Synthesis and Antiviral Activity of 3'-Heterocyclic Substituted 3'-Deoxythymidines

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Various 3'-deoxythymidine analogues with an heterocyclic five-membered ring in the 3'-erythro position have been synthesized. The pyrrol-1-yl (**3**) and the 1,2,4-triazol-4-yl (**5**) compounds were synthesized from 1-(3-amino-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine. The pyrazol-1-yl (**16a**), imidazol-1-yl (**16b**), and 1,2,4-triazol-1-yl (**16c**) derivatives were obtained by epoxide opening of the corresponding 1-(2,3-anhydro-β-D-lyxofuranosyl)thymines followed by 2'-deoxygenation. Only the 3'-pyrrol-1-yl derivative showed marginal antiviral activity against human immunodeficiency virus.

A prerequisite for a nucleoside to inhibit the reverse transcriptase activity associated with human immunodeficiency virus (HIV) is the intracellular phosphorylation of the compound to its triphosphate. As the genome of HIV does not encode for specific kinases, phosphorylation of nucleosides in HIV-infected cells must depend on the action of cellular kinases. This also means that selectivity can be achieved only at the level of the reverse transcriptase. It is generally assumed that those nucleoside analogues that are efficient inhibitors of HIV multiplication are readily phosphorylated to their triphosphates. However, the phosphorylation kinetics differs from one compound to another, which explains at least in part the differences in anti-HIV potency. It has been suggested that the charge distribution in the azido group might mimic the charge distribution in an O–P–O bond.[1,2] This may lead to a stabilization of 1-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (**1a**)[1,2] once it has been incorporated into DNA.

As part of a program on the synthesis of 3'-modified 2',3'-dideoxynucleosides,[3] we synthesized a series of 3'-deoxythymidine analogues with an heterocyclic five-membered ring in the 3'-erythro position. These compounds (**3**, **5**, **16a–c**, **18**) differ in their electronic properties. When the most polar side of the ring is taken into account, some of these rings (i.e. imidazole) have a charge distribution similar to that of the 3'-azido group; others (i.e. pyrazole) have the opposite distribution. Also the pK_a values of these heterocyclic rings are quite different: some (i.e. imidazole) are partially protonated at a physiological pH, which should extend the possibilities for interaction with enzymes.

Chemistry

Two routes were examined for the synthesis of 3'-deoxythymidines substituted in the 3'-position with an heterocyclic ring. The route depicted in Scheme I starts from 1-(3-amino-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (**2a**), a compound with known cytostatic properties.[4] Its derivatives (**2a–c**) with different protecting groups attached to the 5'-hydroxyl group were synthesized by catalytic reduction of the corresponding 3'-azido analogues.[5] Benzoyl migration during the reduction of the azido function of 1-(5-*O*-benzoyl-3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (**1c**) was neglegible.

As the reaction of amines with 2,5-dimethoxytetrahydrofuran (**6**) is acid catalyzed,[6] **2c** was first converted into its hydrochloride salt. Reaction of 1-(5-*O*-benzoyl-3-amino-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine hydrochloride with **6** in DMF for 30 min at 100 °C gave, after debenzoylation with ammonia in methanol, 40% of 1-(3-pyrrol-1-yl-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-thymine (**3**). In an attempt to synthesize 1-[3-(1,2,4-triazol-4-yl)-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]thymine (**5**), **2a** was heated in pyridine in the presence of *N,N'*-diformylhydrazine (**7**). However, under these circumstances, only formylation of the amino group of **2a** took place, yielding 1-(3-formamido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (**4**) in quantitative yield. Therefore, the reaction was repeated with dimethylformamide azine hydrochloride[7] (**8**). Heating the acetate of 1-(5-*O*-trityl-3-amino-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine with **8** in dimethylformamide, followed by detritylation with acetic acid in methanol, afforded 48% of 1-[3-(1,2,4-triazol-4-yl)-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]thymine (**5**) and 37% of the 3'-formamido derivative **4**.

The synthesis of **16a–c** is summarized in Scheme II. This reaction scheme involves ring opening of an epoxide followed by deoxygenation. This route was followed because our efforts to introduce an heterocyclic ring directly by nucleophilic substitution on 1-(5-*O*-trityl-3-*O*-triflyl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine failed. Reaction of the sodium salt of pyrazole with 1-(5-*O*-trityl-3-*O*-triflyl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine in acetonitrile, for example, only gave β-elimination to afford 1-(5-*O*-trityl-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-thymine. Reaction of 1-(5-*O*-trityl-2,3-anhydro-β-D-lyxofuranosyl)thymine[8] (**9**) with 1*H*-1,2,4-triazole in dimethylformamide in the presence of sodium hydride gave 45% of 1-[5-*O*-trityl-3-(1,2,4-triazol-1-yl)-3-deoxy-β-D-arabinofuranosyl]thymine (**11c**) and 25% of 1-[5-*O*-trityl-2-(1,2,4-triazol-1-yl)-2-deoxy-β-D-xylofuranosyl]thymine (**12c**). Both compounds showed a comparable ^{1}H NMR spectrum, and final proof for their structures was provided after detritylation to **13c** and **14**. The position of the triazole ring was also proven by deoxygenation of the secondary alcohol of **11c** and **12c** followed by detritylation

