Aminolysis and alkaline hydrolysis of protected 1-hydroxybenzotriazol-1-yl esters of adenosine 5′-phosphorothioate and -phosphorodithioate

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When 2′,3′-bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)adenosine 9, which was prepared from adenosine in six steps and in ~70% overall yield, was treated with putative tri(benzotriazol-1-yl) phosphorothioate 11 and the products then worked up with aq. triethylamine, the triethylammonium salt of the protected benzotriazol-1-yl ester 6a of adenosine 5′-phosphorothioate was obtained in high yield. The corresponding adenosine 5′-phosphorodithioate derivative 6b was prepared, also in high yield, by a modification of the latter procedure involving work-up with hydrogen sulfide and triethylamine. Reaction between the phosphorothioate derivative 6a and methylamine or morpholine gave the phosphorothioamidates 12a and 13a, respectively, in high yields; in the same way, the phosphorodithioate derivative 6b was converted into the phosphorodithioamidates 12b and 13b. Treatment of the phosphorothioate derivative 6a first with aq. alkali and then with aq. acid gave a mixture of adenosine 5′-phosphorothioate 15a and adenosine 3′,5′-cyclic phosphorothioate 17a. When the phosphorodithioate derivative 6b was treated in the same way, adenosine 3′,5′-cyclic phosphorodithioate 17b was obtained in good yield.

Introduction

Over thirty years ago, Cramer and his co-workers showed[1-3] that ribonucleoside 5′-phosphorimidazolides **1a** behaved as activated nucleotides in that they reacted with primary amines, alcohols and monoalkyl phosphates to give the corresponding phosphoramidates, phosphodiesters and P^1,P^2-dialkyl pyrophosphates, respectively. Hoard and Ott then showed[4] that 2′-deoxyribonucleoside 5′-phosphorimidazolides **1b** reacted with inorganic pyrophosphate to give the corresponding 2′-deoxyribonucleoside 5′-triphosphates **2a**. Orgel and his co-workers[5] later demonstrated that nucleoside 5′-phosphoro-2-methylimidazolides are an effective group of activated nucleotides in the template-directed chemical synthesis of oligonucleotides.

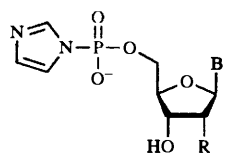
Nucleoside 5′-phosphorothioates have also been activated as their imidazolides. Thus Eckstein and Gindl reported[6] that the nucleoside 5′-thiophosphorimidazolides **3a** and **3b** reacted with inorganic pyrophosphate to give the corresponding nucleoside 5′-α-thiotriphosphates **2b** (R = OH and H, respectively). We recently demonstrated[7] that a nucleoside 3′-thiophosphoro-(1,2,4-triazolide) **4a** and the related 1-hydroxybenzotriazole derivative **5a**, which may both be regarded as activated forms of protected 2′-deoxyribonucleoside 3′-phosphorothioates, can easily be prepared in good yield. We further showed that the corresponding activated forms (**4b** and **5b**, respectively) of protected thymidine phosphorodithioates can also be readily prepared.[7] The 1-hydroxybenzotriazole derivatives **5a** and **5b** proved[7] both to be somewhat easier to isolate and more reactive towards methylamine than were the 1*H*-1,2,4-triazole derivatives **4a** and **4b**.

Activated derivatives of adenosine 5′-phosphorothioate and -phosphorodithioate are potentially of value in the synthesis of analogues of biologically important molecules derived from adenosine 5′-phosphate. The latter class of compounds[8] includes adenosine 3′,5′-cyclic phosphate, adenosine 5′-diphosphate (ADP), adenosine 5′-triphosphate (ATP), and certain nucleotide coenzymes (including NAD, NADP and coenzyme A). We have already reported, in outline,[7,9] procedures for the preparation of the benzotriazol-1-yl esters (**6a** and **6b**, respectively) of protected adenosine 5′-phosphorothioate and -phosphorodithioate and their reactions with amines. We now describe, in detail, procedures for the preparation of these 1-hydroxybenzotriazole esters **6a** and **6b** and their reactions both with amines and with aq. alkali.

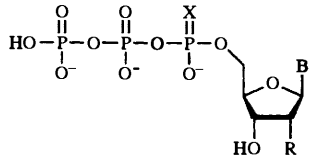
Results and discussion

2′,3′-Bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)adenosine[7] **9**, the protected adenosine derivative required in this study was prepared from adenosine **7** in six steps and in almost 70% overall yield according to the procedure indicated in outline in Scheme 1 and described in detail in the Experimental section. The 9-phenylxanthen-9-yl (Px)[10] group was used to protect the 6-amino function of the adenine residue in order to ensure that the activated adenosine 5′-phosphorothioate and -phosphorodithioate derivatives (**6a** and **6b**, respectively) were sufficiently lipophilic to enable them to be purified without difficulty by short-column chromatography (SCC) on silica gel; this protecting group is easily removable under mild conditions of acidic hydrolysis.[10] The methoxyacetyl protecting group,[11] which was chosen to protect the 2′- and 3′-hydroxy functions of the ribose moiety, is removable by ammonolysis or alkaline hydrolysis under very mild conditions indeed.

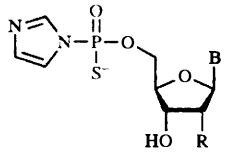
The procedures used[7] for the conversion of the adenosine building block **9** into the activated adenosine 5′-phosphorothioate **6a** and -phosphorodithioate **6b** derivatives are indicated in outline in Scheme 2. The building block **9** was first treated with a three-fold excess of a reagent of putative structure **11**, prepared by allowing thiophosphoryl trichloride to react with three molecular equivalents each of 1-hydroxybenzotriazole and triethylamine in tetrahydrofuran (THF). After the reaction had been allowed to proceed at 0 °C for 30 min, the products were worked up in two different ways. In the first work-up procedure (Scheme 2, step ii), an excess each of triethylamine and water was added. Fractionation of the products by chromatography on silica gel, followed by evaporation of the appropriate fractions, gave the triethylammonium salt of the activated phosphorothioate **6a** which was isolated as a precipitated solid in ~93% yield. In the second work-up procedure (Scheme 2, step iii), the initial phosphorylation products were treated with triethylamine and hydrogen sulfide under anhydrous conditions. The products were then processed



