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Sulfamide Replacement of the Phosphodiester Linkage in Dinucleotides: Synthesis and Conformational Analysis.[1]

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Abstract: Dinucleotide analogues d(TnsnT) and d(TnsnA), in which the phosphodiester linkage of d(TpT) and d(TpA) is replaced by sulfamide $[-NHSO_2NH-]$, have been synthesised. Conformational analysis using ^3J vicinal coupling constants shows that this modification results in a shift from a predominantly southern (C2′–endo) to northern (C3′–endo) ribose ring conformation. The effect of temperature and concentration changes on ribose conformation, reveals that the modified dinucleosides have a greater propensity to base stack intramolecularly, and in the case d(TnsnA), self–associate.

INTRODUCTION

Novel oligonucleotide analogues that can form stable duplexes and triplexes with nucleic acids, are important synthetic targets, because of their use as therapeutic agents.[2] In addition these molecules are useful as probes for hybridisation screening.[3] Furthermore they are of fundamental value as models for the study of nucleic acid structure, function[4,5] and evolution.[6] Key to the successful future design of useful oligonucleotide analogues is an understanding of the effect of structural modifications on the conformation and physical properties of these molecules.

From a therapeutic point of view factors other than the ability to hybridise with mRNA or dsDNA are important, such as stability, particularly to nuclease enzymes, solubility, cell wall permeability, and ability to activate the RNase H cleavage of the mRNA strand in heteroduplexes.[2] To some extent these later criteria can be fulfilled by a chimeric approach.[7] This generally involves the synthesis of oligonucleotide analogues incorporating a central sequence of DNA, or phosphorothioate DNA, which can serve to activate RNase H, flanked by alternatively–modified oligonucleotides which form stable duplexes with mRNA, but in addition are stable to exonucleases, thereby protecting the central sequence from digestion. We were particularly interested in synthesising sulfamide linked oligonucleosides to fulfil this role. It is anticipated that the sulfamide group which is neutral, but isosteric to the parent phosphodiester, might adopt similar conformations, whilst alleviating the electrostatic repulsion which exists between the negatively charged phosphate groups in the complementary strands of parent duplexes. In addition we envisaged that the 3′–amino substituent, which is more electropositive than oxygen, would give rise to a preferential northern (N) C3′–endo ribose conformation. A similar *gauche* effect operating in N3′→ P5′ phosphoramidates [8] is thought, in part, to contribute to the

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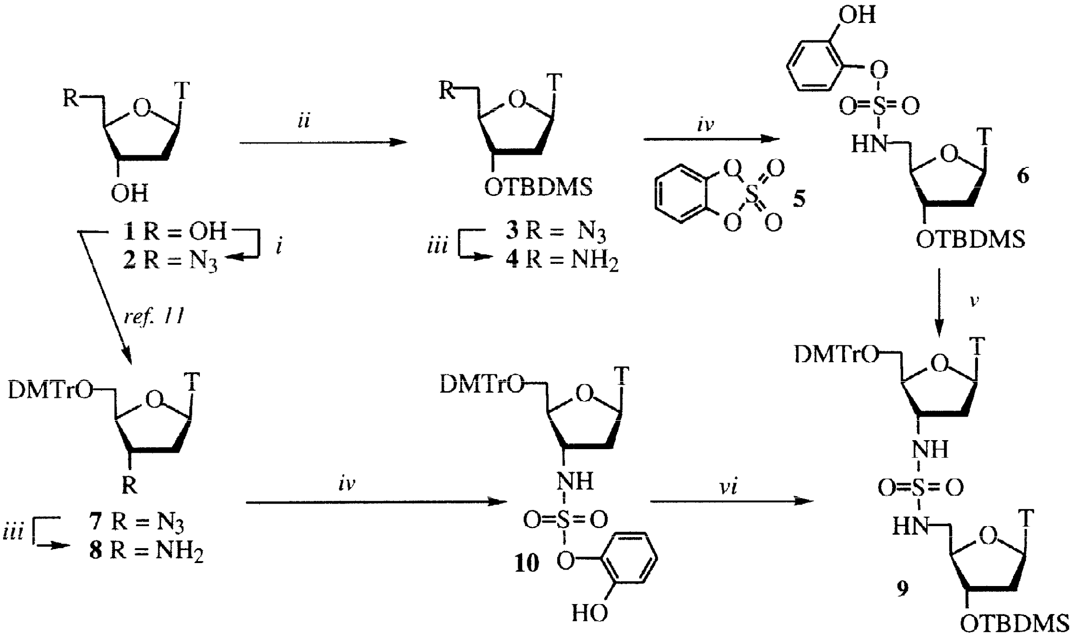
thermodynamic stability of the resulting heteroduplexes formed with complementary RNA, by preorganising[9] the phosphoramidate DNA into the required conformation for a preferred A–type duplex.

RESULTS AND DISCUSSION

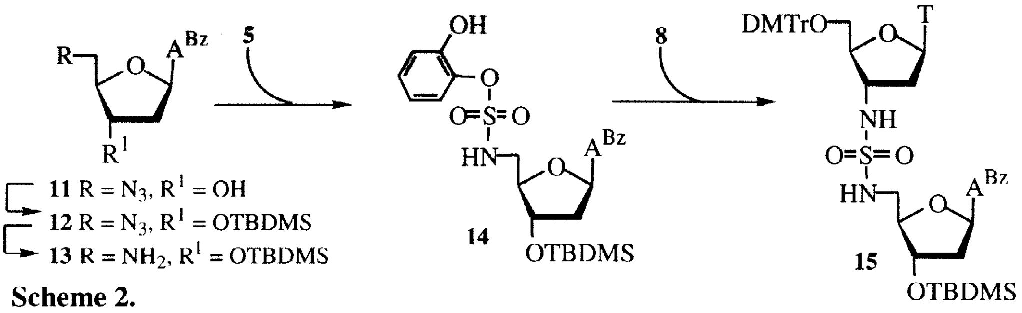
Synthesis of dinucleosides d(TnsnT) and d(TnsnA).

The synthesis of appropriately protected sulfamide linked dinucleosides for conversion into the 3′–phosphoramidites, necessary for incorporation into DNA, was required, as well as fully deprotected dinucleosides for NMR conformational studies. Accordingly the dinucleoside d(TnsnT) in fully protected form was prepared as shown below (Scheme 1). Firstly, Thymidine **1** was converted to 5′–azido–5′–deoxythymidine **2** using established chemistry.[10] The 3′–hydroxyl was then protected with a TBDMS group to give 5′–azido–3′–*O*–(*tert*–butyldimethylsilyl)–5′–deoxythymidine **3**, hydrogenation of which gave the 5′–amine **4**.[11] 3′–azido–5′–*O*–dimethoxytrityl–3′–deoxythymidine **7** was also prepared from thymidine and similarly reduced to 3′–amine **8**.[12]



Scheme 1. *Reagents: i*, PPh_3, CBr_4, NaN_3, DMF; *ii*, TBDMSCl, imidazole; *iii*, H_2, 10% Pd-C; *iv*, catechol sulfate **5**, Et_3N; *v*, 3′–amine **8**, dioxane reflux; *vi*, 5′–amine **4**, dioxane reflux.