(1) Camerman, A.; Mastropaolo, D.; Camerman, N. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8239.
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(6) Sundberg, R. J. *Comprehensive Heterocyclic Chemistry*, 1st ed.; Katritzky, A. R., Rees, C. W., Eds.; Pergamon Press: Oxford, 1984; Part 3, Chapter 6.
(7) Bartlett, R. K.; Humphrey, I. R. *J. Chem. Soc. C* **1967**, *17*, 1664.
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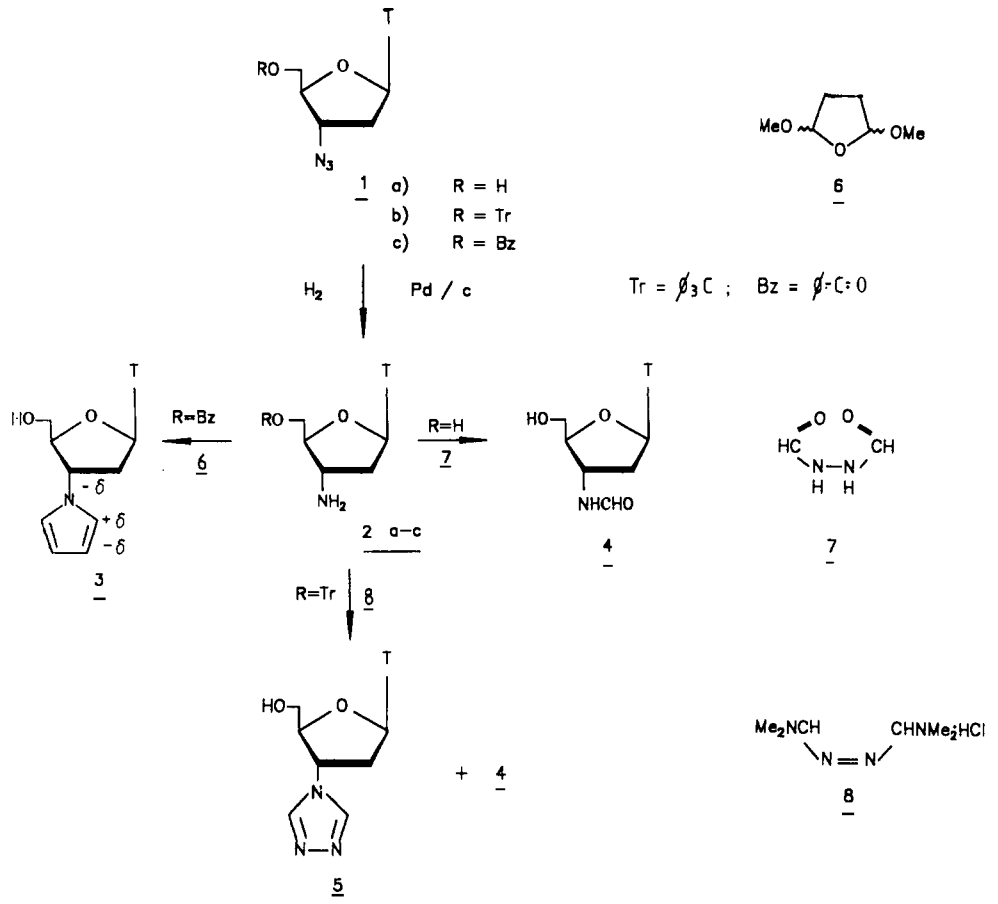
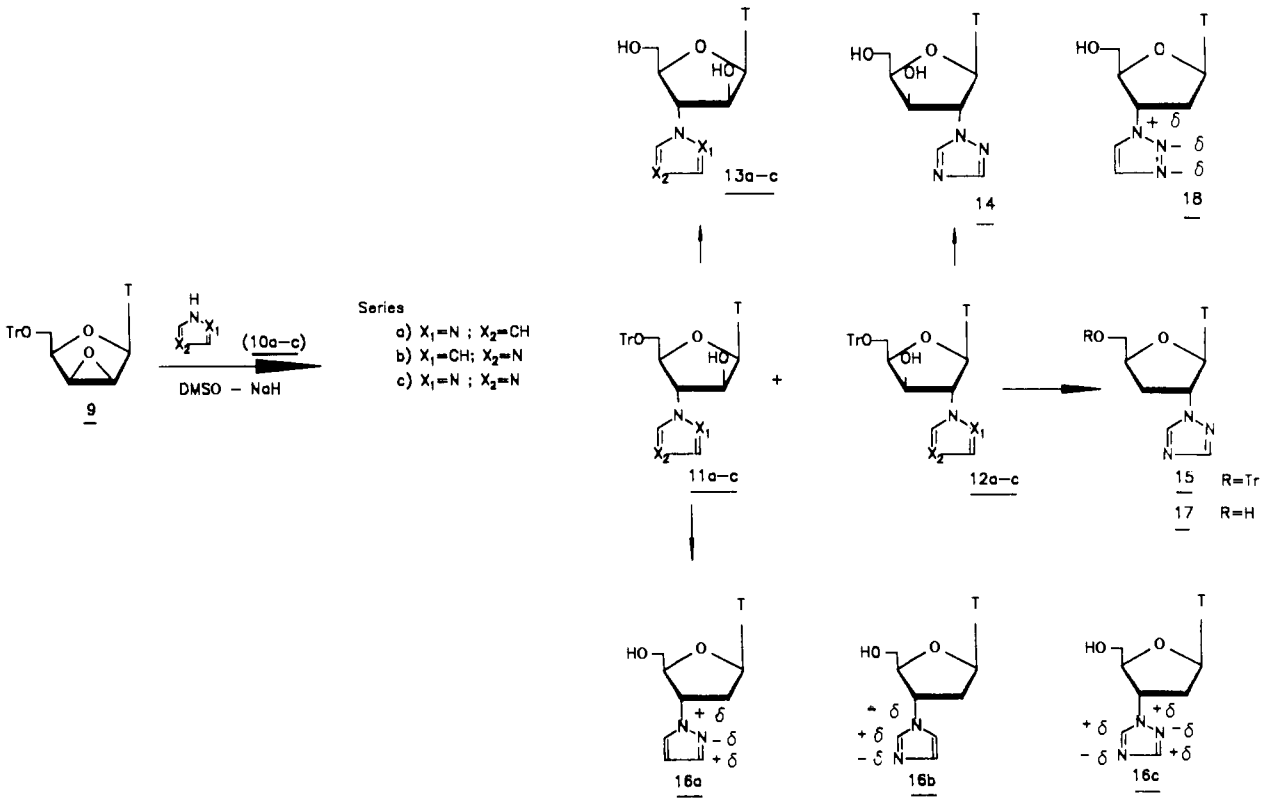
0022-2623/90/1833-0868$02.50/0

Scheme I



Scheme II



to **16c** and **17**. Detritylation was performed with acetic acid in methanol. The ^{1}H NMR of **13c** showed a resonance signal for H-2′ at δ 4.75 ppm, H-3′ at δ 4.85 ppm, and H-1′ at δ 6.26 ppm with a $J_{1',2'}$ value of 6.4 Hz. The ^{1}H NMR spectrum of **14** showed H-3′ at δ 4.50 ppm, H-2′ at δ 5.13 ppm, and H-1′ at δ 6.14 ppm with a $J_{1',2'}$ value of 4.4 Hz.

