MHz, $CDCl_3$, DEPT) C 18.20; CH 138.85, 134.89, 71.40, 67.92; CH_2 117.63, 114.80, 41.59, 41.36, 37.30, 30.09; CH_3 26.06, -4.27, -4.59; MS (CI, CH_4) 285.2254 (M + H). Anal. Calcd for $C_{16}H_{32}O_2Si$: C, 67.55; H, 11.34. Found: C, 67.85; H, 11.23.

(3*R*,5*R*)-3-Benzoyl-5,6-epoxy-1-phenylhexanol (6). (3*S*,5*R*)-5,6-Epoxy-1-phenyl-3-hexanol (474 mg, 2.47 mmol, 1.0 equiv), benzoic acid (361 mg, 2.96 mmol, 1.2 equiv), and triphenylphosphine (766 mg, 2.96 mmol, 1.2 equiv) were dissolved in 10 mL of THF. The mixture was cooled to 0 °C and DEAD (466 μL, 2.96 mmol, 1.2 equiv) was added dropwise via syringe. After being stirred for 5 h, the reaction mixture was concentrated under reduced pressure. Flash chromatography (SiO_2, 10% ethyl acetate/hexanes) gave 631 mg (2.13 mmol, 86%) of the product as a colorless oil: $[\alpha]^{25}_D$ = +28.6° (*c* = 1.28, $CHCl_3$); IR (neat) 3060, 3027, 2997, 2923, 2861, 1715, 1601, 1584, 1496, 1452, 1359, 1314, 1274, 1176, 1112, 1070, 1026, 840, 750, 713, 701, 674 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.11–8.08 (m, 2 H), 7.58–7.16 (m, 8 H), 5.38 (m, 1 H), 3.08 (m, 1 H), 2.84–2.68 (m, 3 H), 2.48 (dd, *J* = 2.7, 5.0 Hz, 1 H), 2.28–1.88 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$, DEPT) C 166.0, 141.1, 130.2; CH 132.9, 129.5, 128.3, 128.3, 128.2, 125.9, 72.2, 48.9; CH_2 46.1, 37.1, 35.8, 31.7; MS (EI) 296.1421, 191, 174, 156, 143, 133, 130, 105, 91, 77. Anal. Calcd for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80. Found: C, 77.19; H, 6.83.

(4*S*,6*R*)-8-Phenyl-1-octene-4,6-diol (7). (3*R*,5*R*)-3-Benzoyl-5,6-epoxy-1-phenylhexanol (96.2 mg, 0.325 mmol, 1.0 equiv) was dissolved in 10 mL of THF under argon, and the solution was cooled to -78 °C. To this solution were added 1.56 mL (1.62 mmol, 5 equiv) of 1.04 M vinyllithium solution and 200 μL (1.63 mmol, 5 equiv) of $BF_3{\cdot}OEt_2$. After stirring for 90 min, the reaction was quenched with 2 mL of MeOH, and the solution was warmed to rt. The reaction mixture was then treated with 3 mL of 15% NaOH solution and 1 mL of 30% H_2O_2 solution, and stirring was continued for 2 h. The reaction mixture was then extracted (3 × Et_2O), dried ($MgSO_4$), and concentrated under reduced pressure. Purification by flash chromatography (SiO_2, 30% ethyl acetate/hexanes) gave the product (50.3 mg, 0.229 mmol, 70%) as a colorless oil: $[\alpha]^{25}_D$ = +17.2° (*c* = 1.23, $CHCl_3$); IR (neat) 3355, 3063, 3026, 2938, 2861, 1642, 1603, 1496, 1454, 1326, 1093, 996, 916, 842, 748, 700 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.31–7.13 (m, 5 H), 5.79 (m, 1 H), 5.15–5.10 (m, 2 H), 3.93–3.84 (m, 2 H), 3.59 (br s, 1 H), 3.25 (br s, 1 H), 2.77 (ddd, *J* = 6.1, 9.5, 13.8 Hz, 1 H), 2.68 (ddd, *J* = 7.1, 9.1, 13.8 Hz, 1 H), 2.23 (m, 2 H), 1.88–1.47 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$, DEPT) C 141.8; CH 134.1, 128.3, 128.3, 125.7, 71.9, 71.8; CH_2 118.2, 42.5, 42.1, 39.5, 31.5; MS (EI) 202.1360 (M - H_2O), 184, 117, 104, 92, 91. Anal. Calcd for $C_{14}H_{20}O_2$: C, 76.33; H, 9.15. Found: C, 76.17; H, 8.95.

(4*R*,6*R*)-6-(2-Phenylethyl)-3,4,5,6-tetrahydro-4-hydroxy-2*H*-pyran-2-one (8). (4*S*,6*R*)-8-Phenyl-1-octene-4,6-diol (74.8 mg, 0.340 mmol) was dissolved in 5 mL of CH_2Cl_2/MeOH (4:1). The solution was cooled to -78 °C and ozone was bubbled through until a blue color persisted. The solution was then degassed with air, followed by addition of 0.5 mL of dimethyl sulfide and warming to rt. After being stirred for 5 h, the reaction mixture was concentrated under reduced pressure. NMR of the crude residue showed it to be a 1:1 mixture of the anomeric methyl acetals. This crude mixture was dissolved in 0.05 M H_2SO_4 and stirred for 3 h at rt. This reaction mixture was then extracted (4 × Et_2O), dried ($MgSO_4$), and concentrated under reduced pressure to give a mixture of the crude lactols. The crude lactols were dissolved in 5 mL of MeOH/H_2O (9:1), followed by addition of $NaHCO_3$ (1.15 g, 13.7 mmol) and bromine (175 μL, 3.4 mmol). After stirring for 4 h, the reaction was quenched with excess $Na_2S_2O_3$ solution, extracted (4 × Et_2O), dried ($MgSO_4$), and concentrated under reduced pressure. Purification by flash chromatography (Si_2O, 60% ethyl acetate/hexanes) gave 33.5 mg (0.152 mmol, 45% yield based on starting alkene) of the product[22] as a crystalline solid: mp 106–107 °C; $[\alpha]^{23}_D$ = +67.2° (*c* = 0.67, $CHCl_3$); IR (KBr) 3401, 3059, 3028, 2936, 2867, 1723, 1495, 1450, 1431, 1388, 1315, 1257, 1181, 1154, 1071, 1051, 755, 703, 601 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.30–7.18 (m, 5 H), 4.70 (m, 1 H), 4.36 (m, 1 H), 2.87 (ddd, *J* = 5.5, 11.3, 13.7 Hz, 1 H), 2.78–2.69 (m, 2 H), 2.63 (ddd, *J* = 1.5, 3.6, 17.6 Hz, 1 H), 2.26 (br s, 1 H), 2.08–1.83 (m, 3 H), 1.75 (ddd, *J* = 3.2, 11.3, 14.4 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$, DEPT) C 170.7, 141.2; CH 128.7, 128.6, 126.3, 75.2, 62.9; CH_2 38.8, 37.5, 36.2, 31.3; MS (EI) 220.1112 (M^+), 202, 142, 129, 117, 104, 92, 91, 73, 43. Anal. Calcd for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32. Found: C, 70.87; H, 7.17.

