Novel Analogs of Nucleoside 3′,5′-Cyclic Phosphates. I. 5′-Mono- and Dimethyl Analogs of Adenosine 3′,5′-Cyclic Phosphate

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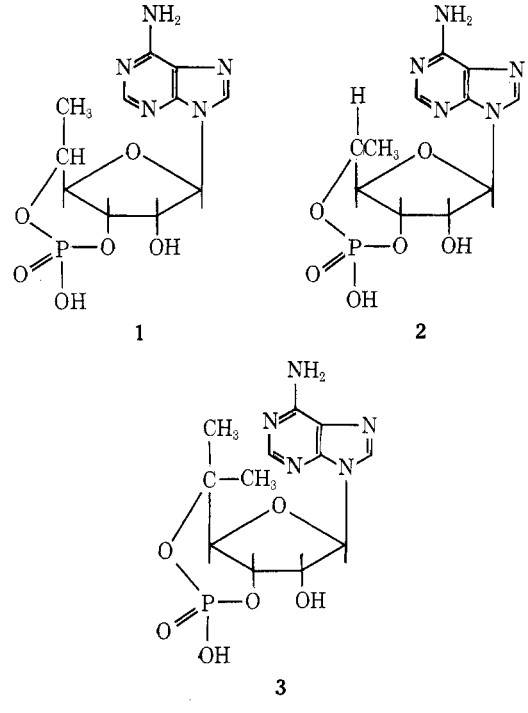
Oxidation of N^6-benzoyl-2′,3′-*O*-isopropylideneadenosine by the DMSO–DCC method gives pure N^6-benzoyl-2′,3′-*O*-isopropylideneadenosine-5′-aldehyde as a stable hydrate. Isolation of the latter aldehyde is facilitated by intermediate formation of a crystalline *N,N*′-diphenylimidazolidine derivative. Reaction of the free nucleoside aldehyde with methylmagnesium chloride gives a roughly 3:2 mixture of protected 6-deoxyhexofuranosyl nucleosides with the β-D-allo and α-L-talo configurations. Phosphorylation of the pure isomers, or of a mixture of both, with bis(2,2,2-trichloroethyl)phosphorochloridate, followed by removal of the protecting groups, gives the corresponding epimeric 6-deoxyhexofuranosyl nucleoside 5′-phosphates. Cyclization of the latter compounds using dicyclohexylcarbodiimide gives the corresponding crystalline 3′,5′-cyclic phosphates which are $C_{5'}$-monomethylated analogs of adenosine 3′,5′-cyclic phosphate. The reaction of methylmagnesium chloride upon a suitably blocked adenosine-5′-carboxylic acid ester gives derivatives of 5′,5′-dimethyladenosine. Phosphorylation of the latter followed by cyclization gives 5′,5′-dimethyladenosine 3′,5′-cyclic phosphate.

The plethora of biological roles played by adenosine 3′,5′-cyclic phosphate (cAMP) has led to unusually intense investigations concerning this key substance.[2] With a particular view to introducing increased selectivity of biological function, considerable activity has centered upon the chemical synthesis of analogs of cAMP. Such studies have led to the preparation of a large number of analogs bearing varied substituents principally at C_6[3] and C_8[3c,4] of the purine ring. Several modifications of the phosphoryl group have also been described leading to phosphonate,[5] thiophosphate,[6] and phosphoramidate[7] analogs. Relatively few modifications have, however, been made on the sugar itself, although the 2′-deoxyribo-,[8] arabino-,[9] and xylofuranose[10] analogs of cAMP have been described. The present work was directed toward determining the effect of adding one or two methyl groups to the 5′ carbon of cAMP. An examination of the biological properties of such molecules should cast some light upon the steric requirements of $C_{5'}$ of cAMP for binding to specific enzymes or receptor proteins. In this paper we describe the synthesis of the 3′,5′-cyclic phosphates of 9-(6-deoxy-β-D-allofuranosyl)adenine (**1**), 9-(6-deoxy-α-L-talofuranosyl)adenine (**2**), and 9-(6-deoxy-5-methyl-β-D-*ribo*-hexofuranosyl)adenine (5′,5′-dimethyladenosine, **3**). The biological properties of these substances will be described elsewhere.[11] Both aspects of the work have been recently summarized.[12]



1 **2**

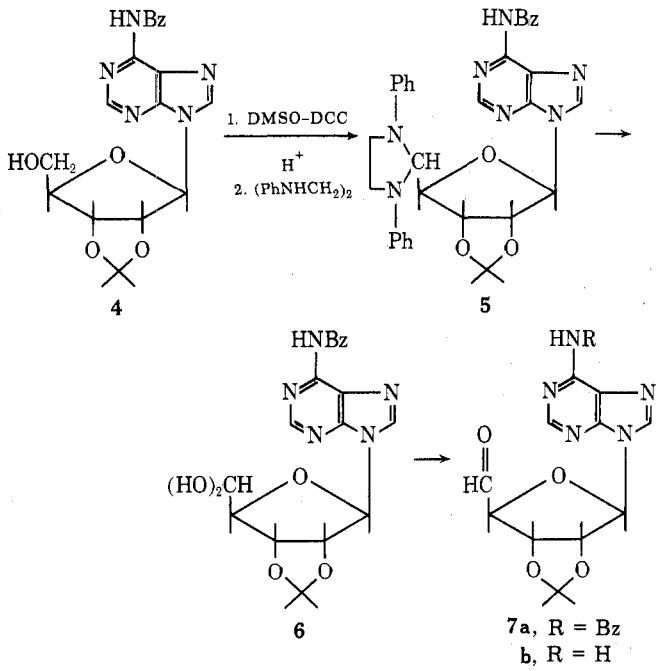
3

The chemical synthesis of **1**, **2** and **3** logically divides itself into three stages, namely, synthesis of the parent nucleosides, phosphorylation of the 5′-hydroxyl groups, and intramolecular cyclization to the 3′,5′-cyclic phosphates.

Preparations of both the parent nucleosides for **1** and **2**, 9-(6-deoxy-β-D-allofuranosyl)adenine[13] and its α-L-talofuranosyl epimer,[14] have been described by Reist, *et al.*, *via* condensation of N^6-benzoyladenine with derivatives of the appropriate sugars. The latter were, in turn, prepared *via* multistep processes starting from L-rhamnose. Our own continuing interest in the preparation and reactions of nucleoside 5′-aldehydes[15] suggested a much more direct route to these 5′-*C*-methyl nucleosides *via* a Grignard reaction. While we have in the past made considerable use of 2′,3′-*O*-isopropylideneadenosine-5′-aldehyde (**7b**) as an intermediate in condensation reactions,[16] this compound is difficult to isolate in pure form. This problem is particularly acute since we have shown that such aldehydes readily epimerize at $C_{4'}$ or eliminate the acetonide function, giving 3′,4′-unsaturated aldehydes upon attempted chromatography.[17]

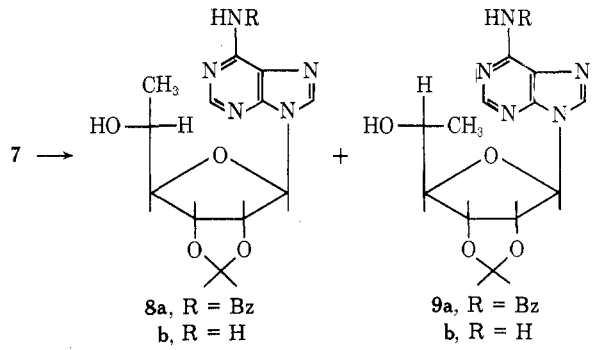
In order to both simplify the isolation of the nucleoside aldehyde and to minimize potential side reactions during the Grignard step we decided to use N^6-benzoyl-2′,3′-*O*-isopropylideneadenosine-5′-aldehyde (**7a**) as the key intermediate. The parent nucleoside N^6-benzoyl-2′,3′-*O*-isopropylideneadenosine (**4**) was prepared in 67% yield by essentially the method of Chládek and Smrt.[18] Spectral and analytical data on **4** clearly showed it to have the correct structure, but the observed melting point was 20° higher than that previously reported.[18] Oxidation of **4** was conveniently achieved by treatment with dimethyl sulfoxide and dicyclohexylcarbodiimide in the presence of dichloroacetic acid.[19] The resulting protected aldehyde (**7a**) was readily isolated as its crystalline 1,3-diphenylimidazolidine derivative (**5**) in 69% yield.[20] Treatment of this derivative (**5**) with Dowex 50 (H^+) resin in aqueous tetrahydrofuran at room temperature readily regenerated the aldehyde as its pure, stable hydrate (**6**) in 79% yield. This simple method appears to provide, for the first time, a general route for the preparation of pure protected nucleoside 5′-aldehydes and has greatly facilitated the study of their chemistry.[21]

The reaction of the aldehyde hydrate **6** with methylmagnesium chloride in tetrahydrofuran was examined

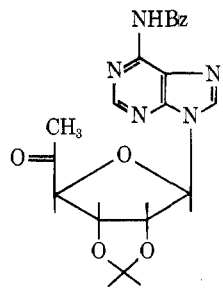


under a variety of conditions and led to the isolation of a mixture of the two expected products N^6-benzoyl-9-(6-deoxy-2,3-*O*-isopropylidene-β-D-allofuranosyl)adenine (**8**) and its α-L-talofuranosyl epimer (**9**) in a ratio of roughly 3:2. These reactions, however, appeared to require a large excess (~10 equiv) of Grignard reagent and were accompanied by significant debenzoylation of the adenine ring. Attempts to inhibit the debenzoylation reaction by using lower temperatures failed, since no significant reaction took place at 0°. If, however, the aldehyde hydrate **6** was azeotroped with benzene using a Dean-Stark apparatus, the insoluble starting material went quite rapidly into solution in the form of the free aldehyde **7**. Evaporation of the benzene left **7** as a foam, the nmr of which showed it to be roughly 85% in the aldehydo form, the expected aldehyde proton appearing as a singlet at 9.29 ppm. In addition, a crystalline 2,4-dinitrophenylhydrazone derivative was obtained starting from the hydrate **6**. Unlike the hydrate **6**, the free aldehyde **7** rapidly reacted with methylmagnesium chloride at low temperatures, and by conducting the homogeneous reaction at −70° in tetrahydrofuran the undesired debenzoylation reaction was avoided. While it is important that the dehydration of **6** to **7** be relatively efficient, we found that prolonged azeotropic treatment with benzene leads to the appearance of a less polar product which is tentatively suggested to be the cyclic trimer of **7**.

Utilizing the low-temperature Grignard reaction described above, it was possible to obtain a mixture of the desired alcohols **8a** and **9a** in a combined yield of 60%. Separation of the isomers by crystallization was inefficient and, while a complete separation could be effected by preparative tlc, it was impractical since it was essential to use very light loading of the plates. Chromatography of the mixture on a column of silicic acid quite readily separated a considerable portion of the less polar isomer **8a** in pure form followed by a mixture of **8a** and **9a** and finally by some pure **9a**. By repeating the chromatography of the mixed fractions it was possible to largely separate **8a** and **9a** as the pure isomers. The purity of isolated **8a** and **9a** could be readily demonstrated by nmr spectroscopy, particularly by examination of the signals for $C_{6'}H_3$ which appeared as three-proton doublets ($J_{5',6'}$ = 6 Hz) at 1.01 and 1.09 ppm, respectively. In addition, removal of the protecting groups from both **8a** and **9a** by treatment first with methanolic ammonium hydroxide and then with 90% trifluoroacetic acid gave the chromatographically homogeneous and separable parent nucleosides. The nucleoside from **8a** was identical with an authentic sample of 9-(6-deoxy-β-D-allofuranosyl)adenine kindly provided by Dr. E. Reist. Finally, both **8a** and **9a** were degraded to the parent 6-deoxy sugars by more vigorous acidic hydrolysis. In this way the less polar nucleoside **8a** was degraded to 6-deoxy-D-allose, which was chromatographically identical with an authentic sample prepared by similar treatment of methyl 6-deoxy-2,3-*O*-isopropylidene-β-D-allofuranoside kindly provided by Dr. Leon Goodman. The more polar isomer **9a** gave, upon similar treatment, a single less polar sugar which was identical in its chromatographic behavior with an authentic sample of 6-deoxy-L-talose.[23] The above degradations confirm that the crystalline, less polar isomer has the D-allo configuration (**8a**) while the more polar isomer is the L-talo epimer (**9a**).



