Analogues of the Antibiotic Puromycin as Potential Prodrugs of 3′-Amino-3′-deoxythymidine

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Condensation of L- and D-3′-amino-2′,3′-dideoxynucleosides **2-5** with *N*-BOC-protected aminoacids **6** and **13** using dicyclohexylcarbodiimide and *N*-hydroxysuccinimide in DMF is reported to give the *N*-BOC-protected acylamino aminonucleosides **7-9** and **14** in 51-81% yield. After deprotection using trifluoroacetic acid the corresponding unprotected new analogues of puromucin **10-12** and **15** were obtained in 43-56% yield. These compounds did not show any significant antiviral activity using HIV (stain HTLV-III B)-infected MT-4 cells as target system.

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Puromycin (**1**) is an aminoacyl aminonucleoside structurally similar to aminoacyl *t*-RNA. It is a broad spectrum antibiotic which inhibits protein synthesis *in vivo* and *in vitro* [1-5]. Also analogues of puromycin are inhibitors of protein synthesis which applies for some cytidyl derivatives [6]. Analogues of puromycin are normally prepared from the corresponding puromycin aminonucleoside (PAN) which can be obtained from puromycin itself [7]. PAN can then be coupled with different aminoacids by standard methods for peptide synthesis. This explains why the majority of analogues synthesized so far have structures similar to puromysin itself, only with another aminoacyl group replacing the *p*-methoxy-L-phenylalanyl moiety.

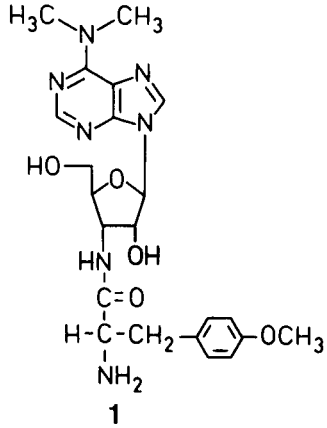

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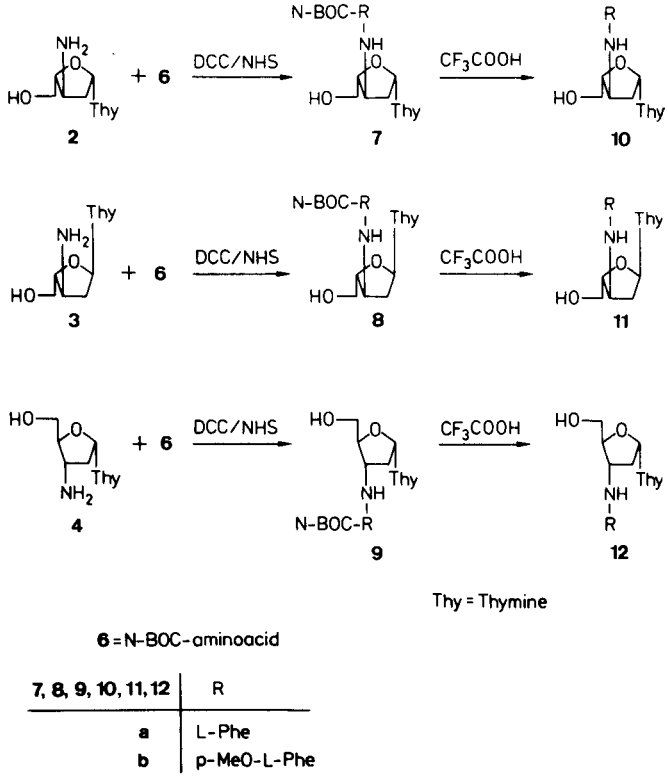
Figure 1

In our laboratories a new method for preparation of L- and D-3′-amino-2′,3′-dideoxynucleosides has been developed [8,9], which opens up an easy synthesis of new 2′,3′-dideoxynucleoside analogues of puromycin. A French group [10] has recently shown its interest in this class of compounds by synthesizing thymidine analogues. In this work we now synthesize a new series of 2′-deoxy-puromycin analogues with thymine as the nucleobase because such compounds may have potential activity against human immunodeficiency virus (HIV). It has been reported that 3′-amino-3′-deoxythymidine (NDT) does not inhibit HIV [11] although its corresponding triphosphate strongly inhibits HIV reverse transcriptase [12,13]. It has been unsuccessfully attempted [14] to induce anti-HIV activity by derivatizing the amino group into amido, thioamido and thioureido substituents with electronic and

