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Total synthesis of 2'-*O*-methyl-β-L-arabinosyluridine and reassignment the nucleoside from *Penicillium sp.* as 2'-*O*-methyl-β-L-uridine

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Abstract

In order to validate the structure of a rarely reported naturally occurring nucleoside isolated from the broth of *Penicillium sp.* (NO. 64), practical syntheses of 2'-*O*-methyl-β-L-arabinosyluridine, 2'-*O*-methyl-α-L-arabinosyluridine, and 2'-*O*-methyl-β-L-uridine were accomplished. Comparing their nuclear magnetic resonance (NMR) spectra and physical data, its structure was reassigned as 2'-*O*-methyl-β-L-uridine instead of former reported 2'-*O*-methyl-β-L-arabinosyluridine.

Keywords

Naturally occurring nucleoside; L-arabinosyluridine; 2'-*O*-methyl-β-L-uridine; total synthesis; *Penicillium sp.*

Introduction

Naturally occurring nucleosides have played extraordinarily important role for discovering new pharmaceutical and chemical identities [1-3]. In the past century, large amount nucleosides have been isolated and identified from various natural resources, such as plants, microorganisms, and recent marine origins. Reprehensive examples are antiviral drug Vidarabine (Ara-A) and Cytarabine (Ara-C) from sponges, which are still prescribed in clinical practice nowadays [4].

Examination the chemical structures of all reported naturally occurring nucleosides showed that almost all of them are D-nucleosides [5]. Actually, D-nucleosides are also the main components of DNA and RNA. But in recent years, L-nucleosides have attracted tremendous interests of medicinal chemists, because of their excellent absorption, distribution, metabolism, and excretion (ADME) properties [6]. Since then, amount of nucleosides with the unnatural β-L-configuration have been synthesized and their biological activities were evaluated [7]. Many have been found to possess very potent antiviral activities [8]. The most noble compound is Lamivudine (3TC, Scheme 1), which is the first-line drug for treating HBV at the moment [9].

In 2017, identification of 2'-*O*-methyl-β-L-arabinosyluridine (compounds **1**, Figure 1) from the broth of *Penicillium sp.* (NO. 64) was reported by Guo *et al* [10]. We immediately realized that discovering L-nucleoside from nature was a breakthrough, which might change our acknowledge about nucleoside's natural occurrence. In addition, as we all know, L-arabinopyranoside and L-arabinofuranoside are widely

presented in nature, especially presence as the component of bacterial cell wall [11]. However, to the best our knowledge, there is very rare example of L-nucleoside reported as natural product. Because only small amount of nucleoside **1** was isolated, its biological activity was not reported by the authors. As our continuous study on total synthesis of naturally occurring nucleosides, we started to carry out its synthesis and investigate its biological activities [12-17].

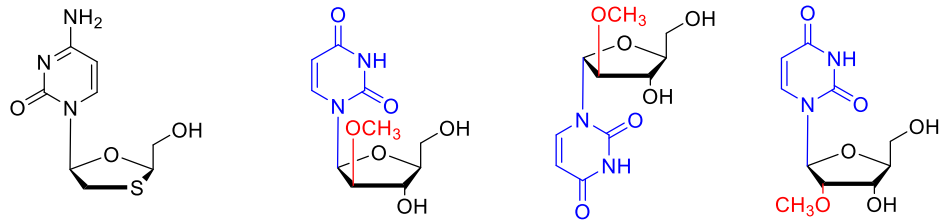


Figure 1. Chemical structures of Lamivudine and the synthesized L-nucleosides in present paper

From the synthetic point view, synthesis of β-L-arabinosyluridine could be carried out in two different approaches shown in Figure 2. The most straightforward approach is selective methylation of β-L-arabinosyluridine, which can be synthesized from 2,3,5-tri-*O*-benzyl-α-L-arabinofuranosyl chloride and uracil (Route A, Figure 2). But, the glycosylation always affords a mixture of anomers and their separation is notorious. In addition, this approach needs tedious protection and deprotection manipulations. Thus, the overall efficiency is low. The other convenient approach is synthesis nucleoside **1** using Vorbrüggen glycosylation of corresponding properly protected 2-*O*-methyl-L-arabinofuranose with uracil directly (Route B, Figure 2). However, because of 2-*O*-methyl group lacking of neighboring participation effect, an anomeric mixture of nucleosides **1** and **2** will be formed inevitably. Considering that the corresponding α-configuration nucleoside was also not reported before and the α-configuration

nucleosides were attracted much interests in recent years, we decided to use the second approach to synthesize **1** and **2** at the same time [18-21].

