

Chemical Synthesis of DNA and DNA Analogues

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Several years ago, an Account from this laboratory emphasized how chemically synthesized deoxyoligonucleotides could be used to deduce some of the key mechanisms whereby proteins recognize unique DNA sequences.¹ However, the chemical synthesis of DNA during this period was extremely laborious and time-consuming. For example, preparation of the *lac* operator used in these earlier studies (a 21 base paired DNA duplex) required the equivalent of four years of highly skilled and intense effort.^{2,3} This was the norm. Generally, deoxyoligonucleotides 10-15 monomers in length could be synthesized with difficulty by those who were familiar with polynucleotide chemistry. However, very few seriously considered that these procedures could ever be used routinely by the large number of researchers who wished to prepare DNA for various biochemical studies. The state of the art at that time can perhaps best be summarized by quoting the concluding remarks from a review on DNA synthesis by V. Amarnath and A. Broom:⁴ "Chemical methods for the synthesis of oligonucleotides have undergone dramatic improvement in the last two decades, but such goals as the synthesis of a tRNA molecule or the facile preparative synthesis of DNA genes still shimmer in the distance.... We have a long way to go."

Because of developments in DNA sequencing and recombinant DNA methodologies, the potential applications for synthetic DNA were growing rapidly during this same time period. For example, synthetic DNA was needed to sequence vast stretches of DNA, clone and express genes, study the biophysical properties and structures of polynucleotides and polynucleotide-protein complexes, isolate genes, map chromosomes, and carry out site-directed mutagenesis on various proteins. As a consequence, several laboratories, including the author's, focused on developing new polynucleotide synthesis methodologies that would meet this challenge. The objective was to devise new procedures for preparing polynucleotides so that they would be readily accessible and not represent the rate-limiting step for biological and biochemical experiments.

By 1981 this goal had been achieved, as methods were available that could be used for rapid (one day or less) preparation of deoxyoligonucleotides the size of a *lac* operator.^{5,6} These procedures, which are based in part on the earlier work of R. Letsinger,⁷ are generally known today as the phosphoramidite method of DNA synthesis. This approach, which is currently widely used, perhaps has been so successful because it is readily adaptable to automated DNA synthesis machines⁸ and

leads to very high yields of relatively pure polynucleotides having 100 or more mononucleotides. These achievements are due primarily to the stability of the deoxynucleoside phosphoramidites, the high yields per nucleotide addition (greater than 99% per cycle), and the very short reaction times (less than 1 min).

Other DNA synthesis chemistries have also been explored and improved significantly during the past decade. These include the phosphate triester approach⁹⁻¹³ and a reexamination, primarily by two laboratories,^{14,15} of an H-phosphonate coupling methodology as first proposed by A. Todd.¹⁶ However, the overall yields, speed of synthesis, and quality of DNA from these procedures have yet to match the phosphoramidite methodology. This Account focuses on the phosphoramidite method we have developed for DNA synthesis and also presents a perspective on our current work with various DNA analogues. Articles that provide extensive reviews on DNA chemistry are recommended for those who wish to examine this field more thoroughly.¹⁷⁻²⁰

Because DNA is a linear polymer composed of mononucleotide units, our strategy was strongly influenced by earlier success in the polypeptide field. In a manner similar to solid-phase peptide synthesis,²¹ we imagined

