Pergamon

Tetrahedron Letters 39 (1998) 8271–8274

TETRAHEDRON LETTERS

Phosphodisulfide Bond:A New Linker for the Oligonucleotide Conjugation

Marcel Chassignol and Nguyen T. Thuong*

Centre de Biophysique Moléculaire, UPR n°4301 du CNRS affiliated to the University of Orléans, Rue Charles Sadron, 45071 Orléans Cedex 02, France

Received 13 May 1998; accepted 1 September 1998

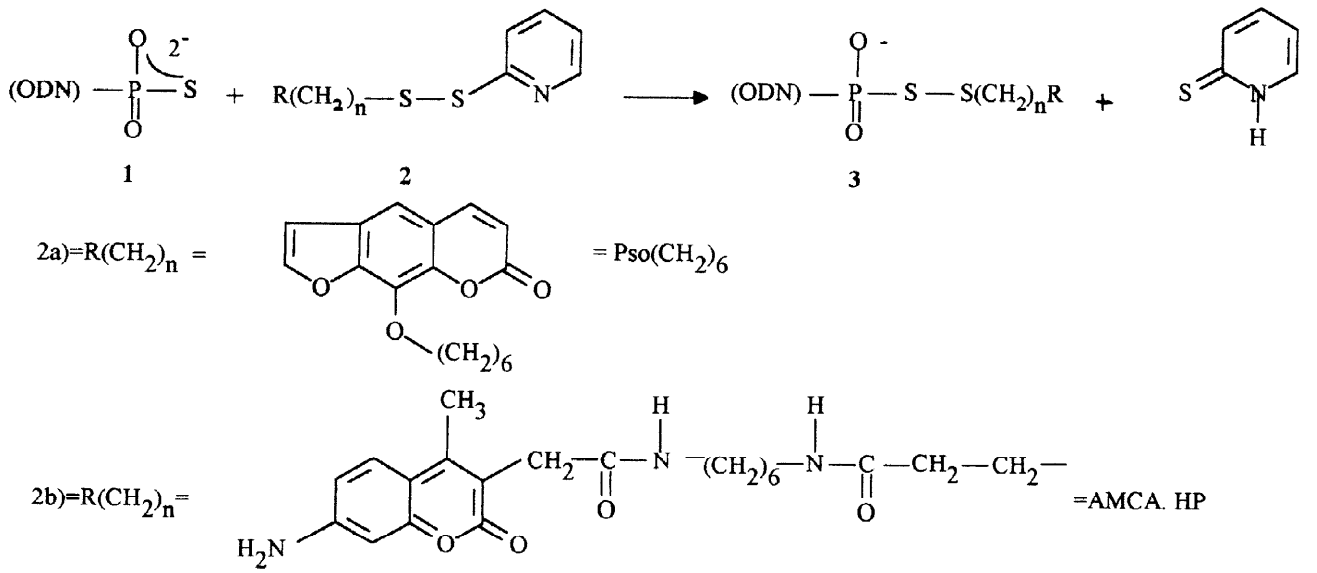
Abstract : Oligonucleotide thiophosphates react with 2-pyridyl-disulfide derivatives to give phosphodisulfide which can, upon reduction, be easily cleaved to give the starting oligonucleotide with a terminal thiophosphate group.

Very often disulfide bonds are used to covalently join an oligonucleotide to a dye [1]or an enzyme[2-6]. However,since thiol derivatives can easily form symmetric dimers in the presence of air oxygen, the method implies the regeneration of alkylsulfhydryl compounds. They need to be regenerated just before coupling, a process which is not easy to carry out when weak amounts of products are involved. To circumvent this drawback, we report here the synthesis of conjugated oligonucleotides **3** via a phosphodisulfide bridge which can be obtained by reaction of phosphorothioate oligonucleotide **1** with an alkyl-2-pyridyl disulfide derivative **2** (Scheme 1).

