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THE SYNTHESIS OF 5′-*C*-ALKYL ANALOGS OF ADENOSINE

THOMAS E. WALKER, HARTMUT FOLLMANN*, AND HARRY P. C. HOGENKAMP
Department of Biochemistry, The University of Iowa, Iowa City, Iowa 52242 (U. S. A.)
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ABSTRACT

Adenosine-5′-carboxaldehyde (**1a**) was treated with nitromethane under alkaline conditions, to give the two stereoisomeric 5′-*C*-(nitromethyl) derivatives (**2** and **3**) of adenosine. Catalytic hydrogenation of **2** gave 9-(6-amino-6-deoxy-β-D-allofuranosyl)adenine (**4**), which, on treatment with nitrous acid, yielded 9-(β-D-allofuranosyl)hypoxanthine (**6**). Similar treatment of **3** gave the α-L-*talo* nucleosides **5** and **7**. Reaction of 2′,3′-*O*-*p*-anisylidene adenosine-5′-carboxaldehyde (**1b**) with ethoxycarbonylmethylene–triphenylphosphorane afforded 9-(ethyl 5,6-dideoxy-β-D-*ribo*-hept-5-enofuranosyluronate)adenine (**8**), which was hydrolyzed to the corresponding uronic acid (**9**). Catalytic hydrogenation of **8** gave 9-(ethyl 5,6-dideoxy-β-D-*ribo*-heptofuranosyluronate)adenine (**10**). Reduction of **8** with lithium aluminum hydride yielded two new analogs of adenosine: 9-(5,6-dideoxy-β-D-*ribo*-heptofuranosyl)adenine (**12**) and 9-(5,6-dideoxy-β-D-*ribo*-hept-5-enofuranosyl)adenine (**13**).

INTRODUCTION

The extremely mild, Pfitzner–Moffatt oxidation of nucleosides to derivatives containing aldehyde or ketone groups in the sugar moiety has provided a completely new approach to the synthesis of nucleoside and nucleotide analogs[1]. For instance, Rosenthal *et al.*[2] treated 9-(3,5-*O*-isopropylidene-β-D-*threo*-2-pentulofuranosyl)-adenine with nitromethane to yield, after removal of the protecting group, 9-[2-(nitromethyl)-β-D-lyxofuranosyl]adenine. Jones and Moffatt[3] described the synthesis of 6′-deoxyhomouridine-6′-phosphonic acid and 6′-deoxyhomoadenosine-6′-phosphonic acid by reaction of the protected nucleoside-5′-carboxaldehydes with a Wittig reagent (diphenyl triphenylphosphoranylidenemethylphosphonate). More recently, Howgate and Hampton[4] prepared the two stereoisomeric 5′-*C*-monomethyl derivatives of adenosine by treating 2′,3′-*O*-isopropylideneadenosine-5′-carboxaldehyde with methylmagnesium iodide.

Analogs of adenosine that have modifications at C-5′ are extremely useful as intermediates in the synthesis of analogs of 5′-deoxyadenosylcobalamin[5], a compound that functions as a coenzyme in reactions involving the transfer of hydrogen between

