Pergamon

Tetrahedron Letters 43 (2002) 3251–3254

TETRAHEDRON LETTERS

A convenient method for the conversion of β-thymidine to α-thymidine based on TMSOTf-mediated C1′-epimerization

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Received 23 January 2002; revised 20 February 2002; accepted 22 February 2002

Abstract—A practically useful method for the synthesis of α-thymidine from β-thymidine was developed by trimethylsilyl triflate-mediated C1′-epimerization. When 5′-*O*-diphenylacetyl-3′-*O*-toluoylthymidine was trimethylsilylated and successively treated with TMSOTf in acetonitrile, the resulting α-thymidine derivative was crystallized and isolated without silica-gel column chromatography. Deprotection gave unprotected α-thymidine in an overall yield of 50% from β-thymidine. © 2002 Published by Elsevier Science Ltd.

Recently, much attention has been paid to the antisense and antigene strategies which enable us to regulate selectively malignant genes and mRNAs, respectively, for gene therapy.[1] Particularly, Imbach and other researchers have reported that α-oligodeoxynucleotide derivatives, which have α-configuration at the anomeric center of nucleotide components, possess promising hybridization affinity toward RNAs as antisense molecules.[2,3] Their studies overturned the long-held stereotype that α-deoxynucleosides are useless byproducts produced by glycosylation of deoxyriboside derivatives with silylated or metallated bases.[4] Development of highly efficient methods for the synthesis of α-deoxynucleosides is now required. Although several methods have been reported for the predominant synthesis of α-deoxynucleosides to date,[5,6] these methods involve multi-step reactions and do not attain ideal selectivity. Compared with these de novo syntheses of α-deoxynucleosides, simple TMSOTf-mediated C1′-epimerization[7] of commercially available β-deoxynucleosides, which was reported by Saneyoshi,[8] would provide a more straightforward route to α-deoxynucleosides on a large scale.

However, it is extremely difficult to separate the desired 3′,5′-*O*-diacylated α-thymidine derivatives from the still remaining 3′,5′-*O*-diacylated β-thymidine derivatives when the conventional toluoyl group was chosen as the 5′- and 3′-hydroxyl protecting groups.[8] This previous method required a tedious procedure for purification of α-thymidine so that its overall yield is low (ca. 20%) in our hands. Quite recently, we have reported the TMSOTf-mediated β–α epimerization of 5′-*O*-carbamoylated or 5′-*O*-thiocarbamoylated β-thymidine derivatives.[9] It turned out that these C1′-epimerizations are essentially associated with not the neighboring group participation of the carbonyl oxygen or sulfur of the 5′-*O*-carbamoyl or thiocarbamoyl group but the steric hindrance of the 5′-*O*-acyl group. However, the reactions required for introduction of the carbamoyl or thiocarbamoyl group into the 5′-hydroxyl group are sluggish and the reagents are expensive. In the same paper,[9] we also reported a procedure for isolation of 5′-*O*-pixyl-α-thymidine from a 75:25 mixture of 3′,5′-*O*-ditoluoylated α- and β-thymidines, which involves tedious steps for elimination of the 5′-*O*-pixylated β-isomer by crystallization and chromatograpy. However, 5′-*O*-DMTr-α-thymidine as a more generally accessible starting material for the synthesis of α-DNA derivatives could not be isolated by a similar method, since 5′-*O*-DMTr-β-thymidine could not be crystallized in any organic solvents tested. In this paper, we wish to report a more practical and general method for the conversion of β-thymidine to α-thymidine.

Based on the previous results,[9] we chose various acyl groups, i.e. acetyl (Ac), phenylacetyl (PA), diphenylacetyl (DPA), and triphenylacetyl (TPA) as the 5′-*O*-protectors of β-thymidine to see if the steric bulkiness of the 5′-*O*-protector affects the α/β ratio in the epimerization. The 3′,5′-*O*-diacylated thymidine derivatives **2a**–**j**(β) were synthesized according to Method A or Method B, as shown in Scheme 1. Method A involves a two-step acylation of β-thymidine. Method B was used for the synthesis of 3′,5′-*O*-protected thymidine

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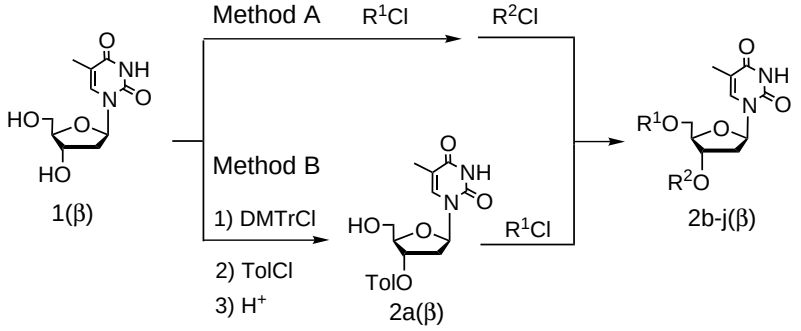
0040-4039/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0040-4039(02)00411-2

derivatives which could not be obtained by Method A. These results are summarized in Table 1.

