Syntheses of 6,8-Disubstituted-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphates

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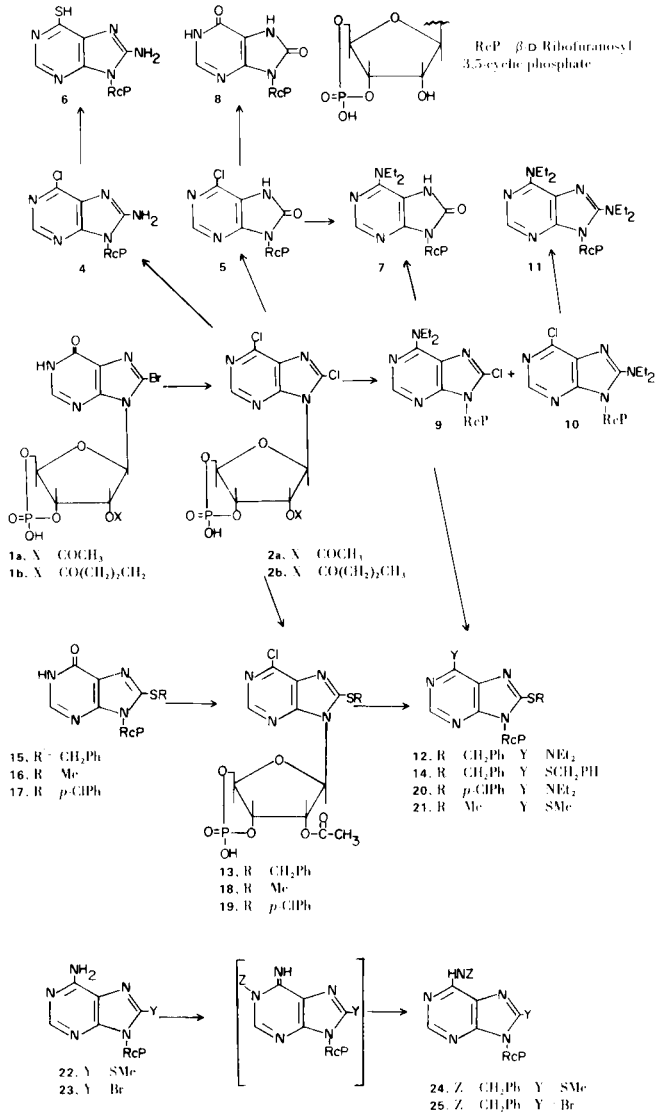
A series of 6,8-disubstituted-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphates were prepared employing preformed 9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate precursors. Three synthetic approaches were utilized to accomplish the syntheses. The first approach involved a study of the order of nucleophilic substitution, 6 *vs* 8, of the intermediate 6,8-dichloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphates (**2**) with various nucleophilic agents to yield 8-amino-6-chloro-, 8-chloro-6-(diethylamino)-, 6-chloro-8-(diethylamino)-, 6,8-*bis*-(diethylamino)- and 8-(benzylthio)-6-chloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**4**, **9**, **10**, **11**, **13**) respectively and 6-chloro-9-β-D-ribofuranosylpurin-8-one 3′,5′-cyclic phosphate (**5**) and 8-amino-9-β-D-ribofuranosylpurine-6-thione 3′,5′-cyclic phosphate (**6**). The order of substitution was compared to similar substitutions on 6,8-dichloropurines and 6,8-dichloropurine nucleosides. The second scheme utilized nucleophilic substitution of 6-chloro-8-substituted-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphates obtained from the corresponding 8-substituted inosine 3′,5′-cyclic phosphates by phosphoryl chloride, 6,8-*bis*-(benzylthio)-, 6-(diethylamino)-8-(benzylthio),8-(*p*-chlorophenylthio(-6-(diethylamino)- and 6,8-*bis*-(methyl-thio)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphates (**14**, **12**, **20**, and **21**) respectively, were prepared in this manner. The final scheme involved N^1-alkylation of an 8-substituted adenosine 3′,5′-cyclic phosphate followed by a Dimroth rearrangement to give 6-(benzylamino)-8-(methylthio)- and 6-(benzylamino)-8-bromo-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**24** and **25**).

Previous synthesis of analogs of adenosine 3′,5′-cyclic phosphate (cAMP), modified in the aglycon, have involved either the tedious route of cyclization of derivatized nucleosides-5′-phosphate, or modification at one reactive site in a preformed 3′,5′-cyclic nucleotide such as the 6-amino group of cAMP or the halo groups of 8-bromoadenosine 3′,5′-cyclic phosphate and 6-chloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (1-5). We wished to prepare a series of 6,8-disubstituted purine 3′,5′-cyclic nucleotides using preformed 3′,5′-cyclic phosphate precursors. We have investigated three different approaches to these compounds: 1, the synthesis and subsequent reactions of 6,8-dichloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate; 2, the synthesis and reactions of 6-chloro-8-substituted-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphates; 3, N^1-alkylation and Dimroth rearrangement of 8-substituted adenosine 3′,5′-cyclic phosphates.

Meyer *et al.*, (1), recently reported on the synthesis of 6-chloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate from 2′-*O*-acetylinosine 3′,5′-cyclic phosphate. Gerster *et al.*, (6), found that upon treatment of 2′,3′,5′-tri-*O*-acetyl-8-bromoguanosine with phosphorus oxychloride, the 8-bromo group was replaced by a chloro group. Treatment of 8-bromoinosine 3′,5′-cyclic phosphate triethylammonium salt (4) with acetic anhydride/pyridine provided 2′-*O*-acetyl-8-bromoinosine 3′,5′-cyclic phosphate triethylammonium salt (**1a**), which, when added to refluxing phosphorus oxychloride, gave the desired 2′-*O*-acetyl-6,8-dichloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**2a**). The 6,8-dichloro nucleotide **2** was found to be labile toward basic hydrolysis, which precluded the purification of the reaction by the usual method of desalting on a charcoal column (1). We had previously postulated that upon treatment with phosphorus oxychloride a 3′,5′-cyclic phosphate is converted to a phosphorochloridate (7). This phosphorochloridate would be expected to be water insoluble, and indeed this property allowed a convenient purification procedure and work-up of the reaction mixture. When the excess phosphorus oxychloride was evaporated from the reaction and the resulting residue was poured onto ice, the presumed 2′-*O*-acetyl-6,8-dichloro-β-D-ribofuranosylpurine 3′,5′-cyclic phosphorochloridate precipitated and could be filtered and purified by washing with cold water. Hydrolysis of the phosphochloridate in sodium acetate buffer followed by a further silica gel purification provided an 18% yield of 2′-*O*-acetyl-6,8-dichloro-



9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**2a**). We subsequently found that 8-bromo-2′-*O*-butyryl-inosine 3′,5′-cyclic phosphate triethylammonium salt (**1b**) gave a higher yield (56%) of the respective dichloronucleotide **2b**.

