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ADENOSINE CYCLIC 3',5'-(R_P)- AND (S_P)-PHOSPHORAMIDATES, THE FIRST REPRESENTATIVES OF NUCLEOSIDE CYCLIC 3',5'-PHOSPHORAMIDATES DERIVED FROM AMMONIA

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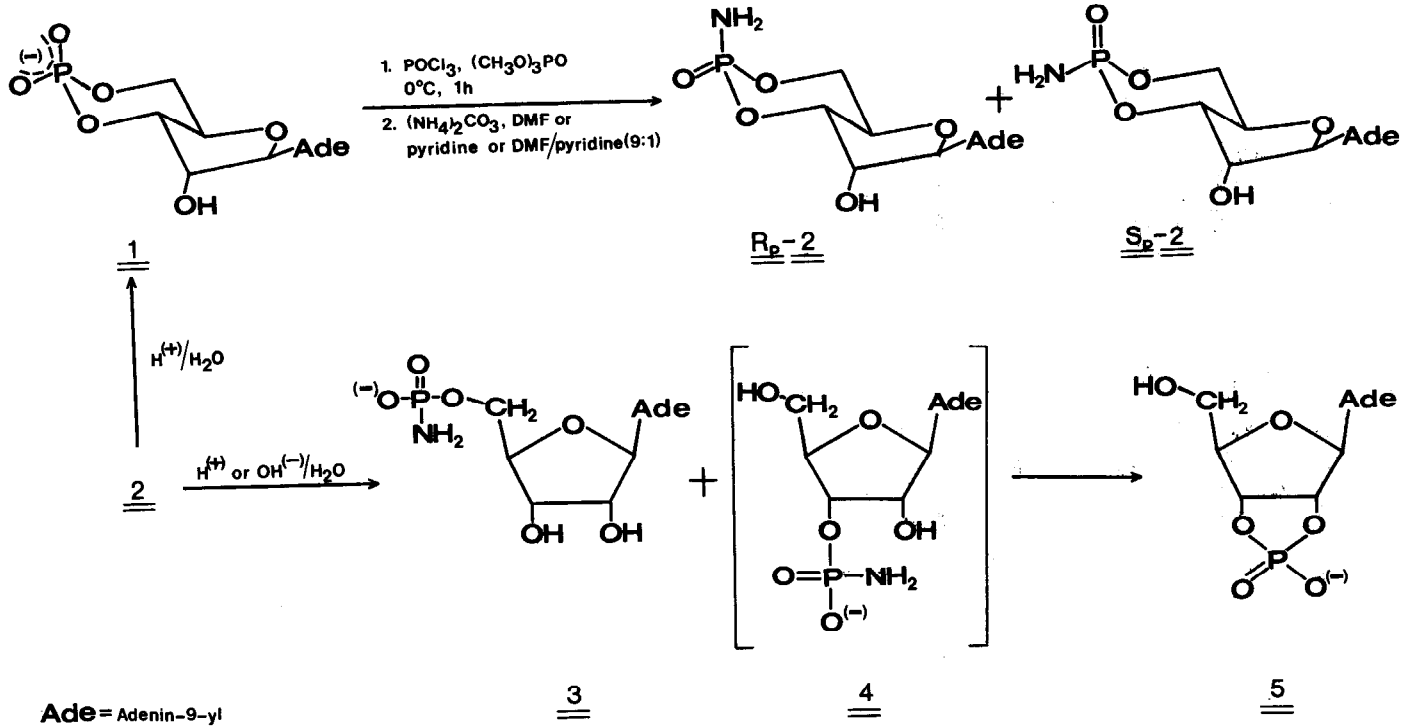
Abstract. Adenosine cyclic 3',5'-phosphoramidate (2) was synthesized by reacting adenosine cyclic 3',5'-phosphate (1) with $POCl_3$ in trimethyl phosphate for 1 h at 0°C, followed by in situ treatment of the reaction mixture with a suspension of $(NH_4)_2CO_3$ in anhydrous DMF or pyridine or DMF/pyridine mixture for 30 min at 25°C. Diastereoisomers (R_P)-2 and (S_P)-2, the relative quantities of which depended on the solvent of $(NH_4)_2CO_3$ treatment, were separated by reversed phase partition chromatography. Hydrolysis of (R_P)-2 and (S_P)2 proceeded with predominant (90% in 0.1 N HCl, 100% in 0.1 N NaOH) P-O-C bond breaking.

