10 in 87% yield. Deprotection of **10** gave the novel dimer **11** (87%). Sequential dimethoxytritylation and phosphitylation of **11** following standard protocols provided protected dimer **13** in an overall yield of 82%.

Dimer **13** was inserted into a 16-mer standard oligonucleotide sequence [d(GpCpGpTpTpTpTpTpTpTpTpTpTpGpCpG)] 1–5 times and into antisense sequences in one or two positions (Table I) via phosphoramidite methodology.[9] The tritylated oligonucleosides possessing T*T linkages [* = 3′-$CH_2N(Me)OCH_2$-5′] were purified by reverse-phase HPLC and exhibited a single band on polyacrylamide gel electrophoresis. The structural identity of the oligonucleosides was indirectly confirmed by determining the structure of tetramer TpT*TpT by ^1H and ^{31}P NMR analysis. Furthermore, HPLC analysis of the enzymatic degradation[10] of d(GpCpGpTpTpTpTpT*TpTpTpTpTpGpCpG) indicated the expected ratios of nucleosides and the T*T dimer.

Hybridization studies indicated that incorporation of 1–5 modified linkages into the standard sequence had remarkably little effect on the stability of the duplexes formed between the oligonucleosides and their RNA complement (average ΔT_m/modification = −0.3 °C compared to the parent DNA:RNA duplex; data not shown).[11] Moreover, the studies suggest that the uniform distribution of T*T (oligonucleoside **i**) provided a more stable oligonucleoside/RNA duplex (ΔT_m/modification = +0.1 °C). The antisense sequences **ii** and **iii** with one or two linkage changes were slingtly stabilized compared to their unmodified parent oligonucleotide. On examination of the base pair specificity of the 5′-T of the T*T dimer in **ii**, it was found that when matched to A in the RNA complement (T-rA) the duplex was more stable than duplexes having thymine mismatched with cytosine, guanine, or uracil (ΔT_m: T-rC, −10.1 °C; T-rG, −3.9 °C; T-rU, −10.3 °C). The average ΔT_m/mismatch (−7.3, ±3.4) was greater than the average ΔT_m/mismatch (−5.5, ±3.3) of the duplexes with thymine in the unmodified parent DNA against its mismatches in the RNA complement. These data indicate that the Watson–Crick base pair specificity of oligonucleosides containing T*T dimers is as good as or better than wild type DNA. Nuclease resistance study in HeLa cellular extracts showed that the half-life of full-length oligonucleoside **i** was 16 h, whereas the unmodified parent oligonucleotide had a $T_{1/2}$ of 0.5 h. The 3′-capped oligonucleoside **iv** had a $T_{1/2}$ of 14 h in 10% fetal calf serum.[12]

The synthesis of a T*T dimer possessing an achiral, neutral linkage replacing the negatively charged phosphodiester moeity of a natural oligonucleotide has been accomplished. Certain T*T-containing oligonucleosides were synthesized and were found to hybridize to their complementary RNAs as effectively as the unmodified parent DNAs. These oligonucleosides exhibit significant resistance to nucleases while maintaining a high level of base pair specificity.

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Supplementary Material Available: Synthetic procedures and listings of spectroscopic and analytical data for compounds **1–5**, **7**, **9**, **11**, and **13** (5 pages). Ordering information is given on any current masthead page.

(9) Oligonucleoside was synthesized on an ABI 380 B DNA synthesizer following the standard protocol (the average coupling efficiency for the T*T dimer in i was 98.6% and the overall yield was 86.7%).

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Synthesis and Radical-Induced Ring-Opening Reactions of 2′-Deoxyadenosine-2′-spirocyclopropane and Its Uridine Analogue. Mechanistic Probes for Ribonucleotide Reductases[1]

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Ribonucleoside di- and triphosphate reductases are metaloenzymes that catalyze the reduction of ribonucleotides to their 2′-deoxy-DNA components.[2] Inhibition of these reductases interferes with the replication of genetic material required for cancer cell division or viral genome biosynthesis. Stubbe and co-workers[3] have pursued elegant and extensive studies on molecular mechanisms of action of these enzymes. The first step in her working hypothesis involves the enzymatic removal of the H3′ atom from a nucleoside 5′-di- or triphosphate to give a C3′ radical intermediate. This radical then undergoes conversion into the corresponding 2′-deoxynucleotide via a series of enzyme-mediated steps that culminate in the return of the initially abstracted H3′ atom to C3′ with concomitant regeneration of the biological radical initiator.[3] Indirect evidence for the involvement of such radical species has been obtained by studies with isotopically labeled substrates and mechanism-based inhibitors.[3,4] However, direct attempts to observe the involvement of radicals in the dynamic enzyme process have been unsuccessful.

