Unusual radical 6-*endo* cyclization to carbocyclic-ENA and elucidation of its solution conformation by 600 MHz NMR and *ab initio* calculations†

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In our previous paper (*J. Am. Chem. Soc.*, 2007, **129**, 8362), we reported the synthesis of 7′-Me-Carba-LNA and 8′-Me-Carba-ENA thymidine through 5-hexenyl or 6-heptenyl radical cyclization. Both 5-hexenyl and 6-heptenyl radical cyclized exclusively in the *exo* form, giving unwanted exocyclic C7′-methyl group. In the present study, we showed that the regioselectivity of the 5-hexenyl radical cyclization could be favorably tuned by introduction of a hydroxyl group β to the olefinic double bond, yielding about 9% of the 6-*endo* cyclization product. Possible pathways to give 6-*endo* cyclization product **9** compared to the intermediates responsible to give the 5-*exo* cyclization product **5** has been discussed. Based on this unique 6-*endo* cyclization strategy, a carbocyclic ENA modified thymidine (carba-ENA) has been successfully synthesized, which also enabled us to perform its full solution conformation analysis by using NMR (^{1}H at 600 MHz) observables for the first time.

Introduction

The conformationally constrained oligonucleotides have been attracting much interest in the field of antisense[1,2] and siRNA technology[3] in the past two decades. The development of the 2′,4′-fused five-membered Locked Nucleic Acid (LNA, Fig. 1)[4–6] and six-membered Ethylene-bridged Nucleic Acid (ENA)[7] modified nucleotides have considerably boosted research in this field. These ribonucleotide analogues consist of a rigid bicyclic systems constraining sugar conformation to 3′-*endo* form by a methylene (for LNA) or ethylene linkage (for ENA) between the 2′-oxygen and 4′-carbon of the ribose ring. Oligonucleotides containing LNA or ENA are preorganized in the A-type canonical structure, and thus typically have high affinity and specificity toward complementary RNA strand. Subsequently, other locked nucleotides with 2′,4′-linkage such as PrNA[8] and aza-ENA[9] have been incorporated into oligonucleotides, and all of them, just like LNA or ENA, have shown typically high affinity toward complementary RNA sequence. They also have shown relatively greater nuclease resistance.[8] This presumably led Nielsen *et al.*[10] to design and synthesize the 6-membered locked 2′,4′-carbocyclic-ENA uridine (carba-ENA-U) through the ring-closing methathesis.[10] This work[10] however did not include any studies on the nuclease stability of carba-ENA-U containing oligonucleotides. Independently, we have reported[11] the synthesis of five- as well as six-membered 2′,4′-carbocyclic analogues (7′-Me-carba-LNA and 8′-Me-carba-ENA) of thymidine *via* 5-hexenyl or 6-heptenyl radical cyclization, respectively. In both 5-hexenyl (for carba-LNA analog) and 6-heptenyl (for the carba-ENA analog) radical, the radical cyclization occurred exclusively in the *exo* form giving exocyclic equatorial methyl group (Fig. 2). It has also been found[11] that these 7′-Me-carba-LNA and

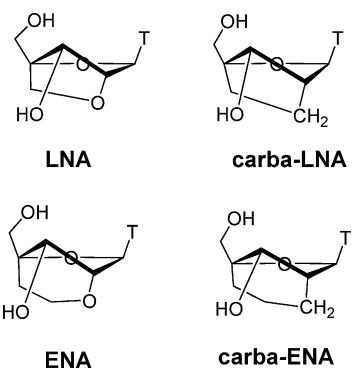

Fig. 1 Locked Nucleic Acid (LNA) and Ethylene-bridged Nucleic Acid (ENA) modifications and their carbocyclic analogues carba-LNA and carba-ENA.

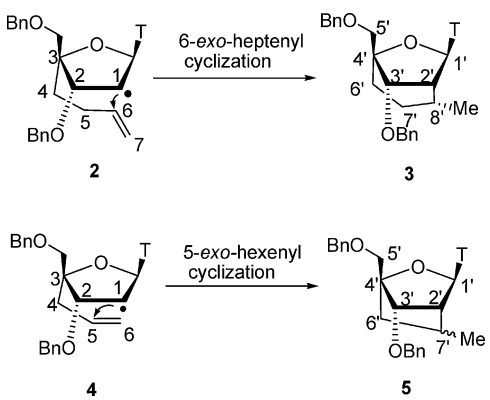

Fig. 2 Radical cyclization of 5-hexenyl and 6-heptenyl radical nucleosides following exclusively *exo*-cyclization pathway.[11]

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† Electronic supplementary information (ESI) available: ^{1}H, ^{13}C, COSY, HMQC, HMBC, DEPT, 1D-NOE NMR spectra of compounds **1**, **7**–**14**. Comparison of sugar conformation parameters of carba-ENA-T (**1**) with that of carba-ENA-U, 8′-Me-carbo-ENA-T, ENA-T and aza-ENA T. Coordinates of structure of compound **1** in pdb format.. See DOI: 10.1039/b813870b

8′Me-carba-ENA containing oligonucleotides at the 3′-end are fully resistant for over 48 h in the human blood serum.[11] Through single LNA substitution at identical site as that with the carbocyclic counterpart it has been shown that the 7′-Me-carba-LNA and 8′-Me-carba-ENA containing oligonucleotides are considerably more stable to nucleases in the human serum[11] than that of the identically LNA-substituted oligo-DNA sequences which in average survive for less then 9 h.

