

3'-叠氮 *D/L*-核苷的合成任 行<sup>a,b</sup> 陶京朝<sup>\*,a</sup> 安浩云<sup>\*,b</sup><sup>(a)</sup> 郑州大学化学与分子工程学院 郑州 450001<sup>(b)</sup> 郑州格然林医药科技有限公司 郑州 450016

**摘要** 报道了两种正交保护的 3'-叠氮-3'-脱氧-*D/L*-核糖关键中间体的合成, 此二关键中间体与各种嘧啶、吡啶、嘌呤相关的杂环碱基进行糖苷化, 得到了相应的 3'-叠氮-3'-脱氧 *D* 核苷衍生物以及 3'-叠氮-3'-脱氧 *L* 核苷衍生物. 合成了药物相关的 3'-叠氮-3'-脱氧-6-氮杂尿苷、4-脱氧尿苷、2-硫代尿苷、3-去氮尿苷、硝基吡啶核苷、异胞苷衍生物. 31 个最终产品中的 14 个是新化合物, 其结构均得以确证. 所有最终产品都是用本文描述的平行法从相应的新关键中间体合成而得. 此二关键中间体可以用于合成含有任何不同碱基的新型核苷衍生物.

**关键词** 叠氮核苷; *L*-核苷; *D*-核苷; 合成; 糖苷化

Synthesis of 3'-Azido-*D/L*-nucleosidesRen, Hang<sup>a,b</sup> Tao, Jingchao<sup>\*,a</sup> An, Haoyun<sup>\*,b</sup><sup>(a)</sup> College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou 450001<sup>(b)</sup> Zhengzhou Granlen PharmaTech, Ltd., Zhengzhou 450016

**Abstract** Two orthogonally protected 3'-azido-3'-deoxy-*D/L*-ribosides were synthesized and successfully glycosylated with various pyrimidine, pyridine and purine related heterocyclic bases. 3'-Azido-3'-deoxy-*D*-pyrimidine nucleosides, purine nucleosides as well as 3'-azido-3'-deoxy-*L*-nucleosides were synthesized. 3'-Azido-3'-deoxy-6-azauridine, 4-deoxyuridine, 2-thiouridine, 3-deazauridine, nitropyridinone and isocytidine derivatives were also synthesized as the drug analogues to explore their biological properties. 14 final products are novel and well characterized, while all of the 31 final products were synthesized by the new strategy from the corresponding novel key intermediates. The two key intermediates can be utilized to make all kinds of novel nucleoside derivatives by glycosylating with various heterocyclic bases.

**Keywords** azido-nucleoside; *L*-nucleoside; *D*-nucleoside; synthesis; glycosylation

## 1 Introduction

A number of nucleoside anticancer and antiviral drugs play an important role in human health.<sup>[1]</sup> Azido-modified nucleosides, such as zidovudine, are efficient reverse transcriptase (RT) inhibitors and have been used as efficient antiviral agents.<sup>[2]</sup> A recently discovered azido-nucleoside drug, azvudine,<sup>[3]</sup> is in clinical studies for the treatment of human immunodeficiency virus (HIV) and demonstrates activity against other viruses. Therefore, more azido-modified nucleoside drugs shall be identified to address long running unmet medical needs. Due to synthetic challenges of azido-modified nucleosides, the true scope of their biological activity has not been thoroughly investigated.<sup>[4]</sup> In addition, these azido group-containing molecules and

their corresponding amino group-containing derivatives can be efficiently utilized for further conjugation, and in return, the conjugated molecules<sup>[5]</sup> can be used to accelerate anti-sense, small RNA and genomic related technologies and further advance of modern diagnostic application.

To further expand the application of small molecule and genomic drug discovery, it is essential to explore the synthesis of azido-modified nucleosides. Herein, we report the efficient synthesis of 3'-azido-modified *D*-nucleosides (compounds **1**~**24**, Figure 1) and *L*-nucleosides (compounds **25**~**31**, Figure 1). To avoid tedious synthesis starting from nucleosides, we utilized readily available *D*- and *L*-azido-modified ribosides **32** and **43** as starting materials.

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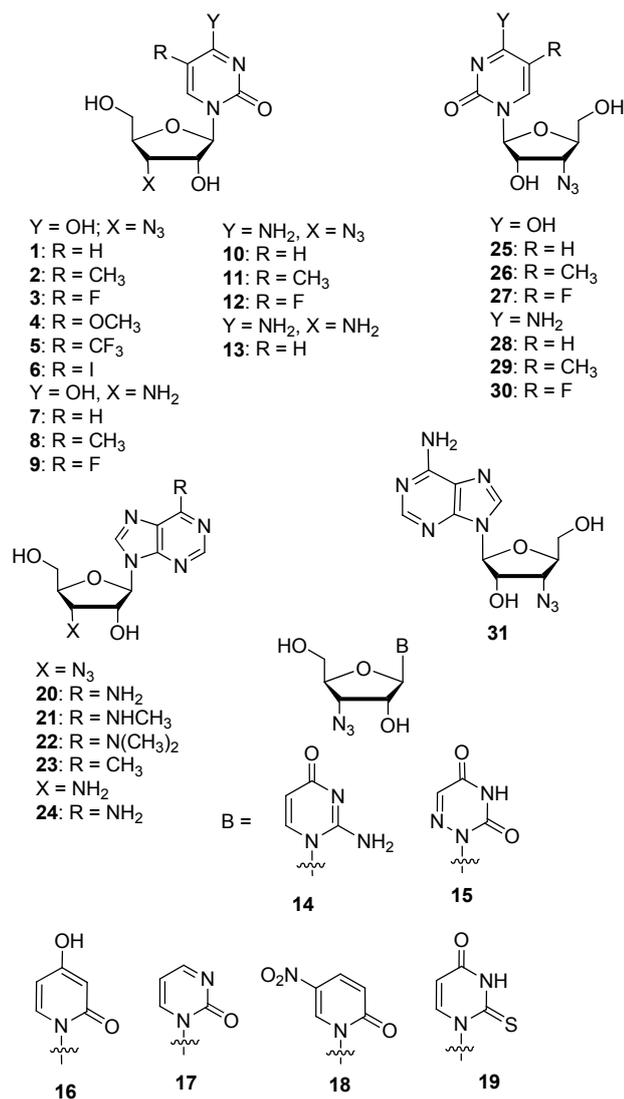
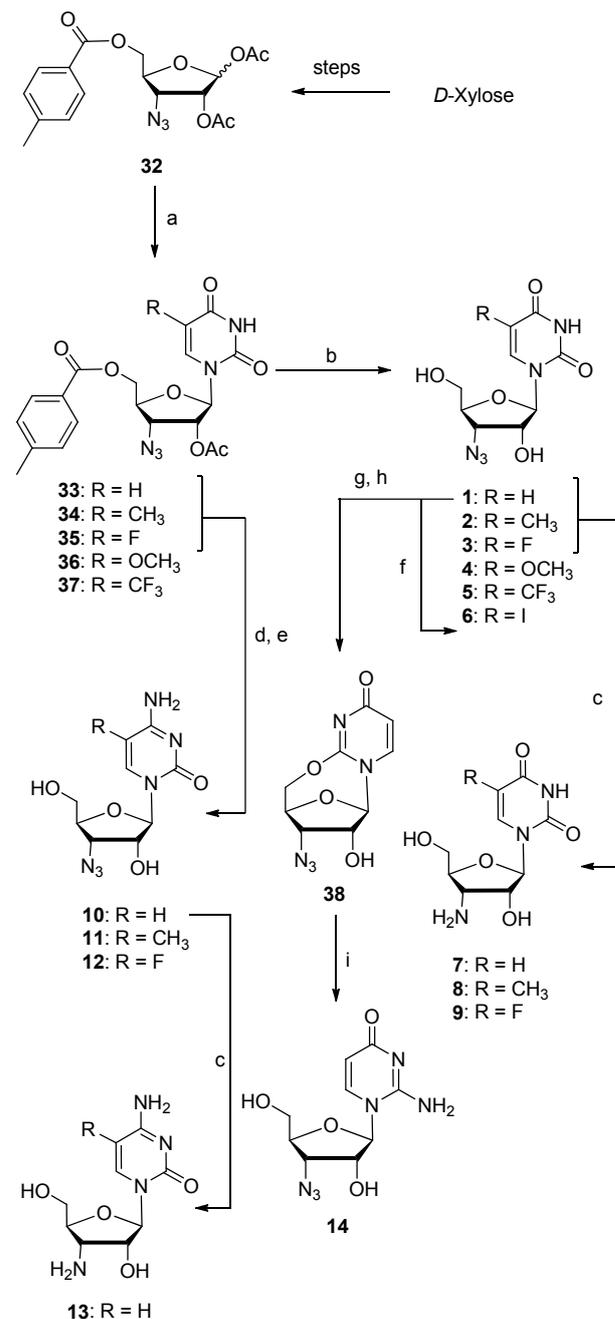


