Study on Disulfur-backboned Nucleic Acid: Part 2. Efficient Synthesis of 3′,5′-Dithiothymidine

Hua Wang, Changmei Cheng,* Hongchao Zheng, Xiaohong Liu, Chang Fang, Shanshan Xu, and Yufen Zhao
Key Laboratory of Bioorganic Phosphorus Chemistry of Education Ministry, Department of Chemistry, School of life Sciences and Engineering, Tsinghua University, 100084 Beijing, P. R. China

(Received November 22, 2004; CL-041408)

A novel and convenient procedure for synthesizing 3′,5′-dithiothymidine was described. In this procedure, DBU was used to form the intramolecular ring of 2,3′-anhydrothymidine and then the thioacetic acid was used as solvent as well as the nucleophilic reagent to produce *S*-acetyl-3′-thiothymidine. A very efficient deprotection step was applied to afford the target compound, which can avoid the oxidization of the thiol groups. And the key intermediate, 5′-*O*-tosyl-2,3′-anhydrothymidine, was found to have regioselectivity for different nucleophiles.

The thionucleosides, which have one or more of the sugar hydroxyl replaced by thiol are considered to be important monomers of oligonucleotide in antisense and RNAi fields.[1a,b] Meanwhile, the nucleosides with metal binding sites at the 3′- and 5′-positions would be built up into a single DNA strand with cooperative participation of metal coordination to replace the covalent phosphodiester linkage in natural DNA.[2] Recently, we started a project to synthesize a series of disulfur-backbones of novel nucleic acids (Figure 1), and to apply them to the study of antisense and the synthesis of the metal-mediated DNA strand. In 1996, Reeses' group[3] reported a procedure to synthesize 3′,5′-dithiothymidine in 5 steps with a good yield, and this procedure was simplified by Mitsuhiko Shinoya's group[4] in 2002. Although their method were pretty good, the use of the sodium salt of 4-methoxy-α-toluenethiol led both 3′ and 5′ hydroxy groups to be changed to thiol groups. In our study on synthesis, we applied a new efficient strategy to form 3′,5′-dithiothymidine step by step, and discovered that the intermediate 5′-*O*-tosyl-2,3′-anhydrothymidine **4** can be regioselectively attacked on 3′- or 5′-position by different nucleophiles. And a 5-minute-deacetylation reaction was also applied in the procedure, which can efficiently avoid the oxidization of the thiol groups.

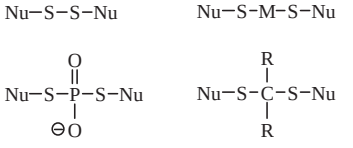
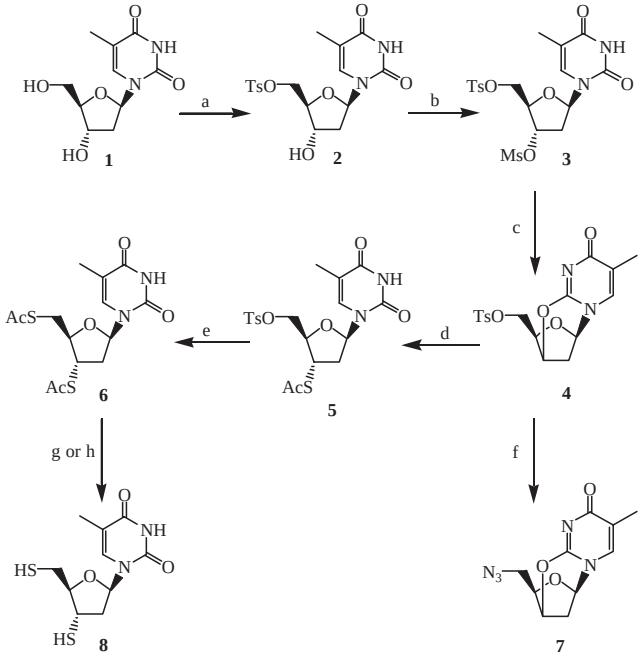


Figure 1. Some Backbones of Novel Nucleic Acids.

The formation of intramolecular ring of 2,3′-anhydrothymidine provides a much convenient procedure to inverse the configuration of 3′-position. We first used 2′-deoxythymidine **1** to react with *p*-tosyl chloride and afforded 5′-*O*-tosyl-2′-deoxythymidine **2** as a single product in 91% yield. The 3′-tosylate isomer was not found as an earlier report.[5] And then the treatment of **2** with MsCl in cooled pyridine afforded the desired 3′-mesylate product **3** in 95% yield. When **3** was heated with 1.4 equiv. DBU in acetonitrile solution, the 5′-*O*-tosyl-2,3′-anhydronucleoside **4** was obtained and isolated as a white solid in 77% yield. In our recent study, we found that DBU is an ideal base that would be applied in the preparation of other 2,3′-anhydronucleosides.



