mixed, and absorbance at 450 and 630 nm was measured with a microplate reader (EL340, Bio-TEK Instruments, Winooski, VT).

Experimental Data Analysis. The dose–effect relationships of at least five different concentrations of each compound (plus no-drug control) were analyzed by the median-effect plot[25,26] using computer software[27] for automated analysis. The analysis provided anti-HIV-1 EC_{50} (median-effect concentrations), IC_{50} (median-inhibitory concentrations), and other dose–effect-related parameters.

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Synthesis and Anti-HIV Activity of Several 2′-Fluoro-Containing Pyrimidine Nucleosides

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Several 2′-fluoroarabino-2′,3′-dideoxy- and 2′-fluoro-2′,3′-unsaturated 2′,3′-dideoxy pyrimidine nucleoside analogues are reported. The saturated analogues 1-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)thymine (2′-*threo*-FddT, **33**), 1-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)uracil (2′-*threo*-FddU, **22**) were readily prepared from the corresponding 2′-deoxy-2′-fluoroarabinosyl nucleoside analogue by radical deoxygenation of the 3′-OH. The unsaturated compounds 1-(2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)thymine (2′-Fd4T, **40**) and 1-[5-*O*-(monomethoxytrityl)-2-fluoro-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl]uracil (**39**) were synthesized by an elimination reaction of the *O*-2,3′-anhydro-2′-fluoro-*lyxo* derivatives under basic conditions. The cytidine analogues **28** and **41** were prepared by amination of the corresponding uridine derivatives; compounds **28** and **41** were deprotected to give 1-(2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)cytidine (2′-*threo*-FddC, **29**) and 1-(2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)cytosine (2′-Fd4C, **42**), respectively. All of these novel compounds were evaluated in vitro against human immunodeficiency virus (HIV) (LAV isolate). 2′-*threo*-FddC (**29**) was the most active of the newly synthesized substances against HIV with an ID_{50} of 0.8 μg/mL; ddC had an ID_{50} of 0.007 μg/mL. Because of its potency in the initial tests, **29** was further evaluated in both T cells and macrophage/monocyte cell lines, with several different isolates of HIV. Although 2′-*threo*-FddC (**29**) exhibited good antiviral activity in these systems, it was less active than AZT in these assays. At 1 μM the inhibition of CFU-GM by **29** was found to be 35–40%; this is slightly higher than seen with AZT.

Introduction

An extensive effort has been conducted during the last few years to discover chemotherapeutic agents to counteract acquired immune deficiency syndrome (AIDS), which results from infection with human immunodeficiency virus (HIV).[1] 3′-Azido-3′-deoxythymidine (AZT, 1) is currently the only drug available for the treatment of HIV infection.[2] There are, however, significant side effects with AZT; the most severe of these side effects are associated with bone marrow toxicity.[3,4] Long-term treatment of patients with AZT can also result in the emergence of low-sensitivity viral strains.[5] There is, therefore, a need for other effective but less toxic chemotherapeutic alternatives to AZT.

Several different classes of nucleoside analogues have already been identified as anti-HIV agents (Figure 1). These compounds fall into three main structural types: (1) the 3′-substituted purine or pyrimidine nucleosides such as AZT (1), 3′-azido-2′,3′-dideoxyuridine (AZU, **2**),[6] 3′-azido-2′,3′-dideoxyguanosine (AZG, **3**),[7] and 3′-fluoro-3′-deoxythymidine (3′-FddT, **4**),[8] (2) saturated dideoxy purine or pyrimidine nucleosides such as 2′,3′-dideoxyadenosine (ddA, **5**), or 2′,3′-dideoxyinosine (ddI, 6), or 2′,3′-dideoxycytidine (ddC, 7);[9] and (3) 2′,3′-unsaturated pyrimidine nucleosides as exemplified by 1-(2,3-dideoxy-β-D-

† Oncogen.

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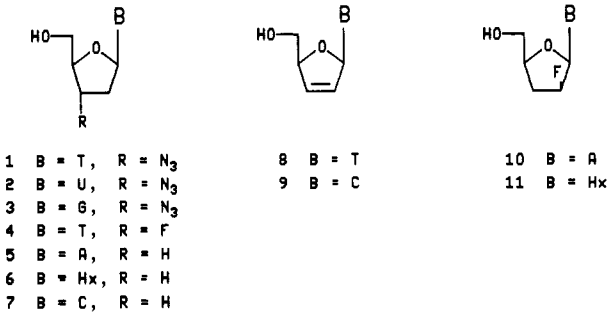
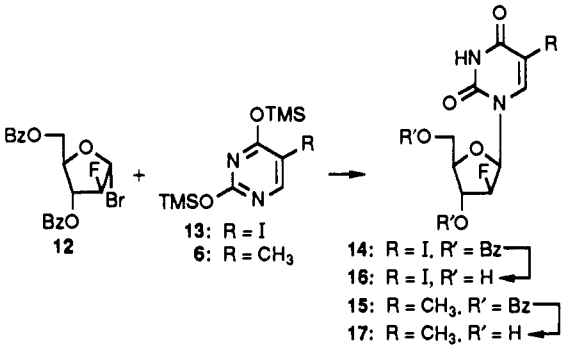


Figure 1.

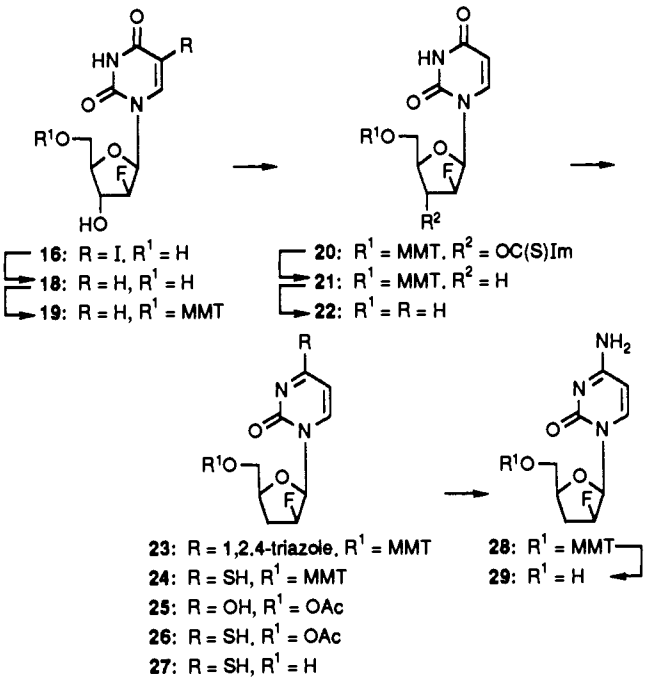
glycero-pent-2-enofuranosyl)thymine (d4T, 8),[10,11] or 1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)cytosine (d4C, **9**).[12] All of these compounds appear to act by a similar mechanism of action to that of AZT. The nucleoside analogue must first be metabolized to the corresponding 5'-*O*-triphosphate. This triphosphate can then act as either a terminator of DNA chain elongation or as an inhibitor of the unique viral enzyme reverse transcriptase (RT), or both.[13] Those HIV isolates that showed reduced sensitivity to AZT remained sensitive to ddC and d4T.[5]

Although the initial in vitro and clinical data demonstrated that the 2',3'-unsubstituted dideoxynucleosides do not cause the same dose-limiting toxicity associated with AZT, other significant side effects were observed. DdC exhibits peripheral neuropathy as the dose-limiting toxicity.[14] Therefore, other substituted dideoxynucleoside analogues need to be considered as anti-HIV compounds. Earlier work with DNA viruses had indicated that the introduction of a 2'-fluoroarabino substituent sometimes enhanced antiviral activity and stabilized the glycosidic bond to enzymatic hydrolysis.[15] A strongly electron withdrawing group such as fluorine atom may also stabilize the glycosidic bond to acid, relative to those structures that do not have a 2'-fluoro substituent.[16] Recently, 9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)adenine (2'-*threo*-FddA, **10**) and 9-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)hypoxanthine (2'-*threo*-FddI, **11**) have been prepared,[17,18] and shown to have significantly im-

Scheme I



Scheme II



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proved acid and purine nucleoside phosphorylase stability compared to ddA and ddI.[17] In this paper, we report on the synthesis and anti-HIV activity of several 2′-fluoro-arabino-2′,3′-dideoxy and 2′-fluoro-2′,3′-2′,3′-unsaturated-2′,3′-dideoxy pyrimidine nucleoside analogues.

