hypoxanthine, 1:9 for *lin*-naphthoxanthine, and 1:3 for *lin*-naphthohypoxanthine. The oxidation for xanthine and hypoxanthine was monitored at 293 nm, while the oxidation for *lin*-naphthoxanthine and *lin*-naphthopoxanthine was monitored at 260 and 267 nm, respectively. Duplicate assays were run for each sample.

In a study of the possible inhibitory effect of *lin*-naphthoxanthine and *lin*-naphthohypoxanthine on uric acid formation from xanthine and hypoxanthine, duplicate samples were run at each of the concentrations given above for xanthine and hypoxanthine at known inhibitor concentrations. Inhibitor concentrations were varied from 8.2×10^{-4} to 2.5×10^{-3} mM for *lin*-naphthoxanthine and 1.52×10^{-3} to 4.57×10^{-3} mM for *lin*-naphthohypoxanthine. Final assay mixtures had a total volume of 3 mL in a cuvette with a 1.0-cm light path. Each assay was initiated by addition of 5 μL of the Sigma buttermilk xanthine oxidase solution, which was a dilution in a ratio of 1:1 in phosphate buffer. The assays were monitored at 293 nm.

Acknowledgment. This work was supported by Research Grant GM-05829 from the National Institute of Health. The mass spectral data processing equipment employed in the present study was provided by Research Grants CA 11388 and GM 16864 from the National Cancer Institute and the National Institute of General Medical Sciences, respectively, of the National Institutes of Health, U.S. Public Health Service. We are pleased to acknowledge our colleagues Professor Gregorio Weber and Dr. David Jameson for their help in the fluorescence studies and Mr. Chris A. Buhr for his invaluable assistance in preparing and maintaining large quantities of starting materials.

Registry No. 5, 80954-04-1; **6**, 80964-05-2; **7**, 57297-31-1; **8**, 57297-36-6; **9**, 80964-06-3; **10**, 4082-23-9; **11**, 4082-21-7; **12a**, 80964-07-4; **12b**, 80964-08-5; **13a**, 80964-09-6; **13b**, 80964-10-9; **14**, 80964-11-0; **15**, 80964-12-1; **16**, 80964-13-2; **17**, 80964-14-3; **18**, 80964-15-4; **19**, 80964-16-5; **20**, 80964-17-6; **21**, 80976-40-5; **22a**, 80964-18-7; **22b**, 80964-19-8; **23**, 80964-20-1; **24**, 80964-21-2; **25**, 80964-22-3; **26**, 80964-23-4; **27**, 80964-24-5; **28**, 80964-25-6; **29**, 80964-26-7; **30**, 80964-27-8; **31**, 80964-28-9; **32a**, 80964-29-0; **32b**, 80964-30-3; **33a**, 80964-31-4; **33b**, 80964-32-5; **34a**, 80964-33-6; **34b**, 80964-34-7; **35a**, 80964-35-8; **35b**, 80964-36-9; **36**, 80964-37-0; **37**, 80964-38-1; **38**, 80964-39-2; **39**, 80964-40-5; **40**, 80964-41-6; **41**, 80964-42-7; **42**, 80964-43-8; bis(trimethylsilyl)acetylene, 14630-40-1; 1,5-hexadiyne, 628-16-0; hypoxanthine, 68-94-0; *lin*-benzohypoxanthine, 53449-18-6; xanthine, 69-89-6; dimethyl acetylenedicarboxylate, 762-42-5; urea, 57-13-6; formamide, 75-12-7; hexamethyldisilazane, 999-97-3; hydroxylamine HCl, 5470-11-1.

Foreshortened Nucleotide Analogues as Potential Base-Pairing Complements for *lin*-Benzoadenosine

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Abstract: Syntheses of foreshortened nucleotide analogues of uridine have been carried out to test the possibility of base pairing with the linearly extended nucleoside *lin*-benzoadenosine. Phosphorylation of *N*-(β-D-ribofuranosyl)formamide (F) provided the 5-monophosphate, which could be dephosphorylated by the action of either alkaline phosphatase or, surprisingly, 5′-nucleotidase. Additional phosphorylations by the method of Hoard and Ott afforded the 5-di- and triphosphates. The diphosphate, 5-FDP, was found not to undergo polymerization with polynucleotide phosphorylase. Syntheses of the self-complementary dinucleoside monophosphates FpA and Fp(*lin*-benzo-A) are described. The foreshortened analogue was protected as its 2-(methoxytetrahydropyranyl)-5-(*tert*-butyldiphenylsilyl) derivative, while 5′-AMP and *lin*-benzo-AMP were protected by a new and easy method as the corresponding 2′,3′-di-*O*-(*tert*-butyldimethylsilyl) nucleotides. Condensation of the fully protected F and 5′-monophosphate moieties with DCC provided the desired (3→5′)-linked nucleotides, which, on treatment with phosphodiesterase I, were hydrolyzed back to F and the corresponding 5′-monophosphate.

Introduction

The complementarity of base pairing in the hydrogen-bonded associations of guanine with cytosine and adenine with thymine or uracil has provided the structural basis for much of molecular biology. It has now been almost 30 years since Watson and Crick recognized the two salient features of this scheme: first, that good linear hydrogen bonds can be formed in these matches, and second (and more importantly), that the same geometry accommodates A–T(U), T(U)–A, G–C, and C–G within the interior of a helix formed by two complementary strands of polymeric DNA or RNA.[1,2] While alternative base-pairing schemes are known to exist,[3] the same stringent geometrical constraints are imposed and must be satisfied in order for a stable duplex to exist.

In conjunction with our continuing investigations concerning the synthesis and utility of dimensional probes for the study of enzyme–coenzyme binding sites,[4] we have been interested in examining the possibility of base pairing with the linearly extended benzonucleotides. *lin*-Benzoadenine nucleotides (e.g., **4**, R = phosphoribosyl) are defined by the formal insertion of a benzene ring (actually four carbon atoms) into the center of the adenine ring system (**2**) and consequently are 2.4 Å wider than adenine. In a base-pairing scheme analogous to that of A–U, one might envision that *lin*-benzoadenosine could hydrogen bond to uridine or ribothymidine, but a double helix made up of stretched cross sections consisting of tricyclic plus monocyclic bases would be distorted from the normal. By contrast, a perfectly proportioned double helix can be constructed with an acyclic, or "zero-ring", complementary partner. The ideal complement would, by ne-

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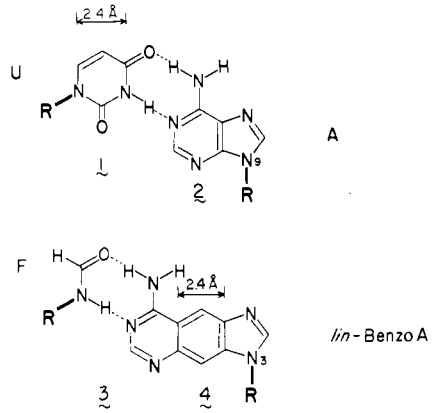


Figure 1. Base-pairing scheme of uridine (**1**) with adenosine (**2**) as compared to hypothetical base-pairing scheme of *N*-ribosylformamide (**3**) with *lin*-benzoadenosine (**4**).

cessity, possess appropriate hydrogen-bonding functionalities in geometries accessible for base pairing but would be 2.4 Å narrower or shorter than uridine, and thus would be a foreshortened analogue of uridine.

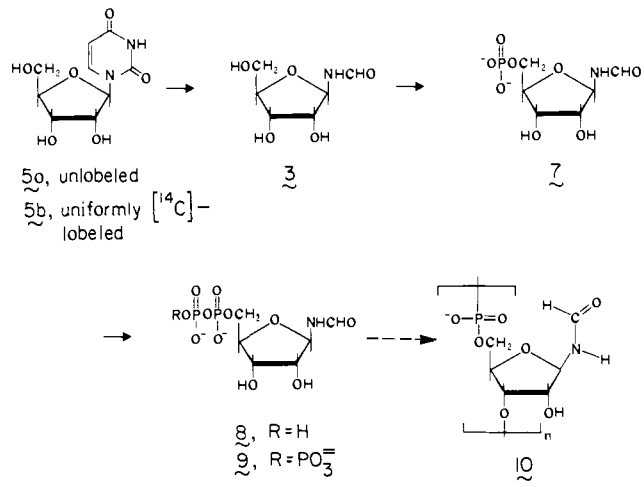
Accordingly, the complement that best fits these criteria is *N*-(β-D-ribofuranosyl)formamide (*N*-ribosylformamide, 1-(formylamino)-1-deoxyribose; **3**), which will hereafter be abbreviated simply as "F". In the combination of tricyclic and acyclic units shown in Figure 1, the distance and angle between N–$C_{1'}$ bonds are identical with those in a normal complementary pair of bicyclic adenosine and monocyclic uridine. In addition, the placement of a hydrogen at the carbonyl carbon, a position that corresponds to C-6 of uridine, ensures that rotation about the glycosidic linkage and positioning in the accompanying syn–anti conformational equilibrium will resemble that of uridine as closely as possible. For example, while uridine is known to exist primarily in the anti conformation,[5] 6-methyluridine exists almost exclusively in the syn conformation.[6]

We therefore set out to synthesize derivatives of **3** that are suitable for detecting its base pairing with *lin*-benzoadenosine, or perhaps even with adenosine itself, which might form a similar, shrunken cross section. Of the three or four levels at which positive evidence for the base pairing of natural nucleotides has been obtained, we have concentrated our efforts on two: polymer–polymer interactions and self-complementary oligonucleotide "minihelix" formation.

