Improved synthesis of oligonucleotides with an allylic backbone. Oligonucleotides containing acyclic, achiral nucleoside analogues: *N*-1 or *N*-9-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]nucleobases

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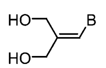
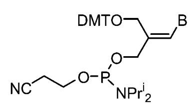
An improved phosphoramidite method is described to prepare oligonucleotides modified with the acyclic, achiral monomers **1**. Examination of dimers, prepared on solid support or in solution, showed that phosphortriester dimers containing the allylic unit **1** were unstable towards bases, whereas phosphordiester dimers were stable. Phosphordiester dimers were obtained by replacing cyanoethyl phosphoramidites **2** with phosphoramidites **3**, which gave phosphordiesters directly upon oxidation. The phosphordiester dimers were found to be stable towards capping and oxidation, but were somewhat labile towards acids. By reducing the contact time to acids during detritylation it was possible to prepare oligonucleotides containing 4 or 8 modified A, G or T units. The modified oligonucleotides hybridized to complementary DNA and RNA, although with reduced affinity (ΔT_m per modification −1 to −5 °C).

Introduction

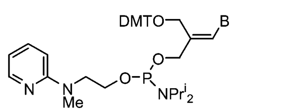
Modified oligonucleotides are of current interest as potential therapeutic agents (antisense or antigene drugs).[1,2] The oligonucleotides need modification to be resistant to nucleases, but the modified units should retain the high selectivity and strong binding of natural DNA and RNA, based on Watson–Crick (or Hoogsteen) bonding. For some years we have studied modified nucleosides where the sugar unit is replaced by a simple achiral unit, *N*-1 or *N*-9-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]nucleobases **1a**–**f** (Fig. 1).[3–7]



1a B = thymine
1b B = adenine
1c B = cytosine
1d B = 5-methylcytosine
1e B = guanine
1f B = uracil



2a B = thymine
2b B = *N*-6-(4-tertbutylbenzoyl)-adenine
2d B = *N*-4-(dibutylaminomethylen)-5-methylcytosine
2e B = *N*-2-isobutyrylguanine



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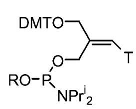

2a′ R = 2-cyanoethyl
3a′ R = *N*-methyl-*N*-(2-pyridyl)-2-aminoethyl

Fig. 1

The central C=C bond restricts the molecules conformationally, and molecular modelling and geometry calculations indicate that **1a** should be able to mimic thymidine in both A- and B-type helices.[4] Synthetic problems concerning the preparation of the monomers **1a**–**f** have been solved,[6,7] but incorporation into DNA *via* the phosphoramidites **2** was troublesome, and DNA oligomers containing only one or two thymine monomers have hitherto been studied.[5] The resistance of these oligomers to snake venom phosphordiesterase was satisfactory, but the binding affinity towards complementary DNA and RNA strands was found to be reduced compared to native DNA (ΔT_m − 2 to −6.5 °C per modification).[5]

In our previous study,[5] the phosphoramidite **2a** was shown to couple efficiently when activated with tetrazole. An aqueous wash was introduced to remove unwanted phosphitylation at the bases. Oxidation with Bu^tOOH instead of I_2–H_2O–Py was necessary since the latter reagent cleaved the allylic C–O bond. However, after detritylation with CCl_3COOH–CH_2Cl_2, the next coupling gave a low yield (DMT efficiency 80–85%), independent of the identity of the next phosphoramidite, and prolonged or repeated couplings did not improve the yield. Removal of the cyanoethyl groups on the phosphortriesters flanking the modified unit was problematic, probably due to competing removal of the allylic modified unit. Thus, aqueous NH_3 mainly cleaved the sequences at the position of the modification, whereas deprotection with neat, dry Pr^i_2NH (a poorer nucleophile), followed by aq. NH_3, did give some full length product (10–20% after ion exchange purification and desalting).

This paper describes our attempts to optimise the coupling and deprotection conditions. Dimers derived from **2a**, or from the α-isomer **2a′** to obtain symmetrical dimers to ease the interpretation of NMR spectra, have been prepared in solution or on solid support, and their sensitivity to cleavage under different conditions examined by ^{31}P and ^{1}H NMR. It is shown that oxidation with Bu^tOOH and capping with NMI–Ac_2O–Py does not result in cleavage, but that detritylation with 3% CCl_3COOH in CH_2Cl_2 and in particular removal of the cyanoethyl group with dry

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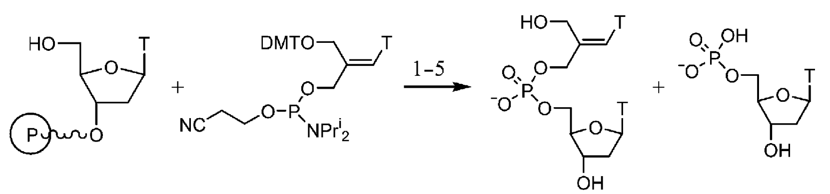
Pr^i_2NH results in partial cleavage. To avoid the latter step, the phosphoramidites **3a** and **3a′** with *N*-methyl-*N*-(2-pyridyl)-2-aminoethyl instead of 2-cyanoethyl have been tested in dimer experiments. The *N*-methyl-*N*-(2-pyridyl)-2-aminoethyl protecting group has been introduced by Beaucage *et al.* and shown to eliminate spontaneously upon oxidation of phosphorus by a cyclodeesterification reaction.[8] Pure modified phosphordiester dimers are formed from phosphite dimers prepared from **3a′** and are shown to be stable to aq. NH_3, but acids used for detritylation results in minor cleavage. The phosphoramidites **3a**, **3b**, and **3e** have been used to prepare some more extensively modified oligonucleotides, which are shown by UV melting experiments to pair with both DNA and RNA, although with reduced affinity compared to unmodified sequences.

Results and discussion

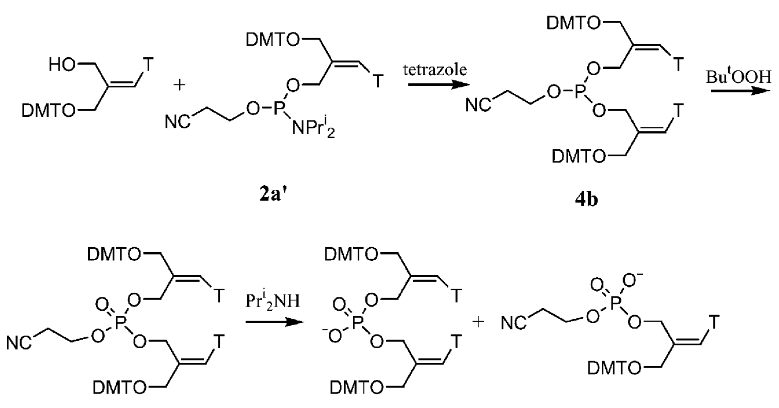
Dimer experiments with cyanoethyl phosphoramidites 2a and 2a′

