Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron 60 (2004) 389–395
Tetrahedron

Nucleoside H-phosphonates. Part 19: Efficient entry to novel nucleotide analogues with 2-pyridyl- and 4-pyridylphosphonothioate internucleotide linkages

Tommy Johansson and Jacek Stawinski*

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

Received 14 August 2003; revised 16 October 2003; accepted 6 November 2003

Abstract—Synthetic and ^{31}P NMR spectroscopy studies resulted in the development of efficient protocols for the stereospecific synthesis of a novel type of nucleotide analogues, 2-pyridyl- and 4-pyridylphosphonothioates. The underlying chemistry involves formation of the P–C bond via a base-promoted reaction of suitably protected dithymidine H-phosphonothioates with *N*-methoxypyridinium tosylate in acetonitrile, or with trityl chloride in pyridine, to produce high yields of nucleotide analogues with a 2-pyridyl- or 4-pyridyl moiety directly bound to the phosphorus centre.

1. Introduction

Pyridylphosphonate derivatives represent an important class of organophosphorus compounds with a wide range of technical applications, for example, corrosion inhibitors,[1] sensitisers for photovoltaic cells,[2] dispersing and emulsifying agents,[3] lubricant additives,[4] etc. Apart from these, they also show diverse biological activity and are known as potent insecticides,[5] fungicides,[6] and herbicides.[7] Recently, 2-pyridylphosphonates emerged as broad spectrum drugs with anti-proliferating and anti-PAF activity,[8] as inhibitors of fructose-1,6-bisphosphates[9] (and thus of potential use in diabetics therapy), and as lucitropic agents for the treatment of cardiovascular diseases.[10] These can probably be traced back to a structural similarity of 2-pyridylphosphonates to α-aminophosphonates[11] (P-analogues of α-amino acids), that are potent inhibitors of proteases[12] and exhibit pronounced antineoplastic activity.[13]

This diverse array of biological activity of simple dialkyl pyridylphosphonates recently prompted us to explore the possibility of incorporating this functionality into nucleic acid fragments, kindled with the hope that it may confer novel properties that could be useful when designing new antisense and antigene therapeutics. For this purpose, we have developed efficient methods for the conversion of dinucleoside H-phosphonates into 4-pyridyl-,[14] 3-pyridyl-,[15] and 2-pyridylphosphonate[16] analogues. As an extension of these studies, we attempted to develop a synthetic method for a new nucleotide analogue in which the phosphoryl oxygen atom of the pyridylphosphonate moiety was replaced by sulfur. Since a 2-pyridylphosphonate moiety may act as a bidentate chelating ligand[17] for transition metals, the replacement of oxygen by sulfur may significantly change its affinity for particular types of metal cations and thus be of importance in designing new artificial nucleases,[18] and reporter groups for investigation of electron transfer (ET) phenomena in nucleic acids.[19]

In contrast to pyridylphosphonates, their thiophosphonate counterparts are rare compounds. Also their biological properties are largely unexplored, apart from a few reports in the patent literature where these compounds have been advocated as activators and enhancers in certain compositions of herbicides and insecticides.[20] These most limited applications are probably due to the lack of convenient methods for the preparation of pyridylphosphonothioates. The only method of synthetic value affords the target thiophosphonates in mediocre yields,[21] and requires lengthy thiation of their oxygen congeners with P_2S_5 in toluene under reflux. Recently, an approach based on the metalation-induced rearrangement of 3-pyridyl phosphorothioates was reported,[22] but this produced mixtures of 2- and 4-pyridylphosphonothioates (3:1) in rather low yields (ca. 30%).

In this paper, ^{31}P NMR spectroscopy investigations and synthetic studies on the formation of 2-pyridyl- and 4-pyridylphosphonothioates from the corresponding H-phosphonothioate diesters, are described.

Keywords: H-Phosphonates; H-Phosphonothioates; Pyridylphosphonothioates.
* Corresponding author. Tel.: +46-8-16-24-85; fax: +46-8-15-49-08; e-mail address: js@organ.su.se

0040–4020/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2003.11.010

2. Results and discussion

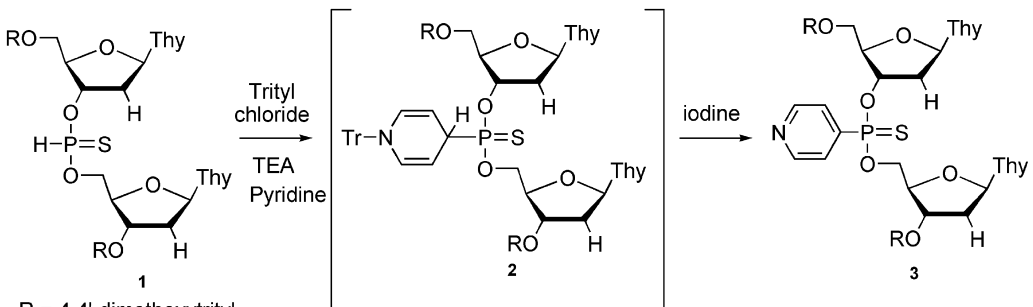
As viable means to synthesize 4-pyridyl- and 2-pyridylphosphonothioates, we considered 1,8-diazabicylo[5.4.0]undec-7-ene (DBU)-promoted reactions of dinucleoside H-phosphonothioates with the pyridine-trityl chloride reagent system and with *N*-methoxypyridinium salts, respectively, analogously to the recently developed methods for the preparation of pyridylphosphonate derivatives.[14] Despite apparent similarities between H-phosphonate and H-phosphonothioate diesters, the latter ones are more prone to side reactions and, under basic conditions, undergo a ligand exchange process[23] that may scramble substituents around the phosphorus center.

