A novel acyl protecting group for cytosine and adenine has been prepared from 4-(chloromethyl) benzoic acid. Reaction of the acid with morpholine produces 4-(4-morpholinyl)methylbenzoic acid which is converted to its acid chloride with thionyl chloride. This may be used to acylate cytidine and adenosine under standard conditions. This ionizable protecting group has the ability to solublize protected oligomers, which allows their purification with ion-exchange chromatography on S-Sepharose. Solid phase synthesis has been performed using this protecting group on morpholine nucleosides. Morpholine nucleoside carbamates were synthesized in high yields. The results revealed that high purities of the hexamers were obtained. These hexamers, which have the protecting groups intact, provide the potential for further segment condensation to make large size oligonucleotide analogues.

The success of the solid phase synthesis was dependent upon use of a selectively cleavable anchor which allowed the finished oligomer to be released from the resin with protecting groups intact. Attempts to modify the anchor to make it more efficient were unsuccessful. It was found that DBU degrades derivatized polystyrene resin and should not be used at early stages in solid phase synthesis.
Advances in Solid Phase Synthesis of Neutral Oligonucleotide Analogues

by
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This thesis is dedicated to my husband Guangliang.

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I thank Jane Hogeland and Jeff Nelson for their many hours of helpful discussion and all the assistance they provided me on this project.

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I thank Dr. Gould also for his helpfulness with my project.
Table of Contents

1  Introduction .............................................................. 1
2  Results and Discussion .................................................. 16
3  Experimental .............................................................. 49

Bibliography ........................................................................ 70
# List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Oligonucleotide analogues</td>
<td>3</td>
</tr>
<tr>
<td>II. Amide and peptide series</td>
<td>4</td>
</tr>
<tr>
<td>III. 4-(4-Morpholinyl)methylbenzoyl group</td>
<td>6</td>
</tr>
<tr>
<td>IV. Linkages for anchoring in solid phase peptide synthesis</td>
<td>8</td>
</tr>
<tr>
<td>V. Linkages for anchoring in solid phase oligonucleotide synthesis</td>
<td>12</td>
</tr>
<tr>
<td>VI. Morpholine nucleotide carbamates</td>
<td>15</td>
</tr>
<tr>
<td>VII. Solid phase synthesizer</td>
<td>25</td>
</tr>
<tr>
<td>VIII. Mass spectrum of crude hexamer 55</td>
<td>29</td>
</tr>
<tr>
<td>IX. Ion-exchange chromatography of hexamer 55 (old deprotection method)</td>
<td>30</td>
</tr>
<tr>
<td>X. Ion-exchange chromatography of hexamer 55 (new deprotection method)</td>
<td>34</td>
</tr>
<tr>
<td>XI. Mass spectrum of purified hexamer 55</td>
<td>36</td>
</tr>
<tr>
<td>XII. $^1$H NMR spectrum of purified hexamer 55</td>
<td>37</td>
</tr>
<tr>
<td>XIII. Ion-exchange chromatography of hexamer 60</td>
<td>41</td>
</tr>
<tr>
<td>XIV. Mass spectrum of hexamer 60</td>
<td>42</td>
</tr>
<tr>
<td>XV. $^1$H NMR spectrum of hexamer 60</td>
<td>43</td>
</tr>
</tbody>
</table>
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Common strategies for solid phase peptide synthesis</td>
<td>9</td>
</tr>
<tr>
<td>II. Protocol for the solid phase synthesis</td>
<td>22</td>
</tr>
<tr>
<td>III. Monitoring the coupling reactions</td>
<td>26</td>
</tr>
<tr>
<td>IV. Swelling the resin 27.</td>
<td>27</td>
</tr>
<tr>
<td>V. Deprotection of trityl group from 27.</td>
<td>27</td>
</tr>
<tr>
<td>VI. Deprotection of monomer 39.</td>
<td>31</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

Natural occurring oligonucleotides and chemically synthesized oligonucleotide analogues are of interest because of their biological significance. They can control genetic expression at the level of transcription or translation, based upon the sequence specificity of their binding to nucleic acids. Thus, the transfer of genetic information can be inhibited by duplex formation from a complementary antisense agent and a targeted segment of mRNA via Watson-Crick base pairing[1, 2, 3, 4, 5, 6, 7]. However, there are potential problems for the use of naturally occurring oligonucleotides as “antisense” reagents. One problem is that they cannot easily penetrate cell membranes due to the charges on the phosphate groups. Additionally, they are prone to degradation because of their sensitivity to cellular nucleases. Thus the synthesis of oligonucleotide analogues with more desirable properties has become very important for the medicinal chemist.

From the structural point of view, there are two general approaches for modification of oligodeoxynucleotides 1. In the first approach, the basic nucleoside unit (sugar plus base) is retained with little or no modification and the phosphodiester group can be replaced by a new, achiral linkage which is not recognized
by nucleases. This strategy has the advantage of availability of the nucleoside building blocks. Walker et al.\[8\] have demonstrated this approach by construction of uncharged nucleic acid analogues 4, which gave evidence of base stacking as in natural nucleotides. Two years later, Weller and Summerton\[9\] reported on the successful binding of carbamate linked oligonucleosides 5 to complementary DNA and RNA. Several other examples have also been found to be effective gene regulators and potential antiviral agents, e.g. methanephosphonates 2 \[10, 11, 12, 13\] and phosphoramidates 3 \[14, 15, 16, 17, 18\], which have been examined in both biophysical and biological test systems.

In the second approach for oligonucleotide modification, the sugar and phosphate group are replaced by a new backbone entirely. This approach has been explored to a much lesser extent. In the pioneering work on polyvinyl compounds conducted by Pitha et al.\[19\], species such as 6 formed a complex with complementary DNA homopolymers. The binding was not perfect due to the unfavorable spacing of the bases and atactic nature of the polymer\[19\]. More recently, binding has been found with other nonsugar backbones including the polyphosphates 7 \[20\], and the polylysines 8 and polyethyleneimines 9 \[21\].

Our group has recently completed modeling studies on a variety of nonsugar backbones of the amide type (such as polyamides, polycarbamates) for use as single stranded nucleic acid binding agents \[22\]. One of the more attractive structural types which emerged from the modeling study was the nylon backbond, i.e. oligomers derived from the amino acids 10a (amide - 5(1)) and 10b (amide - 6(1)). An interesting feature of the modeling is the predicted target preference in the amide - 5(1) and amide - 6(1) series. The amide - 5(1) series showed no low energy conformers when targeted against A - form nucleic acid (RNA), but appeared to be capable of binding with B - form nucleic acid (DNA). The R - series was predicted to show more favorable binding to DNA than the S - series. The amide - 6(1) series was predicted to bind to both A - and B - form targets (RNA
1. R = O\textsuperscript{-} oligodeoxyribonucleotide
2. R = CH\textsubscript{3} methanephosphonates
3. R = NR'R\textasciitilde, R', R\textasciitilde = H or alkyl group phosphoramidates

Base = A, T, C, G

4. acetate-linked analogue
5. carbamate-linked analogue

Figure I  Oligonucleotide analogues
Figure I (Cont)

6 polyvinyls

7 polyphosphates

8 polylysines

9 polyethyleneimines

Figure II Amide and peptide series

10a n = 1, amide-5(1)

10b n = 2, amide-6(1)

11 peptide-6(1)
and DNA). The peptide - 6(1) series 11 also appears to be a strong candidate. It has a strongly ordered backbone with low overall strain energy.

Recently, both amide - 5(1) and amide - 6(1) series have been synthesized, and preliminary biophysical studies have begun. The S - series of amide - 5(1) does not form a duplex with DNA. The results from amide - 6(1) series showed interactions between the neutral analogue and d(G)₆ as evidenced by a hypochromic effect at 272 nm. A major problem, which has thus far prevented extensive studies of the binding to nucleic acids in this series, is that their synthesis is relatively difficult, and has not provided adequate quantities of material.

Early results on the synthesis of acyclic analogues show that hexamers can be synthesized by solution phase chemistry, but with great difficulty [23, 24]. These oligoamide analogues become very insoluble in most organic solvents, especially when attempting to prepare oligomers higher than trimers. Purification of the oligomers by silica gel chromatography is very difficult due to the high polarity of these oligoamides. The use of reverse phase or ion-exchange chromatography is impossible because these compounds are not soluble in water. Clearly, some of these problems might be avoided by the application of solid phase chemistry. The purification and characterization after each intermediate coupling reaction can be avoided, and another great advantage of solid phase synthesis is its speed. However, the application of solid phase synthesis would also require suitable methods for the purification and characterization of the final products, and at present these are not well developed.

In order to facilitate the move from solution phase chemistry to solid phase chemistry for the preparation of amide type oligonucleotide analogues, we envisioned the use of a new protecting group for the nucleic acid bases. From work with other neutral oligonucleotide analogues, it was known that oligomers with intact base protecting groups are strongly preferred for FAB mass spectrometry, the primary means of characterization of these oligomers [25]. The new base pro-
tecting group must have the ability to solubilize the oligomers during the coupling reactions and later for purification, and allow the fully protected oligonucleotide analogues to be characterized by FAB mass spectrometry. An initial attempt by Huang, et al.[23] to make the bases more lipophilic by using the t-butylbenzoyl protecting group was largely unsuccessful. We chose to develop a new protecting group which would be compatible with the developed synthetic methods of the amide series but which would later promote solubility in water so as to enable aqueous strategies of purification. As we decided to keep the concept of the acyl protecting group for the bases, one choice of a water solubilizing protecting group appeared to be the 4-(4-morpholinyl)methylbenzoyl group 12 (Figure III). As this protecting group represents only a small change from the benzoyl group, which is the usual protecting group for the cytosine and adenine bases, we expected that this new group would be stable under conditions of synthesis of acyclic amide analogues[23, 24], and would be soluble in dilute aqueous acid, which would allow purification of the oligomer by reverse phase or even ion-exchange chromatography. An important concern however, was the need to devise a means of cleaving a fully protected oligomer from the solid support without also removing the acyl protecting groups.

Solid phase synthesis has shown great advantages for the synthesis of polypep-
tides and polyoligonucleotides. Solid phase synthesis was introduced by Merrifield in 1963 [26] for peptide synthesis and other workers have since extended this concept to nucleic acid synthesis. The basic idea of the solid phase approach involves covalent attachment (anchoring) of the growing peptide or oligonucleotide chain to an insoluble polymeric support (resin carrier), so that unreacted soluble reagents can be removed by simple filtration and washing without manipulative losses. Subsequently, the insoluble peptide - or oligonucleotide - resin is extended by a series of addition cycles. After the desired sequence of amino acids or nucleotides has been linked together on the support, a reagent can be applied to cleave the chain from the support and liberate the finished peptide or oligonucleotide into solution. However, in most syntheses of peptides or oligonucleotides the desired product is the fully deprotected species. Typically, the reaction step which cleaves the full length molecule from the resin will also remove any side chain protecting groups on the individual amino acids or bases.

The more common protocols for peptide synthesis are shown in Table 1. The original method of Merrifield employed the Boc group as the “temporary protecting group”, which is cleaved by trifluoroacetic acid following each addition step. The reactive side chains are typically protected as the benzyl esters, and are cleaved with hydrogen fluoride (HF), which also cleaves the benzylic ester bond linking the first amino acid to the resin. A more recent method uses the Fmoc group for “temporary” protection, and cleaves the side chain t-butyl esters and Boc group as well as the linkage to the anchor with trifluoroacetic acid. In both cases the final product is fully deprotected. Although these protocols are highly efficient, recent studies on the synthesis of very long peptides have shown that a stepwise assembly of the protected oligopeptide is beneficial. This approach demands that fully protected oligomers be obtainable after solid phase synthesis and, for this purpose, selectively cleavable anchors have been developed.

Recently, several groups have reported work on selectively cleavable anchors,
Figure IV  Linkages for anchoring in solid phase peptide synthesis
Table I. Common strategies for solid phase peptide synthesis

<table>
<thead>
<tr>
<th>Resin</th>
<th>Alpha deprotection</th>
<th>Side-chain blocking</th>
<th>Cleavage reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Boc</td>
<td>TFA, HCl</td>
<td>Benzyl</td>
</tr>
<tr>
<td>14</td>
<td>Fmoc</td>
<td>Piperidine</td>
<td>t-Butyl</td>
</tr>
</tbody>
</table>

or handles [27, 30, 31, 32, 33, 34, 35, 36]. Marshall [37] and Liener [38] introduced a benzylated phenol-sulfide anchoring linkage 15 (4-hydroxyphenylthiomethyl) for the preparation of cyclic peptides. The first amino acid was attached as the phenyl ester and the chain elongated by cleavage of the temporary Boc protecting group and addition of the next amino acid. Removal of oligonucleotide from the resin was accomplished by oxidation of the sulfide to sulfone with hydrogen peroxide [39] in dioxane followed by removal of the terminal Boc group. The activated peptide ester was allowed to self-condense, and the intramolecular cyclization to the product was promoted by shaking the polymer 18 hours in 2% triethylamine/dimethylformamide (DMF). The use of this type of anchor in conventional acyclic peptide synthesis was described by Johnson and Jacob [40]. They reported the peptide linkage to be stable during the oxidation from sulfide to sulfone. However, cysteine, methionine and tryptophan were affected by this treatment.

