Synthesis of a Dinucleoside Monophosphate Analogue Containing 6-*N*-(2-Aminoethyl)-2'-Deoxyadenosine. A Novel Approach to Sequence Specific Cross-linking in Synthetic Oligonucleotides

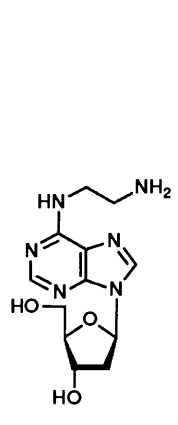
Richard Cosstick* and Mark E. Douglas
The Robert Robinson Laboratories, Department of Chemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, UK

The incorporation of 6-*N*-(2-aminoethyl)-2'-deoxyadenosine **1** into 6-*N*-(2-aminoethyl)-2'-deoxyadenylyl-(3'-5')-thymidine **2** using a phosphoramidite intermediate is described. The trifluoroacetyl group is demonstrated to be an ideal protecting group for the amino function whilst the 4,4'-dimethoxytrityl group is utilised to block the 5'-hydroxy function. This study serves as a model investigation for the incorporation of compound **1** into oligodeoxynucleotides to investigate their potential for forming sequency specific interstrand cross-links.

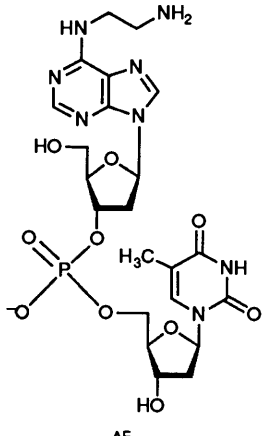
The Watson–Crick hydrogen bonds and the stacking forces that maintain the double helical structures in nucleic acids are relatively weak and whilst the double helix is energetically favoured, strand dissociation occurs readily at elevated temperature. However, in relation to their function the double helical nucleic acid structures are perfectly designed since many fundamental biological processes such as DNA replication and transcription necessitate the transient melting or unwinding of the duplex structures to provide a single stranded DNA substrate for the DNA or RNA polymerases. It is to be expected therefore, that the introduction of covalent interstrand cross-links in DNA would seriously impair these processes and indeed it is established that bifunctional alkylating agents such as 2-halogenoethylnitrosoureas which are able to form interstrand cross-links inhibit nucleic acid synthesis.[1] Furthermore the efficacy of these compounds as anticancer drugs against L1210 leukemia correlates with the extent of DNA cross-linking.[1] The heterogeneity of reaction mixtures of globally alkylated DNA make it difficult to discern the effects of a site specific cross-link upon the processing of DNA. Partly for this reason we have become interested in the synthesis of stabilised nucleic acid duplexes in which the two strands are 'stapled' together at predetermined sites through a covalent interstrand cross-link. It is envisaged that cross-linked synthetic oligonucleotides that could be incorporated into bacteriophage DNA would be an ideal probe for studying the biological activity of cross-linked DNA. Additionally, these 'stapled' oligonucleotides would appear to have several other useful and interesting applications such as stabilising cruciforms and other thermodynamically unfavourable structures, and the synthesis of highly stable oligonucleotide duplexes that could be used in affinity chromatography for isolating proteins that bind specific DNA sequences (*e.g.*, repressor proteins).

The aim of our studies is to synthesise appropriately modified nucleosides which once introduced into chemically prepared oligonucleotides could be triggered to bring about interstrand cross-linking. Unfortunately there are considerable problems in the incorporation of potentially reactive nucleosides into oligonucleotides since the reactive group would be exposed to acids, bases, nucleophiles and electrophiles during the synthesis. Principally for this reason there are comparatively few published studies on sequence specific cross-linking. However, notable work has been reported by Webb and Matteucci[2] which involves the incorporation of 4-*N*,4-*N*-ethanodeoxycytidine into an oligodeoxynucleotide as an electrophilic base analogue. Interstrand cross-linking is achieved by using Watson–Crick base pairing to position a nucleophilic deoxycytidine residue from the complementary strand in close proximity with 4-*N*,4-*N*-ethanodeoxycytidine. However, the sensitivity of this modified nucleoside towards nucleophiles precludes its incorporation into oligodeoxynucleotides containing bases other than thymine since synthesis with the other three deoxynucleosides necessitates prolonged treatment with aqueous ammonia. This sequence limitation severely restricts the potential and application of this technique. Surprisingly, attempts to develop this cross-linking system using 6-*N*,6-*N*-ethanodeoxyadenosine in which the electrophile and nucleophile are in closer proximity were less successful, although this is almost certainly because the 6-*N*,6-*N*-ethanodeoxyadenosine analogue is significantly less susceptible to nucleophilic attack than the 4-*N*,4-*N*-ethanodeoxycytidine.[3] More recently Pieles *et al.*[4] have developed a cross-linking procedure which uses oligodeoxynucleotides derivatised with a psoralen intercalator which is able to undergo a 2 + 2 cycloaddition reaction with a neighbouring pyrimidine residue in the opposite strand. The full potential of this cross-linking procedure is yet to be evaluated but the presence of the psoralen intercalator will undoubtedly cause a major distortion to the helix.

