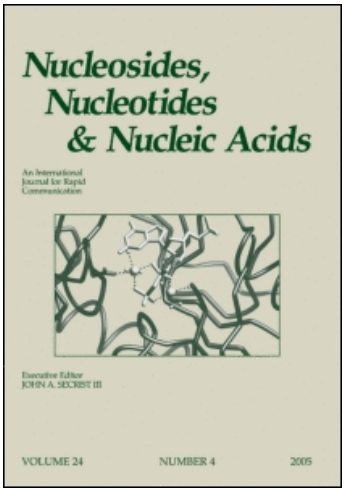

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Synthesis and Biological Evaluation of 3-Deazacytidine and 3-Deazauridine Derivatives

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SYNTHESIS AND BIOLOGICAL EVALUATION OF 3-DEAZACYTIDINE AND 3-DEAZAURIDINE DERIVATIVES.

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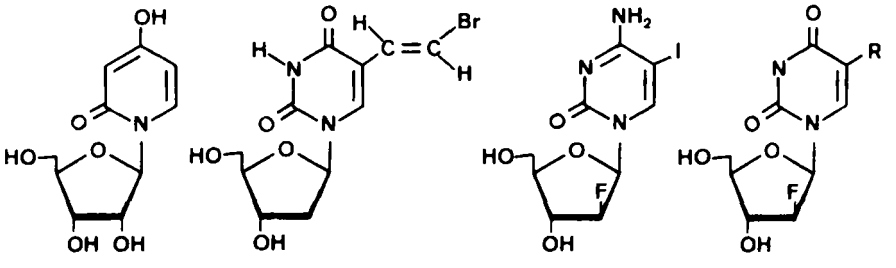
Abstract The synthesis of new 5-and 6- subsituted 3-deazapyrimidine ribonucleosides has been performed by reacting the silylated bases and 1-O-acetyl-2, 3, 5-tri-O-benzoyl-β-D-ribofuranose in dichloroethane in the presence of stannic chloride.
None of these compounds exhibited any antiviral activity in vitro against HSV1 and RV31.

3-Deazauridine (4-hydroxy-1-(β-D-ribofuranosyl)-2-pyridone)1, a synthetic analogue of uridine[1] is cytotoxic to tumor cells in vitro and in vivo, and its activity in tumor bearing animals has led to phase I clinical trials to determine its toxic effects in humans[2].



1 **2** **3**

4 : R = CH_3

5 : R = CH_2CH_3

0732-8311/86/0502-0125$3.50/0

Pyrimidine nucleoside analogues have proved to be of great interest particularly as antiviral compounds. Among the most potent antiviral derivatives known to date, are those substituted at the 5 position of the pyrimidine ring like BVDU[3,4] 2 or FIAC[5] 3 and analogues like FMAU 4 or the ethyle derivative 5 showed also very interesting activities against HSV I and HSV 2[6].

