AN ABSTRACT OF THE THESIS OF

Jeffrey S. Nelson for the degree of Doctor of Philosophy in Chemistry presented on February 23, 1994.

Title: Solid-Phase Synthesis and Biophysical Testing of Uncharged Acyclic Oligonucleotide Analogues.

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Abstract approved: _____
Dwight D. Weller

Alkylation of the tosylates of either (R)- or (S)-N-*t*-Boc-2,2-dimethyl-4-hydroxymethyl-1,3-oxazoline with the potassium salt of cytosine in dimethyl sulfoxide proved to be an effective method for attachment of a heterocyclic base to an acyclic backbone precursor. This intermediate was conveniently converted into two different classes of optically pure activated subunits. One class of the activated subunits was subjected to oligomerization via solid-phase methods, to enable highly efficient syntheses of both configurations of hexamers containing a modified urethane-derived backbone. The other class of activated subunits will be subjected to oligomerization in due course, to similarly enable the synthesis of hexamers containing a modified peptide-derived backbone. This solid-phase approach combines the use of a cleavable anchor to allow the release of the fully protected oligonucleotide analogue from the solid support, and an ionizable base protecting group to facilitate isolation, purification, and characterization of the oligomers. The cleavable anchor consists of an N-protected hydroxyprolyl-proline ester, which upon deprotection cyclizes to a diketopiperazine with the release of the oligomeric alcohol. Use of 4-(4-morpholinyl)methylbenzoyl protecting groups allowed purification of the completed oligomers via ion-exhange chromatography. Following ammonolytic removal of the protecting groups, each hexamer was repurified and

subsequently desalted on a polypropylene column. Thermal denaturation studies were then undertaken to test each enantiomerically pure hexamer for its ability to hybridize complementary RNA or DNA. The (R)-configuration urethane hexamer **28** was found to bind its complementary DNA target $p(dG)_6$ with a T_m = 19°C in a low salt buffer.

Solid-Phase Synthesis and Biophysical Testing of Uncharged Acyclic Oligonucleotide Analogues.

by

Jeffrey S. Nelson

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Chairman of the Department of Chemistry

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Dean of Graduate School

Date thesis is presented February 23, 1994

Typed by Jeffrey S. Nelson

To Kelly and Marcus:

Who contributed enormously to my happiness
through the duration of my graduate career
through their never failing love and support.
The joy and laughter we shared during the past years
has continually reminded me of the aspects in life
which are most important and dear to me.

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SOLID-PHASE SYNTHESIS AND BIOPHYSICAL TESTING OF UNCHARGED ACYCLIC OLIGONUCLEOTIDE ANALOGUES.

I. INTRODUCTION

Therapeutic agents which are capable of specifically targeting a pathogen but which are not accompanied by serious dose-limiting toxicities, continue to be of great interest to both the chemical and pharmaceutical communities. In order to induce a desirable chemical response, many drugs target a specific enzyme, receptor, or ion channel in which the mode of action may not be completely understood.[1] Designing drugs which are capable of binding to a particular protein can be further complicated, due to the structural complexity of the protein target. Hence, there have been concerted efforts to develop drugs which would inhibit a disease-causing protein by targeting its precursor messenger RNA (mRNA), since it is believed that mRNA may be a more vulnerable link in the genetic cycle.[2] There appear to be considerable advantages in developing therapeutic agents which manipulate genetic expression at the level of mRNA translation, as opposed to specifically targeting a protein. Perhaps the most striking of these is efficiency, since each mRNA gives rise to multiple protein copies. Consequently, this strategy has stimulated considerable interest in the possibility of utilizing nucleic acids or modified oligonucleotides as potential therapeutic agents, in order to bring to fruition the promise of the antisense concept.

The applicability of this method was first successfully demonstrated by Zamecnik and Stephenson,[3] in which Rous sarcoma virus 35S RNA (the sense strand) was targeted with a tridecamer complementary to 13 nucleotides of the 3'- and 5'-reiterated terminal sequences of Rous sarcoma virus 35S RNA (the antisense agent), thereby inhibiting virus replication. The results of their early biological experiments suggested that antisense

oligonucleotides were finding their way into cells, because they had affected the functions of viral nucleic acids in the cytosol.[4] This was further suggested in experiments with radiolabelled oligonucleotides and their derivatives, in which the radioactive material had accumulated in the cell nuclei and cytoplasm.[5,6,7] Intracellular localization of the oligonucleotides was demonstrated in one of these experiments, via reactive alkylating oligonucleotide derivatives.[5] It was found that oligothymidylates reacted with the desired cellular RNA target (polyA). It was further suggested that complex formation was a necessary condition in order for reaction to occur, since modification was found to be temperature dependent. An interaction with cellular DNA was also observed in these experiments, and the specificity of this reaction suggested that at least a part of the process was sequence-specific.

Kinetic studies have suggested that oligonucleotides are taken up in cells in a saturable manner, and that the process is hampered by temperature decrease and by inhibitors of endocytosis, suggesting an endocytotic mechanism of uptake.[8] The aforementioned studies suggest that there are mechanisms (possibly endocytosis) which enable cells to internalize oligonucleotides, although the efficiency of such uptake is quite poor. To improve it, one can either attempt to employ a more efficient cellular uptake mechanism, or to develop artificial approaches in order to bypass the natural mechanisms.[4]

An antisense agent should satisfy three basic requirements if it is to effectively suppress gene expression.[2] First and foremost the drug should be sequence-specific, in order to selectively bind the target mRNA molecule. Secondly, the agent should be stable *in vivo*. Due to the susceptibility of native oligonucleotides toward nuclease degradation, unmodified nucleic acids do not appear to be suitable therapeutic targets. The ideal candidate therefore appears to be an oligonucleotide analogue which possesses sufficient stability toward nuclease degradation, so as not to compromise its efficacy. Lastly, the desired compound should be water soluble, but should also be capable of cellular uptake.

Several extensive reviews have recently described the various types of antisense oligonucleotides which have been synthesized and tested with these criteria in mind.[1,2,9,10,11,12] This thesis will not discuss in great detail the various approaches which have been investigated to date, but will instead focus on a class of analogues in which the ribose and phosphodiester moieties have been replaced by an uncharged acyclic backbone. Uncharged analogues appear to be particularly attractive in regard to cell membrane permeation, and have been reported to possess improved cellular uptake properties.[13,14]

The pioneering work in the development of these modified acyclic analogues was that of Pitha and Pitha, who investigated poly(N1-vinyluracil) **1**, poly(N1-vinylcytosine) **2** and related species.[15] Poly(N1-vinyluracil) was prepared by radical polymerization of N1-vinyl-4-ethoxy-2-pyrimidinone **3** followed by acid catalyzed hydrolysis of the resulting polymer.[16] 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.[17] The interaction of **1** with adenine derivatives and the interaction of **2** with polyguanylic acid were 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 analogues to form hydrogen-bonded complexes with complementary oligonucleotides is remarkable given the atactic nature of the backbone and due to the improper spacing of bases along the analogue backbone, as illustrated in Figure 1. 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 analogue-nucleic acid complex.

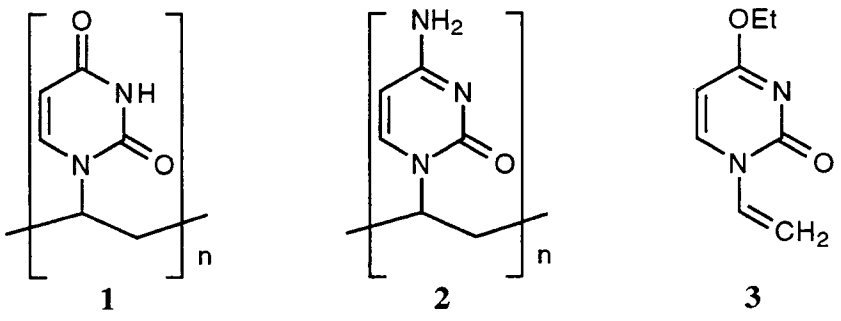
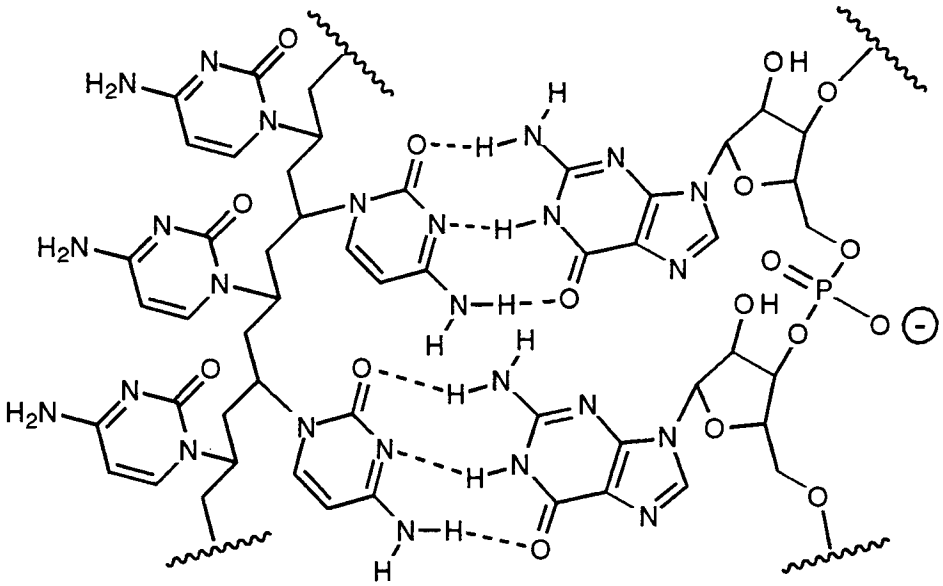
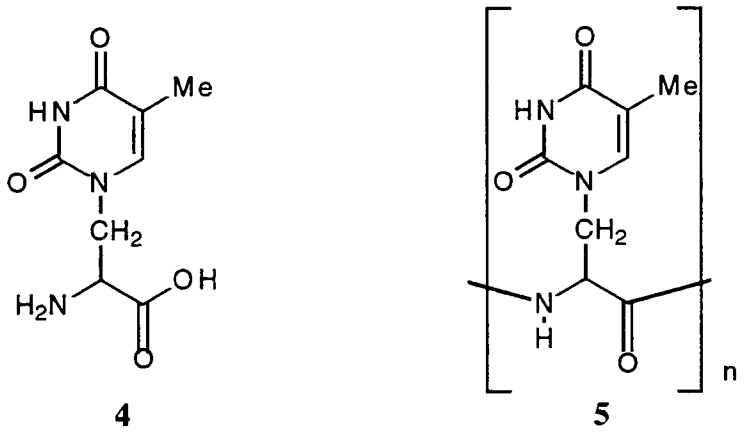


Figure 1. Interaction of Poly(N1-vinylcytosine) with Poly G



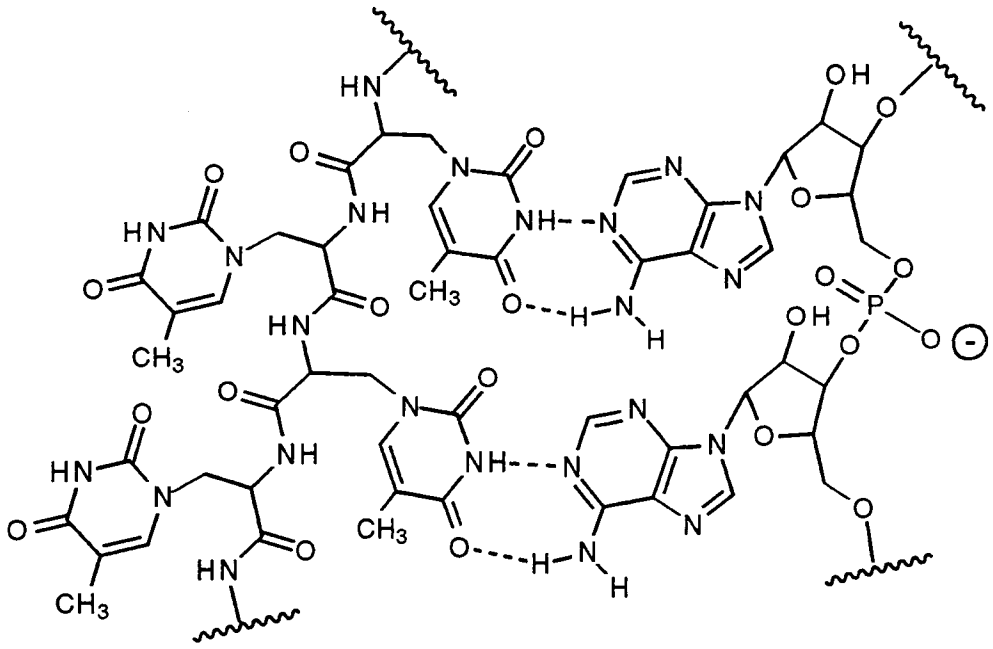
There are many examples of modified nucleic acids in the literature which do not employ the use of a stereochemically defined backbone and, consequently, diastereomeric mixtures are generated during their chemical syntheses. Since each diastereomer may have its own distinct binding affinity for its complementary nucleic acid sequence, it is advantageous to develop oligomers possessing either a diastereomerically pure or an achiral backbone. This ensures that the agent maintains a uniform binding constant to its target sequence, thereby maximizing its efficacy. The earliest example in the literature in which this stereochemical issue is considered is that by Jones and coworkers, who address the importance of obtaining a polypeptide of known stereochemistry in their uncharged, acyclic class of peptide analogues.[18] This is exemplified in the resolution of

DL-ß-(thymin-1-yl)alanine **4**, prior to polymerization. The resulting optically active amino acid polymers showed no evidence of base stacking however, 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 as illustrated in Figure 2, that due to the spacing of the bases on the backbone, only every other base is properly positioned for binding a complementary nucleic acid.



4 **5**

Figure 2. Interaction of Poly-β-(thymin-1-yl) alanine with Poly A

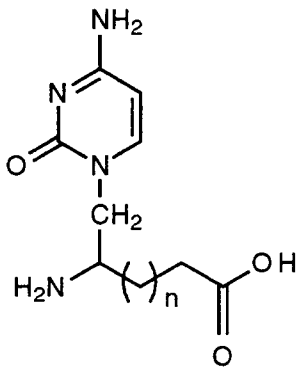


One feature common to both these early investigations is that they rely on polymerization reactions. Few examples in the literature to date have investigated systematic approaches toward planning and constructing these acyclic analogues. This is a key consideration if these analogues are to have significant therapeutic utility, since the ultimate future objective is to develop an analogue oligomer capable of sequence-specific binding to a particular gene 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 analogue 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 analogue backbone subunits and the mode of attachment of heterocyclic bases along the polyamide backbone.

Preliminary studies were performed by Prof. Dwight D. Weller in collaboration with Dr. James Summerton of Antivirals, Inc. using space filling (CPK) models to analyze the association of acyclic analogues and complementary nucleic acids. The feasibility of replacing the sugar-phosphate backbone of the nucleic acids with a polyamide-type backbone has been further investigated by Prof. Weller and Dr. Wilma Olson of Rutgers University, using molecular modeling techniques to examine the ability of the acyclic backbone to adopt low energy conformations that conform to the nucleic acid A- and B-form helices.[19]

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 analogue/nucleic acid duplex. The optimum length of the monomeric subunit

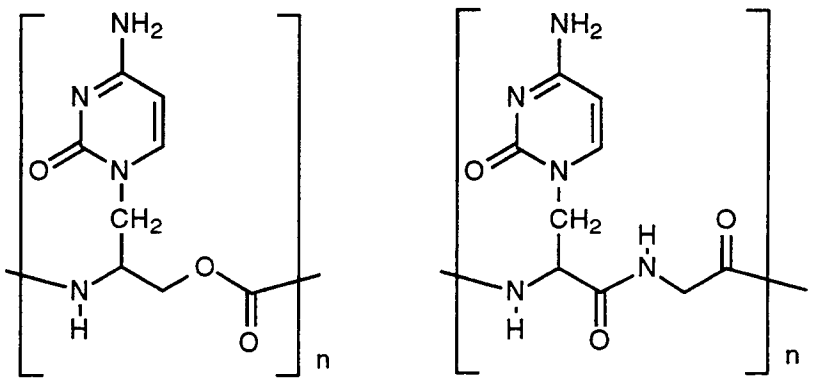
appears to be five or six atoms including the terminal amine and carboxyl group of each subunit. Several acyclic backbone possibilities of the amide type (nylons, polyurethanes, polypeptides) were considered, and we have thus far only reported the synthesis and oligomerization of hexamers derived from amino acid analogues **6** and **7**.[20] Due to problems associated with the solubility and purification of these oligomers in organic solvents, we have addressed the need for more efficient assembly of short oligomers via solid-phase synthesis (*vida infra*).[21,22]



6, n = 1
7, n = 2

The modeling studies further suggested that two of the more promising backbone types appear to be the polyurethanes **8** (due to their relatively low strain energy) and the polypeptides **9** (due in part, to their ability to adopt a low energy helical conformation incorporating an intermolecular 1,7-hydrogen bond).[19] The interactions of **8** and **9** with poly dG are shown in Figures 3 and 4, respectively. Of particular interest is the effect of stereogenicity on the ability of these two classes of analogues to pair with complementary RNA or DNA, since the modeling studies suggested a stereochemical preference with certain backbone types. Consequently we undertook a program to first develop stereochemically pure homooligomers containing only cytosine heterocyclic bases. Relatively shorter oligomers could then be utilized during biophysical testing procedures (due to the additional hydrogen bond in the GC base pair, as previously demonstrated in

the binding studies of the morpholine-derived oligomers),[23] which would simplify matters considerably in regard to chemical synthesis.



