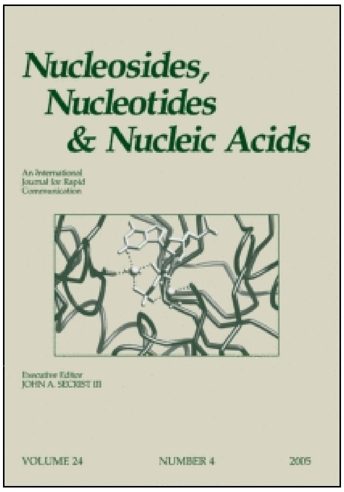

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REACTION OF N^3-BENZOYL-3',5'-*O*-(DI-*TERT*-BUTYLSILANEDIYL)URIDINE WITH HINDERED ELECTROPHILES: INTERMOLECULAR N^3 To 2'-*O* PROTECTING GROUP TRANSFER

Lei Zhu[a]; Osvaldo dos Santos[a]; Nadrian C. Seeman[a]; James W. Canary[a]
[a] Department of Chemistry, New York University, New York, NY, U.S.A.

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REACTION OF N^3-BENZOYL-3′,5′-*O*-(DI-*TERT*-BUTYLSILANEDIYL)URIDINE WITH HINDERED ELECTROPHILES: INTERMOLECULAR N^3 TO 2′-*O* PROTECTING GROUP TRANSFER

Lei Zhu, Osvaldo dos Santos, Nadrian C. Seeman,* and James W. Canary*,†

Department of Chemistry, New York University,
New York, NY 10003

ABSTRACT

The compound N^3-benzoyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)uridine **2** was alkylated with various alkyl iodides in CH_3CN in the presence of base. Normal 2′-*O*-alkylated products were obtained with methyl or benzyl iodide. If hindered alkyl iodides with β-branching such as 2-ethylbutyl iodide were used as electrophiles under the same conditions, N^3-alkyl-2′-*O*-benzoyl uridine derivatives were produced. This unexpected transformation is usually dormant with reactive alkylating agents, but expressed with sterically hindered, less reactive electrophiles. This unwanted reaction gives isomeric products whose spectra differ in only subtle ways from target compounds.

Key Words: Alkylation reaction; Protecting group; 2′-*O*-Alkyl ribonucleosides

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*Corresponding authors. Fax: (212) 260-7905
†E-mail: james.canary@nyu.edu

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INTRODUCTION

Antisense agents are oligonucleotide analogs capable of inhibiting certain points of genetic information flow via base pair interactions with DNA or mRNA. These molecules have been explored extensively because of their potential as therapeutic agents, as well as their increasing use as biotechnological tools.[1] To achieve the desired interaction with target fragments, antisense oligonucleotides have to be able to penetrate cellular membranes, resist degradation by nucleases and bind strongly with target oligonucleotides.[1] Several strategies have been developed to achieve these goals. There are early reports in which nucleobases have been modified to increase the nuclease resistance but at the expense of selectivity.[2] More recently, the polyanionic backbone of traditional antisense nucleotides has been recognized as a disadvantage in intracellular uptake and the cause of limited affinity to targeted natural oligonucleotide fragments.[3] The negatively charged structure is also sensitive to nucleases.[3] Replacement of the nucleotide backbone has led to PNA (peptide nucleic acid) technology and more radical structures such as positively charged guanidinium linkages.[3–5] Currently there are also groups developing oligonucleotides with 3′-3′-internucleotide linkages at the end of the sequence, to achieve resistance to exonucleases.[6] A very large number of modified species have been produced and characterized by the anti-sense and gene therapy enterprises.[7]

In ribonucleotides, functionalization of the free 2′-OH provides the locus for another strategy to design antisense agents.[8] It should be possible to modify the 2′-*O* position with a variety of functional entities, such as positively charged groups, fluorescent probes and other species. Our interest in the synthesis of 2′-*O*-alkyl ribonucleoside derivatives relates in part to DNA nanotechnology.[9–11] One of our projects would require branched, highly functionalized groups within the alkyl group such as in compound **1** (Fig. 1a). An initial approach to the synthesis of this compound necessitated the alkylation of a ribosyl 2′-OH with a *sec*-butyl group. However, published alkylations have been achieved only with relatively reactive electrophiles: for example, methyl iodide has been used frequently to methylate the ribosyl 2′-OH in reaction with silver oxide,[12–14] sodium hydride[15] or 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine (BEMP).[16] Reactive benzyl and allyl halide derivatives[17–20] often have been incorporated into oligonucleotides via 2′-*O*-alkylation as antisense groups or fluorescent probes.[18] Protecting group agents such as alkoxymethyl halides[21] and alkyl bromoacetates[22–24] also have worked well, owing to their high reactivities. Treating 2,2′-anhydrouridine with boron alkoxides has provided an alternative access to 2′-*O*-alkyluridine with less reactive electrophiles,[25,26] but high temperature ($>100°C$) required in this reaction