Figure 1 3'-Azido-3'-deoxy-D/L-nucleosides

## 2 Results and discussion

Some 3-azido- and amino-nucleosides were synthesized by traditional methods starting from nucleosides.<sup>[6]</sup> The methods do not allow the late-stage functionalization for the synthesis of a structurally diverse nucleoside library. Therefore, we decided to utilize a well-protected 3'-azido-3'-deoxyribose which can be glycosylated with any heterocyclic base, enabling the synthesis of all possible azido-nucleoside derivatives. The reported literature for the synthesis of benzoyl and acetyl protected 3'-azido-3'-deoxyribose suffer from the formation of the elimination side product in over 40% in the azido nucleophilic substitution step.<sup>[6,7]</sup> We constructed 1,2-di-*O*-acetyl-5-*O*-(4-methylbenzoyl)-*D*-ribose (**32**) (Scheme 1) in large scale from *D*-(+)-xylose with less than 20% eliminated side product in the key step. The developed process for the synthesis of fully-protected 3'-azido-ribose **32** was also adopted for the synthesis of new 1,2-di-*O*-acetyl-5-*O*-(4-methylbenzoyl)-*L*-ribose (**43**). With these two orthogonally protected

ribose in hands, we were able to synthesize various new azido-modified nucleosides.



Reagents and conditions: (a) Base, BSA, CH<sub>3</sub>CN, r.t.; TMSOTf, r.t.; (b) NH<sub>3</sub>/MeOH, r.t.; (c) Pd/C, H<sub>2</sub>; (d) POCl<sub>3</sub>, 1,2,4-triazole, Et<sub>3</sub>N, r.t.; (e) NH<sub>3</sub>/MeOH, r.t.; (f) ICl, NaN<sub>3</sub>, CH<sub>3</sub>CN; (g) TsCl, pyridine; (h) DBU, CH<sub>3</sub>CN, reflux; (i) NH<sub>4</sub>OH, r.t.

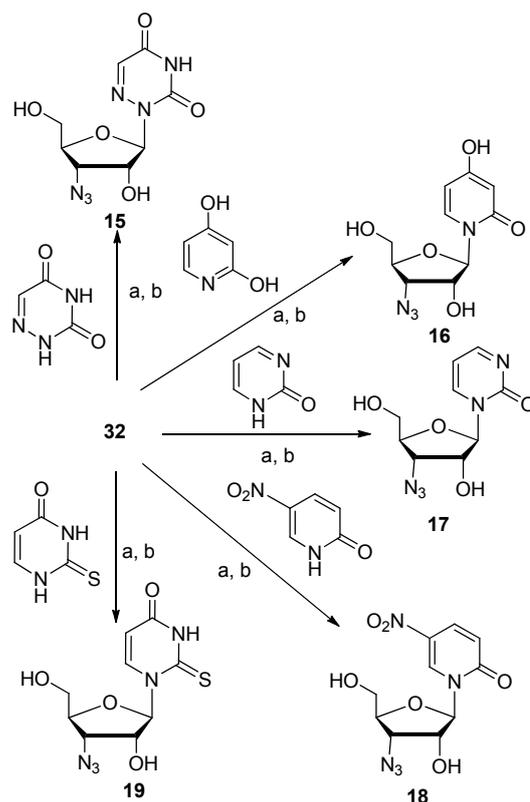
**Scheme 1** Synthesis of 3'-azido-3'-deoxy-*D*-pyrimidine nucleosides **1**~**14**

The orthogonally protected 3'-azido-3'-deoxyribose **32** was glycosylated with uracil, 5-methyluracil, 4-fluorouracil, 5-methoxyuracil and 5-trifluoromethyl uracil under TMS-Tf conditions to provide the corresponding protected nucleosides **33**~**37**. They were deprotected under saturated ammonia solution in methanol to give the desired uridine

and 5-substituted uridine derivatives **1**~**5**. 3'-Azido-3'-deoxyuridine (**1**) was iodinated, resulting in the formation of 5-iodouridine derivative **6**. Compounds **1**~**3** were hydrogenated with Pd/C as the catalyst to generate desired 3'-amino-3'-deoxyuridine derivatives **7**~**9**. The protected key intermediates **33**~**35** (R=H, CH<sub>3</sub> and F) were converted to the corresponding cytidine derivatives **10**~**12** utilizing POCl<sub>3</sub>/triazole method.<sup>[8]</sup> The 3'-azido-3'-deoxycytidine (**10**) was hydrogenated to 3'-amino-3'-deoxycytidine (**13**). 3'-Azido-3'-deoxyuridine (**1**) reacted with *p*-methylphenylsulfonyl chloride to generate the 5'-tosylate, which was cyclized with the oxygen atom at the 2-position on the uracil ring to form 2,5'-anhydro-uridine **38**. Uridine **38** was then treated with ammonium hydroxide to complete the synthesis of 3'-azido-3'-deoxy-isocytidine (**14**).

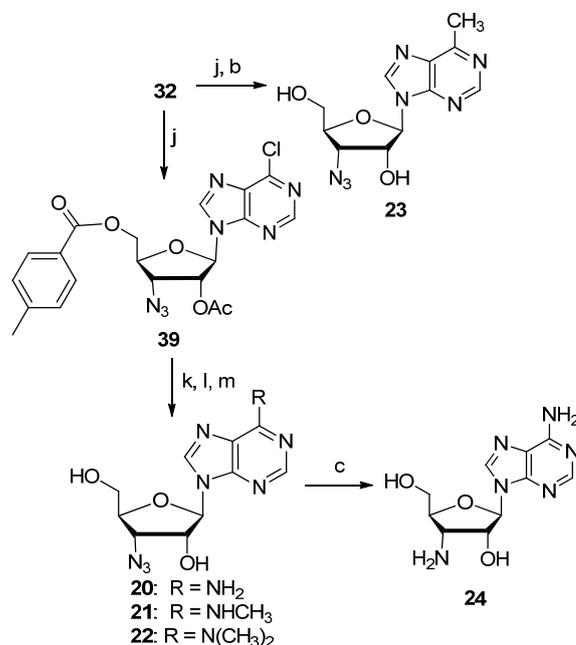
6-Azauridine and 2',3',5'-tri-*O*-acetyl-6-azauridine, azaribine, are all antimetabolic antineoplastic agents.<sup>[9]</sup> The synthase inhibitor 3-deazauridine<sup>[10]</sup> and the DNA methylation inhibitor 4-deoxyuridine<sup>[11]</sup> have been used as anti-cancer drugs. 2-Thiouridine and 5-nitropyridone ribofuranosyl nucleosides are also biologically relevant molecules. In order to explore the biological properties of these drugs with substitution at the 3'-position with an azide, we synthesized the 3'-azido-3'-deoxy derivatives of these drugs. The orthogonally protected azido-ribose **32** was glycosylated with 6-azauracil, and the resulting compound was deprotected with saturated ammonia in methanol to furnish 3'-azido-modified 6-azauridine analogue **15**. Riboside **32** was glycosylated with 2,6-dihydropyridine and 2-hydroxypyrimidine. The resulting protected nucleoside intermediates were deprotected to produce 3'-azido-3'-deoxy-3-deazauridine (**16**) and 3'-azido-3'-deoxy-4-deoxyuridine (**17**), which are the 3'-azido-modified derivatives of 3-deazaurine and 4-deoxyuridine drugs. The same glycosylation and deprotection strategy were used for the synthesis of compounds **18** and **19** where riboside **32** was glycosylated with 5-nitro-pyridin-2-one and 2-thiouracil (Scheme 2).

3'-Azido-3'-deoxyadenosine (**20**) and 3'-amino-3'-deoxyadenosines (**24**) were previously synthesized from adenosine or xylo-adenosine<sup>[4]</sup> using a long synthetic route that required functional groups manipulation and hydroxyl group  $\alpha/\beta$ -conversion. In addition, this strategy does not enable late-stage functionalization to install various heterocycles. Robins and co-workers<sup>[12]</sup> also converted 3'-azido-adenosine to 6-dialkylsubstituted derivatives by the triazole strategy which could be much easier if chloro- is at the 6-position of such adenosine analogue, such as intermediate **39** (Scheme 3). We glycosylated riboside **32** with 6-methylpurine, and the resulted compound was deprotected with saturated ammonia in methanol resulting in the desired 3'-azido-3'-deoxy-6-methylpurine nucleoside **23** which is the derivative of natural product 6-methylpurine riboside. Riboside **32** was also glycosylated with 6-chloro-adenine resulting a versatile intermediate **39**, which can be utilized to make all kinds of 6-modified purine nucleoside derivatives.



