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Synthesis and properties of nucleoside derivatives acylated by chemically stable 2-(trimethylsilyl)benzoyl group

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ABSTRACT

We report the synthesis and properties of nucleoside derivatives acylated by 2-(trimethylsilyl)benzoyl (TMSBz) that proved to be extremely stable under basic conditions when introduced into the 5′-hydroxyl group of thymidine, the 4-amino group of deoxycytidine and the 2′-hydroxyl group of uridine. In particular, 2′-*O*-TMSBz-uridine could be isolated and was more stable in pyridine, while it isomerized in CH_2Cl_2 in the presence of Et_3N to yield a mixture of the 2′-O- and 3′-O-acylated species.

1. Introduction

In the current organic synthesis, acyl groups have been utilized most widely as base-labile protecting groups for chemical conversions of organic compounds[1] and as transient masking groups for generation of genuine drugs in prodrug strategy.[2] In particular, in the chemical synthesis of oligonucleotides, protection of reactive functional groups such as hydroxyl and amino groups has often been performed using various types of acyl groups.[3] In recent years, base-sensitive acyl groups such as phenoxyacetyl and 4-(*tert*-butyl)phenoxyacetyl have been developed for shortening the time required for full-deprotection of all base-labile protecting groups at the last stage of DNA or RNA synthesis.[4] In the present study, our interest is focused on base-stable acyl groups since they could contribute to nucleic acid chemistry, providing a possibility that they could be used as stable functional groups. This idea is based on changing their ubiquitous base-labile properties to unique base-resistant ones.

On the other hand, organosilicon chemistry is now recognized as a powerful synthetic tool for organic synthesis using unique electronic and steric properties of trialkylsilyl groups.[5,6] On the basis of these reasons, we studied 2-(trimethylsilyl)benzoyl (TMSBz) as a sterically hindered acyl group.

In this paper, we report the synthesis and properties of *O*- or *N*-TMSBz-nucleoside derivatives (Fig. 1).

2. Result and discussion

To synthesize *O*- or *N*-TMSBz-nucleoside derivatives, 2-(trimethylsilyl)benzoic acid (**4**) was prepared in an overall yield of 80% from benzaldehyde as the starting material required for introduction of a TMSBz group into functional groups on nucleoside derivatives, according to modification of the method previously reported.[7]

To check the stability of the TMSBz group attached to the primary hydroxyl group of thymidine, 5′-*O*-TMSBz-thymidine (**1**) was synthesized in one step from thymidine (**5**) without protecting the 3′-hydroxyl group using the Mitsunobu reaction,[8] as shown in Scheme 1.

To study the effects of the TMS group of the TMSBz group on the stability, we also synthesized 5′-*O*-(2-methylbenzoyl)thymidine (**6**), 5′-*O*-2-(*tert*-butyl)benzoylthymidine (**7**), and 5′-*O*-[(*Z*)-3-(trimethylsilyl)acryloyl]thymidine (**8**),. Compounds **6–8** were pre-

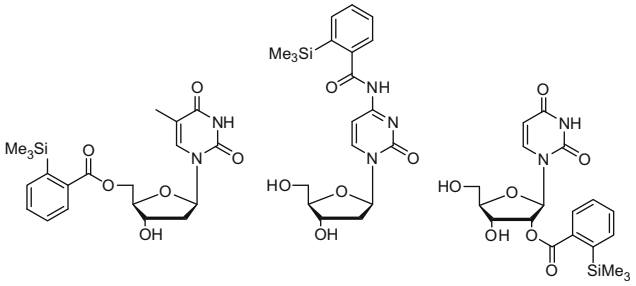

Figure 1. *O*- or 4-*N*-TMSBz nucleoside derivatives.

