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Synthesis and duplex stability of oligonucleotides containing adenine–guanine analogues*,†

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ABSTRACT

The nucleosides N^6-methoxy-2′-deoxyadenosine (d**Z**) and 2-amino-9-(2-deoxy-β-ribofuranosyl)-6-methoxyaminopurine (d**K**) have been synthesised and converted into 5′-*O*-dimethoxytrityl 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidites). These monomers have been used in machine DNA synthesis to give a set of heptadecanucleotides containing up to three analogue nucleotides. The melting transitions (T_m) show that the 17-mer duplexes containing **Z**·**T** and **Z**·**C** base-pairs have closely similar stabilities, as have those containing **K**·**T** and **K**·**C** pairs. They are less stable than the corresponding fully complementary duplexes, but more stable than those containing mismatched pairs. This, in the case of d**Z**, is in accord with the amino-imino tautomeric ratio of ~1:4 observed for the nucleoside in methyl sulfoxide. The application of oligomers containing such "degenerate" bases in oligonucleotide probes and primers is discussed.

INTRODUCTION

The genetic code is degenerate. One consequence is that a unique oligonucleotide sequence cannot be deduced from a peptide sequence within the encoded protein. Thus, oligonucleotides for probing genomic or copy DNA banks for such sequences by hybridisation must, in general, consist of many chains[1]. This multiplicity can be diminished, in part, in a variety of ways. One way is to use deoxyinosine (d**I**) in the probe, since hypoxanthine forms base pairs with all the normal bases[2,3]. Their stabilities, however, vary considerably and indeed do not contribute to the duplex stability as a whole. Nevertheless, use has been made of **I** in designing primers for the polymerase chain reaction[4].

Instead of using a "universal" base with which to replace the normal purines and pyrimidines, **A**, **G**, **C**, and **T**, we thought it more appropriate to seek a pyrimidine analogue to base-pair with **A** and **G**, and correspondingly a purine analogue to pair with **C** and **T**. Thus, initially, we investigated oligonucleotides containing the pyrimidine analogues N^4-methoxycytosine (mo^4C;**M**) (**1**) and the bicyclic base (**P**) **2**[5,6]. These were chosen because the tautomeric state of mo^4C was believed to be of the order of 10–30 in favour of the imino form, that is, it was much closer to unity than **C** or **T** in which the

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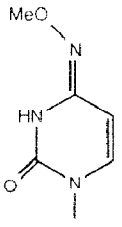
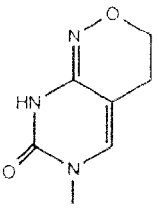
equilibrium constants in favour of the amino and carbonyl tautomers, respectively, are $\sim 10^5$. The preferred *syn* orientation of the methoxyl group in **1**, an impediment to Watson–Crick base pairing, is excluded in **2**, and oligonucleotide duplexes containing the latter base paired to either **A** or **G** showed similar stabilities, comparable to those with the normal complements and much more stable than those with mismatched pairs[6].



1



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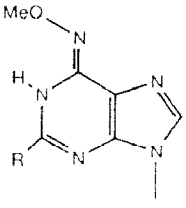
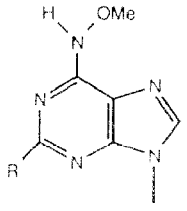


3a



3b

If a purine derivative with comparable degeneracy could be obtained as well, it might be hoped that, by using both, hybridisation probes and primers could be designed with very much reduced multiplicity. With this objective in view, we have begun a study of potential purine analogues **3** incorporated into oligonucleotides and have used, to begin with, as a measure of success, the mid-points of the melting transitions (T_m) of duplexes containing them.

Shugar and co-workers[7] have made a study of N^6-methoxy-2′,3′,5′-tri-*O*-methyladenosine and have found that the methoxyl group is *syn* with respect to N-1. More importantly, they showed that both tautomers **3a,b** (R = H) could be observed by ^{1}H-n.m.r. spectroscopy[8]. In a low dielectric solvent, $CDCl_3$, the amino tautomer is present to the extent of ~70%. Another study[11] of N^6-methoxy-9-methyladenine in Me_2SO gave a value of 29%. During the synthetic work, described below, the ^{1}H-n.m.r. spectrum of N^6-methoxy-3′,5′-di-*O*-*p*-toluoyl-deoxyadenosine in Me_2SO was recorded (Fig. 1). Integration of the resonances for H-2 and H-8 corresponding to the two tautomers gave a value of ~25% for the amino form. Thus, solely from a free energy point of view, an N^6-methoxyadenine residue should show the desired degenerate hydrogen-bonding characteristics. It was felt that the corresponding purine related to guanine, *i.e.*, N^6-methoxy-2,6-diaminopurine (**3**, R = NH_2), should give further base-pair stabilisation.

