Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 8, pp. 1071-1076, 1994

0960-894X/94 $6.00+0.00

0960-894X(94)E0103-L

Synthesis and Physical Properties of Sulfonamide-Containing Oligonucleotides

Eric B. McElroy, Rajanikanth Bandaru, Jianxing Huang and Theodore S. Widlanski*

Department of Chemistry, Indiana University, Bloomington, Indiana 47405

Abstract: *Sulfonamide-linked thymidine dimers have been prepared and incorporated into oligodeoxynucleotides. Oligos containing up to four sulfonamide linkages have been tested for their ability to form duplexes with complementary DNA.*

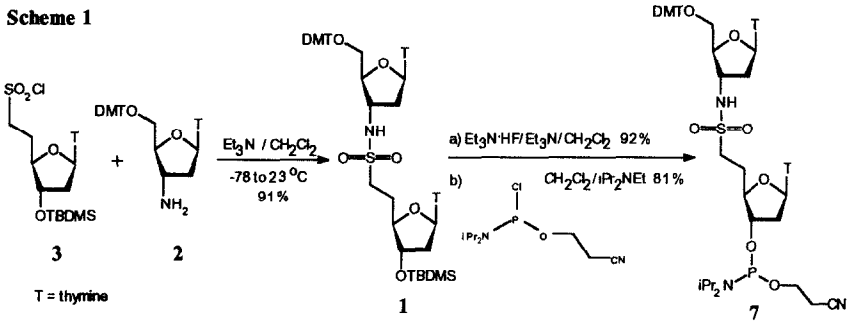
Introduction: Inhibition of gene expression by the use of short oligonucleotides known as 'antisense' oligonucleotides has recently become a highly active and promising field of research.[1] These agents may exert their effect via several different pathways. For example, oligonucleotides that form a duplex with a specific mRNA might trigger RNAseH degradation of the message, or block its interaction with ribosomes.[2] An antisense strategy for the inhibition of DNA transcription has also been reported.[3] One of the potential problems with antisense technology is that nucleases may degrade the antisense oligonucleotide before it can reach its target.[4] In addition, because oligonucleotides are charged, they must be actively transported across the cell membrane.[5]

A method for improving both the nuclease resistance and cellular uptake of oligomers is to neutralize the negatively charged phosphodiester backbone of an oligonucleotide. Uncharged analogs of DNA are relatively lipophilic and may therefore pass more efficiently through the cell membrane. The most widely used uncharged DNA analogue has a methyl phosphonate backbone.[6] However, methyl phosphonates are chiral at phosphorus. Oligomers containing methyl phosphonates are actually complex diastereomeric mixtures, some of which may have reduced effectiveness as antisense agents.[7] In addition, because of the added steric bulk of the methyl group, the methyl phosphonate may not be an accurate homologue of a phosphodiester. Recently, several oligonucleotides with sulfur instead of phosphorus in the backbone have been reported. For example, syntheses of sulfone[8a], sulfonate[8b-d], sulfonamide,[8b-e] sulfamate[8f] and

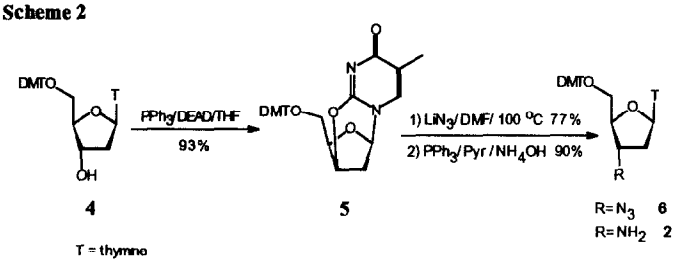
sulfide[8g-i] linked oligonucleotides have been achieved. These linkages have close structural homology to a phosphodiester, yet are uncharged and achiral. In this report we present a new synthesis of sulfonamide-linked dinucleosides, the incorporation of sulfonamide linkages into oligodeoxynucleotides and the physical properties of the resulting DNA.

Synthesis: To prepare the sulfonamide dimer, **1**, 3'-amino-3'-deoxy-5'-*O*-DMT-thymidine, **2**,[9] was synthesized as shown (Scheme 2) from 5'-*O*-DMT-thymidine, **4**. We then prepared the sulfonyl chloride **3** as previously reported.[8c, 10] The coupling of **2** and **3** was achieved in excellent yield by the use of Et_3N in CH_2Cl_2 at low temperatures (Scheme 1). To insert the sulfonamide dimer into an oligonucleotide, the 3'-*O*-TBDMS group was removed and then the 3'-OH was phosphitylated[11] as shown to give the phosphoramidite, **7**.

Scheme 1



Scheme 2



Physical Studies: To test the effect of the sulfonamide linkage on duplex stability, several oligonucleotides of the sequence $dGCT_8CG$ (GCT-#, # denoting the position of sulfonamides, see Figure 1) containing 0-4 sulfonamide linkages were synthesized using standard phosphoramidite chemistry and deprotection conditions. Using phosphoramidite **7** with a 5 min. coupling time, average coupling yields of >95% were routinely obtained. The complementary sequence $dCGA_8GC$ (CGA, **8**) was also synthesized. Oligonucleotides were purified by gel electrophoresis (PAGE) and desalted using a Sephadex® G-25 column before physical studies were performed. Oligos that were synthesized are shown in Table 1.