Scheme 1



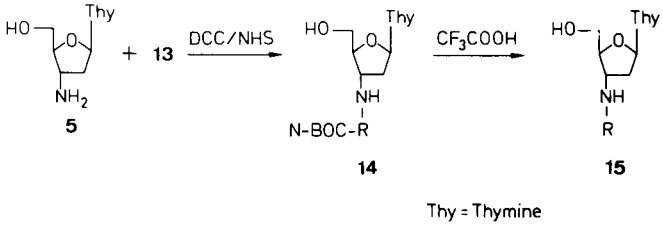
lipophilic properties close to those of fluoro and azido. This should adjust the molecular properties to make a passive transport into the cells possible by the same mechanism as it is known for the AIDS drug AZT.

Now we investigate the corresponding aminoacid derivatives as prodrugs of NDT which may undergo *in vivo* peptidase cleavage by which the aminoacyl group is split off delivering NDT at the proper site for its kinase which is the first step in the inhibition of HIV reverse transcriptase.

We used dicyclohexylcarbodiimide and *N*-hydroxysuccinimide (NHS) for the coupling reactions [15] between the *N*-BOC-protected aminoacids **6, 13** and different 3'-amino-2',3'-dideoxynucleosides **2-5** in dry DMF. After purification we obtained the *N*-BOC-protected acylamino aminonucleosides **7-9** and **14** in 51-81% yield. Deprotection using trifluoroacetic acid afforded the aminoacyl aminonucleosides **10-12** and **15** in 43-56% yield.

It should be emphasized that we have demonstrated the possibility of coupling a nucleoside with a polypeptide through a 3'-amino group. This has been done by carrying out coupling between the *N*-BOC-protected dipeptide **13f** and the 3'-aminonucleoside **5**.

Scheme 2



Thy = Thymine

13 = N-BOC-aminoacid

14,15	R
a	L-Phe
b	p-MeO-L-Phe
c	L-Tyr
d	Gly
e	L-Ala
f	L-Leu-L-Pro

None of the compounds **10, 11, 12** and **15** showed anti-HIV activity at non-cytotoxic concentrations. Moderate cytotoxicity against MT-4 cells was observed for **15a** and **15c** with TD_{50} = 30 μm and 15 μm, respectively. The dipeptide derivative 1-(2,3-dideoxy-3-(L-leucyl-L-prolylamino)-β-D-*erythro*-pentofuranosyl)thymine (**15f**) showed a remarkable cytotoxicity with a TD_{50} value of 0.7 μM which is comparable with those of 3'-isocyano-3'-deoxythymidine [16] and 3'-fluoro-3'-deoxythymidine [17].

EXPERIMENTAL

The nmr spectra were recorded on a Bruker AC 250 FT NMR spectrometer at 250 MHz for ^{1}H nmr and 62.5 MHz for ^{13}C nmr. FAB mass spectra were recorded on a Kratos MS-50 spectrometer. Microanalyses were done at NOVO-NORDISK Microanalytical Laboratory A/S, Novo Allé, DK-2880 Bagsvaerd.

General Procedure for the Preparation of **7-9** and **14**.

The aminonucleosides **2-5** (200 mg, 0.83 mmole) and the *N*-BOC-protected amino acid in question **6** and **13** (0.83 mmole) was suspended in dry DMF (10 ml) and cooled to −20°. After addition of *N*-hydroxysuccinimide (96 mg, 0.83 mmoles) and dicyclohexylcarbodiimide (170 mg, 0.83 mmole) stirring was continued for 24 hours at room temperature. After completing the reaction, the reaction mixture was filtered. The solid material was washed with ethylacetate, and the combined filtrates were evaporated under reduced pressure. The residue was dissolved in ethyl acetate (40 ml) and filtered once again after cooling on ice. The organic phase was washed with cold saturated aqueous sodium hydrogen carbonate (3 x 25 ml) and cold water (2 x 25 ml), dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was chromatographed on a silica gel column (15 g, Merck silica, 230-400 mesh) eluting with methylene chloride/methanol (19:1 v/v). After evaporation of the solvents under reduced pressure the *N*-BOC-protected acylamino aminonucleosides **7-9** and **14** were obtained.

