concentrated, to afford 14.9 mg (81%) of an oil: IR (NaCl) 2780 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.21 (td, 1 H, J = 9, 2 Hz, C_3-H_e), 1.92 (q, 1 H, J = 9 Hz, C_3-H_a), 1.85–1.05 (m, 16 H) 0.86 (t, 3 H, J = 7 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 65.1, 63.8, 51.6, 37.0, 31.1, 30.9, 30.6, 24.8, 20.5, 19.2, 14.6; mass spectrum (CI, NH_3) m/e 168 (MH^+); $[\alpha]_D$ = −111.3° (c 1.3, CH_2Cl_2). Anal. Calcd for $C_{11}H_{21}N$: C, 78.98; H, 12.65. Found: C, 79.10; H, 12.55.

(5R,9R)-(−)-5-Hexylindolizidine (12). Prepared according to the procedure for 11, affording 62 mg (87%) of an oil: IR (NaCl) 2780 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.22 (td, 1 H, J = 9.5, 2 Hz, C_3-H_e), 1.93 (q, 1 H, J = 8.5 Hz, C_3-H_a), 1.90–1.03 (m, 22 H), 0.89 (t, 3 H, J = 7 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 65.1, 63.9, 51.6, 34.7, 31.9, 31.1, 30.9, 30.6, 29.8, 25.9, 24.8, 22.7, 20.5, 14.2; mass spectrum (CI, NH_3), m/e 210 (MH^+); $[\alpha]_D$ = −80.4° (c 1, CH_2Cl_2). Anal. Calcd for $C_{14}H_{27}N$: C, 80.31; H, 13.00. Found: C, 80.11; H, 13.20.

(5S,9R)-(−)-5-Propylindolizine (13). Cyanoamine 8 (71.5 mg, 0.48 mmol) was dissolved in Et_2O (10 mL) and cooled to 0 °C, propylmagnesium chloride (2.1 mL, 7 eq, 1.6 M in Et_2O) was added, and the solution was stirred for 12 h at room temperature. After quenching with saturated NH_4Cl (10 mL) and basification with 1 N NaOH, the aqueous layer was extracted with CH_2Cl_2 (5 × 20 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was flash chromatographed on silica with 90:10 EtOAc–MeOH to afford 13: 61.1 mg (77%), oil; IR (NaCl) 2800 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.83 (td, 1 H, J = 8.5, 3.5 Hz, C_5-H), 2.71 (td, 1 H, J = 9, 3 H, C_3-H_e), 2.53 (q, 1 H, J = 9 Hz, C_3-H_a), 2.35 (m, 1 H, C_9-H), 1.75–0.98 (m, 14 H), 0.81 (t, 3 H, J = 7 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.3, 55.1, 48.8, 31.4, 30.7, 27.7, 25.8, 20.95, 20.91, 19.4, 14.5; mass spectrum (CI, CH_4), m/e 168 (MH^+); $[\alpha]_D$ = −1.7° (c 1.1, CH_2Cl_2). Anal. Calcd for $C_{11}H_{21}N$: C, 78.98; H, 12.65. Found: C, 79.12; H, 12.49.

(5S,9R)-(+)-5-Hexylindolizidine (14). Synthesized according to the procedure for 13 (using n-hexylmagnesium bromide), affording 64 mg (78%) of an oil: IR (NaCl) 2800 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.88 (m, 1 H, C_5-H), 2.78 (td, 1 H, J = 8.5, 3 Hz, C_3-H_e), 2.60 (q, 1 H, J = 8.5 Hz, C_3-H_a), 2.42 (m, 1 H, C_9-H), 1.82–1.20 (m, 19 H), 1.12 (ddd, 1 H, J = 22, 12, 4.5 Hz), 0.86 (t, 3 H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5, 55.1, 48.8, 32.0, 31.3, 30.7, 29.7, 27.73, 27.66, 23.4, 22.7, 20.9, 19.4, 14.2; mass spectrum (CI, NH_3), m/e 210 (MH^+); $[\alpha]_D$ = +8.1° (c 1, CH_2Cl_2). Anal. Calcd for $C_{14}H_{27}N$: C, 80.31; H, 13.00. Found: C, 80.09; H, 12.76.

(5R,9R)-5-Cyano-5-methylindolizidine (15): IR (NaCl) 2830 (s), 2230 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.05 (td, 1 H, J = 8.5, 3.5 Hz, C_3-H_e), 2.28 (q, 1 H, J = 8.5 Hz, C_3-H_a), 2.21 (m, 1 H, C_9-H), 1.91–1.00 (m, 10 H), 1.42 (s, 3 H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 119.1, 62.6, 60.0, 48.1, 37.4, 30.5, 26.5, 22.0, 20.0; mass spectrum (CI, NH_3), m/e 138 (($MH - HCN)^+$).

(5S,9R)-5-Methylindolizidine (16): IR (NaCl) 2810 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.20 (m, 1 H, C_5-H), 2.78 (td, 1 H, J = 9, 3 Hz, C_3-H_e), 2.50 (q, 1 H, J = 9 Hz, C_3-H_a), 2.40 (m, 1 H, C_9-H), 1.81–1.00 (m, 10 H), 0.94 (d, 3 H, J = 7 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 54.6, 50.0, 49.1, 31.43, 31.40, 30.4, 20.8, 19.3, 10.0; mass spectrum (CI, NH_3), m/e 140 (MH^+).

(5R,9R)-5-Methylindolizidine (17): IR (NaCl) 2800 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.18 (td, 1 H, J = 9, 2 Hz, C_3-H_e), 1.96 (m, 1 H, C_5-H), 1.93 (q, 1 H, J = 9 Hz, C_3-H_a), 1.84–1.10 (m, 10 H), 1.06 (d, 3 H, J = 6.5 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 64.8, 58.9, 51.8, 34.3, 31.1, 30.6, 24.8, 21.2, 20.4; mass spectrum (CI, NH_3), m/e 140 (MH^+).

