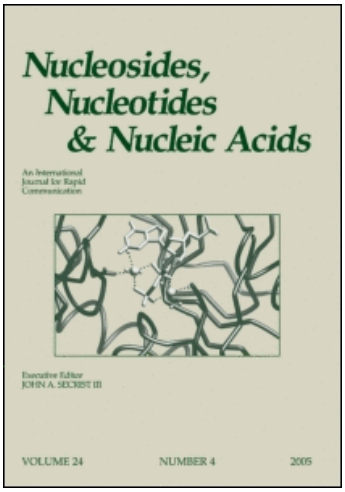

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Synthesis of 5'-Deoxy-5'-nucleosideacetic Acid Derivatives

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SYNTHESIS OF 5'-DEOXY-5'-NUCLEOSIDEACETIC ACID DERIVATIVES

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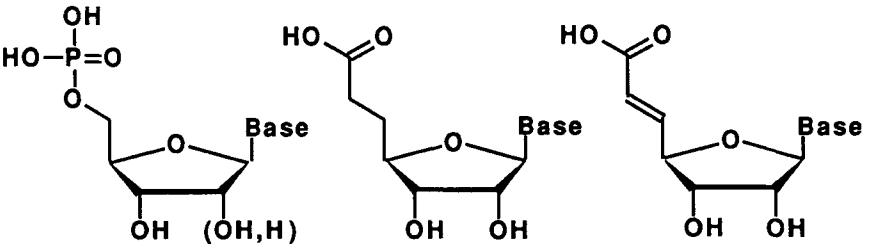
Abstract. The synthesis of several new 5'-deoxy-5'-nucleosideacetic acid derivatives by the reactions of alkoxycarbonylmethylene triphenylphosphoranes with nucleoside 5'-aldehydes is described.

INTRODUCTION

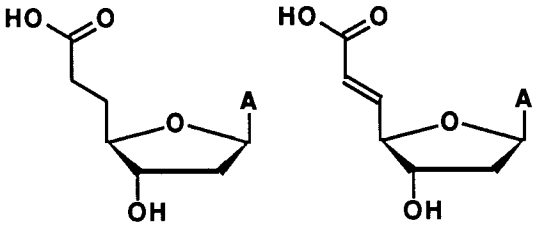
5'-Deoxy-5'-nucleosideacetic acids II-V are isostructural analogs of the natural nucleotides (I) with a carboxylate group in the place of the phosphate group. 5'-Deoxy-5'-adenosineacetic acid (IIa), which was first prepared by Follmann[1] using the Pfitzner-Moffatt oxidation method[2], has been studied as an analog of adenosine-5'-monophosphate (Ia)[3]. The uridylic acid analog IIb has also been prepared[4].

Activated nucleotides, in the presence of complementary oligonucleotide templates, undergo efficient template-directed oligomerization in aqueous solution[5]. In an attempt to evaluate the structural requirements for efficient template-directed oligomerization, the template directed condensation of several nucleotide analogs with altered bases[6] or sugar moieties[7,8] have recently been investigated. In a continuation of this work, we have synthesized the carboxyl analogs of the four ribonucleotides (IIa-d and IIIa-d) and of 2'-deoxyadenylic acid (IVa and Va).

We have studied the oligomerization of adenine derivatives IIa, IIIa, IV and V and guanine derivatives IIc and IIIc in aqueous solution using a water-soluble carbodiimide as a condensing agent. The saturated acid (IV) tends to cyclize to the lactone while IIa and unsaturated acids (IIIa and V) oligomerized efficiently, especially in the presence of poly (U) as a template. Details of the condensation reactions will be published elsewhere[9].