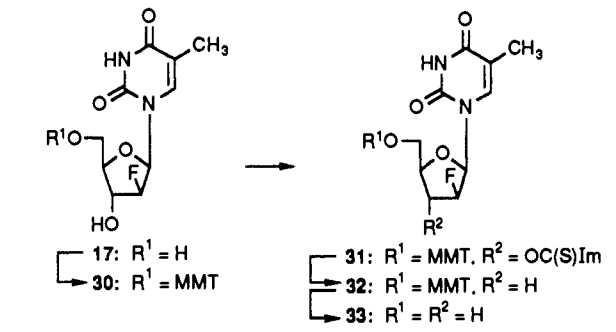
Chemistry

2′-Fluoroarabinosyl nucleoside analogues can be readily prepared by a coupling reaction of an activated base **13** and the bromo sugar **12**.[18,19] The choice of solvent used in the coupling reaction can have a very profound effect on the anomeric ratio of the nucleoside products obtained.[20,21] Deprotection gave the desired nucleoside analogues directly (Scheme I).

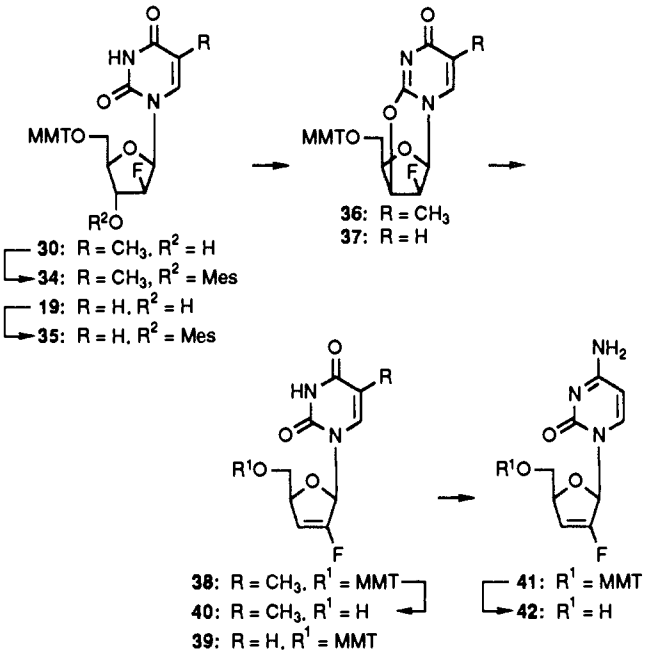
2′-Deoxy-2′-fluoroarabino-5-iodouridine (FIAU, **16**), prepared as shown in Scheme I, was hydrogenolyzed over 10% Pd/C in ethanol–water–triethylamine to give 2′-deoxy-2′-fluoroarabinouridine (FAU, **18**)[22] in 90% yield. Reaction of **18** with monomethoxytrityl chloride (1 equiv) afforded the protected ether **19** as an oil, in 90% yield, after column chromatography (Scheme II). The alcohol **19** was deoxygenated at the 3′-position by reaction with 1,1′-thiocarbonyldiimidazole (1.5 equiv), followed by reduction of the crude imidazolide with tri-*n*-butyltin hydride (5–6 equiv).[23] The deoxygenated product **21** was isolated in 69% yield after column chromatography. The 5′-*O*-monomethoxytrityl group was removed by heating **21** to 55–65 °C in 80% acetic acid to furnish 1-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)uracil (2′-*threo*-FddU, **22**) in 78% yield after recrystallization.

Two separate amination methods for converting the uracil moiety of **21** to the cytidine analogue **28** were investigated (Scheme II). Initially, the protected uridine derivative **21** was treated with excess *p*-chlorophenyl phosphodichloridate and freshly sublimed 1,2,4-triazole for several days to afford the 4-triazole compound **23**.[24] Treatment of the reaction mixture with concentrated ammonia in dioxane gave **28** in 70% yield after chromatography. Compound **28** was deprotected to furnish 2′-*threo*-FddC (**29**) in good yield. In an alternative approach, **21** was reacted with Lawesson's reagent.[25] Rather than the expected product **24**, a mixture of 5′-*O*-deprotected derivatives was formed. The deprotected nucleoside analogue **22** was isolated by silica gel chromatography in 31% yield. The second major component of the reaction mixture was identified later by ^{13}C NMR as 1-(2-fluoro-2,3-dideoxy-β-D-*threo*-pentofuranosyl)-4-thiouracil (**27**). This, to our knowledge, is the first observation of the incompatibility of a MMT protecting group with Lawesson's reagent. The 5′-*O*-MMT protected compound **21** was therefore converted, via **22**, to the 5′-*O*-acetate **25**. Reaction of the acetate **25** with Lawesson's reagent in DME afforded **26**. Ammonolysis of **26** in methanol in a bomb at 100 °C introduced the amino group at the C-4 position

Scheme III



Scheme IV



and removed the 5′-*O*-acetate protecting group in one step. The desired cytidine compound **29** was isolated in 64% overall yield for the latter two steps.[26] For the larger scale aminations the thionation method was found to be more convenient. Ammonolysis of **26** at room temperature for 16 h removed only the 5′-*O*-acetate group to furnish **27** as the final product.

An analogous series of reactions was used to prepare 1-(2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)thymine (2′-*threo*-FddT, **33**) (Scheme III).[26d] FMAU (**17**) was protected as the 5′-*O*-monomethoxytrityl ether **30**. Derivatization of the 3′-O-position as the thiocarbonylimidazolide **31** and direct deoxygenation with tri-*n*-butyltin hydride furnished the protected deoxythymidine analogue **32**. Removal of the ether protecting group afforded the final product **33**, as a white solid, in 25% overall yield for the four steps from FMAU (**17**).

For the preparation of the 2′,3′ unsaturated analogues the trityl ether **30** was converted to the mesylate **34** in nearly quantitative yield on treatment with methane-

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Scheme V

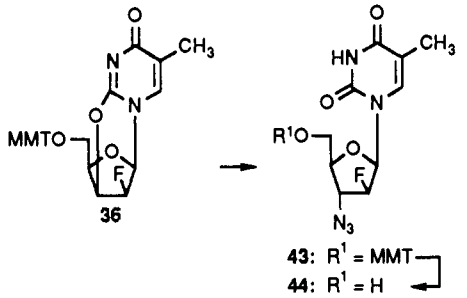

Table I. In Vitro Anti-HIV Activity[a] and Cellular Toxicity[b]

compd	name	ID_{50},[c] μg/mL	$TCID_{50}$,[d] μg/mL
22	2'-*threo*-FddU	>10.0	57.0
33	2'-*threo*-FddT	>25.0	>25.0
	2'-*threo*-FddT[e]	>25.0	8.0
29	2'-*threo*-FddC	2.3	>23.0
	2'-*threo*-fddC[e]	0.9	61.9
40	2'-Fd4T	>25.0	9.7
42	2'-Fd4C	1.3	0.9
	2'-Fd4C[e]	10.9	113.6
44	2'-*ara*-FAZT	>28.5	2.6
1	AZT	0.08–0.25	
	AZT[e]	0.53	
7	ddC	0.007	1.3

[a] The antiviral test was performed against HIV (LAV strain) on CEM cells using 50 tissue culture infectious units. [b] The cellular toxicity was measured in CEM cells after 8 days. [c] The 50% inhibitory dose. [d] The 50% tissue culture inhibitory dose. [e] Thirty tissue culture infectious doses were used.

sulfonyl chloride in pyridine (Scheme IV). Reaction of **34** with sodium hydroxide in ethanol gave the expected *O*-2,3'-anhydro derivative **36** as a white solid after neutralization of the reaction mixture with 80% acetic acid. Deprotonation of **36** at the C-2'-position with potassium *tert*-butoxide in DMSO afforded the olefin **38** in 46% yield. Removal of the 5'-O-protecting group gave **40**, the 2'-fluoro analogue of d4T, as an oil after column chromatography.[27] The corresponding uridine derivative **39** was prepared in a similar manner. Compound **19** was converted, via the mesylate **35**, to the anhydro derivative **37**. Treatment of **37** with potassium *tert*-butoxide in DMSO afforded the protected nucleoside **39**. Conversion of the uracil moiety of **39** to the cytosine base proceeded through the 1,2,4-triazolyl derivative, followed by ammonolysis to afford the cytidine compound **41**. Removal of the monomethoxytrityl protecting group gave Fd4C (**42**) in 19% overall yield from **39**.

Direct hydrogenation of the unsaturated uridine analogue **39** over 10% Pd/C yielded 2'-*threo*-fluoro compound **21** as the only isolated product (66%). The material obtained in this fashion was identical with a sample of **21** prepared from **20** (Scheme II).

