Ribonucleoside Cyclic 3',5'-Phosphoramidates: Synthesis, Stereochemistry, and Conversion into Ribonucleoside Cyclic 3',5'-Phosphorothioates and -[^{18}O]Phosphates

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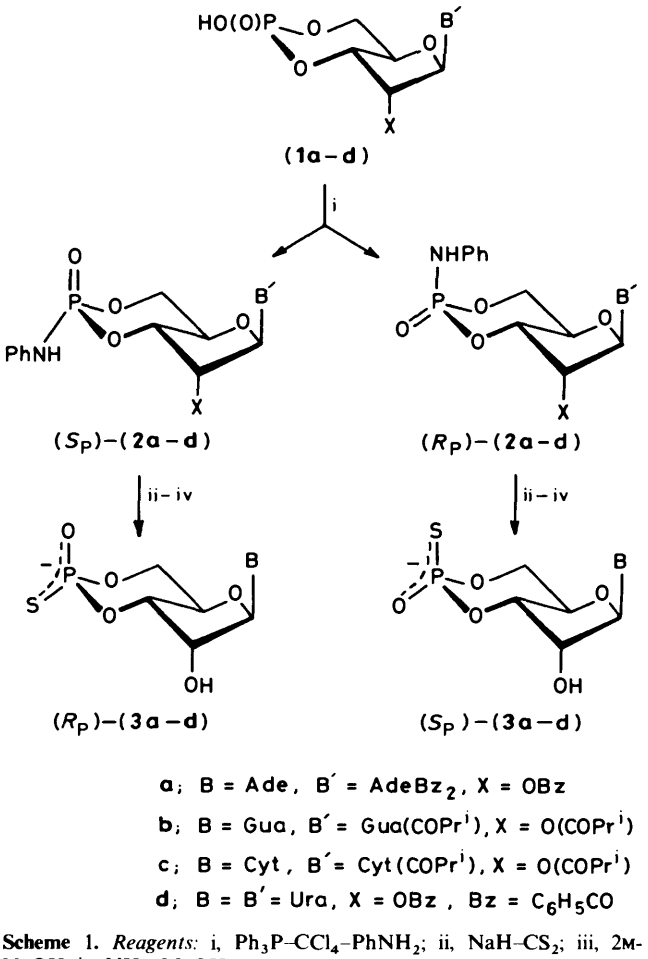
Base- and $O^{2'}$-protected nucleoside cyclic 3',5'-phosphates (**1**) react with aniline in the presence of triphenylphosphine–carbon tetrachloride to give the nucleoside cyclic 3',5'-phosphoranilidates (**2**), which after separation into individual diastereoisomers are converted in stereoretentive manner into the corresponding nucleoside cyclic 3',5'-phosphorothioates (**3**). Isotopomers of adenosine cyclic 3',5'-[^{18}O]phosphates (**9a**) are prepared *via* two independent routes: (a) reaction of adenosine cyclic 3',5'-phosphoranilidates (**2a**) with sodium hydride–[^{18}O]benzaldehyde (retention) or (b) acid-catalysed hydrolysis of adenosine cyclic 3',5'-*N,N*-dimethylphosphoramidates (**10a**) (inversion).

Adenosine cyclic 3',5'-phosphoranilidothioates (S_P)-(**23**) and (R_P)-(**23**), prepared *via* cyclisation of the corresponding 5'-[*O*-(4-nitrophenyl)phosphoranilidothioates] (**22**), have been converted into *P*-achiral adenosine cyclic 3',5'-phosphorodithioate (**26**).

This paper describes the details of the preparation of ribonucleoside cyclic 3',5'-phosphorothioates (**3**) according to the strategy elaborated in this laboratory and presented in a number of earlier communications.[1–3] Our strategy was based on the conversion of base- and 2'-hydroxy-protected nucleoside cyclic 3',5'-phosphates (**1**) into corresponding nucleoside cyclic 3',5'-phosphoranilidates (**2**), their separation into individual diastereoisomers, and stereospecific conversion of each diastereoisomer (**2**) into appropriate nucleoside cyclic 3',5'-phosphorothioate (**3**). The initial protection of the 2'-hydroxy function was essential, because the reaction of unprotected nucleoside cyclic 3',5'-phosphoranilidates with sodium hydride–carbon disulphide did not lead to the desired nucleoside cyclic 3',5'-phosphorothioates. For this reason N^6,N^6,$O^{2'}$-tribenzoyladenosine cyclic 3',5'-phosphate (cAMPBz$_3$) (**1a**), N^2,$O^{2'}$-diisobutyrylguanosine cyclic 3',5'-phosphate [cGMP(COPr^i)$_2$] (**1b**), N^4,$O^{2'}$-di-isobutyrylcytidine cyclic 3',5'-phosphate [cCMP(COPr^i)$_2$] (**1c**), and $O^{2'}$-benzoyluridine cyclic 3',5'-phosphate (cUMPBz) (**1d**) were prepared starting from the readily available ribonucleoside cyclic 3',5'-phosphates (Table 1).

Results and Discussion

Conversion of Protected Ribonucleoside Cyclic 3',5'-Phosphates into the Corresponding Phosphoramidates.—The conversion of compounds (**1a**—**d**) into the corresponding diastereoisomeric phosphoramidates (**2**) and (**10**) was achieved by treatment of each phosphate (**1**) with triphenylphosphine–carbon tetrachloride–amine, following the early reports of Appel[4] and Myshenina *et al.*[5] on dialkyl hydrogen phosphate ⟶ dialkyl phosphoramidate conversion. As reported earlier from this laboratory,[2] aniline and dimethylamine were suitable for conversions (**1**) ⟶ (**2**) or (**1**) ⟶ (**10**), and early results of J. Beres obtained in this laboratory were further developed,[6] demonstrating that benzylamine and piperidine can also be successfully used for the preparation of nucleoside cyclic 3',5'-phosphoramidates by this procedure. Since the ratio of diastereoisomers of ribonucleoside cyclic 3',5'-phosphoranilidates (**2**) prepared from corresponding phosphates (**1a**—**d**) (Scheme 1) in pyridine medium was unsatisfactory, and in the light of increased interest in the accessibility of N^6,N^6,$O^{2'}$-tribenzoyladenosine cyclic 3',5'-(S_P)-phosphoranilidate (S_P)-(**2a**), the precursor of adenosine cyclic 3',5'-(R_P)-phosphorothioate (R_P)-(cAMPS) (**3a**), studies on the mechanism of the Appel reaction were undertaken.[7] It was found that pyridine, when

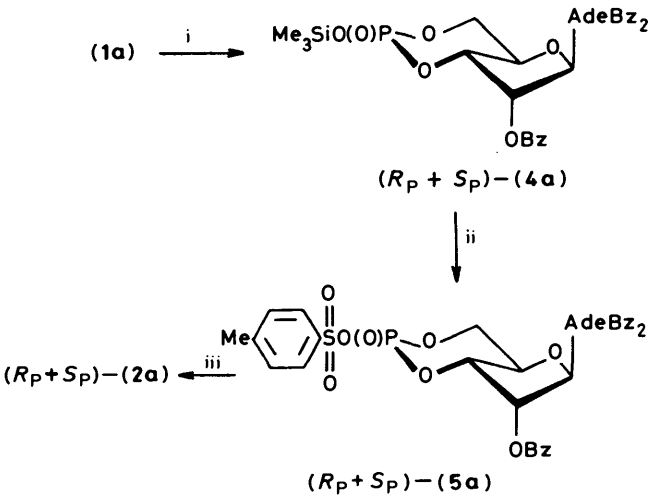


Scheme 1. *Reagents:* i, Ph_3P–CCl_4–$PhNH_2$; ii, NaH–CS_2; iii, 2M-NaOH; iv, NH_3–MeOH

used as the reaction medium, participates in the reaction course as a nucleophile, thus shifting the diastereoisomeric ratio towards a predominance of (R_P)-(**2a**). The use of acetonitrile changed the reaction course towards the formation of (**2a**) in the more desirable ratio (S_P):(R_P) 5:2. Besides aniline, dimethylamine has also been used in the Appel-type reaction of compounds (**1**), leading to nucleoside cyclic 3′,5′-*N*,*N*-dimethylphosphoramidates (cNMPNMe$_2$) (**10a**—**b**). Incorporation of ^{15}N label into the phosphoramidate function of compounds (**2**) and (**10**) was crucial for assignment of the absolute configuration at phosphorus in all diastereoisomers of (**2**) and (**10**) due to the easy measurement of the direct spin–spin coupling constants $^1J(^{31}P-^{15}N)$. Diastereoisomers of compounds (**2**) and (**10**) with an axially oriented amino function (with respect to the chair-shaped dioxaphosphorinane ring *trans*-fused to ribofuranose) possess lower values of spin–spin coupling constants compared with their counterparts with an equatorial amino function.[8] The assignment of axial or equatorial orientation to the amino group attached to phosphorus is, due to the presence of a natural ribose moiety within molecules (**2**) and (**10**), equivalent to assignment of the absolute configuration at phosphorus.

The relative values of chemical shifts in ^{31}P n.m.r. spectra measured for diastereoisomeric pairs of cyclic phosphoramidates (**2**) and (**10**) confirm the absolute configuration assignments.[9] Physicochemical data for phosphoramidates (**2**) and (**10**) are presented in Table 2.

During our studies on the conversion of dialkyl hydrogen phosphates into dialkyl phosphoramidates Michalski *et al.*[10] published their results on the synthesis of mixed phosphoric sulphonic anhydrides and their reactivity with selected nucleophiles, demonstrating excellent phosphorylating properties for these anhydrides. In model studies they demonstrated that reaction of *O*,*O*-diethylphosphoric benzenesulphonic anhydride with aniline occurred with quantitative formation of diethyl phosphoranilidate.[10] This report prompted us to attempt an application of Michalski's approach toward the synthesis of diastereoisomers of compound (**2a**). Thus, compound (**1a**) was treated with hexamethyldisilazane (HMDS) to give a mixture of diastereoisomers of N^6,N^6,$O^{2'}$-tribenzoyladenosine cyclic 3′,5′-trimethylsilylphosphates (**4a**), δ_P −12.2 and −16.4. This mixture on reaction with toluene-*p*-sulphonic anhydride was converted into the corresponding anhydrides (**5a**) (δ_P −21.8) which, without isolation, were treated with aniline. The ^{31}P n.m.r. spectrum of the resulting reaction mixture showed that it contained starting material (**1a**) besides the expected product (**2a**) (Scheme 2). The proportions



Scheme 2. *Reagents:* i, HMDS; ii, toluene-*p*-sulphonic anhydride; iii, $PhNH_2$

of the intensities of signals corresponding to compounds (**1**) and (**2a**) were (S_P)-(**2a**):(R_P)-(**2a**):(**1a**) 4:2:6, respectively. Identification of products was based on the results of ^{31}P n.m.r. investigation and comparison of chemical shifts of genuine samples of both compounds (**2a**) and (**1a**), sequentially introduced into the reaction mixture resulting from the degradation of the sulphonate (**5a**) with aniline.

