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# STUDY ON DISULFUR-BACKBONED NUCLEIC ACIDS: PART 3. EFFICIENT SYNTHESIS OF 3′,5′-DITHIO-2′-DEOXYURIDINE AND DEOXYCYTIDINE

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□ *A general method is described for synthesizing 3′,5′-dithio-2′-deoxypyrimidine nucleosides* ***6*** *and* ***13*** *from normal 2′-deoxynucleosides. 2,3′-Anhydronucleosides* ***2*** *and* ***9*** *are applied as intermediates in the process to reverse the conformation of 3′-position on sugar rings. The intramolecular rings of 2,3′-anhydrothymidine and uridine are opened by thioacetic acid directly to produce 3′-S-acetyl-3′-thio-2′-deoxynucleosides* ***3*** *or* ***5****. To cytidine, $OH^-$ ion exchange resin was used to open the ring and 2′-deoxycytidine* ***10*** *was obtained in which 3′-OH group is in threo- conformation. The 3′-OH is activated by MsCl, and then substituted by potassium thioacetate to form the S,S′-diacetyl-3′,5′-dithio-2′-deoxycytidine* ***12****. The acetyl groups in 3′,5′ position are removed rapidly by EtSNa in EtSH solution to afford the target molecules* ***6*** *and* ***13****. The differences of synthetic routes between uridine and cytidine are also discusssed.*

**Keyword** 3′; 5′-dithio-2′-deoxyuridine; 3′; 5′-dithio-2′-deoxycytidine; 2; 3′-anhydronucleosides; synthesis

## INTRODUCTION

Since the early 1960s, thiol group has been used to replace the hydroxyl in the sugar ring of normal nucleosides.[1] But the work of both 3′,5′ hydroxyl groups were replaced by thiol was rarely mentioned before, not only because of the hard conformation conversion of 3′-position on the sugar ring, but also the easily formation of S-S linkage between bare thiol groups. In 1996, Reese's group developed an efficient route to synthesize the 3′,5′-dithio-2′-deoxythymidine through 2,3′-anhydrothymidine.[2] And this

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procedure was simplified by Mitsuhiko Shinoya's group in 2002.[3] However, the previous work mainly focused on the synthesis of 3′,5′-dithiothymidine, and the other four kinds of dithionucleosides were almost ignored. Since 2004, our group has started a project to synthesize a series of novel disulfur-backbones nucleic acids, and to apply them to the study of antisense and the synthesis of the metal-mediated DNA strand. In the present research, 3′,5′-dithio-2′-deoxyadenosine[4] has been succeffully synthesized and a novel efficient synthetic route toward 3′,5′-dithiothymidine[5] has also been developed. In this article, we report the synthetic process of 3′,5′-dithio-2′-deoxyuridine and deoxycytidine.

## RESULTS AND DISCUSSION

As uridine has a very similar base moiety with thymidine, the similar synthetic route with thymidine was applied in the research. The synthesis of 5′-O-mesyl-2′-deoxy-2,3′-anhydrouridine was followed the procedure reported by Reese.[2] Then there were two methods for synthesis of S,S′-diacetyl-3′,5′-dithio-2′-deoxyuridine, the first one was to open the intramolecular ring in **2** by treatment with thioacetic acid, which served as both solvent and nucleophilic reagent. The product of 5′-O-mesyl-3′-S-acetylthiouridine was then reacted with potassium thioacetate to form **5**. The second method was the reversed order of two steps.

In our previous report,[5] we found that 5′-O-tosyl-2,3′-anhydrothymidine had a completely different regioselectivity to nucleophilic reagent between basic and acidic conditions. In acidic solution, the 3′-position had a higher activity than 5′-position. When it was treated with nucleophilic reagent, the bond of C3′-O2 prefered to break first, to form the 3′-substituted product; while in basic solution, the activity of 5′-position was higher than 3′, so it lead to the only 5′-substituted product, in which the intramolecular ring was not opened. It seemd that the 5′-O-mesyl-2′-deoxy-2,3′-anhydrouridine had the same property as thymidine. When **2** was reacted with AcSH, it can only produce **3**, the 3′-substitution; when reacted with KSAc, it led to **4**, the 5′-substituted product.