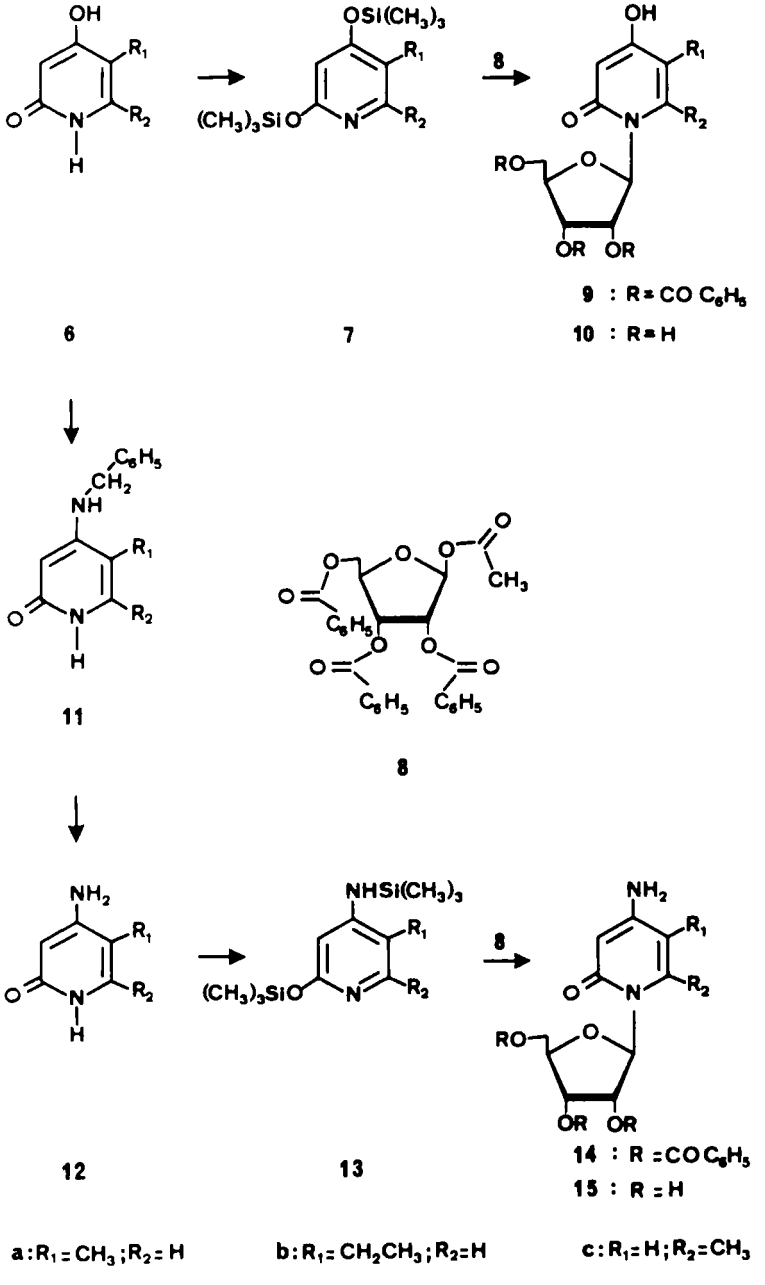
Therefore we have been intrigued to know whether substituents at the 5 position in the 3-deazapyrimidine nucleoside series would be of interest. Two of us described recently[7] a general route to 5 and/or 6 substituted 4-amino or 4-hydroxy pyridine-1H-2 ones which are 3-deaza cytosine and 3-deaza uracile analogues respectively. We report in the present paper the synthesis of unknown 5- and 6-substituted 3-deaza pyrimidine ribonucleoside analogues and biological results in vitro.

Chemistry.

Compounds 6a, 6c, 11a, 11c, 12a and 12c (scheme I) have been described in a previous paper[7]. The synthesis of 12 has been performed from 6 which react with benzylamine to give the corresponding 4-benzylamino derivatives 11. Catalytic debenzylation over palladized charcoal in acetic acid afforded the amino derivatives 12 (scheme I).

5-Ethyl-4-hydroxy-2-oxo-1,2-dihydropyridine 6b was obtained as already described for the homologueous methyl derivative. Butyronitrile reacted with malonylchloride to give 6-chloro-5-ethyl-4-hydroxy-1H-pyridin-2-one which on catalytic hydrogenation with palladized charcoal led to 6b. The latter compound served as starting material for the preparation of 12b via 11b according to the general method[7].

The Hilbert-Johnson nucleoside synthesis with Friedel-Crafts catalyst described by Vorbrüggen and coll[8] is the method of choice to build a nucleoside from a persilylated heterocyclic base and a peracylated sugar. The silylated bases 7 and 13 have been condensed with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose 8 in dichloroethane in the presence of stannic chloride ($SnCl_4$). This method is well known to afford exclusively β-nucleosides according to the proposed mechanism of nucleoside formation[9]. However starting from 13c, the corresponding nucleoside 14c could not be obtained, so far, as pure material. As shown by the NMR spectra in DMSO d_6, (two methyl peaks at 2.00 and 2.04 ppm as well as an exangeable doublet (NH) at 6.84 ppm) 14c was always contaminated with another product which resulted from the condensation of the exocyclic 4-amino group of the base with the sugar and could not be separated from 14c.



Scheme 1

Methanolic ammonia treatment of the protected nucleosides 9 and 14 provided the free nucleosides 10 and 15 in good yield, generally after low pressure liquid chromatography.

