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LIPASE-CATALYZED PROTECTION OF THE HYDROXY GROUPS OF THE NUCLEOSIDES INOSINE AND 2'-DEOXYINOSINE: A NEW CHEMOENZYMATIC SYNTHESIS OF THE ANTIVIRAL DRUG 2',3'-DIDEOXYINOSINE

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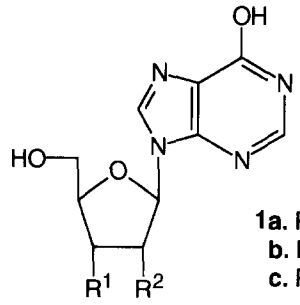
Abstract: The selective acylation of the hydroxy groups of the nucleosides inosine **1a** and 2'-deoxyinosine **1b** has been achieved in the presence of *Candida antarctica* and *Pseudomonas sp.* lipases in organic solvents; starting from the 5'-acetyl derivative of 2'-deoxyinosine, compound **5a**, an efficient chemoenzymatic synthesis of the antiviral drug 2',3'-dideoxyinosine **1c** has been achieved.

2',3'-Dideoxynucleosides have been recently introduced as chemotherapeutic agents with anti-retroviral activity against human immunodeficiency virus (HIV), the causative agent of aquired immune deficiency syndrome (AIDS).[1,2] Among 2',3'-dideoxynucleosides, 2',3'-dideoxyinosine **1c** (DDI, Didanosine) has been approved for treatment of adults and children with advanced HIV infection, who are intolerant to AZT or whose health has been deteriorated by AZT use.[3] A few chemical syntheses of DDI **1c** have already been reported, starting from readily available inosine **1a**[4] or from the relatively expensive 2'-deoxyinosine **1b**.[5]



1a. R1=R2= OH
b. R1= OH, R2= H
c. R1=R2= H

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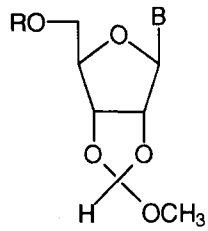
For an alternative synthetic approach to DDI **1c** from inosine **1a**, we unsuccessfully tried to prepare the ketal **2a**[6] that could, in principle, be converted to the unsaturated compound **3a** as described for other nucleosides.[7] We therefore turned our attention to a suitably protected inosine derivative such as, for instance, the 5'-acetate, that could allow the preparation of the compound **2b** and of the unsaturated product **3b** thereof.



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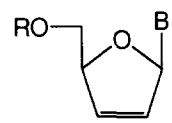
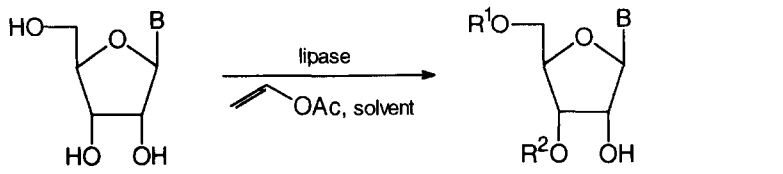


3

B = hypoxantine
a. R= H
b. R= Ac

For this purpose, we considered the enzyme-mediated protection of the ribose moiety, considering that the selective reactions on these hydroxy groups of nucleosides have been widely investigated.[8] However, only sparse applications to inosine **1a** and 2'-deoxynucleoside **1b** can be found in the related literature. We used a few lipases as biocatalysts for the acylation of **1a**[9] and adopted the irreversible transesterification procedure with vinyl acetate in an organic solvent.[10] Due to problem of solubility of inosine **1a**, polar solvents such as pyridine, dimethylsulfoxide or tetrahydrofuran were used, although it is well known that they tend to deactivate the enzyme.[11] This complicated also the recovery of the products, that could hardly be extracted after addition of water to the reaction mixture. In the above conditions, only the *Pseudomonas sp.* lipase (Lipase PS) and the *Candida antarctica* lipase (CAL) exhibited appreciable activity and were used for the reaction.[12] These results are collected in Table 1, which shows that CAL in pyridine or tetrahydrofuran at 60 °C efficiently and selectively allows the preparation of the 5'-acetate **4a**, whereas PS at 30 °C in dimethylsulfoxide or tetrahydrofuran catalyzes the acetylation of the 3'-hydroxy group (compound **4b**). We always observe also the formation of 2'-acetate (23%) that is caused by the well known acyl migration in ribonucleoside derivatives.[13]



1a

4a. R1= Ac, R2= H
b. R1= H, R2= Ac

Table 1. Acylation of inosine 1a in various solvents.[a]

lipase[b]	T (°C)	t (h)	Solvent	Yield[c] (%)	Product
CAL	60	24	Py	87	4a
CAL	60	24	THF	80	4a
CAL	30	--	DMSO	--	--
PS	30	--	Py	--	--
PS	30	60	THF	69	4b[d]
PS	30	96	DMSO[e]	83	4b[d]

[a] These reactions did not take place in absence of enzyme even if stronger conditions were used. [b]Lipases from *Mucor miehei*, *Candida cylindracea* and porcine pancreas gave no reaction under similar conditions. [c]Pure isolated product. [d]We always observe also the formation of 2'-acetate (Ref. 13). [e]Monoacetylation in DMF proceeds as in DMSO.

