Synthesis and nucleic-acid-binding properties of sulfamide- and 3′-*N*-sulfamate-modified DNA †

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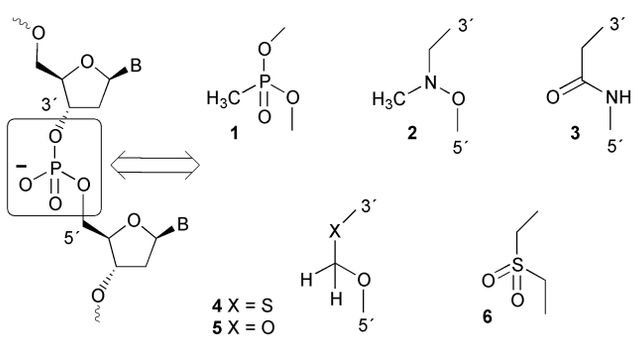
A novel synthetic route for the preparation of sulfamide- and 3′-*N*-sulfamate-modified dinucleosides has been developed. The synthesis utilises 4-nitrophenyl chlorosulfate to prepare 4-nitrophenyl 3′- or 5′-sulfamates (*e.g.*, **18** and **27**), which couple smoothly with the alcohol or amine functionalities of other nucleosides. The conformational properties of the sulfamide- and 3′-*N*-sulfamate-modified dinucleosides d(TnsnT) and d(TnsoT) were compared with the native dinucleotide d(TpT) using NMR and CD spectroscopy. Whilst both modifications result in a shift in the conformational equilibrium of the 5′-terminal ribose rings from C2′-*endo* to a preferred C3′-*endo* conformation, only the 3′-*N*-sulfamate-modified dimer exhibits an increased propensity to adopt a base-stacked helical conformation. Incorporation of the sulfamide- and 3′-*N*-sulfamate modifications into the DNA sequence d(GCGT$_{10}$GCG) allowed the duplex melting temperature to be determined using UV thermal denaturation experiments. This reveals that the sulfamide modification significantly destabilises duplexes with both complementary DNA and RNA. However, the 3′-*N*-sulfamate modification has little effect on duplex stability and even stabilises DNA duplexes at low salt concentration. These results indicate that the 3′-*N*-sulfamate group is one of the most promising neutral replacements of the phosphodiester group in nucleic acids, that have been developed to date, for therapeutic and other important applications.

Introduction

Nucleic acids have been extensively modified by replacing the nucleobase, sugar, phosphodiester linkage or the whole backbone with alternative structures.[1–5] Evaluating the effects of the various structural modifications on the conformation and recognition properties of nucleic acids can provide an alternative perspective from which the structure and function of native DNA and RNA can be better understood. Moreover, if the modifications investigated could potentially have arisen under prebiotic conditions then it is possible to speculate about the origins of the genetic material.[6,7] Modified oligonucleotides have also been developed as antisense or antigene therapeutic agents.[1–3] Similarly, modification strategies can be used to stabilise aptamers[8,9] or ribozymes[10,11] for use *in vivo via* exogenous delivery. Furthermore, modified nucleic acids also have significant potential as diagnostic agents or bio-analytical tools which depend upon hybridisation.[12] For example, novel microarrays have been developed which utilise peptide nucleic acid probes.[13] Here improved stability, affinity and sequence specificity can also be advantageous.

In antisense and antigene research there has been considerable interest in the replacement of the phosphodiester group of oligonucleotides with alternative neutral linkages that are stable to nuclease digestion and increase the lipophilicity of backbone, possibly aiding cellular uptake.[1–3] In addition it was anticipated that introducing neutral linkages would improve affinity for complementary DNA and RNA, by reducing electrostatic repulsion between stands. One of the earliest examples of this class are the methylphosphonate-modified oligonucleotides **1** (Scheme 1).[14,15] Replacement of one of the non-bridging oxygen atoms with a methyl group in **1** results in a chiral phosphorus atom. Although the *R*P-configured methylphosphonates form duplexes which are as stable as isosequential native duplexes, oligonucleotides consisting of a mixture of diastereoisomers have relatively poor affinity for complementary nucleic acids.[14] Despite this, efficient synthetic methodology for the enantioselective synthesis of mixed-



Scheme 1

† Electronic supplementary information (ESI) available: typical melting curves obtained from thermal denaturation of modified oligonucleotides with complementary DNA and RNA. See http://www.rsc.org/suppdata/p1/b1/b110603c/

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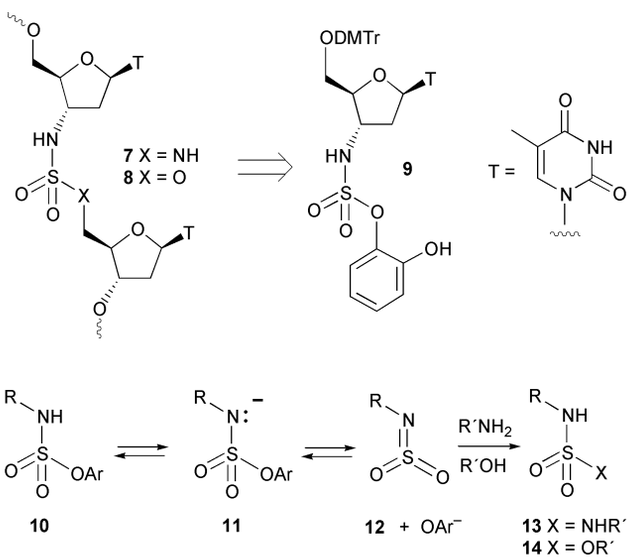
sequence oligomers with all *R*P stereocentres has yet to be developed.[15] Several neutral achiral phosphodiester replacements have also been investigated, including methylene(methylimino) (MMI) **2**,[16] amide **3**,[2] and 3′-thioformacetal **4**,[17] which exhibit good affinity for nucleic acid targets and hold more promise for the development of improved antisense or antigene agents. Other neutral backbone modifications have also been used in the development of oligonucleotides with potentially different modes of therapeutic action. For example, dsDNA possessing an *Eco* RV restriction site was modified with a single neutral dimethylenesulfone linkage **6**.[18] The resulting duplex adopts a bent conformation and functions as a transition-state analogue inhibiting cleavage of the native dsDNA substrate. Extension of this strategy could lead to modified oligonucleotide inhibitors of other enzymes that process native DNA substrates. Furthermore a thrombin binding DNA aptamer, generated by *in vitro* selection, was modified with formacetal linkages **5**.[9] The modified chimeric oligonucleotide aptamer, which adopts a chair-like structure, retains high affinity for the protein target but has considerably improved stability and anticoagulant activity *in vivo*. Finally, methylphosphonate modifications **1** have also been used to probe protein–nucleic acid interactions using a template-directed interference footprinting approach.[19]

Recently we have been involved in the synthesis and evaluation of backbone-modified nucleic acids.[20–22] In particular we have sought to develop modified oligonucleotides with neutral sulfamide **7** and 3′-*N*-sulfamate **8** groups replacing the negatively charged phosphodiester linkage (Scheme 2).[21,22] In



Scheme 2

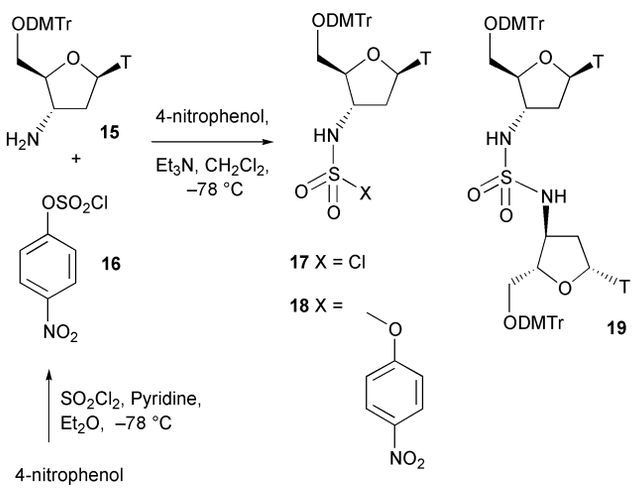
addition to possessing many of the advantageous properties common to other neutral modifications **1**–**6**, these linkages should be easier to prepare and more closely resemble the phosphodiester group both sterically and conformationally. In the case of native nucleic acids anomeric or gauche effects are, at least in part, responsible for the conformational preferences of the phosphodiester P–O_{ester} bonds.[23] Similar stereoelectronic effects are likely to control the conformational properties of the sulfamate and sulfamide linkages in **7** and **8**. In addition the tetrahedral nitrogen atoms, in these linkages, can potentially invert configuration, resulting in different orientations of the N–H bonds.[23] In this paper we describe in detail the novel synthesis of 3′-*N*-sulfamate- and sulfamide-modified oligonucleotides and examine the effects of these modification on the backbone conformation and affinity for complementary DNA and RNA.

Results and discussion

Synthesis of sulfamide- and 3′-*N*-sulfamate-modified dinucleosides

Previously we have shown that the 2-hydroxyphenyl sulfamate **9** (Scheme 2), derived from catechol sulfate, can be coupled with 5′-amino nucleosides to give sulfamide-linked dinucleosides.[22] However, these coupling reactions are slow, requiring heating under reflux in 1,4-dioxane for extended periods and give only moderate yields of sulfamide products. Moreover, treatment of the 2-hydroxyphenyl sulfamate **9** with nucleoside 5′-alcohols in the presence of base fails to give any sulfamate products. Aryl sulfamates **10** are thought to undergo elimination in the presence of base to form *N*-sulfonylamines **12** *via* the anion **11** by an *E*1cB mechanism or directly through an *E*2-type process.[24] The highly electrophilic *N*-sulfonylamine **12** is then trapped by nucleophiles, for example amines or alcohols, resulting in sulfamides **13** or sulfamates **14**, respectively. Given that catechol is a relatively poor leaving group ($pK_{a1} \approx 9.1$)[25] it seems likely that, in the case of the 2-hydroxyphenyl sulfamate **9**, the equilibrium between the sulfamate **10** or the conjugate base **11** and *N*-sulfonylamine **12** lies on the side of the starting material. As a consequence of this only more nucleophilic amines can compete with catecholate, in the reverse reaction, and trap the low concentration of *N*-sulfonylamine to give sulfamide products **13**. With this in mind we reasoned that more reactive aryl sulfamates **10**, sulfamoyl chlorides (RNH-SO_2Cl) or sulfamoyl azides ($RNHSO_2N_3$), would be required as substrates for coupling with alcohols to give sulfamates **14**.

