Journal of Sulfur Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713926081

Preparation of Nucleoside Phosphorothioates, Phosphorodithioates and Related Compounds

Otto Dahl[a]
[a] Institute of Chemistry, University of Copenhagen, The H. C. Ørsted Institute, Copenhagen ø, Denmark

To cite this Article Dahl, Otto(1991) 'Preparation of Nucleoside Phosphorothioates, Phosphorodithioates and Related Compounds', Journal of Sulfur Chemistry, 11: 1, 167 — 188
To link to this Article: DOI: 10.1080/01961779108048765
URL: http://dx.doi.org/10.1080/01961779108048765

Sulfur Reports
Volume 11(1), 1991, pp. 167–192
Photocopying permitted by license only

PREPARATION OF NUCLEOSIDE PHOSPHOROTHIOATES, PHOSPHORODITHIOATES AND RELATED COMPOUNDS

OTTO DAHL
Institute of Chemistry, University of Copenhagen, The H. C. Ørsted Institute, Universitetsparken 5, DK-2100 Copenhagen Ø, Denmark

(Received April 23, 1991)

This review describes synthetic pathways to mono-, di- and oligonucleotides modified in the phosphate group(s) by substitution of one or several oxygen atoms with sulfur. The known sulfur-modified phosphate groups are nucleoside 3′- and 5′-*O*-phosphorothioates, nucleoside thiodi- and triphosphates, nucleoside cyclic phosphorothioates, di- and oligonucleoside *O,O*-phosphorothioates, nucleoside 3′-*S*-phosphorothioates, nucleoside and oligonucleoside 5′-*S*-phosphorothioates, nucleoside *O*-phosphorodithioates, di- and oligonucleoside *O,O*-phosphorodithioates, di- and oligonucleoside *O,S*-phosphorodithioates, thiophosphortriesters, thiophosphoramidates, and methylphosphonothioates.

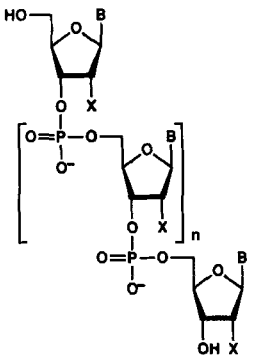
Key words: Phosphorothioates, phosphorodithioates, thiophosphortriesters, thiophosphoramidates, methylphosphonothioates, modified nucleotides, oligonucleotide analogs.

CONTENTS

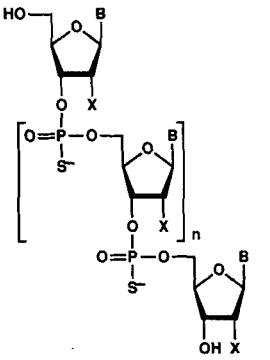
1. INTRODUCTION

For several decades chemists have been able to synthesize shorter DNA or RNA sequences, the oligonucleotides **1**, which have found numerous uses in gene manipulations and other biochemical procedures. In some applications, however, the oligonucleotides are degraded fairly quickly by nucleases, which hydrolyse the phosphordiester bonds. Nucleases are enzymes which are present in all living cells and in most cell lysates and sera. Degradation of oligonucleotides is a serious problem e.g. for *in situ* hybridization experiments and selective inhibition of protein synthesis by "antisense" oligonucleotides.[1] In order to perform such experiments successfully chemists have developed methods to prepare modified oligonucleotides, where the phosphordiester linkage is changed in a way to reduce or prevent cleavage by nucleases. Among the first of these "new generation" oligonucleotides were deoxyribonucleoside phosphorothioates **2**, which have been extensively tested as "antisense" oligonucleotides. The substitution of one of the non-bridging oxygen atoms in the phosphate group with sulfur results in modified oligonucleotides which are largely, but not completely, resistant to nuclease cleavage, and several other modifications have been prepared and examined in recent years in order to improve the compounds' properties as "antisense" oligonucleotides.[2] Many contain sulfur-phosphorus bonds, and the purpose of this review is to describe the synthetic methods used to obtain such compounds and present some of the preparative difficulties involved.

This review will cover the preparation of both monomeric and di- or oligomeric nucleotides contain P–S or P=S bonds, although the emphasis will be on di- and oligomers. Compounds with P–SR groups, where the SR groups only serve as a leaving group, will not be included.



1 DNA: X = H, B = adenine (A), cytosine (C), guanine (G), or thymine (T)
RNA: X = OH, B = A, C, G, or uracil (U)

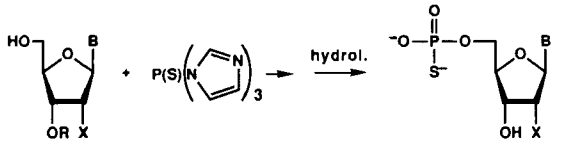


2 Oligonucleoside phosphorothioate

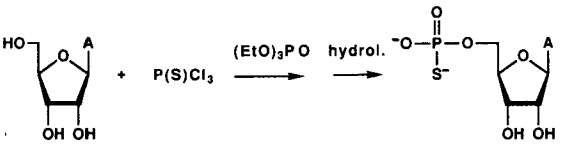
2. PREPARATION OF PHOSPHOROTHIOATES

2.1. Nucleoside 3′- and 5′-Phosphorothioates

The first nucleoside phosphorothioates were prepared by Eckstein in 1966.[3] He obtained thymidine and uridine 5′-*O*-phosphorothioate **3** by thiophosphorylation of the protected nucleosides with thiophosphoryl trisimidazolide. Two years later Murray and Atkinson used thiophosphoryl chloride in triethyl phosphate to obtain adenosine 5′-*O*-phosphorothioate **4** in good yield from unprotected adenosine.[4] The latter method with some modifications (e.g. the use of trimethyl phosphate as solvent for guanosine derivatives) is the method of choice today for the preparation of nucleoside 5′-*O*-phosphorothioates.[5-8]

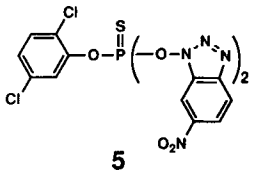


3

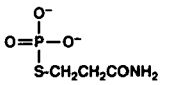


4

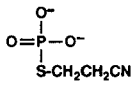
The above methods are unsuitable for thiophosphorylation of the sterically hindered 3′-hydroxy group. Thiophosphoryl chloride in pyridine,[9] more reactive thiophosphoryl derivatives like **5**,[10] or *S*-alkyl thiophosphates like **6**[11] or **7**,[12] both activated by dicyclohexylcarbodiimide, are necessary to give good yields of protected nucleoside 3′-*O*-phosphorothioates. Another method involves phosphorylation with the reactive amidochloridate **8** and introduces sulfur by treatment of the phosphoramidate **9** with sodium hydride and carbon disulfide.[13] The two diastereomers of **9** are easy to separate and the substitution with sulfur occurs with clean retention, making the method attractive to prepare pure R_P or S_P (Cahn-Ingold-Prelog notation for configurations around P) protected nucleoside 3′-*O*-phosphorothioates for use in dimer and oligomer preparations. An alternative method for introducing phosphorothioate groups is the use of phosphoramidites, e.g. **10**, which are very reactive upon activation with tetrazole; the resulting phosphites are oxidized with sulfur and deprotected to give nucleoside 3′- or 5′-*O*-phosphorothioates.[14,15]