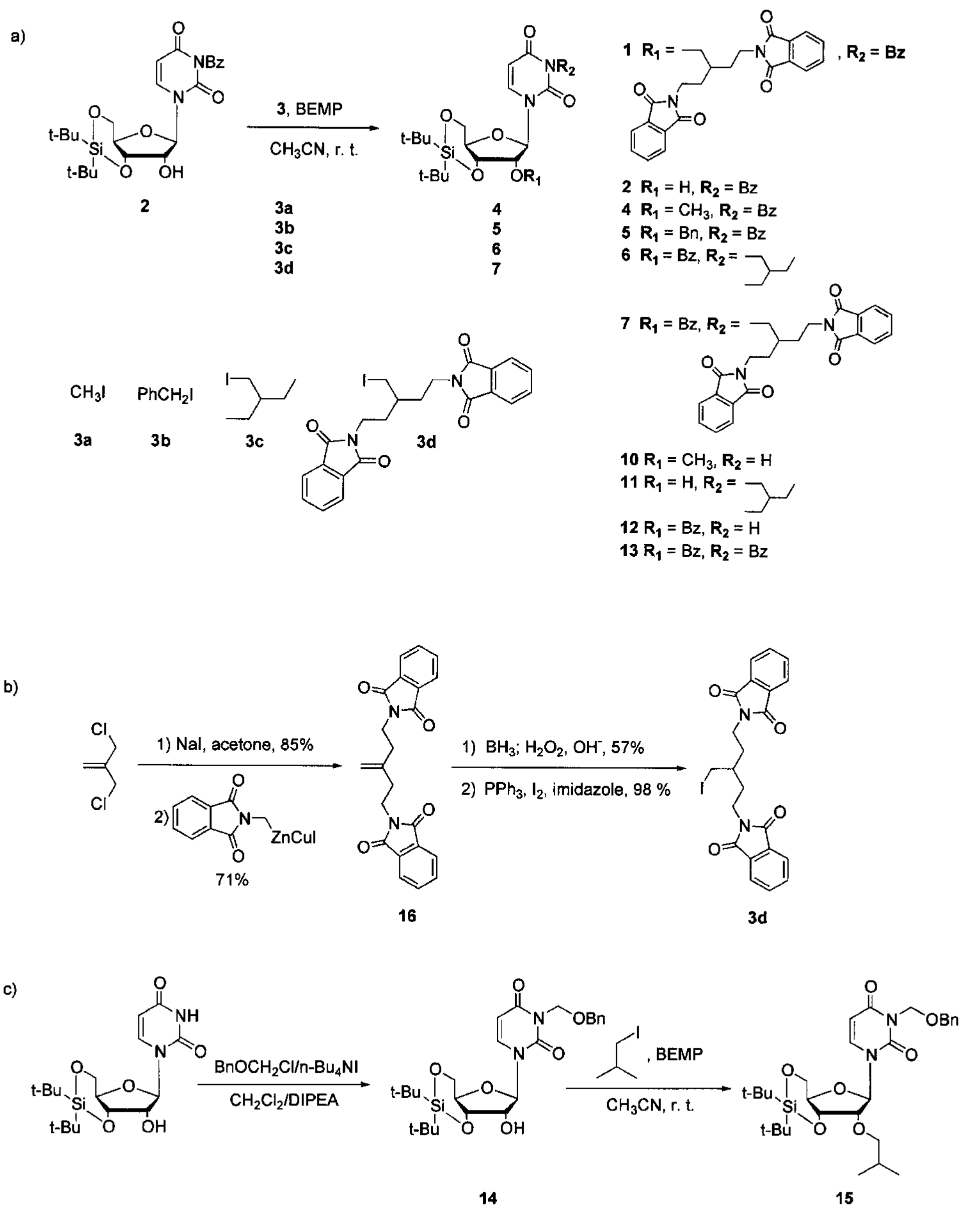

Figure 1. a) Reactions of alkyl iodides with N^3-benzoyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)-uridine; b) Synthesis of iodide **3d**; c) Synthesis of compounds **15**.

limited the types of functional groups which could be incorporated in the alkyl groups. Ribosyl 2′-OH also has been alkylated with primary halides without branching under various base and solvent systems,[23] but alkylation by more hindered electrophiles, such as one with β-substitution, has not been reported.

