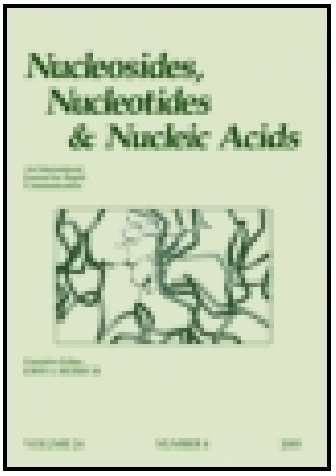

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Diazepinone Nucleosides as Inhibitors of Cytidine Deaminase.

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DIAZEPINONE NUCLEOSIDES AS INHIBITORS OF CYTIDINE DEAMINASE.

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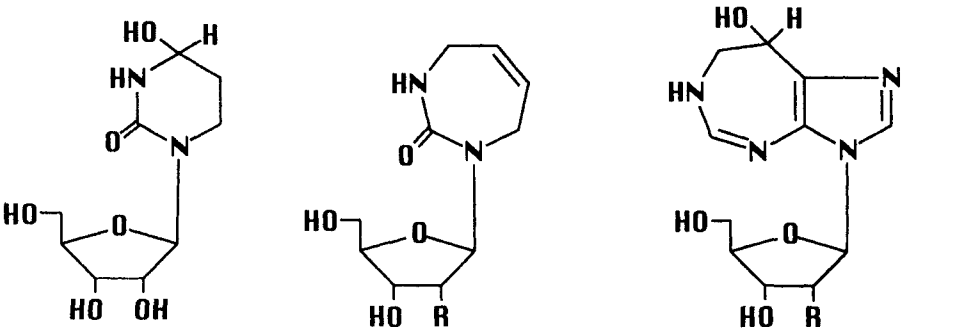
Abstract. The synthesis of 2'-deoxy and 2',3'-dideoxy derivatives of 1-ß-D-ribofuranosyl-1,3,4,7-tetrahydro-2H-1,3-diazepin-2-one (**2**) was undertaken in order to find new cytidine deaminase (CDA) inhibitors and potential adjuvants in anticancer chemotherapy. Replacement of ribose by a 2-deoxyribose moiety led to compound **9** that appeared slightly more potent than **2** (Ki = 2.5 x 10^{-8} M). Remarkably, the corresponding α-2'-deoxynucleoside **10** acted as a very potent inhibitor of human placenta CDA, with a Ki = 7.5 x 10^{-8} M. Attempt to synthesize the 2',3'-dideoxy derivative of **2** led to N-[4,5-dihydroxy-1-(2-oxo-2,3,4,7-tetrahydro[1,3]diazepin-1-yl)-pentyl]-2,2,2-trifluoroacetamide (**13**), which is devoid of CDA inhibitory activity.

In the last years several experiments have revealed that differences exist between normal and cancer cells in terms of enzyme activities involved in pyrimidine and purine metabolisms.[1-5]

Our interests have been focused on adenosine deaminase (ADA) an enzyme that catalyzes deamination of adenosine and 2'-deoxyadenosine to form inosine and 2'-deoxyinosine,[6-11] and on cytidine deaminase (CDA) which catalyzes the deamination of cytidine and 2'-deoxycytidine to uridine and 2'-deoxyuridine, respectively.[12-15]

Inhibition of these enzymes could result in enhanced antiproliferative and antiviral effects of nucleoside analogs which are substrates of ADA or CDA. As expected, inhibitors of ADA and CDA have been shown to prolong the plasma half-life of these drugs.[16] More recently a tumor-selective approach to chemotherapy has been reported, utilizing analogues of deoxycytidine and the CDA inhibitor tetrahydrouridine (**1**, THU, Ki = 10^{-7} M).[17]



THU (**1**) **2** R= OH Coformycin (**3**)
R= H Deoxycoformycin (**4**)

FIGURE 1

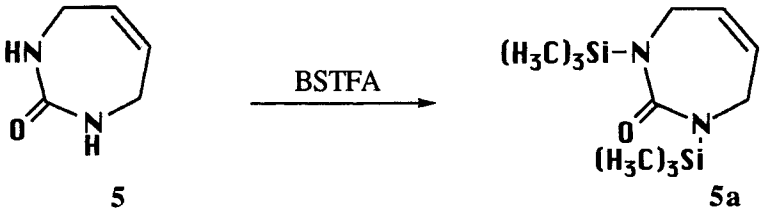
Moreover, Marquez et al. synthesized a series of ß-D-ribofuranosyl cyclic urea nucleosides and demonstrated that the compound with the seven-membered aglycon (**2**) was the more potent, with a Ki = 2.5 x 10^{-8} M on the human liver enzyme (FIGURE 1).[18,19] This behaviour is similar to that observed for the inhibition of ADA: in fact, coformycin (**3**) and 2'-deoxycoformycin (**4**) are very potent inhibitors of this enzyme with Ki = 10^{-11} M and Ki = 10^{-12} M, respectively.[20,21] In addition, we previously reported that the substitution of a ribose by a 2'-deoxyribose resulted in an increase in ADA inhibitory activity also in the 1-deazaadenosine series.[9]