Nucleosides with 5′-sulfamoyl azide groups have been employed previously in the synthesis of 5′-*N*-sulfamate dinucleosides.[26] Nevertheless we chose to avoid this chemistry given that it necessitates the use the explosive reagent sulfuryl chloride azide and yields were low. Also, earlier attempts to synthesise the sulfamoyl chloride **17** (Scheme 3) from 3′-amino

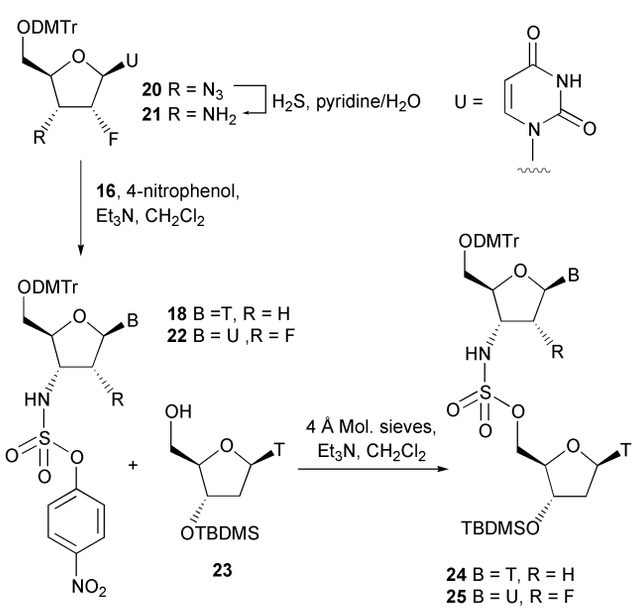


Scheme 3

nucleoside **15** using sulfuryl dichloride had failed. We therefore chose to investigate the reaction of the 3′-amine **15** with 4-nitrophenyl chlorosulfate **16**, anticipating the production of either the sulfamoyl chloride **17** or the 4-nitrophenyl sulfamate **18**. Accordingly, 4-nitrophenyl chlorosulfate **16** was prepared using a modification of an earlier method which involves treating 4-nitrophenol with sulfuryl dichloride and pyridine in diethyl ether at low temperature.[27] The resulting product **16**, is a crystalline solid which is relatively stable and can be safely stored for several months under an inert atmosphere. Although 4-nitrophenyl chlorosulfate **16** has been known since 1948,[28] it has found little practical use in synthesis. Despite this the mechanism of nucleophilic substitution reactions of **16** and other aryl chlorosulfates has been investigated.[29] These studies

show that nucleophilic attack at sulfur can result in either S–Cl or S–OAr bond scission, with S–OAr bond cleavage the major reaction pathway. Yet we found that the reaction of 3′-amine **15** with a slight excess of **16** and triethylamine at room temperature, or at −78 °C, in a variety of different solvents consistently gave the symmetrical sulfamide dimer **19** as the major product (*ca.* 58% yield). A minor amount of the 4-nitrophenyl sulfamate **18** (19% yield) was also produced, but none of the sulfamoyl chloride **17** was ever isolated. It seems likely, if S–OAr bond cleavage is the major reaction pathway, that the sulfamoyl chloride **17** generated *in situ* reacts with the 3′-amine **15** to give the dimer **19**. Presumably the presence of triethylamine, which is necessary to avoid deprotection of the acid-labile dimethoxytrityl group, results in elimination of chloride from the more reactive sulfamoyl chloride **17** to give the *N*-sulfonylamine, which is trapped by the 3′-amine even at −78 °C. Thus the less reactive 4-nitrophenyl sulfamate **18**, which was isolated, is probably derived from the minor reaction pathway involving S–Cl bond cleavage. Attempts to prepare **17** were therefore abandoned. Instead we sought to increase the yield of 4-nitrophenyl sulfamate **18** in order to investigate coupling reactions with the 5′-hydroxy group of other nucleosides. We argued that by adding excess of 4-nitrophenol and triethylamine to the reaction of **15** with **16** it may be possible to intercept any *N*-sulfonylamine with 4-nitrophenoxide, thus preventing formation of the unwanted symmetrical dimer **19**. This proved to be the case and 4-nitrophenyl sulfamate **18** could be prepared in 68% yield, with no dimer **19**, upon addition of 2 equivalents of chlorosulfate **16** to the 3′-amine **15** along with a large excess (10 equivalents) of nitrophenol and triethylamine.

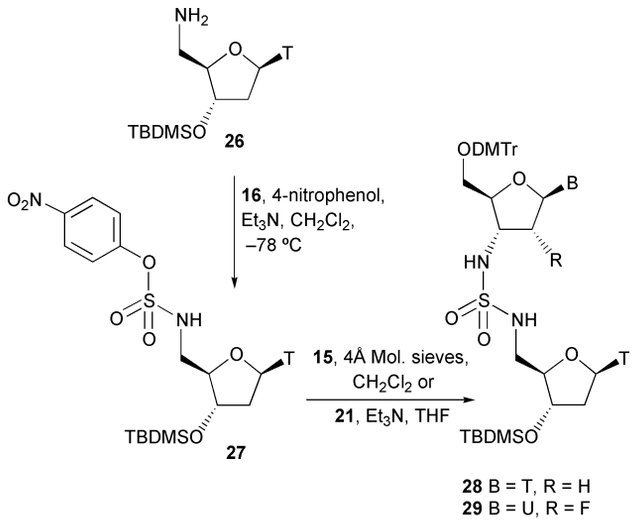
The 4-nitrophenyl sulfamate **18** was then treated with a two-fold excess of 5′-alcohol **23** (Scheme 4) and 5 equivalents



Scheme 4

of triethylamine, resulting in a near quantitative yield of the sulfamate dinucleoside **24** after stirring at room temperature for 12 h. Clearly, nitrophenoxide ($pK_a = 7.1$)[25] is a superior leaving group compared with catecholate and this has a dramatic effect on the rate of nucleophilic substitution. This is consistent with elimination of the aryloxide being the rate-determining step in such reactions.[24] Furthermore, the inclusion of dry 4Å molecular sieves is also critical to the success of this coupling reaction, particularly when performed on a small scale. Without sieves yields were considerably diminished, presumably due to competing hydrolysis and formation of a sulfamic acid side product which was not isolated.

In order to investigate the combined effects of 2′-fluoro as



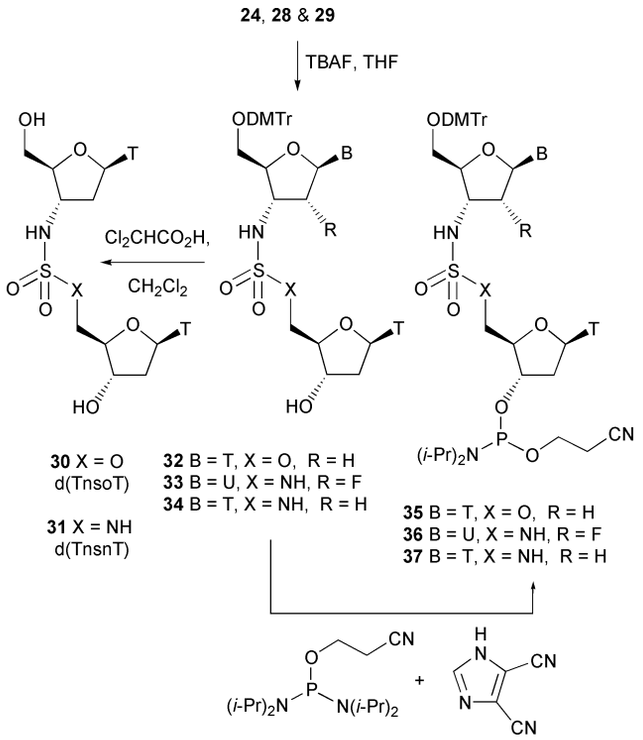
Scheme 5

well as 3′-*N*-sulfamate or sulfamide modifications on DNA duplex stability we also prepared the 3′-azido-2′-fluorouridine derivative **20**.[30] Reduction of the azide to the amine **21** was achieved in near quantitative yield with H_2S in aq. pyridine.[31] Generally we have found this method to be superior, to hydrogenolysis or other methods commonly used in the reduction of 5′- and 3′-azido nucleosides. Sulfomylation of the 3′-amino-2′-fluorouridine derivative **21**, with the 4-nitrophenyl chlorosulfate **16** as described above, was more problematic, resulting in only 59% of sulfamate **22**. For reasons that remain unclear all attempts to couple sulfamate **22** with 5′-alcohol **23** failed to give any 2′-fluoro-3′-*N*-sulfamate dinucleoside **25**. Despite this we were able to prepare the 2′-fluorosulfamide dinucleoside **29** (Scheme 5). This was achieved by sulfonylation of 5′-amino nucleoside **26** with 4-nitrophenyl chlorosulfate **16** (78% yield) to give the 5′-sulfamate **27** followed by coupling with 3′-amino-2′-fluorouridine **21** in the presence of triethylamine (80% yield). Notably, the aminolysis of 4-nitrophenyl sulfamate **27** was complete within 3 h at room temperature in contrast to the rather more vigorous conditions required for aminolysis of the corresponding 2-hydroxyphenyl sulfamate.[22] Similarly, the sulfamide dinucleoside **28** was prepared in 83% yield from 4-nitrophenyl sulfamate **27** and 3′-amine **15**. This was a considerable improvement on the 56% yield obtained previously for the coupling reaction between 3′-amine **15** and the analogous 2-hydroxyphenyl sulfamate.[22] Overall, these findings clearly indicate that 4-nitrophenyl sulfamate nucleosides (*e.g.*, **18** and **27**) are significantly superior building blocks, compared with the 2-hydroxyphenyl sulfamates, for the synthesis of sulfamide- as well as sulfamate-modified dinucleosides.

The synthesis was completed by desilylation of 3′-*N*-sulfamate and 2′-fluorosulfamide dinucleosides **24** and **29**, with TBAF, to give 3′-alcohols **32** and **33** (Scheme 6), which were phosphitylated using 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite and 4,5-dicyanoimidazole resulting in the required phosphoramidites **35** and **36**. These, along with the sulfamide phosphoramidite **37**, which was previously derived from alcohol **34**,[22] were used in the subsequent solid-phase synthesis of modified oligonucleotides. In addition a small portion of the 3′-*N*-sulfamate dinucleoside **32** was detritylated, using dichloroacetic acid, to give the dinucleoside d(TnsoT) **30** which was required for conformational studies.