Adenosine cyclic 3',5'-phosphate derivatives epimeric at the phosphorus are useful compounds for studying the mechanism of the biological action of adenosine cyclic 3',5'-phosphate (1) and the substrate specificities of enzymes which are involved in the metabolism of 1, as well as to probe the conformational properties of the 1,3,2-dioxaphosphorinane ring of 1. Such derivatives may be, for example, the phosphoramidates. However, only few N(P)-substituted phosphoramidate derivatives of 1 have been prepared so far[1-3], and only the N,N-dimethyl phosphoramidate diastereoisomers have been used for biochemical[4,5] and conformational studies[6]. Unsubstituted adenosine cyclic 3',5'-phosphoramidate (2) was postulated as a non-detectable intermediate of the alkaline hydrolysis of P^1-(adenosine 5'-)P^1-amino-triphosphate[7].

This paper reports the synthesis and the products of acid and alkaline hydrolysis of (R_P)-2 and (S_P)-2, the simplest phosphoramidate diastereoisomers of 1. The synthesis was performed as summarized in Scheme[8]. Compound 1 (tri-n-butylammonium salt[3], 0.20 mmol) was reacted for 1 h at 0°C with $POCl_3$ (40.0 µl, 0.44 mmol) in trimethyl phosphate (1.0 ml) under anhydrous conditions. A suspension of $(NH_4)_2CO_3$ (0.8 g, finely powdered then dried for 1 h at 25°C and 0.2 kPa) in dry DMF or pyridine or DMF/pyridine (9:1 v/v) (60.0 ml) was stirred for 50 min at 25°C. After filtration and solvent evaporation the residue[9] was chromatographed on a LiChroprep RP-18 (25-40 µm, Merck) column (1.6x96.0 cm)

Scheme


1. $POCl_3$, $(CH_3O)_3PO$
0°C, 1h
2. $(NH_4)_2CO_3$, DMF or
pyridine or DMF/pyridine(9:1)
Ade
OH
R_p-2
S_p-2
1
2
$H^{(+)}/H_2O$
$H^{(+)}$ or $OH^{(-)}/H_2O$
3
4
5
Ade = Adenin-9-yl

using methanol/deionized H_2O (12:88 v/v), as eluent and 1.2 MPa overpressure (elution rate: 20.0 ml/2.4 min/fraction) to give TLC homogeneous ($\underline{R}_P$)-$\underline{2}$ (from fractions 31-35, as white solid, readily soluble in H_2O) and ($\underline{S}_P$)-$\underline{2}$ (from fractions 37-44, as colorless crystals after recrystallization from 200 fold hot H_2O). Both diastereoisomers can be stored without significant decomposition (<5%) for 2 months at -20°C. Yields were tabulated (Table).

Table

Synthesis and hydrolysis of ($\underline{R}_P$)-$\underline{2}$ and ($\underline{S}_P$)-$\underline{2}$

Solvent of step 2 (Scheme)	SYNTHESIS[a] Overall yield (%)	Rel. % of ($\underline{R}_P$)-$\underline{2}$	($\underline{S}_P$)-$\underline{2}$	Product	HYDROLYSIS[b] in acid[c] ($\underline{R}_P$)-$\underline{2}$	($\underline{S}_P$)-$\underline{2}$ (mol %)	in alkali[d] ($\underline{R}_P$)-$\underline{2}$	($\underline{S}_P$)-$\underline{2}$
DMF	47.8	19.2	80.8	$\underline{5}$	82.7	86.9	70.2	58.8
pyridine	29.3	42.0	58.0	$\underline{3}$	8.6	10.1	29.8	41.2
DMF/pyridine 9:1	29.1	62.9	37.1	$\underline{1}$	8.7	3.0	-	-

[a]Mean values of two parallel experiments determined by UV. [b]5×10^{-3} M solutions in 0.1 N HCl and 0.1 N NaOH, respectively, were hydrolyzed at 37°C. Hydrolyses were followed by TLC on silica gel chromatoplates in n-propanol/conc. NH_4OH/H_2O (11:7:2 v/v). [c]Determined by UV at 260 nm as adenosine 2'(3')-phosphate ($\underline{6}$), adenosine 5'-phosphate ($\underline{7}$) and $\underline{1}$ after 8 h hydrolysis and separation by preparative TLC. In 0.1 N HCl at 37°C during 8 h, $\underline{5}$ and $\underline{3}$ were quantitatively converted to $\underline{6}$ and $\underline{7}$, respectively, but $\underline{1}$ did not change as checked on authentic samples. [d]Determined by UV at 260 nm as $\underline{6}$ and $\underline{3}$ after 2 h hydrolysis and separation by DEAE-cellulose (HCO_3^-) chromatography. In 0.1 N NaOH at 37°C during 2 h, $\underline{5}$ was completely hydrolyzed to $\underline{6}$, but $\underline{3}$ was not altered.

