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Design, synthesis, and evaluation of a novel bridged nucleic acid, 2′,5′-BNA^{ON}, with S-type sugar conformation fixed by *N–O* linkage

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ABSTRACT

We designed a novel 2′-*O*,5′-*N* bridged nucleic acid, 2′,5′-BNA^{ON}, whose sugar puckering was fixed to S-type conformation by an *N–O* linkage. A dimer unit formed from 2′,5′-BNA^{ON}-U and thymidine was synthesized via a coupling reaction between a protected 2′,5′-BNA^{ON}-U monomer and a thymidine derivative. Introduction of 2′,5′-BNA^{ON}-U into DNA was carried out using conventional phosphoramidite chemistry with a DNA synthesizer. The hybridization abilities of 2′,5′-BNA^{ON}-U-modified oligonucleotides against DNA or RNA complement were evaluated.

1. Introduction

Artificial or modified oligonucleotides, which can specifically interact with complementary oligonucleotides and which show resistance to enzymatic degradation are potential tools for genome technology. For this reason, numerous nucleic acid analogues have been synthesized and characterized.

One approach to oligonucleotide modification is replacement of a phosphodiester moiety by another linkage. For example, replacement with a phosphorothioate[1] linkage is known to be a useful modification in several fields. Oligonucleotides in which a phosphodiester linkage is replaced by a carbamate linkage (*O3′–CO–N5′*) also have potential as materials for genome technology because of their resistance to enzymatic digestion (Fig. 1).[2,3] The hybridization abilities of oligonucleotides containing carbamate linkages have been reported previously, and showed interesting tendencies. The simple DNA analogue **II**, which contains a carbamate linkage, showed slightly lower hybridization ability (−3 to −5 °C/modification),[2] while the conformationally flexible analogue **III** showed depressed hybridization ability (−7 to −12 °C/modification)[3] even compared with **II**. These results naturally lead us to an analogue having a carbamate linkage and a conformationally restricted sugar moiety to gain high thermal stability.

Conformational restriction is a powerful strategy for designing artificial nucleic acids possessing excellent hybridization abilities and resistance to enzymatic digestion. We have developed the

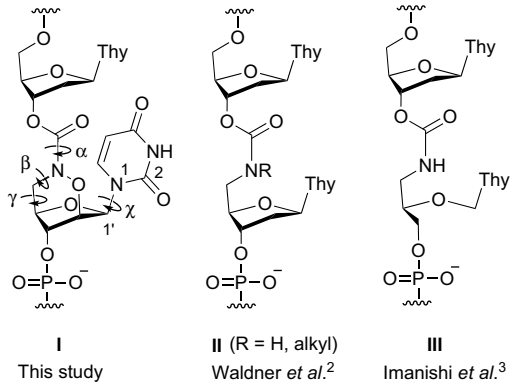

Figure 1. Structures of DNA analogues with carbamate moieties (*O3′–CO–N5′*) replacing the natural phosphodiester linkage. Thy; thymin-1-yl.

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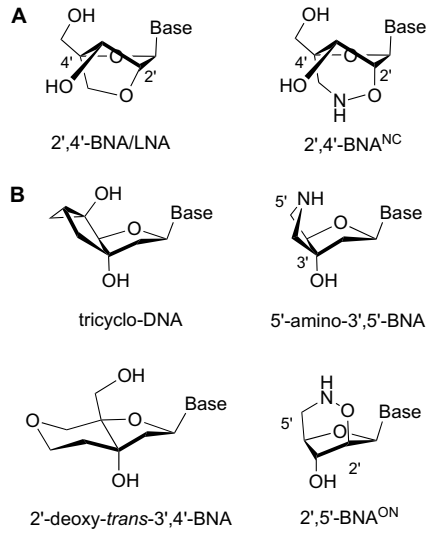
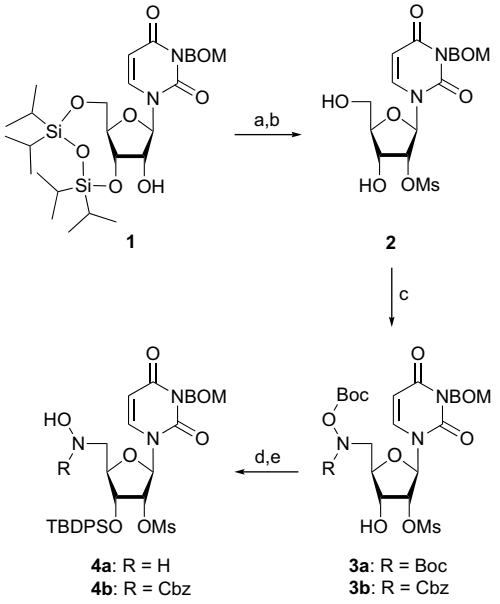


Figure 2. Examples of nucleic acid analogue possessing (A) N-type or (B) S-type sugar conformation.



Scheme 1. Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2, 0 °C; (b) TBAF, THF, 85% over two steps; (c) Boc-NH-*O*-Boc, Ph_3P, DBAD, THF 71% for **3a**, Cbz-NH-*O*-Boc, Ph_3P, DBAD, THF, 65% for **3b**; (d) TBDPSCl, imidazole, DMF; (e) TFA, CH_2Cl_2, 78% for **4a**; 64% for **4b** over two steps.

various types of conformationally restricted nucleic acid analogue,[4–9] and found that 2′,4′-BNAs (*b*ridged *n*ucleic *a*cids, often represented as LNA[10]) whose sugar conformation is fixed to N-type show high binding affinity with the RNA complement (Fig. 2A).[5–7] In particular, the 2′,4′-BNA^{NC}-modified oligonucleotides, which contain an *N*–*O* bond between the 2′- and 4′-positions, showed extremely high tolerance to nuclease and high binding affinity with RNA complement and double-stranded DNA (dsDNA).[7]

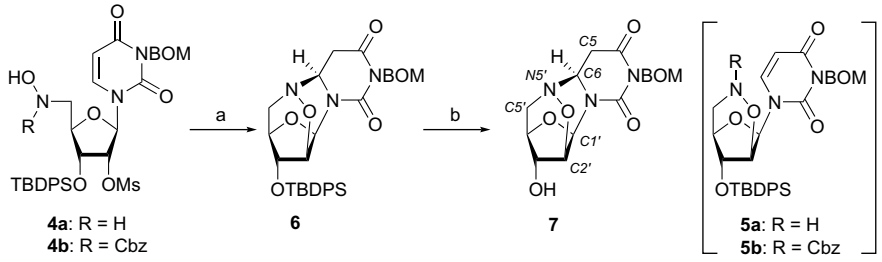
Although the unique characteristics of nucleic acid analogues with S-type sugar conformation have not yet been identified well, BNA with S-type sugar conformation are believed to behave as DNA analogues based on the good hybridizing abilities of tricyclo-DNA,[11] 5′-amino-3′,5′-BNA,[8] and 2′-deoxy-*trans*-3′,4′-BNA.[9] In these analogues, the sugar pucker of the furanose part is restricted to S-type (Fig. 2B). To compare with the hybridization ability of DNA analogues **II** and **III**, we designed a novel bridged nucleic acid **I**, 2′,5′-BNA^{ON}, with S-type sugar pucker and a carbamate linkage (Figs. 1 and 2B). A preliminary molecular modeling study suggested that the sugar puckering of this novel BNA would be rigidly restricted to S-type by an *N*–*O* bond between *C*2′ and *C*5′ and that its γ dihedral angle (*N*5′–*C*5′–*C*4′–*C*3′) would be ca. 15°. It is thought that these conformational characteristics are suitable for DNA duplex formation because these characteristics (e.g., S-type sugar pucker and moderate γ dihedral angle) are found mainly in crystal structures of natural dsDNA.[12]