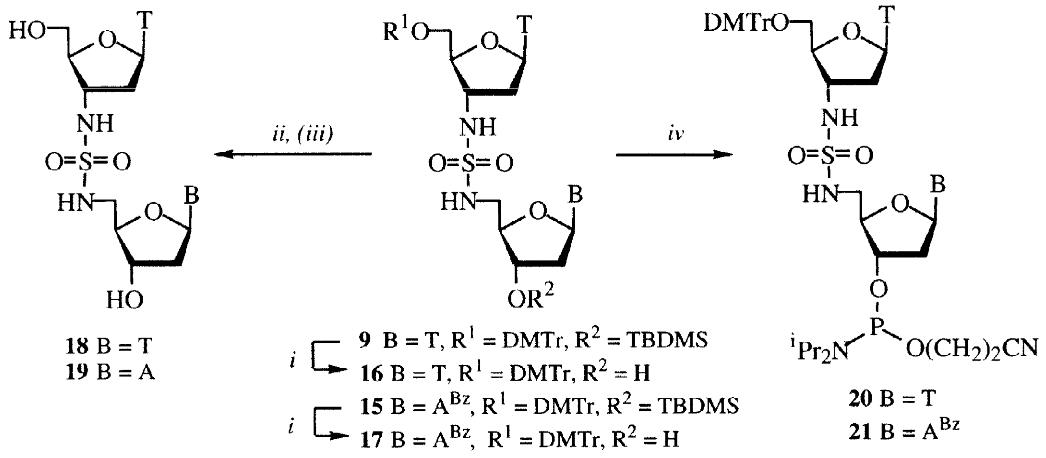
Coupling these amines with conventional sulfonating agents,[13] sulfuryl chloride, sulfuryl chloride azide or sulfonyl diimidazole was unsuccessful so catechol sulfate **5** was used. The strained five membered cyclic sulfate is reactive to attack by nucleophiles,[14] a fact noticed by Dubois *et al.*,[15] who had used this reagent to prepare sulfamic acids and sulfamides. Following this procedure[15] 5′–amine **4** was treated with catechol sulfate and triethylamine to give 2–hydroxyphenyl 5′–sulfamate **6** in 63 % yield. An equimolar mixture of 3′–amine **8** and 5′–sulfamate **6** was then heated under reflux in dioxane to give protected sulfamide linked dinucleoside d(TnsnT) **9** in 56 % yield. Alternatively the 3′–amine **8** was converted to the 2–hydroxyphenyl 3′–sulfamate **10** in 44 % yield, treated with 5′–amine **4** and heated under reflux in dioxane to give the protected dinucleoside **9** in 68 % yield.



Scheme 2.

We also prepared sulfamide linked dinucleoside d(TnsnA) in fully protected form **15** proving that this route was also applicable to nucleosides bearing protected purine bases (Scheme 2). Thus, 5′–amine **13**[16] was prepared from 5′–azide **11**,[10] in an analogous fashion to that described in scheme 1 (**1** → **4**). However, treatment of the 5′–amine **13** with 3′–sulfamate **10** failed to give any of the protected dinucleoside **15**, which was probably due to intramolecular migration of the benzoyl protecting group of **13** to the 5′–amino substituent. This problem was circumvented by converting the 5′–amine into the corresponding 2–hydroxyphenyl 5′–sulfamate **14** (82 % yield), which was successfully coupled with 3′–amine **8** furnishing protected dinucleoside **15** under similar conditions in 38 % yield.

Selective deprotection of the 3′–hydroxyl of dinucleosides **9** and **15** with tetrabutylammonium fluoride gave the 3′–alcohols **16** (99 %) and **17** (94 %). A small portion of each of these was further deprotected, using dichloroacetic acid to remove the dimethoxytrityl groups and, in the later case, concentrated aqueous ammonia solution to effect debenzoylation resulting in dinucleosides d(TnsnT) **18** (95 %) and d(TnsnA) **19** (83 %) required for NMR studies. The high yield for these deprotections suggests that the sulfamide linkage is stable to both the acidic and basic conditions that would be encountered during solid phase oligonucleotide synthesis. Furthermore dinucleosides **18** and **19** are reasonably soluble in water.[17] Finally, phosphitylation of 3′–alcohols **16** and **17** using standard conditions (2–cyanoethyl *N,N,N′,N′*–tetraisopropylphosphorodiamidite and diisopropylammonium tetrazolide)[18] gave the 3′–phosphoramidites **20** and **21** as a mixture of diastereoisomers.



Scheme 3. *Reagents: i,* TBAF; *ii,* $CHCl_2COOH$; *iii*, conc. NH_4OH (4h, 55 °C); iv, $NCCH_2CH_2OP[N(iPr)_2]_2$, diisopropylammonium tetrazolide.

NMR analysis of dinucleosides d(TnsnT) ***18*** *and d(TnsnA)* ***19****.*

The 1D ^1H NMR spectra of d(TnsnT) **18** and d(TnsnA) **19** were fully assigned with the aid of 2D DQF–COSY, TOCSY and NOESY experiments (Table 1). The two spin systems of the 5′– and 3′–terminal ribose rings were assigned by TOCSY and DQF–COSY experiments and distinguished by noticeable shielding of the H3′ and H5′/5″ protons adjacent to the sulfamide linkage. In the case of d(TnsnA) this was confirmed by NOESY experiments connecting base protons H6 of thymine and H8 of adenine to their corresponding ribose rings. Similar NOESY cross–peaks between the ribose ring protons and H6 served to assign the non–exchangeable base protons of d(TnsnT). The H2′ and H2″ protons were also assigned through NOESY cross peaks between H2′ protons and H6 or H8 base protons. Finally H5′ and H5″ protons were assigned according to the Remin and Shugar rule.[19] The coupling constants of d(TnsnT) and d(TnsnA) were determined from 1D spectra and from 2D *J*–resolved spectra where spectral overlap was severe (Table 2).

Table 1. Proton chemical shifts of d(TnsnT) and d(TnsnA).

δ_H[a]	ppm	H1′	2′	2″	3′	4′	5′	5″	6	CH_3	8	2
18	d(*T*nsnT)	6.15	2.56	2.49	4.00[b]	4.00[b]	3.93	3.81	7.69	1.88		
	d(Tnsn*T*)	6.21	2.43	2.38	4.46	4.04	3.41	3.30	7.57	1.87		
19	d(*T*nsnA)	5.86	2.30	2.38	3.82	3.90	3.95	3.80	7.61	1.69		
	d(Tnsn*A*)	6.40	2.85	2.58	4.80	4.31	3.43	3.40			8.24	8.07

a) ^1H chemical shifts recorded in D_2O, with a sample concentration of 18.5 mM, at 30 °C. Data corresponds to italicised nucleoside unit. b) signals could not be separately assigned due to apparent coincidence.

