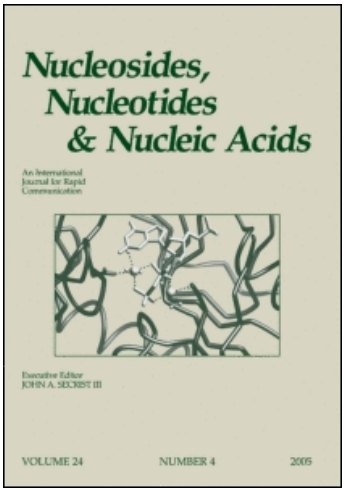

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Synthesis of 5-Methyl-2'-*O*-Deoxycytidine Analogs to Determine Monoclonal Antibody Specificity in the Recognition of the 6-(*p*-Bromobenzoylamino) Caproyl Radical

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SYNTHESIS OF 5-METHYL-2′-*O*-DEOXYCYTIDINE ANALOGS TO DETERMINE MONOCLONAL ANTIBODY SPECIFICITY IN THE RECOGNITION OF THE 6-(*p*-BROMOBENZOYLAMINO) CAPROYL RADICAL.

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Abstract: Eight new *p*-bromobenzoyl derivatives of 5-methyl-2'-*O*-deoxycytidine analogs, substituted at the 4-position, were synthesized. The best conditions for obtaining 5-methyl-4-*N*-aminoalkyl-2′-*O*-deoxycytidine from 3′,5′-di-*O*-acetyl-4-(1,2,4-triazol-1-yl)-2′-*O*-deoxythymidine were studied. The nucleoside analogs were used to identify the fragment of the 6-(*p*-bromobenzoylamino)caproyl radical that binds to the monoclonal antibody obtained against it and to define an affinity scale of monoclonal antibody against them.

In recent years, DNA detection by means of identifying non-radioactive labels has been documented[1-23]. Markers, such as biotin[1-6], 2,4-dinitrophenyl group[6-9], *N*-2-acetylaminofluorene and *N*-2-acetylamino-7-iodofluorene[1, 10-13], 5-bromodeoxyuridine[14-17], biname[18, 19], and digoxigenin[20-23], were linked to nucleic acid precursors and subsequently introduced by classical labeling techniques. In a previous work we reported the obtention of a monoclonal antibody (MAb) against the 6-(*p*-bromo benzoylamino)caproyl radical (BLC)[24]. This marker was introduced at the 4-position of cytidine through a spacer arm of 5-methyl-2'-*O*-deoxycytidine analogs. Several works[21, 25-29] have discussed the effect of spacer arm length on DNA detection level.

The procedure for synthesizing and the characterization of eight new 5-methyl-2'-*O*-deoxycytidine analogs is herein reported. These analogs were used to identify the fragment of the BLC that binds to the MAb and to define an affinity scale of them against the MAb.

RESULTS AND DISCUSSION

Chemical Discussion:

Introduction of the label at the 4-position of thymidine was accomplished by five steps, three of which are carried out one pot (*Scheme 1*).

3'-5'-Di'*O*-acetylthymidine (**4**), and 3'-,5-di-*O*-acetyl-4-(1,2,4-triazol-1-yl)-2'-*O*-deoxythymidine (**5**) were synthesized according to reported procedures[30, 31].

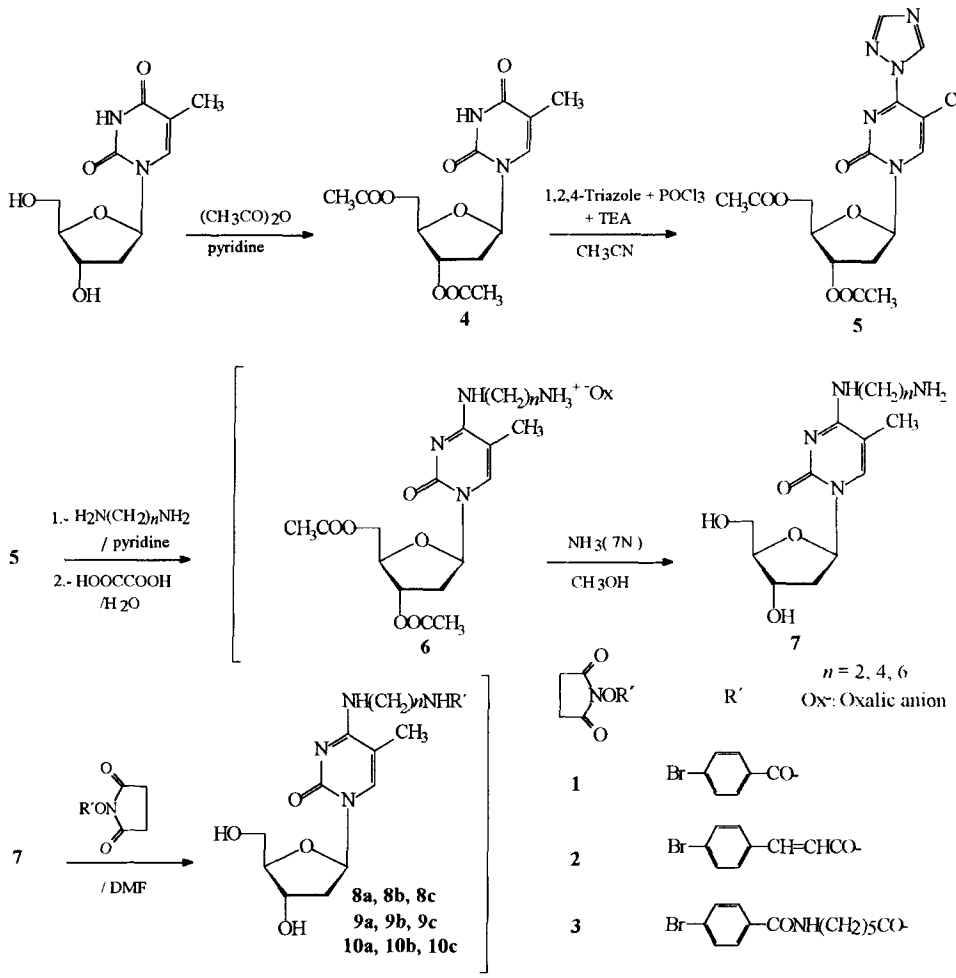
The new analogs of 5-methyl-2'-*O*-deoxycytidine (*Figure 1*) are clustered in three series of compounds: 5-methyl-4-*N*-[n-(*p*-bromobenzoylamino)alk-1-yl]-2′-*O*-deoxycytidine (*series 1*); 5-methyl-4-*N*-[n-(*p*-bromocinnamoylamino)alk-1-yl]-2′-*O*-deoxycytidine (*series 2*) and 5-methyl-4-*N*-{n-[-ε-(*p*-bromobenzoylamino)caproylamino] alk-1-yl}-2′-*O*-deoxycytidine, (*series 3*).

