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# Enzymatic alcoholysis of $SO_2$-uracil analog diacyclonucleosides. Long-distance effect of the substituents on the regioselectivity

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**Abstract**

Diacetoxy $SO_2$-uracil analog diacyclonucleosides have been deprotected via lipase-mediated alcoholysis. The reactivity and regioselectivity of the reaction are dependent on the steric hindrance of distant 5-substituents, even if they are as small as a methyl or a 4,5-trimethylene chain, and, in a lesser extent, the electronic or hydrogen bond interactions of 3-carbonyl and hydroxyl-chain groups. 

*Keywords:* Thiadiazine; Acyclonucleoside; Lipase; Alcoholysis; Regioselectivity

## 1. Introduction

Acyclonucleosides may be described as nucleoside analogues in general in which the ribose moiety has been replaced by a (poly)hydroxylic chain [1]. A research line of our group is aimed at studying the synthesis and biological properties of $SO_2$-uracil (1,1-dioxide-1,2,6-thiadiazine) analog acyclonucleosides [2]. These compounds can be readily obtained as acetoxy derivatives, followed by the deprotection of the hydroxy groups. This deprotection was successful when the acetoxy sugar [3] and monoacyclo [4] moieties of 4-quinolone nucleoside analogs were treated with ammonia-saturated methanol but, except with a few monoacyclic derivatives [2], the system afforded useless decomposition mixtures when it was applied to $SO_2$-uracil analogs.

Nowadays, enzymes are increasingly used in processes in which mild experimental conditions (unstable starting or final products) and/or some selectivity (enantio, regio, etc.) are searched. Because of some favourable features such as availability and activity in organic solvents, lipases are the most widely used enzymes in organic synthesis [5] and they were the selected *reagents* in our attempt to obtain the fully deprotected diacyclonucleoside and the two intermediate monoacetoxy derivatives to test them later as antiviral agents.

We have recently focused our attention on three $SO_2$-uracil sets (Fig. 1): unsubstituted **1a–d**, 5-methyl **2a–d** and 4,5-trimethylene (tetrahydrocyclopenta[*c*]thiadiazine) **3a–d**.

In a previous paper [2], we described a general method to cleave the acetoxy group of

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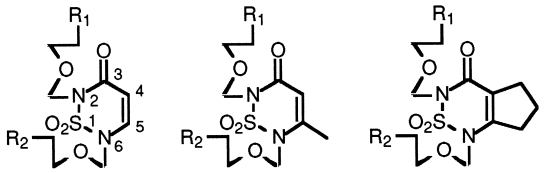



Fig. 1. **a**: $R_1 = R_2 = AcO$; **b**: $R_1 = OH$, $R_2 = AcO$; **c**: $R_1 = AcO$, $R_2 = OH$ **d**: $R_1 = R_2 = OH$.

thiadiazine diacyclonucleosides under very mild conditions and affording nearly quantitative yields of fully deacylated compounds by *Candida antarctica* lipase (CAL from hereon) catalyzed hydrolysis. This reaction was not selective in case of diacyclonucleosides **1a** and **2a**, but all **1b–d** and **2b–d** were obtained when the CAL-mediated hydrolysis of **1a** and **2a** was combined with the alcoholysis of **1a** and **2a** and the acylation of **1d** and **2d**, both regioselective reactions catalyzed by the lipase of *Pseudomonas cepacia* (PSL from now on). The alcoholysis of **2a** and the acylation of **2d** displayed a high regioselectivity and afforded 95:5 and 5:95 **2b:2c** mixtures, respectively [6]. This situation changed with the unsubstituted derivative **1a**. In this case, about 1:1 mixtures **1b:1c** were obtained by both alcoholysis of **1a** and acylation of **1d** [2].