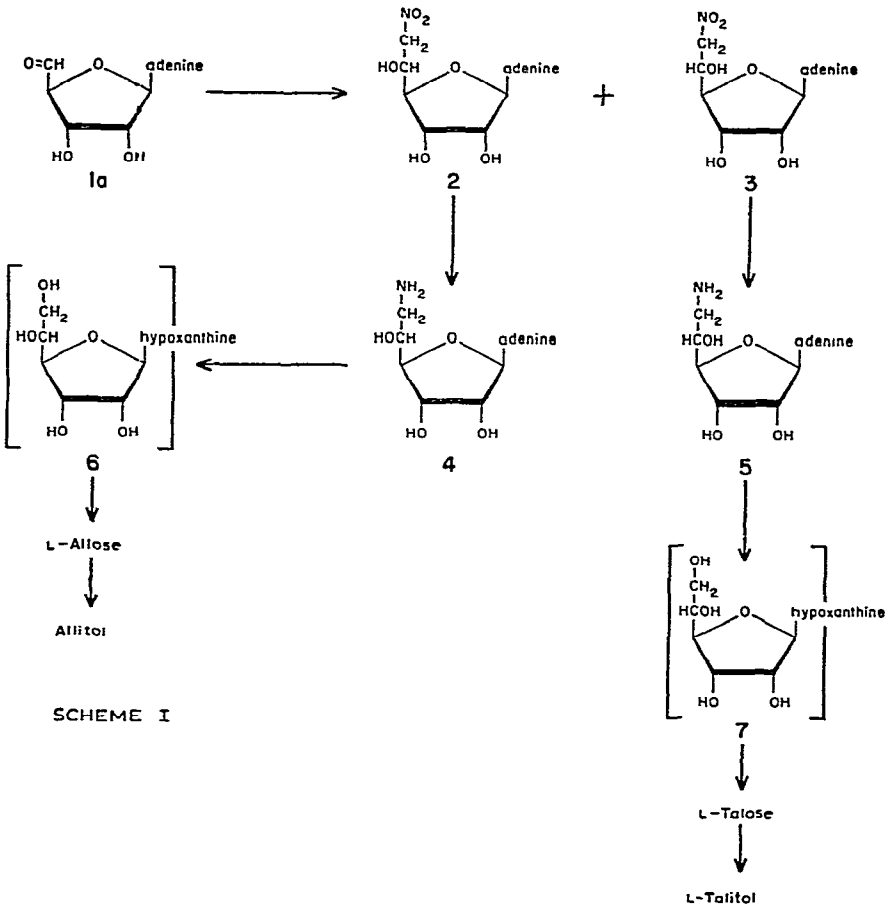
*Present address: Department of Chemistry, Philipps University, 3550 Marburg, Germany.

adjacent carbon atoms of the substrate[6–8], as well as in the reduction of ribonucleotides[9]. In all of these enzymic reactions, the coenzyme functions as an intermediate hydrogen-carrier. Both of the 5′-hydrogen atoms of the coenzyme participate in this process, and thus, any modification at C-5′ should profoundly affect the transfer of hydrogen. Our interest in such analogs of 5′-deoxyadenosylcobalamin led to the synthesis of the analogs of adenosine described herein.

DISCUSSION

Adenosine-5′-carboxaldehyde or its 2′,3′-*O*-*p*-anisylidene derivative were treated with methylmagnesium bromide, nitromethane, or the stabilized Wittig reagent ethoxycarbonylmethylenetriphenylphosphorane. The reaction of 2′,3′-*O*-isopropylideneadenosine-5′-carboxaldehyde with methylmagnesium iodide was described by Howgate and Hampton[4], who isolated the isopropylidene derivatives of 9-(6-deoxy-β-D-allofuranosyl)adenine and 9-(6-deoxy-α-L-talofuranosyl)adenine in 11 and 7% yields, respectively. By treating 2′,3′-*O*-*p*-anisylideneadenosine-5′-carboxaldehyde with an excess of methylmagnesium bromide in boiling tetrahydrofuran, we prepared the corresponding, protected nucleosides. Removal of the protecting group with acetic acid, followed by chromatography on Dowex 1 X-2 (OH^-) ion-exchange resin by the method of Dekker[10] yielded 9-(6-deoxy-β-D-allofuranosyl)adenine and 9-(6-deoxy-α-L-talofuranosyl)adenine, each in a yield of ~5%; these low yields are undoubtedly due to the tendency of nucleoside derivatives to undergo alkaline elimination of the nitrogenous base[11].