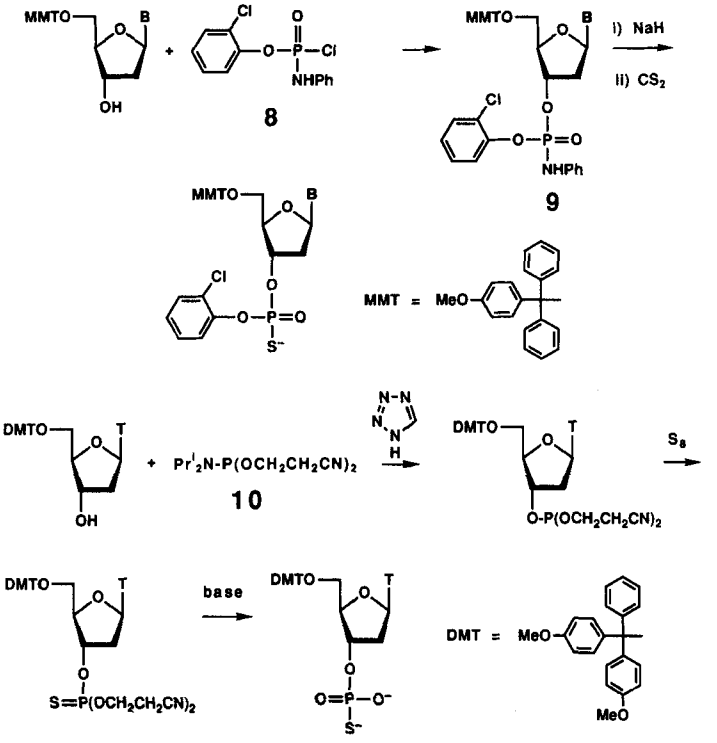
5



6



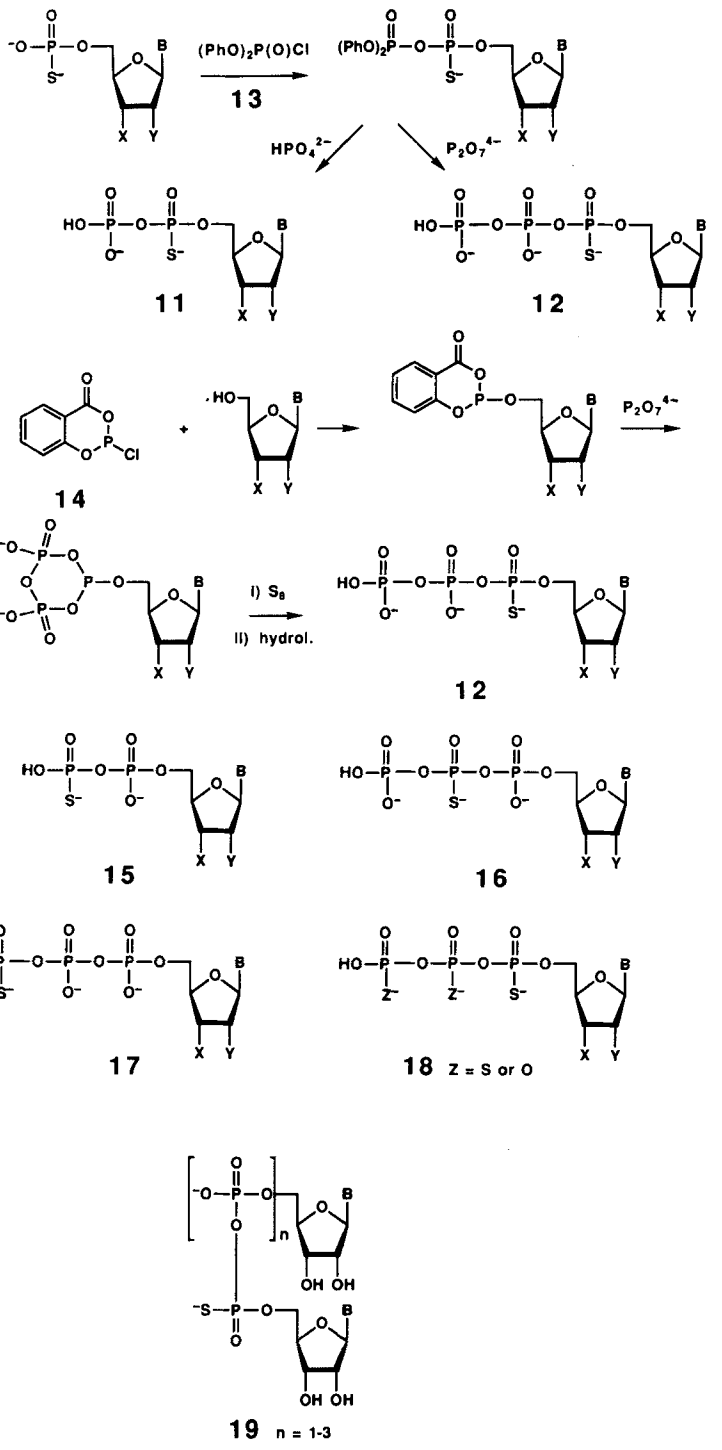
7



2.2. Nucleoside Thiodi- and Triphosphates

Nucleoside 5′-*O*-(1-thiodiphosphates) **11** or nucleoside 5′-*O*-(1-thiotriphosphates) **12** have been prepared from the nucleoside 5′-*O*-phosphorothioates described above by activation with diphenyl phosphorochloridate **13**, followed by reaction with phosphate or pyrophosphate, respectively.[5,16] A similar but simpler procedure to obtain **12** from unprotected nucleosides without isolation of the nucleoside 5′-*O*-phosphorothioate has been described by Goody.[17] These compounds exist as diastereomers due to the chiral phosphorothioate group. The pure or nearly pure R_P and S_P isomers of **12** have been prepared from nucleoside 5′-*O*-phosphorothioates by stereoselective enzymatic phosphorylations.[18] A new efficient method to prepare **12** via tervalent phosphorus chemistry has been described.[19] The nucleoside is phospnitylated with the heterocyclic phosphorochloridite **14**, followed by treatment with pyrophosphate and sulfur; hydrolysis gives **12** in 60–75% yield.

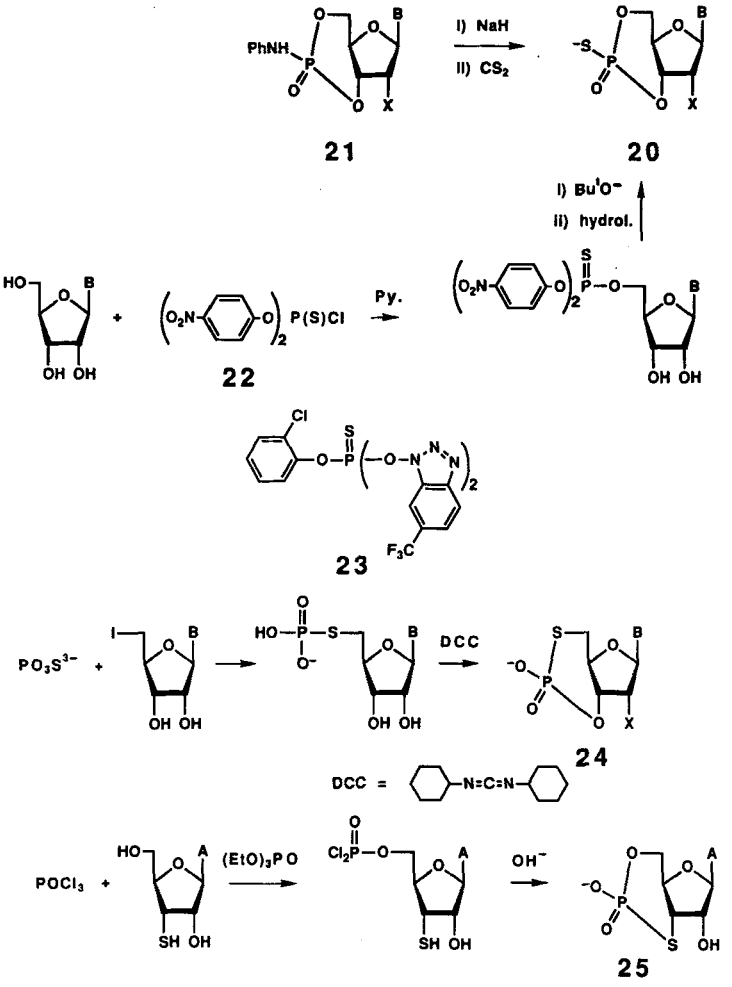
Nucleoside 5′-*O*-(2-thiodiphosphates) **15**, nucleoside 5′-*O*-(2-thiotriphosphates) **16**, and nucleoside 5′-*O*-(3-thiotriphosphates) **17** are also known compounds. The compounds with a terminal phosphorothioate group, **15** or **17**, have been prepared by thiophosphorylation of nucleoside 5′-phosphates or -diphosphates with *S*-(2-carbamoylethyl) phosphorothioate **6**,[20] whereas **16**, with an intermediate phosphorothioate group, has been made by stereoselective enzymatic phosphorylation of **15**.[16] Several di(nucleoside 5′-*O*)-thiooligophosphates **19** have been prepared by methods similar to

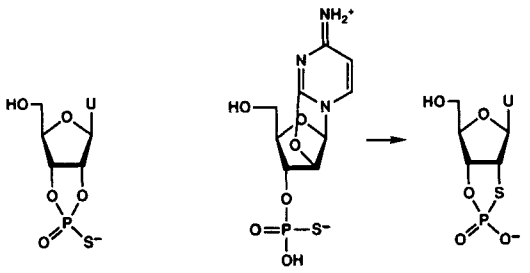


those used for obtaining **11**.[21] Some nucleoside 5′-*O*-(dithiotriphosphates) **18** were also recently described.[22]

2.3. Nucleoside Cyclic Phosphorothioates

Nucleoside 3′,5′-cyclic phosphorothioates are known with sulfur replacing oxygen at all the three different positions. The isomer most studied, **20**, has been prepared as the pure R_P and S_P diastereomers from the cyclic amidates **21** by treatment with sodium hydride and carbon disulfide.[13,23] Other routes to **20** are thiophosphorylation with **22** followed by cyclization of the 5′-*O*-phosphorothioate with *tert*-butoxide,[24] thiophosphorylation with **23**,[25] and with thiophosphoryl chloride in trimethyl or triethyl phosphate, followed by cyclization with potassium hydroxide in acetonitrile.[26] The other isomeric nucleoside 3′,5′-cyclic phosphorothioates, **24**[27] and **25**,[28] have been prepared in low yields by the routes shown. Some deoxynucleoside analogs of **24** have been prepared as well.[9]





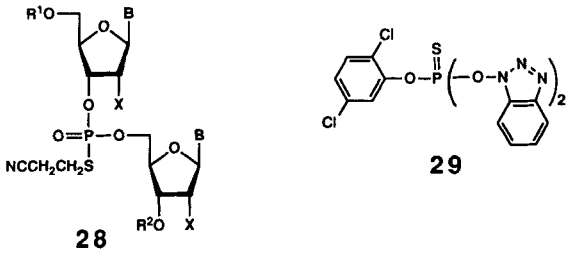
26 27

The uridine 2′,3′-cyclic phosphorothioate **26** is one of the few 2′,3′-cyclic phosphorothioates known.[29] Another is the cytidine derivative **27** with sulfur replacing the 2′-oxygen atom.[30]

2.4. *Di- and Oligonucleoside* O,O-*Phosphorothioates*

Di- and oligonucleoside *O,O*-phosphorothioates are prepared by the same methods and are treated together here; the same holds for ribo- and deoxyribonucleoside derivatives. The nucleoside protecting groups necessary for the selective preparation of 3′,5′-*O,O*-phosphorothioates are generally not specified; the reader is referred to the cited original literature for details.

