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Synthesis and Characterization of a [3-^{15}N]-Labeled Cis-Syn Thymine Dimer-Containing DNA Duplex

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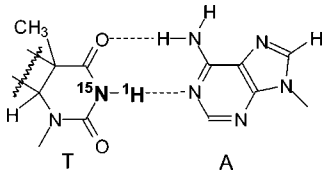

Cis-syn thymine dimers are the major photoproducts of DNA and are the principal cause of mutations induced by sunlight. To better understand the nature of base pairing with cis-syn thymine dimers, we have synthesized a decamer oligodeoxynucleotide (ODN) containing a cis-syn thymine dimer labeled at the N3 of both T's with ^{15}N by two efficient routes from [3-^{15}N]-thymidine phosphoramidite. In the postsynthetic irradiation route, an ODN containing an adjacent pair of [3-^{15}N]-labeled T's was irradiated and the cis-syn dimer-containing ODN isolated by HPLC. In the mixed building block route, a mixture of cis-syn and trans-syn dimer-containing ODNs was synthesized from a mixture of [3-^{15}N]-labeled thymine dimer phosphoramidites after which the cis-syn dimer-containing ODN was isolated by HPLC. The N3-nitrogen and imino proton signals of an ^{15}N-labeled thymine dimer-containing decamer duplex were assigned by 2D ^{1}H−^{15}N heterocorrelated HSQC NMR spectroscopy, and the ^{15}N−^{1}H coupling constant was found to be 1.8 Hz greater for the 5′-T than for the 3′-T. The larger coupling constant is indicative of weaker H-bonding that is consistent with the more distorted nature of the 5′-base pair found in solution state NMR and crystallographic structures.

Introduction

Cis-syn cyclobutane thymine dimers are the major UV photoproducts of DNA and have been correlated with mutations and skin cancer.[1−5] These products arise via a [2 + 2] cycloaddition between the 5,6 double bonds of two adjacent thymidines in DNA (Scheme 1) and change the local structure and properties of the DNA. Two-dimensional NMR studies on DNA duplexes containing a cis-syn thymine dimer have revealed perturbations to the structure and H-bonding of the duplex at the site of dimer.[6−10] In particular, the 3D structures derived from the 2D NOE data indicated that the 5′-T was more distorted and less capable of H-bonding with an A in the complementary strand than the 3′-T. In support of this conclusion, the shift of the N3 imino proton of the 5′-T of the cis-syn thymine dimer-containing DNA duplexes was more upfield than that of the 3′-T, though some or all of this upfield shift has been attributed to shielding effects of the neighboring A, which also causes a large upfield shift on the methyl group of the 5′-T.[10] Intensive theoretical studies have also been carried out on cis-syn dimer containing duplexes with similar conclusions with regard to the distorted nature of the 5′-T, but with differing conclusions as to the extent of DNA bending.[11−16] Most recently, a crystal

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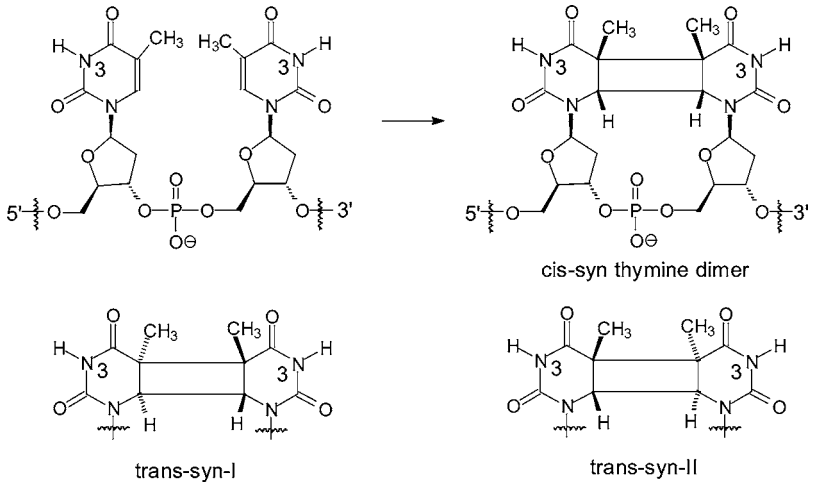

structure was obtained of a cis-syn thymine dimer-containing duplex decamer which corroborates the distorted nature of the base pair with the 5′-T that was deduced from the NMR and theoretical studies.[17]

What remains unresolved about DNA damaged by pyrimidine dimer formation, however, is the extent to which the 5′- and 3′-T's can hydrogen bond to complementary and mismatched bases in a DNA duplex and in the active site of a polymerase. The latter question is of particular interest, given recent results indicating that insertion opposite the more distorted 5′-T of a dimer by polymerase η (eta) proceeds with greater selectivity than opposite the 3′-T.[18,19] Pol η is a member of the Y family of DNA damage bypass polymerases and is the enzyme responsible for the almost error-free bypass of thymine dimers in mammalian cells.[20,21] Loss of pol η activity in humans has been linked to the genetic disease Xeroderma pigmentosum that results in increased sensitivity to sunlight and a much higher incidence of skin cancer.[22−24] Unfortunately, it has not yet been possible to crystallize either yeast or human pol η with DNA, but a crystal structure of the related Y family polymerase Dpo4 revealed the surprising result that insertion opposite the 5′-T occurs via Hoogsteen base pairing, whereas insertion opposite the 3′-T occurs via a normal Watson−Crick base pairing.[25] Recent results showing that 7-deaza-adenosine is inserted with about the same efficiency as adenosine opposite the 5′-T by yeast pol η would suggest that Hoogsteen base pairing is not involved,[19] but this remains to be verified by other methods.

