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Note

Solvent-controlled regioselective protection of 5′-O-protected thymidine

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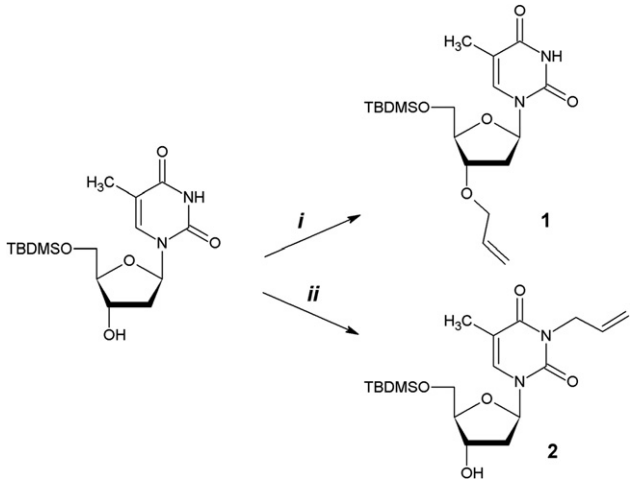
Abstract—This paper describes an efficient procedure for selective 3′-O- or 3-N-protection of 5′-*O*-*tert*-butyldimethylsilylthymidine, depending on the use of aprotic polar solvents with low or high dielectric constant, respectively. These syntheses were activated by either ultrasound or microwaves. Several alkyl bromides offer a convenient route to prepare 3′-O- or 3-N-protected and functionalized thymidine derivatives.

Keywords: Regioselective protection; Nucleoside; Solvent effect; Ultrasound activation; Microwave activation

Thymidine is a key biological compound. A large number of applications in the field of molecular biology rely on the use of thymidine or thymidine derivatives.[1] Insertion of these molecules into polymers or oligomers such as oligonucleotides requires selective protection and functionalization procedures. The latter can be alleviated by the use of regioselective reactions such as those described in this article.

We recently published the dimerization of thymidine using cross-metathesis. As part of this program, we became interested in the preparation of 5′-protected thymidine[2] with an allyl group on the 3′-position (Scheme 1). We first allylated the 3′-hydroxy group using Chattopadhyaya's method[3] with NaH (2.5 equiv) and allyl bromide (2.5 equiv) in THF under ultrasound activation. The 3′-O-alkylated product was obtained in 95% yield after purification. To our surprise, the same reaction when performed in DMF did not lead to O-alkylation but produced the 3′-O, 3-N-dialkylated product instead. The use of 1.2 equiv of NaH and 1.2 equiv of allyl bromide in DMF resulted in the complete reversion of regioselectivity in favor of the N-alkylated product (95%). Structures of allyl-protected thymidines were



Scheme 1. O- and N-Alkylation of 5′-O-protected thymidine. Reagents and conditions: (i) (a) NaH, THF, ultrasound (20 min), (b) allyl bromide, ultrasound (45 min); (ii) (a) NaH, DMF, ultrasound (20 min), (b) allyl bromide, ultrasound (20 min).

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elucidated by NMR analysis (^{1}H, ^{13}C, HMQC, and HMBC). O- and N-Alkylation were demonstrated firstly by long-range coupling observed in HMBC experiments between C-3′/H-3′ and H-α/C-α and between H-α and C-2/C-4 (Tables 1 and 2) and secondly by the disappearance of the OH and NH groups, respectively. In addition, the ^{1}H NMR spectra in DMSO-d_6 displayed signals of the NH and OH hydrogens of compounds **1** and **2**, respectively (Fig. 1).

Table 1. NMR data of compound **1** in DMSO-d_6 (400 MHz for ^{1}H, 100 MHz for ^{13}C)

Position	δ_C	δ_H (mult; J in Hz)	HMBC
1′	83.9	6.12 (dd; 8.5, 5.8)	C-2, 3′, 6
2′	36.3	2.10 (ddd; 13.7, 8.5, 6.0)	C-1′, 3′, 4′
		2.26 (ddd; 13.7, 5.8, 1.8)	C-1′, 3′, 4′
3′	78.6	4.07 (m)	C-1′, 4′, 5′, α
4′	84.1	3.98 (m)	C-3′, 5′
5′	63.3	3.74 (dd; 11.3, 3.9)	C-3′, 4′
		3.78 (dd; 11.3, 4.1)	C-3′, 4′
a	−5.5	0.08 (s)	C-a, c
		0.09 (s)	C-a, c
b	25.7	0.89 br s	C-b, c
c	18.0	—	—
2	150.4	—	—
NH	—	11.36 (br s)	C-2, 4
4	163.6	—	—
5	109.5	—	—
6	135.4	7.49 (d; 0.7)	C-1′, 2, 4, 5, d
d	12.2	1.78 (br s)	C-4, 5, 6
α	69.1	4.01 (d; 5.3)	C-3′, β, γ
β	134.9	5.90 (ddd; 17.1, 10.4, 5.3)	C-α
γ	116.6	5.17 (dd; 10.4, 1.6)	C-α, β
		5.28 (dd; 17.1, 1.6)	C-α, β

