N(3)-Protection of Thymidine with Boc for an Easy Synthetic Access to Sugar-Alkylated Nucleoside Analogs

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The use of Boc as a nucleobase-protecting group in the synthesis of sugar-modified thymidine analogs is reported. Boc was easily inserted at N(3) by a simple and high-yielding reaction and found to be stable to standard treatments for the removal of Ac and ^{t}BuMe$_2$Si (TBDMS) groups, as well as to $ZnBr_2$-mediated 4,4′-dimethoxytrityl (DMTr) deprotection. Boc Protection proved to be completely resistant to the strong basic conditions required to regioselectively achieve *O*-alkylation, therefore, providing synthetic access to a variety of sugar-alkylated nucleoside analogs. To demonstrate the feasibility of this approach, two 3′-*O*-alkylated thymidine analogs have been synthesized in high overall yields and fully characterized.

Introduction. – Boc is a widely used protecting group [1], first developed for the peptide synthesis and successively exploited in a wide range of synthetic organic chemistry procedures. Its application in nucleoside or oligonucleotide chemistry, and related mimics has been reported only in sporadic cases, as for the protection of exocyclic amino groups in cytidine, adenosine, and guanosine derivatives [2], and for the synthesis of modified PNA [3].

To the best of our knowledge, its use for masking the thymine base has been reported only by *Moon* and co-workers, who exploited *N(3)*-Boc-protected thymidine building blocks to obtain a 3′-deoxy,3′-[^{18}F]fluorinated thymidine analog in high radiochemical yield [4]. These researchers prepared the Boc-protected nucleoside by reaction with $(Boc)_2O$ in the presence of stoichiometric amounts of 4-(dimethylamino)pyridine (DMAP) in THF, which required long reaction times (5 h) and led to the target compound in only 53% yield. A similar protocol was applied by *Yu et al.* to protect uracil in the synthesis of 5-fluoro and (*E*)-5-(2-fluorovinyl)arabinosyluridine (Farall and FVAU, resp.) *via* 5-(trimethylstannyl)- and (*E*)-5-[(2-tributylstannyl)vinyl]arabinosyluridine analogs [5]. Also in this case, insertion of the Boc group was obtained in low overall yields (50%), leading to a mixture of *N*- and *O*-derivatized nucleosides.

More efficient procedures to protect thymine and uracil, to attain synthetic access to a variety of sugar-modified thymidine and uridine analogs, have been explored by other research groups. Among others, benzyl (Bn; removable by catalytic hydrogenation) [6], 4-methoxybenzyl (removable by ceric ammonium nitrate treatment) [7], and 4-nitrophenylethyl (removable by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in pyridine) [8] groups have been adopted to this purpose.

In the course of our studies on nucleoside analogs, we recently developed a novel protection for the thymine base in nucleoside and oligonucleotide synthesis, based on

the 2-(phenylsulfanyl)ethyl group, inserted at N(3) by a *Mitsunobu* reaction with 2-(phenylsulfanyl)ethanol [9]. In an efficient 'two-stage' system, after oxidation of the thioether to sulfone, this protecting group could be totally removed by a β-elimination mechanism by using 0.1M aqueous NaOH solution. The use of the 2-(phenylsulfanyl)-ethyl protecting group, stable to strongly basic conditions, allowed us to selectively achieve *O*-alkylation of the ribose moieties in satisfactory yields [10], and, thus, to efficiently obtain the anti-HIV active *Hotoda*'s $^{5'}$TGGGAG$^{3'}$ sequence [11], alkylated at the 5′-OH moiety of the thymidine unit with the 3,4-(dibenzyloxy)benzyl group.

Aiming at expanding the repertoire of available sugar-alkylated thymidine analogs, of interest as potential thymidine kinase inhibitors[1]) and functional building blocks for the synthesis of sugar-modified oligonucleotides, we were intrigued by fast and simple synthetic methods to prepare easy-to-handle nucleoside scaffolds. Following the renovated interest in Boc protection, stimulated by the discovery of novel, very mild procedures for its removal[2]), we decided to re-investigate its applicability as a thymine-protecting group in the chemistry of nucleoside analogs.

Results and Discussion. – As model compounds for this study, we chose 3′-*O*-acetyl-5′-*O*-(4,4-dimethoxytriphenylmethyl)thymidine (**1**; *Scheme 1*) and 3′-*O*-acetyl-5′-*O*-[(*tert*-butyl)dimethylsilyl]thymidine (**4**; *Scheme 2*), which allowed us to explore the compatibility of Boc with the most commonly used protecting groups in nucleoside manipulations, *i.e.*, the acid-labile 4,4′-dimethoxytrityl (= bis(4-methoxyphenyl)phenylmethyl; DMTr), the fluoride-labile ^{t}BuMe$_2$Si (TBDMS) and the base-labile Ac group.