During our efforts to functionalize the five membered 2′,4′-carba-LNA-type thymidine,[12] we found that introducing a 6′-hydroxyl to the 5-hexenyl carbon radical, as in the intermediate **Ts:6′** in Scheme 1, lead to 6-*endo* hexenyl cyclization product **9** (~9%) in conjunction with the competing 5-*exo* cyclized product **7** and **8** (Scheme 1). Here, we report synthesis of carbocyclic ENA thymidine (carba-ENA-T, Fig. 1) through 6-*endo* hexenyl cyclization and its structure by NMR and *ab initio* calculations.

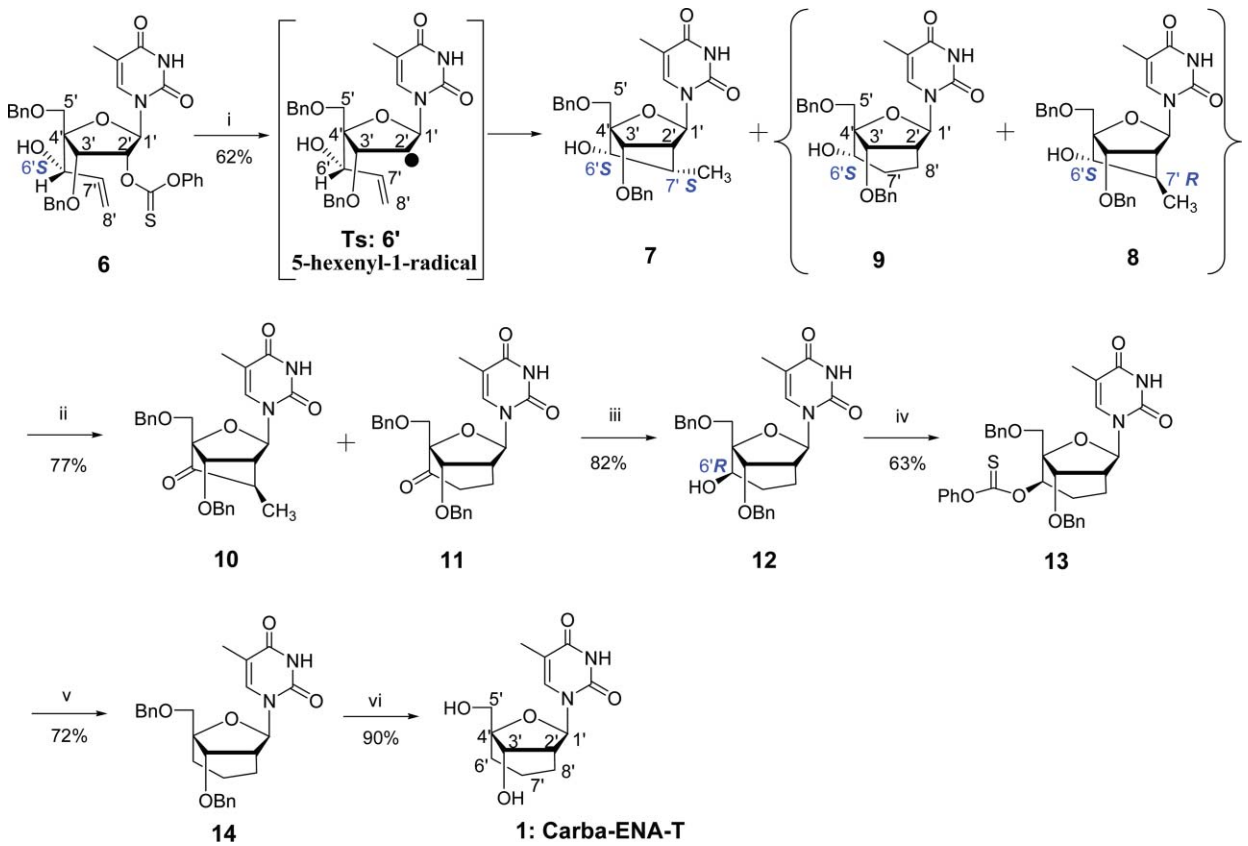
Results and discussion

1. Free radical cyclization

The key intermediate **6** was synthesized according to our previous procedure.[12] The free radical cyclization was carried out in toluene in presence of n-Bu_3SnH and a catalytic amount of AIBN by reflux to give two spots on the TLC. The product with higher R_f was separated by short chromatography and identified as product **7** by NMR (see ESI), which is one of the isomers (7′***S***) formed by the 5-*exo* cyclization reaction. The lower R_f spot was also separated and found it to be a mixture of two components: the 2nd isomer (7′***R***) of 5-*exo* cyclization product **8** and 6-*endo* cyclization product **9**.

The characterization and conformational analysis of **7**, **8** and **9** have been performed using NMR data obtained by ^1H, ^{13}C, DEPT as well as COSY, ^1H-^{13}C HMQC and long range ^1H-^{13}C correlation (HMBC) experiment. $^3J_{HC}$ HMBC correlations between H1′ and C8′ in compound **9** and between H1′ and C7′ in compound **8** (Fig. 3B) unequivocally proves that the oxa-bicyclo [2.2.1] heptane ring system and oxa-bicyclo [3.2.1] octane ring system have been formed for compounds **8** and **9** respectively, which was further confirmed by observation of $^3J_{HH}$ correlation between H7′ and H2′ in compound **8**, $^3J_{HH}$ correlation between H8″ and H2′ in compound **9** in COSY spectrum (Figure S6 in ESI). The endocyclic nature of 7′- and 8′-methylene groups in compound **9** was verified by DEPT and HMQC experiment. In the DEPT spectrum, both C7′ and C8′ appeared as the secondary carbon (Figure S3 in ESI) and in the HMQC spectrum, each of them have two protons attached (Fig. 3A).