1-(2,3-Dideoxy-3-(*N*-*t*-butyloxycarbonyl-L-phenylalanylamino)-β-L-*erythro*-pentofuranosyl)thymine (**7a**).

This compound was obtained in 80% yield (320 mg), mp 136-140°.

1-(2,3-Dideoxy-3-(*N*-*t*-butyloxycarbonyl-*p*-methoxy-L-phenylalanylamino)-β-L-*erythro*-pentofuranosyl)thymine (**7b**).

This compound was obtained in 81% yield (348 mg), mp 140-143°.

1-(2,3-Dideoxy-3-(*N*-*t*-butyloxycarbonyl-L-phenylalanylamino)-α-L-*erythro*-pentofuranosyl)thymine (**8a**).

This compound was obtained in 64% yield (260 mg), mp 135-140°.

1-(2,3-Dideoxy-3-(*N*-*t*-butyloxycarbonyl-*p*-methoxy-L-phenylalanylamino)-α-L-*erythro*-pentofuranosyl)thymine (**8b**).

This compound was obtained in 58% yield (250 mg), mp 144-147°.

1-(2,3-Dideoxy-3-(*N*-*t*-butyloxycarbonyl-L-phenylalanylamino)-α-D-*erythro*-pentofuranosyl)thymine (**9a**).

This compound was obtained in 57% yield (230 mg), mp 138-141°.

1-(2,3-Dideoxy-3-(*N*-*t*-butyloxycarbonyl-*p*-methoxy-L-phenylalanylamino)-α-D-*erythro*-pentofuranosyl)thymine (**9b**).

This compound was obtained in 63% yield (270 mg), mp 140-145°.

1-(2,3-Dideoxy-3-(*N*-*t*-butyloxycarbonyl-L-phenylalanylamino)-β-D-*erythro*-pentofuranosyl)thymine (**14a**).

This compound was obtained in 71% yield (290 mg), mp 135-139°.

1-(2,3-Dideoxy-3-(*N*-*t*-butyloxycarbonyl-*p*-methoxy-L-phenylalanylamino)-β-D-*erythro*-pentofuranosyl)thymine (**14b**).

This compound was obtained in 63% yield (268 mg), mp

138-143°.

1-(2,3-Dideoxy-3-(*N*-*t*-butyloxycarbonyl-L-tyrosylamino)-β-D-*erythro*-pentofuranosyl)thymine (**14c**).

This compound was obtained in 78% yield (326 mg), mp 171-174°.

1-(2,3-Dideoxy-3-(*N*-*t*-butyloxycarbonylglycylamino)-β-D-*erythro*-pentofuranosyl)thymine (**14d**).

This compound was obtained in 51% yield (168 mg), mp 118-120°.

1-(2,3-Dideoxy-3-(*N*-*t*-butyloxycarbonyl-L-alanylamino)-β-D-*erythro*-pentofuranosyl)thymine (**14e**).

This compound was obtained in 60% yield (205 mg), mp 150-152°.

1-(2,3-Dideoxy-3-(*N*-*t*-butyloxycarbonyl-L-leucyl-L-prolylamino)-β-D-*erythro*-pentofuranosyl)thymine (**14f**).

This compound was obtained in 63% yield (284 mg), mp 170-172°.

General Procedure for the Preparation of **10-12** and **15**.

The N-BOC-protected acylamino aminonucleosides **7-9** and **14** were treated with 6 ml of trifluoroacetic acid at 0° for 5 minutes. After evaporation *in vacuo* the residue was chromatographed on a silica gel column (10 g, Merck silica, 230-400 mesh) eluting with methylene chloride/methanol (9:1 v/v). After evaporation of the solvents under reduced pressure a precipitate was formed by treatment with dry diethyl ether. After filtration and washing with dry diethyl ether the final acylamino aminonucleosides **10-12** and **15** were obtained.

1-(2,3-Dideoxy-3-L-phenylalanylamino-β-L-*erythro*-pentofuranosyl)thymine (**10a**).