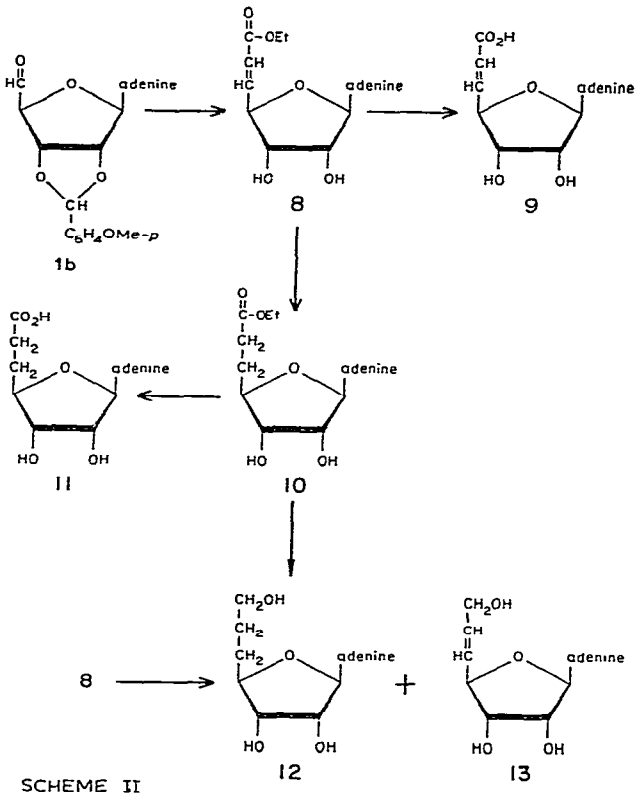
The 5′-*C*-(nitromethyl) derivatives (**2** and **3**) of adenosine were prepared by treating adenosine-5′-carboxaldehyde (**1a**) in 1:1 *p*-dioxane–0.3M aqueous sodium hydroxide with an excess of nitromethane for 8 h at 4° (see Scheme I). Chromatography of the crude reaction-mixture on Dowex 50 afforded two major products that were only poorly resolved; however, one of the products crystallized from the eluate as needles. The other diastereoisomer has not, thus far, crystallized. The configuration at C-5′ of the two products was established by catalytic hydrogenation to the 5′-(aminomethyl) derivatives (**4** and **5**), followed by treatment with nitrous acid. Hydrolysis, with acid, of the 5′-*C*-(hydroxymethyl)inosine isomer (**6**) derived from the crystalline product yielded hypoxanthine and allose, and the isomer derived from the amorphous product (**7**) gave hypoxanthine and talose. The two sugars were identified by paper chromatography, and by gas–liquid chromatography (g.l.c.) of the corresponding hexitol hexaacetates. The gas-chromatographic procedure readily distinguishes between allitol, talitol, glucitol, and mannitol[12]. This sequence of reactions established the identity of the crystalline product as 9-(6-deoxy-6-nitro-β-D-allofuranosyl)adenine (**2**), and that of the amorphous product as 9-(6-deoxy-6-nitro-α-L-talofuranosyl)adenine (**3**). Because neither glucitol nor mannitol could be detected by g.l.c., this reaction sequence also shows that no epimerization had occurred[11] at the C-4′. Attempts to convert the two 5′-*C*-(nitromethyl) derivatives (**2** and **3**) into the 5′-*C*-formyl nucleosides by an acid-catalyzed, Nef reaction were



SCHEME I

unsuccessful; this behavior is probably attributable to steric hindrance, which greatly affects the Nef reaction[13]. Nucleosides **2** and **3** have u.v. absorption spectra similar to that of adenosine; however, in alkaline solution, both nucleosides show a strong, hyperchromic shift. The apparent pK, estimated from the midpoint of the spectral changes, is 9.0. This reversible hyperchromic shift is most probably due to deprotonation of the acidic nitromethyl group in proximity to the purine base. Prolonged incubation of **2** and **3** in alkali causes an irreversible hyperchromic shift probably due to the formation of nitroalkenes. The n.m.r. spectrum of nucleoside **2** is in accord with the structure proposed.

In a second series of reactions (see Scheme II) 2′,3′-*O*-*p*-anisylideneadenosine-5′-carboxaldehyde (**1b**), generated *in situ*, was treated with ethoxycarbonylmethylene-triphenylphosphorane for 36 h at 37°. After removal of the protecting group with 80% acetic acid, one major nucleoside product could be isolated by chromatography on silicic acid. This nucleoside, namely, 9-(ethyl 5,6-dideoxy-β-D-*ribo*-hept-5-eno-furanosyluronate)adenine (**8**) crystallized from ethyl acetate–hexane in 12.5% yield



SCHEME II

(based on the *p*-anisylideneadenosine). Compound **8** is oxidized by sodium periodate, and, during electrophoresis in 0.1M sodium tetraborate, it forms a complex that moves as an anion, indicating the presence of a *cis*-diol grouping. Furthermore, compound **8** has a u.v. absorption spectrum similar to that of adenosine, and its n.m.r. spectrum is in accord with the structure proposed. The n.m.r. data (see Table I) are consistent with the *trans* configuration of the double bond ($J_{5',6'}$ 16 Hz) and with a *trans* relationship for the 3'- and 4'-protons ($J_{3',4'}$ 4 Hz). Howgate *et al.*[14] reported that a similar reaction between 2',3'-*O*-isopropylideneuridine-5'-carboxaldehyde and ethoxycarbonylmethylenetriphenylphosphorane did not yield the unsaturated ester desired.