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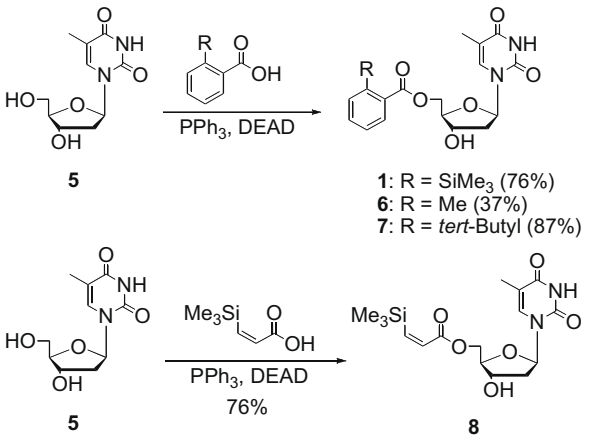


Scheme 1. Synthesis of compound **1**, **6–8**.

pared by use of 2-methylbenzoic acid, 2-*tert*-butyl-benzoic acid,[9] and (*Z*)-3-(trimethylsilyl)acrylic acid.[10]

The stabilities of compounds **1** and **6–8** under basic conditions are summarized in Table 1. In NH_3–EtOH (9:1, v/v) at room temperature or 55 °C, compounds **1** and **7** remained completely unchanged. On the other hand, compound **6** completely hydrolyzed in 24 h (entries 1 and 2). In a 2 M NaOH/EtOH solution, compounds **1** and **7** completely hydrolyzed in 4 h. These results suggested that the effect of the TMS group on the stability of the benzoyl group was almost the same as that of the *tert*-butyl group. The TMSBz and 2-(*tert*-butyl)benzoyl groups could be removed under strong basic conditions if necessary. This highly hindered acyl groups might be used not only as the mother skeleton for further modification of nucleoside or oligonucleotides but also as usual protecting groups under somewhat restricted conditions. On the other hand, compound **8** was hydrolyzed completely in NH_3–EtOH (9:1, v/v) at 55 °C in only 4 h.

To examine the hydrolytic properties of amide derivatives of 2-(trimethylsilyl)benzoic acid, 4-*N*-TMSBz-deoxycytidine (**2**) was synthesized. For introduction of the TMSBz group into the 4-amino group of deoxycytidine, 2-(trimethylsilyl)benzoyl chloride (**9**) was prepared by reaction of compound **4** with $SOCl_2$.[11] The reaction of 3′,5′-*O*-bis(TBDMS)-deoxycytidine (**10**) with the in situ generated acid chloride **9** yielded 3′,5′-*O*-bis(TBDMS)-4-*N*-TMSBz-deoxycytidine (**11**). The TBDMS protecting groups were deblocked by treatment with TEA·3HF to give the desired compound **2**, as shown in Scheme 2. The stability of **2** under various basic conditions is shown in Table 1. This compound proved to be extremely stable under these conditions. In particular, no hydrolyzed product was detected even upon treatment of **2** with 1.5 M NaOMe/MeOH, as shown in entry 5.

These results suggested that this group would remain unchanged under basic conditions generally used in chemical synthesis of oligonucleotides. In addition, this extreme stability of the TMSBz group led us to check if this group is tolerant to 2′-3′ migration when introduced into the 2′-hydroxyl group of ribonucleosides. For this purpose, 2′-*O*-TMSBz-uridine (**3**) was synthesized via a two-step reaction from 3′,5′-*O*-(1,1,3,3-tertraisopropyldisilane-1,3-diyl)uridine (**12**). Compound **12** was treated with compound **9** in CH_2Cl_2 in the presence of pyridine to give the 2′-*O*-acylated product **13** with 60% yield (Scheme 3).

The successive desilylation of compound **13** with TEA·3HF produced a single product, which could be isolated in 86% yield by silica gel column chromatography. The ^1H NMR analysis of this product suggested that the product thus obtained was the desired 2′-*O*-acyl derivative **3** and the 2′-3′ migration of the TMSBz group did not occur at all.

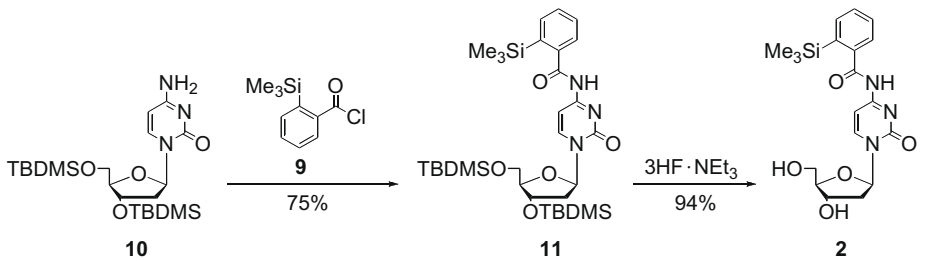
More detailed stability of 2′-*O*-TMSBz-uridine (**3**) was checked under basic conditions by ^1H NMR analysis, where the change in the chemical shift of the α-proton of the TMSBz group was monitored. No migration of the TMSBz group was observed in pyridine or collidine at room temperature for 24 h. To the best of our knowledge, this is the first example of a completely stable acyl group attached to the 2′-hydroxyl group of ribonucleosides with the neighboring unblocked 3′-hydroxyl group in pyridine. When a solution of **3** in pyridine was heated at 100 °C for 1 h, the acyl migration was not detected. The use of triethylamine as a base catalyst resulted in significant migration so that the 3′-*O*-acyl derivative was predominantly formed over compound **3**, as shown in Table 2.