Initial experiments to prepare a dimer from **2a** and CPG-bound dT, using the protocol described earlier,[5] gave *ca.* 25% of the dimer **4a** (δ_P 0.7) and *ca.* 75% of 5′-pdT **5a** (δ_P 4.2), according to ^{31}P NMR in D_2O (Scheme 1). The products were verified by ES MS $^-$ (**4a** found 515.1, calc. 515.1; **5a** found 321.0, calc. 321.0) and CE, where the major peak coeluted with authentic 5′-pdT (δ_P *ca.* 4.3, pH-dependent). Clearly the dimer **4a** largely decomposed, but at which step(s) was unknown. In order to clarify this another dimer **4c** was prepared by solution chemistry (Scheme 2). Since **4c** was rather labile and attempts to purify it failed, all experiments were done on **4c**, freshly prepared from **4b**. The phosphite dimer **4b**, prepared from the α-amidite **2a′**, was stable and could be purified by column chromatography. This difference in stability reflects the huge difference in leaving group ability between a phosphortriester and a phosphite. Oxidation of **4b** (δ_P 141.1) in CH_3CN with Bu^tOOH gave **4c** (δ_P −0.5) without observable byproducts (^{31}P NMR). The solvent was removed *in vacuo* and the residue treated with dry Pr^i_2NH in dry pyridine. The ^{31}P NMR signal from **4c** (δ_P −0.1) was over 2 h at rt replaced by two new signals (δ_P − 1.8 and −2.0) in a *ca.* 2 : 3 ratio. After evaporation the residue was dissolved in DMSO-d_6 and analysed by ^{31}P and ^1H NMR. The phosphorus spectrum showed two products (δ_P −0.5 and −0.9, ratio *ca.* 2 : 3), and the proton spectrum was in accordance with a *ca.* 2 : 3 mixture of **4d** and **5b** (Scheme 2), containing some free thymine base and unidentified products. This shows that the reaction with Pr^i_2NH is far from clean, since in competition with removal of the cyanoethyl group to give **4d**, the allylic modified unit is also removed from the phosphortriester to give **5b**. The dimer **4c** was shown by ^{31}P NMR to be stable to capping conditions (Ac_2O–NMI–Py–THF) for 24 h at rt. However, **4c** partly decomposed under detritylation conditions. When a solution of **4c** in CH_2Cl_2 was treated with 3% CCl_3COOH in CH_2Cl_2, a precipitate formed. The precipitate was dissolved in DMSO-d_6 and analysed by NMR. The phosphorus spectrum showed one signal (δ_P −0.3), while the proton spectrum was in accordance with a *ca.* 4 : 3 mixture of **4e** and **5c** (Scheme 3), containing some free thymine base and unidentified products. In D_2O the phosphorus spectrum showed the expected two signals (δ_P −1.2 and 0.6). The ratio of these two signals changed with time, leaving only the signal at δ_P 0.6 after 3 d. At that time the proton spectrum showed no signals assigned to **4e**, while the signals assigned to **5c** remained. This indicates that **4e** (δ_P −1.2) slowly decomposed to **5c** (δ_P 0.6) in the weakly acidic solution.



2a **4a** 25% **5a** 75%

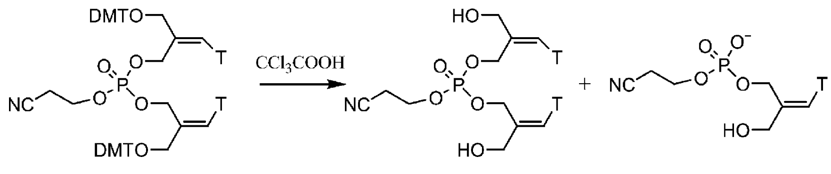
Scheme 1 Reagents: 1. tetrazole, 2. Bu^tOOH, 3. CCl_3COOH, 4. Pr^i_2NH, 5. aq. NH_3.



2a′ **4b**

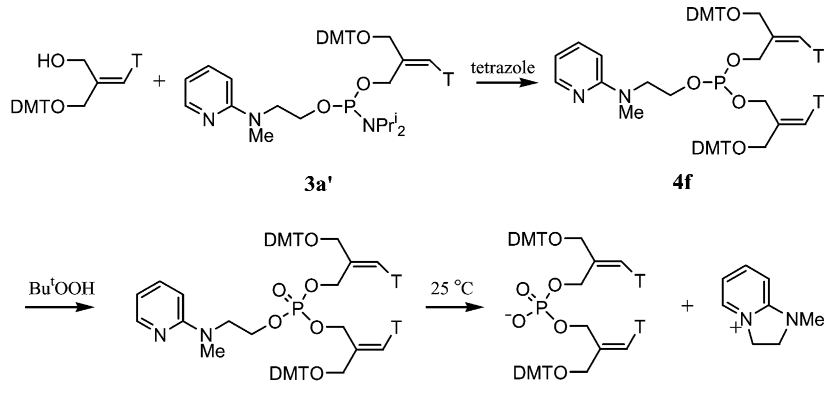
4c **4d** **5b**

Scheme 2



4c 4e 5c

Scheme 3



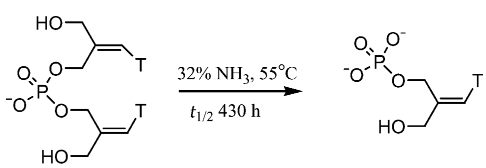
3a' 4f

4g 4d

Scheme 4

Dimer experiments with *N*-methyl-*N*-(2-pyridyl)-2-aminoethyl phosphoramidites 3a and 3a′

The phosphoramidites **3a** and **3a**′ were prepared from crude [*N*-methyl-*N*-(2-pyridyl)-2-aminoethyl] *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite[8] and DMT-protected **1a** in 50–60% yield. The α-amidite **3a**′ was used to prepare the stable symmetrical dimer phosphite **4f** (Scheme 4). Oxidation of **4f** (δ_P 140.9) in CH_3CN with Bu^tOOH gave **4g** (δ_P 0.2), which spontaneously lost the protecting group to give **4d** (δ_P 0.0) with $t_{1/2}$ *ca.* 20 min at 25 °C. Both compounds were formed without byproducts (^{31}P NMR). Extraction of a solution of **4d** in CH_2Cl_2 with aq. $NaHCO_3$–Na_2SO_3 followed by removal of the DMT groups with 80% aq. CH_3COOH gave reasonably pure **4h** (Scheme 5) as the sodium salt in 95% yield. The dimer **4h** was used to evaluate the stability of phosphordiester-linked modified units towards aq. NH_3. A solution of **4h** in 32% aq. NH_3 in a sealed NMR tube was kept at 55 °C, and the ^{31}P NMR spectrum recorded at intervals. A very slow decomposition was observed, **4h** (δ_P 0.5) being transformed to a new compound (δ_P 5.1, t, *J* 7 Hz), probably **5d** (Scheme 5), with $t_{1/2}$ *ca.* 430 h. Since this corresponds to only 0.8% decomposition in 5 h at 55 °C we conclude that the phosphordiester linkage flanking the modified unit is stable enough under normal deblocking conditions to remove the usual base protecting groups.



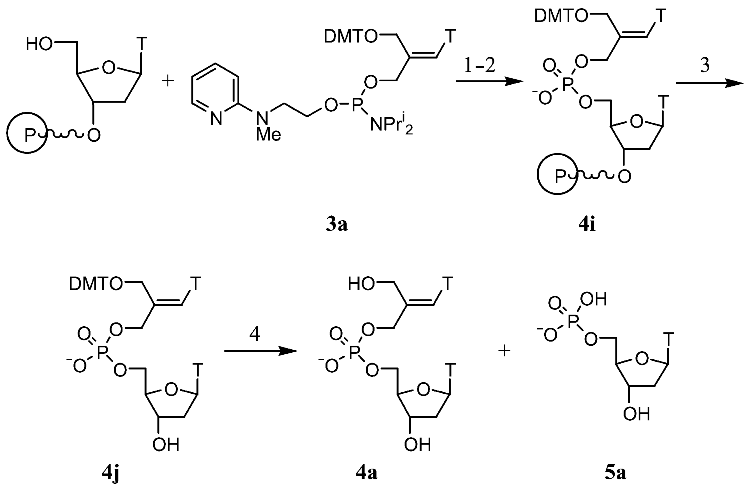
4h 5d

Scheme 5

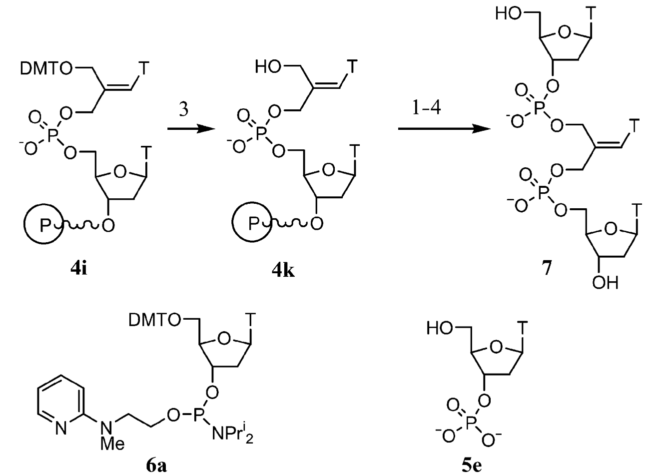
Solid phase experiments with *N*-methyl-*N*-(2-pyridyl)-2-aminoethyl phosphoramidite 3a

