AN ABSTRACT OF THE THESIS OF

Jeffrey S. Nelson for the degree of Master of Science in Chemistry presented on March 31, 1989

Title: Synthesis and Oligomerization of γ,4-Diamino-2-oxo-1(2H)-pyrimidinepentanoic Acid

Redacted for privacy

Abstract approved: _____
Dwight D. Weller

Described herein is the synthesis of an uncharged stereoregular oligonucleotide analog consisting of cytosine bases linked at specific intervals to a polyamide backbone derived from (L)-glutamic acid. Preparation of this analog entailed borane reduction of (L)-pyroglutamic acid to give optically pure lactam alcohol **9**. Tosylation and alkylation with cytosine provided alkylated lactam **10**. The use of amidine-type protecting groups on the amino group of **10** was necessary to allow selective t-butoxycarbonylation of the lactam, which serves to activate the lactam toward ring-opening. The amidine was selectively cleaved with hydrazine derivatives, the cytosine amine group acylated and the lactam ring opened with hydroxide to provide the key subunit **31**.

For the block synthesis of oligomers, two separate series of compounds were developed. In the oligomerization sequence, each series of compounds relied on the activation of monomeric free acid **31** by conversion to the p-nitrophenyl ester, **32**. The activated ester was reacted with 2-(methylamino)ethanol to provide **34**, containing a blocked acid terminus. This end-capping sequence, which provided one of the two series of compounds in the block synthetic pathway, served to prohibit a charged

carboxyl terminus on the completed polymer during biophysical binding studies. The free alcohol was further protected as the t-butylbenzoyl ester **35** which allowed easy chromatographic purification of coupling products. Reaction of **40**, formed by trifluoroacetic acid deprotection of **35**, with active ester **32**, gave the cap-protected dimer **41** in good yield. The same sequence of reactions was followed to provide the desired cap-protected trimer **43**.

Reaction of p-nitrophenyl ester **32** with 1-amino-piperidine led to C-terminal hydrazide monomer **33**, which served as a temporarily masked acid terminus, and which could also be easily purified by flash chromatography. This series was oligomerized in a fashion similar to the former series by reaction of **36** formed by trifluoroacetic acid deprotection of **33**, with active ester **32** to provide the hydrazide masked dimer **37**. This was elaborated to the trimer **39** in the same manner. The carboxyl group was unmasked with aqueous N-bromosuccinimide, converted to the p-nitrophenyl ester, and coupled with the cap-protected trimer free amine **44** to give fully protected hexamer **45**.

Synthesis and Oligomerization of γ,4-Diamino-2-oxo-1(2H)-pyrimidinepentanoic Acid

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Chairman of the Department of Chemistry

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Dean of Graduate School

Date thesis is presented March 31, 1989

Typed by Jeanne Reisner for Jeffrey S. Nelson

To Kelly:

Who contributed enormously to my happiness in the past few years

through her never failing love and friendship.

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INTRODUCTION

Interaction of complementary (anti-sense) oligonucleotides with a specific messenger RNA *in vivo* has been found to be a promising method for inhibiting messenger RNA translation, and thereby inhibiting protein synthesis.[1-3] This anti-sense inhibition of gene expression[4] provides a potential approach to the control of viral infections including the Rous sarcoma virus,[5,6] and HTLV-III/LAV (HIV),[7] the virus that causes AIDS (acquired immune deficiency syndrome).

Early experiments by Izant and Weintraub[8] showed the applicability of this RNA methodology in eukaryotic cells. They demonstrated that the expression of a thymidine kinase gene injected into mouse L cells, is significantly reduced by coinjection of a plasmid that directs the synthesis of anti-sense thymidine kinase RNA. This was similarly demonstrated in *Escherichia coli* by Inouye and coworkers,[9] who found that the expression of the outer membrane protein (OmpF) is normally inhibited by the presence of RNA complementary to OmpF mRNA. This led to the construction of inducible plasmids capable of selectively reducing bacterial gene expression, by directing the synthesis of specific anti-sense RNAs.[10]

Despite the reported inhibitory effects of complementary oligonucleotides toward viral synthesis,[5-7] two serious limitations exist in utilizing anti-sense oligonucleotides as antiviral agents. One problem is the susceptibility of the phosphodiester linkage in the anti-sense oligonucleotides toward degradation by nucleases,[11] which would undoubtedly be expected to reduce their potency and *in vivo* persistence as antiviral agents. The other seriously compromising feature of using oligonucleotides as anti-

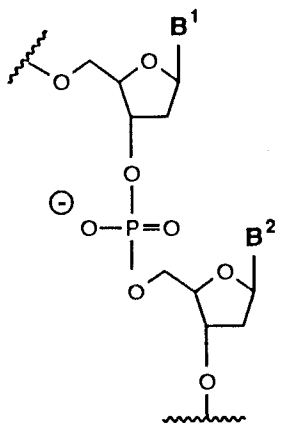
sense agents is their inability to penetrate cell membranes, due to their ionic charge.[12,13] In order to alleviate these potential problems, a number of research groups have developed sequence-specific nonionic nucleic acid analogues that contain an uncharged group in place of the negatively charged phosphodiester group normally found in oligonucleotides.[14,15] Such analogs have been found to bind complementary oligonucleotides in a sequence-specific manner, are relatively resistant to nucleases and can penetrate mammalian cells in culture with relative ease. These features render uncharged anti-sense RNA analogs an attractive alternative to anti-sense oligonucleotides for inhibiting viral infections.

The majority of nonionic oligonucleotide analogs developed to date are those containing the classical nucleosides joined by a modified neutral, phosphorus derived linkage. The most extensively studied derivatives in this class are undoubtedly the phosphotriesters[14] and the methanephosphonates (Figure 1).[15] Both of these phosphate analogs have been shown to exhibit duplex formation with complementary oligonucleotides.[16,17] Recently, oligomeric nucleosides possessing phosphoramidate linkages have been shown to bind to complementary DNA, but as yet these have not been tested as anti-sense agents.[18]

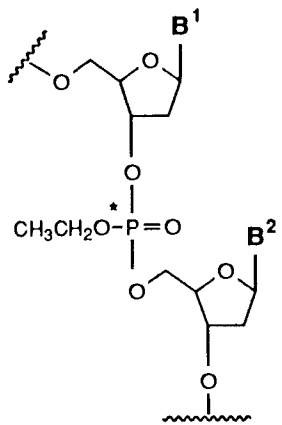
Miller and coworkers have extensively studied oligodeoxyribonucleotide ethyl phosphotriesters and have demonstrated that these analogs are capable of forming hydrogen-bonded complexes with complementary single-stranded regions of transfer RNA,[19] in addition to complementary homopolynucleotides. It has been further found that the complexes formed with the homopolymeric triesters have greater stability than those formed with the parent oligodeoxyribonucleotides, presumably due to the lack of charge repulsion normally present between the phosphate backbones of oligonucleotide duplexes.[19] Furthermore, by virtue of the stability of the triesters toward enzymatic hydrolysis,[14,19] their ability to penetrate cell membranes,[19] and their ability to mask

Figure 1

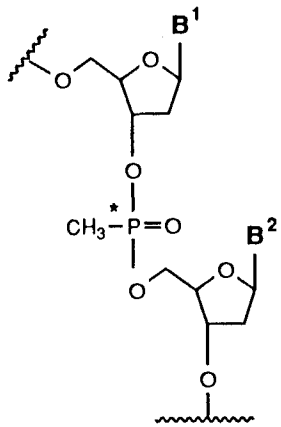
UNCHARGED ANTISENSE MESSENGERS



phosphate



phosphotriesters



methylphosphonate

Advantages- Membrane Permeable

; inhibits protein synthesis from target m-RNA (hemoglobin in erythrocytes)

Limitations- * Stereogenic Atom diastereomers have different affinity for target

single-stranded, exposed regions of complementary polynucleotides, Miller and coworkers have investigated the inhibitory effect of complex formation with oligodeoxyribonucleotide ethyl phosphotriesters on transfer ribonucleic acid amino--acylation.[20] A comprehensive study of the effects of the triesters on synthetase-catalyzed formation of phenylalanyl-tRNA^{Phe} and other aminoacyl-tRNAs, indicated that the triesters did inhibit amino-acylation of tRNA *in vitro*. Miller and coworkers concluded that the inhibition was a consequence of complex formation between the triester and its complementary region of the tRNA. Perhaps the most unattractive feature of phosphotriesters as potential antiviral agents recently discovered by Miller and coworkers, is their sensitivity to nonspecific esterases.[21] This feature further renders them susceptible to nuclease hydrolysis following esterase cleavage, and undoubtedly compromises their effectiveness as antiviral agents.

Miller and coworkers have also extensively investigated the interaction of methanephosphonate analogs with complementary polynucleotides. Substitution of a methyl residue for the nonesterified oxygen of the phosphate group represents the smallest possible structural change which can be made in the nucleic acid backbone which results in the removal of electrostatic charge while retaining the geometrical constraints and relative chemical stability of the phosphodiester linkage.[15] This modification was therefore expected to have a considerably smaller steric perturbation on the conformation of oligomers in solution and on the interaction with complementary polynucleotides, than the corresponding phosphotriesters. Nonionic oligonucleoside methanephosphonates also have the ability to form stable complexes with complementary polynucleotides, the ability to penetrate living cell membranes, and resistance to cellular nuclease hydrolysis.

Miller and coworkers synthesized the methanephosphonate oligomer complementary to the Shine-Dalgarno sequence (-A-C-C-U-C-C-U-) found at the 3' end of

bacterial 16S rRNA.[17] These nonionic oligonucleotide analogs were tested for their ability to inhibit the *in vitro* translation of mRNAs in cell-free systems of *Escherichia coli* and rabbit reticulocytes. In the reticulocyte system there was no significant effect on the translation of globin mRNA, but in the *E. coli* system, RNA-directed protein synthesis was found to be effectively inhibited. According to Miller et al., the specificity of the inhibitory action of the methanephosphonate analogs on protein synthesis strongly suggests that translation is inhibited as a consequence of duplex formation to the single-stranded target region, specifically the Shine-Dalgarno sequence of 16S rRNA. These analogs however, were found to have little or no effect on human cells.

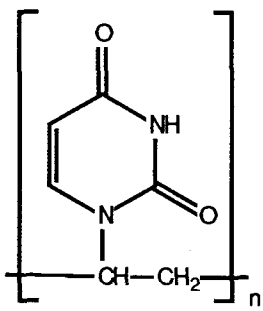
Miller and coworkers have recently found methanephosphonates to have a considerable antiviral effect. For example, oligomers complementary to the initiation codon regions of vesicular stomatitis virus mRNAs inhibit viral but not cellular protein synthesis in infected mouse L cells,[12] and they more recently have shown that methanephosphonate residues complementary to the acceptor splice junction of herpes simplex virus type 1 (HSV-1) cause a dose-dependent inhibition in virus replication.[22] Viral DNA synthesis was reduced 70-75% and there was a 90% reduction in viral protein synthesis, while the same concentration of oligo(deoxynucleoside methanephosphonates) was found to cause a minimal reduction (0-30%) in protein synthesis and growth rates (<40%) of uninfected cells. These results suggest such analogs may be potentially effective in antiviral chemotherapy.

Although the hybridization arrest experiments with the phosphotriesters and methanephosphonates demonstrate the attractiveness of uncharged oligonucleotide analogs in the control of genetic elements *in vivo*, these analogs all contain atactic (stereoisomerically random) backbones. An essential problem with polynucleotide analogs bearing stereoisomerically random backbones is that any given preparation results in a very large number of individual diastereomers, each of which may bind to

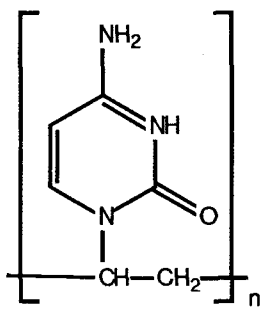
the targeted polynucleotide sequence with its own distinct binding constant. In order to be of considerable therapeutic value, the uncharged polynucleotide analog should exhibit a high degree of sequence specificity. It is therefore desirable that all of the molecules of the analog pair with their complementary target sequences to form duplexes possessing a uniform binding constant. To assure a homogeneous binding constant the analog should, therefore, have a stereochemically defined backbone. Uncharged nucleic acid analogs which replace the phosphorus atom with an achiral linkage have also appeared, as witnessed by the ability of oligo(deoxynucleoside carbamates) to bind complementary DNA and RNA.[23]

Hence, the focus of our research is to develop a class of uncharged polynucleotide analogs possessing a stereochemically defined backbone capable of sequence-specific pairing to complementary polynucleotides with a uniform binding constant. We are particularly interested in those analogs lacking both a phophorus linkage and the cyclic ribose sugar subunit. This so-called acyclic class of uncharged analogs thus far has not been rigorously investigated. Early work in this area was by Pitha and Pitha, who investigated poly(N1-vinyluracil) **1**, poly(N1-vinylcytosine) **2** and related species.[24] Poly(N1-vinyluracil) was prepared by radical polymerization of N1-vinyl-4-ethoxy-2-pyrimidinone **3** followed by acid hydrolysis of the resulting polymer.[25] This method was found to be superior to the direct polymerization of N1-vinyluracil, although this procedure also proved to be successful. Poly (N1-vinylcytosine) containing a small amount of uracil residues was similarly prepared by reaction of poly(N1-vinyl-4-ethoxy-2-pyrimidinone) **3** with ammonia.[26] The interaction of **1** with adenine derivatives and the interaction of **2** with polyguanylic acid was thoroughly investigated. While the observed binding interactions of poly(N1-vinyluracil) to adenosine derivatives were found to be weaker than analogous binding by poly U, the interaction of poly(N1-vinylcytosine) with poly G was found to form

complexes with high stability. The ability of these vinylogous analogs to form hydrogen-bonded complexes with complementary oligonucleotides is remarkable given the atactic nature of the backbone and the improper spacing of bases along the analog backbone. It is unlikely that heteropolymers, if they can be prepared, will show highly sequence specific binding, due to the probable lack of uniform structure in the analog-nucleic acid complex.



1



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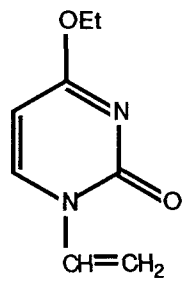
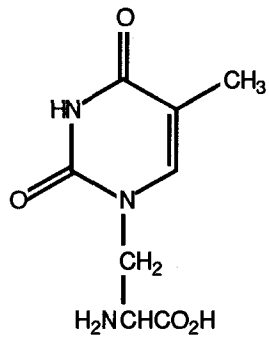


3

Jones and coworkers address the importance of obtaining a polypeptide of known stereochemistry in their uncharged, acyclic class of peptide analogs.[27] This is exemplified in the resolution of DL-ß-(thymin-1-yl)alanine **4**, prior to polymerization. However, the resulting optically active amino acid polymers showed no evidence of base stacking or of interaction with poly A. Jones and coworkers suggest that this may be attributable to the low average molecular weight of the poly-ß-(thymin-1-yl) alanines **5**, which in the optically pure D-series was approximately ten residues long. Note that due to the spacing of the bases on the backbone, only every other base is properly positioned for binding a complementary nucleic acid.

One feature common to all of the existing approaches for constructing uncharged, acyclic oligonucleotide analogs is that they rely on polymerization reactions. No examples in the literature to date have investigated a systematic approach toward planning and constructing these acyclic analogs. This is a key consideration if these

analogs are to have significant therapeutic utility, since the ultimate future objective is to develop an analog oligomer capable of sequence specific binding to a particular gene



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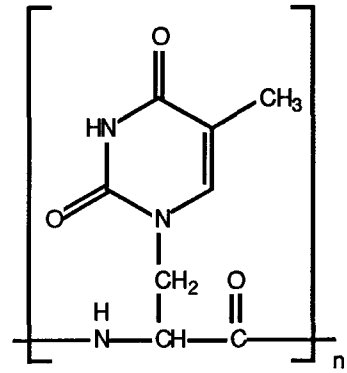


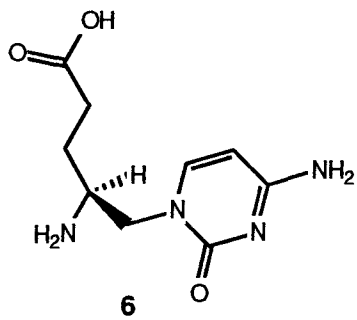
5

message or complementary viral sequence. Developing a method which would allow systematic construction of oligomers was therefore one of the central concerns in our research, and was a notable consideration in choosing the proper oligonucleotide analog backbone. We proposed that this requirement could be satisfied by utilizing a backbone whose subunits are linked by amide or related linkages, whereby standard coupling procedures and, ultimately, solid phase synthesis could be employed. The key questions to be answered were those pertaining to the necessary length of analog backbone subunits and the mode of attachment of heterocyclic bases along the polyamide backbone.

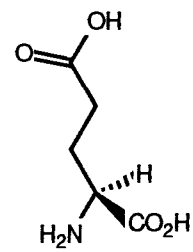
Preliminary studies were performed by Dr. Dwight Weller in collaboration with Dr. James Summerton of Antivirals, Inc. using space filling (CPK) models to analyze the association of acyclic analogs and complementary nucleic acids. Computer modeling has also been utilized since then, by Dr. Weller and Dr. Wilma Olson of Rutgers University. In brief, the modeling studies concluded that the bases should not be directly attached to the polyamide backbone. A methylene spacer between the base and the attachment site was found to alleviate unfavorable nonbonding interactions

between the base and the backbone, while significantly increasing the coplanarity of complementary bases in the putative analog/nucleic acid duplex. The optimum length of the monomeric subunit appears to be five or six atoms including the terminal amine and carboxyl group of each subunit.