Table 2. ^3J vicinal and ^2J geminal coupling constants of constituent ribose rings of d(TnsnT) and d(TnsnA).

J_{HH} (Hz)		$J_{1'2'}$	$J_{1'2''}$	$J_{2'2''}$	$J_{2'3'}$	$J_{2''3'}$	$J_{3'4'}$	$J_{4'5'}$	$J_{4'5''}$	$J_{5'5''}$	Σ1′	%N[a]
18	d(*T*nsnT)	4.6	7.2	14.4	7.5	7.2	___[b]	2.5	4.3	12.7	11.8	66
	d(Tnsn*T*)	6.6	6.6	14.2	6.6	5.1	5.0	3.8	6.0	14.2	13.2	42
19	d(*T*nsnA)	2.4	7.1	13.6	7.6	9.8	8.9	2.4	3.3	12.9	9.5	100
	d(Tnsn*A*)	5.9	6.9	14.3	6.7	4.8	4.6	3.1	3.0	13.6	12.8	49

[a] % Northern conformer of ribose rings determined from Σ1′ values using the sum rule (see below).[22]
[b] could not be determined due to coincidence of H3′ and H4′ proton resonances in d(*T*nsnT) ribose ring.

Ribose ring conformation.

We wished to establish the effect on conformation of replacing the phosphodiester group of dinucleoside phosphates d(TpT) and d(TpA) with a sulfamide group, particularly the extent of the N – S equilibrium, which is known to affect duplex conformation and stability.[8,9] By measuring the coupling constants $J_{1'2'}$, $J_{1'2''}$, $J_{2'3'}$, $J_{2''3'}$ and $J_{3'4'}$ at several different temperatures it is possible to establish the geometries of the N and S conformers as a function of their phase angle of pseudorotation and puckering amplitude,[20] and to establish the molar fractions of each conformer present in the N – S equilibrium using the program PSEUROT.[21] However for d(TnsnT) and d(TnsnA), spectral overlap made it impossible to determine all these coupling constants accurately over a range of different temperatures. Instead we were able to arrive at a more uniform treatment of our data using the graphical method of Altona,[22] which relies on analysis of the relative magnitude of the $J_{1'2'}$ coupling constants and the sums of the H1′ coupling constants (Σ1′) which is related to the percentage of S conformer (% S) by the "sum rule":

$$\% \text{ S} = (\Sigma \text{H1}' - 9.8) / 5.9 \quad \text{where} \quad \Sigma 1' = J_{1',2'} + J_{1',2''}$$

Using this sum rule, we established that replacement of the phosphodiester group of d(TpA) and d(TpT) with sulfamide results in a significant shift in the N – S equilibrium from a predominantly S to N 5′–terminal ribose conformation, as expected. The shift was most noticeable in the d(TnsnA) case, which exists exclusively as the N conformer at 30 °C, compared with d(TpA) which is predominantly in the S conformation (25 % N) at the same temperature (Table 3; see Σ1′ and % N, 30 °C columns). The 3′–terminal ribose rings exhibited a slight increase in the percentage of N conformer at 30 °C, on replacement of the phosphate group of d(TpT) and d(TpA) with sulfamide. In this case the 3′–OH substituent is retained and the slight change in conformation must be due to factors other than the electronic nature of the 3′–substituent and the *gauche* effect.

NOESY experiments at (30 °C) also support these observations. Both 5–terminal nucleosides of d(TnsnT) and d(TnsnA) show strong H6 to H3′ cross–peaks, characteristic of a nucleoside which has a predominantly N ribose conformer, with the base *anti* relative to its ribose ring. Whereas neither the 3′–terminal ribose rings of d(TnsnT) and d(TnsnA) nor the parent dinucleoside phosphates show strong H6 or H8 to H3′ cross–peaks, but all showed strong H6 or H8 to H2′ cross peaks typical of a majority S conformer with the base *anti* to its ribose ring.[23, 24]

Conformational dependence on temperature.

In order to establish whether the shift towards preferred N conformer is due solely to the lower electronegativity of the sulfamide group compared with the phosphate, or in part due to an increase in intramolecular base stacking, the conformation of the ribose rings was monitored with decreasing temperature. It is well established that intramolecular base stacking invariably favours the N ribose conformer in dinucleosides phosphates.[25, 26] From the change in Σ1′ values it is found that both modified dinucleosides tend toward a higher percentage of N conformer with a decrease in temperature from 70 → 10 °C, typical of an increase in intramolecular base stacking, and that the effect is considerably more marked in the case of d(TnsnA). The parent dinucleoside phosphates on the other hand show comparatively little change in Σ1′ values over the same temperature range, indicating intramolecular base stacking is less significant to their overall conformation. This trend can be seen clearly in the splitting patterns of the H1′ protons of modified dinucleosides at 70 and 10 °C (Figure 1). For d(TnsnA) the H1′ signal of the 5′–terminal thymidine collapses from a double of doublets at 70 °C, to an approximate doublet at 10 °C as the the $J_{1'2'}$ coupling becomes small (1.3 Hz), indicative of a H1′–H2′ dihedral angle close to 90 °, which is expected for a N ribose conformer. Similarly the H1′ signals of the 3′–terminal adenosine of d(TnsnA) and the 5′–terminal thymidine of d(TnsnT) both collapse from apparent triplets at 70 °C to double doublets as $J_{1'2'}$ becomes significantly smaller than $J_{1'2''}$, typical of an increase in the percentage of the N conformer. The Σ2′ Σ2′′ and Σ3′ values which can also be related graphically to the extent of the N–S equilibrium,[22] show exactly the same trends (data not shown). Furthermore, the assignment of the H2′ and

Table 3.[a] Conformational change of d(TnsnT) and d(TnsnA) with temperature; comparison with d(TpT) and d(TpA).

	$\Sigma 1'$				$\Delta\Sigma 1'^{b}$ (Hz)	$\%N^{c}$				$\Delta\delta_H{}^{d}$ H1′	CH_3	H6	H2 (ppm)
Temp (°C)	70	50	30	10		70	50	30	10				
*T*nsnA	11.4	10.8	9.5	8.2	– 3.2	73	83	100	100	–0.21	– 0.30	+ 0.19	
Tnsn*A*	13.7	13.7	12.8	11.0	– 2.7	34	34	49	80	–0.07			– 0.37
*T*nsnT	12.3	12.1	11.8	11.2	– 1.1	58	61	66	76	–0.03	– 0.03	+ 0.13	
Tnsn*T*	13.4	13.3	13.2	13.1	– 0.3	39	40	42	44	+0.02	– 0.04	+ 0.06	
*T*pA[e]	14.0	14.1	14.2	14.3	+ 0.3	29	27	25	24	–0.08	– 0.02	– 0.05	
Tp*A*	13.3	13.3	13.3	13.4	+ 0.1	41	41	41	39	–0.05			–0.10
*T*pT[f]	13.7	13.7	13.6	13.4	–0.3	34	34	36	39	- 0.03	– 0.01	+ 0.08	
Tp*T*	13.6	13.6	13.6	13.6	0	36	36	36	36	+0.01	– 0.02	+ 0.06	

a) Table shows change in $\Sigma 1'$ values, % N conformer of ribose rings and chemical shift of H1′ and non–exchangeable base protons with decreasing temperature from 70 to 10 °C. b) $\Delta\Sigma 1' = \Sigma 1'$ (10 °C) – $\Sigma 1'$ (70 °C). c) %N value determined using sum rule.[22] d) Most noticeable changes in chemical shift with decreasing temperature; $\Delta\delta_H = \delta_H$ (10 °C) – δ_H (70 °C). e) Assignment of chemical shifts for d(TpA) agree with literature.[33] f) d(TpT) chemical shifts, coupling constants, % N values are in close agreement with data reported in the literature.[24, 29]