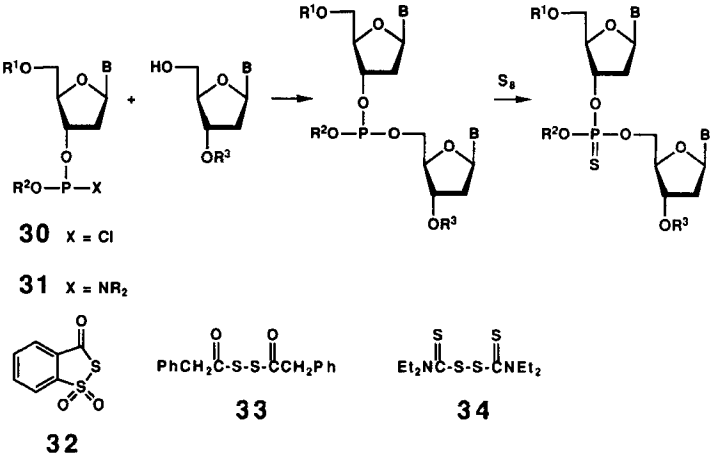
Thiophosphortriester methods have been extensively used to make dimers and shorter oligomers. Early examples include the use of *S*-(2-cyanoethyl) phosphorothioate **7** activated by 2,4,6-triisopropylbenzenesulfonyl chloride to give both ribo- and deoxyribonucleotide dimers **28**,[31,32] however, removal of the cyanoethyl group with triethylamine in aqueous dioxane was accompanied by 20–40% removal of sulfur as well. Sulfur is eliminated because hydroxide ions in the reagent attack phosphorus in competition with the intended attack at the β-hydrogens of the cyanoethyl group. This problem was later solved by the use of *tert*-butylamine in dry pyridine to effect the β-elimination.[12] Thiophosphorylation with *O*-aryl thiophosphorodichloridates is a slow process, but substitution of the chloro groups with hydroxybenzotriazole groups gave activated thiotriesters **29**[33] or the 6-nitrobenzotriazole derivative **5**[10] which reacted much faster. The latter reagent was used to prepare a monomer which coupled within 10 min in a solid support synthesis with a yield of 90% per step.



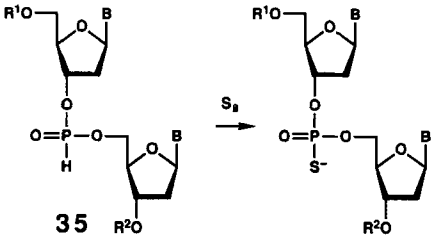
28 29

The phosphite approach was recognized early as an easy route to phosphorothioates;[34] the only modification of the procedure for normal phosphate prep-

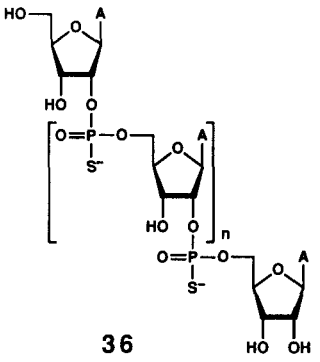
arations is to oxidise the intermediate phosphite with sulfur instead of iodine–water. The phosphorochloridites **30**[35–37] were soon replaced by the more stable phosphoramidites **31**[38,39] as monomers, and long oligodeoxynucleoside phosphorothioates were prepared by solid support syntheses on automatic DNA synthesizers.[40,41] One disadvantage of this method is the use of solutions of sulfur (in carbon disulfide–pyridine) as the oxidation reagent on DNA synthesizers, since precipitation of sulfur easily occurs, resulting in clogging of the valves and tubes of the machine. Several reagents were recently proposed which can deliver sulfur to phosphites and are stable and soluble in common solvents; the best is probably the benzodithiole dioxide **32** which is very soluble in acetonitrile and converts phosphites to phosphorothioates in 30 sec.[42] This reagent has been used to prepare an oligoribonucleoside phosphorothioate as well.[43] The other reagents proposed, **33**[44] and **34**,[45] react more slowly, but are easier to obtain. An oligoribonucleotide containing one phosphorothioate group was recently prepared from **34**.[46]



A third method to obtain oligonucleotides, the H-phosphonate approach, has been much used to prepare oligodeoxynucleoside phosphorothioates with phosphorothioate groups at all positions[47–49] and oligoribonucleoside phosphorothioates as well.[50] This method may be exemplified by the reaction of the H-phosphonate dimer **35** with sulfur. The coupling efficiency (96–97%) is lower than that of the phosphoramidite method (ca. 99%), but recent improvements of the H-phosphonate method [51] may well obviate this difference. The oxidation with sulfur is less troublesome in the H-phosphonate method, since it is performed in one last step following machine synthesis when all the H-phosphonate groups are simultaneously oxidized.



Dimers and oligomers containing 3′,5′-*O*,*O*-phosphorothioate groups exist as diastereomeric mixtures due to the chiral phosphorus atom(s). Compounds with one phosphorothioate group can often be separated in the R_P and S_P isomer by reverse-phase HPLC,[38] but separation is impossible when many phosphorothioate groups are present. Stereoselective syntheses of dimers or trimers have been devised,[12,52,53] and pure R_P and S_P units can be built into longer oligomers.[39,54,55] Enzymatic synthesis of all-R_P oligonucleoside phosphorothioates is possible with mixtures of R_P and S_P nucleoside 5′-*O*-(1-thiotriphosphates) **12** as monomers and DNA or RNA polymerase which only accept the (S_P)-**12** isomers as substrates.[18]

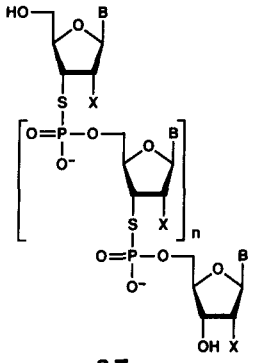


36

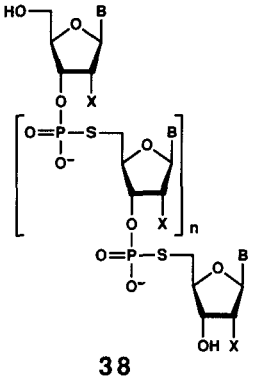
Most natural oligo- and polynucleotides contain 3′,5′-phosphate linkages, an exception being the 2′,5′-phosphates in the antiviral and antitumor agent 2–5A. Phosphorothioate analogs **36** of 2–5A dimers and trimers have been prepared both chemically via phosphites[56] and enzymatically.[57]

2.5. *Nucleoside 3′-S-Phosphorothioates*

Nucleoside phosphorothioates with sulfur at a bridge position, **37** or **38**, have been much less studied than those described in the previous chapters, undoubtedly because they require modified nucleosides as starting materials.