Kaiser and co-workers have reported the usefulness of p-nitrobenzophenone oxime/polystyrene-1% divinylbenzene copolymer [27] (a solid support 16, p-nitrobenzophenoneoxime resin) in peptide synthesis [41, 42, 43]. They demonstrated the cleavage of protected peptides from the oxime resin by amino acid esters in the presence of acetic acid as a catalyst to give peptide esters [42], or by 1-hydroxypiperidine to give 1-piperidinyl esters [43]. The protected peptide 1-piperidyl esters were subsequently converted to the corresponding free carboxylic acids by reduction with zinc in acetic acid. A peptide with 44-amino acid residues
are successfully synthesized utilizing this support [44].

In 1983, Tjoeng and Heavner [45] introduced the photolabile handle [4-(2-chloropropionyl)phenyl]acetic acid. The incorporation of the handle to an aminomethyl resin to form 4-(2-chloropropionyl)phenylacetamidomethyl-resin 17 was achieved via its acid chloride or dicyclohexylcarbodiimide mediated coupling. The first amino acid was attached by displacement of resin bound phenacyl chloride by carboxylate to form the phenacyl ester. The utility of the resin was demonstrated by the stepwise synthesis of two protected peptide segments of thymopoietin II. The peptide was released from the support upon irradiation at a wavelength of 350 nm.

A new base-labile anchoring group 18 (9-hydroxymethyl fluorene resin), derived from 9-(hydroxymethyl) fluorene-4-carboxylic acid for solid phase peptide synthesis, was described by Mutter and Bellof [46]. The fluorenyl ester linkage exhibited properties similar to the now well-known Fmoc protecting group. It is stable to acidic conditions and cleavable by 15% piperidine in DMF. In combination with acid-labile side chain protecting groups, it allows the synthesis of fully protected peptides or peptide segments for further condensation. The model tetrapeptide Leu-Ala-Gly-Val was synthesized using Boc-amino acids in combination with this anchor attached to aminomethylated polystyrene (co - 2% divinyl benzene) resin. The Boc-protected tetrapeptide was released quantitatively from the resin by treatment with 15% piperidine in DMF for 30 minutes. More recently, Felix and co-workers [47] similarly reported a novel bifunctional compound 19 (9-hydroxymethyl-2-fluorenemethyl resin), which is claimed to have increased stability of the linkage during the peptide coupling step, wherein it is exposed to the basic amino groups.

Early this year, Eritja, et al.[48] reported a new approach to the solid phase synthesis of protected peptides by using a base-labile 2-(2-nitrophenyl)ethyl (NPE) - resin 20. They indicated that no special care need be taken during the synthesis
of protected peptides with an NPE - resin, unless L-Pro and D-Pro are the two first amino acids of the sequence. To demonstrate the usefulness of the NPE handle, the following protected peptides, containing a wide range of protecting groups were synthesized: (i) Boc-Leu-Ala-Gly-Val-OH (93% yield after cleavage and 99% purity by HPLC); (ii) Boc-Glu(OBzl)-Ser(Bzl)-Gly-OH (91% yield, 98% purity); (iii) Boc-Lys(Z)-Lys(Z)-Ala-OH (89% yield, 98% purity).

For the solid phase synthesis of oligodeoxynucleotides, the succinoyl group [49] is the most widely used anchor for linking the 3'-OH group of the first nucleoside to the amino function of derivatized solid supports, which are usually controlled pore glass, as in support 21. However, this linkage is cleaved via ammonolysis, which also removes the base protecting groups [50]. Schwyzer, et al.[51] used a very simple and versatile anchor for the synthesis of protected oligonucleotide 3'-phosphates, namely: 2-(4-carboxyphenylmercapto)ethanol 22. After completion of oligonucleotide synthesis, the linker 22 can be oxidized to the sulfoxide stage 23 with NaIO₄ [52] without harming the attached deoxynucleotides. N-chloro succin-imide may also be employed as the oxidizing reagent, as can 3'-chloroperbenzoic acid, to produce the sulfones 24. However, the latter oxidant may also react with the bases and was not generally recommended. The p-carbamoyl substituent enhances the base-catalyzed β-elimination reaction of 23 and 24 considerably. One particular advantage of this support family is the easy accessibility of different oxidation stages. Each degree of oxidation entails a different degree of stability toward base-catalyzed β-elimination.

Early this year, Eritja, et al.[53] introduced a new polymeric support (NPE-resin) 25 for the synthesis of oligonucleotides which contain a 2-(2-nitrophenyl) ethyl linkage which is labile to base through a β-elimination process. It has the capability of providing protected 3'-free hydroxy oligonucleotides. The carbonate linkage of 25 was stable under all the conditions encountered during oligonucleotide synthesis, as well as to a solution of 40% triethylamine in pyridine. The carbonate
Figure V  Linkages for anchoring in solid phase oligonucleotide synthesis
Figure V (Cont)
linkage in DMT-T-NPE-resin was cleaved in less than 1 hour when it was treated with 0.5 M 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU) solution in dioxane or pyridine. They also found that ammonia (5 h, 55°C) and 20% piperdine/DMF (3 h, RT) were able to cleave the nucleoside from the resin. The NPE - resin can also be used as a support for the preparation of oligonucleotides bearing a phosphate group at the 3'- end. Reaction of 2-cyanoethyl phosphoramidates with the primary alcohol functionality on the support and subsequent oxidation gives a phosphate - triester 26 which is stable during the synthesis and is cleaved by conc. ammonia.

Earlier work in our laboratory had focused on the use of a protected dipeptide as the basis for the cleavable anchor 27 [54]. The key to the strategy was the use of a trifunctional amino acid, hydroxyproline, the 4-hydroxyl group of which allowed attachment of the dipeptide to the solid support. The carboxyl group was linked via an amide bond to a proline which was esterfied to the alcohol residue of the first subunit. The nitrogen of the 4-hydroxyproline was blocked with the desired selectively cleavable group, in this case a β-elimination sensitive group such as (phenylsulfonyl)ethoxy carbonyl (PSEC). Treatment of this system with a strong, non-nucleophilic base such as DBU would lead to cleavage of the PSEC group, loss of carbon dioxide and freeing of the secondary amine, thus allowing cyclization to the diketopiperazine with release of the alcohol. The advantage of this type of resin is that the PSEC could be replaced by other types of amino protecting group so as to allow the use a single anchor type which would be cleaved by a wide variety of conditions. As will be seen, the specific disadvantage of this resin is the amount of work involved in attaching the first residue to the column. We decided to employ the resin 27 because of its availability and the local expertise in its use. The deficiencies of its preparation will be addressed later.

The model system chosen for testing the protecting group was the morpholine nucleoside carbamates 28 (Figure VI) employing the new resin 27. The morpholine series was chosen because the coupling chemistry required to join the morpholine
Figure VI Morpholine nucleotide carbamates

nucleoside subunits is similar to the chemistry for the amide 10 and peptide systems 11 respectively, and obviously nearly the same as for the acyclic carbamate systems also under investigation in our group. These nucleosides are relatively easily obtained from ribonucleosides, while synthesis of subunits in the amide series is at present, relatively tedious. Additionally, these oligomers are very water insoluble, and would provide an excellent model system for the evaluation of a water solubilizing protecting group.
Chapter 2

Results and Discussion

The synthesis of the ionizable acyl group for cytosine and adenine protection was begun by treatment of 4-(chloromethyl)benzoic acid 29 with excess morpholine (Scheme I). A small amount of ethanol was added to make the solution homogeneous, and after 36 h at reflux, 4-(morpholinomethyl) benzoic acid 30 was obtained in 86% yield. Compound 30 was then treated with a large excess of thionyl chloride at 60°C to give acid chloride 31. The acid chloride was triturated with anhydrous diethyl ether to give a white solid. The IR spectrum was suggestive of an aromatic acid chloride. The crude acid chloride reacted with cytosine 32 in pyridine - dichloromethane to give N4-[4-(morpholinyl)methyl]benzoyl cytosine 33 in 92% yield (Scheme II). The reaction of the acid chloride with cytidine 34 was carried out under the usual transient protection condition [55]. Formation of the 2',3',5'-O-tris(trimethylsilyl) derivative occurs in minutes upon addition of chlorotrimethylsilane[56] to a suspension of cytidine in pyridine (Scheme III). This labile compound has not been isolated, however N-acylation of 35 is unaffected by the presence of even a substantial excess of chlorotrimethylsilane, and is complete within 4 hours following addition of 31. Conversion of protected cytidine 36 to the morpholine nucleoside by periodate cleavage and reductive amination[57] gave 38. In this procedure, N4-[4-(morpholinyl)methyl] benzoyl cytidine 36 was treated
with sodium periodate and ammonium biborate in methanol to afford the intermediate dialdehyde 37. The precipitated sodium iodate was removed by filtration, and to the solution of 37 was added sodium cyanoborohydride, followed by excess p-toluenesulfonic acid. The desired morpholine nucleoside 38 was obtained in 62% yield as the tosylate salt, which precipitate from the reaction mixture. Ammonium biborate was employed as the nitrogen source for the morpholine ring because of the buffering capability of the biborate salt and the concern for the epimerization of the intermediate dialdehyde. However, use of ammonium carbonate as the nitrogen source also afforded a single diasteromer of morpholine nucleoside 38 in 56% yield. The amine of the morpholine ring of 38 was protected with trityl chloride to give the readily purified derivative 39 in 91% yield. Reaction of 39 with bis(p-nitrophenyl) carbonate gave the activated subunit 40 in 83% yield.

Synthesis of the adenine morpholine nucleoside was performed similarly. Reaction of acid chloride 31 with adenosine under the usual transient protection condition gave 43 (Scheme IV). Reaction of adenine nucleosides with benzoyl chloride is known [58] to give the N,N - dibenzoyl derivative. However, selective hydrolysis of one of the benzoyl groups may be achieved by brief treatment with 2 M aqueous
ammonia. In the case of 43, we have not found any indication of formation of the N,N-dibenzoyl derivative. The ammonia treatment was employed however, as it also effects instant removal of the trimethylsilyl groups. The crude product 43 was obtained in 91% yield and was subsequently used without further purification. The conversion of 43 to the morpholine nucleoside by periodate cleavage and reductive amination followed as for the cytosine case, but due to the failure of the p-toluenesulfonic acid salt to precipitate, HCl was used to facilitate the reductive amination. After removal of solvents, the crude morpholine nucleoside hydrochloride was tritylated on the morpholine nitrogen by treatment with trityl chloride and triethylamine. Chromatographic purification provided the adenine morpholine nucleoside 45 (55% yield from 43). Preparation of the active carbonate 46 was achieved by treatment of 46 with bis(p-nitrophenyl)carbonate employing triethylamine as the catalyst. It should be noted that we also have attempted to prepare acylated adenine. However this has not been successful.