2. Result and discussion

2.1. Synthesis of 2′,5′-BNA^{ON}

To evaluate the characteristics of 2′,5′-BNA^{ON}, we synthesized a uridine analogue 2′,5′-BNA^{ON}-U. Uridine derivative **1**,[13,14] which was prepared from uridine according to a known procedure was transformed to mesylated diol **2** over two steps (Scheme 1). The primary hydroxyl group of **2** was substituted by *N*,*O*-di-Boc-hydroxyamine under Mitsunobu reaction conditions[15] to afford *N*,*O*-di-Boc-5′-deoxy-5′-hydroxyaminouridine derivative **3a**. Protection of the hydroxyl group at the 3′-position with TBDPSCl and imidazole followed by the removal of Boc group on the hydroxyamino group using TFA gave compound **4a**. Next, the reaction to obtain compound **5a**, with a bridged structure between the 2′- and 5′-position of **4a**, was investigated under various reaction conditions (e.g., Et_3N, LDA, KHMDS). However, the only compound that could be identified was not the desired compound **5a** but compound **6** (Scheme 2). We attempted the bridging reaction using *N*-Cbz-protected 5′-deoxy-5′-hydroxyaminouridine derivative **4b** as well, but the desired 2′,5′-BNA^{ON} derivative **5b** was not obtained. The characteristic tetracyclic structure of **6** was determined by X-ray crystallographic analysis after derivatizing to compound **7**, which was provided by treating compound **6** with TBAF (Scheme 2 and Fig. 3).

In order to obtain 2′,5′-BNA^{ON} skeleton, we investigated the possibility of carrying out *N*5–*C*6 bond cleavage of **6** by *N*-alkylative β-elimination (Scheme 3. Table 1). First, a dichloromethane solution of **6** was refluxed in presence of benzyl bromide, but no



Scheme 2. Reagents and conditions: (a) KHMDS, THF, 0 °C, 53%; (b) TBAF, THF, 98%.

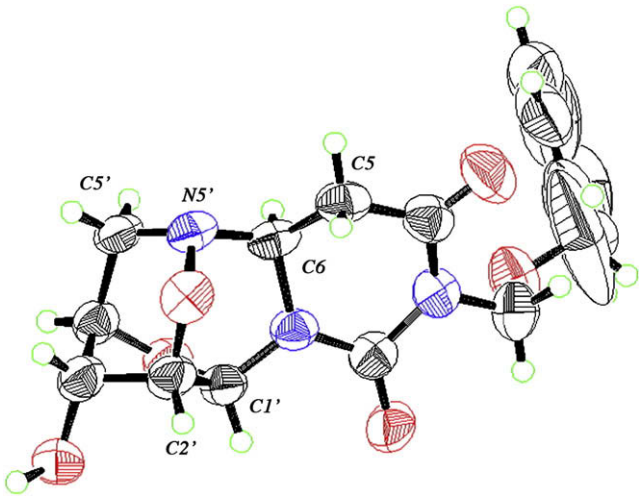
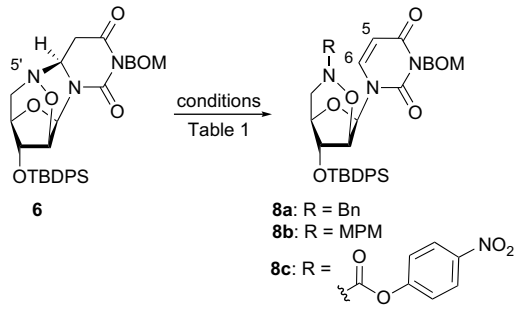


Figure 3. ORTEP plot of compound **7**.

reaction occurred (run 1). Higher-temperature conditions were effective for this reaction, and *N*5′-benzyl derivative **8a** was obtained in 18% and 46% yields, respectively, by refluxing in toluene (run 2) and *o*-xylene (run 3). It was found that benzyl bromide could be replaced by other electrophiles to give methoxybenzyl- and *p*-nitrophenoxycarbonyl derivatives **8b** (run 4) and **8c** (run 5). Evidence for the occurrence of these β-elimination reactions was provided by NMR spectra of **8** showing vinyl signals at the *C*5 and *C*6 positions of the uracil base.

For synthesis of the dimer unit **I** conjugated with a carbamate linker, we attempted to prepare the 5′-amino derivative **12** bearing a free secondary amino group as follows (Scheme 4). After the *N*3-BOM group of **6** was removed via **9** by hydrogenolysis and hydrolysis, the *N*5′–*C*6 bond of **10** was cleaved attending on methoxybenzylation to afford **11**. Unfortunately, treatment of **11** with DDQ induced addition of 5′-amino group to the *C*6 position, reforming **10**. These results suggested that the distance between *N*5′- and *C*6 position is too close to stand **5a** and/or **12**.

As an alternative method, synthesis of the dimer unit **I** with a carbamate structure was performed via compound **8c**, avoiding **12**. First, since the desired **8c** was not obtained efficiently under the conditions described in Table 1, the reaction conditions were optimized. It was found that addition of tetra-*n*-butylammonium iodide and 2,6-di-*tert*-butyl-4-methylpyridine to the reaction mixture worked well, allowing compound **8c** to be obtained in 55% yield (Scheme 5). Treatment with NaH in DMF resulted in a coupling reaction between **8c** and 3-*N*-benzyloxymethyl-5′-*O*-(4,4′-dimethoxytrityl)thymidine[16] to give the fully protected 2′,5′-BNA^{ON}-U dimer unit **13** successfully. After deprotections and the additional suitable protection, the phosphoramidite building block **17** was finally obtained by phosphitylation with a phosphorodiamidite



Scheme 3. β-Elimination reaction.

Table 1
β-Elimination reaction of 5′-amino group from uracil moiety

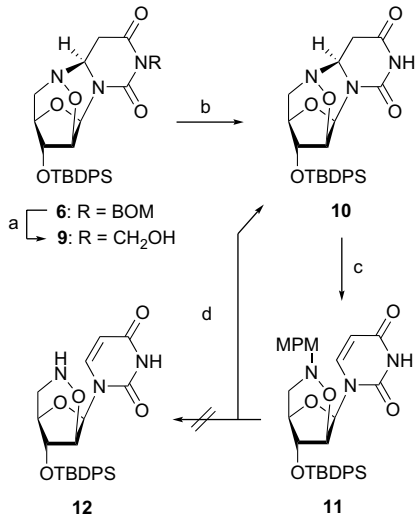
Run	Conditions[a]	Results
1	BnBr, CH_2Cl_2	No reaction
2	BnBr, toluene	**8a** (18%)[b]
3[b]	BnBr, *o*-xylene	**8a** (46%)[b]
4	MPMCl, *o*-xylene	**8b** (50%)[b]
5	*p*-Nitrophenyl chloroformate, *o*-xylene	**8c** (11%)[b]

[a] Reactions were performed under reflux conditions until there was no change on TLC profile.
[b] Isolated yield.

