Study on Disulfur-Backboned Nucleic Acid: Part 1, Efficient Synthesis of 3′,5′-Dithio-2′-Deoxyadenosine

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Abstract: An efficient procedure is established to synthesize 3′,5′-dithio-2′-deoxyadenosine starting from 2′-deoxyadenosine in five steps in 33% overall yield. In this procedure, the key intermediate **9** was synthesized by twice S_N2 reactions with twice 3′-configuration inversions and then it was deprotected to title compound by removing three acyl groups in one pot.

Key words: 3′,5′-dithio-2′-deoxynucleoside, 3′,5′-dithio-2′-deoxyadenosine, disulfur-backboned nucleic acid, configuration inversions, synthesis

3′-Thio-2′-deoxynucleoside and 5′-thio-2′-deoxynucleoside are important monomers[1a,b] that can be used in antisense and RNAi fields. But little work has been devoted to studying 3′,5′-dithio-2′-deoxynucleoside. Recently, we started a project to synthesize a series of disulfur-backbones[2a,b] (Figure 1) of novel nucleic acids and to apply them to the study of antisense, RNAi, nucleic acids probe and the origin of nucleic acid. However, the synthesis of 3′,5′-dithio-2′-deoxy-nucleoside tends to be cumbersome and low-yielding. This is mainly due to the following two reasons. One is the difficult 3′-configuration inversion, which will cause many side reactions. The other is that 3′,5′-dithio-2′-deoxynucleoside is too easily oxided to disulfides.[3] Recently, two approaches to synthesize 3′,5′-dithio-2′-deoxynucleoside have been described. The first one is to synthesize 3′,5′-dithiothymidine starting from thymidine.[2a] Although the method is good, it is not suitable for the synthesis of purine analogues. The second one is to prepare 3′,5′-dithio-2′-deoxyadenosine from 3′,5′-dithiothymidine by nucleobase exchange with adenine.[2b] This procedure took six steps and produced both α- and β-nucleosides which is not easy to be separated.

In this paper, we would like to report an efficient synthesis of 3′,5′-dithio-2′-deoxyadenosine starting from 2′-deoxyadenosine. Firstly, we use TfO- as leaving group and H_2O as nucleophile to realize the first 3′-configuration inversion by a modified procedure (Scheme 1) of Ishido[4] and Herdewijn.[5] Thus, 2′-deoxyadenosine (**1**) was smoothly benzoylated to afford **2** and subsequently **2** was converted into **3** and **4** (3:7 by NMR determination) according to the literature procedure. Then the mixture of **3** and **4** was treated with NaOMe–MeOH 5 minutes at room temperature to selectively remove the ester-benzoyl group and get compound **5** in 70% yield. If the reaction time was longer than 30 minutes, further debenzoylation of the 6-amide would occur.

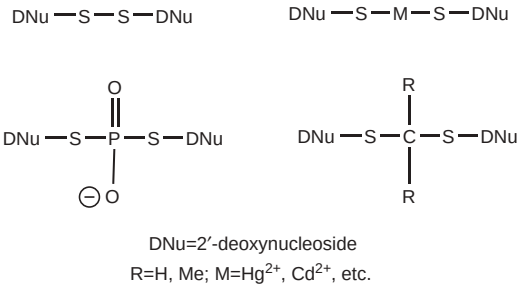
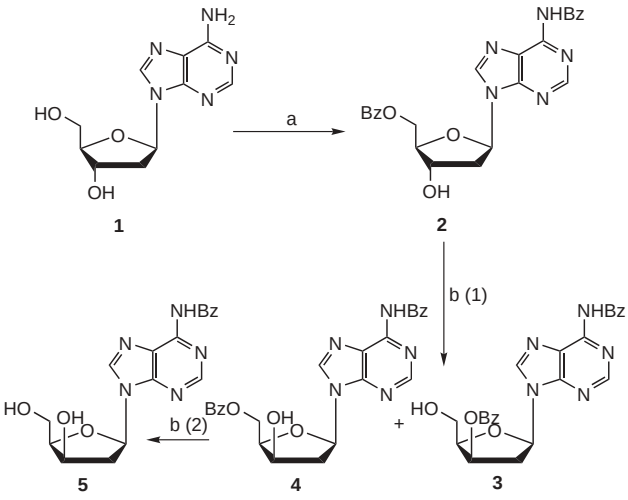


Figure 1 Disulfur-backbones of novel nucleic acid.



