aniline[19,20] afforded in 66% yield synthetically derived (−)-methylenolactocin (mp 82–84 °C; $[\alpha]^{26}_D$ −6.7° (*c* 0.5, CH_3OH)), spectroscopically and chromatographically indistinguishable from the naturally derived substance (mp 82–84 °C; mmp 82–84 °C; $[\alpha]^{26}_D$ −6.8° (*c* 0.5, CH_3OH)).[21,22]

In summary, the feasibility of using chiral olefin diastereofacial differentiation for enantioselective lactone construction has been demonstrated through a high-yield (>20% overall) preparation of enantiopure, natural methylenolactocin. Application of this methodology to the synthesis of congeneric natural products[25] is planned.

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Registry No. (−)-1, 112923-53-2; (−)-**2a**, 98919-68-7; (−)-**2b**, 129098-11-9; **3**, 142188-46-3; (−)-**4**, 142188-47-4; (+)-**5**, 142188-49-6; **6** diacid derivative, 142188-52-1; (−)-**6**, 142188-51-0; (−)-**6** methyl ester, 142235-91-4; 1,1-dibromohexane, 58133-26-9; trichloroacetyl chloride, 76-02-8; (4*R*,5*S*)-3,3-dichloro-5-pentyl-4-phenyl-4-[((1*R*,2*S*)-2-phenylcyclohexyl)oxy]dihydro-2(3*H*)-furanone, 142188-48-5; (4*R*,5*S*)-5-pentyl-4-phenyldihydro-2(3*H*)-furanone, 142188-50-9; (4*S*,5*S*)-5-pentyl-4-phenyldihydro-2(3*H*)-furanone, 142188-53-2.

Supplementary Material Available: Complete experimental procedures with spectral and analytical data for the preparation of compounds 1–6 (5 pages). This material is contained in many 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.

(19) Cf. Gras, J.-L. *Tetrahedron Lett.* **1978**, 2111–2114.
(20) Preliminary results indicate that this modification represents a general improvement over the original procedure(s)[15] in terms of both mildness and yield. The procedure used by Johnson and co-workers for the preparation of (±)-protolichesterinic acid ($MgOCO_2CH_3$; HCHO, $(C_2H_5)_2NH$)[15b] afforded methylenolactocin in less than 20% yield.
(21) A sample of natural methylenolactocin was kindly provided by Professor B. K. Park (Kang Woen National University, Korea). We observed a higher rotation (lower concentration) than that reported.
(22) That the absolute stereochemistry of the natural product is, in fact, as depicted (the formulation consistent with the expected transition-state conformation of enol ether **3**) was established by photochemical decarboxylation (Pyrex filter, acridine, *tert*-C_4H_9SH, C_6H_6, 20 °C, 3 h, 57%)[23] of acid (−)-**6** to yield (*S*)-(−)-γ-nonanolactone ($[\alpha]^{21}_D$ −48.4° (*c* 0.5, CH_3OH); lit.[24] −48.8° (*c* 1, CH_3OH)).
(23) Okada, K.; Okubo, K.; Oda, M. *Tetrahedron Lett.* **1989**, *40*, 6733–6736.
(24) Ravid, U.; Silverstein, R. M.; Smith, L. R. *Tetrahedron* **1978**, *34*, 1449–1452.
(25) Related lactones include the following: protolichesterinic acid (Asahina, Y.; Asano, M. *J. Pharm. Soc. Jpn.* **1927**, *539*, 1–17), nephromopsinic acid (Asano, M.; Azumi, T. *Chem. Ber.* **1935**, *68B*, 995–997), alloprotolichesterinic acid (Asahina, Y.; Yanagita, M. *Chem. Ber.* **1936**, *69B*, 120–125), nephrosterinic acid (Asahina, Y.; Yanagita, M.; Sakurai, Y. *Chem. Ber.* **1937**, *70B*, 227–235), avenaciolide (Brookes, D.; Tidd, B. K.; Turner, W. B. *J. Chem. Soc.* **1963**, 5385–5391), and canadensolide (McCorkindale, N. J.; Wright, J. L. C.; Brian, P. W.; Clarke, S. M.; Hutchinson, S. A. *Tetrahedron Lett.* **1968**, 727–730).

