Recent advances in synthesis of modified oligonucleotides

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With the advent of oligonucleotides as potential therapeutics, the search for modified analogs of nucleic acids having improved properties, such as increased stability against nucleases, improved cellular uptake and enhanced binding affinity to complementary nucleic acids with high specificity, has accelerated rapidly and has attracted considerable attention. Specific binding through natural base pairing has become increasingly important for the development of DNA based diagnostics as well as therapeutics in the form of antisense and antigene oligonucleotides. Modified DNA based oligonucleotides can affect the gene expression at the level of translation as well as transcription. The automation of oligonucleotide synthesis, the development of better coupling reagents as well as support system and efficient scale-ups have expanded the application of these molecules to diverse areas of fundamental as well as applied biological research. The modified molecules are expected to find answers to understanding, unveiling and utilizing the intricate methods of cell functioning at the molecular level and would provide applications in genetic engineering, gene therapy and cellular regulations in an array of conditions. In this review, the synthesis or the assembling of oligonucleotides modified at the backbone, or at the base, or at the sugar residues, has been brought into sharp focus.

Oligonucleotides form stable double helixes under appropriate conditions by binding to single stranded nucleic acids, if these are sequences complementary and antiparallel to the single stranded nucleic acids. This property makes them useful as diagnostic reagents as well as tools in molecular genetics. Certain oligonucleotides bind specifically to a variety of proteins. Biological processes involving such proteins can be affected by the addition of the reacting oligonucleotides. Such properties of the synthetic oligonucleotides make them useful in biology[1-8]. The property of oligonucleotides forming stable double helixes has been used in medicinal biotechnology for their applications as antisense oligonucleotides targeting specific mRNAs. Such bindings have been shown to modulate or inhibit the gene expression at the translation stage (Fig. 1). These molecules are being considered as the potential new generation pharmaceuticals[9-15]. Table 1 lists some such compounds and their present status of use or development as pharmaceuticals by some companies. The information was retrieved from the Internet websites of the companies. On the parallel lines of antisense compounds, if an oligonucleotide is carrying a reactive group, it can modify or attach itself covalently to its binding partner. An oligonucleotide carrying a dye molecule can be localized in a biological system; enzyme bound oligonucleotides can be detected by the enzyme specific catalytic reactions. For many applications in molecular biology, identification and isolation of specific sequences in a genome is a basic requirement, and therefore oligonucleotide probes are used extensively for this purpose[2]. Until recently, synthetic oligonucleotides, tagged with radioisotopes, were the only means to prepare such sensitive probes. These molecules, though useful, suffer from certain inherent drawbacks, like short half-lives, radiation hazards and disposal problems. Many recent studies[16-20] have exposed synthetic oligonucleotides, which include their susceptibility against nucleases, lower stability of the duplexes formed with medium chain length oligonucleotides and their lack of penetration of lipophilic cell membranes.

To overcome the above mentioned obstacles, modifications were introduced in the synthetic oligonucleotides so that (a) non-radioactive labels or fluorophores could be attached to them, (b) they could be able to cross or penetrate the lipophilic cell membrane, (c) be able to hybridize rapidly and selectively to obtain a stable duplex, (d) should be cheaper to prepare in high purity, and (e) should be sufficiently stable towards nucleases. Oligonucleotides thus modified should find numerous applications in molecular biology and biotechnology[21]. Modified oligonucleotides have indeed been designed already for various applications (Table 2).

Table 1. Some antisense oligonucleotides and their status of use as drugs

Given name of the oligonucleotide drug	Targeting disease	Current status of approval as a drug	Ownership Company
Vitravene	Retinitis (AIDS)	Approved	Isis Pharmaceuticals Inc., USA
ISIS 2302	Crohn's disease	Phase II	Isis Pharmaceuticals Inc., USA
ISIS 2302	Rheumatoid arthritis	Phase II	Isis Pharmaceuticals Inc., USA
ISIS 2302	Ulcerative colitis	Phase II	Isis Pharmaceuticals Inc., USA
ISIS 2302	R T rejection	Phase II	Isis Pharmaceuticals Inc., USA
ISIS 3521	Ovarian cancer	Phase II	Isis Pharmaceuticals Inc., USA
ISIS 5132	Cancer	Phase II	Isis Pharmaceuticals Inc., USA
ISIS 2503	Solid tumors	Phase I	Isis Pharmaceuticals Inc., USA
ISIS 13312	Retinitis (AIDS)	Phase I/II	Isis Pharmaceuticals Inc., USA
GEM 91	HIV (AIDS)	Phase I	Hybridon Inc., USA
GEM 92	HIV (AIDS)	Phase I	Hybridon Inc., USA
GEM 132	CMV-Retinitis	Phase II	Hybridon Inc., USA
GEM 231	Tumors	Phase II	Hybridon Inc., USA
G 3139	Cancer	Phase II	Genta Inc., USA
LR 3280	Stent restenosis	Phase I	Lynx Therapeutics Inc., USA
LR 3001	Leukemia	Phase II	Lynx Therapeutics Inc., USA
LR 4437	Tumors	Phase I	Lynx Therapeutics Inc., USA
GPI 2A	AIDS	Phase I	Novopharm Biotech Inc., Canada
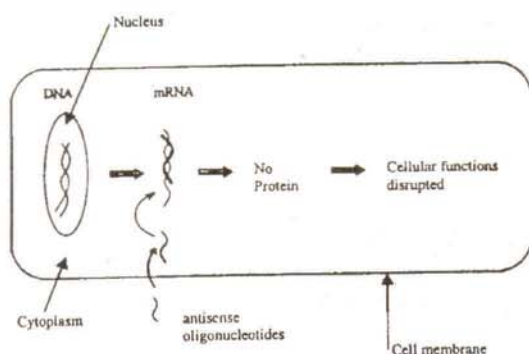
Fig. 1. Schematic representation of action of antisence oligonucleotides.

Table 2. Applications of modified oligonucleotides

Particulars of broad applications	Ref.
Detection of genetic diseases	22
Assignment of gene functioning	23
Elucidation of mechanism of various enzymes	24
Preparation of chemical nucleases	25
Applications in diagnostics	26
Sequencing applications	27–30
Fluorescent microscopy	31
Hybridization affinity chromatography	32
Antisense oligonucleotides	33–37

An oligonucleotide chain consists of purine or pyrimidine bases, a sugar residue and an internucleotide phosphate backbone, all of which are amenable to modifications (Fig. 2). Therefore, one or more sites of modification can be incorporated into the synthetic oligonucleotides. Modified oligonucleotides can be and has been synthesized without much alteration in the commercially available automated DNA synthesizers. The modified nucleotide derivatives are added in an analogous manner as the normal nucleotides are coupled. The derivative with the desired modification or an intermediate, which can also be converted into the desired modification later on, can be used for the synthesis. Several protocols have been developed for the synthesis of these modified analogs of oligonucleotides. Generally, modifications by chemical synthesis at the ends (5'- or 3'-) and at the backbone (phosphate groups) of the oligonucleotides are easier to achieve.

In this paper we have reviewed different types of modifications introduced into the oligonucleotides and the efficacies in achieving the specific goals.

End modifications

End modifications[38-74] are relatively easy to bring about and have advantage of not destabilizing the hybrid, when used as hybridization probes. Besides being used for the
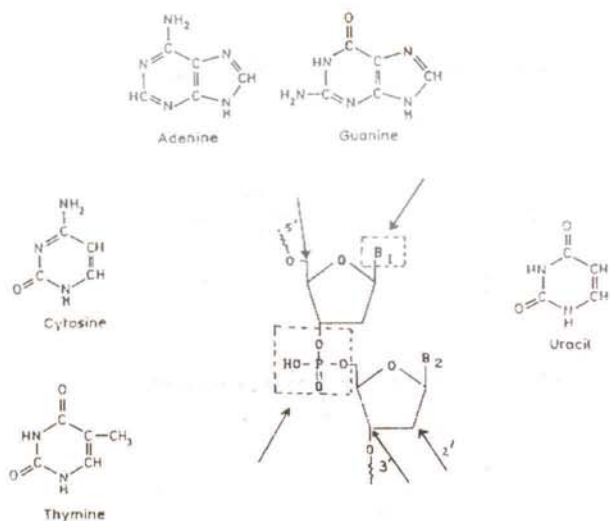
Fig. 2. Sites (marked with arrows) amenable to modifications.

preparation of non-radiolabelled probes, end modified oligonucleotides bearing an intercalator or reactive group at either or both the termini find widespread usage as antisense oligonucleotides. Conjugates of the 3'-end modified oligonucleotides show a marked improvement in cellular stability, while oligonucleotides bearing hydrophobic groups at either of the termini show improved penetration of cellular membrane.

As the requirements of individual modified oligonucleotides are in very small quantities, the solid phase polymer supported synthesis has taken over the synthesis from the solution phase operations. The system is easy to control and provides higher molecular conversions. The polynucleotide chain remains bound to the support throughout the course of chain-elongation and chain-modification, the stepwise separation or purification or characterization can be avoided, the large excess of reagents employed to force the reaction to completion can easily be washed off and the desired product can be obtained in high yields. Further, the repitition of similar or identical steps allows to automate the solid phase synthesis. Two polymer support materials based on controlled pore glass and polystyrene, respectively, both individually and independently, have taken over the use of most of the other support systems. The processes have been standardized to use 20–25 atoms spacer arm for the smooth operation during end modifications. We have recently reviewed the area of polymer supports for the synthesis and modification of oligonucleotides[42].

3'-End modifications :

This type of modifications (Fig. 2) can be introduced using either solution phase, enzymatic or solid phase methods. However, the number of such methods is relatively small. This is due to the fact that most of syntheses are currently carried out on solid supports and during the solid phase synthesis, the 3'-end is unassailable for further manipulations. Some of the support systems are shown in Fig. 3 for the introduction of functionality at 3'-end. These methods can broadly be categorized under following heads :

Support based methods : The limitations imposed by the solid phase methodology on the modification of the 3'-end of the oligonucleotides, is circumvented by modifying the solid support itself. This is generally achieved by attaching a modifying linker to the support. The linker itself may contain the label or the reactive molecule (where the nature of functionalities in the label/hapten or the reactive molecule so permits, without protection, if otherwise then with a suitable protection). The synthesis of desired oligo-
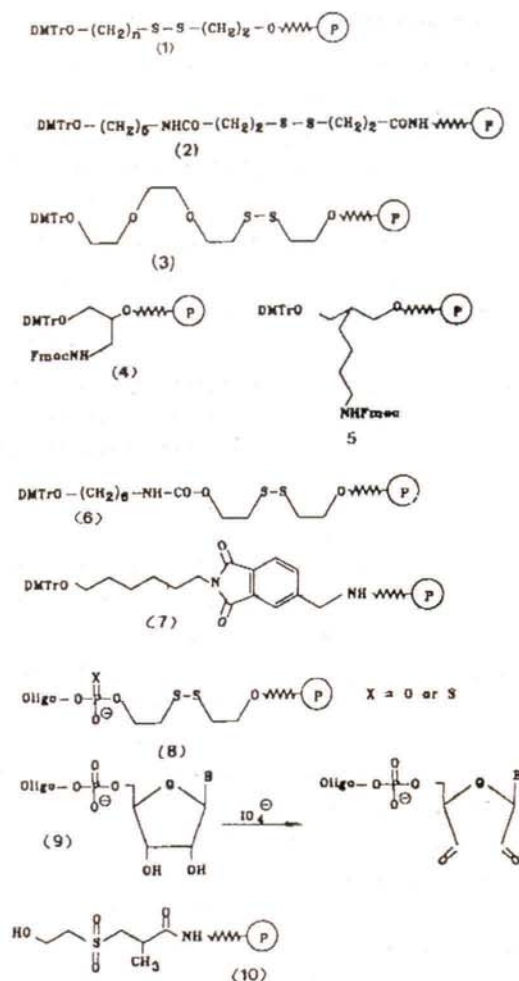

Fig. 3. Reagents for 3'-end modification.

nucleotides on modified support, following standard protocols, gives rise to oligonucleotides with desired functionality at 3'-end during deprotection under specified conditions. The other method relies on the phosphorylation of the support with a building block that would permit chain elongation and upon deprotection, yield a labeled oligomer directly or one bearing a reactive group. Thus methods are available for the introduction of a phosphate[38–47,56], aminoalkyl[48–54,74], peptide-oligonucleotide conjugates[63–65], mercaptoalkyl[55,69–74], thiophosphate[45,56–58], partially protected thiol[72], acridinyl and cholesteryl group[59–62] at the 3'-end of the oligonucleotides.

Another solid support based approach that has been used to incorporate 3'-end modification is based on the use of ribonucleoside attached to the support, which upon deprotection leads to oligonucleotides having a ribose at the 3'-end. The ribonucleoside is then subjected to periodate oxidation to an aldehyde group which, in turn, is converted into an amino group by reductive amination[68].

Solution based methods : Using triester approach a 3'-phosphate group can be introduced readily, the 3'-phosphate can then be condensed with a hydroxyl group in the side-chain of a label like acridine or phenanthroline or its derivative[48,49].

Enzymatic methods : One of the approaches permitting attachment of a label at the 3'-end makes use of T4 RNA ligase to incorporate a ribonucleoside residue at the 3'-end. This residue gives a reactive handle through a sequence of reactions involving periodate oxidation and reductive amination[68]. Likewise methods have been reported for the incorporation of biotin-11dUTP and biotinylated analogs[68a].

5'-End modifications :

The 5'-end modifications (Fig. 2), as a class, have been the subject of maximum number of reports on modifications of oligonucleotides. The main reasons for this are : (i) like the 3'-end modifications discussed above, they do not adversely affect the stability of the hybrid (when used as hybridization probes), and (ii) these are easy to be introduced. Thus using a suitable synthon (amidite or phosphonate), a nucleophilic group or a label can be introduced directly by performing the last coupling reaction with the modified synthon instead of the conventional one. Two types of approaches have been used; first, those incorporating a nucleophile in a protected form, which is labeled in a subsequent post-synthetic step. Second approach is based on direct ligand attachment. Thus, phosphorylated reagents have been used for the introduction of groups like phosphates[75–81], aminoalkyl[82–109] and thioalkyl[110–119]. Some
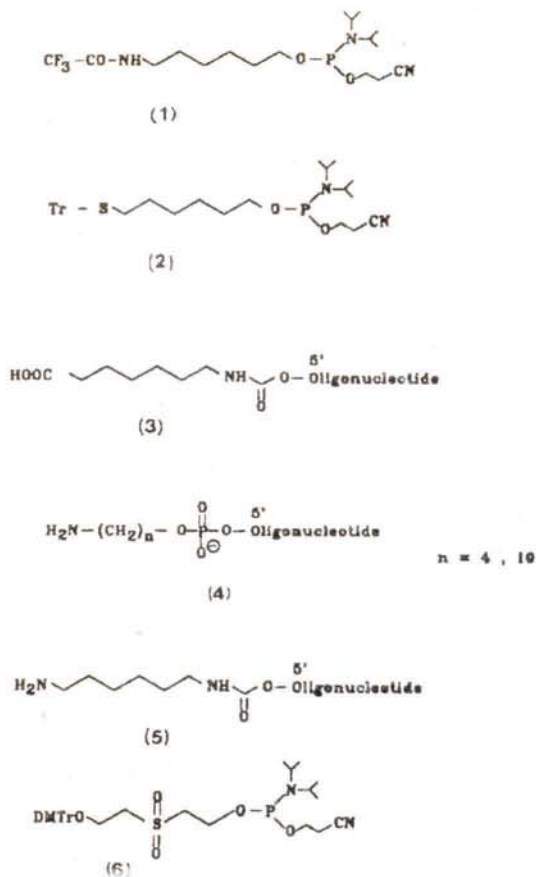

Fig. 4a. Reagents for 5'-end modification.

of the reagents used are shown in Figs. 4 (a and b) for the introduction of ligands diectly or indirectly[120–129].