Table I. ^{1}H NMR Spectral Data of the 3′-Deoxynucleoside Analogues[a,b]

	H-1′	$J_{1',2'}$, Hz	H-3′	H-2″	H-3″	H-4″	H-5″
3	6.30	6.8, 4.6	4.68	6.93 (d)	6.06 (t)	6.06 (t)	6.93 (d)
5	6.28	7.0, 5.5	5.16	8.74	N[c]	N	8.74
16a	6.41	6.8	5.06	N	7.83	6.27 (t)	7.54 (d)
16b	6.28	7.0, 7.2	5.00	7.78	N	7.38	6.96
16c	6.43	6.8	5.26	N	8.07	N	8.64
18	6.43	6.8	5.40	N	N	7.85	8.30

[a] Only the signals which differ significantly from those of thymidine are given. [b] All spectra were taken in DMSO-d_6 with trimethylsilane as internal standard; δ values. [c] N: position of nitrogen atom.

Table II. ^{13}C NMR Data of the Carbohydrate Moiety and the Heterocyclic Five-Membered Ring of the Newly Synthesized Compounds[a]

compd	1′/4′[b]		2′	3′/5′[b]		2″	3″	4″	5″
3	85.3	84.1	38.4	57.3	60.5	119.7	108.9	108.9	119.7
5	84.0	83.4	*c*	53.5	59.7	142.2	N	N[d]	152.2
16a	84.8	84.4	37.4	60.4	61.1	N	139.8	105.6	130.1
16b	84.4	83.4	38.3	54.7	59.8	136.8	N	129.1	117.7
16c	84.5	84.4	33.2	58.7	61.1	N	150.7	N	144.2
18	84.6	84.0	37.3	59.1	60.8	N	N	133.5	124.5
13a	84.4	80.7	74.7	66.5	60.1	N	140.6	106.4	131.3
13b	83.3	79.9	75.2	61.7	59.3	136.5	N	129.6	119.4
13c	83.7	79.8	74.1	64.0	59.6	N	150.7	N	144.8
AZT	84.0	83.5	36.2	60.2	60.8				

[a] Spectra were taken in DMSO-d_6, which was used as internal standard (39.6 ppm). [b] The values for C-1′ and C-4′, on the one hand, and C-3′ and C-5′, on the other hand, were very close to each other; no attempts were made to dissociate them. [c] C-2′ is hidden by the DMSO-d_6 signals. [d] Position of nitrogen atom.

Reaction of **11c** with phenyl chlorothionocarbonate[9] in the presence of 4-(dimethylamino)pyridine followed by reduction with tributyltin hydride in the presence of 2,2′-azobis(2-methylpropionitrile) and 5′-*O*-detritylation gave **16c** in 28% overall yield.

For the synthesis of **17**, the method using 1,1′-carbonyldiimidazole[10] was used. The overall yield of **17** from **12c** was 25%. The position of the 1,2,4-triazole ring in **16c** and **17** is clear from the H-1′ signals in the ^{1}H NMR spectra appearing as a triplet and as a doublet, respectively. The same reaction sequence [(i) oxirane cleavage of **9** with the sodium salt of imidazole (**10b**) and pyrazole (**10a**), (ii) 2′-deoxygenation involving free radicals; (iii) detritylation with 80% methanolic acetic acid] afforded 1-(3-imidazol-1-yl-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine **16b** and 1-(3-pyrazol-1-yl-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine **16a** in moderate overall yield (about 15%). In this case, formation of the compounds **12a,b** resulting from attack of the heterocycle on the 2′ carbon was noted by thin-layer chromatography, but no attempts were made to isolate them. The compounds with the arabino configuration (**11a,b**) were also detritylated, yielding **13a** and **13b**. The final compounds (**13a–c, 14, 16a–c, 17**) were fully identified by ^{1}H NMR (Table I), ^{13}C NMR (Table II), UV, MS, and elementary analysis. 1-[3-(1,2,3-Triazol-1-yl)-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]thymine (**18**) was synthesized as reported previously.[3] The ^{13}C NMR signals of the heterocyclic rings were assigned according to Elguero et al.[11]

Biological Activity

Thymidine derivatives **3–5, 16a–c,** and **18** and the analogues **13a–c, 14,** and **17** were evaluated for their inhibitory effect on HIV-1-induced cytopathogenicity in human MT-4 cells and Moloney murine sarcoma virus (MSV) induced transformation of murine C3H/3T3 embryo fibroblasts. Compound **3** had an inhibitory effect on HIV-1-induced cytopathogenicity in MT-4 cells and MSV-induced transformation of C3H/3T3 cells at subtoxic concentrations. However, its ED_{50} value was much higher than that of 1-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine. None of the other 3′- or 2′-substituted derivatives of thymidine showed an inhibitory activity against HIV-1 or MSV replication at concentrations that were well below the toxicity threshold. The 50% effective doses (ED_{50}) of compounds **2, 13a–c, 14, 16a–c, 17,** and **18** were invariably higher than 100–500 μM. Cytotoxicity was noted with compounds **4** and **5**: their 50% cytotoxic doses (CD_{50}) were 66.1 and 4.82 μM, respectively.

Compounds **3–5, 13a–c, 14, 16a–c, 17,** and **18** showed no antiviral activity when evaluated for their inhibitory effects on the cytopathogenicity of herpes simplex virus type 1 (strain KOS), herpes simplex virus type 2 (strain G), vaccinia virus, vesicular stomatitis virus, Coxsackie virus type B4, Sindbis virus, poliovirus type 1, reovirus

Table III. Inhibitory Effects of the 2′- and 3′-Substituted Thymidine Analogues on HIV-1-Induced Cytopathogenicity in MT-4 Cells and MSV-Induced Transformation of C3H/3T3 Cells

	HIV-1-induced cytopathogenicity in MT-4 cells		MSV-induced transformation of C3H/3T3 cells	
compd	ED_{50},[a] μM	CD_{50},[b] μM	ED_{50},[a] μM	MCC,[c] μM
3	201	>500	91	>100
4	>100	66	>100	>100
5	>5	4.8	>100	>100
13a	>100	>100	>100	>100
13b	>500	>500	>100	>100
13c	>500	>500	>100	>100
14	>500	>500	>100	>100
16a	>500	>500	>100	>100
16b	>500	>500	>100	>100
16c	>500	>500	>100	>100
17	>500	>500	>100	>100
18	>500	>500	>100	>100

[a] Effective dose of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV. [b] Cytotoxic dose of compound, required to reduce the viability of normal uninfected MT-4 cells by 50%. [c] Minimum cytotoxic concentration at which a microscopically alteration of normal cell morphology was noted.

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(10) Prisbe, E. J.; Martin, J. C. *Synth. Commun.* **1985**, *15*, 401.
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type 1, Semliki Forest virus, or parainfluenza virus type 3 (in primary rabbit kidney, HeLa, or Vero cell cultures).

