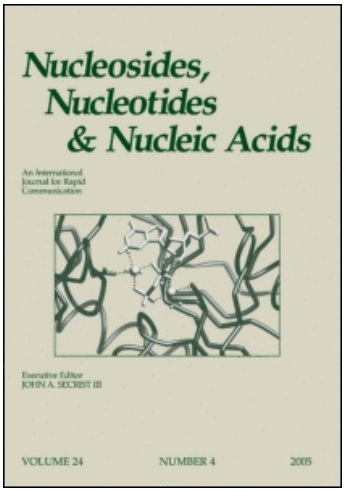

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SYNTHESIS AND ANTI-HIV ACTIVITY OF A NEW HEXOPYRANOSIDE ANALOGUE OF AZT

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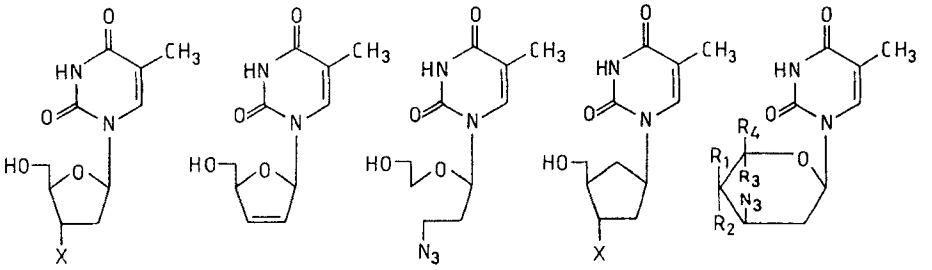
Abstract:
The glycosylation of thymine (**13**) with **12** in the presence of trimethylsilyl triflate promoter afforded the α-, β-L--*ribo*-hexopyranosyl-nucleoside analogue **14a,b**. After removal of the protecting group 1-(3-azido-2,3,6-trideoxy-α- and β-L--*ribo*-hexopyranosyl)-thymine (**15a** and **15b**) were isolated in anomerically pure form in a ratio of 1:20. The anti-HIV activity of the major product **15b** was examined, in comparison with AZT, on H9 lymphoid cell-line.

INTRODUCTION

The discovery of the anti-AIDS activity[1,2] of 3'-azido-3'-deoxythymidine[3] (**2**; AZT, Retrovir, Zidovudine) stimulated considerable interest in the field of the research of pyrimidine nucleosides and deoxy sugar derivatives. The structural modification of thymidine (**1**) involved changes both at the aglycone and carbohydrate portions of the molecule. Thus, besides replacement of the C-3' hydroxyl group of the 2-deoxy-D-ribofuranosyl unit of **1** to give the substituted analogues **2-5**[4-7], the unsaturated (**6**)[8-10], acyclic (**7**)[11] and carbocyclic (**8**, **9**) derivatives [12-14] were also synthesized. Most surprisingly, among the pyranoside analogues of AZT only the preparation of **10** and **11** was reported [15,16].



