16.Biagi, G.L.; Barbaro, A.M.; Sapone, A.; Recanatini, M. Determination of Lipophilicity by Means of Reversed-phase Thin-layer Chromatography, J. Chromatogr. A. 1994, 662, 341-361.

17.Biagi, G.L.; Barbaro, A.M.; Recanatini, M. Determination of Lipophilicity by Means of Reversed-phase Thin-layer Chromatography III. Study of the TLC Equations for a Series of Ionizable Quinolone Derivatives, J. Chromatogr. A. 1994, 678, 127-137.

18.Biagi, G.L.; Barbaro, A.M.; Sapone, A.; Recanatini, M. Thin-layer Chromatographic Study of the Lipophilicity of Triazine Herbicides. Influence of Different Organic Modifiers, J. Chromatogr. 1992, 625, 392-396.

19.Biagi, G.L.; Pietrogrande, M.C.; Barbaro, A.M.; Guerra, M.C.; Borea, P.A.; Cantelli-Forti, G. Study of the Lipophilic Character of a Series of β-Carbolines, J. Chromatogr. 1989, 469, 121-126.

20.Braumann, T. Determination of Hydrophobic Parameters by Reversed-Phase Liquid Chromatography: Theory, Experimental Techniques, and Application in Studies on Quantitative Structure-Activity Relationships, J. Chromatogr. 1986, 373, 191-225.

21.Caffieri, S. Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) Determination of Lipophilicity of Furocoumarins: Relationship with DNA Interaction, J. Pharm. Sci. 2000, 90 (6), 732-739.

22.Biagi, G.L.; Barbaro, A.M.; Sapone, A.; Recanatini, M. Determination of Lipophilicity by Means of Reversed-phase Thin-layer Chromatography II. Influence of the Organic Modifier on the Slope of the Thin-layer Chromatographic Equation, J. Chromatogr. A. 1994, 669, 246-253.

23.Hansch, C.; Leo, A., Hoekman, D. *Exploring QSAR*; American Chemical Society. Press: Washington, 1995: 62 pp.

Andrada, K.; Briñón, M. C. In *Síntesis y Caracterización de Nuevos Análogos de Lamivudina (3TC)*, Asociación Química Argentina (AQA), Buenos Aires, Argentina, Sept 22-24, 2004.

9.Moroni, G.N.; Quevedo, M.A.; Ravetti, S.; Briñón, M.C. Lipophilic Character of Novel Amino Acid Derivatives of Zidovudine with Anti HIV Activity, J. Liq. Chrom. Rel. Technol. 2002, 25 (9), 1345-1365.

10.Teijeiro, S.A.; Moroni, G.N.; Motura, M.I.; Briñón, M.C. Lipophilic Character of Pyrimidinic Nucleoside Derivatives: Correlation Between Shake Flask, Chromatographic (RP-TLC and RP-HPLC) and Theoretical Methods, J. Liq. Chrom. Rel. Technol. 2000, 23 (6), 855-872.

11.Raviolo, M.; Briñón, M.C. Comparative Study of Hydrophobicity Parameters of Novel 5´-Carbamates of Zidovudine, J. Liq. Chrom. Rel. Technol. 2005, 28, 2195-2209.

12.Dross, K.; Sonntag, C.; Mannhold, R. Determination of the Hydrophobicity Parameter $R_{Mw}$ by Reversed-phase Thin-layer Chromatography, R.J. Chromatogr. A. 1994, 673, 113-124.

13.Granero, G.E.; de Bertorello, M.M.; Briñón, M.C. Hydrophobicity Parameters Determined by Reversed-Phase Liquid Chromatography for Some New Isoxazolyl-Naphthoquinones, J. Liq. Chrom. Rel. Technol. 1999, 22 (2), 229-240.

14.Biagi, G.L.; Recanatini, M.; Barbaro, A.M.; Guerra, M.C.; Sapone, A.; Borea, P.A; Pietrogrande, M.C. Chromatographic Parameters as Lipophilicity Indices for QSAR Studies. New Approaches in Chromatography ´91. 1992, 215-234.