Table 2. NMR data of compound **2** in DMSO-d_6 (400 MHz for ^{1}H, 100 MHz for ^{13}C)

Position	δ_C	δ_H (mult, J in Hz)	HMBC
1′	84.9	6.20 (dd; 7.5, 6.3)	C-2, 3′, 6
2′	39.6	2.08 (ddd; 13.3, 7.5, 6.0)	C-1′, 3′, 4′
		2.15 (ddd; 13.3, 6.3, 3.1)	C-1′, 3′, 4′
3′	70.3	4.21 (m)	C-1′
OH	—	5.31 (br s)	—
4′	86.9	3.84 (br dd; 6.3, 3.2)	C-3′, 5′
5′	63.1	3.73 (dd; 11.3, 3.7)	C-3′, 4′
		3.80 (dd; 11.3, 3.1)	C-3′, 4′
a	−5.5	0.07 (s)	C-a, c
		0.08 (s)	C-a, c
b	25.7	0.88 (br s)	C-b, c
c	17.9	—	—
2	150.0	—	—
4	162.2	—	—
5	108.4	—	—
6	134.2	7.56 (d; 1.0)	C-1′, 2, 4, 5, d
d	12.8	1.83 (d; 0.7)	C-4, 5, 6
α	42.4	4.40 (br d; 5.4)	C-2, 4, β, γ
β	132.3	5.81 (ddd; 17.2, 10.5, 5.4)	C-α
γ	116.5	5.05 (dd; 17.2, 1.5)	C-α, β
		5.09 (dd; 10.5, 1.5)	C-α, β

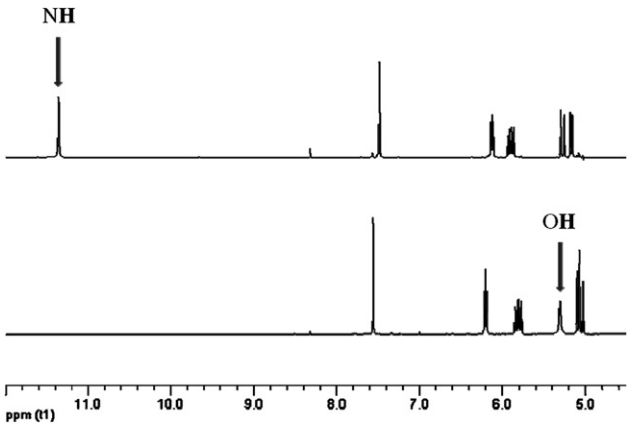


Figure 1. Comparison of ^{1}H NMR spectra of compounds **1** (top) and **2**.

The allylation reaction was also carried out in dioxane, dichloromethane, 1,2-dichloroethane, and dimethyl sulfoxide for comparison. In every case, the reaction was checked by TLC and stopped when there was no more production of product.

In view of these results, it seems that regioselectivity and dielectric constant of solvents are closely linked. Indeed for solvents whose dielectric constant is lower than 10, the O-alkylated product is exclusively obtained, even in the presence of excess sodium hydride and allyl bromide (Table 3). Quasi-stoichiometric conditions (1.2 equiv) lead to a dramatic decrease in yield. However, in the case of higher dielectric constant (DMSO, DMF) and in presence of 1.2 equiv of each reagent, only N-alkylation is observed. An excess of both base and bromide results in the formation of dialkylated thymidine. Finally, a slow alkylation was observed in dichloroethane, with selective formation of the O-alkylated product.

