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Efficient Desulfurization of 2-Thiopyrimidine Nucleosides to the Corresponding 4-Pyrimidinones

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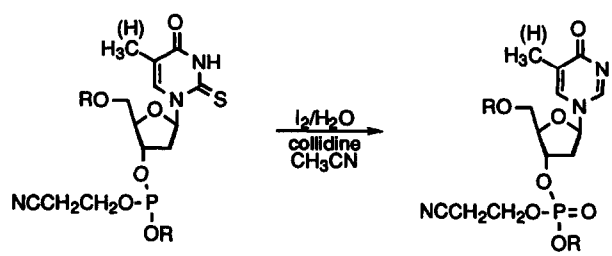
Abstract: **A procedure is described for the preparation of 2'-deoxy-4-pyrimidinone (dH^2U) and 2'-deoxy-5-methyl-4-pyrimidinone (dH^2T) nucleosides. The key transformation is a nearly quantitative desulfurization of the corresponding 2-thio analogue by a brief treatment with a *m*-chloroperbenzoic acid/pyridine solution. The phosphoramidites of these nucleosides have also been synthesized.**

Ligand and protein/DNA interactions can be studied by incorporating nucleoside analogues into DNA sequences at predetermined positions.[1] Hence there is growing interest in the synthesis of such molecules and their incorporation into oligodeoxynucleotides. The 4-pyrimidinones are a class of nucleoside analogues lacking both the N^3-amide hydrogen that participates in Watson-Crick base pairing and the O^2-oxygen situated in the minor groove of B-type DNA.[2] Although the O^2-oxygen does not participate in base pairing, it would normally serve as a hydrogen bond acceptor while interacting with ligands and proteins.[3] In addition, pyrimidinone nucleotides are susceptible to mild acid hydrolysis, thus generating abasic sites at unique positions.[4,5] A recent report[6] describes the desulfurization of 2-thiothymidine (dS^2T) with Raney-nickel to give dH^2T in moderate yield. In this communication, we describe a more efficient and general procedure to effect such a transformation.

While attempting to prepare synthetic oligodeoxynucleotides containing 2-thiopyrimidines, we found the thio-carbonyl moiety to be sensitive to the aqueous iodine oxidation reagent used in the phosphoramidite[7] DNA synthesis method.[8] Further investigation revealed that the major product was the 4-pyrimidinone nucleotide formed via desulfurization (eq. 1).[9,10] Oxidative desulfurization of heterocycles has previously been observed.[11]



(eq. 1)

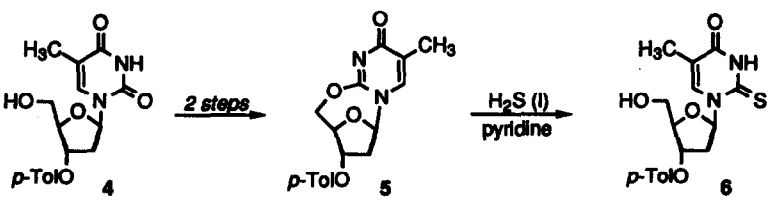
During the evaluation of alternative oxidizing agents, we discovered that treatment of 2-thiopyrimidine nucleosides with *m*-chloroperbenzoic acid/pyridine solutions[12] rapidly produced the 4-pyrimidinone nucleosides in high yield. This desulfurization method represents a significant improvement over current methods. Raney-nickel reductions[6,13] proceed in only moderate yields (30-61%) and are not amenable to post-synthetic modifications of oligodeoxynucleotides. Ogihara and Mitsunobu's dipotassium diazenedicarboxylate treat-

ment and related procedures[14] are quite sluggish (one week) and are not suitable for large-scale preparations[6]. Direct glycosylation methods generally afford complex mixtures of regio- and stereo-isomers.[15]

We have used a modification[9,16] of the silyl Hilbert-Johnson reaction[17] to synthesize β-2'-deoxy-3',5'-*p*-toluoyl-2-thiouridine (3)[18] in 94% isolated yield from the α-chlorosugar (eq. 2). 3'-*p*-Toluoyl-2-thiothymidine

