Supporting information — part (IV) of (IV)

Fine Tuning of Electrostatics Around the Internucleotidic Phosphate through Incorporation of Modified 2', 4'-Carbocyclic-LNA and –ENAs Leads to Significant Modulation of Antisense Properties

Chuanzheng Zhou, Yi Liu, Mounir Andaloussi, Naresh Badgujar, Oleksandr Plashkevych, Jyoti Chattopadhyaya*

Department of Bioorganic Chemistry, Box 581, Biomedical Center,
Uppsala University, SE-751 23 Uppsala, Sweden
jyoti@boc.uu.se

Contents

Discussion about the synthesis of compounds 25-48 (Scheme 4 and 5).

The aldehyde **6** was treated with allyltributyltin in the presence of $MgBr_2 \cdot Et_2O$[41] to give hydroxyl olefine **25** in 93% yield. Compound **25** was subjected to acetolysis followed by a modified Vorbruggen-type coupling, as for compounds **8** and **9** in Scheme 2, to give thymine nucleoside **27** in 88% yield in two steps. Selective deacetylation of 2′-*O*-acetyl group could be done successfully by treating compound **27** with 30% methylamine solution in ethanol at room temperature for 1h to give **28** quantitatively. Esterification of **28** using phenyl chlorothioformate, as for transformation of compound **10** to **11** in Scheme 2, yielded the ester **29** (85%), which was subjected to free radical cyclization utilizing tributyltin hydride in the presence of AIBN to give exclusively 6-*exo* heptenyl cyclization product **30**. The NOESY experiment showed (Fig. SIII.9) the C6′-OH group has the equatorial orientation, which means that the Grignard reaction leading to **25** has the ***S*** configuration at the C6′ center. Deacetylation of **30** was carried out in 30% methylamine solution in ethanol at room temperature overnight to give **31** in 88% yield. The inversion of the equatorial C6′-OH to axial 6′-OH in compound **31** was achieved in high yield (73%) by a two-step procedure involving Dess-martin oxidation (to give **32** as the intermediate) followed by reduction with $NaBH_4$. In ketone **32**, the 8′-methyl group is equatorial, and is sterically far apart from the 6′-keto function, so the bukly 8′-methyl does not impart any stereoselectivity on the reduction at the C6′ center. Since the H^- attack took place on the 6′-keto in **32** from the opposite side of 3′-*O*-Bn to give **36** as the sole product, it was therefore likely to be controlled by the steric bulk of 3′-*O*-Bn group.

Using the same strategy for methylating carba-LNA (shown in Scheme 3), methylated carba-ENA was synthesized. The ketone **32** was first methylated with methyl magnesium iodide to give bifunctional methyl/hydroxyl product at C6′. The methyl anion also attacked the ketone from the opposite side of 3′-*O*-Bn selectively to give only one isomer **33** (6′-***R***) in high yield (90%). Compound **33** was subjected to radical deoxygenation to give two diastereomers of 6′-methyl carba-ENA, **35a** and **35b** (yield 94%, **35a**/**35b** = 1/5).

The 6′-hydroxyl group in diastereomerically pure **31** and **36** was protected with *p*-toluoyl as for compounds **15a-d** in Scheme 2 to give **37** and **38** in 86% and 75% yield respectively. Migration of 6′-*O*-(*p*-tolyl) to primary 5′-OH was observered for both compounds **37** (6′-***S***) and **38** (6′-***R***) during the debenzylation under catalytic hydrogenolysis although they have opposite configuration at C6′. Conformational analysis showed that the $\phi_{C5'\text{-}C4'\text{-}C6'\text{-}O6'}$ are 50º and 51º respectively in compounds **37**

and **38** (Fig SIII.26.) and these very similar torsion angles make migration of 6′-*O*-(*p*-tolyl) to primary 5′-OH geometrically favored in both compounds **37** and **38** during the debenzylation. Just like the debenzylation of compounds **15c** and **15d** (Scheme 2), this side reaction was suppressed by increasing the hydrogenolysis reagents, and by shortening the reaction time. Subsequently, all the C6′ functionalized carba-ENA compounds **37**, **38**, **33**, **35a** and **35b** were transferred to their respective phosphoramdites **44**-**48** by three successive steps (Scheme 5): (i) debenzylation with 20% $Pd(OH)_2$/C and ammonium formate, (ii) 5′-dimethoxytritylation, and (iii) 3′-phosphitylation with 2-cyanoethyl *N*, *N*-diisopropyl phosphoramidochloridite. Here, selective phosphitylation of 3′-OH in compound **41** was also achieved successfully, as from **19a/b** to **20a/b** in Scheme 2, to give phosphoramidite **46** (60%).

Discussion of configuration determination on chiral center by nOe experiments:

The orientation of substituents in the carbocyclic moiety of compound **12a/b**, **14a/b**, **18a/b**, **22a/b**, **30**, **33**, **35a/b** and **36** were assigned by nOe experiments (1D-nOe or 2D ROESY, see Fig. SIII.1 to SIII.13). In compounds **12a**, **14a**, **18a/b**, **22a/b**, the 7′-Me showed strong nOe enhancement (~5-7 %) upon irradiation of H1′, but irradiation of H1′ do not show any nOe enhancement with H7′, suggesting that 7′-Me group in these compound are in close proximity of H1′, and hence (***S)***-configuration could be assigned for C7′. In contradistinction, the nOe enhancement of 5% for H7′ was observed upon irradiation of H1′ in **12b** and **14b**, confirming the ***R***-configuration for C7′. Irradiation of H6′ in compounds **12a** or **14b** leads to strong nOe enhancement (4-7%) for their H7′, but not for 7′-Me, suggesting that the H6′ and H7′ are *cis* to each other, and hence ***S***- and ***R***-configuration are assigned for C6′ for **12a** and **14b** respectively. By comparing the observation that irradiation on H7′ in **18a** leads to nOe enhancement for 6′-Me (3%, Fig. SIII.5) and none for 6′-OH, but, in contradistinction, in **18b** leads to strong nOe enhancement (5%, Fig. SIII.6) for 6′-OH and none for 6′-Me, it was concluded that the C6′ is ***S*** in **18a**, but ***R*** in **18b**. Similarly, (***S)***- and (***R)***-configuration for C6′ in **22a** and **22b** respectively could be shown by irradiation of H7′ in that the former (Fig. SIII.7) showed stronger nOe enhancement for H6′ (4%) than the latter and, *vice versa*, with 6′-Me in **22b** (Fig. SIII.8).

For functionalized carba-ENAs **30**, **33**, **35a/b**, **36** the observation of strong nOe between 8′-Me with H1′ together with weaker nOe between H8′ and H1′ suggested the (***S)***-configuration for C8′ (Fig. SIII.9 to SIII.13). In 2D ROESY spectrum, H8′ show medium nOe effect with H6′ in compound **30** (Fig SIII.9), and nOe effect with 6′-OH in compound **36** (Fig. SIII.13), suggesting, C6′ is ***S*** in **30**, and ***R*** in **36**. For compound **33**, irradiation on H7′′ (axial proton attached to C7′) led to nOe enhancement

for 6′-Me and 8′-Me (Fig. SIII.10), while irradiation on H7′ (equatorial proton attached to C7′) led to nOe enhancement for H8′ and 6′-OH, which suggested ***R***-configuration at C6′. The C6′ configuration for compounds **35a** and **35b** can be similarly assigned by irradiation of H7″ (axial proton attached to C7′). For **35a** (Fig. SIII.11), nOe enhancement for 6′-Me (2.6%) and 8′-Me (2.9%) were observed while for **35b** (Fig. SIII.12), nOe enhancement for H6′ (2.7%) and 8′-Me (2.4%) were observed. Hence, ***S*** and ***R*** stereochemistries were assigned for C6′ of **35a** and **35b** respectively.

Discussion about oligonucleotides synthesis and purification.

Standard DNA synthesis cycle (1 μmol scale) and common reagents were used. For incorporating modified nucleotides, a coupling time of 10 min were used. All the modified building blocks except **20b** and **46** gave satisfactory coupling yield (40-80%). **20b** and **46** only gave 1-5% of coupling yields even when longer coupling time (15-20 min) was used. In phosphoramidites **20b** and **46**, the unprotected 6′-OH can attack the 3′- *O*- phosphoramidite in the presence of tetrazole to form 6-membered dioxaphosphorinane ring, and this intramolecular reaction prevails over the intermolecular coupling, which could cause the low coupling yields for compounds **20b** and **46**.

The selected 15mer DNA sequence is targeted to the coding region of SV40 large T antigen. The sequences, modification site and structure of modification are shown in Table 1. For AONs **1-5**, **22-36** and **45-52**, cleavage from the support and deprotection was carried out by just treating the solid support with 33% aqueous ammonia at room temperature for 12 h, while for AONs **6-21** and **37-44**, deprotection of the 6′-*O*-(*p*-toluoyl) group was found very difficult. The full deprotection was realized by incubating the solid support with 33% aqueous ammonia at 55˚C for 72h. The fully deprotected AONs were then purified by 20% denatured PAGE and their integrity was confirmed by MALDI-TOF mass measurement (Table 1).

Discussion about how the position and orientation of substitutents on carbocyclic moiety affects Tm values.

The T_m values in Table 1 show that methylation at C7′ of carba-LNA or C8′ of carba-ENA results in much more pronounced effect than methylation at C6′. The global conformation of resulting DNA-RNA duplex containing LNA[1] and ENA[2,3] modification resembles the A-form of RNA more

than the B-form of DNA.[4] In the RNA-RNA or DNA-RNA duplexes, a water network can be formed in the minor groove by the 2′-OH with heteroatom of the nucleobase, O3′, and O5′, O4′, and phosphate anion oxygen of the adjacent 3′-end nucleotides directly or through water bridge.[5,6] Extensive hydration of individual hydrogen bond acceptors and donors in oligoucleotides generally increase thermodynamic stability of the corresponding duplexes.[5,7] The unusually extensive hydration pattern in LNA[8,9] or 2′-MOE[10] integrated duplex has been shown to contribute substantially to the stability of the duplex. In the 7′-methyl carba -LNA and 8′-methyl carba -ENA, the bulky methyl, locating in the central of the minor groove of DNA·RNA duplex, will disturb the hydration network in the minor groove. This is especially so when it directs at the 3′-phoshate, it can also break the hydrogen bond network around the sugar-phophate backbone considerably. On the other hand, the steric effect imparted by 7′ or 8′ methyl can also contribute to the duplex instability, but since the AON/RNA duplex has relatively wide minor groove, the steric effect of the substitutents at C7′ of carba-LNA or C8′ of carba-ENA may not be so significant. So, it is very likely that the subsitituents at C7′ of carba-LNA or C8′ of carba-ENA exert their effect on AON/RNA duplex stability predominantly by changing the hydration pattern in the minor groove and the backbone. In the AON/DNA duplex which has a relatively narrow and shallow minor groove, the steric effect should be much more pronounced and it is indeed found to be the case. For example, the carba-ENA U destabilizes AON/DNA duplex by -1˚C /per modification,[3] while 8′-methyl carba-ENA T (type **IX** in Table 1) give a larger decrease, -5 ˚C per modification. This large difference should be attributed in part to the steric clash resulting from the 8′-methyl group in that the narrow groove have to be extended slightly, which results in a local or global conformational change, to accommodate the bulky methyl at the cost of the thermal stability.

Comparing to the 7′ substituents at carba-LNA or 8′substitution at carba-ENA which are located at the center of minor groove of AON/RNA duplex, substituents at 6′ of carba-LNA and carba-ENA are located at the border of grooves, close to phosphate linkage. 6′-methyl does not destabilize the AON/RNA duplex greatly, which means that the hydration is not the predominant factor in this case modulating the thermal stability. It is known that anionic phosphate oxygens are the most hydrated atoms in nucleotide[11,12] and each charged oxygen is hydrated by three hydration sites. The strong ability of hydration of anionic phosphate oxygens makes their hydration pattern much more difficult to be interrupted by surrounding substituents, which could be the reason why we see an insignificant T_m variation by 6′-hydrophobic substitution. When the 6′-methyl points at the 3′-phosphate linkage it can even stabilize the AON/RNA duplex. This unusual observation can be

interpreted by the steric effect. The steric clash between the bulky 6′-methyl and 3′-phosphate could drive the flexible phosphate to adopt a more rigid conformation, and hence formation of duplex is entropically less unfavorable. This result also implies that nucleotide with both conformationally constrained sugar and phosphate moiety could have unusual higher affinity toward complementary oligonucleotides, which is in progress in our lab.[13]

Individual reaction steps, work-up and the product characterization for compounds 13b, 14b, 15b/c/d, 16b/c/d, 17a/b/c/d, 19a/b, 20a/b, 23a/b, 24a/b, 25 – 48.

(1R, 3R, 4R, 5R, 7S)-7- benzyloxy-1-benzyloxmethyl-5-methlyl-6-one-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1] heptane(13b). Compound **12b** (342 mg, 0.43 mmol) was dissolved in anhydrous CH_2Cl_2, Dess-Martin periodinane (15% in CH_2Cl_2, 1.8 ml, 0.85 mmol) was added and stirred at room temperature for 2h. Then diluting the reaction mixture with CH_2Cl_2, filtered through celite bar, the filtrate was washed with aqueous $Na_2S_2O_3$ twice, saturated $NaHCO_3$ once and NaCl once. After anhydrousing over $MgSO_4$, it was applied to silica gel short column chromatography (EtOAc/cyclohexane 2/8-4/6) to give 248 mg of **13b** (73 %). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.76 (1H, s, N<u>H</u>), 7.72 (1H, s, H6), 7.20-7.33 (10H, m, Bn), 5.54 (1H, s, H1′), 4.61-4.49 (4H, m, BnC<u>H</u>$_2$), 4.18 (s, 1H, H3′), 4.0 (d, J_{gem} = 11.7 Hz, 5′H), 3.90 (d, J_{gem} = 11.7 Hz, 5′′H), 2.99 (1H, s, H2′), 2.46 (1H, m, H7′), 1.49 (3H, s, T-C<u>H</u>$_3$), 1.36 (3H, d, $J_{7'CH3,\ 7'H}$ = 7.6 Hz, 7′C<u>H</u>$_3$). ^{13}C NMR (600 MHz, $CDCl_3$): δ 208.5 (C=O), 163.7 (C4), 149.9 (C2), 137.3, 136.3, 135.6, 128.66, 128.62, 128.5, 128.24, 128.21, 127.9, 127.5, 109.9, 88.5 (C1′), 74.1 (Bn<u>C</u>H$_2$), 86.1 (C4′), 72.5 (Bn<u>C</u>H$_2$), 63.3 (C5′), 49.6 (C2′), 43.1 (C7′), 14.3 (7′<u>C</u>H$_3$), 12.0 (T-<u>C</u>H$_3$). MALDI-TOF *m/z*: $[M+H]^+$ 477.2, calcd 477.2.