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The Automated Synthesis of Sulfur-Containing Oligodeoxyribonucleotides Using 3H-1,2-Benzodithiol-3-one 1,1-Dioxide as a Sulfur-Transfer Reagent

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Several polysulfides were tested as potential sulfur-transfer reagents during the automated synthesis of oligodeoxyribonucleoside phosphorothioates via the "deoxyribonucleoside phosphoramidite" approach. The thiosulfonate 3H-1,2-benzodithiol-3-one 1,1-dioxide (12) was particularly efficient as a sulfurizing reagent. A 0.2 M solution of 12 in acetonitrile converted the dinucleoside monophosphite triesters **13a–d** into the corresponding phosphorothioates **15a–d** within 30 s in near quantitative yields. This reagent led to rapid, efficient (stepwise yields of 99%), and reliable automated synthesis of phosphorothioate oligomers (28-mers) complementary to the mRNA of the HIV-1 *rev* gene, carrying either exclusively or a predetermined number of P(S) linkages. Additionally, oligomers exposed to prolonged treatment (24 h) with the sulfurizing reagent did not show any detectable modification of the nucleosidic bases.

Introduction

Nucleoside phosphorothioates are isoelectronic analogues of natural nucleotides in which a nonbridging oxygen atom of the phosphate group is replaced by a sulfur atom.[1] When introduced on either the α- or β-phosphate of a nucleoside triphosphate this modification confers chirality at phosphorus, thereby providing an excellent tool for the determination of the stereochemical course of many enzymatic nucleotidyl and phosphoryl transfer reactions.[1,2] Moreover, the introduction of the above isoelectronic modification on the internucleotidic phosphate linkages of oligodeoxyribonucleotides considerably enhances their

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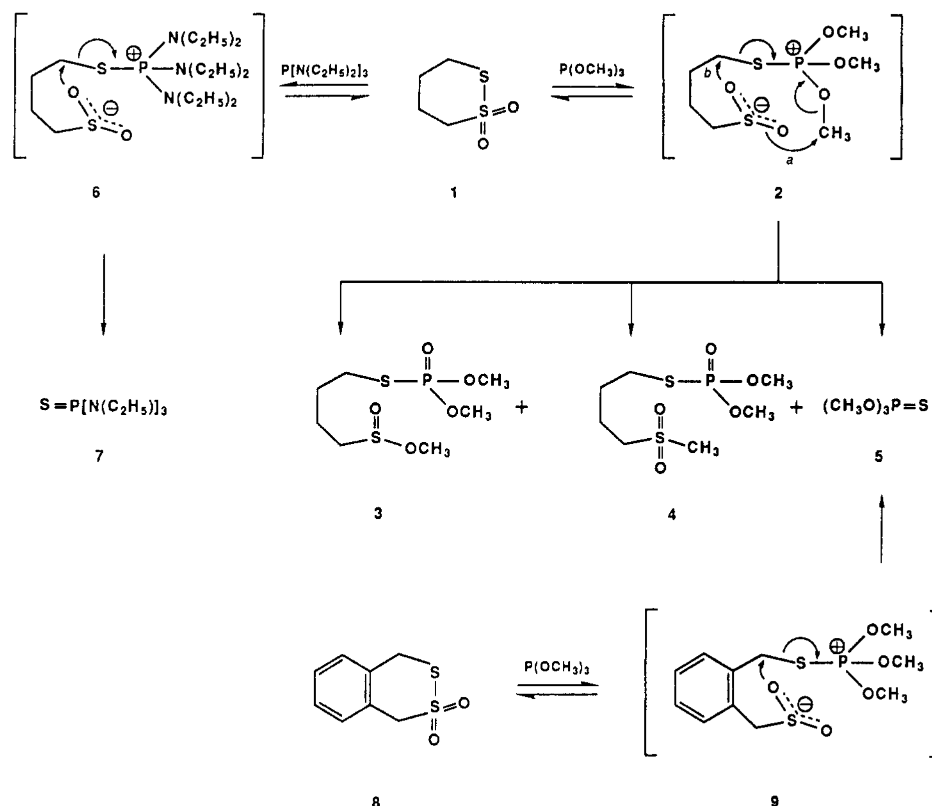
Scheme I



resistance to degradation by nucleases relative to natural oligonucleotides.[2,3] As a consequence of this attribute, oligodeoxyribonucleoside phosphorothioates have demonstrated their usefulness as "antisense" molecules by inhibiting gene expression.[4] The inhibitory mechanism is presumed to occur from the hybridization of a specific messenger RNA (the "sense" molecule) with the complementary ("antisense") DNA sequence. Translation of the message by the ribosomes is thus impaired by either the DNA-RNA duplex[5] or the degradation of the heteroduplex by RNase H[6] or both.

It has been shown that antisense phosphorothioates complementary to the messenger RNA of the HIV-1 *rev* (formerly *art/trs*) gene inhibited the cytopathic effect of the virus in chronically infected H9 cells.[7] By contrast, neither the corresponding sense phosphorothioate nor the natural antisense oligodeoxyribonucleotide was effective in inhibiting viral expression under identical conditions.[7] It has also been demonstrated that the phosphorothioate homodeoxyribooligomer S-dC_{28} blocked the cytopathic effect of HIV-1 in uninfected ATH8 cells (de novo infection) under conditions where the natural homodeoxyribooligomer dC_{28} did not show significant inhibitory effects.[8] These latter findings suggest that oligodeoxyribonucleoside phosphorothioates may represent a new class of experimental chemotherapeutic agents against AIDS.[9] Consequently, the availability of these oligonucleotide analogues is critical for clinical investigations.

The automated synthesis of phosphorothioates can be achieved according to the "H-phosphonate"[10] or the "deoxyribonucleoside phosphoramidite"[11] approach. It has been reported that upon completion of the synthesis, H-phosphonate oligomers could be converted into phosphorothioates by a single sulfurization reaction effected by elemental sulfur (S_8).[12] This methodology is, however, incompatible with the preparation of oligonucleotides carrying predetermined combinations of natural and phosphorothioate linkages. In addition to this inherent limitation, the H-phosphonate approach has not been, in our hands, as efficient as the phosphoramidite methodology for the preparation of phosphorothioate oligomers. Alternatively, the automated synthesis of these analogues using deoxyribonucleoside phosphoramidite synthons[13,14] suffers from a relatively slow (7.5 min)[14] stepwise sulfurization reaction performed by elemental sulfur. This re-

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action has often led to instrument failure as a result of the insolubility of S_8 in most organic solvents.

