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New 1,2,6-Thiadiazine Dioxide Acyclonucleosides: Synthesis and Antiviral Evaluation

Ana I. Esteban,[a] Olga Juanes,[a] Santiago Conde,[a] Pilar Goya,[a] Erik De Clercq[b] and Ana Martínez[a]*

[a]*Instituto de Química Médica (CSIC), Juan de la Cierva, 3. 28006 Madrid, Spain.*

[b]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium.*

Abstract—New acyclonucleosides derived from 1,2,6-thiadiazine dioxide systems have been synthesized. Lipase-mediated deacylation procedure was used to obtain the deprotected derivatives. All the newly prepared compounds were tested as antiviral agents, but none of them showed significant activity.

Introduction

The emergence of Acyclovir, 9-[(2-hydroxyethoxy)methyl] guanine,[1] as an excellent antiviral agent has stimulated the synthesis of a wide variety of acyclic nucleosides modified either in the base moiety or the acyclic part.[2] New compounds have been obtained, not only with potent anti-herpes activity,[3] but also with anti-HIV activity, such as the pyrimidine nucleoside HEPT, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine.[4] It has been found that the nature of the heterocyclic base plays an important biochemical role.[5]

In this context, and continuing our work in this field,[6] we report here the first synthesis of 1,2,6-thiadiazine dioxide acyclonucleosides. In all cases, the side chain introduced was the (hydroxyethoxy)methyl,[7] as present in both acyclovir and HEPT.

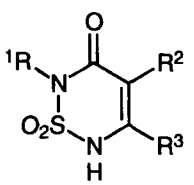
Results and Discussion

Synthesis

The thiadiazine dioxides **1–5** chosen for this first study were related to natural bases cytosine and uracil (Fig. 1) and were prepared according to described procedures (see Experimental section).

R^1=H R^2=NH_2 **1**

R^1=CN R^2=H **2**



R^1=R^3=H R^2=CO_2Et **3**

R^1=Ph R^2=H R^3=CH_3 **4**

R^1=R^2=R^3=H **5**

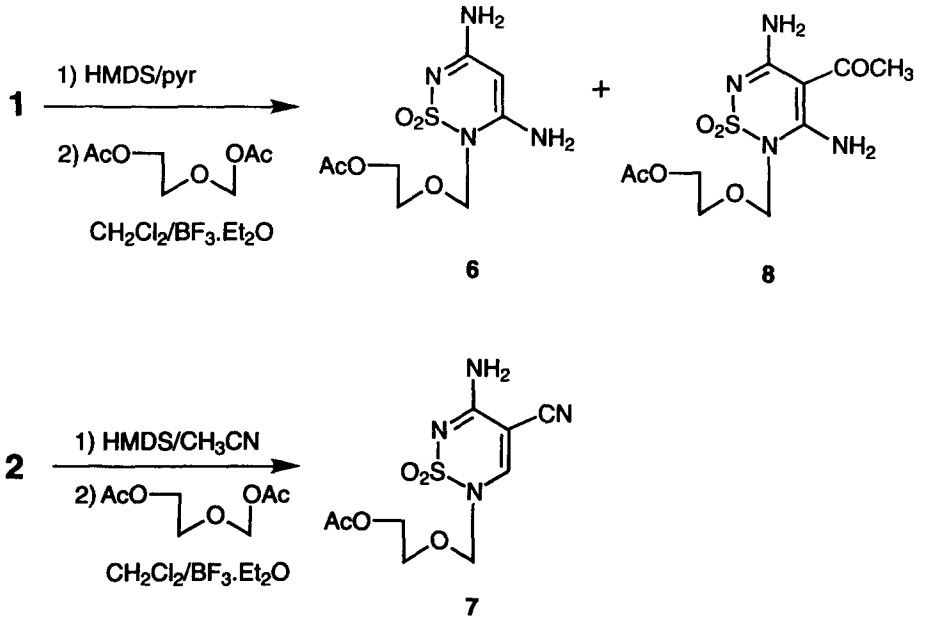
Figure 1.

The synthesis of the acyclonucleosides was achieved using the silylation procedure,[8] thus thiadiazines **1** and **2** were first silylated using hexamethyldisilazane as silylating agent and solvent under a nitrogen atmosphere. Co-solvents were necessary in both cases. In the first one, pyridine was used under the same conditions described for the preparation of related nucleosides,[9] and in the second one, acetonitrile was found to be the most suitable.

