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Aryl H-Phosphonates. 10. Synthesis of Nucleoside Phosphoramidate and Nucleoside Phosphoramidothioate Analogues *via* H-Phosphonamidate Intermediates

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Abstract: Synthesis of a new type of dinucleoside monophosphate analogue with the 3'-5' H-phosphonamidate internucleoside linkage, namely thymidin-3'-yl N(thymidin-5'-yl)-H-phosphonamidate **4**, was achieved *via* the reaction of a suitably protected nucleoside aryl H-phosphonate with a 5'-amino-5'-deoxythymidine derivative. The usefulness of **4** as an intermediate for the preparation of various nucleotide analogues was assessed by converting it into the corresponding dinucleoside phosphoramidate and dinucleoside phosphoramidothioate derivatives bearing the P–N bond in the bridging position of the phosphoramidate linkage.

INTRODUCTION

Chemical modifications at the phosphorus center have been used for years as an efficient tool in investigations of mechanisms of enzymatic reactions[1] and to produce compounds with improved biological properties.[2] The latter aspect became of crucial importance with the advent of the antisense[3] and antigene[4] therapies based on synthetic oligonucleotide analogues, where nuclease resistance, cellular uptake and pharmacokinetic behaviour in organisms of a prospective drug could be modulated by replacing oxygen atoms at the phosphorus center by various heteroatoms, *e.g.* carbon, sulfur, nitrogen, selenium.[5]

Due to advances in phosphorus chemistry of P(III) derivatives,[6,7] the introduction of heteroatoms at non-bridging positions of the phosphodiester bond can be efficiently achieved *via* oxidation of the corresponding phosphite triester or H-phosphonate intermediates. In contrast to these, modifications involving bridging positions of the phosphorus center usually require more elaborated synthetic methods, and probably due to this, such phosphate analogues are less frequently used in biological studies.[8-10] Encouraged by recent reports on favourable antisense properties of oligonucleoside phosphoramidates[11-14] with the nitrogen atom in a bridging

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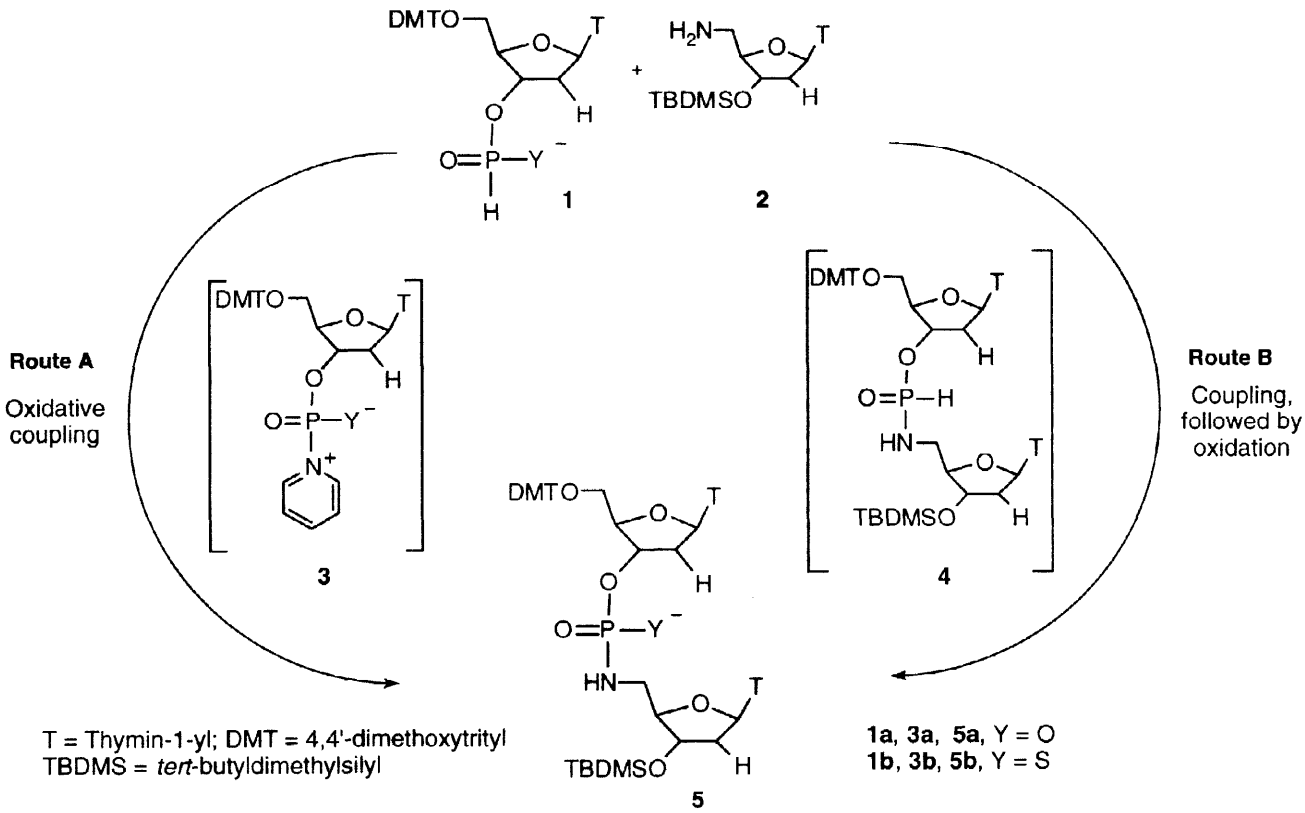
position of the phosphoramidate internucleoside linkage, we recently embarked on studies directed towards the preparation of this type of nucleotide analogues using H-phosphonate chemistry.[15,16]

