# Substituent and Solvent Effects of TMS Triflate Mediated C1′ Epimerization of β-Thymidine to α-Thymidine

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This paper deals with kinetic studies of TMSOTf-mediated C1′ epimerization of β-thymidine to α-thymidine. The effect of neighboring group participation by various 5′-hydroxy protecting groups, such as toluoyl, $Et_2CHC(O)$, $Et_2NC(O)$, and $Et_2NC(S)$, on the β→α conversion is described in detail. The time dependence of the ratio of the α and β anomers in the C1′ epimerization was estimated by $^1H$ NMR. The α/β equilibrium constants $K$ and the rate constants $k_\alpha$ and $k_\beta$ were calculated on the basis of the experimental data. As the result, it was concluded that, in acetonitrile, the α/β equilibrium constants $K$ are thermodynamically affected by steric hindrance from the 5′-hydroxy protecting group. On the other hand, the rate constants $k_\alpha$ and $k_\beta$ are mainly influenced by the stability of the oxonium ion intermediate. In particular, formation of an intramolecularly cyclized iminium ion intermediate from the oxonium ion intermediate, due to the neighboring group participation by the diethylthiocarbamoyl group, tended to decrease the overall reaction rate. Finally, the α/β C1′ epimerization could be carried out with a high α anomer selectivity of 89% through the use of the $Et_2CHC(O)$ group. Thus, 5′-*O*-pixyl-α-thymidine could be synthesized from β-thymidine as a key intermediate for the synthesis of α-DNA in a considerably improved overall yield of 40%.

## Introduction

α-Oligodeoxynucleotides[1−5] and derivatives[6] of nucleotide components with α configurations at the anomeric centers have proven to be promising new sources in the antisense strategy.[7] Imbach and others have reported that these substances are resistant to nucleases[2] in cells and can selectively hybridize with mRNA,[5,6] ssDNA,[2] and dsDNA.[4] However, the α-nucleoside monomers required as the starting materials for the synthesis of α-oligodeoxynucleotides are not available in nature, so must be prepared by chemical approaches. Unfortunately, α-deoxynucleosides have long been viewed only as undesired by-products in the synthesis of β-deoxynucleosides by glycosylation of deoxyriboside derivatives with silylated or metallated bases.[8] Despite this background, several methods for the synthesis of α-deoxynucleosides have been reported.[8−16] However, these methods involve multi-step reactions and do not attain ideal selectivity. Compared with these de novo syntheses of α-deoxynucleosides, simple C1′ epimerization of commercially available β-deoxynucleosides would provide a more straightforward route to α-deoxynucleosides. In 1981, Vorbrüggen reported that treatment of α-(5-ethyldeoxyuridine) with TMSOTf in $CH_3CN$ at 25 °C for 46 h gave a mixture of the α and β anomers, which were recovered in 67% and 27% yields, respectively.[17] Later, Yamaguchi and Saneyoshi applied this C1′ epimerization to the reverse conversion of β-thymidine **1(β)** to α-thymidine **1(α)**, and obtained α-thymidine **1(α)** from 3′,5′-di-*O*-toluoylthymidine **2a(β)**[18] in an overall yield of 25%, as shown in Scheme 1. They used an elevated temperature of 70 °C to accelerate the epimerization. Our own study found that this epimerization resulted in a 75:25 mixture of the α and β anomers **2a(α)** and **2a(β)**, and serious competitive decomposition was observed. Thus, compound **2a(β)** could not be obtained in good yield.

Therefore, in order to identify the essential factors for enhancement of the α/β ratio of **2a(α)** and **2a(β)** in the TMSOTf-mediated epimerization, and also to control the competitive decomposition, we studied the C1′ epimerization of various 5′-*O*-acylthymidine derivatives in more detail. In particular, our interest was focused on the possibility of neighboring group participation by the 5′-*O*-protecting group in this type of epimerization.[19] It was also important to see whether the α/β equilibrium in the Vorbrüggen−Saneyoshi method could be shifted more preferentially to the α isomer and whether the reaction rate could be accelerated. For this purpose, four kinds of acyl groups − toluoyl (Tol), (2-ethyl)butyryl [$Et_2CHC(O)-$], *N*,*N*-diethylcarbamoyl [$Et_2NC(O)-$], and *N*,*N*-diethylthiocarbamoyl [$Et_2NC(S)-$] were chosen. The Tol group was chosen as a reference, since Saneyoshi used this protecting group previously.[18] The latter two were expected to have strong proximal effects on the stabilization of oxonium in-