The structure of ($\underline{R}_P$)-$\underline{2}$ and ($\underline{S}_P$)-$\underline{2}$ was verified by elemental analysis (satisfactory values for ($\underline{S}_P$)-$\underline{2}$ as monohydrate, were obtained), mass spectrometry (m/e $M(TMS)_4^+$ 616), UV (spectra at pHs 2.0, 7.0 and 11.0 were indistinguishable from those of $\underline{1}$ ⇒ unsubstituted adenine ring), ^{31}P NMR (δ/ppm/ 9.49 ($\underline{R}_P$) and 13.13 ($\underline{S}_P$) in DMSO-d_6, downfield from external 85% H_3PO_4, at 101.2 MHz ⇒ P-NH_2 bond[10]) and TLC (R_fs 0.53 ($\underline{R}_P$), 0.51 ($\underline{S}_P$) on PEI-cellulose F_{254}, Merck in deionized H_2O ⇒ neutral molecules, and 0.25 ($\underline{R}_P$), 0.32 ($\underline{S}_P$) on silica gel F_{254}, Merck in chloroform/methanol (7:3 v/v)). The configurations at phosphorus were assigned by ^{31}P NMR. On the basis of literature data[14] ($\underline{S}_P$)-$\underline{2}$ having the amino group at the equatorial position, is expected to absorb at lower field.

Nucleotide products of acid and alkaline hydrolyses of ($\underline{R}_P$)-$\underline{2}$ and ($\underline{S}_P$)-$\underline{2}$ are tabulated in Table. The relative quantities of hydrolysis products are different for the two diastereoisomers both in acid and in alkali. The very fast alkaline hydrolysis (completed within 1 min) proceeded with exclusive P-O-C bond breaking, the products being adenosine cyclic 2',3'-phosphate ($\underline{5}$) and adenosine 5'-phosphoramidate ($\underline{3}$). Compound $\underline{5}$ may be formed from adenosine 3'-phosphoramidate ($\underline{4}$), the unstable product of P-O-$C^{5'}$ bond breaking[11e] (Scheme). The hydrolysis of ($\underline{S}_P$) adenosine cyclic 3',5'-N,N-dimethylphosphoramidate was much slower (approx. 90% conversion in 30 h) and gave a mixture of

adenosine 2'(3')-phosphate(6)/adenosine 5'-N,N-dimethylphosphoramidate(~9:1)[15]. On this basis it seems reasonable to suppose that 2 is hydrolyzed according to an elimination-addition type mechanism via metaphosphorimidate intermediates[16]. The preferential P-O-$C^{5'}$ bond breaking may be interpreted by comparing the leaving group properties of CH_2O^- (or CH_2OH) and CHO^- (or CHOH) groups.

Acid hydrolysis went to completion within 2.5 h and gave 5 as main product as well as 3 and 1 (Scheme). This indicates predominant (>90%) P-O-C bond breaking and only insignificant P-N bond breaking which would have been anticipated[17]. Adenosine cyclic 3',5'-N,N-dimethylphosphoramidate behaves regularly and hydrolyzes with exclusive P-N bond breaking under the same conditions (completed in 9 h)[15].

The observed relative stability towards acid of P-NH_2 bond of 2, a 2-amino-2-oxo-1,3,2-dioxaphosphorinane together with the known instability of P-NH_2 bond of 2-amino-2-oxo-1,3,2-dioxaphospholanes in alkali[11c,11e], suggest the need for detailed kinetic studies to understand the unique behaviors of these six and five-membered phosphodiester-amide rings.

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8. This is a modification of a recently elaborated method of preparation of adenosine cyclic 3',5'-N,N-dimethylphosphoramidate that uses 2,4,6-triisopropylbenzenesulfonylchloride and aqueous dimethylamine instead of $POCl_3$ and $(NH_4)_2CO_3$, respectively[3].
9. The oily evaporational residue was dissolved in DMF (10 ml) and the solution was dropped into dry ether (150 ml). The precipitate was filtered off, dissolved in deionized H_2O (1.0 ml) and applied onto the column.
10. An NH_2-for-OH displacement at tetracoordinate phosphorus atom causes a downfield phosphorus shift of 10-15 ppm[11]. For 1, δ(ppm) is -1.58[12] or -2.6[13].
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