To introduce the 3'-azido substituent, the *O*-2,3'-anhydro derivative **36** was treated with lithium azide in DMF for 62 h at 105 °C, to afford the 3'-azido derivative **43** (Scheme V).[28] The unreacted starting material (16.5%) was removed by trituration and the crude mother liquors were deprotected directly to furnish the desired compound **44** in 31% overall yield from **36**. The corresponding reaction to prepare the protected AZT derivative proceeded in higher yield and shorter reaction time since the 2'-fluoro substituent greatly deactivates the 3'-position to nucleophilic ring opening.[29]

Table II. In Vitro Anti-HIV Activity of 2'-ara-FddC As Compared to AZT on Different Cell Lines

compound	cell line	HIV isolate	ID_{50},[a] μg/mL	$TCID_{50}$,[b] μg/mL
2'-*threo*-FddC (**29**)	CEM	LAV	0.92	61.8
	U937	LAV	0.14	7.7
	Tcell	$HTLV_{IIIB}$	0.33	10.0
	Mø.MO	PDS	1.0	3.3
	PBL	$HTLV_{IIIB}$	<2.0	11.0
AZT (1)	CEM	LAV	0.03–3.0	>3.0
	U937	LAV	0.03	7.5
	Tcell	$HTLV_{IIIB}$	0.033	100.0
	Mø.MO	PDS	0.1	10.0
	PBL	$HTLV_{IIIB}$	0.0003	3.0

[a] The 50% inhibitory dose. [b] The 50% tissue culture inhibitory dose.

Table III. Bone Marrow Toxicity[a] of 2'-*threo*-FddC Compared to AZT

compound	0.1	86.5 ± 12.6
2'-threo-FddC (**29**)	0.1	
	1.0	37.6 ± 5.9
	10.0	0.0
AZT (1)	0.1	74.1 ± 11.3
	1.0	50.4 ± 11.8
	10.0	23.6 ± 5.6

[a] Measured against normal human colony forming units-granulocyte monocyte (CFU-GM). [b] Concentration in μM.

Biological Results and Discussion

The in vitro activity of these various 2'-fluoro-2',3'-dideoxy derivatives was measured against HIV (LAV strain) and compared to AZT and ddC. The in vitro anti-HIV activity was measured in HIV-infected CEM cells; the data in Table I demonstrate the potencies of these fluoro derivatives expressed as an ID_{50} and $TCID_{50}$. Viral replication was measured as the amount of p24 antigen (core protein) in the culture supernatant by using a sandwich ELISA assay.[11a] Both 2'-*threo*-FddU (**22**) and 2'-*threo*-FddT (**33**) showed no activity in HIV-infected CEM cells. 2'-*threo*-FddC (**29**) was active with an ED_{50} of 0.9 μg/mL; the 50% tissue culture inhibitory dose of **29** was 61.9 μg/mL. Both olefinic analogues 2'-Fd4T (**40**) and 2'-Fd4C (**42**) showed no selective antiviral effects against HIV in vitro. 2'-Ara-FAZT (**44**) showed significant cytotoxicity with a $TCID_{50}$ of 2.6 μg/mL; no anti-HIV activity was observed at 28.5 μg/mL or lower. Since 2'-*threo*-FddC (**29**) was the most active of these new analogues, this compound was investigated further (Table II). The results show that 2'-*threo*-FddC (**29**) has activity against HIV in several different cell lines and with several different isolates of the virus.

The compound inhibited the virus in cultures of both monocyte/macrophages and T cells, natural reservoirs of the HIV in humans.[30] In these tests using monocyte/macrophage and T cells, the antiviral endpoint was measured using p24 viral antigen. 2'-*threo*-FddC (**29**) has antiviral potency that is several orders of magnitude less than that of AZT (**1**). The therapeutic indices for AZT are also greater in all of the in vitro experiments shown

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in Table II. The potential toxicity of **29** to normal human granulocyte macrophage progenitor cells was assessed by using a previously described assay.[3] The results in Table III show the bone marrow toxicity of both 2′-*threo*-FddC (**29**) and AZT (**1**). Both of the pyrimidine analogues, **29** and AZT (**1**), exhibit similar bone marrow toxicity. The percentage survival of the colony forming units (CFU-GM) concentrations of approximately 1 μM is of the order of 50%.

The antitumor and antibacterial properties of these 2′-fluorine-containing pyrimidine analogues were also tested.[31] 2′-*threo*-FddC (**29**) did show low to moderate activity against the VP16 resistant cell human carcinoma cell line HCT/VP35. 2′-Fd4C (**42**) was the most potent of these compounds as an antibacterial agent.

In this paper we have detailed synthetic approaches to a series of saturated 2′,3′-dideoxy-2′-fluoro and 2′-fluoro-2′,3′-unsaturated pyrimidine derivatives. In vitro experiments show that 2′-*threo*-FddC (**29**) was the most potent anti-HIV agent of the novel derivatives prepared. 2′-*threo*-FddC (**29**) was effective against several different strains of HIV in a number of different cell lines. The therapeutic in vitro index of **29** was, however, considerably lower than that of AZT.

Experimental Section

Melting points were determined on an Electrothermal capillary melting point apparatus and are uncorrected. Silica gel (100–200 mesh) purchased from E. Merck and Co. was used for the column chromatography. Anhydrous solvents were purchased from Aldrich Co. and used directly. Extracts were dried with anhydrous magnesium sulfate. ^{1}H and ^{13}C NMR spectra were recorded on an AM360 Bruker NMR spectrometer, Varian Gemini 300 NMR spectrometer, or Varian VXR200 spectrometer with TMS as an internal standard; chemical shifts are recorded in parts per million (ppm). IR spectra were recorded on a Perkin-Elmer 1800 FT-IR spectrometer. UV measurements were done on a Hewlett-Packard 8452A spectrophotometer. Mass spectra were obtained on a Kratos MS25 (FAB), Finnegan 4500 (EI and CI), or Kratos MS50 (high-resolution MS). The magic bullet matrix was a 1:3 mixture of dithioerythritol and dithiothreitol. Elemental analyses were obtained by the Analytical Department, Bristol-Myers Squibb Co., Wallingford, CT.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (18). 1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil (**16**) (40.01 g, 107.52 mmol) was suspended in methanol (225 mL) and water (75 mL). The suspension was treated with triethylamine (14.2 mL), and the mixture was hydrogenated over 10% palladium on carbon catalyst (2.07 g) in a Parr apparatus at 40 psi for 18 h. The catalyst was filtered off, and the bulk of the product crystallized upon cooling. Trituration with water and recrystallization from water–methanol gave **18** (23.9 g, 90.3% yield). Mp: 156–157 °C (lit.[22] mp 162 °C). ^{1}H NMR (360 MHz; DMSO-d_6): δ 7.71 (d, $J_{6,5}$ = 9.2 Hz, 1 H, H-6), 6.10 (dd, $J_{1',F}$ = 15.8 Hz, $J_{1',2'}$ = 4.2 Hz, 1 H, H-1′), 5.64 (d, $J_{5,6}$ = 8.0 Hz, 1 H, H-5), 5.02 (dm, $J_{2',F}$ = 52.1 Hz, 1 H, H-2′), 4.20 (dd, $J_{3',F}$ = 19.7 Hz, J = 3.0 Hz, 1 H, H-3′), 3.76 (m, 1 H, H-4′), 3.57 (m, 2 H, H-5′). ^{13}C NMR (50.3 MHz; D_2O): δ 166.1 (C-4), 151.25 (C-2), 142.65 (C-6), 101.68 (C-5), 95.0 (d, $J_{2',F}$ = 191.5 Hz, C-2′), 83.8 (d, $J_{1',F}$ = 16.7 Hz, C-1′), 83.6 (C-4′), 73.55 (d, $J_{3',F}$ = 25.7 Hz, C-3′), 60.64 (C-5′).

1-[5-*O*-(Monomethoxytrityl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]uracil (19). 1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (**18**) (17.68 g, 71.79 mmol) was dissolved in dry pyridine (300 mL) and the mixture stirred for 18 h at room temperature with monomethoxytrityl chloride (32.04 g, 103.75 mmol). The volatiles were removed in vacuo, and the residue was purified by column chromatography on silica gel (20–40% ethyl acetate in dichloromethane followed by 10% ethanol in ethyl acetate) to give **19** as a foam (33.26 g, 89.3%). ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.53 (dd, $J_{6,5}$ = 8.2 Hz, $J_{6,F}$ = 1.7 Hz, 1 H, H-6), 7.41–7.23 (m, 12 H, aromatic), 6.84–6.80 (m, 2 H, aromatic), 6.23 (dd, $J_{1',F}$ = 17.2 Hz, $J_{1',2'}$ = 3.8 Hz, 1 H, H-1′), 5.55 (br d, $J_{5,6}$ = 8.1 Hz, 1 H, H-5), 5.03 (dm, $J_{2',F}$ = 51.9 Hz, 1 H, H-2′), 4.43 (dm, $J_{3',F}$ = 16.3 Hz, 1, H-3′), 4.01 (m, 1, H-4′), 3.78 (s, 3, OCH_3), 3.43 (m, 2 H, H-5′). ^{13}C NMR (75.5 MHz; $CDCl_3$): δ 163.95 (C-4), 150.95 (C-2), 159.8 + 144.57 + 135.8 + 130.93 + 128.91 + 128.56 + 127.77 + 113.79 (aromatic trityl), 141.73 (d, $J_{6,F}$ = 2.3 Hz, C-6), 102.39 (C-5), 95.27 (d, $J_{2',F}$ = 192.5 Hz, C-2′), 87.29 (d, $J_{4',F}$ = 6.8 Hz, C-4′), 84.29 (d, $J_{1',F}$ = 17.3 Hz, C-1′), 83.56 (quaternary trityl), 75.35 (d, $J_{3',F}$ = 24.1 Hz, C-3′), 62.83 (C-5′), 55.58 (OCH_3).