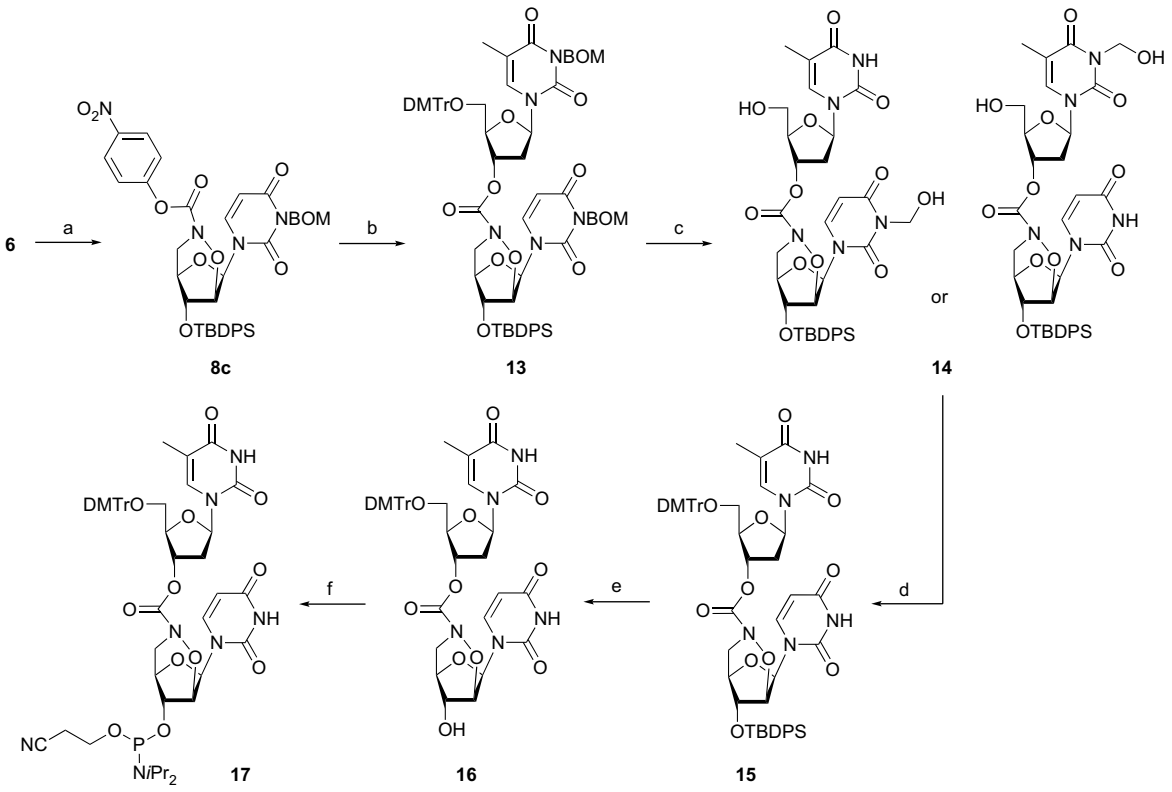
reagent. The 2′,5′-BNA^{ON}-U dimer unit **17** was incorporated into the center of an oligonucleotide **19** using an automated DNA synthesizer and standard phosphoramidite protocols. The modified oligonucleotide **19** was purified by HPLC, and its sequence composition was verified by MALDI-TOF mass spectroscopy.

2.2. Duplex-forming ability

The duplex-forming ability of oligonucleotide **19**, containing 2′,5′-BNA^{ON}, with complementary DNA (**20Dna**) and RNA (**20Rna**) was evaluated by means of UV melting experiments. Single modification of the oligonucleotide led to significant destabilization of its duplex DNA and RNA (ca. −30 °C, Table 2), and the modified oligonucleotides did not show any mismatch recognition abilities (data not shown). These results suggest that the uracil base of 2′,5′-BNA^{ON}-U could not pair with any bases on the opposite strand. One reason for the loss of its hydrogen bonding ability due to 2′,5′-BNA^{ON} modification might be steric and/or electrostatic repulsion between the 6-*H* atom of the thymine base and the 2′-*O* atom of the bridged structure: the torsion angle around glycoside bond χ would be fixed at an unsuitable direction for hybridizations. Nielsen and co-workers[17] reported that the torsion angle of a tricyclic nucleoside containing an *arabino*-type oxy group, deviated slightly from the values of around typically found for nucleosides with S-type conformation, and this resulted in strong destabilization of its duplexes. In addition, the replacement of a phosphate linkage with a carbamate linkage is another possible cause of the destabilization. 2′,5′-BNA^{ON} must have had less flexibility than the expected around the 5′-position, and the linkage described by torsion angle α may have been unfavorably restricted.



Scheme 4. Reagents and conditions: (a) cyclohexene, $Pd(OH)_2$, EtOH, reflux; (b) NaOH, THF, H_2O, THF, 75% over two steps; (c) MPMCl, *o*-xylene, reflux, 45%; (d) DDQ, CH_2Cl_2, H_2O, 0 °C, 50%.



Scheme 5. Reagents and conditions: (a) *p*-nitrophenyl chloroformate, TBAI, 2,6-di-*tert*-butyl-4-methylpyridine, *o*-xylene, reflux, 55%; (b) 3-*N*-BOM-5′-*O*-DMTr-thymidine, NaH, THF, 0 °C, 87%; (c) BCl_3, CH_2Cl_2, −78 °C, 84%; (d) DMTrCl, pyridine, 63%; (e) TBAF, THF, 97%; (f) $(iPr_2N)_2POCH_2CH_2CN$, diisopropylammonium tetrazolide, CH_3CN, THF, 78%.

Table 2
T_m Values of 2′,5′-BNA^{ON}-U oligonucleotides with complementary DNA and RNA[a,b]

Oligonucleotides	T_m Values (°C)	
	20Dna	**20Rna**
5′-d(GCGTTTTTTGCT)-3′ (**18**)	50	48
5′-d(GCGTT**TU**TTGCT)-3′ (**19**)	17 (−33)[c]	18 (−30)[c]

[a] Target sequence: 5′-d(AGCAAAAAACGC)-3′ (**20Dna**) and 5′-r(AGCAAAAAACGC)-3′ (**20Rna**).
[b] Conditions: 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl and 4 μM of each oligonucleotide strand.
[c] Parentheses showed difference between $T_m^{natural}$ and $T_m^{modified}$. **TU**: dimer unit **I** (structure was illustrated in Fig. 1).

3. Conclusion

We synthesized a novel bridged nucleic acid containing a uracil base, 2′,5′-BNA^{ON}-U, whose sugar puckering was fixed to S-type by an *N*–*O* linkage, and successfully incorporated the resulting dimer unit, linked by a carbamate moiety, into an oligonucleotide. The hybridization ability of the 2′,5′-BNA^{ON}-modified oligonucleotide with the complementary single strand DNA/RNA was found to be lower than that of the natural oligonucleotide.[18]

4. Experimental

4.1. Material and methods

Unless otherwise mentioned, all chemicals from commercial sources were used without further purifications. Acetonitrile (MeCN), dichloromethane (CH_2Cl_2), pyridine, and triethylamine (Et_3N) used in reactions were distilled from calcium hydride. *o*-Xylene was dried over calcium hydride, and was distilled. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride just before use. All reactions were performed under a nitrogen atmosphere. Melting points were measured on a Yanagimoto micro melting points apparatus and are uncorrected. ^1H, ^{13}C, and ^{31}P NMR spectra were recorded on JEOL EX-270 (^1H, 270 MHz; ^{13}C, 67.8 MHz) and GX-500 (^1H, 500 MHz; ^{31}P 202 MHz) instruments. Values for δ are in parts per million relative to tetramethylsilane or deuterated solvent as internal standard. ^{31}P NMR spectrum was recorded at 121.5 MHz with 85% H_3PO_4 as external standard. IR spectra were recorded on a JASCO FT/IR-200 spectrometer. FAB-Mass spectra were measured on a JEOL JMS-600 or JMS-700 mass spectrometer. Column chromatography was carried out using Fuji Silysia BW-127ZH, BW-300, and FL-100D. X-ray crystallography was carried out on a Rigaku R-AXIS RAPID-S instrument. DNA synthesis was performed using an Applied Biosystems Expedite™ 8909 synthesizer. MALDI-TOF-MS spectra were recorded in negative ion mode on Brucker Daltonics Autoflex II TOF/TOF instrument. UV melting experiments were carried out using a SHIMADZU UV-1650PC instrument.