Previous work on nucleophilic substitution of various 6,8-dihalo purines has shown that there is some degree of variability in the order of nucleophilic displacement at the 6 and 8 positions (8-13). The variability seems dependent upon several factors, *e.g.*, purinyl-9-substitution, purinyl-2-substitution, the type of nucleophile and reaction conditions such as *p*H.

Sutcliffe and Robins (8) first postulated that 6,8-dichloro-9*H*-purines undergo nucleophilic substitution first in the 6-position with strongly basic nucleophiles because loss of the proton at position 9 ionizes the imidazole ring. The ionization of the ring increases the electron density at position 8 relative to position 6 resulting in rendering the 8 position less susceptible to nucleophilic attack. Electrostatic repulsion of an attacking anionic nucleophile by the ionized ring may also direct nucleophilic attack away from position 8. Alkylation at position 9, by virtue of eliminating the ionization of the imidazole ring, renders position 8 susceptible to nucleophilic attack. Differences, electronic or steric, in alkyl groups at position 9 cause variations in the susceptibility of position 8 to nucleophilic attack. This variation can be seen in the ammonolysis, in alcoholic ammonia, of several 9-alkyl compounds. Sutcliffe (8, 12) found treatment of 2,6,8-trichloro-9-(tetrahydropyran-2-yl)purine yielded 69.5% of the 8-amino-2,6-dichloro isomer and 25% of the 6-amino-2,8-dichloro isomer (8) while under the same conditions, 2,6,8-trichloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine gave 57% of the 8-amino-2,6-dichloro isomer (12). It is noteworthy that substitution of an amino group for the 2-chlorine resulted in deactivating the remaining 6 and 8 chlorine atoms. Indeed, ammonolysis of 2-amino-6,8-dichloro-9-(2,3,5-tri-*O*-acetyl-9-β-D-ribofuranosyl)purine (6) for 5 hours at 5° resulted in only deacetylation in 94% yield.

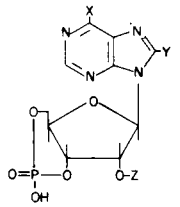
A review of the literature revealed only one 6,8-dichloropurine nucleoside unsubstituted in the 2 position *i.e.*, 6,8-dichloro-9-(2,3,4,6-tetra-*O*-acetyl-9-β-D-glucopyranosyl)purine (14). The reactivity reported for this compound toward ammonolysis (the only example given) appeared to differ from the reactivity observed for the 6 and 8 halogens of 9-alkyl-2,6,8-trichloropurines (8).

Since 2′-*O*-acyl-6,8-dichloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**2**) has two sites for nucleophilic substitution and the literature seemed unclear as to the order of reactivity of these sites, attention was focused as to the order of nucleophilic displacement on **2** in conjunction with its use as a synthetic intermediate.

When 2′-*O*-acetyl-6,8-dichloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**2a**) was treated with methanolic ammonia at 0°, complete reaction occurred within 12 hours. Three compounds, 8-chloroadenosine 3′,5′-cyclic phosphate (**3**), 8-amino-6-chloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**4**) and 6-chloro-9-β-D-ribofuranosylpurine-8-one 3′,5′-cyclic phosphate (**5**), were identified from the reaction mixture. The 8-amino-6-chloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**4**) was isolated in 43% yield and, as determined by pmr of the reaction mixture, was the major isomer (**4**:**3** > 9:1) (15a, b). The structure of **4** was assigned by thin-layer chromatography, uv and pmr spectra comparisions with the known isomeric 8-chloroadenosine 3′,5′-cyclic phosphate (**3**) (16) and by the fact that when **4** was treated with thiourea, 8-amino-9-β-D-ribofuranosylpurine-6-thione 3′,5′-cyclic phosphate (**6**) was isolated. The properties of **6** differed from the known isomeric 8-thioadenosine 3′,5′-

Table I

Uv Spectra and Tlc Mobility of 6,8-Disubstituted-9-β-D-Ribofuranosylpurine 3′,5′-Cyclic Phosphates