For the synthesis of P3'–>N5' phosphoramidate analogues **5**, we designed two routes that use the same starting materials but differ in the method of internucleosidic bond formation (Scheme 1). Route A consists of oxidative coupling of H-phosphonate monoester **1a** or H-phosphonothioate **1b** with aminonucleoside **2** to produce, with intermediacy of a pyridinium adduct of metaphosphate **3**, phosphoramidate analogues **5**.[17] Route B, on the other hand, involves as a key intermediate a new P(III) nucleotide analogue **4** with H-phosphonamidate internucleotide linkage, that can also be considered as a starting material for the preparation of other modified nucleic acids fragments (*e.g.* **5**).

Scheme 1



In this paper we present our synthetic and ^{31}P NMR studies directed towards the development of an efficient method for the synthesis of dinucleoside H-phosphonamidate **4** and its conversion to the corresponding phosphoramidate and phosphoramidothioate analogues **5** bearing the P–N bond in a bridging position of the phosphoramidate internucleotide linkage.

RESULTS AND DISCUSSION

The non-oxidative formation of phosphorus-nitrogen bond using H-phosphonate chemistry seems to be a more challenging task than that of the phosphorus-oxygen one (*e.g.* synthesis of H-phosphonate diesters), as is apparent from our previous studies on the reactions of H-phosphonate derivatives with amino alcohols.[18,19] In a

condensing agent-promoted reaction, the hydroxyl function of amino alcohols was found to be a significantly better nucleophile than the amino group, resulting in almost exclusive formation of derivatives containing P–O rather than P–N bonds.[18,19] Attempted condensations of H-phosphonate monoester **1a** with amines in the presence of pivaloyl chloride (a standard coupling reagent for the synthesis of H-phosphonate diesters), invariably led to low yields (*ca* 10%) of the desired nucleoside H-phosphonamidates.[15] We found that most of the problems connected with the use of coupling agents for the formation of H-phosphonamidates can be alleviated by using aryl H-phosphonates as reactive intermediates.[15] The method consists of the *in situ* formation of a nucleoside aryl H-phosphonate diester, followed by its reaction with primary or unhindered secondary amines and usually affords nucleoside N-alkyl-H-phosphonamidates in good yields.[15]

Although aminonucleosides of type **2** resemble aliphatic amines, they usually react more slowly and cannot be used for the reaction in such excess as for simple aliphatic examples. This called for the development of a new synthetic protocol that would eliminate side reactions, which were tolerable in the instance of simple aliphatic amines, *e.g.* a partial N-acylation or N-phosphorylation of amines by residual condensing agents, *etc.* For this purpose synthetic and ^{31}P NMR studies related to the formation of H-phosphonamidate **4** were undertaken.

Preliminary experiments showed that aminonucleoside **2** (1-2 equiv.), when reacted with the *in situ* produced nucleoside aryl H-phosphonate **6** (*vide infra*), did not afford any detectable amounts of H-phosphonamidate **4**. This we assumed was due to protonation of the amino function of **2**, since the addition of triethylamine (TEA, 5 equiv.) triggered the formation of the expected product **4**.[20] For this reason, in all subsequent experiments involving aminolysis of **6** we used aminonucleoside **2** (1-2 equiv.) in combination with TEA.

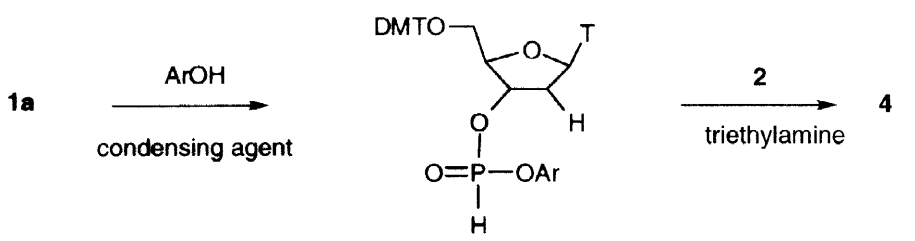
Due to high reactivity, nucleoside aryl H-phosphonates **6**, to be used for the aminolysis with **2**, were always produced *in situ* in methylene chloride – pyridine (9:1, v/v) from nucleoside H-phosphonate **1a** and the corresponding phenol (1.2 - 2 equiv.) using various condensing agents, *i.e.* pivaloyl chloride (Pv-Cl; 1.1 equiv), 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane (NEP-Cl; 2.5 equiv.), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (OXP-Cl; 1.0 equiv.), or diphenyl phosphorochloridate (DPCP; 1.0 equiv.). Although all these reactions rather cleanly produced aryl H-phosphonates **6**, the composition of reaction mixtures resulting from the addition of aminonucleoside **2**, differed significantly, depending on what type of condensing agent was used for the generation of **6**.

