the formation of a site that is apparently similar to a classic blue copper site (a site extremely well-suited for facile electron-transfer reactivity) is apparently associated with a decrease in the catalytic activity of N_2O reductase.

When either ascorbate or dithionite is used to reduce resting N_2O reductase, the resulting "blue" form displays an unusual EPR spectrum that is distinctly different from typical blue copper EPR signals.[36] This EPR spectrum and the blue absorbance persist even in solutions containing excess reductant.[12] Despite the implications of these observations, the vibrational frequencies and relative intensities in the resonance Raman spectrum of the blue form (Figure 6) are essentially identical with those of the "blue" copper chromophore in the resting form (Figure 5) and rather similar to the pattern in the resonance Raman spectra of other blue copper proteins. Furthermore, the CD spectrum (Figure 7) resembles the CD spectra of many blue copper proteins with regard to both the intensities and energies of the bands, although some differences in the signs are evident.[28] We suggest that at least one *oxidized* copper ion may be present in the *reduced* N_2O reductase prepared from either the low- or high-activity resting form. Type 1 copper ions invariably display positive (vs. SHE) reduction potentials, indicating that if the "blue" sites in N_2O reductase are similar, they must be inaccessible to negatively charged exogenous reductants. Because neither reduced form can apparently be reoxidized to the high-activity resting form, irreversible protein conformational changes may accompany reduction. These conformational changes may also render the "blue" sites inaccessible.

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Conclusions

The resonance Raman data indicate that the unusual spectroscopic properties of the *P. perfectomarina* N_2O reductase are associated with Cu–S chromophores. In particular, a $[Cu^{II}S_2(cys)_2N(his)]$ site is suggested to be responsible for the intense 540-nm absorption band of the resting forms; this absorption correlates with catalytic activity, implying that the novel copper site is essential for turnover. No organic coenzyme or Cu^{II}–tyrosine units contribute to the visible absorption spectrum. A copper that is similar to a classical blue copper ion is present in the low-activity form of the resting enzyme, but probably not in the high-activity form. This blue site might be an altered 540-nm chromophore. The incremental activation of the high-activity form by base is not associated with structural perturbations of the 540-nm chromophore, suggesting that the pH-induced changes that give rise to the increased activity are localized on another copper site or the protein moiety. Reduced N_2O reductase also contains a chromophore that resembles an oxidized blue copper ion, which is apparently inaccessible to external reductants.

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A Building Block for the Sequence-Specific Introduction of Cis–Syn Thymine Dimers into Oligonucleotides. Solid-Phase Synthesis of TpT[*c*,*s*]pTpT

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Abstract: We report the synthesis of a synthetic intermediate for the sequence specific incorporation of the cis–syn thymine dimer into oligonucleotides via phosphoramidite-based solid-phase DNA synthesis technology. The required phosphoramidite **1a** was obtained in four steps from the known, pT-O_3-(*tert*-butyldimethylsilyl)thymidylyl-(3′→5′)-thymidine, *R*,*S*-*O*-methyl phosphate, compounds **6a**,**b**. Compounds **6a**,**b** were photolyzed with Pyrex-filtered light in the presence of the triplet sensitizer, acetophenone. The resulting four products, **12a**, **12b**, **8a**, and **8b**, were separated by a combination of flash chromatography and preparative reverse phase gradient HPLC. The structures of the four products were determined by complete deprotection and correlation with authentic cis–syn and trans–syn cyclobutane dimers of TpT, **9** and **13**, obtained from the photosensitized irradiation of thymidylyl-(3′→5′)-thymidine **7**. Compound **8a**, one of the two protected cis–syn isomers epimeric at phosphorus, was converted to the Tp-O_5-DMT derivative **10a**, followed by removal of the *tert*-butyldimethylsilyl group to give **11a**. Compound **11a** was converted to the target pT-O_3-methyl morpholinylphosphonite **1a** which was purified by flash chromatography. The cis–syn thymine dimer containing phosphoramidite was coupled to thymidine derivatized controlled pore glass support and further extended by an additional thymidine. The methyl phosphate protecting groups were removed, and the resulting thymine dimer containing tetranucleotide **14** was obtained after cleavage from the support. Tetrathymidylate **15** was prepared in a similar fashion for comparison purposes. Photolysis of **14** at 254 nm gave **15** as the major photoreversion product, thus establishing the integrity of the cis–syn thymine dimer unit. Both oligonucleotides were characterized by ^1H and ^{31}P NMR spectroscopy, C-18 and anion exchange HPLC chromatography, high resolution gel electrophoresis, and by a chemical degradation sequence. It was also established that the cis–syn cyclobutane dimer of TpT was stable to the conditions required to remove the standard amino protecting groups of A, C, and G. This makes the building block **1a** applicable to the synthesis of cis–syn thymine dimer containing oligonucleotides of heterogeneous sequence.

Exposure of DNA to ultraviolet light leads to the formation of a variety of photoproducts, otherwise known as photolesions.[1] Victims of the inherited disorder *xeroderma pigmentosum* are extremely sensitive to sunlight and have an approximately thou-

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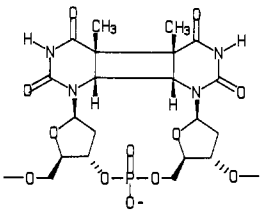
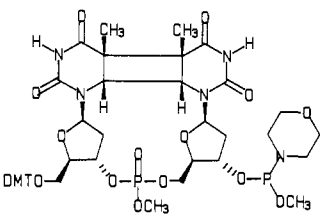
sandfold higher chance of developing skin cancer.[2] This predisposition to skin cancer has been attributed to defects in the biological systems required to repair photolesions and has greatly increased the interest in determining the mechanisms by which DNA photolesions lead to mutations.[3] Much of the work on DNA repair and mutagenesis by ultraviolet light has suffered, however, because of the lack of pure, well-characterized DNA photolesions for use in enzymatic and biological studies. Investigators have been forced to work with complex mixtures of products obtained from the exposure of DNA to ultraviolet light, and it is probable that properties ascribed to one photolesion are actually due to another. In order to circumvent this problem we have undertaken the synthesis of site-specific photolesion-containing oligonucleotides and viruses for physical, enzymological, and mutagenesis studies. This will enable us to determine unambiguously the relationship between the structure, properties, and site of a DNA photolesion and its mutation spectrum.

The cis–syn thymine dimer is the major lesion produced by UV irradiation of DNA and is a member of the general class of cyclobutane pyrimidine dimers.[1] The principal mutagenic role originally ascribed to cyclobutane pyrimidine dimers has recently been called into question by evidence which suggests that the actual premutagenic lesions are of the (6-4) photoproduct class.[4,5] The only report of the preparation of a site-specific photolesion-containing oligonucleotide appeared in 1971 and concerned the incorporation of a cis–syn thymine dimer into positions 5 and 6 of decathymidylate via solution phase, phosphodiester chemistry.[6] A full paper describing the synthesis and characterization of the oligonucleotide never appeared, and the route was never extended to the synthesis of other thymine dimer containing oligonucleotides, probably as a result of the inefficient and laborious nature of the route used. In order to make preparative amounts of photolesion-containing oligonucleotides of any desired sequence available for study, we have undertaken the design and synthesis photolesion "building blocks" suitable for automated solid-phase DNA synthesis. Herein we report the first synthesis of a cis–syn thymine dimer building block, compound **1**, for use with standard phosphoramidite-based coupling chemistry.[7] We also report the synthesis and characterization of tetrathymidylate containing the cis–syn thymine dimer at positions 2 and 3 for use in site-directed mutagenesis studies.



cis-syn



1

Experimental Section

Abbreviations used are as follows: DMT, 4,4′-dimethoxytrityl; TBDMS, *tert*-butyldimethylsilyl; TpT, thymidylyl-(3′→5′)-thymidine; TMP, trimethylphosphate; TPS, 3-(trimethylsilyl)propionic acid, sodium salt; THF, tetrahydrofuran; TBAF, tetrabutylammonium fluoride; DMF, dimethylformamide.