The configuration of C6′ and C7′ in compounds **7**, **8**, **9** was determined by 1D NOE experiments. For compound **7**, irradiation of H1′ leads to NOE enhancement for H2′ (3.1%) and 7′-Me (6.0%), and irradiation of H6′ leads to strong NOE enhancement (6.8%) for H7′, but none for 7′-Me, suggesting both C6′ and C7′ are in ***S***-configuration (Figure S14 in ESI). Since radical cyclization



Scheme 1 *Reagents and conditions*: (i) Bu_4SnH, AIBN, toluene, reflux 4h; (ii) Dess–Martin periodinane, dichloromethane, r.t. 3 h; (iii) $NaBH_4$, ethanol, r.t. 2 h; (iv) phenyl chlorothionoformate, pyridine, r.t. 3 h; (v) Bu_4SnH, AIBN, toluene, reflux 1 h; (vi) 20% $Pd(OH)_2/C$, ammonium formate, methanol, reflux 2 h.

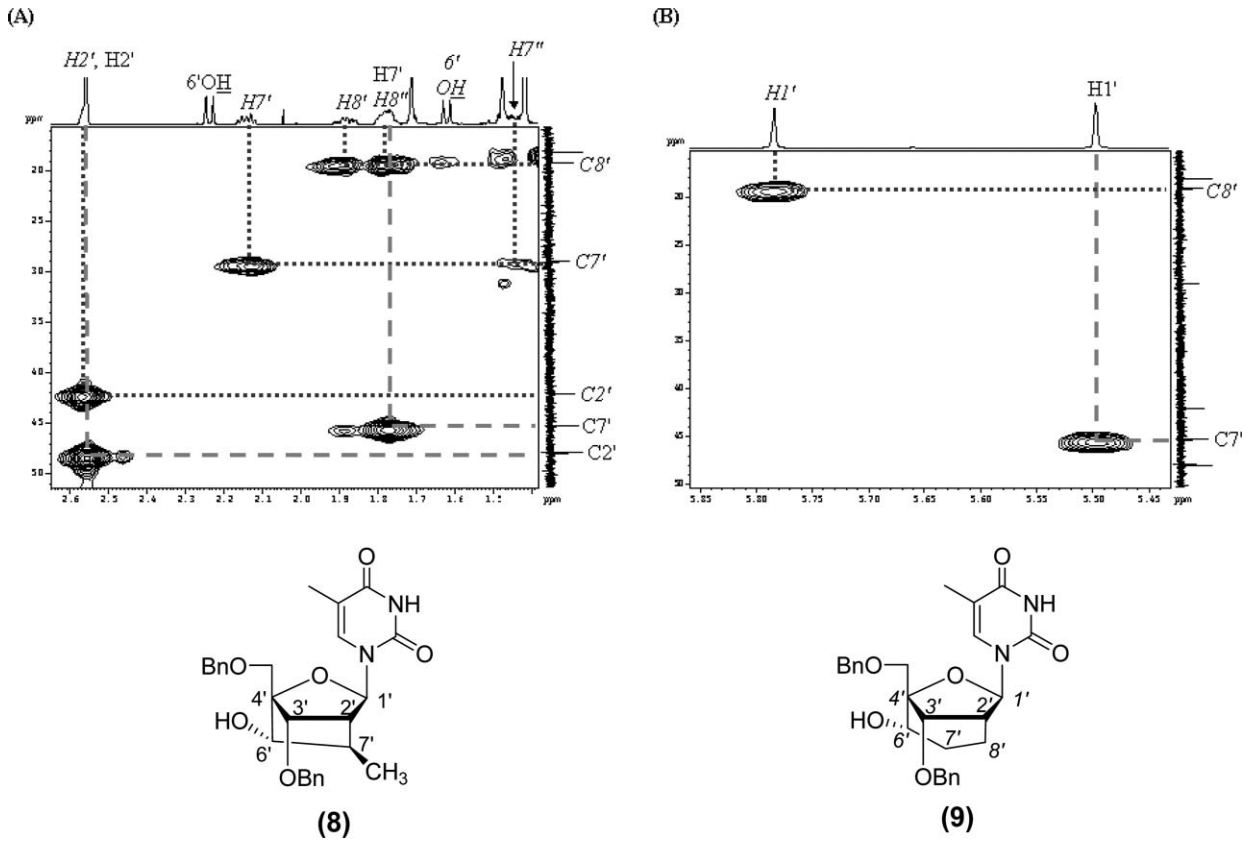


Fig. 3 ^{1}H-^{13}C HMQC (panel A) and HMBC (panel B) spectra of mixture of compounds **8** (dashed line, labeled in normal form) and **9** (dotted line, labeled in *italic* form).

does not change the configuration of distal C6′, compounds **6**, **8** and **9** should have the same C6′-***S*** configuration as that in compound **7**. For compound **8**, irradiation of H1′ leads to NOE enhancement for H2′ (1.8%) and H7′ (5.2%), but none for 7′-Me. (Figure S15 in ESI). Hence, ***R***-configuration was assigned for C7′ of compound **8**.