Insertion of the Boc group was first optimized on **1** (*Scheme 1*), adopting a very simple procedure, involving the use of 2 equiv. of $(Boc)_2O$ in the presence of Et_3N (3 equiv.) and catalytic amounts of DMAP in different solvents.

At this stage, the solvent was found to dramatically affect the course of the reaction. When the above mentioned reagents were dissolved in dioxane or MeCN, no transformation occurred, even forcing the system conditions; in fact, no benefit could be obtained by prolonging the reaction times, or using high temperatures, or adding to the mixtures bases stronger than Et_3N, as, *e.g.*, EtN^iPr_2. On the contrary, in a large variety of other commonly used solvents, the Boc protection was always complete in 1 h at room temperature, with very good-to-excellent yields (see *Table 1*)[3]) [14]. In these

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[1]) For a recent example, see [12].

[2]) For recent examples, see [13].

[3]) Solvent-controlled alkylation of 5′-*O*-protected thymidine derivatives has been described in [14]. Following [14b], when allyl, propargyl, and benzyl bromide were reacted in stoichiometric amounts with 5′-*O*-TBDMS-thymidine in the presence of NaH in THF under ultrasound activation, no reaction was observed. In the same solvent, using 2.5 equiv. of the alkylating agent and of the base, exclusively the 3′-*O*-alkylated derivative was found. On the contrary, only the corresponding *N(3)*-adduct could be isolated from the reaction conducted in DMF. This behavior was explained in terms of different dielectric constants of the solvents, with low dielectric constant solvents favoring the *O*-alkylation, and high dielectric constant solvents promoting the *N*-alkylation. In our case, the dielectric constant of the solvent cannot account for the different course of the Boc insertion on **1**, since the only observed failures occurred in dioxane and MeCN, the first one having a very low (2.21) and the latter a very high (37.5) dielectric constant.

Scheme 1. *Synthesis of Boc-Protected 5′-O-DMTr-Thymidine Derivative* **2** *and Its Conversion into Detritylated Analog* **3a**

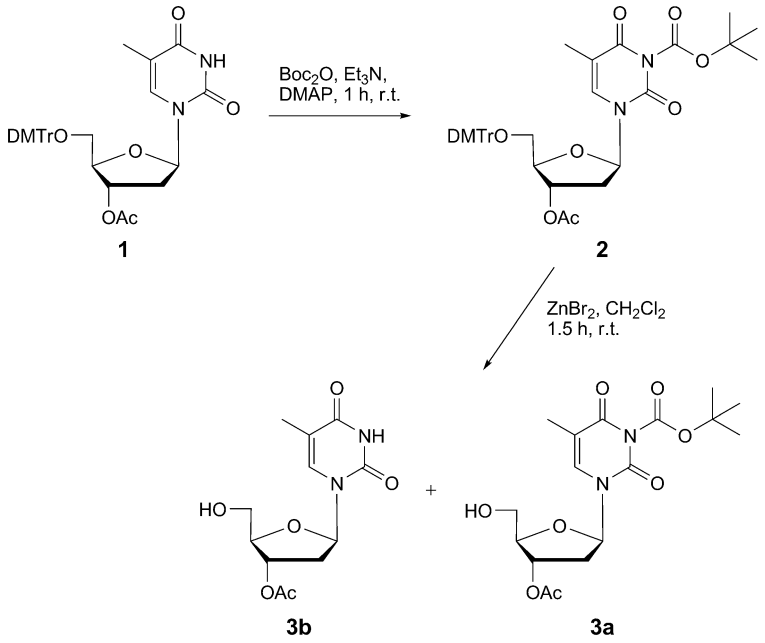


Table 1. *Yields of Insertion of the Boc Group on* **1** *Dependent of the Used Solvent*