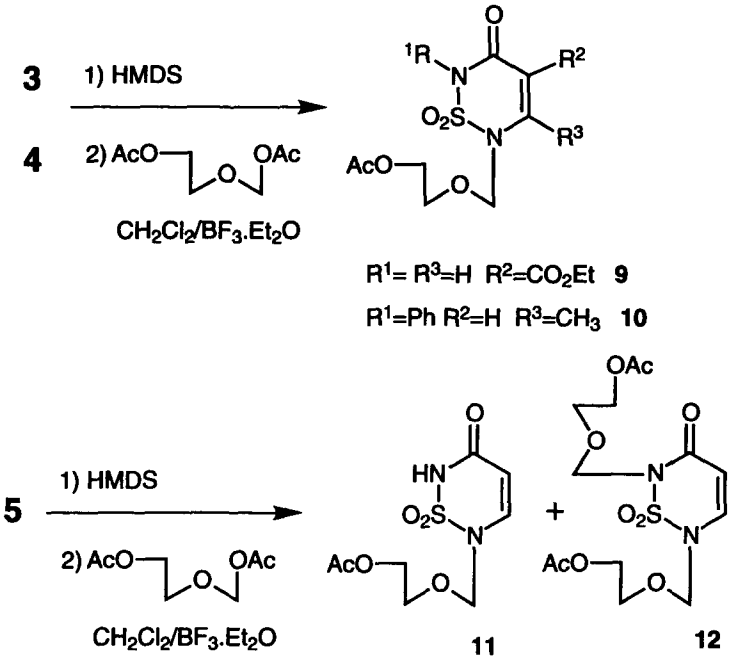
Reaction of these silyl derivatives with 2-acetoxyethyl acetoxymethyl ether in dichloromethane and boron trifluoride as catalyst afforded acyclonucleosides **6** and **7**, respectively (Scheme 1). In the case of diaminothiadiazine **1**, a mixture of the expected compound **6** and the 4-acyl derivative **8** was obtained. This last compound can be generated by a Friedel–Crafts acylation with acetic acid/acetyl chloride and excess Lewis acid. It is known that the 4-position of thiadiazine **1** reacts readily with electrophiles.[10]

Following the same synthetic pathway, 4-oxothiadiazines **3–5** were silylated in the absence of co-solvent and reacted with 2-acetoxyethyl acetoxymethyl ether yielding, in the case of **3** and **4**, the N-6 acyclonucleosides **9** and **10**, respectively (Scheme 2). For compound **5** the reaction product was a mixture of compounds from which predominantly diacyclonucleoside **12** (53%) could be isolated together with traces of the N(6)-monosubstituted derivative **11** (Scheme 2). However, using stannic tetrachloride as the catalyst in the glycosylation step, the overall yield decreases (15%) while the ratio of **11**:**12** changes to 1:3.

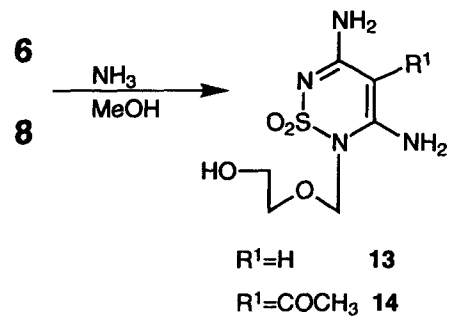
Deprotection of all newly prepared compounds **6–12** was attempted with methanolic ammonia. The procedure was successful with **6** and **8** (Scheme 3), affording yields over 90%, but mixtures of decomposition products were obtained in the other cases.



Scheme 1.



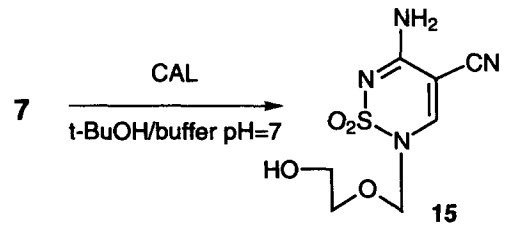
Scheme 2.

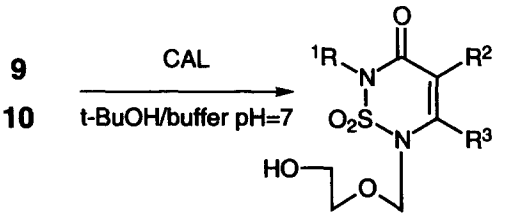


Scheme 3.

In view of these results, a lipase mediated deacylation was attempted. This previously described methodology,[11] has been applied to the regioselective deacylation of a diacyclic nucleoside of the SO_2-analog of 6-methyluracil.[12] Thus, deprotected acyclonucleosides **15–17** were obtained in quantitative yield (Scheme 4) when *Candida antarctica* lipase (CAL) was used under mild hydrolysis conditions (*t*-BuOH:buffer, pH 7, 9:1).

