

Acyclo Nucleosides and Nucleotides: Synthesis, Conformation and Other Properties, and Behaviour in Some Enzyme Systems, of 2',3'-Seco Purine Nucleosides, Nucleotides and 3':5'-Cyclic Phosphates, Analogues of cAMP and cGMP

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2',3'-Seco Cyclic Nucleotides, cAMP and cGMP Analogues, Synthesis, Phosphorylation, NMR and Conformation, Enzymology

The 3':5'-cyclic phosphates of 2',3'-secoadenosine and guanosine, structural analogues of cAMP and cGMP, were synthesized by cyclization of the 5'-phosphates of 2',3'-secoadenosine and guanosine, respectively. The 2',3'-seco-3':5'-cAMP was converted to the IMP analogue by nitrous acid deamination, and to the 8-bromo analogue by bromination.

Chemical phosphorylation of 2',3'-secoadenosine gave four products, the major one of which, in 50% yield, was 2',3'-seco-3':5'-cAMP, identical to that obtained by the cyclization reaction above. The three other products have been tentatively identified.

The conformations in solution of the seco nucleosides, their 5'-monophosphates, and their 3':5'-cyclic phosphates, were determined with the aid of ¹H, ¹³C and ³¹P NMR spectroscopy, particular attention being devoted to orientations about the C–O bonds and the glycosidic bond, and the results compared with crystallographic data available for the 2',3'-seco congener of ribofuranosyl benzimidazole. The findings are briefly discussed in relation to substrate and inhibitor properties in some enzyme systems.

Some of the foregoing compounds have been examined as potential enzyme substrates and inhibitors. In particular, the seco 3':5'-cyclic phosphates are resistant to cAMP cyclic phosphodiesterase of mammalian origin, but are slowly hydrolyzed by purified higher plant cyclic nucleotide phosphodiesterase to the corresponding monophosphates.

Introduction

Periodate oxidation of the *cis*-glycols of ribonucleosides and their 5'-phosphates, followed by borohydride reduction of the resulting 2',3'-dialdehydes to the corresponding tri- or dialcohol (or 2',3'-seconucleosides or nucleotides), has been widely applied, *e.g.* to modification of the 3'-terminal

Abbreviations employed: Secoadenosine, 2',3'-secoadenosine or 9-(1,5-dihydro-4-hydroxymethyl-3-oxapentyl-2-[R]-adenine (with similar connotations for other nucleosides); seco-5'-AMP, 2',3'-seco-5'-AMP, and similarly for seco-5'-GMP and seco-5'-IMP; seco-3',5'-cAMP, 2',3'-seco-3':5'-cAMP; acycloG or acyclovir, 9-(2-hydroxyethoxymethyl)guanine; DHPG, 9-(1,3-dihydroxy-2-propoxymethyl)guanine. For purposes of simplicity, only the abbreviated terms for the acyclonucleosides are employed in the text, with the carbon atoms of the acyclic chains numbered as for the corresponding carbon atoms of the pentose ring, as shown in Scheme 1.

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adenosine of tRNA [1], identification of the 3'-terminal residue of polyribonucleotides [2], removal of the 7-methylguanosine residue from the cap of mRNA [3]. In particular, when the 3'-terminal AMP residue in tRNA^{Phe} is converted to the 2',3'-seco form, the resulting modified tRNA still readily undergoes aminoacylation [1].

At the monomer level, some of these 2',3'-seconucleosides have been found to be enzyme inhibitors [4], presumably because of the ability of the acyclic moiety to adopt a conformation resembling that of the parent ribose ring.

With the advent of acycloG (acyclovir, Scheme 1) as a potent antiherpes agent [5], now licensed for clinical use, and the promising *in vitro* antiviral activity of DHPG [6] and other acyclonucleosides [7–10], it is evident (see Scheme 1) that the seconucleosides, and nucleotides, may be potentially interesting antimetabolites in their own right.

The foregoing prompted us to undertake the preparation of 2',3'-seconucleosides and nucleotides of adenine and guanine (although some of these are

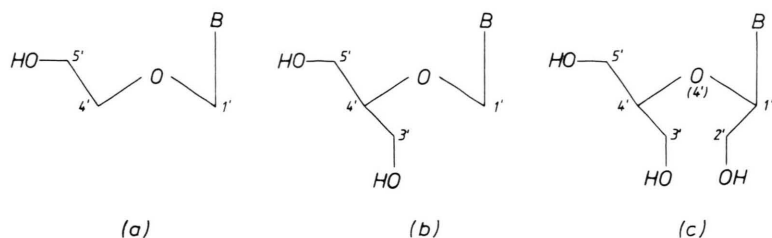


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Scheme 1. Showing formal possible structural resemblance of: (a) acyclovir, B = guanine), (b) DHPG (B = guanine), (c) 2',3'-seconucleoside (B = any base), to the corresponding parent nucleosides. The numbering system of the carbon atoms of the acyclic chains has been maintained to correspond to those of the pentose ring in a nucleoside.

available commercially, with varying degrees of purity), to examine their conformational properties in solution by NMR spectroscopy, and their behaviour in some enzyme systems. Particular attention was directed to preparation of the hitherto unknown 3',5'-cyclic phosphates, which are formal structural analogues of the biologically important so-called second messengers, cAMP and cGMP, and of the recently reported cyclic phosphate of DHPG, also a potent antiviral agent, the mechanism of action of which apparently differs from that of acyclovir and DHPG [11].

Results and Discussion

Chemical syntheses

The 5'-phosphates of adenosine and guanosine were subjected to periodate oxidation as elsewhere described for the nucleosides [12], and the resulting dialdehydes, without isolation, were reduced with sodium borohydride in slightly alkaline medium. The seco 5'-nucleotides were then isolated by ion-exchange chromatography (Scheme 2).

The 2',3'-seconucleotides were converted to the 4-morpholine-N,N'-dicyclohexylcarboxamidinium salts and subjected to cyclization with dicyclohexylcarbodiimide [13] in anhydrous pyridine for seco-5'-AMP, and in anhydrous DMF-pyridine for seco-5'-GMP. The cyclic phosphates were isolated by ion-exchange chromatography in relatively good yield (~75%), one of them (seco-3':5'-cAMP) in crystalline form.