Terminal 5'or 3'thiophosphate oligonucleotides are easily obtained via solid phase synthesis by application of phosphoramidite chemistry using bis(2-cyanoethyl)diisopropylamidophosphite[7] and a 2,2'-dithiodiethyl-derivatized support[8,9], respectively. After purification by reversed-phase HPLC, oligonucleotides **1** can be kept in their monomer form as a solid after lyophilization or in aqueous solution without any special care. The synthesis of conjugated oligonucleotide **3** is carried out with a psoralen derivative used to cross-link either single- or double-stranded target sequences[10,11] and with a 7-amino-4-methylcoumarin-3-acetic (AMCA) derivative used as fluorescent label. The preparation of 2-pyridyldisulfide derivative of psoralen **2a** is achieved by condensation of psoralen sulfide with 2,2'-dipyridyl disulfide,[12] whereas 2-pyridyldisulfide derivative of AMCA-HP **2b** is available from Pierce. Coupling of oligonucleotide **1** in the presence of 15-crown-5 with a slight excess of 2-pyridyldisulfide compounds **2a** or **2b** in methanol[13] affords conjugates **3a** and **3b** with high yield (80%) after 2 or 3 hour reaction time at room temperature.

Fax: 02 38 63 15 17; E mail: chassign@cnrs-orleans.fr

0040-4039/98/$ - see front matter
PII: S0040-4039(98)01854-1



Scheme 1 : Phosphodisulfide bond formation

Reversed-phase HPLC analysis of the crude mixture obtained by coupling Sp-d$^{5'}$($T_4^{Me}CT_4^{Me}C_6T$)$^{3'}$ with compound **2a** shows a main peak corresponding to the 5'-psoralenyl oligonucleotide conjugate (Pso)-$(CH_2)_6$-S-S-pd($T_4^{Me}CT_4^{Me}C_6T$) which has a higher retention time than that of the starting oligonucleotide thiophosphate **1** (Figure 1). Conjugates **3** are easily purified by reversed-phase HPLC.(Table 1).

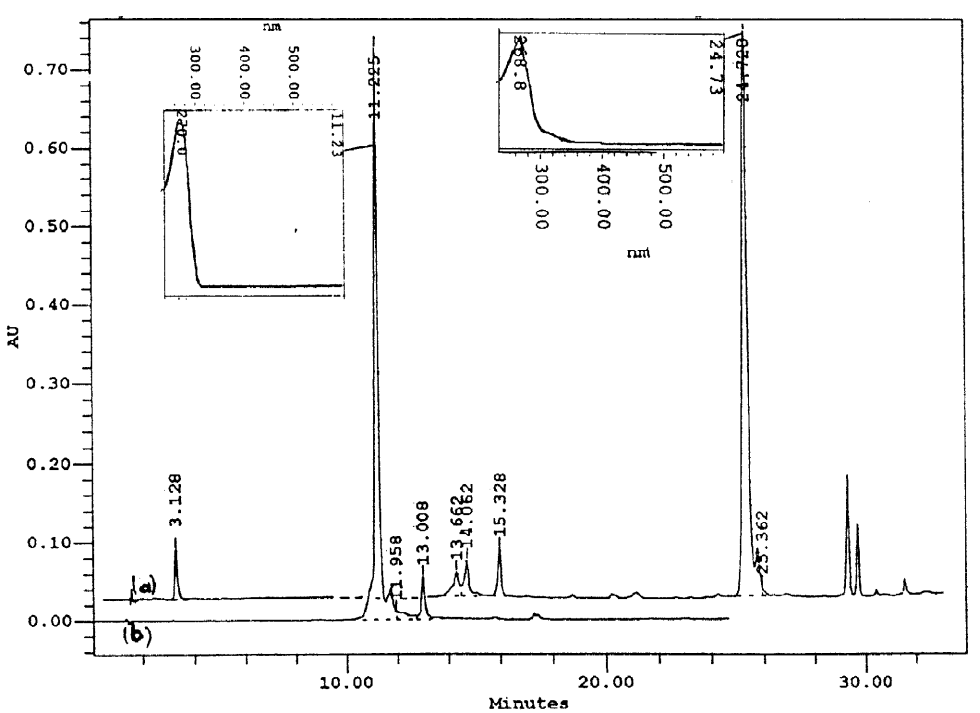


Figure 1: Reversed phase analysis of the crude Pso(-CH_2)$_6$-S-S-pd$^{5'}$($T_4^{Me}CT_4^{Me}C_6T$)$^{3'}$ Rt= 24.72 mn (a) obtained after coupling of spd$^{5'}$($T_4^{Me}CT_4^{Me}C_6T$)$^{3'}$ Rt =11.22 mn (b) with psoralen compound **2a** (for conditions see Table 1). The inserts show the UV visible absorption spectra between λ 240 and λ 600 nm of oligonucleotides **a** (right) and **b** (left).