Sugar modifications

The approach so far discussed suffers from one or the other disadvantages. It has been pointed out that the end modifications which place the functional group at either of the termini are limited in scope as they do not permit interaction of the modifying groups or functionalities with sites located within a complementary sequence. In recent reports, modifications in the sugar moiety have been suggested as the method of choice. The most commonly used position for such modifications is 2'-end (Fig. 2) of ribonucleosides[130–136]. Modifications can also be introduced at other positions (1'- and 4'-)[137–140], but these are not used so frequently as 2'-position is used. The reason may be that modifications at these positions are possible only prior to the attachment of the base to sugar residue. Therefore, only limited number of reports have been published. Recently, ribonucleosides functionalized at the 2'-position have been incorporated into oligonucleotides for post-synthesis conju-
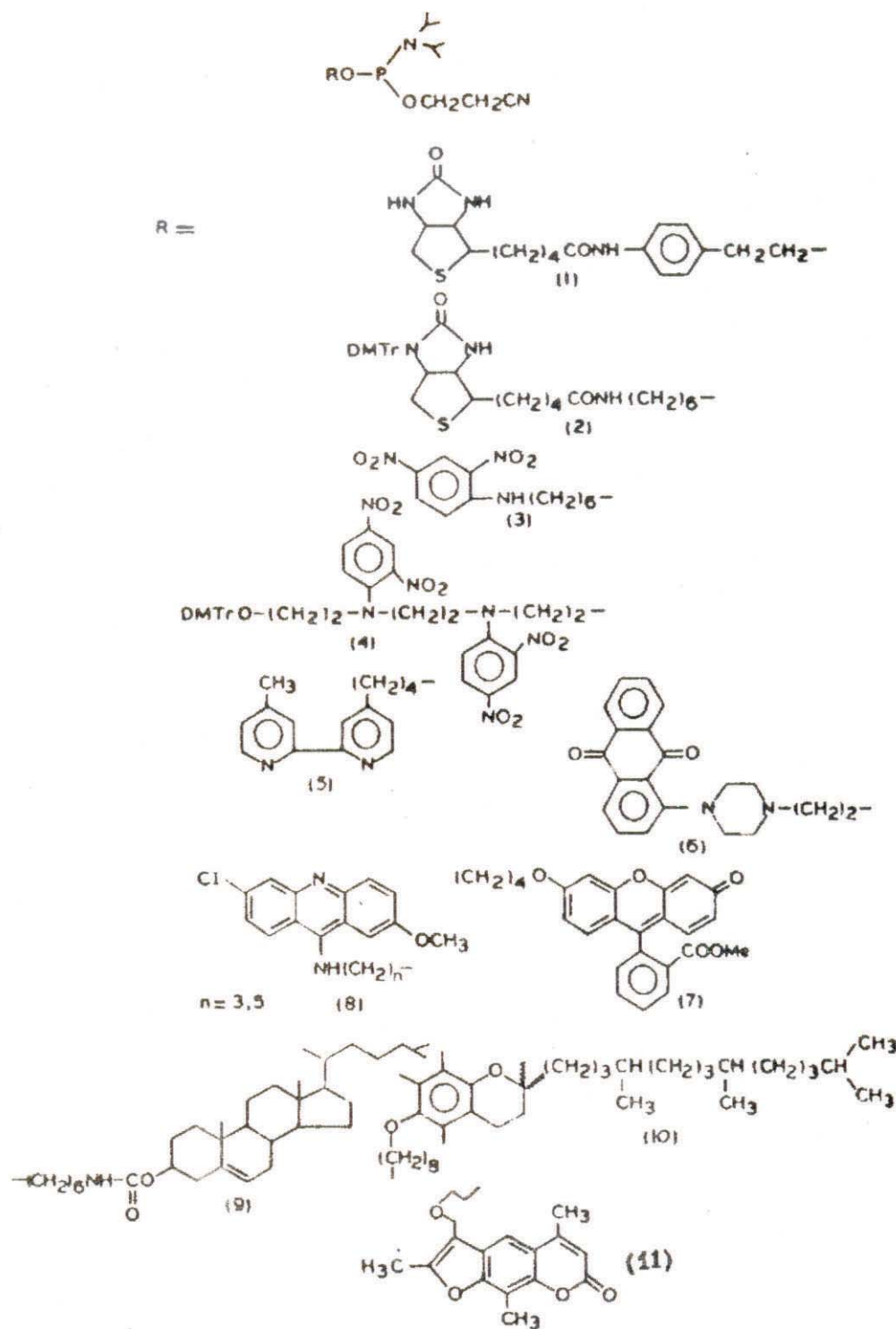
Fig. 4b. Reagents for direct attachment of ligands.

gation of oligonucleotides with intercalators, nucleic acid cleaving agents, cell surface interacting phospholipids and diagnostic agents such as fluorescent labels. Besides, nucleo- sides with modified sugar entities (thiosugars, acyclic, carbocylic, etc. as discussed in later section) are also being used as therapeutics.

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2'-Position :

The modification at 2'-position of the ribonucleosies was introduced in order to impart some stability under physiological conditions. Initially, the modification at this position was confined to 2'-O-methyl derivatives, which also occur naturally, are isolated and characterized. The most striking feature of this modification is that the oligoribonucleotides can be assembled in a similar fashion as oligodeoxyribonucleotides are synthesized and these form stable duplexes with RNA as compared to DNA. Moreover, the same modification improves stability towards nucleases. The monomeric unit can be generated by alkylation of anion resulting from sodium hydride treatment in aprotic solvent at lower temperature. Besides, functional group can also be introduced which can further be used for conjugation purposes. Using 2'-amino linker, several molecules have been conjugated to improve the properties of oligonucleotides required for antisense research, e.g. cholic acid for cellular uptake, pyrene and acridine as intercalators, azides as photoactivable cross-linking agents and polyamines as cleaving agents. Fig. 5 shows some of the groups used for conjugation at 2'-position. The introduction of amino-linker at 2'-position does not affect duplex stability against RNA and even offers some small stabilization as the said modification lies in minor groove. Similarly, mercapto-linker has also been introduced at this position for attaching thiol group specific fluorescent molecules to study uptake of oligonucleotides.

Other positions :

Recently, Matsuda *et al.*[136] have demonstrated a new site for modification, i.e. 1'-position that also lies in the minor groove. This position has been selected for the introduction of an aminoalkyl tether (Fig. 6). It has been illustrated in that work that the reporter or the functional group would be accommodated in the minor groove when oligonucleotide analogs form duplexes with complementary strands.

α-Oligonucleotides : The α-anomer of a 2'-deoxyribosugar has the base inverted with respect to the natural β-anomer. Oligonucleotides containing α-anomeric sugars have been shown to possess resistance against nuclease degradation. Therefore, for a specific purpose, they can be used for attaching intercalators such as acridine, psoralen and phenanthroline derivatives, chain cleavers and other reactive groups[141–143].

Base modifications

Modifications at nucleobases are generally considered the most difficult to be achieved but still are in the great
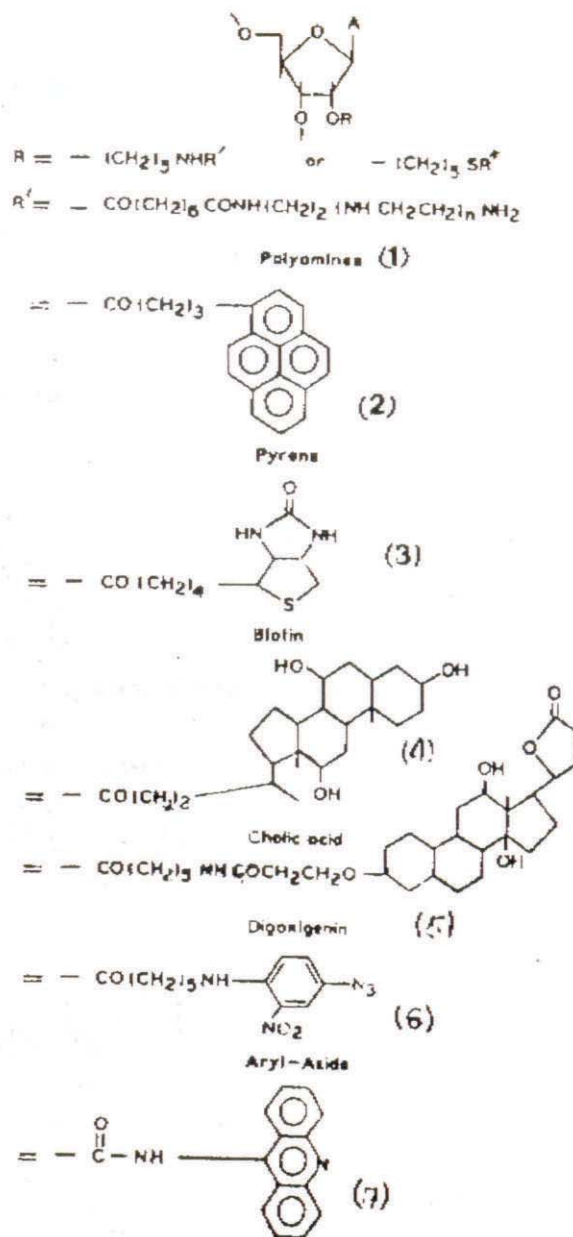
Fig. 5. Modification at 2'-position.

demand. Therefore, one finds a plethora of reports[144–192] for the synthesis of base modified oligonucleotides. Most of the methods rely on the introduction of masked amino, mercapto or carboxyl group(s) through an alkyl chain either through naturally occurring sites in nucleobases or through an electrophilic site (C-5). The linker molecule can also be introduced with a reporter group in the nucleotide before incorporation into growing chain. A variety of intercalating
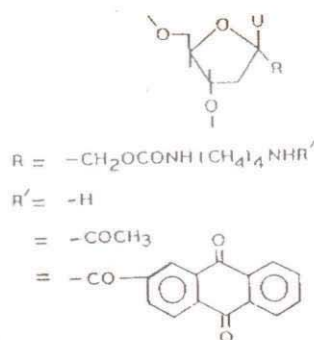
$R = -CH_2OCONH(CH_4)_4NHR'$

$R' = -H$

$= -COCH_3$

$= -CO-$ (anthraquinone structure)

Fig. 6. Modification at 1'-position.

agents, fluorescent groups[147,148], peptides, proteins, cross-linking agents[161] and other groups have been coupled to different positions in pyrimidines and purines. In general, these approaches entail the preparation of a suitable synthon from a duly modified nucleoside. A careful selection of protecting groups is required to avoid the formation of side-products. This strategy allows introduction of any number of modified bases in the oligonucleotide chain at pre-selected positions. Oligonucleotides, incorporating modified bases have extensively been used for the study of DNA-protein interactions[193]. Therefore, modification of the heterocycles requires intelligent designing, since base pairing must be maintained through a combination of proper hydrogen bonding and base stacking interactions.

Pyrimidine modifications :

Modifications in this class (with C-4 and C-5 positions being the preferred sites) are comparatively easy to achieve by making use of mild chemical conditions without the formation of side products.

The C-4 position of thymine and cytosine has extensively been used for introducing functionalities. For the attachment of a linker arm (Fig. 7), this position requires activation, which can be achieved in one of the following ways :
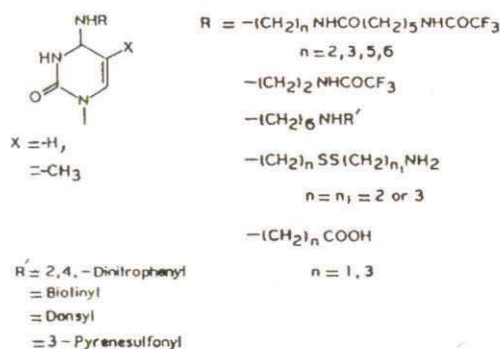

$R = -(CH_2)_n NHCO(CH_2)_5 NHCOCF_3$

$n = 2,3,5,6$

$-(CH_2)_2 NHCOCF_3$

$-(CH_2)_6 NHR'$

$-(CH_2)_n SS(CH_2)_{n_1} NH_2$

$n = n_1 = 2$ or 3

$-(CH_2)_n COOH$

$n = 1,3$

$X = -H,$

$= -CH_3$

$R' = 2,4,-Dinitrophenyl$

$= Biotinyl$

$= Dansyl$

$= 3 - Pyrenesulfonyl$

Fig. 7. Modification at C-4 position in pyrimidines.

(i) A triazole derivative is prepared at C-4 position, which in the subsequent reaction is displaced by nucleophiles. This is used as a general purpose reagent. The other leaving groups such as *p*-toluene sulfonyl[166], *o*-nitrophenyl[167] and 2,4,6-trimethylphenyl[168] have also been used. Recently, MacMillan and Verdine[168,169] have reported the synthesis of oligonucleotides having different linker molecules with reactive group at C-4 position of uracil by making use of *O*-4-trimethylphenyl derivative. In a similar fashion, Brun *et al.*[171] introduced 4-triazolide derivatives during synthesis of oligonucleotides and subsequently labeled them with biotin, using, 1,10-diaminodecane as a linker.

(ii) The second method to obtain C-4 modification involves bisulfite catalyzed addition reaction[177] across the 5,6-double bond that greatly helps in the nucleophilic displacement of amino group at C-4 position of cytosine, termed as transamination reaction. The reaction involves the deamination of cytosine derivative in bisulfite solution, whereas in the presence of primary amines, transaminated product, C-4 substituted cytosine is obtained. Fig. 7 shows some of the modifications that have been introduced at C-4 of cytosine.

(iii) In the third strategy, displacement of sulfur from 4-thiopyrimidine by an alkylamine or direct alkylation with α-haloacetamide derivatives or phenacyl bromides is carried out to prepare a number of conjugates[178].

On the modifications at C-5 position, a relatively large number of methods have been reported in the last few years. This position offers an added advantage of being a major groove modifier and does not interfere in the hybridization process. Oligonucleotides containing 5-fluoro, bromo, alkyl, alkenyl and alkynyl derivatives[194–197] show improved base pairing, which can be explained on the basis of increase in the acidity of the N-3 proton that enhances the base stacking interactions of duplexes. Increased cellular uptake was also observed due to the hydrophobic nature of the above substituents. The method for their introduction involves the reaction of mercuro or halogeno derivatives with an alkynyl or nucleophilic group in the presence of Pd[II] species[146]. The various linker molecules that have been introduced at C-5 position of uracil base residue are listed in Fig. 8. In a novel approach to introduce ribonuclease mimics in the oligonucleotide chain, Bashkin *et al.*[198] have attached an imidazole ring in the side chain of a linker molecule at C-5 position and incorporated this molecule at preselected positions via solid phase methodology.

Purine modifications :

In case of purines, C-2, C-6 and C-8 positions are available for modification. Since C-2 and C-6 positions are in-
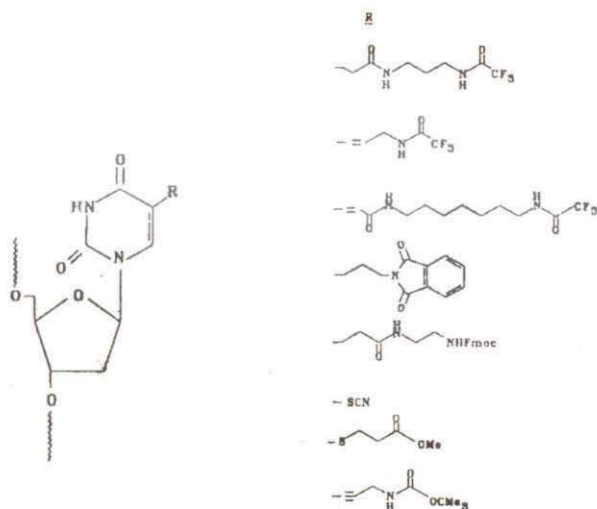
Fig. 8. Modification at C-5 position in uracil.

Fig. 9. Modification at C-2 and C-6 positions in purines.

volved in hydrogen bond formation, substitutions at these positions lead to destabilization. Perhaps for this reason the modifications have not been considered important and therefore, only few reports[189,199–203] are available on this subject. In general, the procedures for conjugation at these positions involve the displacement of halogeno derivatives with a variety of nucleophiles. In order to incorporate post-synthesis modifications, one group of investigators[204] has activated C-6 position of deoxyguanosine with pentafluorophenyl group for the conjugation with an appropriate ligand. Fig. 9 shows C-2 and C-6 modifications in purines. The C-8 position is the only site available, which is not involved in base pairing. The most common method for activation of this position involves the generation of an electrophilic centre by reaction with N-bromosuccinimide. In the subsequent reaction, mercapto or amino group containing alkyl linkers[180,181] easily replaces 8-bromo group. In an attempt to attach a label at C-8 position of deoxyadenosine, investigators[180] have introduced an aminoethyl linker by treatment of 8-bromodeoxyadenosine with protected aminoethylmercaptide. Finally, biotin is attached to the amino linker. Fig. 10 shows some of the labels that have been introduced at C-8 position in purines.