A dimer **4i** was prepared from **3a** (50 μmol) and polystyrene-bound dT (10 μmol) (Scheme 6). After oxidation the support was kept overnight in CH_3CN to allow for complete cycloelimination of the phosphorus protecting group. The support was dried and divided in *ca.* 2 μmol portions. One portion of **4i** was treated with NH_3 to give **4j**, which was analysed by ^{1}H and ^{31}P NMR in D_2O. Apart from **4j** (δ_P 1.2) it contained *ca.* 10% **4a** (δ_P 1.3) and *ca.* 3% **5a** (δ_P 4.9, t, *J* 6 Hz). Spontaneous loss of DMT from a support-bound oligonucleotide on standing is well known, but the formation of **5a** indicates that *ca.* 3% decomposition occurs during coupling. Another portion of **4i** was treated with NH_3, followed by 80% aq. CH_3COOH (steps 3 and 4 in Scheme 6) to give **4a**, shown by ^{31}P NMR to contain *ca.* 10% **5a**. This indicates that the diester dimer **4a**, like the triester dimer **4e** before, is not stable under acidic conditions. A third portion of **4i** was detritylated on the synthesizer with 3% CCl_3COOH in CH_2Cl_2 for 2 min, then immediately washed with 1 M Pr^i_2NH in CH_3CN for 2 min, to give **4k** (Scheme 7). This compound was coupled with the Beaucage phosphoramidite **6a** to give the trimer **7** after oxidation, cycloelimination overnight, detritylation followed immediately by a Pr^i_2NH wash as above, and cleavage from the support by aq. NH_3. NMR analysis in D_2O showed **7** (δ_P 1.1 and 0.1, 1 : 1) contaminated with *ca.* 7% **5a** (δ_P 4.9) and *ca.* 1% of probably 3′-pdT **5e** (δ_P 4.4).

The experiments described above show that phosphoramidites like **3a**, which spontaneously eliminate the phosphorus protection group upon oxidation, are in this context wastly superior to cyanoethyl phosphoramidites like **2a**, because a basic elimination step is avoided. Oligonucleotides which contain allylic phosphortriesters like those described here are cleaved by bases even as weakly nucleophilic as Pr^i_2NH. However, when the phosphate



Scheme 6 Reagents and conditions: 1. tetrazole 10 min, then aq. wash; 2. Bu^tOOH 30 min, then CH_3CN wash, then 20 h wait; 3. 32% aq. NH_3 2 h rt, then evap.; 4. 80% aq. CH_3COOH 30 min, then evap., extraction of DMTOH with ether, evap.



Scheme 7 Reagents: 1. **6a** + tetrazole; 2. Bu^tOOH, then wait; 3. CCl_3COOH, then $Pr^i{}_2NH$; 4. aq. NH_3.

linkages are converted to phosphordiesters, they are highly inert towards bases including aq. NH_3. The allylic phosphordiesters are likewise stable towards other conditions during oligonucleotide synthesis (capping, oxidation) except the acidic treatment during detritylation. The strongly acidic conditions necessary to remove DMT probably leads to protonation of the phosphordiester group,[9] thereby converting it into a good leaving group (like a phosphortriester group). This results in partial cleavage of the allylic P–O bond during detritylation. The cleavage is minimised by a short exposure to 3% CCl_3COOH in CH_2Cl_2 (1–2 min) followed immediately by a $Pr^i{}_2NH$ wash. By this procedure a trimer **7** could be prepared with concomitant cleavage of only 3–4% per detritylation. These improved synthesis conditions were used to prepare some oligonucleotides containing 4 or 8 modified units, as described below.

Solid phase oligonucleotide synthesis using *N*-methyl-*N*-(2-pyridyl)-2-aminoethyl phosphoramidite 3a, 3b, and 3e

The oligonucleotides prepared in this study are the 14-mers $dT_5T^*{}_4T_5$, $dA_5A^*{}_4A_5$, and the 9-mer dG*T*G*A*T*A*T*G*C, where N* are the allylic units corresponding to **1**. Attempts to prepare $dT^*{}_{13}T$ gave a very impure product which was not purified, and attempts to use the 5-methylcytosine amidite **2d** to obtain oligonucleotides containing C* were abandoned because the products even with one modified C were very impure. This is in accord with the high tendency of the cytosine compounds **1c**, **1d**, and their *N*-protected derivatives to cyclise.[7] The A* phosphoramidite **3b** and the G* phosphoramidite **3e** were prepared in the same way as the T* phosphoramidite **3a**, and used together with the Beaucage T phosphoramidite **6a** and the A analogue **6b**[10] which are necessary as the 10th base in the 14-mers. The remaining T and A phosphoramidites were commercial cyanoethyl phosphoramidites

The coupling cycle used to introduce N* was modified in several ways from a normal phosphoramidite cycle. A normal coupling with tetrazole as activator was followed by a 2-min aqueous wash to remove amidites that had reacted at the bases. Capping was performed normally, followed by normal oxidation with Bu^tOOH. A wait step of 0.5 h was then introduced to allow the spontaneous elimination of the phosphorus protection group to occur (experiments with 1 h and 2 h wait did not improve the yields). Detritylation with 3% CCl_3COOH in CH_2Cl_2 and CH_3CN wash for a total of 1 min was immediately followed by a wash with 0.1 M $Pr^i{}_2NH$ in CH_3CN to remove acid which partially cleaves the strand at allylic P–O positions. In case of the 14-mers, a standard cycle was used for the first 4 couplings with normal cyanoethyl phosphoramidites, but for the last 5 unmodified couplings the basic wash after detrylation was included, and the 0.5 h wait was used for the couplings with **6**. A stepwise DMT efficiency of 92–94% was obtained for coupling with the N^* phosphoramidites, and 94–96% for the couplings with **6** and the remaining cyanoethyl phosphoramidites, compared to *ca.* 99% for the first four cyanoethyl phosphoramidites. The support-bound oligonucleotides were left overnight to complete the elimination of the *N*-methyl-*N*-(2-pyridyl)-2-aminoethyl groups and then treated with conc. aq. NH_3 at 55 °C for 8 h (2 h at 25 °C for $dT_5T^*{}_4T_5$). After evaporation the crude oligonucleotides were purified by reverse phase HPLC, and the crude and purified products analysed by ion exchange HPLC. Aproximate yields and MALDITOF MS data are given in Table 1, and an HPLC profile of a crude product is shown in Fig. 2.

Table 1 Yields, purities, and MS data for the modified oligonucleotides

	Scale/μM	Crude yield, OD	Purity (%)	Purified yield, OD	Purity (%)	MS (calc.)
$dT_5T^*_4T_5$	0.25	12	34	2.5	85	4074 (4074)
$dA_5A^*_4A_5$	0.25	21	40	4	85	4202 (4200)
dG*T*G*A*T*A*T*G*C	1.0	51	14	5	90	2514 (2511)

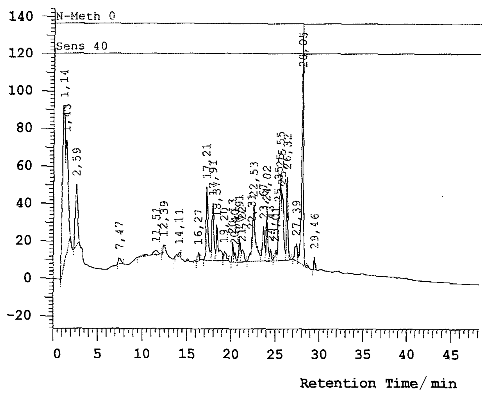


Fig. 2 Ion exchange HPLC profile of crude dG*T*G*A*T*A*T*G*C.

Hybridization studies

The ability of the modified oligonucleotides to bind to complementary DNA and RNA was examined by thermal melting temperature (T_m) measurements at 260 nm. The results are given in Table 2, together with previous results.[5]

From the data in Table 2 it is seen that one modification in the middle of a 14-mer gives a depression of the melting temperature of 5–6 °C, but that four modifications are better tolerated. In particular four modified As are well tolerated with a depression of only 1–2 °C per modification. It was of interest to see whether a fully modified sequence would bind better, as is known in cases where the modified unit deviates strongly from natural nucleosides, *e.g.* phosphonate analogues of PNA[11] and xylo-LNA.[12] However, this is not the case here. The mixed sequence 9-mer with eight modifications binds with a depression of 2–3 °C per modification, only marginally better than those with four modifications.