The reaction of aminonucleoside 2 with nucleoside aryl H-phosphonates 6, produced in situ with the aid of Pv-Cl

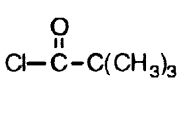
The reaction of aminonucleoside **2** with aryl H-phosphonate **6a** (produced *in situ* with Pv-Cl, *vide supra*) was fast (*ca* 10 min) and produced the desired H-phosphonamidate **4** as a major product (*ca* 70%), although variable amounts of the starting material **1a** could always be detected in the reaction mixture (^{31}P NMR

experiment). This could, in principle, be explained by a partial hydrolysis of **6** due to adventitious water introduced with aminonucleoside **2**, but isolation from the reaction mixture of 5'-N-pivaloylated nucleoside **8** (14%) pointed to another mechanism that simultaneously generated **1a** and **8** under the reaction conditions. Previously, we have observed that nucleoside aryl H-phosphonates of type **6** can be involved in an equilibrium with pivaloate anion,[21] that may produce detectable amounts a pivalic-phosphonic mixed anhydride (*e.g.* **7**). Assuming, that the mixed anhydride **7** can react with amines with incomplete chemoselectivity, attack of the amino group of **2** on the carbonyl center in **7** would result in the formation of both **1a** and **8**. In agreement with this, the reaction of aminonucleoside **2** (1 equiv.) with mixed anhydride **7** [generated from **1a** and a limited amount of Pv-Cl (0.9 equiv.)],[22] in the presence of TEA (5 equiv.), also produced the N-pivaloylated nucleoside **8**[23] (isolated in 16% yield) along with **4**.

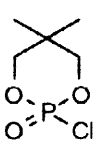
Scheme 2



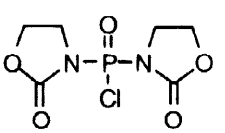
6a, Ar= 2,4,6-trichlorophenyl
6b, Ar = phenyl



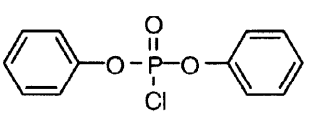
Pv-Cl



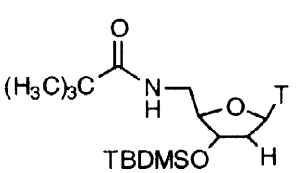
NEP-Cl



OXP-Cl



DPCP



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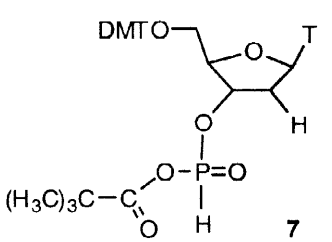

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Attempted synthesis of **4** using less reactive aryl H-phosphonate **6b**, did not result in the anticipated suppression of acylation of **2**. Although in this instance the putative equilibrium with the mixed anhydride **7** should be shifted more towards the nucleoside phenyl H-phosphonate **6b**, lower rate of aminolysis with **2** actually resulted in the increased formation of the N-pivaloylated product **8** (isolated in 29%). Using more phenol (5 equiv.) for the reaction to form **6**, did not give satisfactory results either.[24]

The reaction of aminonucleoside 2 with nucleoside aryl H-phosphonates 6, produced in situ with the aid of chlorophosphates

To avoid the problems associated with the use of Pv-Cl, we decided to try chlorophosphates as condensing agents for the formation of aryl H-phosphonates **6**. To minimise the side-reaction of aminonucleoside **2** with chlorophosphates (or with the corresponding pyrophosphates produced during the course of condensation), sterically hindered 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane (NEP-Cl), was chosen.

The addition of aminonucleoside **2** (2 equiv.) and TEA (5 equiv.) to the *in situ* produced **6a** (*vide supra*) furnished fast and clean formation of the desired H-phosphonamidate **4** (>90%, ^{31}P NMR). The less reactive phenyl H-phosphonate **6b** also reacted smoothly with aminonucleoside **2** (2 equiv.) in the presence of TEA (5 equiv.) to produce the desired product **4**. In these reactions we usually observed formation of some (*ca* 10%) nucleoside H-phosphonate **1a**, and this tendency was more pronounced (*ca* 20% of **1a**) when only 1 equiv. of **2** was used for the aminolysis. Since the presence of molecular sieves decreased the formation of **1a** (<10%) we attributed the generation of **1a** during the course of aminolysis to adventitious water.