Synthesis of 5-methyl-4-N-aminoalkyl-2'-O-deoxycytidine. 4-*N*-Aminoalkyl cytidine has been a valuable intermediate to introduce different labels in pyrimidine bases[5, 8, 26, 29, 32-40]. There are three general procedures for obtaining it: by catalytic transamination of cytidine with sodium bisulfite and different amines[5, 8, 29, 32, 33, 39], by nucleophilic substitution of the sulphur atom on 4-thiouridine or 4-thiothymidine analogs [8, 34] and by nucleophilic substitution of the 1,2,4-triazole group of 4-triazole derivatives of pyrimidine bases of nucleosides[26, 35, 41]. The last procedure was used to obtain 5-methyl-4-*N*-aminoalkyl-2'-*O*-deoxycytidine.

In order to determine the best method for synthesizing this intermediate different conditions for nucleophilic substitution of the triazole group have been tried (*Table 1*). All experiments were accomplished with 3′-*O*-benzoyl-5′-*O*-trityl-4-(1,2,4-triazol-1-yl)thymidine (**12**) to avoid the deacylation side reaction.

1,2-Di-[4*N*-(5-methyl-3′-*O*-benzoyl-5′-*O*-trityl-2′-*O*-deoxycytidyl)]ethane is always obtained alone when the order of addition is amine to nucleoside, regardless of the amount of diamine added (*Table 1*). This compound was characterized by FAB-MS (m/z) = 1 101 $(M + H)^+$. Reversal of the order of addition led to the desired product, 5-methyl-3′-*O*-benzoyl-5′-*O*-trityl-4-*N*-(aminoethyl)-2′-*O*-deoxycytidine (**13**). When the reaction was carried out in acetone, instead of acetonitrile, the rate of reaction decreases significantly. This fact suggests that the slow step in the reaction involves the formation of a polar intermediate (**I**, *Scheme 2*). Only when an excess of diamine was added the rate is increased and the reaction is over. When the reaction is carried out in pyridine, it concludes immediately, although this solvent is less polar than acetonitrile. These results suggest that a proton transfer may be involved in the slow step (*Scheme 2*).

Different 5-methyl-4-*N*-aminoalkyl-2'-*O*-deoxycytidines (**7a, 7b, 7c**) were obtained from **5** through substitution of the 1, 2, 4-triazole group by 1, 2-diaminoethane, 1, 4-diaminobutane or 1,6-diaminohexane, according to the described procedure[24].



SCHEME 1

Label introduction at the 4-position of 5-methyl-4-N-aminoalkyl-2'-O-deoxycytidine intermediates. The reaction was accomplished with the acylating agent (**1, 2 or 3**) and **7(a, b or c)** according to the reported procedure[24]. Different derivatives of the esters of *N*-hydroxysucciminide (*p*-bromobenzoic acid *N*-hydroxysuccinimide ester[24](**1**), *p*-bromo cinnamoic acid *N*-hydroxysuccinimide ester (**2**) and 6-(*p*-bromobenzoylamino)caproic acid *N*-hydroxysuccinimide ester[24](**3**)) were used. They are selective to amine groups and thus make the protection of the hydroxylic group unnecessary. The overall yield of substitution, deprotection and acylation was 43%. All synthesized compounds were fully characterized (IR; NMR; FAB-MS and quantitative analysis)