4.2. 3-*N*-Benzyloxymethyl-2′-*O*-methanesulfonyluridine (2)

To a mixture of **1** (1.65 g, 2.71 mmol) and Et_3N (1.18 mL, 8.33 mmol) in CH_2Cl_2 (33 mL) was added MsCl (0.32 mL, 4.16 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 1 h. After addition of aqueous saturated $NaHCO_3$ to the reaction mixture, the contents were extracted with CH_2Cl_2. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated to give a crude mixture as a yellow oil. To a solution of the crude mixture in THF (29 mL) was added TBAF (1.0 M solution in THF, 5.77 mL, 5.77 mmol), and the solution was stirred at room temperature for 1 h. After concentration, the resulting crude mixture was purified by silica gel column chromatography ($CHCl_3$/EtOH=15:1) to afford **2** (1.02 g, 2.30 mmol, 85% over two steps) as a white powder. Mp 122–124 °C. $[\alpha]_D^{21}$ +33.5 (*c* 0.98, MeOH). IR ν_{max} (KBr): 1090, 1181, 1336, 1703, 2939, 3312 cm^{-1}. ^1H NMR (CD_3OD)

δ 3.24 (3H, s), 3.75 (1H, dd, J=2, 13 Hz), 3.94 (1H, d, J=13 Hz), 4.03 (1H, m), 4.33 (1H, dd, J=5, 6 Hz), 4.65 (2H, s), 5.09 (1H, dd, J=3, 5 Hz), 5.46 (2H, s), 5.73 (1H, d, J=8 Hz), 6.03 (1H, d, J=3 Hz), 7.24–7.32 (5H, m), 8.05 (1H, d, J=8 Hz). ^{13}C NMR ($CDCl_3$) δ_C 38.8, 61.1, 69.2, 71.5, 73.1, 82.7, 85.8, 89.6, 102.2, 128.5, 128.6, 129.2, 139.3, 140.8, 152.3, 164.5. MS (FAB): m/z 443 (MH^+). High-resolution MS (FAB): calcd for $C_{18}H_{23}O_9N_2S$ (MH^+) 443.1124, found: 443.1127.

4.3. 3-*N*-Benzyloxymethyl-5′-*N*,*O*-bis(*tert*-butoxycarbonyl)hydroxyamino-5′-deoxy-2′-*O*-methanesulfonyluridine (3a)

To a solution of **2** (1.78 g, 4.02 mmol) and Ph_3P (1.59 g, 6.03 mmol) in THF (25 mL) was added a solution of Boc-NH-*O*-Boc (2.14 g, 9.17 mmol) in THF (5 mL) and di-*tert*-butyl azodicarboxylate (1.56 g, 6.78 mmol) in THF (4 mL) at 0 °C, and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with EtOAc, washed with H_2O and brine, dried over Na_2SO_4, and evaporated to give a crude product, which was purified by silica gel column chromatography ($CHCl_3$ then $CHCl_3$/MeOH=50:1) to afford **3a** (1.88 g, 2.86 mmol, 71%) as a white solid. Mp 60–61 °C. $[\alpha]_D^{24}$ +38.3 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 1096, 1171, 1277, 1362, 1455, 1668, 1716, 1783, 2980, 3444 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.48 (9H, s), 1.52 (9H, s), 2.61 (1H, br s), 3.23 (3H, s), 3.99 (2H, m), 4.17–4.24 (2H, m), 4.68 (2H, s), 5.05 (1H, dd, J=2, 5 Hz), 5.46 (2H, s), 5.77 (1H, d, J=8 Hz), 5.84 (1H, d, J=2 Hz), 7.22–7.37 (5H, m), 7.51 (1H, d, J=8 Hz). ^{13}C NMR ($CDCl_3$) δ_C 27.6, 28.0, 38.9, 51.2, 69.7, 70.2, 72.3, 80.3, 81.6, 83.7, 85.8, 89.7, 102.2, 127.5, 127.6, 128.2, 137.6, 138.3, 150.6, 152.0, 155.4, 162.2. MS (FAB): m/z 658 (MH^+). High-resolution MS (FAB): calcd for $C_{28}H_{40}O_{13}N_3S$ (MH^+) 658.2282, found: 658.2261.

4.4. 3-*N*-Benzyloxymethyl-3′-*O*-*tert*-butyldiphenylsilyl-5′-deoxy-5′-hydroxyamino-2′-*O*-methanesulfonyluridine (4a)

TBDPSCl (2.00 mL, 7.69 mmol) was added to a solution of **3a** (3.54 g, 5.38 mmol) and imidazole (1.07 g, 15.7 mmol) in DMF (13.5 mL) at 0 °C, and the mixture was stirred at room temperature for 11 h. The reaction mixture was then diluted with Et_2O, washed with H_2O and brine, dried over $MgSO_4$, and was concentrated to give a crude product. This was dissolved in CH_2Cl_2 (50 mL), TFA (6.20 mL, 83.3 mmol) was added, and the mixture was stirred at room temperature for 4 h. The reaction mixture was basified with aqueous saturated $NaHCO_3$ and was extracted with EtOAc. The organic layer was washed with aqueous saturated $NaHCO_3$, H_2O and brine, dried over Na_2SO_4, and evaporated. The resulting crude mixture was purified by silica gel column chromatography (hexane/EtOAc=1:1 and 1:3) to afford **4a** (2.93 g, 4.21 mmol, 78% over two steps) as a white solid. Mp 54–56 °C. $[\alpha]_D^{24}$ +23.2 (c 0.97, $CHCl_3$). IR ν_{max} (KBr): 1104, 1176, 1355, 1672, 1717, 2938 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.12 (9H, s), 2.65 (1H, dd, J=5, 14 Hz), 2.81 (3H, s), 2.93 (1H, d, J=14 Hz), 4.20 (1H, d, J=4 Hz), 4.60 (1H, m), 4.64 (3H, s), 5.14 (1H, dd, J=5, 5 Hz), 5.45 (2H, s), 5.73 (1H, d, J=8 Hz), 5.95 (1H, d, J=5 Hz), 7.25–7.48 (12H, m), 7.65–7.72 (4H, m). ^{13}C NMR ($CDCl_3$) δ_C 19.4, 26.8, 38.1, 53.9, 70.2, 72.0, 72.0, 77.7, 82.6, 90.4, 102.5, 127.6, 127.7, 127.8, 127.9, 128.2, 130.1, 130.2, 132.2, 132.7, 135.7, 135.8, 137.6, 140.1, 150.9, 162.2. MS (FAB): m/z 696 (MH^+). High-resolution MS (FAB): calcd for $C_{34}H_{42}O_9N_3SSi$ (MH^+) 696.2411, found: 696.2430.

4.5. 5′-(*N*-Benzyloxycarbonyl-*O*-*tert*-butoxycarbonyl)-hydroxyamino-3-*N*-benzyloxymethyl-5′-deoxy-2′-*O*-methanesulfonyluridine (3b)

To a solution of **2** (500 mg, 1.13 mmol) and Ph_3P (445 mg, 1.70 mmol) in THF (5 mL) was added a solution of Cbz-NH-*O*-Boc (616 mg, 2.30 mmol) in THF (2.5 ml) and di-*tert*-butyl azodicarboxylate (391 mg, 1.70 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc, washed with H_2O and brine, dried over Na_2SO_4, and evaporated to give a crude product, which was purified by silica gel column chromatography ($CHCl_3$ then $CHCl_3$/MeOH=50:1) to afford **3b** (509 mg, 0.737 mmol, 65%) as an white solid. Mp 54–56 °C. $[\alpha]_D^{21}$ +18.5 (c 0.99, $CHCl_3$). IR ν_{max} (KBr): 1096, 1360, 1667, 1717, 3445 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 3.23 (3H, s), 3.50 (1H, d, J=15 Hz), 4.10 (1H, d, J=15 Hz), 4.21 (2H, m), 4.68 (2H, s), 5.05 (1H, d, J=3 Hz), 5.18 (1H, d, J=12 Hz), 5.24 (1H, d, J=12 Hz), 5.45 (2H, s), 5.69 (1H, d, J=8 Hz), 5.79 (1H, d, J=1 Hz), 7.24–7.41 (11H, m). ^{13}C NMR ($CDCl_3$) δ_C 27.5, 38.8, 51.5, 68.9, 69.8, 70.2, 72.2, 80.0, 81.4, 86.2, 89.9, 102.3, 127.6, 127.6, 127.9, 128.2, 128.4, 134.8, 137.6, 138.2, 150.6, 151.7, 156.0, 162.2. MS (FAB): m/z 692 (MH^+). High-resolution MS (FAB): calcd for $C_{31}H_{38}O_{13}N_3S$ (MH^+) 692.2125, found: 692.2106.

