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The Case for Configurational Stability of H-Phosphonate Diesters in the Presence of Diazabicyclo[5.4.0]undec-7-ene (DBU)

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Abstract—Configurational stability of dinucleoside H-phosphonates and the stereochemical course of their sulfurisation in the presence of diazabicyclo[5.4.0]undec-7-ene (DBU) were investigated using ^{31}P NMR spectroscopy. It was found that under the reaction conditions and irrespective of the type of protecting groups present in the nucleoside moieties, the H-phosphonate diesters investigated did not undergo any detectable epimerisation at the phosphorus centre, and their sulfurisation with elemental sulfur in the presence of DBU, proceeded stereospecifically. Thus, we could not confirm reports from another laboratory on a stereoselective course of sulfurisation of H-phosphonate diesters and the corresponding acylphosphonates in the presence of DBU.

Introduction

In the last decade, the H-phosphonate methodology[1,2] has emerged as a viable alternative to the well established phosphite[3] and phosphotriester[4] approaches for the preparation of biologically important phosphate esters and their analogues. The growing interest in H-phosphonate chemistry[1,2,5] is probably due to the fact that it combines synthetic advantages of other methods based on P(III) and P(V) compounds, for example stability and ease of handling of starting materials, experimental simplicity, high efficiency in the formation of P–O bonds, and versatility in terms of phosphate analogues that can be prepared from a common intermediate. The tautomeric equilibrium of mono- or diesters of phosphonic acid, which is practically completely shifted toward the H-phosphonate form, is most favourable from a synthetic point of view and permits synthesis of phosphorus compounds without recourse to phosphate protecting groups.[1]

A synthesis area where H-phosphonate intermediates proved to be most efficient is preparation of P-chiral phosphorus compounds, for example nucleoside phosphorothioates,[6,7] phosphoroselenoates,[8,9] phosphoroselenothioates,[9] phosphoramidates,[10] and so forth. The advent of antisense[11] and antigene[12] techniques for modulation of gene expression for therapeutical purposes amplified problems connected with the presence of stereogenic phosphorus centre in oligonucleotide analogues,[13] and caused high demand for products with defined chirality[13,14] at each phosphorus centre. Since dinucleoside H-phosphonate and H-phosphonothioates can be separated into diastereomers,[7,15] they are of potential interest as synthons for introduction of a chiral phosphorus centre with known stereochemistry at the predefined position in an oligonucleotide chain. A prerequisite of such an approach is stereospecificity of all transformation involving conversion of a chiral precursor into the final product at the oligonucleotide level.[5,16]

Tetracoordinated P(III) derivatives, for example simple dioxaphosphinanes,[17] phosphinate derivatives,[18] dinucleoside H-phosphonates[7,16,19] are known to be configurationally stable at room temperature and their conversion to tervalent species and vice versa occurs without epimerisation at the phosphorus centre. This constitutes the basis for synthetic applications of these compounds as chiral precursors in various stereospecific transformations. Since some of these reactions are also frequently used in stereochemical correlation analysis for establishing absolute configuration at the phosphorus centre, any departure from the established stereochemical pattern requires meticulous scrutiny to pinpoint a possible source of a stereochemical variation.

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In this respect, two reports by Hata et al.[20] on stereoselective rather than stereospecific sulfurisation of H-phosphonate diesters attracted our attention as exceptions from the commonly observed stereoretentive course of sulfurisation of P(III) compounds. The authors claimed that when a diastereomeric mixture of acylphosphonate **3** was treated with elemental sulfur in the presence of *n*-butylamine and DBU, the exclusive formation of one diastereomer of phosphorothioate **2** (R_P) was observed, that is the transformation appeared to be completely stereoselective.[21] This unexpected course of the reaction Hata et al.[20] explained by DBU-mediated epimerisation of the intermediate H-phosphonate diesters **1**, formed via deacylation of acylphosphonate by *n*-butylamine. According to this mechanism, DBU reacts with H-phosphonate **1** forming a pentacoordinate intermediate that undergoes epimerisation at the phosphorus centre and the produced more thermodynamically stable **1** S_P isomer is oxidised with sulfur to afford R_P phosphorothioate **2**.[22]

Although the proposed mechanism via a pseudorotation pathway seems to be unlikely,[23] the epimerisation of **1** might occur in some other way and thus, the claimed stereoselectivity could point towards general configurational instability of H-phosphonate diesters in the presence of DBU. The latter aspects is of crucial importance since most oxidative transformations of H-phosphonates require strong bases, and for some of them, for example the formation of P–C bonds[24] via H-phosphonate intermediates, DBU was found to be most efficient.

In this paper, we address the problem of possible configurational instability of H-phosphonate diesters in the presence of DBU in light of our ^{31}P NMR spectroscopic studies.

Results and Discussion

To find out if DBU can cause epimerisation of P-chiral H-phosphonate diesters we investigated (i) the stereochemical course of DBU-catalysed sulfurisation of separate diastereomers of dinucleoside H-phosphonate **1**, (ii) the configurational stability of dinucleoside H-phosphonate **1** in the presence of DBU, and (iii) the stereochemical course of sulfurisation of separate diastereomers of dinucleoside acylphosphonate **3** in the presence of *n*-butylamine and DBU.

For these studies, we chose dinucleoside H-phosphonates **1** with the 5′-*O*-dimethoxytrityl and the 3′-*O*-(1,3-benzodithiol-2-yl) protecting groups since it was claimed[20] that presence of these bulky, lipophilic groups, together with a sequence of bases was essential for the observed stereoselectivity of sulfurisation of **1** in the presence of DBU (Chart 1).

