Tetrahedron Vol. 47, No. 14/15, pp. 2579-2590, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00

UNRAVELING THE ORIGIN OF THE MAJOR MUTATION INDUCED BY ULTRAVIOLET LIGHT, THE C→T TRANSITION AT dTpdC SITES. A DNA SYNTHESIS BUILDING BLOCK FOR THE CIS-SYN CYCLOBUTANE DIMER OF dTpdU.

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(*Received in USA* 13 *September* 1990)

Summary: One possible origin of the major mutation induced by ultraviolet light, the C→T transition at dTpdC sites, involves the replicative bypass of the cyclobutane dimer of dTpdC or its deamination product, the cis-syn dimer of dTpdU. In order to investigate the latter proposal, we have designed and synthesized a building block for the incorporation of the cis-syn dTpdU dimer into oligonucleotides by automated solid phase DNA synthesis technology.

Sunlight is a ubiquitous toxic, mutagenic and carcinogenic agent. The mechanisms underlying its effects are poorly understood, though it is reasonably well established that many of them involve photodamaged DNA.[1] Our approach to unravelling the structure-activity relationships of DNA photoproducts is to synthesize site-specifically photodamaged DNA for high resolution structural studies, in vitro repair and replication studies, and in vivo toxicology and mutagenesis studies.[2] The major mutation induced by UV light is the C→T mutation at dTpdC sites[3] and the major photoproducts induced at these sites[4] are illustrated in Figure 1. The primary photoproducts of B form dTpdC sequences are the cis-syn cyclobutane dimer and a presumed azetidine intermediate, both of which result from photo [2+2] reactions. The oxetane product is thermally unstable and irreversibly decomposes to give what is referred to as the (6-4) product because of the bond formed between C6 of the 5'-base and C4 of the 3'-base. We have recently shown that the (6-4) product of dTpdC, like that of dTpdT,[5, 6] is not stable in sunlight and is converted to its Dewar valence isomer.[4] The distribution of the (6-4) and Dewar products depends on the length of exposure to the light, with (6-4) products dominating a short exposure times and Dewar products at long exposure times. The cis-syn

Abbreviations: dT, 2'-deoxythymidine; dC, 2'-deoxycytidine; dU, 2'-deoxyuridine; DMT, dimethoxytrityl; TBDMS, *t*-butyldimethylsilyl; Bn, benzyl; X-dN, 5'-*O*-X deoxynucleoside; dN-X, 3'-*O*-X deoxynucleoside; dNpdN, deoxynucleotidyl-(3'→5')-deoxynucleoside; dNp(X)dN, X-phosphate ester of dNpdN; [c,s], cis-syn photodimer; EtOAc, ethylacetate; TMP, trimethylphosphate; THF, tetrahydrofuran; 3-NBA, 3-nitrobenzoic acid.

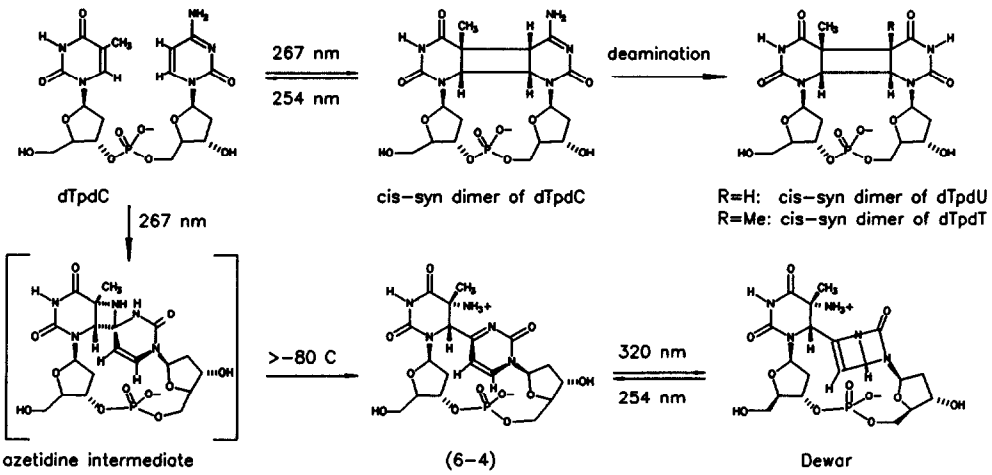

Figure 1. Photochemistry of dTpdC Sites.

cyclobutane dimer of dTpdC is not stable and deaminates with a half life of about one hour at 50 °C to give what is formally the cis-syn cyclobutane dimer of dTpdU.[7] In vivo, the deamination process has been estimated to have a half life of approximately 5 h.[8] Which of these four products or other products leads to the C→T mutation is not known.

Recently, we discovered that the cis-syn cyclobutane dimer of dTpdT (Fig. 1) can be bypassed by *E. coli* DNA polymerase I, and that trans-lesion synthesis occurred in a non-mutagenic manner, that is, adenines were inserted opposite both thymines of the dimer by the polymerase[9] (Fig. 2A, X=T). This result can readily be explained if each thymine of the cis-syn dimer retains the same pattern of hydrogen bond donors and acceptors as in undimerized thymine (illustrated in Fig. 3 for the 3'-thymine, R=CH_3). The cis-syn dimer of dTpdU is identical to that of dTpdT, except that there is a hydrogen in place of the methyl group