1 : X = OH
2 : X = N_3
3 : X = CN
4 : X = H
5 : X = F

6

7

8 : X = H
9 : X = N_3

10 : $R_1 = R_2 = R_3 = H$, $R_4 = CH_2OH$
11 : $R_1 = R_3 = H$, $R_2 = OH$, $R_4 = CH_2OH$

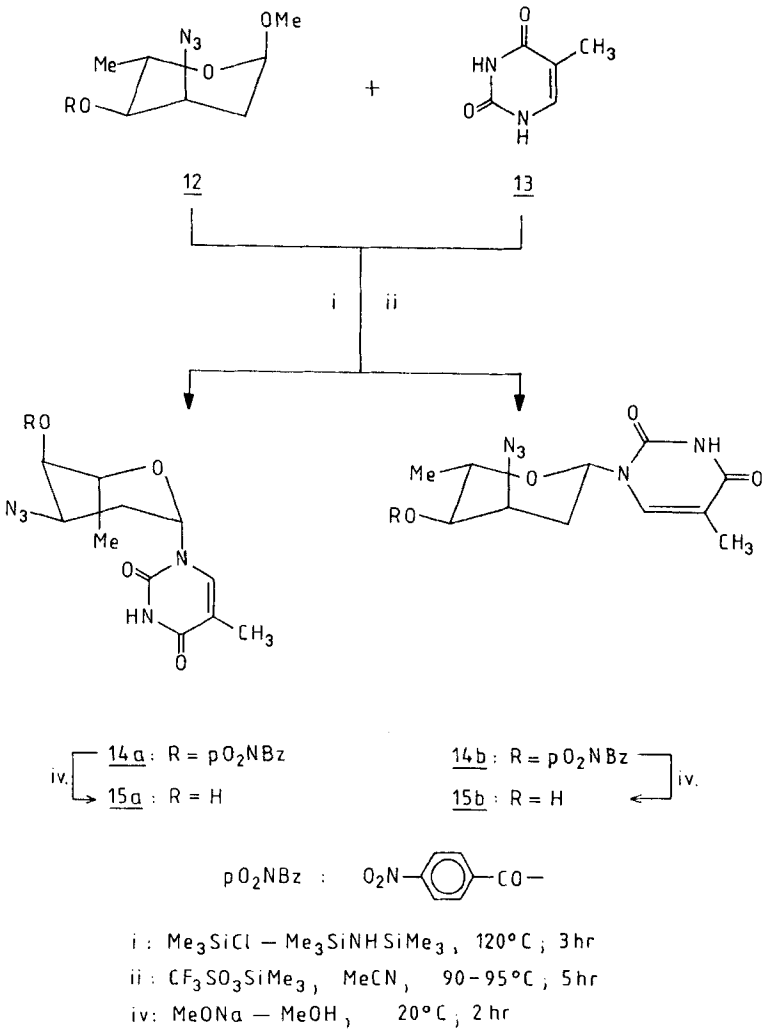
It is to be noted that each of the carbohydrate components of the above nucleosides belongs to the D-sugar series and attached to thymine with a β-glycosidic linkage.

The present paper deals with the synthesis of a new, β-L-*ribo*-hexopyranosyl analogue (**15b**) of AZT and with the potential anti-HIV effect of this nucleoside.

SYNTHESIS AND DISCUSSION

Methyl 3-azido-2,3,6-trideoxy-4-O-(p-nitrobenzoyl)-α-L--*ribo*-hexopyranoside (**12**), the carbohydrate component of **15b**, was synthesized[17,18] from L-rhamnal in eight steps. However, the yield of the key-intermediate, methyl 2,6-dideoxy-3-O--tosyl-α-L-*arabino*-hexopyranoside was found to be unsatisfactory on larger scales. Therefore, the excellent recent method of MONNERET et al[19] for acylation of stannylidene ketals was adopted, allowing the preparation of **12** with a 24% overall yield. For the formation of the nucleoside the method described by DYATKINA et al[20] was found to be the best, and thus a 2:1 mixture of thymine (**13**) and **12** was reacted with an excess of hexamethyldisilazane and chlorotrimethylsilane at 120-130°C for 3 hours (FIG. 1.).

The glycosylation was accomplished in anhydrous acetonitrile in the presence of trimethylsilyl triflate promoter (5 hrs, 90-95°C), and - surprisingly -, according to t.l.c. examination, the reaction resulted in the formation of practically the pure β-anomer **14b**, wich was isolated by column