Sulfurisation of dinucleoside H-phosphonate 1 in the presence of DBU

As we reported in a preliminary account of this work,[25] sulfurisation of mixture of diastereomers of dinucleoside H-phosphonate **1** in pyridine using elemental sulfur (15 equiv) in the presence of *n*-butylamine (10 equiv) and DBU (0.5 equiv) (the reaction conditions from Hata et al.[20]) did not reveal any stereoselectivity in the formation of the corresponding phosphorothioate **2**, as judged form the same ratio of diastereomers in the starting material **1** and the product **2**. The analogous reactions carried out on separate diastereomers of **1** showed that sulfurisation under these reaction conditions was completely stereospecific. Varying amounts of DBU and *n*-butylamine used for the reaction did not produce any noticeable changes in product distribution and the stereochemical outcome of the reaction remained the same as that when only triethylamine was used as a base.

Although these results clearly showed that that sulfurisation of H-phosphonate diesters in the presence of DBU occurred stereospecifically, there was still a possibility that P-chiral H-phosphonates may undergo epimerisation at the phosphorus centre when exposed for a sufficiently long time to DBU.[26] Since sulfurisation of **1** in the presence of DBU in our hands was complete within 5–10 min (^{31}P NMR) while that reported by Hata et al. required 1 h, we investigated, if treatment of separate diastereomers of H-phosphonate diester **1** in pyridine with DBU for at least 1 h would cause their epimerisation.

Stability of dinucleoside H-phosphonate 1 in the presence of DBU. First, chemical stability of dinucleoside H-phosphonate **1** in pyridine in the presence of DBU was investigated. Unfortunately, the addition of **2** equiv of DBU to a pyridine solution of H-phosphonate **1** caused large broadening of the ^{31}P NMR resonances, which prevented any reliable analysis using this spectroscopic method. Since we knew that sulfurisation of **1** under these conditions occurred stereospecifically (vide supra), we decided to make use of this finding to infer the composition of the reaction mixture resulted from treatment of dinucleoside H-phosphonate **1** with DBU.

To this end, a 1:1 diastereomeric mixture of **1** and 2 equiv of DBU were dissolved in pyridine, and after 2 h excess of elemental sulfur was added (Scheme 1). The ^{31}P NMR spectroscopy revealed[27] that the reaction mixture contained several products. These were identified as diastereomeric dinucleoside phosphorothioates **2** ($\delta_P = 57.32$ and 57.37 ppm, 1:1 ratio, ca. 42%), symmetrical dinucleoside phosphorothioates **4** ($\delta_P = 56.9$ ppm, ca. 21%) and **5** ($\delta_P = 58.3$ ppm, ca. 21%), and two isomeric nucleoside H-phosphonate monoesters **6** ($\delta_P = 3.1$ ppm, ca. 7%) and **7** ($\delta_P = 4.7$ ppm, ca. 7%).[28] The ratio of the products varied slightly from experiment to experiment, however, a general tendency observed was that the amount of symmetrical phosphorothioates (**4**+**5**) formed paralleled those of H-phosphonate monoesters (**6**+**7**). Essentially the same results were obtained when the reaction was carried out in dichloromethane.

These results indicated that probably, in the presence of DBU, a significant degradation of dinucleoside H-

phosphonate **1** occurred, and that prior to the addition of sulfur, this reaction mixture contained along with **1**, a significant proportion of symmetrical dinucleoside H-phosphonates **8**, **9**, and nucleoside H-phosphonate monoesters **6**, **7** (Scheme 2). This degradation of **1** was, most likely, due to adventitious water, which in the presence of a very strong base, DBU, caused partial hydrolysis of the parent dinucleoside H-phosphonate **1**, followed by its transesterification with the produced nucleosides. Although, one can envisage the formation of symmetrical H-phosphonates **8** and **9** even under strictly anhydrous via a ligand exchange mechanism,[29] this possibility seemed less likely in light of the observation (vide supra) that amounts of phosphorothioates **4** and **5** (formed most likely from H-phosphonates **8** and **9**, respectively) after sulfurisation paralleled those of H-phosphonates **6** and **7**. The hydrolytic pathway of **1** in the presence of DBU, however, had to be significantly slower than the sulfurisation reaction, since no degradation products could be observed when DBU was added to the reaction mixture containing H-phosphonate **1** and elemental sulfur.

To find out if DBU can cause epimerization of H-phosphonate diesters at the phosphorus centre, separate R_P and S_P diastereomers of dinucleoside H-phosphonate **1** in pyridine were treated with DBU (2 equiv) for 2 h and then sulfurized with elemental sulfur. The ^{31}P