Reagents and conditions: (a) Base, BSA, CH<sub>3</sub>CN, r.t.; TMSOTf, r.t.; (b) NH<sub>3</sub>/MeOH, r.t.

**Scheme 2** Synthesis of 3'-azido-3'-deoxy-*D*-nucleosides **15**~**19** with special heterocyclic bases



Reagents and conditions: (b) NH<sub>3</sub>/MeOH, r.t.; (c) Pd/C, H<sub>2</sub>; (j) Base, DBU, TMSOTf, r.t. ~ 60 °C; (k) NH<sub>3</sub>/MeOH, 80 °C (to **20**); (l) NH<sub>2</sub>Me/MeOH, 80 °C (to **21**); (m) NHMe<sub>2</sub>/MeOH, 100 °C (to **22**)

**Scheme 3** Synthesis of 3'-azido-3'-deoxy-*D*-purine nucleosides **20**~**24**

The key intermediate **39** was synthesized and treated with ammonia resulting in desired compound **20**. Compound **39** was also treated with methylamine and *N,N*-dimethylamine. The chloro at position 6 was replaced and the protecting groups were removed at the same time to furnish the synthesis of *N*<sup>6</sup>-methylated compounds **21** and **22**. Hydrogenation of compound **20** gave 3'-amino-3'-deoxyadenosine **24**. Compounds **20** and **24** were synthesized by Kim and co-workers<sup>[13]</sup> with long route and OH conversion starting from adenosine which can only be used for the synthesis of this compound. Therefore, the key intermediate **39** can be used as a versatile scaffold to make all kinds of 6-*N*-substituted and C-substituted adenosine derivatives by nucleophilic substitution and various C—C bond formation reactions. In addition, the amino and azido groups on the derivatives can be used for various conjugation.

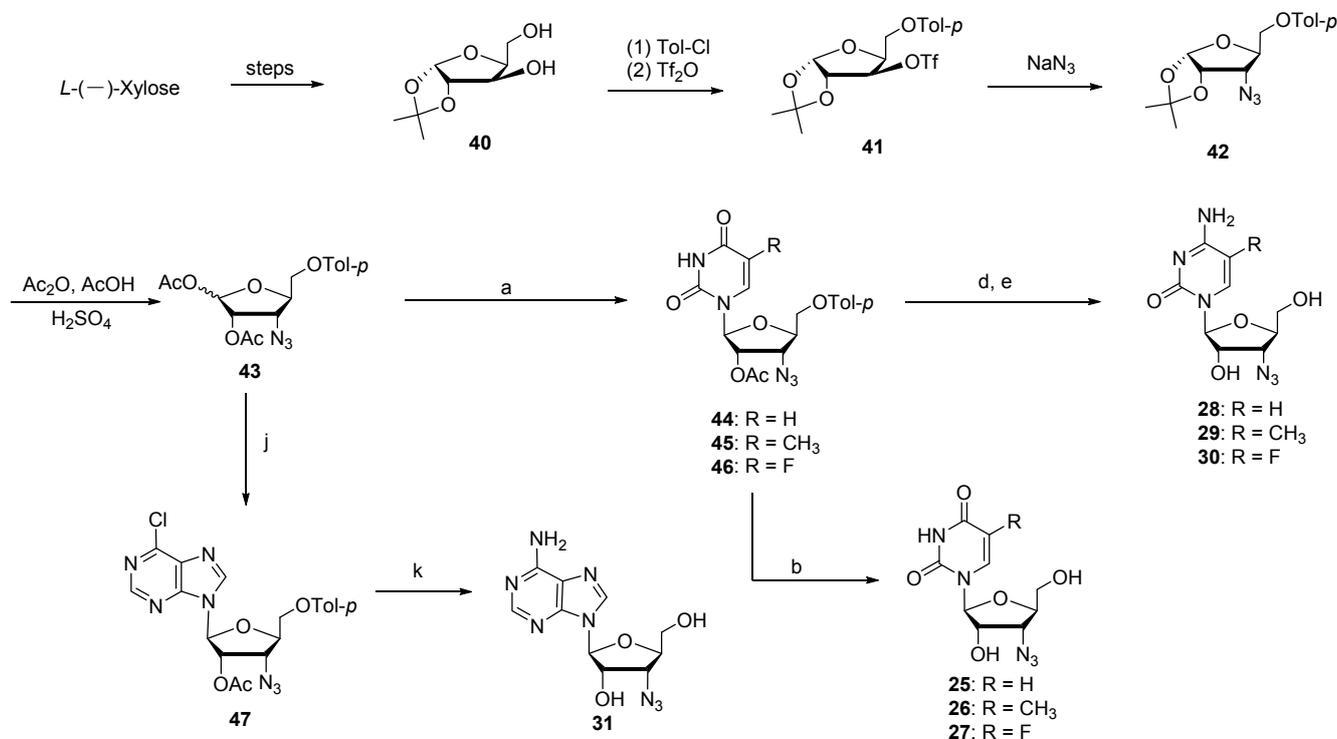
*L*-Nucleosides demonstrate special biological property and have different application compared to the normal *D*-nucleosides.<sup>[14]</sup> With the developed protocol for the synthesis of the protected 3'-azido-3'-deoxyribose **32** ready at Granlen, we transferred the strategy to the synthesis of new 1,2-di-*O*-acetyl-5-*O*-(4-methylbenzoyl)-*L*-ribose (**43**) (Scheme 4). 1,2-*O*-Isopropylidene- $\alpha$ -*L*-xylofuranose (**40**) was synthesized from *L*-(-)-xylose according to the reported protocol.<sup>[15]</sup> Compound **40** was selectively protected and then activated with Tf<sub>2</sub>O giving the active triflate intermediate **41**. Further nucleophilic substitution with sodium azide resulted in the 3'-azido-*L*-ribose **42**. Further treatment with acetic anhydride under acidic condition by

our routine protocol furnished the synthesis of 3'-azido-3'-deoxy-*L*-ribose **43**.

The key intermediate 3'-azido-3'-deoxy-*L*-ribose **43** was glycosylated with uracil, 5-methyluracil and 5-fluoro-uracil. The resulting intermediates **44**~**46** were deprotected with ammonia to give the desired 3'-azido-3'-deoxy-*L*-uridine derivatives **25**~**27**. The key intermediates **44**~**46** were reacted with POCl<sub>3</sub>/triazole, followed by the treatment with ammonia, giving the desired 3'-azido-3'-deoxy-*L*-cytidine derivatives **28**~**30**, respectively. *L*-Riboside **43** was glycosylated with 6-chloropurine, and the key intermediate **47** was obtained. It was further treated with ammonia giving the desired 3'-azido-3'-deoxy-*L*-adenosine (**31**). Therefore, our parallel-last strategy just stated starting from the versatile *D*- and *L*-ribosides **32** and **43** can be utilized broadly to make a variety of *L*-nucleosides with all kinds of heterocyclic bases, and more derivatives can be made from the key intermediates (Scheme 4).

### 3 Conclusions

In conclusion, we have developed the protocol and synthesized the fully-protected 3'-azido-3'-deoxy-*D*-ribose **32** in large scale, and synthesized new protected 3'-azido-3'-deoxy-*L*-ribose **43**. These *D*- and *L*-ribose key intermediates were successfully glycosylated with various substituted uracil, adenine, 6-azauracil, 2-thiouracil, pyrimidone, and pyridinone related heterocyclic bases in a



Reagents and conditions: (a) Bases, BSA, CH<sub>3</sub>CN, r.t.; TMSOTf, r.t.; (b) NH<sub>3</sub>/MeOH, r.t.; (d) POCl<sub>3</sub>, 1,2,4-triazole, Et<sub>3</sub>N, r.t.; (e) NH<sub>3</sub>/MeOH, r.t.; (j) Bases, DBU, TMSOTf, r.t.~60 °C; (k) NH<sub>3</sub>/MeOH, 80 °C.

**Scheme 4** Synthesis of 3'-azido-3'-deoxy-*L*-ribose **43** and nucleosides **25**~**31**

parallel fashion for the synthesis of the diversified nucleoside derivatives after deprotection and simple functional group manipulation. 3'-Azido-3'-deoxy-*D*-nucleoside derivatives **1**~**6**, **10**~**12** and **14**~**23** and *L*-nucleoside derivatives **25**~**31** were obtained and ready for further biological and genomic studies. The key riboside intermediates **32** and **43** reported here can be utilized further for the synthesis of various novel nucleoside derivatives with other heterocyclic bases to expand further research in the related fields.