RESULTS AND DISCUSSION

In our study, Sproat's approach[16] was carried out to access 2′-*O*-alkylated uridine derivatives because of its satisfying yields with various alkyl halides as well as its mild conditions which were compatible with the functional groups we wished to incorporate in the alkyl halides. We investigated the alkylation of 2′-OH of a base-protected nucleoside, N^3-benzoyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)uridine **2**, by four alkyl iodides (**3a**–**3d**) shown in Fig. 1a. The benzoyl protecting group was chosen to prevent N^3-alkylation of uridine.[14,17,21,27] It could be incorporated selectively into the N^3 position by a phase-transfer reaction because of the difference between the acidity of the uracil moiety and that of the ribosyl 2′-OH.[28] It would be removed easily at the deprotection stage of automated oligonucleotide synthesis.

Compound **2** and alkyl halide were dissolved in CH_3CN, followed by the non-nucleophilic base BEMP. The 2′-*O*-alkylation by methyl and benzyl iodides proceeded uneventfully with respectable yields to give compounds **4** and **5**. With the much less reactive 2-ethylbutyl iodide (**3c**) or **3d** (Fig. 1b), superficial analysis of the ^{1}H NMR data seemed to indicate 2′-*O*-alkylation. However, upon closer inspection, we were surprised to find N^3-alkylated products **6** and **7** had been obtained exclusively in ~50% yield in which the benzoyl group had transferred to the 2′-*O*. Products of 2′-*O*-alkylation were not detected. The elimination of hydrogen iodide from **3d** was the major side reaction which compromised the yield of **7**. The alkylated nucleosides were characterized by ^{1}H/^{13}C NMR and FAB-MS (Table 1). All of the ^{1}H NMR peaks were assigned through the use of 2D NMR. The 1.21 and 1.33 ppm downfield shifts of 2′-H in compounds **6** and **7** respectively from 4.47 ppm in compound **2** after alkylation indicated the presence of strongly deshielding

Table 1. Characterization of N^3- vs. 2′-*O*-Alkyl and Acyl Derivatives of Uridine

	2	**4**	**5**	**6**	**7**	**12**
2′-H/ppm^a	4.47	4.00	4.2 ~ 4.6	5.68	5.80	5.68
C=O/ppm^b	168.7	168.6	168.8	165.5	165.1	165.8
FAB-MS (m/z)	489.6 (MH$^+$)	525.8^e (MNa$^+$)	471.3 (**C**)	377.2 (**B**)	487.2^d (**A**)	377.0^c (**B**)
			579.3 (MH$^+$)	451.3 (**C**) 573.3 (MH$^+$)	1024.3^d (MH$^+$)	489.2^c (MH$^+$)

^{a} ^{1}H NMR/$CDCl_3$.
^{b} ^{13}C NMR/$CDCl_3$.
^{c}ESI-MS.
^{d}Dimethoxytrityl derivative.

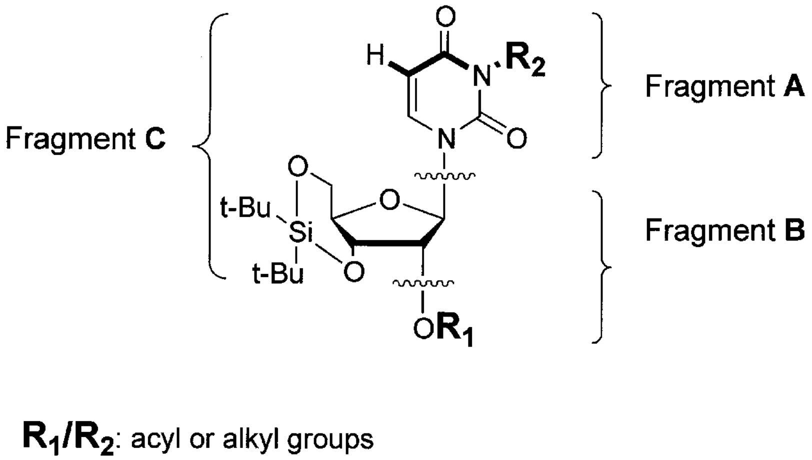

Figure 2. FAB mass spectrometry fragmentation patterns.