1-[5-*O*-(Monomethoxytrityl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]uracil (21). 1-[5-*O*-(Monomethoxytrityl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]uracil (**19**) (12.58 g, 24.26 mmol) and 1,1-thiocarbonyldiimidazole (5.3 g, 29.74 mmol) were dissolved in anhydrous 1,2-dichloroethane (130 mL), and the mixture was heated at reflux under N_2 for 0.5 h. The volatiles were removed in vacuo, and the oily residue was dissolved in dry THF (55 mL). This solution was added dropwise over 0.5 h to a solution of azobisisobutyronitrile (AIBN) (0.4 g, 2.4 mmol) and tri-*n*-butyltin hydride (38.95 g, 133.8 mmol) in dry toluene (250 mL) at reflux under N_2. The mixture was heated at reflux for a further 4 h. The volatiles were removed in vacuo, and the residue was purified on a silica gel column (pentane followed by 10–30% ethyl acetate in dichloromethane). The product **21** was obtained as an oil (8.4 g, 68.9% overall). ^{1}H NMR (360 MHz; $CDCl_3$): δ 9.23 (br s, 1 H, NH), 7.53 (dd, $J_{6,5}$ = 8.2 Hz, $J_{6,F}$ = 1.6 Hz, 1 H, H-6), 7.45–7.21 (m, 12 H, aromatic), 6.83 (m, 2, aromatic), 6.05 (dd, $J_{1',F}$ = 18.0 Hz, $J_{1',2'}$ = 3.2 Hz, 1 H, H-1′), 5.58 (d, $J_{5,6}$ = 8.13 Hz, 1 H, H-5), 5.19 (dm, $J_{2',F}$ = 56.3 Hz, 1 H, H-2′), 4.32 (m, 1 H, H-4′), 3.78 (s, 3 H, OCH_3), 3.32 (m, 2 H, H-5′), 2.47–2.34 (m, 1 H, H-3′a), 2.28–2.03 (m, 1 H, H-3′b); ^{13}C NMR (75.5 MHz; $CDCl_3$): δ 164.15 (C-4), 151.15 (C-2), 159.42 + 144.74 + 144.65 + 135.75 + 128.98 + 128.55 + 127.73 + 113.75 (aromatic trityl), 141.81 (C-6), 102.17 (C-5), 91.08 (d, $J_{2',F}$ = 189.8 Hz, C-2′), 87.05 (quaternary trityl), 86.27 (d, $J_{4',F}$ = 16.3 Hz, C-1′), 77.74 (d, $J_{4',F}$ = 3.9 Hz, C-4′), 65.54 (C-5′), 55.58 (OCH_3), 33.79 (d, $J_{3',F}$ = 21.6 Hz, C-3′).

1-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)uracil (22). 1-[5-*O*-(Monomethoxytrityl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]uracil (**21**) (1.7 g, 3.38 mmol) was dissolved in 80% aqueous acetic acid (80 mL) and stirred for 2.5 h at 55–65 °C. The volatiles were removed in vacuo, and the product **22** (0.5 g) crystallized from methanol–diethyl ether–hexane. Additional material was obtained from the mother liquor by purification on a silica gel column (10% ethanol in ethyl acetate). Combined yield after recrystallization: 0.61 g (78.4%). Mp: 159–162 °C. ^{1}H NMR (360 MHz; DMSO-d_6): δ 7.74 (dd, $J_{6,5}$ = 8.1 Hz, $J_{6,F}$ = 1.8 Hz, 1 H, H-6), 5.97 (dd, $J_{1',F}$ = 16.8 Hz, $J_{1',2'}$ = 3.3 Hz, 1 H, H-1′), 5.62 (dd, $J_{5,6}$ = 8.1 Hz, $J_{5,F}$ = 1.6 Hz, 1 H, H-5), 5.28 (dm, $J_{2',F}$ = 54.8 Hz, 1 H, H-2′), 5.01 (t, $J_{OH,5'}$ = 5.8 Hz, 1 H, OH), 4.10 (m, 1 H, H-4′), 3.52 (m, 2 H, H-5′), 2.54–2.38 (m, 1 H, H-3′a), 2.12–1.98 (m, 1 H, H-3′b). ^{13}C NMR (75.5 MHz; DMSO-d_6): δ 163.6 (C-4), 150.7 (C-2), 141.77 (d, $J_{6,F}$ = 2.8 Hz, C-6), 101.08 (C-5), 91.65 (d, $J_{2',F}$ = 187 Hz, C-2′), 85.0 (d, $J_{1',F}$ = 15.8 Hz, C-1′), 78.03 (C-4′), 62.7 (C-5′), 32.45 (d, $J_{3',F}$ = 19.8 Hz, C-3′). Anal. ($C_9H_{11}FN_2O_4$) C, H, N.

1-[5-*O*-(Monomethoxytrityl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]cytosine (28). 1-[5-*O*-(Monomethoxytrityl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]uracil (**21**) (0.84 g, 1.67 mmol) was stirred in dry pyridine (5 mL) with *p*-chlorophenyl phosphodichloridate (1.23 g, 0.814 mL, 5.0 mmol) and freshly sublimed 1,2,4-triazole (693 mg, 10.0 mmol) for 72 h. This mixture was partitioned between dichloromethane and water. The organic extract was dried and the solvent removed in vacuo to give crude 1,2,4-triazolyl derivative **23**. An analytical sample was purified on a silica gel column (40–60% ethyl acetate in dichloromethane). ^{1}H NMR (200 MHz; $CDCl_3$): δ 9.28 (s, 1 H, triazole), 8.15 (d, $J_{6,5}$ = 7.0 Hz, 1 H, H-6), 8.14 (s, 1 H, triazole), 7.51–7.18 (m, 12 H, aromatic), 6.94 (d, $J_{5,6}$ = 7.4 Hz, 1 H, H-5), 6.87 (d, J = 8.8 Hz, 2 H, aromatic), 6.16 (dd, $J_{1',F}$ = 18.2 Hz, $J_{1',2'}$ = 3.6 Hz, 1 H, H-1′), 5.44 (dm, $J_{2',F}$ = 53.8 Hz, 1 H, H-2′), 4.50 (m, 1 H, H-4′), 3.82 (s, 3 H, OCH_3), 3.39 (d, J = 4.8 Hz, 2 H, H-5′), 2.66–2.14 (br m, 2 H, H-3′). Anal. ($C_{31}H_{28}FN_5O_4 \cdot H_2O$) C, H, N.

(31) The compounds were tested against the following bacteria: *Staphylococcus aureus, Salmonella enteriditis, Pseudomonas aeruginosa, Proteus mirabilis, Bacillus subtilis, Escherichia coli, Haemophilus influenzae, Micrococcus lutea,* and *Neisseria gonnorhoeae.*

Crude **23** was dissolved in dry 1,4-dioxane (15 mL) and stirred for 4 h with 27% aqueous ammonia (8 mL). The volatiles were removed in vacuo, and the residue was purified on a silica gel column (3–10% ethanol in ethyl acetate) to give the product **28** (0.59 g, 70.4%). ^{1}H NMR (200 MHz; $CDCl_3$): δ 7.56 (dd, $J_{6,5}$ = 7.6 Hz, $J_{6,F}$ = 3.0 Hz, 1 H, H-6), 7.50–7.20 (m, 12 H, aromatic), 6.85 (m, 2 H, aromatic), 6.08 (dd, $J_{1',F}$ = 18.2 Hz, $J_{1',2'}$ = 3.6 Hz, 1 H, H-1'), 5.61 (d, $J_{5,6}$ = 7.8 Hz, 1 H, H-5), 5.21 (dm, $J_{2',F}$ = 54.4 Hz, 1 H, H-2'), 4.35 (m, 1 H, H-4'), 3.28 (m, 2 H, H-5'), 2.62–2.06 (br m, 2 H, H-3'). MS (FAB, glycerol): *m/e* 502 (M^+ + H).