Fully deacylated diacyclonucleoside **18** was synthesized following this methodology, while the mono-





R^1=R^3=H R^2=CO_2Et **16**

R^1=Ph R^2=H R^3=CH_3 **17**

Scheme 4.

deprotected nucleosides were achieved with the *Pseudomonas cepacia* lipase (PSL) in alcohol (*n*-BuOH/$^iPr_2O$) (Scheme 5). In this case, the reaction exhibited no regioselectivity and yielded an equimolar mixture of the two isomers **19** + **20**, which could be separated and isolated by column chromatography. Lipase-mediated acylation of compound **18** yielded the same equimolar mixture of isomers **19** and **20**. This fact is in contrast with the high selectivity found in previous studies[12] in which, following the same experimental conditions, the 5-methyl derivative of **12** was selectively deacylated in the N(2)-chain while the N(6)-chain acetyl group remained unchanged.

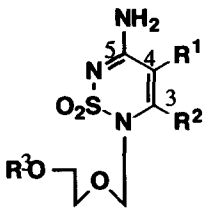
Structural assignments

The structures of all the new compounds were elucidated according to analytical and spectroscopic data, which are gathered in Tables 1–4.

The site of glycosylation was determined on the basis of NOE experiments. Unequivocal assignment of all chemical shifts for all acyclonucleosides (^1H and ^{13}C) was done using sequences of HMQC[13] for one bond correlation and HMBC[14] for long distance correlations. As a general feature, it is worth mentioning that deprotection of the acyclic moiety produced a shielding over the adjacent methylene protons and thus, the AA'BB' present in acetoxyethoxy chain changed to a AA'A"A"' system in the case of the hydroxyethoxy chain.

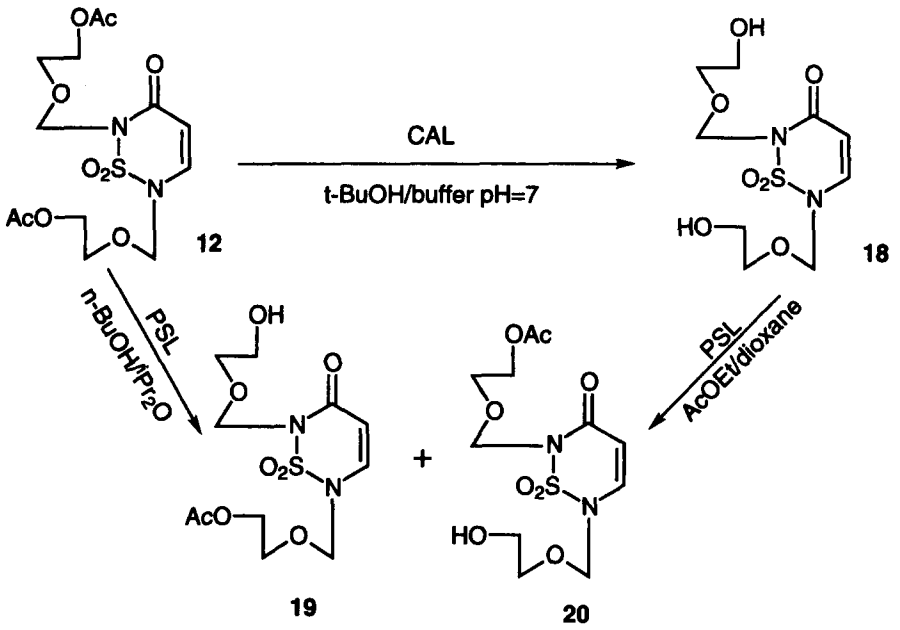
In the case of diacyclonucleoside **12**, the ^{13}C NMR chemical shifts of N-methylene carbons were between δ 79 and 72, which clearly ruled out the possibility that

Table 1. ^1H NMR chemical shifts (ppm) of compounds **6–8** and **13–15** in DMSO-d_6



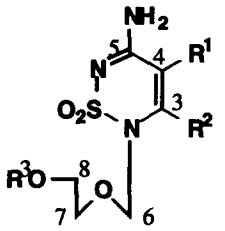
Compound		H-3	H-4	NCH_2O	$AcOCH_2$	CH_2O	CH_3CO	NH_2
R^1=H R^2=NH_2 R^3=Ac	**6**	-	4.70 (s)	5.10 (s)	4.10 (m)	3.70 (m)	1.99 (s)	6.75 (s)
R^1=CN R^2=H R^3=Ac	**7**	8.55 (s)	-	5.13 (s)	4.10 (m)	3.71 (m)	1.99 (s)	8.71(s) 8.19(s)
R^1=$COCH_3$ R^2=NH_2 R^3=Ac	**8**[a]	-	-	5.23 (s)	4.12 (m)	3.73 (m)	1.98 (s)	7.77 (s)
R^1=H R^2=NH_2 R^3=H	**13**	-	4.69 (s)	5.07 (s)	3.51 (m)		-	6.71 (s)
R^1=$COCH_3$ R^2=NH_2 R^3=H	**14**[b]	-	-	5.21 (s)	3.54 (m)		-	7.75 (s)
R^1=CN R^2=H R^3=H	**15**	8.22 (s)	-	5.15 (s)	3.62 (m)		-	-

[a] 2.33 (*s*, 3H, CH_3CO-Het).
[b] 2.32 (*s*, 3H, CH_3CO-Het).