Hydrogenation of nucleoside **8** in ethanol in the presence of 5% palladium-on-barium sulfate as the catalyst gave 9-(ethyl 5,6-dideoxy-β-D-*ribo*-heptofuranosyluronate)adenine (**10**) in 94% yield. Reduction of nucleoside **10** with lithium aluminum hydride afforded **12** as the major product. Hydrolysis of **8** and **10** with Dowex 1 X-2 (OH^-) ion-exchange resin produced the uronic acids **9** and **11**, respectively. Reduction of nucleoside **8** with lithium aluminum hydride gave two nucleosides (**12** and **13**); these were separable by chromatography on Dowex 2 X-8 (OH^-) by the method of Dekker[10]. Each of these nucleosides has a u.v. spectrum similar to that of adenosine,

TABLE I

N.M.R. SPECTRAL DATA[a]

Compound	*Chemical shifts* (δ)[b,c]								
	H-1′	*H*-2′	*H*-3′	*H*-4′	*H*-5′	*H*-6′	*H*-2	*H*-8	*Other*
9-(6-Deoxy-6-nitro-β-D-allofuranosyl)adenine (**2**)	5.87d $J_{1',2'}$ 7	4.80t	4.28 or 4.52	3.90dd	4.52 or 4.28	4.73 4.45	8.12s	8.34s	
9-(Ethyl 5,6-dideoxy-β-D-*ribo*-hept-5-enofuranosyluronate)-adenine (**8**)	5.98d $J_{1',2'}$ 5	4.74t	4.29t $J_{3',4'}$ 4	4.55t $J_{4',5'}$ 6	7.07dd $J_{5',6'}$ 16	6.05dd $J_{4',6'}$ 1.5	8.12s	8.38s	4.14q ($-CH_2-$) 1.23t ($-CH_3$) $J=7$
9-(5,6-Dideoxy-β-D-*ribo*-hept-5-enofuranosyluronic acid)adenine (**9**)[d]	5.91d $J_{1',2'}$ 5			$J_{4',5'}$ 6	6.54dd $J_{5',6'}$ 16	5.95dd $J_{4',6'}$ 1.5	8.10s	8.28s	
9-(Ethyl 5,6-dideoxy-β-D-*ribo*-heptofuranosyluronate)-adenine (**10**)	5.85d $J_{1',2'}$ 5	4.67t	4.10t $J_{3',4'}$ 4.5	4.33sx	1.93sx $J_{5',6'}$ 7	2.35t	8.15s	8.33s	4.01q ($-CH_2-$) 1.13t ($-CH_3$) $J=7$
9-(5,6-Dideoxy-β-D-*ribo*-heptofuranosyluronic acid)-adenine (**11**)[d]	6.00d $J_{1',2'}$ 5				2.05m or 2.36m	2.36m or 2.05m	8.08s	8.25s	
9-(5,6-Dideoxy-β-D-*ribo*-heptofuranosyl)adenine (**12**)	5.85d $J_{1',2'}$ 5	4.66t	4.05t $J_{3',4'}$ 4	3.83m	1.50m	1.68m	8.12s	8.29s	3.44t (H-7′) $J_{6',7'}$ 5
9-(5,6-Dideoxy-β-D-*ribo*-hept-5-enofuranosyl)adenine (**13**)	5.89d $J_{1',2'}$ 5	4.65t	4.13t $J_{3',4'}$ 5	4.32m	5.82m	5.82m	8.12s	8.27s	3.96m (H-7′)

[a]Spectra of solutions in methyl sulfoxide-d_6, except **9** and **11**, which were dissolved in D_2O and 1 equivalent of NaOD. [b]Chemical shifts were measured with respect to TMS or TSP. [c]Peak multiplicities refer to observed splittings after D_2O exchange. [d]No attempt was made to locate H-2′, -3′, and -4′, which were masked by the HOD peak.