The specific target of this work is the 5-atom nylon backbone represented by **6**. Modeling studies found several relatively low energy duplex conformations of this structure (as a hexamer) when paired with complementary DNA but binding to RNA is predicted to be less favorable. In designing a suitable agent for testing the binding abilities of oligomers containing this subunit, the obvious initial choice is a homopolymer. Cytosine (C) was chosen as the base due to its expected high avidity for complementary guanine (G) containing oligonucleotides, relative to the thymine:adenine (T:A) pair. This would allow binding to be demonstrated with relatively shorter oligomers thus minimizing synthetic work. Following successful binding of the homopolymer of C, heteropolymers containing A, T and G would be constructed to test whether the backbone promotes sequence specific binding to complementary oligonucletides.



6

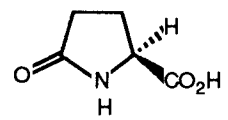


7, (L)-GLU

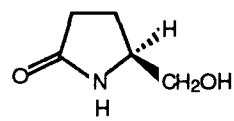
Upon retrosynthetic analysis of the target series, the desired subunit **6** bears a noticeable resemblance to (L)-glutamic acid **7**. Several advantages were foreseen in utilizing an amino acid as a synthetic precursor including availability, low cost, and optical purity. Even more striking was the applicability of (L)-pyroglutamic acid **8** as a

convenient starting material, since the free carboxyl could be reduced to the alcohol without requiring protection of either amine or γ-carboxyl terminus. We speculated that upon tosylation of alcohol **9**, the cytosine subunit could be easily attached by direct alkylation with cytosine and strong base to provide **10**.

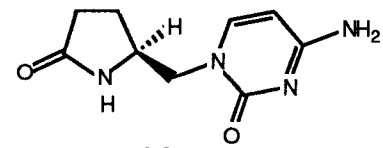
Following benzoylation of the cytosine amino group, the lactam could be activated toward ring-opening by attachment of an electron-withdrawing substituent. The obvious choice for activating the lactam ring for ring-opening was t-butoxycarbonyl (t-BOC) since Grieco and coworkers have previously demonstrated hydroxide-opening of these lactam derivatives.[28] The choice of t-BOC further serves an additional purpose since the amine terminus remains protected for subsequent oligomerization reactions, following ring-opening of fully protected lactam **11**. This would consequently result in a simple and direct route toward construction of the desired monomeric, free acid subunit **12**.



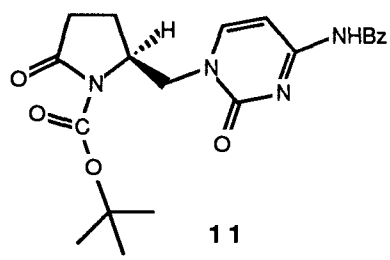
8, (L)-pyroglutamic acid



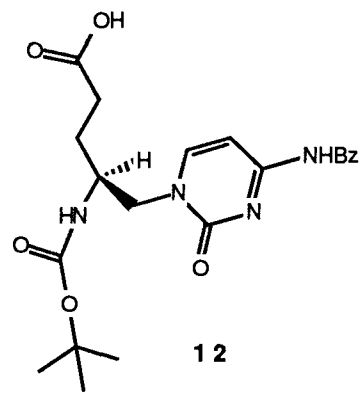
9



10



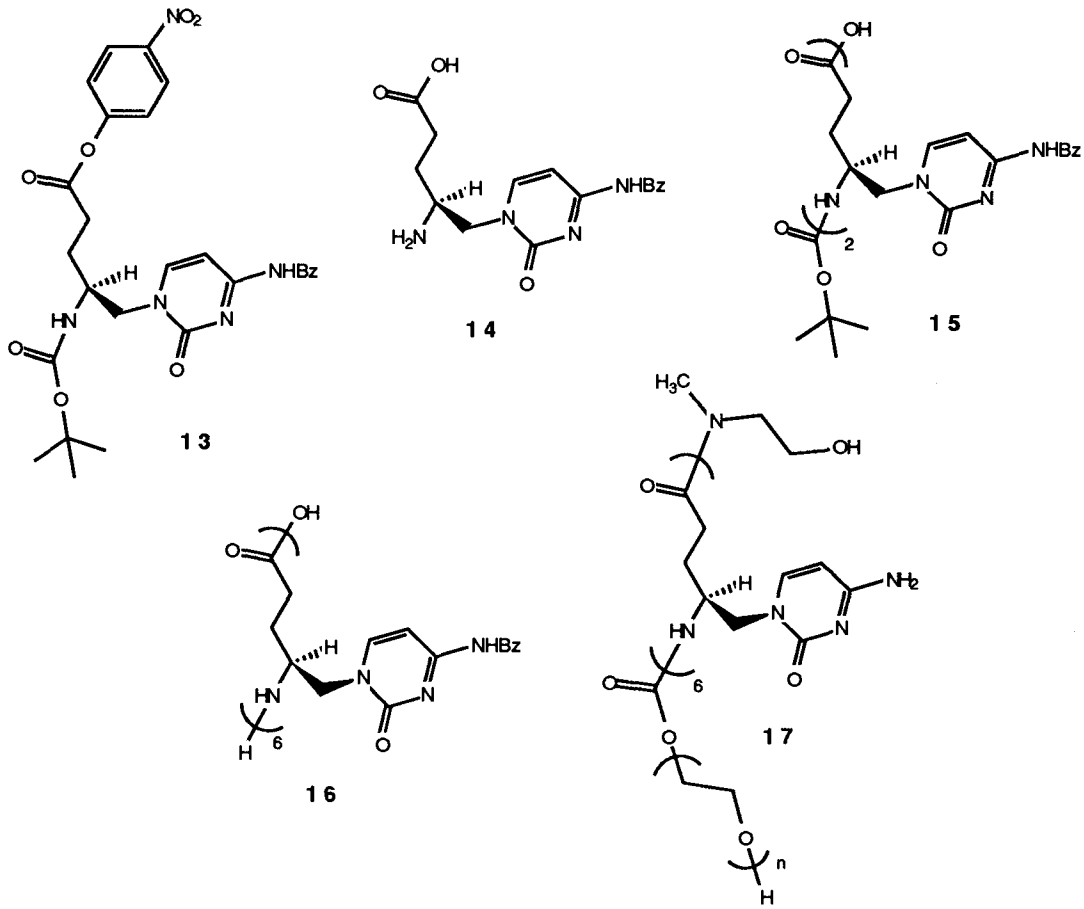
11



12

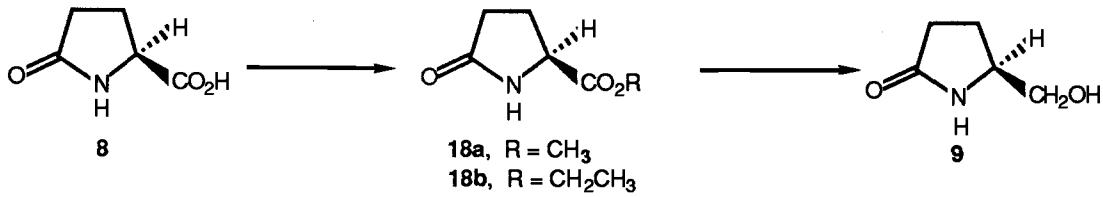
By utilizing standard peptide chemistry, we further speculated this subunit could be oligomerized via the p-nitrophenyl ester **13**, available in principle by reaction of **12**

with p-nitrophenol and dicyclohexylcarbodiimide (DCC). Activated ester **13** would then be coupled with the free amine subunit **14**, derived from t-BOC deprotection of **12** with trifluoroacetic acid, to provide the desired dimer free acid **15**. Similarly, the dimer free acid would be converted to its p-nitrophenyl ester and oligomerized to the trimer level in the same fashion. Utilizing block synthesis, the desired hexamer **16** would then be formed by linking two trimer fragments together, taking advantage of the same series of reactions. Following attachment of an end cap at the acid terminus and a water-solubilizing tail at the amine terminus, the protecting groups would be removed and the hexamer **17** assessed for its interaction with complementary G containing oligonucleotides.



RESULTS AND DISCUSSION

In order to insure the construction of a stereoregular backbone, the optical purity of the intermediates in the synthetic scheme must be established and maintained. The primary objective at the outset was to determine whether reduction of (L)-pyroglutamic acid **8** to (S)-(+)-5-(hydroxymethyl)-2-pyrrolidone **9** could be accomplished without accompanying racemization. The reduction of **8** has been previously demonstrated by several research groups.[29-31] The example of von Hardegger and Ott accomplished reduction of (L)-pyroglutamic acid methyl ester **18a** using lithium aluminum hydride.[29] This reaction proceeded in poor yield however, providing only 34% of the desired alcohol **9** after purification. No method for determination of optical purity was provided, although an optical rotation of +64° (c = 1.76 in ethanol) was reported. Twenty years later, von Faber and Wiegrebe investigated lithium borohydride reduction of **18a**.[30] While improving the yield of alcohol **9** to 60%, they concluded that lithium borohydride reduction was accompanied with a considerable degree of racemization (50% optical purity), as evidenced by an optical rotation of +32.4° (c = 5.0 in ethanol) compared to the +64° reported previously by von Hardegger and Ott.[30] This was confirmed by conversion of the lactam to the natural product (-)-Antofin which was 51% optically pure. In a 1980 paper, Silverman and Levy reported the lithium borohydride reduction of (L)-pyroglutamic acid ethyl ester **18b**.[31] The yield of (S)-(+)-(hydroxymethyl)-2-pyrrolidinone **9** by this route was better yet, providing 74% of the desired alcohol with a reported rotation of +29.0° (c = 5.0 in ethanol). These workers appear however to have been unaware of the rotation reported by von Hardegger and Ott, and the conclusion drawn by von Faber and Wiegrebe. The necessity of obtaining optically pure alcohol led us to investigate a new approach.

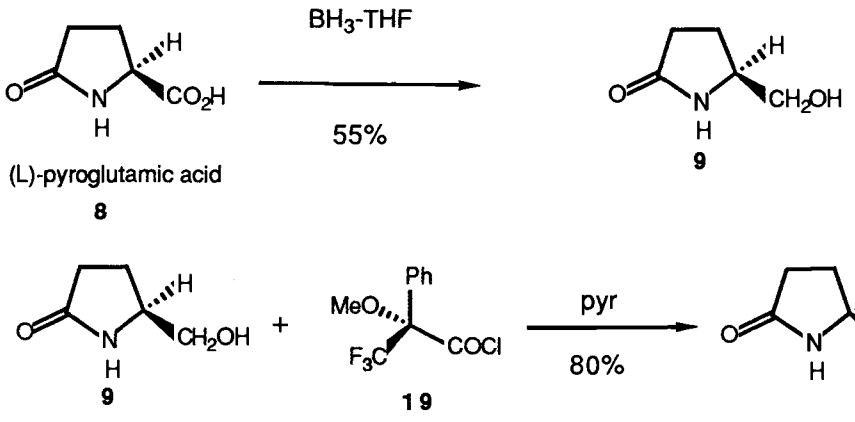


Synthesis of the desired optically pure alcohol was therefore attempted via borane reduction of (L)-pyroglutamic acid. The optical rotation of alcohol **9** derived from borane reduction was disappointing however and found to be +27.6° (c = 0.81 in ethanol) similar to the +29.0° reported by Silverman and Levy via lithium borohydride reduction of the ethyl ester. This was an unsettling discovery, since we expected that borane reduction of (L)-pyroglutamic acid should proceed stereoselectively. Since racemization could not be rationalized the need for an alternative method of optical purity determination became imperative.

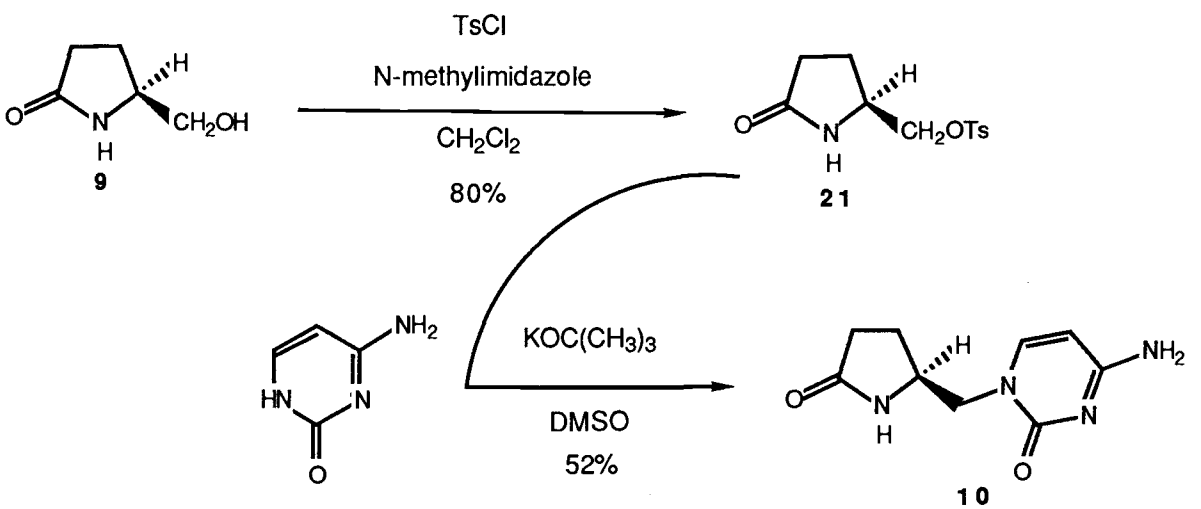
In a 1977 publication, Poindexter and Meyers compared various methods of amino acid reduction with regard to potential racemization.[32] They reported reduction of several optically pure amino acids and compared rotations of alcohols obtained via borane, borohydride, and lithium aluminum hydride reductions. Analysis of optical rotations of the alcohols suggested considerable racemization, although ^{19}F-NMR studies of the Mosher amide derivatives suggested that none of the reduction methods was accompanied by any significant loss of optical purity. Poindexter and Meyers suggest that discrepancies in optical rotations of the amino alcohols were due to trace impurities. This was a reassuring finding, and our attention was centered toward proving enantiomeric purity utilizing high resolution NMR studies.

Alcohol **9** obtained from borohydride reduction of **18b** and from borane reduction of **8** were each reacted with Mosher's reagent **19**,[33] to provide ester **20**. The borane reduction of (D)-pyroglutamic acid was also performed and derivatized as the corresponding ester. Products derived from both borane reductions of (D)- and (L)-

pyroglutamic acid were analyzed separately by 400 MHz NMR spectroscopy, and also investigated as the mixture. The high resolution proton spectra revealed an observable difference in chemical shift of the methylene protons adjacent to the ester functionality for each set of diastereomers. The limit of detection of optical purity using this methodology was determined, and we were able to easily detect a 99:1 mixture of alcohols (Figure 2). The spectra of diastereomeric esters derived from (S)-5-(hydroxymethyl)-2-pyrrolidinone (Figure 2a) and (R)-5-(hydroxymethyl)-2-pyrrolidinone (Figure 2b) are provided, as well as those obtained from 1:1 (Figure 2c) and 99:1 (Figure 2d) enantiomeric mixtures.[34] The chemical shifts of the methylene protons of interest were found to be too close to accurately integrate and were further found to be concentration dependent. Nevertheless, this method was sensitive enought to accurately detect a 99:1 enantiomeric mixture of alcohols. The spectra of diastereomeric esters derived from alcohol **9** obtained via borane and lithium borohydride reductions are also provided (Figure 3). No racemization was detectable in the diastereomer corresponding to borane reduction of naturally occurring (L)-pyroglutamic acid using this method of optical purity determination. However, lithium borohydride reduction of (L)-pyroglutamic acid using the conditions of Silverman and Levy resulted in a mixture of alcohols on the order of our limits of detection (98:2) as revealed in the high resolution proton spectrum of the diastereomeric ester. The ester derived from borane reduction of (D)-pyroglutamic acid was, however, a mixture of diastereomers (as also reported by Silverman and Levy), suggesting that the (D)-pyroglutamic acid (obtained from Fluka) is not optically pure (spectrum not shown). Regardless of this result, borane reduction of (L)-pyroglutamic acid was found to be stereoselective while borohydride reduction of the ester leads to significant racemization.[34]



Our next synthetic objective was to determine whether a pyrimidine base could be attached to the desired backbone directly, without requiring heterocyclic ring-construction. Tosylation of alcohol **9** as described in the literature using pyridine solvent proceeded poorly,[29,30] and instead alcohol activation was achieved by the use of N-methylimidazole in dichloromethane. Alkylation of lactam tosylate **21** proceeded in 40-70% overall yield using cytosine and potassium-t-butoxide in dry dimethylsulfoxide to provide lactam **10**. The alkylation product proved difficult to separate from excess cytosine and large scale alkylation reaction mixtures were carried on as the **10**/cytosine mixture as removal of excess cytosine proved to be more convenient following protection of the base nitrogen. Pure samples of alkylation product **10** were obtainable in small quantities by neutral alumina chromatography.



