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Study on disulfur-backboned nucleic acids: Part IV. Efficient synthesis of 3′,5′-dithio-2′-deoxyguanosine

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Abstract

An efficient and novel method for synthesizing 3′,5′-dithio-2′-deoxyguanosine was described. In this method normal guanosine was used as the starting material. A very efficient procedure was used to synthesize 2́-*O*-tosylguanosine **1**, which used 0.1 eq. DBTO instead of 2 eq. **1** was treated with LTBH to give 9-(2-deoxy-β-D-threo-pentofuranosyl)guanine **2**. **2** could be easily turned to the target compound.

Keywords: 3́,5́-Dithio-2́-deoxyguanosine; Guanosine; Synthesis

The thionucleosides, which have one or more of the sugar hydroxyl replaced by thiol are important monomers [1,2]. They can be used in antisense and RNAi fields. At the same time, the nucleosides with metal binding sites at the 3′- and 5′-positions would be built into single DNA strand with cooperative participation of metal coordination to replace the covalent phosphodiester linkage in natural DNA [3]. Recently we started a project to synthesize a series of disulfur-backbones [4,5] of novel nucleic acids and to apply them to the study of antisense, RNAi, nucleic acids probe and the original of nucleic acid. Our laboratory had found an efficient procedure for synthesizing 3′,5′-dithio-2′-deoxyadenosine [6]. 2′-Deoxyguanosine and 2′-deoxyadenosine have the similar structure. At first we tried to prepare the target compound using the same procedure as 3′,5′-dithio-2́-deoxyadenosine. But 2′-deoxyguanosine was very expensive, meanwhile the yield of 3′-configuration inversion step was very low. Hansske and Robins [7] reported that 2′-*O*-tosyladenosine treated with LTBH then negative ion exchange resin could give 3′-configuration inversion-2′-deoxyadenosine. So we synthesized the target compound from guanosine.

In this paper, we would like to report an efficient synthesis of 3′,5′-dithio-2′-deoxyguanosine starting from guanosine (Scheme 1). Firstly we treated guanosine with DBTO then TsCl to obtain 2′-*O*-tosylguanosine **1**, subsequently **1** was treated with LTBH then negative ion exchange resin to give 9-(2-deoxy-β-D-threo-pentofuranosyl)guanine **2** in 93% yield. In the above step LTBH should be no less than six times of **1**, or else it would cause a side reaction to give guanine (Fig. 1). Then **2** was converted into N^2-benzoyl-9-(2-deoxy-β-D-threo-pentofuranosyl)guanine (**3**) and **3** could be smoothly converted into N^2-benzoyl-9-(3′,5′-di-*O*-methylsulfony-2′-

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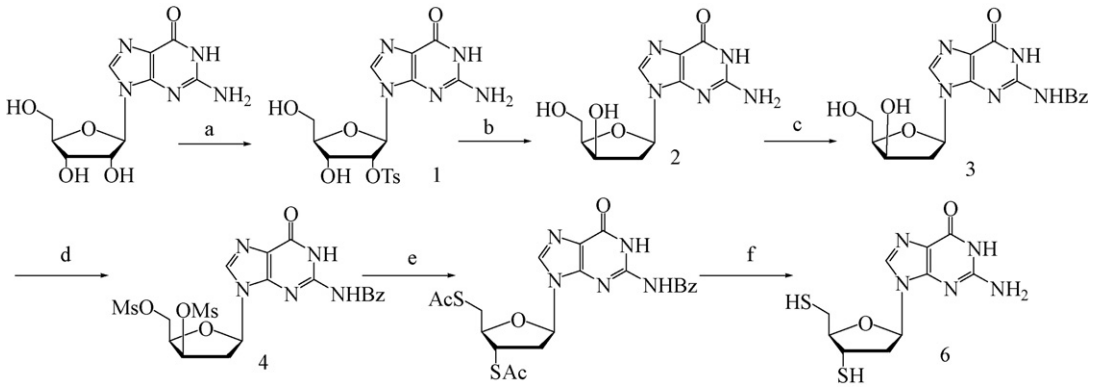

Scheme 1. (a) Bu_2SnO, MeCN, EtN_3, TsCl, (b) $LiEt_3BH$, DMSO, THF, (c) Me_3SiCl, C_5H_5N; BzCl, MeOH, NH_3, MeOH, (d) MsCl, C_5H_5N, (e) AcSK, Dioxane, N_2, 50 °C, 40 h, and (f) EtSH, EtSNa.