and each contains a *cis*-diol grouping, as indicated by their electrophoretic behavior in sodium tetraborate buffer and by a positive benzidine–periodate reaction[15]. Compound **13** is oxidized by osmium tetraoxide, indicating the presence of a double bond. The position of the double bond was established by treatment with osmium tetraoxide followed by oxidation with sodium periodate; approximately one mole of formaldehyde was formed per mole of unsaturated nucleoside. The n.m.r. spectra of **12** and **13** are also consistent with the structures proposed (see Table I).

EXPERIMENTAL

General methods. — Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee. Melting points were measured on a hot stage equipped with a microscope, and are not corrected. Ultraviolet spectra were recorded on a Cary Model 15 spectrophotometer. Nuclear magnetic resonance spectra were recorded with a Varian HR-220 spectrometer, and tetramethylsilane or sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$ as reference standards. Descending chromatography on Whatman No. 1 paper was conducted with the following solvent systems: solvent I, 4:1:5 butyl alcohol–acetic acid–water; II, 7:1:2 2-propanol–2M ammonium hydroxide–water; III, 5% aq. disodium phosphate saturated with isopentyl alcohol; IV, 43:7 butyl alcohol–water; and V, 3:1:1 butyl alcohol–pyridine–water. Nucleosides on chromatograms were located by their absorption of ultraviolet light, or with 0.5% sodium periodate and 0.5% benzidine solutions[15]. Sugars were located by spraying with a 4% solution of *p*-anisidine hydrochloride in butyl alcohol. G.l.c. was performed on a column (5 ft × 0.125 in.) of poly(ethylene glycol) sebacate on Chromosorb Q maintained at 220°, with helium at 25 ml.min^{-1} as the carrier gas[12]. 2′,3′-*O*-*p*-anisylideneadenosine, 2′,3′-*O*-*p*-anisylideneadenosine-5′-carboxaldehyde (**1b**), and adenosine-5′-carboxaldehyde (**1a**) were prepared by published procedures[16,17].

5′-C-(Nitromethyl) derivatives of adenosine (**2** *and* **3**). — A solution of crude adenosine-5′-carboxaldehyde[17] (**1a**, 2.65 g) in 300 ml of 1:1 peroxide-free *p*-dioxane–0.3M sodium hydroxide was cooled to 4°, treated with cold nitromethane (20 ml), and stirred at 4°. After 8 h, the base was neutralized with M hydrochloric acid, and the solution was concentrated to approximately half its volume. During the evaporation, a precipitate formed; this was removed by filtration, and the filtrate was acidified to pH 2.0, and applied to a column (2.5 × 40 cm) of Dowex 50 X-2 (200–400 mesh, pH 3.0). The column was washed with 2–3 liters of water, and then eluted with 0.03M sodium acetate, pH 5.0. Adenosine was eluted first, followed by the two isomers (**2** and **3**), which were only partially resolved. The latter fractions of this second peak contained a single product which, upon concentration of the eluate, crystallized spontaneously. Recrystallization from water gave 300 mg (0.92 mmole, 17%) of pure isomer **2**, dec. 165°; $\lambda_{max}^{H_2O}$ (at pH 1, 256 nm (ε_{mM} 14.6); at pH 7, 259 nm (ε_{mM} 15.0); and at pH 13, 253 nm (ε_{mM} 21.0).

Anal. Calc. for $C_{11}H_{14}N_6O_6$: C, 40.49; H, 4.29; N, 25.77. Found: C, 40.64; H, 4.37; N, 25.68.

The fractions containing the other isomer (**3**) were pooled, concentrated *in vacuo* to ~10 ml, and acidified to pH 2. This solution was rechromatographed on a column (2.5 × 40 cm) of Dowex 50 X-2 as already described. The fractions comprising the main peak were pooled, acidified to pH 2, and readsorbed on a column (1.0 × 20 cm) of Dowex 50 X-2 (H^+) (200–400 mesh). The column was washed with water, the nucleoside was eluted off with 0.1M pyridine, and the elutate was concentrated *in vacuo*, and lyophilized, to yield 70 mg of **3** as a white powder that has thus far resisted crystallization from a variety of solvents; $\lambda_{max}^{H_2O}$ at pH 1, 257 nm; at pH 7, 259 nm; and at pH 13, 255 nm. Paper chromatography in solvents I and III separated nucleosides **2** and **3**. Nucleoside **2**: R_F (solvent I) 0.49, (solvent III) 0.43; nucleoside **3**: R_F (solvent I) 0.55, (solvent III) 0.49.