Compound **7a** (240 mg, 0.49 mmole) provided 160 mg of **10a** (51% yield) mp 168-171°; ^{1}H nmr (DMSO-d_6): δ 1.78 (s, 3 H, CH_3), 2.05-2.21 (m, 2 H, 2'β-H, 2'α-H), 3.03 (m, 2 H, CH_2), 3.55-4.26 (m, 5 H, 3'-H, 4'-H, 5'-H, CH), 6.09 (m, 1 H, 1'-H), 7.23-7.34 (m, 5 H, arom), 7.74 (s, 1 H, 6-H), 8.31 (br s, 3 H, NH_3^+), 8.74 (d, J = 6.5 Hz, 1 H, NH), 11.34 (s, 1 H, NH); ^{13}C nmr (DMSO-d_6): δ 12.1 (CH_3), 36.4, 36.9 (C-2', CH_2), 49.5 (C-3'), 53.5 (CH), 61.3 (C-5'), 83.5 (C-1'), 84.5 (C-4), 109.4 (C-5), 136.0 (C-6), 150.3 (C-2), 163.6 (C-4), 167.5 (CO), 127.1, 128.5, 129.3, 137.7 (aryl); ms: (FAB) m/z (%) 389 (M + 1$^+$, 25).

Anal. Calcd. for $C_{19}H_{24}N_4O_5 \cdot 2CF_3COOH \cdot H_2O$ (634.5): C, 43.54; H, 4.45; N, 8.83. Found: C, 43.16; H, 4.57; N, 8.64.

1-(2,3-Dideoxy-3-*p*-methoxy-L-phenylalanylamino-β-L-*erythro*-pentofuranosyl)thymine (**10b**).

Compound **7b** (280 mg, 0.54 mmole) provided 193 mg of **10b** (52% yield) mp 170-172°; ^{1}H nmr (DMSO-d_6): δ 1.79 (s, 3 H, CH_3), 2.11-2.23 (m, 2 H, 2'β-H, 2'α-H), 2.96 (m, 2 H, CH_2), 3.39-4.28 (m, 4 H, 4'-H, 5'-H, CH), 3.72 (s, 3 H, OCH_3), 5.18 (m, 1 H, 3'-H), 6.11 (t, J = 6.6 Hz, 1 H, 1'-H), 6.89 (d, J = 8.2 Hz, 2 H, arom), 7.13 (d, J = 8.1 Hz, 2 H, arom), 7.75 (s, 1 H, 6-H), 8.76 (d, J = 6.6 Hz, 1 H, NH), 11.31 (s, 1 H, NH); ^{13}C nmr (DMSO-d_6): δ 12.2 (CH_3), 36.3, 36.5 (C-2', CH_2), 49.4 (C-3'), 53.8 (CH), 55.0 (OCH_3), 61.3 (C-5'), 83.5 (C-1'), 84.5 (C-4'), 109.4 (C-5), 136.0 (C-6), 150.4 (C-2), 163.7 (C-4), 168.0 (CO), 113.9, 126.7, 130.4, 158.4 (arom); ms: (FAB) m/z (%) 419 (M + 1$^+$, 28).

Anal. Calcd. for $C_{20}H_{26}N_4O_6 \cdot 2CF_3COOH \cdot 2H_2O$ (682.5): C, 42.23; H, 4.73; N, 8.21. Found: C, 42.44; H, 4.70; N, 8.50.

1-(2,3-Dideoxy-3-L-phenylalanylamino-α-L-*erythro*-pentofuranosyl)thymine (**11a**).

Compound **8a** (200 mg, 0.41 mmole) provided 127 mg of **11a** (49% yield) mp 157-160°; ^{1}H nmr (DMSO-d_6): δ 1.76-1.94 (m, 4 H, CH_3, 2'β-H), 2.46-2.57 (m, 1 H, 2'α-H), 2.99 (d, J = 7.0 Hz, 2 H, CH_2), 3.37-4.25 (m, 5 H, 3'-H, 4'-H, 5'-H, CH), 6.03 (t, J = 6.4 Hz, 1 H, 1'-H), 7.18-7.34 (m, 5 H, arom), 7.48 (s, 1 H, 6-H), 8.29 (br s, 3 H, NH_3^+), 8.80 (d, J = 6.4 Hz, 1 H, NH), 11.33 (s, 1 H, NH); ^{13}C nmr (DMSO-d_6): δ 12.0 (CH_3), 36.8, 36.9 (C-2', CH_2), 49.5 (C-3'), 53.5 (CH), 61.5 (C-5'), 84.1, 84.8 (C-1', C-4'), 109.3 (C-5), 135.7 (C-6), 150.2 (C-2), 163.7 (C-4), 167.8 (CO), 127.0, 128.4, 129.3, 134.6 (arom); ms: (FAB) m/z (%) 389 (M + 1$^+$, 47).