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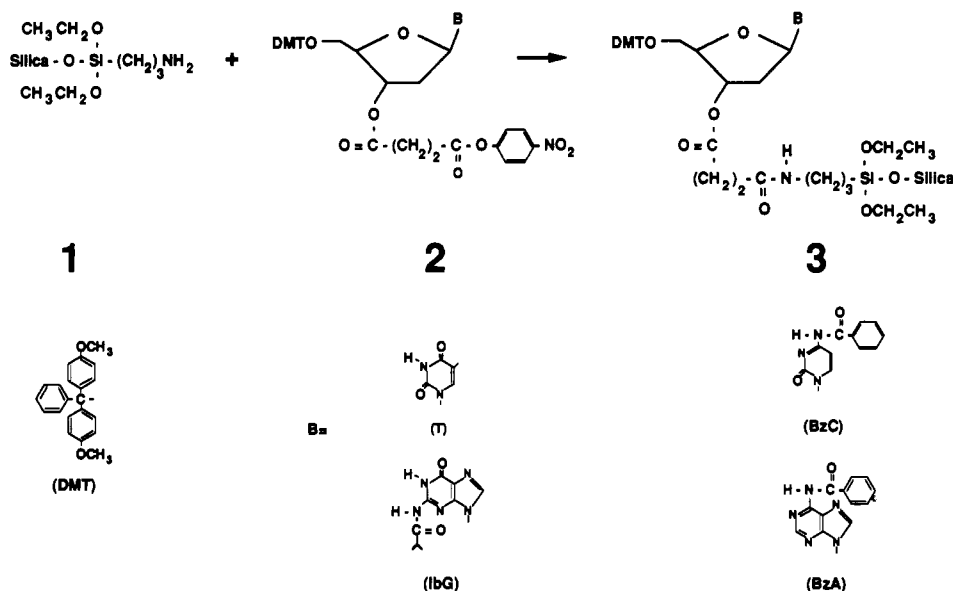


Figure 1. Synthesis of deoxynucleosides covalently attached to silica supports. Abbreviations: DMT, dimethoxytrityl; B can be thymine (T), *N*-benzoylcytosine (BzC), *N*-benzoyladenine (BzA), or *N*-isobutyrylguanaine (IbG).

that a set of mononucleotide synthons could be added sequentially to a growing DNA segment linked covalently to an insoluble support. This approach would have the important advantage that reagents, starting materials, and most side products could be removed simply by filtration. Then, after the synthesis was complete, the deoxyoligonucleotide would be cleaved from the support, isolated, and used for various experiments. Of course we were not the first to attempt polymer-supported polynucleotide synthesis. The initial work²² appeared within two years of the pioneering publications on peptide synthesis.^{23,24} However, inherent in any polymer-support strategy is the assumption that high yields of a growing DNA segment free of side products can be maintained throughout the synthesis. Otherwise, the method fails because these side products and various intermediates will dominate the final reaction mixture and create a very difficult, if not impossible, purification problem. This was the major, unsolved problem with the early polymer-supported DNA synthesis approaches as low repetitive yields and the formation of many side products dominated the strategies. The challenge was to develop a support method that was free of these limitations. This we accomplished by learning how to use silica as an inert matrix for attachment of the growing DNA strand^{5,25} and by discovering a new class of stable tri-valent phosphorus synthons, the deoxynucleoside 3'-phosphoramidites.⁶

Until our investigations, essentially all polymer support research in the polynucleotide field had been completed on organic matrices.^{4,9-12} Because these efforts were uniformly unsuccessful, the results suggested that organic polymers were an undesirable support medium, perhaps because they are highly adsorptive for nucleotides and have poorly defined chromatographic properties. The only exception to organic polymers was

a report from H. Köster where silica was used as a matrix to prepare a dinucleotide.²⁶ The results, however, were not at all encouraging as the yields were even less than those from similar strategies on organic polymers known at the time.²⁷ Despite these disappointing results, we reasoned that silica-based matrices, especially high performance grade silicas, would be more desirable than previously proposed organic supports because they were designed for rapid mass transfer and contained a large number of the theoretical plates. Such properties we considered potentially very important for DNA synthesis where the objective is rapid transfer of reagents throughout the matrix under conditions where little material is adsorbed or trapped within the support. Although rigorous proof that these concepts are valid is still lacking, the high yields of DNA on silica matrices does at least indirectly support the rationale.

Deoxynucleosides linked to silica were prepared as outlined in Figure 1. The initial step involved forming 1 by refluxing (3-aminopropyl)triethoxysilane with the silica matrix in dry toluene for 3 h. The next step was synthesis of 2 by reacting the appropriately protected deoxynucleoside first with succinic anhydride and then with *p*-nitrophenol and dicyclohexylcarbodiimide. Condensation of 1 with the deoxynucleoside 3'-(*p*-nitrophenyl succinate) (2) led to the formation of a silica-linked deoxynucleoside. Underivatized amino groups were blocked against further reaction by acylation with acetic anhydride. Usually the ratio of reagents was adjusted to yield approximately 50 μmol of deoxynucleoside/g of silica, which means that 1–2 μmol of a deoxyoligonucleotide can be prepared from less than 100 mg of silica, a very convenient scale for most work in molecular biology. There have been very few modifications in this initial protocol.^{5,25,28,29} Perhaps the