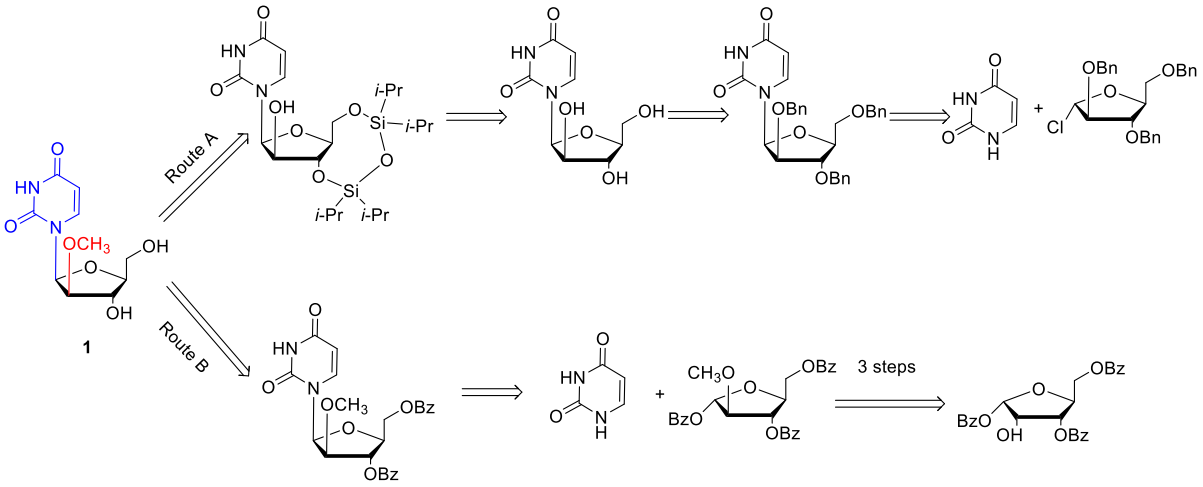
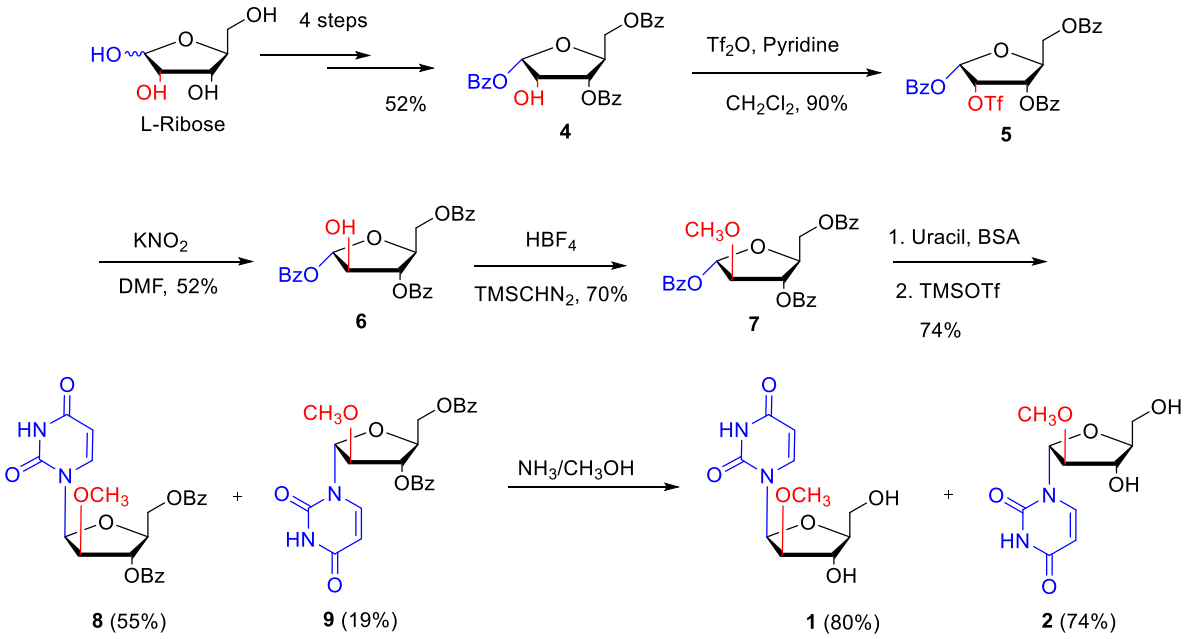


Figure 2. Retrosynthetic analysis of 2'-*O*-methyl-β-L-arabinosyluridine **1**

Results and Discussion



Scheme 1. Synthesis of 2'-*O*-methyl-L-arabinosyluridine **1** and **2** from L-ribose

Our synthesis 2'-*O*-methyl-L-arabinosyluridine started from the preparation of 1,3,5-tri-*O*-benzoyl-α-L-ribofuranose **4**, which was synthesized using L-ribose as starting material in four steps with 52% overall yield according to the reported literature (Scheme 1) [22,23]. Then 2-*O*-trifluoromethylsulfonyl-1,3,5-tri-*O*-benzoyl-α-L-ribofuranose **5** was prepared with 90% yield by esterification with trifluoromethanesulfonic anhydride in a mixture of anhydrous pyridine and DCM. Subsequent nucleophilic substitute of triflate **5** with KNO_2 and simultaneous hydrolysis under H_2O afforded 1,3,5-tri-*O*-benzoyl-α-L-arabinofuranose **6** with 52% yield. Encouragingly, a single crystal of L-arabinofuranose **6** suitable for X-ray crystallography was obtained and its structure was ambiguously confirmed (Figure **3**) [24].

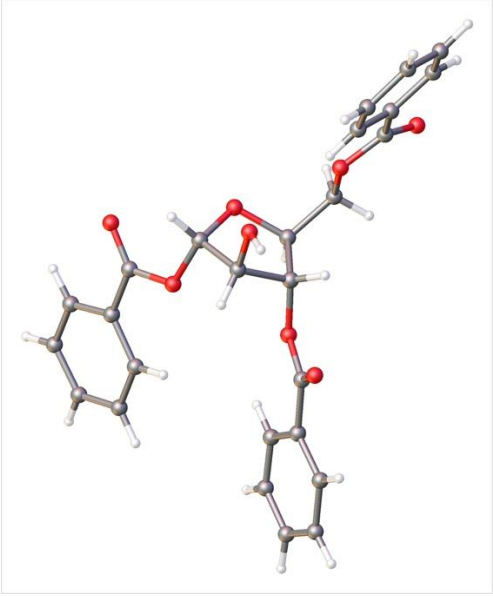


Figure 3. ORTEP molecular structure of 1,3,5-tri-*O*-benzoyl-α-L-arabinofuranose **6**

Next, methylation of 2-OH of compound **6** was investigated. Literature survey revealed that CH_3I/Ag_2O and trimethylsilyl diazomethane ($TMSCHN_2$)/HBF_4 were frequently used for the *O*-methylation of alcohols [25,26]. At first, the method of CH_3I/Ag_2O in

DMF was applied. Because Ag_2O are weak base, transesterification of benzoyl to 2-OH occurred and a complicated mixtures was obtained [27]. Therefore, the method of $TMSCHN_2/HBF_4$ was further employed. In our preliminary experiment, L-arabinose methyl ether **7** was successfully obtained but in low yield accompanied by unreacted starting material. After extensive optimization of solvents, reaction temperature, equivalence ratios of $TMSCHN_2$ and HBF_4, it was found that the best condition was using of 1.0 equiv. 1,3,5-tri-*O*-benzoyl-α-L-arabinofuranose **6**, 2.0 equiv. of $TMSCHN_2$, and 1.0 equiv. of HBF_4 in DCM at room temperature. It afforded methyl ether **7** in 70% yield accompanied with 20% unreacted starting material arabinose **6**.