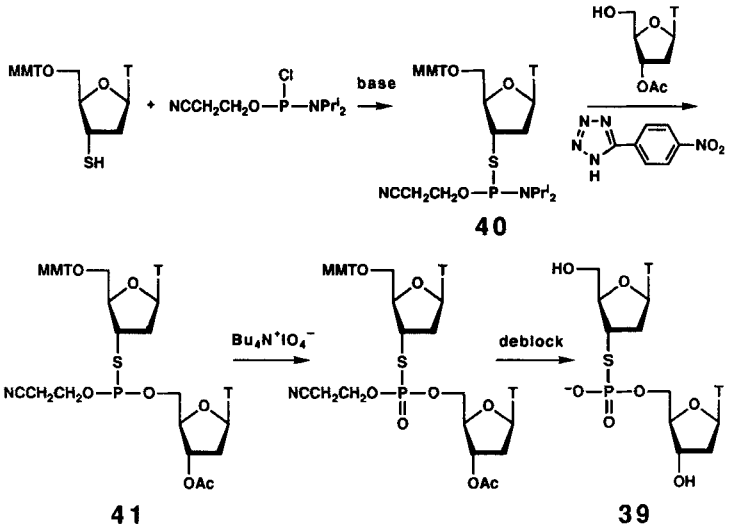


37



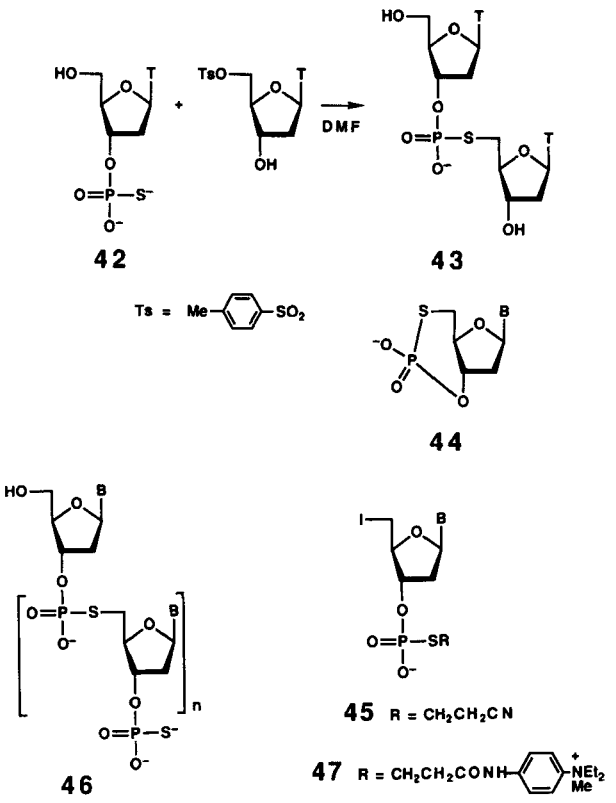
38

Nucleoside 3′-*S*-phosphorothioates **37** have only recently been described by Cosstick and Vyle.[58–60] The TT dimer **39** has been prepared by phosphoramidite chemistry, the thymidine 3′-*S*-thiophosphoramidite **40** being used as the building unit. The reactivity of **40** was much lower than that of the corresponding phosphoramidite, and attempted couplings with 3′-*O*-acetylthymidine with the usual activator, tetrazole, gave no thiophosphite **41**, but only products of ligand reorganisation of **40**. With a stronger activator, 5-(4-nitrophenyl)tetrazole, however, a 75% yield of the dimer **39** could be obtained.[60] The thioamidite **40** was also used to make some oligodeoxynucleotides containing one 3′-*S*-phosphorothioate group by solid support synthesis;[59] the coupling efficiency was 80% after a double coupling. The dimer **39** is quite stable towards hydrolysis, but the P–S bond was cleaved with iodine in aqueous pyridine and with aqueous silver nitrate.



*2.6. Nucleoside 5′-*S*-Phosphorothioates*

Nucleoside 5′-*S*-phosphorothioates **38** have been known since 1962 when Michelson made 5′-thiouridine-2′,3′-cyclic phosphate which was polymerized to a mixture of 2′,5′- and 3′,5′-oligouridine 5′-*S*-phosphorothioates.[61] In most other cases the 5′-*S*-phosphorothioates have been prepared by alkylation of a thiophosphate ion[62] or a nucleoside 3′-*O*-phosphorothioate **42**[9,11,63] with either 5′-iodo-5′-deoxy- or 5′-*O*-tosyl nucleosides, e.g. in the preparation of the dimer **43**. When the same nucleoside contains a 5′-*O*-tosyl group and a 3′-*O*-phosphorothioate it polymerizes to oligomers **38** or cyclizes under high dilution conditions to 5′-*S*-3′,5′-cyclic phosphorothioates **44**.[9] Kumarev and Bogachev prepared oligodeoxynucleoside 5′-*S*-phosphorothioates containing all bases by such alkylation methods and optimised the coupling conditions.[64–67] In DMF the lithium salts of 5′-iodo-5′-deoxynucleoside 3′-*O*-phosphorothioates **45** and **46** with increasing n take an increasing number of hours to react completely, and other solvents or cations gave worse results.[65] However, the reaction could be completed in 3–10 min independent of n when instead of **45** the electroneutral **47** was used, which shows the

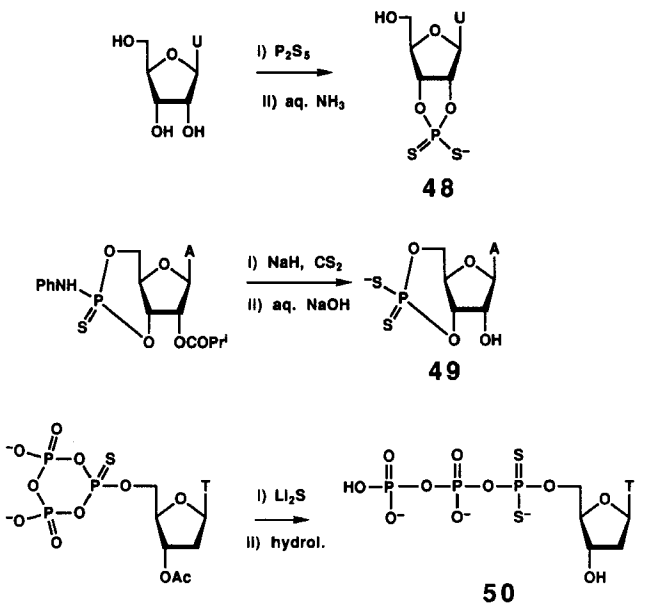


retardation to be due to electrostatic repulsion.[67] Some protected ribonucleoside 5′-*S*-phosphorothioate dimers were recently prepared by similar methods,[68] however, the P–S bond cleaved spontaneously upon removal of the 2′-*O*-protection group, showing that ribonucleoside 5′-*S*-phosphorothioates are much less stable than normal ribonucleotides.

3. PREPARATION OF NUCLEOSIDE PHOSPHORODITHIOATES

3.1. Nucleoside O-Phosphorodithioates

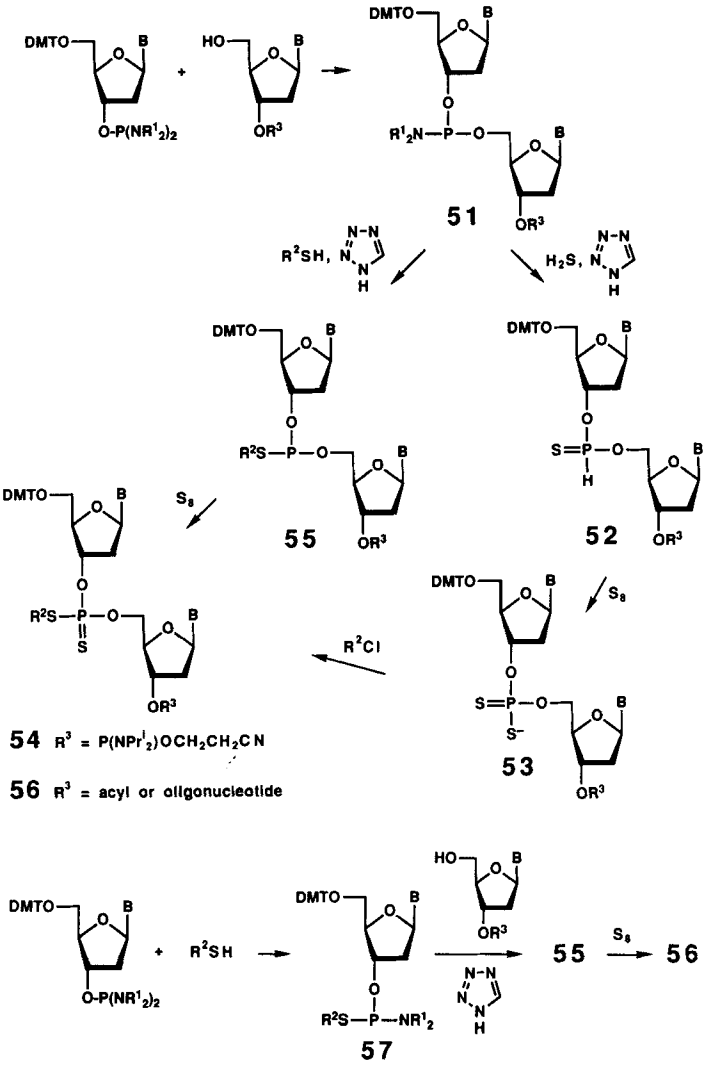
The first report on a nucleoside phosphorodithioate appeared in 1970 when Eckstein prepared the uridine 2′,3′-cyclic phosphorodithioate **48** from 5′-*O*-acetyluridine and phosphorus pentasulfide.[69] The next report came first seventeen years later when Baraniak and Stec published a synthesis of the adenosine 3′,5′-cyclic phosphorodithioate **49**.[23] Apart from these isolated papers and a recent report on thymidine 5′-*O*-(1,1-dithiotriphosphate) **50**, which as anticipated was not a substrate for DNA polymerases,[22] intensive work on phosphorodithioates first began in the last few years and has been concentrated on the preparation of nucleoside dimers and oligomers for use as "antisense" oligonucleotides.