Our approach to sequence specific cross-linking relies on the incorporation of 6-*N*-(2-aminoethyl)-2'-deoxyadenosine (dA^{AE}, **1**) into an oligodeoxynucleotide so that upon hybridisation to a target DNA or RNA sequence the nucleophilic amino group is brought into close proximity with a pyrimidine residue in the complementary strand. Bisulphite which is known to add across the 5,6-double bond in pyrimidines,[5] activates the



dA^{AE} **1**



$d(A^{AE}pT)$ **2**

4-position to nucleophilic attack by the amino function to bring about cross-linking. Detailed studies[5] have revealed that only single stranded regions of nucleic acids undergo reaction with bisulphite and since, for example, a cytosine base opposite a 6-*N*-modified adenine base cannot be involved in a Watson–Crick hydrogen bond it is predicted that only this specific cytosine residue would be activated.

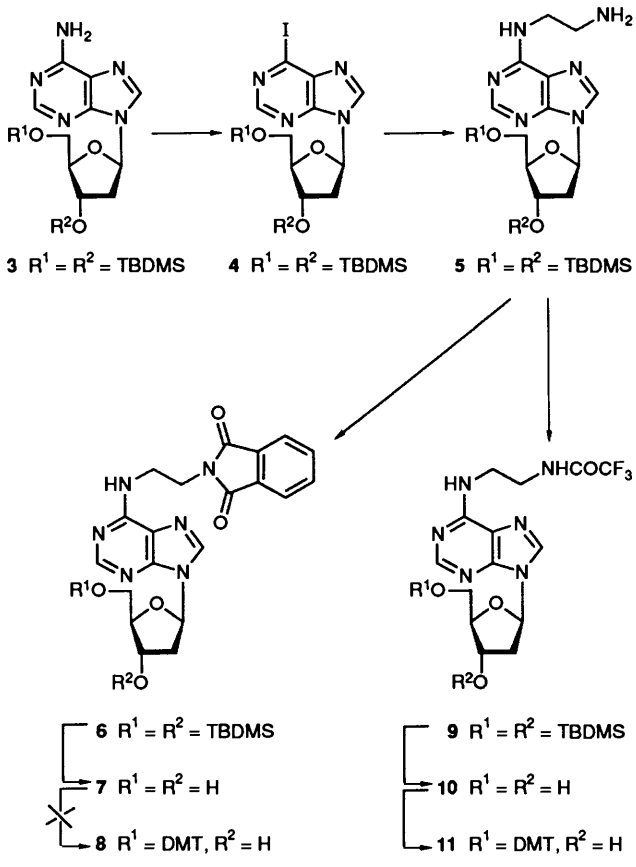
We believe that this cross-linking strategy has a number of attractive features: (i) the procedure is applicable to oligodeoxynucleotides containing all four deoxynucleosides; (ii) the cross-link is unlikely to introduce a gross distortion into the DNA helix; (iii) the nature of the cross-link is closely related to those that are proposed to occur in DNA treated with 2-halogenoethylnitrosoureas.[1] It should be noted however, that this procedure would not be suitable for cross-linking an oligonucleotide to a large DNA fragment since deoxycytidine residues in the single strand regions would be converted to deoxyuridine.

In this paper we report the synthesis of a suitably protected derivative of dA^{AE} and, as a model study for solid-phase synthesis, the preparation and characterisation of 6-*N*-(2-aminoethyl)-2′-deoxyadenylyl-(3′-5′)-thymidine [$d(A^{AE}pT)$], **2** using phosphoramidite chemistry.

Results and Discussion

Our initial objective was the preparation of a 5′-dimethoxytrityl protected derivative of dA^{AE} in which the amino function, which would require protection during oligonucleotide synthesis, was masked by a base labile group (Scheme 1). Two amino protecting groups were investigated: phthalimido (as in **8**) and trifluoroacetyl (as in **11**). Deoxyadenosine was converted to its 3′,5′-di-*O*-*tert*-butyldimethylsilyl (TBDMS) ether **3** in 80% yield using the published procedure of Ogilvie and co-workers.[6] Conversion into the 6-iodopurine nucleoside **4** was accomplished by photoinduced diazotisation with pentyl nitrite and diiodomethane as originally developed by Nair and Richardson.[7] Under optimised conditions of temperature (70 °C) and illumination, compound **4** could be obtained as a yellow oil in 62% yield. We generally found this procedure superior to the more recently published modification which uses trimethylsilyliodide, diiodomethane and pentyl nitrite.[8] Compound **4** has previously been reported by Nogueras *et al.*[9] Displacement of iodide to give the 2-aminoethyl nucleoside **5** was achieved in about 80% yield by treatment with an excess of ethylenediamine in acetonitrile at 60 °C. The polarity of this compound precluded its efficient purification by flash chromatography but the crude material was sufficiently pure to be used in the next stage of the synthesis.

