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SYNTHESIS AND STRUCTURE OF NEW METHYLENE-BRIDGED HEXOPYRANOSYL NUCLEOSIDE (BHNA)

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Abstract – A new member of hexopyranosyl nucleoside family, methylene-bridged hexopyranosyl nucleoside (BHNA), has been synthesized through generation of carbon radical at C6′ in [6′***S***-Me, 7′***S***-Me]-carba-LNA T nucleoside, followed by rearrangement to C4′ radical which was quenched by hydrogen atom to give BHNA. The stereoelectronic requirement for this unusual radical rearrangement has been elucidated by chemical model building and *ab intio* calculations to show that the coplanarity of the single electron occupied *p*-orbital at C6' with $\sigma^*_{O4'\text{-}C4'}$ plays an important role for the rearrangement reaction to take place. The solution structure of BHNA has also been studied using NMR as well as by *ab initio* calculations. The new six-membered pyranosyl ring in BHNA, unlike other known hexopyranosyl nucleosides, adopts a twist conformation, with base moiety occupying the axial position while 3′-hydroxymethyl and 4′-hydroxyl occupying the equatorial position.

INTRODUCTION

The pentose-sugar moiety in natural nucleos(t)ide is very flexible, adopting a dynamic equilibrium of several sugar pseudorotamers.[1,2] In nature, sugar moieties in DNA are present mainly in the South-type pseudorotamers which confer B-form global conformation, whereas the predominant North-type sugars in RNA renders it to a A-form global conformation.[3] It has been also found that some nucleotides are locked in to either South or North sugar conformation such as in ribozymes,[4] or lariat RNAs,[5,6] It is very likely that the conformation of the pentose-sugar moiety is a key factor[7] that determines the global conformation of nucleic acid, and consequently their physical, chemical and biological properties.

Conformationally-constrained nucleotides have attracted considerable interest in the last two decades[8-11] because they enable us to dictate the sugar to take up a specific conformation that, in turn, gives us a

handle to drive the structure and function of nucleic acids. The popular way to constrain the flexible pentose sugar moiety is linking two endocylic sugar carbons by a short covalent linkage.[11] Thus, BNA/LNA,[12-15] ENA[16-19] and their analoges[20-23] were obtained by a covalent bond formation between C2′ and C4′ with alkyl or alkyloxyl linker. Replacement of the pentofuranosyl sugar with hexopyranosyl sugar[24] however gives another type of conformationally-constrained nucleoside because the activation energy barrier for interconversion of conformers in the later is much higher than in the former. Oligonucleotides with this hexopyranosyl type modification give thermodynamically stable duplexes with the target DNA or RNA.[24] It is because, first, the six-membered ring system adopts a rigid chair conformation, requiring a less negative entropy change during the duplex formation; and second, the interstrand phosphate distance in the six-membered pyranosyl-modified system is larger than in the natural nucleic acid duplexes, giving a relatively less interstrand charge repulsion compared to the native counterpart.[25] Many different types of hexopyranosyl nucleosides have been so far synthesized by the groups of Herdewijn[9] and Eschenmoser.[25] These hexopyranosyl nucleosides have also been incorporated in to oligonucleotides, and their affinity toward complementary RNA or DNA, as well as their biochemical features have been studied: oligonucleotides composed of dideoxyglycopyranosyl-4',6'-nucleoside (homo-DNA, Figure 1) is almost linear, and not able to form duplexes with either RNA or DNA,[25,26] while oligonucleotides with 1',5'-anhydrohexitol nucleoside (HNA, Figure 1),[27,28] cyclohexanyl nucleoside (CNA, Figure 1)[29] or cyclohexenyl nucleoside (CeNA, Figure 1)[30-32] modification have improved affinity toward RNA and show improved nuclease resistance compared to the native counterpart. Amongst these, CeNA modified oligonucleotides have attracted more attention because they are able to activate RNase H, resulting in cleavage of the RNA strand in the DNA-RNA heteroduplexes.[30]

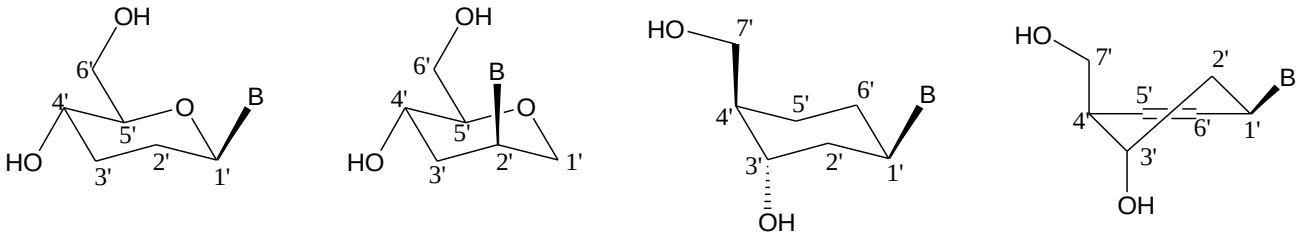


Figure 1. Structures of various pyranosyl nucleosides.

Recently, our group has reported synthesis of conformationally-constrained carba-LNA and carba-ENA nucleosides through radical cyclization reactions.[20,33,34] It is known that carbon radical in the sugar moiety of nucleoside can induce C-O bond scission under anaerobic conditions: For example, C4′ radical leads to

scission of C1′-O4′ bond[35] followed by rearrangement to C1′ radical (Figure 2A). C1′ may lead to scission of C4′-O4′ bond and subsequent rearrangement to C4′ radical (Figure 2B).[36] Accodingly, we argued, if the radical at C6′ in carba-LNA (Figure 2C) were generated under anaerobic conditions, the C6′ radical could be expected (compare with Figure 2A) to induce the scission of C4′-O4′ bond to give rearranged C4′ radical, and subsequent quenching by hydrogen atom. We hypothesized that, if successful, this putative transformation may constitute a new facile route to the synthesis of hexopyranosyl nucleoside.