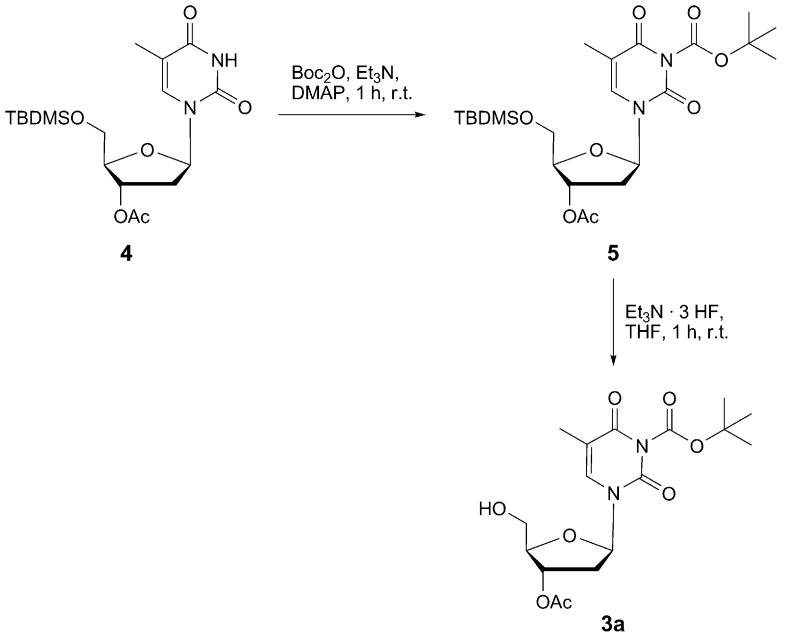
Solvent	Yield [%]	Solvent	Yield [%]
MeCN	0	Et_2O	90
Benzene	92	DMF	90
$ClCH_2CH_2Cl$	92	1,4-Dioxane	0
CH_2Cl_2	92	Pyridine	99

cases, target compound **2** was isolated as a stable compound after a simple workup, in yields always higher than 90%.

The best conditions for the introduction of Boc in **1** were then extended to 3′-*O*-acetyl-5′-*O*-TMDS-thymidine (**4**; *Scheme 2*). This nucleoside, dissolved in pyridine, was, therefore, reacted with $(Boc)_2O$ (2 equiv.) and Et_3N (3 equiv.) in the presence of 0.5 equiv. of DMAP, to afford the target compound **5** in almost quantitative yields.

The ^{1}H- and ^{13}C-NMR spectra of **2** and **5** clearly indicated that the Boc group was selectively inserted at N(3). In fact, no signal attributable to the N–H group could be detected in the ^{1}H-NMR spectra of these nucleosides recorded in (D_6)DMSO; in parallel, the ^{13}C-NMR spectra of both these compounds showed a unique signal at *ca.* 148 ppm, attributed to the sp^2-C-atom of the Boc CO group linked to an N-atom, with the concomitant absence of signals at *ca.* 155 ppm, expected in case of its attachment to an O-atom. In contrast to previous findings [5], we never observed migration of the Boc

Scheme 2. *Synthesis of Boc-Protected 5′-*O*-TBDMS-Thymidine Derivative* **5** *and Its Conversion into* **3a**



group from N(3) to O(4) or O(2), both in this and all of the successive manipulations. In addition, no transposition of the carbamates to the corresponding *O*-^{t}Bu derivatives [2b] was observed, as confirmed by MS analysis of the reaction mixtures and of the isolated reaction products.

With two Boc-protected thymidine derivatives, **2** and **5**, in our hands, we searched, first, for efficient conditions allowing the selective removal of the DMTr group from **2**. Having *a priori* eliminated the use of even diluted, weak protic acids – not able to guarantee the requested selectivity between the two acid-labile protecting groups Boc and DMTr – anhydrous *Lewis* acids, particularly $ZnBr_2$ [15] and $CuSO_4$ [16], were investigated in a variety of reaction systems. The use of the latter always caused partial cleavage of the Boc group. On the contrary, satisfactory results were obtained with anhydrous $ZnBr_2$, which led to **3a** in 84% yields. The isolation of only traces of **3b** from the mixture essentially confirmed that this treatment did not markedly affect the Boc group. The best conditions for the preparation of **3a** involved the use of 2 equiv. of $ZnBr_2$ in anhydrous CH_2Cl_2 under stirring at room temperature for 1.5 h. Prolonging the reaction times, even using a lower excess of the *Lewis* acid, was detrimental for the success of the reaction, causing also partial Boc removal; similar results were obtained when anhydrous MeOH was used in lieu of CH_2Cl_2 as the solvent (*Table 2*).

