Replacement of the phosphodiester linkage in DNA with sulfamide and 3′-*N*-sulfamate groups†

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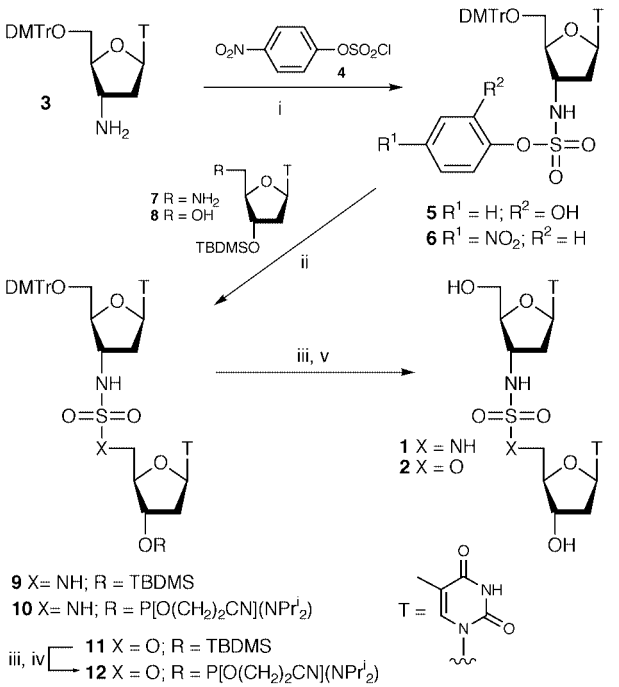
Replacing phosphodiester linkages in DNA with neutral 3′-*N*-sulfamate groups has little effect on the binding affinity for complementary DNA or RNA, whereas incorporation of sulfamide groups into DNA results in considerable destabilisation of isosequential duplexes.

Modified oligonucleotides with neutral linkages replacing the native phosphodiester groups are of interest in the development of improved antisense and antigene agents.[1] Methylphosphonates, one of the earliest examples of this class, can only be efficiently prepared at present as a mixture of diastereoisomers at the phosphorus atom and have low affinity for complementary nucleic acids.[2] More recently neutral methylene(methylimino) (MMI),[3] amide[1] and 3′-thioformacetal[4] modified oligonucleotides have been developed which exhibit improved affinity for complementary nucleic acids. Furthermore, neutral formacetal[5] and dimethylenesulfone[6] linkages have been used effectively in the development of modified DNA aptamers[5] and dsDNA transition state analogues[6] that can bind to specific protein targets.

Earlier we reported the synthesis of dinucleotide analogues in which the phosphodiester linkage is exchanged for a neutral sulfamide group.[7] Here, the effects of the sulfamide **1** and 3′-*N*-sulfamate **2** (Scheme 1) internucleoside linkages on nucleic acid conformation and duplex stability are described. It was envisaged that the block synthesis approach could be used to synthesise chimeric oligodeoxynucleotides in which one or more of the phosphodiester groups are replaced by the neutral linkages. With the sulfamide phosphoramidite **10** in hand[7] a route to the 3′-*N*-sulfamate dinucleosides was required. Although isomeric 5′-*N*-sulfamate dinucleosides have been prepared before, the synthesis uses explosive sulfuryl chloride azide ($ClSO_2N_3$) and the overall yields are low.[8] Therefore, the development of an alternative method was necessary. Initially the coupling of 2–hydroxyphenyl sulfamate **5**[7] with the 5′-alcohol **8** was investigated. However, compound **5** proved insufficiently reactive and failed to give any of the sulfamate dinucleoside **11** despite the earlier observation that **5** reacts smoothly, albeit at high temperature, with the 5′-amine **7** to give the sulfamide dinucleoside **9**. In light of this, the more reactive 4–nitrophenyl sulfamate **6** was prepared by treating the 3′-amine **3** with 4–nitrophenyl chlorosulfate **4**.[9] Coupling of **6** with 5′-alcohol **8** was successful resulting in the sulfamate dinucleoside **11** in excellent yield at room temperature. Similarly the sulfamide dinucleoside **9** could also be prepared more effectively at room temperature from **6** and the 5′-amine **7**. Desilylation of dinucleoside **11** and 3′-phosphitylation gave the required phosphoramidite **12**.