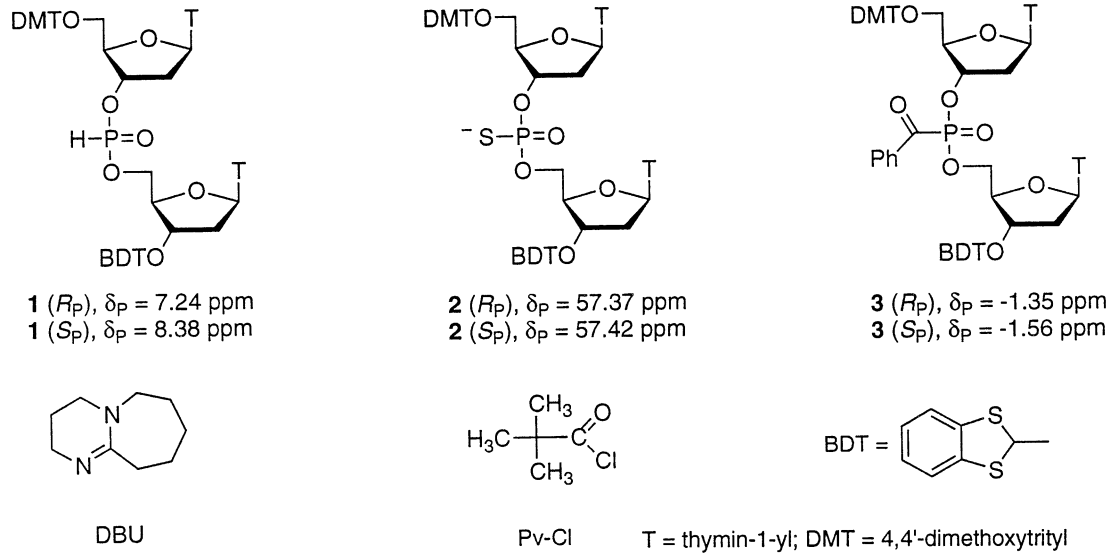
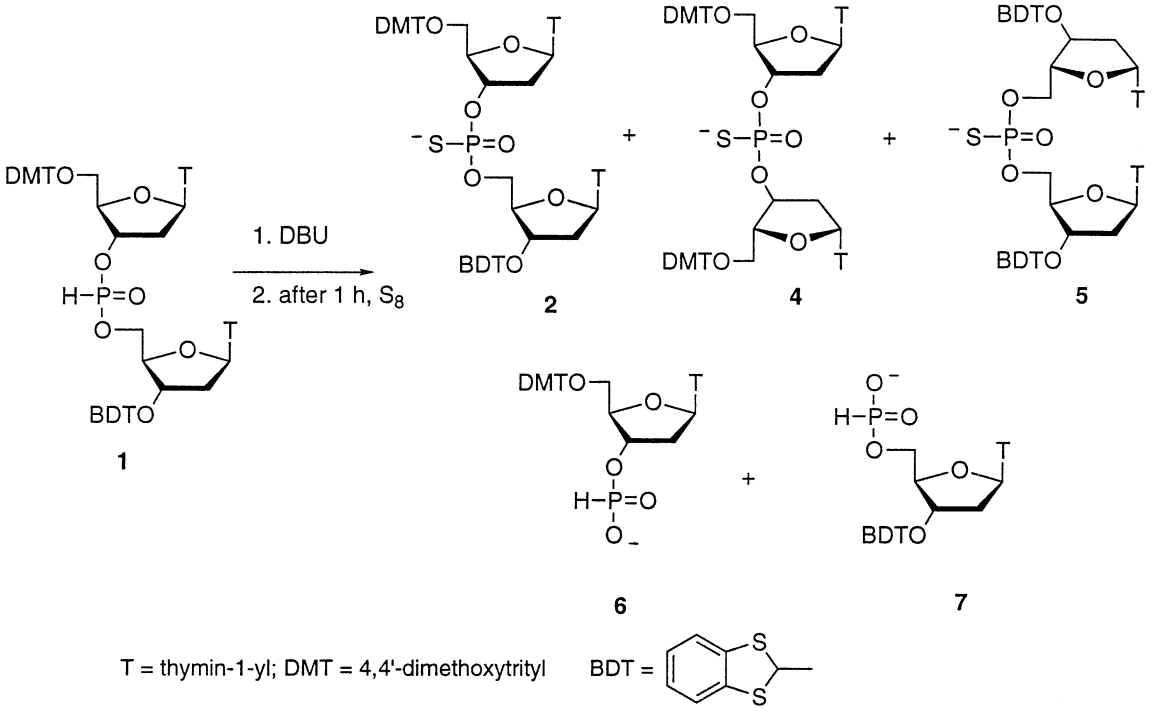


Chart 1.



Scheme 1.

NMR spectroscopy revealed that the same type of products as in the reactions with diastereomeric mixture (1:1) of **1** were formed (phosphorothioates **2**, symmetrical phosphorothioates **4** and **5**, and nucleoside H-phosphonate monoesters **6** and **7**), but the ratio of diastereomeric phosphorothioates **2**R_P/**2**S_P varied depending on which diastereomer of H-phosphonate **1** was used for the reaction. Thus, treatment of **1** R_P with DBU followed by sulfurisation, produced **2** R_P/ **2** S_P in a ratio of ca. 2:3, while from H-phosphonate **1** S_P, this ratio was ca. 6:1.[30]

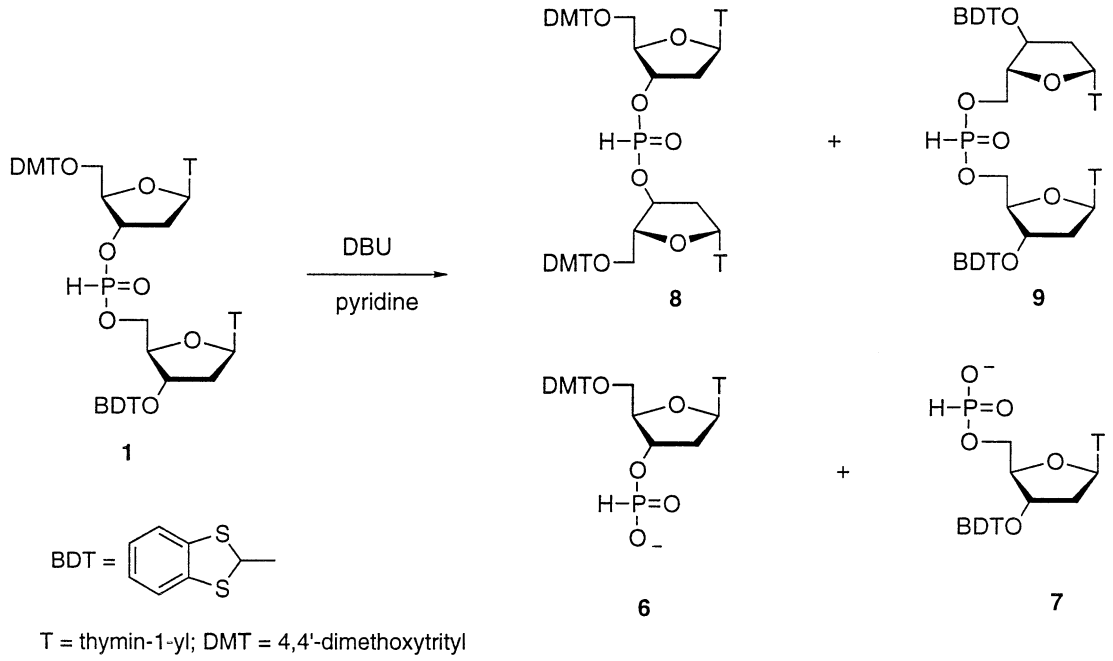
Formation of both diastereomers of phosphorothioate **2** from diastereomerically pure R_P and S_P H-phosphonates **1** at first sight might have suggested a DBU-mediated epimerization of H-phosphonate diester **1**. However, by considering compositions of the whole reaction mixtures, a more likely explanation was that stereochemistry of H-phosphonate **1** diastereomers used for the reaction was eroded due to transesterification with nucleosides, formed as products of hydrolysis the starting material **1**. This was in line with the observation that the ratio between phosphorothioate **2** diastereomers varied and approached value 1:1 with the increasing degree of hydrolysis of dinucleoside H-phosphonate **1**.