Since the overall reaction was performed under strictly anhydrous conditions, the formation of starting material (**1a**) may indicate that when the phosphorus atom is incorporated within a cyclic, and to some extent sterically strained system, as in sulphonate (**5a**), these compounds do not react exclusively as phosphorylating agents as originally suggested,[10] and partial attack of aniline on the sulphur atom of compound (**5a**) can occur.

Assignment of Absolute Configuration at Phosphorus in Diastereoisomers of cAMP Anilidates (**2a**) via *Stereochemical Correlation.*—During the course of our earlier studies 2′-deoxyadenosine cyclic 3′,5′-phosphoranilidates (**8a**) were obtained[11] and separated into diastereoisomeric species. By means of spectroscopic arguments (^{31}P n.m.r. chemical shifts and spin–spin coupling constants) the R_P absolute configuration was assigned to the compound with the lower absolute value of $^1J(^{31}P-^{15}N)$ and which resonated at higher field than its S_P

Table 1. Spectroscopic data for base- and $O^{2'}$-protected nucleoside cyclic 3′,5′-phosphates (1)

Compound	B′	X	δ_P (p.p.m.)	$\lambda_{max.}$ (nm)	Yield (%)
(**1a**)	$AdeBz_2$	OBz	−2.9[a]	230, 276	70.0
(**1b**)	Gua(COPr^i)	OCOPr^i	−1.8[b]	254, 276	74.6
(**1c**)	Cyt(COPr^i)	OCOPr^i	−2.7[b]	278	78.0
(**1d**)	Ura	OBz	−2.8[a]	262	74.4

[a] In $CHCl_3$. [b] In water. Bz = PhCO.

Table 2. Yields and spectroscopic data for nucleoside cyclic 3′,5′-phosphoramidates (**2**) and (**10**)

Expt	Compound	B′	X	δ_P (p.p.m.)[a]		$^1J(^{31}P-^{15}N)$ (Hz)		Yield (%)	
				R_P	S_P	R_P	S_P	R_P	S_P
1	(**2a**)	$AdeBz_2$	OBz	−1.6	1.4	37.5	49.0	20.4[b]	10.7[b]
								11.2[c]	24.9[c]
2	(**2b**)	Gua(COPr^i)	OCOPr^i	−1.4	2.1	39.2	53.7	17.0[b]	7.5[b]
3	(**2c**)	Cyt(COPr^i)	OCOPr^i	−0.6	1.0	38.6	52.7	10.6[b]	7.9[b]
4	(**2d**)	Ura	OBz	−1.3	0.5	40.7	52.7	24.1[b]	7.8[b]
5	(**10a**)	$AdeBz_2$	OBz	6.9	8.0	45.0	54.0	7.0[b]	14.3[b]
6	(**10b**)	Gua(COPr^i)	OCOPr^i	6.2	7.8	46.5	57.0	6.4[b]	17.2[b]

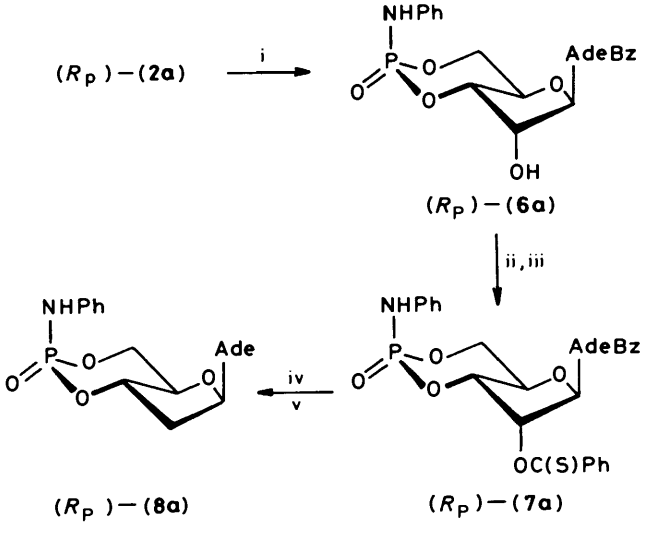
[a] In $CHCl_3$. [b] Pyridine as solvent. [c] Acetonitrile as solvent.

Table 3. Yields and spectroscopic data for nucleoside cyclic 3′,5′-phosphorothioates (3)

Compound	B	δ_P (p.p.m.)[a]		Yield (%)	
		R_P	S_P	R_P	S_P
(3a)	Ade	55.2	53.6	80.0	68.0
(3b)	Gua	56.6	54.9	26.0	22.0
(3c)	Cyt	55.0	53.3	58.0	61.7
(3d)	Ura	55.1	53.3	52.5	44.0

[a] In water.

counterpart. The correctness of this assignment was fully confirmed by an *X*-ray examination of a single crystal of (R_P)-cdAMP anilidate.[12] Since this assignment can be considered as the reference point to all other nucleoside cyclic 3′,5′-phosphoramidates, we decided to correlate the configuration at phosphorus in (R_P)-cdAMP anilidate (**8a**) with that of compound (**2a**), which according to spectroscopic criteria should be R_P. Compound (**2a**) [δ_P −1.6; $^1J(^{31}P-^{15}N)$ 37.5 Hz] was treated with 2M-NaOH for deprotection of its 2′-hydroxy function, and the resulting N^6-benzoyladenosine cyclic 3′,5′-phosphoranilidate (**6a**) was treated with $PhC(Cl)=\overset{+}{N}Me_2\ Cl^-$, followed by hydrogen sulphide–pyridine.[13] N^6-Benzoyl-2′-*O*-thiobenzoyladenosine cyclic 3′,5′-phosphoranilidate (**7a**), although obtained in low yield, was further treated with tributyltin hydride in boiling toluene. After evaporation of the solvent, the reaction mixture was treated with a methanolic solution of ammonia. The final product, isolated by preparative t.l.c. (p.l.c.), appeared to be identical (t.l.c., ^{31}P n.m.r.) with (R_P)-cdAMP anilidate (**8a**) (Scheme 3).



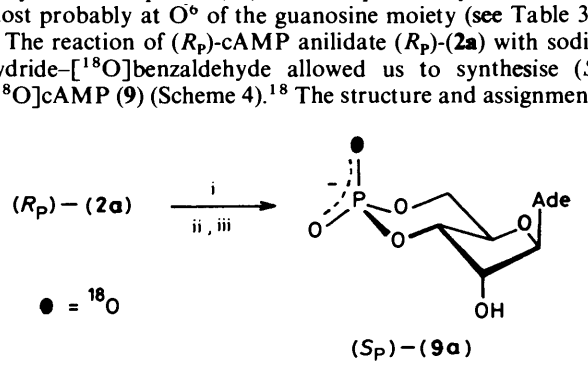
Scheme 3. *Reagents:* i, 2M-NaOH; ii, $PhC(Cl)=\overset{+}{N}Me_2\ Cl^-$; iii, H_2S–C_6H_5N; iv, Bu_3SnH; v, NH_3–MeOH

This stereochemical correlation confirmed the correctness of our assignment of absolute configuration at phosphorus in both isomers of (**2a**) based mainly on criteria of relative values of spin–spin coupling constants, sometimes referred to as Stec's Rule.[14]

Conversion of Ribonucleoside 3′,5′-Phosphoranilidates into the Corresponding Phosphorothioates (**3**) *and* [^{18}O]*Phosphates* (**9**).—The stereoretentive mode of PN ⟶ PX conversion of the Horner–Wadsworth–Emmons-type reaction was confirmed in a number of earlier works from this[15] and other laboratories.[16] Thus, each diastereoisomer of cNMP anilidates (**2**) (Scheme 1) was treated with sodium hydride and after cessation of hydrogen evolution (*ca.* 50 min), carbon disulphide was added to the reaction mixture. The reaction mixture was maintained for 3 h at room temperature and after completion (t.l.c. assay) the protecting groups were removed. Products (**3**) were isolated by column chromatography on DEAE-Sephadex A-25 with a linear gradient of triethylammonium hydrogen carbonate buffer. In no case was cross-contamination of an expected diastereoisomer by its counterpart observed.[1,3]

The synthesis of diastereoisomers of [^{35}S]cAMPS performed according to the same procedure is presented in our earlier communication.[17] It is worthwhile to mention that PN ⟶ PS conversion was attempted in pyridine, 1,2-dimethoxyethane (DME), and dimethylformamide (DMF) solutions, and that DME was the solvent of choice, since in pyridine and in DMF solvents the formation of the product was accompanied by appearance of coloured contaminants. The drastic decrease in the yield of compound (**3b**) can be explained by side-reactions, most probably at O^6 of the guanosine moiety (see Table 3).

The reaction of (R_P)-cAMP anilidate (R_P)-(**2a**) with sodium hydride–[^{18}O]benzaldehyde allowed us to synthesise (S_P)-[^{18}O]cAMP (**9**) (Scheme 4).[18] The structure and assignment of



Scheme 4. *Reagents:* i, NaH–[^{18}O]PhCHO; ii, 2M-NaOH; iii, NH_3–MeOH

isotopic enrichment were confirmed by fast-atom bombardment mass spectrometry and high-field ^{31}P n.m.r. spectroscopy (*vide infra*).

Taking into account that PN ⟶ PX conversion occurs with retention of configuration at phosphorus, and that the configuration of precursors (**2**) was known, we could assign the absolute configurations in the resulting diastereoisomers of cNMPS (**3**), as shown in Table 3.

Solvolysis of (R_P)- *and* (S_P)-*Adenosine Cyclic* 3′,5′-N,N-*Dimethylphosphoramidates* (**10a**).—As mentioned above, (S_P)-[^{18}O]cAMP (**9a**) was obtained from (R_P)-(**2a**) *via* a Horner–Wadsworth–Emmons-type reaction, and this route to stereospecifically and isotopically labelled deoxyadenosine cyclic phosphates was utilised in elegant works of Gerlt *et al.*[16] It was of interest whether diastereoisomers of phosphoramidate (**10a**) would be used as precursors for the synthesis of both diastereoisomers of [^{18}O]cAMP (**9a**). There are several lines of evidence to show that acid-catalysed solvolysis of dialkylphosphoramidates occurs with inversion of configuration.[19] Hydrolysis of compound (**10a**) in tetrahydrofuran (THF) medium with a stoicheiometric amount of methanesulphonic acid at room temperature was very inefficient — after 36 h the reaction mixture still contained unhydrolysed (**10a**). Effective hydrolysis was observed if a large excess of acid and water was used. ^{31}P N.m.r. assay indicated that the hydrolysis of any of diastereoisomers of compound (**10a**) in the presence of a 150 molar excess of methanesulphonic acid and a 600 molar excess of water was complete after 8 h if the reaction mixture was maintained at room temperature. The isolated isotopomers of

[^{18}O]cAMP were studied by ^{31}P n.m.r. spectroscopy according to methodology described by Jarvest and Lowe.[20] Methylation of each diastereoisotopomer of (**9a**) with methyl iodide in the presence of 18-crown-6 in dimethyl sulphoxide (DMSO) solution gave *O*-methyl esters of [^{18}O]cAMP (**11a**)—(**14a**) (Scheme 5). Methylation of cAMP derived from $H_2{}^{18}O$ hydrolysis of (R_P)-(**10a**) led to a mixture of axial and equatorial *O*-methyl cAMP esters (**11a**) + (**12a**) (Figure, part A).