Figure 1. Oligodeoxynucleotide Sequence Used for Duplex Dissociation Studies.

GCT	5' d(	G	C	T	T	T	T	T	T	T	C	G	) 3'
Position #		11	10	9	8	7	6	5	4	3	2	1	
CGA (8)	3' d(	C	G	A	A	A	A	A	A	A	G	C	) 5'

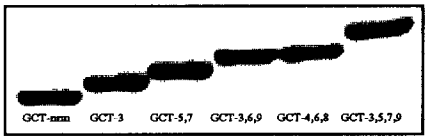


Figure 2. Migratory pattern of oligonucleotides containing sulfonamide linkages. A phosphorimage of a nondenaturing polyacrylamide gel (20%) of radiolabelled oligonucleotides containing 0-4 sulfonamide linkages. Sequences indicated are as described in the text.

The incorporation of the sulfonamide linkage and purity of the oligonucleotides was determined by gel electrophoresis. Several oligonucleotides were 5' end-labelled using γ-^{32}P ATP and T4 polynucleotide kinase. Figure 2 shows the phosphorimage of a gel of oligomers containing 0-4 sulfonamide linkages. As expected, incorporation of sulfonamide linkages into an oligonucleotide results in a decrease in its mobility as the charge to mass ratio is decreased.

Melting curves of the oligonucleotide duplexes were measured in a 10 mM PIPES solution (pH 7.0) containing 1 mM EDTA and the desired NaCl concentration. The strand

dissociation temperatures (Tm's) of the oligonucleotides were determined from the first derivative of the melting curves. Table 1 summarizes the melting data.

A marked decrease in the Tm of the duplexes occurs when 1 or more sulfonamide linkages are present. The effect on the Tm is greatest when the sulfonamide linkage is located within the center of the helix (compare GCT-3 and GCT-6). We had anticipated that the introduction of neutral sulfonamide linkages might stabilize a duplex by lessening charge-charge repulsions along the helix axis.

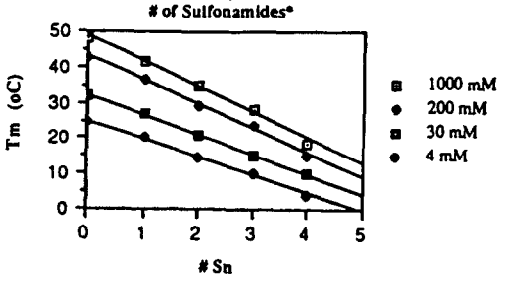
Table 1: Thermal Dissociation (°C) of Duplexes Containing Oligonucleotides with Sulfonamides*

	[Na^+]			
	5 mM	30 mM	200 mM	1000 mM
Normal Oligo				
GCT-Norm	24.6	31.8	42.9	48.1
Sulfonamide Oligos				
GCT-3	23.4	28 4	39.0	44 1
GCT-6	20 2	27 1	36 6	41.5
GCT-3,9	15.9	21 2	30 5	37.7
GCT-4,8	16.3	21.7	30.7	35 1
GCT-5,7	14.5	21 0	29.2	34.6
GCT-3,6,9	9 9	15.1	23.4	28.2
GCT-4,6,8	10.1	15 3	22.5	26.3
GCT-3,5,7,9	4.2	10.1	15.0	18 8

* in 10 mM PIPES, 1 mM EDTA, pH 7.0, total DNA conc. $2x10^{-6}$ M (1:1 ratio GCT CGA)

Figure 3 shows that the Tm of the oligomers is linearly dependent on the number of sulfonamides present. At lower salt concentrations, approximately 5 °C in Tm is lost per sulfonamide. At higher salt concentrations this increases to about 7 °C per sulfonamide. As expected, the ability of salt to stabilize a duplex is reduced as more sulfonamides are incorporated into the oligomer. (Although this tendency seems to vary somewhat as the position of the sulfonamide linkage(s) is changed). The general trend is shown in Table 2.

Figure 3: Tm Dependence on # of Sulfonamides*



* Tm values of GCT-norm, GCT-6, GCT-5,7 GCT-3,6,9 and GCT-3,5,7,9 are shown. [Na^+] is listed in mM.

Table 2: Effect on Sulfonamide Substitution on the Salt Dependence of the Tm *

Oligo	Slope (°C/log [Na^+])
GCT-norm	10.1
GCT-6	9.1
GCT-5,7	8.5
GCT-3,6,9	7.8
GCT-3,5,7,9	6.0

* Slopes are an indication of the Tm's dependence on salt. As shown that dependence decreases for increasing sulfonamide incorporation for the oligos shown.