However, since DBU exhibits a pronounced nucleophilicity towards phosphorus centre,[31] we could not reject a possibility, that P-epimerization of **1** in the above reaction was due (at least partly) to a DBU-catalyzed ligand exchange.[29] To find out if DBU by itself can promote epimerization of H-phosphonate **1** via a ligand exchange mechanism, we had to completely eliminate the hydrolytic path of decomposition of **1**. Since all technical measure to eliminate the presence of adventitious water failed, we tried to use trimethylsilyl chloride (TMS-Cl) to secure the anhydrous reaction conditions. It was gratifying to find, that presence of TMS-Cl (1 equiv) in the reaction mixture completely eliminated the formation of hydrolysis products (compounds **6** and **7**) upon addition of DBU (1 equiv) to a pyridine solution of separated R_P and S_P diastereomers of H-phosphonate **1**, and also preserved their stereochemical integrity. No hydrolysis or P-epimerization of dinucleoside H-phosphonate **1** was observed upon addition of more DBU (total 6 equiv). On this basis we could tentatively conclude that H-phosphonate diesters of type **1** are chemically and configurationally stable in the presence of DBU, provided that the hydrolytic path of their decomposition is eliminated.

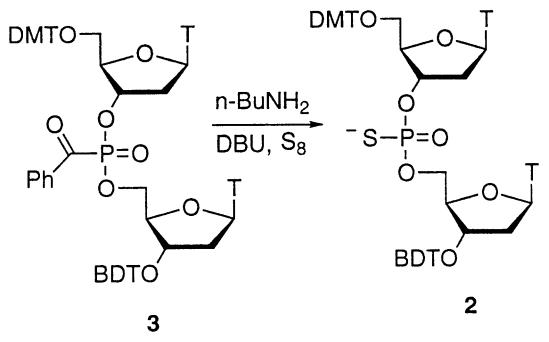
Stereochemical course of sulfurisation of dinucleoside benzoylphosphonate 3

The stereochemistry of sulfurisation of acylphosphonate **3** with elemental sulfur in the presence of *n*-butylamine and DBU was investigated to find out if there is any mechanism operating under the reaction conditions that may lead to epimerisation at the phosphorus centre of acylphosphonate **3** and result in stereoselective formation of only R_P diastereomers of phosphorothioate **2**.[20] To this end, a 1:1 mixture of acylphosphonate **3** diastereomers ($\delta_P = -1.35$ and -1.56 ppm)[32] pyridine was treated with elemental sulfur in the presence of *n*-butylamine (10 equiv) and DBU (0.5 equiv). The reaction was fast and both ^{31}P NMR spectroscopy and TLC analyses indicated the formation of two diastereomers of phosphorothioate **2** in a ratio of ca. 1:1. Thus, we could not confirm the results reported by Hata et al.,[20] that sulfurisation of acylphosphonate **3** under the above conditions occurred stereoselectively (Scheme 3).

As to a possible influence of the amounts of *n*-butylamine and DBU on the stereochemical outcome of the above transformation, additional experiments were



Scheme 2.



T = thymin-1-yl; DMT = 4,4'-dimethoxytrityl

Scheme 3.

carried out. It was found that irrespective of the ratio of *n*-butylamine and DBU used for the reaction, the sulfurisation invariably led to the same equimolar amounts of phosphorothioate 2 diastereomers. When the reaction was carried out in pyridine in the presence of DBU alone (10 equiv) or if DBU was added to the reaction mixture before *n*-butylamine, the formation of phosphorothioate **2** was significantly suppressed due to initiation of an alternative reaction pathway.[33]

Although these experiments showed that the conversion of acylphosphonate **3** into the corresponding phosphorothioate **2** was not stereoselective, we could not exclude a possibility of epimerization of acylphosphonate **3** occurring in the presence of *n*-butylamine and DBU. This point called for clarification since epimerisation, if it occurred, might under certain circumstances lead to a preferential formation of one diastereomer of **1**, and thus be a potential source of stereoselectivity in this type of reactions.

To investigate this problem, we needed separate R_P and S_P diastereomers of acylphosphonate **3**. Unfortunately, in our hands acylphosphonate **3** proved to be much more labile than expected on the basis of literature reports,[20] and the attempted separation of its diastereomers failed due to extensive decomposition of **3** during silica gel chromatography. However, we found that benzoylation of the separate diastereomers of H-phosphonate **1** with benzoyl chloride in methylene chloride produced the desired R_P and S_P diastereomers of **3**, that after purification via precipitation from petroleum ether, gave only one signal in the ^{31}P NMR spectrum.

Using these compounds we found that sulfurisation of a diastereomer of acylphosphonate **3** resonating at higher field in ^{31}P NMR ($\delta_P = -1.56$, probably S_P) with elemental sulfur in the presence of *n*-butylamine and DBU produced only the R_P diastereomer of **2** ($\delta_P = 57.37$ ppm), while the other isomer of **3** (resonating at lower field, $\delta_P = -1.35$, probably R_P), gave exclusively S_P phosphorothioate **2** ($\delta_P = 57.472$ ppm). Thus, these experiments established the conversion of acylphosphonates **3** into the corresponding phosphorothioates **2** as stereospecific and showed that no epimerization occurred during generation of H-phosphonates **1** from acylphosphonate **3** in the presence of *n*-butylamine and DBU.

