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SYNTHESIS OF A DINUCLEOSIDE 3'-*S*-PHOSPHOROTHIOLATE CONTAINING 2'-DEOXY-3'-THIOADENOSINE

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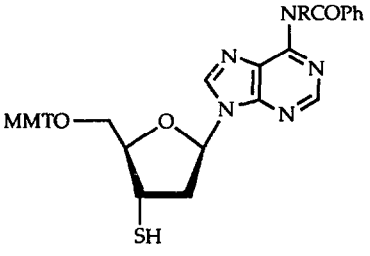
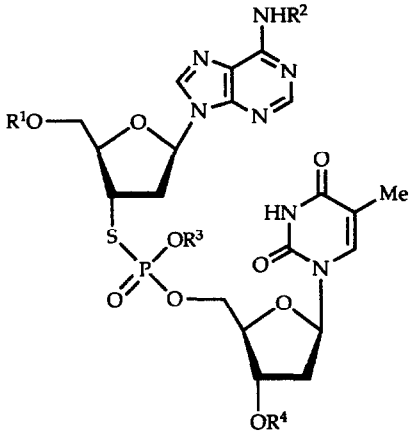
Abstract- *2'-Deoxy-3'-thio-5'-O-(4-monomethoxytrityl)-6-N-benzoyladenosine (**1a**) has been prepared from adenosine in seven steps and, following conversion of to a 3'-S-phosphorothioamidite, used to synthesize a dinucleoside 3'-S-phosphorothiolate containing 2'-deoxy-3'-thioadenosine. The procedure is compatible with automated methods of DNA synthesis and extends the potential of the recently developed phosphorothiolate method for strand specific cleavage of DNA.*

Introduction

In recent years we have reported the synthesis of dinucleosides[1-3] and oligodeoxyribonucleotides[3] containing the novel 3'-*S*-phosphorothiolate linkage. In particular the use of an appropriately protected 2'-deoxythymidine 3'-*S*-phosphorothioamidite has enabled us to prepare, by automated procedures, relatively long oligodeoxyribonucleotides (up to 18 residues) containing a single 3'-*S*-phosphorothiolate modification. We have shown that since the phosphorus-sulphur bond is susceptible to cleavage by either silver ions or aqueous iodine, but resistant to scission by the restriction endonuclease *Eco* RV, this modification can be used as a means to accomplish strand specific and sequence specific cleavage in high molecular weight DNA.[4] Since it may be possible to design useful protocols for engineering DNA based on this procedure it has become necessary to prepare oligodeoxyribonucleotides containing other 3'-thionucleosides so that the cleavage studies can be extended to a larger range of restriction endonucleases. In addition, the eventual synthesis of suitably protected 3'-thio derivatives of all four common deoxyribonucleosides would potentially enable the synthesis of novel anti-sense agents composed entirely of phosphorothiolate linkages. In the present publication we have started to address this

problem and now report the synthesis of suitably protected derivative of 2'-deoxy-3'-thioadenosine, [2'-deoxy-3'-thio-5'-*O*-(4-monomethoxytrityl)-6-*N*-benzoyladenosine, (**1a**)] and its incorporation into 2'-deoxy-3'-thioadenosylyl(3'-5')2'-deoxythymidine [d(AspT)] (**2**) by the phosphoramidite approach.





(**1a**) R=H
(**1b**) R=COPh

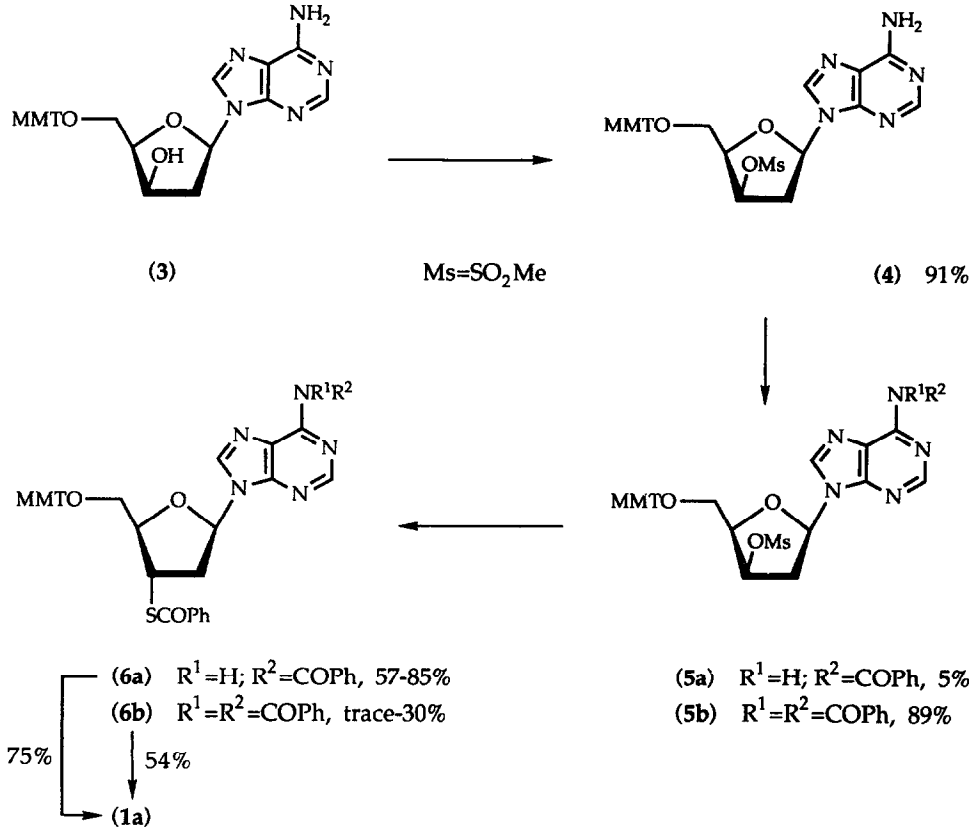
(**2**) $R^1=R^2=R^3=R^4=H$
(**9**) R^1=MMT; R^2=COPh, $R^3=CH_2CH_2CN$; R^4=OAc