1-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)cytosine (29). 1-[5-*O*-(Monomethoxytrityl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]cytosine (**28**) (0.42 g, 0.837 mmol) was dissolved in 80% aqueous acetic acid (15 mL) and stirred for 3 h (60 °C). The volatiles were removed in vacuo, and the residue was filtered through a short silica gel column (5–30% ethanol in ethyl acetate) to give **29**, which was recrystallized from ethanol (89 mg, 46.4%). Mp: 203–205 °C. ^{1}H NMR (360 MHz; DMSO-d_6): δ 7.67 (dd, $J_{6,5}$ = 6.7 Hz, $J_{6,F}$ = 1.5 Hz, 1 H, H-6), 7.19 (br d, 2 H, NH_2), 5.93 (dd, $J_{1',F}$ = 18.7 Hz, $J_{1',2'}$ = 3.5 Hz, 1 H, H-1'), 5.71 (d, $J_{5,6}$ = 6.7 Hz, 1 H, H-5), 5.21 (dm, $J_{2',F}$ = 51.5 Hz, 1 H, H-2'), 4.94 (t, $J_{OH,5'}$ = 5.8 Hz, 1 H, OH), 4.08 (m, 1 H, H-4'), 3.51 (m, 2 H, H-5'), 2.54–2.37 (dddd, $J_{3'a,F}$ = 33.9 Hz, $J_{3'a,3'b}$ = 14.7 Hz, $J_{3'a,4'}$ = 8.0 Hz, $J_{3'a,2'}$ = 5.9 Hz, 1 H, H-3'a), 2.10–1.95 (dddd, $J_{3'b,F}$ = 28.6 Hz, $J_{3'b,3'a}$ = 14.6 Hz, $J_{3'b,4'}$ = 5.3 Hz, $J_{3'b,2'}$ = 2.1 Hz, 1 H, H-3'b). ^{13}C NMR (75.5 MHz; CD_3OD): δ 168.54 (C-4), 158.73 (C-2), 144.18 (C-6), 95.62 (C-5), 92.7 (d, $J_{2',F}$ = 187.8 Hz, C-2'), 88.89 (d, $J_{1',F}$ = 16.5 Hz, C-1'), 79.85 (C-4'), 65.15 (C-5'), 34.24 (d, $J_{3',F}$ = 20.7 Hz, C-3'). UV (MeOH): max 272 nm (ϵ 7906). IR (KBr): max 3470, 3300 (br), 3200 (br), 1645, 1530, 1490, 1400, 1295, 1125, 1075, 858, 818 cm^{-1}. MS (CI; methane): *m/e* 230 (M^+ + H). Anal. ($C_9H_{12}FN_3O_3$) C, H, N.

1-(5-*O*-Acetyl-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)uracil (25). 1-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)uracil (**22**) (2.40 g, 10.43 mmol) was treated with dry pyridine (20 mL) and acetic anhydride (10 mL) and then stirred for 16 h under nitrogen. The volatiles were removed in vacuo, and the product **25** (2.85 g, quantitative yield) solidified after addition and evaporation of toluene (50 mL). This material was used in the next step without purification. ^{1}H NMR (300 MHz; $CDCl_3$): δ 8.90 (br s, 1 H, NH), 7.56 (dd, $J_{6,5}$ = 8.3 Hz, $J_{6,F}$ = 2.1 Hz, 1 H, H-6), 6.01 (dd, $J_{1',F}$ = 20.1 Hz, $J_{1',2'}$ = 2.8 Hz, 1 H, H-1'), 5.75 (d, $J_{5,6}$ = 8.1 Hz, 1 H, H-5), 5.21 (dm, $J_{2',F}$ = 53.7 Hz, 1 H, H-2'), 4.46–4.18 (ABM, 3 H, H-4' + H-5'), 2.52 (dm, $J_{3a',F}$ = 38.4 Hz, 1 H, H-3a'), 2.31–2.07 (m, 4 H, H-3b' + CH_3). ^{13}C NMR (75.5 MHz; $CDCl_3$): δ 171.58 (CH_3CO), 164.65 (C-4), 151.27 (C-2), 141.63 (d, $J_{6,F}$ = 3.1 Hz, C-6), 102.22 (C-5), 90.89 (d, $J_{2',F}$ = 189 Hz, C-2'), 86.71 (d, $J_{1',F}$ = 16.04 Hz, C-1'), 75.72 (C-4'), 65.63 (C-5'), 33.65 (d, $J_{3',F}$ = 20.7 Hz, C-3'), 20.95 (CH_3).

1-(5-*O*-Acetyl-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-4-thiouracil (26). 1-(5-*O*-Acetyl-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)uracil (**25**) (2.7 g, 9.9 mmol) was dissolved in dry 1,2-dimethoxyethane (50 mL) and heated under reflux for 3 h with Lawesson's reagent (6.0 g, 14.8 mmol). The volatiles were removed in vacuo, and the residue was purified on silica gel column (2–15% ethyl acetate in dichloromethane) to give **26** (2.05 g, 71.8%). Mp: 152–153 °C. ^{1}H NMR (360 MHz; $CDCl_3$): δ 7.52 (d, $J_{6,5}$ = 7.5 Hz, 1 H, H-6), 6.38 (d, $J_{5,6}$ = 7.5 Hz, 1 H, H-5), 6.01 (dd, $J_{1',F}$ = 18.6 Hz, $J_{1',2'}$ = 2.0 Hz, 1 H, H-1'), 5.28 (br d, $J_{2',F}$ = 54.2 Hz, 1 H, H-2'), 4.46–4.20 (ABM, 3 H, H-4' + H-5'), 2.63 (dm, $J_{3a',F}$ = 37 Hz, 1 H, H-3a'), 2.26–2.07 (m, 4 H, H-3b' + CH_3). ^{13}C NMR (75.5 MHz; $CDCl_3$): δ 190.6 (C-4), 171.5 (CH_3CO), 148.4 (C-2), 135.88 (d, J = 2.9 Hz, C-6), 113.55 (C-5), 90.7 (d, $J_{2',F}$ = 190.0 Hz, C-2'), 87.26 (d, $J_{1',F}$ = 16.2 Hz, C-1'), 76.2 (C-4'), 65.54 (C-5'), 33.75 (d, $J_{3',F}$ = 20.5 Hz, C-3'), 21.0 (CH_3). MS (FAB, thioglycerol): 289 (M^+ + H). Anal. ($C_{11}H_{13}FN_2O_4S_1$) C, H, N.

1-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-4-thiouracil (27). 1-(5-*O*-Acetyl-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-4-thiouracil (**26**) (119 mg, 0.413 mmol) was dissolved in methanol saturated with ammonia (10 mL) and stirred at room temperature for 16 h. Evaporation of the solvent and recrystallization from ethanol–diethyl ether gave analytically pure **27** (88 mg, 82%). Mp: 134–137 °C. ^{1}H NMR (300 MHz; CD_3OD): δ 7.68 (dd, $J_{6,5}$ = 7.6 Hz, $J_{6,F}$ = 1.6 Hz, 1 H, H-6), 6.35 (d, $J_{5,6}$ = 7.6 Hz, 1 H, H-5), 5.98 (dd, $J_{1',F}$ = 17.0 Hz, $J_{1',2'}$ = 3.4 Hz, 1 H, H-1'), 5.28 (dm, $J_{2',F}$ = 54.4 Hz, 1 H, H-2'), 4.23 (m, 1 H, H-4'), 3.76–3.63 (m, 2 H, H-5'), 2.62–2.42 (m, 1 H, H-3a'), 2.25–2.09 (m, 1 H, H-3b'). ^{13}C NMR (75.5 MHz; CD_3OD): δ 193.18 (C-4), 150.1 (C-2), 137.73 (C-6), 113.93 (C-5), 92.84 (d, $J_{2',F}$ = 188.5 Hz, C-2'), 88.09 (d, $J_{1',F}$ = 16.5 Hz, C-1'), 79.9 (C-4'), 64.82 (C-5'), 33.96 (d, $J_{3',F}$ = 20.6 Hz, C-3'). MS (CI, methane): 247 (M^+ + H), 129 (4-thiouracil$^+$ + H). Anal. ($C_9H_{11}FN_2O_3S_1 \cdot 75H_2O$) C, H, N.

1-(2,3-Dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)cytosine (29). 1-(5-*O*-Acetyl-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl)-4-thiouracil (**27**) (2.9 g, 10.06 mmol) was dissolved in methanol saturated with ammonia (60 mL). The solution was placed in a bomb and heated to 100 °C for 3 h. The solvent was evaporated and the product crystallized from ethanol–acetone to give analytically pure **29** (2.11 g, 91.7%) identical in all respects with the material prepared from **28**. Anal. ($C_9H_{12}FN_3O_3$) C, H, N.