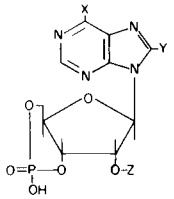


Compound	X	Y	Z (c)	λ max (nm) (ε x 10^{-3}) pH 1	pH 11	RcAMP (a,b) A	B
1a	OH	Br	$COCH_3$	252(14.7	257(13.4)	2.00	2.00
1b	OH	Br	$CO(CH_2)_2CH_3$			2.75	2.20
2a	Cl	Cl	$COCH_3$	248(6.6), 266(10.5), 274sh(8.8)	248(6.6), 266(10.2), 274sh(8.6)	3.03	4.51
2b	Cl	Cl	$CO(CH_2)_2CH_3$			3.24	5.51
3	NH_2	Cl		259.5(15.6)	261(14.2)	1.50	1.63
4	Cl	NH_2		238(4.9), 277(13.4)	257(8.0), 286(12.2)	1.53	1.75
5	Cl	OH		239(3.8), 277(11.1)	261(6.5), 292(10.2)	1.97	2.13
8-Cl-cIMP (b, d, e)	OH	Cl		251	255	1.47	1.13
6	SH	NH_2		234(13.7), 331(17.5), 297sh(10.0)	239(17.0), 312(22.9)	0.76	0.43
8-SH-cAMP (b,f)	NH_2	SH		244(9.9), 308(25.2)	291(22.4)	1.86	1.14
7 (e)	NEt_2	OH		274,300sh	290	2.54	2.38
8	OH	OH		253(11.0), 283sh(5.4)	263(11.7)	1.10	0.14
9	NEt_2	Cl		272(21.1)	276(19.4), 286sh(17.9)	3.71	4.00
10	Cl	NEt_2		290(13.8)	296(13.1), 265sh(6.7)	3.29	3.88
11	NEt_2	NEt_2		289(18.9)	286(20.0)	3.71	4.13
12	NEt_2	SCH_2Ph		291(21.8)	297(17.7)	3.79	5.57
13	Cl	SCH_2Ph	$COCH_3$	294(15.4)	294(15.4)	3.72	6.13
14	SCH_2Ph	SCH_2Ph		313(13.4), 340sh(7.6)	309(20.9)	3.78	9.75
19	Cl	S-*p*-ClPh	$COCH_3$	247(11.0), 293(13.8)	247(11.0), 293(14.0)	3.63	6.25
20	NEt_2	S-*p*-ClPh		290(24.4)	303(17.7)	3.25	4.88
21	SMe	SMe		246(13.7), 309(16.5), 300sh(14.5), 330sh(11.6)	245(15.6), 307(22.2), 301sh(21.6)	2.88	5.51
24	$NHCH_2Ph$	Br		269(24.3)	271(21.7)	3.12	5.29
25	$NHCH_2Ph$	SMe		289(24.6)	286(23.4)	2.97	4.43

(a) RcAMP = mobility relative to that of cAMP in solvent systems A and B. (b) Abbreviations used: cAMP, Adenosine 3′,5′-cyclic phosphate; 8-Cl-cIMP, 8-Chloroinosine 3′,5′-cyclic phosphate: 8-SH-cAMP, 8-Thioadenosine 3′,5′-cyclic phosphate. (c) Z = H unless otherwise indicated. (d) Reference 17. (e) Qualitative uv spectra only. (f) Reference 2.

Table II

Pmr Spectra of Some Isomeric 6,8-Disubstituted and Related 6,8-Disubstituted 9-β-D-Ribofuranosylpurine 3′,5′-Cyclic Phosphates (a, b, c)



Compound	X	Y	Z	δ, H_2	δ, H_1' (d)	δ, $N(CH_2CH_3)_2$ (e)	δ, $N(CH_2CH_3)_2$ (e)
2	Cl	Cl	$COCH_3$	8.95 (s)	6.21 (s) (f)		
3	NH_2	Cl	H	8.27 (s)	5.90 (s)		
4	Cl	NH_2	H	8.41 (s)	6.00 (s)		
5	Cl	OH	H	8.51 (s)	5.75 (s)		
8-Cl-cIMP (c, g)	OH	Cl	H	8.21 (s)	5.86 (s)		
6	SH	NH_2	H	8.11 (s)	5.80 (s)		
8-SH-cAMP (c, h)	NH_2	SH	H	8.24 (s)	6.54 (s) (i)		
9	NEt_2	Cl	H	8.28 (s)	5.90 (s)	3.87 (q)	1.21 (t)
10	Cl	NEt_2	H	8.55 (s)	5.70 (s)	3.49 (q)	1.24 (t)
11	NEt_2	NEt_2	H	8.17 (s)	5.74 (s)	3.91 (q), 3.25 (q)	1.2 (t), 1.12 (t)
12	NEt_2	SCH_2Ph	H	8.26 (s)	5.84 (s)	3.95 (m) (j)	1.24 (t)

(a) 60 MHz spectra were determined on a Perkin Elmer 20A Spectrometer in DMSO-d_6. (b) Proton chemical shifts in parts per million (δ) from internal DSS. (c) Abbreviations used: s, singlet; t, triplet; 8, quartet; m, multiplet; 8-Cl-cIMP, 8-chloroinosine 3′,5′-cyclic phosphate; 8-SH-cAMP, 8-thioadenosine 3′,5′-cyclic phosphate. (d) $J_{1'-2'} < 1$ Hz. For a review of the nmr spectra of 3′,5′-cyclic phosphates see reference 5 p. 238-241. (e) Where applicable. (f) Shifted downfield because of 2′-*O*-acyl group. (g) Reference 17. (h) Reference 2. (i) Shifted downfield because of anistropy effect of 8-thione, Robert A. Long and Leroy B. Townsend, *Chem. Commun.*, 1087 (1970). (j) Superimposed with other signals.

cyclic phosphate (2).

The third cyclic nucleotide formed during the ammonolysis of **2a** was assigned the structure of 6-chloro-9-β-D-ribofuranosylpurin-8-one 3′,5′-cyclic phosphate (**5**). This product was identical by thin-layer chromatography, uv and pmr spectra to the major product isolated from the hydrolyis of **2a** in 1*N* sodium hydroxide at room temperature. The substitution of the 8-chloro group of **2** by OH deactivates the remianing 6-chloro group. Indeed, no ammonolysis product, *i.e.*, 8-hydroxyadenosine 3′,5′-cyclic phosphate (2), could be detected by thin-layer chromatography or uv spectra upon the treatment of **5** with ethanolic ammonia in a bomb for three days at 90°.

Treatment of **5** for 12 hours at 90° with diethylamine in a bomb provided 6-(diethylamino)-9-β-D-ribofuranosylpurin-8-one 3′,5′-cyclic phosphate (**7**) which was identical by thin-layer chromatography and uv spectra to the product obtained from the hydrolysis of 8-chloro-6-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**9**) with sodium acetate:acetic acid at 120° for 24 hours. Vigorous hydrolysis of **5**, *i.e.*, sodium acetate:acetic acid at 120° for three days, gave 9-β-D-ribofuranosylpurine-6,8-dione 3′,5′-cyclic phosphate (**8**) which has been synthesized by two other routes (4). The assignment of the structure of **5** as the 6-chloro-8-hydroxy isomer was further supported by the fact that the uv spectra of **5** was distinctly different from the spectra of 8-chloroinosine 3′,5′-cyclic phosphate (17).