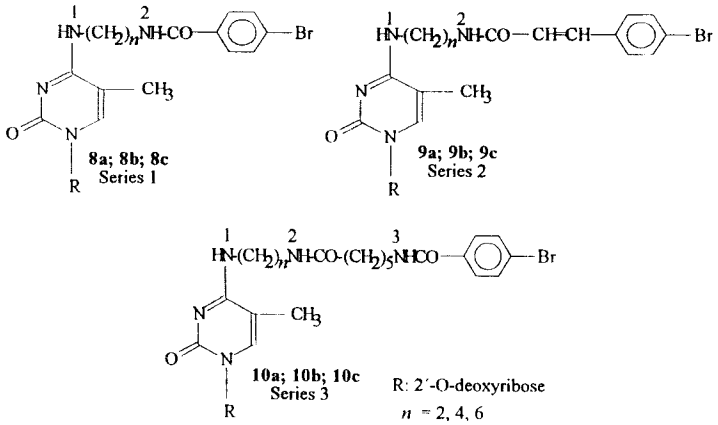
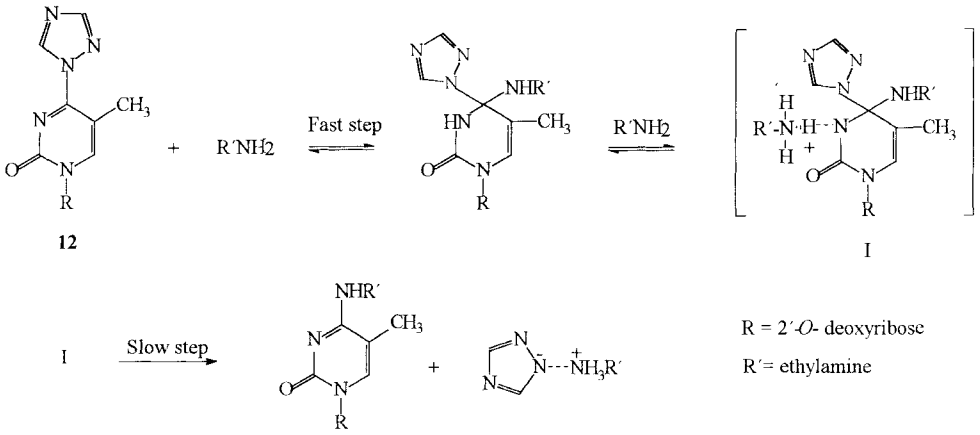


FIG. 1.- General structures of the obtained compounds.

TABLE 1.- Reaction conditions assayed for obtaining 5-methyl-3′-*O*-benzoyl-5′-*O*-trityl-4-*N*-(aminoethyl)-2′-*O*-deoxycytidine (**13**) from compound **12** with 1,2-diaminoethane at room temperature.

N°	Molar relation A:N[a]	Addition order	Solvent	Conversion[b] (%)	Time of reaction (h)	Product
1	1:1	A to N[c]	acetonitrile	100	0,25	*d*
2	2:1	"	"	100	*e*	*d*
3	2:1	N to A[f]	"	100	0,5	**13**
4	4:1	"	"	100	*e*	**13**
5	2:1	"	pyridine	100	*e*	**13**
6	2:1	"	acetone	< 50	6,0	**13**
7	10:1	"	acetone	100	0,5	**13**

[a] A:N: amine-nucleoside ratio. [b] Qualitatively determinated by TLC. [c] Amine to nucleoside. [d] The obtained product was 1,2-di-[4*N*-(5-methyl-3′-*O*-benzoyl-5′-*O*-trityl-2′-*O*-deoxycytidyl)]ethane.[e] Instantaneous reaction. [f] Nucleoside to amine.



SCHEME 2

Immunochemical Discussion:

Competitive Enzyme Linked Immunosorbent Analyses (ELISAs) were performed. The analogs (**8a, 8b, 8c, 9a, 9b**[24], **9c, 10a, 10b, 10c**) and 4-*N*-[6-(*p*-bromobenzoylamino)caproylamino]cytidine[24] (**14**) were used as direct competitors for the MAb binding site (clone 5H11E5). The haptenic group (4-*N*-[6-(*p*-bromobenzoylamino)caproylamino]radical[24]) conjugated to BSA (Bovine Serum Albumin) was immobilized on microtiter PVC plates. Crossreactivity of each compound in relation to **14** was determined by slightly modifying the 10% error method [42, 43]. An immunoaffinity scale was established from the ratios obtained at $2x10^{-8}$ mol/L competitor concentrations[43]. This value lies within the linear response concentration range and consequently can be used for this purpose. Low, medium and high crossreactivities were defined as percents (ratios x 100) < 30 , 30-60, and > 60, respectively (*Table 2*).

From these results, high crossreactivities were obtained for compounds **8b**, **10c**, **8c** and **10a**; medium for **9b** and **10b**; and low for the remaining[43] (*Table 2*). Then, a relative affinity scale **8b > 14 = 10c > 8c > 10a > 9b > 10b > 8a > 9a > 9c** is proposed.

The *p*-bromocinnammoyl radical (**9a** and **9c**) was not recognized by the MAb, except in the compound **9b**, which had a medium value of crossreactivity. Conversely, compounds having *p*-bromobenzoyl radical were well recognized and displayed high crossreactivity values (**8b**, **10c**, **8c**, **10a** and **14**), suggesting that the MAb is quite specific for this group. However, compound **8a** was poorly recognized by the MAb, despite the presence of this radical. This result can be attributed to the short length of the methylenic

TABLE 2.- Crossreactivity values (CR, %) of compounds **8a, 8b, 8c, 9a, 9b**[24], **9c, 10a, 10b, 10c** in relation to **14**, obtained at $2x10^{-8}$ mol/L competitor concentrations.