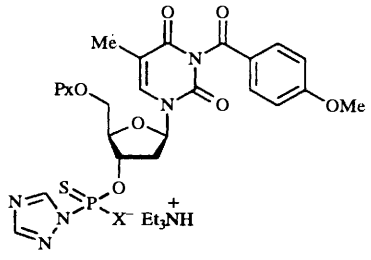
1a; R = OH
b; R = H



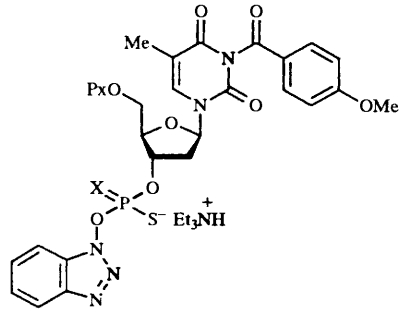
2a; R = H, X = O
b; X = S



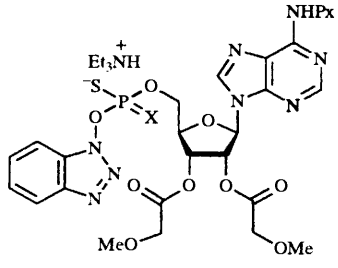
3a; R = OH
b; R = H



4a; X = O
b; X = S

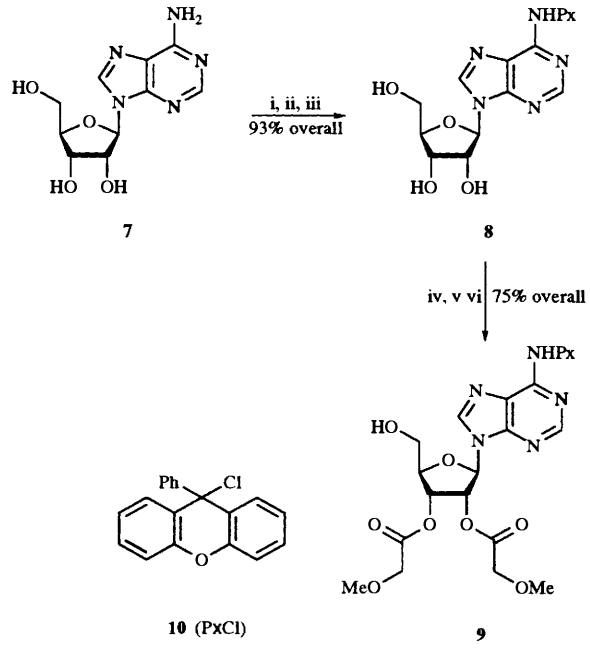


5a; X = O
b; X = S



6a; X = O
b; X = S

Px = 9-phenylxanthen-9-yl



10 (PxCl)

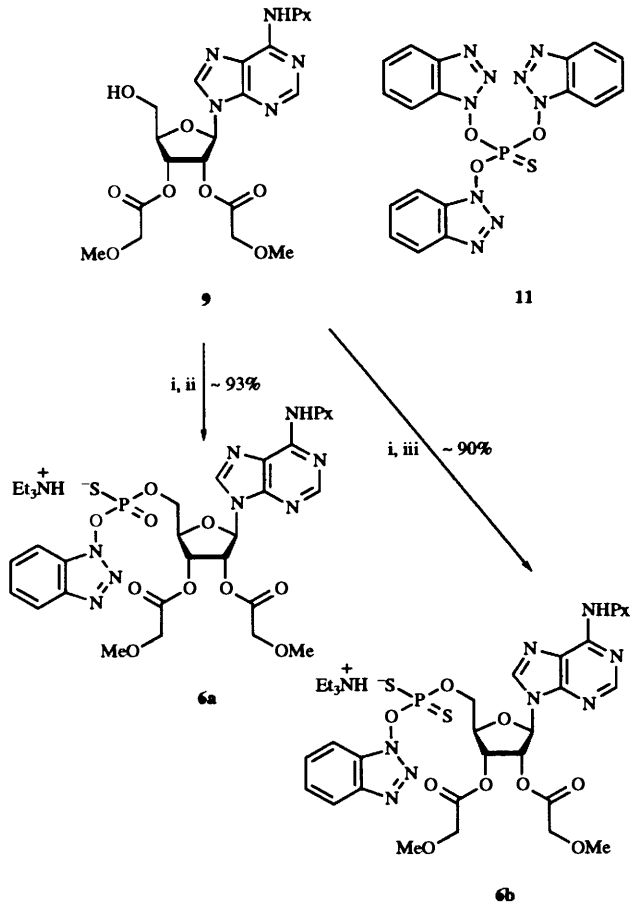
9

Scheme 1 *Reagents and conditions:* i, Ac_2O, C_5H_5N, room temp.; ii, PxCl **10**, C_5H_5N, 90 °C, 2 h; iii, NH_3, MeOH, room temp.; iv, $Me_3C{\cdot}Si(Me_2)Cl$, C_5H_5N, room temp.; v, $(MeOCH_2CO)_2O$, C_5H_5N, room temp.; vi, Et_4NF, AcOH, MeCN, room temp., 48 h

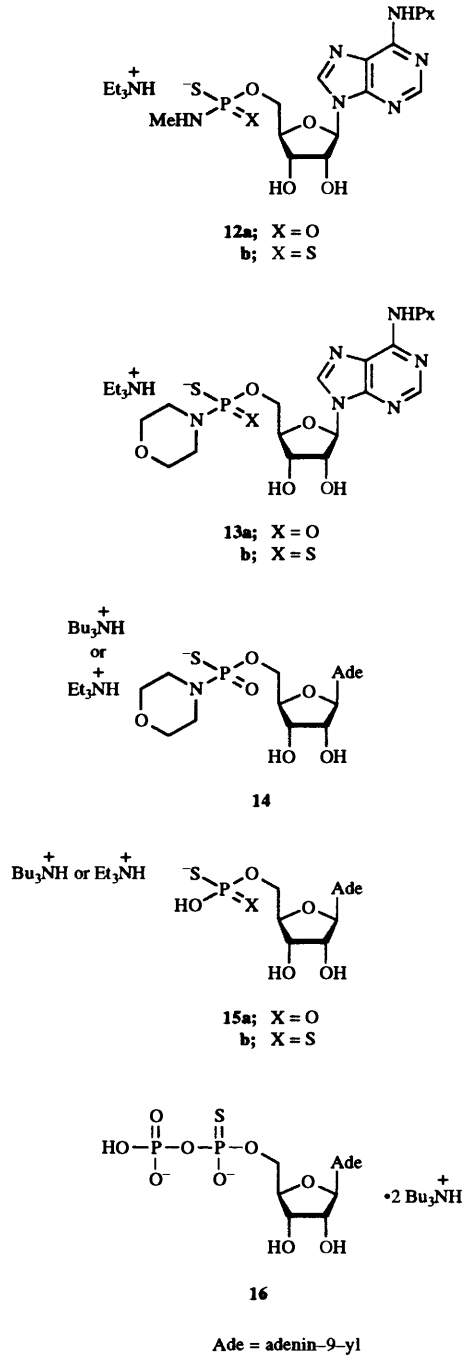
in the same way and fractionated by chromatography on silica gel to give the triethylammonium salt of the activated phosphorodithioate **6b** which was obtained as a precipitated solid in ~90% isolated yield. Both activated esters **6a** and **6b** were found to be homogeneous by reversed-phase HPLC and were characterized on the basis of their ^1H and ^{31}P NMR spectra. The respective chemical shifts of the phosphorus resonance signals in the ^{31}P NMR spectra of the 5′-phosphorothioate **6a** and -phosphorodithioate **6b** derivatives were δ_P 59.9 and 125.5. Although it was clear from its ^1H NMR spectrum that the 5′-phosphorothioate **6a** was a mixture of diastereoisomers, only one resonance signal was observed in its ^{31}P NMR spectrum.