Results and Discussion

Synthesis and Attempted Polymerization of the *N*-(β-D-Ribofuranosyl)formamide 5-Phosphates. The level of complexity at which base pairing is most commonly observed is by the detection of an intermolecular association between two complementary polymers, e.g., between poly(AMP) and poly(UMP). Several physical techniques have proven to be of value in determining whether or not a polymeric complex is formed; UV hypochromism,[7] circular dichroism,[8] digital scanning calorimetry,[9] and resistance to S_1 nuclease digestion[10] have all been used in this respect. We have previously reported that poly(*lin*-benzo-AMP) is obtained by the action of primer-independent polynucleotide phosphorylase from *M. luteus* on *lin*-benzo-ADP in the presence of Mn^{2+} at 37 °C.[11] The polymeric material isolated by gel chromatography showed almost no fluorescence, consistent with quenching due to vertical stacking. We were therefore interested in learning whether this route could be applied similarly to the synthesis of poly(FMP), with which the possibility of double-helix formation with poly(*lin*-benzo-AMP) could be investigated.

Scheme I



The synthesis of *N*-(β-D-ribofuranosyl)formamide (**3a**) was based on that reported by Ducolomb et al., in which the transformation of uridine to **3a** could be carried out on a small scale by the action of trifluoroperacetic acid on uridine, followed by hydrolytic workup and isolation by preparative silica gel chromatography[12,13] (Scheme I). Several modifications of this method allow for the synthesis and isolation of the desired compound on a synthetically useful scale. The procedure affords solely the desired β-anomer of **3**; in contrast, many syntheses of *N*-ribosides possessing an acyclic substituent at C-1 have yielded mixtures of α- and β-anomers that either were not or could not be separated.[14-18] The product **3a** was obtained as a gum, the structure of which was verified by NMR, IR, and high-resolution field desorption mass spectrometry. Facile conversion to the corresponding 2,3,5-tri-*O*-(*p*-nitrobenzoyl) derivative provided a crystalline sample through which the elemental composition could be verified.

An analysis of the ^1H and ^{13}C spectra of tetradeuterio-**3a** provided evidence that supported the structure assignment as a substituted formamide. The ^1H NMR spectrum, taken in $(CD_3)_2SO$, revealed a pair of singlets for the formyl proton and a pair of doublets for the anomeric proton as expected for a rotationally restricted amide. When the sample was heated to 100 °C, the signals in these regions began to coalesce. This is comparable directly to *N*,*N*-dimethylformamide, which coalesces over the range 102–170 °C, with a loss of line separation occurring only above 118 °C.[19] Similarly, the ^{13}C NMR spectrum contains two singlets for the formyl carbon: the major rotamer at δ 165.5 and the minor rotamer at δ 168.5. *N*-Methylformamide analogously exhibits formyl carbon resonances at δ 163.4 and 166.7, corresponding respectively to the *Z* and *E* rotational isomers.[20]

The synthesis of 5-FMP (**7**) was accomplished by using standard monophosphorylation conditions; however, in order to facilitate the chromatographic purification of this non-UV-absorbing in-

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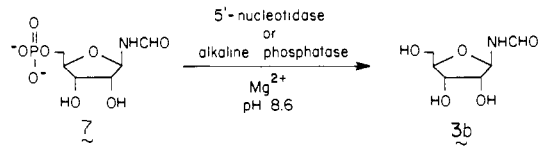
Scheme II

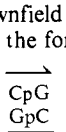


termediate, we incorporated a radiolabel into the compound by using uniformly ^{14}C-labeled uridine (**5b**) as the starting material. We found that the removal of inorganic phosphate before ion-exchange chromatography was essential, owing to the fact that compound **7** eluted from the column at a significantly lower buffer concentration than did heterocyclic mononucleotides. The ^1H NMR spectrum of the isolated product indicated retention of the formyl proton, while the fully decoupled ^{31}P NMR spectrum exhibited a characteristic singlet at δ 4.0. It is of interest that the chemical shift of the ^{31}P signal for mononucleotides seems to be independent of the nature of the aglycone, as the signals of 5′-*lin*-benzo-AMP,[11] 5′-AMP,[21] 5-FMP, and methoxyribosyl 5-monophosphate[22] all fall within ±0.1 ppm of δ 4.0 relative to a standard of 85% H_3PO_4.

As expected, treatment of the monophosphate **7** with alkaline phosphatase at pH 8.6 resulted in its dephosphorylation, which was confirmed by paper chromatography and electrophoresis. Moreover, we were gratified to observe that dephosphorylation was also accomplished by the action of 5′-nucleotidase from *C. atrox* (Scheme II), which is specific for 5′-monophosphates. It therefore seems clear that, contrary to previous reports,[23] the presence of a heterocyclic base at C-1 of the substrate is not an absolute requirement for this enzyme. It should be noted, however, that while **7** is a substrate for 5′-nucleotidase, its hydrolysis proceeds at a much slower rate than that observed for 5′-AMP.

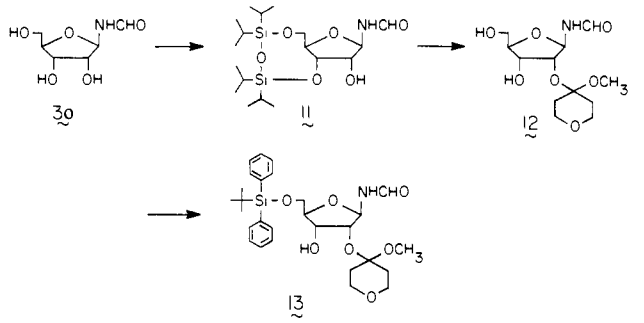
The syntheses of 5-FDP (**8**) and 5-FTP (**9**) were carried out via the 5-phosphorimidazolidate of **7** according to the procedure of Hoard and Ott.[24] Each product eluted from a DEAE-Sephadex anion-exchange column at a characteristic buffer concentration, and the diphosphate **8** was characterized further by paper electrophoresis and quantitative phosphate determination. Attempted polymerization of 5-FDP with polynucleotide phosphorylase (PNPase) under a wide variety of conditions led only to the recovery of unreacted starting material. To date, we know of no similar attempts to polymerize a diphosphate containing an acyclic substituent at C-1, and thus this finding provides some insight into the mechanism of action of the enzyme. It seems probable that the failure of **8** to act as a substrate is due not to a lack of reactivity, which is reflected by the V_{max} for the reaction, but rather to the insufficient hydrophobicity of the formylamino group, which results in a diminished binding ability. The introduction of a hydrophobic substituent on the formamide group may allow us to overcome the lack of binding, and this possibility is currently being examined.

Synthesis of the Self-Complementary Dinucleoside Monophosphates FpA and Fp(*lin*-benzo-A). Another way in which base pairing has been observed between complementary nucleotides is by the synthesis and investigation of short oligonucleotides that contain self-complementary base sequences. For example, ^1H NMR studies of the dinucleoside monophosphate CpG in aqueous solution revealed a notable downfield shift in the hydrogen-bonded N–H protons associated with the formation of the "minihelix"[25]



However, the technique fails to detect complex formation with UpA,[26] a result that is explainable by the fact that G and C are

Scheme III



joined by three hydrogen bonds, while A and U can form only two. A more powerful technique is the complete structure solution of a crystalline "minihelix" by X-ray crystallography. Several studies have succeeded in demonstrating the complementarity of base pairing in these types of complexes, e.g., for ApU,[27] for GpC,[28] and recently, for d-pApTpApT[29] and for d-CpGpCpGpCpG.[30] Therefore, we were interested in preparing the self-complementary dinucleoside monophosphate Fp(*lin*-benzo-A) (**18b**) as a likely model for the perfectly proportioned base-pairing scheme shown in Figure 1. Furthermore, the synthesis of FpA would allow us to examine the possibility of a "shrunken" complex, in which the interior width of the corresponding "minihelix" would be decreased by roughly 2.4 Å.