Internucleotide backbone modifications

Modified internucleotide phosphate residue :

Phosphodiester backbone modifications have shown enhanced stability, affinity and cellular permeation of oligonucleotides. For these reasons, phosphate backbone modifications seem to be the most logical choice for the modification of oligonucleotides. Additionally, several chemical
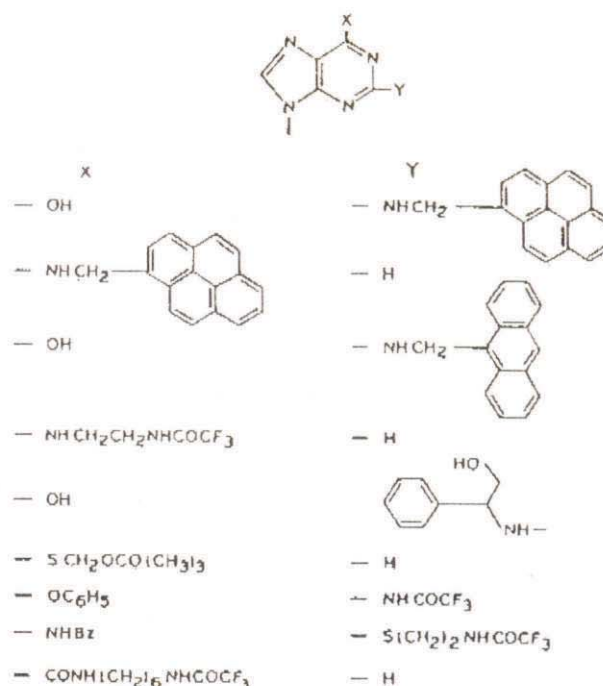
strategies have been employed to replace the extra phosphodiester backbone with different ionic and non-ionic linkages. Most of them have been utilized in the production of dimers rather than fully substituted oligonucleotides. In this manner, they can be introduced during automated DNA synthesis creating an oligonucleotide with alternating phosphodiester and novel linkages. Tremendous advancements in the chemistry have made it possible to produce smaller oligonucleotides fully substituted with novel linkages for various applications. This class of analogs has been most extensively studied. In all, fifteen classes of different
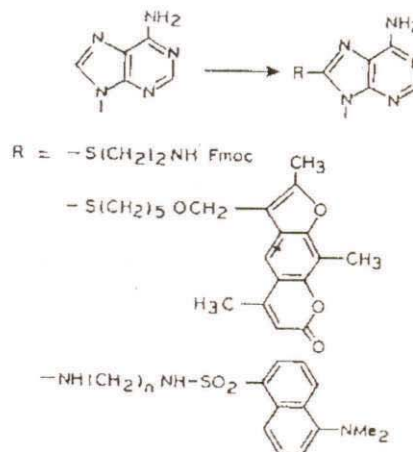

Fig. 10. Modification at C-8 position in purines.

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compounds can be synthesized by substituting each of the four groups attached to phosphorus in the internucleotide bond by O, CH$_3$, NH and S in various combinations (Fig. 11). Furthermore, the use of this approach permits the introduction of reactive sites, suitable for the attachment of different types of ligands, e.g. intercalators, chain cleavers, fluorescent markers, without resort to any extra step in the
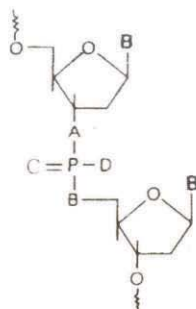
A = O,	B = O,	C = O,	D = O	: Phosphodiester
A = S,	B = O,	C = O,	D = O	: 3'-S-Phosphorothioate
A = O,	B = S,	C = O,	D = O	: 5'-S-Phosphorothioate
A = O,	B = O,	C = S,	D = O ⌉	
A = O,	B = O,	C = O,	D = S	: Phosphorothioate
A = CH$_2$	B = O,	C = O,	D = O	: 3'-Methylphosphonate
A = O,	B = CH$_2$,	C = O,	D = O	: 5'-Methylphosphonate
A = O,	B = O,	C = O,	D = CH$_3$	: Methylphosphonate
A = S,	B = O,	C = S,	D = O	: 3'-S-Phosphorodithioate
A = O,	B = S,	C = S,	D = O	: 5'-S-Phosphorodithioate
A = O,	B = O,	C = S,	D = S	: Phosphorodithioate
A = NH,	B = O,	C = O,	D = O	: 3'-S-Phosphoramidate
A = O,	B = NH,	C = O,	D = O	: 5'-S-Phosphoramidate
A = O,	B = O,	C = O,	D = NH	: Phosphoramidate
A = O,	B = O,	C = S,	D = NH	: Phosphorothioamidate
A = O,	B = O,	C = O,	D = CH$_3$	: Methylphosphonothioate

Fig. 11. Modification at internucleotide linkage.

synthesis cycle. Since most phosphate backbone modifications have been brought about through P–N and P–S linkages, these linkages are discussed further.

Phosphorothioates : Phosphorothioate-oligonucleotides[205–221] have been attractive due to their availability through automated DNA synthesis (Fig. 12). These consist of a sulfur substituted for oxygen at phosphorus, thus retains a formal negative charge, however, less as compared to the phosphodiester homolog which accounts for their somewhat low solubility in water. Phosphorothioate-oligonucleotides are stable to degradation by nucleases but in general hybridize to target sequences with somewhat less affinity as compared to phosphodiester-oligonucleotides at most of the ionic strengths. Besides, the main advantage offered by this linkage is that a suitable label can be attached at pre-selected positions using thiol group specific reagents such as haloacetamides, aziridinylsulfonamide and
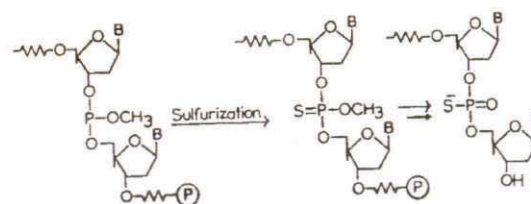

Fig. 12. Synthesis of oligonucleotide-phosphorothioates.

other fluorescent compounds. The hybridization instability has been ascribed to the fact that, since the linkage is chiral, these modified oligonucleotides exist as a mixture of 2^n diastereoisomers (where *n* is the number of linkages), which has been shown that all R_p oligonucleotides hybridize with their targets more strongly (i.e. high T_m) whereas all S_p oligonucleotides hybridize very poorly. The second reason of this instability is their reduced solubility in water.

The chemisty of phosphorothioate-oligonucleotides has been developed and extensively used for various studies. Phosphorothioate-oligonucleotides can essentially be prepared chemically in a manner very similar to the synthesis of unmodified oligonuleotides via phosphotriester, phosphoramidite and H-phosphonate methods. Elemental sulfur in triethylamine/pyridine or carbondisulfide/triethylamine is used in the stepwise oxidation of phosphite triesters in place of aqueous iodine or in a single step at the end of synthesis in H-phosphonate approach. The salient feature of the method of preparation is its adaptability to the normal synthesis cycle, which also allows that phosphorothioate bridges can be incorporated at the designated sites in the oligonucleotides. Some new sulfurization reagents[216,222,223] that are soluble in acetonitrile, the most commonly employed solvent in automated DNA synthesizers, have been developed. Rao *et al.*[224] have recommended the use of the easily accessible, fast and efficient sulfur transfer reagent, dibenzoyl tetrasulfide, from phosphoramidite building blocks. The introduction of these reagents not only dispenses the use of toxic and noxious carbon disulphide but also alleviates the line chocking problem in DNA synthesizers, a common phenomenon associated with the use of colloidal sulfur.

Recently, two groups[210,222] have reported simultaneous introduction of two types of modifications in a single oligonucleotide chain. Oligonucleotide synthesis commenced with the incorporation of a nucleoside-H-phosphonate at 3'-end followed by oxidation with protected hexanediamine in carbon tetrachloride to generate a phosphoramidate linkage. Synthesis was completed using normal nucleoside phosphoramidites approach. The last oxidation step was accomplished with a sulfurization reagent, 3H-1,2-benzo-

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dithiol-3-one-1,1-dioxide, to generate a phosphorothioate linkage which, after final deprotection, was reacted with monobromobimane and the aminoalkyl function was reacted with fluorescein isothiocyanate to yield bifunctionalized oligonucleotide chain that may be used for various applications. Fig. 13 shows some of the ligands that have been introduced through phosphorothioate backbone modifications.

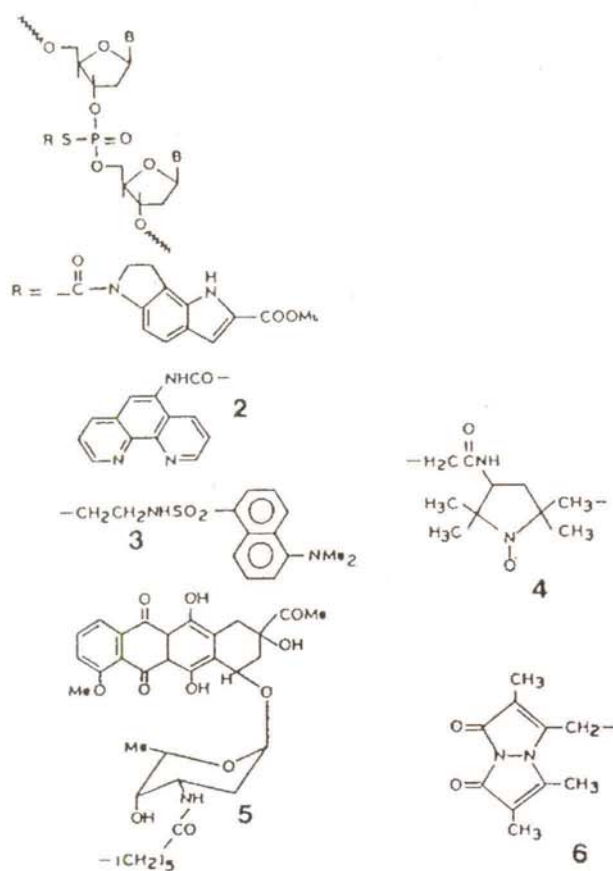*Phosphorodithioates* : Unlike other phosphate backbone modified oligonucleotides, the chirality problems in phosphorodithioate-oligonucleotides[225–237] can be overcome by replacement of both the non-bridged oxygens by sulfur atoms (Fig. 14). The first attempt to synthesize these analogs was based on the condensation of phosphorothioamidite with another nucleoside followed by oxidation of the intermediate triester with sulfur in pyridine. Several alternative approaches have also been used for this purpose. Recently, these methods have been summarized by Marshall and Caruthers[233] and also their potential uses discussed. The phosphorodithioate linkage has found several biochemical properties similar to the unmodified phosphodiester linkage. The linkage is highly stable towards nucleases, achiral and oligonucleotides bind with slightly less affinity than phosphorothioate-oligonucleotides with their targets. The linkage can be used for incorporation of reporter groups useful for making non-radiolabelled probes for a variety of biological applications. Phosphorodithioates can be labeled with higher efficiency and at a much faster rates in comparison to their phosphorothioate analogs. Because of high selectivity and specificity of sulfhydryl group, a variety of thiol specific reporter groups have been conjugated under very mild conditions even at physiological conditions. However, due to poor efficiency of the sulphurization step, the phophorodithioates are usually contaminated with about 10% of the monothiolated analogs. Some of the ligands used for conjugation have been shown in Fig. 15.

Phosphoramidates : The phosphoramidate-oligonucleotides[238–249] are conveniently prepared from both tri- and pentavalent phosphorus compounds (Fig. 16). Mixtures of diastereomers are normally obtained in both of these cases. The most suitable method for obtaining dinucleoside phosphoramidates involves the oxidation of dinucleoside-H-phosphonate in the presence of amines in carbon tetrachloride or iodine as oxidatants. The reaction can easily be extended to polymer support bound di- and oligonucleotide H-phosphonates. The diastereomeric phosphoramidate-oligonucleotides are inert towards nuclease P1 or snake venom phosphodiesterase. The use of the changed oxidation conditions permits the introduction of labels/haptens directly

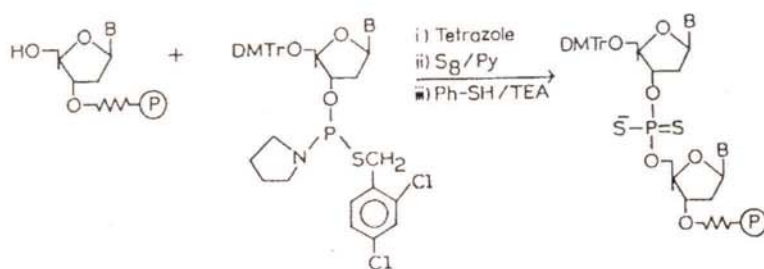Fig. 13. Attachment of ligands at phosphorothioate linkage.

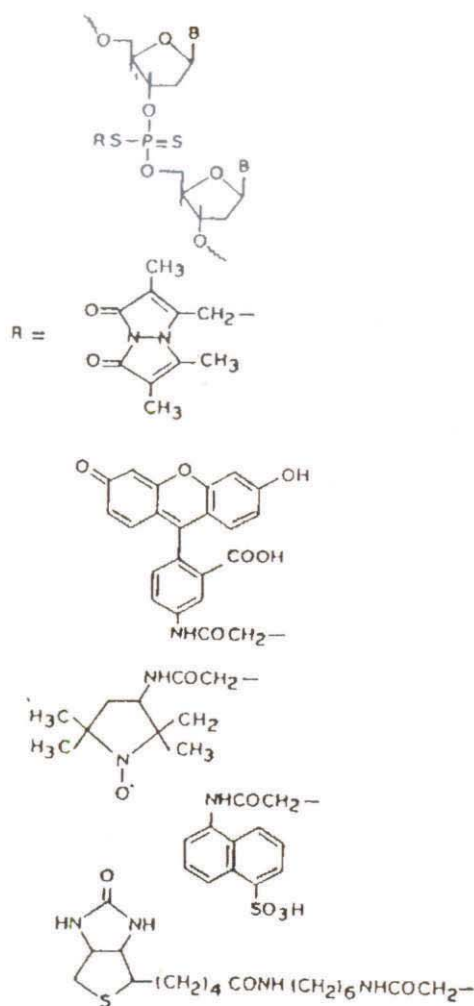Fig. 14. Synthesis of oligonucleotide-phosphorodithioates.

Fig. 15. Attachment of ligands at dithioate linkage.

at designated sites which allow in making oligonucleotide conjugates with intercalating agents. Moreover, a combination of phosphoramidite and H-phosphonate chemistries can also be used to introduce reporter or functional groups at the desired positions in oligonucleotides.

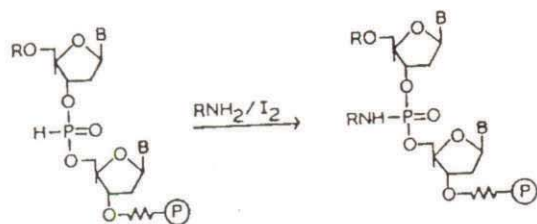The synthesis of an oligonucleotide incorporating a phenanthredinium derivative at P–N linkage has been re-

Fig. 16. Synthesis of oligonucleotide-phosphoramidates.

ported[244]. In an analogous approach, aminoalkylated phosphoramidate[241] was obtained after performing the oxidation of dinucleoside phosphite triester with 1,5-diaminopentane in presence of iodine, which was further used in conjugation reaction with acridine derivative. The method has been found to be very useful for incorporation of multiple reporter groups without affecting duplex stability. In a similar manner, other investigators[247,249] reported the introduction of either (*N*-trifluoroacetyl)hexanediamine linkers at specific sites along the oligonucleotide chains or ethylenediamine or hexamethylendiamine tethers at the 5′-end of oligonucleotides. After final deblocking, these aminoalkylated oligonucleotides were used to couple ligands such as fluorescein isothiocyanate, rhodamine isothiocyanate or activated esters of biotin. The methodology provides
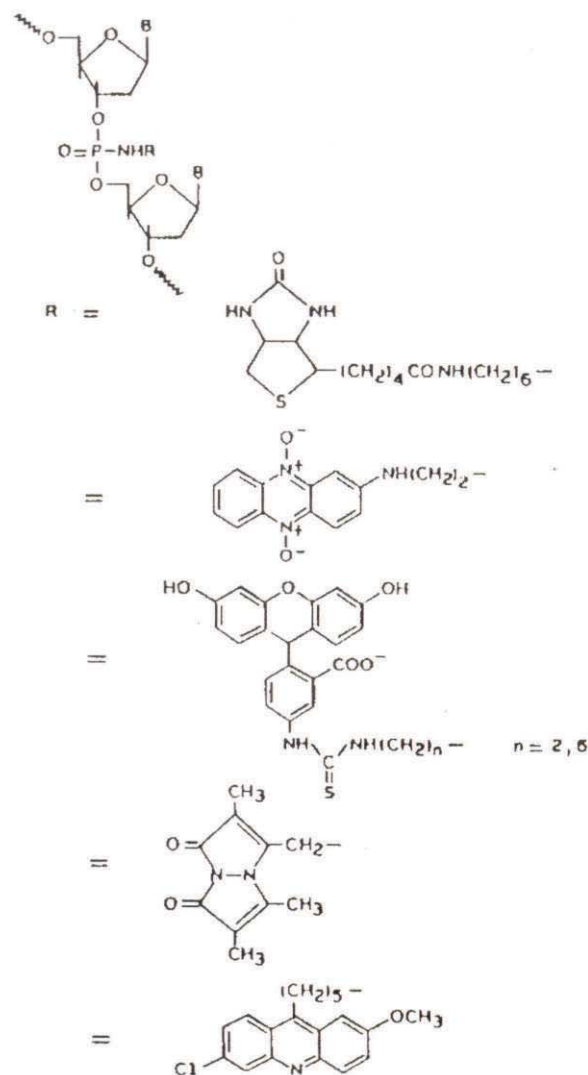
Fig. 17. Attachment of ligands at amidate linkage.

multiple labeling capabilities and thereby increased sensitivity for diagnostic purposes.

