

First Regioselective Enzymatic Alkoxy-carbonylation of Primary Amines. Synthesis of Novel 5'- and 3'-Carbamates of Pyrimidine 3',5'-Diaminonucleoside Derivatives Including BVDU Analogues

Iván Lavandera, Susana Fernández, Miguel Ferrero, and Vicente Gotor*

Departamento de Química Orgánica e Inorgánica, Facultad de Química, Universidad de Oviedo, 33071-Oviedo, Spain

vgs@fq.uniovi.es

Received November 14, 2003

Abstract: The first regioselective enzymatic alkoxy-carbonylation of primary amino groups has been achieved in pyrimidine 3',5'-diaminonucleoside derivatives. Thus, *Candida antarctica* lipase B (CAL-B) catalyzed this reaction with nonactivated homocarbonates allowing the selective synthesis of several *N*-5' carbamates, including (*E*)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) analogues, with moderate-high yields, whereas immobilized *Pseudomonas cepacia* lipase (PSL-C) afforded mixtures of alkoxy-carbonylated regioisomers. To obtain *N*-3' carbamates selectively, a short and efficient chemoenzymatic route was used employing some of the *N*-5'-protected derivatives previously synthesized.

Lipases are one of the most used biocatalysts due to their versatility in accepting a wide range of nucleophiles (alcohols, amines, thiols, water, etc.) and carbonylating agents (esters, anhydrides, carbonates, etc.).¹ The enzymatic alkoxy-carbonylation reaction has been scarcely studied,² despite the biological relevance shown by carbonates and carbamates.³ In this field, our research group has contributed to achieving the first example of alkoxy-carbonylation of amines⁴ and the regioselective synthesis of carbonates in carbohydrates,⁵ nucleosides,⁶ and 1 α ,25-dihydroxyvitamin D₃ A-ring precursors⁷ using vinyl or oxime carbonates. It is noteworthy that through these processes it is possible to introduce functionalities selectively which act as protected or activated groups in alcohols and amines. In the last case, reactions are irreversible since carbamates are not substrates to lipases. Thus, Wong and co-workers⁸ have used nonacti-

vated homocarbonates for preparing chiral protected amines with allyl and benzyloxycarbonyl groups.

Enzymatic reactions have been important processes for achieving nucleoside analogues, relevant compounds due to their inherent value as potential therapeutic agents.⁹ In particular, pyrimidine nucleoside analogues have shown remarkable antiviral¹⁰ and antitumor¹¹ activities. For example, (*E*)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) has been one of them for treating Herpes simplex virus type 1 (HSV-1) and *Varicella zoster* virus (VZV).¹² In recent years, the search for new nucleoside derivatives using this clean, simple, and efficient methodology has received a great deal of attention from organic chemists.¹³ In this context, oxime carbonates have been used with lipases for direct and selective protection of natural nucleosides.⁶

This regioselective process would be more difficult in the case of two primary amines due to the major nucleophilic character of the amino group compared to the hydroxyl group. In fact, to the best of our knowledge, there is no example of a regioselective enzymatic alkoxy-carbonylation in amines. Recently, we have developed the regioselective acylation of 3',5'-diaminonucleosides with lipases and nonactivated esters.¹⁴ In this paper, we report the synthesis of novel pyrimidine aminonucleosides regioselectively protected as carbamates. For that, direct enzymatic alkoxy-carbonylation reaction with *Candida antarctica* lipase B (CAL-B) was carried out to synthesize *N*-5' carbamates, whereas the *N*-3' regioisomers were prepared using a short and efficient chemoenzymatic route.

First, pyrimidine diaminonucleosides were synthesized through efficient routes previously described for us.¹⁴ For the alkoxy-carbonylation reaction, nonactivated carbonates were used as carbonylating agents since amines are much more nucleophilic than alcohols and oxime carbonates react nonenzymatically with the substrates. Specifically, homocarbonates were used because their symmetrical structure gives a single unambiguous product. For 3',5'-diamino-2',3',5'-trideoxyuridine (**1**, Scheme 1), a mixture of THF/Py was chosen to dissolve it, whereas for 3',5'-diamino-3',5'-dideoxythymidine (**2**) and (*E*)-3',5'-diamino-5-(2-bromovinyl)-2',3',5'-trideoxyuridine (**3**), THF was used as solvent. The enzymatic reactions were carried out in the presence of molecular sieves (4 Å),