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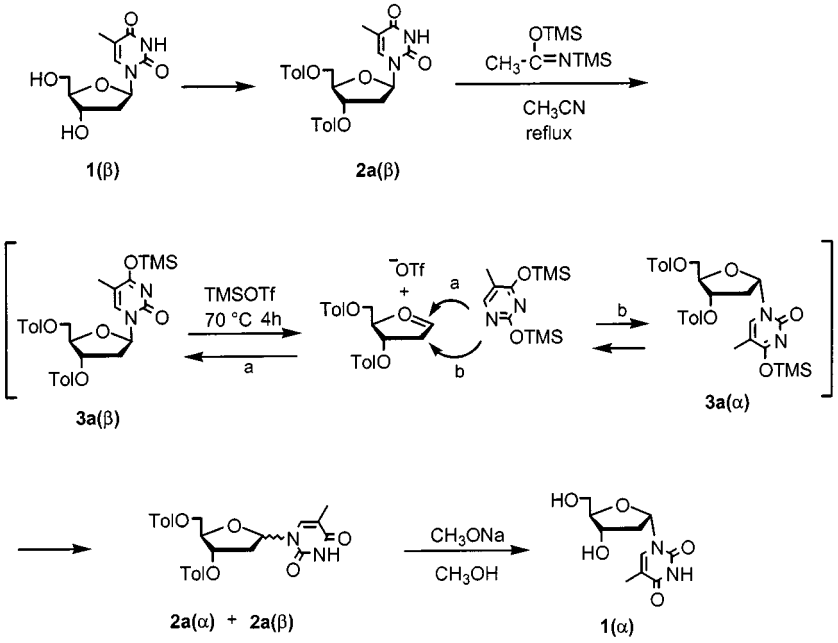
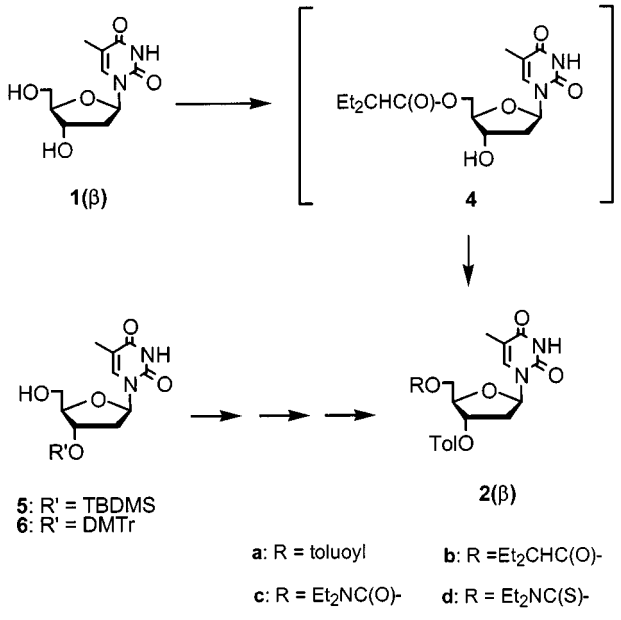



Scheme 1

termediates generated by the TMSOTf-mediated activation of the glycosidic bond. This inherent proximal effect has previously been exploited for the α-selective glycosylation of 1-*O*-acetyl-3-*O*-benzyl-5-*O*-(*N*,*N*-diethylthiocarbonyl)-2-deoxyribofuranose with 2,4-*O*-bis(trimethylsilyl)thymine in the presence of Lewis acids.[20] The observed α selectivity was explained in terms of the formation of a cyclic iminium salt that could be attacked by the silylated thymine from the α face.

In this paper we report that the β/α epimerization of thymidine **1(β)** was greatly affected by the nature of the 5′-*O*-acyl group and the solvent, and also describe its ultimate application to a convenient synthesis of 5′-*O*-pixyl-α-thymidine **9(α)** as a key synthetic intermediate for the synthesis of α-DNA.



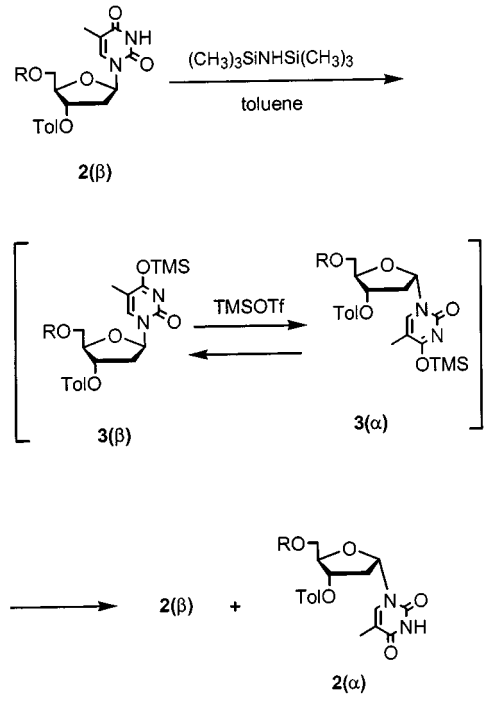

Scheme 2

## Results and Discussion

3′,5′-Di-*O*-toluoylthymidine[21] **2a(β)** was prepared by acylation of **1(β)** with toluoyl chloride. Compound **2b(β)**, with an $Et_2CHC(O)$ group, was synthesized in 73% yield by a two-step procedure from thymidine **1(β)**, by way of the 5′-*O*-acylated species **4**. The other 3′,5′-di-*O*-acylated thymidine derivatives **2c** and **2d** were synthesized from 3′-*O*-(4,4′-dimethoxytrityl)thymidine[22] (**5**) and 3′-*O*-(*tert*-butyldimethylsilyl)thymidine[23] (**6**), respectively, as shown in Scheme 2.

In the β/α epimerization, thymidine derivatives **2a−d** were silylated by treatment with hexamethyldisilazane (HMDS) in toluene under reflux for 1 h. After addition of 1 equiv. of TMSOTf to the silylated thymidine derivatives **3a−d(β)**, the mixture was stirred at 30 °C for 30−96 h (Scheme 3). It was found that when an elevated temperature of 70 °C was employed, as reported by Saneyoshi,[18] the reaction rate was accelerated but the yield of **2a(α)** was reduced because of unfavorable decomposition, giving rise to a considerable quantity of charcoal-like materials.[24] The current reactions were therefore carried out at 30 °C with 1 equiv. of TMSOTf, which produced a marked improvement in the recovery of the α/β mixture. To check solvent effects, the reaction was also examined in $CH_2Cl_2$ in place of $CH_3CN$. At appropriate times, the ratio of **2a−d(α)/2a−d(β)** was estimated by $^1H$ NMR. These results are shown in Figure 1.

