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Synthesis of Trifluorothymidine: Green Glycosylation Condition Using Neither Chloroform nor Transition Metals

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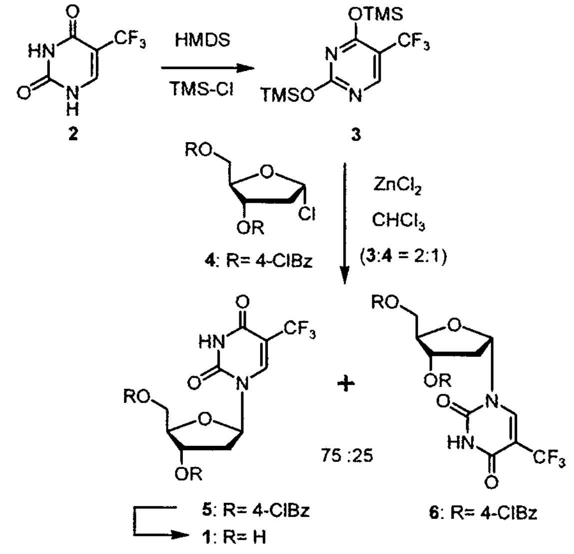
Abstract:

A new green glycosylation condition useful for efficient large-scale preparation of trifluorothymidine 1 is described. The condition requires neither $CHCl_3$ nor transition-metal catalysts for β-selectivity at the anomeric C1-position, which is advantageous for process development of active pharmaceutical ingredients such as 1. Key features of the condition include: (1) only an equimolar amount of trifluorothymine 2 is required, (2) the glycosylation is performed under high concentration, (3) the reaction is carried out at 50 °C to enhance the reaction.

Introduction

Trifluorothymidine **1** has attracted attention for decades due to its structural analogy to natural nucleic acids such as thymidine. Since **1** exhibits antitumor[1] and antiviral activities,[2] intensive investigation has been achieved for development of medical drugs. Particularly, demands for cytomegaloviral disease treatment of AIDS patients are increasing. Conventional processes for preparing **1** involve an enzymatic base exchange of thymidine[3] and trifluoromethylation on the 5-position of 2′-deoxyuridine derivatives using CF_3Cu [4] or $(CF_3)_2Hg$.[5] Those methods were not cost-effective for industrial manufacturing because of low yield and the expensive reagents. The best protocol has been reported by Kawakami,[6] and the key reaction is a glycosylation of chloro sugar **4** (R = 4-chlorobenzoyl)[7] with persilylated trifluorothymine **3** in the presence of $ZnCl_2$ catalyst (Scheme 1). However, a contamination of a significant amount of an α-anomer **6** is a drawback. To increase the stereoselectivity, the following points were indispensable for the reaction condition: (1) an addition of transient metal catalyst like $ZnCl_2$, (2) $CHCl_3$ solvent, and (3) large excess use of **3** (2 equiv was required for a 75:25 ratio of **5**:**6**). To meet the cGMP regulation standard, the residual amount of transient metal catalysts and $CHCl_3$ in an active pharmaceutical ingredient should be strictly controlled at low level. For health and environmental reasons, $CHCl_3$ solvent should be avoided. Additionally, usage of a stoichiometric amount of **3** is preferred for an economical process. Here we report a new glycosylation condition useful for synthesis of **1** using neither $CHCl_3$ nor transition-metal catalysts.

Scheme 1. **Conventional glycosylation method**



Results and Discussion

Effects of Solvents and Additives. To avoid $CHCl_3$, solvent effects were examined in the presence of various additives that were effective for the syntheses of 2′-deoxynucleosides (Table 1). To complete the reaction, 2 equiv of **3** was used in the experiments. Kawauchi[8] reported that CuF_2 had induced high β/α-selectivity even when the stoichiometric amount of **3** was used in the glycosylation. In the presence of excess **3**, the effects of CuF_2 were reduced to show comparable selectivity with the results carried out with no additives (entry 1). Hexamethyldisilazane (HMDS) or triethylamine (Et_3N) were effective in the synthesis of 2′-deoxyadenosine or 2′-deoxycytidine.[9] However, very low selectivity was observed in the reaction of **3** with **4**. $CHCl_3$ and 1,2,4-trichlorobenzene (1,2,4-$Cl_3C_6H_3$) were acceptable