^{15}N NMR has proven to be very useful for studying protonation, tautomerization, and hydrogen bonding to nitrogen and for determining the relative orientations of NH bond vectors in DNA through analysis of residual dipolar coupling.[26−37] ^{15}N-labeling also greatly facilitates the assignment and editing of proton, ^{13}C and ^{15}N spectra. A [3-^{15}N]-labeled thymine dimer would therefore be especially useful for probing the nature of H-bonding interactions between the 5′- and 3′−T's of thymine dimer and complementary and mismatched bases in a duplex and in the active site of a polymerase or repair enzyme by solution and/or solid-state NMR. The most straightforward method to prepare a [3-^{15}N]-labeled thymine dimer-containing duplex would be either to irradiate an ^{15}N-labeled precursor ODN that was prepared by automated DNA synthesis (postsynthetic irradiation route) or to use an ^{15}N-labeled thymine dimer phosphoramidite (building block route). Though uniformly ^{15}N-labeled phosphoramidites are now commercially available (Spectra Stable Isotopes), they are very expensive ($750−$1200/μmol) and because they are uniformly labeled complicate spectral analysis. Recently, a [3-^{15}N]-thymidine phosphoramidite has been synthesized from [3-^{15}N]-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-dyl)thymidine and used to study base pairing to a complementary A in a DNA duplex.[34]

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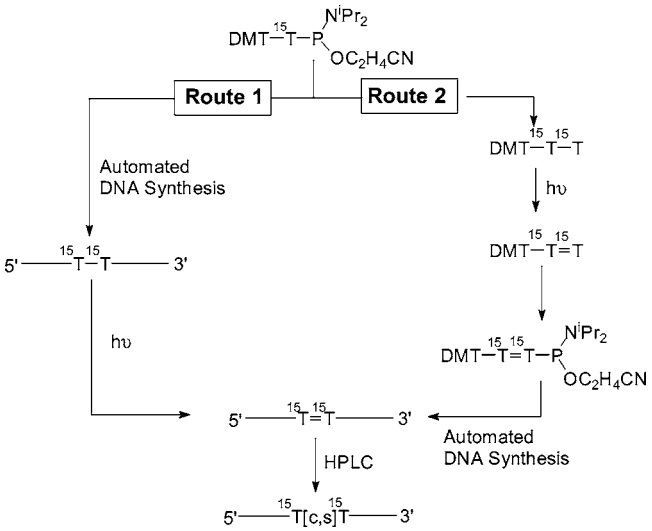
SCHEME 2

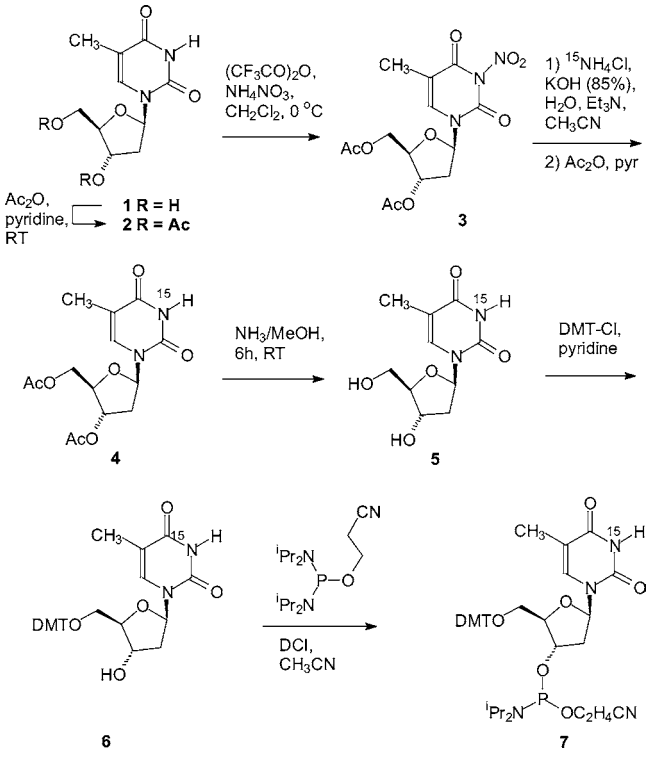


In this paper, we report two efficient routes for the synthesis of ODNs containing cis-syn [3-^{15}N]-thymine dimers from [3-^{15}N]-thymidine phosphoramidite (Scheme 2). In the postsynthetic irradiation route, an ODN containing a [3-^{15}N]-labeled TT site was synthesized from [3-^{15}N]-thymidine phosphoramidite and then irradiated in the presence of a sensitizer to produce the cis-syn dimer which is isolated by HPLC. In the building block route, a mixture of cis-syn and trans-syn [3-^{15}N]-thymine dimer-containing ODNs was synthesized from a mixture of [3-^{15}N]-labeled thymine dimer phosphoramidites after which the cis-syn containing ODN was isolated by HPLC. We also report the assignment and analysis of the ^{15}N shifts and coupling constants of a [3-^{15}N]-thymine dimer-containing decamer duplex leading to the conclusion that the 5′-T is less hydrogen bonded than the 3′-T.