To our surprise, a marked solvent effect between $CH_3CN$ and $CH_2Cl_2$ was observed. In the former, the β→α epimerization reaction rates ranked in the following order:




Scheme 3

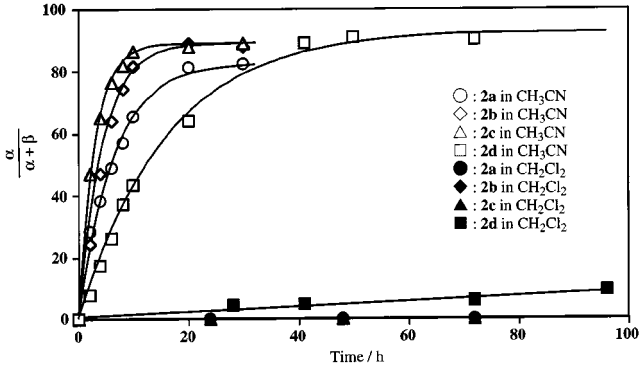



Figure 1. Time dependence of C1′ epimerization of 5′-*O*-acyl-3′-*O*-toluoylthymidine derivatives **2a−d(β)** in acetonitrile and dichloromethane, based on $^1$H NMR analysis; in acetonitrile: open circle **2a(β)**, open diamond **2b(β)**, open triangle **2c(β)**, open square **2d(β)**; in dichloromethane: closed circle **2a(β)**, closed diamond **2b(β)**, closed triangle **2c(β)**, closed square **2d(β)**

**2c(β)** > **2b(β)** > **2a(β)** > **2d (β)**. On the other hand, dramatically different results were obtained in the latter solvent. Namely, no epimerization occurred at all in the cases of **2a−c(β)**, and only **2d(β)** showed a very slow epimerization (α/β ratio = 9:91 after 96 h).

The mixtures of **2a−d(α)** and **2a−d(β)** obtained after 20 h with $CH_3CN$ were purified by silica gel column chromatography without further separation of anomer pairs. The isolated (or recovered) yields of **2a−d(α)** and **2a−d(β)** and their ratios at this time are summarized in Table 1. The mixtures of **2a−d(α)** and **2a−d(β)** could be recovered in 82−91% yields.

The final α/β ratios for **2a(α)** and **2a(β)** in the reaction carried out at 30 °C reached 81:19, which was better than that (75:25) obtained at 70 °C. It was also found that, compared with **2a(β)** with the toluoyl group, the α anomer selectivity in the α/β C1′ epimerization after 20 h was increased to 89:11 and 88:12 by introduction of the $Et_2CHC(O)$ and $Et_2NC(O)$ groups, respectively, at the 5′-hydroxy group of thymidine, as shown in Table 1.

In the case of **2a−c(β)**, all epimerizations in $CH_3CN$ reached equilibrated mixtures within 20 h. The epimerization of **2c(β)** was the fastest among them. Compound **2d(β)**, with the $Et_2NC(S)$ group, underwent very slow epimerization that reached a plateau after 50 h, but α-selective epimerization of 91% was finally accomplished. The equilibrium constants $K$ of these α/β epimerizations were calculated from the data plotted in Figure 1. (These results are also shown in Table 1.) Compound **2d(β)**, with an $Et_2NC(S)$ group, gave the best equilibrium constant $K$ (9.95) but took longer to reach equilibrium.

This result implies that the ultimate α/β ratio in these epimerizations depends not on electronic factors, but on steric factors relating to the 5′-*O*-protecting groups, since compounds **2b−d(β)**, with structurally similar acyl groups, showed very similar α/β ratios (88:12 to 91:9). It is likely that the $Et_2NC(O)$, $Et_2NC(S)$, and $Et_2CHC(O)$ groups interact sterically more strongly than the toluoyl group with the silylated thymine base. Accordingly, the β-thymidine derivatives **2b−d(β)** with these protecting groups are more susceptible to α attack of the silylated thymine and are more thermodynamically unfavorable than compound **2a(β)** with the toluoyl group, as shown in Figure 2.

It is suggested from Taft steric parameters that the $Et_2CHC(O)$ group is larger than the benzoyl group.[25]

Table 1. TMSOTf-mediated C1′ epimerization of **2a−d(β)** in acetonitrile, kinetic data, and $R_f$ values of the products

| Compd. | R | Recovery of **2(α)** and **2(β)** after epimerization (%) | α/β[a] | $R_f$ (TLC) α isomer | $R_f$ (TLC) β isomer | Equilibrium constant $K$ | Rate constant [× $10^6$ $s^{-1}$] $k_\alpha$ | Rate constant [× $10^6$ $s^{-1}$] $k_\beta$ | Half-time [h] |
|---|---|---|---|---|---|---|---|---|---|
| **2a(β)** | toluoyl | 89 | 81:19 | 0.53[b] | 0.59[b] | 4.38 | 36.0 | 7.7 | 6.0 |
| **2b(β)** | $Et_2CHC(O)-$ | 91 | 89:11 | 0.59[c] | 0.64[c] | 8.08 | 56.7 | 7.0 | 4.5 |
| **2c(β)** | $Et_2NC(O)-$ | 82 | 88:12 | 0.35[b] | 0.42[b] | 7.12 | 82.7 | 10.2 | 2.5 |
| **2d(β)** | $Et_2NC(S)-$ | 89<br>77 (50 h) | 64:36<br>91:9 (50 h) | 0.54[b] | 0.59[b] | 9.95 | 15.8 | 1.6 | 12.5 |

[a] The ratio measured after 20 h. [b] $CHCl_3$/EtOAc (1:1, v/v). [c] $CHCl_3$/EtOAc/toluene (1:1:1, v/v/v), developed 3 times.

