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Synthesis and Antiviral Activity of Prodrugs of the Nucleoside 1-[2′,3′-Dideoxy-3′-C-(hydroxymethyl)-β-D-Erythropentofuranosyl] Cytosine

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Abstract—The synthesis and antiviral evaluation of 21 prodrugs of 1-[2′,3′-dideoxy-3′-C-(hydroxymethyl)-β-D-erythropentofuranosyl] cytosine **1** is reported. Cytosine N^4-imine analogues were prepared by condensation of **1** with selected formamide dimethyl acetals. Amino acid substituted prodrugs were prepared from **1** or imine prodrug **2** by coupling with either *N*-*tert*-butoxycarbonyl (*t*-Boc)-L-valine or *N*-*t*-Boc-L- phenylalanine in the presence of dicyclohexycarbodiimide (DCC) and 4-dimethylaminopyridine (4-DMAP). Deprotection of the *t*-Boc protecting group was achieved with trifluoroacetic acid (TFAA) in methylene chloride. Cytosine N^4-amide analogues were prepared by reaction of **1** with appropriate anhydrides in aqueous dioxane. Triacylated analogue **22** was prepared by reaction of **1** with four equivalents of benzoyl chloride in pyridine. Prodrugs were evaluated for activity against duck hepatitis B virus, herpes simplex virus types 1 and 2, human cytomegalovirus, and human immunodeficiency virus. A number of analogues were found comparable in activity to **1** with the cytosine N^4-imine series more active than the amino acid substituted and cytosine N^4-amide prodrugs. Slight to moderate cellular toxicity was observed with some analogues.

Introduction

The need for effective therapeutics for the treatment of viral infections such as those caused by human immunodeficiency virus (HIV), hepatitis B virus, and herpes viruses, has prompted the synthesis and development of numerous biologically active nucleoside analogues.[1,2] Zidovudine (3′-azido-3′-deoxythymidine, AZT) was the first nucleoside analogue approved for the treatment of AIDS, but has undesirable side effects that include bone marrow suppression, leucopenia, and anemia.[3] It has been demonstrated that AZT therapy can result in the emergence of viral resistance,[4] requires frequent dosing due to its short plasma half-life,[5] and does not involve crossing of the blood–brain barrier.[6] In this regard, novel and safe nucleosides with enhanced pharmacological properties are urgently needed.

The mechanism of action of most nucleoside analogues is dependent, at least in part, upon their phosphorylation by viral/cellular nucleoside kinases. This results in the formation of the triphosphate form of the nucleoside analogue, which is then responsible for inhibition of the viral nucleic acid polymerase leading to the disruption of viral nucleic acid replication. AZT is phosphorylated by a host cell nucleoside kinase to the triphosphate form which inhibits the reverse transcriptase of HIV. Furthermore, AZT has a 3′-azido substituent instead of a 3′-hydroxyl group. Because of the lack of a 3′-hydroxyl group, when AZT is incorporated into DNA, 3′ to 5′ phosphodiester linkages cannot be made leading to the termination of the nascent DNA chain.

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Although much work has targeted the treatment of infections caused by HIV, nucleoside analogues display activity against other viruses as well. Other nucleoside analogues, such as (E)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) which is selectively potent against herpes simplex type 1 (HSV-1),[7] are active against herpes viruses, which encode a thymidine kinase that selectively phosphorylates some nucleoside analogues. Another nucleoside analogue, 5-iodo-2′-deoxyuridine (IDU), is effective for the treatment of herpes simplex keratitis although with lesser selectivity.[8] In the area of hepatitis, nucleoside analogues such as 3-TC[9] have shown promise as inhibitors of viral replication.

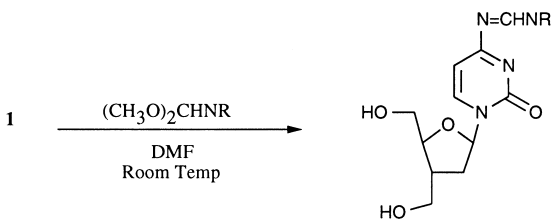
In the search for nucleoside analogues with greater antiviral activity and enhanced selectivity, the incorporation of a hydroxymethylene group at the 3′ position of the sugar moiety of the nucleoside analogue has been considered.[10,11] It has been known for some time that branch-chained sugar nucleosides are biologically active,[12] possibly because of greater recognition of these substrates by polymerase enzymes as compared to non-branched nucleoside analogues.[13] Oxetanocin, a naturally occurring purine nucleoside, and its analogues are prototypical of this class which demonstrates efficacy against infections caused by HIV-1,[14] and herpes viruses.[15] Another branch-chained sugar containing nucleoside analogue, 1-[2′,3′-dideoxy-3′-C-(hydroxymethyl)-β-D-erythropentofuranosyl] cytosine **1** (Figure 1), was found to be a potent inhibitor of HIV-1 replication in vitro (inhibition of HIV replication by 50% at a concentration of 0.01 μM).[11] Due to the branch-chained nature of its sugar moiety, **1** was investigated further for activity against a broader spectrum of viruses.