The relative ratio of compounds **7**/**8**/**9** was 80:11:9. Thus the radical cyclization of **Ts: 6′** gives 9% of 6-*endo* cyclization product **9** compared to **4** → **5** (Fig. 2), in which compound **5**occurs as an exclusive product owing to the participation of 5-*exo* cyclization pathway. This difference suggested that the 6′-hydroxyl group affects the outcome of the regioselectivity of free radical cyclization to some extent. It is well known that cyclization of 5-hexenyl-1-radical is a kinetically controlled process and the *exo* cyclization product, cyclopentane, is preferred (*exo*/*endo* > 98/2).[13] The effect of substituents especially alkyl group on regioselectivity of 5-hexenyl-1-radical cyclization has been studied.[14] Generally, alkylation at C1 and C5 increase the selectivity on 6-*endo* cylization[15] and methylation at C2, C3, C4 and C6 enhance the 5-*exo* cylization.[16,17] In some cases, the 6-*endo* product can however be found as the major product.[15] But unfortunately, the mechanism behind the regioselectivity upon alkyl substitutation is still not clear, and the role of 4-hydroxyl substitution has never been illustrated heretofore.

It is however likely two possible effectors could contribute to the enhanced 6-*endo* hexenyl cyclization by the 6′-OH in **Ts: 6′** (Scheme 1). First, it is known that β-hydroxyl or alkyloxyl has a marked stabilizing effect on a carbon radical (β-Oxygen Effect):[18] Thus, in the intermediate **15** of 6-*endo* cyclization, the 6′-OH located at the β position to radical at C7′ can have a stabilizing effect by inductive effect and/or by resonance delocalization of the charge into the $\sigma^*_{(O6'\text{-}C6')}$ orbital[19,20] since the single occupied *p* orbital and the empty $\sigma^*_{(O6'\text{-}C6')}$ orbital orientate periplanar with respect to each other (Fig. 4).

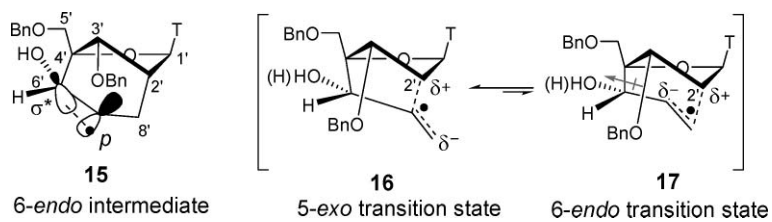


Fig. 4 Graphical representations of the molecular structures of 6-*endo* cyclization intermediate (**15**), 5-*exo* cyclization transition state **16** and 6-*endo* cyclization transition state **17**.

Second possibility is that the 6′-hydroxyl could stabilize transition state of 6-*endo* radical cyclization. During the attack of carbon radical to olefin, the C2′ radical behaves as a nucleophile and becomes slightly positively charged,[14] whereas the terminal carbon in the olefinic moiety becomes fractionally negative to give 5-*exo* (**16**) and 6-*endo* (**17**) cyclization transition states (Fig. 4). When R = H, QM calculations[21] reveal that strain engendered in accommodating the required disposition of reactive centers is greater for the 6-*endo* transition state **17** than for the 5-*exo* transition state **16**, and as a result the 5-*exo* cyclization is kinetically predominant. While, when R = OH, the 6′-hydroxyl can stabilize inductively the developing negative charge on the olefin moiety in the 6-*endo* transition structure **17**, and thus lowering the activation energy of 6-*endo* cyclization, as a result, more 6-*endo* cyclization product (9%) was obtained. Due to the discovery of the 6-*endo* cyclization through the stabilizing effect of the 6′-hydroxy group, we are now attempting to prepare other derivatives with electron-withdrawing groups at C6′ in order to increase the yield of the cyclization step.

2. Radical deoxygenation to give carba-ENA-T (1)

Compound **8** and **9** appeared to have nearly the same polarity and efforts to separate them using column chromatography have failed. Therefore, we treated the mixture with Dess–Martin periodinane to obtain ketones **10** and **11**, which can be separated easily by silica gel column chromatography. Compound **11** was then reduced to alcohol again with $NaBH_4$. This reduction was highly stereoselective to give compound **12** with C6′(***R***)-OH stereochemistry as the only product with high yield (82%). Thus the oxidation followed by reduction constitutes an inversion of the configuration of C6′(***S***)-OH in **9** to C6′(***R***)-OH **12** (in Scheme 1) efficiently. Compound **12** was converted to its 6′-*O*-phenoxythiocarbonyl derivative **13** (63%) followed by standard radical deoxygenation[22] to give intermediate **14** (72%), which was debenzylated with 20% $Pd(OH)_2/C$, ammonium formate in methanol to give the title compound **1** in 90% yield.

3. Molecular structure of carba-ENA-T based on NMR and *ab initio* calculations

The product **1**, carba-ENA-T, has been characterized using NMR and *ab initio* optimized molecular modeling. All the peaks in ^1H and ^{13}C NMR spectra have been assigned through DEPT, COSY, HMQC and HMBC experiments performed using 500 and 600 MHz NMR (see Figure S26 to S31 in ESI). In order to reconstruct the solution structure of carba-ENA-T, we have utilized vicinal proton couplings analysis using data from homodecoupling ^1H NMR experiments and the results of the theoretical simulations. Theoretical vicinal proton coupling constants have been back-calculated using Haasnoot-de Leeuw-Altona generalized Karplus equation[23,24] from the corresponding torsional angles of the *ab initio* optimized molecular structures obtained utilizing HF/6–31G* or B3LYP/6–31++G* geometry optimization by GAUSSIAN 98.[25] As shown in Table 1, the experimental vicinal coupling constants of compound are well reproduced by this theoretical approach (Table 1). This indicates that the modified nucleoside **1** is indeed in rigid locked conformation and its average molecular structure observed experimentally is close to that of the minimized theoretical structure.

