Aryl H-phosphonates. Part 13.[1] A new, general entry to aryl nucleoside phosphate and aryl nucleoside phosphorothioate diesters

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The reaction of nucleoside H-phosphonate monoesters with phenols in the presence of a condensing agent, followed by oxidation of the *in-situ*-generated aryl nucleoside H-phosphonate diesters with iodine–water or with elemental sulfur, provides a new, 'one-pot', efficient entry to nucleoside phosphate or nucleoside phosphorothioate diesters bearing diverse aryl moieties.

With the approval of the first oligonucleotide analogue as an antiviral drug (Vitravene of Isis Pharmaceuticals–Ciba Vision),[2] and with several other oligonucleotide-based therapeutics being at various stages of clinical evaluation, the development of a cost-effective method for oligonucleotide synthesis on a multi-kilogram scale becomes a matter of high importance. Although for current pharmaceutical and diagnostic uses oligonucleotides are produced on a large scale by simple scaling up the existing solid-phase protocol based on phosphoramidite chemistry,[3] it seems that more suitable for this purpose would be a solution synthesis employing phosphotriester chemistry. Some recent studies in this area clearly indicate that phosphotriester methodology has still a lot to offer when it comes to a large-scale synthesis of oligonucleotides[4] or their phosphorothioate analogues[5,6] in the anticipated industrial settings.

When the internucleotide bond is to be formed *via* phosphotriester methodology, the corresponding aryl nucleoside phosphodiesters are used as starting materials,[7] since it was found that only aromatic phosphate protecting groups secure proper stability and electrophilicity of phosphorus centres.[8] Although aryl nucleoside phosphates can be prepared using the appropriate aryl dihydrogen phosphates,[9–13] aryl chlorophosphates,[13–16] their azolides,[17,18] or hydroxybenzotriazolides,[19] these syntheses always require dedicated phosphorylating reagents that in most instances have to be prepared prior to use. This might appear to be rather inconvenient particularly when a number of different aryl nucleoside phosphate diesters are required. For the preparation of aryl nucleoside phosphorothioates, the situation is even less satisfactory, since there is no method of synthetic value, comparable in terms of efficiency or availability of the phosphorylating agents, to those developed for their oxo congeners. One should note, however, that aryl phosphorothioates as nucleotidic components seemed particularly appealing, as using electron-deficient aryl derivatives in combination with highly chemoselective condensing agent[20,21] may appear to be an attractive alternative to the S-protection required in some methods for oligonucleoside phosphorothioate synthesis.[22–24]

These facts, and the growing interest in recent years in the phosphotriester method as means of synthesising oligonucleotides and their analogues on a large scale, prompted us to search for a convenient method permitting preparation of aryl nucleoside phosphates and phosphorothioates bearing aryls derived from phenols of a wide range of acidity (pK_a 1.0–10.0). As part of our studies in developing new, efficient methods for the preparation of biologically important phosphate esters and their analogues using H-phosphonate methodology, we report here on a new, general entry to aryl nucleoside phosphates and their phosphorothioate analogues. As a viable approach we considered the *in situ* generation of bifurcated intermediates, the aryl nucleoside H-phosphonates **3**, followed by their oxidative transformation to the corresponding aryl nucleoside phosphates **4** or aryl nucleoside phosphorothioates **5** (Scheme 1). The method seemed to be experimentally simple and should alleviate problems of preparing a separate phosphorylating/thiophosphorylating reagent for each aryl derivative of types **4** and **5**.

Results and discussion

In the transformations depicted in Scheme 1 the most critical steps were oxidation of an aryl H-phosphonate intermediate with iodine–water or elemental sulfur to produce a phosphodiester **4** or a phosphorothioate **5**, respectively.

To optimise these steps, nucleoside H-phosphonate **1a** was treated with the appropriate phenols **2a**–**j** in the presence of diphenyl phosphorochloridate (DPCP), and oxidation of the product aryl nucleoside H-phosphonate **3**[25] was followed by ^{31}P NMR spectroscopy.

The generation of intermediates **3**, using previously developed reaction conditions,[25] was uneventful and produced the desired aryl H-phosphonate **3** practically as the sole nucleotidic product. However, oxidation of compounds **3** effected by the addition of iodine (1.5 equiv.) in pyridine–water (9 : 1, v/v)[25] to the reaction mixtures, although rapid in all instances (<3 min), produced phosphates **4** in variable amounts, depending on the kind of the aryl moiety present in the starting H-phosphonate **3**.

For aryl H-phosphonates **3aa**–**ac**, oxidation with an aqueous solution of iodine proceeded cleanly and the corresponding aryl nucleoside phosphates **4aa**–**ac** were obtained in >90% yield after simple work-up and purification by silica gel column chromatography (see Experimental section). The structure of products **4aa**–**ac** was confirmed with spectral (^{1}H, ^{31}P NMR, MS), and chromatographic (TLC) analysis.

In the instance of aryl H-phosphonates **3ad**–**ah** bearing

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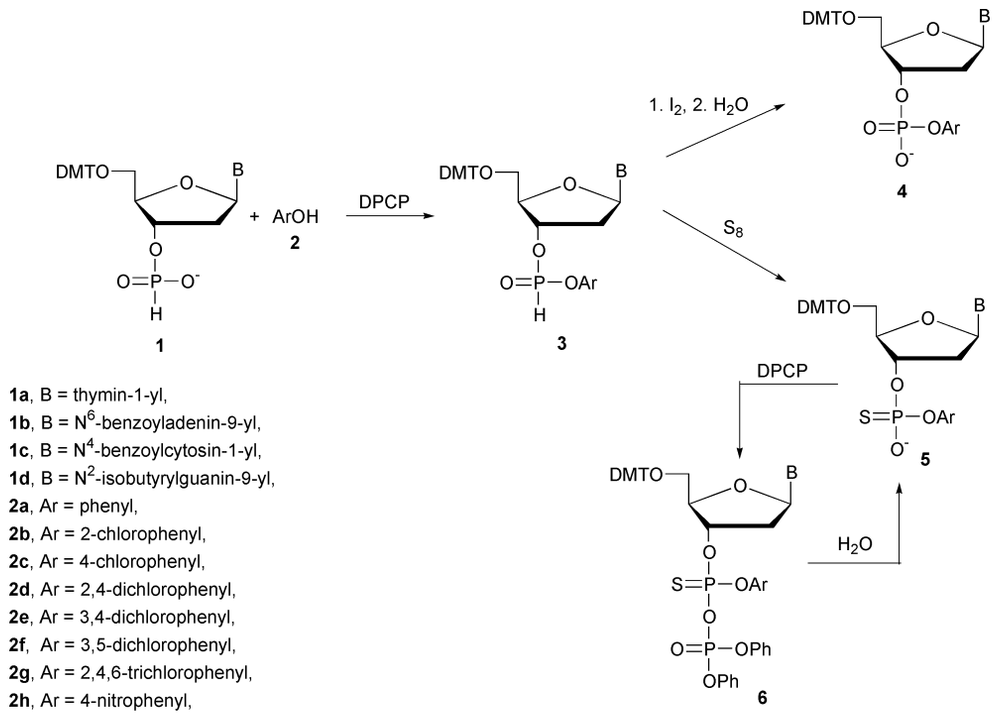