Conclusion

This paper describes our efforts to improve the preparation of oligonucleotides modified with the acyclic, achiral monomers **1**. Previous attempts to incorporate this monomer by standard phosphoramidite chemistry gave low yields, and we have examined the reasons for the low yields by dimer experiments analysed by NMR. It is shown that cyanoethyl phosphoramidites **2** gave phosphortriesters which largely decomposed during removal of the cyanoethyl group with base, because the allylic backbone in the phosphortriester, *e.g.* **4c**, was very sensitive to basic conditions. A simple allyl group is known to be removable with aq. ammonia from allyl dinucleoside phosphates,[13] and in diallyl 2-cyanoethyl phosphate, the cyanoethyl group is removed cleanly to give diallyl phosphate with aq. ammonia.[14] It was unexpected that the allylic backbone was more sensitive to base than a simple allyl group. Since phosphordiester anions containing two modified units, *e.g.* **4h**, are very resistant to bases, we decided to replace the cyanoethyl protection group with a group which spontaneously eliminated after oxidation, without base treatment. The *N*-methyl-*N*-(2-pyridyl)-2-aminoethyl group introduced by Beaucage *et al.*[8] was chosen, and NMR experiments showed that phosphoramidites **3** containing this protection group gave high yields of modified dimers. The dimers were stable to oxidation and capping conditions, as well as aq. ammonia necessary to remove base protection groups. However the strong acid conditions necessary to remove the DMT protection groups resulted in some cleavage, probably because protonation of the phosphordiester group leads to some elimination of the allylic modified unit. By introducing a basic wash immediately after the DMT removal step the cleavage could be reduced to 3–4% per coupling cycle.

Using these improved conditions we were able to prepare oligonucleotides modified with 4 or 8 modifications from monomers **3a**, **3b**, and **3e** containing T, A and G. The corresponding modified ^{Me}C monomer could not be incorporated in useful amounts, probably because it cyclised after removal of the DMT group. The oligonucleotides modified with 4 or 8 consecutive modifications hybridized to both DNA and RNA complements, but with reduced melting temperatures compared to unmodified DNA. The reduction in T_m per modification for the mixed 9-mer with 8 modifications (2–3 °C) was less than previous results with

Table 2 Hybridization data (T_m, °C) for modified and unmodified oligodeoxyribonucleotides with DNA and RNA complements[a]

	dA_{14}	ΔT_m[b]	rA_{14}	ΔT_m[b]	$dA_6A^*A_7$[c]	ΔT_m[b]	$dA_5A^*_4A_5$	ΔT_m[b]
dT_{14}	36	—	34	—	31	−5	27	−2
rU_{14}	12	—	N.d.	—	12	0	9	−1
$dT_7T^*T_6$	31[5]	−5	28[5]	−6	N.d.	—	N.d.	—
$dT_{11}T^*_2T$	31[5]	−2.5	29[5]	−2.5	N.d.	—	N.d.	—
$dT_5T^*_4T_5$	22	−3.5	15	−5	N.d.	—	11	—
	dGCATATCAC	ΔT_m[b]	rGCAUAUCAC	ΔT_m[b]				
dGTGATATGC	33	—	31	—				
dG*T*G*A*T*A*T*G*C	8	−3	12	−2				

[a] T_m was determined by measuring absorbance at 260 nm against increasing temperature (1 °C steps) on equimolar mixtures (3 μM in each strand) of the modified oligomer and its complementary DNA or RNA strand in medium salt buffer (10 mM Na_2HPO_4, 100 mM NaCl, 0.1 mM EDTA, pH 7.0). [b] Change in T_m per modification. [c] Prepared from **2b** as described earlier.[5]

one modification (5–6 °C), and the purines A and G seem to be better tolerated than T.

Our original goal—to prepare a simple acyclic, achiral substitute for the natural nucleosides—has been achieved, but with limited success. The monomers **1** are not simple to prepare, and they are difficult to incorporate as phosphoramidites in oligonucleotides because of their allylic structure. Since their affinities towards DNA and RNA are lower than unmodified DNA, we have decided to stop further work in this area.

Experimental

The compounds **1a**,[6] 6-*N*-(4-tert-butylbenzoyl)-9-[3-hydroxy-2(hydroxymethyl)prop-1-enyl]adenine,[6] 1-[3-benzyloxy-2-(benzyloxymethyl)prop-1-enyl]-*N*-isobutyrylguanine,[6] [*N*-methyl-*N*-(2-pyridyl)-2-aminoethyl] *N*,*N*,*N'*,*N'*-tetraisopropylphosphorodiamidite,[8] **6a**,[8] and **6b**[10] were prepared according to literature procedures. Other chemicals were 97–99% pure from Aldrich, Fluka, Sigma, or Merck. Solvents were HPLC grade from LABSCAN. CH_2Cl_2, DMF, pyridine, Et_3N and CH_3CN were dried over molecular sieves (4 Å from Grace Davison) to a water content below 20 ppm, measured on a Metrohm 652 KF-Coulometer. TLC was run on Merck 5554 silica 60 aluminium sheets, LC on either Merck 9385 silica 60 (0.040–0.063 mm) for normal gravity chromatography, or Merck 15111 silica 60 (0.015–0.040 mm) for dry column vacuum chromatography.[15] RP HPLC purifications were done on a Waters Prep LC 4000 System using a Xterra MS C18-column (10 μm, 7.8 × 150 mm) with a gradient of 1.38% CH_3CN per. min and UV detection at 254 nm. Ion exchange HPLC analysis were performed on a LaChrom D-7000 System using a Gen-Pak Fax column (4.6 × 100 mm) with a gradient of 1.07% 2 M NaCl per. min and UV detection at 254 nm. NMR spectra (reference tetramethylsilane for δ_H and δ_C, external 85% H_3PO_4 for δ_P, *J* values are given in Hz) were run on a Varian Mercury 300 MHz spectrometer. FAB MS data were obtained on a JEOL HX 110/110 Mass Spectrometer with m-NBA as the matrix, ES MS data on a Micromass Q-Tof Mass Spectrometer, and MALDITOF MS data on a Bruker Ultraflex II TOF/TOF System using a HPA matrix. Thermal melting temperature measurements were performed on a Cary 300 Version 9.00 UV spectrometer.

(*E*)- and (*Z*)-1-[2-(Dimethoxytrityloxymethyl)-3-hydroxyprop-1-enyl]thymine

To a solution of dry **1a** (0.212 g, 1.00 mmol) in dry pyridine (4 ml) was added dropwise a solution of DMTCl (0.34 g, 1.00 mmol) in dry pyridine (4 ml), and the mixture was stirred in the dark under N_2 for 2 h at rt. Sat. aqueous $NaHCO_3$ (1 ml) was added and the solvents removed *in vacuo.* The residue was dissolved in a mixture of CH_2Cl_2 (25 ml) and water (10 ml), the phases were separated, the CH_2Cl_2 phase extracted with water (2 × 3 ml), and the combined aq. phases extracted with CH_2Cl_2 (5 ml). The CH_2Cl_2 phase was dried ($MgSO_4$) and the solvent removed *in vacuo* to give the crude product (0.54 g), a mixture of hydrolysed DMTCl, the bis-, and the two mono-dimethoxytrityl derivatives, which was resolved into the components by normal gravity column chromatography, eluted with EtOAc–MeOH–Py 98 : 1 : 1 *v*/*v*/*v*, to give the di-DMT derivative (0.176 g, 22%, $R_f = 0.61$), the (*E*)-product (0.178 g, 35%, $R_f = 0.45$), and the (*Z*)-product (0.135 g, 26%, $R_f = 0.29$), all colourless solids. (***E***): NMR (DMSO-d$_6$): δ_H 11.39 (1H, s, NH), 7.43–6.89 (14H, m, Ar + H-6), 6.65 (1H, s, NCH=C), 4.90 (1H, t, *J* 5.3, OH), 3.92 (2H, d, *J* 5.3, C*H*$_2$OH), 3.74 (6H, s, OCH_3), 3.70 (2H, s, C*H*$_2$ODMT), 1.78 (3H, d, *J* 1.2, T–CH_3). FAB$^+$ MS: 515.2 (M + H$^+$ calc. 515.2). (***Z***): NMR (DMSO-d$_6$): δ_H 11.30 (1H, s, NH), 7.31–6.86 (14H, m, Ar + H-6), 6.49 (1H, s, NCH=C), 5.15 (1H, t, *J* 5.0, OH), 4.16 (2H, d, *J* 5.0, C*H*$_2$OH), 3.73 (6H, s, OCH_3), 3.47 (2H, s, C*H*$_2$ODMT), 1.60 (3H, d, *J* 1.2, T–CH_3). δ_C 164.1, 158.9, 150.4, 144.5, 140.5, 136.1, 135.5, 130.1, 128.2, 127.3, 124.8, 113.5, 110.6, 87.3, 63.6, 58.9, 55.5, 12.6. FAB$^+$ MS: 515.2 (M + H$^+$ calc. 515.2). The *Z* configuration of the latter product was determined by ^{1}H NMR NOE effects from NC*H*=C to C*H*$_2$OH, and 1-alkylation of T by NOE effects from NC*H*=C to H-6.