The stability of the TMSBz group of compound **3** under more basic conditions was examined. As shown in Table 1, the 2′-*O*-acyl derivative **3** proved to be significantly more unstable than the 5′-

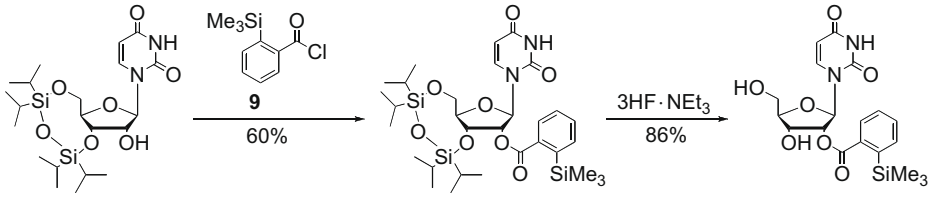
Table 1
Stability of nucleoside derivatives masked with 2-substituted benzoyl groups under basic conditions

Entry	Conditions	Temp	Time for complete hydrolysis					
			1	**6**	**7**	**8**	**2**	**3**
1	NH_3–EtOH (9:1, v/v)	rt	Stable (24 h)	24 h	Stable (24 h)	12 h	nd	48 h
2	NH_3–EtOH (9:1, v/v)	55 °C	Stable (24 h)	10 h	Stable (24 h)	4 h	Stable (24 h)	8 h
3	0.5 M NaOH/EtOH	rt	24 h	1 h	24 h	nd	nd	nd
4	2 M NaOH/EtOH	rt	4 h	15 min	4 h	nd	Stable (24 h)	nd
5	1.5 M NaOMe/MeOH	rt	nd	nd	nd	nd	Stable (24 h)	nd

nd: not determined.



Scheme 2. Synthesis of compound **2**.



12 13 3

Scheme 3. Synthesis of compound **3**.

Table 2
2′, 3′-Acyl migration of the TMSBz group of 3[a]

Entry	Base/solvent	Temp	Time (h)	Ratio of 2′-*O*-acyl and 3′-*O*-acyl derivatives
1	Pyridine	rt	24	100:0
2	2,4,6-Collidine	rt	24	100:0
3	Pyridine	100 °C	1	100:0
4	Et_3N/CH_2Cl_2[b]	rt	4	30:70

[a] Analyzed by ^1H NMR.
[b] 1 equiv of Et_3N was added.

O-acyl derivative **1**. Since the 2′-OH group is known to be more acidic (ca. pK_a 12.17–12.62)[12] because of the surrounding electron-withdrawing substituents (base moiety, franose ring, 3′-oxygen), we concluded that the 2′-oxygen residue might serve as a better eliminating group than the 5′-oxygen. In NH_3–EtOH, the migrated product, 3′-*O*-TMSBz-uridine, was observed by the TLC analysis. Compound **13** underwent 1.4 times slower degradation than compound **3** (data not shown), possibly because the former has a steric effect due to the TIPS group at the 3′-position.

It has generally been recognized that the *tert*-butyl group causes more steric congestion than the trimethylsilyl group, although the trimethylsilyl group occupies a bigger space in organic compound.[6b] However, the result obtained in this study suggested that the stability of compound **1** having the TMSBz group was almost identical to that of compound **7** having the *tert*-butyl. This discrepancy might be explained in terms of the stabilization effect of once-generated carbonyl adducts of the hydroxyl ion with TMSBz esters due to the coordination of the oxygen anion with the silicon atom of the TMS group. The optimized geometry of methyl 2-(trimethylsilyl)benzoate was calculated with the aid of GAUSSIAN 03 programs. In the most stable geometry-optimized structure of this compound, the distance between the silicon atom and the carbonyl oxygen (Si–O) was 2.94 Å, which is shorter than the sum of van der Waals radii (3.35 Å) for silicon and oxygen atoms (See Supplementary data). This result implies the formation of the intermediate as mentioned above.