Here, we report the synthesis of new bicyclo[2. 2.1]-2′,5′-methylene-bridged hexopyranosyl nucleoside **8** (BHNA, Scheme 1) through radical rearrangement under anaerobic conditions. The stereoelectronic requirement for the radical rearrangement from C6′ to C4′ has been elucidated by *ab initio* calculations, and the structure of BHNA has also been studied by high-resolution NMR.

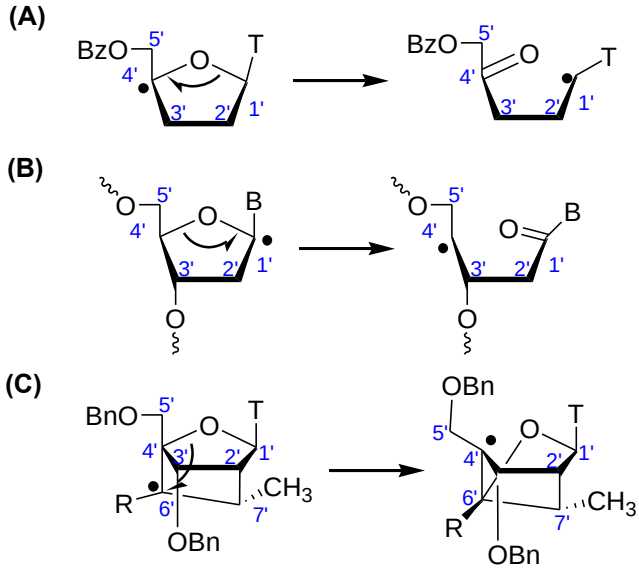


Figure 2. Sugar-carbon radical induced scission of C4′-O4′ or C1′-O4′ bond and subsequent radical rearrangement in nucleosides and nucleotides.