Results and Discussion

Both the postsynthetic and mixed building block routes to the [3-^{15}N]-labeled thymine dimer-containing ODNs proceeded from the [3-^{15}N]-thymidine phosphoramidite **7**, which was prepared in six steps from thymidine (Scheme 3) by adapting routes used for the synthesis of [3-^{15}N]-uridine and its derivatives.[38−40] Thus, thymidine **1** was first acetylated with acetic anhydride to give **2**, which was then nitrated at N3 to give **3**.[41,42] Treatment of **3** with $^{15}NH_4Cl$ (1.3 equiv), KOH, and Et_3N in CH_3CN-H_2O for 6 days at room temperature afforded **4** together with partially and fully deacetylated products. To increase the yield of **4**, the mixture of products was reacetylated in situ prior to chromatographic isolation. Compound **4** was then deacetylated with saturated ammonia in methanol to give [3-^{15}N]-labeled thymidine **5**. The 5′-hydroxyl group of **5** was then protected by reaction with 4,4′-dimethoxytrityl chloride to afford **6** which was then converted to the phosphoramidite **7** by standard procedures. Phosphoramidite **7** was then used to

SCHEME 3



prepare d(CGAA[3-^{15}N]T(cis-syn)[3-^{15}N]TAAGC) by the postsynthetic irradiation and mixed building block routes. This sequence was chosen to allow direct comparison of H-bonding and structural data obtained from solution NMR studies of its duplex form with that of the crystal structure.[17]

Postsynthetic Irradiation Route. For the postsynthetic irradiation route, phosphoramidite **7** was used to incorporate [3-^{15}N]-labeled thymidine into the central two nucleotides of 10-mer ODN using solid-phase automated DNA synthesis on a 1 μmol scale. Following automated synthesis, the ODN was released from the support, completely deprotected with concentrated ammonia, and purified by reversed-phase chromatography. The ODN was then irradiated with a 450 W medium-pressure Hannovia mercury lamp for 1 h in the presence of the triplet sensitizer acetophenone to produce the ^{15}N-labeled cis-syn thymine dimer-containing ODN as the major product (Figure 1). To help confirm the identity of the cis-syn thymine dimer-containing ODN, the chromatographic behavior of the [3-^{15}N]-

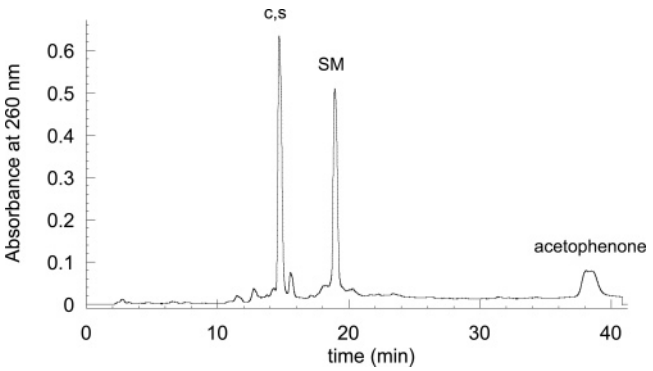


FIGURE 1. HPLC analysis of the acetophenone-sensitized irradiation products of d(CGAA[3-^{15}N]T[3-^{15}N]TAAGC). Abbreviations: c,s, cis-syn photoproduct-containing ODN; SM, starting material (ODN). See the Experimental Section for details.

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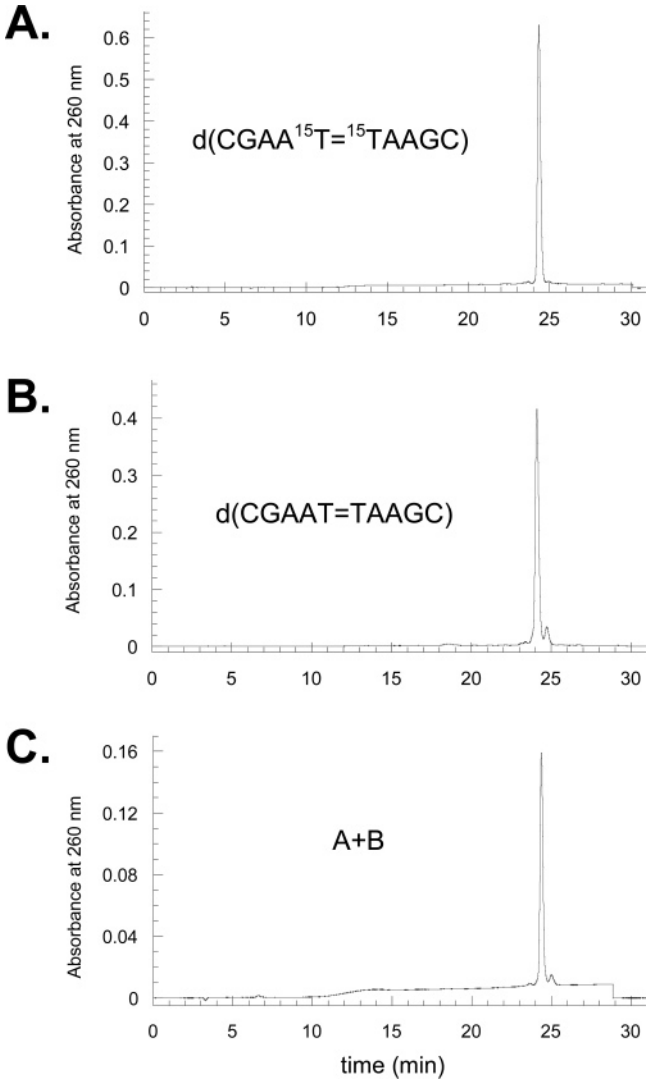

FIGURE 2. HPLC analysis of the major product isolated from the irradiation of d(CGAA[3-^{15}N]T[3-^{15}N]TAAGC). Panel A: the major product from irradiation. Panel B: authentic cis-syn dimer-containing decamer. Panel C: co-injection of the authentic product and that from the major irradiation product. See the Experimental Section for details.