Scheme 1. Synthetic route for 3′,5′-dithiothymidine. a) TsCl, Pyridine, 91%; b) MsCl, pyridine, 95%; c) DBU, CH_3CN, 50 □, 77%; d) AcSH, reflux, N_2, 12 h, 73%; e) AcSK, Dioxane, N_2, 90 h, 73%; f) NaN_3, DMF, 60 °C, 91%; g) $LiAlH_4$, THF, HCl, N_2, 91%; h) EtSNa, EtSH, N_2, 5 min, 83%.

In an earlier approach to the thionucleosides, Imazama et al.[6] reported that the thioacetic aicd was a good nucleophilic reagent that can be used to attack the 2′-position of 2,2′-cyclo-3′,5′-di-*O*-acetyluridine and formed 2′-*S*-acetylnucleoside. But the side reaction between thioacetic acid and DMF, which afforded dimethylthioformamide as a by-product, led a low yield (30%) in the preparation of the desired compound. In our study, we found that the unsolvable 2,3′-anhydrothymidine can easily dissolved into thioacetic acid when heated, so we used AcSH to dissolve **4** directly and heated the mixture to reflux under N_2 atmosphere. After stirring for 12 h, compound **4** had been completely converted. Evaporated the solvent, the 3′-*S*-acetyl compound **5** was isolated by chromatography in 73% yield. Although the polymers of thioacetic acid were also obtained in the reaction, they were in low polarity and can be easily separated on silica gel.[7]

We found that, thioacetic acid only attacked the 3′-postion, not the 5′-position of the intermediate **4**. But when we elongated the reaction time to 3 days, the 3′,5′-*S*-acteylthymidine **6** was also obtained, which were identified by mass spectra. However, the 3 days reaction led the mixture to a black-viscous syrup, whose TLC was so complex, from which **6** could not be easily separated. Thus, we had to synthesize compound **6** step by step. We employed AcSK as a nucleophile to attack the 5′-tosyl group. Compound **4** reacted with AcSK readily in the solution of dioxane at 50 °C for 90 h, afforded *S*,*S*′-diacetyl-3′,5′-dithiothymidine **6** in 73% yield.[8]

When we used NaN_3 as the nucleophilic reagent to react with compound **4**, the 5′-azido derivate **7** was unexpectedly formed as a sole product at 60 °C, without the founding of 3′-azido or the 3′,5′-diazido isomers in the solution.[9] However, a recent study reported[10] that when 5′-*O*-mesyl-2,3′-anhydrothymidine was heated with NaN_3 in DMF solution at 130 °C, the 3′,5′-diazidothymidine was formed in a high yield. Presumably, under basic conditions, the 5′-position has a higher activity than the 3′-position and gives 5′-substituted derivative as the main product at a low temperature; while in thioacetic acid solution, the acidic surrounding enhances the activity of 3′-position, so the nucleophile selectively attacks the 3′-position. Such regioselective reaction of **4** attracted much attention from us, and the further studies had been carried on.

Two new methods were employed to synthesize the target compound **8**. And we believed that both of these methods are efficiently and conveniently. Firstly, we successfully reduced the acetyl groups of **6** with $LiAlH_4$ to afford 3′,5′-dithiothymidine in 91% yield.[11] Secondly, in our previous report, a more convenient method was developed to remove the acetyl groups of 3′,5′-dithioadenosine,[12] and we found that this method was also suitable for thymidine. So we treated compound **6** with EtSNa in the solution of EtSH under N_2 atmosphere. The reaction was completed only in 5 min, and then the mixture was neutralized with acetic acid and was extracted with chloroform. After evaporating the solvent, the residue was saturated with ethyl ether to precipitate compound **8** in 83% yield. We found the solid state of 3′,5′-dithiothymidine can effectively prevent the free thiol groups from being oxidized to disulfide bonds and can be preserved safely under N_2 for long.[13]

In conclusion, we presented a novel and efficient route to synthesize the 3′,5′-dithiothymidine starting from thymidine. We believed that the methods that we have described in this article should have more general applications in the preparation of other 2′-deoxyribonucleoside analogs, which containing one or more thiol groups at the 3′- and 5′-positions of the deoxyribose moieties. The further work to develop antisense drugs and to study the origin of nucleic acid is now in progress.

This work was supported by the National Natural Science Foundation of China (No. 20272031) and the Basic Science Research Foundation of Tsinghua University (JC 2001046).