Materials and methods are as follows: $O_{5'}$-dimethoxytritylthymidine $O_{3'}$-(methyl-*N*,*N*-dimethyl)phosphoramidite was from Beckman. Polynucleotide kinase was from BRL. γ-^{32}P-ATP was from Amersham. Moisture sensitive reactions were conducted in oven-dried apparatus under positive nitrogen pressure. Solid starting materials were rendered anhydrous by pumping overnight under high vacuum. Acetonitrile was dried by distillation from calcium hydride; THF by distillation from sodium/benzophenone; pyridine, DMF, and methylene chloride by storage over 4Å sieves. TLC plates were Kieselgel 60, F254 plates from Merck. Photolysis was conducted in an Ace Pyrex immersion well reactor with a 450-W medium pressure mercury lamp. Analytical reverse phase C-18 chromatography was performed on a Waters μBondapak C-18 column (3.9 × 300 mm, 5 μm μPorasil support, 10% C-18 plus a TMS cap). Preparative reverse phase C-18 chromatography was performed on a Rainin Dynamax column (2 cm × 30 cm). Anion exchange chromatography was performed on an analytical Nucleogen DEAE 60-7 column (4 × 125 mm). Gradient HPLC was performed on a system composed of 2 Waters Series 6000 pumps, a Rheodyne 7125 sample injector with 20, 200, and 2000 μL sample loops, and an ISCO V-4 variable wavelength detector with a 5-mm pathlength heat exchanger HPLC flow cell. Gradient formation and peak integration was controlled by an Apple IIe based system, incorporating an Adalab data aquisition card, Chromadapt interface module, and Chromatochart software. Doubly distilled water was departmental distilled water redistilled through a Corning MP-1 still. Activated carbon for desalting was Aldrich nucleotide desalting grade. Flash chromatography was performed with 40–63 μm silica from Baker. Solid-phase DNA synthesis was conducted on a SAM I Series 2 automated DNA synthesis machine. High resolution electrophoresis was conducted on a BRL sequencing gel system, Model SO, on 34 × 40 cm plates with an ISCO Model 494 high voltage power supply. 300-MHz ^{1}H and 121.5-MHz ^{31}P experiments were performed on a Varian XL-300 instrument with a 5-mm multinuclear proton observe, proton decouple probe. UV spectra were taken on a Bausch and Lomb Spectronic 1001 spectrophotometer interfaced to an Apple IIe microcomputer with Bausch and Lomb wavelength scanning software, version 2.02. Infrared spectra were taken on a Perkin Elmer 283B calibrated with polystyrene. Mass spectrometry was performed on a Finnigan 3300 GC-MS adapted for FAB-MS. High resolution FAB-MS was conducted at the Midwest Center for Mass Spectrometry, University of Nebraska.

Photolysis of HO-TPO(OMe)T-OTBDMS, 6a,b. A nitrogen purged 30% aqueous acetonitrile solution of HO-TPO(OMe)T-OTBDMS,[8] **6a,b** (0.83 g, 1.23 mmol), containing acetophenone (0.862 mL, 7.39 mmol) was photolyzed for 2.5 h in a 0.4-L capacity Pyrex immersion well photolysis apparatus fitted with a 450-W Hanovia medium pressure lamp under tap water cooling. Isocratic analytical reverse phase HPLC chromatography at 30% water/methanol showed the presence of four major products A, B, C, and D with retention times of 7.2, 8.0, 8.4, and 9.2 min, respectively, at a detection wavelength of 230 nm. The reaction solution was then concentrated to a white solid in vacuo and flash chromatographed by using a step gradient of 0–10% methanol/methylene chloride to afford pure B in 27% yield. The three remaining compounds were then separated by preparative isocratic reverse phase HPLC chromatography on a Dynamax C-18 column at 30% water/methanol to afford A, C, and D at 24, 28, and 34 min in yields of 15%, 16%, and 14%, respectively.

HO-T[*t*,*s*]PO(OMe)T-OTBDMS, A = 12a: mp 194 °C dec; ^{1}H NMR (300 MHz $CDCl_3$, ppm from TMS) 5.73 (dd, J = 10.7, 4.7 Hz, $H_{1'}$), 5.25 (dd, J = 10.3, 5.0 Hz, $H_{1'}$), 3.82 (d, J = 11.2 Hz, $POCH_3$), 1.54 and 1.48 (s, C_5CH_3), 0.90 (s, *tert*-butyl CH_3) 0.11 and 0.10 (s, $SiCH_3$); ^{31}P NMR (121.5 MHz, ppm from TMP) −5.19; UV (methanol) no λ_{max} > 200 nm; IR (KBr) 3445, 3082, 2952, 2854, 1709, 1450, 1358, 1259, 1228, 1102, 1024, 837, 780; FAB-MS (glycerol) 675 (M + 1, 38), 577 (81), 451 (36), 445 (31), 333 (100), 319 (48); high resolution FAB-MS calcd for $C_{27}H_{44}N_4O_{12}PSi^+$ 675.2463, found 675.2478; TLC (10% $MeOH/CH_2Cl_2$) R_f 0.50.

HO-T[*c*,*s*]PO(OMe)T-OTBDMS, B = 8a: mp 165–180 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$, ppm from TMS) 6.15 (dd, J = 9.3, 5.9 Hz, $H_{1'}$), 5.36 (dd, J = 10.3, 4.4 Hz, $H_{1'}$), 3.78 (d, J = 11.2 Hz, $POCH_3$), 1.49 and 1.38 (s, C_5CH_3), 0.89 (s, *tert*-butyl CH_3), 0.084 and 0.082 (s, $SiCH_3$); ^{31}P NMR (121.5 MHz, $CDCl_3$, ppm from TMP) −5.88; UV (methanol) no λ_{max} > 200 nm; IR (KBr) 3400, 3209, 3076, 2926, 2850, 1706, 1441, 1368, 1247, 1027, 830, 774; FAB-MS: 675 (M + 1, 60), 577 (100), 543 (22), 451 (43), 333 (97), 319 (61); high resolution

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FAB-MS calcd for $C_{27}H_{44}N_4O_{12}PSi^+$ 675.2463, found 675.2434; TLC (10% MeOH/CH_2Cl_2) R_f 0.35.

HO-T[*t*,*s*]PO(OMe)T-OTBDMS, C = 12b: mp 172 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$, ppm from TMS) 5.98 (dd, J = 10.3, 5.4 Hz, $H_{1'}$), 5.30 (dd, J = 10.7, 4.9 Hz, $H_{1'}$), 3.81 (d, J = 11 Hz, $POCH_3$), 1.53 and 1.48 (s, C_5CH_3), 0.88 (s, *tert*-butyl CH_3), 0.08 (s, $SiCH_3$); ^{31}P NMR (121.5 MHz, ppm from TMP) −3.46; UV (methanol) no λ_{max} > 200 nm; IR (KBr) 3440, 3089, 2951, 2851, 1712, 1461, 1407, 1355, 1254, 1228, 1101, 1027, 834, 779; FAB-MS (glycerol) 675 (M + 1, 25), 577 (54), 451 (26), 333 (100), 319 (41); high resolution FAB-MS calcd for $C_{27}H_{44}N_4O_{12}PSi^+$ 675.2463, found 675.2463; TLC (10% MeOH/CH_2Cl_2) R_f 0.50.

