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Synthesis and Structure of *S*-Nucleosidyl *S*-Aryl Disulfides and their Reaction with Phosphites

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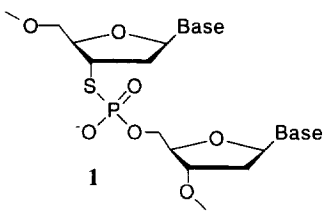
Abstract: A general procedure for the preparation of *S*-nucleosidyl *S*-aryl disulfides from the corresponding thioesters is described. This procedure has been used for the preparation of a ribonucleoside disulphide (**8d**), a key intermediate for the synthesis of oligoribonucleotides containing 3'-*S*-phosphorothiolate linkages. The regioselectivity of the Arbusov reaction of **8d** with phosphites has been examined. The X-ray structure of 3'-deoxy-3'-*S*-(2-nitrophenyldisulfanyl)thymidine (**9b**) is also reported.

INTRODUCTION

The chemistry of *S*-nucleosidyl *S*-aryl disulfides derived from thiosugars has not been widely investigated, and these compounds have received very little attention in the literature.[1, 2] However, we have shown that *S*-nucleosidyl *S*-nitrophenyl disulfides are excellent substrates for Michaelis-Arbusov reactions with phosphites and this reaction represents the best procedure for the preparation of 3'-*S*-phosphorothiolate-containing nucleotides (*e.g.* **1**).[3, 4] Because these analogues are being increasingly used for the study of recognition processes that occur between nucleic acids and their processing enzymes,[5, 6, 7] it has become essential to optimise the preparation of these synthetically challenging compounds. To date, our systematic investigation of this type of Michaelis-Arbusov reaction has concentrated mainly on the scope and reactivity of the phosphite component of the reaction.[4] We now wish to report more thoroughly on the synthesis, structure and reactivity of *S*-nucleosidyl *S*-aryl disulfides, and in particular draw attention to an efficient and general method for their preparation.



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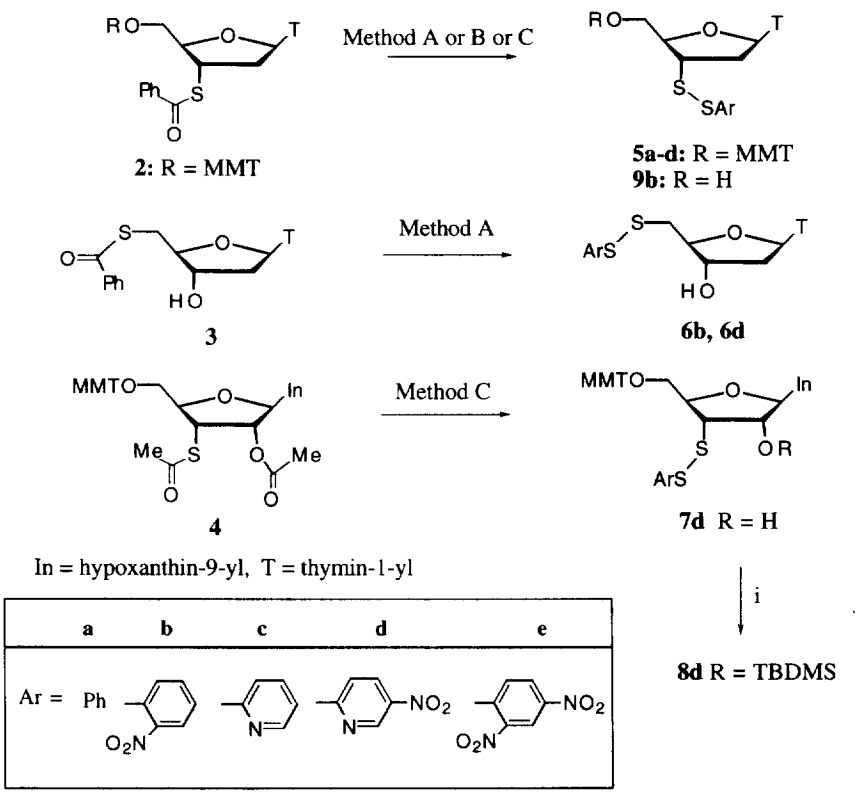
RESULTS AND DISCUSSION