1a, B = thymin-1-yl,
1b, B = N^6-benzoyladenin-9-yl,
1c, B = N^4-benzoylcytosin-1-yl,
1d, B = N^2-isobutyrylguanin-9-yl,
2a, Ar = phenyl,
2b, Ar = 2-chlorophenyl,
2c, Ar = 4-chlorophenyl,
2d, Ar = 2,4-dichlorophenyl,
2e, Ar = 3,4-dichlorophenyl,
2f, Ar = 3,5-dichlorophenyl,
2g, Ar = 2,4,6-trichlorophenyl,
2h, Ar = 4-nitrophenyl,
2i, Ar = 2,3,4,5,6-pentachlorophenyl,
2j, Ar = 2,3,4,5,6-pentafluorophenyl,
3-6aa, B = thymin-1-yl, Ar = phenyl,
3-6ab, B = thymin-1-yl, Ar = 2-chlorophenyl,
3-6ac, B = thymin-1-yl, Ar = 4-chlorophenyl,
3-6ad, B = thymin-1-yl, Ar = 2,4-dichlorophenyl,
3-6ae, B = thymin-1-yl, Ar = 3,4-dichlorophenyl,
3-6af, B = thymin-1-yl, Ar = 3,5-dichlorophenyl,
3-6ag, B = thymin-1-yl, Ar = 2,4,6-trichlorophenyl,
3-6ah, B = thymin-1-yl, Ar = 4-nitrophenyl,
3-6ai, B = thymin-1-yl, Ar = 2,3,4,5,6-pentachlorophenyl,
3-6aj, B = thymin-1-yl, Ar = 2,3,4,5,6-pentafluorophenyl,
3-6bc, B = N^6-benzoyladenin-9-yl, Ar = 4-chlorophenyl,
3-6cc, B = N^4-benzoylcytosin-1-yl, Ar = 4-chlorophenyl,
3-6dc, B = N^2-isobutyrylguanin-9-yl, Ar = 4-chlorophenyl,
3-6bh, B = N^6-benzoyladenin-9-yl, Ar = 4-nitrophenyl,
3-6ch, B = N^4-benzoylcytosin-1-yl, Ar = 4-nitrophenyl,
3-6dh, B = N^2-isobutyrylguanin-9-yl, Ar = 4-nitrophenyl,

Scheme 1 Abbr. DMT = 4,4′-dimethoxytrityl; DPCP = diphenyl chlorophosphate.

more electron-deficient aryl moieties, ^{31}P NMR spectroscopy revealed, together with the desired phosphodiesters **4ad**–**ah**, the presence (10–15%) also of the parent nucleoside H-phosphonate **1a** [δ_P 2.89, dd ($^1J_{HP}$ 628.5 Hz, $^3J_{HP}$ 9.3 Hz)], from which aryl nucleoside H-phosphonates **3ad**–**ah** had been generated. These suggested that aryl nucleoside H-phosphonates **4ad**–**ah**, because of their enhanced reactivity as compared with **4aa**–**ac**, apparently underwent a partial hydrolysis under the reaction conditions. To eliminate this undesired reaction path, we attempted oxidation of **3ad**–**ah** with anhydrous iodine in pyridine, followed by the addition (after 20 s) of water. Indeed, this stepwise oxidation protocol completely eliminated hydrolysis of aryl H-phosphonate intermediates **3ad**–**ah** during oxidation (^{31}P NMR), and provided an efficient access to nucleoside phosphates **4ad**–**ah** bearing electron-deficient aromatic rings. The two-step oxidation procedure worked well also for aryl H-phosphonates **3aa**–**ah**, and thus all aryl nucleoside phosphodiesters **4aa**–**ah** could be obtained in high yields (>90%, after column chromatography) using one general protocol.

With the most acidic phenols investigated (**2i** and **2j**, pK_a < 6), we encountered some problems in preparing the corresponding aryl nucleoside phosphates **4ai** and **4aj**, partly due to the high susceptibility of aryl H-phosphonate intermediates **3ai** and **3aj** to disproportionation,[25,26] and partly due to the hydrolytic instability of products **4ai** and **4aj**.[27]

For the transformation of the *in-situ*-produced aryl nucleoside H-phosphonates **3** to the corresponding aryl nucleoside phosphorothioates of type **5**, we chose elemental sulfur as an oxidising agent. We found that synthesis of phosphorothioates **5** can be carried out as a four-components–one-pot reaction, by allowing nucleoside H-phosphonate **1a** to react with phenols **2** and DPCP in the presence of elemental sulfur. This protocol simplified the experimental procedure and permitted synthesis of all phosphorothioate diesters, including those derived (**5ai** and **5aj**) from the most acidic phenols investigated (**2i** and **2j**) (*vide infra*).

By monitoring these four-components–one-pot reactions by ^{31}P NMR spectroscopy we observed that in the instance of phenols **2a**–**h**, the formation of the corresponding aryl H-phosphonate **3aa**–**ah** (*ca.* 5 min) was faster than its sulfurisation, which required for completion respectively 120 min for **3aa**, 30 min for **3ab**, 20 min for **3ac** and 10 min for **3ad**, **3ae**, **3af**, **3ag** and **3ah**. For the most acidic phenols in the series (**2i**, **2j**), ^{31}P NMR spectroscopy did not reveal any transient formation of the putative intermediates **3**, but instead showed direct transformation of H-phosphonate **1** into products **5**. This indicated that, in these instances, the rates of formation of aryl H-phosphonates **3ai**–**aj** were apparently lower than those for their sulfurisation, and explained why compounds **3ai** and **3aj** did not undergo a noticeable disproportionation[25,26] under the reaction conditions used.

In all these reactions, however, apart from the desired phosphorothioates **5**, additional nucleotidic products were formed (30–40%), as was apparent from the presence in the ^{31}P NMR spectra of two groups of resonances at $\delta_P \approx 46$ and -25 ($^2J_{PP}$ 18.6–22.0 Hz). Since these signals originated from a single phosphorus species (2D ^{31}P NMR spectroscopy), which upon hydrolysis afforded the corresponding aryl nucleoside phosphorothioate **5** and diphenyl hydrogen phosphate, we tentatively identified these compounds as mixed pyrothiophosphates **6** (^{31}P NMR data in Table 1). Their presence in the reaction mixtures were most likely due to a subsequent reaction of the produced phosphorothioates **5** with the excess of DPCP

Table 1 ^{31}P NMR data of bifurcated intermediate aryl nucleoside H-phosphonates **3** and pyrothiophosphates of type **6**