Acknowledgment. Support has been provided by the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the National Institutes of Health (GM43854-01). G.G. acknowledges support as an NSF predoctoral fellow. We would like to thank Dr. David L. Coffen of Hoffmann-La Roche Inc. for the generous gift of BINAP ligand.

Nucleoside H-Phosphonates. 13. Studies on 3*H*-1,2-Benzodithiol-3-one Derivatives as Sulfurizing Reagents for H-Phosphonate and H-Phosphonothioate Diesters†

Jacek Stawiński* and Mats Thelin

Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

Received December 20, 1990 (Revised Manuscript Received May 2, 1991)

Formation of O-oxidized products during sulfurization of H-phosphonothioate and H-phosphonate diesters with 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (1) was found to be due to generation of the O-oxidizing agents, most likely 3*H*-2,1-benzoxathiol-3-one 1-oxide (4) and 3*H*-2,1-benzoxathiol-3-one (5), during the course of the reactions. Another source of the side products formation may be the disproportionation of 1 that occurs in the presence of triethylamine. To overcome these problems, a new sulfur-transferring reagent, 3*H*-1,2-benzodithiol-3-one (3), has been developed. Under aqueous reaction conditions, which are compatible with both solution- and solid-phase synthesis of oligonucleotides, the reagent 3 furnished clean and fast conversion of H-phosphonothioate and H-phosphonate diesters into the corresponding phosphoro*di*- and phosphoro*mono*thioates.

Introduction

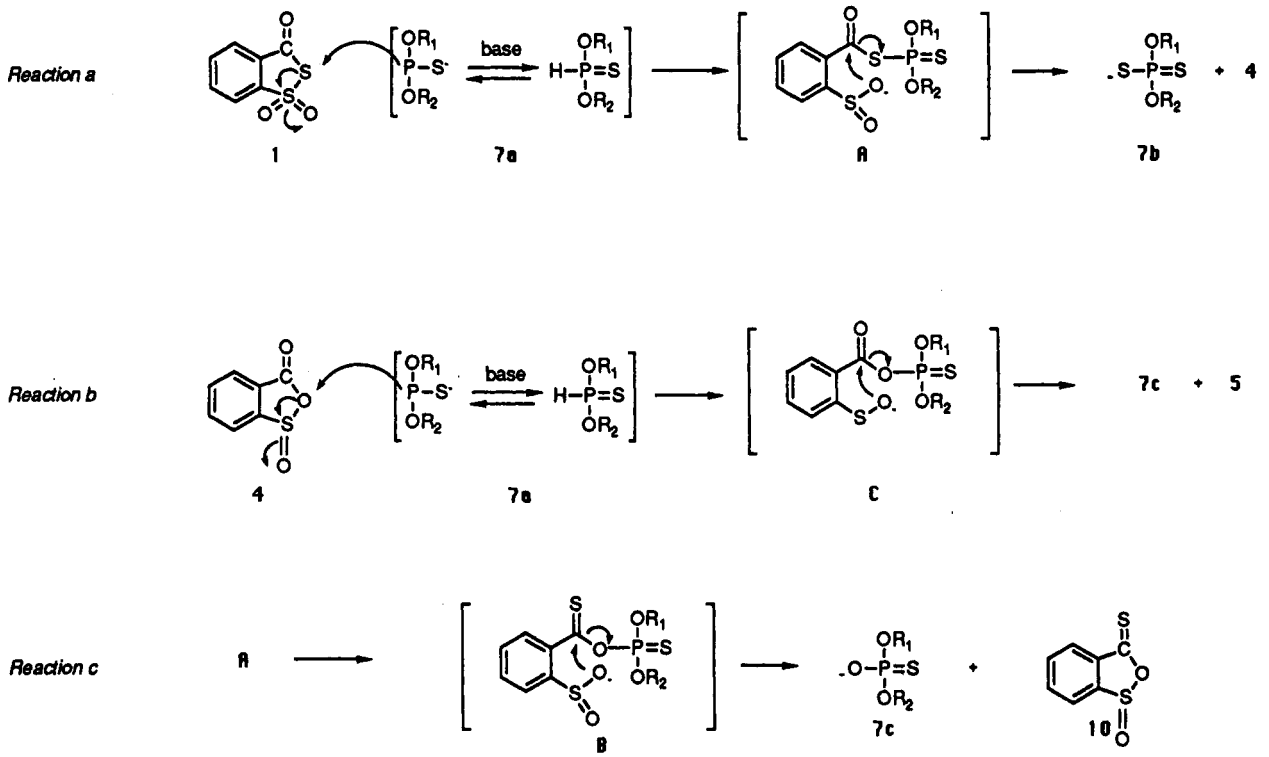
Studies on nucleoside H-phosphonates as starting materials for oligonucleotide synthesis[1] have shown that this class of compounds also can be used for the preparation of various oligonucleotide analogues.[2] One most important class among such analogues constitutes oligonucleotides

† The H is being used to emphasize that the phosphonate is unsubstituted.

(1) Garegg, P. J.; Regbert, T.; Stawinski, J.; Strömberg, R. *Chemica Scr.* **1985**, *25*, 280. Garegg, P. J.; Lindh, I.; Regberg, T.; Stawinski, J.; Strömberg, R.; Henrichson, C. *Tetrahedron Lett.* **1986**, *28*, 4055. Froehler, B. C.; Ng, P. G.; Matteucci, M. D. *Nucl. Acids Res.* **1986**, *14*, 5399.