I II a-d III a-d

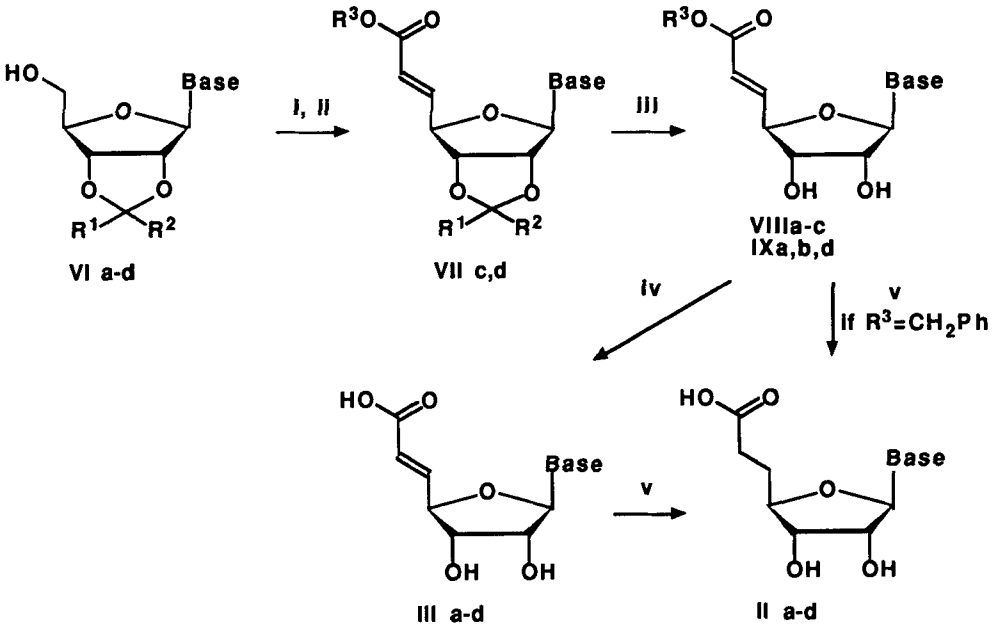


IV V

a: adenine; b: uracil; c: guanine; d: cytosine

RESULTS AND DISCUSSION

Synthesis of adenylic acid analogs IIa and IIIa, and uridylic acid analogs IIb and IIIb were carried out as previously reported[1,4,10] (Scheme 1). The cis-diol was protected by an ethoxymethylene group[9] in the case of adenosine (VIa) or an isopropylidene group[4] in the case of uridine (VIb). 2′,3′-O-Ethoxymethylene adenosine VIa was oxidized to the corresponding 5′-aldehyde by treatment with dimethyl sulfoxide (DMSO) and dicyclohexylcarbodiimide (DCC) in the presence of dichloroacetic acid (Pfitzner-Moffatt oxidation). Ethoxycarbonylmethylene triphenylphosphorane was added to the resulting solution of the 5′-aldehyde. Treatment with aqueous acetic acid or formic acid removed the cis-diol protecting group yielding the α,β-unsaturated acid ethyl ester (VIIIa) which was isolated by silica gel column chromatography in 35% yield (lit.[1] 12.5% yield). Reaction of the 5′-aldehyde of VIa with benzyloxycarbonylmethylene triphenylphosphorane, and removal of the cis-diol protecting group yielded the corresponding benzyl ester IXa in 31% yield (lit.[10] 34.6% yield). Pfitzner-Moffatt oxidation, condensation, and acid hydrolysis of 2′,3′-O-isopropylidene uridine (VIb) was



	R1	R2	R3	Base
VIa	EtO	H	-	adenine
VIb	Me	Me	-	uracil
VIc	Me	Me	-	N-dimethylaminomethyleneguanine
VId	EtO	H	-	N-dimethylaminomethylenecytosine
VIIc	Me	Me	Et	N-dimethylaminomethyleneguanine
VIId	EtO	H	Bzl	N-dimethylaminomethylenecytosine
VIIIa-c	H	H	Et	a: adenine b: uracil c: guanine d: cytosine
IXa,b,d	H	H	Bzl	
IIIa-d	H	H	H	
IIa-d	H	H	H	

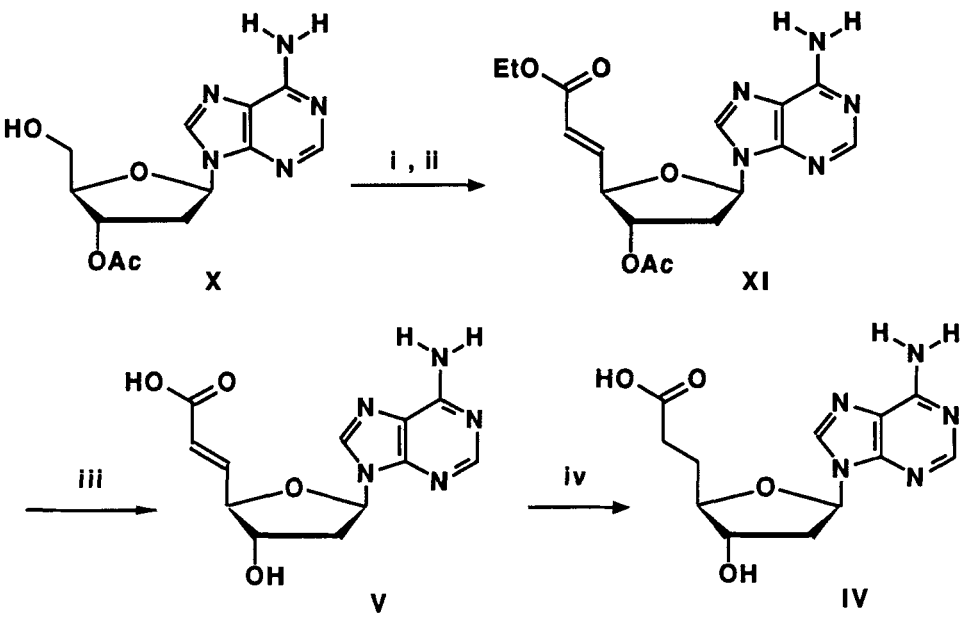
Reagents: i: DMSO, DCC, acid catalyst; ii: $R^3O_2CCH{=}PPh_3$; iii: acid hydrolysis; iv: base hydrolysis; v: H_2/Pd-C.

SCHEME 1

carried out as described above. Condensation of the 5'-aldehyde with ethoxycarbonylmethylene- or benzyloxycarbonylmethylene-triphenylphosphorane resulted in a 44% yield of ethyl ester VIIIb or a 77% yield of the benzyl ester IXb, respectively. The above compounds all showed satisfactory physical properties, with the ^{1}H-NMR data consistent with a *trans* relationship of the olefinic bond. VIIIa and IXa had physical properties identical with those previously reported[1,10].