A review of the methods that have been used in the chemical synthesis of oligonucleotides reveals that three approaches have met with wide success: (1) condensation of a 3′-hydroxyl and a 5′-phosphate,[31] (2) condensation of a 3′-phosphate and a 5′-hydroxyl,[32] and (3) condensation of a 3′-hydroxyl and a 5′-hydroxyl via phosphite coupling followed by oxidation to the corresponding phosphate.[33] Inasmuch as we have previously reported the synthesis of the 5′-monophosphate of *lin*-benzoadenosine,[11] the first of these methods appeared most appealing. Accordingly, we required both a derivative of **3** in which the 2- and 5-hydroxyl groups were protected from the condensation reaction and a mononucleotide that was protected at the 2′- and 3′-hydroxyls. While the common nucleotides are generally stable to standard acidic and basic deprotection conditions, we felt it wise to utilize protecting groups that could be cleaved under mild conditions, owing to the potential lability of the *N*-formyl riboside and its likely propensity to undergo facile anomerization.[34]

Using the procedure of Markiewicz and Wiewiórowski,[35] we could readily convert **3a** to the 3,5-disiloxanyl-protected **11** (Scheme III), which was subsequently treated with Reese's 5,6-dihydro-4-methoxy-2*H*-pyran[36] and deprotected with tetrabutylammonium fluoride[51] in dry tetrahydrofuran to provide the 2-(methoxytetrahydropyranyl)-protected **12**. This sequence does not require the separation or identification of 2- and 3-isomers and has been used in the synthesis of 2′-(methoxytetrahydropyranyl)adenosine.[37] Further reaction with *tert*-butyldiphenylsilyl

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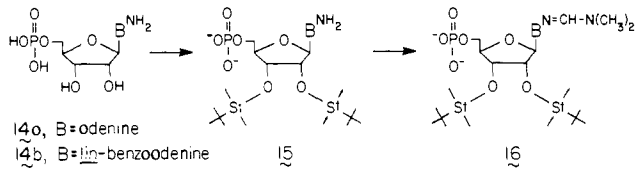
Scheme IV



14a, B=adenine
14b, B=lin-benzoadenine 15 16

chloride[38] gave the desired 2,5-protected **13** as a colorless, UV-absorbing gum (the incorporated UV absorption made the product easy to follow during chromatography), the structure of which was confirmed by 360 MHz ^{1}H NMR, high-resolution field desorption mass spectrometry, and elemental analysis.

Our requirement for suitably protected ribonucleotides provided us with an opportunity to introduce a new and easy method for obtaining these synthetically useful derivatives. Classically, the simultaneous protection of both the 2′- and 3′-hydroxyls of ribonucleotides has involved either protection at the nucleoside level with a methoxymethylidene group, followed by phosphorylation and purification by ion-exchange chromatography,[39] or perbenzoylation at the nucleotide level followed by deprotection of the phosphate group with acetic anhydride.[40] While the use of silyl reagents has been reported for the protection of both nucleosides[41,42] and 2′-deoxyribonucleotides,[43] they have not been used for the protection of 5′-mononucleotides. We can now report that the reaction of 5′-AMP (**14a**) with an excess of *tert*-butyldimethylsilyl chloride and imidazole in DMF, followed by aqueous workup and precipitation from pyridine solution, provided the corresponding 2′,3′-di-*O*-(*tert*-butyldimethylsilyl) nucleotide (**15a**) in a single step (Scheme IV). This compound was of analytical purity, and the location of the silyl groups was confirmed by its ^{1}H NMR spectrum, which is completely analogous to that reported for 2′,3′-di-*O*-(*tert*-butyldimethylsilyl)adenosine.[41] Similarly 5′-*lin*-benzo-AMP (**14b**) was converted to its corresponding 2′,3′-bissilyl ether (**15b**). In each case, treatment of the protected nucleotide in pyridine with an excess of fluoride ion resulted in complete removal of the silyl groups within 1 h as evidenced by TLC.

While it has been reported that protection of the exocyclic amine of adenosine is unnecessary prior to attempting condensation reactions,[44] we have observed that the corresponding amine of *lin*-benzoadenosine is substantially more reactive in similar situations.[45] Therefore, to avoid the possibility of competing N-phosphorylation, both **15a** and **15b** were protected via their corresponding (dimethylamino)methylene derivatives, **16a** and **16b**, using the method of Holý et al.[46] These compounds ran as single spots on TLC and were used without further purification.

The condensation of suitably protected derivatives of F and *lin*-benzo-AMP (or AMP) is diagrammed in Scheme V. Following DCC-mediated coupling, the crude product was fully deprotected by using methanolic NH_4OH to remove the (dimethylamino)methylene group, fluoride ion to desilylate, and finally dilute aqueous acid to effect removal of the methoxytetrahydropyranyl group. Purification by anion-exchange and paper chromatography provided the desired dinucleoside monophosphates **18a** and **18b** in pure form. Analysis by TLC and paper electrophoresis gave results consistent with the assigned structures; moreover, treatment with phosphodiesterase I, which is known to cleave 3′→5′-linked oligonucleotides,[47] yielded a mixture of **3a** and **14a** (or **14b**) as judged by TLC and electrophoresis.

Conclusion

The synthesis of *N*-(β-D-ribofuranosyl)formamide derivatives that are potentially capable of hydrogen bonding with a suitable derivative of *lin*-benzoadenosine should allow us to determine whether or not the aforementioned "unnatural" base-pairing scheme is, in fact, viable. The preparation of 5-FDP and its subsequent failure to polymerize with PNPase rules out, for the time being, investigation at the polymer–polymer level; however, this route has provided useful information concerning the substrate tolerance of both 5′-nucleotidase and PNPase. Utilization of the *tert*-butyldimethylsilyl protecting group at the ribonucleotide level of oligonucleotide synthesis offers a favorable alternative to previously reported procedures. Finally, synthesis of the self-complementary dinucleoside monophosphate Fp(*lin*-benzo-A) provides a valuable model with which the notion of a perfectly proportioned alternative helix can be tested.

Experimental Section

General Comments. *N*,*N*-Dimethylformamide (DMF) was purified before use by stirring over KOH pellets, distilling from BaO, and distilling again from CaH_2. Pyridine was purified by distillation from CaO. Methanol and ethanol used were of anhydrous grade. Uridine was obtained from Aldrich Chemical Co. and [^{14}C]uridine (uniformly labeled) was obtained from New England Nuclear. Both $POCl_3$ and $PO(OEt)_3$ were freshly distilled prior to use. All evaporations were performed in vacuo at a water bath temperature of not greater than 30 °C.

Thin-layer chromatography (TLC) was performed on three different supports: silica gel (0.25 mm with fluorescent indicator, Macherey-Nagel), cellulose sheets (no. 13254 with fluorescent indicator, Eastman), and cellulose paper (ascending, 0.33-mm Whatman 3MM chromatography paper). Column chromatography was performed by using three different stationary phases: silica gel (0.05–0.2 mm, Brinkman), cellulose (Whatman CF 11 cellulose powder) and DEAE-Sephadex (A-25, Pharmacia). The solvent systems employed were as follows: solvent A, 75:16:9 EtOAc–2-propanol–H_2O; solvent B, 9:1 acetone–H_2O; solvent C, 5:3 isobutyric acid–0.5 M NH_4OH; solvent D, 4:1:1 1-butanol–glacial HOAc–H_2O; solvent E, 17:3 $CHCl_3$–EtOH; solvent F, 19:1 $CHCl_3$–MeOH; solvent G, 9:1 $CHCl_3$–MeOH; solvent H, 1:1 EtOAc–MeOH; solvent I, 7:1:2 2-propanol–concentrated NH_4OH–H_2O. Carbohydrates were visualized with either the $NaIO_4$–benzidine or the $AgNO_3$–ethanolic NaOH treatment.[48] Sugar phosphates were visualized with a modified Haynes–Ischerwood spray reagent.[48] Paper electrophoresis on cellulose was carried out at 300 V with either 0.05 M sodium citrate buffer (pH 5.1) or 0.05 M ammonium bicarbonate buffer (pH 7.5). Anion-exchange chromatography was carried out at 4 °C using a linear gradient of triethylammonium bicarbonate (TEAB) buffer, pH 7.5.

Melting points were determined on a Büchi capillary melting point appartus and are uncorrected. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Varian EM-390 or a Nicolet NTC-360 spectrometer using tetramethylsilane with deuterated organic solvents or acetone (δ 2.07) with D_2O solutions as internal standards. ^{31}P and ^{13}C nuclear magnetic resonance spectra were recorded on Varian XL-100-15 and Nicolet NTC-360 spectrometers, respectively. Mass spectra were run on a Varian MAT-731 high-resolution spectrometer coupled with a 620i computer and a Statos recorder. Ultraviolet absorption spectra were obtained on a Beckman Acta MVI spectrophotometer.