Results and discussion

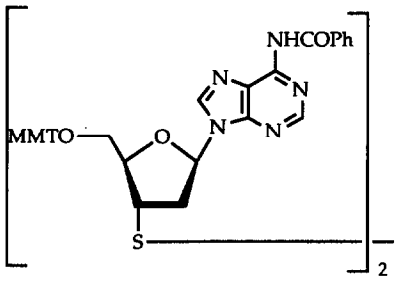
It is surprising that although 3'-thio derivatives of adenine nucleotides are simple and biologically interesting analogues they have received comparatively little attention. There are two published procedures for the preparation of 3'-thioadenosine,[5,6] but to our knowledge there is no reported synthesis of 2'-deoxy-3'-thioadenosine. The literature revealed that a suitably protected derivative of this thio nucleoside (**1a**) was likely to be available from 9-(2-deoxy-5-*O*-(4-monomethoxytrityl)-β-D-*threo*-pentofuranosyl)adenine (**3**) and the hydride-shift rearrangement developed by Haanske and Robins[7] was used as the key step in a three stage conversion of adenosine to (**3**) in an overall yield of 42%.[8] Treatment of (**3**) with excess methanesulphonyl chloride in dry pyridine gave the mesylate (**4**) in 91%. Consistent with our previous work on the synthesis of oligodeoxyribonucleotides and established practice, we chose to protect the exocyclic amino group on adenine as the benzoyl amide. Thus, slow addition of a four-fold excess of benzoyl chloride to a cooled solution of (**4**) in dry pyridine gave the 6,6-di-*N*-benzoyl derivative (**5b**) in 89% yield after chromatography. The more slowly eluting 6-*N*-benzoyl derivative (**5a**) was isolated as a minor component (up to 5% yield).

Following the procedure that we had used previously for the synthesis 3'-thio-2'-deoxythymidine derivatives,[1,2] (**5b**) was treated with a large excess of sodium thiobenzoate in dry DMF at 90°C for two hours. Under these conditions displacement of the methane sulphonate group was accompanied by partial debenzoylation to give the 6-*N*-benzoyl nucleoside (**6a**) in up to 85% yield and a trace of the 6,6-di-*N*-benzoyl nucleoside (**6b**). Unfortunately, these results were not reproduced consistently and in some instances the yield

of (**6b**) was much higher (up to 30%) with the yield of (**6a**) correspondingly reduced (57%). However, as expected both (**6a**) and (**6b**) could be selectively hydrolysed to (**1a**) (yields of 75% and 54% respectively) using a solution of sodium hydroxide in rigorously degassed aqueous ethanol at 5°C. The reaction time was particularly critical for the hydrolysis of (**6b**) since prolonged treatment resulted in oxidation of (**1a**) to the corresponding dimeric disulphide (**7**) whilst premature termination of the reaction yielded significant quantities of the 6,6-di-*N*-benzoyl thiol (**1b**).



Divakar *et al.* have previously reported the value of ^{13}C NMR in distinguishing between some 2'-thioribonucleosides and their dimeric disulphides and therefore the ^{13}C NMR spectra of the thiol (**1a**) and disulphide (**7**) were recorded and compared. The chemical shifts of the C-3' resonance signals of (**1a**) and (**7**) are 42.51 and 47.40 ppm respectively. The downfield shift of the disulphide resonance signal is consistent with the results obtained with 2'-thioribonucleosides, although the magnitude of the shift difference (5 ppm) is only about half of that observed by Divakar *et al.* In the ^1H NMR spectrum of (**1a**) recorded in $CDCl_3$, the thiol proton was clearly observed as a sharp doublet at 1.68 ppm. However, we have found that 2'-deoxy-3'-thionucleosides are most conveniently distinguished from their dimeric disulphides by TLC using Ellman's reagent[9] to stain the faster moving thiol.