## 4 Experimental

### 4.1 Instrument and reagent

Starting materials and reagents were obtained from commercial suppliers and were used without further purification unless otherwise stated. <sup>1</sup>H NMR spectra were obtained with a Bruker Avance 400 spectrometer using DMSO-*d*<sub>6</sub> downfield with respect to an internal  $\delta$  as solvents. Chemical shifts are reported as standard of tetramethylsilane (TMS). Product purity was tested by an Agilent 1260 analytical HPLC system. LC-MS spectra were measured on an Agilent 6120 LC-MS spectrometer. TLC was performed on silica gel GF254. Flash chromatography was performed on silica gel 200~300 mesh (Yantai Silica Gel Co. LTD). 1,2-Di-*O*-acetyl-5-*O*-(4-methylbenzoyl)-*D*-ribose (**32**) was prepared from *D*-xylose by adopting literature strategy with modification and different protecting scheme.<sup>[7]</sup> 6-Methylpurine was prepared from 6-chloropurine according to procedures described in the literature.<sup>[16]</sup>

### 4.2 Experimental procedures

4.2.1 General procedure for the glycosylation of uracil, 5-methyluracil, 4-fluorouracil, 5-methoxyuracil, and 5-trifluoromethyluracil with 1,2-di-*O*-acetyl-5-*O*-(4-methylbenzoyl)ribose (**32**)

To a suspension of uracil, 5-methyluracil, 4-fluorouracil, 5-methoxyuracil or 5-trifluoromethyluracil (9.6 mmol, 1.2 equiv.) in anhydrous acetonitrile (100 mL) was added *N,O*-bis(trimethylsilyl)acetamide (7.8 mL, 31.8 mmol, 4.0 equiv.) dropwise with stirring. The mixture was stirred for 1 h at room temperature. Then 1,2-di-*O*-acetyl-5-*O*-(4-methylbenzoyl)ribose (**32**, 3.0 g, 7.95 mmol, 1.0 equiv.) was added, and the reaction mixture was cooled to 0 °C followed by addition of trimethylsilyl triflate (4.30 mL, 23.85 mmol, 3.0 equiv.). The mixture was stirred overnight at room temperature. Upon completion of the reaction as monitored by thin-layer chromatography (TLC), the reaction mixture was poured into ice water and then treated with a mixture of saturated sodium bicarbonate and ethyl acetate. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate. The combined organic phases were dried over anhydrous sodium sulfate. The drying agent was filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column using petroleum ether-ethyl acetate as eluents providing products **33**~

**37** as white solids in 82%~93% yields.

4.2.2 General procedure for the conversion of compounds **33**~**37** to compounds **1**~**5**

The obtained compounds **33**~**37** were dissolved in NH<sub>3</sub>-MeOH, and the reaction mixture was allowed to stir at room temperature overnight. Upon completion of the reaction as monitored by TLC, the solution was concentrated in vacuum under reduced pressure, and the residue was purified on a silica gel column resulting in compounds **1**~**5** in 78%~95% yields as white solids.

3'-Azido-3'-deoxyuridine (**1**): gray solid, 76% yield in two steps with an HPLC purity of 98.7%.  $R_f=0.4$  (dichloromethane/methanol,  $V:V=10:1$ ). m.p. 130~135 °C (dec.); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$ : 3.48~3.70 (m, 2H, CH<sub>2</sub>), 3.80~4.10 (m, 2H, 2',3'-H), 4.42 (m, 1H, 4'-H), 5.28 (br, 1H, 5'-OH), 5.66 (d,  $J=8.0$  Hz, 1H, ArH), 5.76 (d,  $J=5.2$  Hz, 1H, 2'-OH), 6.17 (s, 1H, 1'-H), 7.87 (d,  $J=8.4$  Hz, 1H, ArH), 11.29 (s, 1H, NH); ESI-MS  $m/z$ : 269.9 [M+H]<sup>+</sup>, 291.9 [M+Na]<sup>+</sup>.

3'-Azido-3'-deoxy-5-methyl uridine (**2**):<sup>[17]</sup> light yellow foam, 77% yield in two steps with an HPLC purity of 99.8%.  $R_f=0.6$  (dichloromethane/methanol,  $V:V=10:1$ ). ESI-MS  $m/z$ : 284.0 [M+H]<sup>+</sup>, 305.9 [M+Na]<sup>+</sup>.

3'-Azido-3'-deoxy-5-fluorouridine (**3**): light yellow foam, 73% yield in two steps with an HPLC purity of 97.7%.  $R_f=0.2$  (petroleum ether-ethyl acetate,  $V:V=1:1$ ). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$ : 3.55~3.75 (m, 2H, CH<sub>2</sub>), 3.91~4.07 (m, 2H, 3,4'-H), 4.37~4.45 (m, 1H, 2'-H), 5.42 (br, 1H, 5'-OH), 5.70~5.75 (m, 1H, 2'-OH), 6.18 (d,  $J=5.2$  Hz, 1H, 1'-H), 8.27 (d,  $J=7.2$  Hz, 1H, ArH), 11.62 (s, 1H, NH); ESI-MS  $m/z$ : 287.8 [M+H]<sup>+</sup>.

3'-Azido-3'-deoxy-5-methoxy uridine (**4**): light yellow solid, 72% yield over two steps with an HPLC purity of 95.7%.  $R_f=0.7$  (dichloromethane/methanol,  $V:V=10:1$ ). m.p. 150~155 °C (dec.); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$ : 3.56~3.64 (m, 4H, CH<sub>3</sub>, CH), 3.66~3.78 (m, 1H, CH), 3.90~3.98 (m, 1H, 4'-H), 4.08 (t,  $J=5.2$  Hz, 1H, 3'-H), 4.42~4.52 (m, 1H, 2'-H), 5.42 (t,  $J=4.4$  Hz, 1H, 5'-OH), 5.79 (d,  $J=4.8$  Hz, 1H, 2'-OH), 6.14 (d,  $J=5.6$  Hz, 1H, 1'-H), 7.61 (s, 1H, ArH), 11.48 (s, 1H, NH); ESI-MS  $m/z$ : 299.8 [M+H]<sup>+</sup>, 322.7 [M+Na]<sup>+</sup>; HRMS calcd for C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>NaO<sub>6</sub> [M+Na]<sup>+</sup> 322.0764, found 322.0761.

3'-Azido-3'-deoxy-5-trifluoro methyluridine (**5**): yellow foam, 65% yield over two steps with an HPLC purity of 95.8%.  $R_f=0.4$  (dichloromethane/methanol,  $V:V=15:1$ ). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$ : 3.50~3.85 (m, 2H, CH<sub>2</sub>), 3.90~4.10 (m, 2H, 4'-H, 3'-H), 4.40~4.50 (m, 1H, 2'-H), 5.53 (t,  $J=4.4$  Hz, 1H, 5'-OH), 5.69 (d,  $J=2.4$  Hz, 1H, 2'-OH), 6.28 (d,  $J=4.8$  Hz, 1H, 1'-H), 8.83 (s, 1H, ArH), 11.41 (s, 1H, NH); ESI-MS  $m/z$ : 359.7 [M+Na]<sup>+</sup>.

4.2.3 Synthesis of 3'-azido-3'-deoxy-5-iodouridine (**6**)

Iodine monochloride (2.4 g, 14.8 mmol, 2.0 equiv.) was added to a suspension of sodium azide (1.4 g, 22.2 mmol, 3.0 equiv.) in acetonitrile (50 mL) at ice-bath temperature with stirring. This mixture was stirred for another 5 min, and a solution of 3'-azido-3'-deoxyuridine **1** (2.0 g, 7.4

mmol) in acetonitrile (60 mL) was then added. The resulting reaction mixture was warmed to 25 °C and stirred for 24 h. Upon completion of the reaction as monitored by TLC, solvent was evaporated and the crude product was purified by flash chromatography on a silica gel column using dichloromethane-methanol as eluent to afford 1.5 g compound **6** as a white solid in 70% yield with an HPLC purity of 97.2%.  $R_f=0.4$  (dichloromethane/methanol,  $V:V=10:1$ ). m.p. 170~175 °C (dec.); UV-vis (MeOH)  $\lambda_{\max}$ : 285 nm;  $^1\text{H NMR}$  (DMSO- $d_6$ , 400 MHz)  $\delta$ : 3.55~3.75 (m, 2H, CH<sub>2</sub>), 3.92~3.97 (m, 1H, 4'-H), 4.03 (t,  $J=5.2$  Hz, 1H, 3'-H), 4.41~4.46 (m, 1H, 2'-H), 5.43 (t,  $J=4.8$  Hz, 1H, 5'-OH), 5.70 (d,  $J=4.4$  Hz, 1H, 2'-OH), 6.17 (d,  $J=5.2$  Hz, 1H, 1'-H), 8.45 (s, 1H, ArH), 11.72 (s, 1H, NH); ESI-MS  $m/z$ : 395.7 [M+H]<sup>+</sup>, 418.6 [M+Na]<sup>+</sup>; HRMS calcd for C<sub>9</sub>H<sub>10</sub>IN<sub>3</sub>NaO<sub>5</sub> [M+Na]<sup>+</sup> 417.9624, found 417.9627.