The molecular structure obtained from *ab initio* geometry optimization is shown in Fig. 5. The six-membered carbocyclic moiety adopts a perfect chair conformation. The furanose ring of carba-ENA-T (Table S1) is found to be locked in the North-type conformation characterized by pseudorotational phase angle $P = 19.6°$ and the puckering amplitude $\Psi_m = 45.9°$. The sugar pucker parameters are very similar to that of carba-ENA-U,[10] 8′-Me-carba-ENA-T,[11] ENA-T[26] and aza-ENA-T,[9] which suggests the 2′,4′-linkage induced locking of the sugar is so strong that the sugar pucker becomes rigid and not sensitive to a large extent to substitutions at the base or/and at the 2′,4′-linkage.

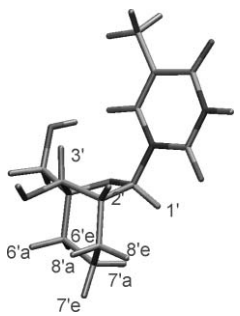


Fig. 5 Molecular structure of carba-ENA-T optimized *ab initio* [only B3LYP geometry is shown since it is identical to the HF optimized geometry (RMSD < 0.05 Å)]. The PDB coordinates are presented in the Electronic Supplementary information (ESI).

Table 1 Experimental and calculated vicinal $^3J_{HH}$ coupling constants and torsion angles of carbo-ENA-T (compound **1**, Scheme 1)

Torsion	$\Phi_{H,H}$, (cal.^3J, *Hz*), HF/6–31G**	$\Phi_{H,H}$ (cal.^3J, *Hz*), B3LYP/6–31++G**	vicinal proton coupling	$^3J_{H,H}$ exp. (Hz)	$\Phi_{H,H}$(°) exp.
H1′-C1′-C2′-H2′	91.89°(1.0)	91.75°(1.0)	$^3J_{H\text{-}1',H\text{-}2'}$	1.0	84.3 to 92.6
H2′-C2′-C3′-H3′	47.55°(5.7)	47.22°(5.8)	$^3J_{H\text{-}2',H\text{-}3'}$	5.2	47.2 to 55.2
H2′-C2′-C7′-H8′e	−51.24°(4.6)	−51.26°(4.6)	$^3J_{H\text{-}2',H\text{-}8''}$	3.7	−61.1 to −52.5
H2′-C2′-C7′-H8′a	65.58°(2.1)	65.52°(2.1)	$^3J_{H\text{-}2',H\text{-}8'}$	2.0	61.0 to 72.0
H6′e-C6′-C7′-H7′a	41.29°(6.7)	42.83°(6.6)	$^3J_{H\text{-}6'',H\text{-}7''}$	5.0	46.1 to 54.0
H6′e-C6′-C7′-H7′e	−74.24°(0.9)	−72.73°(1.1)	$^3J_{H\text{-}6'',H\text{-}7'}$	1.8	−87.5 to −67.1
H6′a-C6′-C7′-H7′a	159.54°(12.0)	161.29°(12.2)	$^3J_{H\text{-}6',H\text{-}7''}$	11.0	157.5 to 172.1
H6′a-C6′-C7′-H7′e	44.01°(6.3)	45.74°(5.9)	$^3J_{H\text{-}6',H\text{-}7'}$	7.0	48.0 to 56.0
H7′a-C7′-C8′-H8′e	−42.39°(6.4)	−43.67°(6.2)	$^3J_{H\text{-}7'',H\text{-}8''}$	5.5	−53.1 to −45.3
H7′a-C7′-C8′-H8′a	−160.40°(11.9)	−161.91°(12.1)	$^3J_{H\text{-}7'',H\text{-}8'}$	12.0	−155.2 to −146.7
H7′-C7′-C8′-H8′e	73.66°(1.0)	72.44°(1.1)	$^3J_{H\text{-}7',H\text{-}8''}$	1.8	61.6 to 72.9
H7′e-C7′-C8′-H8′a	−44.34°(6.1)	−45.80°(5.8)	$^3J_{H\text{-}7',H\text{-}8'}$	7.0	−48.2 to −40.5

Conclusion

In this study, we have devised effective control switch to tune the radical cyclization reaction from 5-*exo* pathway to 6-*endo* pathway. It has been achieved by the introduction of one hydroxyl group at C6′ to the 5-hexenyl carbon radical which has led to enhancement of the regioselectivity of the radical center and ultimately led to the 6-*endo* radical cyclization. Radical deoxygenation of this 6-*endo* cyclization product results in carba-ENA-T in high yield. The molecular structure of carba-ENA-T has been studied using experimental NMR observables and Karplus empirical approaches, as well as theoretical *ab initio* calculations. The furanose ring of carba-ENA-T has been shown to be locked in the North-type conformation ($P = 19.6°$, $\Psi_m = 45.9°$) and the six-membered carbocyclic moiety has been found to adopt a perfect chair conformation. Work is now in progress to synthesize carba-ENA nucleosides (compound **1** and its analogs) through the free-radical addition to the terminal -CH=N- or to an aldehyde or an aziridinylimine function as radical acceptors.