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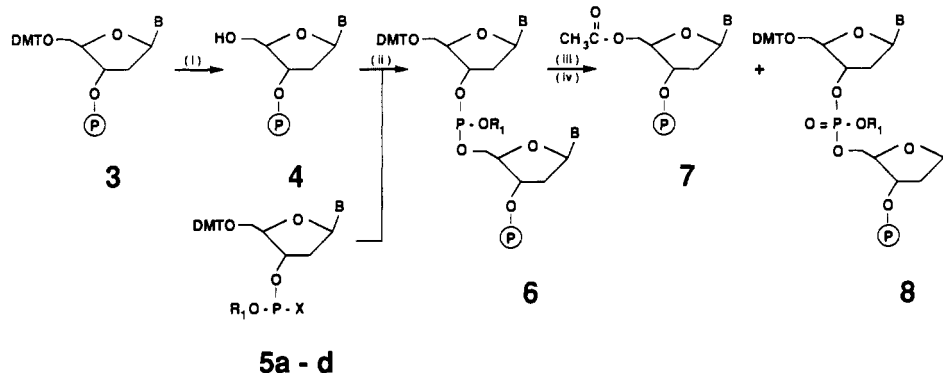


Figure 2. Synthesis of DNA on silica supports using activated deoxynucleoside phosphites as synthons. Steps i-iv correspond to those listed in Table I. Abbreviations: D , the silica matrix, including the succinate linkage as shown in compound **3**, Figure 1; X, chloro (**5a**), tetrazolyl (**5b**), *N,N*-dimethylamino (**5c**), *N,N*-diisopropylamino (**5d**); R_1 , methyl or β -cyanoethyl.

only one of significance was replacement of Vydac and Fractosil with controlled-pore glass³⁰ as the silica matrix. This change increased the repetitive yields by about 1%/cycle,^{31,32} which can be significant when synthesizing larger oligomers. Another recent development was the discovery that polystyrene-divinylbenzene cross-linked polystyrene,³³ especially supports with cross-linking much higher than tested previously,³⁴ can be used for synthesizing DNA via the phosphoramidite method. These results suggest that more studies are needed to define the characteristics required for an optimal DNA synthesis support as matrices other than silica can also be used. Perhaps the key feature is a relatively impenetrable support with major surface channels to enhance synthesis capacity. A support of this type (organic or inorganic) may be accessible to reagents but lack the excessive porosity that leads to irreversible entrapment of reagents in the matrix.

A second key factor was the development of deoxynucleoside phosphoramidites as mononucleotide synthons. Our work began by adapting deoxynucleoside 3'-chlorophosphites, as developed by R. Letsinger and his colleagues,⁷ to polymer support synthesis (Figure 2). Initially, appropriately protected deoxynucleoside 3'-chlorophosphites (**5a**)²⁵ and 3'-tetrazolylphosphoramidites (**5b**)⁵ were reacted with deoxynucleosides that were covalently attached to silica supports (**4**) to generate yields exceeding 90% for the dinucleoside phosphite triester (**6**). The problem, however, was that **5a** and **5b** were unstable, which means they had to be prepared immediately prior to use. They also could not be synthesized in high yield from dichlorophosphites and deoxynucleosides because a 3'-3' deoxydinucleoside phosphite formed with this bifunctional reagent. As a result of these various limitations, the deoxynucleoside 3'-*N,N*-dimethylphosphoramidites (**5c**) were investigated.⁶ Very early in this work, they were found to be quite stable to normal organic synthesis conditions and could be isolated in 90-93% yield merely by routine aqueous extraction and precipitation from pentane. These synthons also had several additional attractive features that made them ideal for DNA synthesis. For example,