Although the formation of **4** was rather clean, the reaction mixtures always contained significant amounts of bis-neopentylene pyrophosphate (formed during generation of **6**), which was difficult to remove during work-up. Silica gel chromatography turned out to be of little help as H-phosphonamidate **4** underwent partial decomposition on the column.[25] For these reasons we searched for another condensing agent that would not only provide efficient formation of **4**, but also enable purification of the product *via* aqueous extraction.

To this end we investigated two chlorophosphates, bis(2-oxo-3-oxazolidinyl)phosphinic chloride (OXP-Cl) and diphenyl phosphorochloridate (DPCP). Both of them showed less tendency than NEP-Cl to form the corresponding pyrophosphates,[26] and the phosphate anions produced were hydrophilic enough to be removed from the reaction mixtures *via* an aqueous extraction. They also showed a similar efficiency (practically quantitative yields) in terms of the H-phosphonamidate **4** produced.[27] However, due to better solubility in organic solvents and higher efficiency in promoting formation of **6** in the presence of just a few equivalents of pyridine, the choice of OXP-Cl *vs* DPCP, was resolved in favour of the latter one.

To suppress hydrolysis of aryl H-phosphonate **6** by adventitious water (and thus increase the yield of **4**), we attempted to carry out the aminolysis with **2** in the presence of trimethylsilyl chloride (TMS-Cl). The addition of TMS-Cl (2 equiv.) and TEA (7 equiv.) to aryl H-phosphonate **6a** produced the corresponding silyl phosphite, which upon addition of aminonucleoside **2** (1 equiv) afforded H-phosphonamidate **4**. The reaction was fast and clean (^{31}P NMR). Aryl H-phosphonate **6b**, bearing a less reactive phenyl group, under analogous reaction conditions produced only traces of H-phosphonamidate **4** overnight.

Optimisation of the reaction conditions led to the protocol (see the Experimental Part) that produced dinucleoside H-phosphonamidate **4** as chromatographically and ^{31}P NMR homogeneous species, after aqueous work-up.

Studies on oxidative transformations of H-phosphonamidate 4 to produce nucleotide analogues 5

The synthetic utility of dinucleoside H-phosphonamidate **4** as starting material for the preparation of other nucleotide analogues was assessed by subjecting it to oxidation under various experimental conditions. In all these reactions H-phosphonamidate **4** purified by aqueous extraction was used[28] (see the Experimental Part). Comparing to dinucleoside H-phosphonate diesters,[29,30] oxidation of **4** was found to be significantly slower, although clean. In pyridine-water (98:2, v/v) in the presence of TEA (5 equiv.) it took almost 1 h to convert **4** into dinucleoside phosphoramidate **5a** (isolated in 86%, calculated on **2**), while H-phosphonate diesters usually afford the corresponding phosphate in less than 1 min under these conditions. This could be connected with lower acidity of H-phosphonamidates, which can make the formation of the corresponding phosphite anion more difficult.

To enhance the susceptibility of H-phosphonamidate **4** to oxidation, we converted it into the corresponding trimethylsilyl phosphite. Unfortunately, in contrast to dialkyl silyl phosphites, which upon treatment with iodine in aqueous pyridine immediately afford the corresponding phosphates,[29,30] the silyl phosphite obtained from **4** underwent fast hydrolysis to produce the parent nucleotide **4**. However, a stepwise addition of iodine to the silyl phosphite derived from **4**, followed by treatment with aqueous pyridine, furnished fast (less than 5 min) and clean formation of **5a** (^{31}P NMR experiment; isolated yield *ca* 85%).

Sulfurization of **4** in pyridine with elemental sulfur (3 equiv.) in the presence of TEA (5 equiv.) produced (*ca* 3.5 h) dinucleoside phosphoramidothioate **5b** in total 78% yield (after silica gel chromatography). The analogous reaction in the presence of DBU (1.5 equiv) went to completion within 5 min giving product **5b** in 77% yield. Also the trimethylsilyl phosphite derivative, formed *in situ* from **4** and TMS-Cl in the presence of TEA, afforded quickly (less than 5 min) and cleanly dinucleoside phosphoramidothioate **5b** (^{31}P NMR experiment; isolated yield *ca* 80%).

In conclusion, we have synthesised dinucleoside H-phosphonamidate **4**, which can be perceived both as a new P(III) nucleotide analogue and a possible starting material for the preparation of other phosphate analogues, *e.g.* **5a**, **5b**. Due to mildness of the reaction conditions, the synthetic protocol developed can be considered as a general method for the preparation of natural product analogues with the P–N bond in the bridging position of the phosphoramidate linkage. Further studies on chemical properties of H-phosphonamidates of type **4** are in progress in our laboratories.