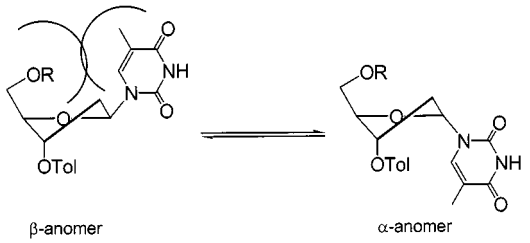



Figure 2. Interaction of the 5′-acyl moiety with the thymine base

Therefore, it is implied that the use of sterically hindered protecting groups here resulted in better α/β ratios.

The 5′-*O*-(*N*,*N*-diethylthiocarbamoyl)thymidine derivative **2d(β)** gave the best result as far as the α anomer selectivity was concerned, but the C1′ epimerization rate in $CH_3CN$ was the slowest. To clarify the reason for the differences in reaction rate between **2d(β)** and the other three compounds **2a−c(β)**, we determined the rate constants $k_\alpha$ and $k_\beta$, respectively, of the β→α epimerization and of its α→β reverse reaction. To this end, the equation $\ln(K'/\{K' - x_\alpha\}) = (k_\alpha + k_\beta)t$ was used [$x_\alpha = \alpha/(\alpha + \beta)$, $K' = K/(K + 1)$] (see Figure 3).

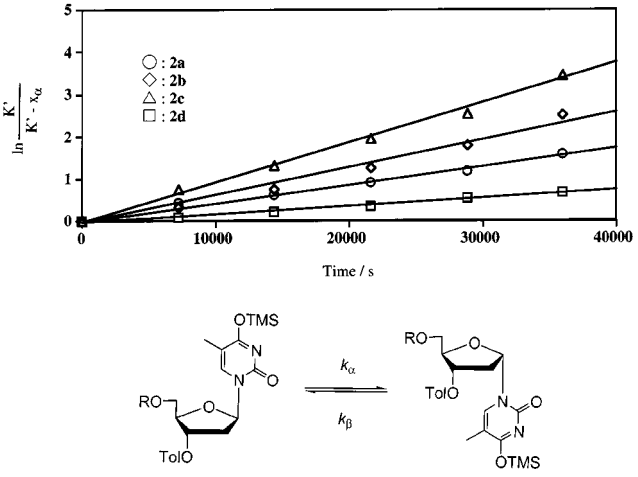
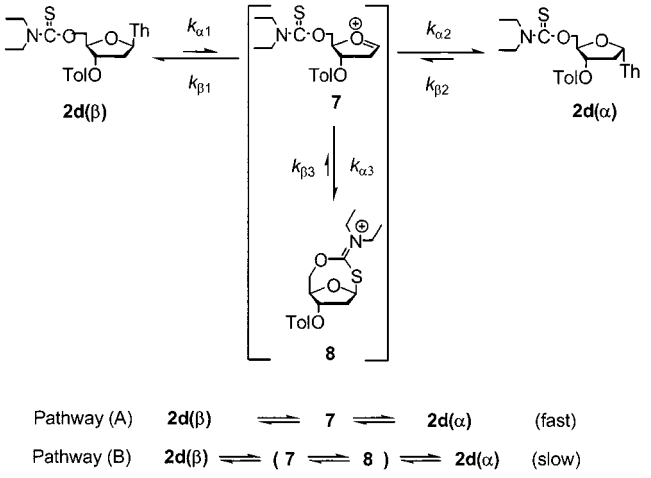



Figure 3. Kinetics of C1′ epimerization of **2a−d** in acetonitrile

As shown in Table 1, it was found that $k_\alpha$ and $k_\beta$ for **2d(β)** were very small compared with those of the other three compounds **2a−c(β)**. If there is a possibility that the sulfur atom of the $Et_2NC(S)$ group may attack the C1′ carbon atom, it is reasonable to assume that two kinds of reaction pathways (A) and (B) may simultaneously exist in the α/β equilibrium, as shown in Scheme 4. The rate constants $k_{\alpha3}$ and $k_{\beta3}$ between the oxonium intermediate **7** and the iminium ion intermediate **8** require discussion. Our results suggest that the constant $k_{\beta3}$ is much smaller than $k_{\alpha3}$ and that this additional slow step affects the overall reaction rate so that the rate constants $k_\alpha$ and $k_\beta$ are decreased.

In the first reaction pathway (A), the C1′ epimerization between the β anomer **2d(β)** and the α anomer **2d(α)** arises from formation of the oxonium ion intermediate **7**. In particular, the epimerization and the reverse reaction at 30 °C in polar solvents such as acetonitrile proceed through the solvated oxonium ion intermediate.