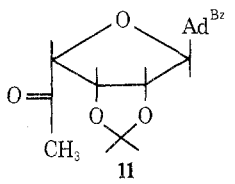
In an effort to modify the ratio of the Grignard products we have examined the oxidation of a mixture of **8a** and **9a** using the DMSO-DCC method.[19] Using pyridinium trifluoroacetate as the proton source, this oxidation led to a single product that was clearly the methyl ketone **10** by



the presence of a three-proton singlet at 1.93 ppm in its nmr spectrum in $CDCl_3$. The product could not, however, be crystallized and was contaminated by a little dicyclohexylurea. Attempted chromatography led to extensive isomerization (roughly 80% by nmr) to a different methyl ketone, presumably the 4′ epimer **11**, arising by mechanisms similar to those attending chromatography of nucleoside 5′-aldehydes.[17] These mixed ketones could not be separated and gave a noncrystalline mixture of 2,4-dinitrophenylhydrazones that could be distinguished only by nmr. The crude, unepimerized ketone was directly reduced with sodium borohydride to regenerate only **8a** and **9a** in a ratio of roughly 1:2. The reduction of **10** with sodium borohydride does not show sufficient stereoselectivity to make this a useful method for more selective preparation of **8a** or **9a**. As yet other more sterically demanding reducing agents have not been examined.

The availability of the largely epimerized ketone arising from attempted chromatography of **10** did, however, give us confidence that the aldehyde **7** did not undergo epim-

erization during the Grignard reaction. Thus, reduction with sodium borohydride of crude epimerized **10** led to the formation of four alcohols, two minor isomers of which were identical by tlc with **8a** and **9a**. The other alcohols, which were totally absent from the original Grignard reaction mixture, were isolated by chromatography and are undoubtedly N^6-benzoyl-9-(6-deoxy-β-D-gulofuranosyl)adenine (**12**) and its 6-deoxy-α-L-mannofuranosyl isomer (**13**). In support of this, both **12** and **13** were hydrolyzed with acid as above to liberate the corresponding 6-deoxyhexoses. The sugar arising from the less polar isomer was shown to be chromatographically identical[22] with 6-deoxy-L-mannose, while that from the more polar isomer was identical with 6-deoxy-D-gulose.[23] The configurations of the four isomers (**8a, 9a, 12,** and **13**) thus appear to be certain.



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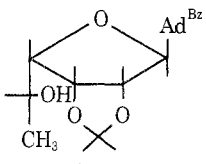

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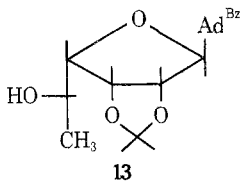

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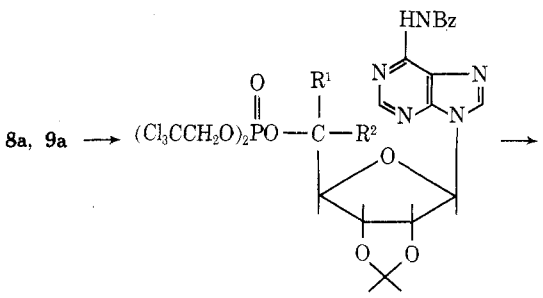
Following the completion of this stage of our work the preparation of the debenzoylated alcohols **8b** and **9b** was described by Howgate and Hampton[24] *via* reaction of crude 2′,3′-*O*-isopropylideneadenosine-5′-aldehyde with methylmagnesium iodide in dioxane–tetrahydrofuran–ether at 20°. The heterogeneous reaction, however, required 21 days for completion, and separation of **8b** and **9b** in yields of 11 and 7% required column chromatography on silicic acid, preparative tlc using 27 developments, and ion exchange chromatography. The *N*-benzoyl derivatives **8a** and **9a** prepared in the present work are thus much easier to separate and offer the additional advantage of not requiring further protection prior to phosphorylation. Debenzoylation of **8a** with methanolic ammonium hydroxide gave crystalline **8b** with a melting point similar to that described by Howgate and Hampton.[24] The chemical shifts shown in the nmr spectrum of our sample were, however, very different from those described by Hampton and Howgate. This is undoubtedly due to a bulk susceptibility effect resulting from their use of an external standard[25] of tetramethylsilane.

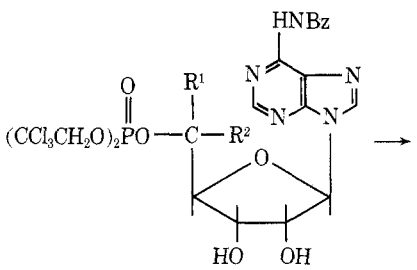
Phosphorylation of the free 5′-hydroxyl group of pure **8a** was readily accomplished by the 2-cyanoethyl phosphate–dicyclohexylcarbodiimide method of Tener.[26] Following removal of the protecting groups by treatment with 9 *N* ammonium hydroxide and then 90% trifluoroacetic acid, the product was purified by ion exchange chromatography giving 9-(6-deoxy-5-*O*-phosphoryl-β-D-allofuranosyl)adenine (**16a**) as the crystalline free acid in 47% yield. Alternatively the phosphorylation of **8a** was achieved using an excess of bis(2,2,2-trichloroethyl)phosphorochloridate in pyridine.[27] By a simple partitioning process the desired 5′-*O*-bis(2,2,2-trichloroethyl)phosphoryl derivative **15a** was isolated as a chromatographically pure foam in quantitative yield.

The great advantage of the use of bis(2,2,2-trichloroethyl)phosphorochloridate, however, lies in its use with the crude mixture of alcohols (**8a, 9a**) resulting from the Grignard reaction. Phosphorylation of this mixture provides a mixture of epimeric phospho triesters (**15a, 15b**) that can be quite easily separated into the pure compounds by chromatography on a column of silicic acid. A single column permits complete resolution of roughly 75% of the mixture and rechromatography of the mixed fractions completes the separation. In this way, phosphorylation of a mixture of **8a** and **9a** that was somewhat enriched in **8a** gave pure **15a** and **15b** in yields of 51 and 22%, respectively. Since the chromatographic separation of **15a** and **15b** is considerably more facile than that of the parent alcohols **8a** and **9a** this becomes the method of choice for preparing the isomeric phosphates.

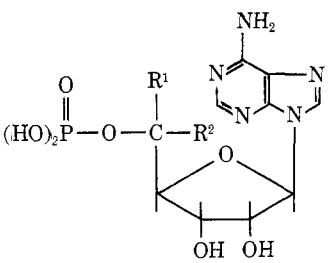
Brief treatment of the D-allo isomer **14a** with 90% trifluoroacetic acid at room temperature removed the isopropylidene group, giving the crystalline diol **15a** in a yield of 89%. Removal of the trichloroethyl protecting groups was accomplished by treatment with finely divided zinc powder and acetic acid in dimethylformamide at 0°, this procedure giving better results than the use of zinc in aqueous pyridine (100°) or acetic acid (50°), or a zinc–copper couple in dimethylformamide at 50°. Following removal of zinc salts by ion exchange, the crude product was treated with ammonium hydroxide to remove the N^6-benzoyl group and purified by ion exchange chromatography. This effected removal of a small amount (12%) of a monoanion (presumably a monotrichloroethyl ester of **16a**) and led to the isolation of the triethylammonium salt of 9-(6-deoxy-β-D-allofuranosyl)adenine 5′-phosphate (**16a**) in a yield of



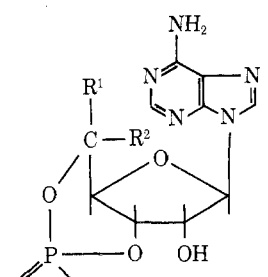
14a, R^1 = Me; R^2 = H
b, R^1 = H; R^2 = Me
c, R^1 = R^2 = Me



15a, R^1 = Me; R^2 = H
b, R^1 = H; R^2 = Me
c, R^1 = R^2 = Me



16a, R^1 = Me; R^2 = H
b, R^1 = H; R^2 = Me
c, R^1 = R^2 = Me



1, R^1 = Me; R^2 = H
2, R^1 = H; R^2 = Me
3, R^1 = R^2 = Me

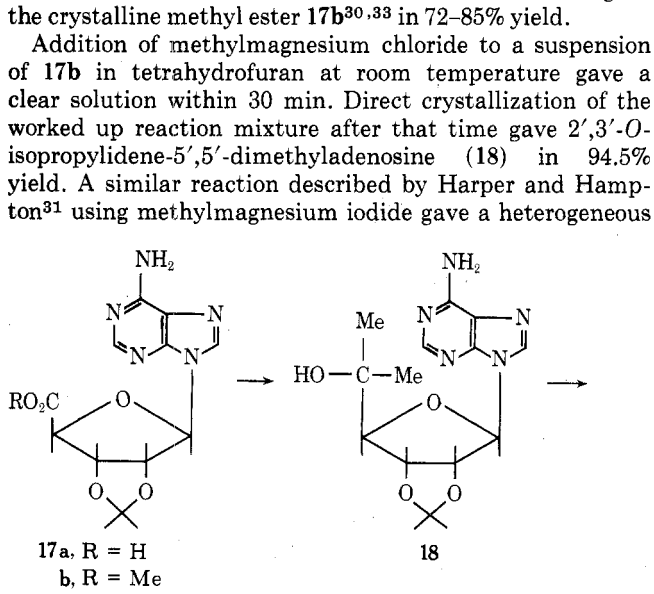
77%. For analytical purposes the latter compound was easily isolated as the crystalline free acid.

In a similar way the L-talo phospho triester **14b** was deacetonated and then without purification subjected to the treatment with zinc and acetic acid in dimethylformamide followed by ammonium hydroxide. Ion exchange chromatography then gave the pure triethylammonium salt of **16b** in an overall yield of 52%. Once again this compound could be isolated as the crystalline free acid with excellent recovery, but the triethylammonium salt was used as such in the next step.

Intramolecular cyclization of both **16a** and **16b** was carried out using dicyclohexylcarbodiimide in pyridine under high dilution conditions according to the general method of Smith, *et al.*[28] Both reactions were essentially quantitative and the pure 3′,5′-cyclic phosphates (**1** and **2**) were isolated as the crystalline free acids in yields of 100 and 84%, respectively. As expected, paper electrophoresis at pH 7.5 showed both **1** and **2** to behave as monoanions and, similar to cAMP itself, the nmr spectra of both compounds showed sharp singlets for $C_{1'}H$.[29]

In order to complete the synthesis of all possible 5′-methylated derivatives of cAMP we also wished to prepare 5′,5′-dimethyladenosine 3′,5′-cyclic phosphate (**3**). The parent nucleoside, 5′,5′-dimethyladenosine, has been prepared by Nutt and Walton *via* condensation of adenine with an appropriately substituted derivative of 5,5-dimethyl-D-ribofuranose.[30] We were, however, more interested in a route to such compounds *via* alkylation at a nucleoside level by a Grignard reaction on a suitable nucleoside uronic acid ester. Such a reaction has recently been described by Harper and Hampton[31] but their results differ sufficiently from our own to justify some comment. Oxidation of 2′,3′-*O*-isopropylideneadenosine with potassium permanganate according to Schmidt, *et al.*,[32] gave the 5′-carboxylic acid **17a** in 74% yield and methylation of the latter with diazomethane in methanol–dioxane–ether gave the crystalline methyl ester **17b**[30,33] in 72–85% yield.