*9-(6-Amino-6-deoxy-β-*D*-allofuranosyl)adenine* (**4**). — To a solution in ethanol (200 ml) of 410 mg (1.26 mmoles) of the crystalline isomer later shown to be **2** was added 400 mg of Adams' catalyst, and the mixture was hydrogenated in a Parr apparatus at 50 lb. in.$^{-2}$ for 60 h. The suspension was then filtered, the filtrate concentrated, and the concentrate applied to a column (2.5 × 40 cm) of Dowex 2 X-8 (OH^-) (200–400 mesh). Elution with 60% aqueous methanol, followed by evaporation of the solvent, gave 220 mg (59%) of a new nucleoside; this reacted with ninhydrin, and behaved as a cation during electrophoresis in phosphate buffer, pH 7. It has thus far resisted crystallization, either as the free base or as its hydrochloride; $\lambda_{max}^{H_2O}$ at pH 1, 256 nm; and at pH 13, 259 nm; R_F (solvent I), 0.18, (solvent III) 0.59. By using an identical procedure, nucleoside **3** (50 mg) was hydrogenated to 9-(6-amino-6-deoxy-β-L-talofuranosyl)adenine (**5**); $\lambda_{max}^{H_2O}$ at pH 1, 258 nm; and at pH 13, 260 nm; R_F (solvent I) 0.21, (solvent III) 0.61.

*Characterization of the 5′-*C*-(nitromethyl) derivatives of adenosine* (**2** *and* **3**). — Nucleosides **4** and **5** (derived from **2** and **3**, respectively, by catalytic hydrogenation) were deaminated with nitrous acid by to the method of MacNutt[18]. To a solution of **4** (100 mg, 0.3 mmole) in water (3 ml) were added sodium nitrite (0.75 g) and acetic acid (1.25 ml). After stirring for 6 h at room temperature, an additional 0.2 g of sodium nitrite and 0.75 ml of acetic acid were added, and the mixture was stirred for another 14 h. For desalting, the mixture was treated with acid-washed charcoal (5 g), the suspension was filtered, and the charcoal pad was washed with water. The nucleoside was then eluted with 1:1 ethanol–0.3M ammonium hydroxide, and the eluate was evaporated to dryness *in vacuo*; $\lambda_{max}^{H_2O}$ at pH 1, 251 nm; at pH 7, 249 nm; and at pH 13, 254 nm; R_F (solvent I) 0.23, (solvent III) 0.70.

To 5 ml of a solution containing ~10 mg of **6** was added Dowex 50 X-2 (H^+) (10 ml; 200–400 mesh), and the mixture was heated for 90 min at 100°. The resin was removed by filtration and washed with water, and the filtrate and washing were combined and evaporated to dryness. Paper chromatography in solvent V showed that the reaction product was identical with allose ($R_F = 0.21$). Reduction of the sugar with sodium borohydride gave only allitol, identified by g.l.c. of the peracetate[12] (retention time relative to that of ribitol pentaacetate, 2.17).

By use of an identical procedure, **5** was deaminated to **7**; $\lambda_{max}^{H_2O}$ at pH 1, 248 nm;

at pH 7, 248 nm; and at pH 13, 252 nm; R_F (solvent I) 0.26, (solvent III) 0.71. Hydrolysis of **7** with Dowex 50 (H^+) and paper chromatography in solvent V gave talose (R_F 0.29). Reduction of the sugar with borohydride gave talitol, identified by g.l.c. of the hexaacetate. (Retention time relative to that of ribitol pentaacetate, 2.51).