Previously we have prepared *S*-nucleosidyl *S*-aryl disulfides by sulfenylation of an isolated thionucleoside with the appropriate sulfenyl chloride. However, this strategy has two potential disadvantages: (i) the thionucleosides are susceptible to oxidation, and can undergo conversion to their corresponding symmetrical disulfides during isolation and storage; (ii) arenesulfenyl chlorides are known to react with the heterocyclic base of pyrimidine nucleosides to produce either the *N*-3-arenesulfenylated product or a *C*-5 substituted product.[8] Additionally, the arenesulfenyl chlorides react efficiently with unprotected hydroxyl groups to produce sulfinate esters.[9] To avoid these problems we chose to investigate a one-pot procedure in which the thionucleoside would be generated *in situ* from a precursor thioester in the presence of a mild sulfenylating agent. Aryl disulfides, such as 2,2'-dithiodipyridine, appeared to be ideal sulfenylating reagents since they react very specifically with thiol groups and have been widely used to quantify and derivatise thiol functions.[10]



Scheme 1 General procedures for the preparation of *S*-nucleosidyl *S*-aryl disulfides. *Reagents and conditions*: Method A- 20% *n*-$BuNH_2$ in DCM, diaryldisulphide; Method B- K_2CO_3 sat. MeOH/H_2O, diaryldisulphide; Method C- sequential treatment with K_2CO_3 sat. MeOH/H_2O or NaOH in EtOH/H_2O, neutralisation with AcOH, then diaryldisulphide in DCM; (i) *t*-butyldimethylsilyl triflate, pyridine.

For the preparation of the 2'-deoxynucleoside disulfides (**5** and **6**) two reagents were initially examined for the *in situ* generation of the thiol, either, a 20% solution of *n*-butyl amine in CH_2Cl_2 (Method A), or a saturated solution of K_2CO_3 in methanol/H_2O (Method B); in each case the reaction contained 3 - 5

equivalents of the diaryl disulfide (**Scheme 1**). Reactions were complete within 2 hr and the isolated yields of the *S*-nucleosidyl *S*-aryl disulfides were generally better than 70% (see **Table 1**). Under both sets of reaction conditions no products resulting from sulfenylation of the nucleobase were obtained, although small quantities of the symmetrical nucleoside disulfides were isolated. It was noted that the nitro-containing diaryl disulfides were very insoluble in methanol and these sulfenylating agents could not be used in Method B. Due to the extreme insolubility of bis(2,4-dinitrophenyl) disulfide it has proved impossible to prepare *S*-nucleosidyl *S*-dinitrophenyl disulfides [*e.g.* (**5e**)] using this reagent.

In the preparation of the ribonucleoside disulphide (**7d**) poor yields were sometimes obtained using Method B, due to slow removal of the 2'-*O*-acetyl group. The best yields of **7d** were obtained using Method C, in which sulfenylation of the thionucleoside was accomplished after neutralisation of the basic solution with acetic acid and removal of the solvents. It was noted that **7d** underwent slow decomposition to give the symmetrical dinucleoside disulfide and 2-mercapto-2-nitropyridine. This instability was not observed with the 2'-deoxynucleoside disulfides, and it is possible that in the ribose derivative, decomposition is facilitated by proton transfer from the 2'-hydroxyl group to the pyridyl nitrogen atom. Silylation of **7d**, using TBDMS triflate in pyridine, gave the fully protected disulfides **8d** in about 88% yield. Once silylated, the ribonucleoside disulfide was not readily decomposed.

Thioester	Aryl Disulfide	Method	Product	Yield (%)
2	Phenyl disulfide	B	**5a**	63
2	2-nitrophenyl disulfide	A	**5b**	73
2	2,2'-Dithiodipyridine	A	**5c**	85
2	2,2'-Dithiobis(5-nitropyridine)	C	**5d**	70
3	2-nitrophenyl disulfide	A	**6b**	74
3	2,2'-Dithiobis(5-nitropyridine)	A	**6d**	73
4	2,2'-Dithiobis(5-nitropyridine)	C	**7d**	85

Table 1 Synthesis of *S*-nucleosidyl *S*-aryl disulfides from the corresponding nucleoside thioesters.