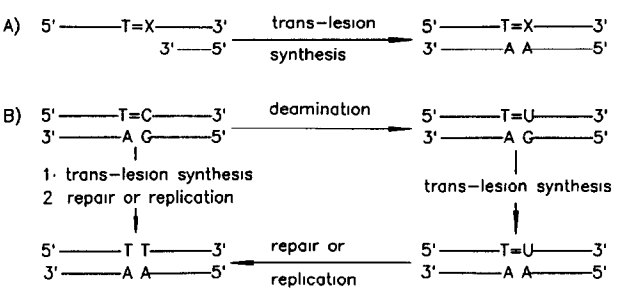

Figure 2. A) Major products of trans-lesion synthesis of cis-syn dimers where X=T or U. B) Two proposed pathways for the origin of the C→T mutation induced by ultraviolet light.

at C5 of the 3'-pyrimidine (Fig. 1). We therefore reasoned that a polymerase should bypass the cis-syn dimer of dTpdU as it does that of dTpdT and introduce pdAs opposite the dimer (Fig. 2A, X=U). This hypothesis suggested that a possible mechanism for the origin of the uv-induced C→T mutation at dTpdC sites involves bypass of the dTpdU cis-syn dimer which results from deamination of the uv-induced dTpdC cis-syn dimer (Figure 2B). After repair or further replication of the bypass product, a sequence results in which the original C has been mutated to a T. Such a mechanism is different from that in which the dTpdU cis-syn dimer is photoreactivated to dTpdU prior to replication.[8] It has also been suggested that trans-lesion synthesis past the dTpdC cis-syn dimer would also result in the C→T mutation, as saturation of the 5,6 double bond of C in the dimer may favor the tautomeric form which is complementary to A (Figure 3, tautomer C).[10]

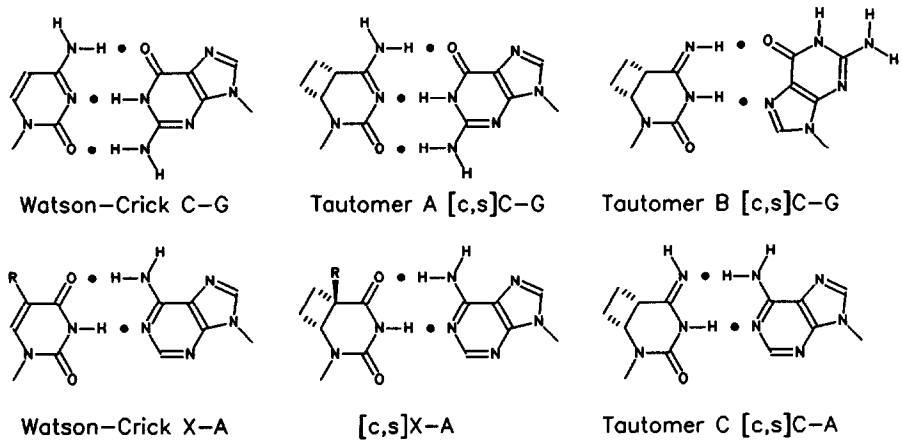

Figure 3. Standard Watson-Crick base pairing interactions (R=CH_3 for X=T and R=H for X=U) along with possible base pairing schemes for the dX ring of the dTpdX cis-syn cyclobutane dimers.

Because the cis-syn dimer of dTpdC is not stable and deaminates to the corresponding dimer of dTpdU, we decided to first study the action of DNA polymerases on templates containing the dTpdU dimer. Rather than delay our studies until a building block for the dTpdU cis-syn dimer could be developed, a template containing the dTpdU dimer was prepared by acetophenone sensitized photolysis of an oligonucleotide containing an isolated dTpdU site as the only dipyrimidine site, followed by HPLC purification of the desired product. This general procedure has been successfully used for the preparation of oligonucleotides containing site-specific dTpdT cis-syn dimers.[11, 12] In accord with our expectation, *E. coli* DNA polymerase I inserted only pdAs opposite the dimer (unpublished results). Quite unexpectedly, however, the dTpdU dimer was much more rapidly bypassed than the dTpdT

dimer, almost by an order of magnitude. In order to elucidate the structural basis for this effect we require large amounts of dTpdU dimer-containing duplex oligonucleotides for high field NMR and X-ray crystal structure analysis. The sensitized photolysis route that we used to prepare the template for the polymerase studies was unsuitable, as it proceeded in low yield and the desired product could only be purified in small quantities by reverse phase HPLC. We therefore decided to synthesize a building block for the incorporation of the dTpdU cis-syn dimer into oligonucleotides by solid phase DNA synthesis.

	R1	R2	R3
a	H	(-)	H
b	DMT	Me	TBDMS
c	DMT	Me	Bn
d	H	Me	TBDMS
e	H	Me	Bn
f	H	Me	H
g	DMT	Me	H
h	-	-	TBDMS
i	-	-	Bn

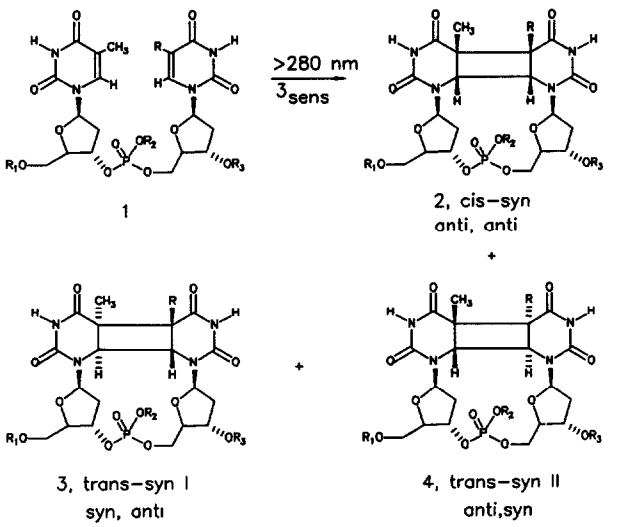

Figure 4. Cyclobutane dimers formed on triplet sensitized photolysis of dTpdT (R=CH_3) and dTpdU sites (R=H). Below each product are the glycosyl bond conformations of **1** leading to that product.

