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Synthesis of Oligonucleotides Containing Thiazole and Thiazole *N*-Oxide Nucleobases

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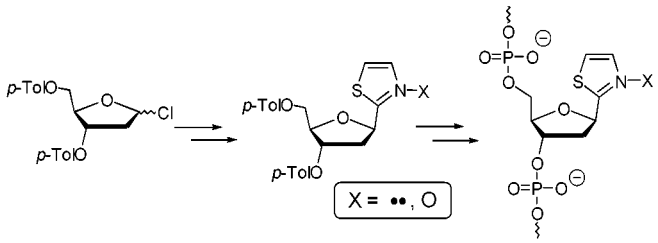
ABSTRACT



The thiazole C-nucleoside analogue was synthesized by the Hantzsch cyclization method to form the thiazole ring and was then converted to the thiazole *N*-oxide C-nucleoside analogue by peracid oxidation of the heterocycle nitrogen. Incorporation of the thiazole and thiazole *N*-oxide phosphoramidites into DNA was successful though significant deoxygenation of the *N*-oxide occurred during DNA assembly. The mechanism proposed for the reduction of the thiazole *N*-oxide to thiazole involves the formation of an *N*-oxide phosphite ester.

Many naturally occurring and synthetic C-nucleosides show biological activity and are synthetic targets of interest for their structural and biochemical properties as well as antitumor and antiviral activity.[1,2] More recently, the synthesis of a variety of C-nucleosides has been undertaken to explore the biophysical and biochemical properties of DNA. These studies have been aimed at exploring the role that hydrogen-bonding, base stacking, and dipolar interactions have on both duplex stability (thermodynamic properties) and the templating ability (enzymatic properties) of oligonucleotides.[3]

A relatively unexplored area of oligonucleotide duplex structure and stability lies in the design of nucleobase analogues that contain both a pronounced directional dipole and the capability to form specific hydrogen bonds with one of the four canonical bases in DNA. One functional group that would introduce a pronounced directional dipole, as well as potential for hydrogen-bonding specificity, is the aromatic *N*-oxides. Such an analogue would provide a means to both examine dipole−dipole interactions and provide a means of introducing functionality capable of forming specific and strong hydrogen bonds. In addition to the unconventional base-pairing selectivity introduced, such nucleoside analogues containing specific hydrogen-bonding capabilities could play an important role in deciphering the importance of DNA polymerase contacts with nucleosides being replicated. Crystallographic studies have shown that the N3 of purines and O2 of pyrimidines make H-bonding contacts with the active site of a number of DNA polymerases.[4−6] Pyrimidine nucleotide triphosphate analogues lacking O2 of the base were not incorporated by Klenow fragment or by *Taq* DNA polymerase and at higher concentrations actually inhibit polymerase activity.[7] We envisioned that nucleoside ana-

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logues containing an *N*-oxide functionality, in a structural position analogous to the O2 of the pyrimidine base, would provide critical data pertaining to the importance of this contact in DNA polymerase catalysis. We have chosen thiazole as our heterocyclic base; it has been previously proven to be stable to the conditions required for oligonucleotide synthesis[8] and is readily oxidized to the *N*-oxide.[9]

Numerous reports have provided synthetic details for the preparation of the thiazole ring in tiazofurin and related analogues.[10−12] The thiazole ring was constructed from ribofuranosylcyanide using the classical Hantzsch method,[13] but with modifications to allow the preparation of 2-(2′-deoxy-β-D-ribofuranosyl)thiazole and its *N*-oxide. Reaction of chlorosugar **1** with excess sodium cyanide in dry DME provided the nitrile **2** in a 3.2:1 β:α mixture of anomers in 83% yield. As previously reported separation of these anomers proved difficult.[10] Quantitative conversion of **2** to the thioamide by treatment with hydrogen sulfide and a catalytic amount of DMAP allowed ready purification of the β-anomer to provide compound **3**. The thioamide **3** was refluxed with 50% aqueous chloroacetaldehyde in 2:1 THF:ethanol,[14] but the reaction was low-yielding (28%) and chromatographic separation of the thiazole nucleoside (**4**) from side-products proved difficult. However, reaction of **3** with bromoacetaldehyde diethyl acetal and a catalytic amount of 4M HCl/dioxane in refluxing acetone[15] provided compound **4** in improved yield (70%), with ready purification from side-products.