Compounds		Series 1			Series 2			Series 3		
	14	**8a**	**8b**	**8c**	**9a**	**9b**	**9c**	**10a**	**10b**	**10c**
CR (%)	100	13	113	83	10	37	< 1	61	35	101

chain, which might reduce the hydrophobic interactions between the MAb and the compound.

EXPERIMENTAL

Chemical Procedures:

Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra (δ, ppm) were recorded at 250 MHz on a Brüker Model AC 250F spectrometer (TMS as internal reference). The assignment for ^{1}H NMR (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and AA'BB', *p*-substituted aromatic system) was based also on COSY experiments. The labeling used to describe the kinds of carbon in ^{13}C NMR (DEPT experiments) is: p, primary; s, secondary; t, tertiary and q, quaternary. Fast-atom-bombardment mass spectra (FAB-MS) were recorded in the positive-ion mode on a JEOL spectrometer model JMS-HX-110. IR spectra were recorded in KBr tablets on a Carl Zeiss SPECORD 71 IR spectrophotometer (Keys: s, strong; m, medium and w, weak). Melting points (uncorrected) were taken on an Electrothermal apparatus. Elemental analysis were performed on a Carlo Erba Model EA-1108 analyzer. Pyridine, dimethylformamide (DMF) and acetonitrile were dried and stored according to the described procedure[24]. 1,6 Diaminohexane, 1,4-diaminobutane, 1,2-diaminoethane and triethylamine (TEA) were dried over KOH, distilled and stored over KOH. All reagents were obtained from commercial suppliers. Thin layer chromatography (TLC) was performed on precoated aluminum sheets of silica gel 60 F_{254} (*E. Merck*).

***p*-Bromobenzoic acid *N*-hydroxysuccinimide ester (1)**: was reported elsewhere[24].

***p*-Bromocinnamoic acid *N*-hydroxysuccinimide ester (2)**: This procedure was similar to that used for synthesizing **1** and **3**[24]. Yield: 94 %, m.p.: 230-34°C. IR: 3400s, 3320s; 2980s, 2850s; 1620s; 1580s; 1440w; 1320m; 1240m; 1090m; 1040w; 900w; 640m. Anal. calcd. for $C_{13}H_{10}N_1O_4Br$: C: 48.48; H: 2.48; N: 4.34. Found: C: 48.45; H: 2.41; N: 4.38.

6-(*p*-Bromobenzoylamino)caproic acid *N*-hydroxysuccinimide ester (3): was reported elsewhere[24].

5-Methyl-4-*N*-[n-(aminoalkyl)]-2′*O*-deoxycytidine (7a, 7b, 7c). General Procedure: this procedure was similar to that used for synthesizing 5-methyl-4-*N*-(4-aminobutyl)-2′-*O*-deoxycytidine[24].

Acylation of 5-methyl-4-*N*-[n-(aminoalkyl)]-2′*O*-deoxycytidine. General Procedure: this procedure was similar to that used for synthesizing 5-methyl-4-*N*-{4-[ε-(*p*-bromobenzoylamino)caproylamino]-but-1-yl}-2′-*O*-deoxycytidine[24].

5-Methyl-4-*N*-(-*p*-bromobenzoylaminoethyl)-2′-*O*-deoxycytidine (8a): Yield: 67 %, m.p.: 118-20 °C. IR: 3350s, 3050m, 2950m, 1660s, 1620s, 1560s, 1510s; 1440m; 1360m; 1300m; 1100m; 1020m; 820w; 790w; 780m. ^{1}H-NMR (DMSO-d_6): 8.8 (1H, t, 2-N*H*); 7.83-7.64 (4H, AA'BB', *p*-BrBz); 7.6 (1H, s, *6*-C*H*); 7.3 (1H, t, 1-N*H*); 6.2 (1H, t, *1′*-C*H*); 5.2 (1H, d, *3′*-O*H*); 5.0 (1H, t, *5′*-O*H*); 4.2 (1H, s, *3′*-C*H*); 3.7 (1H, s, 4′-C*H*); 3.55 (2H, s, 5′-CH_2); 3.5-3.3 (4H, m, 2 CH_2NH); 2.2-1.9 (2H, m, *2′*-CH_2); 1.8 (3H, s, CH_3). ^{13}C-NMR (DMSO-d_6): 165.44 (q, *4-C*); 162.92 (q, *p*-BrBz-*C*O); 154.84 (q, *2-C*O); 137.32 (t, *6-C*H); 133.30 (q, *C*arom-CO); 131.14 and 129.26 (2 t, *C*Harom); 124.75 (q, *C*arom-Br); 101.64 (q, *5-C*); 86.97 (t, *4′-C*H); 84.48 (t, *1′-C*H); 70.30 (t, *3′-C*H); 61.25 (s, *5′-C*H_2); 40.21 (3 s, 2 CH_2NH + *2′-C*H_2); 12.84 (p, *C*H_3). FAB-MS *(m/z)* = 463 $(M+H)^+$. Anal. calcd. for $C_{27}H_{38}N_5O_6Br$: C: 48.29; H: 4.97; N: 12.12.- Found: C: 48.25; H: 4.99; N: 12.05.

