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Hydrolipophilic anomalies of some guanine derivatives

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Summary — The hydrolipophilic properties of some guanine derivatives, acyclovir, deoxyacyclovir and their acetyl congeners, were studied. The partition coefficients *n*-octanol/water (*P*) were determined by the shake-flask method and compared with calculated values obtained by Rekker's method. Additionally, the hydrolipophilic properties were determined by RPLC (reversed-phase liquid chromatography) method. It was established that the RPLC method does not give reliable results for hydrolipophilic properties of the tested guanine derivatives compared with the experimentally determined log*P* value. The calculated log*P* values of investigated derivatives differ significantly from those determined by experiment. The observed differences were discussed in terms of a keto–enol equilibrium and the reduction of contact lipophilic surface area.

guanine derivative / acyclovir / deoxyacyclovir / partition coefficient / RPLC method / Σf-value / Rekker approach

Introduction

The hydrolipophilic character of a compound is conventionally expressed as partition coefficient (*P*) and has an important role in phenomena of physicochemical, biological and environmental interest. Besides the classical determination of partition coefficients by the shake-flask method, other approaches can be used: filter probes, the generator column method and others [1]. These methods are rather laborious and require an analytical method and relatively large quantities of compound; they are sensitive to impurities and degradation. To avoid these problems, different reversed-phase HPLC (high performance liquid chromatography) retention parameters are often used to estimate partition coefficients [2–8].

Researchers using HPLC techniques apply different approaches. Some use untreated octadecylsilane as a stationary phase in reversed-phase liquid chromatography (RPLC) [2–5] with different aqueous phases containing organic modifiers (ie, methanol, acetonitrile, tetrahydrofuran, etc) as mobile phases. Others use different methods to block the active silanol sites (ie, ammonium salts [3] or capcell pak C_{18} column [2]). It was also reported that the partition coefficient in *n*-octanol/water (*P*) can be directly measured by the RPLC method, with an *n*-octanol-coated stationary phase and an *n*-octanol-saturated aqueous mobile phase [6, 7] where in some cases a good correlation with log*P* was obtained.

In estimating partition coefficients by HPLC, a choice must be made between using a capacity factor (*k*) determined at a particular mobile phase organic volume fraction [2, 4, 8] or linearly extrapolating the capacity factor to 0% organic (k_w) volume fraction [3, 5]. The linear extrapolation technique has been described as inappropriate for many large organic molecules [4], because plots of log *k* versus percentage MeOH in the mobile phase for some compounds intersect with each other, especially in the regions of low methanol concentrations [2]. A linear extrapolation can thus only be performed over a limited (and usually impractical) range [8]; the linear extrapolation technique is time consuming; and the logarithm of *k* is related quadratically to the mobile phase organic volume fraction [9, 10]. It seems that the choice of the correct capacity factor is questionable and can (to some extent) depend on the hydrolipophilic properties and the structure of the compounds involved in the study.

The log*P* are generally calculated according to Rekker, Hansch or other more sophisticated methods [11, 12]. However, for morphine [13] it was found that the metabolite morphine glucuronide has higher lipophilicity than the calculated value shows. The existence of a conformation with a reduced hydrophilic surface exposed to the solvent was suggested to explain this observation. Therefore the calculation of log*P* of complex structures may have a restricted

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value because none of the available methods can incorporate the effects of molecular conformation and hydrogen bonds into the calculation procedure. On the other hand, the specific geometry of organic phase, ie, the cell membrane, in comparison with approximately homogeneous octanol phase is also an important source of discrepancy between observed and determined log*P* values [14]. These two examples show the limitation of calculation approach and clearly indicate that all differences between experimental and calculated log*P* values should be carefully analyzed because they may reflect unknown and important properties of a complex molecule in a biological or model system.

In this study we determined hydrolipophilic properties of guanine derivatives: acyclovir (ACV), deoxyacyclovir (DCV) and their *O*-acetyl (OAcACV and OAcDCV), *N*-acetyl (NAcACV and NAcDCV) and *N*,*O*-diacetyl (diAcACV and diAcDCV) derivatives by conventional shake-flask method (*P*) and by RPLC, using octadecylsilane as a stationary phase and an aqueous mobile phase with methanol (MeOH) as the organic modifier. The log*P* values were also calculated by Rekker's method. Finally the results obtained by these different methods were compared and relative differences were discussed in view of the above-mentioned discrepancies. The possible explanation of differences due to a conformation in which a reduced molecular surface is accessible to the solvent is also proposed.