#### 4.2.4 General procedure for the synthesis of compounds 7~9

Compound **1** or **2** or **3** was dissolved in anhydrous methanol and triethylamine (1.0 equiv.). Catalyst 10% Pd/C (50% w/w) was added, and the mixture was stirred for 5 h under H<sub>2</sub> (344.7 kPa) atmosphere. Upon completion of the reaction as monitored by TLC, the catalyst was filtered off through Celite. The solvent was evaporated and the crude product was purified by flash chromatography on a silica gel column using dichloromethane-methanol as eluent giving compounds **7**~**9**, respectively, in 80%~86% yields as white solids.

3'-Amino-3'-deoxyuridine (**7**):<sup>[18]</sup> white foam, 86% yield with an HPLC purity of 96.0%.  $R_f=0.30$  (dichloromethane/methanol,  $V:V=5:1$ ). UV-vis (MeOH)  $\lambda_{\max}$ : 265 nm; ESI-MS  $m/z$ : 244.0 [M+H]<sup>+</sup>.

3'-Amino-3'-deoxy-5-methyl uridine (**8**):<sup>[17]</sup> white foam, 83% yield with an HPLC purity of 96.6%.  $R_f=0.40$  (dichloromethane/methanol,  $V:V=5:1$ ). UV-vis (MeOH)  $\lambda_{\max}$ : 268 nm; ESI-MS  $m/z$ : 258.0 [M+H]<sup>+</sup>.

3'-Amino-3'-deoxy-5-fluoro uridine (**9**):<sup>[19]</sup> white solid, 80% yield with an HPLC purity of 95.0%.  $R_f=0.45$  (dichloromethane/methanol,  $V:V=5:1$ ). UV-vis (MeOH)  $\lambda_{\max}$ : 270 nm; ESI-MS  $m/z$ : 261.9 [M+H]<sup>+</sup>.

#### 4.2.5 General procedure for the synthesis of 3'-azido-3'-deoxycytidine derivatives 10~12

To a solution of 1,2,4-triazole (5 equiv.) in dry dichloromethane was added phosphorus oxychloride (3.0 equiv.) followed by triethylamine (3.0 equiv.) at 0 °C and the reaction mixture was stirred for 30 min at 0 °C. A solution of compound **33** or **34** or **35** (1.0 equiv.) in dry dichloromethane was then added dropwise. The mixture was moved to room temperature and was stirred for 1 h. Upon completion of the reaction as monitored by TLC, the reaction mixture was poured into ice water and was treated with saturated sodium bicarbonate and ethyl acetate. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate. The combined organic phases were dried over anhydrous sodium sulfate. The drying agent was filtered off, and the filtrate was concentrated under reduced

pressure. The residue was dissolved in NH<sub>3</sub>-MeOH, and the reaction mixture was allowed to stir at room temperature overnight. Upon completion of the reaction as monitored by TLC, the solution was concentrated in vacuum under reduced pressure, and the residue was purified on a silica gel column resulting in compounds **10**~**12**, respectively, in 76%~85% yields as white solids.

3'-Azido-3'-deoxycytidine (**10**):<sup>[20]</sup> light yellow solid, 85% yield with an HPLC purity of 98.2%.  $R_f=0.2$  (dichloromethane/methanol,  $V:V=5:1$ ). UV-vis (MeOH)  $\lambda_{\max}$ : 270 nm; ESI-MS  $m/z$ : 268.8 [M+H]<sup>+</sup>.

3'-Azido-3'-deoxy-5-methyl cytidine (**11**): white foam, 76% yield in two steps with an HPLC purity of 98.3%.  $R_f=0.45$  (dichloromethane/methanol,  $V:V=5:1$ ). UV-vis (MeOH)  $\lambda_{\max}$ : 280 nm;  $^1\text{H NMR}$  (DMSO- $d_6$ , 400 MHz)  $\delta$ : 1.82 (s, 3H, CH<sub>3</sub>), 3.50~3.72 (m, 2H, CH<sub>2</sub>), 3.84~3.92 (m, 1H, 4'-H), 3.97 (t,  $J=5.2$  Hz, 1H, 3'-H), 4.32~4.40 (m, 1H, 2'-H), 5.25 (t,  $J=5.2$  Hz, 1H, 5'-OH), 5.77 (d,  $J=4.8$  Hz, 1H, 2'-OH), 6.07 (d,  $J=5.2$  Hz, 1H, 1'-H), 6.84 (s, 1H, NH), 7.33 (s, 1H, NH), 7.64 (s, 1H, ArH); ESI-MS  $m/z$ : 383.3 [M+H]<sup>+</sup>, 565.5 [2M+H]<sup>+</sup>.

3'-Azido-3'-deoxy-5-fluorocytidine (**12**): white solid, 79% yield in two steps with an HPLC purity of 99.0%.  $R_f=0.45$  (dichloromethane/methanol,  $V:V=5:1$ ). m.p. 165~170 °C (dec.); UV-vis (MeOH)  $\lambda_{\max}$ : 239, 280 nm;  $^1\text{H NMR}$  (DMSO- $d_6$ , 400 MHz)  $\delta$ : 3.54~3.61 (m, 1H, CH), 3.69~3.76 (m, 1H, CH), 3.90~3.98 (m, 2H, 3'-H, 4'-H), 4.30~4.36 (m, 1H, 2'-H), 5.37 (t,  $J=5.2$  Hz, 1H, 5'-OH), 5.70~5.74 (m, 1H, 2'-OH), 6.15 (d,  $J=5.2$  Hz, 1H, 1'-H), 7.54 (s, 1H, NH), 7.78 (s, 1H, NH), 8.16 (d,  $J=7.2$  Hz, 1H, ArH); ESI-MS  $m/z$ : 287.1 [M+H]<sup>+</sup>, 309.1 [M+Na]<sup>+</sup>, 325.0 [M+K]<sup>+</sup>. HRMS calcd for C<sub>9</sub>H<sub>11</sub>FN<sub>6</sub>NaO<sub>4</sub> [M+Na]<sup>+</sup> 309.0724, found 309.0725.

#### 4.2.6 Synthesis of 3'-amino-3'-deoxycytidine (13)

This compound was synthesized as described in Scheme 1 from compound **10** (2.0 g, 7.5 mmol). 1.4 g of compound **13**<sup>[19]</sup> was obtained as a white foam in 75% yield with an HPLC purity of 97.3%;  $R_f=0.30$  (dichloromethane/methanol,  $V:V=2:1$ ). UV-vis (MeOH)  $\lambda_{\max}$ : 270 nm; ESI-MS  $m/z$ : 243.0 [M+H]<sup>+</sup>.