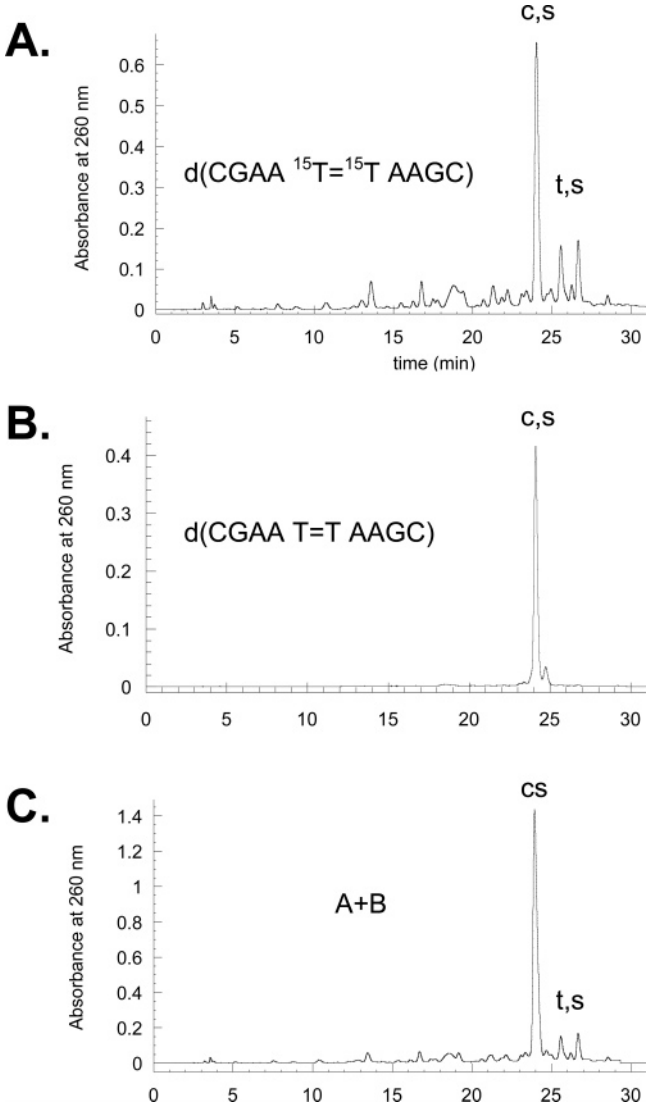

FIGURE 3. HPLC analysis of the products from the mixed building block approach. Panel A: products of the mixed building block approach. The two minor products with retention times greater than the major product are most likely the trans-syn-I and trans-syn-II products. Panel B: authentic sample of cis-syn dimer-containing ODN. Panel C: co-injection of the authentic and synthetic ODNs. See the Experimental Section for details.

labeled ODN, d(CGAA[3-^{15}N]T(cis-syn)[3-^{15}N]TAAGC) was compared to an authentic sample of d(CGAAT(cis-syn)TAAGC) that was prepared from a pure cis-syn dimer building block and whose crystal structure has been solved[17] (Figure 2). Co-injection of both samples resulted in the appearance of single coeluting peak.

Mixed Building Block Route. Our original synthesis of a cis-syn thymine dimer phosphoramidite building block required six steps from a noncommercially available phosphoramidite. It also required a tedious and low-yielding chromatographic separation of six cis-syn, trans-syn-I, trans-syn-II stereoisomers that result from irradiation of a diastereomeric mixture of R_P and S_P methyl phosphate esters of 3′-TBDMS-thymidylyl-(3′→5′)thymidine.[43] More recently, we have developed a three-step mixed building block approach from commercially available T phosphoramidite in which the stereoisomers are not separated until after DNA synthesis and deprotection. At this point, only three stereoisomers have to be separated since deprotection removes the chirality at phosphorus (Ren, Y., Ph.D. Thesis, Washington University, St. Louis, December, 2000). Thus, [3-^{15}N]-thymidine phosphoramidite **7** was coupled with [3-^{15}N]-labeled thymidine to produce the dinucleotide DMT-TpT **8** (Scheme 4). This dinucleotide was irradiated with a 450-W medium-pressure Hanovia mercury lamp in the presence of the triplet sensitizer acetophenone for 3 h to give a mixture of cis-syn, trans-syn-I, and trans-syn-II cyclobutane thymine dimers **9**. The mixture was not separated, but phosphorylated to give the mixed phosphoramidite building block **10**. The mixed building block was used to incorporate the mixture of thymine dimer isomers into the same 10-mer ODN prepared by the postsynthetic irradiation. The resulting mixture of [3-^{15}N]-labeled cis-syn, trans-syn-I, and trans-syn-II dimer-containing ODNs were separated by a reverse phase chromatography. The major peak was identified as the cis-syn thymine dimer-