i : $Me_3SiCl - Me_3SiNHSiMe_3$, 120°C ; 3hr

ii : $CF_3SO_3SiMe_3$, MeCN, 90-95°C ; 5hr

iv: MeONa — MeOH, 20°C ; 2 hr

FIG.1. Synthesis of new pyranoside analogue of AZT

chromatography (see Experimental) with the eluent system C. An alternative and more convenient way of the separation of **14b** from the unreacted **13** involved extraction with the hot solvent systems B and C, and the unchanged **13** was recycled after recrystallization from water. Zemplen transesterification of the samples of **14a,b**, obtained either by chromatography or by this latter extraction procedure, afforded **15b** with good yield. In the IR spectrum of **15b** the intense absorption at 2105 cm^{-1} indicates the presence of the azido group in the molecule. The EI(+) ionization mass spectrum of **15b** (accomplished with the application of ammonium chloride) showed the appearance of the pseudo-molecular ion $M+H]^{+}$ at m/z = 282 with 10 % intensity. The fragmentations characteristic of this structure are depicted on FIG. 2., and the relative intensity values of the peaks are given in brackets.

The ^{1}H-NMR data obtained for **15b** showed the β-L-configuration of the anomeric linkage ($J_{1',2'a}$ = 12 Hz), the *axial* disposition of the protons H-1′, H-4′ and H-5′, and thus the $^{1}C_{4}$ conformation of the carbohydrate unit. Column chromatography (Silica gel 60, eluent system C) of the mother liquor of **15b**, obtained by means of the extraction procedure, allowed the isolation of ca. 5 % of the α-L-nucleoside **15a**.

With respect to this the β-L- and α-L-products are present in the reaction mixture in a ratio of 20:1. Such a highly stereoselective glycosylation with a 2-deoxysugar derivative is quite surprising. However, it is anticipated that the α-L-nucleoside is less stable than the β-anomer under the applied conditions of the reaction and isolation. The ^{1}H-NMR measurements clearly showed that in the α-anomer the carbohydrate unit is present in the $^{4}C_{1}$ conformation ($J_{4',5'}$ = 2.5 Hz),bearing the H-1′proton also in *axial* position ($J_{1',2'a}$= 9.5Hz).

BIOLOGICAL RESULTS

When H9 cells were infected with HIV and examined for IF*, 100 % of the cells became positive for viral antigen on day 12 after infection (Fig.3.). At the same time, the percentage of