Addition of methylmagnesium chloride to a suspension of **17b** in tetrahydrofuran at room temperature gave a clear solution within 30 min. Direct crystallization of the worked up reaction mixture after that time gave 2′,3′-*O*-isopropylidene-5′,5′-dimethyladenosine (**18**) in 94.5% yield. A similar reaction described by Harper and Hampton[31] using methylmagnesium iodide gave a heterogeneous reaction requiring 7 days for completion. Isolation of the product by preparative tlc then gave **18** in 24–35% yields and with a melting point almost 50° lower than we have found.



Phosphorylation of the tertiary hydroxyl group of **18** using both bis(2,2,2-trichloroethyl)phosphorochloridate[27] and *o*-phenylene phosphorochloridate[34] has been examined under a wide range of conditions but without notable success. Using the former reagent it could be seen that the primary site of phosphorylation was the N^6-amino function of the adenine ring giving a product that was isolated by preparative tlc, shown to be a single compound by nmr, and having λ_{max} (MeOH) 262 nm and a shoulder at 267 nm. A compound with the same spectrum was obtained by phosphorylation of 2′,3′,5′-tri-*O*-acetyladenosine.

Continued reaction of **18** with a larger excess of phosphorylating agent did give indication of the formation of diphosphorylated (presumably N^6,5′-O) and triphosphorylated (presumably N^6,1,5′-O) species, the latter being decomposed to the former upon storage in aqueous pyridine. The presumed N^6-phosphorylated species, however, proved to be unexpectedly stable and could not be selectively removed by acidic hydrolysis either before or after treatment with zinc and acetic acid in dimethylformamide.

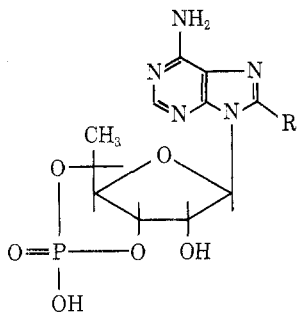
Because of these difficulties, **18** was benzoylated using benzoyl chloride in pyridine and any 5′-*O*-benzoyl group was selectively cleaved with sodium hydroxide in aqueous pyridine by the general method of Ralph and Khorana.[35] Following this treatment the desired N^6-benzoyl derivative **19** was isolated by chromatography on silicic acid in 84% yield. While **19** was not crystalline, its structure is assumed by the presence of a single benzoyl group (nmr and analysis) and a uv maximum at 280 nm (ϵ 20,200). Phosphorylation of **19** using a large excess of bis(2,2,2-trichloroethyl)phosphorochloridate in pyridine was slow at room temperature and required almost 7 days for complete disappearance of the starting material. Examination of the mixture by tlc showed the formation of three less polar products, the two faster moving of which (presumably *N*-phosphoryl compounds) degraded to give the slower spot upon treatment with aqueous pyridine. Following this treatment the desired bis(2,2,2-trichloroethyl)-N^6-benzoyl-2′,3′-*O*-isopropylidene-5′,5′-dimethyladenosine 5′-phosphate (**14c**) was isolated as an analytically and spectroscopically pure foam in 68% yield.

After several trials it was concluded that the most effective sequence for removal of the protecting groups involved initial treatment with zinc dust and formic acid[36] in dimethylformamide followed by removal of the isopropylidene and N^6-benzoyl groups with 90% trifluoroacetic acid and ammonium hydroxide. Ion exchange chromatography of the product led to the isolation of the triethylammonium salt of 5′,5′-dimethyladenosine 5′-phosphate (**16c**) in 60% yield. Despite being chromatographically homogeneous, **16c** could not be obtained in crystalline form and for analytical purposes it was isolated as its barium salt. Intramolecular cyclization of **16c** was achieved under the usual conditions and gave the crystalline free acid form of the 3′,5′-cyclic phosphate **3** in 93% yield.

An examination of the nmr spectra of the various phosphate derivatives shows that there is no difference in the chemical shifts (1.68 and 1.69 ppm) of the $C_{6'}H_3$ groups in the acyclic compounds **16a** and **16b**. In the cyclic phosphates, however, the equatorial methyl group in the D-allo compound **1** appears at much higher field (1.38 ppm) than does the axial methyl group in the L-talo isomer **2** which is at 1.75 ppm.[37] In addition, the equatorial methyl group in **1** shows a small (1 Hz) but significant coupling to phos-

phorus, while this cannot be seen with its axial counterpart in **2**. The dimethyl compound **3** shows characteristic resonances for both types of methyl group, the high-field signal (1.83 ppm) once again showing marked broadening due to phosphorus coupling while the low field methyl group (1.50 ppm) is very sharp.

In view of the interesting biological activities shown by certain 8-substituted derivatives of adenosine 3′,5′-cyclic phosphate,[3c,4] we have also prepared two such derivatives from **1**. Thus the reaction of **1** with bromine in a buffered aqueous solution at pH 3.9 gave the crystalline 8-bromo derivative **20a** in 43% yield. Subsequent treatment of **20a** with benzyl mercaptan in the presence of sodium methoxide led to the formation of 8-benzylthio-9-(6-deoxy-β-D-allofuranosyl)adenine 3′,5′-cyclic phosphate (**20b**) which was isolated in crystalline form following ion exchange chromatography.



20a, R = Br
b, R = SCH_2Ph

A brief survey of some of the biological properties of the alkylated analogs of adenosine 3′,5′-cyclic phosphate described in this paper has been presented.[12] Details of this work will be presented shortly.[11]

Experimental Section

General Methods. Nuclear magnetic resonance (nmr) spectra were obtained using a Varian HA-100 spectrometer and are reported in parts per million downfield from an internal standard of tetramethylsilane. Thin layer chromatography (tlc) was conducted using 0.25-mm layers of silica gel HF from Analtech Corp., and preparative tlc using 20 × 100 cm glass plates coated with a 1.3-mm layer of Merck silica gel GF. Merck silica gel with 0.05–0.20-mm particles was used for column chromatography. Cation exchange procedures were done using Dowex 50W (X8) resin with 50–100 mesh particles. Elemental and other instrumental analyses were obtained by the staff of the Analytical Laboratories of Syntex Research. Melting points were obtained using a hot-stage microscope and are corrected.

N^6-Benzoyl-2′,3′-*O*-isopropylideneadenosine (4). This compound was prepared by the method of Chládek and Smrt[18] and crystallized from ethanol in 67% yield: mp 151–153° (reported mp 132–133°); λ_{max} (MeOH) 280 nm (ε 20,600), 230 (13,150); nmr ($CDCl_3$) 1.36 and 1.61 (s, 3, CMe_2), 3.74 (dd, 1, J_{gem} = 12.5, $J_{4',5'a}$ = 2.5 Hz, $C_{5'a}$**H**), 3.95 (dd, 1, $J_{4',5'b}$ = 2 Hz, $C_{5'b}$**H**), 4.50 (m, 1, $C_{4'}$H), 5.04 (dd, 1, $J_{2',3'}$ = 6, $J_{3',4'}$ = 1 Hz, $C_{3'}$H), 5.20 (dd, 1, $J_{1',2'}$ = 4 Hz, $C_{2'}$H), 5.96 (d, 1, $C_{1'}$H), 7.5 and 8.0 (m, total 5, Ar), 8.08 and 8.70 ppm (s, 1, C_2H, C_8H).

Anal. Calcd for $C_{20}H_{21}N_5O_5$ (411.41): C, 58.38; H, 5.14; N, 17.02. Found: C, 58.40; H, 5.46; N, 17.00.

N^6-Benzoyl-5′-deoxy-2′,3′-*O*-isopropylidene-5′,5′-(*N*,*N*′-diphenylethylenediamino)adenosine (5). A solution of **4** (82 g, 200 mmol) and dicyclohexylcarbodiimide (124 g, 600 mmol) in rigorously anhydrous dimethyl sulfoxide (450 ml) was stirred with ice cooling while dichloroacetic acid (8.0 ml, 100 mmol) was added dropwise. The mixture was then stirred at 20° for 90 min, at which time tlc using 1-butanol–acetone–chloroform (5:15:80) showed the reaction to be complete. A solution of oxalic acid dihydrate (50 g, 400 mmol) in methanol (200 ml) was slowly added, and after 30 min at 20° the mixture was filtered and the crystalline residue of dicyclohexylurea was washed with cold methanol. *N*,*N*′-Diphenylethylenediamine (50 g, 230 mmol) was added to the combined filtrate and washings and the resulting solution was stored at 20° for 1 hr. Water was then added to slight turbidity and after scratching and storage 87.7 g of crystals was obtained. The mother liquors were partitioned between water and chloroform and the organic phase was washed twice with water and evaporated. Crystallization from ethanol gave a further 23.61 g of crude **5**. Recrystallization of the combined crystalline crops from ethanol gave 82.9 g (69%) of pure **5**: mp 132–135° (unchanged upon recrystallization); λ_{max} (MeOH) 252 nm (ε 43,000), 281 (22,700); $[\alpha]^{23}D$ 34.8° (*c* 0.1, MeOH); nmr ($CDCl_3$) 3.60 (m, 4, NCH_2's), 5.72 (d, 1, $J_{4',5'}$ = 2.5 Hz, $C_{5'}$H), 6.14 (d, 1, $J_{1',2'}$ = 1.5 Hz, $C_{1'}$H), 7.79 and 8.69 ppm (s, 1, C_2H and C_8H).

Anal. Calcd for $C_{34}H_{33}N_7O_4$ (603.66): C, 67.64; H, 5.51; N, 16.24. Found: C, 67.29; H, 5.52; N, 16.19.

N^6-Benzoyl-2′,3′-*O*-isopropylideneadenosine-5′-aldehyde (6). A. As the Aldehyde Hydrate. Dried Dowex 50 (H^-) resin (56 g) was added to a solution of **5** (36.6 g, 60.6 mmol) dissolved in 1:1 aqueous tetrahydrofuran (3200 ml) and stirred at 20° for 1 hr. The resin was then removed by filtration and washed with tetrahydrofuran (4 × 100 ml). The combined filtrates were evaporated to roughly half their volume and the resulting white, amorphous solid was removed, washed with water, and dried *in vacuo* at 40°, giving 20.28 g (79%) of **6** as a stable hydrate. This material gave a single spot upon tlc using CCl_4–acetone (3:2): λ_{max} (MeOH) 280 nm (ε 20,300), 230 (sh, 13,900); $[\alpha]^{23}D$ −66.2° (*c* 0.5, MeOH); ORD (MeOH) $[\Phi]_{278}$ (tr) −1800°, $[\Phi]_{249}$ 0°; nmr (DMSO-d_6) 1.35 and 1.56 (s, 3, CMe_2), 4.11 (dd, 1, $J_{3',4'}$ = 1.5, $J_{4',5'}$ = 4 Hz, $C_{4'}$H), 4.87 (dt, 1, $J_{H,OH}$ = 6 Hz, becoming doublet with D_2O, $C_{5'}$H), 5.08 (dd, 1, $J_{2',3'}$ = 6 Hz, $C_{3'}$H), 5.36 (dd, 1, $J_{1',2'}$ = 3 Hz, $C_{2'}$H), 6.15 and 6.28 [d, 1, $C_{5'}(OH)_2$], 6.27 (d, 1, $C_{1'}$H), 7.6 and 8.0 (m, 5, Ar), 8.64 and 8.74 (s, 1, C_2H and C_8H), 11.4 ppm (br s, 1, NH).