Starting from the monoacetate **4a** enzymatically obtained from inosine **1a** in the presence of CAL, the corresponding 5'-*O*-acetyl-2',3'-*O*-(methoxymethylene)inosine **2b** was prepared in 89% yield, but this compound failed to afford the corresponding unsaturated compound **3b** and only a complex mixture of products was recovered.

We then examined the acylation of 2'-deoxyinosine **1b** and found that CAL in pyridine or tetrahydrofuran at 60 °C efficiently yields the 5'-acetate **5a** and that, as for inosine **1a**, PS affords the 3'-acetate **5b** in tetrahydrofuran, as evidenced by the results of the enzymatic reactions collected in Table 2.

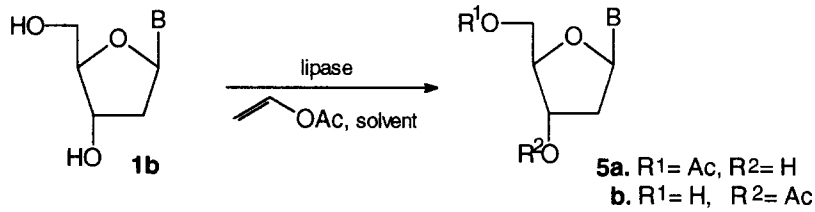
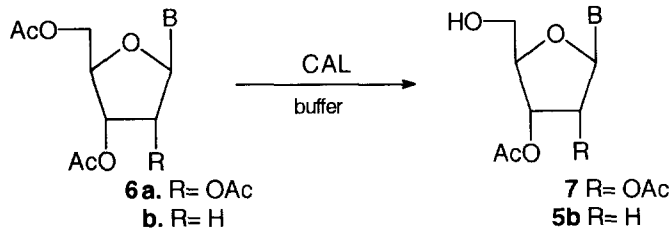


Table 2. Acylation of 2'-deoxyinosine 1b in various solvents.[a]

Lipase[b]	T (°C)	t (h)	Solvent	Yield[c] (%)	Product
CAL	60	20	Py	84	5a
CAL	60	20	THF	82	5a
CAL	30	--	DMSO[d]	--	--
PS	60	--	Py	--	--
PS	60	50	THF	72	5b
PS	30	--	DMSO[d]	--	--

[a] These reactions did not take place in absence of enzyme even if stronger conditions were used. [b]Lipases from *Mucor miehei*, *Candida cylindracea* and porcine pancreas gave no reaction under similar conditions. [c]Pure isolated product. [d] Also DMF gave no reaction.

We have also carried out the enzymatic hydrolysis of the triacetate **6a** and diacetate **6b** in the presence of CAL in aqueous buffer at pH 7 or in water-saturated chloroform and the selective deprotection to the acetates **7** and **5b** was found to be complementary to the lipase-catalyzed acylation in organic solvents.[14]