The reaction described above was extended to the preparation of guanylic acid and cytidylic acid analogs. Pfitzner-Moffatt oxidation of 2',3'-O-isopropylidene guanosine followed by treatment with ethoxycarbonylmethylene triphenylphosphorane did not yield the desired α,β-unsaturated ester. Employing different acid catalysts (trichloroacetic acid, dichloroacetic acid, phosphoric acid, pyridinium trifluoroacetate), increasing the amount of the Wittig reagent (up to 3 equivalents), and protecting the 2-amino group of guanine with a benzoyl group were all unsuccessfull. However, it was found that protection of the 2-amino group of guanine with a dimethylaminomethylene group (VIc) was effective and allowed the protected α,β-unsaturated ester VIIc to be obtained in a 46% yield. Deprotection of the amino protecting group and of the cis-diol protecting group could be carried out simultaneously by hydrolysis with aqueous formic acid. In this way, the α,β-unsaturated ester VIIIc was obtained in an 86% yield. Similarly, oxidation of N^4-dimethylaminomethylene 2',3'-O-isopropylidene cytidine (VId) to the corresponding aldehyde, followed by treatment with benzyloxycarbonylmethylene triphenylphosphorane yielded the protected α,β-unsaturated ester VIId in 34% yield. This compound was extremely labile on silica gel so we believe that the actual yield was substantially higher than the yield observed after chromatography. Removal of the protecting groups with aqueous formic acid yielded α,β-unsaturated ester IXd quantitatively. α,β-Unsaturated esters VIIIc and IXd showed satisfactory physical properties, and the ^{1}H-NMR indicated a *trans* configuration of the double bond ($J_{5',6'}$=16Hz).

Hydrolysis of α,β-unsaturated esters VIIIa-c and IXa,b,d was carried out using a 1M sodium hydroxide/ethanol mixture to give the α,β-unsaturated acids IIIa-d in good yields (50 to 99%). The reactions were monitored by thin layer chromatography and were found to be complete within an hour. Isolation of the resulting acids IIIa-d was carried out by either adjusting the pH of the reaction mixture to approximately 4 and collecting the precipitates thus formed, or by applying the reaction mixture to a column of an anionic exchange resin and eluting with dilute formic acid.



Reagents: i: DMSO, DCC, pyridine, trifluoroacetic acid; ii: $EtO_2CCH{=}PPh_3$; iii: base hydrolysis; iv: H_2/Pd-C.

SCHEME 2

Saturated acids IIa-c were obtained in good yields by hydrogenolysis of α,β-unsaturated acids IIIa-c using 10% palladium-on-carbon as the catalyst. The reactions were carried out at room temperature and atmospheric pressure for up to six hours with monitoring of the consumption of hydrogen using a gas burette. Acids IIa,d were also obtained by hydrogenolysis of α,β-unsaturated acid benzyl esters IXa,d using 10% palladium-on-carbon in a Skita apparatus at 4 atm. and at 50°-60°C for 6 to 8 hours. The acids IIa-d were isolated as in the case of α,β-unsaturated acids IIIa-d.

Syntheses of 2'-deoxyadenylic acid analogs IV and V were attempted in a similar manner by Pfitzner-Moffatt oxidation of 3'-O-acetyl-2'-deoxyadenosine (X), followed by treatment with ethoxycarbonylmethylene triphenylphosphorane (Scheme 2). The nature of the acid catalyst was found to have a large effect on the yield of the α,β-unsaturated ester XI, possibly due to the greater susceptibility of carbon-nitrogen bond cleavage in deoxynucleotides. While only trace amounts of XI could be detected using

dichloroacetic acid, by the use of the milder acid pyridinium trifluoroacetate, XI could be obtained in 25%yield. The ^{1}H-NMR spectra of XI was also consistent with a *trans* relationship of the olefinic bond. Base hydrolysis of XI removed the 3'-O-acetyl protecting group and hydrolyzed the ethyl ester group to yield the unsaturated acid V (77% yield). Hydrogenolysis of the resulting V over palladium-on-carbon proceeded smoothly to yield the saturated acid IV in 93% yield.

EXPERIMENTAL

Melting points were measured on a Laboratory Devices MELTEMP melting point apparatus and are uncorrected. ^{1}H-NMR were obtained on a Nicolet 360MHz FT-NMR using tetramethylsilane as an internal standard unless otherwise stated. UV spectra were obtained on a Beckman Model 35 spectrophotometer. Silica gel column chromatography was performed on Merck, Kiesel gel 60 (230-400 mesh) using the following solvent systems: (A) ethyl acetate - methanol (15:2); (B) ethyl acetate - methanol (10:1); (C) chloroform - methanol (15:2); (D) chloroform - methanol (10:1); (E) chloroform - acetone - methanol (10:5:1). Anion exchange column chromatography was performed on Biorad AG 1-X2, (200-400 mesh, formate form). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee. Mass spectra were obtained from the mass spectrometry facility at the University of California, Riverside.

Reagent grade chemicals were used without further purification. 2',3'-O-Ethoxymethylene adenosine[11], 2',3'-O-isopropylidene uridine[12], 2',3'-O-isopropylidene guanosine[13], N^2-dimethylaminomethylene-2',3'-O-isopropylidene guanosine[14], N^4-dimethylaminomethylene-2',3'-O-ethoxymethylene cytidine[14], and 3'-O-acetyl-2'-deoxyadenosine[15] were prepared according to published procedures.