Cpd no.	δ_P (ppm)	$^1J_{HP}$ (Hz)	$^3J_{HP}$ (Hz)	Cpd no.	δ_P (ppm)	$^2J_{PP}$ (Hz)	$^3J_{HP}$ (Hz)
3aa	4.44, 4.47[a]	712.6 (d), 732.6 (d)	8.3 (dd)	**6aa**	47.43, 47.80[a] −25.28	21.7 (2d) 21.7 (d)	10.2 (2d)
3ab	2.62, 2.70[a]	738.3 (d)	9.2 (dd)	**6ab**	46.75, 46.81[a] −25.02	20.2 (2d) 20.2 (d)	9.2 (2d)
3ac	4.63	737.9 (d)	8.3 (dd)	**6ac**	46.49, 46.68[a] −25.68	21.2 (2d) 21.2 (d)	10.3 (2d)
3ad	3.94, 4.37[a]	745.2 (d), 729.5 (d)	7.0 (dd)	**6ad**	46.30, 46.65[a] −25.89	21.0 (2d) 21.0 (d)	10.00 (2d)
3ae	3.44, 3.50[a]	736.3 (d), 739.0 (d)	7.3 (dd)	**6ae**	46.48, 46.91[a] −25.96	21.0 (2d) 21.0 (d)	11.0 (2d)
3af	3.36, 3.42[a]	740.9 (d), 738.1 (d)	7.3 (dd)	**6af**	46.16, 46.58[a] −26.44	20.2 (2d) 20.2 (d)	10.1 (2d)
3ag	2.44, 2.87[a]	769.3 (d), 760.6 (d)	8.3 (dd)	**6ag**	46.05, 46.41[a] −25.41	20.4 (d) 20.4 (d)	9.3 (2d)
3ah	4.07	750.8 (d)	8.3 (dd)	**6ah**	46.18, 46.56[a] −26.06	18.6 (d) 18.6 (d)	9.3 (2d)
3ai	1.21, 1.63[a]	768.4 (d), 764.7 (d)	9.2 (dd)	**6ai**	46.88, 47.23[a] −26.1	19.3 (2d) 19.3 (d)	11.0 (2d)
3aj	3.61, 3.77[a]	767.4 (d), 763.8 (d)	dm	**6aj**	49.02, 49.17[a] −26.20	20.2 (2m) 20.2 (d)	m[b]
3bc	3.42, 3.55[a]	732.6 (d), 729.9 (d)	8.7 (dd)	**6bc**	46.17, 47.08[a] −25.90	22.0 (2d) 22.0 (d)	9.7 (2d)
3bh	2.90, 2.97[a]	742.7 (d), 740.0 (d)	8.2 (dd)	**6bh**	44.78, 45.74[a] −25.91	21.1 (2d) 21.1 (d)	10.1 (2d)
3cc	3.55, 3.61[a]	730.8 (d), 733.6 (d)	7.3 (dd)	**6cc**	45.35, 45.77[a] −25.95	20.2 (2d) 20.2 (d)	10.1 (2d)
3ch	2.99, 3.05[a]	743.6 (d)	8.2 (dd)	**6ch**	45.51, 45.71[a] −25.94	20.2 (2d) 20.2 (d)	10.1 (2d)
3dc	3.65, 3.98[a]	732.6 (d), 730.8 (d)	7.3 (dd)	**6dc**	45.77, 46.76[a] −25.94	19.2 (2d) 19.2 (d)	9.6 (2d)
3dh	3.50, 3.85[a]	743.6 (d), 742.7 (d)	8.25 (dd)	**6dh**	44.44, 45.49[a] −25.89	17.9 (2d) 17.9 (d)	9.6 (2d)

[a] Two diastereoisomers. [b] Multiplet with several P–F couplings.

(Scheme 1) used for the formation of aryl H-phosphonates **3**.[28] Since mixed anhydrides **6** were readily (5 min) and cleanly hydrolysed with the added water (5 molar equiv.) to produce aryl nucleoside phosphorothioates, their formation did not affect the overall yields (>80% after silica gel chromatography) of isolated aryl nucleoside phosphorothioates **5aa**–**aj**.

To confirm the generality of the developed protocols, the above transformations were carried out on suitably protected H-phosphonate monoesters derived from 2′-deoxyadenosine **1b**, 2′-deoxycytidine **1c**, and 2′-deoxyguanosine **1d**, using two arbitrarily chosen phenols, 4-chlorophenol **2c** and 4-nitrophenol **2h**. In all instances the reactions proceeded similarly to that described for thymidine derivative **1a**, and the products, aryl nucleoside phosphate diesters **4bc**, **4cc**, **4dc**, **4bh**, **4ch**, **4dh** and the corresponding phosphorothioate diesters, **5bc**, **5cc**, **5dc**, **5bh**, **5ch** and **5dh**, were obtained in high yields (see Experimental section).

In conclusion, we have developed a new, efficient method for the synthesis of aryl nucleoside phosphate and phosphorothioate diesters, based on H-phosphonate chemistry. Starting from one type of substrates—easily accessible nucleoside H-phosphonates and phenols—and using simple 'one-pot' reaction, it was possible by changing the oxidation protocol to obtain, in high yields, aryl nucleoside phosphate or phosphorothioate diesters, derived from phenols of a wide range of acidity.[29] The method seems to be rather general and thus applicable to the preparation of other phosphorus-containing natural products and their analogues, or various alkyl aryl phosphates and phosphorothioates for synthetic and biochemical applications.

Experimental

^{1}H and ^{31}P NMR spectra were recorded at 300 MHz and 121 MHz, respectively, on a Varian Unity BB VT spectrometer. The ^{31}P NMR 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). Mass spectra were recorded with liquid secondary-ion mass technique (LSIMS) using Cs^+ (12 keV) for ionisation. Molecular mass of compounds containing chlorine atom(s) was calculated and measured for ^{35}Cl isotope. TLC analyses were carried out on Merck silica gel 60 F_{254} precoated plates using the following solvent systems: (A) *i*-PrOH–NH_4OH–water 85 : 10 : 5 (v/v/v), (B) CH_2Cl_2–CH_3OH–Et_3N 85 : 10 : 5 (v/v/v). TLC mobilities (R_{fTPH}) are reported relative to 5′-*O*-(dimethoxytrityl)thymidine 3′-H-phosphonate. Pyridine (LabScan Ltd.) was stored over molecular sieves 4 Å until the amount of water was below 20 ppm (Karl Fischer coulometric titration). Dichloromethane (POCH, Poland) was dried with P_2O_5, distilled, and stored over molecular sieves 4 Å until the amount of water was below 10 ppm. Phenols **2a**–**j**, diphenyl chlorophosphate (DPCP) and sulfur were commercial grade from Aldrich. Nucleoside H-phosphonates of type **1** were obtained according to the published method.[30] In all experiments 0.5 mmol of substrate **1** was used and the yields of products **4** and **5** were not optimised. 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 ^{1}H-coupled and ^{1}H-decoupled spectra, by spiking the reaction mixtures with appropriate species and, if possible, by isolation of the compound in question from reaction mixtures. Multiplicity of some signals in ^{1}H NMR spectra of products **5** and **6** is due to P-diastereomers.

General procedure for synthesis of aryl nucleoside phosphates of type 4

Nucleoside H-phosphonate **1** (0.5 mmol) and phenol **2** (1.5 molar equiv.) were made anhydrous by evaporation (twice) of added pyridine, dissolved in CH_2Cl_2 containing pyridine

[9 : 1 (v/v)] (0.5 mmol of **1**/5 mL of solvent), and then DPCP (1.5 molar equiv.) was added. When the formation of aryl H-phosphonate **3** was complete (15 min), iodine (1.5 molar equiv.) followed by (after 20 s) water (5 molar equiv.) was added. After 5 min the excess of iodine was neutralised with ethanethiol, the reaction mixture was diluted with CH_2Cl_2 (15 mL/0.5 mmol of nucleotide), and the organic phase was extracted with saturated aq. $NaHCO_3$ (one third of volume of organic layer) and dried with anhydrous Na_2SO_4. The solvent was removed by evaporation and the resulting glassy residue was dissolved in the minimum volume of CH_2Cl_2 and loaded on a silica gel column prepared in the same solvent. Products of type **4** were isolated using linear gradient of methanol (0–10%) in CH_2Cl_2 containing triethylamine (1% of volume). Fractions containing pure product were collected and, after evaporation of solvents, triethylammonium salts of aryl nucleoside phosphate diesters of type **4** were obtained as non-hygroscopic, dry foams, which were solidified by freeze-drying from benzene. Purity of the isolated compounds was >97% (^{1}H NMR spectroscopy).

Phenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphate triethylammonium salt 4aa. Yield 0.377 g, 94%; $R_{f\text{TPH}}$ 1.26 (A), 1.74 (B); δ_H ($CDCl_3$) 1.28 (3H, s, 5-CH_3), 1.85 (9H, t, J = 6.9 Hz, CH_2CH_3), 2.35, 2.64 (2H, 2m, 2′-H_2), 2.82 (6H, q, J = 6.9 Hz, CH_2CH_3), 3.35 (2H, m, 5′-H_2), 3.37 (6H, s, 2 × OCH_3), 4.42 (1H, m, 4′-H), 5.11 (1H, m, 3′-H), 6.46 (1H, m, 1′-H), 6.79–7.37 (18H, m, ArH of DMT and Ph groups), 7.59 (1H, s, 6-H), 8.62 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) −6.14 (d, $^3J_{HP}$ = 6.1 Hz); [M^-] *m*/*z* 699.2106. Calc. for $[C_{37}H_{36}N_2O_{10}P]^-$: 699.2108.

2-Chlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphate triethylammonium salt 4ab. Yield 0.376 g, 90% {for 5′-*O*-[*o*-(dibromomethyl)benzoyl]thymidine derivative 95%[17]}; $R_{f\text{TPH}}$ 1.30 (A), 1.71 (B); δ_H ($CDCl_3$) 1.27 (3H, s, 5-CH_3) 1.28 (9H, t, J = 7.2 Hz, 3 × CH_2CH_3), 2.38, 2.68 (2H, 2m, 2′-H_2), 3.02 (6H, q, J = 7.2 Hz, 3 × CH_2CH_3), 3.38 (2H, m, 5′-H_2), 3.77 (6H, s, 2 × OCH_3), 4.31 (1H, m, 4′-H), 5.18 (1H, m, 3′-H), 6.47 (1H, m, 1′-H), 6.79–7.36 (17H, m, ArH of DMT and 2-chlorophenyl groups), 7.96 (1H, s, 6-H), 8.47 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) −6.59 (d, $^3J_{HP}$ = 7.3 Hz); [M^-] (*m*/*z*) 733.1716. Calc. for $[C_{37}H_{35}ClN_2O_{10}P]^-$: 733.1718.

4-Chlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphate triethylammonium salt 4ac. Yield 0.385 g, 92% (95%[31]); $R_{f\text{TPH}}$ 1.37 (A), 1.71 (B); δ_H ($CDCl_3$) 1.18 (3H, s, 5-CH_3), 1.3 (9H, t, J = 7.2 Hz, 3 × CH_2CH_3), 2.35, 2.64 (2H, 2m, 2′-H_2), 3.05 (6H, q, J = 7.2 Hz, 3 × CH_2CH_3), 3.23, 3.41 (2H, 2m, 5′-H_2), 3.79 (6H, s, 2 × OCH_3), 4.22 (1H, m, 4′-H), 5.07 (1H, m, 3′-H), 6.45 (1H, m, 1′-H), 6.79–7.37 (17H, m, ArH of DMT and 4-chlorophenyl), 7.59 (1H, s, 6-H), 8.62 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) −6.30 (d, $^3J_{HP}$ = 6.1 Hz); [M^-] *m*/*z* 733.1716. Calc. for $[C_{37}H_{35}ClN_2O_{10}P]^-$: 733.1718.

2,4-Dichlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphate triethylammonium salt 4ad. Yield 0.409 g, 94%; $R_{f\text{TPH}}$ 1.44 (A), 1.71 (B); δ_H ($CDCl_3$) 1.34 (3H, s, 5-CH_3), 1.35 (9H, t, J = 7.5 Hz, 3 × CH_2CH_3) 2.40, 2.66 (2H, 2m, 2′-H_2), 3.00 (6H, q, J = 7.5 Hz, 3 × CH_2CH_3), 3.40 (2H, m, 5′-H_2), 3.78 (6H, s, 2 × OCH_3), 4.39 (1H, m, 4′-H), 5.15 (1H, m, 3′-H), 6.45 (1H, m, 1′-H), 6.79–7.60 (16H, m, ArH of DMT and 2,4-dichlorophenyl), 7.60 (1H, s, 6-H), 8.63 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) −7.14 (d, $^3J_{HP}$ = 6.1 Hz); [M^-] *m*/*z* 767.1339. Calc. for $[C_{37}H_{34}Cl_2N_2O_{10}P]^-$: 767.1328.

3,4-Dichlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphate triethylammonium salt 4ae. Yield 0.392 g, 90%; $R_{f\text{TPH}}$ 1.48 (A), 1.71 (B); δ_H ($CDCl_3$) 1.18 (9H, t, J = 7.2 Hz, 3 × CH_2CH_3), 1.22 (3H, s, 5-CH_3), 2.54, 2.66 (2H, 2m, 2′-H_2), 2.90 (6H, q, J = 7.2 Hz, 3 × CH_2CH_3), 3.17, 3.25 (2H, m, 5′-H_2), 3.68 (6H, s, 2 × OCH_3), 4.15 (1H, 2m, 4′-H), 4.98 (1H, m, 3′-H), 6.35 (1H, m, 1′-H), 6.69–7.28 (16H, m, ArH of DMT and 3,4-dichlorophenyl groups), 7.49 (1H, s, 6-H), 8.60 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) −6.64 (d, $^3J_{HP}$ = 6.4 Hz); [M^-] *m*/*z* 767.1329. Calc. for $[C_{37}H_{34}Cl_2N_2O_{10}P]^-$: 767.1328.

3,5-Dichlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphate triethylammonium salt 4af. Yield 0.387 g, 89%; $R_{f\text{TPH}}$ 1.56 (A), 1.77 (B); δ_H ($CDCl_3$) 1.28 (9H, t, J = 7.2 Hz, 3 × CH_2CH_3), 1.35 (3H, s, 5-CH_3), 2.09, 2.36 (2H, 2m, 2′-H_2), 3.00 (6H, q, J = 7.2 Hz, CH_2CH_3), 3.38 (2H, m, 5′-H_2), 3.76 (6H, s, 2 × OCH_3), 4.28 (1H, m, 4′-H), 5.10 (1H, m, 3′-H), 6.44 (1H, m, 1′-H), 6.78–7.36 (16H, m, ArH of DMT and 3,5-dichlorophenyl groups), 7.59 (1H, s, 6-H), 8.76 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) −7.70 (d, $^3J_{HP}$ = 7.3 Hz); [M^-] *m*/*z* 767.1315. Calc. for $[C_{37}H_{34}Cl_2N_2O_{10}P]^-$: 767.1328.

2,4,6-Trichlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphate triethylammonium salt 4ag. Yield 0.407 g, 90%; $R_{f\text{TPH}}$ 1.44 (A), 1.81 (B); δ_H ($CDCl_3$) 1.26 (9H, t, J = 7.2 Hz, 3 × CH_2CH_3), 1.30 (3H, s, 5-CH_3), 2.43, 2.86 (2H, 2m, 2′-H_2), 2.99 (6H, q, J = 7.2 Hz, 3 × CH_2CH_3), 3.47 (2H, m, 5′-H_2), 3.77 (6H, s, 2 × OCH_3), 4.44 (1H, m, 4′-H), 5.30 (1H, m, 3′-H), 6.50 (1H, m, 1′-H), 6.80–7.40 (15H, m, ArH of DMT and 2,4,6-trichlorophenyl groups), 7.65 (1H, s, 6-H), 11.94 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) −7.06 (d, $^3J_{HP}$ = 6.1 Hz); [M^-] *m*/*z* 801.0935. Calc. for $[C_{37}H_{33}Cl_3N_2O_{10}P]^-$: 801.0938.