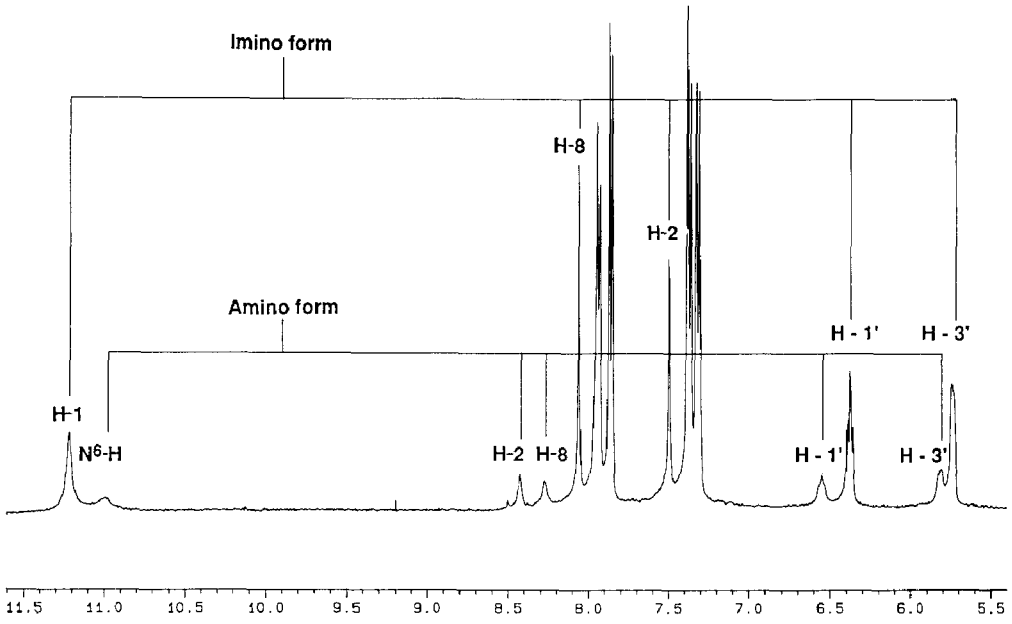


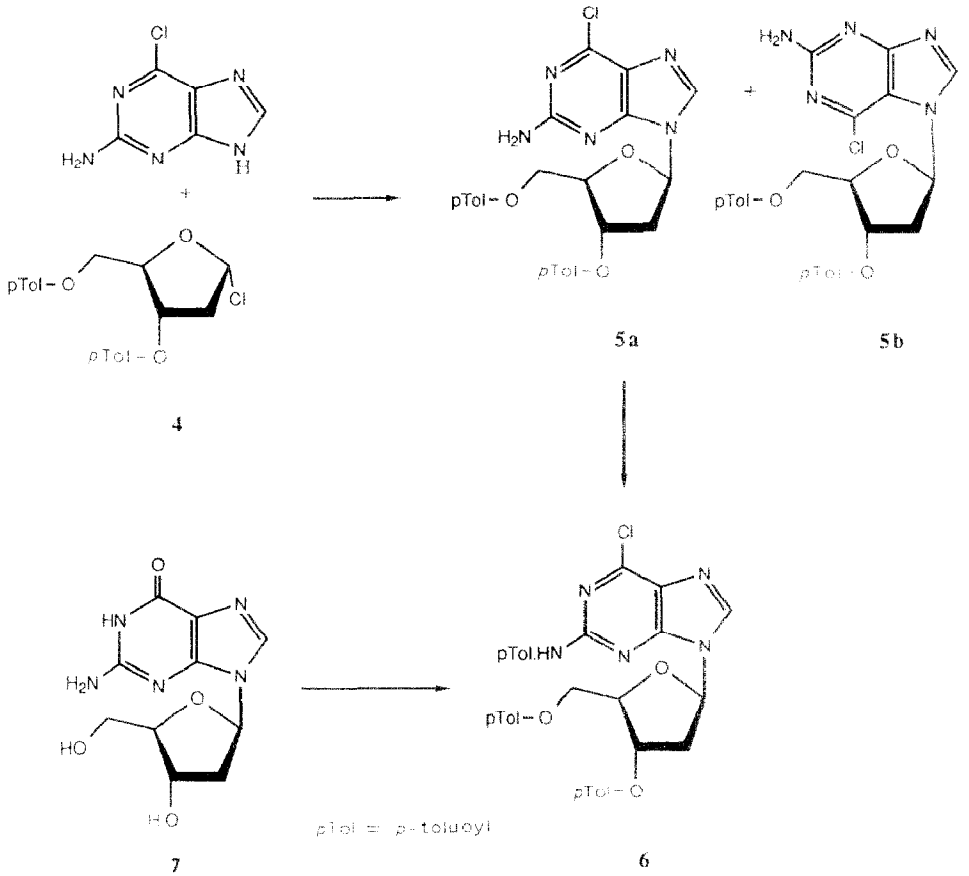
Fig. 1. 400-MHz ^{1}H-n.m.r. spectrum (external Me_4Si) of 2′-deoxy-N^6-methoxy-3′,5′-di-*O*-*p*-toluoyladenosine in Me_2SO at 30°. An equilibrium mixture of imino and amino species is shown by the presence of two sets of proton signals for the base and the sugar (the assignments are based on ref. 13).