***N*-(β-D-Ribofuranosyl)formamide (3a).** A solution of freshly distilled trifluoroacetic acid (41 mL) and 30% H_2O_2 (61 mL) was stirred for 10 min, uridine (25 g, 0.1 mol; **5a**) was added, and the loosely stoppered reaction was stirred at room temperature for 21 h. The resulting colorless solution was evaporated in vacuo to afford a syrupy residue, which was dried overnight at 0.1 torr in a desiccator containing KOH pellets. The white foam was dissolved in 200 mL of H_2O and was then heated in a

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(45) For example, the attempted synthesis of *lin*-benzo-ADP from *lin*-benzo-AMP via the imidazolidate procedure (cf. ref 24) yielded primarily a compound whose UV spectrum is consistent with that expected for an N-phosphorylated product. This unusual reactivity is due likely to proximity effects that result from the strong stacking interactions experienced by members of the "stretched" nucleotide series.
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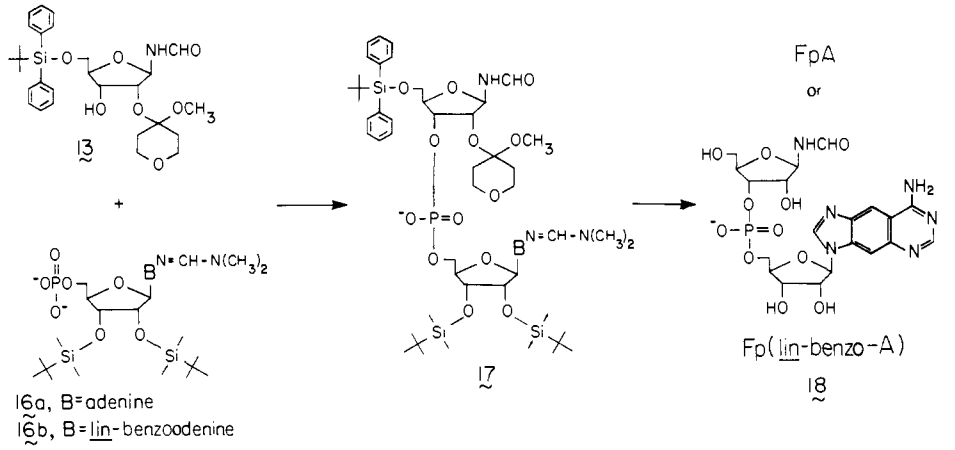
Scheme V



water bath until the temperature of the solution had reached 60 °C. The reaction was maintained at this temperature with occasional swirling for 10 min and was finally evporated in vacuo to afford the crude **3** as a white foam which was used directly for chromatographic purification.

To a solution of the crude **3** in 200 mL of MeOH was added 50 g of silica gel; the mixture was vigorously swirled and was finally evaporated to leave the reaction mixture adsorbed onto the support. This material was added to the top of a 48 × 5 cm column of silica gel packed in solvent A, the column was eluted with the same solvent system at a flow rate of 1–2 drops/s, and fractions of 25 mL were collected. The UV absorbance of each tenth fraction was determined by spotting an aliquot onto a silica gel plate and observing under UV light; the desired product (**3**), which is non-UV absorbing, elutes immediately after unreacted uridine, which absorbs heavily. Fraction 110 was still heavily absorbing, while fractions 150 on were only very slightly absorbing. TLC on silica gel using solvent A and the $NaIO_4$–benzidine visualization procedure confirms that fractions 150–250 contained only a trace of uridine and mainly the desired product. Elution was continued and fractions of 1 L were collected, which were evaporated to about 50 mL and checked for product by TLC. The first four 1-L fractions, together with tube fractions 150–250, were pooled and evaporated thoroughly in vacuo to afford a yellow gum, which was stored in the freezer prior to additional purification.

A solution of the yellow gum in 200 mL of MeOH was evaporated to about 15-mL total volume and then applied to a 46 × 4.5 column of cellulose powder packed in solvent B. The column was eluted with the same solvent system at a flow rate of 1 drop/s and fractions of 25 mL were collected. After 100 fractions had been collected, every second fraction was checked for carbohydrate content by spotting an aliquot onto cellulose paper and developing with the $AgNO_3$–ethanolic NaOH procedure. Fractions 32–48 gave a strongly positive result, and TLC on silica gel using solvent C and the $NaIO_4$–benzidine visualization procedure indicated these to contain essentially pure **3**. The fractions were combined and evaporated in vacuo to afford, after drying at 0.1 torr overnight, *N*-(β-D-ribofuranosyl)formamide (2.42 g, 13%) as a gum which varied in color from yellow-green to colorless: R_f 0.2 (solvent A), 0.5 (solvent C); ^{1}H NMR[49] (sample coevaporated with D_2O, dissolved in $(CD_3)_2SO$) δ 3.40 (m, 2, 5-CH_2), 3.70 (m, 3, 2-, 3-, and 4-CH's), 4.85 (d, 0.42, J = 5 Hz, 1-CH, rotamer 1), 5.25 (d, 0.58, J = 5 Hz, 1-CH, rotamer 2), 8.05 (s, together with adjacent signal integrates to 1 H, formyl CH, rotamer 2), 8.07 (s, together with adjacent signal integrates to 1 H, formyl CH, rotamer 1); ^{1}H NMR (same sample at 100 °C) δ 3.35 (d, 1, 5-C*H*(H)), 3.40 (s, 1, 5-CH(*H*)), 3.70 (m, 3, 2-, 3-, and 4-CH's), 4.85 (br s, 0.42, 1-CH, rotamer 1), 5.25 (br s, 0.58, 1-CH, rotamer 2), 8.05 (s, 1, formyl CH); ^{13}C NMR (D_2O) δ 62.34, 71.09, 74.79, 83.11, 84.33, 84.63, 88.00 (ribosyl C's), 165.53 (C=O, rotamer 2), 168.52 (C=O, rotamer 1); IR (film) 1690 cm^{-1} (C=O). Field desorption mass spectrum: m/e 178 (MH^+). High-resolution field desorption mass spectrum: exact mass calcd for $C_6H_{12}NO_5$ 178.0716, obsd 178.0719.

[^{14}C]-*N*-(β-D-Ribofuranosyl)formamide (3b). The synthesis of ^{14}C-labeled **3b** was carried out exactly as described for **3a** except that in addition to 25 g of uridine, uniformly labeled [^{14}C]uridine (0.25 mCi, 0.12 mg) in 2.5 mL of 7:3 EtOH–H_2O was added to the trifluoroperacetic acid solution. The product thus obtained (2.67 g, 15%) had a specific activity of 1.67 μCi/mmol. Ascending paper chromatography using solvent D indicated that greater than 90% of the radioactivity coeluted with **3a** (R_f 0.42), while less than 10% eluted with a UV-absorbing impurity at a slower R_f (0.32).

***N*-[(2,3,5-Tri-*O*-(*p*-Nitrobenzoyl))-β-D-ribofuranosyl]formamide.** *N*-(β-D-Ribofuranosyl)formamide (130 mg, 0.73 mmol) was dried by coevaporation with dry pyridine (2 × 25 mL), followed by dissolution in dry pyridine (25 mL). A solution of *p*-nitrobenzoyl chloride (620 mg, 3.3 mmol) in dry pyridine (7 mL) was added dropwise by syringe, and the desiccated reaction was stirred at room temperature for 22 h. The vessel was cooled to 0 °C, the reaction was poured onto 20 g of ice, and the resulting mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2 (75 mL), extracted with saturated aqueous $NaHCO_3$ (3 × 40 mL) and H_2O (2 × 40 mL), dried over Na_2SO_4, and evaporated to a brown glass. Purification by silica gel column chromatography using 0–5 % EtOH in $CHCl_3$ as the eluting solvent followed by evaporation of the appropriate fractions gave a foam (158 mg, 35%). Crystallization from a minimal volume of $CHCl_3$ followed by recrystallization from acetone–petroleum ether provided a sample in analytically pure form: mp 121–123 °C; R_f 0.35 (solvent E); ^{1}H NMR ($CDCl_3$) δ 4.65 (m, 3, 4-CH and 5-CH_2), 5.56–6.15 (m, 3, 1-, 2-, and 3-CH's), 6.55 (br d, 1, J = 10 Hz, NH), 8.00–8.35 (m, 13, formyl CH and aryl CH's); IR (KBr) 1740 (ester C=O), 1690 (formamide C=O), 1535 (aryl NO_2), 1280 cm^{-1} (ester COC). Field desorption mass spectrum: m/e 625 (MH^+).