Conclusions

In conclusion, we have shown that sulfurisation of H-phosphonate diester **1** in the presence of *n*-butylamine and DBU as well as the generation this species from the corresponding benzoylphosphonate **3** occurred stereospecifically, most likely with retention of configuration. Thus, we tentatively conclude that H-phosphonate diesters of type **1** are chemically and configurationally stable in the presence of DBU, provided that the hydrolytic path of their decomposition is eliminated.

Experimental

The ^{31}P NMR spectra were recorded on Jeol GSX-270 FT or Varian 300 MHz spectrometer at 25 °C in 5 mm tubes using 25 μmols of phosphorus-containing compounds in 0.5 mL of $CDCl_3$. The spectra were referenced by 2% H_3PO_4 in D_2O as external standard (coaxial inner tube). Pyridine (LabScan, Ireland) was stored over molecular sieves (4 Å), and pivaloyl chloride (Aldrich, Sweden) and diazabicyclo[5.4.0]undec-7-ene (DBU, from Aldrich), were freshly distilled. Starting materials, 5′-*O*-dimethoxytritylthymidine 3′-H-phosphonate[34] and 3′-*O*-(1,3-benzodithiol-2-yl)thymidine[35] were obtained according to published procedures.

The reference compounds used for the assignment of ^{31}P NMR resonances, were obtained as follows: 5′-*O*-dimethoxytritylthymidine 3′-H-phosphonate **6** and 3′-*O*-(1,3-benzodithiol-2-yl)thymidine 5′-H-phosphonate **7**, by the reaction of the appropriate nucleosides with diphenyl H-phosphonate;[34] bis(5′-*O*-dimethoxytritylthymidin-3′-yl) H-phosphonate **8** and bis(3′-*O*-(1,3-benzodithiol-2-yl)thymidin-5′-yl) H-phosphonate **9**, by treatment of the appropriate nucleoside (2 equiv) with diphenyl H-phosphonate in pyridine; bis(5′-*O*-dimethoxytritylthymidin-3′-yl) phosphorothioate **4** and bis(3′-*O*-(1,3-benzodithiol-2-yl)thymidin-5′-yl) H-phosphonate **5**, by sulfurisation of the corresponding H-phosphonate precursors, **8** and **9**, respectively.

The assignments of signals in the ^{31}P NMR spectra to particular products or intermediates were done on the basis of their chemical shifts, multiplicity of the signals in ^1H-coupled and ^1H-decoupled spectra, by spiking the reaction mixtures with appropriate species and, if possible, by isolation of the compound in question from reaction mixtures. The assignment of proton and carbon resonances of **1**–**3** was done on the basis of known or expected chemical shifts in conjunction with ^1H–^1H, ^1H–^{13}C, and DEPT correlated NMR spectroscopy.

5′-*O*-Dimethoxytritylthymidin-3′-yl 3′-*O*-(1,3-benzodithiol-2-yl)thymidin-5′-yl H-phosphonate 1. To a solution of 5′-*O*-dimethoxytritylthymidine 3′-H-phosphonate **6**

(triethylammonium salt, 2.11 mmol) and 3′-*O*-(1,3-benzodithiol-2-yl)thymidine (2.11 mmol, 1.00 equiv) in pyridine (100 mL) was added pivaloyl chloride (4.23 mmol, 2.00 equiv). When the reaction was complete (~1 h, TLC analysis), the mixture was quenched with methanol (5 mL), partitioned between 5% aq $NaHCO_3$ (100 mL) and CH_2Cl_2 (100 mL). The organic layer was dried with Na_2SO_4, evaporated and purified by silica gel chromatography using a stepwise gradient of ethyl acetate (0–100%) in CH_2Cl_2. Yield: 90%. The R_P and S_P diastereomers of **1** were separated using the same chromatographic system.

1. (R_P) (faster moving isomer). Yield 35%, white solid. Purity >98% (^{1}H NMR spectroscopy).

^{31}P NMR:. (δ in ppm $CDCl_3$) 7.24 (d, $^1J_{PH}$ = 717 Hz).

^{1}H NMR. (δ in ppm $CDCl_3$. Subscripts 'a' and 'b' denote resonances in the thymidyl 3′-yl and thymidyl 5′-yl units, respectively) 9.36 (s, 1H, NH), 9.34 (s, 1H, NH), 7.54 (s, 1H, H_a-6), 7.40–7.10 (m, 13H, ArH), 7.07 (s, 1H, H_b-6), 6.84 (d, 4H, J = 9 Hz, CH_3OCH=CH), 6.77 (d, 1H, J = 718 Hz, P-H), 6.75 (s, 1H, SCHS), 6.43 (m, 1H, H_a-1′), 6.11 (t, 1H, J = 7 Hz, H_b-1′), 5.22 (m, 1H, H_a-3′), 4.27–4.09 (m, 5H, H_b-3′, 2×H-4′, H_b 5′), 3.79 (s, 6H, 2×CH_3O), 3.44 (m, 2H, H_a-5′), 2.60–2.42 (m, 2H, H_a-2′), 2.50–2.19 (m, 2H, H_b-2′), 1.81 (s, 3H, C_{5b}–CH_3), 1.40 (s, 3H, C_{5a}–CH_3).

