Pergamon

Tetrahedron Vol. 50, No. 48, pp. 13865-13870, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00934-1

Regioselective Lipase-Mediated Acylation-Deacylation in Thiadiazine Diacyclonucleosides.

Ana I. Esteban, Olga Juanes, Ana Martínez*, Santiago Conde

Instituto de Química Médica (C.S.I.C.). Juan de la Cierva 3. 28006 Madrid. Spain.

Abstract.- The first thiadiazine acyclonucleosides has been prepared, and so acyclic glycosylation of the SO_2 analog of 6-methyluracil has been achieved. Regioselective deprotection of thiadiazine diacyclonucleoside has been performed by a combined strategy of enzyme-catalyzed hydrolysis-acylation.

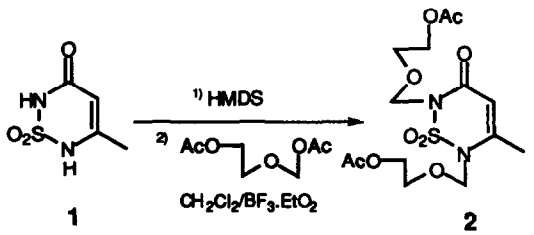
Nucleosides analogues in which the heterocyclic and/or the sugar moiety have been modified are important targets when seeking compounds with biological activity.[1] Recently, acyclonucleosides, in which the ribose moiety has been replaced by a polyhydroxylic chain,[2] have been shown very useful in antiviral therapy.[3] The nature of the heterocyclic base has been found to play an important biochemical role[4] and they are also described changes in the activity when the sugar moiety is di-, mono- or unacylated.[5]

Modification of only one out of several hydroxy or ester groups of very similar reactivity in a molecule is a task as frequent as difficult to solve in organic synthesis. The difficulty of these processes becomes considerably increased in nucleoside chemistry because the derivatives and intermediates are usually unstable compounds and its manipulation requires mild experimental conditions. Application of enzymes to nucleoside chemistry[6] gives good results as they present a high catalytic activity under very mild experimental conditions, combined with great selectivity and specificity. As far as our knowledge, the only one precedent of enzymatic acylation of acyclonucleosides was described by our group.[7]

Continuing with our work on nucleosides studies,[8] here we report the synthesis and the regioselective lipase-mediated deprotection of a diacyclic nucleoside of the SO_2 analog of 6-methyluracil.[9]

Results and Discussion

The glycosylation method used was the silyl procedure,[10] and thus, thiadiazine **1** was first silylated with hexamethyldisilazane under a nitrogen atmosphere in the same conditions used for the obtention of glucosyl derivatives.[11] Reaction of this silyl derivative with 2-acethoxyethyl acethoxymethyl ether[12] in dichloromethane and boron trifluoride as catalyst, afforded the diacyclonucleoside **2** as the only product (Scheme 1).



Scheme 1

One of the target of this work is the difficult problem of distinguishing between two acetyl groups situated at the end of identical linear chains with very similar chemical environments. Lipase-catalyzed methodology looked like a suitable pathway to solve this aim.

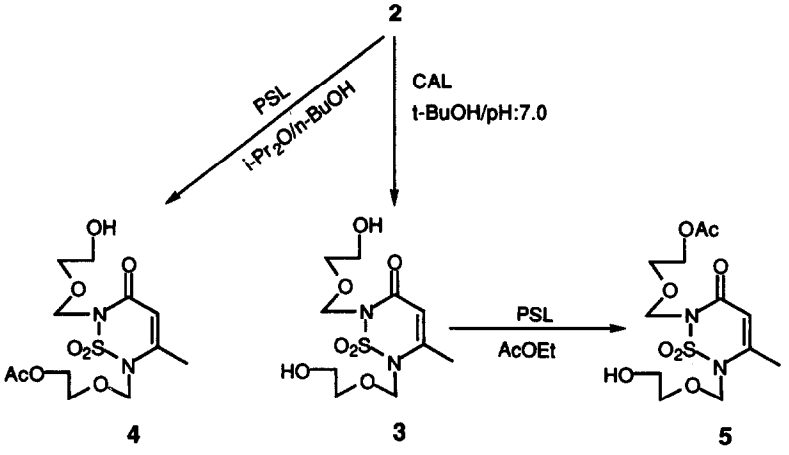
We made an initial screening of several commercial lipases[13] in a standard experiment: lipases (10 mg/mL) were added to a solution of **2** (10 mM) and n-BuOH (60 mM) in anhydrous i-Pr_2O (1.5 mL in screw-cap vials of 2mL). The resulting suspensions were incubated in an orbital shaker at 45°C and 250 r.p.m. Aliquots were taken and analyzed by HPLC (acetonitril/phosphate buffer pH 7.1 30:70) and TLC (CH_2Cl_2/MeOH 10:1) (Table 1). No conversion was detected in a blank reaction without enzyme.