The acetyl groups of **5** can be easily removed by strong base, such as NaOMe in MeOH, MeOH-$NH_3$. But the bare thiol groups can be easily oxidized to form disulfur bond in these conditions. Our group has developed a facile method to deprotect the acetyl groups that can efficiently prevent the oxidization between thiol groups. **5** was treated with EtSNa in the solution of EtSH under $N_2$ atmosphere. The acetyl groups were completely removed in **2** to **3** minutes. Then the mixture was neutralized with 0.1N HCl, and extracted with dichloromethane. After evaporating the solution to dry, the residue was saturated with ethyl ether to precipitate 3′,5′-dithio-2′-deoxyuridine in 85% yield. Under $N_2$ atmosphere and -20°C the solid state of **6** can be stored for three months.

However, the successful route for synthesizing dithiothymidine and uridine is not suitable for the synthesis of dithiocytidine.

The $N^4$-benzoyl-3′,5′-di-O-mesyl-2′-deoxycytidine was readily prepared in two steps.[6] However, the 2,3′-anhydro product cannot be obtained by heating with $Et_3N$ and EtOH. A stronger base DBU had to be used, which has a weak nucleophilic property, to produce **9**. When **9** was treated with refluxing AcSH, the desired 3′-substituted product also cannot be obtained as uridine and thymidine. When the reaction time was elongated to **3** days, the solution became a black-viscous syrup. Some 5′-substituted compound can be found in its ESI-MS detection, but it still had no trace of the formation of 3′-substituted production. Apparently, under AcSH acid condition, the 3′-position of anhydrocytidine had less activity than the 3′-position of anhydrothymidine and uridine, even than its 5′-position.

So the synthetic route had to be changed. Previous report showed that the bond of C3′-O2 in anhydronucleosides can be breaked by $OH^-$ ion exchange resin.[7] **9** was heated with Dowex 1×2, $OH^-$ ion exchange resin on 50°C in the solution of EtOH: $H_2O$ = 1:1 for 5 hours, and **10** was obtained in 75% yield. The threo-conformation of 3′-hydroxyl group was confirmed by NOE $^1$H-NMR spectra. **11** was obtained in a high yield by treatment of **10** with 1.5 mol equiv. of MsCl. Then the 3′,5′-dimesyl groups were readily substituted to thioacetyl groups by reacted with KSAc. With the $SN_2$ nucleophilic substitution, the 3′-S-acetyl group was reversed to a normal conformation.

The deprotection step of **12** was quite similar with **5**. Under $N_2$ atmosphere, the acetyl groups were rapidly removed by adding of the solution of EtSNa in EtSH. However, the benozyl group was unexpectedly resisted to the EtSNa/EtSH system without leaving. Amplified the usage amount of EtSNa to **5** mol equiv., and also elongated the reaction time to 1hr. But he benozyl group was still stable. So it was believed some stonger bases other than EtSNa could remove the Benozyl group.

So many different properties of cytidine caused our strong curiosity. First is the difference in preparation of 2′-deoxy-2,3′-anhydronucleoside. The intramolecular ring of thymidine and uridine can be easily formed by treating with $Et_3N$/EtOH; while to cytidine, there was a stronger base DBU, other than $Et_3N$, can react. A proposed reaction mechanism (Figure 1) accounts for it. In the process of the formation of 2,3′-anhydronucleosides, base was used to deproton of –NH in the base group to form an enolate intermediate. In thymidine and uridine, the –NH groups were connected with two C = O groups, while in cytidine, the –NH group group was bonded with a C = O and a C = N. So the proton's acidity in cytidine was not so strong as that in thymidine and uridine, which needed a stronger base such as DBU to realize the deproton step.