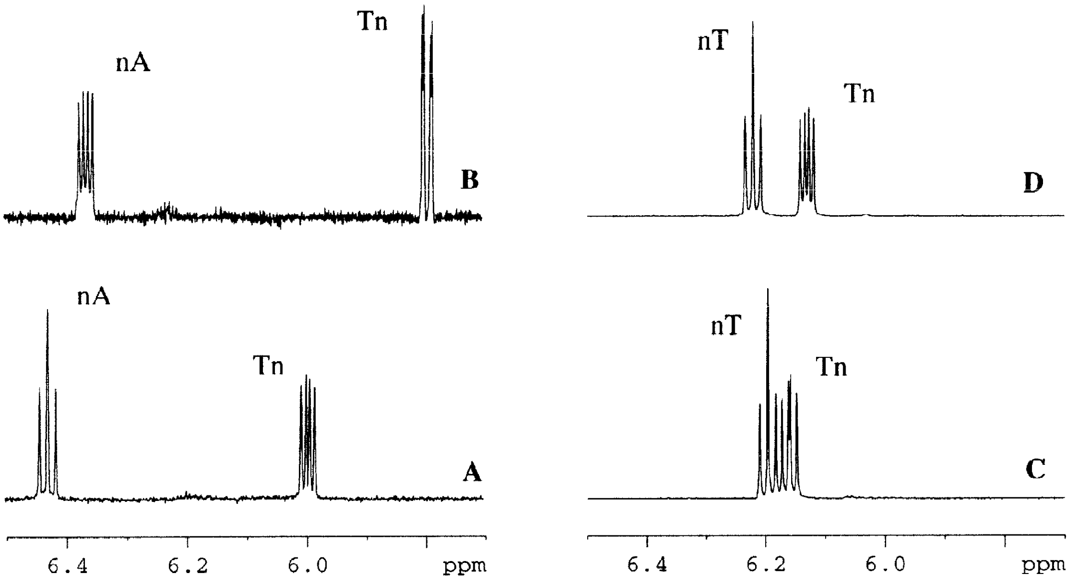


Figure 1. H1′ region of ^1H NMR spectra of d(TnsnA) at 70 °C (**A**), 10 °C (**B**) and d(TnsnT) at 70 °C (**C**), 10 °C (**D**). 5′–terminal ribose H1′ signals (Tn) are upfield of 3′–terminal ribose H1′ signals (nA and nT).

H2'' in d(TnsnT) and d(TnsnA) was confirmed by the rule of Altona,[22] which states that for predominantly N–type ribose conformers $\Sigma 2'' > \Sigma 2'$ ($\Sigma 2'' = J_{1'2''} + J_{2'2''} + J_{2'',3}$ and $\Sigma 2' = J_{1'2'} + J_{2'2''} + J_{2',3'}$).

To confirm that the increase in % N conformer for the sulfamide dinucleosides, upon decreasing temperature, is in part due to an increase in base stacking, the chemical shifts of the modified and parent dinucleotides were monitored as a function of temperature (see $\Delta\delta_H$ table 3). It was apparent that H1', CH_3, H6 and H2 protons of d(TnsnA) showed the most significant change in chemical shift. This can be attributed to these protons being most sensitive to the ring–current magnetic anisotropy effect of purine and pyrimidine bases adopting a base stacked conformation on decreasing temperature.[27, 28] The chemical shift of the H6 proton of the 5'–terminal thmidine of d(TnsnT) was also affected, showing a significant downfield shift (0.13 ppm) on decreasing temperature from 70 to 10 °C, which agrees with the view that H6 is in a deshielded region when stacked with another pyrimidine.[24, 29, 30] On the whole the proton chemical shifts of the parent dinucleoside phosphates were unchanged or comparatively little affected by the same changes in temperature.

It is well established that the electrostatic/hydrophobic attraction between stacked purine and pyrimidine bases is a major stabilising factor in duplex formation.[20, 31] It has also been established that oligonucleotide analogues with structural features that enhance stacking interactions, generally form more stable heteroduplexes with complimentary DNA and RNA and are amongst the more promising candidates for the second generation oligonucleotide therapeutics.[32] The fact that the sulfamide modified dinucleosides more readily adopt stacked conformations than the parent dinucleoside phosphates is therefore very encouraging.

Conformational dependence on concentration.

It is possible that the more marked change in conformation of d(TnsnA) with temperature is due to a greater propensity of the d(TnsnA) to adopt a stacked conformation compared with d(TnsnT), given that the pyrimidine–purine stack is more stable than the pyrimidine–pyrimidine stack.[20, 26] However, d(TnsnA) is self complimentary and therefore potentially capable of self–association through base pairing resulting in an antiparallel miniduplex. In order to determine whether self–association is also a contributing factor to the overall conformation of d(TnsnA), we examined the effect of concentration change on ribose conformation and chemical shift for both modified dinucleosides (Table 4).

Table 4.[a] Conformational change of d(TnsnT) and d(TnsnA) with concentration.

	Σ1'				ΔΣ1'[b] (Hz)	%N[c]		$\Delta\delta_H$[d] H1'	CH_3	H6	H2 (ppm)
Conc. (mM)	0.5	2.0	10	18.5		0.5	18.5				
*T*nsnA	10.2	10.1	9.7	9.5	- 0.7	93	100	–0.04	- 0.14	+ 0.01	
Tnsn*A*	14.0	13.9	13.3	12.8	- 1.2	29	49	–0.03			- 0.17
*T*nsnT	12.0	12.0	11.8	11.8	- 0.2	63	66	–0.01	0	0	
Tnsn*T*	13.3	13.3	13.2	13.2	- 0.1	41	42	0	–0.01	–0.01	

a) Change in Σ1' values, % N conformer of ribose rings and chemical shift with increasing concentration from 0.5 to 18.5 mM. at 30 °C. b) ΔΣ1' = Σ1' (18.5 mM) – Σ1' (0.5 mM). c) % N value determined using sum rule above.[22] d) $\Delta\delta_H = \delta_H$ (18.5 mM) – δ_H (0.5 mM).