To alleviate these problems, sulfur-transfer reagents were designed to meet the following criteria: (i) the reagent should be easily prepared and stable under normal laboratory conditions; (ii) the solubility of the reagent and its stability in various solvents for prolonged period of time should not affect the performance of the instrument during oligonucleotide synthesis; (iii) the sulfur-transfer reaction should be rapid to minimize the synthetic cycle time and should yield to a quantitative formation of phosphorothioates; and (iv) to preserve the genetic identity of the synthetic DNA, the reagent must not generate nucleosidic modifications during the cumulative sulfurization reactions.

The rationale leading to the preparation of these compounds along with their properties are reported herein.[15]

Results and Discussion

From the various polysulfides considered as potential sulfur-transfer reagents, the cyclic thiosulfonate **1**[16] was first investigated. It was envisioned that this compound would be susceptible to nucleophilic attack at the sulfenyl sulfur by trialkyl phosphites and nucleoside phosphite triesters, leading to the cleavage of the polarized sulfur–sulfur bond. The ambident sulfinate anion **2** thus generated would then complete the sulfur-transfer reaction via an intramolecular cyclization (Scheme I, path b).

This approach was evaluated by the addition of trimethyl phosphite to a 2-fold excess of the thiosulfonate **1** in deuteriochloroform ($CDCl_3$). ^{31}P NMR analysis of the reaction mixture displayed resonances at δ 32 ppm, accounting for 92% of the resonances observed. The absence of a resonance at δ 73 ppm indicated that the desired trimethyl thiophosphate (**5**) was not generated under these conditions. The signals at δ 32 ppm tentatively represent the *O,O,S*-trialkyl phosphorothiolates **3** and/or **4** arising from the intramolecular dealkylation of the quasi-phosphonium complex **2** by the ambident sulfinate anion (Scheme I, path a). This assignment is consistent with the data reported by Michalski et al.[17] describing the preparation of *O,O,S*-trialkyl phosphorothiolates from the reaction of trialkylphosphites with certain thiosulfonates. Interestingly, according to ^{31}P NMR spectroscopy, the reaction of tris(diethylamino)phosphine with **1** under the above conditions also resulted in the formation of a phosphonium salt structure (**6**) (δ 65 ppm) which gradually converted to tris(diethylamino)phosphine sulfide (**7**) (δ 79 ppm).[16] The formation of **7** presumably resulted from the nucleophilic attack of the sulfinate anion **6** at the electrophilic center α to the sulfenyl sulfur. This experiment thus suggests that the reaction of **1** with trimethyl phosphite could have generated trimethyl thiophosphate if the intermediate sulfinate anion **2** had preferentially attacked the electrophilic center α to the sulfenyl sulfur atom (Scheme I, path b). To test this hypothesis, the structure of the thiosulfonate **1** was modified as the benzo analogue **8**[18] which was prepared by reacting o-xylene-α-α'-dithiol with hydrogen peroxide in glacial acetic acid. In addition to providing rigidity to the molecule, the fused phenyl ring created electrophilic centers (benzylic) adjacent to both sulfur atoms. The reaction of trimethyl phosphite with **8** under the conditions described for **1** afforded the desired trimethyl thiophosphate **5** (δ 73 ppm) in yields greater than 90%.

The suitability of **8** toward solid-phase synthesis of oligodeoxyribonucleoside phosphorothioates according to the phosphoramidite methodology was then evaluated by reacting, for 1 min at ambient temperature, a 1.0 M solution of **8** in dichloromethane with the dinucleoside phosphite triester **13a**. The extent of the sulfurization reaction was measured by the oxidation of unreacted **13a** with aqueous iodine.[19] After standard deprotection,[20] the composition of the reaction products was analyzed by HPLC. The phosphorothioate dimer S-d(TpT) was produced in only ca. 25% yield as a mixture of *S*p and *R*p diastereoisomers.[13]

In an attempt to enhance the sulfurization kinetics, the structure **8** was redesigned as the thiosulfonate **12** by the fusion of the sulfonyl moiety to the aromatic ring and the introduction of a carbonyl group at the benzylic position adjacent to the sulfenyl sulfur (Scheme II). It was rationalized that the increased electrophilicity of the sulfonyl group created by the electron-withdrawing effect of the carbonyl group at the ortho position would destabilize further, relative to **8**, the sulfur–suflur linkage in **12** thereby facilitating its cleavage by nucleoside phosphite triesters. Additionally, as in **9** (Scheme I), the intramolecular nucleophilic attack by the sulfinate anion at the proximal electrophilic center required to complete the sulfur-transfer reaction would be accelerated as a consequence of the inductive effect created by the carbonyl group. The known thiosulfonate **12**[21] was prepared by the oxidation of 3*H*-1,2-benzodithiol-3-one (**10**)[22] with trifluoroperoxyacetic acid.[23]

The efficacy of **12** as a sulfur-transfer reagent was then evaluated during the automated preparation of the dinucleoside phosphorothioates **15a–d**. A 0.2 M solution of **12** in acetonitrile was reacted with **13a** (2 μmol) for 30 s at ambient temperature. To assess the extent of the sulfurization reaction, any unreacted **13a** was oxidized with aqueous iodine. After deprotection, HPLC analysis revealed that the phosphorothioate dimer S-d(TpT) was obtained in greater than 99% yield. The sulfurization of **13b–d** under similar conditions was equally successful. In addition to being soluble in a variety of organic solvents at various concentrations (up to 2 M), the sulfur-transfer reagent **12** was stable in acetonitrile solution for prolonged period of time without losing significant activity.[24]

The properties of the cyclic mixed anhydride **16** (Scheme II) generated during the sulfurization reaction were investigated. Specifically, **16** was isolated from the reaction of an equimolar amount of the thiosulfonate **12** with trimethyl phosphite in methylene chloride. The preparation of **16** was also accomplished[25,26] by the reaction of the disodium salt of *o*-sulfinobenzoic acid with oxalyl chloride.