The activated 5′-phosphorothioate and -phosphorodithioate derivatives (**6a** and **6b**, respectively) were found to react readily with primary and secondary amines to give phosphorothio- and phosphorodithio-amidates. Thus when the activated phosphorothioate derivative **6a** was allowed to react with a large excess of methylamine [7] in ethanol solution at room temperature, the 5′-(*N*-methylphosphorothioamidate) of 6-*N*-(9-phenylxanthen-9-yl)adenosine was obtained. Following work-up and chromatography of the products on silica gel, the triethylammonium salt **12a** was isolated as an HPLC-homogeneous precipitated solid in ~90% yield. The latter product was identified as an approximately 1 : 1 mixture of diastereoisomers (with respect to the asymmetric phosphorus centre) on the basis of its ^1H and ^{31}P ($\delta_P[(CD_3)_2SO]$ 60.1, 60.3) NMR spectra. The corresponding 5′-phosphorothiomorpholidate **13a** was obtained [9] by allowing the activated phosphorothioate **6a** to react with a large excess of morpholine in THF solution at room temperature. The products were worked up and purified in the same way to give the triethylammonium salt of 6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-phosphorothiomorpholidate **13a** as an HPLC-homogeneous precipitated solid in ~94% isolated yield. It was again established by ^1H and ^{31}P ($\delta_P[(CD_3)_2SO]$ 60.8, 61.0) NMR spectroscopy that an approximately 1 : 1 mixture of diastereoisomers had been obtained. The activated phosphorodithioate derivative **6b** was similarly allowed to react with alcoholic methylamine [7] and morpholine [9] in THF solution to give 6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-(*N*-methylphosphorodithioamidate) and -phosphorodithiomorpholidate, respectively. The latter products were isolated as HPLC-homogeneous triethylammonium salts **12b** and **13b** in ~86 and 97% yield, respectively; they were again characterized on the basis of their ^1H and ^{31}P ($\delta_P[(CD_3)_2SO]$ 108.1 for **12b**, 114.1 for **13b**) NMR spectra.

We previously reported [9] that when 6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-phosphorothiomorpholidate **13a** was treated with acetic acid–water (95 : 5 v/v) at room temperature for 2 min, fully unprotected adenosine 5′-phosphorothiomorpholidate **14** was obtained in high yield. Further hydrolysis in acetic acid–water (20 : 80 v/v) solution led [9] to the formation of adenosine 5′-phosphorothioate **15a** in very high yield. It had

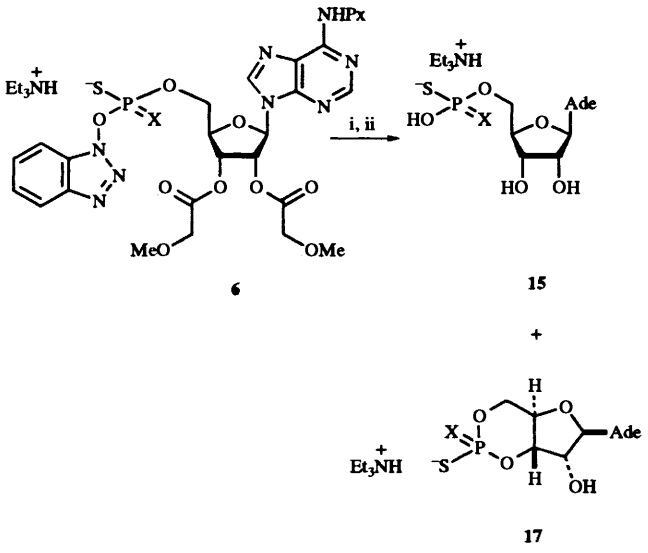


Scheme 2 *Reagents and conditions:* i, reagent (with putative structure **11**) prepared at 0 °C from $PSCl_3$ (3.0 mol equiv.), Et_3N (9.0 mol equiv.), 1-hydroxybenzotriazole (9.0 mol equiv.) and THF, 0 °C, 30 min; ii, aq. Et_3N, 0 °C, 10 min; iii, Et_3N, H_2S, 0 °C, 30 min



previously been found [12] that when the tributylammonium salts of adenosine 5′-phosphorothiomorpholidate **14** and orthophosphoric acid were allowed to react together in *N*,*N*-dimethylformamide (DMF) or pyridine solution, a mixture of adenosine 5′-phosphorothioate **15a** and adenosine 5′-α-thiodiphosphate **16** was obtained. This corresponds to the preparation of adenosine 5′-diphosphate (ADP) from adenosine 5′-phosphoromorpholidate.[13] Thus, adenosine 5′-phosphorothiomorpholidate **14** [and hence its 6-*N*-(9-phenylxanthen-9-yl) derivative **13a**] appears to be a potentially useful intermediate in the synthesis of thiophosphoryl analogues of unsymmetrical pyrophosphates. When 6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-phosphorodithiomorpholidate **13b** was treated with acetic acid–water (95:5 or 20:80 v/v), adenosine **7** was obtained[9] as the sole nucleoside or nucleotide product. It therefore appears that phosphorodithiomorpholidates are very unlikely to prove to be useful intermediates in the synthesis of dithiophosphoryl analogues of condensed phosphates.

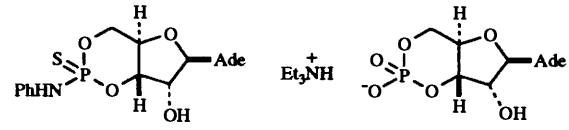
We next turned our attention towards the alkaline hydrolysis of the activated 5′-phosphorothioate and -phosphorodithioate derivatives (**6a** and **6b**, respectively). The activated phosphorothioate derivative **6a** was treated with an excess of sodium hydroxide in aq. 1,4-dioxane solution at room temperature (Scheme 3), and the products were then acidified to remove the 6-*N*-(9-phenylxanthen-9-yl) protecting group. The ^{31}P NMR spectrum (in D_2O) of the crude products consisted of three resonance signals at δ_P 45.7, 55.2 and 56.5. Five main components (t_R 4.07, 7.56, 9.57, 11.23 and 11.89 min) were detected in the products by reversed-phase HPLC. The products were then partially fractionated by chromatography on DEAE-Sephadex A-25. The component with t_R 4.07 min (which accounted for ~22% of the total absorbance at 260 nm of the nucleotide components in the HPLC eluate) was identified as adenosine 5′-phosphorothioate[14] **15a** on the basis of ^1H and ^{31}P (δ_P 45.7) NMR spectroscopic data; the components with t_R 11.23 and 11.89 min (which accounted for ~22 and 56%, respectively, of the total nucleotide absorbance) were similarly identified as the two diastereoisomers of adenosine 3′,5′-cyclic phosphorothioate[15] **17a**. The ^{31}P NMR chemical shifts of the minor and major diastereoisomers were δ_P 56.5 and 55.2, respectively. The component with t_R 9.5 min was believed, on the basis of HPLC evidence, to be 1-hydroxybenzotriazole or its triethylammonium salt. The fifth component (t_R 7.56 min), which accounted for less than 6% of the total absorption of the eluate at 260 nm, has not been identified. The formation of a diastereoisomeric mixture of cyclic phosphorothioates **17a** was not unexpected. Saponification of the methoxyacetate esters occurred very rapidly under the reaction conditions, and it is not surprising that neighbouring-group