The 2-nitrophenyl-containing disulfides proved to be particularly crystalline. After removal of the monomethoxytrityl group and crystallisation from cyclohexane-dichloromethane, (**9b**) was obtained as yellow prisms and the structure was determined by X-ray crystallographic analysis (**Figure 1**).[11] The structure of this type of nucleoside analogue has not previously been determined. The sugar adopts a *C*-2'-endo, *C*-3'-exo conformation with these two atoms being displaced respectively by 0.40Å above and 0.19Å below the C1'-O4'-C4' plane. The O5'-C5'-C4'-C3' torsion angle is 59° which places O5' directly above the sugar in the standard gauche, gauche conformation. This nucleoside also adopts the frequently observed anti conformation about the glycosidic bond with a O4'-C1'-N1-C6 torsion angle of 53°. The C3'-S-S-C dihedral angle of 85.4° suggests that internucleoside disulfide linkages maybe useful phosphate isosteres.

In studying the reactivity of 3'-*S*-nucleosidyl *S*-aryl disulfides, the Arbusov reaction with phosphites is of particular interest since it provides the most general and efficient route to 3'-*S*-phosphorothiolate nucleotides. Of the 3'-*S*-nucleosidyl *S*-aryl disulfides that we have previously prepared, the dinitrophenyl

disulfide (**5e**) was shown to be the most reactive. However, in comparative reactions with P(OMe)3 (3 equivalents in THF at room temperature) the nitropyridyl disulfide (**5d**) was significantly more reactive (reaction complete in 2-3 hours) than the dinitrophenyl disulfide (reaction complete in about 12 hours). Under identical conditions complete reaction with the pyridyl disulfide (**5c**) took about 48 hours.

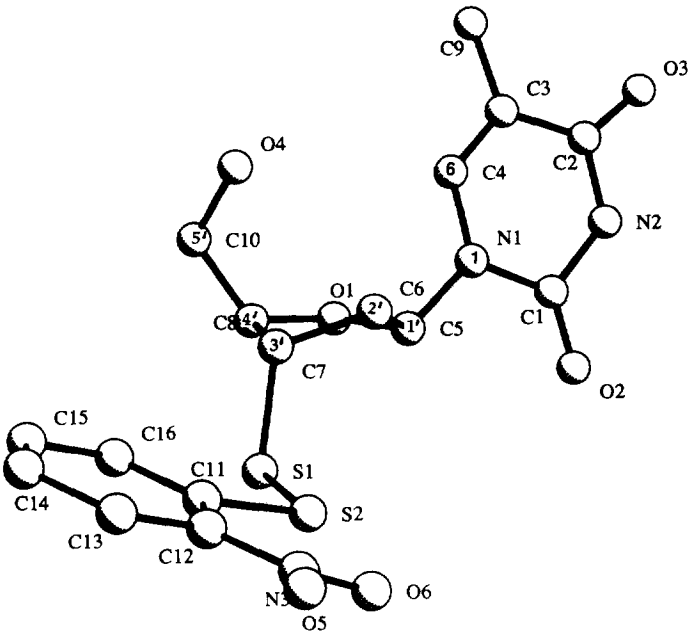
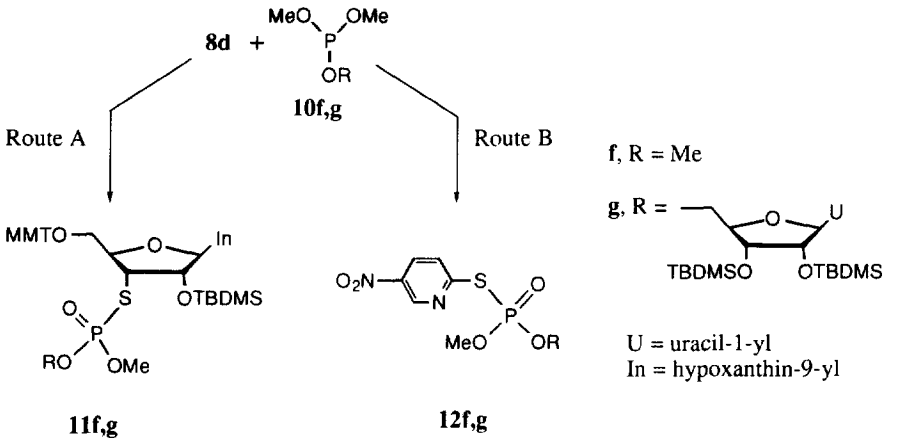


Figure 1 X-ray structure of 3'-Deoxy-3'-*S*-(2-nitrophenyldisulfanyl)thymidine (**9b**).