The solution phase oligomerization of morpholine nucleoside carbamates related to 40 and 47 is well documented [57], and hence we immediately turned our attention to the preparation of the oligomers by solid phase synthesis. The func-
Scheme III
Scheme IV
Scheme V
<table>
<thead>
<tr>
<th>Steps</th>
<th>Time</th>
<th>Repetitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 DCM (0.5 mL)</td>
<td>1 m</td>
<td>3</td>
</tr>
<tr>
<td>2 2% DCA/DCM (0.5 mL)</td>
<td>1 m</td>
<td>1</td>
</tr>
<tr>
<td>3 2% DCA/DCM (0.5 mL)</td>
<td>7 m</td>
<td>1</td>
</tr>
<tr>
<td>4 DCM (0.5 mL)</td>
<td>1 m</td>
<td>4</td>
</tr>
<tr>
<td>5 10% Et$_3$N/DCM (0.5 mL)</td>
<td>1 m</td>
<td>2</td>
</tr>
<tr>
<td>6 DCM (0.5 mL)</td>
<td>1 m</td>
<td>1</td>
</tr>
<tr>
<td>7 DMA (0.5 mL)</td>
<td>1 m</td>
<td>3</td>
</tr>
<tr>
<td>8 30 μmol subunit/150 μL DMA + 30 μmol Et$_3$N</td>
<td>5 h</td>
<td>1</td>
</tr>
<tr>
<td>9 DMA (0.5 mL)</td>
<td>1 m</td>
<td>1</td>
</tr>
<tr>
<td>10 DMA (0.5 mL)/Et$_3$N (50 μL)/ Ac$_2$O (50 μL)</td>
<td>9 m</td>
<td>1</td>
</tr>
<tr>
<td>11 DMA (0.5 mL)</td>
<td>1 m</td>
<td>3</td>
</tr>
<tr>
<td>12 repeat step 1 - 11 for each subunit</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table II. Protocol for the solid phase synthesis

Functionalized resin 27 was synthesized beginning with esterification of the morpholine nucleoside 47 with N-Fmoc proline (Scheme V). After removal of the Fmoc group from 48 by non-nucleophilic means, the free amine was treated with 49 to provide the dipeptide 50. Conversion to the p-nitrophenyl carbonate 51, followed by reaction with 1% crosslinked aminomethyl polystyrene 52 provided the resin 27. The loading of resin 27 was 169 μmol/g dry resin. This was measured by quantitation of trityl group removed from the column by treatment with 2% dichloroacetic acid in dichloromethane. The analysis was performed by UV at 431.5 nm in 20% trifluoroacetic acid/chloroform.

The solid phase synthesis of the hexamer 55 was performed following the protocol in Table 2. The dried, functionalized resin 27 was first deprotected by 2% dichloroacetic acid in dichloromethane. After neutralization by 10% triethylamine
in dichloromethane, the deprotected resin 27 was reacted with the activated subunit 40 at room temperature to form supported dimer 53 (Scheme VI) in the manual solid phase synthesizer (Figure VII). The procedure was repeated four more times as per Table 2 to form the supported hexamer 54. The oligomer was cleaved from the solid support by DBU/diethyl malonate/DMF solution to give crude product 55. Diethyl malonate has been found to be an effective scavanger for the electrophilic alkene formed in this deprotection [59]. After evaporation, the oily product was precipitated from dichloromethane with a large excess of diethyl ether. The crude product was obtained in 78% overall yield. Examination of the crude product by FAB mass spectrometry suggested that the major product was not hexamer 55 but rather the corresponding pentamer.

In an attempt to identify this material, the crude product was fully deprotected by treatment with 2% dichloroacetic acid/dichloromethane (0.5 h), followed by ammonolysis in concentrated NH₃:DMF (1:1 v/v, 15 h at room temperature). The resultant material was then compared with fully deprotected morpholine nucleoside tetramer and hexamer made previously by solution phase reactions [57]. The solid phase synthetic product eluted midway between the tetramer and hexamer on ion-exchange chromatography on S-Sepharose using pH 2.5 phosphate buffer and a linear gradient of KCl (0 - 1.4 M) at flow rate 2.2 mL/min, suggesting that the product was indeed pentamer.

At this point we analyzed the solid phase coupling reactions by measuring the trityl species contained in the detritylation steps (2% dichloroacetic acid/dichloromethane) and subsequent washes. The amount of trityl group was measured by UV at 431.5 nm in 20% trifluoroacetic acid/chloroform, and is shown in Table III. We expected that the amount of trityl group would decrease slowly from the first to the last coupling cycle. It is clear from Table III, however, that the first deprotection step produced an abnormally low amount of trityl residue. We surmised that the problem might be due to inefficient swelling of the dry resin 27. Indeed, the
Scheme VI
Figure VII  Solid phase synthesizer
observation of 2.5 μmol/50 mg of trityl species, only corresponds to a loading of 50μmol/g dry resin, far less than initially measured for the wet resin immediately after its preparation.

We proceeded to determine the best solvent for swelling the dry resin 27, the accurate loading of the resin, as well the best reagent for detritylation. Dry resin 27 (10 mg) was transferred to six different vials. To each vial was added 1 mL of solvent and the mixture kept at ambient temperature for 15 hours. The resin was then washed and treated with different trityl deprotection reagents. The filtrates of the deprotection steps and the following washes were collected. The trityl content was measured by UV and the results are described in Table IV. From the data, it appeared that most solvents appeared to be good choices for dry resin swelling, suggesting that in the initial synthesis of hexamer 55 the resin was swollen for an insufficient amount of time. The resin 27 was next swollen in different vials by neat dimethylacetamide and dimethylacetamide + 20% toluene, then detritylated by 2% dichloroacetic acid (DCA)/dichloromethane (DCM) or 2% dichloroacetic acid + 10% trifluoroethanol (TFE)/dichloromethane, respectively. The results shown in Table V reveal that the differences in these sequences are very small. Dimethylacetamide was chosen for swelling the resin as it is also the prefered coupling solvent. Dichloroacetic acid/dichloromethane was chosen for the

<table>
<thead>
<tr>
<th>No. of coupling</th>
<th>Amount of -Tr based on 50 mg of resin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>2.5 μmol</td>
</tr>
<tr>
<td>2nd</td>
<td>9.5 μmol</td>
</tr>
<tr>
<td>3rd</td>
<td>8.5 μmol</td>
</tr>
<tr>
<td>4th</td>
<td>6.4 μmol</td>
</tr>
<tr>
<td>5th</td>
<td>6.2 μmol</td>
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</table>

Table III. Monitoring the coupling reactions.
<table>
<thead>
<tr>
<th>Solvent</th>
<th>Deprotection reagent</th>
<th>μmol of -Tr (10 mg resin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM</td>
<td>2% formic acid + 10% TFE/DCM</td>
<td>1.45</td>
</tr>
<tr>
<td>DCM</td>
<td>2% DCA + 10% TFE/DCM</td>
<td>1.61</td>
</tr>
<tr>
<td>DMA</td>
<td>2% DCA + 10% TFE/DCM</td>
<td>1.59</td>
</tr>
<tr>
<td>DMA + 20% Toluene</td>
<td>2% DCA + 10% TFE/DCM</td>
<td>1.68</td>
</tr>
<tr>
<td>DMA + 20% Pyridine</td>
<td>2% DCA + 10% TFE/DCM</td>
<td>1.45</td>
</tr>
<tr>
<td>pyridine</td>
<td>2% DCA + 10% TFE/DCM</td>
<td>1.65</td>
</tr>
</tbody>
</table>

Table IV. Swelling the resin 27.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Deprotection reagent</th>
<th>μmol of -Tr (10 mg resin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMA</td>
<td>2% DCA + 10% TFE/DCM</td>
<td>1.66</td>
</tr>
<tr>
<td>DMA</td>
<td>2% DCA/DCM</td>
<td>1.69</td>
</tr>
<tr>
<td>DMA + 20% Tol</td>
<td>2% DCA + 10% TFE/DCM</td>
<td>1.70</td>
</tr>
<tr>
<td>DMA + 20% Tol</td>
<td>2% DCA/DCM</td>
<td>1.72</td>
</tr>
</tbody>
</table>

Table V. Deprotection of trityl group from 27.
deprotection of the trityl group. The loading of the resin is then 169 μmol/g dry resin, consistent with the original assay of the resin.

The synthesis of hexamer was repeated using the conditions outlined above. The functionalized resin 27 was swollen in neat dimethylacetamide at ambient temperature for 15 hours, and the synthesis performed by the procedure described in Table 2. Because the temperature of the laboratory is quite variable we decided for this synthesis to maintain the temperature of the coupling reaction at 30°C. This was done by using a water circulating pump to deliver liquid to rubber tubing wrapped around the synthesis apparatus. The oligomer was cleaved from the solid support by DBU/diethyl malonate/DMF solution to give crude product in DMF. After precipitation with dichloromethane/diethyl ether, a white solid was obtained in 95% overall yield. The negative ion FAB mass spectrum of the crude hexamer (Figure VIII) showed a major hexamer peak and some fragmentation.

For purification, the oligomer was treated with 2% dichloroacetic acid/dichloromethane to remove the trityl group, followed by evaporation and precipitation from dichloromethane/diethyl ether. Ion-exchange chromatography was performed on S-Sepharose (sulfopropyl type resin from Pharmacia) using a phosphate buffer (pH = 2.5) and a linear gradient of KCl (0 - 1.4 M) in 84 minutes at a flow rate of 2.2 mL/min. The trace revealed a major peak, with a significant impurity as a slower running shoulder (Figure IX). The fractions containing each peak were collected and desalted by neutralization to pH = 8 with 0.01 N NaOH and extraction into 20% isopropanol/chloroform.

Retritylation was achieved by treatment of each sample with trityl chloride and triethylamine in DMF. Both products were precipitated from dichloromethane/diethyl ether. The retritylated samples were again analyzed by negative ion FAB mass spectrometry and the results revealed that the major peak corresponds to hexamer 55, while the second peak corresponds to mass 2200 and as yet remains unknown (Figure IX).
Figure VIII Mass spectrum of crude hexamer 55
Figure IX  Ion-exchange chromatography of hexamer 55
(old deprotection method)
Based upon TLC and the initial negative ion FAB mass spectrum, the crude hexamer sample appeared to be very pure. Missing in that FAB mass spectrum is a peak due to contamination at 2199 (M-1). However, following detritylation, a significant amount of impurity is evident in the ion-exchange chromatography. If we assume that the crude hexamer did not originally contain the 2199 MW impurity, then there appeared to be at least two possibilities.

1. Hexamer 55 is decomposing on the ion-exchange column, i.e. it may be not stable to pH 2.5 phosphate buffer.

2. Hexamer 55 is unstable to the detritylation process performed before ion-exchange chromatography.

In order to examine these possibilities, a stability study was performed with the monomer of cytosine morpholine nucleoside, 39. We wanted to ascertain the stability of the cytosine base protecting group N4-(morpholinomethyl) benzoyl group to pH 2.5 phosphate buffer and to the detritylation conditions. Small portions of the monomer 39 were put in four different vials. To each vial was added 1 mL of trityl deprotection reagents (2% formic acid/CH₂Cl₂, 2% acetic acid/CH₂Cl₂, 2% DCA/CH₂Cl₂ and 2% TFA/CH₂Cl₂). The deprotection was followed by TLC (10% MeOH/CHCl₃). The results are shown in Table VI.

Only a single product spot is observed in these deprotection systems, except that the product formed during 2% formic acid/dichloromethane deprotection is

<table>
<thead>
<tr>
<th>Time</th>
<th>2% formic acid</th>
<th>2% acetic acid</th>
<th>2% DCA</th>
<th>2% TFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 h</td>
<td>fully deprot.*</td>
<td>1/4 deprot.</td>
<td>fully deprot.</td>
<td>fully deprot.</td>
</tr>
<tr>
<td>1 h</td>
<td>fully deprot.*</td>
<td>1/4 deprot.</td>
<td>fully deprot.</td>
<td>fully deprot.</td>
</tr>
<tr>
<td>4 h</td>
<td>fully deprot.*</td>
<td>1/3 deprot.</td>
<td>fully deprot.</td>
<td>fully deprot.</td>
</tr>
<tr>
<td>24 h</td>
<td>fully deprot.*</td>
<td>fully deprot.</td>
<td>fully deprot.</td>
<td>fully deprot.</td>
</tr>
</tbody>
</table>

Table VI. Deprotection of monomer 39.
different from that produced by the other three deprotection reagents. By TLC this material was less polar than the product from the other reactions. The 2% acetic acid/dichloromethane deprotection process was very slow and it took almost 24 hours to complete. The detritylations by 2% dichloroacetic acid/dichloromethane and 2% trifluoroacetic acid/dichloromethane were very rapid. The products are stable to prolonged exposure to the deprotection conditions. When these products were retritylated the starting morpholine nucleoside 36a was produced cleanly. From these results we infer that the cytosine base protecting N4-(4-morpholinyl) methylbenzoyl group is stable to those conditions.