Scheme 1 *Reaction conditions*: (a) (1) BzCl, C_5H_5N; (2) Me_3SiCl, BzCl, MeOH, NH_3, 80%; (b) (1) $(CF_3SO_2)_2O$, C_5H_5N, CH_2Cl_2, H_2O; (2) MeOH, MeONa, 70%.

Secondly, compound **9** was synthesized as shown in Scheme 2. In this procedure, we employed MsO- as a leaving group and AcSK as a nucleophile to realize the second 3′-configuration inversion (from **6** to **9**). Thus, compound **5** was converted into compound **6** by treatment with MsCl in pyridine. Then, compound **6** and AcSK were mixed in dioxane and the suspensions were heated to a certain temperature. We found that even though the suspension was heated at 40 °C for more than 4 days, no change occurred. When compound **6** was heated at 50 °C

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for 3 days, only 5′-MsO- had been replaced by -SAc, while 3′-MsO- still kept unchanged. As a result, compound **8** was isolated in 81% yield. When the heating temperature reached 60 °C, we found that both 3′-MsO- and 5′-MsO- were replaced by -SAc after 36 hours. But if the heating temperature increased to 70 °C or more, compound **4** underwent elimination to form compound **7** and the compound **9** could not be generated. The side products **7** and **8** had been identified by mass spectra and the 3′-configuration of **10** was determined by nuclear Overhauser effects (^{1}H NOE). Irradiation of the 3′-proton of the deoxyribose moiety gave a significant nuclear Overhauser enhancement of 5.1% at the 2′H$_\beta$-proton, and nearly gave no enhancement at the 4′-proton (0.1%) and at the 2′H$_\alpha$-proton (0.3%, Figure 2).

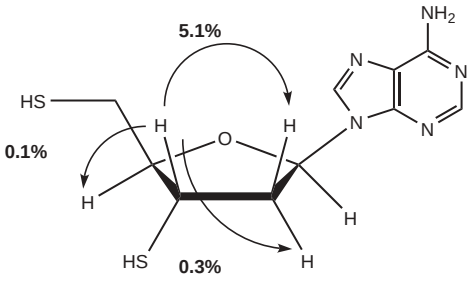
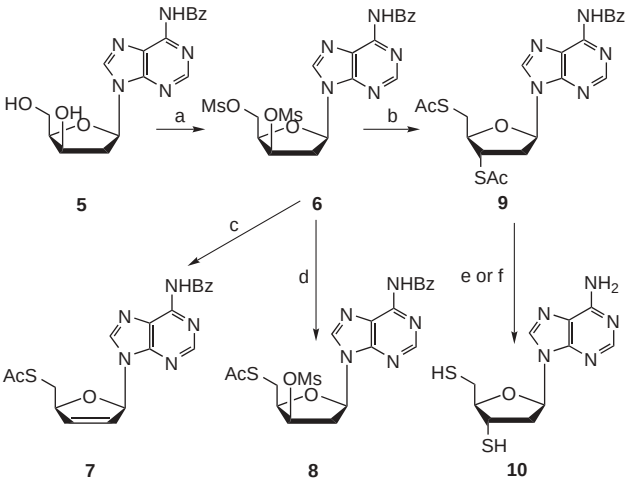


Figure 2 Illustration of nuclear Overhauser enhancements for **10**.

Finally, we synthesized the target compound **10** by two ways (Scheme 2). Initially, we successfully used $LiAlH_4$ as reducing agent to deprotect compound **9** in 4 hours, but the work up was time consume. In another procedure, we treated compound **9** with EtSNa–EtSH under nitrogen atmosphere. The reaction completed in five minutes and all three acyl groups were removed at the same time. Then the mixture was neutralized with acetic acid and exacted with chloroform. After the solution was dried and evaporated, the residual oil was saturated with diethyl ether to precipitate compound **10** in 85% yields. This procedure not only had a good yield, but also avoided the formation of disulfide bonds. We also used EtONa–EtOH instead of EtSNa–EtSH to treat compound **9** and as a result, the compound **9** was converted to some side products that may be a series of disulfide-linked oligonucleosides.