Since it is our intention to apply this Arbusov reaction in a repetitive manner to the automated synthesis of oligonucleotides containing multiple phosphorothiolate linkages, it is important that this reaction proceeds with very high regioselectivity (Route A, **Scheme 2**). If attack of the phosphite occurs at the aryl sulfur atom (as in route B **Scheme 2**), even to only a minor extent, it will lead to significant chain termination over multiple cycles of oligomer synthesis. It seemed probable that the regioselectivity would be least satisfactory in reactions involving the ribonucleoside disulfide (**8d**) where the sterically demanding TBDMS group could hinder attack at the 3'-sulfur atom. The regioselectivity of the reaction between P(OMe)3 (**10f**) and the nucleoside disulfide (**8d**) was therefore invesigated by ^{31}P NMR spectroscopy. The spectrum of the crude reaction mixture showed the presence of only one P-S-containing species, corresponding to the dimethyl ribonucleoside phosphorothiolate [**11f**, δ (^{31}P) = 29.9 ppm]. No resonance corresponding to the dimethylpyridyl phosphorothiolate [**12f**, δ (^{31}P) = 22.2 ppm] was observed (detection limit about 2%). However, when the more sterically hindered 2',3'-di-*O*-(tert-butyldimethylsilyl)uridin-5'-yl dimethyl phosphite (**10g**) was used as the nucleophile the reaction was less regioselective. Analysis by ^{31}P NMR spectroscopy revealed that the crude reaction mixture contained between 2-10% of the pyridyl phosphorothiolate [**12g**, δ (^{31}P) = 20.0, 20.2 ppm] in addition to the desired diribonucleoside phosphorothiolate [**11g**, δ (^{31}P) = 27.7 ppm]. The best regioselectivity was achieved using only one equivalent of the nucleoside phosphite.



Scheme 2

CONCLUSIONS

Our results show that a range of *S*-nucleosidyl *S*-aryl disulfides can be prepared in good yield in a one-pot procedure from the corresponding thioesters, using aryl disulphides as sulfenylating agents. The method is compatible with purine and pyrmidine nucleosides, and does not require protection of the hydroxyl groups. The procedure is also complementary to that reported by Reese and coworkers[1,2] which is used to prepare *S*-nucleosidyl *S*-aryl disulfides from thioethers using sulfenyl chlorides under acidic conditions. In particular, the 2-thio-5-nitropyridyl disulphides react rapidly with phosphites and the reaction generally shows good regioselectivity. We believe this reaction provides the method of choice for preparing a range of phosphorothiolate analogues. The ribonucleoside disulphide **8d** is a key intermediate for the synthesis of oligoribonucleotides containing 3'-*S*-phosphorothiolate linkages and phosphorothiolate analogues of cAMP and its use for these purposes is currently being explored.

EXPERIMENTAL

FAB mass spectra were recorded on a VG Analytical 7070E mass spectrometer operating with a PDP 11/250 data system and an Ion Tech FAB ion gun working at 8 Kv. High resolution FAB mass spectra were obtained on a VG ZAB/E spectrometer at the EPSRC Mass Spectrometry Service Centre (Swansea U.K.) and reported masses are accurate to ± 5 ppm. 3-Nitrobenzyl alcohol was used as a matrix for all FAB spectra. ^{1}H NMR spectra were measured on either a Bruker AMX400 or Bruker AC200 spectrometer and chemical shifts are given in ppm downfield from an internal standard of tetramethylsilane. ^{31}P NMR and spectra are referenced to 85% phosphoric acid.