Subsequent, vorbrüggen glycosylation of uracil with methyl ether **7** was performed in MeCN/BSA/TMSOTf. It afforded the β-L-arabinosyluridine **8** and α-L-arabinosyluridine **9** (approximation ratio of 3:1) as expected in 74% yield. Careful recrystallization of the mixed isomers can give part of nucleoside **8** as a white solid. The remaining residue containing the mixed isomers **8** and **9** was difficult to separate using silica gel chromatography. Thus, the mixture was subjected to a saturated solution of ammonia in methanol directly to afforded nucleoside **1** and **2**, which can be separated successfully by HPLC. Discrimination of the α-anomer and β-anomer was done based on $^3J_{H1',H2'}$ coupling constant. The resonances for the anomeric hydrogens of α/β-L-arabinosyluridine appeared as a doublet ($^3J_{H1',H2'}$ = 2.9 Hz) at δ 5.81 ppm and a doublet ($^3J_{H1',H2'}$ = 5.6 Hz) at δ 6.13 ppm, respectively [28]. It is noteworthy that the small value of $^3J_{H1',H2'}$ is a characteristic trans-relationship between H_1' and H_2' in ribose nucleoside [29].

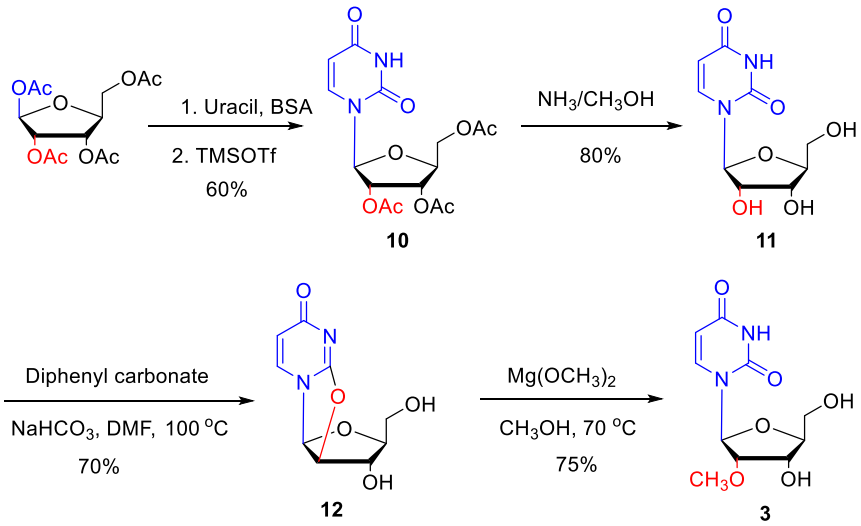
Table 1 ^{13}C Chemical shifts of nucleosides **1**, **2**, **3** and the reported nucleoside[a]

	1/CD_3OD $[\alpha]^{20}_{,D}$ -136[b]	**2/** CD_3OD $[\alpha]^{20}_{,D}$ -16.7[b]	**3/**CD_3OD $[\alpha]^{20}_{,D}$ -44[b]	Reported/CD_3OD $[\alpha]^{20}_{,D}$ -68[a]
1	59.0	58.5	58.8	58.8
2	61.8	62.8	61.6	61.8
3	74.6	75.5	69.7	69.8
4	85.1	90.3	85.0	85.2
5	85.6	91.5	86.1	86.2
6	87.0	92.0	88.8	88.9
7	101.3	100.2	102.5	102.8
8	144.1	143.1	142.4	142.6
9	152.2	152.1	152.1	152.0
10	166.2	166.4	166.2	166.6

[a]Nucleoside reported (ref. 10), [b]Recorded at *c* = 0.050 in CH_3OH.

Further comparing the reported ^{13}C NMR chemical shift of 2'-*O*-methyl-β-L-arabinosyluridine isolated from the broth of *Penicillium sp.* (NO. 64) with the synthetic sample, it clearly showed different data (Table 1). This inconsistent means that the nucleoside structure from the broth of *Penicillium sp.* (NO. 64) is not 2'-*O*-methyl-β-L-arabinosyluridine indeed.

Revisiting the two-dimensional NMR data, we supposed that the uridine might be 2'-*O*-methyl-β-L-uridine instead of the former reported 2'-*O*-methyl-β-L-arabinosyluridine. Although 2'-*O*-methyl-β-D-uridine was commercially available, 2'-*O*-methyl-β-L-uridine was not reported yet. In order to further verify its structure, synthesis of 2'-*O*-methyl-β-L-uridine was accomplished (Scheme **2**).



Scheme 2. Synthesis of 2'-*O*-methyl-β-L-uridine **3**

Synthesis of 2'-*O*-methyl-β-L-uridine started from 1,2,3,5-tetra-*O*-acetyl-β-L-ribofuranose, which was conveniently prepared using L-ribose as starting material in 3 steps. Vorbrüggen glycosylation between 1,2,3,5-tetra-*O*-acetyl-β-L-ribofuranose and uracil gave L-nucleoside **10** in 60% yield. After Zemplén saponification, L-uridine **11** was obtained in 80% yield. Next, 2,2'-anhydro-β-L-arabinosyluridine **12** was prepared in 70% yield by refluxing nucleoside **11** with diphenyl carbonate. In addition, a single crystal of nucleoside **12** suitable for X-ray crystallography was also obtained and its structure was ambiguously confirmed. The molecules form stacked dimers which are linked by two hydrogen bonds (Figure 4).[30] At last, 2'-*O*-methyl-β-L-uridine **3** was prepared by refluxing nucleoside **12** with freshly prepared magnesium methoxide in 75% yield.