1-[5-*O*-(Monomethoxytrityl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]thymine (30). 1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)thymine (**17**) (10.4 g, 40.0 mmol) and monomethoxytrityl chloride (13.9 g, 45.0 mmol) were dissolved in anhydrous pyridine (150 mL) and heated at 65–75 °C for 6 h. The reaction mixture was poured onto ice–water (1.5 L) with vigorous stirring. The resulting precipitate was filtered off (21.1 g) but turned into oil on standing. This material (**30**) was used in the next step without purification. ^{1}H NMR (200 MHz; $CDCl_3$): δ 8.83 (br s, 1 H, NH), 7.5–7.18 (m, 13 H, aromatic + H-6), 6.84 (d, 2 H, aromatic), 6.27 (dd, $J_{1',F}$ = 18.8 Hz, $J_{1',2'}$ = 3.2 Hz, 1 H, H-1'), 5.04 (dm, $J_{2',F}$ = 51.6 Hz, 1 H, H-2'), 4.46 (dd, $J_{3',F}$ = 20.2 Hz, J = 4.0 Hz, 1 H, H-3'), 4.01 (q, J = 4.3 Hz, 1 H, H-4'), 3.80 (s, 3 H, OCH_3), 3.51–3.37 (m, 2 H, H-5'), 1.75 (br s, 3 H, CH_3). ^{13}C NMR (75.5 MHz; $CDCl_3$): δ 164.45 (C-4), 151.0 (C-2), 159.43 + 144.72 + 144.68 + 136.93 + 135.75 + 130.94 + 128.94 + 128.55 + 127.74 + 113.78 (aromatic trityl), 110.82 (C-5), 95.86 (d, $J_{2',F}$ = 193.7 Hz, C-2'), 87.22 (C-4'), 84.14 (d, $J_{1',F}$ = 16.1 Hz, C-1'), 83.55 (quaternary trityl), 75.84 (d, $J_{3',F}$ = 26.1 Hz, C-3'), 63.09 (C-5'), 55.57 (OCH_3), 12.49 (CH_3).

1-(2,3-Dideoxy-2-fluoro-*threo*-pentofuranosyl)thymine (33). 1-[5-*O*-(Monomethoxytrityl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]thymine (**30**) (7.0 g, 13.14 mmol) and 1,1-thiocarbonyldiimidazole (2.67 g, 15.0 mmol) were heated at 80 °C for 2.5 h in dry DMF (50 mL), and the volatiles were removed in vacuo. The residue was then heated at reflux in dry toluene (380 mL) with AIBN (0.12 g), bis(tributyltin) oxide (29 mL), and polymethylhydrosiloxane (29 mL) under N_2 for 3.5 h. The volatiles were removed in vacuo, and the residue was triturated with hexane (250 mL) and then cooled to −70 °C. The supernatant was discarded, and the residue was purified on a silica gel column (25–50% ethyl acetate in dichloromethane). 1-[5-*O*-(Monomethoxytrityl)-2,3-dideoxy-2-fluoro-*threo*-pentofuranosyl]thymine (**32**) was crystallized from dichloromethane–diethyl ether. This material (2.7 g) was dissolved in 80% aqueous acetic acid (20 mL) and stirred for 3 h (45–55 °C). The volatiles were removed in vacuo, and the residue was crystallized from dichloromethane–diethyl ether–hexane to give **33** (0.43 g). Additional material was obtained from the silica gel chromatography on the mother liquor (10% ethanol in ethyl acetate). Recrystallization of the combined lots gave pure **33** (0.81 g, 25.2% overall from **30**). Mp: 162–164 °C. ^{1}H NMR (360 MHz; DMSO-d_6): δ 7.61 (br s, 1 H, H-6), 5.95 (dd, $J_{1',F}$ = 16.6 Hz, $J_{1',2'}$ = 3.8 Hz, 1 H, H-1'), 5.26 (dm, $J_{2',F}$ = 54.9 Hz, 1 H, H-2'), 4.07 (m, 1 H, H-4'), 3.62–3.48 (m, 2 H, H-5'), 2.53–2.37 (m, 1 H, H-3a'), 2.13–1.98 (m, 1 H, H-3b'), 1.77 (br s, 3 H, CH_3). ^{13}C NMR (75.5 MHz; CD_3OD): δ 167.11 (C-4), 152.81 (C-2), 139.64 (C-6), 110.80 (C-5), 92.96 (d, $J_{2',F}$ = 189.1 Hz, C-2'), 87.47 (d, $J_{1',F}$ = 16.5 Hz, C-1'), 79.69 (C-4'), 64.72 (C-5'), 33.89 (d, $J_{3',F}$ = 30.6 Hz, C-3'), 12.52 (CH_3). MS (FAB; glycerol): *m/e* 245 (M^+ + H), 127 (thymine$^+$ + H). Anal. ($C_{10}H_{13}FN_2O_4$) C, H, N.

1-[3-*O*-(Methylsulfonyl)-5-*O*-(monomethoxytrityl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]thymine (34). 1-[5-*O*-(Monomethoxytrityl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]-thymine (**30**) (10.0 g, 18.78 mmol) in dry pyridine (65 mL) was treated dropwise at 0 °C with methanesulfonyl chloride (6 mL, 61.4 mmol), and the mixture was kept at 0 °C overnight. The volatiles were removed in vacuo, and the residue was purified on a silica gel column (25–45% ethyl acetate in dichloromethane) to give **34** as an oil (7.0 g, 61%). ^{1}H NMR (200 MHz; $CDCl_3$): δ 8.40 (br s, 1 H, NH), 7.55–7.20 (m, 13 H, aromatic + H-6), 6.87

(br d, 2 H, aromatic), 6.25 (dd, $J_{1',F}$ = 19.6 Hz, $J_{1',2'}$ = 3.6 Hz, 1 H, H-1'), 5.38 (dd, $J_{3',F}$ = 17.6 Hz, $J_{3',4'}$ = 3.4 Hz, 1 H, H-3'), 5.29 (dd, $J_{2',F}$ = 48 Hz, J = 3.6 Hz, 1 H, H-2'), 4.17 (m, 1 H, H-4'), 3.81 (s, 3 H, OCH_3), 3.51 (m, 2 H, H-5'), 3.06 (s, 3 H, OSO_2CH_3), 1.74 (br s, 3 H, CH_3). ^{13}C NMR (75.5 MHz; $CDCl_3$): δ 164.6 (C-4), 159.54 + 144.39 + 136.83 + 130.9 + 128.87 + 128.59 + 127.68 + 113.8 (aromatic trityl), 151.14 (C-2), 135.39 (C-6), 111.35 (C-5), 93.56 (d, $J_{2',F}$ = 195.5 Hz, C-2'), 87.60 (C-4'), 83.76 (d, $J_{1',F}$ = 17.0 Hz, C-1'), 80.9 (trityl quaternary), 80.3 (d, $J_{3',F}$ = 30.0 Hz, C-3'), 61.71 (C-5'), 55.58 (OCH_3), 38.67 (OSO_2CH_3), 12.5 (CH_3).

O-2,3'-Anhydro-1-[5-O-(monomethoxytrityl)-2-deoxy-2-fluoro-β-D-lyxofuranosyl]thymine (36). 1-[3-O-(Methylsulfonyl)-5-O-(monomethoxytrityl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]thymine (**34**) (2.7 g, 4.42 mmol) was dissolved in ethanol (200 mL) and heated at reflux for 2 h with aqueous 1 N NaOH (4.6 mL). The volatiles were evaporated, and the residual solid was washed into a filtering funnel with cold water. Yield: 1.7 g (74.6%). ^1H NMR (200 MHz; $CDCl_3$): δ 7.5–7.2 (m, 12 H, aromatic), 6.92 (br s, 1 H, H-6), 6.80 (br d, 2 H, aromatic), 5.37 (dt, $J_{2',F}$ = 52.0 Hz, $J_{2',1'}$ = $J_{2',3'}$ = 3.4 Hz, 1 H, H-2'), 5.36 (m, 1 H, H-1'), 5.03 (m, 1 H, H-3'), 4.33 (m, 1 H, H-4'), 3.77 (s, 3 H, OCH_3), 3.36 (br d, $J_{5',4'}$ = 6.3 Hz, 2 H, H-5'), 1.94 (br s, 3 H, CH_3). ^{13}C NMR (75.5 MHz; DMSO-d_6): δ 170.94 (C-4), 153.38 (C-2), 158.77 + 144.29 + 144.14 + 137.38 + 130.42 + 128.31 + 127.34 + 113.614 (aromatic trityl), 134.73 (C-6), 117.61 (C-5), 86.44 (quaternary trityl), 83.83 (d, $J_{1',F}$ = 19.5 Hz, C-1'), 82.29 (d, $J_{2',F}$ = 204.5 Hz, C-2'), 80.67 (d, $J_{4',F}$ = 6.9 Hz, C-4'), 75.79 (d, $J_{3',F}$ = 15.2 Hz, C-3'), 61.73 (C-5'), 55.15 (OCH_3), 12.99 (CH_3). MS (FAB; magic bullet): m/e 515 (M^+ + H).

