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Synthesis of Sulfamide Linked Dinucleotide Analogues

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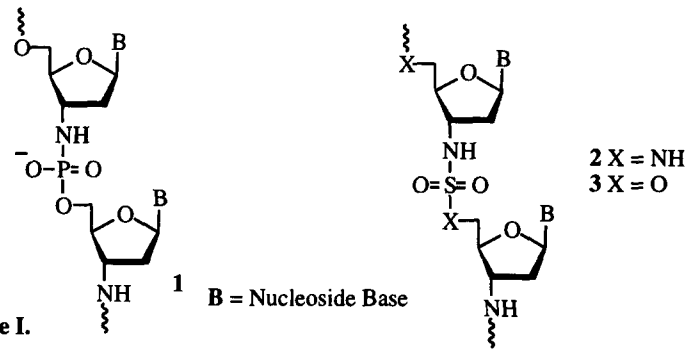
Abstract: Novel sulfamide [–$NHSO_2NH$–] linked dinucleotide analogues d(TnsnT) and d(TnsnA) have been synthesised from 3′– and 5′–amino nucleosides. Treatment of these amino nucleosides with catechol sulfate results in the formation of 2–hydroxyphenyl sulfamate esters which couple smoothly in good yields with either 5′– or 3′–amines of similar nucleosides. NMR studies showed that the 3′–sulfamide group results in a preferential C3′–endo (Northern) sugar conformation.

Oligonucleotides and their analogues have emerged as a new class of therapeutic agents.[1] It was supposed that their main mode of action, the so called "antisense mechanism", would depend on their recognition and hybridisation to complementary mRNA target sequences, resulting in the inhibition of translation, by either sterically blocking the binding of the ribosome and initiation factors to mRNA, or alternatively by recruiting the enzyme RNase H to cleave the mRNA strand of the heteroduplex.[2,3] The primary criteria in the design of oligonucleotide therapeutics are therefore high affinity and specificity for the target mRNA sequence, stability to intra and extra cellular nucleases, efficient cellular uptake, and ability to serve as a substrate for RNase H. To these ends many oligonucleotide analogues have been synthesised and investigated, the results of most of which are covered in extensive reviews.[2-5] The first generation oligonucleotide therapeutics of choice, phosphorothioate DNA, have been evaluated extensively and recent clinical trials have shown encouraging results in the treatment of human myelogenous leukaemia.[6] One of the more promising candidates for the second generation therapeutics are the oligodeoxyribonucleoside N3′→ P5′ phosphoramidates **1**.[7-11] These have been shown to form unusually stable duplexes with nucleic acids, exhibiting an increase in T_m of approximately 2 °C per modification compared with the corresponding parent duplex.[8] Replacement of the 3′–oxygen with the more electropositive amino substituent changes the sugar conformation from C2′–endo (S) to C3′–endo (N); this preorganises the phosphoramidate DNA into the required conformation for a preferred A type heteroduplex with complementary RNA, thereby increasing the thermodynamic stability of the resulting duplex.[10, 12] Favourable solvation of the 3′–NH substituent is also believed to stabilise the A type heteroduplex.[11]

Our research effort in this area has focused on the synthesis and evaluation of modified oligonucleotides, where the phosphodiester linkage is replaced by sulfamide [–$NHSO_2NH$–] **2** and sulfamate [–$NHSO_2O$–] **3** groups (Scheme I).