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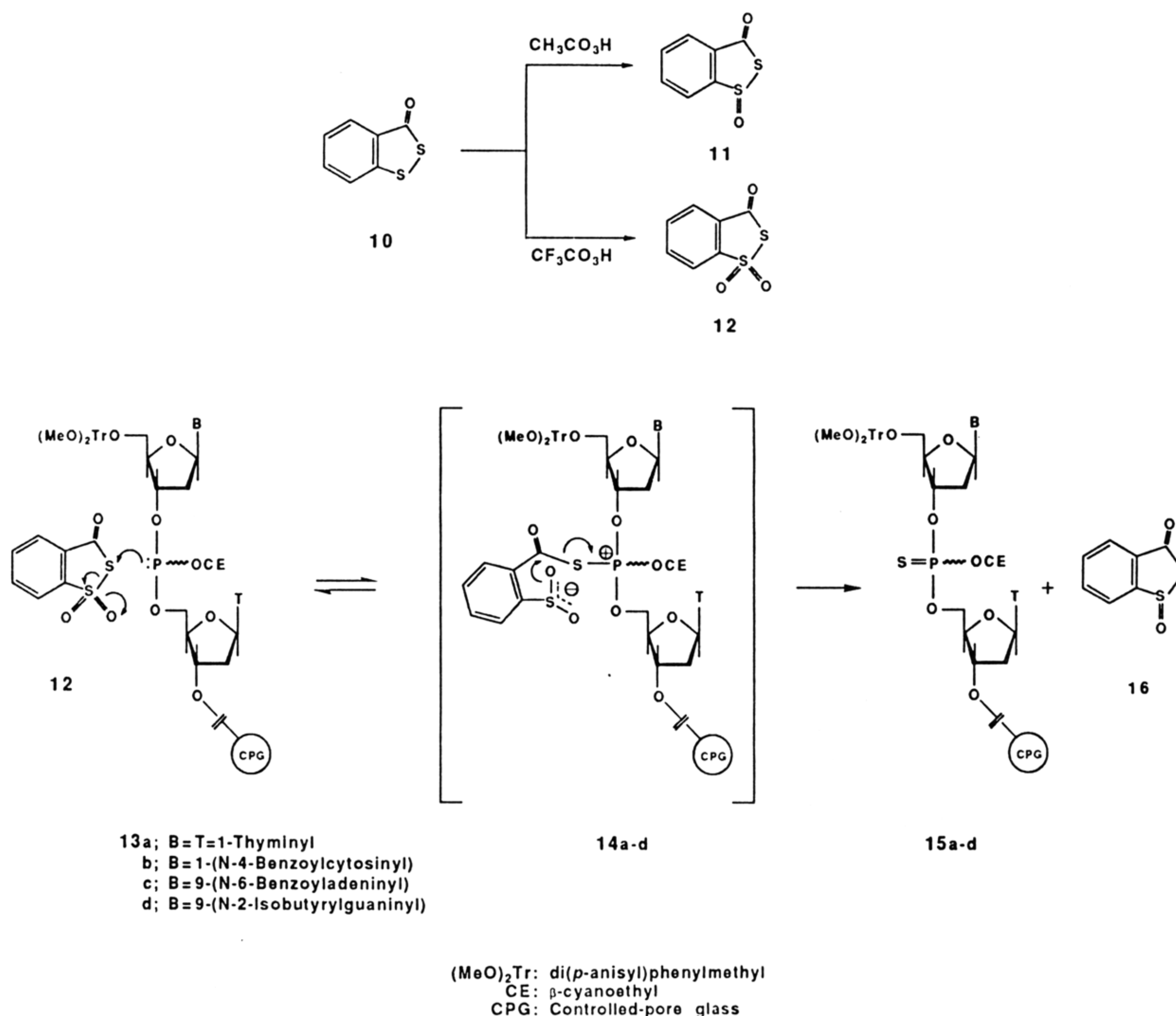
Scheme II



(MeO)$_2$Tr: di(*p*-anisyl)phenylmethyl
CE: β-cyanoethyl
CPG: Controlled-pore glass

The cyclic mixed anhydrides resulting from these different preparations were found to be identical, according to ^{13}C NMR spectroscopy. Interestingly, the reaction of trimethyl phosphite with 2 molar equiv of **16** in deuterated chloroform resulted in the immediate and quantitative formation of trimethyl phosphate as ascertained by ^{31}P NMR spectroscopy. Furthermore, a 0.2 M solution of **16** in acetonitrile quantitatively oxidized **13a** to the corresponding dinucleoside phosphate triester within 30 s at ambient temperature. However, despite its potent oxidizing properties, the susceptibility of **16** toward moisture precludes its use as a reliable alternative to iodine in routine solid-phase DNA syntheses. It is worth mentioning that the potential competition between the thiosulfonate **12** and the cyclic mixed anhydride **16** during the sulfurization of **13a–d** was not observed presumably because of the overall low concentration of **16** relative to **12**.

To further evaluate the usefulness of **12** as a sulfurizing reagent, an oligodeoxyribonucleoside phosphorothioate (28-mer) complementary to the messenger RNA of the HIV-1 *rev* gene was synthesized. A 99% stepwise yield was obtained throughout the synthesis according to "trityl color" determination.[27] After standard deprotection,[20] the crude oligomer was loaded onto a 20% polyacrylamide–7 M urea gel next to an oligomer having the same sequence but composed of only natural phosphodiester linkages. After electrophoresis, visualization of the gel under UV light indicated that the preparation of the phosphorothioate oligomer was as efficient as the preparation of the natural oligomer when comparing the ratio of product vs failure sequences (see Figure 1). Part of the crude phosphorothioate oligomer was then purified by HPLC and analyzed by ^{31}P NMR spectroscopy. More than 96% of the resonances observed accounted for the phosphorothioate diester (P(S)) linkages (δ 52 ppm) whereas less than 4% of the resonances corresponded to natural phosphoric diester (P(O)) linkages (δ –4 ppm) (data not shown).

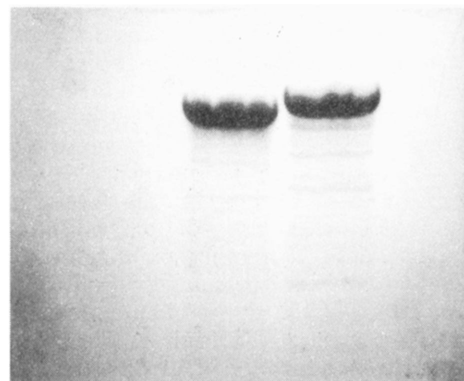


Figure 1. Electrophoretic mobility of crude and fully deprotected synthetic oligomers. Left lane: d(TCGTCGCTGTCTCCGCTTCTTCCTGCCA). Right lane: S-d(TCGTCGCTGTCTCCGCTTCTTCCTGCCA).