* IF = immunofluorescence

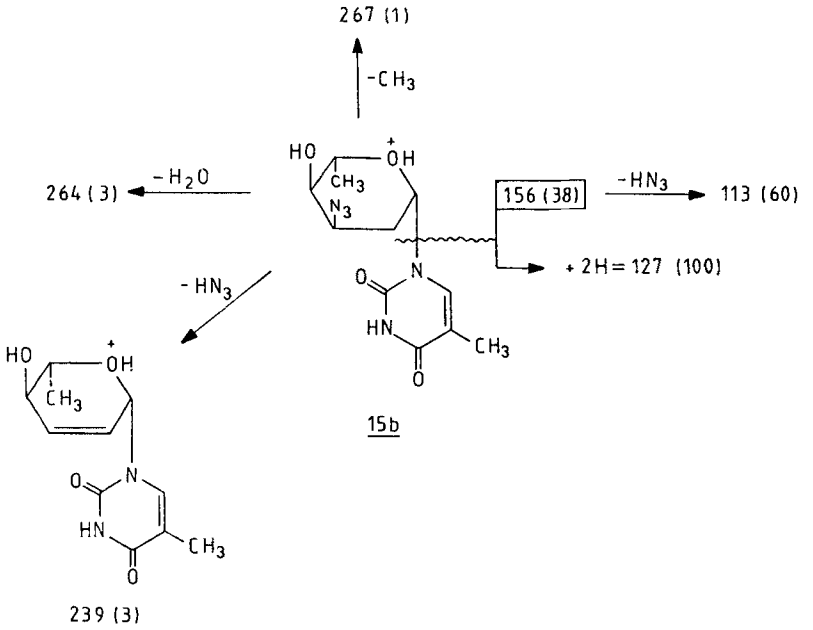


FIG.2. Mass spectrometric fragmentation of **15b**

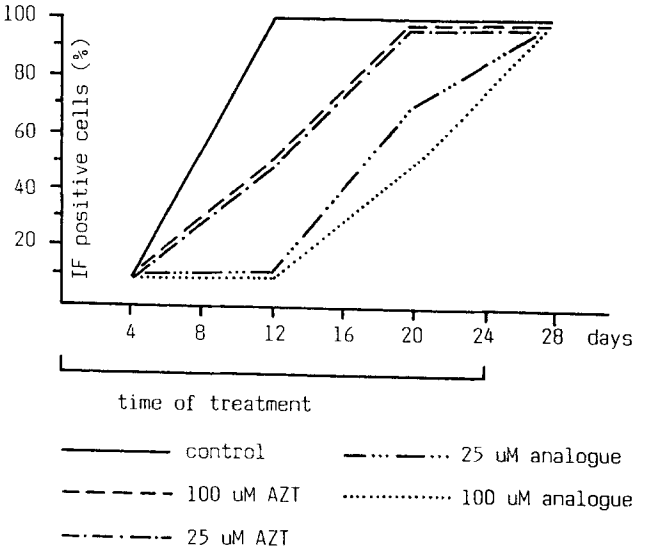


FIG.3. Effect of AZT and a new hexopyranoside analogue (**15b**) on the replication of HIV-I in H9 cells.

IF-positive cells was 50 % in cultures treated with different concentrations of AZT and 10 % in those received the **15b** derivative in two different concentrations.

On day 20 postinfection, 100% of cells treated with AZT was infected, whereas the percentage of IF-positive cells was 70%, and 50% in cultures treated with 25 μM and 100 μM of the derivative, respectively.

On day 28, 100% of the cells became positive for viral antigen in cultures treated with **15b**.

The **15b** inhibited the HIV replication more effectively than AZT. In addition a dose dependent inhibition of IF was observed in the HIV-infected H9 cells incubated in the presence of different concentrations of the new compound.

The **15b** exerted no cytotoxic effect on the cells as estimated by the light microscopic examination of the uninfected H9 cultures treated by the same drug concentrations as the HIV-infected cultures.

The results concerning the *in vitro* reclustering activity of **15b** on MT4 cells, as well as the *in vitro* experiments carried out on BALB/C mices infected with Rauscher leukemia virus will be published in a separate communication.

EXPERIMENTAL PART

Chemical examinations

Melting points were determined in capillary tubes and they are not corrected. Specific optical rotations were determined with a Perkin-Elmer 241 automatic polarimeter. The ^{1}H-NMR spectra were recorded with a BRUKER WP 200 SY instrument at 200 MHz by using tetramethylsilane as an internal standard. The IR spectra were obtained with a Perkin-Elmer 283 B instrument. The mass spectra were recorded on a VG-7035 spectrometer (VG-Analytical Ltd., England), direct sample application mixed with ammonium chloride in EI(+) operation mode (30 eV ionization energy, temperature of the ion source 220°C). T.l.c. and column chromatoghraphy were carried out on DC-Alurolle Silica gel $60F_{254}$ (Merck) and Silicagel 60 (0.2-0.5 mm), respectively, with the following solvent/eluent systems (V/V): (A) 2:1 benzene-methanol; (B) 4:1 benzene-methanol; (C) 9:1

benzene-methanol; (D) 96:4 chloroform-methanol. Detection of the thinlayer chromatograms was carried out in UV light.

1-(3-Azido-2,3,6-trideoxy-β-L-*ribo*-hexopyranosyl)-thymine **(15b; Method A)**

A mixture of **13** (0.30 mM) and **12** (0.15 mM) in hexamethyldisilazane (1.5 ml) was treated with 0.15 ml of chlorotrimethylsilane at 120-130°C for 3 h, until the reaction mixture become homogeneous. After evaporation and coevaporation with abs. toluene, the residue was dissolved in abs. acetonitrile (3 ml) and reacted with trimethylsilyl trifluoromethanesulfonate (0.15 ml) at 90°C for 3 h. 0.15 ml of this glycosylating promoter was added again and the reaction was continued for an additional 2 h. The reaction mixture was evaporated by means of co-distillation with dry benzene and the residue was purified by column chromatography on a Silica gel 60 column (30 x 3 cm) with the eluent system C (3-4 ml/min). Combination and evaporation of the fractions 24-32 in *vacuo* gave crystalline **14b** (78%), m.p. 166-169°C; R_f(D) = 0.54.