We assumed that synthesis of 4-pyridylphosphonothioates **3** could be effected via in situ generation of *N*-tritylpyridinium cation (from e.g., pyridine and trityl chloride), followed by an attack of a phosphorus nucleophile (e.g., H-phosphonothioate) at the less sterically hindered C-4 carbon of the pyridine ring (Scheme 1). Indeed, the reaction of dinucleoside H-phosphonothioate **1** in pyridine with trityl chloride (Tr-Cl, 1.2 equiv.) in the presence of DBU (2.4 equiv.) was rapid (ca. 10 min, ^{31}P NMR spectroscopy) but, apart from the expected 1,4-dihydropyridine intermediate **2** (70%, 2 signals at ca. 94 ppm) and the desired product **3** (10%, 2 signals at ca. 84 ppm), also afforded 5′-*O*-dimethoxytritylthymidin-3′-yl 3′-*O*-dimethoxytritylthymidin-5′-yl phosphorothioate diesters (20%, signals at 56.5 and 56.5 ppm), apparently due to oxidation of the starting material **1**. Use of more DBU (8 equiv.) speeded up the reaction and reduced the amount of the oxidation product to ca. 7%, but simultaneously another side products appeared (ca. 6%; signals at ca. 87 and 88 ppm). We hypothesized that formation of the phosphorothioate diesters under the reaction conditions was probably due to air oxidation of a highly reactive tervalent phosphite form generated from H-phosphonothioate **1** in the presence of a strong base (DBU). To remedy this problem, we replaced DBU by triethylamine (TEA), assuming that in the presence of a weaker base a concentration of a phosphite form generated from H-phosphonothioate **1** should be lower, and thus formation of the oxidation product should be suppressed.

Indeed, when dinucleoside H-phosphonothioate **1** (a diastereomeric mixture, 1:1; δ_P=69.9 and 71.6 ppm, $^1J_{PH}$=675, 674 Hz, $^3J_{PH}$=9.8 Hz, dq) was treated in pyridine with Tr-Cl (1.2 equiv.) in the presence of TEA (2.4 equiv.), the reaction was still rapid (<10 min) and produced 1,4-dihydropyridine derivatives **2** (δ_P=93.9, 94.5 ppm) practically quantitatively (Scheme 1). No dinucleoside phosphorothioate diesters or any other side products could be detected by ^{31}P NMR spectroscopy. Here, however, an unexpected problem arose: 1,4-dihydropyridylphosphonothioate intermediate **2** turned out to be rather stable and, in contrast to the 1,4-dihydropyridylphosphonate, that collapsed spontaneously to the corresponding pyridylphosphonate derivative,[14] it underwent only slow conversion into the product, 4-pyridylphosphonothioate diester **3** (δ_P=83.0, 84.3 ppm; ca. 20% conversion after a few hours).

To facilitate rearomatization of the 1,4-dihydropyridine intermediate and to convert it into 4-pyridylphosphonothioate **3**, we elaborated in situ oxidation of **2** with iodine. Although phosphorothioate diesters undergo rapid desulfurization in the presence of iodine,[24] uncharged phosphorus compounds bearing the thiophosphoryl function are resistant towards desulfurization (e.g., phosphorothioate triesters,[24] H-phosphonothioate diesters **1**[25]) and thus these reaction conditions should not affect the integrity of the produced pyridylphosphonothioates **3**. To check the efficacy of this approach, the reaction mixture containing 1,4-dihydropyridine intermediate **2** (obtained as described above) was treated with iodine (2 equiv.) in pyridine. ^{31}P NMR spectroscopy revealed rapid formation of the desired product **3** (ca. 50% after 10 min), however, with time, signals from dinucleoside phosphorothioate diesters (18%; 2 signals at ca. 56 ppm)[26] also appeared in the ^{31}P NMR spectrum. In this instance, the phosphorothioate diesters could be formed due to a possible reversibility of the dihydropyridine intermediate **2** formation[14] that can generate small equilibrium amounts of the starting material **1**. This, in the presence of iodine and adventitious water could produce, via the intermediacy of phosphorothioiodidates, the corresponding dinucleoside phosphorothioates.

We thought that this problem could be overcome by using more iodine for the reaction. The higher iodine concentration should increase the rate of rearomatization of **2** and thus lessen the problem of reversibility of the 1,4-dihydropyridine intermediate formation. It was rewarding



Scheme 1.

to see that addition of 4 equiv. of iodine to the reaction mixture containing 1,4-dihydrointermediate **2**, afforded, after 15 min the desired 4-pyridylphosphonothioate diester **3** exclusively. In a preparative run, using the developed reaction conditions, 4-pyridylphsophonothioates **3** were obtained in ca. 80% yield, after silica gel column chromatography (see the Section 4).

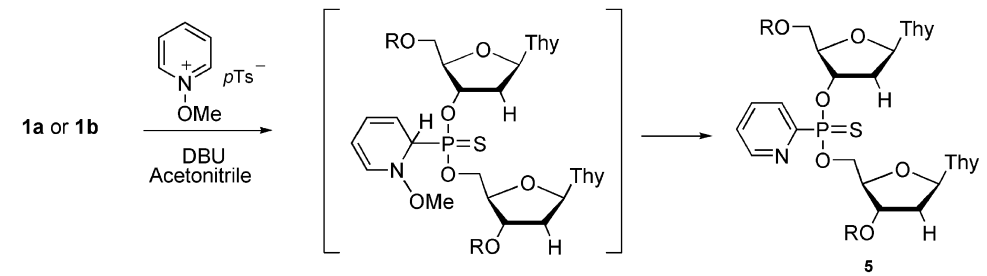
The stereochemical course of the reaction sequence as in Scheme 1 was investigated by performing this transformation on the separate diastereomers of dithymidine H-phosphonothioate **1**.[27] It was found that H-phosphonothioate diester **1a** (R_P diastereomer[27,28] resonating at higher field in the ^{31}P NMR spectrum) afforded pyridylphosphonothioate **3a** (the diastereomer resonating at higher field) with the intermediacy of 1,4-dihydropyridylphosphonothioate **2a**, while the diastereomer **1b** (S_P diastereomer,[27,28] resonating at lower field in the ^{31}P NMR spectrum) gave pyridylphosphonothioate **3b** (resonating at lower field), with the intermediacy of **2b**. Thus, the transformation was found to be stereospecific and, assuming the reaction pathway shown in Scheme 1, it most likely proceeded with an overall retention of configuration. On this basis we tentatively assigned the configuration at the phosphorus center in pyridylphosphonothioate **3a** as R_P, and that in **3b** diastereomer, as S_P.