Anal. Calcd for $C_{20}H_{21}N_5O_6$ (427.41): C, 56.20; H, 4.95; N, 16.39. Found: C, 56.28; H, 5.12; N, 16.19.

Treatment of **6** with 2,4-dinitrophenylhydrazine in dimethyl sulfoxide[38] gave a crystalline dinitrophenylhydrazone: mp 218–220° from ethanol–ethyl acetate; λ_{max} (dioxane) 277 nm (ε 25,900), 351 (21,600); $[\alpha]^{23}D$ −114.7° (*c* 0.6, dioxane).

Anal. Calcd for $C_{26}H_{23}N_9O_8$ (589.51): C, 52.97; H, 3.93; N, 21.38. Found: C, 53.18; H, 4.00; N, 21.65.

B. As the Free Aldehyde. A suspension of the aldehyde hydrate **6** (427 mg, 1 mmol) in benzene (75 ml) was heated under reflux for 1 hr using a Dean-Starke apparatus. The clear benzene solution was then evaporated to dryness and dried under high vacuum, leaving the free aldehyde **7** as a white foam. The nmr spectrum of this material in DMSO-d_6 showed the presence of 20% residual **6** and 80% **7**: nmr 4.78 (d, 1, $J_{3',4'}$ = 2 Hz, $C_{4'}$H), 5.39 (d, 1, $J_{2',3'}$ = 6 Hz, $C_{2'}$H), 5.50 (dd, 1, $C_{3'}$**H**), 6.54 (s, 1, $C_{1'}$H), 7.5 and 8.0 (m, total 5, Ar), 8.57, 8.59 (s, 1, C_2H and C_8H), 9.30 (s, 1, $C_{5'}$H). Addition of D_2O slowly regenerated the spectrum of **6**. A satisfactory elemental analysis was not obtained.

Reaction of 7a with Methylmagnesium Chloride. The aldehyde hydrate **6** (2.15 g, 5 mmol) was converted to the free aldehyde **7** as above. The residue obtained after evaporation of the benzene was dissolved in anhydrous tetrahydrofuran (75 ml) and cooled to −78°. A solution of methylmagnesium chloride in tetrahydrofuran (20 ml of 3.6 *M*) was added and after 1 hr at −78° the mixture was added to saturated aqueous ammonium chloride (500 ml) containing acetic acid (5 ml). The mixture was extracted twice with ethyl acetate (450 ml) and the extracts were washed with aqueous sodium bicarbonate and water, dried ($MgSO_4$), and evaporated, leaving 2.30 g of a foam containing the desired **8a** and **9a** (3:2 by nmr) and about 30% unreacted **6**. While pure **8a** could be obtained by repeated crystallization, the process was wasteful and gave no pure **9a** (see below).

N^6-Benzoyl-9-(6-deoxy-2,3)-*O*-isopropylidene-β-D-allofuranosyl)adenine (8a). The crude Grignard product (2.22 g) from above was chromatographed on a column of silicic acid (300 g) using a linear gradient (4 l.) of 0–40% acetone in carbon tetrachloride–chloroform (1:1). The early peak fractions contained 0.70 g (34%) of pure **8a** (tlc carbon tetrachloride–acetone, 1:1): mp 103–104° from acetone; λ_{max} (MeOH) 280 nm (ε 18,800), 230 (sh, 12,100); $[\alpha]^{23}D$ −69.4° (*c* 0.6, MeOH); ORD (MeOH) $[\Phi]_{298}$ (tr) −3600°, $[\Phi]_{278}$ (pk) −40°, $[\Phi]_{264}$ (tr) −1500°, $[\Phi]_{255}$ 0°, $[\Phi]_{252}$ (pk) 500°, $[\Phi]_{244}$ 0°, $[\Phi]_{220}$ (tr) −15,600°; nmr (DMSO-d_6) 1.01 (d, 3, $J_{5',6'}$ = 6 Hz, $C_{6'}H_3$), 1.33 and 1.54 (s, 3, CMe_2), 3.70 (m, 1, $C_{5'}$H), 3.94 (dd, 1, $J_{3',4'}$ = 2.5, $J_{4',5'}$ = 6 Hz, $C_{4'}$H), 5.04 (dd, 1, $J_{2',3'}$ = 5.5 Hz, $C_{3'}$H and $C_{5'}$OH), 5.37 (dd, 1, $J_{1',2'}$ = 3 Hz, $C_{2'}$H), 6.21 (d, 1, $C_{1'}$H), 7.5 and 8.0 (m, total 5, Ar), 8.63 and 8.72 ppm (s, 1, C_2H and C_8H).

Anal. Calcd for $C_{21}H_{23}N_5O_5$ (425.43): C, 59.28; H, 5.45; N, 16.46. Found: C, 59.08; H, 5.64; N, 16.58.

N^6-Benzoyl-9-(6-deoxy-2,3-*O*-isopropylidene-α-L-talofurano-

syl)adenine (9a). Continued elution of the above column gave 0.54 g (26%) of a mixture of **8a** and **9a** and then 0.48 g (23%) of unreacted **6**. Rechromatography of the mixture did not effect resolution but did give, in the late fractions, some chromatographically homogeneous **9a** as a foam that could not be obtained crystalline from several solvents: λ_{max} (MeOH) 280 nm (ϵ 18,600), 226 (sh, 13,100); $[\alpha]^{23}D$ −51.5° (*c* 0.2, MeOH); ORD (MeOH) $[\Phi]_{300}$ (tr) −3400°, $[\Phi]_{282}$ 0°, $[\Phi]_{262}$ (pk) 2500°, $[\Phi]_{260}$ (tr) 2300°, $[\Phi]_{244}$ (pk) 4700°, $[\Phi]_{227}$ 0°, $[\Phi]_{220}$ (tr) −8700°; nmr (DMSO-d_6) 1.09 (d, 3, $J_{5',6'}$ = 6 Hz, $C_{6'}H_3$), 1.32 and 1.55 (s, 3, CMe_2), 3.8 (m, 1, $C_{5'}H$), 4.05 (dd, 1, $J_{3',4'}$ = 2.5, $J_{2',3'}$ = 6 Hz, $C_{3'}H$), 5.20 (dd, 1, $J_{1',2'}$ = 3 Hz, $C_{2'}H$), 6.24 (d, 1, $C_{1'}H$), 7.5 and 8.0 (m, total 5, Ar), 8.73 ppm (s, 2, C_2H and C_8H).

Anal. Calcd for $C_{21}H_{23}N_5O_5$ (425.43): C, 59.28; H, 5.45; N, 16.46. Found: C, 59.47; H, 5.62; N, 16.23.

Samples of pure **8a** and **9a** (1–5 mg) were treated overnight with methanol–concentrated NH_4OH (1:1, 0.2 ml) and then evaporated to dryness. The residue was then treated with trifluoroacetic acid–water (9:1) for 10 min at 23°, evaporated, and retreated with 9 *N* NH_4OH. Examination by paper chromatography using 2-propanol–concentrated NH_4OH–H_2O (7:1:2) showed that **8a** and **9a** were respectively degraded to compounds giving clearly resolved single spots with R_f values relative to adenosine of 1.05 and 1.18. Similar examinations using saturated ammonium sulfate–2-propanol–water (2:28:70) gave single, separable spots with R_{Ad} 1.06 and 1.02, respectively. In each case the compound derived from **8a** was chromatographically identical with an authentic sample of 9-(6-deoxy-β-D-allofuranosyl)adenine.

Also, samples of **8a** and **9a** (2 mg) were treated with dioxane–1 *N* hydrochloric acid (1:1, 0.1 ml) in sealed tubes at 100° for 10 min. Evaporation of the solvent and paper chromatography using 1-butanol–acetic acid–water (5:2:3) showed that **8a** was degraded to a single sugar, detected by use of a silver nitrate spray,[39] with the same R_f (1.09 relative to glucose) as an authentic sample of 6-deoxy-D-allose.[40] Similar treatment of **9a** gave a spot with R_f 1.18 relative to glucose, identical with an authentic sample of 6-deoxy-L-talose.[23]

N^6-Benzoyl-9-(6-deoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hex-5-ulofuranosyl)adenine (10). A mixture of **8a** and **9a** (430 mg, 1 mmol), dicyclohexylcarbodiimide (618 mg, 3 mmol), and pyridine (0.08 ml, 1 mmol) was dissolved in anhydrous dimethyl sulfoxide (3 ml) and benzene (3 ml). Trifluoroacetic acid (0.04 ml, 0.5 mmol) was added and the mixture was stored for 18 hr at room temperature. A methanolic solution of oxalic acid (2 mmol) was then added and after 30 min the mixture was filtered. The filtrate was partitioned between ethyl acetate and water and the organic phase was washed twice with water, dried ($MgSO_4$), and evaporated. The residue was dissolved in ethyl acetate, filtered to remove some dicyclohexylurea, and evaporated, leaving crude **10** as a foam (490 mg) that showed a single ultraviolet-absorbing spot on tlc [ethyl acetate–acetonitrile (9:1)] but containing some dicyclohexylurea: nmr (DMSO-d_6) 1.41 and 1.60 (s, 3, CMe_2), 1.91 (s, 3, $COCH_3$), 4.65 (d, 1, $J_{3',4'}$ = 2.5 Hz, $C_{4'}H$), 5.35 (d, 1, $J_{2',3'}$ = 6 Hz, $C_{2'}H$), 5.55 (dd, 1, $C_{3'}H$), 6.24 (s, 1, $C_{1'}H$), 7.5 and 8.0 (m, total 5, Ar), 8.13 and 8.63 ppm (s, 1, C_2H and C_8H).

Treatment of this material with 2,4-dinitrophenylhydrazine and a trace of concentrated hydrochloric acid in dimethyl sulfoxide[38] gave a crystalline dinitrophenylhydrazone: mp 141–143° from ethanol–ethyl acetate; λ_{max} 357 nm (ϵ 17,600), 276 (24,600).

Anal. Calcd for $C_{27}H_{25}N_9O_8$ (603.53): C, 53.73; H, 4.17; N, 20.89. Found: C, 53.78; H, 4.31; N, 20.35.

Attempted chromatography of **10** (355 mg) on a column of silicic acid (75 g) using a gradient of 0–40% acetone in CCl_4–$CHCl_3$ (1:1) gave 279 mg of a foam that behaved as a single spot of tlc. The nmr spectra of both this material and its amorphous dinitrophenylhydrazone show it to be a 1:5 mixture of **10** and an isomeric methyl ketone, presumably **11**. The nmr ($CDCl_3$) of **11** showed 1.37 and 1.51 (s, 3, CMe_2), 2.26 (s, 3, $COCH_3$), 4.98 (d, 1, $J_{3',4'}$ = 4 Hz, $C_{4'}H$), 5.5 (m, 2, $C_{2'}H$ and $C_{3'}H$), 6.18 (s, 1, $C_{1'}H$), 7.5 and 8.0 (m, total 5, Ar), 8.06 and 8.72 ppm (s, 1, C_2H and C_8H).