Conformational properties of sulfamide- and 3′-sulfamate-modified dinucleosides

The conformational properties of the sulfamide dimer d(TnsnT) **31** were previously compared with those of the native dinucleotide d(TpT) using NMR spectroscopy.[22] Here the



Scheme 6

conformational effects of replacing the phosphodiester group of d(TpT) with a 3′-*N*-sulfamate group (**30**) are similarly examined. Accordingly the ^{1}H NMR spectrum of d(TnsoT) **30**, in D_2O, was fully assigned with the aid of COSY and NOESY experiments. From this the vicinal coupling constants $J_{1'2'}$ and $J_{1'2''}$ were then measured at various temperatures. The sum of these coupling constants (Σ1′) were used to determine the approximate extent of the equilibrium, between the northern (*N*) or C3′-*endo* and the southern (*S*) or C2′-*endo* conformation of the sugar rings using the method of Altona.[32] This revealed (Table 1) that the 5′-terminal ribose ring of d(*T*nsoT) exists predominantly in a northern sugar conformation (69% *N* at 30 °C), which was also found to be the case with d(*T*nsnT) (66% *N* at 30 °C). In contrast the 5′-terminal ribose unit of the native dinucleoside phosphate d(*T*pT) preferentially adopts a southern conformation (36% *N* at 30 °C). The observed shift towards a preferred *N*-conformation is normally attributed to a reduction in the gauche effect between the 3′-substituent and the deoxyribose O4′ atom, given that the 3′-amino group in d(*T*nsoT) and d(*T*nsnT) is more electropositive than the 3′-oxygen atom of d(*T*pT),[33–35] although other factors, such as aqueous solvation, may also contribute to this conformational effect.[23] The 3′-terminal ring of d(Tnso*T*) was 44% *N* at 30 °C, which is similar to that observed previously for d(Tnsn*T*) (42% at 30 °C) and slightly higher than for d(Tp*T*) (36% at 30 °C). Furthermore we observed that the % of *N*-conformer for the 5′-terminal ring of d(*T*nsoT) increases upon lowering the temperature from 63% *N* at 70 °C to 78% *N* at 10 °C. A similar increase in % *N* had previously been observed for d(*T*nsnT). This was attributed to a possible increased propensity of the sulfamide modified dinucleoside to adopt a base-stacked conformation at lower temperatures, given that intramolecular base stacking is known to favour the *N*-ribose conformation in dinucleoside phosphates.[36,37]

To further investigate the hypothesis that base stacking occurs in the modified dinucleosides, circular dichrosim (CD) spectroscopy was employed. It is well established that the intensity of the CD signal of di- and oligonucleotides is highly dependent on the proportion of molecules that exist in an helical base-stacked conformation, as opposed to a random conformation.[38,39] Accordingly the CD spectra of d(TnsoT), d(TnsnT) and d(TpT) were recorded across a range of different temperatures (Fig. 1). The spectra for all three dimers were similar with a strong positive band at 280 nm, a strong negative band at 255 nm, and a weak positive band at 225 nm. This suggests that all of the dinucleosides adopt similar overall conformations. In addition for each sample the major bands at 280 and 255 nm showed a significant decrease in intensity as the temperature was increased. This shift is attributed to a change from a predominately base-stacked conformation at lower temperature to a random conformation, with little or no base stacking, at higher temperatures.[39] Significantly the change in the signal intensity of the major bands for d(TnsoT) was twice the magnitude of that observed for the native d(TpT) across a similar temperature range. On the other hand the change in intensity of the bands for d(TnsnT) was very similar to d(TpT). Thus d(TnsoT) appears to have a greater propensity to adopt a base-stacked helical conformation at lower temperature than do either d(TnsnT) or d(TpT). In summary both 3′-*N*-sulfamate

Table 1 Conformation of the sugar rings in 3′-*N*-sulfamate dinucleoside d(TnsoT) **30**, between 70 and 10 °C, compared with the sulfamide dinucleoside d(TnsnT) **31** and the native dinucleotide d(TpT)[22]

	Σ1′ [a]/Hz (%*N*) [b]			
Temp./°C⟶	70	50	30	10
d(*T*nsoT) [c] **30**	12.0 [a] (63) [b]	11.8 (66)	11.6 (69)	11.1 (78)
d(Tnso*T*)	12.9 (47)	13.1 (44)	13.1 (44)	12.7 (51)
d(*T*nsnT) **31**[22]	12.3 (58)	12.1 (61)	11.8 (66)	11.2 (76)
d(Tnsn*T*)	13.4 (39)	13.3 (40)	13.2 (42)	13.1 (44)
d(*T*pT)	13.7 (34)	13.7 (34)	13.6 (36)	13.4 (39)
d(Tp*T*)	13.6 (36)	13.6 (36)	13.6 (36)	13.6 (36)

[a] The sum of the H1′ vicinal coupling constants $\Sigma 1' = J_{1'2'} + J_{1'2''}$. [b] The % of *N*-conformer in the *N*–*S* conformational equilibrium was determined from the Altona sum rule[32] % $S = 100(\Sigma 1' - 9.8)/5.9$. [c] Each row refers to the sugar ring italicised with the 5′- and 3′-terminal sugars indicated to the left and right, respectively.

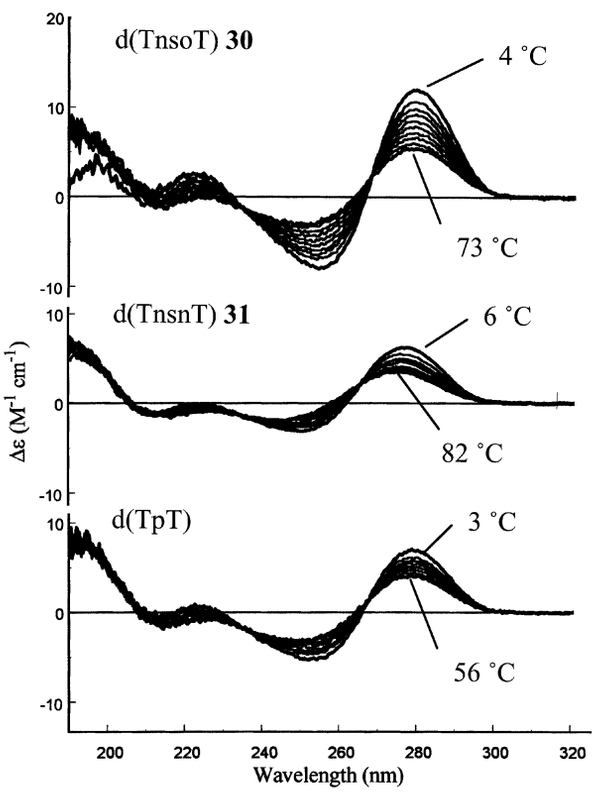


Fig. 1 The effects of changing temperature on the CD spectra of d(TnsoT) **30**, d(TnsnT) **31** compared with that of the native d(TpT).

Table 2 Electrospray mass spectrometry data for native **38** and modified oligonucleotides **39–45**

Oligo.	Electrospray MS/Da (Calc.d)	(Found)
38 d(GCGT$_{10}$GCG)	4875.2	4875.3
39 d(GCGT$_4$TnsoTT$_4$GCG)[a]	4874.3	4874.2
40 d(GCGTnsoTTTTnsoTTTTnsoTGCG)	4872.5	4872.5
41 d(GCGTnsoTTnsoTTnsoTTnsoTTnsoTGCG)	4870.7	4870.6
42 d(GCGT$_4$TnsnTT$_4$GCG)[b]	4873.3	4872.8
43 d(GCGTnsnTTTTnsnTTTTnsnTGCG)	4869.5	4869.7
44 d(GCGT$_4$U^{F}nsnTT$_4$GCG)[c]	4877.3	4876.7
45 d(GCGU^{F}nsnTTTU^{F}nsnTTTU^{F}nsnTGCG)	4881.4	4881.1

[a] A single 3′-*N*-sulfamate nucleoside linkage is indicated by the abbreviation nso. [b] Sulfamide linkage (nsn). [c] 2′-Fluorouridine (U^F).

and sulfamide modification of the dinucleoside phosphate d(TpT) result in a shift towards a preferred C3′-*endo* deoxyribose conformation. However, only the 3′-*N*-sulfamate modification appears to increase the extent with which the bases can adopt a stacked conformation. It is now well established that modifications to oligodeoxynucleotides that increase the proportion of C3′-*endo* deoxyribose rings and increase the propensity of the single strand to base stack are better preorganised into an helical conformation required for the formation of stable hybrid duplexes with complementary RNA as well as DNA.[1] It can therefore be expected that sulfamide- and, particularly, 3′-*N*-sulfamate-modified oligodeoxynucleotides will be well disposed to form duplexes with complementary nucleic acids.

Modified-oligonucleotide synthesis and nucleic-acid-binding studies

To investigate the effects of 3′-*N*-sulfamate and sulfamide modifications on the stability of nucleic acid duplexes we chose to incorporate the modified linkages into the DNA sequence d(GCGT$_{10}$GCG) using the block-synthesis approach. This sequence has been used extensively by others and gives fairly representative results for comparison of the effects of modifications on duplex melting temperatures.[40] Accordingly, chimeric oligodeoxynucleotide 16mers, with between one and five central 3′-*N*-sulfamate (nso) or sulfamide (nsn) linkages **39–43** (Table 2), were synthesised. The synthesis was carried out using an automated DNA synthesiser with standard nucleoside phosphoramidites and the modified dimer phosphoramidites **35** and **37**. The 2′-fluorouridine-modified sulfamide dimer **36** was also used to synthesise oligonucleotides **44** and **45** containing 1 and 3 central 2′-fluorouridine-sulfamide modifications (U^{F}nsnT). Standard phosphoramidite protocols for DNA synthesis were used throughout, with the added precaution of increased coupling times of 15 minutes for the modified dimers. Coupling efficiencies for monomer and modified dimers was in excess of 98% as determined by measuring the release of the dimethoxytrityl cation spectrophotometrically. Following deprotection and cleavage from the solid support, with aq. ethanolic ammonia, the oligonucleotide ammonium salts were purified by RP-HPLC and converted to the sodium forms using an ion-exchange resin. All the modified oligonucleotides were subjected to electrospray mass spectrometry and the observed masses of the parent ions were all in close agreement with the calculated values (Table 2).

Following this the UV absorption at 260 nm *vs.* temperature curves for equimolar mixtures (3 μM strand concentrations) of the native d(GCGT$_{10}$GCG) **38** and modified oligonucleotides (**39–45**) with complementary DNA and RNA (CGCA$_{10}$CGC) were recorded. All mixtures were prepared in phosphate buffer (pH 7.0) adjusted to either 0.02, 0.1 or 1.0 M total sodium ion concentration. Each UV melting curve exhibited typical hyperchromicity, between 10 and 25%, with a single transition consistent with the melting of a duplex to single strands. Typical melting curves obtained from thermal denaturation with complementary DNA and RNA are shown in Fig. 2 and 3, respectively, in the electronic supplementary information that accompanies this paper. †The maximum of the first derivative of these curves was then used to determine the duplex melting temperatures (T_ms) (Table 3). This revealed that modified oligonucleotides with 1 and 3 central sulfamide linkages (**42** and **43**) form less stable duplexes with complementary DNA, exhibiting a drop in T_m per modification (ΔT_m/mod.), compared with the native duplex, of *ca.* −3.2 °C at 0.02 and 0.1 M salt concentration [Na$^+$]. Increasing the salt concentration to 1.0 M Na$^+$ results in a more marked drop in T_m of the sulfamide-modified duplexes, compared with the native duplex, of up to −5.5 °C ΔT_m/mod. Furthermore, 2′-fluorouridine-sulfamide modifications in oligonucleotide **44** and **45** produced an even larger drop in T_m of between −5.8 and −6.4 °C per modification with DNA at 0.1 M Na$^+$. In contrast, oligonucleotides with 1, 3 or 5 sulfamate modifications (**39**, **40** and **41**) formed duplexes with complementary DNA that were moderately more stable than the native duplex at low salt concentration (0.02 M Na$^+$) with an observed increase in T_m of +0.3 °C per modification. At 0.1 M Na$^+$ the 3′-*N*-sulfamate-modified oligonucleotides **39–41** formed duplexes of comparable stability to the native duplex, whilst at higher salt concentration (1.0 M Na$^+$) the T_m dropped by −0.4 °C for one 3′-sulfamate modification.