Some of the strategies used for the introduction of various ligands through amino tethers using phosphoramidate linkage are shown in Fig. 17.

Methylphosphonates : Like phosphorothioates, the methylphosphonate-oligonucleotides[250-261] are obtained in diastereomers. Being uncharged, oligonucleotides are less water-soluble than their charged counterparts. It has been shown that the linkage is very stable with respect to cellular nucleases and can enter cells by passive diffusion mechanism. The charged non-bridging oxygens are replaced by neutral methyl groups to obtain methylphosphonate-oligonucleotides by which the cell uptake as well as improved half-life is achieved. Like thioates, methylphosphonate-oligonucleotides can form stable duplexes at physiological ionic strength or above but with slightly less affinity as compared to unmodified oligonucleotides. These modified analogs can be obtained from both phosphotriester and phosphoramidite methods. However, the method of choice utilizes methyl-phosphonoamidites on solid supports (Fig. 18). The main advantage of the method is its adaptability to the conventional synthesis cycle and its ability to introduce one or multiple linkages at any preselected position. Recently, several attempts have been made for stereoselective synthesis of methylphosphonate-oligonucleotides. Becuase of their lability under basic medium required for removal of protecting groups from nucleic bases and cleavage of oligonucleotides from the polymer support, several alternative deprotection conditions have been established.
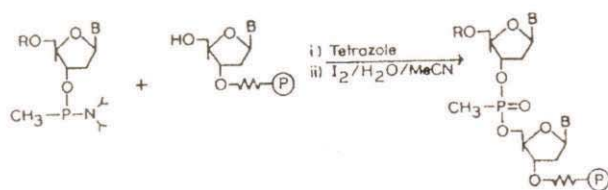

Fig. 18. Synthesis of oligonucleotide-methylphosphonates.

Alkyl phosphotriesters : Oligonucleotide-phosphotriesters, like methylphosphonates, lack the usual negative charge on the oxygen and pose chirality probem. However, these[262-267] can be obtained via several routes currently being used for synthesis of oligonucleotides, viz. phosphotriester, phophoramidite and H-phosphonate approaches (Fig. 19). Because of the instability of these analogs under basic conditions, not much work has been done to produce them stereoselectively.

Replacement of phosphate oxygens involved in the bridge : To circumvent the chirality problems associated

with methylphosphonates, phosphorothioates, amidates and triesters, new achiral linkages have been introduced, viz. 3'-NH$_2$-5'-O-phosphoramidate[268-270], 3'-O-5'-S-phosphorothioate[271], 3'-S-5'-O-phosphorothioate[272] and 3'-CH$_2$-5'-O-phosphonate[273] etc. Several routes have been developed to obtain the desired modification. The analogs so obtained
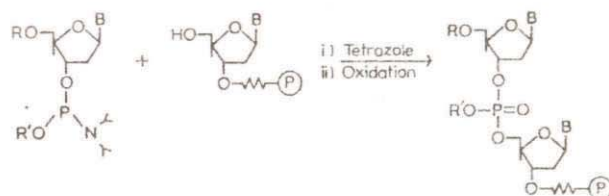

Fig. 19. Synthesis of oligonucleotide-phosphotriesters.

have been shown to possess variable resistance against nucleases but little is known of their hybridization properties.

Dephospho internucleotide analogs :

An ideal way to obtain non-ionic oligonucleotides is to replace ionic phosphodiester linkages by some other different group. Recently, this area has picked up momentum to develop oligonucleotide analogs devoid of phosphodiester or related linkages. They have been developed in order to possess the following advantages of (a) being stable against nucleases, (b) showing enhanced cell-uptake, and (c) having enhanced binding affinity (hybridization) with complementary sequences due to reduced charge repulsion. Most of them have been developed without affecting the natural 3'-5' distance as well as orientation of bases for effective hybridization. Another advantage of dephospho-linkages is that the internucleoside bridges are electroneutral and they do not pose chirality problem.

Siloxane bridges : The internucleoside linkage in silyl-oligomers quite closely resembles the phosphate bridges in the sense that in both the cases, the central atoms have tetrahedral symmetry. Initially, the synthesis was confined to dimers in solution which involved the reaction of a 5'-protected nucleoside with dichlorodiphenyl- or dichlorodicyclohexylsilane followed by reaction with 3'-protected nucleoside to obtain 3'-5'-dimer in 50% yield[274-276] (Fig. 20). In order to improve the yields of the dimers and extension of chains upto hexamers, various modifications in the protocol were suggested. However, low water solubility and instability under acidic conditions have restricted their effective use for biological applications. Because of this low solubility, efforts to detect hybridization properties were unsuccessful.

Carbonate bridges : The first dinucleotide with a carbonate linkage was reported almost 29 years ago by Mertes
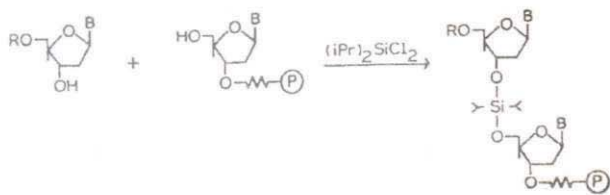
Fig. 20. Preparation of siloxane-bridged dimer.

and Coates[277] and Tittensor[278,279] using phosgene for making this linkage. Oligonucleotides with carbonate linkages have been described upto trimers using (trichloroethoxy)carbonyl chloride as an improved condensing reagent (Fig. 21). However, these were obtained in only 50% yields because inadequately protected nucleosides were employed as this linkage was found to be labile in basic conditions. Hence, special conditions are required for the removal of protecting groups at the end of the synthesis. Such base lability limits the utility of these analogs in a physiological context. Since, upto trimers have been constructed, no hybridization studies have been reported.
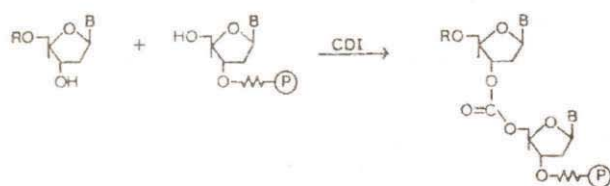

Fig. 21. Preparation of carbonate-bridged dimer.

Carboxymethylester bridges : The utility of this linkage[280–285] was first demonstrated by Halford and Jones by synthesizing several polynucleotides (poly T and poly U) by polymerization reaction. Subsequently, improvements were made in the protocol and synthesis of oligonucleotides was carried out by stepwise coupling reactions using suitable protecting groups (Fig. 22). The oligonucleotides with carboxymethylester linkages were found to hybridize with their complementary sequences as well as with natural oligonucleotides with low affinity. In spite of these properties, they were not found suitable for biological applications because of their poor solubility in water and ready hydrolysis even under mild conditions (half-life at pH 7.5 is 7 h).
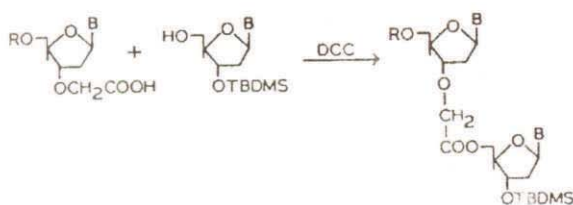

Fig. 22. Preparation of carboxymethylester-bridged oligomer.

Acetamidate bridges : In order to overcome the stability problem of carboxymethylester linked oligonucleotides[286,287], the 5'-ester oxygen was replaced by an amino group and so the acetamidate linkage was obtained (Fig. 23). Polycondensation was achieved using triphenylphosphine and 2,2'-dipyridyl disulfide employing dimer with acetamidate linkage. Condensation with monomer was not successful due to intramolecular reaction, which yielded 3',5'-bridged lactam molecule. Although the stability was achieved over a wide range of pH, the solubility in water and strong affinity (hybridization) could not be obtained. Moreover, another limitation was encountered, that the said oligomers were able to get adsorbed strongly on to glass and plastic surfaces.
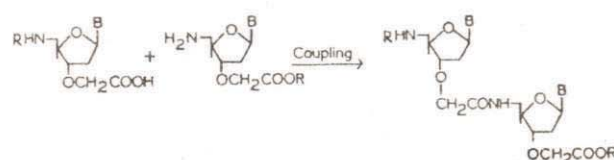

Fig. 23. Preparation of acetamidate-linked oligomer.

Carbamate bridges : In order to improve the properties of acetamidate linked oligonucleotides, carbamate linkage[288–295] was introduced which proved somewhat superior in stability over a greater pH range, resistance against nucleases but found to have low solubility in water. A number of protocols have been established to obtain carbamate linked dimers, trimers and hexamers in higher yields using a variety of condensing reagents to couple 5'-amino-5'-deoxynucleoside with 5'-O-protected nucleoside (Fig. 24). Carbamate-linked oligonucleotides, in some cases, have shown greater affinity towards their complementary sequences as compared to natural oligomers, while in some, they have not entered into base pairing at all. In order to improve the solubility in water, one group has reported the attachment of polyethyleneglycol (PEG) which enhances the solubility of the conjugate upto 50 μM at pH 7.5.
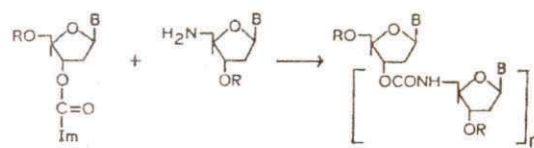

Fig. 24. Preparation of carbamate-linked dimer.

In another variation of carbamate linkage, ribonucleotide-derived morpholino-type oligonucleotides have been synthesized[293,294] in which both phosphodiester linkage as well as sugar moieties are modified. These analogs have shown

interesting binding properties. They bind to DNA much stronger than their unmodified molecules but show no affinity towards RNA.

Thioether bridges : Kawai and Just[296] were the first to report the synthesis of thioether-bridged oligodeoxynucleotides[296–302] (Fig. 25). Subsequently, Schneider and Benner[297] and Cao and Matteucci[301] reported the synthesis of different sulfide-linked dinucleotides. The linkage was differed only in the position of the sulfur atom within two nucleosides. The linkage was found to be neutral, nuclease resistance but the duplexes lacked in thermal stability. Therefore, not much work has been carried out using this linkage.
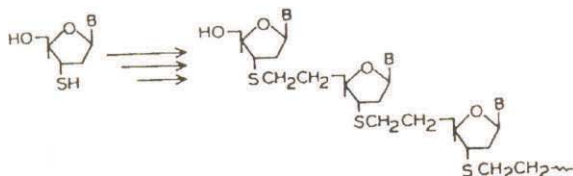

Fig. 25. Preparation of thioether-bridged oligomer.

Sulfone, sulfonate, sulfonamide, sulfate, sulfite and sulfamate bridges : Sulfur, at the central position of the backnone in place of phosphorus, is an isosteric replacement, which offers a variety of options for modified isosteric linkages[303–312] (Fig. 26). Sulfate and sulfite linkages, being labile to nucleophiles, have not found useful in making modified oligonucleotides. Other modifications that include sulfone, sulfonate ester, sulfonamide and sulfamate bridges are usually stable to nucleases but their affinity towards base pairing has not yet been reported in detail, however, these linkages are achiral, polar and neutral at physiological pH. The synthesis was not compatible to the solid phase methodology. The solution phase synthesis of dinucleosides containing these linkages is described using either Michaelis-Arbusov reaction or phosphotriester approach[311]. Widlanski et al.[303–306] and Reynolds et al.[307–309] described the synthesis of both sulfonate and sulfonamide linked nucleosides. Because of the low affinity, T_m value decreases by 1–3° per modification.
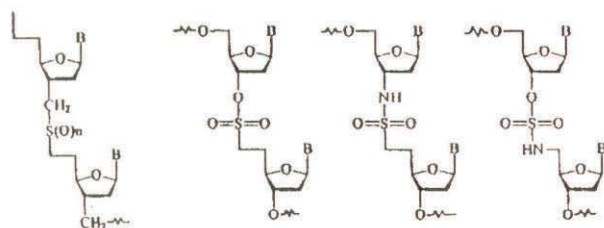

Fig. 26. Preparation of sulfone-, sulfonate-, sulfonamide- and sulfamate-bridged dimers.

The other achiral linkage, sulfamate-linkage, has been described by Huie et al.[310]. They have successfully synthesized a guanosine-adenosine dimer connected by a sulfamate-linkage. The incorporation of this dimer slightly decreased the stability of the duplex. Enzymatic resistance was, however, achieved by incorporating the sulfamate-linkage.

Acetal bridges : The synthesis and hybridization properties of oligonucleotides containing formacetal-linked nucleosides have been reported by both Matteucci et al.[313–317] and Veeneman et al.[318,319] (Fig. 27). The synthesis of dimers was achieved by alkylation of 3′-hydroxyl of a 5′-protected nucleoside with chlorodimethylsulfide. The resulting 3′-methylthioacetal derivative is then coupled with a 3′-protected nucleoside to give the dimer. the use of 5′-deoxy-5′-thionucleoside instead of 3′-protected nucleoside in the coupling reaction yields 5-thioformacetal-linked dimers. Similarly, 3-thioformacetal-linked dimers can be prepared by coupling 5′-O-chloromethylnucleosides with protected 3′-thionucleosides. It has been demonstrated that oligonucleotides containing formacetal-linked dimers hybridize with somewhat lower affinity as compared to thioformacetal-linked dimers containing oligonucleotides with complementary natural RNA and DNA[320–322].

In an alternative approach, Jones et al.[316,317] reported the synthesis of riboacetal-linked dimer, in which aldehyde group introduced at 5′-position was coupled, under acid
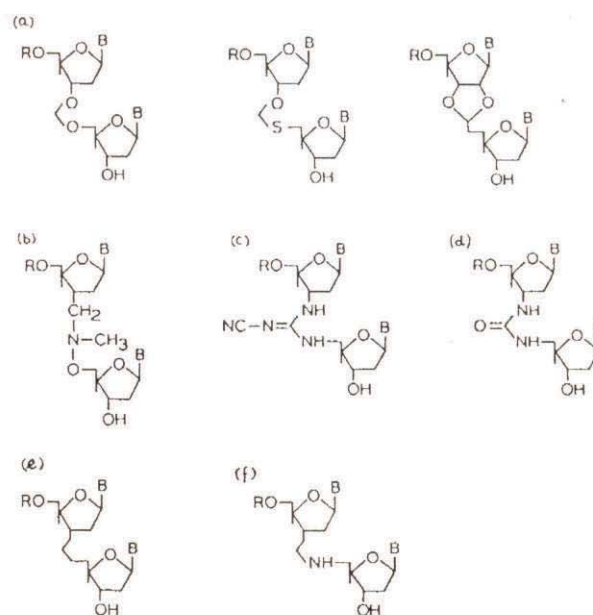

Fig. 27. Preparation of (a) formacetal-, thioformacetal- and riboacetal-bridged dimers, (b) methylhydroxylamine-bridged dimer, (c) N-cyanoguanidine-linked dimer, (d) urea-linked dimer, (e) alkane-linked dimer, and (f) amine-bridged dimer.

catalysis, to 2′,3′-hydroxyl groups of the 5′-protected ribonucleoside. The synthesized dimer was then incorporated into oligonucleotides, but showed some decrease in thermal stability against single stranded RNA. However, a 15-mer sequence showed 100-fold increase in affinity for a double stranded DNA target.

Methylhydroxylamine bridges : The first report citing the synthesis of oligonucleotides with methylhydroxylamine-linked thymidine dimers was published by Vasseur *et al.*[323–325] (Fig. 27). The linkage was neutral polar and resistant against nucleases but showed no or little effect in the hybridization studies.