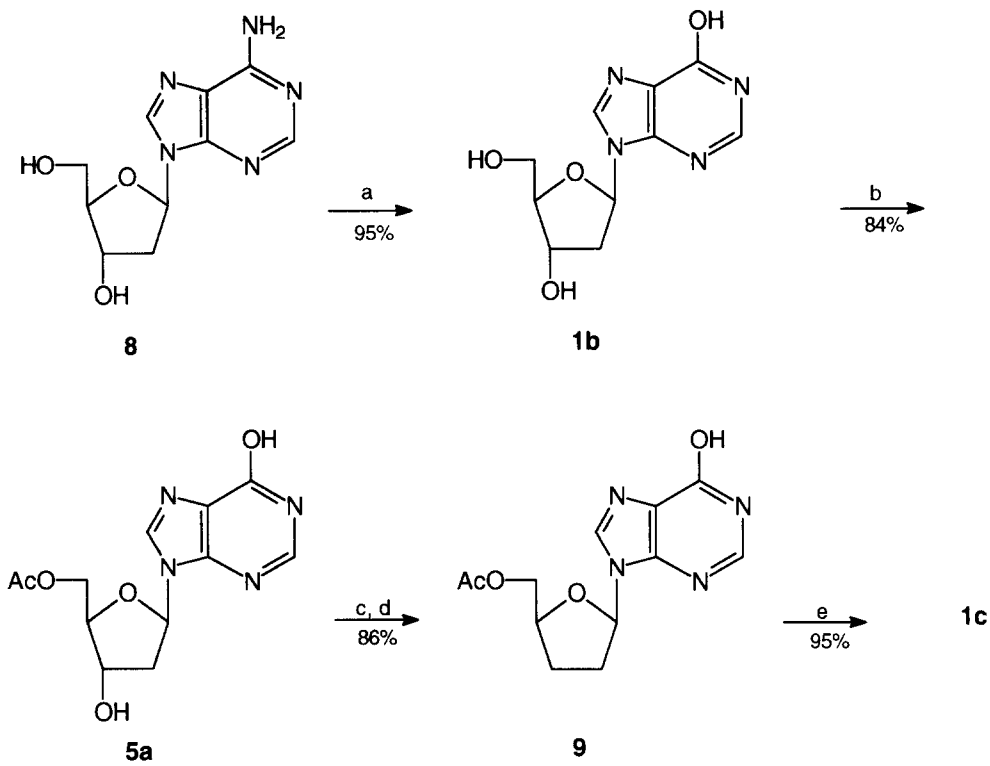


6a. R= OAc
b. R= H

7 R= OAc
5b R= H

In any event, we could study a new chemoenzymatic synthesis of DDI **1c** starting from the 5'-acetate of the 2'-deoxyinosine **5a** enzymatically prepared by the transacetylation procedure. Monoacetate **5a** has been converted into the 3'-*O*-phenoxythiocarbonyl derivative[15] which, without further purification, was reacted with tributyltin hydride in toluene in presence of 2,2'-azobis-(2-methylpropionitrile) yielding the 5'-acetate of DDI **9**.[16] Quantitative deacylation with methanol saturated with ammonia afforded crystalline DDI **1c** in 68% yield from 2'-deoxyinosine **1b**. This starting material was relatively expensive but we could prepare it efficiently from readily available 2'-deoxyadenosine **8** by a deamination reaction efficiently catalyzed by adenosine deaminase (ADA).[17] The overall sequence for the chemoenzymatic synthesis of DDI **1c** could therefore start from 2'-deoxyadenosine **8** and is depicted in Scheme.

Scheme



(a) ADA, water, RT, 1h; (b) CAL, vinyl acetate, pyridine, 60°, 20 h; (c) phenyl chlorothionoformate, DMAP, CH_3CN, RT, 18 h; (d) n-Bu $_3$SnH, AIBN, toluene, 80°, 3 h; (e) NH $_3$, MeOH, 0°, 3 h .

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References and Notes

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6. The experimental conditions were as reported: Griffin, B. E.; Jarman, M.; Reese, C. B.; Sulston, J. E. *Tetrahedron*, **1967**, *23*, 2301. We tried to carry out the reaction using trimethylorthoformate as reagent and solvent, but inosine **1a** was insoluble in this reaction medium and compound **2a** was formed in very poor yield; addition of dimethylsulfoxide did not improve the yields and complicated the work-up.
7. Shiragami, H.; Irie, Y.; Shirae, H.; Yokozeki, K.; Yasuda, N. *J. Org. Chem.* **1988**, *53*, 5170.
8. For a recent review see: Prasad, A. K.; Wengel, J. *Nucleosides & Nucleotides* **1996**, *15*, 1347.
9. The lipase used were obtained as follows: from *Pseudomonas sp.* (Lipase PS, Amano Pharmaceutical Co.), from *Candida antarctica* and *Mucor miehei* (Novo Nordisk), from *Candida rugosa* and porcine pancreas (Sigma).