Because of its potential use in the conformational analysis of the compounds, the 3':5'-cyclic phosphate of secoadenosine was converted to its 8-bromo analogue by treatment with Br₂ in aqueous medium

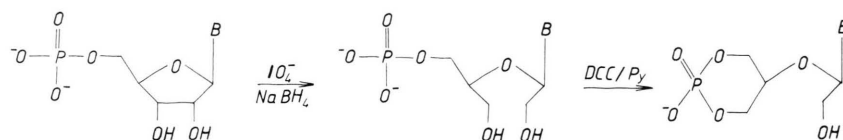
[14]. Treatment of seco-3':5'-cAMP with sodium nitrite in weakly acid medium gave the corresponding inosine analogue (Scheme 3). These again were isolated by ion-exchange chromatography to yield chromatographically homogeneous products (see Table I). As expected, all products exhibited UV absorption spectra similar to those of the parent nucleotides (Table II), while the NMR spectra were

Table I. *R_f* values of 2',3'-seconucleotides on F-254 cellulose with solvent systems: (A) isopropanol-water-NH₄OH (4:4:1, v/v), (B) 1 M ammonium acetate-ethanol (2:5), (C) isopropanol-1% (NH₄)₂SO₄ (7:3), (D) isopropanol-water-acetic acid (4:4:1).

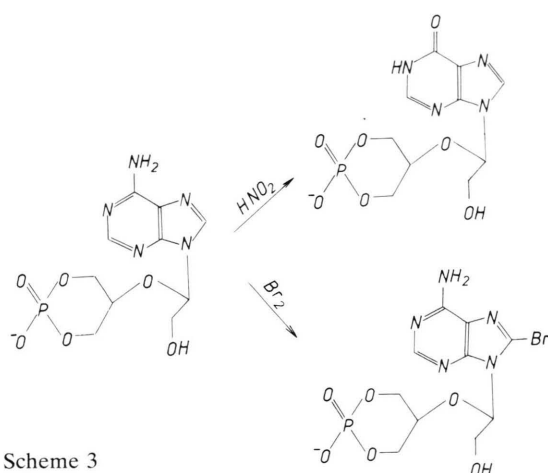
Nucleotide	A	B	C	D
AMP	0.53	0.07	0.15	0.56
Seco-5'-AMP	0.63	0.15	0.31	0.64
2',3'-dialdehyde-5'-AMP	0.49	—	—	—
Seco-3':5'-cAMP	0.70	0.44	0.46	0.72
8-Br-seco-3':5'-cAMP	0.85	0.49	0.49	0.75
Seco-3':5'-cIMP	0.85	0.36	0.35	0.62
GMP	0.56	0.07	0.09	0.38
Seco-5'-GMP	0.71	0.13	0.24	0.54
Seco-3':5'-cGMP	0.82	0.36	0.35	0.55

Table II. UV spectral data for 2',3'-seconucleotides.

Nucleotide	λ_{\max} ($\epsilon_{\max} \times 10^{-3}$)		
	pH 1	pH 7	pH 13
Seco-5'-AMP	258 (14.5)	260 (14.7)	—
Seco-3':5'-cAMP	257 (13.1)	260 (13.4)	—
8-Br-seco-3':5'-cAMP	264 (16.9)	265 (16.3)	—
Seco-3':5'-cIMP	250 (10.5)	249 (11.2)	253 (11.5)
Seco-5'-GMP	255 (10.4)	252 (11.4)	265 (9.5)
Seco-3':5'-cGMP	265 (11.5)	252 (12.6)	261 (10.1)



Scheme 2



Scheme 3

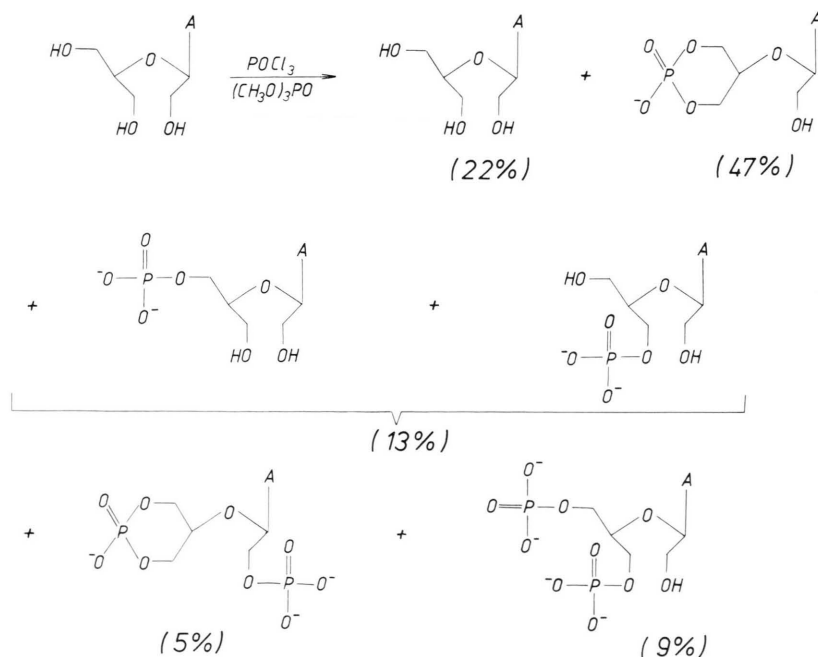
fully consistent with the proposed structures (see below).

Again, with a view to facilitating conformational analysis of the foregoing compounds, the 2',3'-di-deutero derivative of seco-5'-AMP was prepared with the use of NaB^2H_4 in $^2\text{H}_2\text{O}$ [15].

Particularly interesting were the results for the chemical phosphorylation of secoadenosine with POCl_3 in $(\text{CH}_3\text{O})_3\text{PO}$ according to the procedure of Yoshikawa *et al.* [16], which normally converts

nucleosides to their 5'-phosphates. With secoadenosine, the principal product of phosphorylation was the 3':5'-cyclic phosphate ($\sim 50\%$). Three additional products (Scheme 4) were isolated by ion-exchange chromatography (see Experimental). One was presumably the enantiomeric mixture of seco-5'-AMP and seco-3'-AMP (13% yield), chromatographically identical with the product of oxidation-reduction of 5'-AMP. The second, following treatment with alkaline phosphatase, yielded 2',3'-seco-3':5'-cAMP, and must therefore be the latter additionally phosphorylated at 2'. The third, which migrated chromatographically at a rate corresponding to the presence of two phosphate groups, was hydrolyzed by alkaline phosphatase to secoadenosine, and was therefore considered to be 2',3'-secoadenosine-3',5'-bismonophosphate.