(1R, 3R, 4R, 5R, 6R, 7S)-7- benzyloxy-1-benzyloxymethyl-6-hydroxyl-5-methly-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1] heptane(14b). The ketone **13b** (110 mg, 0.23 mmol) was dissolved in 95% ethanol (3 mL) and $NaBH_4$ (10 mg, 0.26 mmol) was added. The mixture was allowed to stir at room temperature for 1h. Then solvent was removed and the residue was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$, dried over $MgSO_4$ and evaporated to give crude product, which was subjected to short column chromatography on silica gel (ethyl acetate in cyclohexane, 20-40% v/v) to give **14b** (100 mg, 92%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.60 (1H, broad, N<u>H</u>), 7.72 (s, 1H, H6), 7.36-7.21 (m, 10H, aromatic), 5.25 (1H, s, H1′), 4.66-4.43 (4H, m, BnC<u>H</u>$_2$), 4.13 (1H, d, J_{gem} = 11.4 Hz, H5′), 4.08

(1H, s, H3′), 4.03 (1H, d, J_{gem} = 11.4 Hz, H5″), 3.87 (1H, dddd, $^{3}J_{6'H,\ 6'OH}$ = 12.9 Hz, $^{W}J_{6'H,\ 3'H}$ = 1.6 Hz, $^{3}J_{6'H,\ 7'H}$ = 7.9 Hz, H6′), 2.78 (1H, d, $^{3}J_{6'H,\ 6'OH}$ = 12.9 Hz, 6′OH), 2.67 (1H, s, H2′), 2.42 (1H, m, H7′), 1.48 (3H, s, T-C$\underline{H}_3$), 1.36 (3H, d, $J_{7',\ 7'Me}$ = 7.6 Hz, 7′-Me). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.8 (C4), 149.9 (C2), 137.6, 136.1, 128.7, 128.6, 128.5, 128.1, 127.9, 109.3 (C5), 88.4 (C1′), 86.3 (C1′), 79.6 (C3′), 76.1 (C6′), 73.9, 73.05 (Bn-$\underline{C}H_2$), 66.1 (C5′), 47.7 (C2′), 38.8 (C7′), 12.1 (7′-Me), 11.9 (T-Me). MALDI-TOF *m/z*: $[M+Na]^+$ found 501.217, calcd 501.200.

(1R, 3R, 4R, 5R, 6S, 7S)-7-benzyloxy-1-benzyloxymethyl-5-methyl-6-(4-methylbenzoate)-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1]heptane (15b). The compound **12b** (485 mg, 1.01 mmol) was coevaporated with dry pyridine twice and dissolved in dry CH_2Cl_2 /pyridine (7/1). The mixture was cooled to 0 ˚C and 4-methyl benzoyl chloride (0.19 mL, 1.01 mmol) was added. Then it was allowed to stir at r.t. overnight. Pyridine was recovered under reduced pressure and the residue was diluted with CH_2Cl_2, washed with saturated solution of $NaHCO_3$, dried with $MgSO_4$ and concentrated under reduced pressure to give crude product, which was subjected to short column chromatography on silica gel (20-30 % ethyl acetate in cyclohexane, v/v) to give **15b** (410 mg, 69%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.05 (s, 1H, N$\underline{H}$), 7.86 (2H, d, J = 8.5 Hz, aromatic), 7.69 (1H, s, H6), 7.27-7.12 (12H, m, aromatic), 5.54 (1H, s, H1′), 5.21 (1H, d, J = 4.0 Hz, H6′), 4.56-4.39 (4H, m, C$\underline{H}_2$Bn), 4.04 (1H, s, H3′), 3.82 (2H, dd, J_{gem} = 11.5 Hz, H5′, H5″), 2.56 (1H, s, J = 3.5 Hz, H2′), 2.33 (3H, s, tol-C$\underline{H}_3$), 2.06 (1H, m, H7′), 1.35 (6H, m, T-C$\underline{H}_3$, 7′-C$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.7 (C=O), 164.1 (C4), 150.1 (C2), 144.1, 137.5, 137.2, 136.1, 129.8-127.5 (aromatic), 109.5 (C5), 88.5, 82.3, 79.2, 73.9, 72.5, 71.6, 66.0, 48.3, 42.1, 21.7 (Tol-Me), 18.2 (7′-Me), 11.9 (T-Me). MALDI-TOF *m/z*: $[M+H]^+$ found 597.2, calcd 597.2.

(1R, 3R, 4R, 5S, 6R, 7S)-7-benzyloxy-1-benzyloxymethyl-5-methyl-6-(4-methylbenzoate)-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1]heptane (15c). The compound **14a** (167 mg, 0.35 mmol) was coevaporated with dry pyridine twice and dissolved in dry pyridine (4 mL). The mixture was cooled to 0 ˚C and 4-methyl benzoyl chloride (0.06 mL, 0.42 mmol) was added. Then it was allowed to stir at r.t. 2 h. Recovered the pyridine under reduced pressure, added saturated solution of $NaHCO_3$, extracted with CH_2Cl_2. Organic layer was dried with $MgSO_4$ and concentrated under reduced pressure to give crude product, which was subjected to short column chromatography on silica gel (25-30 % ethyl acetate in cyclohexane, v/v) to give **15c** (175 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.64 (s, 1H,

NH), 7.80 (2H, d, *J* = 8.0 Hz, aromatic), 7.72 (1H, s, H6), 7.31-7.14 (12H, m, aromatic), 5.73 (1H, s, H1′), 4.77 (1H, d, *J* = 3.5 Hz, H6′), 4.62-4.54 (4H, m, CH_2Bn), 4.21 (1H, s, H3′), 3.98 (1H, d, J_{gem} = 11.0 Hz, H5′), 3.89 (1H, d, J_{gem} = 11.5 Hz, H5″), 2.82 (1H, d, *J* = 4.0 Hz, H2′), 2.76 (1H, m, H7′), 2.41 (3H, s, tol-CH_3), 1.53 (3H, s, T-CH_3), 1.44 (3H, d, *J* = 7.5 Hz, 7′-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.0 (C=O), 163.9 (C4), 149.8 (C2), 143.9, 137.6, 137.4, 136.1, 129.8, 129.1, 128.6, 128.4, 128.0, 127.9, 127.8, 127.5, 126.9, 109.5 (C5), 88.9 (C4′), 83.7 (C1′), 80.1 (C6′), 78.5(C3′), 73.9 (CH_2Bn), 72.0 (CH_2Bn), 65.7 (C5′), 48.1 (C2′), 38.8 (C7′), 21.7(Tol-Me), 13.4 (7′-Me), 11.9 (T-Me). MALDI-TOF *m/z*: $[M+H]^+$ found 597.2, calcd 597.2.

(1R, 3R, 4R, 5R 6R, 7S)-7-(benzyloxy)-1-((benzyloxy)methyl)-5-methyl-6-(4-methylbenzoate)-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1]heptane (15d). The compound **14a** (264 mg, 0.55 mmol) was coevaporated with dry pyridine twice and dissolved in dry pyridine (5 mL). The mixture was cooled to 0 ˚C and 4-methyl benzoyl chloride (0.094 mL, 0.72 mmol) was added. Then it was allowed to stir at r.t. overnight. Recovered the pyridine under reduced pressure, added saturated solution of $NaHCO_3$, extracted with CH_2Cl_2. Organic layer was dried with $MgSO_4$ and concentrated under reduced pressure to give crude product, which was subjected to short column chromatography on silica gel (20-30 % ethyl acetate in cyclohexane, v/v) to tive **15d** (207 mg, 63%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.71 (s, 1H, NH), 7.81 (2H, d, *J* = 8.0 Hz, aromatic), 7.75 (1H, s, H6), 7.38-7.12 (12H, m, aromatic), 5.38 (1H, s, H1′), 5.31 (1H, d, *J* = 7.8 Hz, H6′), 4.67-4.55 (4H, m, CH_2Bn), 4.15 (1H, s, H3′), 4.03 (1H, d, J_{gem} = 9.0 Hz, H5′), 3.98 (1H, d, J_{gem} = 10.8 Hz, H5″), 2.77 (1H, s, H2′), 2.68 (1H, m, H7′), 2.42 (3H, s, tol-CH_3), 1.53 (3H, s, T-CH_3), 1.23 (3H, d, *J* = 7.2 Hz, 7′-CH_3); ^{13}C NMR (125 MHz, CDCl3): δ 166.3 (C=O), 164.3 (C4), 150.4 (C2), 144.4, 137.9, 137.7, 136.4, 130.3, 129.6, 128.9, 128.8, 128.5, 128.3, 128.2, 127.9, 127.2, 109.8 (C5), 88.8 (C4′), 87.6 (C1′), 79.4, 75.4, 74.4, 72.9, 66.3 (C5′), 48.6 (C2′), 39.4 (C7′), 22.1 (Tol-Me), 13.7 (7′-Me), 12.4 (T-Me). MALDI-TOF *m/z*: $[M+H]^+$ found 597.2, calcd 597.2.

(1R, 3R, 4R, 5R, 6S, 7S)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hyroxyl -5-methyl-6-(4-methylbenzoate)-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1]heptane (16b). To a solution of compound **15b** (494 mg, 0.83 mmol) in dry methanol (10 mL) was added 20% $Pd(OH)_2$/C (1 g) and ammonium formate (2 g) and reflux for 4h. The suspension was filtered over celite bar and organic phase was evaporated to give crude **15b′** which was co-evaporated twice with dry pyridine and dissolved in the

same solvent. 4,4′-Dimethoxytrityl chloride (302 mg, 0.89 mmol) was added and stirred overnight at r.t.. The solvent was removed. The residue was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$, dried over $MgSO_4$, evaporated and applied to column chromatography on silica gel (methanol in CH_2Cl_2 containing 1% pyridine, 0.5-1.5%, v/v) to obtain **16b** (372 mg, 63 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.93 (2H, d, *J* = 8.5 Hz, aromatic), 7.83 (1H, s, H6), 7.47-6.83 (15H, m, aromatic), 5.57 (1H, s, H1′), 5.18 (1H, d, *J* = 3.5 Hz, H6′), 4.36 (1H, s, H3′), 3.78 (6H, s, MeO), 3.73 (1H, dd, J_{gem} = 11.5 Hz, H5′), 3.60 (1H, dd, J_{gem} = 11.5 Hz, H5″), 2.55 (1H, s, H2′), 2.44 (3H, s, tol-C$\underline{H}_3$), 2.09 (1H, m, H7′), 1.48 (3H, d, *J* = 7.5 Hz, 7′-C$\underline{H}_3$), 1.45 (3H, s, T-C$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.5, 163.8, 158.7, 149.9 (C2), 144.3,, 143.9, 135.4, 135.3, 130.0, 129.8, 129.1, 128.1, 128.0, 127.2, 127.0, 113.4, 109.6 (C5), 88.9 (C1′, C4′), 87.3, 82.2 (C6′), 74.4 (C3′), 59.2 (C5′), 55.2, 46.9 (C7′), 42.6 (C2′), 21.7 (Tol-Me), 18.4 (7′-Me), 12.3. MALDI-TOF *m/z*: $[M+K]^+$ found 757.3, calcd 757.3.

(1R, 3R, 4R, 5S, 6R, 7S)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hyroxyl -5-methyl-6-(4-methylbenzoate)-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1]heptane (16c). To a solution of compound **15c** (163 mg, 0.27 mmol) in dry methanol (5 mL) was added 20% $Pd(OH)_2$/C (135 mg) and ammonium formate (340 mg, 5.4 mmol) and reflux for 2h. Then the same amout of 20% $Pd(OH)_2$/C and ammonium formate was added and reflux for another 2 h. The suspension was filtered over celite and organic phase was evaporated. The residue was applied to column chromatography on silica gel (methanol in CH_2Cl_2 containing 1% pyridine, 0.5-4%, v/v) to give **15c′** (64 mg, 56%) and a side product **15c″** (38 mg) which was formed by transfer the 6′-O-tolyl to primary 5′-OH. **15c′**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.86 (2H, d, *J* = 7.2 Hz, aromatic), 7.80 (1H, s, H6), 7.18 (2H, d, *J* = 7.8 Hz, aromatic), 5.63 (1H, s, H1′), 4.69 (1H, s, H6′), 4.33 (1H, s, H3′), 4.05 (2H, dd, H5′, H5″), 2.83 (1H, m, H7′), 2.72 (1H, s, H2′), 2.37 (3H, s, tol-C$\underline{H}_3$), 1.82 (3H, s, T-C$\underline{H}_3$), 1.41 (3H, d, *J* = 7.2 Hz, 7′-C$\underline{H}_3$). ^{13}C NMR (150 MHz, $CDCl_3$): δ 166.2, 164.8 (C4), 150.1 (C2), 144.3, 136.6 (C6), 129.7, 129.2, 126.7, 109.6 (C5), 88.8 (C4′), 83.7 (C1′), 80.3 (C6′), 72.2 (C3′), 57.7 (C5′), 49.9(C2′), 38.7 (C7′), 21.6 (Me-Tol), 13.3 (7′-Me), 12.5. MALDI-TOF *m/z*: $[M+H]^+$ found 417.1, calcd 417.1. The side product **15c″**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.99 (2H, d, *J* = 8.4 Hz, aromatic), 7.33 (1H, s, H6), 7.29 (2H, d, *J* = 8.4 Hz, aromatic), 5.54 (1H, s, H1′), 5.19 (1H, d, J_{gem} = 13.2 Hz, H5′), 4.61 (1H, d, J_{gem} = 13.2 Hz, H5″), 4.01 (1H, s, H3′), 3.42 (1H, s, H6′), 2.74 (1H, d, *J* = 3.6 Hz, H2′), 2.55 (1H, m, H7′), 2.43 (3H, s, tol-C$\underline{H}_3$), 1.45 (3H, s, T-C$\underline{H}_3$), 1.39 (3H, d, *J* = 7.2 Hz, 7′-CH_3). ^{13}C NMR (150 MHz, CDCl3): δ168.2, 163.9 (C4), 150.1 (C2), 145.2, 134.9 (C6), 130.0, 129.6, 126.0, 109.6 (C5), 87.1 (C4′), 83.7 (C1′), 80.9

(C6′), 73.7 (C3′), 61.2 (C5′), 49.1 (C2′), 41.8 (C7′), 21.7 (Me-Tol), 13.3 (7′-Me), 11.9 (T-Me). MALDI-TOF *m/z* : $[M+H]^+$ found 417.1, calcd 417.1. 50 mg (0.12 mmol) of **15c′** was co-evaporated twice with dry pyridine and dissolved in the same solvent (3 mL). 4,4′-Dimethoxytrityl chloride (50 mg, 0.15 mmol) was added and stirred 1h at r.t. and then another portion of 4,4′-Dimethoxytrityl chloride (50 mg, 0.15 mmol) was added and stirred futher 3 h at r.t.. The solvent was removed and obtained residue was diluted with CH_2Cl_2, washed with saturatured $NaHCO_3$, dried over $MgSO_4$, evaporated and applied to column chromatography on silica gel (methanol in CH_2Cl_2 containing 1% pyridine, 0.5-1.5%, v/v) to obtain **16c** (72 mg, 83 %). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.52 (1H, s, H3), 7.75 (1H, s, H6), 7.63 (2H, d, J = 7.8 Hz, aromatic), 7.44-6.76 (15H, m, aromatic), 5.70 (1H, s, H1′), 4.71 (1H, s, H6′), 4.47 (1H, s, H3′), 3.78 (6H, s, MeO), 3.69 (1H, d, J_{gem} = 11.4 Hz, H5′), 3.53 (1H, d, J_{gem} = 11.4 Hz, H5″), 2.77 (1H, d, J = 4.2 Hz, H2′), 2.69 (1H, m, H7′), 2.41 (3H, s, tol-C<u>H</u>$_3$), 1.67 (3H, s, T-C<u>H</u>$_3$), 1.48 (3H, d, J = 7.25 Hz, 7′-C<u>H</u>$_3$); ^{13}C NMR (150 MHz, $CDCl_3$): δ165.3, 163.7, 158.6, 149.8 (C2), 144.3, 144.0, 135.5, 135.4, 135.3, 130.1, 129.9, 129.6, 129.1, 128.0, 127.1, 126.7, 113.3, 109.8 (C5), 87.9, 86.8, 83.9, 80.1, 73.5, 58.8, 55.2, 50.2, 38.9, 21.7, 13.4, 12.6. MALDI-TOF *m/z*: $[M+K]^+$ found 757.2, calcd 757.3.