Anal. Calcd for $C_{27}H_{20}N_4O_{14}$: C, 51.92; H, 3.23; N, 8.97. Found: C, 52.09; H, 3.39; N, 9.07.

[^{14}C]-*N*-(β-D-Ribofuranosyl)formamide 5-Monophosphate (7). [^{14}C]-*N*-(β-D-Ribofuranosyl)formamide (458 mg, 2.6 mmol) was dried by coevaporation with dry pyridine (1 × 20 mL) and dry DMF (1 × 20 mL), the residual gum was dissolved in distilled $PO(OEt)_3$ (20 mL), and the resulting solution was cooled to 0 °C in an ice bath under a dry N_2 atmosphere. After $POCl_3$ (1.0 mL, 11 mmol) was added dropwise to the stirred solution via syringe, the reaction was allowed to proceed at 0 °C. After 3 h, the reaction was poured onto ice water (125 mL) and the resulting solution was extracted with Et_2O (3 × 75 mL). The aqueous phase was adjusted to pH 7 with 1 M TEAB buffer, the solution was cooled in an ice bath, and the pH was adjusted to 12 (pH meter) with 185 mM $Ba(OH)_2$ (requires at least 800 mL). Carbon dioxide was bubbled through the resulting cold mixture until pH 7 was reached; then the precipitated inorganic phosphate was removed by filtration, the solid was washed with cold H_2O (150 mL), and the combined filtrates were evaporated to dryness with care to minimize bumping. The residue was coevaporated with MeOH (3 × 100 mL) to remove remaining buffer, and the crude monophosphate was converted to the pyridinium form with a Dowex 50W-X8 column and finally to the triethylammonium form by addition and evaporation of 1 M TEAB (300 mL). The product was purified by chromatography on a column (21 × 6 cm) of DEAE-Sephadex (A-25) using a linear gradient of 3 L of 0.005 M → 3 L of 0.25 M TEAB as the eluting solvent. Both the radioactivity and absorbance at 262 nm of every fifth fraction were determined, which indicated the elution of a single major, non-UV-absorbing product at a buffer concentration of about 0.2 M. The appropriate fractions were evaporated to dryness and coevaporated with MeOH (3 × 100 mL), converted to the Li^+ form (Dowex 50W-X8), dissolved in H_2O (5 mL), and treated with EtOH (40 mL). The precipitate that had formed after 15 min was removed by centrifugation, and the supernatant was evaporated, coevaporated with 1:1 MeOH–pyridine, and dried overnight in vacuo to afford 161 mg (24%) of a white powder: R_f 0.35 (paper, solvent C); comigrates with glucose 1-phosphate (G-1-P) on paper electrophoresis; ^{1}H NMR

(49) See Czarnik (Czarnik, A. W. Ph.D. Thesis, University of Illinois, Urbana, IL, 1981) for spectra and further details.

(D_2O) δ 3.8–4.3 (m, 2-, 3-, and 4-CH's and 5-CH_2), 4.8–5.5 (1-CH, obscured by broad HOD line), 8.05 (one major and one minor singlet, formyl CH); ^{31}P NMR (D_2O) δ 3.3 (s, ca. 20% inorganic phosphate), 4.0 (s, $CH_2OPO_3^{2-}$).

A 7 mM solution of the monophosphate in 100 mM Tris–acetate buffer (pH 8.6) containing 25 mM $MgCl_2$ was treated with alkaline phosphatase (26 units in 150-μL total reaction volume), and the reaction was followed by TLC using solvent C and by electrophoresis. Following 12 h at room temperature, complete conversion of the monophosphate to the riboside was observed. An identical reaction mixture was treated with 5′-nucleotidase (2 units in 150-μL total conversion volume); incubation at 37 °C for 24 h resulted in greater than 60% conversion to the riboside.

[^{14}C]-*N*-(β-D-Ribofuranosyl)formamide 5-Diphosphate (8). A solution of the monophosphate (25 μmol) in H_2O was converted to the pyridinium form with a Dowex 50W-X8 column and then to the tributylammonium form by the addition of freshly distilled tributylamine (18.5 μL). The solution was evaporated and dried by coevaporation with pyridine (2 × 10 mL) and dry DMF (1 × 10 mL), and the residue was completely dissolved in dry DMF (0.5 mL). 1,1-Carbonyldiimidazole (30 mg, 180 μmol) was added, and the desiccated reaction was stirred at room temperature for 4 h. Methanol (13 μL) was added and the reaction was stirred for 30 min, followed by the addition of a solution of tributylammonium phosphate (1.5 mmol) in DMF (1.5 mL). The reaction was desiccated and stirred at room temperature for 11 h, and the resulting yellow solution was evaporated to dryness. The product was purified by chromatography on a column (21 × 6 cm) of DEAE-Sephadex (A-25) using a linear gradient of 2 L of 0.05 M TEAB → 2 L of 0.6 M TEAB as the eluting solvent. The radioactivity of every fifth fraction was determined, which indicated the elution of the major product at a buffer concentration of about 0.4 M. The appropriate fractions were evaporated to dryness and coevaporated with MeOH (2 × 100 mL). The residue was dissolved in H_2O (0.5 mL) to provide a 22 mM solution of the diphosphate (based on the known specific activity of **3b**), which was stored in the freezer prior to use; yield 11 μmol (44%): R_f 0.29 (paper, solvent C); relative migration on paper electrophoresis (pH 5.1), 1.2 (G-1-P = 1.0).

Quantitative phosphate analysis by the method of Fiske and Subbarow[50] indicated that the sample contained 11 μmol of easily hydrolyzable phosphate and 22 μmol of total phosphate.

Attempted Polymerization of [^{14}C]-FDP. An 11 mM solution of diphosphate **8** in 67 mM Tris-HCl buffer (pH 8.1) containing 1.67 mM $MnCl_2$ and 9.2 μM ApApApA (as primer) was treated with polynucleotide phosphorylase from *M. luteus* (30 units in 24-μL total reaction volume). After incubation at 37 °C for 25 h, the reaction was analyzed by paper chromatography using solvent C, which indicated starting material as the only radioactive species present (greater than 90% radiochemical yield). Variations in the reaction concentrations of all five components similarly resulted in no observable polymerization, as did the use of PNPase from *E. coli* or attempted copolymerization with a known substrate (5′-*lin*-benzo-ADP). In each attempt, the activity of the enzyme was ensured with a control reaction containing 5′-ADP.

[^{14}C]-*N*-(β-D-Ribofuranosyl)formamide 5-Triphosphate (9). To an anhydrous solution of the tributylammonium salt of the monophosphate (50 μmol, prepared as described above) in dry DMF (1 mL) was added 1,1-carbonyldiimidazole (60 mg, 360 μmol), and the desiccated reaction was stirred at room temperature for 4 h. Methanol (26 μL) was added and the reaction was stirred for 30 min, followed by the addition of a solution of tetrakis(tributylammonium) pyrophosphate (300 μmol) in DMF (0.5 mL). The reaction was desiccated and stirred at room temperature for 14 h, and the resulting yellow solution was evaporated to dryness. The product was purified by chromatography on a column (21 × 6 cm) of DEAE-Sephadex (A-25) using a linear gradient of 2 L of 0.05 M TEAB → 2 L of 0.8 M TEAB as the eluting solvent. The radioactivity of every fifth fraction was determined, which indicated the elution of the major product at a buffer concentration of about 0.6 M. The appropriate fractions were evaporated to dryness and coevaporated with MeOH (3 × 100 mL). The residue was dissolved in H_2O (1.0 mL) to provide a 22 mM solution of the triphosphate (based on the known specific activity of **3b**), which was stored in the freezer prior to use; yield 22 μmol (44%).