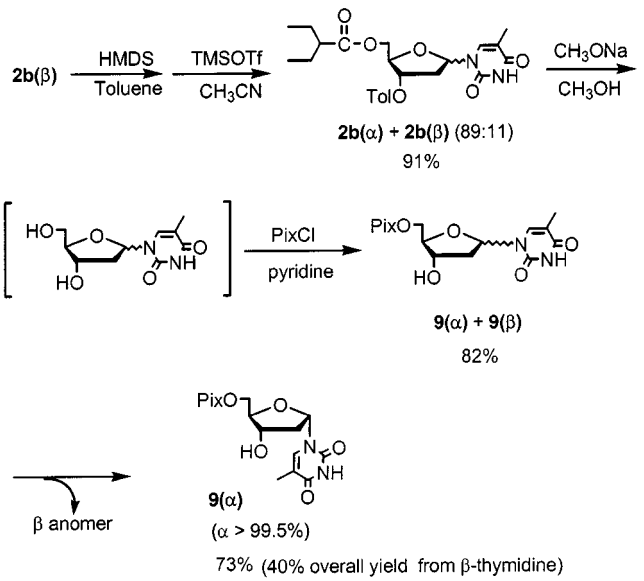



Scheme 4

In the second reaction pathway (B), the C1′ epimerization is retarded by formation of the cyclic iminium ion intermediate **8**. Such a cyclic intermediate would be expected to have a considerable degree of stability[20] and assumed to reduce the effective concentration of the reactive intermediate **7**. It is predicted that attack by the most nucleophilic $Et_2NC(S)$ group on the oxonium C1′ carbon atom is more likely than that by the less nucleophilic Tol, $Et_2NC(O)$, and $Et_2CHC(O)$ groups. In general, neighboring group participation by acyl groups is less effective in polar solvents,[26] because the iminium ion intermediates can be stabilized by solvation. Therefore, the compound **2d(β)**, with the $Et_2NC(S)$ group as a strong nucleophile, underwent the slowest epimerization in $CH_3CN$, as shown in Figure 1.

When the C1′ epimerization of **2a−c(β)** was carried out in less polar dichloromethane, no reaction occurred. Only the C1′ epimerization of **2d(β)** in dichloromethane proceeded, extremely slowly, as shown in Figure 1. Considerable degradation of the starting materials accompanied the reaction. These results can be explained as follows. In the less polar solvent $CH_2Cl_2$, the oxonium ion intermediate **7** cannot be stabilized because of weaker solvation, so that the first cleavage of the glycosidic bond does not occur favorably. It is likely that the oxonium ion **7** can, in the case of **2d(β)**, be trapped by the thiocarbonyl group and be converted into a thermodynamically more stable cyclic intermediate **8**. Therefore, the α anomer product was formed through attack of **8** with the silylated thymine only in this case.

From a practical point of view, α-thymidine would best be obtained from **2a(β)** or **2b(β)**, as multi-step reactions were required for preparation of compounds **2c(β)** and **2d(β)** (see Scheme 2). However, it turned out that it was not easy to separate the pure α anomers from the mixtures of **2a(α)/2a(β)** and **2b(α)/2b(β)** obtained by epimerization, owing to the close $R_f$ values of the compounds on TLC, as shown in Table 1. Therefore, we decided to remove the 3′- and 5′-protecting groups from the most highly α-enriched (89:11) mixture of **2b(α)/2b(β)** and protect the 5′-hydroxy




Scheme 5

group of the resulting **1(α)/1(β)** mixture in situ with pixyl chloride. As a result, 5′-*O*-pixylated α-thymidine **9(α)** was successfully separated from its β anomer **9(β)**[27] and could finally be crystallized from methanol (Scheme 5). Thus, compound **9(α)** was obtained in an overall yield of 40% from β-thymidine **1(β)**, while the β anomer could not be detected by $^1$H NMR, which suggested it was more than 99.5% pure. This compound can be directly used for the synthesis of α-oligodeoxynucleotides. The 5′-*O*-pixylated α-thymidine **9(α)** could, in our hands, also be obtained in an overall yield of 15−20% from thymidine by the original method.[18] Therefore, we had been able to improve the isolated yield of **9(α)** significantly. Development of this new route to the key synthetic intermediate **9(α)** would facilitate the synthesis of a variety of modified α-oligonucleotides.

## Conclusion

In conclusion, we have shown that α/β C1′ epimerization of thymidine derivatives at 30 °C is possible, and that the α anomer selectivity can be improved to ca. 90% through the use of $Et_2CHC(O)-$, $Et_2NC(O)-$, and $Et_2NC(S)-$ as 5′-*O*-protecting groups. Use of the thiocarbamoyl group, which was effective for irreversible selective glycosylation,[20] resulted in slow epimerization in acetonitrile but only effected epimerization in dichloromethane because of its strong neighboring group participation ability. It was also shown that the kinetic rates in the C1′ epimerization are considerably affected by solvent effects. The α/β ratio was highly dependent on the thermodynamic stability of the α- and β-thymidine derivatives. The main factor determining the α/β ratio is not neighboring group participation, but the steric hindrance due to the 5′-protecting group.

These results suggest that it may be possible to enhance the ratio of α and β anomers through a choice of more hindered 5′-*O*-protecting groups, which should inhibit attack of the silylated thymine from the β face. Further studies in this direction are now underway.

## Experimental Section

**General Remarks:** $^1$H and $^{13}$C NMR spectra were recorded at 270 and 68 MHz, respectively. The chemical shifts were measured from tetramethylsilane for $^1$H NMR spectra, $CDCl_3$ (δ = 77) for $^{13}$C NMR. TLC was performed with Kieselgel 60-F-254 (0.25 mm). Column chromatography was performed with silica gel C-200 or C-300 purchased from Wako Co. Ltd., and an aquarium minipump could conveniently be used to attain sufficient pressure for rapid chromatographic separation. Pyridine was distilled twice from *p*-toluenesulfonyl chloride and from calcium hydride and then stored over molecular sieves (4 Å). HMDS and TMSOTf were purchased from Shinetsu Chemical Co. Ltd. Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology at Nagatsuta.