Results and discussion

The experimentally determined and calculated values (Rekker's method) of log*P* for the tested guanine derivatives as well as their differences are given in table I. Our experimentally determined $\log P_{oct}$ of ACV is in good accordance with experimentally determined value ($\log P_{oct}(ACV) = -1.47$) reported by other authors [15]. It is also evident that there is no difference between the calculated and experimentally determined values for ACV, NAcACV and DCV. In the series of ACV derivatives these values differ significantly in the case of OAcACV and diAcACV. The calculated log*P* values of OAcACV and diAcACV are higher by the same amount than their experimentally determined values indicating that both compounds are more hydrophilic than calculated log*P* values show.

We can conclude that *O*-acetylation is the reason for the equal reduction of lipophilicity in both cases. This observation can be explained by either the reduction of contact surface area between *O*-acetylated derivative and solvent or the presence of different tautomeric forms. The contact surface area reduction may be the consequence of conformational changes (ie, extended and folded) due to a different intramolecular interaction caused by *O*-acetylation. Esterification of terminal hydroxy group probably creates a condition for reduction of a solvent-accessible surface. We suggest that *O*-acetyl ACV derivatives prefer the folded conformation where their lipophilic surface is reduced as shown in figure 1. Structural formulae of ACV and DCV are presented in figure 2. On the other hand, *N*-acetylation in NAcACV does not show this discrepancy because the *N*-acetyl group cannot form a folded conformation (it is too short). As regards the differences between calculated and experimentally determined log*P* values for ACV and NAcACV, it can again be stressed that *N*-acetylation does not influence the possible keto–enol contribution to log*P*. These observations are confirmed in the case of diAcACV, for which all the effects are summarized in table I. A keto–enol equilibrium could be the

Table I. Log*P* values determined experimentally in *n*-octanol/water and calculated by Rekker's method and differences between these values.

Compound	*LogP (exp)*	*Δ*	*LogP (calc)*	*Δ'*	*Δ' – Δ*
ACV	–1.56	–	–1.55	–	–
NAcACV	–1.30	0.26	–1.26	0.29	0.03
OAcACV	–1.08	0.48	–0.58	0.97	0.49
diAcACV	–0.83	0.73	–0.29	1.26	0.53
DCV	–1.08	–	–1.05	–	–
NAcDCV	–1.33	–0.25	–0.76	0.29	0.54
OAcDCV	–0.61	0.47	–0.08	0.97	0.50
diAcDCV	–1.05	0.03	0.21	1.29	1.23

Δ: the difference between experimentally determined log*P* values = log*P* (acetylated ACV or DCV derivatives) – log*P* of parent molecule (ACV or DCV); Δ': the difference between calculated log*P* values = log*P* (acetylated ACV or DCV derivatives) – log*P* of parent molecule (ACV or DCV); Δ' – Δ: the difference between calculated and experimentally determined log*P* values.

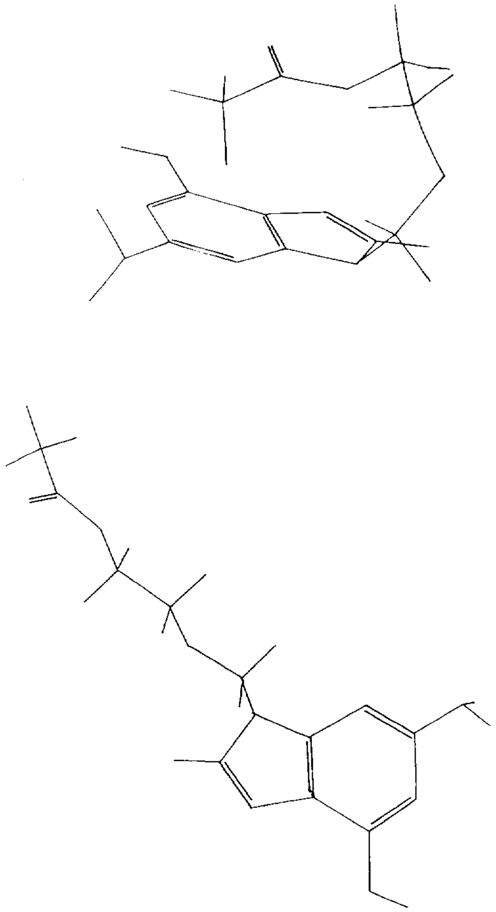

Fig 1. Molecule of OAcACV in extended and folded conformations.

