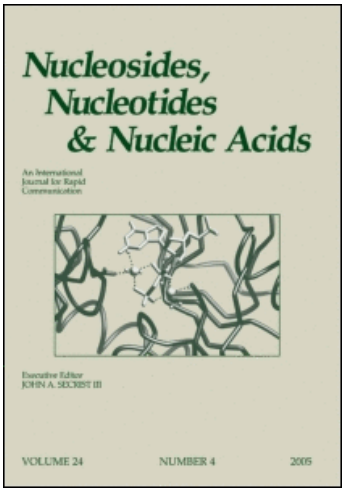

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SYNTHESES OF 2',3'-DIDEOXY-*D*-*C*-NUCLEOSIDES FROM γ-LACTONE

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SYNTHESES OF 2',3'-DIDEOXY-*D*-*C*-NUCLEOSIDES FROM γ-LACTONE

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Abstract: 2',3'-Dideoxy-*D*-*C*-nucleosides, 2,4-diamino-5-(2,3-dideoxy-*D*-glycero-pentofuranosyl)pyrimidines (**11** and **12**), 4-amino-8-(2,3-dideoxy-*D*-glyceropento-furanosyl)pyrazolo[1,5-a]-1,3,5-triazines (**17** and **18**), 4-amino-7-(2,3-dideoxy-*D*-glyceropentofuranosyl)-5H-pyrrolo[3,2-d]pyrimidines (**2** and **25**), 7-(2,3-dideoxy-*D*-glyceropentofuranosyl)-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidines (**30** and **31**) have been synthesized from γ-lactone **4**. These 2',3'-dideoxy-nucleosides were evaluated against HBV and HIV. No significant antiviral activities were found up to 100 μM.

A number of 2',3'-dideoxynucleosides, such as 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxycytidine (ddC), 2',3'-didehydrodideoxythymidine (d4T) and 2',3'-dideoxyinosine (ddI) have shown potent anti-HIV activities.[1] The mechanism of action of these agents involves the sequential phosphorylation to the nucleoside 5'-triphosphate which inhibits the viral reverse transcriptase (RT) as well as acts as a chain terminator.[2] The structure-activity relationships have shown that the presence of a 2,3-dideoxyribo-furanosyl moiety is required to exhibit the potent anti-HIV activity.[1] However, in the case of purine nucleosides such as ddI and 2',3'-dideoxyadenosine (ddA), the 2',3'-dideoxy-modification makes the glycosyl bond unstable under the acidic environment, and thus the bioavailability by oral administration becomes limited.[3] Furthermore, phosphorylases may play an additional role to degrade these nucleosides. In order to circumvent these undesirable chemical properties, the synthesis of 2'-fluoro-nucleosides has been devised.[4,5]

An alternate way to stabilize the glycosyl bond is to synthesize *C*-nucleosides which possess the isosteric C-C bond instead of the C-N bond in *N*-nucleosides. A number of biologically active synthetic as well as natural *C*-nucleosides have been reported.[6] For example, pseudoisocytidine, a *C*-nucleoside analogue of 5-azacytidine exhibited excellent antitumor activity *in vitro*[7] and has undergone clinic trials;[8] 9-deazaadenosine has demonstrated antitumor activity;[9,10] and 9-deazainosine also exhibited antiparasitic

activity.[11,12] Two 2',3'-dideoxy-*C*-nucleosides, 2',3'-dideoxy-pseudoisocytidine (**1**),[13] and 2',3'-dideoxy-9-deazaadenosine (*C*-ddA, **2**)[6,14] have been synthesized as potential antiviral agents (Fig. 1). Compound **2** exhibited moderate anti-HIV activity (ED_{50} 4 μM).[6] These two 2',3'-dideoxy-*C*-nucleosides were previously synthesized by two different routes[6,13,14]. Tam *et al.*[14] treated 9-deazaadenosine with 2-acetoxyisobutyryl bromide to give a mixture of acetylated bromo derivatives. This mixture was converted to an olefin derivative with zinc-copper couple followed by hydrogenation to give *C*-ddA (**2**). *C*-ddA was also synthesized by Watanabe and co-workers[6] from 7-benzyloxymethyl-2'-deoxy-9-deazaadenosine by a selective deoxygenation of the 3'-OH. These methods used the corresponding *C*-nucleosides as the starting material, which do not allow the flexibility for the synthesis of other pyrimidine and purine 2',3'-dideoxy *C*-nucleosides. Herein we wish to report a versatile method for the synthesis of 2',3'-dideoxy-*D*-*C*-nucleosides from γ-lactone **4**, as well as anti-HBV and anti-HIV activities of the synthesized 2',3'-dideoxy-*D*-*C*-nucleosides.

RESULTS AND DISCUSSION

1. SYNTHESIS

For the synthesis of pyrimidine analogues **11** and **12**, 2,3-dideoxy-*D*-glyceropentanoic acid γ-lactone (**4**), prepared by the reported method[15] from 1,2:5,6-di-*O*-isopropylidene-*D*-mannitol in four steps, was treated with trityl chloride in pyridine at refluxing temperature to give 5-*O*-trityl derivative **5** in 87% yield (Scheme 1). The lactone **5** was reduced by DIBAL-H in toluene at -78 °C to give lactol **6** in 91% yield, which was converted to an anomeric mixture of acetonitrile derivatives **7** in 92% yield by Wittig reaction with sodium diethoxy cyanomethylenephosphonate in DME at rt. Formylation of **7** with ethyl formate and *t*-BuOK in a mixture of ether and a catalytic amount of absolute ethanol[16,17] gave a crude potassium enolate **8**, which, without purification, was treated with methyl iodide in DMF to obtain methyl enolate **9**. ^{1}H NMR spectrum showed that the compound **9** was a mixture of α- and β-isomers which was reacted with guanidine in refluxing ethanolic NaOEt to obtain 2,4-diaminopyrimidine derivatives **10** as an anomeric mixture. Detritylation of **10** with methanolic hydrogen chloride followed by preparative TLC separation gave free nucleosides **11** and **12**. The structure and stereochemistry of the compounds **11** and **12** have been determined by ^{1}H NMR spectra, in which the upfield chemical shift of 4'-H (δ 3.93) in **11** (β) was observed in comparison to that (δ 4.08) of **12** (α).[13,18,19]