(1R, 3R, 4R, 5R, 6R, 7S)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hyroxyl -5-methyl-6-(4-methylbenzoate)-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1]heptane (16d). To a solution of compound **15a** (124 mg, 0.21 mmol) in dry methanol (4 mL) was added 20% $Pd(OH)_2/C$ (0.3 g) and ammonium formate (0.76 , 12 mmol) and reflux for 1.5 h. The suspension was filtered over celite bar and organic phase was evaporated to give **15d′** which was co-evaporated twice with dry pyridine and dissolved in the same solvent. 4,4′-Dimethoxytrityl chloride (90 mg, 0.26 mmol) was added and stirred 3h at r.t.. The solvent was removed and residue was diluted with CH_2Cl_2, washed with saturatured $NaHCO_3$, dried over $MgSO_4$, evaporated and applied to column chromatography on silica gel (methanol in CH_2Cl_2 containing 1% pyridine, 0.5-1.5%, v/v) to obtain **16d** (101 mg, 68 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.71 -6.75 (18H, m), 5.30 (2H, m, H1′, H6′), 4.38 (1H, d, J = 4.0 Hz, H3′), 3.74-3.57 (9H, m, MeO, H5′, H5″, 3′-OH), 2.67 (2H, m, H2′, H7′), 2.42 (3H, s, tol-C<u>H</u>$_3$), 1.68 (3H, s, T-C<u>H</u>$_3$), 1.19 (3H, d, J = 7.5 Hz, 7′-C<u>H</u>$_3$); ^{13}C NMR (125 MHz, CDCl3): δ165.3, 163.7, 158.7, 149.7 (C2), 144.2, 144.1, 135.4, 135.3, 135.2, 130.0, 129.9, 129.7, 129.2, 128.05, 128.00, 127.0, 126.5, 113.3, 109.7 (C5), 88.7 (C1′), 87.5, 86.8 (C4′), 75.4 (C6′), 74.2 (C3′), 58.9 (C5′), 55.2, 50.5 (C2′), 39.0 (C7′), 21.7 (Me-Tol), 13.5 (7′-Me), 12.2 (T-Me). MALDI-TOF *m/z*: $[M+K]^+$ found 757.3, calcd 757.3.

(1R, 3R, 4R, 5S, 6S, 7S)- 1-(4,4′-Dimethoxytrityloxymethyl) –6,7-dihyroxyl-5, 6-dimethyl-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1] heptane (19a). To a solution of compound **18a** (189 mg, 0.386 mmol) in dry methanol (5 mL) was added 20% $Pd(OH)_2/C$ (0.58 g) and ammonium formate (1.45 g, 23.2 mmol) and reflux for 1.5h. The suspension was filtered over celite and organic phase was evaporated. The residue was co-evaporated twice with dry pyridine and dissolved in the same solvent. 4,4′-Dimethoxytrityl chloride (130 mg, 0.38 mmol) was added and stirred 2h at r.t.. The solvent was removed. The residue was diluted with CH_2Cl_2, washed with saturatured $NaHCO_3$, dried over $MgSO_4$, evaporated and applied to column chromatography on silica gel (methanol in CH_2Cl_2 containing 1% pyridine, 1-3%, v/v) to obtain **19a** (199 mg, 84 %). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.79 (1H, s, H6), 7.48-6.86 (13H, m, aromatic), 5.70 (1H, s, H1′), 4.28 (1H, d, J = 1.5 Hz, H3′), 3.81 (6H, s, MeO), 3.72 (1H, d, J_{gem} = 11.5 Hz, H5′), 3.56 (1H, d, J_{gem} = 11.5 Hz, H5″), 2.73 (1H, s, 6′-OH), 2.50 (1H, m, H2′), 2.43 (1H, m, H7′), 1.59 (3H, s, T-C$\underline{H_3}$), 1.85 (3H, s, 6′-CH_3), 1.13 (3H, d, J = 7.5 Hz, 7′-C$\underline{H_3}$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 163.9, 158.7, 149.8, 144.4 (C2), 135.6, 135.5, 135.4, 130-127.2 (aromatic), 113.4, 109.8 (C5), 90.2, 87.2, 83.0 (C1′), 77.2 (C6′), 71.8 (C3′), 58.3 (C5′), 55.2, 50.7(C2′), 39.7(C7′), 24.1 (6′-Me), 12.3 (T-Me), 8.1 (7′-Me). MALDI-TOF *m/z* : $[M+Na]^+$ found 637.3, calcd 637.3.

(1R, 3R, 4R, 5S, 6R, 7S)- 1-(4,4′-Dimethoxytrityloxymethyl) –6,7-dihyroxyl-5, 6-dimethyl-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1] heptane (19b). To a solution of compound **18b** (230 mg, 0.46 mmol) in dry methanol (5 mL) was added 20% $Pd(OH)_2/C$ (0.70 g) and ammonium formate (1.74 g, 27.6 mmol) and reflux for 3h. The suspension was filtered over celite bar and organic phase was evaporated. The residue was co-evaporated twice with dry pyridine and dissolved in the same solvent. 4,4′-Dimethoxytrityl chloride (171 mg, 0.50 mmol) was added and stirred 2h at r.t.. The solvent was removed. The residue was diluted with CH_2Cl_2, washed with saturatured $NaHCO_3$, dried over $MgSO_4$, evaporated and applied to column chromatography on silica gel (methanol in dichloromethane containing 1% pyridine, 1-3%, v/v) to obtain **19b** (180 mg, 63 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83 (1H, s, H6), 7.49-6.85 (13H, m, aromatic), 5.51 (1H, s, H1′), 4.46 (1H, s, H3′), 3.81 (7H, m, H5′, MeO), 3.67 (1H, d, J_{gem} = 11.5 Hz, H5′), 2.64 (1H, d, J = 3.5 Hz, H2′), 2.55 (1H, m, H7′), 1.56 (3H, s, T-C$\underline{H_3}$), 1.19 (3H, d, J = 7.5 Hz, 7′-C$\underline{H_3}$), 1.08 (3H, s, 6′-C$\underline{H_3}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.9, 159.1, 149.8, 145.0 (C2), 135.9, 135.8, 130.5-127.5 (aromatic), 113.8, 109.8 (C5), 88.6, 87.2, 83.4,

79.7, 73.5, 59.0, 55.6, 51.5, 44.4, 17.5, 12.2 (T-Me), 10.5 (7′-Me). MALDI-TOF *m/z*: $[M+Na]^+$ found 637.2, calcd 637.3.

(1R, 3R, 4R, 5S, 6S, 7S)- 1-(4,4′-Dimethoxytrityloxymethyl)-5, 6-dimethyl-7-hydroxyl-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1] heptane (23a) and (1R, 3R, 4R, 5S, 6R, 7S)- 1-(4,4′-Dimethoxytrityloxymethyl)-5, 6-dimethyl-7-hydroxyl-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1] heptane (23b). A mixture of **22a** and **22b** (64 mg, 0.135 mmol) was dissolved in dry methanol (2 mL), to which was added 20% $Pd(OH)_2/C$ (0.14 g) and ammonium formate (0.34 g, 2.7 mmol) and reflux for 1.5h. The suspension was filtered over celite and organic phase was evaporated. The residue was co-evaporated twice with dry pyridine and dissolved in the same solvent. 4,4′-Dimethoxytrityl chloride (71 mg, 0.21 mmol) was added and stirred 4h at r.t.. The solvent was removed and the residue was diluted with CH_2Cl_2, washed with saturatured $NaHCO_3$, dried over $MgSO_4$, evaporated and applied to column chromatography on silica gel (methanol in dichloromethane containing 1% pyridine, 0.5-1.5%, v/v) to obtain a mixture of **23a** and **23b** (59 mg, 73 %, **23a**/**23b** = 5/3). ^{1}H NMR (600 MHz, CDCl3) **23a:** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.39 (1H, brs, NH), 7.80(1H, s, H6), 7.49-6.84 (10H, m, aromatic), 5.66 (1H, s, H1′), 4.29 (1H, s, H3′), 3.79 (6H, s, MeO), 3.55 (1H, d, J_{gem} = 11.0 Hz, H5′), 3.26 (1H, d, J_{gem} = 11.0 Hz, H5″), 2.72 (1H, m, H7′), 2.45(1H, m, H2′), 2.15 (1H, m, H6′), 1.60 (3H, s, T-CH_3), 1.09 (3H, d, $J_{7'\text{-Me, }7'}$ = 7.5 Hz, 7′-CH_3), 0.79 (3H, d, $J_{6',\ 6'\text{-CH3}}$ = 7.5 Hz, 6'-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.0 (C4), 158.7, 149.7(C2), 144.5, 136.1-127.1 (aromatic), 113.4, 113.3, 109.4 (C5), 90.2, 86.8, 83.6, 72.3, 60.5, 55.2, 50.8 (C2′), 35.0 (C6′), 31.1 (C7′), 12.3 (T-CH3), 10.0 (7′-CH_3), 8.0 (6′-CH_3). **23b:** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.39 (1H, brs, NH), 7.75(1H, s, H6), 7.49-6.84 (10H, m, aromatic), 5.67 (1H, s, H1′), 4.26 (1H, s, H3′), 3.79 (6H, s, MeO), 3.58 (1H, d, J_{gem} = 11.5 Hz, H5′), 3.46 (1H, d, J_{gem} = 11.5 Hz, H5″), 2.51 (1H, m, H2′), 2.22 (1H, m, H7′), 1.64 (3H, s, T-CH_3), 1.46 (1H, m, H6′), 1.24 (3H, d, $J_{7'\text{-Me, }7'}$ = 7.5 Hz, 7′-CH_3), 0.87 (3H, d, $J_{6',\ 6'\text{-CH3}}$ = 7.5 Hz, 6'-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.0 (C4), 158.7, 149.7(C2), 144.6, 136.1-127.1 (aromatic), 113.33, 113.31, 109.4 (C5), 89.6, 86.7, 83.7, 74.0, 60.0, 55.2, 50.8 (C2′), 46.9 (C6′), 38.0 (C7′), 15.5 (7′-CH_3), 13.8 (6′-CH_3), 12.3 (T-CH_3). MALDI-TOF *m/z*: $[M+Na]^+$ found 621.2, calcd 621.3.

3,5-Di-*O*-benzyl-4-*C*-(4-hydroxy-butylenyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (25). To the suspension of the crude aldehyde **6** (7.06 g, 17.74 mmol) and $MgBr_2 \cdot Et_2O$ (14.43 g, 55.88 mmol) in dry CH_2Cl_2 (210 mL) was added dropwise the allyltributyltin (10.9 mL, 35.48 mmol) and stirred at r.t. overnight under N_2. The reaction was quenched with 1N HCl solution, extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentracted under reduced pressure. The residue was purified by column chromatography on silica gel (2-5% acetone in petroleum ether, v/v) to give **25** (7.23 g, 92.6%) as light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.23-7.27 (10H, m), 5.88 (1H, m, H8), 5.78 (1H, d, $J_{H1,\ H2}$ = 4.0 Hz, H1), 5.10 (1H, dd, J = 2.0 Hz, J = 17.0 Hz, H9a), 5.03 (1H, d, J = 10.0 Hz, H9b), 4.83 (1H, d, J_{gem} = 11.5 Hz, C$\underline{H_2}$Bn), 4.66 (1H, dd, $J_{H1,\ H2}$ = 4.0 Hz, $J_{H2,\ H3}$ = 5.5 Hz, H2), 4.53 (1H, d, J_{gem} = 12.0 Hz, C$\underline{H_2}$Bn), 4.48 (1H, d, J_{gem} = 11.5 Hz, C$\underline{H_2}$Bn), 4.46 (1H, H6), 4.45 (1H, d, $J_{H2,\ H3}$ = 5.5 Hz, H3), 4.41 (1H, d, J_{gem} = 12.0 Hz, C$\underline{H_2}$Bn), 3.81 (1H, d, J = 11.0 Hz, H5a), 3.59 (1H, d, J = 11.0 Hz, H5b), 3.25 (1H, s, 6-OH), 2.59 (1H, dd, J = 7.0 Hz, J = 14.0 Hz, H7a), 2.04 (1H, m, H7b), 1.58 (3H, s, Me), 1.34 (3H, s, Me). ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.1, 137.1 (Bn), 136.3 (C8), 128.6-127.6 (aromatic), 116.3 (C9), 113.5 (isopropyl), 104.7 (C1), 87.3 (C4), 78.8 (C2), 78.7 (C6), 73.6, 72.9 (C$\underline{H_2}$Bn), 72.2 (C3), 68.5 (C5), 34.7 (C7), 26.8, 26.3. MALDI-TOF *m/z*: $[M+K]^+$, found 479.5, calcd 479.5.

