Synthesis of an Oxyamide linked Nucleotide Dimer and Incorporation into Antisense Oligonucleotide Sequences

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Two derivatives of thymidine, a 5′-protected 3′-hydroxylamine and 5′-carboxy-3′-protected compound, were prepared and coupled to form an oxyamide-linked dinucleotide analogue; this dimer was incorporated into an oligodeoxynucleotide then shown to anneal to complementary DNA with nearly the same affinity as the natural sequence.

Structural modifications of oligonucleotides are becoming increasingly important as possible clinical applications of antisense DNA emerge.[1,2] Backbone modifications wherein phosphate linkages are replaced by neutral functionalities are particularly important as they are frequently associated with advantageous physical properties. Such analogues are less susceptible to degradation by nucleases, more likely to cross cell membrane due to their enhanced lipophilicity, and encounter diminished charge–charge repulsion on binding to complementary strands.

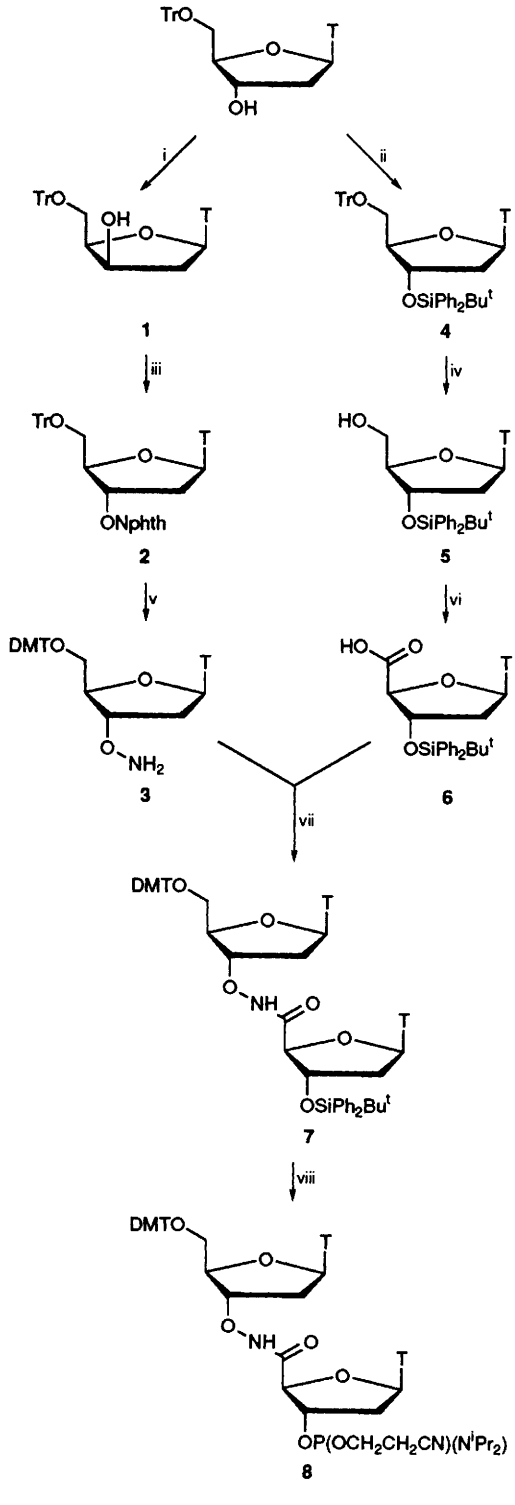
Some DNA surrogates with surprisingly little similarity to natural oligonucleotides have interesting and potentially useful bioactivities. Notable amongst these are 'peptide nucleic acids', PNAs.[3,4] These analogues anneal strongly to DNA, although the interactions by which they do so are not yet elucidated.[5] This communication describes a synthesis of a dinucleotide surrogate which, unlike PNAs, retains the ribose functionalities but it is linked by an amide bond. The effects of incorporating this into small DNA fragments are discussed.