We had previously designed and synthesized the dTpdT cis-syn dimer building block **13** (R=CH_3, Fig. 7)[13] which we used to prepare multimilligram amounts of a decamer containing a site-specific dTpdT cis-syn dimer for 2D NMR and melting temperature studies.[14] The key step in that synthesis was to photolyze dTp(Me)dT-TBDMS (**1d**, R=CH_3, Fig. 4). Unfortunately, this derivative was synthesized by conventional phosphoramidite chemistry which led to the formation of almost equal amounts of two diastereomers, epimeric at phosphorus, which could not be separated efficiently on a preparative scale by either flash chromatography or HPLC. As a consequence, sensitized photolysis of the epimeric mixture of dTpdT derivatives led to epimeric pairs of cis-syn and trans-syn photodimers **2d** and **3d** (R=CH_3). Fortunately, one of the cis-syn isomers could be easily separated from the three other isomers and unreacted starting material by flash chromatography, whereas the remaining

photoproducts required time-consuming separation by preparative reverse phase chromatography. This synthetic route was acceptable for the preparation of the dTpdT cis-syn dimer building block, but was not ideal for the preparation of the trans-syn dTpdT building block because of the tedious reverse phase chromatography step necessary to obtain the pure trans-syn isomers **3d** ($R=CH_3$).[15]

Application of the same synthetic route to the synthesis of the dTpdU cis-syn dimer building block turned out to be even less practical because of the formation of an additional trans-syn isomer (Fig. 4). dTpdU ($R=H$), unlike dTpdT ($R=CH_3$), leads to two trans-syn isomers,[7] isomer I resulting from dimerization of the dTp unit in the syn glycosyl conformation, as occurs for dTpdT, and isomer II resulting from dimerization of the pdU unit in the syn glycosyl conformation. Instead of having to separate the cis-syn dimers from six compounds (including starting material) following sensitized photolysis of a dinucleotide precursor epimeric at phosphorus, as we had to in the dTpdT case, we would have to separate the dTpdU cis-syn dimers from a mixture of eight compounds. In order to facilitate the preparative isolation of pure photoproducts in both the dTpdT and dTpdU series we decided to explore methods for the synthesis of diastereomerically pure phosphotriester derivatives of dinucleotides.

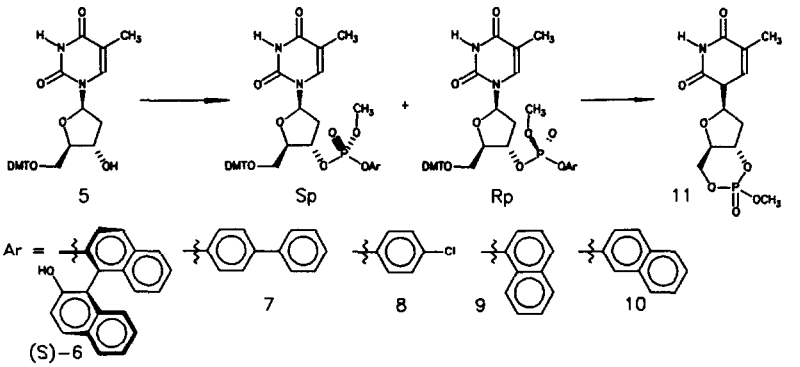

Figure 5. Structures of the aryl phosphotriesters studied and the cyclization product **11** whose absolute configuration at phosphorus has been previously established.

Appropriately substituted phosphates can undergo nucleophilic substitution reactions in high yield and often with a high degree of inversion[16] via an $S_N2(P)$ reaction, suggesting that this might be a *suitable reaction to explore for internucleotide phosphotriester bond formation. There has been a report* of the synthesis TBDMS-dAp(Ph)dA-TBDMS, epimeric at phosphorus, by nucleophilic displacement of the *p*-nitrophenyl group from TBDMS-dAp(Ph)(*p*-NO_2Ph), epimeric at phosphorus, with the lithium salt of dA-TBDMS.[17] There have been no reports, however, of attempts to produce diastereomerically pure

phosphotriester linkages by utilizing diastereomerically pure phosphotriester derivatives in such a coupling reaction, presumably because the ultimate fate of most phosphotriester derivatives of oligonucleotides is to be hydrolyzed to phosphodiesters. Diastereomerically pure or enriched nucleotide derivatives have been utilized in the synthesis of internucleotide thiophosphate[18, 19] and phosphonate[20] linkages. Although essentially exclusive inversion of configuration was observed to occur in the thiophosphate cases, diastereomerically pure starting material was difficult to obtain. In the phosphonate case, diastereomerically pure starting material could be obtained, but the coupling reaction only proceeded with about 80% inversion, presumably because of competing epimerization of the starting material by the nitrophenoxide released in the coupling reaction.

We were therefore faced with two issues: (1) how to prepare diastereomerically pure nucleoside phosphotriesters, and (2) whether the coupling reaction would be stereoselective enough to be useful. Initially we decided to explore the feasibility of using a chiral auxiliary for the direct preparation of a diastereomerically pure nucleoside phosphotriester and examined the chemistry of 1,1'-bi-2-naphthol, a readily available chiral phenol. The (*R*)- and (*S*)-binaphthol derivatives **6** were prepared by reaction of the methyl ester of the cyclic phosphate of binapthol with the sodium salt of DMT-dT (**5**). The highest diastereoselectivity obtained was 1:15 ***Sp*-6**:***Rp*-6** for the (*S*)-binapthol derivative. Unfortunately, the diastereomeric products could not be separated chromatographically and the subsequent coupling step (Figure 6) never proceeded in greater than about 30% yield (a full account of these results will be described elsewhere). Rather than search for other auxiliaries which might be more stereoselective, we decided instead to find a phosphotriester derivative of DMT-dT whose diastereomers could be easily separated by flash chromatography. Of the four derivatives prepared (Fig. 5, **7-10**), the diastereomers of the α-naphthol derivative **9** could be most easily separated by flash chromatography on silica gel and were labelled A and B in order of their elution from the column (Note: all further products are assigned the label of the diastereomer of **9** from which they were derived).

