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SYNTHESIS OF A NOVEL *TRANS*-3',4'-BNA MONOMER BEARING A 4,8-DIOXA-5-AZABICYCLO[5.3.0]DECANE SKELETON

Tetsuya Kodama, Kensaku Sugaya, Takeshi Baba, Takeshi Imanishi, and Satoshi Obika*

Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan
E-mail: obika@phs.osaka-u.ac.jp

Abstract – A novel *trans*-3',4'-BNA monomer, in which sugar conformation was restricted to S-type by a *trans*-fused 3-oxa-4-azapentylene bridge between C3' and C4' position, was designed and synthesized. The *trans*-fused 7-membered cyclic structure within a 4,8-dioxa-5-azabicyclo[5.3.0]decane skeleton was prepared by means of intramolecular substitution reaction using potassium carbonate as a base. We found that all of protecting groups (benzyl, benzyloxymethyl, and benzyloxycarbonyl group) were able to be removed smoothly by DDQ oxidation followed by boron trichloride treatment without cleavage of N-O linkage to afford the desired *trans*-3',4'-BNA monomer.

INTRODUCTION

Chemically modified nucleic acid analogues that interact strongly with a complementary strand are of interest in medicinal chemistry and genome technology.[1,2] We have synthesized a series of 2',4'-*O*,4'-*C*-bridged nucleic acids (2',4'-BNAs[3–6]/LNA[7]), of which sugar puckering is exactly restricted to N-type, and have shown that they confer high binding affinity towards complementary RNA strands and superior resistance to enzymatic digestion.

Oligonucleotides containing a nucleoside analogue with S-type sugar puckering are expected to form stable B-type duplexes with complementary DNA and act as DNA mimics, and are also fascinating materials due to their potential applications in such technologies as DNA microarray[8] and decoy strategy.[9] Therefore, nucleoside analogues bearing a restricted S-type sugar conformation have been designed and synthesized to date, and some of them were introduced into oligonucleotides and the hybridizing properties were evaluated.[10-16] However, these oligonucleotide analogues showed only small increase or

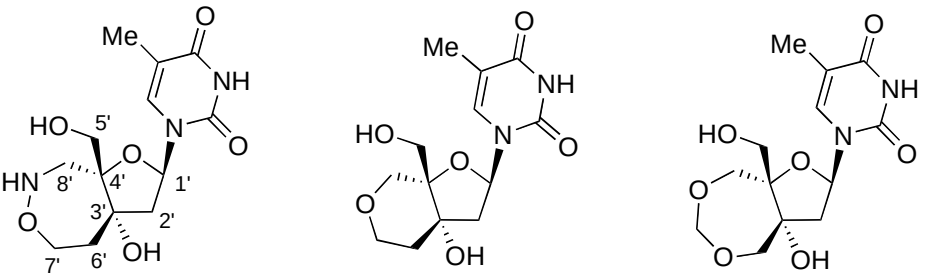
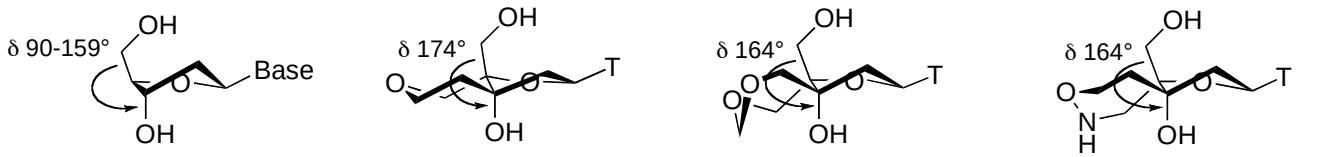


Figure 1. Structure of *trans*-3',4'-BNA analogues.

considerable decrease in the duplex stability, probably due to unfavorable restriction of the sugar conformation and/or steric repulsion caused by the additional structural components.