Scheme 3 *Reagents and conditions:* i, NaOH, aq. 1,4-dioxane, room temp.; ii, dil. hydrochloric acid (pH 2), room temp

attack of the 3'-hydroxy function (presumably as its conjugate base) on the activated 5'-phosphorothioate would compete favourably with the external attack of hydroxide ions. As fractionation of the products proved to be difficult, this does not constitute a useful preparation either of adenosine 5'-phosphorothioate **15a** or of adenosine 3',5'-cyclic phosphorothioate **17a**. In any case, the latter nucleotide analogues are readily obtainable by other routes.[15,16]

On the other hand, the triethylammonium salt of 2',3'-bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)adenosine 5'-phosphorodithioate *O*-(benzotriazol-1-yl) ester **6b** proved to be a useful intermediate in the synthesis of adenosine 3',5'-cyclic phosphorodithioate **17b**. When the activated phosphorodithioate derivative **6b** was treated (Scheme 3) with a large excess of sodium hydroxide in 1,4-dioxane–water (3:2 v/v) at room temperature for 16 h and the products then acidified to remove the 6-*N*-(9-phenylxanthen-9-yl) protecting group, reversed-phase HPLC analysis of the products revealed three main components (t_R 6.10, 9.19 and 13.19 min). The components with t_R 6.10 and 9.19 min (which accounted for ~5 and 14%, respectively, of the total absorbance at 260 nm) were identified as adenosine **7** and 1-hydroxybenzotriazole on the basis of their retention times. The component with t_R 13.19 min (which accounted for ~72% of the total absorbance) was identified as adenosine 3',5'-cyclic phosphorodithioate **17b** on the basis of its ^{1}H and ^{31}P (δ_P[D_2O] 114.5) NMR spectra and on its conversion (see below) into adenosine 3',5'-cyclic phosphate **19**. Following fractionation of the crude products by chromatography on DEAE-Sephadex A-25, adenosine 3',5'-cyclic phosphorodithioate **17b** was isolated as its pure triethylammonium salt in what appeared to be a very satisfactory yield (1140 A_{260} units from ~0.1 mmol of starting material **6b**). No adenosine 5'-phosphorodithioate **15b** was detected in the final products. A possible explanation for this is that a small quantity of the 6-*N*-(9-phenylxanthen-9-yl) derivative of adenosine 5'-phosphorodithioate was indeed formed by the action of alkali on the activated phosphorodithioate derivative **6b** and that it underwent conversion[17] into adenosine under the acidic conditions used to remove the 6-*N*-(9-phenylxanthen-9-yl) protecting group. The first step (Scheme 3, step i) in the conversion of the activated phosphorodithioate derivative **6b** into adenosine 3',5'-cyclic phosphorodithioate **17b** was allowed to proceed for 16 h at room temperature. However, a much shorter reaction time would have been adequate as the deacylation and cyclization processes were found to have



18 **19**

Ade = adenin-9-yl

proceeded to a considerable extent even when the duration of the alkaline hydrolysis step was limited to only 2 min (see Experimental section).

To the best of our knowledge, there is only one previous report in the literature relating to the synthesis of adenosine 3',5'-cyclic phosphorodithioate **17b**. Thus Baraniak and Stec reported[18] that the cyclic phosphoranilidothioate **18** can be converted into the cyclic phosphorodithioate **17b** in three steps and in 61% overall yield. The starting material **18** itself was prepared from adenosine in four steps. The triethylammonium salt of the cyclic phosphorodithioate **17b** was characterized by the latter workers only on the basis of its ^{31}P NMR spectrum (δ_P[D_2O] 110). No ^{1}H NMR spectrum or other relevant data were provided. We found that when the triethylammonium adenosine 3',5'-cyclic phosphorodithioate **17b**, which we had prepared, was desulfurized by treatment[19] with a large excess of iodine in the presence of 1-methylimidazole in aq. THF, the principal product was adenosine 3',5'-cyclic phosphate **19**. The latter material was isolated as its triethylammonium salt following fractionation of the crude products by chromatography on DEAE-Sephadex A-25; it was found to be identical (^{1}H and ^{31}P NMR, reversed-phase HPLC) with authentic triethylammonium adenosine 3',5'-cyclic phosphate[20] **19**.

Experimental

Mps were measured with a Büchi melting point apparatus and are uncorrected. ^{1}H NMR spectra, unless otherwise stated, were measured at 360.1 MHz with a Bruker AM 360 spectrometer; ^{13}C NMR spectra were measured at 90.6 MHz with the same spectrometer. Tetramethylsilane was used as an internal standard, and *J* values are given in Hz. ^{31}P NMR spectra were measured at 145.8 Hz, also with a Bruker AM 360 spectrometer; 85% orthophosphoric acid was used as an external standard. UV spectra were measured with a Perkin-Elmer Lambda-3 spectrophotometer. Merck silica gel 60 F_{254} TLC plates were developed in solvent systems A [chloroform–methanol (9:1 v/v)] and B [chloroform–methanol (19:1 v/v)]. HPLC was carried out on a Jones APEX Octadecyl 5μ column which was eluted with 0.1 mol dm^{-3} triethylammonium acetate buffer (pH 7.0)/acetonitrile mixtures: program 1 involved a linear gradient over a period of 10 min (flow rate 1.5 cm^3 min^{-1}), starting with buffer–acetonitrile (6:4 v/v) and ending with buffer–acetonitrile (3:7 v/v); program 2 involved a linear gradient over a period of 15 min (flow rate 1.5 cm^3 min^{-1}), starting with buffer–acetonitrile (19:1 v/v) and ending with buffer–acetonitrile (4:1 v/v), followed by a second linear gradient over a period of 10 min (same flow rate), ending with buffer–acetonitrile (1:1 v/v). The liquid chromatograph was fitted with a UV detector set at 260 nm. Merck silica gel H was used for SCC. Anion-exchange chromatography on DEAE Sephadex A-25 was carried out with linear gradients of triethylammonium hydrogen carbonate buffer (pH 7.5). Acetonitrile, pyridine, triethylamine and morpholine were dried by heating, under reflux, with calcium hydride for 3–5 h and were then distilled at atmospheric pressure; THF was dried over sodium benzophenone and was then distilled at atmospheric pressure; DMF was dried by distillation over calcium hydride under reduced (water-pump) pressure. The latter solvents and reagents were stored over molecular sieves (no. 4 Å).