	Rt (min)
$Spd^{5'}(T_4^{Me}CT_4^{Me}C_6T)^{3'}$	11.22
$Pso(CH_2)_6$-S-$Spd^{5'}(T_4^{Me}CT_4^{Me}C_6T)^{3'}$	24.73
AMCA HP-S-$Spd^{5'}(T_4^{Me}CT_4^{Me}C_6T)^{3'}$	14.75
$Spd^{5'}(T_4^{Me}C_2T^{Me}CTC_3TC_3T^{Me}CT)^{3'}$	16.28
$Pso(CH_2)_6$-S- $Spd^{5'}(T_4^{Me}C_2T^{Me}CTC_3TC_3T^{Me}CT)^{3'}$	24.75
$d^{5'}(CT_5C_2T_2CTCG)^{3'}pS$	17.68
$d^{5'}(CT_5C_2T_2CTCG)^{3'}pS$-S-$(CH_2)_6$-Pso	23.69

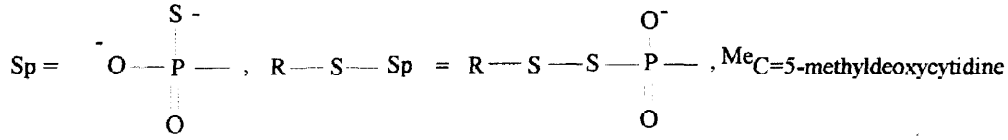


Table 1: Retention times (Rt) of oligonucleotides obtained by analysis on a Lichrospher 100 RP 18 (5µm) column, 4 x 125 mm (Merck) using the following eluents: 5% CH_3CN for 2 min. then a linear gradient of CH_3CN from 5 to 29 % for 20 min and 29 to 100% for 10 min, in 0.1 M aqueous triethylammonium acetate buffer pH 7, with a flow of 1 ml/min.

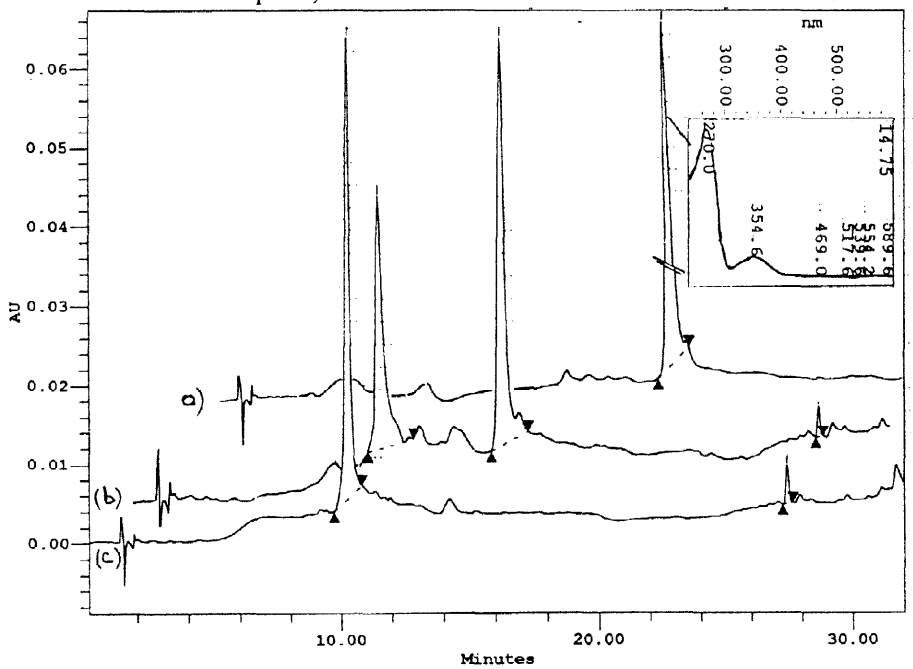


Figure 2: Reversed phase analysis on a Lichrospher 100 R.P. 18 (5µm) column (125mm x 4 mm) performed as described in Table 1: (**a**) AMCA-HP-S-S-$pd^{5'}(T_4^{Me}CT_4^{Me}C_6T)^{3'}$, (**b**) the mixture obtained after reaction with one equivalent of tris-carboxyethylphosphine (TCEP) for 10 mn and (**c**) for 60 mn. Insert absorption spectrum of the conjugated oligonucleotide exhibits the expected absorbance ratio at λ = 270 nm and λ = 354 nm in accordance with the published value of 8.000 $M^{-1}cm^{-1}$ for the λ max of AMCA.