A standard method, which has been described previously[11] for determining the inhibition of virus-induced cytopathogenic effects (CPE) by antiviral compounds was used to evaluate the ribofuranoside analogues against herpes simplex virus, type 1 (HSV-1) and against rhinovirus (RV31). Compounds 10a (100 μg/mL), 10b (100 μg/mL), 15a (100 μg/mL), 10c (120 μg/mL) and 15b (120 μg/mL) were not active at the highest non toxic concentration.

From this study, it can be infered that modifications at the 5 position in the 3-deaza pyrimidine ribonucleoside series do not bring about any antiherpetic activity. However, one cannot rule out that antiviral activity could be observed in the 3-deaza-5-substituted pyrimidine 2'-deoxyribonucleoside series. This will be the subject of further investigations since the absence of an hydroxyl fonction at the 2' position of the sugar is a characteristic feature of numerous antiviral nucleosides like BVDU, FIAC or FMAU.

EXPERIMENTAL SECTION.

Chemistry.

The melting points were measured with a Kofler hot stage apparatus and are uncorrected. Proton NMR spectra were obtained with a Varian XL 100 (100 MHz) spectrometer operating in the Fourier transform mode unless otherwise stated. ^{1}H NMR spectra (400 MHz) were obtained with a Brucker AM 400W spectrometer. Chemical shits (δ) in ppm are reported relative to tetramethylsilane. Microanalyses were performed by the "Service de Microanalyses", CNRS-ICSN, 91190 Gif sur Yvette, France and are within + 0.4 % the theoretical values.

The preparative chromatographies were carried out in glass columns packed with 230-400 mesh silica gel (Kieselgel 60, Merk) under pressure (1-6 bars), using the following solvent systems : (A) (Toluene-ethylacetate 8 : 2), (B) ethylacetate, (C) methylene chloride-ethanol 9 : 1 or (D) methylene chloride-ethanol 8 : 2. (vol/vol).

5-Ethyl-4-hydroxy-1H-pyridin-2-one (6b).

Malonyl chloride (100 g) and n-butyronitrile (70mL) were stirred in the dark for 6 days at O°C and for 24 hours at room temperature. Water (500 mL)

was then added slowly to the precipitate (the addition of water is exothermic). The mixture was cooled and filtered. A further amount of product could be precipitated from the mother liquor by adding concentrated Na OH to a pH of 3-4. The solid crude product was then taken up in 1.5 L of boiling water and a new precipitate was obtained on cooling. This solid material was washed with water (2 L), air dried, and recrystallized from ethanol to afford 25.6 g of pure 6-chloro-5-ethyl-4-hydroxy-1H-pyridin-2-one (6) (R_1 = CH_2CH_3, R_2 = Cl) (21 % yield) which sublimated at 220°C.

The chlorine removal was currently performed on 8.5 g of 6 (R_1 =CH_2CH_3; R_2 = Cl) in methanol (200 mL) in the presence of triethylamine (10 mL) and 30 % palladium on charcoal (2 g) under a hydrogen atmosphere with stirring for 5 hours. After usual work up, the solid residue was recrystallized from water to give **6b** (R_1 = CH_2CH_3;R_2 = H) in 81 % yield ; sublimate from 240°; ^{1}H NMR DMSOd_6 δ 10.68(s,br,2H,NH,OH); 6.99(s,1H,H3); 5.59(s,1H,H6); 2.31(q,2H, CH_2); 1.07(t,3H,CH_3);

Anal. Calcd. for $C_7H_9NO_2$: C,60.42; H,6.52; N,10.07. Found : C,60.20; H,6.46; N,10;08.

4-Benzylamino-5-ethyl-1H-pyridin-2-one **(11b).**

A solution of **6b** (30 mmol) in benzylamine (30 mL) was heated under reflux and nitrogen for 40 hours. The excess benzylamine was distilled in vacuo and **6b** crystallized from dichlorobenzene. yield 75 %. An analytical sample was obtained by recrystallization from AcOEt. m.p. 207-208°C ^{1}H NMR DMSO d_6 δ 10.08(s,1H,NH); 7.34(m,5H,); 6.83(s,1H,H3); 6.67(t,1H,NH benzyl); 5.00(s,1H,H6); 4.34(d,2H,CH_2-benzyl,J = 6Hz); 2.39(q,2H,CH_2ethyl); 1.14(t,3H,CH_3ethyl).