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Table 1. **Effects of solvents and additives in the glycosylation of 4 with 3**[a]

		additives[b]			
entry	solvents	CuF_2	HMDS	Et_3N	no additives
1	$CHCl_3$	93.8 (98.8)	62.4 (98.7)	29.2 (96.6)	93.6 (98.9)
2	o-$Cl_2C_6H_4$	86.2 (97.6)	22.8 (97.5)	36.4 (85.3)	88.6 (97.6)
3	1,2,4-$Cl_3C_6H_3$	89.8 (94.4)	29.7 (96.9)	41.4 (83.3)	94.5 (94.4)
4	toluene	88.2 (95.5)	18.8 (95.0)	45.7 (60.0)	87.5 (95.5)
5	$PhNO_2$	79.7 (98.8)	22.6 (98.4)	5.0 (83.1)	73.2 (98.8)
6	1,4-dioxane	75.0 (98.4)	41.5 (87.8)	25.0 (87.3)	26.1 (92.6)
7	CH_3CN	65.5 (98.7)	24.9 (98.2)	41.1 (96.6)	49.7 (98.7)

[a] Conditions: **3**/**4** mole ratio, 2:1; rt, 22 h; CuF_2, 0.1 equiv; HMDS and Et_3N, 1 equiv. [b] Ratio of **5** (%) and conversion (%) shown in parentheses

Table 2. **Effect of reaction temperature in the glycostlation of 4 with 3**[a,b]

entry	reaction temperature °C	ratio of **5** (%)	conversion (%)
1	rt	94.5	94.4
2	40	91.6	95.7
3	50	90.1	98.4
4	60	91.0	98.7
5	70	87.2	98.8

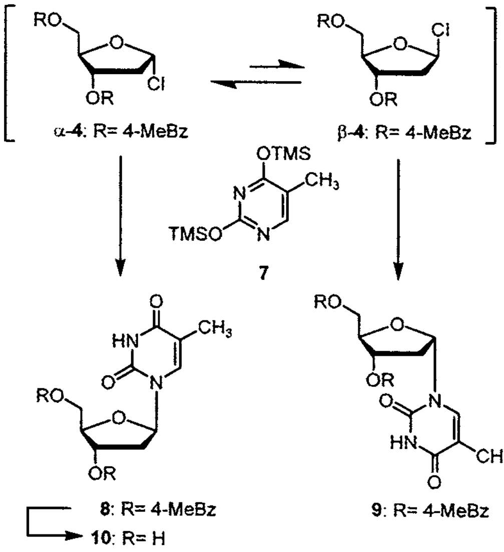
[a] Conditions: **3**/**4** mole ratio, 2:1; 4 h; solvent, 1,2,4-$Cl_3C_6H_3$. [b] Experiments were performed by a direct addition of **4** to liquid **3**.

Table 3. **Effect of substrate concentration in the glycosylation of 4 with 3**[a]

entry	mol equiv of solvent	ratio of **5** (%)	yield **5** + **6** (%)
1	0.0[b]	93.8	84.3
2	2.77	89.6	83.5
3	7.69	86.5	82.3
4	19.1	82.9	80.6
5	58.0	78.9	−

[a] Conditions: **3**/**4** mole ratio, 1:1; 50 °C, 4 h; solvent, o-$Cl_2C_6H_4$. [b] The experiment was performed by direct addition of **4** to liquid **3**.

