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Tetrahedron Letters
Tetrahedron Letters 45 (2004) 6729–6731

Efficient desulfurization of 2-thiopyrimidine nucleosides to the corresponding 4-pyrimidinone analogues using *trans*-2-(phenylsulfonyl)-3-phenyloxaziridine

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Received 31 May 2004; revised 7 July 2004; accepted 13 July 2004
Available online 29 July 2004

Abstract—A brief treatment of 2-thiopyrimidine nucleosides (s^2U^*) with *trans*-2-phenylsulfonyl-3-phenyloxaziridine (PSO) results in efficient substrate desulfurization leading to the corresponding 4-pyrimidinone analogues (H^2U^*). The key transformation proceeds through oxidation of the 2-thiocarbonyl group to a sulfur oxyacid derivative and subsequent elimination of sulfur dioxide. 4-Pyrimidinone 1-β-D-riboside (H^2U) has been transformed into the respective phosphoramidite, a ready-to-use monomer for the introduction of a modified nucleoside into an oligonucleotide chain. Moreover, the effective desulfurization of the 2-thiouridine nucleotide could be achieved directly at the oligonucleotide level, by treatment of the TdA(s^2U)dGdC oligonucleotide with PSO, as verified by MALDI-TOF mass spectrometry.

The synthesis of modified nucleosides and their incorporation into oligonucleotide sequences is an important strategy for the elucidation of structure–function relationships of nucleic acids.[1–3] A variety of nucleoside derivatives have been prepared through deletion or by changing the nature of the functional groups present on the heterocyclic bases.[2–4] One simple base modification among uridine nucleotides that has dramatic effect on nucleoside conformation is the replacement of oxygen at C-2 with sulfur.[5] 2-Thiopyrimidine nucleosides are known to adopt preferentially a rigid C3′-*endo* sugar ring conformation,[6,7] so in RNA duplexes, a modified s^2U-A base pair is more stabilized than the unmodified one.[8–10] Furthermore, due to steric hindrance and the weaker H-bonding ability of sulfur relative to oxygen, 2-thiouridine destabilizes the U-G wobble base pair compared to uridine.[8–10] 4-Pyrimidinone nucleosides are a class of nucleoside analogues lacking both the N^3-amide hydrogen and the 2-carbonyl function,[11–14] and thus they do not form the conventional wobble U-G base pair. An incorporation of a 2-thiouridine and a 4-pyrimidinone-1-β-D-riboside, instead of uridine, in the site specific position within the oligonucleotide chain could provide useful models for the study of biologically important U-G wobble interactions.[15,16]

In the present report we describe a very efficient transformation of 2-thiopyrimidine nucleosides into the corresponding 4-pyrimidinone analogues by a brief treatment with *trans*-2-phenylsulfonyl-3-phenyloxaziridine (PSO).[17] Previously it had been reported that the desulfurization of 2-thiopyrimidine nucleosides proceeds in moderate yield under reductive conditions by dipotassium diazenedicarboxylate treatment[11] or Raney-nickel reduction.[12] Oxidative desulfurization of the 2-thiopyrimidine moiety has been observed on treatment with hydrogen peroxide,[11] aqueous iodine,[13] *m*-chloroperbenzoic acid/pyridine[13] or dimethyldioxirane.[14] Oxaziridine-type oxidizing reagents (2-(phenylsulfonyl)-3-(3-nitrophenyl)oxaziridine and 10-camphorsulfonyl oxaziridine) were applied recently in the oxidation step of oligonucleotide synthesis in H-phosphonate[18] and phosphoramidite[19] approaches.

During our evaluation of 2-thiouridine stability under different oxidizing conditions, used for automated oligonucleotide synthesis, we discovered that treatment of a 2-thiopyrimidine nucleoside with PSO led to complete loss of sulfur giving the 4-pyrimidinone analogue quantitatively.[20]

Keywords: 2-Thiopyrimidine nucleosides; Oxidative desulfurization; 4-Pyrimidinone 1-β-D-ribosides; Oxaziridine.
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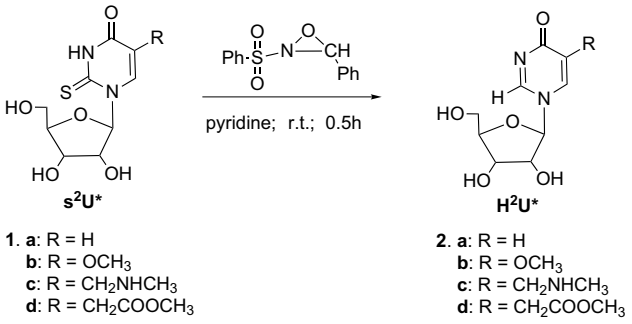
doi:10.1016/j.tetlet.2004.07.052



Scheme 1.

