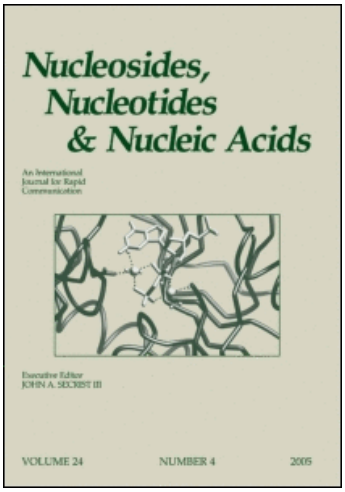


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of Oligonucleotides Covalently Linked to Intercalating Agents and to Reactive Groups

Ulysse Asseline[a]; Nguyen T. Thuong[a]
[a] Centre de Biophysique Moléculaire, C.N.R.S. lA, Orléans Cedex 2, France

To cite this Article Asseline, Ulysse and Thuong, Nguyen T.(1991) 'Synthesis of Oligonucleotides Covalently Linked to Intercalating Agents and to Reactive Groups', Nucleosides, Nucleotides and Nucleic Acids, 10: 1, 359 — 362
To link to this Article: DOI: 10.1080/07328319108046479
URL: http://dx.doi.org/10.1080/07328319108046479

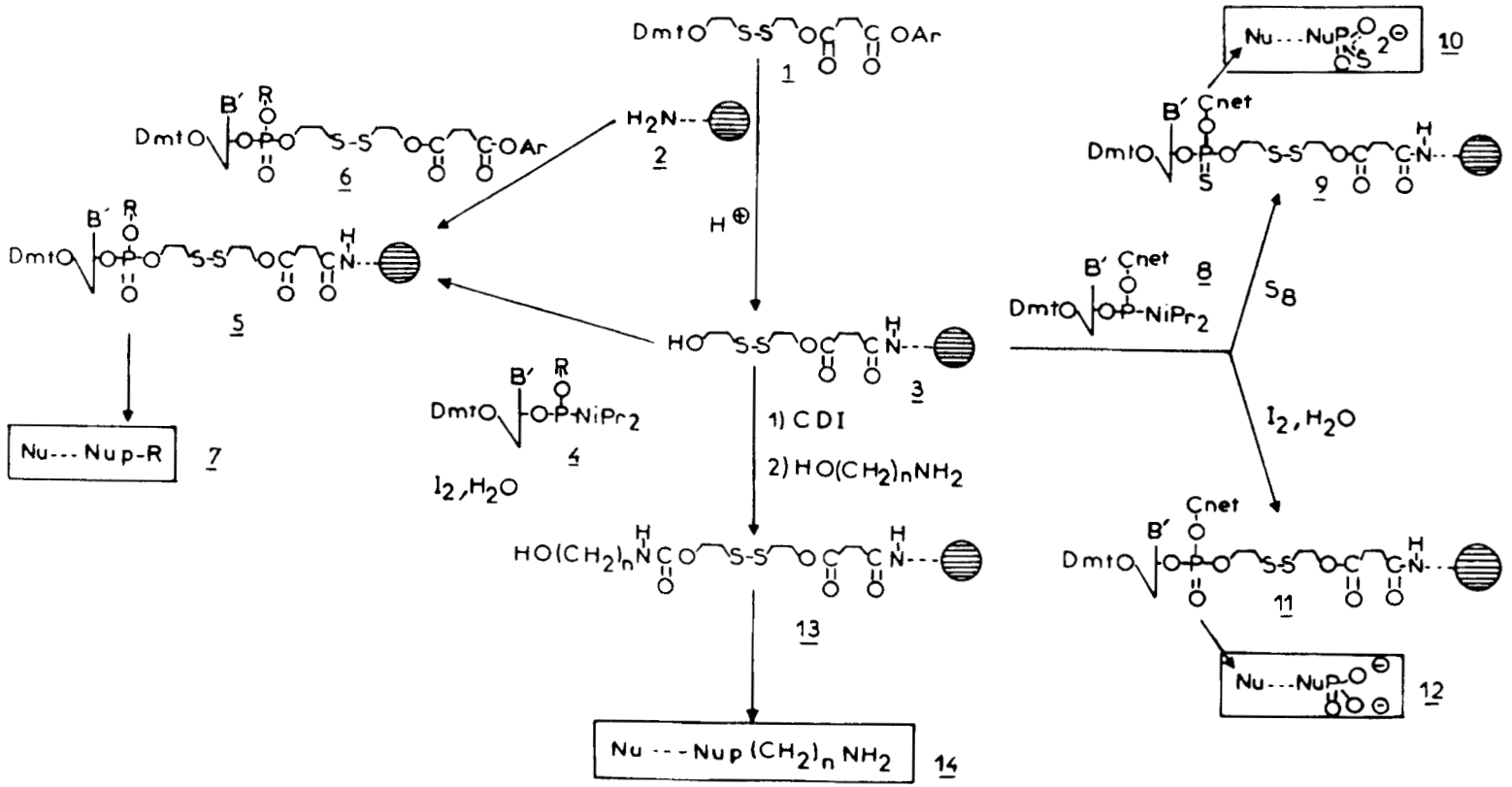
NUCLEOSIDES & NUCLEOTIDES, 10(1-3), 359-362 (1991)

