] Optically pure α-amino acid derivatives containing nucleic acid bases, such as lysine, ornithine and alanine derivatives, were polymerized to give optically active polypeptides.[235,236,238,239]

In spite of the availability of acyclic analogues, information concerning the interactions of these agents with natural polynucleotides and biological activities are relatively sparse. It has been shown that most of these acyclic analogues exhibit base stacking and possess local ordered secondary structures (or helical structures in some cases). For instance, UV and CD spectroscopic studies on polyethylenimine analogues indicated that the polymers are helical with significant base stacking.[223,228] On the other hand, the screening of different base-containing polyethylenimines (PEI) showed that the uracil-containing derivative was significantly active against the virion-associated transcriptase of influenza A/Victoria/75.[228] However, poly(vinyluracil) was not active against influenza A/Victoria/75 when tested in a similar manner.[228] This was attributed to the closer spacing of the bases in the poly(vinyluracil) as compared to the more polynucleotide-like base spacing of PEI. This prohibits an interaction of the former species with an adenine-containing moiety that may play a role in the viral replication mechanism.

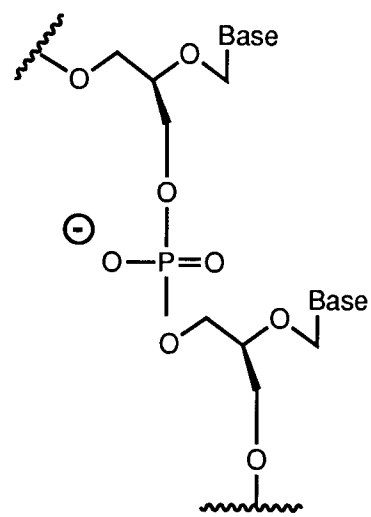
It must be noted that all the acyclic analogues developed so far depend upon polymerization reactions to form the backbones. Although copolymers are obtainable, simple polymerization processes restrict the synthesis of sequence-specific analogues and such processes generally result in backbones which are never completely

stereoregular. Additionally, most of the pendant side chains on these acyclic analogues incorporate a relatively long tether to attach the base. This could result in mispairing interactions between strands due to the additional degrees of freedom introduced. More importantly, the biological activities of the acyclic analogues discussed herein were most likely derived by induction of interferons instead of hybrid arrest (antisense) inhibition. It has been known that a complex of poly(I) and poly(C) is an effective interferon inducer, although with relatively high levels of toxicity.[252] Therefore, in order to achieve true antisense inhibition with acyclic analogues, strategies must be developed for the construction of sequence-specific oligomers.

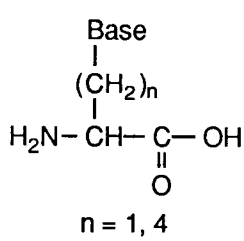
Recent studies by Benner *et al.*[254] and Ogilve *et al.*[255,256] on glyceronucleotides (Figure III) demonstrated the only case for sequence-specific construction of the acyclic analogues. Although the availability of optically active glycerol derivatives could eliminate the diastereomerism problems that generally arise from the oligomerization attempts, all of the oligomers prepared showed little or no tendency to form a stable duplex with natural DNA. This is probably due to the flexibility of backbone chosen,which exacts a very large entropy cost upon duplex formation, as compared to the natural oligonucleotides.

Figure III

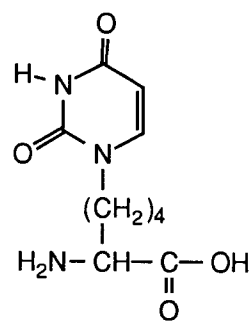
Glyceronucleotides



Upon inspection of the structural features of known acyclic analogues, the polyamides seemed to suggest a logical approach for the sequence-specific construction of oligomers or polymers, given the analogy of the easy assembly of amide bonds in polypeptides by means of available peptide synthesis methodology. This also provides a much more rigid backbone as compared with the glyceronucleosides. Early studies of polypeptide backbones were conducted by Jones *et al.*[238,250,251] and Pandit *et al.*[239,244-249] Several purine and pyrimidine base-substituted amino acids of structure **24** were synthesized; however, few of them were elaborated to the corresponding polypeptides. Base stacking was not observed for polymer of **25**; however, weak complexation with poly(A) was reported for this polymer, as evidenced by a slight hypochromicity.[239] This polymer also stimulated phenylalanine incorporation in the *in vitro* template-directed synthesis of polyphenylalanine. A similar approach by Jones and coworkers led to the construction of the oligothymine derivative **26**, prepared from the corresponding optically pure amino acid. Interestingly, **26** showed no secondary structure or base stacking and failed to complex with poly(A).[238]



24



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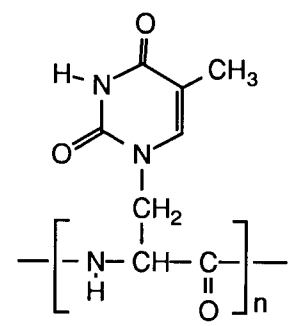


26

I planned to develop methodologies which allow the construction of acyclic oligonucleotide analogues in a sequence-specific manner. Despite the disappointing results of the Jones and Pandit groups, I felt that oligomers prepared from amide-linked

nucleic acid base-containing subunits are excellent candidates for acyclic antisense agents. Recent computer modeling studies had compared the relative binding capabilities of the analogue types in Figure IV with A and B form DNA.[257] Several interesting points were noted, which are summarized here. First, in order to achieve efficient binding to the target oligonucleotide, the most favorable length for the backbone in each subunit was found to be either five atoms or six atoms. A methylene group linkage between bases and backbone is strongly preferred. This arrangement releases the unfavorable nonbonding interactions between bases and backbone, and also improves the co-planarity of the complementary bases during the duplex formation. The most promising candidates are the nylon-5(1), nylon-6(1) and peptide series. Among those candidates, we are particularly interested in the analogues with polyamide backbones. Of these, the nylon-5(1) series is predicted to bind only to DNA (B-form) but not RNA (A-form). The nylon-6(1) series should form complexes with both nucleic acid types.

Figure IV. Backbone Types Studied by Computer Modeling.

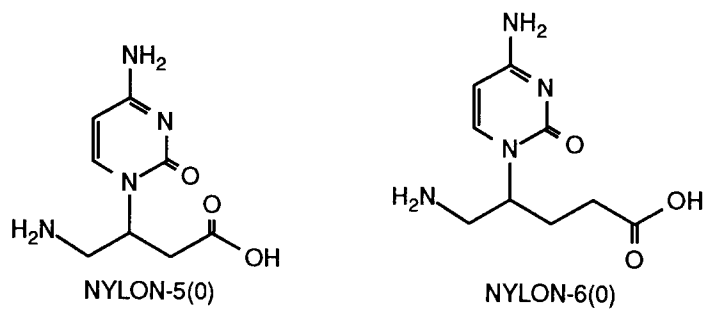
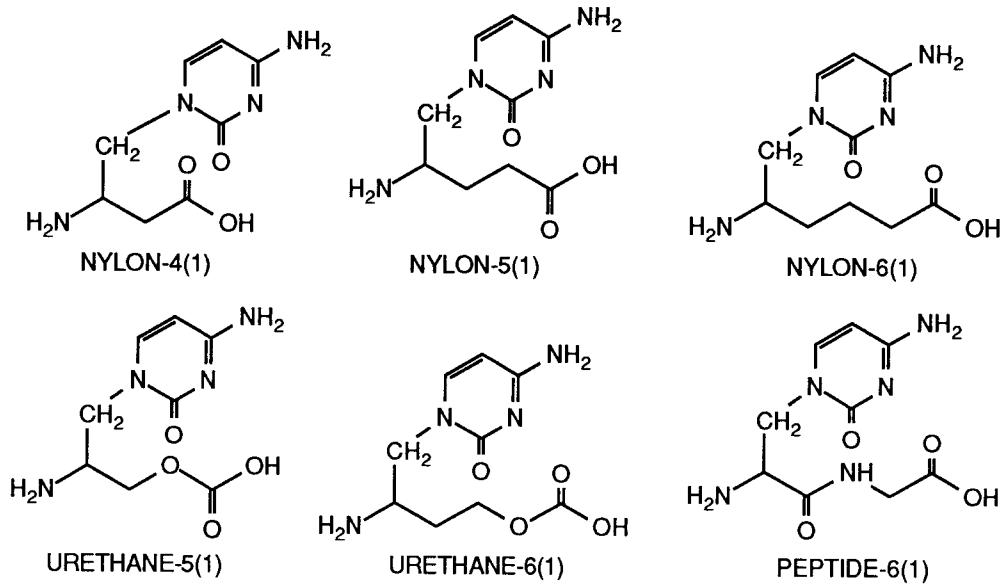
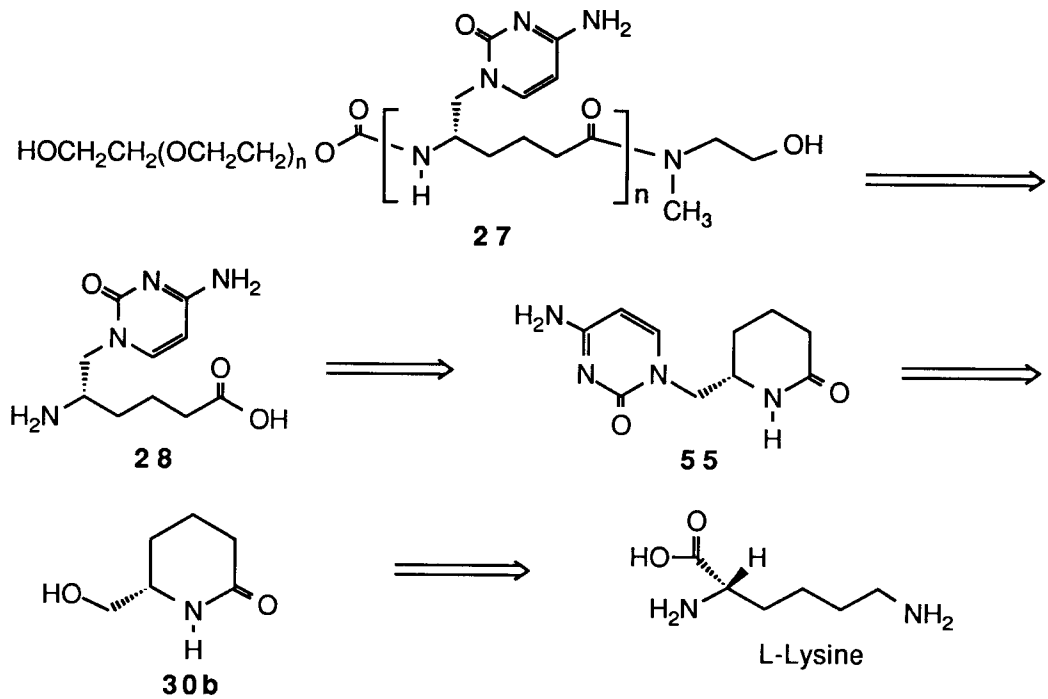


Figure IV. Backbone Types Studied by Computer Modeling (continued).



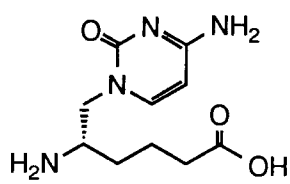
The specific analogues targeted in this work are the so-called nylon-6(1) series (Figure III). The nomenclature refers to polyamide subunits which have six atoms along the backbone and one methylene spacer between the base and backbone. In principle, the nylon-6(1) nucleic acid analogues can be assembled by formal block peptide synthesis from suitably protected monomeric subunits (Scheme I). The first goal in this series is the construction of a homopolymer of cytosine, derivative **27**. Cytosine was chosen because it forms three hydrogen bonds with a guanine moiety in the complementary target strand. This allows shorter oligomers to be used in a test to demonstrate the binding potential of these analogues with nucleic acids. In this approach, lactam alcohol **30b** derived from L-lysine serves as a key intermediate to build up required monomeric subunits, which are in turn oligomerized to form the desired oligomer **27**.

Scheme I Retrosynthetic Analysis of Nylon-6(1) Nucleic Acid Analogue.

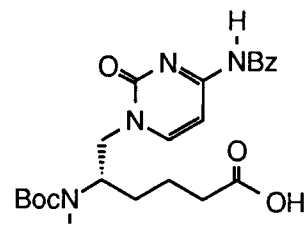


RESULTS AND DISCUSSION

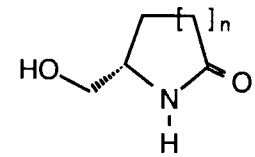
Oligomers derived from **28** are related to both peptides and oligonucleotides, and we borrowed upon protecting group methodologies used for those systems. The initial target was the protected derivative **29**. The readily cleavable *tert*-butoxycarbonyl (Boc) group should be suitable for both solution or solid phase oligomerization. While the N-benzoyl moiety on the base is not strictly required due to the low nucleophilicity of this amino group, the benzamide is expected to confer favorable solubility and chromatographic behavior upon oligomeric species. The key synthetic goal in the preparation of subunits **29** appeared to be the mating of cytosine with a chiral backbone precursor. The chiral precursor necessary for the synthesis of stereoregular nylon-6(1) nucleic acid analogues is alcohol **30b**, which was previously unknown. It is critical that the chiral precursor **30b** be as pure as possible in order to ensure the construction of stereoregular nylon backbones. Incorporation of optically impure subunits into an oligomer will lead to a complex mixture of diastereomeric products. Therefore, the preparation of optically pure (S)-(+)-6-hydroxymethyl-2-piperidone **30b** was the initial synthetic goal.



28



29

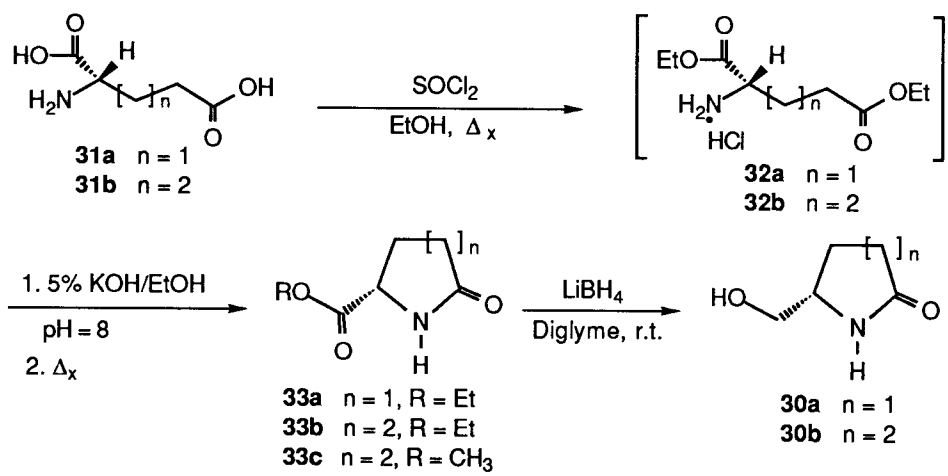


30a n = 1
30b n = 2

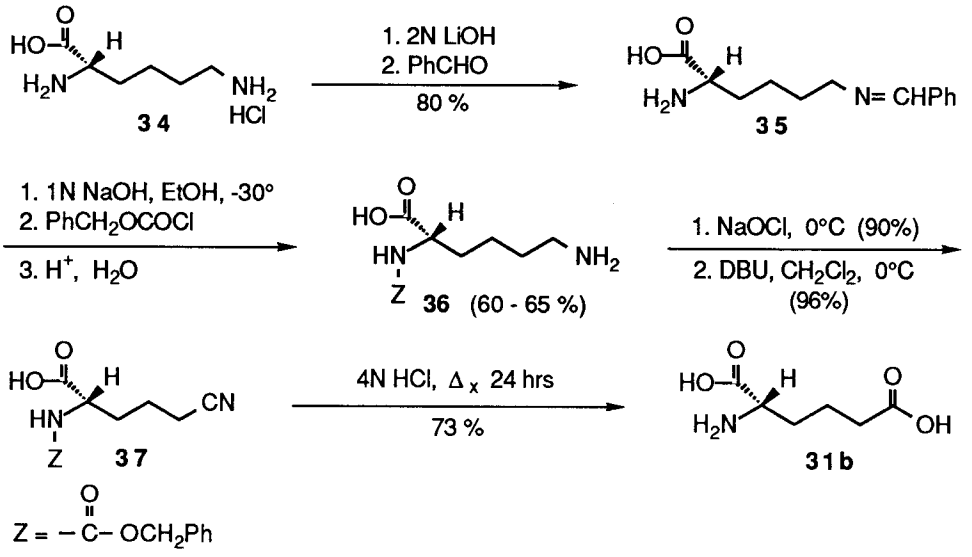
The first attempt of the synthesis of this material followed the procedure reported for the corresponding five-member ring homologue, **30a** (Scheme II, n = 1).[258,259] L-α-Aminoadipic acid **30b** was prepared from L-lysine according to the literature

procedures (Scheme III).[260] Upon esterification, cyclization and $LiBH_4$ reduction (Scheme II, n = 2), **30b** was obtained in 62% yield, calculated from L-α-aminoadipic acid. Since lactam alcohol **30b** was previously unknown, the determination of optical purity of this material cannot rely on the measurement optical rotation. In previous investigations, it has been shown that high field ^{1}H-NMR analysis of the Mosher's derivative of the alcohol **30a** could provide an alternative method for the optical purity determination.[261,263] The detection limit for the minor enantiomer in a mixture was found to be approximately one percent. Therefore, lactam alcohol **30b** was treated with Mosher's reagent[262] to provided Mosher's ester **38** (Scheme IV). Analysis by ^{1}H-NMR of the Mosher's ester **38** revealed the reaction had proceeded in only 50% **ee**. Racemization was also discovered in the previous investigation for the preparation of the alcohol **30a**, however, to a much lesser extent (96% **ee**).[263] Such a large amount of racemization of the monomer precursor would certainly preclude the preparation of stereoregular oligomers.

Scheme II Synthesis of ω-Hydroxymethyl Lactams.

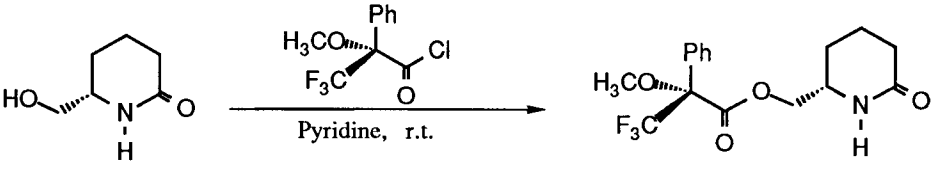


Scheme III. Synthesis of α-Aminoadipic Acid.



34 **35** **36** (60 - 65 %) **37** **31b**

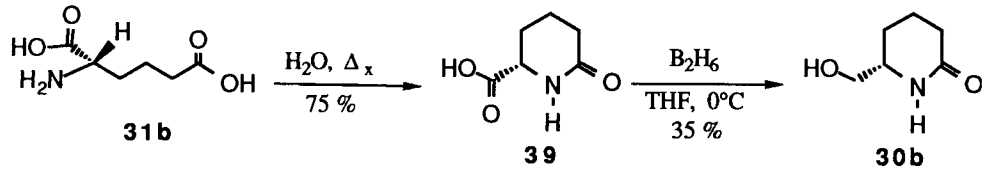
Scheme IV Mosher's Ester Derivatization.



30b **38**

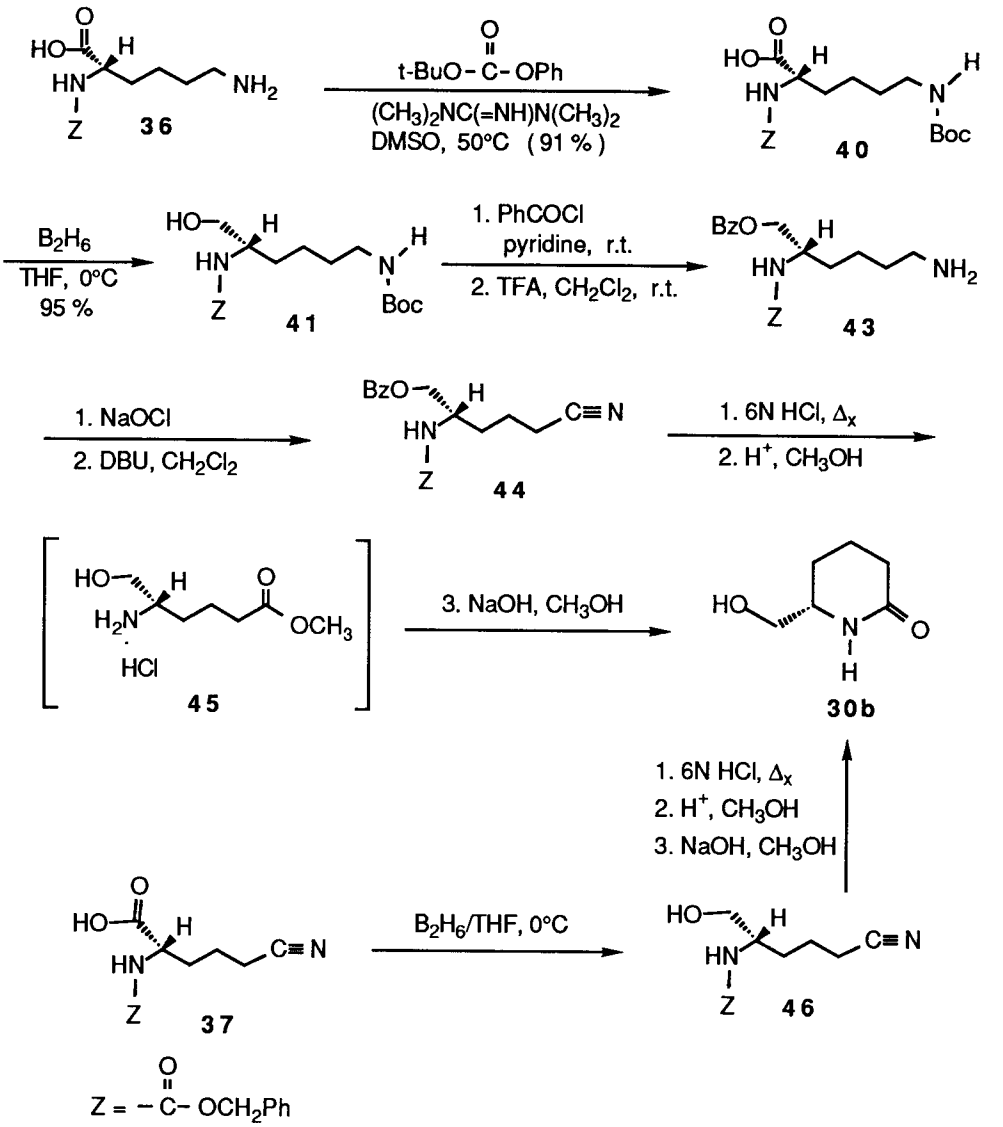
In the previous study, it was found that the borane reduction of (L)-pyroglutamic acid provided optically pure alcohol **30a** in moderate yield.[261,263] To implement this procedure, L-α-aminoadipic acid was cyclized in refluxing H_2O to provided optically pure lactam acid **39** (Scheme V) in 73% yield.[264,265] Subsequent treatment of the acid **39** with borane/THF provided optically pure **30b**, although in only 35% yield for larger scale operations. Since α-aminoadipic acid, the precursor for lactam acid **31b**, is a costly material, more economically efficient routes were required.

Scheme V Alternative Preparation of **30b** via Borane Reduction.



My first thought was to perform the borane reduction at earlier stage in the α-aminoadipic acid synthesis with the hope to improve the yield for larger scale preparations. In the event, protected L-lysine **40**,[266] prepared from L-α-Z-lysine **36**, was reduced to give alcohol **41** with borane/THF without racemization in 95% yield (Scheme VI). Alcohol **41** was protected with benzoyl group and the ω-amino group was deprotected with TFA to give amine **43**. This amine was further elaborated to nitrile **44** in high yields by treatment with aqueous sodium hypochlorite and subsequent dehydrochlorination with DBU. Acid hydrolysis and esterification of nitrile **44** gave the hydrochloride salt of β-amino alcohol **45**. Subsequent neutralization of **45** and intramolecular cyclization gave the desired lactam alcohol **30b** in 45-55% yield calculated from nitrile **44**. The lactam alcohol **30b** derived from this route was proven to be optically pure by ^{1}H-NMR analysis of its Mosher's derivative. This successful trial of the preparation of lactam alcohol **30b** led me to attempt to modify the preparation procedure as shown in Scheme V. Because of reports that the nitrile group is relatively inert to borane reduction,[267] nitrile **37** was treated with borane/THF to provide alcohol **46**, which was found to be optically pure by ^{1}H-NMR analysis of its Mosher's derivative. As before, upon acid hydrolysis, esterification, and neutralization-cyclization of the alcohol **46** gave optically pure lactam alcohol **30b** in 35% yield from nitrile **37**.

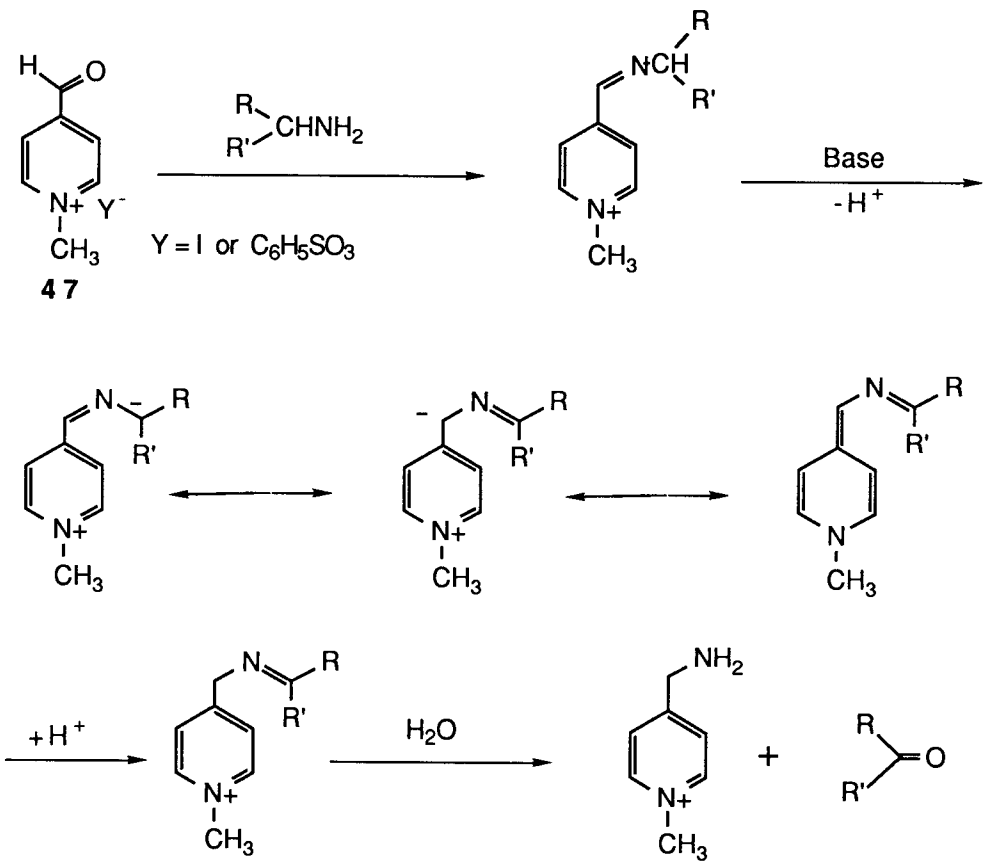
Scheme VI Alternative Synthesis of (S)-(+)-6-Hydroxymethyl-2-piperidone, **30b**.