In the β–α epimerization, thymidine derivatives **2a**–**j**(β) were silylated by treatment with 5 equiv. of hexamethyldisilazane (HMDS) in CH_3CN under reflux for 1 h. After 2 equiv. of TMSOTf was added to the resulting 4-*O*-(trimethylsilyl)thymidine derivatives **3a**–**j**(β), the mixture was stirred at 30°C for 20 h, as shown in Scheme 2. At appropriate times, aliquots were taken

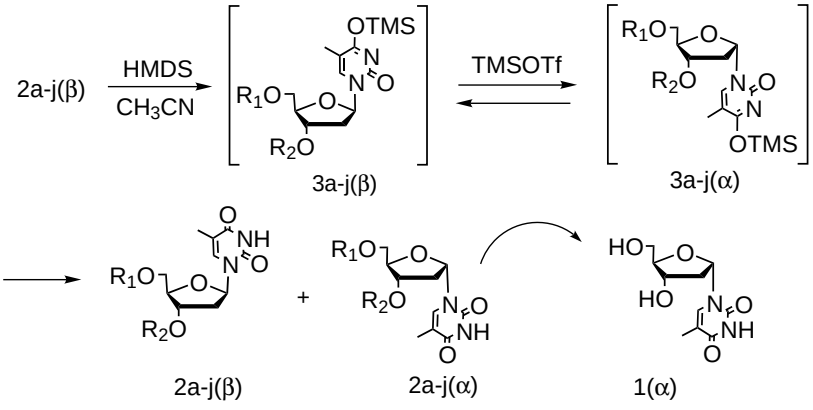


Scheme 1.

Table 1. Synthesis of 3′,5′-*O*-diacylated thymidine derivatives **2a**–**j**(β)

Compd	R^1	R^2	Method	Solvent	First acylation	Second acylation	Yield from **1**(β) (%)	Yield from **2a**(β) (%)
2a(β)	H	Tol	–	Py	–	–	68[a]	–
2b(β)	Ac	Tol	B	Py	Ac_2O (1.5 equiv., rt, 2 h)	–	–	92
2c(β)	PA	Tol	B	Py	MPACl (1.5 equiv., rt, 0.5 h)	–	–	73
2d(β)	Tol	Tol	A	Py	TolCl (2.2 equiv., rt, 1 h)	–	96 [82[b]]	–
2e(β)	DPA	Tol	A	Py:CH_2Cl_2 (9:1, v/v)	DPACl (1.2 equiv., rt, 1 h)	TolCl (1.5 equiv., rt, 3 h)	78 [55[b]]	–
2f(β)	TPA	Tol	A	Py:CH_2Cl_2 (9:1, v/v)	TPACl (1.5 equiv., 60°C, 2 h)	TolCl (1.5 equiv., rt, 3 h)	46	–
2g(β)	DPA	H	A	Py:CH_2Cl_2 (9:1, v/v)	DPACl (1.2 equiv., rt, 1 h)	–	77	–
2h(β)	DPA	Ac	A	Py:CH_2Cl_2 (9:1, v/v)	DPACl (1.2 equiv., rt, 1 h)	Ac_2O (1.5 equiv., rt, 2 h)	74	–
2i(β)	DPA	Bz	A	Py:CH_2Cl_2 (9:1, v/v)	DPACl (1.2 equiv., rt, 1 h)	BzCl (1.5 equiv., rt, 1 h)	81	–
2j(β)	DPA	DPA	A	Py	DPACl (2.2 equiv., rt, 1 h)	–	86	–

[a] The yield was that for conversion of **1**(β) to **2a**(β) via a three-step reaction.
[b] Isolated yield by crystallization without using silica-gel column chromatography.



Scheme 2.

and quenched by addition of $CHCl_3$ and water. The ratio of **2a**–**j**(α) and **2a**–**j**(β) in the organic phase was estimated by ^1H NMR.

In all cases, the epimerization in CH_3CN reached an equilibrated state within 20 h, and the equilibrium constants K of these α–β epimerizations were calculated. These results are shown in Table 2. The mixtures of **2a**–**j**(α) and **2a**–**j**(β) obtained after 20 h were purified by silica gel column chromatography without further separation of a pair of anomers. As shown in Table 2, the mixtures of **2b**–**f**(α) and **2b**–**f**(β) could be recovered in 80–93% yields. When 3′-*O*-toluoylthymidine **2a**(β) or 5′-*O*-diphenylacetylthymidine **2g**(β) was used in the epimerization, unfavorable decomposition predominantly occurred over the epimerization, presumably due to much faster dehydration.[10] It was also found that, compared with the α-anomer selectivity of compound **2b** (α:β = 82:12) having the acetyl group, those of compounds **2e** and **2f** having the $Ph_2CHC(O)$- and $Ph_3CC(O)$- groups were increased up to 88:12 and 91:9, respectively.