General Procedures for *S*-Nucleosidyl *S*-Aryl Disulfides

Method A.- The thiobenzoate and diaryl disulfide (5 eq) were dissolved in DCM and *n*-$BuNH_2$ added so that the solution contained 20% *n*-$BuNH_2$. The reaction was stirred for between 30 min and 2 hours and the solvent evaporated *in vacuo*. In some cases excess diaryl disulfide was removed by dissolving the residue in a small amount of DCM and filtering off the insoluble diaryl disulfide. The crude product was purified by

silica gel column chromatography eluting with a gradient of MeOH in DCM. Appropriate fractions were pooled and evaporated *in vacuo* to give the product.

Method B.-The thiobenzoate and diaryl disulfide (5 eq) were dissolved in a saturated solution of K_2CO_3 in MeOH : H_2O (95 : 5, approximately 20 mL/g of nucleoside) (20-30 ml). The reaction was stirred for 2 hours and the solvent evaporated *in vacuo*. The residue was partitioned between DCM and $NaHCO_3$. The organic phase was washed with water, dried (Na_2SO_4), filtered and evaporated *in vacuo*. The crude product was purified by silica gel column chromatography eluting with a gradient of MeOH in DCM. Appropriate fractions were pooled and evaporated *in vacuo* to give the product.

Method C.-The thioester was dissolved in either a saturated solution of K_2CO_3 in MeOH : H_2O (95 : 5), or a mixture of aqueous NaOH (10 M) and EtOH (5 : 95) (approximately 20 mL/g of nucleoside). The reaction mixture was stirred for between 4-16 hrs, carefully neutralised with acetic acid and the solvent evaporated *in vacuo*. The residue was immediately dissolved in DCM containing and 2,2'-dithiobis(5-nitropyridine) (5 eq), stirred for a further hour and then evaporated *in vacuo*. The crude product was purified by silica gel column chromatography eluting with a gradient of MeOH in DCM. Appropriate fractions were pooled and evaporated *in vacuo* to give the product as a yellow amorphous solid.

3'-Deoxy-5'-*O*-monomethoxytrityl-3'-*S*-(phenyldisulfanyl)thymidine (5a).

Method B, 63%. (Found: *m/z* (FAB$^+$) 639.1977. (M+H)$^+$, $C_{36}H_{35}N_2O_5S_2$ requires 639.1987); ^1H (200 MHz, $CDCl_3$): 9.14 (1H, bs, NH), 7.66 (1H, s, H6), 7.20-7.46 (17H, m, Ar), 6.83 (2H, d, J = 8.8Hz, *o*-anisyl), 6.20 (1H, dd, J = 8.8, 4.4 Hz, H1'), 4.05 (1H, m, H3'), 3.79 (4H, m, OMe, H4'), 3.54 (1H, dd, J = 11, 2.4 Hz, H5'), 3.32 (1H, dd, J = 11, 2.7 Hz, H5''), 2.43-2.65 (2H, m, H2', H2''), 1.43 (3H, s, CH_3); *m/z* (FAB+) 639 (M+H$^+$, 0.9%), 273 (MMT$^+$, 100%).

3'-Deoxy-5'-*O*-Monomethoxytrityl-3'-*S*-(2-nitrophenyldisulfanyl)thymidine (5b).

Method A, 73%. (Found: C, 63.1; H, 4.82; N, 6.20. $C_{36}H_{33}N_3O_7S_2$ requires C, 63.2; H, 4.87; N, 6.15%); ^1H (200 MHz, $CDCl_3$), 8.28 (1H, d, J = 8.1 Hz, H3 Ar), 8.21 (1H, d, J = 7.7 Hz, H6 Ar), 7.62 (1 H, s, H6), 7.59 (1H, t, J = 8.2 Hz, H4 Ar), 7.20-7.40 (13H, m, MMT, Ar), 6.81 (2H, d, J = 8.8 Hz, *o*-anisyl), 6.20 (1H, t, J = 5.1 Hz, 1'-H), 4.08 (1 H, m, 4'-H), 3.77 (3H, s, OMe), 3.75 (1H, m, 3'-H), 3.60 (1H, m, 5'-H), 3.38 (1 H, m, 5''-H), 2.50 (2H, m, H2', 2''), 1.43 (3 H, s, Me5); *m/z* (FAB$^+$) 684 (M+H$^+$, 0.7 %), 273 (MMT$^+$, 100%).

3'-Deoxy-5'-*O*-monomethoxytrityl-3'-*S*-(pyridyl-2-disulfanyl)thymidine (5c).

