**Supporting Information**

**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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S1. Synthesis and characterization of intermediate products.

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**S1. Synthesis and characterization of intermediate products.**

**S1.1. 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 (Jung et al., 1999; Ma et al., 1996).

Yield: 52%, R*f* =0.3 (PE:EA=3:1), m.p. 130-131 °C, [α]= -72.0 (*c* = 0.050, CH3OH).

1H NMR (400 MHz, CDCl3) *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) .

13C NMR (101 MHz, CDCl3) *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 C26H22O8, [M+H]+: 463.1393, found: 463.1378.

**S1.2. 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 MgSO4. 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, [α]= -60.0 (*c* = 0.050, CH3OH).

1H NMR (400 MHz, CDCl3) *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).

13C NMR (101 MHz, CDCl3) *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 C27H21F3O10S, [M+H]+: 595.0886, found: 595.0876.

**S1.3. Synthesis of1,3,5-tri-*O*-benzoyl-α-L-arabinofuranose (6)**

The triflate **5** (5.34 g, 9 mmol, 1 eq.) was dissolved in DMF (90 mL) and KNO2 (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 (Na2SO4) 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, [α]= -64.8 (*c* = 0.054, CH3OH).

1H NMR (400 MHz, CDCl3) *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).

13C NMR (101 MHz, CDCl3) *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 C26H22O8, [M+H]+:463.1393, found: 463.1395.

**S1.4. Synthesis of1,3,5-tri-*O*-benzoyl-2-*O*-methyl-α-L-arabinofuranose (7)**

A mixture of arabinofuranose **6** (1.85 g, 4 mmol, 1 eq.) and HBF4 (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, [α]= -70.7 (*c* = 0.058, CH3OH).

1H NMR (400 MHz, CDCl3) *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).

13C 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 C27H24O8, [M+H]+: 477.1549, found: 477.1541.

**S1.5. 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 MgSO4, 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.

**S1.5. 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, [α]= -40.0 (*c* = 0.050, CH3OH).

1H NMR (400 MHz, CDCl3) *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).

13C NMR (101 MHz, CDCl3) *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 C24H22N2O8, [M+H]+: 467.1454, found: 467.1454.

**S1.6. 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, [α]= -21.2 (*c* = 0.052, CH3OH).

1H NMR (400 MHz, CDCl3) *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).

13C NMR (101 MHz, CDCl3) *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 C15H18N2O9, [M+H]+: 371.1091, found: 371.1092.

**S1.7. 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, [α]= -8.0 (*c* = 0.050, CH3OH).

1H 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).

13C 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 C9H12N2O6, [M+H]+: 245.0774, found: 245.0779.

**S1.8. 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 CH3OH to afford **12** (0.63 g, 70%) as a white solid.

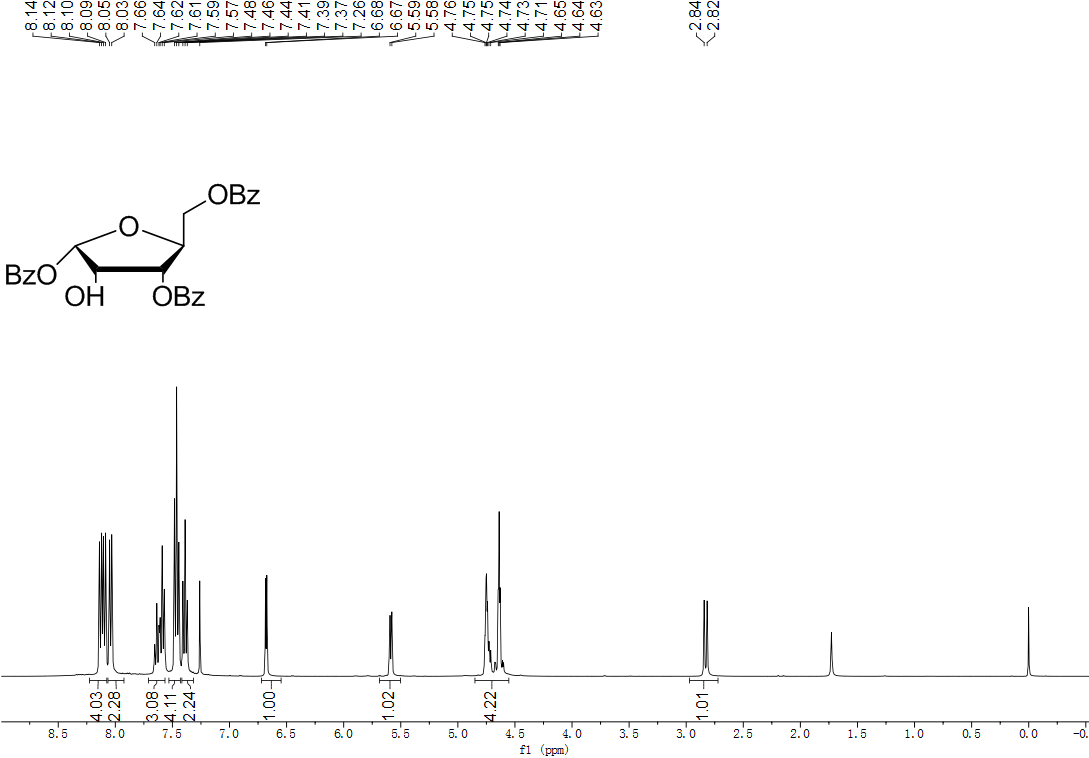
Yield: 70%, R*f* = 0.35 (DCM:MeOH:Ammonium = 40:10:1), m.p. 243-244 °C, [α]= -48.0 (*c* = 0.050, CH3OH)

1H 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).

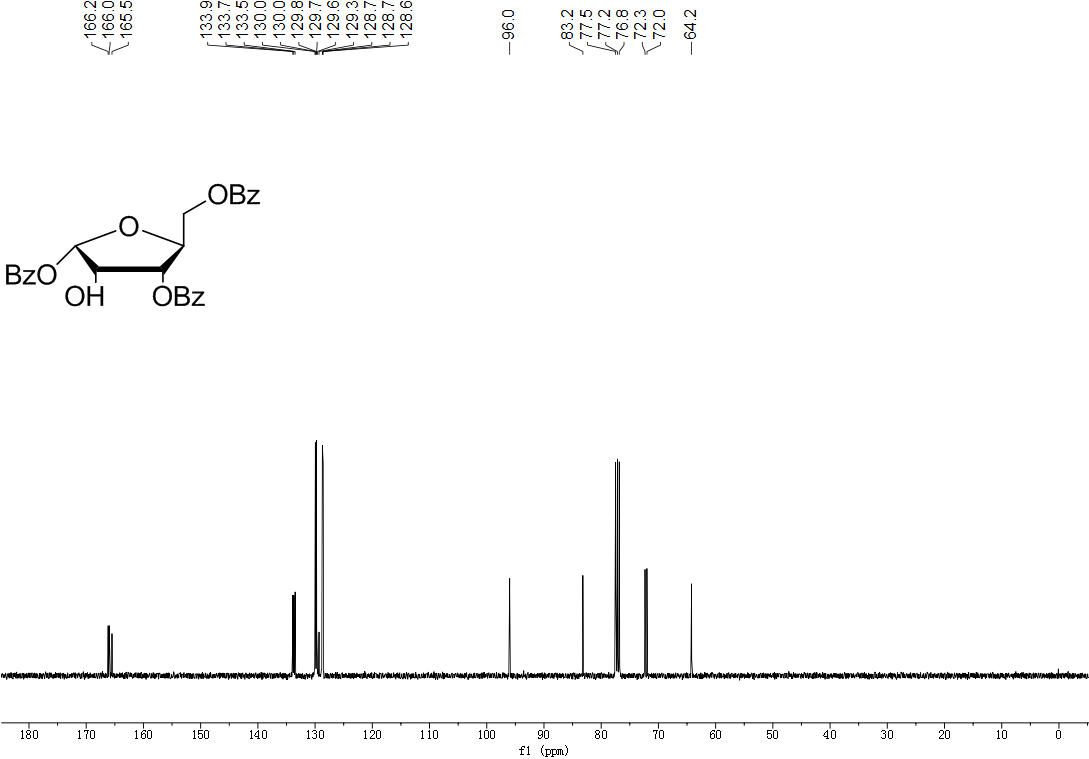
13C NMR (101 MHz, DMSO) *δ*: 171.2, 159.8, 136.9, 108.6, 90.0, 89.2, 88.8, 74.8, 60.9.