N-*Cyanoguanidine bridges :* N-Cyanoguanidine-linked oligonucleotides (Fig. 27) were proposed by Pannecouque *et al.*[326–331]. These analogs have shown improved nuclease stability but paired less strongly than their unmodified complementary DNA and RNA. However, these analogs have strong affinity towards their modified complementary DNA and lower towards RNA.

Urea bridges : Like carbamate-linked oligonucleotides, urea-linked thymidine dimers, introduced by Waldner *et al.*[332], imparted similar type of properties to the oligonucleotides (Fig. 27). The said modification[332,333] imparted less destabilizing effect in DNA/DNA than in DNA/RNA duplexes. Dimers were prepared by condensing 3′-carbamato-3′-deoxythymidine to 5′-amino-5′-deoxythymidine followed by conversion into phosphoramidite.

Alkane bridges : Alkane-linked dimers were reported by Lee and Weimer[334] based on Wittig type of reaction (Fig. 27). Only thymidine dimers were incorporated into oligonucleotides, which led to the severe reduction in the affinity, i.e. thermal stability of DNA/DNA and DNA/RNA duplexes[334–336].

Amine bridges : Introduced by Saha *et al.*[337,338], the dimers with amine-linkage were prepared by coupling a 3′-amino-3′-deoxy- or 5′-amino-5′-deoxynucleoside with the corresponding alkyl extended aldehydes and reduction of the intermediate imines (Fig. 27). The incorporation led to the significant reduction in the duplex stability whereas nuclease resistance was increased.

Miscelleneous modifications

The use of carbocyclic nucleoside analogs was first reported by Perbost *et al.*[339] and Szemzo *et al.*[340,341]. The oligomers containing these carbocyclic nucleosides (thymidylates) are fairly stable against nuclease degradation. They can even form stable duplexes or triplexes depending upon the ratio of the two individual components. The duplexes have more stability than their unmodified coun-

terparts. In the last few years, a number of reports on their syntheses and incorporation into synthetic oligonucleotides have been published. The routes to prepare these monomeric units are very time-consuming and laborious with very low overall yields[339–345].

In another approach, Bellone *et al.*[346,347] have described the introduction of 4′-thiothymidine into thymidine oligomers by substituting the 4′-oxygen in an attempt to restore hybridization properties and to enhance the nuclease resistance (Fig. 28). However, there is a decrease (3–5°) in the thermal stability as compared to normal oligonucleotides[346–353]
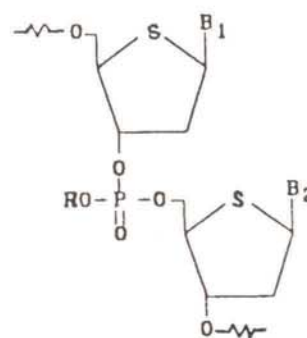
Fig. 28. Preparation of 4′-thiothymidine oligomer.

On the parallel lines, Altmann *et al.*[354] and Hung *et al.*[355] have described the synthesis of N-acetyl-4′-azathymidine (4-hydroxyproline derivative) (Fig. 29). The incorporation into oligoribonucleotides[356,357] has shown increased resistance against nucleases, however, their thermal stability decreased by 0.5–1.5° per modification depending upon the site of incorporation.
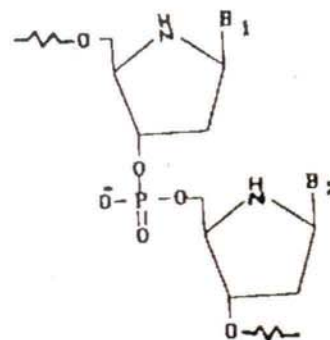
Fig. 29. Preparation of 4′-azathymidine oligomer.

Similarly, in search for some suitable antisense molecules that could obviate the disadvantages of the unmodified oligonucleotides, several groups[358–365] have proposed the syn-

thesis of acyclic nucleoside derivatives, which can be used for oligomer synthesis or incorporation into unmodified oligomers. The first report using glycerol moiety was discussed by Usman et al.[358]. The proposed modification dramatically decreases the affinity towards complementary target. However, the oligomers synthesized using modified building blocks were found 500-times more stable against snake venom phosphodiesterase than the normal oligonucleotides. Other investigators have also reported various types of acyclic analogs but such products were found to have less affinity towards duplex formation, which has made their use as antisense constructs limited. In the same way, various groups[366-368] have also reported the use of serine and phenylserine type of acyclic moieties for the attachment of nucleobases (Fig. 30). After preparation of phosphoramidites, synthesis was performed in an analogous manner as the normal synthesis.
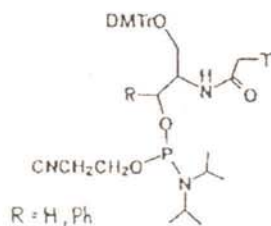

Fig. 30. Serine and phenylserine-linked thymine monomers for oligomerization.

In another elegant approach ring-enlarged hexopyranose nucleosides[369-371] and their oligomers have been proposed by Eschenmoser and Dobler[359]. In this series, the 4'-6'-linkage (homo-DNA) has been the most intensely studied one. The proposed modification enhances the stability of oligomers against nucleases, however, the oligomers form duplexes with less thermal stability.

Very recently, Barawkar and Bruice[372] have reported the incorporation of guanidinium linked dimers (cationic internucleosidic linkage) into oligonucleotides (Fig. 31). With two positive charges at the terminal, oligomers show enhanced binding with complementary DNA and RNA in the absence of salt. In 10 mM sodium chloride, binding is similar to normal DNA : DNA. The 5'- and 3'-capping with this type of linkage provides better protection to hydrolysis by exonuclease S1. Moreover, due to lower net charge on the oligomer chains, the cellular uptake increases.

Peptide nucleic acids (PNA)

Recently, a new class of analogs of oligonucleotides has been reported by replacing the sugar phosphate backbone in DNA by an amide backbone[373-384] (based on amino ac-
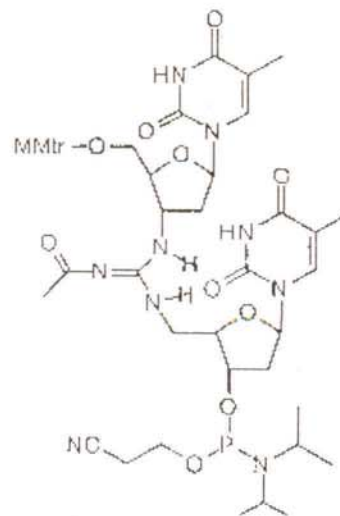
Fig. 31. Monomeric unit for the incorporation of cationic internucleosidic linkage.

ids) without compromising the base-pairing efficiency and selectivity (Fig. 32). The most remarkable feature of PNA is the simplicity of its repeat unit, i.e. nucleobase containing unit, which is based on 2-aminoethylglycine. It consists of an achiral, acyclic non-ionic, dipeptide-like unit prepared from 1,2-diaminoethane and glycine. The nucleobases are attached to the central nitrogen by an amide bond. The synthesis of PNA is based on the method of peptide synthesis via Merrifield method. The monomeric units can be polymerized easily using coupling reagents commonly employed in peptide synthesis. Several reports have described the oligomerization of these molecules[385-389].
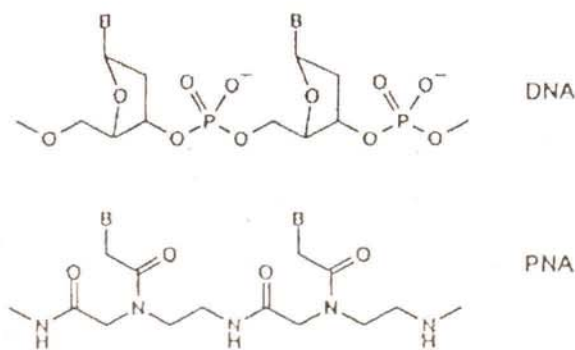
Fig. 32. Structure of DNA and PNA.

Unlike oligonucleotides, PNA oligomers are quite stable under strong acidic conditions (e.g. HF). In addition to their resistance against nucleases and a number of proteases and peptidases[390] in cellular and serum extracts, they have shown much higher affinity as compared to any other analogs and

unmodified oligonucleotides. They bind to DNA by strand displacement mechanism[391–393]. The properties of these molecules have made them ideal for antisense and antigene research as inhibitors of gene expression. Other applications where these molecules look promising are their use as diagnostic and biomolecular probes. PNAs have been found to form very stable Watson-Crick duplexes with DNA and RNA. This affinity is much higher as compared to DNA for DNA or RNA. This enhanced affinity could be due to the absence of repulsion between charged backbones in DNA. The structure of PNA is so flexible that it can form duplexes in parallel or antiparallel direction. Despite all these advantages, the major problem encountered in handling these molecules is their solubility in aqueous medium. To overcome this hurdle, several modifications[394–403] have been tried. These include incorporation of side-chain (basic or acidic) containing amino acids and charged groups (sulfate, phosphate, carboxylate, porphyrin (404) etc.). The most recent trend in this direction is to make PNA-DNA hybrid chains[394] where solubility problem can be taken care of by the charged DNA chain, and lipophilicity to cross cell membrane (for antisense applications) can be taken care of by polyamide PNA chain. Several reports have appeared with different modifications in PNA-DNA structure.

Concluding remarks

Research on modified oligonucleotides is exponentially increasing with ever increasing speed with a view to find newer molecules for understanding, unveiling and utilizing the intricate methods of cell functioning at molecular level. The speed of synthesis has been greatly increased by designing various support system[42]. The investigations encompassing end modifications, introduction of new functionalization at certain sites of the sugar, base modifications or introduction of modified internucleotide residues of phosphorus as well as other novel modifications as described in the text have resulted in enriching the chemistry of handling complex molecules in a wide range of conditions. The new class of peptide nucleic acid analogs replacing sugar phosphate backbone with aminoethylglycine have promises of more effective utilization as inhibitors of gene expexression. Such knowledge and developments would facilitate and broaden our abilities to applications in genetic engineering, gene therapy and cellular regulation in an array of conditions, many of which are yet not well understood and would be unveiled in future.

References

1. J. Goodchild, *Bioconj. Chem.*, 1990, **1**, 165.

2. U. Englisch and D. H. Gauss, *Angew. Chem., Int. Ed. Engl.*, 1991, **90**, 613.

3. R. S. Varma, *Synlett*, 1993, 621.

4. M. H. Caruthers, *Acc. Chem. Res.*, 1991, **24**, 278.

5. P. S. Miller in "Oligonucleotides : Antisense Inhibitors of Gene Expression", ed. J. S. Cohen, CRC Press, Boca Raton, 1989.

6. E. Uhlmann and A. Peyman, *Chem. Rev.*, 1990, **90**, 543.

7. S. Agrawal and Q. Zhao, *Curr. Opinion Chem. Biol.*, 1998, **2**, 519.

8. N. Kashihara, Y. Maeshima and H. Makino, *Exp. Nephrol.*, 1998, **6**, 84.

9. P. D. Cook, *Anti-cancer Drug Design*, 1991, **6**, 585.

10. C. K. Mirabelli, C. F. Bennette, K. Anderson and S. T. Crooke, *Anti-cancer Drug Design*, 1991, **6**, 647.

11. L. J. Maher, D. Wold and P. B. Dervan, *Science*, 1989, **245**, 725.

12. C. Helene, *Anti-cancer Drug Design*, 1991, **6**, 569.

13. J. W. Nyce, *Expert Opinion Invest. Drugs*, 1997, **6**, 1149.

14. A. Chonn and P. R. Cullis, *Curr. Opinion Biotechnol.*, 1995, **6**, 698.

15. H. H. Engelhard, M. Egli and J. M. Rozental, *Ped. Neurosurgery*, 1998, **28**, 279.

16. H. M. Weintraub, *Sci. Am.*, 1990, **262**, 40.

17. C. F. Bennette, M. Y. Chiang, H. C. Chan, J. E. E. Shoemaker and C. K. Mirabelli, *Mol. Pharm.*, 1992, **41**, 1023.

18. R. W. Wagner, M. D. Matteucci, J. L. Lewis, A. J. Gutierrez, C. Moulds and B. C. Froehler, *Science*, 1993, **260**, 1560.

19. J. F. Milligen, M. D. Matteucci and J. C. Martin, *J. Med. Chem.*, 1993, **36**, 1923.

20. C. Epple and C. Leumann, *Chem. Biol.*, 1998, **5**, 209.

21. A. Balland, M. Courtney, S. Jallat, L. H. Tessier, P. Sondermeye, H. de la Salle, R. Harvey, E. Degryse and P. Tolstoshev, *Biochimie*, 1985, **67**, 725.

22. R. B. Wallace, A. B. Studenski and A. Murasugi, *Biochimie*, 1985, **67**, 755.

23. R. A. Cardullo, S. Agrawal, C. Flores, P. C. Zamecnik and D. E. Wolf, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 8790.

24. B. A. Connolly, B. V. L. Potter, F. Eckstein, A. Pingoud and L. Grotjahn, *Biochemistry*, 1984, **23**, 3443.

25. D. S. Sigman, A. Mazumdar and D. M. Perrin, *Chem. Rev.*, 1993, **93**, 2295.

26. U. Landegren, R. Kaiser, C. T. Caskey and L. Hood, *Science*, 1988, **242**, 229.

27. W. Ansorge, B. Sproat, J. Stegemann, C. Schwager and M. Zenke, *Nucl. Acids Res.*, 1987, **15**, 4593.

28. J. A. Brumbaugh, L. R. Middendorf, D. L. Grone and J. L. Ruth, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 5610.

29. B. S. Beck and F. M. Pohl, *EMBO J.*, 1984, **3**, 2905.

30. S. Ferraboli, R. Negri, E. D. Maauro and S. Barlati, *Anal. Biochem.*, 1993, **214**, 566.

31. S. L. Loke, C. A. Stein, X. H. Zhang, K. Mori, M. Nakanishi, C. Subasinghe, J. S. Cohen and L. M. Neckers, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 3474.

125

32. A. I. Lamond, B. S. Sproat, U. Rider and J. Hamm, *Cell*, 1989, **58**, 383.

33. J. S. Cohen in Ref. 5.

34. S. T. Crooke, *Ann. Rev. Pharmacol. Toxicol.*, 1992, **32**, 329.

35. J. A. H. Murray in "Antisense RNA and DNA", Willey-Liss, New York, 1992.

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

37. P. C. Zamecnik and M. L. Stephenson, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 280.

38. W. T. Markiewicz and T. K. Wyrzykiewicz, *Nucl. Acids Res.*, 1989, **17**, 7149.

39. E. Felder, R. Schwyzer, R. Charubala, W. Pfleiderer and B. Schultz, *Tetrahedron Lett.*, 1984, **25**, 3967.

40. V. A. Efimov, A. A. Buryakova, S. V. Reverdatto, O. G. Chakhmakhcheva and Yu. A. Ovchinnikov, *Nucl. Acids Res.*, 1983, **11**, 8369.

41. P. Kumar, N. K. Bose and K. C. Gupta, *Tetrahedron Lett.*, 1991, **32**, 967.

42. P. K. Ghosh, P. Kumar and K. C. Gupta, *J. Indian Chem. Soc.*, 1998, **75**, 206.

43. U. Asseline and N. T. Thuong, *Tetrahedron Lett.*, 1989, **30**, 2521.

44. U. Asseline, E. Bonfils, R. Kurfurst, M. Chassignol, V. Roig and N. T. Thuongf, *Tetrahedron*, 1992, **48**, 1233.

45. U. Asseline and N. T. Thoung, *Nucleosides Nucleotides*, 1991, **10**, 359.

46. N. T. Thuong and U. Asseline in "Oligonucleotides and Analogues : A Practical Approach", ed. F. Eckstein, IRL Press, Oxford, 1991, pp. 283.

47. S. M. Gryaznov and R. L. Letsinger, *Tetrahedron Lett.*, 1992, **33**, 4127.

48. U. Asseline, *Nucleosides Nucleotides*, 1986, **5**, 45.

49. J. C. Francois, T. Saison-Behmoaras, M. Chassignol, N. T. Thuong, J. S. Sun and C. Helene, *Biochemistry*, 1988, **27**, 2272.