4-Nitrophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphate triethylammonium salt 4ah. Yield 0.377 g, 89%; $R_{f\text{TPH}}$ 1.48 (A), 1.68 (B); δ_H ($CDCl_3$) 1.29 (3H, s, 5-CH_3), 1.32 (9H, t, J = 7.5 Hz, 3 × CH_2CH_3), 2.43, 2.81 (2H, 2m, 2′-H_2), 3.47 (2H, m, 5′-H_2), 3.05 (6H, q, J = 7.5 Hz, 3 × CH_2CH_3), 3.69 (6H, s, 2 × OCH_3), 4.44 (1H, m, 4′-H), 5.30 (1H, m, 3′-H), 6.50 (1H, m, 1′-H), 6.80–7.40 (17H, m, ArH of DMT and 4-nitrophenyl groups), 7.65 (1H, s, 6-H), 8.63 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) −6.78 (d, $^3J_{HP}$ = 6.1 Hz); [M^-] *m*/*z* 744.1957. Calc. for $[C_{37}H_{35}N_3O_{12}P]^-$: 744.1958.

4-Chlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)-*N*^{6}-benzoyl-2′-deoxyadenosin-3′-yl phosphate triethylammonium salt 4bc. Yield 0.370 g, 78%; $R_{f\text{TPH}}$ 1.82 (A), 1.77 (B); δ_H ($CDCl_3$) 1.28 (9H, t, J = 7.2 Hz, 3 × CH_2CH_3), 2.87 (2H, m, 2′-H_2), 3.05 (6H, q, J = 7.2 Hz, 3 × CH_2CH_3), 3.34 (2H, m, 5′-H_2), 3.76 (6H, s, 2 × OCH_3), 5.15 (1H, m, 4′-H), 6.53 (1H, m, 3′-H), 6.53 (1H, m, 1′-H), 6.74–7.63 (22H, m, ArH of DMT, *N*^{6}-benzoyl and 4-chlorophenyl groups), 8.02 (1H, s, 2-H), 8.05 (1H, s, 8-H), 9.14 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) −7.31 (d, $^3J_{HP}$ = 6.4 Hz); [M^-] *m*/*z* 846.2096. Calc. for $[C_{44}H_{38}ClN_5O_9P]^-$: 846.2096.

4-Nitrophenyl 5′-*O*-(4,4′-dimethoxytrityl)-*N*^{6}-benzoyl-2′-deoxyadenosin-3′-yl phosphate triethylammonium salt 4bh. Yield 0.374 g, 78%; $R_{f\text{TPH}}$ 1.74 (A), 1.77 (B); δ_H ($CDCl_3$) 1.32 (9H, t, J = 7.4 Hz, 3 × CH_2CH_3), 2.90 (2H, m, 2′-H_2), 3.06 (6H, q, J = 7.4 Hz, 3 × CH_2CH_3), 3.40 (2H, m, 5′-H_2), 3.76 (6H, s, 2 × OCH_3), 4.40 (1H, m, 4′-H), 5.16 (1H, m, 3′-H), 6.52 (1H, m, 1′-H), 6.76–8.20 (22H, m, ArH of DMT, *N*^{6}-benzoyl and 4-nitrophenyl groups), 8.14 (1H, s, 2-H), 8.71 (1H, s, 8-H), 9.07 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) −7.31 (d, $^3J_{HP}$ = 6.4 Hz); [M^-] *m*/*z* 857.2352. Calc. for $[C_{44}H_{38}N_6O_{11}P]^-$: 857.2336.

4-Chlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)-*N*^{4}-benzoyl-2′-deoxycytidin-3′-yl phosphate triethylammonium salt 4cc. Yield 0.384 g, 83%; $R_{f\text{TPH}}$ 1.78 (A), 1.81 (B); δ_H ($CDCl_3$) 1.35 (9H, t, J = 7.5 Hz, 3 × CH_2CH_3), 2.30, 2.95 (2H, 2m, 2′-H_2), 3.07 (6H, q, J = 7.5 Hz, 3 × CH_2CH_3), 3.39 (2H, m, 5′-H_2), 3.78 (6H, s, 2 × OCH_3), 4.35 (1H, m, 4′-H), 5.04 (1H, m, 3′-H), 6.28 (1H, m, 1′-H), 6.80–7.91 (22H, m, ArH of DMT, Bz and 4-chlorophenyl groups), 8.15 (1H, s, 6-H), 8.84 (1H, br s, NH, exch. D_2O). As judged from a COSY experiment, signals of H-5

overlapped with aromatic proton resonances; δ_P (CH_2Cl_2) −6.84 (d, $^3J_{HP}$ = 6.4 Hz); [M$^-$] *m/z* 822.1983. Calc. for [$C_{43}H_{38}ClN_3O_{10}P$]$^-$: 823.1984.

4-Nitrophenyl 5′-*O*-(4,4′-dimethoxytrityl)-*N*^{4}-benzoyl-2′-deoxycytidin-3′-yl phosphate triethylammonium salt 4ch. Yield 0.380 g, 81%; R_{fTPH} 1.70 (A), 1.77 (B); δ_H ($CDCl_3$) 1.35 (9H, t, *J* = 7.4 Hz, 3 × CH_2CH_3), 2.46, 2.99 (2H, 2m, 2′-H_2), 3.10 (6H, q, *J* = 7.4 Hz, 3 × CH_2CH_3), 3.46 (2H, m, 5′-H_2), 3.78 (6H, s, 2 × OCH_3), 4.44 (1H, m, 4′-H), 5.33 (1H, m, 3′-H), 6.32 (1H, t, *J* = 6.3 Hz, 1′-H), 6.80–8.15 (22H, m, ArH of DMT, Bz and 4-nitrophenyl groups), 8.19 (1H, d, *J* = 7.5 Hz, 6-H), 8.57 (1H, br s, NH, exch. D_2O). As judged from a COSY experiment, signals of H-5 overlapped with aromatic proton resonances; δ_P (CH_2Cl_2) −7.26 (d, $^3J_{HP}$ = 6.4 Hz); [M$^-$] *m/z* 833.2221. Calc. for [$C_{43}H_{38}N_4O_{12}P$]$^-$: 833.2224.

4-Chlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)-*N*^{2}-isobutyryl-2′-deoxyguanosin-3′-yl phosphate triethylammonium salt 4dc. Yield 0.382 g, 82%; R_{fTPH} 1.59 (A), 1.68 (B); δ_H ($CDCl_3$) 0.83, 1.08 [6H, 2d, *J* = 6.9 Hz, $CH(CH_3)_2$], 1.21 (9H, t, *J* = 7.2 Hz, 3 × CH_2CH_3) 2.24, 2.62 (2H, 2m, 2′-H_2), 2.96 (6H, q, *J* = 7.2 Hz, 3 × CH_2CH_3), 3.05 [1H, m, *J* = 6.9 Hz, $CH(CH_3)_2$], 3.19, 3.36 (2H, 2m, 5′-H_2), 3.75 (6H, s, 2 × OCH_3), 4.24 (1H, m, 4′-H), 5.46 (1H, m, 3′-H), 6.13 (1H, t, *J* = 6.6 Hz, 1′-H), 6.70–7.36 (17H, m, ArH of DMT and 4-chlorophenyl groups), 7.72 (1H, s, 8-H), 9.52 (1H, br s, NH, exch. D_2O), 12.03 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) −6.88 (d, $^3J_{HP}$ = 7.3 Hz); [M$^-$] *m/z* 828.2204. Calc. for [$C_{41}H_{40}ClN_5O_{10}P$]$^-$: 828.2201.

