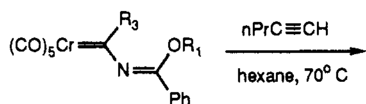
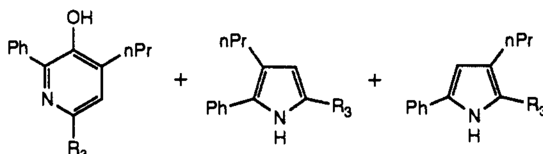


16a R_3 = Ph, R_1 = Me
16b R_3 = Ph, R_1 = C(O)CH_3
16c R_3 = tBu, R_1 = Me
16d R_3 = tBu, R_1 = C(O)CH_3



complex			
16a	5g 51 %	6g 4 %	
16b	5g 31 %	6g 9 %	
16d	5e 37 %	6e 26 %	13e 13 %

spectral data were obtained at the Midwest Center for Mass Spectrometry, an NSF Regional Instrument Facility (CHE-8211164). The NMR instruments used were funded in part by the NSF Chemical Instrumentation Program and by the NCI via the University of Chicago Cancer Research Center (CA-14599).

Supplementary Material Available: ^{1}H and ^{13}C NMR, IR, and mass spectral data for all new compounds (**4a–d, 5e,g, 6e,g, 13a–f,** and **23**) (7 pages). Ordering information is given on any current masthead page.

3*H*-1,2-Benzodithiole-3-one 1,1-Dioxide as an Improved Sulfurizing Reagent in the Solid-Phase Synthesis of Oligodeoxyribonucleoside Phosphorothioates

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Oligodeoxyribonucleoside phosphorothioates are isoelectronic analogues of natural phosphodiesters in which one of the oxygen atoms that does not participate in the internucleotidic linkage is replaced by a sulfur atom.[1] Unlike natural oligomers, phosphorothioate oligodeoxyribonucleotides are resistant to degradation by nucleases[1] and, hence, have demonstrated their usefulness as "antisense" molecules by inhibiting gene expression in vitro.[2] The inhibitory mechanism is presumed to occur by binding specific messenger RNAs (the "sense" molecules) as DNA–RNA duplexes thereby impairing the translation of the messages by the ribosomes[3] and/or from the degradation of the heteroduplexes by RNase H.[4]

In experiments using "antisense" DNA fragments as potential therapies against AIDS,[5] it has been shown that phosphorothioate oligomers inhibited the cytopathic effect of HIV-1 in chronically infected H9 cells.[6] These results suggest that oligonucleoside phosphorothioates may represent a new class of therapeutic agents. Consequently, the availability of these analogues is urgent and crucial for clinical evaluation. Our efforts at improving their preparation are reported herein.

The automated synthesis of phosphorothioate DNA[7-11] according to the "phosphoramidite" approach[12] involves a stepwise sulfurization reaction effected by a solution of elemental sulfur (S_8). This relatively slow (7.5 min)[8] sulfur-transfer reaction has, in our laboratory, led to instrument failure as a result of the insolubility of S_8 in most organic solvents.[13] To circumvent these problems, a novel sulfurizing agent was designed according to the following criteria: (i) The reagent must be readily prepared and easily handled under laboratory conditions. (ii) The stability and solubility of the reagent in various solvents and concentrations must be compatible with automated oligonucleotide synthesis. (iii) The reagent must exhibit fast sulfurization reaction kinetics, and most importantly, it must quantitatively convert phosphite triesters into phosphorothioate triesters without nucleosidic modifications to ensure the genetic integrity of the synthetic DNA.