The small isotope effect (1.4 Hz) observed for isomer (**12a**)

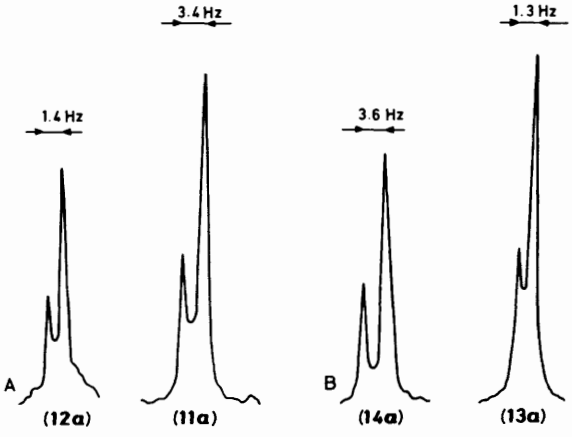
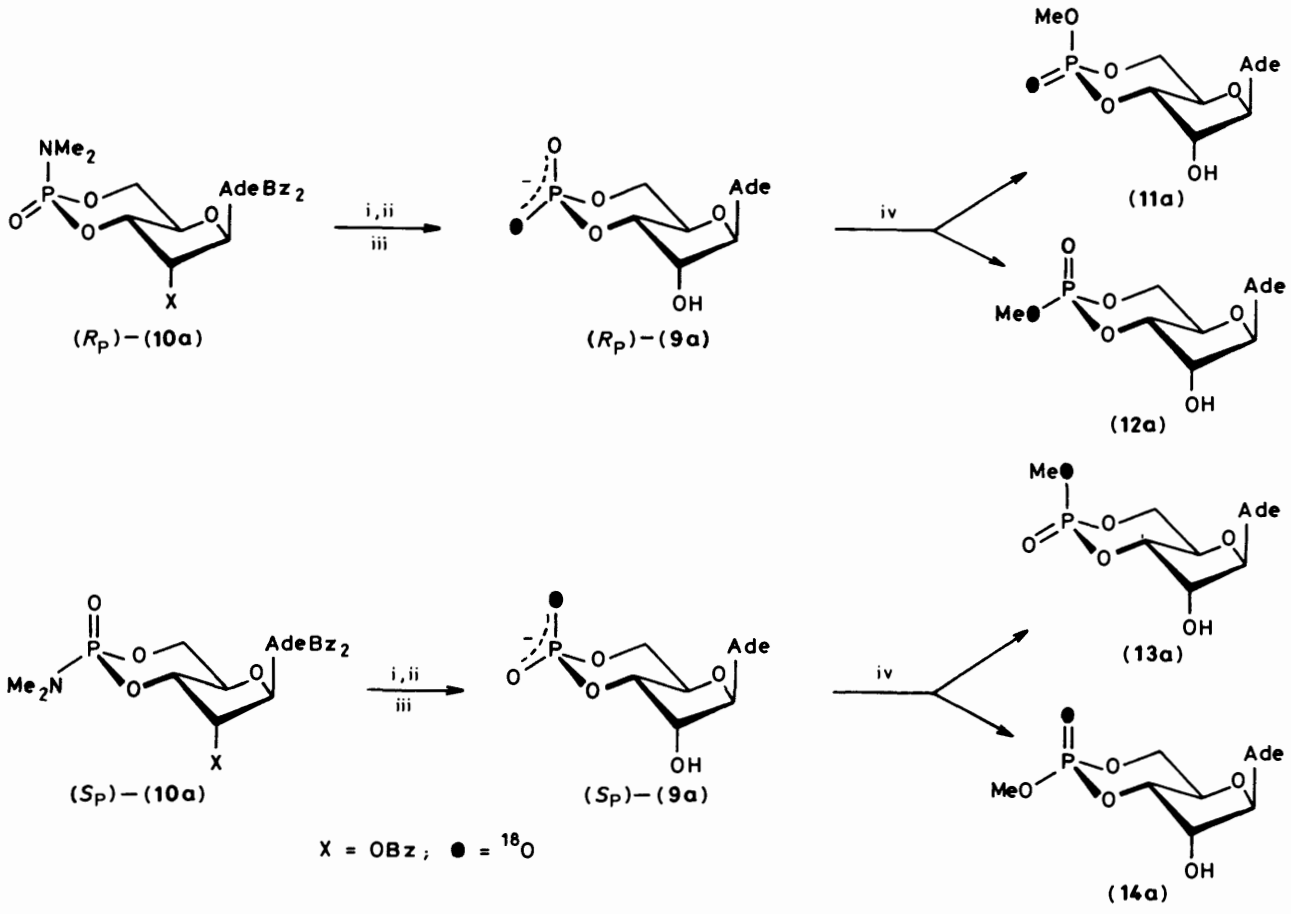


Figure. ^{31}P N.m.r. spectra (81 MHz) of *O*-methyl esters of [^{18}O] cAMP, (**11a**)—(**14a**), recorded in $(CD_3)_2SO$ in the presence of 8-hydroxyquinoline. A and B are explained in the text

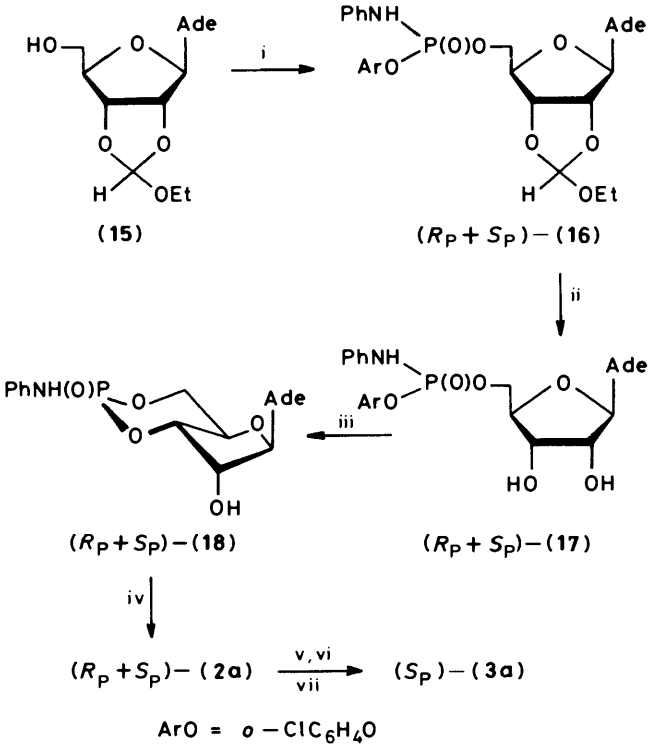
(low-field signals $\delta_P \sim -2.5$) and the higher isotope effect (3.4 Hz) associated with the high-field resonance of isomer (**11a**) ($\delta_P \sim -3.7$) clearly indicate that ^{18}O was introduced into the equatorial position only. Conclusions about the incorporation of ^{18}O into an axial position comes from an analysis of ^{31}P n.m.r. spectra of the methylated products of hydrolysis of (S_P)-(**10a**) with $H_2{}^{18}O$ (Figure, part B). Thus, the above analysis indicates that the process of acid-catalysed hydrolysis of both diastereoisomers of compound (**10a**) is stereospecific and occurs with inversion of configuration at phosphorus.

The yields of isolated [^{18}O]cAMP (**9a**) were 30% for the S_P-isomer and 36% for the R_P one, respectively, which indicates that acid hydrolysis of cNMP dimethylamidates (**10**) in $H_2{}^{18}O$-containing medium can be considered as complementary to the method presented above for the synthesis of diastereoisotopomers of cNMP (**9**) *via* a Horner–Wadsworth–Emmons-type reaction of *N*-metallated cNMP anilidates with $C^{18}O_2{}^{16}$ or $PhCH^{18}O$.[18]

Synthesis of $N^6,N^6,O^{2'}$*-Tribenzoyladenosine Cyclic* 3′,5′*-Phosphoranilidates* (**2a**) *and Adenosine Cyclic* 3′,5′*-Phosphoranilidothioates* (**23**) *from Acyclic Precursors.*—(a) *Phosphorylation of* 2′,3′*-ethoxymethylenadenosine* (**15**). Early attempts to synthesise $N^6,N^6,O^{2'}$-tribenzoyladenosine cyclic 3′,5′-phosphoranilidates (**2a**), intermediates for the stereospecific synthesis of diastereoisomers of cAMPS (**3a**), involved the reaction of 2′,3′-ethoxymethylenadenosine (**15**) with 2-chlorophenylphosphoranilidochloridate[1] and, after deprotection of 2′- and 3′-hydroxy groups, separation of diastereoisomers of adenosine 5′-[*O*-(2-chlorophenyl)phosphoranili-

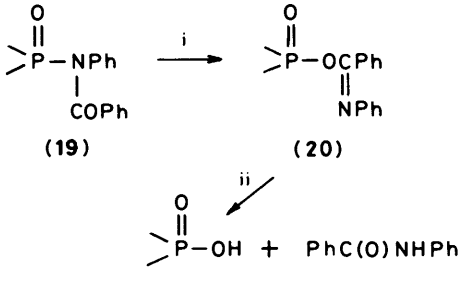


Scheme 5. *Reagents:* i, 2M-NaOH; ii, NH_3–MeOH; iii, [^{18}O]H_2O–Me SO_3H; iv, CH_3I. For (**10b**) see Table 2



Scheme 6. *Reagents:* i, 2-$ClC_6H_4O(PhNH)P(O)Cl$–1,2,4-triazole; ii, 80% AcOH; iii, Bu^{t}OK–DMSO; iv, PhCOCl; v, NaH–CS_2; vi, 2M-NaOH; vii, NH_3–MeOH

date] (**17**). The diastereoisomers underwent stereospecific cyclisation on treatment with potassium t-butoxide to give individual diastereoisomers of adenosine cyclic 3′,5′-phosphoranilidates (**18**) (Scheme 6). It appeared, however, that the reaction of (**18**), containing an unprotected 2′-hydroxy function, with NaH–CS_2 does not afford the expected cAMPS. As the most feasible explanation we considered the involvement of hydroxy groups in a reaction with NaH–CS_2, and for this reason their benzoylation was undertaken. The diastereoisomeric mixture of adenosine cyclic 3′,5′-phosphoranilidates (**18**) (R_P:S_P *ca.* 5:7) in pyridine solution was treated with an excess of benzoyl chloride. Surprisingly, the ratio of expected diastereoisomers of N^6,N^6,$O^{2'}$-tribenzoyladenosine cyclic 3′,5′-phosphoranilidate (**2a**) (overall yield 32%) was (R_P:S_P *ca.* 10:1), and after aqueous work-up of the reaction mixture compound (**1a**) was isolated as the predominant, but undesired, side-product. This result prompted us to investigate further model studies on benzoylation of dialkyl phosphoranilidates, which indicate that in pyridine benzoyl chloride causes the formation of an *O*,*O*-dialkyl *N*-benzoylphosphoranilidate (**19**), which undergoes slow rearrangement to the phosphoric