Although **1** was found to be highly water soluble (S. C. Mauldin, unpublished observation), it was our concern that it might not be transported into the CNS efficiently. Considerable evidence indicates that transport of certain nucleosides into the CNS occurs.[16,17] However, there appears to be a preference for transport of thymidine and uridine analogues rather than cytidine derivatives. For example, AZT readily enters the cerebrospinal fluid whereas ddC is more limited in its ability to penetrate the blood–brain barrier.[16] Based upon the relationship of lipophilicity to membrane permeability[18,19] many examples have been reported regarding modification of nucleosides in an effort to enhance CNS penetration. These include 5′ esterification of the parent nucleoside,[20] phosphate modification of nucleotides,[21,22] or derivatization of the nucleoside base.[23]

We chose to modify **1** to provide a variety of prodrugs with derivitization at the cytidine amino group, deoxy sugar 5′ and 6′ alcohols, or a combination of both in the hopes that such a strategy would provide useful information relating to the three potential H-donor sites of **1** (cytidine NH_2, 5′, 6′ OH). Initially, we sought a method for selective derivatization of the cytosine amine and were especially intrigued by the formation of *N*,*N*-dialkylaminomethylene derivatives of 2′,3′-dideoxycytidine (ddC) and its 3′-fluoro derivative as reported by Kerr and Kalman.[23] We prepared a series of these derivatives for compound **1**. Additionally, we incorporated amino acid substitution into the nucleosides since the amino acid transport system for delivery into the CNS is well defined.[20] A number of cytosine amine (N^4-substituted) analogues and a triacylated derivative were also synthesized for comparison.[24,25] This paper describes the preparation and antiviral activities of the foregoing various prodrugs of **1**.

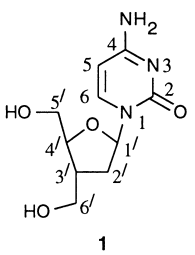
Chemistry

Parent nucleoside **1** was prepared according to the procedure of Svansson et al.[11] The preparation of the cytosine N^4-substituted imines was accomplished as shown in Scheme 1. The requisite formamide acetals were



Compd	NR	% Yield
2	$N(CH_3)_2$	80
3	$N(i\text{pr})_2$	55
4	$N(\text{pr})_2$	80
5	$N(CH_2)_4$	90
6	$N(CH_2)_5$	82
7	$N(CH_2)_6$	76
8	$N(CH_2CH_2)_2O$	66

Scheme 1.

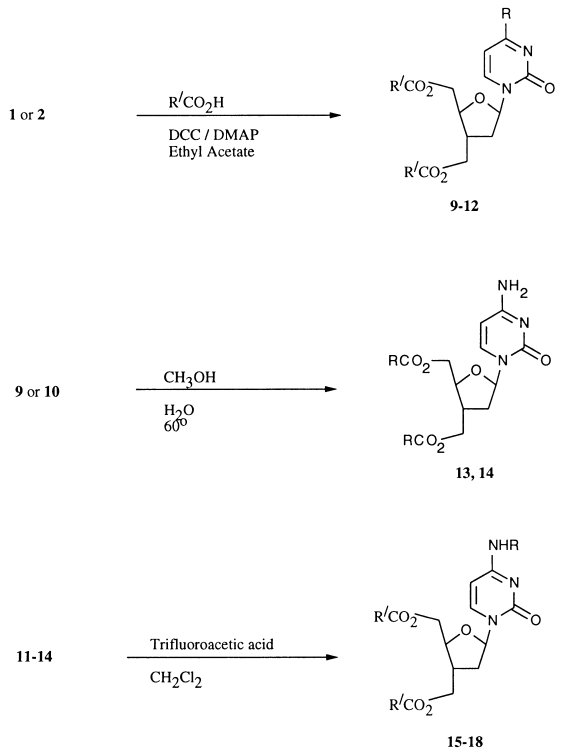


1

Figure 1. Numbering system of **1**.

prepared from intermediate dialkylformamides and dimethyl sulfate at room temperature. Reaction of the intermediate salts with sodium methoxide provided the amide-acetals in 20–40% yield after distillation.[23,26] These were condensed with **1** to provide imines **2**–**8**. In this way, a variety of straight-chain and cyclic analogues were prepared for biological comparison.

The amino acid (α-L-valyl and α-L-phenylalanyl) esters **9**–**18** containing either a free or substituted N^4-cytosine amino function were prepared by coupling of either **1** or **2** with 3.5 and 2.2 equivalents, respectively, of the desired *N-tert*-butoxycarbonyl-α-L-amino acid in a manner analogous to that reported by Aggarwal et al.[20] (Scheme 2).



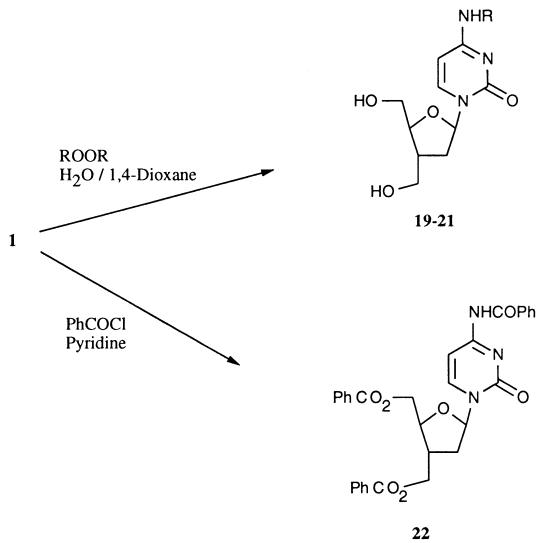
Compd	R	R′	% Yield
9	N=CH(CH$_3$)$_2$	NHBOC-L-Valyl	22
10	N=CH(CH$_3$)$_2$	NHBOC-L-Phe	86
11	NHBOC-L-Valyl	NHBOC-L-Valyl	54
12	NHBOC-L-Phe	NHBOC-L-Phe	41
13	NHBOC-L-Valyl		12
14	NHBOC-L-Phe		17
15	H[a]	L-Valyl	49
16	H[a]	L-Phe	45
17	L-Valyl[a]	L-Valyl	80
18	L-Phe[a]	L-Phe	84

[a]Obtained as trifluoroacetate salt.

Scheme 2.