On increasing the concentration from 0.5 to 18.5 mM it is found for d(TnsnA) that the conformation of the constituent ribose rings tends toward a higher fraction of the N conformer, whereas little change is apparent for d(TnsnT). Similarly the proton chemical shifts of d(TnsnT) were not affected by concentration, whereas both CH_3 and H2 protons in d(TnsnA) experience noticeable upfield shifts. This suggests that the conformation of d(TnsnA) is affected by self–association. Moreover, the fact that the 3′–terminal ribose conformation is shifted considerably towards the N–conformation might suggest that conformational transmission is taking place due to an increasing population of a A–type miniduplex at higher concentration.

In summary replacement of the phosphodiester linkage of d(TpT) and d(TpA) with sulfamide groups results in a significant increase in the % of N ribose conformer, and an increase in intramolecular base stacking. Both factors are known to contribute to more stable heteroduplexes with RNA, which is the goal of this research. Currently we are engaged in the synthesis of oligonucleotides incorporating sulfamide linkages and wish to establish whether the conformational characteristics observed in modified dinucleosides translate to more stable oligomer duplexes. The results from this work will be published elsewhere.

EXPERIMENTAL

General directions.

^1H and ^{13}C NMR were recorded on Jeol GSX 270, Varian VXR 400, or Bruker DRX500 spectrometers and were indirectly referenced to TMS using residual solvent signal. ^{31}P NMR spectra were recorded on a Jeol GSX 270, with H_3PO_4 as an external standard. FAB mass spectra were obtained on a ZAB–SE VG Analytical Fisons Instrument and MALDI–TOF mass spectra were recorded on a VG TOF SPEC. Flash silica column chromatography was carried out over Merck Kieselgel 60 (230–400 mesh). Reactions involving anhydrous conditions were carried out in flame–dried glassware under a positive pressure of argon. All solvents were distilled before use, reagents were purified and solvents dried using standard procedures.[34]

NMR studies of modified and parent dinucleotides.

d(TpT) and d(TpA) were purchased as ammonium salts from Sigma. Samples were typically dissolved in 0.5 ml of 99.9 % D_2O and a trace of 3–trimethylsilylpropionate–2,2,3,3,–d_4 sodium salt (TSP) was added as an internal reference (set at 0 ppm). Spectra were recorded at 500 MHz on a Bruker DRX500, at 30 °C except where stated for variable temperature experiments. A ^1H spectral width of 5000 Hz was used throughout. 1D spectra were collected using 32k data points with a relaxation delay of 1 second. 2D TOCSY, DQF–COSY and NOESY spectra were collected using 8k data points in t_2 and 256 t_1 increments, typically zero–filled to 8k x 512 data points, with a relaxation delay of 2 seconds. Suppression of residual solvent signal (HDO) was achieved using presaturation during the relaxation delay. NOESY spectra had a typical mixing time of 900 ms. 1D spectra were resolution enhanced by the sine transformation method and 2D data was typically processed with pre–multiplication of the time–domain data by a sine–bell squared function shifted by $\pi/2$.

2-Hydroxyphenyl 3′-*O*-(*tert*-butyldimethylsilyl)-5′-deoxythymidine-5′-*N*-sulfamate (6). Catechol sulfate **5**[15] (870 mg, 5.06 mmol) was added to a solution of 5′–amine **4**[11] (1.19 g, 3.35 mmol) and triethylamine (0.7 ml, 5.03 mmol) in dry DMF (9.0 ml) at 0 °C, under argon. The mixture was allowed to warm to room temperature and stirred for 2.5 h. The DMF was evaporated under reduced pressure and the residue was dissolved in ethyl acetate (10 ml) and washed with brine (3 x 10 ml). The combined brine washings were re-extracted with ethyl acetate (10 ml) and the combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. Purification by column chromatography eluting with a gradient of 0 to 7 % methanol in dichloromethane gave **6** (1.11 g, 63 %) as gum. δ_H (400 MHz, $CDCl_3$) 0.05 and 0.06 (each 3H, s, $SiCH_3$), 0.86 (9H, s, $C(CH_3)_3$), 1.86 (3H, s, CH_3), 2.15 (1H, ddd, *J* 13.5, 7.0, 3.5 Hz $C2'\mathit{H}_aH_b$), 2.61 (1H, ddd, *J* 13.5, 7.4, 6.8 Hz, $C2'H_a\mathit{H}_b$), 3.41–3.53 (2H, m, H5′ and H5′′), 3.94 (1H, m, H4′), 4.45 (1H, m, H3′), 5.80 (1H, dd, *J* 7.0, 6.8 Hz, H1′), 6.63 (1H, brs, SO_2NH), 6.83 (1H, m, Ar), 6.97 (1H, dd, *J* 8.1, 1.5 Hz, Ar), 7.00 (1H, s, H6) 7.11 (1H, m, Ar), 7.25 (1H, dd, *J* 8.1, 1.5 Hz, Ar) and 9.35 (1H, brs, NH); δ_C (68 MHz, $CDCl_3$) –4.9 ($Si(CH_3)_2$), 12.3 (CH_3), 17.9 ($Si\mathit{C}(CH_3)_3$), 25.7 ($SiC(\mathit{C}H_3)_3$), 39.0 (C2′), 45.3 (C5′), 72.1 (C3′), 85.3 and 90.0 (C4′ and C1′), 111.6 (C5), 118.0, 120.6, 123.2 and 128.1 (catechol–CH), 137.6 (catechol–C2), 138.7 (C6), 148.5 (catechol–C1), 150.7 (C2) and 163.9 (C4); *m/z* (FAB+) 528 ($[M+H]^+$, 50), and 402 (100 %); HRMS (FAB^+): calculated for $[M+H]^+$ 528.1836; observed, 528.1850.

2-Hydroxyphenyl N^6-benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′,2′-dideoxyadenosine-5′-*N*-sulfamate (14) was prepared from 5′-amine **13**[16] (400 mg, 0.86 mmol), using the above procedure, (451 mg, 82 %) as a gum. δ_H (270 MHz, $CDCl_3$) 0.10 and 0.11 (each 3H, s, $SiCH_3$), 0.91 (9H, s, $C(CH_3)_3$), 2.25 (1H, dd, *J* 13.5, 6.2, 2.5 Hz, $C2'\mathit{H}_aH_b$), 2.84 (1H, dd, *J* 13.5, 8.0, 6.0 Hz, $C2'H_a\mathit{H}_b$), 3.58 (2H, m, H5′ and H5′′), 4.07 (1H, m, H4′), 4.61 (1H, m, H3′), 6.26 (1H, dd, *J* 8.0, 6.2 Hz, H1′), 6.76 (1H, m, catechol-CH), 6.87 (1H, dd, *J* 8.2, 1.6 Hz, catechol–CH), 7.00 (1H, m, catechol–CH), 7.27 (1H, dd, *J* 8.2, 1.5 Hz, catechol–CH), 7.48–7.64 (3H, m, benzoyl), 8.03 (1H, s, H2), 8.07 (2H, m, benzoyl), 8.41 (1H, s, H8), 9.15 and 9.32 (each 1H, brs, NH); δ_C (68 MHz, $CDCl_3$) -4.9 and –4.8 (2 x $SiCH_3$), 17.9 ($Si\mathit{C}(CH_3)_3$), 25.7 ($SiC(\mathit{C}H_3)_3$), 40.4 (C2′), 45.5 (C5′), 72.9 (C3′), 86.3 and 86.7 (C4′ and C1′), 118.1, 120.4, 123.3 (catechol–CH), 127.9 (C5), 128.1 (catechol–CH), 128.8 (2 x, $C5'H_a\mathit{H}_b$), 3.77 (6H, s, 2 x OCH_3), 4.20 (1H, m, H4′), 4.36 (1H, m, H3′), 6.43 (1H, dd, *J* 5.5, 8.6 Hzbenzoyl), 132.9 and 133.2 (benzoyl), 137.9 (catechol-C2), 142.6 (C8), 148.7 (catechol-C1), 149.5, 150.2 and 152.2 (C2, C4 and C6), and 165.2 (C=O); *m/z* (FAB^+) 663 ($[M+ Na]^+$, 100), 641 ($[M+H]^+$, 40), and 531 (35 %); HRMS (FAB^+): calculated for $[M+H]^+$ 641.2214; observed, 641.2240.