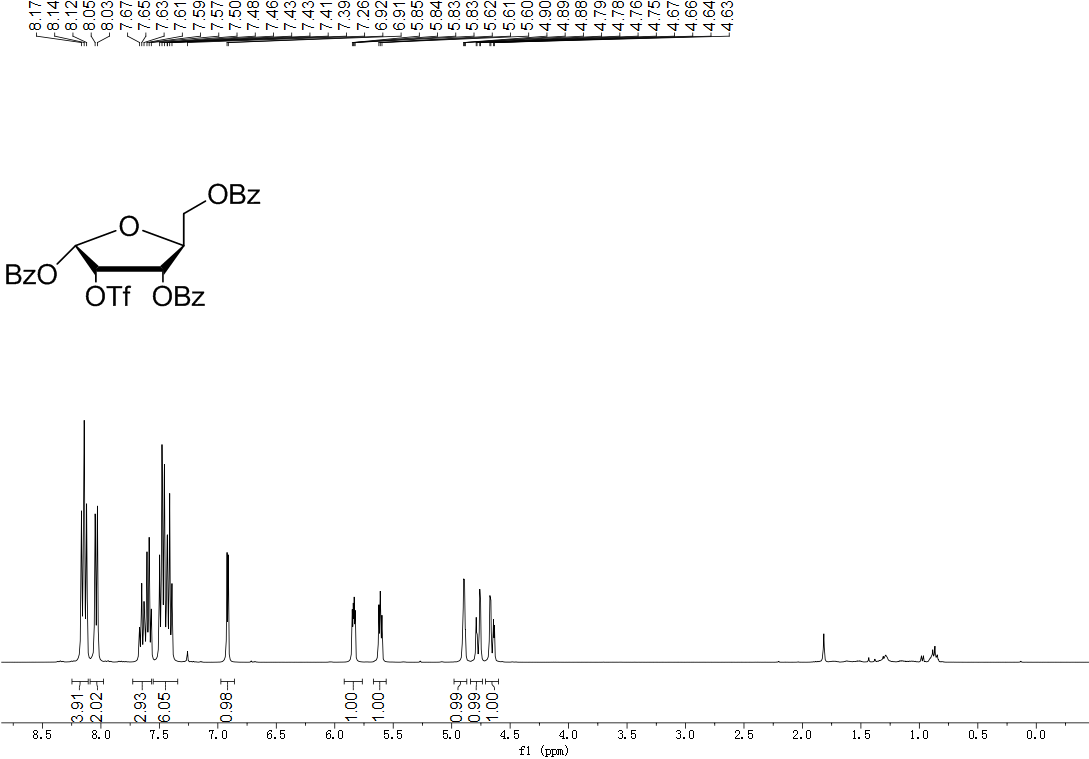
HRMS (ESI): M/Z calculated for C9H10N2O5, [M+H]+: 227.0668, found: 227.0669.

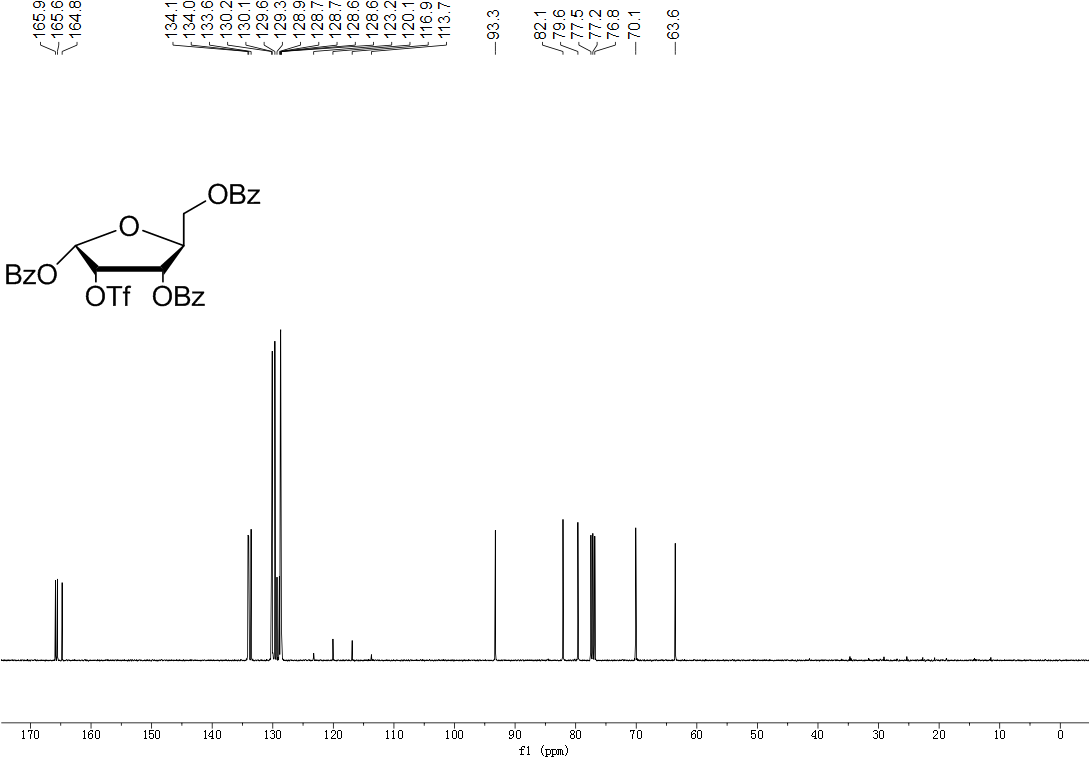
**S2. NMR spectra.**

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


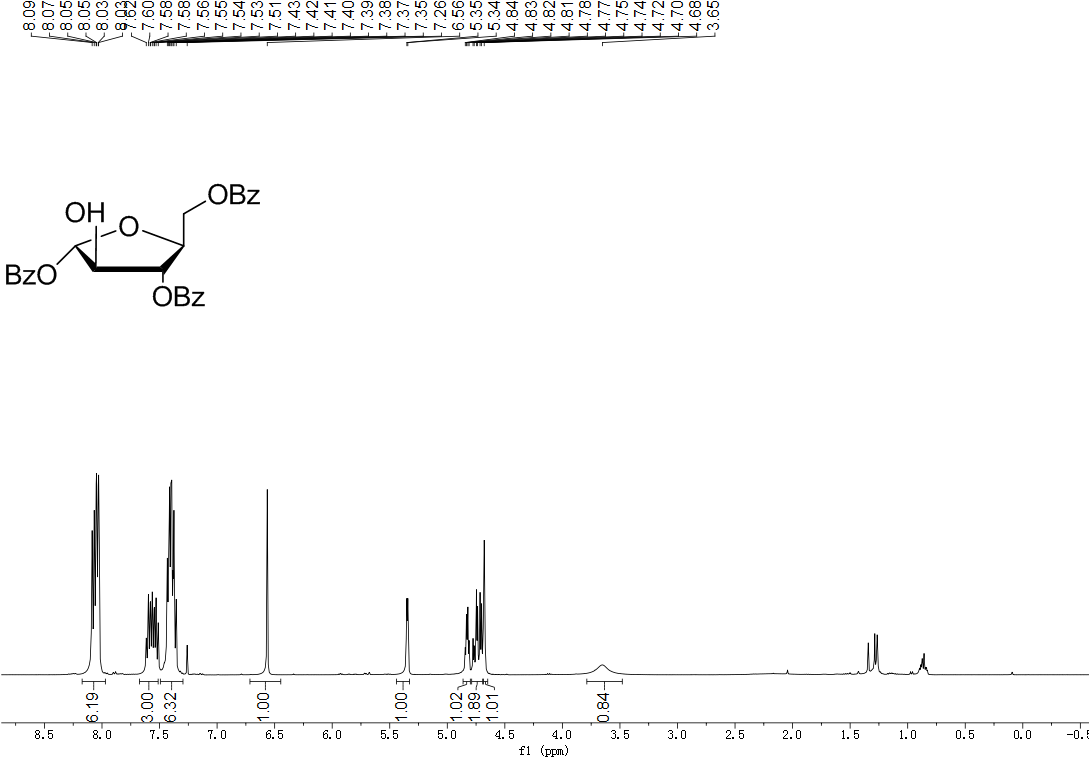
**13C NMR spectrum of 1,3,5-tri-*O*-benzoyl-α-L-ribofuranose (4)**