Recently, we synthesized several nucleic acid analogues with S-type sugar puckering, a series of *trans*-3',4'-BNA,[17-19] which have an additional *trans*-fused ring between C3' and C4' (Figure 1). In previous work, we showed that 2'-deoxy-*trans*-3',4'-BNA monomer (**2**) was successfully incorporated into oligonucleotide, and the oligonucleotide formed duplexes with DNA and RNA complements as stable as natural DNA did.[19] Because of the possibility of creating superior artificial S-type nucleic acids by fixing the sugar conformation but employing a more appropriate bridge structure, we reconsidered the sugar restriction strategy. First, because it was expected that the entropic advantage of conformational fixing during duplex formation was canceled out by unfavorable interactions, e.g. disturbance of the hydration network due to the additional hydrophobic bridge structure of **2**, we planned to use a relatively hydrophilic bridge structure for sugar restriction. Second, bridge size would be enlarged for fine adjustment of sugar conformation. In B-type DNA duplex, S-type sugar conformation[20] was mainly found and the pseudorotation phase angle (*P*)[21] and a dihedral angle (δ) were in range of 86°-189° and 90°-159°, respectively (Figure 2).[22] On the other hand, the 2'-deoxy-*trans*-3',4'-BNA monomer (**2**) showed small difference in these numbers; 194° (*P*) and 174° (δ).[19] In previous study, X-ray crystallography revealed that the *P* (174°) and δ (164°) of **3** with a 7-membered-bridge structure was similar with those of B-form



B-form DNA[22]
mainly S-type
(*P* = 86 to 189°)

crystal structure of **2**[19]
S-type
(C3'-*exo*, *P* = 194°)

crystal structure of **3**[17]
S-type
(C2'-*endo*, *P* = 174°)

calculated structure of **1**
S-type
(C2'-*endo*, *P* = 173°)

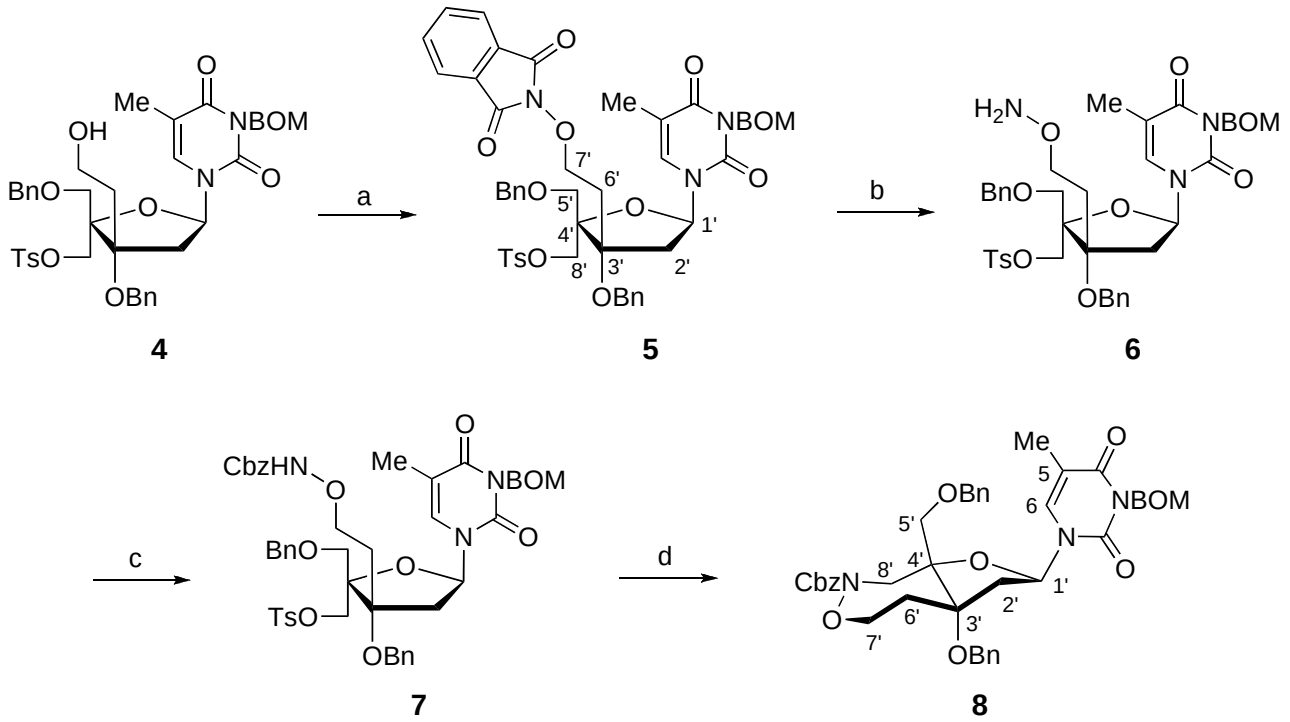
Figure 2. Selected parameters in sugar conformation. T; thymin-1-yl.