HO-T[*c*,*s*]PO(OMe)T-OTBDMS, D = 8b: mp 182 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$, ppm from TMS) 6.22 (t, J = 7.0 Hz, $H_{1'}$), 5.92 (t, J = 6.3 Hz, $H_{1'}$), 3.80 (d, J = 12 Hz, $POCH_3$), 1.54 and 1.48 (s, C_5CH_3), 0.89 (s, *tert*-butyl CH_3), 0.08 and 0.07 (s, $SiCH_3$); ^{31}P NMR (121.5 MHz, ppm from TMP) −3.48 ppm; UV (methanol) no λ_{max} > 200 nm; IR (KBr) 3404, 3196, 3078, 2929, 2851, 1703, 1445, 1364, 1262, 1026, 835, 780; FAB-MS (glycerol) 675 (M + 1, 27), 577 (62), 451 (29), 333 (100), 319 (44); high resolution FAB-MS calcd for $C_{27}H_{44}N_4O_{12}PSi^+$ 675.2463, found 675.2498; TLC (10% MeOH/CH_2Cl_2) R_f 0.50.

Structure Correlation of A, B, C, and D. An authentic mixture of cis–syn and trans–syn cyclobutane dimers of TpT, compounds **9** and **13**, was prepared by acetophenone-sensitized photolysis of TpT **7** according to the procedure of Liu and Yang.[9] Compounds A, B, C, and D were each separately deprotected by the following sequence of steps. The methyl-protecting group was removed by treatment at 60 °C with 50% pyridine/water for 12 h. The solvent was removed in vacuo, and the residue was treated with 80% acetic acid/water at 90 °C for 1 h to remove the *tert*-butyldimethylsilyl group. After removing the solvent in vacuo the residue was dissolved in methanol and chromatographed against the authentic mixture in 12% acetic acid/30% water/butanol on silica gel thin-layer plates.[9] The cyclobutane photodimers were visualized by exposure of the TLC plate to 254-nm light from a hand-held lamp for 5–10 min which causes their photoreversion to UV absorbant TpT. It was found that deprotected A and C cospotted with the trans–syn dimer **13**; and B and D cospotted with the cis–syn dimer **9**.

DMTO-T[*c*,*s*]PO(OMe)T-OTBDMS, 10a. 4,4′-Dimethoxytrityl chloride (139 mg, 0.41 mmol) was added to a solution of **8a** (230 mg, 0.34 mmol) in 3 mL of pyridine and allowed to stir at room temperature for 4 h. The solution was then concentrated to a gum in vacuo, stripped down 3 times with toluene, and flash chromatographed with a step gradient of 0–6% methanol/0.5% pyridine/methylene chloride. Concentration of appropriate fractions gave a gum which was then dissolved in methylene chloride and precipitated from hexane to give the product in 60% yield (200 mg, 0.20 mmol) as a white powder: mp 190–195 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$, ppm from TMS) 6.15 (t, J = 6.4 Hz, $H_{1'}$), 6.03 (t, J = 6.4 Hz, $H_{1'}$), 3.79 (s, $ArOCH_3$), 3.47 (d, J = 11.2 Hz, $POCH_3$), 1.14 (s, C_5CH_3), 0.90 and 0.87 (s, *tert*-butyl CH_3), 0.12 and 0.11 (s, $SiCH_3$); ^{31}P NMR (121.5 MHz, $CDCl_3$, ppm from TMP) −5.28; FAB-MS (glycerol) 303 (34), 242 (100), 131 (24); UV (MeOH, λ_{max}) 229 (ϵ 16 000); IR (KBr, cm^{-1}) 3066, 2960, 2865, 1722, 1709, 1608, 1507, 1461, 1445, 1255, 1176, 1097, 1029, 835, 778; TLC (5% methanol/methylene chloride) R_f 0.30.

DMTO-T[*c*,*s*]PO(OMe)T-OH, 11a. Compound **10a** (200 mg, 0.2 mmol) in 7 mL of tetrahydrofuran was stirred with 5 equiv of tetrabutylammonium fluoride (1.0 mL of 1 M solution in THF, 1.0 mmol) and 50 equiv of acetic acid (0.6 mL, 10.5 mmol) at room temperature for 24 h. The reaction was then diluted with 15 mL of methylene chloride and extracted 2 times with water, 2 times with saturated sodium bicarbonate, and once with saturated sodium chloride solution. The organic layer was filtered through cotton wool, dried with sodium sulfate, filtered, and concentrated in vacuo, and the residue was flash chromatographed with a 0–10% methanol/0.5% pyridine/methylene chloride step gradient in 2% methanol increments. Concentration of the appropriate fractions afforded the product as a white solid in 73% yield (130 mg, 0.15 mmol): mp 174–180 °C dec; ^{1}H NMR (300 MHz, 55 °C, $CDCl_3$, ppm from TMS) 6.02 (dd, J = 6.5, 4.0 Hz, $H_{1'}$), 5.90 (dd, J = 8.2, 5.5, $H_{1'}$), 3.79 (s, $ArOCH_3$), 3.50 (d, J = 11.2 Hz, $POCH_3$), 1.06 and 1.04 (s, C_5CH_3); ^{31}P NMR (121.5 MHz, $CDCl_3$, ppm from TMP) −5.82; IR (KBr, cm^{-1}) 1712, 1608, 1508, 1462, 1251, 1178, 1030, 827; UV (MeOH, λ_{max}) 231 (ϵ 21 000); TLC (10% methanol/methylene chloride) R_f 0.25.

DMTO-T[*c*,*s*]PO(OMe)T-OP(OMe)(NC_4H_8O), 1a. Morpholine (0.040 mL, 0.43 mmol) was added to methyldichlorophosphite (0.02 mL, 0.21 mmol) in 1 mL of anhydrous methylene chloride at 0 °C and stirred for 30 min at this temperature and then 30 min at room temperature. A solution of DMTO-T[*c*,*s*]PO(OMe)T-OH, **11a** (120 mg, 0.15 mmol), and diisopropylethylamine (0.07 mL, 0.43 mmol) in 2 mL of anhydrous methylene chloride was then added and allowed to stir for 15 min. The reaction was then concentrated in vacuo, and the residue was immediately subjected to flash chromatography with 10% triethylamine/45% ethylacetate/methylene chloride. Concentration of the appropriate fractions afforded the product as a white solid in 53% yield (80 mg, 0.079 mmol): ^{1}H NMR (300 MHz, $CDCl_3$, ppm from TMS) 6.06 (t, J = 6 Hz, $H_{1'}$), 6.00 (m, $H_{1'}$), 3.79 (s, $ArOCH_3$), 1.16 and 0.93 (s, C_5CH_3); ^{31}P NMR (121.5 MHz, ppm from TMP) 142.64 and 142.45 (s, phosphoramidite P), −5.25, −5.31 (s, phosphotriester P); TLC (10% triethylamine/45% EtOAc/CH_2Cl_2) R_f 0.30.