Such intermediates seem to undergo hydrolysis of the TMSBz ester functions more slowly as compared to the corresponding adducts formed from the 2-(*tert*-butyl)benzoyl esters that do not have such a coordination effect at the stage of an intermediate resulted from the attack of the nucleophile on the carbonyl carbon.

To the best of our knowledge, the TMSBz group is the most chemically stable under basic conditions in nucleic acid chemistry so that 2′-*O*-TMSBz-uridine could be isolated by chromatography without migration of the TMSBz group. Several researchers reported substituted benzoyl groups attached to the 2′-hydroxyl group of ribonucleoside derivatives.[13] However, all such precedents are known to isomerize even in pyridine to yield 2′- and 3′-O-acylated mixtures. The extreme stability observed for the TMSBz group would be useful as the mother skeleton for further modification of nucleoside or oligonucleotides with a wide variety of functional groups at the 5′-, 3′-, and 2′-positions as well as the exo amino groups of nucleosides using appropriately modified TMSBz groups.

3. Conclusions

Nucleoside derivatives substituted with the TMSBz group were synthesized and found to be rather resistant to basic conditions. The TMSBz and 2-(*tert*-butyl)benzoyl groups showed very high stability compared with the 2-methylbenzoyl and (*Z*)-3-(trimethylsilyl)acryloyl group. Particularly, when the TMSBz group was introduced into the 4-amino group of deoxycytidine, it showed extremely high stability even under basic conditions such as a 1.5 M NaOMe/MeOH solution. The 2′-3′ *O*-acyl migration of the 2′-*O*-TMSBz group introduced into uridine was analyzed. As the result, this acyl group showed no acyl migration to the 3′-hydroxyl group in a solution of pyridine or 2,4,6-collidine. On the other hand, the migrated product, 3′-*O*-TMSBz-uridine, was predominantly produced in a Et_3N–CH_2Cl_2 solution. Further studies on the synthesis of oligonucleotides incorporating nucleoside derivatives protected with the TMSBz group and its derivatives at various positions are currently in progress in our lab. These results will be reported in the near future.

4. Experimental

4.1. General remarks

^1H and ^{13}C NMR spectra were recorded at 270 and 68 MHz, respectively. The chemical shifts were measured from tetramethylsilane for ^1H NMR spectra, $CDCl_3$ (77 ppm) for ^{13}C NMR spectra. Column chromatography was performed with silica gel C-200 purchased from Wako Co. Ltd, and a minipump for a goldfish bowl was conveniently used to attain sufficient pressure for rapid chromatographic separation. ESI mass spectrometry was performed using of Mariner™ (PerSeptive Biosystems Inc.).

4.1.1. Synthesis of compound 4[7]

n-BuLi (19 mL, 30 mmol) was added dropwise to a solution of *N*,*N*,*N*′-trimethylethylenediamine (4.3 mL, 33 mmol) in anhydrous THF (80 mL) at 0 °C. After the mixture was stirred at 0 °C for 15 min, benzaldehyde (3.0 mL, 30 mmol) was added. Then, the mixture was stirred at 0 °C for 30 min and an additional amount of *n*-BuLi (56 mL, 90 mmol) was added dropwise. After being stirred at 0 °C for 1 h, the mixture was treated with TMSCl (23.3 mL, 180 mmol) and stirred at 0 °C for an additional 2 h. The mixture was quenched by adding 1 M HCl (50 mL) and stirring was continued for 15 min. The resulting mixture was extracted with Et_2O. The organic extracts were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The concentrated organic solution was dissolved in acetone–H_2O (6:1, v/v, 84 mL), and $KMnO_4$ (9.5 g, 60 mmol) was added. The mixture was stirred at room temperature for 24 h and partitioned between Et_2O and a 1.5 M KOH aqueous solution. The aqueous layer was collected

and treated with a 3 M HCl aqueous solution. The resulting mixture was extracted with Et_2O. The organic extracts were combined, concentrated, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield compound **4** (4.65 g, 80%). This product was identified by ^1H NMR analysis compared with that of the authentic sample: ^1H NMR ($CDCl_3$) δ 0.37 (9H, s), 7.49 (1H, t, J = 7.5 Hz), 7.58 (1H, t, J = 7.5 Hz), 7.75 (1H, d, J = 7.5 Hz), 8.20 (1H, d, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 0.0, 128.6, 130.6, 132.1, 133.8, 135.3, 143.5, 173.7. HRMS (ESI) calcd for $[C_{10}H_{14}O_2Si+H]^+$ 195.0841, found 195.0722.