(*E*)- and (*Z*)-6-*N*-(4-tert-Butylbenzoyl)-9-[2-(dimethoxytrityloxymethyl)-3-hydroxyprop-1-enyl]adenine

To a solution of dry 6-*N*-(4-tert-butylbenzoyl)-9-[3-hydroxy-2(hydroxymethyl)prop-1-enyl]adenine (0.271 g, 0.710 mmol) in dry pyridine (20 ml) was added dropwise a solution of DMTCl (0.287 g, 0.85 mmol) in dry pyridine (4 ml), and the mixture was stirred in the dark under N_2 for 3 h at rt. Sat. aqueous $NaHCO_3$ (1 ml) was added and the solvents removed *in vacuo.* The residue was dissolved in acetone (15 ml), filtered, and acetone removed *in vacuo* to give the crude product (0.58 g), a mixture of hydrolysed DMTCl, the bis-, the two mono-dimethoxytrityl derivatives, and the starting material. This mixture was resolved into the components by normal gravity column chromatography, eluted with first EtOAc–hexane–MeOH–Et_3N 49 : 49 : 1 : 1 *v*/*v*/*v*/*v*, then EtOAc–MeOH–Et_3N 98 : 1 : 1 *v*/*v*/*v*, and last EtOAc–MeOH–Et_3N 79 : 20 : 1 *v*/*v*/*v*, to give the di-DMT derivative (0.164 g, 23%, $R_f = 0.35$ in EtOAc–hexane–MeOH–Et_3N 49 : 49 : 1 : 1 *v*/*v*/*v*/*v*), the (*E*)-product (0.150 g, 31%, $R_f = 0.55$ in EtOAc–MeOH–Et_3N 98 : 1 : 1 *v*/*v*/*v*), the (*Z*)-product (0.119 g, 24%, $R_f = 0.16$ in EtOAc–MeOH–Et_3N 98 : 1 : 1 *v*/*v*/*v*), and the starting material (0.028 g, 10%, $R_f = 0.36$ in EtOAc–MeOH–Et_3N 79 : 20 : 1 *v*/*v*/*v*), all colourless solids. (***E***): NMR (DMSO-d$_6$): δ_H 11.13 (1H, s, NH), 8.79 (1H, s, H-2), 8.53 (1H, s, H-8), 8.00 (2H, d, *J* 8.2, tert-butyl*benzoyl*), 7.59–7.25 (12H, m, Ar + C=CH), 6.94 (4H, d, *J* 8.8, Ar), 5.07 (1H, t, *J* 5.3, OH), 4.01 (2H, d, *J* 5.3, C*H*$_2$OH), 3.87 (2H, s, C*H*$_2$ODMT), 3.75 (6H, s, OCH_3), 1.34 (9H, s, Bu^t). The (*E*) konfiguration was proven by NOE (irradiation at C*H*$_2$OH gave 5% enhancement of H-8). NMR ($CDCl_3$): δ_H 9.08 (1H, s, NH), 8.79 (1H, s, H-2), 8.13 (1H, s, H-8), 7.97 (2H, d, *J* 8.2, tert-butyl*benzoyl*), 7.56–7.24 (11H, m, Ar), 6.94 (1H, s, C=CH), 6.86 (4H, d, *J* 8.5, Ar), 4.31 (1H, t, *J* 6.7, OH), 4.08 (2H, s, C*H*$_2$ODMT), 3.96 (2H, d, *J* 6.7, C*H*$_2$OH), 3.80 (6H, s, OCH_3), 1.37 (9H, s, Bu^t). δ_C 164.5, 158.8, 156.8, 153.0, 151.8, 150.3, 144.7, 143.4, 139.0, 135.8, 130.7, 130.1, 128.1, 127.9, 127.2, 126.0, 122.7, 116.9, 113.4, 87.3, 64.5, 57.6, 55.4, 35.3, 31.2. FAB$^+$ MS: 684. 5 (M + H$^+$ calc. 684.3). (***Z***): NMR (DMSO-d$_6$): δ_H 11.08 (1H, s, NH), 8.67 (1H, s, H-2), 8.25 (1H, s, H-8), 7.99 (2H, d, *J* 8.5, tert-butyl*benzoyl*), 7.59–7.07 (11H, m, Ar), 7.03 (1H, s, C=CH), 6.77 (4H, d, *J* 8.4, Ar), 5.35 (1H, t, *J* 5.3, OH), 4.30 (2H, d, *J* 5.3, C*H*$_2$OH), 3.70 (6H, s, OCH_3), 3.63 (2H, s, C*H*$_2$ODMT), 1.34 (9H, s, Bu^t). The (*Z*) konfiguration was proven by NOE (irradiation at C*H*$_2$ODMT gave 2% enhancement of H-8). NMR ($CDCl_3$): δ_H 9.0 (1H, br s, NH), 8.75 (1H, s, H-2), 7.97

(2H, d, J 8.2, tert-butyl*benzoyl*), 7.96 (1H, s, H-8), 7.55–7.18 (11H, m, Ar), 7.07 (1H, s, C=CH), 6.77 (4H, d, J 9.1, Ar), 4.49 (2H, s, CH_2OH), 3.83 (2H, s, CH_2ODMT), 3.75 (6H, s, OCH_3), 1.37 (9H, s, Bu^t). δ_C 164.5, 158.9, 156.8, 153.2, 151.9, 149.8, 144.4, 142.6, 136.8, 135.4, 131.0, 130.1, 128.2, 128.1, 127.9, 127.3, 126.1, 122.4, 118.3, 113.5, 87.4, 63.8, 59.7, 55.5, 35.4, 31.4. FAB$^+$ MS: 684.6 (M + H$^+$ calc. 684.3).

1-[3-Hydroxy-2-(hydroxymethyl)prop-1-enyl]-*N*-2-isobutyrylguanine

To a stirred solution of 1-[3-benzyloxy-2-(benzyloxymethyl)prop-1-enyl]-*N*-isobytyrylguanine (0.703 g, 1.44 mmol) in dry CH_2Cl_2 (25 ml) under N_2 at 0 °C was added dropwise BCl_3 (1 M in CH_2Cl_2, 15 ml, 15 mmol). After stirring for 1 h at 0 °C, MeOH–CH_2Cl_2 (1 : 1, v/v, 5 ml) was added, and the solvents removed *in vacuo.* The residue was dissolved in MeOH–CH_2Cl_2 (1 : 1, v/v, 20 ml) and solid $NaHCO_3$ was added to pH 7. The solids were removed by filtration and washed with MeOH–CH_2Cl_2 (1 : 1, v/v, 25 ml). The combined filtrates were concentrated *in vacuo*, and the residue partitioned between H_2O (75 ml) and $CHCl_3$ (50 ml). The aqueous phase was extracted with $CHCl_3$ (2 × 50 ml) and evaporated *in vacuo.* Purification of the residue by normal gravity column chromatography (elution with CH_2Cl_2–MeOH 9 : 1 and 4 : 1, v/v) yielded the title compound as colourless crystals (0.390 g, 88%, R_f = 0.13 in CH_2Cl_2–MeOH (9 : 1)), mp 205–206 °C (Found: C, 50.5; H, 5.5; N, 22.5. $C_{13}H_{17}N_5O_4$ requires C, 50.8; H, 5.6; N, 22.8%). NMR (DMSO-d$_6$): δ_H 12.07 and 11.76 (2 × 1H, 2 × s, NH), 8.05 (1H, s, H-8), 6.80 (1H, br s, NCH=C), 5.19 and 5.06 (2 × 1H, 2 × t, 2 × J 5, OH), 4.21 (2H, dd, J 5 and 1.8, CH_2C=CH), 3.99 (2H, d, J 5, CH_2C=CH), 2.74 (1H, septet, J 6.7, CH(CH_3)$_2$), 1.11 (6H, d, J 6.7, CH(CH_3)$_2$). δ_C 180.1, 154.9, 148.7, 148.2, 139.5, 139.2, 119.7, 115.6, 60.8, 56.2, 34.7, 18.8. FAB$^+$ MS: 308.2 (M + H$^+$ calc. 308.1).