#### 4.2.7 Synthesis of 3'-deoxy-3'-azido-isocytidine (14)

To a solution of 3'-azido-3'-deoxyuridine (**1**) (5.0 g, 18.6 mmol) in dry pyridine (70 mL) at 0 °C with stirring was added *p*-toluenesulfonyl chloride (4.3 g, 22.32 mmol, 1.2 equiv.). The reaction mixture was stirred overnight at room temperature. Upon completion of the reaction as monitored by TLC, the reaction mixture was poured into ice water and was treated with saturated sodium bicarbonate and ethyl acetate. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate. The combined organic phases were dried over anhydrous sodium sulfate. The drying agent was filtered off, and the filtrate was concentrated under reduced pressure. The residue was then dissolved in acetonitrile (100 mL), and 1,8-diazabicyclo[5.4.0]undec-7-ene (5.6 mL, 37.2 mmol, 2.0 equiv.) was added to the solution. The reaction mixture was heated to

reflux for 1 h. Upon completion of the reaction as monitored by TLC, the hot solution was immediately filtered through a Celite pad, and the filtrate was evaporated to dryness. The residue was purified on a silica gel column to provide 3.0 g of product **38**. The obtained compound **38** was dissolved in 30 mL of MeOH, and 30 mL of ammonium hydroxide was added. The reaction mixture was allowed to stir at room temperature overnight. Upon completion of the reaction as monitored by TLC, the solution was concentrated in vacuum under reduced pressure, and the residue was purified on a silica gel column resulting in 2.6 g of compound **14** as a white solid in 52% yield for three steps with an HPLC purity of 95.8%.  $R_f=0.2$  (dichloromethane/methanol,  $V:V=5:1$ ). m.p. 164~169 °C (dec.).  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$ : 3.62~3.70 (m, 3H, 4'-H, CH<sub>2</sub>), 4.02 (t,  $J=2.8$  Hz, 1H, 3'-H), 4.30~4.45 (m, 1H, 2'-H), 5.35 (t,  $J=4.8$  Hz, 1H, 5'-OH), 5.54 (d,  $J=7.6$  Hz, 1H, ArH), 5.76 (d,  $J=6.0$  Hz, 1H, 2'-OH), 6.17 (d,  $J=4.8$  Hz, 1H, 1'-H), 6.84 (s, 2H, NH<sub>2</sub>), 7.61 (d,  $J=8.0$  Hz, 1H, ArH); ESI-MS  $m/z$ : 269.1  $[\text{M} + \text{H}]^+$ . HRMS calcd for C<sub>9</sub>H<sub>12</sub>N<sub>6</sub>NaO<sub>4</sub>  $[\text{M} + \text{Na}]^+$  291.0818, found 291.0815.

#### 4.2.8 Synthesis of 3'-azido-3'-deoxy-*D*-nucleosides **15**~**19**

These compounds were synthesized by the glycosylation of 6-azauracil, 2,6-dihydropyridine, 2-hydroxypyrimidine, 5-nitro-pyridin-2-one and 2-thiouracil with **32**. The resulted compounds were further deprotected resulted in final products **15**~**19**.

3'-Azido-3'-deoxy-6-azauridine (**15**): yellow foam, 71% yield in two steps with an HPLC purity of 98.3%.  $R_f=0.5$  (dichloromethane/methanol,  $V:V=10:1$ ). UV-Vis (MeOH)  $\lambda_{\text{max}}$ : 262 nm;  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$ : 3.40~3.60 (m, 3H, CH<sub>2</sub>, 4'-H), 3.88~4.00 (m, 2H, 2',3'-H), 4.54~4.58 (m, 1H, 5'-OH), 4.85 (br, 1H, 2'-OH), 5.91 (d, 1H,  $J=3.6$  Hz, 1'-H), 6.10 (br, 1H, NH), 7.53 (s, 1H, ArH); ESI-MS  $m/z$ : 271.1  $[\text{M} + \text{H}]^+$ , 293.0  $[\text{M} + \text{Na}]^+$ .

3'-Azido-3'-deoxy-3-deaza uridine (**16**): white foam, 68% yield over two steps with an HPLC purity of 98.0%.  $R_f=0.1$  (petroleum ether/ethyl acetate,  $V:V=1:1$ ).  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$ : 3.53~3.69 (m, 2H, CH<sub>2</sub>), 3.87~3.92 (m, 1H, 4'-H), 4.05 (t,  $J=4.8$  Hz, 1H, 3'-H), 4.40~4.45 (m, 1H, 2'-H), 5.26 (t,  $J=4.8$  Hz, 1H, 5'-OH), 5.65 (d,  $J=8.0$  Hz, 1H, 2'-OH), 5.76 (d,  $J=5.6$  Hz, 1H, 1'-H), 6.15 (d,  $J=5.2$  Hz, 1H, ArH), 7.86 (d,  $J=8.0$  Hz, 1H, ArH), 11.34 (s, 1H, ArH); ESI-MS  $m/z$ : 269.8  $[\text{M} + \text{H}]^+$ , 291.9  $[\text{M} + \text{Na}]^+$ .

3'-Azido-3'-deoxy-4-deoxy uridine (**17**): light yellow foam, 78% yield over two steps with an HPLC purity of 95.2%.  $R_f=0.6$  (dichloromethane/methanol,  $V:V=10:1$ ).  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$ : 3.59~3.66 (m, 1H, CH), 3.80~3.92 (m, 2H, CH, 4'-H), 4.08~4.13 (m, 1H, 3'-H), 4.38~4.43 (m, 1H, 2'-H), 5.39 (t,  $J=0.8$  Hz, 1H, 5'-OH), 5.75 (d,  $J=2.4$  Hz, 1H, 2'-OH), 6.36 (d,  $J=5.2$  Hz, 1H, 1'-H), 6.48~6.52 (m, 1H, ArH), 8.52~8.58 (m, 1H, ArH); ESI-MS  $m/z$ : 254.0  $[\text{M} + \text{H}]^+$ , 275.9  $[\text{M} + \text{Na}]^+$ .

1-(3-Azido-3-deoxy- $\beta$ -*D*-ribofuranosyl)-5-nitropyridine-

2(1*H*)-one (**18**): white solid, 65% yield over two steps with an HPLC purity of 95.7%.  $R_f=0.5$  (dichloromethane/methanol,  $V:V=10:1$ ). m.p. 150~155 °C (dec.).  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$ : 3.62~3.70 (m, 1H, CH), 3.87~4.00 (m, 2H, CH, 4'-H), 4.14~4.21 (m, 1H, 3'-H), 4.37~4.43 (m, 1H, 2'-H), 5.61 (t,  $J=4.4$  Hz, 1H, 5'-OH), 5.90 (d,  $J=1.6$  Hz, 1H, 2'-OH), 6.43 (d,  $J=5.2$  Hz, 1H, 1'-H), 6.51 (d,  $J=10.0$  Hz, 1H, ArH), 8.15 (dd,  $J_1=2.8$  Hz,  $J_2=10.2$  Hz, 1H, ArH), 9.63 (d,  $J=2.8$  Hz, 1H, ArH); ESI-MS  $m/z$ : 319.9  $[\text{M} + \text{Na}]^+$ . HRMS calcd for C<sub>10</sub>H<sub>11</sub>N<sub>5</sub>NaO<sub>6</sub>  $[\text{M} + \text{Na}]^+$  320.0607, found 320.0605.

3'-Azido-3'-deoxy-2-thiouridine (**19**): white solid, 72% yield over two steps with an HPLC purity of 98.4%.  $R_f=0.4$  (dichloromethane/methanol,  $V:V=10:1$ ). m.p. 155~160 °C (dec.). UV-vis (MeOH)  $\lambda_{\text{max}}$ : 273 nm;  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$ : 3.50~3.70 (m, 2H, CH<sub>2</sub>), 3.73~3.80 (m, 2H, 4',3'-H), 4.45~4.48 (m, 1H, 2'-H), 5.44 (t,  $J=5.2$  Hz, 1H, 5'-OH), 5.99 (d,  $J=8.4$  Hz, 1H, ArH), 6.23 (d,  $J=5.6$  Hz, 1H, 2'-OH), 6.54 (d,  $J=3.2$  Hz, 1H, 1'-H), 8.15 (d,  $J=8.2$  Hz, 1H, ArH), 12.65 (s, 1H, NH); ESI-MS  $m/z$ : 285.8  $[\text{M}]^+$ . HRMS calcd for C<sub>9</sub>H<sub>11</sub>N<sub>5</sub>NaO<sub>4</sub>S  $[\text{M} + \text{Na}]^+$  308.0429, found 308.0429.

#### 4.2.9 Synthesis of 6-chloro-9-(2-*O*-acetyl-5-*O*-*p*-toluoyl-3-azido-3-deoxy- $\beta$ -*D*-ribofuranosyl)-9*H*-purine (**39**)

To a precooled (0 °C) mixture of 1,2-di-*O*-acetyl-5-*O*-(4-methylbenzoyl)ribose (**32**, 7.5 g, 19.9 mmol, 1.0 equiv.) and 6-chloropurine (3.38 g, 21.9 mmol, 1.1 equiv.) in anhydrous acetonitrile (100 mL) was added dropwise a solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (9.0 mL, 9.13 g, 60.0 mmol, 3.0 equiv.) in anhydrous acetonitrile (30 mL), followed by addition of trimethylsilyl triflate (14.5 mL, 17.8 g, 80.0 mmol, 4.0 equiv.). The reaction mixture was then stirred at 60 °C for 4 h. Upon completion of the reaction as monitored by TLC, the reaction mixture was poured into ice water and was treated with saturated sodium bicarbonate and ethyl acetate. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate. The combined organic phases were dried over anhydrous sodium sulfate. The drying agent was filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column using petroleum ether-ethyl acetate ( $V:V, 5:1$  to  $3:1$ ) as eluents providing product **39** (8.4 g, 89%) as a white solid. ESI-MS  $m/z$ : 272.1  $[\text{M}]^+$ .