In order to determine the stereoselectivity of nucleophilic displacement reactions at the phosphorus center of each diastereomer of **9** and its absolute configuration, each diastereomer of **9** was treated independently with the sodium salt of dT-TBDMS (**12h**,

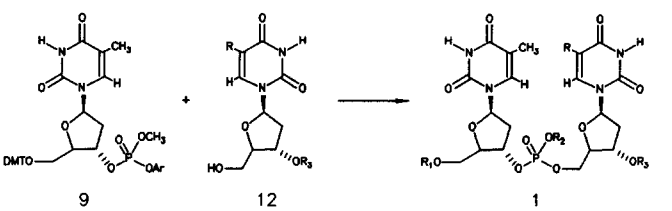

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Figure 6. The coupling reaction and a further product whose absolute configuration at phosphorus has been previously established (**1f**, R = CH_3).

R=CH_3 Fig. 6). The coupling reaction was complete in about one hour at room temperature in THF and each diastereomer led to only one of the diastereomers of **1b** as judged by both ^1H and ^{31}P NMR. Removal of both the DMT and TBDMS groups led to dTp(Me)dT (**1f**, R=CH_3), for which the absolute configuration at phosphorous has been previously established.[21] Assuming the displacement reaction proceeded with inversion, *Rp* can be assigned to the A diastereomer and *Sp* to the B diastereomer (see Table). In further confirmation of this assignment, the DMT group of each diastereomer of **9** was removed, and the products independently cyclized under the same conditions as used in the coupling reaction to give **11** (Fig. 5), whose absolute configuration at phosphorus has also been previously assigned.[22] Only one of the two diastereomers of **11** was detected by ^1H NMR spectroscopy in each of the crude cyclization mixtures, indicating that the cyclization proceeded with high stereoselectivity.

^{31}P NMR vs 85% H_3PO_4	dTp(Me)dT (**1h**, R=CH_3) in DMSO-d_6	cdTMP(Me) (**11**) in acetone-d_6
Sp	0.69[21], 0.45[23] 0.515, B diastereomer	-6.4[24] (-3.5 in D_2O)[22] -4.58, B diastereomer
Rp	0.60[21], 0.36[23] 0.44, A diastereomer	-4.7[24] (-2.7 in D_2O)[22] -2.84, A diastereomer

Having established the high stereoselectivity of the coupling reaction, dU-TBDMS and dU-Bn (**12h** and **12i**, R=H) were likewise coupled with both the *Rp* and *Sp* diastereomers of **9** and as expected only pure diastereomers of DMT-dTp(Me)dU-TBDMS and DMT-dTp(Me)dU-Bn (**1b** and **1c**, R=H) were obtained. Because we had previously experienced difficulty in the acetophenone-sensitized photodimerization of dinucleotide derivatives containing a 5'-DMT group,[13,25] the DMT group was selectively removed from **1b** and **1c** (R=H) with 80% acetic acid/H_2O to give dTp(Me)dU-TBDMS and dTp(Me)dU-Bn (**1d** and **1e**, R=H). These derivatives were then subjected to acetophenone-sensitized photolysis with Pyrex-filtered light. Whereas the cis-syn isomers **2d** and **2e** (R=H) could be obtained in pure form upon flash chromatography, the two trans-syn isomers could not. In the original synthesis of the dTpdT cis-syn dimer building block,[13] the next step was to reintroduce the 5'-DMT group and following that, remove the 3' protecting group. Rather than follow this route it was decided to make the synthesis of the dTpdU cis-syn dimer building block more efficient by removing the 3'-protecting group prior to photolysis, in the same step in which the DMT group is removed. This was most easily effected by hydrogenation of DMT-dTp(Me)dU-Bn (**1c**, R=H) to give dTp(Me)dU (**1f**, R=H). Sensitized photolysis of this product led to the cis-syn product **2f** (R=H) whose structure was confirmed by removal of the methyl phosphate protecting group (vide infra) to give a product matching the ^1H NMR spectrum reported[7] for the dTpdU cis-syn dimer (**2a**, R=H). Unfortunately neither the *Rp* or *Sp*

diastereomer of dTp(Me)[c,s]dU (**2f**, R=H) could be purified to homogeneity by flash chromatography, but after treatment of the *Rp* diastereomer with DMT-Cl in pyridine, DMT-dTp(Me)[c,s]dU (**2g**, R=H) could be obtained in pure form. Treatment of **2g** (R=H) with N,N-diisopropylmethylphosphonoamidic chloride led to the dTpdU cis-syn dimer building block **14** (R=H, Fig. 7) as a diastereomeric mixture, epimeric at the phosphoramidite phosphorus.