RESULTS AND DISCUSSION

The most obvious route to N^6-methoxy-2,6-diaminopurine nucleosides is from deoxyguanosine. Sugar-protected O^6-sulphonylated intermediates have been used in nucleophilic displacements at that site[9,10]. Unfortunately, reaction with methoxyamine was temperamental and, although products of the form **3** (R = HNAcyl) were obtained[11], we preferred the earlier methods involving 6-chloro intermediates[12]. The nucleosides corresponding to **8** and **11** have also been synthesised by other workers, using a Dimroth rearrangement route[13,14].

2-Amino-6-chloropurine was coupled in high yield with 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-ribosyl chloride, using the phase-transfer method of Seela and co-workers[15,16], which leads to inversion at C-1 with high stereospecificity. The major product was the 9-β-nucleoside **5a** together with a minor product assigned the 7-β structure **5b**. In order to establish the structure of the major product **5a**, it was further acylated to the N^2,3′,5′-tri-*p*-toluoyl derivative **6**. Deoxyguanosine (**7**) was tri-*p*-toluoylated and then converted, although in poor yield, into the 6-chloro compound **6**, identical to that from the glycosylation route.

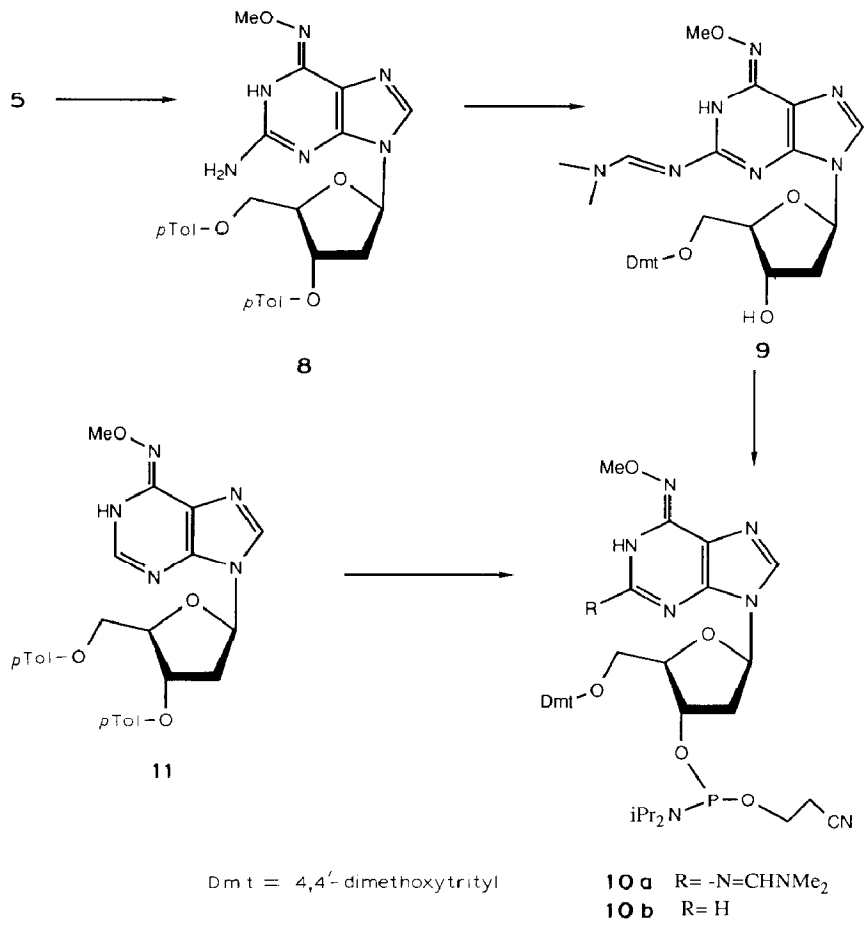
Conversion of **5a** into the N^6-methoxy derivative **8** was best effected by methoxyamine in dry ethanol, following Giner-Sorolla and co-workers[12]. We found that, in this series, as with 2,6-diaminopurine nucleosides, N^2-acyl groups require vigorous conditions for their removal[9]. The nucleoside **8** was therefore deacylated and converted into