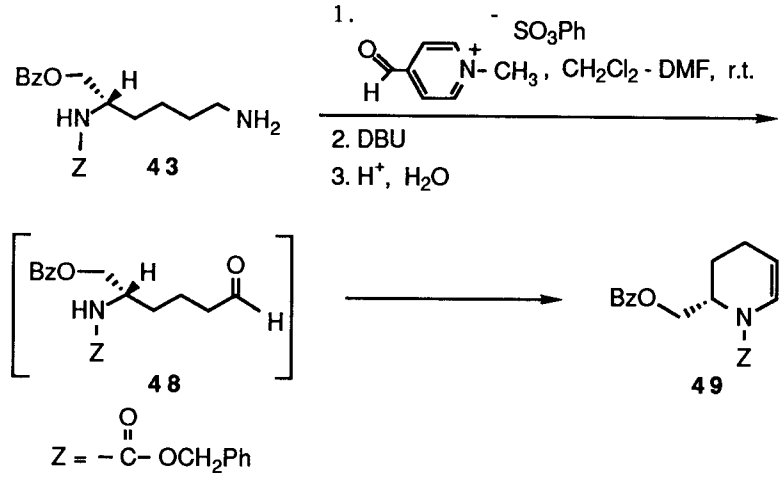
The excellent yields of borane reduction of **40** and **37** led me to further modify the synthetic design so that both the total yields and the convenience of operation would be improved. Rapoport and coworkers[268] have reported an interesting biomimetic conversion of amines to the corresponding carbonyl compounds. This process involves treatment of primary amines with 4-formyl-1-methylpyridinium benzenesulfonate **47**.

Following imine formation, prototropic rearrangement and hydrolysis, primary amines provide aldehydes or ketones (Scheme VII) in high yield.

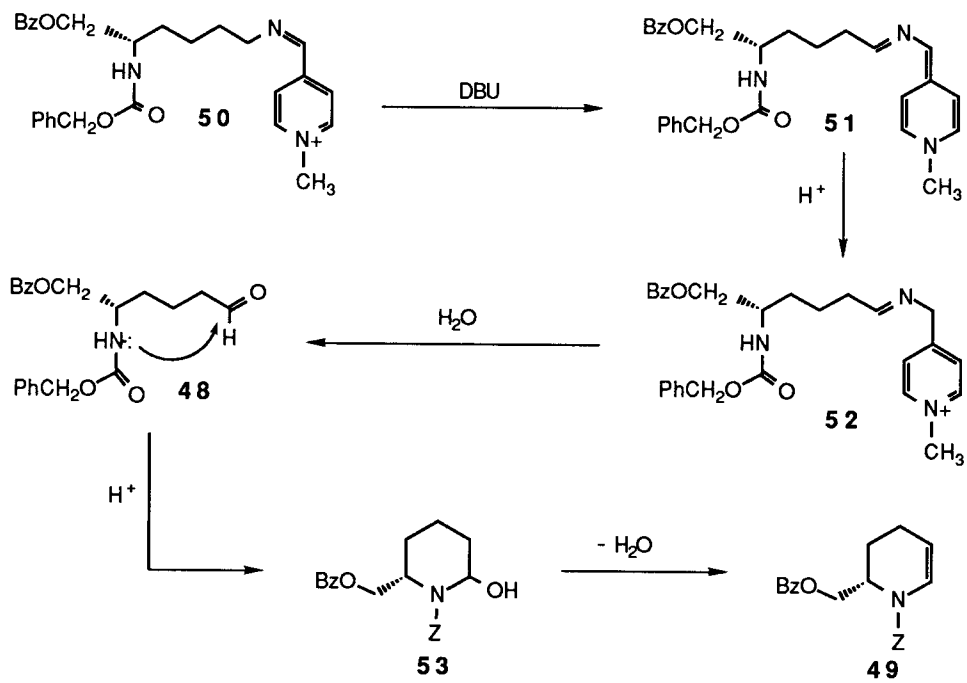
Scheme VII Transamination Reaction.



Unfortunately, no aldehyde **48** was found when **43** was subjected to the transamination reaction under the conditions reported in the literature. The product isolated from this transamination reaction was found to be enamine **49** (31% yield). The formation of enamine **49** is probably due to rapid intramolecular cyclization and dehydration of the aldehyde (Scheme VIII).



Scheme VIII Proposed Mechanism of Enamine **48** Formation.



At this stage, I decided to reinvestigate the $LiBH_4$ reduction of lactam ester **33b** in order to determine the cause of the racemization. If the problem could be corrected, a greatly improved preparation of lactam alcohol **30b** would result. The first action was to investigated the reduction step. Lactam acid **39**, prepared in optically pure form from

L-α-aminoadipic acid by literature procedures,[264,265] was converted to its corresponding esters **33b** and **33c** by diazoalkane treatment and then reduced with $LiBH_4$. Because diazoalkane treatment of the acid will not epimerize the α-carbon of the acid, this is a decisive test of the $LiBH_4$ reduction. Happily, optically pure lactam alcohol **30b** was obtained in 70% yield according to this process for both esters. This method demonstrates that the reduction step in scheme II is not responsible for the loss of optical purity; rather the lactam ester had already been degraded.

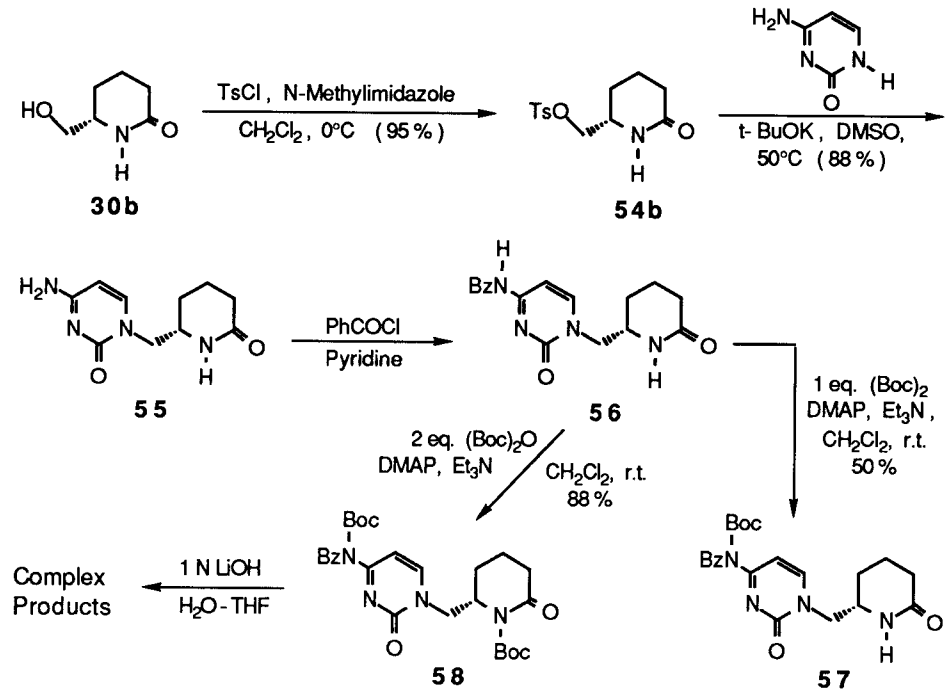
The source of racemization in the preparation of **30b** was quickly determined. Isolation of the intermediate amine **32b**, derivatization with Mosher's reagent and NMR analysis revealed that the first step in Scheme II had proceeded without effect on the stereogenic center. The original literature procedure[269] for the cyclization of **32a** to **33a** calls for neutralization of the esterification reaction with potassium hydroxide to pH 8, evaporation, and thermal cyclization. This technique ultimately provided **30b** of 50% optical purity. However, if the amine **32b** is isolated by an aqueous extractive workup, this material may then be cyclized to optically pure **33b**. The cyclization can be done in refluxing ethanol (8 hours) or neat at 100°C (2 hours) without loss of optical purity. The racemization is then explained by an excess of inorganic base from the neutralization step present during the cyclization step. With this slight modification of the workup, the original method is very efficient for the conversion of L-α-aminoadipic acid into **30b**, affording 67% overall yield of optically pure material.

The initial strategy for the preparation of the cytosine-containing subunits is shown in Scheme IX. Reaction of the alcohol with tosyl chloride and N-methylimidazole in methylene chloride gave the tosylate **54b**. Treatment of the tosylate with the potassium salt of cytosine in dimethyl sulfoxide provided the alkylated species **55** in high yield. Introduction of the base protecting group proceeded smoothly upon treatment of **55** with benzoyl chloride in pyridine. Incorporation of a

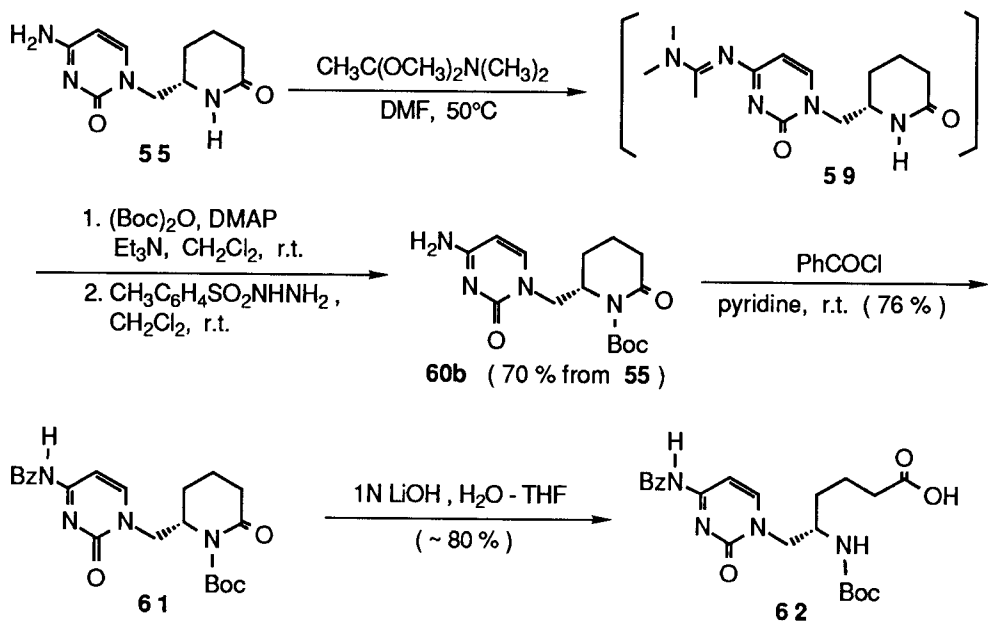
tert-butoxycarbonyl group on the lactam nitrogen would activate the ring toward hydroxide opening and conveniently provide the nascent amino group with the desired protection. Reactions of benzamide **56** with excess di-*tert*-butyl dicarbonate in the presence of 4-(dimethylamino)pyridine produced the bis-acylation product **58**. Attempted nucleophilic opening of the lactam in imide **58** gave complex mixtures of products (Scheme IX). Selective acylation at the lactam nitrogen was not possible as the benzamide moiety proved more reactive than the lactam towards the acylation reagent, resulting in the formation of **57** as evidenced by ^{1}H-NMR analysis. An upfield shift of the methine proton (δ 3.68) of the acylation product **57**, relative to the starting lactam **56** (δ 3.72) was observed. The chemical shift of this methine proton on the lactam ring is expected to shifted down-field if the acylation does occur at the lactam nitrogen, as is the case for the double acylation product **58** (δ 4.65).

To avoid the competing reaction at cytosine during lactam activation I turned to amidines for protection of the base amino group, a moiety suggested by Caruthers for use in oligonucleotide synthesis.[270] Reaction of **55** with N,N-dimethylacetamide dimethyl acetal produced the amidine **59**. In analogy to the known sensitivity of acylated cytosines to hydrazine,[271] the amidine group was selectively cleaved by reaction with tosylhydrazide to give **60b**. Following benzoylation, the lactam of **61** underwent selective hydrolysis by lithium hydroxide[272] to give the desired subunit **62** (Scheme X).

Scheme IX Preparation of the Cytosine-Containing Subunit Precursor.

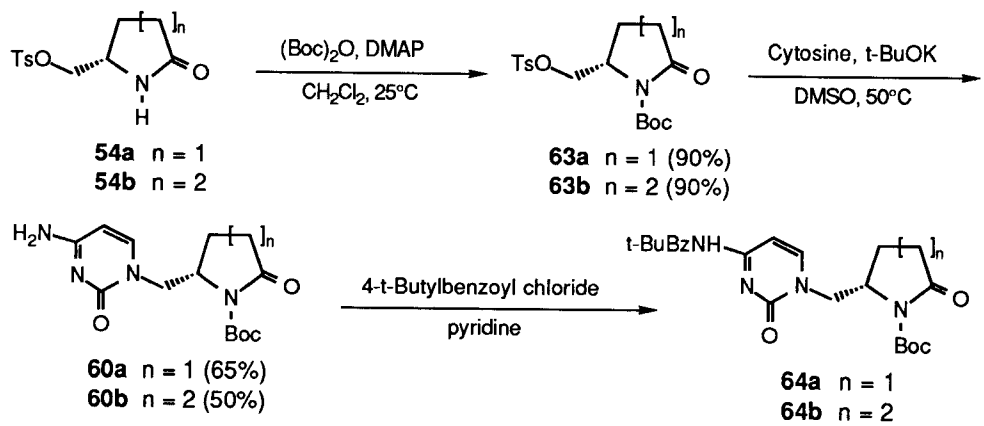


Scheme X Preparation of Monomeric Subunits *via* Amidine Protection.

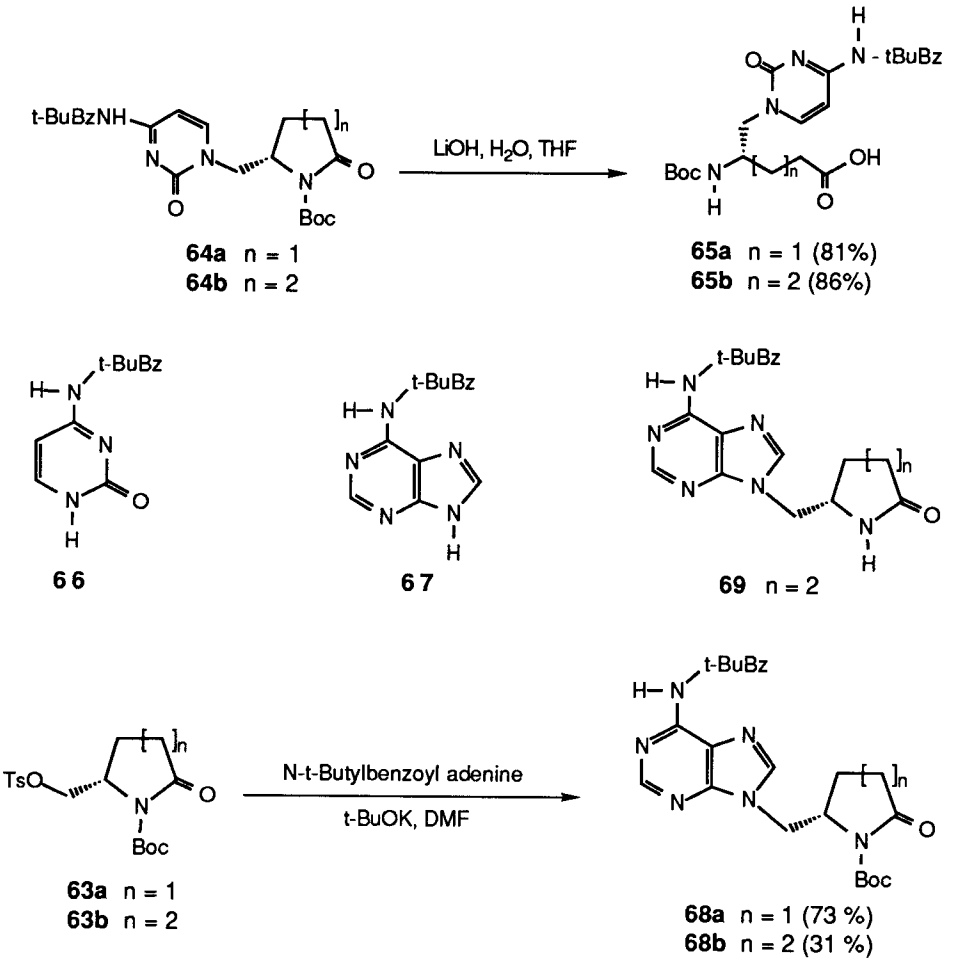


A significant improvement in the preparation of the subunits for the nylon analogues was achieved by early introduction of the *tert*-butoxycarbonyl group, thus avoiding the amidine protection/deprotection sequence. Acylation of the lactam tosylate **54** provided **63**, which underwent alkylation under rigorously anhydrous conditions to **60** (Scheme XI). Reaction with tert-butylbenzoyl chloride in pyridine produced **64** and hydrolysis gave the superior subunits **65**. The change to the more lipophilic *tert*-butylbenzoyl group was necessitated by the extreme polarity of these materials and their subsequent oligomerization products. In the case of **63a**, the alkylation could be successfully performed with the potassium salt of N-*tert*-butylbenzoylcytosine **66** to provide **64a** directly in 70% yield. However, the lactam tosylate **63b** did not effectively alkylate the less reactive acylated cytosine derivative and **64b** was obtained in only 5% yield by this procedure. When the reaction was run at room temperature for 48 hours, the yield was marginally improved (19%) and considerable amounts of the lactam tosylate **54b** could be isolated, suggesting that the *tert*-butoxycarbonyl group on the lactam is thermally labile. Alkylation of *tert*-butylbenzoyladenine **67** by **63a** is also efficient, affording 73% of **68a** while **63b** yielded only 25% of **69** at 75°C and 31% of **68b** at room temperature.

Scheme XI Preparation of Monomeric Subunit Precursors *via* Direct Alkylation.



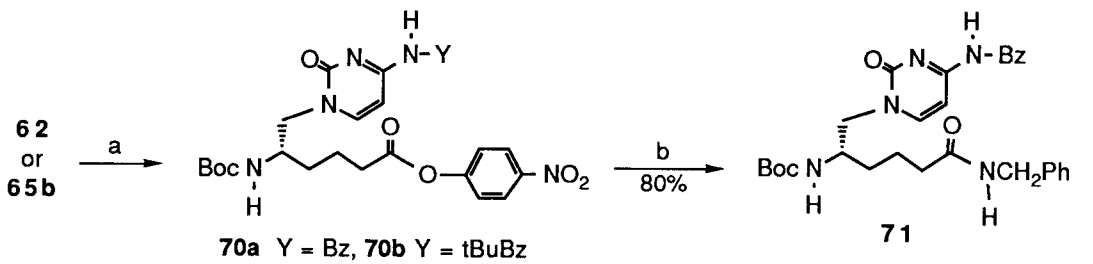
Scheme XI (continued)



For the assembly of the oligomers, the general strategy for oligopeptide synthesis was applied. This involves coupling of a nucleophilic subunit (an amine) with an electrophilic subunit (activated acid) to form an amide linkage. In order to find the proper activation/coupling procedure, several activation methods were evaluated for the formation of benzylamide **71** (Scheme XII). The results are summarized in Table I. The *p*-nitrophenyl ester **70** was conveniently prepared by carbodiimide coupling. Ester **70** reacted in reasonable yield (80%) with benzylamine to give **71**, and this active ester was chosen as the standard. Satisfactory coupling yields were also obtained using

active esters derived by reaction of the **62** with bis-(succinimidyl carbonate) and bis-(oxazolidinyl)phosphoryl chloride in 78% and 67% yields, respectively. The use of succinimidyl ester for coupling was halted after isolation of N-benzoyloxysuccinimide, suggestive that a serious side reaction, namely cleavage of the base protecting groups by N-hydroxysuccinimide, was occurring.

Scheme XII Benzylamide Formation.

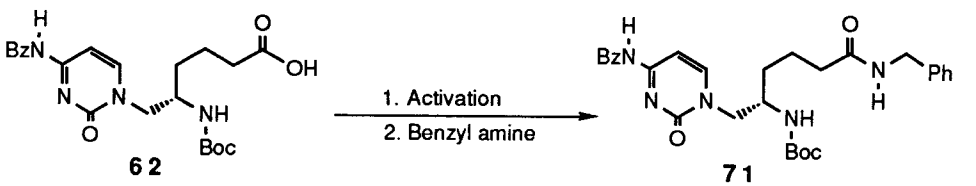


a) $CH_3CH_2N{=}C{=}NCH_2CH_2CH_2N(CH_3)_3I$, CH_2Cl_2, DMAP, p-nitrophenol. b) DMF, benzylamine

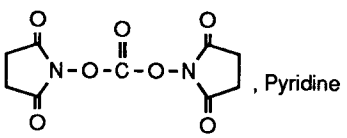
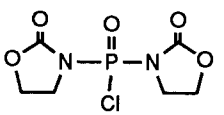
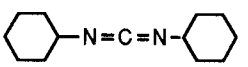
The extreme polarity of the model amide **71** prompted a shift to the *tert*-butylbenzoyl-protected subunit **65**. Additionally, the prospect of working with oligomers containing a free carboxylic acid terminus was unattractive, and it appeared that a carboxyl masking group would be beneficial for the synthesis of oligomers by a block approach. Ideally, the acid protecting group would be relatively nonpolar, would be insensitive to trifluoroacetic acid cleavage of the tert-butoxycarbonyl group, and would not be prone to lactam formation with the free amino group. Protection of carboxylic acids as their hydrazides has been previously demonstrated, and such groups are cleaved by mild oxidative methods.[273] The 1-aminopiperidine was chosen in order to increase the nonpolar character of the hydrazide. It was necessary to purify the commercially available 1-aminopiperidine by recrystallization of the oxalate salt in order to remove contamination by piperidine. Because of the low nucleophilicity of 1,1-

dialkylhydrazines, the secondary amine is a very effective competitor for activated acid derivatives. The hydrazide **72** was formed in reasonable yields by reaction of **70b** with 1-aminopiperidine in dimethylformamide (Scheme XIII).

Table I Summary of Methods for the Activation of Monomer Acid.

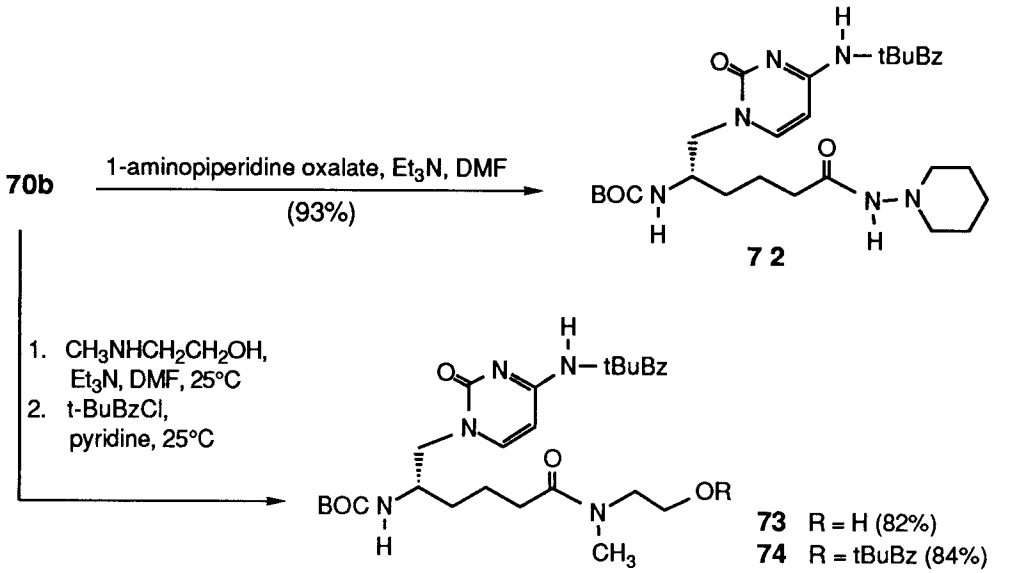


62 **71**

	REAGENTS FOR ACTIVATION	YIELDS	COMMENTS
1.	 , Pyridine	75 - 80 %	Easy work-up Side reaction in dimerization stage
2.	 (BOP-Cl)	65 - 70 %	Problems in separation Low yield in dimerization reaction
3.	 , PNP , DMAP	> 80 %	Difficult to remove dicyclohexyl urea from the reaction mixture
4.	 , PNP, DMAP	> 80 %	Easy work-up and separation

** PNP = p-Nitrophenol

Scheme XIII Protected Monomer Preparation.

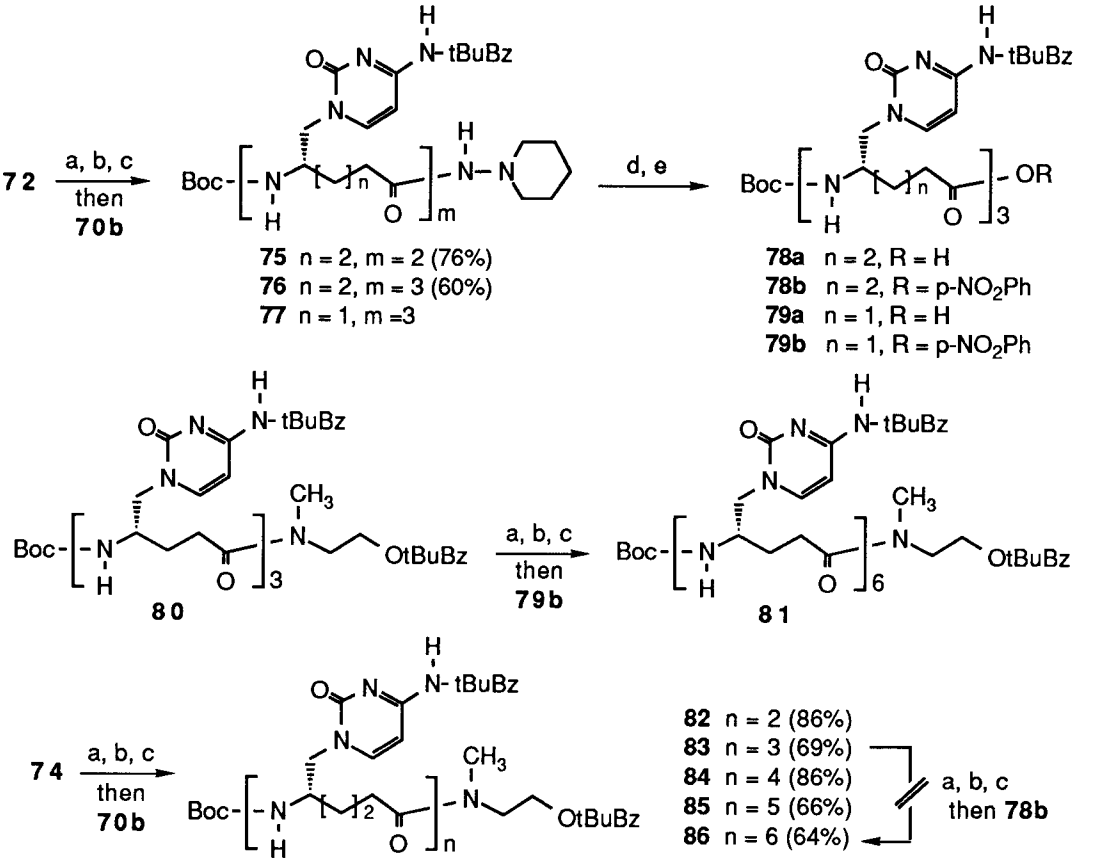


Prior to initiating chain extension we decided to adjust the functionality at the C-terminal subunit of the oligomers. During biophysical testing of the interaction of the nylon oligomers with nucleic acids a charged carboxylate terminus is undesirable. Reaction of **70b** with 2-(methylamino)ethanol gave the alcohol **73**, which was converted into the more lipophilic ester **74** (Scheme XIII). This neutral cap on the C-terminus would be relatively nonpolar during oligomer synthesis, and, after ammonolysis of the ester, should aid in solubilizing the oligomer in water during testing.