10. (a) Degueil-Castaing, M.; De Jeso, B.; Drouillard, S.; Maillard, B. *Tetrahedron Lett.* **1987**, *28*, 953. (b) Wang, Y.-F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200.
11. Koskinen, A. M. P.; Klibanov, A.M., In *Enzymatic Reactions in Organic Media*; Chapman & Hall: London, 1996.
12. Tipically, inosine **1a** or 2'-deoxyinosine **1b** (1.2 mmol), vinyl acetate (2.5 mmol) and 1 g of CAL (or lipase PS) was suspended in 20 ml of pyridine (or THF) under nitrogen atmosphere. The mixture was allowed to react at the temperature and for the time indicated in Table1 for **1a** and Table 2 for **1b**, monitoring the reactions by TLC ($CHCl_3$-MeOH, 4:1). The enzyme was filtered off and washed with MeOH, the solvents were distilled under vacuum and the products were purified by flash chromatography (CH_2Cl_2-MeOH 9:1). The reaction of **1a**, lipase PS in DMSO (or DMF) was carried out with addition of enzyme (200 mg) each day. DMSO was removed by lyophilization.
13. Reese, C. B., Trentham, D. R. *Tetrahedron Lett.* **1965**, *29*, 2467.
The diastereoisomeric mixture shows: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.35 (1H, s, H-2), 8.10 (1H, s, H-8), 6.02 (1H, d, J=7.0 Hz, H-1'), 5.37 (1H, dd, J=2.8, 5.6 Hz, H-3'), 4.89 (1H, dd, J=5.6, 7.0 Hz, H-2'), 4.26 (1H, ddd, J=2.8, 2.8, 2.8 Hz, H-4'), 3.85 (1H, dd, J=2.8, 12.6 Hz, H-5'a), 3.78 (1H, dd, J=2.8, 12.6 Hz, H-5'b), 2.17 (3H, s, OCOCH_3) for the 3'-acetate **4b** (77%) and δ 8.35 (1H, s, H-2), 8.17 (1H, s, H-8), 6.22 (1H, d, J=5.6 Hz, H-1'), 5.62 (1H, dd, J=5.6, 5.6 Hz, H-2'), 4.62 (1H, dd, J=4.9, 5.6 Hz, H-3'), 4.15 (1H, ddd, J=2.8, 2.8, 4.9 Hz, H-4'), 3.89 (1H, dd, J=2.8, 11.9 Hz, H-5'a), 3.77 (1H, dd, J=2.8, 11.9 Hz, H-5'b), 2.08 (3H, s, OCOCH_3) for the 2'-isomer (23%).
14. A typical hydrolysis was performed dissolving compounds **6a** or **6b** (1.2 mmol) in acetone (2 ml) and adding to this solution 1 g of CAL dispersed in 0.1 M phosphate buffer pH=7 (10 ml). The mixture was stirred at room temperature (24 h for both **6a** and **6b**), monitoring the reactions by TLC ($CHCl_3$-MeOH, 4:1). Products were extracted with dichloromethane, followed by usual work-up and flash chromatography (CH_2Cl_2-MeOH, 9:1) affording **7** and **5b** (80 and 84% respectively). Alternatively, to compounds **6a** or **6b** (1.2 mmol) in water-saturated chloroform (10 ml) 1 g of CAL was added. The mixture was stirred at room temperature (48 h for both **6a** and **6b**) affording, after usual work-up, compounds **7** and **5b** in comparable yields.
15. The experimental procedure was in accord to the condition reported by Robbins, M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059.
16. All compounds had spectral data consistent with the proposed structures. Compound **5a**: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.20 (1H, s, H-2), 8.04 (1H, s, H-8), 6.42 (1H, dd, J=6.3, 6.3 Hz, H-1'), 4.57 (1H, ddd, J=4.2, 4.9, 6.3 Hz, H-3'), 4.31 (1H, dd, J=4.2, 11.9 Hz, H-5'a), 4.28 (1H, dd, J=4.9, 11.9 Hz, H-5'b), 4.14 (1H, ddd, J=4.2, 4.2, 4.9 Hz, H-4'), 2.83 (1H, ddd, J=6.3, 6.3, 14.0 Hz, H-2'a), 2.34 (1H, ddd, J=4.9, 6.3, 14.0 Hz, H-2'b), 2.02 (3H, s, OCOCH_3). Compound **9**: (CD_3OD, 500 MHz) δ 8.23 (1H, s, H-2), 8.03 (1H, s, H-8), 6.30 (1H, dd, J=5.4, 5.4 Hz, H-1'), 4.41 (1H, dddd, J=3.4, 4.7, 6.0, 8.6 Hz, H-4'), 4.30 (1H, dd, J=3.4, 12.0 Hz, H-5'a), 4.27 (1H, dd, J=4.7, 12.0 Hz, H-5'b), 2.61-2.56 (2H, m, H-2'a and H-2'b), 2.23-2.10 (2H, m, H-3'a and H-3'b) 2.01 (3H, s, OCOCH_3).
17. 2'-deoxyadenosine (**8**, 1.00 g) in water (50 ml) was treated with 1% by weight (10 mg) of adenosine deaminase (Sigma, type II, 2.2 units/mg protein) at room temperature. The reaction was monitored by TLC ($CHCl_3$-MeOH, 4:1) and was complete in 1h. The solution was lyophilized and the residue recrystallized from methanol to give 0.95 g (95%) of 2'-deoxyinosine **1b**, spectroscopically identical to an authentic sample.