6-*N*-(9-Phenylxanthen-9-yl)adenosine 8

Redistilled acetic anhydride (9.95 cm^3, 0.105 mol) was added to a suspension of dry adenosine (5.655 g, 21.2 mmol) in pyridine (70 cm^3), and the reactants were stirred at room temperature. After 6 h, methanol (5 cm^3) was added, the products were stirred for a further period of 10 min, and were then evaporated under reduced pressure. The residue was dissolved in chloroform (100 cm^3), and the solution was extracted with saturated aq. sodium hydrogen carbonate (200 cm^3). The aqueous layer was separated, and back-extracted with chloroform (2 × 50 cm^3). The combined organic layers were dried ($MgSO_4$), and concentrated under reduced pressure. The residue was crystallized from absolute ethanol to give 2',3',5'-tri-*O*-acetyladenosine as prisms (7.99 g, 96%).

A solution of 9-chloro-9-phenylxanthene[21] **10** (0.76 g, 2.6 mmol) in dry pyridine (10 cm^3) was added dropwise to a dry solution of 2',3',5'-tri-*O*-acetyladenosine (0.79 g, 2.0 mmol) in pyridine (10 cm^3) at room temperature. After the resulting solution had been heated at 90 °C for 2 h, it was cooled to room temperature. Water (0.5 cm^3) was then added. After 10 min, the products were concentrated under reduced pressure, and the residue was dissolved in chloroform (50 cm^3). The resulting solution was extracted with saturated aq. sodium hydrogen carbonate (100 cm^3). The aqueous layer was separated, and back-extracted with chloroform (2 × 20 cm^3). The combined organic extracts were dried ($MgSO_4$), and evaporated under reduced pressure. After toluene (2 × 20 cm^3) had been added, and then removed by evaporation, the residue was dissolved in ~8 mol dm^{-3} methanolic ammonia solution (20 cm^3) at room temperature. After 16 h, the products were evaporated under reduced pressure and the residue was fractionated by SCC on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (96:4 v/v), were combined, and evaporated under reduced pressure. Crystallization of the residue from benzene gave the *title compound* **8** (1.02 g, 97%) (Found: C, 66.7; H, 5.1; N, 13.5. $C_{29}H_{25}N_5O_5$ requires C, 66.5; H, 4.8; N, 13.4%), mp 160 °C; R_f 0.35 (system A); $\delta_H[(CD_3)_2SO]$ 3.54 (1 H, m), 3.66 (1 H, m), 3.96 (1 H, m), 4.16 (1 H, m), 4.64 (1 H, m), 5.23 (2 H, m), 5.48 (1 H, d, *J* 6.1), 5.89 (1 H, d, *J* 6.0), 6.99 (2 H, m), 7.18–7.38 (9 H, m), 7.49 (2 H, m), 7.84 (1 H, s), 8.38 (1 H, s) and 8.50 (1 H, s); $\delta_C[(CD_3)_2SO]$ 58.4, 61.5, 70.5, 73.2, 85.7, 87.8, 115.9, 121.2, 123.2, 126.2, 126.7, 126.9, 127.0, 128.1, 128.5, 140.5, 146.8, 148.5, 150.2, 151.0 and 152.9.

2',3'-Bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)-adenosine 9

tert-Butylchlorodimethylsilane (0.249 g, 1.65 mmol) was added to a dry, stirred solution of 6-*N*-(9-phenylxanthen-9-yl) adenosine **8** (0.78 g, 1.5 mmol) in pyridine (15 cm^3) at room temperature. After 16 h, saturated aq. sodium hydrogen carbonate (1 cm^3) was added to the cooled (ice–water-bath) solution. The products were then evaporated under reduced pressure and the residue was partitioned between chloroform (50 cm^3) and saturated aq. sodium hydrogen carbonate (100 cm^3). The aqueous layer was separated, and back-extracted with chloroform (2 × 10 cm^3). The combined organic extracts were dried ($MgSO_4$), and concentrated under reduced pressure. After toluene (2 × 20 cm^3) had been added, and then removed by evaporation, the residue was fractionated by SCC on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (98:2 v/v), were combined, and evaporated under reduced pressure to give a glass (0.911 g), R_f 0.48 (system A).

Methoxyacetic anhydride (1.0 g, 6.15 mmol) was added to a dry, stirred solution of the latter material (0.785 g) in pyridine (12 cm^3) at room temperature. After 2 h, water (1.0 cm^3) was added. After a further period of 10 min, the products were concentrated under reduced pressure to a small volume and partitioned between chloroform (50 cm^3) and saturated aq. sodium hydrogen carbonate (100 cm^3). The aqueous layer was separated, and back-extracted with chloroform (2 × 20 cm^3). The combined organic layers were dried ($MgSO_4$), and concentrated under reduced pressure. After toluene (2 × 20 cm^3) had been added, and then removed by evaporation, the residue was fractionated by SCC on silica gel: the appropriate fractions, which were eluted with chloroform–light petroleum (distillation range 40–60 °C), were combined, and evaporated under reduced pressure to give a glass (0.894 g), R_f 0.55 (system B).

The latter material (0.697 g) was dissolved in the solution obtained by adding acetic acid (0.31 cm^3, 5.4 mmol) to 1.0 mol dm^{-3} tetraethylammonium fluoride in acetonitrile (5.4 cm^3, 5.4 mmol) at room temperature. After the reactants had been stirred at room temperature for 48 h, the products were concentrated under reduced pressure and the residue was partitioned between chloroform (50 cm^3) and saturated aq. sodium hydrogen carbonate (100 cm^3). The aqueous layer was separated, and back-extracted with chloroform (2 × 20 cm^3). The combined organic layers were dried ($MgSO_4$), and evaporated under reduced pressure. The residue was fractionated by SCC on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (99:1 v/v), were combined, and evaporated under reduced pressure to give a solid. Crystallization of this material from ethyl acetate gave the *title compound* **9** [0.505 g, 75% overall yield for the three steps starting from 6-*N*-(9-phenylxanthen-9-yl)adenosine **8**] (Found: C, 62.1; H, 5.7; N, 9.35. $C_{35}H_{33}N_5O_9 \cdot 0.75\,CH_3CO_2C_2H_5$ requires C, 62.2; H, 5.4; N, 9.5%), mp 165–166 °C; R_f 0.33 (system B); $\delta_H[(CD_3)_2SO]$ 3.21 (3 H, s), 3.34 (3 H, s), 3.60–3.75 (2 H, m), 3.97 (1 H, d, *J* 17.0), 4.07 (1 H, d, *J* 17.0), 4.13 (1 H, d, *J* 16.9), 4.20 (1 H, d, *J* 16.9), 4.25 (1 H, m), 5.54 (1 H, m), 5.63 (1 H, dd, *J* 2.7 and 5.4), 6.06 (1 H, m), 6.22 (1 H, d, *J* 6.7), 6.97 (2 H, t, *J* 7.4), 7.15–7.35 (9 H, m), 7.48 (2 H, d, *J* 7.8), 7.85 (1 H, s), 8.53 (1 H, s) and 8.54 (1 H, s); $\delta_C[(CD_3)_2SO]$ 58.4, 58.6, 61.0, 68.4, 68.7, 71.5, 72.4, 83.6, 85.1, 115.9, 121.1, 123.2, 126.3, 126.70, 126.73, 127.0, 127.1, 128.2, 128.5, 140.3, 146.9, 148.5, 150.2, 151.4, 153.1, 169.0 and 169.4.