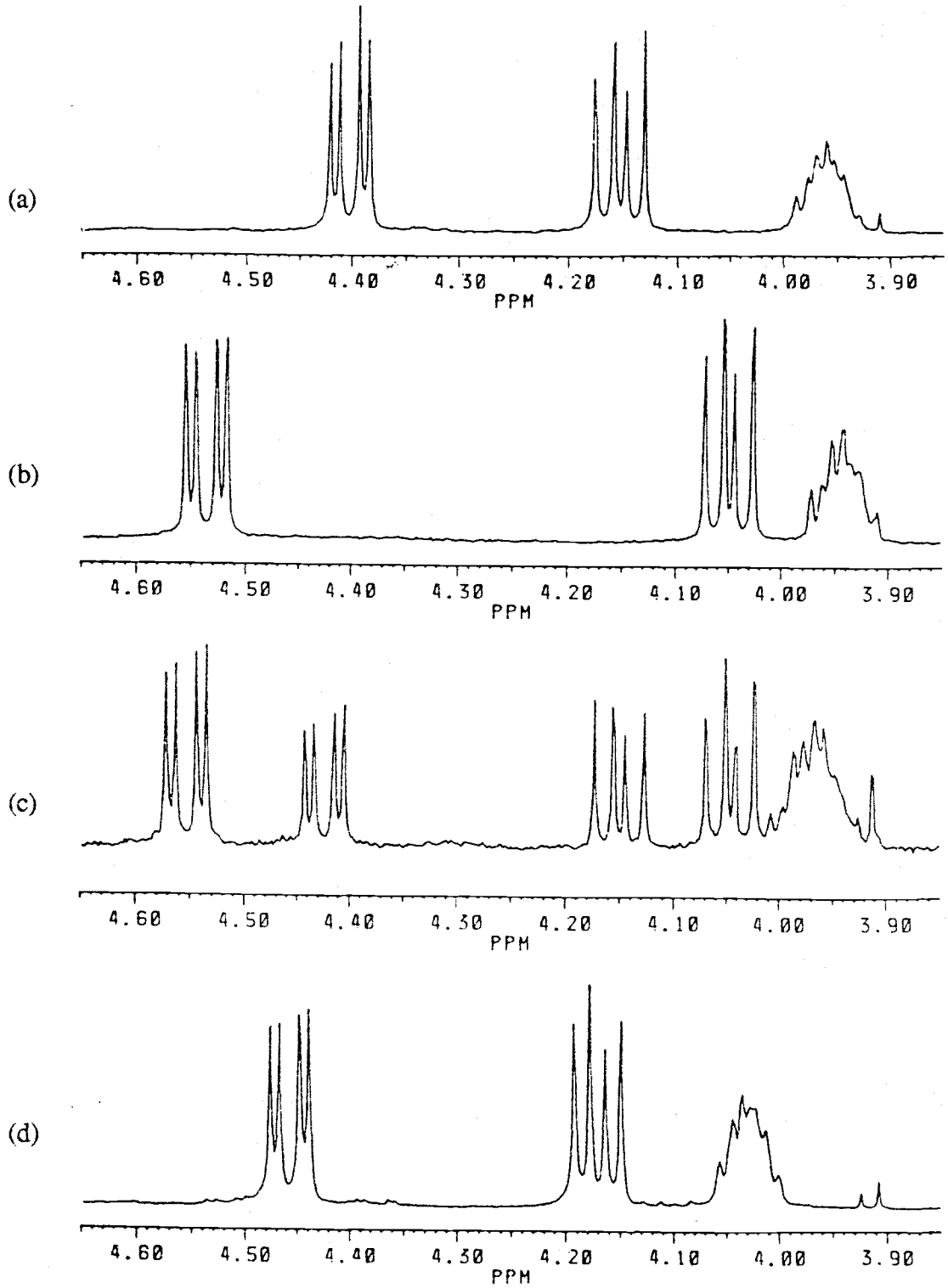


Figure 2. Moshers esters from: (a) pure (S)-**9**; (b) pure (R)-**9**; (c) 1:1 mixture of (S)-**9** and (R)-**9**; (d) 99:1 mixture of (S)-**9** and (R)-**9**.

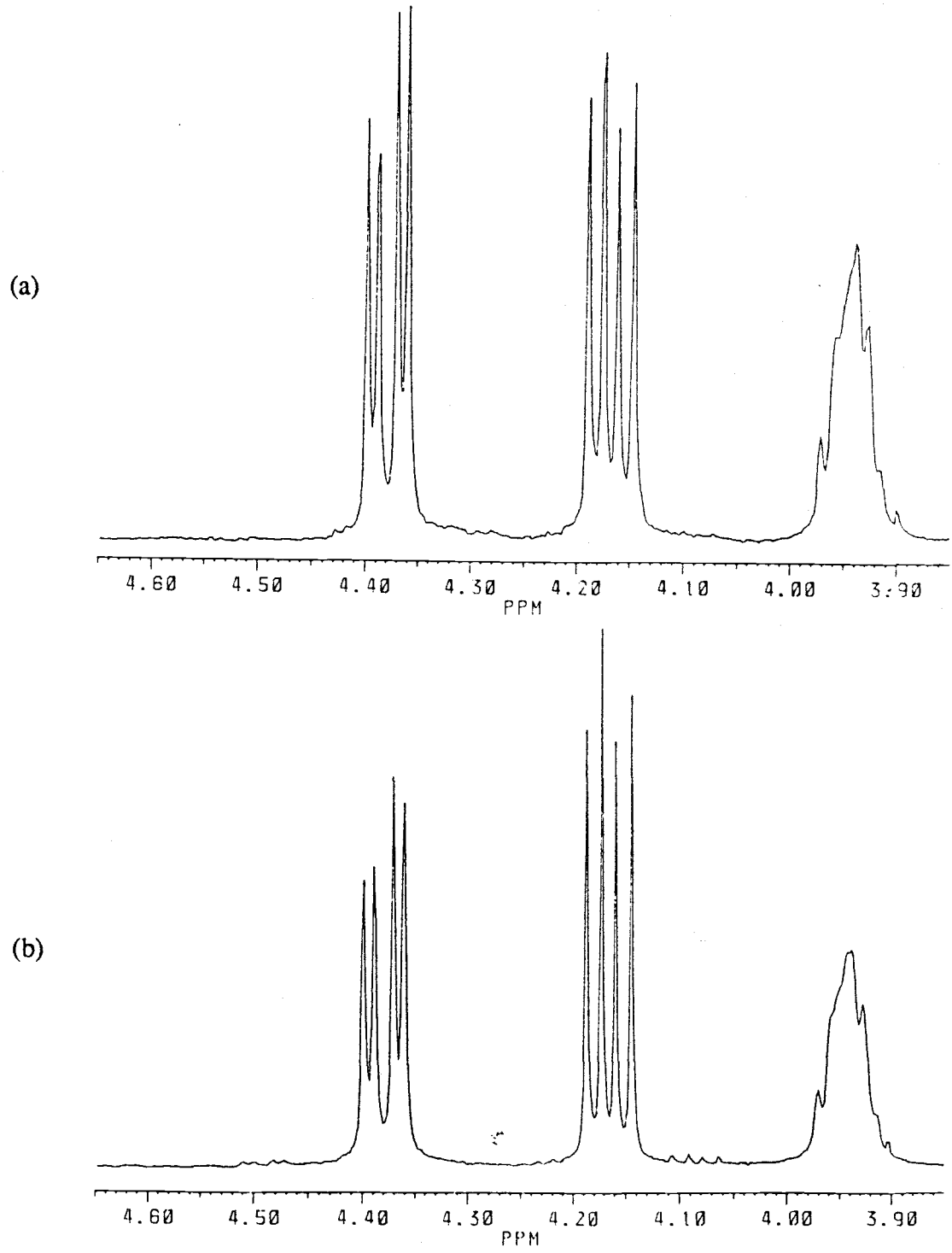
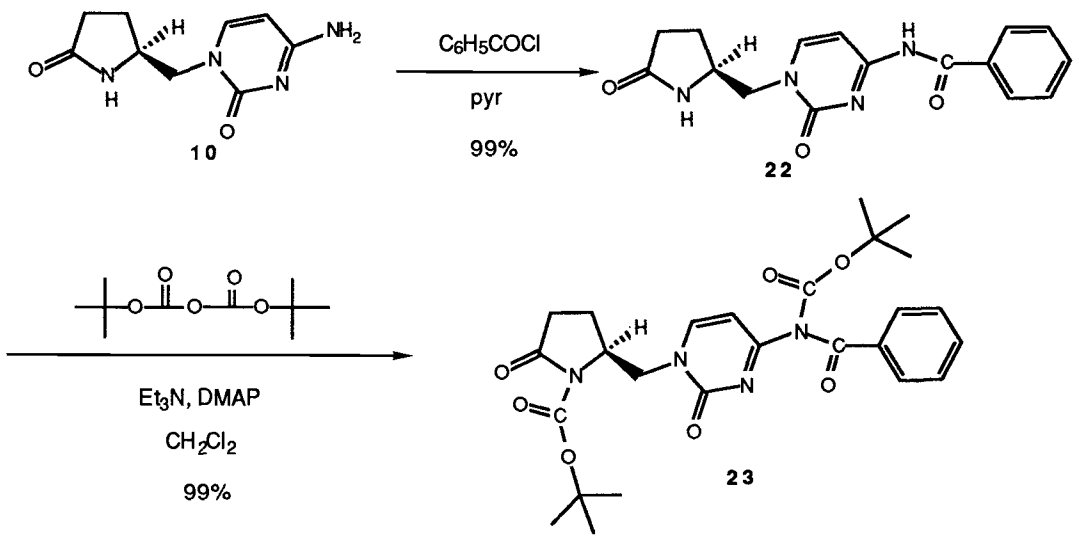


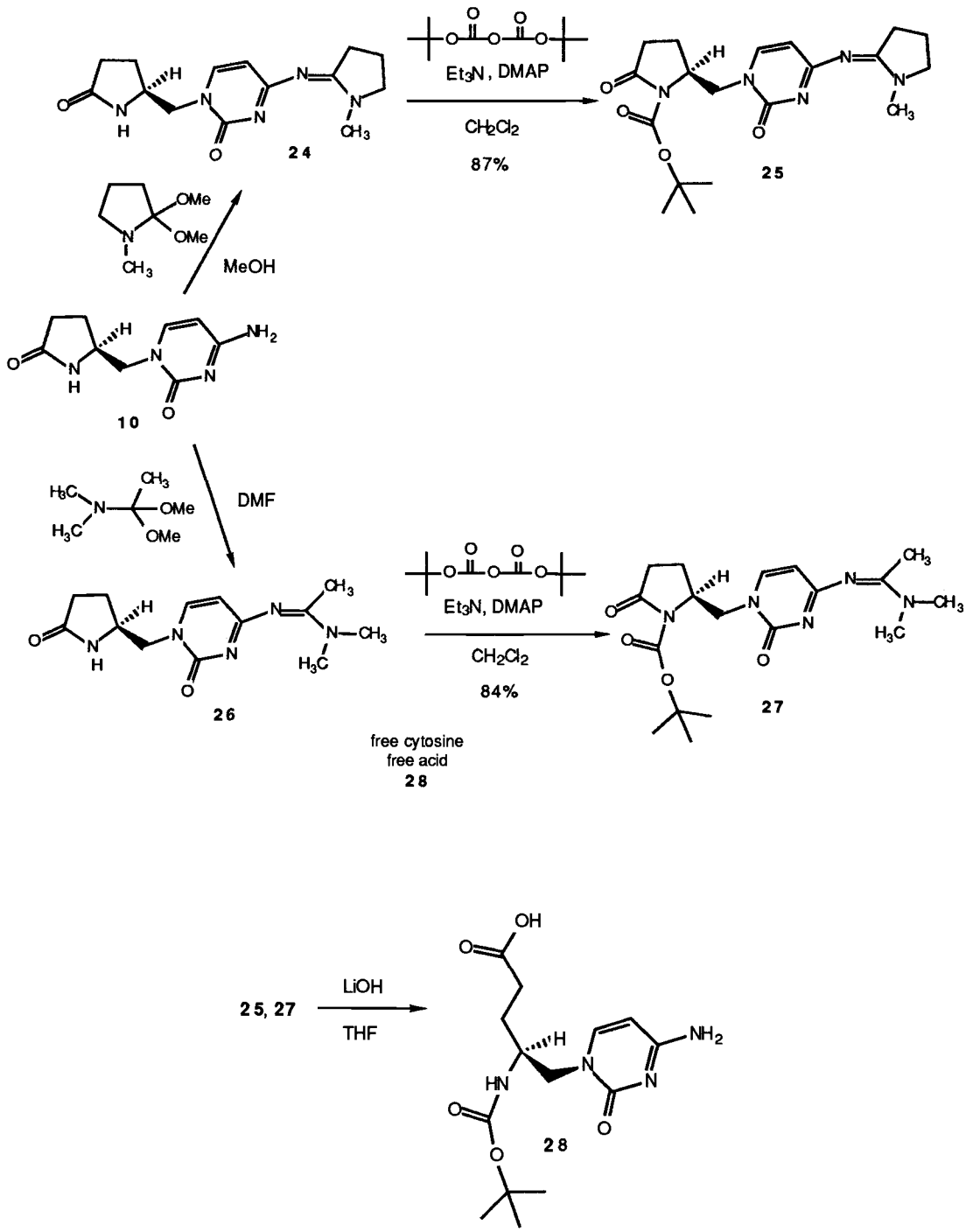
Figure 3. Moshers esters from: (a) reduction of pyroglutamic acid **8** with borane; (b) reduction of pyroglutamate ester **9** with lithium borohydride.

Having developed a direct route to cytosine adduct **10**, we now directed our attention to the hydroxide-opening of the lactam ring. Attachment of an electron-withdrawing substituent to the lactam nitrogen was required in order to activate the ring toward hydroxide-opening. The obvious choice of lactam activating group was t-butoxycarbonyl (t-BOC), since Grieco and coworkers have previously demonstrated ring-opening of lactams so activated in high yield using lithium hydroxide in tetrahydrofuran.[28] Alkylation product **10** was reacted with benzoyl chloride in pyridine to provide benzoylation product **22** in nearly quantitative yield. The choice of benzoyl protecting group on cytosine, we hypothesized, would allow selective t-butoxycarbonylation of the lactam nitrogen. The t-BOC group would be removed at each stage in the coupling sequence to unmask the backbone amine group while the benzoyl moiety would provide more permanent protection of the cytosine amino group. The benzoyl group would be removable at the final stages of the synthesis by ammonolysis in a manner analogous to its use in oligonucleotide synthesis. Unfortunately, reaction of **22** with di-t-butyl dicarbonate and base in dichloromethane led to acylation on both cytosine nitrogen and lactam ring, giving an unexpected and undesired product **23**, in 99% yield.



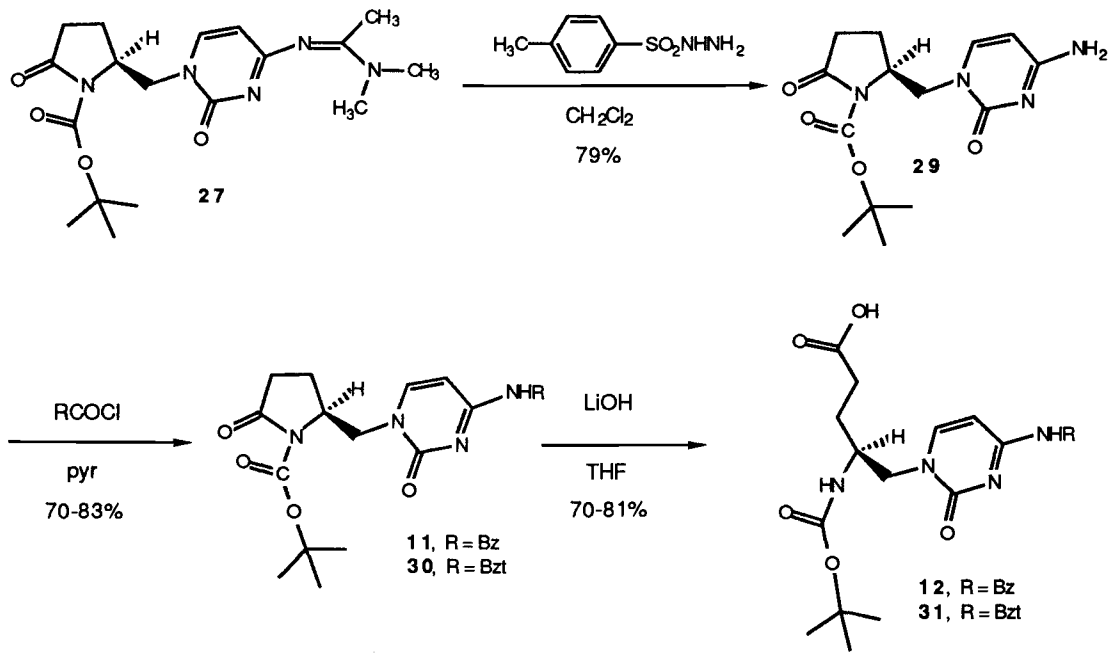
It became evident that a different protecting group needed to be attached to the cytosine moiety, which would allow selective t-BOC attachment to the lactam and which could furthermore, be easily detached at a convenient point in the synthesis. Caruthers and coworkers[35] have investigated various amidine groups for both pyrimidine and purine base protection. They found that for protection of cytosine, amidines derived from 1-methyl-2-pyrrolidinone proved to be the most convenient due to their stability to silica gel chromatography. Reaction of compound **10** with 1-methyl-2,2-dimethoxypyrrolidine[35] in methanol indeed yielded a chromatographically stable amidine, **24**, in nearly quantitative yield. This compound was most conveniently taken forward for t-BOC protection of the lactam ring where purification was found to be considerably easier. Acylation of lactam nitrogen proceeded smoothly by reacting the intermediate amidine with di-t-butyl dicarbonate, 4-(dimethylamino)pyridine, and triethylamine in dichloromethane to provide compound **25**. This compound was purified on silica, and the yield was 87% overall for the two reactions. However, we found 1-methyl-2,2-dimethoxypyrrolidine to be not only difficult to prepare but extremely sensitive to hydrolysis, and we set out to determine whether or not one of the commercially available alternatives suggested by Caruthers et al. could permit the same sequence of reactions. We therefore reacted compound **10** with N,N-dimethyl-acetamide dimethyl acetal in methanol or dimethylformamide, to provide the intermediate amidine **26**. Lactam derivatization as before resulted in 84% for the two steps following purification of **27** on silica.