4-Nitrophenyl 5′-*O*-(4,4′-dimethoxytrityl)-*N*^{2}-isobutyryl-2′-deoxyguanosin-3′-yl phosphate triethylammonium salt 4dh. Yield 0.348 g, 74%; R_{fTPH} 1.52 (A), 1.65 (B); δ_H ($CDCl_3$) 1.03 [6H, d, *J* = 6.9 Hz, $CH(CH_3)_2$], 1.27 (9H, t, *J* = 7.5 Hz, 3 × CH_2CH_3), 2.24, 2.62 (2H, 2m, 2′-H_2), 3.01 (6H, q, *J* = 7.5 Hz, 3 × CH_2CH_3), 3.07 [1H, m, *J* = 6.9 Hz, $CH(CH_3)_2$], 3.26 (2H, m, 5′-H_2), 3.76 (6H, s, 2 × OCH_3), 4.24 (1H, m, 4′-H), 5.42 (1H, m, 3′-H), 6.14 (1H, t, *J* = 6.3 Hz, 1′-H), 6.71–8.10 (17H, m, ArH of DMT and 4-nitrophenyl groups), 7.72 (1H, s, 8-H), 9.40 (1H, br s, NH, exch. D_2O), 12.03 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) −7.69 (d, $^3J_{HP}$ = 7.3 Hz); [M$^-$] *m/z* 839.2442. Calc. for [$C_{41}H_{40}N_6O_{12}P$]$^-$: 839.2442.

General procedure for synthesis of aryl nucleoside phosphorothioates of type 5

Nucleoside H-phosphonate **1** (0.5 mmol), the appropriate phenol **2** (1.5 molar equiv.) and sulfur (3 molar equiv.) were made anhydrous by evaporation (twice) of added excess of pyridine, dissolved in CH_2Cl_2 containing pyridine [9 : 1 (v/v)] (0.5 mmol of **1**/5 mL of solvent) and then, to the stirred mixture, was added DPCP (1.5 molar equiv.). After sulfurisation of the produced aryl H-phosphonate **3** was complete (*ca.* 120 min for **3aa**, 30 min for **3ab**, **3ac**, **3bc**, **3cc**, **3dc**, 20 min for **3ad**–**af**, and 10 min for **3ag**–**3aj**, **3bh**, **3ch**, **3dh**), water and Et_3N (5 molar equiv. each) were added to hydrolyse the pyrothiophosphate of type **6**. After 5 min the reaction mixture was diluted with CH_2Cl_2 (15 mL/0.5 mmol of nucleotide), and the organic phase was extracted with saturated aq. $NaHCO_3$ (one-third of volume of organic layer). Further work-up and isolation of pure triethylammonium salt of aryl nucleoside phosphorothioates of type **5** was as described above for aryl nucleoside phosphates of type **4**. Compounds **5** were obtained as white solids (purity >97%, ^{1}H NMR spectroscopy).

Phenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphorothioate triethylammonium salt 5aa. Yield 0.372 g, 91%; R_{fTPH} 1.44 (A), 1.94 (B); δ_H ($CDCl_3$) 1.21 (9H, t, *J* = 6.9 Hz, 3 × CH_2CH_3), 1.33, 1.35 (3H, 2s, 5-CH_3), 2.34, 2.57 (2H, 2m, 2′-H_2), 2.89 (6H, q, *J* = 6.9 Hz, 3 × CH_2CH_3), 3.36, 3.56 (2H, 2m, 5′-H_2), 3.77 (6H, s, 2 × OCH_3), 4.29, 4.37 (1H, 2m, 4′-H), 5.46 (1H, m, 3′-H), 6.17 (1H, m, 1′-H), 6.48–7.42 (18H, m, ArH of DMT and phenyl groups), 7.61 (1H, s, 6-H), 8.63 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) 53.61, 53.77 (d, $^3J_{HP}$ = 9.4 Hz); [M$^-$] *m/z* 715.1877. Calc. for [$C_{37}H_{36}N_2O_9PS$]$^-$: 715.1879.

2-Chlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphorothioate triethylammonium salt 5ab. Yield 0.392 g, 92%; R_{fTPH} 1.52 (A), 1.87 (B); δ_H ($CDCl_3$) 1.30 (3H, s, 5-CH_3), 1.31 (9H, t, *J* = 7.2 Hz, 3 × CH_2CH_3), 2.38, 2.59 (2H, 2m, 2′-H_2), 3.08 (6H, q, *J* = 7.2 Hz, 3 × CH_2CH_3), 3.80 (6H, s, 2 × OCH_3), 3.96 (2H, m, 5′-H_2), 4.17 (1H, m, 4′-H), 5.27 (1H, m, 3′-H), 6.21 (1H, m, 1′-H), 6.41–7.36 (17H, m, ArH of DMT and 2-chlorophenyl groups), 7.60 (1H, m, 6-H), 8.12 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) 53.67, 53.74 (2d, $^3J_{HP}$ = 9.2 Hz); [M$^-$] *m/z* 749.1490. Calc. for [$C_{37}H_{35}ClN_2O_9PS$]$^-$: 749.1489.

4-Chlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphorothioate triethylammonium salt 5ac. Yield 0.324 g, 76%; R_{fTPH} 1.56 (A), 1.87 (B); δ_H ($CDCl_3$): 1.29 (9H, t, *J* = 7.5 Hz, 3 × CH_2CH_3), 1.34, 1.38 (3H, s, 5-CH_3), 2.37, 2.65 (2H, 2m, 2′-H_2), 3.04 (6H, q, *J* = 7.5 Hz, 3 × CH_2CH_3), 3.37, 3.51 (2H, 2m, 5′-H_2), 3.77 (6H, s, 2 × OCH_3), 4.28, 4.40 (1H, 2m, 4′-H), 5.41 (1H, m, 3′-H), 6.47 (1H, m, 1′-H), 6.81–7.41 (17H, m, ArH of DMT and 4-chlorophenyl groups), 7.60 (m, 1H, 6-H), 8.32 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) 54.82, 54.90 (2d, $^3J_{HP}$ = 11.0 Hz); [M$^-$] *m/z* 749.1490. Calc. for [$C_{37}H_{35}ClN_2O_9PS$]$^-$: 749.1489.

2,4-Dichlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphorothioate triethylammonium salt 5ad. Yield 0.412 g, 93%; R_{fTPH} 1.59 (A), 1.87 (B); δ_H ($CDCl_3$) 1.23 (9H, t, *J* = 7.5 Hz, 3 × CH_2CH_3), 1.36 (3H, s, 5-CH_3), 2.45, 2.85 (2H, 2m, 2′-H_2), 3.08 (6H, q, *J* = 7.5 Hz, 3 × CH_2CH_3), 3.40, 3.59 (2H, 2m, 5′-H_2), 3.76 (6H, s, 2 × OCH_3), 4.44, 4.57 (1H, 2m, 4′-H), 5.62, 5.76 (1H, m, 3′-H), 6.47 (1H, m, 1′-H), 6.52–7.44 (16H, m, ArH of DMT and 2,4-dichlorophenyl groups), 7.67 (1H, m, 6-H), 8.63 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) 55.02, 55.73 (2d, $^3J_{HP}$ = 9.7 Hz); [M$^-$] *m/z* 783.1120. Calc. for [$C_{37}H_{34}Cl_2N_2O_9PS$]$^-$: 783.1100.