8 **9**

Figure 3. Interaction of Polyurethane **8** with Poly dG

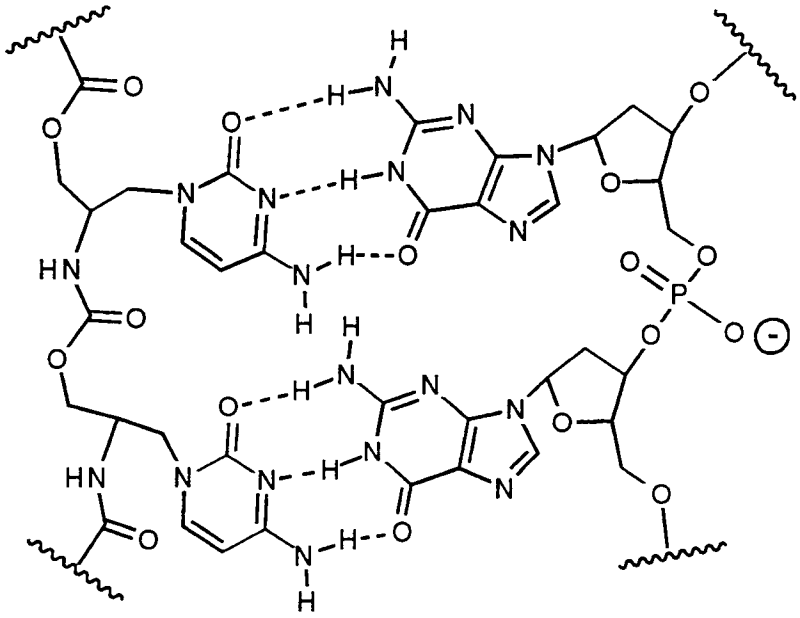
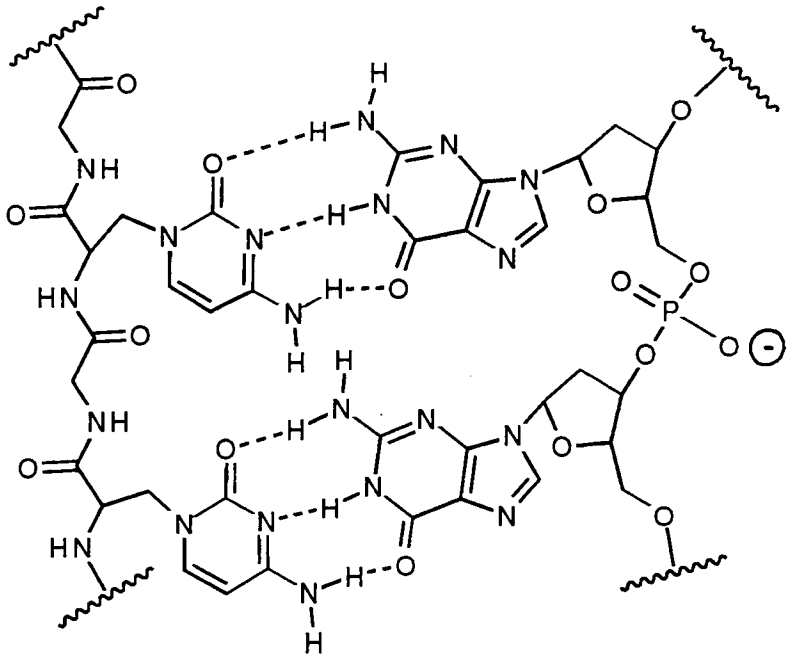
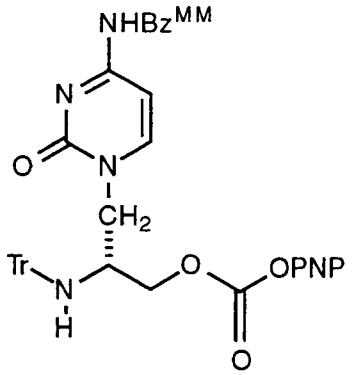


Figure 4. Interaction of Polypeptide **9** with Poly dG



We envisioned that compounds **10** and **12** (and their enantiomers, **11** and **13**) could be conveniently synthesized from (L)-serine {and (D)-serine, respectively}. Each configuration could thereby be obtained in optically pure form, so as to satisfactorily address the stereochemical ramifications, and standard peptide coupling procedures would enable the systematic assembly of subunits. These activated subunits could then be assembled via solid-phase synthesis, to afford the urethane-derived oligomers **14** and **15**, and the peptide-based oligomers **16** and **17**. Should any of the proposed backbone types prove to pair with its nucleic acid complement, this methodology could be easily extended to the remaining heterocyclic bases (G, A, and T), to allow the synthesis of heteropolymers.



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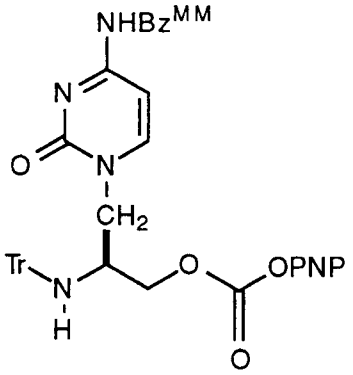

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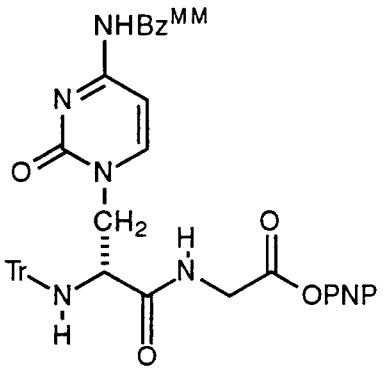

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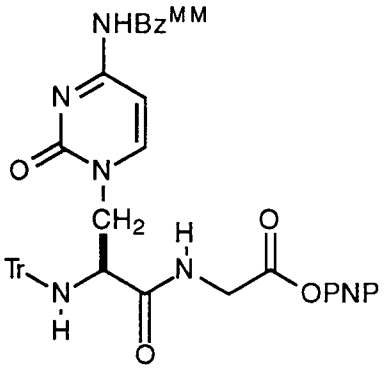

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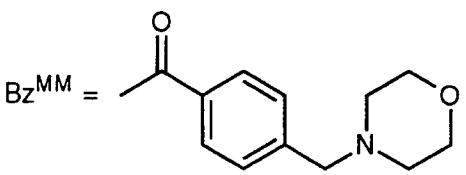
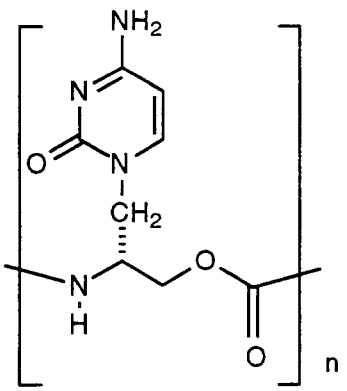
Tr = —C(Ph)$_3$





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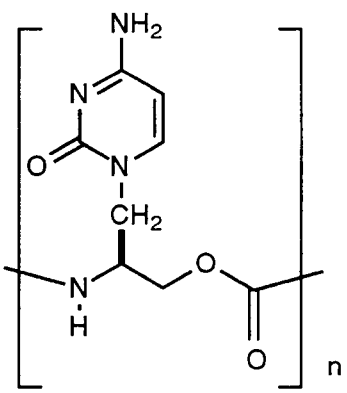

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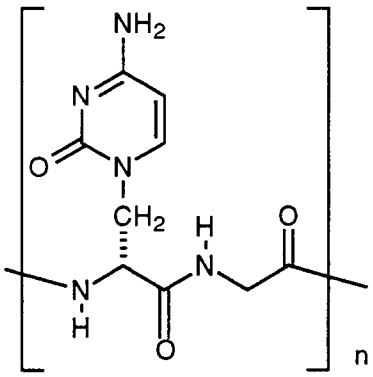

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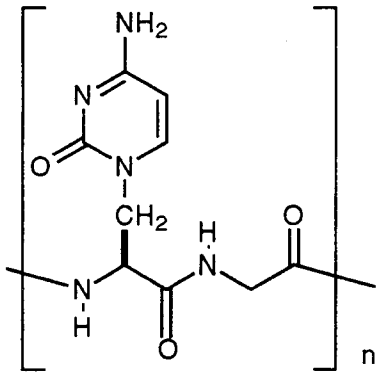
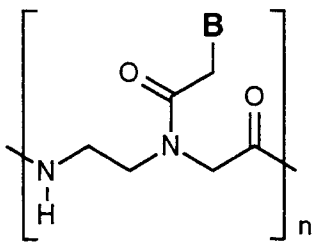


17

At the outset of this project, there were no examples in the literature in which an analogue (possessing either an achiral or a stereochemically defined backbone) was assembled in a systematic fashion *and* which was reportedly capable of sequence-specific pairing with its nucleic acid complement. Since then, however, one noteworthy class of compounds employing an achiral backbone, **18**, has appeared in the literature.[24] These so-called peptide nucleic acids (PNAs) reportedly have strong binding affinity to complementary nucleic acids,[25,26] and are also capable of inducing strand displacement of one of the two strands in double-stranded DNA, under low salt conditions.[24,27] Other recent publications by Nielsen and coworkers have followed,[28] including an account in which the sequence-specific inhibition of DNA restriction enzyme cleavage was obtained with the complementary PNA.[29] These results indicate that PNAs can be utilized as sequence-specific blockers of DNA recognizing proteins, and further suggest the applicability of using modified, acyclic analogues as code blockers.



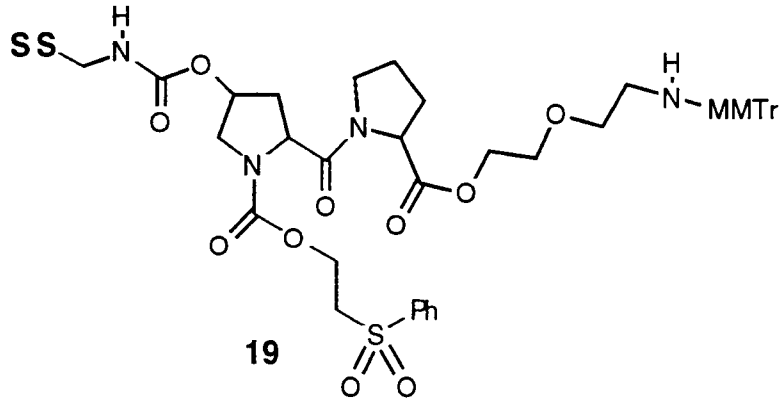
18

B = Adenine, Guanine, Cytosine, Thymine

II. RESULTS AND DISCUSSION

A. Development of a Universal Solid-Support

Previous difficulties associated with characterization of fully deprotected hexamers **6** and **7** by mass spectrometry, coupled with the fact that most prexisting solid-phase synthetic methods for nucleic acids entail the ammonolytic liberation of the base-protected species from the resin, led to a search for a new method to enable incorporation of a cleavable anchor (which would be suitable for the removal of fully protected analogue oligomers from solid supports). Universal solid-support **19** was consequently developed, and has proven to be an efficient and versatile means for the solid-phase assembly of uncharged nucleic acid analogue subunits.[30]

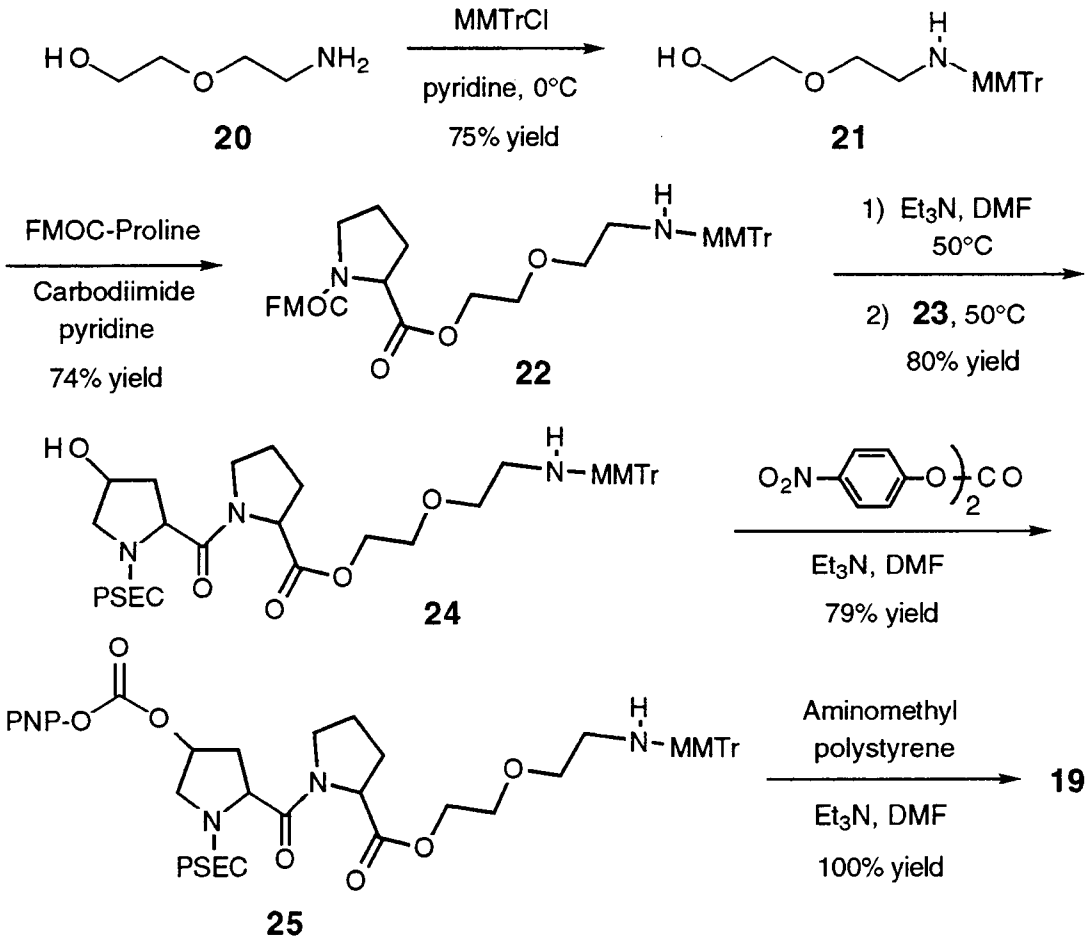


19

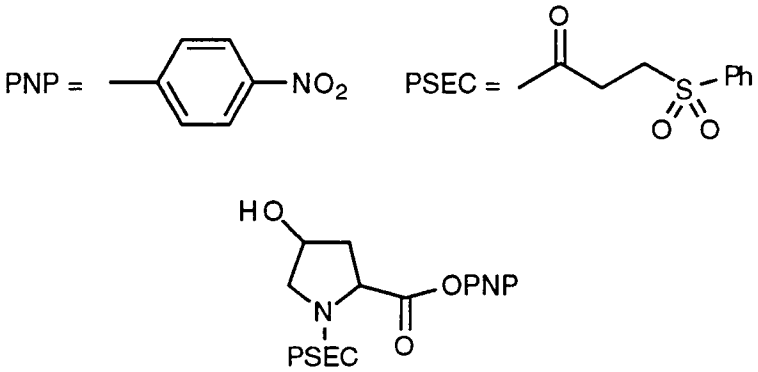
The strategy for the synthesis of **19** is outlined in Scheme I. The starting material utilized in the synthesis of **19** was 2-(2-aminoethoxy)ethanol **20**. Reaction of **20** with monomethoxytrityl chloride in pyridine, resulted in 75% yield of the protected amine **21**. This protected amine was then stirred with FMOC-proline and 1-(3-dimethylamino propyl)-3-ethylcarbodiimide methiodide in pyridine, to afford the proline ester product **22** in 74% yield. The FMOC-protected amino acid ester **22** was then dissolved in DMF containing triethylamine and heated gently to facilitate FMOC removal. To this amine-

containing solution was added active ester **23**, resulting in the formation of dipeptide product **24** in 80% yield. The synthesis and characterization of compound **23** is discussed elsewhere.[22] Subsequent reaction of the free hydroxyl of **24** with bis(*p*-nitrophenyl) carbonate in the presence of triethylamine in DMF provided 79% yield of mixed carbonate **25**. Attachment of this cleavable anchor to aminomethyl polystyrene gave rise to universal solid-support **19**. The loading capacity of resin **19** was quantitated via monomethoxytrityl assay at 476 nm in 20% trifluoroacetic acid/chloroform following protecting group removal, and was found to be 290 mmol/g of dry resin.

Scheme I



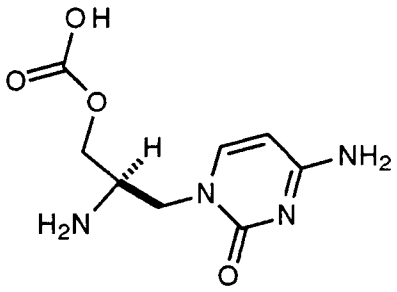
Scheme I, Continued



23

B. Preparation of Activated Subunits

With universal support **19** at our disposal, attention was directed toward the synthesis, oligomerization, and biophysical evaluation of two novel classes of stereochemically defined nucleic acid analogues, specifically those derived from the nonisolable five-atom carbonic acid **26** (and its enantiomer), and those derived from the modified six-atom alanylglycine **27** (and its enantiomer). I will focus first on the synthesis of the urethane-derived hexamer **28** (and **29**), and will later describe subsequent efforts toward the peptide-derived class of analogues **16** (and **17**), both conveniently available from a common synthetic intermediate, compound **30** (and its enantiomer).



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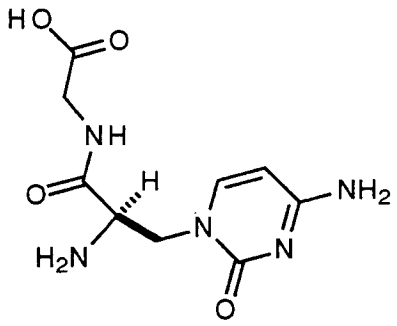

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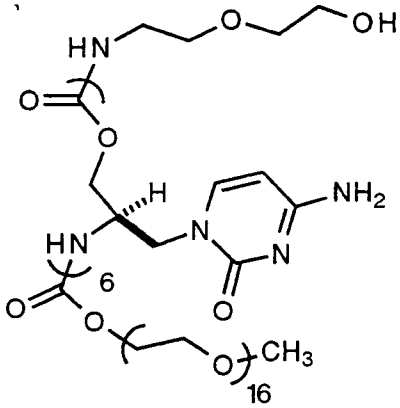

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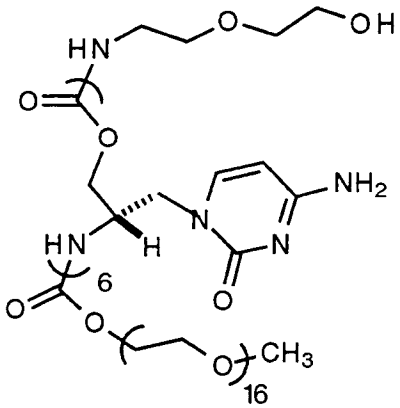

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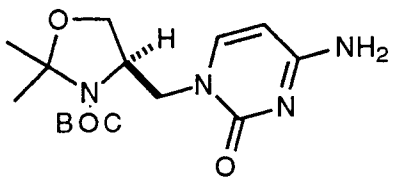
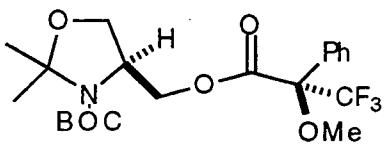


30

The strategy for synthesis of intermediate **30** and its conversion to activated monomer **10** is outlined in Scheme II. (R)-N-*t*-butoxycarbonyl-2,2-dimethyl-4-hydroxymethyl-1,3-oxazoline **31**, was prepared from (L)-serine in the usual manner.[31,32] Optical purity of alcohol **31** was established by reacting separately with each configuration of Mosher's reagent,[33] and analyzing the corresponding diastereomer via high resolution NMR spectroscopy. Analysis of the ^1H spectra of diastereomeric esters **32** and **33** revealed noticeable changes in chemical shift of two of the five oxazoline ring/exocyclic methylene protons, and neither diastereomer was found to be contaminated with the other, within the limits of detection (≤1%).