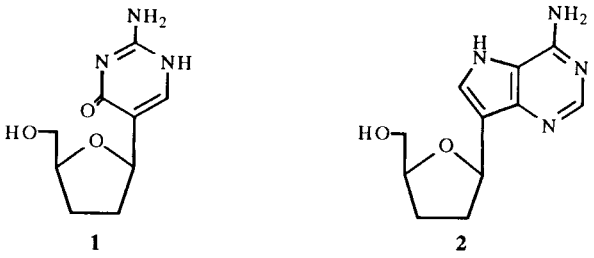
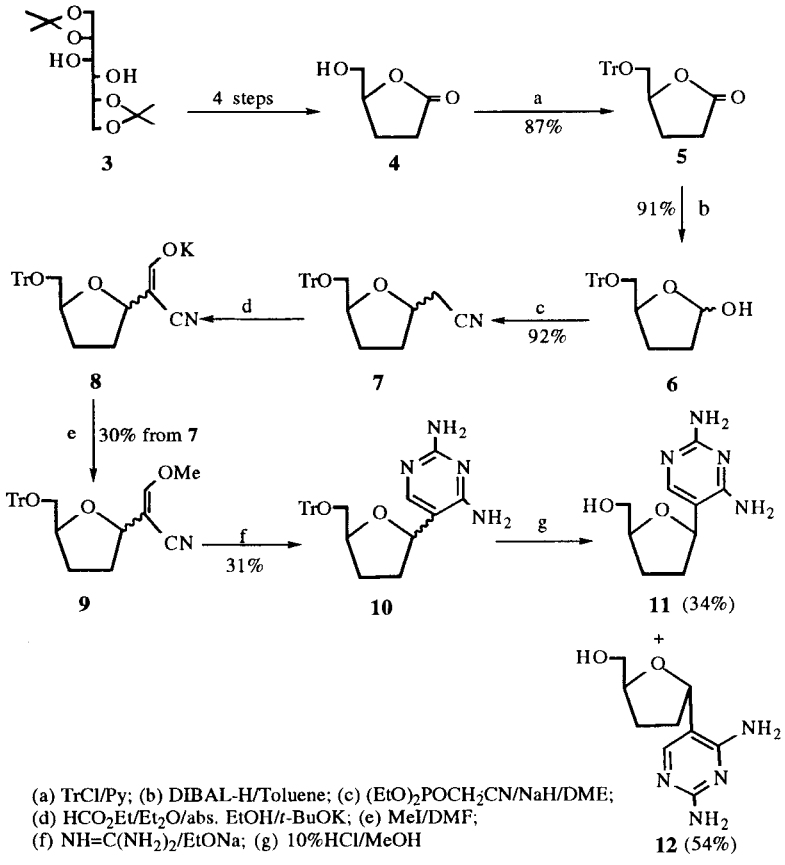


Figure 1



Scheme 1

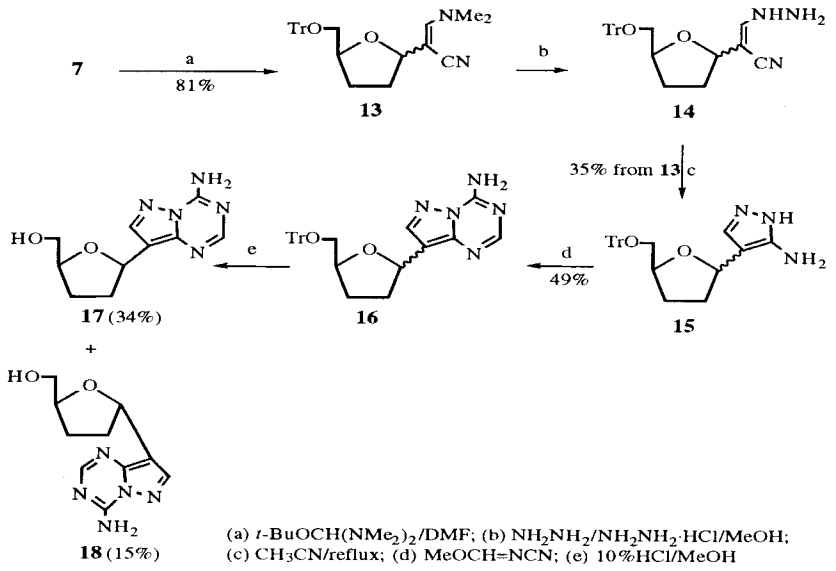
4-Amino-8-(2,3-dideoxy-β-*D*-glyceropentofuranosyl)pyrazolo[1,5-a]-1,3,5-triazines (**17** and **18**) were synthesized from the key intermediate **7** (Scheme 2).[20] The acetonitrile derivative **7** was reacted with bis(dimethylamino)-*t*-butoxymethane in DMF at rt followed by the condensation with hydrazine to obtain the pyrazole derivative **15** as an α- and β- mixture. The pyrazole derivative **15** was treated with methyl *N*-cyano-formimidate in refluxing benzene to give an anomeric mixture **16** which was deprotected and subsequently separated by preparative TLC to afford the free nucleoside **17** (34%) and **18** (15%). The compounds **17** and **18** were characterized by ^{1}H NMR spectroscopy as described for **11** and **12**.