Another difference has the reactive preference between 3′ and 5′ position. It was clearly that anhydrothymidine and uridine had a significant

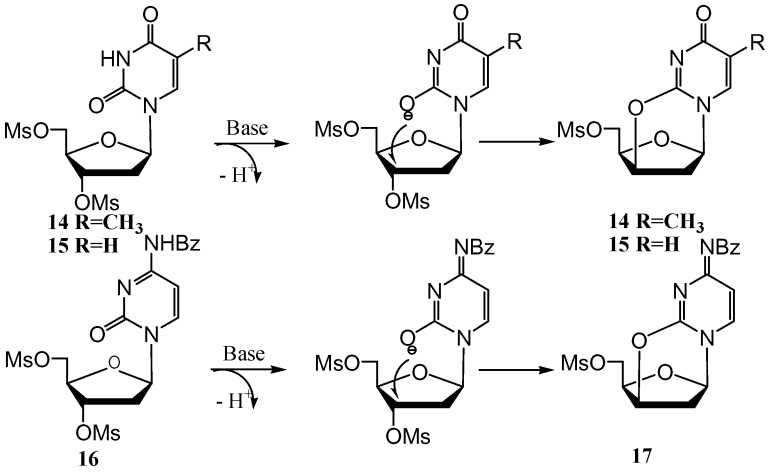



**FIGURE 1** The differences between 2′-deoxy-uridine and cytidine in the process of formating 2,3′-anhydronnucleosides.

regioselectivity to nucleophilic reagents under acidic and basic conditions, which was not seen to anhydrocytidine. Another mechanism was suggested (Figure 2) to try to explain it. Firstly, it was believed that the 5′-position has a stronger reactive activity than the 3′-position. So in basic condition, the nucleophilic reagent prefered to choose the C5′ atom to react. But when in acidic condition, the nitrogen atom in base group prefered to accept a proton to form an aminium cation. In anhydrothymidine and uridine, it was the N3 atom to accept the proton. The positive charge on N3 can be delocalized to O2 by resonance effection, and made a strong electron attraction on C3′, by which the 3′-position was activated with a stronger reactivity than 5′-position. While in anhydrocytidine, it was the N4 to accept the proton. Because of the conjugation influence between N4 = C and C5 = C, the positive charge can be easily delocalized to the base ring, and had less influence with the C3′. So the reactive activity of 3′-position in anhydrocytidine still remained less than that of 5′-position.

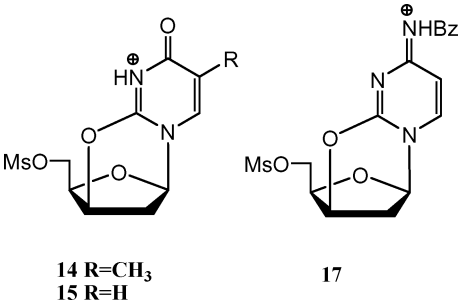
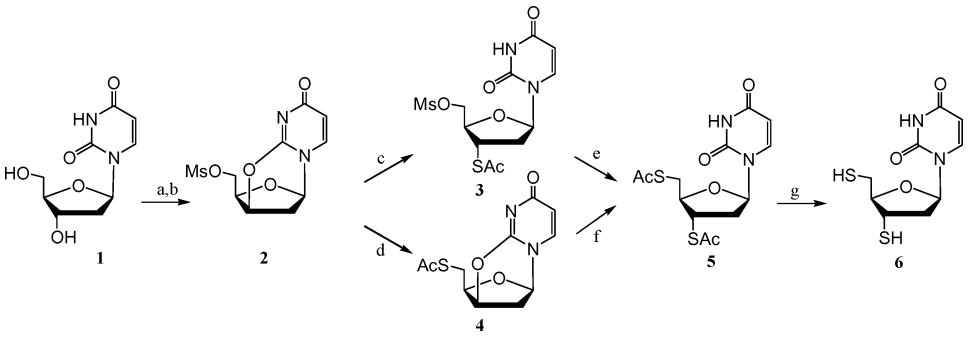