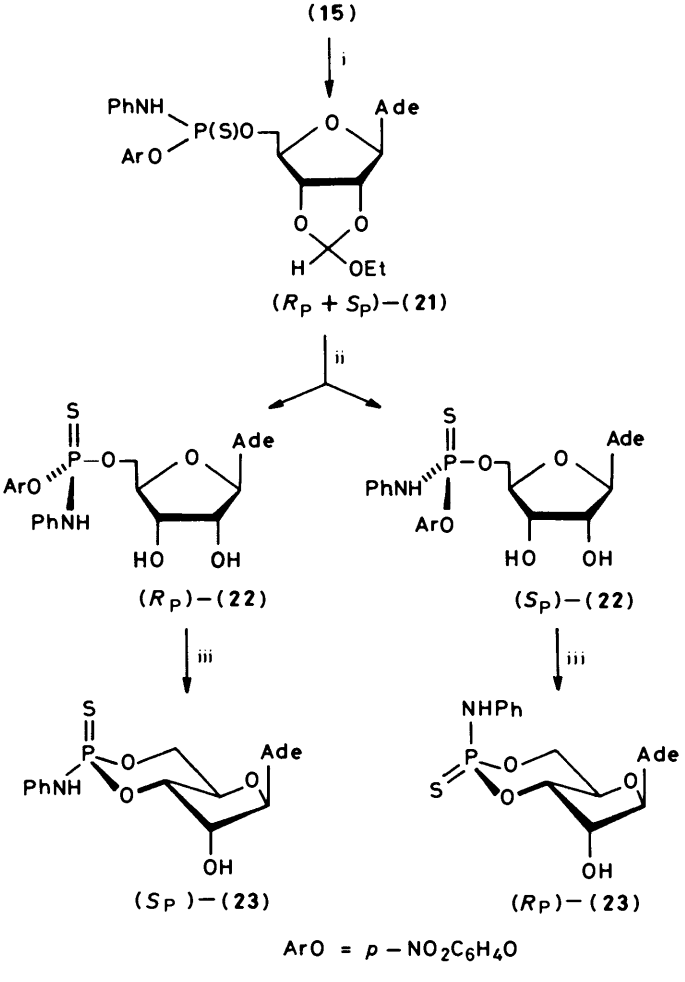


Scheme 7. *Reagents:* i, C_6H_5N; ii, water

benzimidic anhydride (**20**); this process is accelerated by the presence of water, which further attacks the electrophilic centre at the iminocarboxyl group. Dialkyl hydrogen phosphate and benzanilide are exclusive end-products of the reaction (Scheme 7).[21]

Such a course of events explains the aforementioned strategy involving protected nucleotides (**1**) as the substrates for the stereospecific synthesis of both diastereoisomers of desired ribonucleoside 3′,5′-cyclic phosphorothioates (**3**).

(b) *Phosphorothioylation of 2′,3′-ethoxymethylenadenosine* (**15**). As compared with dialkyl phosphoranilidates, dialkyl phosphoranilidothioates have an advantage being convertible into dialkyl phosphorodithioates, dialkyl [^{18}O] phosphorothioates, and dialkyl phosphoroselenothioates.[15] Having in mind the synthesis of new analogues of cAMP, we treated 2′,3′-ethoxymethylenadenosine (**15**) with 4-nitrophenylphosphoranilidochloridothioate in the presence of triethylamine and tetrazole.[22] The diastereoisomeric mixture of 2′,3′-ethoxymethylenadenosine 5′-[*O*-(4-nitrophenyl)phosphoranilidothioates] (**21**), after isolation from the reaction mixture, was treated with 80% acetic acid to give adenosine 5′-[*O*-(4-nitrophenyl)-phosphoranilidothioates] (**22**). The mixture was separated by column chromatography into 'fast' (R_P)-(**22**) (δ_P 61.4) and 'slow' (S_P)-(**22**) (δ_P 61.6) diastereoisomers. Each individual diastereoisomer of compound (**22**) in reaction with Bu^{t}OK in DMF solution was converted into the corresponding adenosine cyclic 3′,5′-phosphoranilidothioate (**23**) (Scheme 8). ^{31}P N.m.r. analysis of reaction mixture indicated that the process of

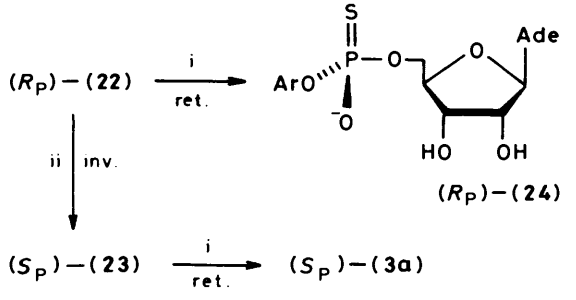


Scheme 8. *Reagents:* i, 4-$NO_2C_6H_4O(PhNH)P(S)Cl$–tetrazole–NEt_3; ii, 80% AcOH; iii, Bu^{t}OK–DMF

cyclisation (22) ⟶ (23) was stereospecific. The absolute configuration at phosphorus in compounds (23) was assigned on the basis of an empirical rule concerning the order of chemical shifts within the diastereoisomeric pairs of 2-anilino-4-methyl-2-oxo-1,3,2-dioxaphosphorinanes; 2-anilino-4-methyl-2-oxo-1,3,2-dioxaphosphorinane with an equatorially oriented exocyclic anilino function resonates at lower field than that with the axial anilino group, with respect to the 'plane' of the 1,3,2-dioxaphosphorinane ring system.[9] Thus, an isomer (23) with chemical shift δ_P 64.7 was assigned to have S_P configuration while its counterpart, resonating at δ_P 61.6, was assigned the R_P configuration. The veracity of this assignment was confirmed by stereoretentive conversion of the low-field isomer of compound (23) into (S_P)-(3a).

The elucidation of the stereochemistry of cyclisation (22) ⟶ (23) required the assignment of absolute configuration at phosphorus in diastereoisomers (22). Owing to earlier results reported from Eckstein's laboratory[23] the absolute configurations at phosphorus in diastereoisomers of adenosine 5′-[*O*-(4-nitrophenyl)phosphorothioate] (24) are known.

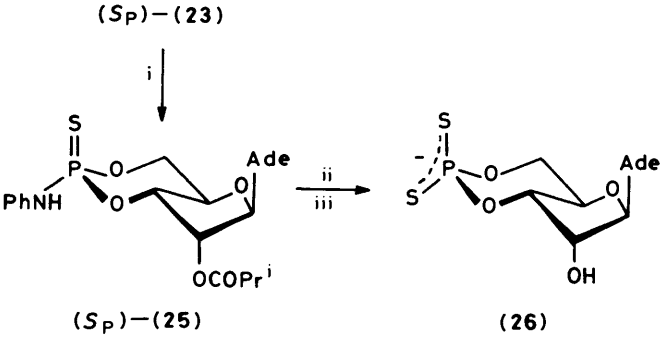
Reaction of each diastereoisomer (22) with sodium hydride–carbon dioxide proceeded smoothly without protection of the 2′-hydroxy function, and it was found that compound (22), precursor of (R_P)-(23) (see Scheme 8) in reaction with NaH–CO_2 gave (S_P)-(24), while its isomer gave (R_P)-(24). Since the conversion (22) ⟶ (24) proceeded with retention of configuration, the cyclisation (22) ⟶ (23), which is stereospecific, must occur with inversion of configuration (Scheme 9).



Scheme 9. *Reagents:* i, NaH–CO_2; ii, Bu^{t}OK–DMF

The relationship shown in Scheme 9 very clearly shows that intramolecular nucleophilic substitution at the phosphorus atom of compounds (22) proceeds according to an addition–elimination-type mechanism, without involvement of an intermediate metaphosphoramidate.[24]

The most striking feature observed during reaction of compounds (22) with potassium t-butoxide was the epimerisation of (R_P)-(23) in the course of its isolation by p.l.c. on silica gel plates with chloroform–methanol (9:1) as developing system. The reaction of (R_P)-(22) with Bu^{t}OK gave (S_P)-(23) (δ_P 64.7) as the main product. Isolation of compound (S_P)-(23) by p.l.c. did not change the chemical shift, and the reaction of (S_P)-(23) with NaH–CO_2 gave (S_P)-(3a), as expected. However, cyclisation of (S_P)-(22) led to (R_P)-(23) (δ_P 61.6). The ^{31}P n.m.r. spectrum of the raw reaction mixture did not contain the signal corresponding to (S_P)-(23). Purification of (R_P)-(23) by p.l.c. under the conditions specified above left the isomer (S_P)-(23). Since the 2′-hydroxy group of compounds (23) was not protected, the working hypothesis for epimerisation (R_P)-(23) ⟶ (S_P)-(23) implies the involvement of this group *via* a penta-co-ordinate strained intermediate which, *via* a permutational process, undergoes isomerisation followed by collapse with formation of the thermodynamically stable product. This hypothesis requires further experimental confirmation. Isolated (S_P)-(23) was used for the synthesis of the new analogue of cAMP, adenosine cyclic 3′-5′-phosphorodithioate (26). Its preparation in the reaction with NaH–CS_2 required former protection of 2′-hydroxy function, achieved by treatment of (S_P)-(23) with isobutyric anhydride. The resulting $O^{2'}$-isobutyryl-



Scheme 10. *Reagents:* i, $(Pr^iCO)_2O$; ii, NaH–CS_2; iii, 2M-NaOH

adenosine cyclic 3′-5′-phosphoranilidothioate (25) (Scheme 10), on treatment with NaH–CS_2, gave $O^{2'}$-isobutyryladenosine cyclic 3′,5′-phosphorodithioate, which under work-up procedure was deprotected to afford the desired dithiophosphate (26). Its electrophoretic mobility was identical with that of cAMP and cAMPS, and its ^{31}P n.m.r. chemical shift was consistent with those reported for other dialkyl phosphorodithioates (δ_P 110).

Conclusions

An increasing interest in the stereochemical approach to the elucidation of the mechanisms of action of nucleolytic enzymes, involving those responsible for the biosynthesis and biodegradation of cyclic nucleotides, prompted us to undertake some efforts towards the design of a general method for the synthesis of nucleoside cyclic 3′,5′-phosphoranilidates (2), which after separation into individual diastereoisomers can be stereospecifically converted into desired (R_P)- and (S_P)-nucleoside cyclic 3′,5′-phosphorothioates (3) and -[^{18}O]-phosphates (9). Preparation of nucleoside cyclic 3′,5′-phosphoranilidates (2), which seems to be straightforward, includes activation of an $N,O^{2'}$-protected (except for the uridine derivative, which requires $O^{2'}$-protection only) nucleoside 3′,5′-phosphate (1) with triphenylphosphine–carbon tetrachloride and subsequent reaction with aniline. The essential influence of the reaction medium on the diastereoisomeric ratio of the resulting products (2) has been observed. This observation creates the basis for partial control of stereoselectivity of this process (see entry 1 in Table 2).