Removal of the TBDMS groups from **5** was carried out first by treatment with standard 1M Bu_4NF (TBAF) solution in THF, which, quite unexpectedly, was found to partially cleave the Boc group. On the contrary, full selectivity was obtained with the

Table 2. *Conditions Tested for the DMTr Removal from Nucleoside* **2** *and Relative Yields*

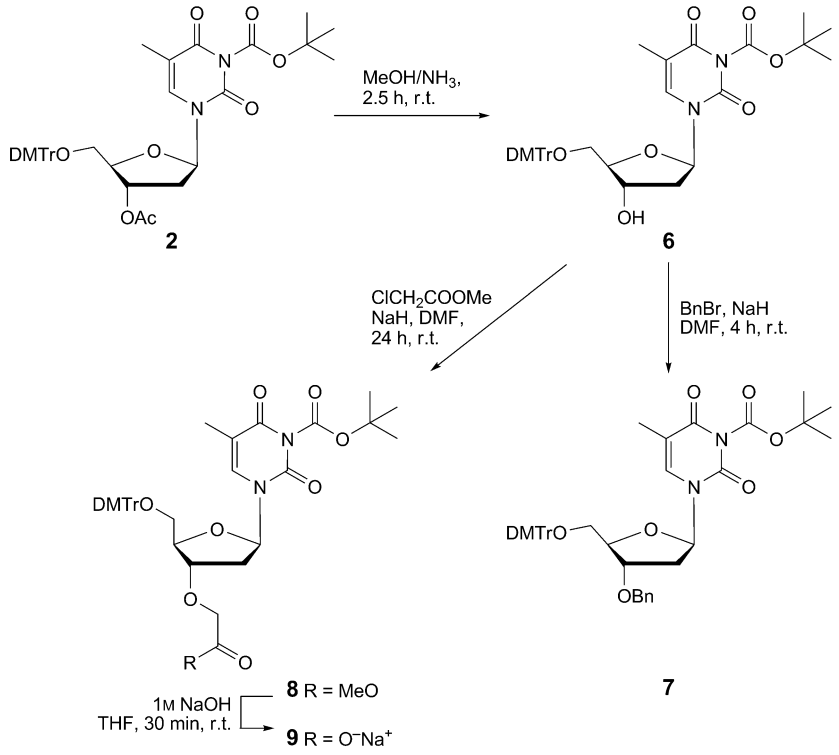
Equiv. of $ZnBr_2$	Solvent	Reaction time [h]	Yield after isolation [%]	
			3a	**3b**
2.5	CH_2Cl_2	1.5	84	9
3.0	CH_2Cl_2	2.5	60	32
1.5	CH_2Cl_2	2.5	60	32
2.5[a])	MeOH	1.2	55	0

[a]) $ZnBr_2$ was added as a sat. MeOH soln.

milder desilylating reagent $Et_3N \cdot 3\,HF$ [17], which gave the desired Boc-protected nucleoside **3a** in quantitative yields.

To prepare 3′-*O*-alkylated thymidine analogs, the Ac group at the 3′-OH moiety had to be removed first. For this step, a standard treatment with concentrated aqueous NH_3 was tested on **2**, which, after 2.5 h at room temperature, fully converted the starting material to desired **6** (*Scheme 3*). A model reaction for the 3′-*O*-alkylation was carried out first by reacting **6** with BnBr and NaH (1.2 equiv. each) in DMF, giving the target adduct **7** in 85% yields. Then, the alkylation reaction on **6** was carried out with

Scheme 3. *Synthesis of 3′*-O-*Alkylated Thymidine Analogs* **7**–**9**



$ClCH_2COOMe$. Due to the lower reactivity of alkyl chlorides compared to bromides, a higher excess of the alkylating agent and the base (2.5 equiv.) was here required to lead the reaction to completion, which, after chromatography, gave methyl ester **8** in 82% yields.

Hydrolysis of **8** with 1M NaOH (30 min, room temperature) led to target 3′-*O*-carboxymethyl sodium salt **9**, obtained in 82% yields as a pure compound (TLC and NMR) after a simple extraction (66% overall yield for four steps starting from **1**). This thymidine analog, as a convenient precursor of 3′-*O*-(carboxymethyl)thymidine [18], is of interest as a versatile building block for backbone-modified oligonucleotides. Its use as a useful monomer linker to be attached at the NH_2 end of a PNA chain in the synthesis of DNA-PNA chimera[4]) is currently under way in our laboratory and will be reported elsewhere.

Conclusions. – In this study, we have described the use of Boc to conveniently protect the thymine nucleobase for a simple and general synthetic access to a variety of sugar-alkylated thymidine analogs. Conditions for its quantitative installation on sugar-protected thymidine derivatives were found, as well as for the selective removal of the commonly used protecting groups DMTr, TBDMS, and Ac from the corresponding Boc-protected nucleosides. These high-yielding procedures, in principle, allow us to easily obtain useful and versatile nucleoside building blocks for several base-promoted sugar modifications. To demonstrate the feasibility of this approach, two sugar-alkylated thymidine analogs, **7** and **9**, have been synthesized and characterized.