In contrast to the foregoing is the report [11] that phosphorylation of DHPG by the same procedure gives only a 7% yield of the cyclic phosphate; no mention is made of other products. Furthermore, in a patent application, R. L. Tolman & M. Malcolm (European Patent Appln. 074,306) report that similar phosphorylation of the adenosine analogue of DHPG leads to an enantiomeric mixture of the monophosphates (30%) and the bismonophosphate (34%), with no mention of other products.



Scheme 4. Products of chemical phosphorylation of 2',3'-secoadenosine.

Behaviour towards acid and alkali

It was noted some years ago [13] that purine nucleoside 3':5'-cyclic phosphates are considerably more stable to acidic hydrolysis than the corresponding monophosphates, with slow release of the purine ring.

Treatment of seco-5'-AMP and seco-3':5'-cAMP with 0.01 N H₂SO₄ at 80 °C for 30 min led to appearance of only traces of adenine. Under more drastic conditions, 1 N HCl at 100 °C for 60 min, both compounds are degraded to several products, the principal one being adenine, with a $t_{1/2}$ for seco-3':5'-cAMP of 60 min and for seco-3':5'-cGMP about 5 min. Under comparable conditions the $t_{1/2}$ values for cAMP and cGMP are about 30 min [13].

By contrast, under alkaline conditions, 1 N NaOH at 100 °C, seco-5'-AMP and seco-3':5'-cAMP are degraded with $t_{1/2}$ values of about 30 min, whereas seco-3':5'-cGMP is fully stable.

Barium ions are known to hydrolyze 3':5'-cAMP and 3':5'-cGMP to a mixture of 3'- (80%) and 5'- (20%) monophosphates [13]. In 0.2 M Ba(OH)₂ at 100 °C hydrolysis is complete in 30 min. Under the same conditions seco-3':5'-cAMP and seco-3':5'-cGMP are hydrolyzed, also to the monophosphates, to the extent of only 10% and 15%, respectively.

The behaviour towards acid of seco-5'-AMP requires some comment in the light of a recent report by Bayard *et al.* [17]. These authors converted the terminal AMP residue of (2'-5')oligoadenylate to the 2',3'-seco derivative. Subsequent treatment with 0.05 N H₂SO₄ at 80 °C for 30 min was reported to lead to cleavage of the C(4')-O bond of the seco residue to release 9-(1,2-dihydroxyethyl)adenine and the 2'-O-phosphoglycerol derivative of (2'-5')oligoadenylate, which exhibited potent antiviral activity. No analytical evidence was offered for the structure of the product. The foregoing led us to examine the behaviour of seco-5'-AMP in 0.05 N H₂SO₄ at 80 °C for 30 min. This led to release of about 10% adenine and, after 2 h, to about 50% adenine, with no other detectable products. It is unfortunate that the foregoing authors did not attempt to detect the presumed released 9-(1,2-dihydroxyethyl)adenine.

Enzymatic trials

Under conditions where 5'-AMP was completely dephosphorylated by purified snake venom 5'-nucleotidase, there was no detectable hydrolysis of

seco-5'-AMP. The latter was also fully resistant to rabbit muscle 5'-AMP deaminase, nor did it detectably inhibit the latter.

The 3':5'-cyclic phosphates of secoadenosine and guanosine were not substrates for purified beef heart cyclic nucleotide phosphodiesterase. It should be noted, in this connection, that the 3':5'-cyclic phosphate of DHPG has also been reported inactive in this respect [11].

By contrast, highly purified potato tuber cyclic nucleotide phosphodiesterase [18] hydrolyzed 2',3'-seco-3':5'-cAMP to the monophosphate at about 10% the rate for cAMP. This suggests that the compound may also be a substrate for the unusual rat liver cCMP cyclic nucleotide phosphodiesterase described by Helfman and Kuo [19], the broad specificity of which resembles in many respects that of the potato enzyme.

Furthermore, it was shown by Smith *et al.* [13] that crude snake venom contains an activity which very slowly hydrolyzes nucleoside 3':5'-cyclic phosphates. We have confirmed this finding, using cAMP as substrate. Incubation of 3 mg cAMP with 1 mg venom in 0.02 M Tris-HCl buffer pH 9 at 37 °C for 3 h led to appearance of about 10% nucleoside and traces of AMP. Similar slow hydrolysis was observed with 2',3'-seco-3':5'-cAMP.

NMR spectroscopy (spectral assignments)

The ¹H and ¹³C chemical shifts of the various seco analogues in neutral ²H₂O are listed in Table III. The values of the coupling constants ¹H-¹H, and the vicinal coupling constants ¹H-¹³C and ¹H-³¹P, required for conformational analyses, are exhibited in Table IV.

The values of the chemical shifts of the "sugar" protons for all the analogues in general parallel those of the parent nucleosides, nucleotides and 3':5'-cyclic nucleotides. With the exception of H(1'), these protons undergo shielding by 0.3–0.8 ppm as a result of scission of the C(2')–C(3') bond. Shielding of H(1') does not exceed 0.1 ppm.

Valid assignments for the signals of the systems H(3'), H(3'') and H(5'), H(5'') were based on an analysis of the spectra for seco-5'-AMP partially deuterated at the 2' and 3' positions by the use of NaB²H₄ during the reduction step in preparation of the compound (see above and Experimental). However, assignments of individual pairs of geminal protons at

Table III. Chemical shifts for protons (in ppm vs internal TSP) and carbons (in ppm vs internal dioxane) for various 2',3'-seco derivatives, in neutral $^2\text{H}_2\text{O}$.

2',3'-Seco Analogue	H(2)	H(8)	H(1')	H(2')	H(2'')	H(3')	H(3'')	H(4')	H(5')	H(5'')
Secoadenosine	8.25	8.37	6.03	4.13	4.10	3.84	3.72	3.65	3.47 ^a	3.47 ^a
Seco-5'-AMP	8.25	8.41	6.05	4.07 ^a	4.07 ^a	3.88	3.73 ^b	3.77 ^b	3.73 ^b	3.73 ^b
Seco-3':5'-cAMP	8.25	8.40	6.01	4.14	4.09	4.52	4.41	3.77	3.94	4.27
8-Br-seco-3':5'-cAMP	8.21	—	6.07	4.27 ^a	4.27 ^a	4.53	4.41	3.73	3.97	4.26
Seco-5'-GMP ^c	—	8.04	5.86	4.03	4.00	3.86	3.75	3.79	3.74 ^a	3.74 ^a
Seco-3':5'-cGMP	—	8.03	5.83	4.07	4.01	4.49	4.37	3.74	3.97	4.28
Seco-3':5'-cIMP ^d	8.37	8.20	6.02	4.12	4.06	4.48	4.39	3.77	3.95	4.26