they were stable to moisture and oxidation under neutral or moderately basic conditions, they could be stored indefinitely as solids, and they were easily activated by weak acids to form **6** in essentially quantitative yields (98-100%). Although a large number of acids such as amine hydrochlorides, halogenated acetic acids, and sulfonic acids were found to activate **5c**, the acid of choice was tetrazole, a commercially available, non-hygroscopic solid. All the other acids tested were hygroscopic and therefore considered unreliable for routine use, primarily because adsorbed water quenched the phosphoramidite and led to lower repetitive yields. Evidence now exists^{6,35} that activation via amine hydrochlorides or tetrazole leads to the formation of phosphorochloridites and phosphorotetrazolides, respectively, which then further react with the nucleoside 5'-hydroxyl to generate **6**. However, the first step in this activation process, presumably protonation on either phosphorus or nitrogen, still remains unresolved.

Originally the deoxynucleoside 3'-phosphoramidite of choice was **5c**, but now it has been largely replaced by the *N,N*-diisopropylphosphoramidite (**5d**),³⁶ primarily because the former was somewhat unstable toward long-term storage. For all of the initial work with deoxynucleoside phosphoramidites, the methyl substituent was used as R_1 , simply because excellent yields of unmodified deoxyoligonucleotides were obtained.³⁷ Because of the work of H. Köster,³⁸ this group has now been replaced by β -cyanoethyl, a protecting group first shown to be compatible with phosphite chemistry by K. Ogilvie.³⁹ The β -cyanoethyl derivative, despite its reduced reactivity,^{40,41} has one major advantage over methyl. Thiophenoxide, which is used to remove the methyl protecting group, can be safely eliminated from the deprotection protocol. This is because the β -cyanoethyl group is removed under basic conditions^{22,42}—the standard procedure for excising DNA from the

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Table I
Chemical Steps for One Synthesis Cycle

step	reagent or solvent ^a	purpose	time, min
i	(a) dichloroacetic acid in CH ₂ Cl ₂ (1:100, v/v)	detritylation	3
	(b) CH ₂ Cl ₂	wash	0.5
	(c) acetonitrile	wash	1.5
	(d) dry acetonitrile	wash	1.5
ii	(a) activated nucleotide in acetonitrile ^b	add nucleotide	5
	(b) acetonitrile	wash	0.5
iii	(a) DMAP/THF/lutidine ^c (6:90:10, v/v/v), 0.1 mL of acetic anhydride	cap	2
	(b) THF/lutidine/H ₂ O (2:2:1, v/v/v)	wash	1
iv	(a) I ₂ solution ^d	oxidation	1
	(b) acetonitrile	wash	0.5
	(c) CH ₂ Cl ₂	wash	0.5

^a Multiple washes with the same solvent involve filtration between wash steps. Each step volume is 1 mL unless otherwise indicated. ^b For each micromole of deoxynucleoside attached covalently to silica gel, 0.1 M tetrazole (0.2 mL) and 0.1 M deoxynucleoside phosphoramidite (0.2 mL) are premixed in acetonitrile. ^c DMAP, (dimethylamino)pyridine; THF, tetrahydrofuran. ^d THF/lutidine/H₂O (2:2:1, v/v/v) containing 0.2 M iodine.

support and deprotection of the purine and pyrimidine blocking groups.

These developments led to the synthesis cycle outlined in Figure 2 and Table I where the addition of one deoxynucleotide to the support requires four steps. (i) Removal of the dimethoxytrityl group from 3 to yield 4 is usually completed with protic acids such as dichloroacetic acid in an inert solvent.⁴³ These conditions are preferred because detritylation proceeds rapidly (2 min or less) even as the DNA segment becomes quite large. This step must be carefully controlled because deoxyadenosine is very susceptible to depurination under acidic conditions, a side reaction that leads to chain cleavage and an overall reduction in yield. Recently various new protecting groups have been shown to stabilize deoxyadenosine toward depurination.⁴⁴⁻⁴⁷ Perhaps some of these may eventually replace the widely used amides (Figure 1) and thus lead to an increase in quality of synthetic DNA. (ii) The second step is condensation of 5c or 5d with 4 to yield 6. Synthesis proceeds by dissolving the deoxynucleoside phosphoramidite and tetrazole in acetonitrile, mixing, and adding the solution to the support-bound deoxynucleoside. Yields per condensation exceed 99%, and polynucleotides containing up to 175 mononucleotides have been reported.³² (iii and iv) The final two steps are capping and oxidation. The objective of the former, which involves a reaction with acetic anhydride and (*N,N*-dimethylamino)pyridine, is to acylate any DNA segments (7) that fail to react during condensation and to remove deoxynucleoside phosphite adducts from the

(43) Lewis acids can be used as well and have the advantage that depurination of deoxyadenosine does not occur during detritylation. Their use appears to be limited to segments up to 15 in length, primarily because of slower detritylation rates with longer compounds.