Table 3 Duplex melting temperatures of 3′-*N*-sulfamate and sulfamide-modified oligonucleotides with complementary DNA and RNA

	T_m[a] (ΔT_m/mod.)[b]/°C			
	DNA[c]			RNA[d]
Oligo.	0.02 M [Na$^+$]	0.1 M [Na$^+$]	1.0 M [Na$^+$]	0.1 M [Na$^+$]
38	43.2[a]	55.0	66.4	49.0
39	43.6[a] (+0.4)[b]	55.1 (+0.1)	66.0 (−0.4)	47.8 (−1.2)
40	44.0 (+0.3)	55.5 (+0.2)	N.D.[e]	48.0 (−0.3)
41	44.8 (+0.3)	53.3 (−0.3)	N.D.	46.0 (−0.6)
42	40.0 (−3.2)	51.7 (−3.3)	60.9 (−5.5)	45.9 (−3.1)
43	33.7 (−3.2)	45.0 (−3.3)	54.6 (−3.9)	41.0 (−2.7)
44	N.D.	49.2 (−5.8)	N.D.	N.D.
45	N.D.	35.8 (−6.4)	N.D.	N.D.

[a] Duplex melting temperature (T_m). [b] Figures in parentheses represent the change in T_m per modification (ΔT_m/mod.) compared with the native duplexes. [c] Data recorded with complementary DNA d(CGCA$_{10}$CGC) at low, intermediate and high salt concentration. [d] Data recorded with complementary RNA r(CGCA$_{10}$CGC) at intermediate salt concentration. [e] N.D.: T_m not determined.

The observed changes in ΔT_m per modification with change in salt concentration for 3′-*N*-sulfamate-modified DNA duplexes can be explained by the reduction in net negative charge of the oligonucleotide backbone upon introduction of neutral linkages. The resulting reduction in electrostatic repulsion between complementary strands will, when all other things are equal, stabilise the duplex that is formed. Clearly this effect will be more significant at low salt concentration when fewer sodium cations are present to mask the negatively charged phosphodiester groups. In the case of the sulfamide modifications (**42** and **43**) any favourable effects due to reduction in backbone negative charge are probably outweighed by an unfavourable change in conformation, which results in a suboptimal structure for formation of a Watson–Crick base-paired duplex. Nevertheless, the destabilisation of the duplex formed between sulfamide-modified oligonucleotides **42** or **43** and DNA is still greatest at higher salt concentration when electrostatic repulsion is less significant.

The sulfamide modifications in oligonugleotides **42** and **43** has a similar effect on duplex stability with complementary RNA at 0.1 M salt concentration, resulting in a drop in T_m of *ca.* −3.0 °C per modification. In the case of the 3′-*N*-sulfamate-modified oligonucleotides the heteroduplexes formed with RNA were slightly less stable than the isosequential modified DNA duplexes. At intermediate salt concentration with 3 and 5 sulfamate modifications a moderate drop in T_m per modification of −0.3 and −0.6 °C, respectively, was observed with a larger drop in T_m of −1.2 °C recorded for one modification. In light of the earlier NMR observations with 3′-*N*-sulfamate dinucleoside d(TnsoT), it seems likely that the more 3′-*N*-sulfamate linkages incorporated into the oligodeoxynucleotide the higher the proportion of C3′-*endo* deoxyribose rings that are present. As a consequence of this, oligonucleotides which are more highly substituted with 3′-*N*-sulfamate groups are more likely to adopt an overall *A*-type conformation which is preferred for binding to complementary RNA. Furthermore we anticipate that oligodeoxynucleotides that are fully modified with, or possess a longer continuous stretch of, 3′-*N*-sulfamate modifications will have greater conformational homogeneity and increased affinity for RNA.

Taken overall these results clearly show that 3′-*N*-sulfamate-modified oligonucleotides **39**–**41** hybridise with both complementary RNA and DNA with similar binding affinity as native DNA. On the other hand the sulfamide congeners **42** and **43** have significantly lower affinity for both complementary nucleic acids. Given that both modifications are neutral and result in a similar shift in the sugar pucker towards a preferred C3′-*endo* conformation it seems that other conformational factors are responsible for the significant differences in affinity. Indeed the additional incorporation of an highly electronegative 2′-fluorine substituent in sulfamide-modified oligonucleotides **44** and **45** would be expected to increase further the bias of the modified sugar rings towards a locked C3′-*endo* conformation. Yet this combination of modifications proved to be most detrimental to the formation of duplexes with complementary DNA. Furthermore CD spectra recorded for both sulfamide and 3′-*N*-sulfamate dinucleosides **30** and **31** indicate that the 3′-sulfamate-modified oligonucleotides are probably better preorganised into a base-stacked helical conformation, in the single stranded state. This would reduce the loss of entropy on binding to complementary RNA and DNA and account for the greater stability of 3′-*N*-sulfamate-modified duplexes. Also the nitrogen atoms in the 3′-*N*-sulfamate and -sulfamide linkages are likely to be tetrahedral in geometry and thus are able to adopt different configurations that will differ in the orientation of the N–H bonds.[23] Given that there are two such nitrogen atoms in the sulfamide linkage and given that the energy barrier to inversion of configuration is likely to be low,[23] it is tempting to postulate that the sulfamide linkage will be intrinsically more flexible than the 3′-sulfamate linkage. If that were the case it would also point towards there being a larger loss of entropy on formation of a duplex with sulfamide modified oligonucleotides.

It is also interesting to note that 3′-*N*-sulfamate-modified oligonucleotides display higher affinity for complementary DNA than do oligonucleotides incorporating isomeric 5′-*N*-sulfamate linkages,[26] with a 5′-amino rather than a 3′-amino substituent. According to an earlier report a DNA duplex with 5′-*N*-sulfamate modifications exhibited in a drop in T_m of −1.5 °C per modification.[26] It is likely that the 5′-amino substituent common to both sulfamide and 5′-*N*-sulfamate modifications is responsible for the destabilisation of duplexes with complementary nucleic acids. A similar but more dramatic effect has been observed with isosteric and isoelectronic phosphoramidate DNA.[35,41] 3′-*N*-Phosphoramidate oligonucleotides form very stable duplexes with complementary DNA and RNA, but the isomeric 5′-*N*-phosphoramidates with the linkage in the opposite orientation are unable to base pair or form duplexes under any conditions.[35,41] A number of explanations have been put forward to account for this. First, modelling studies suggest that the 5′-NH group is less well solvated than the 3′-NH group.[41] Secondly, from X-ray crystallographic data of a 3′-*N*-phosphoramidate-modified duplex, it is postulated that a steric clash between the 5′-amino hydrogen atom and the H2′ atom of an adjacent deoxyribose ring would destabilise a typical *A*-type duplex.[35] Similarly a steric clash of 5′-NH with the pyrimidine H6 or deoxyribose O4′ atoms would destabilise a *B*-type duplex.

Conclusions

Following on from earlier work[22] we have developed an improved synthesis of sulfamide dinucleosides and a new synthetic route for the preparation of 3′-*N*-sulfamate-modified dinucleosides. The synthesis uses 4-nitrophenyl chlorosulfate, a simple yet hitherto little utilised reagent, to prepare nucleosides with 4-nitrophenyl 3′- and 5′-sulfamate groups (**18**, **22** and **27**) which couple smoothly with alcohol or amine functionalities of other nucleosides. Whilst yields are typically high, coupling times with alcohols were in excess of 12 hours. As a result of this it is likely that more reactive aryl sulfamates, for example 2,4-dinitrophenyl sulfamates corresponding to **18**, are required as monomers for future iterative solid-phase synthesis of the most promising 3′-*N*-sulfamate-modified oligonucleotides. Using NMR and CD we have also established the effects of both sulfamide and 3′-*N*-sulfamate modifications on dinucleotide conformation. Notably both modifications result in a shift from a preferred C2′-*endo* to C3′-*endo* sugar conformation, but only the 3′-*N*-sulfamate modification results in an increase in the proportion of base-stacked dimers.

Modified sulfamide and 3′-*N*-sulfamate dinucleoside phosphoramidites were incorporated into a DNA 16mer using the block-synthesis approach. This enabled the effects of both modifications on duplex stability to be determined using UV thermal denaturation experiments. Whilst the sulfamide modification results in a significant destabilisation of duplexes, the 3′-*N*-sulfamate group has little effect compared with the native duplexes and even stabilises DNA duplexes at low salt concentration. Moreover the affinity of 3′-*N*-sulfamate-modified oligonucleotides for complementary DNA and RNA compares favourably with other neutral linkages that have been used to replace the phosphodiester group in oligonucleotides. In fact the methylene(methylimino)- (MMI)[16] **2**, amide-[2] **3** and 3′-thioformacetal-[17] **4** modified oligonucleotides (Scheme 1), which are amongst the best neutral modifications of this type that have been developed, show similar or only moderately higher affinity for complementary nucleic acids.[40] Moreover the chemistry described in this paper indicates that 3′-*N*-sulfamate-modified oligonucleotides can be prepared more easily and economically than some of the other modified oligonucleotides with neutral linkages. Thus 3′-*N*-sulfamate-modified oligonucleotides are worthy of further development for application as antisense or antigene therapeutic agents. In addition the 3′-*N*-sulfamate group is sterically, electronically and probably conformationally more similar to the phosphodiester group than are all the other neutral linkages that have been developed so far. This is reflected in minimal disruption of nucleic acid duplexes as evidenced from the T_m results presented here. As a consequence the 3′-*N*-sulfamate modification may find use as a general phosphodiester replacement for other applications. For example, it might be possible to probe important contacts between the nucleic acid backbones and specific amino acid residues in nucleic acid–protein complexes by selectively replacing phosphodiester groups with the 3′-*N*-sulfamate linkages.[19] Alternatively, aptamers selected *in vitro* for binding to therapeutically relevant protein targets could be stabilised against nuclease digestion by replacing phosphodiester groups, which were not involved in electrostatic contacts

with the protein residues, with 3′-*N*-sulfamate groups.[8,9] Similarly it may be possible to stabilise hammerhead ribozymes using 3′-*N*-sulfamate groups, whilst retaining catalytic activity, for use *in vivo*.[10,11]

Experimental

General procedures

^{1}H NMR spectra were recorded at 270, 400 or 500 MHz. ^{13}C NMR spectra were recorded at 67.9, 100.6 or 125.7 MHz. Chemical shifts are reported in parts per million relative to Me_4Si or residual solvent signal. *J*-values are given in Hz. ^{19}F NMR spectra were recorded at 188.2 MHz unreferenced. ^{31}P NMR spectra were recorded at 161.9 or 202.4 MHz with H_3PO_4 as external standard. FAB mass spectra were obtained on a ZAB-SE VG Analytical Fisons Instrument. IR spectra were acquired on a Perkin-Elmer 783 or 1710 FT instrument for samples as KBr disks or $CHCl_3$ solutions. Melting points were determined using a Cambridge Instrumental microscope with a Reichert-Jung heating mantle and are uncorrected. Flash silica column chromatography was carried out over Merck Kieselgel 60 (230–400 mesh), and Merck Kieselgel 60 F254 0.25 mm plates were used for analytical TLC. 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.[42] Petroleum ether refers to the fraction with distillation range 40–60 °C.