Scheme 1 depicts the synthesis of the dithymidine surrogate. 5′-Tritylated *ara*-thymidine **1** was prepared from 5′-trityl-thymidine[6] according to a literature procedure, *i.e. via* an anhydro intermediate.[7] Mitsunobu inversion[8] at the 3′-position with *N*-hydroxyphthalimide (HONphth) introduced the required masked hydroxylamine functionality giving the inverted product **2**.[9,10] Transposition of the trityl group into the more fragile dimethoxytrityl (DMT) ether functionality, and removal of the phthalyl group gave the desired amine **3** in 26% overall yield from **1**.

An acid component for coupling to amine **3** was prepared by first adding a diphenyl-*tert*-butylsilyl group to the 3′-hydroxy group of 5′-trityl thymidine to give the derivative **4**. Diethylaluminium chloride selectively removed the trityl group from the 5′-oxygen,[11] exposing it for oxidation. Ruthenium tetraoxide with $S_2O_8^{2-}$ as a co-oxidant has been recommended for oxidation of the 5′-hydroxy of nucleosides,[12] and gave good results in our system. The pH of this reaction was maintained at 10 because more alkaline conditions caused loss of the silyl functionality. Overall the yield of acid **6** was 50% from 5′-tritylthymidine.

Coupling of the amine **3** with the acid **6** proceeded smoothly; the conversion to product **7** was 57% but the yield based on starting materials consumed was 72% (*i.e.* significant amounts of starting material were recovered in this reaction). Replacement of the silyl group of ether **7** with a phosphoramidite gave the dinucleotide analogue **8** which is suitably protected for incorporation into DNA strands *via* the automated phosphoramidite methodology.[13]

An ABI 380B DNA synthesizer was used to prepare T_{10} analogues each containing an oxyamide linkage. At the end of



Scheme 1 Syntheses of the oxyamide oligomimetic. *Reagents and conditions*: i, ref. 7; ii, Ph_2Bu^tSiCl, imidazole, DMF; iii, HONphth, PPh_3, DEAD, 4 Å sieves; iv, Et_2AlCl, CH_2Cl_2; v, TsOH, MeOH then DMTCl, py then NH_2NH_2, THF; vi, $RuCl_3$ (catalyst), $K_2S_2O_8$, 1 mol dm^{-3} KOH (aq), THF, pH 10; vii, benzotriazol-1-yloxytris(dimethylamino)phosphoniumhexafluorophosphate (BOP), BtOH, *N*-methylmorpholine, DMF; viii, NBu_4F, THF then $ClP(OCH_2CH_2CN)(NPr^i_2)$, NPr^i_2Et, CH_2Cl_2; Tr = trityl, Ts = tosyl.

the automated sequence the 5′-dimethoxytrityl group was retained, facilitating purification of the oligomers and simultaneous removal of the dimethoxytrityl protecting group by loading the samples onto NENSORB reverse-phase columns, treating with TFA, then eluting with 35% aqueous methanol. Purities of the products were verified using analytical HPLC. Table 1 shows the 'oligomimetics' produced, where **T*T** is used to identify the oxyamide dimer derived from the protected fragment **8**.

Melting temperatures were determined (UV) by annealing the modified T_{10} oligomers to $CGCA_{10}CGC$. The flanking CGC functionalities were included to prevent formation of partial double strands by annealing in such a way that overhang and sliding occurs. Oxyamide linked **T*T** dimers have one atom less than the natural TT phosphates in the backbone chain. This shortening effect is somewhat offset by the planarity of the amide linkage; nevertheless, it might be expected that the analogues would anneal less strongly, particularly if the **T*T** dimer were located in a central position of the oligomer. In fact, there was no significant difference in the melting temperatures observed for the positional isomers of the oxyamide dimer. Natural T_{10} did bind to its complement more strongly than any of the analogues, but the drop in melting temperature was less than that which would be observed if two mismatched bases were introduced.