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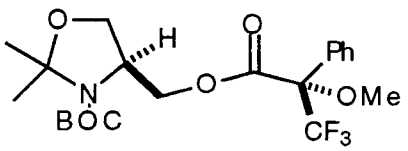
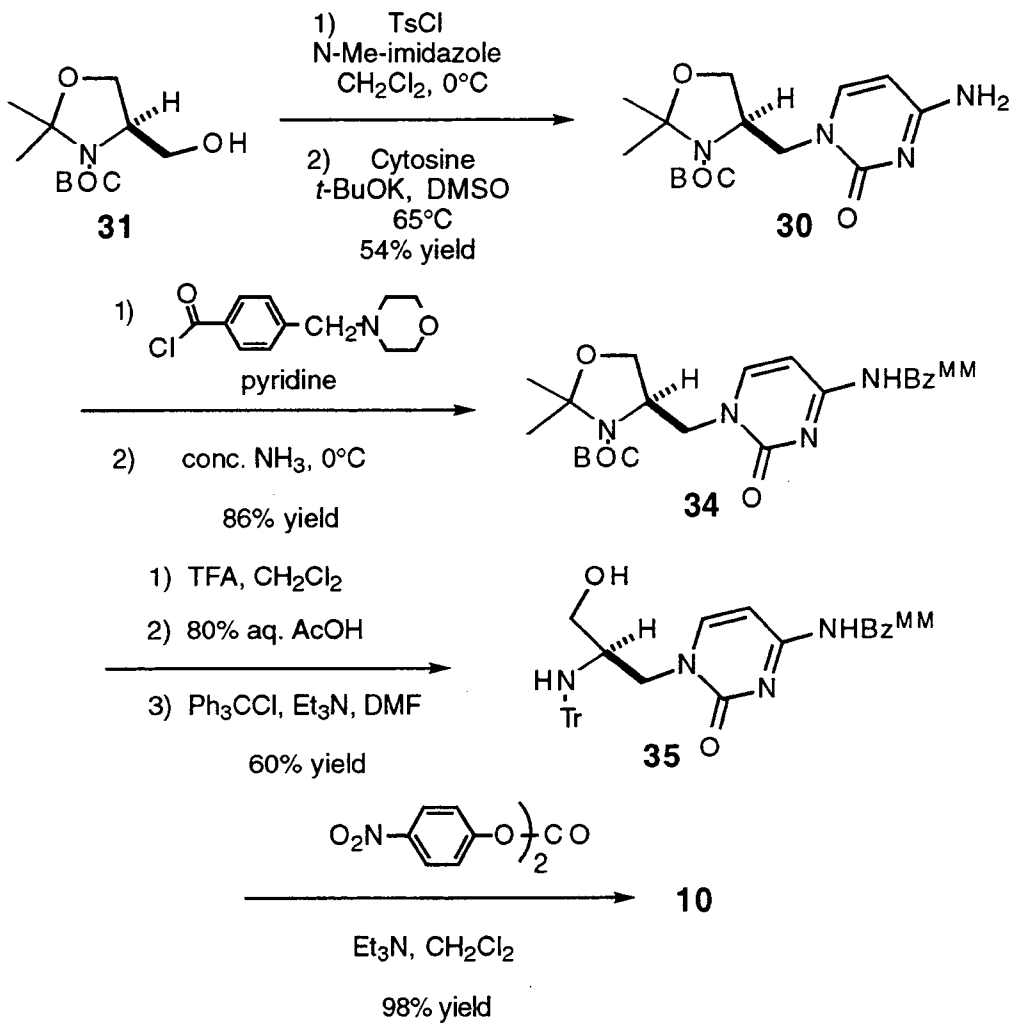


33

Reaction of **31** with tosyl chloride and N-methylimidazole in methylene chloride proceeded smoothly to provide pure tosylate, which when reacted with the potassium salt of cytosine in dimethyl sulfoxide gave rise to alkylated species **30** in 72% yield. Acylation of cytosine with the morpholine-derived ionizable protecting group in pyridine,[16,17] proceeded smoothly to provide 86% yield of fully protected hemiaminal **34**. An ionizable protecting group for cytosine was desirable to enable purification of the protected hexamers via ion-exchange chromatography. Following cleavage of both *t*-BOC and hemiaminal protecting groups, the resulting amine was tritylated to provide the

desired alcohol **35** in 60% yield. Reaction of alcohol **35** with bis-*p*-nitrophenyl carbonate and triethylamine in methylene chloride served both as a method to elongate the backbone by one carbon, and to activate the carboxyl terminus as the *p*-nitrophenyl ester, affording the activated monomer **10** in 98% yield. Enantiomeric carbonate **11** was similarly obtained following the same sequence of synthetic transformations as described in the synthesis of **10**, with the exception that D-serine was substituted for L-serine as starting material. Optical purity of **11** was confirmed by comparing the rotation values of **10** and **11**, which were found to be of equal and opposite magnitude.

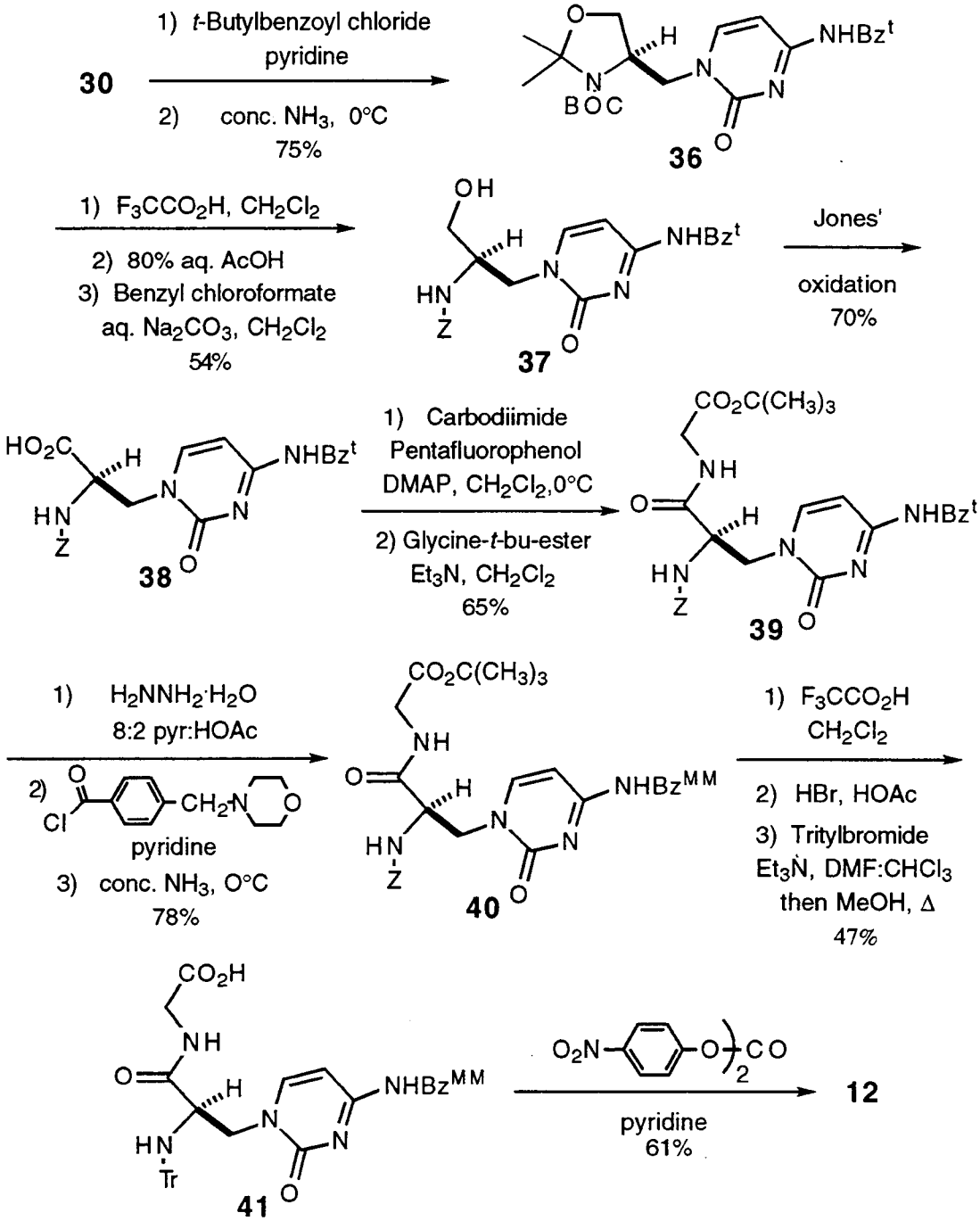
Scheme II



The synthesis of the activated alanylglycine subunit **12** (and its enantiomer, **13**) was similarly undertaken and is outlined in Scheme III, in which intermediate **30** (and its enantiomer) was again utilized as the starting material. Acylation of **30** with *t*-butylbenzoyl chloride in pyridine resulted in 75% yield of fully protected hemiaminal **36**. Cleavage of both *t*-BOC and hemiaminal protecting groups and subsequent reaction of the free amine with benzyl chloroformate and aqueous sodium carbonate in methylene chloride provided 54% yield of N-benzyloxycarbonyl-amino alcohol **37**. The choice of protecting group on the amine terminus was important since it needed to withstand the reaction conditions of the oxidation, and due to an earlier report that CBZ-protected amino alcohols proceed with little or no racemization.[34] Jones' oxidation of **37** afforded the desired N-benzyloxycarbonyl-amino acid **38** in 70% yield.

The activation method of choice was conversion of **38** to the pentafluorophenyl ester, since the reaction of N-benzyloxycarbonyl-S-benzyl-cysteine pentafluorophenyl esters with glycine and other amino acids, has been shown to proceed quickly with minimal racemization during peptide bond formation.[35] (Less reactive active esters reportedly result in considerably more racemization.) Reaction of the pentafluorophenyl ester with glycine-*t*-butyl ester resulted in an overall coupling yield of 65% of the fully protected dipeptide derivative **39**. Selective N-debenzoylation of the cytosine protecting group,[36] and acylation with the ionizable protecting group resulted in 78% yield of the dipeptide analogue **40**. Both *t*-butyl ester and N-benzyloxycarbonyl protecting groups were cleaved under acidic conditions, and the nitrogen terminus was tritylated[37] to afford 47% yield of carboxylic acid **41**. The carboxylic acid terminus was subsequently activated as the *p*-nitrophenyl ester, to afford 61% of the desired activated monomeric subunit **12**. (D)-serine was similarly converted to the enantiomeric *p*-nitrophenyl ester **13**, following the same sequence of synthetic transformations.

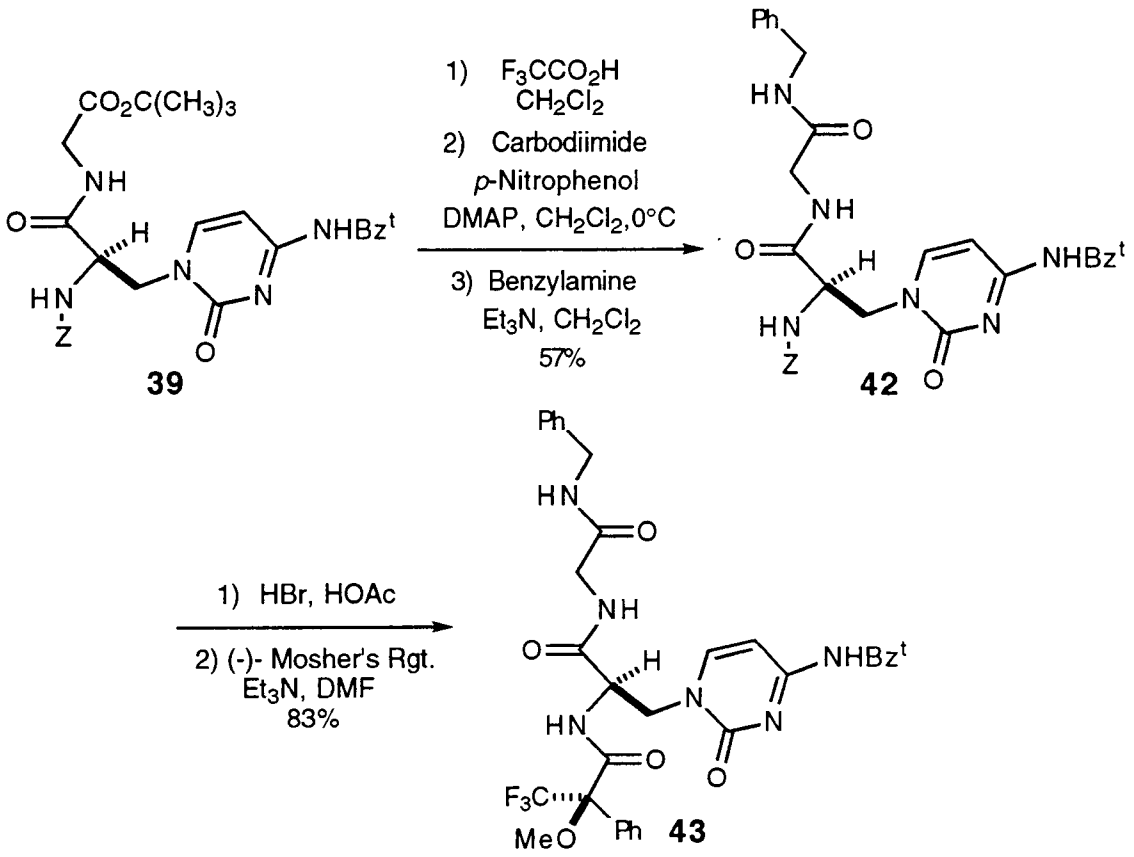
Scheme III



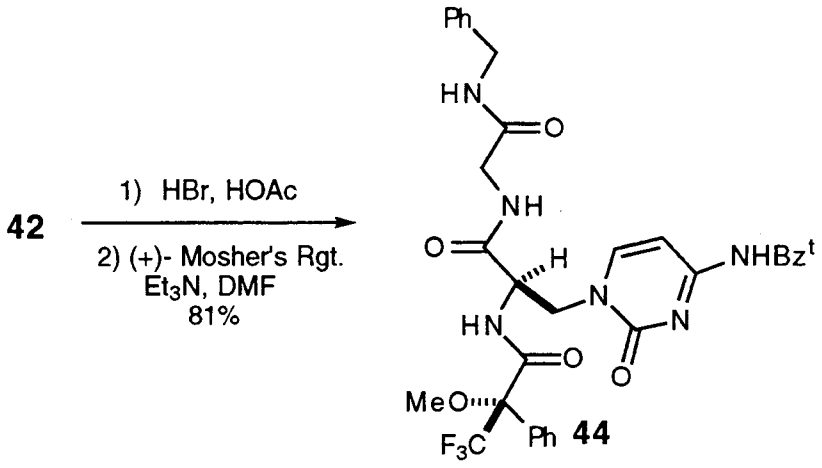
The stereochemical purity of compound **39** (following Jones' oxidation and peptide bond formation) was similarly investigated, despite the previous report of Jones' oxidation on N-CBZ-amino alcohols proceeding with little or no racemization.[34]

Conversion of **39** to the Mosher's derived amides **43** and **44** is shown in Scheme IV. High resolution NMR spectroscopic analysis of diastereomeric amides **43** and **44** revealed noticeable changes in the chemical shift of the methine proton at the stereocenter of the subunit, and one of the methylene protons adjacent to the chiral center. Consequently, it was confirmed that no racemization had occurred during either the oxidation or coupling (within the limits of detection, ≤1%), as evidenced by the absence of any contaminating diastereomeric protons.

Scheme IV



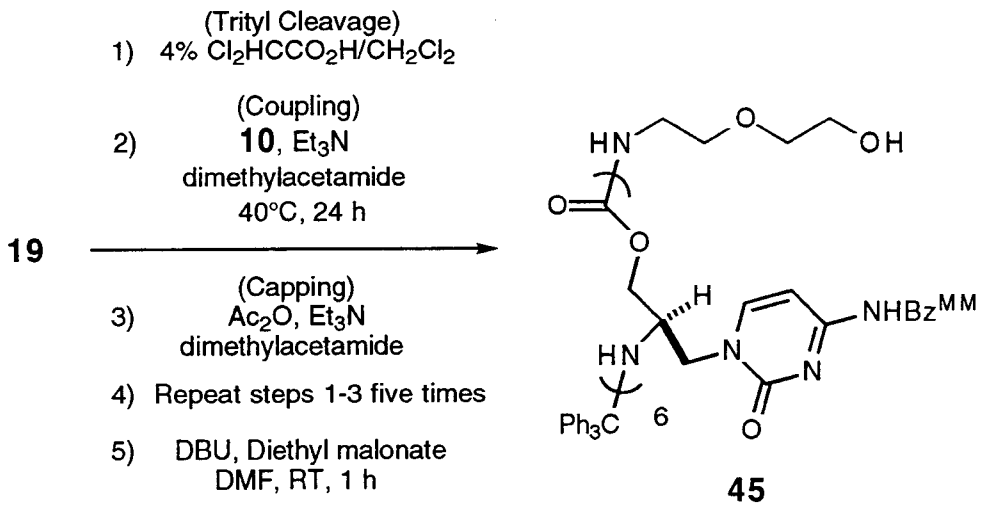
Scheme IV, Continued



C. Oligomer Synthesis

Activated monomer **10** was then utilized in solid-phase synthesis as described in Scheme V. The apparatus utilized in the solid-phase syntheses is shown in Figure 5. Cleavage of monomethoxytrityl on universal support **19**, coupling of the resulting amine with activated monomer **10**, and capping of any unreacted amine with acetic anhydride resulted in convenient stepwise elongation of subunits which, following five repetitions and subsequent cleavage of fully protected hexamer from the solid support with DBU in DMF, provided a highly efficient oligomerization (72% yield) of the protected hexamer **45** (and similarly hexamer **46**, utilizing activated monomer **11**). Compounds **45** and **46** were then subjected to mass spectral analysis. Positive FABMS of **45** (and **46**) revealed a single high molecular weight ion cluster at the expected 2827, corresponding to $[M+H]^+$. Structural confirmation of the corresponding free amines (following detritylation and purification), was similarly obtained via positive FABMS.

Scheme V



Scheme V, Continued

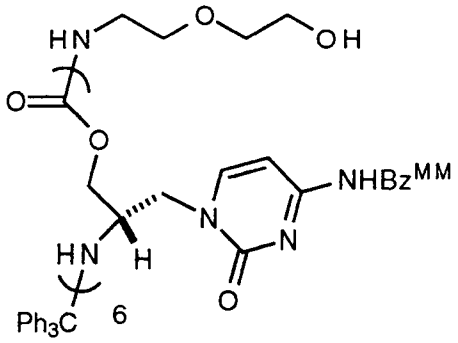


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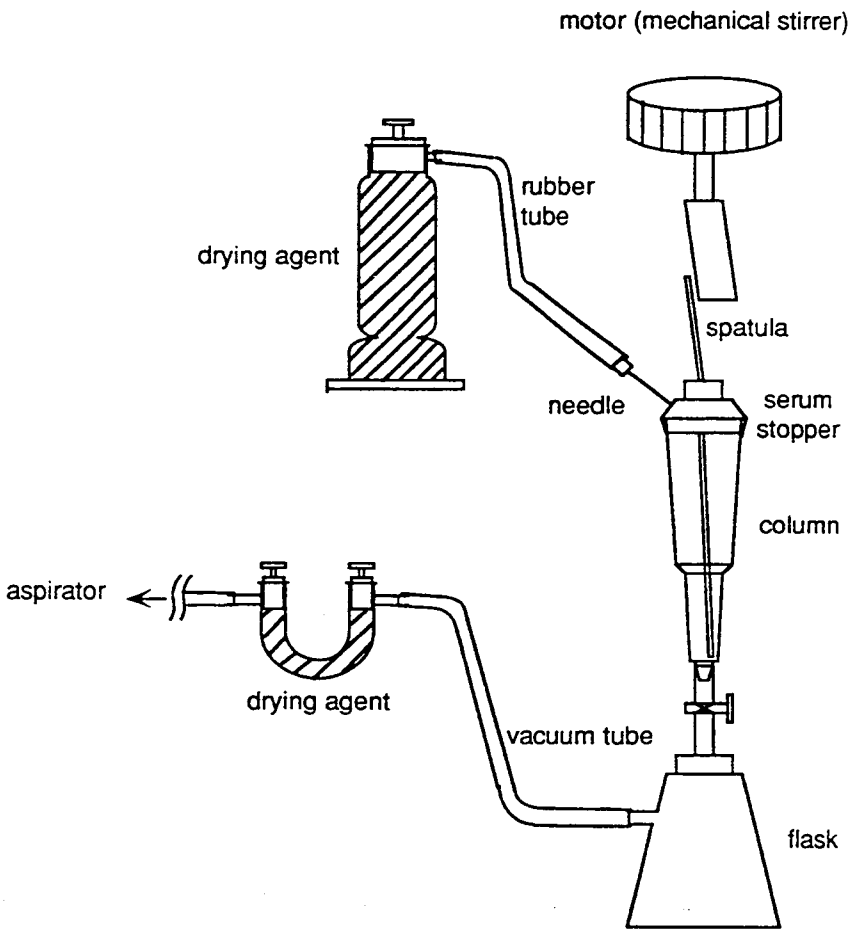
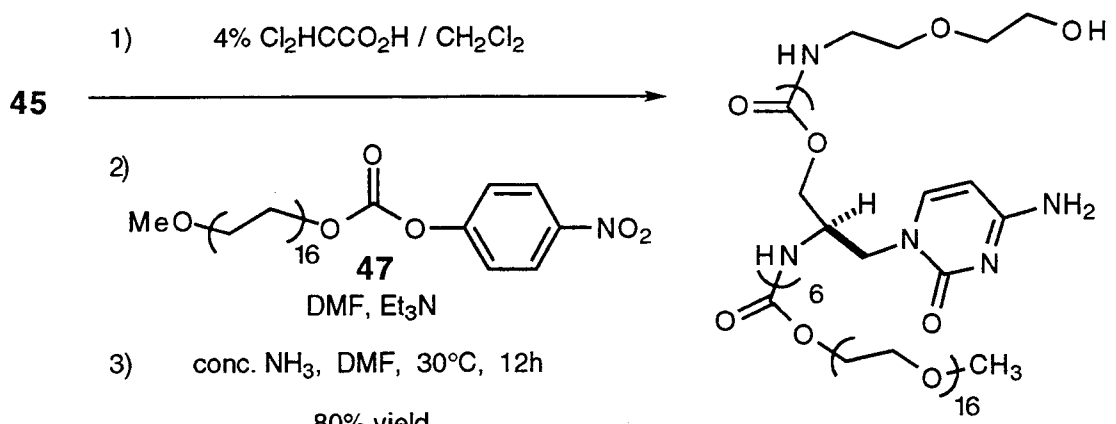
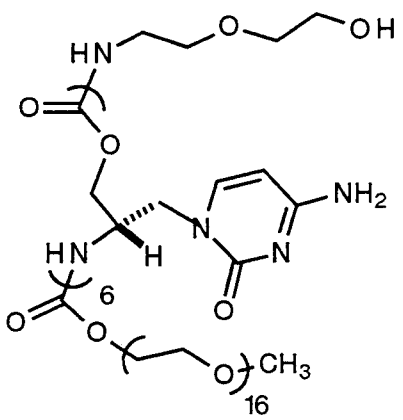
Figure 5. Apparatus for Solid-Phase Synthesis



Hexamer **45** was then converted to a compound suitable for biophysical evaluation as shown in Scheme VI. Reaction of **45** with dichloroacetic acid resulted in detritylation, and the amine terminus was subsequently capped by treating with the mixed *p*-nitrophenyl carbonate (derived from polyethylene glycol monomethyl ether, average MW = 750 g/mol), **47**.[23] Ion-exchange chromatographic purification on S-Sepharose, ammonolysis, repurification, and subsequent desalting on polypropylene, provided the desired compound **28**. The same sequence of synthetic transformations gave rise to hexamer **29** (derived from monomeric subunits of the opposite configuration), readily obtainable from D-serine in an analogous fashion.