DNA.[17] Based on the results, we here designed a novel *trans*-3',4'-BNA monomer **1** bearing a relatively hydrophilic 3-oxa-4-azapentylene bridge between C3' and C4' position (Figure 1). An *ab initio* calculation using 6-31G* basis set for the *trans*-3',4'-BNA monomer **1** estimated the *P* and δ value to be 173° and 164°, which met our expectation (Figure 2).

In this paper, we describe the synthesis of a novel *trans*-3',4'-BNA monomer **1**, via a cyclization reaction using potassium carbonate to form a *trans*-fused 7-membered bridge structure, 1,2-oxazepane.

RESULTS AND DISCUSSION

The desired *trans*-3',4'-BNA **1** was synthesized from compound **4**, which was prepared from thymidine according to previously reported methods.[19] Compound **4** was treated under Mitsunobu reaction conditions using *N*-hydroxyphthalimide in THF to give phthalimide **5** in 90% yield (Scheme 1). The obtained phthalimide **5** was converted to a primary amine **6** using hydrazine hydrate. First, we attempted the bridge-forming reaction of amine **6** under various basic conditions, but the *trans*-3',4'-BNA structure did not obtained. Previously, in synthesis of 2',4'-BNA^{NC}, acylation or carbamation of its primary amino group was effective for the similar cyclization reaction to form the corresponding bridged structure whereas the cyclization of a naked amine derivative was failed.[6] Based on this result, the amino group of **6** was protected with benzyloxycarbonyl (Cbz) group to give compound **7**. Then, a cyclization reaction of

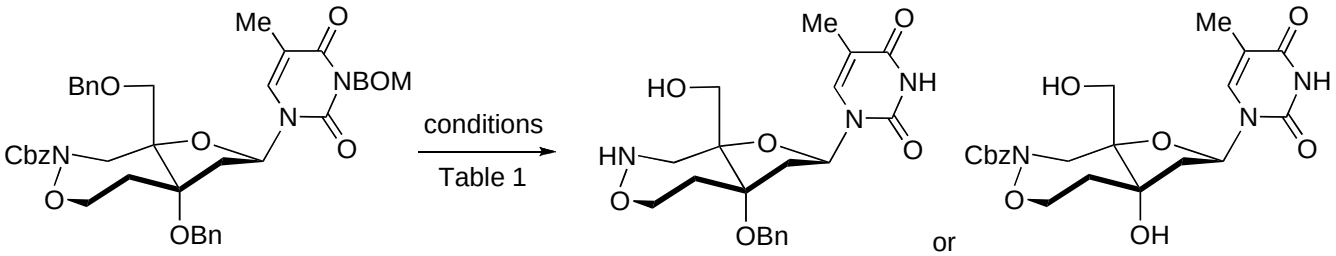


4 **5** **6** **7** **8**

Scheme 1. *Reagents and conditions*: a) *N*-hydroxyphtalimide, Ph_3P, DEAD, THF, rt, 90%; b) hydrazine hydrate, CH_2Cl_2, rt, 93%; c) CbzCl, pyridine, rt, 98%; d) K_2CO_3, DMF, 80 °C, 67%.

7 was investigated, with the result that the intramolecular substitution reation proceeded by treatment with K_2CO_3 in DMF at 80 °C to form compound **8** bearing the desired bridged structure in 67% yield. Neither $CsCO_3$, sodium hexamethyldisilazide, nor sodium hydride gave sufficient results.