^{13}C NMR. (δ in ppm $CDCl_3$. Subscripts 'a' and 'b' denote resonances in the thymidyl 3′-yl and thymidyl 5′-yl units, respectively) 163.91, 163.90 (2×C4), 158.94 (1C of DMT), 150.76, 150.43 (2×C2), 144.08 (1C of DMT), 135.81 (C_b6), 135.72, 135.48 (2C of BDT), 135.20 (C_a6), 135.07 (2C of DMT), 130.21, 130.18, 128.22, 128.18 (8C of DMT), 127.43 (1C of DMT), 125.96 (2C of BDT), 122.39, 122.23 (2C of BDT), 113.47 (4C of DMT), 111.95, 111.52 (2×C5), 89.54 (SCHS), 87.45 (C^{DMT}), 85.70 (C_b1′), 84.61 (d, J = 6.9 Hz, C_a4′), 84.38 (C_a1′), 82.35 (d, J = 6.9 Hz, C_b4′), 77.62 (C_a3′), 74.10 (C_b3′), 64.57 (C_b5′), 63.31 (C_a5′), 55.39 (2×CH_3O), 39.53 (C_a2′), 38.26 (C_b2′), 12.53 (C_b5–CH_3), 11.84 (C_a5–CH_3).

1. (S_P) (slower moving isomer). Yield 40%, white solid. Purity >98% (^{1}H NMR spectroscopy).

^{31}P NMR. (δ in ppm $CDCl_3$) 8.38 (d, $^1J_{PH}$ = 724 Hz)

^{1}H NMR. (δ in ppm $CDCl_3$. Subscripts 'a' and 'b' denote resonances in the thymidyl 3′-yl and thymidyl 5′-yl units, respectively) 9.04 (s, 1H, NH), 8.97 (s, 1H, NH), 7.53 (s, 1H, H_a-6), 7.38–7.10 (m, 13H, ArH), 6.98 (s, 1H, H_b-6), 6.83 (d, 4H, J = 9 Hz, CH_3OCH=CH), 6.80 (d, 1H, J = 717 Hz, P-H), 6.75 (s, 1H, SCHS), 6.43 (m, 1H, H_a-1′), 6.13 (t, 1H, J = 7 Hz, H_b-1′), 5.24 (m, 1H, H_a-3′), 4.21 (m, 1H, H_a-4′), 4.15–4.08 (m, 4H, H_b-3′, H_b-4′, H_b 5′), 3.78 (s, 6H, 2×CH_3O), 3.44 (m, 2H, H_a-5′), 2.57–2.42 (m, 2H, H_a-2′), 2.50–2.07 (m, 2H, H_b-2′), 1.81 (s, 3H, C_{5b}–CH_3), 1.40 (s, 3H, C_{5a}–CH_3).

^{13}C NMR. (δ in ppm $CDCl_3$. Subscripts 'a' and 'b' denote resonances in the thymidyl 3′-yl and thymidyl 5′-yl units, respectively) 163.93, 163.82 (2×C4), 158.92 (2C of DMT), 150.74, 150.43 (2×C2), 144.13 (1C of DMT), 135.70, 135.43 (2C of BDT), 135.36 (C_b6), 135.21 (C_a6), 135.12, 135.10 (2C of DMT), 130.20, 130.18, 128.23, 128.17 (8C of DMT), 127.42 (1C of DMT), 126.02 (2C of BDT), 122.31, 122.14 (2C of BDT), 113.46 (4C of DMT), 111.90, 111.68 (2×C5), 89.41 (SCHS), 87.40 (C^{DMT}), 85.13 (C_b1′), 84.85 (C_a4′), 84.37 (C_a1′), 82.36 (d, J = 6.1 Hz, C_b4′), 77.16 (C_a3′), 73.66 (C_b3′), 64.79 (d, J = 6.1 Hz, C_b5′), 63.17 (C_a5′), 55.37 (2×CH_3O), 39.30 (C_a2′), 38.43 (C_b2′), 12.56 (C_b5–CH_3), 11.81 (C_a5–CH_3).

5′-*O*-Dimethoxytritylthymidin- 3′-yl 3′-*O*-(1,3-benzodithiol-2-yl)thymidin-5′-yl phosphorothioate 2, triethylammonium salts. To a solution of separate R_P and S_P diastereomers of **1** (0.102 mmol) in pyridine (2 mL) was added *n*-butylamine (1.02 mmol, 10 equiv), DBU (0.053 mmol, 0.52 equiv) and elemental sulfur (1.53 mmol, 15.0 equiv). The reaction was complete within 5 min (TLC analysis) but it was left for 1 h, (according to Hata's procedure) before the mixture was partitioned between 5% aq $NaHCO_3$ (10 mL) and CH_2Cl_2 (10 mL), The organic layer was dried with Na_2SO_4, evaporated and purified by silica gel chromatography using CH_2Cl_2/methanol 9:1.

2. (S_P) (faster moving isomer), obtained form **1** (R_P). Yield 88%, white solid. Purity >98% (^{1}H NMR spectroscopy).

^{31}P NMR. (δ in ppm $CDCl_3$) 57.42.

^{1}H NMR. (δ in ppm $CDCl_3$. Subscripts 'a' and 'b' denote resonances in the thymidyl 3′-yl and thymidyl 5′-yl units, respectively) 7.62–7.03 (m, 15H, 2×H6, ArH), 6.82 (d, 4H, J = 9 Hz, CH_3OCH=CH), 6.62 (s, 1H, SCHS), 6.43 (m, 1H, H_a-1′), 6.25 (m, 1H, H_b-1′), 5.32 (m, 1H, H_a-3′), 4.30 (m, 1H, H_b-3′), 4.22 (m, 1H, H_a-4′), 4.13 (m, 1H, H_b-4′), 3.93 (m, 2H, H_b-5″), 3.77 (s, 6H, 2×CH_3O), 3.42 (m, 2H, H_a-5′), 2.63 (q, 6H, J = 7 Hz, N-CH_2), 2.67–2.27 (m, 2H, H_a-2′), 2.35–1.96 (m, 2H, H_b-2′), 1.93 (s, 3H, C_{5b}-CH_3), 1.38 (s, 3H, C_{5a}-CH_3), 1.08 (t, 9H, J = 7 Hz, N-C-CH_3).