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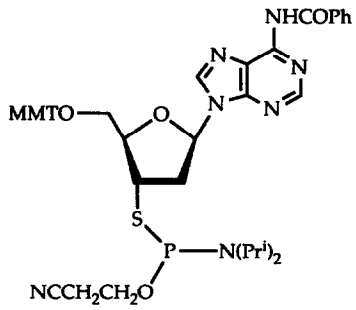

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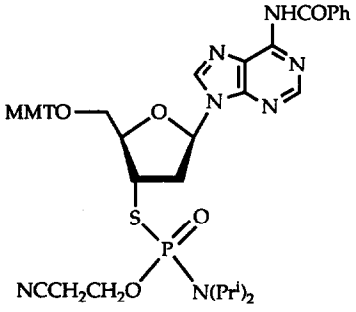

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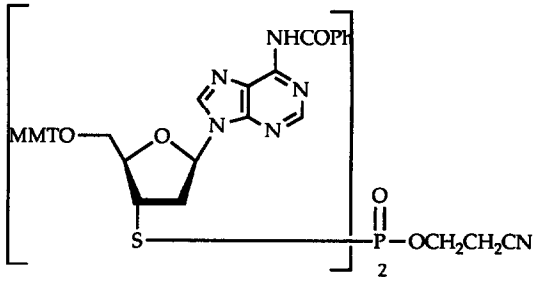

(11)

The phosphorothioamidite (**8**) was prepared as previously described[2] by reaction of the thiol (**1a**) with (2-cyanoethyl)-*N*,*N*-diisopropylaminochlorophosphine and isolated as a mixture of diastereoisomers (61% yield) which could be separated by careful chromatography. Conversion to the fully protected dinucleoside phosphorothiolate (**9**) was achieved by slow addition of a solution of the phosphorothioamidite (**8**) (2 equivalents) to a suspension of 3'-*O*-acetyl-2'-deoxythymidine and 5-(4-nitrophenyl)tetrazole (4 equivalents) in anhydrous acetonitrile. The intermediate phosphorothioite was oxidised *in situ* with tetra-*n*-butylammonium periodate and following chromatography the product was isolated in 30% yield [the yield was only 17% when a stoichiometric amount of (**8**) was used]. It has previosly been observed[2,3,10] that *N*,*N*-diisopropylaminophosphorothioamidites are surprisingly unreactive and we have found it necessary to use 5-(4-nitrophenyl)tetrazole as the activating agent which is more acidic than the conventially used tetrazole. Unfortunately, the thioalkyl group is particularly susceptible to displacement from the phosphorus centre under these acidic conditions; therefore coupling reactions with the phosphorothioamidite are accompanied by the formation of many side-products and give low yields. In particular we have identified the symmetrical dithiolate (**10**), which results from the liberated thio nucleoside reacting with (**8**) and the phosphorothioamidate (**11**) derived from oxidation of unactivated (**8**).

Deprotection of (**9**) was accomplished by sequential treatment with 80% acetic acid and concentrated aqueous ammonia (50°C, 12 hours). The crude d(AspT) purified by elution from

a DEAE Sephadex column with a gradient of triethylammonium bicarbonate (TEAB) and finally converted to the sodium salt by passage down an ion-exchange column. The purified product was characterised by ^{1}H nmr, ^{31}P nmr and reverse-phase hplc (Fig. 1).