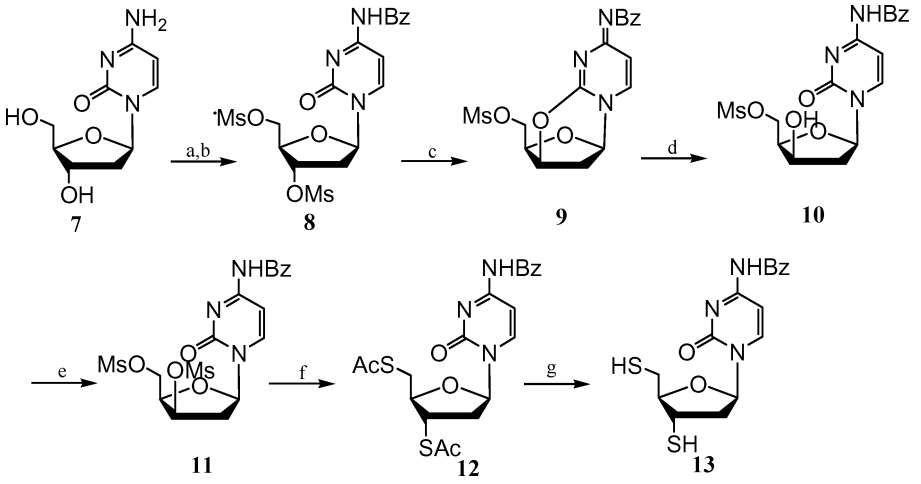

**FIGURE 2** The difference between 2,3′-anhydronuridine and cytidine in the process of protonation.



**SCHEME 1** Synthetic route for 3′,5′-dithio-2′-deoxyuridine. a) MsCl, pyridine, 97%; b) $Et_3N$, EtOH, 92%; c) AcSH, reflux, $N_2$, 77%; d) KSAc, DMF, $N_2$, 73%; e) KSAc, DMF, $N_2$, 70%; f) AcSH, reflux, $N_2$, 76%; g) EtSNa, EtSH, 85%.

## SUMMARY AND CONCLUSIONS

Our work presented an efficient route to synthesize the 3′,5′-dithio-2′-deoxyuridine (Scheme 1) and deoxycytidine (Scheme 2). The use of 2,3′-anhydronucleosides as intermediate molecules provided a general method for 3′ conformation conversion for pyrimidine nucleosides' modification. The regioselectivity of 5′-mesyl-2,3′-anhydrothymidine and anhydrouridine can be applied as a useful synthetic intermediate. By choosing basic or acidic conditions, we can easily make selective substitution on 3′ or 5′ position on sugar rings could easily made. These work should be an important supplement to nucleoside modification. The further work to study the properties of 3′,5′-dithio-2′-deoxynucleosides being carried on.



**SCHEME 2** Synthetic route for 4-benzoyl-3′,5′-dithio-2′-deoxycytidine. a) 1) $Me_3SiCl$, pyridine, BzCl; 2) $H_2O$, $NH_3 \bullet H_2O$, 96%; b) MsCl, pyridine, 92%; c) DBU, $CH_3CN$, 87%; d) Dowex, 1×2, OH ion exchange resin, EtOH/$H_2O$, 75%; e) MsCl, pyridine, 91%; f) KSAc, DMF, $N_2$, 70%; g) EtSNa, EtSH, $N_2$, 80%.

## EXPERIMENTAL

### General

All solvents were freshly distilled. DMF 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). Optical rotation data was obtained on Automatic Polarimeter WZZ-2A. UV$\lambda_{max}$ and $\varepsilon$ data was obtained on Ultrospec 4000 UV/Visible Spectrophotometer. $^1$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. The compound **2**, **8** were prepared by literature procedures.