Solid-Phase DNA Synthesis. Solid-phase continuous flow DNA synthesis was automated with the use of a Biosearch SAM I Series II DNA synthesis machine. Controlled pore glass (CPG) supported thymidine (120 mg, 26 μmol/gm) was prepared according to described procedures[10] and packed into a 3 × 48 mm metal HPLC column which was then connected to the synthesizer. The synthesis protocol was adapted from that described by Biosearch for large scale DNA synthesis and involved the following sequence of steps at a flow rate of 2.5 mL/min (a program listing for the SAM I Series 2 is available on request): (1) acetonitrile wash, 4:30; (2) deblock: 0.122 M dichloroacetic acid/methylene chloride, 3:30; (3) acetonitrile wash, 7:30; (4) couple: alternating 80 mM phosphoramidite and 0.57 M tetrazole, flow = 1.25 mL/min, 0:45; followed by a coupling time of 3:00; (5) acetonitrile wash, 6:00; (6) oxidize: 0.18 M iodine in 0.44% 2,6-lutidine/10% water/THF, 1:20; (7) acetonitrile wash, 7:00; (8) cap: alternating 0.26 M 4-(dimethylamino)pyridine/6.2% pyridine/THF and 0.61 M acetic anhydride/THF, 0:45. The supported oligonucleotide was then deblocked and washed with acetonitrile for 8 min. Removal of the methyl phosphate protecting groups was accomplished by treatment of the bound oligomer with 20% thiophenol/40% triethylamine/THF in a polyethylene microcentrifuge tube for 45 min at room temperature, followed by five 1-mL THF washes, eight 1-mL methanol washes, and three 1-mL ether washes. The support was dried under vacuum and then treated with concentrated aqueous ammonia for 1 h at room temperature to release the deprotected oligomer from the resin. The ammonia solution was removed from the support and evaporated to dryness on a Savant Speedvac, first under water aspirator pressure and then under high vacuum to yield the crude oligomer as a pellet. The crude oligomer was then dissolved in doubly distilled water and purified by HPLC on a Nucleogen DEAE 60-7 preparatory column with a 0–20% 1 M KCl/20% acetonitrile/20 mM potassium phosphate buffer, pH 7.0/20 min gradient. The desired fractions were combined, and the organic solvents were removed by partial evaporation in vacuo. The aqueous fraction was then desalted by applying to an activated charcoal column, Aldrich desalting grade, washing with excess doubly destilled water, and eluting with 10% concentrated aqueous ammonia/methanol. The fractions containing UV activity at 260 nm were combined and concentrated to dryness in vacuo. Analytical C-18 chromatography was performed with a 0–40% methanol/75 mM KH_2PO_4 buffer/30 min gradient. NMR samples were prepared by three evaporations of the oligonucleotide from 99.5% D_2O and dissolution in "100%" D_2O from Merck.

TpT[*c*,*s*]pTpT, 14. The DMTO-T[*c*,*s*]PO(OMe)T-OP(OMe)-(NC_4H_8O) phosphoramidite, **1a** (80 mg), was dissolved in 2.5 mL of acetonitrile to give a 30 mM solution. A 40-min coupling time was required to effect a 30% coupling yield. The desired product eluted from the Nucleogen anion exchange column at 3.8 min and at 20.2 min from the analytical C-18 column: ^{1}H NMR (300 MHz, D_2O, ppm from TPS) d, 7.72, 7.64 (s, H_5), 6.30 (m, T1 and T4 $H_{1'}$), 5.92 and 5.75 (m, T2 and T3 $H_{1'}$), 1.90 and 1.87 (s, T1 and T4 CH_3); ^{31}P NMR (121.5 MHz, D_2O, ppm from TMP) −3.82, −4.06, and −4.54.

TpTpTpT, 15. Prepared according to the general procedure. The desired product eluted from the Nucleogen anion exchange column at 4.1 min and at 29.4 min on the C-18 column: ^{1}H NMR (300 MHz, D_2O, ppm from TPS) d 7.66 and 7.64 (s, H_6), 6.25 (m, $H_{1'}$), 1.88 (s, CH_3); ^{31}P NMR (121.5 MHz, D_2O, ppm from TMP) −4.17, −4.20, −4.26.

Photoreversion of 14 to 15. A 1 mM aqueous solution (20 μL) of compound **14** was exposed to 254-nm light from a Model UVGL-25 Mineralight lamp for 15 min at a distance of 0.4 cm. Analytical C-18 HPLC chromatography (0–40% MeOH/75 mM KH_2PO_4/30 min, detected at 254 nm) of the photoreaction mixture showed major peaks at 20.2 min (**14**), 21.0 min, 22.0 min, and 29.4 min (**15**).

Chemical Degradation Studies. Each oligomer (12 μg) was endlabeled by treatment with polynucleotide kinase in a 10 mM $MgCl_2$, 5 mM DTT, 70 mM Tris-HCl pH 7 buffer for 0.5 h at 37 °C in the presence of 20 μCi of γ-^{32}P-ATP and used without further purification. Approximately

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3.4 μCi of endlabeled oligonucleotide was incubated in the dark with 1 mg of sodium borohydride in 100 μL of 5 mM potassium phosphate (pH 8.3), 5 mM sodium chloride, and 50 mM thymidine at 0 °C for 16 h. The reaction was quenched by the addition of 250 μL of 0.7 mM sodium acetate/acetic acid, pH 5 for 1 h with occasional vortexing. The oligonucleotides were then applied to a 2 cm × 0.4 cm bed of activated charcoal, prewashed with 10 mL of water, washed with 10 mL of water, eluted with 10% concentrated ammonia/methanol, and concentrated to dryness. The lyophilized reaction products were then taken up in 24 μL of water, and 4 μL aliquots were individually incubated with 50 μL of freshly prepared 1 M aniline–acetic acid, pH 4.5 for specific times and temperatures. The reactions were then quickly cooled to −78 °C and lyophilized followed by three lyophilizations from 100 μL of water and two from 100 μL ethanol. The resulting residues were dissolved in 10 μL of water to which was added 5 μL of 1 mM EDTA, 50 mM Tris·HCl, pH 8.3 in 80% aqueous formamide and 2 μL of 0.25% bromophenol blue, 0.25% xylene cyanole, and 30% glycerol. The samples were run, along with controls, on a 1:20 crosslinked, 20% acrylamide gel, 100 mM Tris–borate buffer, pH 7.8, for 3.3 hours at 1500 V at 4 °C. The gel was then exposed to Kodak X-OMAT AR film for 11.5 h at −20 °C and developed with Kodak GBX developer and fixer.

Results and Discussion

Building Block Design and Synthetic Strategy. Three additions to the cis–syn thymine dimer of thymidylyl-(3′→5′)-thymidine are required in order to convert it into a building block suitable for 3′→5′ phosphoramidite-based solid-phase DNA synthesis methodology: (1) a $O_{3'}$ phosphoramidite group for coupling to the $O_{5'}$ hydroxyl of a solid phase supported growing oligonucleotide chain, (2) an $O_{5'}$ protecting group to prevent polymerization during the coupling step, which can easily be removed for subsequent chain extension, and (3) a protecting group for the internucleotide phosphate linkage. Compound **1** was eventually chosen as the building block based on consideration of the chemical reactivity of the cis–syn thymine dimer unit and the method to be used for its purification.

One major concern was the stability of the N_3–C_4 imide bond of the cyclobutane thymine dimer unit to the conditions which would be used to deprotect the final, fully protected oligonucleotide. It is known that reaction of the cyclobutane dimer of thymine with hydroxide[11] and borohydride[12] leads to cleavage of this bond. Methyl phosphoramidite chemistry was chosen because of the relatively mild conditions of thiophenol/triethylamine required to remove the resulting *O*-methyl phosphotriester protecting groups.[10] The morpholinylphosphonite group was chosen as it is known to be stable to silica gel chromatography in the presence of amines,[13] thus allowing purification and characterization of the building block prior to its introduction into an oligonucleotide.

The synthetic approach to **1** was based on the fact that the cis–syn dimer of TpT, compound **9**, can be obtained in 80% yield by triplet-sensitized photolysis of TpT, compound **7**,[9] and that it was expected that derivatives of TpT should also undergo the same reaction. The original plan, then, was to triplet sensitize the photodimerization of $O_{5'}$-DMT-thymidylyl-(3′→5′)-thymidine, *O*-methyl phosphate, **5**, and then convert the resulting cis–syn thymine dimer containing product **8** to the methyl morpholinylphosphonite **1**. The route eventually employed is contained in Scheme I.