4.1.2. Synthesis of compound 1

Thymidine (242 mg, 1.0 mmol) was dissolved in anhydrous DMF (1.0 mL) solution under argon atmosphere. PPh_3 (262 mg, 1.0 mmol) was added and stirred vigorously for 1 h. Compound **4** (233 mg, 1.2 mmol) and diethyl azodicarboxylate (0.46 mL, 1.2 mmol) dissolved in dry DMF (1.0 mL) was added using a syringe. The reaction mixture was stirred for 4 h at room temperature and quenched by adding water (2 mL) and extracted with ethyl acetate. The combined organic layer was washed with water and brine. The organic solution was collected, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to produce a suspension, which was purified by C-200 silica gel column chromatography with $CHCl_3$–MeOH to yield **1** as a white solid (320 mg, 76%): ^1H NMR ($CDCl_3$) δ 0.32 (9H, s), 1.62 (3H, s), 2.15–2.25 (1H, m), 2.41–2.50 (1H, m), 4.24–4.25 (1H, m), 4.52–4.54 (1H, m), 4.57–4.66 (2H, m), 6.32 (1H, t, J = 6.5 Hz), 7.42 (1H, t, J = 7.4 Hz), 7.54 (1H, t, J = 7.4 Hz), 7.73 (1H, d, J = 7.6 Hz), 7.96 (1H, d, J = 8.0 Hz), 8.24 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 0.0, 12.0, 43.4, 64.2, 71.6, 84.5, 84.9, 111.3, 128.8, 129.3, 131.9, 134.5, 135.0, 135.7, 143.4, 150.6, 163.9, 167.6. HRMS (ESI) calcd for $[C_{20}H_{26}N_2O_6Si+Na]^+$ 441.1452, found 441.1446.

4.1.3. Synthesis of compound 6

Thymidine (2.4 g, 15 mmol) was dissolved in anhydrous DMF (10 mL) solution under argon atmosphere. PPh_3 (3.9 g, 15 mmol) was added and stirred vigorously for 1 h at room temperature. 2-Methylbenzoic acid (2.0 g, 15 mmol) and diethyl azodicarboxylate (2.7 mL, 15 mmol) dissolved in dry DMF (10 mL) was added via a syringe. The reaction mixture was stirred for 12 h at room temperature and quenched by addition of water (20 mL) and extracted with ethyl acetate. The combined organic layer was washed with water, and brine. The organic solution was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure resulted in suspension, which was purified by C-200 silica gel column chromatography with $CHCl_3$–MeOH to give **6** as a white solid (2.2 g, 37%): ^1H NMR ($CDCl_3$) δ 3.30 (3H, s), 2.11–2.22 (1H, m), 2.43–2.52 (1H, m), 3.17 (1H, d, J = 3.2 Hz), 4.24–4.28 (1H, m), 4.50–4.52 (1H, m), 4.54–4.58 (2H, m), 6.33 (1H, d, J = 6.2 Hz), 7.20–7.23 (2H, m), 7.28 (1H, m), 7.42 (1H, t, J = 7.6 Hz), 7.85 (1H, d, J = 7.6 Hz), 9.18 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 12.83, 22.00, 39.60, 65.20, 71.31, 84.61, 84.81, 110.77, 127.03, 130.10, 130.97, 132.66, 133.29, 136.71, 140.27, 151.40, 164.61, 167.57. HRMS (ESI) calcd for $[C_{18}H_{20}N_2O_6+Na]^+$ 383.1219, found 383.1236.