Anal. Calcd. for $C_{14}H_{16}N_2O$: C,73.65; H,7.06; N,12.27; Found : C,73.85. H,6.99; N,12.04.

4-Amino-5-ethyl-1H-pyridin-2-one **(12b).**

To a solution of **11b** (2.3 g) in AcOH (200 mL) was added 1 g of palladium on charcoal (30 %). This mixture was stirred under hydrogen atmosphere for 24 hours. At this time the mixture was filtered and the catalyst washed with hot ethanol. The filtrate was concentrated in vacuo and subjected to colomn chromatography. The colomn was first washed with solvent D and **12b** was then eluted with a 50 % solution of CH_2Cl_2 in ethanol. yield 87 % m.p. 218-219°C (dioxane) ^{1}H NMR DMSOd_6 δ 9.92 (s,2H, NH); 6.83(s,1H,H3); 5.86(s,2H,NH_2); 5.31(s,1H,H6); 2.27(q,2H,CH_2); 1.07(t,3H,CH_3)

Anal. Calcd. for : $C_7H_{10}N_2O.\ H_20$: C,53.83; H,7.74; N,17.94; Found : C,53.75; H,7.76; N,17.70.

Silylations : The bases (10 mmol) were routinely silylated by heating (130°) with an excess of hexamethyldisilazane (30 mL) (HMDS) and a catalytic amount of ammonium sulfate[10] (10 mg). After 24 hours, a clear solution was obtained and the excess of HMDS distilled in vacuo. Residual HMDS was then removed by codistillation with dry xylene in vacuo. The silylated bases were then immediately used in the nucleoside formation step assuming that this reaction was quantitative.

General procedure for nucleoside formation :

The persilylated base (10 mmol) in distilled 1,2-dichloroethane (30 mL) was stirred with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (Pfanstiehl Lab. Inc.) (10 mmol) in the presence of $Sn\ Cl_4$ (1 mL) under exclusion of moisture. After 18 hours at room temperature the reaction mixture was diluted with CH_2Cl_2 (50 mL) and shaken with saturated $NaHC0_3$ (50 mL) and the resulting emulsion filtered over Celite. The organic phase was dried (Na_2SO_4) and distilled under reduced pressure. The residue was routinely subjected to column chromatography to eliminate the free sugar and purify the 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl nucleoside which was crystallized as indicated when possible.

Deprotection was carried out in methanol which had been previously saturated with ammonia at 0°C under exclusion of moisture. The tri-O-benzoylated nucleoside (1 g) was stirred in methanolic ammonia solution (40 ml) for 3 days at room temperature.

4-Hydroxy-5-methyl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1H-pyridin-2-one **(9a)**.

The silylated pyridine **7a** (10 mmol) obtained as a colorless oil reacted with **8** as described in the general procedure. The nucleoside **9a** was purified on a silica gel column with solvent (A), as a colorless hard foam. Crystallization from a mixture of cyclohexane-toluene afforded **9a** in a 40 % yield ; m.p. 136-138 °; ^1H NMR $CDCl_3$ δ 9.16(s,1H,OH); 8.20-7.30(m,15H,$3C_6H_5$); 7.21(s,1H,H6); 6.70(d,1H,H1',J = 5Hz); 6.04(s,1H,H3); 5.90(t,1H,H3'; 5.70(t,1H,H2'); 4.91(m,1H,H5'); 4.64(m,1H,H5''); 4.71(m,1H,H4'); 1.62(s,3H,CH_3).

Anal. Calcd. for $C_{32}H_{27}NO_9$: C,67.48 ; H,4.78 ; N,2.46. Found : C,67.77 ; H,4.91 ; N,2.49.

4-Hydroxy-5-methyl-1-(β-D-ribofuranosyl)-1H-pyridin-2-one **(10a).**

This compound was obtained by treatment of **9a** with methanolic ammonia and purified by column chromatography with solvent C which yielded a hard foam in 57 % yield. ^{1}H NMR (DMF d_7) δ 7.73(d,1H,H6,J = 1Hz) ; 6.11(d,1H,H1',J = 4Hz) ; 5.26(broad m,3H,OH2'OH3',OH5') ; 4.21(m,2H,H2',H3') ; 3.97(m,1H,H4') ; 3.78(t, 2H,CH_2) ; 3.44(brd m,1H,OH) ; 1.92(d,3H,CH_3J = 1Hz).