Formylation[19] of **7** with ethyl formate and NaH in a mixture of absolute EtOH and Et_2O gave intermediate **19** which was reacted with aminoacetonitrile hydrochloride in refluxing EtOH in the presence of sodium acetate to give **20** (Scheme 3).[21] *N*-protection of **20** with ethoxycarbonyl chloride and DBN in methylene chloride followed by the cyclization catalyzed by DBN gave an α- and β- mixture **22**. The removal of the carboethoxy group of **22** with sodium carbonate in methanol gave an inseparable α- and β- mixture **23** in 88% yield. Treatment of **23** with formamidine acetate in boiling ethanol gave the pyrrolo-pyrimidine derivative **24** in 95% yield. Compounds **2** (26%) and **25** (35%)[14,6] were obtained by the deprotection of **24** with methanolic hydrogen chloride followed by preparative TLC separation. The structure and stereochemistry of compounds **2** and **25** were confirmed by the method described above.

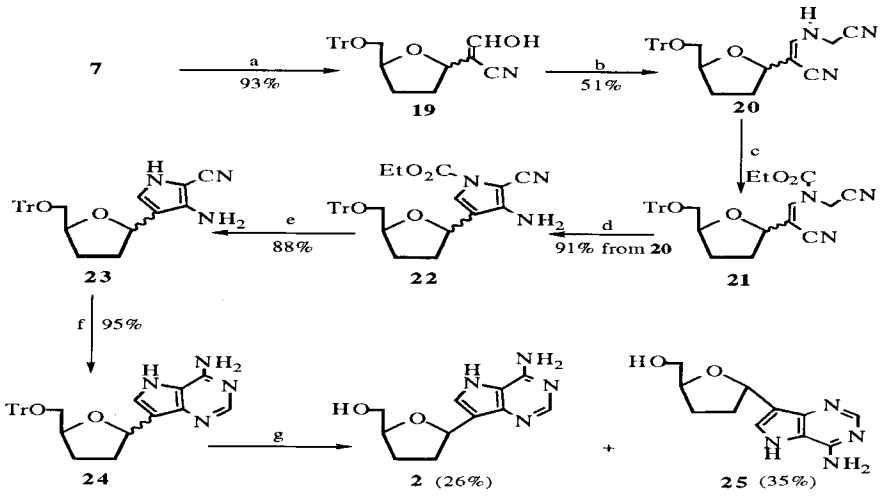
The similar procedure[22,23] was used for the synthesis of 2',3'-dideoxy-9-deazainosine **30** and **31** (Scheme 4). Cyanoaldehyde **19** was condensed with ethyl glycinate hydrochloride in a mixture of water, methanol and sodium acetate to obtain the enamine **26**. *N*-Protection of **26** with ethoxycarbonyl group followed by the cyclization catalyzed by sodium methoxide in methanol gave the pyrrole derivative **28** as a mixture of α- and β- anomers. Compound **28** was treated with formamide acetate in boiling ethanol to give pyrrolo[3,2-d]pyrimidine **29** in 75% yield. The deprotection of compound **29** followed by preparative TLC separation afforded 2',3'-dideoxy-9-deazainosine **30** (70%) and **31** (23%). Again, the assignment for the structure and stereochemistry of compounds **30** and **31** was consistent with those described in Scheme 1-3 (Table 1). The chemical and physical properties of all final nucleosides were consistent with those of their *L*-isomers recently reported by our laboratory.[24]

2. BIOLOGICAL STUDIES

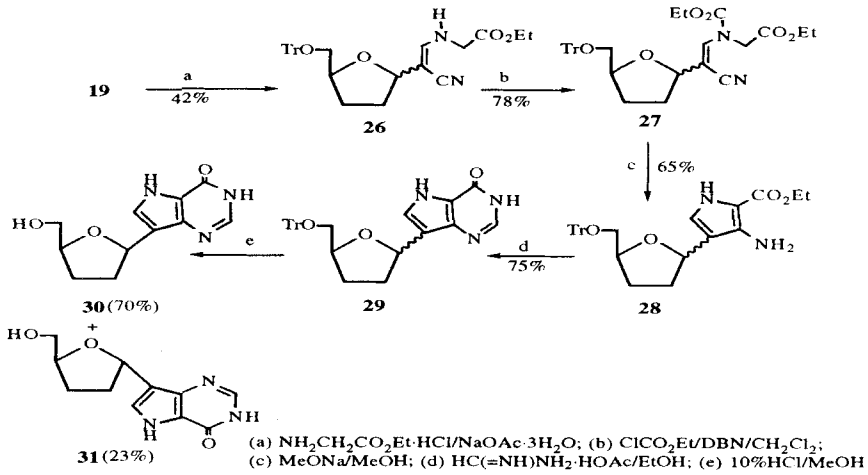
The anti-hepatitis B virus of synthesized *C*-nucleosides were evaluated in 2.2.15 cells and the anti-HIV activity in human peripheral blood mononuclear (PBM) cells infected with



Scheme 2



Scheme 3



Scheme 4

Table 1. Characteristic ^1H NMR Data of 4'-H

Compound	δ	Compound	δ
11	3.93	**12**	4.08
17	3.94	**18**	4.10
2	4.01	**25**	4.12
30	3.95	**31**	4.09

HIV-1 cells. No significant activities against HBV and HIV were found for these *C*-nucleosides up to 100 μM. The toxicities of these *C*-nucleosides have also been assessed in 2.2.15 cells, PBM cells and CEM cells. No significant toxicities were observed for any of these nucleosides with concentrations up to 100 μM.

EXPERIMENTAL SECTION

Melting points were determined on a Mel-temp II laboratory device and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Bruker 250 or 300 MHz spectrometer with tetramethylsilane as the internal reference; chemical shifts (δ) were reported in parts per million. UV spectra were obtained on a Beckman DU-7 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital

polarimeter. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. TLC were performed on Uniplates (silica gel) purchased from Analtech Co.