(1) (a) Faber, K. *Biotransformations in Organic Chemistry*, 4th ed.; Springer: Berlin, 2000. (b) Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis*; Weinheim: Wiley-VCH: 1999.
 (2) Gotor, V. *Bioorg. Med. Chem. Lett.* **1999**, *7*, 2189–2197.
 (3) Parrish, J. P.; Salvatore, R. N.; Jung, K. W. *Tetrahedron* **2000**, *56*, 8207–8237.
 (4) Pozo, M.; Pulido, R.; Gotor, V. *Tetrahedron* **1992**, *48*, 6477–6484.
 (5) (a) Pulido, R.; Gotor, V. *Carbohydr. Res.* **1994**, *252*, 55–68. (b) Pulido, R.; Gotor, V. *J. Chem. Soc., Perkin Trans. 1* **1993**, 589–592.
 (6) (a) Moris, F.; Gotor, V. *J. Org. Chem.* **1992**, *57*, 2490–2492. (b) Moris, F.; Gotor, V. *Tetrahedron* **1992**, *48*, 9869–9876.
 (7) (a) Díaz, M.; Gotor-Fernández, V.; Ferrero, M.; Fernández, S.; Gotor, V. *J. Org. Chem.* **2001**, *66*, 4227–4232. (b) Gotor-Fernández, V.; Ferrero, M.; Fernández, S.; Gotor, V. *J. Org. Chem.* **1999**, *64*, 7504–7510. (c) Ferrero, M.; Fernández, S.; Gotor, V. *J. Org. Chem.* **1997**, *62*, 4358–4363.
 (8) (a) Takayama, S.; Lee, S. T.; Hung, S.-C.; Wong, C.-H. *Chem. Commun.* **1999**, 127–128. (b) Orsat, B.; Alper, P. B.; Moree, W.; Mak, C.-P.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 712–713.

(9) (a) Galmarini, C. M. *Electron. J. Oncol.* **2002**, *1*, 22–32. (b) Robins, R. K.; Revankar, G. R. In *Antiviral Drug Development*; De Clercq, E., Walker, R. T., Eds.; Plenum Press: New York, 1998; p 11.
 (c) Mansour, T. S.; Storer, R. *Curr. Pharm. Des.* **1997**, *3*, 227–264.
 (10) (a) Czernecki, S.; Valery, J. M. In *Carbohydrates in Drug Design*; Witczak, Z. J., Nieforth, K. A., Eds.; Dekker: New York, 1997; p 495. (b) De Clercq, E. Pyrimidine Nucleoside Analogues as Antiviral Agents. *NATO ASI Ser., Ser. A* **1984**, *73*, 203–230.
 (11) Plunkett, W.; Gandhi, V. *Cancer Chemother. Biol. Response Modif. Annu.* **2001**, *19*, 21–45.
 (12) (a) De Clercq, E. *Biochem. Pharmacol.* **1984**, *33*, 2159–2169. (b) De Clercq, E.; Descamps, J.; De Somer, P.; Barr, P. J.; Jones, A. S.; Walker, R. T. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2947–2951.
 (13) (a) Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319–4347. (b) Ferrero, M.; Gotor, V. *Monatsh. Chem.* **2000**, *131*, 585–616.
 (14) (a) Lavandera, I.; Fernández, S.; Ferrero, M.; De Clercq, E.; Gotor, V. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1939–1952. (b) Lavandera, I.; Fernández, S.; Ferrero, M.; Gotor, V. *J. Org. Chem.* **2001**, *66*, 4079–4082.

SCHEME 1



- 1, R= H a, R¹= Et 4a-c, R= H
 2, R= Me b, R¹= CH₂=CHCH₂ 5a-c, R= Me
 3, R= (E)-BrCH=CH c, R¹= Bn 6a-c, R= (E)-BrCH=CH

TABLE 1. Regioselective Enzymatic Alkoxy-carbonylation of 3',5'-Diaminonucleosides with CAL-B

entry	sub-strate	alkoxy-carbonylating agent			T (°C)	t (h)	isol yields of N-5' (%) ^a
		R ¹	equiv	solvent			
1	1	Et	10	THF/Py ^b	50	72	67
2	1	CH ₂ =CHCH ₂	8	THF/Py ^b	40	72	69
3	1	Bn	20	THF/Py ^b	60	168	45 ^c
4	2	Et	10	THF	50	42	62
5	2	CH ₂ =CHCH ₂	8	THF	40	24	64
6	2	Bn	20	THF	60	174	50 ^c
7	3	Et	10	THF	50	135	59
8	3	CH ₂ =CHCH ₂	8	THF	40	96	69
9	3	Bn	20	THF	60	180	50 ^c

^a Isolated yields by flash chromatography. ^b Ratio THF/Py 4.5:1 (v/v). ^c In addition to N-5'-alkoxy-carbonylated derivative starting material was recovered.

which catalyzed these processes.¹⁵ To reach high conversions, ratios of alkoxy-carbonylating agents have been established.