Triethylammonium salt of 2',3'-bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)adenosine 5'-phosphorothioate *O*-(benzotriazol-1-yl) ester 6a

Thiophosphoryl trichloride (0.15 cm^3, 1.5 mmol) and triethylamine (0.63 cm^3, 4.5 mmol) were added to a stirred suspension of 1-hydroxybenzotriazole (0.608 g, 4.5 mmol) in dry THF (15 cm^3) at 0 °C (ice–water-bath). After 30 min, a solution of 2',3'-bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)adenosine **9** (0.33 g, 0.49 mmol) in dry THF (20 cm^3) was added. After the reactants had been stirred for 30 min at 0 °C, triethylamine (2.5 cm^3, 17.9 mmol) and water (1.0 cm^3, 55.5 mmol) were added. After 10 min, the products were filtered and the residue was washed with THF (20 cm^3). The combined filtrate and washings were concentrated under reduced pressure. The residue was dissolved in chloroform (50 cm^3) and the solution was extracted with 0.5 mol dm^{-3} aq. triethylammonium hydrogen carbonate (pH 7.5; 2 × 100 cm^3). The aqueous extracts were back-extracted with chloroform (2 × 20 cm^3). The combined organic layers were dried ($MgSO_4$), and concentrated under reduced pressure. The residue was fractionated by SCC on silica gel: the appropriate fractions, which were eluted with ethyl acetate–ethanol (98:2 v/v), were combined, and evaporated under reduced pressure. A solution of the residue in chloroform (10 cm^3) was added dropwise to stirred light petroleum (distillation range 30–40 °C; 300 cm^3) to give the title compound **6a** (0.452 g, ~93%) as a precipitated solid, t_R 12.04 min (program 1); $\delta_H[(CD_3)_2SO]$ includes the following signals: 3.21 (3 H, 2 s), 3.35 (2 H, s), 4.03 (2 H, m), 4.20 (2 H, m), 4.35 (2 H, m), 4.51 (1 H, m), 5.72 (1 H, m), 6.04 (1 H, m), 6.25 (1 H, d, *J* 6.3), 6.97 (2 H, m), 7.15–7.55 (13 H, m), 7.75 (1 H, m), 7.84 (1 H, 2 s), 7.98 (1 H, d, *J* 8.4), 8.50 (1 H, 2 s) and 8.66 and 8.68 (1 H, 2 s); $\delta_P[(CD_3)_2SO]$ 59.9.

Triethylammonium salt of 2′,3′-bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-phosphorodithioate *O*-(benzotriazol-1-yl) ester 6b

Thiophosphoryl trichloride (0.30 cm^3, 3.0 mmol) and triethylamine (1.25 cm^3, 9.0 mmol) were added to a stirred suspension of 1-hydroxybenzotriazole (1.216 g, 9.0 mmol) in dry THF (30 cm^3) at room temperature. After 30 min, the reaction mixture was cooled to 0 °C (ice–water-bath) and a solution of 2′,3′-bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)adenosine **9** (0.668 g, 1.0 mmol) in dry THF (50 cm^3) was added. After the reactants had been stirred at 0 °C for 30 min, dry triethylamine (5.0 cm^3, 36 mmol) was added and hydrogen sulfide was bubbled through the reaction mixture for 30 min, followed by nitrogen for a further period of 30 min. The products were then worked up in the same way as in the above preparation of the corresponding phosphorothioate derivative **6a**. The material obtained was fractionated by SCC on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (98:2–96:4 v/v), were combined, and evaporated under reduced pressure. A solution of the residue in chloroform (10 cm^3) was added dropwise to stirred light petroleum (distillation range 30–40 °C; 300 cm^3) to give the title compound **6b** (0.89 g, ~90%) as a precipitated solid, t_R 13.53 min; $\delta_H[(CD_3)_2SO]$ includes the following signals: 3.19 (3 H, s), 3.35 (3 H, s), 3.97 (1 H, d, *J* 7.0), 4.06 (1 H, d, *J* 7.0), 4.17 (1 H, d, *J* 6.9), 4.23 (1 H, d, *J* 6.9), 4.45 (2 H, m), 4.57 (1 H, m), 5.74 (1 H, dd, *J* 2.8 and 5.3), 5.99 (1 H, m), 6.25 (1 H, d, *J* 6.5), 7.15–7.5 (13 H, m), 7.71 (1 H, d, *J* 8.3), 7.82 (1 H, s), 7.98 (1 H, d, *J* 8.4), 8.52 (1 H, s) and 8.77 (1 H, s); $\delta_P[(CD_3)_2SO]$ 125.5.

Triethylammonium salt of 6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-(*N*-methylphosphorothioamidate) 12a

A solution of methylamine in absolute ethanol (8.0 mol dm^{-3}; 20 cm^3) was added to the triethylammonium salt of 2′,3′-bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-phosphorothioate *O*-(benzotriazol-1-yl) ester **6a** (0.20 g, ~0.2 mmol), and the resulting solution was stirred at room temperature. After 16 h, the products were evaporated under reduced pressure. The residue was dissolved in chloroform (50 cm^3) and the solution was extracted with 0.5 mol dm^{-3} aq. triethylammonium hydrogen carbonate buffer (pH 7.5; 2 × 50 cm^3). The combined aqueous extracts were back-extracted with chloroform (2 × 20 cm^3). The combined organic layers were dried ($MgSO_4$), and concentrated under reduced pressure. The residue was fractionated by SCC on silica gel: the column was eluted first with chloroform and then with chloroform containing increasing proportions of ethanol. The appropriate fractions which were eluted with chloroform–ethanol (85:15 to 60:40 v/v) were combined, and evaporated under reduced pressure. A solution of the residue in chloroform (5 cm^3) was added dropwise to stirred light petroleum (distillation range 30–40 °C; 200 cm^3) to give the title compound **12a** (0.135 g, ~90%) as a precipitated solid, t_R 5.86 min (program 1); $\delta_H[(CD_3)_2SO]$ includes the following signals: 2.35 (~1.5 H, d, *J* 13.5), 2.37 (~1.5 H, d, *J* 13.4), 3.83 (2 H, m), 4.07 (1 H, m), 4.17 (1 H, m), 4.67 (1 H, m), 5.32 (1 H, br), 5.49 (1 H, d, *J* 5.0), 5.90 (1 H, d, *J* 6.2), 6.97 (2 H, m), 7.15–7.27 (7 H, m), 7.32 (2 H, m), 7.46 (2 H, d, *J* 7.8), 7.81 (1 H, s), 8.31 (1 H, s), 8.69 (~0.5 H, s) and 8.71 (~0.5 H, s); $\delta_P[(CD_3)_2SO]$ 60.1 and 60.3.