Lithium hydroxide ring-opening of both amidine **25** and **27** resulted in cytosine deprotection, in addition to lactam-opening. Attempts to benzoylate the cytosine amino group of the unstable acid **28** were unsuccesful. It therefore became necessary to selectively deprotect the amidine prior to ring-opening, and reprotect cytosine with a protecting group capable of withstanding the ring-opening conditions. Selective



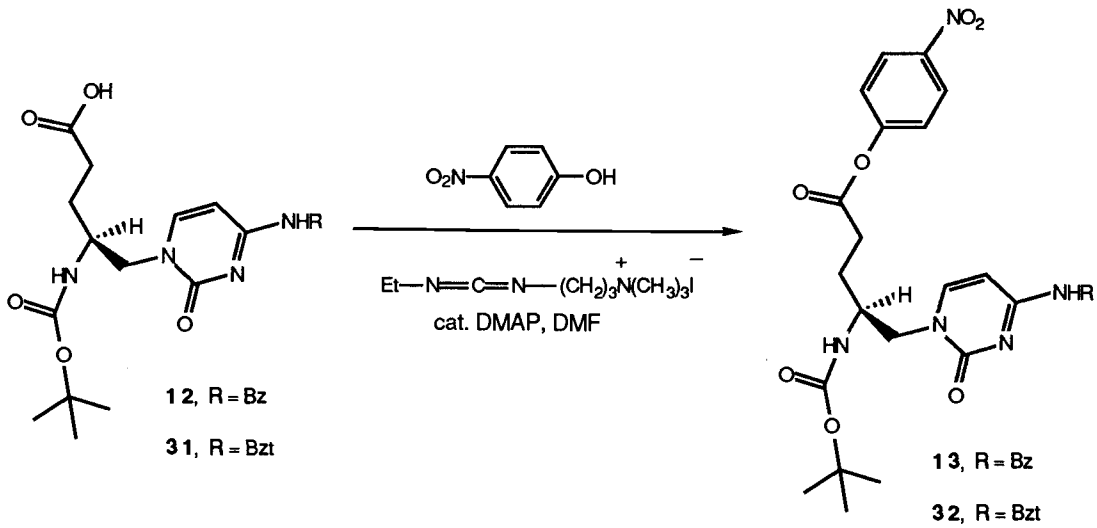
amidine deprotection[36] was accomplished with p-toluenesulfonhydrazide in dichloromethane to provide 79% purified yield of lactam-protected, free cytosine adduct **29**. Reprotection of cytosine with either benzoyl chloride or 4-t-butylbenzoyl chloride in pyridine resulted in greater than 80% purified yield of the desired amide **11** or **30**.

Ring-opening of both the benzoyl-protected cytosine adduct **11**, and 4-t-butylbenzoyl-protected cytosine adduct **30** proceeded smoothly at room temperature, using a 3-fold excess of lithium hydroxide in tetrahydrofuran. The desired free acid **31** was obtained in 81% yield after purification. The 4-t-butylbenzoyl derivative **31** displayed improved solubility and chromatographic properties as compared to the benzoyl protected adduct **12**. For this reason, and to aid in the purificaton of cytosine-protected oligomers, 4-t-butylbenzoyl chloride was the preferred reagent for reprotection of cytosine.



The optimum coupling procedures for formation of the intersubunit amide linkage employed the p-nitrophenyl ester.[37,38] Other activation methods were attempted including disuccinimidyl carbonate, carbonyl diimidazole, and bis-(oxazolidinyl)phosphorochloridate, but each of these methods proved to be unsuccessful. The p-nitrophenyl ester **32** was made by reacting carboxylic acid **31** with a carbodiimide, p-nitrophenol, and a catalytic amount of 4-(dimethylamino)pyridine in

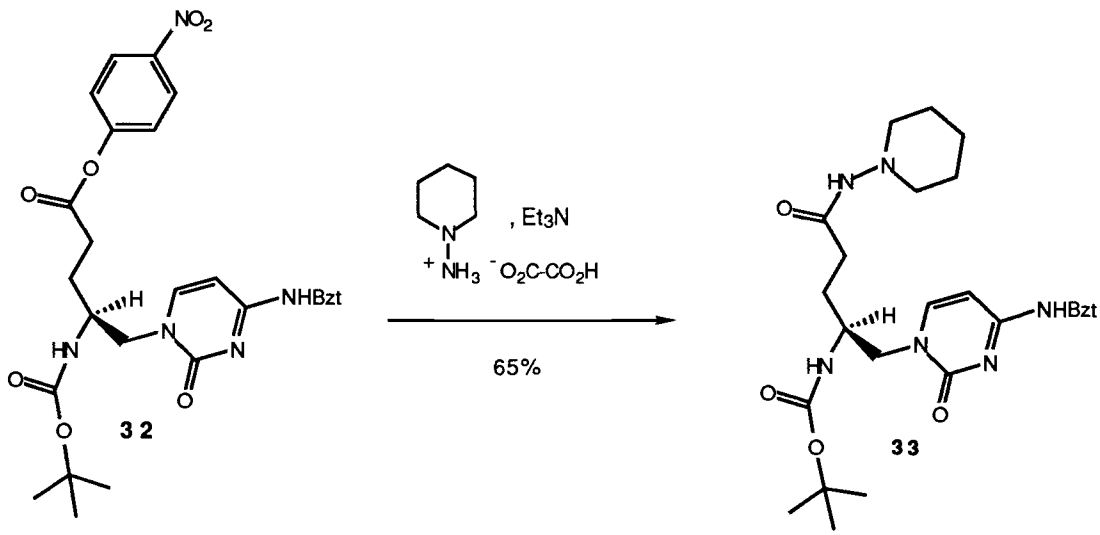
tetrahydrofuran or dimethylformamide. The latter was found to be the solvent of choice, as the active ester could be taken on directly to the coupling reaction without purification. While solubility of the active ester in tetrahydrofuran was not a problem, the solubility of the amine component in the coupling reaction was improved in dimethylformamide. Since complete removal of 1,3-dicyclohexylurea from the reaction product also proved to be difficult, we chose to use a water soluble carbodiimide (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide) in the p-nitrophenyl esterification. This allowed easy removal of diimide by-products in an aqueous extraction.



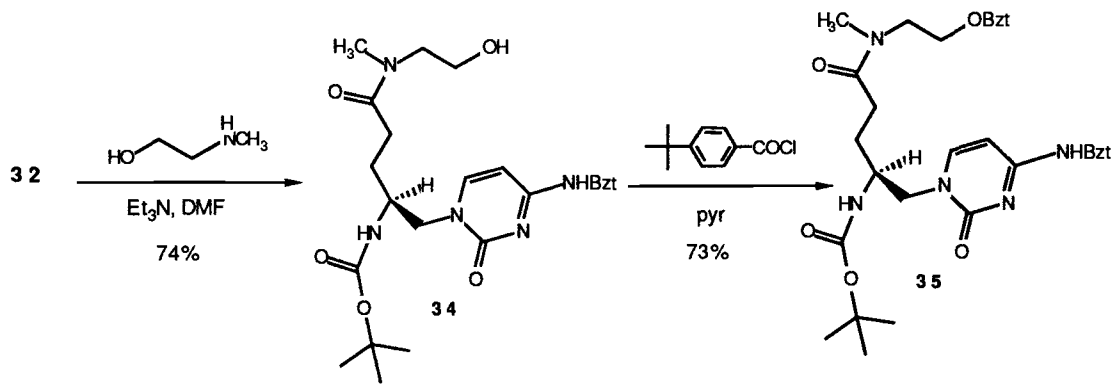
Amide formation was originally demonstrated by coupling the activated monomer with benzylamine. This model worked nicely, so we attempted the dimerization of the monomeric p-nitrophenyl ester and the cytosine-protected monomer free amine, following t-BOC deprotection of **31**. While this reaction appeared to be successful based upon thin layer chromatography, separation and purification of the dimer free acid proved very difficult, due to the high polarity of this compound. This necessitated the investigation of carboxylic acid protecting groups in order to improve the mobility of the coupling product on silica and increase the solubility of these

substrates in organic solvents. The protecting group had to withstand reaction with trifluoroacetic acid, be inert toward lactam formation with the free backbone amine, and be conveniently deprotected later in the synthetic sequence.

Protection of carboxylic acids as their hydrazides has been previously demonstrated,[39,40] and such groups are known to be cleaved by a variety of mild oxidative methods. We chose 1-aminopiperidine over both hydrazine and 1,1-dimethylhydrazine, due to the increased aliphatic character of the product. As obtained from Aldrich Chemicals, 1-aminopiperidine is contaminated by a considerable amount of piperidine. Coupling with the activated monomer led to a 1:1 mixture of the undesired amide obtained from piperidine coupling and the desired hydrazide protected monomer **33**. To avoid this problem, the 1-aminopiperidine was purified as its oxalic acid salt.[41] Reaction of 1-aminopiperidine oxalic acid salt and the activated monomer **32** in dimethylformamide provided **33** in 65% yield following flash chromatography. Attempts to remove the hydrazide with both lead tetraacetate and manganese dioxide proved unsuccessful, but reaction of **33** with N-bromosuccinimide and pyridine in 75% aqueous tetrahydrofuran, led to complete transformation to the free acid monomer **31**.

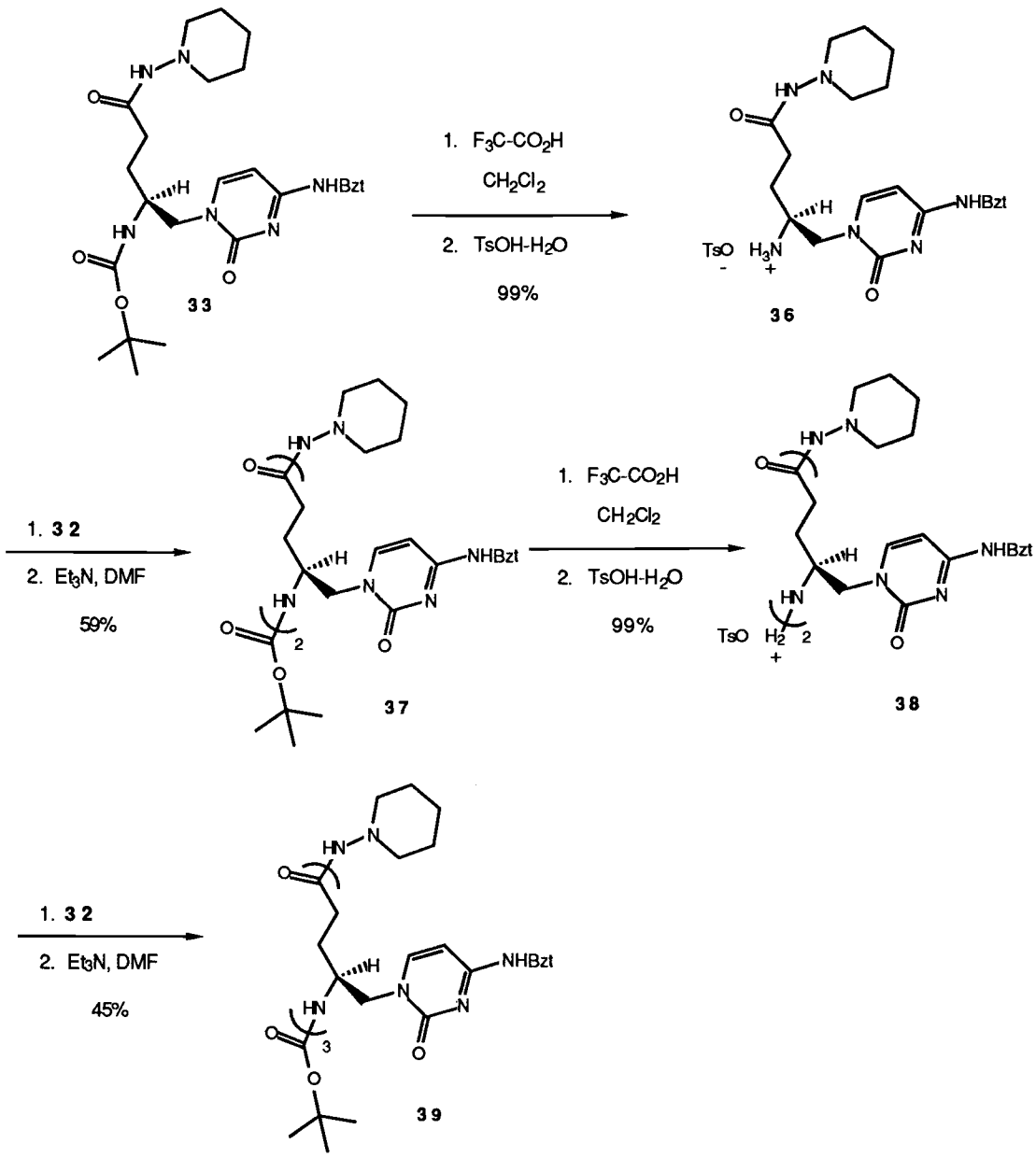


Our focus was now shifted toward attachment of a cap at the carboxyl terminus which, following oligomerization and deprotection, would serve to avoid a charged, free acid species. A secondary consideration was that the cap should aid in the water solubility of these substrates for the binding studies. On the other hand, during the oligomerization steps and the accompanying purification of the coupling products by silica gel chromatography, the cap must be in a relatively nonpolar form. The use of 2-(methylamino)ethanol satisfied these requirements. Reaction of **32** with 2-(methylamino)ethanol led to 74% of the desired product **34**. This alcohol was not routinely purified but was instead protected for use in oligomerizations as the t-butylbenzoyl ester by reaction with t-butylbenzoyl chloride in pyridine, to provide 73% of cap-protected monomer **35**.