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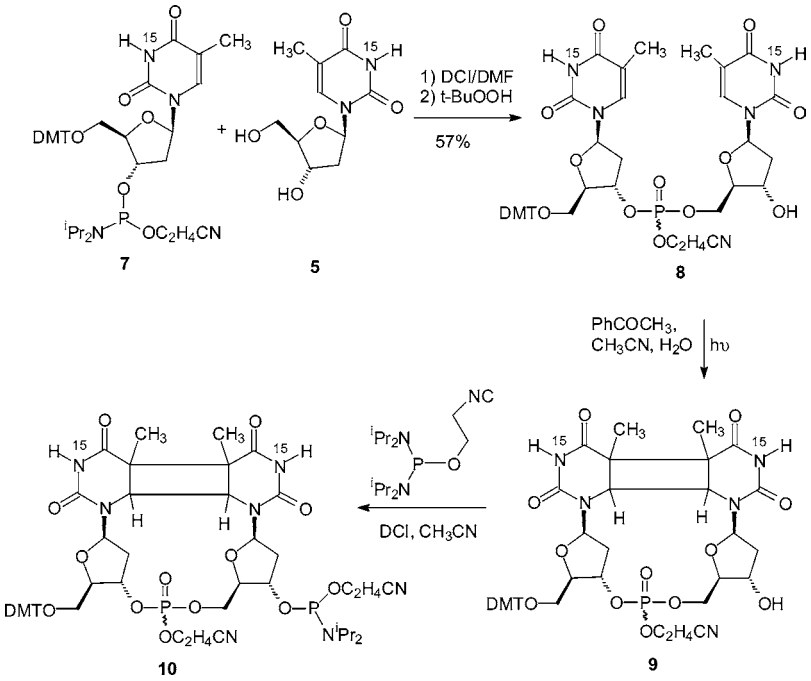
SCHEME 4



containing ODN by its coelution with a sample of authentic ODN (Figure 3).

NMR Spectroscopy. A complementary DNA duplex was prepared, and the ^{15}N shifts were assigned by correlation with the imino protons which have previously been assigned by 2D NOE at 5 °C in the closely related dodecamer sequence d(GCACGAAT[c,s]TAAG)·d(CTTAATTCGTGC).[10] In the dodecamer sequence, which shares the same d(GAAT[c,s]-TAAG) core, the more upfield proton at δ 11.87 was assigned to the 5′-T whereas the more downfield proton at δ 12.93 was assigned to the 3′-T. Figure 4 shows the HSQC spectrum collected at 25 °C in which the more downfield proton at δ 12.72 assigned to the 3′-T correlates with the more upfield nitrogen at δ 153.38. Likewise, the more upfield proton at δ 11.68 assigned to the more distorted 5′-T correlates with the more downfield nitrogen at δ 155.05.

The coupling constant for the ^{15}N-^{1}H of the 5′-T was found to be 89.09 Hz (Figure 5) and is 1.8 Hz greater than that of 87.27 Hz observed for the 3′-T. The larger coupling constant is consistent with weaker H-bonding for the 5′-T than for the 3′-T based on a recent study of the correlation between ^{15}N−^{1}H coupling constants and H-bonding strength for a series of substituted uridines in duplex DNA.[44] In that study the theoretically calculated H-bonding energy was found to linearly correlate with the ^{15}N−^{1}H coupling constant as well as with the pK_a and the chemical shift of the imino proton. From their linear fit parameters an increase of 1.8 Hz in the N−H coupling constant of the 5′-T compared to the 3′-T corresponds to a loss of 0.39 kcal/mol in the strength of the H-bond. When using their linear fit parameters for proton chemical shift, the decrease of 1.06 ppm in chemical shift of the N3H of the 5′-T corresponds to a 0.76 kcal/mol loss in H-bond strength which is about two times larger than that calculated from the difference in the N−H coupling constant data. Though we do not yet know how well any of these parameters apply to thymines with a saturated 5,6 double bond, it is likely that part of the large upfield shift of the N3H of the 5′-T relative to the 3′-T is due to shielding by the neighboring base pair has been previously suggested.[10] None-the-less, it appears from the increase in the ^{15}N−^{1}H coupling of the 5′-T of the dimer relative to the 3′-T that the 5′-T is less H-bonded than the 3′-T and could explain in part the 1.5 kcal/mol destabilization caused by cis-syn dimer formation.[45] The greater selectivity in the insertion of A opposite the 5′-T than the 3′-T by polymerase η[19] would therefore appear

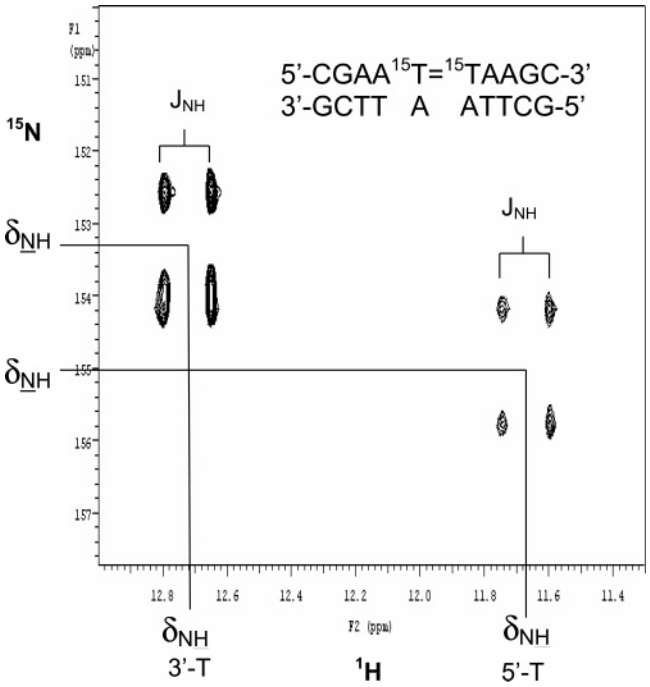


FIGURE 4. Section of the proton-coupled ^{15}N−^{1}H HSQC spectrum of d(CGAA[3-^{15}N]T═[3-^{15}N]TAAGC)·d(GCTTAATTCG) showing the shift correlations and coupling constants for the N3H bonds of the thymine dimer.