2',3'-Seco Analogue	C(2)	C(4)	C(5)	C(6)	C(8)	C(1')	C(2')	C(3')	C(4')	C(5')
Secoadenosine	86.07	82.35	51.95	88.97	74.23	17.52	-4.29	-6.15	13.44	-5.67
Seco-3':5'-cAMP	86.21	82.13	52.05	89.04	73.98	16.57	-4.16	1.05	4.21	2.45
8-Br-seco-3':5'-cAMP	86.14	83.66	52.99	87.92	60.91	19.03	-5.65	0.57	3.82	2.45

^a Mean chemical shifts for "deceptively simple" type system.^b Approximate values, because of signal overlapping.^c Spectrum run on Bruker-500 (see Experimental).^d Spectrum of free acid, at pH 3.5.Table IV. Values of $J(^1\text{H}, ^1\text{H})$, $J(^1\text{H}, ^{31}\text{P})$, and some vicinal coupling constants $J(^1\text{H}, ^{13}\text{C})$, in Hz, for various 2',3'-seco derivatives, from spectra recorded as in Table I.

2',3'-Seco analogue	$J(^1\text{H}, ^1\text{H})$										$J(^1\text{H}, ^{31}\text{P})$				$J(^1\text{H}, ^{13}\text{C})$			
	1',2'1',2''2',2''	3',3''	3',4'3'',4'4',5'4',5''5',5''	3',5'	3',P	3'',P	5',P	5'',P	1',4	1',8	4',1'1',4'	1',4	1',8	4',1'1',4'	1',4	1',8	4',1'1',4'	1',4
Secoadenosine	5.2 6.0 -12.1 -12.3 4.0 5.3 5.2 ^a 5.2 ^a	a	0	—	—	—	—	—	2.8	4.3	4.6	2.5 ^b						
Seco-5'-AMP	5.4 ^a 5.4 ^a a -12.0 ^c 4.5 ^c 4.5 ^c c c c	0	—	—	6.1 ^d	6.1 ^d												
Seco-3':5'-cAMP	5.3 5.7 -12.2 -12.9 2.5 1.8 2.7 2.0 -12.7 2.6	18.9	4.4	19.9	3.5	2.8	4.2	4.5	2.5 ^b									
8-Br-seco-3':5'-cAMP	6.5 ^a 6.5 ^a a -12.9 2.4 1.7 2.6 2.0 -12.7 2.7	19.1	4.3	19.8	3.7	5 ^b	4.3	4.5 ^b	b									
Seco-5'-GMP ^c	5.1 5.9 -12.2 -12.0 3.3 4.5 5.5 ^a 5.5 ^a	a	0	—	—													
Seco-3':5'-cGMP	5.4 5.6 -12.2 -13.0 2.4 1.8 2.6 2.0 -12.7 2.6	18.5	4.8	20.0	3.6													
Seco-3':5'-cIMP	5.2 5.7 -12.2 -13.0 2.4 1.9 2.5 2.0 -13.0 2.6	19.6	4.0	20.0	3.5													

^a Mean coupling constants for "deceptively simple" type system.^b Approximate values (or values not listed) because of large number of couplings.^c Approximate values, or not determined, because of signal overlapping.^d From spectrum of free acid at pH 3.5.^e From spectrum recorded on Bruker-500.

2', 3' and 5' were ambiguous, with the exception of the cyclic phosphates, where this ambiguity was limited to the geminal protons at C(2').

Chemical shifts of the sugar carbon atoms were determined from an analysis of the ^{13}C spectrum under conditions of coupling to protons, or selective decoupling of the latter. The sugar carbons of the compounds investigated follow a pattern analogous to those of the parent compounds, and exhibit shielding ranging from 1 ppm to 12 ppm. Changes in chemical shifts of heterocyclic base carbons did not

exceed 1 ppm. Similar values of the NMR parameters were obtained for the various compounds in the form of the free acids at pH 3.5.

Conformational analysis

The seconucleosides possess three CH_2OH groups which may rotate about the $\text{C}(1')-\text{C}(2')$, $\text{C}(4')-\text{C}(3')$ and $\text{C}(4')-\text{C}(5')$ bonds (Scheme 1). Conformational analyses were carried out with the aid of the modified six-parameter Karplus relationship of Haasnoot *et al.* [20], which furnishes the de-

As might have been anticipated, there is substantial free rotation about the C-C bonds, most pronounced in the case of C(1')-C(2'). Some preference may be seen for the conformation with a *gauche* orientation of hydroxyls on proximate carbons. Similar behaviour is exhibited by the 2',3'-seco derivative of benzimidazole riboside [21]. Changes of the heterocyclic base in the series adenine, guanine, ben-

Conformation of the 3':5'-cyclic phosphate ring. The six-membered ring of the seconucleoside 3',5'-cyclic phosphates displays several vicinal coupling constants, $^1\text{H}-^1\text{H}$ and $^1\text{H}-^{31}\text{P}$, from which the conformation of the system may be determined. Application of the Karplus relationship, as modified by Haasnoot *et al.* [20], to the couplings between H(4') on the one hand, and H(3'), H(3'') and H(5), H(5'') on the other, indicates that the phosphate ring is in the chair form with the O(4') oriented axially, *gauche*



Scheme 5. Newman projections along: (a) C(1')-C(2'), (b) C(3')-C(4'), (c) C(4')-C(5') for a 2',3'-seconucleoside or nucleotide.

Table V. Populations (in %) of individual classical conformers about C–C bonds in various 2',3'-seco derivatives. The two sets of values correspond to the two possible assignments of the ^1H signals for the geminal protons at C(2'), C(3') and C(5').

2',3'-Seco analogue	C(1')–C(2')			C(3')–C(4')			C(4')–C(5')		
	g^+	t	g^-	g^+	t	g^-	g^+	t	g^-
Secoadenosine	42	30	28	47	14	39	a	a	38
	28	42	30	50	31	19			
Seco-5'-AMP ^b	41	29	30	50	c	c	c	c	c
	27	41	32						
Seco-5'-GMP	41	29	30	60	5	35	a	a	34
	27	41	32	63	21	16			
Seco-3':5'-cAMP	38	32	30	Cyclic phosphate in chair form					
	31	38	31						
8-Br-seco-3':5'-cAMP	a	a	12	Cyclic phosphate in chair form					
Seco-3':5'-cGMP	36	33	31	Cyclic phosphate in chair form					
	32	37	31						
Seco-3':5'-cIMP	38	30	32	Cyclic phosphate in chair form					
	30	38	32						

^a The mean values of coupling constants (due to the “deceptively simple” type system) permit determination of the population of only one of the conformers.