2,3-Dideoxy-5-*O*-trityl-*D*-glyceropentanoic acid γ-lactone (5). A mixture of 2,3-dideoxy-*D*-glyceropentanoic acid γ-lactone (**4**, 24.5 g, 0.20 mol), trityl chloride (80.0g, 0.30 mol) and pyridine (150 mL) was refluxed for 16 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (30% EtOAc in hexanes) to give compound **5** (65.0 g, 87.0%) as a light yellow solid: mp 142-143 °C; $[\alpha]^{25}_D$ +11.1° (c 0.15, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.20-7.51 (m, 15H, Tr), 4.65 (m, 1H, H-4), 3.43, 3.16 (2xm, 2H, H-5), 2.01-2.73 (m, 4H, H-2, H-3). Anal. Calcd for $C_{24}H_{22}O_3$: C, 80.04; H, 6.18. Found: C, 80.31; H, 6.18.

2,3-Dideoxy-5-*O*-trityl-*D*-glyceropentanoic acid γ-lactol (6). To a solution of **5** (6.0 g, 16.8 mmol) in toluene (100 mL) at -78 °C, DIBAL-H (18.7 mL, 28 mmol, 1.5 M in hexanes) was added slowly, stirred at the temperature for 1 h and the reaction was quenched with MeOH (10 mL). The reaction mixture was warmed to rt, diluted with EtOAc (100 mL), washed with 1N HCl (100 mL), sat. $NaHCO_3$ solution, brine and dried (Na_2SO_4). The solvent was removed to give compound **6** as a slight yellow solid (5.5 g, 91.0%): ^{1}H NMR ($CDCl_3$) δ 7.24-7.59 (m, 15H, Tr), 5.64, 5.54 (2xm, 1H, H-1), 4.50, 4.30 (2xm, 1H, H-4), 3.95, 3.68 (2xs, 1H, OH, D_2O exchangeable), 3.30, 3.12 (m, 2H, H-5), 1.71-2.20 (m, 4H, H-2, H-3). Anal. Calcd for $C_{24}H_{24}O_3$: C, 79.98; H, 6.71. Found: C, 79.93; H, 6.80.

2-(2,3-Dideoxy-5-*O*-trityl-*D*-glyceropentofuranosyl)acetonitrile (7). To a solution of diethoxy cyanomethylenephosphonate (4.85 mL, 30 mmol) in DME, NaH (1.1 g, 28 mmol) was added portionwise at 0 °C and stirred at rt for 30 min. A solution of **6** (5.2 g, 14.6 mmol) in DME (50 mL) was added to the above mixture and stirred at rt for 2 h. The solvent was evaporated and the residue was dissolved in EtOAc (100 mL), washed with brine and dried (Na_2SO_4). The solvent was removed and the residue was purified by silica gel column chromatography (20% EtOAc in hexanes) to give compound **7** (5.1 g, 92.0%) as a light yellow syrup: ^{1}H NMR ($CDCl_3$) δ 7.20-7.46 (m, 15H, Tr), 4.11-4.35 (m, 2H, H-1', H-4'), 3.09-3.10 (m, 2H, H-5'), 2.23-2.61 (m, CH_2CN), 1.72-2.07 (m, 4H, H-2', H-3'). Anal. Calcd for $C_{26}H_{25}NO_2$: C, 81.29; H, 6.68; N, 3.63. Found: C, 81.44; H, 6.56; N, 3.65.

Potassium 2-(2,3-dideoxy-5-*O*-trityl-*D*-glyceropentofuranosyl)-2-formyl acetonitrile enolate (8). To a suspension of *t*-BuOK (5.6 g, 50 mmol) in anhydrous Et_2O (180 mL) and absolute EtOH (2 mL), a mixture of **7** (7.6 g, 20 mmol) and ethyl formate (10 mL) in Et_2O (50 mL) was added and stirred at rt for 16 h. The solvent was evaporated to dryness under reduced pressure to give the crude compound **8** as a brown syrup (9.0 g) which was used in the next reaction without further purification.

2-(2,3-Dideoxy-5-*O*-trityl-*D*-glyceropentofuranosyl)-3-methoxy acrylonitrile (9). To a DMF (50 mL) solution of **8** (9.0 g), methyl iodide (10 mL) was added dropwise and the reaction mixture was stirred at rt for 24 h and evaporated to dryness. The residue was purified by silica gel column chromatography (20% EtOAc in hexanes) to give compound **9** (2.5 g, 30.0%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 7.20-7.48 (m, 15H, Tr); 6.78, 6.77 (2xs, 1H, CH); 4.93, 4.79 (2xt, J=6.3 Hz, 1H, H-1'), 4.10, 4.35 (2xm, 1H, H-4'), 3.81, 3.78 (2xs, 3H, MeO), 3.08-3.11 (m, 2H, H-5'), 1.94-2.15 (m, 4H, H-2', H-3'). Anal. Calcd for $C_{28}H_{27}NO_3 \cdot 0.75H_2O$: C, 76.61; H, 6.54; N, 3.19. Found: C, 76.60; H, 6.46; N, 2.98.

2,4-Diamino-5-(2,3-dideoxy-β-*D*-glyceropentofuranosyl)pyrimidine (11) and 2,4-diamino-5-(2,3-dideoxy-α-*D*-glyceropentofuranosyl)pyrimidine (12). A mixture of **9** (2.6 g, 6 mmol) and guanidine hydrochloride (2.2 g, 20 mmol) in ethanolic sodium ethoxide (80 mL, 1N) was refluxed for 24 h, cooled to rt, neutralized with 1N HCl and evaporated to dryness. The residue was purified by silica gel column chromatography (80% EtOAc in hexanes) to give compound **10** (1.1 g, 31.0%) as a white foam. The compound **10** (100 mg, 0.22 mmol) was dissolved in methanolic hydrogen chloride (10%, 5 mL), stirred at rt for 15 min and neutralized with sat. $NaHCO_3$ to pH 7. After evaporation of the solvent, the residue was purified by preparative TLC (20% MeOH in $CHCl_3$) to obtain compounds **11** (16 mg, 34.0%) and **12** (25 mg, 54.0%) as a white solid after freeze drying. Compound **11**: $[\alpha]^{25}_D$ +11.60° (c 0.30, MeOH); UV (H_2O) λ_{max} 269.5 (ε 6020, pH 2), 277.2 nm (ε 5870, pH 7), 285.2 nm (ε 7500, pH 11); ^{1}H NMR($CDCl_3$) δ 7.58 (s, 1H, H-6), 6.27 (br s, 2H, NH_2, D_2O exchangeable), 5.78 (br s, 2H, NH_2, D_2O exchangeable), 5.00 (br s, 1H, OH, D_2O exchangeable), 4.52 (t, J=7.4 Hz, 1H, H-1'), 3.93 (m, 1H, H-4'), 3.51-3.37 (m, 2H, H-5'), 1.82-1.88 (m, 4H, H-2', H-3'). Anal. Calcd for $C_9H_{14}N_4O_2 \cdot 0.9H_2O$: C, 47.76; H, 7.03; N, 24.76. Found: C, 47.82; H, 6.90; N, 24.56.