*9-(Ethyl 5,6-dideoxy-β-*D*-ribo-hept-5-enofuranosyluronate)adenine* (**8**). — 2′,3′-*O*-*p*-Anisylideneadenosine (7.7 g, 20 mmoles) was dissolved in methyl sulfoxide (100 ml) and dry pyridine (1.6 ml; 20 mmoles), and trifluoroacetic acid (0.75 ml; 10 mmoles), and *N,N′*-dicyclohexylcarbodiimide (12.4 g; 60 mmoles) was added. The mixture was kept for 12 h at room temperature, and ethoxycarbonylmethylene-triphenylphosphorane (10 g; 29 mmoles) was then added. After stirring for an additional 24 h at 37°, oxalic acid (7.2 g) was added to decompose the excess of *N,N′*-dicyclohexylcarbodiimide. Ethyl acetate (500 ml) was added, and the dicyclohexylurea was removed by filtration. The filtrate was evaporated to dryness, the residue was dissolved in 80% acetic acid (150 ml), and the solution was kept for 24 h at 37°. The solvent was then evaporated, and the oily residue was partitioned between water and benzene, with filtration. Paper chromatography in solvent IV showed that the aqueous phase contained adenine, adenosine, and a new nucleoside. The aqueous phase was extracted repeatedly with ethyl acetate, and the extracts were combined, dried (sodium sulfate), concentrated to a small volume, and the residue chromatographed on a column (2.5 × 40 cm) of silicic acid (Mallinckrodt). The column was washed with ethyl acetate, and nucleoside (**8**) was eluted with 19:1 ethyl acetate–methanol. Crystallization from ethyl acetate–hexane gave 850 mg (12.5%) of **8**, m.p. 178–183° (softens at 100°); $\lambda_{max}^{H_2O}$ at pH 1, 257 nm (ε_{mM} 15.3); at pH 7, 259 nm (ε_{mM} 15.3); and at pH 13, 259 nm (ε_{mM} 15.6); R_F (solvent I) 0.79, (solvent III) 0.48, (solvent IV) 0.60.

Anal. Calc. for $C_{14}H_{17}N_5O_5$: C, 50.15; H, 5.07; N, 20.90. Found: C, 50.07; H, 5.06; N, 20.77.

*9-(Ethyl 5,6-dideoxy-β-*D*-ribo-heptofuranosyluronate)adenine* (**10**). — Compound **8** (500 mg, 1.5 mmoles) in ethanol (100 ml) was hydrogenated in the presence of 5% palladium-on-barium sulfate (220 mg) for 12 h at room temperature and atmospheric pressure. The catalyst was removed by filtration, and washed with ethanol, and the filtrate and washings were evaporated to dryness. The oily residue crystallized from ethyl acetate–hexane; yield 500 mg (94%); m.p. 86–91°; $\lambda_{max}^{H_2O}$ at pH 1, 257 nm (ε_{mM} 14.9); at pH 7, 259 nm (ε_{mM} 15.1); and at pH 13, 259 nm (ε_{mM} 15.2); R_F (solvent I) 0.75, (solvent III) 0.59, (solvent IV) 0.55.

Anal. Calc. for $C_{14}H_{19}N_5O_5 \cdot H_2O$: C, 47.32; H, 5.92; N, 19.72. Found: C, 47.22; H, 5.52; N, 19.63.

*9-(5,6-Dideoxy-β-*D*-ribo-hept-5-enofuranosyluronic acid)adenine* (**9**). — To a solution of **8** (100 mg, 0.3 mmole) in 60% methanol (25 ml) was added Dowex 1 X-2 (OH^-) (50–100 mesh; 25 ml), and the mixture was stirred for 12 h at room temperature. The resin was then filtered off, and washed with 60% methanol, and **9** was eluted batchwise with five 25-ml portions of 0.1M formic acid. The product crystallized during concentration of the combined eluates *in vacuo* at 45°, and was recrystallized from water; yield 59 mg (65%); dec. 265°; $\lambda_{max}^{H_2O}$ at pH 1, 257 nm (ε_{mM} 14.8); at pH 7,

259 nm (ε_{mM} 15.1); and at pH 13, 259 nm (ε_{mM} 15.1); R_F (solvent I) 0.60, (solvent II) 0.30, (solvent III) 0.54, (solvent IV) 0.01.

Anal. Calc. for $C_{12}H_{13}N_5O_5$: C, 46.91; H, 4.23; N, 22.80. Found: C, 46.72; H, 4.25; N, 22.67.