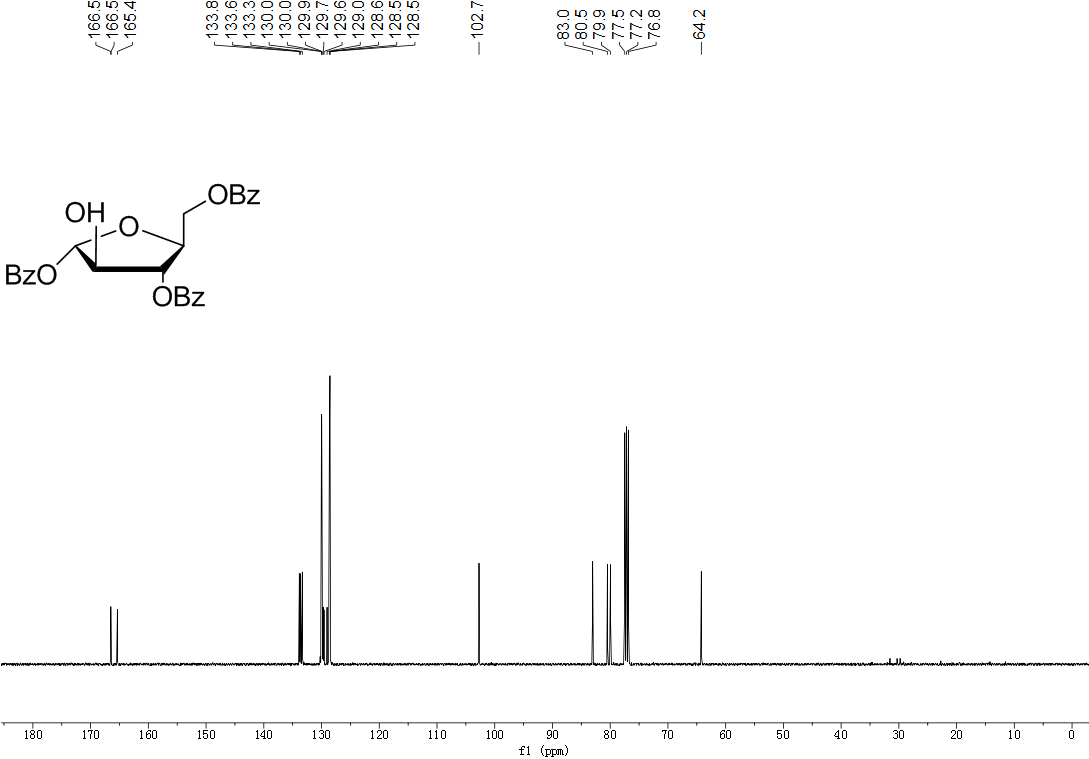
**1H NMR spectrum of 2-*O*-trifluoromethylsulfonyl-1,3,5-tri-*O*-benzoyl-α-L-ribofuranose (5)** 

**13C NMR spectrum of 2-*O*-trifluoromethylsulfonyl-1,3,5-tri-*O*-benzoyl-α-L-ribofuranose (5)**

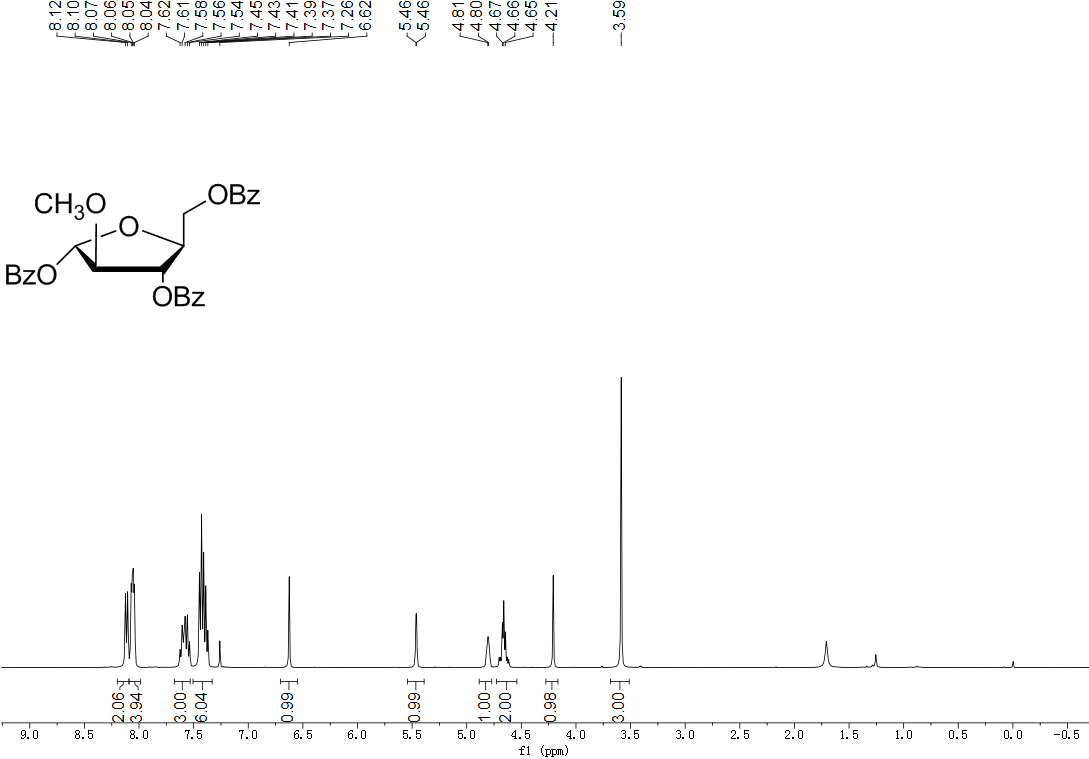
**1H NMR spectrum of 1,3,5-tri-*O*-benzoyl-α-L-arabinofuranose (6)**

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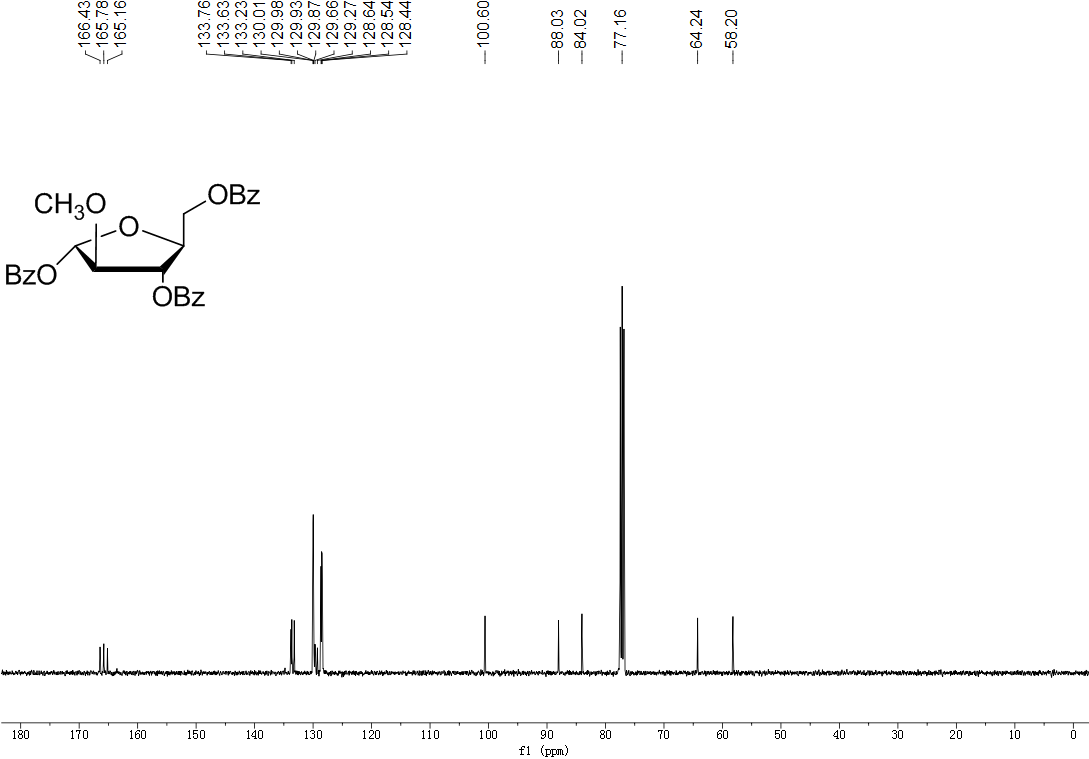
**13C NMR spectrum of 1,3,5-tri-*O*-benzoyl-α-L-arabinofuranose (6)**