EXPERIMENTAL PART

Reactions were carried out in 10-mm NMR tubes and spectra were recorded on a Jeol GSX-270 FT or Varian 300 or 400 MHz spectrometer. For ^{31}P NMR experiments 2% H_3PO_4 in D_2O was used as an external standard (coaxial inner tube). The values of the chemical shifts for the intermediates produced *in situ*, varied (±1 ppm) in some experiments, depending on the reaction conditions. The assignment of proton and carbon

resonances was done on the basis of known or expected chemical shifts in conjunction with ^1H-^1H and ^1H-^{13}C correlated NMR spectroscopy. Multiplicity of some signals was due to diastereomeric mixtures of isolated products **4** and **5b**. Some resonances have not been resolved and they are listed as a group of signals.

Pyridine (Merck, distilled from CaH_2) was stored over molecular sieves (4Å). Methylene chloride was distilled from CaH_2 before use, and pivaloyl chloride and DBU (Aldrich) were freshly distilled. Acetonitrile and 2-oxo-3-oxazolidinyl)phosphinic chloride (OXP-Cl) were commercial grade from Lancaster. 5'-O-dimethoxytritylthymidine 3'-H-phosphonates **1a**,[31] 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane (NEP-Cl)[32] and 5'-amino-5'-deoxy-3'-O-*tert*-butyldimethylsilylthymidine[33,34] were prepared according to published procedures. The reference compound **8**, 5'-amino-5'-deoxy-5'-N-pivaloyl-3'-O-*tert*-butyldimethylsilylthymidine, was prepared by reacting 5'-amino-5'-deoxy-3'-O-*tert*-butyldimethylsilylthymidine with pivaloyl chloride (1.5 equiv.) in pyridine, followed by silica gel chromatography purification. Compounds **5a** and **5b** obtained from H-phosphonamidate **4** (see below) were identical to those prepared by another route.[16]

Preparation of 5'-O-dimethoxytritylthymidin-3'-yl N(3'-tert-butyldimethylsilyl-thymidin-5'-yl)-H-phosphonamidate 4

5'-O-Dimethoxytritylthymidine 3'-H-phosphonate **1a** ($TEAH^+$ salt, 0.390 g, 0.55 mmol) and 2,4,6-trichlorophenol (0.118 g, 0.60 mmol) were rendered anhydrous by evaporation of added pyridine, dissolved in methylene chloride (5 mL) containing pyridine (81 µL, 1.0 mmol), and then diphenyl chlorophosphate (104 µl, 0.5 mmol) was added. When the condensation was completed (*ca* 30 min, TLC analysis[35]) trimethylsilyl chloride (127 µL, 1.5 mmol) and triethylamine (488 µL, 3.5 mmol) were added. After 5 min, 5'-amino-5'-deoxy-3'-O-*tert*-butyldimethylsilylthymidine **2** (previously dried by evaporation of added pyridine) (0.178 g, 0.5 mmol) in pyridine (3 mL) was transferred to the reaction vessel *via* cannulation using argon. When the aminolysis was completed (*ca* 20 minutes, TLC analysis), the reaction mixture was diluted with methylene chloride (30 mL) and washed repeatedly with 0.5 M $NaHCO_3$ (5 x 30 mL). The organic layer was dried over Na_2SO_4, the solvent removed under vacuum, and the residue was coevaporated with a toluene - acetonitrile mixture (1:1, v/v). Crude **4** (purity >95%) was obtained as a foam in quantitative yield. **HRMS** $[M + Na]^+$, found 968.3636. $C_{47}H_{60}N_5O_{12}PSiNa$ requires 968.3643.

^1H NMR (δ in ppm, $CDCl_3$) 7.62-7.01 & 6.89-6.77 (m, 15H, 2 x H6 & ArH) 7.03 & 7.07 (2d, J=651.1 Hz, 650.3, 1H, PH), 6.47 (m, 1H, H_a1'), 5.78 (m, 1H, H_b-1'), 5.21 (m, 1H, H_a3'), 4.59-3.08 (m, 14H, H_b3', 2 x H4', NH, 2 x CH_3O, 2 x H5' & H5"), 2.86-2.12 (m, 4H, 2 x H2' & H-2"), 1.88 (s, 3H, $C5_a$-CH_3), 1.33 &1.37 (2s, 3H, $C5_b$-CH_3), 0.88 (m, 9H, $(CH_3)_3C$), 0.08 (m, 6H, 2 x CH_3Si).

^{31}P NMR (δ in ppm, $CDCl_3$) 14.17 & 14.08, (m with $^1J_{PH}$=643 Hz).