The target chosen for the examination of the oligomerization process was a protected hexamer of **27** (Scheme I, n = 6), and it was originally attemped to employ a block synthesis strategy. Hydrazide **72** was treated with 25% trifluoroacetic acid in dichloromethane to effect removal of the *t*-butoxycarbonyl group (Scheme XIV). Reaction with active ester **70b** produced the dimer **75** in 76% yield. Repetition of this procedure produced the hydrazine capped trimer **76** in 60% yield. By the same

procedure, the corresponding end capped species **74** afforded C-terminal dimer **82** and the trimer **83** in 86% and 69% yields, respectively.

Scheme XIV Oligomerization Reactions.



a) CH_2Cl_2/TFA (4:1) b) DMF, TsOH; Evaporate. c) Et_3N/DMF or DMSO
d) NBS, pyridine, H_2O, THF, 25°C.
e) $CH_3CH_2N{=}C{=}NCH_2CH_2CH_2N(CH_3)_3I$, CH_2Cl_2, DMAP, p-nitrophenol.

A previous investigation on the corresponding five-atom backbone analogues had demonstrated that oxidative cleavage of the trimer hydrazide **77** by N-bromosuccinimide affords trimer acid **79a** quantitatively.[263,273] This acid was converted into the *p*-nitrophenyl ester **79b** and reacted with 1.2 equivalents of the C-

terminal trimer amine derived from acid cleavage of **80**. The hexamer **81** was isolated in 50% yield from the trimer hydrazide **77**. Unexpectedly, the six-atom backbone series was not efficiently prepared by the coupling of trimers **76** and **83**. Deprotection of the hydrazide **76** and activation of the acid to **78b** proceeded smoothly by TLC. However, no trace of hexamer **86** could be obtained from the reaction of trimer active ester **78b** and the amine derived from C-terminal trimer **83**. Isolated instead were acid **78a** along with the amine component. Therefore, the stepwise addition of monomer units to trimer **83** was continued.

The trimer **83** was subjected to the deprotection/coupling scheme to produce the tetramer **84** in 86% yield. Further reaction to pentamer **85** and hexamer **86** proved difficult in this series, primarily due to problems of solubility with their immediate precusors, the tetramer amine and pentamer amine, respectively. As a result, these coupling reactions were carried out in dimethyl sulfoxide. Unfortunately, the oligomeric amines are also relatively insoluble in this medium, and it is difficult to achieve completeness in the coupling reaction with **70b**. Samples of **85**, and finally the **86**, were obtained in 66% and 64% crude yields, respectively. In contrast to the lower oligomers, the pentamer **85** and the hexamer **86** were not satisfactorily purified by routine column chromatography or preparative TLC on silica due to the contamination by the amine component from the coupling. To obtain pure materials for characterization, these compounds required further purification on a silica gel HPLC column.

The fully protected oligomers **75**, **76** and **82 - 86** are very polar amorphous solids with adequate solubility in dimethylformamide, dimethyl sulfoxide, and methanol/chloroform mixtures. While characterization of synthetic intermediates through the monomer level was routine, upon chain elongation, the NMR spectra become extremely complex. The complexity is compounded by slow rotation about the

tertiary amide at the C-terminus. The structure of the lower oligomers was succesfully investigated by proton-proton two dimensional correlation spectroscopy (COSY). The signals for the 5-H and 6-H of the cytosine moieties of the oligomers are normally not resolvable in routine 1-D proton NMR. The downfield protons (6-H) of the cytosine ring are buried underneath, and hardly separate out from the aromatic signals of *tert*-butylbenzoyl groups. However, it is quite possible to assign the 5-H and 6-H protons coupling pairs of the cytosines by the homonuclear COSY experiments. For instance, it is quite clear that three pairs of cytosine's 5-H and 6-H couplings can be identified in the COSY spectra of trimer **83** (Fig. V). By this technique, the structures (length) of the dimers and trimers were evident because two and three pairs of cytosine proton couplings were identified in the spectra of **82** and **83**, respectively. Unfortunately, this method was unsuitable for the oligomers higher than trimer.

Upon closer inspection of the ^{1}H-NMRs of these oligomers, it was found that the signals of the C-terminal end capped α-methylene group (δ 2.29 - 2.46) of the amide linkages along the backbone was distinct from other α-methylene groups (δ 1.85 - 2.17). Although the base line of the spectra are strongly perturbed by the signals from the residual non-deuterated NMR solvent and the associated water molecules in these highly hygroscopic oligomers, identification of the chain length of the oligomers by relative integration of these two types of α-methylene groups is still possible. By this technique, the length of the oligomers was further confirmed (Table II).

Figure V COSY Expansion Plot of Trimer **83**.

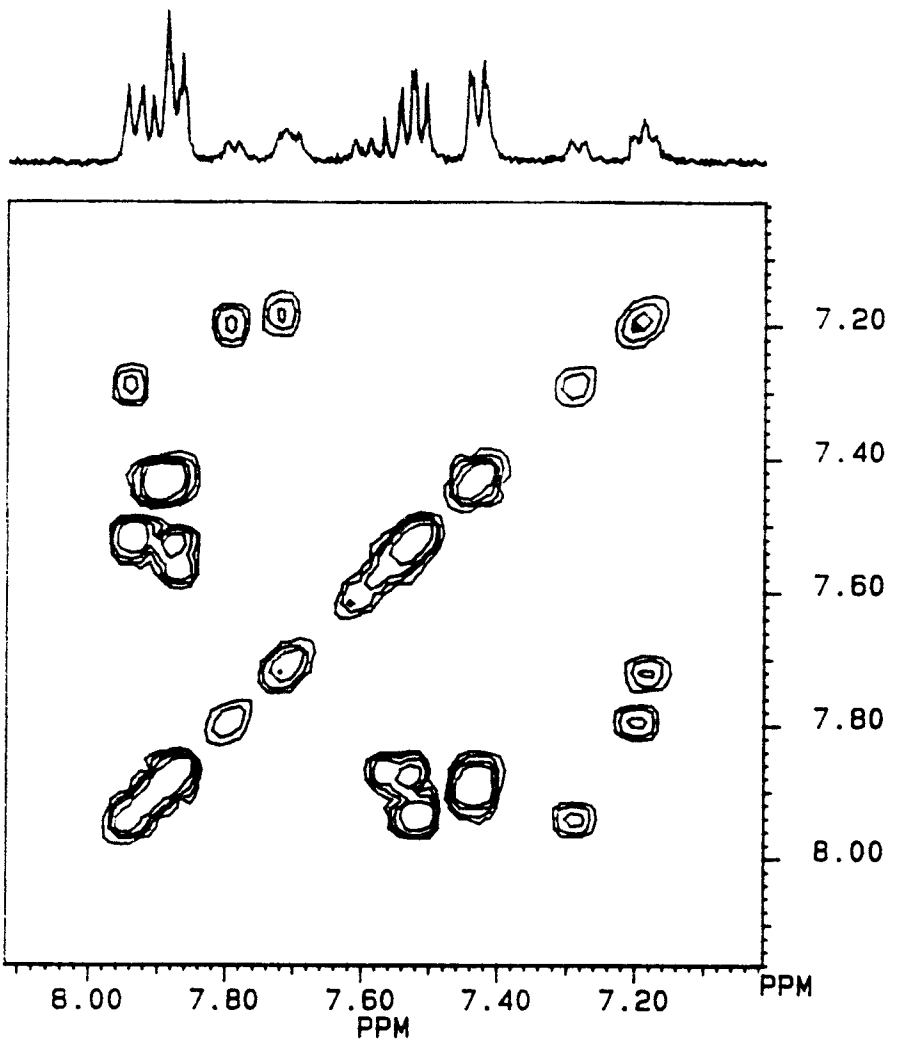
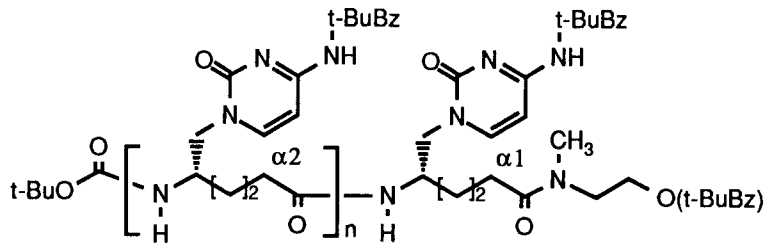


Table II. Relative Integration for the α-Methylene Groups.



Compound Entry	Chemical Shifts at δ 2.29 - 2.46 (α1)	Chemical Shifts at δ 1.85 - 2.17 (α2) found	calcd
82 (n = 1)	2.00	2.00	2.0
83 (n = 2)	2.00	3.95	4.0
84 (n = 3)	2.00	5.48	6.0
85 (n = 4)	2.00	7.44	8.0
86 (n = 5)	2.00	9.34	10.0

It also relied heavily on fast atom bombardment mass spectrometry (FAB MS) for analysis of the oligomers. Although both positive and negative ion FAB gave useful data, negative ion technique appears to be superior for the nylon(6)-1 oligomers. Unlike the carbamate-linked oligonucleosides **10** and **11**,[274-276] in which sequence information could be obtained due to the occurrence of characteristic cleavage modes, few peaks besides the parent clusters could be routinely identified for the nylon oligomers. One peak commonly seen was at 270 in the negative ion FAB (272 in positive FAB) for *t*-butylbenzoylcytosine. Exact mass analysis of the parent ion (M-H) was obtained for monomers and all oligomers. The results are shown in Table III.

Table III. Exact Mass Determination of Oligomers by Negative Ion FABMS.

Compound Entry	Calculated Mass (M-H)	Observed Mass (M-H)
75	963.5456	963.5425
76	1345.7416	1345.7298
82	1098.6028	1098.5870
83	1480.8033	1480.8091
84	1863.0036	1862.9994
85	2245.2040	2245.2024
86	2627.4046	2627.3954

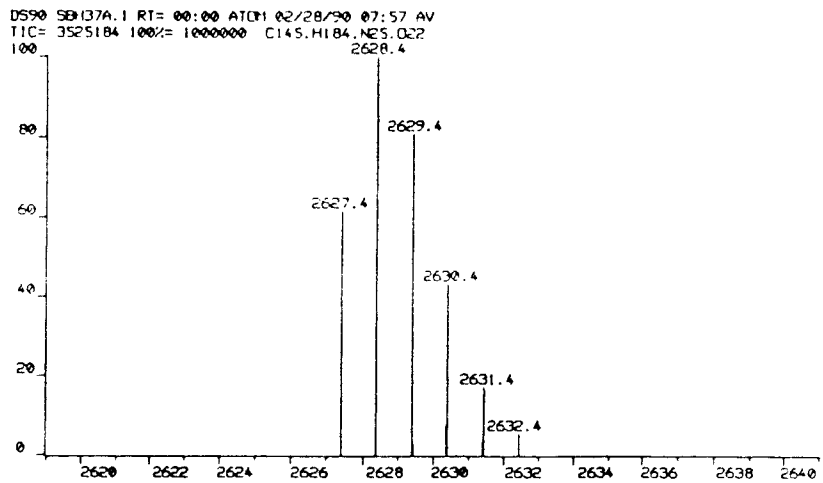
The major peak of the molecular cluster $(M-H)^-$ in the negative ion FABMS analysis for the oligomers higher than trimer were routinely observed with one mass unit higher than the calculated mass. Theoretical calculations of the molecular cluster for the oligomers were performed with the software (DS90) provided with the mass spectrometer (KRATOS MS50RF). The peak pattern, and peak mass of the molecular clusters were closely matched the theoretical calculations. A typical example is shown in Figure VI.

Although the synthesis of **81** is not yet optimal, it nevertheless has provided small quantities of pure material suitable for biophysical testing. Following analogy to published work on the nucleoside carbamates, a polyethylene glycol 1000 (PEG) "tail" was appended to the molecule as shown in Scheme **XV**. Activation of the PEG hydroxyl by conversion to the *p*-nitrophenyl carbamate **87**,[178] and reaction with deprotected hexamer amine gave **88**, which was not isolated, but treated directly with concentrated ammonia in dimethylformamide to obtain the fully deprotected species **27**. This was purified by ion exchange chromatography on a cation exchange column to provide a small quantity of pure material. The sample was characterized by ultraviolet

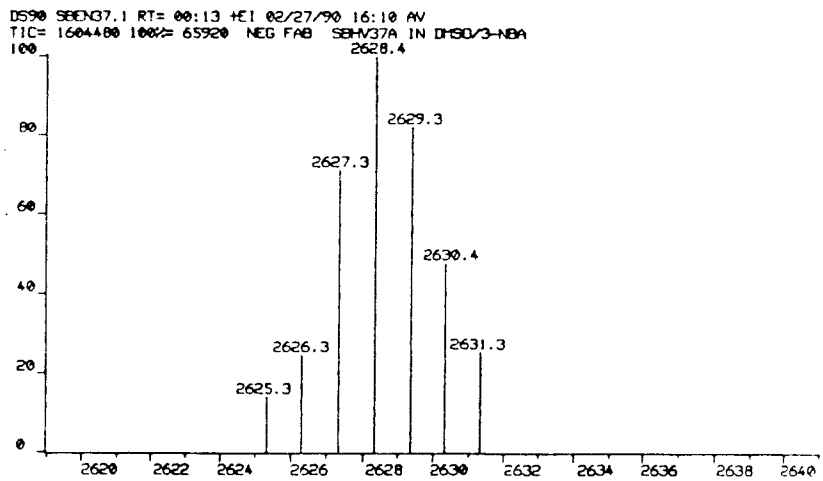
spectroscopy. The loss of benzoyl groups was evidenced by the absence of absorption in the 295-305 nm region and the presence of the usual cytosine chromophore at 271 nm.

Figure VI Mass Pattern Match for the Molecular Cluster of Hexamer **86**.

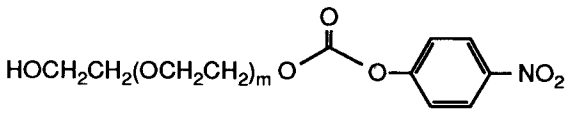
A.



B.

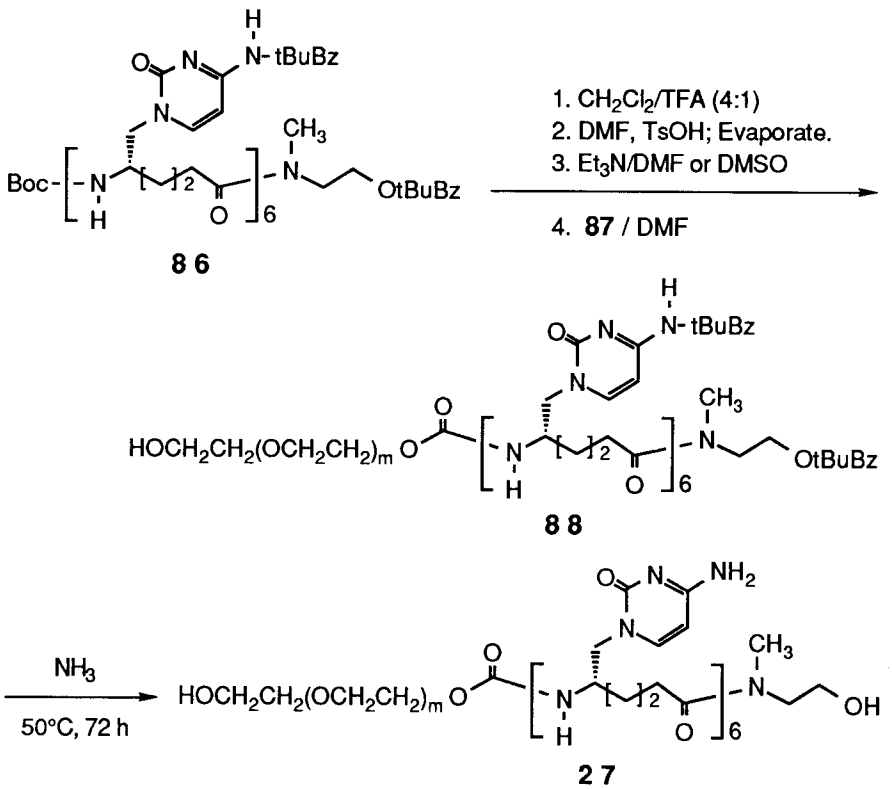


A. Theoretical Calculations. B. Actural Mass Spectrum.



87

Scheme XV Attachment of PEG Tail and Deprotection Rreactions.

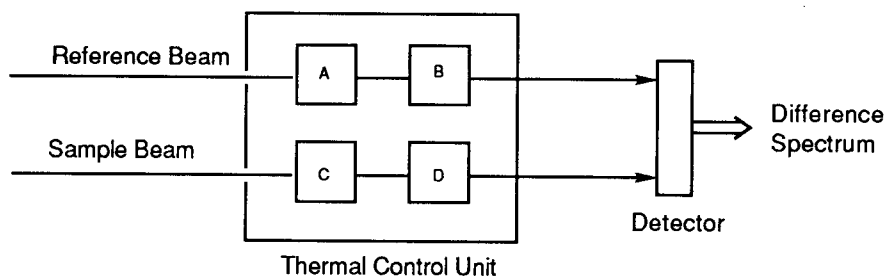


86 88 27

The binding ability of this tailed hexamer **27** with its complementary nucleic acid, $p(dG)_6$, was studied by analysis of the ultraviolet absorption spectrum and thermal denaturation of the complex. The thermal denaturation experiment was performed by obtaining a series of difference ultraviolet spectra between mixed and unmixed samples of **27** and $(dG)_6$ from 15°C to 90°C over a range of 260 to 320 nm. The schematic representation of the difference spectra experiment is shown in Figure VII. In the

sample beam of the spectrometer are placed two cuvettes. One contains buffer alone while the other contains buffers, the neutral analogue and the target. In the reference beam of spectrometer are also placed two cuvettes, containing the neutral analogue and the target in buffers separately. The total amounts of the neutral analogue and the target are identical in both beams. The output is then a difference spectrum where both hypochromic and hyperchromic effects can be seen, depending on the wavelength. After annealing of the neutral analogue and the target, the spectrum is recorded at 15°C and, if pairing is evident, the samples are heated to melt the complex. Thus, a plot of the absorbance difference (Δ absorbance) at the wavelength of maximum hypochromicity against the temperature can be obtained to determine the T_m value of the complex.

Figure VII Schematic Representation of Difference UV Spectrum Measurement.

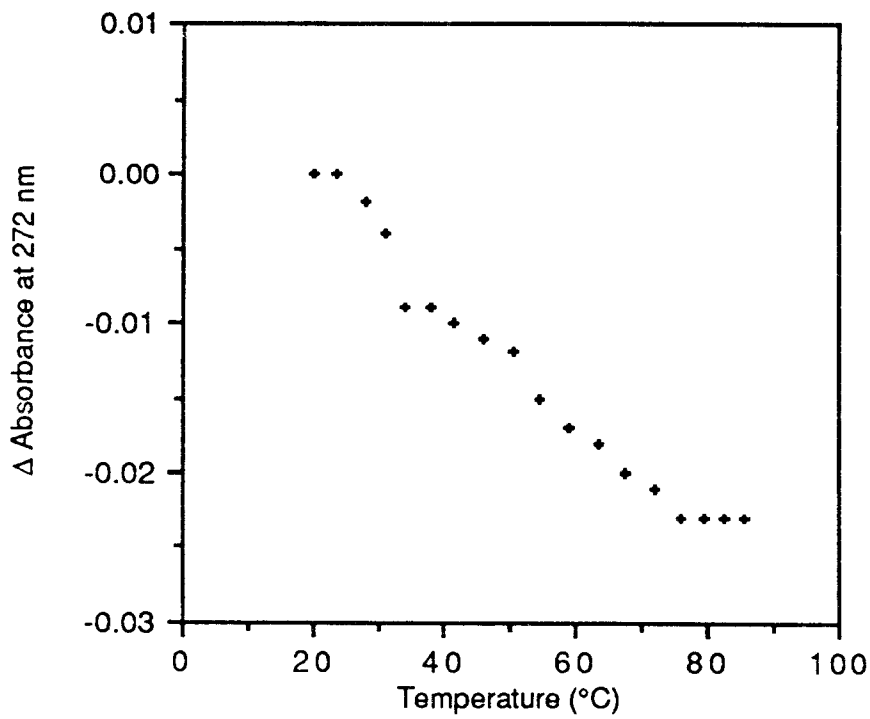


Cuvette A: Neutral Analogue/Buffer. Cuvette B: Target/Buffer.
Cuvette C: Neutral Analogue + Target/Buffer. Cuvette D: Buffer Only.

The difference spectrum of the complex of **27** with $p(dG)_6$ clearly demonstrated the interactions between the neutral analogue **27** and the DNA as evidence that a hypochromic effect at 272 nm was observed. The hypochromicity of this complex was estimated to be ~10% at 272 nm. A plot of the absorbance difference (Δ absorbance) at 272 nm vs. temperature (°C) was obtained (Figure VIII), which reflects a hypochromic

effect. However, the T_m value of this thermal denaturation can not be obtained, as a non-ideal sigmoidal curve was derived; it was estimated to be 48°C. This may possibly be attributed to nonequivalent sample concentrations in the sample beam and the reference beam for the ultraviolet spectra measurements. The concentration differences may arise from the sample transfer by using polyethylene pipet tips for automatic micropipets, as the neutral analogues will often not transfer completely due to the hydrophobic interactions between the neutral analogue and polyethylene.

Figure VIII Thermal Denaturation of **27** with $p(dG)_6$.



In conclusion, the hexamer **86** can be synthesized by solution phase chemistry but inefficiently, due to the solubility problems associated with the coupling reactions when attempting to prepare oligomers higher than tetramer. To improve the availability of the nylon-6(1) analogues, a thorough investigation of the coupling reactions is

required and alternative synthetic strategies may be necessary. More sophisticated NMR markers on the oligomer (in the case present herein is the N-methyl group on the C-terminus) should give proper structural confirmation by easier and clear identification of the marker with accurate integrations. Alternatively, attachment of a colored cap to the oligomer's N-terminus and subsequent determination of the ratio of the cap and bases spectrophotometrically, a method applied for molecular weight determination used by Jones and coworkers,[238] may also provide adequate results.

Although the preliminary binding studies provide evidence for association of **27** with $p(dG)_6$, more complete characterization of interactions must be pursued, e.g. a mixing curve experiment. More careful experimental techniques, such as using different solvents for the sample preparation to solve the pipetting problems associated with solution transfer of neutral oligonucleotide analogues and obtaining a smooth thermal denaturation curve by carried out the experiment with small temperature increments, will be necessary for future studies.

EXPERIMENTAL

Methylene chloride (CH_2Cl_2), pyridine, dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were distilled from powdered calcium hydride (CaH_2) and stored over 3Å/4Å molecular sieves. Methanol (CH_3OH) and ethanol (EtOH) were distilled from the corresponding magnesium alkoxides and stored over 4Å molecular sieves. Anhydrous tetrahydrofuran (THF) and diethyl ether (Et_2O) were freshly distilled from sodium/benzophenone prior to use. All other reagents were purified by distillation or recrystallization prior to use whenever necessary. All moisture-sensitive reagents were transferred in a dry box or via a syringe under a positive pressure of nitrogen or argon atomsphere. All moisture-sensitive reactions were carried out under a positive pressure of inert gas. Column chromatography was performed by using silica gel 60 (Merck, 340 - 400 mesh ASTM) or basic alumina Brockman Activity I (Fisher Scientific, 80 - 200 mesh). Chromatography solvents were distilled before use. Analytical TLC was conducted on precoated Merck silica gel 60 F_{254}, J. T. Baker silica gel IB-F or Merck aluminium oxide 60 F_{254} (neutral, type E) plates. Preparative TLC was performed on precoated Merck silica gel 60 F_{254} (1 or 2 mm thickness) plates. The ^{1}H-NMR and ^{13}C-NMR, including 2D experiments, were taken on either AM-400 or AC-300 Bruker spectrometers with tetramethylsilane (TMS) as internal standard. The chemical shifts (δ) are reported in parts per million (ppm) downfield from TMS. Infrared spectra (IR) were obtained on Perkin-Elmer model 727B or Nicolet 5DXB FT-IR spectrometers. Mass spectra were recorded on Kratos MS50RF, Varian CH7 or Finnigan 4000 mass spectrometers. Optical rotations were measured with a Perkin-Elmer model 243 polarimeter. Melting points were determined on a Buchi capillary melting point apparatus and are not corrected.

(S)-(+)-6-oxo-piperidine-2-carboxylic acid, ethyl ester (**33b**).

Method A: (S)-(+)-α-Aminoadipic acid **31b** (2.0 g, 12.4 mmol) was suspended in dry EtOH (20 mL) and cooled in an ice bath. To this cold solution was dropwise added freshly distilled $SOCl_2$ (2.4 mL, 32.9 mmol). After 1.5 h at room temperature, then 1 h at reflux, the reaction mixture was evaporated to dryness. The residue was redissolved in $CHCl_3$, and the solution washed with 0.15 M NaOH (1x 50mL), saturated $NaHCO_3$ (1x 50 mL), H_2O (1x 50 mL) and brine (2x 50 mL) then dried over anhydrous Na_2SO_4. Following removal of solvent under reduced pressure the residual oil was heated under N_2 at 100°C for 2 h, then Kugelrohr distilled (170°C, 0.8 torr) to give 1.42 g (67% yield) of product as a light yellowish oil. ^1H NMR (300 MHz, $CDCl_3$): δ 6.19 (1H, br s), 4.24 (2H, q, J = 7.1 Hz), 4.08 (1H, m), 2.36 - 2.44 (2H, m), 2.18 - 2.28 (1H, m), 1.72 - 1.98 (3H, m), 1.30 (3H, t, J = 7.1 Hz). IR (neat): 3234, 3104, 2964, 1735, 1669 cm^{-1}. EIMS, m/z (rel. intensity): 171(M+, 5.3), 144(2.4), 99(6.4), 98(100), 70(6.3), 61(5.9), 56(3.0), 55(35.5). $[\alpha]_D^{22}$ = + 21.7 (c = 0.3, EtOH); - 13.7 (c = 0.3, $CHCl_3$). HRMS (EI): calcd for $C_8H_{13}NO_3$ (M+) 171.0895; found 171.0895.

Method B: The (S)-(+)-lactam acid **39** [264,265] (28 mg, 0.196 mmol) was dissolved in CH_3OH (0.5 mL), diluted with ether (2 mL) and cooled in an ice bath. To this solution was added dropwise a freshly prepared solution of diazoethane in ether until no further gas evolution was observed and the yellow color of the solution remained for a few min. The excess diazoethane was quenched by addition of several drops of 10% AcOH/CH_3OH. Removal of volatiles followed by column chromatography on silica (2% $CHCl_3$/CH_3OH) gave 34 mg (100% yield) of product as a light yellowish oil.

(S)-(+)-6-oxo-piperidine-2-carboxylic acid, methyl ester (**33c**).

The (S)-(+)-lactam acid **39** (66 mg, 0.46 mmol) was dissolved in CH_3OH (0.5 mL), diluted with ether (2 mL) and cooled in an ice bath. To this solution was added

dropwise a freshly prepared solution of diazomethane in ether until no further gas evolution was observed and the yellow color of the solution remained for a few min. The excess diazomethane was quenched by addition of few drops of 10% AcOH/CH_3OH. Removal of volatiles followed by column chromatography on silica (2% $CHCl_3/CH_3OH$) gave 72 mg (100% yield) of product as a clear oil. $[\alpha]_D^{22}$ = – 10.7 (c = 0.57, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 6.30 (1H, br s), 4.11 (1H, br t, J = 6.0 Hz), 3.79 (3H, s), 2.40 (2H, m), 2.21 (1H, m), 1.72 - 1.94 (3H, m).

(S)-(+)-Ethyl pyroglutamate (**33a**).