#### 4.2.10 Synthesis of 3'-azido-3'-deoxyadenosine (**20**)

6-Chloro-9-(2-*O*-acetyl-5-*O*-*p*-toluoyl-3-azido-3-deoxy- $\beta$ -*D*-ribofuranosyl)-9*H*-purine (**39**) (3.0 g, 6.4 mmol) was dissolved in methanol (30 mL) and the solution was saturated with dry ammonia gas at 0 °C. The reaction mixture was stirred for overnight at 80 °C, and was concentrated under reduced pressure. The resulting residue was purified by flash chromatography on a silica gel column to afford 1.3 g of compound **20** as a white solid in 70% yield with an HPLC purity of 96.7%.  $R_f=0.20$  (dichloromethane/methanol,  $V:V=10:1$ ). m.p. 200~205 °C (Lit.<sup>[21]</sup> m.p. 208~212 °C (dec.)). UV-Vis (MeOH)  $\lambda_{\text{max}}$ : 260 nm;

ESI-MS  $m/z$ : 292.9  $[M+H]^+$ .

#### 4.2.11 Synthesis of 3'-azido-3'-deoxy-*N*<sup>6</sup>-methyl-adenosine (**21**)

6-Chloro-9-(2-*O*-acetyl-5-*O*-*p*-toluoyl-3-azido-3-deoxy- $\beta$ -*D*-ribofuranosyl)-9*H*-purine (**39**, 4.6 g, 9.7 mmol) was dissolved in methylamine (60 mL) solution in methanol. The reaction mixture was stirred at 80 °C overnight. Upon completion of the reaction as monitored by TLC, the reaction solvent was evaporated under reduced pressure. The resulting residue was purified by flash chromatography on a silica gel column to afford 2.4 g of compound **21**<sup>[22]</sup> as a white solid in 82% yield with an HPLC purity of 98.5%.  $R_f=0.6$  (dichloromethane/methanol,  $V:V=10:1$ ). m.p. 165 ~ 170 °C (dec.); UV-vis (MeOH)  $\lambda_{max}$ : 261 nm; ESI-MS  $m/z$ : 307.1  $[M+H]^+$ , 329.1  $[M+Na]^+$ .

#### 4.2.12 Synthesis of 3'-azido-3'-deoxy-*N*<sup>6</sup>,*N*<sup>6</sup>-dimethyladenosine (**22**)

6-Chloro-9-(2-*O*-acetyl-5-*O*-*p*-toluoyl-3-azido-3-deoxy- $\beta$ -*D*-ribofuranosyl)-9*H*-purine (**39**, 4.2 g, 8.9 mmol) was dissolved in dimethylamine (20 mL) solution in methanol. The reaction mixture was heated at 100 °C overnight. Upon completion of the reaction as monitored by TLC, the reaction solvent was evaporated under reduced pressure. The resulting residue was purified by flash chromatography on a silica gel column to afford 2.4 g of compound **22** as a white solid in 86% yield with an HPLC purity of 98.5%.  $R_f=0.7$  (dichloromethane/methanol,  $V:V=10:1$ ). m.p. 185 ~ 190 °C (dec.) (Lit.<sup>[12]</sup> m.p. 199 ~ 201 °C).

#### 4.2.13 Synthesis of 6-methylpurine- $\beta$ -*D*-(3-azido-3-deoxy)riboside (**23**)

This compound was synthesized from the glycosylation of 6-methylpurine (1.4 g, 8.8 mmol, 1.1 equiv.) with **32** (3.0 g, 8.0 mmol) and the resulted compound was further deprotected resulted in 1.6 g of final product **23** as a white solid in 72% total yield with an HPLC purity of 99.2%.  $R_f=0.6$  (dichloromethane/methanol,  $V:V=10:1$ ). m.p. 170 ~ 173 °C (dec.); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$ : 2.73 (s, 3H, CH<sub>3</sub>), 3.56 ~ 3.65 (m, 1H, CH), 3.69 ~ 3.76 (m, 1H, CH), 4.00 ~ 4.05 (m, 1H, 4'-H), 4.31 ~ 4.36 (m, 1H, 3'-H), 4.99 ~ 5.05 (m, 1H, 2'-H), 5.27 (t,  $J=5.2$  Hz, 1H, 5'-OH), 6.03 (d,  $J=5.6$  Hz, 1H, 2'-OH), 6.29 (d,  $J=5.6$  Hz, 1H, 1'-H), 8.75 (s, 1H, ArH), 8.80 (s, 1H, ArH); ESI-MS  $m/z$ : 292.1  $[M+H]^+$ , 314.1  $[M+Na]^+$ . HRMS calcd for C<sub>11</sub>H<sub>13</sub>N<sub>7</sub>NaO<sub>3</sub>  $[M+Na]^+$  314.0978, found 314.0979.

#### 4.2.14 Synthesis of 3'-amino-3'-deoxyadenosine (**24**)

This compound was synthesized from compound **20** (2.0 g, 6.8 mmol) resulting in 1.6 g of compound **24** as a gray solid in 87% yield with an HPLC purity of 96.6%.  $R_f=0.60$  (dichloromethane/methanol,  $V:V=2:1$ ). m.p. 230 ~ 235 °C (dec.) (Lit.<sup>[21]</sup> > 230 °C); UV-Vis (MeOH)  $\lambda_{max}$ : 258 nm; ESI-MS  $m/z$ : 267.1  $[M+H]^+$ , 329.1  $[M+Na]^+$ .

#### 4.2.15 Synthesis of 1,2-di-*O*-acetyl-5-*O*-(4-methylbenzoyl)-*L*-riboside (**43**)

1,2-*O*-Isopropylidene- $\alpha$ -*L*-xylofuranose (**40**) was synthesized from *L*-(-)-xylose by two steps in 98% total yield

according to procedures described in the literature.<sup>[15]</sup> This material (100 g, 0.53 mol) was dissolved in 800 mL of dry pyridine and the mixture was cooled to 0 °C. Then 4-methylbenzoyl chloride (76.3 mL, 0.58 mol, 1.1 equiv.) was added dropwise with stirring. The reaction mixture was stirred for 2 h. Upon completion of the reaction as monitored by TLC, the reaction mixture was poured into ice water and was treated with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column to provide product 1,2-*O*-isopropylidene-5-*O*-(4-methylbenzoyl)- $\alpha$ -*L*-xylofuranose as a colorless oil. It was then dissolved in 200 mL of dry pyridine and 600 mL of dichloromethane. The mixture was cooled to -20 °C. Then trifluoromethanesulfonic anhydride (96.5 mL, 0.58 mol, 1.1 equiv.) was added dropwise with stirring. The reaction mixture was stirred for 2 h at -20 °C. Upon completion of the reaction as monitored by TLC, the reaction mixture was poured into saturated sodium bicarbonate and was treated with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column to provide 178 g of product 1,2-*O*-isopropylidene-5-*O*-(4-methylbenzoyl)-3-*O*-trifluoromethanesulfonyl- $\alpha$ -*L*-xylofuranose (**41**) in 76.5% yield in two steps as a white solid. This material was dissolved in 1500 mL of dry *N,N*-dimethylformamide (DMF), and sodium azide (52.0 g, 0.81 mol, 2.0 equiv.) was added. The reaction mixture was stirred for 72 h at room temperature. The reaction mixture was poured into water and was treated with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column to provide 74.0 g of product 3-azido-1,2-*O*-isopropylidene-5-*O*-(4-methylbenzoyl)-3-*O*-eoxy- $\alpha$ -*L*-xylofuranose (**42**) in 55% yield as a colorless oil. This material was dissolved in 200 mL of acetic acid and 100 mL of acetic anhydride, and 3.0 mL of concentrated sulfuric acid was added under stirring. The reaction mixture was stirred at room temperature overnight, and treated with 200 mL water. It was neutralized with sodium bicarbonate carefully and extracted with dichloromethane. The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column using petroleum ether-ethyl acetate as an eluent to give 63.0 g white sticky product **43** in 75% yield.

#### 4.2.16 Synthesis of 3'-azido-3'-deoxy-*L*-nucleoside derivatives **44** ~ **46** and deprotected compounds **25** ~ **27**

These compounds were synthesized by the glycosylation of uracil, 5-methyl uracil and 4-fluorouracil with **43**, respectively, resulting in compounds **44** ~ **46**. Further deprotection resulted in final products **25** ~ **27** as white solids in 65% ~ 72% overall yield.