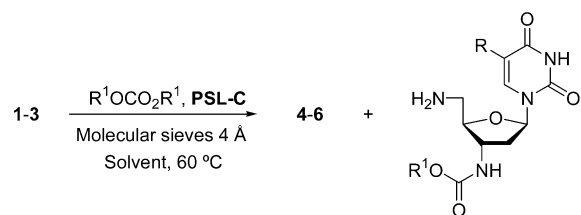
We focused on CAL-B as biocatalyst since this enzyme has demonstrated an excellent regioselectivity toward the amino group at the 5' position in the enzymatic acylation of diaminonucleosides.¹⁴ The study begins with the di-amino derivative of 2'-deoxyuridine (**1**). Thus, when the reaction was carried out with 10 equiv of diethyl carbonate at 50 °C, the formation of a single product was observed (monitored by TLC), which corresponded to monoalkoxy-carbonylated derivative at N-5', **4a** being isolated with 67% yield after flash chromatography (entry 1, Table 1). We also confirmed that no reaction occurred in the absence of the enzyme.¹⁶ The structure of this compound was ascertained from its ¹H NMR (MeOH-*d*₄) spectrum, which showed a downfield shift corresponding to both H_{5'} protons from 2.8 to 3.07 ppm in diaminonucleoside **1** to 3.63 ppm in **4a**. Furthermore, H_{3'} did not display any significant change. In addition, heteronuclear correlation ¹H-¹³C experiments 2D HMBC showed a cross-peak between H_{5'} and C=O, which corresponds to correlation H_{5'}-CNCO via ³J_{CH}. It is noteworthy that neither N-3',5'- nor N-3'-alkoxy-carbonylated derivatives were formed, despite the fact that both amino groups are primaries.

To confer versatility to this enzymatic reaction, other alkoxy-carbonyl moieties were introduced. Thus, 8 equiv

(15) To see an explanation for this effect: Lavandera, I.; Fernández, S.; Ferrero, M.; Gotor, V. *Tetrahedron* **2003**, *59*, 5449–5456.

(16) Detailed conditions: corresponding diaminonucleosides **1–3** and homocarbonates were dissolved in THF/Py (for **1**) or THF (for **2** and **3**) at 60 °C during 8 days and no reactions were observed.

SCHEME 2



- a, R¹= Et 7a-b, R= H
 b, R¹= CH₂=CHCH₂ 8a-b, R= Me
 9a-b, R= (E)-BrCH=CH

TABLE 2. Regioselective Enzymatic Alkoxy-carbonylation of 3',5'-Diaminonucleosides with PSL-C

entry	sub-strate	alkoxy-carbonylating agent			solvent	t (h)	isolated yields (%)	
		R ¹	equiv	solvent			N-3'	N-5'
1	1	Et	50	THF/Py ^a	176	27	50	
2	1	CH ₂ =CHCH ₂	40	THF/Py ^a	117	13	42	
3	2	Et	50	THF	30	22 ^b	20 ^b	
4	2	CH ₂ =CHCH ₂	40	THF	30	26	56	
5	3	Et	50	THF	135	31 ^{b,c}		
6	3	CH ₂ =CHCH ₂	40	THF	96	24	62	

^a Ratio THF/Py 4.5:1 (v/v). ^b Starting material was recovered. ^c Isolated as a mixture of products **6a/9a** 1:1 by ¹H NMR.

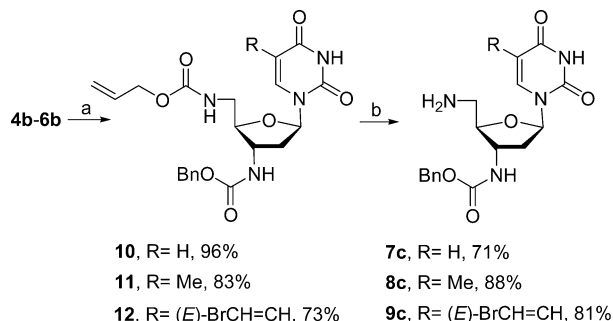
of the more reactive diallyl carbonate at 40 °C were used to alkoxy-carbonylate with total regioselectivity **1** at the 5' position, yielding **4b** with 69% (entry 2, Table 1). On the other hand, when dibenzyl carbonate was used, it was necessary to increase the carbonate ratio to 20 equiv and the temperature up to 60 °C due to the lower reactivity of this agent.¹⁷ After 7 days, the N-Cbz-derivative **4c** was isolated exclusively with 45% yield, recovering a substantial amount of unreacted starting material (entry 3, Table 1).

When similar processes were carried out with thymidine and (E)-5-(2-bromovinyl)-2'-deoxyuridine derivatives **2** and **3**, respectively, comparable behavior was observed. CAL-B showed total regioselectivity toward the 5'-NH₂, isolating exclusively compounds **5** and **6** with moderately high yields (entries 4–9, Table 1) and recovering part of unreacted diaminonucleosides with dibenzyl carbonate as carbonylating agent.

Then, we tried to find a biocatalyst which alkoxy-carbonylated the amino group in the 3' position. For that, immobilized *Pseudomonas cepacia* lipase (PSL-C), which has shown an excellent regioselectivity in the acetylation of 3'-NH₂ in 3',5'-diaminonucleosides, was chosen (Scheme 2).¹⁴ The results are summarized in Table 2.