5-Ethyl-4-hydroxy-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1H-pyridin-2-one **(9b).**

Silylated **7b** was condensed with **8** to yield **9b** according to the general procedure. After work up and purification by chromatography with solvent A, **9b** was obtained in 55 % yield as an amorphous solid. ^{1}H NMR (DMSO-d_6) δ 9.38(s,1H,OH) ; 8.10-7.30(m,16H,3C_6H_5,H6) ; 6.36(d,1H,H1',J = 4Hz) ; 5.98(m,2H,H2',H3') ; 5.66(s,1H,H3) ; 4.70(m,3H,H5',H4') ; 2.19(q,2H,CH_2) ; O.95(t,3H,CH_3).

Anal. Calcd. for $C_{33}H_{29}NO_9$: C,67.91 ; H,5.01 ; N,2.40. Found : C,67.51 ; H,5.06 ; N,2.37.

5-Ethyl-4-hydroxy-1-(β-D-ribofuranosyl)-1H-pyridin-2-one **(10b).**

The deprotection of **9b** gave crude **10b**, which after purification by column chromatography with solvent C and subsequent crystallization in absolute ethanol, afforded **10b** in 45 % yield ; m.p. 238°C ; ^{1}H NMR 400 MHz (DMSO d_6). δ 10.82 (s,1H,OH) ; 7.70 (s,1H,H6) ; 6.03 (d,1H,H1',J = 4.3Hz) ; 5.66 (s,1H,H3) ; 5.34 (d,1H,OH2',J = 5.5Hz) ; 5.18 (t,1H,OH5',J =4.9Hz) ; 5.05 (d,1H,OH3',J = 4.9Hz) ; 4 (m,2H,H2',H3') ; 3.90 (m, 1H,H4',$J_{3'4'}$ = 5Hz,$J_{4'5'}$ = $J_{4'5''}$ = 3Hz) ; 3.75 (m,1H,H5') ; 3.62 (m,1H,H5",$J_{5'5''}$ = 12.2Hz) ; 2.36 (q,2H,CH_2,J = 7.3Hz) ; 1.12 (t,3H,CH_3).

Anal. Calcd. for $C_{12}H_{17}NO_6$: C,53.10 ; H,6.26 ; N,5.16. Found : C,52.91 ; H,6.33 ; N,5.11.

4-Hydroxy-6-methyl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1H-pyridin-2-one **(9c).**

Compound **9c** was prepared from **7c** and **8** (see the general procedure). After work up the residue was subjected to column chromatography. The column was first washed with solvent A and solvent C eluted then **9c** as a hard foam which we failed to crystallize ; yield : 37 %. ^{1}H NMR (DMSO-d_6) δ 10.78 (s,1H,OH) ; 7.1-6.5 (m,15H,phenyl) ; 5.65-5.35 (m,3H,H1',H2',H3') ; 5.88 (m,1H,H5) ; 5.62 (m,1H,H3) ; 4.66 (m,3H,H4',H5',H5") ; 2.36 (s,3H,CH_3).

Anal. Calcd. for $C_{32}H_{27}NO_9$: C,67.48 ; H,4.78 ; N,2.46. Found : C,67.14 ; H,4.86 ; N,2.37.

4-Hydoxy-6-methyl-1-(β-D-ribofuranosyl)-1H-pyridin-2-one (10c).

Compound 9c was deprotected as described above to yield 10c which could not be crystallized. An analytical sample was obtained after column chromatography with solvent C., yield 85 %. ^{1}H NMR (DMSO-d6) δ 5.84 (m,1H,H5) ; 5.66 (d,1H,H1',J = 4.5Hz) ; 5.51 (d,1H,H3,J_{3-5} = 2.7Hz) ; 4.88 (br,3H,3OH) ; 4.70 (t,1H,H2') ; 4.19 (t,1H,H3') ; 3.80 (m,1H,H4') ; 3.55 (m,2H,H5',H5") ; 2.35 (s,3H,CH_3).
Anal. Calcd. for $C_{11}H_{15}NO_6$: C,51.36 ; H,5.88 ; N,5;45. Found : C,51.29 ; H,6.05 ; N,5.18.