1-[3,5-Di-*O*-benzyl-4-*C*-(4-*O*-acetyl-butylen-yl)-2-*O*-acetyl-β-D-ribofuranosyl]-thymine (27). The compound **27** was synthesized from **25** (3.18 g, 7.23mmol) using the same procedure as that used for synthesis of compound **9** from **7**. **27** (3.41g, 88%) was obtained after column chromatography on silica gel (15-25% acetone in petroleum ether, v/v) as white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.51 (1H, brs, N$\underline{H}$), 7.44 (1H, s, H6), 7.32 (10H, m), 6.31 (1H, d, $J_{H1',\ H2'}$ = 6.0 Hz, H1′), 5.69 (1H, ddd, $J_{H7'',\ H8'}$ = 8.5 Hz, $J_{H8',\ H9''}$ = 9.0 Hz, $J_{H8',\ H9'}$ = 17.0 Hz, H8′), 5.48 (1H, dd, $J_{H6',\ H7'}$ = 2.0 Hz, $J_{H6',\ H7''}$ = 9.0 Hz, H6′), 5.35 (1H, dd, $J_{H1',\ H2'}$ = 6.0 Hz, $J_{H2',\ H3'}$ = 6.0 Hz, H2′), 5.01 (1H, d, $J_{H8',\ H9'}$ = 17.0 Hz, H9′), 4.98 (1H, d, $J_{H8',\ 9''}$ = 9.0 Hz, H9″), 4.58 (1H, d, J_{gem} = 11.5 Hz, C$\underline{H_2}$Bn), 4.54 (2H, d, J_{gem} = 11.5 Hz, C$\underline{H_2}$Bn), 4.50 (1H, d, $J_{H2',\ H3'}$ = 6.0 Hz, H3′), 4.45 (1H, d, J_{gem} = 11.5 Hz, C$\underline{H_2}$Bn), 3.79 (1H, d, J_{gem} = 10.0 Hz, H5′), 3.62 (1H, d, J_{gem}= 10.0 Hz, H5″), 2.73 (1H, d, $J_{H7',\ H7''}$ = 14.0 Hz, H7′), 2.30 (1H, dt, $J_{H7'',\ H8'}$ = 8.5 Hz, $J_{H6',\ H7''}$ = 9.0 Hz, $J_{H7',\ H7''}$ = 14.0 Hz, H7″), 2.06 (3H, s, acetyl), 1.88 (3H, s, acetyl), 1.51 (3H, s, T-C$\underline{H_3}$). ^{13}C NMR (125 MHz, $CDCl_3$) : δ 170.7, 169.9 (C=O acetyl), 164.3 (C4), 151.1 (C2), 137.4, 137.4 (Bn), 135.8 (C6), 134.5 (C8′), 129.1-128.1 (aromatic), 117.9 (C9′), 112.0 (C5), 88.0

(C4′), 86.6 (C1′), 79.0 (C3′), 75.6 (CH_2Bn), 75.1 (C2′), 74.2 (CH_2Bn), 72.5 (C6′), 71.2 (C5′), 35.4 (C7′), 21.2, 21.0 (CH_3, acetyl), 12.4 (T-CH_3). MALDI-TOF *m/z*: $[M+H]^+$, found 593.2, calcd 593.6.

1-[3,5-Di-*O*-benzyl-4-*C*-(4-*O*-acetyl-butylen-yl)-2-*O*-hydroxyl-*β*-D-ribofuranosyl]-thymine (28).

27 (3.41 g, 5.75 mmol) was dissolved in methanol (16mL) and methylamine solution (116 mL). The mixture was stirred at r.t. for 1h. The solvent was evaporated. The residue was purified by column chromatography on silica gel (1-1.2% methanol in CH_2Cl_2, v/v) to obtain **28** (3.16g, 100%) as white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34 (10H, m), 7.26 (1H, s, H6), 6.99 (1H, d, $J_{H1', H2'}$ = 7.0 Hz, H1′), 5.68 (1H, m, H8′), 5.46 (1H, dd, $J_{H6', H7'}$ = 2.5 Hz, $J_{H6', H7''}$ = 10.0 Hz, H6′), 5.01 (1H, dd, $J_{H9', H9''}$ = 0.5 Hz, $J_{H8', H9'}$ = 14.0 Hz, H9′), 4.98 (1H, d, $J_{H8', H9''}$ = 9.5 Hz, H9″), 4.70 (1H, d, J_{gem} = 11.0 Hz, CH_2Bn), 4.60 (1H, d, J_{gem} = 11.0 Hz, CH_2Bn), 4.58 (1H, d, J_{gem} = 11.5 Hz, CH_2Bn), 4.55 (1H, d, J_{gem} = 11.5 Hz, CH_2Bn), 4.39 (1H, t, $J_{H2', H3'}$ = 6.0 Hz, $J_{H1', H2'}$ = 7.0 Hz, H2′), 4.28 (1H, d, $J_{H2', H3'}$ = 6.0 Hz, H3′), 3.76 (1H, d, *J* = 10.0 Hz, H5′), 3.62 (1H, d, *J* = 10.0 Hz, H5″), 2.70 (1H, m, H7′), 2.32 (1H, dt, $J_{H7'', H8'}$ = 9.0 Hz, $J_{H6', H7''}$ = 10.0 Hz, $J_{H7', H7''}$ = 14.0 Hz, H7″), 1.95 (3H, s, acetyl), 1.57 (3H, s, T-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) : δ 169.8 (C=O acetyl), 163.6 (C4), 150.8 (C2), 137.0, 136.6 (Bn), 135.6 (C6), 134.1 (C8′), 128.7-127.6 (aromatic), 117.6 (C9′), 111.4 (C5), 88.9 (C1′), 87.2 (C4′), 80.3 (C3′), 75.6 (CH_2Bn), 74.7 (C2′), 74.0 (CH_2Bn), 72.5 (C6′), 71.6 (C5′), 35.0 (C7′), 20.9 (CH_3, acetyl), 12.1 (T-CH_3). MALDI-TOF *m/z*: $[M+H]^+$, found 551.2, calcd 551.6.

1-[3,5-Di-*O*-benzyl-4-*C*-(4-*O*-acetyl-butylen-yl)-2-*O*-phenoxythiocarbonyl -*β*-D-ribofuranosyl]-thymine (29). The compound **29** was synthesized from **28** (3.41g, 6.19mmol) using the same procedure as that used for synthesis of compound **11** from **10**. Column chromatography on silica gel (15-25% acetone in petroleum ether, v/v) to obtain **29** (3.59g, 85 %) as yellow foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.91 (1H, brs, NH), 7.28 (1H, s, H6), 7.23, 6.91 (15H, m, aromatic), 6.42 (1H, d, $J_{H1', H2'}$ = 6.5 Hz, H1′), 4.39 (1H, dd, $J_{H2', H3'}$ = 5.5 Hz, $J_{H', H2'}$ = 6.5 Hz, H2′), 5.59 (1H, m, H8′), 5.35 (1H, dd, $J_{H6', H7'}$ = 2.5 Hz, $J_{H6', H7''}$ = 8.0 Hz, H6′), 4.92 (1H, d, $J_{H8', H9'}$ = 13.0 Hz, H9′), 4.89 (1H, d, $J_{H8', H9''}$ = 6.0 Hz, H9″), 4.68 (1H, d, J_{gem} = 10.5 Hz, CH_2Bn), 4.61 (1H, d, $J_{H2', H3'}$ = 5.5 Hz, H3′), 4.60 (1H, d, J_{gem} = 11.5 Hz, CH_2Bn), 4.50 (1H, d, J_{gem} = 11.5 Hz, CH_2Bn), 4.46 (1H, d, J_{gem} = 10.5 Hz, CH_2Bn), 3.73 (1H, d, *J* = 10.0 Hz, H5′), 3.59 (1H, d, *J* = 10.0 Hz, H5″), 2.67 (1H, m, H7′), 2.27 (1H, m, H7″), 1.91 (3H, s, acetyl), 1.45 (3H, s, T-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) : δ 194.6 (C=S), 169.0 (C=O

acetyl), 163.1 (C4), 153.4 (C2), 136.8, 136.7 (Bn), 135.5 (C6), 134.0 (C8′), 129.6-126.9, 121.6 (aromatic), 117.6 (C9′), 111.8 (C5), 88.2 (C1′), 85.3 (C4′), 82.3 (C2′), 78.5 (C3′), 75.7, 74.2 ($\underline{C}H_2$Bn), 72.5 (C6′), 72.1 (C5′), 35.5 (C7′), 21.3 ($\underline{C}H_3$, acetyl), 12.4 (T-$\underline{C}H_3$). MALDI-TOF *m/z*: $[M+H]^+$, found 687.2, calcd 687.8.

(1R, 2R, 4S, 5S, 7R, 8S)-8-benzyloxy-5-benzyloxymethyl-2-methyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octan-4-yl acetate (30). 29 (2.0 g, 2.91mmol) was dissolved in dry toluene (100 mL) and purged with dry nitrogen for 30 min. Bu_3SnH (1.56 mL, 5.82 mmol) was dissolved in dry toluene (20 mL), and AIBN (237 mg, 0.874 mmol) was dissolved in dry toluene (10 mL). 2 mL of up AIBN solution was added dropwise to the mixture at 80 ˚C in over 30 min, then 4 mL of Bu_3SnH solution was added dropwise to the mixture in over 30 min. The solution was stirred for 30 min. Another 4 mL of AIBN solution and 8 mL of Bu_3SnH solution was added dropwise in over 30 min, the reaction was stirred for 1h at 80 ˚C. The remaining solution of AIBN and Bu_3SnH was added dropwise in over 30 min. After further stirred for 1 h, the reaction was cooled and solvent removed. The residue was purified by column chromatography on silica gel (25-50% ethyl acetate in petroleum ether, v/v) to obtain **30** (1.18 g, 76 %) as white foam. ^{1}H NMR (500MHz, $CDCl_3$): δ 9.19 (1H, brs, N$\underline{H}$), 7.98 (1H, s, H6), 7.30 (10H, m), 5.85 (1H, s, H1′), 5.46 (1H, dd, $J_{H6', H7'}$ = 6.0 Hz, $J_{H6', H7''}$ = 10.5 Hz, H6′), 4.58 (1H, d, J_{gem} = 11.5 Hz, C$\underline{H}_2$Bn), 4.56 (1H, d, J_{gem} = 11.5 Hz, C$\underline{H}_2$Bn), 4.52 (1H, d, J_{gem} = 11.5 Hz, C$\underline{H}_2$Bn), 4.47 (1H, d, J_{gem} = 12.0 Hz, C$\underline{H}_2$Bn), 4.34 (1H, d, $J_{H2', H3'}$ = 5.0 Hz, H3′), 3.93 (1H, d, J_{gem}= 11.0 Hz, H5′), 3.62 (1H, d, J_{gem} = 11.0 Hz, H5″), 2.32 (1H, d, $J_{H2', H3'}$ = 5.0 Hz, H2′), 2.27 (1H, $J_{H8', H7'}$ = 6.5 Hz, $J_{H8', H7''}$ = 12.5 Hz, $J_{8'\text{-Me}, H8'}$ = 7.0 Hz, m, H8′), 2.13 (1H, $J_{H6', H7'}$ = 6.0 Hz, $J_{H8', H7'}$ = 6.5 Hz, $J_{H7', H7''}$ = 12.5 Hz, m, H7′), 2.05 (3H, s, acetyl-Me), 1.38 (3H, s, T-C$\underline{H}_3$), 2.32 (1H, $J_{H6', H7''}$ = 10.5 Hz, $J_{H8', H7''}$ = 12.5 Hz, $J_{H7', H7''}$ = 12.5 Hz, m, H7″), 1.15 (3H, d, $J_{8'\text{-Me}, H8'}$ = 7.0 Hz, 8′-C$\underline{H}_3$). ^{13}C NMR (150MHz, $CDCl_3$): δ 170.2 (C=O acetyl), 164.4 (C4), 150.2 (C2), 137.2, 137.1 (Bn), 136.1 (C6), 129.4-127.6 (aromatic), 109.5 (C5), 85.3 (C4′), 84.9 (C1′), 73.9 (C3′), 73.7, 72.2 ($\underline{C}H_2$Bn), 71.3 (C6′), 67.4 (C5′), 47.6 (C2′), 33.1 (C7′), 24.1 (C8′), 21.0 ($\underline{C}H_3$- acetyl), 18.2 ($\underline{C}H_3$, C8′), 11.7 (T-$\underline{C}H_3$). MALDI-TOF *m/z*: $[M+H]^+$, found 535.2, calcd 535.6.

(1R, 2R, 4S, 5S, 7R, 8S)-8-benzyloxy-5-benzyloxymethyl-4-hydroxy-2-methyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (31). Compound **30** (2.77 g, 5.18 mmol) was dissolved in methanol (13 mL) and 30% methylamine in ethanol (104 mL). The mixture was stirred at r.t. overnight. The solvent was

evaporated. The residue was purified by column chromatography on silica gel (0.8-2% methanol in CH_2Cl_2, v/v) to obtain **31** (2.24 g, 88 %) as white foam. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.00 (1H, brs, NH), 7.99 (1H, s, H6), 7.30 (10H, m), 5.80 (1H, s, H1′), 4.60 (1H, d, J_{gem} = 11.4 Hz, CH$_2$Bn), 4.54 (1H, d, J_{gem} = 12.0 Hz, CH$_2$Bn), 4.34 (1H, d, J_{gem} = 11.4 Hz, CH$_2$Bn), 4.43 (1H, d, J_{gem} = 12.0 Hz, CH$_2$Bn), 4.31 (1H, d, $J_{H2', H3'}$ = 4.8 Hz, H3′), 4.12 (1H, d, J_{gem} = 11.4 Hz, H5′), 3.95 (1H, dd, $J_{H6', H7'}$ = 6.6 Hz, $J_{H6', H7''}$ = 10.8 Hz, H6′), 3.72 (1H, d, J_{gem} = 11.4 Hz, H5″), 2.28 (1H, d, $J_{H2', H3'}$ = 4.8 Hz, H2′), 2.21 (1H, $J_{H8', H7'}$ = 6.6 Hz, $J_{H8', H7''}$ = 12.0 Hz, $J_{8'\text{-Me}, H8'}$ = 6.6 Hz, m, H8′), 2.13 (1H, $J_{H6', H7'}$ = 6.6 Hz, $J_{H8', H7'}$ = 6.6 Hz, $J_{H7', H7''}$ = 13.2 Hz, m, H7′), 1.84 (1H, br, 6′-OH), 1.39 (3H, s, T-CH$_3$), 1.15 (3H, d, $J_{8'\text{-Me}, H8'}$ = 6.6 Hz, 8′-CH$_3$), 1.11 (1H, $J_{H6', H7''}$ = 10.8 Hz, $J_{H8', H7''}$ = 12.0 Hz, $J_{H7', H7''}$ = 13.2 Hz, H7″). ^{13}C NMR (150MHz, $CDCl_3$): δ 164.1 (C4), 150.2 (C2), 137.5, 137.4 (Bn), 136.3 (C6), 128.6-127.4 (aromatic), 109.5 (C5), 86.7 (C4′), 84.8 (C1′), 74.0 (C3′), 73.6, 72.1 (CH$_2$Bn), 68.9 (C6′), 68.1 (C5′), 47.7 (C2′), 37.8 (C7′), 24.1 (C8′), 18.5 (8′-CH$_3$), 11.8 (T-CH$_3$). MALDI-TOF *m/z*: $[M+H]^+$, found 493.267, calcd 493.233.