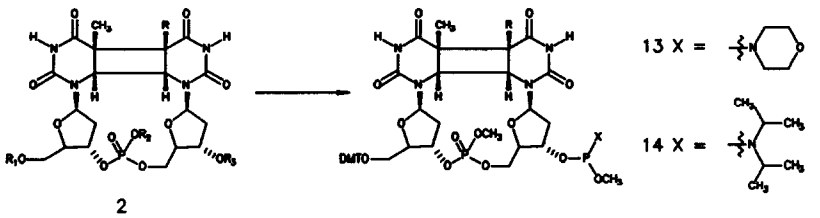

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Figure 7. Steps leading from the cis-syn cyclobutane dimer derivatives to the cis-syn cyclobutane dimer building block.

Before the building block **14** (R=H) could be used to site-specifically introduce dTpdU into an oligonucleotide, its stability to oligonucleotide synthesis chemistry had to be established. The major concern was that the dTpdU cis-syn dimer would not be stable to concentrated ammonia at 55 °C for the 5-8 hours, the conditions used to remove the benzoyl and isobutyryl groups used to protect the amino groups of the nucleotides in standard automated DNA synthesis methodology. Though the C4 carbonyls of the dTpdT cis-syn dimer are known to be susceptible to hydrolysis under strongly basic conditions at elevated temperatures, they are none-the-less stable to the standard deprotection conditions.[13] It was possible, however, that the C4 carbonyl of the dU ring of the dTpdU cis-syn dimer might be more susceptible to nucleophilic attack because it lacks the flanking C5 methyl group present in the dTpdT dimer. Indeed, subjecting the methyl phosphate ester of the dTpdU cis-syn dimer (**2f**, R=H) to concentrated ammonia at room temperature for 2.5 h led to approximately 10% degradation to one major product, in addition to approximately 50% of the desired demethylated cis-syn dimer (**2a**, R=H). Clean demethylation of **2f** (R=H) could be achieved by heating with a 50/50 mixture of diethylamine and isopropanol at 55 °C for 5 h. Unfortunately, N-6-benzoyldeoxycytidine is stable to these conditions, suggesting that these conditions would not be suitable for removal of the standard base protecting groups. Further heating the fully deprotected dTpdU cis-syn dimer **2a** (R=H) under conditions known to remove the benzoyl and isobutyryl groups (conc. ammonia, 55 °C, 5.5 h) led to approximately 25% degradation to one major product, ruling out the use of the standard deprotection conditions in the synthesis of dTpdU cis-syn dimer-containing oligonucleotides.

We are now in the process of scaling up the synthesis of the dTpdU cis-syn dimer building block and exploring alternate base protecting groups for the large scale synthesis of d(CGTAT[c,s]UATGC)·d(GCATXATACG) (X=A or G) for high field NMR studies and for the synthesis of longer fragments of DNA for repair enzyme, polymerase and in vivo mutagenesis studies. Synthetic studies are also in progress aimed at dTpdC cis-syn dimer building block that has a C4 amino protecting group capable of preventing deamination during oligonucleotide synthesis and purification, yet be rapidly and quantitatively removed to generate the dTpdC cis-syn dimer immediately prior to a biological assay. The synthetic methodology developed for the synthesis of diastereomerically pure internucleotide phosphotriester linkages in preparative amounts may also find useful application to the synthesis of oligonucleotides stereospecifically derivatized at phosphorus.

Experimental.

Materials and Methods. All reactions were conducted under anhydrous conditions in an argon atmosphere unless otherwise noted. Methylene chloride, pyridine, triethylamine, acetonitrile and diisopropylethylamine were dried by distillation from CaH_2. THF was dried by distillation from sodium/benzophenone. ^1H and ^{13}C NMR spectra were acquired on a Varian Gemini spectrometer and ^{31}P NMR spectra on a Varian XL-300 spectrometer. ^{31}P spectra were referenced against neat TMP in a coaxial insert.

DMT-dTp(CH_3)(α-Nap), 9. To a well cooled solution of triethylamine (50 mmol, 7 mL) in dry methylene chloride (25 mL) was added dropwise methyl dichlorophosphite (2 mL, 20 mmol). After stirring for 30 m at room temperature DMT-dT 5 (5.45 g, 10 mmol) and triethylamine (50 mmol, 7 mL) in 25 mL of dry methylene chloride. This was immediately followed by a solution of α-naphthol (3.0 g, 20 mmol) and triethylamine (50 mmol) in methylene chloride (15 mL). After stirring at room temperature for 1h the solvent was evaporated and the yellowish residue extracted with a mixture of sat. aq. NaCl and EtOAc. The organic extract was dried (Na_2SO_4) and evaporated. The residue was flash chromatographed on a silica gel column (EtOAc/hexanes, 3:2) to give 3.67 g of the intermediate phosphite as a white foam (79%). The phosphite (3.67 g, 4.9 mmol) was dissolved in dry methylene chloride (25 mL) and *m*-chloroperbenzoic acid (1.72 g, 10 mmol) was added in portions. The solvent was evaporated and the residue was extracted with a mixture of sat. aq. NaCl and EtOAc. The extract was dried (Na_2SO_4), evaporated and flash chromatographed on a silica gel column (EtOAc/Hexanes, 7:3) to give the A diastereomer of **9** (1.55 g, 41%), Rf 0.41 (EtOAc/Hexanes, 7:3), and B (0.96 g, 25%), Rf 0.26. **A Diastereomer (*Rp*):** ^1H NMR (300 MHz, acetone-d_6, referenced to $CD_2\underline{H}COCD_3$ at δ 2.04): δ 10.05 (bs, 1H, NH), 8.18, 7.97, 7.80 (m, 1H each, ArH), 7.57, 7.48, 7.32, 7.25 (m, 14H, ArH), 6.86 (m, 4H, ArH), 6.37 (t, J=6.7, 1H, H1'), 5.40 (m, 1H), 4.35 (q, J=3.2 Hz, 1H), 3.87 (d, J=11.5 Hz, 3H, $POCH_3$), 3.75 (s, 6H, $ArOCH_3$), 3.44 (m, 1H), 2.55 (m, 2H), 1.45 (s, 3H, TCH_3). ^{31}P NMR (121.5 MHz, acetone-d_6, ppm from TMP) -7.67. FAB-MS (3-NBA): m/z 764($[M]^+$, 0.1) 787 ($[M+Na]^+$, 0.5); (3-NBA/LiI): m/z 771 ($[M+Li]^+$, 50) 777 ($[M+2Li-H]^+$, 0.4). Anal. Calcd. for $C_{42}H_{41}O_{10}N_2P$: C, 65.96; H, 5.40; N, 3.66. Found: C, 65.48; H, 5.59; N, 3.58. **B Diastereomer (*Sp*):** ^1H NMR (300 MHz, acetone-d_6, referenced to $CD_2\underline{H}COCD_3$ at δ 2.04): δ 10.06 (s, 1H, NH), 8.14, 7.96, 7.56 (m, 1H each, ArH), 7.6-7.2 (m, 14H, ArH), 6.85 (d, J= 8 Hz, 4H, ArH), 6.37 (t, J=7 Hz, 1H, H1'), 5.37 (m, 1H), 4 18 (q, J=3.5, 1H), 3.91 (d, J=11.5 Hz, 3H, $POCH_3$), 3.74 (s, 6H, $ArOCH_3$), 3.34 (d, J=3.5 Hz, 2H), 2 63 (m, 2H), 1.45 (s, 3H, TCH_3) ^{31}P NMR (121.5 MHz, acetone-d_6, ppm from TMP): δ -7.71. FAB-MS (3-NBA): m/z 764 ($[M]^+$, 0.75); 787 ($[M+Na]^+$, 0.25);