3,4-Dichlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphorothioate triethylammonium salt 5ae. Yield 0.395 g, 89%; R_{fTPH} 1.67 (A), 1.90 (B); δ_H ($CDCl_3$) 1.26 (9H, t, *J* = 7.5 Hz, 3 × CH_2CH_3), 1.34, 1.38 (3H, 2s, 5-CH_3), 2.39, 2.66 (2H, 2m, 2′-H_2), 3.00 (6H, q, *J* = 7.5 Hz, 3 × CH_2CH_3), 3.38, 3.52 (2H, 2m, 5′-H_2), 3.76 (6H, s, 2 × OCH_3), 4.30, 4.39 (1H, 2m, 4′-H), 5.41 (1H, m, 3′-H), 6.47 (1H, m, 1′-H), 6.79–7.45 (16H, m, ArH of DMT and 3,4-dichlorophenyl groups), 7.59, 7.61 (1H, 2br s, 6-H), 9.13 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) 53.28, 53.33 (d, $^3J_{HP}$ = 10.1 Hz); [M$^-$] *m/z* 783.1110. Calc. for [$C_{37}H_{34}Cl_2N_2O_9PS$]$^-$: 783.1100.

3,5-Dichlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphorothioate triethylammonium salt 5af. Yield 0.404 g, 91%; R_{fTPH} 1.67 (A), 1.94 (B); δ_H ($CDCl_3$) 1.30 (9H, t, *J* = 7.2 Hz, 3 × CH_2CH_3), 1.35, 1.39 (3H, 2s, 5-CH_3), 2.42, 2.68 (2H, 2m, 2′-H_2), 3.05 (6H, q, *J* = 7.2 Hz, 3 × CH_2CH_3), 3.39, 3.56 (2H, 2m, 5′-H_2), 3.77, 3.78 (6H, 2s, 2 × OCH_3), 4.39, 4.41 (1H, 2m, 4′-H), 5.44 (1H, m, 3′-H), 6.48 (1H, m, 1′-H), 6.81–7.31 (16H, m, ArH of DMT and 3,5-dichlorophenyl groups), 7.62, 7.63 (1H, 2s, 6-H), 8.26 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) 53.25 (d, $^3J_{HP}$ = 10.1 Hz); [M$^-$] *m/z* 783.1087. Calc. for [$C_{37}H_{34}Cl_2N_2O_9PS$]$^-$: 783.1100.

2,4,6-Trichlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphorothioate triethylammonium salt 5ag. Yield 0.438 g, 95%; R_{fTPH} 1.67 (A), 1.87 (B); δ_H ($CDCl_3$) 1.30 (9H, t, *J* = 7.2 Hz, 3 × CH_2CH_3), 1.35, 1.36 (3H, 2s, 5-CH_3), 2.43, 2.81 (2H, 2m, 2′-H_2), 3.07 (6H, q, *J* = 7.2 Hz, 3 × CH_2CH_3), 3.46

(2H, m, 5′-H_2), 3.76 (6H, s, 2 × OCH_3), 4.45 (1H, m, 4′-H), 5.62 (1H, m, 3′-H), 6.46 (1H, m, 1′-H), 6.48–7.41 (15H, m, ArH of DMT and 2,4,6-trichlorophenyl groups), 7.70 (1H, br s, 6-H), 11.58 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) 53.58, 53.74 (2d, $^3J_{HP}$ = 9.7 Hz); [M^-] *m/z* 817.0706. Calc. for [$C_{37}H_{33}Cl_3N_2O_9PS]^-$: 817.0710.

4-Nitrophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphorothioate triethylammonium salt 5ah. Yield 0.410 g, 95%; R_{fTPH} 1.63 (A), 1.87 (B); δ_H ($CDCl_3$) 1.27 (9H, t, *J* = 7.2 Hz, 3 × CH_2CH_3), 1.35, 1.40 (3H, s, 5-CH_3), 2.39, 2.66 (2H, 2m, 2′-H_2), 2.99 (6H, q, *J* = 7.2 Hz, 3 × CH_2CH_3), 3.41 (2H, m, 5′-H_2), 3.77 (6H, s, 2 × OCH_3), 4.29, 4.38 (1H, m, 4′-H), 5.41 (1H, m, 3′-H), 6.47 (1H, m, 1′-H), 6.81–7.41 (17H, m, ArH of DMT and 4-nitrophenyl groups), 7.62 (1H, m, 6-H), 8.62 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) 55.00, 55.66 (2d, $^3J_{HP}$ = 9.7 Hz); [M^-] *m/z* 760.1730. Calc. for [$C_{37}H_{35}N_3O_{11}PS]^-$: 760.1730.

Pentachlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphorothioate triethylammonium salt 5ai. Yield 0.420 g, 85%; R_{fTPH} 1.70 (A), 1.94 (B); δ_H ($CDCl_3$) 1.12 (9H, t, *J* = 7.2 Hz, 3 × CH_2CH_3), 1.34, 1.38 (3H, 2br s, 5-CH_3), 2.46, 2.84 (2H, 2m, 2′-H_2), 2.69 (6H, q, *J* = 7.2 Hz, 3 × CH_2CH_3), 3.52 (2H, m, 5′-H_2), 3.76, 3.78 (6H, 2s, 2 × OCH_3), 4.44, 4.53 (1H, 2m, 4′-H), 5.56, 5.66 (1H, 2m, 3′-H), 6.50 (1H, m, 1′-H), 6.80–7.42 (13H, m, ArH of DMT), 7.66, 7.68 (1H, 2br s, 6-H), 8.68 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) 52.61, 53.67 (2d, $^3J_{HP}$ = 9.2 and 11.0 Hz); [M^-] *m/z* 884.9914. Calc. for [$C_{37}H_{31}Cl_5N_2O_9PS]^-$: 884.9931.

Pentafluorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphorothioate triethylammonium salt 5aj. Yield 0.409 g, 90%; R_{fTPH} 1.63 (A), 1.87 (B); δ_H ($CDCl_3$) 1.24 (9H, t, *J* = 7.5 Hz, 3 × CH_2CH_3), 1.35, 1.37 (3H, 2br s, 5-CH_3), 2.42, 2.76 (2H, 2m, 2′-H_2), 2.91 (6H, q, *J* = 7.5 Hz, 3 × CH_2CH_3), 3.47 (2H, m, 5′-H_2), 3.78 (6H, s, 2 × OCH_3), 4.43 (1H, m, 4′-H), 5.40, 5.41 (1H, 2m, 3′-H), 6.51 (1H, m, 1′-H), 6.81–7.44 (13H, m, ArH of DMT), 7.63, 7.68 (1H, 2br s, 6-H), 8.64 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) 54.89, 55.85 (2m); [M^-] *m/z* 805.1413. Calc. for [$C_{37}H_{31}F_5N_2O_9PS]^-$: 805.1408.

4-Chlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)-*N*^{6}-benzoyl-2′-deoxyadenosin-3′-yl phosphorothioate triethylammonium salt 5bc. Yield 0.396 g, 82%; R_{fTPH} 2.00 (A), 1.94 (B); δ_H ($CDCl_3$) 1.28 (9H, t, *J* = 7.5 Hz, 3 × CH_2CH_3), 2.87, 2.94 (2H, 2m, 2′-H_2), 3.04 (6H, q, *J* = 7.5 Hz, 3 × CH_2CH_3), 3.37, 3.44 (2H, m, 5′-H_2), 3.76 (6H, s, 2 × OCH_3), 4.47, 4.56 (1H, 2m, 4′-H), 5.44 (1H, m, 3′-H), 6.57 (1H, m, 1′-H), 6.75–7.61 (22H, m, ArH of DMT, Bz and 4-chlorophenyl groups), 8.02, 8.04 (1H, 2s, 2-H), 8.16, 8.19 (1H, 2s, 8-H), 9.07 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) 53.10, 53.13 (2d, $^3J_{HP}$ = 9.2 Hz); [M^-] *m/z* 862.1854. Calc. for [$C_{44}H_{38}ClN_5O_8PS]^-$: 862.1867.