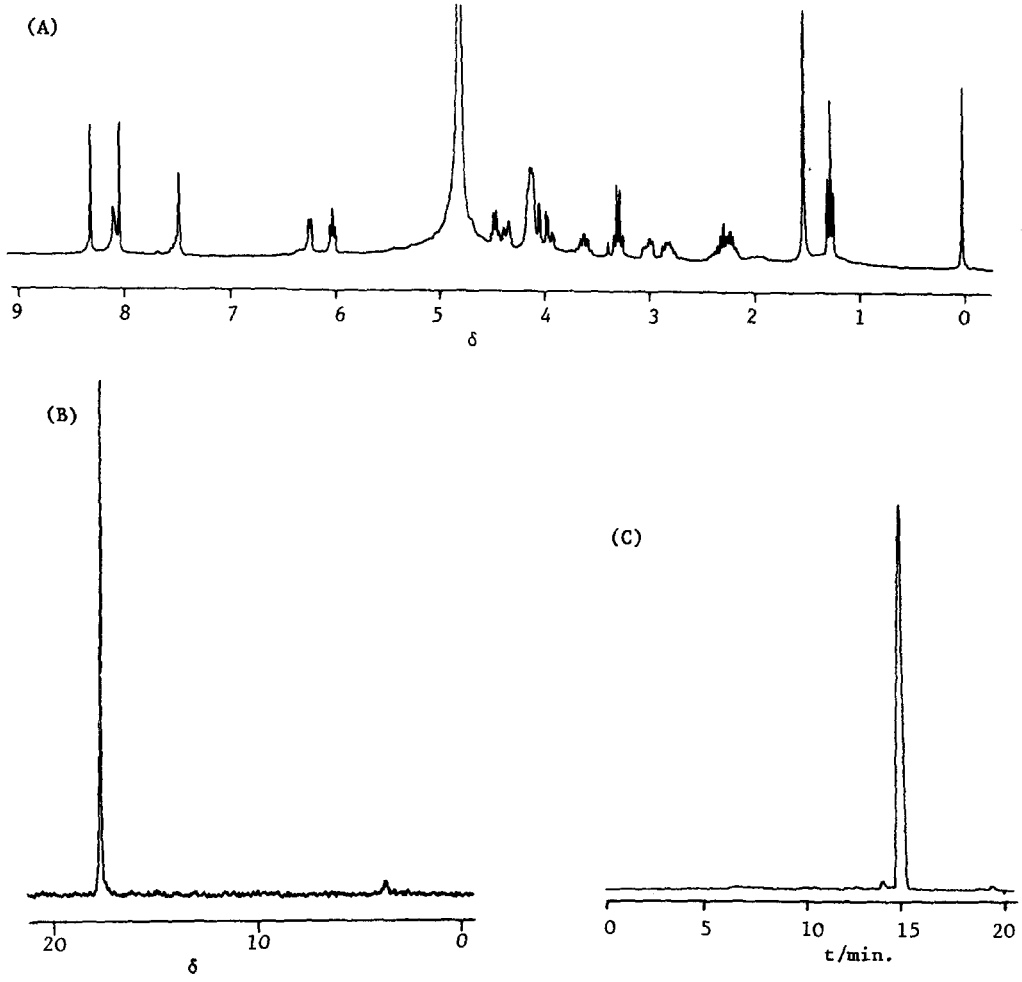

Figure 1. Characterisation of d(AspT). (A) ^{1}H Nmr spectrum (δ 4.8 HOD and δ 1.3 and 3.3 residual triethylammonium salts). (B) ^{31}P Nmr spectrum. (C) Hplc chromatogram obtained on a C-18 reverse-phase column using a 20 min linear gradient of 5-20% CH_3CN in 100 mM triethylammonium acetate.

In conclusion, adenosine is a cheap and convenient starting material for the efficient preparation of a 2'-deoxy-3'-thioadenosine derivative suitably protected for oligonucleotide synthesis. Incorporation into a dinucleoside phosphorothiolate and subsequent deprotection is demonstrated under conditions that are compatible with automated DNA synthesis. The

study extends both the the diversity of oligodeoxynucleotides that can be prepared and the potential of the phosphorothiolate method for strand specific cleavage of DNA.

Experimental

2-Cyanoethyl-*N,N*-diisopropylaminochlorophosphite[11] and sodium thiobenzoate[2] were prepared as previously described. Pyridine was refluxed and distilled from ninhydrin and then freshly distilled from potassium hydroxide pellets. Acetonitrile and *N,N*-diisopropylethylamine were refluxed and distilled from calcium hydride. Dichloromethane was refluxed and distilled from phosphorus pentoxide and passed through basic alumina immediately before use. *N,N*-Dimethylformamide (DMF) was stored over barium oxide for at least 3 days and then distilled under redued pressure (20 mm Hg). FAB mass spectra were recorded on a VG Analytical 7070E mass spectrometer operating with a PDP 11/250 data system and an Ion Tech FAB ion gun working at 8 Kv. High resolution FAB mass spectra were obtained on a VG ZAB/E spectrometer at the SERC Mass Spectrometry Service Centre and reported masses are accurate to ± 5 ppm. 3-Nitrobenzyl alcohol was used as a matrix unless stated otherwise. ^{1}H and ^{13}C Nmr spectra were measured on either a Bruker WM250 or Bruker AC200 spectrometer. Chemical shifts are given in ppm downfield from an internal standard of tetramethylsilane for spectra recorded in $CDCl_3$; spectra recorded in D_2O are referenced to sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4. Proton decoupled ^{31}P nmr spectra are referenced to 85% phosphoric acid. Analytical tlc was performed on Alugram sil G-UV_{254} plates. Thiol containing nucleosides were visualised by spraying with a 0.1% (w/v) solution of Ellman's reagent in a 1:1 mixture of ethanol-0.45 M aqueous Tris-HCl pH 8.5, all other nucleosides were visualised as a black spot by spraying with a solution of 5% (v/v) sulphuric acid and 3% (w/v) phenol in ethanol and charing at 120°C.

9-[2-Deoxy-3-*O*-methanesulphonyl-5-*O*-(4-monomethoxytrityl)-β-D-*threo*-pentofuranosyl]-adenine (4).

To a solution of (3) (1.0 g, 1.91mmol) in dry pyridine (20 ml) at 0°C, methanesulphonyl chloride (purified by passage through a column of basic alumina) (0.48 ml, 6.48 mmol) was added and the solution stored overnight at 4°C. The reaction mixture was poured into water (150 ml), extracted twice with chloroform and the combined extracts dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography: the appropriate fractions, eluted with chloroform-methanol (98:2-96:4) were combined and evaporated to give the product (4) (1.05 g, 91%) as a white solid. (Found: C, 61.67; H, 5.18; N, 11.57. $C_{31}H_{31}N_5O_6S$ requires C, 61.88; H, 5.19; N, 11.64%); δ_H ($CDCl_3$) 2.73 (3H, s, $MeSO_2$), 2.93 (2H, m, H-2', H-2''), 3.35 (1H, m, H-5'), 3.67 (2H, m, H-5''), 3.80 (3H, s, OMe), 4.40 (1H, m, H-4'), 5.44 (1H, m, H-3'), 5.70 (2H, br s, NH_2), 6.48 (1H, t, J=5.0 Hz, H-1'), 6.84 (2H, d, J 8.8 Hz, *o*-anisyl), 7.25-7.44 (12H, m, Ar), 8.01 (1H, s, H-2) and 8.34 (1H, s, H-8); m/z (FAB^+) 602 ($M+H^+$, 27%).