NMR and CD studies of modified and native dinucleotides

1D and 2D NMR spectra for the 3′-*N*-sulfamate-modified dinucleoside d(TnsoT) **30** were recorded at 500 MHz and assigned in an analogous fashion to that described in our earlier paper.[22] CD spectra were measured with either a JASCO 600 or 720 spectropolarimeter complemented with ordinary UV adsorption measurements employing an AVIV 17DS spectrophotometer. Sample temperatures were controlled by a Hewlett-Packard 89090A temperature unit *via* a temperature probe. Samples were dissolved in buffer adjusted to pH 7.0 containing 3.75 mM NaH_2PO_4, 1 mM EDTA and 0.1 M NaCl.

Oligonucleotide synthesis and electrospray mass spectrometry

Solid-phase supports and standard nucleoside phosphoramidites were purchased from Glen Research (Virginia, USA). 0.1 M Phosphoramidite solutions in anhydrous acetonitrile were prepared and oligonucleotides were synthesised automatically on an ABI (Foster City, CA, USA) 392 DNA/RNA synthesiser using standard procedures. 1 μM Syntheses were performed for native and 0.2 μM for oligonucleotides incorporating modified dimers. Coupling wait times were extended to 60 s for native monomer phosphoramidites and 15 min for modified dimer phosphoramidites **35**–**37**. 4,5-Dicyanoimidazole (0.25 M in acetonitrile) was used as the activator. Coupling efficiencies for oligonucleotide synthesis were measured using an on-line dimethoxytrityl cation detector. Final deprotection was carried out using a mixture of conc. aq. ammonia and absolute ethanol (3 : 1 v/v) for 4 hours at 55 °C. Solutions were evaporated to dryness before being reconstituted in Ultrapure™ water and filtered using a Millex®-GS 0.22 μm filter unit. Purification was achieved by HPLC using a Hamilton polystyrene reversed-phase PRP-1 HPLC column (250 × 21.5 mm) and the following elution protocol: Solvent A was 10 mM aq. tetrabutylammonium phosphate (TBAP), pH 7.5; solvent B was 10 mM TBAP, pH 7.5, in MeCN–water (8 : 2, v/v); elution with a convex gradient of 5–95% of solvent B in A in for 40 min at a flow rate of 5 mL/min^{-1}. Fractions containing the oligonucleotide products **38**–**45** were evaporated to dryness and then re-dissolved in Ultrapure™ water and desalted on a Sep-Pak C18 cartridge. The desalted oligonucleotide was then exchanged for the Na^+-form using Dowex® 50WX8 wet ion-exchange resin stirring for 45 min at 4 °C before being filtered and evaporated to dryness. Two ten-fold dilutions were then carried out for each sample, and the absorbance (*A*) was determined using a UV spectrophotometer. Electrospray ionisation mass spectrometry of oligonucleotides was performed on a Q-Tof (Micromass, Altrincham, Manchester, UK). The electrospray capillary potential was 2.5–3.2 kV and the cone was held at 47 V. All samples were introduced *via* constant infusion at 0.5 μL min^{-1} using a syringe driver (Harvard Apparatus). Samples were dissolved in a water–acetonitrile solution (typically 1 : 1 v/v) with 0.1% ammonium acetate. For all infusion experiments a scan rate of 100 *m*/*z*-units per second was used and the data were collected in 'multi-channel analyser' mode. Data were typically accumulated for 2 minutes depending on the signal intensity.

UV thermal denaturation experiments

Phosphate buffer solutions were prepared in doubly distilled water and consisted of 10 mM NaH_2PO_4 (pH 7.0), 0.1 mM EDTA and a total sodium ion concentration of either 0.02, 0.1 or 1.0 M, following addition of NaCl. Buffers for experiments involving RNA were prepared in water pretreated with diethyl pyrocarbonate (DEPC) and heated at 180 °C overnight. For all UV thermal denaturation experiments 9 nmol of each strand was mixed and the solution was evaporated. Samples were then reconstituted in the appropriate buffer (3 mL) and transferred to a 1 cm quartz cuvette containing a stirrer bar. A thin layer of silicone oil was placed over the surface of the sample to eliminate evaporation. Absorbance *vs* temperature profiles were then recorded on an HP845A diode array spectrophotometer with an HP89089A temperature-control unit connected to a Peltier heating block controlled *via* a temperature probe placed directly into sample. Samples were heated at 1.0 °C min^{-1} and absorbance values at 260 nm were collected every 0.1 °C. The first derivative of the absorption *vs.* temperature curve was calculated and used to determine duplex melting temperatures (T_ms). All data acquisition and manipulation was carried out using HP UV Chemstation software.

4-Nitrophenyl chlorosulfate 16[27,28]

A solution of 4-nitrophenol (1.39 g, 10 mmol) and pyridine (0.81 mL, 10 mmol) in Et_2O (10 mL) was added dropwise to a solution of SO_2Cl_2 (0.80 mL, 10 mmol) in Et_2O (10 mL) at −78 °C under argon. The mixture was allowed to warm to room temperature, stirred for 4 h, and then filtered through Celite. The solvent was evaporated under reduced pressure and the resulting orange crystalline solid was recrystallised at −78 °C from a mixture of CH_2Cl_2 and petroleum ether to give **16** (1.82 g) as a low-melting, pale yellow solid. The mother liquor was evaporated and the residue was purified by flash chromatography (CH_2Cl_2–petroleum ether 1 : 1) to give further product **16** (147 mg) in 83% overall yield: ν_{max}(KBr)/cm^{-1} 3080, 3120 (Ar–CH), 1535 (NO_2), 1490 (Ar), 1430 (SO_2), 1350 (NO_2), 1210, 1180, 1150 (SO_2); δ_H (270 MHz; $CDCl_3$) 7.61 (2H, d, *J* 9.2, H2/6), 8.39 (2H, d, *J* 9.2, H3/5); δ_C (67.9 MHz; $CDCl_3$) 122.9 (C2/6), 126.0 (C3/5), 147.5 (C4), 153.5 (C1); *m*/*z* (FAB$^+$) 238 ([M(^{35}Cl) + H]$^+$, 100%), 240 ([M(^{37}Cl) + H]$^+$, 35); HRMS *m*/*z* (FAB$^+$) 237.9590 ([M(^{35}Cl) + H]$^+$, [$C_6H_5ClNO_5S^{35}$]$^+$ requires *m*/*z*, 237.9577).

4-Nitrophenyl 5′-*O*-(4,4′-dimethoxytrityl)-3′-deoxythymidine-3′-sulfamate 18

A solution of 3′-amine **15** (54 mg, 0.10 mmol), 4-nitrophenol (139 mg, 1.00 mmol) and Et_3N (170 μL, 1.22 mmol) in CH_2Cl_2

(0.4 mL) was added dropwise to a solution of chlorosulfate **16** (48 mg, 0.20 mmol) in CH_2Cl_2 (0.1 mL) at −78 °C under argon. After being stirred for 30 min the reaction mixture was allowed to warm to room temperature, diluted with CH_2Cl_2 (5 mL), and washed with 1 M aq. NaH_2PO_4 (3 × 5 mL). The aqueous layers were then re-extracted with CH_2Cl_2 (2 × 5 mL) and the combined organic extracts were dried over $MgSO_4$ and evaporated under reduced. The resulting residue was purified by flash chromatography, eluting with 10% EtOAc in CH_2Cl_2 followed by 1⟶2% MeOH in CH_2Cl_2 to give sulfamate **18** (51 mg, 68%) as a white foam, ν_{max}(KBr)/cm^{-1} 2940–3400 br (NH), 1693 br (C=O), 1530, 1349 (NO_2), 1255, 1180 (SO_2); δ_H (500 MHz; $CDCl_3$) 1.49 (3H, d, *J* 1.1, CH_3), 2.43 (1H, ddd, *J* 13.5, 5.4 and 1.4, H_a2'), 2.50 (1H, ddd, *J* 13.5, 9.4 and 7.3, H_b2'), 3.40 (1H, dd, *J* 10.8 and 2.5, H_b5'), 3.56 (1H, dd, *J* 10.8 and 2.8, H_a5'), 3.78 (6H, s, 2 × OCH_3), 4.34 (1H, ddd, *J* 2.8, 2.5 and 2.0 Hz, H4′), 4.38 (1H, ddd, *J* 7.3, 2.0 and 1.4, H3′), 6.55 (1H, dd, *J* 9.4 and 5.4, H1′), 6.82–6.87 (4H, m, ArH), 7.23–7.33 (9H, m, ArH), 7.37–7.41 (2H, m, ArH), 7.59 (1H, d, *J* 1.1, H6), 8.20 (2H, d, *J* 9.1, ArH), 8.37 and 8.88 (each 1H, br, NH); δ_C (125.7 MHz; $CDCl_3$) 11.8 (CH_3), 37.9 (C2′), 55.3 (2 × OCH_3), 56.1 (C3′), 64.1 (C5′), 84.2, 85.0, 87.5 [C1′, C4′, O*C*(Ar)$_3$], 113.2 (C5), 113.4, 122.6, 125.5, 127.3, 127.8, 128.0, 128.1, 129.1, 130.0, 130.1, 134.5, 135.0, 135.1 (12 × ArC and C6), 144.1, 146.0 (2 × ArC), 151.5 (C2), 154.1, 158.9 (2 × ArC), 162.5 (C4).

Symmetrical dimer 19

In the absence of excess of Et_3N and 4-nitrophenol the reaction of 3′-amine **15** and chlorosulfate **16**, according to the procedure described above, gave the symmetrical dimer **19** as the major product (58% yield), ν_{max}(KBr)/cm^{-1} 2860 br (NH), 1700 br (C=O), 1610, 1510 (Ar), 1255 (SO_2); δ_H (400 MHz; $CDCl_3$) 1.59 (6H, s, 2 × CH_3), 2.45 (4H, m, 2 × H2′/H2″), 3.20 (2H, m, 2 × H_a5'), 3.40 (2H, m, 2 × H_b5'), 3.73 (12H, s, 4 × OCH_3), 3.80 (2H, m, 2 × H3′), 4.08 (2H, m, 2 × H4′), 6.25 (2H, br s, $NHSO_2NH$), 6.40 (2H, m, 2 × H1′), 6.79–7.37 (26H, m, DMTr), 7.56 (2H, 2 × H6), 9.38 (2H, 2 × imide NH); δ_C (100.6 MHz; $CDCl_3$) 12.2 (2 × CH_3), 30.1 (2 × C2′), 55.0 (2 × C3′), 55.6 (4 × OCH_3), 64 (2 × C5′), 85.0 (br, 2 × C1′, 2 × C4′), 87.4 (2 × O*C*Ar$_3$), 113.6, 113.7, 127.5, 128.1, 128.2, 128.4, 129.5, 130.4, 135.6, 144.6 (Ar, 2 × C5, 2 × C6), 151.8 (2 × C2), 159.1 (2 × Ar), 164.0 (C4); *m*/*z* (FAB$^+$) 1172 ([M + Na]$^+$, 35%), 1149 ([M + H]$^+$, 35) and 303 (Ar$_3$C$^+$, 100); HRMS *m*/*z* (FAB$^+$) 1171.4019 ([M + Na]$^+$, $C_{62}H_{64}N_6O_{14}S{\cdot}Na^+$ requires *m*/*z*, 1171.4099).