As to the synthesis of dinucleoside 2-pyridylphosphonothioates **5**, a protocol consisting of a reaction of H-phosphonothioate diesters **1** in acetonitrile with *N*-methoxypyridinium *p*-toluenesulfonate (2 equiv.) in the presence of DBU (4 equiv.), was designed (Scheme 2). The reaction was rapid (<5 min), but along with the desired 2-pyridylphosphonothioates **5** (δ_P=78.2 and 79.3 ppm; ca. 50%), ^{31}P NMR spectroscopy revealed the presence in the reaction mixture of the isomeric 4-pyridylphosphonates **3** (δ_P=83.0 and 84.3 ppm; ca. 15%) and also other side products resonating close to 2-pyridylphosphonatates **5** (two signals at ca. 78 ppm and two signals a ca. 80 ppm; total, 25%). When the order of addition of *N*-methoxypyridinium *p*-toluenesulfonate and DBU was changed that is DBU was added before the pyridinium salt, the reaction showed higher chemoselectivity (65% of 2-pyridylphosphonothioates **5** and 5% of 4-pyridyl isomers **3**), however, the amount of the unidentified side products increased (ca. 30%). Premixing of *N*-methoxypyridinium *p*-toluenesulfonate with DBU, followed by the addition of H-phosphonothioate **1**, severely decreases the formation of the desired 2-pyridylphosphonothioate derivatives, apparently due to the known instability of *N*-alkoxypyridinium salts under basic conditions.[29]

To remedy these problems, we first wanted to identify structures of the side products formed. On the basis of their chemical shift values (that were close to those of dinucleoside pyridylphosphonothioates **5**), multiplicity of the signals in the ^{31}P NMR spectra, and by considering viable reaction pathways, we assumed that the observed side products were, most likely, isomeric nucleoside methyl 2-pyridylphosphonothioates.[30] This was consistent with a putative decomposition pathway of *N*-methoxy-1,2-dihydropyridine intermediate **4** (Scheme 2) that along with 2-pyridylphophonothioates **5** should also generate a methoxide anion. Since, H-phosphonothioate diesters are susceptible to nucleophilic substitution at the phosphorus center,[23] this could cause a partial replacement of the 5′- or 3′-nucleosidic unit in H-phosphonothioate **1** by the methoxide anion, and ultimately lead to the formation of the observed side products.

Various approaches were tried to suppress the transesterification phenomena of **1** by the generated methoxide anion. The most successful one consisted of adding DBU before *N*-methoxypyridinium *p*-toluenesulfonate and decreasing the amount of the latter one to 1.2 equiv.[31] Under these conditions, the formation of methyl nucleoside pyridylphosphonothioates was completely eliminated, and 2-pyridylphosphonothioates **5** were formed as major products (95%, ^{31}P NMR spectroscopy). No further attempts were made to eliminate the formation of 4-pyridylphosphonothioates **3** (ca. 5%) as these were easily removed during silica gel chromatography.

A mechanism for the investigated reaction (Scheme 2) is probably similar to that of 4-pyridylphosphonothioates **3** formation, and involves intermediacy of the corresponding 1,2-dihydropyridine derivatives **4**. However, in contrast to 1,4-dihydropyridylphosphonate **2**, 1,2-dihydropyridine intermediate **4** could not be detected by ^{31}P NMR spectroscopy, probably due to its high lability under basic conditions. Since intermediate **4** spontaneously collapsed to 2-pyridylphosphonothioate **5** with expulsion of a methoxide group, this reaction did not require a separate rearomatization step. When carried out on a preparative scale under the



Scheme 2.

optimised conditions, the reaction of H-phosphonothioate **1** with *N*-methoxypyridinium salt in the presence of DBU afforded dinucleoside 2-pyridylphosphonothioates **5** in ca. 80% yield, after silica gel column chromatography.

In the context of regioselectivity of the above reaction, that is formation of 2-pyridyl- versus 4-pyridylphosphonothioate derivatives (vide supra), an interesting observation was made. In all instances, when the reactions of H-phosphonothioates **1** with *N*-methoxypyridinium *p*-toluenesulfonate were promoted by DBU, the major products formed were 2-pyridyl derivatives **5**. The other positional isomer, 4-pyridylphosphonothioates **3**, was detected only in small amounts (5–15%, vide supra) in these reaction mixtures. By contrast, use of triethylamine instead of DBU, afforded 4-pyridylphosphonothioates **3** as the major products (80%; ^{31}P NMR spectroscopy).[32] Although the precise factors that produce the strikingly different results in the two reactions are not known, it seems that kinetic versus thermodynamic control may be responsible for this phenomenon. Since, the base participates, most likely, in the collapse of *N*-methoxy-1,2-dihydropyridine derivative **4**, this process should be fast in the presence DBU, and thus formation of the kinetic products,[33] 2-pyridylphosphonothioate **5**, should be favored. In the presence of triethylamine, however, the collapse of 1,2-dihydropyridine derivative **4**, is expected to be slower, and thus, this initially formed intermediate may isomerise to the thermodynamically more stable one, the 1,4-dihydropyridyl derivative,[33] from which elimination of methoxide anion can occur. This ultimately will lead to the formation of isomeric 4-pyridylphosphonothioates **3**.

The stereochemical course of the formation of 2-pyridylphosphonothioates **5** (Scheme 2) was elucidated analogously to that of 4-pyridylphosphonothioates using ^{31}P NMR spectroscopy. The exclusive formation of 2-pyridylphosphonothioate **5a** ('fast' diastereomer, resonating at higher field in the ^{31}P NMR spectrum) from H-phosphonothioate **1a** (R_P diastereomer), and **5b** ('slow' diastereomer, resonating at lower field in the ^{31}P NMR spectrum) from H-phosphonothioate **1b** (S_P diastereomer), established this reaction as stereospecific. Assuming a mechanism as proposed in Scheme 2, the reaction in this instance also most likely occurs with overall retention of configuration at the phosphorus center and the produced pyridylphosphonothioates **5a** and **5b**, should have R_P and S_P configurations, respectively.

