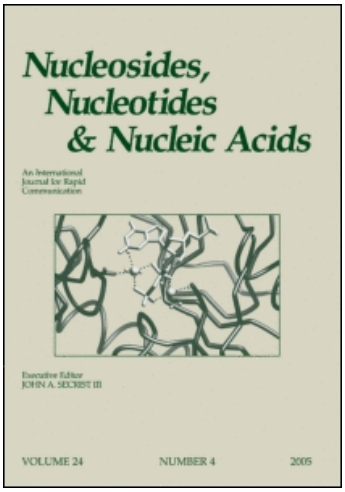

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Synthesis of Novel Isocytosine Pseudonucleotide Analogues

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SYNTHESIS of NOVEL ISOCYTOSINE PSEUDONUCLEOTIDE ANALOGUES

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Abstract: Ethyl dialkylphosphonoacetates were prepared from the corresponding dimethylalkylphosphites via the Arbuzov reaction with ethyl bromoacetate. The phosphonoacetates so produced were converted into enaminoacetates by reaction with DMF dimethylacetal and these were used as bidentate electrophiles for the synthesis of phosphonopyrimidones. Several of these compounds were tested for biological activity but none were found to possess antiviral activity.

Introduction

The recent publication of the synthesis of phosphonate substituted heterocycles from functionalized phosphonates[2] suggested to us a method for preparing unusual nucleotide analogues as part of our program to develop nucleoside and nucleotide analogues having antiviral activity[3].

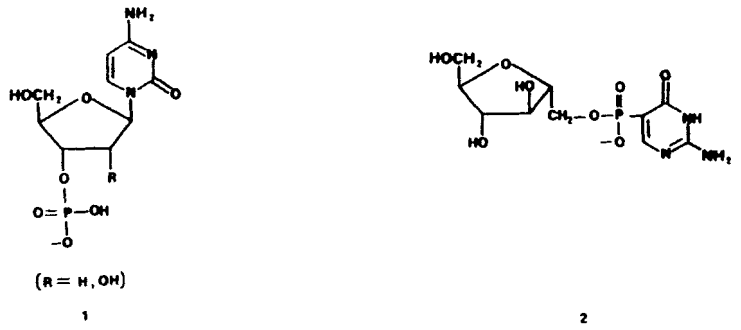
Nucleotides consist of three main units , a phosphate, a sugar and a base linked together as shown in structure **1**. Our plan was to alter this order of blocks in the nucleotide unit by replacing the phosphate with a phosphonate and to rearrange the blocks as in **2** where the phosphonate group is between the carbohydrate moiety and the heterocyclic base (See **Figure 1**). Compounds of the general structure **2** possess some of the gross structural features of natural nucleotides and might possess interesting biological activity.

RESULTS and DISCUSSION

General

The general route to the new nucleotides, described in this manuscript, is illustrated in **Scheme 1**. The phosphodichloridite procedure of linking nucleosides via the 5' and 3' hydroxyls has found extensive use in the synthesis of oligonucleotides[4]. We employed this general method to prepare the desired trialkylphosphite starting materials **20** and **21** from methyl dichlorophosphite (3)

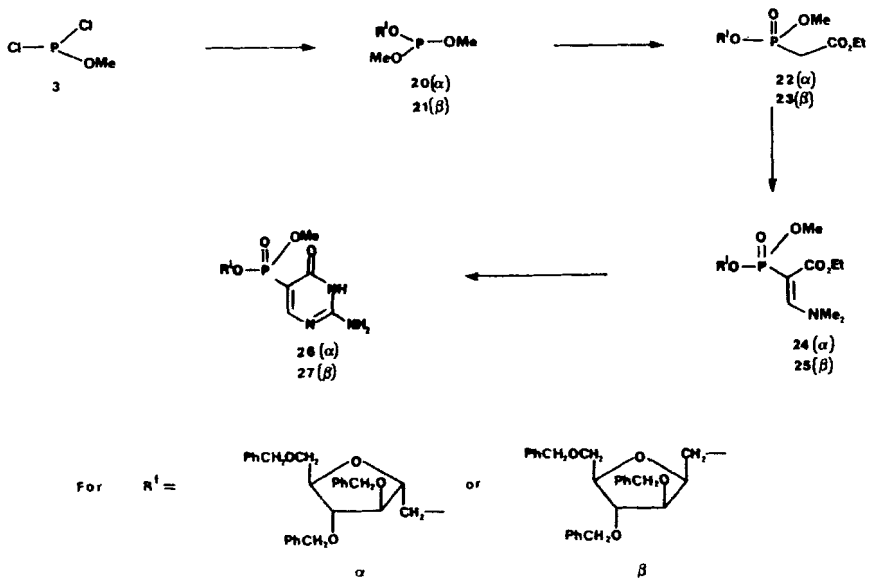
PHOSPHATE--SUGAR--BASE SUGAR--PHOSPHONATE--BASE



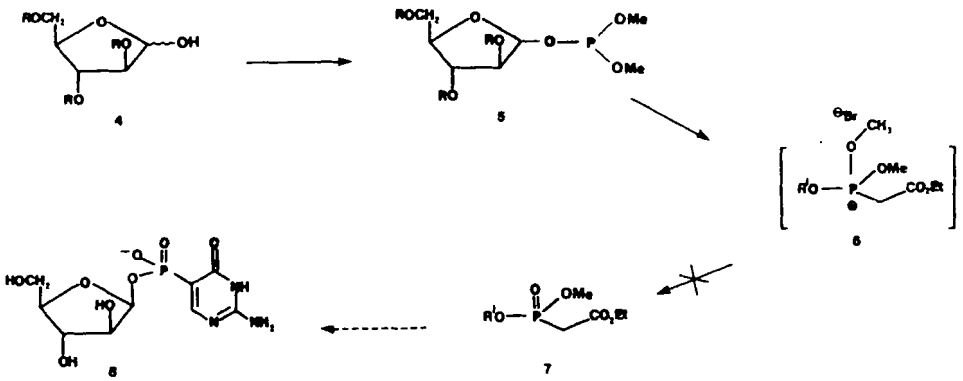
(R = H, OH)

1 2

Figure 1



Scheme 1



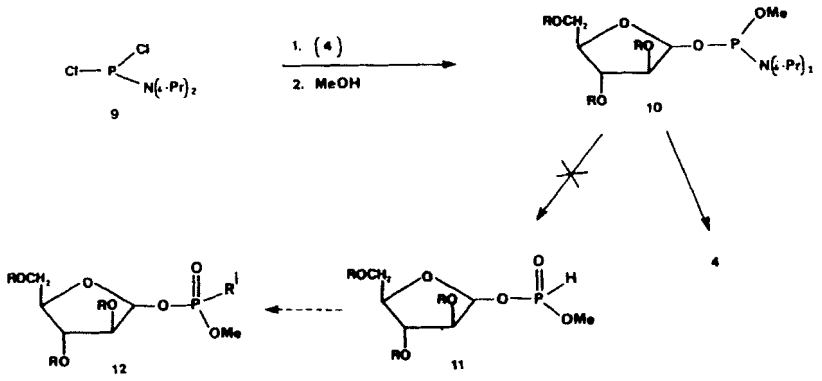
Scheme 2

and the appropriate alcohols (**18** and **19**). Arbuzov reactions with ethyl bromoacetate afforded the phosphonoacetates **22** and **23** in good yield and condensation with DMF-dimethylacetal gave the enaminophosphonate esters **24** and **25**. These were purified by flash chromatography over silica gel and used in the next reaction. Guanidine was generated from guanidine hydrochloride and sodium ethoxide, and reacted with the enaminophosphonate esters to give the corresponding isocytosines[2], **26** and **27** in good yields.