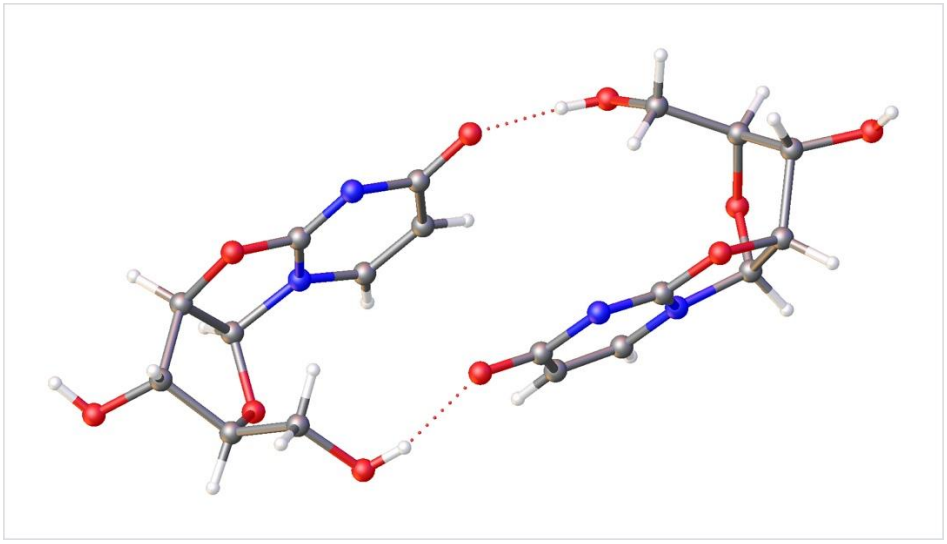


Figure 4. ORTEP molecular structure of 2,2'-anhydro-β-L-arabinosyluridine **12**

After 2'-*O*-methyl-β-L-uridine was obtained, we were glad to find that all the NMR spectroscopy of synthesized are in accordance with the nucleoside isolated from *Penicillium sp.* (NO. 64) (Table 1). Furthermore, the specific optical rotations of nucleosides isolated from *Penicillium sp.* (NO. 64) and synthesized by L-ribofuranose are $[\alpha]^{20}_{,D}$ -68 and $[\alpha]^{20}_{,D}$-44 (c=0.050) respectively. Therefore, we reassigned the nucleoside isolated from *Penicillium sp.* (NO. 64) as 2'-*O*-methyl-β-L-uridine. To the best of our knowledge, it is still the first naturally occurring L-nucleoside and its synthesis was firstly accomplished.

Conclusion

In summary, total synthesis of 2'-*O*-methyl-β-L-arabinosyluridine, 2'-*O*-methyl-α-L-arabinosyluridine, and 2'-*O*-methyl-β-L-uridine were accomplished in present paper. The key intermediates were confirmed by X-ray crystallography. The developed protocol for synthesis 2'-*O*-methyl-β-L-arabinosyluridine and 2'-*O*-methyl-α-L-arabinosyluridine could be extended to preparation other 2'-*O*-methyl-nucleosides. After detailed comparison of their spectra, the nucleoside isolated from *Penicillium sp* (64) was reassigned as 2'-*O*-methyl-β-L-uridine instead of the reported 2'-*O*-methyl-β-

L-arabinosyluridine. To the best of our knowledge, this is the first reported naturally occurring L-nucleoside. Studies on its biological activity and biosynthetic pathway in *Penicillium sp.* (NO. 64) are undergoing.

Experimental

General

All reagents and catalysts were purchased from commercial sources (Energy Chemical Co. Ltd. or Sigma-Aldrich Co. Ltd.) and used without purification. DCM, MeCN, DMF, and Pyridine were dried with CaH_2 and distilled prior to use. Thin layer chromatography was performed using silica gel GF-254 plates (Qing-Dao Chemical Company, China) with detection by UV (254 nm) or charting with 10% sulfuric acid in ethanol. Column chromatography was performed on silica gel (200-300 mesh, Qing-Dao Chemical Company, China). NMR spectra were recorded on a Bruker AV400 spectrometer, and chemical shifts (δ) are reported in ppm. ^{1}H NMR and ^{13}C NMR spectra were calibrated with TMS as an internal standard, and coupling constants (*J*) are reported in Hz. The ESI-HRMS were obtained on a Bruker Dalton microTOFQ II spectrometer in positive ion mode. Melting points were measured on an electrothermal apparatus uncorrected. Optical rotation was measured on a Rudolph Autopol IV at a wavelength of 589 nm.

Synthesis of 1,3,5-tri-*O*-benzoyl-α-L-ribofuranose (4)

1,3,5-Tri-O-benzoyl-α-L-ribofuranose was synthesized according to the procedure reported[22,23].

Yield: 52%, R_f =0.3 (PE:EA=3:1), m.p. 130-131 °C, $[\alpha]^{20}_{D}$= -72.0 (*c* = 0.050, CH_3OH). ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 8.12-8.09 (m, 4H), 8.04 (d, *J* = 7.6 Hz, 2H), 7.66-7.57 (m, 3H), 7.46 (t, *J* = 7.7 Hz, 4H), 7.39 (t, *J* = 7.7 Hz, 2H), 6.68 (d, *J* = 4.4 Hz, 1H), 5.59 (d, *J* = 6.5 Hz, 1H), 4.76-4.71 (m, 2H), 4.65-4.63 (m, 2H), 2.83 (d, *J* = 10.5 Hz, 1H) .