0022-3263/91/1956-5169$02.50/0

Scheme I



in which one or two nonbridging oxygens at the phosphorus center have been replaced by sulfur. The favorable chemical and biological properties of oligonucleotide phosphorothioates and phosphoro*di*thioates, e.g., higher resistance to biological degradation by nucleases,[3] formation of stable duplexes with cellular DNA and RNA,[4,5] and inhibition of translation of complementary DNA,[4] make them a valuable research tool in molecular biology and may constitute a basis for their further chemotherapeutic applications.[5]

Sulfurization of the corresponding H-phosphonate[6] and H-phosphonothioate[7] diesters is a convenient and promising way for preparation of phosphoro*mono*- and phosphoro*di*thioates. For that purpose we have recently reported using 3*H*-1,2-benzodithiol-3-one 1,1-dioxide[8] (1), a reagent developed by Beaucage at al.[9] for the transformation of phosphite triesters into phosphorothioates. However, in contradistinction to sulfurization of nucleoside H-phosphonothioate diesters with elemental sulfur, the reagent 1 produced also phosphoro*mono*thioates (O-oxidation).[8] The amount of the O-oxidized compounds varied, yielding in some experiments up to 60% of this side product. Although we did not observe the O-oxidized products in preliminary experiments on sulfurization of nucleoside H-phosphonate diesters, our later studies have shown that this kind of side products can also be formed during this reaction.

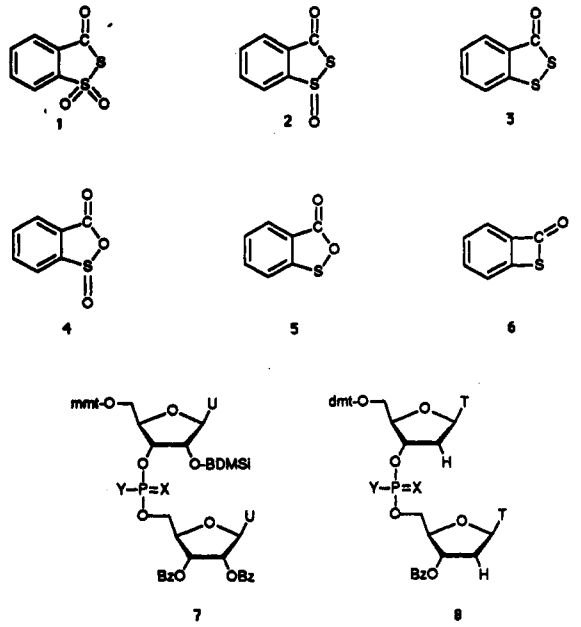
This paper presents studies directed toward (i) identification of a source of the O-oxidized products formed under certain conditions during sulfurization with the reagents 1 and 2, and (ii) development of a new reagent suitable for sulfurization of H-phosphonothioate and H-phosphonate diesters.

Results and Discussion

We have previously found[8] that 1 can act as a sulfurizing reagent for H-phosphonothioate and H-phosphonate diesters in acetonitrile in the presence of triethylamine. We suggest that the mechanism of sulfurization (Scheme I, reaction a) is similar to that proposed by Beaucage et al.[9] for phosphite triesters and involves inter alia generation of an oxidizing species (presumably compound 4) from the sulfurizing reagent. Assuming such a mechanism, one can explain formation of the O-oxidized byproduct (**7c**) as a result of a competitive reaction of the H-phosphonothioate **7a** with 3*H*-2,1-benzoxathiol-3-one 1-oxide (4 Scheme I, reaction b). Alternatively, **7c** may also be formed, if the intermediate A before cyclization would undergo partial rearrangement to the intermediate B (Scheme I, reaction c).

Sources of O-Oxidation during Sulfurization Reactions. Participation of reaction b (Scheme I) in the formation of the O-oxidized products can be easily evaluated by carrying out the sulfurization of H-phosphonothioate **7a** with 0.5 equiv of 1 in acetonitrile in the presence of 2 equiv of triethylamine (TEA). If compound 4 (which according to reaction a is supposed to be formed in equimolar amounts from 1 during sulfurization) acts as an oxidizing agent, one should expect a complete conversion of the starting material **7a** into a mixture of the *di*- and *mono*thiophosphates **7b** and **7c**. This was indeed observed, but the ratio of the S-oxidized to the O-oxidized products was almost 1:2. This can be rationalized by assuming that

(2) Froehler, B. C. *Tetrahedron Lett.* **1986**, *27*, 5575.
(3) Eckstein, F. *Ann. Rev. Biochem.* **1985**, *54*, 367.
(4) Matsukara, M.; Zon, G.; Shinozuka, K.; Robert-Gurogg, M.; Shimada, T.; Stein, C. A.; Mitsya, H.; Wong-Staal, F.; Cohen, J.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 4244.
(5) Zon, G. *Pharm. Res.* **1988**, *5*, 539.
(6) Kume, A.; Fujii, M.; Sekine, M.; Hata, T. *J. Org. Chem.* **1984**, *49*, 2139.
(7) Stawinski, J.; Thelin, M.; Zain, R. *Tetrahedron Lett.* **1989**, *30*, 2157.
(8) Stawinski, J.; Thelin, M.; von Stedingk, E. *Nucleosides Nucleotides* **1990**, in press (presented at the 9th International Round Table: Nucleosides, Nucleotides and Their Biological Applications, June 29–August 3, 1990, Uppsala, Sweden).
(9) Iyer, R. P.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 1253.



7a, 8a, Y=H, X=S

7b, 8b, Y=SH, X=S

7c, 8c, Y=OH, X=S

7d, 8d, Y=H, X=O

7e, 8e, Y=OH, X=O

Abbreviations: mmt - 4-monomethoxytrityl; dmt - 4,4'-dimethoxytrityl; BDMSI - t-butyldimethylsilyl; Bz - benzoyl; U - uracil; T - thymine

3*H*-2,1-benzoxathiol-3-one (compound 5), which probably is formed in reaction b, also can act as an oxidizing agent. Since a similar ratio of **7b** to **7c** was observed with 1.5 equiv of 1, the reactivities of compounds 4 and 5 seem to be comparable to that of 1. With a larger excess of 1 (5 equiv) the amount of the O-oxidized product was only slightly reduced (ca. 45% of **7c** was formed).