The presence of the P(O) linkages was unrelated to the use of **12** as the sulfurizing reagent. P(O) linkages (5%

(27) The periodic release of the dimethoxytrityl cation was spectrometrically measured using an extinction of 72000 at 498 nm.

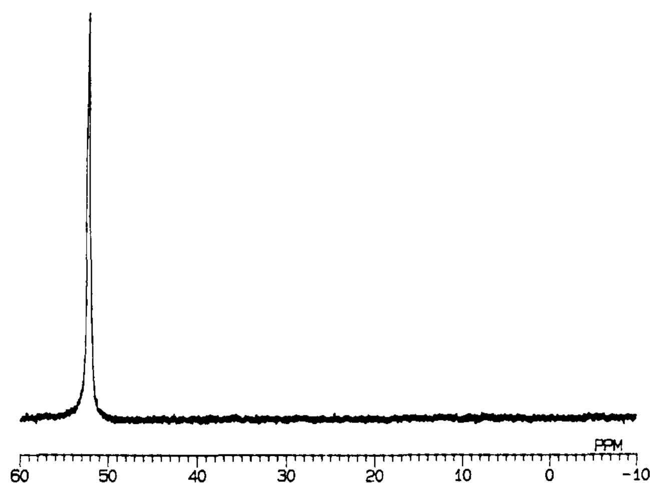


Figure 2. ^{31}P NMR spectrum of the oligodeoxyribonucleoside phosphorothioate S-d(TCGTCGCTGTCTCCGCTTCTTCC-TGCCA). The spectrum was recorded on a JEOL GSX 500 NMR spectrometer operating under the conditions delineated in the Experimental Section. D_2O was used as the solvent and trimethyl phosphate in D_2O as an external reference (0 ppm).

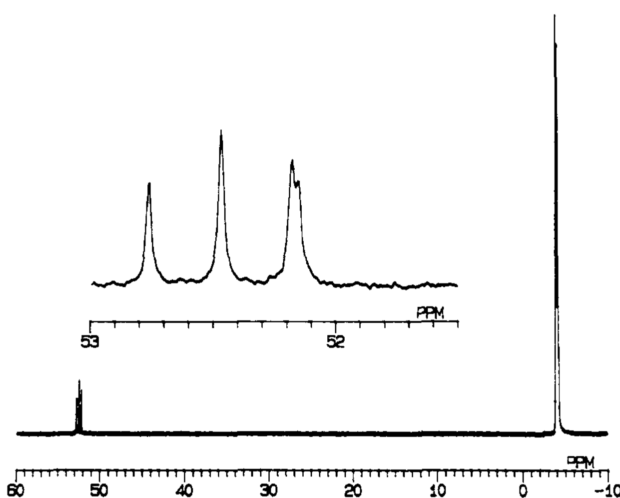


Figure 3. ^{31}P NMR spectrum of the oligodeoxyribonucleoside phosphorothioate d(T_{PS}CGTCGCTGTCTCCGCTTCTTCC-TGCC$_{PS}$A). The spectrum was recorded as described in Figure 2.

or less) were also observed when S_8 was used as sulfur-transfer reagent during the solid-phase synthesis of oligodeoxyribonucleoside phosphorothioates according to the phosphoramidite methodology.[14] Incidentally, the formation of a small percentage of P(O) linkages mainly resulted from the "capping" step preceding the sulfurization reaction during the synthetic cycle. Thus, elimination of this step during the preparation of the above phosphorothioate oligomer reduced the content of P(O) linkages to less than 1% as estimated by ^{31}P NMR spectroscopy.

The capping step is, however, an essential reaction of the solid-phase DNA synthesis cycle. It involves the acetylation of unreacted (failure) sequences after chain elongation to permit a better separation of the desired oligomer from the failure sequences at the end of the synthesis.[20] To prevent premature oxidation of the phosphite link, the capping step was performed after the sulfurization reaction. Under these conditions, less than 1% of P(O) linkage were detected by ^{31}P NMR analysis of the HPLC-purified phosphorothioate oligomer described above (Figure 2).

The biological significance of a small percentage of P(O) linkages carried by an antisense oligodeoxyribonucleoside phosphorothioate was investigated by the preparation of three oligomers.[28] One of the antisense phosphorothioate DNA was synthesized according to the phosphoramidite approach using the thiosulfonate **12** as sulfurizing agent.[29] ^{31}P NMR spectroscopy of the purified oligomer indicated the presence of ca. 2% of P(O) linkages. The same antisense and the corresponding sense phosphorothioate oligomers were prepared according to the H-phosphonate approach using S_8 as the sulfur-transfer reagent. Although the isolated yields of the oligomers prepared by the latter method were about half of that obtained using the phosphoramidite methodology, their ^{31}P NMR spectra indicated the absence of P(O) linkages. These phosphorothioate oligomers were then tested for the inhibition of expression of the *myc* gene product in peripheral blood mononuclear cells. Both antisense oligomers led to the same percentage of inhibition of the *myc* gene expression at identical concentrations, whereas the sense oligomer had no detectable inhibitory effect under the same conditions.[30] This experiment provided the evidence that a small percentage of P(O) linkages (ca. 2%) did not alter the relative effectiveness of oligodeoxyribonucleoside phosphorothioates as antisense molecules.

The versatility of the phosphoramidite approach in conjunction with the sulfur-transfer reagent **12** was demonstrated by the synthesis of an oligomer carrying a P(S) link at its 5′ and 3′ end. ^{31}P NMR analysis of the purified oligonucleotide displayed the proper P(S) resonances as sets of doublets (δ 52.8, 52.5, 52.2, and 52.1 ppm) in correct integrated ratio relative to the P(O) resonances (Figure 3). The resonances at δ 52.5 and 52.2 or 52.1 ppm correspond to the P(S) link located at the 5′-end of the oligomer. This assignment was supported by the preparation of the same oligomer carrying only one phosphorothioate diester function at its 5′-terminus and by recording its ^{31}P NMR spectrum after standard deprotection and purification by HPLC. Similarly, the resonances at δ 52.8 and 52.2 or 52.1 ppm were assigned to the P(S) link positioned at the 3′-end of the oligomer.

