Highly efficient synthesis of phosphorodithioates derived from 3′-thiothymidine by anhydro-ring opening of 2,3′-anhydro-5′-*O*-tritylthymidine with *O,O*-disubstituted phosphorodithioic acids

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Thymidine 3′-*S*-phosphorodithioate 4 and dithymidine-3′-*S*-phosphorodithioate 7 derived from 3′-thiothymidine are synthesized in excellent yield under mild conditions by the nucleophilic ring opening of 2,3′-anhydrothymidine with phosphorodithioic acids.

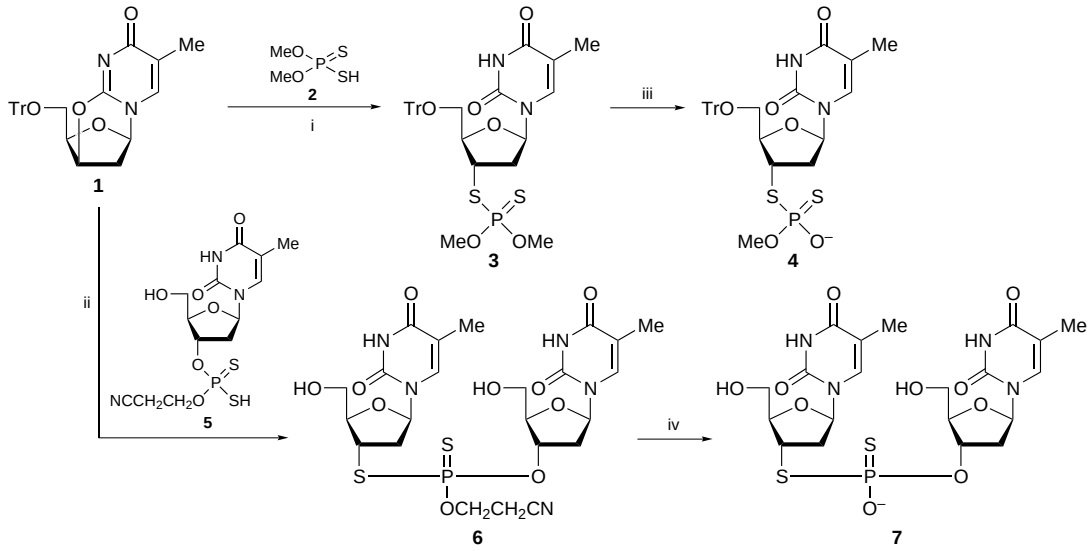
The rapid development of antisense chemotherapy[1] and studies to elucidate the mechanism of rybozyme action[2] have encouraged organic chemists to undertake the synthesis of oligonucleotide analogues in which the sugar residues and internucleotide linkages are modified. Interest in oligonucleotides containing 3′-*S*- or 5′-*S*-phosphorothioate linkages has recently increased, but methods presented to date, although elegant, are laborious and rather difficult to carry out.[3–9]

5′-Deoxy-5′-*S*-nucleosidyl phosphates have been prepared from 5′-iodo-2′-deoxynucleosides by condensation with trisodium phosphorothioate.[3] 2′-Deoxy-5′-*O*-thymidyl-5′-*S*-thymidyl phosphorothioate has been obtained by allowing thymidine 5′-phosphorothioate to react with 5′-iodothymidine.[4] A variation of this approach is the use of 5′-*O*-tosyl nucleoside for condensation with mono- or di-esters of phosphorothioic acid.[5] Cosstick has synthesized dithymidine phosphate analogues containing 3′-thiothymidine by phosphitylation of 5′-*O*-monomethoxytrityl-3′-thiothymidine.[6] The resulting dinucleosidyl phosphorothioite has been oxidized to the corresponding 3′-phosphorothioate. Dithymidine-3′-*S*-phosphorodithioate has been prepared, in an analogous fashion, as a mixture of diastereoisomers and separated after deprotection.[7]

More recently Cosstick has devised a synthesis of internucleoside 3′-phosphorothiolate linkages *via* electrophilic 3′-*S*-thymidinethiol derivatives, specifically, mixed disulfides with one strongly electronegative substituent, which were allowed to react with 3′-*O*-(*tert*-butyldimethylsilyl)thymidin-5′-yl trimethylsilyl phosphite.[8] A similar strategy has been used by Liu and Reese[9] in investigating the chemistry of RNA. Okruszek *et al.* have found that 3′-*O*-phosphorodithioates react in DMF solution with 5′-bromo-5′-deoxythymidine to give corresponding dinucleoside-5′-*S*-phosphorodithioate.[10] In spite of the elegance of Cosstick's, Reese's and Okruszek's approaches, formation of phosphorothiolate linkages requires laborious operations and depends on access to 3′-*S*- or 5′-*S*-thionucleosides and 5′-bromonucleoside which are not readily available. For this reason we have sought an alternative strategy avoiding 3′-*S*- or 5′-*S*-thionucleosides. Our long-standing interest in the chemistry of sugar thiophosphates[11] and modified nucleotides[12] also stimulated this work.