Table 1.- Transesterifications with n-BuOH. Conversions[a] after 2 hours

Enzyme[b]	Recovered **2**	Intermediate **4 + 5**	Final **3**
CAL	2	13	85
MML	16	45	39
PSL	16	64	20
PPL	83	14	3

[a] Determined by HPLC. [b] No reaction was detected when both CRL enzymes were used.

The yeast lipase CAL displayed high activity but no selectivity, so, it was the enzyme selected to obtain the free acyclonucleoside **3**. A mild hydrolysis in a t-BuOH - buffer pH:7.0 (9:1) system was also checked and it proved to be even more efficient than alcoholysis, affording a nearly quantitative yield of pure derivative **3** in a 5 h reaction (Scheme 2).



Scheme 2

The bacterial lipase PSL appeared as the most suitable enzyme to isolate the intermediate products **4** and **5** (Table 1). First, it was studied the kinetic course of the reaction by HPLC (see Figure 1). Curves showed that the highest rate of the mixture **4 + 5** was located at 2 hours. It was found a regioisomer ratio **4**:**5** of 95:5 when the reaction was stopped at this time and the crude analyzed by ^{1}H-NMR. Pure monoacetylated diacyclonucleoside **4** could be finally isolated by CCTLC.

Regioisomer **5** was obtained following an alternative approach, the direct acylation of dihydroxy compound **3**. We based on the idea that if the enzyme is able to discriminate between both acetyl groups, so N(2)-chain would also be preferentially fitted to the active center of PSL when acting as the nucleophile in acylating reactions. A first reaction carried out in a vinyl acetate medium displayed no selectivity and diacetylnucleoside **2** was obtained in 15 minutes as the only reaction product. However, when ethyl acetate was used as solvent and acyl donor, the monoacylated acyclonucleosides **4** + **5** were obtained in a ratio 5:95 (estimated by ^{1}H NMR). Compound **5** was obtained pure by CCTLC isolation.

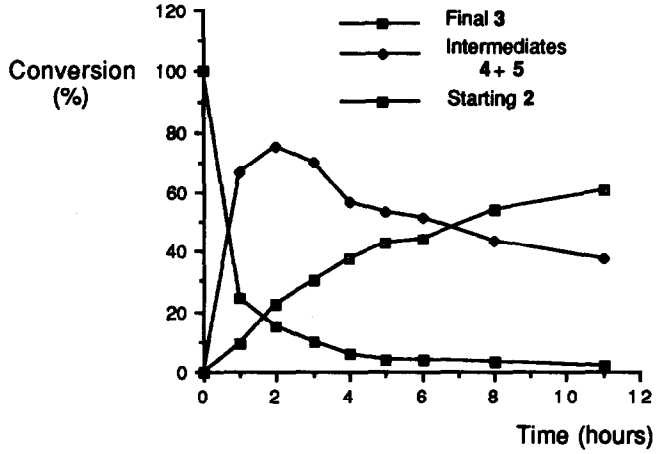


Figure 1.- Alcoholysis of **2** with n-BuOH in i-Pr_2O catalyzed by PSL

Deprotection of acetyl groups of compound **2** was also essayed by chemical pathway, using methanolic ammonia but in all cases only decomposition products were observed.

Structural Assignments.

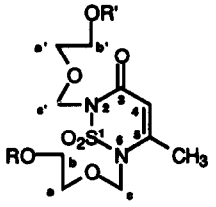
The structures of compounds **2-5** were established according to their analytical and spectroscopycal data which are gathered in Tables 2 and 3.