We next examined the stability of the glycosyl linkage and carbamate linkage in a dimer 56. The dimer was synthesized by coupling of 39 and 40 employing triethylamine as a catalyst in DMF solution. Then it was treated with 2% trifluoroacetic acid/dichloromethane, 2% dichloroacetic acid/dichloromethane, 2% acetic acid/dichloromethane and 2% formic acid/dichloromethane for 24 hours. The solutions were evaporated and the residue precipitated from dichloromethane/diethyl ether. No precipitate was obtained from 2% acetic acid/dichloromethane treatment and little precipitate was obtained from 2% formic acid/dichloromethane
treatment. A normal amount of precipitate was formed in both 2% trifluoroacetic acid/dichloromethane and 2% dichloroacetic acid/dichloromethane treatment. The three precipitates were chromatographed on S-Sepharose using the conditions described above. The results (not shown) indicated a major impurity peak in 2% formic acid/dichloromethane graph, but we can clearly see a deprotected product dimer peak. There is a small shoulder after the major deprotected dimer peak in 2% dichloroacetic acid/dichloromethane. The chromatographic pattern is similar to the chromatogram of the hexamer. The detritylated dimer samples were treated with pH 2.5 phosphate buffer or pH 5.0 succinic buffer for 24 hours and then applied to the ion-exchange column. The traces revealed that the major peaks from both reactions are identical with the major peaks obtained from the acid deprotection treatment. Therefore the carbamate linkage and the glycosyl linkage between two monomers is stable to ion-exchange buffer., but the results with the 2% dichloroacetic acid/dichloromethane suggest these detritylation conditions were causing some decomposition.

We considered 2% trifluoroacetic acid/dichloromethane potentially too harsh for deprotection of oligomers containing purine nucleoside, so we further examined the 2% dichloroacetic acid/dichloromethane deprotection. The work-up procedure for detritylation consisted of evaporation of the 2% dichloroacetic acid/dichloromethane reaction solution and precipitation of the residue from dichloromethane/diethyl ether. However, once dichloromethane was evaporated, dimer (or hexamer) would be exposed to neat dichloroacetic acid at water bath temperature (50 - 60°C). We changed the work-up procedure by instead adding a large excess of diethyl ether to the 2% dichloroacetic acid/dichloromethane solution to precipitate the product. The new work-up procedure was applied to both dimer 56 and hexamer 55. After work-up and ion-exchange chromatography, both compounds showed only a single peak (Figure X).

Although no shoulder is evident, the hexamer peak in Figure X shows some tail-
Figure X  Ion-exchange chromatography of hexamer 55
(new deprotection method)
ing. To determine whether this arises from a small tailing peak, the fractions containing the tailing part were collected and extracted with 20% isopropanol/chloroform followed by precipitation from dichloromethane/diethyl ether to give a white solid. The solid was again chromatographed under the same conditions, and the graph we obtained is identical with the original one, showing only small tailing. Finally, the fractions containing the hexamer peak were collected and desalted. The detritylated hexamer 55 was detritylated again to afford hexamer 55 in 81% yield. The negative ion FAB mass spectrum showed a single high MW ion envelope at the expected m/e 2848 (M-1) (Figure XI). The 300 MHz $^1$H NMR spectrum is consistent with 55 (Figure XII). Final confirmation of the structure of the hexamer 55 was obtained by complete removal of all protecting groups (detritylation, ammonolysis) and comparison with hexamer which had made previously by solution phase chemistry assembly of dimers [57].

It is interesting to note that the synthesis of hexamer 55 was affected by the temperature of the coupling reaction. When the oligomerization was performed at unregulated room temperature (T ~ 15 – 25°C), the ion-exchange chromatogram showed large contamination by smaller fragments which are presumably due to incomplete coupling reactions. When the oligomerization was performed at 30°C, the ion-exchange graph revealed a single peak with no contamination by shorter species.

The glycosyl linkage in adenine nucleosides is less stable toward acid than that of the corresponding cytosine series. To demonstrate the applicability of the N4-(morpholinomethyl) benzoyl protecting group to adenine nucleosides, the dimer 57 (Scheme VIII) was reacted with 2% trifluoroacetic acid/dichloromethane and 2% dichloroacetic acid /dichloromethane, and after 0.5 hour and 24 hour treatments showed exclusively the deprotected dimer peak. The deprotected dimer was treated with pH 2.5 phosphate buffer for 24 hours. The ion-exchange again revealed a single peak of deprotected dimer. Thus, like the cytosine series, the glycosyl linkages,
Figure XI  Mass spectrum of purified hexamer 55
Figure XII  $^1$H NMR spectrum of hexamer 55
carbamate linkages and adenine base protecting groups, are adequately stable to the acidic detritylation and ion-exchange processes.

Next, hexamer 60 was synthesized (Scheme IX). The swollen resin 27 was deprotected by 2% dichloroacetic acid/dichloromethane. The detritylated resin was coupled with the activated subunit 46 in the presence of triethylamine by the procedure described in Table 2, to form supported dimer of morpholine nucleoside benzoyl cytosine and morpholine nucleoside N4-(morpholinomethyl) benzoyl adenine 58. The procedure was repeated four more times to give supported hexamer 59 which had one cytosine base and five adenine bases. The oligomer was cleaved from the solid support with DBU/diethyl malonate [59] to give crude hexamer 60 in 84% overall yield (Scheme IX). The crude hexamer 60 was detritylated by 2% dichloroacetic acid/dichloromethane in 20 minutes, precipitated with ether, and the deprotected hexamer purified by ion-exchange chromatography on S-Sepharose. The graph revealed a major peak with a small amount of contamination by shorter species (Figure XIII). Isolation of the deprotected oligomer from the eluant was achieved by neutralization (0.1 N NaOH to pH 8) and extraction with 20% isopropanol/chloroform. Retritylation afforded 60 in 72% overall yield.
The negative ion FAB mass spectrum showed a single high MW ion envelope at the expected m/e 2968 (Figure XIV). The 300 MHz $^1$H NMR spectrum was also consistent with hexamer 60 (Figure XV).

The combination of the ionizable base protecting group and the selectively cleavable anchor for solid phase attachment proved convenient for the efficient synthesis, purification and characterization of base-protected hexameric neutral oligonucleotide analogues. The retritylated final product is a suitable substrate for activation and further couplings. However, the anchor which we employed was not entirely satisfactory. The synthesis of functionalized resin 27 (Scheme V) began with morpholine subunit 47 and built up the anchor in a stepwise fashion. This is not a very efficient method with respect to the first subunit of the oligomer. We have attempted to make construction of the anchor more efficient. Instead of starting from 47, we hoped to attach subunit 47 at the last step, directly before anchoring to the solid support.

The new approach is outlined in Scheme X. A highly functionalized hydroxyproline derivative 64 would be reacted with 47 to give ester 65. This would then reacted with the aminomethyl polystyrene 52 to yield resin 66. Removal of the Fmoc by non-nucleophilic means and attachment of PSEC-glycine 67 would give the resin 68. In this scheme there would be only one reaction involving 47 which required purification. The remaining reactions, which would complete the preparation of the dipeptide 68, would be done on the column and should be efficiently accomplished. The PSEC protecting group is applicable to the chemistry at hand, however this approach allows the choice of the anchor protecting group to be made at the last stage.

The new approach (Scheme X) began with N-Fmoc-4-hydroxyproline 61, which was reacted with diphenyldiazomethane [60] to give N-Fmoc-4-hydroxyproline benzhydrylester 62. This was activated with bis(p-nitropheryl)carbonate to form fully protected hydroxyproline 63. Compound 63 was deprotected with 25% tri-
Hi.

1. Cl₂HCCO₂H, CH₂Cl₂
2. 46. Et₃N, DMF

Reapt

84% overall yield

Scheme IX
Figure XIII  Ion-exchange chromatography of hexamer 60
Figure XIV  Mass spectrum of hexamer 60
Figure XV  $^1$H NMR spectrum of hexamer 60
Scheme X
fluoroacetic acid/dichloromethane, and then coupled with morpholine nucleoside 47 using a carbodiimide to provide 65. We also tried to make 65 by reacting 64 with thionyl chloride or oxalyl chloride, then coupling with 47, but the results indicated that either no product was formed or the starting material 64 had lost the p-nitrophenyl group. The use of triphosgene instead of thionyl chloride gave no recognizable product. Alternative mild reaction conditions, such as triphenylphosphine and carbon tetrachloride were also used, but no reaction occurred. Using carbodiimide as the coupling reagent gave product in 56% yield along with small amount of by-product which had lost the p-nitrophenyl carbonate activating group. Compound 65 was reacted with 1% crosslinked aminomethyl polystyrene resin 52 to provide functionalized resin 66. The loading of resin 66 was measured by quantitation of the trityl cation with UV at 431.5 nm in 20% trifluoroacetic acid/chloroform to give 204 µmol/g dry resin. After short treatment of the resin 66 with DBU/diethylmalonate in DMF, the free amine was reacted with compound 67 to provide dipeptide 68.

To be efficient, the functionalized resin 66 must allow essentially complete recovery of the morpholine subunit 47. For the resin 27, the recovery of 47 was greater than 95% yield after treatment with DBU/diethyl malonate in DMF for 1 hour. The new resin 66 (loading 204 µmol/g dry resin) was treated with DBU/diethyl malonate in DMF for 5 - 15 minutes, then coupled with 67 for 4 - 24 hours followed by the cleavage with DBU/diethyl malonate for 1 hour. The recovery of 47 was consistently 57%. That 43% still remained on the resin was confirmed by treatment with 2% dichloroacetic acid/dichloromethane and UV analysis of the washes. When the new resin was treated with DBU/diethyl malonate twice (10 m, 15 m), then coupled with 67 for 24 hours followed by the cleavage of PSEC group with DBU/diethyl malonate for 1 hour, the recovery of 47 was 75%. Longer cleavage time (30 minutes) actually led to lower yield of released trityl group. Later evidence showed the functionalized resin was seriously degraded by
DBU (vida infra).

We did not believe the problem arose from an inefficient coupling of deprotected resin 66 with 67. We considered it possibile that the Fmoc group was lost during the resin functionalization. During the anchoring step, the amino group is in excess on the resin 52 which could cleave the Fmoc group. The now unprotected proline amine would be reacted by the capping reagent in the next step. In order to avoid this problem, we considered a new protecting group which would not as sensitive to base as the Fmoc group. The optimal candidate appeared to be the (phenylsulfonyl)ethoxy carbonyl (PSEC) group which has a higher pKa than the Fmoc group.

4-Hydroxyproline 74 was reacted with 72 to provide 75 (Scheme XI), followed by protection of the carboxyl group with diphenyldiazomethane [60] to form 76. Activation of 76 with bis(p-nitrophenyl) carbonate gave fully protected 4-hydroxyproline derivative 77. Deprotection of 77 by 25% trifluoroacetic acid/dichloromethane provided 78, and coupling of 78 with morpholine subunit 47 employing 4-dimethylaminopyridine as the catalyst, gave 79. Compound 79 was then reacted with aminomethyl polystyrene 52 to give functionalized resin 80. Treatment of 80 with DBU/diethyl malonate twice (10 m, 1 h), was followed by coupling with 67 for 24 hours in the presence of triethylamine provided resin 68. Surprisingly, removal of the PSEC group (DBU/DEM/DMF, 1 h) released 47 in only 10% yield. We observed that the functionalized resin 68 undergoes degradation upon treatment with DBU/DEM/DMF. After 3 hours treatment, the resin turned to a sticky mass. We concluded that DBU should not be used in an early stage of derivatization of the resin. It is possible that degradation of resin 66 during DBU treatment is ultimately responsibile for the less than adequate release of the nucleoside from resin 68.
Conclusion:

1. The ionizable protecting group [4-(4-morpholinyl)methylbenzoyl] shows great promise for the solubilization of insoluble nucleic acid analogues. We expect this protecting group will apply to other backbones as well, e.g. amide, carbamate and peptide.

2. Application of solid phase synthesis to oligonucleotide analogues protected with the ionizable protecting group was quite satisfactory. It afforded us a high yield of the hexamers with the protecting groups intact which allowed purification and characterization of the oligomers.