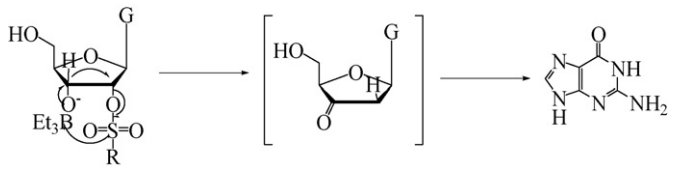

Fig. 1. The side reaction to give guanine.

deoxy-β-D-threo-pentofuranosyl)guanine **4**. We treated **4** with AcSK in anhydrous 1,4-dioxane to give N^2-benzoyl-3′,5′-dithio-3′,5′-di-*S*-acetyl-2′-deoxyguanosine **5**, then **5** was deprotected with EtSNa in EtSH to obtain the target compound **6**, with the total yield of 10%.

1. Experimental

All solvents were freshly distilled. DMSO, MeCN, dioxane and pyridine were dried by standard procedures. All solvents and reagents used can be obtained by commercial sources. TLC analyses were carried out on silica gel F254 and the spots were examined with UV light or by exposure to vaporised iodine. Column chromatography was carried out by using silica gel (100–200 mesh). ^{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*/*z* 6000.

Synthesis of compound **1** [8–11]: A suspension of 28.3 g (100 mmol) of guanosine (dried overnight in vacuo at 80 °C on P_2O_5) and 49.8 g (200 mmol) of dibutyltin oxide in a mixture of 750 mL of anhydrous MeOH and 600 mL of dimethylformamide was stirred for 4 days at room temperature and refluxed for 3 h. The reaction mixture was cooled in an ice bath, and 210 mL of triethylamine and 286 g (1.5 mol) of tosyl chloride were added. After stirring for 15 min, the clear solution was evaporated, coevaporated with pyridine, and dissolved in a mixture of pyridine–acetic anhydride (10:1, 1 L). The reaction mixture was kept overnight at room temperature and 100 mL of MeOH was added. The reaction was stirred for 1 h, evaporated, and diluted with $CHCl_3$ (1 L). The suspension was filtered, and the filtrate was washed with H_2O (3 × 200 mL), dried, and evaporated. The residue was treated with ammonia in methanol (800 mL) overnight at room temperature and evaporated. When the residue was diluted with $CHC1_3$–MeOH (9:1), a crystalline precipitate occurred, which was isolated and washed with MeOH and Et_2O. The mother liquor was further purified by column chromatography ($CHC1_3$–MeOH 85:15 with a little concd. ammonium hydroxide); total yield 26.8 g (61.4 mmol, 61%). m.p. 260–261 °C. ^{1}H NMR (DMSO-d_6): δ 10.66 (s, 1H, NH), 7.70 (s, 1H, 8-CH), 7.46 (d, 2H, J = 7.6 Hz, phenyl), 7.13 (d, 2H, J = 8.3 Hz, phenyl), 6.64 (s, 2H, NH_2), 5.97 (d, 1H, J = 4.8 Hz, OH), 5.85 (d, 1H, J = 7.6 Hz, 1′-H), 5.37 (dd, 1H, J = 5.2, 7.2 Hz, 2′-H), 5.23 (t, 1H, OH), 4.29–4.28 (m, 1H, 3′-H), 3.99–3.98 (m, 1H, 4′-H), 3.61–3.43 (m, 2H, 5′-CH_2), 2.31 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 156.9 (6-C), 153.8 (8-C), 150.7 (5-C),