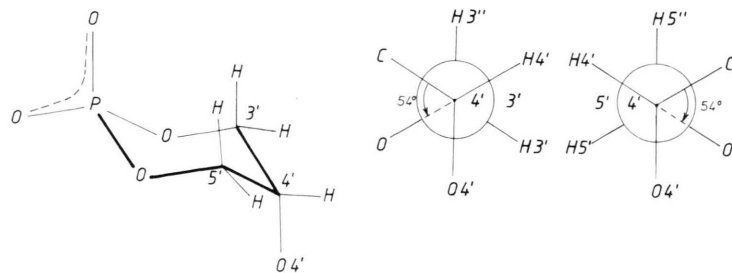
^b Conformer populations about C(1')–C(2') are for the free acid form at pH 3.5.

^c Values not measureable because of difficulties in determining coupling constants due to complexities of spectrum.

relative to the ring oxygens (Scheme 6). The dihedral angles between protons deviate from the classical values of $\pm 60^\circ$ by about 6° , so that the ring is slightly flattened with an endocyclic angle of 54° . A characteristic feature is the long-range coupling between H(3') and H(5'), which are in a zig-zag type structure.

The observed ^1H – ^{31}P coupling constants qualitatively corroborate the foregoing results, viz. large (18.5–20 Hz) coupling constants with the axial protons (*trans*), and small (3.5–4.8 Hz) coupling constants with the equatorial protons (*gauche*). There is, however, a lack of total symmetry for the couplings of phosphorus with H(3'), H(3'') on the one hand, and H(5'), H(5'') on the other, the deviations extending to 1.5 Hz. The dihedral angles derived from a

recently developed Karplus relationship for ^1H – ^{31}P couplings [22] deviate from the tetrahedral angles 180° and 60° non-uniformly, depending on the coupling involved, by 25–30° and 10–15°, respectively, for equatorial and axial protons, hence considerably more than the 6° predicted from proton-proton couplings. This points to the limited applicability of the foregoing relationship, undoubtedly due in part to the fact that its parametrization was based on molecular systems not including closed rings. An additional interpretation for the observed non-symmetrical couplings is the minor involvement of an additional conformation in the dynamic equilibrium, rather of the type screw-boat with O(4') oriented axially, instead of the second chair form with O(4') equatorial. The measured $J(^{31}\text{P}$ – $^{13}\text{C})$, 4.6 Hz for



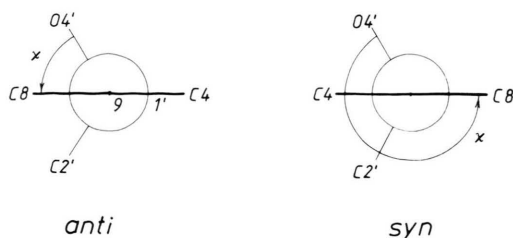
Scheme 6. Conformation of the 3',5'-cyclic phosphate ring, with Newman projections along the C(3')–O(4') bonds.

seco-3':5'-cAMP and 4.7 Hz for its 8-bromo congener, which is directly dependant on the endocyclic angle of the ring, appreciably exceeds that expected, because of two equivalent coupling paths. A similar effect is noted for conventional nucleoside 3':5'-cyclic phosphates [23].

The chair conformation of the cyclic phosphate ring in the seco analogues differs from the chair conformation in conventional 3':5'-nucleotides in that, in the latter, O(4') adopts the orientation equatorial, *trans* relative to the ring oxygens [24, 25]. This is a consequence of steric effects involving the 5-membered sugar ring. The conformations of the seco analogues more closely resemble that of a system such as 3-methoxytrimethylene phosphate [26].

The conformation of the phosphate group itself in the seco congeners may be derived from the above-mentioned relationship between the couplings and dihedral angles of vicinal ^{31}P and ^1H [22], assuming classical conformers about the C(5')–O(5') bond. The mean value of the two ^1H – ^{31}P couplings in seco-AMP (6.1 Hz) points to domination (~65%) of the form *trans* (*gauche'*-*gauche'*), with a transoidal orientation of PO_3^{-2} and C(4'). Rotation about the C(5')–O(5') bond is therefore less hindered than in 5'-AMP, where the population of the form *trans* is 80%.

Conformation about the glycosidic bond. For purine nucleosides, the coupling constants between H(1') and C(4), C(8) should make possible determination of the conformation about the C(1')–N(9) bond. To date, the appropriate Karplus relationship has been sought only for pyrimidine nucleosides [27–29], the most reliable of which is a recent one developed for the individual couplings H(1')– ^{13}C (6) and H(1')– ^{13}C (2). Analysis of the chemical shifts of the sugar protons and carbons, successfully employed for determination of the conformation about the glycosidic bond in purine nucleosides [30], is hardly applicable to seconucleosides because of differences in the sugar moiety and the lack of model *syn* and *anti* analogues. We have therefore made use of the Karplus relationships for pyrimidine nucleosides [29]. The recorded values of the ^1H – ^{13}C couplings in secoadenosine and its 3':5'-cyclic phosphate were not in accord with any single conformation, but could be the averages of two forms with comparable populations, *viz.* *anti*, with the glycosidic bond angle $\chi \sim 60^\circ$, and *syn*, with $\chi \sim 170^\circ$ or 290° (Scheme 7), the value of 290° being most likely because of mutual



Scheme 7. Newman projections along the N(9)–C(1') bond for a 2',3'-seconucleoside or nucleotide.

repulsion between the electronegative O(4') and N(3). This is further supported by the similarity of the couplings for the secoanalogues and the parent purine nucleosides and nucleotides (R. Stolarski, P. Lassota and D. Shugar, in preparation), in which there is a dynamic *syn-anti* equilibrium. The single crystalline structure hitherto reported for a seconucleoside analogue, seco-DRB [21], exhibits two molecules in the asymmetric unit with conformations *anti* ($\chi = 45^\circ$) and *syn* ($\chi = 254^\circ$). These are similar to the above values derived for purine seconucleosides in solution. The differences in the value of ψ for the *syn* form may be a consequence of the above-mentioned repulsion between the ring N(3) of the base and O(4').