(1R, 2R, 5R, 7R, 8S)-8-benzyloxy-5-benzyloxymethyl-2-methyl-4-one-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (32). To 15% Dess Martin in CH_2Cl_2 solution (3.45mL, 1.22 mmol) was added dropwise the solution of compound **31** (500 mg, 1.015 mmol) in dry CH_2Cl_2 (10.0 mL) under nitrogen. The mixture was stirred for 1.5 h at r.t.. The reaction was quenched with saturated $Na_2S_2O_3$ solution and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel (25-33 % ethyl acetate in cyclohexane, v/v) to obtain **32** (450 mg, 90 %) as white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.54 (1H, brs, NH), 8.01 (1H, s, H6), 7.29 (10H, m, aromatic), 6.06 (1H, s, H1′), 4.60 (1H, d, J_{gem} = 11.4 Hz, CH$_2$Bn), 4.59 (1H, d, J_{gem} = 11.5 Hz, CH$_2$Bn), 4.56 (1H, d, J_{gem} = 11.5 Hz, CH$_2$Bn), 4.54 (1H, d, J_{gem} = 11.5 Hz, CH$_2$Bn), 4.54 (1H, d, $J_{H2', H3'}$ = 5.0 Hz, H3′), 4.04 (1H, d, J_{gem} = 11.5 Hz, H5′), 3.92 (1H, d, J_{gem} = 11.5 Hz, H5″), 2.56 (1H, d, $J_{H2', H3'}$ = 5.0 Hz, H2′), 2.50 (1H, m, H8′), 2.45 (1H, m, H7′), 2.43 (1H, m, H7″), 1.39 (3H, s, T-CH$_3$), 1.28 (3H, d, $J_{8'\text{-Me}, H8'}$ = 7.0 Hz, 8′-CH$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 205.6 (C6′), 164.3 (C4), 150.2 (C2), 137.1, 136.7 (Bn), 135.9 (C6), 128.6-127.4 (aromatic), 109.9 (C5), 86.3 (C4′), 85.7 (C1′), 75.9 (C3′), 73.9, 72.3 (CH$_2$Bn), 65.4 (C5′), 48.8 (C2′), 43.0 (C7′), 26.4 (C8′), 18.2 (8′-CH$_3$), 11.8 (T-CH$_3$). MALDI-TOF *m/z*: $[M+H]^+$ found 491.248, calcd 491.218.

(1R, 2R, 4R, 5R, 7R, 8S)-8-benzyloxy-5-benzyloxymethyl-4-hydroxyl-2, 4-dimethyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (33). 3M MeMgI in ether (1.18 mL, 3.54 mmol) was added dropwise to the solution of compound **32** (347 mg, 0.707 mmol) in dry THF (24 mL) at 0 ˚C under nitrogen and stirred at r.t. for 3 h. The reaction was quenched with saturated NH_4Cl solution and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The residue was chromatographyed on silica gel (25-33% ethyl acetate in cyclohexane, v/v) to obtain **33** (322 mg, 90%) as white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.84 (1H, brs, NH), 7.95 (1H, s, H6), 7.30 (10H, m, aromatic), 5.72 (1H, s, H1′), 4.64 (1H, d, J_{gem} = 11.5 Hz, CH$_2$Bn), 4.62 (1H, d, J_{gem} = 11.5 Hz, CH$_2$Bn), 4.59 (1H, d, J_{gem} = 11.5 Hz, CH$_2$Bn), 4.53 (1H, d, $J_{H2', H3'}$ = 5.0 Hz, H3′), 4.52 (1H, d, J_{gem} = 11.5 Hz, CH$_2$Bn), 4.50 (1H, s, C6′-OH), 4.07 (1H, d, J_{gem} = 11.0 Hz, H5′), 3.99 (1H, d, J_{gem} = 11.0 Hz, H5″), 2.37 (1H, ddd, $J_{H7', H8'}$ = 4.5 Hz, $J_{H8', 8'\text{-Me}}$ = 7.0 Hz, $J_{H7'', H8'}$ = 13.5 Hz, H8′), 2.32 (1H, d, $J_{H2', H3'}$ = 5.0 Hz, H2′), 1.72 (1H, dd, $J_{H7', H8'}$ = 4.5 Hz, $J_{H7', H7''}$ = 14.0 Hz, H7′), 1.48 (1H, dd, $J_{H7'', H8'}$ = 13.5 Hz, $J_{H7', H7''}$ = 14.0 Hz, H7″), 1.44 (3H, s, T-CH$_3$), 1.13 (3H, d, $J_{8'\text{-Me}, H8'}$ = 7.0 Hz, 8′-Me), 1.11 (3H, s, 6′-CH$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.1 (C4), 150.0 (C2), 137.4, 136.2 (Bn), 136.2 (C6), 128.7-127.8 (aromatic), 109.5 (C5), 84.3 (C1′), 83.3 (C4′), 75.7 (C3′), 74.3 (C6′), 73.8, 73.3 (CH$_2$Bn), 66.7 (C5′), 47.7 (C2′), 43.3 (C7′), 24.0 (6′-CH$_3$), 22.6 (C8′), 18.5 (8′-CH$_3$), 11.9 (T-CH$_3$). MALDI-TOF *m/z*: $[M+H]^+$ found 507.268, calcd 507.249.

(1R, 2R, 4R, 5R, 7R, 8S)-8-benzyloxy-5-benzyloxymethyl-2,4-dimethyl-4-methoxalyloxy- 7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (34). Methyl oxalyl chloride (0.47 mL, 5.08 mmol) was added to the solution of compound **33** (322 mg, 0.635 mmol) in dry pyridine (11 mL) under nitrogen. The mixture was stirred at 40 ˚C overnight. The reaction was cooled and the solvent was removed. The residue was extracted with CH_2Cl_2, and saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel (24-28 % ethyl acetate in cyclohexane, v/v) to obtain **34** (356 mg, 95 %) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.61 (1H, brs, NH), 7.93 (1H, s, H6), 7.30 (10H, m, aromatic), 5.75 (1H, s, H1′), 4.68 (1H, d, J_{gem} = 12.0 Hz, CH$_2$Bn), 4.66 (2H, s, CH$_2$Bn), 4.55 (1H, d, J_{gem} = 12.5 Hz, CH$_2$Bn), 4.52 (1H, d, $J_{H2', H3'}$ = 5.0 Hz, H3′), 4.13 (1H, d, J_{gem} = 10.5 Hz, H5′), 4.04 (1H, d, J_{gem} = 10.5 Hz, H5″), 3.53 (3H, s, CH_3- methyl oxalyl), 2.95 (1H, dd, $J_{H7', H8'}$ = 5.5 Hz, $J_{H7', H7''}$ = 16.0 Hz, H7′), 2.52 (1H, ddd, $J_{H7', H8'}$ = 5.5 Hz, $J_{H8', 8'\text{-CH3}}$ = 7.0 Hz, $J_{H7'', H8'}$ = 13.0 Hz, H8′), 2.32 (1H, d, $J_{H2', H3'}$ = 5.0 Hz, H2′), 1.56 (3H, s, 6′-CH$_3$), 1.42 (3H, s, T-CH$_3$), 1.41 (1H, dd, $J_{H7'', H8'}$ = 13.0 Hz, $J_{H7', H7''}$ = 16.0 Hz, H7″), 1.11 (3H, d,

$J_{8'\text{-Me, H8}'}$ = 7.0 Hz, 8′-C<u>H</u>$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.0 (C4), 158.3, 155.9 (C=O methyl oxalyl), 150.0 (C2), 138.2, 136.1 (Bn), 137.2 (C6), 128.7-126.7 (aromatic), 109.6 (C5), 86.1 (C6′), 84.1 (C1′), 83.0 (C4′), 74.5 (C3′), 73.9, 72.1 (CH_2Bn), 67.2 (C5′), 53.1 (<u>C</u>H$_3$- methyl oxalyl), 48.2 (C2′), 36.1 (C7′), 22.6 (C8′), 21.8 (6′-<u>C</u>H$_3$), 18.3 (8′-<u>C</u>H$_3$), 11.8 (T-<u>C</u>H$_3$). MALDI-TOF *m/z*: $[M+H]^+$ found 593.2, calcd 593.2.

(1R, 2R, 4S, 5R, 7R, 8S)-8-benzyloxy-5-benzyloxymethyl-2, 4-dimethyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octan (35a) and (1R, 2R, 4R, 5R, 7R, 8S)-8-benzyloxy-5-benzyloxymethyl-2, 4-dimethyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (35b). Compound **34** (356 mg, 0.60 mmol) was dissolved in dry toluene (16 mL) and purged with dry nitrogen for 30 min. AIBN (49 mg, 0.18 mmol) and Bu_3SnH (0.24 mL, 0.90 mmol) were added to the mixture and reflux for 1.5 h. The reaction was cooled to r.t. and evaporated. The residue was chromatographed over silica gel (18-22% ethyl acetate in cyclohexane, v/v) to obtain **35a** (61mg, 16 %) and **35b** (295mg, 78 %) as white solid. **35a:** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.23 (1H, brs, N<u>H</u>), 8.06 (1H, s, H6), 7.32 (10H, m, aromatic), 5.78 (1H, s, H1′), 4.62 (1H, d, J_{gem} = 11.5 Hz, C<u>H</u>$_2$Bn), 4.58 (1H, d, J_{gem} = 11.5 Hz, C<u>H</u>$_2$Bn), 4.55 (1H, d, J_{gem} = 11.5 Hz, C<u>H</u>$_2$Bn), 4.47 (1H, d, J_{gem} = 11.5 Hz, C<u>H</u>$_2$Bn), 4.31 (1H, d, $J_{H2', H3'}$ = 5.0 Hz, H3′), 3.90 (1H, d, J_{gem} = 10.5 Hz, H5′), 3.55 (1H, d, J_{gem} = 10.5 Hz, H5″), 2.28 (1H, d, $J_{H2', H3'}$ = 5.0 Hz, H2′), 2.24(ddd, $J_{H7', H8'}$ = 5.5 Hz, $J_{H8', 8'\text{-CH3}}$ = 7.0 Hz, $J_{H7'', H8'}$ = 12.5 Hz, 1H, H8′), 2.11 (1H, dt, $J_{H6', H7'}$ = 5.5 Hz, $J_{H6', 6'\text{-CH3}}$ = 7.0 Hz, $J_{H6', H7''}$ = 12.5 Hz, H6′), 1.65 (1H, ddd, $J_{H7', H8'}$ = 5.5 Hz, $J_{H6', H7'}$ = 5.5 Hz, $J_{H7', H7''}$ = 13.5 Hz, H7′), 1.41 (3H, s, T-C<u>H</u>$_3$), 1.09 (3H, d, $J_{8'\text{-Me, H8}'}$ = 7.0 Hz, 8′-C<u>H</u>$_3$), 0.97 (1H, dd, $J_{H6', H7''}$ = 12.5 Hz, $J_{H7'', H8'}$ = 12.5 Hz, $J_{H7',7\ H''}$ = 13.5 Hz, H7"), 0.79 (3H, d, $J_{H6', 6'\text{-CH3}}$ = 7.0 Hz, 6'-C<u>H</u>$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.0 (C4), 149.8 (C2), 137.8, 137.5 (Bn), 136.6 (C6), 128.6-127.3 (aromatic), 109.1 (C5), 86.2 (C4′), 84.8 (C1′), 73.7 (C3′), 73.5, 71.8 (<u>C</u>H$_2$Bn), 69.2 (C5′), 48.0 (C2′), 36.5 (C7′), 31.0 (C6′), 25.2 (C8′), 18.8 (8′-<u>C</u>H$_3$), 14.7 (6′-<u>C</u>H$_3$), 11.8 (T-<u>C</u>H$_3$). MALDI-TOF *m/z*: $[M+H]^+$ found 513.279, calcd 513.236. **35b:** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.91 (1H, brs, N<u>H</u>), 8.05 (1H, s, H6), 7.31 (10H, m, aromatic), 5.76 (1H, s, H1′), 4.60 (1H, d, J_{gem} = 12.0 Hz, C<u>H</u>$_2$Bn), 4.57 (1H, d, J_{gem} = 11.5 Hz, C<u>H</u>$_2$Bn), 4.55 (1H, d, J_{gem} = 12.0 Hz, C<u>H</u>$_2$Bn), 4.42 (1H, d, J_{gem} = 11.5 Hz, C<u>H</u>$_2$Bn), 4.20 (1H, d, $J_{H2', H3'}$ = 4.5 Hz, H3′), 3.90 (1H, d, J_{gem} = 10.5 Hz, H5′), 3.69 (1H, d, J_{gem} = 10.5 Hz, H5″), 2.39(1H, ddd, $J_{H7'', H8'}$ = 12.5 Hz, $J_{H8', 8'\text{-CH3}}$ = 7.0 Hz, $J_{H7', H8'}$ = 6.0 Hz, H8′), 2.30 (1H, d, $J_{H2', H3'}$ = 4.5 Hz, H2′), 1.93 (1H, dt, $J_{H6', H7''}$ = 7.5 Hz, $J_{H6', 6'\text{-CH3}}$ = 7.5 Hz, $J_{H6', H7'}$ = 7.0 Hz, H6′), 1.69 (1H, ddd, $J_{H7'', H8'}$ = 12.5 Hz, $J_{H6', H7''}$ = 7.5 Hz, $J_{H7', H7''}$ = 13.5 Hz, H7″), 1.42 (3H, s, T- CH_3), 1.42 (1H, m, $J_{H7', H8'}$ = 6.0

Hz, $J_{H6', H7'}$ = 7.0 Hz, $J_{H7', H7''}$ = 13.5 Hz, H7′), 1.13 (3H, d, $J_{8'\text{-Me}, H8'}$ = 7.0 Hz, 8′-Me), 1.11 (3H, d, $J_{H6', 6'\text{-CH3}}$ = 7.5 Hz, 6′-C$\underline{H}_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.3 (C4), 150.1 (C2), 137.7, 137.5 (Bn), 136.8 (C6), 128.6-127.3 (aromatic), 109.1 (C5), 85.3 (C4′), 84.4 (C1′), 75.6 (C3′), 73.7, 72.4 ($\underline{C}H_2$Bn), 70.3 (C5′), 48.2 (C2′), 35.9 (C6′), 35.0 (C7′), 22.7 (C8′), 19.0 (8′-$\underline{C}H_3$), 17.2 (6′-$\underline{C}H_3$), 11.9 (T-$\underline{C}H_3$). MALDI-TOF *m/z*: $[M+Na]^+$ found 513.279, calcd 513.236.