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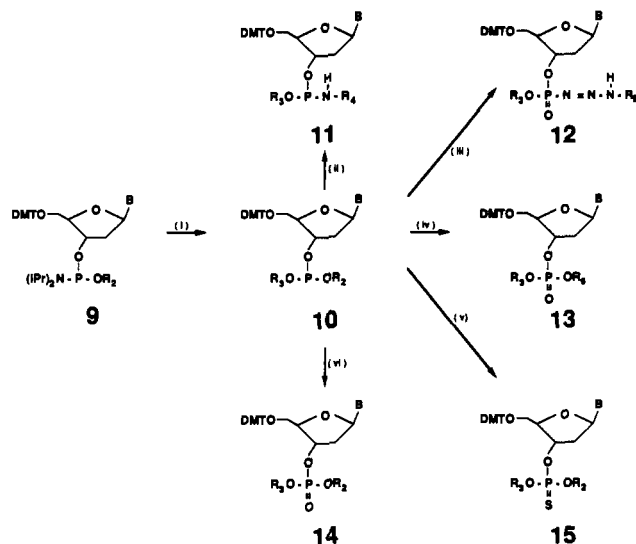


Figure 3. Synthesis of oligonucleotide analogues from a common synthon: (i) tetrazole + 3'-O-acetyldeoxynucleoside; (ii) iodine + *n*-butylamine or *n*-butyl azide; (iii) 3-azido-*N*-ethylcarbazole; (iv) iodine + methanol; (v) sulfur; (vi) *tert*-butyl hydroperoxide. Abbreviations: R₂, 2-cyano-1,1-dimethylethyl; R₃, 3'-O-acetyldeoxynucleoside; R₄, *n*-butyl; R₅, *N*-ethylcarbazole; R₆, methyl; iPr, isopropyl.

bases.⁴⁸ If these adducts are not removed by the capping reagents (or a basic, hydrolytic wash), they are oxidized during the next step of the cycle to stable phosphate triesters or phosphoramidates, which leads to DNA that is useless for biochemical studies.^{8,49} The oxidation step, which uses iodine in 2,6-lutidine, water, and tetrahydrofuran, converts the intermediate phosphite triester (6) to the phosphate triester (8). Oxidation is very rapid (1 min or less), and side products are not generated. These four steps are then repeated until the desired compound is synthesized. Removal of protecting groups with concentrated ammonium hydroxide and purification by polyacrylamide gel electrophoresis or high-performance liquid chromatography then yield the synthetic deoxyoligonucleotide. The only significant change in this protocol has been replacement of (dimethylamino)pyridine, as the capping catalyst, with *N*-methylimidazole. This modification reduces the transition mutation frequency in cloned, synthetic DNA.⁵⁰

The same fundamental approach can be extended to include the synthesis of oligoribonucleotides,⁵¹⁻⁵⁸ 2'-O-