(S)-(+)-Glutamic acid (5.0 g, 33.98 mmol) was converted to its diethyl ester hydrochloride salt by the same procedure as described for the preparation of **33b**. After a similar aqueous workup, the resulting amine was subjected to cyclization under N_2 at 120°C for 4 h and Kugelrohr distilled (170°C, 1 torr) to give 3.85 g (72% yield) of product as a light yellowish oil which solidified upon routine storage in the freezer, mp 49 - 50°C. The spectral data of this ester were identical to those reported in the literature. Lactam **30a** was found to be optically pure, as judged by $LiBH_4$ reduction of the ester **33a**, derivatization with Mosher's reagent, and analysis by ^1H-NMR.

(S)-(+)--α-Aminodiethyladipate, hydrochloride salt (**32b**).

This compound could be isolated by evaporation of the esterification reaction mixture (when preparing **33b**) and purified by recrystallization from $CHCl_3$/hexanes to give pure material as white crystals, mp 123 -123.5°C. ^1H NMR (300 MHz, $CDCl_3$): δ 8.88 (3H, br s), 4.28 (2H, m), 4.12 (3H, m with q, J = 7.1 Hz), 2.40 (2H, t, J = 7.1 Hz), 2.12 (2H, m), 1.72 - 2.02 (2H, m), 1.32 (3H, t, 7.1 Hz), 1.25 (3H, t, 7.1 Hz). IR (KBr): 2995 br, 1743, 1728 cm^{-1}. EIMS, m/z (rel. intensity): 172($(M-Cl)^+$, 16.5), 171($(M-Cl)^+$, 5.5), 144(48.8), 102(3.4), 99(6.1), 98(100), 70(7.2), 56(25.3),

55(36.8). $[\alpha]_D^{22}$ = +13.3 (c = 0.91, $CHCl_3$). Anal. calcd. for $C_{10}H_{20}ClNO_4$: C, 47.32; H, 7.95; N, 5.52; found C, 47.15; H, 8.17; N, 5.47.

A small amount of this amine was converted to its amide with Mosher's reagent upon *in situ* neutralization with 3.0 equivalents of Et_3N by the same procedure as described for the preparation of Mosher's esters **38**.

Mosher's amide of (+/-)-α-aminodiethyl adipate .

^1H NMR (300 MHz, $CDCl_3$): δ 7.38 - 7.70 (11H, m), 7.72 (1H, br d, J = 7.7 Hz), 4.63 (2H, m), 4.04 - 4.28 (8H, m), 3.53 (3H, d, J = 1.4 Hz), 3.38 (3H, d, J = 0.9 Hz), 2.35 (2H, br t, J = 7.3 Hz), 2.27 (2H, br t, J = 7.3 Hz), 1.63 - 2.10 (8H, m), 1.18 - 1.38 (12H, m).

Mosher's amide of (S)-(+)-α-aminodiethyl adipate.

^1H NMR (300 MHz, $CDCl_3$): δ 7.48 - 7.62 (6H, m), 4.63 (1H, m), 4.09 - 4.28 (4H, m), 3.38 (3H, d, J = 1.1 Hz), 2.36 (2H, br t, J = 7.0 Hz), 1.93 - 2.08 (1H, m), 1.63 - 1.84 (3H, m), 1.19 - 1.38 (6H, m).

(S)-(+)-6-hydroxymethyl-2-piperidinone (**30b**).

Method A: To a solution of ethyl ester **33b** (1.42 g, 8.3 mmol) in CH_2Cl_2 (20 mL) was added dropwise a 2 M solution of $LiBH_4$ in THF (4.2 mL, 8.4 mmol). The resulting mixture was stirred at room temperature for 2 h then quenched by dropwise addition of 6 M HCl with cooling in an ice bath until no further gas evolution occurred. The resulting solution was diluted with CH_3OH (50 mL), neutralized by addition of solid K_2CO_3 and filtered. Following removal of solvent, the residual oil was loaded on to a short path silica column and eluted with 10% $CH_3OH/CHCl_3$ (300 mL). The filtrate was concentrated and rechromatographed on silica (5% $CH_3OH/CHCl_3$) to give 950 mg (89% yield) of lactam **30b** as a hygroscopic, white solid, mp 74 °C. Performing the reaction in diglyme has no effect on the yield or optical purity of **30b**.

$[\alpha]_D^{22}$ = + 22.2 (c = 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 6.95 (1H, br. s), 3.70 (1H, dd, J = 11.5, 3.0 Hz), 3.54 (1H, m), 3.45 (1H, m), 3.21 (1H, br. s), 2.36 - 2.46 (1H, m), 2.22 - 2.32 (1H, m), 1.80 - 1.96 (2H, m), 1.64 - 1.80 (1H, m), 1.35 (1H, m). ^{13}C NMR ($CDCl_3$): δ 173.1, 65.8, 54.7, 31.1, 24.2, 19.3 ppm. IR (KBr): 3342, 3278, 3237, 3084, 2966, 2941, 2916, 1670 cm^{-1}. EIMS, m/z (rel. intensity): 129(M+,5.4), 99(5.2), 98(100), 70(6.0), 55(57.4). HRMS (EI): calcd for $C_6H_{11}NO_2$ (M+) 129.0790; found 129.0789.

Method B: The (S)-(+)-lactam acid **39** [264,265] (269 mg, 1.88 mmol) was suspended in dry THF (15 mL) and cooled to 0°C in an ice bath. A 1 M solution of BH_3·THF (3.8 mL, 3.80 mmol) was added dropwise to this cold suspension. The reaction mixture was stirred at 0°C for 1 h followed by dropwise addition of anhydrous MeOH (vigorous gas evolution). Solvents and byproduct methyl borate were removed under reduced pressure. This methanolic work-up was repeated three times. After complete removal of solvents, the residual oil was Kugelrohr distilled (170°C, 25 μtorr) to gave 133 mg (55% yield) of lactam alcohol as a light yellowish oil which solidified upon standing under high vacuum. For larger scale procedures (2 g) the yield was generally 35%.

Method C: A sample of nitrile alcohol **46** (5.15 g, 19.7 mmol) was mixed with 6M HCl (100 mL), and heated to gentle reflux for 24 h. The reaction mixture was cooled to room temperature and extracted with $CHCl_3$ (3 x 30 mL). The aqueous portion was then concentrated to dryness and dried further over P_2O_5 under high vacuum for 12 h. The crude hydrochloride salt of the δ-amino acid was dissolved in dry CH_3OH (20 mL), a solution of methanolic hydrochloric acid (2.5 M, 20 mL) added, and the mixture gently refluxed for 8 h. The reaction mixture was cooled in an ice bath and the solution was adjusted to pH 8-9 by careful addition of 2M $NaOCH_3/CH_3OH$ solution. Precipitated NaCl was filtered and the solution reheated to gentle reflux for one hour. Solvent was evaporated, the residue redissolved in 50% $CH_3OH/CHCl_3$ (100 mL), and

NaCl filtered off. The desalting process was repeated three times with decreasing concentration of CH_3OH in the solvent (20%, 10%, and 0% of $CH_3OH/CHCl_3$). Removal of solvent and column chromatography on silica (1-5% $CH_3OH/CHCl_3$) gave 1.30 g (52% yield) of lactam alcohol **30b**.

General Procedure for the Preparation of Mosher's Esters.

The alcohol of interest (1.0 equiv.) was dissolved in CH_2Cl_2 (2% solution). To this solution was added 1.0 equivalent of (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride and 2.0 equivalent of 4-(dimethylamino)pyridine. After 2 h at room temperature a few drops of H_2O were added with vigorous stirring. Following dilution with $CHCl_3$ (10x volume of the reaction mixture), the mixture was washed with 0.1 M HCl (1x), saturated $NaHCO_3$ solution (1x), brine (1x) and dried over anhydrous Na_2SO_4. The crude product obtained after removal of solvents was subjected to optical purity determination by ^1H NMR analysis without further purification.

Mosher's ester of (S)-(+)-6-hydroxymethyl-2-piperidinone (**38**).

^1H NMR (400 MHz, $CDCl_3$): δ 7.48 (2H, m), 7.43 (3H, m), 6.62 (1H, br s), 4.42 (1H, dd, J = 11.1, 4.3 Hz), 4.14 (1H, dd, J = 11.1, 6.7 Hz), 3.71 (1H, m), 3.53 (3H, s), 2.35 - 2.45 (1H, m), 2.18 - 2.33 (1H, m), 1.85 - 1.97 (2H, m), 1.68 - 1.80 (1H, m), 1.47 (1H, m).

Mosher's ester of (+/-)-6-hydroxymethyl-2-piperidinone (**38**).

^1H NMR (300 MHz, $CDCl_3$): δ 7.38 - 7.53 (10H, m), 5.75 (2H, br s), 4.55 (1H, dd, J = 3.8, 11.1 Hz), 4.43 (1H, dd, J = 4.0, 11.1 Hz), 4.09 (1H, dd, J = 8.0, 11.1 Hz), 4.00 (1H, dd, J = 8.1, 11.1 Hz), 3.64 - 3.79 (2H, m), 3.536 & 3.532 (6H, 2x br s), 2.35 - 2.48 (2H, m), 2.18 - 2.35 (2H, m), 1.86 - 1.98 (4H, m), 1.68 - 1.81 (2H, m), 1.38 - 1.52 (2H, m).

(S)-(-)-α-N-Carbobenzoxy-ε-N-*t*-butoxycarbonyl-2,6-diaminohexan-1-ol (**41**).

The dicyclohexylammonium salt of α-N-Cbz-ε-N-t-Boc-L-lysine **40**[260,266] (16.35 g, 29.1 mmol) was added to a mixture of ether (250 mL) and 10% H_2SO_4 (120 mL) with swirling. The ether layer was washed with 10% H_2SO_4 (2x 40 mL) and dried over anhydrous Na_2SO_4. Solvent was evaporated off under reduced pressure, the residual oil was dried further under high vacuum for 24 h to provide 11.09 g of **40**. This residue was dissolved in dry THF (60 mL) and treated dropwise with a 1M solution of BH_3·THF (60 mL, 60 mmol) at 0°C. After 2 h at 0°C, the reaction was quenched by dropwise addition of 10% AcOH/CH_3OH (15 mL). The resulting reaction mixture was evaporated to dryness, the residual oil was redissolved in EtOAc (500 mL), and washed with saturated $NaHCO_3$ solution (2 x 120 mL), 1M HCl (1 x 120 mL), and brine (1 x 120 mL), then dried over anhydrous Na_2SO_4. Removal of the solvent gave 10.11 g (95% yield) of crude product. A small amount of this crude product was purified by column chromatography on silica (2% to 5% $CH_3OH/CHCl_3$) to provide pure **41** as colorless oil. $[\alpha]_D^{22} = -11.7$ (c = 1.0, EtOAc). ^1H NMR (400 MHz, $CDCl_3$): δ 7.36 (5H, m), 5.10 (2H, s), 4.60 (1H, s), 3.65 (3H, m), 3.02 - 3.27 (2H, m), 2.15 (1H, br s), 1.60 (1H, s), 1.30 - 1.57 (15H, m). IR ($CHCl_3$): 3630, 3621, 3447, 3020, 2939, 2866, 1708, 1603, 1510, 1456, 1393, 1368, 1249, 1217, 1047 cm^{-1}. EIMS; m/z (rel. intensity): 367(12.1), 366(M+; 1.4), 357(14.8), 336(12.8), 335(4.8), 310(12.7), 293(20.0), 267(10.3), 266(19.2), 249(38.9), 248(27.5), 218(12.1), 205(12.2), 204(27.9), 185(23.8), 184(100), 174(45.6), 159(26.0), 157(16.0), 156(10.6), 152(22.1), 150(11.5), 145(17.8), 143(11.8), 141(29.8), 129(23.8), 128(100), 127(10.3), 118(13.4), 117(11.7), 115(25.6), 114(94.5), 108(52.1), 107(40.3), 101(13.7), 100(11.0), 98(22.1), 97(27.5), 96(11.7), 92(85.1), 91(100), 90(15.9), 89(15.9), 86(10.4), 85(38.7), 84(100), 83(24.8), 82(20.1), 81(11.9), 79(37.9), 77(24.0), 72(11.9), 70(14.2), 65(36.6), 60(11.7), 59(31.5), 58(15.7), 57(100),

56(32.7), 55(17.7). HRMS (EI): calcd for $C_{19}H_{30}N_2O_5$ (M+) 366.2154, found 366.2154.

(S)-(-)-α-N-Carbobenzoxy-ε-N-*t*-butoxycarbonyl-2,6-diaminohex-1-yl benzoate (**42**).

To a solution of alcohol **41** (9.95 g, 27.2 mmol) in CH_2Cl_2 (100 mL) was dropwise added distilled benzoyl chloride (4 mL, 34.5 mmol) and dry pyridine (3 mL, 37.3 mmol) at room temperature with stirring. After 8 h at room temperature, the reaction was quenched by addition of H_2O (1 mL) with vigorous stirring. The volatiles were evaporated off, the residual oil redissolved in EtOAc (200 mL) and washed with saturated $NaHCO_3$ solution (2 x 50 mL), 1M HCl (2 x 50 mL), and brine (1 x 50 mL), then dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure and recrystallization from $CHCl_3$-hexanes gave 10.31 g of pure material as white crystals, mp 89 - 90°C. $[\alpha]_D^{22} = -18.2$ (c = 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 8.02 (2H, d, J = 7.3 Hz), 7.58 (1H, t, J = 7.4 Hz), 7.46 (2H, t, J = 7.3 Hz), 7.34 (5H, m), 5.10 (2H, m), 4.93 (1H, br d, J = 8.5 Hz), 4.59 (1H, s), 4.35 (2H, m), 4.08 (1H, m), 3.12 (2H, m), 1.30 - 1.70 (15H, m with a singlet at 1.43). IR (KBr): 3344, 3066, 3034, 2970, 2928, 2854, 1724, 1689, 1535, 1455, 1388, 1364, 1340, 1276, 1172, 1129, 1090, 1050 cm^{-1}. EIMS, m/z (rel. intensity): 470(M+; 2.0), 414(9.1), 397(6.8), 371(7.0), 370(M-100; 19.9), 353(9.8), 352(10.5), 335(9.1), 309(5.4), 308(26.3), 292(10.0), 279(5.2), 275(6.7), 263(10.0), 219(8.8), 218(47.4), 201(17.8), 197(11.9), 185(21.0), 184(72.0), 174(15.5), 164(5.1), 157(11.5), 156(9.0), 152(5.0), 145(9.7), 142(5.0), 141(25.2), 140(12.2), 129(6.0), 128(77.9), 127(6.5), 122(6.0), 108(15.4), 107(10.6), 106(8.0), 105(78.2), 98(11.9), 97(19.9), 96(9.4), 92(14.3), 91(99.1), 85(9.9), 84(100), 83(5.1), 82(5.5), 79(11.4), 77(15.9), 70(5.0), 65(6.8), 59(10.4), 57(47.4).

(S)-(-)-α-N-Carbobenzoxy-1,6-diaminohexa-1-yl benzoate (**43**).

The benzoate **42** (1.54 g, 3.27 mmol) was dissolved in CH_2Cl_2 (20 mL) and cooled in an ice bath. To this cold solution was added dropwise trifluoroacetic acid (1.3 mL, 16.88 mmol). After 3 h, volatiles were removed under reduced pressure at room temperature, the residual oil was redissolved in 0.5 M HCl (50 mL) and extracted with Et_2O (2x 20 mL). The organic layer was dried over anhydrous Na_2SO_4, the solvent evaporated to give 0.28 g (18% yield) of the corresponding trifluoroacetamide as a by-product. This trifluoroacetamide was further purified by recrystallization from $CHCl_3$-hexanes to gave pure material as white crystals, mp 174 - 175°C. ^1H NMR (400 MHz, $CDCl_3$): δ 8.02 (2H, d, J = 7.3 Hz), 7.60 (1H, t, J = 7.3 Hz), 7.47 (2H, t, J = 7.3 Hz), 7.33 (5H, m), 6.62 (1H, s), 5.10 (2H, m), 4.96 (1H, d, J = 9.7 Hz), 4.37 (2H, m), 4.08 (1H, m), 3.38 (2H, m), 1.35 - 1.78 (6H, m). IR (KBr): 3700, 3692, 3443, 3020, 1794, 1722, 1654, 1602, 1546, 1512, 1453, 1381, 1341, 1273, 1271, 1177, 1112, 1097, 1071, 1027 cm^{-1}. EIMS, m/z (rel. intensity): 467(M+1, 5.2), 466(M+, 18.4), 359(15.9), 331(46.3), 288(10.0), 287(60.9), 255(22.5), 216(28.2), 180(49.7), 172(40.8), 152(10.2), 108(19.7), 107(15.5), 105(94.5), 92(17.5), 91(100), 77(17.8). HRMS (EI): calcd for $C_{23}H_{25}F_3N_2O_5$ (M+) 466.1715; found 466.1716.

The aqueous layer was taken to pH ~ 10 by addition of solid K_2CO_3 then extracted with Et_2O (3x 20 mL). This ether solution was dried over anhydrous Na_2SO_4, solvent evaporated and the residue dried further under high vacuum to provided 0.99 g (82% yield) of crude product as a white solid. This crude product was further purified by recrystallization from $CHCl_3$-hexanes to provide pure **43** as white crystals, mp 63 - 63.5 °C. $[\alpha]_D^{22} = -24.2$ (c = 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 8.02 (2H, d, J = 7.3 Hz), 7.58 (1H, t, J = 7.4 Hz), 7.45 (2H, t, J = 7.3 Hz), 7.33 (5H, m), 5.10 (2H, s), 4.92 (1H, d, J = 9.7 Hz), 4.37 (2H, m), 4.08 (1H, m), 2.72 (2H, br s), 1.32 - 1.78 (8H, m). IR (KBr): 3318, 3066, 3035, 2925, 2857, 1724,

1714, 1692, 1541, 1458, 1387, 1291, 1241, 1131, 1071, 1027 cm^{-1}. EIMS, m/z (rel. intensity): 371(M+1, 3.2), 370(M+, 4.3), 249(5.1), 248(27.7), 235(13.7), 218(18.3), 206(12.0), 174(5.3), 162(14.2), 114(5.8), 108(6.4), 107(8.2), 106(9.6), 105(94.5), 100(7.1), 98(27.9), 97(100), 96(19.4), 92(24.5), 91(100), 85(9.6), 84(100), 82(5.7), 79(5.5), 77(23.4), 72(7.9), 70(10.7), 65(11.1), 56(11.1).

Benzoate of (S)-(-)-2-(N-carbobenzoxy)amino-5-hydroxylpentanonitrile (**44**).

Method A: To a mixture of nitrile alcohol **46** (280 mg, 1.07 mmol) and 4-(dimethylamino)pyridine (260 mg, 2.13 mmol) in CH_2Cl_2 (10 mL) was dropwise added distilled benzoyl chloride (150 μL, 1.30 mmol) at room temperature with stirring. After 4 h at room temperature, the reaction was quenched by addition of a few drops of H_2O. The solvent was evaporated off, the residual oil redissolved in EtOAc (100 mL) and washed with 1M HCl (2 x 30 mL), saturated $NaHCO_3$ solution (1 x 30 mL), and brine (1 x 30 mL), then dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure gave 375 mg (96% yield) of crude product as a yellowish oil which solidified upon standing under high vacuum. This crude benzoate was further purified by recrystallization from $CHCl_3$-hexanes to gave 247 mg of pure **44** as white crystals, mp 120 - 122°C. $[\alpha]_D^{22} = -28.8$ (c = 0.198, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 8.01 (2H, d, J = 7.5 Hz), 7.59 (1H, t, J = 7.4 Hz), 7.45 (2H, t, J = 7.7 Hz), 7.36 (5H, br m), 5.09 (2H, s), 4.91 (1H, d, J = 9.4 Hz), 4.32 - 4.41 (2H, m), 4.11 (1H, m), 2.44 (2H, br. t, J = 6.6 Hz), 1.65 - 1.90 (4H, m). IR (KBr): 3289, 3037, 2950, 2924, 2240, 1715, 1675, 1543, 1457, 1270, 1251, 1178, 1118, 1065 cm^{-1}. EIMS, m/z (rel. intensity): 367(7.8), 366(M+, 30.2), 259(12.5), 244(7.9), 231(27.5), 188(23.1), 187(92.1), 181(6.7), 155(32.5), 131(6.3), 119(9.6), 108(69.5), 107(71.4), 106(13.6), 105(100), 97(11.9), 92(34.6), 91(100), 90(5.2), 84(8.2), 79(10.9), 77(35.6), 69(36.4), 65(17.1), 56(4.1), 51(9.3). HRMS(EI): calcd for

$C_{21}H_{22}N_2O_4$ (M+) 366.1579, found 366.1582. Anal. calcd. for $C_{21}H_{22}N_2O_4$: C,68.82; H, 6.06; N, 7.65; found C, 66.66; H, 5.82; N, 7.49.

Method B: To an ice bath-cooled solution of protected amine **43** (0.99 g, 2.67 mmol) in H_2O-dioxane (1:2; 10 mL) was added portionwise a commercial bleach solution (5.25 % NaOCl, 8.5 mL). The reaction mixture was stirred at room temperature for 20 min then acidified to pH 4 by addition of 10% HCl. The resulting mixture was extracted with EtOAc (3x 25 mL), the combined organic solutions washed with H_2O (1x 20 mL), and brine (1x 20 mL), then dried over anhydrous Na_2SO_4. Upon removal of solvent, the residual oil was further dried under high vacuum for 8 h to gave 1.06 g (90% yield) of the corresponding N,N'-dichloro derivative. This compound could be used for dehydrochlorination without further purification. ^1H NMR (400 MHz, $CDCl_3$): δ 8.02 (2H, d, J = 7.3 Hz), 7.58 (1H, t, J = 7.4 Hz), 7.45 (2H, t, J = 7.7 Hz), 7.33 (5H, br s), 5.68 (2H, s), 4.87 (1H, d, J = 9.4 Hz), 4.38 - 4.41 (2H, m), 4.08 (1H, m), 3.63 (2H, t, J = 6.9 Hz), 1.37 - 1.86 (6H, br m). To an ice bath-cooled solution of the N,N'-dichloro derivative (498 mg, 1.13 mmol) in CH_2Cl_2 (10 mL) was dropwise added 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU; 0.4 mL, 2.68 mmol). After 2 h at room temperature, the resulting mixture was diluted with EtOAc (40 mL) then washed with 1M HCl (2 x 15 mL), saturated $NaHCO_3$ solution (1 x 15 mL), and brine (1 x 15 mL), then dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure gave 401 mg (97% yield) of crude product as a yellowish oil which solidified upon standing under high vacuum, pure by ^1H-NMR. This crude benzoate was further purified by recrystallization from $CHCl_3$-hexanes to gave 247 mg of pure **43**.

(S)-(-)-2-(N-carbobenzoxy)amino-5-hydroxylpentanonitrile (**46**).

A 1M solution of BH_3·THF (55.0 mL, 55.0 mmol) was added dropwise to solution of (S)-2-(N-carbobenzoxy)amino-5-cyanopentanoic acid **37**[260] (10.37 g, 37.6 mmol) in THF (30 mL) at 0°C. The reaction mixture was stirred at 0°C for 1 h and the reaction was quenched by dropwise addition of 10% AcOH/CH_3OH (50 mL). The resulting reaction mixture was evaporated to dryness, the residual oil was redissolved in EtOAc (300 mL), and washed with saturated $NaHCO_3$ solution (2 x 80 mL), 1M HCl (1 x 80 mL), and brine (1 x 80 mL), then dried over anhydrous Na_2SO_4. Removal of the solvent gave compound **46** (6.55 g, 66% yield) as a light yellowish oil. A small amount of this crude product was purified by column chromatography on silica (1-5% $CH_3OH/CHCl_3$) to provide the following physical data. $[\alpha]_D^{22} = -19.9$ (c = 1.08, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.36 (5H, br m), 5.10 (2H, s), 4.96 (1H, d, J = 8.4 Hz), 3.65 - 3.78 (2H, m), 3.63 (1H, m), 2.41 (2H, br t, J = 6.6 Hz), 1.92 (1H, br s), 1.56 - 1.84 (4H, m). IR (neat): 3398, 3334, 3065, 3020, 2951, 2249, 1721, 1685, 1074 cm^{-1}. EIMS, m/z (rel. intensity): 263(M+1; 4.6), 262(M+; 13.9), 232(7.3), 231(44.9), 188(11.4), 187(81.2), 108(45.3), 107(13.7), 97(9.1), 92(34.2), 91(100), 90(8.8), 89(8.5), 79(11.2), 77(10.5), 65(27.1). HRMS (EI): calcd for $C_{14}H_{18}N_2O_3$ (M+) 262.1317; found 262.1318.

Enamine (**49**).

To a mixture of **43** (230 mg, 0.62 mmol) in CH_2Cl_2/DMF (6 mL, 3/1) at room temperature was added 4-formyl-1-methylpyridinium benzenesulfonate (200 mg, 0.72 mmol). After complete disappearance of the amine **43**, as monitored by TLC, the reaction mixture was treated with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU; 0.1 mL, 0.67 mmol), stirred for 5 min, and quenched with cold saturated aqueous oxalic acid (6.0 mL). This reaction mixture was stirred for an additional 45 min, diluted with H_2O

(10 mL) and extracted with Et_2O (3x20 mL). The combined organic layers were dried over anhydrous Na_2SO_4. Removal of the solvent and column chromatography on silica gave compound **49** (67 mg, 31% yield) as a light yellowish oil. This compound can be further purified by crystallization from EtOAc/hexanes. mp 114 - 115°C. ^1H NMR (400 MHz, $CDCl_3$): δ 8.04 & 7.99 (2H, 2xd, J = 7.3 Hz), 7.56 (1H, t, J = 7.3 Hz), 7.20 - 7.52 (7H, m), 6.94 & 7.81 (1H, 2xd, J = 9.7 Hz), 4.99 - 5.30 (2H, m), 4.69 - 4.99 (2H, m), 4.37 (2H, m), 1.98 - 2.23 (3H, m), 1.86 (1H, m). IR($CHCl_3$): 3070,3038, 2958, 2939, 2901, 2852, 1754, 1706, 1656, 1498, 1469, 1452, 1416, 1395, 1338, 1328, 1275, 1238, 1216, 1118, 1072 cm^{-1}. EIMS, m/z (rel. intensity): 351(M+1; 10.0), 217(6.8), 216(41.4), 173(7.9), 172(57.9), 149(5,6), 105(30.2), 91(100), 84(5.0), 77(6.9). HRMS(EI): calcd for $C_{21}H_{21}NO_4$ (M+) 351.1470; found 351.1476.

(S)-(+)-6-(Tosyloxy)methyl-2-piperidinone (**54b**).

The lactam alcohol **30b** (1.15 g, 8.90 mmol) was dissolved in CH_2Cl_2 (50 mL), cooled in an ice bath for 30 min, and N-methylimidazole (0.73 mL, 13.3 mmol) was added. To this cold reaction mixture was added dropwise a solution of tosyl chloride (1.87 g, 9.81 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at 0°C for 24 h. The reaction was quenched by addition of H_2O (1.0 mL) with vigorous stirring, diluted with $CHCl_3$ (200 mL), quickly washed with saturated $NaHCO_3$ solution (1 x 50 mL), 1M HCl (2 x 50 mL), and brine (1 x 50 mL), then dried over anhydrous Na_2SO_4. Removal of solvent gave 2.35 g (93% yield) of crude product, which crystallized upon standing under high vacuum. This crude product was subjected to recrystallization from $CHCl_3$-hexanes to gave 1.78 g of pure material as pale yellowish, needle-like crystals, mp 126°C. $[\alpha]_D^{22}$ = + 7.6 (c = 0.197, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.80 (2H, d, J = 8.3 Hz), 7.38 (2H, d, J = 8.2 Hz), 5.85 (1H, br. s), 4.08 (1H, dd, J =

3.9, 9.7 Hz), 3.81 (1H, m), 3.73 (1H, m), 2.47 (3H, s), 2.39 (1H, m), 2.28 (1H, m), 1.98 (2H, m), 1.73 (1H, m), 1.41 (1H, m). IR ($CHCl_3$): 3195, 2956, 2913, 2841, 1675, 1363, 1189, 1171, 1097 cm^{-1}. EIMS, m/z (rel. intensity): 285(3.2), 284(21.4), 283(M+, 2.5), 254(5.3), 253(40.9), 155(13.2), 112(6.8), 111(43.1), 107(4.5), 99(36.3), 98(100), 97(5.9), 92(16.0), 91(89.0), 90(6.7), 89(10.6), 83(9.1), 82(9.4), 70(16.0), 69(9.1), 68(5.7), 65(40.5), 63(8.4), 56(10.6), 55(100). Anal. calcd for $C_{13}H_{17}NO_4S$: C,55.12; H, 6.05; N, 4.95; found C, 54.89; H, 6.01; N, 4.78. HRMS (EI): calcd for $C_{13}H_{17}NO_4S$ (M+) 283.0878; found 283.0878.