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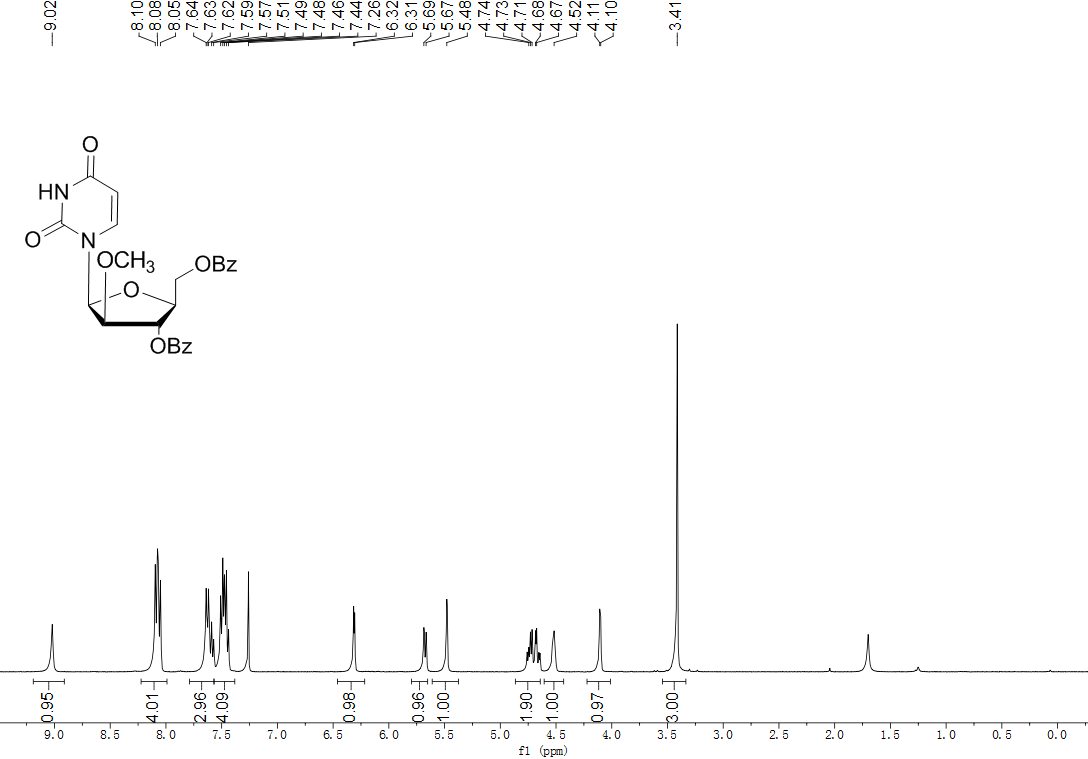
**1H NMR spectrum of 1,3,5-tri-*O*-benzoyl-2-*O*-methyl-α-L-arabinofuranose (7)**

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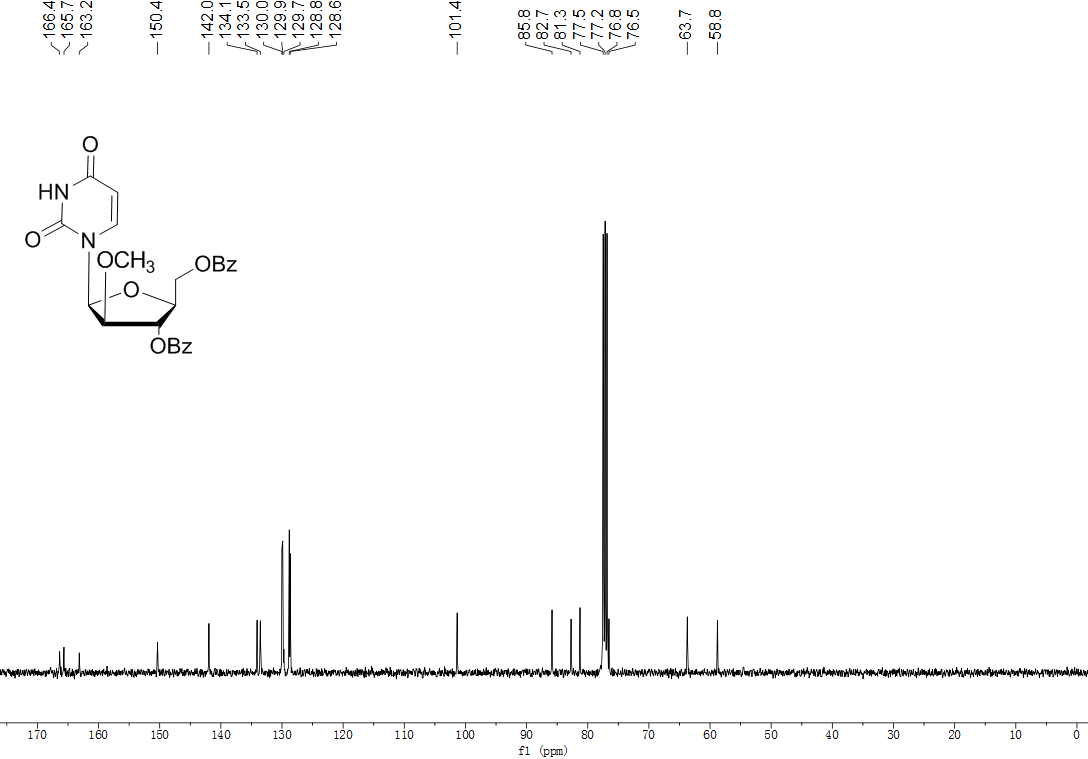
**13C NMR spectrum of 1,3,5-tri-*O*-benzoyl-2-*O*-methyl-α-L-arabinofuranose (7)**

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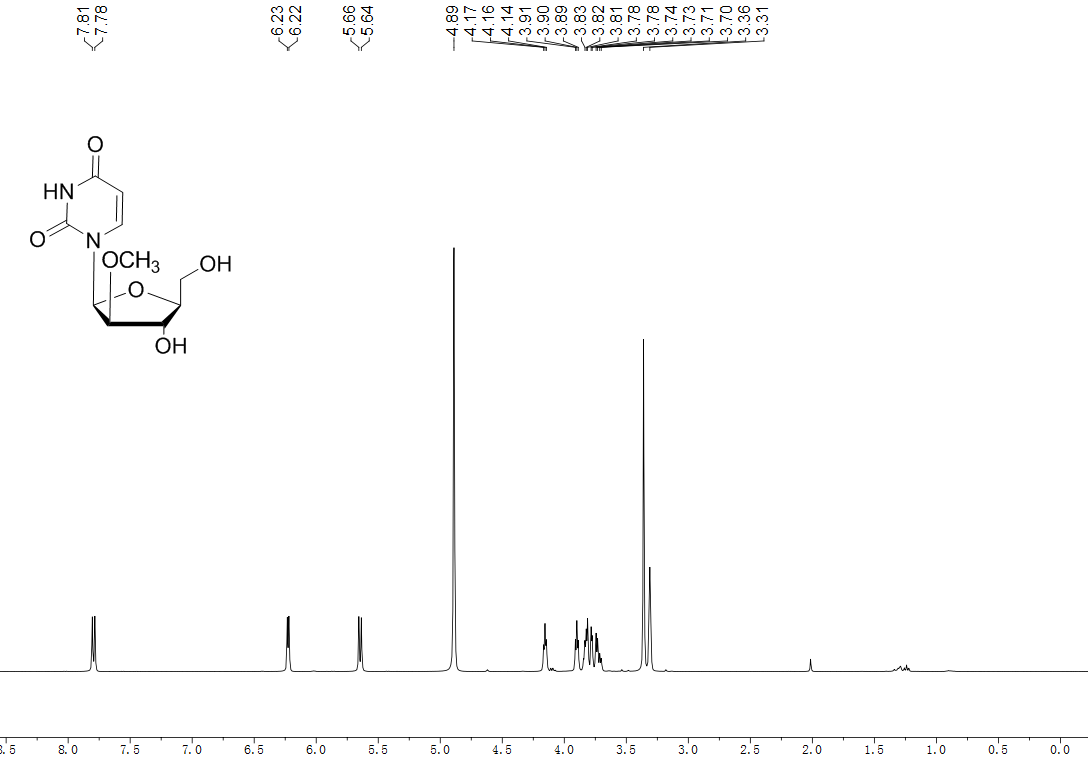
**1H NMR spectrum of 2'-*O*-methyl- 3',5'-di-*O*-benzoyl-β-L-arabinosyluridine (8)**