the N^2-dimethylaminomethylene intermediate, in high yield, and thence into the 5'-*O*-(4,4'-dimethoxytrityl) derivative **9**. This product was converted into the 3'-(2-cyanoethyl *N*,*N*-di-isopropylphosphoramidite "monomer" **10a** in the normal way[17]. Using a similar route from 6-chloropurine, N^6-methoxy-3',5'-di-*O*-*p*-toluoyl-deoxyadenosine (**11**) was obtained and, after deacylation, was converted into the monomer **10b**. In the chloropurine coupling reaction, no evidence of a minor regio-isomer was found.

The analogue phosphoramidites **10a** and **10b** were then used to prepare oligomers by automatic machine synthesis, using the same coupling times used for the normal monomers. They were worked-up in the usual way following treatment with aqueous ammonia, and purified by ion-exchange h.p.l.c. The oligomers synthesised are listed in Table I.

Inspection of Table I shows that the fully complementary duplexes (entries 1,2) are more stable than those containing N^6-methoxyadenine (**Z**) or N^6-methoxy-2,6-diaminopurine (**K**). Indeed, a single **G·T** mismatch (entry 3) reduces the T_m less than does a single **Z·T** or **K·T** pair (entries 6,11). The **Z**-containing duplexes have uniformly lower T_m values than the **K**-series. Turning, therefore, to the latter series, the original



intention of the experiments appears to be borne out, that is, the base pairs **K·T** and **K·C** give closely similar contributions to duplex stability. This is seen clearly in entries (11,12), (13,15), and (16,17) in which one, two, and three **K**-residues are compared. It is evident that the drop in T_m over this series is relatively small and that, compared with the triple mismatch (entry 5), those duplexes with three **K**-residues (entries 16,17) are much more stable. Further experiments will be required to show whether oligomers that contain these residues are useful as hybridisation probes.

The question as to the structures of the base-pairs with **Z** and **K** remains. It has been shown that addition of a thymine derivative in $CDCl_3$ to N^6-methoxy-2',3',5'-tri-*O*-methyl-deoxyadenosine shifts the tautomeric equilibrium of the latter towards the amino form[8]. The free energy difference between the imino and amino forms must be very small. Moreover, the stabilities of duplexes that contain **Z** suggest strongly that the **Z·T** and **Z·C** base-pairs should contain more than one hydrogen bond and are of comparable stability. A similar observation was recently noted by Ueda and co-workers[18], although no T_m values were given. The greater stability of the **K·T** and **K·C**

TABLE I

T_m values (°) of duplexes formed between heptadecamers that contain **Z** and **K** and two complementary duplexes that differ only at position 9 (**T**,**C**). For comparison, T_m values of the fully complementary duplexes and others that contain **G·T** and **A·C** mismatches are included (data from ref. 6)

1.	ACTTGGCCGCCATTTTG TGAACCGGCGGTAAAAC	75
2.	ACTTGGCCACCATTTTG ----------T----------	72
3.	ACTTGGCCGCCATTTTG ----------T----------	70
4.	ACTTGGCCACCATTTTG ----------C----------	64 64
5.	ACTTGGCCACCATTTTG ---------T-------C----C--------	43
6.	ACTTGGCCZCCATTTTG TGAACCGGTGGTAAAAC	65
7.	ACTTGGCCZCCATTTTG ----------C----------	64
8.	ACTTGGCCZCCZTTTTG ----------T----T-----	61
9.	ACTTGGCCZCCZTTTTG ----------C----T-----	57
10.	ACTTGZCCZCCATTTTG ----------C----C----------	52
11.	ACTTGGCCKCCATTTTG TGAACCGGTGGTAAAAC	67
12.	ACTTGGCCKCCATTTTG ----------C----------	66
13.	ACTTGGCCKCCKTTTTG ----------T----T-----	64
14.	ACTTGGCKCCKTTTTG ----------C----T-----	62
15.	ACTTGKCCKCCATTTTG ---------C----C----------	59
16.	ACTTGKCCKCCKTTTTG ----------C-----T-----T-----	58
17.	ACTTGKCCKCCKTTTTG ----------C-----C-----T-----	57