If dimethylamine is used instead of aniline, the corresponding nucleoside cyclic 3′,5′-*N*,*N*-dimethylphosphoramidates (10) are produced. Diastereoisomers of compounds (2) and (10) are separable by chromatographic techniques. Assignment of absolute configuration at phosphorus in diastereoisomers of compounds (2) and (10) was based on the results of spectroscopic investigations using criteria previously developed in this laboratory, mainly on the measurements of absolute values of direct spin–spin couplings between phosphorus and nitrogen-15 nuclei. Conclusions from this assignment are confirmed by chemical transformation of (R_P)-(2a) into the 2′-deoxyadenosine cyclic 3′,5′-(R_P)-phosphoranilidate (8a), formerly studied by *X*-ray crystallography.[12] Besides the conversion of cyclic nucleotides into nucleoside cyclic 3′,5′-phosphoranilidates (2) another approach to the synthesis of precursors of phosphorothioate analogues of cAMP was developed — phosphorothioylation of 2′,3′-ethoxymethylenadenosine (15). This last approach allowed us to develop a

chemical route to both diastereoisomers of adenosine 5′-[*O*-(4-nitrophenyl)phosphorothioate] (**24**). Previously these were available only as the diastereoisomeric mixture, from which the S_P diastereoisomer had been isolated after digestion with snake venom phosphodiesterase.[23] Moreover, studies on cyclisation of 5′-[*O*-(4-nitrophenyl)phosphoranilidothioates] (**22**), which demonstrated the stereoselectivity of this process and led to adenosine cyclic 3′,5′-phosphoranilidothioates (**23**), allowed conclusions to be drawn about the mechanism of intramolecular substitution at phosphorus and the elimination–addition-type mechanism for this reaction to be discounted.

Although comparison of the two routes leading to the precursors of cAMPS, *i.e.* compounds (**2a**) and (**23**), indicates that the first one is the more efficient and generally applicable method of synthesis of nucleoside cyclic 3′,5′-phosphoranilidates, the advantage of adenosine cyclic 3′,5′-phosphoranilidothioates (**23**) available from the second approach should be emphasised. Access to diastereoisomers of compounds (**23**) allows the preparation of adenosine cyclic 3′,5′-[^{18}O]-phosphorothioates and adenosine cyclic 3′,5′-phosphorodithioates (**26**) *via* a Horner–Wadsworth–Emmons-type reaction of compounds (**23**) with NaH–PhCH^{18}O or NaH–CS_2, respectively.

The Horner–Wadsworth–Emmons-type reaction, when applied to all diastereoisomers of nucleoside cyclic 3′,5′-phosphoranilidates (**2**), gave diastereoisomers of nucleoside cyclic 3′,5′-phosphorothioates (**3**). This reaction was shown to be stereospecific, and it was confirmed, *via* cross-correlation, that it occurred, as expected, with retention of configuration. It has been demonstrated that diastereoisomers of compound (**2a**) may be used as the substrate for the synthesis of diastereoisotopomers of adenosine cyclic 3′,5′-[^{18}O]phosphate (**9a**). [^{18}O]Benzaldehyde was used as the source of ^{18}O and it is worthwhile to mention that this aldehyde can be easily prepared *via* acid-catalysed hydrolysis of benzylidenaniline with $H_2{}^{18}O$. To our surprise, however, the conversion (**2a**) ⟶ [^{18}O]cAMP was not fully stereospecific (*ca.* 90%) and the explanation of the partial epimerisation at phosphorus during PN ⟶ P[^{18}O] conversion awaits further studies. As an alternative route to diastereoisotopomers of [^{18}O]cAMP the $H_2{}^{18}O$–acid hydrolysis of phosphoramidate (**10a**) was exploited; it was shown that this reaction, under the described conditions, occurs with inversion of configuration.

Adenosine cyclic 3′,5′-phosphorothioates have found broad application in biochemical studies, and these are described in detail in a number of excellent reviews.[25] Recently, the synthesis of cGMPS diastereoisomers has been reported[26] and their use in biochemical studies was demonstrated.[27] It is expected that the recent discovery of cCMP in living systems, together with isolation of enzymes responsible for its biosynthesis and biotransformation,[28,29] will increase the interest in molecular aspects of their modes of action; phosphorothioate and [^{18}O]-phosphate analogues of cGMP, cUMP, and cCMP, thought to be the best stereochemical probes for elucidation of selected biochemical systems, are available.*

Experimental

^{1}H N.m.r. spectra were recorded at 60 MHz and 220 MHz with Perkin-Elmer R-12 and Bruker WP200SY spectrometers, respectively, with $SiMe_4$ as internal standard. ^{31}P N.m.r. spectra were obtained at 24.3 MHz (Jeol FT-FX-60) and 81 MHz (Bruker WP200SY). ^{13}C N.m.r. spectra were recorded at 15.03 MHz with a Jeol FT-FX-60 spectrometer. Positive chemical-shift values (p.p.m.) are assigned for compounds resonating downfield from 85% H_3PO_4 or Me_4Si, respectively, for ^{31}P or ^{1}H/^{13}C spectra. I.r. spectra were taken on a Carl-Zeiss-Jena Specord 71 spectrometer. Mass spectra were obtained on LKB-2091 (e.i.) and Varian-Mat 7 spectrometers (f.d.–m.s.). U.v. measurements were carried out on a Specord u.v.–vis (Carl-Zeiss-Jena) instrument.

T.l.c. and p.l.c. were performed on silica gel $60F_{254}$ and cellulose F_{254} plates (E. Merck). Silica gel column chromatography was performed on gels 70—230, 230—400 mesh, and Kieselgel 60H (E. Merck), respectively. The following developing solvent systems were applied: (A) $CHCl_3$–EtOH (6:1), (B) $CHCl_3$–MeOH (85:15), (C) Pr^{i}OH–water (85:15), (D) $CHCl_3$–MeOH (9:1), (E) $CHCl_3$–MeOH (12:1), (F) $CHCl_3$–MeOH (20:1), (G) $CHCl_3$–EtOH (98:2), (H) $CHCl_3$–MeOH (95:5), (I) $CHCl_3$–MeOH (30:1), (J) $CHCl_3$–Me_2CO (10:3), (K) 1M-NH_4OAc–EtOH (3:7), (L) Pr^{i}OH–NH_3(aq.)–water (7:1:1). Products were eluted from p.l.c. plates with chloroform–methanol (1:1), unless stated otherwise.

Carbon disulphide was dried over $MgSO_4$. Solvents were of commercial grade and were dried and distilled before use. Pyridine, dried over KOH, was refluxed with $KMnO_4$, distilled, dried over CaH_2, and redistilled. The fraction collected at 114—116 °C was stored over granulated CaH_2. DMF was purified by distillation with benzene and water, then dried over CaH_2 and redistilled. THF was dried over CaH_2, distilled, dried over NaH, distilled, and finally dried over $LiAlH_4$ and redistilled. NaH was used as 50% dispersion in mineral oil; weights given refer to this dispersion. All evaporations under reduced pressure were performed at bath temperature not exceeding 30 °C. cAMP and cGMP were purchased from Sigma Chemical Co.; cCMP and cUMP were prepared according to the methods described in the literature.[31,32]

N^6,N^6,$O^{2'}$-*Tribenzoyladenosine Cyclic* 3′,5′-*Phosphate* (**1a**).—Adenosine cyclic 3′,5′-phosphate (0.66 g, 2 mmol) was added to 0.5M-aqueous triethylamine (10 ml) and the resulting solution was evaporated to dryness under reduced pressure. Traces of water were removed by repeated coevaporation with anhydrous pyridine (3 × 10 ml). The triethylammonium salt of cAMP was dissolved in anhydrous pyridine (20 ml) and the resulting solution, after being cooled to 0 °C, was treated with freshly distilled benzoyl chloride (1.2 ml, 10 mmol). The reaction mixture, protected from light and atmospheric moisture, was left for 2 h at room temperature and was then cooled in ice and treated with water (5 ml). After 15 min the reaction mixture was extracted with chloroform (30 ml). The extract was evaporated to dryness and residual pyridine was removed by coevaporation with toluene (3 × 10 ml). The residue was dissolved in chloroform (50 ml) and the resulting solution was washed successively with 0.5M-HCl (3 × 5 ml) and water (3 × 15 ml) and dried over anhydrous $MgSO_4$. After evaporation of solvent, the solid residue was dissolved in benzene (5 ml). The minute amounts of triethylamine hydrochloride were filtered off and the filtrate was added to stirred diethyl ether (100 ml). The precipitated product was collected by centrifugation, washed with ether, and dried on air (0.90 g, 70%), m.p. 153—155 °C (from THF). T.l.c. on silica gel plates showed a single spot, R_F(C) 0.77; $\lambda_{max.}$(EtOH) 230 and 276 nm; δ_P($CHCl_3$) −2.92; δ_H($CDCl_3$) 8.75 (1 H, s, 8-H), 8.73 and 7.80 (4 H, dd, H_{ortho} in 6-Ph), 7.70—7.32 (9 H, m, ArH), 6.58 (1 H, br s, 1′-H), 6.12 (1 H, d, 2′-H), 5.40 (1 H, m, 3′-H), and 4.71—4.25 (3 H, m, 4′- and 5′-H_2); δ_C($CDCl_3$) only carbonyl carbon atoms: 171.96 (N^6-CO) and 164.53 ($O^{2'}$-CO); $\nu_{max.}$ (NEt_3 salt in $CHCl_3$) 1 710 cm^{-1}(CO); NH, NH_2, and OH frequencies absent.

N^2,$O^{2'}$-*Di-isobutyrylguanosine Cyclic* 3′,5′-*Phosphate* (**1b**).—Guanosine cyclic 3′,5′-phosphate (0.38 g, 1.1 mmol) was passed through an ion-exchange column of Dowex 50W × 8 (tri-n-

* After this manuscript was submitted for publication, the paper by Eckstein and Kutzke, describing the synthesis of all four nucleoside cyclic 3′,5′-phosphorothioates and the conditions for separation of each diastereoisomeric mixture into individual species, appeared.[30]

butylammonium form) and was then lyophilised. To a solution of this salt (0.53 g, 1 mmol) in anhydrous DMF (30 ml) was added 4-(dimethylamino)pyridine (0.25 g, 2 mmol) followed by freshly distilled isobutyric anhydride (3.3 ml, 20 mmol). Under these conditions, and on being vigorously stirred, the resulting mixture became homogenous after *ca.* 0.5 h. The reaction mixture, protected from light and atmospheric moisture, was left for 24 h at room temperature. Then, after being cooled in an ice-bath, the mixture was treated with water (5 ml) and the solution was stirred for 4 h, then concentrated under reduced pressure. The residual oil was extracted with a mixture (1:1) of hexane–diethyl ether (3 × 20 ml) for removal of isobutyric acid. The residual gum was then taken up in propan-2-ol–water (85:15; 2 ml) and applied to a silica gel column (3 × 40 cm; 230—400 mesh). The product was eluted with propan-2-ol–water (85:15) and, after evaporation of solvents, compound (**1b**) was obtained (0.50 g, 74.6%), R_F(C) 0.61; $\lambda_{max.}$(H_2O) 254 and 276 nm; δ_P(H_2O) −1.75.