For the N^4-iminyl- 5′, 6′ disubstituted amino acids, the cytosine imine was found to be quite labile. In fact, **13** was initially formed during purification of **9** and was obtained in a column fraction. Apparently, methanol and the acidity of silica gel were sufficient to cleave the imine to a small extent. This was not observed with the corresponding phenylalanine derivative **10**. It was subsequently found convenient to cleave the imines with warm aqueous methanol and subsequently remove the *N-tert*-butoxycarbonyl protecting groups with TFAA in methylene chloride. In this manner, the diamino acid cytosine derivatives **15** and **16** were obtained as trifluoroacetate salts. Analogously, the trisubstituted *N-tert*-butoxycarbonyl-α-amino acids were deprotected with TFAA to give trisubstituted analogues **17** and **18**. Characteristic of the trisubstituted prodrugs was the ^{1}H NMR resonance of the H-5 proton, found to be considerably further downfield relative to the corresponding 5′,6′ disubstituted cytosine analogues. This effect was attributed to the N^4 neighboring carboxamide deshielding effect.[27] (For example, H-5 for **15** was δ 6.02 ppm; for **17** it was δ 7.21 ppm).

The non-amino acid N^4-amide prodrugs were prepared by classical acylation of **1** analogous to the method of Akiyama, et al.[24] (Scheme 3). Alkylation of **1** with 4 equivalents of benzoyl chloride in pyridine gave the triacylated analogue **22**.



Compd	R	% Yield
19	$COCH_3$	41
20	COPh	28
21	$COC(CH_3)_3$	60
22		54

Scheme 3.

Results and Discussion

Antiviral activity of the prodrugs was determined against duck hepatitis B virus (DHBV), herpes simplex virus (HSV), human cytomegalovirus (HCMV), and human immunodeficiency virus (HIV). Results are shown in Table 1. Initially, **2** was assayed against DHBV. Using **1** as a comparator, the assay was conducted in G2/G3 cells, a human hepatoblastoma cell that constitutively produces DHBV[28] (obtained from W. S. Mason, Fox Chase Cancer Center, Philadelphia, PA). The cells were incubated in test compounds for 11 days, after which time virion DNA in the cell culture fluid was analyzed by polymerase chain reaction (PCR) using primers designed to amplify the pre S region of the viral genome.[29] PCR amplified products were electrophoresed through 1% agarose. Antiviral activity was correlated to a decrease in the amount of PCR product which could be visualized by ethidium bromide staining. Each concentration was performed in duplicate and each compound was compared to its own no-drug control. Parent **1** was found to be active at a concentration of 4.1 μM and prodrug **2** was comparably active at a concentration of 1.7 μM.

More quantitative results against duck hepatitis virus were obtained using a newly developed PCR/PANDEX assay (K. A. Staschke, J. A. Burgess, A. J. Baxter and J. M. Colacino, manuscript in preparation). Briefly, the assay involved a solid-phase capture of a PCR product generated by a primer set designed to amplify a region within the pre-S gene of the DHBV genome. One primer of the set was conjugated to biotin while the other was conjugated to fluoroscein. The resulting PCR product was captured by mixing with avidin coated beads and added to the Pandex 96-well assay plates. The free, unincorporated fluoroscein labeled primers were washed through a cellulose acetate filter in the bottom of the well. The PCR products, which contain fluoroscein labeled primers, were retained by the interaction of the biotin labeled primers with the avidin coated beads and were quantified by fluorescence. DHBV DNA was then quantified by comparing the experiment with a standard curve derived from known amounts of template DHBV DNA.

Table 1. Antiviral activities of various prodrugs of **1** (IC_{50}, μM)

Compd	DHBV	HCMV	HIV-wt	HIV-TIBOr
1	0.2			
2			11.4	9.1
3		13.3	4.5	4.0
4	1.2			
5	0.3	3.4	7.7	6.8
6	0.3	2.1	12.8	4.5
7		2.0	13.1	10.8
8		5.0	3.2	9.4
9	0.5			
10		7.2		
19	2.0	18.7		
21		22.7		

Compounds **2**–**22** were evaluated for their ability to inhibit herpes simplex virus I (HSV-I) Mayo strain induced cytopathic effect (CPE) in BSC-1 cells using Acyclovir as the positive inhibitor control. Acyclovir was found active at a minimum inhibitory concentration of 1.8 μM. Compounds **5**, **6** and **8** were found to be the more active compounds in the HSV-I assay as minimum inhibitory concentrations of 5.0 μM was observed. All test compounds demonstrated similar inhibitory values against herpes simplex virus II (HSV-II) G strain. The inhibitory effect of the prodrugs on HCMV plaque formation in WI-38 cell monolayers was assayed using Ganciclovir as a positive inhibitor control with the more active compounds listed. Analogs were evaluated for activity against wild-type HIV strain IIIb or HIV which is resistant to the non-nucleoside reverse transcriptase inhibitor, TIBO in an XTT assay as previously described.[30,31] An average of two determinations was used and the more active derivatives are shown.

In these antiviral assays, the prodrugs tested displayed only minimal toxicity. Using the XTT assay to evaluate anti-HIV activity in MT4 cells, no toxicity was observed up to 300 μM. In plaque assays to evaluate activity against CMV, slight to moderate toxicity in WI-38 cells was observed for compounds **3**, **5**–**7**,**10**, and **21** at concentrations of 100 μM.

Conclusions

A number of prodrugs of **1** were found to be active in the antiviral assays. Inhibition of DHBV by the imine prodrugs was comparable to **1**. Activity against HSV-1 and 2 was shown by all derivatives in the imine series as well. Certain amino acids in which the cytosine amine was unsubstituted showed some activity, but were in general less active than the imines. Trisubstituted analogue **22** showed some activity, but displayed moderate toxicity. Also, slight to moderate toxicity was observed among the amino acid derivatives.