Unfortunately, PSL-C did not exhibit selectivity in the alkoxy-carbonylation of **1** with 50 equiv of diethyl carbonate at 60 °C affording a mixture of both regioisomers **4a** and **7a** (entry 1, Table 2). Compound **7a** was identified from its ¹H NMR (D₂O) spectrum, which presents a downfield shift of the H_{3'} (from 3.33 ppm in **1** to 4.21 ppm in **7a**), while H_{5'} almost did not change. Moreover, experiments 2D HMBC showed a cross-peak between H_{3'} and C=O corresponding to correlation H_{3'}-CNCO via

(17) The low reactivity of this carbonate has already been reported in the ref 8.

SCHEME 3. Synthesis of 3'-Carbamate Nucleoside Derivatives^a


^a Reaction conditions: (a) BnOCOCl, Na₂CO₃, THF/H₂O, rt, 24 h; (b) PdCl₂(PPh₃)₂, Bu₃SnH, AcOH, CH₂Cl₂, rt, 4 h.

³J_{CH}. To avoid the formation of *N*-5' monocarbonate **4a**, lesser equivalents of diethyl carbonate and/or lower temperatures were used, but in all cases a large amount of starting nucleoside was recovered. With 40 equiv of diallyl carbonate the process takes place with low selectivity, obtaining mainly **4b** (entry 2, Table 2).

Similar results were observed when substrates **2** and **3** were subjected to alkoxy-carbonylation with PSL-C (entries 3–6, Table 2). A mixture of both *N*-3'- and *N*-5'-alkoxy-carbonylated regioisomers was obtained, the latter being the main product when diallyl carbonate was utilized. In the case of dibenzyl carbonate, which had already shown low reactivity with CAL-B, the reaction did not occur.

Since direct enzymatic methodology did not allow for *N*-3'-alkoxy-carbonylated nucleoside derivatives with good yields, an orthogonal protection scheme was designed. The strategy starts with the protection of the 5'-NH₂ group, subsequent derivatization of the *N*-3' amine as a carbamate, and finally selective deprotection of the 5' position.

We decided to synthesize the *N*-3'-Cbz derivatives **7c**–**9c** (Scheme 3) due to the ease of removing this protecting group for further modifications. To selectively protect the 5'-position, reaction of **1** with trityl chloride, a reagent with a very appropriate bulky group to modify that position in natural nucleosides was carried out. However, a mixture of regioisomers was obtained due to the major nucleophilic character of the amino groups.

We made use of the regioselective enzymatic alkoxy-carbonylation shown previously to selectively protect the 5'-NH₂ group as allyloxycarbamate affording derivatives **4b**–**6b**. Treatment of the later compounds with benzyloxycarbonyl chloride at room temperature afforded dicarbamate nucleosides **10**–**12** with high efficiency. Then, selective deprotection of the allyloxycarbonyl group in the conditions described by Guibé and co-workers¹⁸ gave place to *N*-3'-Cbz-protected derivatives **7c**–**9c** through a short and efficient chemoenzymatic route.

Regioselective enzymatic alkoxy-carbonylation of amines using a very simple procedure with nonactivated homocarbonates and CAL-B as biocatalyst have been shown for the first time, synthesizing *N*-5'-carbamate pyrimidine 3',5'-diaminonucleoside derivatives. Allyloxy **8** ben-

zyloxycarbonyl protecting groups, very useful for further modifications, have also been introduced using this enzymatic methodology. The utility of these modified compounds have been presented, achieving the first synthesis of *N*-3'-Cbz-3',5'-diaminonucleoside analogues through a short and efficient chemoenzymatic route with high overall yield. It is noteworthy that a new family derived from BVDU has been obtained. Preliminary studies with some of these carbamate derivatives have been performed against several viruses.¹⁹ They show better results than the unmodified 3',5'-diaminonucleosides.²⁰ The complete study of the biological activity of these new derivatives will be reported in due course.

Experimental Section

General Methods. *Candida antarctica* lipase B (CAL-B, Novozym 435, 7300 PLU/g) was a gift from Novo Nordisk Co. Immobilized *Pseudomonas cepacia* lipase on ceramic particles (PSL-C, 783 U/g) was purchased from Amano Pharmaceutical Co.

General Procedure for the Enzymatic Alkoxy-carbonylation of 3',5'-Diaminonucleosides. Corresponding homocarbonate (diethyl, diallyl, or dibenzyl carbonates) was added to a suspension of diaminonucleoside (20 mg, in the case of **1** it was previously dissolved in 1 mL of dry pyridine), lipase (10 mg of CAL-B or 130 mg of PSL-C), and molecular sieves 4 Å (20 mg) in dry THF (4.5 mL) under nitrogen, and the mixture was stirred at 250 rpm (ratios, temperatures, and reaction times are indicated in Tables 1 and 2). Then, the enzyme and molecular sieves were filtered off and washed with MeOH (3 × 5 mL). The filtrate was evaporated to dryness, and the crude residue was purified by flash chromatography (gradient eluent 10% MeOH/EtOAc–MeOH for compounds **4a**–**c**, **5a**–**c**, and **6a**–**c** and gradient eluent 10% MeOH/EtOAc–MeOH–10% NH₃(aq)/MeOH for compounds **7a,b**, **8a,b**, and **9a,b**).