Microwave irradiation was then used to establish a comparison with ultrasound. Indeed the microwave activation presents some advantages, such as a remarkable decrease in reaction times and in some cases, cleaner reactions and a good selectivity.[4]

In a typical procedure, a solution of TBDMS-thymidine with sodium hydride (1.2 equiv) in aprotic solvent was irradiated for 2 min, then allyl bromide (1.2 equiv) was added, and the mixture was irradiated again for 4 min (Table 4, entry 2) or for 6 min (Table 4, entry 1). In THF compound **1** was obtained in 99% yield (Table 4, entry 1), although in DMF compound **2** was obtained in 96% yield (Table 4, entry 2). We observed the same regioselectivity using either microwave irradiation or ultrasound activation. In order to evaluate to what extent this method can be generalized, we tested different alkyl bromides. The results, summarized in Table 4, show that this methodology permits one to selectively protect in high yields either the 3 or 3′-position of thymidine (entries 5–8) with whichever alkyl

Table 3. Selected results of regioselective allylation (ultrasound activation)

Entry	Solvent (ε_r)	NaH (equiv)	R–Br (equiv)	Activation time		O-Alkylated **1** (%)	N-Alkylated **2** (%)	O,N-Dialkylated (%)
				First	Second			
1	Dioxane (2.21)	2.5	2.5	20 min	45 min	83	0	0
2	THF (7.58)	2.5	2.5	20 min	45 min	95	0	0
3	CH_2Cl_2 (8.93)	2.5	2.5	20 min	1 h 30	87	0	0
4	$ClCH_2CH_2Cl$ (10.56)	2.5	2.5	20 min	3 h	35	0	3
5	DMF (37)	2.5	2.5	20 min	20 min	0	10	80
6	DMF (37)	1.2	1.2	20 min	20 min	0	95	0
7	DMSO (46.7)	1.2	1.2	20 min	20 min	0	96	0

Table 4. Synthesis of 3′-O- and N-alkylated 5′-O-*tert*-butyldimethylsilylthymidine

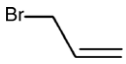
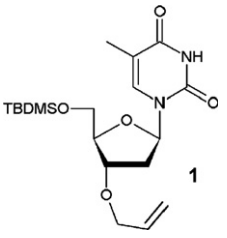
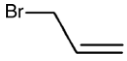
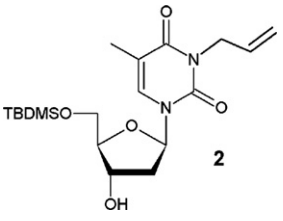
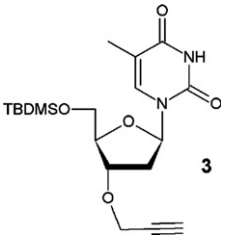
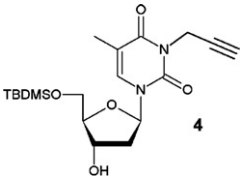
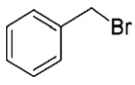
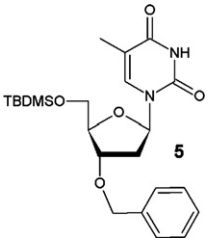
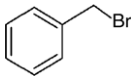
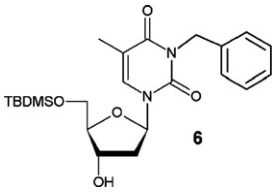
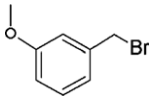
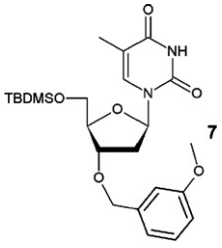
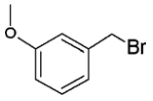
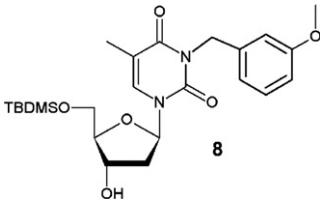
Entry	Solvent	Bromide	Product	Second activation[a] (min)	Isolated yield (%)	HRESIMS (*m*/*z*)
1[2]	THF			6	99	Calcd for $C_{19}H_{32}N_2NaO_5Si$ $[M+Na]^+$: 419.1973, found: 419.1977.
2	DMF			4	96	Calcd for $C_{19}H_{32}N_2NaO_5Si$ $[M+Na]^+$: 419.1973, found: 419.1974.
3[5]	THF			6	99	Calcd for $C_{19}H_{30}N_2NaO_5Si$ $[M+Na]^+$: 417.1816, found: 417.1818.
4	DMF			4	90	Calcd for $C_{19}H_{30}N_2NaO_5Si$ $[M+Na]^+$: 417.1816, found: 417.1819.
5	THF			15	82	Calcd for $C_{23}H_{34}N_2NaO_5Si$ $[M+Na]^+$: 469.2129, found: 469.2133.

