Synthesis of Sulfonate-Linked DNA

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

Department of Chemistry, Indiana University, Bloomington, Indiana 47405

Received March 29, 1994®

Summary: The synthesis of an octamer of a sulfonate-linked DNA is presented. The solid-phase synthesis of DNA containing sulfonate linkages is then demonstrated.

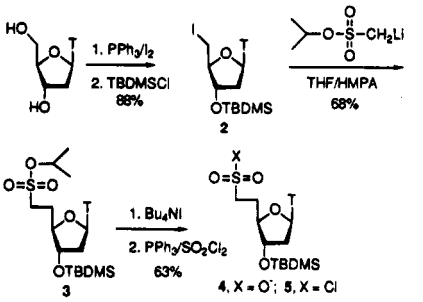
This paper reveals the facile synthesis of an oligonucleotide linked via 3′-*O*-sulfonate esters **1**. This type of DNA is stereoregular and isosteric to naturally occurring DNA. However, the sulfonate-linked nucleosides are nonionic, a feature which may enhance the uptake of these oligomers into cells and may make them useful as probes of protein–DNA and DNA–DNA interactions.[1]

Oligonucleotides known as "antisense" oligonucleotides can inhibit the expression of genetic information.[2] By forming specific, sequence-dependent double helices with complementary pieces of messenger RNA or double-stranded DNA, even relatively short oligonucleotides can interfere with the normal processes required for the expression of a gene. This powerful methodology holds great promise for the development of new therapeutic antiviral and antitumor agents and the amelioration of many disorders caused by the expression of harmful genetic information.

A major drawback of this technique is the vulnerability of exogenous oligonucleotides *in-vivo* degradation by enzymes that cleave phosphate diesters. To enhance the biological stability of oligonucleotides, a number of chemical modifications of the phosphate linking group have been developed.[3] In most cases, these modifications either require the introduction of a chiral center at phosphorus or utilize linking groups that have very different geometries from that of a phosphate diester. The introduction of steric and/or geometric permutations into DNA may compromise the effectiveness of oligomers as "antisense" agents. In addition, the heterogeneity of stereochemically undefined DNA (such as methyl phosphonate-linked DNA) makes these oligomers less desirable for use as probes of DNA structure and function.

A potential solution to this difficulty may be found in the replacement of the phosphate diester with a sulfur-based linkage such as a sulfonate,[4] sulfone,[5] sulfamate,[6] or sulfonamide.[7] Such functionalities are achiral and approximate the geometry of a phosphate diester reasonably well. However, the synthesis of such oligonucleotides has proven difficult. We wanted to develop a synthesis of sulfonate-linked DNA that would be straightforward and, if possible, applicable to automated DNA synthesis. This required that we develop a means to synthesize the isolate activated monomers that could be coupled to a growing DNA chain in an iterative fashion.

Scheme 1



The requisite activated monomer was synthesized as shown (Scheme 1). By use of an improved method for selectively iodinating primary hydroxyl groups of nucleosides, thymidine was converted to its 5′-deoxy 5′-iodo derivative with PPh_3/I_2 in pyridine at 0 °C.[8] This material was not isolated, but silylated to give **2** (88%). Alkylation of **2**, by the anion of isopropyl mesylate,[4] proceeds in good yield (68%–73%) to give the desired sulfonate **3**. The sulfonate ester can be deblocked in quantitative yield with Bu_4NI to give the salt **4**. The sulfonate salt was treated with PPh_3/SO_2Cl_2 to give the labile acid chloride **5** in 65% yield after flash chromatography[9] (37% overall yield from thymidine).