(*E*)- and (*Z*)-2-*N*-Isobutyryl-9-[2-(dimethoxytrityloxymethyl)-3-hydroxyprop-1-enyl]guanine

To a solution of 2-*N*-isobutyryl-9-[3-hydroxy-2-(hydroxymethyl)-prop-1-enyl]guanine (0.383 g, 1.2 mmol) in dry pyridine (15 ml) was added dropwise a solution of DMTCl (0.380 g, 1.1 mmol) in dry pyridine (5 ml), and the mixture was stirred in the dark under N_2 for 48 h at rt. Sat. aqueous $NaHCO_3$ (1 ml) was added and the solvents removed *in vacuo* to give the crude product, a mixture of hydrolysed DMTCl, the bis-, the two mono-dimethoxytrityl derivatives, and the starting material. This mixture was subjected to normal gravity column chromatography, eluted with EtOAc–MeOH–Et_3N (94 : 5 : 1, 89 : 10 : 1 and 79 : 20 : 1, $v/v/v$) to separate the starting material (0.132 g, 35%, R_f = 0.04, the R_f values given are in EtOAc–MeOH–Et_3N (94 : 5 : 1, $v/v/v$)) from the compounds containing DMT. The fractions containing the bis- and the two mono-dimethoxytrityl derivatives were resolved into the components by dry column vacuum chromatgraphy (0–99% EtOAc in toluene, 10% increments, followed by 1–5% MeOH in EtOAc, 1% increments, all containing 1% Et_3N) to give the di-DMT derivative (0.145 g, 13%, R_f = 0.58), the (*Z*)-product (0.196 g, 26%, R_f = 0.26) and the (*E*)-product (0.125 g, 16%, R_f = 0.32), all as slightly yellow foams. (***Z***): NMR (DMSO-d$_6$): δ_H 12.06 and 11.75 (2 × 1H, 2 × s, NH), 7.73 (1H, s, H-8), 7.27–7.14 (5H, m, Ar), 6.94 (8H, AB system, Δ 96.0 Hz, J 8.8, Ar), 6.83 (1H, br s, NCH=C), 5.31 (1H, t, J 5.2, OH), 4.27 (2H, dd, J 5.2 and 1.5, CH_2OH), 3.70 (6H, s, OCH_3), 3.56 (2H, s, CH_2ODMT), 2.74 (1H, septet, J 6.7, CH(CH_3)$_2$), 1.11 (6H, d, J 6.7, CH(CH_3)$_2$). The (*Z*) configuration was proven by NOE (irradiation at CH_2OH gave 5% enhancement of NCH=C). δ_C 180.2, 158.1, 154.8, 148.1, 144.6, 140.2, 138.8, 135.1, 129.5, 128.9, 127.8, 127.4, 126.7, 117.4, 113.1, 85.9, 61.0, 58.6, 55.0, 34.8, 18.8. FAB$^+$ MS: 610.2 (M + H$^+$ calc. 610.3). (***E***): NMR ($CDCl_3$): δ_H 12.24 and 9.90 (2 × 1H, 2 × br s, NH), 7.78 (1H, s, H-8), 7.43–7.14 (9H, m, Ar), 6.80 (4H, d, J 8.8, Ar), 6.71 (1H, s, NCH=C), 4.04 and 3.99 (2 × 2H, 2 × s, CH_2O), 3.76 (6H, s, OCH_3), 2.73 (1H, septet, J 6.8, CH(CH_3)$_2$), 1.20 (6H, d, J 6.8, CH(CH_3)$_2$). FAB$^+$ MS: 610.1 (M + H$^+$ calc. 610.3).

(*E*)-1-[3-(Dimethoxytrityloxy)-2-[2-cyanoethoxy(diisopropylamino)-phosphinoxymethyl]prop-1-enyl]thymine (2a)

To a stirred solution of dry (*Z*)-1-[2-(dimethoxytrityloxymethyl)-3-hydroxyprop-1-enyl]thymine (207 mg, 0.40 mmol) in dry CH_2Cl_2 (10 ml) under N_2 was added dry Et_3N (0.19 ml, 0.80 mmol), followed by 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (114 mg, 0.48 mmol). After 2 h at rt the solution was diluted with CH_2Cl_2 (10 ml), extracted with sat. aq. $NaHCO_3$ (3 × 10 ml), dried ($MgSO_4$) and evaporated *in vacuo* to an oil. Purification by normal gravity column chromatography (elution with EtOAc–Et_3N 99 : 1 v/v) gave the product which was dissolved in EtOAc (2 ml), precipitated from hexane (100 ml) at 0 °C, and lyophilised from dry CH_3CN (10 ml). Yield 194 mg (68%) of a colourless foam, R_f 0.53 (EtOAc–Et_3N 99 : 1 v/v). NMR ($CDCl_3$): δ_H 7.40–7.18 (10H, m, Ar + NH), 7.14 (1H, s, H-6), 6.81 (4H, d, J 8.8, Ar), 6.70 (1H, s, NCH=C), 4.38 (2H, AB of ABX system, Δ = 27.5 Hz, J_{AB} 13.4, J_{AX} = J_{BX} = 8.2, CH_2OP), 3.90–3.53 (6H, m, CH_2ODMT, CH_2CH$_2$CN, CHMe$_2$), 3.78 (6H, s, CH_3O), 2.61 (2H, t, J 6.3, CH$_2$CH_2CN), 1.71 (3H, s, T-CH_3), 1.19 (12H, "t", J 7.0). δ_C 164.1, 158.9, 150.2, 144.6, 140.7, 135.6, 133.3, 133.2, 130.2, 128.2, 128.1, 127.3, 125.3, 113.4, 110.4, 87.1, 64.0, 63.7, 58.9, 58.8, 55.5, 43.5, 43.4, 24.9, 24.8, 20.7, 20.6, 12.6. δ_P 149.8. FAB$^+$ MS: 715.3 (M + H$^+$ calc. 715.3).

(*Z*)-1-[3-(Dimethoxytrityloxy)-2-[2-cyanoethoxy(diisopropylamino)-phosphinoxymethyl]prop-1-enyl]thymine (2a′)

This compound was prepared in the same way as **2a** from (*E*)-1-[2-(dimethoxytrityloxymethyl)-3-hydroxyprop-1-enyl]thymine in 70% yield, R_f 0.13 (EtOAc–hexane–Et_3N 49 : 49 : 2 $v/v/v$). NMR ($CDCl_3$): δ_H 7.39–7.14 (10H, m, Ar + NH), 7.12 (1H, q, J 1.2, H-6), 6.76 (4H, d, J 8.8, Ar), 6.70 (1H, s, NCH=C), 4.11–3.96 (2H, m, CH_2OP), 3.80–3.36 (6H, m, CH_2ODMT, CH_2CH$_2$CN, CH(CH_3)$_2$), 3.72 (6H, s, CH_3O), 2.41 (2H, t, J 6.3, CH$_2$CH_2CN), 1.87 (3H, d, J 1.2, T–CH_3), 1.08–0.95 (12H, m, CH(CH_3)$_2$). δ_P 150.7.

(*E*)-9-[3-(Dimethoxytrityloxy)-2-[2-cyanoethoxy(diisopropylamino)-phosphinoxymethyl]prop-1-enyl]-*N*-6-(4-tert-butylbenzoyl)adenine (2b)

This compound was prepared in the same way as **2a** from (*Z*)-1-[2-(dimethoxytrityloxymethyl)-3-hydroxyprop-1-enyl]-*N*-6-(4-tert-butylbenzoyl)adenine in 72% yield, R_f 0.22 (EtOAc–hexane–Et_3N 49 : 49 : 2 $v/v/v$). NMR ($CDCl_3$): δ_H 9.16 (1H, br s, NH), 8.77 (1H, s, H-2), 8.02 (1H, s, H-8), 7.99 (2H, d, J 8.5, tert-butyl*benzoyl*), 7.54 (2H, d, J 8.5, tert-butyl*benzoyl*), 7.42–7.19

(9H, m, Ar), 7.14 (1H, br s, C=CH), 6.79 (4H, d, *J* 8.2, DMT), 4.53 (2H, AB of ABX system, $\Delta = 30.4$ Hz, J_{AB} 14.1, $J_{AX} = J_{BX} =$ 7.3, CH_2OP), 4.00–3.62 (6H, m, CH_2ODMT, CH_2CH_2CN, $CHMe_2$), 3.76 (6H, s, CH_3O), 2.65 (2H, t, *J* 6.2, CH_2CH_2CN), 1.38 (9H, s, tert-butyl), 1.24 + 1.23 (12H, 2 × d, *J* 7.0, $CHMe_2$). δ_C 164.5, 158.7, 156.5, 153.0, 151.7, 149.7, 144.4, 142.5, 135.3, 135.3, 134.2, 134.1, 130.9, 130.0, 128.0, 127.9, 127.8 127.0, 125.8, 122.2, 118.6, 117.6 113.3, 87.0, 63.8, 63.6, 59.0, 58.7, 58.5, 55.3, 43.4, 43.2, 35.1, 31.2, 24.8, 24.7,24.7, 24.6 20.5, 20.4. δ_P 150.0. FAB$^+$ MS: 884.5 (M + H$^+$ calc. 884.4).