Sulfurization of the H-phosphonate **7d** with 1.5 equiv of 1 proceeded similarly to **7a**, but the amount of the O-oxidized product **7e** was substantially smaller (~10%). With 0.5 equiv of 1, the reaction went to completion, and the ratio of **7c** to **7e** was found to be ~1:1. With less than 0.5 equiv of 1, the reaction did go to completion, but the ratio of **7c** to **7e** remained ~1:1. These may indicate that only compound 4 (but not 5) acts as an oxidizing reagent during sulfurization of H-phosphonate diesters.

To investigate this problem further and to find, if possible, a better sulfur-transferring reagent, we turned our attention to 3*H*-1,2-benzodithiol-3-one 1-oxide (2). Assuming that a similar reaction mechanism is operating as for 1, only one O-oxidizing species, the oxathiolone 5, should be generated from 2. Thus, the reactivity of 5 toward the H-phosphonothioate **7a** should be easily inferred from the amount of **7c** formed during the sulfurization.

The reaction of H-phosphonothioate **7a** with 1.5 equiv of 2 showed kinetics similar to the reaction with 1, but the amount of the O-oxidized product **7c** was only ~20%. Formation of this side product was further reduced (to ~2%) when 5 equiv of 2 was used. With 0.5 equiv of 2 the reaction went to completion and afforded equimolar amounts of **7b** and **7c**. Thus, it seems likely that an O-oxidizing species (presumably the oxathiolone 5) is generated in equimolar amounts from 2 during the sulfurization and that it can act as an oxidizing agent.

The results from sulfurization of the H-phosphonate **7d** with 2 could not be interpreted in such a straightforward manner. With 1.5 equiv of 2 the reaction was clean but

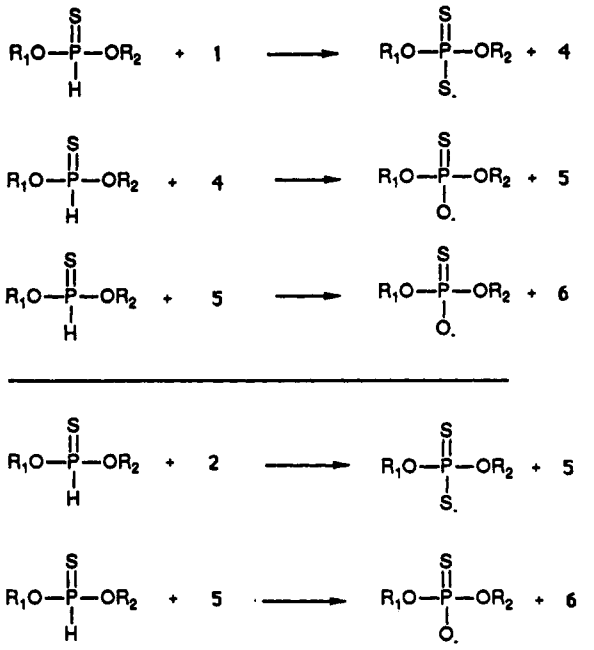


Figure 1. Reactions occurring during sulfurization of H-phosphonothioate diesters with the reagents 1 and 2.

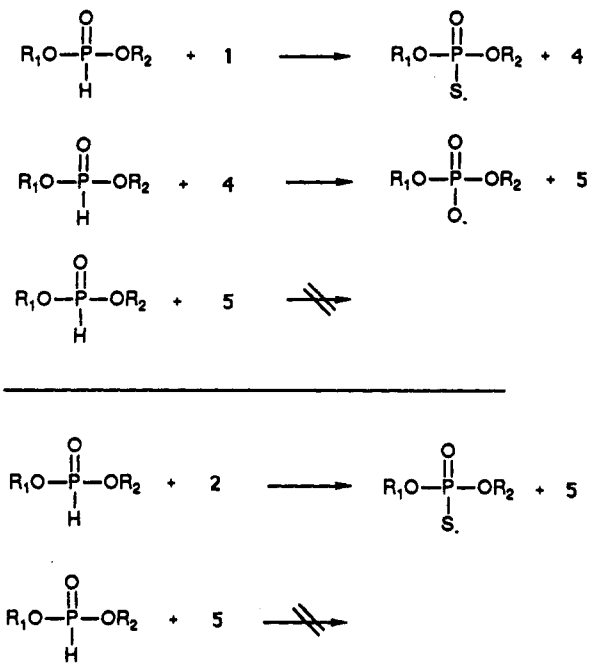


Figure 2. Reactions occurring during sulfurization of H-phosphonate diesters with the reagents 1 and 2.

slower (ca. 3 min) and afforded in addition to **7c** less than 5% of the O-oxidized product **7e**. When the amount of 2 was decreased to 0.5 equiv, the ^{31}P NMR spectrum showed, as expected, that after ca. 3 min half of the H-phosphonate **7d** was sulfurized, but the amount of **7e** only increased to ~10% (upon standing overnight, total 15% of the O-oxidized product). We interpreted these results in the following way. The compound 5, which most likely is formed during the sulfurization reaction, may act as an O-oxidizing reagent for the H-phosphonate **7d**; however, because of the slow reaction with **7d**, a substantial part of it undergoes decomposition. This would explain why only a small amount **7e** is formed during the sulfurization reaction and why it does not increase much even after longer time. Such an interpretation is in agreement with the expected properties of 5.[10] We have also tried to probe

the presence of 5 in the reaction mixture of 7d and 0.5 equiv of 2 by adding after ca. 3 min trimethyl phosphite. Even though this compound is usually quickly oxidized by 5 (see later in the text), no trimethyl phosphate could be detected in the reaction mixture.

The reactions that can occur during the sulfurization of H-phosphonothioate and H-phosphonate diesters with the reagents 1 and 2 are summarized in Figures 1 and 2. Since these explain the formation of the O-oxidized products during sulfurization quite well, it seems to be unlikely that an alternative reaction pathway (Scheme I, reaction c) may be involved in this precess to any significant degree.