Activity against HCMV was noted in the imine series as well as the amino acid series. Slight to moderate toxicity was observed with the L-valyl analogues, making them less desirable than the L-phenylalanyl derivatives in this regard. Significant anti-HIV activity was observed against both wild-type and TIBO-resistant strains in the imine series. The esterified prodrugs were essentially inactive against both HIV strains.

The data obtained in the whole cell assays supports the concept that antiviral activity can be preserved in conjunction with decreasing the polarity of parent **1**. The imine series of prodrugs was more active than the amino acid series of prodrugs, as substitution on the alcohol sites resulted in a decrease in activity in virtually all assays. These results could indicate that intracellular phosphorylation of the 5′ alcohol was necessary to achieve activity. However, it cannot be overlooked that differences in transport, conversion, or extracellular activation could be responsible for the differences in activities as well. The imine series appears attractive for design of prodrugs of **1** since compounds of this type require no derivitization of the 5′ and 6′ alcohols. Further, it has been demonstrated that imines of this type are hydrolytically cleaved to parent.[23] The activity of a number of cytosine N^4-imine analogues is uniform and synthesis of these compounds is highly efficient. Further data on membrane permeability, half-lives, and hydrolytic stability are needed at this point to determine if the prodrugs described here have potential utility as clinical antiviral agents.

Experimental

Melting points were determined with a Thomas–Hoover melting point apparatus and are uncorrected. IR spectra were obtained on a Nicolet 510P FT–IR spectrometer. ^{1}H NMR spectra were obtained on a GE QE-300 spectrometer at 300.15 MHZ in the solvent indicated. Field desorption (FD) mass spectra were recorded on a VG Analytical ZAB-3F instrument. Elemental analyses were performed by the Physical Chemistry Department at Lilly Research Laboratories and were within ±0.4% of the theoretical values. Chromatography was performed using silica gel 60, particle size 0.040–0.063 mm, 230–400 mesh ATM from EM Science. TLC analyses were done on silica gel 60 precoated plates, EM Science, with a layer thickness of 250 μm. Spots were visualized under 254 nm illumination. Products were dried in high vacuum (< 1 mm Hg) for 16 h at either 60 °C for solids melting above 100 °C or at ambient temperature for those melting below 100 °C, foams, or oils.

General procedure for the preparation of the (dialkylamino) methylene derivatives of 1-[2′,3′-dideoxy-3′-C-(hydroxymethyl)-β-D-erythropentofuranosyl] cytosine 1. To a solution of **1** (0.21–0.31 mmol) in dry *N*,*N*-dimethylformamide (DMF) (2–3 mL) was added 5 equiv of the required dialkylformamide dimethylacetal[26] and the mixture was stirred at room temperature under N_2 for 12 h. The solvent was removed under reduced pressure, the residue was either crystallized from ethanol/ethyl ether mixture or chromatographed over silica gel. *N*,*N*-dimethylformamide dimethyl acetal was obtained from Aldrich.

N^4-Dimethylaminomethylene-1-[2′,3′-dideoxy-3′-C-(hydroxymethyl)-β-D-erythropentofuranosyl] cytosine (2). A mixture of **1** (50 mg, 0.21 mmol), *N*,*N*-dimethylformamide dimethyl acetal (125 mg, 1.05 mmol), and DMF (2 mL) was reacted to give after crystallization **2** (49 mg, 80%): mp 198–200 °C; IR (KBr) 3237, 2895, 1650, 1598 cm^{-1} ; ^{1}H NMR (DMSO-d_6): δ 1.90–2.00 (m, 1H), 2.18–2.33 (m, 2H), 3.03 (s, 3H), 3.16 (s, 3H), 3.45 (t, J = 5.1 Hz, 2H), 3.55–3.62 (m, 1H), 3.70–3.84 (m, 2H), 4.74 (t, J = 5.1 Hz, 1H), 5.05 (t, J = 5.1 Hz, 1H), 5.95 (m, 2H), 8.19 (d, J = 7.0 Hz, 1H), 8.60 (s ,1H) FD MS 297. Anal. ($C_{13}H_{20}N_4O_4$) C , H, N.

N^4-Diisopropylaminomethylene-1-[2′,3′-dideoxy-3′-C-(hydroxymethyl)-β-D-erythropentofuranosyl] cytosine (3). A mixture of **1** (75 mg, 0.31 mmol), diisopropylformamide dimethyl acetal (284 mg, 1.62 mmol), and DMF (3 mL) was reacted to give after chromatography (8% methanol in methylene chloride) **3** as a foamy resin, (60 mg, 55%): IR (KBr): 3401 (br, OH), 2978, 1653, 1575 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.23 (d, J = 7.0 Hz, 6H), 1.28 (d, J = 7.0 Hz, 6H),1.89–1.98 (m, 1H), 2.17–2.30 (m, 2H), 3.45 (t, J = 5.5 Hz, 2H), 3.55–3.69 (m, 1H), 3.70–3.76 (m, 1H), 3.78–3.89 (m, 2H), 4.63–4.78 (m, 2H), 5.04 (t, J = 5.5 Hz, 1H), 5.93–5.97 (m, 2H), 8.18 (d, J = 7.0 Hz, 1H), 8.71 (s, 1H); FD MS 353. Anal. ($C_{17}H_{28}N_4O_4$·O.25 H_2O) C, H, N.