15.a) Hansch, C.; Dunn, W.J. Linear Relationship Between Lipophilic Character and Biological Activity of Drugs. J. Pharm. Sci. 1972, 61 (1), 1-19. b) Garst, J.E.; Wilson, W.C. Accurate, Wide-range, Automated, High-performance Liquid Chromatographic Method for the Estimation of Octanol/water Partition Coefficients I: Effect of Chromatographic Conditions and Procedure Variables on Accuracy and Reproducibility of the Method, J. Pharm. Sci. 1984, 73 (11), 1616-1623.

**REFERENCES**

1.Cimpan, G.; Hadaruga, M.; Miclaus, V. Lipophilicity Characterization by Reversed-phase Liquid Chromatography of Some Furan Derivatives, J. Chromatogr. A. 2000, 869, 49-55.

2.Gulyaeva, N.; Zaslavsky, A.; Lechner, P.; Chlenov, M.; McConnell, O.; Chait, A.; Kipnis, V.; Zaslavsky, B. Relative Hydrophobicity and Lipophilicity of Drugs Measured by Aqueous Two-phase Partitioning, Octanol-buffer Partitioning and HPLC. A Simple Model for Predicting Blood-brain Distribution, Eur. J. Med. Chem. 2003, 38, 391-396.

3.Moriguchi, I.; Hirono, S.; Nakagome, I.; Hirano, H. Comparison of Reliability of log P Values for Drugs Calculated by Several Methods, Chem. Pharm. Bull. 1994, 42 (4), 976-978.

4.Dorsey, J.H.; Khaledi, M.G. Hydrophobicity Estimations by Reversed-phase Liquid Chromatography. Implications for Biological Partitioning Processes, J. Chromatogr. A. 1993, 656, 485-499.

5.Nasal, A.; Siluk, D.; Kaliszan, R. Chromatographic Retention Parameters in Medicinal Chemistry and Molecular Pharmacology, Current Medicinal Chemistry. 2003, 10, 381-426.

6.Trinchero, J.; Turk, G.; Ravetti, S.; Briñón, M. C.; Salomón, H. In *Actividad Anti-VIH de Nuevas Prodrogas de Lamivudina (3TC)*, VI Congreso Argentino de SIDA, Buenos Aires, Argentina, Nov 20-23, 2003.

7.Theoretical Estimates of n-octanol/water Partition Coefficients (CLOGP) were Determined using C log P for Windows Version 1.0.0. Copyright©. 1995, Biobyte Corp.

8.(a) Ravetti, S.; Moroni, G.; Briñón, M.C. In *Síntesis y Caracterización de Nuevas Prodrogas de Lamivudina (3TC)*, XXIV Congreso Argentino de Química, Santa Fe, Argentina, Ago 7-9, 2002. (b) Ravetti, S.; Moroni, G.; Briñón, M.C. In *Síntesis y Caracterización Espectroscópica de Nuevas Análogos de Lamivudina (3TC)*, XIV Simposio de Química Orgánica (SAIQO), Santa Fe, Argentina, Nov 9-12, 2003. (c) Ravetti, S.; Crespo

Based on the good correlations obtained between the shake flask and the chromatographic (RP-TLC and RP-HPLC) methods, these latter ones have proven to be reliable and accurate techniques to describe the lipophilic character of this nucleoside family of compounds.

The partition coefficients calculated by the CLOGP program are in good agreement with the measured n-octanol/water partition coefficients, except in the case of cytidine and 3TC-Octa, probably due to their very hydrophilic and lipophilic characteristics, respectively.

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$$\log P_{oct} = 0.996\ (\pm 0.155)\ \log P_{RP\text{-}HPLC} - 0.065\ (\pm 0.158) \quad (10)$$

$$n = 9;\ r = 0.985;\ s = 0.185;\ F = 230.88$$

$$\log P_{oct} = 0.999\ (\pm 0.103)\ \log P_{CLOGP} + 0.004\ (\pm 0.092) \quad (11)$$

$$n = 8;\ r = 0.995;\ s = 0.103;\ F = 566.14$$

Figure 11

**CONCLUSIONS**

All these novel lamivudine derivatives were shown to be considerably more lipophilic than the parent 3TC, in the following order 3TC-Octa > 3TC-Hexa > 3TC-Penta > 3TC-Buta > 3TC-nPro > 3TC-2Pro > 3TC-Etha > 3TC-Metha > 3TC, thus making them potential pharmacological agents in the treatment of AIDS and related diseases, due to their exhibited antiHIV-1 activity (6). It is clear that the chain length of the substituent of the 5'-position influences the lipophilicity, since the addition of a methylene group reduces the side chain polarity, thereby increasing the lipophilicity.