SYNTHESIS OF OLIGONUCLEOTIDES COVALENTLY LINKED TO INTERCALATING AGENTS AND TO REACTIVE GROUPS

Ulysse Asseline and Nguyen T. Thuong
Centre de Biophysique Moléculaire, C.N.R.S.
1A, Avenue de la Recherche Scientifique
45071 - Orléans Cedex 2 (France)

Abstract : The use of modified supports involving 2,2'-dithiodiethanol allows solid-phase synthesis of oligonucleotides derivatized at their 3' end with an acridine or a phenanthroline derivative or with phosphate, thiophosphate and amino group.

The increasing use of synthetic oligonucleotides covalently linked to various ligands (such as non radioactive labels[1], intercalating agents and reactive groups[2]) as tool of molecular biology and as potential agents of gene expression regulation led to the development of various chemical procedures for their synthesis [3-6]. In some cases reactive groups can be directly incorporated at preselected positions of the oligonucleotides during the chain elongation on solid-phase by using modified nucleosides containing the masked reactive groups. In the other cases the substituent groups are not stable under the chemical conditions required for deprotection of the oligodeoxyribonucleotides and therefore need to be coupled with unblocked oligonucleotides. Various methods to obtain automated synthesis of modified oligonucleotides at their 5' end or at the internucleotide bond level were widely reported in the literature, but the solid phase preparation of 3' modified oligodeoxyribonucleotides is more difficult to carry out. We report below the use of modified supports involving the 2,2'-dithiodiethanol group which allow the preparation of oligonucleotides derivatized at their 3' end with an acridine or a phenanthroline derivative or with, phosphate, thiophosphate and primary amino group which can then be reacted with convenient ligands.



Dmt = dimethoxytrityl, B' = protected nucleic bases, H_2N—● = aminopropyl-Fractosil 500 ou CPG/Long Chain Alkylamine ; Ar=p-nitrophenyl ; iPr=Isopropyl ; R=2-methoxy-6-chloro-9-ωpentylaminoacridine or 5-(nonanoamido)-1,10 phenanthroline ; Cnet=cyanoethyl ; Nup=Nucleoside-3'-phosphate ; CDI=Carbonyldiimidazole.

As shown on the scheme the direct incorporation of a substituent group R (2-methoxy-6-chloro-9-pentylaminoacridine or 5-nonanoamido-1,10-phenanthroline) at the 3' end of the oligonucleotide needs the preparation of the nucleoside-3'-acridinyl (or phenanthrolinyl) phosphate attached via the 2,2'-dithiodiethanol derivative to the support 5. This can be achieved by using two ways. The faster method involves the synthesis of the nucleoside phosphoramidite bearing acridine derivative 4 which was then reacted with the hydroxyl of 2,2'-dithiodiethanol immobilized on the support 3 (obtained via the activated ester 1 by analogy with the classical procedures [7]). The second way uses a nucleoside-3'-phosphotriester intermediate $Nu'P(O)(OR)OCH_2CH_2S\text{-}SCH_2CH_2OH$ which was obtained by transesterification of the aryl ester $Nu'P(O)(OR)OAr$ with 2,2'-dithiodiethanol. The hydroxyl function was then used to tether (via succinylated and activated ester intermediate 6) the nucleoside phosphotriester to the support 2 to give 5. Although the latter is long it is a more convenient route because the purification of the phosphotriester derivative is easier than that of the phosphoramidite intermediate and so after fully unblocking of the oligonucleotide and its cleavage from the support by a treatment with dithiothreitol and 0.4 M aqueous NaOH, a much better purity was obtained for 3' derivatized oligonucleotide 7.