Anal. Calcd. for $C_{19}H_{24}N_4O_5 \cdot 2CF_3COOH \cdot H_2O$ (634.5): C, 43.54; H, 4.45; N, 8.83. Found: C, 43.64; H, 4.44; N, 8.58.

1-(2,3-Dideoxy-3-*p*-methoxy-L-phenylalanylamino-α-L-*erythro*-pentofuranosyl)thymine (**11b**).

Compound **8b** (180 mg, 0.35 mmole) provided 105 mg of **11b** (43% yield) mp 160-165°; ^{1}H nmr (DMSO-d_6): δ 1.78 (s, 3 H, CH_3), 2.01-2.09 (m, 1 H, 2'β-H), 2.53-2.63 (m, 1 H, 2'α-H), 2.96 (m, 2 H, CH_2), 3.34-4.31 (m, 5 H, 3'-H, 4'-H, 5'-H, CH), 3.73 (s, 3 H, OCH_3), 6.08 (t, J = 6.1 Hz, 1 H, 1'-H), 6.89 (d, J = 8.4 Hz, 2 H, arom), 7.15 (d, J = 8.4 Hz, 2 H, arom), 7.47 (s, 1 H, 6-H), 8.31 (br s, 3 H, NH_3^+), 8.81 (d, J = 6.7 Hz, 1 H, NH), 11.31 (s, 1 H, NH); ^{13}C nmr (DMSO-d_6): δ 12.0 (CH_3), 36.2, 36.9 (C-2', CH_2), 49.5 (C-3'), 53.8 (CH), 55.0 (OCH_3), 61.7 (C-5'), 84.9, 85.1 (C-1', C-4'), 109.3 (C-5), 135.9 (C-6), 150.3 (C-2), 163.9 (C-4), 168.0 (CO), 113.8, 126.7, 130.5, 158.4 (arom); ms: (FAB) m/z (%) 419 (M + 1$^+$, 42).

Anal. Calcd. for $C_{20}H_{26}N_4O_6 \cdot 2CF_3COOH \cdot 2H_2O$ (682.5): C, 42.23; H, 4.73; N, 8.21. Found: C, 42.43; H, 4.57; N, 8.53.

1-(2,3-Dideoxy-3-L-phenylalanylamino-α-D-*erythro*-pentofuranosyl)thymine (**12a**).

Compound **9a** (180 mg, 0.37 mmole) provided 124 mg of **12a** (54% yield) mp 169-172°; ^{1}H nmr (DMSO-d_6): δ 1.78 (s, 3 H, CH_3), 1.98-2.09 (m, 1 H, 2'β-H), 2.56-2.67 (m, 1 H, 2'α-H), 3.02 (d, J = 6.9 Hz, 2 H, CH_2), 3.28-4.28 (m, 5 H, 3'-H, 4'-H, 5'-H, CH), 6.05 (t, J = 6.2 Hz, 1 H, 1'-H), 7.22-7.37 (m, 5 H, arom), 7.47 (s, 1 H, 6-H), 8.29 (br s, 3 H, NH_3^+), 8.80 (d, J = 6.7 Hz, 1 H, NH), 11.29 (s, 1 H, NH); ^{13}C nmr (DMSO-d_6): δ 12.1 (CH_3), 36.8, 37.0 (C-2', CH_2), 49.5 (C-3'), 53.5 (CH), 61.6 (C-5'), 84.8, 85.0 (C-1', C-4'), 109.4 (C-5), 135.8 (C-6), 150.3 (C-2), 163.8 (C-4), 168.0 (CO), 127.1, 128.5, 129.3, 134.8 (arom); ms: (FAB) m/z (%) 389 (M + 1$^+$, 48).

Anal. Calcd. for $C_{19}H_{24}N_4O_5 \cdot 2CF_3COOH$ (616.5): C, 44.81; H, 4.25; N, 9.09. Found: C, 44.88; H, 4.61; N, 9.27.

1-(2,3-Dideoxy-3-*p*-methoxy-L-phenylalanylamino-α-D-*erythro*-pentofuranosyl)thymine (**12b**).