Synthesis of Dicarbamate Nucleosides 10–12. To a solution of *N*-5'-protected nucleosides **4b**–**6b** (20 mg, 1 equiv) in a mixture of H₂O (1 mL) and THF (0.5 mL) were added sodium carbonate (1.2 equiv) and benzyloxycarbonyl chloride (1.2 equiv). The mixture was stirred at room temperature during 24 h. The solvents were evaporated under vacuum, and the crude residue was purified by flash chromatography (gradient eluent 20% hexane/EtOAc–EtOAc).

Synthesis of Monocarbamate Nucleosides 7c–9c. Substrates **10**–**12** (20 mg, 1 equiv), PdCl₂(PPh₃)₂ (0.02 equiv), and acetic acid (2.4 equiv) were dissolved in dry CH₂Cl₂ under nitrogen. With a syringe, Bu₃SnH (1.1 equiv) was then added rapidly in one portion. The reaction was stirred at room temperature during 4 h. The solvent was evaporated under vacuum, and the crude residue was purified by flash chromatography (gradient eluent MeOH–5% NH₃(aq)/MeOH).

3'-Amino-5'-ethoxycarbonylamino-2',3',5'-trideoxyuridine (4a): ¹H NMR (MeOH-*d*₄, 200 MHz) δ 1.43 (t, 3H, H_{4'}, ³J_{HH} = 7.1 Hz), 2.36–2.49 (m, 2H, H₂), 3.50 (m, 1H, H₃), 3.63 (m, 2H, H₅), 3.89 (m, 1H, H₄), 4.27 (q, 2H, H_{3'}, ³J_{HH} = 7.1 Hz), 5.88 (d, 1H, H₅, ³J_{HH} = 8.1 Hz), 6.29 (dd, 1H, H₁, ³J_{HH} = 6.8 Hz, ³J_{HH} = 5.1 Hz), 7.92 (d, 1H, H₆, ³J_{HH} = 8.1 Hz); MS (ESI⁺, *m/z*) 337 [(M + K)⁺, 8], 321 [(M + Na)⁺, 100], 299 [(M + H)⁺, 31].

5'-Allyloxycarbonylamino-3'-amino-2',3',5'-trideoxyuridine (4b): ¹H NMR (MeOH-*d*₄, 300 MHz) δ 2.36–2.53 (m, 2H, H₂), 3.50 (m, 1H, H₃), 3.59–3.72 (m, 2H, H₅), 3.90 (m, 1H, H₄), 4.74 (d, 2H, H_{3'}, ³J_{HH} = 5.4 Hz), 5.37 (dd, 1H, H_{5'}, ³J_{HH} = 10.5 [²J_{HH}] 1.4 Hz), 5.50 (dd, 1H, H_{5'E}, ³J_{HH} = 17.1 [²J_{HH}] 1.4 Hz), 5.87 (d, 1H, H₅, ³J_{HH} = 8.0 Hz), 6.06–6.19 (m, 1H, H_{4'}), 6.29 (dd, 1H, H₁, ³J_{HH} = 6.8 Hz, ³J_{HH} = 4.6 Hz), 7.92 (d, 1H, H₆,

(19) The antiviral assays are being performed in the Rega Institute for Medical Research (Louvain, Belgium) by Prof. Erik De Clercq.

(20) Lavandera, I.; Fernández, S.; Ferrero, M.; De Clercq, E.; Gotor, V. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 833–836.

(18) Dangles, O.; Guibé, F.; Balavoine, G.; Lavielle, S.; Marquet, A. *J. Org. Chem.* **1987**, *52*, 4984–4993.

$^3J_{\text{HH}} = 8.0$ Hz); MS (ESI⁺, *m/z*) 349 [(M + K)⁺, 20], 333 [(M + Na)⁺, 100], 311 [(M + H)⁺, 42].

3'-Amino-5'-benzyloxycarbonylamino-2',3',5'-trideoxyuridine (4c): ¹H NMR (MeOH-*d*₄, 200 MHz) δ 2.41 (m, 2H, H₂), 3.48 (m, 1H, H₃), 3.67 (m, 2H, H₅), 3.88 (m, 1H, H₄), 5.21–5.36 (m, 2H, H_{3'}), 5.79 (d, 1H, H₅, $^3J_{\text{HH}} = 8.1$ Hz), 6.27 (dd, 1H, H₁, $^3J_{\text{HH}} = 6.3$ Hz, $^3J_{\text{HH}} = 4.9$ Hz), 7.48–7.55 (m, 5H, H_{ar}), 7.87 (d, 1H, H₆, $^3J_{\text{HH}} = 8.1$ Hz); MS (ESI⁺, *m/z*) 399 [(M + K)⁺, 19], 383 [(M + Na)⁺, 100], 361 [(M + H)⁺, 41].