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7 ***S*-acetyl-5′-*O*-tosyl-3′-thiothymidine 5** A solution of 200 mg (0.44 mmole) of compound **4** in 10 mL of thioacetic acid was heated under N_2 atmosphere to reflux for 12 h. The solvent was evaporated in vacuo and the residue was chromatographed on silica gel (eluent firstly with CH_2Cl_2:Petroleum ether = 5:1; secondly with CH_2Cl_2:Methanol = 20:1) to give 0.22 g of compound **5** yielding 91.7%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.40 (1H, s, NH), δ 7.81 (2H, d, J = 8.3 Hz, Ar–H), δ 7.47 (1H, s, 6-H), δ 7.38 (2H, d, J = 8.0 Hz, Ar–H), δ 6.23 (H, dd, J = 6.3, 6.2 Hz, 1′-H), δ 4.36 (1H, dd, J = 1.8, 11.0 Hz, 5′-H), δ 4.26 (1H, dd, J = 3.1, 10.9 Hz, 5″-H), δ 4.12 (1H, ddd, J = 2.2, 3.1, 2.8 Hz, 4′-H), δ 4.00 (1H, ddd, J = 6.5, 6.1, 9.0 Hz, 3′-H), δ 2.46 (3H, s, Ts-Me), δ 2.37 (2H, m, 2′-H), δ 2.36 (3H, s, Ac–Me), δ 1.95 (3H, s, 5-Me); ESIMS m/z 477 $(M + Na)^+$.
8 ***S*,*S*′-diacetyl-3′,5′-dithiothymidine 6** To a solution of 95 mg (0.21 mmole) of **5** in 20 mL of Dioxane, 47 mg (0.42 mmole) potassium salt of thioacetate was added to form a suspension. The mixture was stirring at 50 °C under N_2 atmosphere for 90 h, and then it was filtrated and evaporated. The residue was chromatographed on silica gel (eluent CH_2Cl_2:Methanol = 20:1) to afford 55 mg of compound **6** in 73% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.91 (1H, s, NH), δ 7.34 (1H, s, 6-H), δ 6.13 (1H, dd, J = 5.4, 6.7 Hz, 1′-H), δ 4.03 (1H, ddd, J = 3.6, 7.5, 7.1 Hz, 4′-H), δ 3.81 (1H, ddd, J = 8.2, 8.2, 8.4 Hz, 3′-H), δ 3.38 (1H, dd, J = 3.6, 14.3 Hz, 5′-H), δ 3.27 (1H, dd, J = 6.6, 14.5 Hz, 5″-H), δ 2.48 (2H, m, 2′-H), δ 2.40 (3H, s, Ac–Me), δ 2.39 (3H, s, Ac–Me), δ 1.98 (3H, s, 5-Me); ESIMS m/z 381 $(M + Na)^+$.
9 **5′-Azido-2,3′-anhydothymidine 7** A solution of 20 mg (0.05 mmole) of compound **4** and 13-mg sodium azide in 10-mL DMF was heated at 60 °C under stirring for 2 days. The solvent was taken away by forming azeotrope with toluene in vacuo. The residue was washed with ethyl ether, to give 11.8 mg compound **7** (91%). ^{1}H NMR (300 MHz, DMSO-*d*6) δ 7.60 (1H, s, 6-H), δ 5.88 (1H, d, J = 3.8 Hz, 1′-H), δ 5.27 (1H, s, 3′-H), δ 4.37 (1H, ddd, J = 2.4, 5.2, 7.2 Hz, 4′-H), δ 3.52 (2H, ddd, J = 5.2, 7.2, 13.0 Hz, 5′-H), δ 2.59 (1H, d, J = 12.7 Hz, 2′-H), δ 2.44 (1H, d, J = 13.4 Hz, 2″-H), δ 1.76 (3H, s, 5-Me); ESIMS m/z 272 $(M + Na)^+$.
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13 **3′,5′-dithiothymidine 8, Method A:** A suspension of 100 mg (2.6 mmole) of $LiAlH_4$ in 10 mL anhydrous THF was cooled to 0 °C and a solution of 80 mg (0.22 mmole) of compound **6** in 10-mL anhydrous THF was added drop wise under N_2. The reaction mixture was stirring for 4 h at 0 °C, then the 1 N HCl was added to adjust the pH of the mixture to 3. Extracted with 20 mL of chloroform, and the organic layers were dried over anhydrous $MgSO_4$. Evaporated the chloroform, the residue was chromatographed on silica gel (eluent CH_2Cl_2:Methanol = 20:1) to give 55.7 mg of compound **8** in 91% yield; **Method B:** To a solution of 50 mg (0.14 mmole) compound **6** in 5-mL ethyl thiol, was added 13-mg (0.16 mmole) sodium salt of ethyl thiol under Nitrogen. The suspension was stirring for 5 min at rt, then was neutralized with acetic acid. Then extracted with 20 mL of ethyl acetate, dried over anhydrous $MgSO_4$ and evaporated the organic layer. The residual oil was saturated with 50-mL ethyl ether and the precipitate was collected by filtration, washed with cold ethyl ether (4 × 1 mL), to give 32 mg of the compound **8** in 83% yield.

Published on the web (Advance View) February 23, 2005; DOI 10.1246/cl.2005.432