pairs implies a further stabilisation. Although further work will be necessary to substantiate the case, it seems probable that the purine bases can take up the amino and imino tautomeric forms required for Watson–Crick pairing with thymine and cytosine (Fig. 2). It is noteworthy that, in the case of the related base 2,6-diaminopurine (**D**), oligomer duplexes containing the base pairs **D·T** and **D·C** have widely different stabilities[9]. This emphasises the levelling effect achieved by the introduction of the N^6-methoxyl function; the somewhat lowered stability observed, compared with the normal base-pairs **A·T** and **G·C**, can then be ascribed *inter alia* to the necessity for the *syn* → *anti* configurational change of the methoxyl group. Base-pairs with more than one hydrogen bond cannot be formed with either Watson–Crick or wobble structures with the methoxyl group *syn*.

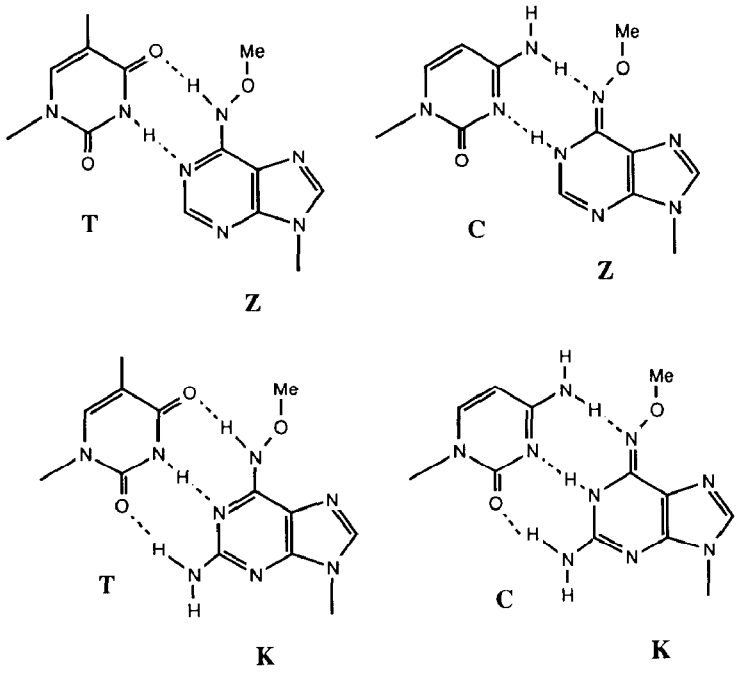


Fig. 2. Bases **Z** and **K** and their amino and imino tautomeric forms pairing with thymine and cytosine in a Watson–Crick manner.

EXPERIMENTAL

General. — 2-Amino-6-chloropurine and 6-chloropurine were purchased from Aldrich Chemical Co. 2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-ribosyl chloride (2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentosyl chloride*) was a generous gift from Dr. R. Hinman and Pfizer Inc., and was also synthesised by the method of Hoffer[20]. Flash-column chromatography and t.l.c. were done using Kieselgel 60 H (7736) and 60 F_{254} (Merck), respectively, with chloroform–methanol mixtures unless otherwise stated. ^{1}H-N.m.r. spectra (external Me_4Si) were recorded with Bruker WM 250 MHz and AM 400 MHz spectrometers. Mass spectra were recorded with a Kratos M350 instrument, and melting points were measured on an Electrothermal apparatus and are uncorrected.

2-Amino-6-chloro-9-(2-deoxy-3,5-di-O-p-*toluoyl-β-*D-*ribofuranosyl)purine* (**5a**). — A suspension of finely powdered KOH (3.2 g, 58 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) (0.376 g, 1.16 mmol) was stirred in anhydrous acetonitrile (240 mL) at room temperature under argon. After 15 min, 2-amino-6-chloropurine (2.0 g, 11.6 mmol) was added and stirring was continued for 10 min. 2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-ribosyl chloride (4.88 g, 12.0 mmol) was added and, after 40 min, the suspension was filtered and taken to dryness. The crude product (6.0 g) was purified by

* In this paper, derivatives of 2-deoxy-D-*erythro*-pentose are named as derivatives of 2-deoxy-D-ribose.