The literature suggested that the phthalimido group would meet our requirements for the protection of the aminoethyl side chain. This group had previously been used by Gibson and Benkovic[10] for *N*-protection of 5-(3-aminopropyl)-2′-deoxyuridine and is known to be removed under the ammonolysis conditions used for the deprotection of the nucleoside bases. Thus treatment of **5** with 2 equiv. of *N*-carboethoxyphthalimide and 1 equiv. of triethylamine in chloroform gave the 6-*N*-(2-phthalimidoethyl) nucleoside **6** in 72% yield. At this stage it was necessary to remove the TBDMS groups to allow the introduction of the 4,4′-dimethoxytrityl (DMT) group at the 5′-position. Using anhydrous tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) it was evident that removal of the silyl protecting groups from **6** was accompanied by complete degradation of the phthalimidoethyl group to give a mixture of polar products that stained purple with ninhydrin. Whilst we have been unable to isolate and identify the products, it is possible that under the basic conditions degradation results from either an acyl transfer from the phthalimido nitrogen to



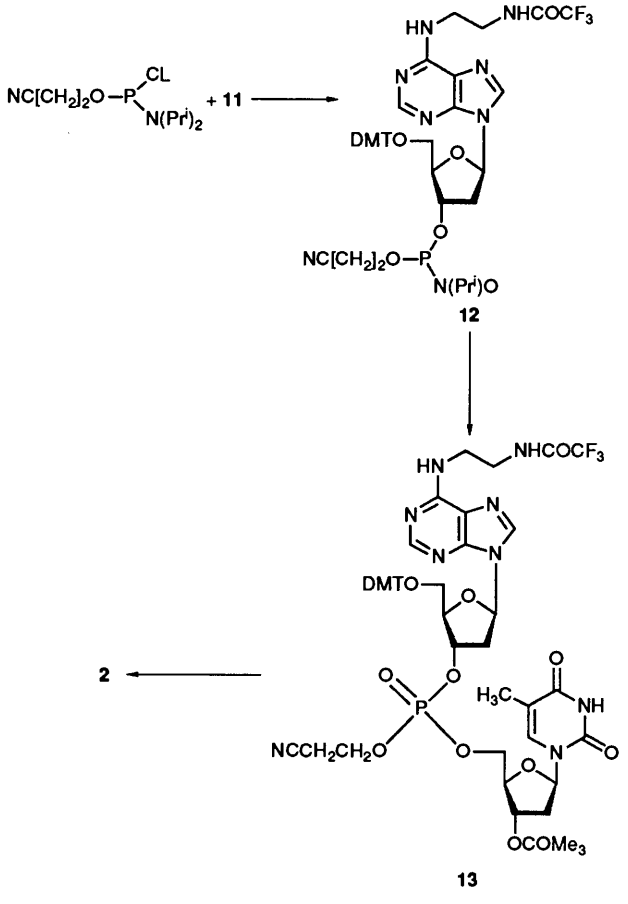
Scheme 1

the 6-N-position of the purine, or a β-elimination reaction of the 6-*N*-(2-phthalimidoethyl) group. Unfortunately attempts to deprotect **6** under acidic conditions using 80% acetic acid in water at 90 °C gave an unacceptable degree of depurination of the nucleoside. Previously Chladek and co-workers[11] have used TBAF in THF–pyridine–water (8:1:1) to moderate its basicity and under these conditions silyl ethers can be cleaved in the presence of a base labile fluorenylmethoxycarbonyl group. Thus treatment of **6** with TBAF in THF–pyridine–water (18:1:1) for 16 h at room temperature gave the desilylated nucleoside **7** in moderate yield (51%), after chromatography. Surprisingly attempts to dimethoxytritylate **7** with 4,4′-dimethoxytrityl chloride (DMTCl), 4-(dimethylamino)pyridine (DMAP) and triethylamine in pyridine were unsuccessful and the starting material could be recovered from the reaction mixture. In an endeavour to enhance the rate of the reaction, DMTCl was used in conjunction with silver nitrate, but even at elevated temperatures we were unable to observe any of the desired reaction. It is possibly that the 2-phthalimidoethyl group is partially occluding the 5′-hydroxy function and inhibiting the reaction with the sterically demanding dimethoxytrityl cation. We have not investigated the use of the recently reported dimethoxytrityl tetrafluoroborate for the preparation of **8**.[12]

It was apparent that the phthalimido group was not suitable for the protection of the 6-*N*-(2-aminoethyl) group and it was appropriate therefore to investigate the utility of the trifluoroacetyl group for this purpose. We rationalised that this latter group should be superior for two reasons: (i) its reduced steric bulk was less likely to inhibit reactions at the 5′-position and (ii) the 6-*N*-(2-trifluoroacetamidoethyl) nucleoside would almost certainly be less susceptible to β-elimination or other undesirable reactions with TBAF. The fully protected 6-*N*-(2-trifluoroacetamidoethyl) nucleoside **9** was prepared in 70% yield by treatment of **5** with excess of *S*-ethyl tri-

fluorothioacetate in methanol. This procedure is analogous to that previously used by Eckstein and Imazawa[13] for the protection of 2'-aminonucleosides. During the course of this work, several other groups have reported the suitability of the trifluoroacetyl group for the protection of nucleosides containing amino alkyl groups attached to the heterocycle.[14] We were gratified to observe that **9** was smoothly and rapidly desilylated with TBAF in anhydrous THF to give the deprotected nucleoside **10** in 90% yield. This result is in stark contrast to that obtained for the analogous reaction of the phthalimido derivative **6**. Dimethoxytritylation of compound **10** was accomplished using 2 equiv. of the DMTCl in pyridine–triethylamine in the presence of a catalytic amount of DMAP to give the 5'-protected nucleoside **11** in 69% yield.