Compound **9b** (200 mg, 0.39 mmole) provided 126 mg of **12b** (49% yield) mp 158-162°; ^{1}H nmr (DMSO-d_6): δ 1.77 (s, 3 H, CH_3), 1.99-2.09 (m, 1 H, 2'β-H), 2.56-2.67 (m, 1 H, 2'α-H), 2.95 (m, 2 H, CH_2), 3.32-4.30 (m, 5 H, 3'-H, 4'-H, 5'-H, CH), 3.74 (s, 3 H, OCH_3), 6.06 (t, J = 6.2 Hz, 1 H, 1'-H), 6.90 (d, J = 8.5 Hz, 2 H, arom), 7.15 (d, J = 8.5 Hz, 2 H, arom), 7.49 (s, 1 H, 6-H), 8.22 (br s, 3 H, NH_3^+), 8.79 (d, J = 6.7 Hz, 1 H, NH), 11.33 (s, 1 H, NH); ^{13}C nmr (DMSO-d_6): δ 12.0 (CH_3), 36.1, 36.8 (C-2', CH_2), 49.5 (C-3'), 53.7 (CH), 55.0 (OCH_3), 61.7 (C-5'), 84.9, 85.0 (C-1', C-4'), 109.3 (C-5),

135.8 (C-6), 150.3 (C-2), 163.8 (C-4), 168.0 (CO), 113.9, 126.5, 130.4, 158.4 (arom); ms: (FAB) m/z (%) 419 (M + 1$^+$, 48).

Anal. Calcd. for $C_{20}H_{26}N_4O_6 \cdot 2CF_3COOH$ (646.5): C, 44.59; H, 4.37; N, 8.67. Found: C, 44.27; H, 4.77; N, 8.86.

1-(2,3-Dideoxy-3-L-phenylalanylamino-β-D-*erythro*-pentofuranosyl)thymine (**15a**).

Compound **14a** (220 mg, 0.45 mmole) provided 155 mg of **15a** (56% yield) mp 165-168°; ^{1}H nmr (DMSO-d$_6$): δ 1.78 (s, 3H, CH_3), 2.01-2.33 (m, 2 H, 2′β-H, 2′α-H), 3.03 (m, 2 H, CH_2), 3.39-4.42 (m, 5 H, 3′-H, 4′-H, 5′-H, CH), 6.15 (t, J = 6.9 Hz, 1 H, 1′-H), 7.18-7.38 (m, 5 H, arom), 7.74 (s, 1 H, H-6), 8.33 (br s, 3 H, NH_3^+), 8.75 (d, J = 7.6 Hz, 1 H, NH), 11.31 (s, 1 H, NH); ^{13}C nmr (DMSO-d$_6$): δ 12.2 (CH_3), 36.9, 37.0 (C-2′, CH_2), 49.3 (C-3′), 53.6 (CH), 61.2 (C-5′), 83.5 (C-1′), 84.5 (C-4′), 109.4 (C-5), 136.0 (C-6), 150.3 (C-2), 163.7 (C-4), 167.4 (CO), 127.1, 128.4, 129.4, 134.8 (arom); ms: (FAB) m/z (%) 389 (M + 1$^+$, 23).

Anal. Calcd. for $C_{19}H_{24}N_4O_5 \cdot 2CF_3COOH$ (616.5): C, 44.81; H, 4.25; N, 9.09. Found: C, 44.60; H, 4.54; N, 8.83.

1-(2,3-Dideoxy-3-*p*-methoxy-L-phenylalanylamino-β-D-*erythro*-pentofuranosyl)thymine (**15b**).

Compound **14b** (210 mg, 0.41 mmole) provided 151 mg of **15b** (56% yield) mp 173-175°; ^{1}H nmr (DMSO-d$_6$): δ 1.79 (s, 3 H, CH_3), 2.03-2.28 (m, 2 H, 2′β-H, 2′α-H), 2.98 (m, 2 H, CH_2), 3.43-4.34 (m, 5 H, 3′-H, 4′-H, 5′-H, CH), 3.74 (s, 3 H, OCH_3), 6.14 (t, J = 6.8 Hz, 1 H, 1′-H), 6.90 (d, J = 8.6 Hz, 2 H, arom), 7.14 (d, J = 8.6 Hz, 2 H, arom), 7.75 (s, 1 H, 6-H), 8.30 (br s, 3 H, NH_3^+), 8.77 (d, J = 7.5 Hz, 1 H, NH), 11.32 (s, 1 H, NH); ^{13}C nmr (DMSO-d$_6$): δ 12.1 (CH_3), 36.1, 36.9 (C-2′, CH_2), 49.3 (C-3′), 53.8 (CH), 55.0 (OCH_3), 61.3 (C-5′), 83.5 (C-1′), 84.5 (C-4′), 109.4 (C-5), 136.0 (C-6), 150.4 (C-2), 163.7 (C-4), 167.5 (CO), 113.9, 126.6, 130.5, 158.5 (arom); ms: (FAB) m/z (%) 419 (M + 1$^+$, 42).