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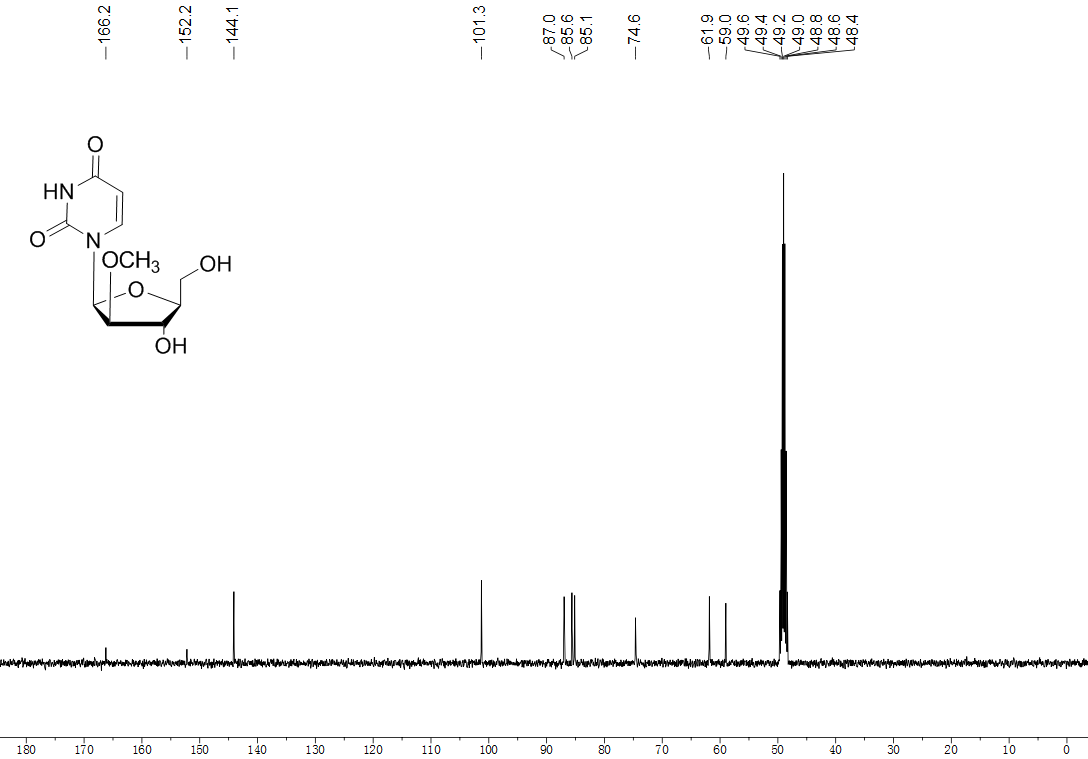
**13C NMR spectrum of 2'-*O*-methyl- 3',5'-di-*O*-benzoyl-β-L-arabinosyluridine (8)**

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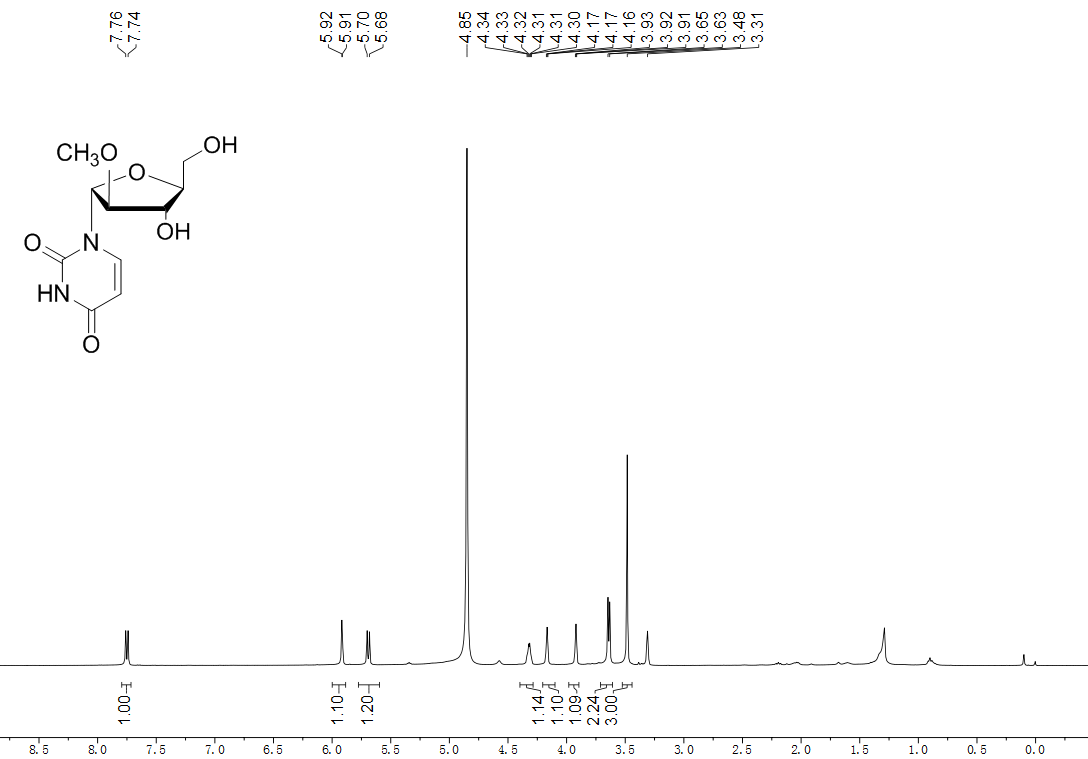
**1H NMR spectrum of 2'-*O*-methyl-β-L-arabinosyluridine (1)**

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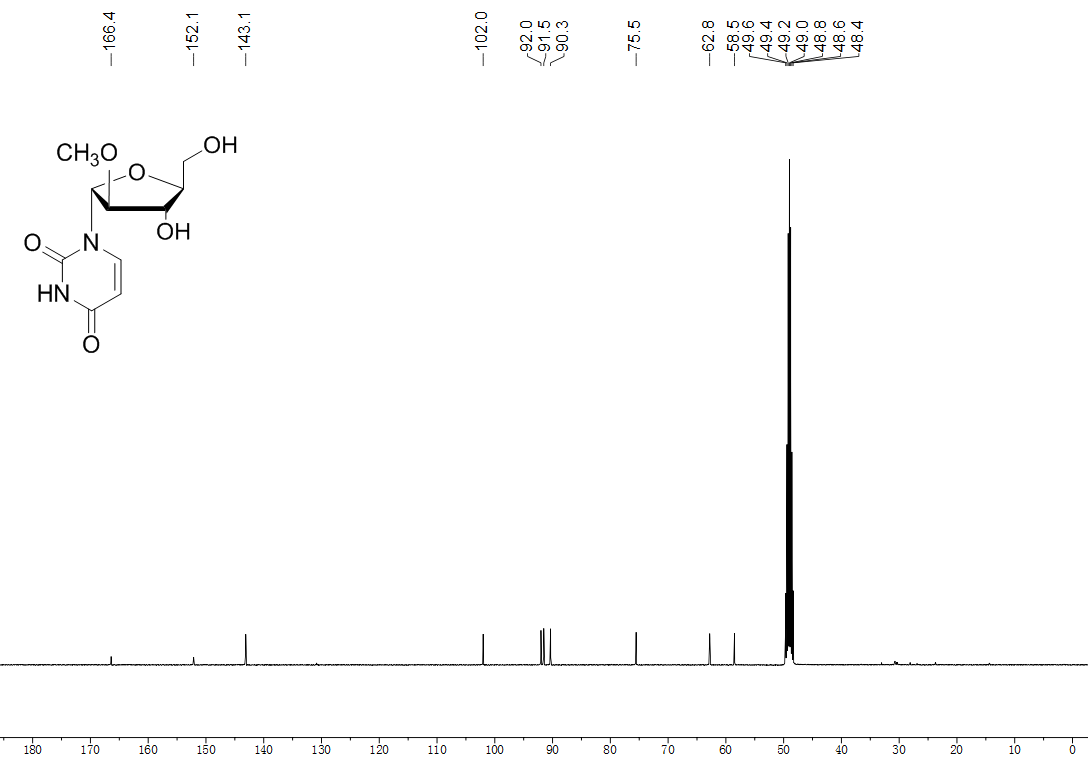
**13C NMR spectrum of 2'-*O*-methyl-β-L-arabinosyluridine (1)**