To investigate the potential occurrence of nucleosidic modification during the sulfurization step, a DNA sequence (28-mer)[31] carrying exclusively P(O) linkages with equal number of the four nucleosidic bases was synthesized. The fully protected oligomer, covalently attached to the solid support, was incubated at ambient temperature with a 0.2 M solution of **12** in acetonitrile for 24 h. An identical experiment in which **12** was omitted was also performed. After deprotection and purification, the oligomers were subjected to enzymatic digestion with snake venom phosphodiesterase and alkaline phosphatase.[32] Nucleosidic base modification was not observed from HPLC analysis of the hydrolysates as only peaks corresponding to the four nucleosides were detected.[33]

(28) S-d(AACGTTGAGGGGCAT) ("antisense" strand); S-d-(ATGCCCCTCAACGTT) ("sense" strand).

(29) During the preparation of the oligomer, the "capping" step was deliberately performed before the sulfurization reaction to generate detectable P(O) linkages.

(30) Stetler-Stevenson, M. A.; Kelly, C., personal communication.

(31) d(TACCGTAGCTAAGGTCATGCAAGTTCCG)

(32) Seela, F.; Kaiser, K. *Nucl. Acids Res.* **1987**, *15*, 3113–3129.

(33) This experiment was not performed with a DNA sequence bearing exclusively P(S) linkages since it hs been reported that the digestion of these oligonucleotides by snake venom phosphodiesterase (SVP) is very slow. Specifically, natural oligodeoxyribonucleotides were hydrolyzed by SVP ca. 3500 times faster than the corresponding phosphorothioate analogues.[14] Moreover, prolonged digestion of oligomers with SVP may allow a contaminating activity such as adenosine deaminase to complicate the interpretation of the results.[36]

In summary, it has been demonstrated that due to its solubility in common organic solvents, its high efficiency as a sulfur-transfer agent in addition to its rapid sulfurization kinetics and facile adaptation to automation, the thiosulfonate **12** is the reagent of choice for the preparation of oligodeoxyribonucleoside phosphorothioates via the phosphoramidite approach.

Experimental Section

Materials and Methods. Reagents and solvents were obtained from commercial suppliers and were used without further purification. The preparation of 1,2-dithiane 1,1-dioxide (**1**) was achieved according to the procedure reported by Harpp et al.[16] 3*H*-2,1-Benzoxathiol-3-one 1-oxide (**16**) was prepared from the disodium salt of *o*-sulfinobenzoic acid and oxalyl chloride according to the procedure described by Lin'kova et al.[26] *o*-Sulfinobenzoic acid was prepared as reported by Douglass et al.[25]

Column chromatography was performed with silica gel 60 (230–400 mesh) (EM Science). Thin-layer chromatography (TLC) was performed on plastic plates coated with a 0.2 mm thick layer of silica gel 60 F_{254} (EM Science).

Melting points are uncorrected and were determined on a Büchi 510 melting point apparatus. NMR spectra were recorded with either a General Electric Model GN 300 or a JEOL GSX 500 NMR spectrometer operating in the presence of broad-band decoupling at 7.05 Tesla (300 MHz for ^1H) or 11.75 Tesla (500 MHz for ^1H), respectively. ^{13}C NMR spectra were recorded in $CDCl_3$ using the middle line of resonances obtained from the solvent (77 ppm) as internal reference. ^{31}P NMR spectra were recorded in deuterated solvents using, as indicated, either 85% phosphoric acid or trimethyl phosphate as an external reference.

Electron-ionization mass spectra were recorded with a Hewlett-Packard 5995 gas chromatograph/mass spectrometer equipped with an HP 59970C MS Chem Station data system. The ionization potential was 70 eV, and the ionizing current was 220 μA.

Solid-phase oligodeoxyribonucleotide syntheses were performed using an Applied Biosystems 380B DNA synthesizer. β-Cyanoethyl deoxyribonucleoside phosphoramidites and all the reagents pertaining to the automated preparation of oligonucleotides were purchased from Applied Biosystems Inc. and used as recommended by the manufacturer. The sulfurization reaction required for the preparation of oligodeoxyribonucleoside phosphorothioates was effected by a 0.2 M solution of the crystalline thiosulfonate **12** (mp 102.5–103 °C) in acetonitrile (Burdick & Jackson, <0.03% water). The time allotted for the reaction was set at 30 s. The reagent was kept in an amber glass bottle,[34] which was placed on the DNA synthesizer at port no. 16.

Deprotected oligonucleotides bearing the "dimethoxytrityl" group at the 5'-end were purified by reverse-phase HPLC using a Hamilton PRP-1 column (10 mm o.d. × 270 mm) under the following conditions: linear gradient of 20% acetonitrile (MeCN)/0.1 M triethylammonium acetate pH 7.0 (TEAA) to 30% MeCN/TEAA in 10 min at a flow rate of 3 mL/min and then held isocratically for 10 min. Purified oligomers were detritylated using 80% acetic acid (30 min), and the solution was extracted three times with an equal volume of ethyl acetate. The aqueous phase was evaporated to dryness under reduced pressure. Phosphorothioate oligomers were stored in water at −20 °C whereas natural oligomers were kept dry at −20 °C.

Fully deprotected oligomers were electrophoresed on 20% polyacrylamide–7 M urea gels (40 cm × 20 cm × 0.75 mm) prepared, as described by Maniatis et al.,[35] using electrophoresis purity reagents (Bio-Rad). Upon exposure to UV light (254 nm) synthetic oligomers appear as dark blue bands when the gel is applied against a fluorescent background (silica gel 60 F_{254} TLC plate).