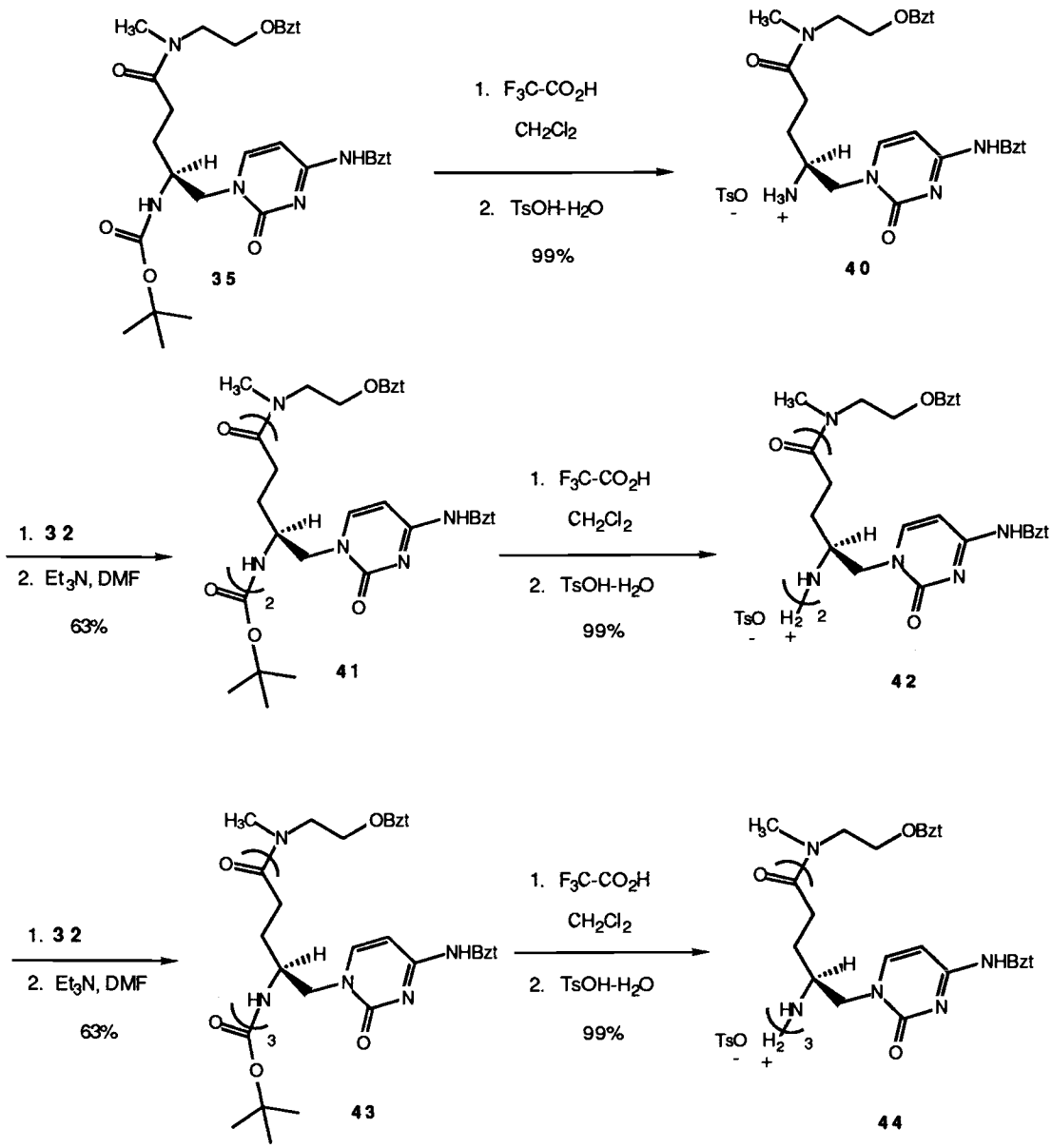


Our attention was now aimed at elongating both the hydrazide-protected series and the cap-protected series to their respective trimers. This we hoped, would allow a direct route toward construction of the desired hexamers by hydrazide trimer deprotection, activation, and coupling with the cap-protected trimer free amine. It would also allow ready synthesis of a nonamer should the hexamer fail to show binding to nucleic acid targets. Therefore, hydrazide monomer **33** was reacted with an excess of trifluoroacetic acid in dichloromethane at room temperature. Forty minutes later, an equivalent of p-toluenesulfonic acid was added to provide the hydrazide protected

monomer amine salt **36**. This served to allow complete removal of trifluoroacetic acid upon high vaccum evaporation and subsequent coevaporation with anhydrous dimethylformamide. This amine salt was reacted with activated monomer **32** to provide 59% yield of the hydrazide protected dimer **37** following flash chromatography. The same deprotection and coupling steps were repeated on the dimer to provide 45% yield of the purified hydrazide trimer **39**. In a similar fashion, the cap-protected dimer **41**

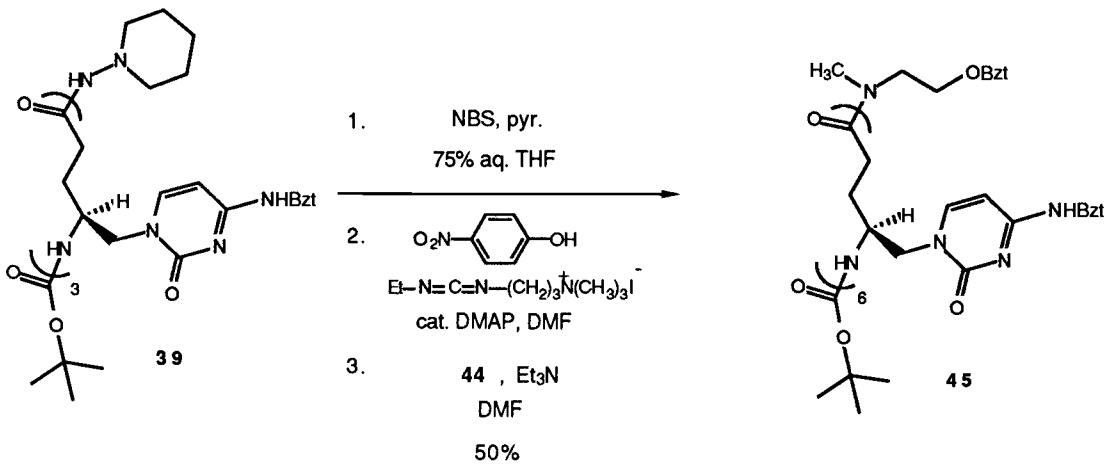


was made by reacting cap-protected monomer amine salt **40** and activated monomer **32**. The yield of **41** was 63% after purification, while the purified yield of cap-protected trimer **43** obtained through the same series of reactions, was 58%. Deprotection of the amine provided the amine salt **44**.



Hydrazide trimer **39** was then reacted with N-bromosuccinimide and pyridine in aqueous tetrahydrofuran to provide a quantitative amount of the trimer free acid, which

was taken on directly to the activation to be used in the hexamerization attempt. The acid was activated in the usual fashion with carbodiimide to give a trimer p-nitrophenyl ester. This was also not isolated but reacted directly with amine salt **44** to provide 50% purified yield of completely protected hexamer **45**.



The characterization of synthetic intermediates through the monomeric stage was quite straightforward. The high resolution nuclear magnetic resonance (NMR) and Fourier transform infrared (IR) spectra of both cap-protected monomer and hydrazide-protected monomer were found to be informative, but nonetheless, considerably complex. We further found that IR and NMR spectra of oligomers were in accord with the information obtained at the monomeric level, but were too complex to completely confirm the structure. We speculated that elemental analysis of these polar, high molecular weight species could potentially be a difficult task. The combination of these considerations forced us to rely considerably on the information obtained from both low and high resolution negative and positive ion fast atom bombardment (FAB) mass spectrometry (MS). Both high resolution negative and positive ion FAB MS provided confirmatory evidence for both the cap-protected series and the hydrazide-protected compounds. The high resolution mass spectral data for both series of oligomers is

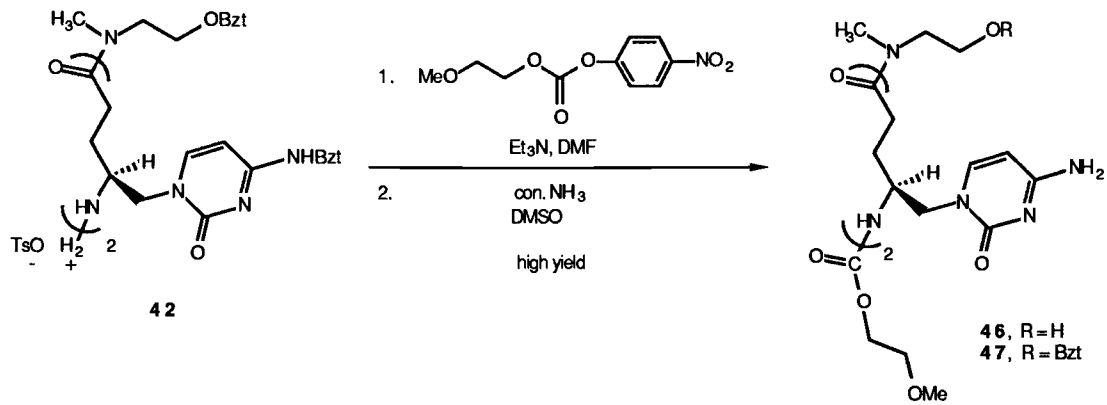
included below in Table 1. In addition, the low resolution mass spectral evidence obtained from negative and positive ion FAB was also considerably informative. A molecular ion peak was detectable at the monomer, dimer, trimer, and hexamer level. Fragmentation was minimal, but the most characteristic was a peak at 270 in the negative ion FAB or 272 in the positive ion FAB, corresponding to fragmentation along the backbone attachment site of the t-butylbenzoyl protected cytosine subunits. The relative intensity of this peak furthermore, grew with the increasing number of cytosine subunits, as would be expected. Mass spectrometry, therefore, became the key tool for confirming the structure of the highly complex oligomer analogs.

Table 1. High Resolution Mass Spectral Data of Oligomers

Compound #	FAB HRMS	m/z	Calculated	Observed
33	Negative	$(M-H)^-$	567.3295	567.3288
35	Positive	(MH^+)	704.4023	704.4009
37	Negative	$(M-H)^-$	935.5143	935.5179
41	Positive	(MH^+)	1072.6051	1072.5780
39	Positive	(MH^+)	1305.7147	1305.7005
43	Positive	(MH^+)	1440.7719	1440.7720
45	Negative	$(M-H)^-$	2543.3107	2543.3340

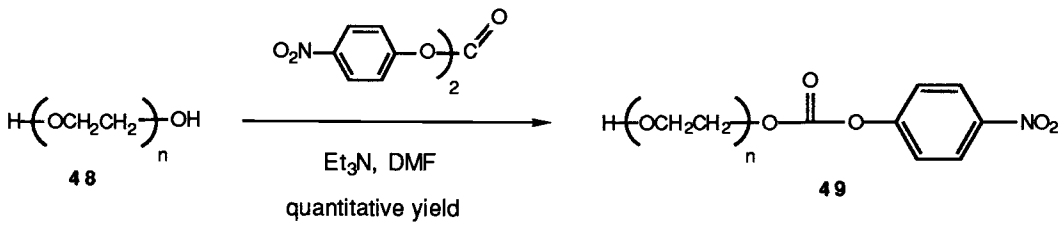
A method was still required however which could somehow provide information regarding the number of attached cytosine residues following removal of t-butylbenzoyl protecting groups as fully deprotected structures proved intractable to mass spectral analysis. Thus, the dimer amine **42** was converted into a carbamate derivative of 2-(methoxy)ethanol. The hypothesis was that following removal of protecting groups, the

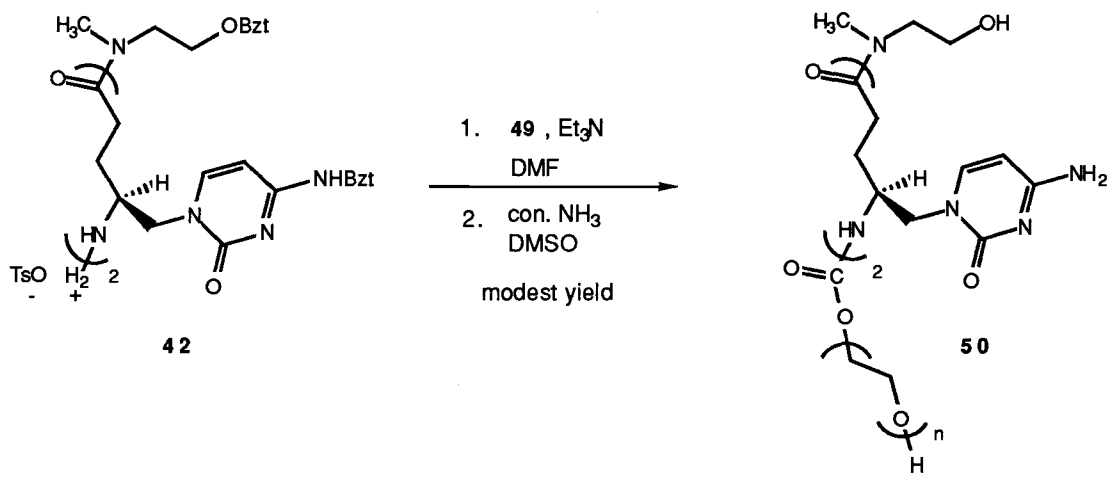
methylene protons on the carbon adjacent to the carbamate oxygen would display a clean triplet in the high resolution NMR spectrum, considerably different in chemical shift to the protons along the analog or polyethylene glycol backbones. Non-solubilized, completely deprotected, capped dimer **46** was synthesized by reacting the cap-protected dimer free amine with 2-(methoxy)ethyl p-nitrophenyl carbonate followed by t-butylbenzoyl deprotection with concentrated ammonia at 45 °C in a sealed vial for 24 hours. Two products were isolated from ion exchange chromatography, and one of those was the desired completely deprotected dimer **46**. The side product corresponded to the t-butylbenzoyl ester **47**. Apparently, the t-butylbenzoyl ester was cleaved more slowly than the t-butylbenzamide protecting groups on the cytosines. The undesired product could, however, be converted to the desired, non-solubilized and completely deprotected dimer by further reaction of **47** with concentrated ammonia in a sealed vial at elevated temperatures for an additional 72 hours. Compound **46** exhibited the predicted spectroscopic properties, rendering this an effective method for determining the relative number of cytosine residues attached, by comparing integrations in the high resolution proton spectrum.



The mixed carbonate **49** derived from polyethyleneglycol 1000 **48** (n ~ 23) and bis-p-nitrophenyl carbonate was synthesized, to provide a method of attaching a water

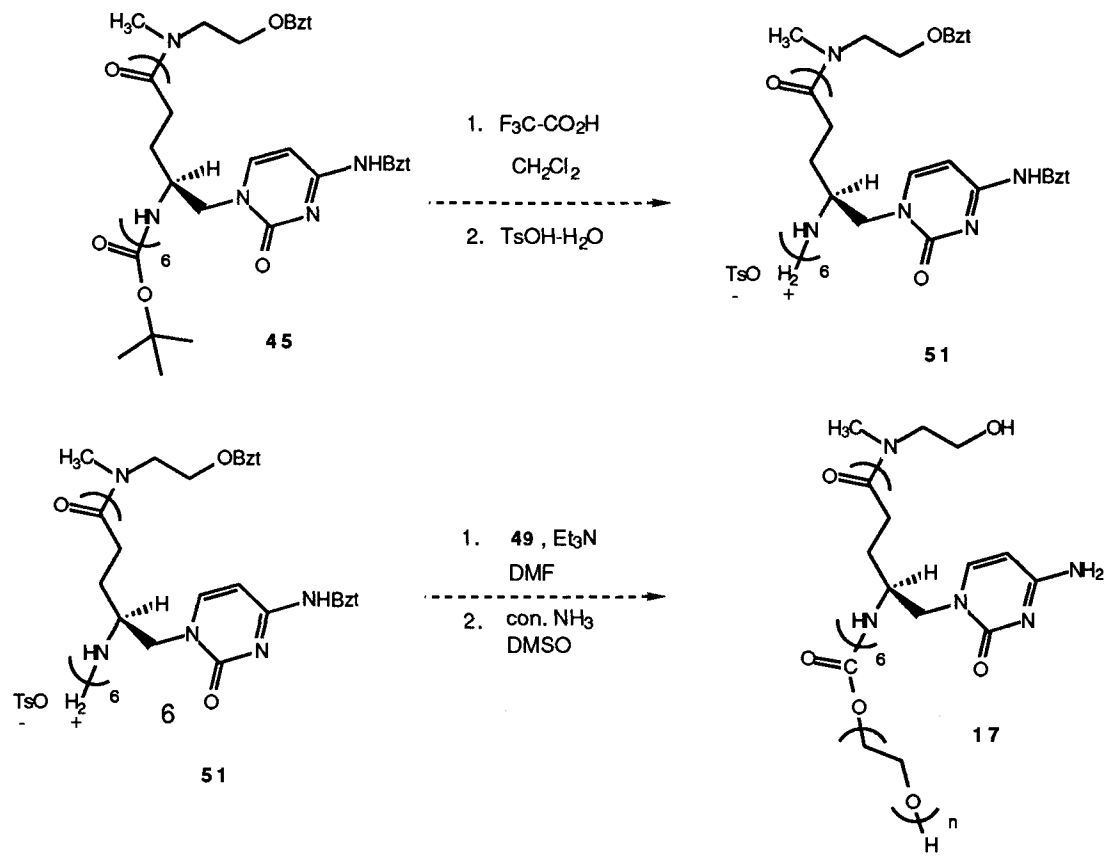
solubilizing tail to the amine terminus of the oligonucleotide analogs. This too, was demonstrated at the dimer level by reacting the cap-protected dimer amine salt **42** with **49**. The resulting solubilized, t-butylbenzoyl protected dimer was reacted directly with concentrated ammonia in a sealed vial at elevated temperatures for 72 hours, to provide a nearly quantitative yield of **50** following ion exchange chromatograhy. The high resolution NMR spectrum of this compound was less conclusive than in the non-solubilized series but exhibited nonetheless, the predicted spectroscopic properties necessary to determine the number of cytosine residues attached, following removal of t-butylbenzoyl protecting groups.





Following the success of these model systems, research attention will now focus toward attaching the solubilizing tail to the hexamer, which upon protecting group removal, will provide a product suitable for biophysical studies. This shall be achieved

by the standard method of t-BOC deprotection of hexamer **45**, and reaction of the resulting amine salt **51** with the solubilizing, mixed-carbonate **49**. The t-butylbenzoyl protecting groups in the hexameric solubilized series will similarly be cleaved by reaction with concentrated ammonia in a sealed vial at elevated temperatures for 72 hours. The resulting solubilized hexamer **17** will be purified utilizing ion exchange chromatography and can be characterized by a variety of methods. Presumably, integration of the aromatic protons in the high resolution proton spectrum will account for all six cytosine subunits. However, assuming the methylene group adjacent to the carbamate linkage along the polyethylene glycol chain does not exhibit the anticipated clean triplet, alternative methods need to be investigated to confirm the subunit length. We anticipate the COSY spectrum will adequately confirm the hexamer structure, but if this information proves inconclusive, the method of molecular weight determination



used by Jones and coworkers[27] whereby a colored cap (2,4-dinitrobenzene group) is attached to the amine terminus and the ratio of colored cap and cytosines determined spectrophotometrically may be employed. The solubilized hexamer **17** will then be assessed for its ability to bind the complementary hexamer of deoxyribonucleic acid containing only guanosine bases (hexa dG) by measuring UV absorbance versus temperature and by photometric mixing curves. Similar studies will be performed on poly(guanylic) acid.