Scheme 2. **Hubbard's theory**



for the reaction in this screening result (entries 1 and 3). Without any additives, aromatic solvents such as toluene or o-dichlorobenzene (o-$Cl_2C_6H_4$) demonstrated moderate β-selectivity (entries 2 and 4). Hubbard[10] explained the β/α-selectivity in the synthesis of thymidine **10** by the theory shown in Scheme 2. The solvent effect in the glycosylation is attributable to the different rate of the anomerization of **4**, as the reaction occurs in inversion of the configuration at the C1-carbon of **4**, and the reactivity of the nucleoside base determines the intrinsic selectivity that the substrate shows. Solvent effects in glycosylation of **3** with **4** were precisely examined for the first time here, and the experimental results performed with **3** also supported Hubbard's theory. Suppression of the unfavorable anomerization in less polar solvents such as toluene, o-$Cl_2C_6H_4$, or 1,2,4-$Cl_3C_6H_3$ should be the first step in developing a highly stereoselective reaction condition. Second, the reaction rate should be increased. Despite the polarity of $CHCl_3$, the reaction resulted in high β-selectivity, probably because the reaction rate in $CHCl_3$ would be larger than the rate of anomerization. The theory clearly elucidates the reason for the high stereoselectivity induced by the excess addition of **3** as the effect of the increased reaction rate. Trifluorothymine **2** is a less reactive base than thymine due to the electron-withdrawing substituent, CF_3. The excess addition of **3** was needed for the conventional method to afford the high β-selectivity.[6] Since our focus was usage of a stoichiometric amount of **3** for an economical process, we examined heating effects to enhance the reaction.

Effect of Reaction Temperature. Since 1,2,4-$Cl_3C_6H_3$ demonstrated the highest β-selectivity, the temperature effect was examined in this solvent (Table 2). Raising the reaction temperature resulted in a low ratio of **5**, but the conversion rate increased moderately. In the course of the experiments, we observed concentration-dependency of the induction of the stereoselectivity. High concentration of **3** will increase the reaction rate, and the condition will simultaneously decrease the unfavorable solvent effect. To evaluate the concept, the concentration effect was examined using o-$Cl_2C_6H_4$ as a reaction solvent.

Effect of Substrate Concentration. On the basis of the preliminary observation of the concentration effect, the glycosylation of **3** with **4** (**3**:**4** = 1:1) was performed in various amounts of the solvent, o-$Cl_2C_6H_4$. Clear evidence of the concentration-dependency was observed (Table 3). Accompanied with an increase of concentration, the **5**/**6** ratio increased to 93.8:6.2 (entry 1). Even though the experiment was performed with only 1 equiv of **3**, the result surpassed the reported highest **5**/**6** ratio (93:7) that was carried out with highly excessive **3** (4 equiv). Although the heating indeed increases the reaction rate, it would likely induce anomerization of **4**. The high selectivity would be partially attributable to the insoluble property of **4** in the liquid **3**. By heating, the powder, **4** gradually dissolves into the liquid **3** and therefore the reaction is maintained in a state where excess **3** exists with a small amount of the dissolved **4**. However, as the reaction mixture finally solidified by crystallization of **5** and **6** in the neat condition (entry 1), the condition was

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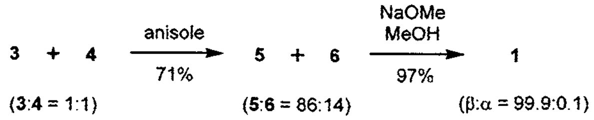
Table 4. **Solvent effect at high concentration in the glycosylation of 4 with 3**[a]

entry	solvent	mol equiv of solvent	ratio of **5** (%)	yield **5** + **6** (%)
1	toluene	2.9	90.5	87
2	ClC_6H_5	3.0	90.7	85
3	*o*-$Cl_2C_6H_4$	3.8	89.6	83
4	1,2,4-$Cl_3C_6H_3$	2.4	92.5	83
5	anisole	1.0	91.9	85
6	anisole	2.6[b]	94.0	88
7	$^{i}Pr_2O$	1.9	82.8	78
8	AcOEt	3.0	81.3	82
9	$C_6H_5NO_2$	2.9	80.9	82
10	2-butanone	3.3	72.0	77
11	$CHCl_3$	3.8	87.7	86

[a] Conditions: **3**/**4** mole ratio, 1:1; 50 °C, 4 h. [b] The experiment was performed by a dropwise addition of anisole into a mixture of **4** and **3**.