145.5 (2-C), 136.3 (phenyl), 132.2 (phenyl), 130.1 (phenyl), 127.4 (phenyl), 117.4 (4-C), 87.1 (1′-C), 84.1 (2′-C), 80.0 (4′-C), 70.2 (3′-C), 61.7 (5′-C), 21.6 (CH_3); ESI-MS: *m/z* 438 $(M+H)^+$, 460 $(M+Na)^+$, 152 $(Base+H)^+$.

Synthesis of compound **2**: A solution of 4.37 g (10 mmol) of **1** in 70 mL of anhydrous Me_2SO under dry nitrogen was treated with 100 mL of 1 mmol/L LTBH/THF and the resulting solution was stirred at room temperature overnight. Careful quenching with 5 mL of H_2O was followed by concentration of the solution to syrup in vacuo. The residue was dispersed in 100 mL H_2O and treated with some Dowex 1 × 2(OH^-) resin at room temperature for 1 h and resulting suspension was filtrated. The filtrate was concentrated in vacuo. The residue was purified by column chromatography (CH_2Cl_2–MeOH 5:1 with some ammonia) to give 2.65 g 9-(2-deoxy-β-D-threo-pentofuranosyl)-guanine with yield of 93%. Decompose at 195–198 °C. ^{1}H NMR (DMSO-d_6): δ 7.95 (s, 1H, 8-CH), 6.53 (s, 2H, NH_2), 6.02 (dd, 1H, *J* = 2.0, 8.1 Hz, 1′-H), 4.33–4.30 (m, 1H, 3′-H), 3.90–3.85 (m, 1H, 4′-H), 3.75–3.56 (m, 2H, 5′-H, 5″-H), 2.74–2.65 (m, 1H, 2″-H), 2.18–2.13 (m, 1H, 2′-H); ESI-MS: *m/z* 268 $(M+H)^+$, 290 $(M+Na)^+$, 152 $(Base+H)^+$.

Synthesis of compound **3**: A solution of 231 mg (0.8 mmol) of **2** in 5 mL anhydrous pyridine was treated with 0.38 mL (2.94 mmol) trimethylchlorosilane (TMSCl 98%) and the resulting solution was stirred at room temperature for 1 h, cooled with ice bath to 0 °C. Then 0.12 mL benzoyl chloride (0.99 mmol, 99%) was added dropwise, and after removing the ice bath, the solution was stirred at room temperature for 6 h. Careful quenching with 1 mL H_2O was followed by treating with NH_3–MeOH (2 mL) for 15 min, concentrated the solution to syrup in vacuo. The residue was dispersed in 4 mL ice water to give some solid, then filtrated, washed with ice water and Et_2O to give 291 mg **3** with yield of 98%. m.p. 276–278. ^{1}H NMR (DMSO-d_6): δ 8.27 (s, 1H, 8-CH), 8.04 (d, 2H, *J* = 7.2 Hz, phenyl), 7.69 (t, 1H, *J* = 7.5 Hz, phenyl), 7.57 (t, 2H, *J* = 7.2, 7.5 Hz, phenyl), 6.22 (dd, 1H, *J* = 8.3, 1.7 Hz, 1′-H), 4.39–4.37 (m, 1H, 3′-H), 3.96–3.94 (m, 1H, 4′-H), 3.77–3.59 (m, 2H, 5′-H, 5″-H), 2.80–2.71 (m, 1H, 2″-H), 2.30–2.25 (m, 1H, 2′-H); ESI-MS: *m/z* 394 $(M+Na)^+$, 256 $(Base+H)^+$.