Deprotection conditions for a fully-protected *trans*-3',4'-BNA **8** was next investigated, and the results were shown in Table 1. Reductive de-benzylation was attempted initially. Compound **8** was subjected to palladium hydroxide and ammonium formate (Run 1) or hydrogen gas (Run 2), but thymine base and N-O bond were found to undergo decomposition. Neither sodium in liquid ammonia (Run 3) nor lithium naphthalenide in THF (Run 4) gave the corresponding deprotected compounds. Acidic conditions were next examined. Whereas methanesulfonic acid (MsOH) as a strong Brønsted acid (Run 5) and timethylsilyl iodide (TMSI) as a Lewis acid (Run 6) did not produce the desired compound, use of boron trichloride at 0 °C afforded compound **9** on which a benzyl group at the *O*-3' position remained in 19% yield (Run 7). Oxidative deprotection of **8** was attempted with excess 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in wet dichloromethane under reflux.[23] In this conditions, Cbz-protected compound **10** was obtained in good yield, although the reaction took a lot of



8 **9** **10**

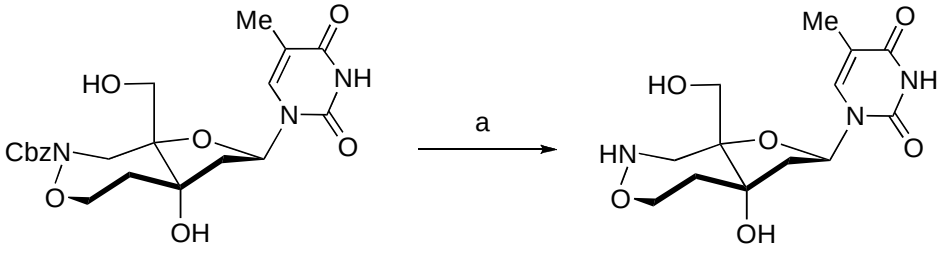
Table 1. Deprotection reaction of compound **8**.

Run	Conditions	Result (isolated yield)
1	HCO_2NH_4, $Pd(OH)_2$-C, EtOH, reflux	decomposition[a]
2	H_2, $Pd(OH)_2$-C, EtOH, rt	decomposition[a]
3	Na, NH_3, –78 °C	no reaction
4	lithium-naphthalenide, THF, –25 °C	complex mixture[b]
5	MsOH, CH_2Cl_2, –78 °C to reflux	complex mixture[b]
6	TMSI, CH_2Cl_2, –78 °C to rt	decomposition[a]
7	BCl_3, CH_2Cl_2, –78 °C to 0 °C	**9** (19%)
8	DDQ, wet CH_2Cl_2, reflux, 3 days	**10** (64%)
9	DDQ, wet $ClCH_2CH_2Cl$, reflux, 6 h	**10** (57%)

[a]Thymine base and N-O bond were found to undergo decomposition. [b]A complex mixture of many nucleoside analogues and unknown compounds was obtained.

time to be completed (Run 8). By using wet 1,2-dichloroethane as a solvent in place of dichloromethane, the oxidative deprotection reaction proceeded smoothly to give **10** in a moderate yield (Run 9).

We finally accomplished synthesis of the desired *trans*-3',4'-BNA **1** by treatment of compound **10** with boron trichloride in dichloromethane at 0 °C. The ^{1}H NMR spectrum of **1** showed similar features to that of compound **3**; e.g. H-1' (δ 6.32, dd, J = 6 and 9 Hz) for **1** and (δ 6.32, dd, J = 6 and 9 Hz) for **3**.[19] This suggests the newly designed *trans*-3',4'-BNA **1** is restricted to S-type conformation as expected by *ab initio* calculation.



10 **1**

Scheme 2. *Reagents and conditions*: (a) BCl_3, CH_2Cl_2, 0 °C, 70%.

CONCLUSION

We designed a novel *trans*-3',4'-BNA monomer bearing a 4,8-dioxa-5-azabicyclo[5.3.0]decane skeleton which consists of a S-type-restricted sugar moiety and a relatively hydrophilic *trans*-fused bridge structure. The *trans*-fused 7-membered cyclic structure was prepared by intramolecular substitution reaction of Cbz-protected amino group. Deprotection of fully protected *trans*-3',4'-BNA monomer was performed by subjection to DDQ followed by boron trichloride.

EXPERIMENTAL

Unless otherwise mentioned, all chemicals from commercial sources were used without further purification. Acetonitrile (MeCN), 1,2-dichloroethane, dichloromethane (CH_2Cl_2), and pyridine used in reaction were distilled from calcium hydride. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride just before use. All reactions were performed under an atmosphere of nitrogen. Melting points were measured on a Yanagimoto micro melting points apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on JEOL EX-270 (^{1}H, 270 MHz; ^{13}C, 67.8 MHz), GX-500 (^{1}H, 500 MHz), or Varian INOVA-600 (^{13}C, 150.8 MHz) instruments. Values for δ are in ppm relative to tetramethylsilane or deuterated solvent as internal standard. The numbering used for NMR assignments is shown in Scheme 1. The IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. The 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, FL-100D silica gel for normal phase, and Nacalai Tesque Cosmosil 75C_{18}-OPN for reverse phase.