4.6. 5′-(*N*-Benzyloxycarbonyl)hydroxyamino-3-*N*-benzyloxymethyl-3′-*O*-*tert*-butyldiphenylsilyl-5′-deoxy-2′-*O*-methanesulfonyluridine (4b)

TBDPSCl (0.425 mL, 1.64 mmol) was added to a solution of **3b** (377 mg, 0.545 mmol) and imidazole (186 mg, 2.73 mmol) in DMF (3 mL) at 0 °C. After stirring at room temperature for 10 h, the reaction mixture was diluted with Et_2O, washed with H_2O and brine, dried over Na_2SO_4, and was concentrated to give a crude product. To a solution of the obtained product in CH_2Cl_2 (10 mL) was added TFA (1.1 mL) and the mixture was stirred at room temperature for 2 h. The reaction mixture was basified with aqueous saturated $NaHCO_3$ at 0 °C, and then extracted with CH_2Cl_2. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated. The resulting crude mixture was purified by silica gel column chromatography (hexane/EtOAc=2:3) to afford **4b** (291 mg, 0.352 mmol, 64% over two steps) as a white solid. Mp 47–50 °C. $[\alpha]_D^{24}$ +31.0 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 1105, 1355, 1457, 1671, 1715, 2938, 3292 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.03 (9H, s), 2.65 (3H, s), 3.27 (1H, dd, J=15, 5 Hz), 3.41 (1H, dd, J=15, 4 Hz), 4.14 (1H, d, J=8 Hz), 4.47 (1H, dd, J=4, 5 Hz), 4.54 (2H, s), 4.94 (1H, m), 4.95 (1H, d, J=12 Hz), 5.02 (1H, d, J=12 Hz), 5.33 (2H, s), 5.54 (1H, d, J=8 Hz), 5.87 (1H, d, J=6 Hz), 7.08–7.37 (17H, m), 7.54–7.61 (4H, m). ^{13}C NMR ($CDCl_3$) δ_C 19.4, 26.8, 37.9, 51.4, 68.2, 70.2, 71.9, 76.9, 82.7, 90.0, 102.6, 127.6, 127.7, 127.8, 127.9, 127.9, 128.2, 128.3, 128.4, 130.1, 130.3, 132.1, 132.4, 135.3, 135.7, 135.7, 137.5, 139.8, 151.1, 157.3, 162.1. MS (FAB): m/z 830 (MH^+). High-resolution MS (FAB): calcd for $C_{42}H_{48}O_{11}N_3SSi$ (MH^+) 830.2779, found: 830.2754.

4.7. (2*S*,8*R*,10*R*,11*S*,12*S*)-5-(Benzyloxymethyl)-11-*tert*-butyldiphenylsilyloxy-9,13-dioxa-1,5,7-triazatetracyclo-[8.3.1.0^{2,7}.0^{8,12}]tetradecane-4,6-dione (6)

To a solution of **4a** (1.42 g, 2.04 mmol) in THF (20 mL) at 0 °C was added KHMDS (0.5 M in toluene, 6.45 mL, 3.23 mmol), and the mixture was stirred at the same temperature for 30 min. Aqueous saturated NH_4Cl was added to the reaction mixture, and the resulting mixture was diluted with EtOAc and separated. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated. The obtained crude product was purified by silica gel column chromatography ($CHCl_3$) to afford **6** (655 mg, 1.09 mmol, 53%) as a white solid. Mp 184–186 °C. $[\alpha]_D^{24}$ −44.5 (c 0.98, $CHCl_3$). IR ν_{max} (KBr): 1111, 1274, 1357, 1444, 1681, 1728, 2861, 2939 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.08 (9H, s), 2.68 (1H, dd, J=4, 16 Hz), 2.91 (1H, d, J=14 Hz), 2.93 (1H, d, J=16 Hz), 3.32 (1H, d, J=14 Hz), 4.11–4.17 (3H, m), 4.22 (1H, d, J=4 Hz), 4.67 (2H, AB, J=13 Hz), 5.31 (1H, d, J=10 Hz), 5.41 (1H, d, J=10 Hz), 6.48 (1H, d, J=5 Hz), 7.24–7.48 (11H, m), 7.64–7.68 (4H, m). ^{13}C NMR ($CDCl_3$) δ_C 19.0, 26.7, 38.0, 60.2, 69.1, 70.2, 72.0, 75.2, 77.6, 80.4, 81.7, 127.4, 127.5, 127.9, 128.0, 128.4, 130.1, 130.2, 132.6, 132.9, 135.5, 138.5, 151.5, 168.2. MS (FAB): m/z 600

(MH^+). High-resolution MS (FAB): calcd for $C_{33}H_{38}O_6N_3Si$ (MH^+) 600.2530, found: 600.2521.

4.8. (2*S*,8*R*,10*R*,11*S*,12*S*)-5-(benzyloxymethyl)-11-hydroxy-9,13-dioxa-1,5,7-triazatetracyclo[8.3.1.0^{2,7}.0^{8,12}]tetradecane-4,6-dione (7)

To a solution of **6** (36.0 mg, 0.060 mmol) in THF (2 mL) was added TBAF (1.0 M solution in THF, 0.09 mL, 0.09 mmol), and the solution was stirred at room temperature for 30 min. After concentration of the reaction mixture, the resulting crude mixture was purified by silica gel column chromatography ($CHCl_3$ and $CHCl_3$/EtOH=15:1) to afford **7** (21.2 mg, 0.059 mmol, 98%) as a white solid. Mp 179–182 °C. $[\alpha]_D^{21}$ −17.7 (*c* 0.99, $CHCl_3$). IR ν_{max} (KBr): 1274, 1446, 1680, 1728, 2961, 3303 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.45 (1H, d, *J*=7 Hz), 2.68 (1H, dd, *J*=4, 16 Hz), 2.98 (1H, d, *J*=16 Hz), 3.08 (1H, dd, *J*=4, 14 Hz), 3.54 (1H, d, *J*=14 Hz), 4.09 (2H, m), 4.25 (1H, d, *J*=4 Hz), 4.33 (1H, d, *J*=5 Hz), 4.67 (2H, s), 5.31 (1H, d, *J*=10 Hz), 5.40 (1H, d, *J*=10 Hz), 6.35 (1H, d, *J*=5 Hz), 7.16–7.29 (5H, m). ^{13}C NMR ($CDCl_3$) δ_C 38.1, 60.2, 69.1, 70.3, 72.1, 73.7, 77.4, 79.8, 81.3, 127.4, 127.5, 128.3, 138.3, 151.5, 168.1. Some of the white solid **7** were recrystallized from acetone for X-ray crystallographic analysis.