On these bases, the synthesis of 2'-deoxy and 2',3'-dideoxy derivatives of compound **2** was undertaken in order to find new adjuvants in anticancer chemotherapy. Moreover, since these compounds are analogues of natural nucleosides, they will be evaluated with regard to their antitumor and antiviral potential.

CHEMISTRY

The synthesis of 2' deoxynucleosides **9** and **10** was carried out according to SCHEMES 1 and 2.

Activation of the base 1,3,4,7-tetrahydro-2H-1,3-diazepin-2-one (**5**)[19] was performed by reacting **5** with bis(trimethylsilyl)trifluoroacetamide (BSTFA) at room temperature for 2 h. The crude silylated derivative **5a**[19] was then added of freshly prepared 1-chloro-2-deoxy-3,5-di-O-*p*-toluoyl-α-D-*erythro*-pentofura



5 5a

BSTFA= $CF_3C[=NSi(CH_3)_3]OSi(CH_3)_3$

SCHEME 1

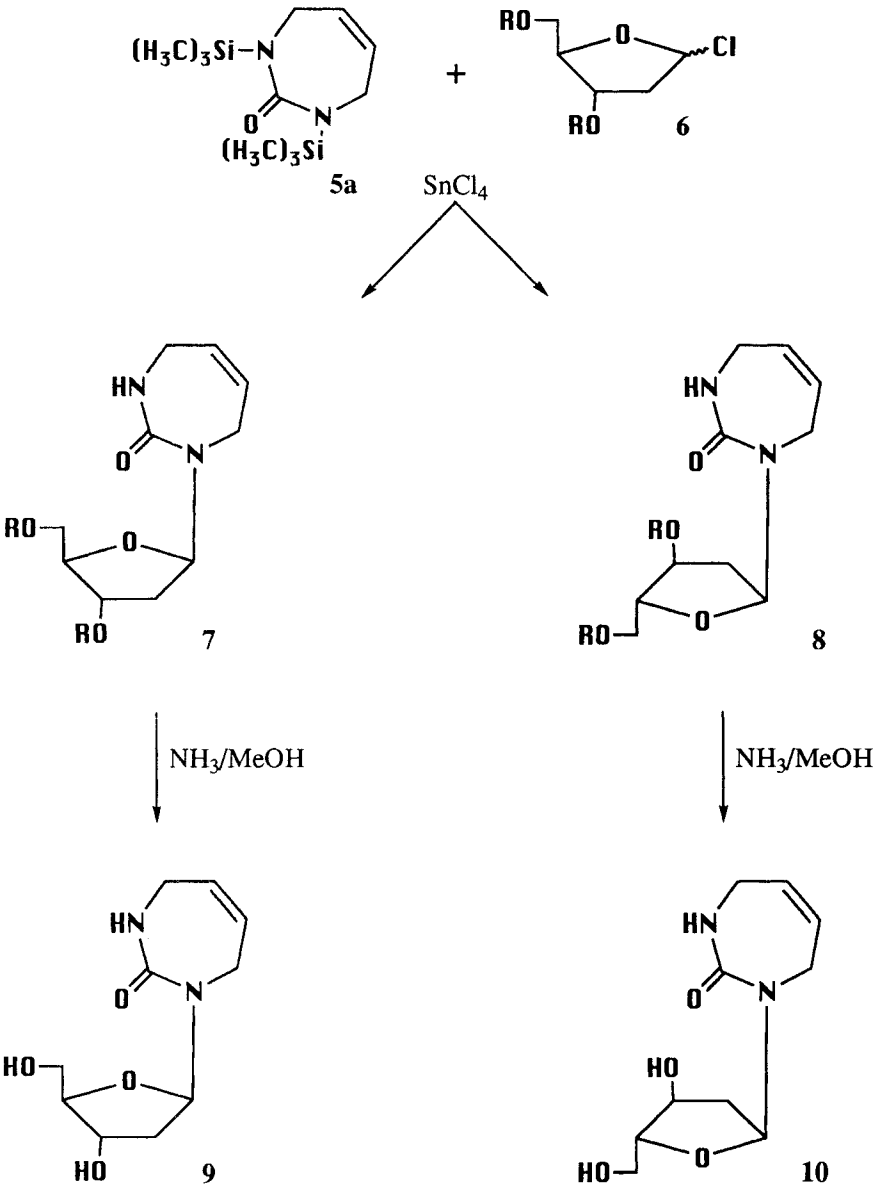
nose (**6**)[22] in dry acetonitrile in the presence of freshly distilled stannic chloride at room temperature for 24 h to give compounds **7** and **8** in 68% total yield. Deblocking of **7** and **8** with methanolic ammonia at room temperature afforded 1-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-1,3,4,7-tetrahydro-2H-1,3-diazepin-2-one (**9**) and its α anomer **10**, in 50% and 40% yield, respectively (SCHEME 2).