The correlation between the extrapolated $R_M$ values obtained using different organic solvents is another important factor, supporting the reliability of using the extrapolated $R_M$ values as an expression of the partitioning between an aqueous mobile phase and the silicone oil of the stationary phase. In fact, the nature of the organic modifier does not substantially affect the extrapolated $R_M$ values. Although the RP-TLC and RP-HPLC experiments were carried out using from 40% and from 50% of organic modifier, respectively, it is assumed that the linearity of the resulting relationships is maintained even at low concentrations of the organic modifier in the mobile phase.

**Log P calculations**

In drug design, and in the Quantitative Structure-Activity Relationship (QSAR), it is useful to predict the log P of unknown drug molecules. For comparative purposes, log P values for carbonate lamivudine derivatives were calculated by the CLOGP program (Table 2) (7). Equation 7 shows that the calculated CLOGP values for lamivudine derivatives are a reliable index for these compounds. However, the CLOGP program does not correctly estimate the partition coefficient of **1** (log P = -2.130) (23) and **10** (log P = 1.720) due to their high hydrophilicity and lipophilicity, respectively.

$$\text{CLOGP} = 1.045\ (\pm\ 0.107)\ \log P_{oct} + 0.009\ (\pm\ 0.096) \qquad (7)$$

$$n = 8;\ r = 0.994;\ s = 0.108;\ F = 566.32$$

**Correlation between different techniques**

Table 2 shows the log P values obtained from the RP-TLC and RP-HPLC techniques, as well as those for the shake flask and the CLOGP program methods. In addition, the corresponding deviations (Δlog P) between the conventional shake flask method and the other different techniques studied (log $P_{RP\text{-}TLCMe2CO}$, log $P_{RP\text{-}TLCMeOH,}$ log $P_{RP\text{-}HPLC}$ and log $P_{CLOGP}$) are also shown. Figure 11 shows the log P relationship between the conventional shake-flask method and the chromatographic and theoretical ones. The linear regressions are described by eqs. 8-11, which can be considered as Collander type equations.

$$\log P_{oct} = 0.975\ (\pm\ 0.141)\ \log P_{RP\text{-}TLC\ Me2CO} + 0.009\ (\pm\ 0.143) \qquad (8)$$

$$n = 9;\ r = 0.987;\ s = 0.169;\ F = 268.54$$

$$\log P_{oct} = 0.963\ (\pm\ 0.169)\ \log P_{RP\text{-}TLC\ MeOH} + 0.014\ (\pm\ 0.173) \qquad (9)$$

$$n = 9;\ r = 0.981;\ s = 0.203;\ F = 180.28$$

$$\text{Intercept}_{\text{RP-TLC MeOH}} = -105.809\ (\pm 19.328)\ \text{slope}_{\text{RP-TLC MeOH}} - 0.854\ (\pm 0.527) \quad (5)$$

$$n = 9;\ r = 0.980;\ s = 0.217;\ F = 168.94$$

$$\text{Intercept}_{\text{RP-HPLC MeOH}} = 32.628\ (\pm 9.796)\ \text{slope}_{\text{RP-HPLC MeOH}} + 1.776\ (\pm 0.182) \quad (6)$$

$$n = 9;\ r = 0.948;\ s = 0.039;\ F = 61.22$$

Figures 10a-c

It has been previously reported that one of the basic features of the chromatographic assays of lipophilicity is the relationship between the slopes and intercepts of the chromatographic equations (9,16). The physicochemical parameters $R_{Mw}$, log $k'_w$ and the intercepts of linear regression of eqs. 4-6, can be considered to be measures of the partitioning of compounds between a polar mobile phase (buffer) and a non-polar stationary phase (octadecanol in RP-TLC and octadedecilsilane in RP-HPLC) (9,16).