Conclusion

The azido and fluorine group are the only substituents in the 3'-position of thymidine that have proved compatible with potent anti-HIV activity. The activity of 3'-deoxythymidine itself seems to vary significantly from one cell line to another. This is most probably due to differences in phosphorylation kinetics. The potent anti-HIV agents AZT (1) and its 3'-fluoro analogue are efficiently phosphorylated in HIV-infected cells. From our investigations it is evident that there are considerable constraints in the modifications allowed at the 3'-position of thymidine. Of the various heterocycles substituted in this position only the pyrrole ring appeared compatible with marginal anti-HIV activity. Also no anti-HIV activity was found for the compounds (14, 17) with the triazole substituent in the 2'-position. Whether the lack of anti-HIV activity of the 3'-heterocyclic substituted thymidine derivatives resides in an inefficient intracellular phosphorylation to their 5'-triphosphate forms, inefficient interaction of these 5'-triphosphates with the HIV-associated reverse transcriptase, or both remains the subject of further investigation.

Experimental Section

Melting points were determined in capillary tubes with a Büchi-Tottoli apparatus and are uncorrected. Ultraviolet spectra were recorded with a Philips PU 8700 UV/Vis spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were determined with a JEOL FX 90Q spectrometer with tetramethylsilane as internal standard for the ^{1}H NMR spectra and DMSO-d_6 (39.6 ppm) for the ^{13}C NMR spectra (s = singlet, d = doublet, t = triplet, br s = broad signal, m = multiplet). Electron impact mass spectra (70 eV) were recorded with an AEI MS-12 apparatus. Precoated Merck silica gel F254 plates were used for TLC, and the spots were examined with UV light and sulfuric acid–anisaldehyde spray. Column chromatography was performed on Merck silica gel (0.063–0.200 mm). Anhydrous solvents were obtained as follows: acetonitrile was obtained by distillation after reflux overnight with calcium hydride; pyridine was refluxed overnight in the presence of potassium hydroxide and distilled; dichloromethane was stored for 1 week on anhydrous calcium chloride, filtered, and distilled; water was removed from *N,N*-dimethylformamide by distillation with benzene followed by distillation in vacuo; dimethyl sulfoxide was refluxed overnight on calcium hydride and then fractionally distilled at reduced pressure.

Antiretroviral Assay Procedures. The anti-HIV-1 assays were carried out with the HTLV-III$_B$ strain and were based on the inhibition of HIV-1-induced cytopathic effect in human MT-4 cells as previously described.[12] The anti-MSV-assays were based on the inhibition of MSV-induced transformation of murine embryo fibroblast C3H/3T3 cells.[12]

1-(5-*O*-Benzoyl-3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (1c). To a solution of 6.67 g (25 mmol) of 1-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (**1a**; AZT) in 100 mL of anhydrous pyridine was added dropwise 4.08 mL (37.5 mmol) of benzoyl chloride. The mixture was kept at room temperature for 16 h, and then another 2.04 mL (19 mmol) of benzoyl chloride was added. After 2 h, TLC analysis ($CHCl_3$–MeOH, 9:1) revealed the complete disappearance of **1a**; 5 mL of MeOH was added and the reaction mixture was evaporated. The residual oil was taken up in $CHCl_3$ (200 mL) and washed with H_2O (200 mL). The aqueous layer was extracted with $CHCl_3$ (100 mL) and the combined organic layer was dried on Na_2SO_4, filtered, and evaporated. After chromatographic purification ($CHCl_3$–MeOH, 95:5), 8.3 g (90%) of **1c** was obtained as a foam; UV (MeOH) λ_{max} 266 nm; ^{1}H NMR δ 1.63 (d, 3 H, CH_3), 2.30–2.63 (m, H-2' partially hidden by DMSO-d_6), 4.14 (m, 1 H, H-4'), 4.42–4.85 (m, 3 H, H-3' and H-5'), 6.18 (t, 1 H, H-1'), 7.27–7.85 (m, 4 H, aromatic H and H-6), 7.88–8.28 (m, 2 H, aromatic H), 11.30 (br s, 1 H, NH) ppm.

1-(5-*O*-Benzoyl-3-amino-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (2c). N_2 was bubbled through a solution of 500 mg (1.39 mmol) of **1c** in 20 mL of MeOH for 15 min. Then 50 mg of 10% Pd/C was added and the mixture was hydrogenated for 16 h at 50 psi. The catalyst was removed by filtration, the solution was evaporated, and the mixture was purified by column chromatography ($CHCl_3$–MeOH, 90:10), giving 354 mg (76%) of **2c** as a foam; UV (MeOH) λ_{max} 269 nm; ^{1}H NMR δ 1.63 (d, 3 H, CH_3), 2.00–2.37 (m, 2 H, H-2'), 3.62 (m, 1 H, H-3'), 3.78–4.14 (m, 3 H, H-4' and NH_2), 4.55 (m, 2 H, H-5'), 6.18 (t, 1 H, H-1'), 7.31–7.82 (m, 4 H, aromatic H and H-6), 7.85–8.17 (m, 2 H, aromatic H), 11.27 (br s, 1 H, NH) ppm.

1-(3-Pyrrol-1-yl-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (3). An amount of 460 mg (1.4 mmol) of 1-(5-*O*-benzoyl-3-amino-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (**2c**) was dissolved in HCl–methanol and evaporated to dryness. The resulting foam was dissolved in a solution of 2,5-dimethoxytetrahydrofuran (mixture of isomers) (200 mg, 1.51 mmol) in 10 mL of DMF and heated for 30 min at 100 °C. After cooling to room temperature, the solvent was evaporated and the residual oil was dissolved in methanol saturated with ammonia. After 16 h at room temperature, the reaction mixture was evaporated and purified by column chromatography ($CHCl_3$–MeOH, 97:3). Two fractions were isolated: the first contained 110 mg (27%) of pure **3** and the second contained 70 mg of **3** contaminated with some minor impurities (TLC analysis). The first fraction was dissolved in hot benzene, Et_2O was added and crystallization started on cooling: mp 156–158 °C; UV (MeOH) λ_{max} 267 nm, log ε 4.01; MS *m/e* 291 (M^+); ^{1}H NMR δ 1.82 (d, 3 H, CH_3), 2.31–2.75 (m, H-2' partially hidden by DMSO-d_6), 3.57 (m, 2 H, H-5'), 4.08 (m, 1 H, H-4'), 5.22 (t, 1 H, OH), 7.83 (d, 1 H, H-6), 11.34 (br s, 1 H, NH) ppm; ^{13}C NMR δ 12.6 (CH_3), 110.0 (C-5), 136.9 (C-6), 150.8 (C-2), 164.4 (C-4) ppm. Anal. ($C_{14}H_{17}N_3O_4$) C, H, N.