The anomeric configuration of compounds **7** and **8** was assigned by applying n.O.e. difference spectroscopy. Saturation of H-1' of **7** resulted in n.O.e.s of the H-2'a and H-4' signals (3.0 % and 2.0 %, respectively) while there was none at H-2'b signal, thus establishing a ß-D-configuration. Saturation of H-1' of **8** yielded n.O.e.s of the H-2'b signal (1.3 %) while there was none at H-4' signal, thus establishing an α-D-configuration (TABLE 1).

On these basis, compounds **9** and **10** were identified as ß and α anomers, respectively.

The condensation between **5a** and 1-methoxy-2,3-dideoxy-5-O-(4-methylbenzoyl)-D-*glycero*-pentofuranose (**11**)[23] in the presence of trimethylsilyl triflate (TMS-TF) in dry methylene chloride gave a derivative which was afterwards identified as N-[4,5-dihydroxy-5-O-*p*-toluoyl-1-(2-oxo-2,3,4,7-tetrahydro[1,3]diazepin-1-yl)-pentyl]-2,2,2-trifluoroacetamide (**12**). Deprotection of **12** with methanolic ammonia at room temperature for 24 h gave the corresponding N-[4,5-dihydroxy-1-(2-oxo-2,3,4,7-tetrahydro[1,3]diazepin-1-yl)-pentyl]-2,2,2-trifluoroacetamide (**13**). (SCHEME 3).

The structure of compounds **12** and **13** was elucidated by means of NMR techniques and chemical reactions. In fact the proton NMR spectrum of **12**, taken in DMSO-d_6, showed, in addition to the expected signals for the dideoxy



R= *p*-toluoyl

SCHEME 2

TABLE 1. N.O.E.-data % of compounds **7** and **8** upon irradiation of H-1' (DMSO-d_6, 25 °C, 300 MHz)

Cpd	H-2'a	H-2'b	H-4'
7	3.0	*	2.0
8	*	1.3	*

*: no detectable intensity enhancement (< 0.5%)

nucleoside, a triplet centered at δ = 5.08 ppm and a doublet at δ = 9.62 ppm, both of them exchangeable with D_2O.

Deblocking in the presence of K_2CO_3 in ethanol led to compound **13** whose ^{1}H-NMR spectrum presented now three exchangeable protons, two of them overlapped at δ = 4.95 ppm and the other again at δ = 9.62 ppm.

To confirm the presence of an additional hydroxyl group, compound **13** was treated with *p*-nitrobenzoylchloride in dry pyridine at room temperature for 3 hours. This reaction yielded two compounds identified as N-[4,5-dihydroxy-4,5-O-di-*p*-nitrobenzoyl-1-(2-oxo-2,3,4,7-tetrahydro[1,3]diazepin-1-yl)-pentyl]-2,2,2-trifluoroacetamide (**14**) and N-[4,5-dihydroxy-4,5-O-di-*p*-nitrobenzoyl-1-(3-*p*-nitrobenzoyl-2-oxo-2,3,4,7-tetrahydro[1,3]diazepin-1-yl)-pentyl]-2,2,2-trifluoro acetamide (**15**).