Attempts To Eliminate the O-Oxidation Processes Occurring during Sulfurization. When analyzing the reactions shown in Figures 1 and 2 it seems that the simplest way to avoid O-oxidation of 7a or 7d during sulfurization with 1 would be to inactivate the sulfoxide 4 via its hydrolysis. To this end we carried out the sulfurization of 7a with 1.5 equiv of 1 in 2% aqueous acetonitrile in the presence of 2 equiv of TEA. As expected, the reaction afforded the desired *di*thio compound 7b as the major product (75% as judged from the ^{31}P NMR spectrum), but ca. 25% of the O-oxidized product 7c was still formed. Since formation of the *mono*thioate 7c was reduced, but not eliminated, this may indicate that the rate of hydrolysis of the presumed intermediate 4 and the rate of O-oxidation are comparable under the reaction conditions. This is, however, apparently not the case during sulfurization of the H-phosphonate 7d where the O-oxidation of 7d seems to be considerably slower than hydrolysis of 4. In consequence, only formation of the S-oxidized product 7c could be detected during sulfurization of 7d with 1 under aqueous conditions (^{31}P NMR analysis).

These findings substantiated our assumption concerning the reaction pathway leading to the O-oxidized products and also provided an explanation for their variable amounts during sulfurization. Since 4 is prone to hydrolysis, the presence of traces of water in the reaction mixtures may affect the amounts of O-oxidized products. It should be stressed, however, that under strictly anhydrous conditions (acetonitrile + 1.5 equiv of 1 + 2 equiv of TEA), the O-oxidized products were formed in invariable amounts, both from the H-phosphonothioate 7a (~60% of 7c) and the H-phosphonate 7d (~10% of 7e).

Sulfurization of 7a with 2 in 2% aqueous acetonitrile/TEA did not eliminate formation of the O-oxidized product 7c, and in addition, formation of a new side product (ca. 30%) was also observed under these reaction conditions (^{31}P NMR, δ ~90 ppm).

Although sulfurization in aqueous acetonitrile with the reagent 1 offered some advantages over the anhydrous conditions, there was one major disadvantage with the reagent system. We have found that addition of TEA to a solution of 1 in acetonitrile produced a yellow precipitate within 5–10 s, irrespective of the presence or absence of water. This alone precludes using the reagent (under the reaction conditions specified above) in a machine-assisted solid-phase synthesis of oligonucleotides. In addition, when a mixture of 1.5 equiv of 1 and 2 equiv of TEA in anhydrous acetonitrile was left standing for 5 min and then used for sulfurization of 7d, the major product (~90%) was the O-oxidized product (the phosphorodiester 7e).[12] The observed predominant O-oxidation in this reaction can be explained by probable dismutation of the reagent 1 in the presence of TEA, which generates an oxidizing agent (presumably 4) and elemental sulfur. This was further substantiated by ^{13}C NMR spectroscopy, which revealed that, indeed, the reagent 1 was completely converted into 4 upon the treatment with triethylamine in anhydrous acetonitrile.

Although the disproportionation of 1 is fast, it must be slower than the sulfurization reaction, since addition of TEA to a reaction mixture containing 7d and the reagent 1 caused preferentially (in anhydrous acetonitrile) or exclusively (in 2% aqueous acetonitrile) sulfurization. In contradistinction, we did not observe any disproportionation of the reagent 2. Even upon keeping 2 in acetonitrile (or in 2% aqueous acetonitrile) with TEA overnight, its sulfur-transferring properties were the same as those for the freshly prepared solutions.

Sulfur-Transferring Properties of 3*H*-1,2-Benzothiol-3-one (3). Since the formation of O-oxidized products during sulfurization seemed most likely to be due to generation of the oxidizing species (presumably 4 and 5) from 1 or 2, we turned our attention to another reagent, 3*H*-1,2-benzothiol-3-one (3). Though the dithiolone 3 has been used as a starting material for the preparation of 1 and 2, its chemical properties and, particularly, its ability to transfer sulfur have not been investigated. A distinctive feature of the reagent 3 is that it cannot generate O-oxidizing species during sulfurization[13] and thus, in principle, should act exclusively as a sulfur-transferring reagent.

Sulfurization of 7a with 1.5 equiv of the reagent 3 in acetonitrile/TEA proceeded at a rate similar to that as with the reagents 1 and 2. As expected, no O-oxidized product 7c could be detected during sulfurization, but unfortunately, formation of a small amount (~5%) of unidentified side product(s) was observed (^{31}P NMR, resonances at ca. 90 ppm). With 0.5 equiv of 3, the sulfurization did not go to completion, but the amount of side products increased to ~10%. A similar phenomenon was observed during the reaction of 3 with the H-phosphonate 7d (^{31}P NMR, additional resonances at ca. 22 ppm, ca. 5%). Replacement of anhydrous acetonitrile by 2% aqueous acetonitrile in these reactions resulted in higher amounts of the side products[14] (ca. 40 and 10% in the case of 7a and 7d, respectively).

Comparison of 1–3 as Sulfur-Transferring Reagents under Various Experimental Conditions. At that stage of the investigation none of the reagents 1–3 gave completely satisfactory results, so we made an attempt to change the reaction conditions for sulfurization. Since dismutation of 1 seemed to be promoted by triethylamine, we decided to check if a basic solvent could substitute a strong base (TEA) in the sulfurization reaction. To this end, we carried out sulfurization of 7a and 7d with 1.5 equiv of the reagents 1–3 in anhydrous pyridine. All the reactions, with the exception of 7a + 1, proved to be substantially slower than those in acetonitrile/TEA. The ^{31}P NMR spectra recorded during sulfurization of the H-phosphonothioate 7a with 1 and 2 showed rather complicated and difficult to interpret patterns of signals.

(10) Vasileva, T. P.; Bystrova, V. M.; Kildisheva, O. V. *Izv. Acad. Nauk SSSR, Ser. Khim.* **1988**, *7*, 1633.

(11) Iyer, R. P.; Philips, L. R.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Org. Chem.* **1990**, *55*, 4693.

(12) A similar experiment in which an anhydrous solvent was replaced by 2% aqueous acetonitrile did not produce any S- or O-oxidized products from the H-phosphonate 7d.

(13) We assume that during sulfurization with 3 benzothiet-2-one (6) is generated. It may undergo dimerization or oligomerization under anhydrous reaction conditions or hydrolyze in the presence of water to afford thiosalicylic acid. See also: Wentrup, C.; Bender, H.; Gross, G. *J. Org. Chem.* **1987**, *52*, 3838.

(14) Though no attempts have been made to identify these side products, they probably arise from a nucleophilic attack of the phosphorus on the sulfur in position 1. This would explain the increased formation of the side products under more basic reaction conditions.