As a model study for the solid-phase synthesis of longer oligodeoxynucleotides we chose to prepare the dinucleoside monophosphate d(A^{AE}pT) using the phosphoramidite approach (Scheme 2). This method originally developed by Caruthers and co-workers[15] is a very efficient procedure for the synthesis of oligodeoxynucleotides and importantly is compatible with automated methods of solid-phase synthesis. The nucleoside phosphoramidite **12** was prepared by treatment of **11** with 2 equiv. of 2-cyanoethyl-*N*,*N*-diisopropylaminochlorophosphine and excess *N*,*N*-diisopropylethylamine in dry dichloromethane.[16] After flash chromatography and precipitation from pentane, compound **12** was obtained in 62% yield as a mixture of diastereoisomers and was greater than 95% pure as determined by ^{31}P NMR. The fully protected dinucleoside phosphate **13** was prepared by reaction of **12** with *ca.* 0.8 mol equiv. of 3'-*O*-acetylthymidine and 3 mol equiv. of tetrazole in dry acetonitrile at room temperature for 20 min. *In situ* oxidation of the resulting dinucleoside phosphite with iodine in THF–2,6-lutidine–water (8:1:1) gave **13** (mixture of diastereoisomers), in 75% yield after chromatography and precipitation from pentane. Quantitative deprotection of **13** could be achieved in two steps; unblocking of the 5'-hydroxy function was accomplished with acetic acid–water (8:2) at 35 °C for 30 min and after evaporation of the acetic acid, the remaining protecting groups were removed by a 3 h treatment with concentrated aqueous ammonia. After removal of the residual 4,4'-dimethoxytrityl alcohol by extraction with diethyl ether, reverse phase liquid chromatography (Experimental section) indicated that the d(A^{AE}pT) was greater than 95% pure. For spectroscopic analysis small quantities of d(A^{AE}pT) (*ca.* 1 mg) were purified by reverse phase chromatography. Cleavage of the d(A^{AE}pT) with snake venom phosphodiesterase in 4-(2-hydroxyethy)-1-piperazineethane sulphonic acid (HEPES) buffer pH 7.4 and analysis of the digest by liquid chromatography revealed the presence of thymidine-5'-*O*-monophosphate and dA^{AE} in the expected ratio of 1:1 (the identity of the digestion products was confirmed by co-injection with authentic samples).



Scheme 2

Conclusion

The incorporation of dA^{AE} into a dinucleoside phosphate has been accomplished for the first time. This study demonstrates that the protecting group chemistry is compatible with automated solid-phase oligonucleotide synthesis, and the preparation of self-complementary dodecanucleoside undecaphosphates containing dA^{AE} opposite pyridine residues for cross-linking studies is currently being investigated.

Experimental

S-ethyl trifluorothioacetate,[17] pentyl nitrite[18] and 2-cyanoethyl-*N*,*N*-diisopropylaminochlorophosphite[16] were prepared as previously described. Pyridine and 2,6-lutidine were 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. Liquid chromatography was performed on a Varian Star 9010 solvent delivery system equipped with a Varian Star 9050 variable wavelength UV detector and a Varian 4400 integrator. Elution was carried out with a linear 20 min gradient of 0–30% acetonitrile in 50 mmol dm^{-3} potassium phosphate buffer pH 6.0 at a flow rate of 1 cm^3 min^{-1}. Analytical columns (250 × 4.6 mm) were packed with 5μ octadecylsilyl silica.

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 ±5ppm. 3-Nitrobenzyl alcohol was used as a matrix unless stated otherwise. ^{1}H 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 $CDCl_3$ and $(CD_3)_2SO$; spectra recorded in D_2O are referenced to sodium [2,2,3,3-$^2H_4$]-3-(trimethylsilyl)propionate. Proton decoupled ^{31}P and ^{19}F NMR spectra are referenced to 85% phosphoric acid and trifluoroacetic acid, respectively. All *J* values are in Hz. Analytical TLC was performed on Alugram sil G-UV_{254} plates developed in one of the following eluents; system A [CH_2Cl_2–MeOH (9:1)], system B [CH_2Cl_2–ethyl acetate–triethylamine (45:45:10)], system C [toluene–ethyl acetate (60:40)]. Nucleosides containing primary amino groups were visualised by spraying with commercial ninhydrin spray (BDH) and heating at 120 °C until the purple colouration developed. 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. Enzymes were purchased from Sigma.

6-*Iodo*-9-[3,5-*di*-O-(tert-*butyldimethylsilyl*)-β-D-*ribofuranosyl*]*pyrine* **4**.—A solution of the silylated nucleoside **3** (500 mg, 1.04 mmol), and diiodomethane (5.89 cm^3, 73 mmol) in pentyl nitrite (2.67 cm^3, 21.8 mmol) was stirred under nitrogen at 70 °C and illuminated by a tungsten lamp (unfrosted, operating at 110 W output and mounted 0.5 m from the reaction vessel) for 1 h. The resulting red solution was allowed to cool and concentrated under reduced pressure. The residue was dissolved in dichloromethane (40 cm^3) washed with saturated aqueous sodium metabisulphite (10 cm^3) and with water (10 cm^3). The aqueous washings were then back extracted with dichloromethane (2 × 5 cm^3) and the combined organic fractions dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel: appropriate fractions, eluted with CH_2Cl_2–MeOH (90:10), were pooled and concentrated under reduced pressure to give the title compound as a pale yellow gum (380 mg, 62%); *m/z* (FAB) 591 $(M + 1)^+$; δ_H($CDCl_3$, 200 MHz), 0.53 (12 H, 2s, Me), 0.93 (18 H, 2s, Bu^t), 2.44 (1 H, m, 2′-H), 2.62 (1 H, m, 2″-H), 3.78 (2 H, m, 5′-H and 5″-H), 4.02 (1 H, m, 4′-H), 4.58 (1 H, m, 3′-H), 6.45 (1 H, t, *J* 6.4, 1′-H), 8.43 [1 H, s, purinyl (Pu) H] and 8.56 (1 H, s, Pu H).