EXPERIMENTAL

Proton (^1H) nuclear magnetic resonance spectra (NMR) were recorded on an AM-400 Bruker spectrophotometer. Chemical shifts (δ) are reported as parts per million downfield from tetramethylsilane as internal standard. Infrared spectra (IR) were recorded on Perkin-Elmer model 727B or Nicolet 5DXB FT-IR spectrometers. Melting points were determined on a Buchi capillary melting point apparatus and are uncorrected. Dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), methylene chloride (CH_2Cl_2), and pyridine were distilled from powdered calcium hydride and stored over 3Å molecular sieves. Tetrahydrofuran (THF) and ether (Et_2O) were distilled immediately prior to use from sodium/benzophenone. Flash column chromatography on silica was performed using E. Merck Silica Gel 60 with particle size 0.040-0.063 mm (340-400 mesh ASTM).[42] Chromatographic solvents ($CHCl_3$, MeOH) were distilled before use. Standard procedure for medium pressure ion-exchange chromatography involved dissolving the crude product (up to 20 mg) in 50 mL of (pH 2.0) phosphoric acid solution and applying to a column of Pharmacia sulfopropyl Sepharose cation exchange resin (45-165 μm). The column was then eluted with 1000 mL of potassium chloride solution (0.0 - 1.0 M gradient). Retention times were not completely reproducible since the gradient was approximate. For desalting, the column was washed first with (pH 2.0) phosphoric acid solution and then with water. Product fractions were diluted with an equal volume of water, reapplied to the cation exchange column, and washed with 150 mL of H_2O. The column was then eluted with 2:1 aqueous NH_4OH (pH 11.0) / acetonitrile solution with the desired product coming off soon thereafter. The combined product fractions were then evaporated to dryness to provide the desired compound. This generally was found to still contain an appreciable amount of salt, but could be removed by repeating the

desalting procedure or dissolving the product in 2,2,2-trifluoroethanol and filtering the undissolved salt. Reactions were run under a positive nitrogen or argon pressure, with the exception of those performed in aqueous media. An appendix containing Chemical Abstracts structural nomenclature is also provided.

(S)-(+)-5-(hydroxymethyl)-2-pyrrolidinone (**9**). A solution of BH_3·THF (23 mL, 23 mmol) in a large 3-neck flask equipped with an external stirrer, was immersed in an ice bath. 2-Pyrrolidone-5-carboxylic acid (1.0 g, 7.7 mmol) was mixed with 10 mL of anhydrous THF and this suspension was added slowly over 15 min to the cold BH_3·THF solution using an addition funnel. This suspension was occasionally stirred with a long syringe through the septum on the addition funnel to prevent the stopcock from clogging. Upon addition of the acid, violent gas evolution was observed. The reaction mixture was allowed to stir for 30 min at 0 °C and an additional 60 min at 25 °C. The reaction mixture was immersed again in an ice bath and 30 min later, quenched with addition of 30 mL of anhydrous methanol. Vigorous gas evolution was again observed, and all solids dissolved upon addition of methanol. The reaction mixture was evaporated under reduced pressure, the residual oil taken up three times in dry methanol and evaporated in order to remove trimethyl borate. The residual oil was dissolved in methanol and stirred with Dowex-50 cation exchange resin to remove over-reduced material. After filtration methanol was removed under reduced pressure. The remaining residual oil was purified by Kugelrohr distillation (158 °C, 1 mm Hg) to give 48.8 mg of a clear oil, which solidified on cooling (55% after purification). Physical properties and spectroscopic data of **9** were identical to those of a sample prepared by the method of Silverman and Levy,[31] with the exception of optical rotation, which was found to be +27.6 (c=1.7, ethanol).

(S)-(+)-5-methyl-[α-methoxy-α-(trifluoromethyl)phenyl acetate]-2-pyrrolidinone (**20**). Compound **9** (73.5 mg, 0.64 mmol), obtained from borane reduction, was dissolved in 260 μL of dry pyridine. (+)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride[33] (200 μL 1.0 mmol) was added dropwise, and the reaction mixture allowed to stir overnight. Upon addition of the acid chloride, a brown precipitate formed nearly instantaneously and the solution turned yellow in appearance. After 8 h, the reddish-brown solution was quenched by the addition of CH_2Cl_2 (5 mL) and H_2O (1 mL). The organic layer was then extracted three times with 2 mL portions of 1% $NaHCO_3$, once with brine, and dried over Na_2SO_4. The CH_2Cl_2 was removed *in vacuo* and the residue dried on high vacuum.

^1H NMR ($CDCl_3$) δ 1.74-1.84 (1H, m), 2.18-2.31 (3H, m), 3.53 (3H, s), 3.90-3.97 (1H, m), 4.14-4.19 (1H, dd, J=11.1, 5.9 Hz), 4.36-4.40 (1H, dd, J=11.4, 4.1 Hz), 6.50-6.66 (1H, bs), 7.33-7.44 (3H, m), 7.48-7.51 (2H, m).

IR (NaCl) cm^{-1} 3230 bd, 3050, 2980, 2870, 1760, 1710, 1500, 1460, 1430, 1280, 1180, 1120, 1020.

Preparation of the Mosher's ester derivative of the alcohol obtained via lithium borohydride reduction by the method of Silverman and Levy,[31] followed the same procedure. The NMR and IR spectra of this ester were identical to those reported above except this derivative was found to be contaminated with 5% of the other diastereomer as evidenced by additional peaks in the ^1H NMR spectrum.

^1H NMR ($CDCl_3$) δ 4.06-4.11 (dd, J=11.1, 6.0 Hz), 4.47-4.51 (dd, J=11.3, 4.0 Hz).

(S)-(+)-5-(tosyloxy methyl)-2-pyrrolidinone (**21**). Tosyl chloride (5.20 g, 27.2 mmol) was dissolved in 20 mL of dry CH_2Cl_2. In a different flask, 3.20 g (27.8 mmol) of alcohol **9** was suspended in 20 mL of dry CH_2Cl_2, 2.43 mL (30.5 mmol) of

N-methylimidazole was added, and the mixture cooled to 0 °C. Following dropwise addition of the tosyl chloride solution to this cold suspension, the ice bath was removed and the reaction mixture was allowed to stir at 25 °C for 18 h. H_2O (5 mL) was then added, followed by an additional 100 mL of CH_2Cl_2. The organic layer was extracted three times with 30 mL portions of 0.5 M HCl followed by three extractions with 30 mL portions of 2% $NaHCO_3$. The organic layer was dried over Na_2SO_4 and evaporated to dryness to provide 5.86 g (80% yield) of tosylate **21**, which was recrystallized in ethanol. Physical properties and spectroscopic data of **21** were identical to those reported by von Hardegger and Ott.[29]

Cytosine adduct (**10**). Cytosine (2.13 g, 19.2 mmol) and 3.44 g (12.8 mmol) of tosylate **20** were dried separately by coevaporation with anhydrous DMF. Potassium-t-butoxide (2.0 g, 20.0 mL of 0.1 g/mL of t-butoxide/DMSO solution) was added by cannulation to the round bottom flask containing the predried cytosine and swirled to promote solution. The reaction mixture was allowed to stir for 18 h at 25 °C. The homogeneous yellow solution was quenched by addition of 1 mL of acetic acid in 135 mL of 20% MeOH/$CHCl_3$ to give a milky white suspension. The suspension was filtered and the precipitate was found to contain predominantly unreacted cytosine and the potassium salt of p-toluene sulfonic acid, with only small amounts of product **10**. The filtrate was evaporated *in vacuo* and the residue dried on high vacuum. Pure samples of alkylation product were obtained on a small scale by neutral alumina chromatography (1:1 - 3:1 MeOH/$CHCl_3$), and recrystallization in MeOH containing a very small amount of water to provide a white, crystalline solid, mp 298 °C (d). Overall yield of purified **10** was 1.38 g (52%) with an additional 5-10% generally being recoverable from the filtered solids.

^{1}H NMR (d_6 DMSO) δ 1.67-1.76 (1H, m), 1.96-2.17 (3H, m), 3.53-3.57 (1H, dd, J=13.0, 5.6 Hz), 3.73-3.78 (1H, dd, J=13.0, 6.0 Hz), 3.80-3.86 (1H, m), 5.62-5.63 (1H, d, J=7.1 Hz), 6.98-7.07 (1H, bd, J=36.8 Hz), 7.50-7.52 (1H, d, J=7.2 Hz).

IR (KBr pellet) cm-1 3376, 3101, 1702, 1677, 1635, 1622, 1520, 1500, 1277, 1262.

MS (Negative FAB HRMS) m/z, $(M-H)^-$ calculated 207.0882, observed 207.0883.

$[\alpha]_D^{22}$ = 113.3, C=0.0015 in H_2O).

UV (H_2O) λ_{max} = 274, ε = 20,400 at pH = 7.0.

Benzoylated lactam (**22**). To 76 mg (0.365 mmol) of **10**, was added 0.7 mL of anhydrous pyridine followed by 47 mL (0.402 mmol) of benzoyl chloride. The reaction mixture was stirred for 8 h and became milky white in appearance. Excess benzoyl chloride was quenched by addition of 0.5 mL of H_2O. The reaction mixture was evaporated to dryness and dissolved in 30 mL of 20% isopropanol/$CHCl_3$ and extracted twice with 10 mL of saturated bicarbonate and once with 10 mL of 0.5 M HCl. The aqueous layers were extracted with 20% isopropanol/$CHCl_3$ three times each and the combined organic layers were dried over Na_2SO_4, and evaporated to dryness. Chromatography on silica (1.25 - 10% MeOH/$CHCl_3$) provided 99% of **22**, which was recrystallized from $CHCl_3$/hexanes, mp 216 °C (d).

^{1}H NMR ($CDCl_3$) δ 1.82-1.95 (1H, m), 2.28-2.44 (3H, m), 3.81-3.87 (1H, dd, J=13.5, 7.4 Hz), 4.09-4.14 (1H, dd, J=13.2, 4.2 Hz), 4.15-4.24 (1H, m), 6.88 (1H, bs), 7.49-7.53 (3H, m), 7.59-7.61 (1H, d, J=7.3 Hz), 7.67-7.69 (1H, d, J=7.2 Hz), 7.92-7.94 (2H, d, J=7.6 Hz), 9.12-9.46 (1H, bs).

IR (KBr pellet) cm^{-1} 1697, 1693, 1687, 1655, 1646, 1639, 1625, 1568, 1559, 1552, 1485, 1357, 1304, 1279, 1262, 1246.

MS (Negative FAB HRMS) m/z, $(M-H)^-$ calculated 311.1144, observed 311.1138.

$[\alpha]_D^{22}$ = +69.5 (c = 0.002, MeOH).

UV (MeOH) λ_{max} = 306 and 260, ε = 5275 and 15,280 at pH = 7.0, λ_{max} = 318, ε = 16,560 at pH = 12.0.

Bis-(t-Butoxycarboxylated) product (**23**). To a stirring solution of **22** (55 mg, 0.173 mmol) in CH_2Cl_2 at 25 °C was added 24 μL (0.173 mmol) of Et_3N, 80 μL (0.345 mmol) of di-t-butyl dicarbonate, and 19 mg (0.173 mmol) of 4-(dimethylamino)pyridine. After 2 h at 25 °C, the volatiles were removed *in vacuo*, and the residue purified by chromatography on silica (5% MeOH/$CHCl_3$). This resulted in a quantitative yield of **23** which displayed the following NMR spectrum.

^{1}H NMR ($CDCl_3$) δ 1.31 (9H, s), 1.53 (9H, s), 2.06-2.14 (2H, m), 2.40-2.47 (1H, m), 2.53-2.62 (1H, m), 3.90-3.95 (1H, dd, J=13.5, 4.9 Hz), 4.23-4.29 (1H, dd, J=13.3, 7.0 Hz), 4.46-4.52 (1H, m), 7.13-7.15 (1H, d, J=7.3 z), 7.45-7.49 (2H, t, J=7.8 Hz), 7.55-7.57 (1H, d, J=7.3 Hz), 7.58-7.60 (1H, m), 7.86-7.90 (2H, dd, J=9.1, 1.4 Hz).

Amidine (**25**). Alkylation product **10** (0.50 g, 2.40 mmol) was carefully dried by three coevaporations with dry pyridine then mixed with dry methanol (5 mL). To the stirring suspension was added dropwise 0.47 mL of N-methyl-2,2-dimethoxypyrrolidine at 25 °C and the reaction mixture gently warmed to 60 °C until the starting material had completely dissolved. The warm bath was then removed, and the reaction mixture was allowed to stir for 1 h while gradually coming to 25 °C. The reaction mixture was concentrated *in vacuo* and purified by chromatography on silica (20% MeOH/$CHCl_3$) to provide 0.692 g (2.39 mmol) of **24** (99% yield). The crude product was then dissolved

in 5 mL of dry CH_2Cl_2, 0.33 mL (2.39 mmol) of Et_3N was added, followed by 1.10 mL (4.78 mmol) of di-t-butyl dicarbonate, and finally 0.258 g (2.39 mmol) of 4-(dimethylamino)pyridine. Upon addition of the catalyst, the solution changed from light yellow to intensely dark orange in color with vigorous gas evolution. Complete reaction occurred within 2 h and following removal of volatiles, the lactam-protected adduct was purified by chromatography on a large excess of silica (100:1, 10% MeOH/$CHCl_3$) to provide 0.863 g of an amorphous solid **25**, in 86.5% overall yield.

^1H NMR ($CDCl_3$) δ 1.54 (9H, s), 2.01-2.13 (4H, m), 2.36-2.46 (1H, m), 2.54-2.64 (1H, m), 3.06 (3H, s), 3.13-3.26 (2H, m), 3.45-3.49 (2H, t, J=7.2 Hz), 3.92-3.97 (1H, dd, M=13.6, 4.5 Hz), 4.20-4.25 (1H, dd, J=13.6, 7.0 Hz), 4.45-4.50 (1H, m), 5.99-6.00 (1H, d, J=7.1 Hz), 7.22-7.23 (1H, d, J=7.1 Hz).

MS (Negative FAB HRMS) m/z, $(M-H)^-$ calculated 388.1985, observed 388.1983.

Amidine (**27**). Compound **10** (0.195 g, 0.938 mmol) was dried by three coevaporations with dry pyridine, 3 mL of DMF was added and the mixture gently warmed to 40 °C while stirring, to completely dissolve the starting material. N,N-dimethylacetamide dimethyl acetal (0.275 mL 1.88 mmol) was added dropwise and the reaction mixture was allowed to stir for 8 h at 40 °C. Solvent was removed by high vacuum, the crude **26** was redissolved in 3 mL of dry CH_2Cl_2, 0.130 mL (0.938 mmol) of Et_3N was added, followed by 0.431 mL (1.88 mmol) of di-t-butyl dicarbonate, and finally 0.101 g (0.938 mmol) of 4-(dimethylamino)pyridine. Upon addition of catalyst, violent gas evolution occurred and the reaction was complete within 2 h. The reaction mixture was evaporated to dryness and purified by chromatograhy on silica (0 - 20% MeOH/$CHCl_3$) providing 0.297 g (0.788 mmol) of an amorphous solid **27**, in 84% overall yield.

^{1}H NMR ($CDCl_3$) δ 1.54 (9H, s), 2.05-2.26 (2H, m), 2.31 (3H, s), 2.39-2.46 (1H, m), 2.55-2.65 (1H, m), 3.10 (6H, s), 3.89-3.94 (1H, dd, J=13.7, 4.8 Hz), 4.23-4.28 (1H, dd, J=13.7, 6.6 Hz), 4.46-4.51 (1H, m), 5.89-5.91 (1H, d, J=7.0 Hz), 7.20-7.22 (1H, d, J=6.7 Hz).

Selective amidine deprotection (**29**). Amidine **27** (100 mg, 0.265 mmol) was dissolved in 2 mL of anhydrous CH_2Cl_2 and 197 mg (1.06 mmol) of p-toluenesulfonhydrazide was added in one portion. The reaction mixture was stirred at 25 °C for 24 h at which point the reaction had gone virtually to completion. The reaction mixture was evaporated to dryness and purified by chromatography on silica (0 - 20% MeOH/$CHCl_3$), providing 65 mg (0.211 mmol) of **29**, mp 177-179 °C, in 79% yield.

^{1}H NMR (d_6 DMSO) δ 1.38 (9H, s), 1.72-1.77 (1H, m), 2.02 (2.10 (1H, m), 2.23-2.33 (2H, m), 3.74-3.79 (1H, dd, J=13.6, 7.3 Hz), 3.88-3.93 (1H, dd, J=13.5, 5.6 Hz), 4.44-4.49 (1H, m), 5.62-5.64 (1H, d, J=7.2 Hz), 6.98-7.08 (2H, bd, J=38.0 Hz), 7.44-7.45 (1H, d, J=7.3 Hz).

IR (KBr pellet) cm^{-1} 3428, 3354, 1778, 1725, 1709, 1654, 1523, 1494, 1390, 1372, 1313, 1257, 1154.

MS (Negative FAB HRMS) m/z, $(M-H)^-$ calculated 307.1406, observed 307.1407.

$[\alpha]_D^{22}$ = +60.7 (c = 0.0015, MeOH).