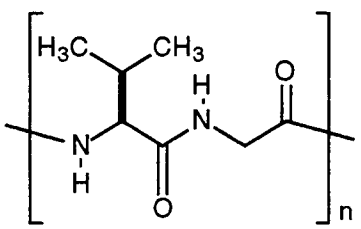
Scheme VI





Activated peptide subunit **12** was similarly taken on to solid-phase synthesis utilizing universal support **19** (as described previously in Scheme V, for the synthesis of hexamers **45** and **46**). Due to a shortage of material however, the oligomerization in the peptide series was attempted on a 5 mmol scale in which only a two-fold excess of the activated subunit was employed. The coupling reactions were monitored utilizing UV spectroscopy (by quantitating trityl at 432 nm). The combined Cl_2HCCO_2H washes and CH_2Cl_2 rinses from each trityl deprotection reaction were evaporated *in vacuo*, and dissolved in 20% F_3CCO_2H for UV assay. The initial attachment proceeded in 93% yield, but after the second coupling only 23% remained attached to the solid-support. Following the third coupling, a dismal 3% remained attached to the solid-support.

It was unclear at this point whether a poor coupling reaction was the source of our problem, or whether we were simply working with unrealistically small amounts of material (and solvent) in the coupling reactions. What had become evident, however, was that more of the activated subunits **10** and **11** would be needed to enable the synthesis of oligomers **16** and **17**. During the time allotted to remake the activated subunits, I opted to simultaneously investigate the proposed coupling reaction, through the use of a model system which resembled the desired oligomers. Consequently, attention was shifted to the valylglycine model oligomer **48**, which was used to probe the proposed coupling reaction and ascertain if a similarly hindered primary amine could be efficiently coupled with an activated glycine residue.



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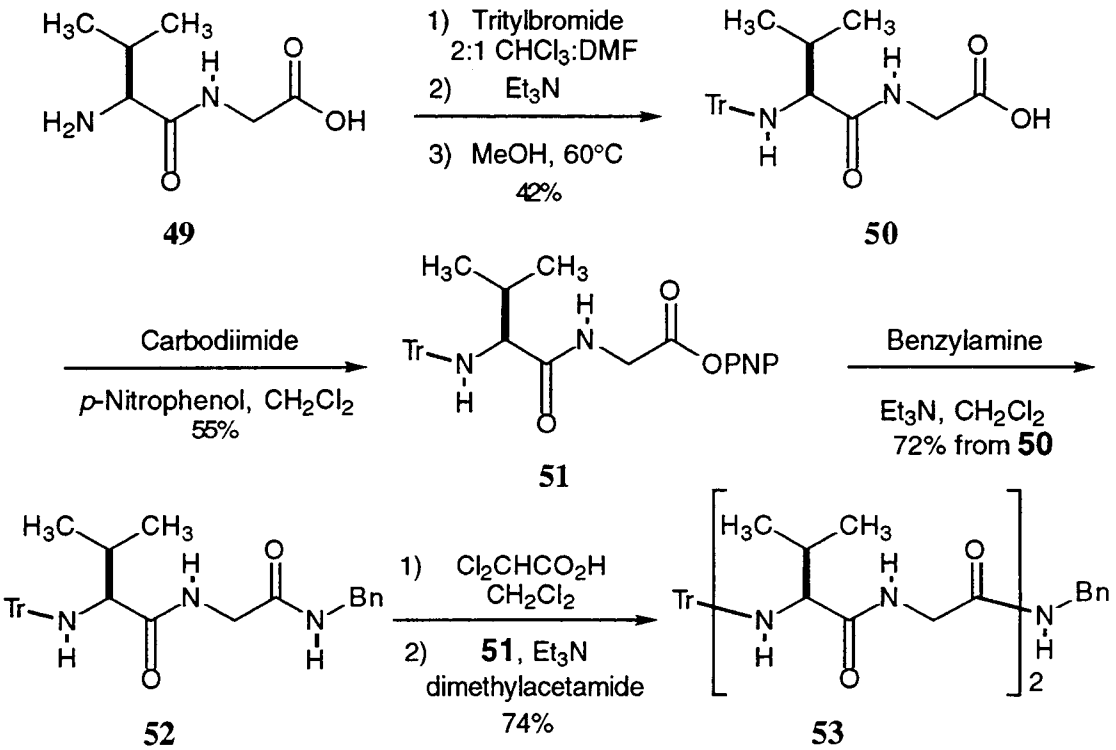
The synthesis of the activated subunit utilized in the model system is outlined in Scheme VII. Valylglycine **49** was reacted with trityl bromide and triethylamine, which following methanolysis afforded 42% yield of N-trityl-valylglycine **50**. Activation of the carboxylic acid as the *p*-nitrophenyl ester resulted in 55% yield of the activated model subunit **51**. The carboxyl terminus of the model was capped by reacting the activated subunit directly with benzylamine, thereby affording intermediate **52** in 72% overall yield from compound **50**. The trityl protecting group was removed upon treatment with 4% $Cl_2HCCO_2H/CHCl_2$. The amine salt was precipitated from 1:1 Et_2O:hexanes, and was reacted with the activated model subunit **51** and triethylamine, to afford 74% yield of the desired model oligomer **53**.

Scheme VII

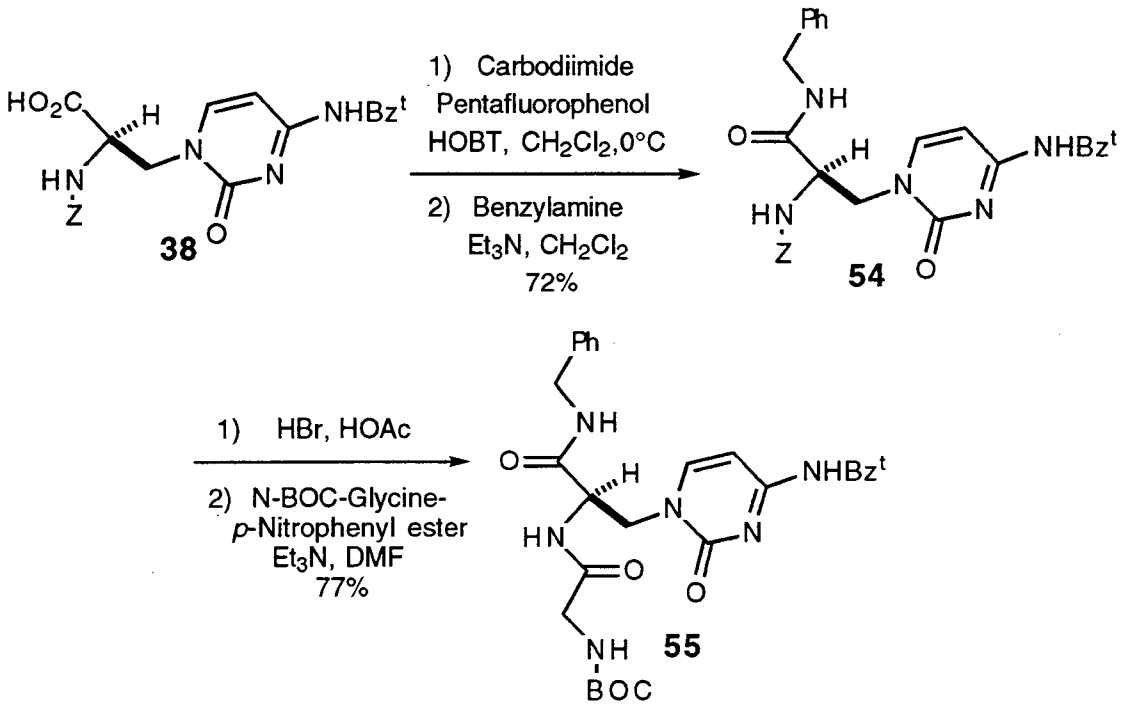


Having demonstrated that a similarly hindered primary amine could be reacted with an activated glycine residue in solution, attention was shifted toward solid-phase

assembly utilizing universal solid-support **19**. The standard protocol previously described in the synthesis of the urethane hexamers (Scheme V) was again followed, with the exception that the model activated subunit **51** was employed. After three couplings (quantitating each trityl deprotection reaction via UV at 432 nm), it was found that 93% of the model oligomer remained attached to the solid-support. The results from the model study, therefore, implied that it should be possible to adequately couple the modified alanylglycine subunits in the proposed manner.

It has recently been demonstrated that the amine terminus of the modified alanyl subunit benzylamide **54**, can also be effectively coupled with an activated glycine residue as outlined in Scheme VIII. Consequently, this result further suggests that the proposed methodology could be similarly applied to activated subunits **12** and **13**, and enable the solid-phase synthesis of the peptide-derived oligomers **16** and **17**.

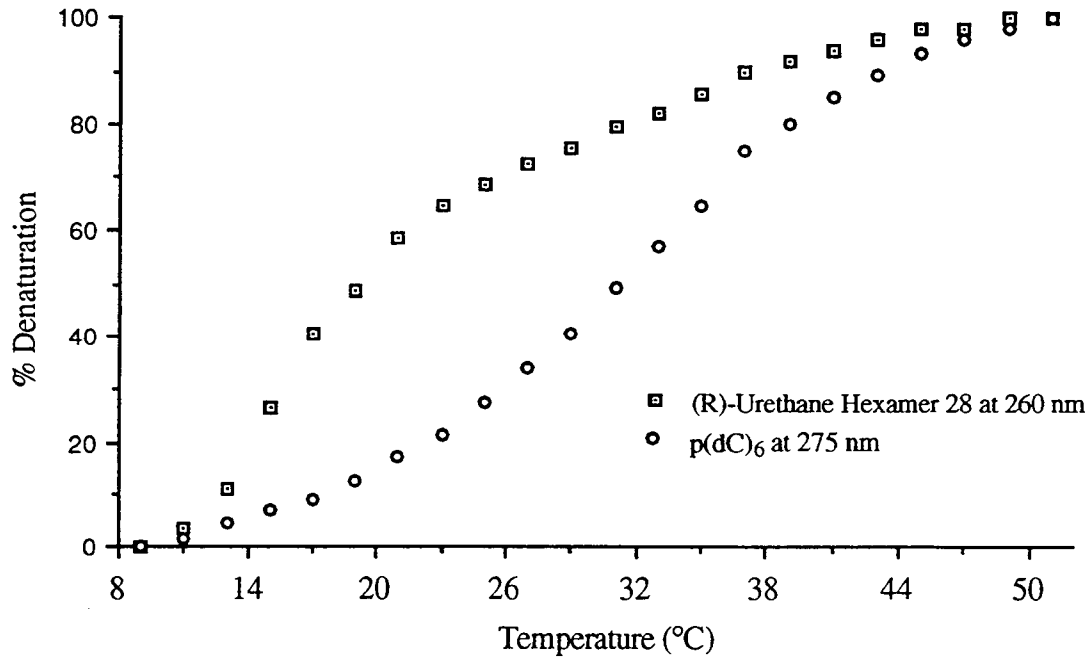
Scheme VIII



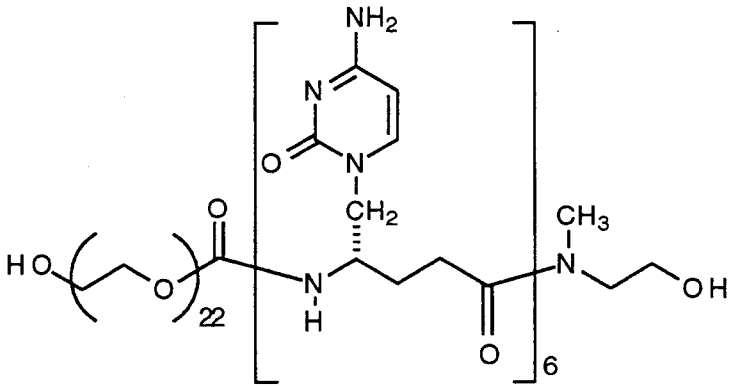
D. Biophysical Testing

Each enantiomer of urethane-derived hexamer was analyzed for its ability to bind the complementary DNA target $p(dG)_6$ or RNA (poly G) utilizing thermal denaturation experiments under low salt conditions. Neither **28** nor **29** was found to pair with complementary RNA. Experimental results also indicated that **29** did not pair with its target DNA. However, the (R)-configuration hexamer **28** was found to bind to its DNA complement with T_m = 19°C, as evidenced by the characteristic melting curve shown in Figure 6. The observed binding affinity of **28** with $p(dG)_6$, albeit weaker than the binding of the control oligomer, $p(dC)_6$ (in which T_m = 32°C), demonstrates the plausibility of hybridizing uncharged oligonucleotide analogues with native nucleic acids.

Figure 6. Thermal Denaturation of (R)-Urethane Hexamer **28** and $p(dC)_6$ vs. $p(dG)_6$



Although both the (S)- and (R)-configurations of the 5-atom nylon-derived hexamers (**56** and **57**, respectively) have been made previously,[20,38] neither of these analogues has to date been analyzed for its ability to bind with complementary DNA or RNA. Consequently, we report here for the first time the results from the corresponding thermal denaturation experiments, in which neither **56** nor **57** was found to pair with its complementary DNA [p(dG)$_6$)] or RNA [poly G] target under low salt conditions.



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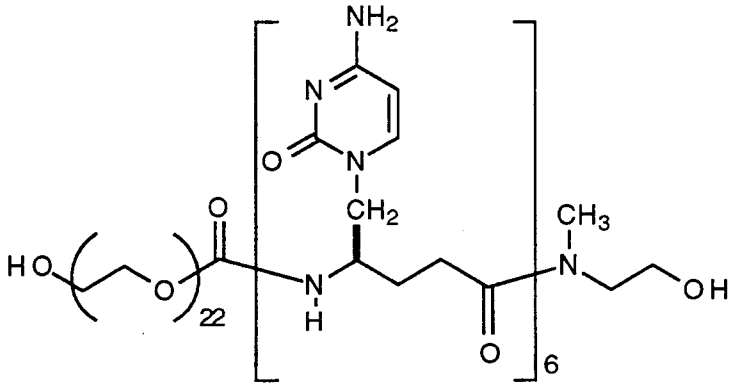

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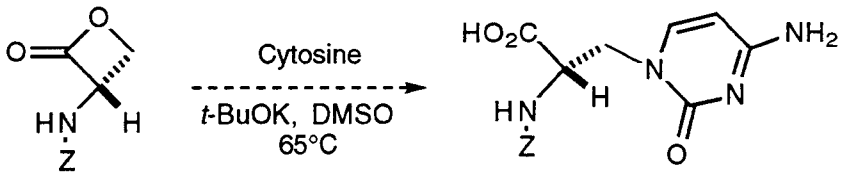
III. CONCLUSIONS

Universal solid-support **19** has been developed to enable the solid-phase synthesis of uncharged oligonucleotide analogues, and has been found to be a versatile means of assembling a variety of amide- and carbamate-derived backbones. The synthesis of mixed *p*-nitrophenyl carbonates **10** and **11** is also described herein, and these activated subunits have been utilized in the solid-phase synthesis of the urethane-derived hexamers **45** and **46** (employing universal solid-support **19**). The synthesis of *p*-nitrophenyl esters **12** and **13** has also been described, and in due course, solid-phase methods will be employed to assemble these (or related) activated subunits and complete the synthesis of the peptide-derived oligomers **16** and **17**. Both of the (R)-configuration activated subunits **10** and **12** have been synthesized from a common synthetic intermediate **30**, and conversely, both of the (S)-configuration activated subunits **11** and **13** have been synthesized from its enantiomer.[31,32] Each class of the analogue subunits has also been shown to be stereochemically pure, which is an important consideration if the completed analogue oligomers are to possess a uniform binding constant for their complementary nucleic acid targets.

The synthesis of the urethane-derived analogues was found to be convergent and highly efficient, but in hindsight, there appear to be alternative (and more convergent) routes toward the synthesis of the peptide-derived analogues. Hence, our original plan to utilize a common synthetic intermediate for the synthesis of both the urethane- and peptide-derived analogues might not have been the optimal method. The synthetic route could perhaps be simplified considerably via an alkylation reaction, as envisioned in Scheme IX, whereby cytosine could be attached to (and ring-open) the known lactone **58**.[39] Nevertheless, there appears to be one advantage for synthesizing the peptide-derived subunits as described herein, and that is the ability to convert (L)-serine into the unnatural (D)-configuration of the alanylglycine-derived peptide backbone.