Conceptually, thiosulfonates[14-16] are attractive reagents for sulfur-transfer reactions. These compounds are susceptible to nucleophilic attack by phosphite triesters at the sulfenyl sulfur leading to the cleavage of the polarized sulfur–sulfur bond and the generation of a sulfinate anion[15] (Figure 1). This anion would then trigger an intramolecular cyclization[16] to complete the sulfur-transfer reaction with enhanced kinetics.[17] Selected thiosulfonates were therefore prepared and evaluated with respect to the criteria outlined above (data not shown). Thiosulfonate **1**[18] fulfilled all requirements. The compound, isolated in large quantities (20 g), was prepared in 50% yield by the oxidation of 3*H*-1,2-benzodithiole-3-one[19] using trifluoroperoxyacetic acid.[20]

The efficacy of the sulfurizing reagent was tested during the automated solid-phase synthesis of the dinucleoside phosphorothioate **3** (Figure 1). A 0.2 M solution of **1** in acetonitrile[21] was used to sulfurize **2** during a period of 30 s. To assess the extent of the reaction, excess **1** was immediately washed away with

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(13) Alternatively, the sulfurization of "H-phosphonate" oligomers can be manually achieved outside the instrument by using a solution of S_8. This approach, however, suffers from the inherent limitation that oligonucleotides carrying predetermined combinations of natural and phosphorothioate linkages cannot easily be prepared.
(14) Michalski et al.[15] reported that the reaction of certain thiosulfonates with trialkylphosphites led to *O,O,S*-trialkyl phosphorothiolates. These observations prompted us to design thiosulfonates which upon reaction with trialkylphosphites would generate the desired *O,O,O*-trialkyl phosphorothioates.
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(21) For optimum results, **1** is dissolved in dry acetonitrile prior to use and the solution kept in an amber "siliconized" glass bottle.

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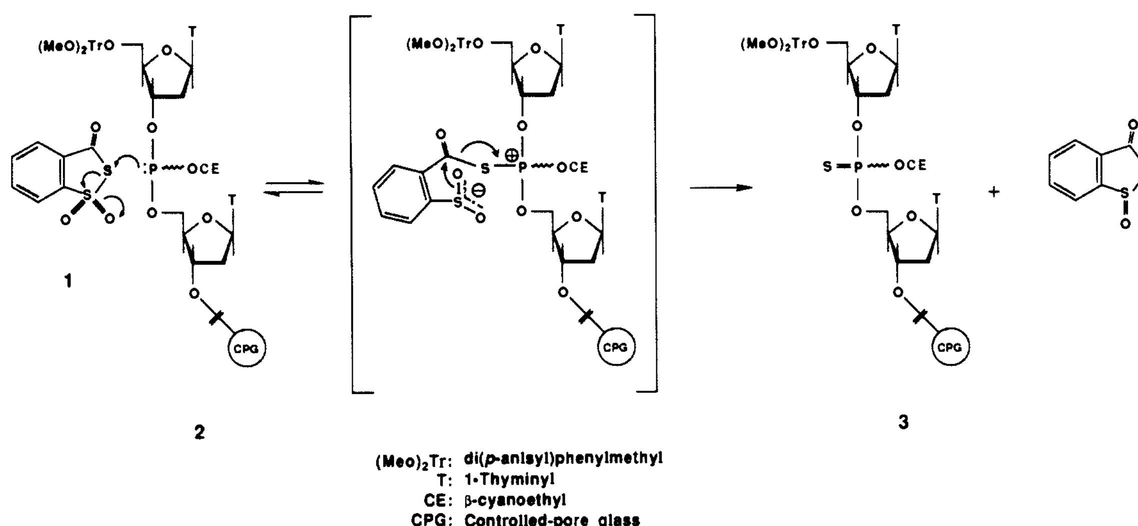

Figure 1. Sulfurization of a dinucleoside phosphite triester by 3*H*-1,2-benzodithiole-3-one 1,1-dioxide (**1**) as a model experiment for the preparation of oligodeoxyribonucleoside phosphorothioates.