To facilitate spectral characterization of the produced 4- and 2-pyridylphosphonothioate diesters, compounds **3a**, **3b**, **5a** and **5b**, were subjected to detritylation with 80% aqueous acetic acid. The unprotected compounds, **3c**, **3d**, **5c** and **5d**, respectively, showed the expected pattern of signals in the ^{1}H NMR spectra, characteristic for the respective isomeric 4- and 2-pyridylphosphonothioate derivatives.

3. Conclusions

We have developed simple and efficient methods for the preparation of new types of nucleotide analogues bearing 2- and 4-pyridylphosphonothioate internucleotide linkages. The methods make use of easily available starting materials, H-phosphonothioate diesters, and afford the target pyridylphosphonothioates in high yields under mild reaction conditions. The underlying chemical reactions are stereospecific and can be extended to the preparation of other types of biologically important phosphorus compounds bearing these types of modifications.

4. Experimental

4.1. Material and methods

^{1}H and ^{31}P NMR spectra were recorded on a Varian Unity 400 BB VT spectrometer. The ^{31}P NMR spectroscopy experiments were carried out at 25 °C in 5 mm tubes using 0.1 M concentrations of phosphorus-containing compounds in appropriate solvents (0.6 mL), and the spectra were referenced to 2% H_3PO_4 in D_2O (external standard). TLC analyses were carried out on Merck silica gel 60 F_{254} precoated plates using the following solvent systems: (A) CH_3Cl/CH_3OH 8:2 (v/v); (B) toluene/ethyl acetate 8:2 (v/v). Pyridine (LabScan Ltd.) and anhydrous acetonitrile (LabScan Ltd.) were stored over molecular sieves 4 Å. 1,8-Diazabicylo[5.4.0]undec-7-ene (DBU) and triethylamine (from Aldrich) were freshly distilled. The starting materials for the synthesis, dinucleoside H-phosphonothioates **1** were obtained according to published procedure.[34]

The assignments of signals in the ^{31}P NMR spectra to particular products or intermediates were carried out on the basis of their chemical shifts, multiplicity of the signals in ^{1}H-coupled and ^{1}H-decoupled spectra, by spiking the reaction mixtures with appropriate species and, if possible, by isolation of a compound in question from reaction mixtures. The assignment of proton and carbon resonances of **3** and **5** was carried out on the basis of known or expected chemical shifts in conjunction with ^{1}H–^{1}H, ^{1}H–^{13}C, and DEPT correlated NMR spectroscopy. Subscripts 'a' and 'b' in the ^{1}H NMR spectroscopy data refer to the protons in nucleosid-3′-yl and nuclosid-5′-yl units, respectively.

4.2. General procedure for the preparation of 4-pyridylphosphonothioates 3a and 3b

The separate diastereomers of 5′-*O*-dimethoxytritylthymidin-3′-yl 3′-*O*-dimethoxytritylthymidin-5′-yl H-phosphonothioate **1** (0.26 mmol) were rendered anhydrous by repeated evaporation of added acetonitrile (3×10 mL), and the residue was treated in pyridine (5 mL) with trityl chloride (1.2 equiv.) and triethylamine (2.4 equiv.). When the starting material **1** disappeared (<10 min, TLC analysis), iodine was added (4 equiv.), and after 15 min the reaction mixture was concentrated, partitioned between a solution of aq. $Na_2S_2O_3/NaHCO_3$ (3:1, v/v; 20 mL) and CH_2Cl_2 (20 mL), and the aqueous phase was washed with CH_2Cl_2 (2×20 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated and the residue was purified by silica gel column chromatography using a stepwise gradient of methanol (1–2%) in toluene/ethyl acetate (1:1, v/v) containing 0.01% TEA. Purity of the isolated compounds >98% (^{1}H NMR spectroscopy).

4.2.1. 5′-*O*-Dimethoxytritylthymidin-3′-yl 3′-*O*-dimethoxytritylthymidin-5′-yl pyridyl-4-phosphonothioate 3a (from faster moving H-phosphonate diastereomer 1a). White solid (0.263 mg), yield 82%. HRMS $[M+H]^+$ found: 1227.4069; $C_{67}H_{66}N_5O_{14}PS$ requires: 1227.4065.

^{1}H NMR. δ_H (in ppm, $CDCl_3$) 8.97 and 8.96 (2×s, 2H, 2×NH), 8.71 (m, 2H, pyr-H2, pyr-H6), 7.52–7.57 (m, 3H, H_a6, pyr-H3, pyr-H5), 7.44–7.14 (m, 18H, ArH), 7.09 (s, 1H, H_b6), 6.85–6.82 (m, 8H, ArH *ortho* to OMe), 6.36 (m, 1H, H_a1′), 6.23 (m, 1H, H_b1′), 5.46 (m, 1H, H_a3′), 4.24 (d, J=6.2 Hz, 1H, H_b3′), 3.95 (m, 2H, 2×H4′), 3.93–3.70 (m, 2H, H_b5′), 3.80, 3.79, 3.77 (3×s, 12H, 4×CH_3O), 3.35–3.23 (m, 2H, H_a5′), 2.49–2.30 (m, 2H, H_a2′), 2.03–1.98, 1.77–1.72 (2×m, 2H, H_b2′), 1.77 (s, 3H, C_b5–CH_3), 1.47 (s, 3H, C_a5–CH_3).

^{31}P NMR. δ_P ($CDCl_3$) 83.04 ppm.