Compound **8** is not the first dinucleotide analogue with an amide bond connecting the two units,[14] but it has attributes not shared with some of the other members of this category.[15–20] Notably, the 3′-oxygen is retained so that it both increases the nucleophilicity of the amine functionality and projects it away from the encumbering effects of the ribose fragment. These properties are particularly attractive with respect to projects involving generation of oxyamide linked oligomimetics by the techniques typically used in solid-phase peptide syntheses.

Table 1 Melting temperatures for DNA and analogues annealed to $CGCA_{10}CGC$[a]

Entry	Sequence	T_m[b]/°C
1	T_{10}	25 ± 0.5
2	T^*TT_8	18 ± 1.0
3	$T_2T^*TT_6$	20 ± 1.0
4	$T_4T^*TT_4$	19 ± 0.5

[a] Melting temperatures were determined by adding equimolar amounts of the complementary strands in a 10 mmol dm^{-3} phosphate buffer (pH 7.0), 10 mmol dm^{-3} $MgCl_2$ and 20 mmol dm^{-3} NaCl. The solution was heated to 95 °C, slowly cooled to 25 °C over 30 min, then to 4 °C over 45 min. UV absorbances at 260 nm were recorded with increasing temperature at regular intervals of 1 °C. Temperature and normalized absorbances were plotted to give melting curves. [b] Determined *via* UV absorbance, errors quoted represent the range of variation over at least three experiments for each of the entries.

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References

1 H. M. Weintraub, *Sci. Am.*, 1990, 40.
2 M. D. Matteucci and N. Bischofberger, *Ann. Rep. Med. Chem.*, 1991, **26**, 287.
3 M. Egholm, O. Buchardt, P. E. Nielson and R. H. Berg, *J. Am. Chem. Soc.*, 1992, **114**, 1895.
4 P. Garner and J. U. Yoo, *Tetrahedron Lett.*, 1993, **34**, 1275.
5 M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden and P. E. Nielsen, *Nature*, 1993, **365**, 566.
6 J. P. Horwitz, J. A. Urbanski and J. Chua, *J. Org. Chem.*, 1962, **27**, 3300.
7 J. P. Horwitz, J. Chua, J. A. Urbanski and M. Noel, *J. Org. Chem.*, 1963, **28**, 942.
8 O. Mitsunobu, *Synthesis*, 1981, 1.
9 K. Kondo, T. Ogiku and I. Inoue, *ACS Symp. Ser.*, 1985, **16**, 93.
10 E. D. Clercq, I. Inoue and K. Kondo, *Chem. Abstr.*, 1991, **114**, 122980n.
11 H. Koster and N. D. Sinha, *Tetrahedron Lett.*, 1982, **23**, 2641.
12 R. S. Varma and M. E. Hogan, *Tetrahedron Lett.*, 1992, **33**, 7719.
13 M. H. Caruthers, *Acc. Chem. Res.*, 1991, **24**, 278.
14 R. S. Varma, *Synlett*, 1993, 621.
15 M. J. Gait, S. Jones and R. T. Walker, *J. Chem. Soc., Perkin 1*, 1974, **4**, 1684.
16 J. Lebreton, A. D. Mesmaeker, A. Waldner, V. Fritsch, R. M. Wolf and S. M. Freier, *Tetrahedron Lett.*, 1993, **34**, 6383.
17 I. Idziak, G. Just, M. J. Damha and P. A. Glannaris, *Tetrahedron Lett.*, 1993, **34**, 5417.
18 W. S. Mungall and J. K. Kaiser, *J. Org. Chem.*, 1977, **42**, 703.
19 J. M. Coull, D. V. Carlson and H. L. Weith, *Tetrahedron Lett.*, 1987, **28**, 745.
20 E. P. Stirchak, J. E. Summerton and D. D. Weller, *J. Org. Chem.*, 1987, **52**, 4202