^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 166.2, 166.0, 165.5, 133.9, 133.7, 133.5, 130.0 (Cx4), 129.8, 129.7, 129.6, 129.3, 128.7 (Cx4), 128.6 (Cx2), 96.0, 83.2, 72.3, 72.0, 64.2.

HRMS (ESI): M/Z calculated for $C_{26}H_{22}O_8$, $[M+H]^+$: 463.1393, found: 463.1389.

Synthesis of 2-*O*-trifluoromethylsulfonyl-1,3,5-tri-*O*-benzoyl-α-L-ribofuranose (5)

A mixture of 1,3,5-tri-*O*-benzoyl-α-L-ribofuranose **4** (4.62 g, 10 mmol, 1 eq.) and anhydrous pyridine (11.7 mL, 14.6 mmol, 14.6 eq.) in anhydrous DCM (150 mL) was stirred under ice bath for 10 min. Trifluoromethanesulfonic anhydride (12 mmol, 2 mL) was then added dropwise with vigorous stirring. The obtained reaction mixture was stirred for another 1 hr at 0 °C and then stirred at room temperature for 3 hrs. Then, the reaction was quenched by the addition of 200 mL of ice water. The aqueous solution was extracted with DCM (3×200 mL) and the combined extract was washed with a saturated solution of sodium carbonate (3×200 mL) and brine (3×200 mL), respectively. The organic phase was dried with anhydrous $MgSO_4$. After filtered and evaporated under reduced pressure, the obtained syrup was purified by column chromatography on silica gel to give **5** as a white solid **(**5.34 g, 90%).

Yield: 90%, R_f = 0.4 (PE:EA = 5:1), m.p. 53-54 °C, $[\alpha]^{20}_D$= -60.0 (*c* = 0.050, CH_3OH).

^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 8.14 (t, *J* = 8.9 Hz, 4H), 8.04 (d, *J* = 7.9 Hz, 2H), 7.67-7.57 (m, 3H), 7.52-7.36 (m, 6H), 6.92 (d, *J* = 4.3 Hz, 1H), 5.84 (dd, *J* = 6.2, 3.3 Hz, 1H), 5.65-5.57 (m, 1H), 4.94-4.85 (m, 1H), 4.77 (dd, *J* = 12.3, 2.8 Hz, 1H), 4.65 (dd, *J* = 12.3, 3.3 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 165.9, 165.6, 164.8, 134.1, 134.0, 133.6, 130.2 (Cx2), 130.1 (Cx2), 129.6 (Cx2), 129.3, 128.9, 128.7 (Cx4), 128.6 (Cx3), 118.5 (q, *J* = 319.8 Hz), 93.3, 82.1, 79.6, 70.1, 63.6.

HRMS (ESI): M/Z calculated for $C_{27}H_{21}F_3O_{10}S$, $[M+H]^+$: 595.0886, found: 595.0889.

Synthesis of 1,3,5-tri-*O*-benzoyl-α-L-arabinofuranose (6)

The triflate **5** (5.34 g, 9 mmol, 1 eq.) was dissolved in DMF (90 mL) and KNO_2 (3.83 g, 45 mmol) was added. The reaction mixture was stirred at 40 °C for 24 h. Water (100 mL) was added and the resulting mixture was extracted with DCM (3×100 mL). The organic layer was combined, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel to give a white solid **6** (2.16 g, 52%).

Yield: 52%, R_f =0.3 (PE:EA=3:1), m.p. 81-82 °C, $[\alpha]^{20}_{,D}$= -64.8 (*c* = 0.054, CH_3OH).

^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 8.09-8.03 (m, 6H), 7.62-7.51 (m, 3H), 7.43-7.35 (m, 6H), 6.56 (s, 1H), 5.35 (d, *J* = 3.4 Hz, 1H), 4.83 (dd, *J* = 9.1, 4.1 Hz, 1H), 4.76(dd, *J* = 11.9, 4.0 Hz, 1H),4.72-4.62 (m, 2H), 3.65 (s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 166.5 (Cx2), 165.4, 133.8, 133.6, 133.3, 130.0 (Cx4), 129.9 (Cx2), 129.7, 129.6, 129.0, 128.6 (Cx2), 128.5 (Cx4), 102.7, 83.0, 80.5, 79.9, 64.2.

HRMS (ESI): M/Z calculated for $C_{26}H_{22}O_8$, $[M+Na]^+$:485.1212, found: 485.1209.

Synthesis of 1,3,5-tri-*O*-benzoyl-2-*O*-methyl-α-L-arabinofuranose (7)

A mixture of arabinofuranose **6** (1.85 g, 4 mmol, 1 eq.) and HBF_4 (42% aqueous, 4 mmol) in DCM (16 mL) was vigorously stirred at ice bath for 10 min. Trimethylsilyl diazomethane (1.8M hexane solution, 4.48 mL, 8 mmol) was dropwise added during 10 min. The mixture was stirred at 0°C for further 1 h, then poured into water (100 mL), and extracted with DCM (3×100 mL). The organic layer was washed with water (3×100 mL), dried over magnesium sulfate, and concentrated. The residue was purified by column chromatography on silica gel to give a white solid **7** (1.07 g) and arabinofuranose **6** (0.37 g, 70%).

Yield: 70%, R_f = 0.5 (PE:EA=4:1), m.p. 123-124 °C, $[\alpha]^{20}_{,D}$= -70.7 (*c* = 0.058, CH_3OH).

^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 8.11 (d, *J* = 7.6 Hz, 2H), 8.07-8.04 (m, 4H), 7.62-7.54 (m, 3H), 7.45-7.37 (m, 6H), 6.62 (s, 1H), 5.45 (d, *J* = 2.0 Hz, 1H), 4.81-4.80 (m, 1H), 4.70-4.62 (m, 2H), 4.21 (s, 1H), 3.59(s, 3H).