Ring opening of cyclopropylcarbinyl radicals to the corresponding 3-butenyl radicals occurs extremely rapidly. This radical clock[5a] has been used as a mechanistic probe to implicate radical intermediates in reaction pathways by detection of ring-opened products.[5b,c] The enhanced rates of ring opening of rigid spiro cyclopropylcarbinyl radicals have been attributed to the greater relief of ring strain and more favorable orbital alignment.[6] These considerations guided our design[7] of 2′-deoxynucleoside-2′-spirocyclopropanes as novel mechanistic probes for ribonucleotide reductases. We now describe the synthesis of 2′-deoxyadenosine-2′-spirocyclopropane (**5a**) and 2′-deoxyuridine-2′-spirocyclopropane (**5b**), their conversion to the thionoester (**7a** and **7b**) precursors of cyclopropylcarbinyl radicals, and the characterization of the respective 3-butenyl (**8** and **10**) and cyclonucleoside (**9** and **11**) ring-opening products.

Simmons-Smith[8] and related carbenoid methods for the synthesis of cyclopropanes failed to give the desired spirocyclopropyl nucleoside analogues. Treatment of 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-2′-methyleneadenosine[9] (**1a**) with excess diazomethane in diethyl ether for 48 h at ambient temperature gave a separable mixture of the microcrystalline

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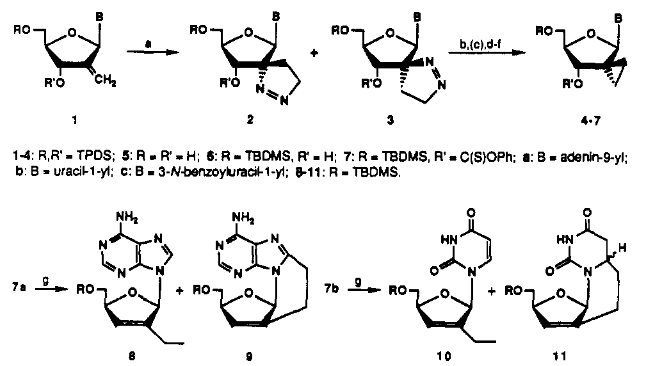
Scheme I[a]



[a](a) CH_2N_2/Et_2O. (b) $h\nu/PhC(O)Ph/MeCN/C_6H_6$. (c) $NH_3/MeOH$. (d) $Bu_4N^+F^-/THF$. (e) TBDMSCl/imidazole/DMF. (f) PhOC(S)Cl/DMAP/MeCN. (g) $Bu_3SnH/AIBN/C_6H_6/\Delta$.

2′-deoxynucleoside-2′-spiropyrazoline derivatives **2a** (88%) and **3a** (4%) (Scheme I). The stereochemistry of **2a**(2′*R*) and **3a**(2′*S*) was assigned from 2D ROESY NMR experiments with each compound. Thus, diazomethane cycloaddition occurred preferentially from the less hindered α-face to give **2a** as the major isomer, analogous with our results with protected 3′-ketonucleosides and a bulky reducing agent[10a] or methyltriphenylphosphorane.[10b] Benzophenone-sensitized photolysis[11] of **2a**/**3a** in acetonitrile/benzene (1:1) provided the 2′-spironucleoside **4a** (92%). Deprotection (TBAF/THF) gave microcrystalline 2′-deoxyadenosine-2′-spirocyclopropane (**5a**, 90%).[12]

Analogous treatment of **1c**[13] with diazomethane/ether gave spiropyrazolines **2c** (63%) and **3c** (28%). Photolysis of **2c**/**3c** and deprotection (NH_3/MeOH, TBAF/THF) gave crystalline 2′-deoxyuridine-2′-spirocyclopropane (**5b**, 50% from **2c**/**3c**).[12] Compounds **5a** and **5b** are the first examples of nucleoside analogues containing the novel spirocyclopropane–sugar moiety.