N^4,$O^{2'}$-*Di-isobutyrylcytidine Cyclic* 3′,5′-*Phosphate* (**1c**).—Cytidine cyclic 3′,5′-phosphate[31] (0.31 g, 1 mmol) was added to 0.5M-aqueous triethylamine (5 ml) and the resulting solution was evaporated to dryness under reduced pressure. The residual oil was dissolved in dry pyridine (10 ml) and the solution was concentrated to give a foam. This procedure was repeated twice and the residual gum was then left under high vacuum at room temperature for 2 h. To the triethylammonium salt of cCMP was added dry pyridine (15 ml) and the mixture was heated to ~120 °C under nitrogen to achieve maximum dissolution. The still heterogeneous solution was cooled and freshly distilled isobutyric anhydride (3.3 ml, 20 mmol) was added. The solution was heated at 120 °C for 5—10 min and then left at room temperature for 24 h under dry nitrogen, protected from the light. The mixture was cooled in an ice-bath, water (5 ml) was added, and the solution was stirred for 4 h, then concentrated under reduced pressure, and residual oil was shaken and decanted three times with a mixture (1:1) of hexane–diethyl ether (3 × 20 ml) in order to remove an excess of isobutyric acid. The gum was dissolved in chloroform (5 ml) and this solution was dropped into a mixture (1:1) of hexane–diethyl ether (150 ml). The precipitated pale-yellow product was filtered off, washed with diethyl ether, and dried under reduced pressure (0.426 g, 78%). T.l.c. on silica gel plates showed a single spot, R_F(C) 0.55; $\lambda_{max.}$(H_2O) 278 nm; δ_P(H_2O) −2.66; f.d.–m.s. [free acid of (**1c**)] *m/z* 445 ($M^{+\cdot}$).

$O^{2'}$-*Benzoyluridine Cyclic* 3′,5′-*Phosphate* (**1d**).—The triethylammonium salt of uridine cyclic 3′,5′-phosphate[32] (0.41 g, 1 mmol) was dissolved in dry pyridine (10 ml) and the solution was concentrated to give a foam. This was repeated twice and the residue was then left at high vacuum at room temperature for 2 h. Dry pyridine (15 ml) was added and the resulting solution, after being cooled to 0 °C, was treated with freshly distilled benzoyl chloride (0.6 ml, 5 mmol). The reaction mixture, protected from light and atmospheric moisture, was stirred for 4 h at room temperature and then, after being cooled to −5 °C, was treated with water (3 ml). The solution was stirred during 15 min, and then extracted with chloroform (3 × 15 ml). The extract was evaporated to dryness and residual pyridine was removed by coevaporation with toluene (3 × 5 ml). The residue was dissolved in chloroform (4 ml) and this solution was dropped into diethyl ether (150 ml). The precipitated product (**1d**) was filtered off, and washed with diethyl ether (5 ml). Further purification was achieved by means of column chromatography on silica gel (70—230 mesh) with elution by chloroform, chloroform–methanol (4:1), and finally chloroform–methanol (3:1). The yield of triethylammonium salt of compound (**1d**) was 0.38 g (74.4%); R_F(C) 0.64; $\lambda_{max.}$(MeOH) 264 nm; δ_P($CHCl_3$) −3.22.

General Procedure for Conversion of Ribonucleoside Cyclic 3′,5′-*Phosphates into Ribonucleoside Cyclic* 3′,5′-*Phosphoranilidates* (**1**) ⟶ (**2**).—A solution of a cyclic phosphate (**1**) (3 mmol) in pyridine (5 ml) was evaporated to dryness under reduced pressure. To the residue was added solid triphenylphosphine (2.83 g, 10.8 mmol) and the mixture was dried by repeated coevaporation with pyridine (3 × 5 ml) under high vacuum. The residual solid was then dissolved in pyridine (15 ml) and to this solution was added carbon tetrachloride (0.87 g, 9 mmol), followed by freshly distilled aniline (1.62 g, 18 mmol) added dropwise. This reaction mixture was left for 12 h at room temperature, cooled to 0 °C, and treated with water (5 ml, dropwise); products were extracted with chloroform (3 × 20 ml). The extracts were washed with water (3 × 10 ml) and dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The crude product (**2**) was purified on a silica gel (70—230 mesh; 200 g) column with chloroform–methanol (20:1) as eluting system. The following procedures were applied for separation of diastereoisomeric mixtures of compounds (**2**).

(a) In the case of (R_P + S_P)-(**2a**), the eluted diastereoisomeric mixture [R_F(F) 0.28 and 0.25] was separated by subsequent chromatography on silica gel (Kieselgel 60H; 120 g) with chloroform–ethanol (98:2) as eluant. Fractions containing 'fast' (S_P)-(**2a**) were collected, the solvent was evaporated off, and the residual solid (0.28 g) was dissolved in chloroform (2 ml) and the solution was added dropwise to a stirred mixture of diethyl ether–hexane (1:1; 150 ml). The precipitated product (S_P)-(**2a**) was collected by centrifugation (0.23 g, 10.7%); δ_P($CDCl_3$) 1.41; f.d.–m.s. *m/z* 716 ($M^{+\cdot}$).

Similarly, fractions containing 'slow' (R_P)-(**2a**) were evaporated to give the product (0.59 g), which was crystallised from chloroform (0.44 g, 20.4%), m.p. 155—157 °C; δ_P ($CDCl_3$) −1.62; f.d.–m.s. *m/z* 716 ($M^{+\cdot}$).

(b) In the case of (R_P + S_P)-(**2b**), separation into individual diastereoisomers was achieved by p.l.c. (system H). (S_P)-(**2b**) was obtained in 7.5% yield; R_F(H) 0.27; $\lambda_{max.}$(MeOH) 225, 257, and 274 nm; δ_P($CDCl_3$) 2.12; and (R_P)-(**2b**) in 17% yield; R_F(H) 0.21; $\lambda_{max.}$(MeOH) 225, 257, and 274 nm; δ_P($CDCl_3$) −1.44.

(c) In the case of (R_P + S_P)-(**2c**), separation into individual diastereoisomers was performed by p.l.c. (system E) to afford (R_P)-(**2c**) in 10.6% yield; R_F(E) 0.70; δ_P($CHCl_3$) −0.64; f.d.–m.s. *m/z* 520 ($M^{+\cdot}$); and (S_P)-(**2c**) in 7.9% yield; R_F(E) 0.60; δ_P($CHCl_3$) 0.97; f.d.–m.s. *m/z* 520 ($M^{+\cdot}$).

(d) In the case of (R_P + S_P)-(**2d**), the eluted diastereoisomeric mixture was separated by subsequent chromatography on silica gel (Kieselgel 60H) with chloroform–methanol (30:1) as eluant. (R_P)-(**2d**) was obtained in 24.1% yield; R_F(E) 0.74; δ_P($CHCl_3$) −1.34; e.i.–m.s. *m/z* 485 ($M^{+\cdot}$); and (S_P)-(**2d**) in 7.8% yield; R_F(E) 0.67; δ_P($CHCl_3$) 0.54; e.i.–m.s. *m/z* 485 ($M^{+\cdot}$).

Synthesis of N^6,N^6,$O^{2'}$-*Tribenzoyladenosine Cyclic* 3′,5′-*N,N-Dimethylphosphoramidates* (**10a**) *and* N^2,$O^{2'}$-*Di-isobutyrylguanosine Cyclic* 3′,5′-*N,N-Dimethylphosphoramidates* (**10b**).—The analogous procedure to that described for the synthesis of compounds (**2**) was applied, with dimethylamine in pyridine solution instead of aniline.

(a) In the case of (R_P + S_P)-(**10a**), the diastereoisomeric mixture was separated by chromatography on silica gel [60H; chloroform–ethanol (98:2)]. (R_P)-(**10a**) was obtained in 7% yield, R_F(G) 0.25; δ_P($CHCl_3$) +6.93; f.d.–m.s. *m/z* 670 ($M^{+\cdot}$); and (S_P)-(**10a**) in 14.3% yield, R_F(G) 0.28; δ_P($CHCl_3$) +7.98; f.d.–m.s. *m/z* 670 ($M^{+\cdot}$).

(b) In the case of (R_P + S_P)-(**10b**), the diastereoisomeric mixture was separated by p.l.c. [chloroform–methanol (95:5)]. (R_P)-(**10b**) was obtained in 6.4% yield, R_F(H) 0.29;

$\lambda_{max.}$(MeOH) 251 and 279 nm; $\delta_P(CHCl_3)$ 6.21; f.d.–m.s. *m/z* 514 ($M^{+\cdot}$); and (S_P)-(**10b**) in 17.2% yield R_F(H) 0.35; $\lambda_{max.}$(MeOH) 257 and 277 nm; $\delta_P(CHCl_3)$ 7.83; f.d.–m.s. *m/z* 514 ($M^{+\cdot}$).

General Procedure for the Conversion of Ribonucleoside Cyclic 3',5'-Phosphoranilidates into Ribonucleoside Cyclic 3',5'-Phosphorothioates (**2**) ⟶ (**3**).—Compound (R_P)-(**2**) (0.5 mmol) was dissolved in dry DME and the solution was treated with NaH (0.05 g). The resulting mixture was vigorously stirred for 45 min at room temperature and then for 10 min at 35 °C. After the mixture had been cooled, CS_2 (0.38 g, 5 mmol) was added and the reaction mixture was stirred for 3 h at room temperature. Then dry pentane (20 ml) was added, and the precipitate was isolated by centrifugation and very carefully transferred into a mixture of ethanol (5 ml) and 2M-sodium hydroxide (2 ml). After 5 min an excess of Dowex 50W × 8 (pyridinium form) ion-exchange resin was added for removal of sodium ions. The resin was filtered off, and washed with methanol, and the filtrate was concentrated. The solid residue was dissolved in methanol saturated (at 0 °C) with dry ammonia (50 ml). The mixture was left for 24 h at room temperature, and was then concentrated, and the residue was dissolved in water (10 ml). This solution was extracted with diethyl ether (3 × 5 ml) and the aqueous fraction was chromatographed on a DEAE-Sephadex A-25 column. The product was eluted with a linear gradient of triethylammonium hydrogen carbonate buffer (0.05—0.6M). The pooled fractions of compound (S_P)-(**3**) were evaporated and buffer was removed by repeated evaporations with ethanol.

In an analogous way (S_P)-(**2**) was converted into (R_P)-(**3**). The yields and δ_P chemical shifts of isomers (S_P)-(**3**) and (R_P)-(**3**) are given in Table 3.