Attempts to Prepare Nucleotide Analogue 8

Our first proposed target was **8** (See **Scheme 2**) and this we envisaged making from the dimethylphosphite **5** via the type of reactions outlined in **Scheme 1**. Although we were able to prepare **5**, from tri-O-benzylarabinofuranose **4** and methyl dichlorophosphite, and characterize it by ^{1}H, ^{13}C and ^{31}P NMR, its reaction with ethyl bromoacetate was unsuccessful because of degradation of the molecule. This, in retrospect, is not that surprising if one considers the highly sensitive nature of the hemi-acetal phosphite **5**, and the intermediate salt **6** which is formed by the initial attack of **5** on ethyl bromoacetate. The quaternary phosphorus salt would be a very good leaving group and with the possible participation of the ring oxygen to assist in the departure of the C-1 substituent, then one can at least propose an explanation for the failure to detect any of the desired Arbuzov reaction product.

Attempts to circumvent this problem by use of phosphoramidite chemistry (See **Scheme 3**) were unsuccessful. We appeared to form amidite **10** (from ^{1}H, ^{13}C and ^{31}P NMR data) but attempts to hydrolyse it to **11** were unsuccessful. When a THF solution of **10** was exposed to 50% aqueous acetic acid, the sugar **4**, resulting from hydrolysis of the acetal, was recovered. Identification of compounds was made on the basis of comparisons of both TLC and 1H NMR spectra of both the crude and chromatographed products with those of the starting materials. Exposure of **10** in THF to 1.5 eq. of tetrazole and 3 eq. of water mainly resulted in the recovery of **4**. Treatment of **10** in THF



Scheme 3

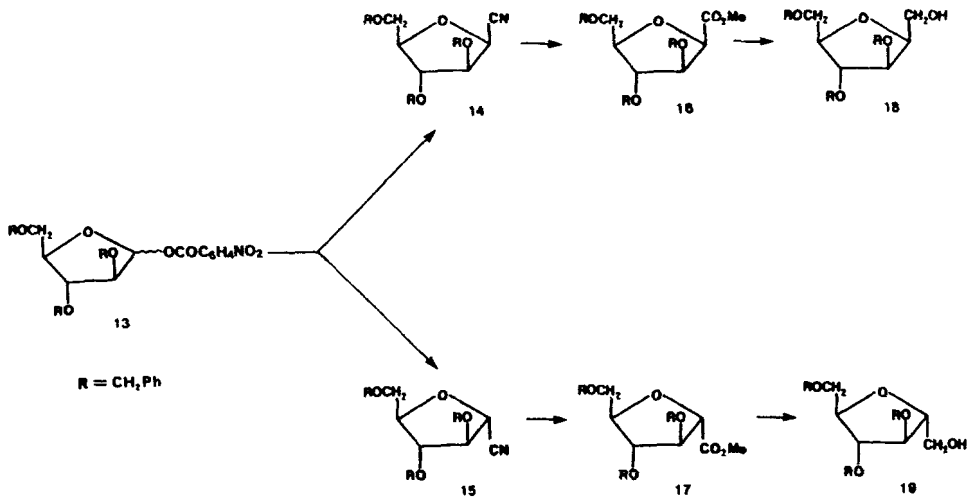
with 20 eq. of water which contained 10% acetic acid allowed the isolation of both the unreacted amidite **10** and some of the sugar **4**. This suggests that hydrolysis of the acetal is much more facile than the desired hydrolysis of the phosphoramidite. Had we been able to form **11**, our plan was to deprotonate it and condense the resulting anion with electrophiles to prepare compounds of type **12**.

In order to overcome the instability problems associated with intermediates **5** and **10**, we attempted the preparation of a one carbon homologue of the original target molecule **4**. This was accomplished by first carrying out a one carbon chain extension at the C-1 position of a protected arabinofuranose.

Arabinose Derived Analogues

Alcohols **18** and **19** (See **Scheme 4**) were prepared from 2,5-anhydro-3,4,6-tri-O-benzyl-D-mannononitrile (**14**) and 2,5-anhydro-3,4,6-tri-O-benzyl-D-gluconononitrile (**15**). The nitriles were prepared according to a literature procedure[5] by reaction of commercially available tri-O-benzyl-1-O-p-nitrobenzoyl-arabinofuranose, **13**, with trimethylsilyl-triflate and trimethylsilylcyanide in acetonitrile. The *ca.* 2:1 mixture of diastereoisomers were separated by HPLC.

Methanolysis of each of the isomers under acidic conditions, followed by hydrolysis, afforded the esters **16** and **17** in 92% and 76% yield respectively. Reduction of these compounds with Super-Hydride[6] afforded alcohols **18** and **19** in good yield. A LAH reduction of the alpha isomer was carried out but the product yield (55%) was much lower than the reduction with Et_3BHLi (92%). The alcohols **18** and **19** were converted to phosphites **20** and **21**. The phosphites were carefully purified by column chromatography before use in the Arbuzov reaction. The column of silica gel was washed with 68:28:4 hexane:ethyl acetate:triethylamine before applying the crude mixture and the products were then eluted with the same solvent system. We found that using crude preparations of phosphites **20** and **21** gave poor yields of the Arbuzov reaction products but use of the chromatographed compounds led to the formation of phosphonates **22** and **23** in good yield.



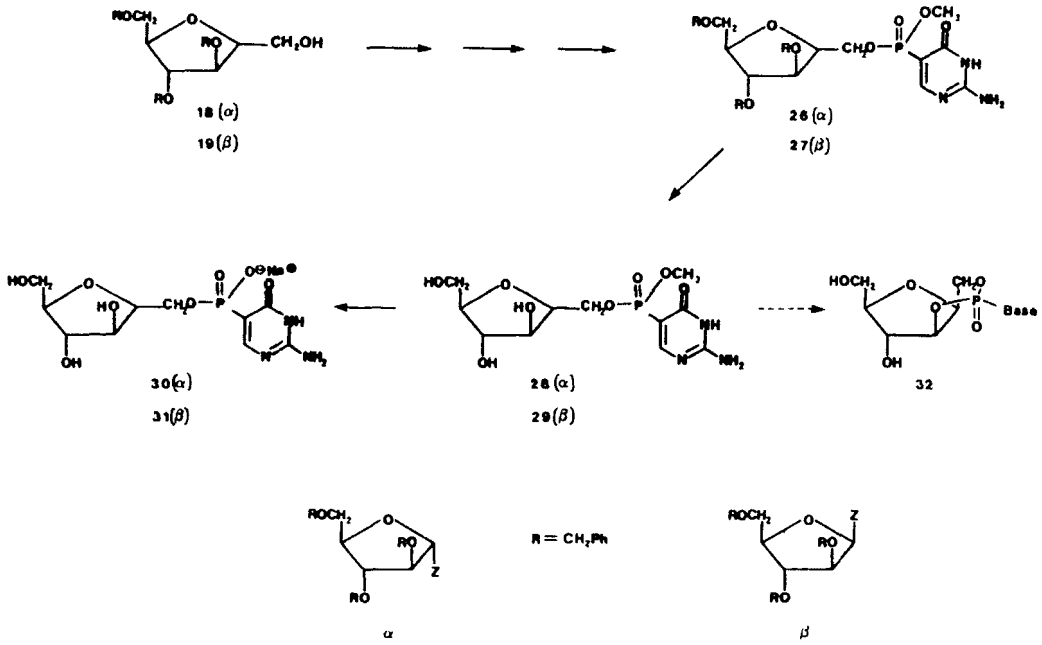
Scheme 4

The phosphites **20** and **21** are chiral molecules and have interesting NMR spectra. As is characteristic of methyl phosphites, the CH_3 signal is coupled to the phosphorus nucleus. This results in a splitting of the signal to give a doublet at *ca.* 3.5 ppm with a coupling constant of *ca.* 10 Hz. The ^{31}P NMR signal is observed at 141 ppm. In marked contrast, the ^{31}P signals of phosphonates **22** and **23** are well upfield of that for the parent trialkylphosphites and are observed at *ca.* 22 ppm. The phosphorus atom is now chiral, due to its 4 different substituents, and so we see two signals, in a 1:1 ratio, for each of the diastereoisomers of **22** and **23**. Two doublets, one for each of the diastereoisomers of **22** or **23**, appear at *ca.* 3.0 ppm and are attributable to the CH_2 of the acetate portion of **22** and **23**. The coupling constant between the CH_2 protons and the phosphorus is quite large i.e. *ca.* 21-22 Hz.