^{13}C NMR (δ in ppm, $CDCl_3$), 164.56-164.43 (2 x C4), 151.23-150.79 (2 x C2), 158.88, 158.77, 144.35 (DMT), 138.26-135.08 (2 x C6 & 2C of DMT), 130.28-127.42 & 113.74-113.39 (13C of DMT), 112.04-111.23 (2 x C5), 88.72-84.41 (2 x C4', 2 x C1', C^{DMT}), 75.87-72.20 (2 x C3'), 63.65, 45.94 (2 x C5'), 55.56 (2 x CH_3O),

41.93-39.73 (2 x C2'), 26.10 (3 x $CH_3{}^{t\text{-Bu}}$), 18.28 & 18.27 ($C^{t\text{-Bu}}$), 12.76 &12.12 (2 x C5-CH_3), -4.17, -4.20, -4.34, -4.36 (2 x CH_3Si).

Preparation of 5'-O-dimethoxytritylthymidin-3'-yl N(3'-tert-butyldimethylsilyl-thymidin-5'-yl)phosphoramidate, triethylammonium salt 5a

To H-phosphonamidate **4** (0.5 mmol) dissolved in pyridine (10 mL), triethylamine (348 μL, 2.5 mmol), water (200 μL) and iodine (0.190 g, 0.75 mmol) were added, and the reaction mixture was stirred at ambient temperature. When the reaction was finished (ca 1 hour, TLC analysis), the mixture was concentrated in vacuum, the residue dissolved in methylene chloride (20 mL) and extracted with 10% $Na_2S_2O_3$ in brine (30 mL). The aqueous layer was extracted with methylene chloride (3 x 30 mL), the combined organic phase was washed with 1 M TEAB (pH 7.0, 50 mL), dried over Na_2SO_4 and the solvent evaporated. The residue was coevaporated with added toluene and chromatographed on a silica gel column using a stepwise gradient of methanol (0-5%) in methylene chloride containing 0.5% TEA to afford **5a** as a foam (0.46 g, 86% yield). Purity > 98% (^{1}H NMR spectroscopy). **HRMS** $[M]^-$, found 960.3620. $C_{47}H_{59}N_5O_{13}PSi$ requires 960.3616.

^{1}H NMR (δ in ppm, $CDCl_3$) 12.60 (s, 1H, $(CH_3CH_2)_3N$-$\underline{H}^+$), 9.72 (s, 1H, NH), 9.51 (s, 1H, NH), 7.62-7.15 & 6.85-6.74 (m, 15H, 2 x H6 & ArH), 6.37 (dd, J=5.6 & 8.0 Hz, 1H, H_a-1'), 6.22 (t, J=6.8 Hz, 1H, H_b-1'), 4.93 (m, 1H, H-3'$_a$), 4.30 (m, 2H, H_a-4', H_b-3'), 3.87-2.91 (m, 19H, H_b-4', NH, 2 x CH_3O, 2 x H-5' & H-5'', $(CH_3\underline{CH_2})_3N$-$H^+$), 2.71-2.01 (m, 4H, 2 x H-2' & H-2''), 1.84 (s, 3H, C5-CH_3), 1.29 (m, 12H, $(\underline{CH_3}CH_2)_3N$-$H^+$, C5-$CH_3$), 0.86 (s, 9H, $(CH_3)_3C$) , 0.06 (s, 6H, 2 x CH_3Si).

^{31}P NMR (δ in ppm, $CDCl_3$) 8.33.

^{13}C NMR (δ in ppm, $CDCl_3$) 164.70 (2 x C4), 158.69, 144.47, 135.52 (5C of DMT), 151.09 (2 x C2), 135.72 (2 x C6), 130.30, 128.42, 128.00, 127.13, 113.34 (13C of DMT), 111.10 & 111.05 (2 x C5), 87.70 (d, J=30.5 Hz, C4'), 87.02 (C^{DMT}), 85.42 (d, J=17.0 Hz, C4'), 84.76 (2 x C1'), 74.57, 73.14 (2 x C3'), 64.15 & 44.31 (2 x C5'), 55.41 (2 x CH_3O), 46.13 (3 x $CH_2{}^{TEAH+}$), 40.54 & 40.15 (2 x C2'), 26.05 (3 x $CH_3{}^{t\text{-Bu}}$), 18.17 ($C^{t\text{-Bu}}$), 12.85 &12.02 (2 x C5-CH_3), 10.99 (3 x $CH_3{}^{TEAH+}$), -4.34 (2 x CH_3Si).

Preparation of 5'-O-dimethoxytritylthymidin-3'-yl N(3'-tert-butyldimethylsilyl-thymidin-5'-yl)phosphoramidothioate, triethylammonium salt 5b

To a stirred solution of H-phosphonamidate **4** (0.5 mmol) in pyridine (10 mL) was added elemental sulfur (48 mg, 1.5 mmol) and TEA (348 μL, 2.5 mmol). Upon completion of the reaction (*ca* 3.5 h, TLC analysis) the volatile components were removed in vacuum, the residue coevaporated with toluene, and partitioned between methylene chloride (4 x 30 mL) and saturated $NaHCO_3$ (30 mL). The combined organic phase was washed with 1 M TEAB (pH 7.0, 50 mL), dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography using methylene chloride containing 3% methanol and 0.1% TEA to afford the title compound as a foam (0.42 g, 78% yield). Purity > 98% (^{1}H NMR spectroscopy). **HRMS** $[M]^-$, found 976.3692. $C_{47}H_{59}N_5O_{12}PSSi$ requires 976.3688.