The slopes indicate the rate at which the solubility of the compounds increases in the mobile phase as the percentage of organic solvent increases. Hence, it is reasonable that the most lipophilic compound (more sensitive to a decrease in the polarity of the mobile phase, and therefore having a higher slope) exhibits a higher $R_{Mw}$ and greater log $k'_w$ values. Conversely, a more hydrophilic compound with a lower slope and less sensitivity to a decrease in the mobile phase polarity, will have a lower $R_{Mw}$ and smaller log $k'_w$ values. These features account for the linear correlations between the intercepts and slopes, as observed in eqs. 4-6. A strong slope vs intercept relationship can only be found when dealing with strictly congeneric compounds. The deviation from linearity of cytidine was also attributed to the different structural features possessed by this nucleoside.

**Relationship between log k'$_w$ with $R_{Mw}$**

Other interesting linear correlations were observed between log k'$_w$ and $R_{Mw}$ (eqs. 2 and 3, Figures 8 and 9).

$$\log k'_w = 0.087 (\pm 0.018) R_{MwMe2CO} + 1.026 (\pm 0.039) \quad (2)$$

$$n = 9; r = 0.974; s = 0.028; F = 130.99$$

$$\log k'_w = 0.114 (\pm 0.016) R_{MwMeOH} + 0.966 (\pm 0.034) \quad (3)$$

$$n = 9; r = 0.988; s = 0.019; F = 289.17$$

Figures 8 and 9

These results show a very good correlation between log k'$_w$ and the $R_{Mw}$ values obtained from two different chromatographic systems. It is interesting to point out, that the correlations described by eqs. 2 and 3 hold over a wide range of lipophilicity. This justifies our confidence in the use of chromatographic data as lipophilic parameters (5,16-20). Also, cytidine was omitted in Figures 8 and 9 due to its large deviation.

**Relationship between slopes and intercepts (RP-TLC and RP-HPLC)**

The "congenerity" of substances can be expressed as the linearity between the extrapolated parameter log k'$_w$ (or $R_{Mw}$) and the slope (1,16-18). This is due to the complex nature of the factors that contribute to retentions in reverse phase chromatographic methods, which are drastically different from those in the transfer of solutes between an isotropic aqueous and an organic solvent. In this way, Figures 10a-c show good linear relationships between the slopes and the intercepts ($R_{Mw}$ and log k'$_w$) of the RP-TLC and RP-HPLC equations.

$$\text{Intercept}_{\text{RP-TLC Me2CO}} = -111.513 (\pm 13.874) \text{ slope}_{\text{RP-TLC Me2CO}} - 1.110 (\pm 0.386) \quad (4)$$

$$n = 9; r = 0.990; s = 0.191; F = 359.28$$

The correlation between the log k´ values and the composition of the mobile phase was established, and showed a linear relationship, as can be seen in Table 1 and in Figure 4. Table 2 summarizes the extrapolated log k´ at 0% methanol (log $k'_w$).

Figure 4

From Table 1 it is possible to note that the slope is negative in all cases, being related to the hydrophobic surface of the molecule which interacts with the non-polar stationary phase (21,22).

**Relationship between $R_{Mw}$ or log $k_w'$ with log $P_{oct}$ values**

Assuming the extra thermodynamic linear free-energy relationship, one may expect the standard log $P_{oct}$ data to be linearly related to the partition chromatographic parameters, $R_{Mw}$ and log $k'_w$, obtained from RP-TLC and RP-HPLC, respectively.

The above considerations seem to point to the reliability of the $R_M$ or log k´ values as a measure of the partitioning between an aqueous mobile phase and a non-aqueous stationary phase. Thus, resulting linear dependences were observed in the correlations between $R_{MwMe2CO}$ and $R_{MwMeOH}$ with log $P_{oct}$ obtained by the shake-flask method, which have been represented in Figures 5 and 6.

Figures 5 and 6

In a similar way, good correlations can be noted between the log $k'_w$ and log $P_{oct}$ values of the newly synthesized series of 5'-carbonates of lamivudine (Figure 7).