For formation of the *N*-oxide (**5**), compound **4** was treated with 2 equivalents of *m*CPBA[9] in THF (72 h) to provide the *N*-oxide **5** in 48% yield. Longer reaction times provided little additional product, although unreacted thiazole **4** was readily recycled. However a more efficient method using 1.1 equivalents each of trifluoroacetic anhydride (TFAA) and hydrogen peroxide urea complex (UHP) was utilized,[16] which provided a much higher yield (97%) after only 3 h at room temperature. Removal of the hydroxyl protecting groups with NH_4OH proved destructive to the thiazole ring of **4**. Therefore, the esters of **4** and **5** were dissolved in 50 mM sodium methoxide/methanol to provide the free nucleoside analogues **6** and **7** in 74% and 83% yields, respectively.

The structure and absolute configuration of **4**, **5**, and **6** were confirmed by X-ray crystallography (Figure 1). Structural analysis indicates a C2′ endo-sugar conformation (P=∼180). The thiazole ring is planar with the sulfur positioned over the oxygen of the furanose ring. The dihedral

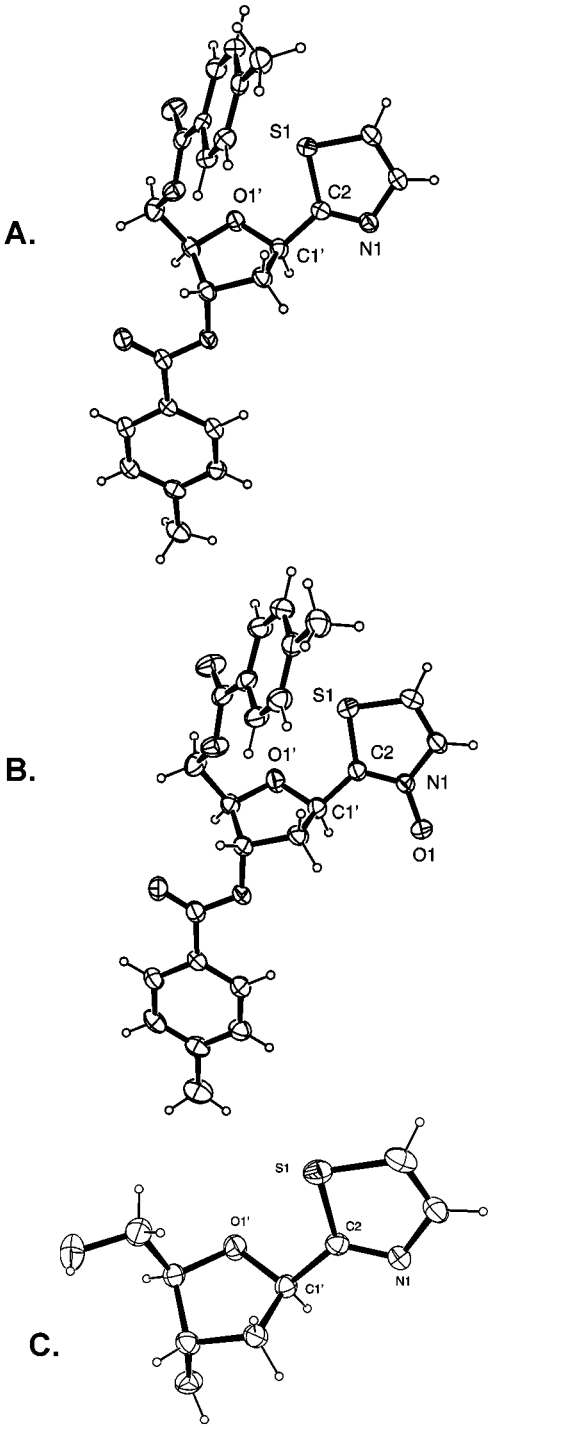

Figure 1. X-ray crystallography ORTEP diagrams of (A) 2-(3′,5′-*O*-*p*-toluoyl-2′-deoxy-β-D-ribofuranosyl)thiazole, (B) 2-(3′,5′-*O*-*p*-toluoyl-2′-deoxy-β-D-ribofuranosyl)thiazole-*N*-oxide, and (C) 2-(2′-deoxy-β-D-ribofuranosyl)thiazole.

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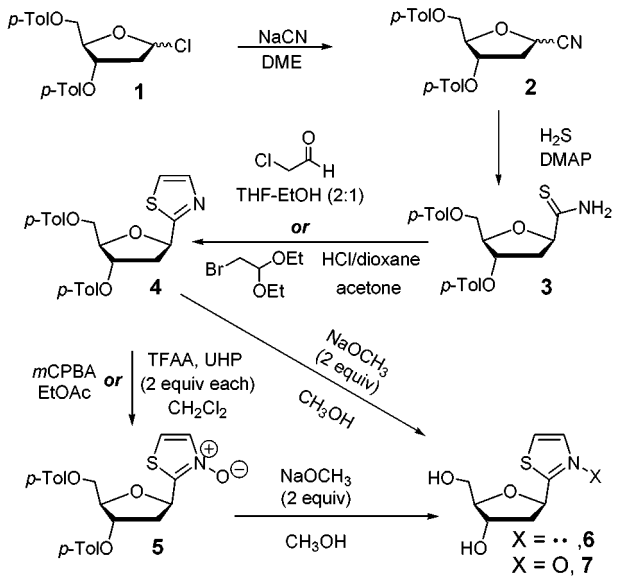
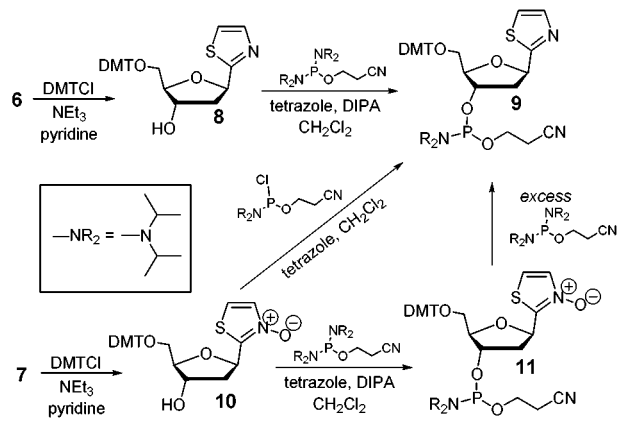
Scheme 1



angles, χ (O1′−C1′−C2−S1), for the toluoyl protected thiazole nucleoside, the toluoyl protected thiazole *N*-oxide nucleoside, and the free thiazole nucleoside are 21.6°, 23.0°, and 32.6°, respectively, which is comparable to other crystal structure data obtained for similar thiazole nucleosides.[17]

For incorporation into oligonucleotides, nucleoside analogues **6** and **7** were converted to their protected nucleoside phosphoramidites (Scheme 2). The 5′-hydroxyl of each was