*9-(5,6-Dideoxy-β-*D*-ribo-heptofuranosyluronic acid)adenine* (**11**). — A solution of **10** (450 mg, 1.27 mmoles) was hydrolyzed with Dowex 1 X-2 (OH^-) as already described. Compound **11** crystallized, and was recrystallized from water; yield 350 mg (82%); m.p. 233–234° (dec.); $\lambda_{max}^{H_2O}$ at pH 1, 257 nm (ε_{mM} 14.4); at pH 7, 259 nm (ε_{mM} 14.8); and at pH 13, 259 nm (ε_{mM} 14.8); R_F (solvent I) 0.57, (solvent II) 0.31, (solvent III) 0.60, (solvent IV) 0.03.

Anal. Calc. for $C_{12}H_{15}N_5O_5$: C, 46.60; H, 4.85; N, 22.66. Found: C, 46.41; H, 4.83; N, 22.46.

*9-(5,6-Dideoxy-β-*D*-ribo-heptofuranosyl)adenine* (**12**) *and 9-(5,6-dideoxy-β-*D*-ribo-hept-5-enofuranosyl)adenine* (**13**). — To a suspension of lithium aluminum hydride (150 mg, 4 mmoles) in dry ether (5 ml) was added a solution of **8** (335 mg, 1 mmole) in dry tetrahydrofuran (25 ml), and the mixture was boiled under reflux for 2.5 h. The excess of the reductant was then decomposed with ethyl acetate (3 ml) and M acetic acid (15 ml). The precipitate was removed by filtration, and washed with tetrahydrofuran and methanol, and the combined filtrate and washings were evaporated to dryness. The residue was dissolved in 60% methanol (5 ml), and the solution made neutral with M sodium hydroxide, and applied to a column (2.5 × 30 cm) of Dowex 2 X-8 (OH^-) (200–400 mesh). Elution with 60% methanol gave two, minor, u.v.-absorbing peaks, followed by two well-resolved, u.v.-absorbing compounds which were eluted in fractions (12 ml) 45–70 and 100–140, respectively. The fractions containing peaks III and IV were separately pooled, evaporated to dryness, and the products crystallized from water.

Material III, 75 mg (25%), had m.p. 179–180°; $\lambda_{max}^{H_2O}$ at pH 1, 257 nm (ε_{mM} 14.6); at pH 7, 259 nm (ε_{mM} 14.8); and at pH 13, 259 nm (ε_{mM} 14.9); R_F (solvent I) 0.54, (solvent III) 0.57, (solvent IV) 0.19.

Anal. Calc. for $C_{12}H_{17}N_5O_4$: C, 48.81; H, 5.76; N, 23.73. Found: C, 48.70; H, 5.97; N, 23.52.

Material IV, 30 mg (10%), had m.p. 187–189°; $\lambda_{max}^{H_2O}$ at pH 1, 257 nm (ε_{mM} 14.5); at pH 7, 259 nm (ε_{mM} 14.8); and at pH 13, 259 nm (ε_{mM} 14.9); R_F (solvent I) 0.49, (solvent III) 0.50, (solvent IV) 0.16.

Anal. Calc. for $C_{12}H_{15}N_5O_4$: C, 49.15; H, 5.12; N, 23.89. Found: C, 48.91; H, 5.16; N, 23.66.

The compounds in peaks III and IV were identified as **12** and **13**, respectively, by their n.m.r. spectra (see Table I). The identity of peak IV as **13** was also established by oxidation with osmium tetraoxide followed by oxidation with sodium periodate; one mole of formaldehyde[19] was formed per mole of IV (*i.e.*, **13**). By using the procedure already described, a solution of **10** (100 mg; 0.28 mmole) in dry tetrahydrofuran (15 ml) was reduced with lithium aluminum hydride (50 mg, 1.3 mmoles) in dry ether (5 ml). The mixture was then applied to a column (2.5 × 50 cm) of Dowex 2

X-8 (OH^-) (200–400 mesh). Elution with 60% methanol gave one major peak in fractions (12 ml) 40–52. These fractions were combined, and evaporated to dryness, and the nucleoside was crystallized from water (60 mg, 69%). This product was identical with **12** obtained by reduction of **8**.

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