IR-spectrum(KBr): 2105 (ν_{N3}), 1600-1720 (thymine $\nu_{C=O}$ and ester $\nu_{C=O}$), 1605 ($\nu_{C=C}$), 1522 (ν_{as} NO_2), 1345 ($\nu_s NO_2$), 945 (thymine) cm^{-1}.

^{1}H-NMR (200 MHz, MeOD) spectrum δ ppm: 8.41-8.23 (4H, m, pNO_2--phenyl); 7.54 (1H, q, -CH=), $J_{CH,CH3}$ = 1.2 Hz, 6.02 (1H, dd, H-1′), $J_{1',2'a}$ = 11.0 Hz, $J_{1',2'e}$ = 2.7 Hz, 5.11 (1H, dd, H-4′, $J_{3',4'}$ = 3.2 Hz, $J_{4',5'}$ = 9.8 Hz), 4.54 (1H, ddd, H-3′), 4.33 (1H dq, H-5′), 2.35 (1H, ddd, H-2′a), $J_{2'a,3'}$ = 3.2 Hz, $J_{2'a,2'e}$ = 13.9 Hz, 2.12 (1H, ddd, H-2′e), 1.90 (3H, d, thymine, CH_3), 1.28 (3H, d, CH_3-5′), $J_{Me,H-5'}$ = 6.1 Hz.

Analysis: for $C_{18}H_{18}N_6O_7$(430.37)

Calculated C % = 50.23	Found C % = 50.67
H % = 4.21	H % = 4.11
N % = 19.52	N % = 19.34

0.80 mM of **14b** obtained as described above was dissolved in abs. methanol (50 ml) and the solution was treated with 1.5 ml of 0.1 M sodium methoxide in methanol at room temperature for 2 hrs. The pH was then adjusted to 6 by the addition of acetic acid, the solution was evaporated and the residue was dried in a vacuum desiccator over potassium hydroxide and

calcium chloride. The obtained colourless crystals were washed with a small volume of cold dry ether and then purified by means of column chromatography on Silica gel 60 with the eluent system C to give 67 % of **15b** m.p. 224-225°C (dec.), R_f(B) = 0.35; $[\alpha]^{20}_D$ -52.6° (c = 0.27, water).

IR-spectrum (KBr): 3400-3370 (ν_{OH}), 3320 (ν_{NH}), 2982 ($\nu_s CH_3$), 2930 ($\nu_{as} CH_2$), 2105 (ν_{N3}), 1680-1610 (thymine $\nu_{C=O}$ and $\nu_{C=C}$), 940 (thymine) cm^{-1}.

^{1}H-NMR-spectrum (200 MHz, MeOD) δ ppm: 7.48 (1H, q, CH=), $J_{H,CH3}$ = 1.2 Hz, 5.86 (1H, dd, H-1′), $J_{1',2'a}$ = 12 Hz, $J_{1',2'e}$= 2.5 Hz, 4.12 (1H, ddd, H-3′), $J_{3',4'}$ = 3.2 Hz, $J_{3',2'e}$ = 3 Hz, $J_{3',2'a}$ = 3 Hz, 3.51 (1H, dd, H-4′), $J_{4',5'}$ = 9.5 Hz, 2.13 (1H, ddd, H-2′a), $J_{2'a,2'e}$ = 12 Hz, 1.97 (1H, ddd, H-2′e), 1.87 (3H, d, thymine CH_3), 1.27 (3H, d, CH_3-5′, $J_{5',6'}$ = 6.2 Hz).