flash-column chromatography, the faster-running major component was collected, and the product (3.22 g, 51%) was crystallised from acetonitrile to give **5a** as needles, m.p. 187–188°; λ_{max} (95% EtOH) 222, 242, and 310 nm (broad); ε_{mM} 4.44, 4.45, and 3.85. ^{1}H-N.m.r. data (Me_2SO): δ 2.37 (s, 3 H, CH_3), 2.40 (s, 3 H, CH_3), 2.69–2.79 (m, 1 H, H-2'a), 3.19–3.31 (m, 1 H, H-2'b), 4.51–4.65 (m, 3 H, H-4',5'a,5'b), 5.73–5.76 (m, 1 H, H-3'), 6.40 (t, 1 H, *J* 6.6 Hz, H-1'), 7.02 (s, 2 H, NH_2-2), 7.03–7.39 (m, 4 H, Ar), 7.82–7.95 (m, 4 H, Ar), 8.35 (s, 1 H, H-8).

Anal. Calc. for $C_{26}H_{24}ClN_5O_5$: C, 59.8; H, 4.6; N, 13.4; *m/z* (M$^+$) 521.1427. Found: C, 59.3; H, 4.8; N, 13.4; M$^+$ 521.1485.

2-Amino-6-chloro-7-(2-deoxy-3,5-di-O-p-*toluoyl-β*-D-*ribofuranosyl)purine* (**5b**). The slower running, minor component from the chromatography above was collected and the product (0.95 g, 15%) was isolated as a foam with λ_{max} (95% EtOH) 238 and 322 nm (broad), ε_{mM} 4.56 and 3.69. ^{1}H-N.m.r. data (Me_2SO): δ 2.36 (s, 3 H, CH_3), 2.38 (s, 3 H, CH_3), 2.81–2.91 (m, 1 H, H-2'a), 3.04–3.13 (m, 1 H, H-2'b), 4.49–4.64 (m, 3 H, H-4',5'a,5'b), 5.69 (t, 1 H, *J* 3.12 Hz, H-3'), 6.67 (t, 1 H, *J* 6.4 Hz, H-1'), 6.74 (s, 2 H, NH_2-2), 7.25–7.37 (m, 4 H, Ar), 7.77–7.94 (m, 4 H, Ar), 8.72 (s, 1 H, H-8).

Anal. Calc. for $C_{26}H_{24}ClN_5O_5$: C, 59.8; H, 4.6; N, 13.4; *m/z* (M$^+$) 521.1427. Found: C, 59.3; H, 4.5; N, 13.2; M$^+$ 521.1467.

6-Chloro-9-(2-deoxy-3,5-di-O-p-*toluoyl-β*-D-*ribofuranosyl)-2*-p-*toluamidopurine* (**6**). — (*a*) A solution of 2'-deoxyguanosine (1.0 g, 3.7 mmol) in pyridine (15 mL) was treated dropwise with *p*-toluoyl chloride (1.71 g, 11.0 mmol). After 8 h at 40°, the solvent was evaporated *in vacuo* and a solution of the residue in CH_2Cl_2 was washed with aqueous $NaHCO_3$, water, and dried. Removal of the solvent and chromatography gave the tri-*p*-toluoyl derivative (1.0 g, 43%). ^{1}H-N.m.r. data (Me_2SO): δ 2.36 (s, 3 H, CH_3), 2.39 (s, 3 H, CH_3), 2.40 (s, 3 H, CH_3), 2.74–2.82 (m, 1 H, H-2'a), 3.18–3.29 (m, 1 H, H-2'b), 4.51–4.68 (m, 3 H, H-4',5'a,5'b), 5.74 (d, 1 H, *J* 5.6 Hz, H-3'), 6.42–6.49 (m, 1 H, H-1'), 7.25–7.40 (m, 4 H, Ar), 7.68–7.97 (m, 4 H, Ar), 8.28 (s, 1 H, H-8), 11.76 (b, 1 H, NH), 12.34 (b, 1 H, NH).