Compound **12**: $[\alpha]^{25}_D$ +3.60° (c 0.28, MeOH); UV (H_2O) λ_{max} 270.2 nm (ε 5880, pH 2), 277.5 nm (ε 6200, pH 7), 285.5 nm (ε 7840, pH 11); ^{1}H NMR ($CDCl_3$) δ 7.62 (s, 1H, H-6), 6.33 (br s, 2H, NH_2, D_2O exchangeable), 5.85 (br s, 2H, NH_2, D_2O exchangeable), 4.69 (t, J=5.2 Hz, 1H, H-1'), 4.08 (m, 1H, H-4'), 3.49-3.54 (m, 2H, H-5'), 1.71-2.10 (m, 4H, H-2', H-3'). Anal. Calcd for $C_9H_{14}N_4O_2 \cdot 1.25H_2O$: C, 46.45; H, 7.14; N, 24.08. Found: C, 46.26; H, 6.86; N, 24.42.

3-Dimethylamino-2-(2,3-dideoxy-5-*O*-trityl-*D*-glyceropentofuranosyl) acrylonitrile (13). A mixture of **7** (8.0 g, 21 mmol) and bis(dimethylamino)-*tert*-butoxymethane (10 g, 57 mmol) in DMF (30 mL) was stirred at rt for 24 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (30% EtOAc in Hexane) to give a pale yellow syrup **13** (7.4 g, 81.0%):

UV (MeOH) λ_{max} 275 nm; IR (neat) 2187, 1635 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.18-7.46 (m, 15H, Tr), 6.48, 6.43 (2xs, 1H, H-2), 4.24-4.36 (m, 1H, H-1'), 4.03-4.10 (m, 1H, H-4'), 3.35-3.11 (m, 2H, H-5'), 3.01, 2.97 (2xs, 6H, Me), 1.76-2.08 (m, 4H, H-2', H-3'). Anal. Calcd for $C_{29}H_{30}N_2O_2 \cdot 0.25H_2O$: C, 78.64; H, 6.89; N, 6.32. Found: C, 78.51; H, 6.94; N, 6.22.

3-Amino-2-(2,3-dideoxy-5-*O*-trityl-*D*-glyceropentofuranosyl)pyrazole (15). A mixture of **13** (3.5 g, 8 mmol), MeOH (20 mL), anhydrous hydrazine (6 mL), H_2O (1 mL) and hydrazine hydrochloride (0.80 g) was refluxed for 24 h. The mixture was concentrated to dryness under reduced pressure and the residue partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with H_2O, dried (Na_2SO_4) and evaporated to dryness to obtain **14** as a pale yellow syrup. The crude **14** was dissolved in CH_3CN (80 mL) and refluxed for 2 h. The mixture was evaporated to dryness and the residue was purified by silica gel column chromatography (80% EtOAc in Hexane) to give a white foam **15** (1.20 g, 35.0%) as a mixture of α- and β-isomers: ^{1}H NMR ($CDCl_3$) δ 7.16-7.49 (m, 16H, H-2, Tr), 4.84, 4.95 (2xt, J=6.1, 8.0 Hz, 1H, H-1'), 4.31, 4.19 (2xm, 1H, H-4'), 3.70 (br s, 3H, NH, NH_2, D_2O exchangeable), 3.08-3.22 (m, 2H, H-5'), 1.97-2.08 (m, 4H, H-2', H-3'). Anal. Calcd for $C_{27}H_{27}N_3O_2 \cdot 1.25H_2O$: C, 72.39; H, 6.63; N, 9.38. Found: C, 72.42; H, 6.97; N, 9.18.

4-Amino-8-(2,3-dideoxy-5-*O*-trityl-*D*-glyceropentofuranosyl)pyrazolo-[1,5-a]-1,3,5-triazine (16). A mixture of the pyrazole derivative **15** (650 mg, 1.5 mmol) and methyl *N*-cyanoformimidate (0.3 g, 3.0 mmol) in benzene (20 mL) was refluxed for 16 h. The solvent was evaporated and the residue was purified by silica gel column chromatography (80% EtOAc in hexanes) to give a white foam **16** (357 mg, 49%) as an α- and β- mixture: UV (MeOH) λ_{max} 270.5 nm; ^{1}H NMR ($CDCl_3$) δ 8.16, 8.13 (2xs, 1H, H-2); 8.08, 8.05 (2xs, 1H, H-7), 8.70 (br s, 2H, NH_2, D_2O exchangeable), 7.18-7.46 (m, 15H, Tr), 5.28-5.33(m, 1H, H-1'), 4.46, 4.29 (2xm, 1H, H-4'), 3.09-3.29 (m, 2H, H-5'), 1.89-2.33 (m, 4H, H-2', H-3'). Anal. Calcd for $C_{29}H_{27}N_5O_2 \cdot 1.0H_2O$: C, 70.29; H, 5.89; N, 14.13. Found: C, 70.49; H, 5.85; N, 14.17.