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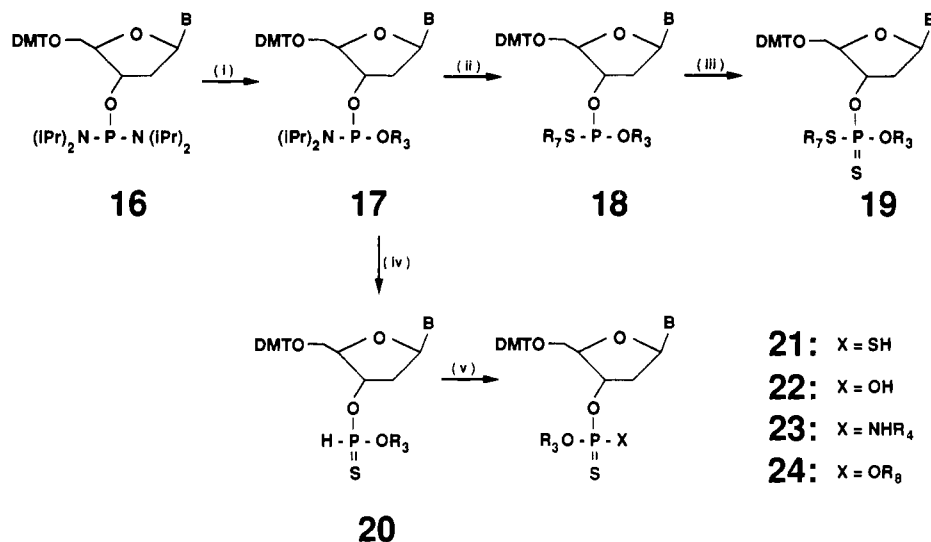


Figure 4. Synthesis of deoxyoligonucleotide dithioates from deoxynucleoside diamidites: (i) tetrazole + 3'-O-acetyldeoxynucleoside; (ii) 4-chlorobenzyl mercaptan or 2,4-dichlorobenzyl mercaptan + tetrazole; (iii) sulfur; (iv) tetrazole + H₂S; (v) sulfur, X = SH; aqueous iodine, X = OH; iodine + *n*-butylamine, X = NHR₄; iodine + 9-anthracenylmethanol, X = OR₉. Abbreviations: R₇, 2,4-dichlorobenzyl or 4-chlorobenzyl; R₉, 9-anthracenylmethyl.

methyloligoribonucleotides,^{59,60} oligoarabino-nucleotides,⁶¹ and α-DNA.⁶² In all cases, synthesis of the internucleotide linkage proceeds rapidly and with high yields. Problems, however, still exist for the oligoribonucleotides, primarily because there is currently no completely satisfactory 2'-protecting group. Several additional analogues can be synthesized as well by simply changing the oxidant. These include the phosphorothioates,^{63,64} phosphoroselenoates,⁶⁵ and certain types of phosphoramidates,⁶⁶ but the yields of the latter derivatives are only 40–80%. Another important adaptation is to use the deoxynucleoside 3'-methylphosphonamidites. With this synthon, the same cycle leads to the formation of a methylphosphonite linkage which can be oxidized with aqueous iodine or sulfur to yield oligonucleotide methylphosphonates^{67,68} or methylphosphonothioates,⁶⁹ respectively. Through the elegant studies of P. O. P. Ts'o and P. S. Miller with methylphosphonate DNA⁷⁰ and several laboratories with phosphorothioate DNA,⁷¹ some of these analogues are beginning to show potential as therapeutic drugs.

A further variation on this theme is outlined in Figure 3. Certain deoxynucleoside 3'-phosphoramidites (9),

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having either 2-cyano-1,1-dimethylethyl as shown or *o*-methylbenzyl as the phosphorus protecting group, can be used to prepare a large number of analogues modified at phosphorus.⁷² These derivatives first react with a suitably protected deoxynucleoside and tetrazole to yield dinucleoside phosphite triesters (10), which can then be oxidized under variable conditions to generate a large variety of analogues. For example, oxidation with *n*-butyl azide or iodine and *n*-butylamine produces the *n*-butylphosphoramidate (11) quantitatively, whereas oxidation with an aryl azide such as 3-azido-*N*-ethylcarbazole yields the deoxydinucleoside phosphoroazoamidate (12). When an alcohol or water is substituted for the amine, iodine oxidation generates the deoxydinucleoside phosphate triester (13) or natural diester internucleotide linkage, respectively. On the basis of ³¹P NMR analysis, this reaction appears to proceed through the formation of first the iodo-phosphonium iodide and then the phosphoryl iodide via elimination selectively of the tertiary alkyl or benzyl protecting group. The resulting phosphoryl iodide can then react with various nucleophiles to generate amidates or esters. The deoxydinucleoside phosphite triester (10) can also be oxidized with *tert*-butyl hydroperoxide or sulfur to yield phosphate (14) or phosphorothioate (15) triesters, respectively, without loss of the protecting group. This approach is thus quite flexible as phosphoramidates, phosphoroazoamidates, phosphate triesters, and phosphorothioates, as well as the natural internucleotide linkage, can be introduced into any specific phosphate of an oligonucleotide merely by changing the oxidation conditions. These synthons should become very useful for many applications in biochemistry and molecular biology. This is because synthetic DNA usually must be further modified by addition of a fluorescent or radioactive probe, biotin, antigens, or some other reporter group before it is used in a biochemical assay. By using phosphoramidites such as 9 and varying the oxidation conditions, many different probes as amines, azides, or alcohols can be introduced into synthetic DNA through a common in-