6-*N*,*N*-Dibenzoyl-9-[2-deoxy-3-*O*-methanesulphonyl-5-*O*-(4-monomethoxytrityl)-β-D-*threo*-pentofuranosyl]adenine (5b).

To a solution of (**4**) (1.30 g, 2.17 mmol) in dry pyridine at 0°C (5 ml) freshly distilled benzoyl chloride (1.26 ml, 10.8 mmol) was added and after 4 hours at this temperature the mixture was poured into ice-water (200 ml) and extracted twice with chloroform. The organic phase was washed with saturated sodium bicarbonate, brine, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography: the appropriate fractions, eluted with dichloromethane-methanol (98:2) were combined and evaporated to give the product (**5b**) (1.57 g, 89%) as a white solid. (Found: C, 66.40; H, 4.79; N, 8.73. $C_{45}H_{39}N_5O_8S$ requires C, 66.74; H, 4.85; N, 8.65%); δ_H ($CDCl_3$) 2.69 (3H, s, $MeSO_2$), 2.96 (2H, m, H-2', H-2''), 3.35-3.69 (2H, m, H-5', H-5''), 3.80 (3H, s, OMe), 4.40 (1H, m, H-4'), 5.46 (1H, m, H-3'), 6.56 (1H, t, J=5.1Hz,, H-1'), 6.84 (2H, d, J 8.8 Hz, *o*-anisyl), 7.20-7.51 (18H, m, Ar), 7.83 (4H, d, J=6.9 Hz, *o*-benzoyl), 8.29 (1H, s, H-2) and 8.61 (1H, s, H-8); m/z (FAB+) 810 ($M+H^+$, 4%).

The slower eluting monobenzoate (**5a**) was also isolated as a minor product (0.06 g, 4%); (Found: C, 64.50; H, 4.94; N, 9.67. $C_{38}H_{35}N_5O_7S$ requires C, 64.67; H, 5.00; N, 9.92%); δ_H ($CDCl_3$) 2.73 (3H, s, $MeSO_2$), 2.97 (2H, m, H-2', H-2''), 3.36 (1H, m, H-5'), 3.69 (1H, m, H-5''), 3.79 (3H, s, OMe), 4.41 (1H, m, H-4'), 5.48 (1H, m, H-3'), 6.59 (1H, t, J=5.0 Hz, H-1'), 6.84 (2H, d, J 8.8 Hz, *o*-anisyl), 7.20-7.58 (15H, m, Ar), 8.02 (2H, d, J=6.9 Hz, *o*-benzoyl) 8.34 (1H, s, H-2), 8.77 (1H, s, H-8) and 9.28 (1H, br s, NH); m/z (FAB+) 706 ($M+H^+$).

2'-Deoxy-3'-*S*-thiobenzoyl-5'-*O*-(4-monomethoxytrityl)-6-*N*-benzoyladenosine (6a)

The nucleoside (**5b**) (0.3g, 0.425 mmol) and sodium thiobenzoate (0.54 g, 3.4 mmol) were dissolved in dry DMF (10 ml) and heated at 90°C for 1.5 hours. After cooling to room temperature the mixture was diluted with dichloromethane (50 ml) and the solution washed with a mixture of saturated brine and saturated sodium bicarbonate solution (1:1, 100 ml). The aqueous layer was extracted with dichloromethane, the organic layers combined, dried (Na_2SO_4), evaporated and solvent residues removed by co-evaporation with toluene. The residue was purified by column chromatography: the appropriate fractions, eluted with chloroform were combined and evaporated to give the product (**6a**) (57-85%) as a white solid. (Found: C, 70.79; H, 5.09; N, 9.45. $C_{44}H_{37}N_5O_5S$ requires C, 70.66; H, 4.99; N, 9.36%); δ_H ($CDCl_3$) 2.72 (1H, m, H-2'), 3.25 (1H, m, H-2''), 3.50 (2H, m, H-5', H-5''), 3.73 (3H, s, OMe), 4.33 (1H, m, H-4'), 4.59 (1H, m, H-3'), 6.49 (1H, dd, J=6.6, 4.1 Hz, H-1'), 6.77 (2H, d, J=8.9 Hz, *o*-anisyl), 7.18-7.66 (18H, m, Ar), 7.91 (2H, d, J=8.7 Hz, *o*-benzoyl), 8.03 (2H, d, J=6.6 Hz, *o*-thiobenzoyl),8.35 (1H, s, H-2), 8.81 (1H, s, H-8) and 9.09 (1H, br s, NH); m/z (FAB+) 748 ($M+H^+$, 10%).