acetonitrile and unreacted **2** was oxidized with aqueous iodine.[22] After standard deprotection, HPLC analysis showed that the phosphorothioate dimer S-d(TpT) was generated in greater than 99% yield as a mixture of R_p and S_p diastereoisomers.[7] Less than 1% of the natural phosphodiester d(TpT) was detected. Under similar conditions, an oligodeoxyribonucleoside phosphorothioate (28-mer) complementary to the messenger RNA of the HIV-1 *rev* gene[6,23] was synthesized with a 99% stepwise yield according to "trityl color" determination. ^{31}P NMR analysis of the fully deprotected and HPLC-purified oligonucleotide indicated that more than 96% of the resonances observed accounted for P(S) (δ 52 ppm) linkages whereas less than 4% of the resonances corresponded to P(O) (δ −4 ppm) linkages.[7,24] To demonstrate the versatility of the synthetic approach, a similar oligomer bearing only two P(S) links at predetermined positions was also prepared.[25] The purified oligonucleotide displayed the proper P(S) resonances in correct integrated ratio relative to the P(O) resonances according to ^{31}P NMR.

Finally, a random DNA sequence (28-mer)[26] bearing exclusively P(O) linkages and an equal number of the four nucleosidic bases was synthesized to investigate potential nucleosidic modification during the sulfurization step. The fully protected oligomer covalently attached to the solid support was incubated with a 0.2 M solution of **1** in acetonitrile for 24 h at ambient temperature. After deprotection and purification, the oligonucleotide was subjected to enzymatic degradation with snake venom phosphodiesterase and alkaline phosphatase. No evidence of nucleosidic base modification was detected from HPLC analysis of the hydrolysates as only peaks corresponding to the four nucleosides were observed.

We have demonstrated that, because of its solubility in common organic solvents, its rapid sulfurization kinetics, and its facile automation, the thiosulfonate **1** is a superior reagent relative to S_8 for the preparation of oligodeoxyribonucleoside phosphorothioates via the "phosphoramidite" approach. The high efficiency of the stepwise sulfurization has allowed the preparation of oligomers carrying either exclusively or a predetermined combination of P(S) linkages, with no observable modification of the nucleosidic bases. One can then speculate that the use of the thiosulfonate **1** in conjunction with the "deoxynucleoside phosphoramidite" approach may become the method of choice for the large-scale preparation of oligodeoxyribonucleoside phosphorothioates required for therapeutic applications.

Acknowledgment. Research funding earmarked for AIDS Targeted Antiviral Research to W.E. and R.P.I. from the Office of the Director, NIH, is gratefully acknowledged.

Supplementary Material Available: Detailed preparation of **1** and ^{31}P NMR spectra of oligodeoxyribonucleoside phosphorothioates[23,25] (3 pages). Ordering information is given on any current masthead page.

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(25) d(T_{PS}CGTCGCTGTCTCCGCTTCTTCCTGCC$_{PS}$A).
(26) d(TACCGTAGCTAAGGTCATGCAAGTTCCG).

Organic Molecules Dimerize with High Structural Recognition When Each Possesses a Large Lipophilic Surface Containing Two Preorganized and Complementary Host and Guest Regions[1]

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We report here the unprecedented phenomenon of two identical molecules with large preorganized and complementary surfaces strongly binding each other in $CHCl_3$ in the absence of pole–pole, pole–dipole, metal ligation, hydrogen-bonding, or hydrophobic forces. As in an analogous synthesis,[2] **5**[3] was treated with 2,3-dichloroquinoxaline to give the mobile system **1** ⇄ **4** (37%).[4] Above −38 °C, only the *vase* form is detected (^{1}H NMR). Below −38 °C, only the *kite* conformation of **1** is detected (^{1}H NMR).[2a] No **1·1** was observed at any temperature (^{1}H NMR). Similarly, **6**[3] was converted to **2**[4] (30%). Octol **7**[4] was prepared[3] (87%) from 2-ethylresorcinol[5] and hexanal and similarly converted[2] to **3**[4] (16%).

Molecular models (CPK) of **2** and **3** indicate that the extra alkyl groups sterically inhibit *vase* formation. The *kite* conformation of **2** possesses a roughly planar (15 × 20 Å) rectangular face

(1) We warmly thank the U.S. Public Health Service for supporting Grant GE-12640.
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