***N*-(3,5-*O*-(Tetraisopropyldisiloxane-1′,3′-diyl)-β-D-ribofuranosyl)formamide (11).** *N*-(β-D-Ribofuranosyl)formamide (600 mg, 3.4 mmol) was dried by coevaporation with dry pyridine (2 × 5 mL) and dry DMF (1.5 mL) and then was dissolved in dry DMF (5 mL). Imidazole (1.01 g, 15 mmol) was added and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[37] (1.16 g, 3.7 mmol) was added to the stirred solution via syringe and the desiccated reaction was allowed to proceed with stirring at room temperature. After 2.5 h, the reaction was quenched by the addition of H_2O (30 mL), which resulted in the precipitation of a heavy oil. The mixture was extracted with $CHCl_3$ (5 × 50 mL), and the combined organic layers were dried over Na_2SO_4 and evaporated to afford a light yellow gum. Purification was accomplished by chromatography on a column of silica gel (45 × 4.5 cm) using 0–4% MeOH in $CHCl_3$ as the eluting solvent. The fractions were checked for organic material by charring an aliquot of each on a glass-backed silica gel plate with 5% H_2SO_4 in MeOH and heat. The appropriate fractions were pooled, evaporated to dryness, and dried at 0.01 torr and room temperature for 24 h to afford the single major product as a colorless gum (823 mg, 59%): R_f 0.43 (solvent F); ^{1}H NMR ($CDCl_3$) 1.10 (m, 28, CH_3 and CH_3CH), 1.65 (contaminant), 3.15 (pseudo t, 1, 2 OH, exchangeable with D_2O), 3.95 (m, 4, 2- and 4-CH's and 5-CH_2), 4.35 (m, 1, 3-CH), 5.00 (dd, 0.37, J = 4 and 10 Hz, coalesces to d, J = 4 Hz, with D_2O, 1-CH, rotamer 1), 5.40 (dd, 0.63, J = 4 and 7 Hz, coalesces to d, J = 4 Hz, with D_2O, 1-CH, rotamer 2), 7.10 (d, 0.63, J = 7 Hz, exchangeable with D_2O, NH, rotamer 2), 7.55 (d, 0.37, J = 10 Hz, exchangeable with D_2O, NH, rotamer 1), 8.00 (s, 0.63, formyl CH, rotamer 2), 8.10 (s, 0.37, formyl CH, rotamer 1). Chemical ionization mass spectrum: m/e 420 (MH^+). High-resolution chemical ionization mass spectrum: exact mass calcd for $C_{18}H_{38}NO_6Si_2$ (MH^+) 420.2237, obsd 420.2206.

***N*-[2-*O*-4′-(4′-Methoxytetrahydropyranyl)-β-D-ribofuranosyl]formamide (12).** To a solution of *N*-[3,5-*O*-(tetraisopropyldisiloxane-1′,3′-diyl)-β-D-ribofuranosyl]formamide (1.05 g, 2.5 mmol) and *p*-toluenesulfonic acid monohydrate (19 mg, 0.1 mmol) in dry DME (12 mL) was added 4-methoxy-5,6-dihydro-2*H*-pyran[51] (2.3 g, 20 mmol), and the resulting solution was desiccated and stirred at room temperature. After 30 min, TLC on silica using solvent F and the H_2SO_4/heat treatment indicated the formation of a single, faster running product. The reaction was neutralized with dilute aqueous NH_4OH, evaporated to dryness, and coevaporated with toluene (3 × 50 mL); then the residue was mostly dissolved in toluene (15 mL) and the decanted solution was evaporated thoroughly to dryness. To a solution of the crude residual product in dry THF (6.0 mL) was added a solution of 1 M tetrabutylammonium fluoride in dry THF[51] (5.0 mL), and the desiccated reaction was stirred at room temperature. After 30 min, TLC using solvent G indicated complete conversion to a slower running product. The reaction was quenched with 125 mL of a solution of 5:1:1 pyridine–MeOH–H_2O and the mixture was passed through a short Dowex column (pyr^+ form), evaporated to dryness, partially dissolved in H_2O (20 mL), and extracted with $CHCl_3$ (5 × 20 mL). The aqueous phase was evaporated, coevaporated with $CHCl_3$, and loaded onto a silica gel column (40 × 4.5 cm) packed in $CHCl_3$. Elution with 0–15% MeOH in $CHCl_3$ and evaporation of the appropriate fractions afforded, after drying at 0.01 torr for 24 h, a colorless gum[52] (324 mg, 44%): R_f 0.18 (solvent G); ^{1}H NMR (D_2O) δ 1.85 (m, 4, pyran OCH_2's), 3.20 (s, 3, OCH_3), 3.70–4.30 (m, 9, 2-, 3-, and 4-CH's, 5-CH_2, and pyran OCH_2CH_2's), 4.60 (HOD), 5.10 (d, 0.36, J = 6 Hz, 1-CH, rotamer 1), 5.50 (d, 0.64, J = 5 Hz, 1-CH, rotamer 2), 8.10 (s, together with adjacent signal integrates to 1 H, formyl CH, rotamer 2), 8.13 (s, together with adjacent signal integrates to 1 H, formyl CH, rotamer 1). Chemical ionization mass spectrum: m/e 292 (MH^+). High-resolution chemical ionization mass spectrum: exact mass calcd for $C_{12}H_{22}NO_7$ (MH^+) 292.1396, obsd 292.1391.

***N*-[5-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-4′-(4′-methoxytetrahydropyranyl)-β-D-ribofuranosyl]formamide (13).** A mixture of *N*-[2-*O*-(4′-methoxytetrahydropyranyl)-β-D-ribofuranosyl]formamide (230 mg, 0.79 mmol) and imidazole (120 mg, 1.8 mmol) was dried by coevaporation with dry DMF (1 × 5 mL) and dissolved in dry DMF (2.3 mL). To this solution was added freshly distilled *tert*-butyldiphenylsilyl chloride[51] (226 μL, 0.87 mmol), and the resulting solution was desiccated and stirred at room temperature for 6 h. The reaction was then added dropwise to H_2O (30 mL) and the resulting mixture was stirred for 15 min, after which the clear supernatant was removed by decantation. The residual gum was dried in vacuo, dissolved in $CHCl_3$, and applied to a preparative silica gel TLC plate, which was eluted twice with Et_2O. Excision of the UV absorbing band at ca. R_f 0.3 and elution with 10% MeOH in $CHCl_3$ provided the product as a colorless gum (292 mg, 70%) that could not be induced to crystallize: R_f 0.79 (solvent G), 0.16 (Et_2O); ^{1}H NMR ($CDCl_3$–CD_3OD) δ 1.10 (2 close singlets, 9, $C(CH_3)_3$, rotamers 1 and 2), 1.85 (m, 4, pyran OCH_2's), 3.22 (s, 0.37, OCH_3, rotamer 1), 3.27 (s, 0.63, OCH_3, rotamer 2), 3.5–4.4 (m, 9, 2-, 3-, and 4-CH's, 5-CH_2, and pyran OCH_2CH_2's), 5.15 (d, 0.37, J = 7 Hz, 1-CH, rotamer 1), 5.70 (d, 0.63, J = 4 Hz, 1-CH, rotamer 2), 7.40 (m, 6, Ar H), 7.65 (m, 4, Ar H), 7.90 (s, 0.63, formyl CH, rotamer 2), 8.20 (s, 0.37, formyl CH, rotamer

(50) Clark, J. M.; Switzer, R. L. "Experimental Biochemistry"; W. H. Freeman: San Francisco, CA, 1977; pp 166–167.

(51) Obtained from Aldrich Chemical Co.

(52) A chloroform solution of this compound deposited colorless crystals, mp 122–123 °C, on standing at 4 °C for several weeks.

1). Field desorption mass spectrum: m/e 530 (MH^+). High-resolution field desorption mass spectrum: exact mass calcd for $C_{28}H_{40}NO_7Si$ 530.2574, obsd 530.2544.

Anal. Calcd for $C_{28}H_{39}NO_7Si$: C, 63.49; H, 7.43; N, 2.64. Found: C, 63.26; H, 7.20; N, 2.37.

2′,3′-Di-*O*-(*tert*-butyldimethylsilyl)adenosine 5′-Monophosphate Monoimidazolium Salt (15a). A mixture of adenosine 5′-monophosphoric acid (694 mg, 2.0 mmol) and imidazole (2.04 g, 30 mmol) was coevaporated with pyridine (1 × 40 mL) and then partially dissolved in dry DMF (20 mL). To the mixture was added *tert*-butyldimethylsilyl chloride (3.0 g, 20 mmol), and the resulting solution was desiccated and stirred at room temperature for 30 h, after which the reaction was evaporated in vacuo at 30 °C. The resulting oily residue was dissolved completely in pyridine (30 mL); then H_2O (40 mL) was added and the reaction was stirred for ca. 2 min. The solution was extracted with petroleum ether (1 × 100 mL) and EtOAc (3 × 100 mL) and the pooled EtOAc layers were evaporated to dryness; then to a solution of the residue in pyridine (100 mL) was added H_2O (100 mL), and the resulting solution was stirred overnight (14 h). After evaporation to dryness, the residue was coevaporated thoroughly with pyridine (3 × 50 mL) and dissolved completely in dry pyridine (20 mL). A solution of 1:1 ether–acetone (800 mL) was added dropwise over several hours to the vigorously stirred pyridine solution, and the resulting precipitate was collected by centrifugation, washed with ether (3 × 20 mL), and dried in vacuo overnight at 35 °C to afford the product as a white powder (729 mg, 57%): mp 125–128 °C (turns to a glass); R_f 0.7 (solvent D); ^1H NMR ($(CD_3)_2SO$) δ −0.35 (s, 3, $SiCH_3$), −0.10 (s, 3, $SiCH_3$), 0.10 (2 close singlets, 6, 2 $SiCH_3$), 0.70 (s, 9, $C(CH_3)_3$), 0.90 (s, 9, $C(CH_3)_3$), 4.00 (m, 3, 4′-CH and 5′-CH_2), 4.30 (d, 1, J = 4 Hz, 3′-CH), 4.80 (dd, 1, J = 4 and 7 Hz, 2′-CH), 5.85 (d, 1, J = 7 Hz, 1′-CH), 7.20 (br s, 2, exchangeable with D_2O, NH_2), 7.25 (s, 2, imidazolium CH=CH), 8.05 (s, 1, H-2 of adenine), 8.30 (s, 1, imidazolium CH), 8.40 (s, 1, H-8 of adenine), 10.70 (br s, 3, acidic H's).