The modified support derivatized with 2,2'-dithodiethanol 3 can also be used to obtain oligonucleotides bearing various functional groups at their 3' ends which can be subsequently coupled with different chemical reagents.

The synthesis of oligonucleotides with 3' end phosphorothioate function 10 was achieved via the nucleoside 3'-thiophosphate derivatized support 9 which was obtained by coupling the hydroxyl group of the support 3 with 5'-O-dimethoxytrityl protected nucleoside-3'-(β -cyanoethyl) diisopropylamidophosphite 8 followed by the addition of sulfur to the intermediate phosphite. After elongation of the oligonucleotide chain by using the classical phosphoramidite procedure the unblocking of the oligonucleotide and its cleavage from the support were achieved simultaneously by treatment with a mixture of dithiotreitol and concentrated aqueous ammonia. The obtained 3'-phosphorothioate containing oligonucleotides 10 can be then reacted with halogenoalkyl derivatives in aqueous or organic media (in the presence of crown ether

to solubilize the oligonucleotide) to afford the oligonucleotide 3'-phosphorothioloester Nup---NupsR.

The direct use of 2,2'-dithiodiethanol derivatized support 3 to carrying out chain elongation 11 leads after full unblocking of the oligonucleotide and its releasing from the support by the previously described treatment, to oligonucleotide with 3' terminal phosphate 12 which can be coupled with amino compound leading to phosphoramidate derivative.

Immobilization of an aliphatic aminoalcohol on the previously described 2,2'-dithiodiethanol derivatized support 3 via a carbamate linkage afford the support 13 with a free hydroxyl group. After assembly of the oligonucleotide chain the carbamate linkage can be cleaved from the support (simultaneously with the unblocking of the oligonucleotide) by treatment with aqueous ammonia and dithiothreitol to generate 3'-primary amino derivatized oligonucleotide 14 which can then be reacted with electrophilic agents.

The above mentioned methods were developed with natural (β-D) and unnatural (α-D, β-L and α-L) oligodeoxyribonucleotides. The use of these derivatized supports, involving a disulfide bond, stable under an acidic or alkaline medium, allows moreover to make other chemical reactions at the 5' end of the oligonucleotides. The development of oligodeoxyribonucleotides biderivatized with two identical or different groups is now in progress.

Acknowledgments : This work was supported by the Federation Nationale des Centres de Lutte Contre le Cancer, Rhône-Poulenc Santé and Biomerieux.

References

1) Matthews J.A. and Kricka L.J. Analytical Biochemistry (1988) 169, 1-25.
2) Oligodeoxynucleotides Antisense Inhibitors of Gene Expression In Topics in Molecular and Structural Biology. N° 12. Ed. by J.S. Cohen Mac Millan Press (1989).
3) Knorre D.G., Vlassov V.V. and Zarytova V.F. in Topics in Molecular and Structural Biologics N° 12. Ed. by J.S. Cohen Mac Millan Press (1989) 172-196.
4) Nguyen T. Thuong, Asseline U. and Montenay-Garestier T. In Topics in Molecular and Structural Biologics N° 12. Ed. by J.S. Cohen Mac Millan Press (1989) 25-51.
5) Shabarova Z. Biochimie 70 (1988) 1323-1334.
6) Chu B. and Orgel L. Nucl. Acids Res., 11 (1983) 6513-6529.
7) Asseline U. and Nguyen T. Thuong, Tetrahedron Lett. 30 (1989) 2521-2525.