9-(Tetrahydropyran-2-yl and β-D-ribofuranosyl)-2,6,8-trichloropurines perferentially undergo attack with strongly basic nucleophiles such as hydroxide, alkoxide and alkylmercaptides at position 8 to yield 8-substituted followed by 6,8-disubstituted derivatives (8, 12). An exception to this is the alkylamines, which yield 6-(alkylamino)-2,8-dichloro and 6,8-*bis*-(alkylamino)-2-chloro derivatives (12). Montgomery *et al.* (13) found that 9-benzyl-6,8-dichloropurine, upon treatment with aqueous dimethylamine, gave 79% yield of the 6-(dimethylamino)-8-chloro isomer. Gerster *et al.* (6), upon treatment of 2-amino-6,8-dichloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine with alkylamines, isolated, in 37 to 58% yields, 6-(alkylamino)-8-chloro isomers.

When 2′-*O*-butyryl-6,8-dichloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**2b**) was treated with diethylamine in refluxing ethanol overnight followed by

hydrolysis of the 2′-*O*-acyl group with 2*N* ammonium hydroxide, two major products were isolated: 6,8-*bis*-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**11**) and 8-chloro-6-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**9**) were obtained in yields of 23% and 46%, respectively, after anion exchange chromatography of the reaction. When **2a** was treated with diethylamine in DMF at room temperature for four days, the same products **11** and **9** were obtained in yields of 28% and 35%, respectively, However, when the DMF reaction was quenched after 1 hour and the residue treated with 2*N* ammonium hydroxide to remove the 2′-*O*-acyl group, anion exchange chromatography yielded only a small amount of **11** ($< 3\%$) plus 40% of **9** and a new product, 6-chloro-8-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**10**) in 41% yield. Both **9** and **10** were stable to 2*N* ammonium hydroxide at room temperature for at least 15 hours. The 8-chloro-6-(diethylamino) isomer **9** was not aminated to the 6,8-*bis*-(diethylamino)purine nucleotide (**11**) upon treatment with diethylamine in DMF for four days, whereas the 6-chloro-8-(diethylamino) isomer **10** was completely converted to **11** overnight. Therefore, while nucleophilic attack took place at both the 6 and 8 positions with alkylamines, only the 6-chloro-8-dialkylamino isomer was a precursor for the *bis*-(dialkylamino)purine nucleotide **11**. The structure of 8-chloro-6-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**9**) was verified by treatment of **9** with benzylmercaptan to give 8-(benzylthio)-6-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**12**) which was also synthesized from 8-(benzylthio)-6-chloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**13**). The structure of **10** was assigned by comparing its uv and pmr spectra and thin-layer chromatography mobility with that of its isomer **9**.

Two routes were utilized to secure 2′-*O*-acetyl-8-(benzylthio)-6-chloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**13**). The first route involved treatment of **2a** with an ethanolic sodium acetate solution of benzylmercaptan at room temperature overnight. Several products were detected, the main product, which was isolated in 51% yield, was 2′-*O*-acetyl-8-(benzylthio)-6-chloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**13**). One of the other products formed during the reaction was found to be 6,8-*bis*(benzylthio)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**14**). Further reaction of **13** under reflux conditions with benzylmercaptan resulted in the formation of **14** as the major reaction product.

The second route to the 8-(benzylthio)-6-chloro nucleotide **13** is representative of the second method of synthesis of 6,8-disubstituted-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphates, *viz.* from 8-substituted inosine 3′,5′-cyclic phosphates (4) whose 8-substituents are stable to refluxing phosphorus oxychloride. This method was particularly suitable for 8-(substituted-thio)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphates. For example, acetylation of the triethylammonium salt of 8-(benzylthio)-inosine 3′,5′-cyclic phosphate (**15**) with acetic anhydride gave the respective 2′-*O*-acetyl derivative. The 2′-*O*-acetyl-8-(benzylthio)inosine 3′,5′-cyclic phosphate was then treated with refluxing phosphorus oxychloride. The resulting phosphorichloridate of 2′-*O*-acetyl-8-(benzylthio)-6-chloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**13**) was isolated by virtue of its insolubility in ice-water. Hydrolysis of the phosphorochloridate of **13** in sodium acetate buffer and further purification on a silica gel column resulted in a 51% yield of 2′-*O*-acetyl-8-(benzylthio)-6-chloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate sodium salt (**13**). Similar chlorinations with phosphorus oxychloride of the 2′-*O*-acetyl derivatives of 8-(methylthio) and 8-(*p*-chlorophenylthio)inosine cyclic phosphates (**16** and **17**) gave the corresponding 6-chloro-8-(methylthio) (**18**) and 6-chloro-8-(*p*-chlorophenylthio)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphates (**19**), respectively.

The 2′-*O*-acetyl-6-chloro-8-(substituted-thio)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphates (**13**, **18**, and **19**) were found to undergo facile nucleophilic attack by alkylthio and alkylamino nucleophiles. Amination of the 2′-*O*-acetyl-8-(benzylthio)-6-chloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**13**) with diethylamine gave the 8-(benzylthio)-6-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**12**) which was identical by thin-layer chromatography and uv spectra to the thiation product from 8-chloro-6-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**9**) and therefore established the structure of the 8-chloro-6-(diethylamino)purine (**9**) and 8-(benzylthio)-6-chloropurine (**13**) nucleotides obtained from 2′-*O*-acetyl-6,8-dichloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**2a**). An analogous amination of 2′-*O*-acetyl-6-chloro-8-(*p*-chlorophenylthio)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**19**) gave 8-(*p*-chlorophenylthio)-6-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphates (**20**). Compound **20** was also synthesized from **9** by treatment with *p*-chlorobenzenethiol. 6,8-*Bis*-(methylthio)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**21**) was obtained by treatment of 2′-*O*-acetyl-6-chloro-8-(methylthio)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**18**) with an aqueous methylmercaptan solution at 50° for three days.