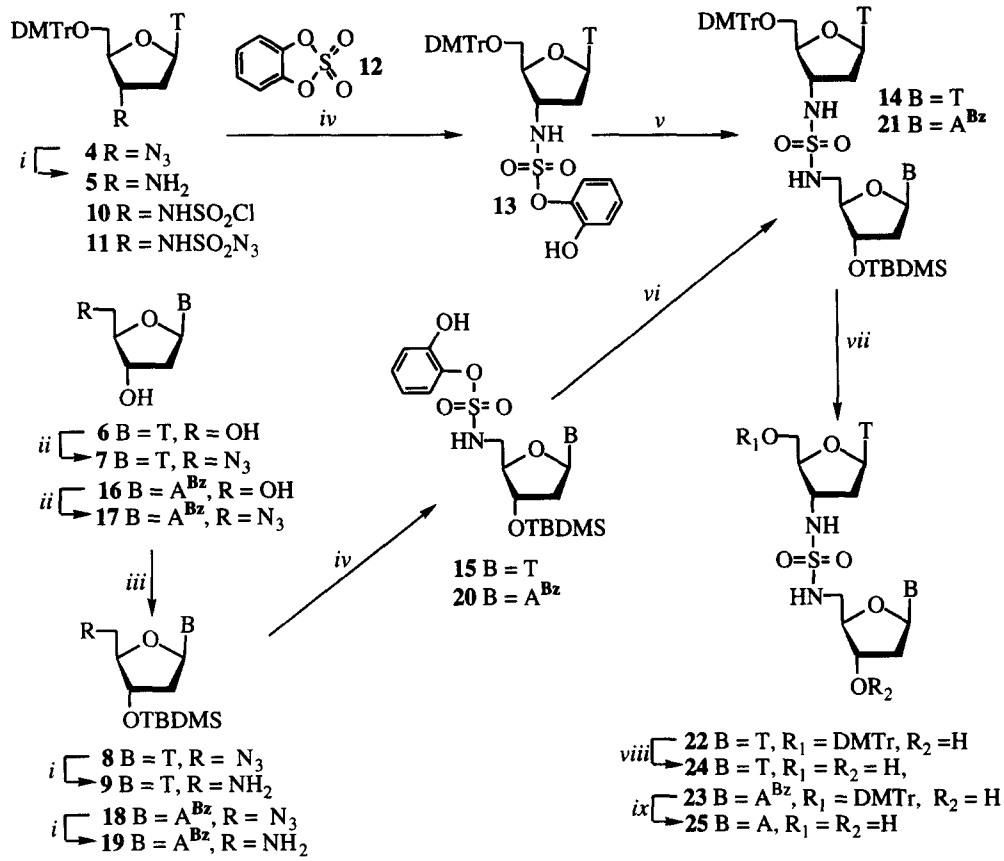


Scheme I.

We chose these groups because they are isosteric to the phosphate group, which might allow them to adopt conformations similar to parent phosphodiester. Moreover we anticipated that the neutral 3′–sulfamide and sulfamate groups would give rise to a preferential N–type ribose conformer, as well as alleviating the electrostatic repulsion which exists between the negatively charged phosphate groups in the complementary strands of parent duplexes. Both these factors could give rise to a more stable heteroduplex with RNA. Finally they would be more lipophilic than the parent oligonucleotides, which might aid cellular uptake.

Herein we report the synthesis of sulfamide linked dinucleotide analogues. Accordingly 3′–azido–5′–*O*–dimethoxytrityl–3′–deoxythymidine **4** was prepared from thymidine **6** and then reduced to 3′–amino–5′–*O*–dimethoxytrityl–3′–deoxythymidine **5**.[9] Similarly 5′–amino–3′–*O*–*tert*–butyldimethylsilyl–5′–deoxythymidine **9** was prepared from thymidine via 5′–azido–5′–deoxythymidine **7**,[13] which was TBDMS protected to give 5′–azido–3′–*O*–*tert*–butyldimethylsilyl–5′–deoxythymidine **8**, followed by reduction to give the amine **9**.