4-Amino-8-(2,3-dideoxy-β-*D*-glyceropentofuranosyl)pyrazolo[1,5-a]-1,3,5-triazine (17) and 4-amino-8-(2,3-dideoxy-α-*D*-glyceropentofuranosyl) pyrazolo[1,5-a]-1,3,5-triazine (18). A mixture of **16** (370 mg, 0.77 mmol) and methanolic hydrogen chloride (10%, 10 mL) was stirred at rt for 10 min and neutralized with sat. $NaHCO_3$. The solvent was evaporated and the residue was purified by preparative TLC (10% MeOH in CH_2Cl_2) to afford individual isomers **17** (62 mg, 34.0%) and **18** (27 mg, 15.0%) as a white solid after recrystallization from MeOH-CH_2Cl_2-hexanes. Compound **17**: mp 176-178 °C; $[\alpha]^{25}_D$ +16.10° (c 0.50, MeOH); UV (H_2O) λ_{max} 266.0 nm (ε 5840, pH 2), 273.0 nm (ε 8820, pH 7), 273.2 nm (ε 9720, pH 11); ^{1}H NMR

(DMSO-d_6) δ 8.85 (br s, 1H, NH, D_2O exchangeable), 8.34 (br s, 1H, NH, D_2O exchangeable), 8.17 (s, 1H, H-2), 8.02 (s, 1H, H-7), 5.01 (t, *J*=7.0 Hz, 1H, H-1'), 4.76 (t, *J*=5.8 Hz, 1H, 5'-OH, D_2O exchangeable), 3.94 (m, 1H, H-4'), 3.45 (m, 2H, H-5'), 1.83-2.16 (m, 4H, H-2', H-3'). Anal. Calcd for $C_{10}H_{13}N_5O_2 \cdot 0.5\,H_2O$: C, 49.18; H, 5.77; N, 28.67. Found: C, 49.37; H, 5.45; N, 28.42.

Compound **18**: mp 150-152 °C $[\alpha]^{25}_D$ -0.75 ° (c 0.50, MeOH); UV (H_2O) λ_{max} 264.5 nm (ε 6250, pH 2), 273.2 nm (ε 7820, pH 7), 273.5 nm (ε 10720, pH 11); ^{1}H NMR (DMSO-d_6) δ 8.63 (br s, 1H, NH, D_2O exchangeable), 8.33 (br s, 1H, NH, D_2O exchangeable), 8.14 (s, 1H, H-2), 8.03 (s, 1H, H-7), 5.14 (t, *J*=7.0 Hz, 1H, H-1'), 4.67 (t, *J*=5.8 Hz, 1H, 5'-OH, D_2O exchangeable), 4.10 (m, 1H, H-4'), 3.40 (m, 2H, H-5'), 1.74-2.22 (m, 4H, H-2', H-3'). Anal. Calcd for $C_{10}H_{13}N_5O_2 \cdot 0.46\,H_2O$: C, 49.70; H, 5.76; N, 28.76. Found: C, 49.70; H, 5.41; N, 28.36.

2-Formyl-2-(2,3-dideoxy-5-*O*-trityl-*D*-glyceropentofuranosyl)-acetonitrile (19). To a suspension of NaH (1.6 g, 60% in mineral oil) in anhydrous Et_2O (200 mL) and absolute EtOH (2 mL), a mixture of compound **7** (20 g, 48 mmol) and ethyl formate (10 mL) in Et_2O (50 mL) was added dropwise, stirred at rt for 16 h and water (200 mL) was added. The aqueous layer was separated, neutralized to pH 6-7 with 1 N HCl and then extracted with $CHCl_3$ (3x100 mL). The combined $CHCl_3$ solution was dried (Na_2SO_4) and evaporated to dryness to give crude compound **19** (21.0 g, 93.0%) which was used in the next reaction without further purification.

3-Amino-1-carboethoxy-4-(2,3-dideoxy-5-*O*-trityl-*D*-glyceropento-furanosyl)-1H-2-cyanopyrrole (22). A mixture of the crude **19** (4.1 g, 10 mmol), MeOH (40 mL), H_2O (2 mL), aminoacetonitrile hydrochloride (2.8 g, 20 mmol) and NaOAc·$3H_2O$ (2.1 g, 12.6 mmol) was stirred at 60 °C for 2 h. The solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc (100 mL), washed with brine, dried (Na_2SO_4) and evaporated to dryness. The residue was purified by flash silica gel chromatography (30% EtOAc in hexanes) to afford **20** (2.3 g, 51.0%) as a white foam. To a mixture of enamine **20** (1.8 g, 4 mmol) and DBN (1.2 g, 10 mmol) in CH_2Cl_2 (20 mL), ethyl chloroformate (0.9 g, 8 mmol) was added at 0 °C and stirred at rt for 24 h to obtain **21** as a crude mixture. To the mixture, additional DBN (0.5 g, 5 mmol) was added, stirred at rt for 24 h, diluted with CH_2Cl_2 (50 ml), washed with water and dried (Na_2SO_4). Solvents were removed and the residue was purified by silica gel column chromatography (30% EtOAc in hexanes) to afford an anomeric mixture **22** (1.9 g, 91.0%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 7.24-7.48 (m, 15H, Tr), 7.12, 7.10 (m, 1H, CH), 4.93, 4.78 (2xt, *J*=6.8, 7.0 Hz, 1H, H-1'), 4.42 (m, 4H, OCH_2, NH_2, D_2O exchangeable), 4.27 (m, 1H, H-4'), 3.16 (m, 2H, H-5'), 1.70-2.20 (m, 4H, H-2', H-3'), 1.42 (t, *J*=6.8 Hz, 3H, Me). Anal. Calcd for $C_{32}H_{31}N_3O_4 \cdot 0.5H_2O$: C, 72.44; H, 6.07; N, 7.92. Found: C, 72.65; H, 6.03; N, 7.59.