2'-Deoxy-3'-*S*-thiobenzoyl-5'-*O*-(4-monomethoxytrityl)-6-*N*,*N*-dibenzoyladenosine (6b) was also obtained in varying yield (trace-30%) as a white solid. (Found: C, 71.94; H, 4.85; N, 8.24. $C_{51}H_{44}N_5O_6S$ requires C, 71.90; H, 4.85; N, 8.22%); δ_H ($CDCl_3$) 2.69 (1H, m, H-2'), 3.26 (1H, m, H-2''), 3.35-3.55 (2H, m, H-5', H-5''), 3.70 (3H, s, OMe), 4.32 (1H, m, H-4'), 4.58 (1H, m, H-3'), 6.49 (1H, dd J=6.6, 4.2 Hz, H-1'), 6.75 (2H, d, J 8.0 Hz, *o*-anisyl), 7.18-7.66 (21H, m, Ar), 7.82 (4H, d, J=7.3 Hz, *o*-benzoyl), 7.89 (2H, d, J=8.7 Hz, *o*-thiobenzoyl), 8.41 (1H, s, H-2) and 8.63 (1H, s, H-8); m/z (FAB+) 852 ($M+H^+$).

2'-Deoxy-3'-thio-5'-*O*-(4-monomethoxytrityl)-6-*N*-benzoyladenosine (1a)

To a solution of (**6a**) or (**6b**) (0.48 mmol) in argon saturated ethanol (60 ml) at 5°C aqueous sodium hydroxide (0.4 ml, 10 M) was added with vigorous stirring. After 2 hours the mixture was partitioned between chloroform (300 ml) and saturated sodium bicarbonate (200 ml). the aqueous layer was extracted further with chloroform (2 X 100 ml) the organic extracts combined, dried (Na_2SO_4) and evaporated to a foam. The crude material was purified by column chromatography: the appropriate fractions, eluted with chloroform-methanol (98:2) were combined and evaporated to give the product (**1a**) [74% from (**6a**) and 54% from (**6b**] as a white solid. Rf 0.48 (dichloromethane-methanol, 95:5); (Found: $(M+1)^+$, 644.2331. $C_{37}H_{34}N_5O_3S$ requires.$(M+1)^+$, 644.2331); δ_H ($CDCl_3$) 1.65 (1H, d, J=7.0 Hz, SH), 2.52 (1H, m, H-2'), 3.08 (1H, m, H-2''), 3.39-3.60 (2H, m, H-5', H-5''), 3.78 (3H, s, OMe), 3.84 (1H, m, H-3'), 4.01 (1H, m, H-4'), 6.39 (1H, dd, J=7.0, 2.2 Hz, H-1'), 6.81 (2H, d, J=8.8 Hz, *o*-anisyl), 7.21-7.60 (15H, m, Ar), 8.02 (2H, d, J=6.7 Hz, *o*-benzoyl) 8.30 (1H, s, H-2), 8.78 (1H, s, H-8) and 9.20 (1H, br s, NH); δ_C ($CDCl_3$) 35.09, 42.51, 55.32, 61.91, 84.65, 87.04, 88.60, 113.41, 123.65, 127.29, 128.12, 128.54, 129.04,130.49, 132.92, 133.95, 135.30, 141.76, 144.10, 149.75, 151.14, 152.87, 158.92, 164.79.

***Bis*-[2'-deoxy-3'-thio-5'-*O*-(4-monomethoxytrityl)-6-*N*-benzoyladenosine] disulphide (7)** was also isolated from the column in about 5% yield. Rf 0.31 (dichloromethane-methanol, 95:5); δ_H ($CDCl_3$) 2.72 (2H, m, H-2'), 3.11 (2H, m, H-2''), 3.35-3.56 (4H, m, H-5', H-5''), 3.74 (6H, s, OMe), 3.92 (2H, m, H-3'), 4.17 (2H, m, H-4'), 6.27 (2H, m, H-1'), 6.77 (4H, d, J 7.0 Hz, *o*-anisyl), 7.16-7.60 (30H, m, Ar), 8.02 (2H, d, J 8.0 Hz, *o*-benzoyl), 8.21 (2H, s, H-2), 8.71 (2H, s, H-8) and 9.15 (2H, br s, NH); δ_C ($CDCl_3$) 38.11, 47.40, 55.24, 63.17, 84.72, 84.84, 86.98, 113.24, 123.52, 127.15, 127.94, 128.32, 128.82, 130.28, 132.74, 133.71, 135.00, 141.73, 143.88, 149.67, 151.01,152.61, 158.71,164.72; m/z (FAB$^+$) 1307 ($M+Na^+$), 1285 ($M+H^+$).