Dialkyldisulfide derivatized oligonucleotide **3b** can be quantitatively reduced[14] to the corresponding thiol containing ligand and oligonucleotide **1** (Figure 2). The easy cleavage of the phosphodisulfide bond enables the use of 5'conjugated oligonucleotides as primers for DNA polymerases for the preparation of DNA fragments. After reduction of the conjugate **3b**, the obtained 5'-thiophosphate can be easily oxidized into a phosphate which could be useful for cloning.

Acknowledgements This work was supported by the «*Agence Nationale de Recherche contre le SIDA*» and Rhône-Poulenc Rorer.

References and notes

1. Zuckermann, R.; Corey, D.; Schultz, P. *Nucleic Acids Res.* **1987**, *15*, 5305 -5321.
2. Corey, R. D. ; Schultz, P. G. *Science* **1987**, *238*, 1401-1403.
3. Chu, B. C. F.; Orgel, L. E. *Nucleic Acids Res.* **1988**, *15*, 3671-3691.
4. Murakami, A.; Tada, J.; Yamagata, K.; Takamo, J. *Nucleic Acids Res.* **1989**, *16*, 5587-5595.
5. Zuckermann, R. N.; Schultz, P. G. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 1766-1770.
6. Corey, D. R.; Munoz-Medellin, D., Huang, A. *Bioconjugate Chem.* **1995**, *6*, 93-100.
7. Thuong, N. T.; Chassignol, M. *Tetrahedron Lett.* **1987**, *28*, 4157-4160.
8. Asseline, U. ; Thuong, N. T. *Tetrahedron Lett.* **1989**, *30*, 2521-2524.
9. Thuong, N. T.; Asseline, U. In *Oligonucleotides and Analogues: A practical approach*, F/.Eckstein ed.I.R.L.Press Oxford **1991**, 283-306.
10. Costes, B.; Girodon, E.; Ghanem, N.; Chassignol, M.; Thuong, N. T.; Dupret, D.; Goossens, M. *Human Molecular Genetics* **1993**, *2*, 393-397.
11. Takasugi, M.; Guendouz, A.; Chassignol, M.; Decout, J. L.; Lhomme, J.; Thuong, N. T.; Hélène, C. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 5602-5606.
12. 1.8 mmol of Pso$(CH_2)_6$I in a mixture of MeOH(2 ml) and DMF(6 ml) was added to a methanolic solution (3 ml) of NaSH (2 mmol) (obtained by bubbling H_2S through a sodium methoxide solution). The mixture was kept at room temperature under argon with magnetic stirring. After 4 h the iodinated compound was fully transformed into the thiol derivative and disulfide-containing dimer which can be easily separated by flash chromatography on silica gel using a CH_2Cl_2/MeOH mixture as eluent. Analytical TLC was carried out on Merck 5554 Kieselgel 60 F 254 plates and eluted with CH_2Cl_2/acetone/H_2O (95:4:1, v/v/v), thiol: Rf=0.57, yield 73% (0.43g); disulfide derivative, Rf=0.38 (0.15g). A mixture of thiol compound (0.75 mmol) obtained above and 2,2'-dipyridyl disulfide (3.7 mmol) in CH_2Cl_2 (5 ml) and MeOH (5ml) was kept at room temperature for 3 h. Psoralen-2-pyridyl disulfide **2a** was purified by flash chromatography on silica gel, yield 80% (0.26 g), Rf = 0.44 using CH_2Cl_2/acetone/H_2O (95:4:1 v/v/v).
13. The oligonucleotide-thiophosphate 1 (sodium salt) (34 nmol) was dissolved in MeOH (0.2 ml) in the presence of 15-crown-5 followed by addition of 2-pyridyl disulfide derivative **2** (45 nmol) and the mixture was incubated with stirring at room temperature for 4 h. The excess of compound **2** was removed by gel filtration G 25 from Pharmacia and the conjugated oligonucleotide was purified by HPLC.
14. Burns, J.A., Butler , J.C., Moran, J., and Whitesides, G.M., *J. Org. Chem.* **1991**,*56*, 2648-2650.