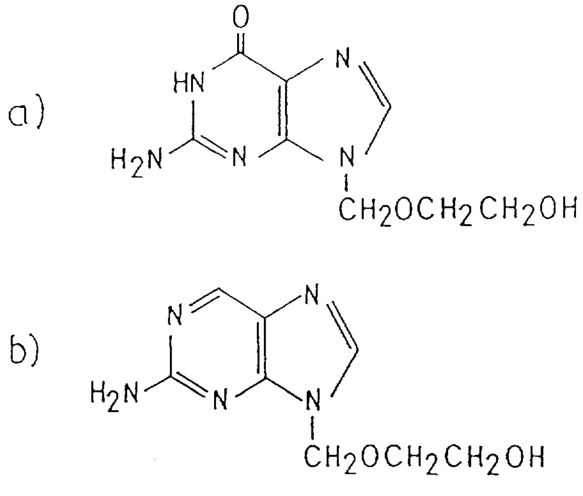

Fig 2. Structural formulas of **a**) ACV and **b**) DCV.

reason for the observed discrepancies only in the ACV series. However, the *O*-acetylation (OAcACV) is in the side chain far from purine ring and therefore cannot influence the equilibrium. *N*-Acetylation of ACV (close to the purine ring) could influence the keto–enol equilibrium, but it was found that experimental and calculated log*P* values agree.

Using ALCHEMY III software we found that extended and folded conformations of OAcACV and diAcACV differ slightly in potential energy calculated in a vacuum. The potential energies for OAcACV in extended and folded conformations are 23.2 and 24.2 kcal/mol, respectively. The difference in potential energies for both conformations in the case of diAcACV is also in the same range (≤ 1 kcal/mol). These small differences indicate that both conformations are possible in the gas phase. Therefore we assume that conversion from the extended to the folded form depends mostly on the environment (ie, solvent). Because of the reduction of lipophilicity by approximately 0.5 in both cases (the value for hydrophobic fragmental constant for CH_2 = 0.512), we assume that in the folded conformation the methyl group of the acetyl moiety and some of the hydrophobic surface of the purine ring are excluded from interaction with solvent (fig 1).

Similar effects were observed by Carrupt et al [13], who found that morphine metabolites (morphine 3- and 6-glucuronide) diffuse through hematoencephale barrier in larger amounts in spite of their hydrophilic character. They explained this phenomenon by different conformations of morphine 3- and 6-glucuronide, ie, the stretched conformation, which is more hydrophilic, and the folded one, which is more lipophilic. Further support for this assumption was given by Braumann [5], who reported that the calculated Hansch π substituent constants are not applicable to the description of the hydrophobicity of complex structures (ie, substituted cyclic nucleotides) where strong perturbing effects are exerted by substituents on the electrons of the heterocyclic ring. The additional electrons donated to the ring could be effectively delocalized, resulting in different tautomeric forms of cyclic nucleotides, which by an increase in the (hydrophobic) surface area enhances the overall hydrophobicity of the purine base.

When applying these observations to DCV derivatives we found that as well as the observed conformation phenomena in ACV series, there is still some unknown contribution that drastically reduces the lipophilicity of NAcDCV. In this series all the experimentally determined values log*P* for acetylated DCV derivatives are lower than the calculated ones. OAcDCV has the same reduction of $\log P$ as its OAcACV counterpart and the reduction of solvent-accessible surface can also be proposed as an explanation of this phenomenon. *N*-Acetylation causes reduction of lipophilicity which cannot be explained by the change of exposed surface, but rather by the above-mentioned electronic changes in the heterocyclic ring after acetylation [5]. This reaction causes the unexpected rise in hydrophilicity. It can be noticed that these effects are additive. Thus both conformational and electronic effects are summarized in the case of diAcDCV. Therefore diAcDCV is 18 times more polar than calculation shows.