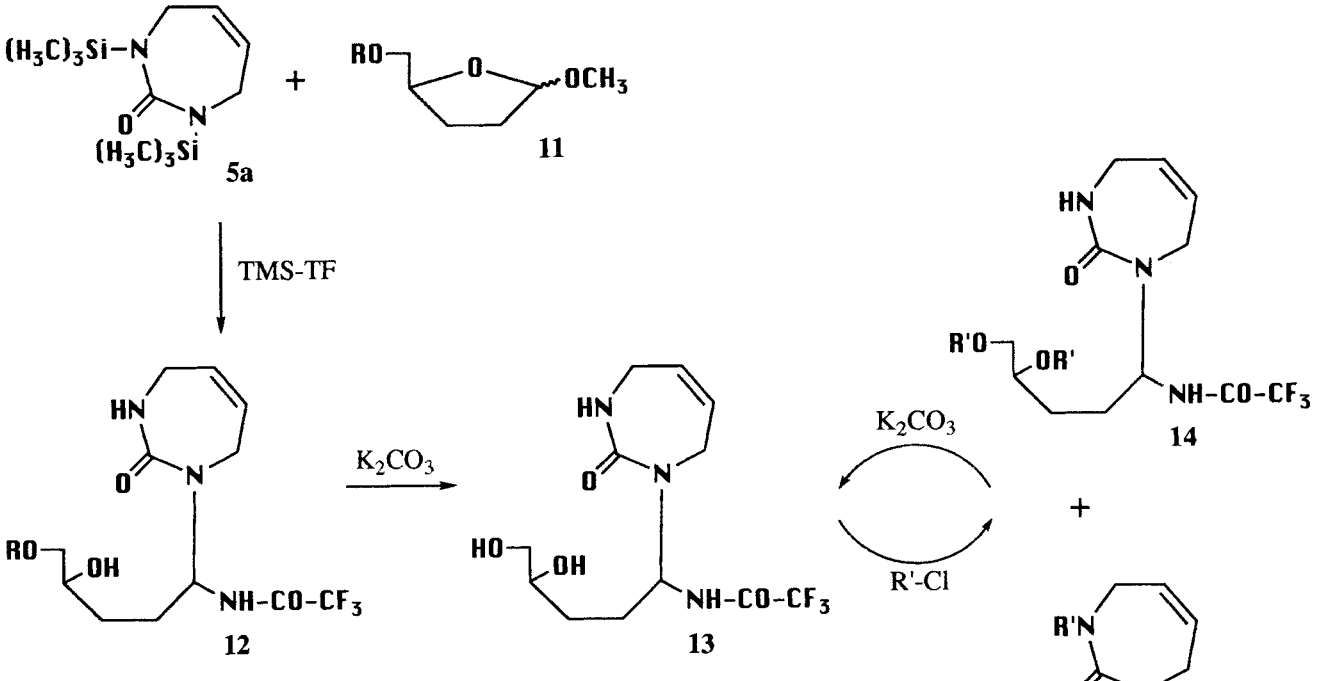
Treatment of both **14** and **15** with K_2CO_3 in ethanol led again to compound **13** (SCHEME 3). The ^{1}H-NMR of compound **14** clearly showed the presence of two overlapped *p*-nitrobenzoyl groups and the disappearance of the multiplet at δ = 4.95 ppm, in comparison with the starting material **13**.

Moreover, the multiplet assigned to $H_{4'}$ in the supposed nucleoside presented a strong downfield shift (from δ = 3.65 ppm to δ = 5.50 ppm).

From these data it was possible to conclude that the sugar was cleaved on the $C_{1'}$-O bond with formation of a 4,5-dihydroxypentyl chain. Accordingly, the two hydroxyl groups explained the presence of:

i) signals at δ = 5.08 and at δ = 4.95, both of them exchangeable with D_2O, in the ^{1}H-NMR spectra of compounds **12** and **13**, respectively;

ii) two *p*-nitrobenzoyl groups in compound **14**;



R= *p*-toluoyl
R'= *p*-nitrobenzoyl
TMS-TF= trimethylsilyl triflate

SCHEME 3

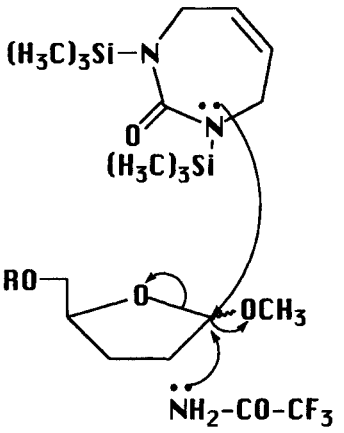


FIGURE 2

iii) the strong downfield shift of the multiplet assigned to $H_{4'}$ proton in the ^{1}H-NMR spectrum of compound **14**, due to benzoylation of the hydroxyl group directly bond to $C_{4'}$.

To clarify the structure of the substituent on $C_{1'}$, IR and ^{3}C-NMR spectra of compound **13** were investigated.