In the ^{13}C NMR spectrum of derivative **2**, the chemical shifts of N-methylene carbons (*C*-c and *C*-c') are between 77 and 72 ppm, which clearly ruled out the possibility that an O-substitution have taken place. So, compound **2** was identified as the N(2),N(6)-diacyclonucleoside derivative. The O-CH_2- protons linked to N(6) were assigned by means of a NOE experiment. Thus, irradiation of 5-methyl group (δ 2.22) showed a 10% NOE effect on the singlet at 5.14 ppm. For the unequivocal assignment of all chemical shifts (^{1}H and ^{13}C) of both acethoxy methoxy chains a sequence of HMQC[14] for one bond correlation and HMBC[15] for long distance correlation experiments was performed. As a result, we could observe in the ^{1}H NMR spectrum that substitution at N(2) produced a deshielding of the C*H*$_2$ protons directly linked and reduced the difference in the chemical shifts split pattern of the AA'BB' system of the acethoxymethoxy chain (Table 4). This is probably due because to the fact that the N(2)-chain is affected by the anisotropy of the adjacent C=O group. Nevertheless, if we considered the ^{13}C NMR spectrum, we could observe the opposite effect, *i.e.*, the signals of the N(6)-chain are more deshielded than those of the chain locates on N(2). This fact could be explained if we considered the different β effect exerted by a C=O or a CH_3 moiety on the carbons chemical shifts.[16]

The structure of the monoacylated acyclonucleoside **4** was determined using the AA'BB' system chemical shift difference of the acylated chain (see Table 4). The value of Δδ led us to established that the remainder acetyl

group is linked to the chain located on N(6). The unequivocal assignment of all signals of ^{1}H and ^{13}C spectra was done by means of NOE and HMQC experiments. Data are collected on tables 2 and 3.

Table 2.- ^{1}H NMR chemical shifts and coupling constants of compounds 2-5



Comp.	Solvent	H-4	CH_3-5	H-a	H-b	H-c	H-a'	H-b'	H-c'	CH_3CO	$J_{H4,CH3}$
	$CDCl_3$	5.68	2.22	3.69	4.14	5.14	3.77	4.14	5.28	2.00	1.0
R=R'=Ac		(q)	(d)	(m)	(m)	(s)	(m)	(m)	(s)	2.01	
2	MeOH	5.80	2.30	3.74	4.17	5.25	3.79	4.17	5.28	2.00	0.9
		(q)	(d)	(m)	(m)	(s)	(m)	(m)	(s)	2.01	
R=Ac	$CDCl_3$	5.67	2.21	3.64	4.13	5.13	---3.66---		5.29	1.99	1.1
R'=OH		(q)	(d)	(m)	(m)	(s)	(m)		(s)	(s)	
4	MeOH	5.76	2.25	3.70	4.13	5.21	---3.61---		5.27	2.05	0.8
		(q)	(d)	(m)	(m)	(s)	(m)		(s)	(s)	
R=OH	$CDCl_3$	5.72	2.27	---3.68---		5.21	3.82	4.18	5.33	2.05	-
R'=Ac		(q)	(d)	(m)		(s)	(m)	(m)	(s)	(s)	
5	MeOH	5.97	2.51	---3.84---		5.46	4.00	4.38	5.49	2.23	0.9
		(q)	(d)	(m)		(s)	(m)	(m)	(s)	(s)	
R=R'=OH	MeOH	6.09	2.63	---3.95---		5.59	---3.95---		5.63	-	1.0
3		(q)	(d)	(m)		(s)	(m)		(s)		

Table 3.- ^{13}C NMR chemical shifts of compounds 2-5

Comp.	Solvent	C-3	C-4	C-5	CH_3-5	C-a	C-b	C-c	C-a'	C-b'	C-c'	C=O	CH_3CO
R=R'=Ac	$CDCl_3$[a]	161.44	107.08	150.62	19.51	66.99	62.87	75.99	67.48	62.57	72.13	170.76	20.66
2	MeOH[b]	163.53	107.74	153.58	19.62	68.22	64.30	77.40	68.66	64.16	73.45	172.73	20.80
R=Ac	$CDCl_3$	161.53	107.29	150.60	19.63	67.16	62.73	76.12	71.30	61.46	72.32	170.81	20.79
R'=OH 4	MeOH	163.83	107.94	153.72	19.75	68.42	64.37	77.52	72.59	62.08	73.89	172.56	20.92
R=OH	$CDCl_3$	161.72	107.28	150.67	19.68	70.74	61.32	76.37	67.57	62.98	72.15	170.98	20.82
R'=Ac 5	MeOH	163.64	107.44	153.74	19.66	71.98	61.76	77.74	68.61	64.27	73.38	172.73	20.73
R=R'=OH 3	MeOH	163.76	107.52	153.72	19.64	72.02	61.78	77.75	72.38	61.91	73.66	-	-

[a] 170.61 (C=O), 20.71 (CH_3CO); [b] 172.58 (C=O), 20.84 (CH_3CO)

The same reasoning was applied to the structure elucidation of compound **5**. A perfect coherence in all data results was found, and so, the acetoxyethoxy methoxy chain is directly linked to N(2).