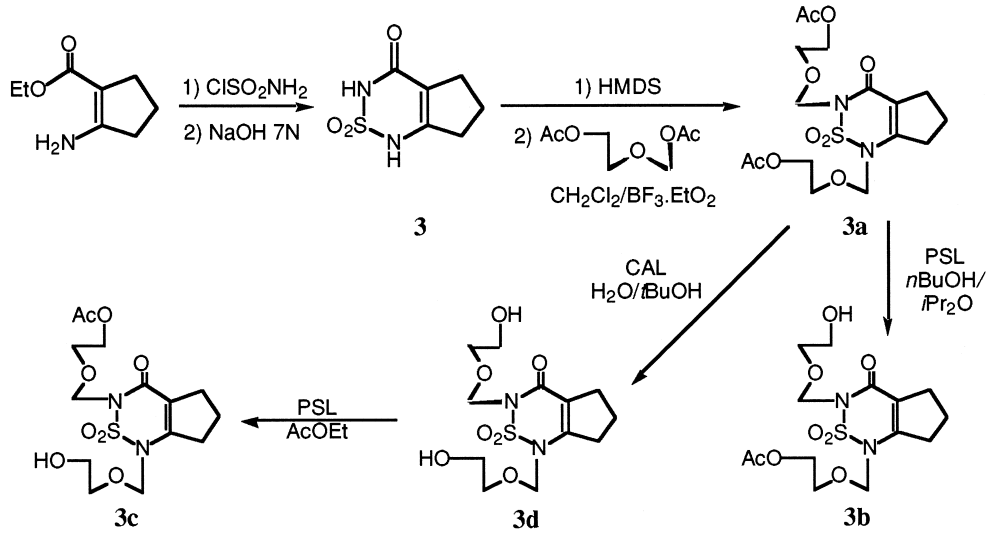
These results suggested that the regioselectivity of the reaction was dramatically affected by the C-5 substituent. In this paper we reported our studies with the new diacyclonucleoside **3a** in order to confirm that the reactivity and regioselectivity of the PSL-catalyzed alcoholysis of the diacyclonucleosides **1a–3a** depends on the 5- or 4,5-small ring substituents located at a 7–9 atoms distance of the side chains cleaving bonds.

## 2. Results and discussion

### *2.1. Synthesis and structural elucidation of new compounds*

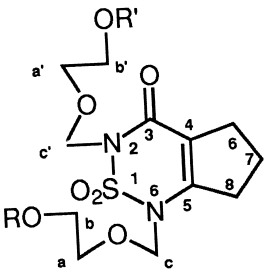
Following our research on potential antiviral compounds, we synthesized the 4,5-trimethylene thiadiazine derivative **3** by condensation of the corresponding $\beta$-enaminoester and sulfamoyl chloride in basic medium. The diacyclonucleoside **3a** was obtained in good yield following the silylation procedure [7] and then **3b–d** using enzymatic catalyzed reactions (Scheme 1).

The structures of compounds **3** and **3a–d** were established according to their analytical




Scheme 1. Synthesis of diacyclonucleosides of 4,5-trimethylenethiadiazine derivatives **3a–c**.

Table 1
$^{1}H$ and $^{13}C$ NMR chemical shifts of compound **3a–d** in $CDCl_3$




| Compound | H-6 | H-7 | H-8 | H-a | H-b | H-c | H-a′ | H-b′ | H-c′ | $CH_3CO$ |
|---|---|---|---|---|---|---|---|---|---|---|
| R = R′ = Ac **3a** | 2.74 (t) | 2.08 (m) | 2.87 (t) | 3.74 (m) | 4.19 (m) | 5.12 (s) | 3.81 (m) | 4.19 (m) | 5.34 (s) | 2.04, 2.05 |
| R = Ac, R′ = OH **3b** | 2.73 (t) | 2.09 (m) | 2.89 (t) | 3.74 (m) | 4.18 (m) | 5.15 (s) | 3.74 (m) | 3.74 (m) | 5.38 (s) | 2.06 (s) |
| R = OH, R′ = Ac **3c** | 2.75 (t) | 2.06 (m) | 2.88 (t) | 3.68 (m) | 3.68 (m) | 5.14 (s) | 3.81 (m) | 4.20 (m) | 5.34 (s) | 2.04 (s) |
| R = R′ = OH **3d** | 2.70 (m) | 2.06 (m) | 2.87 (m) | 3.69 (m) | 3.69 (m) | 5.14 (s) | 3.69 (m) | 3.69 (m) | 5.36 (s) | – |