Thus, 2-thiouridine **1a** and its 5-substituted analogues **1b–d**, commonly found at the wobble position of the anticodon loop of tRNA,[16] were studied in the desulfurization process (Scheme 1). Treatment of 2-thiouridine[21] with an excess of PSO solution in pyridine (minimum 2 equiv) for 30 min at room temperature afforded 4-pyrimidinone **2a** in 81% isolated yield.[22] Modified 2-thiouridines **1b–d** underwent similar desulfurization in quantitative yields. The courses of the reactions were monitored by ^{1}H NMR spectroscopy. A significant upfield shift of the resonance signal of 1′-H was observed during the **1** → **2** transformation (from 7.2–7.0 ppm for **1b–d** to 5.9–5.7 ppm for **2b–d**).

Unexpectedly, the same quantitative transformation of s^2U to H^2U was observed when the PSO-assisted oxidation was carried out in the presence of oxygen or nitrogen nucleophiles (methanol, water, *n*-propylamine), so it was possible to perform efficient desulfurization of 2-thiopyrimidine nucleosides in aqueous media. An aqueous solution of the 2-thiouridine was treated with 3 equiv of PSO dissolved in acetonitrile (30 min, 25 °C) and, after washing with ethyl acetate, was concentrated in vacuo. The crude reaction product was purified by silica gel column chromatography in chloroform/methanol solution and pure derivative **2a** was isolated in 79% yield.

It is noteworthy that common DNA and RNA nucleosides were not affected by PSO under these reaction conditions.

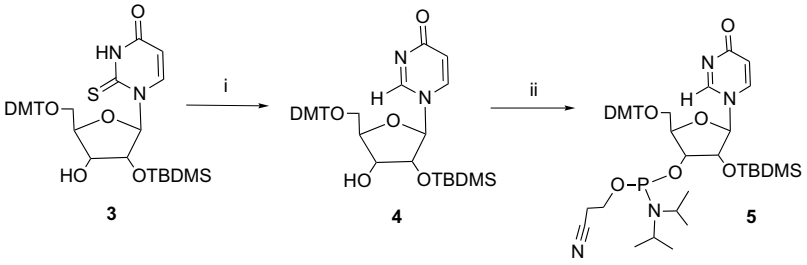
We suggest that the PSO-assisted desulfurization of 2-thiouridines proceeds via the initial formation of an sulfur oxyacid[14] followed by subsequent decomposition to the 4-pyrimidinone nucleosides. To confirm this, 2′,3′,5′-*O*-tribenzoyl-2-thiouridine in anhydrous methylene chloride or acetonitrile was treated with 2 equiv of PSO under a stream of argon. The emerging argon was analyzed for sulfur oxides. Using known procedures,[23] we showed that sulfur dioxide was the only gaseous reaction product. Moreover, the solid reaction residue, separated on a silica gel column, gave in quantitative yield the tribenzoyl derivative of the corresponding 4-pyrimidinone ribonucleoside together with the sulfonimine $PhSO_2N = CHPh$.

PSO-assisted desulfurization was also used for transforming 5′-*O*-(dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-2-thiouridine **3**[24,25] into **4**, which allowed us to prepare the phosphoramidite **5**, ready to use as a monomer for the introduction of a 4-pyrimidinone nucleoside into an oligonucleotide chain (Scheme 2). Desulfurization of **3** was performed by its brief treatment (30 min) with two molar equivalents of PSO in anhydrous methylene chloride (after silica gel column chromatography the isolated yield of **4** was 77%). Pyrimidinone derivative **4** was then easily converted into the respective phosphor-amidite **5** by the reaction with 2-cyanoethyl diisopropylchlorophosphoramidite.[26] The structure of phosphoramidite **5** was confirmed by ^{31}P NMR and HR mass spectrometry.[27]

The desulfurization procedure shown here represents a significant improvement over current methods.[11–13] High yields, mild reaction conditions, the stability of common nucleosides and oligonucleotides to the action of oxaziridine-type oxidizing agents[18,19] encouraged us to apply this method to the post-synthetic modification of oligonucleotides containing 2-thiopyrimidine nucleosides. The preliminary experiment was performed on the model pentamer TdA(s^2U)dGdC. The reaction substrate and products were analyzed by MALDI TOF mass spectrometry (Fig. 1).