Enzymatic degradation of desalted (Sephadex G-50, Pharmacia) DNA was achieved with phosphodiesterase (*Crotalus durissus*, Boehringer) and bacterial alkaline phosphatase (Sigma) according to a published procedure.[32]

Benzo-2,3-dithiane 2,2-Dioxide (8). *o*-Xylene-α,α'-dithiol (Aldrich) (10 g, 58 mmol) was dissolved by heating with 80 mL of glacial acetic acid. The solution was then allowed to cool at ambient temperature in a water bath. Aqueous 30% hydrogen peroxide (Aldrich) (20 mL, 176 mmol) was added, dropwise, over a period of 15 min. The turbid reaction mixture was stirred for 2 h at the water bath temperature to ensure an internal temperature not exceeding 35 °C. The mixture was then stirred at ambient temperature for 18 h. The greenish solution was filtered, and most of the acetic acid was removed under reduced pressure at a temperature lower than 35 °C. To the residue was added water (150 mL) followed by solid sodium bicarbonate until complete neutralization. The suspension was extracted with dichloromethane (ca. 200 mL). The aqueous phase was further extracted with CH_2Cl_2 (100 mL). The organic fractions were combined, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure, yielding 10.3 g of a light brown solid. The material (3.85 g) was purified on a silica gel column (3 cm × 30 cm). Elution with $CHCl_3$ afforded **8** (1.6 g), which was recrystallized from methylene chloride–hexane. The colorless but light-sensitive crystals melted at 100–102 °C (lit.[18] mp 101 °C). ^{13}C NMR ($CDCl_3$): δ 38.2, 61.4, 128.2, 128.3, 128.7, 129.7, 130.5, 130.6. MS (70 eV): *m/z* 202 (M + 2) (0.09), 201 (M + 1) (0.1), 200 (M) (0.8), 135 (100, base peak), 104 (63), 78 (28).

Further elution of the silica gel column with $CHCl_3$–EtOAc (98:2) yielded benzo-2,3-dithiane 2-oxide (1.9 g), mp 138.5–139 °C dec. ^{13}C NMR ($CDCl_3$): δ 32.9, 59.4, 127.6 (two signals at this frequency were resolved by attached proton test (APT)), 128.3, 129.1, 131.6, 135.3. MS (70 eV): *m/z* 186 (M + 2) (0.1), 185 (M + 1) (0.2), 184 (M) (1), 135 (100, base peak), 104 (76), 78 (40).

3*H*-1,2-Benzodithiol-3-one (10). The compound was prepared according to a modification of the procedure reported by McKibben and McClelland.[22] To a mechanically stirred suspension of 2-thiolbenzoic acid (Aldrich) (50 g, 0.32 mol) and concentrated sulfuric acid (500 mL) in a three-necked round-bottom flask (1 L) was added 47 mL of thiolacetic acid (Aldrich) (50 g, 0.66 mol), dropwise over a period of 40 min at ambient temperature (water bath). The temperature of the bath was then increased to 50 °C, and the brown reaction mixture was stirred for 2 h. The reaction was stopped by pouring the mixture onto crushed ice (6 L). The precipitate was filtered through a fritted-glass Buchner (2 L, coarse porosity), thoroughly washed with water and suspended in chloroform (300 mL). A saturated solution of sodium bicarbonate (150 mL) was added, and the mixture was vigorously shaken and filtered. The solid material isolated was triturated with boiling chloroform (100 mL) and filtered. The trituration process was repeated, and the filtrates were combined together. The aqueous phase was separated, and the organic phase was extracted once more with a saturated solution of sodium bicarbonate (100 mL) and twice with water (100 mL). The organic layer was then dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure. The yellow material (50.4 g) dissolved upon heating with hexane (800 mL). The solution was decanted from dark insoluble material, brought to reflux, and allowed to cool first at ambient temperature and then kept in the refrigerator (10 °C). The yellow crystals were isolated by filtration, washed with cold hexane, and dried under vacuum. Yield: 44.4 g (0.26 mol, 82%). This material is suitable for the preparation of **12**.

A sample of **10** recrystallized from 95% ethanol melted at 75–76 °C and was submitted to spectral analysis. ^{13}C NMR ($CDCl_3$): δ 193.5, 148.2, 133.5, 129.0, 127.2, 125.6, 124.6. MS (70 eV): *m/z* 170 (M + 2) (9), 169 (M + 1) (9), 168 (M) (100, base peak), 140

(34) The bottle was first cleaned by immersion in concentrated sulfuric acid for several hours (ca. 15 h), washed with water, and dried. The bottle was then "siliconized" by treatment with a 10% solution of dichlorodimethylsilane in dichloromethane for 3–5 min, rinsed with methanol, and dried in an oven at 110 °C. These precautionary measures also apply to bottles that have been washed with strong alkali and/or with detergents. Prior to the attachment of the reagent bottle to port no. 16, the Teflon capillary line was either replaced or thoroughly rinsed by passing acetonitrile. Storing solutions of the sulfurizing reagent in bottles containing metallic substances must be avoided to ensure optimal performance of the reagent.

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(36), 104 (49), 96 (60), 76 (46), 69 (52), 50 (60).

3*H*-1,2-Benzodithiol-3-one 1,1-Dioxide (12). To a stirred suspension of 3*H*-1,2-benzodithiol-3-one (**10**) (39.2 g, 0.23 mol) in trifluoroacetic acid (250 mL) was added 40 mL of a 30% aqueous solution of hydrogen peroxide within 1 min at ambient temperature (water bath). Cooling by adding ice to the water bath became necessary to maintain the internal reaction temperature at 40–42 °C. After 30 min an additional 40 mL of 30% H_2O_2 was rapidly added, and the reaction mixture was stirred at the same internal temperature for half an hour. The third and last portion of 30% H_2O_2 (40 mL) was then added, and the solution was warmed to stabilize the internal reaction temperature at 40–42 °C. The reaction was monitored by TLC ($CHCl_3$) for the disappearance of the slow moving thiosulfinate 11 (R_f = 0.27). The reaction mixture was then filtered, and the filtrate was added to 3.5 L of crushed ice. The white precipitate was filtered through a 2-L fritted-glass Buchner of coarse porosity and thoroughly washed with water until the filtrate was neutral and free of peroxides. The solid material was then transferred into a 500-mL separatory funnel to which were added dichloromethane (200 mL) and a 1% solution of sodium bisulfite (100 mL). After vigorous shaking, the organic phase was separated, washed with water (100 mL), and dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure, and the amorphous solid was dried under vacuum. The crude compound (20.8 g, 45%) was dissolved in boiling dichloromethane, treated with activated charcoal, and filtered through Celite. Hexane (ca. 50 mL) was added to the boiling filtrate (ca. 70 mL), and the solution was first allowed to cool at ambient temperature and then to 10 °C in the refrigerator. The crystalline thiosulfonate **12** (17.2 g), mp 102.5–103 °C (lit.[21] mp 98–99 °C), was collected by filtration through a sintered-glass funnel, washed with cold hexane, and dried under vacuum. ^{13}C NMR ($CDCl_3$): δ 182.9, 148.3, 136.5, 134.5, 130.0, 125.6, 121.9. MS (70 eV): *m/z* 202 (M + 2) (4), 201 (M + 1) (4), 200 (M) (45), 136 (100, base peak), 108 (41), 104 (37), 76 (92), 69 (31).