### 5′-S-Acetyl-2,3′-anhydro-1-(2′-deoxy-$\beta$-D-xylofuranosyl) uracil 4

5′-O-mesyl-2,3′-anhydro-1-(2′-deoxy-$\beta$-D-xylofuranosyl) uracil 2 (576 mg, 2.00 mmol), potassium thioacetate (456 mg, 4 mmol) and dry DMF (25 mL) were stirred together at 50°C for 30 hours. The resulting solution was evaporated in reduced pressure. The residual oil was dispersed by 50 mL $CH_2Cl_2$ and 50 mL $H_2O$. The organic layer was then separated, washed twice by two portions of 30 mL sodium bicarbonate, and then concentrated. The residue was applied to column chromatography, which was eluted with $CH_2Cl_2$-MeOH (98:2 to 90:10 v/v), yielding 390 mg (1.46 mmol, 73%) of compound **4**. $^1$H NMR (300 M Hz, DMSO-d6): $\delta$ 7.11 (d, $J = 7.22$ Hz, 1H, 6-H), 6.02 (d, $J = 7.57$ Hz, 1H, 5-H), 5.52 (d, $J = 3.78$ Hz, 1H, 1′-H), 5.19 (s, 1H, 3′-H), 4.33 (ddd, $J = 2.06, 7.22, 7.22$ Hz, 1H, 4′-H), 3.25 (dd, $J = 7.22, 14.10$ Hz, 1H, 5′-H), 3.03 (dd, $J = 7.22, 14.10$ Hz, 1H, 5′-H), 2.68 (d, $J = 12.73$ Hz, 1H, 2′-H), 2.49 (ddd, $J = 3.44, 2.75, 12.73$ Hz, 1H, 2′-H), 2.36 (s, 3H, Ac-Me). ESI-MS: m/e = 269 $[M + H]^+$, 291 $[M + Na]^+$, 307 $[M + K]^+$.

### S,S′-Diacetyl-3′,5′-dithio-2′-deoxyuridine 5

***Method A***

Compound **4** (270 mg, 1.00 mmol) was dissolved in 20 mL AcSH. The reaction mixture was heated to reflux under nitrogen for 12 hours. The solution was evaporated, extracted by 50 mL $CH_2Cl_2$ and **5** portions of 30 mL sodium bicarbonate. The organic layer was separated, dried, and concentrated. The residual oil was applied to column chromatography (eluted with petrol ether-EtOAc, firstly 80:20, then 30:70 v/v), to give 260 mg (0.76 mmol, 76%) of compound **5**.

***Method B***

5′-O-mesyl-2,3′-anhydro-1-(2′-deoxy-$\beta$-D-xylofuranosyl) uracil 2 (576 mg, 2.00 mmol) was dissolved in 30 ml thioacetate acid under $N_2$ atmosphere. The reaction mixture was stirred, heated to refluxing temperature (100°C) for 15 hours. The extra AcSH was evaporated in reduced pressure. The residue was added 50 mL $CH_2Cl_2$, and then washed 5 times by portions of 30 mL sodium biocarbonate. The organic layer was seperated, dried and concentrated. The residual oil was purified by a fast column chromatography, eluted with petrol ether-EtOAc (firstly 80:20, then 30:70 v/v). Concentration of the appropriate fraction gave crude compound **3** as a yellow solid. (560 mg, approximately in 77% yield). Compound **3** (360 mg, 1.00 mmol), potassium thioacetate (228 mg, 2 mmol) and 20 mL DMF was stirred together at 50°C for 32 hours. The extra solvent was removed in reduced pressure. The residue was dissolved by 50 mL $CH_2Cl_2$ and washed twice with 30 mL sodium bicarbonate. The $CH_2Cl_2$ layer was dried and concentrated, purified by silica column chromatography, which was eluted with $CH_2Cl_2$-methanol (98:2 to 95:5 v/v), yielding 239 mg (0.70 mmol, 70%) of compound **5**. $[\alpha]^{20}_D$ +74.5° (c 1.00, $CH_2Cl_2$). UV($CH_2Cl_2$)$\lambda_{max}$ ($\varepsilon$) 313 nm (368). $^1$H NMR (300 MHz, CDCl3): $\delta$ 9.64 (s, 1H, -NH), 7.51 (d, $J$ = 7.84 Hz, 1H, 6-H), 6.09 (s, 1H, 1′-H), 5.80 (d, $J$ = 7.89 Hz, 1H, 5-H), 4.03 (s, 1H, 3′-H), 3.79 (m, 1H, 4′-H), 3.37 (dd, $J$ = 3.44, 14.45 Hz, 1H, 5′-H), 3.24 (dd, $J$ = 6.19, 14.45 Hz, 1H, 5′-H), 2.46 (m, 2H, 2′-H), 2.37 (s, 3H, Ac-Me), 2.36 (s, 3H, Ac-Me). ESI-MS: m/e = 367 $[M + Na]^+$, 383 $[M + K]^+$.