Scheme 3. **Hundred-gram-scale synthesis of 1**



not scalable without modification. The selectivity of the second concentrated condition (entry 2) was rather low (an 89.6:10.4 ratio of **5**:**6**) for process development, even though stirring of the reaction mixture was kept smooth. Investigation of solvent that maintains smooth stirring without a significant decrease of selectivity was attempted next.

Solvent Effect at High Concentration. In the course of the experiment, the glycosylation of **4** with **3** (1:1 ratio of **3**:**4**) was performed in the presence of only a minimum amount of solvent necessary for smooth stirring (Table 4). The aromatic solvents substituted with electron-donating groups such as Me, Cl, or MeO demonstrated high β-selectivity and good yields (entries 1−6). The results surpassed the selectivity observed in $CHCl_3$ (entry 11). The relatively high solubility of **4** in $CHCl_3$ at 50 °C would be inducible of anomerization compared with that of the nonpolar aromatic solvents. However, even with the equimolar usage of **3** and **4**, all the results except 2-butanone (entry 10) surpassed the selectivity reported in the conventional method.[6] Among the solvents, 1,2,4-$Cl_3C_6H_3$ demonstrated the highest β-selectivity. However, for safety and environmental reasons, anisole was preferred for process development. To reduce the solvent effect, portionwise addition of anisole was examined (entry 6). Initially, direct glycosylation of **4** with **3** was performed in neat condition, and anisole was added dropwise as the reaction proceeded. Using the procedure, the β-selectivity (94.0%) was comparable with the result in neat condensation shown as entry 1 in Table 3. The condition requires neither $CHCl_3$ nor transition-metal catalysts and will be suitable to scale-up.

Large-Scale Synthesis of Trifluorothymidine 1. The green glycosylation condition was applied to large-scale preparation of **1** (Scheme 3). Trifluorothymine **2** was converted to **3** in HMDS at 90 °C in the presence of TMS-Cl. Condensation of **4** with an equimolar amount of **3** was performed at 45−49 °C for 3.5 h in the presence of a small amount of anisole (96% w/w of **4**) to afford a mixture of **5** and **6** in 71% isolated yield[11] (85.7:14.3 ratio of **5:6** in HPLC assay and 85% HPLC yield before isolation) without purification. In the course of the reaction, anisole and **4** were added in two portions, and smooth stirring was maintained. Even though both the yield and the ratio of **5** were decreased compared with the result of entry 6 in Table 4, they surpassed the result of the conventional procedure[6] (75:25 ratio of **5:6**, 78% HPLC yield) that requires $CHCl_3$ solvent, excess amount of **3**, and $ZnCl_2$. Removal of the protective groups was carried out with 2.5 equiv of NaOMe in MeOH at 4 °C for 3.5 h. Neutralization with HCl and an elimination of the resulting methyl 4-chlorobenzoate by washing with AcOBu were successively performed to give **1** in 97% yield (99.92:0.08 ratio of β-**1**:α-**1** in HPLC assay).

Conclusions

In summary, we have developed a new green condition that is applicable to synthesis of trifluorothymidine **1**. The condition requires neither $CHCl_3$ solvent nor transition-metal catalysts to generate high stereoselectivity even when the equimolar amount of the nucleoside base **3** against the glycosyl donor **4** was used. The β-anomer of the protected trifluorothymidine **5** was synthesized in a stereoselective manner. An enhancement of the reaction by performing at 50 °C under a highly concentrated condition resulted in a 94.0:6.0 ratio of **5**:**6** (β:α). The process was scalable to produce **1** in hundred-gram scale by maintaining smooth stirring in the presence of a small amount of anisole. The selectivity and the yield (85.7:14.3 ratio of **5**:**6** in HPLC assay and 85% HPLC yield) surpassed the results of the conventional method.[6] The process will be expedient for manufacturing of active pharmaceutical ingredients such as **1**.