Reduction of Crude 11. Sodium borohydride (5 mg) was added to a solution of crude **11** (70 mg) in ethanol (1 ml) at 0–5°. The mixture was kept at *ca.* 5° for 30 min, neutralized with dilute acetic acid, and partitioned between ethyl acetate and water. The ethyl acetate layer was extracted with sodium bicarbonate solution and water, dried ($MgSO_4$), and evaporated to dryness, giving a mixture of **8a, 9a, 12,** and **13** (65 mg) distinguishable by tlc using acetone–carbon tetrachloride–chloroform (3:3:4). Preparative chromatography on two 20 × 20 × 1 cm silica gel plates afforded a mixture of **8a** (allo) and **9a** (talo) (9.8 mg, 14%) as the least polar component. The band of medium polarity gave **12** (gulo, 15.5 mg, 22%) slightly contaminated with **13**: nmr ($CDCl_3$) 1.28 (d, 3, $J_{5',6'}$ = 6 Hz, $C_{6'}H_3$), 1.41 and 1.58 (s, 3, CMe_2), 4.17 (m, 2, $C_{4'}H$ and $C_{5'}H$), 5.35 (m, 1, $C_{3'}H$), 5.55 (d, 1, $J_{2',3'}$ = 6 Hz, $C_{2'}H$), 6.06 (s, 1, $C_{1'}H$), 7.5 and 8.0 (m, total 6, Ar and C_2H or C_8H), 8.24 ppm (s, 1, C_2H or C_8H).

The most polar band gave **13** (manno, 17.0 mg, 24%), which was crystallized from chloroform–hexane: mp 209–210°; nmr ($CDCl_3$) 1.26 (d, 3, $J_{5',6'}$ = 5.5 Hz, $C_{6'}H_3$), 1.37 and 1.54 (s, 3, CMe_2), 4.20 (m, 2, $C_{4'}H$ and $C_{5'}H$), 5.15 (m, 1, $C_{3'}H$), 5.57 (d, 1, $C_{2'}H$, $J_{2',3'}$ = 6 Hz), 6.06 (s, 1, $C_{1'}H$), 7.5 and 8.0 (m, total 6, Ar and C_2H or C_8H), 8.23 ppm (s, 1, C_2H or C_8H).

Anal. Calcd for $C_{21}H_{23}N_5O_5$ (425.43): C, 59.28; H, 5.45; N, 16.46. Found: C, 59.38; H, 5.58; N, 16.64.

Samples of **12** and **13** were hydrolyzed with acid as above for **8a** and **9a** and the resulting sugars were chromatographically identical with 6-deoxygulose[23] and 6-deoxymannose, respectively, on tlc using the solvent system methanol–2-propanol–ethyl acetate (15:15:70).[22]

A sample of the nonepimerized ketone (**10,** 5 mg) was treated with sodium borohydride in a similar manner. Examination of the reaction product by tlc using the same solvent system as above showed that only the allo (**8a**) and talo (**9a**) isomers were present in a ratio of 1:2 by quantitative extraction of the spots with methanol followed by an ultraviolet determination.

N^6-Benzoyl-9-[6-deoxy-5-*O*-bis(2,2,2-trichloroethyl)phosphoryl-2,3-*O*-isopropylidene-β-D-allofuranosyl]adenine (14a). A solution of bis(2,2,2-trichloroethyl)phosphorochloridate (6.25 g, 16 mmol) in pyridine (10 ml) was added over 15 min to a stirred solution of pure **8a** (1.40 g, 3.3 mmol) in anhydrous pyridine (5 ml) at 0°. The mixture was then stirred at room temperature for 1.25 hr, and, after cooling to 0°, water (10 ml) was slowly added. After 1 hr the solvent was evaporated *in vacuo* and the residue was coevaporated with toluene to remove pyridine. The residue was dissolved in chloroform, washed with aqueous sodium bicarbonate and then water, dried ($MgSO_4$), and evaporated to give 2.6 g (quantitative) of **14a** as a white foam giving a single spot on tlc using CCl_4–acetone (7:3). Crystallization of **14a** has not been achieved: λ_{max} (MeOH) 280 nm (ϵ 21,300), 232 (sh, 13,600); $[\alpha]^{23}D$ −25.6° (*c* 0.1, MeOH); ORD (MeOH) $[\Phi]_{300}$ (tr) −3600°, $[\Phi]_{273}$ (pk) 0°, $[\Phi]_{262}$ (tr) −1000°, $[\Phi]_{256}$ 0°, $[\Phi]_{242}$ (pk) 4200°, $[\Phi]_{220}$ 0°, $[\Phi]_{216}$ (tr) −14,200°; nmr ($CDCl_3$) 1.43 (d, 3, $J_{5',6'}$ = 6 Hz, $C_{6'}H_3$), 1.38 and 1.61 (s, 3, CMe_2), 4.1 (m, 1, $C_{4'}H$), 4.54 and 4.62 (d, 2, $J_{P,H}$ = 6 Hz, CH_2OP), 4.9 (m, 1, $C_{5'}H$), 5.26 (dd, 1, $J_{2',3'}$ = 6, $J_{3',4'}$ = 4 Hz, $C_{3'}H$), 5.41 (dd, 1, $J_{1',2'}$ = 2.5 Hz, $C_{2'}H$), 6.13 (d, 1, $C_{1'}H$), 8.5 and 8.0 (m, total 5, Ar), 8.13 and 8.77 (s, 1, C_2H and C_8H), 9.06 ppm (br s, 1, NH).

Anal. Calcd for $C_{25}H_{26}N_5O_8PCl_6$ (768.25): C, 39.07; H, 3.41; N, 9.11; P, 4.03. Found: C, 38.83; H, 3.39; N, 8.77; P, 4.38.

N^6-Benzoyl-9-[6-deoxy-5-*O*-bis(2,2,2-trichloroethyl)phosphoryl-2,3-*O*-isopropylidene-α-L-talofuranosyl]adenine (14b) and Its β-D-Allo Isomer (14a). A mixture of **8a** and **9a** (3.46 g, 8.1 mmol, somewhat enriched in **8a**) was dried by several evaporations of its solution in pyridine. This material was then phosphorylated as above using bis(2,2,2-trichloroethyl)phosphorochloridate (15.4 g, 40 mmol) at 0° for 30 min and then at room temperature for 30 min. After treatment with water and partitioning as above a crude product (6.7 g) showing two spots in tlc with CCl_4–acetone (7:3) was obtained. This material was chromatographed on a column containing 540 g of silicic acid deactivated with 6% water and using a linear gradient 0–50% acetone in chloroform (3.6 l. total). This column gave first 2.83 g of the pure D-allo isomer **14a** followed by 0.96 g of a mixture of **14a** and **14b** and finally 0.91 g of the pure L-talo isomer **14b.** Rechromatography of the mixed fractions by preparative tlc using four developments with CCl_4–acetone (7:3) gave a further 0.31 g (total yield 3.14 g, 51%) of pure **14a** identical with that above, and a further 0.47 g (total yield 1.38 g, 22%) of pure **14b.** The α-L-talo isomer **14b** was chromatographically and spectroscopically homogeneous but could not be crystallized: λ_{max} (MeOH) 280 nm (ϵ 20,400), 230 (sh, 13,000); $[\alpha]^{23}D$ −12.3° (*c* 0.24, MeOH); ORD (MeOH) $[\Phi]_{302}$ (tr) −3200°, $[\Phi]_{279}$ 0°, $[\Phi]_{244}$ (pk) 6800°, $[\Phi]_{223}$ 0°, $[\Phi]_{210}$ (tr) −8800°; nmr ($CDCl_3$) 1.53 (d, 3, $J_{5',6'}$ = 6.5 Hz, $C_{6'}H_3$), 1.38 and 1.62 (s, 3, CMe_2), 4.3 (m, 1, $C_{4'}H$), 4.46 (d, 4, $J_{P,H}$ = 6.5 Hz, CH_2OP), 4.75 (m, 1, $C_{5'}H$), 5.07 (dd, 1, $J_{2',3'}$ = 6.5 Hz, $J_{3',4'}$ = 4 Hz, $C_{3'}H$), 5.36 (dd, 1, $J_{1',2'}$ = 2.5 Hz, $C_{2'}H$), 6.19 (d, 1, $C_{1'}H$), 7.5 and 8.0 (m, total 5, Ar), 8.15 and 8.79 (s, 1, C_2H and C_8H), 8.98 ppm (br s, 1, NH).

Anal. Calcd for $C_{25}H_{26}N_5O_8PCl_6$ (768.25): C, 39.07; H, 3.41; N, 9.11; P, 4.03. Found: C, 39.34; H, 3.55; N, 8.90; P, 4.24.

The same compound could also be obtained in 85% yield by phosphorylation of pure **9a** as above.

N^6-Benzoyl-9-[6-deoxy-5-*O*-bis(2,2,2-trichloroethyl)phosphoryl-β-D-allofuranosyl]adenine (15a). A solution of **14a** (1.31 g, 1.7 mmol) in trifluoroacetic acid–water (9:1, 15 ml) was kept at room temperature for 20 min and then evaporated to dryness. The residue was coevaporated with ethanol several times and then triturated with ether, giving 1.17 g of crystalline product which was recrystallized from acetone–hexane, giving 1.10 g (89%) of **15a**: mp 181–182°; λ_{max} (MeOH) 279 nm (ε 21,300), 230 (sh, 13,800); $[\alpha]^{23}D$ −30.1° (*c* 1.0, MeOH); nmr (DMSO-d_6) 1.39 (d, 3, $J_{5',6'}$ = 6 Hz, $C_{6'}H_3$), 4.77 (d, 4, $J_{H,P}$ = 6 Hz, CH_2OP), 5.44 (d, 1, $J_{3',4'}$ = 5 Hz, $C_{3'}H$), 5.62 (d, 1, $J_{1',2'}$ = 6 Hz, $C_{2'}H$), 6.02 (d, 1, $C_{1'}H$), 7.5 and 8.0 (m, total 5, Ar), 8.65 and 8.70 ppm (s, 1, C_2H and C_8H).

Anal. Calcd for $C_{22}H_{22}N_5O_8PCl_6$ (728.2): C, 36.28; H, 3.05; N, 9.62; P, 4.27. Found: C, 36.14; H, 2.98; N, 9.55; P, 4.13.

9-(6-Deoxy-5-*O*-phosphoryl-β-D-allofuranosyl)adenine (16a). **A.** *Via* **15a.** Glacial acetic acid (16 ml, 272 mmol) was added dropwise over 8 min to a stirred, ice-cooled solution of **15a** (4.42 g, 6 mmol) in dimethylformamide (40 ml) containing 7.0 g (108 mmol) of finely divided zinc dust. After stirring at 0° for 40 min the mixture was filtered, the solid was washed with DMF, and the combined filtrates were evaporated to dryness *in vacuo*. Water (200 ml) and Dowex 50 (NH_4^+) resin (20 ml) were added to the residue and once the precipitate had dissolved the entire mixture was added to the top of a column containing 100 ml of fresh Dowex 50 (NH_4^+) resin. The column was eluted with water and the effluents were evaporated to dryness. The residue was dissolved in 9 *N* ammonium hydroxide (80 ml) and stored at room temperature for 16 hr. The solvent was evaporated *in vacuo* and the residue was applied to a 4 × 45 cm column of DEAE Sephadex (HCO_3^-) which was eluted with a linear gradient of triethylammonium bicarbonate (6 l., 0.005–0.25 *M*). A small peak (10,800 OD units, 12%) of a monoanion was first eluted followed by a major peak (70,720 OD units, 77%) of an electrophoretically and chromatographically homogeneous dianion (**12a**). Evaporation of the latter peak and coevaporation with methanol left 2.78 g of the triethylammonium salt of **16a** suitable for the next step. For analytical purposes a small sample of the triethylammonium salt was dissolved in 90% ethanol and brought to pH 2 with ethanolic hydrochloric acid. The resulting solid was collected by centrifugation and crystallized from aqueous ethanol, giving **16a** as the dihydrate: mp 181–186° dec; λ_{max} (H_2O) 259 nm (ε 15,600); $[\alpha]^{23}D$ −35.2° (*c* 0.15, water); ORD (H_2O) $[\Phi]_{268}$ (tr) −3900°, $[\Phi]_{252}$ 0°, $[\Phi]_{248}$ (pk) 850°, $[\Phi]_{232}$ 0°; nmr (pyridine-d_5–D_2O) 1.68 ppm (d, 3, $J_{5',6'}$ = 6 Hz, C_6H_3), 4.56 (m, 1, $C_{4'}H$), 5.1–5.9 (m, 3, masked by D_2O), 6.73 (d, 1, $J_{1',2'}$ = 6 Hz, $C_{1'}H$), 8.53 and 9.14 (s, 1, C_2H and C_8H).