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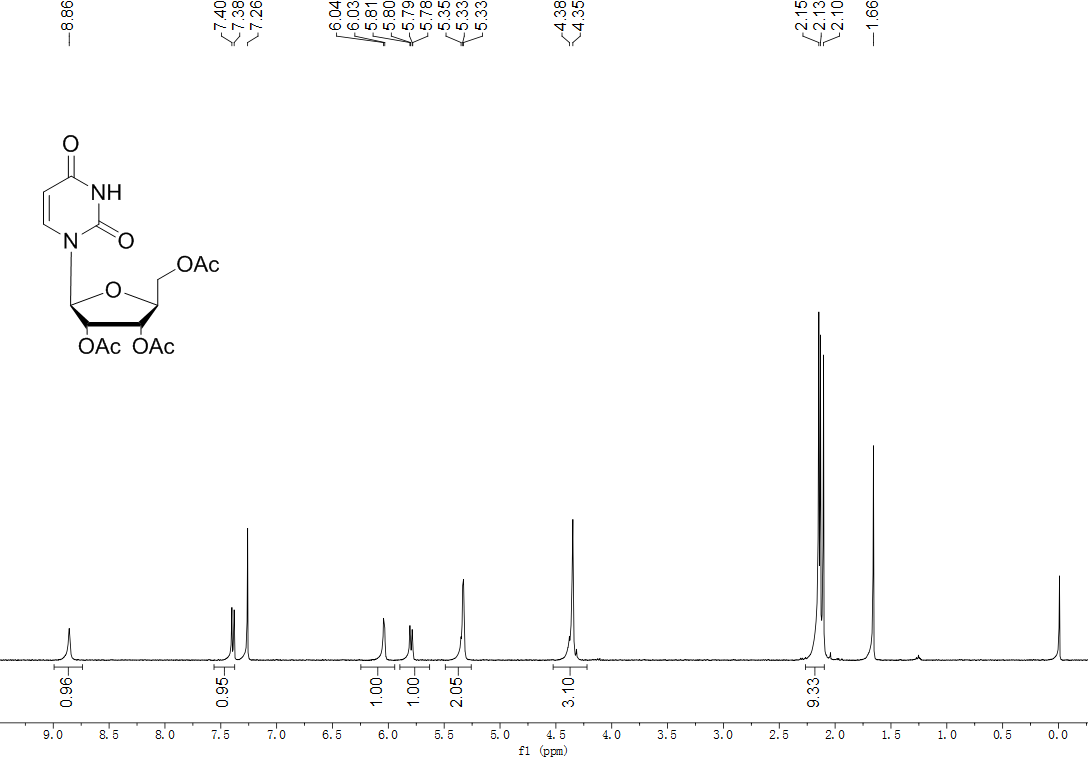
**1H NMR spectrum of 2'-*O*-methyl-α-L-arabinosyluridine (2)**

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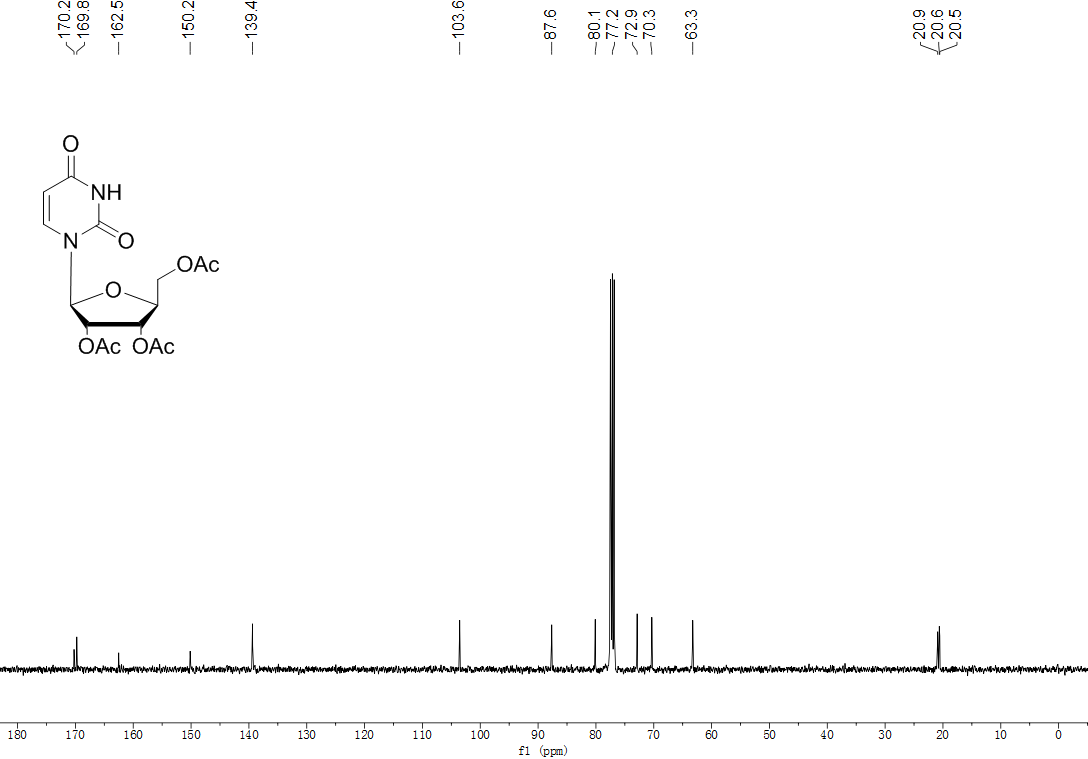
**13C NMR spectrum of 2'-*O*-methyl-α-L-arabinosyluridine (2)**

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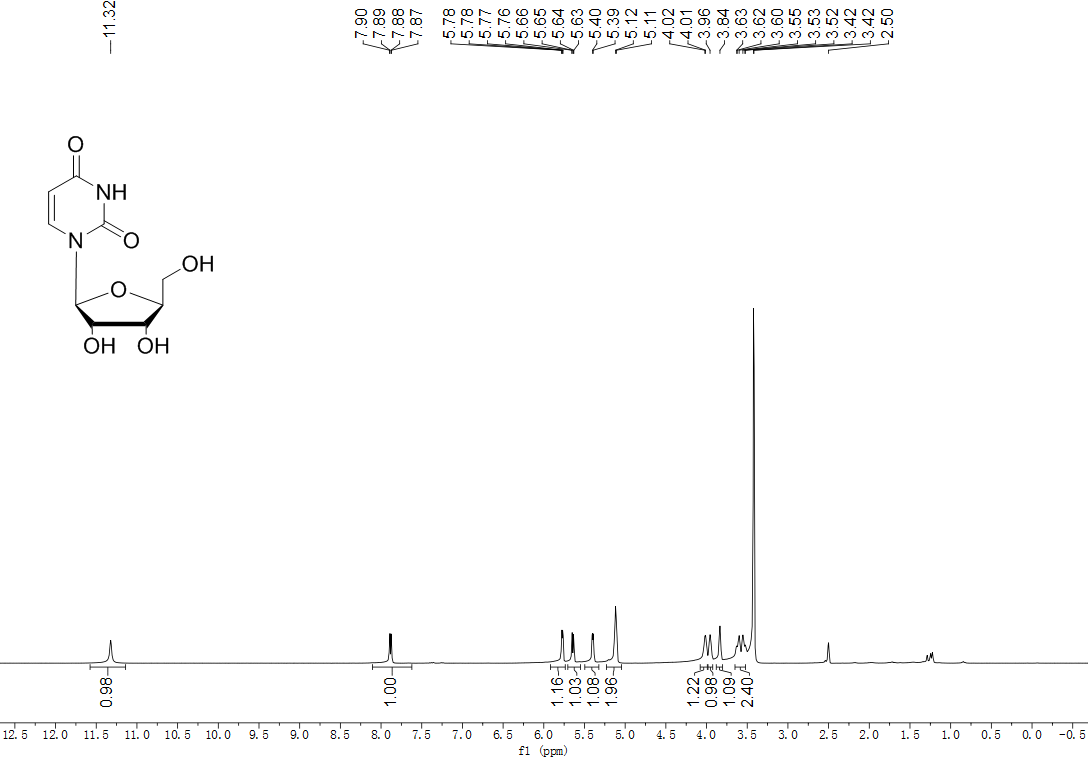
**1H NMR spectrum of 2',3',5'-tri-O-acetyl-β-L-uridine (10)**