3′-Amino-5′-*O*-(4,4′-dimethoxytrityl)-2′-fluoro-2′,3′-dideoxyuridine 21

A solution of 2′-fluoro-3′-azide **20**[30] (900 mg, 1.57 mmol) in 60% aq. pyridine (20 mL) was saturated with H_2S gas and left for 18 h at room temperature. CH_3OH (15 mL) was then added and the reaction mixture was filtered through Celite, evaporated under reduced pressure, and purified by flash chromatography, eluting with a gradient of 0⟶3% CH_3OH in CH_2Cl_2, to give 2′-fluoro-3′-amine **21** (851 mg, 99%) as a yellow solid, mp 107–109 °C; δ_H (270 MHz; $CDCl_3$) 3.46 (1H, dd, *J* 11.0 and 2.0 Hz, H_b5'), 3.60–3.69 (2H, m, H3′ and H4′), 3.72 (6H, s, 2 × OCH_3), 3.82 (1H, d, *J* 11.0, H3′), 4.78 (1H, dd, $^2J_{HF}$ 52.4, $^3J_{HH}$ 3.9, H2′), 5.26 (1H, d, *J* 8.4, H5), 5.94 (1H, d, *J* 16.4, H1′), 6.78 (4H, d, *J* 9.0, ArH), 7.17–7.34 (9H, m, ArH), 8.01 (1H, d, *J* 8.4, H6); δ_C (67.9 MHz; $CDCl_3$) 51.8 (d, $^2J_{CF}$ 18.3, C3′), 55.2 (2 × OCH_3), 60.5 (C5′), 83.3 (C4′), 87.0 (*C*Ar$_3$), 88.5 (d, $^2J_{CF}$ 36.7, C1′), 95.9 (d, $^1J_{CF}$ 183.3, C2′), 102.5 (C5), 113.3, 123.7, 127.1, 127.9, 128.1, 130.1, 135.2, 135.3, 135.9, 139.8, 144.3, 149.6, 150.1, 158.7 (12 × ArC, C2, C6), 163.3 (C4); *m*/*z* (FAB$^+$) 680 ([M + Cs]$^+$, 16%), 570 ([M + Na]$^+$, 27), 548 ([M + H]$^+$, 100), 303 (100); HRMS *m*/*z* (FAB$^+$) 548.2187 ([M + H]$^+$, $[C_{30}H_{31}FN_3O_6]^+$ requires *m*/*z*, 548.2197).

4-Nitrophenyl 5′-*O*-(4,4′-dimethoxytrityl)-2′-fluoro-2′,3′-dideoxyuridine-3′-sulfamate 22

A solution of 4-nitrophenyl chlorosulfate **16** (261 mg, 1.10 mmol) in dry CH_2Cl_2 (0.5 mL) was added rapidly to a mixture of 2′-fluoro-3′-amine **21** (275 mg, 0.50 mmol), 4-nitrophenol (696 mg, 5 mmol), Et_3N (0.83 mL, 6 mmol) and activated 4Å molecular sieves (≈ 0.5 g) in dry CH_2Cl_2 (2.5 mL) under argon. After stirring of the mixture at room temperature for 1 h an additional equivalent of 4-nitrophenyl chlorosulfate (119 mg, 0.50 mmol) and Et_3N (70 μL, 0.5 mmol) in dry CH_2Cl_2 (230 μL) was added to the reaction mixture. After a further 2.5 h of stirring at room temperature, CH_2Cl_2 (20 mL) was added and the mixture was washed with 1 M aq. NaH_2PO_4 (3 × 30 mL). The aqueous layers were then re-extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic extracts were dried over $MgSO_4$ and evaporated under reduced. Purification by flash chromatography, eluting with 10% EtOAc in CH_2Cl_2 followed by a gradient of 10⟶50% acetone in CH_2Cl_2, gave 2′-fluoro-3′-sulfamate **22** as a pale yellow foam (154 mg, 59% based on unrecovered starting material **21**), ν_{max}(KBr)/cm^{-1} 3310–2860 br (NH), 1690 (C=O), 1530, 1350 (NO_2), 1255, 1180 (SO_2); δ_H (500 MHz; $CDCl_3$) 3.61 (1H, dd, *J* 11.4 and 2.8, H_b5'), 3.76 (1H, dd, *J* 11.4 and 1.8, H_a5'), 3.78 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 4.12 (1H, ddd, *J* 10.0, 2.8 and 1.8, H4′), 4.73 (1H, ddd, $^3J_{HF}$ 25.2, $^3J_{HH}$ 9.8 and 4.3, H3′), 5.26 (1H, dd, $^2J_{HF}$ 52.0, $^3J_{HH}$ 4.3, H2′), 5.29 (1H, d, *J* 8.2, H5), 5.99 (1H, d, $^3J_{HF}$ 18.6, H1′), 6.82 (4H, m, ArH), 7.23–7.40 (11H, m, ArH), 7.89 (1H, d, *J* 8.2, H6), 8.21 (2H, d, *J* 9.1, ArH), 8.74 (1H, br s, NH); δ_C (125.7 MHz; $CDCl_3$) 53.9 (d, $^2J_{CF}$ 16.9, C3′), 55.2 (OCH_3), 60.2 (C5′), 80.7, 87.5 [C3′, *C*(Ar)$_3$], 89.3 (d, $^2J_{CF}$ 35.4, C1′), 93.3 (d, $^1J_{CF}$ 183.3, C2′), 102.8 (C5), 113.2, 113.3, 122.2, 125.6, 127.3, 128.1, 128.2, 130.2, 130.3, 134.8, 134.9, 140.2, 144.0, 146.1, 149.8, 154.0, 158.8, 158.9 (16 × ArC, C5, C6), 162.7 (C4); δ_F (188.2 MHz; $CDCl_3$) −194.6 (ddd, *J* 52.0, 25.2 and 18.6); *m*/*z* (FAB$^+$) 881 ([M + Cs]$^+$, 100%), 749 ([M + H]$^+$, 75); HRMS *m*/*z* (FAB$^+$) 881.0852 ([M + Cs]$^+$, $C_{36}H_{33}FN_4O_{11}S{\cdot}Cs^+$ requires *m*/*z*, 881.0905).

5′-*O*-(4,4′-Dimethoxytrityl)-3′-deoxythymidine-3′-sulfamoyl-[3′(*N*)⟶5′(*O*)]-3′-*O*-(*tert*-butyldimethylsilyl)thymidine 24

A mixture of sulfamate **18** (32 mg, 0.043 mmol), 5′-alcohol **23** (31 mg, 0.087 mmol) and activated 4Å molecular sieves (≈ 50 mg) in dry CH_2Cl_2 (110 μL) was stirred at room temperature for 1 h in a sealed Wheaton vial under argon. Et_3N (31 μL, 0.22 mmol) was then added and the reaction mixture immediately developed a deep yellow colour. After 12 h the reaction mixture was filtered, and purified by flash chromatography, eluting with 10% EtOAc in CH_2Cl_2 followed by a gradient of 1⟶3% MeOH in CH_2Cl_2, to give the sulfamate dimer **24** (40 mg, 97%) as a white foam, ν_{max}(KBr)/cm^{-1} 3470–3191 br (NH), 1698 (C=O str), 1610, 1510 (Ar), 1275 (Si–C), 1252, 1178 (SO_2); δ_H (500 MHz; $CDCl_3$) 0.07 and 0.08 (each 3H, s, $SiCH_3$), 0.88 [9H, s, $C(CH_3)_3$], 1.42 (3H, d, *J* 1.2, CH_3), 1.76 (3H, d, *J* 1.2, CH_3), 2.21 (1H, ddd, *J* 13.5, 7.3 and 3.9, H_a2' or H_b2'), 2.41 (1H, ddd, *J* 13.5, 9.1 and 7.5, H_b2' or H_a2'), 2.54 (1H, ddd, *J* 13.5, 5.4 and 1.6, H_a2' or H_b2'), 2.65 (1H, ddd, *J* 13.5, 6.7 and 6.7, H_b2' or H_a2'), 3.36 (2H, m, H_a5'/H_b5'), 3.78 (6H, s, 2 × OCH_3), 4.06 (1H, ddd, *J* 5.3, 3.9 and 3.9, H4′), 4.26 (2H, m, H4′ and H_b5'), 4.32 (1H, br s, H3′), 4.35 (1H, dd, *J* 10.7 and 4.0, H_a5'), 4.53 (1H, ddd, *J* 7.7, 3.9 and 3.3, H3′), 5.72 (1H, dd, *J* 6.7 and 6.7, H1′), 6.48 (1H, dd, *J* 9.1 and 5.4, H′), 6.82 (4H, m, ArH), 6.96 (1H, d, *J* 1.2, H6), 7.21–7.31 (9H, m, ArH), 7.51 (1H, d, *J* 1.2, H6), 7.61 (1H, br s, SO_2NH), 9.64 and 9.83 (each 1H, br s, imide NH); δ_C (125.7 MHz; $CDCl_3$) −4.9, −4.8 (2 × $SiCH_3$), 11.7, 12.2 (2 × CH_3), 17.9 [Si*C*(CH$_3$)$_3$], 25.7 [SiC(*C*H$_3$)$_3$], 38.1, 39.1 (2 × C2′), 55.2 (2 × OCH_3), 55.7 (HNC3′), 64.0, 69.0 (2 × C5′), 72.3 (OC3′), 84.2, 84.3, 85.0, 87.2, 90.9 [2 × C4′, 2 × C1′, O*C*(Ar)$_3$], 110.8,

112.5 (2 × C5), 113.3, 127.2, 128.0, 128.1, 130.0, 135.0, 135.1, 135.2, 138.6, 144.2, 150.4, 151.5, 158.8 (9 × ArC, 2 × C2, 2 × C6), 163.5, 163.9 (2 × C4); *m/z* (FAB^+) 984 ($[M + Na]^+$, 52%), 961 (M^+, 17) and 303 (CAr_3^+, 100); HRMS *m/z* (FAB^+) 984.3470 ($[M + Na]^+$, $C_{47}H_{59}N_5O_{13}SSi{\cdot}Na^+$ requires *m/z*, 984.3497).