^{13}C NMR (101 MHz, CDCl3) *δ*: 166.4, 165.8, 165.2, 133.8, 133.6, 133.2, 130.0 (Cx4), 129.9 (Cx2), 129.7, 129.3, 128.6 (Cx2), 128.5 (Cx2), 128.4 (Cx2), 100.6, 88.0, 84.0, 77.2, 64.2, 58.2.

HRMS (ESI): M/Z calculated for $C_{27}H_{24}O_8$, $[M+H]^+$: 477.1549, found: 477.1544.

Synthesis of 2'-*O*-methyl- 3',5'-di-*O*-benzoyl-β-L-arabinosyluridine (8)

To a solution of uracil (0.27 g, 2.4 mmol) in dry MeCN (10 mL) was added BSA (1.95 g, 2.4 mL, 9.6 mmol) and stirred under nitrogen for 1 h at room temperature. After addition of **7** (0.95 g, 2 mmol), TMSOTf (1.78 g, 1.5 mL, 8 mmol) was added to the mixture at ice bath. The mixture was stirred for 15 min before heating to 80 °C for 12 hrs. After cooling, the reaction mixture was poured into water (30 mL) and extracted with EtOAc (3 × 30 mL). The combined extract was washed third with a saturated solution of sodium carbonate (3 × 30 mL) and brine (3 × 30 mL), respectively. The organic phase was dried with anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel to give crude products. The crude products were recrystallized from a mixture of EA and PE to give a white solid **8** (0.51 g, 55%). The filtrated stock solution containing isomers of nucleoside **9** was evaporated under reduced pressure and used directly without further purification.

2'-*O*-methyl- 3',5'-di-*O*-benzoyl-β-L-arabinosyluridine 8

Yield: 55%, R_f = 0.3 (PE:EA=1:1), m.p. 223-224 °C, $[\alpha]^{20}_{D}$= -40.0 (*c* = 0.050, CH_3OH).

^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 9.02 (s, 1H), 8.10-8.05 (m, 4H), 7.64-7.57 (m, 3H), 7.51-7.44 (m, 4H), 6.31 (d, *J* = 3.7 Hz, 1H), 5.68 (d, *J* = 8.1 Hz, 1H), 5.48 (s, 1H), 4.84-4.64 (m, 2H), 4.52 (s, 1H), 4.10 (d, *J* = 3.6 Hz, 1H), 3.41 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 166.4, 165.7, 163.2, 150.4, 142.0, 134.1, 133.5, 130.0 (Cx2), 129.9 (Cx2), 129.7, 128.8 (Cx3), 128.6 (Cx2), 101.4, 85.8, 82.7, 81.3, 76.5, 63.7, 58.8.

HRMS (ESI): M/Z calculated for $C_{24}H_{22}N_2O_8$, $[M+H]^+$: 467.1454, found: 467.1450.

Synthesis of 2'-*O*-methyl-β-L-arabinosyluridine (1)

A solution of **8** (0.37g, 0.8 mmol) in methanolic ammonia (MeOH saturated with NH_3 at 0 °C, 6 mL) was placed in an autoclave and stirred at 130 °C for 12 hrs. After cooling, the mixture was concentrated to dryness and the residue was purified by column chromatography on silica gel to give **1** (0.16 g, 80%) as a white solid.

Yield: 80%, R_f =0.3 (DCM:MeOH = 10:1), m.p. 167-168 °C, $[\alpha]^{20}_{,D}$= -136.0 (*c* = 0.050, CH_3OH).

^{1}H NMR (400 MHz, CD_3OD) *δ*: 7.79 (d, *J* = 8.1 Hz, 1H), 6.23 (d, *J* = 5.2 Hz, 1H), 5.65 (d, *J* = 8.1 Hz, 1H), 4.16 (t, *J* = 4.4 Hz, 1H), 3.90(t, *J* = 4.5 Hz, 1H), 3.83-3.78 (m, 2H), 3.72 (dd, *J* = 11.8, 5.0 Hz, 1H), 3.36 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) *δ*: 166.2, 152.2, 144.1, 101.3, 87.0, 85.6, 85.1, 74.6, 61.8, 59.0.

HRMS (ESI): M/Z calculated for $C_{10}H_{14}N_2O_6$, $[M+H]^+$: 259.0930, found: 259.0930.

Synthesis of 2'-*O*-methyl-α-L-arabinosyluridine (2)

The nucleoside **2** was synthesized as described for **1** starting from crude nucleoside **9**. The reaction mixture was concentrated to dryness and the residue was purified by column chromatography on silica gel to give a crude product. The nucleoside **2** (0.04 g, 14%) was obtained by preparative HPLC purification from crude product.

Yield: 14% (two steps), R_f =0.2 (DCM:MeOH = 10:1), $[\alpha]^{20}_{,D}$= -16.7 (*c* = 0.050, CH_3OH).

^{1}H NMR (400 MHz, CD_3OD) *δ*: 7.75 (d, *J* = 8.1 Hz, 1H), 5.92 (d, *J* = 5.2 Hz, 1H), 5.69 (d, *J* = 8.1 Hz, 1H), 4.34-4.30 (m, 1H), 4.17(t, *J* = 2.7 Hz, 1H), 3.92 (t, *J* = 2.3 Hz, 1H), 3.64 (d, *J* = 5.9 Hz, 1H), 3.48 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) *δ*: 166.4, 152.1, 143.1, 102.0, 92.0, 91.5, 90.3, 75.5, 62.8, 58.5.

ESI-TOF-MS: M/Z calculated for $C_{10}H_{14}N_2O_6$, $[M+H]^+$: 259.0930, found: 259.0936.