The sulfonamide linkage as a replacement for the phosphodiester has several attractive features such as the achiral nature of the sulfonamide, the lack of charge and its stability towards nucleases.[9d] [12] However, our experiments indicate that sulfonamide-linked oligodeoxynucleotides do not form stable duplexes with complementary DNA. Though the incorporation of a sulfonamide linkage has a relatively modest effect on the stability of a duplex, the incorporation of the number of sulfonamide linkages necessary to impart nuclease resistance gives an oligonucleotide that hybridizes very poorly. While this result is discouraging, it does not mean that sulfonamide-linked oligonucleotides will necessarily be poor antisense agents, because the *in vivo* target is likely to be RNA not DNA. Tm studies of the corresponding RNA-DNA hybrids are ongoing.

This research was funded by a grant from the National Institutes of Health (R01 GM45572-02). We are also indebted to Drs. John Richardson and Keith Iams for helpful discussions.

References and Notes

1 a) *Antisense RNA and DNA*; Murray, J. A. H., Ed ; Wiley-Liss, Inc., New York, **1992**. b) Uhlmann, E., Peyman, A. *Chem. Rev.* **1990**, *90*, 543-584. c) Goodchild, *Bioconjugate Chem.* **1990**, *1*, 165-187. d) *Oligodeoxynucleotides, Antisense Inhibitors of Gene Expression*; Cohen, J. S., Ed.; CRC Press, Inc., Boca Raton, FL, **1989**.

2 a) Cazenave, C.; Hélène, C. *Antisense Nucleic Acids and Proteins*; Mol, J. N. M.; van der Krol, A. R., Eds.; Marcel Dekker, New York, **1991**, pp. 47-93. b) Hélène, C.; Toulmé, J. -J *Biochim. Biophys. Acta* **1990**, *1049*, 99-125.

3 Hélène, C.; Thuong, N. T.; Harel-Ballan, A. *Annals N. Y. Acad. Sci.* **1992**, *660*, 27-36.

4 Tidd, D. M. *Antisense RNA and DNA*; Murray, J. A. H., Ed.; Wiley-Liss, Inc., New York, **1992**, pp. 227-240.

5 a) Yakubov, L. A.; Deeva, E. A.; Zarytova, V. F.; Ivanova, E. M.; Ryte, A. S ; Yurchenko, L. V.; Vlassov, V. V *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 6454-6458. b) Loke, S. L.; Stein, C. A.; Zhang, X. H.; Mori, K.; Nakanishi, M.; Subasinghe, C.; Cohen, J. S.; Neckers, L. M *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 3474-3478.

6 For a recent review see: Miller, P. S. *Antisense RNA and DNA*; Murray, J. A. H., Ed.; Wiley-Liss, Inc., New York, **1992**, 241-253.

7 Bower, M.; Summers, M. F., Powell, C.; Shinozuka, K ; Regan, J. B.; Zon, G.; Wilson, W. D. *Nucleic Acids Res.* **1987**, *15*, 4915-4930.

8 a) Huang, Z.; Schneider, K. C ; Benner, S. A. *J. Org. Chem.* **1991**, *56*, 3869-3882. b) Musicki, B.; Widlanski, T. S *Tetrahedran Lett.* **1991**, *32*, 1267-1270. c) Huang, J.; Widlanski, T. S. *J. Am. Chem. Soc.* submitted **1993**. d) McElroy, E. B.; Iams, K. P ; Huang, J.; Ellington A. D.; Widlanski, T. S. *Science*, submitted **1993**. e) Reynolds, R. C.; Crooks, P. A.; Maddry, J. A.; Akhtar, M. S.; Montgomery, J. A.; Secrist III, J. A. *J. Org. Chem.* **1992**, *57*, 2983-2985. f) Huie, E. M.; Kirshenbaum, M. R.; Trainor, G. L. *J. Org. Chem.* **1992**, *57*, 4569-4570. g) Kawai, S. H.; Wang, D., Just, G. *Can. J. Chem.* **1992**, *70*,1573-1580. h) Meng, B.; Kawai, S. H.; Wang, D , Just, G ; Giannaris, P. A.; Damha, M. J. *Angew. Chem. Int. Ed. Engl.* **1993**. *32*, 729-731. i) Kawai, S. H.; Wang, D , Giannaris, P. A.; Damha, M. J.; Just, G. *Nucleic Acids Res.* **1993**, *21*, 1473-1479.

9 a) Huie, E. M.; Trainor, G. L. **PCT Int. Appl. WO 91 15,500**. b) Weis, A. L.; Hausheer, F. H., Chaturvedula, P. V. C.; Delecki, D J.; Cavanaugh, P F. Jr., Moskwa, P. S.; Oakes, F. T. **PCT Int. Appl. WO 92 02,534**.

10 Huang, J., Widlanski, T. S. *Tetrahedran Lett.* **1992**, *33*, 2657-2660.

11 Sinha, N. D.; Biernat, J.; Köster, H. *Tetrahedron Lett.* **1983**, *24*, 5843-5846.

12 Perrin, K. A.; Iams K. P.; Huang, J.; McElroy, E. B.; Widlanski, T. S. *J. Am. Chem. Soc.* submitted **1993**.

(*Received 29 September* 1993)