| | C-3 | C-4 | C-5 | C-6 | C-7 | C-8 | C-a | C-b | C-c | C-a′ | C-b′ | C-c′ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R = R′ = Ac **3a**[a] | 160.35 | 116.18 | 152.59 | 28.51 | 20.26 | 32.33 | 67.44 | 62.70 | 76.43 | 67.12 | 63.00 | 72.29 |
| R = Ac, R′ = OH **3b**[b] | 160.48 | 116.40 | 152.69 | 28.52 | 20.25 | 32.22 | 71.30 | 62.91 | 76.92 | 67.32 | 61.81 | 72.51 |
| R = OH, R′ = Ac **3c**[c] | 160.38 | 116.29 | 152.55 | 28.59 | 20.62 | 32.50 | 70.83 | 63.05 | 77.11 | 67.55 | 61.48 | 72.30 |
| R = R′ = OH **3d** | 160.37 | 116.43 | 152.57 | 28.53 | 20.73 | 32.39 | 71.15 | 61.51 | 76.99 | 70.80 | 61.22 | 72.14 |

[a]170.90, 170.69 ($COCH_3$); 20.79, 20.71 ($COCH_3$).
[b]170.93 ($COCH_3$); 20.32 ($COCH_3$).
[c]170.91 ($COCH_3$); 20.32 ($COCH_3$).

Table 2
AA′BB′ system chemical shifts difference of compounds **3a–c**

| Compound | $\Delta\delta$ N(2) | $\Delta\delta$ N(6) |
|---|---|---|
| **3a** | 0.39 | 0.46 |
| **3b** | – | 0.44 |
| **3c** | 0.39 | – |

$\Delta\delta = \delta_b - \delta_a$ ($\delta$ in ppm).

Table 3
Alcoholysis[a] of **1–3a**

| Derivative | **a** | **b** | **c** | **d** |
|---|---|---|---|---|
| **1**[b] | 20 | 15 | 17 | 24 |
| **2**[c] | 22 | 60 | 3 | 9 |
| **3**[c] | 82 | 18 | n.d. | n.d. |

Results after 1 h.
[a] **1–3a** (10 mM), *n*-BuOH (60 mM) and PSL (10 mg/ml) in *i*-$Pr_2O$ at 45°C.
[b] Preparative scale, isolated product (%).
[c] Analytical scale, HPLC data (%).
n.d., not detected.

and spectroscopic data which are gathered in Table 1 and in the experimental part. For the unequivocal assignment of all chemical shifts, bidimensional NMR experiments (NOESY, HMQC [8] and HMBC [9]) were performed. As in previous diacyclonucleosides, it can be observed that substitution at N(2) produced a deshielding of the $CH_2$ protons directly linked, probably due to the anisotropy of the adjacent