^{13}C NMR. δ_C (in ppm, $CDCl_3$) 163.87 and 163.84 (2×C4), 158.95, 158.93 and 158.91 (4C of DMT), 150.57, 150.44, 150.39, 150.33 (pyr-C2, pyr-C6, 2×C2), 144.91 and 144.19 (2C of DMT), 141.15 (d, J=151 Hz, pyr-C4), 136.02 (2C of DMT), 135.78 (C_b6), 135.21 (C_a6), 135.09 and 135.05 (2C of DMT), 130.33, 130.27, 130.13, 128.22, 128.19, 128.12 (16C of DMT), 127.35 (2C of DMT), 123.88 (d, J=9.9 Hz, pyr-C3, pyr-C5), 113.58 and 113.48 (8C of DMT), 111.88 and 111.27 (2× C5), 87.59 and 87.34 (2×C DMT), 86.51 (C_b1′), 84.57 and 84.51 (C_a4′, C_a1′), 84.19 (d, J=8.4 Hz, C_b4′), 78.40 (d, J=4.6 Hz, C_a3′), 74.30 (C_b3′), 66.78 (d, J=6.1 Hz, C_b5′), 63.20 (C_a5′), 55.38 (4×CH_3O), 39.31 (C_a2′), 39.08 (C_b2′), 12.48 (C_b5–CH_3), 11.91 (C_a5–CH_3).

4.2.2. 5′-*O*-Dimethoxytritylthymidin-3′-yl 3′-*O*-dimethoxytritylthymidin-5′-yl pyridyl-4-phosphonothioate 3b (from slower moving H-phosphonate diastereomer 1b). White solid (0.245 g), yield 77%. HRMS $[M+H]^+$ found: 1227.4071; $C_{67}H_{66}N_5O_{14}PS$ requires: 1227.4065.

^{1}H NMR. δ_H (in ppm, $CDCl_3$) 8.77–8.74 (m, 3H, pyr-H2, pyr-H6, NH), 8.58 (s, 1H, NH), 7.58–7.52 (m, 3H, H_a6, pyr-H3, pyr-H5), 7.42–7.14 (m, 18H, ArH), 6.93 (s, 1H, H_b6), 6.86–6.79 (m, 8H, ArH *ortho* to OMe), 6.40 (m, 1H, H_a1′), 6.19 (m, 1H, H_b1′), 5.40 (m, 1H, H_a3′), 4.22 (s, 1H, H_a4′), 4.11 (d, J=6.6 Hz, 1H, H_b3′), 3.95 (s, 1H, H_b4′), 79, 3.76, 3.75 (3×s, 12H, 4×CH_3O), 3.78–3.70 (m, 2H, H_b5′), 3.48–3.39 (m, 2H, H_a5′), 2.36–2.32 (m, 2H, H_a2′), 1.84–1.76, 1.46–1.38 (2×m, 2H, H_b2′), 1.76 (s, 3H, C_b5–CH_3), 1.45 (s, 3H, C_a5–CH_3).

^{31}P NMR. δ_P ($CDCl_3$) 84.32 ppm.

^{13}C NMR. δ_C (in ppm, $CDCl_3$) 163.81 and 163.64 (2×C4), 158.93 (4C of DMT), 150.64, 150.49, 150.37, 150.29 (pyr-C2, pyr-C6, 2×C2), 144.83 and 144.20 (2C of DMT), 141.06 (d, J=150 Hz, pyr-C4), 135.95 and 135.94 (2C of DMT), 135.26, 135.12, 135.05 (2C of DMT, 2×C6), 130.28, 130.24, 130.15, 128.27, 128.23, 128.20, 128.15 (16C of DMT), 127.39 and 127.34 (2C of DMT), 123.99 (d, J=9.2 Hz, pyr-C3, pyr-C5), 113.53 (8C of DMT), 111.93 and 111.32 (2×C5), 87.55 and 87.48 (2×C DMT), 85.39 (C_b1′), 85.32 (d, J=3.1 Hz, C_a4′), 84.50 (C_a1′), 83.96 (d, J=8.4 Hz, C_b4′), 78.90 (d, J=4.6 Hz, C_a3′), 74.03 (C_b3′), 66.85 (C_b5′), 63.21 (C_a5′), 55.39 and 55.36 (4× CH_3O), 39.15 (2×C2′), 12.60 (C_b5–CH_3), 11.87 (C_a5–CH_3).

4.2.3. Thymidin-3′-yl thymidin-5′-yl pyridyl-4-phosphonothioates 3c and 3d. The diastereomers of fully protected 4-pyridylphosphonothioates **3a** and **3b** (0.130 mmol) were dissolved separately in 80% acetic acid (aq) (15 mL) and were left while stirring for 4 h. Water (15 mL) was then added and the mixture was washed with diethyl ether (3×30 mL). The aqueous layer was separated, evaporated to dryness and the product was freeze-dried from benzene-methanol (4:1, v/v).

Compound **3c** (from faster moving diastereomer **3a**). White solid (0.069 g), 85%. Anal. calcd for $C_{25}H_{30}N_5O_{10}PS$: C, 48.15; H, 4.85; N, 11.23. Found: C, 47.95; H 4.95; N 11.00.

^{1}H NMR. δ_H (in ppm, CD_3OD), 8.78 (m, 2H, pyr-H2, pyr-H6), 7.89 (m, 2H, pyr-H3, pyr-H5), 7.81 (d, J=1.3 Hz, 1H, H6), 7.52 (d, J=1.1 Hz, 1H, H6), 6.34 (m, 1H, H_a1′), 6.27 (t, J=6.7 Hz, 1H, H_b1′), 5.42 (m, 1H, H_a3′), 4.46–4.42 (m, 1H, H_b3′), 4.46–4.33 (m, 1H, H_b5′), 4.15–4.10 (m, 2H, 2×H4′), 3.78–3.69 (m, 2H, H_a5′), 2.63–2.58, 2.43–2.36 (2×m, 2H, H_a2′), 2.33–2.28 (m, 2H, H_b2′), 1.90 (d, J=1.1 Hz, 3H, C5–CH_3), 1.83 (d, J=1.1 Hz, 3H, C5–CH_3).

^{31}P NMR. δ_P (CD_3OD) 82.40 ppm.

Compound **3d** (from slower moving diastereomer **3b**). White solid (0.065 g), 80%. Anal. calcd for $C_{25}H_{30}N_5O_{10}PS$: C, 48.15; H, 4.85; N, 11.23. Found: C, 47.99; H 4.98; N 11.06.