3. Attempted modification of the solid anchor suggests that DBU should not be used in an early stage of derivatization of the resin, but is acceptable for use at the last step. Further work needs to be done before a completely satisfactory anchor is at hand.
Chapter 3

Experimental

Methylene chloride (CH₂Cl₂), dimethylacetamide (DMA) and dimethylformamide (DMF) were distilled from powdered calcium hydride (CaH₂). Pyridine was distilled over powdered barium oxide (BaO). These solvents were stored over 4Å molecular sieves. Trimethylchlorosilane was distilled from powdered CaH₂ prior to use. Diethyl ether was freshly distilled from sodium/benzophenone prior to use also. All other reagents were purified by distillation or recrystallization prior to use whenever necessary. The moisture sensitive reactions were carried out under a nitrogen atmosphere. Column chromatography was performed using silica gel 60 (Merck, 340-400 mesh ASTM). Chromatography solvents were distilled before use. ¹H NMR and ¹³C NMR were taken on a Bruker AC-300 spectrometer. All the NMR samples were prepared in DMSO-d₆. Infrared spectra (IR) were obtained either neat (if oils) or as KBr pellets (if solids) on a Nicolet 5DXB FT-IR spectrometer. Mass spectra were recorded on Kratos MS50RF, Varian CH7 or Finnigan 4000 mass spectrometers. Optical rotations were measured with a Perkin-Elmer model 243 polarimeter. Melting points were determined on a Buchi capillary melting point apparatus and are uncorrected. S-Sepharose was purchased from Sigma.
4-(4-Morpholinyl)methylbenzoic acid (30)

4-(Chloromethyl)benzoic acid 29 (5 g, 29.3 mmol) was mixed with morpholine (25 mL, 293 mmol). Ethanol (3 mL) was added to make the mixture homogeneous and the solution was heated at 110 °C for 36 h. The excess morpholine was evaporated and the residue was dissolved in 14 mL water. The solution was adjusted to pH 2 with 1M HCl acid, and the aqueous solution washed twice with diethyl ether (75 mL). The aqueous layer was then concentrated to dryness. The residue was dissolved in a minimum amount of water and 1M NaOH was used to adjust the pH to 5.8. The solution was concentrated to 10 mL to precipitate the product, which was further purified by recrystallization from EtOH to give 6.19 g (88%) of compound 9: m.p. 185.0-185.5 °C. \(^1\)H NMR \(\delta\) 12.9 (1H, bs, exchanges with D\(_2\)O); 7.90 (2H, m); 7.42 (2H, m); 3.57 (4H, m); 3.52 (2H, s); 2.35 (4H, m). \(^{13}\)C NMR \(\delta\) 167.2, 143.2, 129.5, 129.3, 128.9, 66.2, 62.0, 53.2. IR \(\nu\) 3455.7, 3411.5, 1619.0, 1568.0, 1361.7, 1121.5 cm\(^{-1}\). Positive FAB mass spectrum [M+H]+, 222.1 (100), 135.3 (35), 86.1 (10), 177.1 (5). Anal. calcd. for C\(_{12}\)H\(_{15}\)NO\(_3\)·H\(_2\)O: C, 60.24; H, 7.16; N, 5.85. Found: C, 60.28; H, 7.19; N, 5.98.

N4-[4-(4-Morpholinyl)methylbenzoyl] cytosine (33)

4-(4-Morpholinyl)methylbenzoic acid 30 (250 mg, 1.13 mmol) was dissolved in 0.5 mL of thionyl chloride and stirred at 60 °C (sand bath) for 85 min. After the solution cooled, anhydrous Et\(_2\)O (2 mL) was added and a white precipitate of acid chloride 31 was obtained. The ether was decanted. Another 2 mL of anhydrous Et\(_2\)O was added to the flask and decanted again. After the white solid had dried under vacuum for 1 hour, anhydrous methylene chloride (3 mL) and anhydrous pyridine (1 mL) were added to the flask. Cytosine (37.7 mg, 0.34 mmol) was added to the mixture which was then stirred for 30 min. Evaporation of the solvent gave an off-white solid which was dissolved in 2 mL of water. The solution
was adjusted to pH 8 with 1M NaOH to precipitate the product 33 (77.0 mg, 72%). An analytical sample was recrystallized from EtOH, m.p. 293-294 °C. $^{1}$H NMR δ 11.4 (1H, bs, exchanges with D$_2$O); 7.98 (2H, m); 7.86 (1H, d, J = 7.04 Hz); 7.43 (2H, m); 7.20 (1H, bs); 3.58 (4H, m); 3.53 (2H, s); 2.36 (4H, m). $^{13}$C NMR δ 167.5, 163.8, 155.9, 147.0, 143.0, 132.1, 128.7, 128.4, 95.5, 66.2, 61.9, 53.2. IR ν 2853.5, 2810.6, 1695.8, 1616.3, 1457.9, 1245.8 cm$^{-1}$. Positive FAB mass spectrum [M+H]$^+$, 315.2 (100), 204.2 (15), 118.1 (10). Anal. calcd. for C$_{16}$H$_{18}$N$_4$O$_3$: C, 61.14; H, 5.77; N, 17.82. Found: C, 61.18; H, 5.88; N, 17.69.

**N4-[4-(4-Morpholinyl)methylbenzoyl] cytidine (36)**

4-(Morpholinomethyl)benzoic acid 30 (1.50 g, 6.78 mmol) and 3.5 mL of thionyl chloride were mixed, and the reaction was stirred at 60°C for 85 min. The excess thionyl chloride was evaporated and the residue was coevaporated several times with anhydrous CH$_2$Cl$_2$ to remove traces of thionyl chloride. The acid chloride 31 was dried over high vacuum for 1 h.

To cytidine 34 (329.5 mg, 1.36 mmol) which had been dried three times by coevaporation of pyridine, was added dry pyridine (5 mL) and trimethylchlorosilane (1.5 mL, 11.74 mmol). The reaction mixture was allowed to stir at room temperature for 1 h.

To the acid chloride 31 was added dry pyridine (0.5 mL), followed by transfer of the cytidine-TMS 35 solution to the flask under nitrogen. Stirring was continued for 3 h, at which time the mixture was cooled in an ice bath and 5 mL of water was added. After 5 min, 6 mL of concentrated ammonium hydroxide was added and the mixture was stirred vigorously at room temperature for 15 min. The reaction was then evaporated to near dryness and the residue was dissolved in 75 mL of water. The solution was basified to pH 8 with 1M NaOH and then washed 4 times with 50 mL-portions of n-BuOH (saturated with H$_2$O). After evaporation, the residue was dissolved in a small amount of EtOH and added to a large excess of EtOAc to form
a white precipitate. After drying under vacuum, 544.7 mg (87%) of compound 36 was obtained. An analytical sample was recrystallized from MeOH, m.p. 139-140 °C. [α]D = +28.9° (c 1.16, H2O). 1H NMR δ 11.2 (1H, bs, exchanges with D2O); 8.50 (1H, d, J = 7.44 Hz); 7.99 (2H, m); 7.45 (2H, m); 7.32 (1H, d, J = 7.40 Hz); 5.80 (1H, d, J = 2.24 Hz); 5.54 (1H, d, J = 4.49 Hz, exchanges with D2O); 5.22 (1H, t, J = 4.76 Hz, exchanges with D2O); 5.09 (1H, d, J = 5.32 Hz, exchanges with D2O); 3.98 (2H, m); 3.84 (1H, m); 3.76 (1H, dd, J = 11.86 Hz); 3.63 (1H, dd, J = 11.86 Hz); 3.58 (4H, m); 3.54 (2H, s); 2.36 (4H, m). 13C NMR δ 167.2, 163.0, 154.6, 145.2, 143.2; 131.9, 128.8, 128.5, 96.0, 90.2, 84.2, 78.3, 68.6, 66.2, 61.9, 59.9, 53.2. IR ν 3600 - 3100, 1649.0, 1562.9, 1489.6, 1255.7, 1109.8 cm⁻¹. Positive FAB mass spectrum [M+H]⁺, 447.1 (90), 315.1 (100), 204.1 (55), 229.1 (20), 204.1 (55), 118.1 (25), 89.1 (25). Anal. calcd. for C21H26N4O7: C, 56.50; H, 5.87; N, 12.55. Found: C, 56.62; H, 5.79; N, 12.33.

**Morpholine nucleoside C (39)**

Compound 36 (600 mg, 1.24 mmol) and ammonium bborate (696.0 mg, 2.64 mmol) were suspended in methanol (30 mL), treated with sodium periodate (282.5 mg, 1.32 mmol) and stirred for 2.25 h. The solution was filtered and the solid washed with a small amount of methanol. The filtrate and wash were combined and added to a flask containing sodium cyanoborohydride (51.0 mg, 0.81 mmol). After 20 min, an additional (24 mg, 0.38 mmol) of the hydride reagent was added and the solution stirred for 15 min longer. p-Toluenesulfonylic acid (480 mg, 2.52 mmol) was added to the reaction vessel, and after the evolution of gas had ceased, an additional amount of p-TsOH (240 mg, 1.26 mmol) was added to the flask. Precipitation of the product began as the second aliquot of acid was added. A third portion of p-TsOH (120 mg, 0.63 mmol) was added to the reaction vessel. After stirring overnight the solution was filtered and the solid washed with water, methanol, and finally ether. The solid was dried under vacuum and afforded 444.5
mg (62%) of the desired morpholine salt 38, m.p. 248-253 °C (d). \( ^1H \) NMR \( \delta \)

11.3 (1H, bs, exchanges with \( \text{D}_2\text{O} \)); 8.20 (1H, d, \( J = 7.65 \) Hz); 8.00 (2H, m); 7.45 (4H, m); 7.40 (1H, d, \( J = 7.5 \) Hz); 7.12 (2H, m); 6.01 (1H, d, \( J = 8.06 \) Hz); 5.04 (1H, t, \( J = 6.44 \) Hz, exchanges with \( \text{D}_2\text{O} \)); 4.00 (1H, m); 3.59 (4H, m); 3.52 (2H, m); 3.42 (1H, bd, \( J = 12.49 \) Hz); 3.4 - 3.2 (4H, m); 3.08 (1H, t, \( J = 10.83 \) Hz); 2.88 (1H, t, \( J = 12.14 \) Hz); 2.42 (4H, m); 2.28 (3H, s).

The crude tosylate salt 38 (500 mg, 0.8 mmol) was dried by coevaporation from DMF (5 mL) twice. The dried solid was dissolved in DMF (10 mL) containing triethylamine (0.2 mL, 2.2 mmol) and the solution was treated with trityl chloride (278.8 mg, 1.0 mmol). After 30 min, the clear solution was diluted with chloroform (150 mL) and washed with 5% aqueous sodium bicarbonate. The organic layer was dried over sodium sulfate, then evaporated to dryness. The residue was dissolved in a minimum volume of chloroform, then added to hexane to afford an off-white precipitate. The solid was collected by filtration and the residue dried under vacuum to afforded 540 mg of crude product 39. The crude product was chromatographed (SiO\(_2\), 0-2% MeOH/CHCl\(_3\)) to give 506.4 mg (91%) white solid 39, and an analytical sample was recrystallized from i-PrOH, m.p. 196-197 °C. \( [\alpha]_D \) = +113° (c 0.53, CHCl\(_3\)). \( ^1H \) NMR \( \delta \)

11.2 (1H, bs, exchanges with \( \text{D}_2\text{O} \)); 7.95 (3H, m); 7.6-7.1 (18H, m), 6.12 (1H, d, \( J = 8.35 \) Hz); 4.78 (1H, t, \( J = 5.68 \) Hz, exchanges with \( \text{D}_2\text{O} \)); 4.21 (1H, m); 3.57 (4H, m); 3.52 (2H, s); 3.5 - 3.2 (3H, m); 3.11 (1H, d, \( J = 11.14 \) Hz); 2.35 (4H, m); 1.39 (1H, t, \( J = 10.94 \) Hz); 1.24 (1H, t, \( J = 10.22 \) Hz). \( ^{13}C \) NMR \( \delta \)

167.2, 163.0, 153.6, 145.1, 143.2, 131.8, 128.9, 128.7, 128.5, 127.8, 126.3, 96.4, 81.2, 79.2, 76.8, 76.4, 66.2, 62.0, 61.9, 53.2, 52.3, 49.4. IR \( \nu \)

3410.8, 1654, 1559.8, 1487.4, 1263.0 cm\(^{-1}\). Negative FAB mass spectrum [M-\( \text{H}^-\)], 670.3 (95), 459.1 (30), 305.1 (100) and positive FAB mass spectrum [M+\( \text{H}^+\)], 672.3 (75), 594.3 (10), 460.1 (15), 428.2 (30), 357.2 (40), 307.1 (10), 243.2 (100), 165.1 (10). Anal. Calcd. for C\(_{40}\)H\(_{41}\)N\(_5\)O\(_5\): C, 71.52; H, 6.15; N, 10.27. Found: C, 71.21; H, 6.20; N, 10.27.
Activated morpholine nucleoside C (40)