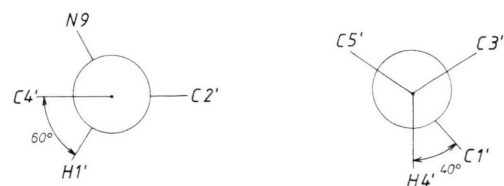
8-Br-seco-3':5'-cAMP displays a ^1H – ^{13}C coupling constant which diverges from those for nucleosides unsubstituted at C(8). The Br substituent may, of course, affect these coupling constants, particularly H(1')– ^{13}C (8). On the other hand, the value of the coupling constant H(1')– ^{13}C (4), and the similarities of both constants to those found in 8-bromo-adenosine which is predominantly *syn* [30], point to the conformation *syn* with $\chi \sim 270^\circ$.

Conformations about the bonds C(1')–O(4') and O(4')–C(4'). The conformations about the C–O bonds in the acyclic chain, which determine the overall conformation of a seconucleoside, were evaluated from the coupling constants for H(4')–C(1') and C(4')–H(1'), shown in Table IV. Many such coupling constants have hitherto been reported for both linear and cyclic etheric systems $^{13}\text{COC}^1\text{H}$ and $^1\text{HCO}^{13}\text{C}$ [31, 32]. The relationship between coupling constants and dihedral angles between ^1H and ^{13}C is of the Karplus type, with values of 2.7 Hz for an angle of 60° (*gauche* form) and 9–11.5 Hz for 180° (*trans* form). For the present compounds, one of the carbons is linked to nitrogen, which may affect

the values of the coupling constants. Nonetheless, ^1H - ^{13}C coupling constants in the systems $\text{H}_2\text{XC}-\text{CH}_2-\text{CH}_3$ and $\text{CH}_3-\text{CXH}-\text{CH}_3$ ($\text{X}=\text{CH}_3$ or NH_2) were found to be modified at most by only 0.3 Hz when CH_3 is replaced by NH_2 [33]. An analogous effect may be anticipated for couplings in systems with oxygen, such as the seconucleosides. The observed $J[\text{C}(4')-\text{H}(1')]\sim 2.5$ Hz in seco-adenosine and its 3':5'-cyclic phosphate points to a transoidal orientation of $\text{C}(4')$ and $\text{C}(2')$, as illustrated in Scheme 8. Also permissible is a conformation with a *trans* orientation of $\text{C}(4)$ and $\text{N}(9)$ (in both instances with a 60° angle between $\text{C}(4')$ and $\text{H}(1')$). From the value 4.5 Hz for $J[\text{C}(1')-\text{H}(4')]$, it follows that the conformation about $\text{C}(4')-\text{O}(4')$ is dislocated about 20° relative to the transoidal orientation of $\text{C}(1')$ and $\text{C}(5')$ or $\text{C}(3')$. This ambiguity is due to the fact that only single values of the coupling constants about the two $\text{C}-\text{O}$ bonds can be recorded. By analogy with the solid-state data for seco-DRB [21], it may be assumed that the predominant structure is an extended one with a transoidal orientation of $\text{C}(2'), \text{C}(4')$, and an orientation of $\text{C}(1'), \text{C}(5')$ deviating about 20° from transoidal.

Departure from a fully transoidal conformation about $\text{C}(4')-\text{O}(4')$ was also noted in one of the two independent molecules of seco-DRB in the solid state [21], about the $\text{C}-\text{O}$ bonds in the disaccharide methyl- β -cellobioside [32], as well as in acyclovir [34]. An alternative interpretation is that the higher value of $J[\text{H}(1'), \text{C}(4')]$ is due to participation of a conformation with a *gauche* orientation of $\text{C}(1')$ and $\text{C}(3'), \text{C}(5')$, with a 180° angle between $\text{H}(4')$ and $\text{C}(1')$. While such a conformation is less likely, because of steric factors, it cannot be fully excluded.

From the foregoing conformational analysis, it appears that the acyclic chain in seconucleosides is predominantly in a single extended form, in contrast to the equilibrium of many conformations of the side-chain CH_2OH groups, and of the heterocyclic base



Scheme 8. Newman projections along the $\text{C}(1')-\text{O}(4')$ bond (left) and the $\text{C}(4')-\text{O}(4')$ bond (right) for a 2',3'-seconucleoside or nucleotide.

about the glycosidic bond. With such a conformation, the molecule resembles the structure of an α -nucleoside, except for the non-typical orientation of the 2'- CH_2OH group (see Scheme 8). It nonetheless appears likely that additional conformations about the $\text{C}-\text{O}$ bonds exist, of comparable energy (differences of the order of several kcal/mol). Quantum mechanical calculations of the structurally similar acyclovir are in line with such reasoning (M. Geller *et al.* in preparation), and calculations on other acyclonucleosides are presently under way in this laboratory. It is therefore possible that, in enzymatic reactions, the seco analogues may also mimic the structures of β -nucleosides and nucleotides with differing orientations of the 2'-OH and 3'-OH, *i.e.* ribo, arabinosyl, xylo and lyxo.

Conformation and biological activity

What is now of fundamental interest is the conformation adopted by a given acyclonucleoside on interaction with an enzyme for which it is a substrate or inhibitor, and the extent to which the acyclic chain approaches the conformation of the parent 5-membered sugar ring. Several model systems now exist for such studies, *e.g.* acycloG and DHPG, which are good substrates for herpes simplex type 1 thymidine kinase [5, 6], and a number of pyrimidine acyclonucleosides which are good inhibitors of uridine, but not thymidine, phosphorylase [35–37]. In particular, since DHPG is phosphorylated even more effectively than acycloG by the herpes thymidine kinase, it would be of interest to determine whether 2',3'-seco-guanosine (see Scheme 1) is also a substrate (or inhibitor).

At first sight, it would appear that rotation about $\text{C}-\text{C}$ and $\text{C}-\text{O}$ bonds would enable a seconucleoside or nucleotide to readily adopt the conformation of the parent nucleoside or nucleotide. But our conformational analysis points rather to predominance of extended conformations of the acyclic chains. The resulting atypical orientation of the heterocyclic base, and steric effects related to the orientation of the 2'- CH_2OH group (see above) may account for the lack of substrate and/or inhibitory properties of these analogues in some enzyme systems. Steric hindrance by the 2'- CH_2OH was previously proposed by Lerner and Rossi [4] as the source of the differences in inhibitory properties of such compounds in the adenosine deaminase system.

One of the best examples of the ability of a seco-nucleotide to mimic the properties of the parent nucleotide is forthcoming from the demonstration [1] that tRNA^{Phe}, in which the terminal AMP residue is converted to seco-AMP, undergoes aminoacylation, known to occur specifically at the 3'-OH, as effectively as the parent tRNA. In the presence of borate, which complexes with *cis*-glycols, aminoacylation of the parent tRNA is reduced, but that of the tRNA with the terminal seco-AMP residue is unaffected. This is in accord with our suggestion regarding the differing orientation of the 2'-CH₂OH relative to the 3'-CH₂OH. It is further supported by the fact that the chromatographic mobility of a seconucleotide is unaffected by inclusion of borate in the development solvent, since the 2'- and 3'-OH groups are not suitably orientated to complex with borate.