Scheme 5.

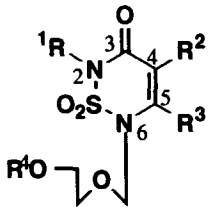
Table 2. ^{13}C NMR chemical shifts (ppm) of compounds 6–8 and 13–15 in DMSO-d_6



Compound		C-3	C-4	C-5	C-6	C-7	C-8	C=O	CH_3CO
R^1=H R^2=NH_2 R^3=Ac	6	156.51	71.31	163.58	72.99	66.04	62.97	170.60	20.95
R^1=CN R^2=H R^3=Ac	7[a]	155.26	78.41	159.49	80.27	66.89	62.94	171.13	20.79
R^1=$COCH_3$ R^2=NH_2 R^3=Ac	8[b]	158.62	91.21	164.32	74.18	66.56	62.88	170.88	20.84
R^1=H R^2=NH_2 R^3=H	13	156.75	71.22	163.56	73.64	69.88	59.99	-	-
R^1=$COCH_3$ R^2=NH_2 R^3=H	14[c]	158.58	91.10	164.15	74.21	69.96	59.63	-	-
R^1=CN R^2=H R^3=H	15[d]	156.40	81.26	162.13	82.30	73.11	62.78	-	-

[a] 114.52 (*C*N).
[b] 29.68 ($\underline{C}H_3CO$-Het), 195.36 ($CH_3\underline{C}O$-Het).
[c] 29.46 ($\underline{C}H_3CO$-Het), 194.91 ($CH_3\underline{C}O$-Het).
[d] 116.13 (*C*N).

Table 3. ^{1}H NMR Chemical shifts (ppm) and coupling constants (Hz) of compounds **9–12** and **16–20**



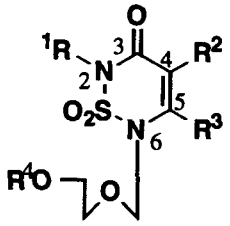
Compound		H-4	H-5	N(6)				N(2)				Solvent
				NCH_2O	R^4OCH_2	CH_2O	CH_3CO	NCH_2O	R^4OCH_2	CH_2O	CH_3CO	
R^1=R^3=H R^2=CO_2Et R^4=Ac	**9**[a]	-	8.12 (s)	5.07 (s)	4.15 (m)	3.76 (m)	1.98 (s)	-	-	-	-	$CDCl_3$
R^1=Ph R^2=H R^3=CH_3 R^4=Ac	**10**[b]	5.79 (d)	2.27 (d)	5.20 (s)	4.18 (m)	3.77 (m)	2.00 (s)	-	-	-	-	$CDCl_3$
R^1=R^2=H R^3=H R^4=Ac	**11**[c]	5.59 (d)	7.34 (d)	5.24 (s)	4.39 (m)	3.98 (m)	2.12 (s)	-	-	-	-	CD_3OD
R^1=Acethox. R^2=R^3=H R^4=Ac	**12**[d]	5.78 (d)	7.09 (d)	5.11 (s)	4.20 (m)	3.76 (m)	2.05 (s)	5.36 (s)	4.20 (m)	3.83 (m)	2.08 (s)	$CDCl_3$
R^1=R^3=H R^2=CO_2Et R^4=H	**16**[e]	-	8.34 (s)	5.14 (s)	—3.59— (m)		-	-	-	-	-	CD_3OD
R^1=Ph R^2=H R^3=CH_3 R^4=H	**17**[f]	6.01 (d)	2.35 (d)	5.30 (s)	—3.56— (m)		-	-	-	-	-	DMSO
R^1=Hydroxy. R^2=R^3=H R^4=H	**18**[g]	5.78 (d)	7.72 (d)	5.19 (s)	—3.36— (m)		-	5.23 (s)	—3.36— (m)		-	DMSO
R^1=Hydroxy. R^2=R^3=H R^4=Ac	**19**[h]	5.79 (d)	7.11 (d)	5.13 (s)	4.22 (s)	3.77 (m)	1.99 (s)	5.39 (s)	—3.75— (m)		-	$CDCl_3$
R^1=Acethox. R^2=R^3=H R^4=H	**20**[i]	5.77 (d)	7.12 (d)	5.15 (s)	—3.70— (m)		-	5.37 (s)	4.21 (m)	3.83 (m)	2.00 (s)	$CDCl_3$