### S,S′-3′,5′-Dithio-2′-deoxyuridine 6

The reaction system was operated under nitrogen protection. To a stirred solution of compound **5** (103 mg, 0.30 mmol) in 15 mL EtSH was added 50 mg (0.60 mmol) EtSNa. The reaction was taken place at room temperature for 5 minutes. Then 0.1 N HCl solution was added to ajust the pH to neutral. Evaporated the EtSH, extracted with 20 mL $CH_2Cl_2$ and 15 mL $H_2O$. Concentrated the $CH_2Cl_2$ layer, the residual oil was poured into 20 mL cold ethyl ether. Collected the white precipitate, and washed with cold ethyl ether, yielding 66 mg (0.254 mmol, 85%) compound **6**. Compound **6** was soon kept in a case with nitrogen atomosphere at -20°C. $^1$H NMR (300 MHz, DMSO-d6): $\delta$ 7.44 (d, $J$ = 7.23 Hz, 1H, 6-H), $\delta$ 6.13 (d, $J$ = 3.44 Hz, 1H, 1′-H), $\delta$ 5.83 (d, $J$ = 7.22 Hz, 1H, 5-HH), $\delta$ 3.89 (s, 1H, 4′-H), $\delta$ 3.46 (m, 1H, 3′-H), $\delta$ 3.08 (m, 1H, 5′-H), 2.92 (m, 1H, 5′-H), $\delta$ 2.55–2.38 (m, 2H, 2′H). ESI-MS: m/e = 283 $[M + Na]^+$.

### $N^4$-Benzoyl-5′-O-mesyl-2,3′-anhydro-1-(2′-deoxy-$\beta$-D-xylofuranosyl) cytosine 9

To a stirred suspension of $N^4$-benzoyl-3′,5′-di-O-dimesyl-2′-deoxycytidine **8** (2.85 g, 5.82 mmol) in dry $CH_3CN$ (50 mL) was added dropwise a solution

of 1 mL (6.58 mmol) DBU in 5 mL $CH_3CN$ at room temperature Compound **8** was dissolved with adding of the DBU solution. The mixture was stirred for 2 hours. The solution was evaporated. The residual oil was extracted twice with 50 mL sodium bicarbonate and 50 mL $CH_2Cl_2$. The organic layer was dried and evaporated in reduced pressure to give 1.98 g (5.06 mmol, 87%) crude product **9**. A part of crude product was recrystallized in $CH_3CN$ to give pure compound **9** for $^1$H NMR detectation. The main crude product was directly used to prepare compound **10**. $^1$H NMR (300 MHz, DMSO-d6): $\delta$ 7.90 (d, $J = 7.57$ Hz, 2H, phenyl-H), 7.62 (d, $J = 7.22$ Hz, 1H, 6-H), 7.48 (t, $J = 7.57$, 6.88 Hz, 1H, phenyl-H), 7.39 (t, $J = 7.57$, 7.22 Hz, 2H, phenyl-H), 6.41 (d, $J = 7.22$ Hz, 1H, 5-H), 5.94 (d, $J = 3.44$ Hz, 1H, 1′-H), 5.36 (s, 1H, 4′-H), 4.50–4.44 (m, 2H, 3′-H, 5′-H), 4.26–4.19 (dd, $J = 7.57$, 12.04 Hz, 1H, 5′-H), 3.14 (s, 3H, Ms-Me), 2.63 (d, $J = 12.73$ Hz, 1H, 2′-H), 2.53–2.46 (m, 1H, including the peak of DMSO, 2′-H). ESI-MS: m/e = 392 $[M + H]^+$, 414 $[M + Na]^+$, 430 $[M + K]^+$.