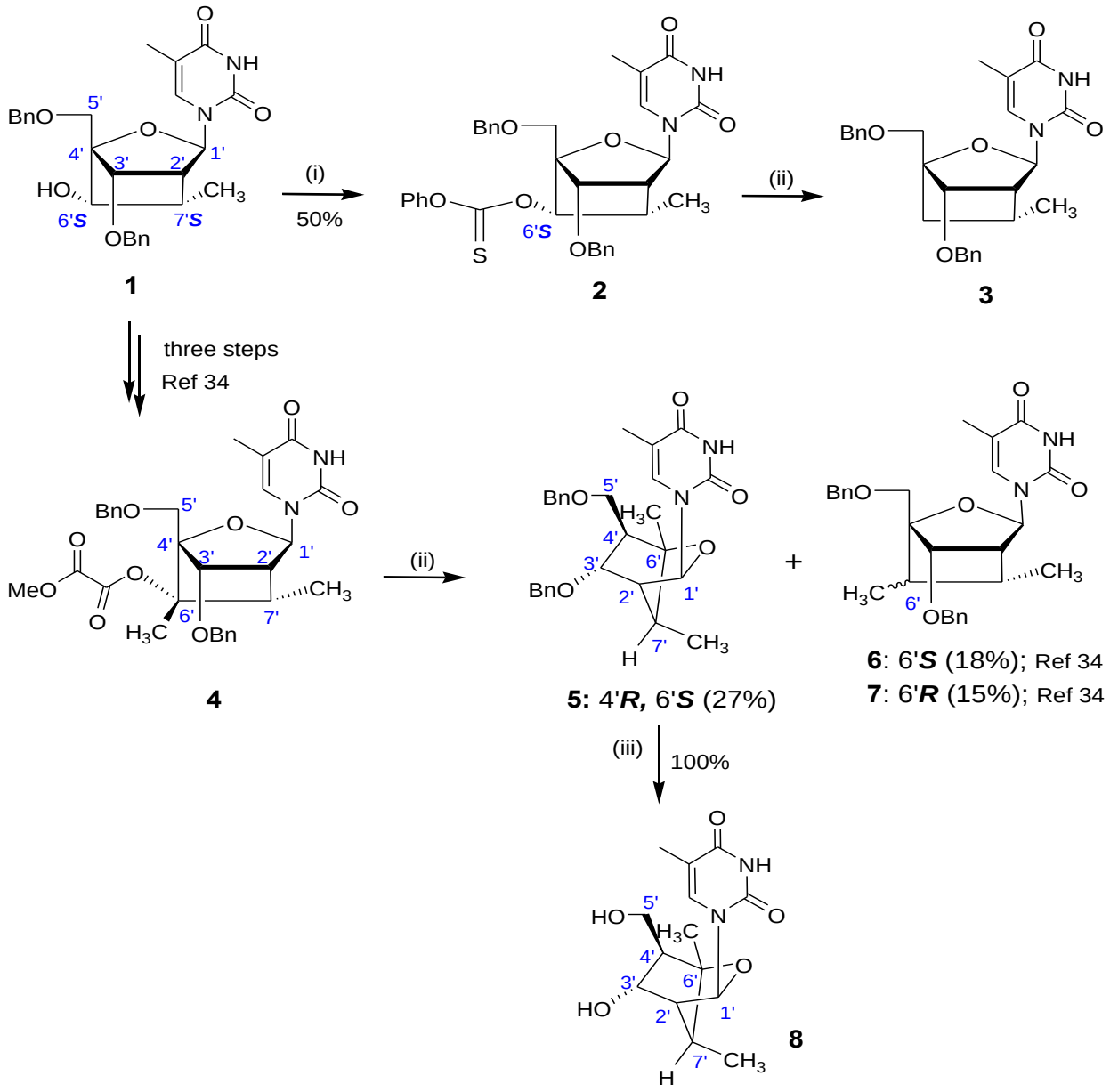
RESULTS AND DISCUSSION

(A) Synthesis of BHNA 8

The synthesis started from a known compound **1**,[33,34] 6′*S*-OH, 7′*S*-Me-carba-LNA T (Scheme 1). Compound **1** was reacted with phenyl chlorothionoformate in pyridine at rt to give radical precursor **2**. Treatment of **2** with Bu_3SnH in presence of a catalytic amount of AIBN in anhydrous toluene unfortunately gave only C6′ reduced product **3** in a poor yield (17%), and most of the starting material **2** was recovered (46%). This suggested that the *in situ* generated secondary radical at C6′, under this condition, is not sufficiently reactive to undergo rearrangement to C4′ radical, and therefore was quenched very fast by the surrounding $H^{\bullet}$. We argued that a more stable tertiary C6′ radical could increase

the possibility of radical rearrangement from C4′ to C6′.

Therefore, radical precursor **4**[34] was synthesized starting from compound **1.** Treatment of compound **4** with Bu_3SnH in presence of a catalytic amount of AIBN in anhydrous toluene, under an identical condition as used for compound **3** from **2**, gave hithertofore an uncharacterized[34] radical rearranged product, protected BHNA **5**, in 27% yield along with the known C6′ reduced products **6** (18%) and **7** (15%).[34] The protected BHNA **5** was debenzylated using 20% $Pd(OH)_2/C$ and ammonium formate under reflux in methanol to give fully deprotected BHNA **8** quantitatively.



Scheme 1. *Conditions and reagents:* (i) Phenyl chlorothionoformate, anhydrous pyridine, rt, 3 h; (ii) Bu_3SnH, AIBN, anhydrous toluene, 80 ˚C, 30 min; (iii) 20% $Pd(OH)_2/C$, ammonium formate, MeOH, reflux, 1.5 h. Synthesis of intermediates **4** was published in our earlier paper.[34]

(B) NMR characterization of BHNA thymidines 5 and 8

The key intermediates **5**, and final product **8** were characterized by ^{1}H, ^{13}C, DEPT, COSY, ^{1}H-^{13}C HMQC, HMBC, 1D-nOe NMR experiments as well as mass spectroscopy.

For compound **5**, the formation O1'-C6' bond can not be confirmed by HMBC experiment because the cross peak between H1' and C6' is unfortunately absence, but the structure of **5** is clearly evidenced by several 2D NMR experiments and mass spectroscopy: Although compound **5** has the same mass as that of diastereomer **6** or **7**, the comparison of their respective NMR data clearly shows that compound **5** vis-à-vis **6**/**7** are structurally very different: (1) The COSY spectrum of compound **5** shows that the cross peak between H1' and H2', H2' and H3', H3' and H4' can be observed (Figure S27), which suggests that the covalent linkage along C1'-C2'-C3'-C4' pathway are conserved. (2) The presence of cross peak between H2' and H7' in the COSY spectrum (Figure S27), and the long-range connectivity evidenced by the cross peak between H-6'Me and C7' as well as C4', cross peak between C6' and H7'-Me in HMBC spectrum (Figure S29) suggests that the covalent linkage along C2'-C7'-C6'-C4' pathway is also conserved in compound **5**. (3) The obvious difference between compound **5** and **6/7** is that in compound **5** *the C4′ is a tertiary carbon with H4′ attached and C6′ is a quaternary carbon* (DEPT 45, 90 and 135 spectra in Figure S24), whereas in compound **6/7**, *the C6′ is a tertiary carbon with H6′ attached and C4′ is a quaternary carbon* (DEPT 45, 90 and 135 spectra in Figure S21). (4) That the C4' is tertiary is also further proven by C4'-H4' connectivity in ^{1}H-^{13}C HMQC spectra (Figure S26), which is also solidly backed up H3'-H4' connectivity in the COSY spectrum (Figure S27), which are clearly absent in **6**/**7** (Figures S15, 16 and 17).

Thus, all the above evidences put together led us argue that quaternary C6' in compound **5** should be attached to O4' beside C4', C7' and 6'-Me, and consequently, the hyper-constrained bicyclo[2.2.1]-2′,5′-methylene-bridged hexopyranosyl nucleoside, BHNA **5**, must have been obtained. This structure of **5** was also supported by 1D-nOe experiment (Figure 3 and S30) as well as by detailed comparison of the experimental and simulated $^{3}J_{HH}$ (see part D).

The configuration of C4′ and C7′ in protected BHNA **5** was evidenced by 1D nOe (nuclear Overhauser effect) experiments (Figure 3 and S30) and the results are as follows: (1) 7′-Me but not H7′ shows nOe upon irradiation at H1′, suggesting H7′ oriented away from H1′ (Figure S30). (2) Irradiation at H4′ shows more nOe enhancements at H7′ (3.9%) compared to H3′ (1.2%), suggesting H4′ and H3′ are *trans* orientated while H4′ and H7′ are *cis* orientated (Figure 3). (3) Similarly, irradiation at H7′ shows enhancements at H4′ (4.1%) and H2′ (2.0%). These results are consistent with the spatial atomic distances ($d_{H4'\text{-}H3'}$ = 2.93Å, $d_{H4'\text{-}H7'}$ = 2.38Å, $d_{H7'\text{-}H2'}$ = 2.65Å) obtained from *ab initio* geometry optimization (Figure 3).