Because of the good cAMP-dependent protein kinase activation by 8-(substituted-thio)adenosine 3′,5′-cyclic phosphates (2), we have been particularly interested in a one-step reaction from an 8-(substituted-thio)adenosine 3′,5′-cyclic phosphate to a 6,8-disubstituted derivative. We have recently reported on the Dimroth rearrangement

of N^1-alkoxyadenosine 3′,5′-cyclic phosphates to give N^6-alkoxyadenosine 3′,5′-cyclic phosphates (18). As a third method for obtaining 6,8-disubstituted cyclic nucleotides we investigated the applicability of the Dimroth rearrangement upon 1-alkyl-8-substituted-adenosine 3′,5′-cyclic phosphates. Accordingly, 8-(methylthio) (**22**) and 8-bromo- (**23**) adenosine 3′,5′-cyclic phosphates were alkylated with α-bromotoluene in DMSO, utilizing 1,8-diazabicyclo[5. 4.0]-undec-7-ene as a proton acceptor and as a solublizing agent for the cyclic nucleotide. The 1-alkyl-8-substituted nucleotides were rearranged by heating with aqueous carbonate/bicarbonate to give the 6-(benzylamino)-8-substituted-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphates (**24** and **25**). Difficulties in obtaining complete alkylation and in purification of the products, as well as loss of starting material in the formation of side products resulted in variable isolated yields of the desired 6-(monoalkylamino)-8-substituted purine 3′,5′-cyclic nucleotides; however, ease of synthesis made this method very favorable when compared to the aforementioned methods from 8-substituted-inosine 3′,5′-cyclic phosphates.

EXPERIMENTAL

Evaporations were performed *in vacuo* at $<40°$. Uv spectra were determined on a Cary 15 spectrometer. Silica gel for column chromatography was E. M. Reagent Silica Gel 60 (particle size 0.063-0.200 mm). The eluates from column chromatography were monitored at 254 nm to detect the presence of uv absorbing compounds. Unless otherwise stated, analytical samples were dried at 80-100°, 0.01 mm from 12 hours. Tlc of the ammonium salt of the compounds was run on E. Merck Silica Gel F-254 plates and developed with solvents system *A* acetonitril:0.1*M* ammonium chloride (4:1) or *B* methanol:chloroform (35:65). Elemental analyses were by Galbraith Laboratories, Inc., Knoxville, Tenn.

2′-*O*-Acetyl-8-bromoinosine 3′,5′-Cyclic Phosphate Triethylammonium Salt (**1a**).

A solution of 8-bromoinosine 3 ,5 -cyclic phosphate (4) (19 g., 71 mmoles) in methanol containing 10 ml. of triethylamine was evaporated to dryness. The residue was dissolved in 200 ml. of dry pyridine and 150 ml. of acetic acid and stirred overnight at room temperature. The solvent was removed *in vacuo* and the gum which remained crystallized upon scratching. The crude crystals were filtered, washed with ethyl acetate and dissolved in a small volume of methanol. Ethyl acetate was added to the methanol until crystals formed. The crystals were filtered and dried to yield 27 g. (69%) of **1a**.

Anal. Calcd. for $C_{18}H_{27}N_5O_8BrP$: C, 39.14; H, 4.92; N, 12.68; Br. 14.46. Found: C, 38.85; H, 4.65; N, 12.55; Br, 14.66.

2′-*O*-Acetyl-6,8-dichloro-9-β-D-ribofuranosylpurine 3′,5′-Cyclic Phosphate Sodium Salt (**2a**).

A stirrred mixture of 2′-*O*-acetyl-8-bromoinosine 3′,5′-cyclic phosphate triethylammonium salt (**1a**, 2 g., 3.6 mmoles) in 150 ml. of phosphorus oxychloride was placed into a 160° oil bath and refluxed for 10 minutes. The resulting solution was cooled and the liquid evaporated until a light oil remained. The oil was added dropwise to a mechanically stirred ice-water mixture. The resulting fine suspension was filtered and the solid washed with an ice-water mixture. The wet solid was dissolved in 15 ml. of *p*H 5 0.5*N* sodium acetate and extracted 3 times with 25 ml. of ether. The aqueous solution was evaporated to dryness and the residue co-distilled with ethanol until dry. The final dry residue was stirred with chloroform and filtered. The filter cake was washed several times with chloroform to ensure complete removal of product from residue salts. The filtrate and washings were evaporated to a small volume and placed onto a column of 30 g. silica gel (packed in chloroform). The column was washed with 1 l. of chloroform and then the product was eluted with methanol-chloroform (20: 80). The fractions containing product were pooled and evaporated. The residue was dissolved in a small volume of methanol and 20 volumes of ether added. This suspension was evaporated, ether added and the suspension again evaporated to dryness to yield 300 mg. (18%) of **2a**.

Anal. Calcd. for $C_{12}H_{10}N_4O_7Cl_2NaP \cdot 1\ 1/4\ H_2O$: C, 30.68; H, 2.68; N, 11.93; C. 15.09; P, 6.59. Found: C, 30.48; H, 2.27; N. 11.64; Cl, 15.16; P, 6.57.

8-Bromo-2′-*O*-butyrylinosine 3′,5′-Cyclic Phosphate Triethylammonium Salt (**1b**).

A solution of 8-bromoinosine 3′,5′-cyclic phosphate (4) (5 g., 9.8 mmoles) in methanol containing 3 ml. of triethylamine was evaporated to dryness. Ethanol was added to the residue and the resulting crystals were filtered, washed with ether and dried. A solution of the dry triethylammonium salt and 4-dimethylaminopyridine (0.2 g., 1.6 mmoles) in 50 ml. of DMF and 30 ml. of butyric anhydride was stirred for 3 hours. The solvent was evaporated and the residue was co-distilled 2 times with ethanol. The residue was dissolved in ethanol and ethyl acetate added until crystals formed. The crystals were filtered, washed with ethyl acetate, ether and dried to give 4.6 g. (81%) of **1b**.

2′-*O*-Butyryl-6,8-dichloro-9-β-D-ribofuranosylpurine 3′,5′-Cyclic Phosphate Sodium Salt (**2b**).

A solution of 8-bromo-2′-*O*-butyrylinosine 3′,5′-cyclic phosphate triethylammonium salt (**1b**, 4.5 g., 9.4 mmoles) was refluxed for 4 minutes in 30 ml. of phosphorus oxychloride and worked up as for compound **2a** to yield 2.65 g. (56%) of **2b**.

Anal. Calcd. for $C_{14}H_{14}N_4O_7Cl_2NaP \cdot H_2O$: C, 34.04; H, 3.30; N, 11.10; Cl, 14.45. Found: C, 34.09; H, 3.27; N, 11.36 Cl, 14.37.