Table I. Sulfurization of the H-Phosphonothioate 7a with 1–3 under Various Reaction Conditions

reaction system[a]	time[b]	composition of the reaction mixture[c] (%) 7b	7c	other byproducts
	Reagent 1			
CH_3CN/TEA	<30 s	40	60	
2% aq Ch_3CN/TEA	<30 s	75	25	
anhyd pyridine	<30 s	40	60	
2% aq pyridine	<30 s	100		
	Reagent 2			
CH_3CN/TEA	<30 s	80	20	
2% aq CH_3CN/TEA	<30 s	55	15	30
anhyd pyridine	5 min	80	20	*d*
2% aq pyridine	15 min	70	15	15 ($\delta \sim 90$ and 93)
	Reagent 3			
CH_3CN/TEA	<30 s	95		5 ($\delta \sim 90$)
2% aq CH_3CN/TEA	<30 s	70		30 ($\delta \sim 90$)
anhyd pyridine	20 min	95	5	
2% aq pyridine	<30 s	100		

[a] Reactions were carried out by a ddition of 1.5 equiv of the reagent into the 12.5 mM solution of a nucleotidic substrate in the appropriate solvent system. [b] TLC analysis. [c] ^{31}P NMR analysis (δ in ppm). [d] Several intermediates were formed during this reaction; however, addition of water converted them into **7b** and **7c**.

Table II. Sulfurization of the H-Phosphonate 7d with 1–3 under Various Reaction Conditions

solvent system[a]	time[b]	composition of the reaction mixture[c] (%) 7c	7e	other byproducts
	Reagent 1			
CH_3CN/TEA	30 s	90	10	
2% aq CH_3CN/TEA	30 s	100		
anhyd pyridine	30 min	70	30	
2% aq pyridine	2–4 h	97		3 ($\delta \sim 2$ and 5)
	Reagent 2			
CH_3CN/TEA	3 min	95	5	
2% aq CH_3CN/TEA	2 min	98		2 ($\delta \sim 25$)
anhyd pyridine	120 min	40	50	10 (several)
2% aq pyridine	2–3 h	85	10	5 ($\delta \sim 5$)
	Reagent 3			
CH_3CN/TEA	30 s	95		5 ($\delta \sim 20$)
2% aq CH_3CN/TEA	30 s	90		10 ($\delta \sim 20$)
anhyd pyridine	90 min	100		
2% aq pyridine	20 min	100		

[a] Reactions were carried out by addition of 1.5 equiv of the reagent into the 12.5 mM solution of a nucleotidic substrate in the appropriate solvent system. [b] TLC analysis. [c] ^{13}P NMR analysis (δ in ppm).

However, addition of water to the reaction mixtures only produced the expected S- and O-oxidized products, and their ratios were the same as in the corresponding reactions in acetonitrile/TEA (see Table I). Sulfurization of the H-phosphonate **7d** with **1** and **2** in pyridine gave even worse results (Table II). The reactions were rather slow, and the amounts of the O-oxidized product **7e** increased to 30 and ~50%, respectively. In the latter case, also several other side products (ca. 10%) were formed. That may indicate that the O-oxidizing species, generated from the sulfurizing reagents **1** and **2**, are more reactive in pyridine than they are in acetonitrile/TEA and that this also may stimulate some other side reactions.

The dithiolone **3** reacted rather slowly with **7a** and **7d** in pyridine, and it took ca. 20 and 90 min, respectively, to drive the reactions to completion (Tables I and II). As with the reactions in acetonitrile/TEA, no intermediates could be detected using ^{31}P NMR. However, in contradistinction to the sulfurization of **7a** with **3** in the acetonitrile/TEA system, formation of the O-oxidized product (**7c**, ~5%) was observed. Since neither **3** nor **6** can act as O-oxidizing agents, formation of **7c** can be rationalized by assuming an isomerization process, analogous to the reaction c in Scheme I. It is noteworthy that, despite a rather long reaction time (Table II), no side products were formed during the sulfurization of the H-phosphonate **7d** with the reagent **3**.

Table III. ^{31}P NMR Data for Some Products Formed during the Sulfurization Reactions[a]

compd	δ (ppm)	$^1J_{PH}$(Hz)	$^3J_{PH}$(Hz)
7a	74.39	686.0	12.2
	73.54	670.0	12.2
7b	117.10		13.4, 12.2
7c	57.74		7.9
	58.48		11.0
7d	9.13	720.0	9.2
	9.24	734.0	7.9
7e	−1.22		*b*
8a	73.00	672.6	11.0, 9.8
	71.50	675.1	9.8, 8.5
8b	116.80		11.0, 6.1
8c	59.46		8.5
	59.02		7.3
8d	9.69	715.9	8.5, 9.8
	8.10	718.7	8.5, 7.3
8e	−1.10		8.1

[a] Spectra recorded in pyridine. 2% H_3PO_4 in D_2O as an external reference (coaxial inner tube). [b] Unresolved multiplet.

Because the reactions of **7a** and **7d** with **1–3** in anhydrous pyridine proved to be unsatisfactory (both in terms of yields and the reaction times), we tried to use 2% aqueous pyridine as a solvent. We expected that abstraction of the phosphorus-bound hydrogen in H-phosphonothioate and H-phosphonate diesters should be facilitated under these conditions, and in consequence, it should result in faster sulfurization. In addition, hydrolysis of the O-oxidizing species generated from the sulfurizing reagents should be faster in aqueous pyridine than it was in 2% aqueous acetonitrile, and thus, formation of the O-oxidized products should be eliminated.

The sulfurization of **7a** with 1.5 equiv of **1** or **3** in 2% aqueous pyridine was fast (Table I) and afforded the desired compound **7b** as the sole nucleotidic product. The absence of the O-oxidized product in the reaction of **7a** with **1** may indicate that either the sulfurization is substantially faster than the O-oxidation or that hydrolysis of the O-oxidizing species (presumably **4**) is extremely fast under the reaction conditions. Since the rate of sulfurization in aqueous pyridine is apparently similar to that in acetonitrile, the second explanation seems to be most likely. The reagent **2**, however, under the same conditions (Table I) produced a complicated mixture of products, as judges from ^{31}P NMR spectra.