Experimental

Synthesis of (1R, 3R, 4R, 5S, 6S, 7S)-7-benzyloxy-1-benzyloxmethyl-6-hydroxyl-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1] heptane(7), (1R, 3R, 4R, 5R, 6S, 7S)-7- benzyloxy-1-benzyloxmethyl-6-hydroxyl-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1] heptane(8) and (1R, 4S, 5R, 7R, 8S)-8-benzyloxy-5-benzyloxmethyl-4-hydorxyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]oct-4-one (9)

4.2 g (5.5 mmol) of **6**[12] was dissolved in 200 mL of dry toluene which was purged by N_2 for ca 30 min. The mixture was heated under reflux and Bn_3SnH (1.85 ml in 20 mL dry toluene), AIBN (0.55 g in 20 mL dry toluene) was added dropwise in 2h. The reaction was found to be incomplete after 30 min (TLC). So another part of Bn_3SnH (0.9 mL in 10 mL toluene) and AIBN (0.25 g in 10 mL toluene) was added dropwise in 1 h and continued reflux for further 1 h. The solvent was evaporated and the residue was applied to silica short column chromatography (EtOAc/cyclohexane, 2/8 to 6/4) to give 1.3 g (49%) of compound **7**, 0.32 g (12%) of mixture of **8** and **9** (**8**/**9** = 11:9) and recovered 0.7 g of substrate **6**. **7**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.15 (d, 3H, $J_{7'CH3,\,7'H}$ = 7.6 Hz, 7′CH_3), 1.50(s, 3H, 5-CH_3), 2.25 (d, 1H, $J_{6'H,\,6'OH}$ = 11.6 Hz, 6′OH), 2.66 (d, 1H, J = 2.75 Hz, 2′H), 2.72 (m, 1H, H7′), 3.81 (d, 1H, J = 11.0 Hz, 5′H), 3.91(d, 1H, J = 11.0 Hz, 5″H), 4.42–4.61 (m, 4H, BnCH_2), 5.75 (s, 1H, H1′), 7.23–7.34 (m, 10H), 7.72 (s, 1H, H6), 8.87 (broad, 1H, N^3H). ^{13}C NMR ($CDCl_3$): δ 8.4 (7′CH$_3$), 12.1 (5-CH$_3$), 33.1(C7′), 47.8(C2′), 66.2(C5′), 71.9(C6′), 72.1 (Bn CH$_2$), 73.9 (Bn CH$_2$), 76.8 (C3′), 83.8 (C1′), 89.1 (C4′), 109.6 (C5), 127.6, 127.9, 128.1, 128.5, 128.6, 136.0 (C6), 137.0, 137.6, 149.9 (C2), 164.0(C4). MALDI-TOF MS m/z: [M + Na]$^+$ 501.2, calcd 501.2.Though we isolated a mixture of **8** and **9**, their proton and carbon NMR peaks could be assigned clearly, so here they are given separately. **8**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.31 (d, 3H, $J_{7'CH3,\,7'H}$ = 7.2 Hz, 7′CH_3), 1.47 (s, 3H, 5-CH_3), 1.77 (m, 1H, 7′H), 2.23 (1H, $J_{6'H,\,6'OH}$ = 11.0 Hz, 6′OH), 2.55 (s, 1H, 2′H), 3.83 (d, 1H, J = 11.3 Hz, 5′H), 3.93 (d, 1H, J = 11.3 Hz, 5′H), 4.00 (dd, 1H, $J_{6'H,\,6'OH}$ = 11.0 Hz, $J_{6'H,\,7'H}$ = 3.5 Hz, 6′H), 4.04 (s, 1H, 3′H), 4.40–4.62 (m, 4H, BnCH_2), 5.50 (s, 1H, H1′), 7.22–7.34 (m, 10H), 7.75 (s, 1H, H6), 8.71 (s, 1H, N^3H).^{13}C NMR (600 MHz, $CDCl_3$): δ 12.0 (5-CH$_3$), 18.1 (7′CH$_3$), 42.1 (C7′), 45.3 (C2′), 65.8 (C5′), 72.4(Bn CH$_2$), 73.9(Bn CH$_2$), 79.0 (C3′), 81.0 (C6′), 88.6 (C1′), 88.9 (C4′), 109.5 (C5), 127.5, 127.9, 128.0, 128.1, 128.5, 128.6, 135.9, 136.8, 137.5, 149.9, 163.8. **9**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.71 (s, 3H, 5-CH_3), 1.45 (m, 1H, H7′), 1.62 (d,1H, $J_{6'H,\,6'OH}$ = 10.2 Hz, 6′OH), 1.77 (m, 1H, 8′H), 1.88 (m, 1H, 8″H), 2.15 (m, 1H, H7′), 2.56 (s, 1H, H2′), 3.72 (d, 1H, J = 11.0 Hz, 5′H), 4.00 (m, 1H, H6′), 4.15 (d, 1H, J = 11.0 Hz, 5′H), 4.33 (d, 1H, J = 4.9 Hz, 3′H), 4.40–4.63 (m, 4H, BnCH_2), 5.78 (s, 1H, H1′), 7.22–7.34 (m, 10H), 8.00 (s, 1H, H6), 8.71 (s, 1H, N^3H). ^{13}C NMR (600 MHz, $CDCl_3$): δ 11.8 (5-CH$_3$), 19.1 (C8′), 29.0(C7′), 42.1 (C2′), 68.1 (C5′), 68.9 (C6′), 72.0(Bn CH$_2$), 73.6 (Bn CH$_2$), 73.8 (C3′), 87.5(C4′), 87.7(C1′), 109.4, 127.4, 127.8, 127.9, 128.0, 128.5, 128.6, 136.2, 137.40, 137.47, 150.1, 163.9. MALDI-TOF MS m/z: [M + H]$^+$ 479.2, calcd 479.2.