To a solution of the above derivative (0.5 g, 0.8 mmol) in dry acetonitrile (10 mL) was added tetramethylammonium chloride (0.26 g), *N,N*-dimethylbenzylamine (0.18 mL), and phosphoryl chloride (0.67 mL). The solution was boiled under reflux for 1 h, the solvent was evaporated *in vacuo*, and a solution of the residue in $CHCl_3$ was added to ice–water. The $CHCl_3$ solution was washed with aqueous $NaHCO_3$, water, and dried. Removal of the solvent then chromatography gave **6** as a pale-yellow foam (0.12 g, 23%). ^{1}H-N.m.r. data (Me_2SO): δ 2.35 (s, 3 H, CH_3), 2.38 (s, 3 H, CH_3), 2.40 (s, 3 H, CH_3), 2.76–2.81 (m, 1 H, H-2'a), 3.40–3.46 (m, 1 H, H-2'b), 4.57–4.71 (m, 3 H, H-4',5'a,5'b), 5.90 (d, 1 H, *J* 3.0 Hz, H-3'), 7.24–7.39 (m, 4 H, Ar), 7.76–7.96 (m, 4 H, Ar), 8.74 (s, 1 H, H-8), 11.23 (s, 1 H, NH).

Anal. Calc. for $C_{34}H_{30}ClN_5O_6$: *m/z* (M$^+$) 639.1884. Found: M$^+$ 639.1905.

(*b*) Compound **5a** (0.42 g, 0.77 mmol) was treated with *p*-toluoyl chloride (0.92 g, 0.143 mmol) in anhydrous pyridine (15 mL) for 6 h. After the usual work-up, the product was purified by chromatography to afford **6** as a foam that was identical with the product in (*a*).

6-Chloro-9-(2-deoxy-3,5-di-O-p-*toluoyl-β-*D-*ribofuranosyl)purine*. — 6-Chloropurine (2.5 g) was treated with 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-ribosyl chloride, as described above for **5a**, to give the title compound (4.13 g, 48%), m.p. 119° (from acetonitrile). ^{1}H-N.m.r. data ($CDCl_3$): δ 2.39 (s, 3 H, CH_3), 2.44 (s, 3 H, CH_3), 2.83–2.92 (m, 1 H, H-2'a), 3.10–3.22 (m, 1 H, H-2'b), 4.61–4.83 (m, 3 H, H-4',5'a,5'b), 5.81–5.84 (m, 1 H, H-3'), 6.53–6.59 (m, 1 H, H-1'), 7.18–7.29 (m, 4 H, Ar), 7.83–7.98 (m, 4 H, Ar), 8.28 (s, 1 H, H-8), 8.66 (s, 1 H, H-2).

Anal. Calc. for $C_{26}H_{23}ClN_4O_5$: C, 61.6; H, 4.5; N, 11.1; *m/z* (M^+) 506.1356. Found: C, 61.3; H, 4.5; N, 10.9; M^+ 506.1311.

2-Amino-9-(2-deoxy-3,5-di-O-p-*toluoyl-β-*D-*ribofuranosyl)-6-methoxyaminopurine* (**8**). — To a solution of **5a** (0.2 g, 0.368 mmol) in dry EtOH (2 mL) was added methoxyamine[21] (0.5 mL), and the sealed vessel was heated at 90° for 4 h. The solvent was evaporated and a solution of the product in $CHCl_3$ was chromatographed to give **8** (100 mg, 49%) as a foam. ^{1}H-N.m.r. data (Me_2SO): δ 2.38 (s, 3 H, CH_3), 2.40 (s, 3 H, CH_3), 2.62–2.70 (m, 1 H, H-2'a), 3.00–3.12 (m, 1 H, H-2'b), 3.73 (s, 3 H, $NOCH_3$), 4.46–4.64 (m, 3 H, H-4',5'a,5'b), 5.66–5.69 (m, 1 H, H-3'), 6.18–6.24 (m, 1 H, H-1'), 6.58 (b, 2 H, NH_2), 7.31–7.38 (m, 4 H, Ar), 7.72 (s, 1 H, H-8), 7.86–7.98 (m, 4 H, Ar), 9.84 (s, 1 H, NH).

Anal. Calc. for $C_{27}H_{28}N_6O_6$: *m/z* (M^+) 532.2070. Found: M^+ 532.2025.

2'-Deoxy-N^6-*methoxy-3',5'-di*-O-p-*toluoyladenosine* (**11**). — 6-Chloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-ribofuranosyl)purine was treated with methoxyamine in dry EtOH, as described above, to obtain **11**, as a colourless crystalline powder, m.p. 213°. ^{1}H-N.m.r. data (Me_2SO): imino tautomer, δ 2.38 (s, 3 H, CH_3), 2.40 (s, 3 H, CH_3), 2.68–2.75 (m, 1 H, H-2'a), 3.16–3.21 (m, 1 H, H-2'b), 3.76 (s, 3 H, $NOCH_3$), 4.49–4.62 (m, 3 H, H-4',5'a,5'b), 5.75 (b, 1 H, H-3'), 6.38 (t, 1 H, *J* 6.6 Hz, H-1'), 7.30–7.38 (m, 4 H, Ar), 7.49 (s, 1 H, H-2), 7.84–7.95 (m, 4 H, Ar), 8.07 (s, 1 H, H-8), 11.25 (b, 1 H, NH); amino tautomer, δ 5.61 (b, H-3'), 6.54 (b, H-1'), 8.27 (s, H-8), 8.42 (s, H-2), 11.00 (b, NH).