Scheme 2



dimethoxytritylated in anhydrous pyridine using 1.0 equiv of Et_3N and dimethoxytrityl chloride (DMTCl) to provide DMT ethers **8** and **10** in 70% and 90% yield, respectively. For complete conversion, compound **7** required treatment with a second equiv of Et_3N and DMTCl. Phosphitylation of thiazole **8** was accomplished by treatment with 2-cyanoethyl tetraisopropylphosphorodiamidite (3 equiv), diisopropylamine (DIPA; 1 equiv) and tetrazole (1 equiv) in dry CH_2Cl_2. After a 2 h reaction time, CH_3OH was added and the product purified to provide thiazole phosphoramidite **9** in 89% yield. These conditions were initially used for the phosphitylation of thiazole *N*-oxide nucleoside **10** and resulted in a complex reaction mixture containing significant amounts of the deoxygenated thiazole amidite **9** as indicated by TLC. Attempted phosphitylation of the *N*-oxide **10** with the more reactive chlorophoramidite reagent also led to a mixture of products containing mostly starting material as well as thiazole phorphoramidite **9**. Thus a modified protocol was developed whereby phosphordiamidite reagent is added portionwise to avoid large excesses. To a solution of DMT-*N*-oxide **10** in CH_2Cl_2 was added DIPA (1.1 equiv), tetrazole (1.1 equiv), 2-cyanoethyl tetraisopropylphosphorodiamidite (1.3 equiv) and was reacted for 30 min. A second portion of 2-cyanoethyl tetraisopropylphosphorodiamidite (1.3 equiv) was added. Following an additional 20 min of reaction, CH_3OH was added to quench the reaction and thiazole *N*-oxide phosphoramidite **11** was purified by flash chromatography in 67% yield.

For oligonucleotide synthesis, each nucleoside phosphoramidite was dissolved in dry acetonitrile to a 0.10 M concentration and used to prepare several self-complementary oligonucleotides based on the Dickerson dodecamer sequence as well as oligonucleotides to examine the enzymatic properties of each analogue. Due to the observed ammonia lability of thiazole, phenoxyacetyl (Pac), 4-isopropylphenoxyacetyl, and acetyl *N*-protection for dA, dG, and dC phosphoramidites, respectively, were used for oligonucleotide assembly (Glen Research, Sterling, VA). The synthesis was performed on a 1.0 μmol scale, and all coupling steps except for the thiazole analogues were >98% by trityl assay (vide infra). Base and phosphodiester deprotection was accomplished using 50 mM potassium carbonate in methanol (200 μL) at room temperature for 3 h. Acetic acid (1.5 equiv) in water (200 μL) was added, and the resulting solutions were evaporated to dryness.

The deprotected oligonucleotides were analyzed by anion-exchange HPLC. Figure 2 shows the resulting HPLC profile for the oligonucleotide sequence 5′-d(CGCXAATTTGCG)-3′, where X represents a nucleoside having either thiazole or thiazole *N*-oxide as heterocyclic base. Analysis of the products by MALDI spectroscopy indicates that the thiazole nucleoside is completely stable to the steps used for oligonucleotide synthesis and deprotection. However, three product peaks are evident for the thiazole *N*-oxide oligonucleotide. The mass of the peaks corresponds to an octanucleotide (Figure 2B, peak III) as well as intact dodecanucleotides containing either thiazole (Figure 2B, peak I) or thiazole *N*-oxide (Figure 2B, peak II). Both the problem in the synthesis of the thiazole *N*-oxide phosphoramidite **11** and the deoxygenation of thiazole *N*-oxide during solid-phase oligonucleotide synthesis suggest that the activated P(III) reagents are causing the reduction. A common method for reduction of aromatic *N*-oxides is the reaction with phosphorus trichloride.[18,19] Thus, it appears that for deoxygenation

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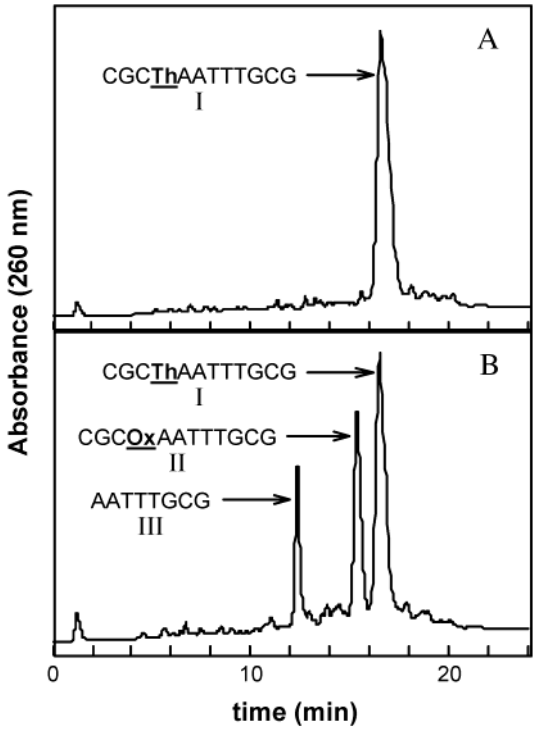