4-Nitrophenyl 3′-*O*-(*tert*-butyldimethylsilyl)-5′-deoxythymidine-5′-sulfamate 27

A solution of 5′-amine **26** (71 mg, 0.20 mmol), 4-nitrophenol (278 mg, 2.00 mmol) and Et_3N (334 μL, 2.40 mmol) in dry CH_2Cl_2 (0.8 mL) was added dropwise over a period of 5 min to a solution of chlorosulfate **16** (95 mg, 0.40 mmol) in dry CH_2Cl_2 (0.2 mL) at −78 °C under argon. After 1 h the reaction mixture was allowed to warm to room temperature, diluted with CH_2Cl_2 (5 mL), and washed with 1 M aq. NaH_2PO_4 (2 × 25 mL). The aqueous layers were then re-extracted with CH_2Cl_2 (3 × 15 mL) and the combined organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure. Purification by flash chromatography, eluting with EtOAc–petroleum ether (1 : 4⟶1 : 1) gave sulfamate **27** (76 mg, 68%) as a white foam, $\nu_{max}(CHCl_3)/cm^{-1}$ 3675 br (NH), 1696 (C=O), 1521, 1350 (NO_2) 1180 (SO_2); δ_H (270 MHz; $CDCl_3$) 0.04 and 0.06 (each 3H, s, $SiCH_3$), 0.86 [9H, s, $C(CH_3)_3$], 1.85 (3H, d, *J* 0.8, CH_3), 2.17 (1H, ddd, *J* 13.7, 7.0 and 3.9, H_a2'), 2.62 (1H, ddd, *J* 13.7, 6.9 and 6.9, H_b2'), 3.50 (2H, m, H_25'), 3.99 (1H, dd, *J* 7.6 and 3.8, H4′), 4.47 (1H, ddd, *J* 7.4, 3.9 and 3.8, H3′), 5.78 (1H, dd, *J* 7.0 and 6.9, H1′), 7.01 (1H, d, *J* 0.8, H6), 7.43 (2H, d, *J* 9.4, ArH), 8.22 (2H, d, *J* 9.4, ArH); δ_C (67.9 MHz; $CDCl_3$) −4.9, −4.7 [2 × $Si(CH_3)_2$], 12.2 (CH_3), 17.8 [$SiC(CH_3)_3$], 25.6 [$SiC(CH_3)_3$], 39.1 (C2′), 45.1 (C5′), 72.2 (C3′), 85.1, 90.1 (C1′ and C4′), 111.5 (C5), 122.4, 125.4 (ArC), 138.6 (C6), 145.8 (ArC), 150.4 (C2), 154.7 (ArC), 163.6 (C4); *m/z* (FAB^+) 579 ($[M + Na]^+$, 11%), 557 ($[M + H]^+$, 34), 431 ($[M - C_5H_5N_2O_2]^+$, 28), 418 ($[M - C_6H_4NO_3]^+$, 7); HRMS *m/z* (FAB^+) 557.1766 ($[M + H]^+$, $[C_{22}H_{32}N_4O_9SSi]^+$ requires *m/z*, 557.1738).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-fluoro-2′,3′-dideoxyuridin-3′-ylsulfamido-[3′(*N*)⟶5′(*N*)]-3′-*O*-(*tert*-butyldimethylsilyl)-5′-deoxythymidine 29

Et_3N (5 μL, 0.036 mmol) was added to a solution of 2′-fluoro-3′-amine **21** (20 mg, 0.036 mmol) and 5′-sulfamate **27** (29 mg, 0.052 mmol) in dry THF (200 μL) sealed in a Wheaton vial under argon. After 3 h the reaction mixture was purified by flash chromatography, eluting with EtOAc–CH_2Cl_2 (1 : 1), to give sulfamide dimer **29** (28 mg, 80%) as a white solid, mp 135–137 °C; δ_H (400 MHz; CD_3OD) 0.16 (6H, s, 2 × $SiCH_3$), 0.95 [9H, s, $C(CH_3)_3$], 1.90 (3H, d, *J* 1.0, CH_3), 2.18–2.30 (2H, m, T-$H_2$2′), 3.15 (1H, dd, *J* 14.1 and 5.0, T-H_b5′), 3.21 (1H, dd, *J* 14.1 and 4.5, T-H_a5′), 3.59 (2H, m, U-$H_2$5′), 3.80 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.94 (1H, m, T-H4′), 4.14 (1H, m, U-H4′), 4.43 (1H, ddd, $^3J_{HF}$ 27.1, $^3J_{HH}$ 10.6 and 4.0, U-H3′), 4.50 (1H, m, T-H3′), 5.17 (1H, d, *J* 8.0, U-H5), 5.30 (1H, dd, $^2J_{HF}$ 52.0, $^3J_{HH}$ 4.0, U-H2′), 5.98 (1H, d, $^3J_{HF}$ 17.6, U-H1′), 6.22 (1H, dd, *J* 6.5 and 7.5, T-H1′), 6.89 (4H, d, *J* 9.0, ArH), 7.21–7.49 (9H, m, ArH), 7.54 (1H, d, *J* 1.0, T-H6), 8.13 (1H, d, *J* 8.0, U-H6); δ_C (100.6 MHz; CD_3OD) −4.1 (2 × $SiCH_3$), 12.9 (T-CH_3), 19.2 [$SiC(CH_3)_3$], 26.7 [$SiC(CH_3)_3$], 41.1 (T-C2′), 45.5 (T-C5′), 54.4 (d, $^2J_{CF}$ 18.3, U-C3′), 56.2 (2 × OCH_3), 61.9 (U-C5′), 74.3, 82.2, 87.1, 87.3 (T-C3′, U-C4′, T-C4′ and T-C1′), 88.8 [$C(Ar)_3$], 91.5 (d, $^2J_{CF}$ 34.6, U-C1′), 94.7 (d, $^1J_{CF}$ 183.4, U-C2′), 102.9, 112.3 (T-C5 and U-C5) 114.7, 116.9, 127.5, 128.5, 129.4, 129.9, 131.9, 132.0, 136.9, 137.1, 138.7, 142.8, 146.3 (11 × ArC, U-C6 and T-C6), 152.1, 152.8 (2 × C2), 160.6, 160.7 (2 × ArC), 166.4, 166.8 (2 × C4); *m/z* (FAB^+) 987 ($[M + Na]^+$, 71%), 964 ($[M]^+$, 37); HRMS *m/z* (FAB^+) 964.3474 (M^+, $C_{46}H_{57}FN_6O_{12}SSi^+$ requires *M*, 964.3509).

5′-*O*-(4,4′-Dimethoxytrityl)-3′-deoxythymidin-3′-ylsulfamido-[3′(*N*)⟶5′(*N*)]-3′-*O*-(*tert*-butyldimethylsilyl)-5′-deoxythymidine 28[22]

A mixture of sulfamate **27** (50 mg, 0.09 mmol), 3′-amine **15** (98 mg, 0.18 mmol) and activated 4Å molecular sieves (≈ 75 mg) in dry CH_2Cl_2 (225 μL) was sealed in a Wheaton vial under argon. After being stirred for 12 h the reaction mixture was filtered and subjected directly to flash chromatography, eluting with EtOAc–CH_2Cl_2 (1 : 1), to give sulfamide dimer **28** (72 mg, 83%) as a white foam which was identical to a sample that had been prepared by an alternative route.[22]

5′-*O*-(4,4′-Dimethoxytrityl)-3′-deoxythymidin-3′-ylsulfamoyl-[3′(*N*)⟶5′(*O*)]-thymidine 32

A solution of 1 M TBAF in THF (1.68 mL, 1.68 mmol) was added to a solution of sulfamate dimer **24** (538 mg, 0.56 mmol) in dry THF (1 mL) and the resulting mixture was stirred for 5 h. EtOAc (60 mL) was then added and the mixture was washed with 1 M aq. KH_2PO_4 (3 × 40 mL). The aqueous layers were then re-extracted with EtOAc (2 × 50 mL) and the combined organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure. Purification by flash chromatography, eluting with 1⟶5% MeOH in CH_2Cl_2, gave dimer-3′-alcohol **32** (444 mg, 94%) as a white foam, ν_{max}(KBr)/cm^{-1} 3450 (OH), 3196 (NH), 1700 (C=O), 1610, 1510 (Ar), 1254 and 1177 (SO_2); δ_H (500 MHz; acetone-d_6) 1.48 (3H, d, *J* 1.2, CH_3), 1.78 (3H, d, *J* 1.2, CH_3), 2.25 (2H, dd, *J* 6.9 and 5.1, $H_2$2′), 2.55 (1H, ddd, *J* 14.0, 6.7 and 5.2, H_a2′), 2.61 (1H, ddd, *J* 14.0, 8.1 and 6.7, H_b2′), 3.43 (1H, dd, *J* 10.7 and 2.9, H_b5′), 3.45 (1H, dd, *J* 10.7 and 3.7, H_a5′), 4.07 (1H, ddd, *J* 4.8, 3.7 and 3.5, H4′), 4.19 (1H, m, H4′), 4.27 (1H, dd, *J* 10.9 and 4.8, H_b5′), 4.35 (1H, dd, *J* 10.9 and 3.5, H_a5′), 4.40–4.46 (2H, m, 2 × H3′), 6.25–6.30 (2H, m, 2 × H1′), 6.89 (4H, m, ArH), 7.21–7.39 (7H, m, ArH), 7.45 (1H, d, *J* 1.3, H6), 7.49 (2H, m, ArH), 7.59 (1H, d, *J* 1.2, H6); δ_C (125.7 MHz; acetone-d_6) 12.2, 12.5 (2 × CH_3), 38.8, 40.2 (2 × C2′), 55.0 (HNC3′), 55.5 (2 × OCH_3), 64.0, 70.4 (2 × C5′), 71.8 (OC3′), 84.2, 84.7, 85.0, 85.8 (2 × C4′, 2 × C1′), 87.5 [$OC(Ar)_3$], 111.1, 111.2 (2 × C5), 114.0, 127.7, 128.7, 129.0, 131.0 (5 × ArC), 136.3, 136.5, 136.5, 136.6 (2 × ArC, 2 × C6), 145.8 (ArC), 151.2 (2 × C2), 159.7 (ArC), 164.1 (2 × C4); *m/z* (FAB^+) 870 ($[M + Na]^+$, 25%), 847 ($[M + H]^+$, 15); HRMS *m/z* (FAB^+) 870.2650 ($[M + Na]^+$, $C_{41}H_{45}N_5O_{13}S{\cdot}Na^+$ requires *m/z*, 870.2632).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-fluoro-2′,3′-dideoxyuridin-3′-ylsulfamido-[3′(*N*)⟶5′(*N*)]-5′-deoxythymidine 33

A solution of sulfamide dinucleoside **29** (319 mg, 0.331 mmol) in dry THF (3 mL) was treated with 1 M TBAF in THF (1 mL, 1 mmol). After being stirred for 1.5 h the reaction mixture was worked up and purified as described above (**24**⟶**32**) to give the dimer-3′-alcohol **33** (272 mg, 97%) as a white solid, mp 205–208 °C; δ_H (270 MHz; $CDCl_3$) 1.86 (3H, s, CH_3), 2.24 (2H, m, $H_2$2′), 3.10 (1H, dd, *J* 14.1 and 5.1, H_b5′), 3.45 (1H, dd, *J* 14.1 and 3.9, H_a5′), 3.56 (2H, br s, H_25'), 3.75 (6H, s, 2 × OCH_3), 3.87 (1H, dd, *J* 8.8 and 4.5, T-H4′), 4.06 (1H, m, U-H4′), 4.25–4.44 (2H, m, 2 × H3′), 5.14 (1H, d, *J* 7.8, H5), 5.21 (1H, dd, $^2J_{HF}$ 52.0, $^3J_{HH}$ 3.9, U-H2′), 5.94 (1H, d, $^3J_{HF}$ 17.6, U-H1′), 6.12 (1H, t, *J* 6.6, T-H1′), 6.80–6.83 (4H, d, *J* 9.0, ArH), 7.16–7.41 (10H, m, ArH and T-H6), 8.04 (1H, d, *J* 7.8, U-H6); δ_C (67.9 MHz; $CDCl_3$) 11.2 (CH_3), 38.7 (T-C2′), 43.5 (T-C5′), 52.1 (d, $^2J_{CF}$ 18.3, U-C3′), 54.4 (2 × OCH_3), 59.6 (U-C5′), 70.5 (T-C3′), 80.1, 84.0, 85.2, 86.6 (T-C1′, 2 × C4′, $OCAr_3$), 88.9 (d, $^2J_{CF}$ 36.7, U-C1′), 92.5 (d, $^1J_{CF}$ 187.0, U-C2′), 101.4, 110.5 (2 × C5), 112.7, 126.5, 127.3, 127.7, 129.7, 129.8, 134.6, 134.8 (6 × ArC, 2 × C6), 136.4, 140.1, 143.8 (3 × ArC), 149.9 and 150.4 (2 × C2), 158.2, 158.3 (2 × ArC), 163.9, 164.4 (2 × C4); *m/z* (FAB^+) 873 ($[M + Na]^+$, 15%), 850 (M^+, 40); HRMS *m/z*

(FAB$^+$) 873.2549 ([M + Na]$^+$, $C_{40}H_{43}FN_6O_{12}S{\cdot}Na^+$ requires *m/z*, 873.2541).