Method A, 85%. (Found: C, 65.61; H, 5.21; N, 6.58. $C_{35}H_{33}N_3O_5S_2$ requires C, 65.76; H, 5.20; N, 6.57%); ^1H (200 MHz, $CDCl_3$), 8.53 (1H, bs, NH), 8.42 (1H, d, J = 7.5 Hz, Ar), 7.20-7.68 (15H, m, 12MMTH, 2Ar, H6), 7.11 (1H, t, J = 7.7 Hz, Ar), 6.82 (2H, d, J = 8.8 Hz, *o*-anisyl), 6.21 (1H, t, J = 5.6 Hz, H1'), 4.08 (1H, m, H3'), 3.90 (1H, m, H4'), 3.80 (3H, s, OMe), 3.55 (1H, m, H5'), 3.45 (1H, m, H5''), 2.61 (2H, m, H2' and H2''), 1.41 (3H, s, CH_3); *m/z* (FAB$^+$) 640 (M+H$^+$, 0.3%), 273 (MMT, 100%).

3'-Deoxy-5'-*O*-monomethoxytrityl-3'-*S*-(5-nitropyridyl-2-disulfanyl)thymidine (5d).

Method C, 70%. (Found: C, 61.21; H, 4.74; N, 8.15. $C_{35}H_{32}O_7N_4S_2$ requires C, 61.39; H, 4.71; N, 8.18%); ^1H (200 MHz, $CDCl_3$), 9.20 (1H, d, J = 2.75, H6 Ar), 8.86 (1H, bs, NH), 8.31 (1H, dd, J = 2.75, 9.00 Hz, H4 Ar), 7.70 (2H, m, 3H Ar, H6), 7.20-7.44 (12H, m, MMT), 6.82 (2H, d, J = 8.8 Hz, *o*-anisyl), 6.23 (1H, t, J = 5.5 Hz, H1'), 4.07 (1H, m, H3'), 3.91 (1H, m, H4'), 3.80 (3H, s, OMe), 3.63

(1H, m, H5'), 3.40 (1H, m, H5"), 2.05 (2H, m, H2', H2"), 1.44 (3H, s, 5Me); *m/z* (FAB+) 685 ($M+H^+$, 0.7%), 273 (MMT^+, 100%).

5'-Deoxy-5'-*S*-(2-nitrophenyldisulfanyl)thymidine (6b).

Method A, 74%. (Found: C, 47.0; H, 4.3; N, 9.7% $C_{16}H_{17}N_3O_6S_2$ requires C, 46.7; H, 4.1; N, 10.2%); ^1H (400 MHz, $(CD_3)_2CO$): 9.75 (1H, br.s, NH), 8.07 (1H, dd, *J* = 8.4, 1.2 Hz, H3 Ar), 7.96 (1H, dd, *J* = 8.4, 1.3 Hz, H6 Ar), 7.53 (1H, dt, *J* = 1.3, 8.0 Hz, H4 Ar), 7.19 (1H, dt, *J* = 1.2, 8.0 Hz, H5 Ar), 7.15 (1H, s, H6), 5.95 (1H, t, *J* = 7.6 Hz, H1'), 4.35 (1H, bs, 3'OH), 4.09 (1H, m, H4'), 3.72 (1H, m, H3'), 2.90 (1H, m, H5'), 2.86 (1H, m, H5"), 2.04 (1H, m, H2'), 1.92 (1H, m, H2"), 1.48 (3H, s, 5Me); *m/z* (FAB^+) 412 ($M+H^+$, 12%), 290 (M^+-Aryl, 3%), 127 (Base+$2H^+$, 27%).