Figure 7

Cytidine (points shown in Figures 5-7 as ■ shaped points), was omitted from the regression analyses because of its large deviation, due to the different structural features possessed by this nucleoside.

Table 2

Inspection of Table 2 shows that although some differences were obtained in the $R_{Mw}$ values of **2**-**10** which were determined with acetone and methanol as the organic modifier, a good correlation between $R_{MwMeOH}$ and $R_{MwMe2CO}$ was observed (Eq. 1).

$$R_{MwMeOH} = 0.755\ (\pm 0.076)\ R_{MwMe2CO} + 0.556\ (\pm 0.161) \quad (1)$$

$$n = 9;\ r = 0.967;\ s = 0.278;\ F = 99.53$$

In this way, Biagi et al studied the RP-TLC lipophilicity behavior for different series of compounds, concluding that $R_{Mw}$ values were not dependent on the nature of the organic modifier (16-19). However, Nasal et al have observed that the retention values depend on the organic modifier in an aqueous eluent (5). This can be explained taking into account the fact that the physicochemical properties of acetone are distinctly different from those of water, whereas methanol is very similar. Accordingly, methanol has to be viewed as the most commonly used modifier for the chromatographic determination of lipophilicity. For this reason, Braumann (20) recommends methanol as the best modifier, due to its physicochemical similarity to water. Hence, it provides the strongest hydrogen-bond donor and acceptor properties of all the modifiers used, so its addition to an aqueous phase over a wide range of volume fractions has only a moderate effect on the ordering of water molecules. These considerations are substantiated by our present investigations, since some differences between $R_{MwMe2CO}$ and $R_{MwMeOH}$ values have been found (Table 2), as we also observed for other zidovudine derivative series (20).

**Reversed-phase high performance liquid chromatography (RP-HPLC)**

Since no considerable differences were found in RP-TLC between the use of acetone or methanol as the organic modifier, and also taking into account Braumann´s recommendations, only methanol was used as the organic phase in RP-HPLC analysis.

**Reversed-phase thin layer chromatography (RP-TLC)**

The chromatographic value, $R_M$, was determined using acetone-buffer pH 7.4 and methanol-buffer pH 7.4 as mobile phases. The $R_M$ values decreased linearly with increasing organic modifier content of the mobile phase. Table 1 shows parameters obtained from the linear relationships between $R_M$ values and acetone and also with methanol organic modifier concentrations, including the values of the intercept (a ± st), slope (b ± st), standard error (s) and correlation coefficient (r) obtained from the corresponding linear relationship, for all compounds studied (**1**-**10**).

Table 1

From the regression analyses (Figs. 2 and 3), it can be seen that **1-10** exhibited a good linear correlation between the $R_M$ values and the range of the organic modifier content of the mobile phases (40-80). The RP-TLC experiments were not performed for organic modifier values of below 40% in the mobile phase in order to avoid experimental errors when measuring low $R_F$ values. These results have shown that each compound of the carbonate lamivudine series presents a linear relationship between the $R_M$ value and the organic solvent concentration in the mobile phase (acetone and methanol). These features confirm that the separation mechanism is a partition of the analyte between the mobile and stationary phases.

Figures 2 and 3

The intercepts of the equations reported in Table 1 represent the theoretical $R_M$ values at 0% acetone or methanol. These extrapolated $R_M$ values at 0% of organic modifier could be considered as a measure of the partitioning of the compounds between an aqueous buffer mobile phase and the non-polar stationary phase, in a standard system where all the compounds could be compared according to their lipophilic character. These results, summarized in Table 2, made it possible to compare the assayed compounds on the basis of their intrinsic lipophilicity.

**Shake flask octanol-water partition coefficients**

Partition coefficients of **1**-**10** were measured by means of the shake flask method (15) using *n*-octanol as the non-polar phase and buffer pH 7.40 as the polar phase, with each phase being previously saturated with the other one. The concentration of samples in both the *n*-octanol and the buffer phases was determined by UV spectrophotometric analyses (Shimadzu UV-260) and applying the following equations which have been previously reported (9,10),

$$P = \left[ \frac{A_w^i - A_w^f}{A_w^f} \right] \qquad\qquad P = \left[ \frac{A_o^f}{A_o^i - A_o^f} \right]$$

where $A_w^i$, $A_o^i$ and $A_w^f$, $A_o^f$ represent the absorbance at 272.0 nm for each compound in the aqueous phase (*w*) and the organic phase (*o*), before (*i*) and after (*f*) distribution, respectively.