Triethylammonium salt of 6-*N*-(9-phenylxanthen-9-yl)-adenosine 5′-phosphorothiomorpholidate 13a

Morpholine (0.70 cm^3, 8.0 mmol) was added to a stirred solution of 2′,3′-bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-phosphorothioate *O*-(benzotriazol-1-yl) ester **6a** (0.20 g, ~0.20 mmol) in dry THF (4 cm^3) at room temperature. After 16 h, the products were concentrated under reduced pressure (water-pump, followed by high-vacuum pump), and then worked up as in the above preparation of the corresponding 5′-(*N*-methylphosphorothioamidate) **12a**. The residue was fractionated by SCC on silica gel: the column was eluted first with chloroform and then with chloroform containing increasing proportions of ethanol. The appropriate fractions which were eluted with chloroform–ethanol (80:20 v/v) were combined, and evaporated under reduced pressure. A solution of the residue in chloroform (5 cm^3) was added dropwise to stirred light petroleum (distillation range 30–40 °C; 200 cm^3) to give the title compound **13a** (0.151 g, ~94%) as a precipitated solid, t_R 6.29 min (program 1); $\delta_H[(CD_3)_2SO]$ includes the following signals: 3.87 (2 H, m), 4.06 (1 H, m), 4.17 (1 H, m), 4.65 (1 H, m), 5.33 (1 H, m), 5.52 (1 H, m), 5.91 (1 H, d, *J* 6.1), 6.97 (2 H, m), 7.16–7.35 (9 H, m), 7.45 (2 H, d, *J* 7.8), 7.81 (1 H, s), 8.35 (1 H, br s), 8.68 (~0.5 H, s) and 8.70 (~0.5 H, s); $\delta_P[(CD_3)_2SO]$ 60.8 and 61.0.

Triethylammonium salt of 6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-(*N*-methylphosphorodithioamidate) 12b

A solution of methylamine in absolute ethanol (8.0 mol dm^{-3}; 20 cm^3) was added to the triethylammonium salt of 2′,3′-bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-phosphorodithioate *O*-(benzotriazol-1-yl) ester **6b** (0.20 g, ~0.2 mmol). The resulting solution was stirred at room temperature for 16 h, and the products were worked up, and chromatographed on silica gel as in the above preparation of the corresponding *N*-methylphosphorothioamidate **12a**. The appropriate fractions which were eluted with chloroform–ethanol (92:8 to 90:10 v/v) were combined, and evaporated under reduced pressure. A solution of the residue in chloroform (5 cm^3) was added dropwise to stirred light petroleum (distillation range 30–40 °C; 200 cm^3) to give the title compound **12b** (0.129 g, ~86%) as a precipitated solid, t_R 7.61 min (program 1); $\delta_H[(CD_3)_2SO]$ includes the following signals; 2.39 (3 H, d, *J* 15.0), 3.71 (1 H, m), 3.91 (1 H, m), 4.09 (1 H, m), 4.15 (1 H, m), 4.67 (1 H, m), 5.19 (1 H, d, *J* 4.3), 5.41 (1 H, d, *J* 6.7), 5.92 (1 H, d, *J* 6.8), 6.97 (2 H, m), 7.16–7.35 (9 H, m), 7.48 (2 H, m), 7.80 (1 H, m), 8.32 (1 H, s) and 8.94 (1 H, s); $\delta_P[(CD_3)_2SO]$ 108.1.

Triethylammonium salt of 6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-phosphorodithiomorpholidate 13b

Morpholine (0.70 cm^3, 8.0 mmol) was added to a stirred solution of the triethylammonium salt of 2′,3′-bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-phosphorodithioate *O*-(benzotriazol-1-yl) ester **6b** (0.20 g, ~0.2 mmol) in dry THF (4 cm^3) at room temperature. After 16 h, the products were worked up as in the above preparation of the corresponding 5′-phosphorothiomorpholidate **13a**. The appropriate fractions which were eluted with chloroform–ethanol (92:8 to 88:12 v/v) were combined, and evaporated under reduced pressure. A solution of the residue in chloroform (5 cm^3) was added dropwise to stirred light petroleum (distillation range 30–40 °C; 200 cm^3) to give the title compound **13b** (0.158 g, ~97%) as a precipitated solid, t_R 8.04 min; $\delta_H[(CD_3)_2SO]$ includes the following signals: 2.9–3.1 (4 H, m), 3.96 (1 H, m), 4.08 (1 H, m), 4.15 (1 H, dd, *J* 1.8 and 4.8), 4.66 (1 H, dd, *J* 5.0 and 6.5), 5.91 (1 H, d, *J* 6.7), 6.97 (2 H, m), 7.15–7.35 (9 H, m), 7.48 (2 H, m), 7.80 (1 H, s), 8.35 (1 H, s) and 8.87 (1 H, s); $\delta_P[(CD_3)_2SO]$ 114.1.

Alkaline hydrolysis of triethylammonium salt of 2′,3′-bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-phosphorothioate *O*-(benzotriazol-1-yl) ester 6a

A solution of the starting material **6a** (0.10 g, ~0.10 mmol) in 0.5 mol dm^{-3} aq. sodium hydroxide–1,4-dioxane (2:3 v/v; 10 cm^3) was kept at room temperature. After 16 h, the products were concentrated to dryness under reduced pressure (oil-pump). The residue was suspended in distilled water (10 cm^3) at room temperature and the stirred mixture was carefully

acidified to pH 2 (pH meter) by the addition of 1.0 mol dm^{-3} hydrochloric acid. After 40 min, the products were neutralized with conc. aq. ammonia (*d* 0.88) and then were extracted with chloroform (2 × 20 cm^3) and diethyl ether (20 cm^3). The aqueous layer was concentrated under reduced pressure. HPLC analysis (program 2) of the residue obtained revealed five main components: t_R 4.07 min (17.1%), 7.56 min (5.7%), 9.57 min (12.6%, corresponding to 1-hydroxybenzotriazole or its triethylammonium salt), 11.23 min (17.4%) and 11.89 min (43.5%). The latter material was fractionated on a column (18 cm × 2.5 cm diameter) of DEAE-Sephadex A-25. The column was eluted with aq. triethylammonium hydrogen carbonate buffer (pH 7.5; linear gradient from 0.001 to 0.25 mol dm^{-3} over 1000 cm^3), and fractions of 15–17 cm^3 were collected. After HPLC (program 2) analysis, fractions 37–45 (average buffer concentration 0.14 mol dm^{-3}) and fractions 51–55 (average buffer concentration 0.20 mol dm^{-3}) were combined.