2-Hydroxyphenyl 5′-*O*-(4,4′-Dimethoxytrityl)-3′-deoxythymidine-3′-*N*-sulfamate (10) was prepared from 3′-amino-5′-*O*-Dimethoxytrityl-3′-deoxythymidine **8**[12] (845 mg, 1.55 mmol), using the above procedure, (488 mg, 44 %) as a gum. δ_H (270 MHz, $CDCl_3$) 1.43 (3H, s, CH_3), 2.35–2.50 (2H, m, H2′ and H2′′), 3.36 (1H, dd, *J* 10.6, 2.4 Hz, $C5'\mathit{H}_aH_b$), 3.45 (1H, dd, *J* 10.6, 2.2 Hz, $C5'H_a\mathit{H}_b$), 3.77 (6H, s, 2 x OCH_3), 4.20 (1H, m, H4′), 4.36 (1H, m, H3′), 6.43 (1H, dd, *J* 8.6, 5.5 Hz, H1′), 6.76–7.38 (17H, m, Ar) and 7.55 (1H, d, *J* 1.2 Hz, H6); δ_C (68 MHz, CD_3OD) 12.0 (CH_3), (C2′), 55.5 (C5′), 55.7 (2 x OCH_3), 64.1 (C3′), 85.7, 85.8 and 88.2 ($O\mathit{C}Ar_3$, C4′ and C1′), 111.7 (C5), 114.2, 118.4, 120.7, 124.5, 128.0 128.6, 128.9, 129.5, 131.5, 136.9, 137.4, 139.6, 146.1, 150.8, 152.2 and 160.3 (16 x Ar) and 166.2 (C4); *m/z* (FAB^+) 738 ($[M+ Na]^+$, 45), 716 ($[M+H]^+$, 17), 629 (25), 466 (100 %); HRMS (FAB^+): calculated for $[M+H]^+$ 716.2278; observed, 716.2257.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-deoxythymidinylsulfamido-[3′(*N*)→5′(*N*)]-3′-*O*-(*tert*-butyldimethylsilyl)-5′-deoxythymidine (9). 5′-Sulfamate **6** (331 mg, 0.628 mmol) and 3′-amino-5′-*O*-(dimethoxytrityl)-3′-deoxythymidine **8**[12] (375 mg, 0.691 mmol) were heated under reflux in dry dioxane (3.15 ml) for 4 h, under argon. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography, eluting with 5 % methanol in dichloromethane to give **9** (337 mg, 56 %) as a gum. The title compound **9** was similarly prepared from 3′-sulphamate **10** (56 mg, 78.3 μmol) and 5′-amine **4** (30 mg, 84.5 μmol) in dioxane (1.35 ml) with heating under reflux for 2 h, (62 mg, 68 %). δ_H (400 MHz, CDCl$_3$) 0.07 and 0.08 (each 3H, s, SiCH$_3$), 0.86 (9H, s, C(CH$_3$)$_3$), 1.38 and 1.83 (each 3H, s, CH$_3$), 2.07 (1H, m, C2′*H*$_a$H$_b$), 2.45 (2H, m, H2′ and H2″), 2.83 (1H, m, C2′H$_a$*H*$_b$), 3.22 (2H, m, H5′ and H5″), 3.32 (1H, m, C5′*H*$_a$H$_b$), 3.41 (1H, m, C5′H$_a$*H*$_b$), 3.76 (6H, s, 2 x OCH$_3$), 3.93 (1H, m, H4′), 4.25 (2H, m, H4′ and NHC*H*3′), 4.52 (1H, m, OCH3′), 5.58 (1H, t, H1′), 5.66 (1H, t, H1′), 6.45 and 6.71 (each 1H, m, SO$_2$NH), 6.83 (4H, d, *J* 8.8 Hz, Ar), 6.91 (1H, s, H6), 7.19 - 7.40 (9H, m, Ar), 7.55 (1H, s, H6) and 9.50 (1H, br, N*H*); δ_C (68 MHz, CDCl$_3$) -4.8 and -4.7 (2 x SiCH$_3$), 11.8 and 12.2 (2 x CH$_3$), 17.9 (Si*C*(CH$_3$)$_3$), 25.7 (SiC(*C*H$_3$)$_3$), 38.6 and 38.8 (2 x C2′), 54.7 (HNC5′), 55.3 (2 x OCH$_3$), 60.4 (OC5′), 64.0 and 73.0 (2 x C3′), 77.2, 84.5, 84.7, 86.1 and 87.1 (O*C*Ar$_3$, 2 x C1′ and 2 x C4′), 111.3 and 112.1 (2 x C5), 113.2, 113.4, 127.2, 127.8, 128.0, 128.2, 129.1 and 130.1 (8 x Ar) 135.2 and 135.3 (2 x C6), 144.3 (Ar), 150.8 and 151.2 (2 x C2), 158.8 (Ar), and 163.6 and 163.7 (2 x C4); *m/z* (FAB$^+$) 1093 ([M+Cs]$^+$, 20), 791 (15), 393 (15) and 303 (100 %); HRMS (FAB$^+$): calculated for [M+Cs]$^+$ 1093.2814; observed, 1093.2820.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-deoxythymidinylsulfamido-[3′(*N*)→5′(*N*)]-*N*^{6}-benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′,2′-dideoxyadenosine (15) was prepared using the above procedure from 5′-sulphamate **14** (197 mg, 0.308 mmol) and 3′-amine **8** (185 mg, 0.341 mmol) in dioxane (1.5 ml) with heating under reflux for 4 h, (127 mg, 38 %) as a gum. δ_H (270 MHz, CDCl$_3$) 0.07 and 0.08 (each 3H, s, SiCH$_3$), 0.88 (9H, s, C(CH$_3$)$_3$), 1.37 (3H, s, CH$_3$), 2.20 (1H, m, C2′*H*$_a$H$_b$), 2.39 (2H, m, H2′ and H2″), 3.00 (1H, m, C2′H$_a$*H*$_b$), 3.24–3.47 (4H, m, 2 x H5′ and 2 x H5″), 3.72 and 3.73 (each 3H, s, OCH$_3$), 4.04 (1H, m, H4′), 4.13 (1H, m, H4′), 4.23 (1H, m, NHC*H*3′), 4.63 (1H, m, OCH3′), 5.98 (1H, d, *J* 