The phosphonoacetates were converted into their corresponding enaminophosphonoacetates, **24** and **25**, which were isolated in 89% and 88% yields, respectively, after flash chromatography. In the ^1H NMR spectra of **24** and **25**, the β hydrogen of the α,β unsaturated ester - phosphonate system appears at *ca.* 7.4 ppm as a doublet with $J_{H\text{-}P}$ = 14-15 Hz. In the P^{31} NMR spectra, two sets of signals are observed i.e. an intense 1:1 pair at *ca.* 26 ppm and a *much* weaker intensity pair at 21.5 ppm. This may be due to the presence of the Z isomer (**Figure 2**) as a minor component of the reaction mixture, although we have no conclusive proof of this. Cyclization with guanidine[2] then gave the isocytosines **26** and **27**. These compounds were characterized by ^1H, ^{31}P and ^{13}C NMR, by their UV spectra, and by FAB MS.

Debenzylation of **26** with boron trichloride gave **28** in an overall yield of 44% after a series of purification steps (See **Scheme 5**). Conventional procedures such as column chromatography over silica gel, preparative thin layer chromatography or recrystalizations were unsuccessful and unsuitable

Figure 2



Scheme 5

for this compound due to the polar nature of this molecule. Eventually we found that chromatography on cellulose plates[7] and extraction of the cellulose with water afforded a partially purified sample of **28**. Chromatography on C-18 sep-pak columns[8] effected the separation of salts from the product. Evaporation of the aqueous fractions gave **28** as an off-white foam. The ^{1}H, ^{31}P, ^{13}C NMR spectra, UV spectra and Chemical ionization MS were all consistent with the proposed debenzylated product. Deprotection of the phosphonate by nucleophilic attack of *t*-butylamine on the methyl group, followed by sodium ion exchange, over Dowex resin[9] gave **30**.

Surprisingly, debenzylation of the β isomer with boron trichloride and purification[10] of the crude reaction products, resulted not only in the deprotection of the sugar ring, but also in loss of the methyl phosphonate protecting group. The NMR (^{1}H, ^{31}P, ^{13}C) data suggests the presence of two isomeric compounds in a ratio of *ca.* 3:1. Compounds **31** and **32** are both possible reaction products.

The two diastereoisomers of 32 could account for the two sets of signals observed in the NMR spectra. However, a FAB mass spectrum of the isolated product was not consistent with structure 32 but rather lent support to the presence of the sodium salt of the fully deprotected isocytosine 31. The minor compound present was not identified.

CONCLUSIONS

α and β Arabinose derived nucleotide analogues have been prepared based on cyclization reactions of enaminophosphonate - esters with guanidine. Compounds 22, 23, 24, 25, 26, 27, 28, 30 and 31 were submitted for biological testing, but none were found to be active against either HSV - 1 or HSV - 2.

Experimental

General

Except where stated to the contrary, the following particulars apply: All apparatus was oven - dried for at least 3 h (110°C) and cooled in a desiccator over Drierite. Solvents for chromatography were distilled prior to use. Solvents for reactions were distilled from appropriate drying agents and were either used immediately or stored over molecular sieves. During product isolation, organic extracts were dried over anhydrous magnesium or sodium sulphate, and the solvents were evaporated under vacuum by use of a Buchi rotary evaporator or a Salvant Speed Vac. Melting points were determined on a Fischer - Johns apparatus and are uncorrected. Commercial thin layer chromatography (TLC) plates (silica gel Merck $60F_{-254}$ and Merck cellulose F, 0.1mm) were used. TLC plates were examined under ultraviolet radiation (254 nm) and the silica plates were heated (with a heat gun or in an 80°C oven) after being dipped in a solution (100 mL of 10% H_2SO_4 in H_2O) of ammonium molybdate (2.5 g) and ceric sulphate (1.0 g). Infrared (IR) spectra were recorded on a Perkin - Elmer 521 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded om either a Varion XL - 200 or on a Varion XL - 300. The following abbreviations are used with respect to NMR spectra: s = singlet; d = doublet; t = triplet; q = quartet; br = broad; J = coupling constant; δ = chemical shift. The spectra are referenced internally against TMS or against the solvent reference peak and externally against DSS (2,2-dimethyl-2-silapentane-5-sulphonate sodium salt) in the case of C^{13} spectra run in D_2O, or against 85% phosphoric acid for ^{31}P NMR spectra. Mass spectra (MS) were recorded with a Dupont Instruments 21-492-B mass spectrometer (for low resolution EI) or Hewlitt-Packard 5984A mass spectrometer (chemical ionization) or a Vacuum Generators ZAB-2F instrument (FAB MS). Ultraviolet (UV) spectra were obtained on a Hewlitt-Packard 8451-A which has a reported resolution of 2 nm. Microanalysis were performed by Canadian Microanalytical Services of Vancouver, B.C.

Materials

Methyl dichlorophosphite (Cl_2POCH_3), N,N-diisopropylmethylphosphonamidic chloride, trimethylsilyl-cyanide (TMSCN), trimethylsilyltriflate (TMSTf), dimethylformamide dimethyl acetal, N-benzyl glycine ethyl ester and Super Hydride (1 M solution in THF) were purchased from Aldrich and stored in a refrigerator. were purchased from Aldrich Ethyl bromoacetate was purchased from Aldrich, distilled, and stored in a desiccator in the refrigerator. Ammonium molybdate(VI) tetrahydrate, cerium sulphate hydrate, and guanidine hydrochloride, were purchased from Aldrich and the protected Arabinofuranoses **4** and **13** were purchased from Pfanstiehl and all were stored at room temperature.

Compound 5: Dimethylphosphite of 2,3,5-tri-O-benzyl-D-arabinofuranose

A solution of 2,3,5-tri-O-benzyl-D-arabinofuranose (549 mg, 1.31 mmol) in THF (1.0 mL plus 1.0 mL rinse) was slowly added over *ca.*10 min to a cold (-78°C) solution of diisopropylethylamine (2.0 mL, 1.5 g, 11.5 mmol) and methylphosphorodichloridite (0.3 mL, 420 mg, 3.2 mmol) in THF (1.5 mL), under an argon atmosphere. The reaction mixture was stirred for 15 min before the dropwise addition of methanol (0.5 mL) over *ca.*5 min. The mixture was warmed to room temperature, diluted with a saturated solution of sodium bicarbonate (15 mL), and extracted with methylene chloride (2x30 mL). The combined organic extracts were washed with bicarbonate solution (15 mL), dried, and evaporated. Flash chromatography of the crude residue over silica gel (2x10 cm) in 1:1:6 CH_2Cl_2 : ethyl acetate : hexane containing 2% Et_3N afforded dimethylphosphite **5** (593 mg, 1.15 mmol, 88% yield) as an opaque oil: R_f=0.5 (1:1:6 CH_2Cl_2: ethyl acetate: hexane containing 2% Et_3N); ^1H NMR (200 MHz, $CDCl_3$) 7.30(m, 15 H), 5.65(m, 1H), 4.60(m, 6H), 4.13(m, 3H), 3.62(m, 2H), 3.49(d, J=10.3 Hz), 3.47(d, J=10.5 Hz). The signals at 3.49 and 3.47 together integrate to 6H. ^{31}P NMR (121 MHz, $CDCl_3$) 140(br); 13C NMR (75.4 MHz, $CDCl_3$) 138.07, 138.02, 137.56, 128.4, 128.3, 127.8, 127.6, 95.0 (d, J=17.5 Hz), 84.24, 84.18, 82.5, 80.9, 73.3, 72.35, 72.27, 49.3 (d, J=7.6 Hz).