3',5'-Di-*O*-benzyl-3-*N*-benzyloxymethyl-3'-*C*-[2-(phthalimidyloxy)ethyl]-4'-*C*-(tosyloxymethyl)thymidine (5). To a solution of **4**[19] (454 mg, 0.589 mmol) in THF (10 mL) was added *N*-hydroxyphthalimide (115 mg, 0.707 mmol) and Ph_3P (185 mg, 0.707 mmol) at rt, and the resulting mixture was stirred for 15 min. Diethyl azodicarboxylate (2.2 M in toluene, 0.320 mL, 0.71 mmol) was added to the reaction mixture on an ice-cooled bath. After stirred for 20 min, the resulting mixture was concentrated under reduced pressure. The obtained crude mixture was separated between Et_2O and H_2O, and the organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography [hexane:AcOEt (2:3)] to give **5** (485 mg, 90%) as white powder: mp 58–61°C. $[\alpha]_D^{23}$ –3.7 (*c* 1.00, $CHCl_3$). IR ν_{max} (KBr): 1095, 1177, 1273, 1363, 1464, 1495, 1599, 1666, 1709, 1734, 1789, 2925, 3031 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.51 (3H, d, 5-*Me*, *J* = 1 Hz), 2.21 (1H, dd, H-2', *J* = 10, 14 Hz), 2.29 (3H, s, tosyl-*Me*), 2.33-2.56 (2H, m, H-6'), 2.80 (1H, dd, H-2', *J* = 5, 14 Hz), 3.78 (2H, s), 4.10 (1H, d, OCH_2, *J* = 10 Hz), 4.15-4.36 (2H, m, H-7'), 4.32 (1H, d, OCH_2, *J* = 10 Hz), 4.49 (1H, d, OCH_2, *J* = 11 Hz), 4.50 (2H, s), 4.57 (2H, s), 4.61 (1H, d, OCH_2, *J* = 11 Hz), 5.36 (2H, s, NCH_2O), 6.12 (1H, dd, H-1', *J* = 5, 10 Hz), 7.10-7.31 (17H, m), 7.51 (1H, d, H-5, *J* = 1 Hz), 7.63-7.80 (6H, m). ^{13}C-NMR ($CDCl_3$):δ 12.9, 21.7, 30.5, 40.0, 64.6, 70.0, 70.4, 71.5, 72.1, 73.8, 74.0, 83.9, 85.8, 88.4, 110.0, 123.5, 127.0, 127.5, 127.5, 127.6, 128.0, 128.1, 128.4, 128.6, 128.7, 129.6, 132.0, 134.3, 134.5, 136.5, 136.9, 137.8, 144.8, 150.8, 163.1, 163.2. FAB-MS: *m/z* 916 (MH^+). High-resolution FAB-MS: Calcd for $C_{50}H_{50}N_3O_{12}S$ (MH^+): 916.3125. Found: 916.3115.