1-[5-O-(Monomethoxytrityl)-2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl]thymine (38). O-2,3'-Anhydro-1-[5-O-(monomethoxytrityl)-2-deoxy-2-fluoro-β-D-lyxofuranosyl)thymine (**36**) (412 mg, 0.8 mmol) and *t*-BuOK (199.8 mg, 1.78 mmol) were suspended in dry DMSO (7 mL) and stirred for 0.5 h at room temperature under an inert atmosphere. The mixture was poured onto ice-water and acidified to pH 5.0 with 80% acetic acid. The precipitate was filtered off (0.2 g), but became an oil upon standing. Additional material was obtained from ethyl acetate extraction of the water layer. The crude **38** was purified on silica gel column (45–50% ethyl acetate in hexane) to give pure **38** (190 mg, 46.1%). ^1H NMR (360 MHz; $CDCl_3$): δ 8.22 (br s, 1 H, NH), 7.47 (s, 1 H, H-6), 7.4–7.20 (m, 12 H, aromatic), 6.92 (dd, J = 4.5 Hz, J = 1.4 Hz, 1 H, H-1'), 6.80 (m, 2 H, aromatic), 5.69 (d, J = 1.3 Hz, 1 H, H-3'), 4.93 (m, 1 H, H-4'), 3.77 (s, 3 H, OCH_3), 3.35 (AB of ABX, 2 H, H-5'), 2.03 (br s, 1 H, CH_3). ^{13}C NMR (75.5 MHz; $CDCl_3$): δ 164.47 (C-4), 153.81 (d, $J_{2',F}$ = 282.9, C-2'), 151.64 (C-2), 159.56 + 144.20 + 144.09 + 135.74 + 130.96 + 129.08 + 128.88 + 128.605 + 128.47 + 127.75 + 113.67 (aromatic trityl), 135.20 (C-6), 112.72 (C-5), 107.1 (d, $J_{3',F}$ = 7.3 Hz, C-3'), 87.30 (quaternary trityl), 83.1 (d, $J_{1',F}$ = 28.1 Hz, C-1'), 81.1 (d, $J_{4',F}$ = 9.5 Hz, C-4'), 65.18 (C-5'), 11.43 (CH_3).

1-(2,3-Dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)thymine (40). 1-(5-O-Monomethoxytrityl-2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)thymine (**38**) (0.3 g, 0.583 mmol) was stirred for 1.5 h at 60 °C in 80% acetic acid (5 mL), and the volatiles were removed in vacuo. The residue was purified on silica gel column (3–5% ethanol in 1:1 ethyl acetate–dichloromethane) to give the title nucleoside **40** (40 mg, 28.3%). Mp 129–131 °C dec. ^1H NMR (200 MHz; DMSO-d_6): δ 7.89 (br s, 1 H, H-6), 6.75 (m, 1 H, H-1'), 5.99 (s, 1 H, H-3'), 5.16 (t, $J_{OH,5'}$ = 5.4 Hz, 1 H, OH), 4.80 (m, 1 H, H-4'), 3.61 (m, 2 H, H-5'), 1.76 (br s, 3 H, CH_3). ^{13}C NMR (300 MHz; $CDCl_3$): δ 163.31 (C-4), 152.78 (d, $J_{2',F}$ = 282.9 Hz, C-2'), 150.63 (C-2), 135.84 (C-6), 111.63 (C-5), 106.41 (d, $J_{3',F}$ = 7.3 Hz, C-3'), 83.31 (d, $J_{1',F}$ = 28.1 Hz, C-1'), 81.93 (d, $J_{4',F}$ = 9.1 Hz, C-4'), 63.46 (C-5'), 12.45 (CH_3). MS (FAB; magic bullet): 243 (M^+ + H). Anal. ($C_{10}H_{11}FN_2O_4$) C, H, N.

1-[3-O-(Methylsulfonyl)-5-O-(monomethoxytrityl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]uracil (35). 1-[5-O-(Monomethoxytrityl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]uracil (**19**) (43.0 g, 82.92 mmol) was dissolved in dry pyridine (200 mL) and treated at 0 °C with methanesulfonyl chloride (10.36 g, 7.0 mL, 90.44 mmol) for 16 h. The excess reagent was destroyed with water (2 mL) while the temperature was kept around 0 °C. The mixture was then poured onto ice–water with stirring. The precipitate (**30**) (50.0 g, quantitative) was isolated by filtration and dried, but it became an oil upon standing. This material was used without purification in the next step. ^1H NMR (200 MHz; $CDCl_3$): δ 8.52 (br s, 1 H, NH), 7.54–7.20 (m, 13 H, aromatic + H-6), 6.84–6.89 (m, 2 H, aromatic), 6.23 (dd, $J_{1',F}$ = 18.4 Hz, $J_{1',2'}$ = 3.4 Hz, 1 H, H-1'), 5.58 (dd, $J_{5,6}$ = 8.2 Hz, J = 2.0 Hz, 1 H, H-5), 5.36 (dm, $J_{3',F}$ = 20.6 Hz, 1 H, H-3'), 5.30 (dm, $J_{2',F}$ = 50.6 Hz, 1 H, H-2'), 4.20 (m, 1 H, H-4'), 3.81 (s, 3 H, OCH_3), 3.52 (m, 2 H, H-5'), 3.07 (s, 3 H, OSO_2CH_3). ^{13}C NMR (75.5 MHz; $CDCl_3$): δ 164.04 (C-4), 151.01 (C-2), 159.56 + 144.32 + 144.27 + 137.23 + 135.26 + 130.96 + 128.9 + 128.67 + 127.92 + 113.91 (aromatic trityl), 141.14 (d, J = 1.5 Hz, C-6), 102.89 (C-5), 93.25 (d, $J_{2',F}$ = 195.4 Hz, C-2'), 87.70 (C-4'), 83.91 (d, $J_{1',F}$ = 16.9 Hz, C-1'), 81.03 (quaternary trityl), 80.04 (d, $J_{3',F}$ = 29.4 Hz, C-3'), 61.64 (C-5'), 55.60 (OCH_3), 38.72 (OSO_2CH_3).

O-2,3'-Anhydro-1-[5-O-(monomethoxytrityl)-2-deoxy-2-fluoro-β-D-lyxofuranosyl]uracil (37). 1-[3-O-(Methylsulfonyl)-5-O-(monomethoxytrityl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]uracil (**35**) (20.0 g, 33.5 mmol) was dissolved in ethanol (400 mL), treated with 1 N aqueous NaOH (35 mL), and heated at reflux for 4 h. The mixture was cooled to 5 °C, and the pH was adjusted to 7.5 with 80% acetic acid. The resulting precipitate was isolated by filtration, washed with methanol–water, and dried to give **37** (14.0 g, 83.5%). ^1H NMR (360 MHz; DMSO-d_6): δ 7.76 (d, $J_{6,5}$ = 7.5 Hz, 1 H, H-6), 7.37–7.2 (m, 12 H, aromatic), 6.88 (d, 2 H, aromatic), 6.03 (br d, J = 3.8 Hz, 1 H, H-1'), 5.91 (dt, $J_{2',F}$ = 50.0 Hz, J = 3.6 Hz, 1 H, H-2'), 5.89 (d, $J_{5,6}$ = 7.3 Hz, 1 H, H-5), 5.43 (t, J = 2.7 Hz, 1 H, H-3'), 4.60 (m, 1 H, H-4'), 3.72 (s, 3 H, OCH_3), 3.13 (m, 2 H, H-5'). ^{13}C NMR (75.5 MHz; DMSO-d_6): δ 170.32 (C-4), 153.67 (C-2), 158.79 + 144.3 + 144.16 + 134.69 + 130.43 + 128.35 + 128.23 + 127.38 + 113.64 (aromatic trityl), 141.69 (C-6), 109.33 (C-5), 86.44 (trityl quaternary), 83.93 (d, $J_{1',F}$ = 19.5 Hz, C-1'), 82.33 (d, $J_{2',F}$ = 204.1 Hz, C-2'), 80.68 (d, $J_{4',F}$ = 6.9 Hz, C-4'), 76.03 (d, $J_{3',F}$ = 15.3 Hz, C-3'), 61.69 (C-5'), 55.15 (OCH_3). Anal. ($C_{29}H_{25}FN_2O_5$) C, H, N.

1-[5-O-(Monomethoxytrityl)-2-fluoro-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl]uracil (39). O-2,3'-Anhydro-1-[5-O-(monomethoxytrityl)-2-deoxy-2-fluoro-β-D-lyxofuranosyl]uracil (**37**) (4.2 g, 8.39 mmol) and *t*-BuOK (2.1 g, 18.75 mmol) in dry DMSO (120 mL) were stirred at room temperature for 1 h. This mixture was poured onto ice–water (600 mL) and then extracted with ethyl acetate. The organic phase was dried then evaporated to give **39** (4.0 g, 95.2%), which was used in the next reaction without purification. ^1H NMR (360 MHz; $CDCl_3$): δ 8.41 (br s, 1 H, NH), 7.92 (d, $J_{6,5}$ = 8.1 Hz, 1 H, H-6), 7.35–7.19 (m, 12 H, aromatic), 6.88 (m, 1 H, H-1'), 6.83 (d, 2 H, aromatic), 5.62 (s, 1 H, H-3'), 5.04 (d, $J_{5,6}$ = 8.1 Hz, 1 H, H-5), 4.89 (m, 1 H, H-4'), 3.43 (m, 2 H, H-5').