Scheme IX

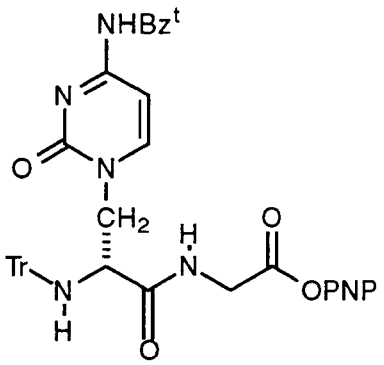


58 **59**

Another important consideration in the synthetic design of the peptide-derived analogues, is the mode of subunit assembly during the solid-phase synthesis. The choice to develop activated dipeptide subunits (such as **12** and **13**) for the synthesis of the desired hexamers, is preferable to the alternative approach (in which an activated alanyl-derived monomer *and* an activated glycine residue would be employed), since only six coupling reactions would be required during the solid-phase synthesis (as compared to twelve in the alternative route). The proposed method for solid-phase synthesis of the peptide-derived hexamers is advantageous, furthermore, since it utilizes an activated glycine residue in all six of the dipeptide subunit coupling reactions. Six of the twelve coupling reactions, in the alternative approach, would involve an activated modified alanine residue (and the remaining six would involve an activated glycine residue). Consequently, an *in situ* activation would be required for each alanyl-derived subunit (prior to coupling with the free amine of the terminal glycine residue), since a more reactive active ester than *p*-nitrophenyl would be required (e.g., pentafluorophenyl), in order to prevent racemization during these particular coupling reactions.[35]

The proposed coupling reaction has been investigated in solution, and although it is believed that an activated dipeptide subunit is the method of choice, we have recently discovered that deprotected uncharged higher oligomers (22mers in another class of analogues) are suitable for mass spectral analysis, if laser desorption mass spectrometry (LDMS) is employed (unpublished results). In an effort to further simplify the synthesis of the peptide-derived analogues, we therefore propose to utilize activated dipeptide

subunits **60** and **61** (employing a *t*-butyl benzoyl protecting group on cytosine), since the morpholine-derived protecting group has been found to no longer be necessary. Efforts to synthesize the peptide-derived analogues **16** and **17** via solid-phase methods are ongoing, and we hope to report on their biophysical (binding) properties in the near future.



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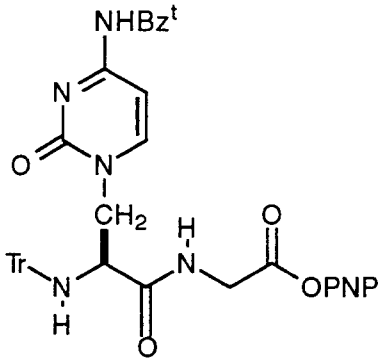

61

Computer modeling studies had previously suggested that the S-configuration urethane-derived hexamer **29**, would be a more promising molecule than the R-configuration hexamer **28** for binding to complementary DNA.[19] Discrepancies between the computer assisted predictions of duplex stability and the experimental binding results imply perhaps, that simple searching and analysis of potential duplex conformations may not be capable of determining whether uncharged oligonucleotide analogues are capable of pairing with complementary nucleic acids. Nevertheless, the positive binding results described herein for **28** (and the aforementioned successes reported in the literature[24-29]), suggest that uncharged acyclic oligonucleotide analogues have potential merit as single-stranded nucleic acid binding agents for diagnostic and therapeutic antisense applications.

IV. EXPERIMENTAL

Methylene chloride (CH_2Cl_2), pyridine, dimethylformamide (DMF), dimethylacetamide (DMA), and dimethyl sulfoxide (DMSO) were distilled from powdered calcium hydride (CaH_2) and stored over 3Å/4Å molecular sieves. Methanol (MeOH) and ethanol (EtOH) were distilled from the corresponding magnesium alkoxides and stored over 3Å molecular sieves. Diethyl ether (Et_2O) was freshly distilled from sodium/benzophenone prior to use. All other reagents were purified by distillation or recrystallization prior to use whenever necessary. All moisture sensitive reagents were transferred in a dry box or via a syringe under a positive pressure of nitrogen. All moisture sensitive reactions were carried out under a positive pressure of inert gas. Column chromatography was performed by using silica gel 60 (Merck, 340 - 400 mesh ASTM). Chromatography solvents were distilled before use. Analytical TLC was conducted on precoated Merck silica gel 60 F_{254}, J. T. Baker silica gel IB-F or Merck aluminium oxide 60 F_{254} (neutral, type E) plates. The ^{1}H-NMR spectra (including 2D experiments) were taken on either AM-400 or AC-300 Bruker spectrometers with tetramethylsilane (TMS) as internal standard. The chemical shifts (δ) are reported in parts per million (ppm) downfield from TMS. Infrared spectra (IR) were obtained on a Nicolet 5DXB FT-IR spectrometer. Mass spectra were recorded on a Kratos MS50RF mass spectrometer. Ultraviolet spectra (UV) were obtained on an IBM 9420 UV-Vis spectrophotometer. Optical rotations were measured with a Perkin-Elmer model 243 polarimeter. Melting points were determined on a Buchi capillary melting point apparatus and are not corrected. S-Sepharose was purchased from Sigma. Thermal denaturation experiments were performed utilizing a Carey 1 type UV-Vis spectrophotometer (Varian).

2-[2-(Monomethoxytrityl)aminoethoxy]-ethanol (21). To a 0°C solution of monomethoxytrityl chloride (3.09 g, 10.0 mmol) in anhydrous pyridine (20 mL), was added 2-(2-aminoethoxy)ethanol **20** (3.15 g, 30.0 mmol). The reaction mixture was allowed to warm to RT over 30 min, and solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (300 mL), washed with H_2O (2 x 100 mL) and satd $NaHCO_3$ (2 x 100 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated *in vacuo*. The crude product was chromatographed twice (SiO_2, 33 - 67% ethyl acetate/hexane) to afford 2.83 g (75% yield) of **21** as an amber-colored glass. In order to prevent decomposition, three drops of N,N-diethylaniline were added to the sample prior to loading on the column. ^1H NMR (300 MHz, DMSO-d_6): δ 7.39 (2H, d, J = 7.53 Hz), 7.31 - 7.26 (6H, m), 7.17 (2H, t, J = 7.11), 6.86 (2H, d, J = 8.80 Hz), 4.56 (1H, t, J = 5.48 Hz, exchanged with D_2O), 3.72 (3H, s), 3.50 (2H, t, J = 6.10 Hz), 3.44 (2H, t, J = 5.20 Hz), 3.35 (2H, t, J = 5.05 Hz), 2.60 (1H, t, J = 7.87 Hz), 2.13 (2H, q, J = 13.45, 6.15 Hz). IR (Neat): 3423, 3326, 3058, 3027, 3001, 2927, 2909, 2864, 1606, 1508, 1450, 1298, 1248, 1210, 1181, 1125, 1064, 1034, 905, 831, 767, 706 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 378.2 ($[M+H]^+$, 3.2), 300.2 (24.4), 274.1 (37.8), 273.1 (100.0), 165.1 (7.8), 136.0 (6.7). HRMS (Pos. FABMS): calcd for $C_{24}H_{28}NO_3$ $[M+H]^+$ 378.2069, found 378.2068.

FMOC-proline-2'-[2'-(monomethoxytrityl)aminoethoxy]-ethanoate (22). Compound **21** (0.973 g, 2.58 mmol) was combined with FMOC-proline (1.22 g, 3.63 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (1.15 g, 3.87 mmol) in pyridine (12 mL) and allowed to stir at RT for 2 h. Additional carbodiimide (0.340 g, 1.14 mmol) was then added and the reaction mixture was allowed to stir an additional 2 h. A small portion of H_2O (0.5 mL) was added, and the solvents were removed under reduced pressure. The residue was dissolved in EtOAc (300 mL),

washed with H_2O (100 mL) and satd $NaHCO_3$ (2 x 100 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated *in vacuo*. The crude product was chromatographed (SiO_2, 33 - 67% ethyl acetate/hexane) to afford 1.34 g (74% yield) of **22** as a colorless glass. In order to prevent decomposition, three drops of N,N-diethylaniline were added to the sample prior to loading on the column. ^1H NMR (300 MHz, DMSO-d_6): δ 7.88 (2H, dd, J = 11.63, 7.57 Hz), 7.53 - 7.66 (2H, m), 7.44 - 7.21 (14H, m), 7.16 (2H, t, J = 7.12 Hz), 6.82 (2H, t, J = 8.21 Hz), 4.30 - 4.05 (6H, m), 3.70 (3H, s), 3.55 - 3.42 (4H, m), 3.36 (2H, t, J = 6.89 Hz), 2.46 (1H, buried beneath DMSO), 2.20 - 2.05 (3H, m), 1.90 - 1.70 (3H, m). IR (KBr): 2951, 2880, 1746, 1705, 1606, 1508, 1449, 1417, 1349, 1296, 1275, 1248, 1179, 1121, 1087, 1033, 831, 763, 738, 705 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 697.3 ($[M+H]^+$, 4.0), 620.3 (3.9), 619.3 (9.1), 589.3 (2.2), 274.1 (31.1), 273.1 (100.0), 179.1 (18.9), 178.1 (11.1), 165.1 (7.8), 137.0 (8.9), 136.0 (14.4). Anal. calcd for $C_{44}H_{44}N_2O_6 \cdot 0.5\ H_2O$: C, 74.87; H, 6.43; N, 3.97. Found: C 74.83; H, 6.21; N, 3.53. HRMS (Pos. FABMS): calcd for $C_{44}H_{45}N_2O_6$ $[M+H]^+$ 697.3277, found 697.3281.

Anchor alcohol (24). Compound **22** (2.00 g, 2.90 mmol) was dissolved in DMF (40 mL) containing Et_3N (10 mL), and heated at 50°C for 1 h. To this solution was added **23**[22] (1.50 g, 3.23 mmol) and the reaction mixture was evaporated under reduced pressure at RT to a final volume of approximately 5 mL. This concentrated solution was heated at 50°C for 2 h, at which time the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (200 mL) and washed successively with 0.15 M NaOH (4 x 50 mL), H_2O (100 mL), and satd NaCl (100 mL). After drying over anhydrous Na_2SO_4, the solution was filtered, and evaporated under reduced pressure. The crude product was then chromatographed (SiO_2, 0 - 40% acetone/$CHCl_3$) to afford 1.80 g (80% yield) of **24** as an amorphous white solid. ^1H NMR (300 MHz, DMSO-d_6): δ

7.89 (2H, t, J = 7.04 Hz), 7.74 (1H, t, J = 7.02), 7.70 - 7.62 (2H, m), 7.39 (4H, d, J = 7.68 Hz), 7.28 (6H, t, J = 7.08 Hz), 7.17 (2H, t, J = 7.20 Hz), 6.85 (2H, d, J = 8.70 Hz), 5.05 (1H, dd, J = 13.72, 4.00 Hz), 4.44 (1H, t, J = 7.21 Hz), 4.37 - 4.12 (5H, m), 4.10 - 3.95 (2H, m), 3.72 (3H, s), 3.65 - 3.45 (5H, m), 3.30 (2H, m), 3.02 (1H, dd, J = 10.63, 4.92 Hz), 2.83 (1H, br d), 2.54 (1H, buried beneath DMSO), 2.19 - 1.96 (4H, m), 1.92 - 1.68 (4H, m). IR (KBr): 3416, 2949, 2916, 2876, 1743, 1708, 1653, 1608, 1509, 1448, 1427, 1363, 1317, 1249, 1181, 1146, 1086, 1032, 1006, 831, 765, 730, 701 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 801.5 (2.7), 800.5 ($[M+H]^+$, 5.3), 722.3 (2.4), 528.4 (3.4), 526.4 (3.1), 307.1 (24.4), 289.1 (13.3), 274.1 (22.2), 273.1 (100.0), 165.1 (5.6), 138.0 (17.8), 137.0 (33.3), 136.0 (44.4). Anal. calcd for $C_{43}H_{49}N_3SO_{10}$: C, 64.56; H, 6.17; N, 5.25; S, 4.01. Found: C, 64.92; H, 5.95; N, 5.34; S, 3.94. HRMS (Pos. FABMS): calcd for $C_{43}H_{50}N_3SO_{10}$ $[M+H]^+$ 800.3217, found 800.3220.

Activated anchor subunit (25). Protected dipeptide **24** (1.70 g, 2.10 mmol) and bis(*p*-nitrophenyl) carbonate (1.40 g, 4.6 mmol) were dissolved in DMF (15 mL), and Et_3N (0.20 mL) was added. The reaction mixture was allowed to stir at RT for 1 h. To this solution, H_2O (1 mL in 2 mL of DMF) was added and the reaction was stirred an additional 1 h at RT. Solvents were removed under reduced pressure, the residue was dissolved in EtOAc (300 mL) and washed with 0.15 M NaOH (4 x 50 mL), H_2O (2 x 50 mL), and satd NaCl (100 mL). The resulting solution was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was chromatographed (SiO_2, 0 - 20% acetone/$CHCl_3$) to afford 1.70 g (79% yield) of mixed carbonate **25**. ^1H NMR (300 MHz, DMSO-d_6): δ 8.33 (2H, dd, J = 9.12, 3.38 Hz), 7.91 (2H, dd, J = 6.95, 4.61, Hz), 7.80 - 7.55 (5H, m), 7.38 (4H, d, J = 7.73 Hz), 7.28 (6H, t, J = 6.75 Hz), 7.17 (2H, t, J = 6.81 Hz), 6.85 (2H, d, J = 8.80 Hz), 5.21 (1H, br d), 4.57 (1H, t, J = 7.84 Hz), 4.40 - 4.25 (2H, m), 4.17 (1H, dd, J = 12.00, 5.36 Hz), 4.05 (1H,

dd, J = 12.20, 4.85 Hz), 3.78 - 3.68 (5H, m, with 3H, s, at 3.71), 3.65 - 3.45 (6H, m), 3.30 (2H, m), 2.54 (1H, buried beneath DMSO), 2.20 - 2.00 (4H, m), 2.00 - 1.70 (4H, m). IR (KBr): 3458, 2957, 2916, 2902, 2875, 1768, 1743, 1712, 1659, 1612, 1599, 1526, 1448, 1425, 1351, 1318, 1255, 1220, 1192, 1145, 1086, 1064, 1034, 858, 832, 766, 729, 702 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 966.3 (1.2), 965.3 ($[M+H]^+$, 2.0), 887.3 (1.7), 693.2 (1.5), 691.2 (1.1), 307.1 (16.7), 289.1 (10.0), 274.1 (22.2), 273.1 (100.0), 165.1 (5.6), 138.0 (14.4), 137.0 (27.8), 136 (38.9). Anal. calcd for $C_{43}H_{49}N_3SO_{10}$: C, 62.23; H, 5.43; N, 5.81; S, 3.32. Found: C, 61.83; H, 5.28; N, 5.56; S, 3.14. HRMS (Pos. FABMS): calcd for $C_{50}H_{53}N_4SO_{14}$ $[M+H]^+$ 965.3279, found 965.3283.

Preparation of universal solid-support (19). 1% crosslinked aminomethyl polystyrene (60.0 mg, loading of 1.21 mmol/g) was treated according to the sequence of steps described in Table 1. A 10 mL Biorad disposable polypropylene column and vacuum filtration were utilized, following each rinse or reaction step (see Figure 5).

Table 1. Protocol for the Preparation of Universal Solid-Support **19**

Steps	Time	Repetitions
1) DMF (1.0 mL)	15 min	1
2) 10% Diisopropylethylamine in DMF (0.5 mL)	10 min	1
3) DMF (1.0 mL)	1 min	5
4) Activated Anchor Subunit **25** (36.6 mg, 0.038 mmol) in DMF (1.0 mL)	30 min	1
5) DMF (1.0 mL)	1 min	5
6) *p*-Nitrophenyl methyl carbonate (15.0 mg, 0.076 mmol) in DMF (1.0 mL)	1 h	1
7) DMF (1.0 mL)	1 min	5

The resin was then thoroughly dried under high vacuum to afford 80.0 mg of resin **19**.

Determination of monomethoxytrityl binding to resin 19 (loading). In a small vial, 34.5 mg resin **19** was swelled in 1 mL dimethylacetamide at RT for 15 h. The mixture was transferred to a 10 mL Biorad disposable polypropylene column and filtered. The resin was washed with CH_2Cl_2 (3 x 0.5 mL) and then stirred in 2% $CHCl_2CO_2H/CH_2Cl_2$ (0.5 mL) for 1 min. After filtration, 2% $CHCl_2CO_2H/CH_2Cl_2$ (0.5 mL) was added and stirred for 7 min. The solid was washed with CH_2Cl_2 (3 x 0.5 mL). The acid filtrates and three washes were combined and evaporated *in vacuo*. The monomethoxytrityl cation concentration was quantitated by UV at 476 nm in 20% $CF_3CO_2H/ CHCl_3$ (ε = 74,613). The loading capacity of dried resin **19** was found to be 290 μmol/g.

Alkylation product (30). Hemiaminal alcohol **31**[31,32] (0.65 g, 2.81 mmol) was dissolved in CH_2Cl_2 (3.50 mL) and cooled in an ice bath for 30 min, followed by addition of N-methylimidazole (0.27 mL, 3.37 mmol). To this cold reaction mixture was added dropwise a solution of tosyl chloride (0.59 g, 3.09 mmol) in CH_2Cl_2 (3.50 mL). The reaction mixture was stirred at 0°C for 30 min and allowed to stir for an additional 20 h while slowly warming to RT. The reaction was quenched by addition of H_2O (1.0 mL) with vigorous stirring, diluted with CH_2Cl_2 (10 mL), washed with 2% aq. $NaHCO_3$ solution (3 x 3 mL), 0.5 M HCl (3 x 3 mL), and satd NaCl (1 x 5 mL). The solution was dried over anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure to provide 1.03 g of a white solid. This crude product was recrystallized from $CHCl_3$/hexanes to afford 0.76 g (71% yield) of pure tosylate as white crystals, mp 114°C. $[\alpha]_D^{22}$ = -42.6 (c = 0.50, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$): δ 7.80 (2H, d, J = 6.76 Hz), 7.35 (2H, d, J = 6.73 Hz), 4.16 - 3.78 (5H, m), 2.46 (3H, s), 1.56 - 1.46 (6H, 4 x s), 1.45 - 1.40 (9H, 2 x s). IR (KBr): 2956, 1698, 1369, 1177, 1093, 982, 827 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 386 ($[M+H]^+$, 11.4), 330

(47.1), 286 (38.6), 272 (100.0), 270 (32.9), 254 (10.7), 158 (14.3), 155 (13.6), 100 (20.7), 91 (14.0), 57 (70.0). Anal. calcd for $C_{18}H_{27}NSO_6$: C, 56.08; H, 7.06; N, 3.63; S, 8.32. Found: C, 56.09; H, 7.11; N, 3.63; S, 8.25. HRMS (Pos. FABMS): calcd for $C_{18}H_{28}NSO_6$ $[M+H]^+$ 386.1637, found 386.1637.

Cytosine (2.31 g, 20.8 mmol) was mixed with potassium-*tert*-butoxide (2.32 g, 20.8 mmol) in DMSO (30 mL) and the resulting mixture was swirled with gentle heating until a homogeneous solution formed. A solution of tosylate (4.00 g, 10.4 mmol) in DMSO (30 mL) was then added dropwise, and the reaction mixture was gently warmed and allowed to stir at 65°C for 12 h. The reaction was quenched with 20% AcOH/$CHCl_3$ (80 mL). Following removal of solvents under reduced pressure, the residual oil was taken up in 20% MeOH/CH_2Cl_2 (400 mL) and washed with 1M NaOH (2 x 70 mL). The combined aqueous washes were back-extracted with CH_2Cl_2 (150 mL). The combined organic layers were then dried over anhydrous Na_2SO_4, filtered, and solvent was removed *in vacuo* to afford 3.10 g of a white solid. This material was subsequently purified by column chromatography (SiO_2, 10 - 20% MeOH/$CHCl_3$) to provide 2.56 g (76% yield) of white solid **30**. Analytically pure material was obtained from recrystallization in MeOH, to afford fine white crystals, mp 251-252°C (dec). $[\alpha]_D^{22}$ = + 116.8 (c = 0.25, MeOH). ^1H NMR (300 MHz, DMSO-d_6): δ 7.34 (1H, d, J = 7.1 Hz), 7.04 & 6.97 (2H, 2 br s, exchanged with D_2O), 5.62 (1H, d, J = 6.8 Hz), 4.25 - 4.05 (1H, m), 4.00 - 3.75 (3H, m), 3.65 - 3.40 (1H, m), 1.53 & 1.48 (6H, 2 x s), 1.39 & 1.32 (9H, 2 x s). IR (KBr): 3387, 3110, 3091, 1709, 1696, 1666, 1629, 1493, 1477, 1388, 1382, 1372, 1357, 1264, 1159, 1093 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 326 (13.6), 325 ($[M+H]^+$, 72.9), 269 (17.1), 225 (39.3), 211 (100.0), 193 (6.4), 167 (7.1), 136 (7.1), 125 (10.7), 112 (32.1), 57 (33.6). Anal. calcd for $C_{15}H_{24}N_4O_4$: C, 55.54; H, 7.46; N, 17.27. Found C, 55.38; H, 7.61; N, 17.29. HRMS (Pos. FABMS): calcd for $C_{15}H_{25}N_4O_4$ $[M+H]^+$: 325.1875, found 325.1876.