In summary, we have developed an efficient route for the preparation of 3′,5′-dithio-2′-deoxyadenosine (**10**) starting from 2′-deoxyadenosin in five steps in 33% overall yield. Furthermore, this method is also suitable for the preparation of other 3′,5′-dithio-2′-deoxynucleosides. The further work to develop antisense drug and to study the origin of nucleic acid is on progress.



Scheme 2 *Reaction conditions*: (a) MsCl, C_5H_5N, 91%; (b) AcSK, Dioxane, N_2, 60 °C, 36 h, 77%; (c) AcSK, Dioxane, N_2, 70 °C or more; (b) AcSK, Dioxane, N_2, 50 °C, 3 days, 81%; (e) $LiAlH_4$, THF, HCl, N_2, 71%; (f) EtSH, EtSNa, N_2, 85%.

[1]H NMR spectra were obtained on JOEL JNM-ECA600 and JOEL JNM-ECA300 spectrometers. Mass spectra were recorded with a Bruker ESQUIRE-LC ion trap spectrometer equipped with a gas nebulizer probe, capable of analyzing ions up to *m/e* 6000. Dichloromethane was treated for 16 hours with P_2O_5 and distilled; pyridine was refluxed overnight over potassium hydroxide and distilled; methanol was dried by magnesium overnight at refluxing temperature and distilled; 1,4-dioxane was dried by sodium and distilled; THF was freshly distilled under nitrogen after sodium treatment. Thin layer chromatography (TLC) was performed on silica gel F254 and the spots were examined with UV light and sulfuric acid–ethanol spray. All experiments were carried out in dried glassware. The –30 °C and 0 °C external bath temperatures designated are approximate as achieved by a dry ice-acetone or ice baths. Column chromatography was carried out using silica gel (100–200 mesh).

N^6,5′-Dibenzoyl-2′-deoxyadenosine (2)

To a stirred solution of 2′-deoxyadenosine (2.0 g, 8.0 mmol) in pyridine (30 mL) was added dropwise a solution of benzoyl chloride (1.0 mL, 8.8 mol) in pyridine (20 mL) at room temperature in 30 min. To this solution were added in turn chlorotrimethylsilane (5.1 mL, 40 mmol) and benzoyl chloride (2.8 mL, 24 mmol) dropwise. After further stirring for 1.5 h, the resulting mixture was quenched with MeOH (10 mL), and treated with ammoniacal MeOH (sat. at 0 °C, 60 mL) dropwise at 0 °C. The resulting solution was, after stirring for 30 min at r.t., evaporated and the residue was distributed between $CHCl_3$ and H_2O. The syrup obtained by evaporating the organic solution after drying over anhyd $MgSO_4$, was purified by column chromatography (CH_2Cl_2–MeOH 20:1), yielding 2.94 g (6.4 mmol, 80%) of compound **2**. ^{1}H NMR (DMSO-d_6): δ = 11.15 (1 H, s, NH), 8.67 (1 H, s, adenine), 8.61 (1 H, s, adenine), 7.40–8.10 (10 H, m, phenyl), 6.48 (1 H, dd, *J* = 6.5, 6.5 Hz, 1′-H), 5.56 (1 H, d, *J* = 4.1 Hz, 3′-OH), 4.69 (1 H, ddd, *J* = 4.1, 4.1, 6.2 Hz, 4′-H), 4.57 (1 H, dd, *J* = 4.1, 12.0 Hz, 5′-H), 4.46 (1 H, dd, *J* = 6.2, 12.0 Hz, 5″-H), 4.19 (1 H, dddd, *J* = 3.3, 4.1, 4.1, 4.7 Hz, 3′-H), 3.01 (1 H, ddd, *J* = 3.4, 6.5, 12.4 Hz, 2′-H), 2.47 (1 H, ddd, *J* = 4.8, 6.5, 12.4 Hz, 2″-H). ESI-MS: *m/e* = 460 $[M + H]^+$, 240 $[adenine + H]^+$.