1-[5-O-(Monomethoxytrityl)-2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl]cytosine (41). 1-[5-O-(Monomethoxytrityl)-2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl]uracil (**39**) (4.0 g, 8.0 mmol) was dissolved in dry pyridine (40 mL) and treated with *p*-chlorophenyl phosphodichloridate (4.9 g, 3.26 mL, 20 mmol) and 1,2,4-triazole (2.77 g, 40.0 mmol). The mixture was stirred under argon for 72 h at room temperature and subsequently partitioned between water and dichloromethane. The organic layer was dried, and the solvent was removed under reduced pressure. The residue was dissolved in dry 1,4-dioxane (100 mL) and treated with concentrated aqueous ammonia (50 mL). The reaction mixture was stirred for 5 h at room temperature. The volatiles were removed in vacuo, and the residue was purified on a silica gel column (5–12.5% of ethanol in ethyl acetate) to give **41** (0.75 g, 18.8% overall). ^1H NMR (200 MHz; DMSO-d_6): δ 7.71 (d, $J_{6,5}$ = 7.4 Hz, 1 H, H-6), 7.38–7.19 (m, 12 H, aromatic), 6.88 (m, 3 H, aromatic + H-1'), 6.07 (m, 1 H, H-3'), 5.37 (d, $J_{5,6}$ = 7.4 Hz, 1 H, H-5), 4.90 (m, 1 H, H-4'), 3.75 (s, 3 H, OCH_3), 3.60 (m, 2 H, H-5').

1-(2,3-Dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)cytosine (42). 1-[5-O-(Monomethoxytrityl)-2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl]cytosine (**41**) (0.75 g, 3.30 mmol) dissolved in 80% AcOH (5 mL) was stirred for 5 h at room temperature. The volatiles were removed in vacuo, and the product was purified on a silica gel column (5–25% of methanol in dichloromethane) to give **42** (0.24 g, 70.4%). ^1H NMR (360 MHz; DMSO-d_6): δ 7.85 (d, $J_{6,5}$ = 7.4 Hz, 1 H, H-6), 7.32 (br d, 2 H, NH_2), 6.84 (br s, 1 H, H-1'), 5.93 (s, 1 H, H-3'), 5.76 (d, $J_{5,6}$ = 7.4 Hz, 1 H, H-5), 5.05 (t, J = 5.3 Hz, 1 H, OH), 4.75

(m, 1 H, H-4′), 3.55 (m, 3 H, H-5′). MS (FAB; glycerol): m/e 288 ($M^+ + H$), 112 (cytosine$^+ + H$). Anal. ($C_9H_{10}FN_3O_3$) C, H, N.

1-(3-Azido-2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)thymine (44). *O*-2,3′-Anhydro-1-[5-*O*-(monomethoxytrityl)-2-deoxy-2-fluoro-β-D-lyxofuranosyl]thymine (**36**) (0.85 mg, 1.65 mmol) was dissolved in dry DMF (25 mL) and stirred with lithium azide (0.98 g, 20 mmol) for 62 h at 105 °C. A few crystals of potassium carbonate were added, and the mixture was partitioned between water and ethyl acetate. Unreacted starting material (0.14 g) crystallized out upon trituration with diethyl ether–methylene chloride. The mother liquor (crude **43**, 0.7 g) was dissolved in 80% acetic acid (5 mL) and stirred for 6 h at 35 °C. After removal of the volatiles in vacuo, the crude product was purified on silica gel column (50% ethyl acetate in dichloromethane) to give **44** (0.15 g, 31.9% overall). ^{1}H NMR (360 MHz; DMSO-d_6): δ 11.46 (br s, 1 H, NH), 7.60 (s, 1 H, H-6), 6.14 (dd, $J_{1',F}$ = 10.9 Hz, $J_{1',2'}$ = 5.4 Hz, 1 H, H-1′), 5.37 (dt, $J_{2',F}$ = 54.0 Hz, $J_{2',1'} = J_{2',3'}$ = 5.4 Hz, 1 H, H-2′), 5.34 (br s, 1 H, OH), 4.51 (ddd, $J_{3',F}$ = 22.4 Hz, $J_{3',4'}$ = 7.5 Hz, $J_{3',2'}$ = 5.3 Hz, 1 H, H-3′), 3.82 (m, 1 H, H-4′), 3.68 (m, 2 H, H-5′), 1.77 (br s, 3 H, CH_3). ^{13}C NMR (50.3 MHz; acetone-d_6): δ 137.02 (C-6), 110.23 (C-5), 95.11 (d, $J_{2',F}$ = 195.0 Hz, C-2′), 83.21 (d, $J_{1',F}$ = 16.8 Hz, C-1′), 81.35 (d, J = 0.4 Hz, C-4′), 64.21 (d, $J_{3',F}$ = 25.0 Hz, C-3′), 60.95 (C-5′), 12.51 (CH_3). Anal. ($C_{10}H_{12}FN_5O_4$) C, H, N.

1-[5-*O*-(Monomethoxytrityl)-2,3-dideoxy-2-fluoro-β-D-*threo*-pentofuranosyl]uracil (21). Method B. 1-[5-*O*-(Monomethoxytrityl)-2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl]uracil (**39**) (0.45 g, 0.9 mmol) was dissolved in ethanol (60 mL) and hydrogenated over 10% palladium on carbon catalyst (85 mg) for 2 h. Filtration and evaporation of the solvent in vacuo yielded the crude product **21**, which was subsequently purified on a silica gel column (15–25% ethyl acetate in dichloromethane). Yield: 300 mg (66.4%). MS (EI): m/e 502 (M^+). This compound is identical with **21** obtained through the deoxygenation method described earlier.

Antiviral Assays. Antiviral activity against HIV activity was measured as described earlier.[10] The assay for the granulocyte-macrophage CFU inhibition was also previously described.[3] The CEM/LAV assay (Table III) uses a T cell line, CEM-SS, infected with HIV-1, LAV strain. The antiviral endpoint was p24 (viral antigen) and was measured 6 days postinfection; the endpoint for cellular toxicity was inhibition of cellular DNA synthesis. The U937/LAV test used a monocytic cell line; the antiviral endpoint was again p24 viral antigen measured 6 days postinfection; the cellular toxicity endpoint was inhibition of cellular DNA synthesis. The T cell/HTLV$_{IIIB}$ assay used freshly isolated T cells, stimulated with the mitogen PHA, and subsequently infected with HIV (HTLV III$_B$ strain). The antiviral endpoint was measured with p24 viral antigen at 10 days postinfection. Cell viability was taken as a measure of the toxicity endpoint. The macrophage monocyte (Mø.MO/PDS assay used freshly isolated monocyte/macrophage cells infected with a monocytotropic strain of HIV (Pan Data Strain, PDS). The antiviral endpoint was p24 viral antigen measured three (3) weeks postinfection, the toxicity endpoint was cell viability. The PBL/HTLV$_{IIIB}$ test used freshly isolated peripheral blood lymphocytes, stimulated with PHA and infected with HTLV III$_B$ strain. The antiviral endpoint was viral RNA and p24 viral antigen; the toxicity endpoint was cell viability.

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2-(4-Amino-4-carboxybutyl)aziridine-2-carboxylic Acid. A Potent Irreversible Inhibitor of Diaminopimelic Acid Epimerase. Spontaneous Formation from α-(Halomethyl)diaminopimelic Acids

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2-(4-Amino-4-carboxybutyl)aziridine-2-carboxylic acid (**3**) (aziridino-DAP) was identified as the product of spontaneous hydrolysis of α-(halomethyl)diaminopimelic acids (α-halomethyl-DAPs) **2a–c**. Under physiological conditions, **3** is an extremely potent irreversible inhibitor of the bacterial enzyme diaminopimelic acid epimerase (DAP-epimerase; EC 5.1.1.7). This unusual mode of action of an α-halomethyl amino acid with a *non*-pyridoxal enzyme is investigated. Synthesis and characterization of **2a–c** and **3**, kinetics of spontaneous formation of **3** from α-halomethyl-DAPs, and kinetics of enzyme inhibition by both **3** and by α-halomethyl-DAPs are reported.

The unusual amino acids D-alanine, D-glutamic acid, and D,L-diaminopimelic acid (**1**, D,L- or *meso*-DAP) are important components of bacterial cell wall.[1] Their biosynthesis is restricted to bacteria in general (and Gram negatives in particular *for most meso*-DAP incorporating bacteria[2]). Therefore, specific inhibition of the biosynthesis of these crucial cell-wall components could lead to a new generation of antibacterials.

Studies on alanine racemase (EC 5.1.1.1), which converts L-alanine to D-alanine, are numerous;[3–7] little work, however, has been reported on diaminopimelate epimerase (EC 5.1.1.7),[8] the enzyme which converts L-DAP to *meso*-DAP. As part of our program on the design of enzyme-activated irreversible inhibitors aimed at specific bacterial pathways, we prepared α-(halomethyl)diaminopimelic acids **2** (see

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