Stereospecific Synthesis of Adenosine 3′,5′-(S_p)- and -(R_p)-Cyclic Phosphorothioates (cAMPS)

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Summary *NNO*$^{2'}$-Tribenzoyladenosine 3′,5′-cyclic phosphate (**3**) was converted into a diastereoisomeric mixture of *NNO*$^{2'}$-tribenzoyladenosine 3′,5′-cyclic phosphoranilidates (**4**); after separation of these diastereoisomers, the corresponding adenosine 3′,5′-cyclic phosphorothioates (**1**, cAMPS) were obtained and the absolute configuration at the P atom in the diastereoisomers of (**4**) and (**1**) was established by ^{31}P n.m.r. spectroscopy.

THE easy assignment of *cis–trans* geometry to chiral 2-anilido-2-oxo-1,3,2-dioxaphosphorinans[1] and the recently demonstrated simple separation of diastereoisomeric nucleoside phosphoranilidates,[2] as well as the stereospecific conversion of organic phosphoranilidates into the corresponding phosphorothioates, [3,4] have provided a means for the determination of the absolute configuration at the P atom of diastereoisomers of adenosine 3′,5′-cyclic phosphorothioate (**1**, cAMPS). Although the chemical synthesis of a mixture of the two diastereoisomers of cAMPS has already been described,[5] various attempts to separate these isomers, which would be of considerable interest for enzyme mechanistic studies, have not been successful.[6]

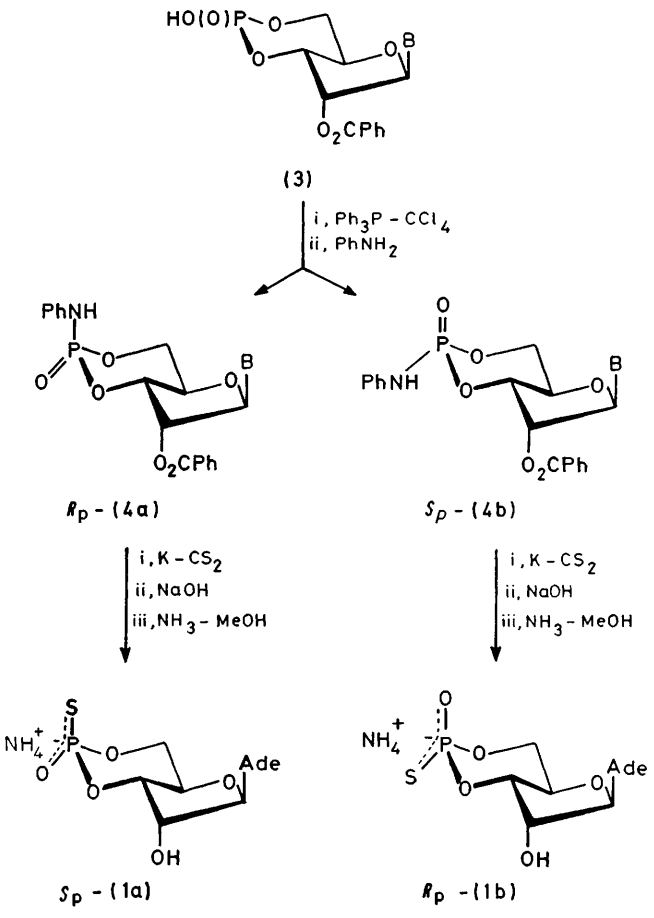
The stereospecific synthesis of both diastereoisomers of (**1**) has been achieved as follows. Treatment of a pyridine solution of adenosine 3′,5′-cyclic phosphate (**2**, cAMP, 2 mmol) with benzoyl chloride (30 mmol) gave the *NNO*$^{2'}$-tribenzoyladenosine 3′,5′-cyclic phosphate (**3**) in 78% yield. Reaction of (**3**) (0·86 mmol) with triphenylphosphine–carbon tetrachloride (2·58 mmol) in pyridine containing aniline (5·16 mmol)[7] gave a mixture of diastereoisomers (2:1) of the phosphoranilidate (**4**) in 27% yield. These diastereoisomers were separated by preparative t.l.c.† (system A); (**4a**) $\delta(^{31}P)$ +3·22 p.p.m., (**4b**), $\delta(^{31}P)$ −0·81 p.p.m.‡ Each diastereoisomer of (**4**) (0·28 mmol) was treated in dimethoxyethane solution with 2 mol. equiv. of potassium followed by carbon disulphide (1 ml).[3] Removal of the excess of potassium and evaporation left the diastereoisomeric potassium *NNO*$^{2'}$-tribenzoyladenosine 3′,5′-cyclic phosphorothioates, which, without isolation, were converted by means of 2N NaOH and methanolic ammonia[8] into stereoisomers of (**1**). These were purified by preparative t.l.c.† (system B). In this way (**4a**) was converted into cAMPS-(**1a**) in 66% yield [ammonium salt, $\delta(^{31}P)$ −53·58 p.p.m. (H_2O)] and (**4b**) gave cAMPS-(**1b**)