Sulfurization of the H-phosphonate **7d** in 2% aqueous pyridine with **1** was substantially slower than in anhydrous pyridine. No O-oxidized product was formed under these conditions, but it took ca. 3 h to drive the reaction to completion (Table II). The reagent **3**, on the other hand, was found to be more reactive in 2% aqueous pyridine than in the anhydrous solvent (Table II). With 1.5 equiv of the reagent **3**, clean formation of the phosphorothioate **7c** was furnished within 20 min.

Both reagents **1** and **3** are stable in aqueous pyridine, at least for several hours. When **1** or **3** (1.5 equiv), after being kept in 2% aqueous pyridine overnight, was used for sulfurization of **7a**, clean conversion into the phosphoro*di*thioate **7b** was observed. This demonstrates that under these reaction conditions both reagents should be

compatible with the requirements for the solid-phase synthesis of phosphorothioate diesters. Even though most of our investigations have been carried out on the ribodinucleoside H-phosphonothioate **7a** and H-phosphonate **7d**, we have found that the reagents **1** and **3** were equally suited for the sulfurization of the deoxyribodinucleoside H-phosphonothioate **8a** and H-phosphonate **8d** (data not shown).

Reactivity of 1–3 toward Trimethyl Phosphite. As a final stage of these studies, we compared the sulfur-transferring properties of the reagents **1–3** toward trimethyl phosphite. These can be relevant to the sulfurization procedures used in the phosphoramidite approach to the synthesis of oligonucleotide analogues.[11] Sulfurization of trimethyl phosphite with 1 equiv of **1** in anhydrous acetonitrile was complete in less than 2 min (time necessary to record the ^{31}P NMR spectrum) and afforded only the desired product, trimethyl phosphorothioate (^{31}P NMR, δ 73.53, $^{3}J_{PH}$ = 13.4 Hz). This is in agreement with data reported by Beaucage et al.[11] Assuming that the mechanisms for sulfurization of H-phosphonate diesters and phosphite triesters are similar, in this case two O-oxidizing species (presumably **4** and **5**) should also be generated during the sulfurization. Indeed, stepwise addition of more trimethyl phosphite[15] (2 equiv) to the above reaction mixture resulted in rapid formation of trimethyl phosphate, and the final ratio of the S-oxidized to the O-oxidized products was found to be ca. 1:2.

The reagent **2** also reacted fast with trimethyl phosphite (less than 2 min, ^{31}P NMR experiments). However, in this case, almost equimolar amounts of trimethyl phosphorothioate and trimethyl phosphate (^{31}P NMR, δ 3.06, $^{3}J_{PH}$ = 11.0 Hz) were formed, irrespective of the amounts of trimethyl phosphite used. This may be indicative of a competitive O-oxidation of trimethyl phosphite by, presumably, the oxathiolone **5**.

The dithiolone **3** proved to be the least reactive among the investigated reagents. Although the reaction time was rather long (ca. 1 h), the sulfurization of trimethyl phosphite produced exclusively trimethyl phosphorothioate.

Since the reaction pathways for sulfurization and oxidation of H-phosphonate diesters and trimethyl phosphite with the reagent **1** seem to be similar, we decided to investigate the latter reaction further in order to substantiate our hypothesis about the possible intermediacy of compounds **4** and **5**. Although ^{31}P NMR spectroscopy was extremely useful in these investigations, it did not provide us with information concerning the fate of reagent **1** during the sulfurization. Since compounds **1** and **4** have distinctive ^{13}C NMR resonances, we decided to follow the reaction of trimethyl phosphite with **1** by ^{13}C NMR spectroscopy in order to find out if **4** is indeed formed during the sulfurization. To this end the reaction of **1** with 1 equiv of trimethyl phosphite was carried out in the deuterated acetonitrile, and the ^{13}C NMR spectra were immediately recorded. It was found that signals assigned to the reagent **1** completely disappeared and seven new resonances, which one should expect for the compound **4**, appeared. Unfortunately, we were not able to obtain any direct evidence from ^{13}C NMR for the possible intermediacy of compound **5**. Upon addition of the second equivalent of trimethyl phosphite to the above reaction mixture, we could not detect any signals in the region of 120–190 ppm assignable the compound **5**.[16] The signals originating from **4**, however, visibly decreased in intensities, and a large number of low-intensity signals between 120–140 ppm appeared. When the third equivalent of trimethyl phosphite was added, the signals from **4** disappeared, but a complicated pattern of signals around 120–140 ppm remained. As it was observed before, the reaction of **1** with more than 1 equiv of trimethyl phosphite was accompanied by formation of an insoluble substance.

Also the ^{13}C NMR data of the reaction of **2** with trimethyl phosphite did not reveal any presence of **5** in the reaction mixture. During the reaction of 1 equiv of trimethyl phosphite with **2**, only signals from **2** and the products (trimethyl phosphorothioate and trimethyl phosphate) could be detected in the ^{13}C NMR spectra, together with a large number of low-intensity signals in the range of 120–140 ppm. Similar to **1**, formation of an insoluble material during the course of the reaction was observed. However, in contradistinction to the former reaction, this time precipitation started immediately after addition of the first equivalent of trimethyl phosphite.

The above results were interpreted in a following way. Since the sulfurization of trimethyl phosphite with reagent **1** is faster than oxidation by the in situ generated compound **4**, the presence of the latter should be detectable by ^{13}C NMR spectroscopy. It was indeed observed upon the reaction of **1** with 1 equiv of trimethyl phosphite. Addition of the second equivalent of trimethyl phosphite caused formation of trimethyl phosphate (^{31}P NMR and ^{13}C NMR), but we could not observe any new signals in the ^{13}C NMR spectra assignable to compound **5**. Since compound **4** was still present in the reaction mixture and the ratio of S- to O-oxidized products was 1:1 (^{31}P NMR, ^{13}C NMR data), this may indicate that under the reaction conditions **5** is more reactive than **4** as an oxidizing agent. Thus, its presence in the reaction mixture cannot be detected, probably because it is immediately consumed in the oxidation reaction.[17] In principle, compound **6** should be formed in such a reaction but since **6** is known to be unstable and prone to polymerization even at low temperatures, a polymeric material should be the most likely product. We always observed formation of an insoluble material in the reaction of **1** with more than 1 equiv of trimethyl phosphite, and ^{13}C NMR also showed the presence of several low-intensity signals in the range 120–140 ppm. This may be indicative of a transient formation of **5** that is transformed during the course of the reaction to **6** and, finally, to a polymeric substance.