Anal. Calcd. for $C_{20}H_{26}N_4O_6 \cdot 2CF_3COOH$ (646.5): C, 44.59; H, 4.37; N, 8.67. Found: C, 44.92; H, 4.78; N, 8.90.

1-(2,3-Dideoxy-3-L-tyrosylamino-β-D-*erythro*-pentofuranosyl)thymine (**15c**).

Compound **14c** (240 mg, 0.48 mmole) provided 169 mg of **15c** (56% yield) mp 178-181°; ^{1}H nmr (DMSO-d$_6$): δ 1.79 (s, 3H, CH_3), 2.03-2.28 (m, 2 H, 2′β-H, 2′α-H), 2.91 (d, J = 6.8 Hz, 2 H, CH_2), 3.34-4.37 (m, 5 H, 3′-H, 4′-H, 5′-H, CH), 6.16 (t, J = 6.8 Hz, 1 H, 1′-H), 6.72 (d, J = 8.4 Hz, 2 H, arom), 7.01 (d, J = 8.4 Hz, 2 H, arom), 7.76 (s, 1 H, 6-H), 8.27 (br s, 3 H, NH_3^+), 8.78 (d, J = 7.6 Hz, 1 H, NH), 11.33 (s, 1 H, NH); ^{13}C nmr (DMSO-d$_6$): δ 12.1 (CH_3), 36.2, 36.9 (C-2′, CH_2), 49.2 (C-3′), 53.9 (CH), 61.3 (C-5′), 83.5 (C-1′), 84.6 (C-4′), 109.4 (C-5), 136.0 (C-6), 150.4 (C-2), 163.7 (C-4), 167.6 (CO), 115.2, 124.7, 130.3, 156.5 (arom); ms: (FAB) m/z (%) 405 (M + 1$^+$, 32).

Anal. Calcd. for $C_{19}H_{24}N_4O_6 \cdot 2CF_3COOH$ (632.5): C, 43.68; H, 4.14; N, 8.86. Found: C, 43.83; H, 4.33; N, 9.01.

1-(2,3-Dideoxy-3-glycylamino-β-D-*erythro*-pentofuranosyl)thymine (**15d**).

Compound **14d** (120 mg, 0.30 mmole) provided 69 mg of **15d** (43% yield) mp 134-137°; ^{1}H nmr (DMSO-d$_6$): δ 1.80 (s, 3 H, CH_3), 2.08-2.34 (m, 2 H, 2′β-H, 2′α-H), 3.57-4.39 (m, 6 H, 3′-H, 4′-H, 5′-H, CH_2), 6.22 (t, J = 6.7 Hz, 1 H, 1′-H), 7.79 (s, 1 H, 6-H), 8.14 (br s, 3 H, NH_3^+), 8.88 (d, J = 7.3 Hz, 1 H, NH), 11.33 (s, 1 H, NH); ^{13}C nmr (DMSO-d$_6$): δ 12.2 (CH_3), 36.8 (C-2′), 40.2 (CH_2), 49.5 (C-3′), 61.3 (C-5′), 83.6 (C-1′), 84.8 (C-4′), 109.5 (C-5), 136.1 (C-6), 150.4 (C-2), 163.7 (C-4), 165.8 (CO); ms: (FAB) m/z (%) 299 (M + 1$^+$, 37).

Anal. Calcd. for $C_{12}H_{18}N_4O_5 \cdot 2CF_3COOH$ (526.4): C, 36.51; H, 3.83; N, 10.64. Found: C, 36.69; H, 4.20; N, 10.67.

1-(2,3-Dideoxy-3-L-alanylamino-β-D-*erythro*-pentofuranosyl)thymine (**15e**).