6-N-(2-*Phthalimidoethyl*)-5′,3′-*di*-O-(tert-*butyldimethylsilyl*)-2′-*deoxyadenosine* **6** via *the Amino Nucleoside* **5**.—To a stirred solution of the iodopurine **4** (0.85 g, 1.44 mmol) in acetonitrile, heated to 60 °C in a flask protected from the light, ethylenediamine (1.8 cm^3, 27 mmol) was added dropwise over a period of 5 min. After a further 5 min, the volatile materials were removed under reduced pressure and the residual gum was dissolved in dichloromethane, washed with saturated aqueous sodium hydrogen carbonate (20 cm^3), water (10 cm^3) and dried (Na_2SO_4). After filtration, the solution was chilled at −11 °C overnight and precipitated ammonium iodides were removed by further filtration. Solvent removal under reduced pressure gave the amino nucleoside as a pale yellow gum (0.6 g, 80%); R_f 0.12 (system A; positive test with ninhydrin); *m/z* (FAB) 523 $(M + 1)^+$; δ_H(250 MHz, $CDCl_3$), 0.08 (12 H, 2s, Me), 0.8 (18 H, 2s, Bu^t), 2.19 (2 H, br s, NH_2; D_2O exchangeable), 2.41 (1 H, m, 2′-H), 2.62 (1 H, m, 2″-H), 2.99 (2 H, m, CH_2), 2.90 (2 H, m, CH_2), 3.77 (2 H, m, 5′-H and 5″-H), 3.99 (1 H, m, 4′-H), 4.59 (1 H, m, 3′-H), 6.1 (1 H, br s, 6-NH; D_2O exchangeable), 6.2 (1 H, t, *J* 6.3, 1′-H), 8.06 (1 H, s, Pu H) and 8.35 (1 H, s, Pu H). The material was sufficiently pure to use in the next stage.

The amino nucleoside **5** (60 mg, 1.15 mmol) and *N*-carboethoxyphthalimide (50 mg, 2.34 mmol) were dissolved in dry chloroform (25 cm^3) and triethylamine (0.17 cm^3, 1.27 mmol) was added. After being stirred for 2 h in a sealed flask, the reaction mixture was washed with water (2 × 30 cm^3 aliquots), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was subjected to chromatography on silica gel; appropriate fractions, eluted with toluene–ethyl acetate (60:40), were pooled and concentrated under reduced pressure to give a clear gum from which residual solvents were repeatedly co-evaporated with ethanol to give the title compound as a colourless solid (53 mg, 72%), m.p. 173 °C (precipitated from toluene–hexane); R_f 0.5 (system C) [Found: $(M + 1)^+$, 653.3303. $C_{32}H_{49}N_6O_5Si_2$ requires $(M + 1)^+$, 653.3303]; δ_H($CDCl_3$, 200 MHz), 0.10 (12 H, 2s, Me), 0.93 (18 H, s, Bu^t), 2.30–2.48 (1 H, m, 2′-H), 2.55–2.70 (1 H, m, 2″-H), 3.82 (2 H, m, 5′-H and 5″-H), 3.91–4.12 (5 H, m, CH_2CH_2 and 4′-H), 4.60 (1 H, m, 3′-H), 6.28 (1 H, br s, 6-NH D_2O exchangeable), 6.41 (1 H, t, *J* 7.1, 1′-H), 7.74 (4 H, m, ArH), 8.07 (1 H, s, 2-H) and 8.23 (1 H, s, 8-H).

6-N-(2-*Phthalimidoethyl*)-2′-*deoxyadenosine* **7**.—Nucleoside **6** (560 mg, 0.85 mmol) was dissolved in THF (25 cm^3) and pyridine (1.97 cm^3) water (1.97 cm^3) then TBAF (1 mol dm^{-3} in THF; 9.22 cm^3) were added sequentially with vigorous stirring. The mixture was set aside at room temperature overnight, saturated aqueous sodium hydrogen carbonate (25 cm^3) was added and the mixture extracted with ethyl acetate (20 × 25 cm^3 aliquots) until no UV absorbing material remained in the aqueous phase. The organic phase was dried (Na_2SO_4) concentrated under reduced pressure to give a colourless solid which was purified by column chromatography; the appropriate fractions, eluted with CH_2Cl_2–MeOH (100:0–82:18) were combined and evaporated under reduced pressure to give the product **7** as a colourless solid, (183 mg, 51%) [Found: $(M + 1)^+$, 425.1573. $C_{20}H_{21}N_6O_5$ requires $(M + 1)^+$, 425.1573]; R_f 0.39 (system A); δ_H[$(CD_3)_2SO$, 200 MHz], 2.25 (1 H, m, 2′-H), 2.71 (1 H, m, 2″-H), 3.71 (2 H, m, 5′-H and 5″-H), 3.83 (5 H, m, 4′-H and CH_2CH_2), 4.40 (1 H, m, 3′-H), 5.22 (1 H, t, 5′-OH; D_2O exchangeable), 5.33 (1 H, d, 3′-OH; D_2O exchangeable), 6.34 (1 H, t, *J* 6.7, 1′-H), 7.81 (4 H, s, ArH), 8.05 (1 H, s, Pu H) and 8.32 (1 H, s, Pu H).