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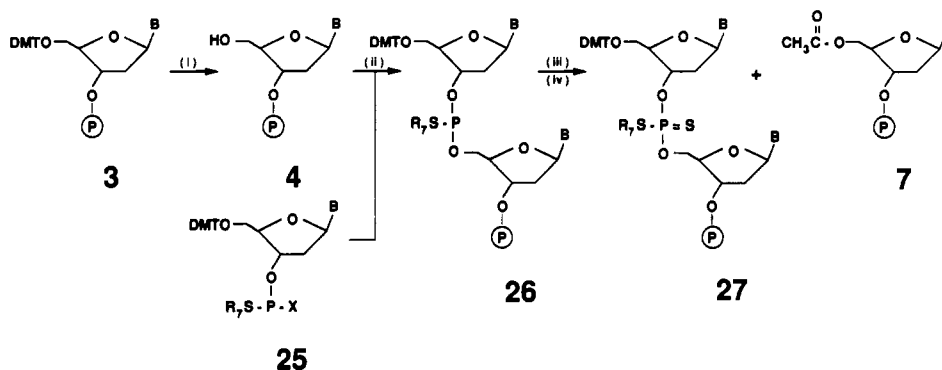


Figure 5. Synthesis of deoxyoligonucleotide dithioates from deoxynucleoside 3'-phosphorothioamidites: (i) 2% dichloroacetic acid; (ii) tetrazole; (iii) sulfur; (iv) (dimethylamino)pyridine + acetic anhydride. Abbreviations: X, *N,N*-dimethylamino or pyrrolidino.

intermediate at a site (phosphorus) that is independent of sequence.

Within the past three years, we have focused our attention on the development of methodologies for synthesizing phosphorodithiolate internucleotide linkages.^{73,74} This analogue initially appealed to us as an attractive synthetic objective because, in a manner similar to natural DNA, it is achiral and anionic. This is not the case with the currently popular phosphorothioate and methylphosphonate derivatives where P-chiral internucleotide linkages generate a large number of diastereomers, a potentially serious disadvantage for many biological studies. Now that phosphorodithioate DNA has been synthesized and tested in various biochemical assays, we are even more excited about its potential, as oligonucleotides containing this linkage are completely resistant to nucleases and form duplexes with normal DNA.

The initial pathway⁷³ we developed for synthesizing the phosphorodithioate internucleotide linkage is outlined in Figure 4. The first step leading to the synthesis of 19, the completely protected deoxydinucleoside phosphorodithioate, is condensation of a deoxynucleoside phosphorodiamidite (16) with a 3'-protected deoxynucleoside in the presence of tetrazole to yield 17. This deoxydinucleoside phosphoramidite is then converted to the thiophosphite triester (18) by condensation with either 4-chlorobenzyl mercaptan or 2,4-dichlorobenzyl mercaptan using tetrazole as catalyst. Oxidation with sulfur generates the completely protected dinucleoside phosphorodithioate (19). If the deoxydinucleoside phosphoramidite is sulfurized with H₂S and tetrazole, the H-phosphonothioate that is generated (20) can be oxidized to several analogues. For example, treatment with either sulfur or aqueous iodine yields the phosphorodithioate (21) or phosphorothioate (22), respectively. Alternatively, phosphorothioamidates (23) and phosphorothioate triesters (24) are formed by iodine oxidation in the presence of amines or alcohols. These dinucleotides (19, 23, 24, and also 21 when it is further protected with α ,2,4-trichlorotoluene) can then be converted to the 3'-phosphoramidites and used for preparing modified DNA via the cycle presented in Table I. In retrospect, the most attractive feature of this pathway is its versatility, as appendant R groups (R₄ and R₈) can be variable and

include a large variety of reporter groups. This approach is therefore most attractive for selectively inserting analogues such as 23 or 24 into DNA rather than for routine synthesis of dithioate DNA. This is because all 16 dimers are needed in order to create the flexibility for incorporating a dithioate linkage into any predetermined position.