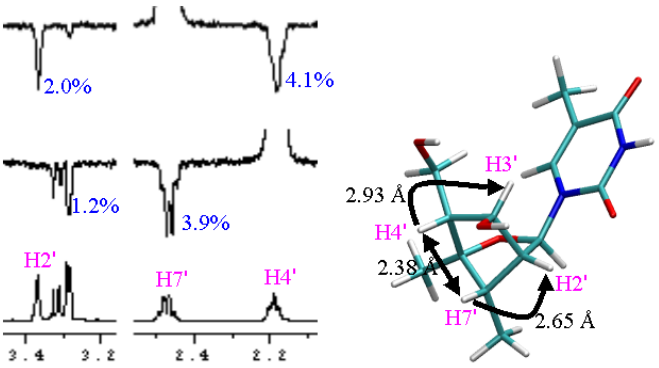


Figure 3. 1D nOe spectra of protected BHNA **5** (left) and molecular structure of BHNA **8** (right) obtained from *ab initio* geometry optimization (HF/6-31G**).

(C) Mechanism of Radical Rearrangement

Treatment of radical precursor **2** or **4** with Bu_3SnH in presence of a catalytic amount of AIBN in anhydrous toluene gives the putative radical intermediate **2a** or **4a**, which could either be reduced directly by $H^{\bullet}$ to give C6′ reduced product (Path "A" in Figure 4), or/and it could induce scission of C4′-O4′ bond

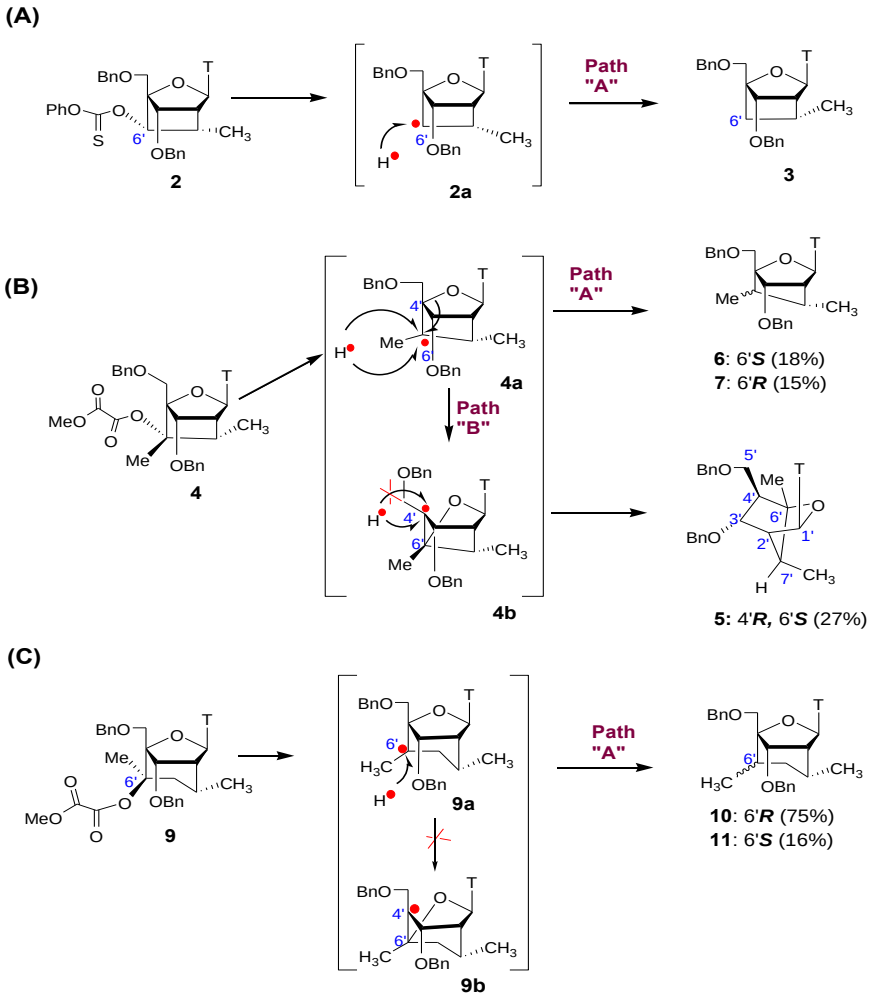


Figure 4. Structure of putative radical intermediates and plausible reaction pathway for rearrangement reaction from compound **4** to **5**.

to give C4′ radical, which upon quenching by H$^{\bullet}$ could give hexopyranosyl nucleoside (Path "B" in Figure 4). Actually, radical **2a** only followed the Path "A" to give C6′ reduced product **3**. Whereas radical **4a** gave both diastereomeric mixture of C6′ reduced compounds **6**(6'*S*) and **7**(6'*R*) by Path "A", and the diastereomerically pure rearranged product **5** through radical **4b**. The diastereospecific nature of **5** (4′*R*, 6′*S*) suggests that the H$^{\bullet}$ atom quenches the C4' radical in **4b** exclusively from below the plane of the sugar-ring. These different fates of radical **2a** and **4a** suggest that a stable tertiary radical is indeed a prerequisite to ensure the rearrangement to take place through path "B", in competition with path "A" giving the reduced product.