Reaction of 1-(3-Amino-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (2a) with *N,N'*-Diformylhydrazine (7). A mixture of 100 mg (0.44 mmol) of **2a** and 80 mg (0.9 mmol) of **7** in 20 mL of anhydrous pyridine was refluxed overnight. The solvent was evaporated and the mixture was purified by column chromatography yielding 110 mg (0.41 mmol, 93%) of 1-(3-*N*-formamido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (**4**): UV (MeOH) λ_{max} 266 nm; MS *m/e* 269 (M^+); ^{1}H NMR δ 1.78 (d, 3 H, CH_3), 2.19 (m, 2 H, H-2'), 3.62 (d, 2 H, H-5'), 3.77 (m, 1 H, H-4'), 4.37 (m, 1 H, H-3'), 5.07 (t, 1 H, OH), 6.17 (t, 1 H, H-1'), 7.75 (d, 1 H, H-6), 8.02 (d, 1 H, C*H*O), 8.47 (d, 1 H, N*H*CHO, D_2O exchangable), 11.23 (br s, 1 H, NH) ppm; ^{13}C NMR δ 12.6 (CH_3), 37.8 (C-2'), 48.1 (C-3'), 61.5 (C-5'), 84.4 and 85.1 (C-1' and C-4'), 110.3 (C-5), 137.1 (C-6), 151.0 (C-2), 162.3 (C-4), 164.7 (NH*C*HO) ppm.

1-[3-(1,2,4-Triazol-4-yl)-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]thymine (5). An amount of 460 mg (0.7 mmol) of **2b** was converted into its acetate by dissolving it in a solution of acetic acid (1 mL) and methanol (19 mL). The mixture was evaporated and coevaporated twice with toluene. The acetate of **2b** was dissolved in 20 mL of DMF, 200 mg (1.1 mmol) of *N,N*-dimethylformamide azine hydrochloride (**8**) was added, and the solution was heated for 24 h at 100 °C. Then, another 200 mg of **8** was added. After heating for 48 h at the same temperature, TLC analysis ($CHCl_3$–MeOH, 8:2) showed disappearance of all the starting material. However, partial detritylation also occurred. Therefore, the DMF was evaporated and the reaction mixture was taken up in 20 mL of a mixture of HOAc–MeOH (8:2) and heated for 1 h at 100 °C. After evaporation and chromatographic purification, two products were isolated: 70 mg (37%) of the 3'-*N*-formyl derivative **4** and 104 mg (48%) of **5** which was crystallized from acetonitrile: mp 223–224 °C; UV (MeOH) λ_{max} 266 nm, log ε 4.01; MS *m/e* 293 (M^+); ^{1}H NMR δ 1.80 (d, 3 H, CH_3), 2.74 (m, H-2' partially hidden by DMSO-d_6), 3.60 (m, 2 H, H-5'), 4.17 (m, 1 H, H-4'), 5.23 (t, 1 H, OH), 7.80 (d, 1 H, H-6), 11.24 (br s, 1 H, NH) ppm; ^{13}C NMR δ 12.2 (CH_3), 109.3 (C-5), 136.3 (C-6), 150.3 (C-2), 163.7 (C-4) ppm. Anal. ($C_{12}H_{15}N_5O_4 \cdot {}^1/_4H_2O$) C, H, N.

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1-(5-*O*-Trityl-3-pyrazol-1-yl-3-deoxy-β-D-arabinofuranosyl)thymine (11a). To a solution of 175 mg (3 mmol) of pyrazole in 10 mL of DMSO was added 60 mg (1.5 mmol) of a 60% suspension of NaH. The mixture was stirred for 1 h, and then 250 mg (0.50 mmol) of 1-(5-*O*-trityl-2,3-anhydro-β-D-lyxofuranosyl)thymine (**9**) was added. The solution was heated for 16 h at 110 °C, cooled to room temperature, and neutralized with acetic acid. The solvent was removed by a short-path vacuum distillation (70–80 °C) and the reaction mixture was purified by column chromatography ($CHCl_3$–MeOH, 95:5) 240 mg (84% yield) of **11a** was obtained; UV (MeOH) λ_{max} 266 nm; ^1H NMR δ 1.63 (s, 3 H, CH_3), 3.51 (m, 2 H, H-5′), 4.35 (m, 1 H, H-4′), 4.68 (m, 1 H, H-2′), 4.86 (m, 1 H, H-3′), 6.02 (d, 1 H, OH), 6.28 (m, 2 H, H-1′ and H-4″), 7.10–7.90 (m, 18 H, trityl, H-6, H-3″, and H-5″), 11.34 (br s, 1 H, NH) ppm. The structure of this compound was fully identified after detritylation.

1-(3-Pyrazol-1-yl-3-deoxy-β-D-arabinofuranosyl)thymine (13a). A 210-mg sample of **11a** (0.44 mmol) was heated for 1 h at 100 °C in a mixture of HOAc–MeOH (8:2). After cooling, evaporation, and chromatographic purification, 97 mg (72%) of **13a** was obtained. The compound was dissolved in MeOH and precipitated by addition of Et_2O: UV (MeOH) λ_{max} 267 nm, log ε 3.98; MS *m/e* 308 (M^+); ^1H NMR δ 1.79 (d, 3 H, CH_3), 3.55 (m, 2 H, H-5′), 4.16 (m, 1 H, H-4′), 4.65–4.81 (m, 2 H, H-2′ and H-3′), 5.25 (t, 1 H, 5′-OH), 5.94 (d, 1 H, 2′-OH), 6.17–6.32 (m, 2 H, H-1′ and H-4″), 7.55 (s, 1 H, H-5″), 7.71 (s, 1 H, H-3″), 7.87 (d, 1 H, H-6), 11.27 (s, 1 H, NH) ppm; ^{13}C NMR δ 12.8 (CH_3), 106.4 (C-4″), 108.7 (C-5), 131.3 (C-5″), 138.8 (C-6), 140.6 (C-3″), 151.2 (C-2), 164.8 (C-4) ppm. Anal. ($C_{13}H_{16}N_4O_5 \cdot H_2O$) C, H, N.,

1-(5-*O*-Trityl-3-imidazol-1-yl-3-deoxy-β-D-arabinofuranosyl)thymine (11b). To a solution of 350 mg (5.1 mmol) of imidazole in 20 mL of DMSO was added 110 mg (2.75 mmol) of a 60% dispersion of NaH in oil. The mixture was stirred for 1 h, and then 500 mg (1.9 mmol) of **9** was added. The solution was heated for 16 h at 100 °C. The reaction mixture was cooled to 75 °C and the solvent was removed by short-path vacuum distillation. Then the reaction mixture was purified by column chromatography ($CHCl_3$–MeOH, 98:2–95:5). The title compound was isolated in a 524-mg yield (95%): UV (MeOH) λ_{max} 266 nm; ^1H NMR δ 1.61 (s, 3 H, CH_3), 3.50 (m, 2 H, H-5′), 4.60 (m, 1 H, H-2′), 4.81 (m, 1 H, H-3′), 5.94 (d, 1 H, OH), 6.26 (d, 1 H, H-1′), 6.98 (s, 1 H, H-5″), 7.10–7.90 (m, 18 H, trityl, H-6, H-2″, and H-4″), 11.36 (br s, 1 H, NH) ppm.