Crystallographic data (excluding structure factors) of **7** has been deposited at the Cambridge Crystallographic Data Center as supplementary publication no. CCDC 704648. Copy of the data can be obtained, free of charge via www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi (or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

4.9. 5′-Benzylamino-3-*N*-benzyloxymethyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*,5′-*N*-*cyclo*-5′-deoxyarabinouridine (8a)

A mixture of **6** (32.5 mg, 50.1 mmol), BnBr (0.034 mL, 0.250 mmol) and *o*-xylene (0.4 mL) was refluxed for 48 h. The resulting mixture was mixed with aqueous saturated $NaHCO_3$ over an ice bath and was diluted with EtOAc. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated. The resulting crude product was purified by silica gel column chromatography ($CHCl_3$) to afford **8a** (16.0 mg, 0.0232 mmol, 46%) as colorless oil. $[\alpha]_D^{21}$ +58.6 (*c* 0.71, $CHCl_3$). IR ν_{max} (KBr): 1077, 1110, 1453, 1665, 1711, 2939 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.08 (9H, s), 2.28 (1H, d, *J*=12 Hz), 3.04 (1H, dd, *J*=6, 12 Hz), 3.48 (1H, d, *J*=12 Hz), 3.75 (1H, d, *J*=12 Hz), 4.07 (1H, d, *J*=2 Hz), 4.21 (1H, d, *J*=5 Hz), 4.65 (2H, s), 4.81 (1H, s), 5.34 (1H, d, *J*=10 Hz), 5.44 (1H, d, *J*=10 Hz), 5.67 (1H, d, *J*=8 Hz), 5.99 (1H, d, *J*=2 Hz), 7.04–7.69 (20H, m), 7.83 (1H, d, *J*=8 Hz). ^{13}C NMR ($CDCl_3$) δ_C 18.9, 26.8, 58.5, 62.4, 69.9, 71.9, 72.8, 79.3, 79.8, 89.5, 99.0, 122.4, 127.5, 127.6, 127.7, 127.8, 128.0, 128.1, 129.4, 129.9, 130.1, 130.1, 132.2, 133.0, 135.1, 135.4, 135.5, 137.7, 141.6, 150.7, 163.2. MS (EI): *m*/*z* 689 (M^+, 7), 400 (100). High-resolution MS (EI): calcd for $C_{40}H_{43}O_6N_3Si$ 689.2921, found: 689.2917.

4.10. 3-*N*-Benzyloxymethyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*,5′-*N*-*cyclo*-5′-deoxy-5′-(4-methoxyphenyl)methylaminoarabinouridine (8b)

A mixture of **6** (21.0 mg, 0.035 mmol), MPMCl (0.047 mL, 0.334 mmol), and *o*-xylene (0.3 mL) was refluxed for 22 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc, and washed with aqueous saturated $NaHCO_3$, H_2O and brine, dried over Na_2SO_4, and evaporated. The resulting crude product was purified by silica gel column chromatography (EtOAc/*n*-hexane=1:5 and 1:3) to afford **8b** (12.0 mg, 16.7 mmol, 50%) as a pale yellow oil. $[\alpha]_D^{25}$ +115.3 (*c* 0.80, $CHCl_3$). IR ν_{max} (KBr): 1078, 1110, 1249, 1452, 1665, 1710, 2941 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.08 (9H, s), 2.25 (1H, d, *J*=12 Hz), 3.00 (1H, dd, *J*=4, 12 Hz), 3.40 (1H, d, *J*=13 Hz), 3.72 (3H, s), 3.67–3.77 (2H, m), 4.06–4.13 (1H, m), 4.20 (1H, d, *J*=4 Hz), 4.66 (2H, s), 4.81 (1H, s), 5.33 (1H, d, *J*=10 Hz), 5.44 (1H, d, *J*=10 Hz), 5.64 (1H, d, *J*=8 Hz), 5.98 (1H, d, *J*=3 Hz), 6.69–6.99 (4H, m), 7.25–7.70 (15H, m), 7.79 (1H, d, *J*=8 Hz). ^{13}C NMR ($CDCl_3$) δ_C 19.1, 26.8, 55.1, 58.4, 61.5, 69.9, 71.9, 79.2, 79.9, 89.4, 98.9, 113.3, 126.9, 127.5, 127.7, 127.8, 127.9, 128.1, 130.0, 130.1, 130.7, 132.2, 133.1, 135.4, 135.5, 137.9, 141.4, 150.6, 158.9, 163.1. MS (FAB): *m*/*z* 720 (MH^+). High-resolution MS (FAB): calcd for $C_{41}H_{46}O_7N_3Si$ (MH^+) 720.3105, found: 720.3124.

4.11. (2*S*,8*R*,10*R*,11*S*,12*S*)-11-*tert*-butyldiphenylsilyloxy-9,13-dioxa-1,5,7-triazatetracyclo[8.3.1.0^{2,7}.0^{8,12}]tetradecane-4,6-dione (10)

A mixture of **6** (78.2 mg, 0.130 mmol), 20% $Pd(OH)_2$ on carbon (64.5 mg), cyclohexene (0.54 mL, 5.34 mmol), and ethanol (4 mL) was refluxed for 3.5 h. After cooling to room temperature, the reaction mixture was filtered, and the filtrate was concentrated to give a crude product. To a solution of the crude product was added an aqueous solution of NaOH (16 mg in 0.25 mL of H_2O) over an ice bath, and the resulting mixture was stirred for 2 h at the same temperature before being diluted AcOEt, washed with H_2O and brine, dried over Na_2SO_4, and evaporated. The resulting crude product was purified by silica gel column chromatography (EtOAc/*n*-hexane=1:2 and 1:1) to afford **10** (47.8 mg, 99.7 mmol, 75% over two steps) as a white solid. Mp 107–110 °C. $[\alpha]_D^{25}$ −45.8 (*c* 1.00, $CHCl_3$). IR ν_{max} (KBr): 1115, 1286, 1446, 1713, 2937, 3046, 3221 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.06 (9H, s), 2.75 (1H, dd, *J*=4, 17 Hz), 2.98 (1H, m), 3.04 (1H, dd, *J*=12, 17 Hz), 3.36 (1H, d, *J*=15 Hz), 4.11 (1H, m), 4.16 (1H, s), 4.27 (1H, d, *J*=5 Hz), 4.66 (1H, dd, *J*=4, 12 Hz), 6.46 (1H, d, *J*=5 Hz), 7.39–7.73 (10H, m). ^{13}C NMR ($CDCl_3$) δ_C 19.1, 26.8, 37.6, 60.3, 70.6, 75.2, 77.7, 79.6, 81.6, 127.8, 127.9, 130.0, 130.1, 132.4, 132.8, 135.4, 150.8, 168.4. MS (FAB): *m*/*z* 480 (MH^+). High-resolution MS (FAB): calcd for $C_{25}H_{30}O_5N_3Si$ (MH^+): 480.1955, found: 480.1931.

4.12. 3′-*O*-*tert*-Butyldiphenylsilyl-2′-*O*,5′-*N*-*cyclo*-5′-deoxy-5′-(4-methoxyphenyl)methylaminoarabinouridine (11)

A mixture of **10** (20.0 mg, 41.7 mmol), MPMCl (0.045 mL, 0.334 mmol), and *o*-xylene (0.3 mL) was refluxed for 32 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc, and washed with aqueous saturated $NaHCO_3$, H_2O and brine, dried over Na_2SO_4, and evaporated. The resulting crude product was purified by silica gel column chromatography (EtOAc/*n*-hexane=1:2) to afford **11** (11.0 mg, 18.3 mmol, 45%) as pale yellow oil. $[\alpha]_D^{24}$ +126.5 (*c* 1.01, $CHCl_3$). IR ν_{max} (KBr): 1112, 1271, 1462, 1686, 2945, 3045, 3165 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.08 (9H, s), 2.25 (1H, d, *J*=12 Hz), 3.01 (1H, dd, *J*=5, 12 Hz), 3.41 (1H, d, *J*=12 Hz), 3.74 (1H, d, *J*=12 Hz), 3.76 (3H, s), 4.07 (1H, s), 4.21 (1H, d, *J*=5 Hz), 4.76 (1H, s), 5.62 (1H, dd, *J*=2, 8 Hz), 6.00 (1H, d, *J*=3 Hz), 6.76 (2H, d, *J*=9 Hz), 7.00 (2H, d, *J*=9 Hz), 7.37–7.86 (10H, m), 7.85 (1H, d, *J*=8 Hz), 8.89 (1H, s). ^{13}C NMR ($CDCl_3$) δ_C 19.1, 26.8, 55.2, 58.1, 61.6, 79.2, 79.8, 89.1, 99.0, 113.5, 113.6, 126.9, 127.8, 127.9, 129.6, 130.0, 130.1, 130.7, 132.2, 132.9, 135.4, 135.5, 142.8, 149.9, 159.0, 163.7. MS (FAB): *m*/*z* 600 (MH^+). High-resolution MS (FAB): calcd for $C_{33}H_{38}O_6N_3Si$ (MH^+): 600.2530, found: 600.2512.