Treatment of the sulfonyl chloride **5** with pyridine and diisopropylethylamine in the presence of 5′ MMTr thymidine **6** gave the sulfonate-linked dimer **7** in 94% isolated yield. The 3′ end of the dinucleoside could be deblocked by treatment with an excess of triethylammonium fluoride to give a 5′ protected-sulfonate-linked dinucleoside **8**. A second round of coupling and deblocking to give a trimer **10** could then be effected. Subsequent iterations of this process were performed to give an octamer (Scheme 2). The coupling of the incoming acid chloride and the 3′ OH of the growing chain is almost quantitative even on the very small scales used (typically between 10 and 20 mg of oligomer). *Isolated* yields of the coupling products were typically better than 90% except for the longer oligomers which were very difficult to solubilize. The high yields obtained during the coupling steps indicate that it may be possible to adapt this methodology to the solid-phase synthesis of short, sulfonate-linked oligomers, despite the fact that the synthesis proceeds in the 3′ to 5′ direction rather than

® Abstract published in *Advance ACS Abstracts,* June 15, 1994.

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

(2) For a recent comprehensive review of this field see: *Antisense RNA and DNA*; Murray, J. A. H., Ed.; Wiley-Liss: New York, 1992.

(3) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543.

(4) Musicki, B.; Widlanski, T. S. *J. Org. Chem.* **1990**, *55*, 4231. Musicki, B.; Widlanski, T. S. *Tetrahedron Lett.* **1991**, *32*, 1267.

(5) Huang, Z.; Schneider, K. C.; Benner, S. A. *J. Org. Chem.* **1991**, *56*, 3869. Schneider, K. C.; Benner, S. A. *Tetrahedron Lett.* **1990**, *31*, 335.

(6) Huie, E. M.; Kirshenbaum, M. R.; Trainor, G. L. *J. Org. Chem.* **1992**, *57*, 4569.

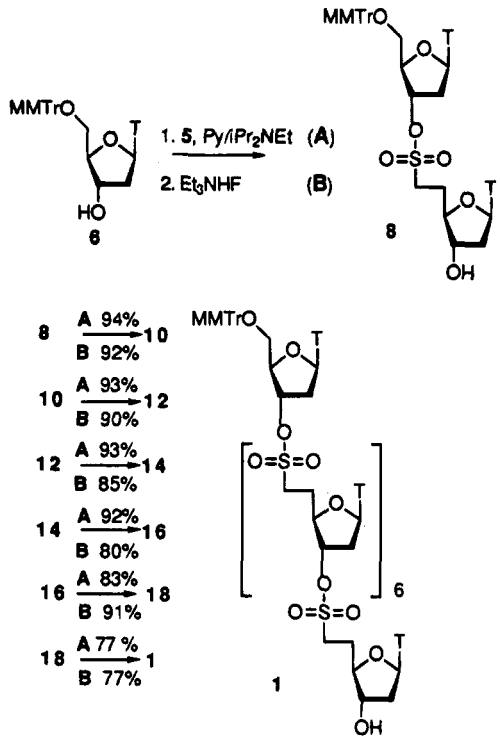
(7) Crooks, P. A.; Reynolds, R. C.; Maddry, J. A.; Rathore, A.; Akhtar, M. S.; Montgomery, J. A.; Secrist, J. A., III. *J. Org. Chem.* **1992**, *57*, 2830. Reynolds, R. C.; Crooks, P. A.; Maddry, J. A.; Akhtar, M. S.; Montgomery, J. A.; Secrist, J. A., III. *J. Org. Chem.* **1992**, *57*, 2983. McElroy, E. B.; Bandaru, R.; Huang, J.; Widlanski, T. S. *Bioorg. Med. Chem. Lett.* **1994**, *4*(8), 1071.

(8) In our hands, the use of Ph_3PCH_3I for this iodination (Verheyden, J. P. H.; Moffatt, J. G. *J. Org. Chem.* **1970**, *35* 2319) gave relatively low yields. The use of PPh_3/I_2 in pyridine gives the 5′ iodide in high yield while avoiding the use of the unstable phosphonium salt.

(9) Huang, J.; Widlanski, T. S. *Tetrahedron Lett.* **1992**, *33*, 2657. The acid chloride **5** decomposes relatively quickly upon contact with silica gel but is stable for more than a month when stored dry at −20 °C.

0022-3263/94/1959-3520$04.50/0

Scheme 2



the traditional 5′ to 3′ direction currently employed for DNA synthesis.