2′-*O*-acyl groups. Alkyl groups would not induce such drastic shifts of 2′-H, as observed in compounds **4** and **5**, where no deshielding effect was observed. The approximate 3-ppm shifts of the benzoyl carbonyl carbon in ^{13}C NMR spectra of **6** and **7** also indicated the transfer of benzoyl group to a different chemical environment. FAB-MS data (Table 1) further confirmed the structures by characteristic fragmentation resulting from loss of the base. In Fig. 2, fragment **A** represents the base portion of the molecule (noted as BH_2^+ by McCloskey),[29] establishing the connection between $\mathbf{R_2}$ and N^3 in compound **7** (Table 1), and fragment **B** confirms the identity of 2′-*O*-substituents in compound **6** (Table 1).[29] Compounds **8** and **9** in Fig. 3 were obtained by desilating compound **7** with HF-pyridine.[30] In compound **8**, both 2′-H and anomeric 1′-H were downfield shifted relative to **9** in the ^{1}H NMR spectra. 2D NMR and deuterium exchange experiments confirmed that **8**/**9** were isomers generated from 2′,3′-*O*-migration, which was precedented for ribosyl 2′-*O*-acyl groups.[31] This migration event provided further evidence for the structure of compound **7**. Debenzoylations were performed on compounds **4** and **6** to give compounds **10** and **11** respectively (Fig. 1a). Compared with **4** (2′-H, 4.00 ppm), the chemical shift of 2′-H in **10** (2′-H, 4.00 ppm) did not change upon debenzoylation while in compound **11** (2′-H, 4.31 ppm), the 2′-H shifted 1.37 ppm further upfield compared to **6** (2′-H, 5.68 ppm); these data indicated clearly that the benzoyl group was originally connected to the 2′-*O* in **6**.

A control experiment was performed in which **2** was treated with BEMP in the absence of alkyl halides. A substantial amount of **2** was transformed after 3 days into another species, which was identified as compound **12** (Fig. 1a). The presence of N^3-H in **12** was evidenced by the "**W**" shaped long range coupling ($^4J_{NH\text{-}H5} = 2.0\,Hz$) in ^{1}H NMR between N^3-H

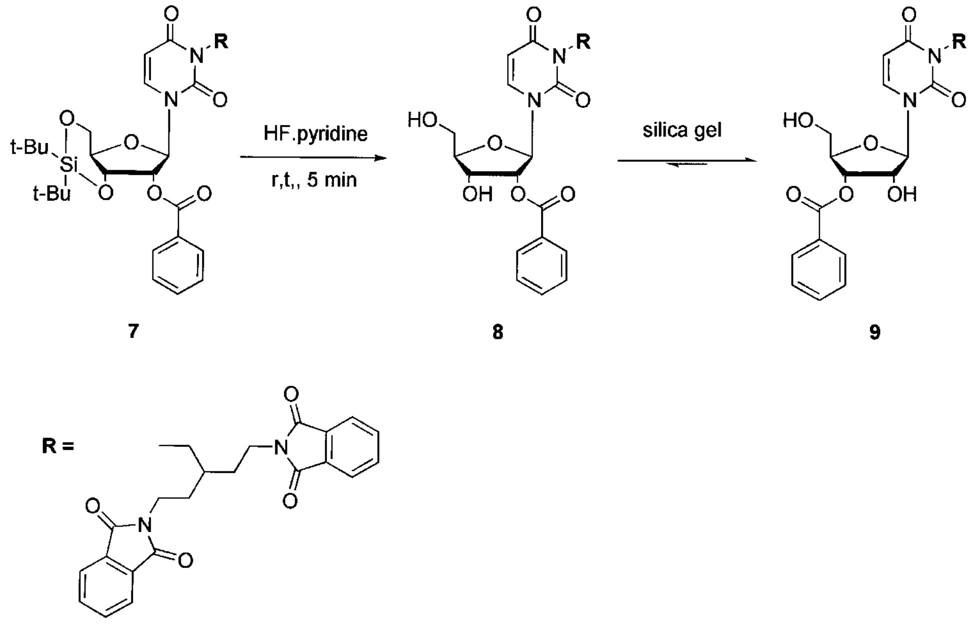

Figure 3. Ribose 2′-O-benzoyl migration in compound **7**.

and C^5-H on the uracil moiety. The structure of **12** was established by the data presented in Table 1. In this reaction, the ribosyl 2′-OH (pKa $\sim$ 13)[32] is deprotonated by BEMP in solution. In the absence of a strong electrophile, intermolecular nucleophilic substitution occurs, during which the benzoyl group transfers to 2′-O. Uracil N^3-H (pKa $\sim$ 9)[28] is more acidic than 2′-OH which renders the anionic uracil more stable than ribose 2′-alkoxide. The intermolecular nature of the transformation is further supported by the ^{1}H NMR spectrum of the crude reaction product in the formation of **7** which contained peaks consistent with structure of N^3-benzoyl-3′,5′-O-(di-*tert*-butylsilanediyl)-2′-O-benzoyluridine (**13**). The 2′-O-alkylation and benzoyl transfer are competing transformations so that whichever holds the kinetic advantage will result. With hindered alkylating agents, the benzoyl transfer occurs, and the freed N^3-position of the uracil moiety is alkylated. We suggest that this pathway is always available in base-catalyzed alkylating systems with electrophilic protecting groups, but it has not been reported previously because of the high reactivities of commonly used alkylating agents.[17,20,21,33]

Finally, a base-protecting group immune to nucleophilic attack should enable alkylation of ribosyl hydroxyl(s) with a broader range of alkylating agents. The benzyloxymethyl group was chosen to protect the uracil-N^3 to give compound **14** as the precursor for the desired 2′-O-alkylation. Reaction of **14** with the simplest β-substituted alkyl halide, 1-methylpropyl iodide, gave compound **15** (Fig. 1c), representing a successful alkylation with β-substitution on the ribosyl-2′-OH. The poor yield of this reaction resulted from competing elimination of β-substituted iodides inherently compromised the applicability of this approach to alkylation of ribosyl-2′-OH with sterically hindered entities.