5′-*O*-(4,4′-Dimethoxytrityl)-3′-deoxythymidin-3′-ylsulfamoyl-[3′(*N*)⟶5′(*O*)]-3′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]thymidine 35

A solution of 0.375 M 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphoramidite in CH_2Cl_2 (1.76 mL, 0.66 mmol) was added to a mixture of 3′-alcohol **32** (335 mg, 0.40 mmol) and 4,5-dicyanoimidazole (47 mg, 0.40 mmol) under argon. After stirring of the mixture for 12 h CH_2Cl_2 (20 mL) was added and the mixture was washed with saturated aq. $NaHCO_3$ (3 × 20 mL) followed by saturated aq. NaCl (20 mL). The aqueous layers were then re-extracted with CH_2Cl_2 (2 × 60 mL) and the combined organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure. Purification by flash chromatography, eluting with 1% Et_3N in CH_2Cl_2 then 1% Et_3N in EtOAc, gave a mixture of phosphoramidite diastereoisomers **35** (307 mg, 74%), as a white powder; ν_{max}(KBr)/cm^{-1} 3190 (NH), 2255 (CN), 1690 (C=O), 1610, 1512 (Ar), 1255, 1180 (SO_2); δ_H (500 MHz; $CDCl_3$) 1.18 [12H, m, 2 × NCH(CH_3)$_2$], 1.46 (3H, br s, 5-CH_3), 1.80 (3H, d, 5-CH_3), 2.36–2.73 (6H, m, CH_2CN, 2 × $H_2$2′), 3.39 (2H, m, $H_2$5′), 3.56–3.64 [2H, m, 2 × NCH(CH_3)$_2$], 3.69–3.75 (1H, m, OCH_aH$_b$CH_2CN), 3.80 (6H, s, 2 × OCH_3), 3.84–3.91 (1H, m, OCH$_a$$H_bCH_2$CN), 4.23–4.29 (2H, m, 2 × H4′), 4.31–4.46 (3H, m, HNC3′H, $H_2$5′), 4.61–4.69 (1H, m, OC3′H), 5.84 and 5.89 (each 0.5H, dd, H1′ diastereoisomers), 6.42–6.47 (1H, m, H1′), 6.92 (4H, d, *J* 8.8, ArH), 7.02 and 7.05 (each 0.5H, s, H6 diastereoisomers), 7.22–7.42 (9H, m, ArH), 7.51 (1H, s, H6); δ_C (100.6 MHz; $CDCl_3$) 11.6, 11.9 (2 × 5-CH_3), 20.5 (*C*H_2CN), 24.7, 25.2 [2 × NCH(*C*H_3)$_2$], 38.3, 38.5 (2 × C2′), 43.2, 43.4, 43.5 [2 × N*C*H(CH_3)$_2$ diastereoisomers], 55.4 (2 × OCH_3 and NC3′), 58.1, 58.3 (O*C*H_2CH_2CN diastereoisomers), 64.1, 69.4 (2 × OC5′), 73.5, 73.6 (OC3′ diastereoisomers), 84.0, 84.4, 87.3, 89.5 [2 × C1′, 2 × C4′, *C*(Ar)$_3$], 111.1, 111.3, 112.5 (2 × C5 diastereoisomers), 113.5 (ArC), 118.2 (CN), 127.3, 128.2, 130.2 (4 × ArC), 135.1, 135.3, 135.4 (2 × C6, ArC), 144.4 (ArC), 150.7, 151.5 (2 × C2), 158.9 (ArC), 163.6, 163.9, 164.1 (C4 diastereoisomers); δ_p (161.9 MHz; $CDCl_3$) 149.2, 149.9 (P diastereoisomers); *m/z* (FAB$^+$) 1086 ([M + K]$^+$, 55%), 303 (CAr_3^+, 100); HRMS *m/z* (FAB$^+$) 1086.3446 ([M + K]$^+$, $C_{50}H_{62}N_7O_{14}PS{\cdot}K^+$ requires *m/z*, 1086.3450).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-fluoro-2′,3′-dideoxyuridin-3′-ylsulfamido-[3′(*N*)⟶5′(*N*)]-3′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-5′-deoxythymidine 36

A solution of 0.375 M 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphoramidite in CH_2Cl_2 (0.895 mL, 0.34 mmol) was added to a mixture of 3′-alcohol **33** (168 mg, 0.197 mmol) and 4,5-dicyanoimidazole (23 mg, 0.197 mmol). After stirring of the solution for 12 h CH_2Cl_2 (10 mL) was added and the mixture was worked up and purified as described above (**32**⟶**35**) to give a mixture of phosphoramidite diastereoisomers **36** (102 mg, 49%) as a white powder, ν_{max}(KBr)/cm^{-1} 3240 (NH), 2260 (CN), 1690 (C=O), 1610, 1512 (Ar), 1255, 1180 (SO_2); δ_H (500 MHz; $CDCl_3$; 323 K) 1.12–1.20 [12H, m, 2 × NCH(CH_3)$_2$], 1.82 (3H, br s, CH_3], 2.37–2.70 (4H, m, CH_2CN, $H_2$2′), 3.40–3.65 [6H, m, 2 × $H_2$5′ and 2 × NCH(CH_3)$_2$], 3.72 (7H, m, 2 × OCH_3 and OCH_aH$_b$CH_2CN), 3.88 (1H, m, H4′), 4.00 (2H, m, NHC3′H, OCH$_a$$H_bCH_2$CN), 4.38 (1H, br d, H3′), 4.70 (1H, br d, H3′), 5.21 (1H, m, H5), 5.26 (1H, d, $^2J_{HF}$ 52, H2′), 5.93 (1H, m, H1′), 6.15 (1H, m, H1′), 6.82 (4H, d, ArH), 7.05–7.45 (9H, m, ArH), 7.87 (1H, m, H6); δ_C (100.6 MHz; $CDCl_3$; 298 K) partial spectrum 12.5 (5-CH_3), 20.4 (*C*H_2CN), 24.6 [NCH(*C*H_3)$_2$], 43.2, 43.3 [2 × N*C*H(CH_3)$_2$, 45.8 (T-C5′), 52.4 (U-C3′), 55.2 (2 × OCH_3), 57.8, 59.8 (O*C*H_2CH_2CN and U-C5′), 80.9, 83.9, 87.2, 88.9 (T-C3′, U-C4′, T-C4′, T-C1′, *C*Ar$_3$), 102.1 (C5), 113.7, 127.5, 128.4, 128.6, 130.5, 130.7, 134.5, 144.2, 150.4, 159.0, 159.1, 162.7 (ArC); δ_p (202.4 MHz; $CDCl_3$; 287 K) 149.2, 150.8 (P diastereoisomers); *m/z* (FAB$^+$) 1089 ([M + K]$^+$, 90%), 303 (CAr_3^+, 100); HRMS *m/z* (FAB$^+$) 1089.3377 ([M + K]$^+$, $C_{49}H_{60}N_8O_{13}FPS{\cdot}K^+$ requires *m/z*, 1089.3359).

3′-Deoxythymidin-3′-ylsulfamoyl-[3′(*N*)⟶5′(*O*)]-thymidine d(TnsoT) 30

3% $CHCl_2CO_2H$ in dry CH_2Cl_2 (0.5 mL) was added to the 3′-alcohol **32** (35 mg, 0.041 mmol) under argon. The orange reaction mixture was purified immediately by flash chromatography, eluting with a gradient of 0⟶10% MeOH in CH_2Cl_2, to give d(TnsoT) **30** (17.5 mg, 78%) as a white foam, ν_{max}(KBr)/cm^{-1} 3420 (OH), 3211 (NH), 1691 (C=O), 1275, 1180 (SO_2); δ_H (500 MHz; D_2O; 303 K) 1.84 (3H, s, CH_3 5′-Tnso*T*-3′), 1.85 (3H, s, CH_3 5′-*T*nsoT-3′), 2.40 (2H, m, $H_2$2′ Tnso*T*), 2.45–2.50 (2H, m, $H_2$2′ *T*nsoT), 3.78 (1H, dd, *J* 12.8 and 3.7, H_b5′ *T*nsoT), 3.91 (1H, dd, *J* 12.8 and 2.7, H_a5′ *T*nsoT), 3.99–4.06 (2H, m, H3′ and H4′ *T*nsoT), 4.19 (1H, ddd, *J* 4.9, 3.8 and 2.6, H4′ Tnso*T*), 4.39 (1H, dd, *J* 11.1 and 3.8, H_b5′ Tnso*T*), 4.46 (1H, dd, *J* 11.1 and 2.6, H_a5′ Tnso*T*), 4.58 (1H, ddd, *J* 5.7, 5.7 and 4.9, H3′ Tnso*T*), 6.07 (1H, dd, *J* 6.5 and 5.1, H1′ *T*nsoT), 6.28 (1H, dd, *J* 6.5 and 6.5, H1′ Tnso*T*), 7.51 (1H, s, H6 Tnso*T*), 7.68 (1H, s, H6 *T*nsoT); δ_C (125.7 MHz; D_2O) 11.5, 11.6 (2 × CH_3), 37.1, 38.3 (2 × C2′), 51.8 (NHC3′), 59.6, 68.7 (2 × C5′), 69.5 (OC3′), 82.9, 83.8 (2 × C4′), 85.0 (2 × C1′ coincident), 111.0, 111.3 (2 × C5), 137.0, 137.1 (2 × C6), 151.1, 151.4 (2 × C2), 165.8, 166.1 (2 × C4); *m/z* (FAB$^+$) 568 ([M + Na]$^+$, 35%); HRMS *m/z* (FAB$^+$) 568.1350 ([M + Na]$^+$, $C_{20}H_{27}N_5O_{11}S{\cdot}Na^+$ requires *m/z*, 568.1325).

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