The IR spectrum of compound **13** showed the presence of two carbonyl groups with $\sqrt{}_{max}$ 1603 and 1735, respectively, and one of them was assigned to the ureic CO of the diazepinone. These carbonyl carbons were identified also by ^{13}C-NMR spectrum of the same compound: a singlet at δ = 164.3 ppm was again assigned to the ureic CO whereas a quartet at δ = 156.17 ppm with J = 36.0 Hz was afterwards assigned to CO of the trifluoroacetamidic group.

Since prior to the coupling reaction, 1,3,4,7-tetrahydro-2H-1,3-diazepin-2-one (**5**) was activated using bis(trimethylsilyl)trifluoroacetamide (BSTFA) as silylating agent to give **5a** (SCHEME 1),[19] it was postulated that the C_1 carbon underwent nucleophilic attack both from the silylated base **5a** and from a trifluoroacetamido group arising from BSTFA (FIGURE 2).

^{19}F-NMR spectrum of compound **13** confirmed the presence of a fluorine signal at δ = 279.63 ppm.

Moreover a trifluoroacetamido group on C_1 in compound **13** could explain the presence of:

i) an exchangeable doublet at δ = 9.57 ppm corresponding to the amidic proton in the ^{1}H-NMR spectrum;

TABLE 2. CDA inhibitory activity of tetrahydrodiazepinone derivatives.

Compound	Ki (μM), CDA (human placenta)
1	1.66
2	0.075
9	0.025
10	0.075
13	inactive

Cytidine deaminase activity was measured by the decrease in absorbance at 282 nm with cytidine as substrate (See experimental section).

ii) a signal at δ = 156.17 (quartet, J = 36.0 Hz, long range coupling with the three fluorine atoms) corresponding to the carbon of the amidic carbonyl group in the ^{13}C-NMR spectrum;

iii) a signal at δ = 116.19 ppm (quartet, J = 288.7 Hz) corresponding to the carbon of the trifluoromethyl group in the ^{13}C-NMR spectrum.

BIOLOGICAL EVALUATION

The synthesized nucleoside analogs were evaluated as inhibitors of cytidine deaminase purified from human placenta, following the experimental procedure described earlier,[15] and the results are reported in TABLE 2.

As already reported by Marquez *et al.* in studies on mouse kidney and human liver CDA,[19] compound **2** is a potent inhibitor of the human placenta enzyme (Ki = 7.5 x 10^{-8} M).

Substitution of ribose by a 2-deoxyribose moiety led to compound **9** that appeared slightly more potent than **2**. This finding confirms our rationale based on the results obtained in the 1-deazaadenosine series[9] and on the higher activity of 2'-deoxycoformycin (**4**) in comparison with that of coformycin (**3**).[20,21]

Surprisingly, the corresponding α-2'-deoxynucleoside **10** acted as a very potent inhibitor of human placenta CDA, with a Ki similar to that of the ß-ribofuranosyl derivative **2**. This finding is not in agreement with previous hypotheses that the α-anomers should not present any relevant inhibitory activity on cytidine deaminase.[18] Moreover, since the reaction between **5a** and tetraacetyl ribose in our hand gave only the ß-isomer **2**, as already described by

Marquez *et al*,[19] we could not test the α-ribofuranosyl derivative of **2**, in order to confirm the result obtained with the α-2'-deoxynucleoside derivative **10**.

The opening of the sugar in the condensation of **5a** with the 2,3-dideoxyribose **11** led to the N-[4,5-dihydroxy-1-(2-oxo-2,3,4,7-tetrahydro [1,3]diazepin-1-yl)-pentyl]-2,2,2-trifluoroacetamide (**13**), that was inactive as CDA inhibitor, in agreement with the very low activity showed by some previously reported N-alkyl and N-aryl derivatives of cytosine and uracil.[15,25,26]

On the basis that coformycin (**3**) and deoxycoformycin (**4**), which are very potent inhibitors of adenosine deaminase (ADA), present a seven-membered ring in their chemical structure, the tetrahydrodiazepinone derivatives **2**, **9**, **10**, and **13** were also evaluated as inhibitors of ADA from calf intestine and found inactive.

EXPERIMENTAL SECTION

Chemistry.

Melting points were determined with a Buchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Varian VXR 300 MHz spectrometer. IR spectra were recorded on a Perkin-Elmer Model 297 spectrophotometer. TLC were carried out on pre-coated TLC plates with silica gel 60 F-254 (Merck). For column chromatography, silica gel 60 (Merck) was used. Elemental analyses were determined on a Carlo Erba model 1106 analyser.

1,3-Di(trimethylsilyl)-1,3,4,7-tetrahydro-2*H*-1,3-diazepin-2-one (5a).