Table 4.- AA'BB' system chemical shifts difference of compounds **2,4** and **5**

Comp.	Solvent	Δδ N(2)	Δδ N(6)
2	$CDCl_3$	0.37	0.45
	MeOH	0.38	0.43
4	$CDCl_3$	-	0.49
	MeOH	-	0.43
5	$CDCl_3$	0.36	-
	MeOH	0.38	-

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

Conclusions.

We have reported the first acyclonucleosides of 1,2,6-thiadiazine 1,1-dioxides. A highly selective enzymatic strategy is used for the obtention of monoacylated diacyclonucleosides. So, monoacylated regioisomers and fully deacylated thiadiazine diacyclonucleosides have been obtained by the combined use of two enzymes, first a non selective one (CAL) to eliminate the two acetyl groups, and then a regioselective one (PSL) to catalyze two opposite reactions, the alcoholysis of the N(2)-chain acetyl group from the diacetyl compound **2** to obtain a first regioisomer **4**, and the acetylation of dihydroxy compound **3** on the same position to afford the second regioisomer **5**.

EXPERIMENTAL

Column Chromatography was performed on Merck silicagel 60 (70-230 mesh). ^1H 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 μs (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 16kHz, acquisition time 0.99 s, pulse width 9 μs (57°) and data size 32 K.

2,6-Di[(2-acetoxyethoxy)methyl]-5-methyl-1,2,6-thiadiazin-3(2H)-one 1,1-dioxide (**2**).- To a solution in dichloromethane (25 ml) of the silyl derivative of **1**[9] prepared by refluxing the base (0.48 g, 0.003 mol) in hexamethyldisilazane (9 ml) and ammonium sulphate (catalytic amounts) under nitrogen, the 2-acethoxyethyl acethoxymethyl ether[12] (0.47 g, 0.004 mol) dissolved in dichloromethane (25 ml) was added. The mixture was cooled, and $BF_3.Et_2O$ (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 CH_2Cl_2:MeOH (50:1) to give **2** (0.34 g, 28 %) as a colorless syrup. Anal. Calc. for $C_{14}H_{22}N_2O_9S$: C, 42.60; H, 5.60; N, 7.10; S, 8.10. Found: C, 42.50; H, 5.63; N, 7.01; S, 7.84.

2,6-Di[(2-hydroxyethoxy)methyl]-5-methyl-1,2,6-thiadiazin-3(2H)-one 1,1-dioxide (**3**).- CAL (254 mg) was added to a solution of **2** (100 mg, 0.254 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 r.p.m.) for 5 hours. Then the enzyme was filtered off, washed with acetone and the combined filtrates evaporated to dryness under reduced pressure, to give compound **3** (75 mg, 95 %) as a colorless syrup. Anal. Calc. for $C_{10}H_{18}N_2O_7S$: C, 38.71; H, 5.81; N, 9.03; S, 10.32. Found: C, 38.53; H, 5.64; N, 8.87; S, 9.95.

6-[(2-Acetoxyethoxy)methyl]-2-[(2-hydroxyethoxy)methyl]-5-methyl-1,2,6-thiadiazin-3(2H)-one 1,1-dioxide (**4**).- Lipase PS (254 mg) was added to a solution of **2** (100 mg, 0.254 mmol) and n-BuOH (140 mL, 1.523 mmol) in i-Pr_2O (25.4 mL). The mixture was incubated (45°C, 250 r.p.m.) for 2 hours. 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 **4** (45 mg, 60 %). Anal. Calc. for $C_{12}H_{20}N_2O_8S$: C, 40.91; H, 5.68; N, 7.95; S, 9.09. Found: C, 41.18; H, 5.90; N, 7.83; S, 9.09.