(1R, 2R, 4R, 5S, 7R, 8S)-8-benzyloxy-5-benzyloxymethyl-4-hydroxy-2-methyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (36). $NaBH_4$ (36 mg, 0.93 mmol) was added to the solution of compound **32** (167mg, 0.341mmol) in ethanol (10mL). The reaction was stirred at r.t. overnight. The solvent was removed and residue was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated. **36** (138 mg, 82 %) was obtained after column chromatography on silica gel (0.9-1.1% methanol in CH_2Cl_2, v/v). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.05 (1H, brs, H3), 7.96 (1H, s, H6), 7.31 (10H, m), 5.74 (1H, s, H1′), 4.62 (1H, d, J_{gem} = 11.5 Hz, C$\underline{H}_2$Bn), 4.61 (1H, d, J_{gem} = 11.5 Hz, C$\underline{H}_2$Bn), 4.58 (1H, d, J_{gem} = 11.5 Hz, C$\underline{H}_2$Bn), 4.47 (1H, d, J_{gem} = 11.0 Hz, C$\underline{H}_2$Bn), 4.41 (1H, d, $J_{H2', H3'}$ = 4.5 Hz, H3′), 4.15 (1H, d, J = 11.5 Hz, H5′), 3.82 (1H, d, J = 11.5 Hz, H5″), 3.81 (1H, d, $J_{6'\text{-OH}, H6'}$ = 12.0 Hz, C6′-OH), 3.64 (1H, dd, $J_{H6', H7'}$ = 5.0 Hz, $J_{H6', H7''}$ = 5.0 Hz, $J_{6'OH,H6'}$ = 12.0 Hz, H6′), 2.42 (1H, m, $J_{H7', H8'}$ = 5.5 Hz, $J_{H8', 8'\text{-CH3}}$ = 7.0 Hz, $J_{H7'', H8'}$ = 12.5 Hz, H8′), 2.38 (1H, d, $J_{H2', H3'}$ = 4.5 Hz, H2′), 1.87 (1H, dd, $J_{H7', H8'}$ = 5.5 Hz, $J_{H6', H7'}$ = 5.0 Hz, $J_{H7', H7''}$ = 15.0 Hz, H7′), 1.69 (1H, m, $J_{H7'', H8'}$ = 12.5 Hz, $J_{H6', H7''}$ = 5.0 Hz, $J_{H7', H7''}$ = 15.0 Hz, H7″), 1.45 (3H, s, T-C$\underline{H}_3$), 1.16 (3H, d, $J_{8'\text{-Me}, H8'}$ = 7.0 Hz, 8′-Me). ^{13}C NMR (150 MHz, $CDCl_3$): δ 164.2 (C4), 150.0 (C2), 137.3 (C6), 136.2, 136.1 (Bn), 128.7-127.8 (aromatic), 109.5 (C5), 84.4 (C1′), 81.6 (C4′), 75.7 (C3′), 73.8, 73.2 ($\underline{C}H_2$Bn), 71.8 (C6′), 69.0 (C5′), 48.1 (C2′), 36.1 (C7′), 22.3 (C8′), 18.6 (8′-$\underline{C}H_3$), 11.9 (T-Me). MALDI-TOF *m/z*: $[M+H]^+$ found 493.267, calcd 493.233.

(1R, 2R, 4S, 5S, 7R, 8S)-8-benzyloxy-5-benzyloxymethyl-4-(4-methylbenzoate)-2-methyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (37). Compound **31** (211 mg, 0.428 mmol) was dissolved in dry pyridine (4 mL). The 4-toluoyl chloride (113 μL, 0.856 mmol) was added to this mixture at 0 ˚C and stirred at r.t. overnight. The reaction was quenched with saturated $NaHCO_3$ solution and exacted thrice with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The residue was chromatographyed on silica gel (25-30% ethyl acetate in cyclohexane, v/v) to give **37** (225 mg, 86%) as white foam. ^{1}H NMR (500MHz, $CDCl_3$): δ 8.86 (1H, brs, NH), 8.03 (1H, d, J = 1.0 Hz, H6), 7.93

(2H, d, J = 8.0 Hz, toluoyl), 7.17 (12H, m, aromatic), 5.92 (1H, s, H1′), 5.44 (1H, dd, $J_{H6', H7'}$ = 6.0 Hz, $J_{H6', H7''}$ = 10.0 Hz, H6′), 4.60 (1H, d, J_{gem} = 11.5 Hz, C$\underline{H}_2$Bn), 4.56 (1H, d, J_{gem} = 11.5 Hz, C$\underline{H}_2$Bn), 4.49 (1H, d, J_{gem} = 11.5 Hz, C$\underline{H}_2$Bn), 4.48 (1H, d, J_{gem} = 11.0 Hz, C$\underline{H}_2$Bn), 4.40 (1H, d, $J_{H2', H3'}$ = 4.5 Hz, H3′), 4.03 (1H, d, J_{gem} = 11.0 Hz, H5′), 3.70 (1H, d, J_{gem} = 11.0 Hz, H5″), 2.42 (3H, s, Tol-CH_3), 2.36 (1H, d, $J_{H2', H3'}$ = 4.5 Hz, H2′), 2.34 (1H, m, H8′), 2.25 (1H, m, $J_{H6', H7'}$ = 6.0 Hz, $J_{H7', H8'}$ = 6.5 Hz, $J_{H7', H7''}$ = 12.0 Hz, H7′), 1.44 (1H, dd, $J_{H6', H7''}$ = 10.0 Hz, $J_{H7', H7''}$ = 12.0 Hz, H7″), 1.36 (3H, s, T-C$\underline{H}_3$), 1.18 (3H, d, $J_{8'\text{-Me}, H8'}$ = 7.0 Hz, 8′-C$\underline{H}_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.7 (C=O toluoyl), 164.1 (C4), 150.0 (C2), 144.0, 137.3, 137.2 (Bn), 136.2 (C6), 129.8-127.1 (aromatic), 109.5 (C5), 85.7 (C4′), 85.0 (C1′), 74.1 (C3′), 73.8, 72.2 (C$\underline{H}_2$Bn), 71.6 (C6′), 67.6 (C5′), 47.8 (C2′), 33.2 (C7′), 24.2 (C8′), 21.7 (C$\underline{H}_3$-Tol), 18.3 (8′-C$\underline{H}_3$), 11.7 (T-C$\underline{H}_3$). MALDI-TOF *m/z*: $[M+H]^+$ found 611.2, calcd 611.3.

(1R, 2R, 4R, 5S, 7R, 8S)-8-benzyloxy-5-benzyloxymethyl-4-(4-methylbenzoate)-2-methyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (38). Compound **36** (263 mg, 0.534 mmol) was dissolved in dry pyridine (6 mL). The 4- toluoyl chloride (0.14 mL, 1.07 mmol) was added to this mixture and stirred at 40 ˚C overnight. The reaction was quenched with saturated $NaHCO_3$ solution and exacted thrice with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The residue was chromatographed on silica gel (22-30% ethyl acetate in cyclohexane, v/v) to give **38** (244 mg, 75 %) as white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.74 (1H, brs, N$\underline{H}$), 7.99 (1H, s, H6), 7.76 (2H, d, J = 8.0 Hz, tol), 7.31 (10H, m, aromatic), 7.76 (2H, d, J = 8.0 Hz, tol), 5.82 (1H, s, H1′), 5.12 (1H, d, $J_{H6', H7'}$ = 3.5 Hz, H6′), 4.63 (1H, d, J_{gem} = 11.0 Hz, C$\underline{H}_2$Bn), 4.57 (2H, s, C$\underline{H}_2$Bn), 4.54 (1H, d, J_{gem} = 11.0 Hz, C$\underline{H}_2$Bn), 4.40 (1H, d, $J_{H2', H3'}$ = 3.5 Hz, H3′), 3.84 (2H, s, H5′, H5″), 2.63 (1H, m, H8′), 2.48 (1H, d, $J_{H2', H3'}$ = 3.5 Hz, H2′), 2.36 (3H, s, CH_3-tol), 1.91 (1H, m, H7′), 1.82 (1H, m, H7″), 1.43 (3H, s, T-C$\underline{H}_3$), 1.17 (3H, d, $J_{8'\text{-Me}, H8'}$ = 6.5 Hz, 8′-Me). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.9 (C=O), 164.1 (C4), 150.0 (C2), 143.7 (tol), 137.6, 137.3 (Bn), 136.3 (C6), 130.0-127.9 (aromatic), 109.5 (C5), 84.6 (C1′), 82.3 (C4′), 74.8 (C3′), 73.9, 73.0 (C$\underline{H}_2$Bn), 70.6 (C6′), 68.9 (C5′), 48.0 (C2′), 33.1 (C7′), 22.7 (C8′), 21.7 (C$\underline{H}_3$-tol), 18.6 (8′-C$\underline{H}_3$), 11.8 (T-C$\underline{H}_3$). MALDI-TOF *m/z*: $[M+H]^+$ found 611.2, calcd 611.3.

(1R, 2R, 4S, 5S, 7R, 8S)- 5-(4, 4′-Dimethoxytrityloxymethyl)-8-hydroxyl-4-(4-methylbenzoate)-2-methyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (39). To a solution of compound **37** (225 mg, 0.368 mmol) in dry methanol (13 mL) was added 20% $Pd(OH)_2$/C (276 mg) and ammonium formate

(697 mg, 11.1 mmol) and refluxed for 1h. Then the same amount of 20% $Pd(OH)_2/C$ and ammonium formate was added and refluxed for another 1h. The suspension was filtered over celite bar and organic phase was evaporated. The residue was co-evaporated twice with dry pyridine and dissolved in the same solvent. 4,4′-Dimethoxytrityl chloride (249 mg, 0.736 mmol) was added and stirred overnight at r.t.. The solvent was removed. The residue was chromatographed on silica gel (0.33-0.4% methanol in dichloromethane containing 1% pyridine, v/v) to obtain **39** (180 mg, 67 %) as yellow foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83 (2H, aromatic), 7.44 (1H, s, H6), 7.30 (11H, m, aromatic), 6.75 (4H,dd, aromatic), 5.84 (1H, s, H1′), 5.58 (1H, dd, $J_{H6', H7'}$ = 6.0 Hz, $J_{H6', H7''}$ = 10.0 Hz, H6′), 4.39 (1H, d, $J_{H2', H3'}$ = 5.0 Hz, H3′), 3.79 (1H, d, J_{gem} = 10.0 Hz, H5′), 3.74 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 3.34 (1H, d, J_{gem} = 10.0 Hz, H5″), 2.52 (1H, m, H8′), 2.42 (3H, s, $\underline{C}H_3$- tol), 2.35 (2H, m, H2′ and H7′), 1.50 (3H, s, T-$\underline{C}H_3$), 1.35 (1H, m, H7″), 1.18 (3H, d, $J_{8'\text{-Me}, H8'}$ = 6.5 Hz, 8′-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.7 (C=O), 163.9 (C4), 158.7, 158.6, 149.7 (C2), 144.0, 143.9, 135.2 (C6), 135.0, 134.7, 130.0-127.1, 113.4 (aromatic), 109.6 (C5), 87.2, 85.4 (C1′), 84.7 (C4′), 71.0 (C6′ and C3′), 62.4 (C5′), 55.1 (OMe), 50.3 (C2′), 33.1 (C7′), 23.7 (C8′), 21.7 (T-$\underline{C}H_3$), 18.2 (8′-$\underline{C}H_3$), 12.2 (T-$\underline{C}H_3$). MALDI-TOF *m/z*: $[M+Na]^+$ found 755.4, calcd 755.3.

(1R, 2R, 4R, 5S, 7R, 8S)-5-(4, 4′-Dimethoxytrityloxymethyl)-8-hydroxyl-4-(4-methylbenzoate)-2-methyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (40). To a solution of compound **38** (287 mg, 0.47 mmol) in dry methanol (14 mL) was added 20% $Pd(OH)_2/C$ (352 mg) and ammonium formate (899 mg, 14.1 mmol) and refluxed for 1h. Then the same amount of 20% $Pd(OH)_2/C$ and ammonium formate was added and refluxed for another 1 h. The suspension was filtered over celite bar and organic phase was evaporated. The residue was co-evaporated twice with dry pyridine and dissolved in the same solvent. 4,4′-Dimethoxytrityl chloride (318 mg, 0.94 mmol) was added and stirred overnight at r.t.. The solvent was removed and the obtained residue was chromatographed on silica gel (0.5% methanol in dichloromethane containing 1% pyridine, v/v) to obtain **40** (188 mg, 55 %) as yellow foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.72 (1H, s, H6), 7.67-6.72 (17H, m, aromatic), 5.76 (1H, s, H1′), 5.25 (1H, d, $J_{H6', H7'}$ = 4.5 Hz, H6′), 4.60 (1H, bs, H3′), 3.71 (6H, s, $OC\underline{H}_3$), 3.51 (1H, d, J_{gem} = 11.0 Hz, H5′), 3.43 (1H, d, J_{gem} = 11.0 Hz, H5″), 2.45 (2H, m, H8′ and H2′), 2.44 (3H, s, $C\underline{H}_3$-tol), 2.03 (1H, dd, J = 5.0 Hz, J = 16.0 Hz, H7′), 1.81 (1H, m, H7″), 1.49 (3H, s, T-$C\underline{H}_3$), 1.20 (3H, d, $J_{8'\text{-Me}, H8'}$ = 7.0 Hz, 8′-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.7 ($\underline{C}$=O), 163.7 (C4), 158.5, 149.7 (C2), 144.4 (tol), 144.1, 135.4 (C6), 135.2, 134.8, 130.1-126.4, 113.2 (aromatic), 109.9 (C5), 86.7, 84.8

(C1′), 81.4 (C4′), 71.9 (C6′), 70.3 (C3′), 61.9 (C5′), 55.1 (OMe), 49.9 (C2′), 32.3 (C7′), 22.0 (C8′), 21.7 ($\underline{C}H_3$-tol), 18.4 (8′-$\underline{C}H_3$), 12.3 (T-$\underline{C}H_3$). MALDI-TOF *m/z*: $[M+Na]^+$ found 755.4, calcd 755.3.

(1R, 2R, 4R, 5R, 7R, 8S)-5-(4, 4′-Dimethoxytrityloxymethyl)-8-hydroxyl-4-hydroxyl-2,4-dimethyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (41). To a solution of compound **33** (168 mg, 0.332 mmol) in dry methanol (10 mL) was added 20% $Pd(OH)_2/C$ (166 mg) and ammonium formate (418 mg, 6.6 mmol) and reflux for 3 h. Then the same amount of 20% $Pd(OH)_2/C$ and ammonium formate was added and reflux for another3 h. The suspension was filtered over celite bar and organic phase was evaporated. The residue was co-evaporated twice with dry pyridine and dissolved in the same solvent. 4,4′-Dimethoxytrityl chloride (168 mg, 0.5 mmol) was added and stirred overnight at r.t.. After removing the solvent, the residue was chromatographed.on silica gel (5.2-5.8% methanol in dichloromethane containing 1% pyridine, v/v) to obtain **41** (163 mg, 78 %) as. yellow foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.80 (1H, brs, NH), 7.88 (1H, s, H6), 7.32, 6.84 (13H, m, aromatic), 5.64 (1H, s, H1′), 4.93 (1H, d, $J_{H2', H3'}$ = 5.0 Hz, H3′), 3.79 (3H, s, OC$\underline{H}_3$), 3.78 (3H, s, OC$\underline{H}_3$), 3.76 (1H, d, J_{gem} = 11.0 Hz, H5′), 3.51 (1H, d, J_{gem} = 11.0 Hz, H5″), 2.52 (1H, m, H8′), 2.36 (1H, d, $J_{H2', H3'}$ = 5.0 Hz, H2′), 1.66 (1H, dd, $J_{H7', H8'}$ = 5.5 Hz, $J_{H7', H7''}$ = 14.5 Hz, H7′), 1.44 (1H, t, $J_{H7'', H8'}$ = 13.5 Hz, $J_{H7', H7''}$ = 14.0 Hz, H7″), 1.32 (3H, s, T-C$\underline{H}_3$), 1.15 (3H, d, $J_{8'\text{-Me}, H8'}$ = 7.0 Hz, 8′-C$\underline{H}_3$), 0.95 (3H, s, 6′-C$\underline{H}_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.2 (C4), 158.7, 158.6, 149.9 (C2), 144.3, 136.0, 135.4 (C6), 135.2, 130.1-125.3, 113.4 (aromatic), 109.7 (C5), 87.2, 84.6 (C1′), 82.5 (C4′), 75.8 (C6′), 70.4 (C3′), 60.5 (C5′), 55.3 (OMe), 50.1 (C2′), 43.1 (C7′), 24.6 (6′-$\underline{C}H_3$), 22.1 (C8′), 18.5 (8′-$\underline{C}H_3$), 11.9 ($\underline{C}H_3$-T). MALDI-TOF *m/z*: $[M+Na]^+$ found 651.2, calcd 651.3.