Anal. Calcd for $C_{11}H_{16}N_5O_7P\cdot 2H_2O$ (397.30): C, 33.25; H, 5.08; N, 17.63; P, 7.80. Found: C, 33.65; H, 4.71; N, 17.42; P, 7.74.

B. Directly from 8a. A solution of **8a** (1.5 g, 3.5 mmol), 2-cyanoethyl phosphate (from 7.0 mmol of barium salt),[26] and dicyclohexylcarbodiimide (2.92 g, 14 mmol) in anhydrous pyridine (50 ml) was kept at room temperature for 2 days. Water (20 ml) was then added and after 1 hr the mixture was filtered and the solvent was evaporated. The residue was treated with 9 *N* ammonium hydroxide (50 ml) at room temperature for 5 days and then at 50° for 1 hr. After evaporation of the solvent the residue was treated with trifluoroacetic acid–water (9:1, 20 ml) for 15 min. The solvent was evaporated *in vacuo* and the residue was triturated with ether, giving a solid that was dissolved in water, adjusted to pH 8, and applied to a 4 × 47 cm column of DEAE Sephadex (HCO_3^-). The column was eluted with a linear gradient of triethylammonium bicarbonate (8 l. 0.005–0.25 *M*), giving a small peak (2750 ODU at 259 nm, 5%) followed by a large peak containing 30,400 ODU at 259 nm (56%) of chromatographically homogeneous **16a.** Evaporation of the pooled peak followed by repeated coevaporation with methanol left 1.05 g of the triethylammonium salt of **16a.** This was dissolved in 50% aqueous ethanol (6 ml) and adjusted to pH 2 with 1 *N* hydrochloric acid, giving 650 mg (47% overall) of crystalline **16a** identical with that above.

9-(6-Deoxy-5-*O*-phosphoryl-α-L-talofuranosyl)adenine (16b). A solution of **14b** (1.34 g, 1.75 mmol) in 90% trifluoroacetic acid (15 ml) was stored at room temperature for 20 min and then evaporated to dryness. The residue was triturated with ether, giving 1.0 g of crude diol **15b** which was directly dissolved in dimethylformamide (10 ml) and stirred at 0° in the presence of finely divided zinc (1.75 g, 27 mmol) while glacial acetic acid (4.0 ml, 67 mmol) was added dropwise over 10 min. After stirring at 0° for 30 min the solvent was evaporated and the residue was stirred with water in the presence of Dowex 50 (NH_4^+) resin (20 ml) until the precipitates had dissolved. The mixture was then added to a column of Dowex 50 (NH_4^+) resin (35 ml) and the column was washed with water. The effluents were evaporated to dryness and the residue was treated with 9 *N* ammonium hydroxide for 16 hr. After evaporation of the solvent the residue was chromatographed on a 3 × 43 cm column of DEAE Sephadex (HCO_3^-) using a linear gradient of triethylammonium bicarbonate (5 l., 0.005–0.25 *M*) giving a small peak (2800 ODU, 10%) of a monoanion followed by a large peak (13,920 ODU, 52%) of chromatographically homogeneous **16b.** Evaporation of the pooled peak followed by repeated coevaporation with methanol left the triethylammonium salt of **16b** which was suitable for further work. A portion of this salt was dissolved in 50% ethanol and adjusted to pH 2 with hydrochloric acid, giving crystalline **16b** as the free acid hydrate, mp 184–186° dec, with excellent recovery: λ_{max} (H_2O) 260 nm (ε 15,600); $[\alpha]^{23}D$ −48.3° (*c* 0.1, H_2O); ORD (H_2O) $[\Phi]_{272}$ (tr) −4700°, $[\Phi]_{255}$ 0°, $[\Phi]_{242}$ (pk) 1800°; nmr (pyridine-d_5–D_2O) 1.69 (d, 3, $J_{5',6'}$ = 6 Hz, $C_{6'}H_3$), 6.71 (d, 1, $J_{1',2'}$ = 3.5 Hz, $C_{1'}H$), 8.51 and 9.24 ppm (s, 1, C_2H and C_8H).

Anal. Calcd for $C_{11}H_{16}N_5O_7P\cdot H_2O$ (379.27): C, 34.83; H, 4.78; N, 18.47; P, 8.16. Found: C, 34.77; H, 4.58; N, 18.57; P, 7.70.

9-(6-Deoxy-β-D-allofuranosyl)adenine 3′,5′-Cyclic Phosphate (1). 4-Morpholine-*N,N′*-dicyclohexylcarboxamidine (357 mg, 1.22 mmol) was added to a stirred suspension of the triethylammonium salt of **16a** (1.22 mmol) in pyridine (25 ml). When a clear solution resulted the solvent was evaporated *in vacuo* and the residue was coevaporated several times with anhydrous pyridine. The final residue was dissolved in pyridine (120 ml) and added dropwise over 2 hr to a solution of dicyclohexylcarbodiimide (495 mg, 2.2 mmol) in pyridine (120 ml) under reflux. The solution was then heated under reflux for a further 2 hr and evaporated to dryness. The residue was partitioned between water and ether and filtered, and the aqueous phase was applied to a 3.2 × 42 cm column of DEAE Sephadex (HCO_3^-). Elution with a linear gradient of triethylammonium bicarbonate (5 l., 0.005–0.15 *M*) gave a single peak of chromatographically and electrophoretically homogeneous **1.** The pooled peak was evaporated to dryness and coevaporated several times with methanol. The residue was dissolved in 50% ethanol (4 ml) and adjusted to pH 2 with hydrochloric acid, giving 425 mg (100%) of crystalline **1** as the free acid with no definite melting point: λ_{max} (pH 11) 259 nm (ε 14,100); $[\alpha]^{23}D$ −22.9° (*c* 0.3, H_2O); ORD (H_2O) $[\Phi]_{272}$ (tr) −2100°, $[\Phi]_{257}$ 0°, $[\Phi]_{230}$ (pk) 5100°; nmr (pyridine-d_5–D_2O) 1.38 (dd, 3, $J_{5',6'}$ = 5 Hz, $J_{P,H}$ = 1 Hz, $C_{6'}H_3$), 6.54 (s, 1, $C_{1'}H$), 8.55 and 8.61 ppm (s, 1, C_2H and C_8H).

Anal. Calcd for $C_{11}H_{14}N_5O_6P$ (343.24): C, 38.49; H, 4.11; N, 20.40; P, 9.03. Found: C, 38.21; H, 4.25; N, 20.21; P, 8.84.

9-(6-Deoxy-α-L-talofuranosyl)adenine 3′,5′-Cyclic Phosphate (2). An anhydrous solution of the 4-morpholine-*N,N′*-dicyclohexylcarboxamidine salt of **16b** (from 0.6 mmol of the triethylammonium salt) in pyridine (60 ml) was prepared as above. This solution was added over 1 hr to a refluxing solution of dicyclohexylcarbodiimide (270 mg, 1.2 mmol) in pyridine (60 ml) and heating was continued for a further 1 hr. After evaporation of the solvent the residue was partitioned between water and ether and filtered, and the aqueous phase was chromatographed on a 3.2 × 37 cm column of DEAE Sephadex (HCO_3^-) using elution with a linear gradient of triethylammonium bicarbonate (5 l., 0.005–0.15 *M*). The single peak which resulted (8290 ODU at 259 nm, 90%) was evaporated to dryness, coevaporated with methanol, and crystallized by acidification to pH 2 of a solution of the residue in 50% ethanol with hydrochloric acid. In this way 173 mg (84%) of free acid **2** was obtained as needles with no definite melting point: λ_{max} (pH 11) 259 nm (ε 14,900); $[\alpha]^{23}D$ −65.2° (*c* 0.14, H_2O); ORD (H_2O) $[\Phi]_{272}$ (tr) −5100°, $[\Phi]_{242}$ (pk) −600°; nmr (pyridine-d_5–D_2O) 1.75 (d, 3, $J_{5',6'}$ = 6 Hz, $C_{6'}H_3$), 6.56 (s, 1, $C_{1'}H$), 8.57 ppm (s, 2, C_2H and C_8H).

Anal. Calcd for $C_{11}H_{14}N_5O_6P$ (343.24): C, 38.49; H, 4.11; N, 20.40; P, 9.03. Found: C, 38.44; H, 4.25; N, 20.18; P, 8.87.

Methyl 9-(2,3-*O*-Isopropylidene-β-D-ribofuranosyluranoate)-adenine (17b). Methylation of the 5′ carboxylic acid (**17a,** 4.72 g, 14 mmol, prepared in 74% yield by the method of Schmidt, *et al.*[32]) by treatment with an excess of ethereal diazomethane in dioxane (1 l.) and methanol (1.5 l.) essentially according to Harper and Hampton[31] gave **14b** in yields of 72–85%, mp 244–245° (reported mp 245–248°,[31] 244°[33]).

2′,3′-*O*-Isopropylidene-5′,5′-dimethyladenosine (18). A 3 *M* solution of methylmagnesium chloride in tetrahydrofuran (4.8 ml, 14.3 mmol) was added dropwise over 10 min at room temperature to a stirred suspension of **17b** (478 mg, 1.43 mmol) in tetrahydrofuran under nitrogen. After 30 min the resulting yellow solution was quenched by addition of saturated aqueous ammonium chloride (10 ml) at 0°. The mixture was filtered and the filtrate was evaporated to give **18** as a crystalline residue (554 mg, 94.5%), mp

268–271°. Recrystallization from acetone–hexane raised the melting point to 272–273° with good recovery (reported[31] mp 225–227°, 24–35% yield): λ_{max} (MeOH) 259 nm (ϵ 15,400); $[\alpha]^{23}D$ −64.0° (*c* 0.2, MeOH); ORD (MeOH) $[\Phi]_{276}$ (tr) −3200°, $[\Phi]_{225}$ 0°, $[\Phi]_{238}$ (pk) 1600°, $[\Phi]_{228}$ 0°; nmr (DMSO-d_6) 1.12 and 1.16 (s, 3, $C_{5'}Me_2$), 1.32 and 1.56 (s, 3, acetonide), 3.92 (d, 1, $J_{3',4'}$ = 3 Hz, $C_{4'}H$), 4.95 (dd, 1, $J_{2',3'}$ = 6 Hz, $C_{3'}H$), 5.13 (dd, 1, $J_{1',2'}$ = 4 Hz, $C_{2'}H$), 5.28 (s, 1, $C_{5'}OH$), 6.06 (d, 1, $C_{1'}H$), 7.33 (s, 2, NH_2), 8.10 and 8.36 ppm (s, 1, C_2H and C_8H).

Anal. Calcd for $C_{15}H_{21}N_5O_4$ (335.38): C, 53.74; H, 6.31; N, 20.88. Found: C, 53.64; H, 6.19; N, 20.94.