Sensitized Photodimerization of TpT Derivatives. Previously unreported compound **5a,b** (**a** and **b** refer to epimers at phosphorus) was prepared in two steps from phosphoramidite **2**[13] and $O_{3'}$-TBDMS-thymidine, **3**,[14] according to a general procedure.[15] Acetophenone-sensitized photolysis of either compound **5a,b** or

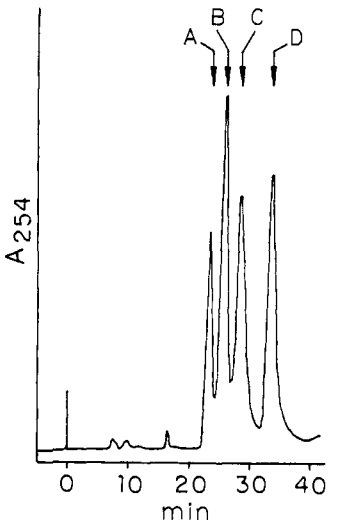
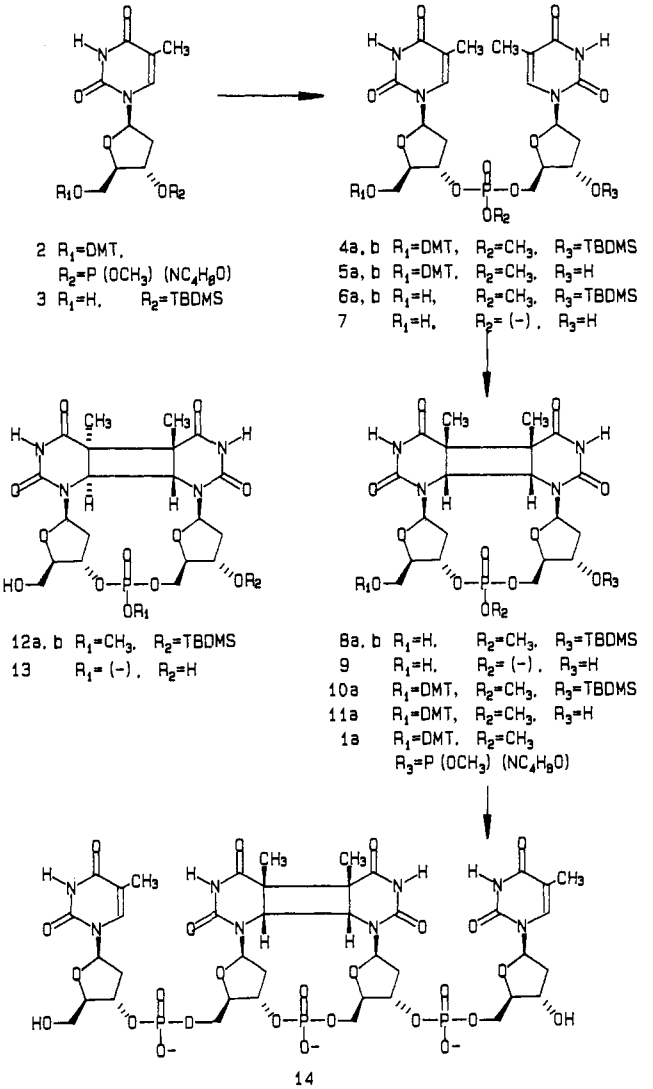


Figure 1. Preparative isocratic reverse phase HPLC trace of the mixture of compounds **12a** = A, **12b** = C, **8a** = B, and **8b** = D resulting from the sensitized photolysis of compound **6a,b**. In practice compound B was removed by silica gel flash chromatography prior to preparative reverse phase HPLC, leading to base line separation of the other three components. Details can be found in the Experimental Section.

Scheme I



4a,b under conditions which had been successfully used for TpT[9] led to complex nonresolvable mixtures. This suggested that the

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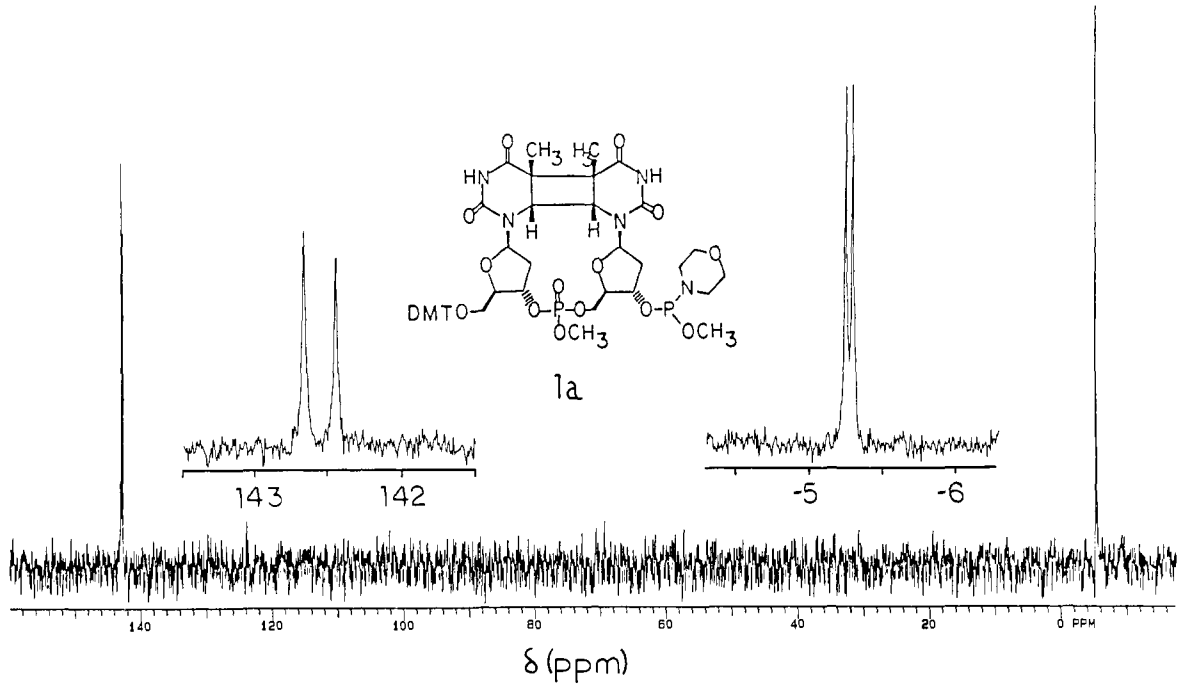


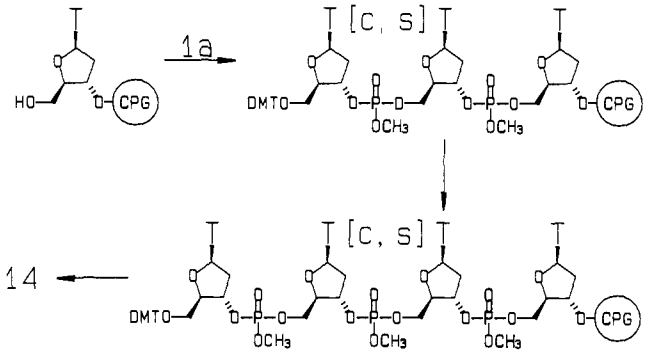
Figure 2. ^{31}P spectrum (121.5-MHz) of the cis–syn target phosphoramidite building block **1a** in $CDCl_3$, relative to TMP. The high barrier to inversion at the phophoramidite leads to a pair of diastereomers on the NMR time scale.