8-Amino-6-chloro-9-β-D-ribofuranosylpurine 3′,5′-Cyclic Phosphate (**4**).

A solution of 2′-*O*-acetyl-6,8-dichloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate sodium salt (**2a**, 1 g., 2.1 mmoles) in 60 ml. of methanol saturate at 0° with ammonia was stirred at room temperature overnight in a bomb. The solvent was evaporated and the residue dissolved in water and placed onto a Dowex 50 x 8 (H^+, 100-200 mesh, 5 x 30 cm) column. The column was washed with water to elute first 6-chloro-9-β-D-ribofuranosylpurin-8-one 3′,5′-cyclic phosphate (**5**) then **4** followed by 8-chloroadenosine 3′,5′-cyclic phosphate (**3**). The appropriate fractions were pooled and evaporated. Methanol was added to the residue. The solid which formed was filtered and dried to yield 350 mg. (43%) of **4**. An analytical sample was obtained by recrystallization from water.

Anal. Calcd. for $C_{10}H_{11}N_5O_6ClP \cdot H_2O$: C, 31.46; H, 3.43; N, 18.35; Cl, 9.28. Found: C, 31.24; H, 3.58; N, 18.30; Cl, 9.31.

8-Amino-9-β-D-ribofuranosylpurine-6-thione 3′,5′-Cyclic Phosphate (**6**).

A solution of 8-amino-6-chloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**4**, 600 mg., 1.6 mmoles), thiourea (300 mg., 4.7 mmoles) and 5 drops formic acid in 20 ml. of water was refluxed for 1 hour. The solution was cooled to room temperature and the solid which had precipitated was filtered, washed with water and dried to give 447 mg. (78%) of crude **6**. An analytical sample was obtained by acidifying a basic solution of the crude product with 1*N* hydrochloric acid to *p*H 2. The solid was filtered, washed with water and dried.

Anal. Calcd. for $C_{10}H_{15}N_5OPS$: C, 33.24; H, 3.34; N, 19.38; S, 8.87. Found: C, 33.26; H, 3.36; N, 19.13; S, 8.78.

6-Chloro-9-β-D-ribofuranosyl purin-8-one 3′,5′-Cyclic Phosphate Sodium Salt (**5**).

A solution of 2′-*O*-acetyl-6,8-dichloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate sodium salt (**2a**, 1 g., 2.1 mmoles) in 20 ml. of 1*N* sodium hydroxide was stirred at room temperature overnight. A small amount of solid was filtered off and the filtrate was passed through a Dowex 50 x 8 (H^+, 100-200 mesh, 4 x 10 cm) column. The column was washed with water and the appropriate fractions were evaporated to dryness. The residue was dissolved in water and passed through a Dowex 50 (Na^+, 100-200 mesh, 4 x 5 cm) column. The column was washed with water and the appropriate fractions were evaporated to dryness. The residue was co-distilled with absolute ethanol. The final dry residue was dissolved in methanol and 4 g. of silica gel added. The solvent was evaporated and the dry powder applied to a 20 g. column of silica gel (packed in chloroform). The column was eluted with methanol chloroform (20:80). Appropriate fractions were pooled, evaporated and the resulting residue dissolved in methanol. Addition of ether to the methanol precipitated 319 mg. (33%) of **5**.

Anal. Calcd. for $C_{10}H_9N_4O_7ClNaP \cdot 3\ 1/2\ H_2O$: C, 26.70; H, 3.58; N, 12.46. Found: C, 26.69; H, 3.26; N, 12.40.

6,8-*Bis*-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-Cyclic Phosphate (**11**) and 8-Chloro-6-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-Cyclic Phosphate (**9**).

A solution of 2′-*O*-acetyl-6,8-dichloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate sodium salt (**2a**, 2 g., 4.2 mmoles) in 20 ml. of diethylamine and 30 ml. of DMF was stirred at room temperature for 4 days. The solvent was evaporated. The residue was taken up in water and after adjusting to *p*H 7 with 1*N* hydrochloric acid the solution was placed onto a Dowex 1 x 2 (formate, 100-200 mesh, 70 ml.) column. The column was washed with water and then eluted with a 1.4 l. gradient of 0-4*N* formic acid to elute **11** and then **9**. The appropriate fractions were pooled, evaporated to dryness, co-distilled twice with methanol and the dry residues taken up in methanol and 20 volumes of ethanol added. The solids which formed were collected on a filter, washed with ethanol and dried to give 0.54 g. (25%) of **11** and 0.66 g. (35%) of **9**.

Anal. Calcd. for $C_{18}H_{29}N_6O_6P$: C, 47.36; H, 6.40; N, 18.41. Found: C, 47.17; H, 6.32; N, 18.21.

Anal. Calcd. for $C_{14}H_{19}N_5O_6ClP$: C, 40.05; H, 4.56; N, 16.68; Cl, 8.44. Found: C, 39.77; H, 4.51; N, 16.45; Cl, 8.52.

6-Chloro-8-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-Cyclic Phosphate (**10**).

A solution of 2′-*O*-butyryl-6,8-dichloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate sodium salt (**2b**, 0.5 g., 1.05 mmoles) in 10 ml. of diethylamine and 20 ml. of DMF was stirred at room temperature for 1 hour. The solvent was evaporated and the residue dissolved in 50 ml. of 2 *N* ammonium hydroxide. After stirring for 1 hour at room temperature, the solvent was evaporated and the residue chromatographed on a Dowex 1 x 2 (35 ml.) column as for compound **9** to yield **11** (<3% by uv), 173 mg. of **9** (41%) and 176 mg. of **10** (40%).

Anal. Calcd. for $C_{14}H_{19}N_5O_6ClP$: C, 40.05; H, 4.56; N, 16.68. Found: C, 40.25; H, 4.63; N, 16.42.

2′-*O*-Acetyl-8-(benzylthio)-6-chloro-9-β-D-ribofuranosylpurine 3′,5′-Cyclic Phosphate Sodium Salt (**13**).

A.