6-N-(2-*Trifluoroacetamidoethyl*)-5′,3′-*di*-O-(tert-*butyldimethylsilyl*)-2′-*deoxyadenosine* **9** via *the Amino Nucleoside* **5**.—To a stirred solution of the amino nucleoside **5** (prepared as described under the synthesis of **6**) (0.52 g, 1 mmol) in methanol, *S*-ethyl trifluorothioacetate (0.26 cm^3, 2.0 mmol) was added dropwise by syringe, and the solution stirred in a fume hood for 14 h. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel, the appropriate fractions, eluted with CH_2Cl_2–MeOH (98:2), were pooled and concentrated under reduced pressure to give a colourless solid (0.43 g, 70%) [Found: $(M + 1)^+$, 619.3071. $C_{26}H_{46}F_3N_6O_4Si_2$ requires $(M + 1)^+$, 619.3071]; m.p. 153–156 °C (precipitated from ethyl acetate-heptane); δ_H(250 MHz, $CDCl_3$), 0.04 (6 H, s, Me), 0.06 (6 H, s, Me), 0.87 (18 H, 2s, Bu^t), 2.03 (1 H, m, 2″-H), 2.61 (1 H, m, 2′-H), 3.57–3.82 (6 H, m, 5′-H, 5″-H and CH_2CH_2), 3.99 (1 H, m, 4′-H), 4.57 (1 H, m, 3′-H), 6.41 (1 H, t, *J* 6.9, 1′-H), 6.69 (1 H, br s, 6-NH; D_2O exchangeable), 8.11 (1 H, s, Pu H), 8.50 (1 H, s, Pu H) and 9.48 (1 H, s, amide NH D_2O exchangeable); δ_F(235.3 MHz, $CDCl_3$), −1.56 (s).

6-N-(2-*Trifluoroacetamidoethyl*)-2′-*deoxyadenosine* **10**.—TBAF (1 mol dm^{-3} in THF; 14 cm^3) was added to the fully protected nucleoside **4** (0.86 g, 1.39 mmol) and the solution stirred at room temperature. After 5 min, ethyl acetate (15 cm^3) was added to the reaction mixture which was washed with saturated aqueous sodium hydrogen carbonate (25 cm^3) and water (10 cm^3). The aqueous fractions were back extracted with ethyl acetate (20 × 25 cm^3), the organic extracts were combined and dried (Na_2SO_4). After concentration under reduced pressure, the residue was purified by chromatography on silica gel, the appropriate fractions, eluted with CH_2Cl_2–MeOH (100:0–95:5), were combined and evaporated under reduced pressure to give the title compound as a colourless solid (0.49 g, 90%) [Found: $(M + 1)^+$, 391.1342. $C_{14}H_{18}F_3N_6O_4$ requires $(M + 1)^+$, 391.1342]; R_f 0.17 (system A); δ_H[$(CD_3)_2SO$, 250 MHz], 2.40 (1 H, m, 2′-H), 2.75 (1 H, m, 2″-H), 3.57 (1 H, m, 6-NCH_2), 3.70–3.75 (4 H, m, 5′-H, 5″-H and CH_2NHCO), 4.05 (1 H, m, 4′-H), 4.57 (1 H, m, 3′-H), 6.42 (1 H, dd, *J* 7.9 and 6.0, 1′-H), 8.22 (1 H, s, Pu H) and 8.27 (1 H, s, Pu H).

6-N-(2-*Trifluoroacetamidoethyl*)-5′-O-*dimethoxytrityl*-2′-*deoxyadenosine* **11**.—Compound **10** (374 mg, 0.958 mmol) was dried by co-evaporation of residual moisture with pyridine (× 3) and finally dissolved in dry pyridine (30 cm^3) containing

distilled triethylamine (0.26 cm^3), 4,4′-dimethoxytrityl chloride (649 mg, 1.91 mmol) and a catalytic amount of DMAP (*ca.* 10 mg). After being stirred at room temperature for 12 h, the reaction mixture was evaporated to dryness under reduced pressure, the residue was dissolved in dichloromethane, washed with saturated aqueous sodium hydrogen carbonate (2 × 40 cm^3), water (40 cm^3) and dried (Na_2SO_4). The solution was concentrated under reduced pressure and the residue purified by chromatography on silica gel; the appropriate fractions, eluted with CH_2Cl_2–MeOH (100:0–95:5) containing *ca.* 0.5% triethylamine, were combined, evaporated under reduced pressure, dissolved in a minimum of dichloromethane and precipitated from 150 cm^3 of rapidly stirred dry pentane to give **11**, as a fine colourless powder (460 mg, 69%) [Found: $(M + 1)^+$, 693.2648. $C_{35}H_{36}N_6O_6F_3$ requires $(M + 1)^+$, 693.2648]; R_f 0.69 (system A); δ_H(200 MHz, $CDCl_3$); 2.48–2.62 (1 H, m, 2′-H), 2.80 (1 H, m, 2″-H), 3.40 (2 H, m, 5′-H and 5″-H), 3.58 (2 H, m, 6-NCH_2), 3.75 (6 H, s, OMe), 3.82 (2 H, m, CH_2NHCO), 4.16 (1 H, m, 4′-H), 4.69 (1 H, m, 3′-H), 6.25 (1 H, br t, 6-NH), 6.43 (1 H, t, *J* 8.2, 1′-H), 6.78–6.87 (4 H, m, ArH), 7.19–7.42 (9 H, m, ArH), 7.96 (1 H, s, Pu H), 8.28 (1 H, s, Pu H) and 9.46 (1 H, br s, amide NH).