[2'-Deoxy-3'-thio-5'-*O*-(4-monomethoxytrityl)-6-*N*-benzoyladenosine]-3'-*S*-(2-cyanoethyl)-*N,N*-diisopropylphosphorothioamidite (8)

2-Cyanoethyl *N,N*-diisopropylaminochlorophosphoramidite (0.23 ml, 13 mmol) was added dropwise over 2 minutes to a stirred solution of (**1a**) (0.42 g, 0.65 mmol) and *N,N*-diisopropylethylamine (0.58 mL, 3.25 mmol) in dichloromethane (2 ml) at room temperature. The reaction was stirred for a further 1 hour, quenched with methanol (0.04 ml) and taken up into ethyl acetate (40 ml). This solution was washed with saturated solutions of sodium bicarbonate (2 x 15 ml) and sodium chloride (2 x 15 ml), dried and evaporated. The residual gum was subjected to column chromatography on silica gel eluting with 40-60 petroleum ether-dichloromethane-ethyl acetate-triethylamine (5:4:5:1). Fractions containing individual diastereoisomers were pooled, evaporated, dissolved in ethyl acetate (4 ml) and precipitated into vigorously stirred 40-60 petroleum ether . The pure diastereoisomers were collected by filtration and dried under vacuum (total yield 0.33 g, 61%).

Fast diastereoisomer (27%): Rf 0.87 (hexane-dichloromethane-ethyl acetate-triethylamine, 5:5:4:1); δ_H ($CDCl_3$) 1.13 (6H, d, J 6.0 Hz, $(CH_3)_2C$), 1.18 (6H, d, J 6.0 Hz $(CH_3)_2C$), 2.43 (2H, t, CH_2CN), 2.68-3.10 (2H, m, H-2´ and H-2´´), 3.38-3.70 (6H, m, CH_2O, (CH)N, ,H-5´ and

H-5´´), 3.77 (3H, s, CH_3O), 3.83 (1H, m, H-3´) 4.23 (1H, m, H-4´), 6.41 (1H, m, H-1´), 6.79 (2H, d, J 7.0 Hz, *o*-anisyl) 7.23-7.63 (15H, m, ArH), 8.02 (2H, d, J 8.0 Hz, *o*-benzoyl), 8.30 (1H, s, H-2), 8.76 (1H, s, H-8) and 9.08 (1H, br s, NH); δ_P ($CDCl_3$): 164.5; m/z (FAB$^+$) 844 (M+H$^+$).

Slow diastereoisomer (34%): Rf 0.79 (hexane-dichloromethane-ethyl acetate-triethylamine, 5:5:4:1); δ_H ($CDCl_3$) 1.06 (6H, d, J 6.0 Hz, $(CH_3)_2C$), 1.17 (6H, d, J 6.0 Hz $(CH_3)_2C$), 2.56 (2H, t, CH_2CN), 2.71-3.12 (2H, m, H-2´ and H-2´´), 3.41 (2H, m, H-5´ and H-5´´), 3.70 (4H, m, CH_2O, and (CH)N), 3.77 (3H, s, CH_3O), 3.83 (1H, m, H-3´) 4.23 (1H, m, H-4´), 6.45 (1H, m, H-1´), 6.77 (2H, d, J 7.0 Hz, *o*-anisyl) 7.20-7.59 (15H, m, ArH), 8.01 (2H, d, J 7.0 Hz, *o*-benzoyl), 8.28 (1H, s, H-2), 8.76 (1H, s, H-8) and 9.10 (1H, br s, NH); δ_P ($CDCl_3$): 161.1; m/z (FAB$^+$) 844 (M+H$^+$).

Fully protected d(AspT) (9)

A mixture of 5-(4-nitrophenyl)tetrazole (337 mg, 1.76 mmol) and 3'-*O*-acetyl-2'-deoxythymidine (100 mg, 0.35 mmol) were suspended in dry acetonitrile (6ml) and a solution of (8) (743 mg, 0.88 mmol) also in dry acetonitrile added dropwise over 15 minutes to the stirred mixture. After an additional 15 minutes 2,6-lutidine (0.1 ml) was added and followed by a solution of tetra-*n*-butylammonium periodate (761 mg, 1.76 mmol) in dichloromethane (6 ml) and the solution stirred for 7 minutes. The reaction mixture was diluted with dichloromethane (50 ml) and washed sequentially with aqueous solutions of sodium metabisulphite, saturated sodium bicarbonate and sodium chloride. The organic layer was dried (Na_2SO_4), evaporated and the crude product purified by column chromatography: the appropriate fractions, eluted with a gradient of dichloromethane-methanol (98:2-96:4) were combined and evaporated to give the product (9) as separate diastereoisomers. (total yield 226 mg, 30%)

Fast diastereoisomer: Rf 0.37 (dichloromethane-methanol, 95:5); (Found: $(M+1)^+$ 1043.3163. $C_{52}H_{52}N_8O_{12}PS$ requires. $(M+1)^+$, 1043.3163); δ_H ($CDCl_3$) 1.85 (3H, s, CH_3 dT), 2.08 (3H, s, CH_3CO), 2.22-2.37 (2H, m, H-2´ and H-2´´dT), 2.69-2.86 (3H, m, CH_2CN and H-2´ dA), 3.31-3.52 (3H, m, H-2´´,H-5´ and H-5" dA), 3.79 (3H, s, CH_3O), 4.12-4.38 (7H, m, CH_2O, H5´, H5´´, H-4´ dT and H-3´, H-4´ dA), 5.24 (1H, m, H-3´dT), 6.27 (1H, t, J=6.1 Hz, H-1' dT), 6.43 (1H, dd, J=5.1, 7.9 Hz, H-1´ dA), 6.80 (2H, d, J=8.5 Hz, *o*-anisyl), 7.23-7.61 (16H, m, ArH, H6), 8.04 (2H, d, J=7.3 Hz, *o*-benzoyl) 8.22 (1H, s, H-2), 8.76 (1H, s, H-8), 8.94 (1H, s, NH), 9.19 (1H, s, NH); δ_P ($CDCl_3$) 25.8.