Experimental Part

General. TLC: SiO_2 plates from *Merck* (*60*, *F254*); visualization with UV light and then by treatment with a 10% $Ce(SO_4)_2/H_2SO_4$ aq. soln. Column chromatography (CC): silica gel (SiO_2; *Kieselgel 40*, 0.063–0.200 mm; *Merck*). NMR Spectra: *Bruker WM-400* or *Varian XR-200* spectrometers; chemical shifts in ppm with respect to the residual solvent signal; *J* values in Hz; peak assignments on the basis of standard $^1H,^1H$-COSY and HSQC experiments. ESI-MS: *Waters Micromass ZQ* instrument, equipped with an electrospray source; in the positive-ion and/or negative-ion mode.

Procedure for the Insertion of Boc on **1**. *Synthesis of* **2**. *3′-O-Acetyl-5′-O-[bis(4-methoxyphenyl)-(phenyl)methyl]thymidine* (**1**; 53 mg, 0.090 mmol), dissolved in the appropriate solvent, was reacted with $(Boc)_2O$ (40 mg, 0.18 mmol), Et_3N (38 µl, 0.27 mmol), and 4-(dimethylamino)pyridine (DMAP; 5.5 mg, 0.045 mmol). After 1 h under stirring at r.t., the solvent was removed under reduced pressure, and the residue was purified by CC (SiO_2; hexane/AcOEt 7:3 (*v*/*v*)). The yields obtained for each reaction are compiled in *Table 1*.

*3′-O-Acetyl-5′-O-[bis(4-methoxyphenyl)(phenyl)methyl]-3-[(*tert*-butoxy)carbonyl]-2′-deoxy-3,4-dihydrothymidine* (**2**). Foam. R_f (hexane/AcOEt 1:1) 0.6. ^1H-NMR ($CDCl_3$, 200 MHz): 7.61 (*d*, *J* = 1.2, H–C(6)); 7.39–6.81 (*m*, 13 arom H of DMTr); 6.40 (*dd*, *J* = 7.4, 6.0, H–C(1′)); 5.45–5.41 (*m*, H–C(3′)); 4.14–4.12 (*m*, H–C(4′)); 3.82 (*s*, 2 MeO); 3.46 (*ABX*, *J* = 2.8, 10.4, CH_2(5′)); 2.46–2.40 (*m*, CH_2(2′)); 2.10 (*s*, MeCO); 1.60 (*s*, ^{t}Bu); 1.35 (*d*, *J* = 1.0, Me of Thy). ^{13}C-NMR ($CDCl_3$, 50 MHz): 170.2 (Me*C*O); 161.1 (C(4)); 158.7, 144.2, 135.0, 130.0, 128.1, 128.0, 127.1, 113.2 (arom. C of DMTr); 148.5 (C(2)); 148.0 (N–CO–N); 135.3 (C(6)); 111.3 (C(5)); 87.2 (C or DMTr); 86.6 (Me_3C); 84.6 (C(1′)); 84.1 (C(4′)); 75.3 (C(3′)); 63.6 (C(5′)); 55.2 (MeO); 38.1 (C(2′)); 27.4 (Me_3C); 21.0 (*Me*CO); 11.7 (Me of Thy). ESI-MS (pos.): 686.1 ($[M+H]^+$).

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[4]) For an example of linker monomers in the synthesis of DNA-PNA, see [19].

Procedure for the Removal of the DMTr Group from **2**. *Synthesis of* **3a**. Compound **2** (30 mg, 0.044 mmol) was dissolved in 1 ml of anh. CH_2Cl_2, then dry $ZnBr_2$ (25 mg, 0.110 mmol) was added. The mixture was stirred at r.t. for 1.5 h, then the reaction was quenched by addition of few drops of MeOH. The crude product was diluted with CH_2Cl_2 and washed twice with H_2O. The org. phase was dried (Na_2SO_4), concentrated under reduced pressure, and purified by CC (SiO_2; hexane/AcOEt 2:3). Products **3a** and **3b** were isolated in 84 and 9% yield, resp.

Other reaction conditions were tested (see *Table 2*), but only lower yields of **3a** were obtained under all other conditions.