N^4-Di-*n*-propylaminomethylene-1-[2′,3′-dideoxy-3′-C-(hydroxymethyl)-β-D-erythropentofuranosyl] cytosine (4). A mixture of **1** (50 mg, 0.21 mmol), di-*n*-propylformamide dimethylacetal (175 mg, 1.00 mmol), and DMF (2 mL) was reacted to give following crystallization **4** as a white solid (59 mg, 80%): mp 145–147 °C; IR (KBr): 3296 (br,OH), 2965, 2899, 1609 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 0.86 (dt, J = 7.4 Hz, 6H), 1.60 (q, J = 7.4 Hz, 4H), 1.89–1.99 (m, 1H), 2.17–2.30 (m, 2H), 3.36–3.46 (m, 6H), 3.54–3.61 (m, 1H), 3.70–3.76 (m, 1H), 3.79–3.85 (m, 1H), 4.74 (t, J = 5.2 Hz, 1H), 5.04 (t, J = 5.2 Hz, 1H), 5.95 (m, 2H), 8.18 (d, J = 7.4 Hz, 1H), 8.62 (s, 1H); FD MS 353. Anal. ($C_{17}H_{28}N_4O_4$) C, H, N.

N^4-Pyrrolidinomethylene-1-[2′,3′-dideoxy-3′-C-(hydroxymethyl)-β-D-erythropentofuranosyl] cytosine (5). A mixture of **1** (50 mg, 0.21 mmol), *N*-(dimethoxymethyl) pyrrolidine (160 mg , 1.10 mmol), and DMF (3 mL) was reacted to give following crystallization **5** (60 mg, 90%): mp 210-212 °C; IR (KBr): 3228 (br,OH), 2891, 1614 cm^{-1} ; ^{1}H NMR (DMSO-d_6): δ 1.89 (m, 5H), 2.17–2.31 (m, 2H), 3.39–3.51 (m, 4H), 3.55–3.77 (m, 2H), 3.65 (m, 2H), 3.83 (m, 1H), 4.74 (t, J = 5.2 Hz, 1H), 5.05 (t, J = 4.8 Hz, 1H), 5.95 (m, 2H), 8.19 (d, J = 7.4 Hz, 1H), 8.78 (s, 1H); FD MS 323. Anal. ($C_{15}H_{22}N_4O_4$) C, H, N.

N^4-Piperidinomethylene-1-[2′,3′-dideoxy-3′-C-(hydroxymethyl)-β-D-erythropentofuranosyl] cytosine (6). A mixture or **1** (50 mg, 0.21 mmol), *N*-(dimethoxymethyl) piperidine (175 mg, 1.10 mmol), and DMF (2 mL) was reacted to give following crystallization **6** (58 mg, 82%): mp 170–174 °C ; IR (KBr): 3295, 2922, 1651 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.49–1.70 (m, 6H) , 1.93 (m, IH), 2.24 (m, 2H), 3.44 (t, J = 5.2 Hz, 2H), 3.53 (m, 2H), 3.61 (m, 1H), 3.65–3.76 (m, 3H), 3.82 (m, 1H), 4.76 (t, J = 5.5 Hz, 1H), 5.04 (t, J = 5.5 Hz, 1H), 5.94 (m, 2H), 8.18 (d, J = 7.4 Hz, 1H), 8.62 (s, 1H); FD MS 337. Anal. ($C_{16}H_{24}N_4O_4$) C, H, N.

N^4-Hexamethyleneiminylmethylene-1-[2′,3′-dideoxy-3′-C-(hydroxymethyl)-β-D-erythropentofuranosyl] cytosine (7). A mixture of **1** (50 mg, 0.21 mmol), *N*-(dimethoxymethyl)hexamethyleneimine (190 mg, 1.10 mmol), and DMF (2 mL) was reacted to give following crystallization **7** (56 mg, 76%): mp 194–196 °C; IR (KBr): 3249, 2936, 1647 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.52 (m, 4H), 1.72 (m, 4H), 1.93 (m, 1H), 2.24 (m, 2H), 3.45 (t, J = 5.2 Hz, 2H), 3.60 (m, 5H), 3.69–3.76 (m, 1H), 3.82 (m, 1H), 4.74 (t, J = 5.2 Hz, 1H), 5.04 (t, J = 5.2 Hz, 1H), 5.95 (m, 2H), 8.19 (d, J = 7.0 Hz, 1H), 8.65 (s, 1H); FD MS 351. Anal. ($C_{17}H_{26}N_4O_4$) C, H, N.

N^4-Morpholinomethylene-1-[2′,3′-dideoxy-3′-C-(hydroxymethyl)-β-D-erythropentofuranosyl] cytosine (8). A mixture of **1** (50 mg, 0.21 mmol), *N*-(dimethoxymethyl) morpholine (177 mg, 1.37 mmol), and DMF (3 mL) was reacted to give after crystallization **8** (47 mg, 66%): mp 225–228 °C; IR (KBr): 3279, 2908, 1652 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.94 (m, 1H), 2.25 (m, 2H), 3.45 (t, J = 5.2 Hz, 2H), 3.55–3.77 (m, 10H), 3.83 (m, 1H), 4.75 (t, J = 5.2 Hz, 1H), 5.05 (t, J = 5.2 Hz, 1H), 5.94 (m, 2H), 8.22 (d, J = 7.4 Hz, 1H), 8.67 (s, 1H) ; FD MS 339. Anal. ($C_{15}H_{22}N_4O_4$) C, H, N.