6-N-(2-*Trifluoroacetamidoethyl*)-2′-*deoxyadenosine*-5′-O-*dimethoxytrityl*-3′-O-(2-*cyanoethyl*-N,N-*diisopropyl*)*phosphoramidite* **12**.—Compound **11** (440 mg, 0.63 mmol), previously dried by co-evaporation of residual moisture with pyridine, was dissolved in dry dichloromethane (15 cm^3) and 2-cyanoethyl-*N*,*N*-diisopropylaminochlorophosphine (0.28 cm^3, 1.26 mmol) was added by syringe during 10 min to the stirred reaction mixture. After 25 min, the reaction mixture was quenched by addition of methanol (1 cm^3), poured into ethyl acetate (100 cm^3), washed sequentially with saturated aqueous sodium hydrogen carbonate (2 × 100 cm^3), saturated brine (2 × 100 cm^3), water (2 × 100 cm^3) and dried (Na_2SO_4). The solution was concentrated under reduced pressure and purified by chromatography on silica gel; appropriate fractions, eluted with dichloromethane–ethyl acetate–triethylamine (45:45:10), were concentrated under reduced pressure to a foam which was dissolved in dry ethyl acetate (4 cm^3) and precipitated from 500 cm^3 of rapidly stirred pentane to give **12** (mixture of diastereoisomers) as a colourless powder (350 mg, 62%); R_f 0.48 (system B); *m*/*z* (FAB) 891 $(M - 1)^-$; δ_H(200 MHz, $CDCl_3$), 1.05–1.35 [12 H, m, $(CMe_2)_2$], 2.47–2.78 (3 H, m, CH_2CN and 2′-H), 2.82–3.00 (1 H, m, 2″-H), 3.29–3.44 (2 H, m, 5′-H and 5″-H), 3.53–3.74 (6 H, m, Pr^iCH, OCH_2 and 6-NCH_2), 3.79 (6 H, m, OMe), 3.81–3.95 (3 H, m, CH_2NHCO and 4′-H), 4.31 (1 H, m, 3′-H), 6.13 (1 H, br t, 6-NH), 6.46 (1 H, m, 1′-H), 6.78–6.85 (4 H, m, ArH), 7.21–7.44 (9 H, m, ArH), 8.02 (1 H, 2s, Pu H), 8.30 (1 H, s, Pu H) and 9.58 (1 H, br s, amide NH); δ_P(80.1 MHz, $CDCl_3$), 148.78 (s) and 148.89 (s).

Fully Protected d(A^{AE}pT) **13**.—A solution of the phosphoramidite **12** (100 mg, 0.11 mmol), in dry acetonitrile (5 cm^3) was added to 3′-*O*-acetylthymidine (26.5 mg, 0.093 mmol) and tetrazole (23 mg, 0.33 mmol), also in dry acetonitrile (3 cm^3). The mixture was stirred for 20 min and a solution of iodine in 2,6-lutidine–water–THF (4% w/v I_2 in 1:1:8 v/v) was added until the iodine colouration persisted. After a further 5 min, dichloromethane (50 cm^3) was added and the mixture washed sequentially with saturated aqueous sodium metabisulphite (5 cm^3), sodium hydrogen carbonate (10 cm^3), brine (10 cm^3) and water (10 cm^3), and dried (Na_2SO_4). The solution was concentrated under reduced pressure and the residue purified by chromatography on silica gel, the appropriate fractions, eluted with CH_2Cl_2–MeOH (100:0–96:4) containing *ca.* 0.5% triethylamine, were combined and evaporated under reduced pressure. The residue was dissolved in the minimum quantity of dry ethyl acetate and precipitated from rapidly stirred pentane to give the title compound **13** (mixture of diastereoisomers) as a colourles powder (91 mg, 75%), R_f 0.3 (system A); *m*/*z* (FAB) 1090 $(M - 1)^-$; δ_H(200 MHz, $CDCl_3$), 1.65 (3 H, s, Me), 2.10 and 2.17 (1 H, 2s, MeCO), 2.24 (2 H, m), 2.66–2.78 (4 H, m), 3.44–3.60 (4 H, m), 3.78 (6 H, s, OMe), 3.84 (2 H, m), 4.10–4.38 (6 H, m), 5.28 (2 H, m), 6.20–6.46 (3 H, m, 1′-H and 6-NH), 6.81 (4 H, m, ArH), 7.26 [10 H, m, pyrimidinyl (Py) 6-H and ArH], 7.94 and 7.96 (1 H, 2s, Pu H), 8.20 and 8.25 (1 H, 2s, Pu H) and 9.30 (1 H, br s, amide NH); δ_P(80.1 MHz, $CDCl_3$), −3.417 and −3.724.