In contradistinction, generation of a radical at the C6′ center of the 6-membered carbocyclic nucleoside **9** (Figure 4C), under an identical condition, gave only the C6′ reduced products, **10** (6′*R*, 75%) and **11** (6'*S*, 16%), without any trace of the rearranged product.[34]

Crich et al. have shown that the C4' radical of 2',3'-dideoxythymidine is known to rearrange to C1' radical by scission of the C1'-O4' bond to give the acyclic product, but this reaction does not take place with 2'-deoxy-3'-methoxythymidine.[35] They suggested that different stereoelectronic effect in these two radical intermediates leads to their different fates. Accordingly, the structures of tertiary radicals **4a** and **9a** were investigated to show how the nature of different stereoelectronic effects causes their different fates.

Molecular structure (from *ab initio* HF/6-31G** geometry optimization) of the radical **4a** revealed (Table S1) that the radical formation expectedly changes sp^3 character of C6' to the sp^2 leading to formation of near-planar [C4'-C6'(Me)-C7'] geometry with the unpaired electron located on the *p*-orbital perpendicular to the plane. The [O4'-C4'-C6'-C(Me]) torsion in **4a** is 80.8º (Figure 5C and Table S2) and the single electron occupied *p*-orbital of C6' is nearly coplanar ($\phi = 5º$) with the $\sigma^*_{O4'-C4'}$ orbital. This coplanarity favors the rupture of C4'-O4' bond and formation of new C6'-O4'.[37] In contradistinction, the sp^2 configured radical at C6' of the 6-membered carba-ENA radical **9a** is also formed with the [O4'-C4'-C6'-C(Me)] torsion of 154.5º (Figure 5F) which results in a dihedral angle of 51º between the single electron occupied *p*-orbital at C6' and the $\sigma^*_{O4'-C4'}$ orbital. Energy calculations utilizing DFT B3LYP/6-311+G(d, p) (as implemented in GAUSSIAN98) show that the activation energy from **9a** to **9b** (Figure 4C) is 2.7 kcal/mol, which is somewhat higher than the activation energy from **4a** to **4b** (Figure 4B) [0.2 kcal/mol, Table S1], thereby showing that the formation of **4b** from **4a** is energetically more favored than that of the rearrangement of **9a** to **9b**. We thus demonstrate that stereoelectronic requirement of coplanarity of single electron occupied *p*-orbital at C6 with σ* orbital of O4'-C4' bond is a prerequisite for the rupture O4'-C4' bond, and the energy penalty involved in that process seems to be a key determinant for the rearrangement reaction to take place in **4a** to **4b**, but not in **9a** to **9b**.