(3-NBA/LiI): m/z 771 ($[M+Li]^+$, 0.75), 777 ($[M+2Li-H]^+$, 0.15). Anal. Calcd. for $C_{42}H_{41}O_{10}N_2P$: C, 65.96; H, 5.40; N, 3.66. Found: C, 65.48; H, 5.59; N, 3.58. Found: C, 64.37; H, 5.60; N 3.77.

dU-Bn, 12l (R=H). To a suspension of NaH (40% in oil, 800 mg, 20 mmol) in 10 mL of THF was added a solution of DMT-dU[28] (2.48 g, 4.68 mmol) in 5 mL of THF at room temperature. After stirring for 1 h, benzyl bromide (1 mL, 8 mmol) was added and the mixture was stirred for an additional 6 h. After quenching any unreacted NaH with 1 mL of sat. aq. NaCl soln., the mixture was extracted with a mixture of sat. aq. NaCl/EtOAc. The extract was dried ($MgSO_4$), evaporated and the residue flashed chromatographed on silica gel with 3:2 EtOAc/hexanes to give DMT-dU-Bn in 78% yield (Rf 0.57, 3:2 EtOAc/hexanes). 966 mg (1.55 mmol) of this product was dissolved in 30 mL of 80% acetic acid/H_2O and stirred for 8 h. The solvent was evaporated under reduced pressure and the residue was flash chromatographed on a silica gel column (EtOAc) to give dU-Bn (407 mg, 82.5%) as a white powder. Rf 0.27 (EtOAc). ^{1}H NMR (300 MHz, methanol-d_4, referenced to $CD_2\underline{H}OD$ at δ 3.30): δ 7.97 (d, J=8.1 Hz, 1H, H6), 7.35-7.25 (m, 5H, ArH), 6.25 (dd, J=8.0, 5.9 Hz, 1H, H1'), 5.69 (d, J=8.1 Hz, 1H, UH5), 4.56 (AB quartet, CH_2Ph), 4.25 (m, 1H), 4.10 (m, 1H), 3.71 (m, 2H), 2.44 (m, 1H), 2.15 (m, 1H). Anal. Calcd. for $C_{16}H_{16}N_2O_5$: C, 60.37; H, 5.69; N, 8.80. Found: C, 60.49; H, 5.87; N, 8.52.

DMT-dTp(Me)dU-Bn, 1c (R=H). A Diastereomer (*Rp*): To a suspension of sodium hydride (40% in oil, 192 mg, 4.8 mmol) in dry THF (5 mL) was added a solution of dU-Bn (**12l**, 383 mg, 1.03 mmol) in THF (15 mL) at room temperature (the THF had to be heated slightly to promote dissolution of the dU-Bn). After stirring for 1 h at room temperature, a solution of the A diastereomer of DMT-dTp(Me)(α-Nap) (**9**, 835 mg, 1.09 mmol) in THF (5 mL) was added and the mixture stirred at room temperature for 45 min. The reaction flask was then cooled in an ice bath and a minimum amount of sat. aq. NH_4Cl (approx. 1 mL) was added to quench the unreacted sodium hydride. The resulting clear solution was filtered through a short column of silica gel and eluted with EtOAc and then MeOH/EtOAc (1:9). The combined eluants were evaporated and the residue was flash chromatographed on a silica gel column (EtOAc) to give the desired product **1c (R=H)** as a white foam (714 mg, 72%). R_f 0.15 (EtOAc). ^{1}H NMR (300 MHz, acetone-d_6, referenced to $CD_2\underline{H}COCD_3$ at δ 2.04): δ 10.32, 10.29 (s, 1H each, NH), 7.67 (d, J=8.2 Hz, 1H, UH5), 7.58 (s, 1H, TH6), 7.47 (d, J=7.2 Hz, 2H, ArH), 7.4-7.2 (m, 12H, ArH), 6.90 (d, J=7.1 Hz, 4H, ArH), 6.35 (t, J=7.6 Hz, 1H, H1'), 6.25 (dd, J=6.1,2.0 Hz, 1H, H1'), 5.62 (d, J=8.1, 1H, UH5), 5.24 (m, 1H), 4.57 (AB quartet, 2H, CH_2Ph), 4.25 (m, 5H), 3.76 (s, 6H, $ArOCH_3$), 3.76 (d, J=11.4 Hz, 3H, $POCH_3$), 3.41 (m, 2H), 2.59(m, 2H), 2 52 (m, 1H), 2.23 (m, 1H), 1.45 (s, 3H, TCH_3). ^{31}P NMR (121.5 MHz, acetone-d_6, ppm from TMP): δ -3.2. FAB-MS (glycerol): m/z 939.3 ($[M+H]^+$, 0.3), 961($[M+Na]^+$ 0.1). Anal. Calcd. for $C_{46}H_{51}O_{14}N_4P$: C, 61.40; H, 5.47; N, 5.96. Found: C, 60.89; H, 5.49; N, 5.87.