It is known that 2,3′-anhydrothymidine reacts with a variety of nucleophiles, usually under harsh conditions.[13] We discovered that 2,3′-anhydrothymidine reacts rapidly with phosphorus dithioacids [RR′P(S)SH] at ambient temperature in almost quantitative yield, and the ring opening proceeds with inversion of configuration at the 3′-carbon. Protonation of the anhydro-ring oxygen and the high nucleophilicity of phosphorus dithioacids make this procedure efficient and mild. Phosphorus dithioacids, including those derived from nucleosides,[14] are readily available.

Our methodology is exemplified by reactions of 5′-*O*-trityl-2,3′-anhydrothymidine[15] **1** with *O,O*-dimethyl phosphorodithioate[16] **2**‡ and with *O*-(5′-*O*-tritylthymidin-3′-yl) *O*-(2-cyanoethyl) phosphorodithioate **5**§ (Scheme 1). The dithioic acid **5** was prepared *in situ* by treating its DBU salt **5a** with excess of toluene-*p*-sulfonic acid monohydrate. Water introduced with



Scheme 1 *Reagents and conditions*: i, THF, 2 h, 20 °C; ii, MeCN, TsOH, 2 h, 20 °C; iii, *tert*-butylamine, 8 h, 20 °C; iv, THF–DBU (9 : 1), 1 h, 20 °C

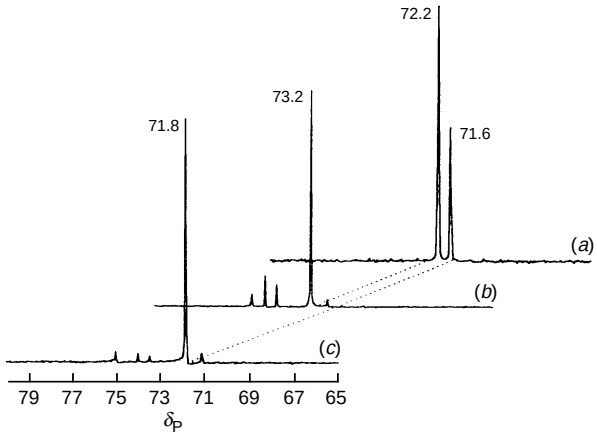


Fig. 1 The ^{31}P NMR spectra of (*a*) **4**, (*b*) **4b** (slow diastereoisomer) and (*c*) **4a** (fast diastereoisomer)

toluene-*p*-sulfonic acid does not interfere with the ring opening reaction but effects the removal of the trityl group. The analogous reaction with the DBU salt of **5a** requires severe conditions.

Both reactions proceed in THF or MeCN solution at 20 °C and are completed within 2 h yielding phosphorodithioates **3** and **6** in almost quantitative yield.

The demethylation of phosphorodithioate **3** by *tert*-butylamine and removal of the 2-cyanoethyl group of phosphorodithioate **6** leads to the corresponding salts **4a**, **b** and **7a**, **b**, respectively. These stable compounds contain a chiral phosphorus centre and are formed as a mixture of diastereoisomers. The 1 : 1 mixture of diastereoisomers **4** was separated into the 'fast' **4a** and 'slow' **4b** diastereoisomers by silica gel chromatography. ^{31}P NMR spectra of phosphorodithioates **4**, **4a** and **4b** are shown in Fig. 1.

We are currently exploring the use of nucleoside anhydrides to construct analogues of oligonucleotides containing 3′-*S*- or 5′-*S*-phosphorothiolate linkages by this methodology.

These studies were supported by grants No 3T09A 146 08 (M. M.) and No 3T09A 155 10 (W. D. and I. T.) of the Committee of Scientific Research, Poland.

Notes and References

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‡ *Experimental procedure*: to a solution of 2,3′-anhydro-5′-*O*-tritylthymidine **1** (0.1 mmol) in 10 ml of dry THF was added a solution of *O*,*O*-dimethyl phosphorodithioate **2** (0.1 mmol) in 10 ml of dry THF and the mixture was kept for 2 h at 20 °C. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (230–400 mesh, Merck 9835) using CH_2Cl_2–Me_2CO (10 : 2 v/v) as eluent to give **3** (80%); $\delta_P(C_6D_6)$ 96.55. Compound **3** was dissolved in *tert*-butylamine, the reaction mixture was stirred for 8 h at room temperature and was solvent evaporated *in vacuo*. The product **4** was isolated by silica gel chromatography [CH_2Cl_2–Me_2CO–Et_3N, 10 : 2 : 0.5 (v/v/v)] as a mixture of diastereoisomers (1 : 1) of the *tert*-butylammonium salt of **4** (95%); $\delta_P(C_6D_6)$ 72.2, 71.6. The diastereoisomers were resolved by column chromatography using CH_2Cl_2–Me_2CO–Et_3N [10 : 2 : 0.5 (v/v/v)] as eluent and collecting small fractions. Fast diastereoisomer **4a**: $\delta_P(C_6D_6)$ 71.7. Slow diastereoisomer **4b**: $\delta_P(C_6D_6)$ 73.2.