Compound **14e** (145 mg, 0.35 mmole) provided 90 mg of **15e** (49% yield) mp 177-181°; ^{1}H nmr (DMSO-d$_6$): δ 1.36 (d, J = 7.0 Hz, 3 H, CH_3), 1.79 (s, 3 H, CH_3), 2.05-2.34 (m, 2 H, 2′β-H, 2′α-H), 3.34-4.41 (m, 5 H, 3′-H, 4′-H, 5′-H, CH), 6.19 (t, J = 6.7 Hz, 1 H, 1′-H), 7.78 (s, 1 H, 6-H), 8.16 (br s, 3 H, NH_3^+), 8.85 (d, J = 7.5 Hz, 1 H, NH), 11.33 (s, 1 H, NH); ^{13}C nmr (DMSO-d$_6$): δ 12.2 (CH_3), 17.1 (CH_3), 36.9 (C-2′), 48.3, 49.5 (C-3′, CH), 61.4 (C-5′), 83.6 (C-1′), 84.7 (C-4′), 109.5 (C-5), 136.1 (C-6), 150.4 (C-2), 163.7 (C-4), 169.3 (CO); ms: (FAB) m/z (%) 313 (M + 1$^+$, 36).

Anal. Calcd. for $C_{13}H_{20}N_4O_5 \cdot 2CF_3COOH$ (540.4): C, 37.79; H, 4.10; N, 10.37. Found: C, 38.11; H, 4.53; N, 10.49.

1-(2,3-Dideoxy-3-L-leucyl-L-prolylamino-β-D-*erythro*-pentofuranosyl)thymine (**15f**).

Compound **14f** (220 mg, 0.40 mmole) provided 135 mg of **15f** (48% yield) mp 185-188°; ^{1}H nmr (DMSO-d$_6$): δ 0.93 (m, 6 H, $(CH_3)_2CH$), 1.55 (m, 2 H, CH_2), 1.78 (s, 3 H, CH_3), 1.97-2.24 (m, 4 H, 2′β-H, 2′α-H, CH_2), 3.37-4.32 (m, 10 H, 3′-H, 4′-H, 5′-H, 2 x CH_2, 2 x CH), 6.21 (t, J = 6.7 Hz, 1 H, 1′-H), 7.78 (s, 1 H, 6-H), 8.11 (br s, 3 H, NH_3^+), 8.20 (m, 1 H, NH), 8.42 (d, J = 7.5 Hz, 1 H, NH), 11.31 (s, 1 H, NH); ^{13}C nmr (DMSO-d$_6$): δ 12.2 (CH_3), 21.1 (CH_3), 23.1, 23.3 (CH_3, CH_2), 24.6 ($CH(CH_3)_2$), 29.2 (CH_2), 36.9 (C-2′), 40.3 (CH_2), 46.7 (CH_2), 49.1, 49.4 (C-3′, CH), 59.6 (CH), 61.3 (C-5′), 83.5 (C-1′), 85.0 (C-4′), 109.4 (C-5), 136.0 (C-6), 150.3 (C-2), 163.6 (C-4), 167.5 (CO), 170.9 (CO); ms: (FAB) m/z (%) 452 (M + 1$^+$, 62).

Anal. Calcd. for $C_{21}H_{33}N_5O_6 \cdot 2CF_3COOH \cdot H_2O$ (697.6): C, 43.05; H, 5.35; N, 10.04. Found: C, 42.96; H, 5.24; N, 9.66.

Anti-HIV Assay Procedure.

Puromycin analogues were examined for possible antiviral activity using HIV (strain HTLV-IIIB)-infected MT-4 cells as target system. For screening studies MT-4 cells were incubated with virus for two hours, washed and thereafter added in a proportion of 1:10 to uninfected MT-4 cells, which had been preincubated with test compounds in growth medium for two hours. Compounds were screened using a concentration in the medium of 0.33 m*M*. Cultures were maintained for one week in parallel with virus-infected control cultures without compound added. Expression of HIV in the culture medium was quantitated by HIV antigen detection ELISA. Compounds mediating less than 20% reduction of antigen expression were considered without biological activity. Compounds mediating a reduction of 20% or more were examined for cytotoxic potential using concentration-dependent inhibition of MT-4 cell proliferation as measure of cytotoxicity. A 20% inhibition of cell growth relative to control cultures was considered significant. The compounds were finally reexamined for antiviral activity in the highest sub-toxic concentration observed.

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