Experimental

Melting points (uncorr.) were measured on a Boetius microscope hot stage. Elementary analyses were performed by the Institute of Organic Chemistry, Academy of Sciences.

UV spectra were recorded on Zeiss (Jena, GDR) VSU-2P and Specord UV-VIS instruments, using 10-mm pathlength cuvettes.

¹H NMR spectra for most compounds were recorded on a Bruker 270 AM. Exceptions were 2',3'-seco-5'-GMP and 2',3'-seco-3',5'-cGMP, the spectra of which were obtained with Bruker 500 AM and Bruker 200 instruments. ¹³C and ³¹P spectra were run on the Bruker 270 AM at frequencies of 67.93 and 109.35 MHz, respectively. All spectra were recorded at concentrations of 0.02 M in aqueous solution (pH ~7 and 3.5) at 30 °C. ¹H chemical shifts were measured *vs* internal TSP (sodium 2,2,3,3-tetradeutero-3-(trimethylsilyl)-propionate) to an accuracy of ±0.005 ppm, and ¹³C chemical shifts *vs* internal dioxane to an accuracy of ±0.01 ppm. Coupling constants were measured to an accuracy of ±0.2 Hz.

DMF was purified by azeotropic distillation with water and benzene, then under reduced pressure and stored over 4 Å molecular sieves. Pyridine was distilled over CaH₂ and stored over molecular sieves. ²H₂O (99.7% mol ²H) was from the Institute of Nuclear Studies (Swierk, Poland). Nucleosides and nucleotides were commercial products, checked for purity by chromatography and UV spectrophotometry.

Materials for column chromatography included 200–400 mesh Dowexes (Bio Rad, Richmond, VA., USA) and DEAE A-25 Sephadex (Pharmacia, Uppsala, Sweden), with the use of an LKB 2070 Ultrarac II instrument recording at 254 nm. Thin-layer chromatography was performed with Merck (Darmstadt, GFR) F₂₅₄ cellulose plates, using solvent systems as listed in Table I, which provides *R_f* data. Ultraviolet absorption spectral data are presented in Table II, and detailed NMR data in Tables III, IV, V.

Snake venom 5'-nucleotidase (5'-ribonucleotide phosphohydrolase, EC 3.1.3.5), rabbit muscle 5'-AMP deaminase (5'-AMP aminohydrolase, EC 3.5.4.6), beef heart 3':5'-cyclic nucleotide phosphodiesterase (3':5'-cyclic-nucleotide-5'-nucleotidohydrolase EC 3.1.4.17) and bovine spleen purine nucleoside phosphorylase (purine nucleoside: orthophosphate ribosyltransferase, EC 2.4.2.1) were products of Sigma (St. Louis, MO., USA). Potato tuber cyclic nucleotide phosphodiesterase was a purified preparation elsewhere described [18].

Enzyme assays. Tests for substrate and inhibitory properties of the various compounds were performed with the aid of assays for enzyme activity described elsewhere for 5'-nucleotidase [38], 5'-AMP deaminase [39], cyclic nucleotide phosphodiesterase [18], purine nucleoside phosphorylase [40].

Seco-5'-AMP. To a solution of 3.47 g (10 mmol) 5'-AMP (free acid) in 60 ml water, brought to neutrality by addition of triethylamine, was added 3.40 g (15 mmol) of HIO₄ · H₂O. The solution was stirred at room temperature for 1 h, with addition of triethylamine to maintain the pH near neutrality, followed by addition of 0.8 ml ethylene glycol to terminate the reaction. This was followed by the portionwise addition, with stirring for three h, of 1.52 g (40 mmol) NaBH₄, and stirring continued for an additional 3 h at room temperature. The solution was then diluted to 300 ml and loaded on a 4.5 × 25 cm column of Dowex 1 × 8 (HCOO⁻). On elution with a linear gradient of H₂O–1 M formic acid, the nucleotide was eluted at about 0.35 M formic acid. The pooled fractions of the nucleotide were brought to dryness under reduced pressure, and the residue taken up in a small volume of water. Addition of acetone led to precipitation of 2.25 g (65%) of the chromatographically homogeneous product, as the free acid.

Seco-3':5'-cAMP. The foregoing seco-5'-AMP (420 mg, 1.2 mmol) was converted to the 4-mor-

pholine-N,N'-dicyclohexylcarboxamidinium salt, which was dried by evaporation from anhydrous pyridine, and then dissolved in 100 ml anhydrous pyridine. This solution was added dropwise to a boiling solution of 740 mg (3.6 mmol) DCC in 110 ml anhydrous pyridine. The solution was refluxed for an additional 2 h, and the reaction terminated by addition of 5 ml water. The mixture was brought to dryness, and the residue brought to dryness several times from water to remove residual pyridine, then taken up in 300 ml water and filtered through Celite. The solution was loaded on a 2.5 × 40 cm column of Dowex 1 × 8 (HCOO⁻), and elution conducted with a linear gradient of H₂O–0.5 M formic acid (2 l). The fractions containing the nucleotide (at ~ 0.3 M) were pooled, brought to dryness, the residue dissolved in a small volume of water (~ 5 ml) and precipitated by dropwise addition of 20 ml *n*-propanol-acetone (1:1), to yield 425 mg (77%) of product in the form of fine needles, m.p. 186–195° (dec.) *Elem. anal.*: Calculated for C₁₀H₁₄N₅O₆P·H₂O: C = 34.39%; H = 4.61%, N = 20.05%. Found: C = 34.25%; H = 4.68%; N = 19.91%.

Seco-5'-GMP. To a solution of 2.4 g (6 mmol) of 5'-GMP·Na₂ in 20 ml water was added 1.7 g (7.45 mmol) of HIO₄·H₂O. The solution was brought to near neutrality with triethylamine, stirred for 1 h, and the reaction terminated by addition of 0.5 ml ethylene glycol. This was followed by portionwise addition of NaBH₄ (1.9 g, 50 mmol) with monitoring the course of the reaction chromatographically. Following stirring for 8 h, water was added to bring the volume to 300 ml and the mixture deposited on a 3 × 60 cm column of DEAE Sephadex (HCO₃⁻). On elution with a 0–0.8 M linear gradient of H₂O-triethylammonium carbonate (2 l), the product was located in fractions at about 0.7 M. The pooled fractions were brought to dryness, and the residue brought to dryness several times from water and ethanol. The product was converted to the sodium salt with the aid of Dowex 50W(Na⁺) and precipitated from aqueous solution by addition of acetone to yield 1.73 g (72%) of the disodium salt.