^{1}H NMR. δ_H (in ppm, CD_3OD), 8.76 (m, 2H, pyr-H2, pyr-H6), 7.89 (m, 2H, pyr-H3, pyr-H5), 7.80 (d, J=0.9 Hz, 1H, H6), 7.42 (d, J=1.3 Hz, 1H, H6), 6.30 (m, 1H, H_a1′), 6.22 (t, J=6.9 Hz, 1H, H_b1′), 5.40 (m, 1H, H_a3′), 4.42–4.36 (m, 3H, H_b3′, H_b5′), 4.32 (m, 1H, H_a4′), 4.10 (m, 1H, H_b4′), 3.84 (m, 2H, H_a5′), 2.42–2.29 (2×m, 2H, H_a2′), 2.31–2.20 (m, 2H, H_b2′), 1.89 (d, J=1.1 Hz, 3H, C5–CH_3), 1.83 (d, J=1.1 Hz, 3H, C5–CH_3).

^{31}P NMR. δ_P (CD_3OD) 83.22 ppm.

4.3. General procedure for the preparation of 2-pyridylphosphonothioates 5a and 5b

To a solution of separate diastereomers of 5′-*O*-dimethoxytritylthymidin-3′-yl 3′-*O*-dimethoxytritylthymidin-5′-yl H-phosphonothioate **1** (0.26 mmol) in acetonitrile (10 mL) DBU (4 equiv.) and *N*-methoxypyridinium tosylate (1.2 equiv.) were added. *Caution*: the reagents have to be added swiftly and in the above mentioned order to ensure the efficient formation of 2-pyridylphosphonothioate **5**. After 5 min (^{31}P NMR) the reaction mixture was concentrated, partitioned between 10% aq. $NaHCO_3$ (20 mL) and CH_2Cl_2 (20 mL), and the aqueous layer was extracted with CH_2Cl_2 (2×20 mL). The organic layer was dried over anhyd Na_2SO_4, concentrated and the residue was purified by silica gel column chromatography using toluene/ethyl acetate/methanol (49:49:2, v/v/v). Purity of the isolated compounds >98% (^{1}H NMR spectroscopy).

4.3.1. 5′-*O*-Dimethoxytritylthymidin-3′-yl 3′-*O*-dimethoxytritylthymidin-5′-yl pyridyl-2-phosphonothioate 5a (from faster moving H-phosphonate diesters 1a). White solid (0.253 g), yield 79%. HRMS $[M+H]^+$ found: 1227.4070; $C_{67}H_{66}N_5O_{14}PS$ requires: 1227.4065.

^{1}H NMR. δ_H (in ppm $CDCl_3$) 8.55 and 8.48 (2×s, 2H, 2×NH), 8.43 (d, 1H, J=4.8 Hz, pyr-H6), 7.81 (m, 1H, pyr-H3), 7.71 (m, 1H, pyr-H4), 7.54 (s, 1H, H_a6), 7.45–7.15 (m, 20H, ArH, pyr-H5, H_b6), 6.83 (m, 8H, ArH *ortho* to OCH_3), 6.41–6.34 (m, 2H, 2× H1′), 5.47 (m, 1H, H_a3′), 4.34 (d, J=6.2 Hz, 1H, H_b3′), 4.14 (s, 1H, H_a4′), 4.05–3.67 (m, 2H, H_b5′), 3.85 (s, 1H, H_b4′), 3.79, 3.77 (2×s, 12H, 4× CH_3O), 3.33 (m, 2H, H_a5′), 2.45–2.31 (m, 2H, H_a2′), 2.07–1.87 (m, 2H, H_b2′), 1.73 (s, 3H, C_b5–CH_3), 1.43 (s, 3H, C_a5–CH3).

^{31}P NMR. δ_P ($CDCl_3$) 78.15 ppm.

^{13}C NMR. δ_C (in ppm, $CDCl_3$) 163.86 and 163.78 (2×C4), 158.91 (4C of DMT), 154.55 (d, J=192 Hz, pyr-C2), 150.49, 150.43, 150.20 (2×C2, pyr-C6), 145.04 and 144.30 (2C of DMT), 136.51 (d, J=13.0 Hz, pyr-C4), 136.25 and 136.44 (2C of DMT), 136.00 (Cb6), 135.34 (2C of DMT), 135.21 (C_a6), 130.31, 130.18, 128.40, 128.22, 128.18 (16C of DMT), 127.32 (2C of DMT), 127.04 (d, J=29.8 Hz, pyr-C3), 126.28 (pyr-5H), 113.58 and 113.46 (8C of DMT), 111.75 and 111.15 (2×C5), 87.57 and 87.29 (2×C DMT), 85.80 (C_b1′), 84.80 (C_a4′), 84.52 (C_a1′, C_b4′) 78.50 (C_a3′), 74.91 (C_b3′), 67.02 (C_b5′), 63.44 (C_a5′), 55.40 (4×CH_3O), 39.42 (C_a2′, C_b2′), 12.34 (C_b5–CH_3), 11.82 (C_a5–CH_3).

4.3.2. 5′-*O*-Dimethoxytritylthymidin-3′-yl 3′-*O*-dimethoxytritylthymidin-5′-yl pyridyl-2-phosphonothioate 5b (from slower moving H-phosphonate diesters 1b). White solid (0.255 mg), yield 80%. HRMS $[M+H]^+$ found: 1227.4067; $C_{67}H_{66}N_5O_{14}PS$ requires: 1227.4065.

^{1}H NMR. δ_H (in ppm, $CDCl_3$) 8.58 (d, 1H, J=4.4 Hz, pyr-H6), 8.35, 8.24 (2×s, 2H, 2×NH), 7.94 (m, 1H, pyr-H3), 7.79 (m, 1H, pyr-H4), 7.56 (s, 1H, H_a6), 7.41–7.17 (m, 20H, ArH, pyr-H5, H_b6), 6.82 (m, 8H, ArH *ortho* to OCH_3), 6.43–6.33 (m, 2H, 2×H1′), 5.46 (m, 1H, H_a3′), 4.23 (d, J=5.6 Hz, 1H, H_b3′), 4.15 (s, 1H, H_a4′), 3.79–3.69 (m, 3H, H_b4′, H_b5′), 3.79, 3.76 (2×s, 12H, 4×CH_3O), 3.41 (m, 2H, H_a5′), 2.44–2.25 (m, 2H, H_a2′), 1.93–1.63 (m, 2H, H_b2′), 1.76 (s, 3H, C_b5–CH_3), 1.56 (s, 3H, C_a5–CH_3).