Figure 2. (A) Chromatogram of the Dickerson dodecamer containing thiazole C-nucleoside (Th); MALDI $(M - H)^-$ 3593, (calcd) 3593. (B) Chromatogram of the Dickerson dodecamer containing thiazole *N*-oxide C-nucleoside (Ox); I. MALDI $(M - H)^-$ 3593, (calcd) 3593; II. MALDI $(M - H)^-$ 3613 (calcd) 3609; III. MALDI $(M - H)^-$ 2426 (calcd) 2422. Conditions: Hydrocell NS 1000 (anion exchange) 150 × 4.6 mm column; solvent A 25 mM CHES pH 8, 30% MeOH; solvent B 25 mM CHES/1 M $(NH_4)_2SO_4$ pH 8, 30% MeOH; 0−100% B over 60 min, flow rate = 1 mL/min.

to occur the *N*-oxide must first be covalently attached to the P(III) moiety. In Scheme 3 we suggest a mechanism (A) whereby the thiazole *N*-oxide, whether resin bound or in solution, reacts with activated phosphoramidite reagent to give phosphitylated *N*-oxide species **12**. Once attached to the oxygen, the phosphorus can donate its electrons to reduce the thiazole and create a metaphosphate-like intermediate. We favor this mechanism over direct nucleophilic reduction by the P(III) reagents (B) as we find the thiazole *N*-oxide phosphoramidite to be stable to long-term storage.[20] This explains the decreased coupling efficiency of the thiazole

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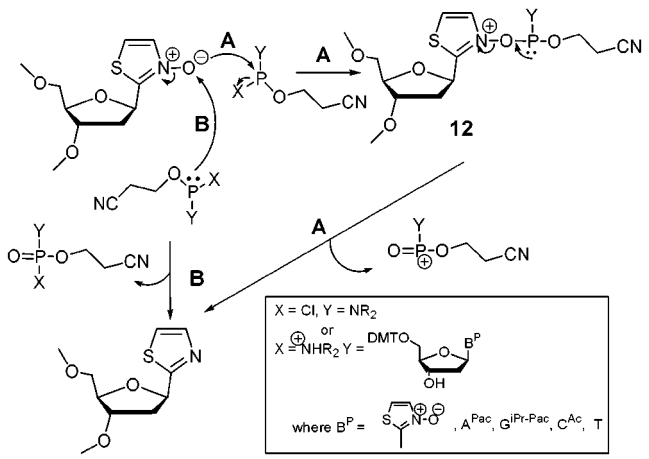
Scheme 3



N-oxide phosphoramidite and therefore the large peak corresponding to the octanucleotide seen in the HPLC chromatogram. The tetrazole-activated *N*-oxide may be reacting with the phosphorus of other *N*-oxide phosphoramidite molecules, thus decreasing the concentration of the active phosphorus species.

In summary, we have successfully synthesized nucleosides bearing thiazole and thiazole *N*-oxide as the heteroaromatic base. These nucleosides were incorporated into oligonucleotides both for duplex melting studies and to study the properties of each nucleoside when transcribed. The thermodynamic and enzymatic behavior of oligonucleotides containing thiazole and thiazole *N*-oxide analogues are currently being investigated.

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Supporting Information Available: Experimental details for Schemes 1 and 2, full characterization of compounds **2**−**11**, X-ray crystallographic information for Figure 1, and MALDI mass spectra of the oligonucleotides synthesized. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Thiazole *N*-oxide phosphoramidite **11** is stable for 1 year when stored at −20 °C.