These results imply that the ultimate α/β ratio in these epimerizations highly depends on the steric factor on the 5′-*O*-protectors. It is likely that the $Ph_2CHC(O)$-, and $Ph_3CC(O)$-groups interact more sterically with the silylated thymine base than the acetyl group so that the β-thymidine derivatives **2e**(β) and **2f**(β) having these protecting groups are more thermodynamically unfavorable than compound **2a**(β) having the acetyl group, as shown in Fig. 1.

On the other hand, when 5′-*O*-diphenylacetylthymidine derivatives **2h**–**j** having an Ac, Bz, or DPA group at the 3′-position were similarly treated with TMSOTf, the α/β ratio varied from 82:18–88:12. In these cases, however, there is no clear-cut relationship between the α-selectivity and the steric hindrance of the 3′-protecting group. It seems to us that the α/β ratio is dependent on other factors such as the electronic effect of the 3′-protecting and the stacking effect of the 3′-protecting group with the silylated thymine base residue group which can stabilize the whole conformation of α-thymidine derivatives preferencially over β-thymidine derivatives.

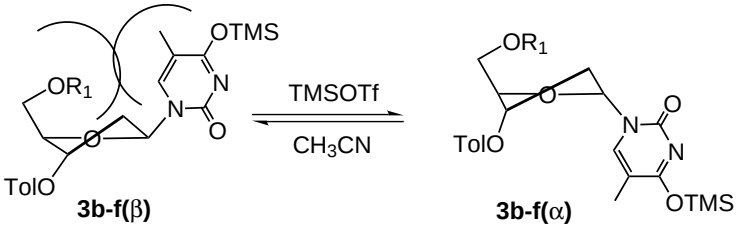
Since the epimerization is a reversible process, the α/β ratio essentially depends on the thermodynamical stability of both isomers. It seems to us that 91:9 is the ultimate α/β ratio in this kind of epimerization.

As the starting material, compound **2e**(β) was prepared in a higher yield (78%) than **2f**(β) (46%). It was also found that compound **2e**(α) could be easily crystallized in ethanol from the 88:12 mixture of **2e**(α) and **2e**(β) obtained by the C1′-epimerization without column chromatography. This finding eliminates the need for

Table 2. C1′-epimerization of 3′,5′-*O*-diacylated thymidine derivatives **2a**–**j**(α/β)

Compd	R^1	R^2	Recovery of **2**(α)/**2**(β) (%)	α:β[a]	R_f (TLC)[b] of α- and β-epimers		Equilibrium constant K
					α	β	
2a(α/β)	H	Tol	Decomp.	–	–	–	–
2b(α/β)	Ac	Tol	86	82:18	0.29	0.37	4.59
2c(α/β)	PA	Tol	90	83:17	0.55	0.62	4.81
2d(α/β)	Tol	Tol	93	85:15	0.56	0.63	5.54
2e(α/β)	DPA	Tol	93	88:12	0.63	0.68	7.62
2f(α/β)	TPA	Tol	80	91:9	0.84	0.89	9.00
2g(α/β)	DPA	H	Decomp.	–	–	–	–
2h(α/β)	DPA	Ac	87	82:18	0.42	0.44	4.43
2i(α/β)	DPA	Bz	89	88:12	0.64	0.71	7.47
2j(α/β)	DPA	DPA	88	86:14	0.69	0.77	6.14

[a] The ratio measured after 20 h.
[b] TLC was preformed three times by use of $CHCl_3$–AcOEt–toluene (1:1:1, v/v/v).



5′-*O*-Steric effect Ac < MPA < Tol < DPA < TPA

Figure 1.

column chromatographic separation which is virtually impossible since both epimers have very similar R_f values, as shown in Table 2. Thus, the α-isomer **2e**(α) could be isolated as pure crystals (mp 201°C) in 76% yield. From the mother solution, the **2e**(β)-enriched mixture could be recovered and reused for the further epimerization. As far as the difference in the R_f value between the two epimers is concerned, **2d** and **2j** showed significant differences, but it turned out that multi-time separation was required to purify the α-isomers. This bothersome process is apparently unsuitable for the large-scale synthesis of α-thymidine.

Simple deacylation of the β-free **2e**(α) with 0.1 M NaOH gave the unprotected α-thymidine in 86% yield. As a result, α-thymidine could be prepared in a considerably improved overall yield of 50% from β-thymidine. In consideration of the recovery of **2e**(β) after crystallization of **2e**(α), the optimized overall yield is expected to be ca. 65%. α-Thymidine thus obtained was found to be highly pure from ^{1}H NMR (270 MHz), since the β-isomer could not be detected under the conditions of the NMR measurement.

The present method would provide a large-scale synthesis of α-thymidine which is a key synthetic material for the synthesis of α-DNA derivatives. Further studies to apply of our method to the synthesis of other α-deoxyribonucleosides are under way.

Acknowledgements

This work was supported by a Grant from 'Research for the Future' Program of the Japan Society for the Promotion of Science (JSPS-RFTF97I00301).

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