[a] 4.20 (*q*, 2H, J = 7.5 Hz, CH_2CH_3), 1.18 (*t*, 3H, J = 7.5 Hz, CH_2CH_3).
[b] $J_{CHO,H4}$ = 1 Hz, 7.45–7.39 (*m*, 5H, Ph).
[c] $J_{H4,H5}$ = 8 Hz.
[d] $J_{H4,H5}$ = 8.3 Hz.
[e] 4.21 (*q*, 2H, J = 7.1 Hz, CH_2CH_3), 1.20 (*t*, 3H, J = 7.1 Hz, CH_2CH_3).
[f] $J_{CHO,H4}$ = 1 Hz, 7.55–7.29 (*m*, 5H, Ph).
[g] $J_{H4,H5}$ = 8.3 Hz.
[h] $J_{H4,H5}$ = 8.3 Hz.
[i] $J_{H4,H5}$ = 8.3 Hz.
Acethox. = acethoxyethoxymethyl; Hydroxy. = hydroxyethoxymethyl.

an O-substitution had taken place. So, compound **12** was confirmed as a N(2),N(6)-diacyclonucleoside. The O-CH_2- protons linked to N(6) were assigned by means of a NOE experiment. Thus, irradiation of H-5 (δ 7.09) showed a 12% NOE effect on the singlet at δ 5.20. The remaining protons were attributed by means of two-dimensional experiments (HMQC and HMBC). As a result, we could observe in the ^{1}H NMR spectrum that substitution at N(2) produced a deshielding of the CH_2 protons directly linked and reduced the difference in the chemical shifts split pattern of the AA'BB' system of the acetoxy-methoxy chain (Table 3). This is probably due to the influence of the adjacent C=O group anisotropy.

The structures of the monoacylated diacyclonucleosides **19** and **20** were determined using the AA'BB' system chemical shift difference of the acylated chain (see Table 5). The value of Δδ led us to establish the position of the remaining acetyl group. The unequivocal assignment of all signals was done by means of NOE and HMQC experiments.

Table 4. ^{13}C NMR Chemical shifts (ppm) of compounds **9–12** and **16–20**



Compound		C-3	C-4	C-5	N(6)					N(2)					Solvent
					NCH_2O	R^4OCH_2	CH_2O	CH_3CO	CH_3CO	NCH_2O	R^4OCH_2	CH_2O	CH_3CO	CH_3CO	
R^1=R^3=H R^2=CO_2Et R^4=Ac	**9**[a]	166.42	99.21	151.77	78.97	62.97	67.54	20.60	170.89	-	-	-	-	-	$CDCl_3$
R^1=Ph R^2=H R^3=CH_3 R^4=Ac	**10**[b]	161.20	107.45	149.77	76.12	62.43	66.88	19.18	170.42	-	-	-	-	-	$CDCl_3$
R^1=R^2=H R^3=H R^4=Ac	**11**	172.81	101.25	143.21	79.22	64.20	67.60	20.75	184.30	-	-	-	-	-	CD_3OD
R^1=Acethox. R^2=R^3=H R^4=Ac	**12**	161.94	104.79	140.24	78.75	62.80	67.19	20.66	170.76	72.26	62.49	67.46	20.61	170.60	$CDCl_3$
R^1=R^3=H R^2=CO_2Et R^4=H	**16**[c]	162.47	101.20	153.57	81.02	62.30	72.31	-	-	-	-	-	-	-	CD_3OD
R^1=Ph R^2=H R^3=CH_3 R^4=H	**17**[d]	160.69	106.04	151.08	76.42	59.36	70.27	-	-	-	-	-	-	-	DMSO-d_6
R^1=Hydroxy. R^2=R^3=H R^4=H	**18**	170.49	92.74	142.13	80.40	62.10	72.50	-	-	79.50	61.90	71.80	-	-	CD_3OD
R^1=Hydroxy. R^2=R^3=H R^4=Ac	**19**	161.98	105.19	140.00	78.83	62.67	67.41	20.84	184.70	72.50	61.58	71.33	-	-	$CDCl_3$
R^1=Acethox. R^2=R^3=H R^4=H	**20**	162.05	104.94	140.26	79.38	61.48	72.39	-	-	75.87	67.67	70.96	20.83	184.38	$CDCl_3$

[a] 14.08 ($\underline{C}H_3$-CH_2), 61.90 (CH_3-$\underline{C}H_2$), 169.22 ($\underline{C}$OOEt).
[b] 20.45 (CH_3-5), 129.03 (*Co*), 129.38 (C*m*), 129.61 (C*p*), 131.27 (C*i*).
[c] 14.94 ($\underline{C}H_3$-CH_2), 63.01 (CH_3-$\underline{C}H_2$), 168.94 ($\underline{C}$OOEt).
[d] 18.59 ($\underline{C}H_3$-5), 128.94 (*Co*), 129.37 (C*m*), 129.17 (C*p*), 131.27 (C*i*).
Acethox. = acethoxyethoxymethyl; Hydroxy. = hydroxyethoxymethyl.