1-(3-Imidazol-1-yl-3-deoxy-β-D-arabinofuranosyl)thymine (13b). To 20 mL of a mixture of HOAc–MeOH (8:2) was added 240 mg of **11b**. The solution was heated for 1 h at 100 °C. The liquids were evaporated and coevaporated twice with toluene. The residue was purified by column chromatography ($CHCl_3$–MeOH, 90:10), yielding 110 mg (88%) of title compound **13b**, which was dissolved in MeOH and precipitated by addition of Et_2O: UV (MeOH) λ_{max} 267 nm, log ε 3.97; MS *m/e* 308 (M^+); ^1H NMR δ 1.80 (d, 3 H, CH_3), 3.70 (d, 2 H, H-5′), 4.15 (m, 1 H, H-4′), 4.62 (m, 1 H, H-2′), 4.82 (m, 1 H, H-3′), 5.32 (t, 1 H, 5′-OH), 6.01 (d, 1 H, 2′-OH), 6.26 (d, 1 H, H-1′), 6.97 (s, 1 H, H-5″), 7.40 (s, 1 H, H-4″), 7.71 (d, 1 H, H-6), 7.77 (s, 1 H, H-2″), 11.26 (br s, 1 H, NH) ppm; ^{13}C NMR δ 12.8 (CH_3), 110.9 (C-5), 119.4 (C-5″), 129.6 (C-4″), 136.5 (C-2″), 138.5 (C-6), 151.2 (C-2), 165.7 (C-4) ppm. Anal. ($C_{13}H_{16}N_4O_5 \cdot {}^1/_4H_2O$) C, H, N.

1-[5-*O*-Trityl-3-(1,2,4-triazol-1-yl)-3-deoxy-β-D-arabinofuranosyl]thymine (11c) and 1-[5-*O*-Trityl-2-(1,2,4-triazol-1-yl)-2-deoxy-β-D-xylofuranosyl]thymine (12c). To a solution of 250 mg of 1*H*-1,2,4-triazole (3.85 mmol) in 20 mL of DMF was added 180 mg (4.4 mmol) of NaH (60% dispersion). The mixture was stirred for 15 min at room temperature, and then 1 g (2.1 mmol) of **9** was added. The mixture was stirred for 16 h at 100 °C. After cooling, 1 mL of HOAc was added, and the solvent was evaporated. The residual oil was taken up in 200 mL of EtOAc and washed with 200 mL of H_2O. The organic layer was dried and evaporated. Column chromatography yielded 500 mg (45%) of **11c** and 280 mg (25%) of **12c**. **11c**: ^1H NMR δ 1.70 (s, 3 H, CH_3), 3.50 (m, 2 H, H-5′), 4.36 (m, 1 H, H-4′), 4.73 (m, 1 H, H-2′), 5.12 (m, 1 H, H-3′), 6.17 (d, 1 H, OH), 6.34 (d, 1 H, H-1′), 6.77–7.74 (m, 16 H, trityl and H-6), 8.12 (s, 1 H, H-3″), 8.69 (s, 1 H, H-5″), 11.29 (br s, 1 H, NH) ppm. **12c**: ^1H NMR δ 1.66 (s, 3 H, CH_3), 3.50 (m, 2 H, H-5′), 4.46 (m, 1 H, H-4′), 4.68 (m, 1 H, H-3′), 5.11 (m, 1 H, H-2′), 6.00 (d, 1 H, OH), 6.15 (d, 1 H, H-1′), 6.73–7.86 (m, 16 H, trityl and H-6), 8.10 (s, 1 H, H-3″), 8.61 (s, 1 H, H-5″), 11.27 (br s, 1 H, NH) ppm. Both compounds were fully identified after deprotection.

1-[3-(1,2,4-Triazol-1-yl)-3-deoxy-β-D-arabinofuranosyl]thymine (13c). Detritylation of 450 mg of **11c** (0.8 mmol) was carried out in 10 mL of a mixture HOAc–MeOH (8:2) at 100 °C in 75 min. After evaporation, coevaporation with toluene, and chromatographic purification ($CHCl_3$–MeOH, 90:10), 206 mg (80%) of **13c** was obtained. This product was crystallized from MeOH–Et_2O: mp 216–218 °C; UV (MeOH) λ_{max} 266 nm, log ε 4.03; MS *m/e* 309 (M^+); ^1H NMR δ 1.77 (d, 3 H, CH_3), 3.62 (d, 2 H, H-5′), 4.18 (m, 1 H, H-4′), 4.75 (m, 1 H, H-2′), 4.85 (m, 1 H, H-3′), 5.29 (t, 1 H, 5′-OH), 6.04 (d, 1 H, 2′-OH), 6.26 (d, 1 H, H-1′), 7.73 (d, 1 H, H-6), 8.07 (s, 1 H, H-3″), 8.70 (s, 1 H, H-5″), 11.30 (s, 1 H, NH) ppm; ^{13}C NMR δ 12.4 (CH_3), 108.2 (C-5), 138.0 (C-6), 144.8 (C-5″), 150.7 (C-3″), 152.2 (C-2), 164.0 (C-4) ppm. Anal. ($C_{12}H_{15}N_5O_5$) C, H, N.