To 0.3 g (2.7 mmol) of **5** in 30 ml of dry acetonitrile were added 2.7 ml of bis(trimethylsilyl)trifluoroacetamide (BSTFA) and the mixture was stirred at room temperature for 2 hours. After removing the solvent under *vacuum*, compound **5a** was obtained as a light yellow oil which was dissolved in 5 ml of dry methylene chloride and used for the coupling reactions without further purification.

1-(2-Deoxy-3,5-di-O-*p*-toluoyl-ß-D-*erythro*-pentofuranosyl)-1,3,4,7-tetrahydro-2*H*-1,3-diazepin-2-one (7)
1-(2-Deoxy-3,5-di-O-*p*-toluoyl-α-D-*erythro*-pentofuranosyl)-1,3,4,7-tetrahydro-2*H*-1,3-diazepin-2-one (8).

To a ice-cooled solution of **5a**[19] in 5 ml of dry methylene chloride were added 1.14 g (2.93 mmol) of freshly prepared 1-chloro-2-deoxy-3,5-di-O-*p*-toluoyl-D-

erythro-pentofuranose (**6**)[22] and 0.5 ml of $SnCl_4$. The suspension was stirred at room temperature for 36 hours, then the solvent was removed, the residue neutralized with a saturated sodium bicarbonate solution and extracted with chloroform. The organic layer was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was chromatographed on silica gel column eluting with $CHCl_3$-MeOH (99-1) to give 2.9 g (68%) of a mixture of ß and α anomers **7** and **8**. Separation of anomers was achieved by flash chromatography: elution with cC_6H_{12}-$CHCl_3$ (80:20) gave 0.34 g (27%) of **7** and 0.24 g (19%) of **8** as vetrous chromatographically pure solids.

7: ^{1}H NMR (Me_2SO-d_6) δ 2.26 (m, 2H, H-2'a and H-2'b), 2.41 (s, 6H, CH_3), 3.62 (m, 4H, CH_2-4 and CH_2-7), 4.28 (m, 1H, H-4'), 4.52 (m, 2H, CH_2-5',), 5.48 (m, 1H, H-3'), 5.69 (m, 2H, C*H*=C*H*), 5.96 (m, 1H, H-1'), 6.27 (m, 1H, NH), 7.37 (d, 4H, J = 8.3 Hz, H-arom), 7.92 (d, 4H, J = 8.3 Hz, H-arom). Anal. Calcd. for $C_{26}H_{28}N_2O_6$: C, 67.23; H, 6.08; N, 6.03. Found: C, 67.52; H, 6.21; N, 5.89.

8: ^{1}H NMR (Me_2SO-d_6) δ 2.04 (m, 1H, H-2'b), 2.40 (s, 6H, CH_3), 2.79 (m, 1H, H-2'a), 3.58 (m, 2H, CH_2-4), 3.82 (m, 2H, CH_2-7), 4.41 (m, 2H, CH_2-5',), 4.60 (m, 1H, H-4'), 5.47 (m, 1H, H-3'), 5.78 (m, 2H, C*H*=C*H*), 5.97 (m, 1H, H-1'), 6.25 (m, 1H, NH), 7.37 (m, 4H, H-arom), 7.90 (d, 4H, J = 7.3 Hz, H-arom). Anal. $C_{26}H_{28}N_2O_6$: C, 67.23; H, 6.08; N, 6.03. Found: C, 67.49; H, 6.22; N, 5.87.

1-(2-Deoxy-ß-D-*erythro*-pentofuranosyl)-1,3,4,7-tetrahydro-2*H*-1,3-diazepin-2-one (9).

To 0.3 g (0.65 mmol) of **7** was added 80 mL of methanol saturated at 0 °C with ammonia and the mixture was set aside at room temperature for 24 h. The reaction mixture was evaporated and the residue was flash chromatographed over silica gel eluting with $CHCl_3$-MeOH (from 97:3 to 95:5) to give 0.074 g (50%) of **9** as a chromatographically pure solid.

^{1}H NMR (Me_2SO-d_6) δ 1.73 (m, 1H, H-2'b), 1.89 (m, 1H, H-2'a), 3.45 (m, 2H, CH_2-5',), 3.57 (m, 3H, CH_2-4 and H-4'), 3.64 (m, 2H, CH_2-7), 4.08 (m, 1H, H-3'), 5.79 (m, 3H, H-1' and C*H*=C*H*), 6.05 (m, 1H, NH). ^{13}C NMR (Me_2SO-d_6) δ 37.16 (C-2'), 40.61 (C-4 or C-7), 42.26 (C-7 or C-4), 62.26 (C-5'), 70.89 (C-3'), 85.83 (C-1' and C-4'), 126.92 (C-5 or C-6), 127.84 (C-6 or C-5), 164.63 (C-2). Anal. $C_{10}H_{16}N_2O_4$: C, 52.62; H, 7.07; N, 12.27. Found: C, 52.88; H, 7.21; N, 12.02.