The base protected morpholine nucleoside 39 (500 mg, 0.74 mmol) was evaporated once from DMF. Bis(p-nitrophenyl)carbonate (386.6 mg, 1.27 mmol) was added to the reaction vessel and the mixture was again evaporated from DMF. The residue was dissolved in DMF (6 mL) and treated with triethylamine (0.42 mL, 3.49 mmol). The solution was stirred at room temperature for 1.5 h and chloroform (150 mL) was added to dilute the solution. The solution was washed with 75 mL-portions of 0.01 N NaOH four times (until the yellow color disappeared), followed by distilled water (3×75 mL), then sat'd NaCl solution (75 mL), and the resulting solution dried over sodium sulfate. The solvent was evaporated and the residue was chromatographed (SiO₂, 0-2% MeOH/CHCl₃) and precipitated from CHCl₃/hexane. Thorough drying under vacuum gave 511.3 mg (83%) of the activated morpholine nucleoside 40, and an analytical sample was recrystallized from i-PrOH, m.p. 168.5-169.5 °C. [α]D = +184° (c 0.50, CHCl₃). ¹H NMR δ 11.2 (1H, bs, exchanges with D₂O); 8.30 (2H, m); 7.95 (2H, m); 7.91 (1H, d, J = 7.63 Hz); 7.5 - 7.1 (20H, m); 6.21 (1H, d, J = 8.30 Hz); 4.64 (1H, m); 4.36 (1H, dd, J = 11.45 Hz); 4.26 (1H, dd, J = 11.52 Hz); 3.57 (4H, m); 3.52 (2H, s); 3.31 (1H, d, J = 9.67 Hz); 3.12 (1H, d, J = 10.67 Hz); 2.35 (4H, m); 1.46 (1H, t, J = 11.12 Hz); 1.31 (1H, t, 10.53 Hz). ¹³C NMR δ 167.3, 166.8, 163.1, 155.2, 153.7, 151.8, 145.2, 144.8, 143.2, 131.8, 129.0, 128.7, 128.5, 127.9, 126.4, 125.4, 122.6, 96.6, 81.0, 76.4, 73.5, 68.9, 66.2, 61.7, 53.2, 52.1, 48.4. IR ν 1772.2, 1676.7, 1526.3, 1487.2, 1348.7, 1216.4 cm⁻¹. Negative FAB mass spectrum [M-H]⁻, 836.1 (100), 670.1 (10), 612.0 (10), 459.1 (10), 305(50) and positive FAB mass spectrum [M+H]⁺, 837.4 (75), 759.4 (10), 593.3 (30), 522.3 (10), 460.3 (10), 445.3 (90), 444.1 (100), 205.0 (10), 165.6 (30). Anal. calcd. for C₄₇H₄₄N₆O₉·H₂O: C, 66.03; H, 5.42; N, 9.83. Found: C, 66.16; H, 5.30; N, 10.04.
N6-[4-(4-Morpholinyl)methylbenzoyl] adenosine (43)

4-(4-Morpholinyl)methylbenzoic acid 30 (1.06 g, 4.79 mmol) and thionyl chloride (3.5 mL) were stirred at 60°C for 85 min. The excess thionyl chloride was removed by coevaporation several times with anhydrous dichloromethane to remove traces of thionyl chloride. The acid chloride 31 was dried over high vacuum for 1 h.

To adenosine 41 (256.0 mg, 0.96 mmol) which had been dried three times by evaporation of pyridine, was added dry pyridine (20 mL) and trimethylchlorosilane (1.1 mL, 8.64 mmol), and the mixture was stirred at room temperature for 1 h.

To the acid chloride 31 was added dry pyridine (0.5 mL), then the adenosine-TMS 42 solution was transferred to the flask under nitrogen. Stirring was continued for 4 h, then the mixture was cooled in an ice bath and water (5 mL) was added. After 5 min, concentrated ammonium hydroxide (10 mL) was added and the mixture was stirred vigorously at room temperature for 30 min. The solution was then evaporated to near dryness and the residue was dissolved in 75 mL of water. The solution was washed 4 times with 100 mL portions of n-BuOH (saturated with H₂O first). After the n-BuOH solution was collected, it was evaporated and the residue was dissolved in a small amount of EtOH and added to a large excess of EtOAc to form an off-white precipitate. After drying under vacuum, 468.2 mg (96%) of crude product 33b was obtained and was used immediately for the next step. An analytical sample was prepared by column chromatography (SiO₂, 5% MeOH/CHCl₃ to 17% MeOH/CHCl₃), followed by recrystallization from MeOH, m.p 126 - 126.5°C. [α]D = -34.5° (c 1.00, H₂O). ¹H NMR δ 11.2 (1H, bs, exchanges with D₂O); 8.76 (1H, s); 8.72 (1H, s); 8.02 (2H, m); 7.48 (2H, m); 6.04 (1H, d, J = 5.78 Hz); 5.57 (1H, d, J = 6.02 Hz, exchanges with D₂O); 5.27 (1H, d, J = 4.84 Hz, exchanges with D₂O); 5.14 (1H, t, J = 4.54 Hz, exchanges with D₂O); 4.65 (1H, dd, J = 5.50 Hz); 4.18 (1H, dd, J = 4.22 Hz); 3.98 (1H, m); 3.68 (1H, m); 3.61 (4H, m); 3.60 (1H, m); 3.58 (2H, s); 2.39 (4H, m). ¹³C NMR δ 165.4, 151.6,
150.4, 143.1, 142.8, 139.1, 132.1, 128.8, 128.5, 115.3, 87.5, 85.7, 73.6, 70.4, 66.2, 61.9, 61.3, 53.2. IR ν 3600 - 3200, 2863.7, 2816.3, 1700, 1613.6, 1583.3, 1414.9, 1222 cm⁻¹. Positive FAB mass spectrum [M+H]⁺, 471.2 (90), 386.2 (10), 339.2 (100), 277.2 (15), 253.2 (20), 221.2 (5), 204.2 (30). Anal. calcd. for C₂₂H₂₆N₆O₆·1/2H₂O: C, 55.10; H, 5.67; N, 17.53. Found: C, 54.76; H, 5.24; N, 17.31.

**Morpholine nucleoside A (45)**

Compound 43 (100 mg, 0.18 mmol) and ammonium bborate (116.0 mg, 0.44 mmol) were suspended in methanol (6 mL), treated with sodium periodate (48.0 mg, 0.22 mmol) and stirred for 2 h. The solution was filtered and the solid washed with a small amount of methanol. The filtrate and wash were combined and added to a flask containing sodium cyanoborohydride (25.0 mg, 0.40 mmol). After an hour, the solution was treated with 0.5 M HCl to pH 4 and the solution was stirred for 1 h longer, then evaporated to dryness and dried under vacuum overnight. The crude product was co-evaporated with DMF (4 mL) and triethylamine (0.24 mL), then were added DMF (8 mL), Et₃N (0.24 mL) and trityl chloride (80.0 mg, mmol) and the mixture stirred for 30 m. The solution was diluted with chloroform (50 mL) and washed with 5% aqueous sodium bicarbonate (25 mL). The organic solution was dried over sodium sulfate and evaporated to dryness. The residue was chromatographed on silica gel (0 - 40% acetone/EtOAc). The resulting solid was dissolved in a minimum amount of CHCl₃, and added to a large excess of hexane. The solid was collected by filtration and the residue dried under vacuum to afford 75.9 mg (55%) of product 36b. An analytical sample was obtained by recrystallization from diethyl ether, m.p 168 - 168.5 °C. [α]D = + 78.5° (c 0.47, CHCl₃). ¹H NMR δ 11.2 (1H, bs, exchanges with D₂O); 8.75 (1H, s); 8.46 (1H, s); 7.98 (2H, m); 7.5 - 7.1 (17H, m); 6.41 (1H, d, J = 8.45 Hz); 4.79 (1H, t, J = 5.76 Hz, exchanges with D₂O); 4.28 (1H, m); 3.59 (4H, m); 3.55 (2H, s); 3.5 - 3.3 (3H, m); 3.16 (1H, bd, J = 11.41 Hz); 2.38 (4H, m); 2.06 (1H, t, J = 10.16 Hz); 1.45
Activated morpholine nucleoside A (46)

The base protected morpholine nucleoside 45 (500 mg, 0.86 mmol) was evaporated once from DMF. Bis(p-nitrophenyl)carbonate (471.9 mg, 1.55 mmol) was added to the reaction vessel and the mixture was again evaporated from DMF. The residue was dissolved in DMF (6 mL) and to this solution triethylamine (0.5 mL, 4.23 mmol) was added slowly. The solution was stirred at room temperature for 90 min and diluted with chloroform (100 mL). The chloroform solution was washed with 0.01 N NaOH four times, twice with H₂O and the resulting solution dried over sodium sulfate. The solvent was evaporated and the residue was chromatographed (SiO₂, 0 - 10% acetone/EtOAc) and precipitated from CHCl₃/hexane. After thorough drying under vacuum, 627.0 mg (85%) of the activated morpholine nucleoside A 37b was obtained. After recrystallization from Et₂O, the analytical sample gave m.p 149 - 150°C. [α]D = + 52° (c 0.47, CHCl₃). ¹H NMR δ 11.2 (1H, bs, exchanges with D₂O); 8.76 (1H, s); 8.48 (1H, s); 8.28 (2H, m); 7.99 (2H, m); 7.6 - 7.2 (19H, m); 6.50 (1H, d, J = 8.28 Hz); 4.70 (1H, m); 4.34 (1H, dd, J = 10.04 Hz); 4.23 (1H, dd, J = 10.80 Hz); 3.57 (4H, m); 3.55 (2H, s); 3.42 (1H, bd, J = 10.70 Hz); 3.17 (1H, bd, J = 10.63 Hz); 2.37 (4H, m); 2.16 (1H, t, J = 10.65 Hz); 1.56 (1H, t, J = 11.47 Hz). ¹³C NMR δ 165.8, 164.0, 155.4, 151.7, 150.8, 149.9, 145.2, 142.8, 142.1, 129.0, 128.8, 128.5, 127.9, 126.4, 125.4, 125.2, 122.5, 118.2, 102.4, 79.6, 76.5, 73.6, 68.8, 66.2, 61.9, 53.2, 51.4, 48.5. IR ν 1772.4, 1613.7, 1525.5, 1450.5, 1247.1 cm⁻¹. Negative FAB mass spectrum [M-H]⁻, 860 (100), 694.3 (10), 657.3 (20),
612.3 (10). Anal. Calcd. For C\textsubscript{48}H\textsubscript{44}N\textsubscript{8}O\textsubscript{8}·1/2H\textsubscript{2}O: C, 66.27; H, 5.21; N, 12.88; Found : C, 65.97; H, 4.84; N, 12.72.

**Determination of trityl binding to resin 27 (loading)**

In a small vial, 10 mg resin 27 was swelled in 1 mL DMA at room temperature for 15 h. The mixture was transferred to a 10 mL Biorad disposable polypropylene column and filtered. The resin was washed with DCA (3×0.5 mL) and then stirred in 0.5 mL 2% DCA/DCM for 1 min. After the filtration, another 0.5 mL 2%DCA/DCM was added for 7 min stirring. The solid was washed with DCM (3×0.5 mL). The acid filtrates and three washes were combined and evaporated to dryness. The trityl concentration was measured by UV at 431.5 nm in a 20%CF\textsubscript{3}COOH/CHCl\textsubscript{3} solution (ε = 33,135). This gave a loading of 169 μmol/g dried resin.

**Synthesis of dimer 56, 57**

Compound 39 (20.0 mg, 0.03 mmol) was treated with 2% DCA/DCM (5 mL) for 30 min at ambient temperature. After evaporation, the resulting residue was twice coevaporated from DMF. The solid was dissolved in DMF (3 mL) and to this solution the activated monomer 40 (18.5 mg, 0.03 mmol) was added. The solids were dissolved and the triethylamine (0.1 mL, 0.72 mmol) was added to the reaction vessel. The solution was stirred at room temperature overnight. The solvent was evaporated and the residue chromatographed on silica gel (4% MeOH/CHCl\textsubscript{3}). The product 56 was precipitated from CHCl\textsubscript{3}/hexane to give a white solid. 300 MHz \textsuperscript{1}H NMR and FAB mass spectrum identified the product. Dimer 57 was synthesized by coupling of 45 and 46 using the procedure described above.
Stability studies of monomers and dimers

Monomers and dimers were tested in 2% formic acid/DCM, 2% acetic acid/DCM, 2% DCA/DCM and 2% TFA/DCM respectively for different time periods as previously described. Phosphate buffer (pH 2.5), acetate buffer (pH 4.5) and succinic buffer (pH 5.5) were also used for testing the stabilities. Following analysis by TLC or ion-exchange chromatography we found that the base protecting group, glycosyl linkage and carbamate linkage are stable to the conditions at room temperature with the exception of 2% formic acid/DCM treatment.

Synthesis of oligomers

50 mg resin 25 (8.5 μmol) was swelled with DMA (1 mL) in a small vial at room temperature for 15 h. The mixture was transferred to a 10 mL Biorad disposable column (Figure VII), DMA was filtered off and the sequence of steps in Table 2 was performed at room temperature except step 8 (30 °C).