Synthesis of 2',3',5'-tri-O-acetyl-β-L-uridine (10)

2',3',5'-Tri-*O*-acetyl-β-L-uridine was synthesized as described for 2'-*O*-methyl-3',5'-di-*O*-benzoyl-arabinosyluridine to give a white solid **10** (60%).

Yield: 60%, R_f = 0.6 (PE:EA = 1:3), m.p. 55-56 °C, $[\alpha]^{20}_D$= -21.2 (*c* = 0.052, CH_3OH).

^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 8.86 (s, 1H), 7.39 (d, *J* = 8.2 Hz, 1H), 6.04 (d, *J* = 4.7 Hz, 1H), 5.79 (dd, *J* = 8.1, 2.0 Hz, 1H), 5.46-5.21 (m, 2H), 4.38-4.32 (m, 3H), 2.32-2.04 (m, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 170.2, 169.8 (Cx2), 162.5, 150.2, 139.4, 103.6, 87.7, 80.1, 72.9, 70.3, 63.3, 20.9, 20.6, 20.5.

HRMS (ESI): M/Z calculated for $C_{15}H_{18}N_2O_9$, $[M+H]^+$: 371.1091, found: 371.1090.

Synthesis of β-L-uridine (11)

The uridine **11** was synthesized as described for **1** starting from **10** to give a white solid.

Yield: 80%, R_f = 0.3 (DCM:MeOH = 6:1), m.p. 168-170 °C, $[\alpha]^{20}_D$= -8.0 (*c* = 0.050, CH_3OH).

^{1}H NMR (400 MHz, DMSO) *δ*: 11.32 (s, 1H), 7.88 (dd, *J* = 8.1, 2.0 Hz, 1H), 5.77 (dd, *J* = 5.1, 1.7 Hz, 1H), 5.64 (dd, *J* = 8.0, 1.9 Hz, 1H), 5.40 (d, *J* = 3.6 Hz, 1H), 5.11 (d, *J* = 4.5 Hz, 2H), 4.02 (d, *J* = 3.5 Hz, 1H), 3.96 (s, 1H), 3.84 (s, 1H), 3.69-3.51 (m, 2H).

^{13}C NMR (101 MHz, DMSO) *δ*: 163.2, 150.8, 140.8, 101.8, 87.8, 84.9, 73.6, 70.0, 60.9.

HRMS (ESI): M/Z calculated for $C_9H_{12}N_2O_6$, $[M+H]^+$: 245.0774, found: 245.0771.

Synthesis of 2,2'-anhydro-β-L-arabinosyluridine (12)

To a solution of uridine **11** (0.98 g, 4.0 mmol) in anhydrous DMF (2 mL) was added diphenyl carbonate (0.94 g, 4.4 mmol) and sodium bicarbonate (0.02 g, 0.24 mmol)

under nitrogen. The mixture was heated at 100 °C for 4 hrs under nitrogen. Then, the mixture was cooled to room temperature and diethyl ether (40 mL) was added. After stirring a further period of 30 mins, the mixture was filtered and the residue was recrystallized from CH_3OH to afford **12** (0.63 g, 70%) as a white solid.

Yield: 70%, R_f = 0.35 (DCM:MeOH:Ammonium = 40:10:1), m.p. 243-244 °C, $[\alpha]^{20}_{,D}$= -48.0 (*c* = 0.050, CH_3OH)

^{1}H NMR (400 MHz, DMSO) *δ*: 7.84 (d, *J* = 7.4 Hz, 1H), 6.31 (d, *J* = 5.6 Hz, 1H), 5.90 (s, 1H), 5.84 (d, *J* = 7.4 Hz, 1H), 5.20 (d, *J* = 5.6 Hz, 1H), 4.99 (s, 1H), 4.38 (s, 1H), 4.07 (s, 1H), 3.29-3.17 (m, 2H).

^{13}C NMR (101 MHz, DMSO) *δ*: 171.2, 159.8, 136.9, 108.6, 90.0, 89.2, 88.8, 74.8, 60.9.

HRMS (ESI): M/Z calculated for $C_9H_{10}N_2O_5$, $[M+H]^+$: 227.0668, found: 227.0671.

Synthesis of 2'-*O*-methyl-β-L-uridine (3)

2,2′-Anhydrouridine **12** (0.57 g, 2.5 mmol) was added to a freshly prepared solution of 12% magnesium methoxide (7 mL, 9.8 mmol) in anhydrous methanol. The reaction mixture was refluxed under nitrogen. Once TLC monitoring showed disapperance of the starting material, the reaction was quenched with *sat.* NH_4Cl. After cooling, the mixture was concentrated to dryness and the residue was purified by column chromatography on silica gel to give nucleoside **3** as a white solid (0.48 g, 75%).

Yield: 75%, R_f = 0.3 (DCM:MeOH = 10:1), m.p. 158-159 °C, $[\alpha]^{20}_{,D}$= -44.0 (*c* = 0.050,CH_3OH).

^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.10 (d, *J* = 8.1 Hz, 1H), 5.94 (d, *J* = 3.4 Hz, 1H), 5.69 (d, *J* = 8.1 Hz, 1H), 4.24 (t, *J* = 5.6 Hz, 1H), 4.04 – 3.94 (m, 1H), 3.91-3.82 (m, 2H), 3.74 (dd, *J* = 12.3, 2.6 Hz, 1H), 3.52 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) *δ*: 166.2, 152.1, 142.4, 102.5, 88.8, 86.1, 85.0, 69.7, 61.6, 58.8.

HRMS (ESI): M/Z calculated for $C_{10}H_{14}N_2O_6$, $[M+H]^+$: 259.0930, found: 259.0935.