Mosher's ester (32). Alcohol **31** (52.5 mg, 0.227 mmol) was dissolved in anhydrous CH_2Cl_2 (2.5 mL). (-)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride[33] (48.0 μL, 0.250 mmol) was added dropwise, followed by 4-dimethylaminopyridine (55.5 mg, 0.454 mmol), and the reaction mixture was allowed to stir at RT. After 2.5 h a few drops of H_2O were added with vigorous stirring. The reaction mixture was diluted with $CHCl_3$ (25 mL) and extracted with 0.1 M HCl (2 x 15 mL), satd $NaHCO_3$ (2 x 15 mL), and satd NaCl (1 x 20 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated *in vacuo* to afford 98.0 mg (96% yield) of colorless oil **32**. ^1H NMR (300 MHz, $CDCl_3$): δ 7.51 - 7.40 (5H, m), 4.63 - 4.51 (1H, m), 4.23 - 4.15 (1H, m), 4.05 - 3.96 (1H, m), 3.95 - 3.86 (1H, m), 3.79 (1H, dd, J = 9.17, 4.12 Hz), 3.53 (3H, s), 1.56 - 1.46 (15H, m with singlets).

Mosher's ester (33). By the same procedure as for **32**, reaction of alcohol **31** (59.0 mg, 0.255 mmol) with (+)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride[33] (54.0 μL, 0.281 mmol) and 4-dimethylaminopyridine (62.3 mg, 0.510 mmol), resulted in 114 mg (100% yield) of colorless oil **33**. ^1H NMR (300 MHz, $CDCl_3$): δ 7.51 - 7.40 (5H, m), 4.52 - 4.47 (1H, m), 4.24 - 4.16 (1H, m), 4.14 - 4.00 (1H, m), 3.90 (1H, dd, J = 9.29, 4.62 Hz), 3.75 (1H, dd, J = 9.06, 9.06 Hz), 3.54 (3H, s), 1.58 - 1.46 (15H, m with singlets). Dramatic changes in chemical shift of two of the five oxazoline ring/exocyclic methylene protons were observed [shifted from 4.63 - 4.51 (1H, m) and 4.05 - 3.96 (1H, m) in **32**, to 4.52 - 4.47 (1H, m) and 4.14 - 4.00 (1H, m) in **33**]. Within the limits of detection (≤1%), neither diastereomer was found to be contaminated with the other as evidenced by the absence of additional diastereomeric protons.

Cytosine adduct with ionizable protecting group (34). Freshly prepared 4-(4-morpholinyl)methylbenzoyl chloride[22] (255 mg, 0.930 mmol) and cytosine hemiaminal **30** (200 mg, 0.620 mmol) was taken up in anhydrous pyridine (2 mL). The reaction mixture was allowed to stir at RT for 18 h. The reaction mixture was cooled in an ice bath and quenched by addition of H_2O (0.5 mL). After 5 min at 0°C, conc. NH_4OH (0.5 mL) was added and the reaction mixture allowed to stir an additional 15 min at 0°C. Solvents were evaporated under reduced pressure and the resulting crude solid was taken up in 1% MeOH/$CHCl_3$ (10 mL) and filtered to remove any remaining undissolved solids prior to silica gel purification. Solvents were again removed *in vacuo* and the resulting solid was purified by column chromatography (SiO_2, 1 - 5% MeOH/$CHCl_3$) to provide 280 mg (86% yield) of **34** as a white solid, mp 216°C (d). $[\alpha]_D^{22}$ = +92.0 (c = 0.05, MeOH). ^1H NMR (300 MHz, DMSO-d_6): δ 11.12 (1H, br d, exchanged with D_2O), 7.97 (2H, d, J = 8.06 Hz), 7.91 (1H, m), 7.44 (2H, d, J = 8.14 Hz), 7.28 (1H, m), 4.31 (1H, m), 4.13 (1H, m), 3.92 (2H, m), 3.71 (1H, dd, J = 12.93, 8.07 Hz), 3.59 (4H, m), 3.53 (2H, s), 2.36 (4H, m), 1.41 - 1.28 (24 H, m with singlets). IR (KBr): 3429, 2950, 1693, 1673, 1667, 1629, 1559, 1483, 1425, 1394, 1342, 1295, 1256, 1118, 1076 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 528 ($[M+H]^+$, 100.0), 428 (28.1), 204 (60.0), 147 (31.5), 136 (61.8), 118 (51.7), 105 (29.2), 90 (36.0). Anal. calcd for $C_{27}H_{37}N_5O_6$: C, 61.46; H, 7.07; N, 13.27. Found: C, 61.39; H, 7.02; N, 13.19. HRMS (Pos. FAB): calcd for $C_{27}H_{38}N_5O_6$ $[M+H]^+$ 528.2822, found 528.2823.

N-Tr-Alcohol (35). BOC-hemiaminal **34** (50.0 mg, 0.095 mmol) was taken up in CH_2Cl_2 (0.7 mL). To this solution was added CF_3CO_2H (0.70 mL) at RT with stirring. After 1 h, the reaction mixture was evaporated under reduced pressure. The resulting oily residue was dissolved in 80% aq. AcOH (2 mL), allowed to stir at RT for 12 h, and TsOH·H_2O (36.1 mg, 0.190 mmol) was added prior to evaporating the reaction mixture

in vacuo. Residual H_2O and AcOH was removed by three consecutive coevaporations with anhydrous DMF, and the sample was placed under high vacuum for 12 h prior to reprotection of the amine. The crude amine salt was dissolved in 0.5 mL anhydrous DMF, trityl chloride (79.0 mg, 0.280 mmol) was added, followed by the addition of Et_3N (0.13 mL, 0.950 mmol). After stirring at RT for 1 h, the reaction mixture was diluted with $CHCl_3$ (15 mL) and extracted with satd $NaHCO_3$ (3 x 6 mL) and satd NaCl (1 x 6 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated *in vacuo*. Purification of the residual oil (SiO_2, 1 - 2.5% MeOH/$CHCl_3$) resulted in 36.1 mg (60% yield) of white solid **35**, mp 149-151°C. $[\alpha]_D^{22}$ = -20.0 (c = 0.05, CH_2Cl_2). ^1H NMR (400 MHz, DMSO-d_6): δ 11.24 (1H, s, exchanged with D_2O), 8.18 (2H, d, J = 7.2 Hz), 8.00 (2H, d, J = 8.2 Hz), 7.45 (2H, d, J = 8.3 Hz), 7.38 (6H, d, J = 7.3 Hz), 7.27 - 7.14 (10 H, m), 4.43 (1H, t, J = 5.05 Hz, exchanged with D_2O), 3.93 (1H, m), 3.75 (1H, m), 3.58 (4H, m), 3.54 (2H, s), 2.92 (2H, br m, 1H exchanged with D_2O), 2.60 - 2.50 (1H, m, confirmed by COSY and high temperature NMR to be buried beneath DMSO solvent peak), 2.37 (4H, m), 2.20 - 2.10 (1H, m). IR (KBr): 3406, 2955, 2929, 2856, 1697, 1653, 1624, 1485, 1449, 1365, 1350, 1246, 1116 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 669 ($[MH+K]^+$, 3.9), 630 ($[M+H]^+$, 6.4), 628 (5.2), 552 (3.3), 386 (6.7), 315 (5.3), 307 (4.0), 259 (6.1), 244 (68.9), 243 (100.0), 165 (66.7). Anal. calcd for $C_{38}H_{39}N_5O_4 \cdot 0.5\ H_2O$: C, 71.45; H, 6.31; N, 10.97. Found: C, 71.53; H, 6.18; N, 10.64. HRMS (Pos. FAB): calcd for $C_{38}H_{40}N_5O_4$ $[M+H]^+$ 630.3080, found 630.3078.

Activated monomer (10). N-Trityl-alcohol **35** (57.2 mg, 0.091 mmol) and bis(*p*-nitrophenyl) carbonate (50.0 mg, 0.163 mmol) was taken up in anhydrous CH_2Cl_2 (0.15 mL). Et_3N (50 mL, 0.362 mmol) was added and the reaction mixture allowed to stir at RT for 2 h. The reaction mixture was then diluted with $CHCl_3$ (20 mL) and extracted with 0.01 M NaOH (4 x 5 mL), H_2O (2 x 5 mL), and satd NaCl (1 x 5 mL). The

organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Purification on silica (1 - 2.5% MeOH/$CHCl_3$) resulted in 72.3 mg (100% yield) of white solid **10**, mp 133-134°C. $[\alpha]_D^{22}$ = +25.37 (c = 0.95, CH_2Cl_2). ^1H NMR (300 MHz, DMSO-d_6): δ 11.25 (1H, s), 8.28 (2H, d, J = 7.91 Hz), 7.38 (6H, d, J = 7.71 Hz), 7.29 - 7.16 (10 H, m with d, J = 7.58 Hz), 4.08 (1H, m), 3.67 (1H, m), 3.58 (4H, m), 3.54 (2H, s), 3.21 (2H, br m), 2.60 - 2.50 (1H, m, buried beneath DMSO), 2.37 (4H, m), 1.75 (1H, m). IR (KBr): 3390 br, 1772, 1697, 1686, 1664, 1658, 1623, 1594, 1557, 1525, 1487, 1448, 1370, 1348, 1316, 1301, 1249, 1213, 1114 cm^{-1} Pos. FABMS, m/z (rel. intensity): 796 (2.1), 795 ($[M+H]^+$, 4.3), 551 (2.4), 328 (2.3), 315 (2.1), 307 (2.1), 289 (2.2), 267 (2.4), 244 (28.9), 243 (100.0), 165 (37.8), 136 (26.7). Anal. calcd for $C_{45}H_{42}N_6O_8 \cdot H_2O$: C, 66.49; H, 5.46; N, 10.34. Found: C, 66.39; H, 5.28; N, 9.97. HRMS (Pos. FAB): calcd for $C_{45}H_{43}N_6O_8$ $[M+H]^+$ 795.3142, found 795.3143.

Activated monomer (11). Spectroscopic properties of D-serine derived activated monomer **11** were identical to those for L-serine derived intermediate **10**, with the exception of optical rotation which was of equal and opposite magnitude. $[\alpha]_D^{22}$ = -25.76 (c = 0.66, CH_2Cl_2).

N-CBZ-Alcohol (37). Compound **36** (0.78 g, 1.61 mmol) was dissolved in CH_2Cl_2 (10 mL) and trifluoroacetic acid (10 mL) was added at RT with stirring. The reaction mixture was allowed to stir for 1 h and following removal of solvent, the residue was dissolved in 80% aq. AcOH (20 mL) and allowed to stir at RT for 12 h. The reaction mixture was then evaporated under reduced pressure and the residual oil was coevaporated twice with hexane (50 mL portions). The residue was then thoroughly dried under high vacuum for 12 h, anhydrous CH_2Cl_2 (10 mL) and 1 M aq. Na_2CO_3 (6.44 mL, 6.44 mmol) were added, followed by dropwise addition of benzyl

chloroformate (0.32 mL, 2.25 mmol) at RT with vigorous stirring. The reaction mixture was allowed to continue stirring vigorously for 12 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and extracted with 5% aq. $NaHCO_3$ (3 x 20 mL), 0.5 M HCl (1 x 20 mL), and satd NaCl (1 x 20 mL). Following drying over Na_2SO_4 and removal of solvent, the colorless syrup was purified by column chromatography (SiO_2, 1 - 2.5% MeOH/$CHCl_3$) to afford 0.50 g (54% yield) of an amorphous white solid **37**, mp 121°C. $[\alpha]_D^{22}$ = +129.0 (c = 0.10, MeOH). ^1H NMR (300 MHz, DMSO-d_6): δ 11.08 (1H, s), 7.99 - 7.95 (3H, m with 2H, d, J = 8.39 Hz), 7.54 (2H, d, J = 8.49 Hz), 7.34 (6H, m), 5.02 - 4.88 (3H, m with 2H, AB_q, J_{AB} = 12.77 Hz, $\Delta\nu_{AB}$ = 25.57 Hz), 4.21 (1H, dd, J = 13.1, 3.60 Hz), 3.94 (1H, m), 3.58 - 3.38 (3H, m), 1.31 (9H, s). IR (KBr): 3367, 3334, 2965, 1686, 1658, 1653, 1627, 1560, 1538, 1523, 1502, 1497, 1488, 1373, 1250, 1093 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 480 (34.0), 479 ($[M+H]^+$, 89.8), 272 (5.4), 161 (100.0), 146 (5.7), 118 (4.8), 91 (30.6). Anal. calcd for $C_{26}H_{30}N_4O_5$: C, 65.26; H, 6.32; N, 11.71. Found: C, 65.24; H, 6.21; N, 11.94. HRMS (Pos. FAB): calcd for $C_{26}H_{31}N_4O_5$ $[M+H]^+$ 479.2294, found 479.2299.

N-CBZ-Carboxylic acid (38). Compound **37** (159 mg, 0.33 mmol) was dissolved in acetone (2 mL) and added dropwise to a solution of Jones' reagent[36] (1.5 mL) in acetone (1 mL) at 0°C. Additional acetone (1 mL) was added to the flask which previously contained compound **37**, swirled, and added to the reaction vessel. The reaction mixture was allowed to stir at 0°C for 8 h, at which time isopropanol (4 mL) was added. After 30 min at 0°C, the reaction mixture was diluted with acetone (20 mL) and, following thorough mixing, the organic layer was decanted. This procedure was repeated three times and the organic washes were combined and evaporated *in vacuo*. The residue was taken up in H_2O and extracted with EtOAc (3 x 40 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The resulting white solid was dried

further under high vacuum and then purified chromatographically (SiO_2, 1.25 - 20% MeOH/$CHCl_3$) to afford 115 mg (70% yield) of a white solid **38**, mp 219-220°C(d). $[\alpha]_D^{22}$ = +116.0 (c = 0.05, MeOH). ^1H NMR (300 MHz, DMSO-d_6): δ 11.04 (1H, s), 7.97 (2H, d, J = 8.04 Hz), 7.53 (2H, d, J = 8.27 Hz), 7.36 - 7.16 (6H, m), 6.82 (1H, br d), 4.93 (2H, AB_q, J_{AB} = 12.68 Hz, $\Delta\nu_{AB}$ = 29.42 Hz), 4.54 (1H, m), 4.18 (1H, m), 3.56 (1H, t, J = 12.02 Hz), 1.31 (9H, s). IR (KBr): 3600 - 3100 br, 2903, 1701, 1691, 1651, 1644, 1642, 1640, 1638, 1627, 1623, 1611, 1608, 1571, 1564, 1561, 1487, 1428, 1425, 1363, 1325, 1297, 1254 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 494 (20.4), 493 ($[M+H]^+$, 61.3), 310 (38.8), 272 (20.4), 216 (39.5), 161 (100.0), 91 (57.9). Anal. calcd for $C_{26}H_{28}N_4O_6 \cdot 0.5\ H_2O$: C, 62.26; H, 5.83; N, 11.17. Found: C, 62.25; H, 5.65; N, 11.14. HRMS (Pos. FAB): calcd for $C_{26}H_{29}N_4O_6$ $[M+H]^+$ 493.2087, found 493.2088.

N-CBZ-Dipeptide (39). Carboxylic acid **38** (0.56 g, 1.14 mmol), pentafluorophenol (2.09 g, 11.4 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (0.68 g, 2.27 mmol) were dissolved in CH_2Cl_2 (20 mL) and cooled to 0°C. 4-Dimethyl-aminopyridine (61 mg, 0.57 mmol) was added and the reaction mixture was allowed to stir 8 h under inert atmosphere while slowly warming to RT. Glycine-*t*-butyl ester hydrochloride (0.21 g, 1.25 mmol) was then added, followed by the addition of Et_3N (1.58 mL, 11.4 mmol). After 4 h, the reaction mixture was diluted with $CHCl_3$ (300 mL) and extracted with H_2O (1 x 75mL), 0.15 M NaOH (3 x 75 mL), and satd NaCl (1 x 100 mL). Each aqueous extract was then back-extracted with $CHCl_3$ (200 mL). The combined organic extracts were then dried over Na_2SO_4, filterered, and evaporated *in vacuo*. The crude product was purified via column chromatography (SiO_2, 1.25 - 2.5% MeOH/$CHCl_3$) to provide 0.44 g (64% yield) of white solid **39**, mp 119-120°C. $[\alpha]_D^{22}$ = +59.52 (c = 0.50, CH_2Cl_2). ^1H NMR (300 MHz, DMSO-d_6): δ 11.12 (1H, s), 8.48 (1H, t, J = 5.46 Hz), 8.05 - 7.92 (3H, m with 2H, d, J = 8.29 Hz at 7.98), 7.69 (1H, d, J

= 9.32 Hz), 7.54 (2H, d, J = 8.35 Hz), 7.40 - 7.18 (6H, m), 4.98 (2H, AB_q, J_{AB} = 12.70 Hz, $\Delta\nu_{AB}$ = 32.50 Hz), 4.67 - 4.51 (1H, m), 4.40 (1H, dd, J = 13.51, 3.43 Hz), 3.85 - 3.60 (3H, m), 1.41 (9H, s), 1.31 (9H, s). IR (KBr): 3284 br, 2966. 1724, 1689, 1674, 1666, 1629, 1554, 1484, 1425, 1365, 1301, 1252, 1157, 1113, 1051, 791, 747, 698 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 607.3 (18.2), 606.3 ($[M+H]^+$, 48.2), 550.3 (24.7), 272.1 (10.6), 162.1 (12.9), 161.1 (100.0), 91.1 (30.6), 57.1 (9.4). Anal. calcd for $C_{32}H_{39}N_5O_7$: C, 63.46; H, 6.49; N, 11.56. Found: C, 63.34; H, 6.75; N, 11.34. HRMS (Pos. FAB): calcd for $C_{32}H_{40}N_5O_7$ $[M+H]^+$ 606.2927, found 606.2927.