**3′,5′-Di-*O*-toluoylthymidine [2a(β)]:**[18,21] Toluoyl chloride (680 mg, 4.4 mmol) was added to a solution of thymidine (485 mg, 2 mmol) in dry pyridine (10 mL). The solution was stirred at room temperature for 1 h. The mixture was partitioned between $CHCl_3$ (50 mL) and 5% $NaHCO_3$ (50 mL). The organic layer was collected, washed with 5% $NaHCO_3$ (50 mL), dried with $Na_2SO_4$, and filtered, and the solvents were evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (30 g) with $CHCl_3$/MeOH (100:1, v/v) to give **2a(β)** as a foam (919 mg, 96%). $^1$H NMR (270 MHz, $CDCl_3$): δ = 1.62 (s, 3 H), 2.31−2.69 (m, 2 H), 2.42, (s, 3 H), 2.43 (s, 3 H), 4.52 (m, 1 H), 4.63−4.80 (m, 2 H), 5.63 (m, 1 H), 6.46 (dd, *J* = 8.9, 5.6 Hz, 1 H), 7.25−7.96 (m, 9 H), 8.86 (br., 1 H). $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 12.2, 21.8, 21.8, 38.1, 64.2, 74.9, 82.8, 84.9, 111.6, 126.2, 126.5, 129.2, 129.4, 129.4, 129.7, 134.3, 144.4, 150.1, 163.2, 165.8, 165.9. $C_{26}H_{26}N_2O_7$ (478.5): C 65.26, H 5.48, N 5.85; found C 65.43, H 5.36, N 6.11.

**5′-*O*-(2-Ethylbutyryl)-3′-*O*-toluoylthymidine [2b(β)]:** 2-Ethylbutyryl chloride (444 mg, 3.3 mmol) was added to a solution of thymidine (727 mg, 3 mmol) in dry pyridine (30 mL). The solution was stirred at room temperature for 2 h and toluoyl chloride (510 mg, 3.3 mmol) was then added. The mixture was stirred at room temperature for 1 h, and the reaction was quenched by addition of water (2 mL). After having been kept for 10 min, the mixture was partitioned between $CHCl_3$ (100 mL) and $NaHCO_3$ (5%, 50 mL). The organic layer was collected, washed with 5% $NaHCO_3$ (50 mL), dried with $Na_2SO_4$, and filtered, and the solvents were evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (15 g) with hexane/$CHCl_3$ (20:80, v/v) to give **2b(β)** as a foam (1.01 g, 73%). $^1$H NMR (270 MHz, $CDCl_3$): δ = 0.92 (t, *J* = 7.4 Hz, 6 H), 1.55−1.67 (m, 4 H), 1.97 (s, 3 H), 2.19−2.70 (m, 6 H), 4.30−4.63 (m, 2 H), 4.43 (m, 1 H), 5.43 (m, 1 H), 6.43 (dd, *J* = 8.7, 5.4 Hz, 1 H), 7.25−7.95 (m, 5 H), 9.53 (br., 1 H). $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 11.9, 11.9, 12.6, 21.8, 24.9, 25.0, 37.8, 48.9, 63.6, 74.5, 82.3, 84.8, 111.5, 126.1, 129.1, 129.7, 134.4, 144.4, 150.3, 163.6, 165.8, 175.4. $C_{24}H_{30}N_2O_7$ (458.5): C 62.87, H 6.59, N 6.11; found C 62.26, H 6.64, N 6.19.

**5′-*O*-(*N*,*N*-Diethylcarbamoyl)-3′-*O*-toluoylthymidine [2c(β)]:** Sodium hydride (1.5 g, 37.5 mmol) was added to a solution of 3′-*O*-(4,4′-dimethoxytrityl)thymidine (8.17 g, 15 mmol) in dry THF (30 mL). The solution was stirred at room temperature for 30 min and *N*,*N*-diethylcarbamoyl chloride (4.07 g, 30 mmol) was then added. After having been stirred at room temperature for 1 d, the

mixture was quenched by addition of phosphate buffer (0.2 M, pH = 6.0, 200 mL). The mixture was extracted with $CHCl_3$ (100 mL × 2), and the organic layer was collected. Trifluoroacetic acid (6 mL) was added to the solution, and the mixture was stirred at room temperature for 1 h. After neutralization by addition of saturated $NaHCO_3$, the mixture was extracted with $CHCl_3$ (100 mL). The organic layer was collected, dried with $Na_2SO_4$, and filtered, and the solvents were evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (50 g) with hexane/EtOAc (30:70, v/v). The product was dissolved in dry pyridine (75 mL). Toluoyl chloride (2.55 g, 16.5 mmol) was added to the mixture. After having been stirred at room temperature for 1 h, the mixture was partitioned between $CHCl_3$ (100 mL) and 5% $NaHCO_3$ (100 mL). The organic layer was collected, dried with $Na_2SO_4$, and filtered, and the solvents were evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (50 g) with hexane/EtOAc (50:50, v/v) to give **2c(β)** as a foam (3.65 g, 53%). $^1$H NMR (270 MHz, $CDCl_3$): δ = 1.14 (t, *J* = 7.1 Hz, 6 H), 1.94 (s, 3 H), 2.12−2.71 (m, 2 H), 2.43 (s, 3 H), 3.31 (br., 4 H), 4.43−4.48 (m, 3 H), 5.49 (m, 1 H), 6.39 (dd, *J* = 8.7, 5.4 Hz, 1 H), 7.25−7.95 (m, 5 H), 9.15 (br., 1 H). $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 12.6, 21.8, 37.9, 64.5, 74.6, 82.9, 85.1, 111.3, 126.2, 129.1, 129.7, 134.5, 144.3, 150.2, 155.0, 163.5, 165.8. $C_{23}H_{29}N_3O_7$ (459.5): C 60.12, H 6.36, N 9.14; found C 59.97, H 6.31, N 9.11.