1-[2-(1,2,4-Triazol-1-yl)-2-deoxy-β-D-xylofuranosyl]thymine (14). Deprotection of 250 mg of **12c** (0.44 mmol) was carried out in 10 mL of a mixture of HOAc–MeOH (8:2) at 100 °C in 90 min. The solvents were evaporated and after chromatographic purification ($CHCl_3$–MeOH, 90:10) 130 mg (91%) of **14** was obtained. The product was taken up in MeOH and precipitated by addition of Et_2O: UV (MeOH) λ_{max} 267 nm, log ε 3.99; MS *m/e* 309 (M^+); ^1H NMR δ 1.82 (d, 3 H, CH_3), 3.77 (d, 2 H, H-5′), 4.38–4.61 (m, 2 H, H-4′ and H-3′), 4.95 (t, 1 H, 5′-OH), 5.13 (t, 1 H, H-2′), 5.83 (br s, 1 H, 3′-OH), 6.14 (d, 1 H, H-1′), 7.82 (d, 1 H, H-6), 8.09 (s, 1 H, H-3″), 8.64 (s, 1 H, H-5″), 11.33 (br s, 1 H, NH) ppm. ^{13}C NMR 12.6 δ (CH_3), 59.5 (C-5′), 69.2 (C-2′), 73.2 (C-3′), 82.8 (C-4′), 86.3 (C-1′), 110.0 (C-5), 136.1 (C-6), 144.8 (C-5″), 150.6 (C-3″), 152.3 (C-2), 163.8 (C-4) ppm. Anal. ($C_{12}H_{15}N_5O_5$) C, H, N.

1-[3-(1,2,4-Triazol-1-yl)-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]thymine (16c). General Procedure for the Conversion of 11a–c into 16a–c. To a solution of 950 mg (1.72 mmol) of **11c** in 50 mL of acetonitrile at 0 °C was added 1.5 g (15 mmol) of 4-(dimethylamino)pyridine and 360 μL of phenyl chlorothiocarbonate (2.6 mmol). The mixture was stirred for 1 h at room temperature. After addition of 5 mL of MeOH, the solvent was evaporated and the residual oil was taken up in 200 mL of EtOAc. This solution was washed with 200 mL of 0.1 N HCl and with 200 mL of H_2O, dried on Na_2SO_4, filtered, and evaporated. Flash chromatography yielded 1.15 g (98%) of 1-[5-*O*-trityl-3-(1,2,4-triazol-1-yl)-2-*O*-(phenoxythionocarbonyl)-3-deoxy-β-D-arabinofuranosyl]thymine as a pure compound (TLC analysis). This compound was dissolved in 20 mL of toluene, nitrogen was bubbled through, and 20 mg of 2,2′-azobis(2-methylpropionitrile) (AIBN) and 900 μL (3 mmol) of *n*-tributyltin hydride were added. This mixture was heated at 100 °C for 2.5 h under a N_2 atmosphere. After cooling to room temperature, the solvent was evaporated and the mixture was separated chromatographically ($CHCl_3$–MeOH, 95:5), affording 350 mg (38%) of 1-[5-*O*-trityl-3-(1,2,4-triazol-1-yl)-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]-thymine: UV (MeOH) λ_{max} 267 nm; ^1H NMR ($DCCl_3$) δ 1.67 (d, 3 H, CH_3), 2.44–3.15 (m, 2 H, H-2′), 3.52 (m, 2 H, H-5′), 4.30 (m, 1 H, H-4′), 5.14 (m, 1 H, H-3′), 6.48 (t, 1 H, H-1′), 6.78–7.72 (m, 16 H, trityl and H-6), 7.91 (s, 1 H, H-3″), 7.96 (s, 1 H, H-5″), 9.93 (br s, 1 H, NH) ppm. Detritylation of the obtained material (340 mg, 0.64 mmol) was performed with 20 mL of a mixture HOAc–MeOH (8:2) for 1 h at 100 °C. After cooling, evaporation, and chromatographic purification ($CHCl_3$–MeOH, 90:10), 140 mg (75%) of **16c** was obtained (28% overall yield from **11c**). The product was crystallized from MeOH: mp >250 °C; UV (MeOH) λ_{max} 266 nm, log ε 4.03; MS *m/e* 293 (M^+); ^1H NMR δ 1.81 (d, 3 H, CH_3), 2.44–2.71 (m, H-2′ partially hidden by DMSO), 3.66 (m, 2 H, H-5′), 4.12 (m, 1 H, H-4′), 5.23 (t, 1 H, OH), 7.80 (d, 1 H, H-6), 11.27 (br s, 1 H, NH) ppm; ^{13}C NMR δ 12.5 (CH_3), 110.0 (C-5), 136.5 (C-6), 152.3 (C-2), 164.9 (C-4) ppm. Anal. ($C_{12}H_{15}N_5O_4$) C, H, N.

1-[2-(1,2,4-Triazol-1-yl)-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]thymine (17). To a solution of 800 mg (1.45 mmol) of **12c** in anhydrous pyridine was added 540 mg (3.33 mmol) of 1,1′-carbonyldiimidazole. The solution was kept at 90 °C for 3 h. After cooling, the pyridine was evaporated and coevaporated with toluene (3×). The residual oil was taken up in 50 mL of dry MeOH. This solution was stirred for 1 hour at 60 °C and kept at room temperature for 16 h. MeOH was evaporated and the

mixture was purified by flash column chromatography [(1) $CHCl_3$, (2) $CHCl_3$–MeOH, 95:5]; the main reaction product was isolated in 44% yield (400 mg, 0.64 mmol). After it was dissolved in 20 mL of toluene, nitrogen was bubbled through the solution. A catalytic amount of AIBN and 800 μL of *n*-tributyltin hydride (2.6 mmol) were added. The mixture was heated for 2 h at 100 °C under a N_2 atmosphere. After cooling, the solvent was evaporated and the residual oil was purified by column chromatography: 240 mg (70%) of 1-[5-*O*-trityl-2-(1,2,4-triazol-1-yl)-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]thymine (**15**) was obtained as a foam. UV (MeOH) λ_{max} 266 nm; ^1H NMR ($CDCl_3$) δ 1.54 (s, 3 H, CH_3), 2.59 (m, 2 H, H-3′), 3.55 (m, 2 H, H-5′), 4.56 (m, 1 H, H-4′), 5.16 (m, 1 H, H-2′), 5.87 (d, 1 H, H-1′), 6.82–7.65 (m, 15 H, trityl), 7.77 (s, 1 H, H-6), 7.97 (s, 1 H, H-3″), 8.50 (s, 1 H, H-5″), 9.90 (br s, 1 H, NH) ppm. The trityl group of **15** was removed by heating the product (240 mg, 0.45 mmol) for 1 h at 100 °C in a mixture of HOac–MeOH (8:2). After evaporation and chromatographic purification, 106 mg (82%) of the title product **17** was obtained (overall yield 25% from **12c**). The product was taken up in MeOH and precipitated by addition of Et_2O. UV (MeOH) λ_{max} 2.66 nm, log ε 3.95; MS *m/e* 293 (M^+); ^1H NMR δ 1.81 (d, 3 H, CH_3), 2.30–3.19 (m, H-3′ partially hidden by DMSO), 3.69 (m, 2 H, H-5′), 4.57 (m, 1 H, H-4′), 5.01–5.57 (m, 2 H, H-2′ and 5′-OH), 5.98 (d, 1 H, J = 4.0 Hz, H-1′), 7.86 (d, 1 H, H-6), 8.06 (s, 1 H, H-3″), 8.59 (s, 1 H, H-5″), 11.30 (br s, 1 H, NH) ppm; ^{13}C NMR δ 12.5 (CH_3), 31.9 (C-3′), 62.1 (C-5′), 63.1 (C-2′), 81.2 (C-4′), 89.3 (C-1′), 109.9 (C-5), 136.3 (C-6), 144.4 (C-5″), 150.7 (C-3″), 152.3 (C-2), 164.2 (C-4) ppm. Anal. ($C_{12}H_{15}N_5O_4 \cdot H_2O$) C, H, N.