N^4-Dimethylaminomethylene-1-[2′,3′-dideoxy-5′,6′-*O*-(*N*-α-*tert*-butoxycarbonyl-L-valyl)-β-D-erythropentofuranosyl] cytosine (9) and 1-[2′,3′-dideoxy-5′,6′-*O*-(*N*-α-*tert*-butoxycarbonyl - L - valyl) - β- D - erythropentofuranosyl] cytosine (13). To a stirring solution of **2** (135 mg, 0.46 mmol), 4-dimethylaminopyridine (167 mg, 1.37 mmol), and *N*-*tert*-butoxycarbonyl-L-valine (218 mg, 1.00 mmol) in dry ethyl acetate (20 mL) under N_2 was added dicyclohexylcarbodimide (227 mg, 1.10 mmol). The mixture was stirred at room temperature for 36 h and the solids were filtered. The filtrate was concentrated in vacuo and the residue was chromatographed (2.5% methanol in methylene chloride). Initially obtained was **9** as a foam (70 mg, 22%): IR (KBr): 3380, 2970, 1725, 1715 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.90 (d, J = 2.6 Hz, 3H), 0.92 (d, J = 2.6 Hz, 3H), 0.98 (d, J = 3.7 Hz, 3H), 1.00 (d, J = 3.7 Hz, 3H), 1.45 (s, 18H), 2.13 (m, 2H), 2.30 (m, 1H), 2.46 (m, 2H), 3.18 (s, 6H), 4.18 (m, 6H), 4.45 (m, 1H), 5.02 (br, 1H,), 5.10 (d, J = 8.5 Hz, 1H), 6.11 (m, 1H), 6.22 (br, 1H), 7.88 (d, J = 6.6 Hz, 1H), 8.86 (s, 1H); FD MS 696. Anal. ($C_{33}H_{54}N_6O_{10}$) C, H, N. Further elution provided **13** as a white foam filtered with the aid of ethyl ether (35 mg, 12%); IR (KBr): 3386, 2968, 1747, 1717 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.90 (d, J = 4.0 Hz, 3H), 0.92 (d, J = 4.0 Hz, 3H), 0.98 (d, J = 2.6 Hz, 3H), 1.00 (d, J = 2.6 Hz, 3H), 1.44 (s, 18H), 2.12 (m, 2H), 2.29–2.52 (m, 3H), 4.19 (m, 6H), 4.37–4.50 (m, 1H), 5.30 (d, J = 8.8 Hz, 2H), 5.94 (d, J = 7.4 Hz, 1H), 6.04 (m, 1H), 7.83 (d, J = 6.6 Hz, 1H); FD MS 640. Anal. ($C_{30}H_{49}N_5O_{10}\cdot 0.5C_4H_{10}O$) C, H, N.

N^4-(Dimethylaminomethylene)-1-[2′,3′-dideoxy-5′,6′-*O*-(*N*-α-*tert*-butoxycarbonyl-L-phenylalanyl)-β-D-erythropentofuranosyl] cytosine (10). A mixture of dicyclohexylcarbodiimide (227 mg, 1.10 mmol), compound **2** (135 mg, 0.46 mmol), 4-dimethylaminopyridine (167 mg, 1.37 mmol), and *N*-*tert*-butoxycarbonyl-L-phenylalanine (266 mg, 1.00 mmol) in anhydrous ethyl acetate (20 mL) was stirred for 36 h at room temperature. The solids were filtered and the filtrate was concentrated and chromatographed (2.5% methanol in methylene chloride) to give a white amorphous solid. Crystallization from ethyl ether/hexanes gave **10** as a white solid (310 mg, 86%): mp 120–125 °C; IR (KBr): 3349, 2927, 1737, 1691 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.42 (s, 18H), 1.85 (brm, 1H), 2.02–2.29 (m, 2H) , 3.05 (m, 4H), 3.17 (s, 6H), 3.78 (m, 1H), 3.95 (d, J = 5.9 Hz, 2H), 4.22 (m, 2H), 4.54 (m, 2H), 5.05 (m, 2H), 5.99 (dd, J = 3.3, 6.6 Hz, 1H), 6.17 (d, J = 7.0 Hz, 1H), 7.11–7.34 (m, 10H), 7.74 (d, J = 7.4 Hz , 1H), 8.86 (s, 1H); MS FD 792. Anal ($C_{41}H_{54}N_6O_{10}$) C , H , N.

N^4-(*N*′-α-*tert*-butoxycarbonyl-L-valyl)-1-[2′,3′-dideoxy-5′,6′-*O*-(*N*″-α-*tert*-butoxycarbonyl-L-valyl)-β-D-erythropentofuranosyl] cytosine (11). To a solution of **1** (120 mg, 0.50 mmol), 4-dimethylaminopyridine (275 mg, 2.25 mmol), *N*-*tert*-butoxycarbonyl-L-valine (380 mg, 1.75 mmol), and anhydrous ethyl acetate (25 mL) under N_2 was added diclohexylcarbodiimide (365 mg, 1.77 mmol). The solution was stirred 2 days at room temperature, filtered, and concentrated. The residue was chromatographed (2% methanol in methylene chloride) to give **11** as a white foam (225 mg, 54%); IR (KBr): 3448, 2976, 1719 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.01 (m, 18H), 1.44 (m, 27H), 2.12–2.50 (m, 6H), 4.18 (m, 6H), 4.30–4.68 (m, 2H), 5.02 (m, 3H), 6.06 (m, 1H), 7.49 (t, J = 9.9 Hz, 1H), 8.20 (d, J = 6.6 Hz, 1H), 8.78 (s, 1H) ; MS FD 840. Anal. ($C_{40}H_{66}N_6O_{13}$) C, H, N.