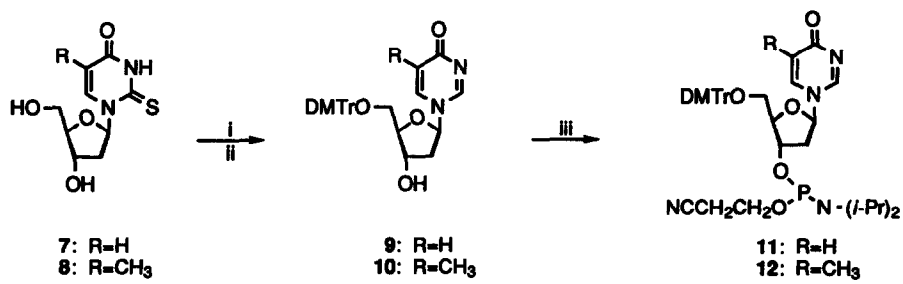
(eq. 2)

(6)[19] was synthesized from thymidine by a variation of Faeber and Scheit's procedure[20] utilizing sulfhydrolysis of the cyclic 2,5'-anhydro intermediate (5) with liquid H_2S/pyridine under pressure (eq. 3). The 2-thiopyrimidines were deprotected by transesterification with 0.1M NaOMe to give (7)[21] and (8),[22] which were



(eq. 3)

next treated with 4,4'-dimethoxytrityl chloride to give the 5'-*O*-4,4'-dimethoxytrityl ethers (scheme 1). After purification, treatment with 0.1M *m*-chloroperbenzoic acid in CH_2Cl_2/pyridine for ten minutes afforded the 4-pyrimidinones 9[23] and 10[24] in 88% and 98% isolated yield, respectively. The diester 3 also undergoes a similar desulfurization in quantitative yield.[25] The work-up involves quenching the excess reagent with 10% aqueous $NaHSO_3$, washing the organic layer with 5% aqueous $NaHCO_3$, drying over Na_2SO_4, and purifying by silica gel chromatography. Reaction of 9 and 10 with β-cyanoethyl-bis-(diisopropylamino)-phosphoramidite[26] and diisopropylammonium tetrazolide[27] gave the dH^2U and dH^2T phosphoramidites, 11[28] and 12[29], in good yield. The phosphoramidite 12 was recently used to synthesize an oligonucleotide.[6]



Scheme 1. Synthetic route for the preparation of the dH^2U and dH^2T phosphoramidites. Reaction conditions were as follows: i) 4,4'-dimethoxytrityl chloride, DMAP, pyridine; ii) 0.1M MCPBA in CH_2Cl_2/pyridine (9:1), 10 min; iii) $(i\text{-}Pr_2N)_2POCH_2CH_2CN$, diisopropylammonium tetrazolide, CH_2Cl_2.

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18. ^1H NMR ($CDCl_3$, 300.7 MHz) δ= 9.8(s,1H,NH) 7.9(dd,4H,Ar) 7.7(d,1H,H_6) 7.3(d,4H,Ar) 7.0(dd,1H,$H_{1'}$) 5.8(dd,1H,H_5) 5.6(m,1H,$H_{3'}$) 4.8(ddd,2H,$H_{5'}$) 4.6(d,1H,$H_{4'}$) 3.1(ddd,1H,$H_{2'}$) 2.4(d,6H,CH_3) 2.2(m,1H,$H_{2''}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ= 174.3, 160.3, 144.6, 144.5, 139.4, 130.0, 129.9, 129.3, 129.1, 126.3, 126.2, 106.8, 89.8, 83.2, 74.0, 63.6, 38.1.

19. ^{1}H NMR ($CDCl_3$ + CD_3OD, 300.7 MHz) δ= 8.1(s,1H,H_6) 7.8(d,2H,Ar) 7.3(d,2H,Ar) 7.1(dd,1H,$H_{1'}$) 5.5(m,1H,$H_{3'}$) 4.3(dd,1H,$H_{4'}$) 3.9(d,2H,$H_{5'}$) 2.8(m,1H,$H_{2'}$) 2.4(s,3H,CH_3) 2.3(m,1H,$H_{2''}$) 1.9(s,3H,CH_3). ^{13}C NMR ($CDCl_3$ + CD_3OD, 75.5 MHz) δ= 174.4, 166.5, 160.9, 144.5, 136.9, 129.7, 129.2, 126.3, 116.3, 89.8, 85.9, 74.6, 61.7, 38.0, 21.6, 12.6.