Table 5. AA'BB' system chemical shifts difference of compounds **12**, **19** and **20**

Comp.	Solvent	Δδ N(2)	Δδ N(6)
12	$CDCl_3$	0.37	0.44
19	$CDCl_3$	-	0.45
20	$CDCl_3$	0.38	-

$\Delta\delta = \delta_B - \delta_A$ (δ in ppm) (A = O-CH_2-CH_2-OAc; B = O-CH_2-CH_2-OAc)

Biological evaluation

The new 1,2,6-thiadiazine dioxide acyclonucleosides synthesized (**6–20**) were evaluated for their antiviral activity in a wide variety of assay systems: herpes simplex virus type 1 (strains KOS, F. McIntyre), herpes simplex virus type 2 (strains G, 196, Lyons), thymidine kinase-deficient (TK^-) herpes simplex virus type 1 (strains B 2006, VMW 1837), vaccinia virus and vesicular stomatitis virus in E_6SM cells; vesicular stomatitis virus, poliovirus type 1 and Coxsakie B4 virus in HeLa cells; parainfluenza virus type 3, reovirus type 1, Sindbis virus, Coxsackie B4 virus and Semliki forest virus in Vero Cells; HIV types 1 and 2 in T-lymphocyte (MT-4) cells. However, no antiviral activity was noted in any system (at compound concentrations up to 400 μg mL^{-1}).

Experimental

Melting points were determined with a Reichert-Jung Thermovar and are uncorrected. CC was performed on Merck silica gel 60 (70–230 mesh).^{1}H NMR spectra were obtained at 298 K using TMS as internal standard on a Varian-Gemini 200 and a Varian XL-300, operating at 200 and 300 MHz, respectively.^{13}C NMR spectra were recorded with a Varian-Gemini 200 and a Bruker AM-200, operating at 50 MHz and using TMS as internal reference. *Candida antarctica* lipase used was the Novo Nordisk's immobilized preparation Novozym 435. *Pseudomonas cepacia* lipase used was Amano's preparation Lipase PS.

General procedure for glycosylation

To a solution in CH_2Cl_2 (25 mL) of the silyl derivative of the 1,2,6-thiadiazine 1,1-dioxide (**1–5**) (1 mmol) prepared by refluxing the base in hexamethyldisilazane (3 mL) under nitrogen using suitable catalyst and co-solvents, 2-acetoxyethyl acetoxymethyl ether[7] dissolved in CH_2Cl_2 (25 mL) was added. The mixture was cooled, and BF_3·Et_2O (1.40 mmol) was added under vigorous stirring and exclusion of moisture. The resulting mixture was stirred at room temperature for 1–6 h, and was then shaken with saturated $NaHCO_3$ solution (50 mL). The organic phase was separated, dried over sodium sulfate, and evaporated under reduced pressure. The residue was chromatographed on a silica gel column, using as eluent mixtures of solvents in the proportions indicated for each particular case.

2-[(2-Acetoxyethoxy)methyl]-3,5-diamino-1,2,6-thiadiazine 1,1-dioxide (6) and 2-[(2-acetoxyethoxy)methyl]-4-acetyl-3,5-diamino-1,2,6-thiadiazine 1,1-dioxide (8). Following the general procedure, the silyl derivative of **1**[15] (1.62 g, 10 mmol) [catalyst: trimethylchlorosilane, TCS, (1 mL); cosolvent: pyridine (30 mL)], was treated with 2-acetoxyethyl acetoxymethyl ether (1.76 g, 10 mmol), at room temperature for 6 h. After work-up, the syrupy residue obtained was chromatographed on a silica gel column. Thus, eluting with CH_2Cl_2:MeOH, 50:1, the 4-acyl nucleoside **8** (0.124 g, 5%) was obtained: mp 159–160 °C. (Found: C, 37.46; H, 5.10; N, 17.33; S, 10.20. $C_{10}H_{16}N_4O_6S$ requires C, 37.50; H, 5.00; N, 17.50; S, 10.00%).

When CH_2Cl_2:MeOH, 20:1 was used as eluent, compound **6** (0.230 g, 7%) was isolated: mp 139–140 °C (Found: C, 34.45; H, 5.39; N, 19.91; S, 11.43. $C_8H_{14}N_4O_5S$ requires C, 34.53; H, 5.07; N, 20.14; S, 11.55%).

2-[(2-Acetoxyethoxy)methyl]-5-amino-4-cyano-1,2,6-thiadiazine 1,1-dioxide (7). According to the general procedure, the silyl derivate of **2**[9] (0.172 g, 1 mmol) [catalyst: TCS (1 mL), co-solvent: acetonitrile (2 mL)], was treated with 2-acetoxyethyl acetoxymethyl ether (0.176 g, 1 mmol), for 2 h at room temperature. After work-up, the residue crystallized from dichloromethane to give **7** (0.091 g, 32%): mp 117–118 °C. (Found: C, 37.25; H, 3.89; N, 19.40; S, 11.21. $C_9H_{12}N_4O_5S$ requires C, 37.50; H, 4.20; N, 19.40; S, 11.10%).