**Statistics**

All statistical procedures were run with Sigma Plot 8.0 for Windows, and Statistica for Windows R 4.5 programs. Deviations are given as 95% confidence intervals.

**RESULTS AND DISCUSSION**

In this paper, the lipophilicity of carbonate derivatives of lamivudine (**2-10**) shown in Figure 1 were analyzed by reversed-phase thin layer chromatography (RP-TLC), reversed-phase high performance liquid chromatography (RP-HPLC), the conventional shake-flask (log $P_{oct}$), and the theoretical CLOGP methods. The methodology was similar to that of chromatographic work previously carried out in our laboratory, which used RP-TLC and RP-HPLC analyses for different nucleoside derivatives of AZT, and provided the corresponding lipophilic parameters (9-11).

hydrophobic constant ($R_M$), since they have the considerable advantage of high stability, thus allowing their use over a large range of organic modifier contents. All compounds (**1**-**10**) were dissolved in methanol, reaching a final concentration of 1 mg/mL in the corresponding solvent. In order to determine the thermodynamically true solvent front position, potassium iodide (KI) was used. [8-11] A 50 µl aliquot of the test solution of each compound was applied to the plates in random positions. Methanol-buffer pH 7.40 and acetone-buffer pH 7.40 mixtures were used as mobile phases, with modifier contents being between 40 and 80 % (v/v) in 10 % increments. Finally, plates were dried at 40 ºC in an oven and developed with UV radiation. The thermodynamically true $R_M$ values were calculated according to the equation $R_M = \log [(1/R_f)-1]$ (9-11,13,14) where $R_f$ is the ratio of the migration distance of the analyte to that of the marker (KI) front distance from the start point.

**Reversed-phase high-performance liquid chromatography**

The high performance liquid chromatographic (HPLC) measurements of **1-10** were performed on an Agilent Series 1100 chromatograph, using an UV detector at $\lambda$ = 272 nm equipped with a Phenomenex® column, Hypersil ODS 10µ particle diameter of 250 mm in length, and 4.6 mm internal diameter, packed with a $C_{18}$ (octadecyl silane) chemically bonded non-polar stationary phase. Data were produced by means of a Peak Simple Chromatography Data System.® Methanol-buffer pH 7.4 mixtures were used as mobile phases, with a methanol content between 50% and 90% (v/v) in 10 % increments, at a flow-rate of 1 mL/min. The solutions were injected into the column by a 20 µL loop. Experiments were performed at room temperature. The capacity factor k´, was determined from the equation $k' = (tr-t_0)/t_0$, where tr is the retention time of the solute and $t_0$ is the hold-up time defined as the retention time of a non-retained compound (9-11,13,14).

## EXPERIMENTAL

### Chemicals

Cytidine (1-β-D-ribofuranosylcytosine, Cyt, **1**) was obtained from Sigma Co. Lamivudine (2´,3´-dideoxytiacytidine, 3TC, **2**) was a generous gift from Filaxis (Buenos Aires, Argentina). The novel compounds 3TC-Metha (2´,3´-dideoxy-3´-thiacytidin-5´-yl *O*-methyl carbonate, **3**), 3TC-Etha (2´,3´-dideoxy-3´-thiacytidin-5´-yl *O*-ethyl carbonate, **4**), 3TC-nPro (2´,3´-dideoxy-3´-thiacytidin-5´-yl *O*-propyl carbonate **5**), 3TC-2Pro (2´,3´-dideoxy-3´-thiacytidin-5´-yl *O*-2propyl carbonate, **6**), 3TC-Buta (2´,3´-dideoxy-3´-thiacytidin-5´-yl *O*-butyl carbonate, **7**), 3TC-Penta (2´,3´-dideoxy-3´-thiacytidin-5´-yl *O*-pentyl carbonate, **8**), 3TC-Hexa (2´,3´-dideoxy-3´-thiacytidin-5´-yl *O*-hexyl carbonate, **9**) and 3TC-Octa (2´,3´-dideoxy-3´-thiacytidin-5´-yl *O*-octyl carbonate, **10**) were prepared under our previously developed methodology (8). The structures of assayed compounds are shown in Figure 1.