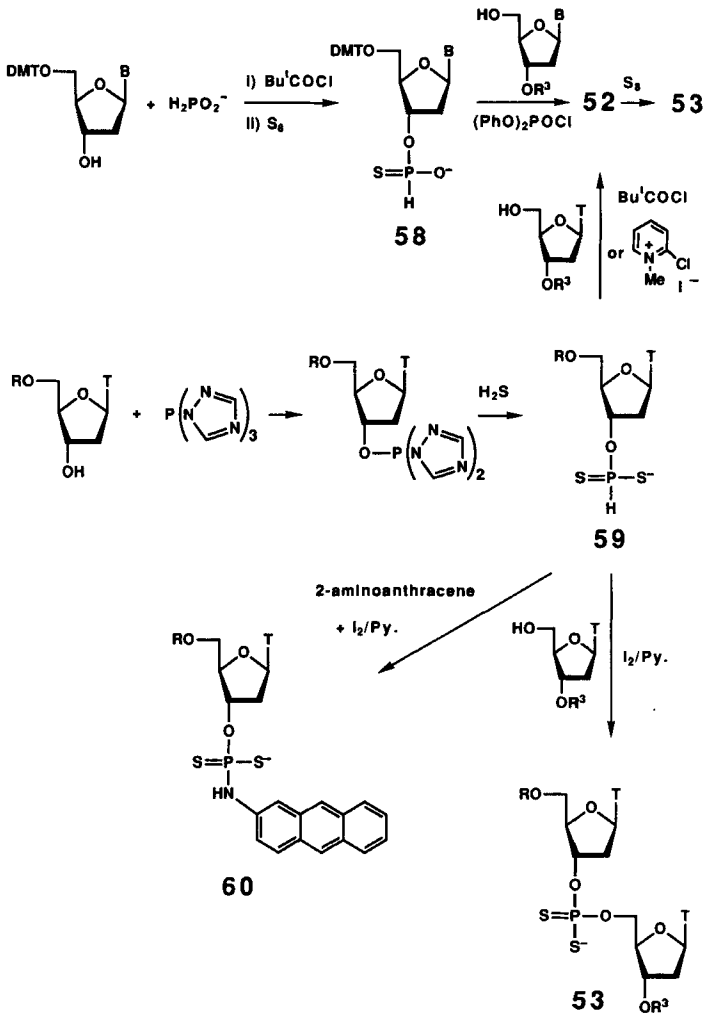
3.2. *Di- and Oligonucleoside* O,O-*Phosphorodithioates*

Several methods for the preparation of deoxynucleoside phosphorodithioate dimers have been investigated. In the first method, published in 1988 by Caruthers *et al.*,[70] a dithymidine phosphoramidite **51** (R^1 = Pr^i, B = T) was treated with hydrogen sulfide and tetrazole to obtain a H-phosphonothioate **52** which, after oxidation with sulfur, gave a dithymidine phosphorodithioate **53**. The charged phosphorodithioate group could be protected by alkylation, and the dimer transformed into a building block **54** subsequently used to obtain an oligonucleotide containing one phosphorodithioate group. Alternatively, **51** could be treated with a thiol and tetrazole to give a thiophosphite **55**, which with sulfur gave a protected phosphorodithioate **56**.[71,72] Attempts to use **51** in a solid support synthesis to obtain oligonucleotide phosphorodithioates by this route gave low yields, probably because the phosphoramidite hydrolyses too easily.[72] An alternative route from **52** to **56**, briefly described by van Boom *et al.*,[73] is to treat **52** with *N*-(alkylthio)succinimides.

A more successful route to di- and oligonucleoside phosphorodithioates is the one shown via the thiophosphoramidites **57**. Thiophosphoramidites are easy to prepare and are fairly stable; the first examined (**57**, R^1 = Pr^i)[72,74–76] were very unreactive (1/300 as reactive as the corresponding phosphoramidites[76]), but smaller amino groups (dimethylamino or pyrrolidino) increased the reactivity to a level satisfactory for solid support synthesis.[76,77] By this method many oligodeoxynucleoside phosphorodithioates have been prepared, but the coupling efficiencies (96–98% after double couplings) and the purity of the products (they contain 2–15% phosphorothioate groups, probably because of hydrolysis of tervalent intermediates) are not quite satisfactory.[78,79] The thiophosphoramidite method has been used to prepare ribonucleoside phosphorodithioate dimers as well.[80]

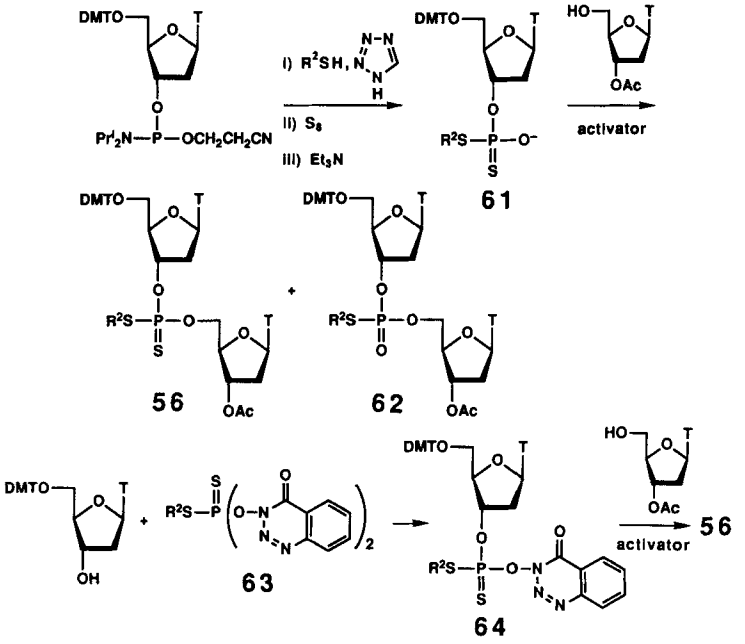


Methods analogous to the H-phosphonate route to unmodified oligonucleotides have also been developed. Nucleoside 3′-*O*-H-phosphonothioates **58**, like the oxygen analogues, can be activated with acid chlorides to give nucleoside H-phosphonothioate dimers **52**, which with sulfur gives the phosphorodithioates **53** as described above.[81,82] The use of pivaloyl chloride as the activator gave undesirable by-products, but diphenyl phosphorochloridate apparently activated the oxygen atom of **58** cleanly. Nucleoside 3′-*O*-H-phosphonodithioates **59**, which like **58** are easy to obtain, do not suffer from this ambiguity and can be activated with pivaloyl chloride[83] or *N*-methyl-2-chloropyridinium iodide[84] to give **52**. A dimer, prepared in this way, has been treated with **59** to give a thymidine phosphorodithioate trimer.[85] Interestingly, **59** could be coupled with 3′-*O*-acetylthymidine to the dimer phosphorodithioate **53** directly by treatment with an



equivalent amount of iodine in pyridine.[84] An excess of iodine gave "overoxidised products" which, however, could be reduced again with sodium bisulfite. With 2-aminoanthracene instead of 3′-*O*-acetylthymidine the thymidine 3′-*O*-dithiophosphoramidate **60** was similarly obtained.

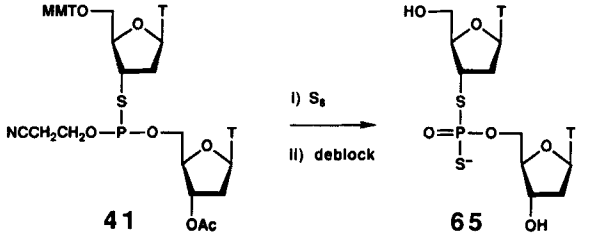
In two recent papers the phosphortriester approach to oligonucleotides was modified to obtain nucleoside phosphorodithioate dimers. Caruthers *et al.* prepared the protected thymidine 3′-*O*-phosphorodithioate **61** and optimized the conditions for its coupling with 3′-*O*-acetylthymidine.[86] Like other triester methods, the coupling rate was rather low due to the deactivating effect of the P=S group, and the selective activation of O versus S is a potential problem; however, with 2,4,6-triisopropylbenzenesulfonyl chloride and *N*-methylimidazole as activators in methylene chloride or THF, a nearly quantitative yield of **56** was obtained in 1.5 h with only 1% of the unwanted dimer



62 being formed. Dahl *et al.* have examined another route via active dithiophosphortriesters **63**.[87] The synthesis of active esters derived from 1-hydroxybenzotriazole was unsuccessful with proper *S*-protecting groups (4-chlorobenzyl or 2,4-dichlorobenzyl), but the corresponding 3-hydroxy-4-oxo-3,4-dihydrobenzotriazine derived esters **63** were reactive enough to give an active monomer **64** in 20 min, and the latter with 3′-*O*-acetylthymidine and *N*-methylimidazole in pyridine was sufficiently reactive to give a dimer phosphorodithioate **56** in 1 h.[87,88] These triester methods have yet to be evaluated for solid support synthesis of oligonucleoside phosphorodithioates.

3.3. Di- and Oligonucleoside O,S-*Phosphorodithioates*

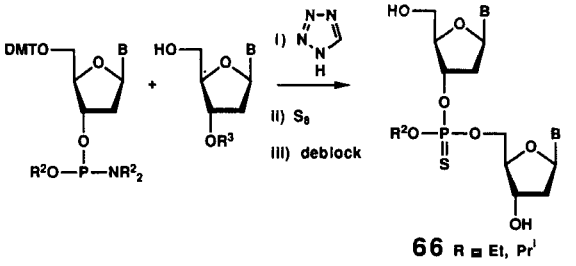
Nucleoside phosphorodithioates with sulfur replacing oxygen in the 3′- or 5′-position are virtually unknown. Cosstick and Vyle has recently described the dithymidine 3′-*S*-phosphorodithioate **65**, prepared by oxidation of the previously described thymidine 3′-*S*-thiophosphite **41** with sulfur.[60] A corresponding 5′-*S*-phosphorodithioate has not yet been described, but should be easily obtainable from **61** and 5′-iodo-5′-deoxythymidine.



4. MISCELLANEOUS NUCLEOTIDE ANALOGS CONTAINING P–S OR P=S BONDS

4.1. Thiophosphortriesters

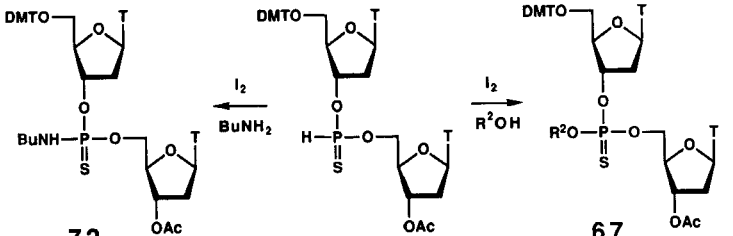
There are four possible types of dinucleoside thiophosphortriesters, since each of the four different oxygen atoms in the natural dinucleoside 3′,5′-*O*,*O*-phosphortriesters can be replaced by sulfur. Although examples of all are known only the *O*-alkyl dinucleoside 3′,5′-*O*,*O*-phosphorothioates **66** have been evaluated as nucleoside analogs; the other three types have been used only as intermediates during preparation of dinucleoside phosphates or phosphorothioates and are not treated here.