5 - Methyl - 4 - *N* - [- 4 - (*p*-bromobenzoylamino) but -1- yl]-2′- *O*- deoxycytidine (8b)*:* Yield: 47 %, m.p.: 128-30 °C IR: 3400s, 3100s, 2950s, 2900s, 1640s, 1560m; 1520m; 1470m; 1360m; 1320m; 1100w, 1060w; 1020w; 860w; 760w. ^{1}H-NMR (DMSO-d_6): 8.55 (1H, t, 2-N*H*); 7.8-7.6 (4H, AA'BB', *p*-BrBz); 7.6 (1H, s, *6*-C*H*); 7.15 (1H, t, 1-N*H*); 6.2 (1H, t, *1′*-C*H*); 5.2 (1H, d, *3′*-O*H*); 5.0 (1H, t, *5′*-O*H*); 4.2 (1H, t, *3′*-C*H*); 3.78 (1H, m, *4′*-CH); 3.6 (2H, m, *5′*-CH_2); 3.3 (4H, m, 2 CH_2NH); 2.0 (2H, m, *2′*-CH_2); 1.85 (3H, s, CH_3); 1.55 (4H, s, 2 CH_2). ^{13}C-NMR (DMSO-d_6)*:* 165.05 (q, *4-C*); 162.57 (q, *p*-BrBz-*C*O); 154.94 (q, *2-C*O); 137.11 (t, *6-C*H); 133.65 (q, *C*arom-CO); 131.14 and 129.19 (2 t, *C*Harom); 124.60 (q, *C*arom-Br); 101.58 (c, *5-C*); 86.94 (t, *4′-C*H); 84.43 (t, *1′-C*H); 70.35 (t, *3′-C*H); 61.31 (s, *5′-C*H_2); 39.99 (s, *2′-C*H_2); 39.59 and 38.28 (2s, *C*H_2NH-1 and *C*H_2NH-2); 26.44 and 26.08 (2 s, *C*H_2); 13,05 (p, *C*H_3). FAB-MS *(m/z)* = 496 $(M+H)^+$. Anal. calcd. for $C_{27}H_{38}N_5O_6Br$: C: 50.93; H: 5.45; N: 11.31.- Found: C: 50.97; H: 5.49; N: 11.20.

5 - Methyl - 4 - *N* - [- 6 - (*p* - bromobenzoylamino) hex-1- yl]-2′-*O*-deoxycytidine (8c): Yield: 49 %, m.p.: 117-20 °C. IR: 3350s, 3100m, 2950s, 2850s, 1680s, 1640s, 1560s, 1510s; 1440s; 1360m; 1320m; 1100s; 1080s; 1060m; 1020m; 860w; 800m; 760m. ^{1}H-NMR (DMSO-d_6): 8.5 (1H, t, 2-N*H*); 7.8-7.6 (4H, AA'BB', *p*-BrBz); 7.55 (1H, s, *6-*

CH); 7.1 (1H, t, 1-N*H*); 6.2 (1H, t, *1'*-C*H*); 5.15 (1H, d, *3'*-O*H*); 4.95 (1H, t, *5'*-O*H*); 4.2 (1H, s, *3'*-C*H*); 3.75 (1H, m, *4'*-C*H*); 3.6 (2H, m, *5'*-CH_2); 3.4-3.2 (4H, m, 2 CH_2NH); 2.2 and 1.9 (2H, m, *2'*-CH_2); 1.8 (3H, s, CH_3); 1.6-1.5 and 1.3-1.2 (8H, m, 4 CH_2). ^{13}C-NMR (DMSO-d_6): 165.44 (q, *4*-C); 162.92 (q, *p*-BrBz-CO); 154.93 (q, *2*-CO); 137.12 (t, *6*-CH); 133.83 (q, Carom-CO); 131.10 and 129.19 (2 t, CHarom); 124.52 (q, Carom-Br); 101.53 (q, *5*-C); 87.01 (t, *4'*-CH); 84.57 (t, *1'*-CH); 70.38 (t, *3'*-CH); 61.38 (s, *5'*-CH_2); 40.06 (s, *2'*-CH_2); 40.06 and 39.15 (2 s, CH_2NH-1 and CH_2NH-2); 28.87; 28.47; 26.12 and 26.07 (4 s, 4 CH_2); 12.99 (p, CH_3). FAB-MS (*m/z*) 524 $(M+H)^+$. Anal. calcd. for $C_{27}H_{38}N_5O_6Br$: C: 52.80; H: 5.92; N: 10.70.- Found: C: 52.78; H: 5.88; N: 10.66.