Table 4 (*continued*)

Entry	Solvent	Bromide	Product	Second activation[a] (min)	Isolated yield (%)	HRESIMS (m/z)
6	DMF		 **6**	4	90	Calcd for $C_{23}H_{34}N_2NaO_5Si$ $[M+Na]^+$: 469.2129, found: 469.2135.
7	THF		 **7**	18	92	Calcd for $C_{24}H_{36}N_2NaO_6Si$ $[M+Na]^+$: 499.2235, found: 499.2240.
8	DMF		 **8**	4	82	Calcd for $C_{24}H_{36}N_2NaO_6Si$ $[M+Na]^+$: 499.2235, found: 499.2240.

[a] First activation with NaH: 2 min, 200 W, 40 °C.

bromide is used, for example, allyl or propargyl. It is worth noting that chloride derivatives were not used since they proved unreactive even after several hours of microwave or ultrasound activation. Tables 5 and 6 summarize the NMR data for compounds **3**–**8**.

In conclusion, a fast and efficient regioselective protection method for generation of N- and 3′-O-substituted thymidine was developed. Microwave irradiation decreases reaction time, but conserves the same regioselectivity as sonication. This new product bank could be of great interest in organic synthesis in order to insert thymidine into complex compounds or to generate synthetic oligomers.[5]

1. Experimental

1.1. General methods

All solvents and chemicals were commercially available, and unless otherwise stated, were used as received. Reactions were monitored by thin-layer chromatography (TLC) on precoated 0.2-mm Silica Gel 60 F_{254} (E. Merck) plates and visualized in two ways: (1) with an ultraviolet light source at 254 nm, or (2) by spraying with sulfuric acid (6 N) and heating to 200 °C. Microwave irradiations were performed by the means of an Ethos 1600 MicroSynth reactor from Milestone. The temperature was measured with a fiberoptic thermometer (ATC-FO)/Ethos. ^{1}H NMR spectra were recorded at 400.13 MHz with a Bruker DPX spectrometer. Chemical shifts (δ) are expressed in ppm with Me_4Si as the internal standard ($\delta = 0$). Data are reported as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, and br, broad), coupling constants (Hz), and assignment.

1.2. General method for ultrasound activation: example with O-allylation (Table 3)

To 5′-*O*-*tert*-butyldimethylsilylthymidine (2.32 g, 6.52 mmol) in dry THF (30 mL) was added NaH (60%, 652 mg, 2.5 equiv, 16.3 mmol), and the mixture was activated by ultrasound activation (20 min). Allyl bromide (1.410 mL, 2.5 equiv, 16.3 mmol) was then added, and the mixture was activated by ultrasound (20 min). After workup (NH_4Cl–H_2O) and purification by chromatography ($CHCl_3$ as eluent) **1** was obtained: 2.45 g (95%).

1.3. General method for microwave activation: example with O-allylation (Table 4)

To a solution of 5′-*O*-*tert*-butyldimethylsilylthymidine (1.27 g, 3.57 mmol) in dry THF (15 mL) was added