Each step was performed with agitation afforded by a spatula which penetrated a small septum stoppered on top of the column. Reagent were introduced by syringe through the serum stopper. A motor with a cam was used to rock the spatula gently back and forth to mix the suspension. The entire coupling sequence was repeated 4 more times. Both hexamer 55 and hexamer 60 were cleaved from the solid support by washing the resin with DMF (0.5 mL) 3 times in 3 min, then treated with a solution of DBU (60 μL), diethyl malonate (30 μL) and DMF (1 mL) at room temperature for 1 h with agitation. The solutions were filtered and the resins were washed with DMF (0.5 mL) 3 times. The filtrates were evaporated to dryness at low temperature. The oils obtained were dissolved in a minimum amount of CH2Cl2 and precipitated by addition of a large excess of anhydrous ether. After centrifugation, the solvent was decanted and the precipitation repeated once again. The crude hexamer 55 was dried under vacuum to give 22.9 mg (95%)
white solid, and the crude hexamer 60 gave 21.0 mg (84%) white solid.

**Purification and characterization of hexamer 55 and 60**

The crude hexamer 55 (2.6 mg) was treated with 2% DCA/DCM (0.5 mL) for 30 min. A large excess of anhydrous Et$_2$O was added to the reaction vessel to afford a white precipitate. After centrifugation, the solvent was decanted and the residue was dried. Ion exchange of this detritylated crude hexamer 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) revealed a single peak with no contamination by shorter species. The product-containing fractions were combined, adjusted to pH 8 with 1 M NaOH and extracted with 20% i-PrOH/CHCl$_3$. After drying the organic layer over anhydrous sodium sulfate, the solution was evaporated and the product was precipitated using CH$_2$Cl$_2$/Et$_2$O. After drying under vacuum, the pure hexamer was dissolved in DMF (0.5 mL) and trityl chloride (1.2 mg) and triethyl amine (1.0 μL) were added and stirred overnight. The solution was diluted with CHCl$_3$ (10 mL) and washed twice with 5 mL-portions of 5% NaHCO$_3$. The organic layer was dried over sodium sulfate. Evaporation of the solvent and precipitation of the tritylated product several times from CH$_2$Cl$_2$/Et$_2$O afforded 2.1 mg (81%) of hexamer 49a. Negative FAB mass spectrum showed a single high MW ion at the expected m/z 2848 (M-H). $^1$H NMR $\delta$ = 11.3 (6H, bs, exchanges with D$_2$O); 8.20 (6H, m); 8.00 (12H, m); 7.7 - 7.1 (28H, m); 6.16 (1H, m); 5.75 (5H, m); 5.70 (1H, m); 4.94 (1H, m); 4.3 - 3.9 (25H, m); 3.8 (6H, m); 3.6 - 3.4 (32H, m); 3.2 - 2.8 (12H, m); 2.5 - 2.2 (20H, m); 1.5 - 1.3 (2H, m).

Final confirmation of the structure was obtained by complete removal of all protecting groups (detritylation, ammonolysis) and comparison with the same material derived from deprotection of the fully benzoylated hexamer made previously [57]. The ammonolysis was done by treatment of the detritylated hexamer with 1:1 DMF/NH$_4$OH at room temperature for 24 h. The two materials were indist-
ingshable by ion exchange chromatography.

The crude hexamer 60 (6.0 mg) was treated with 2% DCA/DCM (0.5 mL) for 20 min. After precipitation from CH₂Cl₂/Et₂O, ion exchange of this detritylated crude hexamer on S-Sepharous using phosphate buffer (pH 2.5) and a gradient of KCl (0 - 1.4 M in 84 min at a flow rate 2.2 mL/min) revealed a major peak with small contamination by short species. The fractions containing product were combined and adjusted to pH 8 with 1 M NaOH, then extracted with 20% i-PrOH/CHCl₃. After the detritylation, 4.3 mg (72%) of hexamer 60 was obtained. Negative FAB mass spectrum showed a single high MW ion at the expected m/z 2969 (M - H). ¹H NMR δ = 11.2 (6H, bs, exchanges with D₂O); 8.7 - 8.5 (10H, m); 8.1 - 7.9 (12H, m); 7.5 - 7.0 (29H, m); 6.42 (1H, m); 6.0 - 5.6 (6H, m); 4.55 (1H, m); 4.3 - 4.0 (20H, m); 3.75 (6H, m); 3.6 - 3.4 (32H, m); 3.2 - 2.8 (12H, m); 2.4 - 2.3 (20H, m); 1.5 - 1.3 (1H, m); 1.2 - 1.0 (1H, m).

4-PNP-N-FMOC-hydroxyproline morpholine nucleoside C ester (65)

p-Nitrophenyl hydroxyproline 64 (50 mg, 0.096 mmol) and 1-(3-dimethylamino propyl)-3-ethylcarbodiimide methiodide (31.4 mg, 0.10 mmol) were added to the flask which contained protected morpholino nucleoside cytosine 47 (56.3 mg, 0.098 mmol) and 4-dimethylaminopyridine (1 mg, 0.008 mmol). Methylene chloride (4 mL) was added to the reaction vessel and the solution was stirred for 20 h at room temperature until esterification was complete. The solution was diluted with chloroform (50 mL) and washed with water (20 mL) twice. The organic layer was dried over sodium sulfate and concentrated to dryness. The crude product was purified by column chromatography (SiO₂, 0 - 1.5% MeOH/CHCl₃). After drying under vacuum, 64.5 mg (56%) of product 65 was obtained. The product was precipitated from CHCl₃/Hexane to give a white solid 65, m.p 157.5 - 158.0 °C [α]D = +65.5° (c 0.42, CHCl₃). ¹H NMR δ 11.2 (1H, bs, exchanges with D₂O);
8.33 (2H, m); 7.95 (2H, m); 7.85 (3H, m) 7.8 - 7.1 (26H, m); 6.11 (1H, m); 5.17 (1H, m); 4.5 - 4.2 (4H, m); 4.2 - 4.0 (2H, m); 3.7 (1H, m); 3.6 (1H, m); 3.32 (1H, m); 3.0 (1H, t, J = 13.71 Hz); 2.05 (1H, m); 1.34 (1H, t, J = 11.32 Hz); 1.20 (1H, t, J = 11.67 Hz). $^{13}$C NMR $\delta$ 171.9, 166.8, 163.6, 155.6, 153.7, 151.2, 145.8, 145.4, 143.6, 142.3, 140.7, 133.1, 132.7, 128.9, 128.4, 127.9, 127.6, 127.1, 126.4, 125.4, 125.0, 122.5, 120.1, 96.5, 80.9, 77.7, 76.9, 75.8, 73.7, 66.9, 66.7, 57.2, 56.6, 52.6, 51.4, 48.5, 46.5, 35.7. IR $\nu$ 1764.6, 1704.0, 1671.1, 1525.7, 1482.5, 1349.4, 1221.9, 1197.4 cm$^{-1}$. Positive FAB mass spectrum [M+H]$^+$, 1073 (90), 995.2 (25), 857 (30), 829 (15), 780 (20), 616 (20), 243 (100), 165.1 (20). Anal. Calcd. for C$_{62}$H$_{52}$N$_6$O$_{12}$: C, 69.39; H, 4.88; N, 7.83. Found: C, 69.33; H, 4.85; N, 7.66.

**Preparation of functionalized resin (66)**

Aminomethyl polystyrene 52 (63.3 mg) (loading 1.21 mmol/g) was treated by the following sequence in a 10 mL Biorad disposable polypropylene column.

<table>
<thead>
<tr>
<th>Steps</th>
<th>Times</th>
<th>Repetitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 DMF (1 mL)</td>
<td>15 m</td>
<td>1</td>
</tr>
<tr>
<td>2 DIPEA (0.5 mL)</td>
<td>10 m</td>
<td>1</td>
</tr>
<tr>
<td>3 DMF (1 mL)</td>
<td>1 m</td>
<td>5</td>
</tr>
<tr>
<td>4 activated carbonate 65 (40.9 mg) in DMF (1 mL)</td>
<td>30 m</td>
<td>1</td>
</tr>
<tr>
<td>5 DMF (1 mL)</td>
<td>1 m</td>
<td>5</td>
</tr>
<tr>
<td>6 p - nitrophenyl methyl carbonate (15.0 mg)</td>
<td>1 h</td>
<td>1</td>
</tr>
<tr>
<td>7 DMF (1 mL)</td>
<td>1 m</td>
<td>5</td>
</tr>
</tbody>
</table>

DIPEA = Diisopropylethylamine

After drying, 81.9 mg resin 66 was obtained (loading 204 $\mu$mol/g).
Cleavage of monomer (47) from resin (66)

The resin 66 (38 mg, loading 204 \( \mu \text{mol/g dry resin} \)) obtained above was treated as follows:

<table>
<thead>
<tr>
<th>Steps</th>
<th>Times</th>
<th>Repetitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMA (1 mL)</td>
<td>15 h</td>
</tr>
<tr>
<td>2</td>
<td>DMF (0.5 mL)</td>
<td>3 m</td>
</tr>
<tr>
<td>3</td>
<td>DBU (60 ( \mu \text{L} ))/DEM (30 ( \mu \text{L} ))/DMF (1 mL)</td>
<td>10 m</td>
</tr>
<tr>
<td>4</td>
<td>DBU (60 ( \mu \text{L} ))/DEM (30 ( \mu \text{L} ))/DMF (1 mL)</td>
<td>15 m</td>
</tr>
<tr>
<td>5</td>
<td>DMF (0.5 mL)</td>
<td>3 m</td>
</tr>
<tr>
<td>6</td>
<td>DMA (0.5 mL)</td>
<td>3 m</td>
</tr>
<tr>
<td>7</td>
<td>67 (17.0 mg) Et(_3)N (40 ( \mu \text{mol} ))/DMA (0.2 mL)</td>
<td>24 h, 30°C</td>
</tr>
<tr>
<td>8</td>
<td>DMA (0.5 mL)</td>
<td>1 m</td>
</tr>
<tr>
<td>9</td>
<td>DMA (0.5 mL)/Et(_3)N (50 ( \mu \text{L} ))/Ac(_2)O (50 ( \mu \text{L} ))</td>
<td>10 m</td>
</tr>
<tr>
<td>10</td>
<td>DMA (0.5 mL)</td>
<td>3 m</td>
</tr>
<tr>
<td>11</td>
<td>DMF (0.5 mL)</td>
<td>3 m</td>
</tr>
<tr>
<td>12</td>
<td>DMF (1 mL)/DBU (60 ( \mu \text{L} ))/DEM (30 ( \mu \text{L} ))</td>
<td>1 h</td>
</tr>
<tr>
<td>13</td>
<td>DMF (0.5 mL)</td>
<td>3 m</td>
</tr>
</tbody>
</table>

The filtrates of step 12 and step 13 were collected and evaporated. The residue was redissolved in chloroform (20 mL) and washed with water (10 mL) twice. The organic layer was dried over sodium sulfate and residue was dissolved in 20% CF\(_3\)COOH/CLCl\(_3\) to measure the trityl absorption at 431.5 nm. The trityl group left on the resin was determined by treatment with 2% DCA/DCM and UV analysis of the washes.
2-Phenylsulfonyl ethanol (71)

The literature procedure was improved. A tungstic acid catalyst solution was prepared by slurrying WO$_3$·H$_2$O (37.0 mg, 0.15 mmol) in 12 mL of distilled water and then dissolving it by adding a few drops of 1M NaOH to bring the pH to 11.3. The pH was then reduced to 5.6 with a few drops of glacial acetic acid to give a translucent solution. This was immediately added into a 100 mL round-bottom flask, followed by 2-phenylthioethanol 70 (5 mL, 0.037 mol). The rapidly stirred heterogeneous mixture was heated to 63° C. The heating mantle was removed and 3.7 mL (0.037 mol) of 30% hydrogen peroxide was slowly added dropwise over 5 min through a pressure equalizing funnel. The reaction mixture required continuous cooling to maintain the temperature in the 63 - 67 °C range. A clear solution resulted after 3.1 mL of hydrogen peroxide was added. After a total of 10 min, a starch-iodide test was negative. The addition was resumed until a total of 6.8 mL (0.068 mmol) had been added after total of 30 min, at which point the solution became cloudy. Only intermittent cooling was needed during this addition to maintain the temperature at 70 - 75 °C. Hydrogen peroxide was then added dropwise with slight heating to a total of 7.6 mL (0.076 mole) over a 30 min period while testing with starch-iodide paper to find the point where there was a slight excess over the theoretical stoichiometric amount (0.074 mole) of hydrogen peroxide needed to obtain the sulfone. The reaction was stopped when the color of starch-iodide test persisted for 2 h. Two layers formed on cooling to 25 °C, with the pH of the water layer being 4.5. A trace of hydrogen peroxide was destroyed with addition of 0.1 g of sodium bisulfite. The product was isolated and purified by dissolving the organic layer in benzene, extracting the aqueous layer with benzene, drying the combined organic extracts over Na$_2$SO$_4$ and evaporation of the benzene to give 2-phenylsulfonyl ethanol (71) 4.25 g (77%). $^1$H NMR $\delta$ 7.90 (2H, m); 7.72 (1H, m); 7.64 (2H, m); 4.89 (1H, bs, exchanges with D$_2$O); 3.68 (2H, t, J = 6.33
Hz); 3.46 (2H, t, $J = 6.36$ Hz).