Tp(Me)dU, 1f (R=H). A Diastereomer (*Rp*): The A diastereomer of DMT-dTp(Me)dU-Bn (**1c**, 1.160 g, 2.12 mmol) was dissolved in 45 mL of 3.5:1 methanol/acetone and approximately 100 mg of 10% Pd-C was added. After stirring overnight (15 h) under an atmosphere of hydrogen, the catalyst was filtered off and the solvent was evaporated under reduced pressure. The residue was flash chromatographed on a silica gel column (MeOH/EtOAc, 1:4) to afford 494 mg (71%) of the A diastereomer of dTp(Me)dU (**1f**) Rf 0.38 (20% methanol in ethyl acetate). ^{1}H NMR (300 MHz, methanol-d_4, referenced to $CD_2\underline{H}OD$ at δ 3.30): δ 7.78 (s, 1H, TH6), 7.69 (d, J=8.2 Hz, 1H, UH6), 6.28 (dd, 8.4,5.8 Hz, 1H, H1'), 6.24 (t, J=6.7 Hz, 1H, H1'), 5.73 (d, J=8 1 Hz, 1H, UH5), 5.07 (m, 1 H, TH3'), 4.39 (m, 1H), 4.30 (m, 1H), 4.20 (m, 1H), 4.06 (m, 1H), 3.85 (d, J=11.3 Hz, 3H, $POCH_3$), 3.77 (d, J=3.2 Hz, 1H), 2.52 (m, 1H), 2.4-2.2 (m, 3H), 1.86 (s, 3H, TCH_3). ^{31}P NMR (121.5 MHz, methanol-d_4, ppm from TMP): δ -3.25. FAB-MS (3-NBA): 547 ($[M+H]^+$, 65), 569.3 ($[M+Na]^+$, 35).

dTp(Me)[c,s]dU, 2f (R=H). A Diastereomer (*Rp*): The A diastereomer of dTp(Me)dU (**1f**, 497 mg, 0.9 mmol) was

dissolved in 330 mL of 2:1 H_2O/acetonitrile and acetophenone (0.6 mL) and transferred to a Pyrex immersion well photochemical reactor. After purging with argon gas for 35 min., the mixture was irradiated with a 450 W medium pressure Hannovia lamp for 8 h. The solvent was removed under reduced pressure and the residue was flash chromatographed on a silica gel column (30% MeOH/EtOAc) to yield two separate fractions. The less polar fraction, Rf 0.63 (30% MeOH/EtOAc), 112 mg, 22.5%, consisted of trans isomers I and II and the more polar fraction, Rf 0.32, consisted primarily of the cis-syn isomer **2h** (318 mg, 64%). Pure cis-syn isomer (**2f**, R=H) prepared via an alternate route: ^{1}H NMR (300 MHz, methanol-d_4, referenced to $CD_2\underline{H}OD$ at δ 3.30): δ 5.95 (dd, J=9.1,5.7 Hz, 1H, H1'), 5.44 (dd, J=10.2,5.5 Hz, 1H, H1'), 4.64 (dd, J=9.0,5.1 Hz, 1H), 4.24 (m, 2H), 4.12-4.04 (m, 4H), 3.85 (m, 1H), 3.80 (d, J=11.4 Hz, 3H, $POCH_3$), 3.55 (m, 2 H), 3.44 (dd, J=9.0, 2.6 Hz, 1H), 2.78 (m, 1H), 2.30 (m, 1H), 2.17 (m, 1H), 2.08-2.01 (m, 1H) 1.66 (s, 3H, TCH_3). ^{31}P NMR (121.5 MHz, methanol-d_4, ppm from TMP): δ -5.92. ^{13}C NMR (75 MHz, methanol-d_4, referenced to $\underline{C}D_3OD$ at δ 49.0 which obscured δ 48-50): δ 22.2, 34.8, 34.9, 37.8, 43.9, 54.7, 54.8, 63.2, 64.4, 70.0, 70.1, 70.5, 82.7, 82.8, 84.3, 84.4, 86.3, 90.4, 154.0, 155.2, 170.0, 171.0. FAB-MS (3-NBA): m/z 547 ([M+H]$^+$, 10), 569 ([M+Na]$^+$, 60); (3-NBA/Li): m/z 553 ([M+Li]$^+$, 35), 559 ([M+2Li-H]$^+$, 20).