DMT group was interfering in some way with the desired photoreaction, perhaps by influencing the conformation of the molecule or by quenching its excited state. In order to circumvent these problems compound **4a,b** was converted to the known compound **6a,b**.[8] Acetophenone-sensitized photolysis of **6a,b** with Pyrex filtered medium pressure mercury arc light in an immersion well type reactor led smoothly to a mixture of compounds, A, B, C, and D, labeled in the order of their elution from a C-18 column (Figure 1). Compound B was cleanly separated in 27% yield from the other three compounds by silica gel flash chromatography. Compounds A, C, and D were then separated by preparative C-18 HPLC in 15%, 16%, and 14% yields, respectively.

Structure Assignment of Compounds A, B, C, and D. Due to the epimeric nature of the phosphotriester group of **6a,b**, the four compounds, A, B, C, and D, which resulted from its photolysis were expected to be diastereomeric pairs of cis–syn and trans–syn photoproducts. In order to determine the cyclobutane ring stereochemistry of these four products, each was separately deprotected with pyridine/water to remove the phosphate-protecting group,[16] followed by acetic acid at 90 °C to remove the TBDMS group.[17] The deprotected compounds were then compared by TLC to authentic samples of the cis–syn and trans–syn photodimers of TpT, compounds **9** and **13**.[9] As a result, the cyclobutyl methyls of A and C could be assigned trans–syn stereochemistry and those of B and D cis–syn stereochemistry.

Conversion of B to the Target Phosphoramidite 1a. Compound **8a** was converted to the $O_{5'}$-DMT compound **10a** by treatment with DMT-Cl in pyridine. The TBDMS group was then removed with TBAF in the presence of acetic acid to give **11a**, conditions which have been previously shown not to lead to phosphotriester cleavage.[18] The desired phosphoramidite building block, **1a**, was then synthesized by addition of compound **11a** to methyl chloromorpholinylphosphonite, prepared in situ from methyl dichlorophosphite. **1a** was purified by silica gel flash chromatography in the presence of triethylamine and characterized by ^1H and ^{31}P NMR spectra. Four ^{31}P NMR peaks were observed corresponding to the presence of two diastereomers which result from the slow inversion of the phosphoramidite phosphorus relative to the NMR time scale (Figure 2).

Scheme II



Solid-Phase Automated Synthesis of TpT[*c*,*s*]pTpT. In order to demonstrate that compound **1a** would serve as a suitable cis–syn thymine dimer building block, the synthesis of tetrathymidylate, with and without a cis–syn thymine dimer at positions 2 and 3, was undertaken according to standard procedures[10] (Scheme II). Tetrathymidylate was chosen as it has been shown that tetranucleotides can be used to introduce lesions into viruses via gapped duplex structures[19] and that the appropriate gapped viruses can be prepared in a straightforward way.[20]

The synthesis was automated by use of a SAM I Series 2 DNA synthesis machine whose reagent delivery system is of the continuous flow, packed column design. In the first cycle, controlled pore glass derivatized thymidine was condensed with phosphoramidite **1a** in the presence of tetrazole resulting in a twice reproducible coupling yield of 30% based on the detritylation step. This yield could only be achieved with a coupling time of 40 min and could be attributed to a lower than optimal phosphoramidite concentration resulting from adventitious introduction of water into the system. In a subsequent synthesis of another oligonucleotide on an 80-μmol scale we have obtained a 50% coupling

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Table I. ^1H and ^{31}P Chemical Shifts of Thymine and Thymine Dimer Containing Monomers and Oligomers[a]

	GCATTAATGC[b]	TpTpTpT[c]	TpT[d]	T[e]	TpT[c,s]pTpT[c]	T[c,s]pT[f]
H_6	7.37	7.66 (2 H)	7.71	7.68	7.72	
	7.21	7.64 (2 H)	7.69		7.64	
H_1	5.92	6.25 (4 H)[g]	6.34	6.28	6.30 (2 H)[g]	
	5.73		6.23			
					5.92	6.00
					5.75	5.67
CH_3	1.66	1.88	1.91	1.89	1.90	
	1.46		1.90		1.87	
					1.46 (2 CH_3)	1.54
						1.50
^{31}P		−4.15	−4.01		−3.82	
		−4.19			−4.06	
		−4.25			−4.55	−4.52

[a] ^1H NMR in D_2O relative to TPS, ^{31}P NMR in D_2O relative to TMP. [b] Duplex DNA, ref 25. [c] This work. [d] References 23b and 24. [e] Reference 24. [f] Reference 23. [g] Center of a set of overlapping multiplets.

yield with only 3 equiv of phosphoramidite and expect that with a larger excess of phosphoramidite the yield should be close to 100%. The chain was then elongated with another thymidine by the same procedure. The methyl phosphate protecting groups were removed with thiophenol/triethylamine/THF and the deprotected oligonucleotide **14** cleaved from the resin by treatment with concentrated aqueous ammonia. Tetrathymidylate **15** was synthesized in an analogous manner.

Chromatographic Behavior of 14 and 15. The two tetramers have approximately the same retention time on an anion exchange chromatography, as expected based on their equivalent charges. On the other hand, the cis–syn containing dimer had a retention time of 29.4 min on C-18 gradient chromatography, whereas the nondimer-containing tetranucleotide had a retention time of 21.0 min. The large difference in retention time between dimer-containing and nondimer-containing oligonucleotides has been previously noted in photolysis studies on di-, tri-, and tetranucleotides.[21] C-18 chromatography of oligonucleotides is known to be sensitive to changes in the hydrophobicity of the oligonucleotides. This has been used to advantage in the separation of DMT-containing oligonucleotides from non-DMT-containing oligonucleotides which elute earlier. In this case, the cis–syn thymine dimer containing oligonucleotide **14** has an appreciably shorter retention time than the nondimer containing oligonucleotide **15**. This can be ascribed to the loss of two hydrophobic faces of the two internal thymines upon formation of the cis–syn cyclobutane dimer.

Photoreversion of 14 to 15. Irradiation of the cis–syn cyclobutane dimer of TpT **9** at 254 nm leads to its photoreversion to TpT **7** ultimately resulting in a photoequilibrium mixture consisting of approximately 75% TpT and 25% cis–syn and trans–syn photodimers in an approximately 8:2 ratio.[22] Exposure of the cis–syn thymine dimer containing tetramer **14** to 254-nm light from a hand-held UV lamp resulted in its predominant conversion to tetrathymidylate **15** as determined by C-18 HPLC chromatography (Figure 3). This establishes the presence of a cyclobutane dimer unit in the synthetic compound. In addition, two other products were formed in significant amounts which presumably correspond to the formation of a cis–syn cyclobutane thymine dimer unit between thymines 1 and 2 as well as between thymines 3 and **4**. The tetramer containing two cis–syn thymine dimers would not be expected to have appreciable absorbance at the 254-nm detection wavelength used. In principle, photolysis of tetrathymidylate could lead to 10 thymine dimer containing oligonucleotides, resulting from all possible combinations of cis–syn and trans–syn stereoisomers. It would be expected, though, that those products containing trans–syn isomers would be formed in much lower yield, as is the case for photolysis of TpT.