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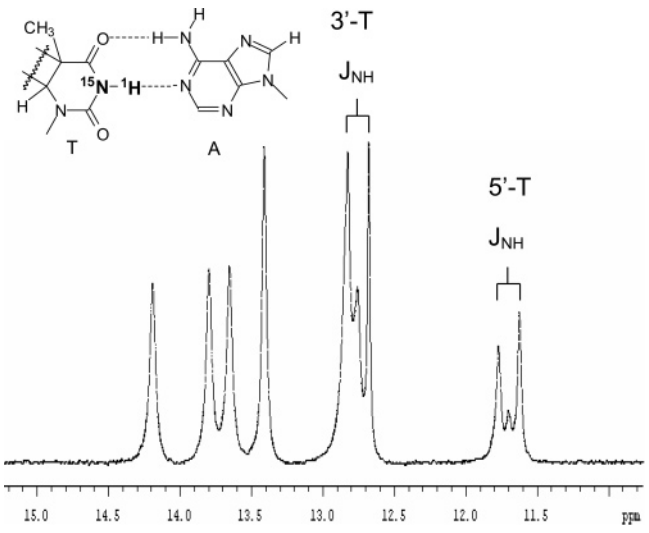

FIGURE 5. Section of the 1D ^{1}H spectrum of d(CGAA[3-^{15}N]T═[3-^{15}N]TAAGC)•d(GCTTAATTCG) at 0.11 Hz digital resolution from which the coupling constants were determined.

not to be associated with H-bonding strength, but more likely due to the constrained nature of the 5′-T in the active site of the polymerase as previously proposed.[46] A more definitive answer, however, will require a study of the H-bonding in a complex between pol η and a thymine dimer-containing template primer.

Conclusion

We have described the use of a [3-^{15}N]-thymidine phosphoramidite to prepare a [3-^{15}N]-labeled cis-syn thymine dimer-containing oligodeoxynucleotide by two routes. The presence of the ^{15}N label greatly facilitated the assignment of the ^{15}N shifts and ^{15}N-^{1}H coupling constants of the dimer from which we concluded that the 5′-T is only slightly less strongly H-bonded than the 3′-T. The availability of the [3-^{15}N]-labeled thymine dimer opens the door to study the sequence context dependence of base pairing of thymine dimers in DNA duplexes, as well as in the active site of DNA polymerases and repair enzymes by both solution and solid-state NMR.

Experimental Section

[3-^{15}N]-3′,5′-Di-*O*-acetylthymidine (4). Water (15 mL), CH_3CN (15 mL), Et_3N (2.40 mL, 17.3 mmol), and a solution of 3′,5′-di-*O*-acetyl-3-nitrothymidine in 30 mL of acetonitrile (**3**, 4.927 g, 13.26 mmol)[41] were added sequentially to a tightly sealed 250-mL round-bottom flask containing $^{15}NH_4Cl$ (940 mg, 17.25 mmol) and KOH (85%, 1.050 g, 15.92 mmol). After being stirred vigorously for 6 days, the solution was first saturated with NaCl and then extracted with CH_2Cl_2. The aqueous layer was evaporated, and the residue was triturated with CH_2Cl_2−MeOH (9:1). The combined organic layers were dried over Na_2SO_4 and evaporated. The resulting residue was dissolved in pyridine (30 mL), and acetic anhydride (50 mmol) was added. After the mixture was stirred for 3 days, 4 mL of methanol was added and the solvent evaporated. The residue was purified by column chromatography with ethyl acetate as the eluant to give **4** (3.037 g, 70%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.94 (s, 3H, C5CH_3), 2.12 (s, 3H, OCOCH_3), 2.14 (s, 3H, OCOCH_3), 2.18 (m, 1H, H2′), 2.47 (m, 1H, H2″), 4.24 (m, 1H, H4′). 4.37 (m, 2H, H5′ and H5″), 5.22 (m, 1H, H3′), 6.33 (t, J = 7.1 Hz, 1H, H1′), 7.28 (s, 1H, ArH), 8.95 (d, J = 90.6 Hz, 1H, ^{15}NH); ^{15}N NMR (90 MHz, $CDCl_3$) δ 155.9; ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 12.0, 20.3, 20.4, 36.8, 64.1, 74.7, 82.2, 85.0, 110.8, 135.8, 150.9, 164.0, 170.4; HRMS (FAB) calcd for $C_{14}H_{18}N^{15}NO_7$ (M + Li)$^+$ 334.1244, found 334.1239.

[3-^{15}N]-Thymidine (5). Ammonia (7 N) in MeOH (10 mL) was added to a solution of [3-^{15}N]-3′,5′-di-*O*-acetylthymidine (**4**, 985 mg, 3.01 mmol) in MeOH (10 mL) and stirred for 6 h at room temperature. The solvent was then removed under vacuum, and the remaining acetamide was removed by heating in a vacuum at 100 °C for 2 h to yield **5** (731 mg, 100%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.77 (s, 3H, C5CH_3), 2.07 (m, 2H, H2′ and H2″), 3.66 (m, 2H, H5′ and H5″), 3.76 (s, 1H, C4′-OH), 4.23 (s, 1H, C3′-OH), 5.03 (m, 1H, H4′), 5.24 (m, 1H, H3′), 6.16 (t, J = 6.8 Hz, 1H, H1′), 7.70 (s, 1H, ArH), 11.29 (d, J = 97.2 Hz, ^{15}NH); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 12.2, 61.3, 70.4, 83.7, 87.2, 109.3, 136.1, 150.4, 163.6, 163.8; HRMS (FAB) calcd for $C_{10}H_{14}N^{15}NO_5$ (M + Li)$^+$ 250.1051, found 250.1045.