(1R, 2R, 4S, 5R, 7R, 8S)- 5-(4,4′-Dimethoxytrityloxymethyl)-2,4-dimethyl-8-hydroxyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octan (42) and **(1R, 2R, 4R, 5R, 7R, 8S)- 5-(4,4′-Dimethoxytrityloxymethyl)-2,4-dimethyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (43).** A mixture of **35a** and **35b** (274 mg, 0.557 mmol) was dissolved in dry methanol (16 mL) to which was added 20% $Pd(OH)_2/C$ (335 mg) and ammonium formate (844 mg, 13.4 mmol) and reflux for 3h. Then the same amount of 20% $Pd(OH)_2/C$ and ammonium formate was added and reflux for another 3h. The suspension was filtered over celite bar and organic phase was evaporated. The residue was co-evaporated twice with dry pyridine and dissolved in the same solvent. 4,4′-Dimethoxytrityl chloride (377 mg, 1.11mmol) was added and stirred overnight at r.t.. Then the solvent was removed and

obtained residue was chromatographed on silica gel (0.32-0.4% methanol in dichloromethane containing 1% pyridine, v/v) to obtain **42** (69 mg, 20 %) and **43** (153 mg, 43 %) as yellow foam. **42**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.61 (1H, brs, H3), 7.78 (1H, s, H6), 7.36- 6.84 (13H, m, aromatic), 5.73 (1H, s, H1′), 4.40 (1H, d, $J_{H2', H3'}$ = 4.0 Hz, H3′), 3.79 (6H, s, OC$\underline{H}_3$), 3.55 (1H, d, J_{gem} = 11.0 Hz, H5′), 3.38 (1H, d, J_{gem} = 11.0 Hz, H5″), 2.48 (1H, m, H8′), 2.27 (1H, d, $J_{H2', H3'}$ = 5.0 Hz, H2′), 2.06 (1H, m, H6′), 1.66 (1H, m, H7′), 1.44 (1H, m, H7″), 1.42 (3H, s, T-C$\underline{H}_3$), 1.13 (3H, d, $J_{8'\text{-Me}, H8'}$ = 7.0 Hz, 8′-CH_3), 1.00 (3H, d, $J_{H6', 6'\text{-CH3}}$ = 7.0 Hz, 6′-C$\underline{H}_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.0 (C4), 158.6, 149.8 (C2), 144.4, 136.0 (C6), 135.6, 135.3, 130.1-123.8, 113.3 (aromatic), 109.3 (C5), 86.7, 85.8 (C1′), 84.7 (C4′), 70.2 (C3′), 64.1 (C5′), 55.2, 50.9 (C2′), 35.2 (C6′), 34.8 (C7′), 22.1 (C8′), 19.0 (8′-$\underline{C}H_3$), 17.1 (6′-CH_3), 12.1 (T-$\underline{C}H_3$). MALDI-TOF *m/z*: $[M+K]^+$ found 651.3, calcd 651.3. **43**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.61 (1H, brs, N$\underline{H}$), 7.83 (1H, s, H6), 7.34, 6.85 (13H, m, aromatic), 5.78 (1H, s, H1′), 4.58 (1H, d, $J_{H2', H3'}$ = 4.5 Hz, H3′), 3.79 (6H, s, OC$\underline{H}_3$), 3.45 (1H, d, J_{gem} = 10.5 Hz, H5′), 3.32 (1H, d, J_{gem} = 10.5 Hz, H5″), 2.32 (1H, m, H8′), 2.27 (1H, s, H2′), 2.05 (1H, m, H6′), 1.63 (1H, m, H7′), 1.37 (3H, s, T-C$\underline{H}_3$), 1.10 (3H, d, $J_{8'\text{-Me}, H8'}$ = 7.0 Hz, 8′-C$\underline{H}_3$), 0.92 (1H, dd, H7″), 0.67 (3H, d, $J_{H6', 6'\text{-CH3}}$ = 6.0 Hz, 6′-C$\underline{H}_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.0 (C4), 158.7, 158.6, 149.8 (C2), 144.2, 135.9 (C6), 135.3, 135.2, 130.0-123.8 (aromatic), 109.4 (C5), 86.9, 86.1 (C4′), 84.9 (C1′), 68.7 (C3′), 63.0 (C5′), 55.2 (OMe), 50.4 (C2′), 36.3 (C7′), 30.6 (C6′), 24.6 (C8′), 18.8 (8′-$\underline{C}H_3$), 14.6 (6′-$\underline{C}H_3$), 12.0 (T-$\underline{C}H_3$). MALDI-TOF *m/z*: $[M+Na]^+$ found 635.3, calcd 635.3.

(1R, 3R, 4R, 5S, 6S, 7S)-7-((2-Cyanoethoxy)-diisopropylamino-phosphinoxy)-1-(4, 4′-dimethoxytrityloxymethyl)-5-methyl-6-(4-methylbenzoate)-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1]heptane (17a). A mixture of tow isomers of **17a** (233 mg, 77%) was obtained from **16a** (237 mg, 0.33mmol) after column chromatography on silica gel (ethyl acetate in cyclohexane containing 1% Et_3N, 20-60%, v/v). ^{31}P NMR (202.4 MHz, $CDCl_3$): δ 149.9, 149.5. MALDI-TOF *m/z*: $[M+H]^+$ found 919.4, calcd 919.4.

(1R, 3R, 4R, 5R, 6S, 7S)-7-((2-Cyanoethoxy)-diisopropylamino-phosphinoxy)-1-(4, 4′-dimethoxytrityloxymethyl)-5-methyl-6-(4-methylbenzoate)-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1]heptane (17b). A mixture of tow isomers of **17b** (126 mg, 67%) was obtained from **16b** (148 mg, 0.206 mmol) after column chromatography on silica gel (ethyl acetate in cyclohexane

containing 1% Et_3N, 20-60%, v/v). ^{31}P NMR (202.4 MHz, $CDCl_3$): δ 150.6, 149.1. MALDI-TOF *m/z*: $[M+H]^+$ found 919.4, calcd 919.4.

(1R, 3R, 4R, 5S, 6R, 7S)-7-((2-Cyanoethoxy)-diisopropylamino-phosphinoxy)-1-(4, 4′-dimethoxytrityloxymethyl)-5-methyl-6-(4-methylbenzoate)-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1]heptane (17c). Tow isomers of **17c** (58 mg, 70%) were obtained from **16c** (65 mg, 0.09 mmol) after column chromatography on silica gel (ethyl acetate in cyclohexane containing 1% Et_3N, 10-50%, v/v). ^{31}P NMR (109.4 MHz, $CDCl_3$): δ 147.4, 147.1. MALDI-TOF *m/z*: $[M+H]^+$ found 919.5, calcd 919.4.

(1R, 3R, 4R, 5R, 6R, 7S)-7-((2-Cyanoethoxy)-diisopropylamino-phosphinoxy)-1-(4, 4′-dimethoxytrityloxymethyl)-5-methyl-6-(4-methylbenzoate)-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1]heptane (17d). 16d (100 mg, 0.139 mmol) was used and the reaction gave two isomers (about 5/1 from on TLC). The major isomer (64 mg, 50%) was seperated by column chromatography on silica gel (ethyl acetate in cyclohexane containing 1% Et_3N, 20-60%, v/v). ^{31}P NMR (242.9 MHz, $CDCl_3$): δ 149.8. MALDI-TOF *m/z*: $[M+H]^+$ found 919.5, calcd 919.4.

(1R, 3R, 4R, 5S, 6S, 7S)- 7-((2-Cyanoethoxy)-diisopropylamino-phosphinoxy)-1-(4, 4′-dimethoxytrityloxymethyl) –6-hyroxyl- 5, 6-dimethyl-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1] heptane (20a). 19a (189 mg, 0.3 mmol) was used and two isomers (total yield 77%) were obtained after column chromatography on silica gel (ethyl acetate in cyclohexane containing 1% Et_3N, 20-50%, v/v). The isomer wiht higher R_f (93 mg): ^{31}P NMR (202.4 MHz, $CDCl_3$) : δ 149.5. MALDI-TOF *M/Z*: $[M+H]^+$ found 815.4, calcd 815.4. The isomer wiht lower R_f (100 mg): ^{31}P NMR (202.4 MHz, $CDCl_3$): δ 149.7. MALDI-TOF *m/z*: $[M+H]^+$ found 815.3, calcd 815.4.

(1R, 3R, 4R, 5S, 6R, 7S)- 7-((2-Cyanoethoxy)-diisopropylamino-phosphinoxy)-1-(4, 4′-dimethoxytrityloxymethyl) –6-hyroxyl- 5, 6-dimethyl-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1] heptane (20b). 19b (170 mg, 0.27 mmol) was used and the reaction gave two isomers (total yield 67%) after column chromatography on silica gel (ethyl acetate in cyclohexane containing 1% Et_3N, 20-50%, v/v). The isomer with higher R_f (67 mg): ^{31}P NMR (202.4 MHz, $CDCl_3$): δ 149.7. MALDI-TOF

M/Z: $[M+H]^+$ found 815.3, calcd 815.4. The isomer with lower R_f (84 mg): ^{31}P NMR (202.4 MHz, $CDCl_3$): δ 150.2. MALDI-TOF *m/z*: $[M+H]^+$ found 815.4, calcd 815.4.

(1R, 3R, 4R, 5R, 6S, 7S)- 7-((2-Cyanoethoxy)-diisopropylamino-phosphinoxy)-1-(4, 4′-dimethoxytrityloxymethyl)-5, 6-dimethyl-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1] heptane (24a) and (1R, 3R, 4R, 5S, 6S, 7S)- 7-((2-Cyanoethoxy)-diisopropylamino-phosphinoxy)-1-(4, 4′-dimethoxytrityloxymethyl)-5, 6-dimethyl-3-(thymin-1-yl)-2-oxa-bicyclo[2.2.1] heptane (24b). A mixture (46 mg, 63%) of tow isomers of **24a** and tow isomers of **24b** (**24a**/**24b** = 8/2)was obained from a mixture of **23a** and **23b** (55 mg, 0.092 mmol) after column chromatography on silica gel (ethyl acetate in cyclohexane containing 1% Et_3N, 20-30%, v/v): ^{31}P NMR (202.4 MHz, $CDCl_3$): δ 150.0, 149.9, 149.8, 149.7. MALDI-TOF *m/z*: $[M+H]^+$ found 799.4, calcd 799.4.

(1R, 2R, 4S, 5S, 7R, 8S)-8-((2-Cyanoethoxy)-diisopropylamino-phosphinoxy)-5-(4, 4′-dimethoxytrityloxymethyl)-4-(4-methylbenzoate)-2-methyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (44). 44 (177 mg, 74%) was obtained from **39** (188 mg, 0.26 mmol) as two isomers after column chromatography on silica gel (20-40% ethyl acetate in cyclohexane containing 1% Et_3N, v/v). ^{31}P NMR (202.4 MHz, $CDCl_3$): δ 151.9, 150.3. MALDI-TOF *m/z*: $[M+H]^+$ found 933.5, calcd 933.4.

(1R, 2R, 4R, 5S, 7R, 8S)-8-((2-Cyanoethoxy)-diisopropylamino-phosphinoxy)-5-(4, 4′-dimethoxytrityloxymethyl)-4-(4-methylbenzoate)-2-methyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (45). 45 (77 mg, 61 %) was obtained as two isomers from **40** (99mg, 0.135 mmol) after 24h reaction followed by column chromatography on silica gel (1% Et_3N, ethyl acetate in cyclohexane containing 1% Et_3N, v/v). ^{31}P NMR (202.4 MHz, $CDCl_3$): δ 152.3, 149.4. MALDI-TOF *m/z*: $[M+H]^+$ found 933.5, calcd 933.4.

(1S, 2R, 4R, 5R, 7R, 8S)-8-((2-Cyanoethoxy)-diisopropylamino-phosphinoxy)-5-(4, 4′-dimethoxytrityloxymethyl)-4-hydroxyl-2,4-dimethyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (46). 46 (125 mg, 60 %) was obtained as two isomers from **41** (157 mg, 0.25 mmol) after column chromatography on silica gel (ethyl acetate in cyclohexane containing 1% Et_3N, 20-45%, v/v). ^{31}P NMR (202.4 MHz, $CDCl_3$): δ 152.1, 151.6. MALDI-TOF *m/z*: $[M+H]^+$ found 829.4, calcd 829.4.

(1R, 2R, 4S, 5R, 7R, 8S)-8-((2-Cyanoethoxy)-diisopropylamino-phosphinoxy)-5-(4, 4′-dimethoxytrityloxymethyl)-2,4-dimethyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (47). 47 (86 mg, 80 %) was obtained as two isomers from **42** (83 mg, 0.135 mmol) after column chromatography on silica gel (25-50% ethyl acetate in cyclohexane containing 1% Et_3N, v/v). ^{31}P NMR (202.4 MHz, $CDCl_3$): δ 149.7, 148.6. MALDI-TOF *m/z*: $[M+H]^+$ found 813.4, calcd 813.4.

(1R, 2R, 4R, 5R, 7R, 8S)-8-((2-Cyanoethoxy)-diisopropylamino-phosphinoxy)-5-(4, 4′-dimethoxytrityloxymethyl)-2,4-dimethyl-7-(thymin-1-yl)-6-oxa-bicyclo[3.2.1]octane (48). 48 (169 mg, 63.8%) was obtained as two isomers from **43** (200 mg, 0.326 mmol) after after column chromatography on silica gel (25-50% ethyl acetate in cyclohexane containing 1% Et_3N, v/v). ^{31}P NMR (202.4 MHz, $CDCl_3$): δ 149.5, 149.4. MALDI-TOF *m/z*: $[M+H]^+$ found 813.4, calcd 813.4.