Anal. Calcd for $C_{25}H_{46}N_7O_7PSi_2$: C, 46.64; H, 7.20; N, 15.23; P, 4.81. Found: C, 46.77; H, 7.28; N, 15.14; P, 4.85.

***N*⁶-[(Dimethylamino)methylene]-2′,3′-di-*O*-(*tert*-butyldimethylsilyl)-adenosine 5′-Monophosphate Pyridinium Salt (16a).** To a solution of 2′,3′-di-*O*-(*tert*-butyldimethylsilyl)adenosine 5′-monophosphate monoimidazolium salt (100 mg, 0.16 mmol) in dry DMF (2.0 mL) was added *N*,*N*-dimethylformamide dimethyl acetal[51] (0.5 mL), and the reaction was allowed to stand at room temperature overnight. After this time, UV spectroscopy demonstrated the complete conversion of starting material (λ_{max} 254 nm, MeOH) to the desired product (λ_{max} 313 nm, MeOH). The solution was evaporated thoroughly at 30 °C, coevaporated with pyridine (2 × 10 mL), and finally passed through a Dowex column (pyr^+ form) at 5 °C using 1:1 pyridine–H_2O as the eluting solvent to afford the pyridinium salt of the fully protected nucleotide in quantitative yield: R_f 0.30 (solvent D), 0.08 (solvent H).

***lin*-Benzoadenosine 5′-Monophosphate Pyridinium Salt (14b).** The preparation of the monophosphate was carried out as previously described,[11] except that the product was isolated by DEAE-Sephadex-chromatography using a linear gradient of 2 L of 0.05 M TEAB → 2 L of 0.5 M TEAB buffer as the eluting solvent. The appropriate fractions were combined, evaporated to dryness, and coevaporated with MeOH (4 × 100 mL) to afford the triethylammonium salt of **14b** as a white solid (77% as determined spectrophotometrically). Passage through a Dowex column (pyr^+ form) and elution with 4:1 H_2O–pyridine provided the required pyridinium salt of *lin*-benzoadenosine 5′-monophosphate, which was used directly in subsequent reactions.

2′,3′-Di-*O*-(*tert*-butyldimethylsilyl)-*lin*-benzoadenosine 5′-Monophosphate Monoimidazolium Salt (15b). A mixture of *lin*-benzo-AMP (216 μmol; **14b**) and imidazole (225 mg, 3.3 mmol) was coevaporated with pyridine (1 × 8 mL) and dry DMF (1 × 8 mL) and then partially dissolved in dry DMF (5 mL). *tert*-Butyldimethylsilyl chloride (324 mg, 2.2 mmol) was added, and the desiccated reaction was stirred at room temperature for 36 h, with care being taken to ensure that all parts of the reaction vessel came in contact with the resulting solution. The light yellow solution was evaporated thoroughly and then the residual oil was dissolved in pyridine (10 mL). This solution was added to H_2O (60 mL) and immediately extracted with petroleum ether (1 × 30 mL), EtOAc (3 × 50 mL), and 20% 1-butanol in EtOAc (3 × 50 mL). The pooled EtOAc layers were evaporated to dryness and dissolved in pyridine (50 mL), H_2O (10 mL) was added, and the reaction was stirred at room temperature for 14 h. The solution was evaporated, coevaporated with dry pyridine (3 × 30 mL), and partially dissolved in pyridine (10 mL). Et_2O (400 mL) was added dropwise with stirring, and the resulting solid was triturated, collected by centrifugation, washed with EtOAc (1 × 25 mL) and Et_2O (2 × 25 mL), and dried in vacuo at 35 °C overnight to afford a cream-colored solid (122 mg, 81%): dec without melting above 185 °C; R_f 0.64 (silica, solvent I); ^1H NMR (CD_3OD) δ −0.80 (s, 3, $SiCH_3$), −0.15 (s, 3, $SiCH_3$), 0.20 (s, 6, 2 $SiCH_3$), 0.70 (s, 9, $C(CH_3)_3$), 0.90 (s, 9, $C(CH_3)_3$), 4.0–5.1 (m, 2′-, 3′-, and 4′-CH's and 5′-CH_2), 5.85 (d, 1′-CH), 7.2–8.9 (m, base and imidazole CH's).

Anal. Calcd for $C_{29}H_{48}N_7O_7PSi_2$: C/N, 3.55. Found: C/N, 3.62.

***N*⁸-[(Dimethylamino)methylene]-2′,3′-di-*O*-(*tert*-butyldimethylsilyl)-*lin*-benzoadenosine 5′-Monophosphate Pyridinium Salt (16b).** Protection of the exocyclic amine of the 2′,3′-bissilyl-*lin*-benzo-AMP (**15b**, λ_{max} 317, 331, 346 nm) was accomplished exactly as described for the corresponding bissilyl-AMP derivative (**16a**) to afford the pyridinium salt of the fully protected nucleotide **16b** in quantitative yield: R_f 0.41 (silica, solvent D); λ_{max} 358 nm (broad, MeOH).

***N*-(β-D-Ribofuranosyl)formamidyl-(3→5′)-adenosine [FpA] (18a).** A mixture of **16a** (51 μmol), **13** (27 mg, 51 μmol), and dry Dowex resin (pyr^+ form, ca. 10 mg) was coevaporated with dry pyridine (5 × 10 mL) and then partially dissolved in dry pyridine (500 μL). *N*,*N*′-Dicyclohexylcarbodiimide[51] (105 mg, 510 μmol) was added, and the desiccated mixture was stirred for 1 h and allowed to stand at room temperature in the dark for 5.5 days. An additional 1.0 mL of dry pyridine was added, and the mixture was swirled and allowed to stand another 2 days. A 1:1 solution of pyridine–H_2O (1.0 mL) was added, and after 2 h the mixture was diluted to 25 mL with MeOH and filtered, concentrated NH_4OH (1.0 mL) was added, and the filtrate was evaporated to dryness. The residue was almost completely dissolved in MeOH (10 mL), concentrated NH_4OH (5 mL) was added, and the resulting mixture was allowed to stand at room temperature for 8 h. The reaction was evaporated to dryness, coevaporated with dry pyridine (3 × 20 mL), and partially dissolved in dry pyridine (5 mL). A solution of 1 M tetrabutylammonium fluoride in THF[51] (765 μL, 5-fold excess) was added, and the solution that resulted within 5 min was allowed to stand desiccated for 5 h, after which TLC on silica using solvent A indicated that desilylation to be complete. The reaction was poured into H_2O (25 mL) and extracted with cyclohexane (3 × 10 mL), and the aqueous layer was passed through a short Dowex column (pyr^+ form) using 1:4 pyridine–H_2O as the eluting solvent. The eluant was evaporated and coevaporated with MeOH (2 × 20 mL), and then the product was extracted from the residual solid with MeOH (50 mL). The solution was evaporated to dryness and then dissolved in 0.01 M HCl (10 mL), and the resulting solution was allowed to stand at room temperature for 3 h. The reaction was neutralized with TEAB buffer and then evaporated, coevaporated with MeOH (2 × 10 mL), and dissolved in cold H_2O (500 mL). Purification by anion-exchange chromatography on a column (21 × 6 cm) of DEAE-Sephadex using a linear gradient of 3 L of 0.005 M TEAB → 3 L of 0.4 M TEAB as the eluting solvent gave a single major UV-absorbing peak, which eluted at a buffer concentration of ca. 0.18 M. The appropriate fractions were pooled and evaporated to dryness, and the residue was further purified by paper chromatography on a 20 × 25 cm sheet of Whatmann 3MM paper and eluting with solvent I. Of the two major UV-absorbing bands (R_f's 0.35 and 0.49), only one (R_f 0.35) gave **3a** on treatment with phosphodiesterase I. Elution with 0.5% concentrated NH_4OH in H_2O provided the desired dinucleoside monophosphate **18a** as a colorless powder after evaporation (3.6 μmol as estimated by UV, 7%): λ_{max}^{pH7} 258 nm; R_f 0.58 (silica, solvent C); relative migration on thin-layer electrophoresis (pH 7.5), 0.59 (5′-AMP = 1.0).