Combined fractions 37–45 were evaporated to dryness under reduced pressure. The residue was redissolved in distilled water (20 cm^3) and the solution was re-evaporated under reduced pressure. The process was repeated once again. The residual material (528 A_{260}-units) was identified as the triethylammonium salt of adenosine 3′,5′-cyclic phosphorothioate **17a**, t_R (program 2) 11.23 min (6.5%) and 11.89 min (92%); $\delta_H(D_2O)$ includes the following signals: 4.23 (1 H, dt, *J* 4.9 and 10.1), 4.33–4.51 (2 H, m), 4.64 (1 H, d, *J* 5.0), 4.86 (1 H, m), 6.05 (1 H, s), 8.10 (1 H, s) and 8.14 (1 H, s); $\delta_P(D_2O)$ 55.2 and 56.5.

Combined fractions 51–55 were worked up as above. The residual material (281 A_{260}-units) was identified as the triethylammonium salt of adenosine 5′-phosphorothioate **15a**, t_R (program 2) 4.07 min; $\delta_H(D_2O)$ includes the following signals: 4.07 (2 H, m), 4.36 (1 H, m), 4.49 (1 H, m), 6.08 (1 H, d, *J* 6.0), 8.18 (1 H, s) and 8.61 (1 H, s); $\delta_P(D_2O)$ 45.7.

Triethylammonium salt of adenosine 3′,5′-cyclic phosphorodithioate 17b

(*a*) A solution of the triethylammonium salt of 2′,3′-bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-phosphorodithioate *O*-(benzotriazol-1-yl) ester **6b** (0.10 g, ~0.10 mmol) in 0.5 mol dm^{-3} aq. sodium hydroxide–1,4-dioxane (2:3 v/v; 10 cm^3) was kept at room temperature. After 16 h, the products were concentrated to dryness under reduced pressure (oil-pump). The residue was suspended in distilled water (10 cm^3) at room temperature and the stirred mixture was carefully acidified to pH 2 (pH meter) by the addition of 1.0 mol dm^{-3} hydrochloric acid. After 40 min, the products were neutralized with conc. aq. ammonia (*d* 0.88) and then were extracted with chloroform (2 × 20 cm^3) and diethyl ether (20 cm^3). The aqueous layer was concentrated under reduced pressure. HPLC analysis (program 2) of the residue obtained revealed three main components: t_R 6.1 min (4.8%, corresponding to adenosine), 9.19 min (13.6%, corresponding to 1-hydroxybenzotriazole or its triethylammonium salt) and 13.19 min (72.4%). The latter material was fractionated on a column (18 cm × 2.5 cm diameter) of DEAE-Sephadex A-25. The column was eluted with aq. triethylammonium hydrogen carbonate buffer (pH 7.5; linear gradient from 0.001 to 1.0 mol dm^{-3} over 1000 cm^3), and fractions of 15–17 cm^3 were collected. After HPLC (program 2) analysis, fractions 37–46 (average buffer concentration ~0.7 mol dm^{-3}) were combined.

Combined fractions 37–46 were evaporated to dryness under reduced pressure. The residue was redissolved in distilled water (20 cm^3) and the solution was re-evaporated under reduced pressure. The process was repeated once again to give the triethylammonium salt of adenosine 3′,5′-cyclic phosphorodithioate **17b** (1140 A_{260}-units), t_R (program 2) 13.19 min; $\delta_H(D_2O)$ includes the following signals: 4.25 (1 H, dt, *J* 4.7 and 9.7), 4.46 (1 H, ddd, *J* 4.6, 9.5 and 23.6), 4.54 (1 H, ddd, *J* 4.5, 9.5 and 10.6), 4.67 (1 H, d, *J* 5.0), 4.98 (1 H, m), 6.06 (1 H, s), 8.15 (1 H, s) and 8.18 (1 H, s); $\delta_P(D_2O)$ 114.5.

(*b*) The triethylammonium salt of 2′,3′-bis-*O*-(methoxyacetyl)-6-*N*-(9-phenylxanthen-9-yl)adenosine 5′-phosphorodithioate *O*-(benzotriazol-1-yl) ester **6b** (0.05 g, ~0.05 mmol) was dissolved in stirred 0.5 mol dm^{-3} aq. sodium hydroxide–1,4-dioxane (2:3 v/v; 5 cm^3) at room temperature. After 2 min, an aliquot (50 mm^3) of the reaction solution was removed and immediately evaporated to dryness under reduced pressure (oil-pump). The residue, which was obtained in ~30 s, was suspended in distilled water (1 cm^3) and treated with 1.0 mol dm^{-3} hydrochloric acid (25 mm^3) at room temperature. After 40 min, the stirred reactants were neutralized with aq. ammonia and extracted with chloroform (2 cm^3). HPLC analysis (program 2) revealed that the chloroform layer contained virtually no UV-absorbing products. HPLC analysis of the aqueous layer revealed three main components: t_R 6.1 min (17.2%), 9.19 min (13.3%) and 13.19 min (48.0%).

Desulfurization of triethylammonium adenosine 3′,5′-cyclic phosphorodithioate 17b

A solution of triethylammonium adenosine 3′,5′-cyclic phosphorodithioate **17b** (~720 A_{260}-units, prepared as above) was dissolved in a solution obtained by mixing freshly prepared 0.2 mol dm^{-3} iodine in THF (7.5 cm^3; 1.5 mmol of iodine) with another solution containing THF (4.5 cm^3), 1-methylimidazole (0.75 cm^3, 9.4 mmol) and water (2.25 cm^3) at room temperature. After 3 h, water (20 cm^3) was added and the resulting mixture was extracted with chloroform (2 × 20 cm^3) and diethyl ether (20 cm^3). The aqueous layer was separated, and evaporated under reduced pressure. HPLC (program 2) of the residue obtained revealed five main components: t_R 2.89 min (15.8%), 3.35 min (9.3%), 8.05 min (13.3%), 8.5 min (11.7%) and 9.46 min (47.7%). The residue was fractionated on a column (18 cm × 2.5 cm diameter) of DEAE-Sephadex A-25. The column was eluted with aq. triethylammonium hydrogen carbonate buffer (pH 7.5; linear gradient from 0.001 to 1.0 mol dm^{-3} over 1000 cm^3), and fractions of 15–17 cm^3 were collected. Following HPLC (program 2) analysis, fractions 9–15 were combined, and evaporated to dryness under reduced pressure. The residue was redissolved in distilled water (20 cm^3) and the solution was re-evaporated under reduced pressure. The residual material (414 A_{260}-units) was identified [^1H and ^{31}P NMR and HPLC (program 2, t_R = 9.46 min)] as the triethylammonium salt of adenosine 3′,5′-cyclic phosphate **19** by comparison with authentic material.

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