Analysis: for $C_{11}H_{15}N_5O_4$ (281.30)

Calculated		Found	
C % =	46.96	C % =	47.10
H % =	5.37	H % =	5.53
N % =	24.90	N % =	24.92

Isolation of **15b** by the extraction procedure (**Method B**)

The glycosylation of **13** with **12** was carried out as described above, followed by evaporation of the reaction mixture under diminished pressure. The residue was suspended with a small volume of ice-cold water, filtered, washed with a small volume of 10 % aqueous sodium hydrogen carbonate, water and then it was air-dried under the IR lamp. The crude solid product was successively extracted with a tenfold volume (by weight) of the hot eluents B and C to ensure the separation of the soluble product **14b** from the unreacted **13**. The residual **13** was crystallized (73 %) and recycled. Upon evaporation of the combined extracts compound **14b** was obtained in crystalline form (64 %). R_f(B) = 0.60.

O-Deacyclation of **14b** was carried out in abs. methanol with a catalytic amount of sodium methoxide, as described above. The residue obtained after evaporation was taken up in the hot B solvent system and after filtration the pure crystalline **15b** precipitated (68 %). The product was dried in vacuo over phosphorus pentoxide and wax at 100 °C, m.p. 223-224°C (dec.), R_f(B) = 0.38. $[\alpha]^{20}_D$ -49.3° (c = 0.32, water).

Analaysis: for $C_{11}H_{15}N_5O_4$ (281.30)

Calculated C % = 46.96	Found C % = 47.01
H % = 5.37	H % = 5.42
N % = 24.90	N % = 25.05

The chromatographic purification (Silica gel 60, eluent system C) of the mother liquor of **15b** gave ca. 5 % of the α-L-nucleoside **15a**, m.p. 313-315 °C (dec.), R_f(B) = 0.26. $[\alpha]^{20}_D$ + 21.8° (c = 0.83, methanol).

^{1}H-NMR spectrum (200 MHz, $CDCl_3$) for **15a** δ ppm: 9.30 (1H, bs, NH), 7.41 (1H, bs, CH=), 5.98 (1H, dd, H-1'), $J_{1',2'a}$ =9.5 Hz, $J_{1',2'e}$ = 4.2 Hz, 4.40 (1H, m, H-3'), 3.93 (1H, dq, H-5'), $J_{4',5'}$ = 2.5 Hz, $J_{5',6'}$ = 6 Hz, 3.77 (1H, bs, H-4'), 2.80 (1H, bs, OH-4'), 2.0-2.20 (2H, m, H-2'a and H-2'e), 1.95 (3H, s, thymine CH_3), 1.35 (3H, d, CH_3-5')

Analysis: for $C_{11}H_{15}N_5O_4$ (281.30)

Calculated C % = 46.96	Found C % = 47.20
H % = 5.37	H % = 5.86
N % = 24.90	N % = 24.54

Biological examinations

Materials and Methods

Cells

The H9 cell line[21] was used in this study. The cells were cultured and maintained in RPMI-1640 medium supplemented with 10 % fetal calf serum (FCS), 100 IU/ml penicillin G and 100 μg/ml streptomycin.

Virus and infection

The HTLV-IIIB strain of HIV-1 was obtained from the culture supernatant of the H9 cell line as previously described[21]. The cells were infected with 100 $TCID_{50}$ of HIV, incubated for 1 h at 37°C. After virus adsorption, infected cells were washed and resuspended in fresh medium adjusted to a concentration of $3x10^5$ cells/ml and cultured at 37°C in a Co_2 incubator.

Assay for HIV-specific antigen expression

Virus-specific antigen expression in HIV-infected H9 cells was determined by indirect cytoplasmic immunofluorescence (IF).