5 - Methyl - 4 - *N* - (*p* -bromocinnamoylaminoethyl) - 2'- *O* - deoxycytidine (9a): Yield: 51 %, m.p.: 158-60 °C. IR: 3390s, 3100m, 2950m, 1680s, 1640s, 1560s, 1520s; 1360m; 1320m; 1240m; 1100m; 1080m; 1020m; 820m; 800m. ^{1}H-NMR (DMSO-d_6): 8.4 (1H, t, 2-N*H*); 7.6-7.5 (4H, AA'BB', *p*-BrBz); 7.6 (1H, s, *6*-C*H*); 7.5 (1H, d, *p*-BrPhC*H*=CH); 7.3 (1H, t, 1-N*H*); 6.6 (1H, d, CH=C*H*CO); 6.2 (1H, t, *1'*-C*H*); 5.2 (1H, d, *3'*-O*H*); 5.0 (1H, t, *5'*-O*H*); 4.2 (1H, s, *3'*-C*H*); 3.75 (1H, m, *4'*-CH); 3.6 (2H, s, *5'*-CH_2); 3.4 (4H, m, 2 CH_2NH); 2.1-1.9 (2H, m, *2'*-CH_2); 1.8 (3H, s, CH_3). ^{13}C-NMR (DMSO-d_6): 165.50 (q, *4*-C); 162.91 (t, CH=CHCO); 154.81 (q, *2*-CO); 137.28 (2 t, CH=CHCO and *6*-CH); 132.02 (q, Carom-CH); 131.78 and 129.37 (2 t, CHarom); 122.93 (t, *p*-BrPhCH=CH); 121.05 (q, Carom-Br); 101.90 (q, *5*-C); 86.99 (t, *4'*-CH); 84.50 (t, *1'*-CH); 70.32 (t, *3'*-CH); 61.28 (s, *5'*-CH_2); 40.22 (s, *2'*-CH_2); 40.06 and 38.04 (2s, CH_2NH-1 and CH_2NH-2); 12.97 (p, CH_3). FAB-MS (*m/z*) 494 $(M+H)^+$. Anal. calcd. for $C_{27}H_{38}N_5O_6Br$: C: 51.14; H: 5.06; N: 11.36.- Found: C: 51.10; H: 5.00; N: 11.30.

5 - Methyl - 4 - *N* - [- 4 -(*p* -bromocinnamoylamino) but-1-yl]-2'-*O*-deoxycytidine (9b): Yield: 59 %, m.p.: 127-30 °C. IR: 3350s, 3100m, 2950s, 2870m, 1660s, 1620s, 1560s, 1520s; 1410m, 1350s; 1240m; 1100s; 1080m; 1060m; 1020m; 980m; 820m; 790m; 760m. ^{1}H-NMR (DMSO-d_6): 8.19 (1H, t, 2-N*H*); 7.57-7.53 (4H, AA'BB', *p*-BrBz); 7.57 (1H, s, *6*-C*H*); 7.41 (1H, d, *p*-BrPhC*H*=CH); 7.20 (1H, t, 1-N*H*); 6.73 (1H, d, CH=C*H*CO); 6.18 (1H, t, *1'*-C*H*); 5.16 (1H, d, *3'*-O*H*); 4.97 (1H, t, *5'*-O*H*); 4.20 (1H, s, *3'*-C*H*); 3.75 (1H, m, *4'*-CH); 3.55 (2H, m, *5'*-CH_2); 3.35 (2H, m, CH_2NHCO); 3.15 (2H, m, CH_2NH-1); 2.03-2.01 (2H, m, *2'*-CH_2); 1.98 (3H, s, CH_3); 1.55 (4H, m, 2 CH_2). ^{13}C-NMR (DMSO-d_6): 164.52 (q, *4*-C); 162.57 (t, CH=CHCO); 154.98 (q, *2*-CO); 137.15 (t, *6*-CH); 137.00 (t, CH=CHCO); 134.22 (q, Carom-CH); 131.75 and 129.33 (2 t, CHarom); 123.24 (t, *p*-BrBzCH=CH); 122.35 (q, Carom-Br); 101.56 (q, *5*-C); 86.96 (t, *4'*-CH); 84.47 (t, *1'*-CH); 70.35 (t, *3'*-CH); 61.31 (s, *5'*-CH_2); 40.08 (s, *2'*-CH_2); 40.00 and 38.42 (2s, CH_2NH-2; CH_2NH-1); 26.32 and 26.03 (2 s, CH_2); 13.05 (CH_3). FAB-

MS (*m/z*) = 522 $(M+H)^+$. Anal. calcd. for $C_{27}H_{38}N_5O_6Br$: C: 53.00; H: 5.56; N: 10.74.- Found: C: 52.96; H: 5.50; N: 10.60.

5 - Methyl - 4 - *N* - [- 6- (*p*-bromocinnamoylamino) hex-1-yl] - 2'-deoxycytidine (9c): Yield: 40 %, m.p.: 118-22 °C. IR: 3400s; 3100m; 2950m; 2850m; 1660s; 1620s; 1560s; 1510s; 1350m; 1240m; 1100m; 1080m, 1060m; 1020m; 820w, 790w. ^{1}H-NMR (DMSO-d_6): 8.1 (1H, t, 2-N*H*); 7.62-7.45 (5H, m, *p*-BrBz); 7.58 (1H, s, *6*-C*H*); 7.4-7.35 (1H, d, *p*-BrPhC*H*=CH); 7.20-7.10 (1H, t, 1-N*H*); 6.7-6.6 (1H, d, CH=C*H*CO); 6.2 (1H, t, *1'*-C*H*); 5.2 (1H, d, *3'*-O*H*); 5.0 (1H, t, *5'*-O*H*); 4.20 (1H, s, *3'*-C*H*); 3.75 (1H, t, *4'*-C*H*); 3.55 (2H, m, *5'*-CH_2); 3.3 (2H, m, CH_2NHCO); 3.15 (2H, m, CH_2NH-1); 2.0-1.9 (2H, m, *2'*-CH_2); 1.85 (3H, s, CH_3); 1.55-1.35 (8H, m, 4 CH_2). ^{13}C-NMR (DMSO-d_6): 164.50 (q, *4*-*C*); 162.55 (q, CH=CH*C*O); 155.02 (q, *2*-*C*O); 137.07 (t, *6*-*C*H); 136.95 (t, *C*H=CHCO); 134.21 (q, *C*arom-CH); 131.76 and 129.31 (2 t, *C*Harom); 123.20 (t, *p*-BrPhCH=*C*H); 122,35 (q, *C*arom-Br); 101.59 (q, *5*-*C*); 86.95 (t, *4'*-*C*H); 84.44 (t, *1'*-*C*H); 70.37 (t, *3'*-*C*H); 61.33 (s, *5'*-CH_2); 39.98 (2 s, *2'*-CH_2 + CH_2NH); 38.54 (s, CH_2NH); 28.95; 28.45; 26.10 and 26.04 (4 s, CH_2); 13.06 (p, CH_3). FAB-MS (*m/z*)= 550 $(M+H)^+$. Anal. calcd. for $C_{27}H_{38}N_5O_6Br$: C: 54.67; H: 6.00; N: 10.20.- Found: C: 54.60; H: 5.96; N: 10.15.