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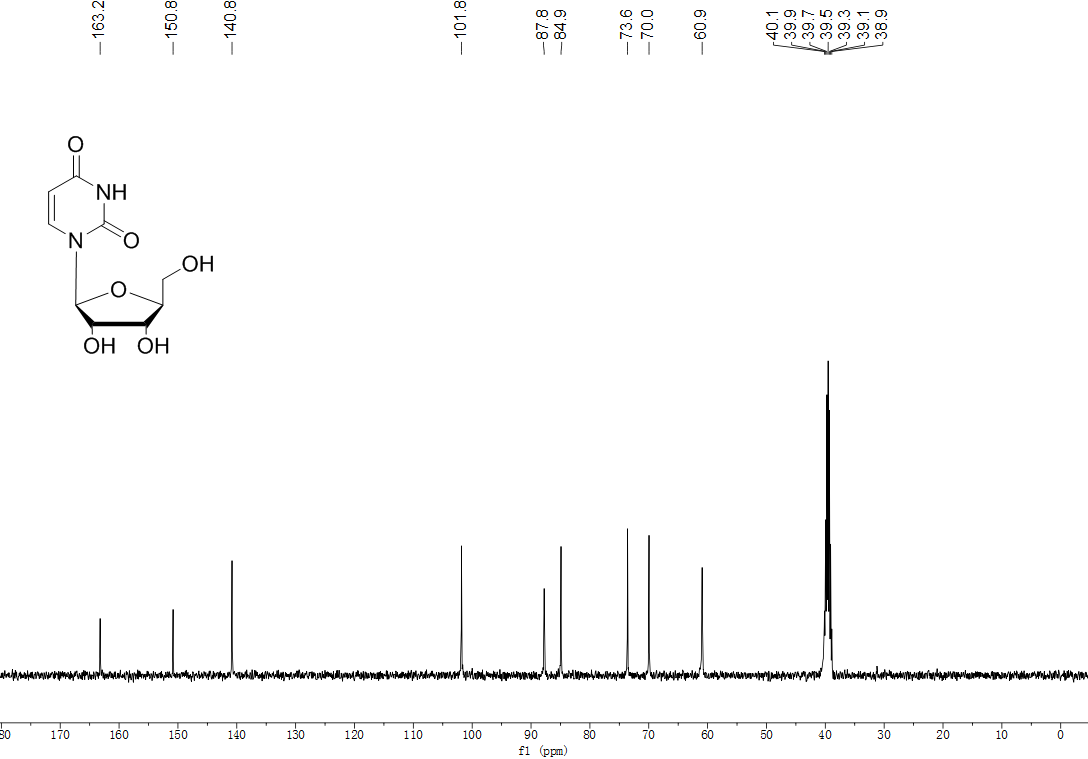
**13C NMR spectrum of 2',3',5'-tri-O-acetyl-β-L-uridine (10)**

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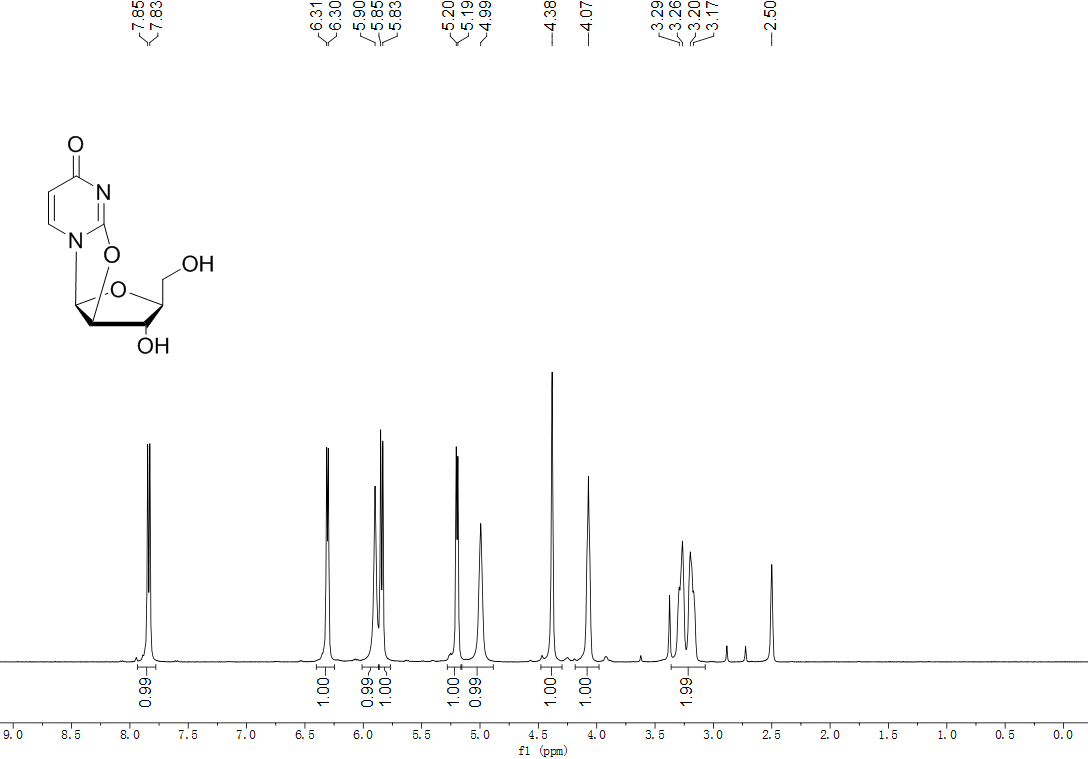
**1H NMR spectrum of β-L-uridine (11)**

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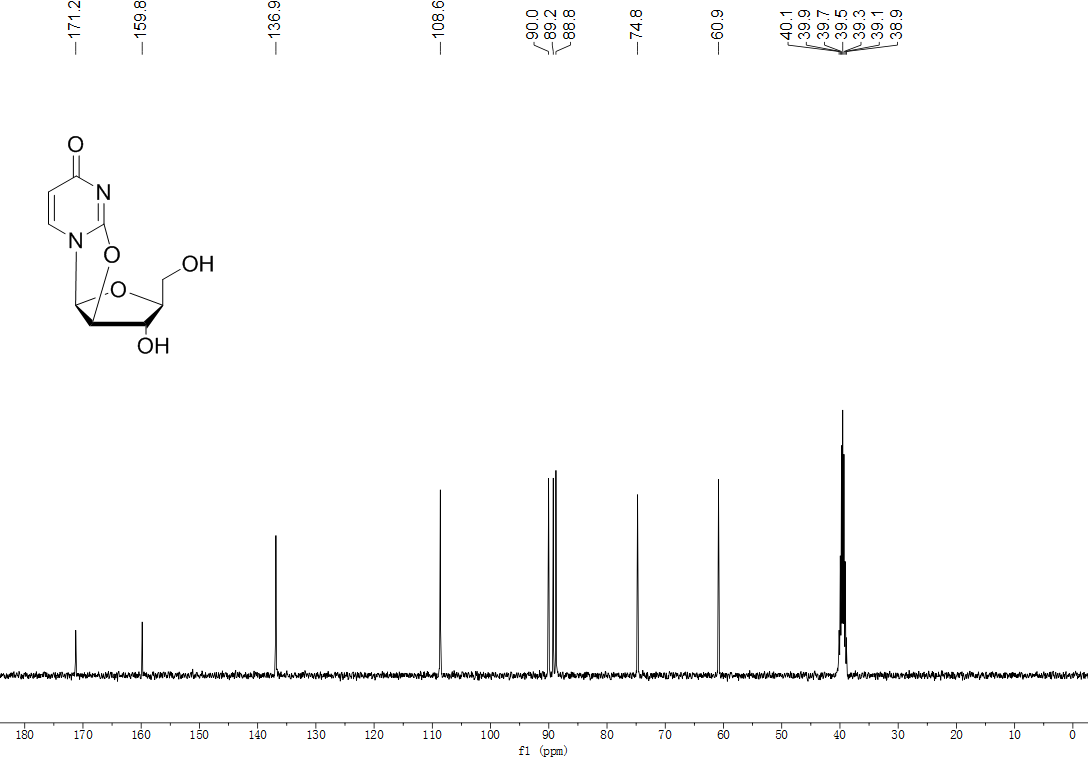
**13C NMR spectrum of β-L-uridine (11)**

