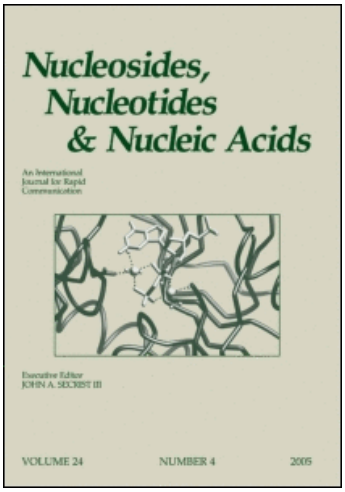


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Preparation and Properties of Oligodeoxynucleoside Phosphorodithioates

Kirsten Bjergarde[a]; Bjarne H. Dahl[a]; Otto Dahl[a]
[a] Department of General and Organic Chemistry, The H. C. Orsted Institute, University of Copenhagen, Copenhagen, Denmark

To cite this Article Bjergarde, Kirsten , Dahl, Bjarne H. and Dahl, Otto(1991) 'Preparation and Properties of Oligodeoxynucleoside Phosphorodithioates', Nucleosides, Nucleotides and Nucleic Acids, 10: 1, 461 — 464
To link to this Article: DOI: 10.1080/07328319108046498
URL: http://dx.doi.org/10.1080/07328319108046498

NUCLEOSIDES & NUCLEOTIDES, 10(1-3), 461-464 (1991)

PREPARATION AND PROPERTIES OF OLIGODEOXYNUCLEOSIDE PHOSPHORODITHIOATES

Kirsten Bjergårde, Bjarne H. Dahl, and Otto Dahl*

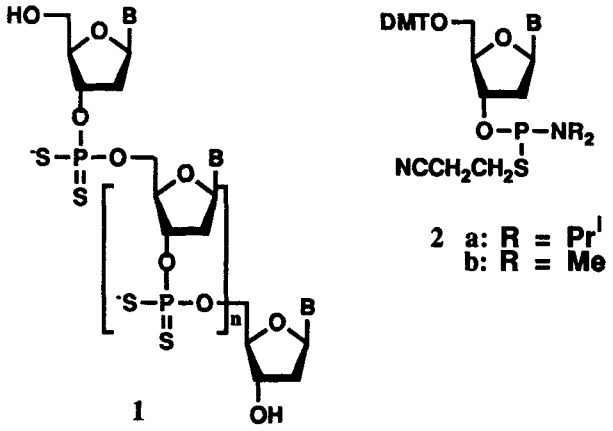
Department of General and Organic Chemistry, The H. C. Ørsted Institute, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark

Abstract: The thiophosphoramidite method for preparation of oligodeoxynucleoside phosphorodithioates is evaluated and four main problems identified. Preliminary results on some properties of phosphorodithioates are given.

Modified oligodeoxynucleotides with improved nuclease resistance are of interest as antisense compounds which can bind to selected mRNA and stop translation of essential proteins of *e.g.* HIV vira.[1] Phosphorodithioates **1**, first prepared in 1988[2], are promising antisense compounds which can be made from thiophosphoramidites **2** like unmodified oligonucleotides.[3] We have encountered several problems in using the thiophosphoramidite method on solid supports and discuss four main problems below.

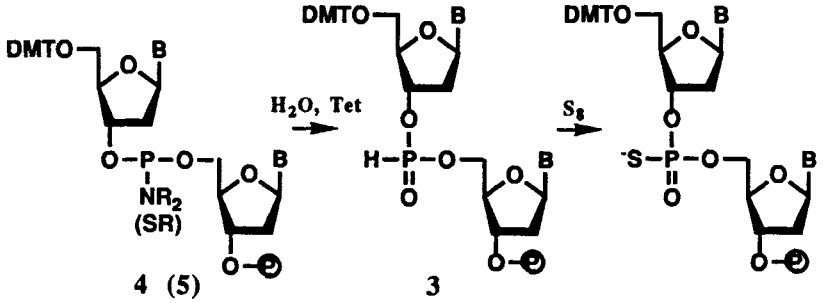
The first problem is the low reactivity of thiophosphoramidites. With tetrazole catalysis the cyanoethyl *N,N*-diisopropylthiophosphoramidite **2a** couples with a rate that is about 300 times less than that of the corresponding phosphoramidite under identical conditions.[4] This problem can be partly overcome by using more reactive thiophosphoramidites like **2b**.[3,4] These are still not so reactive as normal amidites, giving only about 85% coupling after one minute, but two couplings and oxidations with sulphur result in an acceptable 94-98% DMT-efficiency.

The next problem is more serious. In our hands, dithioates prepared on solid supports always contain some monothioate impurities, 4-15% according to ^{31}P NMR. We have examined the likely origin of monothioates and excluded, we think, obvious sources like oxygen in the solvents, impure sulphur, incomplete oxidation with sulphur, and replacement of sulphur with oxygen during capping, detritylation or deblocking with aq. ammonia. Most probably monothioate impurities are formed by oxidation with sulphur of *H*-phosphonates **3**,