3'-Amino-5'-ethoxycarbonylamino-3',5'-dideoxythymidine (5a): ¹H NMR (MeOH-*d*₄, 200 MHz) δ 1.43 (t, 3H, H_{4'}, $^3J_{\text{HH}} = 7.1$ Hz), 2.09 (s, 3H, H₇), 2.42 (m, 2H, H₂), 3.55 (m, 1H, H₃), 3.64 (m, 2H, H₅), 3.84 (m, 1H, H₄), 4.28 (q, 2H, H_{3'}, $^3J_{\text{HH}} = 7.1$ Hz), 6.31 (dd, 1H, H₁, $^3J_{\text{HH}} = 6.8$ Hz, $^3J_{\text{HH}} = 4.9$ Hz), 7.74 (s, 1H, H₆); MS (ESI⁺, *m/z*) 351 [(M + K)⁺, 10], 335 [(M + Na)⁺, 100], 313 [(M + H)⁺, 28].

5'-Allyloxycarbonylamino-3'-amino-3',5'-dideoxythymidine (5b): ¹H NMR (MeOH-*d*₄, 200 MHz) δ 2.08 (s, 3H, H₇), 2.43 (m, 2H, H₂), 3.50 (m, 1H, H₃), 3.66 (m, 2H, H₅), 3.88 (m, 1H, H₄), 4.74 (d, 2H, H_{3'}, $^3J_{\text{HH}} = 5.1$ Hz), 5.36 (dd, 1H, H_{5'z}, $^3J_{\text{HH}} = 10.2$ [$^2J_{\text{HH}}$] 1.2 Hz), 5.49 (dd, 1H, H_{5'E}, $^3J_{\text{HH}} = 17.3$ [$^2J_{\text{HH}}$] 1.2 Hz), 6.03–6.21 (m, 1H, H_{4'}), 6.31 (dd, 1H, H₁, $^3J_{\text{HH}} = 6.6$ Hz), 7.73 (s, 1H, H₆); MS (ESI⁺, *m/z*) 363 [(M + K)⁺, 5], 347 [(M + Na)⁺, 100], 325 [(M + H)⁺, 27].

3'-Amino-5'-benzyloxycarbonylamino-3',5'-dideoxythymidine (5c): ¹H NMR (MeOH-*d*₄, 200 MHz) δ 2.00 (s, 3H, H₇), 2.39 (m, 2H, H₂), 3.50 (m, 1H, H₃), 3.69 (m, 2H, H₅), 3.88 (m, 1H, H₄), 5.21–5.37 (m, 2H, H_{3'}), 6.30 (dd, 1H, H₁, $^3J_{\text{HH}} = 6.4$ Hz, $^3J_{\text{HH}} = 5.1$ Hz), 7.46–7.53 (m, 5H, H_{ar}), 7.71 (s, 1H, H₆); MS (ESI⁺, *m/z*) 413 [(M + K)⁺, 14], 397 [(M + Na)⁺, 100], 375 [(M + H)⁺, 63].

(E)-3'-Amino-5-(2-bromovinyl)-5'-ethoxycarbonylamino-2',3',5'-trideoxyuridine (6a): ¹H NMR (MeOH-*d*₄, 200 MHz) δ 1.46 (t, 3H, H_{4'}, $^3J_{\text{HH}} = 7.1$ Hz), 2.45 (m, 2H, H₂), 3.50 (m, 1H, H₃), 3.68 (m, 2H, H₅), 3.88 (m, 1H, H₄), 4.31 (q, 2H, H_{3'}, $^3J_{\text{HH}} = 7.1$ Hz), 6.28 (dd, 1H, H₁, $^3J_{\text{HH}} = 6.1$ Hz, $^3J_{\text{HH}} = 4.9$ Hz), 7.07 (d, 1H, H₇, $^3J_{\text{HH}} = 13.7$ Hz), 7.56 (d, 1H, H₈, $^3J_{\text{HH}} = 13.7$ Hz), 8.01 (s, 1H, H₆); MS (ESI⁺, *m/z*) 443 [(M⁸¹Br + K)⁺, 15], 441 [(M⁷⁹Br + K)⁺, 13], 427 [(M⁸¹Br + Na)⁺, 100], 425 [(M⁷⁹Br + Na)⁺, 98], 405 [(M⁸¹Br + H)⁺, 73], 403 [(M⁷⁹Br + H)⁺, 75].