Synthesis of Phosphoramidite 10

2,3,5-Tri-O-benzyl-D-arabinofuranose (330 mg, 0.785 mmol) in THF (1.0 mL plus 1.0 mL rinse) was added to a solution of N,N-diisopropylmethylphosphonamidic chloride (0.24 mL, 0.24 g, 1.2 mmol) and N,N-diisopropylethylamine (0.5 mL, 0.37 g, 2.9 mmol) in THF (2.0 mL) and the reaction mixture was stirred at room temperature under an argon atmosphere for 1.5 h. The mixture was filtered to remove a white precipitate and the filter cake was washed with ethyl acetate (*ca.* 2-3 mL). The filtrates were combined and diluted with methylene chloride (*ca.* 30 mL), washed with a saturated aqueous solution of sodium bicarbonate (10 mL) and brine (10 mL), and dried. The solvent was evaporated and flash chromatography of the crude residue over silica gel (2x17 cm) with 6:1 hexane containing 2% Et_3N : ethyl acetate afforded amidite **10** (364 mg, 0.626 mmol, 80% yield) as an opaque oil: Rf = 0.36 (6:1 hexane containing 2% Et_3N :ethyl acetate); ^1H NMR (200 MHz, $CDCl_3$) δ 7.3(m, 15 H), 5.6-5.4(m, 1H), 4.78-4.28(m, 6H), 4.2-3.9(m, 3H), 3.60(m, 4H), 3.42(d, J=13.4 Hz), 3.38(d, J=13.2 Hz), 1.2(m, 12H). The signals at δ 3.42 and 3.38 together integrate to 3H. ^{31}P NMR (121

MHz, $CDCl_3$), δ 150.3, 148.4, 148.3 (in *ca.* 1:1.5:2.1 ratio). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 138.3, 138.2, 138.0, 137.9, 137.7, 137.6, 128.4, 128.3, 127.8, 127.72, 127.68, 127.6, 127.5, 101.8(d, J=18.7 Hz), 101.3(d, J=18.8 Hz), 96.4(d, J=19.1), 96.0(d, J=18.6 Hz), 89.1(m), 84.6, 84.5, 84.2, 84.1, 83.9, 83.7, 83.4, 83.3, 81.5, 81.4, 80.7, 80.5, 73.3, 72.8, 72.7, 72.2, 72.0, 71.9, 71.8, 69.7, 50.6(m), 43.2(m), 24.5(m).

Synthesis of 2,5-Anhydro-3,4,6-tri-O-benzyl-D-mannononitrile (14) and 2,5-Anhydro-3,4,6-tri-O-benzyl-D-gluconononitrile (15)

Compounds **14** and **15** were prepared from commercially available 2,3,5-tri-O-benzyl-1-O-p-nitrobenzoyl-arabino-furanose (**13**) according to the procedure outlined in reference 5. The two diastereoisomers were separated by use of high pressure liquid chromatography as suggested by the authors.

Methyl 2,5-Anhydro-3,4,6-tri-O-benzyl-D-mannonate 16

A stream of hydrogen chloride gas was bubbled through a cold (0°C) solution of alpha-nitrile **14** [930 mg, 2.16 mmol] in methanol-ether [1:1, 40 mL] for 10 min. The reaction mixture was warmed to room temperature and HCl was passed through the solution for a further 15 min. Stirring was continued for 20 min, HCl was passed through the solution for an additional 5 min, and the solution was stirred for 30 min. Ice-water [*ca.* 40 mL] and ether were added and the mixture was stirred for 2.5 h. The aqueous layer was extracted twice with ether [*ca.* 100 mL each] and the combined organic extracts were washed with saturated $NaHCO_3$ [*ca.* 100mL] and brine [*ca.* 100mL], dried, and evaporated. Flash chromatography of the residue over silica gel [2x20 cm] in 1:1:6 dichloromethane:ethyl acetate:hexane afforded ester **16** [919mg, 1.99mmol, 92% yield] as a pale yellow oil: R_f = 0.16 [TLC, silica, 1:1:6 CH_2Cl_2:EtOAc:Hexane]. ^1H NMR (300 MHz, $CDCl_3$) δ 7.30(m, 15H, *Ar-H*), 4.65(m, 1H, *H*-2), 4.46-4.60(m, 6H, CH_2Ph), 4.38(dd, J = 5.3, 9.6 Hz, 1H, *H*-5), 4.33(apparent t, J = 2.2 Hz, 1H, *H*-3), 4.03(dd, J = 4.1, 2.2 Hz, 1H, *H*-4), 3.72(s, 3H, CO_2CH_3), 3.61(dd, J = 5.2, 2.5 Hz, *H*-6a, *H*-6a'). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 138.2, 137.6, 137.3, 128.5, 128.39, 127.98, 127.87, 127.80, 127.76, 127.71, 127.66, 86.5, 83.6, 83.0, 81.4, 73.4, 71.9, 71.7, 69.7, 52.3. IR (film) 1755(s), 1728(s), 1100(s) cm^{-1}. MS [low resolution,EI] m/z : 371[$(M - CH_2Ph)^{\cdot +}$, 6.4%], 91[100% peak, $(CH_2Ph)^{\cdot +}$]. Elemental Analysis : Calcd. C(72.71%), H(6.54%); Found C(72.89%), H(6.49%).

Methyl 2,5-Anhydro-3,4,6-tri-O-benzyl-D-gluconate 17

A stream of hydrogen chloride gas was bubbled through a cold (0°C) solution of beta-nitrile **15** [0.73 g, 1.7 mmol] in methanol - ether [1:1, 30 mL] for 15 min. The clear yellow solution was stirred for an additional 15 min at 0°C and then for 20 min at room temperature. Ice-water (*ca.* 100 mL) and ether (*ca.* 100 mL) were added and the mixture was stirred at room temperature for 2.5 h. The mixture was extracted twice with ether (*ca.* 100 mL each) and the combined organic extracts were washed with saturated $NaHCO_3$ (*ca.* 100mL) and brine (*ca.* 100mL), dried , and evaporated. Flash

chromatography of the residue over silica gel (2x20 cm) in 4:1 hexane-ethyl acetate afforded ester **17** [0.60 g, 1.3 mmol, 76% yield] as a pale yellow oil : R_f = 0.25 [TLC, silica, 3:1 hexane-Ethyl acetate]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30(m, 15H, Ar-*H*), 4.74(d, J = 4.1 Hz, 1H, *H*-1), 4.51(m, 6H, CH_2Ph), 4.24(m, 2H, *H*-2,*H*-3), 4.00(m, 1H, *H*-3), 3.78(dd, J = 5.9, 9.7 Hz, 1H, *H*-5a), 3.74(s, 3H, CH_3), 3.61(dd, J = 7.2,9.7 Hz, *H*-5a'). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.3, 138.2, 137.5, 137.3, 128.46, 128.39, 128.31, 127.90, 127.80, 127.71, 127.60, 83.6, 83.4, 82.7, 80.5, 73.3, 72.1, 71.6, 70.1, 51.9. IR (film) 1765(s), 1735(s) cm^{-1}. MS (low resolution , EI) m/z : No Molecular ion seen; 371[5.1%, (M - $CH_2Ph)^{\cdot +}$], 265[10%, (M - 2xCH_2Ph + $CH_3)^{\cdot +}$, 91[100%, $(CH_2Ph)^{+}$]. Elemental Analysis : Calcd. C (72.71%), H (6.54%), Found C (72.85%), H (6.57%).

2,5-Anhydro-3,4,6-tri-O-benzyl-D-mannitol 18

An excess of Et_3BHLi [10.0mL, 1M in THF, 10.0 mmol] was added dropwise, over 10 min, to a cold (0^{o}C) solution of ester **16** [1.48g, 3.20 mmol] in THF (10 mL) under an argon atmosphere. The solution was stirred at 0^{o}C for 1.2h. A few pieces of ice were first cautiously added to the reaction mixture, followed by addition of dilute HCl (*ca.* 50 mL, 1M in H_2O) and dichloromethane (*ca.* 50 mL). The aqueous layer was extracted with dichloromethane (2 x *ca.*.50 mL) and the combined organic extracts were washed with saturated $NaHCO_3$ (*ca.* 100 mL) and brine (*ca.* 100 mL), dried, and evaporated. Flash chromatography of the residue over silica gel (4x20 cm) in 1:1 hexane-ethyl acetate afforded alcohol **18** [1.24g, 2.85 mmol, 89% yield] as a very slightly impure sample. Kugelrohr distillation [0.05mmHg, 180^{o}C] provided an analytical sample: R_f = 0.34 [TLC, silica, 1:1 hexane-ethyl acetate]; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.30(m, 15 H, *Ar-H*), 4.53(m, 6H, CH_2Ph), 4.26(m, 1H, sugar *H*), 4.14(m, 1H, sugar *H*), 4.06(m, 2H, sugar *H* 's), 3.70(m, 2H, *H*-1a, *H*-1a'), 3.57(m, 2H, *H*-6a, *H*-6a'), 2.05(t, J = 6.2 Hz, 1H, *OH*). Upon D_2O exchange the triplet at δ 2.05 disappeared and the signal at δ 3.70 was simplified. ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.0, 137.7, 137.6, 128.49, 128.44, 128.40, 127.88, 127.84, 127.78, 127.71, 84.6, 84.1, 83.2, 81.9, 73.4, 72.1, 71.9, 70.1, 62.7. IR (film) 3460(s,br) cm^{-1}. MS [low resolution, EI] No Molecular ion. m/z : 343[6.8%, $(M\text{-}CH_2Ph)^{\cdot +}$], 91[100%,$(CH2Ph)^{\cdot +}$].