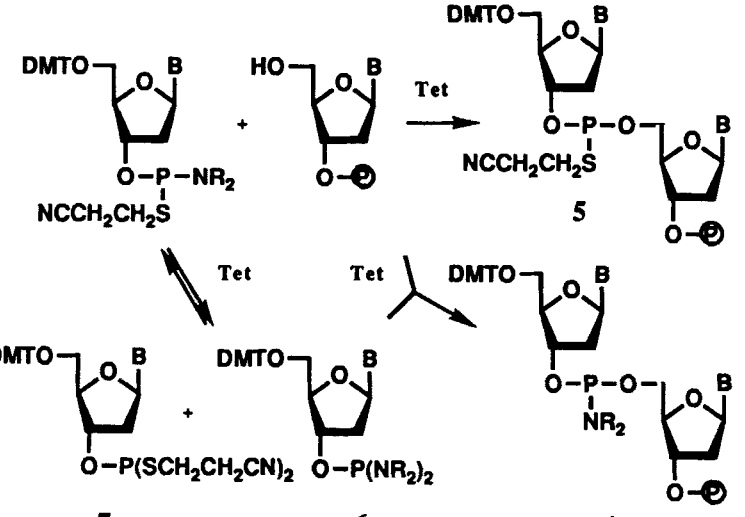


1

2 a: R = Pr^{i}
b: R = Me



4 (5) 3

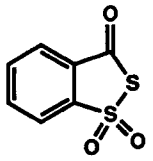


7 6 4

Scheme

the latter appearing in the products as a result of hydrolysis of incorporated phosphoramidites **4**, and maybe also of the primary coupling products, thiophosphites **5** (Scheme). Incorporated phosphoramidites **4** are formed because thiophosphoramidites in the presence of tetrazole are prone to dismutate to nucleoside phosphorodiamidites **6** and dithiophosphites **7**.[4] The diamidites are much more reactive than thioamidites, and even small amounts are likely to give substantial amounts of incorporated phosphoramidites, and hence monothioates after hydrolysis and oxidation with sulphur. Thiophosphites are also hydrolysed in the presence of tetrazole, although more slowly than thiophosphoramidites, and much more slowly than phosphoramidites.

The third problem arises from the necessity to oxidize with elemental sulphur, which is a slow process (5 min is used for each of the two coupling steps in a cycle) and requires a solvent shift to pyridine/carbon disulphide. Both factors increase the danger that some thiophosphite linkages are hydrolysed before they are oxidised. We have tried to use instead the new sulphurization reagent **8**[5] which however gives an increased amount of monothioate impurities, and some disulphides **9**[6] which unfortunately react very slowly.



8

RC(O)SSC(O)R **9**

The fourth problem is the selective removal of the *S*-protecting groups, which is much more difficult than removal of the corresponding *O*-protecting groups. Side reactions like substitution of the RS group at phosphorus (OH^- gives monothioates) or nucleophilic attack at the 5'-carbon (cleavage of the dithioate linkage) are likely to occur if the protecting group is not sufficiently labile. The 2-cyanoethyl group we use can be removed with less than 1% monothioate formation.[4] The 2,4-dichlorobenzyl group[3] is likewise an acceptable protecting group, but removal of a 4-chlorobenzyl group, and particularly a methyl group, with thiophenolate is accompanied by cleavage of the dithioate linkage (*ca.* 3% and 35%, resp.).

These problems with the thiophosphoramidite method makes it worthwhile to explore methods to prepare phosphorodithioates which circumvents labile tertiary thiophosphorus compounds. Caruthers[7] and we[8] have recently published different dithiotriester methods which however still have to be adapted for solid support synthesis.

The properties of oligonucleoside phosphorodithioates have not yet been intensively studied because of our lack of longer, pure oligomers. We have measured the melting points of some 20-mers hybridized to a complementary, unmodified 20-mer DNA (Table). The

Table. T_m for 20-mers, medium salt (140 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.4).

d(ACA CCC AAT TCT GAA AAT GG)	61	°C
d(ACA CCC AAT TCsT GAA AAT GG)	59	-
d(AsCsAs CCC AAT TCT GAA AAT GG)	58	-
d(AsCsA CCC AAT TCT GAA AATs GsG)	58	-
d(AsCsAs CsCsCs AsAsTs TsCsTs GsAsAs AsAsTs GsG)	41	-

reduction of *ca.* 1° pr. dithioate linkage is similar to that found for monothioates. The dithioate linkage is very stable towards nucleases as also found by Caruthers[2,9] and Reese[10]. Compounds with several dithioate linkages at the 5'-end are difficult to kinase with T4 kinase and γ-^{32}P-ATP, although they are labeled under forcing conditions.

Acknowledgements: Financial support from the Danish Natural Science Research Council and from Lundbeckfonden is gratefully acknowledged.

References:

1. Cohen, J. S. (ed.) *Oligodeoxynucleotides: Antisense Inhibitors of Gene Expression.* MacmillanPress: London, 1989.
2. Nielsen, J., Brill, W. K.-D., and Caruthers, M. H., *Tetrahedron Lett.* **29**, 2911 (1988).
3. Brill, W. K.-D., Tang, J.-Y., Ma, Y.-X., and Caruthers, M. H., *J. Am. Chem. Soc.* **111**, 2321 (1989).
4. Dahl, B. H., Bjergårde, K., Sommer, V. B., and Dahl, O., *Acta Chem. Scand.* **43**, 896 (1989).
5. Iyer, R. P., Egan, W., Regan, J. B., and Beaucage, S. L., *J. Am. Chem. Soc.* **112**, 1253 (1990).
6. Kramer, P. C. J., Roelen, H. C. P. F., van den Elst, H., van der Marel, G. A., and van Boom, J. H., *Tetrahedron Lett.* **30**, 6757 (1989).
7. Yau, E. K., Ma, Y.-X., and Caruthers, M. H., *Tetrahedron Lett.* **31**, 1953 (1990).
8. Dahl, B. H., Bjergårde, K., Nielsen, J., and Dahl, O., *Tetrahedron Lett.* **31**, 3489 (1990).
9. Grandas, A., Marshall, W. S., Nielsen, J., and Caruthers, M. H., *Tetrahedron Lett.* **30**, 543 (1989).
10. Porritt, G. M. and Reese, C. B., *Tetrahedron Lett.* **31**, 1319 (1990).