Synthesis of (1R, 3R, 4R, 5R, 7S)-7- benzyloxy-1-benzyloxmethyl-6-one-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1] heptane (10) and (1R, 5R, 7R, 8S)-8-benzyloxy-5-benzyloxmethyl-4-one-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (11)

Mixture of **8** and **9**(342 mg, 0.71 mmol) was dissolved in dry DCM, Dess–Martin periodinane (15% in DCM, 1.8 mL, 0.85 mmol) was added and stirred at r.t. for 2 h. Then diluting the reaction mixture with DCM, filtered through celite bar, the filtrate was washed with aqueous $Na_2S_2O_3$ twice, saturated $NaHCO_3$ solution once and NaCl solution once. After drying over $MgSO_4$, it was applied to silica short column chromatography (EtOAc/cyclohexane 2/8 to 4/6) to give 150 mg of **10** and 112 mg of **11** (overall yield 77%). **10**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.36 (d, 3H, $J_{7'CH3,\,7'H}$ = 7.6 Hz, 7′CH_3), 1.49(s, 3H, 5-CH_3), 2.46(m, 1H, H7′), 2.99 (s, 1H, H2′), 3.90 (d, J = 11.7 Hz, 5′H), 4.0 (d, J = 11.7 Hz, 5″H), 4.18(s, 1H, H3′), 4.49–4.61(m, 4H, BnCH_2), 5.54(s, 1H, H1′), 7.20–7.33(m, 10H, Bn-*Ph*), 7.72(s, 1H, H6), 8.76 (s, 1H, N^3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 12.0 (5-CH_3), 14.3 (7′CH$_3$), 43.1 (C7′), 49.6 (C2′), 63.3 (C5′), 72.5 (BnCH$_2$), 74.1 (BnCH$_2$), 86.1 (C4′), 88.5 (C1′), 109.9, 127.5, 127.9, 128.21, 128.24, 128.5, 128.62, 128.66, 135.6, 136.3, 137.3, 149.9, 163.7, 208.5(C6). MALDI-TOF MS m/z: [M + H]$^+$ 477.2, calcd 477.2. **11**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.38 (s, 3H, 5-CH_3), 2.14 (m, 2H, H8′ and H8″), 2.46 (m, 1H, H7′), 2.72–2.81 (m, 2H, H2′ and H7″), 3,93 (d, J = 11.7 Hz, 5′H), 4.05 (d, J = 11.7 Hz, 5″H), 4.44–4.60 (m, 5H, H3′ and BnCH_2), 5.98 (s, 1H, H1′), 7.22–7.35 (m, 10H), 8.01 (s, 1H, H6), 8.60 (s, 1H, N^3H). ^{13}C NMR ($CDCl_3$): δ11.7 (5-CH_3), 20.7(C8′), 34.5 (C7′), 43.4(C2′), 65.4 (C5′), 72.3 (Bn CH$_2$), 73.9 (Bn CH$_2$), 76.2 (C3′), 87.4 (C4′), 88.9 (C1′), 109.9 (C5), 127.4, 128.0, 128.2, 128.5, 128.6, 135.8, 136.7, 137.1, 150.1(C2), 163.7(C4), 205.6(C6). MALDI-TOF MS m/z: [M + H]$^+$ 477.2, calcd 477.2.

Synthesis of (1R, 4R, 5R, 7R, 8S)-8-benzyloxy-5-benzyloxmethyl-4-hydroxyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (12)

110 mg (0.23 mmol) of compound **11** was dissolved in 95% ethanol, $NaBH_4$ (17 mg, 0.46 mmol) was added in portions in 10 min. The mixture was allowed to stir at r.t. for 2h. The reaction mixture was diluted with saturated $NaHCO_3$, and extracted with DCM. The separated organic phase was dried over $MgSO_4$ and applied to

short column chromatography (EtOAc/cyclohexane 2/8 to 4/6) to give 90 mg of compound **12** (81%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.44(s, 3H, 5-CH_3), 1.79–2.11 (m, 4H, H7′, H7″, H8′, H8″), 2.67 (m, 1H, H2′), 3.67(dd, 1H, $J_{6'H,6'OH}$ = 11.7 Hz, $J_{6'H,7''}$ = 3.2 Hz), 3.81 (d, 1H, $J_{5'H,5''H}$ = 11.0 Hz, H5′), 3.90 (d, 1H, $J_{6'OH,6'H}$ = 11.9 Hz, H6-OH), 4.14 (d, 1H, $J_{5''H,5'H}$ = 11.3 Hz, H5″), 4.40 (d, 1H, $J_{3'H,2'H}$ = 11.7 Hz, H3′), 4.45–4.63 (m, 4H, BnCH_2), 5.75 (s, 1H, H1′), 7.26–7.79 (m, 10H), 7.97(s, 1H, H6), 8.76(s, 1H, N^{3}H).). ^{13}C NMR (500 MHz, $CDCl_3$): δ11.8 (5-CH_3), 17.3 (C8′), 27.2 (C7′), 42.4 (C2′), 69.1 (C5′), 71.7 (C6′), 73.2 (Bn CH_2), 73.7 (Bn CH_2), 75.1 (C3′), 82.2 (C4′), 87.2 (C1′), 109.5 (C5), 127.8, 127.9, 128.1, 128.5, 128.6, 128.7, 136.1, 136.2, 137.3, 150.2 (C2), 163.9 (C4).MALDI-TOF MS *m*/*z*: [M + H]$^+$ 479.6, calcd 479.2.