§ A solution of 2,3′-anhydro-5′-*O*-tritylthymidine **1** (0.1 mmol) and *O*-[(5′-*O*-trityl)-thymidin-3′-yl] *O*-(β-cyanoethyl) phosphorodithioate **5** (0.1 mmol) in 10 ml of MeCN was acidified with toulene-*p*-sulfonic acid monohydrate (0.25 mmol). After 2 h at 20 °C the reaction mixture was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel (230–400 mesh, Merck 9835) using CH_2Cl_2–Me_2CO [10 : 2 (v/v)] as eluent to give **6** as a mixture of diastereoisomers (95%); $\delta_P(C_6D_6)$ 92.401, 91.751 (3 : 4). A solution of **6** (0.1 mmol) in THF–DBU (9 : 1, 10 ml) was left at room temperature for 1 h. The product **7** was a mixture of diastereoisomers (3 : 2) (90%); $\delta_P(C_6D_6)$ 67.53, 67.27 (3 : 2).

1 *Oligonucleotides: Antisense Inhibitors of Gene Expression*, ed. J. S. Cohen, Macmillan, London, 1989.
2 W. A. Picken, D. B. Olsen, F. Benseler and F. Eckstein, *Science*, 1991, **253**, 314.
3 A. Hampton, L. W. Brox and M. Bayer, *Biochemistry*, 1969, **8**, 2303; E. F. Rossomando, G. A. Cordis and G. D. Markham, *Arch. Biochem. Biophys.*, 1983, **220**, 71.
4 A. F. Cook, *J. Am. Chem. Soc.*, 1970, **92**, 190.
5 A. F. Cook, *J. Am. Chem. Soc.*, 1970, **92**, 190; J. Nagyvary, S. Chladek and J. Roe, *Biochem. Biophys. Res. Commun.*, 1970, **39**, 878; S. Chladek and J. Nagyvary, *J. Am. Chem. Soc.*, 1972, **94**, 2079; J. Kresse, K. L. Nagpal, J. Nagyvary and J. T. Uchlic, *Nucleic Acids Res.*, 1975, **2**, 1.
6 J. S. Vyle, X. Li and R. Cosstick, *Tetrahedron Lett.*, 1992, **33**, 3017.
7 R. Costick and J. S. Vyle, *Nucleic Acids Res.*, 1990, **4**, 829.
8 X. Li, G. K. Scott, A. D. Baxter, R. J. Taylor, J. S. Vyle and R. Cosstick, *J. Chem. Soc., Perkin. Trans. 1*, 1994, 2123; L. B. Weinstein, D. J. Earnshaw, R. Cosstick and T. R. Cech, *J. Am. Chem. Soc.*, 1996, **118**, 10 341; A. P.Higson, G. K. Scott, D. J. Earnshaw, A. D. Baxter, R. A. Taylor and R. Cosstick, *Tetrahedron*, 1996, **52**, 1027.
9 X. Liu and C. B. Reese, *Tetrahedron Lett.*, 1996, **37**, 925.
10 A. Okruszek, M. Olesiak and W. J. Stec, *Phosphorus Sulfur Silicon Relat. Elem.*, 1996, **111**, 81.
11 W. Kudelska and M. Michalska, *Tetrahedron*, 1986, **42**, 629 and references cited therein; M. Michalska, E. Brzezinska and P. Lipka, *J. Am. Chem. Soc.*, 1991, **113**, 7945; H. Bielawska and M. Michalska, *J. Carbohydr. Chem.*, 1991, **10**, 107; W. Kudelska and M. Michalska, *Synthesis*, 1995, 1539.
12 W. Dąbkowski, J. Michalski and Q. Wang, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 522; J.Heliński, W. Dąbkowski and J. Michalski, *Nucleosides Nucleotides*, 1993, **12**, 597; W. Dąbkowski, I. Tworowska and R. Saiakhov, *Tetrahedron Lett.*, 1995, **36**, 9223.
13 S. Czernecki and J.-M. Valéry, *Synthesis*, 1991, 239; G. V. Zaitseva, E. I. Kvasyuk, E. V. Vaaks, V. N. Barai, S. B. Bokut, A. I. Zinchenko and I. A. Mikhailopulo, *Nucleosides Nucleotides*, 1994, **13**, 819; B.-Ch. Chen, S. L. Quinlan and J. G. Reid, *Tetrahedron Lett.*, 1995, **36**, 7961.
14 A. Okruszek, A. Sierzchala, M. Sochacki and W. J. Stec, *Tetrahedron Lett.*, 1992, **33**, 7585; A. Okruszek, M. Olesiak, D. Krajewska and W. J. Stec, *J. Org. Chem.*, 1997, **62**, 2269.
15 B. Bennua-Skalmowski and H. Vorbrüggen, *Nucleosides Nucleotides*, 1996, **15**, 739.
16 J. L. Lefferts, K. C. Molloy, J. J. Zuckerman, I. Haiduc, C. Guta and D. Ruse, *Inorg. Chem.*, 1980, **19**, 1662.

Received in Glasgow, UK, 26th August 1997; 7/06211G