66 R = Et, Pr^{i}

The thiophosphortriesters **66** have been prepared by phosphoramidite methods, with sulfur instead of iodine–water being used for the oxidation of the intermediate phosphite. The alkyl group should be small (in order to reduce steric effects), but should not be removed during the deblocking of the protected nucleoside intermediates; methyl esters are too labile, but ethyl[89,90] and isopropyl esters[91,92] **66** are stable enough to survive deblocking with conc. ammonia at room temperature. Several deoxynucleoside dimers have been prepared and the diastereomers separated; an octamer with one ethyl or isopropyl thiophosphortriester linkage has also been made.

The H-phosphonothioate method is also a useful route to thiophosphortriesters as exemplified by the preparation of **67**.[70]

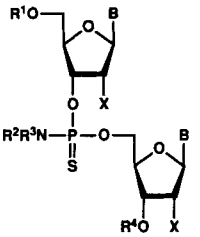


72 **67**

R² = 9-anthracenylmethyl

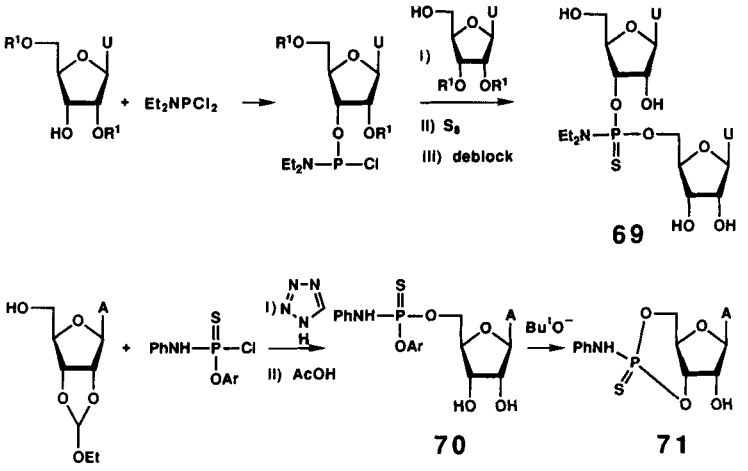
4.2. Thiophosphoramidates

Of the three possible types of thiophosphoramidates only the dinucleoside 3′,5′-*O*,*O*-thiophosphoramidates **68** are known. The first example of a nucleoside dimer of this type was prepared by Nemer and Ogilvie in 1980; they used dichloro(diethylamino)phosphine



68

to prepare a diuridine phosphoramidite which was oxidized with sulfur to yield **69**.[93] Adenosine 5′-*O*-thiophosphoranilidates **70** and the 3′,5′-cyclic analog **71** were prepared by Baraniak and Stec who used a thiophosphoranilidochloridate to introduce the thiophosphoramidate group.[23] A dithymidine thiophosphoramidate **72** has been obtained from a dithymidine H-phosphonothioate by oxidation with iodine in the presence of butylamine.[70]



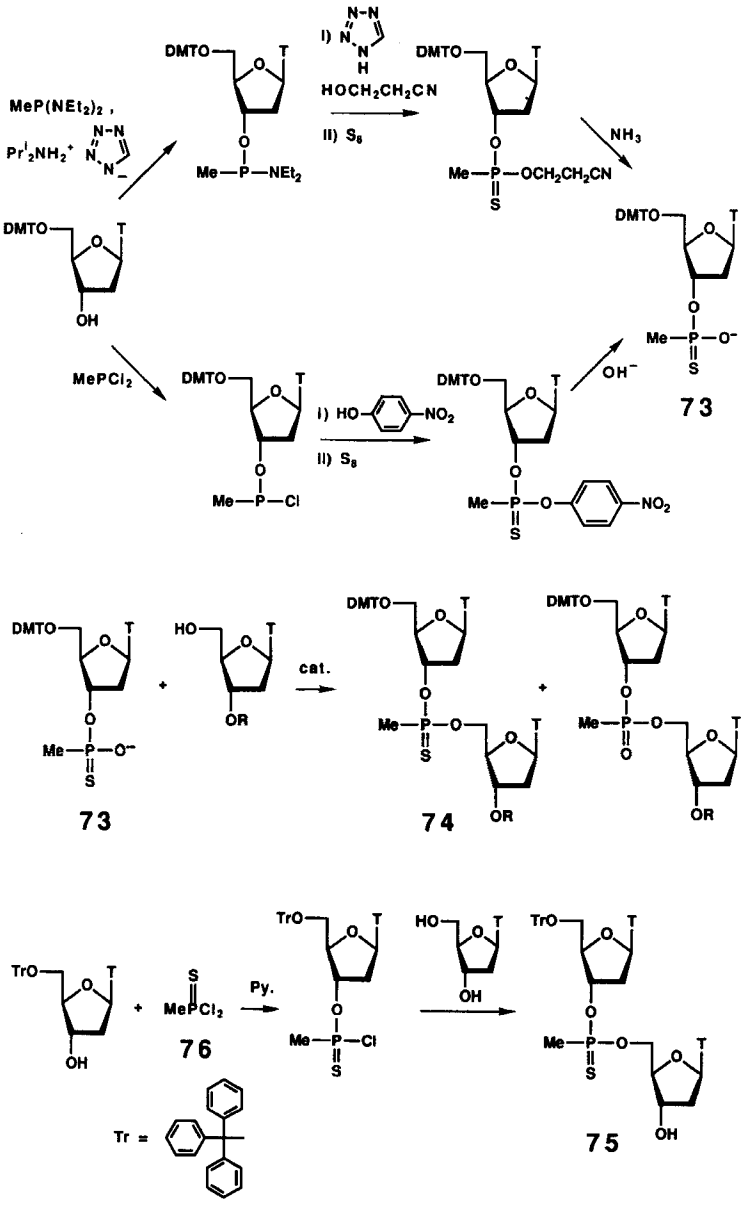
69

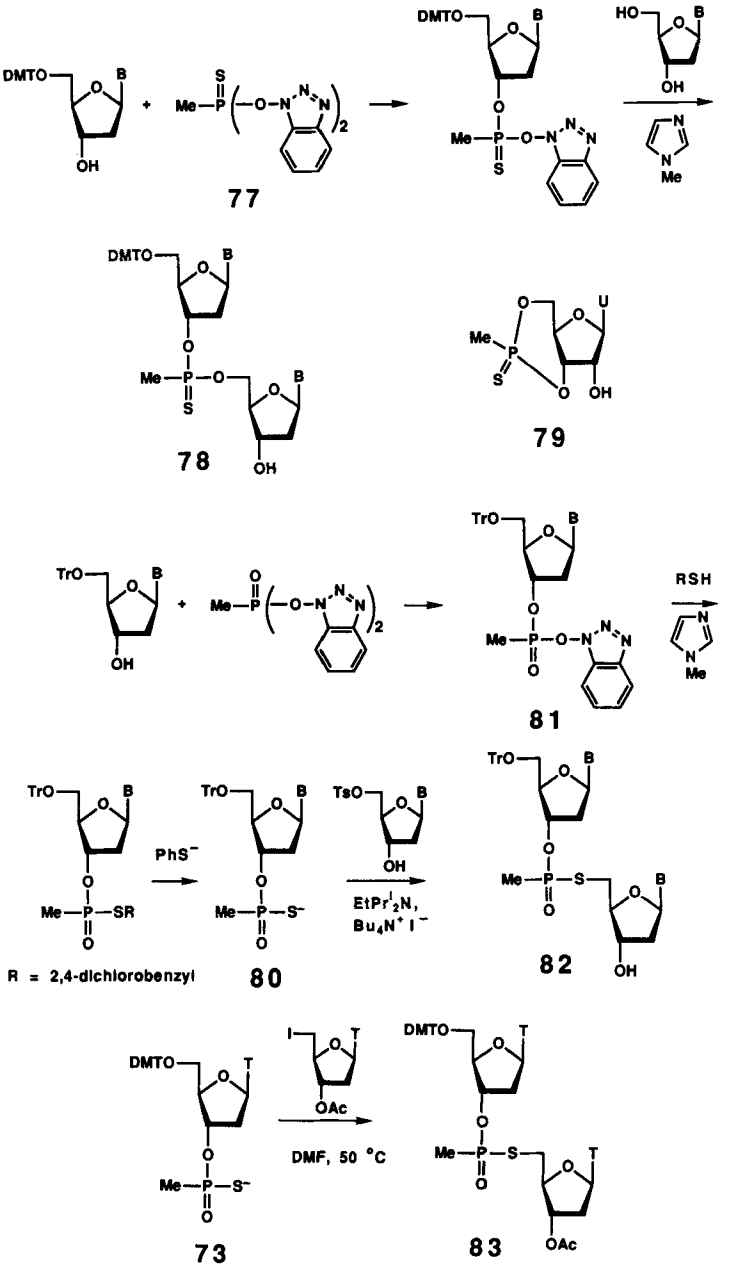
70 **71**

4.3. Methylphosphonothioates

Nucleoside methylphosphonothioates are known with sulfur at both the non-bridging and the 5′-position. Stec *et al.* prepared the thymidine 3′-*O*-methylphosphonothioate **73** by two routes via tervalent intermediates as shown.[94] The ester intermediates could be separated as the pure R_P and S_P isomers which gave pure R_P and S_P-isomers **73** after hydrolysis. Coupling reactions with 3′-*O*-methoxyacetylthymidine were performed under different conditions in order to obtain the dimeric methylphosphonothioate **74**, but the reactions were neither chemo- nor stereoselective. Brill and Caruthers examined several tervalent phosphorus reagents in order to obtain a similar dimer **75**, but were only successful with the methylthiophosphonodichloridate **76**.[95] More reactive than **76** was the active methylthiophospho ester **77**, which was used by van Boom *et al.* to prepare several nucleoside dimers **78**, one of which was built into a hexanucleotide.[96] A 3′,5′-cyclic nucleoside methylphosphonothioate **79** has also been prepared from **77**.[96]