3'-Azido-3'-deoxy- $\beta$ -*L*-uridine (**25**): white solid, 73%

yield in two steps with an HPLC purity of 96.8%.  $R_f=0.5$  (dichloromethane/methanol,  $V:V=10:1$ ). m.p. 135~138 °C; UV-Vis (MeOH)  $\lambda_{\max}$ : 259 nm;  $^1\text{H NMR}$  (DMSO- $d_6$ , 400 MHz)  $\delta$ : 3.49~3.54 (m, 2H, CH<sub>2</sub>), 3.80~4.05 (m, 2H, 4', 3'-H), 4.35~4.50 (m, 1H, 2'-H), 5.29 (t,  $J=5.2$  Hz, 1H, 5'-OH), 5.66 (d,  $J=8.0$  Hz, 1H, ArH), 5.76 (d,  $J=5.2$  Hz, 1H, 2'-OH), 6.17 (d,  $J=5.2$  Hz, 1H, 1'-H), 7.88 (d,  $J=8.0$  Hz, 1H, ArH), 11.48 (s, 1H, NH); ESI-MS  $m/z$ : 270.2 [M+H]<sup>+</sup>, 291.9 [M+Na]<sup>+</sup>.

3'-Azido-3'-deoxy-5-methyl- $\beta$ -L-uridine (**26**): white foam, 77% yield in two steps with an HPLC purity of 98.9%.  $R_f=0.6$  (dichloromethane/methanol,  $V:V=10:1$ ). UV-Vis (MeOH)  $\lambda_{\max}$ : 260 nm;  $^1\text{H NMR}$  (DMSO- $d_6$ , 400 MHz)  $\delta$ : 1.77 (s, 3H, CH<sub>3</sub>), 3.53~3.71 (m, 2H, CH<sub>2</sub>), 3.85~3.89 (m, 1H, 4'-H), 4.07 (t,  $J=5.2$  Hz, 1H, 3'-H), 4.40~4.45 (m, 1H, 2'-H), 5.26 (t,  $J=5.2$  Hz, 1H, 5'-OH), 5.76 (d,  $J=5.6$  Hz, 1H, 2'-OH), 6.11 (d,  $J=5.6$  Hz, 1H, 1'-H), 7.71 (s, 1H, ArH), 11.34 (s, 1H, NH); ESI-MS  $m/z$ : 284.1 [M+H]<sup>+</sup>, 306.1 [M+Na]<sup>+</sup>.

3'-Azido-3'-deoxy-5-fluoro- $\beta$ -L-uridine (**27**): white solid, 70% yield in two steps with an HPLC purity of 97.5%.  $R_f=0.6$  (dichloromethane/methanol,  $V:V=10:1$ ). m.p. 160~163 °C; UV-Vis (MeOH)  $\lambda_{\max}$ : 261 nm;  $^1\text{H NMR}$  (DMSO- $d_6$ , 400 MHz)  $\delta$ : 3.55~3.64 (m, 1H, CH), 3.68~3.76 (m, 1H, CH), 3.90~3.98 (m, 1H, 4'-H), 4.03 (t,  $J=5.2$  Hz, 1H, 3'-H), 4.37~4.52 (m, 1H, 2'-H), 5.44 (t,  $J=4.8$  Hz, 1H, 5'-OH), 5.69~5.73 (m, 1H, 2'-OH), 6.19 (d,  $J=5.6$  Hz, 1H, 1'-H), 8.27 (d,  $J=7.2$  Hz, 1H, ArH), 11.89 (s, 1H, NH); ESI-MS  $m/z$ : 288.2 [M+H]<sup>+</sup>, 310.1 [M+Na]<sup>+</sup>; HRMS calcd for C<sub>9</sub>H<sub>10</sub>FN<sub>5</sub>NaO<sub>5</sub> [M+Na]<sup>+</sup> 310.0564, found 310.0563.

4.2.17 General procedure for the synthesis of 3'-azido-3'-deoxy-L-cytidine derivatives **28**~**30**

Compounds **28**~**30** were synthesized from compounds **44**~**46**, respectively.

3'-Azido-3'-deoxy- $\beta$ -L-cytidine (**28**): white solid, 78% yield in two steps with an HPLC purity of 98.4%.  $R_f=0.3$  (dichloromethane/methanol,  $V:V=5:1$ ). m.p. 140~143 °C (dec.); UV-vis (MeOH)  $\lambda_{\max}$ : 265 nm;  $^1\text{H NMR}$  (DMSO- $d_6$ , 400 MHz)  $\delta$ : 3.53~3.60 (m, 1H, CH), 3.65~3.71 (m, 1H, CH), 3.88~3.97 (m, 2H, 3'-H, 4'-H), 4.33~4.39 (m, 1H, 2'-H), 5.21 (t,  $J=5.2$  Hz, 1H, 5'-OH), 5.70~5.77 (m, 2H, 2'-OH, ArH), 6.10 (d,  $J=5.2$  Hz, 1H, 1'-H), 7.20 (s, 1H, NH), 7.30 (s, 1H, NH), 7.82 (d, 1H,  $J=7.6$  Hz, ArH); ESI-MS  $m/z$ : 269.1 [M+H]<sup>+</sup>.

3'-Azido-3'-deoxy-5-methyl- $\beta$ -L-cytidine (**29**): light yellow solid, 74% yield in two steps with an HPLC purity of 95.3%.  $R_f=0.45$  (dichloromethane/methanol,  $V:V=5:1$ ). m.p. 105~107 °C; UV-vis (MeOH)  $\lambda_{\max}$ : 275 nm;  $^1\text{H NMR}$  (DMSO- $d_6$ , 400 MHz)  $\delta$ : 1.87 (s, 3H, CH<sub>3</sub>), 3.54~3.62 (m, 1H, CH), 3.68~3.77 (m, 1H, CH), 3.90~4.00 (m, 2H, 4', 3'-H), 4.37~4.40 (m, 1H, 2'-H), 5.31 (t,  $J=5.2$  Hz, 1H, 5'-OH), 5.75 (d,  $J=4.4$  Hz, 1H, 2'-OH), 6.14 (d,  $J=5.2$  Hz, 1H, 1'-H), 7.72 (s, 1H, NH), 7.81 (s, 1H, NH), 7.84 (s, 1H, ArH); ESI-MS  $m/z$ : 283.1 [M+H]<sup>+</sup>.

3'-Azido-3'-deoxy-5-fluoro- $\beta$ -L-cytidine (**30**): white

foam, 74% yield in two steps with an HPLC purity of 97.3%.  $R_f=0.5$  (dichloromethane/methanol,  $V:V=5:1$ ). UV-vis (MeOH)  $\lambda_{\max}$ : 239, 280 nm;  $^1\text{H NMR}$  (DMSO- $d_6$ , 400 MHz)  $\delta$ : 3.54~3.62 (m, 1H, CH), 3.70~3.76 (m, 1H, CH), 3.90~3.98 (m, 2H, 3'-H, 4'-H), 4.31~4.37 (m, 1H, 2'-H), 5.38 (br, 1H, 5'-OH), 5.70~5.74 (m, 1H, 2'-OH), 6.15 (br, 1H, 1'-H), 7.61 (s, 1H, NH), 7.81 (s, 1H, NH), 8.17 (d, 1H,  $J=7.2$  Hz, ArH); ESI-MS  $m/z$ : 287.1 [M+H]<sup>+</sup>, 573.2 [2M+H]<sup>+</sup>.

4.2.18 Synthesis of 6-chloro-9-[2-*O*-acetyl-5-*O*-(*p*-toluoyl)-3-azido-3-deoxy- $\beta$ -L-ribofuranosyl]-9*H*-purine (**47**) and 3'-azido-3'-deoxy- $\beta$ -L-adenosine (**31**)

Compound **47** was synthesized from 6-chloropurine (1.80 g, 11.7 mmol, 1.1 equiv.) and **43** (4.0 g, 10.6 mmol, 1.0 equiv.). Further deprotection by Method 6 resulted in 2.2 g of final product **31** as a white solid in 70% over yield with an HPLC purity of 97.0%.  $R_f=0.4$  (dichloromethane/methanol,  $V:V=10:1$ ). m.p.<sup>[21]</sup> 191~195 °C (dec.); UV-vis (MeOH)  $\lambda_{\max}$ : 259 nm; ESI-MS  $m/z$ : 293.1 [M+H]<sup>+</sup>, 315.2 [M+H]<sup>+</sup>.

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**Supporting Information**  $^1\text{H NMR}$ , MS and HRMS spectral data. The Supporting Information is available free of charge via the Internet at <http://sioc-journal.cn>.

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