### N$^4$-Benzoyl-1-(5′-O-mesyl-2′-deoxy-$\beta$-D-xylofuranosyl) cytosine 10

A solution of **9** (1.50 g, 3.84 mmol) in EtOH-$H_2O$ (1:1 v/v, 100 mL) was heated to 50°C. Ion exchange resin (Dowex 1×2, $OH^-$) 20 g was added, and the mixture was stirred at 50°C for 5 hours. Then the resin was filtered off an washed with $H_2O$ and EtOH, the combined filtrate was concentrated in reduced pressure. The residual oil was applied to column chromatography, eluted with $CH_2Cl_2$-MeOH (95:5 v/v) to give 1.18 g **10** (2.88 mmol, 75%). $^1$H NMR (600 MHz, DMSO-d6): $\delta$ 8.32 (d, $J = 7.56$ Hz, 1H, 6-H), 8.00 (d, $J = 7.56$ Hz, 2H, phenyl-H), 7.63 (t, $J = 7.56$ Hz, 1H, phenyl-H), 7.52 (t, $J = 7.56$ Hz, 2H, phenyl-H), 7.37 (d, $J = 7.56$ Hz, 1H, 5-H), 6.06 (d, $J = 6.87$ Hz, 1H, 1′-H), 4.62–4.60 (dd, $J = 3.44$, 11.00 Hz, 1H, 5′-H), 4.57–4.54 (dd, $J = 8.25$, 11.00 Hz, 1H, 5′-H), 4.35 (t, $J = 4.12$ Hz, 1H, 3′-H), 4.29–4.27 (m, $J = 3.44$, 6.87 Hz, 1H, 4′-H), 3.26 (s, 3H, Ms-Me), 2.65–2.61 (ddd, $J = 4.81$, 8.25, 14.43 Hz, 1H, 2′-H), 2.05 (d, $J = 14.43$ Hz, 1H, 2′-H). ESI-MS: m/e = 410 $[M + H]^+$, 432 $[M + Na]^+$.

### N$^4$-Benzoyl-1-(3′,5′-di-O-mesyl-2′-deoxy-$\beta$-D-xylofuranosyl) cytosine 11

To a stirred suspension of **10** (1.23 g, 3.01 mmol) in dry pyridine (30 mL) was added a solution of 0.3 mL (3.90 mmol) MsCl in 3.0 mL pyridine at 0°C. When compound **10** was disappeared in TLC detection, 5 mL ice-water was added to the mixture. Removed the solvent under reduced pressure. The residual oil was poured into 100 mL ice-water. The precipitate was collected, washed with ice water, yielding 1.32 g (2.74 mmol, 91%) of compound **11**. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.11 (d, $J = 7.57$ Hz, 1H, 6-H), 7.95 (d, $J = 7.22$ Hz, 2H, phenyl-H), 7.70–7.60 (m, 2H, phenyl-H),

7.51 (dd, $J = 7.57$, 7.22 Hz, 2H, phenyl-H), 6.24 (d, $J = 5.85$ Hz, 1H, 1′-H), 5.35 (m, 1H, 3′-H), 4.65–4.63 (m, 2H, 4′-H, 5′-H), 4.55–4.50 (m, 1H, 5′-H), 3.13 (s, 3H, Ms-Me), 3.03 (s, 3H, Ms-Me), 2.99–2.91 (m, 1H, 2′-H), 2.72 (d, $J = 15.82$ Hz, 1H, 2′-H). ESI-MS: m/e = 488 $[M + H]^+$, 510 $[M + Na]^+$.