Pure (R_P)-and (S_P)-**80** have been prepared from the active methylphosphonate esters **81** and thiols, followed by *S*-dealkylation with benzenethiolate ions.[97] These products reacted slowly with 5′-*O*-tosylnucleosides to give the 5′-*S*-methylphosphonothioates **82**.[98] A similar dithymidine 5′-*S*-methylphosphonothioate **83** has been prepared by Wickstrom *et al.* from **73** and 5′-deoxy-5′-iodo-3′-*O*-acetylthymidine;[99] the alkylation reaction was very slow at room temperature, but was complete in less than 6 h at 50 °C.





5. CONCLUSION

The last ten years have seen a rapid development of nucleic acid chemistry which has made it possible to prepare a number of modified oligonucleotides and to study their

properties. This is also true for nucleotides modified by introduction of sulfur in the phosphate groups as described above. Such a modification has only minor effects on the structure and polarity of the nucleotides, although enzymes often are sufficiently sensitive to reject them as substrates. The larger and more polarizable sulfur atom(s) makes the modified nucleotides more hydrophobic and also changes the geometry of the thiophosphate group somewhat; in addition a sulfur atom replacing a 3′- or a 5′-oxygen atom increases the intrastrand nucleoside distance. All these changes influence to some degree the ability of sulfur-modified oligonucleotides to bind to DNA or RNA. Chemically, phosphate derivatives with a P=S bond are more stable towards hydrolysis than their unmodified counterparts, whereas the opposite is the case for derivatives with a P–SR group. The ability of modified oligonucleotides to form a double helix with DNA or RNA under physiological conditions, to penetrate cell walls, to resist degradation by nucleases better than unmodified oligonucleotides, and their chemical stability, are the basis for their pharmacological usefulness. The phosphorothioates **2** fulfil these criteria, as may several of the other sulfur-modified oligonucleotides, although the latter have been less well studied so far.

Preparation of nucleoside phosphorothioates and related compounds is not possible from the natural nucleotides, because direct exchange of an oxygen atom with sulfur, using e.g. Lawesson's reagent, would exchange the carbonyl oxygen atoms in the nucleobases as well as the phosphate oxygen atoms. Phosphorylation with thiophosphoryl compounds occurs much more slowly (typically by a factor of 30–60[33,100]) than phosphorylation with phosphoryl compounds, but particularly reactive thiophosphorylation reagents (e.g. **5** and **23**) have been developed for this purpose. Phosphorylation with reagents containing *S*-alkyl groups is not retarded, and provided the alkyl group can be selectively removed (2-cyanoethyl, 2,4-dichlorobenzyl) these reagents function well. A third way to overcome the reactivity problem is to prepare phosphoranilidates (e.g. **9**) and exchange the anilido group with sulfur by treatment with sodium hydride and carbon disulfide. Tervalent phosphorus reagents are far more reactive than their pentavalent counterparts, and for the preparation of nucleoside *O*-phosphorothioates the phosphoramidite method in combination with oxidation with sulfur or the new sulfurization reagents **32–34** is probably the best approach. The surprisingly low reactivity of thiophosphoramidites and the instability of the thiophosphoramidites and thiophosphite intermediates when activated with tetrazole, however, make such methods less suitable for the preparation of nucleoside 3′- or 5′-*S*-phosphorothioates or phosphorodithioates.

Nucleoside phosphorotrithioates and tetrathioates have to my knowledge not been described, and the same holds for several isomers of the described nucleotide analogs. Some of these would seem straightforward to prepare from known precursors. Further work on the preparation and evaluation of such modified nucleotides will undoubtedly remain a very stimulating field of research for the organic chemist in the years to come.

6. REFERENCES

1. J. S. Cohen, *Oligodeoxynucleotides. Antisense Inhibitors of Gene Expression* (The Macmillan Press Ltd, London, 1989).
2. E. Uhlmann and A. Peyman, *Chem. Rev.*, **90**, 543 (1990).

3. F. Eckstein, *J. Am. Chem. Soc.*, **88,** 4292 (1966).
4. A. W. Murray and M. R. Atkinson, *Biochemistry*, **7,** 4023 (1968).
5. K.-F. R. Sheu, J. P. Richard and P. A. Frey, *Biochemistry*, **18,** 5548 (1979).
6. R. S. Brody and P. A. Frey, *Biochemistry*, **20,** 1245 (1981).
7. B. A. Connolly, P. J. Romaniuk and F. Eckstein, *Biochemistry*, **21,** 1983 (1982).
8. B. V. L. Potter and F. Eckstein, *J. Biol. Chem.*, **259,** 14243 (1984).
9. S. Chladek and J. Nagyvary, *J. Am. Chem. Soc.*, **94,** 2079 (1972).
10. J. E. Marugg, C. van den Bergh, M. Tromp, G. A. van der Marel, W. J. van Zoest and J. H. van Boom, *Nucleic Acids Res.*, **12,** 9095 (1984).
11. A. F. Cook, *J. Am. Chem. Soc.*, **92,** 190 (1970).
12. R. Cosstick and D. M. Williams, *Nucleic Acids Res.*, **15,** 9921 (1987).
13. Z. J. Lesnikowski, W. Niewiarowski, W. S. Zielinski and W. J. Stec, *Tetrahedron*, **40,** 15 (1984).
14. N. T. Thuong and M. Chassignol, *Tetrahedron Lett.*, **28,** 4157 (1987).
15. T. Horn and M. S. Urdea, *Tetrahedron Lett.*, **27,** 4705 (1986).
16. F. Eckstein and R. S. Goody, *Biochemistry*, **15,** 1685 (1976).
17. R. S. Goody and M. Isakov, *Tetrahedron Lett.*, **27,** 3599 (1986).
18. F. Eckstein, *Ann. Rev. Biochem.*, **54,** 367 (1985).
19. J. Ludwig and F. Eckstein, *J. Org. Chem.*, **54,** 631 (1989).
20. R. S. Goody and F. Eckstein, *J. Am. Chem. Soc.*, **93,** 6252 (1971).
21. H. F. Haikal, C. Chavis, A. Pompon and J.-L. Imbach, *Bull. Soc. Chim. Fr.*, 521 (1989).
22. J. Ludwig and F. Eckstein, *Proceedings, 9th International Round Table Nucleosides, Nucleotides & their Biological Applications*, p. 87 (Uppsala, 1990).
23. J. Baraniak and W. J. Stec, *J. Chem. Soc. Perkin Trans. I*, 1645 (1987).
24. F. Eckstein and U. Kutzke, *Tetrahedron Lett.*, **27,** 1657 (1986).
25. E. de Vroom, G. A. van der Marel and J. H. van Boom, *Recl. Trav. Chim. Pays-Bas*, **106,** 577 (1987).
26. H.-G. Genieser, W. Dostmann, U. Bottin, E. Butt and B. Jastorff, *Tetrahedron Lett.*, **29,** 2803 (1988).
27. D. A. Shuman, J. P. Miller, M. B. Scholten, L. N. Simon and R. K. Robins, *Biochemistry*, **12,** 2781 (1973).
28. M. Morr, L. Ernst and R. Mengel, *Liebigs Ann. Chem.*, 651 (1982).
29. F. Eckstein and H. Gindl, *Chem. Ber.*, **101,** 1670 (1968).
30. D. Dunaway-Mariano, *Tetrahedron*, **32,** 2991 (1976).
31. P. M. J. Burgers and F. Eckstein, *Biochemistry*, **18,** 592 (1979).
32. P. J. Romaniuk and F. Eckstein, *J. Biol. Chem.*, **257,** 7684 (1982).
33. Ö. Kemal, C. B. Reese and H. T. Serafinowska, *J. Chem. Soc., Chem. Commun.*, 591 (1983).
34. P. M. J. Burgers and F. Eckstein, *Tetrahedron Lett.*, 3835 (1978).
35. J. F. Marlier and S. J. Benkovic, *Tetrahedron Lett.*, **21,** 1121 (1980).
36. M. J. Nemer and K. K. Ogilvie, *Tetrahedron Lett.*, **21,** 4149 (1980).
37. B. Uznanski, W. Niewiarowski and W. J. Stec, *Tetrahedron Lett.*, **23,** 4289 (1982).
38. W. J. Stec, G. Zon, W. Egan and B. Stec, *J. Am. Chem. Soc.*, **106,** 6077 (1984).
39. B. A. Connolly, B. V. L. Potter, F. Eckstein, A. Pingoud and L. Grotjahn, *Biochemistry*, **23,** 3443 (1984).
40. J. Ott and F. Eckstein, *Biochemistry*, **26,** 8237 (1987).
41. C. A. Stein, C. Subasinghe, K. Shinozuka and J. S. Cohen, *Nucleic Acids Res.*, **16,** 3209 (1988).
42. R. P. Iyer, L. R. Phillips, W. Egan, J. B. Egan and S. L. Beaucage, *J. Org. Chem.*, **55,** 4693 (1990).
43. F. Morvan, B. Rayner and J.-L. Imbach, *Tetrahedron Lett.*, **31,** 7149 (1990).
44. P. C. J. Kamer, H. C. P. F. Roelen, H. van den Elst, G. A. van der Marel and J. H. van Boom, *Tetrahedron Lett.*, **30,** 6757 (1989).
45. H. Vu and B. Hirschbein, *Tetrahedron Lett.*, **32,** 3005 (1991); *Sulfurization with TETD: Phosphorothioate Oligonucleotide Synthesis Via Phosphoramidite Chemistry. User Bulletin No. 58* (Applied Biosystems, Foster City, California 1991).
46. G. Slim and M. J. Gait, *Nucleic Acids Res.*, **19,** 1183 (1991).
47. B. C. Froehler, *Tetrahedron Lett.*, **27,** 5575 (1986).
48. *Oligonucleotide Synthesis with Hydrogen-Phosphonate Monomers. User Bulletin No.* 44 (Applied Biosystems, Foster City, California 1987).
49. S. Agrawal, T. Ikeuchi, D. Sun, P. S. Sarin, A. Konopka, J. Maizel and P. C. Zamecnik, *Proc. Natl. Acad. Sci.*, **86,** 7790 (1989).
50. S. Agrawal and J.-Y. Tang, *Tetrahedron Lett.*, **31,** 7541 (1990).
51. V. A. Efimov, I. Y. Dubey and O. G. Chakhmakhcheva, *Nucleosides Nucleotides*, **9,** 473 (1990).
52. M. Fujii, K. Ozaki, A. Kume, M. Sekine and T. Hata, *Tetrahedron Lett.*, **27,** 935 (1986).
53. Z. J. Lesnikowski and M. M. Jaworska, *Tetrahedron Lett.*, **30,** 3821 (1989).