**p-Nitrophenol 2-(phenylsulfonyl)ethyl carbonate (72)**

Phenylsulfonyl ethanol 71 (120 mg, 0.64 mmol) was dissolved in pyridine (10 mL) and p-nitrophenol chloroformate (134 mg, 0.67 mmol) was added to the reaction vessel. The reaction was sonicated for 1 h, followed by stirring for 1 h. The solvent was evaporated and the residue was dissolved in 30 mL CHCl$_3$, washed with 0.1 N HCl until the aqueous layer was acidic, and then washed with 25 mL water, followed with 0.01 N NaOH until the aqueous solution was colorless. The organic layer was dried over Na$_2$SO$_4$. After evaporation and drying under vacuum, the crude product 198.0 mg (88%) was obtained. The product was recrystallized from CHCl$_3$/MeOH to give a white crystalline solid (164.3 mg, 73%), m.p. 78 - 79 °C. $^1$H NMR $\delta$ 8.33 (2H, m); 7.96(2H, m); 7.78(1H, m); 7.76(1H, m); 7.47 (2H, m); 4.52(2H, t, $J = 5.62$ Hz); 3.89(2H, t, $J = 5.63$ Hz). $^{13}$C NMR $\delta$ 155.0, 151.4, 145.2, 139.3, 134.1, 129.5, 127.8, 125.4, 122.4, 62.2, 53.6. IR $\nu$ 1769.8, 1525.1, 1263.0, 1221.6, 1145.8 cm$^{-1}$. FAB FAB mass spectrum [M+H]$^+$, 352 (100), 169 (20), 141 (65), 77.1 (55), 63.1 (10). Anal.calcd. for C$_{15}$H$_{13}$NO$_7$S: C, 51.28; H, 3.73; N, 3.99. Found: C, 51.39; H, 3.69; N, 3.91.

**Carbonate (67)**

p-Nitrophenyl 2-(phenylsulfonyl)ethyl carbonate 72 (32.5 mg, 0.092 mmol) was dissolved in dioxane (0.5 mL) and a solution of glycine (15.0 mg, 0.20 mmol) and LiOH (6.6 mg, 0.16 mmol) in 0.75 mL of water was added to the dioxane reaction vessel. After 30 min, the solution was evaporated to dryness and 0.8 mL of 2 M HCl was added to the reaction vessel. After evaporation of the aqueous solution, the residue was co-evaporated from ethanol 3 times, and then the residue was dried under vacuum overnight. To the crude product, was added 1-(3-dimethylaminopropyl) -3-ethyl carbodiimide methiodide (71.0 mg, 0.24 mmol),
p-nitrophenol (120 mg, 0.86 mmol) and anhydrous CH$_2$Cl$_2$ (5 mL) for overnight stirring. The solution was diluted with CHCl$_3$ (50 mL) and washed four times with 35 mL-portions water. After drying the organic layer over sodium sulfate, the solution was evaporated and purified by column chromatography (SiO$_2$, 60 : 40 EtOAc/hexane or CHCl$_3$). The fractions which contained product were evaporated, and the product was dried overnight to provide a colorless oil 67 (23.7 mg, 63%). $^1$H NMR $\delta$ 8.34 (2H, m); 7.89 (2H, m); 7.78 (1H, m, exchanges with D$_2$O); 7.76 (1H, m); 7.66 (2H, m); 7.42 (2H, m); 4.25 (2H, t, $J = 6.03$ Hz); 4.05 (2H, d, $J = 5.86$ Hz); 3.70 (2H, t, $J = 6.07$ Hz). $^{13}$C NMR $\delta$ 168.5, 155.9, 155.0, 145.1, 139.2, 134.0, 129.5, 127.7, 125.4, 123.0, 57.9, 54.2, 42.5. IR $\nu$ 2924.5, 2851.2, 1777.2, 1726.5, 1524.0, 1491.2, 1348.4, 1294.4, 1143.9 cm$^{-1}$. Positive FAB mass Spectrum [M+H]$^+$, 409 (60), 270.1 (25), 242.1 (80), 187.1 (60), 167.1 (20), 141.0 (55), 77.1 (45).

**Benzhydryl ester (76)**

4-Hydroxyproline 74 (131.0 mg, 1.0 mmol) and LiOH (42.0 mg, 1.0 mmol) were dissolved in water (3 mL) and dioxane (3 mL). To this solution was added p-nitrophenyl 2-(phenylsulfonyl)ethyl carbonate 67 (421.2 mg, 1.2 mmol) in dioxane (3 mL) and the solution stirred at ambient temperature for 12 h. Water (5 mL) and ethanol (5 mL) were added and mixture evaporated. The residue was taken up in water (10 mL) and washed with DCM three times. Then 1 M HCl (1 mL) was added and the solution was evaporated and dried overnight under vacuum. $^1$H NMR $\delta$ 7.92 (2H, m); 7.75 (1H, m); 7.62 (2H, m); 5.05 (1H, bs, exchanges with D$_2$O); 4.24 (2H, t, $J = 5.60$ Hz); 4.05 (1H, m); 3.72 (2H, t, $J = 7.17$ Hz); 3.60 (1H, m); 3.01 (1H, dd, $J = 11.22$ Hz); 2.83 (1H, dd, $J = 12.51$ Hz); 2.05 (1H, m); 1.90 (1H, m).

The residue was co-evaporated twice with DMF, and additional DMF (5 mL) was added to the flask, followed by excess diphenyldiazomethane solution [60]
with stirring for 1 h at 50°C. The solution was evaporated and the residue was chromatographed (SiO2, 0 - 1% MeOH/CHCl3). The fractions containing the product were evaporated and dried under vacuum to obtain 327.7 mg (64%) of the product 66 as a foam. 1H NMR δ 7.9 - 7.6 (5H, m), 7.4 - 7.2 (10H, m); 6.77 (1H, s); 5.13 (1H, m, exchanges with D2O); 4.2 - 4.0 (3H, m); 3.79 (1H, m); 3.71 (2H, m); 3.00 (1H, dd, J = 10.63 Hz); 2.85 (1H, dd, J = 13.10 Hz); 2.11 (1H, m); 1.88 (1H, m). 13C NMR δ 174.5, 153.6, 140.3, 140.1, 139.7, 139.2, 133.9, 129.5, 128.6, 127.9, 127.5, 126.5, 126.3, 125.6, 76.8, 68.3, 67.6. 58.8, 58.2, 58.0, 57.2, 54.6, 54.3, 53.9. IR ν 3400, 1707, 1747, 1425.0, 1319.2, 1147.1 cm⁻¹. Positive FAB mass Spectrum [M + H]⁺, 509 (60), 298 (20), 167 (100), 141.1 (10), 132.9 (30).

Activated benzhydryl ester (77)

The hydroxy ester 76 (286.0 mg, 0.56 mmol) and bis (p-nitrophenyl) carbonate (258.6 mg, 0.84 mmol) were dissolved in DMF (6 mL) and treated with triethylamine (0.1 mL). After 12 h at room temperature, the solvent was evaporated, the residue dissolved in CHCl3 (50 mL) and extracted with 0.01 N NaOH (35 mL) 5 times, followed by water (30 mL) twice. The organic layer was chromatographed (SiO2, CHCl3) to provide 302.2 mg (80%) product 57, m.p. 64 - 65°C, [α]D = -15.3° (c 0.17, CHCl3). 1H NMR δ 8.23 (2H, m); 8.0 - 7.5 (5H, m); 7.4 - 7.2 (12H, m); 6.80 (1H, s); 5.23 (1H, m); 4.27 (3H, m); 3.73 (3H, m); 3.5 (1H, m); 2.6 (1H, m); 2.1 (1H, m). 13C NMR δ 170.1, 155.2, 153.2, 151.3, 145.2, 140.2, 139.9, 139.5, 139.2, 133.9, 129.6, 129.5, 128.6, 127.9, 127.6, 126.5, 126.3, 126.2, 125.4, 122.6, 77.8, 77.1, 59.0, 58.4, 57.5, 54.2, 51.3, 38.7, 34.4. IR ν 1764.6, 1712.8, 1524.8, 1256.9, 1198.5 cm⁻¹. Positive FAB mass Spectrum [M + H]⁺, 675 (90), 509 (70), 460 (80), 167 (100). Anal. Calcd. For C₃₄H₃₀N₂O₁₁S : C, 60.53; H, 4.48; N, 4.15. Found : C, 60.24; H, 4.32; N, 4.07.
**Acid (78)**

The ester 77 (248.0 mg, 0.37 mmol) was dissolved in 5 mL of 25% trifluoroacetic acid/CHCl₂ (v/v). After 1 h at room temperature, the solution was washed with water until the aqueous phase was no longer acidic and the organic layer dried and evaporated. The residue was chromatographed (SiO₂, 25% MeOH/CHCl₃) to give the desired product 78 (155.3 mg, 83%) as a light yellow foam which was used directly in the next step. ¹H NMR δ 8.31 (2H, m); 7.90 (2H, m); 7.7 - 7.5 (5H, m); 5.14 (1H, m); 4.32 (1H, m); 4.17 (1H, m); 4.01 (2H, m); 3.72 (2H, m); 3.63 (1H, m); 3.15 (1H, m); 2.2 (1H, m). ¹³C NMR δ 155.2, 153.0, 151.5, 145.2, 139.7, 139.3, 133.8, 129.5, 127.6, 125.4, 122.6, 79.2, 78.0, 77.3, 58.2, 54.4, 35.9. IR ν 2987.5, 2861.8, 1766.4, 1708.3, 1677.8, 1594.0, 1349.9 cm⁻¹, 1146.1 cm⁻¹. Positive FAB mass Spectrum [M + H]⁺, 509 (70), 329 (80), 176 (100), 167.1 (15), 141.1 (20).

**Hydroxyproline morpholine nucleoside ester (79)**

p-Nitrophenyl hydroxyproline 78 (102.4 mg, 0.2 mmol) and 1-(3-dimethylamino propyl)-3-ethylcarbodiimide methiodide (69.0 mg, 0.23 mmol) were added to a flask which contained morpholine nucleoside 47 (121.1 mg, 0.21 mmol) and 4-(dimethylamino)pyridine (1 mg, 0.008 mmol). Methylene chloride (5 mL) was added to the reaction vessel and the solution was stirred for 20 h at room temperature. The solution was then diluted with CHCl₃ (50 mL) and washed by water (20 mL) three times. The organic layer was dried over Na₂SO₄ and concentrated to near dryness. The crude product was purified by column chromatography (SiO₂, 0 - 1.5% MeOH/CHCl₃). After drying under vacuum, 22.0 mg (10%) of product was obtained. The product was precipitated from CHCl₃/hexane to give a white solid 79, m. p. 113 °C (d). ¹H NMR δ 11.3 (1H, bs, exchanges with D₂O); 8.31(2H, m); 8.10(1H, m); 7.97(4H, m); 7.7 - 7.2(23H, m); 6.85(1H, m); 6.20(1H, d, J = 7.57 HZ); 4.64(1H, m); 4.4 - 4.0(7H, m); 3.3 - 3.1(7H, m); 1.45(1H, m); 1.30(1H, m).
IR $\nu$ 1698.4, 1679.5, 1652.1, 1620.5, 1594.4, 1483.9, 1337.4, 1252.4 cm$^{-1}$. Positive FAB mass Spectrum [M+H]$^+$, 1063.4 (100), 847.3 (20), 677.4 (10), 573.4 (80).
BIBLIOGRAPHY


[56] The trimethylchlorosilane used must be carefully stored and handled. Reagent which has been repeatedly exposed to moist air will give unsatisfactory results.


[59] N-4 acylated cytidine is apparently alkylated by phenyl vinyl sulfone, the elimination product of the PSEC group, in the presence of DBU, see ref.[47].