The reason for observed differences between the experimentally determined and calculated $\log P$ values could also be due to the intermolecular interactions (ie, association) of solute molecules in *n*-octanol or the aqueous phase. We showed previously [16] that there is no intermolecular association of tested guanine derivatives even at concentrations close to saturation.

The hydrolipophilic properties of the tested substances were also determined by the RPLC method. Calculated $\log k$ values at different methanol concentrations in mobile phase are given in table II. Using these results and the least squares linear regression method, extrapolated values for $\log k_w$ (the intercepts on y axis) were obtained (table III, fig 3). In the concentration range 15–40% (v/v) MeOH in mobile phase, a linear relationship occurs between $\log k$ values and methanol concentration in the mobile phase, although some deviations from the linear line (obtained by the least squares linear regression method) can be observed.

Many authors have observed excellent correlations between $\log P_{oct}$ and $\log k_w$ for different types of compound [3, 5]. It was also reported that for large number of substances the capacity factor obtained in 100% water ($\log k_w$) is identical to $\log P$ [5]. Comparing the values for the experimentally determined $\log P$ and for $\log k_w$ the following relationship is obtained:

$\log P(\mathrm{ACV}) < \log P(\mathrm{NAcDCV}) \approx \log P(\mathrm{NAcACV}) < \log P(\mathrm{OAcACV}) \approx \log P(\mathrm{DCV}) \approx \log P(\mathrm{diAcDCV}) < \log P(\mathrm{diAcACV}) < \log P(\mathrm{OAcDCV})$

and

$\log k_w(\mathrm{ACV}) < \log k_w(\mathrm{DCV}) < \log k_w(\mathrm{NAcDCV}) < \log k_w(\mathrm{OAcACV}) < \log k_w(\mathrm{NAcACV}) < \log k_w(\mathrm{OAcDCV}) < \log k_w(\mathrm{diAcDCV}) < \log k_w(\mathrm{diAcACV})$

It is obvious that the relationship obtained for $\log P$ and $\log k_w$ values are significantly different and that one cannot get adequate information about real $\log P$ from the $\log k_w$ values in the case of the tested compounds. Although some plots intersect each other (fig 3), the results in table II showed that no significant improvement in relationship between $\log P$ and $\log k$ can be achieved using a capacity factor (k) determined at a particular mobile phase organic volume fraction.

On the basis of these results, we can establish only that intermolecular interactions between the solute, solvent and stationary phases of treated guanine derivatives in HPLC column are different from the solute–solvent interactions in the *n*-octanol/water system

Table II. Calculated $\log k$ values for tested guanine derivatives at different methanol concentrations (v/v) in mobile phase.

	Percentage MeOH (%)					
	15	*20*	*25*	*30*	*35*	*40*
ACV	–0.071 ± 0.006	–0.184 ± 0.012	–0.309 ± 0.021	–0.410 ± 0.023	–0.565 ± 0.034	–0.638 ± 0.028
NAcACV	0.521 ± 0.033	0.375 ± 0.025	0.132 ± 0.010	–0.021 ± 0.001	–0.191 ± 0.015	–0.359 ± 0.021
AcACV	0.442 ± 0.021	0.330 ± 0.016	0.143 ± 0.009	–0.022 ± 0.002	–0.103 ± 0.008	–0.253 ± 0.013
diAcACV	1.074 ± 0.062	0.889 ± 0.052	0.598 ± 0.039	0.383 ± 0.014	0.195 ± 0.011	–0.027 ± 0.002
DCV	0.217 ± 0.007	0.114 ± 0.006	–0.085 ± 0.005	–0.186 ± 0.007	–0.338 ± 0.014	–0.432 ± 0.021
NAcDCV	0.269 ± 0.008	0.158 ± 0.005	–0.067 ± 0.002	–0.176 ± 0.004	–0.348 ± 0.017	–0.447 ± 0.017
AcDCV	0.823 ± 0.034	0.663 ± 0.034	0.392 ± 0.012	0.261 ± 0.008	0.034 ± 0.002	–0.133 ± 0.004
diAcDCV	0.887 ± 0.029	0.725 ± 0.025	0.427 ± 0.013	0.266 ± 0.009	0.030 ± 0.001	–0.104 ± 0.006

Table III. The equations for log$k$ values of tested guanine derivatives versus percentage MeOH (v/v) in mobile phase obtained by the least squares linear regression method (using the data from table II) with the Pearsons coefficients of correlation (R).