Completely protected lactam (**11**). To compound **29** (0.31 g, 1.03 mmol) dissolved in 10 mL of dry pyridine was added 0.180 mL (1.55 mmol) of benzoyl chloride. The reaction mixture was stirred at 25 °C for 3 h followed by addition of 5 mL of H_2O. The reaction mixture was evaporated to dryness, dissolved in 300 mL of $CHCl_3$, extracted twice with 50 mL portions of saturated $NaHCO_3$, and twice with 50 mL portions of 0.5

M HCl. The organic layer was dried over Na_2SO_4, evaporated to dryness, and chromatographed on silica (0 - 10% MeOH/$CHCl_3$) to provide 0.297 g of product **11** (70% yield), mp 190-191 °C, and 0.069 g of a dibenzoylated cytosine adduct (13% yield).

^1H NMR (d_6 DMSO) δ 1.35 (9H, s), 1.75-1.80 (1H, m), 2.10-2.16 (1H, m), 2.29-2.35 (1H, m), 2.61-2.70 (1H, m), 3.94-3.99 (1H, dd, J=13.6, 7.9 Hz), 4.10-4.15 (1H, dd, J=13.2, 4.7 Hz), 4.55-4.65 (1H, m), 7.25-7.26 (1H, bs), 7.29-7.31 (1H, d, J=7.2 Hz), 7.50-7.54 (2H, t, J=7.6 Hz), 7.61-7.65 (1H, t, J=7.3 Hz), 7.99-8.01 (2H, d, J=7.6 Hz), 8.08-8.10 (1H, d, J=7.3 Hz).

IR (KBr pellet) cm^{-1} 3416, 1783, 1725, 1710, 1696, 1691, 1664, 1658, 1626, 1560, 1487, 1368, 1309, 1250, 1157.

MS (Negative FAB HRMS) m/z, $(M-H)^-$ calculated 411.1669, observed 411.1667.

$[\alpha]_D^{22}$ = +97.0 (c = 0.001, MeOH).

Completely protected lactam (**30**). By the same procedure for the preparation of **11**, 1.030 g (3.34 mmol) of **29** was converted into 1.140 g of product **30** (83% yield), mp 159-161 °C, and 0.153 g of a di-t-butylbenzoylated cytosine adduct (7% yield).

^1H NMR (d_6 DMSO) δ 1.36 (9H, 2), 1.53 (9H, s), 2.13-2.19 (2H, m), 2.45-2.52 (1H, m), 2.62-2.69 (1H, m), 3.90-3.95 (1H, dd, J=13.5, 5.2 Hz), 4.38-4.43 (1H, dd, J=13.5, 6.9 Hz), 4.56-4.60 (1H, m), 7.52-7.55 (3H, d, J=8.3 Hz), 7.63-7.65 (1H, d, J=7.2 Hz), 7.83-7.85 (2H, d, J=8.3 Hz), 8.69-8.86 (1H, bs).

IR (KBr pellet) cm^{-1} 3406, 2968, 1782, 1716, 1710, 1698, 1690, 1687, 1666, 1657, 1654, 1645, 1627, 1559, 1550, 1489, 1370, 1309, 1255, 1155.

MS (Negative FAB HRMS) m/z, $(M-H)^-$ calculated 467.2295, observed 467.2309.

$[\alpha]_D^{22}$ = +88.2 (c = 0.001, MeOH).

Carboxylic Acid (**12**). Compound **11** (0.500 g, 1.21 mmol) was dissolved in 10 mL of THF, cooled to 0 °C, and treated with 3.64 mL (3.64 mmol) of 1M LiOH. After stirring at 0 °C for 15 min, the solution was gradually warmed to 25 °C over 30 min. 1M HCl (3.64 mL) was added and the reaction mixture evaporated to dryness. The residue was purified by chromatography using a small excess of silica (20:1, 20% MeOH/$CHCl_3$). The combined fractions were diluted with an equal volume of $CHCl_3$ and filtered to remove undissolved silica. This solution was concentrated *in vacuo* to approximately 10 mL, and hexanes added dropwise until the solution became cloudy. The flask was placed in a refrigerator and 48 h later, filtered to provide 0.3632 g (0.845 mmol) of pure acid **12** (70% yield), mp 235-236 °C.

^{1}H NMR (d_6 DMSO) δ 1.38 (9H, s), 1.48-1.79 (2H, m), 2.22-2.33 (2H, m), 3.36-3.47 (1H, m), 3.74-3.88 (1H, m), 4.06-4.11 (1H, m), 6.79-6.81 (1H, d, J=9.2 Hz), 7.25-7.28 (1H, bs), 7.49-7.53 (2H, t, J=7.8 Hz), 7.60-7.64 (1H, t, J=7.5 Hz), 7.90-7.91 (1H, d, J=7.2 Hz), 7.98-8.00 (2H, d, J=7.6 Hz), 11.10-11.20 (1H, bs), 12.10-12.20 (1H, bs).

IR (KBr pellet) cm^{-1} 3408, 1709, 1700, 1686, 1682, 1675, 1667, 1652, 1644, 1641, 1638, 1627, 1623, 1578, 1574, 1572, 1560, 1522, 1503, 1491, 1372, 1253, 1150.

MS (Negative FAB HRMS) m/z, $(M-H)^-$ calculated 429.1774, observed 429.1705.

$[\alpha]_D^{22}$ = +118.0 (c = 0.001, MeOH).

UV (MeOH) λ_{max} = 303, 256, and 224, ε = 6900, 17,030, and 14,120 at pH = 7.0, λ_{max} = 316, ε = 12,500 at pH = 12.0.

Carboxylic Acid (**28**). The procedure for lithium hydroxide ring-opening of both amidine **25** and **27** was the same as that described above for ring-opening of **11**.

^{1}H NMR (d_6 DMSO) δ 1.32 (9H, s), 1.46-1.67 (2H, m), 2.02-2.14 (2H, m), 3.57-3.72 (2H, m), 3.84-3.92 (1H, m), 5.59-5.62 (1H, d, J=7.3 Hz), 6.76-6.79 (1H, d, J=9.1 Hz), 6.82-7.13 (2H, bd), 7.37-7.39 (1H, d, J=7.2 Hz).

Carboxylic Acid (**31**). To 0.576 g (1.23 mmol) of **30** in 10 mL of THF was added 3.69 mL (3.69 mmol) of 1M LiOH. The reaction mixture was allowed to stir at 25 °C for 40 min, at which time 4.92 mL (4.92 mmol) of 1M HCl was added. The reaction mixture, which had turned cloudy, was evaporated to dryness. The resulting free acid **31**, was purified by chromatography on silica (20% MeOH/$CHCl_3$). The product residue was dissolved in 20% isopropanol/$CHCl_3$ (200 mL) and extracted with 0.02 M HCl to remove LiCl and dissolved silica. The aqueous layer was back-extracted with 20% isopropanol/$CHCl_3$ until no more product was recovered as evidenced by TLC. The organic layers were dried over Na_2SO_4 and evaporated to dryness on high vacuum with external heating (50 °C), providing 0.485 g (0.996 mmol) of pure acid **31** (81% yield), mp 238-240 °C.

^{1}H NMR (d_6 DMSO) δ 1.28 (9H, s), 1.31 (9H, s), 1.53-1.74 (2H, m), 2.06-2.19 (2H, m), 3.41-3.47 (1H, dd, J=14.4, 7.6 Hz), 3.72-3.83 (1H, m), 4.07-4.11 (1H, dd, J=13.2, 3.7 Hz), 6.82-6.85 (1H, d, J=9.1 Hz), 7.21=7.28 (1H, bs), 7.51-7.53 (2H, d, J=8.6 Hz), 7.89-7.91 (1H, d, J=7.1 Hz), 7.94-7.96 (2H, m), 11.10-11.20 (1H, bs), 12.10-12.20 (1H, bs).

IR (KBr pellet) cm^{-1} 3371, 2968, 1705, 1703, 1680, 1653, 1644, 1626, 1623, 1571, 1560, 1550, 1545, 1522, 1496, 1409, 1390, 1372, 1350, 1299, 1260, 1168, 1114.

MS (Negative FAB HRMS) m/z, $(M-H)^-$ calculated 485.2400, observed 485.2425.

$[\alpha]_D^{22} = +112.8$ (c = 0.0025, MeOH).

Standard procedure for formation of p-nitrophenyl esters to be used in coupling reactions. Dried, free acid **32** (75 mg, 0.154 mmol), 4-nitrophenol (129 mg, 0.925 mmol), 137 mg (0.462 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide, and 1.7 mg (0.015 mmol) of 4-(dimethylamino)pyridine were placed in a 10 mL flask under a N_2 atmosphere in a glove box. Dry DMF (3 mL) was added and the mixture stirred at 25 °C for at least 2 h (generally overnight). All solids dissolved upon completion of reaction. The activated p-nitrophenyl ester, as the DMF solution, was used directly in coupling reactions, without isolation or purification. The formation and utilization of these esters could be monitored by TLC. Exposure of the TLC plate to ammonia fumes produced a yellow spot of liberated ammonium p-nitrophenylate which was easily recognized.

Hydrazide monomer (**33**). 1-Aminopiperidine oxalate salt[41] (59 mg, 0.308 mmol) and 85.5 μL (0.924 mmol) of Et_3N were added to a solution containing 93.6 mg (0.154 mmol) of p-nitrophenyl ester **32** produced by the standard activation. The reaction mixture was allowed to stir for 24 h, evaporated to dryness, and dissolved in 30 mL 20% isopropanol/$CHCl_3$. The organic layer was extracted once with a 5 mL portion of H_2O, six times with 5 mL portions of 0.20 M NaOH, twice with 5 mL portions of 0.02 M HCl, and once with a 5 mL portion of saturated brine. The aqueous layers were each back-extracted once with 20% isopropanol/$CHCl_3$. The combined organic layers were dried over Na_2SO_4 and evaporated to dryness, providing 74 mg (0.129 mmol) of crude hydrazide protected monomer **33** (84% yield). The crude **33** was generally taken on

directly to the next reaction, but could also be purified on silica (1.25 - 10% MeOH/$CHCl_3$) to provide 57 mg of pure **33** (65% purified yield).

^1H NMR (d_6 DMSO) δ 1.27-1.29 (9H, d, J=5.4 Hz), 1.31 (9H, s), 1.51-1.53 (2H, t, J=5.3 Hz), 1.58-1.76 (4H, m), 1.96-2.02 (2H, m), 2.29-2.45 (2H, m), 2.60-2.68 (4H, m), 3.39-3.44 (1H, m), 3.61-3.69 (1H, m), 4.03-4.11 (1H, m), 6.70-6.75 (1H, t, J=8.7 Hz), 7.27-7.29 (1H, d, J=6.2 Hz), 7.52-7.54 (2H, d, J-8.4 Hz), 7.87-7.91 (1H, t, J=7.6 Hz), 7.94-7.96 (2H, d, J=8.2 Hz), 11.10, 11.20 (1H, bs).

IR (KBr pellet) cm^{-1} 3353, 3245, 2956, 2936, 1695, 1685, 1680, 1675, 1658, 1626, 1623, 1559, 1550, 1545, 1522, 1489, 1369, 1350, 1297, 1268, 1255, 1168.

MS (Positive FAB LRMS) m/z (relative abundance) 570 (MH^+ +1, 19.4), 569 (MH^+, 70.9), 469 (17.2), 457 (23.1), 451 (35.8), 307 (47.0), 272 (38.1), 198 (15.7), 146 (47.8), 145 (29.1), 118 (29.1), 105 (15.7), 101 (22.4), 100 (23.9), 99 (35.8), 84 (66.4), 61 (24.6), 57 (100.0), 55 (28.4).

MS (Negative FAB HRMS) m/z, $(M-H)^-$ calculated 567.3295, observed 567.3288.

Amido (**35**). 2-(Methylamino)ethanol (15 μL, 0.185 mmol) was added to active ester **32** in DMF followed by 22 μL (0.154 mmol) of Et_3N. The reaction mixture was allowed to stir at 25 °C for 2 h. The reaction mixture was evaporated to dryness and dissolved in 30 mL of 20% isopropanol/$CHCl_3$. The organic layer was extracted once with a 5 mL portion of H_2O, six times with 5 mL portions of 0.20 M NaOH, twice with 5 mL portions of 0.02 M HCl, and once with a 5 mL portion of saturated brine. The aqueous layers were each back-extracted once with 20% isopropanol/$CHCl_3$. The combined organic layers were dried over Na_2SO_4 and evaporated to dryness, providing 62 mg (0.114 mmol) of the corresponding alcohol (74% crude yield). This alcohol (**34**) was neither purified nor characterized at this stage, but rather protected directly,

and characterized as the 4-t-butylbenzoyl ester **35**. The crude alcohol was dried by two successive coevaporations with 3 mL portions of dry pyridine. This residue was dissolved in 0.60 mL of dry pyridine and 25 μL (0.125 mmol) of 4-t-butylbenzoyl chloride was added at 25 °C. After stirring for 30 min, a small amount of water was added and the reaction mixture evaporated to dryness. The residue was then purified on silica (1.25 - 5% MeOH/$CHCl_3$ gradient) providing 59 mg (0.084 mmol) of **35** (73% yield).

^1H NMR (d_6 DMSO, 327°K) δ 1.28 (9H, s), 1.30 (9H, s), 1.32 (9H, s), 1.62-1.80 (2H, m), 2.31-2.43 (2H, m), 2.96 (3H, s), 3.35-3.47 (2H, m), 3.62-3.70 (1H, m), 3.76-3.84 (1H, m), 4.01-4.09 (1H, m), 4.31-4.45 (2H, m), 6.50-6.60 (1H, bs), 7.19-7.21 (1H, d, J=8.3 Hz), 7.48-7.52 (4H, dd, J=8.4, 1.3 Hz), 7.78-7.80 (1H, d, J=8.3 Hz), 7.85-7.88 (4H, dd, J=8.4, 1.5 Hz).

IR (KBr pellet) cm^{-1} 2964, 2961, 2928, 1730, 1717, 1709, 1694, 1687, 1653, 1644, 1637, 1627, 1622, 1612, 1560, 1493, 1422, 1411, 1366, 1318, 1289, 1270, 1160.

MS (Negative FAB LRMS) m/z (relative abundance) 703 (M^-, 20.0), 702 ($(M-H)^-$, 100.0), 602 (5.2), 542 (3.4), 450 (4.0), 270 (39.7), 201 (6.9), 177 (29.3).

MS (Positive FAB HRMS) m/z, (MH^+) calculated 704.4023, observed 704.4009.

Standard procedure for oligomerization reactions. The same procedure was followed for dimerization of both cap-protected monomer **35** and hydrazide protected monomer **33**. A typical dimerization in the cap-protected series entailed suspending 54 mg (0.076 mmol) of **35** in 0.30 mL of dry CH_2Cl_2, followed by addition of an equal volume (0.30 mL, 3.80 mmol) of dry trifluoroacetic acid. The reaction mixture became homogeneous upon addition of TFA and CO_2 gas evolution was observed. The

reaction mixture was allowed to stir at 25 °C for 40 min, at which time 15 mg (0.076 mmol) of $TsOH \cdot H_2O$ was added. The solution was then evaporated to dryness and coevaporated twice with 3 mL portions of dry DMF to insure removal of TFA and H_2O. The resulting amine salt was set aside for the subsequent coupling reaction. The active ester was prepared on a scale 1.2 times that of the corresponding free amine portion to be used in the dimerization reaction. Therefore, 44 mg (0.091 mmol) of monomer acid was converted into **32**. The DMF solution containing monomeric activated ester **32** was transferred to the flask containing the predried amine salt. To this was added 63 μL (0.456 mmol) of Et_3N, and the reaction allowed to stir at 25 °C for at least 8 h. Upon complete disappearance of amine, the reaction mixture was evaporated to dryness, dissolved in 30 mL of 20% isopropanol/$CHCl_3$, and extracted once with a 5 mL portion of H_2O, six times with 5 mL portions of 0.20 M NaOH, twice with 5 mL portions of 0.02 M HCl, and once with a 5 mL portion of saturated brine. The aqueous layers were each back-extracted with 20% isopropanol/$CHCl_3$, the combined organic layers dried over Na_2SO_4, and evaporated to dryness. The residue was then purified on silica (0.625 - 5% MeOH/$CHCl_3$) resulting in 52 mg (0.048 mmol) of purified cap-protected dimer **41** (63% yield). Yields for coupling reactions generally ranged from 40-70% following chromatography. The same series of reactions resulted in 59% purified yield of hydrazide-protected dimer **37**. The same procedure was followed for trimer formation as described above for dimerization reactions, involving BOC-deprotection of either cap-protected dimer **41**, or hydrazide-protected dimer **37**, and coupling with the monomeric p-nitrophenyl ester. The same extractive workup was also followed, and further purified on silica as described above providing 58% purified yield of cap-protected trimer **43**, and 45% purified yield of hydrazide protected trimer **39**.

37: ^1H NMR (d_6, DMSO) δ 1.26-1.27 (9H, d, J=2.2 Hz), 1.30 (9H, s), 1.31 (9H, s), 1.50-1.80 (6H, m), 1.96-2.15 (4H, m), 2.24-2.46 (4H, m), 2.57-2.69

(4H, m), 3.38-3.44 (2H, m), 3.51-3.65 (1H, m), 3.70-3.81 (1H, m), 4.02-4.07 (2H, m), 6.75-6.81 (1H, m), 7.24-7.32 (2H, m), 7.50-7.54 (4H, dd, J=8.3, 4.3 Hz), 7.72=7.78 (1H, m), 7.84-7.90 (1H, m), 7.94-7.97 (4H, dd, J=8.4, 3.6 Hz).