6-[(2-Acetoxyethoxy)methyl]-4-ethoxycarbonyl-1,2,6-thiadiazin-3(2H)-one 1,1-dioxide (**9**). Following the general procedure, the silyl derivative of the thiadiazine **3**[10] (0.220 g, 1 mmol) [catalyst: $SO_4(NH_4)_2$], was treated with 2-acetoxyethyl acetoxymethyl ether (0.176 g, 1 mmol) for 2 h at room temperature. After work-up, the residue was purified on silica gel CC, eluting with CH_2Cl_2:MeOH 25:1, to yield **9** (0.067 g, 20%): mp 146–148 °C. (Found: C, 39.40; H, 4.60; N, 8.10; S, 9.80. $C_{11}H_{16}N_2O_8S$ requires C, 39.50; H, 4.80; N, 8.30; S, 9.50%).

6-[(2-Acetoxyethoxy)methyl]-2-phenyl-5-methyl-1,2,6-thiadiazin-3(2H)-one 1,1-dioxide (10). Following the general procedure, the silyl derivative of **4**[16] (0.238 g, 1 mmol) [catalyst: TCS (1 mL), co-solvent: acetonitrile (2 mL)], reacted with 2-acetoxyethyl acetoxymethyl ether (0.176 g, 1 mmol), for 2 h at room temperature. After work-up, the residue was purified on silica gel CC, using CH_2Cl_2:MeOH 0.5 % as eluent, to give **10** (0.223 g, 63%) as a syrup. (Found: C, 51.14; H, 5.13; N, 7.66; S, 8.64. $C_{15}H_{18}N_2O_8S$ requires C, 50.85; H, 5.08; N, 7.91; S, 9.04%).

6-[(2-Acetoxyethoxy)methyl]-1,2,6-thiadiazin-3(2H)-one 1,1-dioxide (11) and 2,6-di[(2-acetoxyethoxy)methyl]-1,2,6-thiadiazin-3(2H)-one 1,1-dioxide (12). Following the general method, the silyl derivative of thiadiazine **5**[17] (0.296 g, 2 mmol) [catalyst: TCS (1 mL)], was treated with 2-acetoxyethyl acetoxymethyl ether (0.176

g, 1 mmol) for 3 h at room temperature. After work-up, the residue was purified on silica gel CC, eluting with CH_2Cl_2:MeOH as eluent. The first fractions (CH_2Cl_2:MeOH, 100:1), yielded the diacyclonucleoside **12** (0.395 g, 52%) as a syrup. (Found: C, 40.98; H, 5.60; N, 7.51; S, 8.41. $C_{13}H_{20}N_2O_9S$ requires C, 41.05; H, 5.31; N, 7.36; S, 8.61%). From the last fractions (CH_2Cl_2:MeOH, 20:1) the monoacyclonucleoside **11** (0.005 g, 1%) was obtained as a syrup. (Found: C, 36.12; H, 4.16; N, 10.56; S, 12.20. $C_8H_{11}N_2O_6S$ requires C, 36.50; H, 4.18; N, 10.65; S, 12.17%).

Using $SnCl_4$ in the glycosylation reaction, and following the same purification method, the yields obtained for compounds **11** and **12** were 4 and 11%, respectively.

2-[(2-Hydroxyethoxy)methyl]-3,5-diamino-1,2,6-thiadiazine 1,1-dioxide (13). A solution of compound **6** (0.080 g, 0.279 mmol) in saturated methanolic ammonia solution (15 mL), was stirred at room temperature for 8 h. The solvent was evaporated to dryness to yield **13** (0.066 g, 99%): mp 126–127 °C. (Found: C, 30.26; H, 4.97; N, 23.68; S, 13.55. $C_6H_{12}N_4O_4S$ requires C, 30.51; H, 5.08; N, 23.73; S, 13.56%).

2-[(2-Hydroxyethoxy)methyl]-4-acetyl-3,5-diamino-1,2,6-thiadiazine 1,1-dioxide (14). A solution of compound **8** (0.109 g, 0.341 mmol) in saturated methanolic ammonia solution (15 mL), was allowed to stand at room temperature for 3 h. The solvent was eliminated under reduced pressure to yield **14** (0.088 g, 90%): mp 144–146 ° C. (Found: C, 34.82; H, 5.42; N, 20.44; S, 11.90. $C_8H_{14}N_4O_5S$ requires C, 34.53; H, 5.03; N, 20.14; S, 11.51%).

General procedure for the enzymatic cleavage of acetyl groups

A solution of the acetylated acyclonucleoside in *t*-BuOH:buffer pH 7 (90:10) was incubated with 10 mg mL^{-1} of *Candida antarctica* lipase (CAL), at 45 °C and 250 rpm in an orbital shaker. When all the starting material had disappeared, the enzyme was removed by filtration and washed with methanol. The filtrate was evaporated *in vacuo* and the residue obtained purified using chromatography techniques.