ACV	$\log k = (0.278 \pm 0.023) - (0.023 \pm 0.001)$ (% MeOH), $R = 0.9975$
NAcACV	$\log k = (1.058 \pm 0.033) - (0.036 \pm 0.001)$ (% MeOH), $R = 0.9980$
OAcACV	$\log k = (0.865 \pm 0.039) - (0.028 \pm 0.001)$ (% MeOH), $R = 0.9953$
diAcACV	$\log k = (1.742 \pm 0.042) - (0.044 \pm 0.002)$ (% MeOH), $R = 0.9978$
DCV	$\log k = (0.621 \pm 0.037) - (0.027 \pm 0.001)$ (% MeOH), $R = 0.9954$
NAcDCV	$\log k = (0.716 \pm 0.044) - (0.030 \pm 0.002)$ (% MeOH), $R = 0.9947$
OAcDCV	$\log k = (1.396 \pm 0.046) - (0.038 \pm 0.002)$ (% MeOH), $R = 0.9965$
diAcDCV	$\log k = (1.503 \pm 0.057) - (0.041 \pm 0.002)$ (% MeOH), $R = 0.9954$

$\mathrm{Log}k = \log k_w - k\cdot$(% MEOH (v/v)).

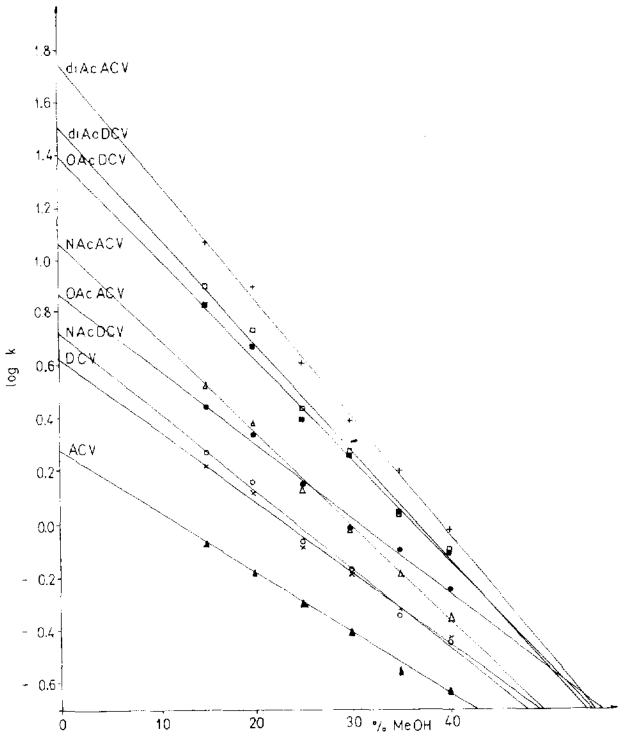

Fig 3. Relationship between log$k$ values of tested substances and methanol concentration (v/v %) in the mobile phase. The points represent experimentally determined values, the lines are obtained by the least squares linear regression method. The equations are given in table III. ▲ ACV; △ NAcACV; ● OAcACV; + diAcACV; x DCV; ○ NAcDCV; ■ OAcDCV; □ diAcDCV.

[17], ie, different solute dipolarity/polarizability, hydrogen bond acidity and basicity in octanol and water/methanol mixtures [18].

We can conclude that the tested guanine derivatives exhibit rather unexpected hydrolipophilic properties due to different conformations. Neither the calculation of log$P$ by Rekker's approach nor the determination of hydrolipophilic properties with RPLC method gives reliable results regarding log$P$ values determined by the conventional shake-flask method. It seems that neither method is appropriate for predicting the log$P$ values of substituted guanines and purines.