5 - Methyl -4 - *N* - { 2 - [ε - (*p* - bromobenzoylamino) caproylamino] - ethyl } -2'-*O*-deoxycytidine (10a) : Yield: 53 %, m.p.: 107-10 °C. IR: 3350s; 3100m; 2980m; 2890m, 1640s; 1550s; 1520s; 1440m; 1340m; 1260m; 1100m; 1020m; 860m; 800m; 760m; 680m. ^{1}H-NMR (DMSO-d_6): 8.55 (1H, t, *3*-N*H*); 8.0 (1H, t, *2*-N*H*); 7.79-7.68 (4H, AA'BB', *p*-BrBz); 7.6 (1H, s, *6*-C*H*); 7.2 (1H, t, 1-N*H*); 6.2 (1H, t, *1'*-C*H*); 5.19 (1H, d, *3'*-O*H*); 5.0 (1H, t, *5'*-O*H*); 4.2 (1H, s, *3'*-C*H*); 3.75 (1H, m, *4'*-C*H*); 3.58 (2H, q, *5'*-CH_2); 3.45 (2H, m, CH_2*N*H-1); 3.21 (4H, m, 2 CH_2NHCO); 2.1-2.0 (2H, m, NHCOCH_2); 2.0-1.9 (2H, m, *2'*-CH_2); 1.85 (3H, s, CH_3); 1.5 and 1.3 (6H, m, 3 CH_2). ^{13}C-NMR (DMSO-d_6): 172.60 (q, NH*C*OCH$_2$); 165.08 (q, *4*-*C*); 162.85 (q, *p*-BrBz-*C*O); 154.88 (q, *2*-*C*O); 137.30 (t, *6*-*C*H); 133.81 (q, *C*arom-CO); 131.11 and 129.18 (2 t, *C*Harom); 124.54 (q, *C*arom-Br); 101.51 (q, *5*-*C*); 87.05 (t, *4'*-*C*H); 84.60 (t, *1'*-*C*H); 70.36 (t, *3'*-*C*H); 61.37 (s, *5'*-CH_2); 40.12 (s, *2'*-CH_2); 40.46; 39.09; 37.87; 35.32 (4s, 2 CH_2NHCO + NHCOCH_2 + CH_2NH); 28.71; 26.07; 24.88 (3 s, CH_2); 12.86 (p, CH_3). FAB-MS (*m/z*) = 581 $(M+H)^+$. Anal. calcd. for $C_{27}H_{38}N_5O_6Br$: C: 51.75; H: 5.85; N: 12.07.- Found: C: 51.70; H: 5.80; N: 12.00.

5 - Methyl - 4 - *N* - { 4 - [ε- (*p* - bromobenzoylamino) caproylamino] - but- 1-yl } -2'-*O*-deoxycytidine (10b) : was reported elsewhere[24].

5 - Methyl - 4 - *N* - { 6 - [ε- (*p* - bromobenzoylamino) caproylamino] - hex- 1-yl } -2'-*O*-deoxycytidine (10c): Yield: 41 %, m.p.: 98-100 °C. IR: 3340s; 3100m; 2950s; 2880s; 1640s; 1560s; 1510s; 1440s; 1350s; 1320s; 1100s; 1060s; 1020s; 860m; 800m;