The sulfamate dinucleoside d(TnsoT) **2** was also prepared and its conformation compared with d(TnsnT) **1**, and the native dinucleoside phosphate d(TpT) using NMR spectroscopy. As expected the sum of the $J_{1'2'}$ and $J_{1'2''}$ vicinal coupling constants ($\Sigma 1'$) for the 5′-terminal ribose ring of d(TnsoT) revealed a higher proportion of the C3′-*endo*, or northern (N), sugar conformation (69% N at 30 °C) than the native d(TpT) which exists predominantly in the C2′-*endo*, southern, conformation (36% N).[7] This can be attributed to the lower electronegativity of the 3′–amino substituent of d(TnsoT) and a reduced *gauche* effect between this substituent and the deoxyribose O4′ atom. Furthermore, the shift in the % of N-conformer with change in temperature for both deoxyribose rings of d(TnsoT) was virtually the same as that observed for d(TnsnT),[7] with the largest change occurring in the 5′-terminal ring (63% N at 70 °C increasing to 78% at 10 °C). The native d(TpT) shows little or no variation in sugar pucker with change in temperature. To investigate if this effect is due, in part, to an increase in base stacking of the modified dimers circular dichroism (CD) spectra of d(TnsoT) d(TnsnT) and d(TpT) were recorded at various temperatures. All spectra revealed the same basic characteristics



Scheme 1 *Reagents and conditions*: i, 4–nitrophenyl chlorosulfate **4**,[9] 4–nitrophenol, Et_3N, CH_2Cl_2; ii, alcohol **8** (2 equiv.), Et_3N, 4Å molecular sieves, CH_2Cl_2; iii, TBAF, THF; iv, 2–cyanoethyl–*N*,*N*,*N*′,*N*′–tetraisopropylphosphorodiamidite, 4,5–dicyanoimidazole, CH_2Cl_2; v, 2% $CHCl_2CO_2H$, CH_2Cl_2. 4,4′–Dimethoxytrityl is abbreviated to DMTr.

† Electronic supplementary information (ESI) available: Experimental details for oligonucleotide synthesis and duplex denaturation experiments. See http://www.rsc.org/suppdata/cc/b0/b001586p/

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Table 1 Electrospray mass spectrometry data and duplex melting temperatures for oligonucleotides **13**–**18**

			T_m[a] (ΔT_m/mod.)[b]/°C			
	Electrospray MS/Da		DNA d(CGCA$_{10}$CGC)			RNA r(CGCA$_{10}$CGC)
	M^+ (calc.)	M^+ (found)	0.02 M [Na^+]	0.1 M [Na^+]	1.0 M [Na^+]	0.1 M [Na^+]
13 d(GCGT$_{10}$GCG)	4875.2	4875.3	43.2	55.0	66.4	49.0
14 d(GCGT$_4$TnsnTT$_4$GCG)	4873.3	4872.8	40.0 (−3.2)[b]	51.7 (−3.3)	60.9 (−5.5)	45.9 (−3.1)
15 d(GCGTnsnTTTTnsnTTTTnsnTGCG)	4869.5	4869.7	33.7 (−3.2)	45.0 (−3.3)	54.6 (−3.9)	41.0 (−2.7)
16 d(GCGT$_4$TnsoTT$_4$GCG)	4874.3	4874.2	43.6 (+0.4)	55.1 (+0.1)	66.0 (−0.4)	47.8 (−1.2)
17 d(GCGTnsoTTTTnsoTTTTnsoTGCG)	4872.5	4872.5	44.0 (+0.3)	55.5 (+0.2)	N.D.[c]	48.0 (−0.3)
18 d(GCGTnsoTTnsoTTnsoTTnsoTTnsoTGCG)	4870.7	4870.6	44.8 (+0.3)	53.3 (–0.3)	N.D.[c]	46.0 (−0.6)

[a] T_m values are accurate to within ± 0.5 °C. [b] The changes in T_m per modification (ΔT_m/mod.) are shown in parentheses.[c] T_m was not determined.