N^4-(*N*′-α-*tert*-butoxycarbonyl-L-phenylalanyl)-1-[2′,3′-dideoxy-5′,6′-*O*-(*N*″-α-*tert*-butoxycarbonyl-L-phenylalanyl)-β-D-erythropentofuranosyl] cytosine (12). A mixture of dicyclohexylcarbodiimide (365 mg, 1.77 mmol), compound **1** (120 mg, 0.50 mmol), 4-dimethylaminopyr-

idine (275 mg, 2.25 mmol), *N*-*tert*-butoxycarbonyl-L-phenylalanine (465 mg, 1.75 mmol), and dry ethyl acetate (25 mL) was stirred at room temperature for 2 days under N_2. The solids were filtered, the filtrate concentrated, and the resin chromatographed to give **12** as an amorphous solid (200 mg, 41%). IR (KBr): 3420, 3370, 2979, 1720 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.39 (m, 27H), 2.24 (m, 3H), 2.99–3.27 (m, 6H), 3.74–3.97 (m, 5H), 4.61 (m, 3H), 5.00 (m, 3H), 5.95 (m, 1H), 7.10–7.35 (m, 15H), 7.47 (m, 1H), 8.03 (d, J = 7.4 Hz, 1H), 8.84 (s, 1H); MS FD 983. Anal. ($C_{52}H_{66}N_6O_{13}$) C, H, N.

1-[2′,3′-Dideoxy-5′,6′-*O*-(*N*-α-*tert*-butoxycarbonyl-L-phenylalanyl)-β-D-erythropentofuranosyl] cytosine (14). Compound **10** (280 mg, 0.35 mmol) was heated and stirred at 60 °C in 1:1 methanol:water (20 mL) for 16 h. The solvent was removed in vacuo and the residue was chromatographed (2% methanol in methylene chloride) to give **14** as a white solid (45 mg, 17%); mp 115–120 °C; IR (KBr): 3430, 3346, 2980, 1749 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.32 (s, 18H), 1.91 (m, 1H), 2.14 (m, 2H), 2.93 (m, 4H), 3.89 (brs, 1H), 4.02 (m, 2H), 4.15–4.31 (m, 4H), 5.78 (d, J = 7.7 Hz, 1H), 5.97 (m, 1H), 7.09–7.40 (m, 14H), 7.64 (d, J = 7.4 Hz, 1H); MS FD 737. Anal. ($C_{38}H_{49}N_5O_{10}$) C, H, N.

1-[2′,3′-Dideoxy-5′,6′-*O*-(α-L-valyl)-β-D-erythropentofuranosyl] cytosine trifluoroacetate (15). A solution of **9** (40 mg, 0.06 mmol) in 1:1 methanol:water (10 mL) was heated at 60 °C for 12 h. The solvent was removed in vacuo and the residue was chromatographed (5% methanol in methylene chloride) to give **13**, which was used as such without further purification. The residue was dissolved in methylene chloride (1 mL) and added to a stirring solution of trifluoroacetic acid (1 mL) and methylene chloride (1 mL). The mixture was stirred 1 h at room temperature, the solvent removed in vacuo and the resulting oil was crystallized from ethanol/ethyl ether to give **15** (22 mg, 49%, from **9**) as an amorphous solid. IR (KBr): 3430, 2980, 1753, 1680 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.97 (m, 12H), 2.17 (m, 2H), 2.26–2.43 (m, 2H), 2.60 (m, 1H), 3.97 (brs, 2H), 4.15 (m, 1H), 4.29 (d, J = 5.2 Hz, 2H), 4.49 (m, 2H), 6.02 (m, 2H), 7.93 (d, J = 7.7 Hz, 1H), 8.20–9.20 (brm, 9H). MS FD 440 (parent ion). Anal. ($C_{20}H_{33}N_5O_6 \cdot 3CF_3COOH$) C, H, N.

1-[2′,3′-Dideoxy-5′,6′-*O*-(α-L-phenylalanyl)-β-D-erythropentofuranosyl] cytosine trifluoroacetate (16). A mixture of **14** (20 mg, 0.027 mmol) and 1:1 trifluoroacetic acid: methylene chloride (2 mL) was stirred 2 h at room temperature and concentrated to dryness. The addition of ethyl ether gave **16** as an amorphous solid (10.8 mg, 45%). IR (KBr): 3425, 3035, 1757, 1681 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.94 (m, 1H), 2.10 (m, 2H), 3.10 (m, 4H), 3.69–3.87 (m, 2H), 4.17–4.28 (m, 2H), 4.28 (m, 1H), 4.36 (m, 2H), 5.90 (m, 1H), 5.98 (d, J = 7.4 Hz, 1H), 7.27 (m, 10H), 7.69 (d, J = 8.1 Hz, 1H), 8.00–8.60 (brs, 9H); MS FD 536 (free base). Anal. ($C_{28}H_{33}N_5O_6 \cdot 3CF_3COOH$) C, H, N.

N⁴-(α-L-valyl)-1-[2′,3′-dideoxy-5′,6′-*O*-(α-L-valyl)-β-D-erythropentofuranosyl] cytosine trifluoroacetate (17). To a solution of trifluoroacetic acid (2 mL) and methylene chloride (2 mL) was added compound **11** (194 mg, 0.23 mmol) in methylene chloride (2 mL). The solution was stirred at room temperature for 1 h and the solvent removed in vacuo to give a resin. The resin was crystallized from ethanol/ethyl ether to give **17** as a white solid (163 mg, 80%): mp 160–165 °C (d); IR (KBr): 3480, 2978, 1753, 1678 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.98 (m, 18H), 2.17 (m, 4H), 2.51 (m, 2H), 3.95 (m, 3H), 4.25 (m, 3H), 4.52 (m, 2H), 6.03 (m, 1H), 7.21 (m, 1H), 8.23 (d, J = 7.7 Hz, 1H) , 8.25–8.59 (br, 9H), 11.45 (br, IH); MS FD (539, free base). Anal. ($C_{25}H_{42}N_6O_7 \cdot 3CF_3COOH$) C, H, N.