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21. ^{1}H NMR ($CDCl_3$, 300.7 MHz) δ= 9.8(s,1H,NH) 7.9(dd,4H,Ar) 7.7(d,1H,H_6) 7.3(d,4H,Ar) 7.0(dd,1H,$H_{1'}$) 5.8(dd,1H,H_5) 5.6(m,1H,$H_{3'}$) 4.8(ddd,2H,$H_{5'}$) 4.6(d,1H,$H_{4'}$) 3.1(ddd,1H,$H_{2'}$) 2.4(d,6H,CH_3) 2.2(m,1H,$H_{2''}$). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ= 174.3, 160.3, 144.6, 144.5, 139.4, 130.0, 129.9, 129.3, 129.1, 126.3, 126.2, 106.8, 89.8, 83.2, 74.0, 63.6, 38.1.

22. ^{1}H NMR ($CDCl_3$ + CD_3OD, 300.7 MHz) δ= 8.0(s,1H,H_6) 6.8(t,1H,$H_{1'}$) 4.3(dd,1H,$H_{3'}$) 3.8(ddd,2H,$H_{5'}$) 3.3(m,1H,$H_{4'}$) 2.4(m,1H,$H_{2'}$) 2.1(m,1H,$H_{2''}$) 1.8(s,3H,CH_3). ^{13}C NMR ($CDCl_3$ + CD_3OD, 75.5 MHz) δ= 173.9, 161.4, 137.2, 115.6, 89.4, 87.3, 69.3, 60.6, 40.5, 12.2.

23. ^{1}H NMR ($CDCl_3$, 300.7 MHz) δ= 8.2(s,1H,H_2) 7.8(dd,1H,H_6) 6.8-7.4(m,13H,Ar) 5.9(m,2H,$H_{1'}$+H_5) 4.8(s,1H,$H_{3'\text{-}OH}$) 4.7(m,1H,$H_{3'}$) 3.8(d,6H,OCH_3) 3.4(ddd,2H,$H_{5'}$) 2.6(m,1H,$H_{2'}$) 2.4(m,1H,$H_{2''}$). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ= 171.4, 158.6, 150.6, 144.2, 138.3, 135.2, 135.0, 130.0, 128.0, 128.0, 127.1, 113.3, 112.7, 91.6, 87.5, 87.0, 71.9, 63.5, 55.2, 42.1.

24. ^{1}H NMR ($CDCl_3$, 300.7 MHz) δ= 8.2(s,1H,H_2) 7.8(s,1H,H_6) 6.8-7.4(m,13H,Ar) 6.0(m,1H,$H_{1'}$) 4.7(d,1H,$H_{3'}$) 4.2(s,1H,$H_{4'}$) 3.8(d,6H,OCH_3) 3.4(d,2H,$H_{5'}$) 2.5(m,2H,$H_{2'}$+$H_{2''}$) 1.6(s,3H,CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ= 172.2, 158.7, 149.7, 149.6, 144.2, 135.3, 135.1, 134.2, 130.1, 128.1, 128.0, 127.1, 113.3, 91.8, 87.6, 87.0, 72.4, 63.9, 55.2, 42.1, 13.7.

25. ^{1}H NMR ($CDCl_3$, 300.7 MHz) δ= 8.3(d,1H,H_2) 7.9(dd,4H,Ar) 7.5(dd,1H,H_6) 7.3(d,4H,Ar) 6.1(d,1H,H_5) 5.9(dd,1H,$H_{1'}$) 5.6(m,1H,$H_{2'}$) 4.7(d,2H,$H_{5'}$) 4.6(m,1H,$H_{4'}$) 2.8(dd,1H,$H_{2'}$) 2.5(m,1H,$H_{2'}$) 2.4(d,6H,CH_3); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ= 170.1, 165.9, 165.7, 150.0, 144.7, 144.6, 136.6, 129.4(m,Ar), 126.3, 126.0, 113.1, 91.0, 83.4, 74.4, 63.7, 39.0, 21.7, 21.6.

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28. ^{31}P NMR ($CDCl_3$, 121.7 MHz) δ= 150.5, 150.2.

29. ^{31}P NMR ($CDCl_3$, 121.7 MHz) δ= 150.3, 150.2.

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