EXPERIMENTAL SECTION

General Methods

CH_3CN was dried by refluxing with CaH_2. ^{1}H NMR and ^{13}C NMR were recorded on Varian-Gemini-200 or 300 MHz spectrometers. MALDI-TOF mass spectra were measured with a Kratos MALDI-I spectrometer using the matrix α-cyano-4-hydroxycinnamic acid. FAB mass spectra were measured using the matrix NBA/$CHCl_3$. ESI-MS spectra were measured at the New York University Protein Analysis Facility, Skirball Institute of Biomolecular Medicine, NYU School of Medicine. Elemental analyses were performed by the Complete Analysis Laboratories, Inc.

N^3-Benzoyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-*O*-methyluridine (4). N^3-Benzoyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)uridine **2** (122 mg, 0.25 mmol) was dissolved in CH_3CN (0.34 mL) under argon and cooled in an ice bath. BEMP (109 μL, 0.375 mmol) was added followed immediately by methyl iodide (15.6 μL, 0.25 mmol) with stirring and exclusion of moisture. Solvent was removed under vacuum after compound **2** was consumed as evidenced by TLC. Extractive isolation with CH_2Cl_2 and water gave a residue that was submitted to silica chromatography (CH_2Cl_2 to 10% ethyl acetate in CH_2Cl_2) to give 89.1 mg of product as a glass. The yield was 71%. IR (NaCl) 1747 (s), 1708 (s), 1678 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.0–7.5 (m, 5H, ArH), 7.39 (d, 1H, J = 9.0 Hz, 6-H), 5.87 (d, 1H, J = 9.0 Hz, 5-H), 5.69 (s, 1H, 1′-H), 4.50 (m, 1H, 4′-H), 4.13 (m, 1H, 3′H), 3.99 (3H, 2′, 5′, 5″-H), 3.60 (s, 3H), 1.08 (s, 9H), 1.04 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 168.6, 162.0, 139.4, 135.2, 130.7, 129.4, 102.7, 92.2, 82.7, 75.0, 67.6, 59.4, 32.0, 27.6, 27.3, 22.8, 20.6; MS (MALDI-TOF) calcd ($M+Na^+$) 525.6, found 525.8.

N^3-Benzoyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-*O*-benzyluridine (5). Co mpound **5** was prepared from 2′-*O*-alkylation of N^3-benzoyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)uridine **2** with benzyl iodide according to the procedure used for the preparation of compound **4** above. The crude product was chromatographed on silica gel (19/1 $CHCl_3$/EtOAc) to yield 80 mg (68%) of pure product. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.9–7.2 (m, 11H, ArH, 6-H), 5.84 (d, 1H, J = 8.2 Hz, 5-H), 5.81 (s, 1H, 1′-H), 4.93 (d, 1H, J = 12.0 Hz), 4.81 (d, 1H, J = 12.0 Hz), 4.51 (m, 1H, 4′-H), 4.23 (m, 1H, 3′-H), 4.16–3.96 (m, 3H, 2′, 5′, 5″-H), 1.14 (s, 9H), 1.10 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ

168.8, 162.2, 149.2, 139.5, 138.2, 135.6, 131.9, 131.0, 129.7, 128.7, 128.1, 128.0, 102.8, 92.3, 80.9, 76.9, 75.4, 73.2, 67.9, 28.0, 27.7, 23.4, 21.0; MS (FAB) calcd (M+H$^+$) 579.24, found 579.34.