^{1}H NMR (δ in ppm, $CDCl_3$) 11.98 (s, 1H, $(CH_2)_3N$-$\underline{H}^+$), 9.99 (m, 2H, 2 x NH), 7.66-7.17 & 6.89 -6.78 (m, 15H, 2 x H6 & ArH), 6.39 (m, 1H, H_a-1'), 6.24 (m, 1H, H_b-1'), 5.29 (m, 1H, H_a-3'), 4.46-4.26 (m, 2H, H_a-4' & H_b-3'), 3.96-3.24 (m, 12H, H_b-4', NH, 2 x CH_3O, 2 x H-5' & H-5''), 3.13 (q, 6H, $(CH_3\underline{CH_2})_3N$-$H^+$), 2.61 (m, 1H, H_a-2'), 2.35 (m, 1H, H_a-2''), 2.23-2.10 (m, 2H, H_b-2' & H_b-2''), 1.87 &1.86 (2s, 3H, $C5_a$-CH_3), 1.30 (m, 12H, $(\underline{CH_3}CH_2)_3N$-$H^+$, $C5_b$-CH_3), 0.87 & 0.86 (2s, 9H, $(\underline{CH_3})_3C$), 0.08 (m, 6H, 2 x CH_3Si).

^{31}P NMR (δ in ppm, $CDCl_3$) 63.70 & 62.90.

^{13}C NMR (δ in ppm, $CDCl_3$) 164.11 & 164.02 (2 x C4), 158.24, 144.14, 135.48, 135.43, 135.16, 135.11 (5C of DMT), 150.58-150.49 (2 x C2), 135.84-135.52 (2 x C6), 129.89, 129.83, 127.98, 127.93, 127.72, 126.75, 113.03 (13C of DMT), 110.92, 110.72 (2 x C5), 87.35-86.94 & 85.62-84.99 (2 x C4'), 86.72 (C^{DMT}), 84.78-84.23(2 x C1'), 76.01-75.53, 72.94 & 72.66 (2 x C3'), 64.02, 44.48, 44.24 (2 x C5'), 55.01 (2 x CH_3O), 45.29 (3 x CH_2^{TEAH+}), 40.58-39.49 (C2'), 25.67 (3 x $CH_3{}^{t\text{-}Bu}$), 17.73 ($C^{t\text{-}Bu}$), 12.72-11.43 (2 x C5-CH_3), 8.61 (3 x CH_3^{TEAH+}), -(4.47-4.72) (2 x CH_3Si).

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REFERENCES AND NOTES

1. Eckstein, F.; Gish, G. *TIBS* **1989**, 97-100.
2. Zon, G. *Pharm. Res.* **1988**, *5*, 539-549.
3. De Mesmaeker, A.; Altmann, K.-H.; Waldner, A.; Wendeborn, S. *Current Opinion Struct. Biol.* **1995**, *5*, 343-355.
4. Thuong, N. T.; Helene, C. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 666-690.
5. Agrawal, S.; Zhao, Q. *Current Opinion Mol. Biol.* **1998**, *2*, 519-528.
6. Stawinski, J. Some Aspects of H-Phosphonate Chemistry. In *Handbook of Organophosphorus Chemistry;* R. Engel, Ed.; Marcel Dekker: New York, 1992; pp. 377-434.
7. Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123-6194.
8. Cosstick, R.; Vyle, J. S. *Tetrahedron Lett.* **1989**, *30*, 4693-4696.
9. Szabó, T.; Kers, A.; Stawinski, J. *Nucleic Acids Res.* **1995**, *23*, 893-900.
10. Gryaznov, S. M.; Letsinger, R. L. *Nucleic Acids Res.* **1992**, *20*, 3403-3409.
11. Gryaznov, S.; Skorski, T.; Cucco, C.; Nieborowska-Skorska, M.; Chiu, C. Y.; Lloyd, D.; Chen, J.-K.; Koziolkiewicz, M.; Calabretta, B. *Nucleic Acids Res.* **1996**, *24*, 1508-1514.
12. Escude, C.; Giovannangeli, C.; Sun, J.-S.; Llyod, D. H.; Chen, J.-K.; Gryaznov, S. M.; Garestier, T.; Helene, C. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 4365-4369.
13. Skorski, T.; Perrotti, D.; Nieborowska-Skorska, M.; Gryaznov, S.; Calabretta, B. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 3966-3971.
14. Rigl, C. T.; Lloyd, D. H.; Tsou, D. S.; Gryaznov, S. M.; Wilson, W. D. *Biochemistry* **1997**, *36*, 650-659.
15. Sobkowska, A.; Sobkowski, M.; Cieslak, J.; Kraszewski, A.; Kers, I.; Stawinski, J. *J. Org. Chem.* **1997**, *62*, 4791-4794.
16. Kers, I.; Stawinski, J.; Kraszewski, A. *Tetrahedron Lett.* **1998**, *39*, 1219-1222.