3-Amino-4-(2,3-dideoxy-5-*O*-trityl-*D*-glyceropentofuranosyl)-1H-2-cyanopyrrole (23). A mixture of enamine **22** (1.35 g, 2.60 mmol) and Na_2CO_3 (30 mg, 0.26 mmol) in MeOH (20 mL) was stirred at rt for 1 h, neutralized with HOAc and concentrated to dryness. The residue was purified by silica gel column chromatography (30% EtOAc in hexanes) to afford an anomeric mixture **23** (1.0 g, 88.0%) as a white foam: ^{1}H NMR ($CDCl_3$): δ 8.05, 8.15 (2xs, 1H, NH), 7.20-7.51 (m, 15H, Tr), 6.51 (dd, H, H-5), 4.92-4.80 (m, 1H, H-1'), 4.30, 4.20 (2xm, 1H, H-4'), 4.06 (br s, 2H, NH_2, D_2O exchangeable), 3.10-3.27 (m, 2H, H-5'), 1.83-2.15 (m, 4H, H-2', H-3'). Anal. Calcd for $C_{32}H_{31}N_3O_2 \cdot 1.0H_2O$: C, 74.50; H, 6.25; N, 8.99. Found: C, 74.88; H, 6.26; N, 8.63.

7-(2,3-Dideoxy-5-*O*-trityl-*D*-glyceropentofuranosyl)-4-amino-5H-pyrrolo[3,2-d]pyrimidine (24). A mixture of **23** (339 mg, 1 mmol) and formamidine acetate (0.3 g, 3 mmol) in ethanol (10 mL) was refluxed for 5 h and concentrated to dryness. The residue was purified by silica gel column chromatography (5% MeOH in $CHCl_3$) to obtain an anomeric mixture **24** (440 mg, 95.0%) as a white foam: UV (MeOH) λ_{max} 270.2 nm; ^{1}H NMR ($CDCl_3$) δ 8.40 (br s, 1H, NH, D_2O exchangeable), 8.01 (s, 1H, H-2), 7.15-7.51 (m, 16H, Tr, H-6), 6.89 (s, 2H, NH_2, D_2O exchangeable), 5.21 (m, 1H, H-1'), 4.39, 4.32 (2xm, 1H, H-4'), 3.11-3.25 (m, 2H, H-5'), 1.76-2.37 (m, 4H, H-2', H-3'). Anal. Calcd for $C_{30}H_{28}N_3O_2$: C, 77.90; H, 6.10; N, 9.08. Found: C, 77.69; H, 6.19; N, 9.37.

7-(2,3-Dideoxy-β-*D*-glyceropentofuranosyl)-4-amino-5H-pyrrolo[3,2-d]pyrimidine (2) and 7-(2,3-dideoxy-α-*D*-glyceropentofuranosyl)-4-amino-5H-pyrrolo[3,2-d]pyrimidine (25). A mixture of **24** (380 mg, 0.82 mmol) and methanolic hydrogen chloride (10%, 10 mL) was stirred at rt for 10 min and neutralized with sat. $NaHCO_3$. The solvent was evaporated and the residue was purified by silica gel column chromatography (10% MeOH in CH_2Cl_2) to afford the desired nucleosides **2** (50 mg, 26.0%) and **25** (68 mg, 35.0%) as a white solid after recrystallization from MeOH-CH_2Cl_2-hexanes. Compound **2**: $[\alpha]^{25}_D$ +16.30 ° (c 0.50, MeOH); UV (H_2O) λ_{max} 274.0 nm (ε 6820, pH 2); 273.5 nm (ε 8240, pH 7) ; 273.2 nm (ε 9720, pH 11); ^{1}H NMR (DMSO-d_6) δ 10.82 (br s, H, NH, D_2O exchangeable), 8.02 (s, 1H, H-2), 7.45 (s, 1H, H-6), 6.76 (s, 2H, NH_2, D_2O exchangeable), 5.85 (br s, 1H, 5'-OH, D_2O exchangeable), 4.99 (dd, *J*=5.5, 8.8 Hz, 1H, H-1'), 4.01 (m, 1H, H-4'), 3.35-3.58 (m, 2H, H-5'), 1.90-2.23 (m, 4H, H-2', H-3'). Anal. Calcd for $C_{11}H_{14}N_4O_2 \cdot 0.7H_2O$: C, 53.52; H, 6.28; N, 22.70. Found: C, 53.41; H, 5.88; N, 22.52.

Compound **25**: $[\alpha]^{25}_D$ -9.90 ° (c 0.13, MeOH); UV (H_2O) λ_{max} 274.2 nm (ε 7840, pH 2), 273.7 nm (ε 9720, pH 7), 273.2 nm (ε 9430, pH 11); ^{1}H NMR (DMSO-d_6) δ 10.91 (br s, 1H, NH, D_2O exchangeable), 8.06 (s, 1H, H-2), 7.42 (s, 1H, H-6), 6.69 (s, 2H, NH_2, D_2O exchangeable), 5.15 (t, *J*=6.9 Hz, 1H, H-1'), 4.65 (t, *J*=5.4 Hz, 1H, 5'-OH,

D_2O exchangeable), 4.12 (m, 1H, H-4'), 3.29-3.50 (m, 2H, H-5'), 1.65-2.22 (m, 4H, H-2' H-3'). Anal. Calcd for $C_{11}H_{14}N_4O_2 \cdot 0.5CH_2Cl_2$: C, 49.92; H, 5.46; N, 20.25. Found: C, 49.98; H, 5.88; N, 21.52.