DMT-dTp(Me)[c,s]dU, 2g (R=H). A Diastereomer (*Rp*): The A diastereomer of dTp(Me)[c,s]dU (**2h**, 74 mg, 0 13 mmol) was dissolved in dry pyridine (5 mL) and dimethoxytritylchloride (60 mg, 0.18 mmol) was added at room temperature. The solution was stirred for 4 5 h. Pyridine was evaporated and the residue was flash chromatographed on a silica gel column (10% MeOH/EtOAc) to afford 54 mg, 57%, of the desired DMT-derivative, **2g**, Rf 0.29 (10% MeOH/EtOAc). ^{1}H NMR (300 MHz, acetone-d_6, referenced to $CD_2\underline{H}COCD_3$ at δ 2.04): δ 9.36, 9.27 (bs, 1H each, NH), 7.50 (d, J=9, 2H, ArH), 7.3-7.2 (m, 7H, ArH), 6.90 (d, J=9, 4H), 5.97 (t, J=7.0 Hz, 1H, H1'), 5.86 (t, J=6.2 Hz, 1H, H1'), 4.93 (m, 1H), 4.71 (dd, J=8.7, 5.3 Hz, 1H), 4.38 (m, 2H), 4.22 (m, 2H), 4.10 (m, 1H), 3.95 (m, 1H), 3.77 (s, 6H, $ArOCH_3$), 3.58 (d, J=11.1 Hz, 3H, $POCH_3$), 3 42 (dd, J=10.4, 3.4 Hz, 1H), 3.24 (dd, J=10.4, 3.8 Hz, 1H), 3.17 (d, J=7.5, Hz, 1H), 2.41 (m, 1H), 2.25 (m, 2H), 1.34 (s, 3H, TCH_3). ^{31}P NMR (121 5 MHz, acetone-d_6, ppm from TMP): δ -5 70. ^{13}C NMR (75 MHz, acetone-d_6, referenced to $\underline{C}D_3C(O)CD_3$ at δ 29.8 which obscured δ 29-30): δ 22.8, 35.7, 39.5, 47.4, 47.8, 51.0, 54.2, 54.3, 55.5, 60.1, 63.3, 68.5, 68.6, 70.0, 77.9, 77 8, 82.1, 82.2, 84.0, 84.1, 86.4, 86.6, 87.0, 113.8, 127.6, 128.5, 129 3, 131.1, 131.2, 136.5, 136.6 145.7, 152.8, 153.1, 159.6, 167.1.

DMT-dTp(Me)[c,s]dU-P(OMe)N(CH(CH3)2), 14 (R=H). A Diastereomer (*Rp*). The A diastereomer of DMT-dTp(Me)[c,s]dU (**2g**, 100 mg, 0.12 mmol) was dissolved in 5 mL of dry methylene chloride and N,N-diisopropylethylamine (0.10 ml, 0.59 mmol) was added. After stirring for 10 min N,N-diisopropylmethylphosphonamidic chloride (45 μL, 0.23 mmol) was added and stirred at room temperature for 15 min. Methylene chloride was evaporated under reduced pressure and the residue extracted with sat. aq. NaCl/EtOAc. The extract was dried (Na_2SO_4), evaporated and flash chromatographed on a silica gel column (85% EtOAc/hexanes, 5% triethylamine) to give the product a as a white foam (68 mg, 59%). The product was obtained as an inseparable mixture of diastereomers, epimeric at the phosphoramidite phosphorus, Rf 0.58 (85% EtOAc/hexanes, 5% triethylamine). ^{31}P NMR (121.5 MHz, acetone-d_6, referenced to TMP): δ 147.60, -5.17, -5.22. ^{1}H NMR (300 MHz, acetone-d_6, referenced to $CD_2\underline{H}C(O)CD_3$ at 2.04): δ 9.39, 9.29 (broad s, 1H each, NH), 7.49 (d, J=9, 2H, ArH), 7.40-7.16 (m, 7H, ArH), 6.89 (d, J=9, 4H, ArH), 6.01 (m, 1H, H1'), 5.87 (t, J=6.4 Hz, 1H, H1'), 4.91 (m, 1H), 4.71 (dd, J=8.8,5.6 Hz, 1H), 4.37 (m, 2H), 4.25 (m, 1H), 4.11 (m, 2H), 4.02 (m, 1H), 3.77 (s, 6H, $ArOCH_3$), 3.61, 3.60 (d, J=11.2 & J=11.4 Hz, 3H total, phosphate $POCH_3$), 3.37, 3.36 (d, J=13.2 & 13.1 Hz, 3H total, phosphoramidite $POCH_3$), 3.26-3.15 (m, 2H), 2 97 (m, 1H), 2.86 (m, 1H), 2.34 (m, 3H) 2.41 (m, 2H), 1.34, 1.33 (s, 3H total, TCH_3), 1.16 (m, 14H). FAB-MS (3-NBA): m/z 1010 3 ([M+H]$^+$, 20).

Acknowledgments.

This investigation was supported by PHS Grant RO1-CA40463, awarded by the National Cancer Institute, DHHS. The assistance of the Washington University High Resolution NMR Service Facility, funded in part through NIH Biomedical Research Support Shared Instrument Grant (1 S10 RR02004), and a gift from the Monsanto Co. is gratefully acknowledged. Mass spectral determinations were performed at the Washington University Mass Spectrometry Resource, which is supported by a grant from the National Institutes of Health (RR00954), and the Midwest Center for Mass Spectrometry, a National Science Foundation Regional Instrumentation Facility (NSF-CHE-8620177).

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25. Contrary to our intial experiences (ref. 13), we have recently found that the 5'-DMT dinucleotide derivatives **1g** (R=H or CH_3) can be photodimerized in the presence of acetophenone and that the cis-syn dimers **2g** (R=H or CH_3) can be chromatographically isolated in high purity and in reasonable yield, thereby making the overall synthesis of the building blocks more efficient by eliminating the need to remove, and then later reintroduce the DMT group.

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