Slow diastereoisomer: Rf 0.32 (dichloromethane-methanol, 95:5); (Found: $(M+1)^+$ 1043.3163. $C_{52}H_{52}N_8O_{12}PS$ requires. $(M+1)^+$, 1043.3163); δ_H ($CDCl_3$) 1.85 (3H, s, CH_3 dT), 2.09 (3H, s, CH_3CO), 2.22-2.37 (2H, m, H-2´ and H-2´´dT), 2.66-2.82 (3H, m, CH_2CN and H-2´ dA), 3.31-3.57 (3H, m, H-2´´,H-5´ and H-5" dA), 3.77 (3H, s, CH_3O), 4.14-4.39 (7H, m, CH_2O, H5´, H5´´, H-4´ dT and H-3´, H-4´ dA), 5.24 (1H, m, H-3´dT), 6.24 (1H, t, J=6.1 Hz, H-1' dT), 6.43 (1H, dd, J=5.1, 7.9 Hz, H-1´ dA), 6.79 (2H, d, J=8.5 Hz, *o*-anisyl), 7.22-7.61 (16H, m, ArH, H6), 8.05 (2H, d, J=7.3 Hz, *o*-benzoyl) 8.23 (1H, s, H-2), 8.75 (1H, s, H-8), 9.36 (1H, s, NH), 9.56 (1H, s, NH); δ_P ($CDCl_3$) 25.4.

Phosphorothioamidate (11) was isolated from the column as a mixture of diastereoisomers (about 30% yield). δ_H ($CDCl_3$) 1.15-1.28 (12H, m, $(CH_3)_2C$), 2.53-2.70 (2H, m,

CH_2CN), 2.84 (1H, m, H-2′), 3.38-3.68 (7H, m, CH_2O, (CH)N, H-2', H-5′ and H-5′′), 3.77 (3H, s, CH_3O), 4.04-4.27 (1H, m, H-3′ and H-4′), 6.45 (1H, m, H-1′), 6.79 (2H, d, J 8.8 Hz, *o*-anisyl) 7.23-7.63 (15H, m, ArH), 8.03 (2H, d, J 6.9 Hz, *o*-benzoyl), 8.26 (1H, s, H-2), 8.74 (1H, s, H-8) and 9.08 (1H, br s, NH); δ_P ($CDCl_3$): 29.98, 30.47; m/z (FAB$^+$) 860 (M+H$^+$).

Symmetrical dithiolate (10) was also isolated in about 5% yield. δ_H ($CDCl_3$) 2.55 (2H, t, CH_2CN), 2.8 (2H, m, H-2′), 3.30-3.48 (6H, m, H-2", H-5′ and H-5′′), 3.74 (6H, s, CH_3O), 4.09-4.37 (6H, m, H-3′, H-4′ and CH_2O), 6.42 (2H, dd, J=5.0, 7.2 Hz, H-1′), 6.77 (4H, d, J 8.8 Hz, *o*-anisyl) 7.23-7.63 (30H, m, ArH), 7.80 (4H, d, J 7.1 Hz, *o*-benzoyl), 8.28 (2H, s, H-2), 8.60 (2H, s, H-8) and 9.20 (2H, br s, NH); δ_P ($CDCl_3$): 50.42.

d(AspT) (2)

The fully protected dinucleotide (**9**) (85mg, 80μmol) was dissolved in a solution of acetic acid (80% in water). After 5 hours at room temperature the solution concentrated to dryness and traces of acetic acid removed by coevaporation with water. The residue was dissolved in concentrated aqueous ammonia solution, transferred to a screw-top vial and heated at 50^{0}C for 12 hours. The solution was evaporated to dryness, dissolved in water (5 ml) and extracted twice with ether. The aqueous layer was purified on a column of DEAE-A25 Sephadex eluting with a gradient of triethylammonium bicarbonate (0.05-0.2 M). Appropriate fractions were pooled, evaporated, coevaporated with water and the product obtained as the sodium salt after passage down a Dowex 50w x 8 (Na$^+$) column (yield 1100 A_{260} units)
δ_H (D_2O) 1.52 (3H, s, CH_3), 2.29 (2H, m, H2', H2" dT), 2.81-2.91 (2H, m, H2', H2" dA), 3.61 (1H, m, H3' dA), 3.97-4.15 (5H, m, H5', H5", H4' dA and H5', H4' dT), 4.36 (1H, m, H5" dT), 4.57 (1H, m, H3' dT), 6.02 (1H, t, J=5.4 Hz, H1' dT), 6.31 (1H, d, J=4.2 Hz H1' dA), 7.47 (1H, s, H6), 8.03 (1H, s, H-2), 8.31 (1H, s, H-8); δ_P (D_2O): 17.32; m/z (FAB$^-$, glycerol matrix) 570 (M-H$^-$).

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