of strong and weak positive bands at 280 and 225 nm, respectively, as well as a negative band at 255 nm, suggesting that the native and the modified dimers are adopting similar overall conformations. Upon increasing temperature all showed a significant decrease in the intensity of the bands at 280 and 255 nm. Notably the change in band intensity for d(TnsoT) was twice the magnitude of that observed for d(TnsnT) and d(TpT) which were virtually identical. This suggests that d(TnsoT) has an higher propensity to adopt a base stacked helical conformation than d(TnsnT) and d(TpT), which base stack to a lesser extent.[10]

Solid phase synthesis of 16-mer chimeric oligodeoxynucleotides, incorporating 3′–*N*-sulfamate or sulfamide linkages, was carried out under standard conditions using the modified dimer phosphoramidites **10** and **12**. The oligonucleotides prepared, **13**–**18**, were characterised by electrospray mass spectrometry and all have the same sequence GCGT$_{10}$GCG[11] (Table 1). The duplex melting temperatures (T_m) of modified oligonucleotides with complementary DNA were then determined by variable temperature UV spectroscopy and compared with the T_m for the native duplex. Modified oligonucleotides with one and three central sulfamide linkages, **14** and **15**, both show a similar change in T_m per modification (ΔT_m/mod.), compared with the native duplex, of −3.2 °C at 0.02 and 0.1 M salt concentration [Na^+]. At 1.0 M salt concentration the modification was even more destabilising. In contrast incorporation of one, three and five sulfamate groups, **16**, **17** and **18**, has little effect on duplex stability with complementary DNA and is even moderately stabilising at low salt concentration with an observed increase in T_m of 0.3 °C per modification. Upon increasing salt concentration the T_m dropped by 0.4 °C for one sulfamate modification. It is likely that the neutral linkages reduce the electrostatic repulsion between strands which is more evident at low salt concentration when fewer cations are present to mask the negatively charged phosphodiester groups. With complementary RNA at 0.1 M salt concentration, the sulfamide group had a similar effect on duplex stability with a drop in T_m of *ca.* 3.0 °C per modification. However the 3′-*N*-sulfamate modified oligonucleotides formed slightly less stable duplexes with RNA than DNA with a drop in T_m of 1.2 °C for one modification and a more moderate drop in ΔT_m/mod of 0.3 and 0.6 °C for three and five modifications respectively. It is probable that oligonucleotides with more 3′-*N*-sulfamate linkages have an higher proportion of C3′-*endo* sugar rings and are therefore more likely to adopt an A–type conformation which is preferred for binding to RNA.

From these results it is clear that 3′-*N*-sulfamate modified oligonucleotides hybridise with complementary nucleic acids with similar binding affinity as native DNA, whereas the sulfamide congeners have significantly lower affinity. It is possible in light of the CD spectra that the sulfamate modified oligonucleotides are more preorganised into a base stacked helical conformation, in the single stranded state, which would reduce the loss of entropy on binding to complementary RNA and DNA and account for the greater stability of the resulting duplexes. Furthermore, the 3′-*N*-sulfamate modified oligonucleotides appear to form more stable duplexes than DNA incorporating the isomeric 5′-*N*-sulfamate modification, which is reported to result in a drop in T_m of 1.5 °C per modification.[8] The destabilisation of duplexes by sulfamide and 5′-*N*-sulfamate modifications may be accounted for by the 5′-amino substituent common to both. In the case of isosteric and isoelectronic N5′→P3′ phosphoramidate DNA, the presence of a 5′-NH group completely abolishes base pairing with complementary nucleic acids.[12] This is attributed to poor hydration and steric clashes between the 5′-amino hydrogen atom and either the H2′ atom of an adjacent deoxyribose ring in an A–type conformation, or the pyrimidine H6 and deoxyribose O4′ atoms in a B-type conformation.[12]

The data reported here suggest that the 3′-*N*-sulfamate modification may be of use in the development of improved antisense or antigene agents. Moreover, given that the 3′-*N*-sulfamate group causes minimal disruption of nucleic acid duplexes, is sterically and electronically more similar to the phosphodiester group than other neutral linkages so far developed, it might also be useful as a general phosphodiester replacement for other applications. For example, one can envisage probing nucleic acid–protein interactions using this modification to determine which phosphodiester groups are involved in electrostatic contacts with protein residues. Alternatively 3′-*N*-sulfamate groups might be used to stabilise aptamers, or hammerhead ribozymes for use *in vivo*.

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