5'-Deoxy-5'-*S*-(5-nitropyridyl-2-disulfanyl)thymidine (6d)

Method A, 73%. (Found: C, 43.3; H, 3.9; N, 13.1% $C_{15}H_{16}N_4O_6S_2$ requires C, 43.7; H, 3.9; N, 13.6%); ^1H (400 MHz, $(CD_3)_2CO$): 9.60 (1H, br.s, NH), 8.89 (1H, d, *J* = 2.8 Hz, H6 Ar), 8.23 (1H, dd, *J* = 8.0, 2.1 Hz, H4 Ar), 7.85 (1H, d, *J* = 8.0 Hz, H3 Ar), 7.15 (1H, s, H6), 5.93 (1H, t, *J* = 7.6 Hz, H1'), 4.12 (1H, m, H4'), 3.74 (1H, m, H3'), 3.00 (1H, m, H5'), 2.95 (1H, m, H5"), 2.07 (1H, m, H2'), 1.92 (1H, m, H2"), 1.48 (3H, s, 5Me); *m/z* (FAB^+) 413 ($M+H^+$, 3%).

3'-Deoxy-5'-*O*-monomethoxytrityl-3'-*S*-(5-nitropyridyl-2-disulfanyl)inosine (7d).

Method C. It was noted that this compound slowly decomposed to give the symmetrical dinucleoside disulfide and therefore it was generally converted to **8d** immediately after isolation.

73%. ^1H (200 MHz, $CDCl_3$): 9.20 (1H, d, *J* = 2.2 Hz, H6 Ar), 8.24 (1H, d, *J* = 9.4 Hz, H4 Ar), 8.12 (1H, s, H8), 8.06 (1H, s, H2), 7.57 (1H, d, *J* = 9.4 Hz, H3 Ar), 7.39-7.14 (12H, m, Aryl H), 6.77 (2H, d, *J*= 8.1 Hz, *o*-anisyl H), 6.27 (1H, s, br, HO2') 6.11 (1H, s, H1'), 4.72 (1H, m, H2'), 4.33 (1H, m, H3'), 4.12 (1H, m, H4'), 3.77 (3H, s, OMe), 3.62 (1H, m, H5'), 3.48 (1H, m, H5") ; *m/z* (FAB^+) 711 ($M+H^+$, 3%).

3'-Deoxy-5'-*O*-monomethoxytrityl-2'-*O*-tert-butyldimethylsilyl-3'-*S*-(5-nitropyridyl-2-disulfanyl)inosine (8d).

To a stirred solution of the disulfide **7d** (880 mg, 1.24 mmoles) in dry pyridine (8 ml) t-butyldimethylsilyl triflate (0.85 ml, 3.72 mmoles) was added dropwise by syringe. After 5 hrs the reaction mixture was partitioned between DCM (40 ml) and saturated aqueous $NaHCO_3$ (40 ml), the DCM layer was washed with water, dried and evaporated *in vacuo*. The crude product was purified by silica gel column chromatography eluting with DCM-MeOH (98 : 2). Appropriate fractions were pooled and evaporated *in vacuo* to give the product as a pale yellow amorphous solid.

(Found: C, 59.49; H, 5.34; N, 10.13% $C_{41}H_{44}N_6O_7S_2Si$ requires C, 59.74; H, 5.38; N, 10.19%); ^1H (200 MHz, $CDCl_3$): 9.13 (1H, d, *J* = 2.2 Hz, H6 Ar), 8.24 (1H, d, *J* = 9.4 Hz, H4 Ar), 8.19 (1H, s, H8), 8.07 (1H, s, H2), 7.67 (1H, d, *J* = 9.4 Hz, H3 Ar), 7.34-7.20 (12H, m, Aryl H), 6.75 (2H, d, *J*= 8.2 Hz, *o*-anisyl H), 6.07 (1H, d, *J* = 2.7 Hz, H1'), 5.00 (1H, m, H2'), 4.56 (1H, m, H3'), 4.05 (1H, m, H4'), 3.75 (3H, s, OMe), 3.73 (1H, m, H5'), 3.73 (1H, m, H5') 0.94 (9H, s, t-Butyl), 0.20 (3H, s, Me), 0.11 (3H, s, Me); *m/z* (FAB^+) 825 ($M+H^+$, 3.5%), 767 ($M-tBu^+$, 3.4%).

2',3'-Di-*O*-tert-butyldimethylsilyluridin-5'-yl dimethyl phosphite (10g).

To a stirred solution of 2',3'-di-*O*-t-butyldimethylsilyluridine (500 mg, 1.05 mmol) and tetrazole (110 mg, 1.8 mmol) in dry dioxane (5 ml) at room temperature, a solution of the dimethoxy(diisopropylamino)phosphane (2.3 mmol), in dry dioxane, was added by syringe. After 80 min the precipitated material was removed by

filtration and washed with ethyl acetate (2 x 10 ml). The combined filtrate was washed with $NaHCO_3$ solution, dried and evaporated. Silica gel chromatography (CH_2Cl_2-petroleum ether 40-60-NEt_3: 1:8:1) gave the nucleoside phosphite (90% yield).

^1H (200 MHz, $CDCl_3$): 7.97 (1H, d, *J*=8.2 Hz, H6), 5.78 (1H, d, *J*=2.7 Hz, H1'), 5.68 (1H, d, *J*=8.2 Hz, H5), 4.11-4.05 (5H, m, H2' ,3' ,4', 5' & 5''), 3.51 (6H, d, J_{PH}=10.4 Hz, OMe), 0.86 (18H, s, t-Butyl), 0.07 (12H, s, Me); ^{31}P (81 MHz, $CDCl_3$): 142.2; *m/z* (FAB$^+$) 565 (M+H$^+$, 6%), 453 (M-Base$^+$, 9%).

Studies on the Arbusov reactions using ^{31}P NMR spectroscopy

Reactions studied by ^{31}P nmr were conducted using 25 mg of the *S*-nucleosidyl *S*-aryl disulfide and between one to three equiv. of the phosphite in THF (3-5 ml). For these studies the phosphorothiolates **12f** and **12g** were generated *in situ* by reaction of 2,2'-dithiobis(5-nitropyridine) with trimethyl phosphite or 2',3'-Di-*O*-tert-butyldimethylsilyluridin-5'-yl dimethyl phosphite (**10g**) respectively.

Fully protected 3'-thioinosinylyl-(3'-5')uridine (11g)

To a stirred solution of the disulphide **8d** (72 mg, 0.0874 mmoles) in DCM (3 ml) the phosphite **10g** was added (148 mg, 0.262 mmoles) and the the resulting red solution stirred overnight. The reaction mixture was partitioned between DCM (20 ml) and saturated aqueous $NaHCO_3$ (20 ml), the DCM layer was washed with water, dried and evaporated *in vacuo*. The crude product was purified by silica gel column chromatography eluting with DCM-MeOH (95 : 5). Appropriate fractions were pooled and evaporated *in vacuo* to give the product as a colourless amorphous solid (62%).

(Found: *m/z* (FAB$^+$) 1241.4749. (M+Na)$^+$, $C_{58}H_{83}N_6O_{13}PSSi_2Na$ requires 1241.4682). ^1H (200 MHz, $CDCl_3$): 9.63 (1H, br s, uridine NH), 8.05 (1H, s, inosine H2 or H8), 8.02 (1H, s, inosine H2 or H8), 7.53-7.20 (13H, m, trityl H & uridine H6), 6.83 (2H, d, *J*=8.9 Hz,*o*-anisyl H), 5.99 (1H, s, inosine H1'), 5.59 (1H, d, *J*=8.0 Hz, uridine H5), 5.47 (1H, d, *J*=2.6 Hz, uridine H1'), 4.76 (1H, d, *J*=4.3 Hz, inosine H2'), 4.46 (1H, m), 4.36 (1H, m), 4.16 (2H, m), 4.07 (2H, m), 3.87 (1H, m), 3.76 (3H, s trityl OMe) 3.57 (2H, m), 3.39 (3H, d, J_{PH}=13.0 Hz, POMe), 0.93 (9H, s, t-Butyl), 0.86 (9H, s, t-Butyl), 0.84 (9H, s, t-Butyl), 0.1 (18H, m, SiMe); ^{31}P (81 MHz, $CDCl_3$): 29.5; *m/z* (FAB$^+$) 1241 (M+Na$^+$, 78%).

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11. Crystal data for **9b**: $C_{16}H_{16}N_3O_6S_2.CH_2Cl_2$; orthorhombic, a=9.56(2) Å, b=12.92(2) Å, c=34.899(9) Å, V=4311 Å^3, z=8, density = 1.526g/cm^3, R=0.10 (R_W=0.096), 2167 reflections. A list of atomic coordinates, thermal parameters, bond lengths and angles has been deposited at the Cambridge Crystallographic Data Centre.

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