N^3-(2-Ethylbutyl)-3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-*O*-benzoyluridine (6). N^3-Benzoyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)uridine **2** (245 mg, 0.5 mmol) and 1-bromo-2-ethylbutane (140 μL, 1.0 mmol) were dissolved in CH_3CN (1.0 mL) with n-Bu_4NI (37 mg, 0.1 mmol) as catalyst. BEMP (217 μL, 0.75 mmol) was added dropwise at –20°C. The solution was stirred for 72 h and then poured onto ice (25 g). Extractive isolation was performed with CH_2Cl_2/hexanes (1/3) and water. The organic layer was dried over Na_2SO_4 and the solvent was removed by reduced pressure. The residue was chromatographed with hexanes/ethyl acetate (from 10/1 to 10/3) to give **6** (124 mg, 43% yield) as a glass. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.1–7.4 (m, 5H, ArH), 7.18 (d, 1H, J = 8.0 Hz, 6-H), 5.90 (s, 1H, 1′-H), 5.81 (d, 1H, J = 8.0 Hz, 5-H), 5.68 (d, 1H, J = 5.6 Hz, 2′-H), 4.50 (m, 1H, 4′-H), 4.30 (m, 1H, 3′-H), 4.08 (m, 2H, 5′, 5″-H), 3.85 (m, 2H), 1.75 (m, 1H), 1.30 (m, 4H), 1.08 (s, 9H), 0.80 (m, 15H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 165.5, 163.0, 151.1, 138.4, 133.9, 130.4, 129.7, 128.8, 103.2, 92.8, 75.9, 75.6, 75.4, 67.8, 45.3, 39.3, 27.9, 27.5, 24.0, 23.9, 23.3, 20.7, 11.2, 11.1; MS (FAB) calcd (M+H$^+$) 573.29, found 573.23; Anal. calcd for $C_{30}H_{44}N_2O_7Si$: C, 62.91; H, 7.91; N, 4.89. Found: C, 63.17; H, 7.90; N, 4.70.

Compound 7. Compound **7** was prepared by treating N^3-benzoyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)uridine **2** with iodide **3d** according to the procedure used for preparation of compound **6** above to give **7** as a white solid in 40 ~ 55% yield. IR (NaCl) 1770 (m), 1713 (s), 1670 (m) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.1–7.3 (14H, ArH, 6-H), 5.95 (s, 1H, 1′-H), 5.85–5.81 (2H, 5-H, 2′-H), 4.53–4.00 (6H, 3′, 4′, 5′, 5″-H, CH_2), 3.70 (m, 4H), 2.10 (m, 1H), 1.79 (m, 4H), 1.10 (s, 9H), 0.80 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.6, 168.5, 165.1, 163.1, 151.2, 138.4, 134.1, 134.0, 133.7, 132.7, 130.4, 128.8, 123.7, 123.6, 102.9, 92.5, 76.9, 75.9, 75.7, 75.3, 67.8, 45.4, 36.3, 32.6, 31.5, 31.4, 28.2, 28.0, 27.4, 23.4, 20.7; MS (MALDI-TOF) calcd (M+Na$^+$) 886.0, found 886.3; Anal. calcd for $C_{45}H_{50}N_4O_{11}Si$: C, 61.19; H, 5.58; N, 6.15. Found: C, 60.96; H, 5.34; N, 6.00.

Compounds 8 and 9. HF.pyridine (100 μL, 3.84 mmol) was carefully diluted with pyridine (0.51 mL) and then added dropwise to a solution of **7** (86.3 mg, 0.10 mmol) in THF (0.6 mL) at 0°C. The mixture was warmed to room temperature, stirred for 5 min, and then quenched with 5% $NaHCO_3$. Extractive isolation was performed with CH_2Cl_2/water. The organic layer was collected, washed with 5% $NaHCO_3$, and dried over sodium sulfate. After evaporating most of the solvents under vacuum, the residue was co-evaporated with CH_3CN repeatedly to remove pyridine and by-product di-