^1H and ^{31}P NMR Spectroscopy of TpT[c,s]pTpT. Expanded portions of the proton spectra of **14**, **15**, and the cis–syn dimer of TpT **9** are shown in Figure 4. The relevant data is tabulated

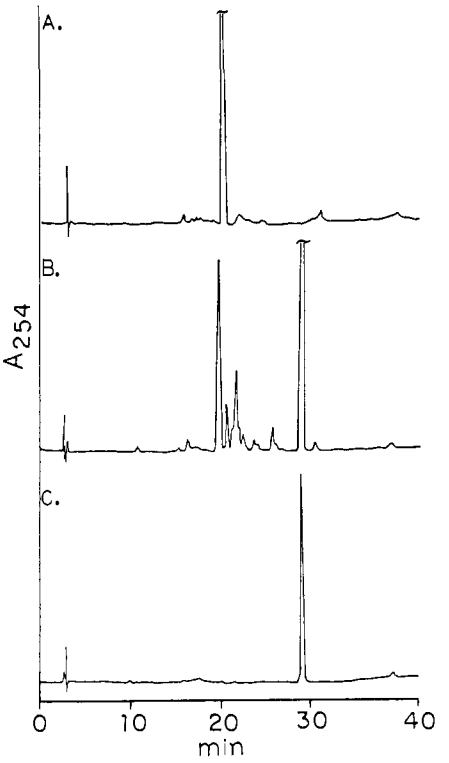

Figure 3. Analytical gradient reverse phase HPLC traces of (A) the dimer-containing synthetic oligonucleotide **14**, (B) the resulting mixture after photolysis at 254 nm, and (C) the nondimer-containing oligonucleotide **15**. Details are in the Experimental Section.

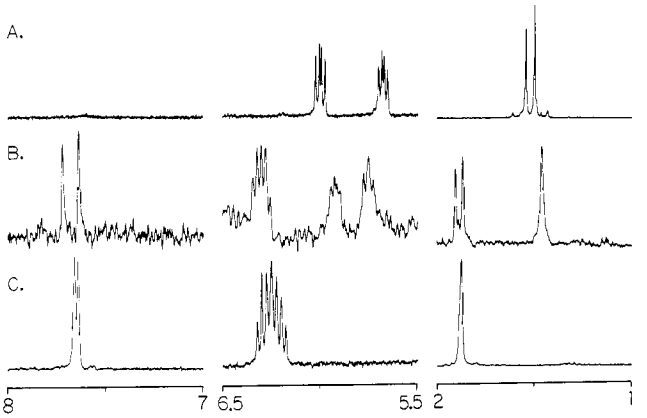

Figure 4. Details of the NMR spectra in D_2O for (A) the cis–syn dimer of TpT **9** (B) the cis–syn thymine dimer containing tetranucleotide **14**, and (C) the nondimer-containing tetranucleotide **15** in the ranges (from left to right) 8.0–7.0, 6.5–5.5, and 2.0–1.0 ppm from TPS.

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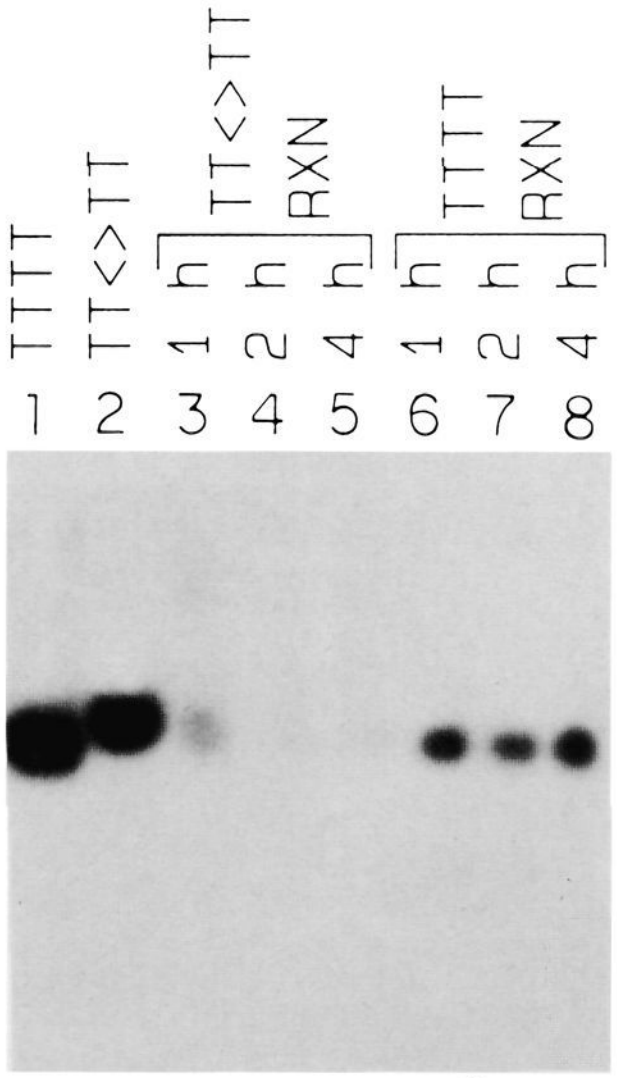

Figure 5. High resolution gel electrophoresis of ^{32}P labeled dimer- and nondimer-containing tetramers **14** and **15** before and after a chemical degradation sequence specific for thymine dimers. Lanes 1 and 2 correspond to untreated oligonucleotides; lanes 3–8 correspond to the results of sodium borohydride treatment followed by heating at 90 °C with aniline·HCl for the indicated times. The anode was at the botton of the gel shown. Complete details can be found in the Experimental Section.

in Table I together with data for related compounds. The shifts of the two $H_{1'}$ protons and the two cyclobutyl methyls of the central cis–syn thymine dimer unit of **14** correspond closely to those found for the cis–syn photoproduct of TpT.[23] The only minor changes in shifts noted for the $H_{1'}$ and methyl proton signals relative to the cis–syn dimer of TpT suggests little if any stacking interactions with the terminal thymines. The H_6, $H_{1'}$, and C_5 methyl proton shifts of both the terminal thymines of **14** and all those of **15** are very similar to those of monomeric thymidine.[24] In contrast, the H_6 signals of T4 and T5 in the fully stacked duplex GCTTAATGC shift upfield by 0.39 ppm, the $H_{1'}$ signals by about 0.47 ppm, and the thymine methyl signals by about 0.33 ppm.[25]

In the ^{31}P NMR spectrum of **14** three peaks are observed at −3.82, −4.06, and −4.54 ppm relative to TMP. The first two can be assigned to the terminal phosphates and the third to the central phosphate of the cis–syn dimer unit, by comparison with the ^{31}P signal of TpT and the cis–syn dimer of TpT.[23b] The similarity of both the $H_{1'}$ coupling pattern and the ^{31}P chemical shift of the cis–syn thymine dimer sugar phosphate unit of **14** to that of the cis–syn thymine dimer of TpT suggests that the conformation of this unit in the tetranucleotide is not greatly affected by the attachment of nucleotides to either end. It will be interesting to see if the conformation of the dimer unit changes significantly when incorporated into duplex DNA.

Electrophoretic Behavior. The oligonucleotides were endlabeled with polynucleotide kinase and γ-^{32}P-dATP and subjected to high resolution 20% polyacrylamide gel electrophoresis. Mobility of oligonucleotides on gels is known to be sensitive to their shape and has been related to the mean square end-to-end distance.[26] The dimer-containing oligonucleotide was found to migrate more slowly than the nondimer-containing oligomer (Figure 5) indicating that dimerization of the central thymines might have led to a shortening or bending of the single-stranded oligonucleotide. Such a propensity to bend in a single-stranded oligomer might lead to bending in double-stranded DNA as has been predicted on the basis of calculations.[27] Bending has been suggested to play an important role in the recognition of the thymine dimer and other lesions by the uvrABC system.[28] It is possible, however, that the retardation of **14** relative to **15** is due to other less well understood effects not relating to length or bending and will be the subject of future investigations.