4-Amino-5-methyl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1H-pyridin-2-one (14a).

Compound 14a was prepared from 13a and 8 as described above. Column chromatography with solvent B was necessary to obtain an analytic sample as a hard foam. yield 91 %.^{1}H NMR ($CDCl_3$) δ 8.21-7.90 (m,15H,3C_6H_5) ; 7.07 (d,1H,H2 J = 0.9Hz) ; 6.74 (d,1H,H1',J = 5.5Hz) ; 5.96 (q,1H,H3',$J_{3'2'}$ =6Hz,$J_{3'-4'}$ = 4.5Hz) ; 5.80 (m,1H,H2',$J_{2'1'}$ = 5.5Hz) ; 5.61 (s,1H,H5) ; 5.61-4.57 (m,3H,H5',H4',H5") ; 4.16 (s,2H,NH_2) ; 1.66 (d,3H,CH_3,J = 0.9Hz).
Anal. Calcd. for $C_{32}H_{28}N_2O_8$: C,67.60 ; H,4.96 ; N,4.93. Found : C,67.45 ; H,5.02 ; N,4.91.

4-Amino-5-methyl-1-(β-D-ribofuranosyl)-1H-pyridin-2-one (15a).

Compound 14a was treated with methanolic ammonia as described above to afford 15a as a foam after column chromatography with solvent C. yield 74 % ^{1}H NMR (DMSO-d6) δ 7.40 (d,1H,H6,J=1Hz) ; 5.95 (m,3H,H1',NH_2) ; 5.33 (s,1H,H3) ; 5.17 (d,1H,OH,J = 5.4Hz) ; 5.06 (t,1H,OH5',J = 5.5Hz) ; 4.90 (d,1H,OH,J =4.5) ; 4.01 (m,2H,H2',H5') ; 3.83 (m,1H,H4') ; 3.62 (m,2H,CH_2) ; 1.88 (d,3H,CH_3).
Anal. Calcd. for $C_{11}H_{16}N_2O_5$: C,51.56 ; H,6.29 ; N,10.93. Found : C,51.22 ; H,6.67 ; N,10.59.

4-Amino-5-ethyl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1H-pyridin-2-one (14b).

Compound 14b was synthesized from 13b and 8 as already described. Chromatography with solvent B afforded 14b as an oil which solidified on

standing; yield 92 %. ^{1}H NMR (DMSO-d6) δ 8.12-7.34 (m,15H,3xC_6H_5) ; 7.23 (d,1H,H6,J = 0.8Hz) ; 6.38 (d,1H,H1',J = 4Hz) ; 6.15 (s,2H,NH_2) ; 6.03 (m;2H,H2',H3') ; 5.38 (s,1H,H3) ; 4.72 (m,3H, H4',H5',H5") ; 2.20 (q,2H,CH_2) ; 0.99 (t,3H,CH_3).

Anal. calcd. for $C_{33}H_{30}N_2O_8$: C,68.03 ; H,5.19 ; N,4.81. Found : C,67.80 ; H,5.28 ; N,4.72.

4-Amino-5-ethyl-1-(β-D-ribofuranosyl)-1H-pyridin-2-one **(15b).**

Compound **14b** led to **15b** which was recrystallized from absolute ethanol ; yield 95 %. m.p.=232°. ^{1}H NMR (DMSO-d6) δ 7.39 (d,1H,H6) ; 5.97 (m,3H,NH_2,H1') ; 5.33 (s,1H,H3) ; 5.19 (d,1H,OH,J = 5.3Hz) ; 5.08 (t,1H,OH5',J = 5Hz) ; 4.91 (d,1H,OH,J = 4.8Hz) ; 4.02 (m,2H,H2',H3') ; 3.84 (m,1H,H4') ; 3.60 (m,2H,H5',H5") ; 2.30 (q,2H,CH_2) ; 1.10 (t,3H,CH_3).

Anal. calcd. for $C_{12}H_{18}N_2O_5$: C,53.32 ; H,6.71 ; N,10.37. Found : C,53.47 ; H,6.68 ; N,10.59.

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