Supporting Information

Supporting information text

Supporting Information File 1:

File Name: NMR spectra, and X-ray crystal data

File Format: .pdf

Title: Supporting Information of Total synthesis of 2'-*O*-methyl-β-L-arabinofuranosyl uracil and reassignment the nucleoside structure from *Penicillium sp.* as 2'-*O*-methyl-β-L-uridine

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References

1. Aksoy, S. C.; Uzel, A.; Bedir, E. *J. Antibiot.* **2016**, *69*, 51-56.
2. Ferrero, M.; Goto, V. *Monatsh. Chem.* **2000**, *131*, 59-90.
3. Li, H.; Zhu, S.; Zheng, G. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 1071-1076.
4. Montaser, R.; Luesch, H. *Future Med. Chem.* **2011**, *3*, 1475-1489.
5. Liu, J.; Ingale, S. A.; Seela, F. *Bioconjugate Chem.* **2018***, 29,* 2265-2277.

6. Ban, J.; Shabbir, S.; Lim, M.; Lee, B.; Rhee, H. *Synthesis* **2017**, *49*, 4299-4302.
7. Wang, P.; Agrofoglio, L. A.; Newton, M. G.; Chu, C. K. *J. Org. Chem.* **1999**, *64*, 4173-4178.
8. Patel, M. J.; Akhani, R. C.; Patel, A. T.; Dedania, S. R.; Patel, D. H. *Enzyme Microb. Technol.* **2017**, *97*, 27-33.
9. Dienstag, J. L.; Schiff, E. R.; Wright, T. L.; Perrillo, R. P.; Hann, H. W. L.; Goodman, Z.; Crowther, L.; Condreay, L. D.; Woessner, M.; Rubin, M. *New Engl. J. Med.* **1999**, *341*, 1256-1263.
10. Li, X.-S.; Tan, M.-H.; Lv, M.-M.; Liu, C.-X.; Guo, Z.-Y.; Zhang, L.; Zou, K.; Chen, H.-Y.; Proksch, P. *Phytochem. Lett.* **2017**, *20*, 285-288.
11. Zhao, H.; Bai, H.; Jing, Y.; Li, W.; Yin, S.; Zhou, H. *Nat. Prod. Res.* **2017**, *31*, 945-950.
12. Ding, H.; Li, W.; Ruan, Z.; Yang, R.; Mao, Z.; Xiao, Q.; Wu, J. *Beilstein J. Org. Chem.* **2014**, *10*, 1681-1685.
13. Sun, Z.; Zhu, Y.; Huang, H.; Song, X.; Xiao, Q. *Chinese J. Org. Chem.* **2016**, *36*, 2729-2734.
14. Hu, C.; Ruan, Z.; Ding, H.; Zhou, Y.; Xiao, Q. *Molecules* **2017**, *22*, 643.
15. Li, C.; Ding, H.; Ruan, Z.; Zhou, Y.; Xiao, Q. *Beilstein J. Org. Chem.* **2017**, *13*, 855-862.
16. Ding, H.; Li, C.; Dong, X.; Cao, B.; Zhang, N.; Hong, S.; Xiao, Q. *Chinese J. Org. Chem.* **2018**, *38*, 3351-3355.
17. Ding, H.; Ruan, Z.; Kou, P.; Dong, X.; Bai, J.; Xiao, Q. *Marine Drugs* **2019**, *17*, 226.
18. Miah, A.; Reese, C. B.; Song, Q.; Sturdy, Z.; Neidle, S.; Simpson, I. J.; Read, M.; Rayner, E. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3277-3284.
19. Suck, D.; Saenger, W. *Acta Crystallogr. Sect. B* **1973**, *29*, 1323-1330.
20. Delbaere, L. T. J.; James, M. N. G. *Acta Crystallogr. Sect. B* **1973**, *29*, 2905-2912.

21. Sweeney, J. B.; Bethel, P. A.; Gill, D. M.; Ochocinska, A. M.; Walsh, A. E. J.; Walton, S. M. *Org. Lett.* **2019**, *21*, 2004-2007.

22. Ma, T.; Pai, S. B.; Zhu, Y. L.; Lin, J. S.; Shanmuganathan, K.; Du, J.; Wang, C.; Kim, H.; Newton, M. G.; Cheng, Y. C. *J. Med. Chem.* **1996**, *39*, 2835-2843.

23. Jung, M. E.; Nichols, C. J.; Kretschik, O.; Xu, Y. *Nucleos. Nucleot. Nucl.* **1999**, *18*, 541-546.

24. Crystallographic Data for Ribose **6**: $C_{26}H_{22}O_8$, M = 462.45, Crystal Dimensions 0.3 × 0.28 × 0.25 mm, Colorless Block, Space Group, P21. CCDC 1940346 Contains the Supplementary Crystallographic Data. The Data Can Be Obtained Free of Charge from The Cambridge Crystallographic Date Centre via. Available online: www.ccdc.cam.ac.uk/date_request/cif (accessed on 7 July 2019).

25. Purdie, T.; Irvine, J. C. *J. Chem. Soc.Trans*. **1903**, *83*, 1021-1037.

26. Shioiri, T.; Aoyama, T.; Snowden, T. Encyclopedia of Reagents for Organic Synthesis. **1990,** 1-16.

27. Shohda, K.; Okamoto, I.; Wada, T.; Seio, K.; Sekine, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1795-1798.

28. Remin, M.; Shugar, D. *J. Am. Chem. Soc.* **1973**, *95*, 8146-8156.

29. Sivets, G. G. *Tetrahedron* **2018**, *74*, 920-931.

30. Crystallographic Data for nucleoside **12**: $C_9H_{10}N_2O_5$, M = 226.19, Crystal Dimensions 0.3 × 0.28 × 0.25 mm, Colorless Block, Space Group, P21. CCDC 1940359 Contains the Supplementary Crystallographic Data. The Data Can Be Obtained Free of Charge from The Cambridge Crystallographic Date Centre via. Available online: www.ccdc.cam.ac.uk/date_request/cif (accessed on 7 July 2019).