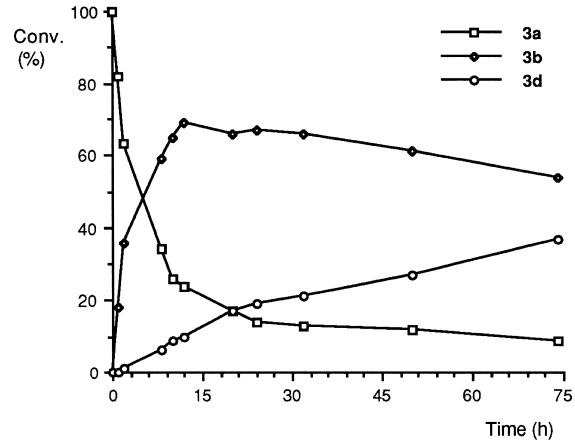


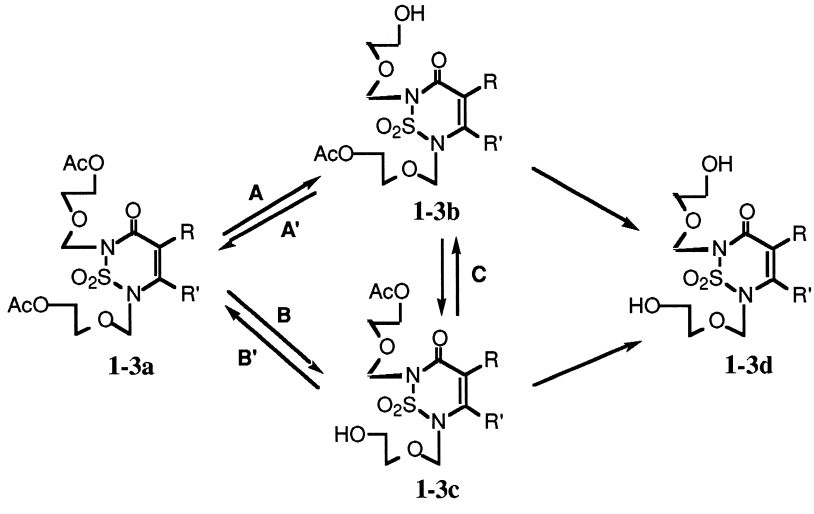

Fig. 2. Alcoholysis of **3a**. General experimental conditions.

C=O group, and, what is more important, reduced the difference in the chemical shifts split pattern of the AA′BB′ system of the acetoxymethoxy chain (Table 2). This chemical shifts difference rule is crucial for the unequivocal assignment of monodeprotected diacyclonucleosides **3b** and **3c**.

## 2.2. Enzymatic reactions

### 2.2.1. Alcoholysis of *3a*

The alcoholysis rate of **3a** was lower than that of **1a** and **2a** (Table 3) but it took place in a more regioselective manner (Scheme 2). The




Scheme 2. The general scheme of PSL-catalyzed alcoholysis.

route **B** was not significant and **3d** was mainly formed via **A**: no **3c** was detected and the increasing slope in the formation curve of **3d** is only slightly higher than the decreasing one of **3b** (Fig. 2). These data point out a high influence of the 5-(or 4,5-)substituents, even when they are as small as a methyl or a trimethylene chain, on the rate and regioselectivity of the reaction.

### 2.2.2. Alcoholysis of *3b* and *3c*

In order to check possible differences between the two regioisomers **3b** and **3c**, they were separately subjected to alcoholysis. The two reactions took place at a slower rate than with **1** and **2** substrates (Table 4) and both **3b** and **3c** were exclusively converted into dihydroxy **3d**. Moreover, their same-order reactivity confirmed that the regioselective reaction is the alcoholysis of **3a** into **3b** while **3c** is not appreciably formed. The presence of a hydroxy group at the end of the 2-chain facilitates a conformation that allows the alcoholysis of the 6-chain acetoxy group.

### 2.2.3. Alcoholysis of *1b* and *1c*

The structural difference between the 2- and 6-side chains in **1a** lies on their relative position to the 3-carbonyl group, so, we wanted to check its possible role in the regioselectivity of the reaction. Unsubstituted intermediates **1b** and **1c** were chromatographically separated [2] and subjected to alcoholysis with *n*-BuOH at the same experimental conditions. The progress curves of both reactions are shown in the Figs. 3 and 4.

In both cases, we found the surprising result that a pure monoacetoxy regioisomer was converted, in addition to the final dihydroxy **1d**, into the other regioisomer and even into the initial diacetoxy **1a**. The conversion of one regioisomer into the other (routes **C**) can take place when a unique molecule of the monoacetoxy substrate undergoes the two steps [in short: (1) alcoholysis of the ester group by the enzyme PSL-Ser-OH and (2) nucleophilic attack to the PSL-Ser-OAc formed] of the enzymatic alcoholysis in two different sites. For example, when the PSL-Ser-O-Ac entity formed from the 6-chain acetoxy group of **1b** is subjected to a nucleophilic attack by the 2-chain hydroxy group from the same molecule (in fact a newly formed

Table 4
**3d** formed[a] by alcoholysis[b] of **3b** and **3c**

| Substrate | 1 h | 3 h | 5 h | 7 h | 20 h | 45 h |
|---|---|---|---|---|---|---|
| **3b** | 6.6 | 16.0 | 19.1 | 23.7 | 39.0 | 50.2 |
| **3c** | 10.5 | 24.6 | 29.9 | 36.4 | 58.2 | 78.5 |

[a]Conversion (%), analytical scale, HPLC data.
[b]General experimental conditions.