tert-butyldifluorosilane (bp. 109°C) to obtain **8** (70 mg) in almost quantitative yield. Compound **8** was highly reactive and could not be purified further. Compound **9** was obtained after **8** was chromatographed on silica gel (CH_2Cl_2/ethylacetate) with 50 ~ 60% isolated yield. **8**: ^1H NMR (300 MHz, $CDCl_3$) δ 8.0–7.3 (14H, ArH, 6-H), 6.15 (d, 1H, J = 5.4 Hz, 1′-H), 5.76 (d, 1H, J = 9.0 Hz, 5-H), 5.67 (dd, 1H, J = 6.0, 5.4 Hz, 2′-H), 4.77 (dd, 1H, J = 6.0, 6.0 Hz, 3′-H), 4.21 (dd, 1H, J = 6.0, 3.0 Hz, 4′-H), 4.05 (dd, 1H, J = 9.0, 3.0 Hz, 5′-H), 4.00 (d, 2H, J = 7.0 Hz), 3.89 (dd, J = 9.0, 3.0 Hz, 5″-H), 3.73 (m, 4H), 2.05 (m, 1H), 1.76 (m, 4H); ^1H NMR (200 MHz, DMSO-d_6) δ 8.1–7.4 (14H, ArH, 6-H), 6.15 (d, 1H, J = 5.4 Hz, 1′-H), 5.74 (d, 1H, J = 9.0 Hz, 5-H), 5.64 (br, s, 1H, OH, D_2O exchangeable), 5.38 (dd, 1H, J = 6.0, 6.0 Hz, 2′-H), 5.30 (br, s, 1H, OH, D_2O exchangeable), 4.40 (br, m, 1H, 3′-H, D_2O exchangeable to triplet), 4.05 (dd, 1H, J = 6.0, 3.0 Hz, 4′-H), 3.90–3.65 (4H, 5′, 5″-H, CH_2), 3.58 (m, 4H), 1.85 (m, 1H), 1.63 (m, 4H); ^{13}C NMR (75.5 MHz $CDCl_3$) δ 168.1, 166.1, 163.0, 151.6, 139.5, 133.9, 132.6, 130.2, 128.8, 123.4, 102.6, 91.3, 85.0, 70.0, 62.0, 45.0, 36.1, 32.6, 31.1, 31.0; MS (ESI) calcd ($M+H^+$) 723.222, found 723.218. **9**: IR(NaCl) 1770 (m), 1710 (s), 1660 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.1–7.4 (14H, ArH, 6-H), 5.86 (d, 1H, J = 5.4 Hz, 1′-H), 5.77 (d, 1H, J = 9.0 Hz, 5-H), 5.60 (dd, 1H, J = 5.4, 1.5 Hz, 3′-H), 5.00 (br, s, 1H, 2′-H, CD_3OD exchangeable to triplet), 4.48 (br, s, 1H, OH, CD_3OD exchangeable), 4.38 (m, 1H, 4′-H), 4.01 (d, 2H, J = 7.0 Hz), 3.95 (2H, 5′, 5″-H), 3.74 (m, 4H), 3.41 (br, s, 1H, OH, CD_3OD exchangeable), 2.07 (m, 1H), 1.78 (m, 4H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 168.7, 166.3, 163.0, 152.1, 140.0, 134.1, 133.9, 133.7, 132.5, 130.2, 129.9, 128.8, 123.5, 102.5, 93.9, 84.2, 73.8, 72.9, 62.6, 53.5, 45.3, 36.2, 32.0, 31.2; MS (MALDI-TOF) calcd ($M+Na^+$) 745.7, found 746.1.

3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-*O*-methyluridine (10). Compound **4** (10 mg) was dissolved in methanol (1.0 mL) and treated with concentrated ammonia (29% in water, 2.0 mL) at room temperature for 4 h. Methanol was removed by vacuum and extraction was performed with CH_2Cl_2 and 5% $NaHCO_3$. The organic layer was washed with 5% $NaHCO_3$ and dried over Na_2SO_4. Compound **10** (7.9 mg) was obtained in almost quantitative yield after evaporation of solvent. ^1H NMR (300 MHz, $CDCl_3$) δ 9.16 (d, 1H, 2.1 Hz, NH), 7.26 (d, 1H, 8.2 Hz, 6-H), 5.76 (dd, 1H, J = 8.2, 2.1 Hz, 5-H), 5.68 (s, 1H, 1′-H), 4.48 (m, 1H, 4′-H), 4.11 (m, 1H, 3′-H), 3.95 (3H, 2′, 5′, 5″-H), 3.62 (s, 3H), 1.08 (s, 9H), 1.02 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 163.3, 149.8, 139.9, 102.8, 91.6, 82.5, 82.3, 74.7, 67.5, 59.4, 29.9, 27.5, 27.2, 23.0, 20.5; MS (MALDI-TOF) calcd ($M+H^+$) 399.5, found 399.4.

N^3-(2-ethylbutyl)-3′,5′-*O*-(di-*tert*-butylsilanediyl)uridine (11). Compound **6** was debenzoylated according to the procedure used for the preparation of compound **10** above to give compound **11** in almost quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, 1H, J = 8.1 Hz, 6-H), 5.75 (d, 1H, J = 8.1 Hz, 5-H), 4.47 (m, 1H, 4′-H), 4.30 (d, 1H, J = 4.0 Hz, 2′-H), 4.03 (3H, 3′, 5′, 5″-H), 3.82 (m, 2H), 2.88 (s, 1H), 1.77 (m, 1H), 1.29 (m, 4H), 1.08 (s, 9H), 1.02 (s, 9H), 0.87 (m, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 163.0, 150.8, 138.1, 102.4, 94.2, 76.1, 74.7, 73.9, 73.7, 76.5, 44.8, 38.8, 31.8, 27.5, 27.4, 23.4, 22.9, 20.6, 14.3, 10.7; MS (MALDI-TOF) calcd ($M+H^+$) 469.7, found 469.8.