Table 5. ^{1}H NMR chemical shifts of alkyl compounds **3–8**

Position	3	4	5	6	7	8
1′	6.10 dd 5.7, 8.6	6.20 dd 6.5, 7.2	6.15 dd 5.7, 8.6	6.20 dd 6.5, 7.2	6.15 dd 5.8, 8.4	6.21 dd 6.5, 7.1
2′	2.10 ddd 6.0, 8.7, 13.7	2.09 ddd 6.5, 7.4, 13.3	2.12 ddd 6.0, 8.6, 13.5	2.09 ddd 6.3, 7.4, 13.2	2.11 ddd 6.0, 8.4, 13.6	2.10 ddd 6.1, 7.4, 13.3
	2.29 ddd 1.6, 5.7, 13.7	2.16 ddd 3.1, 6.2, 13.3	2.33 ddd 1.7, 5.7, 13.5	2.16 ddd 3.2, 6.1, 13.3	2.32 ddd 1.6, 5.7, 13.6	2.16 ddd 3.3, 6.2, 13.3
3′	4.24 m	4.21 m	4.12 dd 2.0, 3.7, 5.8	4.21 m	4.10 m	4.21 m
4′	3.99 br d 3.7, 5.6	3.85 br d 3.0, 6.0	4.06 br dd 3.7, 5.8	3.84 br 3.1, 6.2	4.05 dd 3.6, 5.6	3.84 dd 3.2, 6.2
5′	3.74 dd 3.8, 11.2	3.73 dd 3.8, 11.4	3.72 dd 3.8, 11.2	3.73 dd 3.8, 11.4	3.72 dd 3.7, 11.3	3.73 dd 3.8, 11.4
	3.78 dd 4.0, 11.2	3.80 dd 3.0, 11.4	3.78 dd 4.2, 11.2	3.80 dd 3.1, 11.4	3.77 dd 4.1, 11.3	3.80 dd 3.1, 11.4
3′-OH	—	5.30 d 4.2	—	5.30 d 4.1	—	5.29 d 4.1
a	0.09 s	0.07 s	0.05 s and 0.06 s	0.07 s	0.04 s and 0.05 s	0.07 s
b	0.89 s	0.88 s	0.86 s	0.88 s	0.86 s	0.88 s
3-NH	11.35 s	—	11.36 s	—	11.34 s	—
6	7.48 d 0.7	7.57 d 0.6	7.49 d 0.8	7.58 br s	7.47 d 0.7	7.58 br s
d	1.78 br s	1.84 br s	1.78 br s	1.84 br s	1.77 br s	1.85 br s
α	4.20 dd 2.2, 16.0	4.51 dd 2.4, 15.6	4.53 d 14.0	4.90 d 14.5	4.50 d 14.4	4.94 d 14.6
	4.25 dd 2.2, 16.0	4.55 dd 2.4, 15.6	4.56 d 14.0	4.94 d 14.5	4.53 d 14.3	4.98 d 14.6
γ	3.47 t 2.3	3.10 t 2.3	—	—	—	—
Ar-2	—	—	7.35 m	7.36 m	6.90 br d 1.0	6.81 br s
Ar-3	—	—	7.37 m	7.37 m	—	—
Ar-4	—	—	7.31 m	7.30 m	6.86 br dd 1.5, 7.7	6.81 m
Ar-5	—	—	7.37 m	7.37 m	7.27 br t 7.7, 8.2	7.21 dd 7.5, 8.9
Ar-6	—	—	7.35 m	7.36 m	6.91 br dd 1.0, 8.0	6.80 br d 7.8
3-OCH_3	—	—	—	—	3.75 s	3.72 s

Table 6. ^{13}C NMR chemical shifts of alkyl compounds **3–8**

Position	3	4	5	6	7	8
1′	83.8	85.1	83.9	85.0	84.0	85.0
2′	35.9	39.6	36.2	39.6	36.3	39.5
3′	78.2	70.4	78.5	70.4	78.6	70.3
4′	83.8	87.0	84.0	87.0	84.1	86.9
5′	63.1	63.2	63.2	63.1	63.3	63.1
a	−5.6	−5.5	−5.6	−5.5	−5.5	−5.5
b	25.7	25.8	25.7	25.7	25.7	25.7
c	17.9	18.0	17.9	18.0	17.9	17.9
d	12.1	12.8	12.1	12.8	12.2	12.8
2	150.3	149.6	150.3	150.0	150.4	150.3
4	163.5	161.7	163.5	162.3	163.6	162.5
5	109.5	108.5	109.4	108.5	109.5	108.5
6	135.2	134.6	135.3	134.5	135.4	134.4
α	55.7	30.0	70.0	42.5	69.9	43.5
β	79.9	79.0	—	—	—	—
γ	77.3	73.0	—	—	—	—
Ar-1	—	—	137.9	137.5	139.6	138.5
Ar-2	—	—	127.5	127.1	113.1	113.6
Ar-3	—	—	128.2	128.5	159.3	159.1
Ar-4	—	—	127.4	127.0	113.0	112.2
Ar-5	—	—	128.2	128.5	129.4	129.3
Ar-6	—	—	127.5	127.1	119.7	119.5
3-OCH_3	—	—	—	—	55.0	54.9

NaH (60%, 326 mg, 2.5 equiv, 8.15 mmol), and the mixture was activated by microwave irradiation (2 min, 200 W, 40 °C). Allyl bromide (705 μL, 2.5 equiv, 8.15 mmol) was then added, and the mixture was activated by microwave irradiation (6 min, 200 W, 40 °C). After workup (NH_4Cl–H_2O) and purification by chromatography ($CHCl_3$ as eluent) **1** was obtained: 1.28 g (99%).

Acknowledgments

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Supplementary data

Supplementary data (spectra for compounds **1**,**2**) associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.04.026.

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