Figure 1

Analytical grade *n*-octanol was purchased from Riedel-de Haën; acetone ($Me_2CO$) and methanol (MeOH) from Cicarelli, and HPLC grade methanol from Sintorgan. The water for HPLC was purified using a Milli-Q water-purification system (Millipore)® and mobile phases, as well as all solutions used for HPLC, were filtered through a Millipore® Type FH filter (0.45 μm pore size) and then vacuum degassed. Buffer pH 7.40 (12.5 mM) was prepared with potassium phosphate monobasic and sodium phosphate dibasic dihydrate (potassium phosphate monobasic 15.78%, sodium phosphate dibasic dihydrate 84.22%) in Milli-Q water. All other chemicals were of analytical-reagent grade and used as delivered.

### Reversed-phase thin layer chromatography

Precoated thin layer chromatographic plates (RP-18 HPTLC $F_{254}$, 5 x 10 cm) purchased from Merck (Darmstadt, Germany) were used for the measurements of the chromatographic

## INTRODUCTION

Lipophilicity is a molecular property of solute-solvent interactions, generally characterized in terms of partition coefficients. In order to quantify lipophilicity, the commonly accepted parameter is the logarithm of the partition coefficient, log P, where P is the partition coefficient of the neutral species in equilibrium between two immiscible solvents. Octanol is the most frequently used organic solvent, and the octanol-water partition coefficient is one of the parameters which influence the biological activity of a substance. When determining the partition coefficient by direct measurement using the shake flask equilibration method, problems arise such as poor reproducibility, time consuming experiments, and the fact that a reasonable quantity of the pure compound is needed. The alternative indirect methods are the chromatographic ones, reversed-phase thin layer chromatography (RP-TLC) and reversed-phase high performance liquid chromatography (RP-HPLC) (1-3). To replace log $P_{oct}$ with chromatographic lipophilicity parameters, the partitioning process in reversed-phase chromatography should mimic as closely as possible that occurring in the standard octanol-water partitioning system. In this way, a linear relationship between the retention parameters and the concentration of the organic modifier ($\varphi$) in the aqueous mobile phase has to be established for a successful chromatographic measurement of lipophilicity (4,5).

The purpose of the present study is to investigate the chromatographic behavior of a newly synthesized series of lamivudine derivatives with proven anti-HIV activity (6), by using RP-TLC and RP-HPLC, in order to establish if the linear relationships between $R_M$ = f(φ), and log $k'$ = f(φ), allow the extrapolation procedure (1,2). Also, a comparison with the experimental shake flask log $P_{oct}$ and those values calculated using the CLOGP computer program (7) is included.

**Lipophilicity of 5'-carbonates of lamivudine with antiretroviral activity.**

**Correlation between different methods**

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Short running title: **Lipophilicity of 5'-carbonates of lamivudine**

**ABSTRACT**

This study reports on the lipophilicity of novel 5'-carbonates of lamivudine with anti-HIV activity. Reversed-phase thin layer chromatography (RP-TLC), reversed-phase high performance liquid chromatography (RP-HPLC), conventional shake-flask (log $P_{oct}$), and the theoretical CLOGP methods were used, in order to establish if the linear relationships between the conventional and chromatographic methods permit an extrapolation procedure. The nature of the organic modifiers used in the chromatographic techniques did not substantially affect the measurement of lipophilicity, since a good correlation between experimental log $P_{oct}$ and extrapolated RP-TLC and RP-HPLC values ($R_{Mw}$ and log $k'_w$, respectively) supported the validity of the extrapolation technique.

**Keywords**: Lipophilicity, 5'-Carbonates of lamivudine, RP-TLC, RP-HPLC, log $P_{oct}$, CLOGP.