4.1.4. Synthesis of compound 7

Thymidine (242 mg, 1.0 mmol) was dissolved in anhydrous DMF (1.0 mL) solution under argon atmosphere. PPh_3 (262 mg, 1.0 mmol) was added and stirred vigorously for 1 h. 2-(*tert*-Butyl)benzoic acid (267 mg, 1.2 mmol) and diethyl azodicarboxylate (0.46 mL, 1.2 mmol) dissolved in dry DMF (1.0 mL) was added via a syringe. The reaction mixture was stirred for 4 h at rt and quenched by addition of water (2 mL) and extracted with ethyl acetate. The combined organic layer was washed with water, and brine. The organic solution was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure resulted in suspension, which was purified by C-200 silica gel column chromatography with $CHCl_3$–MeOH to give **7** as a white solid (330 mg, 82%): ^1H NMR ($CDCl_3$) δ 0.48 (9H, s), 0.70 (3H, s), 1.24–1.42 (2H, m), 3.17–3.18 (1H, m), 3.459 (1H, br s), 3.55- 3.65 (2H, m), 4.64 (1H, d, J = 4.1 Hz), 5.36 (1H, t, J = 6.8 Hz), 6.40–6.61 (4H, m), 6.68 (1H, d, J = 8.1), 10.46 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 11.88, 31.19, 35.68, 40.12, 64.59, 71.18, 84.07, 84.71, 111.12, 125.49, 127.07, 128.13, 130.19, 131.91, 135.31, 147.68, 150.74, 164.20, 171.40; HRMS (ESI) calcd for $[C_{21}H_{26}N_2O_6+Na]^+$ 425.1683, found 425.1691.

4.1.5. Synthesis of compound 8

Thymidine (484 mg, 2.0 mmol) was dissolved in anhydrous DMF (2.0 mL) solution under argon atmosphere. PPh_3 (525 mg, 2.0 mmol) was added and stirred vigorously for 1 h. (*Z*)-3-(Trimethylsilyl)acrylic acid (0.27 mL, 3.0 mmol) and diethyl azodicarboxylate (0.91 mL, 3.0 mmol) dissolved in dry DMF (2.0 mL) was added via a syringe. The reaction mixture was stirred for 12 h at room temperature and quenched by addition of water (2 mL) and extracted with ethyl acetate. The combined organic layer was washed with water, and brine. The organic solution was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure resulted in suspension, which was purified by C-200 silica gel column chromatography with $CHCl_3$–MeOH to give **8** as a white solid (320 mg, 76%): ^1H NMR ($CDCl_3$) δ 0.87 (9H, s), 1.89 (3H, s), 2.09–2.20 (1H, m), 2.40–2.49 (1H, m), 2.88 (1H, br s), 4.18–4.20 (1H, m), 4.33–4.46 (3H, m), 6.32 (1H, t, J = 6.6), 6.49 (1H, d, J = 14.3), 6.68 (1H, s, J = 14.0), 7.30 (1H, s), 9.05(1H, br s); ^{13}C NMR ($CDCl_3$) δ 0.00, 12.60, 40.37, 63.86, 71.69, 84.48, 85.16, 111.22, 133.50, 135.24, 150.73, 155.17, 164.14, 166.01; HRMS (ESI) calcd for $[C_{20}H_{26}N_2O_6Si+Na]^+$ 441.1452, found 441.1446.

4.1.6. Synthesis of compound 11

A solution of compound **9** (2.6 g, 12 mmol) in dry CH_2Cl_2 (20 mL) was added to a solution of 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)deoxycytidine (4.56 g, 10 mmol) in dry pyridine (20 mL). The mixture was stirred at room temperature for 2 h, and the mixture was diluted with $CHCl_3$. The $CHCl_3$ solution was washed successively with H_2O and saturated $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C-200 silica gel column chromatography with hexane–ethyl acetate to yield the product **11** (4.74 g, 75%): ^1H NMR (DMSO-d_6) δ 0.03–0.08 (12H, m), 0.25 (9H, s), 0.87–0.89 (18H, m), 2.14–2.23 (1H, m), 2.29–2.39 (1H, m), 3.73–3.85 (2H, m), 3.90–3.92 (1H, m), 4.37 (1H, m), 6.10 (1H, t, J = 5.9 Hz), 7.34 (1H, d, J = 7.3 Hz), 7.46–7.52 (2H, m), 7.62–7.68 (2H, m), 8.25 (1H, d, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ −5.6, −5.5, −5.0, −4.6, 0.1, 17.9, 18.3, 25.7, 25.9, 42.3, 61.9, 70.3, 87.0, 88.0, 126.2, 129.0, 130.9, 135.9, 140.2, 141.2, 144.9, 162.0. HRMS (ESI) calcd for $[C_{31}H_{53}N_3O_5Si_3+H]^+$ 632.3366, found 632.3364.