Cytosine-lactam (**55**).

Cytosine (1.03 g, 9.28 mmol) was mixed with potassium *tert*-butoxide (1.04 g, 9.28 mmol) in DMSO (10 mL). The resulting mixture was swirled with occasional heating until a homogeneous solution formed. This freshly prepared cytosine anion-containing solution was added to the tosylate **54b** (1.66 g, 5.86 mmol) solution in DMSO (15 mL) at room temperature. The reaction mixture was stirred at 50°C for 8 h. The reaction was quenched with 20% AcOH/CH_3OH (10 mL). After removal of solvents under high vacuum, 20% $CH_3OH/CHCl_3$ (150 mL) was added to the residual oil with vigorous stirring. The resulting mixture was allowed to sit at room temperature for 2-3 h. The precipitated tosylate salt and excess cytosine were filtered off and the filtrate was concentrated to dryness. This desalting process was repeated twice. Removal of solvents and column chromatography on basic alumina (5 - 15% $CH_3OH/CHCl_3$) gave 1.14 g (88% yield) of alkylated lactam **55** as a white powder, mp 227°C (dec). $[\alpha]_D^{22}$ = + 17.2 (c = 0.5, CH_3OH). ^1H NMR (400 MHz, DMSO-d6): δ 7.51 (1H, d, J = 7.3 Hz), 7.50 (1H, br s, exchanged with D_2O), 7.07 & 6.98 (2H, 2 br s, exchanged with D_2O), 5.63 (1H, d, J = 7.3 Hz), 4.83 (1H, dd, J = 12.2, 4.3 Hz), 3.52 - 3.63 (2H, m), 2.09 (2H, m), 1.76 (1H, m), 1.67 (1H, m), 1.54 (1H, m), 1.33 (1H, m). IR

(KBr): 3251, 3055 (br), 2952, 2878, 1698, 1670, 1644, 1621, 1561, 1523, 1506, 1482, 1455, 1435, 1418, 1384, 1331, 1306, 1266, 1199, 1172, 1133 cm^{-1}. EIMS, m/z (rel. intensity): 223(1.2), 222(M+, 3.0), 221(4.6), 206(10.1), 205(71.8), 125(100), 112(62.4), 110(6.3), 109(62.9), 98(71.8), 96(22.4), 83(27.2), 81(20.5), 70(10.8), 69(16.1), 56(17.5), 55(81.9), 53(16.1). Neg. FABMS, m/z (rel. intensity): 257(12.6), 222(13.6), 221($(M-H)^-$, 100), 178(7.8), 110(5.7), 75(12.4), 64(6.6). HRMS (Neg. FAB): calcd for $C_{10}H_{13}N_4O_2$ $(M-H)^-$ 221.1038; found 221.1043.

4-(N-Benzoyl)cytosine-lactam (**56**).

To a suspension of cytosine-lactam **55b** (165 mg, 0.74 mmol) in dry pyridine (10 mL) was dropwise added distilled benzoyl chloride (86 μL, 0.74 mmol) at room temperature. The reaction mixture was stirred for 8 h and quenched by addition of H_2O (0.5 mL). Solvents were evaporated off and the residual oil was redissolved in 10% isopropanol/$CHCl_3$ (40 mL), washed with 1M HCl (2 x 10 mL), saturated $NaHCO_3$ solution (1 x 10 mL), and brine (1 x 10 mL), then dried over anhydrous Na_2SO_4. Removal of solvent gave 151 mg (63% yield) of crude product which was further purified by column chromatography on silica (5% $CH_3OH/CHCl_3$) to gave **56** as a white solid, mp 238°C (dec). ^1H NMR (400 MHz, DMSO-d6): δ 11.20 (1H,br s), 8.08 (1H, d, J = 7.3 Hz), 8.01 (2H, d, J = 7.6 Hz), 7.62 (2H, m), 7.52 (2H, t, J = 7.6 Hz), 7.31 (1H, br s), 3.98 (1H, dd, J = 12.9, 6.3 Hz), 3.81 (1H, dd, J = 13.0, 6.0 Hz), 3.72 (1H, m), 2.12 (2H, t, J = 6.4 Hz), 1.76 (2H, m), 1.60 (1H, m), 1.43 (1H, m). IR (KBr): 3180, 3128, 3092, 3072, 2955, 1697, 1669, 1563, 1485, 1446, 1444, 1425, 1376, 1358, 1343, 1329, 1304, 1248 cm^{-1}. EIMS, m/z (rel. intensity): 326(M+, 8.3), 325(5.9), 230(7.7), 229(48.1), 216(18.3), 139(6.1), 124(9.2), 122(4.0), 121(5.1), 111(4.2), 108(6.0), 106(8.2), 105(100), 98(21.9), 83(6.3), 82(4.6), 81(4.5), 77(49.0), 55(25.0), 51(9.5). Neg FABMS, m/z (rel. intensity):

327(6.6), 326(27.0), 325((M-H)$^-$, 100), 282(7.6), 214(15.2), 145(6.7), 139(6.1), 107(34.5), 105(6.1). HRMS (Neg. FAB): calcd for $C_{17}H_{17}N_4O_3$ (M-H)$^-$ 325.1301; found 325.1304.

Mono-(*tert*-butoxycarbonylated) product (**57**).

To a mixture of **56** (35 mg, 0.107 mmol) and 4-(dimethylamino)pyridine (13 mg, 0.108 mmol) in dry DMF at 25 °C was added Et_3N (15 µL, 0.108 mmol) and di-*tert*-butyl dicarbonate (25 µL, 0.109 mmol) simultaneously. After 4 h at room temperature, the volatiles were removed under reduced pressure and the residue purified by column chromatography on silica (5% $CH_3OH/CHCl_3$) to give 30 mg (65% yield) of **57** as a hygroscopic, amorphous solid. ^1H NMR (400 MHz, DMSO-d6): δ 8.15 (1H, d, J = 7.2 Hz), 7.85 (2H, d, J = 7.9 Hz), 7.74 (1H, t, J = 7.9 Hz), 7.59 (3H, t, J = 7.9 Hz), 6.97 (1H, d, J = 7.2 Hz), 3.92 (1H, dd, J = 12.4, 6.8 Hz), 3.76 (1H, dd, J = 12.4, 5.6 Hz), 3.67 (1H, m), 2.11 (2H, t, J = 7.2 Hz), 1.66 - 1.87 (2H, m), 1.58 (1H, m), 1.49 (1H, m), 1.28 (9H, s).

Bis-(*tert*-butoxycarbonylated) product (**58**).

According to the procedure described for **57**, a sample of **56** (18 mg, 55.2 µmol) was treated with two equivalents of the reagent mixture to give 20 mg (69% yield) of **58**. This compound tends to decompose upon routine storage. However, it can be purified by column chromatography on silica (5% $CH_3OH/CHCl_3$). ^1H NMR (400 MHz, DMSO-d6): δ 8.05 (1H, d, J = 7.2 Hz), 7.83 (2H, d, J = 8.0 Hz), 7.75 (1H, t, J = 8.0 Hz), 7.60 (2H, t, J = 8.0 Hz), 6.95 (1H, d, J = 7.2 Hz), 4.65 (1H, m), 3.88 - 4.15 (2H, m), 2.40 - 2.60 (2H, m), 1.62 - 2.00 (4H, m), 1.21 - 1.37 (18H, m).

Cytosine-Boc-lactam (**60b**).

Method A: A sample of N,N-dimethylacetamide dimethyl acetal (0.52 mL, 3.56 mmol) was added to a suspension of cytosine lactam **55** (530 mg, 2.39 mmol) in DMF (20 mL). This reaction mixture was allowed to stir at 40°C for 8 h. Solvent and excess reagent were removed under reduced pressure and the residual amidine derivative further dried under high vacuum overnight. The crude amidine derivative was dissolved in CH_2Cl_2 (30 mL) and treated with 4-(dimethylamino)pyridine (320 mg, 2.62 mmol). To this solution was added dropwise di-tert-butyl dicarbonate (1.1 mL, 4.79 mmol) and Et_3N (0.35 mL, 2.51 mmol) at the same time. The reaction was followed by TLC every 30 min until complete disappearance of starting amidine was achieved. Removal of volatiles and flash column chromatography on silica gave the acylated derivative of **59** as a dark brown oil. This acylated intermediate was immediately redissolved in CH_2Cl_2 (40 mL) and treated with *p*-toluenesulfonhydrazide (1.77 g, 9.50 mmol) and *p*-toluenesulfonic acid monohydrate (227 mg, 1.19 mmol). The resulting mixture was allowed to stirred at room temperature for 24 h. Removal of solvent and column chromatography on silica (2 - 10% $CH_3OH/CHCl_3$) gave 432 mg (56% yield) of cytosine-Boc-lactam **60b** as a fine white powder, mp 190 - 191°C. $[\alpha]_D^{22}$ = + 129.2 (c =0.504, CH_3OH). ^1H NMR (400 MHz, DMSO-d6): δ 7.38 (1H, d, J = 7.2 Hz), 7.04 & 6.94 (2H, 2 br s, exchanged with D_2O), 5.62 (1H, d, J = 7.2 Hz), 4.56 (1H, br. m), 3.90 (1H, dd, J = 13.3, 5.8 Hz), 3.68 (1H, dd, J = 13.4, 8.3 Hz), 2.38 - 2.48 (2H, m), 1.95 (1H, m), 1.80 (1H, m), 1.70 (2H, m), 1.31 (9H, s). IR (KBr): 3357, 3132, 2980, 1756, 1722, 1659, 1628, 1523, 1490, 1441, 1391, 1370, 1302, 1285, 1257, 1158, 1135, 1106, 1062 cm^{-1}. Neg. FABMS, m/z (rel. intensity): 359(17.3), 358(10.3), 357(43.8), 322(17.0), 321($(M-H)^-$, 100), 249(15.2), 221(13.2), 145(7.3), 143(20.3), 110(15.1), 105(7.1). HRMS (Neg. FAB): calcd for $C_{15}H_{21}N_4O_4$ $(M-H)^-$ 321.1563; found 321.1560.

Method B: A sample of Boc-lactam-tosylate **63b** (225 mg, 0.59 mmol) was alkylated with cytosine (99 mg, 0.89 mmol) by the procedure given for the preparation of cytosine-lactam **55** (except the reaction was run at room temperature for 24 h instead of 8 h at 50°C) to provide 81 mg (43% yield) of alkylated Boc-lactam **60b** after column chromatography on silica (5% - 10% $CH_3OH/CHCl_3$). Similarly, tosylate **63a** (1.22g, 3.29 mmol) was converted to 627 mg (62% yield) of **60a** after routine column chromatography on silica (5% - 10% $CH_3OH/CHCl_3$).

4-(N-Benzoyl)cytosine-Boc-lactam (**61**).

To a suspension of cytosine-Boc-lactam **60b** (713 mg, 2.21 mmol) in pyridine (15 mL) was dropwise added distilled benzoyl chloride (0.28 mL, 2.41 mmol). The resulting mixture was stirred at room temperature for 8 h and the reaction was quenched by addition of H_2O (0.5 mL) and solvent was removed under reduced pressure. The residual oil was dissolved in $CHCl_3$ (150 mL), washed with saturated $NaHCO_3$ solution (1 x 50 mL), 0.5 M HCl (2 x 50 mL), and brine (1 x 50 mL), then dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure gave 1.0 g crude product which was further purified by recrystallization from $CHCl_3$-hexanes to gave 890 mg (95% yield) of pure **61**, mp > 260°C. $[\alpha]_D^{22}$ = + 114.8 (c = 0.5, $CHCl_3$). ^1H NMR (400 MHz, DMSO-d6): δ 11.18 (1H, s, exchanged with D_2O), 8.00 (2H, d, J = 7.5 Hz), 7.98 (1H, d, J = 7.2 Hz), 7.63 (1H, t, J = 7.3 Hz), 7.51 (2H, t, J = 7.7 Hz), 7.29 (1H, d, J = 7.2 Hz), 4.70 (1H, m), 4.10 (1H, dd, J = 13.3, 4.9 Hz), 3.97 (1H, dd, J = 13.3, 9.3 Hz), 2.48 - 2.60 (2H, m), 1.70 -2.04 (4H, m), 1.31 (9H, s). IR (KBr): 3270, 3251, 3239, 3148, 3064, 2979, 2935, 2905, 1764, 1728, 1699, 1659, 1624, 1561, 1488, 1454, 1426, 1393, 1371, 1327, 1305, 1275, 1249, 1189, 1149, 1112, 1068 cm^{-1}. EIMS, m/z (rel. intensity): 426(M+, 2.1), 327(2.4), 326(6.1), 325(2.6), 231(2.7), 230(14.9), 229(94.3), 217(2.3), 216(22.3), 200(7.4), 155(2.6),

130(5.6), 125(2.3), 124(12.3), 122(9.5), 111(4.5), 106(8.6), 105(100), 98(25.8), 85(5.5), 84(2.5), 83(6.4), 82(2.4), 81(3.4), 78(2.5), 77(32.4), 70(2.3), 69(2.1), 66(3.0), 58(6.6), 57(20.2), 56(52.5), 55(29.7), 54(3.8), 53(6.1), 51(9.7), 50(6.8). Neg. FABMS, m/z (rel. intensity): 427(14.5), 426(49.7), 425($(M-H)^-$, 100), 325(10.9), 321(5.4), 215(11.2), 214(34.0), 171(11.1), 145(8.8), 143(8.1), 139(6.3), 107(81.2), 106(7.6), 105(7.4). HRMS (Neg. FAB): calcd for $C_{22}H_{25}N_4O_5$ $(M-H)^-$ 425.1825; found 425.1816.

Monomer acid with benzoyl protected cytosine (**62**).

To a solution of N-benzoyl protected cytosine-Boc-lactam **61** (420 mg, 0.99 mmol) in THF (20 mL) was dropwise added 1 M LiOH solution (5 mL). The reaction mixture was allowed to stir at room temperature and monitored by TLC every 10 min to minimize hydrolysis of the benzamide. The reaction was normally done within 30 - 40 min. The reaction was quenched by addition of 10% AcOH/MeOH (5 mL) and solvents were evaporated off under reduced pressure. The residue was redissolved in 20% MeOH/$CHCl_3$ and the precipitated solid was filtered. Removal of solvent and column chromatography on silica (5 - 10% $CH_3OH/CHCl_3$) gave 281 mg (64% yield) of monomer acid **62** as a white solid, mp 211 - 212°C. $[\alpha]_D^{22}$ = + 101.3 (c = 0.157, CH_3OH). ^1H NMR (400 MHz, DMSO-d6): δ 11.18 (1H, br s, exchanged with D_2O), 7.99 (2H, d, J = 7.5 Hz), 7.89 (1H, d, J = 7.3 Hz), 7.62 (1H, t, J = 7.2 Hz), 7.51 (2H, t, J = 7.7 Hz), 7.26 (1H, m; after D_2O exchange turn to br d, J = 6.4 Hz), 6.74 (1H, d, J = 9.3 Hz, exchanged with D_2O), 4.05 (1H, m), 3.77 (1H, m), 2.20 (2H, br. t, J = 7.1 Hz), 1.33 -1.68 (4H, m), 1.27 & 1.20 (9H, 2xs). IR (KBr): 3360, 3318, 3309, 3289, 3249, 3185, 3066, 3007, 2978, 2936, 1713, 1701, 1674, 1626, 1572, 1561, 1531, 1492, 1448, 1422, 1374, 1348, 1301, 1278, 1246, 1204 cm^{-1}. Pos. FABMS: 468(10),467($(M+Na)^+$, 24), 445($(M+H)^+$, 8), 389(15), 367(14), 345(10),

327(8), 263(11), 261(10), 254(11), 251(19), 185(22), 115(84), 113(20), 105(92), 93(96), 91(22), 77(20), 75(53), 61(64), 57(100). Neg. FABMS, m/z (rel. intensity): 445(12), 444(43), 443($(M-H)^-$, 100), 370(12), 369(35), 307(17), 273(9), 214(51), 201(12). HRMS (Neg. FAB): calcd for $C_{22}H_{27}N_4O_6$ $(M-H)^-$ 443.1930; found 443.1933.

Boc-lactam-tosylate (**63a,b**).

To a mixture of lactam tosylate **54a** [277] (1.50 g, 5.58 mmol) and DMAP (818 mg, 6.69 mmol) in CH_2Cl_2 (30 mL) was added di-*tert*-butyl dicarbonate (2.02 mL, 8.80 mmol) and Et_3N (2.15 mL, 15.4 mmol) simultaneously. After stirring for 8 h at room temperature, H_2O (1.0 mL) was added to this reaction mixture with vigorous stirring. The resulting mixture was diluted with $CHCl_3$ (150 mL), washed with 0.5 M HCl (3 x 40 mL), saturated $NaHCO_3$ solution (1 x 40 mL), and brine (1 x 40 mL), then dried over anhydrous Na_2SO_4. Removal of solvent gave 1.97 g (96% yield) of crude product as a yellowish oil. This compound can be further purified by crystallization from EtOAc-hexanes after column chromatography on silica (1% $CH_3OH/CHCl_3$) to provide pure **63a** as white crystals, mp 106 - 107°C. $[\alpha]_D^{22} = -40.3$ (c = 1.024, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.77 (2H, d, J = 8.3 Hz), 7.36 (2H, d, J = 8.2 Hz), 4.32 (1H, m), 4.14 - 4.30 (2H, m), 2.63 (1H, m), 2.45 (3H, s), 2.41 (1H, m), 2.17 (1H, m), 2.00 (1H, m), 1.44 (9H, s). IR (KBr): 3067, 2981, 2956, 2942, 1788, 1775, 1702, 1596, 1474, 1460, 1398, 1371, 1355, 1341, 1318, 1290, 1257, 1206, 1146, 1096, 1044, 1023 cm^{-1}. EIMS, m/z (rel. intensity): 296($(M-73)^+$, 2.1), 270(8.0), 239(2.7), 187(4.7), 184(7.3), 155(15.4), 140(2.3), 98(8.9), 97(10.1), 92(3.1), 91(18.2), 85(4.9), 84(100), 83(3.1), 65(5.2), 57(39.2), 56(8.7), 55(5.9). Anal. calcd for $C_{17}H_{23}NO_6S$: C, 55.27; H, 6.28; N, 3.79; found C, 55.54; H, 6.32; N, 3.66.

By the same procedure, lactam tosylate **54b** (759 mg, 2.68 mmol) was reacted with di-*tert*-butyl dicarbonate (1.23 mL, 5.36 mmol) to provided 911 mg (89% yield) of the corresponding Boc-lactam-tosylate **63b** as a pale yellowish oil upon column chromatography on silica (1% $CH_3OH/CHCl_3$). This compound can be further purified by recrystallization from EtOAc-hexane to provided pure material as white crystals, mp 68 - 69°C. $[\alpha]_D^{22} = -29.9$ (c = 0.408, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.78 (2H, d, J = 8.3 Hz), 7.36 (2H, d, J = 8.2 Hz), 4.38 (1H, m), 4.11 (1H, dd, J = 9.7, 4.0 Hz), 4.02 (1H, dd, J = 9.7, 7.9 Hz), 2.45 (5H, s with m), 1.71 - 2.10 (4H, m), 1.44 (9H, s). IR (KBr): 3309, 2966, 2900, 1735, 1662, 1617, 1481, 1400, 1334, 1188, 1177, 1094 cm^{-1}. All of the attempts to obtain a satisfactory elemental analysis or mass spectrum were unsuccessful due to the thermal instability of this compound, which readily loses the Boc group on standing.

4-(N-*tert*-Butylbenzoyl)cytosine-BOC-lactam (**64a,b**).

Method A: A sample of cytosine-Boc-lactam **60b** (246 mg, 0.76 mmol) was reacted with 4-*tert*-butylbenzoyl chloride (158 μL, 0.84 mmol) to provided 349 mg (95% yield) of product **64b** according to the procedures described for the preparation of compound **61**. This fully protected cytosine lactam was further purified by recrystallization from CHCl3-hexanes to provided **64b** as a hygroscopic, pale brownish color solid, mp > 260°C. $[\alpha]_D^{22} = +115.5$ (c = 1.009, $CHCl_3$). ^1H NMR (400 MHz, DMSO-d6): δ 11.11 (1H, s, exchanged with D_2O), 7.97 (1H, d, J = 7.0 Hz), 7.91 (2H, d, J = 8.4 Hz), 7.53 (2H , d, J = 8.5 Hz), 7.30 (1H, d, J = 7.2 Hz), 4.70 (1H, br s), 4.10 (1H, dd, J = 13.3, 4.8 Hz), 3.96 (1H, dd, J = 13.3, 9.3 Hz), 2.42 - 2.62 (2H, m), 1.69 - 2.03 (4H, m), 1.308 & 1.304 (18H, 2xs). IR (KBr): 3231, 2964, 1766, 1764, 1716, 1670, 1624, 1556, 1489, 1424, 1385, 1367, 1341, 1299, 1256, 1155, 1146, 1065 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 484(4.1), 483($(M+H)^+$, 15.0), 397(6.0),

384(7.3), 308(56.0), 307(96.8), 306(24.9), 305(25.5), 303(11.0), 223(7.8), 162(15.7), 161(100), 146(8.3), 145(7.4), 105(20.0), 100(22.5), 99(34.7), 98(24.7), 93(11.1), 84(24.6), 83(9.9), 81(7.2), 60(10.3), 57(23.6), 56(8.8), 55(19.6). Neg. FABMS, m/z (rel. intensity): 483(16.2), 482(53.9), 481((M-H)$^-$, 100), 381(12.8), 271(6.0), 201(6.9), 107(42.9). HRMS (Neg. FAB): calcd for $C_{26}H_{33}N_4O_5$ (M-H)$^-$ 481.2451; found 481.2448.

Method B: To a solution of 4-(N-*tert*-butylbenzoyl)cytosine **66** (815 mg, 3.0 mmol) and potassium *tert*-butoxide (337 mg, 3.0 mmol) in DMSO (5 mL) was added dropwise a DMSO (5 mL) solution of tosylate **63a** (590 mg, 1.60 mmol). After 10 h at 65°C, the reaction was quenched by addition of 10% AcOH/$CHCl_3$ (10 mL) and diluted with $CHCl_3$ (150 mL). The resulting mixture was washed with 50% saturated NaCl solution (3x 50 mL), saturated $NaHCO_3$ solution (1 x 50 mL), and brine (1 x 50 mL), then dried over anhydrous Na_2SO_4. Removal of solvent and column chromatography on silica (5 - 10% MeOH/$CHCl_3$) gave 532 mg (71% yield) of **64a**. The spectral properties of **64a** were identical to those reported earlier.[263] By the same procedure, **64b** can be obtained from **63b** (150 mg, 0.39 mmol), however, in relatively low yield (19%).

Monomer acid (**65a,b**).

Lactam **64a** (2.0 g, 4.27 mmol) was dissolved in a mixture of THF (12.8mL), *tert*-butanol (8.5 mL), ethanol (4.2 mL) and H_2O (4.2 mL) and stirred in a room temperature bath. To this was added an ice cold solution of 1 M LiOH (29.9 mL) all at once. The pale yellow solution was stirred for 8 min then diluted with H_2O (100 mL) and neutralized by the addition of 80% AcOH (5 mL). The precipitate was filtered, washed with H_2O and dried in vacuuo over P_2O_5 to provide 1.52 g (73% yield) of the acid **65a**, mp 238-240 °C. $[\alpha]_D^{22}$ = +112.8 (c = 0.25, MeOH). ^{1}H NMR (400 MHz,

DMSO-d6) δ 12.15 (1H, br s), 11.15 (1H, br s), 7.95 (2H, m), 7.90 (1H, d, J = 7.1 Hz), 7.52 (2H, d, J = 8.6 Hz), 7.25 (1H, br s), 6.83 (1H, d, J = 9.1 Hz), 4.09 (1H, dd, J = 13.2, 3.7 Hz), 3.72 - 3.83 (1H, m), 3.44 (1H, dd, J = 14.4, 7.6 Hz), 2.06 - 2.19 (2H, m), 1.53 - 1.74 (2H, m), 1.28 & 1.31 (18H, 2 singlets). IR (KBr): 3371, 2968, 1705, 1703, 1680, 1653, 1644, 1626, 1623, 1571, 1560, 1550, 1545, 1522, 1496, 1409, 1390, 1372, 1350, 1299, 1260, 1168, 1114 cm^{-1}. HRMS (Neg. FAB): calcd for $C_{25}H_{33}N_4O_6$ $(M-H)^-$ 485.2400, found 485.2425.

By the same procedure, the 4-(N-4-*tert*-butylbenzoyl)cytosine-Boc-lactam **64b** (255 mg, 0.529 mmol) was converted to 161 mg (61% yield) of the acid **65b**. This carboxylic acid was further purified by column chromatography on silica (5 to 15% $CH_3OH/CHCl_3$) to provided pure material as a white solid, mp 193°C. $[\alpha]_D^{22}$ = +93.7 (c = 0.479, CH_3OH). ^1H NMR (400 MHz, DMSO-d6): δ 11.07 (1H, s, exchanged with D_2O), 7.95 (2H, d, J = 8.6 Hz), 7.88 (1H, d, J = 7.2 Hz), 7.53 (2H ,d, J = 8.4 Hz), 7.27 (1H, br s), 6.77 (1H, d, J = 9.3 Hz), 4.10 (1H, dd, J = 13.3, 4.8 Hz), 3.77 (1H, br m), 2.21 (2H, br t, J = 7.0 Hz), 1.22 - 1.76 (4H, m), 1.31 & 1.27 (18H, 2 singlets). IR (KBr): 3357, 3228, 3149, 3071, 3014, 2967, 2869, 2619, 2566, 2513, 1708, 1699, 1675, 1660, 1644, 1627, 1561, 1527, 1502, 1461, 1425, 1376, 1323, 1299, 1287, 1250, 1200, 1165, 1115 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 501($(M+H)^+$, 1.5), 459(1.7), 421(2.6), 415(6.2), 401(2.6), 307(2.7), 278(1.9), 272(5.5), 162(12.8), 161(100), 160(1.7), 159(3.9), 147(1.8), 146(9.0), 145(6.9), 130(1.8), 118(7.0), 115(2.3), 112(5.8), 105(5.5), 91(5.5), 77(2.1), 73(2.0), 61(3.1), 57(14.4), 55(2.6). Neg. FABMS, m/z (rel. intensity): 501(16.8), 500(54.7), 499($(M-H)^-$, 100), 426(8.1), 425(30.1), 271(8.2), 270(41.8), 143(8.8). HRMS (Neg. FAB): calcd for $C_{26}H_{35}N_4O_6$ $(M-H)^-$ 499.2556, found 499.2559.

4-(N-*tert*-Butylbenzoyl)cytosine (**66**).