A solution of 8-(benzylthio)inosine 3′,5′-cyclic phosphate (**4**) (**15**, 5 g., 11.1 mmoles) in 10% aqueous triethylamine was evaporated to dryness. The residue was co-distilled twice with pyridine. The final residue was stirred in 250 ml. of pyridine and 150 ml. of acetic anhydride overnight. The resulting solution was evaporated to dryness. The residue co-distilled with ethanol until all traces of pyridine were removed and 50 ml. of phosphorus oxychloride were added to the residue. After refluxing for 10 minutes, the reaction was worked up as for compound **2a** to yield 3.2 g. (51%) of **13**.

B.

To a solution of 2′-*O*-acetyl-6,8-dichloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate sodium salt (**2a**, 1 g., 2.1 mmoles) in 25 ml. of *p*H 8.70 0.1*M* sodium acetate was added 2 ml. of benzylmercaptan in 20 ml. of ethanol. The solution was stirred at toom temperature overnight. The solvent was evaporated and residue dissolved in 10 ml. of chloroform. This was applied to a column (2.5 cm) of 15 g. of silica gel (packed in chloroform). The column was washed with chloroform to remove excess benzylmercaptan. The product was then eluted with methanol-chloroform (20:80). The solvent was evaproated, the residue dissolved in ethanol and 20 volumes of ether added. The precipitate was filtered, washed with ether and dried to give 612 mg. (51%) of **13**.

Anal. Calcd. for $C_{19}H_{17}N_9NaO_7PS \cdot 2H_2O$: C, 39.97; H, 3.70; N, 9.81. Found: C, 39.94; H, 3.35; N, 9.92.

6,8-*Bis*(benzylthio)-9-β-D-ribofuranosylpurine 3′,5′-Cyclic Phosphate Sodium Salt (**14**).

A solution of 2′-*O*-acetyl-8-benzylthio-6-chloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate sodium salt (**13**, 750 mg., 1.3 mmoles) and 2 ml. of benzylmercaptan in 30 ml. of 0.25*M* sodium acetate was refluxed overnight. The solvent was evaporated and the residue co-distilled with ethanol until dry. The dry residue was dissolved in methanol and 4 g. of silica gel added. The methanol was evaporated and the residue applied to a column (2.5 cm.) of 15 g. of silica gel (packed in chloroform). The column was eluted with chloroform to remove the contaminating benzylmercaptan followed by methanol-chloroform, 500 ml. each of (5:95 and 10:90) then (15:85) until the product was eluted. The fractions containing the product were pooled and the solvent evaporated. A portion of residue was taken up in ethanol, 20 volumes of ether added and the solid filtered and dried to give 80 mg. of crude 2′-*O*-acetyl-6,8-*bis*-(benzylthio)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate sodium salt. The remainder of the residue was taken up in 20 ml. of 2 *N* ammonium hydroxide and let stand at room temperature for 2 hours. The solvent was evaporated and the residue co-distilled once with water to ensure complete removal of ammonia. The final residue was taken up in 50 ml. of ethanol-water (1:1) and 50 ml. of a saturated sodium chloride solution was added. The resulting gel was filtered, washed well with water and then with ethanol. The final gel was dried under vacuum for 12 hours at 100° to yield 290 mg. (38%) of **14**.

Anal. Calcd. for $C_{24}H_{22}N_4O_6NaPS_2 \cdot 1/2H_2O$: C, 48.84; H, 3.90; N, 9.50; S, 10.87. Found: C, 48.71; H, 4.03; N, 9.53; S, 10.79.

8-(Benzylthio)-6-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-Cyclic Phosphate (**12**).

A solution of 2′-*O*-acetyl-8-(benzylthio)-6-chloro-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate sodium salt (**13**, 3.5 g., 6.1 mmoles) in 90 ml. of 20% aqueous diethylamine was stirred overnight at room temperature. The solvent was evaporated and the residue was triturated 2 times with ether. The final residue was dissolved in water and placed onto a Dowex 50 x 8 [H^+, 100-200 mesh, 2.5 x 10 cm. prewashed with methanol-water (1:1)] column. The column was washed with water followed by 100 ml. of methanol-water (20:80), 100 ml. of methanol-water (1:1) and finally methanol-water (75:25) until all product was eluted. The appropriate fractions were pooled and taken to dryness. The residue was dissolved in boiling ethanol and 5 volumes water were added. The solution was boiled down to 1/5 volume. The crystals, which deposited upon cooling, were filtered and dried to yield 2.4 g. (75%) of **12**.

Anal. Calcd. for $C_{21}H_{26}N_5O_6PS \cdot H_2O$: C, 47.99; H, 5.37; N, 13.32; S, 6.10. Found: C, 47.71; H, 5.52; N, 13.12; S, 6.08.

2′-*O*-Acetyl-6-chloro-8-(*p*-chlorophenylthio)-9-β-D-ribofuranosyl purine 3′,5′-Cyclic Phosphate Sodium Salt (**19**).

8-(*p*-Chlorophenylthio)inosine 3′,5′-cyclic phosphate (4) 6.0 g., 12.2 mmoles) was dissolved in methanol containing 3 ml. of triethylamine. The solution was evaporated to dryness and the residue was dissolved in a mixture of DMF (100 ml.) and acetic anhydride (50 ml.) containing 4-dimethylaminopyridine (305 mg, 2.5 mmoles). The solution was stirred for 2 hours at room temperature, the solvent was evaporated and the residue dissolved in a minimum volume of methanol-water (1:1). This was passed through a Dowex 50 [H^+, 100-200 mesh, 4.5 x 15 cm, prewashed with methanol-water (1:1)] column. The eluate was evaporated to a small volume and the crystals which formed were filtered and washed with water. Recrystallization from water yielded 5.43 g. of 2′-*O*-acetyl-8-(*p*-chlorophenylthio)inosine 3′,5′-cyclic phosphate.

To 2′-*O*-acetyl-8-(*p*-chlorophenylthio)inosine 3′,5′-cyclic phosphate (1.9 g., 3.7 mmoles) and 2,6-lutidine (394 mg., 3.7 mmoles) was added 30 ml. of phosphorus oxychloride. The mixture was placed in a 160° oil bath and, after refluxing for 4 minutes the reaction worked up as for compound **2a** to yield 822 mg. (40%) of **19**.

Anal. Calcd. for $C_{18}H_{14}N_4O_7Cl_2NaPS$: C, 38.93; H, 2.54; N, 10.09. Found: C, 38.72; H, 2.52; N, 10.10.