760m. ^{1}H-NMR (DMSO-d_6): 8.54 (1H, t, *3*-N*H*); 7.79(1H, t, *2*-N*H*); 7.80-7.64 (4H, AA'BB', *p*-BrBz); 7.56 (1H, s, *6*-C*H*); 7.13 (1H, t, 1-N*H*); 6.18 (1H, t, *1'*-C*H*); 5.17 (1H, d, *3'*-O*H*); 4.99 (1H, t, *5'*-O*H*); 4.20 (1H, s, *3'*-C*H*); 3.74 (1H, q, *4'*-CH); 3.55 (2H, s, *5'*-CH_2); 3.29-3.26 (4H, m, 2 CH_2NHCO); 3.05 (2H, q, CH_2NH-1); 2.08-2.01 (4H, m, NHCOCH_2 + 2'-CH_2); 1.95 (3H, s, CH_3); 1.50-1.25 (14H, m, 7 CH_2). ^{13}C-NMR (DMSO-d_6): 171.74 (q, NH*C*OCH$_2$); 164.99 (q, *4*-*C*); 162.52 (q, *p*-BrBz-*C*O); 155.02 (q, 2-*C*O); 137.05 (t, 6-*C*H); 133.65 (q, *C*arom-CO); 131.13 and 129.17 (2 t, *C*Harom); 124.61 (q, *C*arom-Br); 101.60 (q, *5*-*C*); 86.94 (t, *4'*-*C*H); 84.42 (t, 1'-*C*H); 70.36 (t, *3'*-*C*H); 61.31 (s, *5'*-CH_2); 39.64 (s, 2'-CH_2); 39.63; 39.06; 38.19; 35.26 (4s, 3 CH_2NH + CH_2CONH); 30.59; 29.01; 28.72; 28.47; 26.04 (2); 25.00 (7 s, 7 CH_2); 13.06 (p, CH_3). FAB-MS (*m/z*) = 637 (M+H)$^+$. Anal. calcd. for $C_{27}H_{38}N_5O_6Br$: C: 54.74; H: 6.60; N: 11.00.- Found: C: 54.69; H: 6.57; N: 10.93.

3'-*O*-Benzoyl-5'-*O*-trityl-2'-*O*-deoxythymidine (11): 1.1 mL of benzoyl chloride was added to a solution of 5'-*O*-tritylthymidine[44] (3.87 g, 0.008 mol), in anhydride pyridine (41.3 mL). The reaction mixture was stirred at room temperature for 2 hours. Methanol (15 mL) was added and after 30 minutes the solvent was evaporated under reduced pressure. The residue was dissolved in chloroform, washed with 5% sodium bicarbonate solution and the organic phase was dried over Na_2SO_4. The solvent was evaporated and the product was purified by column chromatography (chloroform). Yield: 40%, m.p.: 121-22°C. IR: 3300s, 1700s, 1580m, 1570m, 1550m; 1450m; 1310m; 1270m; 1200m; 1100s; 1080m; 1020m; 900w; 750w. ^{1}H-NMR ($CDCl_3$): 8.05 (1H, s, N*H*); 7.65 (1H, s, 6-C*H*); 7.5-7.0 (20H, m, Tr + Bz); 6.1 (1H, t, 1'-C*H*); 5.7 (1H, s, *3'*-C*H*); 4.3 (1H, s, *4'*-C*H*); 3.5 (2H, s, *5'*-CH_2); 2.66 (2H, m, 2'-CH_2); 1.49 (3H, s, CH_3). FAB-MS (*m/z*) = 590 (M+H)$^+$.

3'-*O*-Benzoyl-5'-*O*-trityl-4-(1,2,4-triazol-1-yl)-2'-*O*-deoxythymidine (12): was obtained in the same conditions described[31]. Yield: 84 %, m.p.: 105-6°C. IR: 1720s, 1680s, 1500s, 1450s; 1430w; 1380m; 1320m; 1280w; 1200m; 1110w; 1080w; 1040m; 980w; 800w; 780m; 750m; 710s. ^{1}H-NMR ($CDCL_3$): 9.3 (1H, s, C*H*-triazole); 8.35 (1H, s, 6-C*H*); 8.19 (1H, s, C*H*-triazole); 7.6-7.1 (20H, m, Tr + Bz); 6.5 (1H, t, 1'-C*H*); 5.7 (1H, m, *3'*-C*H*); 4.49 (1H, s, *4'*-C*H*); 3.6 (2H, d, *5'*-CH_2); 3.1 and 2.5 (2H, m, 2'-CH_2); 2.0 (3H, s, CH_3). FAB-MS (m/z) = 640 (M+H)$^+$.

3'-*O*-Benzoyl-5'-*O*-trityl-4-*N*-(aminoethyl)-2'-*O*-deoxycytidine (13): A solution of **12** (1.278 g, 0.002 mol) in pyridine (8.6 mL) was added dropwise to a cold solution (5°C) of 1,2-diaminoethane (0.23 mL, 0.003 mol) in pyridine (8.6 mL). The reaction mixture was stirred at 5°C for 1 hour. Pyridine was evaporated under reduced pressure and its traces was coevaporated with toluene. The product was purified by column chromatography (chloroform:methanol, from 99:1 to 90:10). Yield: 64 %. IR: 3400m;

3310w; 2900m; 1710s; 1670s; 1480s; 1470s; 1450m; 1310m; 1270m; 1200m; 1100s; 1080s; 1020m; 900w; 750m. ^{1}H-NMR ($CDCl_3$): 7.85 (1H, s, 1-N*H*); 8.05 (1H, d, 6-C*H*); 7.7-7.2 (20H, m, Tr + Bz); 6.6 (1H, t, 1'-CH); 5.7 (1H, m, 3'-C*H*); 4.3 (2H, s, 5'- CH_2); 3.8-3.1 (7H, m, 4'-C*H* + 2 CH_2NH + NH_2); 2.7-2.4 (2H, m, 2'-CH_2); 1.5 (3H, s, CH_3). FAB-MS (m/z) = 633 $(M+H)^+$.

4-*N*-[6-(*p*-Bromobenzoylamino)caproylamino]cytidine (14): was reported elsewhere[24].

Immunochemical procedures:

The competitive ELISAs were performed as previously reported[43].

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