N-CBZ-Dipeptide with ionizable base protecting group (40). Dipeptide **39** (52 mg, 0.086 mmol) was dissolved in 8:2 pyridine:HOAc (0.18 mL). Hydrazine monohydrate (17 μL, 0.34 mmol) was then added, and the reaction mixture was allowed to stir at RT for 22 h. Solvents were removed under reduced pressure and the resulting residue was purified chromatographically (SiO_2, 2.5 - 10% MeOH/ $CHCl_3$) to afford 35 mg (91% yield) of the free cytosine adduct as a beige solid, mp 170-171°C. $[\alpha]_D^{22}$ = 88.38 (c = 0.50, MeOH). ^1H NMR (300 MHz, DMSO-d_6): δ 8.36 (1H, t, J = 5.61 Hz), 7.59 (1H, d, J = 8.79 Hz), 7.44 (1H, d, J = 7.23 Hz), 7.40 - 7.24 (5H, m), 7.15 - 6.95 (2H, br d), 5.60 (1H, d, J = 7.05 Hz), 5.00 (2H, AB_q, J_{AB} = 12.84 Hz, $\Delta\nu_{AB}$ = 22.86 Hz), 4.50 - 4.38 (1H, m), 4.28 - 4.12 (1H, dd, J = 13.37, 9.89 Hz), 1.41 (9H, s). IR (KBr): 3415 br, 3330 br, 1739, 1673, 1652, 1528, 1498, 1377, 1280, 1251, 1228, 1158 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 892.3 (17.1), 891.3 (35.0), 447.1 (25.7), 446.1 ($[M+H]^+$, 48.6), 391.1 (20.7), 390.1 (100.0). Anal. calcd for $C_{21}H_{27}N_5O_6 \cdot H_2O$: C, 54.42; H, 6.31; N, 15.11. Found: C, 54.50; H, 6.38; N, 14.78. HRMS (Pos. FAB): calcd for $C_{21}H_{28}N_5O_6$ $[M+H]^+$ 446.2039, found 446.2038.

Anhydrous pyridine (0.2 mL) was added to a flask containing the free cytosine adduct (35 mg, 0.079 mmol) and freshly prepared 4-(4-morpholinyl)methylbenzoyl

chloride (108 mg, 0.393 mmol). The reaction mixture was allowed to stir at RT under an inert atmosphere for 3 h. The reaction mixture was then externally cooled to 0°C. H_2O (0.30 mL) was added and after 5 min, conc. NH_3 (0.30 mL) was added and allowed to stir for an additional 15 min at 0°C. Solvents were removed *in vacuo*, and the crude product was purified by chromatography (SiO_2, 1.25 - 5.0% MeOH/$CHCl_3$) to afford 30 mg (59% yield) of white solid **40**, mp 170-171°C. $[\alpha]_D^{22}$ = +55.31 (c = 0.50, CH_2Cl_2). ^1H NMR (300 MHz, DMSO-d_6): δ 11.15 (1H, s), 8.48 (1H, t, J = 5.96 Hz), 8.06 - 7.90 (3H, m with 2H, d, J = 8.07 Hz at 7.99), 7.67 (1H, d, J = 9.93 Hz), 7.45 (2H, d, J = 8.27 Hz), 7.40 - 7.18 (6H, m), 4.98 (2H, AB_q, J_{AB} = 12.77 Hz, $\Delta\nu_{AB}$ = 31.29 Hz), 4.65 - 4.50 (1H, m), 4.48 - 4.32 (1H, m), 3.85 - 3.64 (3H, m), 3.58 (4H, m), 3.54 (2H, s), 2.37 (4H, m), 1.41 (9H, s). IR (KBr): 3325 br, 1728, 1691, 1659, 1622, 1546, 1542, 1497, 1455, 1420, 1364, 1301, 1259, 1247, 1156, 1116, 1048, 797, 747, 697 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 650.1 (33.3), 649.1 ($[M+H]^+$, 100.0), 593.0 (26.7), 488.1 (31.1), 432.0 (28.9), 204.1 (34.4), 91.1 (86.7). Anal. calcd for $C_{33}H_{40}N_6O_8$: C, 61.10; H, 6.22; N, 12.95. Found: C, 60.80; H, 6.12; N, 13.06. HRMS (Pos. FAB): calcd for $C_{33}H_{41}N_6O_8$ $[M+H]^+$ 649.2986, found 649.2985.

N-Tr-Dipeptide carboxylic acid with ionizable protecting group (41). Compound **40** (300 mg, 0.462 mmol) was dissolved in CH_2Cl_2 (2.0 mL) at RT. This solution was allowed to stir while F_3CCO_2H (2.0 mL) was slowly added. After 1 h, the reaction mixture was evaporated *in vacuo* and the residue was purified via column chromatography (SiO_2, 10:2:88 MeOH:HOAc:$CHCl_3$ - 20:2:78 MeOH:HOAc:$CHCl_3$) to afford 314 mg (96% yield) of pure carboxylic acid, mp 153°C (d). ^1H NMR (300 MHz, DMSO-d_6): δ 12.30 (1H, br s, exchanges with D_2O), 11.21 (1H, br s, exchanges with D_2O), 8.43 (1H, t, J = 5.42 Hz), 8.08 (2H, d, J = 7.09 Hz), 7.96 (1H, d, J = 7.33 Hz), 7.67 (1H, d, J = 9.20 Hz), 7.64 - 7.48 (2H, br d), 7.40 - 7.18 (6H, m), 4.98 (2H,

AB_q, J_{AB} = 12.70 Hz, $\Delta\nu_{AB}$ = 34.08 Hz), 4.58 (1H, m), 4.41 (1H, dd, 13.19, 3.83 Hz), 3.95 - 3.53 (9H, m), 2.54 - 2.47 (4H, m, buried beneath DMSO). IR (KBr): 3391 br, 1674 br, 1666, 1631, 1565, 1516, 1488, 1457, 1421, 1364, 1307, 1256, 1197, 1127, 1084, 1057, 753 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 615.3 ($[M+Na]^+$, 40.0), 593.3 ($[M+H]^+$, 65.0), 329.1 (40.0), 221.3 (60.0), 219.1 (45.0), 205.1 (45.0), 176.1 (100.0). HRMS (Pos. FAB): calcd for $C_{27}H_{33}N_6O_8$ $[M+H]^+$ 593.2360, found 593.2361.

The carboxylic acid (311 mg, 0.440 mmol) was taken up in HBr·HOAc (4.0 mL). The reaction mixture was allowed to stir at RT for 25 min. Anhydrous Et_2O (10 mL) was added with vigorous stirring and the resulting precipitate was collected by centrifugation. The pellet was triturated three times with additional anhydrous Et_2O (20 mL portions) and the solid was dried under reduced pressure. The dried solid was dissolved in 2:1 DMF:$CHCl_3$ (5.0 mL) and trityl bromide (711 mg, 2.20 mmol) was added in one portion.[37] The reaction mixture was allowed to stir 1 h at RT, Et_3N (0.61 mL, 4.40 mmol) was added and the reaction mixture was allowed to stir an additional 1 h at RT. MeOH (6.0 mL) was then added and the reaction mixture was stirred for 3 h at 60°C. Solvents were removed *in vacuo* and the residue was triturated with anhydrous Et_2O (6 x 15 mL portions). To the solid was added CH_2Cl_2 (30 mL) and pH 6 phosphate buffer (30 mL). The aqueous layer at pH 6 (the isoelectric point) was further extracted with CH_2Cl_2 (2 x 30 mL), and the combined organic layers were extracted with satd NaCl (20 mL). After drying over Na_2SO_4 and removal of solvent under reduced pressure, the crude orange solid (213 mg) was purified by column chromatography (SiO_2, 1.25 - 20.0% MeOH:$CHCl_3$) to afford 150 mg (49% yield) of white solid **41**, mp 190-191°C(d). $[\alpha]_D^{22}$ = 46.09 (c = 0.50, CH_2Cl_2). ^1H NMR (300 MHz, DMSO-d_6): δ 11.26 (1H, br s), 8.32 (1H, d, J = 7.25 Hz), 8.01 (2H, d, J = 8.23 Hz), 7.45 (2H, d, J = 8.32 Hz), 7.37 (1H, d, J = 6.84 Hz), 7.35 - 7.08 (17H, m), 3.95 (1H, dd, J = 13.19, 3.29 Hz), 3.73 (1H, dd, J = 13.20, 8.81 Hz), 3.58 (4H, m), 3.54 (2H, s), 3.10 (1H, dd, J = 17.34, 4.81), 2.88 (1H, dd, J = 16.86, 3.57), 2.36 (4H, m). IR

(KBr): 3402 br, 1697, 1667, 1654, 1621, 1587, 1562, 1486, 1450, 1446, 1420, 1403, 1369, 1309, 1245, 1114, 790, 750, 704 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 739.3 ($[M+K]^+$, 1.1), 723.3 ($[M+Na]^+$, 1.6), 701.3 ($[M+H]^+$, 1.6), 460.1 3.1), 459.1 (1.4), 457.1 (1.7), 413.2 (1.4), 244.1 (25.0), 243.1 (100.0), 165.1 (21.4). Anal. calcd for $C_{40}H_{40}N_6O_6 \cdot H_2O$: C, 66.84; H, 5.89; N, 11.69. Found: C, 66.86; H, 5.28; N, 11.73. HRMS (Pos. FAB): calcd for $C_{40}H_{41}N_6O_6$ $[M+H]^+$ 701.3087, found 701.3085.

N-Tr-Dipeptide activated subunit (12). Anhydrous pyridine (0.10 mL) was added to a mixture of compound **41** (10 mg, 0.0145 mmol) and bis(*p*-nitrophenyl)carbonate (22 mg, 0.0725 mmol). After 20 h at RT, the reaction mixture was diluted with 20% isopropanol/$CHCl_3$ (20 mL) and extracted with 0.15 M NaOH (4 x 5 mL), pH 7 buffer (2 x 5 mL), and satd NaCl (1 x 10 mL). The organic layer was dried over $MgSO_4$, and solvent was removed *in vacuo*. The product was purified on SiO_2 (1.25 - 2.5% MeOH/$CHCl_3$) to afford 7.3 mg (61% yield) of a white solid **12**, mp 128-129°C (d). $[\alpha]_D^{22}$ = 32.06 (c = 0.10, CH_2Cl_2). Compound **12** could also be made from **41** and *p*-nitrophenol via carbodiimide coupling, although the use of 4-dimethylaminopyridine was found to lead to complications during the workup and isolation, and should therefore be avoided. ^1H NMR 400 MHz, DMSO-d_6): δ 11.20 (1H, br s, exchanges with D_2O), 8.33 - 8.21 (3H, m with 2H, d, J = 8.93 Hz at 8.26), 7.99 (2H, d, J = 8.03 Hz), 7.44 (2H, d, J = 8.08 Hz), 7.40 - 7.10 (20H, m), 4.02 - 3.92 (1H, m), 3.80 - 3.60 (2H, m), 3.58 (4H, m), 3.54 (2H, s), 3.42 - 3.32 (2H, m), 2.37 (4H, m). IR (KBr): 3427 br, 1673, 1666, 1660, 1639, 1628, 1560, 1522, 1486, 1456, 1450, 1367, 1359, 1351, 1347, 1302, 1247, 1206, 1136, 1114, 790, 750, 706 cm^{-1}. Pos. FABMS, m/z (rel. intensity): 823.3 (1.5), 822.3 ($[M+H]^+$, 2.7), 821.3 (0.8), 820.3 (1.0), 578.2 (1.1), 244.2 (20.9), 243.2 (100.0), 165.2 (16.3). HRMS (Pos. FAB): calcd for $C_{46}H_{44}N_7O_8$ $[M+H]^+$ 822.3251, found 822.3252.

N-CBZ-Dipeptide benzylamide (42). Compound **38** (56 mg, 0.102 mmol) and *p*-nitrophenol (142 mg, 1.02 mmol) were dissolved in DMF (1 mL) and cooled to 0°C. The 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (76 mg, 0.255 mmol) and 4-dimethylaminopyridine (2 mg, 0.020 mmol) were added and the reaction mixture was allowed to stir for 8 h while gradually warming to RT. Benzylamine (56 μL, 0.510 mmol) and Et_3N (142 μL, 1.02 mmol) were added and the reaction mixture was allowed to stir an additional 8 h. The reaction mixture was diluted with 20% isopropanol/$CHCl_3$ (40 mL) and extracted with 0.15 M NaOH (4 x 10 mL), 0.5 M HCl (1 x 10 mL), and satd NaCl (1 x 10 mL). The resulting product was purified on SiO_2 (1.25 - 2.5% MeOH/$CHCl_3$) to afford 37 mg (57% yield) of **42** as a white solid. ^1H NMR (400 MHz, DMSO-d_6): δ 11.08 (1H, s), 8.41 (1H, t, J = 5.87 Hz), 8.28 (1H, t, J = 5.67 Hz), 7.98 (2H, d, J = 8.31 Hz), 7.94 (1H, d, J = 7.48 Hz), 7.70 (1H, d, J = 8.37 Hz), 7.54 (2H, d, J = 8.47 Hz), 7.40 - 4.18 (11H, m), 4.95 (2H, AB_q, J_{AB} = 12.68 Hz, $\Delta\nu_{AB}$ = 37.08 Hz), 4.54 (1H, m), 4.45 - 4.35 (1H, m), 4.30 (2H, d, J = 5.92 Hz), 4.86 - 4.70 (3H, m with 2H, d, J = 5.81 Hz at 3.76), 1.31 (9H, s). Pos. FABMS, m/z (rel. intensity): 640.2 (10.0), 639.2 ($[M+H]^+$, 24.2), 272.1 (12.2), 161.1 (100.0), 91.1 (66.7), 77.1 (35.6). HRMS (Pos. FAB): calcd for $C_{35}H_{39}N_6O_6$ $[M+H]^+$ 639.2931, found 639.2933.

Mosher's amide (43). Compound **42** (6.4 mg, 0.010 mmol) was dissolved in HBr·HOAc (0.5 mL) and was allowed to stir for 15 min at RT. Anhydrous Et_2O (5 mL) was added to precipitate the amine salt, which was subsequently triturated with Et_2O (4 x 5 mL). The amine salt was dried for 2 h *in vacuo*, and was dissolved in anhydrous pyridine (0.10 mL). (-)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride[33] (2.1 μL, 0.011 mmol) and Et_3N (14 μL, 0.10 mmol) were added and the reaction mixture was allowed to stir for 2 h at RT. A few drops of H_2O were added and the reaction was stirred vigorously for 5 min. The reaction mixture was diluted with $CHCl_3$ (20 mL) and

extracted with 0.10 M NaOH (2 x 5 mL), satd $NaHCO_3$ (2 x 5 mL), and satd NaCl (1 x 5 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo* to afford 6.0 mg (83% yield) of **43** as a white solid, which was examined by NMR without further purification. ^1H NMR (300 MHz, DMSO-d_6): δ 11.12 (1H, s), 8.67 (1H, d, J = 7.92 Hz), 8.44 (1H, t, J = 6.11 Hz), 8.33 (1H, t, J = 5.57 Hz), 8.06 - 7.92 (3H, m with 2H, d, J = 8.51 Hz at 8.00), 7.80 (1H, d, J = 7.19 Hz), 7.62 - 7.48 (3H, m with 2H, d, J = 8.27 Hz at 7.56), 7.42 - 7.18 (8H, m), 7.05 (1H, d, J = 6.80 Hz), 5.02 - 4.92 (1H, m), 4.40 - 3.94 (1H, m), 3.92 - 3.72 (2H, m), 3.48 (3H, s), 1.32 (9H, s).

Mosher's amide (44). By the same procedure as for **43**, reaction of the free amine of **42** with (+)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride[33] (2.1 μL, 0.011 mmol) and Et_3N (14 μL, 0.10 mmol) in pyridine (0.10 mL), resulted in 5.8 mg (81% yield) of **44** as a white solid. ^1H NMR (300 MHz, DMSO-d_6): δ 11.12 (1H, s), 8.87 (1H, d, J = 7.03 Hz), 8.41 (1H, t, J = 5.94 Hz), 8.29 (1H, t, J = 5.62 Hz), 8.04 (1H, d, J = 7.47 Hz), 7.97 (2H, d, J = 8.46 Hz), 7.62 - 7.48 (3H, m with 2H, d, J = 8.40 Hz at 7.53), 7.48 - 7.38 (2H, m), 7.38 - 7.18 (8H, m), 4.92 - 4.82 (1H, m), 4.42 - 4.34 (1H, m), 4.30 (2H, d, J = 5.96 Hz), 4.18 - 4.06 (1H, m), 3.88 - 3.73 (2H, m), 3.47 (3H, s), 1.31 (9H, s). Dramatic changes in the chemical shift of the methine proton at the stereocenter of the subunit, and one of the methylene protons adjacent to the chiral center were observed [shifted from 5.02 - 4.92 (1H, m) and 4.06 - 3.94 (1H, m) in **43**, to 4.92 - 4.82 (1H, m) and 4.18 - 4.06 (1H, m) in **44**]. Within the limits of detection (≤1%), neither diastereomer was found to be contaminated with the other as evidenced by the absence of additional diastereomeric protons.

Solid-phase synthesis of oligomers (45 and 46). Resin **19** (34.5 mg, 10.0 μmol) was swelled with DMA (1 mL) in a small vial at RT for 16 h. The apparatus utilized in the solid-phase syntheses is shown in Figure 5. The mixture was transferred to a 10 mL Biorad disposable column, DMA was removed via filtration, and the sequence of steps in Table 2 was performed at RT with the exception of step 8 (which was run at 40°C). Each step was performed with external agitation utilizing a spatula which had been penetrated through a septum, stoppered at the top of the column. Mixing of the suspension was facilitated by an external motor with a cam, used to gently rock the spatula back and forth. (Vigorous stirring was found to degrade the solid support.) Reagents were introduced via syringe, through the serum stopper. Following oligomerization the resin was rinsed with DMF (3 x 0.5 mL, at 3 min intervals), and the desired hexamer was cleaved from the solid support by treating with a solution of DBU (60 μL), diethyl malonate (30 μL), and DMF (1.0 mL) at RT for 1 h with agitation. The supernatant solution was removed by filtration and the resin was again rinsed with DMF (3 x 0.5 mL). The combined filtrates were evaporated *in vacuo* without external heating, the resulting residual oil was dissolved in a minimum amount of CH_2Cl_2, and precipitated by addition of 1:1 anhydrous Et_2O/hexanes (4 mL). Following centrifugation, the solvent was decanted and the precipitation repeated. The crude hexamer was dried under high vacuum to afford 20.4 mg (72%, or approximately 95% per step based on overall yield) of beige solid **45**. Pos. FABMS of **45** (and **46**) revealed a single high molecular weight ion cluster centered at the expected 2827, corresponding to $[M+H]^+$. Upon ion-exchange analysis on S-Sepharose the product appeared in the chromatographic profile to be one major peak, using 0.02 M phosphate buffer (pH 2.5) and a gradient of KCl (0 - 1.4 M in 84 min at a flow rate of 2.2 mL/min).

Table 2. Protocol for the Solid-Phase Synthesis of Oligomers [a]

Steps		Time	Repetitions
1)	CH_2Cl_2 (1.0 mL)	1 min	3
2)	Trityl Deprotection 4% $CHCl_2CO_2H/CH_2Cl_2$ (0.5 mL)	1 min	1
3)	Trityl Deprotection (Repeat) 4% $CHCl_2CO_2H/CH_2Cl_2$ (0.5 mL)	10 min	1
4)	CH_2Cl_2 (0.5 mL)	1 min	4
5)	10% Et_3N/CH_2Cl_2 (0.5 mL)	1 min	2
6)	CH_2Cl_2 (0.5 mL)	1 min	2
7)	Dimethylacetamide (0.5 mL)	1 min	3
8)	Coupling Reaction (30 μmol subunit + 30 μmol Et_3N in 150 μL DMA)	24 h	1
9)	Dimethylacetamide (0.5 mL)	1 min	1
10)	Capping Sequence (50 μL Et_3N + 50 μL Ac_2O in 0.5 mL DMA)	10 min	1
11)	Dimethylacetamide (0.5 mL)	1 min	3
12)	Repeat steps 1 - 11 for each subunit		

a) All steps 25°C except for step 8, which was at 40°C.