4.13. 3-*N*-Benzyloxymethyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*,5′-*N*-*cyclo*-5′-deoxy-(4-nitrophenoxy)carbonylaminoarabinouridine (8c)

A mixture of **7** (256 mg, 0.427 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (146 mg, 0.711 mmol), tetra-*n*-butylammonium iodide (27 mg, 0.072 mmol), 4-nitrophenyl chloroformate (347 mg, 1.72 mmol), and *o*-xylene (0.2 mL) was refluxed for 66 h. After

cooling to room temperature, the reaction mixture was diluted with EtOAc, and washed with aqueous saturated $NaHCO_3$, H_2O and brine, dried over Na_2SO_4, and evaporated. The resulting crude product was purified by silica gel column chromatography ($CHCl_3$) to afford **8c** (182 mg, 0.238 mmol, 55%) as a white solid. Mp 80–82 °C. $[\alpha]_D^{25}$ +67.0 (*c* 1.10, $CHCl_3$). IR ν_{max} (KBr): 1082, 1120, 1351, 1149, 1671, 1722, 2973 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.12 (9H, s), 3.17 (1H, d, *J*=13 Hz), 4.18 (1H, dd, *J*=5, 13 Hz), 4.27 (1H, d, *J*=1 Hz), 4.41 (1H, d, *J*=5 Hz), 4.59 (2H, s), 5.13 (1H, s), 5.47 (2H, s), 5.77 (1H, d, *J*=8 Hz), 6.09 (1H, d, *J*=3 Hz), 7.05 (2H, d, *J*=9 Hz), 7.26–7.73 (16H, m), 8.11 (2H, d, *J*=9 Hz). ^{13}C NMR ($CDCl_3$) δ_C 19.1, 26.7, 49.8, 69.9, 71.9, 75.7, 78.7, 80.2, 88.7, 100.6, 121.7, 125.1, 127.5, 127.5, 128.0, 128.1, 128.1, 130.4, 130.5, 131.5, 135.4, 137.4, 138.3, 145.0, 150.1, 154.6, 162.4. MS (FAB): *m*/*z* 765 (MH^+). High-resolution MS (FAB): calcd for $C_{40}H_{41}O_{10}N_4Si$ (MH^+) 765.2592, found: 765.2579.

4.14. 3-*N*-Benzyloxymethyl-5′-*O*-dimethoxytritylthymidylyl-(3′-5′-carbamoyl)-5′-amino-3-*N*-benzyloxymethyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*,5′-*N*-*cyclo*-5′-deoxyarabinouridine (13)

To a mixture of NaH (20.4 mg, 0.51 mmol) in THF (1 mL) was slowly added a solution of 3-*N*-benzyloxymethyl-5′-*O*-(4,4′-dimethoxytrityl)thymidine[16] (71.6 mg, 0.108 mmol) in THF (1.2 mL) over an ice bath, and the mixture was stirred at the same temperature for 30 min. A solution of **8c** (69.8 mg, 0.0913 mmol) in THF (1.5 mL) was added to the reaction mixture, and the mixture was stirred for further 3 h. The resulting mixture was diluted with Et_2O, washed with H_2O and brine, dried over Na_2SO_4, and evaporated. The resulting crude mixture was purified by silica gel column chromatography (EtOAc/*n*-hexane=1:2) to give **13** (104 mg, 0.0807 mmol, 87%) as a white solid. Mp 97–100 °C. $[\alpha]_D^{21}$ +25.6 (*c* 1.07, $CHCl_3$). IR ν_{max} (KBr): 1105, 1454, 1667, 1710, 2940 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.10 (9H, s), 1.36 (3H, s), 2.33 (2H, m), 3.02 (1H, d, *J*=12 Hz), 3.32 (1H, d, *J*=10 Hz), 3.41 (1H, d, *J*=10 Hz), 3.77 (7H, s), 3.92 (1H, s), 4.02 (1H, dd, *J*=4, 12 Hz), 4.17 (1H, s), 4.31 (1H, d, *J*=4 Hz), 4.67 (2H, s), 4.69 (2H, s), 5.00 (1H, s), 5.20 (1H, d, *J*=4 Hz), 5.46 (4H, s), 6.02 (1H, d, *J*=3 Hz), 6.24 (1H, t, *J*=8 Hz), 6.80–6.83 (4H, m), 7.20–7.69 (31H, m). ^{13}C NMR ($CDCl_3$) δ_C 12.3, 19.1, 26.8, 38.3, 49.9, 55.2, 63.7, 70.0, 70.7, 72.1, 75.8, 78.2, 78.7, 79.8, 83.7, 84.9, 87.4, 88.8, 110.9, 113.2, 127.1, 127.4, 127.5, 127.6, 127.9, 128.0, 128.1, 128.1, 128.2, 130.0, 130.3, 130.5, 131.5, 132.4, 134.0, 134.9, 135.1, 135.4, 135.4, 135.8, 137.7, 137.8, 138.3, 143.9, 150.4, 150.5, 150.8, 158.5, 158.5, 163.2, 163.2. MS (FAB): *m*/*z* 1312 (MNa^+). High-resolution MS (FAB): calcd for $C_{73}H_{75}O_{15}N_5SiNa$ (MNa^+) 1312.4952, found: 1312.4955.

4.15. Thymidylyl-(3′-5′-carbamoyl)-5′-amino-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*,5′-*N*-*cyclo*-5′-deoxy-3-*N*-hydroxymethylarabinouridine (14)

To a solution of **13** (96.5 mg, 0.0748 mmol) in CH_2Cl_2 (8 mL) at −78 °C was added a solution of BCl_3 (1 M in CH_2Cl_2, 0.748 mL, 0.748 mmol). After stirring for 30 min at −78 °C, MeOH was added to the reaction mixture at the same temperature. The resulting mixture was concentrated to give crude mixture, which was purified by silica gel column chromatography ($CHCl_3$/MeOH=15:1) to give **14** (48.7 mg, 0.0651 mmol, 84%) as a white solid. Mp 105–110 °C. $[\alpha]_D^{24}$ +79.6 (*c* 1.01, $CHCl_3$). IR ν_{max} (KBr): 1108, 1462, 1696, 2940, 3439 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.10 (9H, s), 1.90 (3H, s), 2.28 (2H, m), 3.03 (1H, d, *J*=13 Hz), 3.76 (1H, s), 3.79 (1H, d, *J*=3 Hz), 3.95 (1H, dd, *J*=2, 13 Hz), 4.07 (1H, m), 4.19 (1H, s), 4.36 (1H, m), 5.00 (1H, s), 5.06 (1H, s), 5.48 (2H, s), 5.67 (1H, d, *J*=8 Hz), 5.74 (1H, d, *J*=8 Hz), 6.05 (1H, dd, *J*=3, 13 Hz), 6.12 (1H, t, *J*=6 Hz), 7.42–7.53 (7H, m), 7.61–7.70 (5H, m), 9.35 (1H, br s). ^{13}C NMR ($CDCl_3$) δ_C 13.0, 19.1, 26.7, 37.6, 49.4, 62.2, 64.9, 65.6, 75.8, 78.8, 79.8, 84.6, 85.6, 88.3, 88.7, 100.4, 110.6, 128.0, 128.1, 130.3, 130.4, 131.5, 132.3, 135.0, 135.3, 135.3, 139.0, 140.2, 149.7, 150.3, 150.6, 150.6, 162.9, 163.3, 163.9, 164.2. MS (FAB): *m*/*z* 778 (MH^+). High-resolution MS (FAB): calcd for $C_{37}H_{44}O_{12}N_5Si$ (MH^+) 778.2756, found: 778.2726.