The reaction of **2** with trimethyl phosphite can be interpreted in the same way. However, in contradistinction to **1**, in this case the postulated intermediate **5** should be formed upon addition of the first equivalent of trimethyl phosphite. Since **5** is apparently more reactive than **2** (^{31}P NMR and ^{13}C NMR experiments) it should oxidize trimethyl phosphite and be converted to the compound **6**. In consequence, an insoluble material (apparently a polymeric material derived from **6**) should be formed as soon as the reaction started. This, in fact, was observed.

Conclusions

These studies suggest that formation of O-oxidized products during the sulfurization of H-phosphonothioate

(15) Addition of more than 1 equiv of trimethyl phosphite into a solution of **1** in acetonitrile always resulted in formation of a light yellow precipitation. This, however, did not affect the oxidation of trimethyl phosphite.

(16) The ^{13}C NMR data for **5** are not available, but for this type of compound one should expect 7 resonances in the region of 170–120 ppm. By comparing tendencies in chemical shifts of the carbonyl and tertiary carbons in compounds **1–3** with those of the oxy analogue of **1** and compound **4**, one should expect for the compound **5** two diagnostic signals at ca. 165 and 145 ppm.

(17) It is still possible that **5** cannot be detected due to its low concentration in the reaction mixture.

and H-phosphonate diesters with the reagent 1 in anhydrous acetonitrile/TEA is due to generation of two oxidizing species presumably of type 4 and 5. An additional amount of 4, which may contribute to the formation of the undesired O-oxidized products, can also be formed as a result of dismutation of 1. The reagent 2 produced substantially less O-oxidized products during sulfurization of H-phosphonothioate and H-phosphonate diesters. The dithiolone 3 did not produce any O-oxidized products when applied as a sulfurizing agents for **7a** and **7d**, but it produced a small amounts of unidentified side products.

It was also found that presence of water in the reactions systems had a favorable effect on the sulfurization of **7a** and **7d**. The best results were obtained when 2% aqueous pyridine was used as a solvent. Both reagents, 1 and 3, are stable in this solvent and furnished sulfurization without formation of any O-oxidized products. These conditions are also compatible with the solution and solid phase synthesis of oligonucleotides.

Considering the general properties of 1–3 it seems apparent that the sulfonyl or sulfoxide group, in contradistinction to the 3-keto function,[11] has a rather minor effect on the sulfur-trasferring ability of the reagents as far as H-phosphonates are concerned. A decisive factor in this case seems to be the reactivity of P–H bonds in H-phosphonothioate and H-phosphonate diesters. In addition, since generation of the oxidizing species from the reagents 1 and 2 during sulfurization is due to the presence of the sulfonyl or sulfoxide group, such functional groups seems to be undesired in potential sulfurizing reagents.

Finally, it was found that 3 has following advantages over 1 as sulfur-transferring reagents for H-phosphonothioate and H-phosphonate diesters: (i) it can be prepared in a good yield in a one-step reaction, (ii) the reagent 3 seems to be more stable than 1 and it does not undergo dismutation in the presence of a strong base, (iii) it is, in principle, safer as a sulfur-transferring reagent, since it cannot act as an O-oxidizing agent, and (iv) sulfurization of H-phosphonate diesters in aqueous pyridine is ca. 10 times faster with 3 than it is with the reagent 1.

Experimental Part

Materials and Methods. Pyridine was refluxed with CaH_2 overnight and then distilled and stored over molecular sieves (4 Å) or CaH_2. The same procedure was used for the preparation of anhydrous acetonitrile. TLC was carried out on Merck silica gel 60 F_{254} using chloroform/methanol (9:1, v/v). The sulfurizing reagents 1–3,[11] as well as the H-phosphonothioates **7a** and **8a**,[7] and the H-phosphonates **7d**[18] and **8d**[1] were prepared by standard methods. The reference compounds used for the identification of some reaction products were obtained as follows: **7b** and **8b**, by sulfurization of **7a** and **8a** with elemental sulfur;[7] **7c** and **8c**, by sulfurization of **7d** and **8d** with elemental sulfur;[6] **7e** and **8e**, by oxidation of **7d** and **8d** with iodine in aqueous pyridine.[1,18]

The ^{31}P NMR experiments were carried out at 25 °C in 10-mm tubes using 25 μmol of phosphorus-containing compounds in 2 mL of an appropriate solvent. The products of the reactions (**7b**, **7c**, **7e**, **8b**, **8c**, and **8e**) were isolated by silica gel chromatography and found to be identical (FAB MS, ^1H NMR, ^{31}P NMR, TLC) with the compounds of known structures prepared by other methods.[1,6,7,18]

General Procedure for Sulfurization. A suitably protected H-phosphonothioate **7a** (**8a**) or H-phosphonate **7d** (**8d**) (25 μmol) was rendered anhydrous by evaporation of added acetonitrile or pyridine and then dissolved in the appropriate solvent system (2 mL acetonitrile + 2 equiv of triethylamine; 2% aqueous acetonitrile + 2 equiv of triethylamine; anhydrous pyridine; 2% aqueous pyridine). A sulfurizing reagent (1.5 equiv or as stated in the text, (1–3) was added, and the ^{31}P NMR spectra were recorded immediately. Progress of the reactions were also followed by TLC.

Disproportionation of the Reagent 1. The reagent 1 (38 μmol) was dissolved in anhydrous or 2% aqueous acetonitrile, and then triethylamine (50 μmol) was added. Almost immediately, a yellow precipitate started to form. After 5 min **7d** (25 μmol) was added and the ^{31}P NMR spectra were recorded immediately thereafter (for discussion, see in the text). The ^{13}C NMR spectrum of the supernatant from the reaction in anhydrous acetonitrile showed that signals from 1 were replaced by new ones (δ (in ppm) 166.6, 152.7, 137.5, 127.0, 126.1, and 124.4) corresponding to the compound 4.[11]

Acknowledgment. We are indebted to Prof. Per J. Garegg and Dr. Roger Strömberg for their interest, to the Swedish National Board for Technical Development, and to the Swedish Natural Science Research Council for financial support.

(18) Stawinski, J.; Strömberg, R.; Thelin, M.; Westman, E. *Nucl. Acids Res.* **1988**, *16*, 9285.