Benzyloxycarbonylmethylene triphenylphosphorane was prepared by heating a solution of benzylbromoacetate (10 mmol) and triphenyl phosphine (11 mmol) in acetonitrile (100 ml) at reflux for 30 minutes and then neutralizing with a 1 M methanolic sodium methoxide solution (10 ml)[4]. The resulting solution was used directly for reaction with nucleoside-5'-aldehydes.

Ethyl 1,5,6-trideoxy-1-(adenin-1-yl)-β-D-ribo-hept-5-enofuranuronate (VIIIa).

Dichloroacetic acid (5 mmol) was added to a solution of 2',3'-O-ethoxymethylene adenosine (VIa) (10 mmol) and N,N'-dicyclohexyl carbodiimide (DCC) (30 mmol) in dry dimethylsulfoxide (DMSO) (50 ml). The mixture was stirred at room temperature, and

the extent of the reaction monitored by thin layer chromatography (TLC). When the starting material was practically all consumed (3 hours), pyridine (10 mmol) was added to neutralize the acid catalyst. Ethoxycarbonylmethylene triphenylphosphorane (11 mmol) was added to this mixture, and the reaction mixture was stirred overnight at room temperature. The solution was then diluted with ethyl acetate (200 ml). Oxalic acid (40 mmol) was added at 0°C to quench excess DCC, and the resulting solution was stirred for another 30 minutes. Precipitated N,N'-dicyclohexylurea was removed by filtration. The filtrate was diluted with ethyl acetate (300 ml), washed twice with a saturated sodium hydrogen carbonate solution (250 ml), dried over anhydrous magnesium sulfate, and evaporated to dryness *in vacuo*. The resulting residue was redissolved in 80% aqueous acetic acid (200 ml) to remove the diol protecting group and left overnight at 37°C. The solvent was removed by evaporation *in vacuo*. The residue was then dissolved in ethyl acetate (300 ml) and washed twice with a saturated bicarbonate solution (100 ml). The ethyl acetate layer was dried over anhydrous magnesium sulfate and the solvent was evaporated *in vacuo*. Silica gel column chromatography was performed using solvent system (A). 35% yield. m.p. 174-175°C (from ethyl acetate) (lit.[1] 178-183°C). ^{1}H-NMR (δ, DMSO-d_6) 1.22 (t, 3H, J=7Hz), 4.14 (q, 2H, J=7Hz), 4.28 (m, 1H), 4.54 (m, 1H), 4.74 (m, 1H), 5.97 (d, 1H, J=5Hz), 6.04 (dd, 1H, J=16 and 1Hz), 7.06 (dd, 1H, J=16 and 6Hz), 7.33 (br s, 2H), 8.15 (s, 1H), and 8.37 ppm (s, 1H).

Benzyl 1,5,6-trideoxy-1-(adenin-1-yl)-β-D-ribo-hept-5-enofuranuronate (IXa).

Pfitzner-Moffatt oxidation of 2',3'-O-ethoxymethylene adenosine (VIa) (10 mmol) was carried out as described above. The resulting solution was treated with benzyloxycarbonylmethylene triphenylphosphorane (11 mmol) and allowed to stir overnight at room temperature. The cis-diol protecting group was hydrolyzed with acid and the product worked up as described above. The residue was applied to a silica gel column and eluted with solvent system (A). 31% yield (from VIa). m.p. 145-147°C (from ethyl acetate) (lit[6] 154-155°C). ^{1}H-NMR (δ, DMSO-d_6) 3.39 (br s, 4H), 4.30 (m, 1H), 4.57 (m, 1H), 4.77 (m, 1H), 5.18 (s, 2H), 5.98 (d, 1H, J=5Hz), 6.12 (d, 1H, J=5Hz), 7.14 (dd, 1H, J=6 and 16Hz), 8.3-8.5 (m, 5H), 8.12 (s, 1H), and 8.38 ppm (s, 1H).

Ethyl 1,5,6-trideoxy-1-(uracil-1-yl)-β-D-ribo-hept-5-enofuranuronate (VIIIb).

Pfitzner-Moffatt oxidation of 2',3'-O-isopropylidene uridine (VIb) (1.42 g, 5 mmol) was carried out as described above. The resulting solution was treated with ethoxycar-

bonylmethylene triphenylphosphorane (5 mmol) and allowed to stir at room temperature overnight. After workup of the solution as described above, the cis-diol protecting group was removed by dissolving the residue in 90% aqueous formic acid and allowing the solution to stand overnight at 37°C. The solvent was removed by evaporation *in vacuo*. The residue was dissolved in ethyl acetate (200 ml), washed twice with saturated bicarbonate solution (50 ml), dried over anhydrous magnesium sulfate, and the solvent was removed *in vacuo*. Silica gel column chromatography was performed using solvent system (D). 44% yield. m.p. 161°C (from ethyl acetate). ^{1}H-NMR (δ, DMSO-d_6) 1.23 (t, 3H, J=7Hz), 3.37 (br s, 2H), 3.96 (d, 1H, J=5Hz), 4.15 (q, 2H, J=7Hz), 4.39 (t, 1H, J=6Hz), 5.66 (d, 1H, J=8Hz), 5.76 (d, 1H, J=4Hz), 6.04 (dd, 1H, J=16 and 1Hz), 6.99 (dd, 1H, J=16 and 6Hz), 7.66 (d, 1H, J=8Hz), and 11.40 ppm (br s, 1H). $C_{13}H_{16}N_2O_7$ requires %C; 50.00, %H; 5.17, %N; 8.97. Found: %C; 50.00, %H; 5.09, %N; 8.80.

Benzyl 1,5,6-trideoxy-1-(uracil-1-yl)-β-D-ribo-hept-5-enofuranuronate (IXb).

Pfitzner-Moffatt oxidation, condensation and removal of the cis-diol protecting group of 2',3'-O-isopropylidene uridine (VIb) (1.42 g, 5 mmol) was carried out as described above for the synthesis of VIIIb, except that benzyloxycarbonylmethylene triphenylphosphorane was used instead of ethoxycarbonylmethylene triphenylphosphorane. Silica gel column chromatography was performed using solvent system (D). 71% yield. m.p. 139-141°C (from ethyl acetate). ^{1}H-NMR (δ, DMSO-d_6) 3.37 (br s, 2H), 3.97 (m, 1H), 4.15 (m, 1H), 4.40 (t, 1H, J=6Hz), 5.17 (s, 2H), 5.68 (d, 1H, J=8Hz), 5.77 (d, 1H, J=3Hz), 6.11 (d, 1H, J=16Hz), 7.04 (dd, 1H, J=16 and 6Hz), 7.39 (m, 5H), and 7.66 ppm (d, 1H, J=8Hz). $C_{18}H_{18}N_2O_7$ requires %C; 57.75, %H; 4.85, %N; 7.49. Found: %C; 57.65, %H; 4.75, %N; 7.33.

Ethyl 1,5,6-trideoxy-1-(guanin-1-yl)-β-D-ribo-hept-5-enofuranuronate (VIIIc).

To a solution of N^2-dimethylaminomethylene-2',3'-O-isopropylideneguanosine (VIc) (1.3 mmol) and DCC (0.81 g, 3.9 mmol) in DMSO (10 ml) was added pyridine (0.11 ml, 1.3 mmol) followed by trifluoroacetic acid (0.06 ml, 0.7 ml) at room temperature. The solution was stirred for 5 hours. Ethoxycarbonylmethylene triphenylphosphorane (0.52 g, 1.5 mmol) was added and the mixture was stirred overnight. The reaction mixture was then diluted with ethyl acetate (50 ml), and cooled to 0°C. Oxalic acid (0.6 g) was added and the reaction mixture was stirred for a further 30 minutes. The resulting precipitate was filtered off, and the filtrate diluted with ethyl acetate

(80 ml), washed twice with saturated bicarbonate solution (50 ml), and dried over anhydrous magnesium sulfate. The solvent was then evaporated *in vacuo*. The protected α,β-unsaturated acid ester VIIc was isolated by silica gel column chromatography. It was necessary to isolate the protected derivative at this stage in the synthesis since the unprotected ester is sparingly soluble in organic solvents. Ethyl 2',3'-O-isopropylidene-1',5',6'-trideoxy-1-(N^2-dimethylaminomethylene guanin-1-yl)-β-D-ribo-hept-5-enofuranuronate (VIIc): VIIc was isolated by silica gel column chromatography first using solvent system (B), then on a separate column using solvent system (A). 46% yield. ^{1}H-NMR (δ, $CDCl_3$) 1.24 (t, 3H, J=7Hz), 1.40 (s, 3H), 1.64 (s, 3H), 3.12 (s, 3H), 3.17 (s, 3H), 4.15 (q, 2H, J=7Hz), 4.81 (m, 1H), 4.95 (dd, 1H, J=4 and 6Hz), 5.25 (dd, 1H, J=2 and 6Hz), 5.98 (dd, J=2 and 16Hz), 6.09 (d, 1H, J=2Hz), 7.05 (dd, 1H, J=5 and 16Hz), 7.62 (s, 1H), 8.49 (s, 1H), and 9.52 ppm (br s, 1H, D_2O exchangeable).

The 2-amino and cis-diol protecting groups were removed from VIIc by dissolving in 80% formic acid (25 ml) and allowing the solution to stand overnight at 37°C. The solvent was removed by evaporation *in vacuo*. Recrystallization of the residue from ethanol gave VIIIc in 86% yield from VIIc. m.p. 225°C (decomposition). ^{1}H-NMR (δ, DMSO-d_6) 1.22 (t, 3H, J=7Hz), 4.14 (q, 2H, J=7Hz), 4.1-4.2 (m, 1H), 4.5 (m, 2H), 5.77 (d, 1H, J=5Hz), 6.02 (dd, 1H, J=16 and 1Hz), 6.75 (br s, 2H), 7.03 (dd, 1H, J=16 and 6Hz), 7.92 (s, 1H), and 8.36 ppm (s, 1H). $C_{14}H_{17}N_5O_6 + 0.5H_2O$ requires %C; 47.25, %H; 4.96, %N; 19.68. Found: %C; 46.82, %H; 4.83, %N; 20.11.

Benzyl 1,5,6-trideoxy-1-(cytosin-1-yl)-β-D-ribo-hept-5-enofuranuronate (IXd).

Dichloroacetic acid (0.19 g, 1.5 mmol) in DMSO (5 ml) was added to a solution of N^4-dimethylaminomethylene-2',3'-O-ethoxymethylene cytidine (1.06 g, 3 mmol) and DCC (1.86 g, 9 mmol) in DMSO (10 ml) with stirring. After 2 hours, pyridine (0.3 ml), followed by a freshly prepared solution of benzyloxycarbonylmethylene triphenylphosphorane (4.5 mmol) was added. After the usual workup, isolation of VIId was attempted by silica gel column chromatography using solvent system (B) as eluent. 494 mg of a mixture of products containing VIId was obtained. Since repeated attempts at purification using column chromatography on silica gel always gave a similar mixture of products, further isolation of compound VIId was not attempted. ^{1}H-NMR of the mixture of products indicated the presence of a dimethylamino group (δ, 2.85 and 2.91), a pair of ethyl groups, the methylene of a benzyl group (δ, 5.15), cytosine (δ, 5.73, the signal due

to the methene proton of the ring could not be identified), an olefinic group (δ, 7.0), and a phenyl group (δ, 7.5).

Compound VIId was treated with 80% aqueous acetic acid at 37°C overnight. The solvent was removed by evaporation *in vacuo*, the resulting residue was redissolved in ethyl acetate (100 ml) and washed with saturated sodium hydrogen carbonate (50 ml) to remove any excess acid. The solution was dried over anhydrous magnesium sulfate, and the solvent was evaporated *in vacuo*. The ^{1}H-NMR of the residue indicated the presence of the methylene of a benzyl group (δ, 5.20), cytosine (δ, 5.76, the signal due to the methene proton of the ring could not be identified), an olefinic group (δ, 6.12 and 7.06), and a phenyl group (δ, 7.5). The crude IXd obtained was used for subsequent reactions without further purification.

Ethyl 3-O-acetyl-1,2,5,6-tetradeoxy-1-(adenin-1-yl)-β-D-erythro-hept-5-enofuranuronate (XI).

To a solution of 3'-O-acetyl adenosine (X) (0.88 g, 3 mmol) and DCC (1.86 g, 9 mmol) in DMSO (10 ml) was added dry pyridine (0.24 ml, 3 mmol) followed by trifluoroacetic acid (0.12 ml, 1.5 mmol). The solution was stirred for 2 hours at room temperature. Ethoxycarbonylmethylene triphenylphosphorane (1.05 g, 3 mmol) was added and the reaction was allowed to stir at room temperature for 20 hours. The reaction mixture was worked up in the usual manner and XI was isolated by silica gel column chromatography first using solvent system (A), and then using solvent system (C) on a separate column. 25% yield (liquid). ^{1}H-NMR (δ, $CDCl_3$) 1.30 (t, 3H, J=7Hz), 2.16 (s, 3H), 2.60 (m, 1H), 3.02 (m, 1H), 4.21 (q, 2H, J=7Hz), 4.75 (m, 1H), 5.36 (m, 1H), 5.97 (br s, 2H), 6.12 (dd, 1H, J=16 and 2Hz), 6.50 (dd, 1H, J=9 and 6 Hz), 7.14 (dd, 1H, J=16 and 5Hz), 7.97 (s, 1H), and 8.37 ppm (s, 1H).

General procedure for the base hydrolysis of α,β-unsaturated esters (VIII, IX and XI) to α,β-unsaturated acids (III and V).

The α,β-unsaturated ester (VII, VIII or XI) (1 mmol) was suspended in 1 M aqueous sodium hydroxide (5 ml) and ethanol was added with stirring until the substrate dissolved. The reaction mixture was left at room temperature for up to six hours (the reaction was monitored by thin layer chromatography and found to be practically over in an hour) and worked up by either of the following methods. (A) The

reaction mixture was applied to an anion exchange column. The column was first eluted extensively with water. The acid was collected by successively eluting with 0.3 M aqueous formic acid, and then with 3.0 M aqueous formic acid, monitoring by UV at 254 nm. (B) The pH of the reaction mixture was adjusted to pH 4 using 1 M hydrochloric acid causing the pure acid to precipitate.

1,5,6-Trideoxy-1-(adenin-1-yl)-β-D-ribo-hept-5-enofuranuronic acid (IIIa).

82% yield (workup by method B from IXa). m.p. 247°C (decomposition) (from H_2O) (lit.[1] 265°C (decomposition)). ^{1}H-NMR (δ, NaOH-D_2O) 4.27 (t, 1H, J=5Hz), 4.66-4.70 (m, 2H), 6.00 (d, 1H, J=5Hz), 6.08 (d, 1H, J=16Hz), 6.77 (dd, J=16 and 5Hz), 8.14 (s, 1H), and 8.32 ppm (s, 1H); (δ, DMSO-d_6) 3.39 (br), 4.28 (m, 1H), 4.52 (m, 1H), 4.72 (m, 1H), 5.96 (d, 1H, J=16Hz), 5.97 (d, 1H, J=5Hz), 6.98 (dd, 1H, J=6 and 16Hz), 7.34 (br s, 2H), 8.17 (s, 1H), and 8.39 ppm (s, 1H).

1,5,6-Trideoxy-1-(uracil-1-yl)-β-D-ribo-hept-5-enofuranuronic acid (IIIb).

99% yield (workup by method A from IXb). m.p. 95-98°C (hygroscopic). ^{1}H-NMR (δ, NaOH, D_2O) 4.19 (m, 1H), 4.35 (m, 1H), 4.58 (m, 1H), 5.84 (d, 1H, J=8Hz), 5.97 (m, 1H), 6.15 (d, 1H, J=16Hz), 6.66 (dd, 1H, J=7 and 16Hz), 7.58 ppm (d, 1H, J=8Hz); (δ, DMSO-d_6) 3.5 (br), 3.95 (t, 1H, J=5Hz), 4.14 (t, 1H, J=5Hz), 4.38 (t, 1H, J=6Hz), 5.67 (dd, 1H, J=2 and 8Hz), 5.77 (d, 1H, J=4Hz), 5.96 (d, 1H, J=16Hz), 6.92 (dd, 1H, J=6 and 16Hz), 7.64 (d, 1H, J=8Hz), 8.14 (s, 1H), and 11.38 ppm (s, 1H). $C_{11}H_{12}N_2O_7$ + 1.1 H_2O requires %C; 43.45, %H; 4.71, %N; 9.22. Found: %C; 43.71, %H; 4.29, %N; 8.60.

1,5,6-Trideoxy-1-(guanin-1-yl)-β-D-ribo-hept-5-enofuranuronic acid (IIIc).

50% yield (workup by method A from VIIIc). m.p. >300°C (gradual decomposition) (from H_2O). ^{1}H-NMR (δ, DMSO-d_6) 4.16 (m, 1H), 4.48-4.53 (m, 2H), 5.85 (d, 1H), 5.99 (dd, 1H, J=16 and 1Hz), 7.00 (dd, 1H, J=16 and 6Hz), 8.79 (s, 1H), and 11.46 ppm (s, 1H, D_2O exchangeable). $C_{12}H_{13}N_5O_6$ + 1.5 H_2O requires %C; 41.14, %H; 4.60, %N; 19.99. Found: %C; 41.43, %H; 4.52, %N; 20.09.

1,5,6-Trideoxy-1-(cytosin-1-yl)-β-D-ribo-hept-5-enofuranuronic acid (IIId).

69% yield from VIId without purification of the intermediate α,β-unsaturated ester IXd (workup by method B). UV (λ_{max} in H_2O (ε)) at pH 1, 280 nm (11.5 x 10^3); pH

7.4, 271 nm (8.8 x 10^3); pH12, 273 nm (7.9 x 10^3). m.p. 250°C (decomposition) (from H_2O). ^{1}H-NMR (δ, NaOH, D_2O) 4.06 (m, 1H), 4.18 (m, 1H), 5.89 (d, 1H, J=3Hz), 6.07 (d, 1H, J=8Hz), 6.12 (d, 1H, J=16Hz), 6.70 (dd, 1H, J=16 and 6Hz), and 7.69 ppm (d, 1H, J=8Hz). $C_{11}H_{13}N_3O_6$ requires %C; 46.64, %H; 4.63, %N; 14.84. Found: %C; 46.11, %H; 4.67, %N; 14.62.

1,2,5,6-Tetradeoxy-1-(adenin-1-yl)-β-D-erythro-hept-5-enofuranuronic acid (V).

77% yield (from XI, workup by method B). m.p. 172-173°C (from H_2O). UV (λ_{max} in H_2O (ε)) at pH 1, 262 nm (11.7 x 10^3); pH 7.4, 260 nm (13.5 x 10^3); pH12, 260 nm (14.3 x 10^3). ^{1}H-NMR (δ, DMSO-d_6) 2.3-2.4 (m, 2H), 2.9-3.0 (m, 2H), 4.4-4.6 (m, 2H), 5.7 (br s, 1H), 5.86 (dd, 1H, J=16 and 1Hz), 6.41 (t, 1H, J=6.7Hz), 6.69 (dd, 1H, J=16 and 6Hz), 7.3 (br s, 2H), 8.16 (s, 1H), and 8.35 ppm (s, 1H). $C_{12}H_{13}N_5O_4 + H_2O$ requires %C; 46.60, %H; 4.89, %N; 22.65. Found: %C; 46.45, %H; 4.88, %N; 22.35. MS (MH+) 292.

Hydrogenolysis of the benzylesters IXa,d to acids IIa,d.

The α,β-unsaturated acid benzyl ester (IXa,d) (1 mmol) and 10% palladium on carbon (400 mg) in a mixture of nine parts of 95% ethanol and one part of acetic acid (v/v) was shaken at 4 atmospheres at 50 to 60°C in a Skita apparatus for 6 to 8 hours. The reaction mixture was filtered over celite, and the precipitates were washed with hot water (50 ml, twice). The insoluble material and celite were further extracted with hot water (100 ml) and filtered. The combined filtrates were evaporated *in vacuo* to yield the almost pure acid. Acids IIa,d were suspended in 5 ml of water and dissolved by titrating with 1m sodium hydroxide. The pure acid precipitated by adjusting the pH of the solution to about 4 using 1 M aqueous hydrochloric acid.

1,5,6-Trideoxy-1-(adenin-1-yl)-β-D-ribo-heptofuranuronic acid (IIa).