To a suspension of cytosine (5.0 g, 43 mmol) in pyridine-CH_2Cl_2 (1:4; 50 mL) was added dropwise *tert*-butylbenzoyl chloride (10.6 mL, 56.6 mmol). After 4 h at room temperature, the reaction mixture was added H_2O (10 mL) and $CHCl_3$ (40 mL) with vigorous stirring. The precipitates were collected and washed with H_2O. The organic layer of the filtered solution was seperated and washed with 1M HCl (30 mLx2), brine (30 mLx1) and quickly dried over anhydrous Na_2SO_4 and evaporated to 1/3 volume (~ 20 mL). Hexanes (50 mL) were added and the solution set aside to complete precipitation. The combined precipitates were dried under high vacuum over P_2O_5 for 8 h to provide 11.2 g (92% yield) of desired product, mp > 260°C. ^1H NMR (300 MHz, DMSO-d6): δ 11.59 (1H, br s, exchanged with D_2O), 11.03 (1H, br s, exchanged with D_2O), 7.96 (2H, d, J = 8.42 Hz), 7.87 (1H, d, J = 7.08 Hz), 7.53 (2H, d, J = 8.43 Hz), 7.22 (1H, br s), 1.31 (9H, s). IR (KBr): 3600 - 2100 (br), 1850, 1680, 1580, 1440, 1300, 1240, 1110 cm^{-1}. Neg. FABMS, m/e (rel. intensity): 271(17.9), 270((M-H)$^-$, 100). HRMS (Neg. FAB): calcd for $C_{15}H_{16}N_3O_2$ $(M-H)^-$ 270.1242; found 270.1237.

6-(N-*tert*-Butylbenzoyl)adenine (**67**)

Adenine (1.35 g, 10 mmol) was suspended in 15 mL of pyridine, *tert*-butylbenzoyl chloride (5.91 g, 30 mmol) was added and the mixture heated with reflux for 2 h. The mixture was cooled to room temperature, treated with water (0.54 mL) and the solution again brought to reflux. After 1 h water (0.54) was added and heating continued for 1 h more. To the cooled mixture was added isopropanol/$CHCl_3$ (100 mL) and water (50 mL). After vigorous mixing and seperation of the layers, the water layer was washed with $CHCl_3$ (20 mL) and the combined organic layers dried over anhydrous Na_2SO_4 and evaporated. The residue was dissolved in dry pyridine (50 mL) and treated with

hexane (200 mL). The product precipitates as a pure off-white solid (1.75 g, 59%) mp 258 - 259°C. ^{1}H NMR (300 MHz, DMSO-d6): δ 12.37 (1H, s, exchanged with D_2O), 11.48 (1H, s, exchanged with D_2O), 8.73 (1H, s), 8.50 (1H, d, J = 1.3 Hz; s after D_2O shake), 8.08 (2H, d, J = 8.4 Hz), 7.60 (2H, d, J = 8.4 Hz), 1.34 (9H, s). IR (KBr): 3193, 3111, 2961, 1686, 1551, 1518, 1507, 1457, 1386, 1369, 1342, 1276, 1265, 1193, 1144, 1113, 1095 cm^{-1}. Neg. FABMS, m/z (rel. intensity): 295(21.2), 294((M-H)$^-$, 100), 278(8.3), 201(12.8), 160(11.0), 134(10.4), 133(6.6), 117(11.3). HRMS (Neg. FAB): calcd for $C_{16}H_{16}N_5O$ (M-H)$^-$ 294.1355; found 294.1364. Analytical sample was obtained by recrystallization from $CHCl_3$/hexanes. Anal. calcd for $C_{16}H_{17}N_5O$: C, 65.07; H, 5.80; N, 23.71; found C, 64.84; H, 5.66; N, 23.70.

6-(N-*tert*-Butylbenzoyl)adenine-BOC-lactam (**68a,b**)

According to the same procedure as for **64a**, tosylate **63a** (309 mg, 0.84 mmol) was converted to 302 mg (73% yield) of the corresponding 6-(N-*tert*-butylbenzoyl)adenine derivative **68a**. This compound was purified by column chromatography on silica (5% MeOH/$CHCl_3$) to provide pure material as an amorphous solid. ^{1}H NMR (300 MHz, DMSO-d6): δ 11.09 (1H, s, exchanged with D_2O), 8.70 (1H, s), 8.46 (1H, s), 7.99 (2H, d, J = 8.4 Hz), 7.57 (2H, d, J = 8.5 Hz), 4.46 - 4.68 (3H, m), 2.20 (3H, m), 1.30 - 1.50 (19H, m with 2 singlets). IR (KBr): 3500, 3400, 3250, 3050, 2950, 1720, 1565, 1440 cm^{-1}. Neg. FABMS, m/z (rel. intensity): 493(5.2), 492(16.1), 491((M-H)$^-$, 42.0), 392(7.0), 391(25.2), 306(6.6), 295(21.4), 294(100), 278(10.8), 201(22.1), 185(6.1), 160(15.0), 134(15.3), 133(8.6), 117(13.0), 92(6.2). HRMS (Neg. FAB): calcd for $C_{26}H_{31}N_6O_4$ (M-H)$^-$ 491.2406, found 491.2407.

Similarly, tosylate **63b** (99 mg, 0.26 mmol) yielded 41 mg (31% yield) of **68b** after column purification, except that the reaction was carried out at room temperature for 36 h. ^{1}H NMR (300 MHz, DMSO-d6): δ 11.10 (1H, s, exchanged with D_2O), 8.72 (1H,

s), 8.38 (1H, s), 7.98 (2H, d, J = 8.4 Hz), 7.57 (2H, d, J = 8.4 Hz), 4.74 (1H, m), 4.50 (2H, m), 2.07 (1H, br m), 1.89 (1H, br m), 1.74 (2H, br m), 1.30 - 1.47 (11H, m with s), 1.22 (9H, s). IR (KBr): 3440, 3350, 3100, 3050, 2950, 1760, 1690, 1605, 1585, 1460, 1415, 1350, 1320, 1275, 1155, 1135 cm^{-1}. Neg. FABMS, m/z (rel. intensity): 507(6.0), 506(32.7), 505((M-H)$^-$, 100), 405(19.5), 295(10.9), 294(55.0), 278(6.0), 201(8.2), 172(6.5), 171(54.3), 160(6.0), 151(9.0), 122(8.8), 117(5.9), 92(5.0). HRMS (Neg. FAB): calcd for $C_{27}H_{33}N_6O_4$ (M-H)$^-$ 505.2562, found 505.2574.

When the reaction of **63b** with 6-(N-*tert*-butylbenzoyl)adenine was carried out at 75°C, compound **69** was isolated in 25% yield along with **68b** (19% yield). **69**: ^1H NMR (300 MHz, DMSO-d6): δ 11.09 (1H, s, exchanged with D_2O), 8.74 (1H, s), 8.44 (1H, s), 7.99 (2H, d, J = 8.4 Hz), 7.75 (1H, s, exchanged with D_2O), 7.57 (2H, d, J = 8.4 Hz), 4.42 (1H, dd, J = 14.1, 5.1 Hz), 4.26 (1H, dd, J = 14.0, 6.2 Hz), 3.87 (1H, m), 2.08 (2H, m), 1.72 (2H, m), 1.54 (1H, m), 1.34 (9H, s), 1.28 (1H, m). IR (KBr): 3200, 3075, 2950, 2875, 1650, 1600, 1580, 1520, 1480, 1450, 1405, 1300, 1260, 1160, 1120 cm^{-1}. Neg. FABMS, m/z (rel. intensity): 406(26.7), 405((M-H)$^-$, 100), 404(5.2), 403(5.9), 295(6.1), 294(30.2), 201(5.8), 188(5.8), 151(6.0). HRMS (Neg. FAB): calcd for $C_{22}H_{25}N_6O_2$ (M-H)$^-$ 405.2039; found 405.2043.

Standard procedure for formation of *p*-nitrophenyl esters to be used in coupling reactions.

To a mixture of carboxylic acid **65b** (65 mg, 130 μmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide (58 mg, 195 μmol), *p*-nitrophenol (90 mg, 650 μmol) and 4-(dimethylamino)pyridine (8 mg, 65 μmol) was added CH_2Cl_2 (5 mL). After complete disappearance of the starting carboxylic acid was achieved, TLC showed formation of a less polar material which turned yellow when exposed to ammonia

fumes. The reaction mixture was diluted with 20% isopropanol/$CHCl_3$ (40 mL) and washed with 0.15 M NaOH (3 x 10 mL), 0.5 M HCl (2 x 10 mL), and brine (1 x 10 mL), then dried over anhydrous Na_2SO_4. After removal of solvent, the residual amorphous solid **70b** was dried further under high vacuum for 8 h and redissolved in CH_2Cl_2 or DMF (5 mL) for use in coupling reactions. Small amounts of **70b** were purified by column chromatography (2% $CH_3OH/CHCl_3$) to provided a sample for spectroscopic analysis. ^1H NMR (300 MHz, DMSO-d6): δ 11.06 (1H, s; exchanged with D_2O), 8.31 (2H, d, J = 9.1 Hz), 7.95 (2H, d, J = 8.5 Hz), 7.91 (1H, d, 7.1 Hz), 7.52 (2H, d, J = 8.6 Hz), 7.45 (2H, d, J = 9.0 Hz), 7.29 (1H, d, J = 7.1 Hz), 6.84 (1H, d, J = 9.3; exchanged with D_2O), 4.10 (1H, m), 3.83 (1H, m), 3.43 (1H, m), 2.68 (2H, t, J = 7.1 Hz), 1.70 (2H, m), 1.51 (2H, m), 1.15 - 1.40 (18H, m). Neg. FABMS, m/z (rel. intensity): 622(9.8), 621(35.2), 620($(M-H)^-$; 70.0), 270(16.0), 139(7.4), 138(100), 122(10.2), 42(12.1). HRMS (Neg. FAB): calcd for $C_{32}H_{38}N_5O_8$ $(M-H)^-$ 620.2720, found 620.2727.

Benzylamide monomer (**71**).

Method A: To a mixture of carboxylic acid **62** (21.6 mg, 0.049 mmol), 1,3-dicyclohexylcarbodiimide[278] (13 mg, 0.063 mmol), *p*-nitrophenol (13 mg, 0.093 mmol) and 4-(dimethylamino)pyridine (3 mg, 0.025 mmol) was added CH_2Cl_2 (3 mL). After 12 h at room temperature, the resulting mixture was diluted with 10% isopropanol/$CHCl_3$ (20 mL) and washed with H_2O (2 x 5 mL), 5% aqueous HOAc (2 x 5 mL), and brine (2 x 5 mL), then dried over Na_2SO_4. Following evaporation, the residue was redissolved in CH_2Cl_2 (3 mL) and a sample of distilled benzylamine (10 μL, 0.092 mmol) was added. After 8 h at room temperature, the resulting mixture was diluted with 10% isopropanol/$CHCl_3$ (20 mL) and washed with saturated $NaHCO_3$ solution (2 x 5 mL), 0.5 M HCl (2 x 5 mL), brine (2 x 5 mL) and dried over anhydrous

Na_2SO_4. Removal of solvent and column chromatography on silica (1 - 5% $CH_3OH/CHCl_3$) gave 21 mg (80% yield) of **71**. ^1H NMR (400 MHz, DMSO-d6): δ 11.14 (1H, s; exchanged with D_2O), 8.33 (1H, s; exchanged with D_2O), 8.00 (2H, d, J = 7.5 Hz), 7.90 (1H, d, J = 7.0 Hz), 7.62 (1H, t, J = 7.7 Hz), 7.51 (2H, t, J = 7.6 Hz), 7.18 - 7.38 (6H, m), 6.77 (1H, d, J = 9.4 Hz; exchanged with D_2O), 4.26 (2H, d, J = 5.8 Hz), 4.08 (1H, dd, J = 13.0, 2.6 Hz), 3.79 (1H, m), 3.40 (1H, m), 2.15 (2H, br t, J = 7.5 Hz), 1.35 - 1.70 (4H, m), 1.28 & 1.20 (9H, 2xs). IR (KBr): 3320, 3060, 3020, 2925, 1665, 1640, 1620, 1480, 1370, 1300, 1240, 1165, 1115 cm^{-1}. Neg. FABMS, m/z (rel. intensity): 534(16.8), 533(57.1), 532($(M-H)^-$, 100),458(13.4), 354(19.5), 313(10.7), 273(13.6), 253(10.3), 251(22.2), 249(12.8), 237(33.4), 214(37.4), 197(15.8), 167(18.7), 165(51.3), 163(20.9), 145(34.2), 143(70.4), 139(27.6). HRMS (Neg. FAB): calcd for $C_{29}H_{34}N_5O_5$ $(M-H)^-$ 532.2560; found 532.2574.

Method B: A mixture of carboxylic acid **62** (34 mg, 76.6 μmol), N, N'-disuccinimidyl carbonate [279,280] (DSC, 40 mg, 156 μmol) and pyridine (12 μL, 150 μmol) in DMF (2 mL) was stirred at room temperature. Upon complete consumption of the acid, the reaction was quenched with H_2O (0.5 mL). The reaction mixture was evaporated to dryness and diluted with 10% isopropanol/$CHCl_3$ (20 mL). This solution was washed with H_2O (2 x 5 mL), and brine (5 mL) then dried over Na_2SO_4. Following evaporation, the residue was redissolved in CH_2Cl_2 (2 mL) and a sample of distilled benzylamine (20 μL, 0.18 mmol) was added. After 8 h at room temperature, the resulting mixture was evaporated to dryness and the residue chromatographed on silica (5% $CH_3OH/CHCl_3$) to give 32 mg (78% yield) of product **71**. This procedure was halted due to the isolation of N-hydroxysuccinimidyl benzoate in the subsequent dimerization attempt. Succinimidyl benzoate ^1H NMR (400 MHz, $CDCl_3$): δ 8.16 (2H, d, J = 7.6 Hz), 7.68 (1H, t, J = 7.6 Hz), 7.54 (2H, d, J = 7.6 Hz), 2.92 (4H, s).

Method C: To a mixture of carboxylic acid **62** (18 mg, 40.5 μmol) and N,N'-bis(2-oxo-3-oxazolidinyl)phosphorodiamidic chloride [281] (12 mg, 47.1 μmol) was added CH_2Cl_2 (2 mL) and Et_3N (10 μL, 71.7 μmol). After 12 h at room temperature, a sample of distilled benzylamine (10 μL, 92 μmol) was added and allowed to stirred for an additional 12 h. The resulting mixture was evaporated to dryness and the residue chromatographed on silica (5% $CH_3OH/CHCl_3$) gave 14.2 mg (67% yield) of product **71**.

Hydrazide monomer (**72**).

The carboxylic acid **65b** (28.1 mg, 56 μmol) was converted to its corresponding *p*-nitrophenyl ester **70b** according to the standard procedure. In a separate flask, a suspension of 1-aminopiperidine oxalate [282] (22 mg, 156 μmol) in dry DMF (1 mL) was treated with dry Et_3N (150 μL, 1.08 mmol) and the mixture stirred at room temperature for 30 min. This hydrazine-containing solution was then injected into the freshly prepared solution of activated ester. After 12 h at room temperature, the volatiles were removed under reduced pressure. The residual oil was diluted with 20% isopropanol/$CHCl_3$ (30 mL) and washed with 0.15 M NaOH (3 x 10 mL), 0.1 M HCl (2 x 10 mL), and brine (1 x 10 mL), then dried over anhydrous Mg_2SO_4. Removal of solvent under reduced pressure and drying under high vacuum for 8 h provided 30 mg (93% yield) of crude product. This crude hydrazide derivative was generally subjected to further reaction without purification; however, it could be purified by column chromatography on silica (5% $CH_3OH/CHCl_3$) to give pure **72** as a hygroscopic, yellowish amorphous solid. ^1H NMR (400 MHz, DMSO-d6): δ 11.05 (1H, s), 8.70 & 8.30 (1H, 2xs), 7.95 (2H, d, J = 8.3 Hz), 7.89 (1H, br d, J = 7.2 Hz), 7.53 (2H, d, J = 8.4 Hz), 7.28 (1H, br d, J = 6.8 Hz), 6.75 (1H, m), 4.06 (1H, m), 3.77 (1H, m), 3.42 (1H, m), 2.64 (2H, m), 2.33 (2H, m), 1.96 (2H, m), 1.11 -1.67 (28H, m with

singlets). IR (KBr): 3375, 3220, 3125, 2960, 1755, 1675, 1620, 1590, 1555, 1480, 1370, 1350, 1295, 1260, 1205, 1160, 1115 cm^{-1}. Neg. FABMS, m/z (rel. intensity): 583(20), 582(66), 581($(M-H)^-$, 100), 567(12), 566(21), 526(12), 507(16), 381(12), 271(11), 270(63), 201(13), 153(94), 87(16), 75(12). HRMS (Neg. FAB): calcd for $C_{31}H_{45}N_6O_5$ $(M-H)^-$ 581.3451; found 581.3489.

2-(Methylamino)ethanol capped monomer (**73**).

Carboxylic acid **65b** (65 mg, 130 μmol) was converted into the active ester **70b** by the standard procedure and redissolved in CH_2Cl_2 (5 mL). To this solution was added 2-(methylamino)ethanol (30 μL, 370 μmol) and the resulting mixture was stirred at room temperature for 8 h. Removal of the volatiles and column chromatography on silica (5 to 10% $CH_3OH/CHCl_3$) gave 59 mg (82% yield) of **73** as an amorphous yellowish solid. ^1H NMR (300 MHz, DMSO-d6): δ 11.05 (1H, s; exchanged with D_2O), 7.95 (2H, d, J = 8.4 Hz), 7.89 (1H, d, J = 6.8 Hz), 7.53 (2H, d, J = 8.4 Hz), 7.28 (1H, d, J = 6.9 Hz), 6.77 (1H, d, J = 9.0 Hz; exchanged with D_2O), 4.81& 4.65 (1H, 2 triplets, J = 5.4 Hz), 4.05 (1H, m), 3.78 (1H, m), 3.20 - 3.57 (5H, m), 2.98 & 2.81 (3H, 2 singlets), 2.22 - 2.42 (2H, m), 1.17 - 1.65 (22H, m with 3 singlets at d 1.31, 1.28 and 1.20). Neg. FABMS, m/z (rel. intensity): 558(9.8), 557(40.7), 556($(M-H)^-$, 100), 483(5.3), 482(16.7), 439(6.0), 271(3.7), 270(20.3), 201(4.6), 151(4.2), 42(17.8). HRMS (Neg. FAB): calcd for $C_{29}H_{42}N_5O_6$ $(M-H)^-$ 556.3135; found 556.3151.

Capped Monomer (**74**).

To a mixture of 2-(methylamino)ethanol capped monomer **73** (24.6 mg, 44 μmol) and 4-(dimethylamino)pyridine (11 mg, 90 μmol) in CH_2Cl_2 (2.5 mL) was added 4-*tert*-butylbenzoyl chloride (10 μL, 51.2 μmol) at room temperature with stirring. The

reaction was monitored by TLC every 2 h. After the reaction went to completion, the reaction mixture was diluted with 20% isopropanol/$CHCl_3$ (30 mL) and washed with saturated $NaHCO_3$ solution (2 x 10 mL), 0.5 M HCl (1 x 10 mL), and brine (1 x 10 mL), then dried over anhydrous Na_2SO_4. Removal of solvent and column chromatography on silica (5% $CH_3OH/CHCl_3$) gave 26.5 mg (84% yield) of **74** as a pale yellowish amorphous solid. ^1H NMR (400 MHz, DMSO-d6): δ 11.04 (1H, s; exchanged with D_2O), d 7.95 (2H, d, J = 8.2 Hz), 7.88 (3H, d, J = 7.6 Hz), 7.48 - 7.58 (4H, m), 7.27 (1H, br s), 6.76 (1H, d, J = 9.4 Hz; exchanged with D_2O), 4.32 - 4.47 (2H, m), 4.05 (1H, m), 3.45 - 3.88 (4H, m), 3.05 &2.86 (3H, 2xs), 2.27 - 2.43 (2H, m), 1.15 -1.64 (31H, m with singlets). IR (KBr): 3275, 3050, 2960, 1650, 1550, 1480, 1370, 1300, 1270, 1190, 1170, 1115 cm^{-1}. Neg. FABMS, m/z (rel. intensity): 718(22), 717(64), 716(M-H, 100), 529(15), 470(12), 413(19), 353(27), 289(17), 271(19), 270(45), 211(17), 201(16), 189(18), 177(78), 153(52), 133(16). HRMS (Neg. FAB): calcd for $C_{40}H_{54}N_5O_7$ $(M-H)^-$ 716.4023; found 716.4026.

General procedure for oligomerization.

To a suspension of a terminal capped t-Boc protected subunit (5 μmol) in CH_2Cl_2 (1 mL) was added trifluroacetic acid (0.25 mL). The reaction mixture was allowed to stir at room temperature until complete disappearance of starting material was achieved. The volatiles were evaporated off under reduced pressure at room temperature. To this residual oil was added 1.2 equivalent of *p*-toluenesulfonic acid. The residue was then coevaporated three times with $CHCl_3$ (1 mL) and twice with dry DMF (0.5 mL) and dried further under high vacuum for 8 h. Prior to the coupling reaction, this *p*-toluenesulfonic acid salt of the subunit was dissolved in dry DMF (0.5 mL) and excess dry Et_3N (20 equiv.). To this free amine-containing solution was then added a CH_2Cl_2 solution of *p*-nitrophenyl ester of the acid (2.0 equiv.) which was prepared

separately by the standard procedure. The coupling reaction was followed by TLC until complete disappearance of the amine component was achieved. The reaction was normally done within 8 h. The resulting mixture was evaporated to dryness, the residual oil was diluted with 20% isopropanol/$CHCl_3$ (20 mL) and washed with 0.15 M NaOH (3 x 5 mL), 0.5 M HCl (1 x 5 mL), and brine (1 x 5 mL), then dried over anhydrous Mg_2SO_4. Removal of solvent under reduced pressure and purification either by column chromatography (2 - 10% $CH_3OH/CHCl_3$) or preparative TLC (1 x 2% $CH_3OH/CHCl_3$, 1 x 5% $CH_3OH/CHCl_3$, 1 x 15% $CH_3OH/CHCl_3$) provided the material for spectral analysis and for further coupling reactions. The purity of the oligomers was confirmed by analytical HPLC (30 - 40% CH_3OH/CH_2Cl_2 on a Beckman Ultrasphere-Si column) as a evidenced by symmetrical peak and further characterized by negative FABMS, high field ^{1}H-NMR and COSY experiments.

Hydrazide dimer (**75**).

According to the procedure for oligomerization, the hydrazide monomer **72** (14.5 mg, 24.9 μmol) was coupled with the *p*-nitrophenyl ester **70b** to provided 18.2 mg (76% yield) of hydrazide dimer **75** after purification. ^{1}H NMR (400 MHz, DMSO-d6): δ 11.07 (1H, s), 11.01 (1H, s), 8.72 & 8.30 (1H, 2 singlets), 7.85 - 8.02 (5H, m), 7.73 (2H, m), 7.52 (2H, d, J = 8.4 Hz), 7.44 (2H, d, J = 7.7 Hz), 7.29 (1H, br d, J = 7.2 Hz), 7.20 (1H, br s), 6.66 (1H, m), 3.90 - 4.20 (3H, m), 3.69 (1H, m), 3.42 - 3.59 (2H, m), 2.64 (2H, m), 2.33 (2H, m), 1.89 - 2.16 (4H, m), 1.11 -1.67 (41H, m with singlets). Cytosine 5-H and 6-H coupled pairs by COSY: (δ 7.93 & 7.27), (δ 7.72 & 7.18). Neg. FABMS, m/z (rel. intensity): 965(23), 964(63), 963($(M-H)^-$, 100), 908(15), 306(40), 305(39), 270(28), 199(26), 169(24), 154(15), 153(75), 152(50), 151(38), 122(14). HRMS (Neg. FAB): calcd for $C_{52}H_{71}N_{10}O_8$ $(M-H)^-$ 963.5456, found 963.5425.

Hydrazide trimer (**76**).

According to the procedure for oligomerization, the hydrazide dimer **75** (21 mg, 21.8 μmol) was coupled with the *p*-nitrophenyl ester **70b** to provide 17.6 mg (60 % yield) of hydrazide trimer **76** after purification. ^{1}H NMR (400 MHz, DMSO-d6): δ 11.01 (3H, br s), 8.72 & 8.30 (1H, 2 singlets), 7.83 - 8.10 (8H, m), 7.73 (2H, m), 7.65 (1H, br d, J = 9.6 Hz), 7.52 (2H, d, J = 8.3 Hz), 7.44 (4H, m), 7.29 (1H, br s), 7.19 (2H, m), 6.66 (1H, d, J = 9.1 Hz), 3.87 - 4.22 (6H, m), 3.48 - 3.74 (3H, m), 2.64 (2H, m), 2.32 (2H, m), 1.89 - 2.18 (6H, m), 1.02 -1.70 (54H, m with singlets). Neg. FABMS, m/z (rel. intensity): 1347(40), 1346(85), 1345.6($(M-H)^-$, 100), 1291(24), 1290.5(32), 963(23), 909(10), 908(14), 306(37), 305(36), 270(50), 199(19), 169(17), 153(86), 152(47), 151(32), 122(15).HRMS (Neg. FAB): calcd for $C_{73}H_{97}N_{14}O_{11}$ $(M-H)^-$ 1345.7461; found 1345.7298.

Capped dimer (**82**).

According to the procedure for oligomerization, the capped monomer **74** (78.5 mg, 109 μmol) was coupled with the *p*-nitrophenyl ester **70b** to provide 103.4 mg (86% yield) of capped dimer **82** after purification. ^{1}H NMR (400 MHz, DMSO-d6): δ 11.06 (1H, br s; exchanged with D_2O), 11.00 (1H, br s; exchanged with D_2O), 7.86 - 8.00 (7H, m), 7.74 (1H, d, J = 8.0 Hz), 7.66 (1H, d, J = 8.5 Hz; exchanged with D_2O), 7.40 - 7.59 (6H, m), 7.28 (1H, br s), 7.19 (1H, br s), 6.63 (1H, d, J = 9.8 Hz; exchanged with D_2O), 4.32 - 4.46 (2H, m), 4.16 (1H, m), 3.92 - 4.08 (2H, m), 3.63 - 3.75 (3H, m), 3.43 - 3.52 (2H, m), 3.06 &2.87 (3H, 2 singlets), 2.25 - 2.43 (2H, m), 1.93 - 2.12 (2H, m), 1.10 -1.62 (44H, m with singlets). Cytosine 5-H and 6-H coupled pairs by COSY: (δ 7.93 & 7.28), (δ 7.74 & 7.19). Neg. FABMS, m/z (rel. intensity): 1100(30), 1099(73), 1098.3($(M-H)^-$, 100), 716(11), 306(19), 305(21), 270(47),

199(18), 168(23), 154(14), 153(67), 151(39), 122(16). HRMS (Neg. FAB): calcd for $C_{61}H_{80}N_9O_{10}$ $(M-H)^-$ 1098.6028; found 1098.5870.

Capped trimer (**83**).