N^6-Benzoyl-2′,3′-*O*-isopropylidene-5′,5′-dimethyladenosine (19). Benzoyl chloride (1.08 ml, 9 mmol) was added dropwise to an ice-cooled suspension of **18** (1.3 g, 3.87 mmol) in pyridine (30 ml) and the resulting solution was stirred at room temperature for 1 hr. Aqueous sodium hydroxide (35 ml of 2 *N*) was then added and after 1 hr the red solution was neutralized at 0° with acetic acid. After evaporation of the solvent the residue was dissolved in chloroform, washed with aqueous sodium bicarbonate, 1 *N* hydrochloric acid, and water, dried ($MgSO_4$), and evaporated. The residue (1.98 g) was chromatographed on a column containing 170 g of silicic acid using a linear gradient of acetone in carbon tetrachloride (4 l., 0–40%), giving 1.43 g (84%) of **19** as a white foam: λ_{max} (MeOH) 280 nm (ϵ 20,200), 228 (sh, 13,100); $[\alpha]^{23}D$ −50.5° (*c* 0.27, MeOH); ORD (MeOH) $[\Phi]_{300}$ (tr) −1800°, $[\Phi]_{282}$ 0°, $[\Phi]_{248}$ (pk) 1500°, $[\Phi]_{229}$ 0°; nmr ($CDCl_3$) 1.26 and 1.34 (s, 3, $C_{5'}Me_2$), 1.39 and 1.64 (s, 3, acetonide), 4.17 (d, 1, $J_{3',4'}$ = 1.5 Hz, $C_{4'}H$), 5.15 (m, 2, $C_{2'}H$ and $C_{3'}H$), 5.94 (d, 1, $J_{1',2'}$ = 3.5 Hz, $C_{1'}H$), 7.5 and 8.0 (m, total 5, Ar), 8.05 and 8.75 ppm (s, 1, C_2H and C_8H).

Anal. Calcd for $C_{22}H_{25}N_5O_5$ (439.48): C, 60.10; H, 5.73; N, 15.93. Found: C, 60.25; H, 5.72; N, 15.42.

Bis(2,2,2-trichloroethyl) N^6-Benzoyl-2′,3′-*O*-isopropylidene-5′,5′-dimethyladenosine 5′-Phosphate (14c). A solution of bis(2,2-2-trichloroethyl)phosphorochloridate (12.65 g, 33 mmol) in pyridine (25 ml) was added to an anhydrous solution of **19** (1.43 g, 3.25 mmol) in pyridine (50 ml). The mixture was stirred at room temperature while being monitored by tlc using CCl_4–acetone (7:3). After 7 days **19** had disappeared and water (50 ml) was added dropwise with ice cooling. The mixture was stirred at room temperature for 2 hr and then the solvent was evaporated *in vacuo*. The residue was dissolved in chloroform and washed with ice-cold 1 *N* hydrochloric acid, water, aqueous bicarbonate, and water, dried ($MgSO_4$), and evaporated. The resulting foam (2.72 g) was chromatographed on a column of silicic acid (300 g) using a linear gradient of acetone in CCl_4 (4 l., 0–30%), giving 1.72 g (68%) of chromatographically homogeneous **14c** as a foam: λ_{max} (MeOH) 279 nm (ϵ 18,200), 230 (sh, 11,500); $[\alpha]^{23}D$ −19.0° (*c* 1.0, MeOH); ORD (MeOH) $[\Phi]_{300}$ (tr) −1700°, $[\Phi]_{280}$ 0°, $[\Phi]_{270}$ (pk) 900°, $[\Phi]_{265}$ (tr) 0°, $[\Phi]_{244}$ (pk) 3300°, $[\Phi]_{227}$ 0°; nmr ($CDCl_3$) 1.38 and 1.63 (s, 3, acetonide), 1.65 and 1.67 (s, 3, $C_{5'}Me_2$), 4.11 (dd, 1, $J_{3',4'}$ = 5, $J_{H,P}$ = 3.5 Hz, $C_{4'}H$), 4.55 and 4.57 (d, 2, $J_{P,H}$ = 6 Hz, $POCH_2$), 5.10 (dd, 1, $J_{2',3'}$ = 6.5 Hz, $C_{3'}H$), 5.37 (dd, 1, $J_{1',2'}$ = 3 Hz, $C_{2'}H$), 6.16 (d, 1, $C_{1'}H$), 7.5 and 8.0 (m, total 5, Ar), 8.17 and 8.77 (s, 1, C_2H and C_8H), 9.0 ppm (br s, 1, NH).

Anal. Calcd for $C_{26}H_{28}N_5O_8PCl_6$ (782.24): C, 39.93; H, 3.61; N, 8.95; P, 3.96. Found: C, 40.00; H, 3.75; N, 8.79; P, 4.23.

5′,5′-Dimethyladenosine 5′-Phosphate (16c). Fine zinc dust (2.8 g, 40 mmol) was added to a stirred solution of **14c** (1.56 g, 2 mmol) in dimethylformamide (15 ml) at 0° followed by dropwise addition of 88% formic acid (4.4 ml, 100 mmol). After 45 min at 0° the mixture was filtered, the residue was washed with dimethylformamide, and the filtrates were evaporated to dryness. The residue was dissolved in 90% trifluoroacetic acid and kept at room temperature for 20 min before evaporation to dryness and coevaporation with ethanol. The residue was dissolved in dilute ammonia (pH 11) and passed through a 2.5 × 36 cm colum of Dowex 50 (NH_4^+) resin. The effluent and water wash was evaporated to dryness and then treated with 9 *N* ammonium hydroxide (100 ml) at room temperature for 14 hr to effect debenzoylation. The residue following evaporation was then applied to a column of DEAE Sephadex (HCO_3^-) and washed thoroughly with water to remove benzamide. Elution with a linear gradient of triethylammonium bicarbonate (6 l., 0.005–0.25 *M*) gave a small amount (3,720 ODU at 259 nm, 12%) of a monoanion followed by a major peak containing 18,350 ODU (60%) of the desired dianion. Evaporation of the solvent and repeated coevaporation with methanol left the triethylammonium salt of **16c** (673 mg, 60%) as a chromatographically homogeneous syrup. This material failed to crystallize upon acidification under the usual conditions and for analytical purposes a portion was converted to the barium salt and precipitated from 67% ethanol: λ_{max} (H_2O) 259 nm (ϵ 13,300); $[\alpha]^{23}D$ −36.5° (*c* 0.7, H_2O); ORD (H_2O) $[\Phi]_{272}$ (tr) −3300°, $[\Phi]_{251}$ 0°, $[\Phi]_{244}$ (pk) 500°, $[\Phi]_{240}$ 0°.

Anal. Calcd for $C_{12}H_{16}N_5O_7PBa$ (510.64): C, 28.22; H, 3.16; N, 13.72; P, 6.07. Found: C, 28.08; H, 3.26; N, 13.55; P, 6.19.

5′,5′-Dimethyladenosine 3′,5′-Cyclic Phosphate (3). A suspension of the triethylammonium salt of **16c** (346 mg, 0.6 mmol) in pyridine (25 ml) was stirred with 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine (175 mg, 0.6 mmol) until a clear solution resulted. The solvent was then evaporated and the residue was coevaporated several times with pyridine. A solution of the final residue in pyridine (60 ml) was added dropwise over 1.5 hr to a refluxing solution of dicyclohexylcarbodiimide (270 mg, 1.2 mmol) in pyridine (60 ml) and heating was then continued for a further 1 hr. The solvent was then evaporated and the residue was partitioned between water and ether. The filtered aqueous solution was applied to a 2.5 × 45 cm column of DEAE Sephadex (HCO_3^-) and eluted with a linear gradient of triethylammonium bicarbonate (4 l., 0.005–0.15 *M*), giving essentially a single peak. The pooled peak (8840 ODU at 259 nm) was evaporated to dryness and the residue was dissolved in 50% aqueous ethanol (2 ml). Acidification to pH 2 with hydrochloric acid gave 200 mg (93%) of crystalline **3**: λ_{max} (pH 11) 259 nm (ϵ 13,800); $[\alpha]^{23}D$ −36.4° (*c* 0.5, H_2O); ORD (H_2O) $[\Phi]_{276}$ (tr) −1200°, $[\Phi]_{264}$ 0°, $[\Phi]_{242}$ 2400°; nmr (pyridine-d_5–D_2O) 1.50 (br s, 3, allo $C_{5'}Me$), 1.83 (s, 3, talo $C_{5'}Me$), 4.65 (d, 1, $J_{3',4'}$ = 10 Hz, $C_{4'}H$), 5.07 (d, 1, $J_{2',3'}$ = 5 Hz, $C_{2'}H$), 5.7 (obscured by HDO, $C_{3'}H$), 6.53 (s, 1, $C_{1'}H$), 8.54 ppm (s, 2, C_2H and C_8H).

Anal. Calcd for $C_{12}H_{16}N_5O_6P$ (357.28): C, 40.33; H, 4.49; N, 19.60; P, 8.67. Found: C, 40.23; H, 4.48; N, 19.47; P, 8.57.

8-Bromo-9-(6-deoxy-β-D-allofuranosyl)adenine 3′,5′-Cyclic Phosphate (20a). A solution of **1** (180 mg, 0.5 mmol) and sodium hydroxide (0.25 ml of 2 *N*, 0.5 mmol) in 1 *M* sodium acetate buffer, pH 3.9 (3 ml), was stirred at room temperature while a solution of bromine (0.04 ml, 0.8 mmol) in the same buffer (4 ml) was added until a color persisted and tlc using 1-butanol–acetic acid–water (5:2:3) showed complete conversion of **13a** to a slightly faster spot. The mixture was then kept overnight, diluted to 750 ml with water, and applied to a 2.3 × 45 cm column of DEAE Sephadex (HCO_3^-). Elution with a linear gradient of triethylammonium bicarbonate (4 l., 0–0.15 *M*) gave one major peak which was pooled and evaporated to dryness, leaving 360 mg of a dry residue. This was dissolved in ethanol–water (3 ml, 2:1) and acidified to pH 2 with 3 *N* ethanolic hydrochloric acid, giving 91 mg (43%) of **20a** as white crystals after drying *in vacuo* at 60°: nmr (pyridine-d_5) 1.45 (dd, 3, $J_{5',6'}$ = 6, $J_{H,P}$ = 0.5 Hz, $C_{6'}H_3$), 4.40 (dd, 1, $J_{3',4'}$ = $J_{4',5'}$ = 9.5 Hz, $C_{4'}H$), 5.0 (m, 1, $C_{5'}H$), 5.66 (d, 1, $J_{2',3'}$ = 5 Hz, $C_{2'}H$), 6.50 (s, 1, $C_{1'}H$), 8.42 ppm (s, 1, C_2H). There were also signals corresponding to less than 1 mol of ethanol.

Anal. Calcd for $C_{11}H_{13}N_5O_6PBr \cdot H_2O \cdot \frac{1}{2}EtOH$ (463.19): C, 31.11; H, 3.92; N, 15.12. Found: C, 31.33; H, 3.71; N, 15.32.