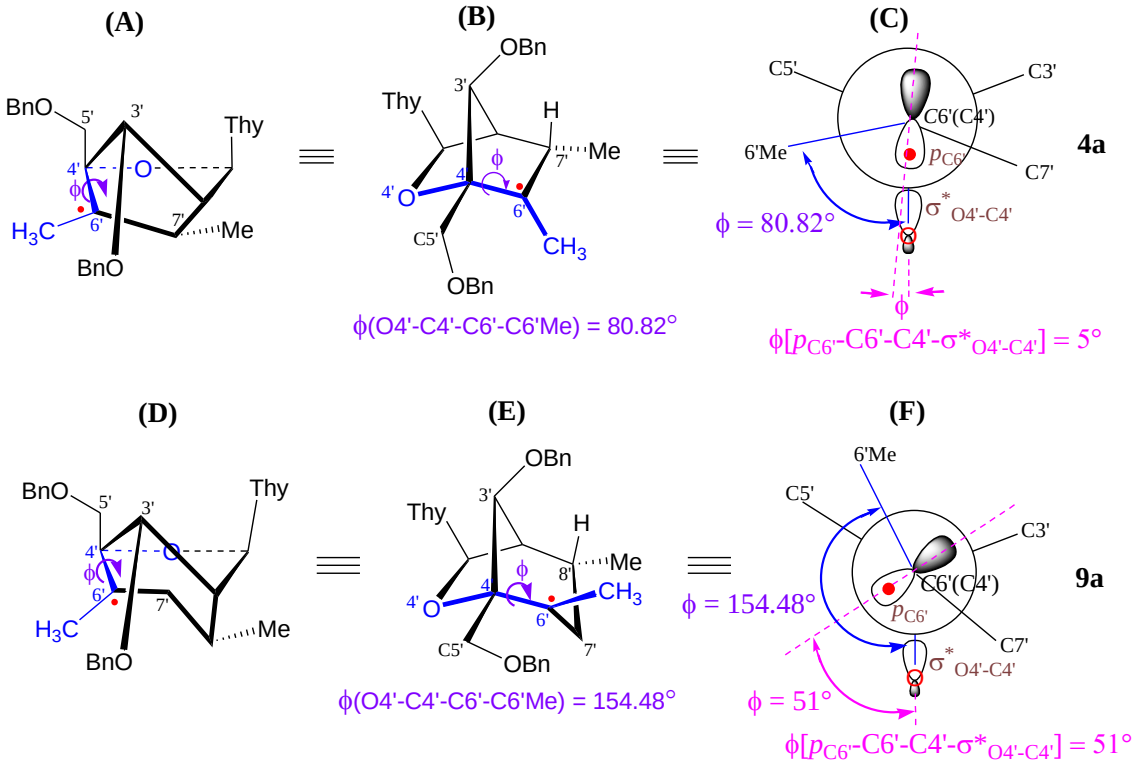


Figure 5. Comparison of the dihedral angles, ϕ[*O*4'-*C*4'-*C*6'-C6'Me] [in (A/B) and (D/E)] with the Newman projections of ϕ[$p_{C6'}$-*C*6'-*C*4'-σ*$_{O4'-C4'}$] [(C) and (F)] for the 5-membered and 6-membered radical intermediates **4a** and **9a**, respectively, through the *ab initio* optimized molecular geometries. Molecular formulae with the radical center at C6' are shown in cartoons (A) and (D) for the 5-membered and 6-membered radical intermediates, while the corresponding Howarth structures are shown in (B) and (E). The comparison of ϕ[*O*4'-*C*4'-*C*6'-C6'Me], in blue, in the 5- and 6-membered radical intermediates [(B) & (E)] shows they are respectively 80.82° and 154.48°. In the Newman projections [(C) and (F)], the C6' atom is located at the front of the panel, and the C4' atom is at the back. The dihedral angle of *O*4'-*C*4'-*C*6'-C6'Me of 80.82° and 154.48° for the 5-membered and 6-membered radical intermediates **4a** and **9a** translates into the dihedral angle between the single electron occupied *p*-orbital of C6'-radical and the σ*$_{O4'-C4'}$ orbital of O4'-C4' bond, ϕ[$p_{C6'}$-*C*6'-*C*4'-σ*$_{O4'-C4'}$], are 5˚ and 51˚ respectively [(C) & (F)].

(D) Structure of BHNA

In order to elucidate the solution structure of BHNA **8**, we have utilized vicinal proton coupling constants from homodecoupling ^{1}H NMR experiments (Figure S35) and the results from the theoretical simulations. Theoretical vicinal proton coupling constants have been back-calculated using Haasnoot-de Leeuw-Altona generalized Karplus equation[38] from the corresponding torsional angles of the *ab initio* optimized molecular structures from HF/6-31G* or B3LYP/6-31++G* geometry optimization by GAUSSIAN 98.[39] As shown in Table 1, the experimental vicinal coupling constants of BHNA **8** are well reproduced by this theoretical approach. This indicates that the BHNA **8** is indeed in rigid locked conformation and its average molecular structure observed experimentally is close to that of the minimized theoretical structure.

Table 1. Comparison of the experimental and theoretical vicinal proton coupling constants ($^3J_{H,H}$) of BHNA **8**.

Torsion (ϕH,H)	ϕH,H(°) HF/6-31G**)	$^3J_{H,H}$, calc. Hz[a]	$\Delta^3J_{H,H}$	ϕH,H(°) B3LYP /6-31++G**	$^3J_{H,H}$, calc. Hz[a]	$\Delta^3J_{H,H}$	Vicinal proton coupling	$^3J_{H,H}$, exp. Hz
H1'-C1'-C2'-H2'	46.4	3.9	1.1	46.3	3.9	1.1	$^3J_{H-1',H-2'}$	2.8
H2'-C2'-C3'-H3'	77.5	1.4	0.6	77.8	1.3	0.5	$^3J_{H-2',H-3'}$	0.8
H2'-C2'-C7'-H7'	70.6	1.6	0.0	70.6	1.7	0.1	$^3J_{H-2',H-7'}$	1.6
H7'-C7'-C7Me-7Me	-68.3 170.7 50.4	6.9 (average)	0.2	-66.9 172.1 51.8	6.7 (average)	0.2	$^3J_{H-7',H-7Me'}$	6.9
H3'-C3'-C4'-H4'	-129.3	4.1	0.7	-131.0	4.4	1.0	$^3J_{H-3',H-4'}$	3.4
H4'-C4'-C5'-H5'	-69.8	0.8	6.4	-71.8	0.7	6.3	$^3J_{H-4',H-5'}$	6.8
H4'-C4'-C5'-H5"	171.2	12.0	(average)	168.8	11.9	(average)	$^3J_{H-4',H-5"}$	7.9

[a] theoretical $^3J_{H,H}$ ($^3J_{H,H}$,calc.)[38] are obtained using Haasnoot-de Leeuw-Altona generalized Karplus equation taking into account β substituent correction.

The molecular structure of BHNA **8** obtained from *ab initio* geometry optimization (HF/6-31G**) is shown in Figure 3. A comparison of the different hexopyranosyl nucleosides shows that BHNA is very similar to cyclohexanyl nucleoside (CNA, Figure 1) in that C4′-hydroxymethyl and C3′-hydroxyl groups are trans orientated in both cases. CNA adopts chair conformation,[40] and its base moiety is orientated equatorially while 3′-hydroxymethyl and 4′-hydroxyl are orientated axially.[40] It may be noted that when CNA was incorporated into an oligonucleotides, a chair inversion occurred and as a result, the base moiety took up the axial position.[29] This suggested that the chair like six-membered ring in CNA is relatively flexible. In contradistinction, the additional methylene bridge between C2′ and C6′ in BHNA makes the six-membered ring completely rigid and it adopts a twist conformation (Figure 3). The base moiety occupies the axial position while 3′-hydroxymethyl and 4′-hydroxyl take up the equatorial position.

The BHNA **8** has also been transformed to the corresponding phosphoramidite and incorporated to oligonucleotides.[41] In view of the rigidity of the bicyclic system in BHNA, it should adopt the same conformation as that in the corresponding nucleoside, with base moiety taking the axial position while both 3′-hydroxymethyl and 4′-hydroxyl taking the equatorial position. Just like CNA, BHNA modified oligodeoxynucleotides are also RNA selective. But unfortunately, the BHNA destabilized both DNA/DNA and DNA/RNA duplexes, unlike CNA which stabilizes the thermal stability of DNA/DNA as well as DNA/RNA.[29] Clearly, our preliminary study[41] on this BHNA modified oligo did not show any improvement on the thermal stability of the DNA/DNA or DNA/RNA duplex, we are however exploring other avenues to reveal some useful applications in the antisense, siRNA or triplexing technologies.