### $N^4$-Benzoyl-S,S′-diacetyl-3′,5′-dithio-2′-deoxycytidine 12

Compound **11** (1.0 g, 2.05 mmol), potassium thioacetate (935 mg, 8.2 mmol) and 30 mL DMF was stirred together at 50°C under $N_2$ atomosphere for 24 hours. The potassium salt was removed by filtration. The filtrate was evaporated to dry under reduced pressure. The residue was dissolved in 50 mL $CH_2Cl_2$, and was washed twice by 30 mL sodium bicarbonate. The organic layer was dried and concentrated, then was applied to a column chromatography, which was eluted with $CH_2Cl_2$-MeOH (98:2 to 95:5 v/v), yielding 640 mg (1.43 mmol, 69.8%) compound **12**. $[\alpha]^{20}_D$ +36.2° (c 1.00, $CH_2Cl_2$). UV($CH_2Cl_2$)$\lambda_{max}$ ($\varepsilon$) 341 (669), 322 nm (618). $^1$H NMR (300 MHz, CDCl3): $\delta$ 8.88 (s, 1H, -NH), 8.16 (d, $J = 7.57$ Hz, 1H, 6-H), 7.91 (d, $J = 7.57$ Hz, 2H, phenyl-H), 7.61 (t, $J = 7.22$ Hz, 2H, phenyl-H), 7.54–7.49 (m, 2H, 5-H, phenyl-H), 6.08 (dd, $J = 4.13$, 6.54 Hz, 1H, 1′-H), 4.12 (ddd, $J = 4.13$, 6.19, 8.26 Hz, 1H, 4′-H), 3.72 (d, $J = 8.60$ Hz, 1H, 3′-H), 3.41–3.27 (m, $J = 4.13$, 6.54, 14.79 Hz, 2H, 5′-H), 2.68–2.55 (m, 2H, 5′-H), 2.40 (s, 3 H, Ac-Me), 2.36 (s, 3 H, AC-Me). ESI-MS: m/e = 448 $[M + H]^+$, 470 $[M + Na]^+$, 486 $[M + K]^+$.

### $N^4$-Benzoyl-S,S′-3′,5′-dithio-2′-deoxycytidine 13

To a solution of compound **12** (100 mg, 0.224 mmol) in 15 mL EtSH was added 68 mg (0.809 mmol) EtSNa under $N_2$ atmosphere at room temperature The compound **12** disappeared in 2 minutes. by TLC detection. 0.1 N HCl was added dropwise to adjust the pH of the solution to neutral. Evaporated the solvent, and extracted the residue with 15 mL $CH_2Cl_2$ and 15 mL $H_2O$ rapidly. A concentration of the $CH_2Cl_2$ layer and 20 mL cold $Et_2O$ was added. The white sediment was collected to yield 65 mg (0.179 mmol, 80.0%) pure compound **13**. Compound 13 was then kept under nitrogen protection at −20°C. $^1$H NMR (300 MHz, CDCl3): $\delta$ 8.98 (s, 1H, -NH), 8.26 (d, $J = 7.57$ Hz, 1H, 6-H), 7.90 (d, $J = 7.56$ Hz, 2H, phenyl-H), 7.62–7.50 (m, 2H, 5-H, phenyl-H), 7.49 (t, $J = 7.56$ Hz, 2H, phenyl-H), 6.13 (dd, $J = 2.75$, 6.54 Hz, 1H, 1′-H), 4.00–3.95 (m, 1H, 4′-H), 3.25–3.20 (m, 1H, 3′-H), 3.15–2.93 (m, 2H, 5′-H), 2.68–2.50 (m, 2H, 2′-H). ESI-MS: m/e = 364 $[M + H]^+$, 386 $[M + Na]^+$.

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