8-Benzylthio-9-(6-deoxy-β-D-allofuranosyl)adenine 3′,5′-Cyclic Phosphate (20b). The chromatographically homogeneous mother liquors from crystallization of free acid **20a** (~0.25 mmol) were evaporated to dryness, suspended in methanol (3 ml), and heated under reflux in the presence of sodium methoxide (54 mg, 1 mmol) and benzyl mercaptan (0.35 ml, 3 mmol). Tlc using 1-butanol–acetic acid–water (5:2:3) showed completion of the reaction in 1.5 hr, and after 2.5 hr the mixture was diluted with water (125 ml) and applied to a 2.3 × 45 cm column of DEAE Sephadex (HCO_3^-). Elution with a linear gradient of triethylammonium bicarbonate (4 l., 0–0.25 *M*) gave a major peak (2330 ODU at 283 nm, ~57%) that was evaporated to dryness. The residue was dissolved in 66% ethanol and acidified to pH 2 with 3 *N* ethanolic hydrochloric acid, giving 41 mg (35%) of **20b** as a chromatographically homogeneous crystalline hydrate, λ_{max} (H_2O) 283 nm.

Anal. Calcd for $C_{18}H_{20}N_5O_6PS \cdot H_2O$ (483.42): C, 44.72; H, 4.58; N, 14.49. Found: C, 44.64; H, 4.52; N, 14.25.

Registry No. **1**, 43076-99-9; **2**, 43077-00-5; **3**, 43077-01-6; **4**, 39947-04-1; **5**, 43077-03-8; **6**, 43077-04-9; **7a**, 43077-06-1; **7a** dinitrophenylhydrazone, 43077-05-0; **8a**, 43077-07-2; **9a**, 43077-08-3; **10**, 43077-09-4; **10** dinitrophenylhydrazone, 43077-10-7; **11**, 43077-11-8; **12**, 43077-12-9; **13**, 43077-13-0; **14a**, 43077-14-1; **14b**, 43077-15-2; **14c**, 43077-16-3; **15a**, 43077-17-4; **16a**, 43077-18-5; **16a** triethylammonium salt, 43077-19-6; **16b**, 43077-20-9; **16b** 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine salt, 43077-21-0; **16c** barium salt, 43077-22-1; **16c** triethylammonium salt, 43077-23-2; **17a**, 19234-66-3; **17b**, 23754-29-2; **18**, 23680-27-5; **19**, 43077-27-6; **20a**, 43077-28-7; **20b**, 43077-60-7; bis(2,2,2-trichloroethyl)phosphorochloridate, 17672-53-6; 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine, 4975-73-9.

References and Notes

(1) Syntex Postdoctoral Fellow, 1970–1972.
(2) See, *e.g.*, (a) P. Greengard, R. Paoletti, and G. A. Robison, Ed., "Advances in Cyclic Nucleotide Research," Vol. I, Raven Press, New York, N. Y., 1972; (b) G. A. Robison, R. W. Butcher, and E. W. Sutherland, "Cyclic AMP," Academic Press, New York, N. Y., 1971; (c) P. Greengard and E. Costa, Ed., "Role of Cyclic AMP in Cell Function," Raven Press, New York, N. Y., 1970.
(3) See, *e.g.*, (a) T. Posternak, I. Marcus, A. Gabbai, and G. Cehovic, *C. R. Acad. Sci., Ser. D*, **269**, 2409 (1969); (b) R. B. Meyer, D. A. Shuman, R. K. Robins, R. J. Bauer, M. K. Dimmitt, and L. N. Simon, *Biochemistry*, **11**, 2704 (1972); (c) G. Weimann, E. Haid, K. Mühlegger, H. U. Bergmeyer, K. Dietmann, G. Michal, and M. Nelboeck-Hochstetter, U. S. Patent 3,712,885 (1973).
(4) K. Muneyama, R. J. Bauer, D. A. Shuman, R. K. Robins, and L. N. Simon, *Biochemistry*, **10**, 2390 (1971).
(5) G. H. Jones, H. P. Albrecht, N. P. Domodaran, and J. G. Moffatt, *J. Amer. Chem. Soc.*, **92**, 5510 (1970).
(6) F. Eckstein, *J. Amer. Chem. Soc.*, **92**, 4718 (1970).
(7) (a) A. Murayama, B. Jastorff, F. Cramer, and H. Hettler, *J. Org. Chem.*, **36**, 3029 (1971); (b) R. B. Meyer, D. A. Shuman, and R. K. Robins, *Tetrahedron Lett.*, 269 (1973).
(8) G. I. Drummond, M. W. Gilgan, E. J. Reiner, and M. Smith, *J. Amer. Chem. Soc.*, **86**, 1626 (1964).
(9) (a) W. W. Lee, L. V. Fisher, and L. Goodman, *J. Heterocycl. Chem.*, **8**, 179 (1971); (b) T. A. Khwaja, R. Harris, and R. K. Robins, *Tetrahedron Lett.*, 4681 (1972).
(10) M. Hubert-Habart and L. Goodman, *Chem. Commun.*, 740 (1969).
(11) D. V. K. Murthy and R. Golling, in preparation.
(12) Symposium on the Chemistry and Biochemistry of Cyclic AMP, Pacific Conference on Chemistry and Spectroscopy, San Francisco, Calif., Oct 1972, Papers 131 (G. H. Jones) and 133 (D. V. K. Murthy and R. Golling).
(13) E. J. Reist, L. Goodman, R. R. Spencer, and B. R. Baker, *J. Amer. Chem. Soc.*, **80**, 3962 (1958).
(14) E. J. Reist, L. Goodman, and B. R. Baker, *J. Amer. Chem. Soc.*, **80**, 5775 (1958).
(15) K. E. Pfitzner and J. G. Moffatt, *J. Amer. Chem. Soc.*, **85**, 3027 (1963).
(16) *E.g.*, G. H. Jones and J. G. Moffatt, *J. Amer. Chem. Soc.*, **90**, 5337 (1968).
(17) G. H. Jones and J. G. Moffatt, Abstracts, 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, CARB 16.
(18) S. Chládek and J. Smrt, *Collect. Czech. Chem. Commun.*, **29**, 214 (1964).
(19) (a) K. E. Pfitzner and J. G. Moffatt, *J. Amer. Chem. Soc.*, **87**, 5661 (1965). (b) For a review on sulfoxide-based oxidations, see J. G. Moffatt in "Techniques and Applications in Organic Synthesis. Oxidation," Vol. II, Marcel Dekker, New York, N. Y., 1971, p 1.
(20) (a) H. W. Wanzlick and W. Löchel, *Chem. Ber.*, **86**, 1463 (1953); (b) N. P. Damodaran, G. H. Jones, and J. G. Moffatt, *J. Amer. Chem. Soc.*, **93**, 3812 (1971).
(21) Unpublished work by G. H. Jones, D. Tegg, G. B. Howarth, N. P. Damodaran, A. Rudinskas, and J. G. Moffatt.
(22) H. Kaufmann, P. Mühlradt, and T. Reichstein, *Helv. Chim. Acta*, **50**, 2287 (1967).
(23) Kindly provided by Professor T. Reichstein.
(24) P. Howgate and A. Hampton, *Carbohyd. Res.*, **21**, 309 (1972).
(25) We would like to thank Dr. M. L. Maddox for clarification of this point.
(26) G. M. Tener, *J. Amer. Chem. Soc.*, **83**, 159 (1961).
(27) A. Franke, K. H. Scheit, and F. Eckstein, *Chem. Ber.*, **101**, 2998 (1968).
(28) M. Smith, G. I. Drummond, and H. G. Khorana, *J. Amer. Chem. Soc.*, **83**, 698 (1961).
(29) (a) C. D. Jardetzky, *J. Amer. Chem. Soc.*, **84**, 62 (1962); (b) B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *ibid.*, **95**, 2873 (1973).
(30) R. F. Nutt and E. Walton, *J. Med. Chem.*, **11**, 151 (1968).
(31) P. J. Harper and A. Hampton, *J. Org. Chem.*, **35**, 1688 (1970).
(32) R. R. Schmidt, U. Schloz, and D. Schwiele, *Chem. Ber.*, **101**, 590 (1968).
(33) H. J. Fritz, R. Machat, and R. R. Schmidt, *Chem. Ber.*, **105**, 642 (1972).
(34) T. A. Khwaja and C. B. Reese, *Tetrahedron*, **27**, 6189 (1971).
(35) R. K. Ralph and H. G. Khorana, *J. Amer. Chem. Soc.*, **83**, 2926 (1961).
(36) It has previously been noted that the cleavage of trichloroethyl esters in different compounds may optimally require different acids. See, *e.g.*, R. R. Chauvette, P. A. Pennington, C. W. Ryan, R. D. G. Cooper, F. L. José, I. G. Wright, E. M. Van Heyningen, and G. W. Huffman, *J. Org. Chem.*, **36**, 1259 (1971).
(37) *Cf.* methyl groups in carbocyclic chair conformations. See L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed, Pergamon Press, London, 1969, p 240.
(38) J. Parrick and J. W. Rasbun, *Can. J. Chem.*, **43**, 3453 (1965).
(39) W. E. Trevelyan, D. P. Procter, and J. S. Harnson, *Nature (London)*, **166**, 444 (1950).
(40) Prepared by treatment as above of methyl 6-deoxy-2,3-*O*-isopropylidene-β-D-allofuranoside[13] kindly provided by Dr. Leon Goodman.

Synthesis and Reactions of Azido Halo Sugars

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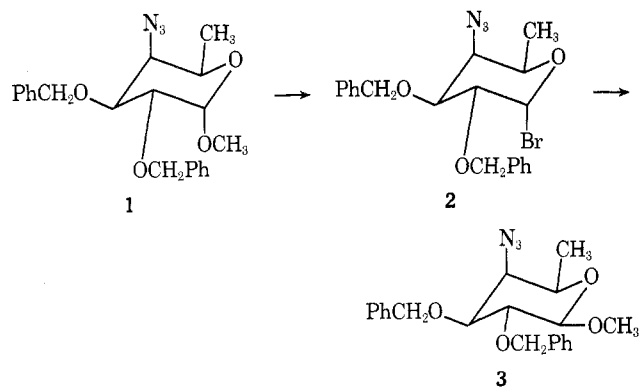
The syntheses of several crystalline 4-azido-4,6-dideoxy-1-halo hexoses, which are useful intermediates in the chemical synthesis of natural products containing amino hexoses, are described. The reactions of these compounds with methanol and ethanol in the presence of silver carbonate are shown to be stereospecific. The uses of azido halo sugars in the synthesis of cardiac glycosides, antibiotics, and amino sugar nucleosides are indicated.

Many amino sugars have been isolated from biologically important natural sources such as antibiotics,[2,3] cell wall polysaccharides,[2] and cardiac glycosides.[4] Because an azide can be conveniently used as an amine precursor, azido halo sugars are extremely useful intermediates for the chemical synthesis of these natural products and their structural anaogs of potential biological activity. However, except for the recent reports on the isolation of 6-azido-1-chloro hexoses by Umezawa and coworkers,[5] azido halo sugars have not been prepared. Earlier attempts to obtain this class of compounds were reported to be unsuccessful.[6,7] We now describe the synthesis and reactions of several crystalline 4-azido-4,6-dideoxy-1-halo sugars as part of our investigation of the chemistry of 4-amino-4,6-dideoxy hexoses and their derivatives.[8]

Treatment of methyl 4-azido-4,6-dideoxy-2,3-di-*O*-benzyl-α-D-galactopyranoside[9] (**1**) with acetyl bromide at room temperature for 30 min gave the crystalline 4-azido-4,6-dideoxy-2,3-di-*O*-benzyl-α-D-galactopyranosyl bromide (**2**). The α configuration for the bromo sugar **2** was indicated by its nmr spectrum, which showed the anomeric proton as a doublet ($J_{1,2}$ = 3.5 Hz) at δ 6.43. Also, reaction of **2** with methanol in the presence of silver carbonate gave clean inversion at the anomeric center, providing methyl 4-azido-4,6-dideoxy-2,3-di-*O*-benzyl-β-D-galactopy-



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