7.8 Hz, SO$_2$NH), 6.22–6.30 (2H, m, 2 x H1′), 6.78–6.81 (4H, m, Ar), 7.13 - 7.58 (13H, m, Ar), 8.05–8.08 (3H, m, Ar), 8.16 (1H, d, *J* 6.3 Hz, NHSO$_2$), 8.77 (1H, s, H8), 9.49 and 9.71 (each 1H, br, NH); δ_C (68 MHz, CDCl$_3$) -4.8 and -4.7 (2 x SiCH$_3$), 11.8 (CH$_3$), 17.9 (Si*C*(CH$_3$)$_3$), 25.8 (SiC(*C*H$_3$)$_3$), 38.9 and 40.5 (2 x C2′), 45.0 and 53.6 (2 x C5′), 55.2 (2 x OCH$_3$), 63.4 and 73.4 (2 x C3′), 84.3, 84.6, 86.8, 86.9 and 87.0 (O*C*Ar$_3$, 2 x C1′ and 2 x C4′), 111.7, 113.3, 113.5, 124.7, 127.1, 128.0, 128.2, 128.4, 128.6, 130.1, 132.8, 133.2, 135.1, 135.3, 135.4, 142.2, 142.8, 144.3, 150.4, 150.7, 151.2, 151.9, 158.7 and 164.0 (24 x Ar) and 165.2 (C=O, benzoyl); *m/z* (FAB$^+$) 1096 ([M+Na]$^+$, 100), 1074 ([M+H]$^+$, 25), 794 (7) and 540 (9 %); HRMS (FAB$^+$): calculated for [M+Na]$^+$ 1096.4035; observed,1096.4060.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-deoxythymidinylsulfamido-[3′(*N*)→5′(*N*)]-5′-deoxythymidine (16). A solution of protected dinucleoside **9** (88 mg, 91.7 μmol) in THF (1.5 ml) was treated with a solution of TBAF in THF (1M; 0.30 ml, 0.30 mmol) and left to stir for 2 h at room temperature, under argon. Ethyl acetate (5 ml) was added and the solution was washed with 1M aqueous sodium phosphate (NaH$_2$PO$_4$) (3 x 20 ml), dried (MgSO$_4$) and evaporated. Purification by column chromatography eluting with a gradient of 5 to 8 % methanol in dichloromethane gave **16** (77 mg, yield 99%) as a gum. δ_H (270 MHz, CD_3OD) 0.91 and 1.28 (each 3H, s, CH_3), 2.41–2.55 (4H, m, 2 x H2′ and 2 x H2′′), 3.06 (1H, dd, *J* 14.1, 6.1 Hz, C5′H_aH_b), 3.19 (1H, dd, *J* 14.1, 4.2 Hz, C5′H_aH_b), 3.45 (2H, m, H5′ and H5′′), 3.76 (6H, s, 2 x OCH_3), 3.89 (1H, m, H4′), 4.01 (1H, m, H4′) 4.22–4.33 (2H, m, 2 x H3′), 6.14 and 6.21 (each 1H, t, H1′), 6.85 (4H, m, Ar), 7.19 – 7.50 (9H, m, Ar), 7.50 and 7.68 (each 1H, d, *J* 1.2 Hz, H6); δ_C (68 MHz, CD_3OD) 12.0 and 12.4 (2 x CH_3), 39.9 and 40.3 (2 x C2′), 45.7 (HNC5′), 54.1 (OC5′), 55.8 (2 x OCH_3), 64.0 (HNC3′), 72.7 (OC3′), 85.2, 86.1, 86.2, 86.8 and 88.1 (O*C*Ar_3, 2 x C4′ and 2 x C1′), 111.7 and 111.8 (2 x C5), 114.2, 128.1, 128.9, 129.5, 131.4, 131.5, 136.9 (7 x Ar) 137.6 and 138.3 (2 x C6), 146.1 (Ar), 152.2 and 152.3 (2 x C2), 160.3 and 166.4 (2 x C4); *m/z* (FAB^+) 869 ($[M+Na]^+$, 30), 441 (80), 303 (100 %); HRMS (FAB^+): calculated for $[M+Na]^+$ 869.2792; observed, 869.2760.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-deoxythymidinylsulfamido-[3′(*N*)→5′(*N*)]-*N*⁶-benzoyl-5′,2′-dideoxyadenosine (17) was prepared from protected dinucleoside **15** (43 mg, 40 μmol) using the above procedure, (36 mg, 94 %) as a gum. δ_H (270 MHz, CD_3OD) 1.33 (3H, d, *J* 1.0 Hz, CH_3), 2.33–2.46 (3H, m, H2′, H2′′ and C2′H_aH_b), 2.92 (1H, m, C2′H_aH_b), 3.25 (1H, dd, *J* 13.4, 4.3 Hz C5′H_aH_b), 3.34 (1H, dd, *J* 13.4, 4.1 Hz, C5′H_aH_b), 3.41 (2H, m, H5′ and H5′′), 3.72 (6H, s, 2 x OCH_3), 3.98 (1H, m, H4′), 4.16 (1H, m, H4′), 4.29 (1H, m, HNC*H*3′), 4.60 (1H, m, HOC*H*3′), 6.05 (1H, dd, *J* 6.6, 4.8 Hz, H1′), 6.44 (1H, dd, *J* 7.8, 6.3 Hz, H1′), 6.82 (4H, dd, *J* 9.0, 1.2 Hz, Ar), 7.16 – 7.67 (13H, m, Ar–H), 8.08–8.11 (2H, m, Ar–H), 8.44 and 8.69 (each 1H, s, H2 and H8); δ_C (68 MHz, CD_3OD) 12.1 (CH_3), 39.9 and 40.7 (2 x C2′), 45.9 and 53.5 (2 x C5′), 55.8 (2 x OCH_3), 63.5 and 73.3 (2 x C3′), 85.1, 86.3, 87.4, 87.7 and 88.1 (O*C*Ar_3, 2 x C1′ and 2 x C4′), 111.6, 114.3, 128.0, 128.9, 129.5, 129.8, 130.5, 131.4, 133.8, 134.2, 135.0, 136.9, 137.3, 145.0, 146.0, 151.5, 151.9, 152.7, 153.0 153.7 and 160.3 (21 x Ar) and 166.4 (C=O, benzoyl); *m/z* (FAB^+) 982 ($[M+Na]^+$, 5), 701 (100), 651 (35), 629 (25 %); HRMS (FAB^+): calculated for $[M+Na]^+$ 982.3170; observed, 982.3197.