*3′-*O*-Acetyl-3-[(*tert*-butoxy)carbonyl]-2′-deoxy-3,4-dihydrothymidine* (**3a**). Oil. R_f (hexane/AcOEt 3:7) 0.4. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.55 (*d*, *J* = 1.2, H–C(6)); 6.25 (*dd*, *J* = 7.4, 6.8, H–C(1′)); 5.34–5.32 (*m*, H–C(3′)); 4.11–4.09 (*m*, H–C(4′)); 3.93–3.91 (*m*, CH_2(5′)); 2.42–2.36 (*m*, CH_2(2′)); 2.09 (*s*, MeCO); 1.93 (*d*, *J* = 1.4, Me of Thy); 1.60 (*s*, ^{t}Bu). ^{13}C-NMR ($CDCl_3$, 100 MHz): 170.6 (Me*C*O); 161.1 (C(4)); 148.5 (C(2)); 147.7 (C of carbamate); 135.3 (C(6)); 110.9 (C(5)); 86.7 (Me_3C); 86.0 (C(1′)); 85.0 (C(4′)); 74.5 (C(3′)); 62.4 (C(5′)); 37.3 (C(2′)); 27.3 (Me_3C); 20.8 (*Me*CO); 12.5 (Me of Thy). ESI-MS (pos.): 791.2 ($[2M+Na]^+$), 423.0 ($[M+K]^+$), 407.1 ($[M+Na]^+$).

Compound **3b** was identified by TLC and NMR comparison with an authentic sample.

Procedure for the Insertion of Boc in **4**. *Synthesis of* **5**. *3′-*O*-Acetyl-5′-*O*-[(*tert*-butyl)dimethylsilyl]thymidine* (**4**; 53 mg, 0.090 mmol) was dissolved in 1 ml of dry pyridine, then $(Boc)_2O$ (40 mg, 0.180 mmol), Et_3N (38 µl, 0.270 mmol), and DMAP (5.5 mg, 0.045 mmol) were added in this order. The mixture was stirred at r.t. for 1 h, the solvent was removed under reduced pressure, and the residue was purified by CC (SiO_2; hexane/AcOEt 7:3). The desired compound **5** was obtained in 98% isolated yield (60 mg, 0.088 mmol).

*3′-*O*-Acetyl-3-[(*tert*-butoxy)carbonyl]-5′-*O*-[(*tert*-butyl)dimethylsilyl]-2′-deoxy-3,4-dihydrothymidine* (**5**). Oil. R_f (hexane/AcOEt 4:6) 0.8. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.54 (*d*, *J* = 1.2, H–C(6)); 6.33 (*dd*, *J* = 5.2, H–C(1′)); 5.25–5.21 (*m*, H–C(3′)); 4.11–4.09 (*m*, H–C(4′)); 3.91–3.89 (*m*, CH_2(5′)); 2.47–2.38 (*m*, CH_2(2′)); 2.08 (*s*, MeCO); 1.93 (*d*, *J* = 1.2, Me); 1.61 (*s*, ^{t}Bu); 0.93 (*s*, ^{t}BuSi); 0.13 (*s*, Me_2Si). ^{13}C-NMR ($CDCl_3$, 50 MHz): 170.6 (Me*C*O); 161.2 (C(4)); 148.5 (C(2)); 147.9 (C of carbamate); 134.3 (C(6)); 110.9 (C(5)); 86.7 (Me_3C); 85.4 (C(1′)); 85.0 (C(4′)); 75.3 (C(3′)); 63.5 (C(5′)); 37.3 (C(2′)); 27.4 (Me_3C); 25.9 (Me_3CSi); 20.9 (*Me*CO); 18.3 (Me_3CSi); 12.5 (Me of Thy); −5.6 (Me_2Si). ESI-MS (pos.): 536.8 ($[M+K]^+$), 520.9 ($[M+Na]^+$), 499.1 ($[M+H]^+$).

Procedure for the Removal of TBDMS Group from **5**. Compound **5** (20 mg, 0.040 mmol), dissolved in 200 µl of anh. THF, was reacted with $Et_3N \cdot 3$ HF (20 µl, 0.120 mmol). The mixture was stirred at r.t. for 1 h, then the soln., diluted with CH_2Cl_2, was washed twice with H_2O; the org. phase was dried (Na_2SO_4), concentrated under reduced pressure, and purified by CC (SiO_2; hexane/AcOEt 2:3). The desired product was obtained in quant. yield, and identified as identical to nucleoside **3a**, isolated from detritylation of **2**.

Procedure for the Removal of the Ac Group from **2**. *Synthesis of* **6**. Compound **2** (61 mg, 0.089 mmol) was dissolved in 800 µl of MeOH, and 200 µl of a NH_4OH soln. (29.1% *p.p.*) were added. After stirring for 2.5 h at r.t., the mixture was concentrated under reduced pressure. The crude product was then washed with CH_2Cl_2/H_2O, and the org. phase was dried (Na_2SO_4) and concentrated under reduced pressure. The desired product **6** was obtained in almost quant. yield without any observable side-reaction.