† T.l.c. was carried out on pre-coated plates, silica gel $60F_{254}$(Merck); solvent system A, $CHCl_3$–MeOH (12:1); B, Pr^iOH–conc. aq. NH_3–H_2O (7:1:1).

‡ Negative chemical shift values are assigned for compounds absorbing at lower field than H_3PO_4.

in 42% yield [ammonium salt, $\delta(^{31}P)$ −55·19 p.p.m. (H_2O)].§

Both diastereoisomers (**1a**) and (**1b**) gave a positive test with $PdCl_2$[9] and showed electrophoretic and chromatographic characteristics identical with a genuine sample of a



Ade = adenin-9-yl
B = *NN*-dibenzoyladenin-9-yl

'*P*-racemic' mixture of cAMPS. The ^{31}P n.m.r. chemical shift values reported for the diastereoisomeric mixture of (**1**) are −53·22 and −54·27 p.p.m.[5]

The elucidation of the absolute configuration at the P atom in both pairs of diastereoisomers of (**4**) and (**1**) is based on the assumption that the 1,3,2-dioxaphosphorinanyl part of (**4**) and (**1**) possesses the chair conformation.[10] We have previously demonstrated in diastereoisomeric chiral 2-X-2-Y-1,3,2-dioxaphosphorinanyl ring systems (X = RNH or R_2N; Y = O, S, or Se) that the isomer absorbing at lower field in the ^{31}P n.m.r. spectrum has an equatorial orientation of the alkyl(aryl)–amino substituent and that the isomer absorbing at higher field has this substituent in an axial position.[1,11] In *P*-chiral ring substituted 2-X-2-Y-1,3,2-dioxaphosphorinans the elucidation of the spatial arrangements of the X and Y groups is equivalent to assignment of the absolute configuration at the P atom. Thus, (**4a**) which absorbs at higher field has the absolute configuration R_p and (**4b**) S_p. This conclusion has been confirmed by ^{15}N labelling experiments and by direct spin–spin coupling constant [^1J (P–^{15}N)] measurements for both (**4a**) and (**4b**). From previous studies,[12] we can conclude that in diastereoisomeric 2-X-2-Y-1,3,2-dioxaphosphorinans the direct spin–spin coupling constant between phosphorus and a magnetically active axially oriented X nucleus ($I = \frac{1}{2}$, X = ^{1}H, ^{13}C, ^{19}F, or ^{77}Se)[12,13] has a lower absolute value than that in the isomer having X in an equatorial disposition. As ^{15}N also has $I = \frac{1}{2}$, using ^{15}N enriched aniline we have synthesized both [^{15}N]-(**4a**) and [^{15}N]-(**4b**) and measured the corresponding values of ^1J(P–^{15}N): 37·5 ± 0·5 Hz for (**4a**) and 49·0 ± 0·5 Hz for (**4b**).¶ As the conversion of metallated phosphoranilidates into phosphorothioates by means of CS_2 proceeds stereospecifically with retention of configuration at the P atom,[3,4] the absolute configuration at this atom in the isomer (**1a**) with higher field ^{31}P n.m.r. absorption [$\delta(^{31}P)$ −53·58 p.p.m.] is S_p and that in (**1b**) [$\delta(^{31}P$ −55·19 p.p.m.] is R_p.

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§ Yields of (**1a**) and (**1b**) have not been optimized.

¶ As model systems both ^{15}N labelled 2-phenylamino-2-oxo-4-methyl-1,3,2-dioxaphosphorinans were obtained and the isomer with equatorial 4-methyl and axial 2-phenylamino groups has $\delta(^{31}P)$ + 4·8 p.p.m. and ^1J(P–^{15}N) 38·0 ± 0·5 Hz. The isomer with 4-methyl and 2-phenylamino groups both equatorial has $\delta(^{31}P)$ + 0·8 p.p.m. and ^1J(P–^{15}N) 52·0 ± 0·5 Hz.

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