^{31}P NMR. δ_P ($CDCl_3$) 79.29 ppm.

^{13}C NMR. δ_C (in ppm, $CDCl_3$) 163.71 (2×C4), 158.93 and 158.87 (4C of DMT), 154.62 (d, J=192 Hz, pyr-C2), 150.51, 150.38, 150.27 (2×C2, pyr-C6), 145.02 and 144.25 (2C of DMT), 136.58 (d, J=13.0 Hz, pyr-C4), 136.22 and 136.13 (2C of DMT), 135.88 (C_b6), 135.34 (2C of DMT), 135.17 (C_a6), 130.34, 130.28, 130.21, 128.35, 128.22 (16C of DMT), 127.37, 127.27 (2C of DMT), 127.33 (d, J=31.3 Hz, pyr-C3), 126.45 (pyr-5H), 113.52 (8C of DMT), 111.65 and 111.37 (2×C5), 87.52 and 87.41 (2×C DMT), 85.17 (d, J=4.6 Hz, C_a4′), 84.98 (C_b1′), 84.60 (C_a1′), 84.42 (d, J=8.4 Hz, C_b4′), 78.67 (C_a3′), 74.62 (C_b3′), 66.65 (C_b5′), 63.20 (C_a5′), 55.41 and 55.38 (4×CH_3O), 39.25 (C_a2′, C_b2′), 12.35 (C_b5–CH_3), 11.81 (C_a5–CH_3).

4.3.3. Thymidin-3′-yl thymidin-5′-yl pyridyl-2-phosphonothioate 5c and 5d. Separate diastereomers of 2-pyridylphosphonothioates **5a** and **5b** were deprotected analogously as it was described above to the 4-pyridyl derivatives **3a** and **3b**.

Compound **5c** (from faster moving diastereomer **5a**). White solid (0.068 g), yield 84%. Anal. calcd for $C_{25}H_{30}N_5O_{10}PS$: C, 48.15; H, 4.85; N, 11.23. Found: C, 48.03; H 4.99; N 11.11.

^{1}H NMR. δ_H (in ppm CD_3OD) 8.76 (d, 1H, J=4.4 Hz, pyr-H6), 8.12 (m, 1H, pyr-H3), 7.99 (m, 1H, pyr-H4), 7.82, 7.67 (2×s, 2H, 2×6), 7.60 (m, 1H, pyr-H5), 6.36–6.27 (m, 2H, 2×H1′), 5.43 (m, 1H, H_a3′), 4.52–4.39 (m, 3H, H_b3′, H_b5′), 4.22 (s, 1H, H_a4′), 4.15 (s, 1H, H_b4′,), 3.80 (m, 2H, H_a5′), 2.61–2.35 (m, 2H, H_a2′), 2.33–2.22 (m, 2H, H_b2′), 1.89, 1.80 (2× s, 6H, 2× C5–CH_3).

^{31}P NMR. δ_P ($CDCl_3$) 78.81 ppm.

Compound **5d** (from slower moving diastereomer **5b**). White solid (0.065 g), yield 80%. Anal. calcd for $C_{25}H_{30}N_5O_{10}PS$: C, 48.15; H, 4.85; N, 11.23. Found: C, 47.99; H 4.93; N 11.05.

^{1}H NMR. δ_H (in ppm, CD_3OD) 8.77 (d, 1H, J=4.4 Hz, pyr-H6), 8.15 (m, 1H, pyr-H3), 7.99 (m, 1H, pyr-H4), 7.82, 7.65 (2×s, 2H, 2×6), 7.60 (m, 1H, pyr-H5), 6.36–6.28 (m, 2H, 2×H1′), 5.42 (m, 1H, H_a3′), 4.55–4.34 (m, 2H, H_b5′), 4.47 (m, 2H, H_b3′), 4.31 (m, 1H, H_a4′), 4.15 (s, 1H, H_b4′), 3.85 (m, 2H, H_a5′), 2.50–2.27 (m, 2H, H_a2′), 2.24–2.19 (m, 2H, H_b2′), 1.89, 1.83 (2×s, 6H, 2×C5–CH_3).

^{31}P NMR. δ_P ($CDCl_3$) 79.26 ppm.

Acknowledgements

We are indebted to Professor P. J. Garegg for his interest in this work. Financial support from the Swedish Natural Science Research Council and the Swedish Foundation for Strategic Research is gratefully acknowledged.

References and notes

1. Redmore, D. (Petrolite Corp.). U.S. 3,673,196, 1972.
2. Ruile, S.; Kohle, O.; Pechy, P.; Grätzel, M. *Inorg. Chim. Acta* **1997**, *261*, 129–140. Grätzel, M.; Kohle, O.; Nazeeruddin, M. K.; Pechy, P.; Rotzinger, F. P.; Ruile, S.; Zakeeruddin, S. M. PCT Int. Appl. 9,529,924, Ecole Polytechnique Federale de Lausanne (epfl), Switz., 1995.
3. Redmore, D. (Petrolite Corp.). U.S. 3,775,057, 1973.
4. Redmore, D. (Petrolite Corp.) U.S. 3,810,907, 1974.
5. Protopopova, G. V.; Reidalova, L. I.; Pavlenko, A. F.; Sologub, L. S.; Kukhar, V. P.; Petrenko, V. S. *Fiziol. Akt. Veshchestva* **1980**, *12*, 16–18.
6. Kukhar, V. P.; Cherepenko, T. I.; Pavlenko, A. F. *Dokl. Akad. Nauk Ukr. RSR, Ser. B: Geol., Khim. Biol. Nauki* **1982**, 60–63. Akiba, K.; Tsuzuki, K. (Toyo Soda Mfg. Co., Ltd, Japan). Jpn Kokai Tokkyo Koho 61,221,102, 1986..