3'-*C*-[2-(Aminoxy)ethyl]-3',5'-di-*O*-benzyl-3-*N*-benzyloxymethyl-4'-*C*-(tosyloxymethyl)thymidine (6). To a solution of **5** (485 mg, 0.505 mmol) in CH_2Cl_2 (5 mL) was added hydrazine monohydrate (32 mg, 0.64 mmol) at rt, and the resulting mixture was stirred for 15 min. The resulting mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography [hexane:AcOEt (1:1)] to give **6** (367 mg, 93%) as white powder: mp 39 – 41 °C. $[\alpha]_D^{24}$ –13.2 (*c* 1.00, MeOH). IR ν_{max} (KBr): 1094, 1176, 1274, 1362, 1455, 1496, 1597, 1667, 1709, 2925, 3031, 3064, 3322 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.57 (3H, d, 5-*Me*, *J* = 1 Hz), 2.20 (1H, dd, H-2', *J* = 10, 14 Hz), 2.24-2.31 (2H, m, H-6'), 2.41 (3H, s, tosyl-*Me*), 2.69 (1H, dd, H-2', *J* = 5, 14 Hz), 3.72-3.86 (2H, m, H-7'), 3.88 (1H, d, *J* = 11 Hz), 3.95 (1H, d, *J* = 11 Hz), 4.16 (1H, d, *J* = 11 Hz), 4.37 (1H, d, *J* = 11 Hz), 4.40 (1H, d, *J* = 11 Hz), 4.48 (1H, d, *J* = 11 Hz), 4.55 (1H, d, *J* = 11 Hz), 4.64 (1H, d, *J* = 11 Hz), 4.64 (2H, s, PhCH_2O), 5.42 (2H, s, NCH_2O), 6.17 (1H, dd, H-1', *J* = 5, 10 Hz), 7.17-7.58 (15H, m), 7.62-7.74 (5H, m). ^{13}C-NMR ($CDCl_3$): δ 13.0, 21.7, 30.9, 40.9, 65.1, 71.8, 72.2, 73.1, 74.6, 85.4, 87.8, 89.8, 110.5, 128.2, 128.4, 128.5, 128.5, 128.7, 128.9, 129.1, 129.1, 129.4, 129.5, 130.8, 133.6, 135.2, 136.5, 138.7, 139.1,

139.4, 146.4, 152.0, 164.9. FAB-MS: *m/z* 786 (MH^+). High-resolution FAB-MS: Calcd for $C_{42}H_{48}N_3O_{10}S$ (MH^+): 786.3052. Found: 786.3060.

3',5'-Di-*O*-benzyl-3'-*C*-[2-(*N*-benzyloxycarbonylaminoxy)ethyl]-3-*N*-benzyloxymethyl-4'-*C*-(tosyloxymethyl)thymidine (7). To a solution of **6** (340 mg, 0.434 mmol) in pyridine (8.7 mL) was added benzyl chloroformate (89.0 mg, 0.521 mmol) on an ice-cooled bath, and the resulting mixture was stirred for 20 min at rt. After addition of saturated aqueous $NaHCO_3$, the mixture was extracted with AcOEt. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography [hexane:AcOEt (2:1)] to give **7** (390 mg, 98%) as white powder: mp 43–46 °C. $[\alpha]_D^{24}$ +2.4 (*c* 1.00, $CHCl_3$). IR ν_{max} (KBr): 1096, 1176, 1242, 1362, 1455, 1496, 1599, 1666, 1709, 1748, 2887, 2956, 3032, 3064, 3268 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.56 (3H, s, 5-*Me*), 2.19 (1H, dd, H-2', *J* = 10, 14 Hz), 2.23-2.45 (2H, m, H-6'), 2.39 (3H, s, tosyl-*Me*), 2.70 (1H, dd, H-2', *J* = 5, 14 Hz), 3.80 (1H, d, *J* = 11 Hz), 3.90 (1H, d, *J* = 11 Hz), 4.02-4.13 (2H, m, H-7'), 4.09 (1H, d, *J* = 10 Hz), 4.35 (1H, d, *J* = 10 Hz), 4.35 (1H, d, *J* = 11 Hz), 4.47 (1H, d, *J* = 11 Hz), 4.49 (1H, d, *J* = 11 Hz), 4.61 (1H, d, *J* = 11 Hz), 4.64 (2H, s, PhCH_2O), 5.17 (2H, s, PhCH_2O), 5.42 (2H, s, NCH_2O), 6.17 (1H, dd, H-1', *J* = 5, 10 Hz), 7.16-7.35 (22H, m), 7.58 (1H, s, H-6), 7.66-7.70 (3H, m). ^{13}C-NMR ($CDCl_3$): δ 13.2, 21.8, 30.5, 40.4, 65.1, 67.7, 71.4, 71.5, 71.7, 72.5, 72.7, 74.2, 84.8, 87.2, 89.1, 110.3, 128.2, 128.3, 128.4, 128.5, 128.6, 128.8, 128.9, 129.1, 129.4, 129.4, 129.5, 130.8, 133.1, 135.5, 137.3, 138.4, 139.0, 139.4, 146.3, 151.8, 158.0, 164.0. FAB-MS: *m/z* 920 (MH^+). High-resolution FAB-MS: Calcd for $C_{50}H_{54}N_3O_{12}S$ (MH^+): 920.3428. Found: 920.3417.