3′-deoxythymidinylsulfamido-[3′(*N*)→5′(*N*)]-5′-deoxythymidine, d(TnsnT) (18). Anhydrous 3% dichloroacetic acid in dichloromethane (200 μl) was added to protected dinucleoside **16** (11 mg, 13.0 μmol). The orange reaction mixture was immediately purified by column chromatography eluting 10 % methanol in dichloromethane to give d(TnsnT) (6.7 mg, 95%) as a gum. δ_C (125 MHz, D_2O) 14.3 and 14.4 (2 x CH_3), 40.1 and 40.9 (2 x C2′), 46.5 (HNC5′), 54.2 (OC5′), 62.9 (HNC3′), 73.6 (OC3′), 86.9, 87.0, 87.8 and 88.3 (2 x C4′ and 2 x C1′), 114.2 and 114.9 (2 x C5), 140.4 and 140.6 (2 x C6), 154.3 and 154.4 (2 x C2), 169.1 and 169.2 (2 x C4); *m/z* (FAB^+): 567 ($[M+Na]^+$, 25), 242 (100), 176 (25 %); HRMS (FAB^+): calculated for $[M+Na]^+$ 567.1485, observed, 567.1460. (For ^{1}H NMR see tables 1 and 2).

3′-Deoxythymidinylsulfamido-[3′(*N*)→5′(*N*)]-2′,5′-dideoxyadenosine, d(TnsnA) (19). Anhydrous 3% dichloroacetic acid in dichloromethane (200 μl) was added to protected dinucleoside **17** (9 mg, 9.40 μmol). The orange reaction mixture was immediately applied to a column of silica gel eluting with 10 % methanol in dichloromethane. The more polar fractions were combined and evaporated under reduced pressure, dissolved in concentrated aqueous ammonia solution (500 μl) and heated to 55 °C for 4h in a sealed Wheaton vial. The reaction mixture was then evaporated under reduced pressure and the residue was purified by column chromatography eluting with 15 % methanol in dichloromethane to give d(TnsnA) **19** (4.3 mg, 83 %) as a gum.

δ_C (125 MHz, D_2O) 14.3 (CH_3), 40.7 and 41.6 (2 x C2′), 45.8 (HNC5′), 52.3 (OC5′), 61.3 (HNC3′), 73.4 (OC3′), 86.8, 87.0, 87.6 and 87.9 (2 x C1′and 2 x C4′), 112.8 (T–C5), 114.9 (A–C5), 139.2 (T–C6), 143.4 (A–C8), 150.9 (A–C4), 153.5 (A–C6), 155.2 (T–C2), 157.8 (A–C2) and 168.4 (T–C4); *m/z* (FAB^+): 554 ($[M+H]^+$, 25), 351 (85), 245 (55), 223 (100 %); HRMS (FAB^+): calculated for $[M+H]^+$ 554.1782; observed, 554.1760. (For ^1H NMR see tables 1 and 2).

5′–*O*–(4,4′–Dimethoxytrityl)–3′-deoxythymidinylsulfamido–[3′(*N*)→5′(*N*)]–3′–*O*–[(2–cyanoethoxy) (*N*,*N*–diisopropylamino) phosphino]–5′–deoxythymidine (20).

A solution of 2–cyanoethyl–*N*, *N*, *N*′, *N*′–tetraisopropylphosphorodiamidite in dry dichloromethane (0.3 M; 150 µl, 45 µmol) was added to a solution of 3′–alcohol **16** (19.6 mg, 23 µmol) and diisopropylammonium tetrazolide (4.0 mg, 23 µmol). This solution was allowed to stand at room temperature for 16 h, under argon then diluted with dichloromethane (2 ml), washed with saturated aqueous sodium hydrogen carbonate solution (2 x 1 ml) and brine (1 ml). The aqueous layers were re–extracted with dichloromethane (2 x 4 ml), and the combined organic solution was dried ($MgSO_4$) and evaporated under reduced pressure. Purification by precipitation three times from hexane (10 ml) at –78 °C gave **20** (19 mg, 79 %) as white powder. δ_H (500 MHz, acetone d_6) 1.20 (12H, m, 2 x $C(CH_3)_2$), 1.48 and 1.80 (each 3H, m, CH_3), 2.34–2.81 (6H, m, CH_2CN, 2 x H2′ and 2 x H2′′), 3.21–3.49 (4H, m, 2 x H5′ and 2 x H5′′), 3.63–3.93 (4H, m, OCH_2CH_2CN and 2 x $NCH(CH_3)_2$), 3.79 (6H, s, 2 x OCH_3), 4.09–4.24 (2H, m, 2 x H4′), 4.31–4.41 (1.5H, m, NHC*H*3′ and OCH3′–diastereomer), 4.62 (0.5H, m, OCH3′–diastereomer), 6.20 (1H, m, H1′), 6.29 (1H, t, H1′), 6.90 (4H, m, Ar), 7.22–7.53 (10H, m, Ar and H6) and 7.62 (1H, 2 x d, H6); ^{31}P NMR (109 MHz, benzene d_6): δ 148.5 and 149.6; *m/z* ($MALDI^+$): 1085 ($[M+K]^+$, 100), 1069 ($[M+Na]^+$, 80 %).

5′–*O*–(4,4′–Dimethoxytrityl)–3′-deoxythymidinylsulfamido–[3′(*N*)→5′(*N*)]–*N*^{6}–benzoyl–3′–*O*–[(2–cyanoethoxy) (*N*,*N*–diisopropylamino) phosphino]–2′,5′–dideoxyadenosine (21).

Using the above procedure (**16** → **20**) 3′–alcohol **17** (6.7 mg, 7.0 µmol) was converted to the title compound **21** (5.3 mg, 65 %) as a white powder. δ_H (500 MHz, acetone d_6) 1.21 (12H, m, 2 x $C(CH_3)_2$), 1.41 and 1.43 (3H, 2 x s, CH_3–diastereomers), 2.41–2.98 (6H, m, CH_2CN, 2 x H2′ and 2 x H2′′), 3.34–3.53 (4H, m, 2 x H5′ and 2 x H5′′), 3.65–3.98 (4H, m, OCH_2CH_2CN and 2 x $NCH(CH_3)_2$), 3.76 (6H, s, 2 x OCH_3), 4.08 (1H, m, H4′), 4.38–4.57 (2H, m, NHC*H*3′ and H4′), 4.84 (1H, m, OCH3′), 6.03 (1H, m, H1′), 6.58 (1H, t, H1′), 6.92 (4H, m, Ar), 7.18–7.68 (13H, m, Ar), 8.21 (2H, m, Ar) 8.44 and 8.73 (each 1H, s, H2 and H8); ^{31}P NMR (109 MHz, benzene d_6): δ 148.4 and 149.6; *m/z* ($MALDI^+$): 1198 ($[M+K]^+$, 100), 1182 ($[M+Na]^+$, 65 %).

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