6-N-(2-*Aminoethyl*)-2′-*deoxyadenylyl*-(3′-5′)-*thymidine* **2**.—Fully protected dinucleotide **13** (5 mg) was dissolved in glacial acetic acid–water (8:2, 10 cm^3) and incubated at 35 °C for 30 min. The orange solution was then evaporated and residual acetic acid co-evaporated with distilled water (3 × 20 cm^3) until no hint of acetic acid could be detected. Concentrated ammonia (*d* 0.88, 3 cm^3) was added to the gum and the flask sealed at room temperature. After 3 h, liquid chromatography indicated that the reaction was complete. Water (10 cm^3) was added and the mixture was extracted with diethylether (2 × 10 cm^3), evaporated and then co-evaporated with water (3 × 10 cm^3) to produce the title compound as a colourless solid in essentially quantitative yield (105 A_{260} units). For spectroscopic analysis small amounts of the product (40–50 A_{260} units) were purified on an octadecylsilyl silica cartridge (SEP-PAK, Millipore) connected to a syringe and eluted with a stepwise gradient of 0–30% acetonitrile in 50 mmol dm^{-3} triethylammonium hydrogen carbonate; t_R 11.9 min (liquid chromatography); *m*/*z* (FAB, glycerol) 597 $(M - 1)^-$; δ_H(250 MHz, D_2O); 1.59 (3 H, s, Me), 2.29 (2 H, m, 2′-H), 2.85 (2 H, m, 2′-H), 3.43 (2 H, m), 3.85–4.02 (4 H, m), 4.14 (2 H, m), 4.27 (1 H, m), 4.37 (1 H, m), 4.56 (1 H, m, 3′-H), 4.68 (1 H + HOD, m), 6.20 (1 H, t, *J* 6.6), 6.39 (1 H, t, *J* 6.5), 7.44 (1 H, s, Py 6-H), 8.25 (1 H, s, Pu) and 8.29 (1 H, s, Pu).

Enzymatic Digestion of 6-N-(2-*Aminoethyl*)-2′-*deoxyadenylyl*-(3′-5′)-*thymidine* **2**.—To a solution of **2** (*ca.* 5 A_{260} optical density units) in HEPES buffer (100 mm^3, 25 mmol dm^3, pH 7.4) snake venom phosphodiesterase (8 units) was added and reaction monitored by liquid chromatography. Digestion products (dA^{AE} and dTMP) were identified by co-injection with standard samples.

Acknowledgements

We are grateful to the SERC for provision of a studentship (M. E. D.) and also wish to thank Mr. A. Mills (Liverpool) and the SERC service at Swansea for obtaining FAB mass spectra. The assistance of Mrs. A. Murphy in the typing of this manuscript is also appreciated.

References

1 J. W. Lown, L. W. McLaughlin and Y-M. Chang, *Bioorg. Chem.*, 1978, **7**, 97.
2 T. R. Webb and M. D. Matteucci, *J. Am. Chem. Soc.*, 1986, **108**, 2764.
3 M. D. Matteucci and T. R. Webb, *Tetrahedron Lett.*, 1987, **28**, 2469.
4 U. Pieles, B. S. Sproat, P. Neuner and F. Cramer, *Nucl. Acids Res.*, 1989, **17**, 8967.
5 R. Shapiro, B. Brauerman, J. B. Louis and R. E. Servis, *J. Biol. Chem.*, 1973, **248**, 4060.
6 K. K. Ogilvie, *Can. J. Chem.*, 1973, **51**, 3799.
7 V. Nair and S. G. Richardson, *J. Org. Chem.*, 1980, **45**, 3969.
8 V. Nair and S. D. Chamberlain, *J. Org. Chem.*, 1985, **50**, 5069.
9 M. Nogueras, M. Lucas and J.-L. Imbach, *Nucleosides, Nucleotides*, 1987, **6**, 369.
10 K. J. Gibson and S. J. Benkovic, *Nucl. Acids Res.*, 1987, **16**, 6455.

11 E. Happ, C. Scalfi-Happ and S. Chladek, *J. Org. Chem.*, 1987, **52**, 5387.
12 C. Bleasdale, S. B. Ellwood and B. T. Golding, *J. Chem. Soc., Perkin Trans. 1*, 1990, 803.
13 F. Eckstein and M. Imazawa, *J. Org. Chem.*, 1979, **44**, 2039.
14 (*a*) J. Telser, K. A. Cruickshank, L. E. Morrison and T. L. Netzel, *J. Am. Chem. Soc.*, 1990, **111**, 6966; (*b*) B. S. Sproat, A. I. Lamond, B. Beijer, P. Neuner and U. Ryder, *Nucl. Acids Res.*, 1989, **17**, 3373; (*c*) F. W. Hobbs, *J. Org. Chem.*, 1989, **54**, 3420.
15 (*a*) S. L. Beaucage and M. H. Caruthers, *Tetrahedron Lett.*, 1981, **22**, 1859; (*b*) L. J. McBride and M. H. Caruthers, *Tetrahedron Lett.*, 1983, **24**, 245.
16 N. D. Sinha, J. Biernat, J. McManus and H. Köster, *Nucl. Acids Res.*, 1984, **12**, 4539.
17 M. Bodanszky, *The Practice of Peptide Synthesis*, Springer-Verlag, New York, 1984, p. 64.
18 A. Vogel, *Vogel's Textbook of Practical Organic Chemistry*, Longmans, London, 1978, p. 409.

Paper 0/05129B
Received 15*th November* 1990
Accepted 18*th December* 1990