^{13}C NMR. (δ in ppm $CDCl_3$. Subscripts 'a' and 'b' denote resonances in the thymidyl 3′-yl and thymidyl 5′-yl units, respectively) 164.10, 164.03 (2×C4), 158.58 (2C of DMT), 150.62, 150.52 (2×C2), 144.30 (1C of DMT), 135.97, 135.52, 135.43, 135.38, 135.31, 135.24 (2C of BDT, 2C of DMT, 2×C6′), 130.08, 128.13, 127.92 (8C of DMT), 127.02 (1C of DMT), 125.61, 125.56 (2C of BDT), 122.37, 122.29 (2C of BDT), 113.21 (4C of DMT), 111.17 (2×C5), 89.45 (SCHS), 86.95 (C^{DMT}), 85.47 (C_a4′), 84.71 (C_b1′), 84.58 (C_a1′), 82.36 (d, J = 8.4 Hz, C_b4′), 77.53 (C_a3′), 77.06 (C_b3′), 64.89 (C_b5′), 63.96 (C_a5′), 55.20 (2×CH_3O), 45.87 (3×CH_2 of TEA), 39.17 (C_a2′), 37.94 (C_b2′), 12.39 (C_b5–CH_3), 11.54 (C_a5–CH_3), 9.46 (3×CH_3 of TEA).

2. (R_P) (slower moving isomer), obtained form **1** (S_P). Yield 88%, white solid. Purity >98% (^{1}H NMR spectroscopy).

^{31}P NMR. (δ in ppm $CDCl_3$) 57.37.

^{1}H NMR. (δ in ppm $CDCl_3$. Subscripts 'a' and 'b' denote resonances in the thymidyl 3′-yl and thymidyl 5′-yl units, respectively) 7.52–6.98 (m, 15H, 2×H6, ArH), 6.75 (d, 4H, $J=9$ Hz, $CH_3OCH{=}CH$), 6.57 (s, 1H, SCHS), 6.35 (m, 1H, H_a-1′), 6.18 (m, 1H, H_b-1′), 5.22 (m, 1H, H_a-3′), 4.40 (m, 1H, H_b-3′), 4.28 (m, 1H, H_a-4′), 4.13 (m, 1H, H_b-4′), 4.00 (m, 2H, H_b-5′), 3.70 (s, 6H, 2×CH_3O), 3.37 (m, 2H, H_a-5′), 2.68 (q, 6H, $J=7$ Hz, N-CH_2), 2.49–2.23 (m, 2H, H_a-2′), 2.39–2.05 (m, 2H, H_b-2′), 1.83 (s, 3H, C_{5b}–CH_3), 1.29 (s, 3H, C_{5a}–CH_3), 1.07 (t, 9H, $J=7$ Hz, N–C–CH_3).

^{13}C NMR. (δ in ppm $CDCl_3$. Subscripts 'a' and 'b' denote resonances in the thymidyl 3′-yl and thymidyl 5′-yl units, respectively) 164.20, 164.12 (2×C4), 158.76 (2C of DMT), 150.80, 150.62 (2×C2), 144.45 (1C of DMT), 136.16, 135.78, 135.51, 135.43, 135.38 (2C of BDT, 2C of DMT, 2×C6′), 130.24, 128.28, 128.08 (8C of DMT), 127.16 (1C of DMT), 125.78, 125.73 (2C of BDT), 122.49, 122.35 (2C of BDT), 113.39 (4C of DMT), 111.38 (2×C5), 89.61 (SCHS), 87.13 (C^{DMT}), 85.21 (C_a4′), 84.74 (C_a1′), 84.63 (C_b1′), 83.56 (C_b4′), 77.18 (C_a3′), 76.87 (C_b3′), 65.26 (C_b5′), 64.11 (C_a5′), 55.33 (2×CH_3O), 45.94 (3×CH_2 of TEA), 39.86 (C_a2′), 38.12 (C_b2′), 12.49 (C_b5–CH_3), 11.71 (C_a5–CH_3), 9.81 (3×CH_3 of TEA).

5′-*O*-Dimethoxytritylthymidin- 3′-yl 3′-*O*-(1,3-benzodithiol-2-yl)thymidin-5′-yl benzoylphosphonate 3. To a solution of separate R_P and S_P diastereomers of **1** (0.203 mmol) in CH_2Cl_2 (4 mL) were added, consecutively, *N,N*-diethylaniline (0.409 mmol, 2.0 equiv), benzoyl chloride (0.809 mmol, 4.0 equiv) and bis(trimethyl)acetamide BSA (0.607 mmol/3.0 equiv). After ca. 2 h (^{31}P NMR) the mixture was added to petroleum ether (150 mL) and the white solid was filtered off and washed with several portions of petroleum ether. The precipitation from petroleum ether was repeated.

3. (R_P), obtained form **1** (R_P). Yield 82%, white solid. Purity >90% (^{1}H NMR spectroscopy).

^{31}P NMR. (δ in ppm $CDCl_3$) −1.35 (q, $^{3}J_{PH}=7$ Hz)); MS (-FAB) *m/z* 1088.3 (M−H).

^{1}H NMR. (δ in ppm $CDCl_3$) 8.94 (m, 2H, 2×NH), 8.12 (d, 2H, $J=7$ Hz, benz-H_A), 7.65 (t, 1H, $J=7$ Hz, benz-H_C), 7.55 (s, 1H, H_a-6) 7.47 (t, 2H, $J=7$ Hz, Benz-H_B), 7.35–7.05 (m, 14H, H_b-6, ArH), 6.81 (d, 4H, $J=9$ Hz, $CH_3OCH{=}CH$), 6.72 (s, 1H, SCHS), 6.46 (m, 1H, H_a-1′), 6.09 (t, 1H, $J=7$ Hz, H_b-1′), 5.29 (m, 1H, H_a-3′), 4.45–4.13 (m, 5H, H_b-3′, 2× H-4′, H_b 5′), 3.76 (s, 6H, 2× CH_3O), 3.45 (m, 2H, H_a-5′), 2.65–2.38 (m, 2H, H_a-2′), 2.49–2.10 (m, 2H, H_b-2′), 1.76 (s, 3H, C_{5b}–CH_3), 1.42 (s, 3H, C_{5a}–CH_3).