*5′-*O*-[Bis(4-methoxyphenyl)(phenyl)methyl]-3-[(*tert*-butoxy)carbonyl]-2′-deoxy-3,4-dihydrothymidine* (**6**). Oil. R_f (hexane/AcOEt 1:1) 0.4. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.65 (*d*, *J* = 1.2, H–C(6)); 7.44–6.84 (*m*, 13 arom. H of DMTr); 6.40 (*dd*, *J* = 7.4, 6.0, H–C(1′)); 4.62–4.60 (*m*, H–C(3′)); 4.10–4.07 (*m*, H–C(4′)); 3.82 (*s*, 2 MeO); 3.45 (*ABX*, *J* = 2.8, 10.4, CH_2(5′)); 2.46–2.38 (*m*, CH_2(2′)); 1.63 (*s*, ^{t}Bu); 1.45 (*d*, *J* = 1.2, Me of Thy). ^{13}C-NMR ($CDCl_3$, 50 MHz): 161.1 (C(4)); 158.7, 144.2, 135.0, 130.0, 128.1, 128.0, 127.1, 113.2 (arom. C of DMTr); 148.5 (C(2)); 148.0 (C of carbamate); 135.3 (C(6)); 110.8 (C(5)); 86.9 (C or DMTr); 86.6 (Me_3C); 86.2 (C(1′)); 85.0 (C(4′)); 72.1 (C(3′)); 63.4 (C(5′)); 55.2 (MeO); 41.1 (C(2′)); 27.4 (Me_3C); 11.7 (Me of Thy). ESI-MS (pos.): 673.0 ($[M+K]^+$), 666.9 ($[M+Na]^+$), 645.0 ($[M+H]^+$).

Procedure for the Synthesis of **7**. Compound **6** (50 mg, 0.078 mmol) was dissolved in 400 µl of anh. DMF and cooled at 0°. After 5 min, BnBr (11 µl, 0.094 mmol) and NaH (60% *p.p.*, 4.0 mg, 0.094 mmol) were added in this order. The mixture was stirred at r.t. for 4 h, then a few drops of MeOH were added at 0° to quench the reaction. The suspension was concentrated under reduced pressure, and the crude was

then washed with CH_2Cl_2/H_2O; the org. phase was dried (Na_2SO_4), concentrated under reduced pressure, and purified by CC (SiO_2; hexane/AcOEt 2:3). The desired product **7** was obtained in 85% yield (49 mg, 0.066 mmol).

*3′-O-Benzyl-5′-O-[bis(4-methoxyphenyl)(phenyl)methyl]-3-[(*tert*-butoxy)carbonyl]-2′-deoxy-3,4-dihydrothymidine* (**7**). Oil. R_f (hexane/AcOEt 1:1) 0.8. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.63 (*d*, *J* = 1.2, H–C(6)); 7.36–6.79 (*m*, 18 arom. H of DMTr and Bn); 6.34 (*dd*, *J* = 7.6, 5.8, H–C(1′)); 4.58–4.40 (*AB*, *J* = 11.8, PhCH_2O); 4.33–4.31 (*m*, H–C(3′)); 4.19–4.17 (*m*, H–C(4′)); 3.79 (*s*, 2 MeO); 3.53–3.26 (*ABX*, *J* = 2.9, 10.6, CH_2(5′)); 2.61–2.17 (*m*, CH_2(2′)); 1.61 (*s*, ^{t}Bu); 1.42 (*d*, *J* = 1.0, Me of Thy). ^{13}C-NMR ($CDCl_3$, 50 MHz): 161.3 (C(4)); 158.7, 144.2, 137.3, 130.0, 128.5, 128.1, 128.0, 127.9, 127.6, 127.1, 113.2 (arom. C of DMTr and Bn); 148.5 (C(2)); 148.0 (C of carbamate); 135.3 (C(6)); 110.9 (C(5)); 86.9 (C or DMTr); 86.2 (C(1′)); 85.2 ((Me$_3$*C*); 84.2 (C(4′)); 78.3 (Ph*C*H$_2$); 71.3 (C(3′)); 63.4 (C(5′)); 55.2 (MeO); 46.2 (C(2′)); 27.4 (*Me*$_3$C); 11.4 (Me). ESI-MS (pos.): 772.67 ([*M* + K]$^+$), 756.77 [*M* + Na]$^+$), 735.91 ([*M* + H]$^+$).