MS (Positive FAB LRMS) m/z (relative abundance) 939 (MH^+ +1, 5.9), 938 (MH^+, 9.7), 838 (3.6), 595 (5.9), 495 (3.5), 369 (2.7), 308 (21.2), 307 (100.0), 272 (15.2), 146 (15.2), 145 (12.1).

MS (Negative FAB HRMS) m/z, $(M-H)^-$ calculated 935.5143, observed 935.5179.

41: ^1H NMR (d_6 DMSO, 348°K) δ 1.25-1.37 (36H, m), 1.60-1.82 (4H, m), 2.03-2.20 (2H, m), 2.30-2.45 (2H, m), 3.19 (3H, s), 3.45-3.55 (2H, m), 3.62-3.83 (3H, m), 3.97-4.06 (2H, m), 4.07-4.16 (1H, m), 4.33-4.43 (2H, m), 6.29-6.41 (1H, bs), 7.10-7.21 (2H, m), 7.46-7.53 (6H, m), 7.80-7.82 (1H, d, J=7.3 Hz), 7.84-7.88 (5H, m), 7.93-7.96 (2H, dd, J=8.5, 3.5 Hz).

MS (Negative FAB LRMS) m/z (relative abundance) 1072 (M^-, 7.8), 1071 ($(M-H)^-$, 20.9), 996 (2.0), 929 (1.6), 270 (100.0), 227 (7.8), 201 (19.4), 177 (17.1).

MS (Positive FAB HRMS) m/z, (MH^+) calculated 1072.6051, observed 1072.5780.

39: ^1H NMR (d_6 DMSO) δ 1.26-1.38 (9H, d, J=3.8 Hz), 1.31 (9H, s), 1.32 (9H, s), 1.34 (9H, s), 1.49-1.81 (6H, m), 1.95-2.17 (6H, m), 2.23-2.49 (6H, m), 2.57-2.70 (4H, m), 3.35-3.50 (3H, m), 3.51-3.82 (3H, m), 4.00-4.12 (3H, m), 6.72-6.78 (1H, m), 7.20-7.31 (3H, m), 7.50-7.56 (6H, m), 7.70-7.90 (6H, m), 7.91-8.00 (6H, m).

MS (Negative FAB LRMS) m/z (relative abundance) 1304.6 (M^-, 45.2), 1303.6 ($(M-H)^-$, 50.0), 1220.5 (4.0), 1143.6 (6.7), 991.5 (2.7), 906.5 (3.0), 367.2 (4.8), 307.1 (6.5), 270.1 (100.0), 227.1 (6.5), 201.1 (29.0).

MS (Positive FAB HRMS) m/z, (MH^+) calculated 1305.7147, observed 1305.7005.

43: ^1H NMR (d_6 DMSO, 348°K) δ 1.24-1.38 (45H, m), 1.59-1.84 (6H, m), 2.05-2.43 (6H, m), 3.20 (3H, s), 3.46-3.57 (2H, m), 3.60-3.85 (4H, m), 3.94-4.20 (4H, m), 4.32-4.41 (2H, m), 6.41-6.52 (1H, bs), 7.08-7.22 (3H, m), 7.44-7.55 (8H, m), 7.79-7.81 (1H, d, J=7.4 Hz), 7.83-7.90 (8H, m), 7.94-7.98 (2H, dd, J=8.3, 3.5 Hz).

MS (Negative FAB LRMS) m/z (relative abundance) 1441 (M^-, 9.9), 1440 ($(M-H)^-$, 40.4), 1279 (4.7), 1071 (4.2), 367 (3.8), 271 (18.3), 270 (100.0), 201 (35.2), 177 (11.3).

MS (Positive FAB HRMS) m/z, (MH^+) calculated 1440.7719, observed 1440.7720.

Hexamer (**45**). Hydrazide protected trimer **39** (14 mg, 0.011 mmol) was dissolved in 0.20 mL of 75% aq. THF. To this stirring solution, 1.4 mL (0.017 mmol) of pyridine was added, followed by 2.3 mg (0.013 mmol) of N-bromosuccinimide. This reaction mixture was allowed to stir 15 min (longer for larger scale reactions). Upon completion of reaction, 0.315 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 extracted twice with 3 mL portions of 0.02 M HCl. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The product was thoroughly dried on high vacuum with external heating (50 °C) before taking on to the activation reaction. Yield of the crude trimer free acid was quantitative, and was taken on directly to the activation stage without characterization.

The same procedure was followed for coupling trimer portions as previously described in formation of both cap-protected dimer **41**, and hydrazide protected dimer

38. This involved BOC-deprotection of cap-protected trimer **43** to provide the trimer amine salt **44**, and activation of trimer free acid, made readily available upon hydrazide deprotection of **39**, by the standard procedure. The same relative amounts of reagents were used to couple the trimer portions, but the standard aqueous workup was avoided. For purification, the DMF reaction mixture was evaporated to dryness and purified directly on silica (1.25 - 20% MeOH/$CHCl_3$), to provide the desired, fully-protected hexamer **45** (50% purified yield).

^1H NMR (d_6 DMSO, 348°K) δ 1.25-1.36 (72H, m), 1.50-1.90 (12H, m), 2.07-2.28 (12H, m), 3.06 (3H, s), 3.45-3.53 (2H, m), 3.54-3.70 (8H, m), 3.75-3.90 (1H, m), 3.91-4.31 (9H, m), 4.32-4.43 (2H, m), 6.30-6.45 (1H, bs), 6.74-6.76 (2H, d, J=7.1 Hz), 7.05-7.22 (6H, m), 7.37-7.56 (14H, m), 7.79-7.81 (1H, d, J=7.1 Hz), 7.84-7.86 (1H, d, J=8.4 Hz), 7.87-7.97 (14H, m), 8.11-8.12 (1H, d, J=7.0), 10.43-10.71 (5H, bs).

MS (Negative FAB LRMS) m/z (relative abundance) 2544 ($(M-H)^-$, 24.5), 2423 (5.9), 2174 (7.0), 271 (27.9), 270 (100.0), 201 (61.2), 177 (28.9).

MS (Negative FAB HRMS) m/z, $(M-H)^-$ calculated 2543.3107, observed 2543.3340.

$[\alpha]_D^{22} = +108.0$.

Non-solubilized Dimer (**46**). Bis-(p-nitrophenyl) carbonate (1.00 g, 3.29 mmol) was dissolved in 10 mL of dry DMF and 0.130 mL (1.64 mmol) of 2-(methoxy)ethanol was added while stirring at 25 °C. Upon addition of 0.243 mL (1.64 mmol) of Et_3N, the solution turned yellow instantaneously. After 8 h, 1.0 mL of H_2O was added, and the reaction mixture evaporated to dryness. The product was only distinguishable from bis-p-nitrophenyl carbonate on TLC by elution with a low concentration (1.25%) of MeOH/$CHCl_3$. The product was purified on silica ($CHCl_3$) to provide the desired

mixed carbonate (quantitative yield). This compound was not fully characterized, but appears pure by 400 MHz ^{1}H NMR.

^{1}H NMR ($CDCl_3$) δ 3.44 (3H, s), 3.70-3.72 (2H, t, J=4.5 Hz), 4.43-4.46 (2H, J=4.5 Hz), 7.39-7.41 (2H, dd, J=7.0, 1.9 Hz), 8.28-8.30 (2H, dd, J=7.0, 2.0 Hz).

The procedure for non-solubilizing tail attachment entailed stirring 5.9 mg (0.005 mmol) of dimeric amine salt **42**, 3.5 mg (0.016 mmol) of mixed carbonate, and 3.8 μL (0.026 mmol) of Et_3N in 0.15 mL dry DMF overnight at 25 °C. This reaction mixture was then transferred to a vial equipped with a teflon-sealed cap. The DMF reaction mixture was cooled down and an equal volume (0.15 mL) of cold, concentrated ammonia (4.35 mmol) was added, the vessel sealed, and stirred at 45 °C for 24 h. The reaction mixture was then evaporated to dryness and purified by medium-pressure ion exchange chromatography. This resulted in the separation of two products, which appear to be a mixture of the desired fully deprotected product **46** and an equal amount of the cytosine-deprotected, 4-t-butylbenzoyl ester **47**, eluting off in that order (quantitative combined yields). Retention time was not completely reproducible, since the gradient in the ion exchange purification was approximate, as previously described. The undesired product was subjected again to NH_4OH deprotection conditions, but substituting DMSO as solvent and stirred for 72 h at 45 °C in the sealed vial. This resulted in complete deprotection, providing the non-solubilized, deprotected dimer **46** in quantitative yield.

46: ^{1}H NMR (d_6 DMSO, 347°K) δ 1.51-1.77 (4H, m), 2.05-2.10 (2H, t, J=8.1 Hz), 2.27-2.36 (2H, m), 2.95-3.10 (1H, bs, beneath H_2O), 3.25 (3H, s), 3.31-3.34 (2H, t, J=5.8 Hz), 3.44-3.46 (2H, t, J=4.9 Hz), 3.47-3.57 (2H, m), 3.66-3.74 (1H, m), 3.74-3.78 (5H, m), 3.78-3.90 (2H, m), 3.91-3.97 (1H, m), 3.98-4.03 (2H,

t, J=5.2 Hz), 5.60-5.62 (2H, d, J=7.1 Hz), 6.60-6.70 (4H, bd, J=13.9 Hz), 6.71-6.85 (1H, bs), 7.36-7.39 (2H, dd, J=7.2, 2.8 Hz), 7.40-7.50 (1H, bs).

47: ^{1}H NMR (d_6 DMSO, 347°K) δ 1.30 (9H, s), 1.50-1.75 (4H, m), 2.05-2.09 (2H, t, J=7.4 Hz), 2.28-2.40 (2H, m), 3.18 (3H, s), 3.24 (3H, s), 3.42-3.45 (2H, t, J=5.0 Hz), 3.61-3.88 (7H, m), 3.94-3.97 (1H, m), 3.98-4.02 (2H, t, J=5.2 Hz), 4.30-4.41 (2H, bm), 5.59-5.62 (2H, d, J=7.4 Hz), 6.60-6.70 (4H, bd, J=12.4 Hz), 6.70-6.82 (1H, bs), 7.34-7.36 (1H, d, J=6.8 Hz), 7.35-7.37 (1H, d, J=6.8 Hz), 7.44-7.46 (1H, bd, J=8.1 Hz), 7.51-7.53 (2H, d, J=8.4 Hz), 7.85-7.87 (2H, d, J=8.5 Hz).

Solubilizing mixed carbonate (**49**). A 0.131 M solution (40 mL, 5.24 mmol) of polyethylene glycol (PEG) 1000 **48** was co-evaporated three successive times with 25 mL portions of dry DMF. To the dried PEG was added 0.797 g (2.62 mmol) of bis-p-nitrophenyl carbonate and dissolved in 20 mL of dry DMF. Following addition of 1.55 mL (10.5 mmol) of Et_3N, the solution was allowed to stir at 25 °C for 24 h under an inert atmosphere. The resulting polyethylene glycol mixed carbonate was stored in DMF solution (approximately 0.105 M solution) and used accordingly for tail attachment in large (50-fold) excess without characterization of further purification.

Solubilized dimer (**50**). Compound **47** (2.46 mL, 0.258 mmol of 0.105 M solution in DMF) was added to a flask containing 5.9 mg (0.005 mmol) of predried dimeric amine salt **42** and 7.7 μL (0.052 mmol) of Et_3N. The reaction mixture was allowed to stir at 25 °C for at least 24 h. Upon completion of reaction, a small amount of H_2O (0.25 mL) containing 2 mg (0.016 mmol) of 4-(dimethylamino)pyridine was added and the reaction mixture evaporated to dryness. The protecting groups were cleaved as previously described for **43**, in either DMF/conc. NH_4OH or DMSO/conc. NH_4OH,

stirring at 45 °C in a sealed, teflon-capped vial. Prolonged reaction (72 h) resulted in the desired solubilized, completely deprotected dimer **50** which was purified by medium-pressure ion exchange chromatography. Yield of **50** was not determined due to the presence of salt following ion exchange purification and a single desalting.

50: ^{1}H NMR (d_6 DMSO, 347°K) δ 1.42-1.74 (4H, m), 1.90-2.18 (2H, m), 2.26-2.40 (2H, m), 2.77-3.28 (1H, bs; 3H, s; and approx. 90H, m; beneath H_2O and PEG peaks), 3.31-3.46 (4H, m), 3.62-3.72 (3H, m), 3.81-3.86 (2H, dd, J=13.3, 4.7 Hz), 3.88-4.05 (3H, m), 5.32 (1H, bs), 5.61-5.62 (1H, d, J=7.0 Hz), 5.62-5.63 (1H, d, J=7.0 Hz), 5.67 (1H, bs), 6.64 (4H, bm), 7.37-7.39 (1H, d, J=7.0 Hz), 7.47-7.49 (1H, d, J=7.3 Hz).

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34. After we had completed this study optically pure (R)- and (S)-**9** became available commercially from Fluka Chemical. These alcohols were also analyzed as the Mosher ester derivatives and appear pure using this method of optical purity determination. The optical rotation of (S)-(+)-5-(hydroxymethyl)-2-pyrrolidinone **9** obtained from Fluka is +38.5°, and we now believe this rotation to be correct rather than the +64° reported by von Hardegger and Ott.

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APPENDIX

APPENDIX A

Chemical Abstracts Names for Key Compounds

10 2(1H)-Pyrimidinone, 4-amino-1-[5-oxo-2-pyrrolidinyl)methyl]-, (S)-

17 Poly(oxy-1,2-ethanediyll), .alpha.-[3,8,13,18,23,28-hexakis[(4-amino-2-oxo-1(2H)-pyrimidinyl)methyl]-34-hydroxy-32-methyl-1,6,11,16,21,26,31-heptaoxo-2,7,12,17,22,27,32-hepaazatetratriacont-1-yl]-, [3S-(3R*,8R*,13R*,18R*,23R*,28R*)]-

22 Benzamide, N-[1,2-dihydro-2-oxo-1-[(5-oxo-2-pyrrolidinyl)methyl]-4-pyrimidinyl]-, (S)-

23 1-Pyrrolidinecarboxylic acid, 2-[[4-[benzoyl[(1,1-dimethylethoxy)carbonyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-5-oxo-, 1,1-dimethylethyl ester, (S)-

24 2(1H)-Pyrimidinone, 4-[(1-methyl-2-pyrrolidinylidene)amino]-1-[(5-oxo-2-pyrrolidinyl)methyl]-, (S)-

26 Ethanimidamide, N'-[1,2-dihydro-2-oxo-1-[(5-oxo-2-pyrrolidinyl)methyl]-4-pyrimidinyl]-N,N-dimethyl-, (S)-

27 1-Pyrrolidinecarboxylic acid, 2-[[4-[[1-(dimethyl amino)ethylidine]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-5-oxo-, 1,1-dimethylethyl ester, (S)-

29 1-Pyrrolidinecarboxylic acid, 2-[4-amino-2-oxo-1(2H)-pyrimidinyl)methyl]-5-oxo-, 1,1-dimethylethyl ester, (S)-

30 1-Pyrrolidinecarboxylic acid, 2-[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-5-oxo-, 1,1-dimethylethyl ester, (S)-

31 1(2H)-Pyrimidinepentanoic acid, .gamma.-[[(1,1-dimethylethoxy)carbonyl]amino]-4-[[4-(1,1-dimethyl ethyl)benzoyl]amino]-2-oxo-, (S)-

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

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

37 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]-2-oxo-4-(1-piperidinylamino)butyl]amino]-4-oxobutyl]-, 1,1-dimethylethyl ester, [S-(R*,R*)]-

39 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*)]]]-

41 2-Oxa-5,10,15-t5riazahexadecan-16-oic acid, 9,14-bix[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-1-[4-(1,1-dimethylethyl)phenyl]-5-methyl-1,6,11-trioxo-, 1,1-dimethylethyl ester, [S-(R*,R*)]-

43 2-Oxa-5,10,15,2-tetraazaheneicosan-21-oic acid, 9,14,19-tris[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-1-[4-(1,1-diemthylethyl)phenyl]-5-methyl-1,6,11,16,tetraoxo-,1,1-dimethylethyl ester, [9S-(9R*,14R*,19R*)]-

45 2-Oxa-5,10,15,20,25,30,35-heptaazahexatriacontan-36-oic acid, 9,14,19,24,29,34-hexakis[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2H)-pyrimidinyl]methyl]-1-[4-(1,1-dimethylethyl)phenyl]-5-methyl01,6,11,16,21,26,31-heptaoxo-, 1,1-dimethylethyl ester, [9S,(9R*,14R*,19R*,24R*,29R*,34R*)]-

46 Carbamic acid, [1-[(4-amino-2-oxo-1(2H)-pyrimidinyl)methyl]-4-[[1-[4-amino-2-oxo-1(2H)-pyrimidinyl)methyl]-4-[(2-hydroxyethyl)methylamino]-4-oxobutyl]amino]-4-oxobutyl]-, 2-methoxyethyl ester, [S-(R*,R*)]-

50 Poly(oxy-1,2-ethanediyll), .alpha.-[3,8-bis[(4-amino-2-oxo-1(2H)-pyrimidinyl)methyl]-12-hydroxy-14-methyl-1,6,11-trioxo-2,7,12-triazabutadecan-1-yl]-, [3S(3R*,8R*)]-