76% yield. m.p. 235-237°C (from H_2O) (lit.[6] 242-243°C, lit.[1] 233-234°C). ^{1}H-NMR (δ, DMSO-d_6) 1.90 (m, 2H), 2.29 (m, 2H), 3.36 (br), 3.86 (m, 1H), 4.07 (t, 1H, J=5Hz), 4.65 (t, 1H, J=5Hz), 5.84 (d, 1H, J=5Hz), 7.29 (br s, 2H), 8.14 (s, 1H), and 8.33 ppm (s, 1H).

1,5,6-Trideoxy-1-(cytosin-1-yl)-β-D-ribo-heptofuranuronic acid (IId).

74% yield from VIId without purification of the intermediate α,β-unsaturated ester. m.p. 230°C (decomposition) (from H_2O). UV (λ_{max} in H_2O (ε)) at pH 1, 280 nm

(11.4 x 10^3); pH 7.4, 272 nm (7.3 x 10^3); pH12, 273 nm (7.3 x 10^3). ^{1}H-NMR (δ, NaOH-D_2O) 1.91 (m, 1H), 2.03 (m, 1H), 2.32 (m, 2H), 3.87 (m, 2H), 4.15 (m, 1H), 5.78 (d, 1H, J=5Hz), 6.06 (d, 1H, J=8Hz), and 7.71 ppm (d, 1H, J=8Hz). $C_{12}H_{15}N_5O_6$ + H_2O requires %C; 40.91, %H; 5.15, %N; 19.88. Found: %C; 40.78, %H; 4.94, %N; 19.79.

General procedure for the hydrogenolysis of α,β-unsaturated acids IIIa-c and V to saturated acids IIa-c and IV.

10% palladium on carbon (100 mg) was added to the substrate (1 mmol) in 90% aqueous acetic acid (9:1). The solution was stirred in the presence of hydrogen in a gas burette at atmospheric pressure until absortion of the hydrogen ceased (up to 6 hr). The reaction mixture was then passed through celite and the solvent removed by evaporation *in vacuo*. The acids were isolated by: (A) dissolving the residue in dilute alkali and applying to an anion exchange column, then eluting stepwise with water, 0.3 M aqueous formic acid, and 3 M aqueous formic acid. The UV absorbing component was collected and the solvent was removed by evaporation *in vacuo* to yield the acid as a solid. (B) suspending the residue in 5 ml of water and dissolving by titrating with 1 M sodium hydroxide. The acid precipitated on adjusting the pH of the solution to about 4 using 1 M aqueous hydrochloric acid.

1,5,6-Trideoxy-1-(uracil-1-yl)-β-D-ribo-heptofuranuronic acid (IIb).

75% yield (workup by method A). m.p. 114-115°C (ref.[4] 115-117°C). ^{1}H-NMR (δ, DMSO-d_6) 1.8-2.0 (m, 2H), 2.25 (m, 2H), 3.77 (m, 2H), 4.05 (t, 1H, J=5Hz), 5.64 (d, 1H, J=8Hz), 5.69 (d, 1H, J=5Hz), 7.80 (d, 1H, J=8Hz), and 8.42 ppm (br s, 0.5H).

1,5,6-Trideoxy-1-(guanin-1-yl)-β-D-ribo-heptofuranuronic acid (IIc).

82% yield (workup by method A). m.p. >300°C (gradual decomposition) (from H_2O). NMR (δ, DMSO-d_6) 1.8-1.9 (m, 2H), 2.2-2.3 (m, 2H), 3.8 (m, 1H), 3.95 (t, 1H, J=5Hz), 4.43 (t, 1H, J=5Hz), 5.83 (d, 1H, J=5Hz), 6.62 (br s, 2H, D_2O exchangeable), and 7.86 ppm (s, 1H). $C_{12}H_{15}N_5O_6$ + 1.5 H_2O requires %C; 40.91, %H; 5.15, %N; 19.88. Found: %C; 40.78, %H; 4.94, %N; 19.79.

1,2,5,6-Tetradeoxy-1-(adenin-1-yl)-β-D-erythro-heptofuranuronic acid (IV).

93% yield (workup by method B). m.p. 159-162°C (from H_2O). UV (λ_{max} in H_2O (ε)) at pH 1, 262 nm (12.0 x 10^3); pH 7.4, 260 nm (13.9 x 10^3); pH12, 260 nm (14.7 x 10^3). ^{1}H-

NMR (δ, DMSO-d_6) 1.9 (m, 2H), 2.3 (m, 3H), 2.8 (m, 1H), 3.78 (m, 1H), 4.30 (m, 1H), 6.29 (t, 1H), 7.28 (s, 2H, D_2O exchangeable), 8.14 (s, 1H), and 8.30 ppm (s, 1H); (d, D2O-NaOH) 1.8-1.9 (m, 2H), 2.2-2.3 (m, 2H), 2.5-2.6 (m, 1H), 2.7-2.8 (m, 1H), 3.95 (m, 1H), 4.42 (m, 1H), 6.36 (t, 1H, J=6Hz), 8.14 (s, 1H), and 8.29 ppm (s, 1H). $C_{12}H_{15}N_5O_4$ +1.5 H_2O requires %C; 44.99, %H; 5.66, %N; 21.87. Found: %C; 45.28, %H; 5.34, %N; 21.81. MS (MH+) 294.

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