A 12 mM solution of FpA in 50 mM Tris–acetate buffer (pH 8.6) containing 13 mM $MgCl_2$ was treated with phosphodiesterase I from *C. adamanteus*[53] (ca. 0.05 unit in 15-μL total reaction volume). After 25 h at 25 °C, 5′-AMP was the only UV-absorbing species present, as confirmed by electrophoresis and TLC in two solvent systems (R_f 0.58, silica, solvent C; R_f 0.09, cellulose, solvent I). Furthermore, TLC using a glass-backed silica plate, elution with solvent A, and visualization by the H_2SO_4/heat treatment indicated the presence of **3a**, whereas none has been observed before incubation with the enzyme.

***N*-(β-D-Ribofuranosyl)formamidyl-(3→5′)-*lin*-benzoadenosine [Fp-(*lin*-benzo-A)] (18b).** A mixture of **16b** (160 μmol, **13** (270 mg, 510 μmol), and dry Dowex resin (pyr^+ form, ca. 50 mg) was coevaporated with dry pyridine (3 × 8 mL) and then completely dissolved (except for the resin) in dry pyridine (1.0 mL). *N*,*N*′-Dicyclcohexylcarbodiimide[51] (330 mg, 1.6 mmol) was added, and the resulting solution was desiccated, stirred for 1 h, and allowed to stand in the dark at room temperature for 4 days. A 1:1 solution of pyridine–H_2O (4 mL) was added and the resulting mixture was allowed to stand for 4 h, after which concentrated NH_4OH (3 mL) was added. After standing at room temperature for 13 h, the reaction was diluted to 40 mL with MeOH; then the solution was decanted, the resin washed with MeOH, and the combined solution and washings were evaporated to dryness. The residue was coevaporated with dry pyridine (3 × 30 mL) and partially dissolved in dry pyridine (15 mL); then a solution of 1 M tetrabutylammonium fluoride in THF[51] (4.2 mL, 5-fold excess) was added, and the reaction was allowed to proceed with

(53) Obtained from Sigma Chemical Co.

occasional swirling at room temperature. After 4 h, the solution was poured into H_2O (100 mL), and the resulting mixture was extracted with cyclohexane (3 × 50 mL). The aqueous phase was passed through a Dowex column (pyr^+ form) using 4:1 H_2O–pyridine as the eluting solvent, and the eluant was evaporated and coevaporated with MeOH (2 × 100 mL). The residue was dissolved in 0.01 M HCl (300 mL) and the reaction was allowed to stand at room temperature for 6 h, after which the solution was neutralized with 1 M TEAB buffer, evaporated to dryness, coevaporated with MeOH (2 × 200 mL), and dissolved in cold H_2O (500 mL). Purification by anion-exchange chromatography on a column (21 × 6 cm) of DEAE-Sephadex using a linear gradient of 3 L of 0.005 TEAB → 3 L of 0.5 M TEAB followed by 2 L of 1 M TEAB as the eluting solvents gave three major UV-absorbing peaks of approximately equal size, which eluted at ca. 0.3, 0.5, and 0.7 M buffer concentration. The peak that eluted at ca. 0.7 M was determined to be the P–O–P-linked *lin*-benzo-AMP dimer, P^1,P^2-di-*lin*-benzoadenosine 5'-pyrophosphate, based on its characteristic UV absorption spectrum.[11] The peak that had eluted first, i.e., that at ca. 0.3 M TEAB, was evaporated to dryness and coevaporated with MeOH (4 × 100 mL) to provide the desired dinucleoside monophosphate **18b** (35 μmol as estimated by UV using ϵ 10.4 mM^{-1} cm^{-1} at 331 nm; 22%), which was both chromatographically and electrophoretically homogeneous without additional purification: λ_{max}^{pH7} 318, 331, 346 nm; R_f 0.38 (cellulose, solvent I), 0.56 (silica, solvent C); relative migration on thin-layer electrophoresis (pH 7.5), 0.48 (5'-*lin*-benzo-AMP = 1.0).

A 23 mM solution of Fp(*lin*-benzo-A) in 50 mM Tris–acetate buffer (pH 8.6) containing 13 mM $MgCl_2$ was treated with phosphodiesterase I from *C. adamanteus*[53] (ca. 0.05 unit in 15-μL total reaction volume). After 19 h at 25 °C, an additional 0.1 unit of enzyme was added, and after a total of 40 h, 5'-*lin*-benzo-AMP was the only UV-absorbing species present as confirmed by electrophoresis and TLC (R_f 0.06, cellulose, solvent I). Furthermore, TLC using a glass-backed silica plate, elution with solvent A, and visualization by the H_2SO_4/heat treatment indicated the presence of **3a**. A control reaction without added enzyme did not show any notable change in its electrophoresis or TLC characteristics within the time limits of this experiment, nor was the presence of **3a** detected using the same procedure as described above.

Acknowledgment. This work was supported by Research Grant GM 05829 from the National Institutes of Health, U.S. Public Health Service. High-resolution and field desorption mass spectral data were obtained in part under a grant from the National Institute of General Medical Sciences (Grant GM 27029). NMR data were obtained with support from the University of Illinois NSF Regional Instrumentation Facility, Grant No. NSF CHE 79-16100. We thank A. Moder for the preparation of *lin*-benzoadenosine and M. d'Alarcao for assistance in obtaining 360-MHz ^1H and ^{13}C NMR spectra.

Registry No. 3a, 54193-15-6; **3b**, 80963-86-6; **5a**, 58-96-8; **5b**, 32456-54-5; **7**, 80963-87-7; **8**, 80963-89-9; **9**, 80963-91-3; **11**, 80963-92-4; **12**, 80963-93-5; **13**, 80963-94-6; **14a**, 61-19-8; **14b**, 67126-65-2; **15a**, 80963-95-7; **15b**, 80975-53-7; **16a**, 80963-97-9; **16b**, 80963-99-1; **18a**, 80964-01-8; **18b**, 80964-00-7; *N*-[(2,3,5-tri-*O*-(*p*-nitrobenzoyl))-β-D-ribofuranosyl]formamide, 80964-02-9.

Communications to the Editor

On the Migration of a HOOC Group in a Wagner–Meerwein Rearrangement in Superacid Solution: Proof by Double Labeling with Carbon-13

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Received July 20, 1981
Revised Manuscript Received January 25, 1982

1,2 Shifts of electron-withdrawing groups (COR, COOR,[1] COSR, $CONR_2$, $PO(OR)_2$, etc.) toward electron-deficient centers have been observed in several carbenium ion rearrangements, including the pinacol,[2] glycidic ester,[3] semipinacol,[4] dienone–phenol,[5] and Wagner–Meerwein rearrangements.[6-8] We have recently shown by double labeling with ^{13}C that the β-hydroxy esters **1** (**b**, R = Me; **c**, R = Et) undergo Wagner–Meerwein

$$\underset{\mathbf{1}}{\mathrm{PhCHOHCMe_2COOR}} \xrightarrow[\sim 0\ ^\circ C]{HSO_3F} \underset{\mathbf{2}}{\mathrm{PhC(COOR)=CMe_2}}$$

a, R = H; **b**, R = Me; **c**, R = Et

rearrangement to **2b,c** by 1,2 shifts of the alcoxycarbonyl groups exclusively.[9] Whereas migrations of COO^- occur in the benzilic acid[10] and the tertiary ketol[11] rearrangements, no Whitmore-type 1,2 shift of a COOH group seems to be known. *We now report what appears to be the first example of a 1,2 shift of a COOH group (or equivalent) toward an electron-deficient center.*

We dissolved the acid **1a**[9] in HSO_3F and SO_2ClF (1:3) at −100 °C and slowly heated to 0–10 °C, where it was kept until the starting material and its unrearranged derivatives had disappeared from the NMR spectrum; numerous new peaks appeared, among which we observed those belonging to derivatives of **2a**; the same signals were formed from the authentic unsaturated acid **2a** by protonation (in the same medium) [^1H NMR δ 8.0–7.5 (m, 5 H), 2.62 (s, 3 H), 2.07 (s, 3 H); ^{13}C NMR δ 186.9 (C(3)), 180.2 (C(1)), 122.2 (C(2)), 28.9 (Me), 25.5 (Me)] and by subsequent cleavage into the corresponding oxocarbonium ion, Ph-$C(CO^+)$=CMe_2 (3)[9,12] [^1H NMR δ 2.85 (s, 3 H), 2.47 (s, 3 H); ^{13}C NMR δ 94.2 (C(2))].[13,14,16] By quenching and extraction, 40–44% yields

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