Protection of **5a** with *tert*-butyldimethylsilyl chloride/imidazole/DMF gave the 5′-*O*-TBDMS (**6a**, 90%) and 3′,5′-bis-*O*-TBDMS (5%) derivatives. Treatment of **6a** with phenyl chlorothionoformate/DMAP/MeCN[14] gave 5′-*O*-TBDMS-2′-deoxy-3′-*O*-(phenoxythiocarbonyl)adenosine-2′-spirocyclopropane (**7a**, 90%).[12] The uridine analogue **7b** was prepared from **5b** in an analogous manner.

Our first biomimetic model reaction utilized the Barton radical-mediated deoxygenation[14,15] (Bu_3SnH/AIBN/benzene/80 °C) of **7a**. We were gratified to discover that 2′-ethyl-2′,3′-unsaturated (**8**, 65%) and 8,2′-ethano-2′,3′-unsaturated cyclonucleoside (**9**, 25%) derivatives were formed. The structure of **8** was apparent from its ^{1}H NMR spectrum, which had an ethyl triplet (δ 1.09) as expected in the product of hydrogen transfer to the primary radical intermediate. Its UV (λ_{max} 260 nm) and MS data and elemental analysis were compatible with those of **8**. Structure **9** was in harmony with its ^{1}H and ^{13}C NMR, UV (λ_{max} 264 nm) and mass spectral data, elemental analysis, and known chemistry involving the preferential addition of radicals at the 8-position of purine nucleosides.[16] Analogous treatment of **7b** gave the 3-butenyl nucleoside **10** (71%)[12] and the UV-transparent 5,6-dihydrouracil cyclonucleoside **11** (25%).[12] These results demonstrate a rational new approach to investigate the proposed radical-mediated conversion of ribonucleotides to their 2′-deoxy analogues by ribonucleotide reductases.

In summary, 2′-deoxynucleoside-2′-spirocyclopropanes have been prepared for the first time, as mechanistic probes for ribonucleotide reductases. A cycloaddition/photolysis route provided these analogues in good yields. Biomimetic radical reactions have yielded products resulting from ring opening of cyclopropylcarbinyl radicals. Studies with other nucleosides and collaborative enzymatic evaluations[7] with 5′-di- and triphosphate esters are in progress.

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Supplementary Material Available: Listings of experimental details and spectral data for compounds **2a,c**, **3a,c**, **4a,b**, **5a,b**, **6a,b**, **7a,b**, and **8–11** (9 pages). Ordering information is given on any current masthead page.

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Novel Synthetic Route to Isolable Pentacoordinate 1,2-Oxaphosphetanes and Mechanism of Their Thermolysis, the Second Step of the Wittig Reaction

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Although there have been many mechanistic studies on the Wittig reaction, most of them have dealt with the formation process of 1,2-oxaphosphetanes for the purpose of elucidating the origin of stereochemistry of the Wittig reaction.[1,2] Attempts to investigate independently the second step of the Wittig reaction have been carried out only by using in situ generated 1,2-oxaphosphetanes,[2,3] in spite of the synthesis of several isolable 1,2-oxaphosphetanes.[4]

In the course of our study to trap an intermediate of the Horner–Emmons reaction, an oxidophosphorane, we have found a novel and general synthetic route to isolable pentacoordinate 1,2-oxaphosphetanes bearing the Martin ligand. We now report on a mechanistic study of their thermolysis, the second step of the Wittig reaction.

Sequential treatment of phosphine oxide **1** with 2 equiv of *n*-BuLi and then with *p,p′*-disubstituted benzophenones (**2**) in THF at 0–50 °C led to the isolation of a good yield of 1,2-oxaphosphetanes **3** via the corresponding dihydroxy derivatives **4** (Scheme I, Table I).[5] Compound **3a** formed as colorless needles, mp 179 °C dec. The structure of **3a** was strongly supported by its ^{31}P (δ_P −35.8) and ^{19}F NMR spectra (double quartet with centers of δ_F −79.6 and −76.5 (J_{FF} = 9.8 Hz)). In the ^{1}H NMR spectrum the signal due to the ortho proton of the Martin ligand[6] was

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