1-(3-Imidazol-1-yl-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (16b). Compound **16b** was synthesized according to the general procedure described for **16c**. Starting from 1.120 g (2 mmol) of **11b**, 70 mg of **16b** (12% overall yield) was obtained. The compound was crystallized from MeOH–Et_2O: mp 248–250 °C; UV (MeOH) λ_{max} 266 nm, log ε 3.98; MS *m/e* 292 (M^+); ^1H NMR δ 1.82 (s, 3 H, CH_3), 2.68 (m, 2 H, H-2′), 3.51 (m, 2 H, H-5′), 4.11 (m, 1 H, H-4′), 5.23 (t, 1 H, OH), 7.78 (m, 2 H, H-6 and H-2″), 11.34 (br s, 1 H, NH) ppm; ^{13}C NMR δ 12.3 (CH_3), 109.3 (C-5), 136.3 (C-6), 150.4 (C-2), 163.7 (C-4) ppm. Anal. ($C_{13}H_{16}N_4O_4 \cdot {}^1/_2H_2O$) C, H, N.

1-(3-Pyrazol-1-yl-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (16a). The procedure for the synthesis of **16c** was followed. Starting from 1.00 g (1.8 mmol) of **11a**, 78 mg of **16a** (15% overall yield) was obtained. The compound was crystallized from acetone: mp 162–164 °C; UV (MeOH) λ_{max} 267 nm, log ε 4.00; MS *m/e* 292 (M^+); ^1H NMR δ 1.80 (s, 3 H, CH_3), 2.39–2.71 (m, H-2′ partially hidden by DMSO), 3.65 (m, 2 H, H-5′), 4.11 (m, 1 H, H-4′), 5.13 (t, 1 H, OH), 7.83 (m, 2 H, H-6 and H-5″), 11.03 (br s, 1 H, NH) ppm; ^{13}C NMR δ 12.4 (CH_3), 109.9 (C-5), 136.5 (C-6), 150.6 (C-2), 164.0 (C-4) ppm. Anal. ($C_{13}H_{16}N_4O_4$) C, H, N.

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Registry No. **1a**, 30516-87-1; **1c**, 106060-78-0; **2a**, 52450-18-7; **2b**, 124355-25-5; **2b** acetate, 124355-26-6; **2c**, 124355-23-3; **2c**·HCl, 124355-43-7; **3**, 124355-24-4; **4**, 123533-11-9; **5**, 124355-28-8; *cis*-**6**, 13269-48-2; *trans*-**6**, 13269-49-3; **7**, 628-36-4; **8**, 124355-27-7; **9**, 115913-84-3; **10a**, 288-13-1; **10b**, 288-32-4; **10c**, 288-88-0; **11a**, 124355-29-9; **11b**, 124355-30-2; **11c**, 124355-32-4; **11c** phenoxythionocarbonyl derivative, 124355-36-8; **12c**, 124355-33-5; **13a**, 124379-59-5; **13b**, 124355-31-3; **13c**, 124355-34-6; **14**, 124355-35-7; **15**, 124355-39-1; **16a**, 124355-42-6; **16b**, 124355-41-5; **16c**, 124355-38-0; **16c** trityl derivative, 124355-37-9; **17**, 124355-40-4; **18**, 122370-58-5.

Studies on Ca^{2+} Channel Antagonists. A 2-Diazo-3,3,3-trifluoropropionamide Derivative Related to Verapamil as a Potential Photoaffinity Probe

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2-(3,4-Dimethoxyphenyl)-2-isopropyl-5-[*N*-[4-(*N*-methyl-2-diazo-3,3,3-trifluoropropionamido)phenethyl]methylamino]valeronitrile (**3**), a potential photoaffinity probe for Ca^{2+} channels related to verapamil (**1**), was prepared from *N*-methyl-4-nitrophenethylamine (**7**) and 2-(3,4-dimethoxyphenyl)-2-isopropyl-5-(methanesulfonoxy)valeronitrile (**12**). Compound **3** showed concentration-dependent negative inotropic effects in rat right myocardial ventricular strips, EC_{50} = (1.05 ± 0.33) × 10^{-7} M (mean ± SD), being slightly less potent than gallopamil (**2**), EC_{50} = (2.18 ± 0.66) × 10^{-8} M. It displaced [^3H]gallopamil in myocardial membranes, K_i = (3.76 ± 1.55) × 10^{-8} M, compared to **2**, K_i = (1.55 ± 0.16) × 10^{-8} M. Photoactivation at 265 nm reduced the recoverable binding of [^3H]gallopamil to 26% compared to no effect on **2**. This agent may be a useful photoaffinity probe to aid in further characterization of Ca^{2+} channels.

Influx of extracellular Ca^{2+} through ion channels is an important process in the excitation coupling of contraction of cardiac, smooth, and skeletal muscle.[1-4] Structurally diverse Ca^{2+} channel antagonists like verapamil (**1**) and gallopamil (**2**), nifedipine, diltiazem, prenylamine, and their congeners are used in the treatment of a variety of cardiovascular diseases.[5,6] These compounds also have been studied widely in efforts to characterize these ion channels in various tissues. Evidence has been accumulated showing separate binding sites for different chemical classes of ligands, and the use of photoaffinity ligands related to nifedipine and verapamil (**1**), and other techniques have provided useful information about the polypeptides associated with Ca^{2+} ion channels.[7-15]

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