Procedure for Synthesis of **8**. Compound **6** (40 mg, 0.062 mmol) was dissolved in 0.5 ml of dry DMF, then $ClCH_2COOMe$ (14 µl, 0.15 mmol) and NaH (60% *p.p.*, 8.0 mg, 0.19 mmol) were added. The mixture was stirred at r.t. for 24 h, then a few drops of MeOH were added at 0° to quench the reaction. The suspension was taken to dryness, then the crude was redissolved in CH_2Cl_2 and washed twice with H_2O; the org. phase was dried (Na_2SO_4), concentrated under reduced pressure, and purified by CC (SiO_2; hexane/AcOEt 3:7). The desired ester **8** was obtained in 82% yield (37 mg, 0.051 mmol).

*5′-O-[Bis(4-methoxyphenyl)(phenyl)methyl]-3-[(*tert*-butoxy)carbonyl]-2′-deoxy-3,4-dihydro-3′-O-(2-methoxy-2-oxoethyl)thymidine* (**8**). Oil. R_f (hexane/AcOEt 1:1) 0.5. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.63 (*s*, H–C(6)); 7.44–6.84 (*m*, 13 arom. H of DMTr); 6.33 (*dd*, *J* = 5.6, 6.0, H–C(1′)); 4.33–4.10 (*m*, H–C(3′), H–C(4′), C*H*$_2$COOMe); 3.81 (*s*, 2 MeO); 3.75 (*s*, COOMe); 3.41 (*ABX*, *J* = 2.8, 10.4, CH_2(5′)); 2.58–2.26 (*m*, CH_2(2′)); 1.61 (*s*, ^{t}Bu); 1.44 (*s*, Me of Thy). ^{13}C-NMR ($CDCl_3$, 50 MHz): 170.7 (*C*OOMe); 161.1 (C(4)); 158.7, 144.2, 135.0, 130.0, 128.1, 128.0, 127.1, 113.2 (arom. C of DMTr); 148.5 (C(2)); 147.3 (C of carbamate); 135.3 (C(6)); 110.6 (C(5)); 86.8 (C or DMTr); 84.9 (Me$_3$*C*); 84.2 (C(1′)); 80.2 (C(4′)); 79.2 (C(3′)); 63.4 (C(5′)); 62.2 (*C*H$_2$COOMe); 55.2 (MeO); 52.2 (COO*Me*); 37.1 (C(2′)); 27.4 (*Me*$_3$C); 11.8 (Me of Thy). ESI-MS (pos.):754.75 ([*M* + K]$^+$), 738.73 ([*M* + Na]$^+$, 716.94 ([*M* + H]$^+$).

Procedure for the Synthesis of the Sodium Salt **9**. Compound **8** (12 mg, 0.017 mmol) was dissolved in 100 µl of dry THF, then NaOH (0.7 mg, 0.019 mmol) in 20 µl of H_2O was added. The mixture was kept under stirring at r.t. for 30 min, then the mixture was taken to dryness, redissolved in CH_2Cl_2, and washed twice with H_2O; the org. phase was dried (Na_2SO_4) and concentrated under reduced pressure. The desired salt **9** was obtained in 82% yield (10 mg, 0.014 mmol).

*Sodium 5′-O-[Bis(4-methoxyphenyl)(phenyl)methyl]-3-[(*tert*-butoxy)carbonyl]-3′-O-(carboxylatomethyl)-2′-deoxy-3,4-dihydrothymidine* (**9**). Oil. R_f (AcOEt) 0.1. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.61 (*s*, H–C(6)); 7.31–6.76 (*m*, 13 arom. H of DMTr); 6.34 (br., H–C(1′)); 4.18–4.16 (*m*, H–C(3′), H–C(4′)); 3.79 (*s*, CH_2CO); 3.73 (*s*, 2 MeO); 3.37–3.35 (*m*, CH_2(5′)); 2.55–2.14 (*m*, CH_2(2′)); 1.49 (*s*, ^{t}Bu); 1.25 (*s*, Me of Thy). ^{13}C-NMR ($CDCl_3$, 50 MHz): 175.6 (COO); 161.1 (C(4)); 158.6, 144.0, 129.9, 127.9, 127.0, 113.2 (arom. C of DMTr); 148.6 (C(2)); 147.8 (C of carbamate); 135.1 (C(6)); 111.1 (C(5)); 86.9 (C or DMTr); 86.5 (Me$_3$*C*); 85.2 (C(1′)); 83.9 (C(4′)); 80.9 (C(3′)); 68.6 (*C*H$_2$COO); 63.8 (C(5′)); 55.1 (MeO); 37.3 (C(2′)); 27.2 (*Me*$_3$C); 11.5 (Me of Thy). ESI-MS (neg.): 700.88 (M^-).

We acknowledge *MIUR* (*PRIN*) for grants in support of this investigation. We also thank *C. I. M. C. F.*, Università degli Studi di Napoli 'Federico II', for the NMR and MS facilities.

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Received March 28, 2011