Ethyl *N*-[2-(2,3-dideoxy-5-*O*-trityl-*D*-glyceropentofuranosyl)-2-cyanovinyl]glycinate (26). A mixture of **19** (4.1 g, 10 mmol), MeOH (40 mL), H_2O (2 mL), ethyl glycinate hydrogen chloride (2.1 g, 15 mmol) and $NaOAc \cdot 3H_2O$ (2.1 g, 12.6 mmol) was stirred at 60 °C for 2 h. The mixture was evaporated under reduced pressure and the residue was dissolved in EtOAc (100 mL), washed with brine, dried (Na_2SO_4) and evaporated to dryness. The resulting syrup was purified by flash silica gel chromatography (20% EtOAc in hexanes) to obtain **26** (2.1 g, 42.0%) as a white foam: ^1H NMR ($CDCl_3$) δ 7.21-7.49 (m, 15H, Tr); 6.5-6.72 (m, 1H, CH), 5.04 (br s, 1H, NH, D_2O exchangeable), 3.78-4.46 (m, 6H, H-1', H-4', CH_2O, NCH_2), 3.06-3.14 (m, 2H, H-5'), 1.70-2.17 (m, 4H, H-2', H-3'), 1.20-1.32 (m, 3H, Me). Anal. Calcd for $C_{31}H_{32}N_2O_4$: C, 74.98; H, 6.49; N, 5.64. Found: C, 74.72; H, 6.53; N, 5.62.

7-(2,3-Dideoxy-5-*O*-trityl-*D*-glyceropentofuranosyl)-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidine (29). To a mixture of enamine **26** (2.0 g, 4 mmol) and DBN (1.2 g, 10 mmol) in CH_2Cl_2 (20 mL), ethyl chloroformate (0.9 g, 8 mmol) was added at 0 °C, stirred at rt for 16 h, diluted with CH_2Cl_2 (50 mL), washed with water and dried (Na_2SO_4). The solvent was removed and the residue was purified by silica gel column chromatography (20% EtOAc in hexanes) to give an anomeric mixture **27** (1.8 g, 78.0%) as a syrup. Enamine **27** (1.65 g, 2.90 mmol) was dissolved in methanolic sodium methoxide (10 mL, 0.5N), stirred at rt for 1 h and neutralized with HOAc. The reaction mixture was evaporated to dryness and the residue was purified by silica gel column chromatography (30% EtOAc in hexanes) to obtain an anomeric mixture **28** (0.9 g, 65.0%) as a white foam. A mixture of **28** (1.0 g, 2 mmol) and formamidine acetate (1.5 g, 20 mmol) in ethanol (20 mL) was refluxed for 4 days and evaporated to dryness. The residue was purified by silica gel column chromatography (5% MeOH in $CHCl_3$) to afford an anomeric mixture **29** (0.72 g, 75.0%) as a white foam: UV (MeOH) λ_{max} 260.2 nm; ^1H NMR ($CDCl_3$) δ 10.98, 10.68, 10.55, 10.30 (4xs, 2H, 2xNH, D_2O exchangeable), 8.19, 8.08 (2xs, 1H, H-2), 7.17-7.50 (m, 16H, Tr, H-6), 5.33 (m, 1H, H-1'), 4.46, 4.30 (2xm, 1H, H-4'), 3.09-3.30 (m, 2H, H-5'), 1.86-2.36 (m, 4H, H-2', H-3'). Anal. Calcd for $C_{30}H_{27}N_3O_3 \cdot 1.0H_2O$: C, 72.71; H, 5.89; N, 8.48. Found: C, 72.90; H, 5.87; N, 8.23.

7-(2,3-Dideoxy-β-*D*-glyceropentofuranosyl)-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidine (30) and 7-(2,3-dideoxy-α-*D*-glyceropentofuranosyl)-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidine (31). A mixture of **29** (700 mg, 1.47 mmol) and methanolic hydrogen chloride (10%, 20 mL) was stirred at rt for 10 min and neutralized

with sat. $NaHCO_3$. The solvent was evaporated and the residue was separated by silica gel column chromatography (10% MeOH in $CHCl_3$) to afford individual isomers **30** (240 mg, 70.0%) and **31** (80 mg, 23.0%) as a white solid after recrystallization from MeOH-$CHCl_3$-hexanes. Compound **30**: $[\alpha]^{25}_D$ +29.70° (c 0.21, MeOH); UV (H_2O) λ_{max} 260.0 nm (ε 7840, pH 2); 261.0 nm (ε 8720, pH 7), 267.0 nm (ε 4720, pH 11); ^1H NMR (DMSO-d_6) δ 11.93 (br s, 2H, 2xNH, D_2O exchangeable), 7.77 (s, 1H, H-2), 7.33 (s, 1H, H-6), 4.97 (m, 2H, H-1', 5'-OH, D_2O exchangeable), 3.95 (m, 1H, H-4'), 3.33 (m, 2H, H-5'), 1.86-2.36 (m, 4H, H-2', H-3'). Anal. Calcd for $C_{11}H_{13}N_3O_3 \cdot 0.25CHCl_3$: C, 50.97; H, 5.03; N, 15.85. Found: C, 51.30; H, 5.29; N, 15.62:

Compound **31**: $[\alpha]^{25}_D$ +2.07° (c 0.23, MeOH); UV (H_2O) λ_{max} 259.0 nm (ε 6820, pH 2), 261.0 nm (ε 7840, pH 7), 267.2 nm (ε 5240, pH 11); ^1H NMR (DMSO-d_6) δ 11.92 (br s, 1H, NH, D_2O exchangeable), 11.83 (br s, 1H, NH, D_2O exchangeable), 7.76 (s, 1H, H-2), 7.27 (s, 1H, H-6), 5.01 (t, 1H, *J*=5.2 Hz, H -1'), 4.62 (t, *J*=7.0 Hz, 5'-OH, D_2O exchangeable), 4.09 (m, 1H, H-4'), 3.31 (m, 2H, H-5'), 1.70-2.16 (m, 4H, H-2', H-3'). Anal. Calcd for $C_{11}H_{13}N_3O_3 \cdot 0.25CHCl_3$: C, 50.97; H, 5.03; N, 15.85. Found: C, 51.24; H, 5.26; N, 15.70.

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