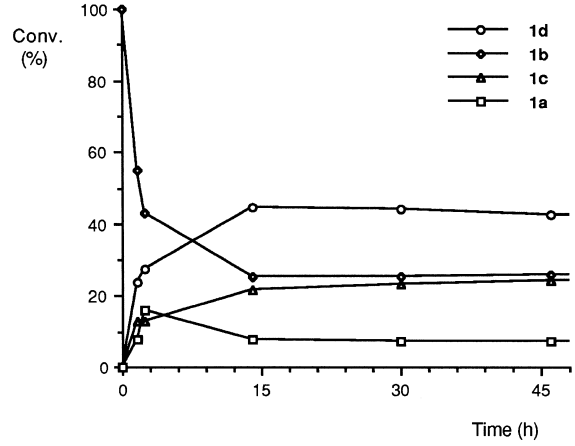



Fig. 3. Alcoholysis of **1b**. General experimental conditions.

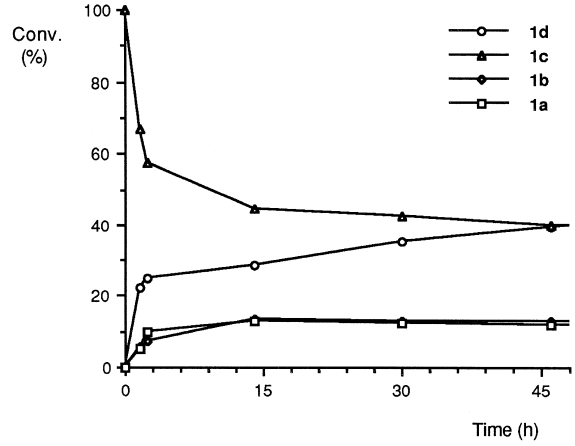



Fig. 4. Alcoholysis of **1c**. General experimental conditions.

dihydroxy **1d**). Original diacetoxy **1a** would be formed when the nucleophilic attack on PSL-Ser-OAc is carried out by a molecule of monohydroxy **1b** or **1c** (routes **A′** and **B′**).

These reactions require a definite conformation which is apparently possible only in the absence of a 5-substituent. The progress curves plotted in the Figs. 3 and 4 are rather different: they point out that the 3-carbonyl group interacts with some point of the enzyme (polar residue or hydrogen bond network) and leads to an enzyme–substrate complex preferred conformation. As a result, the formation of one regioisomer from the other is more favoured in the alcoholysis of **1b**, where at equilibrium the ratio **1b:1c** is about 1:1 while in the case of **1c** is roughly 1:4. Significant amounts of diacetoxy **1a** were produced in both reactions.

## 3. Conclusions

These results point out that the sterical hindrance displayed by the 5-(or 4,5-)substituents influences the substrate conformation inside the active site, although they are as far as 7–9 atoms and nearly 6 and 10 Å [10] from the cleaving bonds of both side chains. Consequently, the rate and regioselectivity of the reaction are affected by them. In absence of these substituents, the electronic interactions of the 3-carbonyl group also have an important effect. Our results are in agreement with two recently published simultaneous articles describing the X-ray structure of crystallized Amano lipase PS [11] and a comparison of three crystal structure [12] obtained by three independent groups from Genzyme and Amano *P. cepacia* lipases. According to them, the serine of the triad lies at the bottom of an ovoid hole 10 Å × 25 Å and about 15 Å deep: although the distance between the 5-substituent and the cleaving bond of the 2-side chain is nearly 10 Å, the active site pocket is deep enough to enclose the whole molecule and the 5-hydrocarbon substituent can interact with the hydrophobic residues surrounding the active site and induce a preferred conformation. At the same time, the presence of the 5-substituent avoids the conformational changes needed to convert one regioisomer into the other.