50. U. Asseline and N. T. Thuong, *Tetrahedron Lett.*, 1990, **31**, 81.

51. P. S. Nelson, R. A. Frye and E. Liu, *Nucl. Acids Res.*, 1989, **17**, 7187.

52. P. S. Nelson, *Nucl. Acids Res.*, 1992, **21**, 6253.

53. C. R. Petrie, M. W. Reed, A. D. Adams and R. B. Meyer, Jr., *Bioconj. Chem.*, 1992, **3**, 85.

54. N. D. Sinha and S. Striepeke in Ref. 46.

55. K. C. Gupta, P. Sharma, S. Sathyanarayana and P. Kumar, *Tetrahedron Lett.*, 1990, **31**, 2471.

56. P. Kumar, K. C. Gupta, R. Rosch and H. Seliger, *Chem. Lett.*, 1997, **12**, 1231.

57. L. Perrouault, U. Asseline, C. Rivalle, N. T. Thuong, E. Bisagni, C. Giovannangeli, T. Le Doan and C. Helene, *Nature*, 1990, **344**, 358.

58. J. S. Sun, C. Giovannangeli, J. C. Francois, R. Kurfurst, T. Montenay-Garestier, U. Asseline, T. Saison-Behamoaras, N. T. Thuong and C. Helene, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 6023.

59. M. W. Reed, A. D. Adams, J. S. Nelson and R. B. Meyer, Jr., *Bioconj. Chem.*, 1991, **2**, 217.

60. W. H. Gmeiner, W. Luo, R. T. Pon and J. W. Lown, *BioMed. Chem. Lett.*, 1991, **1**, 487.

61. H. B. Gamper, M. W. Reed, T. Cox, J. S. Virosco, A. D. Adams, A. A. Gall, J. K. Scholler and R. B. Meyer, Jr., *Nucl. Acids Res.*, 1993, **21**, 145.

62. C. Mackellar, D. Graham, D. W. Will, S. Burgess and T. Brown, *Nucl. Acids Res.*, 1992, **20**, 3411.

63. J. Haralambidis, L. Duncan and G. W. Tregear, *Tetrahedron Lett.*, 1987, **28**, 5199.

64. J. Haralambidis, L. Dunkan, K. Agnus and J. W. Tregear, *Nucl. Acids. Res.*, 1990, **18**, 493.

65. J. Haralambidis, K. Agnus, S. Ponwall, L. Duncan, M. Chai and G. W. Tregear, *Nucl. Acids Res.*, 1991, **18**, 501.

66. C. D. Juby, C. D. Richardson and R. Brousseau, *Tetrahedron Lett.*, 1991, **32**, 879.

67. S. Agrawal, C. Christodoulou and M. J. Gait, *Nucl. Acids Res.*, 1986, **14**, 6227.

68. M. Lemaitre, B. Bayard and B. Lebleu, *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 648.

68a. L. J. Kricka in "Nonisotopic DNA Probe Techniques", Academic, New York, 1992.

69. K. Zuckermann, D. Corey and P. Schultz, *Nucl. Acids Res.*, 1987, **15**, 5305.

70. E. Bonfils, C. Depierreux, P. Midoux, N. T. Thuong, M. Monsigny and A. C. Roche, *Nucl. Acids Res.*, 1992, **20**, 4621.

71. E. Bonfils and N. T. Thuong, *Tetrahedron Lett.*, 1991, **32**, 3053.

72. K. C. Gupta, P. Sharma, P. Kumar and S. Sathyanarayana, *Nucl. Acids Res.*, 1991, **19**, 3019.

73. J. C. Trufferrt, O. Lorthioir, U. Asseline, N. T. Thuong and A. Brack, *Tetrahedron Lett.*, 1994, **35**, 2353.

74. P. Kumar, K. C. Gupta, R. Rosch and H. Seliger, *BioMed. Chem. Lett.*, 1996, **6**, 2247.

75. E. Uhlmann and J. Engels, *Tetrahedron Lett.*, 1986, **27**, 1023.

76. W. Bannwarth and A. Trzeciak, *Helv. Chim. Acta*, 1987, **70**, 175.

77. T. Horn and M. S. Urdea, *DNA*, 1986, **5**, 421.

78. B. A. Connolly, *Tetrahedron Lett.*, 1987, **28**, 463.

79. T. Horn and M. S. Urdea, *Tetrahedron Lett.*, 1986, **27**, 4705.

80. W. Uhlmann and J. Engel, *Chem. Scr.*, 1986, **26**, 217.

81. W. Bannwarth and E. Kung, *Tetrahedron Lett.*, 1989, **30**, 4219.

82. L. M. Smith, S. Fung, M. W. Hunkapiller, T. J. Hunkapiller and L. E. Hood, *Nucl. Acids Res.*, 1985, **13**, 2399.

83. B. S. Sproat, B. Beijer and P. Rider, *Nucl. Acids Res.*, 1987, **15**, 6181.

84. B. A. Connolly, *Nucl. Acids Res.*, 1987, **15**, 3131.

85. N. D. Sinha and R. M. Cook, *Nucl. Acids Res.*, 1988, **16**, 2659.

86. J. L. Ruth, C. Morgan and A. Pasko, *DNA*, 1985, **4**, 93.

87. E. Jablonski, E. W. Moomaw, R. H. Tullis and J. L. Ruth, *Nucl. Acids Res.*, 1986, **14**, 6115.

88. J. Haralambidis, M. Chai and G. W. Tregear, *Nucl. Acids Res.*, 1987, **15**, 4857.

89. W. Ansorge, B. S. Sproat, J. Stegemann, C. Schwager and M. Zenke, *Nucl. Acids Res.*, 1987, **15**, 4593.

90. G. B. Dreyer and P. B. Dervan, *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 968.

91. W. Bannwarth, D. Scmidt, R. L. Stallard, C. Hornung, R. Knorr and F. Muller, *Helv. Chim. Acta*, 1988, **71**, 2085.

92. J. M. Coull, H. L. Weith and R. Bischoff, *Tetrahedron Lett.*, 1986, **27**, 3991.

93. R. Bischoff, J. M. Coull and F. E. Regnier, *Anal. Biochem.*, 1987, **164**, 336.

94. F. F. Chehab and Y. W. Kan, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 9178.

95. L. M. Smith, R. J. Kaiser, J. Z. Sanders and L. E. Hood, *Methods Enzymol.*, 1987, **155**, 260.

96. L. M. Smith, J. Z. Sanders, R. J. Kaiser, P. Hughes, C. Dodd, C. R. Connell, C. Heiner, S. B. H. Kent and L. E. Hood, *Nature*, 1986, **21**, 674.

97. M. L. Dawson, A. N. Jina, S. Torkelson, M. Moore, D. A. Zarling and P. D. Hobbs, *Nucl. Acids Res.*, 1990, **18**, 1099.

98. P. S. Nelson, R. Shreman-Gold and R. Leon, *Nucl. Acids Res.*, 1989, **17**, 7179.

99. J. Van Ness, S. Kalbfeisch, C. R. Petrie, M. W. Reed, J. C. Tabone and N. M. J. Vermeulen, *Nucl. Acids Res.*, 1991, **19**, 3345.

100. J. Van Ness and L. Chen, *Nucl. Acids Res.*, 1991, **19**, 5143.

101. M. Chatterjee and S. E. Rokita, *J. Am. Chem. Soc.*, 1990, **112**, 6397.

102. M. Chatterjee and S. E. Rokita, *J. Am. Chem. Soc.*, 1991, **113**, 5116.

103. R. K. Saiki, P. S. Walsh, C. H. Levenson and H. A. Erlich, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 6230.

104. P. C. Emson, H Arai, S. Agrawal, C. Christodoulou and M. J. Gait, *Methods Enzymol.*, 1989, **168**, 753.

105. Y. Shoji, S. Akhtar, A. Periasamy, B. Herman and R. L. Juliano, *Nucl. Acids Res.*, 1991, **19**, 5543.

106. S. Akhtar, S. Basu, E. Wikstrom and R. L. Juliano, *Nucl. Acids Res.*, 1991, **19**, 551.

107. S. Agarwal, *Tetrahedron Lett.*, 1989, **30**, 7025.

108. L. Watcher, J.-A. Jablonski and K. L. Ramachandran, *Nucl. Acids Res.*, 1986, **14**, 7985.

109. T. Kempe, W. I. Sundquist, F. Chow and S. -L. Hu, *Nucl. Acids Res.*, 1985, **13**, 45.

110. B. S. Sproat, B. Beijer, P. Rider and P. Neuner, *Nucl. Acids Res.*, 1987, **15**, 4837.

111. B. A. Connolly and P. Rider, *Nucl. Acids Res.*, 1985, **13**, 4485.

112. R. K. Gaur, P. Sharma and K. C. Gupta, *Nucl. Acids Res.*, 1989, **17**, 4404.

113. A. Kumar and S. Malhotra, *Nucleosides Nucleotides*, 1992, **11**, 1003.

114. A. Kumar and S. Advani, *Nucleosides Nucleotides*, 1992, **11**, 999.

115. N. T. Thoung and M. Chassignol, *Tetrahedron Lett.*, 1987, **28**, 4157.

116. W. Bannwarth and J. Wippler, *Helv. Chim. Acta*, 1990, **73**, 1139.

117. W. H. A. Kuijpers and C. A. A. van Boeckel, *Tetrahedron*, 1993, **49**, 10931.

118. B. G. de la Torre, A. M. Avino, M. Escrceller, M. Royo, F. Albericio and R. Eritja, *Nucleosides Nucleotides*, 1993, **12**, 993.

119. P. Kumar, D. Bhatia, R. C. Rastogi and K. C. Gupta, *BioMed. Chem. Lett.*, 1996, **6**, 683.

120. A. M. Alves, D. Holland and M. D. Edge, *Tetrahedron Lett.*, 1989, **30**, 3089.

121. A. J. Cocuzza, *Tetrahedron Lett.*, 1989, **30**, 6287.

122. R. T. Pon, *Tetrahedron Lett.*, 1991, **32**, 1715.

123. U. Pieles, B. S. Sproat and G. M. Lamm, *Nucl. Acids Res.*, 1990, **18**, 4355.

124. D. W. Will, C. E. Pritchard and T. Brown, *Carbohydr. Res.*, 1991, **216**, 315.

125. F. Schubert, C. E. Ahlert, D. Cech and A. Rosenthal, *Nucl. Acids Res.*, 1990, **18**, 3427.

126. A. S. Modak, J. K. Gard, M. C. Merriman, K. A. Winkeler, J. K. Bashkin and M. K. Stern, *J. Am. Chem. Soc.*, 1991, **113**, 283.

127. J. Woo and P. B. Hopkins, *J. Am. Chem. Soc.*, 1991, **113**, 5457.

128. P. Kumar, A. K. Sharma and K. C. Gupta, *Nucleosides Nucleotides*, 1996, **15**, 1263.

129. P. Kumar, D. Bhatia, B. S. Garg and K. C. Gupta, *BioMed. Chem. Lett.*, 1994, **4**, 1761.

130. F. Vandendriessche, A. van Aerschot, M. Voortmans, G. Janssen, R. Busson, A. van Overbeke, W. van den Bossche, J. Hoogmartens and P. Herdewijn, *J. Chem. Soc.*, 1993, 1567.

131. K. Yamana, Y. Nishijima, T. Ikeda, T. Gokota, H. Ozaki, H. Nakano, O. Sangen and T. Shimidzu, *Bioconj. Chem.*, 1990, **1**, 319.

132. K. Yamana, T. Gokota, H. Ozaki, H. Nakano, O. Sangen and T. Shimidzu, *Nucleosides Nucleotides*, 1991, **10**, 383.

133. M. Manoharan, C. J. Guinosso and P. D. Cook, *Tetrahedron Lett.*, 1991, **32**, 7171.

134. M. E. Douglas, B. Beijer and B. S. Sproat, *BioMed. Chem. Lett.*, 1994, **4**, 995.

135. K. Kido, H. Inoue and E. Ohtsuka, *Nucl. Acids Res.*, 1992, **20**, 1339.

136. (a) A. Dan, Y. Yoshimura, A. Ono and A. Matsuda, *BioMed. Chem. Lett.*, 1993, **3**, 615; (b) B. Cuenoud, F. Casset, D. Husken, F. Natt, R. M. Wolf, K. -H. Altman, P. Martin and H. E. Moser, *Angew. Chem., Int. Ed. Engl.*, 1998, **37**, 1288.

137. A. Szemzo, J. Szecsi, J. Sagi and L. Otvos, *Tetrahedron Lett.*, 1990, **31**, 1463.

138. B. C. Froehler and D. J. Ricca, *J. Am. Chem. Soc.*, 1992, **114**, 8320.

139. R. D. Elliott, G. A. Rener, J. M. Riordan, J. A. Secrist, L. L. Bennette, W. B. Parker and J. A. Montgomery, *J. Med. Chem.*, 1994, **37**, 739.

140. K. Augustyns, F. Vandendriessche, A. Van Aerschot, R. Busson, C. Urbanke and P. Herdewijn, *Nucl. Acids Res.*, 1992, **20**, 4711.

141. N. T. Thuong and M. Chassignol, *Tetrahedron Lett.*, 1998, **29**, 5905.

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

143. D. Praseuth, M. Chassinol, M. Takasugi, T. Le Doan, N. T. Thuong and C. Helene, *J. Mol. Biol.*, 1987, **196**, 939.

144. S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, **49**, 1925.

145. D. E. Bergstrom and R. L. Ruth, *J. Carbohydrate : Nucleosides Nucleotides*, 1977, **4**, 257.

146. D. E. Bergstrom and R. L. Ruth, *J. Am. Chem. Soc.*, 1976, **98**, 1587.

147. J. Telser, K. A. Cruickshank, L. E. Morrison and T. L. Netzel, *J. Am. Chem. Soc.*, 1989, **111**, 6966.

148. J. Telser, K. A. Cruickshank, K. S. Schanze and T. L. Netzel, *J. Am. Chem. Soc.*, 1989, **111**, 7221.

149. A. H. N. Hopman, J. Weigant, G. I. Tesser and P. Van Duijn, *Nucl. Acids Res.*, 1986, **14**, 6471.

150. A. F. Cook, E. Vuocolo and C. L. Brakel, *Nucl. Acids Res.*, 1988, **16**, 4077.

151. J. J. Kirchner, E. J. Hustedt, B. H. Robinson and P. B. Hopkins, *Tetrahedron Lett.*, 1990, **31**, 593.

152. S. Schmidt, C. D. Pien, H. J. Fritz and D. Cech, *Nucl. Acids Res.*, 1992, **20**, 2421.

153. J. Liu and G. L. Verdine, *Tetrahedron Lett.*, 1992, **33**, 4625.

154. C. J. Marasco, Jr. and J. R. Sufrin, *J. Org. Chem.*, 1992, **57**, 6363.

155. D. Peters, Anna-Britta Hornfeldt, S. Gronowitz and N. G. Johansson, *Nucleosides Nucleotides*, 1992, **11**, 1151.

156. P. Hurskainen, P. Dahlen, J. Ylikoski, M. Kwiatkowski, H. Siitari and T. Lovgren, *Nucl. Acids Res.*, 1991, **19**, 1057.

157. D. H. Bradley and M. M. Hanna, *Tetrahedron Lett.*, 1992, **33**, 6223.

158. D. A. Barawkar and K. N. Ganesh, *BioMed. Chem. Lett.*, 1993, **3**, 347.

159. E. Jablonski, E. W. Moomaw, R. H. Tullis and J. L. Ruth, *Nucl. Acids Res.*, 1986, **14**, 6128.

160. J. L. Ruth, in Ref. 46.

161. K. J. Gibson and S. J. Benkovic, *Nucl. Acids Res.*, 1987, **15**, 6455.

162. R. B. Meyer, Jr., J. C. Tabone, G. D. Hurst, T. M. Smith and H. Gamper, *J. Am. Chem. Soc.*, 1989, **111**, 8517.

163. T. J. Posvic, S. A. Strobel and P. B. Dervan, *J. Am. Chem. Soc.*, 1992, **114**, 5934.

164. K. A. Cruickshank and D. L. Stockwell, *Tetrahedron Lett.*, 1988, **29**, 5221.

165. T. Horn and M. S. Urdea, *Nucl. Acids Res.*, 1989, **17**, 6959.

166. R. Kierzek and W. T. Markiewicz, *Nucleosides Nucleotides*, 1987, **6**, 403.

167. B. S. Sproat, A. I. Lamond, B. Beijer, P. Neuner and U. Ryder, *Nucl. Acids Res.*, 1989, **17**, 3373.

168. A. M. MacMillan and G. L. Verdine, *J. Org. Chem.*, 1990, **55**, 5931.

169. A. M. MacMillan and G. L. Verdine, *Tetrahedron*, 1991, **47**, 2603.

170. W. Bannwarth and D. Schmidt, *BioMed. Chem. Lett.*, 1994, **4**, 977.

171. S. L. Brun, N. Duchange, A. Namane, M. M. Zakin, T. Huynh-Dinh and J. Igolen, *Biochimie*, 1989, **71**, 319.

172. M. S. Urdea, B. D. Warner, J. A. Running, M. Stempien, J. Clyne and T. Horn, *Nucl. Acids Res.*, 1988, **16**, 4937.