2-[(2-Acetoxyethoxy)methyl]-6-[(2-hydroxyethoxy)methyl]-5-methyl-1,2,6-thiadiazin-3(2H)-one 1,1-dioxide (**5**).- Lipase PS (323 mg) was added to a solution of the dihydroxy compound **3** (100 mg, 0.323 mmol) in EtAcO (32.3 mL). The reaction mixture was shaken (45°C, 250 r.p.m.) for 2 hours. Then, the enzyme was filtered off, washed with EtAcO and the combined filtrates evaporated to dryness under reduced pressure. Purification by CCTLC using EtAcO/hexane (3:2) as eluent gave compound **5** (38 mg, 45 %) as a colorless syrup. Anal. Calc. for $C_{12}H_{20}N_2O_8S$: C, 40.91; H, 5.68; N, 7.95; S, 9.09. Found: C, 41.25; H, 5.85; N, 7.97; S, 9.25.

Acknowledgments.- We thank Novo Nordisk Bioindustrial S.A. for its generous gift of Lipozyme and Novozym. Financial support from DIGYCYT (Project no. FAR 90/746) is gratefully acknowledged.

REFERENCES

1. "Nucleosides and Nucleotides as Antitumor and Antiviral Agents"; Chu, C. K.; Baker, D. C., Ed.; Plenum Press: 1993.
2. Chu, C.K.; Cutler, S.J. *J.Heteroclyc.Chem.*, **1986**, *23*, 289.
3. Schaeffer, H.J.; Beauchamp, L.; De Miranda, P.; Elion, G.; Baver, D.J.; Collins, P. *Nature* (London), **1978**, *272*, 583.
4. Bennett, S.M.; Nguyen-Ba, N.; Oglivie, K.K. *J. Med. Chem.*, **1990**, *33*, 2162.
5. Hamamura, E. K.; Prydstasz, M.; Verheyden, J. P. H.; Moffat, J. G.; Yamaguchi, K.; Uchida, N.; Sato, K.; Nomura, A.; Shiratori, O.; Takase, S.; Katagiri, K. *J. Med. Chem.* **1976**, *19*, 654, 667.
6. Hanrahan, J. R.; Hutchinson, D. W. *J. Biotechnol.* **1992**, *23*, 193.
7. De la Cruz, A.; Elguero, J.; Gotor, V.; Goya, P.; Martinez, A.; Moris, F. *Synt. Commun.* **1991**, *21*, 1477.
8. a) De la Cruz, A.; Goya, P.; Elguero, J.; Martinez, A.; Gotor, V.; Moris, F. *J.Chem.Res.* **1992**, (S), 216 b) De la Cruz, A.; Goya, P.; Elguero, J.; Martinez, A. *J.Chem.Soc Perkin Trans. I*, **1993**, 845.
9. Die, R.; Diez, J.; Garcia-Muñoz, G.; Madroñero, R.; Stud, M. *J. Heterocycl. Chem.*, **1972**, *9*, 973
10. Vorbruggen, H.; Niedballa, U. *J.Org.Chem.*, **1974**, *39*, 3664; *ibid.*, **1974**, *39*, 3667.
11. Goya, P.; Martinez, A.; Jimeno, M. L. *J.Chem.Soc. Perkin Trans II*, **1990**, 783.
12. Rosowsky, A.; Kim, S. H.; Wick, M. *J.Med.Chem.*, **1981**, *24*, 1177.
13. PPL: Porcine pancreatic lipase (Sigma); CRL: *Candida rugosa* lipase (formerly called *Candida cylindracea*; Sigma and Lipase AY from Amano); PSL: *Pseudomonas sp.* (Lipase PS from Amano); MML: *Mucor miehei* lipase (Lipozyme IM20, Novo Nordisk); CAL: *Candida antarctica* lipase (Novozym 435, Novo Nordisk). All of them were used without any manipulation. We did not take activities into account.
14. Bax, A.; Subramanian, S. *J.Magn.Res.*, **1986**, 565-569.
15. Bax, A.; Summers, M. F. *J.Am.Chem.Soc.*, **1986**, *108*, 565-569.
16. Kalinowski, H.O.; Berger, S.; Braun, S., *"Carbon-13 Spectroscopy"*, John Wiley Ed., England, **1988**.

(*Received in UK* 12 *September* 1994; *revised* 17 *October* 1994; *accepted* 21 *October* 1994)