7. Malhotra, S. K.; Evoy, I. L. (Dow Chemical Co., USA). Dow Chemical Co. 4,606,757, 1986.
8. Murray, K. J.; Porter, R. A.; Prain, H. D.; Warrington, B. H. (Smith Kline and French Laboratories Ltd, UK). PCT Int. Appl. 9,117,987, 1991.
9. Erion, M. D.; Vanpoelje, P. (Metabasis Therapeutics, Inc., USA). PCT Int. Appl. 0,038,666, 2000. Dang, Q.; Kasibhatla, S. R.; Reddy, K. R.; Erion, M. D.; Reddy, M. R.; Agarwal, A. (Metabasis Therapeutics, Inc., USA). PCT Int. Appl. 0,014, 095, 2000.
10. Murray, K. J.; Porter, R. A.; Warrington, B. H.; Lahouratate, P. (SmithKline Beckman Corp., UK Smithkline Beecham Laboratoires Pharmaceutiques). PCT Int. Appl. 9,319,754, 1993.
11. Kafarski, P.; Lejczak, B. *Phosphor. Sulfur Silicon* **1991**, *63*, 193–215.
12. Bergin, C.; Hamilton, R.; Walker, B.; Walker, B. J. *Chem. Commun.* **1996**, 1155–1156.
13. Chen, R. Y.; Mao, L. J. *Phosphor. Sulfur Silicon* **1994**, *89*, 97–104.
14. Kers, A.; Stawinski, J. *Tetrahedron Lett.* **1999**, *40*, 4263–4266.
15. Johansson, T.; Stawinski, J. *Chem. Commun.* **2001**, 2564–2565.
16. Johansson, T.; Kers, A.; Stawinski, J. *Tetrahedron Lett.* **2001**, *42*, 2217–2220.
17. Huang, Y. S.; Chaudret, B.; Bellan, J.; Mazieres, M. R. *Polyhedron* **1991**, *10*, 2229–2233. Speca, A. N.; Pytlewski, L. L.; Karayannis, N. M. *J. Inorg. Nucl. Chem.* **1972**, *34*, 3671–3679.
18. Häner, R.; Hall, J.; Pfützer, A.; Hüsken, D. *Pure Appl. Chem.* **1998**, *70*, 111–116. Konevetz, D. A.; Beck, I. E.; Beloglazova, N. G.; Sulimenkov, I. V.; Silnikov, V. N.; Zenkova, M. A.; Shishkin, G. V.; Vlassov, V. V. *Tetrahedron* **1999**, *55*, 503–512.
19. Barton, J. K. *Pure Appl. Chem.* **1998**, *70*, 873–879. Kelley, S. O.; Jackson, N. M.; Hill, M. G.; Barton, J. K. *Angew. Chem., Int. Ed.* **1999**, *38*, 941–945. Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253–260.
20. Pitt, L. S.; Large, G. B.; MacDonald, A. A. (Stauffer Chemical Co., USA). Ger. Offen. 2,738,194, 1978.. Gray, R. A.; Hyzak, D. L. (Stauffer Chemical Co., USA). U.S. 4,652,302, 1987.
21. Eliseenkov, V. N.; Bureva, N. V.; Ivanov, B. E. *Khim. Geterotsikl. Soedin.* **1974**, 1354–1356. Haase, M.; Gunther, W.; Gorls, H.; Anders, E. *Synthesis* **1999**, 2071–2081.
22. Onys'ko, P. P.; Suvalova, E. A.; Chudakova, T. I.; Sinitsa, A. D. *Zh. Obshch. Khim.* **1994**, *64*, 610–612.
23. Zain, R.; Stawinski, J. *J. Org. Chem.* **1996**, *61*, 6617–6622.
24. Connolly, B. A.; Potter, B. V. L.; Eckstein, F.; Pingoud, A.; Grotjahn, L. *Biochemistry* **1984**, *23*, 3443–3453.
25. Bollmark, M.; Stawinski, J. *Chem. Commun.* **1997**, 991–992.
26. After 1 h, the reaction mixture consisted of intermediate **2** (21%), 4-pyridylphosphonothioate **3** (61%) and a dinucleoside phosphorothioate diester (18%).
27. Stawinski, J.; Strömberg, R.; Zain, R. *Tetrahedron Lett.* **1992**, *33*, 3185–3188.
28. Stawinski, J.; Thelin, M. *Tetrahedron Lett.* **1992**, *33*, 3189–3192.
29. Katritzky, A. *J. Chem. Soc.* **1956**, 2404–2408. Feely, W.; Lehn, W. L.; Boekelheide, V. *J. Org. Chem.* **1957**, *22*, 1135. Coats, N. A.; Katritzky, A. *J. Org. Chem.* **1959**, *24*, 1836–1837.
30. This assignment was supported by an independent synthesis of methyl 5′-*O*-dimethoxytritylthymidin-3′-yl and methyl 3′-*O*-dimethoxytritylthymidin-5′-yl 2-pyridylphosphonothioates, and their comparison with the side products formed in the investigated reaction.
31. The amount of DBU used in this reaction (4 equiv.) was also critical for efficient formation of pyridylphosphonothioates **5**. When the reaction of H-phosphonothioate **1** was carried out with 1.2 equiv. of *N*-methoxypyridinium *p*-toluenesulfonate in the presence of only 2 equiv. of DBU, complex reaction mixture was formed (pyridylphosphonate **5**, ca. 70% methyl nucleoside 2-pyridylphosphonothioates, ca. 4% dinucleoside hydroxymethylphosphonates, ca. 18% and 4-pyridylphosphonothioates **3**, ca. 8%).
32. The reaction was carried out in acetonitrile using H-phosphonothioate **1**, 2 equiv. of *N*-methoxypyridinium *p*-toluenesulfonate and 4 equiv. of triethylamine (rt, 5 min). Further studies on regioselectivity in this type of reactions are in progress.
33. Katritzky, A. R.; Lunt, E. *Tetrahedron* **1969**, *25*, 4291–4305.
34. Stawinski, J.; Thelin, M. *J. Org. Chem.* **1994**, *59*, 130–136. Stawinski, J.; Thelin, M.; Zain, R. *Tetrahedron Lett.* **1989**, *30*, 2157–2160.