54. R. Cosstick and F. Eckstein, *Biochemistry*, **24,** 3630 (1985).
55. M. Koziolkiewicz, B. Uznanski and W. J. Stec, *Nucleosides Nucleotides*, **8,** 185 (1989).
56. P. S. Nelson, C. T. Bach and J. P. H. Verheyden, *J. Org. Chem.*, **49,** 2314 (1984).
57. K. Kariko, R. W. Sobol, L. Suhadolnik, S. W. Li, N. L. Reichenbach, R. J. Suhadolnik, R. Charubala and W. Pfleiderer, *Biochemistry*, **26,** 7127 (1987).
58. R. Cosstick and J. S. Vyle, *J. Chem. Soc., Chem. Commun.*, 992 (1988).
59. R. Cosstick and J. S. Vyle, *Tetrahedron Lett.*, **30,** 4693 (1989).
60. R. Cosstick and J. S. Vyle, *Nucleic Acids Res.*, **18,** 829 (1990).
61. A. M. Michelson, *J. Chem. Soc.*, 979 (1962).
62. A. Hampton, L. W. Brox and M. Bayer, *Biochemistry*, **8,** 2303 (1969).
63. J. Kresse, K. L. Nagpal, J. Nagyvary and J. T. Uchic, *Nucleic Acids Res.*, **2,** 1 (1975).
64. V. P. Kumarev, V. S. Bogachev, V. F. Kobzev and L. V. Baranova, *Soviet J. Bioorg. Chem.*, **8,** 795 (1982).
65. V. P. Kumarev, V. S. Bogachev, V. F. Kobzev, L. V. Baranova, M. I. Rivkin and V. N. Rybakov, *Soviet J. Bioorg. Chem.*, **8,** 803 (1982).
66. V. S. Bogachev and V. P. Kumarev, *Soviet J. Bioorg. Chem.*, **11,** 821 (1985).
67. V. S. Bogachev, V. P. Kumarev and V. N. Rybakov, *Soviet J. Bioorg. Chem.*, **12,** 64 (1986).
68. C. Sund and J. Chattopadhyaya, *Tetrahedron*, **45,** 7523 (1989).
69. F. Eckstein, *J. Am. Chem. Soc.*, **92,** 4718 (1970).
70. J. Nielsen, W. K.-D. Brill and M. H. Caruthers, *Tetrahedron Lett.*, **29,** 2911 (1988).
71. A. Grandas, W. S. Marshall, J. Nielsen and M. H. Caruthers, *Tetrahedron Lett.*, **30,** 543 (1989).
72. B. H. Dahl, K. Bjergårde, V. B. Sommer and O. Dahl, *Nucleosides Nucleotides*, **8,** 1023 (1989).
73. C. E. Dreef, C. M. Dreef-Tromp, G. A. van der Marel and J. H. van Boom, *Synlett.*, 481 (1990).
74. W. K.-D. Brill, J. Nielsen and M. H. Caruthers, *Tetrahedron Lett.*, **29,** 5517 (1988).
75. N. Farschtschi and D. G. Gorenstein, *Tetrahedron Lett.*, **29,** 6843 (1988).
76. B. H. Dahl, K. Bjergårde, V. B. Sommer and O. Dahl, *Acta Chem. Scand.*, **43,** 896 (1989).
77. W. K.-D. Brill, J.-Y. Tang, Y.-X. Ma and M. H. Caruthers, *J. Am. Chem. Soc.*, **111,** 2321 (1989).
78. M. H. Caruthers, G. Beaton, K. Cummins, D. Dellinger, D. Graff, Y.-X. Ma, W. S. Marshall, H. Sasmor, P. Shankland, J. V. Wu and E. K. Yau, *Nucleosides Nucleotides*, **10,** 47 (1991).
79. K. Bjergårde, B. H. Dahl and O. Dahl, *Nucleosides Nucleotides*, **10,** 461 (1991).
80. K. H. Petersen and J. Nielsen, *Tetrahedron Lett.*, **31,** 911 (1990).
81. J. Stawinski, M. Thelin and R. Zain, *Tetrahedron Lett.*, **30,** 2157 (1989).
82. J. Stawinski, M. Thelin, E. Westman and R. Zain, *J. Org. Chem.*, **55,** 3503 (1990).
83. G. M. Porritt and C. B. Reese, *Tetrahedron Lett.*, **30,** 4713 (1989).
84. W. K.-D. Brill, E. K. Yau and M. H. Caruthers, *Tetrahedron Lett.*, **30,** 6621 (1989).
85. G. M. Porritt and C. B. Reese, *Tetrahedron Lett.*, **31,** 1319 (1990).
86. E. K. Yau, Y.-X. Ma and M. H. Caruthers, *Tetrahedron Lett.*, **31,** 1953 (1990).
87. B. H. Dahl, K. Bjergårde, J. Nielsen and O. Dahl, *Tetrahedron Lett.*, **31,** 3489 (1990).
88. B. H. Dahl, K. Bjergårde, J. Nielsen and O. Dahl, *Nucleosides Nucleotides*, **10,** 553 (1991).
89. K. A. Gallo, K.-L. Shao, L. R. Phillips, J. B. Regan, M. Koziolkiewicz, B. Uznanski, W. J. Stec and G. Zon, *Nucleic Acids Res.*, **14,** 7405 (1986).
90. P. Guga, M. Koziolkiewicz, A. Okruszek, B. Uznanski and W. J. Stec, *Nucleosides Nucleotides*, **6,** 111 (1987).
91. W. J. Stec, G. Zon, K. A. Gallo and R. A. Byrd, *Tetrahedron Lett.*, **26,** 2191 (1985).
92. D. P. Lawrence, C. Wenqiao, G. Zon, W. J. Stec, B. Uznanski and M. S. Broido, *J. Biomol. Struct. Dyn.*, **4,** 757 (1987).
93. M. J. Nemer and K. K. Ogilvie, *Tetrahedron Lett.*, **21,** 4153 (1980).
94. W. Niewiarowski, Z. J. Lesnikowski, A. Wilk, P. Guga, A. Okruszek, B. Uznanski and W. J. Stec, *Acta Biochim. Pol.*, **34,** 217 (1987).
95. W. K.-D. Brill and M. H. Caruthers, *Tetrahedron Lett.*, **28,** 3205 (1987).
96. H. C. P. F. Roelen, E. de Vroom, G. A. van der Marel and J. H. van Boom, *Nucleic Acids Res.*, **16,** 7633 (1988).
97. W. K.-D. Brill and M. H. Caruthers, *Tetrahedron Lett.*, **29,** 1227 (1988).
98. W. K.-D. Brill and M. H. Caruthers, *Nucleosides Nucleotides*, **8,** 1011 (1989).
99. A. V. Lebedev, J. P. Rife, H. W. Seligsohn, G. R. Wenzinger and E. Wickstrom, *Tetrahedron Lett.*, **31,** 855 (1990).
100. J. A. A. Ketelaar, H. R. Gersmann and K. Koopmans, *Recl. Trav. Chim. Pays-Bas*, **71,** 1253 (1952).