[3-^{15}N]-Dimethoxytritylthymidine (6). 4,4′-Dimethoxytrityl chloride (10221 g, 3.61 mmol) was quickly added to a solution of **5** (731 mg, 3.01 mmol) in 15 mL of pyridine and the solution stirred overnight at room temperature. The pyridine was removed under vacuum to yield the crude product as a brown gum which was redissolved in EtOAc and washed with saturated NaCl. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give a brown gum which was purified by flash column chromatography using 80% EtOAc in hexane to give **6** (1.183 g, 72%): ^{1}H NMR (300 MHz, acetone-d_6) δ 1.47 (s, 3H, C5CH_3), 2.34 (m, 2H, H2′ and H2″), 3.38 (d, J = 3.3 Hz, 2H, H5′ and H5″), 3.79 (s, 6H, ArOCH_3), 4.04 (m, 1H, H4′), 4.60 (m,1H, H3′), 6.36 (t, J = 7.2 Hz, 1H, H1′), 6.83−7.57 (m, 13H, ArH), 7.63 (s, 1H, ArH5), 9.9 (d, J = 91.2 Hz, 1H, ^{15}NH); ^{13}C NMR (75.4 MHz, acetone-d_6) δ 12.1, 41.0, 55.4, 64.6, 72.3, 85.3, 87.0, 87.2, 111.0, 113.8, 127.6, 128.6, 128.8, 130.8, 136.3, 136.5, 136.6, 145.7, 151.3, 159.5, 159.5, 164.5, 164.7; HRMS (FAB) calcd for $C_{31}H_{32}N^{15}NO_7$ (M + Li)$^+$ 552.2355, found 552.2351.

[3-^{15}N]-Dimethoxytritylthymidine Phosphoramidite (7). To a solution of **6** (550 mg, 1.01 mmol) in anhydrous CH_3CN were added 4,5-dicyanoimidazole (179 mg, 1.52 mmol) and 2-cyanoethyl tetraisopropylphosphorodiamidite (642 μL, 2.02 mmol) at room temperature. The reaction mixture was stirred for 0.5 h and then extracted with EtOAc and washed with saturated aqueous $NaHCO_3$. The organic layer was further washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography after neutralizing the silica gel with Et_3N using 60% EtOAc in hexane to give **7** (658 mg, 87%): ^{31}P (121.5 MHz, acetone-d6 referenced to trimethyl phosphate TMP) δ 145.78, 145.85; HRMS (FAB) calcd for $C_{40}H_{49}N_3^{15}NO_8P$ (M + Li)$^+$ 752.3405, found 752.3407.

DMT-[3-^{15}N]T-PO(OCH$_2$CH$_2$CN) [3-^{15}N]T (8). To [3-^{15}N]-labeled thymidine (**5**, 278 g, 1.14 mmol) and 4,5-dicyanoimidazole (90 mg, 0.76 mmol) in 2 mL of anhydrous DMF was added dropwise a 2 mL solution of DMT-[3-^{15}N]dT phosphoramidite (**7**, 284 mg, 0.38 mmol) in anhydrous DMF at room temperature. After 5 min, *t*-BuOOH (209 μL, 5−6 M solution in nonane, 1.25 mmol) was added. After 10 min at room temperature, the reaction mixture was extracted with ethyl acetate and washed with saturated $NaHCO_3$ aqueous solution. The organic layer was further washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by column chromatography after neutralizing the silica gel with Et_3N and eluted with 10% methanol in ethyl acetate to afford the title compound (558 mg, 70%) as a yellowish solid: TLC (methanol/ethyl acetate, 5:95) R_f 0.25; ^{31}P (121.5 MHz, acetone-d_6, referenced to TMP as an external reference) δ −4.5 and −4.7; MS (+ESI); HRMS calcd for $C_{40}H_{49}N_3^{15}NO_8P$ (M + Na)$^+$ 926.2774, found 926.2783.

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DMT-[3-^{15}N]T[=]PO(OCH$_2$CH$_2$CN)[3-^{15}N]T (9): To a Pyrex immersion well photochemical reactor were added compound **8** (400 mg, 0.44 mmol), 400 mL of acetonitrile/H_2O (1:1), and acetophenone (0.4 mL, 3.42 mmol). The solution was purged with argon for 40 min and continued while the mixture was irradiated with a 450 W medium-pressure Hanovia mercury lamp for 3 h. Solvents were evaporated under reduced pressure, and the residue was subjected to silica gel flash chromatography after neutralizing the silica gel with Et_3N and eluted with 10% MeOH in ethyl acetate to afford a mixture of dimers **9** (300 mg, 0.33 mmol, 75%) as a yellowish solid: ^{31}P NMR (121.5 MHz, acetone-d_6, referenced to TMP as an external reference) −4.6, −4.8, −5.2, −7.5, −7.8, and −8.2 ppm; HRMS (FAB) calcd for $C_{44}H_{48}N_3{}^{15}N_2O_{14}P$ $(M + H)^+$ 904.2954, found 904.3325.