(E)-5'-Allyloxycarbonylamino-3'-amino-5-(2-bromovinyl)-2',3',5'-trideoxyuridine (6b): ¹H NMR (MeOH-*d*₄, 200 MHz) δ 2.44 (m, 2H, H₂), 3.50 (m, 1H, H₃), 3.68 (m, 2H, H₅), 3.92 (m, 1H, H₄), 4.76 (d, 2H, H_{3'}, $^3J_{\text{HH}} = 5.6$ Hz), 5.38 (dd, 1H, H_{5'z}, $^3J_{\text{HH}} = 10.5$ [$^2J_{\text{HH}}$] 1.5 Hz), 5.50 (dd, 1H, H_{5'E}, $^3J_{\text{HH}} = 17.3$ [$^2J_{\text{HH}}$] 1.5 Hz), 6.05–6.22 (m, 1H, H_{4'}), 6.29 (dd, 1H, H₁, $^3J_{\text{HH}} = 6.1$ Hz), 7.06 (d, 1H, H₇, $^3J_{\text{HH}} = 13.7$ Hz), 7.56 (d, 1H, H₈, $^3J_{\text{HH}} = 13.7$ Hz), 8.01 (s, 1H, H₆); MS (ESI⁺, *m/z*) 439 [(M⁸¹Br + Na)⁺, 97], 437 [(M⁷⁹Br + Na)⁺, 100], 417 [(M⁸¹Br + H)⁺, 89], 415 [(M⁷⁹Br + H)⁺, 86].

(E)-3'-Amino-5'-benzyloxycarbonylamino-5-(2-bromovinyl)-2',3',5'-trideoxyuridine (6c): ¹H NMR (MeOH-*d*₄, 300 MHz) δ 2.42 (m, 2H, H₂), 3.49 (m, 1H, H₃), 3.61–3.75 (m, 2H, H₅), 3.90 (m, 1H, H₄), 5.31 (m, 2H, H_{3'}), 6.27 (dd, 1H, H₁, $^3J_{\text{HH}} = 6.1$ Hz), 7.05 (d, 1H, H₇, $^3J_{\text{HH}} = 13.6$ Hz), 7.45–7.57 (m, 6H, H_{ar}+H₈), 8.00 (s, 1H, H₆); MS (ESI⁺, *m/z*) 489 [(M⁸¹Br + Na)⁺, 32], 487 [(M⁷⁹Br + Na)⁺, 33], 467 [(M⁸¹Br + H)⁺, 94], 465 [(M⁷⁹Br + H)⁺, 100].

5'-Amino-3'-benzyloxycarbonylamino-2',3',5'-trideoxyuridine (7c): ¹H NMR (MeOH-*d*₄, 200 MHz) δ 2.55 (m, 2H, H₂), 3.10 (m, 2H, H₅), 3.92 (m, 1H, H₄), 4.35 (m, 1H, H₃), 5.27 (br s, 2H, H_{3'}), 5.90 (d, 1H, H₅, $^3J_{\text{HH}} = 8.3$ Hz), 6.27 (dd, 1H, H₁, $^3J_{\text{HH}} = 7.1$ Hz), 7.53 (s, 5H, H_{ar}), 7.93 (d, 1H, H₆, $^3J_{\text{HH}} = 8.3$ Hz); MS (ESI⁺, *m/z*) 383 [(M + Na)⁺, 71], 361 [(M + H)⁺, 100].

5'-Amino-3'-benzyloxycarbonylamino-3',5'-dideoxythymidine (8c): ¹H NMR (MeOH-*d*₄, 200 MHz) δ 2.09 (s, 3H, H₇), 2.44–2.67 (m, 2H, H₂), 3.11 (m, 2H, H₅), 3.93 (m, 1H, H₄), 4.37 (m, 1H, H₃), 5.28 (s, 2H, H_{3'}), 6.33 (dd, 1H, H₁, $^3J_{\text{HH}} = 5.6$ Hz),

7.53 (br s, 5H, H_{ar}), 7.73 (s, 1H, H₆); MS (ESI⁺, *m/z*) 397 [(M + Na)⁺, 9], 375 [(M + H)⁺, 63].

(E)-5'-Amino-3'-benzyloxycarbonylamino-5-(2-bromovinyl)-2',3',5'-trideoxyuridine (9c): ¹H NMR (MeOH-*d*₄, 300 MHz) δ 2.55–2.72 (m, 2H, H₂), 3.27–3.42 (m, 2H, H₅), 4.08 (m, 1H, H₄), 4.44 (m, 1H, H₃), 5.29 (br s, 2H, H_{3'}), 6.28 (dd, 1H, H₁, $^3J_{\text{HH}} = 5.7$ Hz), 7.05 (d, 1H, H₇, $^3J_{\text{HH}} = 13.7$ Hz), 7.53 (m, 6H, H_{ar} + H₈), 7.98 (s, 1H, H₆); MS (ESI⁺, *m/z*) 505 [(M⁸¹Br + K)⁺, 3], 503 [(M⁷⁹Br + K)⁺, 3], 489 [(M⁸¹Br + Na)⁺, 30], 487 [(M⁷⁹Br + Na)⁺, 28], 467 [(M⁸¹Br + H)⁺, 100], 465 [(M⁷⁹Br + H)⁺, 99].