^{13}C NMR. 197.00 (d, $J=173.2$ Hz, C=O), 163.77, 163.73 (2×C4), 158.93 (2C of DMT), 150.62, 150.34 (2×C2), 144.20 (1C of DMT), 135.72, 135.55, 135.41, 135.18 (2C of BDT, 2C of DMT, 1C of benzoyl, 2×C6′), 130.21, 128.20 (8C of DMT), 129.93 (2C of benzoyl), 129.27, 129.23 (2C of benzoyl), 127.40 (1C of DMT), 125.99, 125.95 (2C of BDT), 122.33, 122.26 (2C of BDT), 113.49 (4C of DMT), 111.96, 111.57 (2×C5), 89.55 (SCHS), 87.46 (C^{DMT}), 85.44 (C_b1′), 84.85 (C_a4′), 84.37 (C_a1′), 82.36 (d, $J=6.1$ Hz, C_b4′), 79.49 (C_a3′), 74.28 (C_b3′), 66.58 (C_b5′), 63.46 (C_a5′), 55.38 (2×CH_3O), 39.87 (C_a2′), 38.30 (C_b2′), 12.42 (C_b5–CH_3), 11.86 (C_a5–CH_3).

3. (S_P), obtained form **1** (S_P). Yield 88%, white solid. Purity >90% (^{1}H NMR spectroscopy).

^{31}P NMR. (δ in ppm $CDCl_3$) −1.56 (q, $^{3}J_{PH}=7$ Hz); MS (-FAB) *m/z* 1088.5 (M−H).

^{1}H NMR. (δ in ppm $CDCl_3$) 8.82 (s, 1H, NH), 8.74 (s, 1H, NH), 8.17 (d, 2H, $J=7$ Hz, benz-H_A), 7.66 (t, 1H, $J=7$ Hz, benz-H_C), 7.52–6.91 (m, 17H, 2×H6, ArH), 6.81 (d, 4H, $J=9$ Hz, $CH_3OCH{=}CH$), 6.70 (s, 1H, SCHS), 6.41 (m, 1H, H_a-1′), 6.05 (t, 1H, $J=7$ Hz, H_b-1′), 5.30 (m, 1H, H_a-3′), 4.26–4.06 (m, 5H, H_b-3′, 2×H-4′, H_b 5′), 3.76 (s, 6H, 2×CH_3O), 3.44 (m, 2H, H_a-5′), 2.65–2.25 (m, 2H, H_a-2′), 2.45–2.15 (m, 2H, H_b-2′), 1.76 (s, 3H, C_{5b}–CH_3), 1.39 (s, 3H, C_{5a}–CH_3).

^{13}C NMR. 196.90 (d, $J=173.2$, C=O), 163.79, 163.66 (2×C4), 158.92 (2C of DMT), 150.52, 150.18 (2×C2), 144.18 (1C of DMT), 135.57, 135.40, 135.28, 135.16 (2C of BDT, 2C of DMT, 1C of benzoyl, 2×C6′), 130.20, 128.23, 128.20 (8C of DMT), 129.96 (2C of benzoyl), 129.21 (2C of benzoyl), 127.42 (1C of DMT), 126.02, 125.98 (2C of BDT), 122.28 (2C of BDT), 113.48 (4C of DMT), 111.80, 111.52 (2×C5), 89.45 (SCHS), 87.42 (C^{DMT}), 85.12 (C_b1′), 84.96 (C_a4′), 84.47 (C_a1′), 82.41 (C_b4′), 79.96 (C_a3′), 73.96 (C_b3′), 66.21 (C_b5′), 63.22 (C_a5′), 55.38 (2×CH_3O), 39.33 (C_a2′), 38.41 (C_b2′), 12.51 (C_b5–CH_3), 11.82 (C_a5–CH_3).

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21. In the original publications the authors erroneously referred to this and the related reactions as stereospecific instead of stereoselective ones.
22. The reaction occurs with retention of configuration at the phosphorus center, but the priority rules of the CIP notation of absolute configuration lead in this instance to a formal change of the sense of chirality at the P-atom.
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26. At the initial stages of this project, there was a remote possibility that the reported stereoselectivity during sulfurisation of **1** could be due to configurational instability of phosphorothioates **2** in the presence of DBU. In a separate experiment, we found that this was not the case.
27. Before analysis, the reaction mixture was partitioned between dichloromethane and 0.3 M TEAB buffer, to secure good resolution of the ^{31}P NMR resonances from diastereomeric dinucleoside phosphorothioates **2**.
28. The species were identified on the basis of their distinctive chemical shifts, multiplicity of the signals in ^{31}P–^{1}H coupled spectra, and by comparison with authentic samples, prepared as described in the Experimental.
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30. The degree of hydrolysis of H-phosphonate **1**, as judged from the sum of H-phosphonate monoesters **6** and **7** formed under the reaction conditions, was higher for diastereomer **1** R_P (ca. 20%) than for **1** S_P (ca. 10%).
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32. Acylphosphonate diesters resonate in ^{31}P NMR downfield from the corresponding H-phosphonates (usually at −1 to −3 ppm; see, e.g., de Vroom, E.; Spierenburg, M. L.; Dreef, C. E.; van der Marel, G. A.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* 1987, *106*, 65) and thus the chemical shift of purified acylphosphonate **3** reported in ref 20 ($\delta_P = 7.54$ and 8.31 ppm) is probably erroneous and refers most likely to its decomposition product, i.e., H-phosphonate **1** diastereomers.
33. Under such conditions, the major (or the sole) product of the reaction was apparently the corresponding tetranucleoside 1-(phosphinoxy)benzylphosphonate formed via the reaction of H-phosphonate 1 (generated from **3**) with benzoylphosphonate **3**, followed by the rearrangement of the produced symmetrical tetranucleoside bisphosphonate. See also: Fitch, S. J.; Moedritzer, K. *J. Am. Chem. Soc.* 1962, *84*, 1876.
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