DMT-[3-^{15}N]T[=]PO(OCH$_2$CH$_2$CN)[3-^{15}N]T−P(OCH$_2$CH$_2$CN)-(N^{i}Pr$_2$) (10). To 5 mL of an anhydrous acetonitrile solution of the photoproduct mixture **9** (300 mg, 0.33 mmol) and 4,5-dicyanoimidazole (58 mg, 0.49 mmol) was added 2-cyanoethyl tetraisopropylphosphorodiamidite (210 μL, 0.66 mmol) at room temperature. The reaction mixture was stirred for 0.5 h under argon and then extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated, and the residue was purified by column chromatography after neutralizing the silica gel with Et_3N and eluted with 80% ethyl acetate in hexane to afford the photoproduct mixture **10** (232 mg, 0.21 mmol, 64%) as a yellowish solid: ^{31}P NMR (121.5 MHz, acetone-d_6, referenced to TMP as an external reference) 146.15, 146.08, 146.03, 145.92, 145.85, 145.78, 145.71, 145.50, −4.54, −4.63, −7.53, −8.19.

ODN Synthesis and HPLC Analysis and Purification. Oligodeoxynucleotides were synthesized on an automated DNA synthesizer on a 1 μmol scale. The ODNs were cleaved from the support with concentrated ammonium hydroxide (30% aqueous, 1.5 mL) and fully deprotected by heating the solution at 55 °C in a sealed tube for 8 h. The samples were dried in a Speedvac, dissolved in ddH_2O, and purified and analyzed by HPLC on an XTerra MS C18 2.5 μM 10 × 50 mm column at a flow rate of 1 mL/min with UV detection at 260 nm. Unless otherwise indicated, standard conditions for purification and analysis were 5 min of 100% buffer A (5% acetonitrile/0.05 M triethylammonium acetate TEAA, pH ∼ 7), followed by a 50 min gradient of 100% buffer A to 80% buffer A, 20% buffer B (50% acetonitrile, 0.05 M TEAA, pH ∼ 7).

Synthesis of d(CGAA[3-^{15}N]T=[3-^{15}N]TAAGC) by Irradiation of d(CGAA[3-^{15}N]T[3-^{15}N]TAAGC). d(CGAA[3-^{15}N]T[3-^{15}N]TAAGC) was synthesized using phosphoramidite **7** and purified by HPLC under standard conditions. Dimer formation was induced by irradiating d(CGAA[3-^{15}N]T[3-^{15}N]TAAGC) (300 μM) and acetophenone (30 mM) in 15% CH_3CN in ddH_2O with Pyrex filtered light for 1 h at 0 °C. HPLC analysis (Figure 1) was carried out using 5 min 100% solvent A (10% MeOH, 90% 75 mm phosphate buffer, pH ∼7) followed by a 30 min gradient of 100% solvent A to 100% solvent B (40% MeOH, 60% phosphate buffer, pH ∼7). The cis-syn containing ODN was then purified and analyzed (Figure 2) under standard conditions.

Synthesis of d(CGAA[3-^{15}N]T=[3-^{15}N]TAAGC). The title oligodeoxynucleotide was synthesized from the mixed thymidine dimer building block **10**. The desired cis-syn product was purified and analyzed (Figure 3) under standard conditions and used for the preparation of the NMR sample. The cis-syn, trans-syn-I, and trans-syn-II eluted at 24.4, 26.1, and 27.0 min, respectively.

Preparation of the Duplex DNA NMR Sample. A solution of d(CGAA[3-^{15}N]T=[3-^{15}N]TAAGC)·d(GCTTAATTCG) (3.5 mM) was prepared by mixing approximately equimolar amounts of the two ODNs and then using HPLC with detection at 260 nm to determine the ratio of the two strands from (area % of the complementary strand/molar extinction coefficient of the complementary strand)/(area% of the dimer strand/ molar extinction coefficient of the dimer strand). The ratio was adjusted to ∼1.0 by addition of the appropriate strand, and then made up to a final volume of 0.2 mL in 10% D_2O (99.96%) 90% ddH_2O containing 100 mM NaCl, 10 mM sodium phosphate buffer (pH 7), and 0.01% NaN_3. The final pH of the solution was 5.7. ^{15}N NMR was referenced to external $^{15}NH_4Cl$ at δ 23.6.[47]

^{15}N NMR Spectroscopy. NMR experiments were performed at 25 °C on a 600 MHz spectrometer with 3 mm gradient probe (600 MHz ^{1}H, 60.8 MHz ^{15}N). Proton chemical shifts were measured in parts per million (ppm) downfield from an internal TSP standard. Proton spectra were obtained at a spectral width of 14000 Hz and collected into 32K data points with a hard-pulse WATERGATE sequence for water suppression.[48] Proton-nitrogen coupling constants were measured directly from the splitting of the proton resonance signals with a 14000 Hz spectral width collecting into 128K data points (digital resolution 0.11 Hz). A two-dimensional ^{1}H-^{15}N HSQC experiment was collected with spectral width of 14000 and 10000 Hz along the F2 (^{1}H) and F1 (^{15}N) dimensions, respectively. A proton-coupled ^{1}H-^{15}N HSQC spectrum was obtained with a 1500 Hz in F2 and a 5000 Hz in F1 dimension. The 90° ^{1}H pulse width was 6.8 μs and the 90° ^{15}N pulse width was 32 μs. A data matrix with 256 complex points in F1 and 4096 complex points in F2 with 16 scans per t1 value was collected using pulse sequence of Kay and co-workers.[49]

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Supporting Information Available: ^{1}H and assorted ^{13}C, ^{15}N, and ^{31}P NMR spectra of compounds **4**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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