**5′-*O*-(*N*,*N*-Diethylthiocarbamoyl)-3′-*O*-toluoylthymidine [2d(β)]:** Sodium hydride (288 mg, 7.2 mmol) was added to a solution of 3′-*O*-(*tert*-butyldimethylsilyl)thymidine (1.07 g, 3 mmol) in dry THF (10 mL). The solution was stirred at room temperature for 30 min and *N*,*N*-diethylthiocarbamoyl chloride (958 mg, 6 mmol) was then added. After having been stirred at room temperature for 1 d, the mixture was quenched by addition of phosphate buffer (pH = 6.0, 0.2 M, 100 mL). The mixture was extracted with $CHCl_3$ (100 mL × 2), and the organic layer was collected, dried with $Na_2SO_4$, and filtered, and the solvents were evaporated under reduced pressure. The residue was dissolved in dry THF (6 mL). Tetrabutylammonium fluoride hydrate (1.57 g, 6 mmol) was added to the solution. After having been stirred at room temperature for 4 h, the mixture was partitioned between $CHCl_3$ (100 mL) and 5% $NaHCO_3$ (100 mL). The organic layer was collected, dried with $Na_2SO_4$, and filtered, and the solvents were evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (30 g) with hexane/EtOAc (50:50, v/v). The product was subsequently dissolved in dry pyridine (3.7 mL). Toluoyl chloride (324 mg, 2.1 mmol) was added to the mixture. After having been stirred at room temperature for 1 h, the mixture was partitioned between $CHCl_3$ (100 mL) and 5% $NaHCO_3$ (100 mL). The organic layer was collected, dried with $Na_2SO_4$, and filtered, and the solvents were evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (20 g) with $CHCl_3$/MeOH (100:1, v/v) to give **2d(β)** as a foam (798 mg, 56%). $^1$H NMR (270 MHz, $CDCl_3$): δ = 1.15−1.23 (m, 6 H), 1.96 (s, 3 H), 2.20−2.71 (m, 2 H), 2.43 (s, 3 H), 3.46−3.87 (m, 4 H), 4.51 (m, 1 H), 4.83−4.86 (m, 2 H), 5.49 (m, 1 H), 6.40 (dd, *J* = 8.6, 5.6 Hz, 1 H), 7.25−7.95 (m, 5 H), 9.33 (br., 1 H). $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 11.9, 12.6, 13.4, 21.8, 37.6, 43.3, 48.1, 69.8, 74.2, 82.3, 85.0, 111.5, 126.1, 129.1, 129.7, 134.5, 144.3, 150.2, 163.5, 165.7, 186.4. $C_{23}H_{29}N_3O_6S$ (475.6): C 58.09, H 6.15, N 8.84, S 6.74; found C 57.61, H 6.08, N 8.82, S 6.69.

**Synthesis of α-Nucleosides by β/α Epimerization:** A mixture of an appropriate thymidine derivative (2 mmol) and hexamethyldisilazane (HMDS) (8.4 mL, 40 mmol) was refluxed in toluene (8 mL) for 1 h. The mixture was concentrated in vacuo. After the residue had been dissolved in anhydrous $CH_3CN$ or $CH_2Cl_2$ (20 mL), TMSOTf (360 μL, 2 mmol) was added. The mixture was stirred at 30 °C for 30−96 h. At appropriate times, an aliquot was taken from the mixture and analyzed by $^1$H NMR. Each α anomer formed was isolated by silica gel column chromatography and characterized by $^1$H and $^{13}$C NMR as follows.

**3′,5′-Di-*O*-toluoyl-α-thymidine [2a(α)]:**[18,21] $^1$H NMR (270 MHz, $CDCl_3$): δ = 1.87 (s, 3 H), 2.41−3.01 (m, 8 H), 4.46−4.59 (m, 2 H), 4.89 (m, 1 H), 5.61 (m, 1 H), 6.38 (dd, *J* = 7.3, 1.6 Hz, 1 H), 7.21−7.95 (m, 9 H), 9.03 (br., 1 H). $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 12.7, 21.8, 38.9, 64.1, 74.8, 85.3, 87.2, 110.2,126.0, 126.4, 129.3, 129.3, 129.4, 129.6, 135.2, 144.2, 144.7, 150.1, 163.7, 165.4, 166.0. $C_{26}H_{26}N_2O_7$ (478.5): C 65.26, H 5.48, N 5.85; found C 64.73, H 5.41, N 5.95.

**5′-*O*-(2-Ethylbutyryl)-3′-*O*-toluoyl-α-thymidine [2b(α)]:** $^1$H NMR (270 MHz, $CDCl_3$): δ = 0.92 (t, *J* = 7.4 Hz, 6 H), 1.52−1.72 (m, 4 H), 1.96 (s, 3 H), 2.24−2.97 (m, 6 H), 4.30 (m, 2 H), 4.78 (m, 1 H), 5.50 (m, 1 H), 6.33 (dd, *J* = 6.9, 1.6 Hz, 1 H), 7.21−7.81 (m, 5 H), 9.32 (br., 1 H). $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 11.9, 12.7, 21.8, 25.0, 25.0, 38.9, 48.8, 63.7, 74.8, 85.0, 87.2, 110.2, 125.9, 129.3, 129.4, 135.1, 144.7, 150.2, 163.8, 165.4, 175.4. $C_{24}H_{30}N_2O_7{\cdot}0.5H_2O$ (467.5): C 61.66, H 6.68, N 5.99; found C 61.70, H 6.27, N 6.22.