Chemical Degradation Studies. The C_4 carbonyl of thymine dimers has been shown to be more easily attacked by nucleophilic reagents such as sodium borohydride and sodium hydroxide than that of undimerized thymine. This enhanced susceptibility of the C_4 carbon of the thymine dimer toward nucleophilic attack by sodium borohydride[12] has been exploited in the technique of "photofootprinting".[29] This technique involves the determination of the quantum yield of thymine dimer production at a particular site in the presence or absence of such DNA binding molecules as repressors. Reduction of the C_4 carbonyl group to an alcohol with sodium borohydride enhances the rate of aniline hydrochloride catalyzed glycosyl bond hydrolysis relative to that of unmodified nucleotides. Rapid strand cleavage then results from β-elimination of the $O_{5'}$- and $O_{3'}$-phosphates allowing one to determine the sites of thymine dimers by high resolution gel electrophoresis of labeled precursor DNA fragments. When this reaction sequence was applied to the non-thymine dimer and thymine dimer containing oligonucleotides we found that the dimer-containing oligonucleotide underwent sizable degradation under conditions where the nondimer-containing oligonucleotide did not (Figure 5). Interestingly, the degradation required much more drastic conditions than those reported in the literature.[29] The standard treatment with aqueous aniline·HCl at 60 °C for 20 min led to very little cleavage, and it was only when a temperature of 90 °C for 1 h was used that complete cleavage was observed. The reason for the difference is not clear at this moment. It might be that conditions required to get minimal background cleavage in photofootprinting assays with heterogenous DNA lead to only partial cleavage at thymine dimer sites. Alternatively, cis–syn thymine dimers in short oligonucleotides might be much less reactive than those in longer fragments.

Chemical Stability of the Cis–Syn Thymine Dimer. Because the oligonucleotide synthesized was composed of thymine only, the stability of the cis–syn thymine dimer to the more drastic conditions for removal of the commonly used acyl-protecting groups for adenine, cytosine, and guanine was not tested. As a result we have examined the stabiliity of the cis–syn photodimer of TpT to incubation with concentrated ammonium hydroxide at 60 °C for 12 h. Comparison of a sample before and after such treatment by 300-MHz NMR showed that >80% of the sample survived the treatment. A triplet peak at 6.39 ppm in the $H_{1'}$ region of the proton NMR integrated to 10% of the anomeric proton area and must be due to some degradation product resulting from the aminolysis of the diimide unit. A methyl peak at 1.60 ppm was also noticed which integrated for approximately the same percentage of the methyl proton area. The conditions used were 5 °C higher in temperature and almost 6 h longer than what is used in most protocols for the quantitative removal of the benzoyl and isobutyryl groups. In this respect the thymine dimer can be

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considered to be stable to all the standard deprotection conditions used in phosphoramidite based DNA synthesis technology.

Conclusion

We have described the synthesis of a building block for the sequence specific introduction of the cis–syn thymine photodimer into oligonucleotides via phosphoramidite-based solid-phase DNA synthesis methodology. The convergent nature of the route to the building block will enable the preparation of isotopically labeled and structurally modified analogues of this lesion useful for a variety studies. We have also demonstrated the successful use of this building block for the incorporation of the cis–syn thymine dimer into positions 2 and 3 of tetrathymidylate. Site-specific introduction of the cis–syn thymine dimer into a virus can now be attempted by annealing and then ligating this oligonucleotide to a complimentary gapped duplex structure by methods successfully employed for other lesions.[19,20] We have also demonstrated that the cis–syn thymine dimer is stable to the standard conditions required for deprotection of all the bases. Therefore, this building block can be used for the preparation of site specific cis–syn thymine dimer containing oligonucleotides of any sequence, in amounts and purities required for detailed biophysical, enzymological, and mutagenesis studies.

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Registry No. 1, 110270-85-4; **6** (isomer 1), 110350-81-7; **6** (isomer 2), 110311-60-9; **7**, 87367-14-4; **8** (isomer 1), 110312-28-2; **8** (isomer 2), 110312-30-6; **9**, 110312-31-7; **10**, 110270-83-2; **11**, 110270-84-3; **12** (isomer 1), 110243-20-4; **13**, 110312-32-8; **14**, 110270-86-5; **15**, 2476-57-5.

3,4-Connected Carbon Nets: Through-Space and Through-Bond Interactions in the Solid State

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Abstract: A theoretical study of a series of "honeycombed" or "layered" 3,4-connected nets is presented. The most significant feature of this series of nets is that they have infinite stacks of carbon–carbon double bonds in close contact with each other. Two of these nets have structures that not only contain stacks of 3-connected centers but also have infinite 1-dimensional polymeric units related to *cis*-polyacetylene. We employ tight-binding band structure calculations on selected examples of these nets to determine their electronic properties. The consequences of a stacked structure are analyzed by calculating the band structure of infinite layers (stacks) of ethylenes. Some of these carbon nets may be metallic. We also show that through-space and through-bond (hyperconjugative) interactions are important in the solid state, but the overall effect of these interactions varies according to the area of k-space that is being sampled.

Pursuing our continuing interest in alternative structures of diamond and graphite,[1] we decided to study a series of 3,4-connected nets. There are a number of reasons for examining these nets: first, they have an intermediate valency between graphite (3-connected) and diamond (4-connected); second, the density of the nets we will be studying here is intermediate ($\approx$3.0 g/cm^3) between that of diamond (3.51 g/cm^3) and graphite (2.27 g/cm^3);[2] and third, these nets are interesting because they have close intrastack distances (2.3 to 2.8 Å) thereby allowing for greater (especially π) band dispersions (this may make these nets metallic).

Before proceeding to a discussion of the electronic properties of these nets we would like to review briefly the current status of the allotropy of carbon.

The structurally well-characterized allotropes of carbon are restricted to only two main types: diamond (cubic and hexagonal) and graphite (hexagonal and rhombohedral).[3] In cubic (**1**) and hexagonal diamond (**2**) each carbon atom is tetrahedrally surrounded by four other carbon atoms at a distance of 1.545 Å. The structural differences between these two diamond forms can be envisioned in this way: **1** can be imagined to consist of an infinite network of adamantyl moieties, all chair cyclohexane rings, whereas in **2** there are some chair and some boat six-membered rings. Both hexagonal (**3**) and rhombohedral (**4**) graphite contain hexagonal carbon layers linked by van der Waals forces. The difference, though, between these two forms lies in the stacking of these sheets. In **3** every third layer repeats (ABAB...) with the first and second layers displaced away from each other in such a way that one-half of the atoms in each layer are above and below the center of the hexagon in the neighboring layer. **4**, on the other hand, has every fourth layer repeating to give an ABCABC... stacking pattern.

There is some evidence for other carbon allotropes.[4] The best studied of these is karbin, sometimes called chaoite.[5,6] Its structure is thought to contain carbon in alkyne or cumulene needles, **5**. Though there has been much work on karbin, our opinion is that

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(1) Hoffmann, R.; Hughbanks, T.; Kertesz, M.; Bird, P. H. *J. Am. Chem. Soc.* **1983**, *105*, 4831. Hoffmann, R.; Eisenstein, O.; Balaban, A. T. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5588. Balaban, A. T.; Rentia, C. C.; Ciupitu, E. *Rev. Roum. Chim.* **1968**, *13*, 231; erratum: *Ibid.* **1968**, *13*, 1233.

(2) These are theoretical densities calculated from idealized geometries and should be considered as an upper limit to the experimental densities.

(3) Donohue, J. *The Structure of the Elements*; Wiley: New York, 1974.

(4) See the following recent review of experimental and theoretical work: Stankevich, I. V.; Nikerov, M. V.; Bochvar, D. A. *Usp. Khim.* **1984**, *53*, 1101; *Russ. Chem. Rev.* **1984**, *53*, 640.

(5) For a review on karbin see: Mel'nichenko, V. M.; Sladkov, A. M.; Nikulin, Yu. N. *Usp. Khim.* **1982**, *51*, 736; *Russ. Chem. Rev.* **1982**, *51*, 421.

(6) Whittaker, A. G. *Science* **1978**, *200*, 763.

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