Experimental protocols

Reagents and solutions

ACV (9-(2-hydroxyethoxymethyl)guanine, NAcACV (N^2-acetylacyclovir), OAcACV (O-acetylacyclovir), diAcACV (N^2, O-diacetylacyclovir), DCV (2-amino-9-(2-hydroxyethoxymethyl)-9H-purine), NAcDCV (N^2-acetyldeoxyacyclovir), OAcDCV (O-acetyldeoxyacyclovir) and diAcDCV (N^2,O-diacetyldeoxyacyclovir) were synthesized at National Institute of Chemistry (Ljubljana, Slovenia) [19]. Anhydrous n-octanol (99%) purchased (Aldrich) was used for the experiments. The saturation of the solvents used for partition experiments was achieved through shaking of n-octanol with demineralized water for 1 h at room temperature as described previously [20]. The pK_a values of tested substances were determined by the spectrophotometric method and are the following: $pK_{a1} = 2.19$, $pK_{a2} = 9.25$ for ACV; $pK_{a1} = 2.27$, $pK_{a2} = 9.45$ for OAcACV; $pK_a = 8.54$ for NAcACV; $pK_a = 8.18$ for diAcACV; $pK_a = 3.63$ for DCV; $pK_a = 3.87$ for OAcDCV; $pK_a = 2.41$ for NAcDCV; and $pK_a = 2.39$ for diAcDCV.

Determination of P

The experiments were carried out in the same manner as reported previously [16]. Briefly, the same volumes of n-octanol and demineralized water (containing the appropriate amount of tested substances) were shaken for 1 h. After shaking the concentrations of the tested substances in both phases were determined by UV-vis spectrophotometry (Perkin–Elmer Lambda 15). The values for P were calculated by the equations [1] and [2]:

$$P = c_o/c_w \qquad [1]$$

$$P = (A_i - A_f)/A_f \qquad [2]$$

where c_o and c_w represent the concentrations in the organic and aqueous phases, respectively, A_i is the measured absorbance of the aqueous phase before shaking with *n*-octanol, and A_f is the corresponding value determined afler shaking. The results obtained by both approaches were in good agreement. Because of the weak ionization properties of tested compounds determined values for distribution coefficients (D) can be considered as real partition coefficient (P).

Determination of hydrophobic parameters by RPLC method

The RPLC analyses were performed on a system constructed from Knauer HPLC pump 64, injector Knauer A 0258, fitted with 20 μL loop, detector Knauer UV-vis filter photometer, Knauer recorder and Hewlet Packard HP 3396 series II integrator. The column used was Lichrospher RP-18, 5 μm, 125 x 4.6 mm id. The mobile phase consisted of MeOH and corresponding buffer with ionic strength $\mu = 0.1$. Buffers were chosen in such a manner that the ionization of tested substances was prevented [16, 20], ie, for ACV and DCV derivatives phosphate buffers with pH 5.7 and 7 were used, respectively.

The RPLC experiments were performed using mobile phases with methanol concentration varying from 15–40% (v/v). Capacity factor (k) was calculated as $k = (t_r - t_o)/t_o$, where t_r and t_o are the retention times of the sample and unretained solute (methanol), respectively. The experiments for each k determination were repeated at least three times. The content of the drug in methanol solutions was about 10 mg/L.

Calculation of logP by Rekker's method

The log$P$ values for tested derivatives were calculated by Rekker's method. The presented calculation approach gave us log$P$ values for ACV and DCV close to experimental values and enabled us to study the comparison of relative changes in log$P$ values after derivatization, which was the main aim of this work. Assuming that experimentally determined log$P$ value for guanine is –1.00 [11], we can calculate the log$P$ value for ACV in the following manner [12]:

$$\log P(\text{ACV}) = \log P(\text{guanine}) - f(\text{H(neg)}) + f(\text{O(al)}) + 3\,f(\text{CH}_2) + f(\text{OH}) + 6c_M = -1.55$$
$$c_M = 0.219$$

In a similar way, the log$P$ values for NAcACV, OAcACV and diAcACV were calculated (table I). The log$P$ values for DCV and its derivatives were calculated using experimentally determined log$P$ value for pyrimidine (log$P$ = –0.40) given in reference [12]. For instance, log$P$(DCV) was calculated in the following manner:

$$\log P(\text{DCV}) = \log P(\text{pyrimidine}) - 3f(\text{H}) + f(\text{NH}_2\text{(ar)}) + 2f(\text{N}) + 3f(\text{CH}_2) + f(\text{O(al)} + f(\text{OH(al)} + f(\text{CH}) + 18c_M = -1.05$$

The log$P$ values for the acetyl DCV derivatives were calculated in a similar manner.

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