The peak at *m*/*z* 1495 corresponding to s^2U-containing oligonucleotide was shifted to *m*/*z* 1463 after PSO treatment. This result indicated an efficient loss of sulfur atom during oxidation, resulting in the formation of an oligonucleotide with a modified H^2U unit.

Further work on optimization of the desulfurization protocol for 2-thiouridine-containing oligonucleotides, also bound to the solid support is in progress.



Scheme 2. Reagents and conditions: (i) PSO 2 equiv/CH_2Cl_2; rt; 30 min. (ii) DIPEA/CH_2Cl_2; 2-cyanoethyl diisopropylchlorophosphoramidite; argon; rt; 30 min.

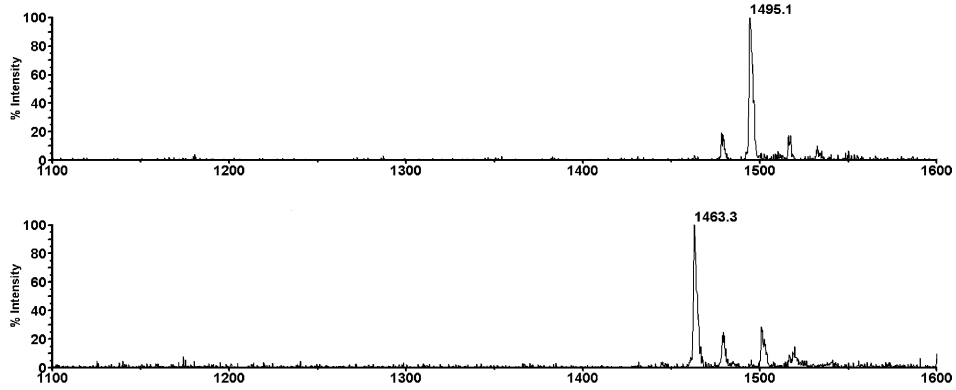


Figure 1. MALDI TOF mass spectra of (a) TdA(s^2U)dGdC in 2,4,6-trihydroxyacetophenone as a matrix; (b) reaction mixture after TdA(s^2U)dGdC (0.2 OD in 10 μL of water) treatment with 0.1 M PSO solution in acetonitrile (10 μL).

Acknowledgements

This work was supported by the State Committee for Scientific Research (project PBZ-KBN-059/T09/07 to E.S.).

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22. ^{1}H NMR (250 MHz, D_2O, δppm) 3.75 (dd, 1H, $^3J_{5''\text{-}4'}$ = 4.0 Hz, $^3J_{5'\text{-}5''}$ = 12.8 Hz, H5″), 3.85 (dd, 1H, $^3J_{5'\text{-}4'}$ = 3.1 Hz, $^3J_{5'\text{-}5''}$ = 12.8 Hz), 4.15–4.25 (m, 2H, H3′, H4′), 4.28–4.36 (m 1H, H2′), 5.55 (d, 1H, $^3J_{1'\text{-}2'}$ = 5.7 Hz, H1′), 6.43 (d, 1H, $^3J_{5\text{-}6}$ = 7.7 Hz, H5), 8.03 (dd, 1H, $^4J_{6\text{-}2}$ = 2.3 Hz, $^3J_{6\text{-}5}$ = 7.7 Hz, H6), 8.60 (d, 1H, $^4J_{2\text{-}6}$ = 2.3 Hz, H2); ^{31}C NMR (D_2O, δ ppm) 60.5, 69.8, 72.8, 74.7, 85.8, 94.6, 112.2, 140.4, 151.9, 173.4; FAB MS: positive ions—*m/z* = 229, $[M+H]^+$; negative ions—*m/z* = 227, $[M-H]^-$.
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27. ^{31}P NMR (benzene, δ ppm); 151.8; 148,6; FAB HRMS: calcd for $C_{45}H_{61}N_4O_8SiPNa$ 867.3897, found *m/z* = 867.3901 $[M+Na]^+$.