Synthesis of hexamers used for biophysical testing (28 and 29). A portion of the crude hexamer **45** (3.6 mg, 1.27 μmol) was detritylated with 4% $CHCl_2CO_2H/CH_2Cl_2$ (0.75 mL). After 15 min, 1:1 Et_2O/hexanes (6 mL) was added to precipitate the resulting amine salt. Following centrifugation, the supernatant was decanted and the remaining precipitate was triturated three times with 2:1:1 CH_2Cl_2:Et_2O:hexanes (4 mL portions) to remove trityl byproducts.

The resulting amine salt was dried *in vacuo* for 8 h, was taken up in 0.02 M pH 2.5 phosphate buffer and chromatographed on S-Sepharose, using phosphate buffer (pH 2.5) and a gradient of KCl (0 - 1.4 M in 84 min at a flow rate of 2.2 mL/min). Fractions 24 - 30 were combined, concentrated to approximately 5 mL, and eluted onto a Waters Sep-Pak® C_{18} cartridge. The sample was desalted and neutralized by flushing successively with 20 mL of H_2O, 6 mL of 1% Et_3N/H_2O, and again with 6 mL of H_2O. The free amine product was then eluted off the C_{18} cartridge by flushing with 80% CH_3CN/H_2O (3 x 3 mL). The 80% CH_3CN/H_2O fractions were combined, CH_3CN was removed under reduced pressure, and the H_2O was removed via lyophilization to afford 83% yield (2.7 mg, 1.05 μmol) of the hexamer amine. Pos. FABMS of the purified hexamer amine revealed a single high molecular weight ion cluster centered at the expected 2585, corresponding to $[M+H]^+$.

This dried solid was then transferred with DMF (120 μL) to a Kontes micro V-vial adapted with a solid-top screw cap and Teflon-faced, styrene-butadiene rubber liner (pressurization vial), followed by 20 μL of polyethylene glycol monomethyl ether-derived mixed carbonate **47**.[23] Et_3N (6 μL) was added, and the reaction mixture allowed to stir at RT for 48 h. This reaction mixture was then taken on directly to ammonolysis by diluting with additional DMF (to a total volume of 1 mL), cooled to 0°C, and followed by addition of cold conc. NH_4OH (1 mL). The vial was sealed and stirred 24 h at 30°C. The reaction mixture was transferred to a round-bottomed flask, and solvents were removed *in vacuo*. The tailed, deprotected product was purified by ion-exchange chromatography on S-Sepharose, using 0.02 M phosphate buffer (pH 2.5) and a gradient of KCl (0 - 1.4 M in 84 min at a flow rate of 2.2 mL/min). Fractions were collected every 1.5 min and the chromatographic profile revealed a major, symmetrical peak at 30 - 36 min (fractions 20 - 24) corresponding to the desired hexameric product.

Fractions 20 - 24 were combined and desalted on chromatographic grade polypropylene (Polysciences), which had been equilibrated with 100% H_2O. The column was flushed with H_2O at a flow rate of 2.2 mL/min for 10.5 min (23.1 mL), 0.5% aq. NH_4OH for 6 min (13.2 mL), and again with H_2O for an additional 6 min (13.2 mL). The sample was then eluted with 80% MeOH/H_2O. The MeOH was evaporated *in vacuo* and the remaining aqueous solution was lyophilized to provide 45% yield (1.0 mg, 0.47 μmol) of white flocculent solid, **28**. The high resolution ^1H NMR of **28** revealed a complex spectrum containing data consistent with the expected structure including the aromatic region, as evidenced by six nearly distinguishable cytosine subunits. ^1H NMR (300 MHz, DMSO-d_6): δ 8.10 (1H, br m), 7.90 - 7.35 (10H, m), 7.35 - 6.70 (14H, m), 5.82 (1H, d, J = 7.69 Hz), 5.80 - 5.50 (5H, m), 4.67 (1H, br t), 4.60 - 3.80 (18H, m), 3.75 - 3.05 (large envelope containing the PEG protons, and several signals of the oligomer), 2.97 (3H, s), 2.45 - 1.85 (8H, m), 1.85 - 1.35 (6H, m). The crude hexamer composed of subunits with the opposite (S)-configuration (5.0 mg, 1.77 μmol), was converted to 53% yield of hexamer **29** (2.0 mg, 0.93 μmol) in an analogous fashion.

Native DNA [$p(dG)_6$ and the control oligomer, $p(dC)_6$] and RNA (poly G) utilized for biophysical testing of polyurethanes **28** and **29** (as well as polyamides **56** and **57**), were purchased from Pharmacia and Sigma Chemical Company, respectively. Hybridization affinities of each hexamer with complementary $p(dG)_6$ and poly G, were assessed via thermal denaturation experiments in a pH 7.2 low salt buffer consisting of 0.02 M NaOH buffered with 0.2 M H_3PO_4 to pH 7.2. Stock solutions of the testing oligomers were prepared by dissolving the oligomers in 80% DMSO/H_2O. The concentration of each stock solution was determined by the nearest neighbor group extinction coefficient method,[40] with actual UV spectroscopy measurements of the diluted stock oligomer solutions. Equimolar amounts of native DNA (or RNA) and test oligomer were then diluted to 1.00 mL in the test buffer (to a final concentration of 10

μM), warmed, and slowly cooled to insure complete annealing of the hexameric analogues to their native nucleic acid complements. UV spectra were recorded in increments of 2°C, monitoring from 9°C to 85°C with 2 min of stabilization time. The thermal melt curve was obtained by plotting UV absorbance changes at 260 nm (or 275 nm) from the recorded spectra versus temperature. T_m values were obtained via direct extrapolation from the corresponding thermal melt or from the 1st derivative of the appropriate thermal denaturation curve.

N-Tr-Valylglycine (50). Valylglycine (0.50 g, 2.87 mmol) and trityl bromide (2.04 g, 6.31 mmol) were dissolved in 2:1 $CHCl_3$:DMF (15 mL) and allowed to stir for 40 min at RT.[37] Et_3N (1.60 mL, 14.48 mmol) was dissolved in 2:1 $CHCl_3$:DMF (3 mL) and added dropwise over 10 min, and stirring was continued for 90 min at RT. MeOH (15 mL) was added and the reaction mixture was stirred for 30 min at 50°C. Solvents were removed *in vacuo* and the residue was triturated thrice with Et_2O (20 mL portions). The resulting residual solid was found however, to be mostly $Et_3NH^+Br^-$, and consequently, the combined ethereal triturates were diluted with Et_2O (to a final volume of 150 mL) and extracted with 10% aq. citric acid (3 x 50 mL), H_2O (2 x 50 mL), and satd NaCl (1 x 50 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo*. Attempts to recrystallize the crude product were unsuccessful, and hence, the product was purified on SiO_2 (1.25 - 15% MeOH/$CHCl_3$ with 1% Et_3N) to afford 0.63 g (42% yield) of **50** as a colorless oil. ^1H NMR (300 MHz, DMSO-d_6): δ 7.50 - 7.12 (16H, m), 3.28 (2H, m), 3.10 - 2.95 (1H, m), 2.87 (1H, d, J = 8.55 Hz), 1.72 - 1.56 (1H, m), 0.83 (6H, d, 6.84 Hz).

N-Tr-Valylglycine activated subunit (51). Compound **50** (30 mg, 0.058 mmol), *p*-nitrophenol (40 mg, 0.290 mmol), and predried 1-hydroxybenzotriazole (3 mg, 0.022 mmol) were dissolved in CH_2Cl_2 (0.15 mL) and were cooled to 0°C. The 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (52 mg, 0.174 mmol) was added and the reaction mixture was allowed to stir at 0°C for 30 min and an additional 4 h at RT. The reaction mixture was diluted with $CHCl_3$ (30 mL) and extracted with 0.10 M NaOH (5 x 5 mL), pH 7 buffer (1 x 5 mL), and satd NaCl (1 x 10 mL). The residue was purified on SiO_2 (1.25 - 2.5% MeOH/$CHCl_3$) to afford 17 mg (55% yield) of **51** as a white solid. ^1H NMR (300 MHz, DMSO-d_6): δ 8.29 (2H, m), 7.93 (1H, t, J = 5.21 Hz), 7.50 - 7.14 (17H, m), 3.62 (2H, d, J = 5.29 Hz), 3.07 (1H, dd, 9.00, 4.40 Hz), 2.88 (1H, d, J = 9.04 Hz), 1.85 - 1.70 (1H, m), 0.87 (6H, d, J = 6.58 Hz). Pos. FABMS, m/z (rel. intensity): 538.2 ($[M+H]^+$, 0.7), 461.2 (2.5), 460.2 (8.0), 314.2 (4.7), 244.2 (63.5), 243.2 (100.0). HRMS (Pos. FAB): calcd for $C_{32}H_{32}N_3O_5$ $[M+H]^+$ 538.2342, found 538.2344.

N-Tr-Valylglycine benzylamide (52). Compound **50** (30 mg, 0.058 mmol), *p*-nitrophenol (40 mg, 0.290 mmol), and predried 1-hydroxybenzotriazole (3 mg, 0.022 mmol) were dissolved in CH_2Cl_2 (0.15 mL) and were cooled to 0°C. The 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (52 mg, 0.174 mmol) was added and the reaction mixture was stirred at 0°C for 30 min and an additional 4 h at RT. Benzylamine (8 μL, 0.070 mmol) and Et_3N (81 μL, 0.580 mmol) were added, and after 8 h at RT the reaction mixture was diluted with $CHCl_3$ (50 mL) and extracted with 0.10 M NaOH (5 x 10 mL), pH 7 buffer (1 x 10 mL), and satd NaCl (1 x 20 mL). The residue was purified on SiO_2 (1.25 - 2.5% MeOH/$CHCl_3$) to afford 21 mg (72% yield) of **52** as a white solid. ^1H NMR (300 MHz, DMSO-d_6): δ 8.11 (1H, t, J = 5.57 Hz), 7.48 (1H, t, 5.00 Hz), 7.46 - 7.12 (16H, m), 4.24 (2H, d, J = 5.91 Hz), 3.40 (1H, m),

3.20 - 3.00 (2H, m), 2.90 (1H, d, J = 8.75 Hz), 1.82 - 1.68 (1H, m), 0.85 (3H, d, J = 6.74 Hz), 0.84 (3H, d, 6.84 Hz). Pos. FABMS, m/z (rel. intensity): 506.3 ($[M+H]^+$, 2.3), 314.2 (4.7), 244.2 (47.1), 243.2 (100.0). HRMS (Pos. FAB): calcd for $C_{33}H_{35}N_3O_2$ $[M+H]^+$ 506.2807, found 506.2806.

N-Trityl-[valylglycine]$_2$ benzylamide (53). Compound **52** (8 mg, 0.0158 mmol) was reacted with 5% Cl_2HCCO_3H/CH_3Cl_3 (0.50 mL) for 15 min at RT. The amine salt was precipitated with 1:1 Et_2O:hexanes (3 mL), and the solid was then triturated twice with 1:1 Et_2O:hexanes (3 mL portions). The amine salt was dissolved in dimethylacetamide (0.10 mL), Et_3N (14 μL, 0.102 mmol) was added, followed by the addition of compound **51** (11 mg, 0.0204 mmol). The reaction mixture was then allowed to stir for 8 h at RT. Solvents were removed *in vacuo* and the residue was purified on SiO_2 (1.25 - 5% MeOH/$CHCl_3$) to afford 5 mg (74% yield) of **53** as a white solid. ^1H NMR (300 MHz, DMSO-d_6): δ 8.26 (2H, m), 7.65 (1H, d, J = 8.14 Hz), 7.52 - 7.12 (22H, m), 4.36 - 4.18 (2H, m), 4.09 (1H, t, J = 7.30 Hz), 3.83 - 3.63 (2H, m), 3.50 - 3.38 (2H, m), 3.14 - 2.96 (2H, m), 2.90 (1H, d, J = 8.57 Hz), 2.00 - 1.86 (1H, m), 1.84 - 1.68 (1H, m), 0.83 (12H, m). Pos. FABMS, m/z (rel. intensity): 662.4 ($[M+H]^+$, 2.4), 584.3 (1.6), 244.2 (21.1), 243.2 (100.0). HRMS (Pos. FAB): calcd for $C_{40}H_{48}N_5O_4$ $[M+H]^+$ 662.3706, found 662.3710.

N-CBZ-Alanyl subunit benzylamide (54). Compound **38** (40 mg, 0.081 mmol) and pentafluorophenol (75 mg, 0.410 mmol) were dissolved in DMF (0.5 mL) and cooled to 0°C. The 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (60 mg, 0.203 mmol) was added, and the reaction mixture was stirred 4 h while gradually warming to RT. Benzylamine (12 μL, 0.113 mmol) and Et_3N (113 μL, 0.810 mmol) were added and the reaction mixture was allowed to stir an additional 8 h at RT. Solvents were

removed *in vacuo* and the residue was dissolved in $CHCl_3$ (20 mL) and extracted with H_2O (1 x 5 mL), 0.15 M NaOH (3 x 5 mL), and satd NaCl (1 x 10 mL). The organic layer was dried over $MgSO_4$ and solvents were removed under reduced pressure. The product was purified on SiO_2 (1.25 - 2.5% MeOH/$CHCl_3$) to afford 34 mg (72% yield) of **54** as a white solid. ^1H NMR (300 MHz, DMSO-d_6): δ 11.11 (1H, s), 8.71 (1H, m), 7.98 (2H, d, J = 8.44 Hz), 7.91 (1H, d, J = 7.21 Hz), 7.69 (1H, m), 7.55 (2H, d, J = 8.54 Hz), 7.50 - 7.14 (11H, m), 4.99 (2H, AB_q, J_{AB} = 12.79 Hz, $\Delta\nu_{AB}$ = 30.54 Hz), 4.66 - 4.52 (1H, m), 4.45 - 4.33 (1H, m), 4.29 (2H, d, J = 5.87 Hz), 3.87 - 3.74 (1H, m), 1.32 (9H, s). Pos. FABMS, m/z (rel. intensity): 583.1 (30.6), 582.1 ($[M+H]^+$, 76.5), 386.2 (15.3), 238.1 (27.1), 161.1 (87.1), 91.1 (54.1), 85.1 (100.0). HRMS (Pos. FAB): calcd for $C_{33}H_{36}N_5O_5$ $[M+H]^+$ 582.2716, found 582.2716.

N-BOC-Glycylalanine-derived subunit benzylamide (55). Compound **54** (10.0 mg, 0.0172 mmol) was dissolved in HBr·HOAc (0.50 mL) and stirred for 15 min at RT. Anhydrous Et_2O (4 mL) was added to precipitate the amine salt, which was subsequently centrifuged and triturated twice with additional anhydrous Et_2O (4 mL portions). The amine salt was dissolved in DMF (0.10 mL), Et_3N (24 μL, 0.172 mmol) was added, followed by the addition of N-BOC-glycine *p*-nitrophenyl ester (5.6 mg, 0.0189 mmol). After stirring for 16 h at RT, the solvents were removed *in vacuo*. The residue was dissolved in $CHCl_3$ (20 mL) and extracted with H_2O (1 x 5 mL), 0.15 M NaOH (3 x 5 mL), and satd NaCl (1 x 10 mL). The organic layer was dried over $MgSO_4$ and solvents were removed under reduced pressure. The product was purified on SiO_2 (1.25 - 2.5% MeOH/$CHCl_3$) to afford 8.0 mg (77% yield) of **55** as a white solid. ^1H NMR (300 MHz, DMSO-d_6): δ 11.07 (1H, s), 8.57 (1H, br m), 8.26 (1H, br m), 7.96 (2H, d, J = 8.37 Hz), 7.87 (1H, d, J = 7.45 Hz), 7.53 (2H, d, J = 8.46 Hz), 7.35 - 7.15 (6H, m), 7.01 (1H, br m), 4.84 - 4.72 (1H, m), 4.40 - 4.20 (3H, m with 2H, d, J =

5.28 Hz at 4.28), 3.97 - 3.83 (1H, m), 3.65 - 3.45 (2H, m), 1.35 (9H, s), 1.31 (9H, s). Pos. FABMS, m/z (rel. intensity): 606.3 (30.6), 605.3 ($[M+H]^+$, 82.4), 594.4 (35.3), 272.1 (20.0), 161.1 (100.0), 91.1 (21.4), 77.1 (21.2), 57.1 (15.3). HRMS (Pos. FAB): calcd for $C_{32}H_{41}N_6O_6$ $[M+H]^+$ 605.3087, found 605.3085.

V. ENDNOTES

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APPENDIX

VI. APPENDIX

Chemical Abstracts Names for Key Compounds

(21). Ethanol, 2-[2-[[(4-methoxyphenyl)diphenylmethyl]amino]ethoxy]-

(22). 1,2-Pyrrolidinedicarboxylic acid, 1-(9*H*-fluoren-9-ylmethyl) 2-[2-[2-[[(4-methoxyphenyl)diphenylmethyl]amino]ethoxy]ethyl] ester

(23). 1,2-Pyrrolidinedicarboxylic acid, 4-hydroxy-, 2-(4-nitrophenyl) 1-[2-(phenylsulfonyl)ethyl] ester

(24). DL-Proline, 1-[4-hydroxy-1-[[2-(phenylsulfonyl)ethoxy]carbonyl]-DL-prolyl]-, 2-[2-[[(4-methoxyphenyl)diphenylmethyl]amino]ethoxy]ethyl ester

(25). DL-Proline, 1-[4-[[(4-nitrophenoxy)carbonyl]oxy]-1-[[2-(phenylsulfonyl) ethoxy]carbonyl]-DL-prolyl]-,2-[2-[[(4-methoxyphenyl)diphenylmethyl]amino]ethoxy] ethyl ester

(30). 3-Oxazolidinecarboxylic acid, 4-[(4-amino-2-oxo-1(2*H*)-pyrimidinyl)methyl]-2,2-dimethyl-, 1,1-dimethylethyl ester, (*R*)-

(34). 3-Oxazolidinecarboxylic acid, 2,2-dimethyl-4-[[4-[[4-(4-morpholinylmethyl) benzoyl] amino]-2-oxo-1(2*H*)-pyrimidinyl]methyl]-, 1,1-dimethylethyl ester, (*R*)-

(35). Benzamide, *N*-[1,2-dihydro-1-[3-hydroxy-2-[(triphenylmethyl)amino]propyl]-2-oxo-4-pyrimidinyl]-4-(4-morpholinylmethyl)-, (*R*)-

(10). Carbonic acid, 3-[4-[[4-(4-morpholinylmethyl)benzoyl]amino]-2-oxo-1(2*H*)-pyrimidinyl]-2-[(triphenylmethyl)amino]propyl 4-nitrophenyl ester, (*R*)-

(36). 3-Oxazolidinecarboxylic acid, 4-[[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2*H*)-pyrimidinyl]methyl]-2,2-dimethyl-, 1,1-dimethylethyl ester, (*R*)-

(37). Carbamic acid, [2-[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2*H*)-pyrimidinyl]-1-(hydroxymethyl)ethyl]-, phenylmethyl ester, (*R*)-

(38). 1(2*H*)-Pyrimidinepropanoic acid, 4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-α-[[(phenylmethoxy)carbonyl]amino]-, (*R*)-

(39). Glycine, *N*-[3-[4-[[4-(1,1-dimethylethyl)benzoyl]amino]-2-oxo-1(2*H*)-pyrimidinyl]-N-[(phenylmethoxy)carbonyl]-D-alanyl]-, 1,1-dimethylethyl ester

(40). Glycine, *N*-[3-[4-[[4-(4-morpholinylmethyl)benzoyl]amino]-2-oxo-1(2*H*)-pyrimidinyl]-N-{(phenylmethoxy)carbonyl]-D-alanyl]-, 1,1-dimethylethyl ester

(12). Glycine, *N*-[3-[4-[[4-(4-morpholinylmethyl)benzoyl]amino-2-oxo-1(2*H*)-pyrimidinyl]-*N*-(triphenylmethyl)-D-alanyl]-, 4-nitrophenyl ester