Anal. Calc. for $C_{27}H_{27}N_5O_6$: *m/z* (M^+) 517.1961. Found: M^+ 517.1972.

*9-[2-Deoxy-5-*O-*(4,4'-dimethoxytrityl)-β-*D-*ribofuranosyl]-2-dimethylaminomethyleneamino-6-methoxyaminopurine 3'-(2-cyanoethyl* N,N-*di-isopropylphosphoramidite)* (**10a**). — Compound **8** was heated at 55° overnight with saturated NH_3/MeOH to give the free nucleoside quantitatively, a solution of which (0.4 g, 1.35 mmol) in anhydrous *N,N*-dimethylformamide (2.5 mL) and *N,N*-dimethylformamide dimethyl acetal (2.5 mL) was stirred at 50° for 2 h. Removal of the solvent and further coevaporation of toluene and acetone from the residue *in vacuo* gave the N^2-dimethylaminomethylene derivative (one spot in t.l.c.). The crude product was treated with 4,4'-dimethoxytrityl chloride (0.54 g, 1.62 mmol) in dry pyridine at room temperature for 1.5 h. Removal of the solvent *in vacuo* then chromatography of the dark-blue foam with CH_2Cl_2–Me_2CO (4:1) afforded the dimethoxytrityl derivative **9** (131 mg) as a pale-yellow foam. ^{1}H-N.m.r. data (Me_2SO): δ 3.01 (s, 3 H, NCH_3), 3.10 (s, 3 H, NCH_3), 3.72 (s, 9 H, 3 OCH_3), 6.77–7.36 (m, 13 H, Ar), 7.77 (s, 1 H, H-8), 8.48 (s, 1 H, N=CHN), 8.88 (s, 1 H, NH).

A solution of **9** (120 mg, 0.19 mmol) in anhydrous tetrahydrofuran (5 mL) and Hunig's base (0.132 mL, 7.6 mmol) was treated, with the exclusion of moisture, with 2-cyanoethyl *N,N*-di-isopropylphosphoramidochloridite (0.66 mL, 0.285 mmol). Reaction was complete in 1 h. The solution was diluted with ethyl acetate, washed with saturated aqueous NaCl, and dried (Na_2SO_4). Chromatography of the product with ethyl acetate–CH_2Cl_2–Et_3N (45:45:10) afforded **10a** (115 mg, 73%). ^{31}P-N.m.r. data ($CDCl_3$): δ 148.93 and 149.15.

2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-N^6-methoxyadenosine 3'-(2-cyanoethyl N,N-di-isopropylphosphoramidite) (**10b**). — Compound **11** was deacylated to the free nucleoside, which was converted into the 5'-*O*-(4,4'-dimethoxytrityl) derivative. This product was chromatographed and the pure compound (51% yield) was converted, as described above, into **10b**. ^{31}P-N.m.r. data ($CDCl_3$): δ 149.08 and 149.22.

Synthesis of oligonucleotides. — The phosphoramidite monomers **10a** and **10b** were incorporated into oligonucleotides, using the normal programme on Applied Biosystems 380B and Pharmacia gene assembler Instruments. Deprotection was complete after treatment with conc. aqueous NH_3 at 55° overnight. Purification was carried out by h.p.l.c. on a Waters system, using a Whatman SAX Partisphere column and potassium phosphate (pH 6.6) gradient in aqueous 60% formamide. The oligomers synthesised are listed in Table I.

Melting transitions of oligonucleotide duplexes. — Melting transitions were measured at 260 nm in 6xSSC buffer[22] at an oligomer strand concentration of ~3μM. Absorbance *vs.* temperature for each duplex was obtained using a Unicam SP500 spectrometer (Pye Unicam, Cambridge, U.K.) fitted with a Gilford 222 photometer and 2527 thermoprogrammer (Gilford Instruments, Oberli, OH). The temperature was increased by 1°/min and melting temperatures (T_m) were determined as the midpoints of the sigmoidal melting curves with an error of ±1°.

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