Seco-3',5'-cGMP. The disodium salt of seco-5'-GMP (490 mg, 1.2 mmol) was converted to the 4-morpholine-N,N'-dicyclohexylcarboxamidinium salt and dissolved in 100 ml of N,N-dimethylformamide pyridine (1:1). The slightly opalescent solution was added dropwise, over a period of 1 h, to a refluxing solution of 740 mg (3.6 mmol) of DCC in anhydrous

pyridine, and heating continued for an additional 2 h. The reaction was terminated by addition of 5 ml water, and the mixture brought to dryness. The residue was taken up in water, brought to dryness, and this procedure repeated several times until there was no detectable odor of pyridine. The final residue was taken up in 200 ml water, filtered, and the filtrate deposited on a 3 × 60 cm column of DEAE Sephadex (HCO₃⁻) and eluted with a 0–0.8 M linear gradient of water-triethylammonium carbonate (2 l). The cyclic phosphate product came through at about 0.6 M. The pooled fractions of the product were brought to dryness, and the residue dried several times from water and ethanol. The product was converted to the sodium salt with Dowex 50W(Na⁺), and precipitated from aqueous solution by addition of acetone, to yield 360 mg (81%) of an amorphous white powder. *Elem. anal.*: Calculated for C₁₀H₁₃N₅O₇PNa₂: C = 32.56%; H = 3.55%; N = 18.97%. Found: C = 32.26%; H = 3.59%; N = 18.68%.

Seco-3',5'-cIMP. To a solution of 347 mg (1 mmol) seco-3',5'-cAMP in 10 ml water was added 280 mg NaNO₂ and 0.4 ml CH₃COOH, and the mixture stirred for 5 h at room temperature. An additional 140 mg NaNO₂ was added and stirring continued overnight. Water was added to a final volume of 100 ml, the solution brought to neutrality by addition of NH₄OH and deposited on a 2.5 × 15 cm column of Dowex 1 × 8(HCOO⁻). Elution with a linear gradient of water-2.5 M formic acid (2 l) led to appearance of the product at about 2 M formic acid. The pooled fractions of the nucleotide were brought to dryness, dissolved in a small volume of water, and precipitated with ethanolic acetone, to yield 187 mg (54%).

8-Br-seco-3':5'-cAMP. To a solution of 210 mg (0.6 mmol) of seco-3':5'-cAMP in 20 ml water was added 200 mg CH₃COONa, followed by 100 μl Br₂ (1.95 mmol), and the mixture stirred for 3 h at room temperature. Excess Br₂ was removed by addition of Na₂S₂O₄. The reaction mixture was brought to 100 ml with water and deposited on a 2.5 × 15 cm column of Dowex 1 × 8(HCOO⁻). Elution with a 0–0.5 M linear gradient of water-HCOOH (2 l) led to appearance of the product at about 0.4 M HCOOH. The pooled fractions of product were brought to dryness and precipitated from aqueous solution by addition of acetone, to yield 189 mg (76%) of a white amorphous powder.

Chemical phosphorylation of secoadenosine. Secoadenosine, prepared as elsewhere described [41], and crystallized from ethanol, m.p. 141–142 °C, was chromatographically homogeneous.

To a stirred suspension of 130 mg (0.48 mmol) of 2',3'-secoadenosine in 4 ml $(\text{CH}_3\text{O})_3\text{PO}$ at 0 °C was added 100 μl (1.09 mmol) POCl_3 portionwise over a period of 30 min. Stirring was continued for an additional 1.5 h, followed by addition of 100 ml iced water with ice, and the pH then brought to neutrality with NaHCO_3 . Vigorous stirring was continued while the solution was brought to room temperature to remove liberated CO_2 , and then deposited on a 3.5×27 cm column of DEAE-Sephadex (HCO_3^-). A water wash released 22% of unreacted nucleoside. The column was then eluted with a 0–0.8 M linear gradient of triethylammonium carbonate (3.6 l), with collection of 19.5 ml fractions. The first peak, identified as seco-3':5'-cAMP (fractions 54–63, 47% yield relative to starting compound), was followed by a second, presumably the enantiomeric mixture of seco-5'-AMP and seco-3'-AMP (fractions 80–94, 13%). A third peak, identified as seco-3':5'-cAMP, additionally phosphorylated at the 2' position (fractions 109–127, 5%), was succeeded by a fourth (fractions 129–151, 9%), considered to be secoadenosine-3',5'-bimonophosphate.

Seco-2',3'-dideutero-5'-AMP. 5'-AMP (694 mg, 2 mmol, in 10 ml H_2O) was oxidized with periodate as described above, the course of the reaction being monitored by TLC (see Table I). Following disappearance of 5'-AMP, the mixture was diluted to 100 ml and loaded on a 3.5×25 cm column of Dowex 1×8 (HCOO^-). Elution with a linear gradient of 0–1 M HCOOH (2 l) led to appearance of the dialdehyde in the region 0.7–0.9 M HCOOH . The pooled fractions were brought to dryness, the residue brought to dryness several times from water, taken up in the minimal quantity of water and pre-

cipitated with ethanol, to yield 510 mg (74%) of a white, amorphous, product, chromatographically homogenous.

The foregoing dialdehyde (1.47 mmol) was taken up in 10 ml $^2\text{H}_2\text{O}$ and lyophilized. The residue was dissolved in 6 ml $^2\text{H}_2\text{O}$ and reduced by portionwise addition, over a period of 2 h, with stirring, of 390 mg (9.3 mmol) NaB^2H_4 . Stirring was continued for 2 h, the pH brought to about 5 by addition of 200 μl HCOOH , the solution diluted with water to 100 ml, and loaded on a 3.5×20 cm column of Dowex 1×8 (HCOO^-). Elution with a linear gradient of 0–0.6 M HCOOH (2 l) released the product at about 0.5 M HCOOH . The pooled fractions were brought to dryness thrice from water, dissolved in 10 ml hot water and, following cooling to room temperature, precipitated by addition of 50 ml acetone. The resultant white, amorphous, product was dried over P_2O_5 under vacuum to yield 380 mg (75%), chromatographically homogeneous, and otherwise identical to the seco-5'-AMP described above.

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