Synthesis of (1R, 4R, 5R, 7R, 8S)-8-benzyloxy-5-benzyloxmethyl-4-(*O*-phenoxythiocarbonyl)-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (13)

80 mg (0.16 mmol) of **12** was coevaporated with dry pyridine twice and dissolved in the same solvent. Phenyl chlorothionoformate (45 ul, 0.32 mmol) was added at r.t. and stirring at r.t. 2h. Then the solvent was evaporated and the residue was diluted with dichloromethane, washed with $NaHCO_3$, brine in turn, dried over $MgSO_4$, applied to short column chromatography (EtOAc/cyclohexane 1/9 to 3/7) to give 61 mg of compound **13** (62%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (s, 3H, 5-CH_3), 1.84 (m, 1H, 8′He), 2.20(m, 2H, H7′, H7″), 2.30 (m, 1H, 8′Ha), 2.69 (s, 1H, H2′), 3.93 (dd, 2H, *J* = 10.7 Hz, H5′ and 5″), 4.41 (d, 1H, *J* = 3.7 Hz, H3′), 5.44 (s, 1H, H6′), 5.84 (s, 1H, H1′), 7.12–7.47 (m, 15H), 7.92 (s, 1H, H6), 8.49 (s, 1H, N^{3}H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 11.8 (5-CH_3), 17.7 (C8′), 23.3 (C7′), 42.7 (C2′), 69.1 (C5′), 72.1 (Bn CH_2), 73.1 (C3′), 73.8 (Bn CH_2), 80.1 (C6′), 82.7 (C4′), 87.1 (C1′), 109.6 (C5), 121.9, 126.6, 127.1, 127.9, 128.2, 128.3, 128.6, 129.5, 135.9, 137.1, 137.7, 150.0 (C2), 153.4, 163.7 (C4), 194.8 (PhO*C*(S)O). MALDI-TOF MS *m*/*z*: [M + H]$^+$ 615.2, calcd 615.2.

Synthesis of (1R, 5R, 7R, 8S)-8-benzyloxy-5-benzyloxmethyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (14)

40 mg (0.065 mmol) of compound **13** was coevaporated with dry toluene twice and dissolved in the same solvent (2 ml), to which was purged dry N_2 for 10 min. Bn_3SnH (50 μl, 0.18 mmol) and AIBN (5 mg) was added. The mixture was heated under reflux for 1h. Then evaporating the solvent and the residue was subjected to short column chromatography (EtOAc/cyclohexane 1/9 to 3/7) to give 22 mg of compound **14** (72%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.34 (dd, 1H, H6′), 1.43 (s, 3H, 5-CH_3), 1.72 (m, 3H, H7′, H7″, H8′), 1.83 (m, 1H, H6″), 1.92 (m, 1H, H8″), 2.58 (s, 1H, H2′), 3.54 (d, 1H, *J* = 11.0 Hz, H5′), 3.67(d, 1H, *J* = 11.0 Hz, H5″), 4.15 (d, 1H, *J* = 4.9 Hz, H3′), 4.44–4.61 (m, 4H, BnCH_2), 5.82 (s, 1H, H1′), 7.26–7.36 (m, 10H), 8.06 (s, 1H, H6), 8.56 (s, 1H, N^3*H*). ^{13}C NMR ($CDCl_3$): δ 11.8 (C5-CH_3), 17.7 (C7′), 20.7 (C8′), 26.8 (C6′), 42.8 (C2′), 70.7 (C5′), 71.7(Bn CH_2), 72.9 (C3′), 73.5 (Bn CH_2), 85.4 (C4′), 87.5 (C1′), 109.1 (C5), 127.3, 127.8, 127.8, 128.0, 128.4, 128.6, 136.5, 137.5, 137.7, 150.1(C2), 164.0(C4). MALDI-TOF MS *m*/*z*: [M + H]$^+$ 463.8, calcd 463.2.

Synthesis of (1R, 5R, 7R, 8S)-8-hydroxy-5-hydroxymethyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (1)

20 mg (0.043 mmol) of compound **14** was dissolved in methanol (2 mL), to which ammonium formate (168 mg) and 20% $Pd(OH)_2$/C (68 mg) was added. The reaction mixture was heated under reflux for 1 h and filtered through celit bar. Evaporation of the solvent gave 11 mg of pure product **1** (90%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.12 (dd, 1H, $J_{6'He,7'Ha}$ = 4.0 Hz, $J_{6'He,6'Ha}$ = 13.1 Hz, 6′He), 1.54 (m, 1H, 8′He), 1.55–1.64 (m, 3H, 7′He, 7′Ha and 6′Ha), 1.87 (m, 1H, 8′Ha), 2.16 (broad, 1H, H2′), 3.41 (dd, 1H, $J_{5'H,5''}$ = 12.3 Hz, $J_{5'H,5'OH}$ = 4.4 Hz), 3.48 (dd, 1H, $J_{5'H,5''}$ = 12.3 Hz, $J_{5''H,5'OH}$ = 4.4 Hz), 4.1 (broad, 1H, H3′), 5.24 (d, 1H, $J_{3'H,3'OH}$ = 4.0 Hz, 3′OH), 5.31 (broad, 1H, 5′OH), 5.61 (s, 1H, H1′), 8.30 (s, 1H, H6), 11.22 (broad, 1H, N^3*H*). ^{13}C NMR (DMSO-d_6): δ12.3 (C5-CH_3), 17.4 (C7′), 20.1 (C8′), 25.4 (C6′), 44.9 (C2′), 61.4 (C5′), 64.0 (C3′), 85.8 (C4′), 86.1 (C1′), 107.0 (C5), 136.3 (C6), 150.1 (C2), 163.9 (C4).MALDI-TOF MS *m*/*z*: [M + H]$^+$, found 282.9, calcd 283.1.

Theoretical calculations

The geometry optimizations of the carba-ENA-T (**1**) have been carried out by GAUSSIAN 98 program package[25] 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[23,24] taking into account β substituent correction in form:

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

where 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.[23]), and $\Delta\chi_i^{group} = \Delta\chi_i^{\alpha-substituent} - P_7 \Sigma \Delta\chi_i^{\beta-substituent}$ where $\Delta\chi_i$ are taken as Huggins electronegativities.[27]

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