**5′-*O*-(*N*,*N*-Diethylcarbamoyl)-3′-*O*-toluoyl-α-thymidine [2c(α)]:** $^1$H NMR (270 MHz, $CDCl_3$): δ = 1.16 (t, *J* = 7.1 Hz, 6 H), 1.88 (s, 3 H), 2.41−2.89 (m, 5 H), 3.31 (br., 4 H), 4.19−4.35 (m, 2 H), 4.77 (m, 1 H), 5.56 (m, 1 H), 6.33 (dd, *J* = 6.9, 1.6 Hz, 1 H), 7.21−7.80 (m, 5 H), 9.29 (br., 1 H). $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 12.7, 21.8, 38.7, 64.5, 74.6, 85.3, 87.1, 110.1, 126.1, 129.2, 129.4, 135.2, 144.6, 150.1, 155.0, 163.8, 165.3. $C_{23}H_{29}N_3O_7$ (459.5): C 60.12, H 6.36, N 9.14; found C 59.94, H 6.34, N 9.18.

**5′-*O*-(*N*,*N*-Diethylthiocarbamoyl)-3′-*O*-toluoyl-α-thymidine [2d(α)]:** $^1$H NMR (270 MHz, $CDCl_3$): δ = 1.20−1.29 (m, 6 H), 1.88 (s, 3 H), 2.41−2.93 (m, 5 H), 3.51−3.88 (m, 4 H), 4.53−4.73 (m, 2 H), 4.85 (m, 1 H), 5.58 (m, 1 H), 6.33 (dd, *J* = 7.1, 1.8 Hz, 1 H), 7.21−7.80 (m, 5 H), 9.08 (br., 1 H). $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 11.9, 12.7, 13.5, 21.8, 38.5, 43.6, 48.2, 69.3, 74.4, 85.0, 86.9, 110.2, 126.0, 129.2, 129.4, 135.2, 144.6, 150.1, 163.7, 165.2, 186.3. $C_{23}H_{29}N_3O_6S$ (475.6): C 58.09, H 6.15, N 8.84, S 6.74; found C 57.75, H 6.48, N 8.51, S 6.50.

**5′-*O*-Pixyl-α-thymidine [9(α)]:** Sodium methoxide (177 mg, 3.28 mmol) was added to a solution of an 89:11 mixture of 5′-*O*-(2-ethylbutyryl)-3′-*O*-toluoyl-α-thymidine [**2b(α)**] and 5′-*O*-(2-ethylbutyryl)-3′-*O*-toluoylthymidine [**2b(β)**] (301 mg of the α/β mixture, 656 μmol) in methanol (20 mL). After having been stirred at room temperature for 1 d, the mixture was quenched by addition of hydrochloric acid in water (1 M, 3.3 mL). The mixture was partitioned between $CHCl_3$ (20 mL) and water (20 mL). The water layer was collected and washed with $CHCl_3$ (20 mL), and the solvents were evaporated under reduced pressure. The residue was rendered anhydrous by repeated coevaporation and finally dissolved in dry pyridine (3.3 mL). 9-Chloro-9-phenylxanthene (288 mg, 984 μmol) was added to the solution. After stirring had been continued for an additional 3 h, the resulting mixture was partitioned between $CHCl_3$ (100 mL) and 5% $NaHCO_3$ (50 mL). The organic layer was collected, dried with $Na_2SO_4$, and filtered, and the solvents were evaporated under reduced pressure to give an 86:14 mixture of **9(α)** and **9(β)** (268 mg, 82%). This mixture was chromatographed four times on a column of silica gel (15 g) with $CHCl_3$/MeOH (100:1, v/v) containing 1% pyridine, to give **9(α)** as a foam [196 mg, 73%

from the mixture of **9(α)/9(β)**, α-purity > 99.5%], m.p. 133 °C (crystallization from methanol). $^1$H NMR (270 MHz, $CDCl_3$): δ = 1.88 (s, 3 H), 2.18−2.80 (m, 2 H), 2.91−3.06 (m, 2 H), 4.36−4.42 (m, 2 H), 6.11 (dd, *J* = 7.7, 2.1 Hz, 1 H), 7.01−7.45 (m, 14 H), 8.69 (br., 1 H). $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 12.5, 40.9, 63.9, 72.4, 76.0, 77.2, 88.3, 88.7, 109.0, 116.3, 122.3, 122.5, 123.6, 126.2, 126.7, 127.8, 128.9, 129.2, 129.3, 137.5, 148.3, 150.7, 151.0, 151.1, 164.4. $C_{29}H_{26}N_2O_6$ (498.5): C 69.87, H 5.26, N 5.62; found C 69.43, H 5.08, N 5.64.

**5′-*O*-Pixylthymidine [9(β)]:**[27] $^1$H NMR (270 MHz, $CDCl_3$): δ = 1.67 (s, 3 H), 2.25−2.49 (m, 2 H), 3.12−3.28 (m, 2 H), 4.00 (m, 1 H), 4.43 (m, 1 H), 6.37 (dd, *J* = 7.6, 5.9 Hz, 1 H), 7.01−7.62 (m, 14 H), 8.82 (br., 1 H). $^{13}$C NMR (68 MHz, $CDCl_3$): δ = 12.2, 41.2, 63.5, 72.7, 85.0, 86.0, 111.1, 116.5, 116.5, 122.0, 122.4, 123.6, 123.7, 126.2, 127.0, 128.0, 128.8, 129.2, 129.4, 129.6, 135.4, 147.9, 150.2, 151.1, 151.4, 163.5. $C_{29}H_{26}N_2O_6{\cdot}0.5H_2O$ (507.5): C 68.63, H 5.36, N 5.52; found C 68.60, H 5.13, N 5.54.

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