(*E*)-1-[3-(Dimethoxytrityloxy){*N*-methyl-*N*-(2-pyridyl)-2-aminoethoxy}(diisopropylamino)phosphinoxymethyl]prop-1-enyl]thymine (3a)

To a stirred solution of dry (*Z*)-1-[2-(dimethoxytrityloxymethyl)-3-hydroxyprop-1-enyl]thymine (103 mg, 0.20 mmol) in dry CH_3CN (5 ml) under N_2 was added [*N*-methyl-*N*-(2-pyridyl)-2-aminoethyl] *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (90 mg, *ca.* 88% pure, 0.27 mmol), followed by tetrazole (9 mg, 0.13 mmol). After 2 h at rt the solution was concentrated *in vacuo*, and the residue dissolved in EtOAc (10 ml), extracted with sat. aq. $NaHCO_3$ (2 × 5 ml), the $NaHCO_3$ phase extracted with EtOAc (5 ml), the EtOAc phase dried ($MgSO_4$) and evaporated *in vacuo* to an oil. The crude oil was purified by normal gravity column chromatography, eluted with EtOAc–heptane–Et_3N 58 : 40 : 2 *v*/*v*/*v*, to give the product (80 mg, 50%) as a colourless oil, R_f 0.50 (EtOAc–Et_3N 98 : 2 *v*/*v*). NMR ($CDCl_3$): δ_H 8.6 (1H, br, NH), 8.12–8.10 (1H, m, Ar), 7.42–7.18 (10H, m, Ar), 7.10 (1H, d, *J* 1.2, H-6), 6.81 (4H, d, *J* 8.8, Ar), 6.65 (1H, s, NCH=C), 6.51–6.47 (2H, m, Ar), 4.33 (2H, AB of ABX system, $\Delta = 22.3$ Hz, J_{AB} 13.8, $J_{AX} = J_{BX} = 7.0$, CH_2OP), 3.86–3.51 (8H, m, CH_2ODMT, NCH_2CH_2O, $CHMe_2$), 3.77 (6H, s, CH_3O), 3.09 (3H, s, NCH_3), 1.71 (3H, d, *J* 1.2, T–CH_3), 1.16 (6H, d, *J* 6.7, $CHMe_2$), 1.15 (6H, d, *J* 6.7, $CHMe_2$). δ_P 148.1. FAB$^+$ MS: 795.3 (M + H$^+$ calc. 796.4).

(*Z*)-1-[3-(Dimethoxytrityloxy){*N*-methyl-*N*-(2-pyridyl)-2-aminoethoxy}(diisopropylamino)phosphinoxymethyl]prop-1-enyl]thymine (3a′)

This compound was prepared in the same way as **3a** from (*E*)-1-[2-(dimethoxytrityloxymethyl)-3-hydroxyprop-1-enyl]thymine in 60% yield as a colourless foam, R_f 0.55 (EtOAc–Et_3N 98 : 2 *v*/*v*). NMR ($CDCl_3$): δ_H 9.2 (1H, br, NH), 8.09 (1H, m, Ar), 7.47–7.19 (11H, m, Ar + H-6), 6.84 (4H, d, *J* 8.8, Ar), 6.81 (1H, s, NCH=C), 6.49 (1H, dd, *J* 5.0 and 7.0, Ar), 6.42 (1H, d, *J* 8.8, Ar), 4.02 (2H, AB of ABX system, $\Delta = 22.4$ Hz, J_{AB} 12.0, $J_{AX} = J_{BX} =$ 6.2, CH_2OP), 3.82–3.62 (6H, m, CH_2ODMT, NCH_2CH_2O), 3.78 (6H, s, CH_3O), 3.54–3.41 (2H, m, $CHMe_2$), 3.01 (3H, s, NCH_3), 1.92 (3H, d, *J* 0.6, T–CH_3), 1.09 (6H, d, *J* 7.0, $CHMe_2$), 1.03 (6H, d, *J* 7.0, $CHMe_2$). δ_P 149.0. FAB$^+$ MS: 796.4 (M + H$^+$ calc. 796.4).

(*E*)-1-[3-(Dimethoxytrityloxy){*N*-methyl-*N*-(2-pyridyl)-2-aminoethoxy}(diisopropylamino)phosphinoxymethyl]prop-1-enyl]-*N*-6-(4-tert-butylbenzoyl)adenine (3b)

This compound was prepared in the same way as **3a** from (*Z*)-1-[2-(dimethoxytrityloxymethyl)-3-hydroxyprop-1-enyl]-*N*-6-(4-tert-butylbenzoyl)adenine in 70% yield as a colourless foam, R_f 0.60 (EtOAc–Et_3N 98 : 2 *v*/*v*). NMR ($CDCl_3$): δ_H 8.9 (1H, br, NH), 8.76 (1H, s, H-2), 8.10–8.08 (1H, m, Ar), 7.97 (1H, s, H-8), 7.96 (2H, d, *J* 8.5, Ar), 7.55 (2H, d, *J* 8.5, Ar), 7.40–7.14 (10H, m, Ar), 7.08 (1H, s, NCH=C), 6.76 (4H, d, *J* 9.1, Ar), 6.50–6.44 (2H, m, Ar), 4.45 (2H, AB of ABX system, $\Delta = 30.7$ Hz, J_{AB} 14.1, $J_{AX} = J_{BX} = 7.0$, CH_2OP), 3.94–3.59 (8H, m, CH_2ODMT, NCH_2CH_2O, $CHMe_2$), 3.75 (6H, s, CH_3O), 3.10 (3H, s, NCH_3), 1.37 (9H, s, tert-Bu), 1.20 (6H, d, *J* 6.7, $CHMe_2$), 1.17 (6H, d, *J* 6.7, $CHMe_2$). δ_P 148.3. FAB$^+$ MS: 965.5 (M + H$^+$ calc. 965.5).

(*E*)-1-[3-(Dimethoxytrityloxy){*N*-methyl-*N*-(2-pyridyl)-2-aminoethoxy}(diisopropylamino)phosphinoxymethyl]prop-1-enyl]-*N*-2-isobutyrylguanine (3e)

This compound was prepared in the same way as **3a** from (*Z*)-1-[2-(dimethoxytrityloxymethyl)-3-hydroxyprop-1-enyl]-*N*-2-isobutyrylguanine in 35% yield after purification by normal gravity column chromatography (first eluted with EtOAc–Et_3N 98 : 2 *v*/*v*, then with EtOAc–MeOH–Et_3N 93 : 5 : 2 *v*/*v*/*v*). Colourless foam, R_f 0.32 (EtOAc–Et_3N 98 : 2 *v*/*v*). NMR ($CDCl_3$): δ_H 8.05–8.03 (1H, m, Ar), 7.59 (1H, s, H-8), 7.45–7.16 (10H, m, Ar), 6.86 (1H, s, NCH=C), 6.78 (4H, d, *J* 9.1, Ar), 6.58–6.49 (2H, m, Ar), 4.38 (2H, AB of ABX system, $\Delta = 35.5$ Hz, J_{AB} 14.7, J_{AX} 8.5, J_{BX} 7.9, CH_2OP), 3.92–3.52 (8H, m, CH_2ODMT, NCH_2CH_2O, $CHMe_2$), 3.77 (6H, s, CH_3O), 3.15 (3H, s, NCH_3), 2.57 (1H, septet, *J* 6.7, $COCHMe_2$), 1.21–1.16 (18H, m, $CHMe_2$). δ_P 148.2. FAB$^+$ MS: 891.1 (M + H$^+$ calc. 891.4).