Using the procedure describe above, multigram quantities of sulfonate-linked dinucleosides may be synthesized easily.[10] This has proven very useful since these dinucleosides may be readily converted to 3″ phosphoramidites and then incorporated into one or more positions of a synthetic DNA oligomer.[11] Synthesis of DNA oligomers (homododecamers of thymidine) containing single sulfonate substitutions at various positions were accomplished as follows: For the synthesis of the oligomer containing a sulfonate at the 1 position (the first position at the 3′ end of the oligo), a sulfonate-linked dinucleoside was coupled via its 3′ hydroxyl group to a controlled pore glass resin using an oxalate linkage[12] and then elongated by automated synthesis using standard solid-phase phosphoramidite chemistry. The oligomer was deblocked by treatment with diisopropylamine. Release of the oligomer from the resin was effected with $MeOH/K_2CO_3$. For the synthesis of other oligonucleotides a controlled pore glass resin containing a 5′ DMT-protected 3′ oxalate-linked thymidine residue was prepared.[12] This charged resin was then elongated using standard phosphoramidite chemistry. The sulfonate linkage was inserted by using a 3″ phosphoramidite of a sulfonate-linked dinucleoside in the place of a normal amidite. This amidite could be prepared by phosphitylation of the 3″ end of the dinucleoside **1** with 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite. The oligomers were deblocked and cleaved as described above and then purified by polyacrylamide gel electrophoresis.

The position of the sulfonate linkage was verified by treatment of the oligomer with ammonium hydroxide (50 °C, overnight). This treatment causes quantitative cleavage of the oligonucleotide at the position containing the sulfonate linkage. The resulting 5′ fragment of the oligomer thus cleaved contains a 3′ cyclonucleotide residue. Interestingly, oligomers containing a 3′ cyclonucleoside are not degraded by the exonuclease function of Klenow Fragment or T4 DNA polymerase but can be hydrolyzed by T7 DNA polymerase (data not shown).

In order to eventually carry out effective automated syntheses of sulfonate-linked DNA, the sulfonate linkage must survive conditions normally required for the removal of base protecting groups and cleavage of the oligomer from a solid support. To test the stability of the solfonate linkage, a sulfonate-linked dinucleoside of thymidine was exposed to various conditions normally used for the deblocking and cleavage of oligomers synthesized on solid supports. The dinucleoside is quite stable toward $MeOH/K_2CO_3$, as well as neat and methanolic diisopropylethylamine. However, as expected, the dinucleoside is not as stable toward basic conditions as sulfonate-linked RNA.[4] The DNA dinucleoside is degraded in concentrated aqueous ammonia fairly quickly and more slowly ($t_{1/2}$ ca. 14 h, 20 °C) in saturated methanolic ammonia.

Because the sulfonate linkage is similar in geometry to a phosphate diester but is nonionic, oligomers containing one or more (or all) neutral sulfonate linkages should be valuable tools for probing the charge dependence of DNA/DNA interactions as well as DNA/protein interactions. In addition, these oligomers may provide a new "antisense" strategy for inhibiting gene expression. Studies of the biological properties of these sulfonate-linked oligonucleotides are ongoing.

Acknowledgment. This research was funded by a grant from the National Institutes of Health (R01 GM45572-02) and by a Camille Dreyfus Teacher-Scholar Award to T.S.W.

Supplementary Material Available: Experimental procedures, compound characterization data, and copies of NMR spectra (21 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(10) In addition to the thymidine dinucleosides (TsT), we have also prepared CsT, GsT, and AsT dinucleosides. All three of these dimers are more stable than the TsT dimer, with the AsT being the most stable among the three.

(11) Depending on the position of the substitution, the melting temperature of a duplex of an oligonucleotide containing a single sulfonate linkage with a complementary oligonucleotide containing all phosphodiester linkages is typically 1–3 °C lower than the corresponding unmodified duplex.

(12) Alul, R. H.; Singman, C. N.; Zhang, G.; Letsinger, R. L. *Nucleic Acid Res.* **1991**, *19*(7), 1527.