Attempted Synthesis of Phosphoranilidates (**2a**) via *Phosphoric Sulphonic Anhydride*.—Compound (**1a**) (0.064 g, 0.1 mmol) was dissolved in benzene (1 ml) and HMDS (0.024 g, 0.15 mmol) was added. The ^{31}P n.m.r. spectrum recorded after 1 h showed two signals corresponding to a diastereoisomeric mixture of *O*-trimethysilyl ester (**4a**) (δ_P −12.2 and −16.4). After removal of the solvent under reduced pressure the residue was dissolved in dry THF (1 ml) and toluene-*p*-sulphonic anhydride (0.32 g, 1 mmol) was added. The ^{31}P n.m.r. spectrum indicated the presence of only one product, (**5a**), with a signal at δ_P −21.8. To the reaction mixture was added aniline (0.56 g, 6 mmol). The precipitate was filtered off and the filtrate was concentrated to small volume and analysed by ^{31}P n.m.r. spectroscopy [δ_P (THF) 0.7, −1.9, and −3.8 in the proportions (S_P)-(**2a**):(R_P)-(**2a**):(**1a**) 4:2:6, respectively]. Identification of products was based on comparison of chemical shifts of genuine samples of both compounds (**2a**) and (**1a**), sequentially introduced into the reaction mixture resulting from the degradation of the mixed anhydride (**5a**) with aniline.

N^6-*Benzoyladenosine Cyclic 3',5'-Phosphoranilidate* (R_P)-(**6a**).—Into a solution of compound (R_P)-(**2a**) (0.13 g, 0.18 mmol) in ethanol (5 ml) was added 2M-NaOH (0.5 ml). After 5 min an excess of sodium ions was removed by Dowex 50W × 8 (pyridinium form) ion-exchange resin. The resin was filtered off and the filtrate was evaporated to dryness. The crude product was chromatographed on p.l.c. plates (silica gel; system D) to give the title compound (0.062 g, 68.1%); R_F(D) 0.35; $\delta_P(CHCl_3)$ −1.57.

N^6-*Benzoyl*-$O^{2'}$-*thiobenzoyladenosine Cyclic 3',5'-Phosphoranilidate* (R_P)-(**7a**).—Into a solution of *N,N*-dimethylbenzamide (0.42 g, 2.85 mmol) in dry methylene dichloride (10 ml) was introduced phosgene (2 ml) at −20 °C. The solution was stirred for 12 h at room temperature,[13] and the solvent and excess of phosgene were removed under reduced pressure. The residue was dissolved in dry methylene dichloride (10 ml) and phosphoranilidate (R_P)-(**6a**) was added as a solid. The mixture was stirred for 12 h, pyridine (1.5 ml) was added, and a stream of dry hydrogen sulphide (generated in a Kipps apparatus) was bubbled through the mixture for 10 min. The resulting solution was washed successively with water (2 × 10 ml), 4M-sulphuric acid (2 × 10 ml), and saturated aqueous sodium hydrogen carbonate, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was dissolved in chloroform and chromatographed on silica gel plates (system D as eluant) to afford the thiobenzoate (**7a**) (0.032 g, 41.8%), R_F(D) 0.50; $\delta_P(CHCl_3)$ −3.02.

2'-Deoxyadenosine Cyclic 3',5'-Phosphoranilidate (R_P)-(**8a**).—To a solution of compound (R_P)-(**7a**) (0.032 g, 0.051 mmol) in refluxing toluene (2.5 ml) was added a solution of tri-n-butyltin hydride (0.015 ml) in toluene (2.5 ml) dropwise during 1 h. The mixture was heated under reflux for an additional 30 min, and then was left at room temperature for 12 h. The solvent was removed under reduced pressure, and the residue was treated with methanol saturated with dry ammonia (5 ml). After 24 h the product was isolated from the reaction mixture by p.l.c. (system D) (0.007 g, 35.2%), R_F(D) 0.12; $\delta_P(C_5H_5N)$ −3.53; $\lambda_{max.}$(EtOH) 260.5 nm. This final product appeared to be identical (t.l.c., ^{31}P n.m.r.) with the genuine (R_P)-cdAMP anilidate (**8a**).[11]

Solvolysis of Compounds (R_P)-(**10a**) *and* (S_P)-(**10a**) *in the Presence of* $H_2{}^{18}O$.—N^6,N^6,$O^{2'}$-Tribenzoyladenosine cyclic 3',5'-(S_P)-*N,N*-dimethylphosphoramidate (**10a**) (0.07 g, 0.11 mmol) was dissolved in a mixture of ethanol (0.5 ml) and pyridine (0.5 ml). This solution was treated at room temperature with 2M-NaOH (0.5 ml) and left for 5 min. An excess of Dowex 50W × 8 (pyridinium form) ion-exchange resin was added for removal of sodium ions. The resin was filtered off, and washed with water, and the filtrate was concentrated under reduced pressure. To the solid residue was added methanol (15 ml) saturated (at 0 °C) with dry ammonia. After 24 h the reaction mixture was concentrated to dryness, and the residue was dissolved in a small amount of methanol and chromatographed on p.l.c. plates with chloroform–ethanol (6:1) as developer. Adenosine cyclic 3',5'-(S_P)-*N,N*-dimethylphosphoramidate was obtained (0.024 g, 64%), R_F(A) 0.33; $\lambda_{max.}$(MeOH) 260 nm; $\delta_P(CHCl_3)$ 8.66.

This last compound (0.057 g, 0.16 mmol) was dissolved in dry THF (4 ml) containing methanesulphonic acid [1.5 ml, 0.023 mol; freshly distilled from $(MeSO_2)_2O$] and $H_2{}^{18}O$ (1.9 ml, 0.092 mol; 73% ^{18}O). The reaction mixture was left at room temperature for 8 h and then concentrated under reduced pressure. The residue was dissolved in THF (3 ml) and neutralised with aqueous ammonia. The product was isolated from reaction mixture by p.l.c. (silica gel; system C) and chromatographed again (Cellulose plates; system L). After lyophilisation, compound (S_P)-(**9a**) was obtained (0.017 g, 30%) as a white powder, R_F(C) 0.47; $\lambda_{max.}(H_2O)$ 261 nm; $\delta_P(H_2O)$ −2.11.

(b) In the same way compound (R_P)-(**10a**) (0.095 g, 0.15 mmol) was converted into adenosine cyclic 3',5'-(R_P)-*N,N*-dimethylphosphoramidate (0.03 g, 58.7%) [R_F(A) 0.31; $\lambda_{max.}$(MeOH) 260 nm; $\delta_P(CHCl_3)$ 7.75] and this compound was hydrolysed with methanesulphonic acid (0.75 ml, 0.011 mol) and $H_2{}^{18}O$ (0.95 ml, 0.046 mol; 73% ^{18}O) to afford phosphate (R_P)-(**9a**) (0.01 g, 36.1%), R_F(C) 0.47; $\lambda_{max.}(H_2O)$ 261 nm; $\delta_P(H_2O)$ −2.11.

Stereochemical Analysis of Phosphates (S_P)-(**9a**) *and* (R_P)-(**9a**).—(a) Compound (S_P)-(**9a**) (0.017 g, 0.05 mmol) was

dissolved in water (5 ml) and the solution was passed through a short column of Dowex 50W × 2 (K^+ form). After concentration to a small volume (~2 ml), the mixture was treated with 18-crown-6 (0.013 g, 0.05 mmol) and concentrated to dryness again under reduced pressure. Traces of water were removed by repeated coevaporation with dry DMF. The residue was dissolved in $(CD_3)_2SO$ (0.4 ml) and MeI (0.1 ml) was added. The reaction mixture was left for 12 h at room temperature, protected from light and moisture. Then an excess of methyl iodide was removed under reduced pressure, 8-hydroxyquinoline (0.003 g) was added, and the solution was filtered into an n.m.r. tube (5 mm). Analysis of the ^{31}P n.m.r. spectrum (Figure) gave the following data:

(**11a**) $\delta_P[(CD_3)_2SO]$ −3.74, Δδ 0.0415
(**12a**) $\delta_P[(CD_3)_2SO]$ −2.49, Δδ 0.0168.

(b) In the same way, compound (R_P)-(**9a**) was converted into a mixture of methyl esters (**13a**) and (**14a**). Analysis of the ^{31}P n.m.r. spectrum gave

(**13a**) $\delta_P[(CD_3)_2SO]$ −3.72, Δδ 0.0143
(**14a**) $\delta_P[(CD_3)_2SO]$ −2.52, Δδ 0.0428.

Adenosine Cyclic 3′,5′-(S$_P$)-[^{18}O]Phosphate (**9a**) *Obtained from Phosphoranilidate* (**2a**).—(a) [^{18}O]*Benzaldehyde*. A solution of benzylideneaniline (3.62 g, 0.02 mol) in dry THF (70 ml) was saturated with dry HCl at room temperature until the precipitation of benzylideneanilinium chloride was completed (20 min). Then $H_2{}^{18}O$ (0.45 g, 0.025 mol; 98% ^{18}O) was added in one portion. The reaction mixture was shaken for 30 min at room temperature and anilinium chloride was filtered off. The filtrate was concentrated and to the residue was added diethyl ether (70 ml). After filtration of the mixture the filtrate was concentrated again and the residue was distilled. [^{18}O]Benzaldehyde was collected, b.p. 62 °C/16 mmHg; n_D^{20} 1.5457 (1.55 g, 73%). The ^{18}O enrichment in benzaldehyde (86.5%) was determined by mass spectrometry (m.i.d.). The measurements were made for ions in the range *m/z* 105—108.

(b) To a solution of compound (R_P)-(**2a**) (0.358 g, 0.5 mmol) in anhydrous THF (10 ml) was added NaH (0.05 g). The resulting mixture was vigorously stirred for 45 min at room temperature and then for 10 min at 35 °C. After the mixture had cooled, [^{18}O]benzaldehyde (0.541 g, 5 mmol; 86.5% enriched in ^{18}O) was added and the reaction mixture was stirred for 2 h at 30 °C. The rest of the procedure was analogous to that of the preparation of ribonucleoside cyclic 3′,5′-phosphorothioates (**3**) (*vide supra*). In this way, compound (S$_P$)-(**9a**) (triethylammonium salt) was obtained (0.060 g, 28%), $\delta_P(H_2O)$ −0.1; R_F(C) 0.47 — identical with commercially available cAMP.

2′,3′-Ethoxymethylenadenosine 5′-[O-(2-Chlorophenyl)phosphoranilidates] (R$_P$ + S$_P$)-(**16**).—A suspension of 2′,3′-ethoxymethylenadenosine (**15**)[33] (0.323 g, 1 mmol) in dry acetonitrile (3 ml) was added to a stirred suspension of 1,2,4-triazole (0.207 g, 3 mmol) and 2-chlorophenyl phosphoranilidochloridate[34] (0.601 g, 2 mmol) in dry acetonitrile (10 ml). After 4 days the solution was concentrated, the residual oil was dissolved in chloroform (30 ml), and the solution was washed successively with 10% aqueous $NaHCO_3$ (3 × 10 ml) and with water (3 × 10 ml). The dried organic layer was concentrated and the crude product was purified on silica gel (70—230 mesh) with chloroform–ethanol (6:1) as eluant. Fractions containing the title product were collected and, after evaporation of the solvent, compound (**16**) was obtained as a mixture of two diastereoisomers (0.353 g, 60%); R_F(A) 0.55; $\delta_P(C_5H_5N)$ −1.77 and −1.91; f.d.–m.s. *m/z* 588 ($M^{+\bullet}$).