## 4. Experimental

### *4.1. Synthesis of substrates*

#### *4.1.1. Chemical synthesis*

Column chromatography was performed on Merck silicagel 60 (70–230 mesh). $^{1}$H NMR spectra were obtained on Varian XL-300 and Gemini-200 spectrometers operating at 300 and 200 MHz, respectively. Typical spectral parameters were: spectral width 10 ppm, pulse width 9 $\mu$s (57°), data size 32 K. NOE difference spectra were measured under the same conditions, using a presaturation time of 3 s. $^{13}$C NMR experiments were carried out on the Varian Gemini-200 spectrometer operating at 50 Mhz. The acquisition parameters were: spectral width 16 kHz, acquisition time 0.99 s, pulse width 9 $\mu$s (57°) and data size 32 K.

*4.1.1.1. 1,5,6,7-Tetrahydrocyclopenta[c]-[1,2,6]thiadiazin-4(3h)-one 2,2-dioxide* ***(3)***. To a cooled solution (5°C) of ethyl 2-amino-1-cyclopentylcarboxylate [13] (2.86 g, 0.02 mol) in benzene (40 ml), freshly sulfamoyl chloride [14] (3 g, 0.03 mol) dissolved in benzene (10 ml) was added. The mixture was stirring for 1 h at room temperature. Then, a solution of sodium hydroxide 7 N (40 ml) was added, and vigorous stirring was continued for 24 h more. The aqueous phase was separated, acidified with hydrogen chloride 12 N until pH = 1 and extracted with dichloromethane (3 × 40 ml). The organic phase was dried over sodium sulphate, and evaporated under reduced pressure. The residue was chromatographed on silica gel column eluting with $CH_2Cl_2$:MeOH (15:1) to give **3** (1.9 g, 51%) as a white solid, m.p.: 178–179°C. $^{1}$H NMR (DMSO-$d_6$, 200 MHz) $\delta$: 2.63 (t, 2H, $J_{CH_2,CH_2}$ = 6.7 Hz, H-7), 2.48 (t, 2H, $J_{CH_2,CH_2}$ =

6.7 Hz, H-5), 2.33 (m, 2H, $J_{CH_2,CH_2}$ = 6.7 Hz, H-6). $^{13}$C NMR (DMSO-$d_6$, 50 MHz) $\delta$: 165.21 (C-4), 159.93 (C-7a), 101.54 (C-4a), 32.51 (C-7), 26.79 (C-6), 20.47 (C-5). Anal. Calc. for $C_6H_8N_2O_3S$: C, 38.25; H, 4.25; N, 14.88; S, 17.00. Found: C, 38.23; H, 4.26; N, 14.89; S, 17.02.

*4.1.1.2. 1,3-Di[(2-acetoxyethoxy)methyl]-5,6,7-trihydrocyclopenta[c][1,2,6]thiadiazin-4-one 2,2-dioxide* ***(3a)***. To a solution in dichloromethane (25 ml) of the silyl derivative of **3** prepared by refluxing the base (0.56 g, 0.003 mol) in hexamethyldisilazane (9 ml) and ammonium sulphate (catalytic amounts) under nitrogen, the 2-acetoxyethyl acetoxymethyl ether [15] (0.42 g, 0.003 mol) dissolved in dichloromethane (25 ml) was added. The mixture was cooled, and $BF_3 \cdot Et_2O$ (0.58 ml, 0.004 mol) was added with vigorous stirring and exclusion of moisture. The resulting mixture was stirred for 3 h at room temperature, and was then shaken with saturated sodium hydrogen carbonate solution (50 ml). The organic phase was separated, dried over sodium sulphate, and evaporated under reduced pressure. The residue was chromatographed on silica gel column eluting with ethyl acetate: hexane (1:1) to give **3a** (0.55 g, 45%) as a colorless syrup. Anal. Calc. for $C_{16}H_{24}N_2O_9S$: C, 45.71; H, 5.75; N, 6.66; S, 7.63. Found: C, 45.68; H, 5.49; N, 6.81; S, 7.84.