Oligonucleotides with a Nuclease-Resistant Sulfur-Based Linkage

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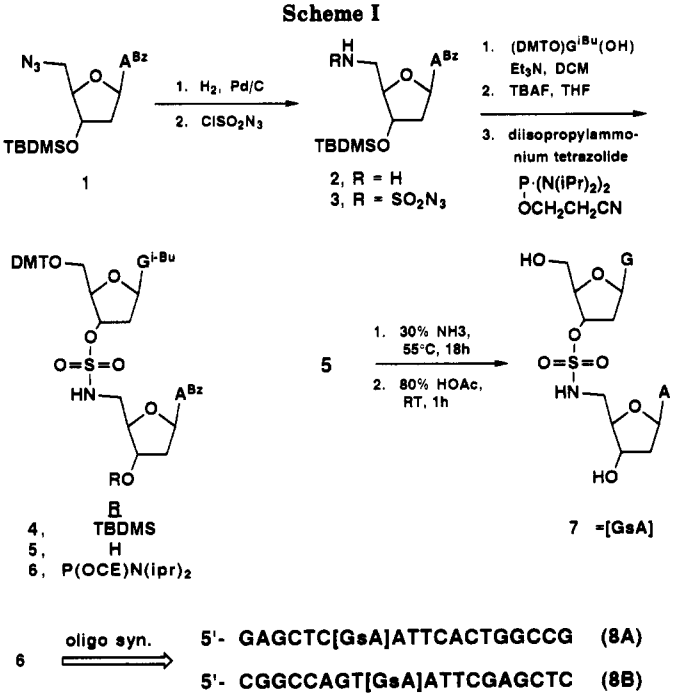
Summary: A pair of complementary oligonucleotides with an *EcoR1* recognition sequence (GÂATTC) bearing a novel sulfamate linkage at the cleavage site have been prepared and shown to be capable of annealing to form a stable duplex that is resistant to *EcoR1* cleavage.

Over the past several years there has been increasing interest in the development of nucleic acids as potential therapeutics.[1] The most common approach, known as antisense therapy, involves the use of short oligonucleotides to target regions of complementary sequence on a large nucleic acid (e.g., mRNA) for the purposes of blocking function (e.g., translation). Numerous structural modifications of oligonucleotides, most centered on the phosphodiester linkage, have been investigated in an attempt to increase lipophilicity and confer nuclease resistance.[2,3]

We have been exploring a class of oligonucleotide analogues which feature the replacement of the internucleotide phosphorus atom with sulfur.[4] For ease of synthesis and chemical stability we have also replaced the 5′-bridging oxygen with nitrogen. The resulting sulfamate linkage is neutral, achiral, and isoelectronic and isostructural with the phosphodiester. We report herein on a pair of oligo-

Scheme I



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(3) Zon, G. *Pharmaceut. Res.* **1988**, *5*, 539–549.
(4) For other analogues with a replacement of phosphorus with sulfur see: (a) Musicki, B.; Widlanski, T. S. *Tetrahedron Lett.* **1991**, *32*, 1267–1270. (b) Musicki, B.; Widlanski, T. S. *J. Org. Chem.* **1990**, *55*, 4231–4233. (c) Huang, Z.; Schneider, K. C.; Benner, S. A. *J. Org. Chem.* **1991**, *56*, 3869–3882. (d) Schneider, K. C.; Benner, S. A. *Tetrahedron Lett.* **1990**, *31*, 334–338. (e) Summerton, J. E.; Weller, D. D. US Patent 5,034,506, 1991.

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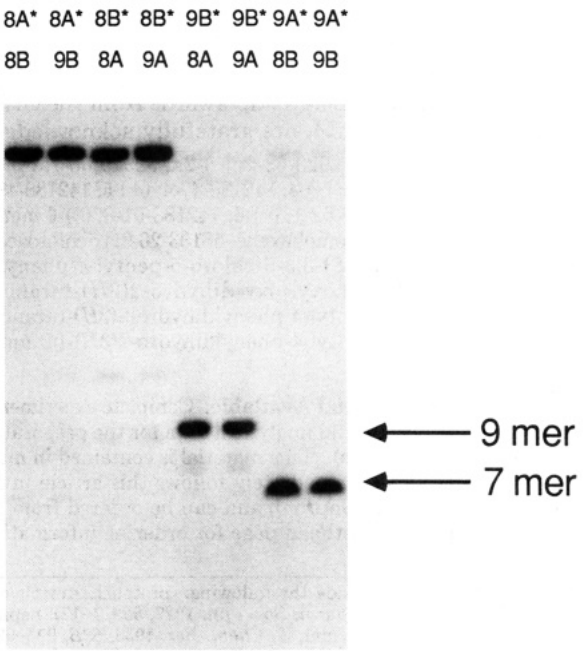

Figure 1. Analysis of the *EcoR1* cleavage of oligonucleotides bearing a sulfamate (**8A** and **8B**) or phosphodiester (**9A** and **9B**) linkage at the cleavage site (GÂATTC). Oligonucleotides were 5′-end-labeled with T4 polynucleotide kinase and γ-ATP and were thereafter ethanol precipitated twice to remove excess γ-ATP. Labeled oligonucleotide (*) was mixed with 10 pmol of cold oligonucleotide in 10 μL of 50 mM Tris-HCl pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$, and 1 mM dithioerythritol and then incubated at 80 °C for 15 min and then cooled over 1 h to 25 °C. The mixture was digested with 5 units of EcoR1 for 60 min at 37 °C. The reaction was stopped with the addition of 1 μL of 0.5 M EDTA. Reaction products were separated on a 19% denaturing polyacrylamide gel. The arrows indicate the 7-nt and 9-nt cleavage fragments.

deoxyribonucleotides bearing such a linkage that are easily synthesized and chemically stable and show that the linkage is nuclease resistant and capable of supporting duplex formation.