2-[2-(Hydroxyethoxy)methyl]-5-amino-4-cyano-1,2,6-thiadiazine 1,1-dioxide (15). Following the general enzymatic procedure, compound **7** (0.029 g, 1 mmol) was incubated during 45 min. Then, the enzyme was removed by filtration and the filtrate evaporated under reduced pressure to yield **15** (0.024 g, 98%), as a syrup. (Found: C, 34.21; H, 4.10; N, 22.90; S, 13.34. $C_7H_{10}N_4O_4S$ requires C, 34.15; H, 4.06; N, 22.76; S, 13.01%).

6-[(2-Hydroxyethoxy)methyl]-4-ethoxycarbonyl-1,2,6-thiadiazin-3(2H)-one 1,1-dioxide (**16**). Following the general enzymatic procedure, compound **9** (0.022 g, 0.065 mmol) was incubated during 45 min. Then, the enzyme was removed by filtration and the filtrate evaporated under reduced pressure to yield **16** (0.015 g, 83%): mp 132–133 °C. (Found: C, 36.51; H, 4.91; N, 9.41; S, 11.10. $C_9H_{14}N_2O_7S$ requires C, 36.73; H, 4.76; N, 9.52; S, 10.88%).

6-[(2-Hydroxyethoxy)methyl]-2-phenyl-5-methyl-1,2,6-thiadiazin-3(2H)-one 1,1-dioxide (17). Method (a). A solution of compound **10** (0.212 g, 0.599 mmol) in saturated methanolic ammonia solution (15 mL) was allowed to stand at room temperature for 4 h. The solvent was eliminated *in vacuo* and the residue was chromatographed on silica gel column eluting with CH_2Cl_2:MeOH (50:1) to yield **17** (0.043 g, 23%); mp 107–108 °C.

Method (b). According to the general enzymatic procedure, compound **10** (0.040 g, 0.104 mmol) was incubated during 1 h. The enzyme was filtered off and the filtrate evaporated *in vacuo* to yield **17** (0.032 g, 90%): mp 107–108 °C. (Found: C, 50.32; H, 5.25; N, 8.82; S, 10.51. $C_{13}H_{16}N_2O_5S$ requires C, 50.00; H, 5.13; N, 8.97; S, 10.25%).

2,6-Di[(2-hydroxyethoxy)methyl]-1,2,6-thiadiazin-3(2H)-one 1,1-dioxide (**18**). Following the general enzymatic procedure, compound **12** (0.140 g, 0.368 mmol) was incubated during 5 h. The enzyme was filtered off and the filtrate evaporated. Compound **18** (0.108 g, 99%) was obtained as a syrup. (Found: C, 36.49 H, 5.65; N, 9.57; S, 10.84. $C_9H_{16}N_2O_7S$ requires C, 36.48; H, 5.40; N, 9.46; S, 10.81%).

6-[(2-Acetoxyethoxy)methyl]-2-[(2-hydroxyethoxy)methyl]-1,2,6-thiadiazin-3(2H)-one 1,1-dioxide (19) and 6-[(2-acetoxyethoxy) methyl]-2-[(2-hydroxyethoxy)methyl]-1,2,6-thiadiazin-3(2H)-one 1,1-dioxide (20). Method (a). To a solution of *n*-butanol (60 mM) and the acetylated acyclonucleoside **12** (0.065 g, 0.254 mmol) in $^iPr_2O$ (17.22 mL) *Pseudomonas* sp. lipase (PSL) (10 mg mL^{-1}) were added. The reaction mixture was incubated at 45 °C in an orbital shaker (250 rpm). After 1 h, the enzyme was filtered off and washed with MeOH. The filtrate was evaporated under reduced pressure and the residue which was a mixture of partially deacylated products was separated by chromatography methods using silica gel and EtOAc:hexane 1:1 as eluent. Compound **19** (0.009 g, 15%) was obtained as a syrup. (Found: C, 39.32; H, 5.18; N, 7.98; S, 9.16. $C_{11}H_{18}N_2O_8S$ requires C, 39.05; H, 5.36; N, 8.28; S, 9.47%). Compound **20** (0.011 g, 18%) was isolated as a syrup. (Found: C, 39.02; H, 5.42; N, 7.98; S, 9.31. $C_{11}H_{18}N_2O_8S$ requires C, 39.05; H, 5.36; N, 8.28; S, 9.47%).

Method (b). A solution of compound **18** (0.004 g, 0.013 mmol) in EtOAc (1.34 mL) and dioxane (0.02 mL) as co-solvent, was incubated during 1 h with the PSL. After the enzyme was filtered off, and the solvent evaporated, the residue was analyzed by HPLC using CH_3CN:buffer pH 7 (25:75) as eluent. An equimolar mixture of the above described acyclonucleosides **19** and **20** was detected.

Biological evaluation

The compounds were evaluated for antiviral activity following well established procedures, as reviewed in Ref. 18.

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