173. R. S. Coleman and J. M. Siedlecki, *J. Am. Chem. Soc.*, 1992, **114**, 9229.

174. T. T. Nikiforov and B. A. Connolly, *Tetrahedron Lett.*, 1992, **33**, 2379.

175. H. Bazin and A. Roget, *Nucleosides Nucleotides*, 1991, **10**, 363.

176. S. Sauvagio, B. Fourque, A. Roget, T. Livache, H. Bazin, C. Chypre and R. Teoule, *Nucl. Acids Res.*, 1990, **18**, 3175.

177. D. E. Draper, *Nucl. Acids Res.*, 1984, **12**, 989.

178. A. Roget, H. Bazin and R. Teoule, *Nucl. Acids Res.*, 1989, **17**, 7643.

179. E. Krzymanska-Olejnik and R. W. Adamiak, *Nucleosides Nucleotides*, 1991, **10**, 595.

180. J. P. Roduit, J. Shaw, A. Chollet and S. A. Biogen, *Nucleosides Nucleotides*, 1987, **6**, 349.

181. U. Pieles, B. S. Sproat, P. Neuner and F. Cramer, *Nucl. Acids Res.*, 1989, **17**, 8967.

182. D. J. Allen, P. L. Darke and S. J. Benkovic, *Biochemistry*, 1989, **28**, 4601.

183. O. Kemal, T. Brown, S. Burgess, J. -D. Bishop and A. J. Leigh-Brown, *Nucleosides Nucleotides*, 1991, **10**, 555.

184. B. R. Shranabasna and L. W. McLaughlin, *Tetrahedron Lett.*, 1992, **33**, 6081.

185. T. R. Waters and B. A. Connolly, *Nucleosides Nucleotides*, 1992, **11**, 985.

186. H. G. Gao, R. Fathi, B. L. Gaffney, B. Goswami, P. -P. Kung, Y. Rhee, R. Jin and R. A. Jones, *J. Org. Chem.*, 1992, **57**, 6954.

187. P. Clivio, J. L. Fourrey, J. Gasche, A. Audic, A. Farve, C. Perrin and A. Woisard, *Tetrahedron Lett.*, 1992, **33**, 65.

188. Y. -Z. Xu, Q. Zheng and P. F. Swann, *Tetrahedron*, 1992, **48**, 1729.

189. H. Lee, M. Hinz, J. J. Stezowski and R. G. Harvey *Tetrahedron Lett.*, 1990, **31**, 6773.

190. A. Ono, N. Haginoya, M. Kiyokawa, N. Minakawa and A. Matsuda, *BioMed. Chem. Lett.*, 1994, **40**, 361.

191. L. M. Khalimskaya, A. S. Levina and V. F. Zarytova, *BioMed. Chem. Lett.*, 1994, **4**, 1083.

192. J. Haralambidis, L. Lagniton and G. W. Tregear, *BioMed. Chem. Lett.*, 1994, **4**, 1005.

193. B. A. Connolly, in Ref. 46.

194. W. Saenger, "Principles of Nucleic Structure", Springer-Verlag, New York, 1984.

195. S. Schmidt, C. D. Pein, H. J. Fritz and D. Cech, *Nucl. Acids Res.*, 1992, **20**, 2421.

196. P. D. Cook, *Anti-cancer Drug Design*, 1991, **6**, 585.

197. B. C. Froehler, S. Wadwani, T. J. Terhorst and S. R. Gerrard, *Tetrahedron Lett.*, 1992, **33**, 5307.

198. J. K. Bashkin, J. K. Gard and A. S. Modak, *J. Org. Chem.*, 1990, **55**, 5125.

199. S. J. Kim, M. P. Stone, C. M. Harris and T. M. Harris, *J. Am. Chem. Soc.*, 1992, **114**, 5480.

200. D. Singh, V. Kumar and K. N. Ganesh, *Nucl. Acids Res.*, 1990, **18**, 3339.

201. C. M. Harris, L. Zhou, E. A. Strand and T. M. Harris, *J. Am. Chem. Soc.*, 1991, **113**, 4328.

202. R. Cosstick and M. E. Douglas, *J. Chem. Soc., Perkin Trans. 1*, 1991, 1035.

203. R. Casale and L. W. McLaughlin, *J. Am. Chem. Soc.*, 1990, **112**, 5264.

204. H. Gao, R. Fathi, B. L. Gaffney, B. Goswami, P. -P. Kung, Y. Rhee, R. Jin and R. A. Jones, *J. Org. Chem.*, 1992, **57**, 6954.

205. G. Gish and F. Eckstein, *Science*, 1988, **240**, 1520.

206. F. Eckstein, *Angew. Chem., Int. Ed. Engl.*, 1975, **14**, 180.

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

208. R. R. Hodges, N. E. Conway and L. W. McLaughlin, *Biochemistry*, 1989, **28**, 261.

209. N. E. Conway and L. W. McLaughlin, *Bioconj. Chem.*, 1991, **2**, 5419.

210. S. Agrawal and P. C. Zamecnik, *Nucl. Acids Res.*, 1990, **18**, 5419.

211. F. Eckstein, *Annu. Rev. Biochem.*, 1985, **54**, 367.

212. F. Eckstein and G. Gerald, *Trends Biochem. Sci.*, 1989, **14**, 97.

213. C. A. Stein, K. Shinozuka, C. Subasinghe and J. S. Cohen, *Nucl. Acids Res.*, 1988, **16**, 3209.

214. M. Mag, S. Kaking and J. W. Engels, *Nucl. Acids Res.*, 1991, **19**, 1437.

215. H. Ozaki and L. W. McLaughlin, *Nucl. Acids Res.*, 1992, **20**, 5205.

216. H. Vu and B. L. Hirschbein, *Tetrahedron Lett.*, 1991, **32**, 3005.

217. L. LaPlanche, T. L. James, C. Powell, D. W. Wilson, B. Uznanski, W. J. Stec, M. F. Summers and G. Zon, *Nucl. Acids Res.*, 1986, **14**, 9081.

218. J. A. Fidanza, H. Ozaki and L. W. McLaughlin, *J. Am. Chem. Soc.*, 1992, **114**, 5509.

219. Z. J. Lesnikowski, *Bioorg. Chem.*, 1993, **21**, 127.

220. N. N. Polushin, A. M. Morocho, B. Chen and J. S. Cohen, *Nucl. Acids Res.*, 1994, **22**, 639.

221. L. Zhou, A. M. Morocho, B. Chen and J. S. Cohen, *Nucl. Acids Res.*, 1994, **22**, 453.

222. R. P. Iyer, L. R. Phillips, W. Egan, J. B. Regan and S. L. Beaucage, *J. Org. Chem.*, 1990, **55**, 4693.

223. F. Morvan, B. Rayner and J. L. Imbach, *Tetrahedron Lett.*, 1990, **31**, 7149.

224. M. V. Rao, C. B. Reese and Z. Zhengyun, *Tetrahedron Lett.*, 1992, **33**, 4839.

225. W. K. D. Brill, J. Nielsen and M. H. Caruthers, *J. Am. Chem. Soc.*, 1991, **113**, 3972.

226. ! Stawinski, M. Thelin and R. Zain, *Tetrahedron Lett.*, 1989, **30**, 2157.

227. K. Bjergarde and O. Dahl, *Nucl. Acids Res.*, 1991, **19**, 5843.

228. G. Beaton, D. Dellinger, W. S. Marshall and M. H. Caruthers, in Ref. 46.

229. M. H. Caruthers, G. Beaton, J. V. Wu and W. Weisler, *Methods Enzymol.*, 1992, **211**, 8.

230. E. L. Hancox, B. A. Connolly and R. T. Walker, *Nucl. Acids Res.*, 1993, **21**, 3485.

231. C. Periguad, G. Gosselin and J. L. Imbach, *Nucleosides Nucleotides*, 1992, **11**, 903.

232. G. Beaton, W. K. -D. Brill, A. Grandas, Y. -X. Ma. J. Nielsen, E. Yau and M. H. Caruthers, *Tetrahedron*, 1991, **47**, 2377.

233. W. S. Marshall and M. H. Caruthers, *Science*, 1993, **259**, 1564.

234. M. K. Ghosh, K. Ghosh, O. Dahl and J. S. Cohen, *Nucl. Acids Res.*, 1993, **21**, 5761.

235. B. H. Dahl, K. Bjergarde, V. B. Sommer and O. Dahl, *Acta Chem. Scand.*, 1989, **43**, 896.

236. N. Farschtschi and D. G. Gorenstein, *Tetrahedron Lett.*, 1988, **29**, 6843.

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

238. R. L. Letsinger, G. Zhang, D. K. Sun, T. Ikeuchi and P. S. Sarin, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 6553.

239. S. M. Gryaznov and V. K. Potapov, *Tetrahedron Lett.*, 1991, **32**, 3715.

240. J. A. Fidanza and L. W. McLaughlin, *J. Org. Chem.*, 1992, **57**, 2340.

241. A. Jager, M. J. Levy and S. M. Hecht, *Biochemistry*, 1988, **27**, 7237.

242. K. Yamana and R. L. Letsinger, *Nucl. Acids Res., Symp. Ser.*, 1985, **16**, 169.

243. R. L. Letsinger, S. A. Bach and J. S. Eadie, *Nucl. Acids Res.*, 1986, **14**, 3487.

244. R. L. Letsinger, C. N. Singman, G. Histand and M. Salunkhe, *J. Am. Chem. Soc.*, 1988, **110**, 4470.

245. M. Mag, R. Schmidt and J. W. Engels, *Tetrahedron Lett.*, 1992, **33**, 7319.

246. S. M. Gryaznov and R. L. Letsinger, *Nucl. Acids Res.*, 1992, **20**, 3403.

247. A. Murakami, M. Nakaura, Y. Nakatsuji, S. Nagahara, O. Tran-Cong and K. Makino, *Nucl. Acids Res.*, 1991, **19**, 4097.

248. M. J. O'Donnell, N. Hebert and L. W. McLaughlin, *BioMed. Chem. Lett.*, 1994, **4**, 1001.

249. S. Agrawal and J. Y. Tang, *Tetrahedron Lett.*, 1990, **31**, 1543.

250. P. S. Miller, C. H. Agris, M. Blandin, A. Murakami, P. M. Reddy, S. A. Spitz and P. O. P. Ts'o, *Nucl. Acids Res.*, 1983, **11**, 5189.

251. M. A. Dorman, S. A. Noble, L. J. McBride and M. H. Caruthers, *Tetrahedron*, 1984, **40**, 95.

252. A. Jager and F. Riftina, *Nucl. Acids Res.*, 1984, **25**, 1437.

253. P. S. Miller and P. O. P. Ts'o, *Anti-cancer Drug Design*, 1987, **2**, 117.

254. P. S. Sarin, S. Agarwal, M. P. Cieira, J. Goodchild, T. Ikeuchhi and P. C. Zamecnik, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 7448.

255. R. T. Hogfere, M. M. Vaghefi, M. A. Reynolds, K. M. Young and L. J. Arnold, Jr., *Nucl. Acids Res.*, 1993, **21**, 2031.

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

257. Z. J. Lesnikowski, M. Jaworska and W. J. Stec, *Nucl. Acids Res.*, 1988, **16**, 11675.

258. Z. J. Lesnikowski, P. J. Wolkanin and W. J. Stec, *Tetrahedron Lett.*, 1987, **28**, 5535.

259. J. F. Cormier and T. Pannunzio, *Tetrahedron Lett.*, 1991, **32**, 7161.

260. J. Nielsen, W. K. -D. Brill and M. H. Caruthers, *Tetrahedron Lett.*, 1988, **29**, 2911.

261. B. H. Dahl, K. Bjergarde, J. Nielsen and O. Dahl, *Tetrahedron Lett.*, 1990, **31**, 3489.

262. C. J. Marcus-Secura, A. M. Woerner, K. Shinozuka, G. Zon and G. V. Quinnan, Jr., *Nucl. Acids Res.*, 1987, **15**, 5749.

263. M. Koziolkiewicz, B. Uznanski and W. J. Stec, *Chem. Scr.*, 1986, **26**, 251.

264. W. J. Stec, G. Zon, K. A. Gallo, R. A. Byrd, K. Shao, B. Uznanski and P. Guga, *Tetrahedron Lett.*, 1985, **26**, 2191.

265. M. Koziolkiewicz, B. Uznanski and W. J. Stec, *Nucleosides Nucleotides*, 1989, **8**, 185.

266. P. Guga, M. Koziolkiewicz, A. Obruszek, B. Uznanski and W. J. Stec, *Nucleosides Nucleotides*, 1987, **6**, 111.

267. B. Uznanski, A. Wilk and W. J. Stec, *Tetrahedron Lett.*, 1987, **28**, 3401.

268. W. Bannwarth, *Helv. Chim. Acta*, 1988, **71**, 1517.

269. J. M. Dagle, D. L. Weeks and J. A. Walder, *Antisense Res. Dev.*, 1991, **1**, 11.

270. M. Mag and J. W. Engels, *Nucleosides Nucleotides*, 1988, **7**, 725.

271. E. R. Felder, *Nucleosides Nucleotides*, 1991, **10**, 495.

272. R. Costick and J. S. Vyle, *Tetrahedron Lett.*, 1989, **30**, 4693.

273. M. Morr, L. Ernst and L. Grotjahn, *Z. Naturforsch., Teil B*, 1983, **38**, 1665.

274. K. K. Ogilvie and J. F. Cormier, *Tetrahedron Lett.*, 1985, **26**, 4159.

275. J. F. Cormier and K. K. Ogilvie, *Nucl. Acids Res.*, 1988, **16**, 4583.

276. A. K. Saha, M. Sardaro, C. Waychunas, D. Delecki, R. Kutny, P. Cavanaugh, A. Yawman, D. A. Upson and L. I. Kruse, *J. Org. Chem.*, 1993, **58**, 7827.

277. M. P. Mertes and E. A. Coats, *J. Med. Chem.*, 1969, **12**, 154.

278. D. S. Jones and J. R. Tittensor, *J. Chem. Soc., Chem. Commun.*, 1969, 1240.

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

280. M. H. Halford and A. S. Jones, *Science*, 1968, **217**, 638.

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

282. M. D. Edge, A. Hodgson, A. S. Jones and R. T. Walker, *J. Chem. Soc., Perkin Trans. 1*, 1972, 1991.

283. M. D. Edge, A. Hodgson, A. S. Jones, M. MacCoss and R. T. Walker, *J. Chem. Soc., Perkin Trans. 1*, 1973, 290.

284. A. S. Jones, M. MacCoss and R. T. Walker, *Biochem. Biophys. Acta*, 1973, **365**, 365.

285. R. C. Bleaney, A. S. Jones and R. T. Walker, *Nucl. Acids Res.*, 1975, **2**, 699.

286. M. J. Gait, A. S. Jones and R. T. Walker, *J. Chem. Soc., Perkin Trans. 1*, 1974, 1684.

287. M. J. Gait, A. S. Jones, M. D. Jones, M. J. Shepherd and R. T. Walker, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1389.

288. W. S. Mungall and J. K. Kaiser, *J. Org. Chem.*, 1977, **42**, 703.

289. J. M. Coull, D. V. Carlson and H. L. Weith, *Tetrahedron Lett.*, 1987, **28**, 745.

290. E. P. Stirchak, J. E. Summerton and D. D. Weller, *J. Org. Chem.*, 1987, **52**, 4202.

291. A. Waldner, A. De Mesmaeker and J. Lebreton, *BioMed. Chem. Lett.*, 1994, **4**, 405.

292. I. Habus, J. Temsamani and S. Agarwal, *BioMed. Chem. Lett.*, 1994, **4**, 1065.

293. E. P. Stirchak, J. E. Summerton and D. D. Weller, *Nucl. Acids Res.*, 1989, **17**, 6129.

294. H. Wang and D. D. Weller, *Tetrahedron Lett.*, 1991, **32**, 7385.

295. D. D. Weller and D. T. Daly, *J. Org. Chem.*, 1991, **56**, 6000.

296. S. H. Kawai and G. Just, *Nucleosides Nucleotides*, 1991, **10**, 1485.

297. K. C. Schneider and S. A. Benner, *Tetrahedron Lett.*, 1990, **31**, 335.

298. Z. Huang, K. C. Schneider and S. A. Benner, *J. Org. Chem.*, 1991, **56**, 3869.

299. S. H. Kawai, D. Wang and G. Just, *Can. J. Chem.*, 1992, **70**, 1573.

300. S. H. Kawai, D. Wang, P. A. Giannaris, M. J. Damha and G. Just, *Nucl. Acids Res.*, 1993, **21**, 1473.

301. X. Cao and M. D. Matteucci, *Tetrahedron Lett.*, 1994, **35**, 2325.

302. B. Meng, S. H. Kawai, D. Wang, G. Just P. A. Giannaris and M. J. Damha, *Nucleosides Nucleotides*, 1991, **10**, 1485.