For construction of the internucleotide sulfamate linkage, a synthetic approach involving the base-catalyzed reaction of a nucleoside bearing a free 3′-hydroxy group with a 2′-deoxynucleoside 5′-sulfamoylazide was developed (Scheme I). *tert*-Butyldimethylsilylation of 5′-azido-N^6-benzoyl-2′,5′-dideoxyadenosine[5] gave **1** which was subjected to catalytic hydrogenation to afford the protected 5′-aminodeoxynucleoside as a *p*-toluenesulfonate salt **2**. Following in situ neutralization with triethylamine, **2** was treated with chlorosulfonylazide (1 M in acetonitrile)[6-9] to afford after flash chromatography the corresponding sulfamoylazide **3** as a stable, colorless solid. Condensation of **3** with 5′-*O*-(dimethoxytrityl)-N^6-isobutyryl-2′-deoxyguanosine in the presence of triethylamine in dichloromethane afforded the protected, sulfamate-linked dinucleoside **4** in 76% yield.

To prepare oligonucleotides bearing a single sulfamate linkage, a block-dinucleotide approach was utilized. Deprotection of **4** with tetrabutylammonium fluoride afforded alcohol **5** which was converted to its 3′-*O*-(β-cyanoethyl)-phosphoramidite **6** under standard conditions.[10] Phosphoramidite **6** was found to be fully functional in automated oligonucleotide synthesis. The fully deprotected block-dimer **7** ([GsA]) was obtained in 69% yield after successive treatment of **5** with methanolic ammonia and aqueous acetic acid.

Reagent **6** was used to prepare a pair of complementary oligonucleotides **8A** and **8B** capable of annealing to form an EcoR1 restriction endonuclease recognition site. In each oligonucleotide, the sulfamate-linkage was positioned between the residues where EcoR1 normally cleaves (GÂATTC). The corresponding unmodified oligonucleotides **9A** and **9B** were also prepared. The nucleotide composition of **8A** and **8B** and integrity of the sulfamate-linkage was confirmed by digestion to the constituent nucleosides followed by HPLC analysis.[11] In each case the sulfamate-linked dinucleoside (**5**) was recovered intact after prolonged treatment with snake venom phosphodiesterase and alkaline phosphatase.

The ability of **8A** and **8B** to anneal to form duplex was examined by thermal denaturation studies at pH 6.8 in phosphate buffer containing 150 mM sodium chloride. For both **8A·8B** and **9A·9B**, a characteristic sigmoid transition was observed. The shape of the curves and the net hypochromicity were nearly identical, suggesting that the introduction of a sulfamate linkage into each strand does not significantly perturb the duplex structure. The small decrease in the estimated transition temperature T_m (from 73 °C for **9A·9B** to 70 °C for **8A·8B**) indicates a slight decrease in the thermodynamic stability of the sulfamate-modified duplex under these conditions. As a point of reference, a single mismatch in a duplex of this length would be expected to lead to a 5–8 °C drop in *Tm*.[12]

Finally, the susceptibility of the linkage to cleavage by the restriction enzyme *EcoR1* was examined. The products resulting from the treatment of the control duplex **9A·9B**, the fully modified duplex **8A·8B**, as well as the two hemimodified duplexes **8A·9B** and **9A·8B**, were analyzed by gel electrophoresis (Figure 1). For each duplex, cleavage on each strand was independently monitored by separately labeling either the **A** or **B** strand. The sulfamate linkage was found to be fully resistant to *EcoR1*. In each of the eight experiments, strands bearing a phosphodiester linkage at the cleavage site were quantitatively cleaved whereas those bearing the sulfamate-linkage remained fully intact.

In summary, we have prepared complementary oligodeoxyribonucleotides, each bearing a single sulfamate linkage, and shown them to be capable of annealing to form a stable duplex. We have demonstrated that the sulfamate linkage is resistant to cleavage by both snake venom phosphodiesterase and *EcoR1* restriction endonuclease. This resistance suggests that sulfamate-modified oligonucleotides may be useful for probing nuclease–DNA interactions. They should also be useful in antisense research and, ultimately, therapeutic applications.

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Supplementary Material Available: Experimental procedures and data for preparations, UV experiments, and degradations (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any