4-Nitrophenyl 5′-*O*-(4,4′-dimethoxytrityl)-N^6-benzoyl-2′-deoxyadenosin-3′-yl phosphorothioate triethylammonium salt 5bh. Yield 0.405 g, 83%; R_{fTPH} 1.93 (A), 1.97 (B); δ_H ($CDCl_3$) 1.32 (9H, t, *J* = 7.5 Hz, 3 × CH_2CH_3), 2.94 (2H, m, 2′-H_2), 3.10 (6H, q, *J* = 7.5 Hz, 3 × CH_2CH_3), 3.39, 3.44 (2H, m, 5′-H_2), 3.76 (6H, s, 2 × OCH_3), 4.50 (1H, m, 4′-H), 5.44, 5.48 (1H, 2m, 3′-H), 6.57 (1H, m, 1′-H), 6.76–8.19 (22H, m, ArH of DMT, Bz and 4-nitrophenyl groups), 8.17, 8.20 (1H, 2s, 2-H), 8.72, 8.73 (1H, 2s, 8-H), 9.09 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) 52.34 (d, $^3J_{HP}$ = 9.2 Hz); [M^-] *m/z* 873.2099. Calc. for [$C_{44}H_{38}N_6O_{10}PS]^-$: 873.2108.

4-Chlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)-*N*^{4}-benzoyl-2′-deoxycytidin-3′-yl phosphorothioate triethylammonium salt 5cc. Yield 0.410 g, 87%; R_{fTPH} 1.89 (A), 1.97 (B); δ_H ($CDCl_3$) 1.30 (9H, t, *J* = 7.5 Hz, 3 × CH_2CH_3), 2.30, 2.98 (2H, 2m, 2′-H_2), 3.06 (6H, q, *J* = 7.5 Hz, 3 × CH_2CH_3), 3.45 (2H, m, 5′-H_2), 3.78 (6H, s, 2 × OCH_3), 4.48 (1H, m, 4′-H), 5.34 (1H, m, 3′-H), 6.33 (1H, t, *J* = 6.2 Hz, 1′-H), 6.82–7.91 (22H, m, ArH of DMT, Bz and 4-chlorophenyl groups), 8.19 (1H, d, *J* = 7.2 Hz, 6-H), 8.70 (1H, br s, NH, exch. D_2O). As judged from a COSY experiment, the signal of H-5 overlaps with aromatic proton resonances; δ_P (CH_2Cl_2) 55.00, 55.66 (2d, $^3J_{HP}$ = 9.7 Hz); δ_P (CH_2Cl_2) 53.06, 53.12 (2d, $^3J_{HP}$ = 11.0 Hz); [M^-] *m/z* 838.1760. Calc. for [$C_{43}H_{38}ClN_3O_9PS]^-$: 838.1755.

4-Nitrophenyl 5′-*O*-(4,4′-dimethoxytrityl)-*N*^{4}-benzoyl-2′-deoxycytidin-3′-yl phosphorothioate triethylammonium salt 5ch. Yield 0.419 g, 88%; R_{fTPH} 1.78 (A), 1.97 (B); δ_H ($CDCl_3$) 1.36 (9H, t, *J* = 7.4 Hz, 3 × CH_2CH_3), 2.46, 2.99 (2H, 2m, 2′-H_2), 3.10 (6H, q, *J* = 7.4 Hz, 3 × CH_2CH_3), 3.46 (2H, m, 5′-H_2), 3.78 (6H, s, 2 × OCH_3), 4.48 (1H, m, 4′-H), 5.33 (1H, m, 3′-H), 6.32 (1H, t, *J* = 6.3 Hz, 1′-H), 6.81–7.94 (22H, m, ArH of DMT, Bz and 4-nitrophenyl groups), 8.16 (1H, m, 6-H), 8.60 (1H, br s, NH, exch. D_2O). As judged from a COSY experiment, the signal of H-5 overlaps with aromatic proton resonances; δ_P (CH_2Cl_2) 52.20, 52.32 (2d, $^3J_{HP}$ = 9.1 Hz); [M^-] *m/z* 849.1978. Calc. for [$C_{43}H_{38}N_4O_{11}PS]^-$: 849.1955.

4-Chlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)-*N*^{2}-isobutyryl-2′-deoxyguanosin-3′-yl phosphorothioate triethylammonium salt 5dc. Yield 0.398 g, 84%; R_{fTPH} 1.70 (A), 1.81 (B); δ_H ($CDCl_3$) 0.99 [m, 6H, $CH(CH_3)_2$], 1.26 (9H, t, *J* = 7.5 Hz, 3 × CH_2CH_3), 2.05, 2.65 (2H, 2m, 2′-H_2), 3.03 (6H, q, *J* = 7.5 Hz, 3 × CH_2CH_3), 3.16 [1H, m, $CH(CH_3)_2$], 3.26, 3.41 (2H, 2m, 5′-H_2), 3.74, 3.75 (6H, 2s, 2 × OCH_3), 4.29 (1H, m, 4′-H), 5.71, 5.87 (1H, 2m, 3′-H), 6.15 (1H, m, 1′-H), 6.69–7.40 (17H, m, ArH of DMT and 4-chlorophenyl groups), 7.74, 7.76 (1H, 2s, 8-H), 8.48 (1H, br s, NH, exch. D_2O), 11.89 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) 53.09, 53.80 (2d, $^3J_{HP}$ = 11.0 Hz); [M^-] *m/z* 844.1969. Calc. for [$C_{41}H_{40}ClN_5O_9PS]^-$: 844.1973.

4-Nitrophenyl 5′-*O*-(4,4′-dimethoxytrityl)-*N*^{2}-isobutyryl-2′-deoxyguanosin-3′-yl phosphorothioate triethylammonium salt 5dh. Yield 0.378 g, 79%; R_{fTPH} 1.67 (A), 1.81 (B); δ_H ($CDCl_3$) 0.93, 1.06 [6H, 2d, *J* = 6.9 Hz, $CH(CH_3)_2$], 1.32 (9H, t, *J* = 7.5 Hz, 3 × CH_2CH_3), 2.57, 2.68 (2H, 2m, 2′-H_2), 2.91, 3.20 [1H, 2m, *J* = 6.9 Hz, $CH(CH_3)_2$], 3.11 (6H, q, *J* = 7.5 Hz, 3 × CH_2CH_3), 3.75 (2H, m, 5′-H_2), 3.80 (6H, s, 2 × OCH_3), 4.32 (1H, m, 4′-H), 5.53, 5.68 (1H, 2m, 3′-H), 6.15 (1H, m, 1′-H), 6.71–7.43 (17H, m, ArH of DMT and 4-nitrophenyl groups), 8.17, 8.20 (1H, 2s, 8-H), 11.23 (1H, br s, NH, exch. D_2O); δ_P (CH_2Cl_2) 52.19, 52.74 (2d, $^3J_{HP}$ = 11.0 and 10.1 Hz); [M^-] *m/z* 855.2221. Calc. for [$C_{41}H_{40}N_6O_{11}PS]^-$: 855.2213.

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