N^6-Benzoyl-9-(2′-deoxy-β-D-threo-pentofuranosyl) Adenine (5)

A suspension of 1.84 g (4.0 mmol) of **2** in anhyd CH_2Cl_2 (100 mL) and pyridine (4 mL) was cooled to –30 °C and 10 mL of trifluoromethanesulfonic anhydride (10% vol. in CH_2Cl_2) was added dropwise. The reaction mixture was stirred in air for 30 min till it became complete clear. After addition of H_2O (2 mL), the emulsion was further stirred for 3 h at r.t. Then 10 mL H_2O was added, and the organic layer was separated, dried, evaporated and co-evaporated with toluene to remove pyridine. The residual oil was diluted with 100 mL of anhyd MeOH and 100 mg sodium in MeOH was added. The suspension was stirred for 5 min at r.t., neutralized with HOAc, evaporated, and purified by column chromatography

(CH_2Cl_2–MeOH 9:1),yielding 1.00 g (2.8 mmol, 70%) of compound **5**. ^{1}H NMR (DMSO-d_6): δ = 11.19 (1 H, s, NH), 8.75 (1 H, s, adenine), 8.67 (1 H, s, adenine), 7.50–8.10 (5 H, m, phenyl), 6.45 (1 H, dd, *J* = 7.9, 7.9 Hz, 1′-H), 5.53 (1 H, d, *J* = 4.1 Hz, 3′-OH), 4.71 (1 H, dd, *J* = 5.7, 5.7 Hz, 5′-OH), 4.40 (1 H, ddd, *J* = 2.3, 7.0, 7.0 Hz, 4′-H), 3.99 (1 H, dddd, *J* = 2.3, 4.1, 8.4, 8.4 Hz, 3′-H), 3.75 (1 H, ddd, *J* = 5.7, 7.0, 11.7 Hz, 5′-H), 3.64 (1 H, ddd, *J* = 5.7, 7.0, 11.7 Hz, 5″-H), 2.81 (1 H, ddd, *J* = 7.9, 8.4, 14.4 Hz, 2′-H), 2.37 (1 H, ddd, *J* = 7.9, 8.4, 14.4 Hz, 2″-H). ESI-MS: *m/e* = 356 $[M + H]^+$, 378 $[M + Na]^+$, 240 $[base + H]^+$.

N^6-Benzoyl-9-(3′,5′-di-*O*-methylsulfonyl-2′-deoxy-β-D-threo-pentofuranosyl) Adenine (6)

To a solution of compound **5** (532.5 mg, 1.50 mmol) in 10 mL pyridine was added dropwise a solution of 0.3 mL (3.90 mmol) MsCl in 2.0 mL pyridine at 0 °C. After removal of the cooling bath, the reaction mixture was further stirred for 12 h at r.t. Then 5 mL ice water and 20 mL $CHCl_3$ were added, and the organic layer was separated, dried, evaporated and co-evaporated with toluene to remove pyridine. The residual oil was purified by column chromatography, eluted with EtOAc, yielding 697.5 mg (1.37 mmol, 91%) of compound **6**. ^{1}H NMR (DMSO-d_6): δ = 11.23 (1 H, s, NH), 8.78 (1 H, s, adenine), 8.53 (1 H, s, adenine), 7.50–8.10 (5 H, m, phenyl), 6.58 (1 H, dd, *J* = 3.0, 7.7 Hz, 1′-H), 4.53–4.70 (2 H, m, 3′-H and 4′-H), 4.51 (1 H, dd, *J* = 4.5, 11.3 Hz, 5′-H), 3.80 (1 H, dd, *J* = 5.9, 11.3 Hz, 5″-H), 3.35 (3 H, s, 3′-Me), 3.24 (3 H, s, 5′-Me), 3.14 (1 H, ddd, *J* = 3.0, 7.5, 15.5 Hz, 2′-H), 2.91 (1 H, ddd, *J* = 7.4, 7.8, 15.5 Hz, 2″-H). ESI-MS: *m/e* = 512 $[M + H]^+$, 534 $[M + Na]^+$, 550 $[M + K]^+$, 240 $[base + H]^+$, 262 $[base + Na]^+$.