CONCLUSION

Synthesis of Methylene-bridged hexopyranosyl nucleoside (BHNA) represents a new C6′ radical rearrangement (intermediate **4a**) to C4′ accompanied by scission of C4′-O1′ bond. The stereoelectronic configuration of the tertiary radical intermediate in the 5-membered system gives a slight energetic edge to drive the rearrangement of the C6' tertiary radical in **4a** compared to that of the corresponding 6-membered counterpart. The structure of BHNA has been studied using NMR as well as by theoretical *ab initio* and Karplus empirical approaches. The six-membered pyranose ring in BHNA is hyper-constrained by the C2′ to C6′ methylene linker, and adopts a twist conformation, with base moiety occupying the axial position while 3′-hydroxymethyl and 4′-hydroxyl occupying the equatorial position. Though the structure of BHNA is similar to another member of hexopyranosyl nucleoside family, but its synthetic rout is novel; BHNA, unlike CNA, does not improve the thermal stability of DNA/DNA and DNA/RNA duplex when it was incorporated into DNA strand, but future work may reveal new applications in the design of gene-directed therapeutics.

EXPERIMENTAL

Experimental procedures for the synthesis of compounds **4**, described in the present synthetic Scheme (Scheme 1), have been reported in our earlier JOC paper.[34]

(1*R*,3*R*,4*R*,5*S*,6*S*,7*S*)-7-benzyloxy-1-benzyloxmethyl-5-methyl-6-*O*-phenoxythiocarbonyl-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1]heptane (2). Compound **1** (175 mg, 0.37mmol) was coevaporated thrice with anhydrous pyridine and dissolved in the same solvent. The solution was cooled to –5 °C, and phenyl chlorothionoformate (0.172 mL, 0.55 mmol) was added dropwise. Then the reaction mixture was allowed to warm to rt and kept at this temperature for 2 h. After recovery of pyridine under reduced pressure, reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, concentrated, and chromatographed over silica gel (0-2 % MeOH in CH_2Cl_2, v/v) to give compound **2** as colorless oil (0.108 mg, 50 %). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.63 (1H, bs, NH), 7.71 (1H, s, H6), 7.10-7.43 (15H, m, aromatic), 5.83 (1H, s, H1′), 5.76 (1H, d, J = 9.60 Hz, H6′), 4.47-4.63 (4H, m, CH_2Bn), 4.12 (1H, s, H2′), 3.91 (1H, d, J_{gem} = 10.8 Hz, H5′), 3.86 (1H, d, J_{gem} = 10.8 Hz, H5″), 3.05 (1H, m, H7′), 2.66 (1H, d, $J_{H2', H7'}$ = 4.2 Hz, 2′H), 1.50 (3H, s, T-CH_3). 1.25 (3H, d, $J_{7'\text{-}CH3, H7'}$ = 6.9 Hz, 7′CH_3). ^{13}C NMR (150 MHz): δ 195.4 (C=S),164.43 (C4), 150.1 (C2), 137.5 (C6), 136.8, 135.8, 129.5, 128.5, 128.6, 128.1, 127.8, 126.7, 126.1, 121.8 (aromatic), 109.5 (C5), 88.3 (C4′), 88.0 (C1′), 83.6 (C6′), 78.2 (C3′), 73.9 (CH_2Bn), 72.3 (CH_2Bn), 66.1 (C5′), 47.9 (C2′), 12.0 (T-CH_3), 8.75 (7′-CH_3). MALDI-TOF *m/z*: $[M+H]^+$ found 615.19, calcd 615.21.

(1*R*,3*R*,4*R*,5*R*,7*S*)-7-benzyloxy-1-benzyloxmethyl-5,6-dimethyl-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1]-

heptane (3). Compound **2** (85 mg, 0.138 mmol) was dissolved in dry toluene (10 mL) and nitrogen was purged for 30 minutes. Bu_3SnH (0.037 mL in 5 mL dry toluene) and AIBN (10 mg in 5 mL dry toluene) was added. The above mixture was heated to 80 ˚C and stirred at this temperature for 30 minutes. Then another portion of Bu_3SnH (0.037 mL in 5 mL dry toluene) and AIBN (10 mg in 5 mL dry toluene) were added over 30 min and continued reaction for further 1 h. Solvent was evaporated and the obtained residue was applyed to short silica column chromatography (CH_2Cl_2/MeOH, 99:1) to give compound **3** (10 mg, and recover 40 mg of substrate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.09 (1H, brs, NH), 7.72 (1H, s, H6), 7.32-7.24 (10H, m, aromatic), 5.75 (1H, s, H1′), 4.59-4.44 (4H, m, CH_2Bn), 4.01 (1H, s, H3′), 3.79 (1H, d, J_{gem} = 10.5 Hz, H5′), 3.69 (1H, d, J_{gem} = 10.5 Hz, H5″), 2.60 (2H, broad, H7′, H2′), 2.06 (1H, t, $J_{H6', H7'}$ = 11.6 Hz, $J_{H6', 6H''}$ = 12.3 Hz, H6′), 1.54 (3H, s, T-CH_3), 1.26 (3H, d, $J_{7'\text{-Me}, H7'}$ = 6.0 Hz, 7′-CH_3), 1.15 (1H, dd, $J_{H6'', H7'}$ = 4.0 Hz, $J_{H6', 6H''}$ = 12.9 Hz, H6"). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.7 (C4), 149.7 (C2), 137.8, 137.5, 136.4 (C6), 128.5-127.5 (aromatic), 109.1 (C5), 88.9 (C4′), 84.2 (C1′), 78.7 (C3′), 73.7, 71.8 (CH_2Bn), 67.6 (C5′), 48.0 (C2′), 37.7 (C6′), 28.8 (C7′), 15.5 (7′Me), 12.1 (T-CH_3), 10.1 (7′-CH_3), 8.0 (6′-CH_3). MALDI-TOF *m/z*: $[M+H]^+$ found 463.1, calcd 463.2.