8-(*p*-Chlorophenylthio)-6-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-Cyclic Phosphate (**20**).

A.

A solution of 2-*O*-acetyl-6-chloro-8-(*p*-chlorophenylthio)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate sodium salt (**19**, 0.8 g., 1.4 mmoles) in 30 ml. of 20% aqueous diethylamine was treated as for compound **12**. The residue from the column was dried to yield 395 mg. (51%) of **20**.

Anal. Calcd. for $C_{20}H_{23}N_5O_6ClPS$: C, 45.15; H, 5.19; N, 13.16. Found: C, 45.29; H, 5.07; N, 13.19.

B.

A solution of 8-chloro-6-(diethylamino)-9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate (**9**, 270 mg., 0.65 mmoles), sodium methoxide (200 mg., 3.7 mmoles) and *p*-chlorobenzenethiol (300 mg., 2.1 mmoles) in 50 ml. of methanol was refluxed overnight. The solvent was evaporated and the residue was triturated 2 times with ether. The final residue was dissolved in water and acidified to *p*H 1 with 2*N* hydrochloric acid. The resulting solid was collected and dissolved in water by the addition of 2*N* sodium hydroxide. The solvent was evaporated and the residue co-distilled 2 times with ethanol. The dry residue was dissolved in methanol and 2 g. silica gel was added. After evaporating the solvent, the residue was put onto a column of 10 g. of silica gel (packed in chloroform). The column was washed with chloroform to remove excess *p*-chlorobenzenethiol and then the product was eluted with methanol-chloroform (30:70). The fractions containing the product were pooled and were evaporated to dryness to give 35 mg. (9%) of **20**.

Anal. Calcd. for $C_{20}H_{22}N_5O_6ClNaPS \cdot 2H_2O$: C, 41.06; H, 4.44; N, 11.97. Found: C, 40.82; H, 4.27; N, 12.38.

6,8-*Bis*-(methylthio)-9-β-D-ribofuranosylpurine 3′,5′-Cyclic Phosphate Sodium Salt (**21**).

8-(Methylthio)inosine 3′,5′-cyclic phosphate (4) (**16**, 2 g., 5.3 mmoles) was treated as for compound **13** up to and including the addition of the oil to the ice-water mixture. The resulting solid which formed was filtered, washed with ice-water, and then dissolved in 50 ml. of *p*H 7 1*M* sodium acetate. The *p*H of the solution was adjusted to *p*H 9 with 2*N* sodium hydroxide and 10 ml. of methylmercaptan was added. The reaction mixture was stirred for 3 days at 50° under a condenser. After cooling to room temperature the volume was evaporated to 1/2. The solid which separated out was filtered and dissolved in 30 ml. of hot water and then 30 ml. of ethanol was added. Upon cooling **21** separated. Compound **21** was filtered, washed with ethanol and dried, yielding 300 mg. (12%).

Anal. Calcd. for $C_{12}H_{14}N_4O_6NaPS_2 \cdot H_2O$: C, 32.28; H, 3.58; N, 12.55. Found: C, 32.07; H, 3.98; N, 12.21.

6-(Benzylamino)-8-(methylthio)-9-β-D-ribofuranosylpurine 3′,5′-Cyclic Phosphate (**24**).

Aliquots of α-bromotoluene (0.25 ml.) were added at times 0, 20 minutes, 40 minutes, 80 minutes and 5 hours to a solution of 8-(methylthio)adenosine 3′,5′-cyclic phosphate (**2**) (1.0 g., 2.49 mmoles) and 1,8-diazabicyclo[5.4.0]undec-7-ene (812 mg., 5.4 mmoles) in 5 ml. of DMSO at 60°. After cooling to room temperature overnight, the reaction mixture was added to 150 ml. of water containing sodium bicarbonate (1.2 g.) and sodium carbonate (0.9 g.). The solution was heated on a steam bath for 2.5 hours. After filtering, the volume was diluted to 1 l., 10 g. of sodium chloride added and the solution extracted 2 times with 50 ml. of chloroform. The aqueous phase was applied to an Amberlite XAD-4 (130 ml., 2.5 x 25 cm) column. The column was washed well with water and the nucleotide eluted with a 5 l. gradient of water *vs* methanol. The solvent was evaporated and the residue suspended in methanol, filtered and dried at room temperature to yield 340 mg. (29%) of **24**.

Anal. Calcd. for $C_{18}H_{20}N_5O_6PS$: C, 46.44; H, 4.33; N, 15.04. Found: C, 46.20; H, 4.19; N, 14.77.

6-(Benzylamino)-8-bromo-9-β-D-ribofuranosylpurine 3′,5′-Cyclic Phosphate (**25**).

A solution of 8-bromoadenosine 3′,5′-cyclic phosphate (2) (1.22 g., 3 mmoles), 1,8-diazabicyclo[5.4.0]undec-7-ene (1.0 ml., 6.0 mmoles) and α-bromotoluene (0.4 ml.) in 8 ml. of DMSO was stirred for 18 hours. An additional aliquot of α-bromotoluene was added and the stirring was continued for 36 hours. The solution was added to 150 ml. of water containing sodium bicarbonate (1.3 g.) and sodium carbonate (1.0 g.) and heated on a steam bath for 2 hours. After adjusting the *p*H to 1.5 with concentrated hydrochloric acid, the solution was absorbed onto a charcoal column (30 ml., Barnebey-Cheney, UU 50-200 mesh) and washed

well with water. The nucleotide was eluted with ethanol-ammonium hydroxide-water (4:5:1). The solvent was evaporated and the residue in a small volume of water was applied to a Dowex 1 x 2 (formate, 100-200 mesh, 2.5 x 3 cm) column. The column was washed with water and then eluted with a 1 l. gradient of 0 to 4 *N* formic acid. The appropriate fractions were pooled and evaporated. The residue was suspended in a small amount of water, filtered and air-dried to yield 142 mg. (9.0%) of **25**.

Anal. Calcd. for $C_{17}H_{17}N_5O_6BrP \cdot 1\ 3/4H_2O$: C, 38.53; H, 3.95; N, 13.21. Found: C, 38.79; H, 3.74; N, 12.92.

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