3',5'-Di-*O*-benzyl-3'-*C*,4'-*C*-(*N*-benzyloxycarbonyl-3-oxa-4-azapentylene)-3-*N*-benzyloxymethylthymidine (8). A mixture of **7** (78 mg, 0.085 mmol) and K_2CO_3 (94 mg, 0.68 mmol) in DMF (3 mL) was stirred for 48 h at 80 °C. After neutralization with diluted aqueous HCl, the mixture was extracted with AcOEt. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [hexane:AcOEt (5:2)] to give **8** (35 mg, 67%) as white powder: mp 49–52 °C. $[\alpha]_D^{23}$ –27.7 (*c* 1.00, $CHCl_3$). IR ν_{max} (KBr): 1183, 1211, 1262, 1300, 1362, 1412, 1453, 1496, 1659, 1707, 2871, 2950, 3031, 3064 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.61 (3H, s, 5-*Me*), 2.00-2.18 (2H, m, H-6'), 2.37 (1H, dd, H-2', *J* = 9, 14 Hz), 2.81 (1H, dd, H-2', *J* = 5, 14 Hz), 3.39 (1H, d, *J* = 12 Hz), 3.63 (1H, d, *J* = 11 Hz), 4.06 (2H, brs, H-7'), 4.20 (1H, d, *J* = 12 Hz), 4.20 (1H, d, *J* = 11 Hz), 4.30 (1H, d, *J* = 11 Hz), 4.52 (2H, s, PhCH_2O), 4.54 (1H, d, *J* = 11 Hz), 4.60 (2H, s, PhCH_2O), 4.85 (1H, d, *J* = 12 Hz), 5.04 (1H, d, *J* = 12 Hz), 5.38 (2H, s, NCH_2O), 6.30 (1H, dd, *J* = 5, 9 Hz), 7.16-7.30 (20H, m), 7.73 (1H, s, H-6). ^{13}C-NMR ($CDCl_3$): δ 13.8, 22.2, 29.3, 39.6, 52.0, 64.2, 68.3, 71.1, 72.8, 74.5, 84.8, 84.9, 86.6, 87.0, 89.8, 110.2, 127.7, 128.1, 128.2, 128.3, 128.6, 128.7, 128.9, 128.9, 129.1, 129.3, 134.8, 135.5, 136.5, 137.2, 137.4, 137.9, 138.5, 146.1, 151.6, 164.0, 184.2. FAB-MS: *m/z* 748 (MH^+). High-resolution FAB-MS: Calcd for $C_{43}H_{46}N_3O_9$ (MH^+): 748.3234. Found: 748.3246.

3'-*O*-Benzyl-3'-*C*,4'-*C*-(3-oxa-4-azapentylene)thymidine (9). To a solution of **8** (15.0 mg, 0.020 mmol) in CH_2Cl_2 (1 mL) was added BCl_3 (1 M in hexane, 0.20 mL, 0.20 mmol) at –78 °C, and the resulting mixture was stirred for 1 h. After stirring for 6 h at 0 °C, MeOH was added to the reaction mixture at the same temperature. The resulting mixture was concentrated to give a crude mixture, which was purified by silica gel column chromatography [$CHCl_3$:MeOH (15:1)] to give **9** (1.5 mg, 19%) as a white foam. ^{1}H-NMR (CD_3OD) δ: 1.79 (3H, d, 5-*Me*, *J* = 1 Hz), 1.89-2.01 (1H, m, H-6'), 2.23-2.29 (1H, m, H-6'), 2.34 (1H, dd, H-2', *J* = 9, 14 Hz), 2.76 (1H, dd, H-2', *J* = 5, 14 Hz), 3.06 (1H, d, H-8', *J* = 13 Hz), 3.20 (1H, d, H-8', *J* = 13 Hz), 3.78-3.89 (1H, m, H-7'), 3.85 (1H, d, *J* = 11 Hz), 3.98-4.08 (1H, m, H-7'), 4.01 (1H, d, *J* = 11 Hz), 4.46 (1H, d, *J* = 11 Hz), 4.56 (1H, d, *J* = 11 Hz), 6.21 (1H, dd, H-1', *J* = 5, 9 Hz), 7.07-7.60 (5H, m), 8.13 (1H, d, H-6, *J* = 1 Hz). FAB-MS: *m/z* 404 (MH^+). High-resolution FAB-MS: Calcd for $C_{20}H_{26}N_3O_6$ (MH^+): 404.1823. Found: 404.1825.