Initially we investigated the reaction of 3′–amine **5** with sulfuryl chloride and sulfuryl chloride azide ($ClSO_2N_3$). However, due probably to the high reactivity of these reagents, we were unable to isolate the corresponding sulfamoyl chloride **10** or sulfamoyl azide **11**.[14] Instead we opted for the milder conditions of Dubois *et al.*[15] Here catechol sulfate **12** is opened by the 3′–amine of **5** in the presence of triethylamine to give the 2–hydroxyphenyl 3′–sulfamate **13** in 44 % yield. An equimolar mixture of sulfamate **13** and 5′–amine **9** were then heated under reflux in dioxane for 2 hr to give the protected sulfamide linked dinucleoside d(TnsnT) **14** in 68 % yield. Evidence suggests that this reaction takes place through initial deprotonation of the sulfamate NH group, followed by elimination of catechol to give a reactive *N*–sulfonylamine intermediate (C3′–N=SO_2), which is trapped by the 5′–amine.[16] The 2–hydroxyphenyl 5′–sulfamate **15** was also synthesised (63 % from amine **9**), treated with the 3′–amine **5** and heated under reflux in dioxane for 4 hr to give protected dinucleoside **14**, this time in 56 % yield. In order to check the generality of this route for the synthesis of dinucleosides incorporating purine as well as pyrimidine bases we also synthesised the protected sulfamide linked dinucleoside d(TnsnA) **21**. An analogous series of reactions **16** → **19**, as those described above, gave 5′–amino–N^6–benzoyl–3′–*O*–*tert*–butyldimethylsilyl–2′,5′–dideoxyadenosine **19**, which was converted into 2–hydroxyphenyl 5′–sulfamate **20** in 82 % yield, and then coupled with 3′–amine **5** to give the protected dinucleoside d(TnsnA) **21** under similar conditions, but in only 38 % yield. The alternative attack of 5′–amine **19** on 3′–sulfamate **13** failed, however, to yield any of the protected dinucleoside d(TnsnA) **21**.



Scheme II. *Reagents: i*, H_2, 10% Pd-C; *ii*, PPh_3, CBr_4, NaN_3, DMF; *iii*, TBDMSCl, Et_3N; *iv*, catechol sulfate **12**, Et_3N; *v*, amine **9**, dioxane reflux; *vi*, amine **5**, dioxane reflux; *vii*, TBAF; *viii*; $CHCl_2COOH$ *ix*, a) $CHCl_2COOH$, b) conc. NH_4OH.

Deprotection of the 3′–hydroxyl of dinucleosides **14** and **21** with tetrabutylammonium fluoride gave the 3′–alcohols d(TnsnT) **22** and d(TnsnA) **23**, facilitating conversion into the corresponding 3′–phosphoramidites required for incorporation into DNA using standard solid phase phophoramidite technology. The fully deprotected dinucleosides d(TnsnT) **24** (85 % from **14**) and d(TnsnA) **25** (78 % from **21**) were prepared by acidolysis of the dimethoxytrityl group and ammonolysis of the benzoyl protecting group in the latter case. These dinucleosides **24** and **25** were subjected to extensive 1D and 2D NMR studies[17] from which the conformations of the ribose rings were deduced using the graphical method of Rinkel and Altona.[18] This showed that replacement of the phosphodiester group of d(TpT) and d(TpA) with sulfamide groups results in a shift in the N–S equilibrium from a predominantly S to N 5′–terminal ribose conformation,[17] although the fraction of N conformer was not as high as in the corresponding phosphoramidate d(TnpT).[10]

In summary 2–hydroxyphenyl 3′– and 5′–sulfamate nucleoside esters react with 5′– and 3′–amino nucleosides, to give sulfamide linked dinucleosides with both pyrimidine and purine bases. The methodology developed here should therefore allow the synthesis of sulfamide linked oligodeoxynucleosides. Currently we

are investigating the incorporation of these dinucleosides into DNA in order to measure the effects of this modification on duplex melting temperatures. In addition we wish to elucidate the structure of modified duplexes by NMR.

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17. New componds were characterised by ^{1}H and ^{13}C NMR spectroscopy and Mass spectrometry. ROESY, TOCSY, DQF–COSY and *J*–resolved spectra (500 MHz) were recorded for compounds **24** and **25**. Selected data: From $^{3}J_{HH}$ vicinal coupling constants the percentage N conformation of the ribose rings in **24** and **25** were calculated;[18] d(*T*nsnT) 65 %, d(Tnsn*T*) 45 %, d(*T*nsnA) >95 % and d(Tnsn*A*) 60 %.
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