According to the procedure for oligomerization, the capped dimer **80** (8.9 mg, 8.1 μmol) was coupled with the *p*-nitrophenyl ester **70b** to provide 8.3 mg (69% yield) of capped trimer **83** after purification. ^1H NMR (400 MHz, DMSO-d6): δ 11.06 (1H, br s; exchanged with D_2O), 11.02 (1H, br s; exchanged with D_2O), 11.00 (1H, br s; exchanged with D_2O), 7.84 - 7.99 (9H, m), 7.79 (1H, br d, J = 6.0 Hz), 7.72 (2H, m), 7.60 (1H, d, J = 9.3 Hz; exchanged with D_2O), 7.53 (4H, m), 7.43 (4H, m), 7.29 (1H, br d, J = 7.2 Hz), 7.19 (2H, br t, J = 7.5 Hz), 6.65 (1H, d, J = 9.9 Hz; exchanged with D_2O), 4.30 - 4.45 (2H, m), 4.16 (1H, m), 3.87 - 4.20 (4H, m), 3.60 - 3.75 (4H, m), 3.40 - 3.53 (2H, m), 3.05 &2.86 (3H, 2xs), 2.30 - 2.45 (2H, m), 1.88 - 2.16 (4H, m), 1.08 -1.65 (57H, m with singlets). Cytosine 5-H and 6-H coupled pairs by COSY: (δ 7.94 & 7.29), (δ 7.79 & 7.19), (δ 7.71 & 7.19). Neg. FABMS, m/z (rel. intensity): 1484(18), 1483(49), 1482(96), 1481($(M-H)^-$, 100), 1480(12), 270(25), 153(23), 152(10). HRMS (Neg. FAB): calcd for $C_{82}H_{106}N_{13}O_{13}$ $(M-H)^-$ 1480.8033; found 1480.8091.

Tetramer (**84**).

According to the procedure for oligomerization, the capped trimer **83** (30 mg, 20.2 μmol) was coupled with the *p*-nitrophenyl ester of the acid **70b** to provide 32.5 mg (86% yield) of capped tetramer after purification. ^1H NMR (400 MHz, DMSO-d6): δ 7.68 - 8.02 (overlapping multiplets) and 7.09 - 7.65 (overlapping multiplets) with relative integration ratio: 1.00/1.11, 6.65 (d, J = 9.8 Hz), 4.30 - 4.50 (m), 3.82 - 4.20 (m), 3.60 - 3.78 (m), 3.05 & 2.88 (2 singlets; 1.75:1.00), 2.29 - 2.46 (m) and 1.85 -

2.17 (m) with relative integration ratio: 1.00/2.74 (i.e. ~2H/6H), 1.00 - 1.65 (m). Neg. FABMS, m/z (rel. intensity): 1867(12), 1866(31), 1865(68), 1864((M-H)$^-$, 100), 1863(91), 1862(18), 1861(10), 1100(21), 1099(46), 1098.7(63), 937(10), 936.6(15), 853.5(10), 526(11), 306(35), 270(34), 199(29), 168(29), 154(19), 153(100), 152(69), 151(54). HRMS (Neg. FAB): calcd for $C_{103}H_{132}N_{17}O_{16}$ (M-H)$^-$ 1863.0036; found 1862.9994.

Pentamer (**85**).

According to the procedure for oligomerization but with DMSO in place of DMF, the capped tetramer **84** (9.0 mg, 4.8 μmol) was coupled with the *p*-nitrophenyl ester **70b** to provided 7.1 mg (66% crude yield) of capped pentamer after standard preprative TLC purification. Further purification of this hexamer by HPLC was required in order to provide a clean sample for characterization. ^{1}H NMR (400 MHz, DMSO-d6): δ 7.72 - 8.05 (overlapping multiplets) and 7.10 - 7.65 (overlapping multiplets) with relative integration ratio: 1.00/1.12, 4.30 - 4.48 (m), 3.83 - 4.22 (m), 3.62 - 3.77 (m), 3.05 & 2.88 (2 singlets; 1.65:1.00), 2.29 - 2.46 (m) and 1.85 - 2.17 (m) with relative integration ratio: 1.00/3.72 (i.e. ~2H/8H), 1.06 - 1.70 (m). Neg. FABMS, m/z (rel. intensity): 2249(14), 2248(30), 2247(61), 2246(M-H, 100), 2245(42), 2244(16), 1766(16), 1765(19), 1764(19), 1703(14), 1702(20), 1701(19), 1469(28), 1468(46), 1467(51), 1466(12), 1454(21), 1453(30), 1319(16), 1293(47), 1292(54), 1291(63), 909(14), 908(23), 498(16), 306(26), 305(28), 270(37), 199(25), 168(30), 154(19), 153(100), 152(82), 151(54). HRMS (Neg. FAB): calcd for $C_{124}H_{158}N_{21}O_{19}$ (M-H)$^-$ 2245.2040; found 2245.2024.

Hexamer (86).

To a solution of hydrazide protected trimer **76** (5 mg, 3.71 μmol) in 0.25 mL of 75% aq. THF was added pyridine (0.45 mL, 5.59 μmol) and 1.0 mg (5.62 μmol) of N-bromosuccinimide. This reaction mixture was stirred at room temperature for 15 min and followed by TLC. Upon completion of reaction, 0.15 mL of 0.1 M HCl was added and the reaction mixture evaporated to dryness. The residue was dissolved in 10 mL of 20% isopropanol/$CHCl_3$ and washed with 0.02 M HCl (2 x 3 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The product was thoroughly dried on high vacuum at 50 °C for 8h before taking on to the activation reaction. Small amount of this trimer acid was subjected to FABMS analysis to verify the formation of **78a**. This crude trimer acid **78a** was taken on directly to the activation stage without further purification.

The standard procedure was followed for coupling trimer portions. This involved Boc-deprotection of cap-protected trimer **83** (6.5 mg, 4.38 μmol) to provide the trimer amine salt, and activation of trimer free acid **78a** by the standard procedure to give **78b**. The activation of the trimer acid went smoothly as evidenced by TLC; however, no hexamer was isolated after the coupling reaction. The corresponding trimer amine (2.5 mg, 1.81 μmol) and trimer acid (4.0 mg, 3.16 μmol) were recovered from the crude reaction mixture by standard preparative TLC purification. Neg. FABMS for trimer amine; m/z (rel. intensity): 1383 (25), 1382 (50), 1381($(M-H)^-$, 59), 270 (25), 153 (100). Neg. FABMS for trimer acid **78a**; m/z (rel. intensity): 1264 ($(M-H)^-$, 90), 365 (39), 307 (49), 270 (100). Instead, hexamer **86** was prepared by the stepwise oligomerization procedure from pentamer **85**. The capped pentamer **85** (9.8 mg, 4.36 μmol) was coupled with the *p*-nitrophenyl ester **70b** in DMSO to provided 7.3 mg (64% yield) of capped hexamer **86** after purification by preparative TLC. Further purification of this hexamer by HPLC was required in order to provide a

clean sample for characterization. ^{1}H NMR (400 MHz, DMSO-d6): δ 8.06 - 8.28 (m), 7.68 - 8.05 (overlapping multiplets) and 7.08 - 7.65 (overlapping multiplets) with relative integration ratio: 0.25/1.00/1.00, 4.25 - 4.50 (m), 3.80 - 4.20 (m), 3.58 - 3.80 (m), 3.05 & 2.88 (2xs; 1.73:1.00), 2.29 - 2.46 (m) and 1.85 - 2.17 (m) with relative integration ratio: 1.00/4.67 (i.e. ~2H/10H), 1.00 - 1.72 (m). Neg. FABMS, m/z (rel. intensity): 2628($(M-H)^-$, 30), 1468(14), 1467(18), 1310(39), 1292(40), 1291(65), 1082(26), 1045(54), 909(21), 908(97), 306(12), 305(17), 270(100). HRMS (Neg. FAB): calcd for $C_{145}H_{184}N_{25}O_{22}$ $(M-H)^-$ 2627.4047; found 2627.3954.

Hexamer (**27**).

According to the standard procedure (see Scheme XV) for oligomerization, the capped hexamer **86** (6.5 mg, 1.03 μmol) was coupled with mixed carbonate **87**[178] to provide tailed hexamer. Volatiles were removed under reduced pressure, the residue treated with 1:1 concentrated NH_3/DMF (2mL) then sealed and stirred at 50°C for 72 h. Solvents were evaporated and the residue was redissolved in 0.3 N KCl solution with phosphate buffer (2 mL, pH 2.1) and loaded on a cation exchange column (fast flow S-Sepharose from Sigma) then eluted with a KCl gradient at pH 2.1. The fractions containing hexamer **27** were combined and repurified by the same procedure. Upon neutralization with 0.15 M NaOH to pH 7, solvent was evaporated under reduced pressure. To this residue was added trifluoroethanol (4 mL), the supernate was transferred into another flask and concentrated to dryness. To this residue was added CH_3OH (2 mL) and the mixture transferred into a centrifuge tube then diluted with Et_2O. This mixture was shaken for 5 min and centrifuged. The supernate was decanted and the pellet was allowed to dried under high vacuum for 8 h. This sample was then subjected to binding test to $p(dG)_6$. Due to the limited amount of sample, the percentage recovery of the material cannot be obtained. The absence of absorbance in the 295-300

nm region and presence of 272 nm absorption in UV indicated complete removal of *tert*-benzoyl base protecting groups.

Procedure for binding test.

The pellet of hexamer **27** was dissolved in DMSO (25 μL) then diluted with 0.05 N phosphate buffer containing 0.001 N EDTA (975 μL) to provide the sample solution. The concetration of this stock solution was 4.07 μM, calculated from the absorbance (0.22) of UV spectra based on $\varepsilon = 9.0 \times 10^3$ for the cytosine ($\lambda_{max} = 271$ nm). The target solution of $p(dG)_6$ was prepared to be 4.00 μM by dilution of 23.6 μL of a $p(dG)_6$ stock solution (1.69×10^{-4} M) to a total volume of 1 mL. The concentration of the $p(dG)_6$ stock solution was determined by UV spectra based on $\varepsilon = 6.52 \times 10^4$ for $p(dG)_6$ at $\lambda_{max} = 250.9$ nm in neutral medium. A double beam ultraviolet spectrometer was used for thermal denaturation studies, 500 μL of the sample solution and the target solution were transferred into separate UV cells (cuvette A and B respectively), and diluted with buffer solution (500 μL) then placed in the UV spectrophotomer for the reference beam. The sample beam also contained two cuvettes, equal amount (500 μL) of sample solution and target solution were mixed in cuvette C, and cuvette D contained buffer solution (1.00 mL) only. Therefore, the output is the difference UV spectrum between mixed and unmixed samples of **27** and $p(dG)_6$. The schematic representation is shown in Figure VII. The samples were warmed to 50°C and cool to room temperature over several hours, then a difference UV spectrum was recorded. In this spectrum, a hypochromic effect at 272 nm and a hyperchromic effect at 297 nm were detected. After annealing of the neutral analogue and the target, a series of difference spectra were recorded over a range of 260 to 320 nm from 20°C in ca. 4°C increments up to 90°C. The thermal denaturation plot was obtained by using the absorbance difference (Δ absorbance) against temperature (°C) at 272 nm as shown in figure VIII.

The determination of T_m value from this graph is difficult due to the non-ideal sigmoidal curve obtained, but can be estimated to be 48°C.

REFERENCES

1. Inouye, M. *Gene* **1988**, *72*, 25.
2. Toulme, J.-J.; Helene, C. *Gene* **1988**, *72*, 51.
3. Stein, C.A.; Cohen, J.S. *Cancer Research* **1988**, *48*, 2659.
4. Zon, G. *Pharm. Research* **1988**, *5*, 539.
5. Miller, P.S.; Ts'o, P.O.P *Ann. Rep. Med. Chem.* **1988**, *23*, 295.
6. Green, P.J.; Pines, O.; Inouye, M. *Ann. Rew. Biochem.* **1986**, *55*, 569.
7. Knorre, D.G.; Vlassov, V.V.; Zarytova, V.F. *Biochimie* **1985**, *67*, 785.
8. Simons, R.W. *Gene* **1988**, *72*, 35.
9. Krinke, L.; Wulff, D.L. *Genes Dev.* **1988**, *1*, 1005.
10. Nordstrom, K.; Wagner, G.H.; Persson, C.; Blomberg, P.; Ohman, M. *Gene* **1988**, *72*, 237.
11. Dempsey, W.B. *Mol. Gen. Genet.* **1987**, *209*, 533.
12. Tomizawa, J. *Molecular Biology of RNA: New Prospectives*, Academic Press, New York, **1987**, 249.
13. Okamoto, K.; Freundlich, M. *Molecular Biology of RNA: New Prospectives*, Academic Press, Orlando, FL, **1987**, 357.
14. Takayama, K.M.; Houba-Herin, N.; Inouye, M. *Mol. Gen. Genet.* **1987**, *210*, 184.
15. Liao, S.-M.; Wu, T.; Chiang, C.H.; Susskind, M.M.; McClure, W.R. *Genes Dev.* **1987**, *1*, 197.
16. Finlay, B.B.; Frost, L.S.; Paranchych, W.; Willetts, N.S. *J. Bacteriol.* **1986**, *167*, 754.
17. Okamoto, K.; Freundlich, M. *Proc. Natl. Acad. Sci.* **1986**, *84*, 5000.
18. Kim, K.; Meyer, R.J. *Nucleic Acids Res.* **1986**, *14*, 8027.

19. Patel, I.; Bastia, D. *Cell* **1986**, *47*, 785.

20. Gerdes, K.; Bech, F.W.; Jorgensen, S.T.; Lobner-Olesen, A.; Molin, S.; von Meyenburg, K. *EBMO J.* **1986**, *5*, 2023.

21. Gerdes, K.; Rasmussen, P.B.; Molin, S. *Proc. Natl. Acad. Sci.* **1986**, *83*, 3116.

22. Hoopes, B.C.; McClure, W.R. *Proc. Natl. Acad. Sci.* **1985**, *82*, 3134.

23. Kumar, C.C.; Novick, R.P. *Proc. Natl. Acad. Sci.* **1985**, *82*, 638.

24. Mizuno, T.; Chou, M.; Inouye, M. *Proc. Natl. Acad. Sci.* **1984**, *82*, 1966.

25. Simons, R.W.; Kleckner, N. *Cell* **1983**, *34*, 683.

26. Mizuno, T.; Chou, M.; Inouye, M. *Proc. Jpn. Acad.* **1983**, *59*, 335.

27. Light, J.; Molin, S. *EMBO J.* **1983**, *2*, 93.

28. Tomizawa, J.; Itoh, T. *Cell* **1982**, *31*, 575.

29. Cesareni, G.; Musing, M.; Polisky, B. *Proc. Natl. Acad. Sci.* **1982**, *79*, 6313.

30. Tomizawa, J.; Itoh, T.; Selzer, G.; Som, T. *Proc. Natl. Acad. Sci.* **1981**, *78*, 1421.

31. Stougaard, P.; Molin, S.; Nordstrom, K. *Proc. Natl. Acad. Sci.* **1981**, *78*, 6008.

32. Easton, A.M.; Sampathkumark, P.; Rownd, R.H. *The Initiation of DNA Replication*, Academic Press, New York, **1981**, 125.

33. Croen, K.D.; Ostrove, J.M.; Dragovic, L.J.; Smialek, J.E.; Straus, S.E. *New England J. Med.* **1988**, *317*, 1427.

34. Roger, J. *Plant Mol. Biol.* **1988**, *11*, 125.

35. Andersen, J.; Delihas, N.; Ikenaka, K.; Green, P.J.; Pines, O.; Ilercil, O.; Inouye, M. *Nucleic Acids Res.* **1987**, *15*, 2089.

36. Chang, D.D.; Clayton, D.A. *Science* **1987**, *235*, 1178.

37. Stevens, J.G.; Wagner, E.K.; Devi-Rao, G.B.; Cook, M.L.; Feldman, L.T. *Science* **1987**, *235*, 1056.

38. Adelman, J.P.; Bond, C.T.; Douglass, J.; Herbert, E. *Science* **1987**, *235*, 1514.

39. Nepveu, A.; Marcu, K.B. *EMBO J.* **1986**, *5*, 2859.

40. Rezaian, M.A.; Symons, R.H. *Nucleic Acids Res.* **1986**, *14*, 3229.

41. Spencer, C.A.; Gietz, R.D.; Hodgetts, R.B. *Nature* **1986**, *322*, 279.

42. Williams, T.; Fried, M. *Nature* **1986**, *322*, 275.

43. Heywood, S.M. *Nucleic Acids Res.* **1986**, *14*, 6711.

44. Adeniyi-Jones, S.; Zasloff, M. *Nature* **1985**, *317*, 81.

45. Farnham, P.J.; Abrams, J.M.; Schimke, R.T. *Proc. Natl. Acad. Sci.* **1985**, *82*, 3978.

46. McCarthy, T.L.; Siegel, E.; Mroczkowski, M.; Heywood, S.M. *Biochemistry* **1983**, *22*, 935.

47. Hirashima, A.; Kawaki, S.; Inokuchi, Y.; Inouye, M. *Proc. Natl. Acad. Sci.* **1986**, *83*, 7726.

48. Coleman, J.; Hirashima, A.; Inokuchi, Y.; Green, P.J.; Inouye, M. *Nature* **1985**, *315*, 601.

49. van der Krol, A.R.; Mol, J.N.M.; Stuitje, A.R. *Gene* **1988**, *72*, 45.

50. Ecker, J.R.; Davis, R.W. *Proc. Natl. Acad. Sci.* **1986**, *83*, 5372.

51. Vlassov, V.V.; Svinarchuk, F.P. *Molekulyarnaya Biologiya* (English Translation) **1987**, *21*, 28.

52. To, R.Y.-L.; Booth, S.C.; Neiman, P.E. *Mol. Celluar Biol.* **1986**, *6*, 4758.

53. McGarry, T.J.; Lindquist, S. *Proc. Natl. Acad. Sci.* **1986**, *83*, 399.

54. Melton, D.A. *Proc. Natl. Acad. Sci.* **1985**, *82*, 144.

55. Harland, R.; Weintraub, H. *J. Cell Biol.* **1985**, *101*, 1094.

56. Kim, S.K.; Wold, B.J. *Cell* **1985**, *42*, 129.

57. Izant, J.G.; Weintraub, H. *Science* **1985**, *229*, 345.

58. Izant, J.G.; Weintraub, H. *Cell* **1984**, *36*, 1007.

59. Rubenstein, J.L.R; Nicolas, J-F.; Jacob, F. *Genetics*, C.R. Seances Acad. Sci., Ser. 3, **1984**, *299*, 271.

60. To, R.Y.-L.; Boorh, S.C.; Neiman, P.E. *Mol. Cell Biol.* **1986**, *6*, 4758.

61. De Benedetii, A.; Pytel, B.A.; Baglioni, C. *Proc. Natl. Acad. Sci.* **1987**, *84*, 658.

62. Chang, L.-J.; Stoltzfus, C.M. *J. Virol.* **1987**, *61*, 921.

63. Gupta, K.C. *J. Biol. Chem.* **1987**, *262*, 7492.

64. Khokha, R.; Waterhouse, P.; Yagel, S.; Lala, P.K.; Overall, C.M.; Norton, G.; Denhardt, D.T. *Science* **1989**, *243*, 947.

65. Wickstrom, E.L.; Bacon, T.A.; Gonzalez, A.; Freeman, D.L.; Lyman, G.H.; Wickstrom, E. *Proc. Natl. Acad. Sci.* **1988**, *85*, 1208.

66. Holt, J.T.; Redner, R.L.; Nienhuis, A.W. *Mol. Cell Biol.* **1988**, *8*, 963.

67. Heikila, R.; Schwab, G.; Wickstrom, E.; Loke, S.L.; Pluznik, D.H.; Watt, R.; Neckers, L.M. *Nature* **1987**, *328*, 445.

68. Zerial, A.; Thuong, N.T.; Helene, C. *Nucleic Acids Res.* **1987**, *15*, 9909.

69. Holt, J.T.; Gopal, T.V.; Moulton, A.D.; Nienhuis, A.W. *Proc. Natl. Acad. Sci.* **1986**, *83*, 4794.

70. Groger, R.K.; Morrow, D.M.; Tykocinski, M.L. *Gene* **1989**, *81*, 285.

71. Zamecnik, P.C.; Stephenson, M.L. *Proc. Natl. Acad. Sci.* **1978**, *75*, 280.

72. Stephenson, M.L.; Zamecnik, P.C. *Proc. Natl. Acad. Sci.* **1978**, *75*, 285.

73. Shibahara, S.; Mukai, S.; Morisawa, H.; Nakashima, H.; Kobayashi, S.; Yamamoto, N. *Proc. Natl. Acad. Sci.* **1989**, *86*, 239.

74. Sarin, P.S.; Agrawal, S.; Civerira, M.P.; Goodchild, J.; Ikeuchi, T.; Zamecnik, P.C. *Proc. Natl. Acad. Sci.* **1988**, *85*, 7448.

75. Agrawal, S.; Goodchild, J.; Civerira, M.P.; Thornton, A.H.; Sarin, P.S.; Zamecnik, P.C. *Proc. Natl. Acad. Sci.* **1988**, *85*, 7079.

76. Goodchild, J.; Agrawal, S.; Civerira, M.; Civerira, M.; Sarin, P.S.; Sun, D.; Zamecnik, P.C. *Proc. Natl. Acad. Sci.* **1988**, *85*, 5507.

77. Goodchild, J.; Letsinger, R.L.; Sarin, P.S.; Zamecnik, M.; Zamecnik, P.C. *Human Retroviruses, Cancer & AIDS: Approach to Prevention & Therapy*, UCLA Symposia on Molecular and Celluar Biology New Series, ed. Bolognesi, D. (Liss. New York), **1988**, *77*, 423.

78. Matsukura, M.; Shinozuka, K.; Zon, G.; Mitsuya, H.; Reitz, M.; Cohen, J.S.; Broder, S. *Proc. Natl. Acad. Sci.* **1987**, *84*, 7706.

79. Zamecnik, P.C.; Goodchild, J.; Taguchi, Y.; Sarin, P.S. *Proc. Natl. Acad. Sci.* **1986**, *83*, 4143.

80. Lemaitre, M.; Bayard, B.; Lebleu, B. *Proc. Natl. Acad. Sci.* **1987**, *84*, 648.

81. Agris, C.H.; Blake, K.R.; Miller, P.S.; Reddy, M.P.; Ts'o, P.O.P. *Biochemistry*, **1986**, *25*, 6268.

82. Miller, P.S.; Agris, C.H.; Aurelian, L.; Blake, K.R.; Murakami, A.; Reddy, M.P.; Spitz, S.A.; Ts'o, P.O.P. *Biochimie* **1985**, *67*, 769.

83. Smith, C.C.; Aurelian, L.; Reddy, M.P.; Miller, P.S.; Ts'o, P.O.P. *Proc. Natl. Acad. Sci.* **1986**, *83*, 2787.

84. Coleman, J.; Hirashima, A.; Inokuchi, Y.; Green,P.J.; Inouye, M. *Nature* **1985**, *315*, 601.

85. Coleman, J.; Green,P.J.; Inouye, M. *Cell* **1984**, *37*, 429.

86. Adams, R.L.P.; Knowler, J.T.; Leader, D.P. in *The Biochemistry of the Nucleic Acids* 10th Ed, **1986**, 96, Champman and Hall, New York.

87. Cazenave, C.; Loreau, N.; Thuong, N.-T.; Toulme, J.J.; Helene, C. *Nucleic Acids Res.* **1987**, *15*, 4717.

88. Dash, P.; Lotan, I.; Knapp, M.; Kandel, E.R.; Goelet, P. *Proc. Natl. Acad. Sci.* **1987**, *84*, 7896.

89. Haeuptle, M.T.; Frank, R.; Dobberstein, B. *Nucleic Acids Res.* **1986**, *14*, 1427.

90. Minshull, J.; Hunt, T. *Nucleic Acids Res.* **1987**, *15*, 6443.

91. Walder, R.Y.; Walder, J.A. *Proc. Natl. Acad. Sci.* **1988**, *85*, 5011.

92. Marcus-Sekura, C.J. *Anal. Biochem.* **1988**, *172*, 289.

93. Dervan, P.B. Science **1986**, *232*, 464.

94. see 4, 72, 75, 78 and 86.

95. Pauwel, R.; Debyser, Z.; Balzarini, J.; Baba, M.; Desmyter, J.; Rayner, B.; Morvan, F. Imbach, J.L.; De Clercq, E. *Nucleosides & Nucleotides* **1989**, *8*, 995.

96. Imbach, J.L.; Rayner, B.; Morvan, F. *Nucleosides & Nucleotides* **1989**, *8*, 627.

97. Thuong, N.T.; Asseline, U.; Roig, V.; Takasugi, M.; Helene, C. *Proc. Natl. Acad. Sci.* **1987**, *84*, 5129.

98. Praseuth, D.; Chassignol, M.; Takasugi, M.; La Doan, T.; Thuong, N.T.; Helene, C. *J. Mol. Biol.* **1987**, *196*, 939.

99. Cazenave, C.; Chevrier, M.; Thuong, N.T.; Helene, C. *Nucleic Acids Res.* **1987**, *15*, 10507.

100. Gagnor, C.; Bertrand, J.R.; Thenet, S.; Lemaitre, M.; Morvan, F.; Rayner, B.; Malvy, C.; Lebleu, B.; Imbach, J.L.; Paoletti, C. *Nucleic Acids Res.* **1987**, *15*, 10419.

101. Lancelot, G.; Guesnet, J.-L.; Thuong, N.T. *Nucleic Acids Res.* **1987**, *15*, 7531.

102. Morvan, F.; Rayner, B.; Imbach, J.L.; Lee, M.; Hartley, J.A.; Chang, D.K.; Lown, J.W. *Nucleic Acids Res.* **1987**, *15*, 7027.

103. Gautier, C.; Morvan, F.; Rayner, B.; Huynh, T.; Igolen, J.; Imbach, J-L.; Paoletti, C.; Paoletti, J. *Nucleic Acids Res.* **1987**, *15*, 6625.

104. Sun, J.S.; Asseline, U.; Rouzaud, D.; Montenary-Garestier, T.; Thuong, N.T.; Helene, C. *Nucleic Acids Res.* **1987**, *15*, 6149.

105. Morvan, F.; Rayner, B.; Imbach, J.L.; Chang, D.K.; Lown, J.W. *Nucleic Acids Res.* **1987**, *15*, 4241.

106. Morvan, F.; Rayner, B.; Imbach, J.L.; Thenet, S.; Bertrand, J.R.; Paoletti, J.; Malvy, C.; Paoletti, C. *Nucleic Acids Res.* **1987**, *15*, 3421.

107. Morvan, F.; Rayner, B.; Imbach, J.L.; Chang, D.K.; Lown, J.W. *Nucleic Acids Res.* **1986**, *14*, 5019.

108. Amini, S.; DeSeau, V.; Reddy, S.; Shalloway, D.; Bolen, J.B. *Mol. Cell Biol.* **1986**, *6*, 2305.

109. Andrus, A.; Geiser, T.; Zon, G. *Nucleosides & Nucleotides* **1989**, *8*, 967.

110. Agrawal, S.; Goodchild, J.; Civerira, M.P.; Sarin, P.S.; Zamecnik, P.C. *Nucleosides & Nucleotides* **1989**, *8*, 819.

111. Spitzer, S.; Eckstein, F. *Nucleic Acids Res* **1988**, *16*, 11691.

112. Brill, W. K.-D.; Nielsen, J.; Caruthers, M.H. *Tetrahedron Lett.* **1988**, *29*, 5517.

113. Marcus-Sekura, C.J.; Woerner, A.M.; Shinozuka, K.; Zon, G.; Quinnan, Jr., G.V. *Nucleic Acids Res* **1987**, *14*, 5749.

114. Uznanski, B.; Wilk, A.; Stec, W.J. *Tetrahedron Lett*, **1987**, *28*, 3401.

115. LaPlanche, L.A.; James, T.J.; Powell,C.; Wilson, W.D.; Uznanski, B.; Stec, W.J.; Summers, M.F.; Zon, G. *Nucleic Acids Res* **1986**, *14*, 9081.