303. B. Musicki and T. S. Widlanski, *J. Org. Chem.*, 1990, **55**, 4231.

304. B. Musicki and T. S. Widlanski, *Tetrahedron Lett.*, 1991, **32**, 1267.

305. E. B. McElroy, R. Bandaru, J. Huang and T. S. Widlanski, *BioMed. Chem. Lett.*, 1994, **4**, 1071.

306. J. Huang, E. B. McElroy and T. S. Widlanski, *J. Org. Chem.*, 1994, **59**, 3520.

307. P. A. Crooks, R. C. Reynolds, J. A. Maddry, A. Rathore, M. S. Akhtar, J. A. Montgomery and J. A. Secrist III, *J. Org. Chem.*, 1992, **57**, 2830.

308. R. C. Reynolds, P. A. Crooks, J. A. Maddry, M. S. Akhtar, J. A. Montgomery and J. A. Secrist III, *J. Org. Chem.*, 1992, **57**, 2983.

309. C. Glemaree, R. C. Reynolds, P. A. Crooks, J. A. Maddry, M. S. Akhtar, J. A. Montgomery and J. A. Secrist III, *Tetrahedron*, 1993, **49**, 2287.

310. E. M. Huie, M. R. Kirshenbaum and G. L. Trainor, *J. Org. Chem.*, 1992, **57**, 4569.

311. J. S. Vyle, X. Li and R. Cosstick, *Tetrahedron Lett.*, 1992, **33**, 3017.

312. T. F. Jenny, N. Previsani and S. A. Benner, *Tetrahedron Lett.*, 1991, **32**, 7029.

313. M. D. Matteucci, *Tetrahedron Lett.*, 1990, **31**, 2385.

314. M. D. Matteucci, *Nucleosides Nucleotides*, 1991, **10**, 231.

315. M. D. Matteucci, K. -Y. Lin, S. Butcher and C. Moulds, *J. Am. Chem. Soc.*, 1991, **113**, 7767.

316. R. J. Jones, K. -Y. Lin, J. F. Milligan, S. Wadwani and M. Matteucci, *J. Org. Chem.*, 1993, **58**, 2983.

317. K. -Y. Lin, J. S. Pudlo, R. J. Jones, N. Boschofberger, M. D. Matteucci and B. C. Froehler, *BioMed. Chem. Lett.*, 1994, **4**, 1061.

318. G. H. Veeneman, G. A. van der Marel, H. van den Elst and J. H. van Boom, *Recl. Trav. Chim. Pays-Bas*, 1990, **109**, 449.

319. G. H. Veeneman, G. A. van der Marel, H. van den Elst and J. H. van Boom, *Tetrahedron*, 1991, **47**, 1547.

320. P. J. L. M. Quaedflieg, G. A. van der Marel, E. Kuyl-Yeheskiely and J. H. van Boom, *Recl. Trav. Chim. Pays-Bas*, 1991, **110**, 435.

321. P. J. L. M. Quaedflieg, C. M. Timmers, V. E. Kal, G. A. van der Marel, E. Kuyl-Yeheskiely and J. H. van Boom, *Tetrahedron Lett.*, 1992, **33**, 3081.

322. P. J. L. M. Quaedflieg, J. A. Pikkemaat, G. A. van der Marel, E. Kuyl-Yeheskiely, C. Altona and J. H. van Boom, *Recl. Trav. Chim. Pays-Bas*, 1993, **112**, 15.

323. F. Debart, J. -J. Vasseur, Y. S. Sanghvi and P. D. Cook, *Tetrahedron Lett.*, 1992, **33**, 2645.

324. F. Debart, J.-J. Vasseur, Y. S. Sanghvi and P. D. Cook, *BioMed. Chem. Lett.*, 1992, **2**, 1479.

325. J. -J. Vasseur, F. Debart, Y. S. Sanghvi and P. D. Cook, *J. Am. Chem. Soc.*, 1992, **114**, 4006.

326. C. Pannecouque, P. Wigerinck, A. van Aerschot and P. Herdewijn, *Tetrahedron Lett.*, 1992, **33**, 7609.

327. F. Vandendriessche, M. Voortmans, J. Hoogmartens, A. van Aerschot and P. Herdewijn, *BioMed. Chem. Lett.*, 1993, **3**, 193.

328. F. Vandendriessche, A. van Aerschot, M. Voortmans, G. Janssen, R. Busson, A. van Overbeke, W. van den Bossche, J. Hoogmartens and P. Herdewijn, *J. Chem. Soc. Perkin Trans. 1*, 1993, 1567.

329. C. Pannecouque, G. Schepers, J. Rozenski, A. van Aerschot, P. Claes and P. Herdewijn, *BioMed. Chem. Lett.*, 1994, **4**, 1203.

330. C. Pannecouque, F. Vandendriessche, J. Rozenski, G. Janssen, R. Busson, A. van Aerschot, P. Claes and P. Herdewijn, *Tetrahedron*, 1994, **50**, 7231.

331. R. O. Dempcy, O. Almarsson and T. C. Bruice, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 7864.

332. A. Waldner, A. De Mesmaeker, J. Lebreton, V. Fritsch and R. M. Wolf, *Synlett*, 1994, 57.

333. K. M. K. Kutterer and G. Just, *BioMed. Chem. Lett.*, 1994, **4**, 435.

334. K. Lee and D. F. Wiemer, *J. Org. Chem.*, 1993, **58**, 7808.

335. J. Lebreton, A. De Mesmaeker and A. Waldner, *Synlett*, 1994, 54.

336. A. De Mesmaeker, A. Waldner, Y. S. Sanghvi and J. Lebreton, *BioMed. Chem. Lett.*, 1994, **4**, 395.

337. A. K. Saha, W. Schairer, C. Waychunas, C. V. C. Prasad, M. Sardaro, D. Upson and L. I. Kruse, *Tetrahedron Lett.*, 1993, **34**, 6017.

338. T. J. Caufield, C. V. C. Prasad, C. P. Prouty, A. K. Saha, M. Sardaro, W. Schairer, A. Yawman, D. Upson and L. I. Kruse, *BioMed. Chem. Lett.*, 1993, **3**, 2771.

339. M. Perbost, M. Lucas, C. Chavis, A. Pompon, H. Baumgartner, B. Rayner, H. Griengl and J. -L. Imbach, *Biochem. Biophys. Res. Commun.*, 1989, **165**, 742.

340. A. Szemzo, A. Azecsi and L. Otvos, *Tetrahedron Lett.*, 1990, **31**, 163.

341. J. Sagi, A. Szemzo, A. Szecsi and L. Otvos, *Nucl. Acids Res.*, 1990, **18**, 2133.

342. B. C. Froehler and D. J. Ricca, *J. Am. Chem. Soc.*, 1992, **114**, 8320.

343. J. Lang and H. E. Moser, *Helv. Chim. Acta*, 1994, **77**, 1527.

344. A. N. Payne and S. M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 1992, 2633.

345. K. -H. Altmann and R. Kesselring, *Synlett*, 1994, 853.

346. L. Bellon, F. Morvan, J. Barascut and J. -L. Imbach, *Biochem. Biophys. Res. Commun.*, 1992, **184**, 797.

347. L. Bellon, J. Barascut, G. Maury, G. Divita, R. Goody and J. -L. Imbach, *Nucl. Acids Res.*, 1993, **21**, 1587.

348. E. L. Hancox, B. A. Connolly and R. T. Walker, *Nucl. Acids Res.*, 1993, **21**, 3485.

349. B. Urbas and R. L. Whistler, *J. Org. Chem.*, 1986, **31**, 813.

350. E. J. Reist, D. E. Gueffroy and L. Goodman, *J. Am. Chem. Soc.*, 1964, **86**, 5658.

351. J. Uenishi, M. Motoyama, Y. Nishiyama and S. Wacabayashi, *J. Chem. Soc., Chem. Commun.*, 1991, 1421.

352. J. A. Secrist III, K. N. Tiwari, J. M. Riordan and J. A. Montgomery, *J. Med. Chem.*, 1991, **34**, 2361.

353. M. R. Dyson, P. L. Coe and R. T. Walker, *J. Med. Chem.*, 1991, **34**, 2782.

354. K. H. Altman, S. M. Freier, U. Pieles and T. Winkler, *Angew. Chem.*, 1994, **106**, 1735.

355. B. Huang, B. Chen and Y. Hui, *Synthesis*, 1993, 769.

356. G. Lowe and T. Vilaivan, *J. Chem. Soc., Perkin Trans. 1*, 1997, 539.

357. G. Ceulemans, A. Van Aerschot, J. Rozenski and P. Herdewijn, *Tetrahedron*, 1997, **53**, 14957.

358. N. Usman, C. D. Juby and K. K. Ogilvie, *Tetrahedron Lett.*, 1988, **29**, 4831.

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

360. M. Aztmah, C. Chavis, M. Lucas, F. Morvan and J. -L. Imbach, *Nucleosides Nucleotides.*, 1992, **11**, 1241.

361. L. Merle, G. Spach, Y. Merle, J. Sagi and A. Szemzo, *Origins Life*, 1993, **23**, 91.

362. F. Vandendriessche, R. Snoeck, G. Janssen, J. Hoogmartens, A. van Aerschot, E. De Clercq and P. Herdewijn, *J. Med. Chem.*, 1992, **35**, 1567.

363. F. Vandendriessche, K. Augustyns, A. van Aerschot, R. Busson, J. Hoogmartens and P. Herdewijn, *Tetrahedron*, 1993, **49**, 7223.

364. P. Nielsen, F. Kirpekar and J. Wengel, *Nucl. Acids Res.*, 1994, **22**, 703.

365. K. Augustyns, A. van Aerschot, A. van Schepdael, C. Urbanke and P. Herdewijn, *Nucl. Acids Res.*, 1991, **19**, 2587.

366. K. S. Ramassamy and W. Seifert, *BioMed. Chem. Lett.*, 1996, **6**, 1799.

367. R. Benhida, M. Devys, J. -L. Fourrey, F. Lecubin and J. -S. Sun, *Tetrahedron Lett.*, 1998, **39**, 6167.

368. V. S. Rana, V. A. Kumar and K. N. Ganesh, *BioMed. Chem. Lett.*, 1997, **7**, 2837.

369. A. Eschenmoser and M. Dobler, *Helv. Chim. Acta*, 1992, **75**, 218.

370. G. Otting, M. Billeter, K. Wuthrich, H. -J. Roth, C. Leumnn and A. Eschenmoser, *Helv. Chim. Acta*, 1993, **76**, 2701.

371. K. Augustyns, A. Van Aerschot and P. Herdewijn, *BioMed. Chem. Lett.*, 1992, **2**, 945.

372. D. A. Barawkar and T. C. Bruice, *Proc. Acad. Sci. USA*, 1998, **95**, 11047.

373. P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, *Science*, 1991, **254**, 1497.

374. P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt in "Antisense Research and Applications", eds. S. T. Crooke and B. Lebleu, CRC Press, Boca Raton, 1993, p. 363.

375. M. Egholm, O. Buchardt, P. E. Nielsen and R. H. Berg, *J. Am. Chem. Soc.*, 1992, **114**, 1895.

376. M. Egholm, P. E. Nielsen, O. Buchardt and R. H. Berg, *J. Am. Chem. Soc.*, 1992, **114**, 9677.

377. K. L. Dueholm, M. Egholm, C. Behrens, L. Christensen, H. F. Hansen, T. Vulpius, K. H. Petersen, R. H. Berg, P. E. Nielsen and O. Buchardt, *J. Org. Chem.*, 1994, **59**, 5767.

378. M. Egholm, C. Behrens, L. Christensen, R. H. Berg, P. E. Nielsen and O. Buchardt, *J. Chem. Soc., Chem. Commun.*, 1993, 800.

379. M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden and P. E. Nielsen, *Nature*, 1993, **365**, 566.

380. P. E. Nielsen, M. Egholm and O. Buchardt, *Bioconj. Chem.*, 1994, **5**, 3.

381. O. Buchardt, M. Egholm, R. H. Berg and P. E. Nielsen, *Trends Biotechnol.*, 1993, **11**, 384.

382. B. Hyrup and P. E. Nielsen, *BioMed. Chem.*, 1996, **4**, 5.

383. P. E. Nielsen and G. Haaima, *Chem. Soc. Rev.*, 1997, 73.

384. E. Uhlmann, A. Peyman, G. Brepohl and D. W. Will, *Angew. Chem., Int. Ed. Engl.*, 1998, **37**, 2796.

385. D. W. Will, G. Breipohl, D. Langer, J. Knolle and E. Uhlmann, *Tetrahedron*, 1995, **51**, 12069.

386. S. A. Thomson, J. A. Josey, R. Cadilla, M. D. Gaul, C. F. Hassman, M. J. Luzzio, A. J. Pipe, K. L. Reed, D. J. Ricca, R. W. Wiethe and S. A. Noble, *Tetrahedron*, 1995, **51**, 6179.

387. L. Christensen, R. Fitzpatrick, B. Gildea, K. H. Petersen, H. F. Hansen, T. Koch, M. Egholm, O. Buchardt, P. E. Nielsen, J. Coull and R. H. Berg, *J. Peptide Sci.*, 1995, **1**, 175.

388. A. Farese, N. Patino, R. Condon, S. Dalleu and R. Guedj, *Tetrahedron Lett.*, 1966, **37**, 1413.

389. T. Koch, H. F. Hansen, P. Andersen, T. Larsen, H. G. Batz, K. Otteson and H. Orum, *J. Peptide Res.*, 1997, **49**, 80.

390. V. V. Demidov, V. N. Potaman, M. D. Frank-Kamenetskii, M. Egholm, O. Buchardt, S. H. Sonnichsen and P. E. Nielsen, *Biochem. Pharmacol.*, 1994, **48**, 1310.

391. V. V. Demidov, M. D. Frank-Kamenetskii, M. Egholm, O. Buchardt and P. E. Nielsen, *Nucl. Acids Res.*, 1993, **21**, 2103.

392. D. Y. Cherny, B. P. Belotserkovskii, M. D. Frank-Kamenetskii, M. Egholm, O. Buchardt, R. H. Berg and P. E. Nielsen, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 1667.

393. P. E. Nielsen, M. Egholm and O. Buchardt, *J. Mol. Recogn.*, 1994, **7**, 165.

394. K. H. Petersen, D. K. Jensen, M. Egholm, P. E. Nielsen and O. Buchardt, *BioMed. Chem. Lett.*, 1995, **5**, 199.

395. G. Haaima, A. Lohse, O. Buchardt and P. E. Nielsen, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1939.

396. A. Puschl, S. Sforza, G. Haaima, O. Dahl and P. E. Nielsen, *Tetrahedron Lett.*, 1998, **39**, 4707.

397. D. A. Stetsenko, E. N. Lubyako, V. K. Potapov, T. L. Azhikina and E. D. Sverdlov, *Tetrahedron Lett.*, 1996, **37**, 3571.

398. L. Kosynkina, W. Wang and T. C. Liang, *Tetrahedron Lett.*, 1994, **35**, 5173.

399. A. Peyman, E. Uhlmann, K. Wagner, S. Augustin, C. Weiser, S. Hein, D. Langner, G. Breipohl and D. W. Will, *Nucleosides Nucleotides*, 1998, **17**, 1997.

400. A. Peyman, E. Uhlmann, K. Wagner, S. Augustin, G. Breipohl, D. W. Will, A. Schafer and H. Wallmeier, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 2636.

401. A. Peyman, E. Uhlmann, K. Wagner, S. Augustin, C. Weiser, D. W. Will and G. Breipohl, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 2809.

402. G. Breipohl, J. Knolle, D. Langner, G. O'Malley and E. Uhlmann, *BioMed. Chem. Lett.*, 1996, **6**, 665.

403. M. Fujii, K. Yoshida and J. Hidaka, *BioMed. Chem. Lett.*, 1997, **7**, 637.

404. P. Bigey, S. H. Sonnichsen, B. Meunier and P. E. Nielsen, *Bioconj. Chem.*, 1997, **8**, 267.