Experimental Section

For characterization of known compounds **1**, **3**, and **5**, see ref 6.

5-Trifluoromethyl-2,4-bis(trimethylsilyloxy)pyrimidine 3. Trifluorothymine **2** (186.2 g, 1.03 mol) and TMS-Cl (6.29 g, 57.9 mmol) in HMDS (353.8 g, 2.19 mol) were heated at 125 °C (90 °C of the internal temperature) for 5 h. After evaporation of the low-boiling point reagents, the residual crude product was purified by distillation (64−70 °C, 0.3−0.4 mmHg) to afford **3** (290.6 g, 0.896 mol; yield: 87%) as a colorless liquid.

3′,5′-*O*-Bis(4-chlorobenzoyl)-α,α,α-trifluoro-*β*-D-thymidine 5. 3,5-*O*-Bis(4-chlorobenzoyl)-2-deoxy-α-D-ribofuranosyl chloride **4** (400 g, 0.931 mol) was added to **3** (349.2 g, 1.08 mol) at 50 °C (25−45 °C of the internal temperature), and 15 min later, anisole (192 g) was added. To the reaction mixture were added **4** (62.5 g, 0.145 mol; total: 462.5 g, 1.08 mol) and anisole (144 g; total: 336 g), and the reaction was continued at 50 °C (45−49 °C of the internal temperature) for 3.5 h. In HPLC assay, the β/α ratio was 85.7:14.3, and the total content [**5** and **6** (α-isomer of **5**)] was

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(11) The low yield was attributable to the isolation loss into the filtrate. The selection of the suitable solvent instead of EtOH should be investigated further for the optimized yield.

528.6 g (HPLC yield: 85%). After cooling, EtOH (1750 mL) was added. The resulting suspension was filtered and washed with EtOH (180 mL), and the obtained wet powder was dried in vacuo (50 °C, 5 h) to afford **5** as a *β*/α mixture (85.7:14.3 ratio of **5**:**6**; 438.4 g, 0.765 mol; yield: 71%) as a colorless solid: HPLC t_R 7.8 min (**6**); t_R 8.7 min (**5**) [column: YMC Pack AM312 (ODS-AM) (150 cm × 6.0 mm) (from YMC, Co. Ltd.), mobile phase: 10 mM $KH_2PO_4-CH_3CN$ (3:7), flow rate: 1.2 mL/min, UV wavelength: 260 nm, column temperature: 40 °C, internal standard: dibutyl phthalate (t_R 11.4 min)].

α,α,α-Trifluoro-*β*-D-thymidine 1. To a solution of **5** (400.0 g of *β*/α mixture, 0.598 mol as **5**) in MeOH (2.6 L) was added 28% NaOMe in MeOH (336.5 g, 1.74 mol) at 4 °C, and 3.5 h later, the reaction mixture was neutralized with 8.6% w/w in MeOH at 5−6 °C. The resulting suspension was filtered, and the filtrate was concentrated. The residual solids were dissolved in acetone (3.5 L), and the precipitates were filtered off. The filtrate was concentrated, and to the residue was added AcOBu (1.3 L). The resulting suspension was filtered and washed with AcOBu (200 mL), and the obtained wet powder was dried in vacuo (40 °C, 4 h) to afford **1** (171.9 g, 0.580 mol; yield: 97%; 99.92:0.08 ratio of *β*-**1**:α-**1**) as a colorless solid: HPLC t_R 5.5 min (1); t_R 7.6 min (α-**1**) [column: YMC Pack AM312 (ODS-AM) (150 cm × 6.0 mm) (from YMC, Co. Ltd.), mobile phase: 10 mM $KH_2PO_4-CH_3CN$ (3:7), flow rate: 0.5 mL/min, UV wavelength: 260 nm, column temperature: 40 °C].

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