116. Eckstein, F. *Ann. Rew. Biochem.* **1985**, *54*, 367.

117. Stec, W.J.; Zon, G.; Egan, W.; Stec, B. *J. Am. Chem. Soc.* **1984**, *106*, 6077.

118. Eckstein, F. Angew. *Chem. Int. Ed. Engl.* **1983**, *22*, 423.

119. Lesnikowski, Z.J.; Jaworska, M.; Stec, W.J. *Nucleic Acids Res* **1988**, *16*, 11675.

120. Maher III, L.J.; Dolnick, B.J. *Nucleic Acids Res* **1988**, *16*, 3341.

121. Lee, B.L.; Murakami, A.; Blake, K.R.; Lin, S.-B.; Miller, P.S. *Biochemistry* **1988**, *27*, 3197.

122. Viari, A.; Ballini, J.P.; Vigny, P.; Blonski, C.; Dousset, P.; Shire, D. *Tetrahedron Lett.* **1987**, *28*, 3349.

123. Agrawal, S.; Goodchild, J. *Tetrahedron Lett.* **1987**, *28*, 3539.

124. Lesnikowski, Z.J.; Wozkanin, P.J.; Stec, W.J. *Tetrahedron Lett.* **1987**, *28*, 5535.

125. Bower, M.; Summers, M.F.; Powell, C.; Shinozuka, K.; Regan, J.B.; Zon, G.; Wilson, W.D. *Nucleic Acids Res* **1987**, *15*, 4915.

126. Marugg, J.E.; de Vroom, E.; Dreef, C.E.; Tromp, M.; van der Marel, G.A.; van Boom, J.H. *Nucleic Acids Res* **1986**, *14*, 2171.

127. Callahan, L.; Han, F.-S.; Watt, W., Duchamp, D.; Kezdy, F.J. *Proc. Natl. Acad. Sci.* **1986**, *83*, 1617.

128. Smith, C.C.; Aurelian, L.; Reddy, M.P.; Miller, P.S.; Ts'o, P.O.P. *Proc. Natl. Acad. Sci.* **1986**, *83*, 2787.

129. Agris, C.H.; Blake, K.R.; Miller, P.S.; Reddy, M.P.; Ts'o, P.O.P. *Biochemistry* **1986**, *25*, 6286.

130. Katti, S.B.; Agarwal, K. *Tetrahedron Lett.* **1986**, *27*, 5327.

131. Miller, P.S.; Reddy, M.P.; Murakami, A.; Blake, K.R.; Lin, S.-B.; Agris, C.H. *Biochemistry* **1986**, *25*, 5092.

132. Stec, W.J.; Zon, G.; Egan, W.; Byrd, R.A.; Phillips, L.R.; Gallo, K.A. *J. Org. Chem.* **1985**, *50*, 3908.

133. Stec, W.J.; Zon, G.; Gallo, K.A.; Byrd, R.A. *Tetrahedron Lett.* **1985**, *26*, 2191.

134. Blake, K.R.; Murakami, A.; Miller, P.S. *Biochemistry* **1985**, *24*, 6132.

135. Blake, K.R.; Murakami, A.; Spitz, S.A.; Glave, S.A.; Reddy, M.P.; Ts'o, P.O.P.; Miller, P.S. *Biochemistry* **1985**, *24*, 6139.

136. Murakami, A.; Blake, K.R.; Miller, P.S. *Biochemistry* **1985**, *24*, 4041.

137. Miller, P.S.; Agris, C.H.; Aurelian, L.; Blake, K.R.; Murakami, A.; Reddy, M.P.; Spitz, S.A.; Ts'o, P.O.P. *Biochimie* **1985**, *67*, 769.

138. Jager, A.; Engels, J. *Tetrahedron Lett.* **1984**, *25*, 1437.

139. Wierzchowski, K.L.; Zielenkiewicz, A.; Miller, P.S. *Biopolymers* **1984**, *23*, 2361.

140. Miller, P.S.; Agris, C.H.; Murakami, A.; Reddy, M.P.; Spitz, S.A.; Ts'o, P.O.P. *Nucleic Acids Res* **1983**, *11*, 6225.

141. Engels, J.; Jager, A. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 912.

142. Jayaraman, K.; McParland, K.; Miller, P.; Ts'o, P.O.P. *Proc. Natl. Acad. Sci.* **1981**, *78*, 1537.

143. Miller, P.S.; McParland, K.B.; Jayaraman, K.; Ts'o, P.O.P. *Biochemistry* **1981**, *20*, 1874.

144. Kan, L.S.; Cheng, D.M.; Miller, S.; Yano, J.; Ts'o, P.O.P. *Biochemistry* **1980**, *19*, 2122.

145. Miller, P.S.; Dreon, N.; Pulford, S.M.; McParland, K.B. *J. Biol. Chem.* **1980**, *255*, 9659.

146. Miller, P.S.; Yano, J.; Yano, E.; Carroll, C.; Jayaraman, K.; Ts'o, P.O.P. *Biochemistry* **1979**, *18*, 5134.

147. Agarwal, K.L.; Riftina, F. *Nucleic Acids Res* **1979**, *6*, 3009.

148. Miller, P.S.; Braiterman, L.T.; Ts'o, P.O.P. *Biochemistry* **1977**, *16*, 1988.

149. Summers, M.F.; Powell, C.; Egan, W.; Byrd, R.A.; Wilson, W.D.; Zon, G. *Nucleic Acids Res* **1986**, *14*, 7421.

150. Gallo, K.A.; Shao, K.-L.; Phillips, L.R.; Regan, J.B.; Koziolkiewicz, M.; Uznanski, B.; Stec, W.J.; Zon, G. *Nucleic Acids Res* **1986**, *14*, 7405.

151. Letsinger, R.L.; Bach, S.A.; Eadie, J.S. *Nucleic Acids Res* **1986**, *14*, 3487.

152. Danilyuk, N.K.; Petrenko, V.A.; Pozdnyakov, P.I.; Popov, S.G.; Sivolobova, G.F.; Shubina, T.N. *Molekulyarnaya Biologiya* (English Translation) **1983**, *17*, 892.

153. Miller, P.S.; Chandrasegaran, S.; Dow, D.L.; Pulford, S.M.; Kan, L.S. *Biochemistry* **1982**, *21*, 5468.

154. Pless, R.C.; Ts'o, P.O.P. *Biochemistry* **1977**, *16*, 1239.

155. Miller, P.S.; Braiterman, L.T.; Ts'o, P.O.P. *Biochemistry* **1977**, *16*, 1988.

156. Barrett, J.C.; Miller, P.S.; Ts'o, P.O.P. *Biochemistry* **1974**, *13*, 4897.

157. Miller, P.S.; Barrett, J.C.; Ts'o, P.O.P. *Biochemistry* **1974**, *13*, 4887.

158. Miller, P.S.; Fang, K.N.; Kondo, N.S.; Ts'o, P.O.P. *J. Am. Chem. Soc.* **1971**, *93*, 6657.

159. Jager, A.; Levy, M.J.; Hecht *Biochemistry* **1988**, *27*, 7237.

160. Froehler, B.; Ng, P.; Matteucci, M. *Nucleic Acids Res* **1988**, *16*, 4831.

161. Letsinger, R.L.; Bach, S.A.; Eadie, J.S. *Nucleic Acids Res* **1986**, *14*, 3487.

162. Froehler, B.C. *Tetrahedron Lett.* **1986**, *27*, 5575.

163. Letsinger, R.L.; Schott, M.E. *J. Am. Chem. Soc.* **1981**, *103*, 7394.

164. Bleaney, R.C.; Jones, A.S.; Walker, R.I. *Nucleic Acids Res* **1975**, *2*, 69.

165. Jones, A.S.; MacCoss, M.D.; Walker, R.T. *Biochim. Biophys. Acta* **1973**, *365*, 365.

166. Edge, M.D.; Hodgson, A.; Jones, A.S.; MacCoss, M.; Walker, R.T. *J. Chem. Soc. Perkin Trans. I* **1973**, 290.

167. Edge, M.D.; Hodgson, A.; Jones, A.S.; Walker, R.T. *J. Chem. Soc. Perkin Trans. I* **1972**, 1991.

168. Halford, M.H.; Jones, A.S. *J. Chem. Soc. (C)* **1968**, 2667.

169. Gait, M.J.; Jones, A.S.; Jones, M.D.; Shepard, M.J.; Walker, R.T. *J. Chem. Soc. Perkin Trans. I* **1979**, 1389.

170. Gait, M.J.; Jones, A.S.; Walker, R.T. *J. Chem. Soc. Perkin Trans. I* **1974**, 1684.

171. Tittensor, J.R. *J. Chem. Soc. (C)* **1971**, 2656.

172. Mertes, M.P.; Coats, E.A. *J. Med. Chem.* **1969**, *12*, 154.

173. Jones, D.S.; Tittensor, J.R. *J. Chem. Soc. (D)* **1969**, 1240.

174. Coull, J.M.; Carlson, D.V.; Weith, L. *Tetrahedron Lett.* **1987**, *28*, 745.

175. Stirchak, E.P.; Summerton, J.E.; Weller, D.D. *J. Org. Chem.* **1987**, *52*, 4202.

176. Mungall, W.S.; Kaiser, J.K. *J. Org. Chem.* **1977**, *42*, 703.

177. Gait, M.J.; Jones, A.S.; Walker, R.T. *J. Chem. Sco. Perkin Trans. I* **1974**, 1684.

178. Stirchak, E.P.; Summerton, J.E.; Weller, D.D. *Nucleic Acids Res.* **1989**, *17*, 6129.

179. Andrus, A.; Geiser, T.; Zon G. *Nucleosides & Nucleotides* **1989**, *8*, 967.

180. Buck, H.M.; Koole, L.H. van Genderen, M.H.P.; Smit, L.; Geelen, J.L.M.C.; Jurriaans, S.; Goudsmit, J. *Science* **1990**, *248*, 208.

181. Quaedflieg, P.J.L.M.; Broeders, N.L.H.L.; Koole, L.H.; van Genderen, M.H.P.; Buck, H.M.; *J. Org. Chem.* **1990**, *55*, 122.

182. Koole, L.H.; Moody, H.M.; Broeders, N.L.H.L.; Quaedflieg, P.J.L.M.; Kuijpers, W.H.A.; van Genderen, M.H.P.; Coenen, A.J.J.M.; van der Wal, S.; Buck, H.M. *J. Org. Chem.* **1989**, *54*, 1657.

183. Quaedflieg, P.J.L.M.; Koole, L.H.; van Genderen, M.H.P.; Buck, H.M.; *Recl. Trav. Chim. Pays-Bas* **1989**, *108*, 421.

184. Moody; H.M. *et al. Nucleic Acids Res* **1989**, *17*, 4769.

185. Pitha, J. *Adv. Polym. Sci.* **1983**, *50*, 1.

186. Chou, H.J.; Froehlich, J.P.; Pitha, J. *Nucleic Acids Res* **1978**, *5*, 691.

187. Vengris, V.E.; Pitha, P. M. and Sensenbrenner, L. *Mol. Pharmacol.* **1978**, *14*, 271.

188. Pitha, J. *Polymer* **1977**, *18*, 425.

189. Noronha-Blob, L.; Vengris, V.E.; Pitha, P. M.; Pitha, J. *J. Med. Chem.* **1977**, *20*, 356.

190. Pitha, J.; Wilson, S.H. *Nucleic Acids Res* **1976**, *3*, 825.

191. Reynolds, F.H.; Pitha, P. M. *Mol. Pharmacol.* **1975**, *11*, 708.

192. Pitha, P. M.; Teich, N.M.; Lowy, D.R.; Pitha, J. *Proc. Natl. Acad. Sci.* **1973**, *70*, 1204.

193. Reynolds, F.; Grunberger, D.; Pitha, J.; Pitha, P. M. *Biochemistry* **1972**, *11*, 3261.

194. Pitha, J.; Pitha, P. M. *Science* **1971**, *172*, 1146.

195. Pitha, J.; Pitha, P. M.; Stuart, E. *Biochemistry* **1971**, *10*, 4595.

196. Pitha, P. M.; Pitha, J. *Biopolymers* **1970**, *9*, 965.

197. Pitha, P. M.; Michelson, A.M. *Biochim. Biophys. Acta* **1970**, *204*, 381.

198. Pitha, J.; Pitha, P. M.; Ts'o, P.O.P. *Biochim. Biophys. Acta* **1970**, *204*, 39.

199. Pitha, J.; Ts'o, P.O.P. *J. Org. Chem.* **1968**, *33*, 1341.

200. Kita, Y; Inaki, Y.; Takemoto, K. *J. Polym. Sci. Polym. Chem. Ed.* **1980**, *18*, 427.

201. Inaki, Y.; Futagawa, H.; Takemoto, K. *J. Polym. Sci. Polym. Chem. Ed.* **1980**, *18*, 2959.

202. Inaki, Y.; Futagawa, H.; Takemoto, K. *Org. Prep. Proc. Int.* **1980**, *12*, 275.

203. Takemoto, K.; Inaki, Y.; Akashi, M. *J. Macromol. Sci.-Chem.* **1979**, *A13*, 519.

204. Akashi, M.; Okimoto, K.; Inaki, Y.; Takemoto, K. *J. Polym. Sci. Polym. Chem. Ed.* **1979**, *17*, 905.

205. Akashi, M.; Takada, H.; Inaki, Y.; Takemoto, K. *J. Polym. Sci. Polym. Chem. Ed.* **1979**, *17*, 747.

206. Akashi, M.; Kita, Y.; Inaki, Y.; Takemoto, K. *J. Polym. Sci. Polym. Chem. Ed.* **1979**, *17*, 301.

207. Akashi, M.; Inaki, Y.; Takemoto, K. *J. Macromol. Sci.-Chem.* **1978**, *A12*, 619.

208. Inaki, Y.; Takada, H.; Kondo, K.; Takemoto, K. *Makromol. Chem.* **1977**, *178*, 365.

209. Akashi, M.; Inaki, Y.; Takemoto, K. *Makromol. Chem.* **1977**, *178*, 353.

210. Takemoto, K.; Akashi, M.; Inaki, Y. *J. Polym. Sci. Polym. Chem. Ed.* **1974**, *12*, 1861.

211. Kondo, K.; Iwasaki, H.; Ueda, N.; Takemoto, K. *Makromol Chem.* **1968**, *120*, 21.

212. Kondo, K.; Tanioku, S.; Takemoto, K. *Macromol. Chem. Rapid Commun.* **1980**, *2*, 203.

213. Kondo, K.; Hisaoka, Y.; Takemoto, K. *Chem. Lett.* **1973**, 125.

214. Overberger, C.G.; Chen, C.C. *J. Polym. Sci. Polym. Chem. Ed.* **1987**, *25*, 389.

215. Overberger, C.G.; Lu, C.X. *J. Polym. Sci. Polym. Chem. Ed.* **1987**, *25*, 1523.

216. Overberger, C.G.; Kikyotani, S. *J. Polym. Sci. Polym. Chem. Ed.* **1983**, *21*, 525.

217. Overberger, C.G.; Kikyotani, S. *J. Polym. Sci. Polym. Chem. Ed.* **1983**, *21*, 541.

218. Overberger, C.G.; Chang, J.Y. *J. Polym. Sci. Polym. Chem. Ed.* **1989**, *27*, 3589.

219. Overberger, C.G.; Lu, C.X. *J. Polym. Sci. Polym. Chem. Ed.* **1985**, *23*, 1321.

220. Seita, T.; Yamauchi, K.; Kinoshita, M.; Imoto, M. *Makromol. Chem.* **1973**, *164*, 15.

221. Seita, T.; Yamauchi, K.; Kinoshita, M.; Imoto, M. *Makromol. Chem.* **1972**, *154*, 263.

222. Overberger, C.G.; Chen, C.C. *J. Polym. Sci. Polym. Chem. Ed.* **1987**, *25*, 373.

223. Lan, M.-J.; Overberger, C.G. *J. Polym. Sci. Polym. Chem. Ed.* **1987**, *25*, 1887.

224. Lan, M.-J.; Overberger, C.G. *J. Polym. Sci. Polym. Chem. Ed.* **1987**, *25*, 1909.

225. Chu, V.P.; Overberger, C.G. *J. Polym. Sci. Polym. Chem. Ed.* **1986**, *24*, 2657.

226. Ludwick, A.G.; Robinson, K.S.; McCloud, Jr. J. *J. Polym. Sci. Polym. Symposium* **1986**, *74*, 55.

227. Ludwick, A.G.; Overberger, C.G. *J. Polym. Sci. Polym. Chem. Ed.* **1982**, *20*, 2123.

228. Ludwick, A.G.; Overberger, C.G. *J. Polym. Sci. Polym. Chem. Ed.* **1982**, *20*, 2139.

229. Overberger, C.G.; Morishima, Y. *J. Polym. Sci. Polym. Chem. Ed.* **1980**, *18*, 1433.

230. Overberger, C.G.; Morishima, Y. *J. Polym. Sci. Polym. Chem. Ed.* **1980**, *18*, 1247.

231. Overberger, C.G.; Morishima, Y. *J. Polym. Sci. Polym. Chem. Ed.* **1980**, *18*, 1267.

232. Overberger, C.G.; Chang, J.Y.; Gunn, V.E. *J. Polym. Sci. Polym. Chem. Ed.* **1989**, *27*, 99.

233. Overberger, C.G.; Chang, J.Y. *J. Polym. Sci. Polym. Chem. Ed.* **1989**, *27*, 4013.

234. Kropachev, V.A.; Aleksiuk, G.P.; Zaviriukha, V.L.; Kovtun, G.I. *Makromol. Chem. Suppl.* **1985**, *9*, 47.

235. Takemoto, K.; Inaki, Y. *Polym. Mat. Sci. Eng.* **1988**, *58*, 250.

236. Kricheldorf, H.R.; Fehrle, M. *Biopolymers* **1982**, *21*, 2097.

237. Draminski, M.; Pitha, J. *Makromol. Chem.* **1978**, *179*, 2195.

238. Buttrey, J.D. Jones, A.S.; Walker, R.T. *Tetrahedron* **1975**, *31*, 73.

239. De Koning, H.; Pandit, U.K. *Rec. Trav. Chim.* **1971**, *91*, 1069.

240. Kaye, H. *J. Am. Chem. Soc.* **1970**, *92*, 5777.

241. Quartin, R.S.; Wetmur, J.G. *Biochemistry* **1989**, *28*, 1040.

242. Pitha, P. M.; Pitha, J.; Rowe, W.P. *Virology* **1975**, *63*, 568.

243. Tennant, R.W.; Hanna, M.G.; Farrelly, J.G. *Proc. Natl. Acad. Sci.* **1974**, *71*, 3167.

244. De Koning, H.; Pandit, U.K. *Rec. Trav. Chim.* **1971**, *90*, 874.

245. Nollet, A.J.H.; Koomen, G.J.; Grose, W.F.A.; Pandit, U.K. *Tetrahedron Lett.* **1969**, 4607.

246. Nollet, A.J.H.; Pandit, U.K. *Tetrahedron Lett.* **1969**, 4605.

247. Nollet, A.J.H.; Pandit, U.K. *Tetrahedron* **1968**, *24*, 5989.

248. Nollet, A.J.H.; Pandit, U.K. *Tetrahedron* **1969**, *25*, 5983.

249. Nollet, A.J.H.; Huting, C.M.; Pandit, U.K. *Tetrahedron* **1969**, *25*, 5971.

250. Doel, M.T.; Jones, A.S.; Walker, R.T. *Tetrahedron* **1974**, *30*, 2755.

251. Doel, M.T.; Jones, A.S.; Taylor, *Tetrahedron Lett.* **1969**, 2285.

252. Absher, M.; Stinebring, W.R. *Nature* **1969**, *223*, 715.

253. see 216, 218, 219, 224, 225, 227, 229, 230, 232 and 233.

254. Schneider, K.C.; Benner, S.A. *J. Am. Chem. Soc.* **1990**, *112*, 453.

255. Usman, N.; Juby, C.D.; Ogilvie, K.K. *Tetrahedron Lett.* **1988**, *29*, 2285.

256. Ogilvie, K.K.; Nguyen-Ba, N.; Gillen, M.F.; Radatus, B.K.; Cheriyan, U.O.; Hanna, H.R.; Smith, S.M.; Galloway, K.S. *Can. J. Chem.* **1984**, *62*, 241.

257. Weller, D.D.; Daly, D.T.; Olson, W.K. *J. Org. Chem.* Submitted for publication.

258. Silverman, R.B.; Levy, M.A. *J. Org. Chem.*, **1980**, *45*, 815.

259. Saijo, S.; Wada, M.; Himizu, J.; Ishida, A. *Chem. Pharm. Bull.*, **1980**, *28*, 1449.

260. Scott, A.I.; Wilkinson, T.J. *Synthetic Comm.* **1980**, *10*, 127.

261. Huang, S.-B.; Nelson, J.S.; Weller, D.D. *Synthetic Comm.* **1989**, *19*, 3485.

262. Dale, J.A.; Dull, D.L.; Mosher, H.S. *J. Org. Chem.*, **1969**, *34*, 2543.

263. Nelson, J.S. Master Thesis, Oregon State University, Corvallis, OR, **1989**.

264. Szirtes, T.; Kisfaludy, L.; Palosi, E.; Szporny, L. *J. Med. Chem.*, **1986**, *29*, 1654 and references cited therein.

265. Greenstein, J.P.; Birnbaum, S.; Otey, M.C. *J. Am. Chem. Soc.*, **1953**, *75*, 1994.

266. Scott, J.W.; Parker, D.; Parrish, R. *Synthetic Comm.* **1981**, *11*, 303.

267. Brown, H.C.; Heim, P.; Yoon, N.M. *J. Am. Chem. Soc.*, **1970**, *92*, 1637.

268. Bukley, T.F.; Rapport, H *J. Amer. Chem. Soc.* **1982**, *104*, 4446.

269. Adkins, H.; Billica, H.R. *J. Am. Chem. Soc.*, **1948**, *70*, 3121.

270. McBride, C.J.; Kienzek, R.; Beancage, S.C.; Caruthers, M.J. *J. Amer. Chem. Soc.* **1986**, *108*, 2040.

271. Letsinger, R.L.; Miller, P.S.; Grams, G.W. *Tetrahedron Lett.* **1968**, 2621.

272. Flynn, D.L.; Zelle, R.E.; Grieco, P.A. *J. Org. Chem.* **1983**, *48*, 2424.

273. Barton, D.H.R.; Girijavallabhan, Sammes, P.G. *J. Chem. Soc., Perkin Trans. I* **1972**, 929.

274. Griffin, D.; Laramee, J.A.; Deinzer, M.L.; Stirchak, E.P.; Weller, D.D. *Biomed. Environ. Mass Spectrom.* **1988**, *17*, 105.

275. Laramee, J.A.; Arbogast, B.C.; Deinzer, M.L. ; Stirchak, E.P.; Weller, D.D. *Org. Mass Spectrom.* **1990**, *25*, 33.

276. Laramee, J.A.; Arbogast, B.C.; Stirchak, E.P.; Weller, D.D.; Deinzer, M.L. *Org. Mass Spectrom.*; submitted.

277. Hardegger, E.; Ott, H. *Helv. Chim. Acta*, **1955**, *38*, 312.

278. Hassner, A.; Alexanian, V. *Tetrhedron Lett.* **1978**, *22*, 4475.

279. Ogura, H; Kobayashi, T.; Shimizu, K.; Kawabe, K.; Takeda, K. *Tetrhedron Lett.* **1979**, *23*, 4745.

280. Ogura, H.; Sato, O.; Takeda, K. *Tetrhedron Lett.* **1978**, *22*, 4817.

281. Diago-Meseguer, J.; Palomo-Coll, A.L. *Synthesis* **1980**, 547.

282. Gosl, R.; Meuwsen, A. *Chemishe Berichte* **1959**, *92*, 2521.

APPENDIX

APPENDIX

Chemical Abstracts Names for Key Compounds

27 Poly(oxy-1,2-ethanediyll), .alpha.-[3,9,15,21,27,33-hexakis[(4-amino-2-oxo-1(2H)-pyrimidinyl)methyl]-34-hydroxy-32-methyl-1,6,11,16,21,26,31-heptaoxo-2,8,14,20,26,32,38-hepaazatetratriacont-1-yl]-, [3S-(3R*,9R*,15R*,21R*,27R*,33R*)]-

55 2(1H)-Pyrimidinone, 4-amino-1-[(6-oxo-2-pipridinyl)methyl]-, (S)-

56 Benzamide, N-[1,2-dihydro-2-oxo-1-[(6-oxo-2-pipridinyl)methyl]-4-pyrimidinyl]-, (S)-

58 1-Pipridinecarboxylic acid, 2-[[4-[benzoyl[(1,1-dimethylethoxy)carbonyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-6-oxo-, 1,1-dimethylethyl ester, (S)-

59 Ethanimidamide, N'-[1,2-dihydro-2-oxo-1-[(6-oxo-2-pipridinyl)methyl]-4-pyrimidinyl]-N,N-dimethyl-, (S)-

60a 1-Pyrrolidinecarboxylic acid, 2-[4-amino-2-oxo-1(2H)-pyrimidinyl)methyl]-5-oxo-, 1,1-dimethylethyl ester, (S)-

60b 1-Piperidinecarboxylic acid, 2-[4-amino-2-oxo-1(2H)-pyrimidinyl)methyl]-6-oxo-, 1,1-dimethylethyl ester, (S)-

64a 1-Pyrrolidinecarboxylic acid, 2-[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-5-oxo-, 1,1-dimethylethyl ester, (S)-

64b 1-Piperidinecarboxylic acid, 2-[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-6-oxo-, 1,1-dimethylethyl ester, (S)-

65a 1(2H)-Pyrimidinepentanoic acid, .gamma.-[[(1,1-dimethylethoxy)carbonyl]amino]-4-[[4-(1,1-dimethyl ethyl)benzoyl]amino]-2-oxo-, (S)-

65b 1(2H)-Pyrimidinehexanoic acid, .delta.-[[(1,1-dimethylethoxy)carbonyl]amino]-4-[[4-(1,1-dimethyl ethyl)benzoyl]amino]-2-oxo-, (S)-

72 Carbamic acid, [1-[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-5-oxo-5-(1-piperidinylamino)pentyl]-, 1,1-dimethylethyl ester, (S)-

74 Benzoic acid, 4-(1,2-dimethylethyl)-, 2-[[5-[[(1,1-dimethylethoxy)carbonyl]amino]-6-[4-[[4-(1,1-dimethylethyl)benzoyl]amino-2-oxo-1(2H)-pyrimidinyl]-1-oxohexyl]methylamino]ethyl ester, (S)-

75 Carbamic acid, [1-[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-5-[[1-[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-2-oxo-5-(1-piperidinylamino)pentyl]amino]-5-oxopentyl]-, 1,1-dimethylethyl ester, [S-(R*,R*)]-

76 Carbamic acid, [1-[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-5-[[1-[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-5-[[1-[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-5-oxo-5-(1-piperidinylamino)pentyl]amino]-5-oxopentyl]amino]-5-oxopentyl]-, 1,1-dimethylethyl ester, [S-[R*,[R*(R*)]]]-

77 Carbamic acid, [1-[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-4-[[1-[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-4-[[1-[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-4-oxo-4-(1-piperidinylamino)butyl]amino]-4-oxobutyl]amino]-4-oxobutyl]-, 1,1-dimethylethyl ester, [S-[R*,[R*(R*)]]]-

82 2-Oxa-5,11,17-triazaoctadecan-18-oic acid, 10,16-bis[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-1-[4-(1,1-dimethylethyl)phenyl]-5-methyl-1,6,12-trioxo-, 1,1-dimethylethyl ester, [S-(R*,R*)]-

83 2-Oxa-5,11,17,23-tetraazatetracosan-24-oic acid, 10,16,22-tris[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-1-[4-(1,1-diemthylethyl)phenyl]-5-methyl-1,6,12,18,tetraoxo-,1,1-dimethylethyl ester, [10S-(10R*,16R*,22R*)]-

86 2-Oxa-5,11,17,23,29,35,41-dotetracontan-42-oic acid, 10,16,22,28,34,40-hexakis[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-1-[4-(1,1-dimethylethyl)phenyl]-5-methyl-1,6,12,18,24,30,36-heptaoxo-, 1,1-dimethylethyl ester, [10S,(10R*,16R*,22R*,28R*,34R*,40R*)]-