Adenosine 5′-[O-(2-Chlorophenyl)phosphoranilidates] (R$_P$ + S$_P$)-(**17**).—A solution of compound (**16**) (0.589 g, 1 mmol) in 80% acetic acid (25 ml) was left at room temperature for 2 days. Then acetic acid was evaporated off and dry methanol (15 ml) was added to the residual oil. After 24 h the reaction mixture was concentrated and the crude product, consisting of a mixture of two diastereoisomers (**17**), was separated by column chromatography (silica gel 230—400 mesh) with chloroform–ethanol (6:1) as eluant. The separation was incomplete and gave pure isomer (R_P)-(**17**) (0.032 g), isomer (S_P)-(**17**) (0.015 g), and the remaining mixture of both diastereoisomers (0.420 g). Total yield of (R_P + S_P)-(**17**) was 0.467 g (87.8%). For (R_P)-(**17**), R_F(A) 0.34; $\delta_P(C_5H_5N)$ −1.77; f.d.–m.s. *m/z* 532 ($M^{+\bullet}$); and for (S_P)-(**17**); R_F(A) 0.30; $\delta_P(C_5H_5N)$ −1.62; f.d.–m.s. *m/z* 532 ($M^{+\bullet}$).

Adenosine Cyclic 3′,5′-Phosphoranilidates (**18**).—To a solution of a mixture of the two diastereoisomers of compound (**17**) (0.80 g, 1.5 mmol) in dry DMSO (5 ml) was added freshly prepared potassium t-butoxide (5.04 g, 45 mmol). The reaction mixture was left at room temperature for 3 days, and was then cooled to 0 °C and Dowex 50W × 8 (pyridinium form) was added to remove the potassium ions. The ion-exchange resin was filtered off, and washed with pyridine, and the filtrate was concentrated. Traces of pyridine were removed by repeated coevaporation with toluene (3 × 5 ml). The residual oil was dissolved in methanol and purified by p.l.c. (system B). In this manner a mixture of both diastereoisomers (**18**) was obtained (0.145 g, 24%), $\delta_P(C_5H_5N)$ 1.53 and −3.00; f.d.–m.s. *m/z* 404 ($M^{+\bullet}$).

N^6,N^6,O$^{2'}$-Tribenzoyladenosine Cyclic 3′,5′-Phosphoranilidates (**2a**) *from Compounds* (**18**).—A mixture of the two diastereoisomers (**18**) (0.60 g, 1.5 mmol) in the ratio (R_P:S_P) 5:7 was dried by repeated co-evaporation with pyridine (3 × 3 ml) and was then dissolved in pyridine (5 ml). To this solution at 0 °C was added freshly distilled benzoyl chloride (0.70 g, 5.0 mmol). The reaction mixture was left for 2 h at room temperature and then was poured into ice–water. The water-insoluble product was filtered off and then extracted with chloroform (3 × 15 ml). The extracts were washed with water and evaporated. The crude product (**2a**) was purified by p.l.c. (system J) (0.342 g, 32%), $\delta_P(C_5H_5N)$ 0.87 and −3.20 in the ratio 1:10 (S_P:R_P).

2′,3′-Ethoxymethylenadenosine 5′-[O-(4-Nitrophenyl)phosphoranilidothioates] (R$_P$ + S$_P$)-(**21**).—To a cooled solution of 4-nitrophenyl phosphoranilidochloridothioate[35] (0.49 g, 1.5 mmol) and tetrazole (0.22 g, 3 mmol) in dry acetonitrile (10 ml) at 0 °C were added triethylamine (0.3 g, 3 mmol) and 2′,3′-ethoxymethylenadenosine (**15**) (0.32 g, 1 mmol). The mixture was stored at room temperature for 4 days. The precipitate was filtered off, then washed with chloroform, and the filtrate was evaporated to dryness. The crude product was chromatographed on a short column of silica gel (230—400 mesh; 70 g) with chloroform–methanol (25:1), followed by chloroform–methanol (9:1) as eluant, to give compounds (**21**) (0.33 g, 53.6%) as a mixture of two diastereoisomers in *ca.* 1:1 ratio; R_F(D) 0.73; $\delta_P(CHCl_3)$ 60.67 and 60.91; f.d.–m.s. *m/z* 615 ($M^{+\bullet}$).

Adenosine 5′-[O-(4-Nitrophenyl)phosphoranilidothioates] (R$_P$)-(**22**) *and* (S$_P$)-(**22**).—A solution of compound (R_P + S_P)-(**21**) (0.615 g, 1 mmol) in 80% acetic acid (25 ml) was left at room temperature for 2 days. The acetic acid was evaporated off under reduced pressure and dry methanol (15 ml) was added to the residual oil. After 24 h the resulting reaction mixture was concentrated. The crude product, consisting of only a mixture of

two diastereoisomers of compound (**22**) (0.52 g, 93%), was separated by column chromatography (Kieselgel 60H; 50 g) in chloroform–methanol (30:1) as eluant. The separation was incomplete and gave pure (R_P)-(**22**) (0.190 g) and (S_P)-(**22**) (0.171 g), and a mixture of both diastereoisomers (0.103 g). Total yield was 0.464 g (83%).

For (R_P)-(**22**) R_F(D) 0.32; $\delta_P(CDCl_3)$ 61.36; f.d.–m.s. *m/z* 559 ($M^{+\cdot}$); and for (S_P)-(**22**) R_F(D) 0.27; $\delta_P(CDCl_3)$ 61.57; f.d.–m.s. *m/z* 559 ($M^{+\cdot}$).

Adenosine Cyclic 3′,5′-Phosphoranilidothioates (S_P)-(**23**) *and* (R_P)-(**23**).—(a) A solution of compound (R_P)-(**22**) (0.25 g, 0.45 mmol) in DMF (15 ml) was added to freshly prepared potassium t-butoxide (1.5 g, 13.5 mmol) and the reaction mixture was left for 48 h at room temperature with exclusion of moisture. After the mixture had been cooled to 0 °C, acetic acid (*ca.* 3 ml) was added for neutralisation. The solution was evaporated under reduced pressure and the residue was treated with pyridine (10 ml). The precipitated inorganic salt was filtered off and the filtrate was concentrated. Traces of pyridine were removed by repeated coevaporation with toluene (3 × 3 ml). The residue was dissolved in methanol and chromatographed (p.l.c., system D) to afford compound (S_P)-(**23**) (0.047 g, 24.9%), R_F(D) 0.41; $\delta_P(C_5H_5N)$ 64.73; f.d.–m.s. *m/z* 420 ($M^{+\cdot}$).

Besides (S_P)-(**23**), an interesting by-product was also isolated (7 mg, 3.7%). This product was characterised as the 2′-formyl derivative of compound (S_P)-(**23**): R_F(D) 0.53; $\delta_P(C_5H_5N)$ 64.71; f.d.–m.s. *m/z* 448 ($M^{+\cdot}$). When dissolved in methanol, after a few hours at room temperature this derivative was transformed into its parent, (S_P)-(**23**).

To confirm the structure of this by-product the reaction of compound (S_P)-(**23**) with formic anhydride was carried out. Chromatographic mobility and u.v. and ^{31}P n.m.r. spectra of the product were identical with those for the by-product from the cyclisation process. It is assumed that the formyl group derives from DMF, which under the reaction conditions formylates the 2′-hydroxy function.

(b) In the same way compound (S_P)-(**22**) (0.25 g, 0.45 mmol) was converted into (R_P)-(**23**) in 16.7% yield; $\delta_P(C_5H_5N)$ 61.62; f.d.–m.s. *m/z* 420 ($M^{+\cdot}$). Compound (R_P)-(**23**) underwent epimerisation to (S_P)-(**23**) during chromatography on silica gel plates.

Adenosine 5′-[O-(4-Nitrophenyl)phosphorothioates] (R_P)-(**24**) *and* (S_P)-(**24**).—To a vigorously stirred solution of compound (R_P)-(**22**) (0.056 g, 0.1 mmol) in anhydrous DMF (3 ml) was added NaH (0.01 g). After 30 min, dry carbon dioxide was passed into the mixture during a further 15 min. To remove excess of NaH the reaction mixture was filtered through Celite, which was then carefully washed with DMF. The combined filtrates were treated with an excess of Dowex 50W × 8 (pyridinium form). The resin was filtered off and washed with pyridine. The filtrate was evaporated to dryness and traces of pyridine were removed by repeated coevaporation with toluene. The residue was dissolved in water (5 ml) and extracted with diethyl ether (3 × 2 ml). The water fraction was concentrated to small volume and the residue was subjected to p.l.c. (Cellulose F; system K). The product was eluted from the adsorbent with water and was then passed through a column with Biogel P-2 to afford compound (R_P)-(**24**) (0.019 g, 38%) R_F(K) 0.58; $\delta_P(D_2O)$ 51.27; $\lambda_{max.}(H_2O)$ 263.5 nm.

In the same way, isomer (S_P)-(**22**) (0.084 g, 0.15 mmol) was converted into compound (S_P)-(**24**) in 34% yield; R_F(K) 0.58; $\delta_P(D_2O)$ 51.00; $\lambda_{max.}(H_2O)$ 263.5 nm.

Conversion of Adenosine Cyclic 3′,5′-Phosphoranilidothioate (S_P)-(**23**) *into Adenosine Cyclic 3′,5′-Phosphorothioate* (S_P)-(**3a**).—The reaction was carried out in a manner analogous to that described for compounds (**24**) (*vide supra*). Thus, compound (S_P)-(**23**) (0.042 g, 0.1 mmol) was converted, by reaction with NaH–CO_2, into (S_P)-(**3a**) in 55% yield. Characteristics of this product are given in Table 3.

O$^{2'}$-Isobutyryladenosine Cyclic 3′,5′-Phosphoranilidothioate (**25**).—Compound (**23**) (0.084 g, 0.2 mmol) was dissolved in DMF (2 ml). To this solution was added freshly distilled isobutyric anhydride (0.16 g, 20 mmol). The reaction mixture was left for 10 h at room temperature, and was then cooled to 0 °C; water (1 ml) was added and the reaction mixture was evaporated under reduced pressure. The crude product was purified by p.l.c. (system D) (0.058 g, 60%), $\delta_P(CHCl_3)$ 66.46.

Adenosine Cyclic 3′,5′-Phosphorodithioate (**26**).—Phosphoranilidothioate (**25**) (0.035 g, 0.072 mmol) was dried by repeated coevaporation with pyridine (3 × 1 ml) under reduced pressure. It was then dissolved in pyridine (2 ml) and the solution was treated with an excess of NaH (20 mg). After 30 min, CS_2 (0.5 ml) was added and the reaction mixture was maintained for 3 h at room temperature, then cooled to 0 °C. Water (2 ml) was added carefully under nitrogen. After 10 min, an excess of the pyridinium form of Dowex 50W × 8 ion-exchange resin was added to remove sodium ions. The resin was filtered off, the filtrate was concentrated under reduced pressure, and the residue was dissolved in water (2 ml) and placed on a DEAE Sephadex A-25 column. The product was eluted with a linear gradient of triethylammonium hydrogen carbonate buffer (0.05—0.8M). After removal of the buffer by repeated evaporation with ethanol, compound (**26**) was obtained (0.02 g, 61%); $\delta_P(H_2O)$ 110; $\lambda_{max.}(H_2O)$ 263 nm.

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