17. Synthesis *via* this route proved to be very efficient and a preliminary account of this work has already been published. See, ref. 16.

18. Kraszewski, A.; Sobkowski, M.; Stawinski, J. *J. Chem. Soc. Perkin Trans. 1* **1993**, 1699-1704.

19. Sobkowski, M.; Stawinski, J.; Sobkowska, A.; Kraszewski, A. *J. Chem. Soc. Perkin Trans. 1* **1994**, 1803-1808.

20. In separate experiments we showed that under analogous reaction conditions aryl H-phosphonate **6** did not react with the added *n*-butylamine hydrochloride overnight.

21. Kers, A.; Kers, I.; Kraszewski, A.; Stawinski, J. *Coll. Czech. Chem. Commun. (Special Issue)* **1996**, *61*, S246-249.

22. This reaction mixture contained the starting H-phosphonate monoester (*ca* 30%), indicating that all Pv-Cl was consumed.

23. When **7** was mixed in methylene chloride-pyridine (9:1, v/v) with adamantoyl-ethyl H-phosphonic anhydride, no acyl exchange was observed by ^{31}P NMR spectroscopy. Thus, it seems that the observed pivaloylation of **2** was probably due to incomplete chemoselectivity of the reaction rather than due to an equilibrium involving **1a** and **7**, which would generate Pv-Cl.

24. In another attempt to suppress the formation of **7**, more Pv-Cl (2 equiv.) was used for the condensation, in order to convert the produced pivalic anion into pivalic anhydride. Unfortunately, although the latter one is rather unreactive towards alcohols, it reacted with aminonucleoside **2** to produce N-pivaloylated compound **8** (TLC experiment).

25. Purified dinucleoside H-phosphonamidate **4** hydrolysed in acetonitrile-methylene chloride (1:1, v/v) in the presence of pyridinium hydrochloride (10 equiv.) and water (10 equiv.) in *ca* 70% within 30 min. Under basic conditions [acetonitrile-methylene chloride (1:1, v/v), TEA (10 equiv.) and water (10 equiv.)], the hydrolysis occurred to *ca* 20% within 90 min (^{31}P NMR experiments).

26. In the instance of NEP-Cl, the activation of **1a** was apparently slower than the subsequent reaction to produce **6**, and thus extensive formation of bis-neopentylene pyrophosphate occurred. As a consequence at least 2 equivalents of NEP-Cl were required to drive the reaction to completion. For the more reactive chlorophosphates, such as OXP-Cl or DPCP, the activation of H-phosphonate monoester **1a** seemed to be faster than the second reaction step, and thus no significant formation of the corresponding pyrophosphates occurred when 1 equivalent of the chlorophosphate was used for the condensation.

27. OXP-Cl and DPCP as well as the corresponding pyrophosphates, did not react readily with phenols under the reaction conditions, but they were very reactive towards amines, rapidly forming N-phosphorylated derivatives. To eliminate this possible side reaction with aminonucleoside **2**, both chlorophosphates were used in equimolar (or slightly less) amounts to generate aryl H-phosphonate **6**.

28. Attempted oxidation or sulfurization of **4** carried out on the reaction mixtures that were not subjected to aqueous work-up, usually gave very low yields of the desired products **5a** or **5b**. Also silylation with TMS-Cl carried out on such reaction mixtures, gave very little of the expected silyl phosphites (^{31}P NMR experiments).

29. Garegg, P. J.; Regberg, T.; Stawinski, J.; Strömberg, R. *Nucleosides Nucleotides* **1987**, *6*, 429-432.

30. Garegg, P. J.; Regberg, T.; Stawinski, J.; Strömberg, R. *J. Chem. Soc. Perkin Trans. 1* **1987**, 1269-1273.

31. Jankowska, J.; Sobkowski, M.; Stawinski, J.; Kraszewski, A. *Tetrahedron Lett.* **1994**, *35*, 3355-3358.

32. McConnell, R. L.; Coover, H. W. *J. Org. Chem.* **1959**, *24*, 630-635.

33. Glinski, R. P.; Khan, M. S.; Kalamas, R. L.; Sporn, M. B. *J. Org. Chem.* **1973**, *38*, 4299-4305.

34. Yamamoto, I.; Sekine, M.; Hata, T. *J. Chem. Soc. Perkin Trans. 1* **1980**, 306-310.

35. A sample of the reaction mixture (5 μL) was quenched with dry methanol (10 μL) prior to analysis to convert a hydrolytically unstable nucleoside aryl H-phosphonate **6a** into the stable methyl ester.