N⁴-(α-L-phenylalanyl)-1-[2′,3′-dideoxy-5′,6′-*O*-(α-L-phenylalanyl)-β-D-erythropentofuranosyl] cytosine (18). To a solution of trifluoroacetic acid (2 mL) and methylene chloride (2 mL) was added **12** (165 mg , 0.17 mmol) and the mixture was stirred 1 h at room temperature. The solvent was removed under reduced pressure and the resulting oil was crystallized from ethanol/ethyl ether to give **18** (144 mg, 84%); mp 195–200 °C (d); IR (KBr): 3428, 3035, 1755, 1677 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.80–2.40 (brm, 3H), 3.14 (m, 6H), 3.65–3.99 (brm, 2H), 4.35 (m, 4H), 5.92 (m, 1H), 7.30 (m, 16H), 8.08 (d, J = 8.1 Hz, 1H), 8.20–8.75 (br, 9H), 11.50 (brs, 1H); MS FD 683 (free base). Anal. ($C_{37}H_{45}N_6O_7 \cdot 3CF_3COOH \cdot 4H_2O$) C, H, N.

General procedure for the preparation of N⁴-amido derivatives of 1. To a solution of **1** (0.30 mmol) in water was added the appropriate anhydride (2.2 equiv) and *p*-dioxane (1.2 mL). After stirring 12 h at room temperature, the solvent was removed under reduced pressure, and the residue chromatographed (7–10% methanol in methylene chloride) to give, after crystallization from ethyl ether, the desired products.

N⁴-Acetyl-1-[2′,3′-dideoxy-3′-C-(hydroxymethyl)-β-D-erythropentofuranosyl] cytosine (19). Compound **1** (75 mg, 0.30 mmol) was reacted with acetic anhydride (0.06 mL, 0.64 mmol) in *p*-dioxane (1.2 mL) and water (0.4 mL) to give **19** (35 mg, 41%): mp 150–155 °C (d); IR (KBr): 3392, 2928, 1697, 1652 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.01 (m, 1H), 2.10 (s, 3H), 2.27 (m, 2H), 3.45 (t, J = 4.8 Hz, 2H), 3.61 (m, 1H), 3.77 (m, 1H), 3.87 (m, 1H), 4.74 (t, J = 5.2 Hz, 1H), 5.09 (t, J = 5.2 Hz, 1H), 5.93 (m, 1H), 7.17 (d, J = 7.4 Hz, 1H), 8.51 (d, J = 7.4 Hz, 1H), 10.83 (s, 1H); MS FD 284. Anal. ($C_{12}H_{17}N_3O_5$) C, H, N.

N^4-Benzoyl-1-[2′,3′-dideoxy-3′-C-(hydroxymethyl)-β-D-erythropentofuranosyl] cytosine (20). Compound **1** (75 mg, 0.30 mmol) was reacted with benzoic anhydride (150 mg, 0.66 mmol) in *p*-dioxane (1.2 mL) and water (0.04 mL) to give **20** (30 mg, 28%) as an amorphous solid. IR (KBr): 3481, 3372, 2933, 1697, 1650 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.06 (m, 1H), 2.30 (m, 2H), 3.46 (brs, 2H), 3.63 (d, $J=9.9$ Hz, 1H), 3.79 (d, $J=12.5$ Hz, 1H), 3.90 (m, 1H), 4.76 (brs, 1H), 5.13 (brs, 1H), 5.97 (m, 1H), 7.33 (d, $J=7.4$ Hz, 1H), 7.52 (m, 5H), 8.59 (d, $J=7.4$ Hz, 1H), 11.20 (brs, 1H). MS FD 346. Anal. ($C_{17}H_{19}N_3O_5$) C, H, N.

N^4-Pivaloyl-1-[2′,3′-dideoxy-3′-C-(hydroxymethyl)-β-D-erythropentofuranosyl] cytosine (21). Compound **1** (75 mg, 0.30 mmol) was reacted with pivalic anhydride (0.14 mL, 0.66 mmol) in *p*-dioxane (1.2 mL) and water (0.04 mL) to give **21** as a white foam (60 mg, 60%); IR (KBr): 3416, 3010, 1657 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.21 (s, 9H), 2.02 (m, 1H), 2.28 (m, 2H), 3.47 (t, $J=5.2$ Hz, 2H), 3.61 (m, 1H), 3.76 (m, 1H), 3.88 (m, 1H), 4.76 (t, $J=5.2$ Hz, 1H), 5.12 (t, $J=5.2$ Hz, 1H), 5.94 (m, 1H), 7.24 (d, $J=7.7$ Hz, 1H), 8.51 (d, $J=7.4$ Hz, 1H), 10.30 (brs, 1H). MS FD 326. Anal. ($C_{15}H_{23}N_3O_5$) C, H, N.

N^4-Benzamido-1-[2′,3′-dideoxy-5′,6′-*O*-(benzoyl)-β-D-erythropentofuranosyl] cytosine (22). To a solution of **1** (50 mg, 0.21 mmol) in dry pyridine (3 mL) was added benzoyl chloride (0.10 mL, 0.80 mmol). The mixture was stirred at room temperature for 2 h, the solvent was removed in vacuo and the residue was partitioned between water (50 mL) and ethyl acetate (50 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated to a resin. Chromatography (1% methanol in methylene chloride) gave **22** as an amorphous resin (62 mg, 54%). IR (KBr): 3420, 1723 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.51 (m, 1H), 2.72 (m, 2H), 4.40 (m, 2H), 4.55 (dd, $J=3.0$, 6.0 Hz, 1H), 4.76 (m, 2H), 6.17 (m, 1H), 7.51 (m, 10H); 8.02 (m, 6H), 8.32 (d, $J=7.0$ Hz, 1H), 8.75 (brs, 1H). MS FD 554. Anal. ($C_{31}H_{27}N_3O_7$) C, H, N.

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