4.1.7. Synthesis of compound 2

Compound **11** (3.26 g, 5.0 mmol) was rendered anhydrous by repeated coevaporation with pyridine, toluene, and CH_2Cl_2, and finally dissolved in anhydrous THF (46 mL). TEA·3HF (4.0 mL, 25 mmol) was added to the solution, and the resulting mixture was stirred at room temperature for 1 h. The mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C-200 silica gel column chromatography with $CHCl_3$–MeOH to yield the product **2** (1.9 g, 94%): ^1H NMR ($CDCl_3$) δ 0.28 (9H, s), 2.32–2.37 (1H, m), 2.53–2.59 (1H, m), 3.72 (1H, br s), 3.90 (2H, m), 4.06–4.07 (1H, m), 4.17 (1H, br s), 4.55–4.57 (1H, m), 6.15 (1H, t, J = 5.9 Hz), 7.41–7.48 (2H, m), 7.57–7.60 (2H, m), 7.68 (1H, d, J = 3.8 Hz), 8.36 (1H, d, J = 7.6 Hz), 8.87 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 0.0, 26.1, 28.5,

29.8, 35.4, 40.2, 40.6, 72.8. HRMS (ESI) calcd for $[C_{19}H_{25}N_3O_5Si+H]^+$ 404.1636, found 404.1612.

4.1.8. Synthesis of compound 13

Compound **12** (4.87 g, 10 mmol) was rendered anhydrous by repeated coevaporation with pyridine and finally dissolved in anhydrous pyridine (20 mL). Compound **9** (2.6 g, 12 mmol) in dry CH_2Cl_2 (20 mL) was added to this solution. After being stirred at room temperature for 6 h, the mixture was partitioned between $CHCl_3$ and saturated $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C-200 silica gel column chromatography with hexane–ethyl acetate to yield the product **13** (3.95 g, 60%): ^{1}H NMR (DMSO-d_6) δ 0.05 (9H, s), 0.52–0.80 (28H, m), 3.70–3.90 (3H, m), 4.37–4.40 (1H, m), 5.34 (1H, d, J = 8.1 Hz), 5.54–5.56 (2H, m), 7.25–7.45 (4H, m), 7.84 (1H, d, J = 7.6 Hz), 11.17 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 0.1, 12.5, 12.87, 12.92, 13.4, 16.76, 16.81, 16.84, 16.9, 17.21, 17.27, 17.34, 17.4, 59.5, 67.8, 75.6, 82.4, 88.8, 102.0, 128.7, 130.0, 131.7, 134.8, 135.5, 139.4, 143.6, 149.5, 162.9, 166.1. HRMS (ESI) calcd for $[C_{31}H_{50}N_2O_8Si_3+Na]^+$ 685.2767, found 685.2712.

4.1.9. Synthesis of compound 3

Compound **13** (1.66 g, 2.5 mmol) was rendered anhydrous by repeated coevaporation with pyridine, toluene, and CH_2Cl_2, and finally dissolved in dry THF (18.4 mL). TEA·3HF (1.6 mL, 12.5 mmol) was added to the solution, and the mixture was stirred at room temperature for 2 h. The mixture was partitioned between $CHCl_3$ and saturated $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by C-200 silica gel column chromatography with $CHCl_3$–MeOH to yield the product **3** (900 mg, 86%): ^{1}H NMR ($CDCl_3$) δ 0.27 (9H, s), 2.32 (1H, br s), 2.39 (1H, br s), 3.86–4.05 (2H, m), 4.21 (1H, s), 4.71 (1H, s), 5.57–5.61 (1H, m), 5.75 (1H, d, J = 8.1 Hz), 6.04 (1H, d, J = 4.9 Hz), 7.41–7.71 (4H, m), 8.05 (1H, d, J = 7.6 Hz), 8.21 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 0.0, 61.8, 69.9, 75.8, 84.9, 89.9, 103.0, 129.0, 130.3, 132.4, 133.6, 135.8, 141.4, 144.0, 150.1, 162.6, 166.9. HRMS (ESI) calcd for $[C_{19}H_{24}N_2O_7Si+H]^+$ 421.1426, found 421.1409.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.07.003.

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