Briefly, acetone-methanol-fixed cells were incubated with 1:500 diluted seropostive anti-HIV human serum (IF titer 1:2000) for 30 min at 37°C. Then, the cells were washed with PBS for 15 min, incubated with the fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human IgG (Hyland, Costa Mesa, CA) for 30 min at 37°C and washed again with PBS. Cells were contained under a fluorescent positive cells was calculated.

Drug treatment

AZT and **15b** were used in concentrations of 25 μM and 100 μM. Cells were pretreated with the drugs for 4 h prior to infection with HIV. After washing the cells, the drugs were added again to the cells in the concentrations described above. Drug-containing growth medium was changed at 4 days intervals.. The cell cultures were treated for 24 days.

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REFERENCES

1. H. Mitsuya, K J. Weinhold, P. A. Furman, M. H. St.Clair,S. N. Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, S. Broder, Proc. Natl. Acad. Sci. U.S.A. 82, 7096 (1985)
2. R. M. Ruprecht, L. G. O'Brien, L. D. Rossoni, S. Nusinoff--Lehrman, Nature, 323, 467 (1986)
3. J. P. Horowitz, J. Chua, N. Noel, J. Org. Chem. 29, 2076 (1964)
4. H. Mitsuya, S. Broder, Proc. Natl. Acad. Sci. U.S.A. 83, 1911 (1986)
5. C. K. Chu, R. F. Schinazi, M. K. Ahn, G. V. Ullas, Z.P. Gu, J. Med. Chem. 32, 612 (1989)
6. J. Calvo-Mateo, M.-J. Camarasa, A. Dias-Oritz, F. G. De las Heras, Tetrahedron Lett. 29, 941 (1988)
7. G. W. J. Fleet, J.-Ch. Son, A. E. Derome, Tetrahedron 44, 625 (1988)

8. J. Balzarini, R. Pauwels, P. Herdewijn, E. DeClercq, D. A. Cooney, G. J. Kang, M. Dalal, D. G. Johns, S. Broder, Biochem. Biophys. Res. Comm. 140, 735 (1986)
9. T. S. Lin, R. R. Schinazi, M. S. Chen, E. Kinney-Thomas, W. H. Prusoff, Biochem. Pharmaco 36, 311 (1987)
10. M. M. Mansuri, J. E. Starrett, Jr., I. Ghazzouli, M. J. Hitchcoch, R. Z. Sterzycki, V. Brankovan, T. S. Lin, E. M. August, W. H. Prusoff, J.-P. Sommadossi, J. C. Martin, J. Med. Chem. 32, 461 (1989)
11. P. Scheiner, A. Geer, A.-M. Bucknor, J.-L. Imbach, R. F. Schinazi, J. Med. Chem. 32, 73 (1989)
12. L. Ötvös, J. Béres, Gy. Sági, I. Tömösközi, L. Gruber, Tetrahedron Lett. 28, 6381 (1987)
13. Y. F. Shealy, C. A. O'Dell, W. M. Shannon, G. Arnett, J. Med. Chem. 29, 483 (1986)
14. M. Bodenteich, H. Griengl, Tetrahedron Lett. 28, 5311 (1987)
15. Z. Kaluza, E. B. Pedersen, c. M. Nielsen, M. Chmielewski, Acta Chem. Scan. 44, 294 (1990)
16. P. Hansen, J. Lau, E. B. Pedersen, c. M. Nielsen, Liebigs. Ann. Chem. 1079 (1990)
17. F. Sztaricskai, I. Pelyvás, L. Szilágyi, R. Bognár, J. Tamás, A. Neszmélyi, Carbohyd. Res. 65, 193 (1978)
18. F. Sztaricskai, M. Menyhárt, R. Bognár, A. Neszmélyi, Carbohydr. Res. 100, C-14, (1982)
19. C. Monneret, R. Gagnet, J. C. Florent, J. Carbohydr. Chem. 6, 221 (1987)
20. N. B. Dyatkina, A. A. Krayevsky, A. V. Azhaev, Bioorg. Khim. 12, 1048 (1986)
21. M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, Science 224, 497 (1984)

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