1-(2-Deoxy-α-D-*erythro*-pentofuranosyl)-1,3,4,7-tetrahydro-2*H*-1,3-diazepin-2-one (10).

To 0.2 g (0.43 mmol) of **8** was added 50 mL of methanol saturated at 0 °C with ammonia and the mixture was set aside at room temperature for 24 h. The

reaction mixture was evaporated and the residue was chromatographed over silica gel preparative TLC eluting with $CHCl_3$-MeOH (90:10) to give 0.050 g (40%) of **10** as a chromatographically pure solid.
^1H NMR (Me_2SO-d_6) δ 1.63 (m, 1H, H-2'b), 2.32 (m, 1H, H-2'a), 3.34 (m, 2H, CH_2-5'), 3.57 (m, 2H, CH_2-4), 3.79 (m, 3H, CH_2-7 and H-4'), 4.08 (m, 1H, H-3'), 5.76 (m, 3H, H-1' and C*H*=C*H*), 6.02 (m, 1H, NH). Anal. $C_{10}H_{16}N_2O_4$: C, 52.62; H, 7.07; N, 12.27. Found: C, 52.82; H, 7.25; N, 12.01.

N-[4,5-dihydroxy-5-O-*p*-toluoyl-1-(2-oxo-2,3,4,7-tetrahydro[1,3]diazepin-1-yl)-pentyl]-2,2,2-trifluoroacetamide (12).
To a solution of **5a** in 5 ml of dry methylene chloride was added 0.74 g (2.96 mmol) of 1-methoxy-2,3-dideoxy-5-O-*p*-toluoyl-D-*glycero*-pentofuranose (**11**)[23] and 0.5 ml of TMS-triflate and the mixture was allowed to stand at room temperature for 24 hours. The solvent was evaporated to dryness and the residue neutralized with a saturated sodium bicarbonate solution and extracted with chloroform. The organic layer was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was flash chromatographed on silica gel column eluting with $CHCl_3$-MeOH (from 99.75:0.25 to 98:2) to give 0.54 g (45%) of **12** as a chromatographically pure solid.
12: ^1H NMR (Me_2SO-d_6) δ 1.47 (m, 2H, CH_2-3), 1.86 (m, 2H, CH_2-2), 2.40 (s, 3H, CH_3), 3.52 (m, 2H, CH_2-4'), 3.65 (m, 1H, H-4), 3.79 (m, 2H, CH_2-7'), 4.14 (m, 2H, CH_2-5), 5.08 (t, 1H, OH-4), 5.69 (m, 3H, C*H*=C*H* and H-1), 5.94 (m, 1H, NH-3'), 7.35 (d, 2H, J = 8.1 Hz, H-arom), 7.91 (d, 2H, J = 8.1 Hz, H-arom), 9.62 (d, 1H, J = 8.1 Hz, CHN*H*). Anal. $C_{20}H_{24}F_3N_3O_5$: C, 54.17; H, 5.46; N, 9.48. Found: C, 54.41; H, 5.67; N, 9.12.

N-[4,5-dihydroxy-1-(2-oxo-2,3,4,7-tetrahydro[1,3]diazepin-1-yl)-pentyl]-2,2,2-trifluoroacetamide (13).
To a solution of 0.5 g (1.13 mmol) of **12** in 10 ml of ethanol was added 0.6 g (4.35 mmol) of a suspension of K_2CO_3 in boiling ethanol and the reaction mixture was stirred at room temperature for 1 hour. The insoluble K_2CO_3 was filtered and the filtrate was evaporated to dryness. The residue was flash chromatographed on silica gel column eluting with $CHCl_3$-MeOH (from 98:2 to 95:5) to yield 0.13 g (35%) of **13** as a chromatographically pure solid.
IR $\sqrt{}_{max}$ 1603 (C=O), 1735 (C=O); ^1H NMR (Me_2SO-d_6) δ 1.34 (m, 2H, CH_2-3), 1.79 (m, 2H, CH_2-2), 3.14-3.90 (m, 7H, CH_2-5, CH_2-4', CH_2-7', H-4), 4.95 (m, 2H, OH-4 and OH-5), 5.65 (m, 1H, H-1), 5.73 (m, 2H, C*H*=C*H*), 5.93 (m, 1H, NH-3'),