(1*S*,3*R*,4*R*,5*S*,6*R*,7*S*)-5-benzyloxy-6-benzyloxmethyl-1,7-dimethyl-3-(thymin-1-yl)-2-oxa-bicyclo-[2.2.1]heptane (5). Compound **4** (210 mg, 0.36 mmol) was dissolved in dry toluene (10 mL) and purged with dry nitrogen for 30 min. AIBN (30 mg, 0.18 mmol) and Bu_3SnH (0.29 mL, 1.08 mmol) were added to the mixture and heated to 80 ˚C for 0.5 h. The reaction was cooled to rt and evaporated. The residue was chromatographed over silica gel (20-35% EtOAc in cyclohexane, v/v) to obtain earlier reported **6** and **7** as a mixture (57 mg, 33.3%, **6**/**7** = 4/3)[34] along with a new hithertofore unidentified compound **5** (46 mg, 27%). Compound **5 :** ^{1}H NMR (500MHz, $CDCl_3$): δ 8.23 (1H, brs, H3), 7.35-7.25 (m, 11H), 5.52 (d, $J_{1', 2'}$ = 3.1 Hz, H1′), 4.57-4.45 (m, 4H, BnCH_2), 3.58 (dd, $J_{5', 4'}$ = 6.9 Hz, J_{gem} = 9.5 Hz, 1H, H5′), 3.36 (s, 1H, H2′), 3.31 (dd, $J_{5'', 4'}$ = 6.9 Hz, J_{gem} = 9.5 Hz, 1H, H5″), 3.28 (d, $J_{3', 4'}$ = 3.2 Hz, 1H, H3′), 2.47 (m, 1H, H7″), 2.19 (m, 1H, H4′), 1.78 (s, 3H, T-Me), 1.37 (s, 3H, 6′-Me), 1.12 (d, $J_{7', 7'Me}$ = 6.9 Hz, 3H, 7′-Me). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 163.5 (C4), 149.7 (C2), 138.03, 138.01, 136.9 (C6), 128.6-127.3 (aromatic), 109.4 (C5), 89.7 (C6′), 88.0 (C1′), 77.2 (C3′), 73.1, 70.8 (CH_2Bn), 68.0 (C5′), 56.6 (C4′), 49.8 (C2′), 45.5 (C7′), 15.4 (6′-Me), 12.4 (T-Me), 8.8 (7′-Me). MALDI-TOF *m/z*: $[M+H]^+$ found 477.5, calcd 477.3.

(1*S*,3*R*,4*R*,5*S*,6*R*,7*S*)-5,6-dihydroxyl-1,7-dimethyl-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1]heptane (8). To a solution of compound **5** (53 mg, 0.11 mmol) in dry MeOH (3 mL) was added 20% $Pd(OH)_2$/C (168 mg) and ammonium formate (423 mg, 6.7 mmol) and reflux for 1.5 h. The suspension was filtered over Celite bar and organic phase was evaporated to give the compound **11** (33 mg, 100%). ^{1}H-NMR (500

MHz, D_2O): δ 7.64 (s, 1H, H6), 5.58 (d, $J_{1',2'}$ = 2.8 Hz, 1H, H1′), 3.77 (dd, $J_{5',4'}$ = 6.8 Hz, J_{gem} = 11.5 Hz, 1H, H5′), 3.58 (d, $J_{3',4'}$ = 3.4 Hz, 1H, H3′), 3.51 (dd, $J_{5'',4'}$ = 7.9 Hz, J_{gem} = 11.5 Hz, 1H, H5″), 2.84 (m, 1H, H2′), 2.39 (dt, $J_{2',7'}$ = 1.6 Hz, $J_{7',7'Me}$ = 6.9 Hz, 1H, H7′), 1.89 (m, 1H, H4′), 1.86 (s, 3H, T-Me), 1.30 (s, 3H, 6′-Me), 1.03 (d, $J_{7',7'Me}$ = 6.9 Hz, 3H, 7′-Me). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 164.1 (C4), 149.6 (C2), 138.3 (C6), 110.2 (C5), 90.9 (C6′), 88.1 (C1′), 69.4 (C3′), 59.8 (C5′), 59.5 (C4′), 52.3 (C2′), 44.9(C7′), 14.2(6′-Me), 11.6 (T-Me), 7.9 (7′-Me). MALDI-TOF *M/S*: $[M+Na]^+$ found 318.9, calcd 319.1.

Theoretical calculations. The geometry optimizations have been carried out by GAUSSIAN 98 program package[39] at the Hartree-Fock level using HF/6-31G** and B3LYP/6-31++G**. The experimental torsion angles have been back-calculated from experimental vicinal proton $^3J_{H,H}$ coupling constants employing Haasnoot-de Leeuw-Altona generalized Karplus equation[38] taking into account β substituent correction in form:

$$^3J = P_1 \cos^2(\phi) + P_2 \cos(\phi) + P_3 + \Sigma \left(\Delta\chi_i^{group} \left(P_4 + P_5 \cos^2(\xi_i \phi + P_6 |\Delta\chi_i^{group}|\right)\right)$$

where $\Delta\chi_i^{group} = \Delta\chi_i^{\alpha\text{- substituent}} - P_7 \Sigma \Delta\chi_i^{\beta\text{- substituent}}$, $\Delta\chi_i$ are taken as Huggins electronegativities[42] and P_1 = 13.70, P_2 = -0.73, P_3 = 0.00, P_4 = 0.56, P_5 = -2.47, P_6 = 16.90, P_7 = 0.14 (parameters from[38]).

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