4.16. 5′-*O*-Dimethoxytritylthymidylyl-(3′-5′-carbamoyl)-5′-amino-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*,5′-*N*-*cyclo*-5′-deoxyarabinouridine (15)

To a solution of **14** (161 mg, 0.207 mmol) in pyridine (1.5 mL) was added 4,4′-dimethoxytrityl chloride (103 mg, 0.305 mmol), and the mixture was stirred at room temperature for 16 h. The reaction solution was mixed with aqueous $NaHCO_3$ over an ice bath and then extracted with AcOEt. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated. The resulting crude product was purified by silica gel column chromatography (EtOAc/*n*-hexane=1:1 and 2:1) to give **15** (137 mg, 0.130 mmol, 63%) as a white solid. Mp 160–163 °C. $[\alpha]_D^{24}$ +65.1 (*c* 0.98, $CHCl_3$). IR ν_{max} (KBr): 1108, 1260, 1456, 1696, 2942, 3063, 3198 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.99 (9H, s), 1.30 (3H, s), 2.28–2.39 (2H, m), 2.81 (1H, d, *J*=13 Hz), 3.33 (1H, d, *J*=8 Hz), 3.42 (1H, d, *J*=8 Hz), 3.69 (6H, s), 3.89 (1H, m), 3.96 (1H, s), 4.09 (1H, s), 4.27 (1H, d, *J*=4 Hz), 4.96 (1H, s), 5.00 (1H, s), 5.49 (1H, d, *J*=8 Hz), 5.97 (1H, d, *J*=3 Hz), 6.21 (1H, dd, *J*=5, 9 Hz), 6.72–6.75 (4H, m), 7.12–7.60 (21H, m), 10.26 (1H, br s), 10.59 (1H, br s). ^{13}C NMR ($CDCl_3$) δ_C 11.7, 19.0, 26.7, 37.5, 49.5, 55.2, 63.7, 75.8, 78.6, 78.8, 79.6, 83.9, 84.1, 87.1, 88.4, 100.8, 111.7, 113.2, 127.0, 127.8, 127.9, 128.0, 129.9, 130.2, 130.4, 131.5, 132.3, 135.0, 135.0, 135.1, 135.3, 135.3, 139.1, 144.0, 150.0, 151.2, 158.4, 158.4, 158.5, 163.1, 163.8. MS (FAB): *m*/*z* 1072 (MNa^+). High-resolution MS (FAB): calcd for $C_{57}H_{59}O_{13}N_5SiNa$ (MNa^+) 1072.3776, found: 1072.3798.

4.17. 5′-*O*-Dimethoxytritylthymidylyl-(3′-5′-carbamoyl)-5′-amino-2′-*O*,5′-*N*-*cyclo*-5′-deoxyarabinouridine (16)

To a solution of **16** (187 mg, 0.178 mmol) in THF (2 mL) was added TBAF (1.0 M solution in THF, 0.21 mL, 0.21 mmol), and the solution was stirred at room temperature for 15 min. After concentration of the reaction mixture under reduced pressure, the resulting crude mixture was purified by silica gel column chromatography (EtOAc/EtOH=15:1) to afford **16** (140 mg, 0.172 mmol, 97%) as a white powder. Mp 100–104 °C. $[\alpha]_D^{25}$ +74.7 (*c* 0.87, $CHCl_3$). IR ν_{max} (KBr): 1110, 1259, 1457, 1694, 2932, 3063, 3200 cm^{-1}. ^1H NMR (acetone-d_6) δ 1.25 (3H, s), 2.36 (1H, d, *J*=7 Hz), 2.37 (1H, d, *J*=7 Hz), 3.22 (1H, dd, *J*=3, 10 Hz), 3.33 (1H, dd, *J*=3, 10 Hz), 3.35 (1H, d, *J*=13 Hz), 3.66 (6H, s), 3.96 (1H, s), 4.17 (1H, dd, *J*=5, 13 Hz), 4.23 (1H, s), 4.58 (1H, d, *J*=5 Hz), 4.78 (1H, s), 5.04 (1H, br s), 5.19 (1H, s), 5.41 (1H, d, *J*=8 Hz), 5.69 (1H, d, *J*=3 Hz), 6.15 (1H, t, *J*=7 Hz), 6.76–6.81 (4H, m), 7.11–7.35 (11H, m), 7.52 (1H, d, *J*=1 Hz), 7.67 (1H, d, *J*=8 Hz), 10.12 (2H, br s). ^{13}C NMR (acetone-d_6) δ_C 11.4, 38.2, 50.0, 54.9, 64.0, 74.2, 77.7, 79.2, 80.1, 83.9, 84.6, 87.1, 88.3, 100.0, 110.6, 113.3, 127.0, 128.0, 128.2, 130.2, 130.2, 135.2, 135.5, 135.6, 140.2, 144.9, 150.1, 150.7, 158.9, 158.9, 163.3. MS (FAB): *m*/*z* 834 (MNa^+). High-resolution MS (FAB): calcd for $C_{41}H_{41}O_{13}N_5Na$ (MNa^+) 834.2599, found: 834.2589.

4.18. 5′-*O*-Dimethoxytritylthymidylyl-(3′-5′-carbamoyl)-5′-amino-2′-*O*,5′-*N*-*cyclo*-5′-deoxy-3′-*O*-(*N*,*N*-diisopropylamino-β-cyanoethoxyphosphino)arabinouridine (17)

2-Cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.07 mL, 0.219 mmol) was added dropwise to a mixture of **16** (105 mg, 0.130 mmol) and *N*,*N*-diisopropylammonium tetrazolide (30.4 mg, 0.151 mmol) in a mixed solution of MeCN and THF (3:1, v/v, 1.6 mL) at room temperature. After stirring for 18 h, the solvent was evaporated and the product was purified by silica gel column chromatography (EtOAc/*n*-hexane=2:1) followed by trituration from *n*-hexane to afford **17** (102 mg, 0.101 mmol, 78%) as a white

powder. Mp 129–133 °C. ^{31}P NMR ($CDCl_3$) δ_P 149.24, 150.88. MS (FAB): *m*/*z* 1034 (MNa^+). High-resolution MS (FAB): calcd for $C_{50}H_{58}O_{14}N_7PNa$ (MNa^+) 1034.3677, found: 1034.3734.

4.19. Synthesis of oligodeoxynucleotides

Synthesis of oligodeoxynucleotides **19** was carried out on a 0.2 mmol scale using commercially available 2-cyanoethyl phosphoramidites, compound **17** (0.1 M in MeCN), and dicyanoimidazole as an activator. The synthesis followed the regular protocol for DNA synthesis: however, for compound **17** a prolonged coupling time of 30 min was used. The 5′-*O*-DMTr-*Off* oligonucleotides were cleaved from the solid support by treatment with concd ammonia at room temperature for 1.5 h, and the protecting groups were removed by heating to 70 °C for 3 h. Purification was carried out using disposable gel filtration cartridges (NAP™-10 column from GE Healthcare) followed by HPLC (Waters XTerra® MS C_{18} 2.5 mm, 50 °C) to afford the pure oligonucleotide **19**. MALDI-TOF-MS using a 1:1 mixture of 3-hydroxypicolinic acid and diammonium hydrogen citrate as the matrix gave the following results: calcd for **19** 3594.62 (MH^-), found 3594.65.

4.20. UV melting experiments

UV melting experiments were carried out in a medium salt buffer containing 10 mM sodium phosphate (pH 7.2) and 100 mM NaCl using 4 mM concentrations of the two complementary sequences. The increase in absorbance at 260 nm as a function of time was recorded while the temperature was raised linearly from 5 to 80 °C at a rate of 0.5 °C min^{-1}. The melting temperature was determined as the local maximum of the first derivatives of the absorbance versus temperature curve.

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