### *4.1.2. Enzymatic synthesis*

*4.1.2.1. 1-[(2-Acetoxyethoxy)methyl]-3-[(2-hydroxyethoxy) methyl] -5,6,7- trihydrocyclopenta-[c]-[1,2,6]thiadiazin-4-one 2,2-dioxide* ***(3b)***. Lipase PS (254 mg) was added to a solution of **3a** (100 mg, 23.6 mmol) and *n*-BuOH (140 ml, 1.523 mmol) in *i*-$Pr_2O$ (25.4 ml). The mixture was incubated (45°C, 250 rpm) for 2 h. Then, the enzyme was removed by filtration, washed with acetone and the combined filtrates evaporated to dryness. The residue was purified by CCTLC (EtAcO/hexane 1:1) isolated the monoacylated derivative **3b** (45 mg, 60%). Anal. Calc. for $C_{14}H_{22}N_2O_8S$: C, 44.39; H, 5.81; N, 7.39; S, 8.45. Found: C, 44.56; H, 5.90; N, 7.73; S, 8.19.

*4.1.2.2. 3-[(2-Acetoxyethoxy)methyl]-1-[(2-hydroxyethoxy) methyl] -5,6,7- trihydrocyclopenta-[c]-[1,2,6]thiadiazin-4-one 2,2-dioxide* ***(3c)***. Lipase PS (30 mg) was added to a solution of the dihydroxy compound **3d** (10 mg, 0.03 mmol) in EtAcO (3 ml). The reaction mixture was shaken (45°C, 250 rpm) for 24 h. Then, the enzyme was filtered off, washed with EtAcO and the combined filtrates evaporated to dryness under reduced pressure. Purification by CCTLC using EtAcO/MeOH (40:1) as eluent gave compound **3c** (3.5 mg, 31%) as a colorless syrup. Anal. Calc. for $C_{14}H_{22}N_2O_8S$: C, 44.39; H, 5.81; N, 7.39; S, 8.45. Found: C, 44.25; H, 5.65; N, 7.57; S, 8.25.

*4.1.2.3. 1,3-di[(2-hydroxyethoxy)methyl]-5,6,7-trihydrocyclopenta[c][1,2,6]thiadiazin-4-one 2,2-dioxide* ***(3d)***. CAL (254 mg) was added to a solution of **3a** (33 mg, 7.8 mmol) in a mixture of *t*-BuOH/citrate–phosphate buffer pH:7.0 (9:1) (25.4 ml) and stirred in a thermostatized orbital shaker (45°C, 250 rpm) for 4 h. Then the enzyme was filtered off, washed with acetone and the combined filtrates evaporated to dryness under reduced pressure, to give compound **3d** (24 mg, 91%) as a colorless syrup. Anal. Calc. for $C_{12}H_{20}N_2O_7S$: C, 42.85; H, 5.99; N, 8.33; S, 9.53. Found: C, 42.65; H, 6.01; N, 8.22; S, 9.76.

### *4.2. Enzymatic reactions*

Analytical HPLC was performed on a Beckman chromatograph using a Waters Delta Pak C-18 column (3.9 × 150 mm), eluted with different proportions of acetonitrile and a 0.05% trifluoroacetic acid aqueous solution at a flow rate of 1 ml/min and UV detector at $\lambda$: 264 nm. Diisopropylether was refluxed on sodium

wire, distilled and stored on molecular sieves 4 Å powder before using. Commercial lipase PS (Amano) from *P. cepacia* was used without any purification.

*4.2.1. General procedure*

The reactions were carried out at an analytical scale in sealed screw-cap 2-ml vials containing 1.5 ml of the reaction mixture. Lipase PS (10 mg/ml) was added to a solution of the diacyclonucleoside derivative (10 mM) and *n*-BuOH (60 mM) in anhydrous diisopropylether. Aliquots were periodically withdrawn and analyzed by HPLC by comparing with authentic samples.

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