5'-Allyloxycarbonylamino-3'-benzyloxycarbonylamino-2',3',5'-trideoxyuridine (10): ²¹¹H NMR (CDCl₃, 200 MHz) δ 2.26–2.39 (m, 2H, H₂), 3.39 (m, 1H, H₅), 3.62 (m, 1H, H₅), 3.89 (m, 1H, H₄), 4.11 (m, 1H, H₃), 4.56 (d, 2H, H_{3'}, $^3J_{\text{HH}} = 5.1$ Hz), 5.11–5.33 (m, 4H, H_{3'z} + H_{5'z} + H_{5'E}), 5.73–6.04 (m, 4H, H₅ + H_{4'} + 2 NH), 6.13 (dd, 1H, H₁, $^3J_{\text{HH}} = 6.2$ Hz), 7.34 (s, 5H, H_{ar}), 7.43 (d, 1H, H₆, $^3J_{\text{HH}} = 8.7$ Hz), 9.75 (s, 1H, NH); MS (ESI⁺, *m/z*) 483 [(M + K)⁺, 67], 467 [(M + Na)⁺, 100].

5'-Allyloxycarbonylamino-3'-benzyloxycarbonylamino-3',5'-dideoxythymidine (11): ²¹¹H NMR (CDCl₃, 200 MHz) δ 1.90 (s, 3H, H₇), 2.18–2.35 (m, 2H, H₂), 3.43 (m, 1H, H₅), 3.62 (m, 1H, H₅), 3.89 (m, 1H, H₄), 4.11 (m, 1H, H₃), 4.57 (d, 2H, H_{3'}, $^3J_{\text{HH}} = 5.1$ Hz), 5.10–5.33 (m, 4H, H_{3'z} + H_{5'z} + H_{5'E}), 5.72 (br s, 1H, NH), 5.80–5.99 (m, 1H, H_{4'}), 6.20 (dd, 1H, H₁, $^3J_{\text{HH}} = 5.9$ Hz), 6.28 (d, 1H, NH, $^3J_{\text{HH}} = 6.4$ Hz), 7.34 (m, 6H, H_{ar} + H₆), 9.77 (s, 1H, NH); MS (ESI⁺, *m/z*) 497 [(M + K)⁺, 100], 481 [(M + Na)⁺, 76].

(E)-5'-Allyloxycarbonylamino-3'-benzyloxycarbonylamino-5-(2-bromovinyl)-2',3',5'-trideoxyuridine (12): ²¹¹H NMR (MeOH-*d*₄, 300 MHz) δ 2.55 (m, 2H, H₂), 3.57–3.74 (m, 2H, H₅), 4.08 (m, 1H, H₄), 4.31 (m, 1H, H₃), 4.75 (br s, 2H, H_{3'}), 5.28–5.37 (m, 3H, H_{3'z} + H_{5'z}), 5.49 (dd, 1H, H_{5'E}, $^3J_{\text{HH}} = 17.3$ [$^2J_{\text{HH}}$] 1.4 Hz), 6.06–6.18 (m, 1H, H_{4'}), 6.30 (dd, 1H, H₁, $^3J_{\text{HH}} = 6.0$ Hz), 7.06 (d, 1H, H₇, $^3J_{\text{HH}} = 13.7$ Hz), 7.54 (m, 6H, H_{ar} + H₈), 7.99 (s, 1H, H₆); MS (ESI⁺, *m/z*) 589 [(M⁸¹Br + K)⁺, 22], 587 [(M⁷⁹Br + K)⁺, 30], 573 [(M⁸¹Br + Na)⁺, 100], 571 [(M⁷⁹Br + Na)⁺, 91], 551 [(M⁸¹Br + H)⁺, 7], 549 [(M⁷⁹Br + H)⁺, 8].

Acknowledgment. We thank Prof. Erik De Clercq for the biological assays of these compounds. We express our appreciation to Novo Nordisk Co. for the generous gift of the CAL-B. Financial support of this work by the Spanish Ministerio de Ciencia y Tecnología (Project PPQ-2001-2683) and by Principado de Asturias (Project GE-EXP01-03) is gratefully acknowledged. S.F. thanks MCYT for a personal grant (Ramón y Cajal Project). I.L. thanks MCYT for a pre-doctoral fellowship.

Supporting Information Available: Complete ¹H, ¹³C, and DEPT NMR spectral data and some 2D NMR experiments are shown in addition to mp, IR, microanalysis, and MS data. The level of purity is indicated by the inclusion of copies of ¹H and ¹³C NMR spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

JO035678M

(21) Structures of compounds 10–12 are labeled as follows:

