Synthesis of 5'-Alkylthio-5'-deoxynucleosides from Nucleosides in a One-pot Reaction [1]

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5'-Alkyl(aryl)thio-5'-deoxynucleosides [alkyl(aryl)nucleoside sulphides] were prepared in high yields by the reaction of nucleosides with dialkyl or diaryl disulphides in the presence of tri-n-butylphosphine. The method is widely applicable to the synthesis of unsymmetrical sulphides.

The synthesis of sulphur-containing nucleosides has been reported by a number of workers. Most of the alkylthionucleosides were synthesized by reactions of *O*-mesyl-, *O*-tosyl-, halogeno-, or cyclo-nucleoside with different alkanethiols,[2] in several steps regardless of the method used. Only one example of a one-step procedure has been reported, by Holy,[3] who used pyridine-2-thiol in the presence of dimethylformamide dialkyl acetals. In a previous paper [1] we described briefly the synthesis of 5'-*S*-alkyl(aryl)thio-5'-deoxyribonucleosides (1) using dialkyl or diaryl disulphides in the presence of tri-n-butylphosphine. We report here the details of the reaction of nucleosides and alcohols with these reagents.

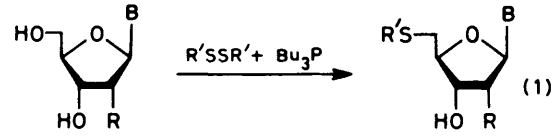
During our study of the synthesis of oligonucleotides *via* the phosphodiester method using 2,2'-dipyridyl disulphide and triphenylphosphine,[4] we noticed a curious phenomenon: during the condensation reaction nucleotidic material seemed to be lost owing to an unidentified side reaction. After several screenings, we found that the nucleoside reacted with di-2-pyridyl disulphide and triphenylphosphine, even in the presence of a phosphate component (nucleotide), to give the corresponding 5'-*S*-(2-pyridylthio)-5'-deoxyribonucleoside. Thus, the reaction of nucleosides with the dialkyl(diaryl) disulphides in the presence of phosphines was established.

When uridine was treated with 5 equiv. of di-2-pyridyl disulphide in the presence of 5 equiv. of tri-n-butylphosphine in dry pyridine at room temperature for 1 h, 5'-*S*-(2-pyridylthio)-5'-deoxyuridine was obtained almost quantitatively. When triphenylphosphine was used the reaction required 2 days and the same compound was obtained in 96% yield; in most cases, tri-n-butylphosphine was found to give the corresponding alkylthionucleosides more rapidly and in higher yields.

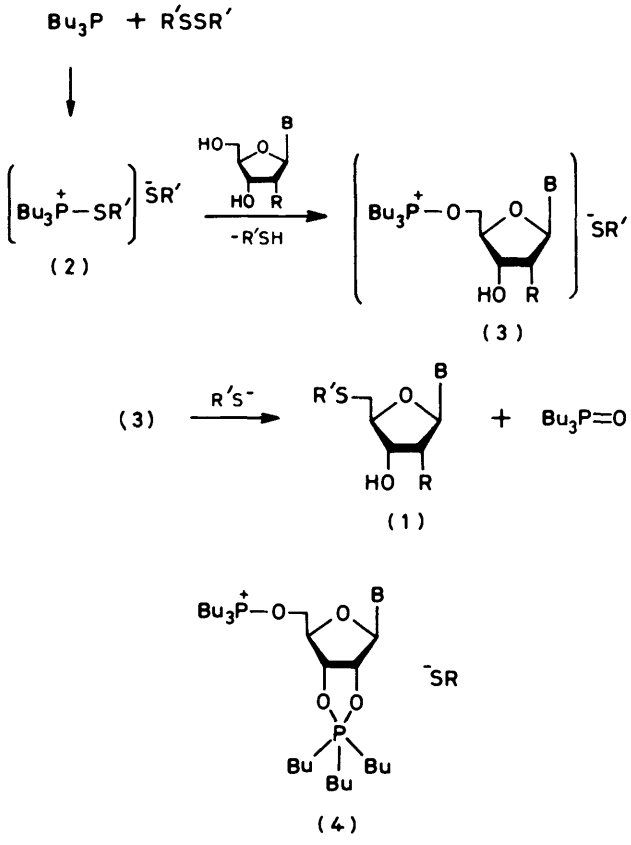
In a similar manner, methylthio-, phenylthio-, and *N,N*-diethylthiocarbamoyl-groups were introduced into the 5'-position of ribo- and deoxyribo-nucleosides, and *RS* groups were selectively introduced into the 5'-position without formation of the cyclo-nucleosides.

The reaction seems to proceed as follows. An alkoxyphosphonium salt of nucleosides (3) was formed from a phosphonium salt (2). The intermediate (3) decomposes on attack of alkyl(aryl)thiolate ion to afford alkyl(aryl)thionucleoside and tri-n-butylphosphine oxide. When an unsymmetrical disulphide, ethyl phenyl disulphide, was employed only the PhS group was introduced to the resulting sulphide, and not the EtS group. This means the counter ion of the alkoxyphosphonium salt (3) should be PhS^- which is more stable than the EtS anion.

We found that a 2',3'-cyclic oxyphosphorane intermediate (4), as described by Mitsunobu,[5] was formed by the reaction of unprotected ribonucleoside with a phosphonium salt (2). The cyclic oxyphosphorane intermediate (4) must act as a protecting group of the 2'- and 3'-hydroxy groups since no reaction



B = nucleoside base; R = H or OH



was observed when 5'-*O*-trityluridine was treated with di-2-pyridyl disulphide and triphenylphosphine.

With deoxyribonucleosides, the yields of the 5'-*S*-alkylthionucleosides gradually decreased as the reaction time was increased. The analogous phosphonium salt is probably formed at the 3'-position of deoxyribonucleoside but it did not afford the 3',5'-bisalkylthio-nucleoside.

The reaction conditions and the results are summarized in Table 1.

Simple alcohols also reacted with disulphide and tri-n-

Table 1. Synthesis of 5′-alkylthio-5′-deoxyribonucleosides (1) and physical characteristics

Nucleoside (mmol)	Disulphide (RSSR) R (mmol) *	Phosphine (mmol) *	Solvent (ml)	Time (h)	Yield (%) detected **	Yield (%) isolated	M.p. (°C)
Uridine (1)	2-Pyridyl (5)	Bu_3P (5)	Pyridine (5)	1	95	78	168
Uridine (0.5)	2-Pyridyl (2.5)	Ph_3P (2.5)	Pyridine (2.5)	48	96	65	166—168
Cytidine (1)	2-Pyridyl (3)	Bu_3P (3)	Pyridine (5)	4	92	87	108—109
Adenosine (2)	2-Pyridyl (6)	Bu_3P (6)	Pyridine (8)	17	Quant.	96	98—100
Guanosine (1)	2-Pyridyl (3)	Bu_3P (5)	Pyridine (5)	20	58	34	139—143
Thymidine (1)	2-Pyridyl (3)	Bu_3P (3)	Pyridine (5)	1.5	41	26	180—181
Deoxyadenosine (1)	2-Pyridyl (3)	Bu_3P (3)	DMF (5)	4	92	87	139—140
Deoxycytidine (1)	2-Pyridyl (3)	Bu_3P (3)	Pyridine (5)	4	81	25	184—185
Uridine (0.5)	Ph (1.5)	Bu_3P (1.2)	Pyridine (2.5)	24	83	56	206—207
Uridine (5)	$Et_2NC(S)$ (25)	Bu_3P (50)	Pyridine (12.5)	6	90	82	155—156
Thymidine (1)	$Et_2NC(S)$ (5)	Bu_3P (10)	Pyridine (2.5)	24	93	72	129—130
Adenosine (1)	Me (5)	Bu_3P (10)	DMF (5)	10 days	73	38	207—208

Nucleoside (mmol)	Disulphide (RSSR) R (mmol) *	U.v. data $\lambda_{max.}$ (Solvent) (ε)	U.v. data $\lambda_{min.}$	Elemental analysis (%) Found (Calc.)	Paper chromatography (R_F) (Solvent)
Uridine (1)	2-Pyridyl (5)	251 (MeOH) (16 500)	226 (MeOH)	C, 50.55; H, 4.5; N, 12.2; S, 9.55 (C, 49.85; H, 4.95; N, 12.46; S, 9.50)	
Uridine (0.5)	2-Pyridyl (2.5)				
Cytidine (1)	2-Pyridyl (3)	251 (0.1M-HCl) (8 600) 281 (0.1M-HCl) (13 100) 310 (0.1M-HCl) (7 700)	234 (0.1M-HCl) 258 (0.1M-HCl) 304 (0.1M-HCl)	C, 48.55; H, 4.8; N, 15.95 (C, 48.91; H, 5.41; N, 15.22)	
Adenosine (2)	2-Pyridyl (6)	252 (MeOH) (22 500) 254 (0.1M-HCl) (12 500) 312 (0.1M-HCl) (5 500)	226 (MeOH) 228 (0.1M-HCl) 288 (0.1M-HCl)	C, 48.65; H, 5.1; N, 21.4 (C, 49.00; H, 5.10; N, 21.43)	
Guanosine (1)	2-Pyridyl (3)	251 (0.1M-HCl) (14 300) 310 (0.1M-HCl) (6 200)	223 (0.1M-HCl) 294 (0.1M-HCl)		0.32 (A) [dG (0.07)]
Thymidine (1)	2-Pyridyl (3)	249 (MeOH) (9 800) 247 (H_2O) (7 700) 270 (H_2O) (6 800)	231 (MeOH) 230 (H_2O) 260 (H_2O)	C, 53.75; H, 5.1; N, 12.6 (C, 53.73; H, 5.11; N, 12.53)	0.72 (B) [dT (0.65) (B)] 0.70 (A) [dT (0.43) (A)]
Deoxyadenosine (1)	2-Pyridyl (3)	251 (0.1M-HCl) (14 300) 310 (0.1M-HCl) (6 200)	223 (0.1M-HCl) 294 (0.1M-HCl)		0.51 (A) [dA (0.16) (A)]
Deoxycytidine (1)	2-Pyridyl (3)	251 (0.1M-HCl) (8 900) 281 (0.1M-HCl) (13 400) 310 (0.1M-HCl) (7 800)	233 (0.1M-HCl) 257 (0.1M-HCl) 303 (0.1M-HCl)	C, 52.15; H, 5.05; N, 17.4; S, 9.7 (C, 52.17; H, 4.97; N, 17.39; S, 9.93)	0.49 (A) [dC (0.09) (A)]
Uridine (0.5)	Ph (1.5)	256 (MeOH) (14 100) 254 (0.1M-HCl) (13 700)	229 (MeOH) 230 (0.1M-HCl)	C, 53.5; H, 4.85; N, 8.35; S, 9.7 (C, 53.57; H, 4.76; N, 8.33; S, 9.52)	0.46 (A) [U (0.12) (A)]
Uridine (5)	$Et_2NC(S)$ (25)	260 (H_2O) (17 400)	232 (H_2O)	C, 45.6; H, 5.7; N, 11.1; S, 16.7 (C, 44.79; H, 5.64; N, 11.19; S, 16.78)	
Thymidine (1)	$Et_2NC(S)$ (5)	270 (H_2O) (17 500)	225 (H_2O)	C, 48.2; H, 6.2; N, 11.25 (C, 48.24; H, 6.21; N, 11.25)	
Adenosine (1)	Me (5)	260 (MeOH)	228 (MeOH)	C, 44.3; H, 5.05; N, 23.25; S, 10.8 (C, 44.44; H, 5.05; N, 23.57; S, 10.77)	0.57 (A) [A (0.16) (A)]

• Equivalents indicate relative to nucleosides. ** The detected yields were estimated from the spot on the paper chromatogram.

Table 2. Synthesis of alkyl 2-pyridyl sulphides

Alkyl group	Isolated yield (%)	Elemental analysis (%) Found (Calc.)
n-C_4H_9	78	C, 64.9; H, 7.9; N, 8.3; S, 19.35 (C, 64.61; H, 7.85; N, 8.37; S, 19.17)
s-C_4H_9	35	C, 65.0; H, 7.8; N, 8.45; S, 19.45 (See in the case of n-C_4H_9)
t-C_4H_9	0	
Crotyl	69	C, 65.6; H, 6.75; N, 8.7; S, 19.4 (C, 65.40; H, 6.72; N, 8.48; S, 19.40)
Gerarryl	76	C, 72.7; H, 8.75; N, 5.65; S, 12.85 (C, 72.81; H, 8.57; N, 5.66; S, 12.96)
Benzyl	92	C, 72.15; H, 5.6; N, 7.25; S, 16.25 (C, 71.57; H, 5.52; N, 6.96; S, 15.95)

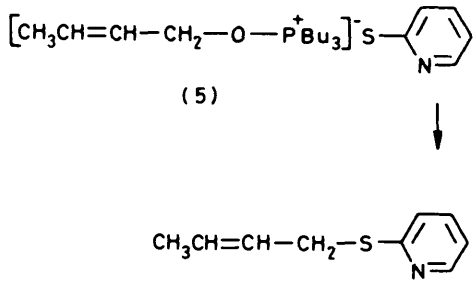
butylphosphine to give the unsymmetrical sulphides in good yields. With n-butyl, s-butyl, and t-butyl alcohols, n-butyl alcohol reacted more smoothly than s-butyl alcohol, and t-butyl alcohol did not react. α,β-Unsaturated alcohols reacted and afforded the corresponding sulphides (see Table 2), *e.g.* crotyl alcohol gave predominantly crotyl 2-pyridyl sulphide. In this reaction no migration of the double bond of the α,β-unsaturated alcohols took place, and the γ-position of crotyl alcohol was unaffected. Therefore, the reaction did not proceed through a carbonium ion intermediate formed from the alkoxyphosphonium salt (5), but rather from attack of a 2-pyridylthiolate ion on the alkoxyphosphonium salt (5) in an S_N2 mechanism.

In conclusion, the present reaction is widely applicable to the synthesis of unsymmetrical sulphides. For example, allyl 2-pyridyl sulphides are known to be useful synthetic intermediates for selective alkylation at the α-position.[6] It is noteworthy that the air-sensitive RS groups can be introduced by use of the disulphides under very mild conditions and the reactions proceed regio- and stereo-selectively.

Experimental

M.p.s are uncorrected. U.v. spectra were recorded on a Hitachi 124 spectrometer. Commercially available triphenylphosphine was recrystallised from ethanol. Di-2-pyridyl disulphide was recrystallised from methanol–water (4 : 3 v/v). Pyridine was purified by distillation in the presence of toluene-*p*-sulphonyl chloride and stored on calcium hydride. Dimethylformamide was purified by distillation under reduced pressure. Bis(*N*,*N*-diethylthiocarbamoyl) disulphide was prepared according to the procedure of Braun.[7] Silica-gel column chromatography was performed on Wakogel C-200 purchased from the Wako Chemical Co. Thin-layer chromatography and paper chromatography were carried out by descending technique using Toyo Roshi No. 51A. Solvent systems used were butan-1-ol–water–concentrated aqueous ammonia (84 : 16 : 1 v/v) (solvent A) and propan-2-ol–concentrated aqueous ammonia–water (7 : 1 : 2 v/v) (solvent B). Molar ratios and physical properties of the resulting compounds are summarised in Table 1.

5′-(2-*Pyridylthio*)-5′-*deoxyuridine*.—*Method A*. To a solution of uridine (244 mg) in dry pyridine (5 ml) was added di-2-pyridyl disulphide (1.10 g) and tri-n-butylphosphine (1.25 ml, 5 mmol). After 1 h the reaction was quenched by addition of water (50 ml). The mixture was concentrated under reduced pressure and the residue was washed with light petroleum. The supernatant was decanted off. The decantation was repeated three times and the residue was crystallised from propan-2-ol to give yellowish crystals (342 mg, quant.), m.p. 168 °C (decomp.).



Method B. To a solution of uridine (122 mg) in dry pyridine were added di-2-pyridyl disulphide (550 mg) and triphenylphosphine (655 mg). The reaction required 2 days at room temperature. After usual work-up, as described above, the title compound was obtained.

5′-(2-*Pyridylthio*)-5′-*deoxycytidine*.—To a solution of cytidine (243 mg) in dry pyridine were added di-2-pyridyl disulphide (660 mg) and tri-n-butylphosphine (0.8 ml). After 4 h, water (50 ml) was added. The mixture was concentrated to dryness under reduced pressure and the residue dissolved in a small amount of EtOH. The solution was added dropwise to vigorously stirred ether (500 ml). The precipitates were filtered off and washed with ether to give yellowish crystals.

5′-(2-*Pyridylthio*)-5′-*deoxyadenosine*.—To a suspension of adenosine (534 mg) in dry pyridine (8 ml) were added di-2-pyridyl disulphide (1.32 g) and tri-n-butylphosphine (1.5 ml). After 17 h water (100 ml) was added and the solution evaporated. The residue was suspended in light petroleum and the precipitate which appeared was decanted off, suspended in ether and refluxed for 30 min. It was then cooled, washed with ether, filtered off and dissolved in a small amount of MeOH. The solution was added dropwise to vigorously stirred ether (500 ml) to give a yellowish powder.

5′-(2-*Pyridylthio*)-5′-*deoxyguanosine*.—To a suspension of guanosine (283 mg) in dry pyridine were added di-2-pyridyl disulphide (660 mg) and tri-n-butylphosphine (1.25 ml). After 20 h water was added, the mixture was concentrated under reduced pressure and the precipitates were decanted off. Decantation was repeated three times and the residue finally dissolved in $CHCl_3$. Reprecipitation was carried out by addition of ether. The title compound (97 mg) was obtained as a white powder.

5′-(2-*Pyridylthio*)-2′,5′-*dideoxythymidine*.—To a solution of thymidine (242 mg) in dry pyridine were added di-2-pyridyl disulphide (660 mg) and tri-n-butylphosphine (0.6 ml). After 1.5 h water was added, the solution was concentrated under reduced pressure and the residue dissolved in $CHCl_3$. Yellowish precipitates (90 mg) were obtained.

5′-(2-*Pyridylthio*)-2′,5′-*dideoxyadenosine*.—To a solution of deoxyadenosine (251 mg) in dry dimethylformamide (5 ml) were added di-2-pyridyl disulphide (660 mg) and tri-n-butylphosphine (0.8 ml). After 4 h, water was added and the title compound was separated by paper chromatography.

5′-(2-*Pyridylthio*)-2′,5′-*dideoxycytidine*.—To a solution of deoxycytidine (227 mg) in dry pyridine (5 ml) were added di-

2-pyridyl disulphide (660 mg) and tri-n-butylphosphine (0.8 ml). After 45 min water was added, the solution was concentrated under reduced pressure and treated with light petroleum to afford a suspension. The supernatant was decanted off and decantation was repeated four times. The precipitates were dissolved in a small amount of MeOH and the solution was added to vigorously stirred ether. The precipitates formed were filtered off, suspended in acetone under reflux, and stored in a refrigerator. The title compound was obtained as pale yellow crystals.

5'-Phenylthio-5'-deoxyuridine.—To a solution of uridine (122 mg) in dry pyridine were added diphenyl disulphide (327 mg) and tri-n-butylphosphine (0.3 ml). After 24 h, water was added. The mixture was concentrated under reduced pressure and dissolved in a small amount of MeOH. The compound was precipitated by addition of ether, then dissolved in hot EtOH. After being cooled the solution was added dropwise to vigorously stirred ether. The title compound precipitated and was recrystallised from water as colourless needles.

5'-(N,N-Diethylthiocarbamoyl)-5'-deoxyuridine.—Uridine (1.22 g) and bis(*N,N*-diethylthiocarbamoyl) disulphide (7.4 g) were dissolved in dry pyridine and the mixture was concentrated. Co-evaporation with dry pyridine was repeated three times. Finally, the residue was dissolved in dry pyridine and tri-n-butylphosphine (12.5 ml) was added at room temperature. After 6 h the mixture was treated with water and a portion was developed by paper chromatography. The title compound (90%; estimated by u.v. absorption) was obtained. The rest of the aqueous solution was concentrated under reduced pressure and the residue was applied to a silica-gel column. The column was first washed with $CHCl_3$ and then developed with $CHCl_3$–MeOH (20 : 1 v/v), and finally eluted by $CHCl_3$–MeOH (8 : 1 v/v). The eluant was concentrated and dissolved in a small amount of water with heating. Insoluble materials were filtered off and the title compound was obtained on cooling.

5'-(N,N-Diethylthiocarbamoyl)-2',5'-dideoxythymidine.—Thymidine (242 mg) and bis(*N,N*-diethylthiocarbamoyl) disulphide (1.48 g) were dissolved in dry pyridine and the mixture concentrated under reduced pressure. Co-evaporation was repeated three times and finally the residue was dissolved in dry pyridine. Tri-n-butylphosphine (2.5 ml) was added, followed by water after 24 h. The solution was then concentrated and applied to a silica-gel column. After washing with $CHCl_3$, the column was eluted with $CHCl_3$–MeOH (20 : 1 v/v) and then with $CHCl_3$–MeOH (9 : 1 v/v). The eluant was concentrated and the residue was recrystallised from water. The title compound was obtained as white crystals.

5'-Methylthio-5'-deoxyadenosine (Vitamin L_2).—To a solution of adenosine (267 mg) in dry dimethylformamide (5 ml) were added dimethyl disulphide (0.78 ml, 5 mmol) and tri-n-butylphosphine (2.5 ml). After 10 days water was added and the solution was concentrated under reduced pressure and dissolved in CCl_4. It was stored in a refrigerator. A sticky material formed which was dissolved in a small amount of MeOH and chromatographed on cellulose plates. The title compound was obtained and recrystallised from MeOH.

Reaction of Ethyl Phenyl Disulphide with Uridine in the Presence of Tri-n-butylphosphine.—To a solution of uridine (25 mg) in dry pyridine (0.5 ml) were added ethyl phenyl disulphide (85 mg) and tri-n-butylphosphine (0.13 ml). After 6 h water was added and the resulting solution was treated in the usual manner (above) to give 5'-phenylthio-5'-deoxyuridine.

Synthesis of Unsymmetrical Sulphides (General Procedure).—To a solution of the appropriate alcohol (5 mmol) in dry pyridine (5 ml) were added triphenylphosphine (15 mmol) and di-2-pyridyl disulphide (15 mmol). After 24 h water was added and the mixture was stirred for 2 h. The resulting solution was concentrated under reduced pressure and dissolved in $CHCl_3$ (30 ml). The $CHCl_3$ solution was washed with three portions of 1M-aqueous sodium hydroxide (3 × 10 ml) and then with water. The $CHCl_3$ layer was concentrated to a small volume and chromatographed on a silica-gel column, with benzene as eluant. The corresponding alkyl 2-pyridyl sulphide was obtained (Table 2).

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