2,5-Anhydro-3,4,6-tri-O-benzyl-D-glucitol 19

An excess of Et_3BHLi [3.5 mL, 1 M in THF, 3.5 mmoL] was added dropwise, over 10 min, to a cold (0^{o}C) solution of ester **17** [0.48g, 1.04 mmol] in THF (5.0 mL) under an argon atmosphere. The solution was stirred at 0^{o}C for 1.5 h. Ice, dilute hydrochloric acid (*ca.* 30 mL) and ether (*ca.* 50 mL) were sequentially added. The aqueous layer was extracted with ether (2 x *ca.* 50 mL) and the combined organic extracts were washed with saturated $NaHCO_3$ (*ca.* 30 mL) and brine (*ca.* 30 mL), dried, and evaporated. Flash chromatography of the residue over silica gel (2 x 20 cm) with 1:1 hexane-ethyl acetate afforded alcohol **19** [0.35g, .806mmol, 77.6% yield] as a pale yellow oil : R_f = 0.15 [TLC, silica, 2:1 hexane-ethyl acetate]; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.30(m, 15H, *Ar-H*), 4.60-4.38(m, 6H, CH_2Ph), 4.08(m, 4H, *H*-2,-3,-4,-5), 3.83(m, 2H, *H*-1a, *H*-1a'), 3.59(d, J = 4.6 Hz, *H*-6a, *H*-6a'), 2.34(t, J = 6.2 Hz, *OH*). Upon addition of D_2O the signal at δ 2.34 disappeared and the signal at δ 3.83 was simplified. ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.9, 137.8, 137.4, 128.47, 128.35,

128.29, 127.89, 127.75, 127.64, 83.8, 83.1, 81.7, 80.2, 73.3, 71.85, 71.79, 70.1, 61.6. IR (film) 3450(s,br) cm^{-1}. MS [low resolution, EI] No Molecular ion observed; m/z : 343[2%, $(M-CH_2Ph)^{\cdot +}$], 91[100%,$(CH_2Ph)^{\cdot +}$].

Compound 20: Dimethylphosphite of 2,5-Anhydro-3,4,6-tri-O-benzyl-D-mannitol

An excess of diisopropylethylamine [3.2 mL, 2.4g, 18.4 mmol] was added dropwise, over *ca.* 5 min, to a cold (-78°C) solution of dichloromethylphosphite [0.5 mL, 0.73g, 5.5 mmol] in THF (3.0 mL) under argon. A solution of alcohol **18** [0.80g, 1.84 mmol] in THF [3.0 mL plus 2x1.0 mL rinse] was then added dropwise ,over *ca.* 15, min and the reaction mixture was stirred for 20 min. Methanol [1.0 mL] was added and stirring was continued at -78°C for 10 min before warming to room temperature. Saturated $NaHCO_3$ (*ca.* 50 mL) was added and the solution was extracted with dichloromethane (2 x *ca.* 50 mL). The combined organic extracts were dried and evaporated. Flash chromatography of the residue over silica gel [2x20 cm, eluted with 68:28:4 hexane-ethyl acetate-triethylamine (solvent system A) prior to loading of crude product onto the column] with solvent system A afforded some of the starting material **18** [0.12g, 0.276 mmol, 15% recovery] along with the desired trialkylphosphite **20** [0.708 g, 1.34 mmol, 73% yield] as a colourless oil : R_f = 0.59 [TLC, silica, solvent system A]. ^1H NMR (200 MHz, $CDCl_3$) δ 7.30(m, 15H, *Ar-H*), 4.52(m, 6H, CH_2Ph), 4.20(m, 2H, sugar *H*'s), 4.07(m, 2H, sugar *H*'s), 3.90(dd, J = 7.5, 6.0 Hz, 2H, *H*-1a, *H*-1a'), 3.57(d, J = 5.7 Hz, 2H, *H*-6a, *H*-6a'), 3.488(d, J = 10.4 Hz, $P\text{-}OCH_3$), 3.482(d, J = 10.2 Hz, $P\text{-}OCH_3$). The signals at δ 3.488 and 3.482 together integrate to 6H. ^{31}P NMR (121 MHz, $CDCl_3$) δ 141 . MS [low resolution] No Molecular ion seen; m/z : 343[2.7%], 91[100%,$(CH_2Ph)^{\cdot +}$].

Compound 21: Dimethylphosphite of 2,5-Anhydro-3,4,6-tri-O-benzyl-D-glucitol

The general procedure described in the above experiment for **20** was also used for the synthesis of **21**. The following quantities of reagents were used: diisopropylethylamine [1.2 mL, 0.89g, 6.9 mmol], dichloromethylphosphite [0.20 mL, 0.28 g, 2.1 mmol], alcohol **19** [0.302 g, 0.695 mmol], THF(4.0 mL) and methanol(.5 mL). Trialkylphosphite **21** was isolated as a colourless oil [0.334g, 0.634 mmol, 92% yield] : R_f = 0.55 [TLC, silica, 60:38:2 hexane-ethyl acetate-triethylamine]. ^1H NMR (200 MHz, $CDCl_3$) δ 7.30(m, 15H, *Ar-H*), 4.50(m, 6H, CH_2Ph), 4.24-3.92(m, 6H, sugar *H*'s), 3.60(dd, J = 5.8, 9.8 Hz, 1H, *H*-6a), 3.49(dd, J = 9.8, 11.1 Hz, 1H, *H*-6a'), 3.48(dd, J = 10.4 Hz, 6H, $P\text{-}OCH_3$). ^{31}P NMR (121 MHz, ,$CDCl_3$) δ 141. MS [low resolution, EI] No Molecular ion seen; m/z : 343[8.6%], 91[100%,$(CH_2Ph)^{\cdot +}$].

Alpha Phosphonoacetates 22

A solution of phosphite **20** [0.13 g, 0.247 mmol] and ethyl bromoacetate [0.30 mL, 0.45 g, 2.7 mmol] in dry acetonitrile [1.0 mL] was stirred, for 19 h, at *ca.* 80°C under an argon atmosphere. The solvent and excess reagent were evaporated and flash chromatography of the crude residue over silica gel (2x15 cm) with 1:1 hexane-ethyl acetate, followed by ethyl acetate neat, afforded phosphonoacetates **22** as an opaque oil [0.137 g, 0.229 mmol, 92% yield] : R_f = 0.13 [TLC, silica, 1:1

hexane-ethyl acetate]. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.30(m, 15H, Ar-*H*), 4.53(m, 6H, CH_2Ph), 4.29-4.13(m, 6H, sugar *H*'s + OCH_2CH_3), 4.08(m, 2H, sugar *H*'s), 3.76(d, J = 10.9 Hz, 3H, $POCH_3$), 3.56(overlapping doublets, J = 5.0, 6.0 Hz, 2H, *H*-6a, *H*-6a'), 2.99(d, J = 21.5 Hz, PCH_2CO_2Et), 2.97(d, J = 21.6 Hz, PCH_2CO_2Et), 1.25(t, J = 7.0 Hz, 3H, $CO_2CH_2CH_3$). The signals at δ 2.99 and 2.97 together integrate to 2H. ^{31}P NMR (121 MHz, $CDCl_3$) δ 22.6, 22.5 (1:1 ratio). ^{13}C NMR (50 MHz, CDCl3) δ 165.65, 165.54, 138.1, 137.7, 137.6, 128.5, 128.42, 128.38, 128.0, 127.8, 127.72, 127.66, 84.5, 84.2, 82.2, 81.5 [apparent triplet - overlapping doublets at δ 81.528(J = 6.0 Hz) and 81.421(J = 4.8 Hz)], 73.4, 71.97, 71.91, 70.1, 65.8[apparent triplet - overlapping doublets at δ 65.906(J = 6.2 Hz) and 65.792(J = 5.4 Hz)], 61.6, 53.13[m, signals at 53.24, 53.14, 53.10. 53.02 - $POCH_3$], 35.2, 32.5, 14.1. IR (film) 1735(s), 1270(s) cm^{-1} . MS [low resolution] m/z : 598[0.1%, $M^{\cdot+}$], 553[0.4%, $(M-OCH_2CH_3)^{\cdot+}$], 507[0.3%, $(M-CH_2Ph)^{\cdot+}$], 91[100%,$(CH_2Ph)^{\cdot+}$].

Beta Phosphonoacetates 23

A solution of phosphite **21** [0.20 g, 0.38 mmol] and ethyl bromoacetate [0.20mL, 0.30 g, 1.8mmol] in dry acetonitrile [1.5 mL] was stirred at *ca.* 80°C, for 5 h, under an argon atmosphere. The solvent and excess reagent were evaporated and flash chromatography of the residue over silica (2 x 20 cm), with 1:1 hexane-ethyl acetate followed by 2:3 hexane-ethyl acetate, afforded phosphonate ester **23** as an opaque oil [0.187 g, 0.312 mmol, 82% yield]. R_f = 0.14 [TLC, silica, 1:1 hexane-ethyl acetate]. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.30(m, 15H, *Ar-H*), 4.62-4.10(m, 12H), 3.99(m, 2H, sugar *H*'s), 3.77(d, J = 11.4 Hz, 3H, $POCH_3$), 3.63(m, 1H, *H*-6a), 3.51(dd, J = 6.7, 9.7 Hz, 1H, *H*-6a'), 2.99(d, J = 21.5 Hz, PCH_2CO_2Et), 2.96(d, J = 21.6 Hz, PCH_2CO_2Et), 1.24, 1.23(overlapping triplets, J = 7.1, 7.1 Hz, 3H, $CO_2CH_2CH_3$). The doublets at δ 2.99 and 2.96 together integrate to 6H. ^{31}P NMR (121 MHz, $CDCl_3$) δ 22.6, 22.2 (1:1 ratio). 13C NMR (75 MHz, $CDCl_3$) δ 165.6(m, signals at 165.66, 165.59, 165.57, 165.53), 138.2, 137.7, 137.5, 128.5, 128.4, 127.88, 127.86, 127.74, 127.68, 127.60, 83.12, 83.07, 83.04, 82.62, 82.56, 79.8(overlapping doublets at 79.97, J = 7.1 Hz and 79.77, J = 8.4 Hz), 73.3, 71.6, 70.4, 65.33(d, J = 6.2 Hz), 64.97(d, J = 5.8 Hz), 61.64, 61.60, 53.22(d, J = 5.8 Hz, $POCH_3$), 52.93(d, J = 5.8 Hz, $POCH_3$), 34.76(d, J = 5.9 Hz, PCH_2CO_2Et), 32.97(d, J = 5.0 Hz, PCH_2CO_2Et), 14.1. IR (film) 1730(s), 1265(s) cm^{-1}. MS [low resolution, EI] No Molecular ion seen; m/z : 553 [0.3%, $(M - OCH_2CH_3)^{\cdot+}$, 325 [1.0%, $(M - 3xCH_2Ph)^{\cdot+}$], 91 [100%,$(CH_2Ph)^{\cdot+}$].

Alpha Enaminophosphonoacetate 24

Dimethylformamide dimethylacetal [0.30 mL, 0.27 g, 2.2 mmol] was added to phosphonoacetates **22** [0.277 g, 0.463 mmol] and the mixture was stirred at reflux, for 2 h, under an argon atmosphere. The excess of reagent was evaporated and flash chromatography of the residue over silica gel [2x20 cm] with 95:5 ethyl acetate-methanol afforded compound **24** [0.269 g, 0.411 mmol, 89% yield] as a yellow oil. R_f = 0.43 [TLC, silica, 9:1 ethyl acetate-methanol]. ^{1}H NMR (200 MHz, $CDCl_3$) δ7.44(d, J = 14.8 Hz, 1H,βH), 7.30(m, 15H, *Ar-H*), 4.53(m, 6H, CH_2Ph), 4.33-4.00(m, 9H), 3.69(d, J = 11.5Hz, $POCH_3$), 3.68(d, J = 12.0 Hz, $POCH_3$),3.57(d, J = 5.7 Hz, 2H, *H*-6a, *H*-6a'), 3.16(low broad signal, $W_{1/2}$ = 6.9 Hz, NMe_2), 3.02(broad signal, $W_{1/2}$ = 16.6 Hz, NMe_2), 1.23(t, J = 6.9 Hz, CH_2CH_3), 1.22(t, J = 6.9 Hz, CH_2CH_3), The signals at δ 3.69 and 3.68 together integrate

to 3H, those at 3.16 and 3.02 to 6H, and those at 1.23 and 1.22 to 3H. 31P NMR (121 MHz, $CDCl_3$) 26.6, 26.5(1:1) and 21.5 in a ratio of 16:1. The ^1H and ^{31}P NMR spectra are consistent with the presence of two diastereoisomers (1:1 ratio) of the *E*-conformation shown by structure **26** as well as with a minor amount of another pair of diastereoisomers due perhaps to the *Z*-conformation. ^{13}C NMR (75MHz, $CDCl_3$) δ 165.31, 165.17, 159.7, 159.5, 138.1, 137.90, 137.86, 137.81, 128.3, 128.2, 127.7, 127.6, 127.5, 84.78, 84.72, 84.65, 84.47, 82.77(d, J = 6 Hz), 82.1, 82.0, 81.85, 81.72, 81.66, 81.55, 80.13(d, J = 8 Hz), 73.3, 71.80, 71.75, 71.69, 70.23, 70.18, 64.91, 64.85, 59.9, 52.36(d, J = 6.5 Hz), 14.3. IR (film) 1740(w), 1690(s), 1610(s) cm^{-1}. MS [low resolution, EI] No Molecular ion seen; m/z : 608 [0.4%, $(M - OCH_2CH_3)^{\cdot +}$], 562 [1.1%, $(M - CH_2Ph)^{\cdot +}$], 91 [100%,$(CH_2Ph)^{\cdot +}$]. Elemental Analysis: Calcd. C(64.31), H(6.78), N(2.14), P(4.74); Found C(64.55), H(6.81), N(2.16), P(4.88).

Beta Enaminophosphonoacetate 25

Using the general procedure described above for the preparation of **24** ,phosphonoacetates **23** [0.18 g, 0.301 mmol] and DMF-dimethylacetal [0.50 mL, 0.45 g, 3.76 mmol] gave compound **25** [0.173 g, 0.265 mmol, 88% yield] as a yellow oil. R_f = 0.39 [TLC, silica, 9:1 ethyl acetate-methanol]. ^1H NMR (200 MHz, $CDCl_3$) δ 7.43(d, J = 14.2 Hz, 1H, *βH*), 7.30(m, 15H, *Ar-H*), 4.50(m, 6H, CH_2Ph), 4.28(m, 2H, sugar *H*'s), 4.13(m, 4H, 2 sugar *H*'s and CH_2CH_3), 4.01(m, 1H, sugar *H*), 3.92(m, 1H, sugar *H*), 3,685(d, J = 11.3 Hz, $POCH_3$), 3.678(d, J = 11.3 Hz, $POCH_3$), 3.56(m, 2H, *H*-6a, *H*-6a'), 3.18(low broad signal, NMe_2), 3.02(broad signal, $W_{1/2}$ = 9.6 Hz, NMe_2), 1.23(t, J = 7.2 Hz, 3H, CH_2CH_3). The signals at δ 3.685 and 3.678 together integrate to 3H, and, those at 3.18 and 3.02 integrate to 6H. ^{31}P NMR (121 MHz, $CDCl_3$) δ 26.8, 26.4 (1:1) and 21.7, 21.5 (1:1) in a ratio of 10:1. The ^1H and ^{31}P NMR spectra are consistent with the presence of two diastereoisomers (1:1 ratio) of the *E*-conformation shown by structure **25** as well as with a minor amount of another pair of diastereoisomers due perhaps to the *Z*-conformation. ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.90, 159.72, 159.64, 159.47, 138.2, 137.9, 137.8, 128.43, 128.37, 128.34, 127.75, 127.65, 127.60, 83.70, 83.65, 82.98, 82.94, 82.40, 82.29, 80.15(apparent triplet; overlapping doublets, J = 8.1, 9.1 Hz), 73.3, 71.67, 71.60, 71.4, 70.5, 63.52(m, coupled to ^{31}P), 60.0, 52.4(m, coupled to ^{31}P, $POCH_3$), 14.3. IR (film) 1682(s), 1605(s) cm^{-1}. MS [low resolution, EI] No Molecular ion seen; m/z : 608 [0.8%, $(M - OCH_2CH_3)^{\cdot +}$, 562 [0.3%, $(M - CH_2Ph)^{\cdot +}$], 91 [100%, $(CH_2Ph)^{\cdot +}$]. Elemental Analysis: Calcd. C(64.31), H(6.78), N(2.14), P(4.74); Found C(63.70), H(6.77), N(2.14), P(4.16).

Alpha Isocytosine 26

A solution of guanidine [0.28 M in absolute ethanol] was prepared as follows: Sodium ethoxide [2.5 mL, 1.87 M in EtOH, 4.7 mmol] was added to a suspension of guanidine hydrochloride [0.335 g, 3.51 mmol] in EtOH (10.0 mL). An aliquot of this cloudy solution [3.2 mL, 0.28 M, 0.90 mmol] was added to enaminophosphonoacetate **24** [0.46 g, 0.70 mmol] in EtOH (5.0 mL). This mixture was stirred at reflux under argon for 4.5 h. Evaporation of the solvent and flash chromatography of the residue over silica gel (2x13 cm) with dichloromethane-methanol (9:1, followed by 8:2) allowed separation of recovered starting material **24** [0.050 g, 11% recovery] from the desired product **26** [0.36 g, 0.58 mmol, 83%] as white foam (mpt = 87-107°C). R_f = 0.6 [TLC, silica, 8:2

dichlormethane-methanol]. 1H NMR (200 MHz, DMSO-d_6) δ 7.98(d, J = 9.1 Hz, *H*-6 base), 7.96(d, J = 9.2 Hz, *H*-6 base), 7.25(m, 15H, *Ar-H*), 4.47(m, 6H, CH_2Ph), 4.08(m, 3H, sugar *H*'s), 3.99(m, 3H, sugar *H*'s), 3.571(d, J = 11.6 Hz, $POCH_3$), 3.566(d, J = 11.5 Hz, $POCH_3$), 3.492(d, J = 6.0 Hz, *H*-6a, *H*-6a'), 3.488((d, J = 6.1 Hz, *H*-6a, *H*-6a'), 3.3(s, overlap of NH_2 with HOD), The signals at δ 3.571 and 3.566 integrate to 3H, those at 3.492 and 3.488 to 2H, and those at 7.98 and 7.96 to 1H. ^{31}P NMR (121 MHz, CD_3OD) δ 23.2(br, $W_{1/2}$ = 40 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) 172.6, 142.0, 138.2, 137.8, 128.2, 127.64, 127.57, 127.51, 127.42, 127.36, 83.9, 83.7, 81.6, 81.21(d, J = 7.1 Hz), 72.2, 70.9, 70.8, 69.9, 64.7(m, coupled to ^{31}P, CH_2), 52.1(m, coupled to ^{31}P, $POCH_3$). UV (absolute EtOH) λ_{max} = 286 nm, ϵ = 5600. MS [FAB+ , glycerol matrix] 714 [M + H^+ + glycerol], 622 [M + H^+].

Beta Isocytosine 27

The general procedure described in the previous experiment was used to prepare **27**. Guanidine [1.2 mL, 0.28M in EtOH, 0.38 mmol] and beta enaminophosphonoacetate **25** [0.17 g, 0.26 mmol] were refluxed in EtOH (2.0 mL) and from the reaction mixture, was isolated starting material [0.020g, 12% recovery] and the product **27** [0.13g, 0.20 mmol, 77% yield] as a white foam(mpt = 63-70°C). R_f = 0.42 [TLC, silica, 9:1 ethyl acetate-methanol]. ^{1}H NMR (300 MHz, DMSO-d_6) 7.98(overlapping doublets, J = 9.4, 9.3 Hz, 1H, *H*-6 base), 7.29(m, 15H, *Ar-H*), 4.63-4.40(m, 6H, CH_2Ph), 4.26-3.93(m, 6H), 3.57(d, J = 11.1 Hz, 3H, $POCH_3$), 3.48(m, 2H, *H*-6a, *H*-6a'), 3.33(s,br,-HOD + NH_2 or *OH*). ^{1}H NMR (200 MHz, CD_3OD) δ 8.01(overlapping doublets, J = 9.3, 9.0 Hz, 1H, *H*-6 base), 7.20(m, 15H, *Ar-H*), 4.48-4.37(m, 4H, CH_2Ph), 4.33(m, 2H, CH_2Ph), 4.16(m, 3H, sugar *H*'s), 3.96(m, 2H, sugar *H*'s), 3.89(m, 1H, sugar *H*), 3.62(d, J = 11.7 Hz, 3H, $POCH_3$), 3.52-3.32(m, 2H, *H*-6a, *H*-6a'). ^{31}P NMR (121 MHz, CD_3OD) δ 21.1(br, $W_{1/2}$=69 Hz). ^{13}C NMR (75 MHz,CD_3OD) δ 167.2, 165.2(low broad signal), 161.1(low broad signal), 139.5, 139.2, 139.1, 129.44, 129.34, 128.96, 128.91, 128.82, 128.68, 84.5, 84.17, 84.11, 83.66, 83.56, 81.16, 81.07, 74.2, 72.77, 72.67, 72.5, 66.1(d, J = 6.5 Hz, $POCH_2$), 65.8(d, J = 4.8 Hz, $POCH_2$), 53.6(apparent triplet; overlapping doublets, J = 4.7, 4.8 Hz, $POCH_3$). UV (Absolute EtOH) λ_{max} = 292 nm, ϵ = 10,000. MS [FAB+, glycerol matrix] 622 [M + H^+], 714 [M+ H^+ + glycerol].

Debenzylation of alpha isocytosine 26

An excess of boron trichloride [4.0 mL, 1.0 M in CH_2Cl_2, 4.0 mmol]was added to a cold (-78°C) suspension of compound **26** [0.220g, 0.35 mmol] in methylene chloride (3 mL) under an argon atmosphere. The reaction mixture was stirred at -78°C for 2 h, warmed to room temperature over 1.5 h and then cooled to -78°C. Methanol (10 mL) was added over *ca.* 5 min and the solution was warmed to room temperature and then evaporated to dryness. The crude residue was taken up in *ca.* 10 mL of methanol, filtered through anhydrous potassium carbonate (*ca.* 0.5 g) and the filtrate was then concentrated to a volume of *ca.* 2 mL. Chromatography on cellulose plates [Merck Cellulose F, 0.1mm thickness; *(ca.*20 mg/plate) 3:1 ethanol-1M ammonium acetate in H_2O, R_f = 0.43] and extraction of the cellulose with water afforded a partially purified sample of product **28**. Chromatography on C-18 sep-pak columns [using H_2O and then 20% CH_3CN in H_2O to effect

separation of the salts from the product] then afforded product **28** [0.055 g, 0.156 mmol, 44% yield] as an off-white foam. R_f = 0.44[TLC, silica, 7:2:1 *iso*-propanol - H_2O -NH_4OH]. The TLC shows the presence of a minor impurity at R_f = 0.22, although the NMR data appears to be consistent with that of only the two diastereoisomers of **28**. MPt (foam) : 105-110°C. ^1H NMR (200 MHz, DMSO-d_6) 7.98(d, J = 9.3 Hz, 1H, *H*-6 base), 7.28(low broad signal, 1H, C-4 *OH* base), 5.21(m, 1H, C-4 or C-3 *OH* of sugar), 5.14(m, 1H, C-4 or C-3 *OH* of sugar), 4.70(t, J = 5.5Hz, 1H, C-6 *OH* sugar), 4.10-3.84(m, 2H), 3.76(m, 3H, includes *H*-3' and *H*-4' of sugar), 3.68-3.52(m, 4H; contains d at 3.58, J = 11.4 Hz, $POCH_3$), 3.52-3.35(m, 2H, *H'*-6a, H'-6a' of sugar), 3.32(s, "3H", HOD + NH_2). ^1H NMR (200 MHz, D_2O) δ 7.994(d, J = 10.1 Hz, *H*-6 base), 7.986(d, J = 10.1 Hz, *H*-6 base), 4.20-3.76(m, 5H), 3.76-3.40(m, 6H; contains d at 3.61, J = 11.7 Hz, $POCH_3$). The signals at δ 7.994 and 7.986 together integrate to 1H. ^{31}P NMR (121 MHz, D_2O) δ 22.6(br, $W_{1/2}$ = 87 Hz). ^{13}C NMR (75 MHz, D_2O) δ 168.1(low intensity multiplet), 166.1(m, contains doublet at 166.1, J = 14 Hz), 161.5, 101.6(low intensity multiplet), 98.8(low intensity multiplet), 85.08, 85.02, 82.13(d, J = 7.0 Hz, *C*-2 of sugar), 82.95(d, J = 6.6Hz, *C*-2 of sugar), 78.76, 78.62, 78.50, 78.46, 68.30(d, J = 5.5 Hz, *C*-1 of sugar), 68.16(d, J = 5.6Hz, *C*-1 of sugar), 63.5(s, *C*-6 of sugar), 55.92(overlapping doublets at 55.95, J = 5.7 Hz and 55.92, J = 5.4 Hz; $POCH_3$). UV (H_2O) : pH = 7, λ_{max} = 288 nm, ϵ= 11,300 ; pH = 1, λ_{max} = 264 nm, ϵ= 8500; pH = 13, λ_{max} = 278 nm, ϵ = 9000. MS [high resolution CI] m/z : 369.1175274 [100%, (calcd. for $C_{11}H_{18}N_3O_8P + NH_4^+$, 369.11799)].

Debenzylation of beta isocytosine 27

The general procedure used for the debenzylation of **26** was also used for **27**: Boron trichloride [4.0 mL, 1.0 M in CH_2Cl_2, 4.0 mmol], and **27** [0.182 g, 0.293 mmol] in CH_2Cl_2 were mixed together and treated according to the procedure described above. Chromatography of the crude residue on C-18 reverse phase sep-pak columns [using H_2O and then 20% CH_3CN in H_2O as the eluting solvent] allowed a crude purification of the reaction products. Chromatography of the residue on cellulose plates, [this time using 1:1 ethanol-1M ammonium acetate as the solvent system; R_f = 0.45] afforded the major product which was contaminated with some ammonium acetate. A portion of this product was passed through a sodium ion-exchange resin and evaporation of the water gave a pale yellow foam. R_f = 0.09[TLC, silica, 7:2:1 *iso*-propanol-H_2O-NH_4OH]. The ^1H, ^{13}C, and ^{31}P NMR spectra were consistent with the presence of two isomeric compounds in a ratio of *ca.* 3:1 along with the acetate salt. ^1H NMR (200 MHz, D_2O) δ 7.83(d, J = 10.2 Hz, 1.0H), 4.40(m, 0.4H), 4.02(m, 1.4H), 3.96(dd, J = 2.3, 3.5 Hz, 0.3H), 3.92-3.72(m, 1.4H; contains dd at 3.86, J = 4.1, 1.8 Hz). 3.72-3.28(m, 3.5H), 1.74(s, acetate *CH3*; *ca.* 30% weight content and 62% molar content). ^{13}C NMR (75 MHz, D_2O) δ 184.0(s,from acetate carbonyl), 169.4(m), 161.5(m), 160.3. The aliphatic region clearly shows two sets of signals in a ratio of *ca.* 3-4:1, major isomer: δ 87.26, 82.33(d, J = 7.8 Hz), 80.4, 79.5, 65.4(d, J = 4.8 Hz), 64.42 ; minor isomer: δ 87.41, 83.13(d, J = 5.7 Hz), 82.22, 79.73, 64.53* (this may be one of the signals of the doublet which corresponds to the d at 65.4 , with the other half of the signal overlapping with the signal at 64.42*, as the difference between the two* signals is 7.9 Hz), 62.8. The signals from δ 88-78 are CH carbons and those from 70-60 are CH_2 carbons, as shown by a DEPT sequence. ^{31}P NMR (121 MHz, D_2O) δ 12.0, 11.3(3:1). UV (H_2O) : pH = 7,

λ_{max} = 288 nm ; pH = 1, λ_{max} = 262 nm ; pH = 13 λ_{max} = 278 nm. [FAB $^+$, glycerol matrix] m/z : 382.04304 [M($C_{10}H_{15}O_8N_3P_1Na_1$) + Na^+ , calcd. 382.03922].

Sodium salt of alpha isocytosine 30

A suspension of **28** [15.3 mg, 0.0435 mmol] in *t*-butylamine (2 mL) was stirred at reflux. The reaction mixture was monitored periodically by TLC (silica, 7:2:1 *iso*-propanol-H_2O-NH_4OH ; R_f of starting material = 0.5 and R_f of amine salt = 0.2) and after 3 days the excess of reagent was evaporated. Ion-exchange chromatography on Dowex, followed by chromatography on a C-18 reverse phase sep-pak afforded the sodium salt **30** as a pale brown foam (9.8 mg, 0.027 mmol, 62% yield). R_f = 0.3[TLC, silica, 7:2:1 *iso*-propanol-H_2O-NH_4OH]. The 1H and 31P NMR show the presence of two compounds in an 8:1 ratio. ^{1}H NMR (200 MHz, D_2O) δ 7.82(d, J = 10.0 Hz, 0.9H), 7.58(d, J =12.0 Hz, 0.1H),3.98(m, 1H), 3.86(t, J = 6.7 Hz, 1H), 3.82-3.62(m, 4H), 3.64-3.20[m, 2H: contains ABX system; δ 3.59(dd, J = 3.2, 12.5 Hz, and δ 3.49(dd, J = 5.2, 12.5 Hz)]. 13 C NMR (75 MHz, D_2O) δ 169.0(m), 161.6(m), 154.1(d, J = 16.7 Hz), 85.0, 83.8(d, J = 7.8Hz), 79.2, 78.9, 66.71(d, J = 4.8 Hz), 63.7. ^{31}P NMR (121 MHz, D_2O) δ 12.0, and 10.1 (8:1). UV (H_2O) pH = 7 λ_{max} = 290 nm, ϵ = 5500; pH = 1 λ_{max} = 262 nm, ϵ = 5800; pH = 13 λ_{max} = 278 nm, ϵ = 5100. MS [FAB $^+$, glycerol matrix] m/z 382.03935 [M($C_{10}H_{15}O_8N_3P_1Na_1$) + Na^+ , calcd. 382.03922], 369.28 (M + H^+), 338.16(R'RPOH + H^+).

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8 Waters C-18 reverse phase sep-pak columns

9 Dowex 50W-X8, Na+ 20-50 Mesh (Baker)

10 Chromatography on cellulose plates using 1:1 ethanol : 1M ammonium acetate in H_2O as the eluting solvent

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