2-Cyanoethyl bis-[(*E*)-2-(dimethoxytrityloxymethyl)-3-(1-thyminyl)prop-2-enyl] phosphite (4b)

To a solution of (*E*)-1-[2-(dimethoxytrityloxymethyl)-3-hydroxy-prop-1-enyl]thymine (0.206 g, 0.40 mmol) and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.075 g, 80% pure, 0.20 mmol) in dry CH_3CN (5 ml) was added tetrazole (0.035 g, 0.50 mmol). The mixture was stirred under N_2 for 2.5 h at rt, and concentrated *in vacuo.* The residue was dissolved in a mixture of EtOAc (20 ml) and sat. aq. $NaHCO_3$ (5 ml), the EtOAc phase extracted with sat. aq. $NaHCO_3$ (5 ml), the combined aq. phases extracted with EtOAc (5 ml), and the combined EtOAc phases dried ($MgSO_4$) and evaporated *in vacuo* to an oil. The crude oil was purified by normal gravity column chromatography, eluted with heptane–EtOAc–MeOH–Py 50 : 43 : 5 : 2 *v*/*v*/*v*/*v*, to give, after lyophilisation from CH_3CN (10 ml) the product (0.183 g, 81%) as a colourless solid, R_f 0.10 (heptane–EtOAc–MeOH–Py 50 : 43 : 5 : 2 *v*/*v*/*v*/*v*). NMR ($CDCl_3$): δ_H 9.0–8.9 (2H, br m, NH), 7.43–7.18 (18H, m, Ar), 6.91 (2H, d, *J* 1.2, H-6), 6.82 (8H, d, *J* 8.8, Ar), 6.62 (2H, s, C=CH), 4.23 (4H, d, *J* 7.0, CH_2OP), 3.77 (16H, s, CH_3O + CH_2ODMT), 3.65 (2H, dt, *J* 7.0 and 6.2, $NCCH_2CH_2O$), 2.35 (2H, t, *J* 6.2, $NCCH_2CH_2O$), 1.86 (6H, br s, T–CH_3). δ_C 163.9, 158.8, 149.9, 144.6, 140.3, 135.8, 134.0 (d, *J* 6), 130.1, 128.2, 128.1, 127.1, 124.5, 117.2, 113.4, 113.3, 110.9, 87.0, 63.1, 57.7 (d, *J* 11), 57.4 (d, *J* 11), 55.4, 20.2 (d, *J* 5), 12.4. δ_P 141.1. FAB$^+$ MS: 1128.6 (M + H$^+$ calc. 1128.4).

Bis-[(*E*)-2-(dimethoxytrityloxymethyl)-3-(1-thyminyl)prop-2-enyl] *N*-methyl-*N*-(2-pyridyl)-2-aminoethyl phosphite (4f)

To a solution of **3a**′ (90 mg, 0.11 mmol) in dry CH_3CN (2 ml) was added (*E*)-1-[2-(dimethoxytrityloxymethyl)-3-hydroxyprop-1-enyl]thymine (51.5 mg, 0.10 mmol) and tetrazole (7 mg, 1.0 mmol).

Table 3 Coupling cycle to introduce N*, 0.25 μmol scale

Reagent	Time
3% CCl_3COOH in CH_2Cl_2	30 s + (5 s wait + 10 s CH_3CN) × 2
0.1 M $Pr^i{}_2NH$ in CH_3CN	(15 s + 10 s wait) × 3
Wash CH_3CN[a]	3 min
0.2 ml 0.05 M amidite in CH_3CN + 0.3 ml	
0.50 M tetrazole in CH_3CN	6 min manually
Wash CH_3CN	1 min
10% H_2O + 2% CAP A[b] in THF	2 min manually
Wash CH_3CN	2 min
CAP A + CAP B[c], 1:1	(30 s + 30 s wait) × 4
Wash CH_3CN	1 min
80% Bu^tOOH in $Bu^t{}_2O$, 0.5 M in CH_2Cl_2–acetone 1 : 1	(6 s + 90 s wait) × 2
Wash CH_3CN	1.5 min
Wait (elimination of phosphate protection)	30 min
Wash CH_3CN	0.5 min

[a] Wash CH_3CN is a combination of flush, wash, and wait steps. [b] CAP A = 10% $(CH_3CO)_2O$ + 10% lutidine in THF. [c] CAP B = 16% *N*-methylimidazole in THF.

The mixture was stirred under N_2 for 2.5 h at rt, and concentrated *in vacuo.* The residue was dissolved in a mixture of EtOAc (20 ml) and sat. aq. $NaHCO_3$ (10 ml), the aq. phase extracted with EtOAc (5 ml), and the combined EtOAc phases dried ($MgSO_4$) and evaporated *in vacuo* to an oil. The crude oil was purified normal gravity column chromatography, eluted with EtOAc–Py 99 : 1 *v*/*v*, to give, after evaporation with CH_3CN (2 × 5 ml) the product (92 mg, 76%) as a colourless foam, R_f 0.42 (EtOAc–Py 99 : 1 *v*/*v*). NMR ($CDCl_3$): δ_H 8.6 (2H, br, NH), 8.04–8.01 (1H, m, Ar), 7.41–7.16 (19H, m, Ar), 6.90 (2H, s, H-6), 6.80 (8H, d, *J* 8.8, Ar), 6.62 (2H, s, C=CH), 6.50–6.46 (1H, m, Ar), 6.36–6.33 (1H, m, Ar), 4.10 (4H, d, *J* 6.4, CH_2OP), 3.78–3.74 (18H, m, CH_3O + CH_2ODMT + NCH_2CH_2O), 3.60 (2H, t, *J* 6.2, NCH_2CH_2O), 2.88 (3H, s, CH_3N), 1.80 (6H, s, T–CH_3). δ_P 141.1. FAB$^+$ MS: 1209.2 (M + H$^+$ calc. 1209.5).

Sodium di-[(*E*)-2-(hydroxymethyl)-3-(1-thyminyl)prop-2-enyl] phosphate (4h)

To a solution of **4f** (0.060 g, 0.050 mmol) in dry CH_3CN (2.0 ml) was added Bu^tOOH (5–6 M in decane, 0.020 ml, 0.10–0.12 mmol). The reaction was monitored by ^{31}P NMR, to give first the trialkyl phosphate **4g** (δ_P 0.2) which slowly eliminated the protection group to give the dialkyl phosphate **4d** as the 1-methyl-2,3-dihydroimidazo[1,2-*a*]pyridinium salt (δ_P 0.0, $t_{1/2}$ *ca.* 20 min at 25 °C). After 20 h at rt the solvent was removed *in vacuo*, the residue was dissolved in CH_2Cl_2 (15 ml) and extracted two times with aq. $NaHCO_3$ + Na_2SO_3 (0.5 M in each, 2 × 5 ml). The aq. phase was extracted with CH_2Cl_2 (5 ml), the combined CH_2Cl_2 phase dried ($MgSO_4$) and the solvent removed *in vacuo.* The residue (**4d**, sodium salt, 0.055 g) was dissolved in 80% aq. acetic acid (1 ml) and kept for 1 h at rt to remove the DMT groups. After concentration *in vacuo*, the residue in H_2O (2 ml) was extracted with ether (4 × 2 ml), and the aq. phase concentrated *in vacuo* to give the product (0.024 g, 95%) as a colourless powder, *ca.* 85% pure according to NMR. NMR (D_2O): δ_H 7.42 (2H, s, H-6), 6.61 (2H, s, C=CH), 4.40 (4H, d, *J* 6.2, CH_2OP), 4.30 (4H, s, CH_2OH), 1.88 (6H, s, T–CH_3). δ_C 167.2, 152.2, 142.8, 137.3 (*J* 7.8), 125.1, 111.6, 61.3, 60.4 (*J* 5.2), 12.0. δ_P 1.1. FAB$^-$ MS: 485.3 (M$^-$ calc. 485.1).

Solid phase oligonucleotide synthesis

The syntheses were performed on 500 Å CPG supports on a Biosearch 8750 DNA Synthesizer. The coupling cycle used for the modified phosphoramidites **3a**, **3b**, and **3e** is given in Table 3.

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