9.57 (d, 1H, J = 8.1 Hz, CHN*H*); ^{13}C NMR (Me_2SO-d_6) δ 27.69 and 27.74 (C-3), 29.80 (C-2), 41.01 and 41.12 (C-7' or C-4'), 42.51 and 42.53 (C-4' or C-7'), 63.06 and 63.21 (C-5 or C-4), 66.18 and 66.29 (C-4 or C-5), 71.02 and 71.19 (C-1), 116.19 (q, J = 288.7 Hz, CF_3), 126.38 (C-5' or C-6'), 128.32 (C-6' or C-5'), 156.17 (q, J = 36.0 Hz, C=O), 164.27 and 164.3 (C-2'); ^{19}F NMR (Me_2SO-d_6) δ 279.63. Anal. $C_{12}H_{18}F_3N_3O_4$: C, 44.31; H, 5.58; N, 12.92. Found: C, 44.65; H, 5.71; N, 12.69.

N-[4,5-dihydroxy-4,5-O-di-*p*-nitrobenzoyl-1-(2-oxo-2,3,4,7-tetrahydro[1,3] diazepin-1-yl)-pentyl]-2,2,2-trifluoroacetamide (14)
N-[4,5-dihydroxy-4,5-O-di-*p*-nitrobenzoyl-1-(3-*p*-nitrobenzoyl-2-oxo-2,3,4,7-tetrahydro[1,3]diazepin-1-yl)-pentyl]-2,2,2-trifluoroacetamide (15).
To an ice-cooled solution of 0.25 g (0.77 mmol) of **13** in 5 ml of dry pyridine was added 0.43 g (2.3 mmol) di *p*-nitrobenzoyl chloride, and the mixture was allowed to stand at room temperature for 2 hours. The solvent was removed by distillation under vacuum, and the residue was purified on a silica gel column. Elution with cC_6H_{12}-AcOEt-C_6H_6 (60:30:10) gave 0.15 g (36%) of **14** and 0.05 g (10%) of **15** as chromatographically pure solids.
14: ^{1}H NMR (Me_2SO-d_6) δ 1.87 (m, 4H, CH_2-3 and CH_2-2), 3.50 (m, 2H, CH_2-4'), 3.70 (m, 2H, CH_2-7'), 4.63 (m, 2H, CH_2-5), 5.50 (m, 1H, H-4), 5.70 (m, 3H, C*H*=C*H* and H-1), 6.0 (m, 1H, NH-3'), 8.17 (m, 4H, H-arom), 8.37 (m, 4H, H-arom), 9.63 (dd, 1H, CHN*H*). Anal. $C_{26}H_{24}F_3N_5O_{10}$: C, 50.09; H, 3.88; N, 11.23. Found: C, 50.22; H, 4.06; N, 11.02.
15: ^{1}H NMR (Me_2SO-d_6) δ 1.74 (m, 2H, CH_2-3), 1.90 (m, 2H, CH_2-2), 3.81 (m, 2H, CH_2-7'), 4.39 (m, 2H, CH_2-4'), 4.71 (m, 2H, CH_2-5), 5.47 (m, 1H, H-4), 5.68 (m, 1H, H-1), 5.80 (m, 2H, C*H*=C*H*), 7.80 (d, 2H, H-arom), 8.10 (d, 2H, H-arom), 8.16 (m, 4H, H-arom), 8.33 (m, 4H, H-arom), 9.96 (m, 1H, CHN*H*). Anal. $C_{32}H_{27}F_3N_6O_{13}$: C, 50.53; H, 3.58; N, 11.5. Found: C, 50.74; H, 3.69; N, 10.87.

Enzyme assays

Cytidine deaminase activity was determined by a direct spectrophotometric assay based on the decrease of absorbance at 282 nm, when the substrates (cytidine and deoxycytidine) are deaminated. The enzyme was obtained from human placenta following a purification methods already reported by Vita *et al.*[15] In the inhibition experiments, cytidine was used as substrate and the reaction was followed at 282 nm using a UVIKON 940 (Kontron Instruments) spectrophotometer. The standard recation mixture consisted of 0.167 mM

cytidine and 100 mM Tris-HCl, pH 7.5, in a final volume of 1.0 ml. The reaction was initiated by the addition of 0.02-0.04 enzyme units. One enzyme unit is defined as the amount of enzyme which catalyzes the deamination of 1μmol of cytidine pre minute at 37 °C.

The method used for the determination of activity against adenosine deaminase has been described by Lupidi *et al.* in a preceding paper.[7]

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