2′-*O*-Benzoyl-3′,5′-(di-*tert*-butylsilanediyl)uridine (12). N^3-Benzoyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)uridine **2** (49 mg, 0.1 mmol) in CH_3CN (0.15 mL), was treated with BEMP (44 μL, 0.15 mmol), at ambient temperature under argon for 3 days. The reaction mixture was poured onto ice (25 g). The crude product was obtained by extractive isolation (CH_2Cl_2/water) and was purified by silica chromatography (hexanes/ethyl acetate) to give 29 mg of product as a white solid. The yield was 60%. IR (NaBr) 1720–1700 (br, s) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.69 (d, 1H, J = 2.0 Hz, NH), 8.15–7.40 (m, 5H, ArH), 7.27 (d, 1H, J = 8.0 Hz, 6-H), 5.81 (dd, 1H, J = 8.0, 2.0 Hz, 5-H), 5.76 (s, 1H, 1′-H), 5.68 (d, 1H, J = 6.0 Hz, 2′-H), 4.52–4.41 (m, 2H, 3′, 4′-H), 4.08 (m, 2H, 5′, 5″-H), 1.08 (s, 9H), 0.82 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 165.8, 163.0, 149.8, 141.6, 134.1, 130.4, 129.5, 128.9, 103.6, 93.2, 75.7, 75.6, 75.3, 67.7, 27.9, 27.5, 23.4, 20.8; MS (ESI) calcd ($M+H^+$) 489.2, found 489.2.

N^3-Benzoylxymethyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)uridine (14). The compounds 3′,5′-*O*-(di-*tert*-butylsilanediyl)uridine (1.92 g, 5.0 mmol) and tetrabutylammonium iodide (3.7 g, 10 mmol) were dissolved in CH_2Cl_2 (20 mL) under argon. DIPEA (~2mL, 10 mmol) was introduced by syringe and the solution was stirred for 15 min followed by addition of benzyl chloromethyl ether (693 μL, 5 mmol) dropwise. The solution was stirred for 3 h before being partitioned between CH_2Cl_2 and water. The organic layer was washed with water 3 times and dried over Na_2SO_4. The solvent was removed under vacuum without heating. The residue was subjected to silica chromatography, eluting with hexanes/ethyl acetate (up to 10/3) to give 1.8 g of white solid (71% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.25 (m, 5H, ArH), 7.17 (d, 1H, J = 8.2 Hz, 6-H), 5.76 (d, 1H, J = 8.2 Hz, 5-H), 5.59 (s, 1H, 1′-H), 5.49 (s, 2H), 4.70 (s, 2H), 4.48 (d, 1H, J = 3.0 Hz, 4′-H), 4.33 (d, 1H, J = 3.0 Hz, 2′-H), 4.12 (m, 1H, 3′-H), 4.01 (m, 2H, 5′-H), 2.71 (s, 1H, OH), 1.09 (s, 9H), 1.04 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 162.6, 150.7, 139.5, 138.1, 128.5, 127.9, 102.4, 94.8, 76.1, 74.9, 73.6, 72.6, 70.5, 67.4, 27.6, 27.4, 22.9, 20.6; MS (MALDI-TOF) calcd ($M+Na^+$) 527.6, found 527.1.

N^3-Benzoylxymethyl-3′,5′-*O*-(di-*tert*-butylsilanediyl)-2′-O-(2-methylpropyl)-uridine (15). Compound **14** (252 mg, 0.5 mmol) and 1-iodo-2-methylpropane (230 μL, 2 mmol) were dissolved in CH_3CN (2.0 mL). BEMP (579 μL, 2 mmol) was added dropwise *in situ*. The solution was stirred for 48 h before being poured onto ice. Extractive isolation was performed with CH_2Cl_2 and water. The organic layer was dried over Na_2SO_4 and solvent was removed at reduced pressure without heating. The residue was chromatographed with hexanes/ethyl acetate (up to 4/1) to give compound **15** (24 mg, 9% yield). ^1H NMR (200 MHz, $CDCl_3$) δ 7.38–7.27 (ArH, 5H), 7.21 (d, 1H, J = 8.2 Hz, 6-H), 5.74 (d, 1H, J = 8.2 Hz, 5-H), 5.70 (s, 1H, 1′-H), 5.50 (s, 2H), 4.70 (s, 2H), 4.50 (m, 1H, 4′-H), 4.19 (m, 1H, 3′-H), 4.02–3.80 (3H, 2′, 5′-H), 3.57 (m, 2H), 1.92 (m, 1H), 1.07 (s, 9H), 1.03 (s, 9H), 0.96 (d, 6H, J = 6.4 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.9, 150.8, 138.4, 138.1, 128.8, 128.1, 102.3, 92.0, 81.7, 75.2, 72.9, 70.8, 68.0, 29.5, 27.9, 27.6, 23.4, 21.0, 19.9; MS (MALDI-TOF) calcd ($M+Na^+$) 583.7, found 583.8.

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