Synthesis of compound **4**: 278 mg (0.75 mmol) of **3** in 5 mL anhydrous pyridine was added dropwise to a solution of 0.15 mL (1.95 mmol) menthane sulfonyl chloride in 1 mL anhydrous pyridine at 0 °C in 30 min. After removing the ice bath the solution was stirred at room temperature for 14 h. Then 3 mL ice water and 10 mL $CHCl_3$ was added, and the organic layer was separated, dried with anhydrous $MgSO_4$ for 30 min, evaporated, and then coevaporated with toluene. The residue was purified by column chromatography (EtOAc) to give 296 mg **4** with yield of 75%. ^{1}H NMR ($CDCl_3$): δ 8.00 (d, 2H, *J* = 7.2 Hz, phenyl), 7.86 (s, 1H, 8-CH), 7.63 (t, 1H, *J* = 7.6 Hz, phenyl), 7.52 (dd, 2H, *J* = 7.2, 7.6 Hz, phenyl), 6.13 (dd, 1H, *J* = 4.1, 7.9, 1′-H), 5.42–5.41 (m, 1H, 3′-H), 4.56–4.50 (m, 2H, 5″-H, 5′-H), 4.47–4.46 (m, 1H, 4′-H), 3.29–3.26 (m, 1H, 2″-H), 3.14 (s, 3H, CH_3), 3.06 (s, 3H, CH_3), 2.95–2.89 (m, 1H, 2′-H); ESI-MS: *m/z* 528 $(M+H)^+$, 550 $(M+Na)^+$, 256 $(Base+H)^+$.

Synthesis of compound **5**: A solution of 264 mg (0.5 mmol) of **4** in 10 mL anhydrous 1,4-dioxane was added to 228 mg (2 mmol) AcSK. The suspension was stirred at 60 °C for 40 h under dry nitrogen, and then evaporated. The residue was distributed between $CHCl_3$ and H_2O. The organic layer was dried with anhydrous for 30 min, and then evaporated to give a syrup. The residue was purified by column chromatography (EtOAc) to give 73 mg **5** with yield of 30%. ESI-MS: *m/z* 488 $(M+H)^+$, 510 $(M+Na)^+$, 256 $(Base+H)^+$.

Synthesis of compound **6**: A solution of 49 mg (0.1 mmol) of **5** in 5 mL EtSH was added to 10 mg (0.12 mmol) EtSNa, the suspension was stirred at room temperature for 5 min under dry nitrogen, then neutralized with HOAc. 20 mL EtOAc was added and the organic layer was separated, dried, and evaporated. The residue 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 24 mg **6** with yield of 80%. ^{1}H NMR (DMSO-d_6 containing one drop of D_2O): δ 7.96 (s, 1H, 8-CH), 6.16 (t, 1H, *J* = 6.5 Hz, 1′-H), 4.40 (ddd, 1H, *J* = 2.8, 4.8, 4.8 Hz, 4′-H), 3.89–3.88 (m, 1H, 3′-H), 3.62 (dd, 1H, *J* = 4.5, 12.0 Hz, 5′-H), 3.55 (dd, 1H, *J* = 4.8, 12.0 Hz, 5″-H), 2.61–2.52 (m, 1H, 3′-H), 2.28 (ddd, 1H, *J* = 3.1, 5.9, 13.1 Hz, 2″-H); ESI-Ms: *m/z* 300 $(M+H)^+$, 322 $(M+Na)^+$.

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[11] (Method b): To stirred a solution of Et_3N (139 mg, 0.75 mmol), guanosine (134 mg, 0.5 mmol) and DBTO (30 mg, 0.1 mmol) in MeCN (dried with P_2O_5, 12 mL) at room temperature for 10 min, then TsCl (115 mg, 0.6 mmol) was added, and the suspension was stirred for about 28 h at the same temperature. The solid materials were collected, then washed with MeCN (dried with P_2O_5, 5 mL) and CH_2Cl_2 (5 mL). The crude product was purified by column chromatography ($CHC1_3$–MeOH 85:15 with a little concd. ammonium hydroxide).