3*H*-1,2-Benzodithiol-3-one 1-Oxide (11). To a stirred suspension of 3*H*-1,2-benzodithiol-3-one (**10**) (10.1 g, 60 mmol) in glacial acetic acid (95 mL) was added 10.2 mL of a 30% aqueous solution of H_2O_2 over a period of 10 min at ambient temperature. After 14 h, TLC ($CHCl_3$) showed incomplete reaction. The mixture was then heated at 40 °C until completion of the reaction (ca. 2 h). After filtration, the filtrate was added to 1 L of crushed ice. The precipitate was collected on a fritted-glass Buchner and thoroughly washed with water. The material was dissolved in chloroform (100 mL) and extracted once with water (50 mL). The organic phase was dried over anhydrous magnesium sulfate and evaporated to dryness under reduced pressure. The residue (8.25 g) was recrystallized from CH_2Cl_2–hexane. The pure compound melted at 103.5–104 °C (lit.[21] mp 101.5–103 °C). ^{13}C NMR ($CDCl_3$): δ 190.4, 151.6, 135.6, 133.0, 130.8, 127.9, 126.7. MS (70 eV): *m/z* 186 (M + 2) (3), 185 (M + 1) (3), 184 (M) (33), 136 (100, base peak), 108 (28), 104 (13), 96 (10), 76 (31), 69 (17).

3*H*-2,1-Benzoxathiolan-3-one 1-Oxide (16). Trimethyl phosphite (1.2 mL, 10 mmol) was added, under an argon atmosphere, to a solution of **12** (2 g, 10 mmol) in 20 mL of CH_2Cl_2, dropwise over a period of 5 min at ambient temperature. The reaction mixture was stirred for 15 min and then evaporated to dryness under reduced pressure. The yellowish oil was triturated with cyclohexane (2 × 50 mL). The moisture-sensitive solid (1.5 g) was recrystallized from CH_2Cl_2–hexane, affording colorless crystals at 10 °C. The compound melted at 81–82 °C (lit.[26] mp 83 °C). ^{13}C NMR ($CDCl_3$): δ 165.3, 151.6, 136.3, 134.2, 128.5, 124.9, 123.4. MS (70 eV): *m/z* 170 (M + 2) (0.4), 169 (M + 1) (0.6), 168 (M) (7), 104 (100, base peak), 76 (72), 50 (44), 38 (12). This material was identical in all respects with that prepared by the published procedure.[26]

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Registry No. 5, 152-18-1; 8, 24765-75-1; 8 monooxide derivative, 127183-91-9; 10, 1677-27-6; 11, 127183-89-5; 12, 66304-01-6; 16, 127183-90-8; S-d(AACGTTGAGGGGCAT), 127279-14-5; S-d(ATGCCCCTCAACGTT), 127279-13-4; d(TACCGTAGCTAAGGTCATGCAAGTTCCG), 127279-16-7; d-(TCGTCGCTGTCTCCGCTTCTTCCTGCCA), 115427-90-2; S-d(TCGTCGCTGTCTCCGCTTCTTCCTGCCA), 127279-15-6; d(T_{PS}CGTCGCTGTCTCCGCTTCTTCCTGCC$_{PS}$A), 124991-77-1; 2-mercaptobenzoic acid, 147-93-3; *o*-xylene-α,α′-dithiol, 41383-84-0.

Synthesis of Carbocyclic Analogues of 2-Deoxy-Kdo

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3(*R*),4(*S*)-Dihydroxy-5(*R*)-(1′(*S*),2′-dihydroxyethyl)-(*S*)-cyclohexanecarboxylic acid (**1**) and 3(*R*),4(*S*)-dihydroxy-5(*R*)-(1′(*S*),2′-dihydroxyethyl)-(*R*)-cyclohexanecarboxylic acid (**2**) have been synthesized as potential inhibitors of the enzyme CMP-Kdo synthetase. The key steps in the synthesis of **1** and **2** were a three-carbon chain extension at C-4 of the protected D-manno derivatives 1-*O*-(*tert*-butyldimethylsilyl)-2,3:5,6-di-*O*-isopropylidene-4-*O*-(phenoxythiocarbonyl)-D-mannitol (**5**) and 1,6-anhydro-2,3-*O*-isopropylidene-4-*O*-(phenoxythiocarbonyl)-β-D-mannopyranose (**11a**) with allyltributylstannane under radical coupling conditions and the intramolecular alkylation of 1,4-dideoxy-4-*C*-[2′-(*tert*-butoxycarbonyl)ethyl]-1-iodo-2,3:5,6-di-*O*-isopropylidene-D-mannitol (**15**) to form the protected products **1** and **2**. Two different routes leading to **1** and **2**, both starting from D-mannose, were used. The two routes converge at 4-*C*-allyl-4-deoxy-2,3:5,6-di-*O*-isopropylidene-D-mannitol (**7a**), obtained as a diastereomeric mixture in one route and as a pure isomer in the other.

Introduction

3-Deoxy-D-manno-2-octulosonic acid (Kdo)[1] is an eight-carbon sugar found in Gram-negative bacteria. It is a constituent of the core region in the outer membrane lipopolysaccharides (LPS),[2] serving as a bridge between lipid A and the polysaccharide portion. Kdo is also found in the capsular polysaccharide (K-antigen) of some Gram-negative bacteria strains,[1,3,4] some protozoans,[5] the green

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