This limitation led us to develop a synthetic method whereby mononucleotide synthons are used to prepare DNA having phosphorodithioate linkages. The objective was to design a pathway compatible with the phosphoramidite method of DNA synthesis. In this way, DNA having any combination of natural and phosphorodithioate linkages could be prepared on DNA synthesis machines with a minimum of additional new steps. This has now been realized by development of deoxynucleoside 3'-phosphorothioamidites as the appropriate intermediates.⁷⁴

Synthesis begins (Figure 5) by condensing a deoxynucleoside 3'-phosphorothioamidite (25) with 4, a deoxynucleoside linked to a silica support, to yield a thiophosphite internucleotide linkage (26). Activation with tetrazole and condensation is complete within 3 min. After oxidation with 5% sulfur in pyridine/carbon disulfide to yield 27, acylation of unreactive deoxynucleoside to 7, and detritylation, the dinucleoside phosphorodithioate can be further condensed in a cyclic manner with either 25 or 5d, to yield oligodeoxynucleotides with normal and phosphorodithioate linkages in any desired sequence. Synthesis of the deoxynucleoside 3'-phosphorothioamidites proved to be especially straightforward. Typically, a protected deoxynucleoside, triethylamine, and either bis(dimethylamino)chlorophosphine or bis(pyrrolidino)chlorophosphine are mixed in solvent and, after 5 min, the mercaptan is added. The reaction mixture is then concentrated, extracted with aqueous bicarbonate, dried, and precipitated to yield 25. These deoxynucleoside 3'-phosphorothioamidites also appear to be stable toward storage and oxidation, which is quite attractive for use in automated DNA synthesizers. Once a DNA segment has been synthesized, thiophenol and ammonium hydroxide are used to remove protecting groups and the DNA is purified by high-performance liquid chromatography or polyacrylamide gel electrophoresis.

Recently we found that phosphorodithioate DNA has several potentially very exciting biological and biochemical applications. For example, these compounds are extremely potent inhibitors of retroviral reverse

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transcriptases (HIV and AMV) and readily substitute for normal DNA in activating RNase H.⁷⁵ Perhaps phosphorodithioate DNA will therefore be useful as a therapeutic drug or as a diagnostic reagent. Additionally, we have shown that the phosphorodithioate linkage is readily alkylated by (iodoacetyl)fluorescein, (iodoacetyl)biotin, and bromobimane while the oligonucleotides are in aqueous buffer.⁷⁶ This means that a large variety of chemically labile reporter groups can be attached to phosphorodithioate DNA postsynthesis and under extremely mild conditions. These preliminary results clearly indicate the potential of dithioate DNA and encourage us to continue to explore various applications.

As a consequence of the research outlined in this Account, DNA, RNA, and a large number of analogues can be synthesized in near quantitative yields. Moreover, we have made considerable progress in structuring these procedures so that different derivatives are prepared by simple variations on standard protocols.

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These developments should provide considerable flexibility in preparing analogues for various biochemical applications. However, several significant challenges still remain. One is developing methods for the large-scale synthesis of oligonucleotides and oligonucleotide analogues. As we learn more about using DNA (or RNA) for therapeutic and diagnostic applications, the synthesis of kilogram amounts of oligonucleotides will become necessary. Another major challenge is to develop synthesis protocols for several additional analogues such as compounds having oligonucleotides linked through sulfur, nitrogen, or carbon to phosphorus. Undoubtedly, some of these will have extremely interesting biochemical and biological properties.

The value of synthetic oligonucleotides as a major research tool in biochemistry and molecular biology is now clear. This realization should continue to stimulate further developments in nucleic acid chemistry and lead to even more fascinating challenges in the years to come.

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