Model of RNA/DNA duplex (A type) containing carba-LNA modification to show the location of subsitutents in the carbocylic moeity.

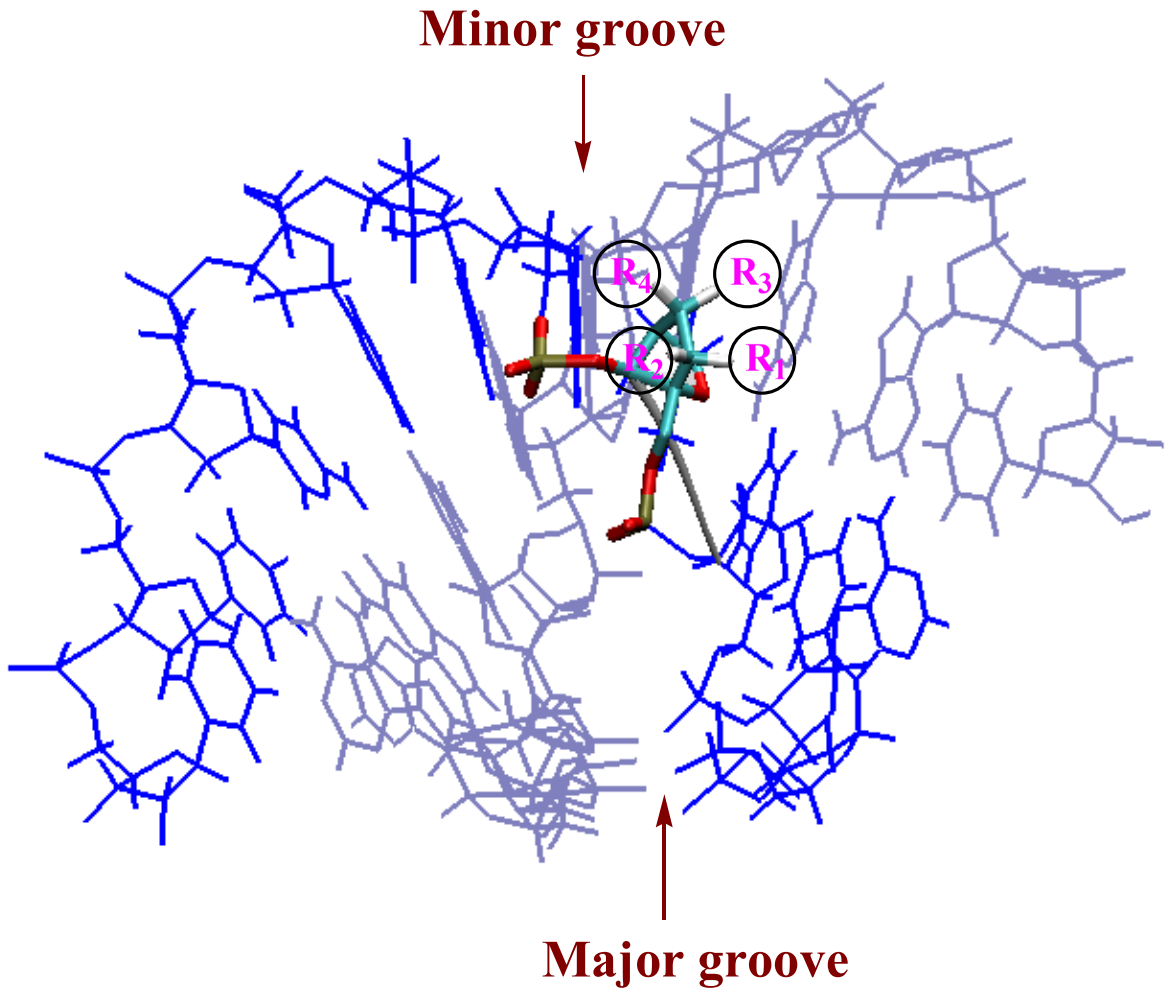


Fig. SIV.1 Model of DNA/RNA duplex (A type) with Type II modification.

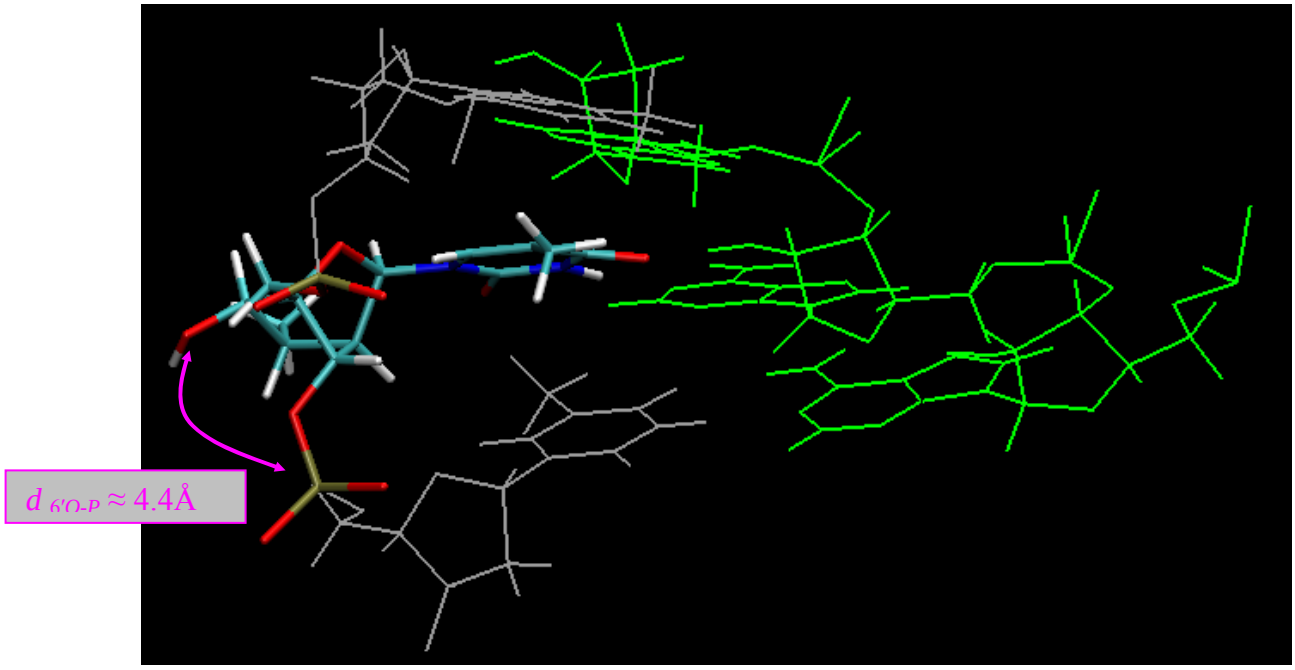


Fig. SIV.2 Model of DNA/RNA duplex (A type) with Type IV modification.

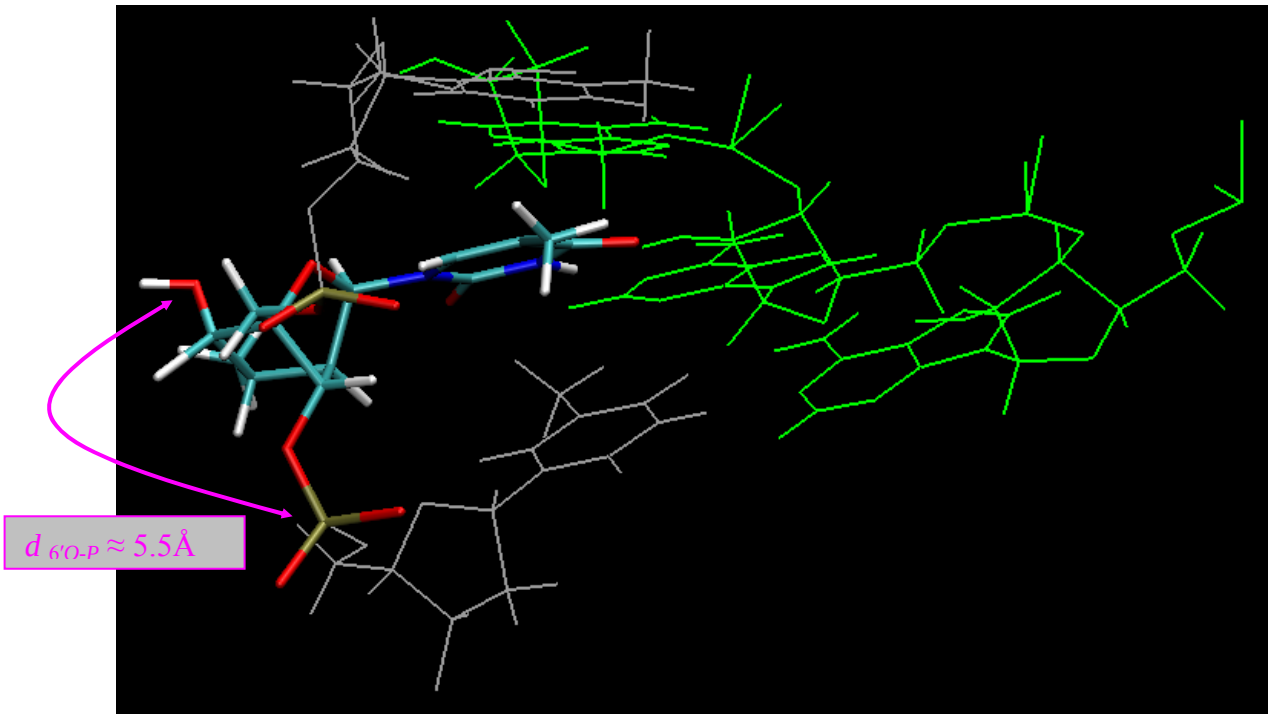
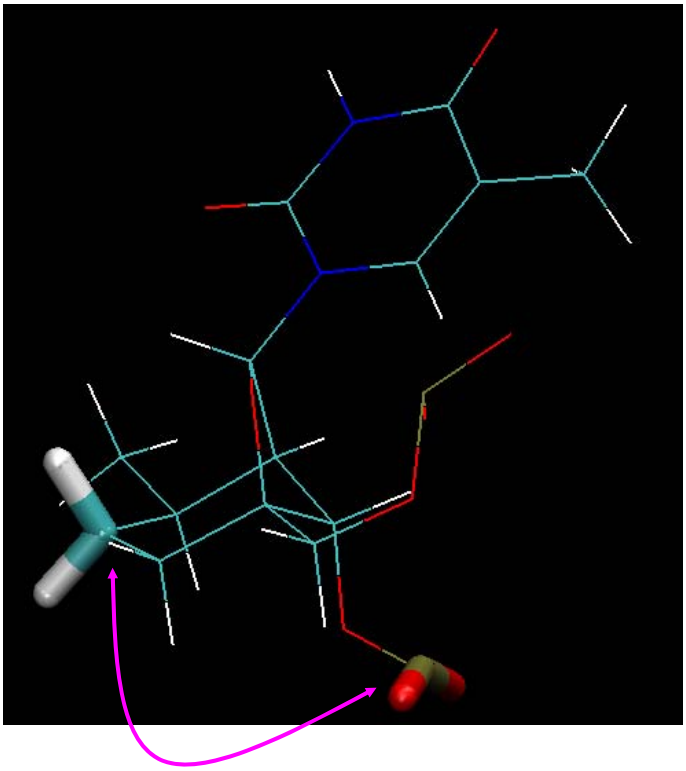


Fig.SIV.3 Model to show the the additional endocyclic methylene in the six-membered carbocycle of carba-ENA orientated away from the 3′-phosphate.



Individual author's contributions on this work

We would like to thank Mr. Puneet Srivastava for providing carba-LNA T and carba-ENA-T phosphoramidite for this study. Chuanzheng Zhou (C. Zhou) has carried through total synthesis of all of the functionalized carba-LNAs and their phosphoramidites, their incorporations into oligos, thermal denaturation studies as well as all of the enzymology study in this manuscript. C. Zhou also takes the major responsibility for data analysis and manuscript writing. Dr Yi Liu has carried out synthesis of all the functionalized carba-ENA nucleosides and their phosphoramidites, their incorporations into oligos, thermal denaturation studies and also participated in the enzymology study and manuscript writing. Dr M Andaloussi prepared compounds **12**, **31** as well as phorsphoramidite **17a** in the preliminary study. Dr N Badgujar was responsible for the scale-up of compound **12**, and he also participated thermal denaturation study. Dr O Plashkevych has performed molecular structure analysis based on theoretical calculations.

Complete Reference 53

Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J., J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; S., R. E.; Pople, J. A. *Gaussian 98 (Revision A.6)*; Gaussian, Inc: Pittsburgh PA, 1998.

References:

(1) Bondensgaard, K.; Petersen, M.; Kingh, S. K.; Rajwanshi, V. K.; Kumar, R.; Wengel, J.; Jacobsen, J. P. *Chem. Eur. J.* **2000**, *6*, 2687-2695.

(2) Morita, K.; Takagi, M.; Hasegawa, C.; Kaneko, M.; Tsutsumi, S.; Sone, J.; Ishikawa, T.; Imanishi, T.; Koizumi, M. *Bioorg. Med. Chem.* **2003**, *11*, 2211-2226.

(3) Albak, N.; Petersen, M.; Nielsen, P. *J. Org. Chem.* **2006**, *71*, 7731-7740.

(4) Gyi, J. I.; Conn, G. L.; Lane, A. N.; Brown, T. *Biochemistry* **1996**, *35*, 12538-12548.

(5) Egli, M.; Usman, N.; Rich, A. *Biochemistry* **1993**, *32*, 3221-3237.

(6) Sundaralingam, M.; Pan, B. *Biophys. Chem.* **2002**, *95*, 273-282.

(7) Tereshko, V.; Gryaznov, S.; Egli, M. *J. Am. Chem. Soc.* **1998**, *120*, 269-283.

(8) Egli, M.; Minasov, G.; Teplova, M.; Kumar, R.; Wengel, J. *Chem. Commun.* **2001**, 651-652.

(9) Pande, V.; Nilsson, L. *Nucleic Acids Res.* **2008**, *36*, 1508-1516.

(10) Teplova, M.; Minasov, G.; Tereshko, V.; Inamati, G. B.; Cook, P. D.; Manoharan, M.; Egli, M. *Nature Structural Biology* **1999**, *6*, 535-539.

(11) Schneider, B.; Patel, K.; Berman, H. M. *Biophys. J* **1998**, *75*, 2422-2434.

(12) Liu, D.; Wyttengach, T.; Bowers, M. T. *J. Am. Chem. Soc.* **2006**, *128*, 15155-15163.

(13) Zhou, C.; Plashkevych, O.; Chattopadhyaya, J. *Manuscript in preparation*.

(14) Kakefuda, A.; Yoshimura, Y.; Sasaki, T.; Matsuda, A. *Tetrahedron* **1993**, *49*, 8513-8528.