N^6-Benzoyl–*S*,*S*′-diacetyl-3′,5′-dithio-2′-deoxyadenosine (9)

To a solution of compound **6** (511 mg, 1.00 mmol) in 20 mL 1,4-dioxane was added 456 mg (4.00 mmol) AcSK. Then the suspension was stirred and heated at 60 °C for 36 h under nitrogen. The resulting solution was evaporated and the residue was distributed between $CHCl_3$ and H_2O. The syrup obtained by evaporating the organic solution after drying over anhyd $MgSO_4$, was purified by column chromatography (eluted with EtOAc), yielded 362.7 mg (0.77 mmol, 77%) of compound **9**. ^{1}H NMR ($CDCl_3$): δ = 11.23 (1 H, s, NH), 8.78 (1 H, s, adenine), 8.28 (1 H, s, adenine), 7.50–8.10 (5 H, m, phenyl), 6.36 (1 H, dd, *J* = 3.9, 7.0 Hz, 1′-H), 4.08–4.23 (2 H, m, 3′-H and 4′-H), 3.44 (1 H, dd, *J* = 3.1, 14.0 Hz, 5′-H), 3.27 (1 H, dd, *J* = 6.0, 14.0 Hz, 5″-H), 3.15 (1 H, ddd, *J* = 4.0, 7.3, 14.0 Hz, 2′-H), 2.62 (1 H, ddd, *J* = 7.0, 7.3, 14.0 Hz, 2″-H), 2.40 (3 H, s, 3′-Me), 2.34 (3 H, s, 5′-Me). ESI-MS: *m/e* = 472 $[M + H]^+$, 494 $[M + Na]^+$, 510 $[M + K]^+$, 240 $[base + H]^+$, 262 $[base + Na]^+$.

3′,5′-Dithio-2′-deoxyadenosine (10)

Method A: A suspension of 30.4 mg (0.800 mmol) of $LiAlH_4$ in anhyd THF (5 mL) was cooled to 0 °C and a solution of 47.1 mg (0.100 mmol) compound **9** in 5 mL THF was added dropwise under nitrogen. After the reaction mixture was stirred for 4 h at 0 °C, the resulting solution was treated with 1 N HCl to adjust the pH value of the solution to 4.0. Then 20 mL $CHCl_3$ was added, and the organic layer was separated, dried, and evaporated. The residual oil was purified by column chromatography (CH_2Cl_2–MeOH 20:1) and 20 mg (0.071 mmol, 71%) of compound **10** was yielded.

Method B: To a solution of compound **9** (47.1 mg, 0.100 mmol) in 5 mL EtSH, was added 10 mg (0.120 mmol) EtSNa under nitrogen. The suspension was stirred for 5 min at r.t. and neutralized with HOAc. Then 20 mL EtOAc was added and the organic layer was separated, dried, and evaporated. The residual oil was saturated with 5 mL Et_2O and the precipitate was collected by filtration and washed with cold Et_2O (4 × 1 mL) to give the title compound **10** (24 mg, 0.085 mmol, 85%).^{1}H NMR (DMSO-d_6 containing one drop of D_2O): δ = 8.37 (1 H, s, adenine), 8.21 (1 H, s, adenine), 6.40 (1 H, dd, *J* = 6.5, 6.5 Hz, 1′-H), 4.52 (1 H, ddd, *J* = 3.4, 3.4, 3.4 Hz, 4′-H), 4.04 (1 H, ddd, *J* = 2.8, 3.0, 5.8 Hz, 3′-H), 3.74 (1 H, dd, *J* = 4.1, 12.4 Hz, 5′-H), 3.64 (1 H, dd, *J* = 4.1, 12.4 Hz, 5″-H), 2.73 (1 H, ddd, *J* = 2.8, 6.2, 13.5 Hz, 2′-H), 2.44 (1 H, ddd, *J* = 6.2, 7.5, 13.8 Hz, 2″-H). ESI-MS: *m/e* = 284 $[M + H]^+$, 306 $[M + Na]^+$.

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