3'-*C*,4'-*C*-(*N*-Benzyloxycarbonyl-3-oxa-4-azapentylene)thymidine (10). To a solution of **8** (70.0 mg, 0.094 mmol) in wet 1,2-dichloroethane (2.0 mL) was added DDQ (213 mg, 0.94 mmol), and the mixture was refluxed for 6 h. After saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ were added to the reaction mixture at rt, the resulting mixture was extracted with AcOEt. The organic layer was washed with H_2O, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography [$CHCl_3$:MeOH (6:1)] to give **10** (23 mg, 57%) as a white solid. Colorless crystals from hexane:AcOEt (1:1): mp 139-141 °C. $[\alpha]_D^{24}$ +100.9 (*c* 0.80, MeOH). IR ν_{max} (KBr): 1034, 1139, 1267, 1356, 1453, 1694, 3351 cm^{-1}. ^{1}H-NMR (CD_3OD): δ 1.80-1.88 (1H, m, H-6'), 1.88 (3H, d, 5-*Me*, *J* = 1 Hz), 2.15-2.25 (1H, m, H-6'), 2.32 (1H, dd, H-2', *J* = 6, 13 Hz), 2.57 (1H, dd, H-2', *J* = 9, 13 Hz), 3.60 (1H, d, *J* = 12 Hz), 3.86 (1H, d, *J* = 12 Hz), 3.90 (1H, d, *J* = 12 Hz), 4.06 (1H, d, *J* = 12 Hz), 4.10-4.32 (2H, m, H-7'), 5.17 (2H, s), 6.42 (1H, dd, H-1', *J* = 6, 9 Hz), 7.28-7.40 (5H, m), 8.19 (1H, d, H-6, *J* = 1 Hz). ^{13}C-NMR (CD_3OD): δ 12.6, 30.9, 34.2, 46.4, 51.1, 67.5, 68.6, 74.0, 82.0, 87.1, 90.3, 110.9, 128.7, 129.0, 129.4, 137.6, 138.7, 152.3, 156.5, 166.3. FAB-MS: *m/z* 448 (MH^+). High-resolution FAB-MS: Calcd for $C_{21}H_{26}N_3O_8$ (MH^+): 448.1720. Found: 448.1710.

3'-*C*,4'-*C*-(3-Oxa-4-azapentylene)thymidine (1). To a solution of **10** (19.0 mg, 0.042 mmol) in CH_2Cl_2 (1 mL) was added BCl_3 (1 M in hexane, 0.13 mL, 0.13 mmol) at 0 °C and the resulting mixture was stirred for 1 h at rt. After AcOEt was added to the reaction mixture at 0 °C, the mixture was extracted with H_2O. The obtained aqueous layer was washed with AcOEt and concentrated to give crude mixture, which was purified by C18 reverse phase column chromatography [MeCN:H_2O (10:1)] to give **1** (9.3 mg, 70%) as a white solid. White powder from MeOH:H_2O (1:9): mp >300 °C. ^{1}H-NMR (D_2O): δ 1.83 (3H, s, 5-*Me*), 1.88-2.05 (2H, m, H-6'), 2.38 (1H, dd, H-2', *J* = 6, 14 Hz), 2.46 (1H, dd, H-2', *J* = 9, 14 Hz), 3.06 (1H, d, *J* = 13 Hz), 3.46 (1H, d, *J* = 13 Hz), 3.76 (1H, d, *J* = 12 Hz), 3.91 (1H, d, *J* = 12 Hz), 4.01-4.66 (2H, m, H-7'), 6.32 (1H, dd, H-1', *J* = 6, 9 Hz), 7.83 (1H, s, H-6). ^{13}C-NMR (5% CD_3CN in D_2O): δ 12.9,

33.4, 43.8, 53.5, 66.9, 71.9, 84.1, 87.9, 91.1, 111.9, 120.2, 138.7, 153.4, 168.3. FAB-MS: *m/z* 314 (MH^+). High-resolution FAB-MS: Calcd for $C_{13}H_{19}N_3O_6$ (MH^+): 314.1354. Found: 314.1349.

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