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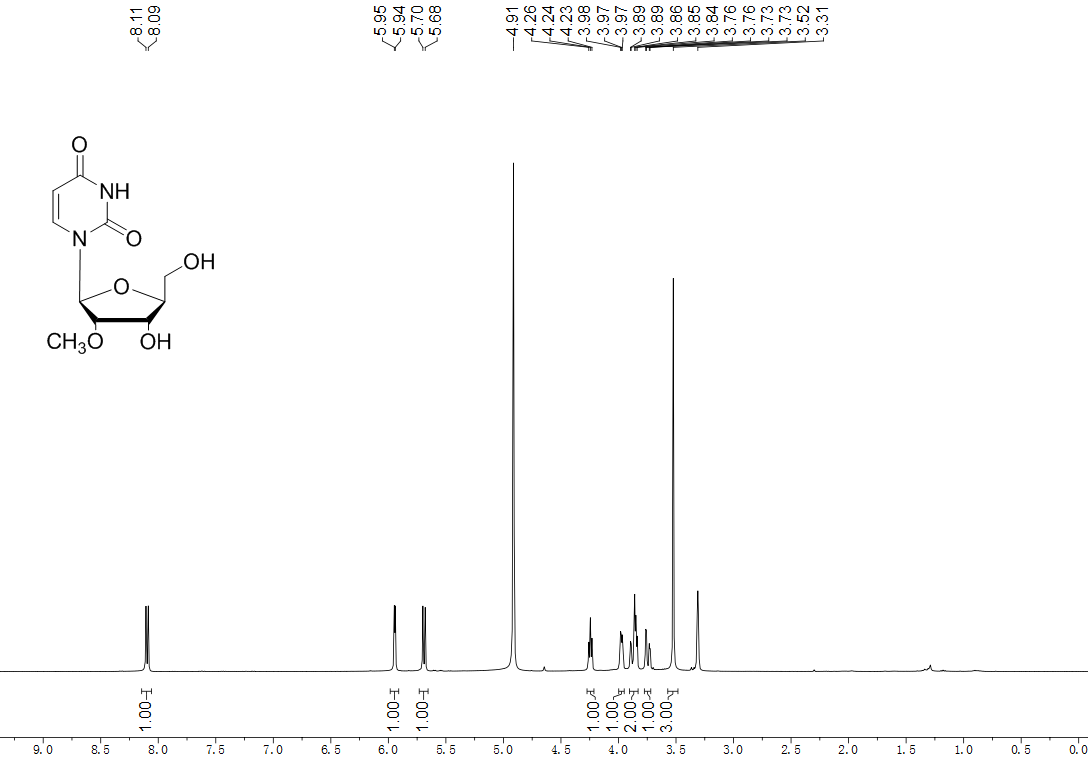
**1H NMR spectrum of 2,2'-anhydro-β-L-arabinosyluridine (12)**

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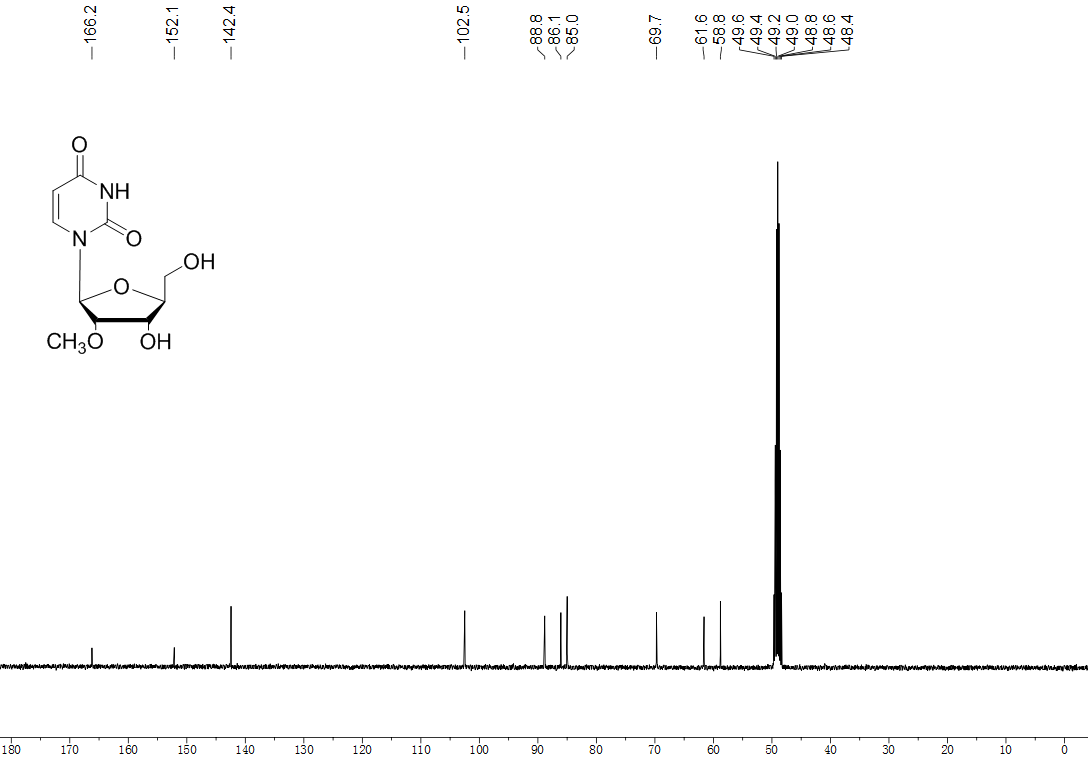
**13C NMR spectrum of 2,2'-anhydro-β-L-arabinosyluridine (12)**

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**1H NMR spectrum of 2'-*O*-methyl-β-L-uridine (3)**

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**13C NMR spectrum of 2'-*O*-methyl-β-L-uridine (3)**



|  |  |
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| **S3. Table S1 Crystal data and structure refinement for 6.** | |
|  | |
| Identification code | 1 |
| Empirical formula | C26H22O8 |
| Formula weight | 462.43 |
| Temperature/K | 296(2) |
| Crystal system | orthorhombic |
| Space group | P2(1)2(1)2(1) |
| a/Å | 5.8763(16) |
| b/Å | 17.706(5) |
| c/Å | 24.914(7) |
| α/° | 90 |
| β/° | 90 |
| γ/° | 90 |
| Volume/Å3 | 2592.2(12) |
| Z | 4 |
| ρcalcg/cm3 | 1.185 |
| μ/mm‑1 | 0.088 |
| F(000) | 968 |
| Crystal size/mm3 | 0.280 x 0.260 x 0.240 |
| Radiation | MoKα (λ = 0.71073) |
| 2Θ range for data collection/° | 5.418 to 49.99 |
| Index ranges | -6 ≤ h ≤ 6, -11 ≤ k ≤ 21, -29 ≤ l ≤ 26 |
| Reflections collected | 10840 |
| Independent reflections | 4518 [R(int) = 0.0398] |
| Data/restraints/parameters | 4518/0/307 |
| Goodness-of-fit on F2 | 1.024 |
| Final R indexes [I>=2σ (I)] | R1 = 0.0698, wR2 = 0.1931 |
| Final R indexes [all data] | R1 = 0.1116, wR2 = 0.2253 |
| Largest diff. peak/hole / e Å-3 | 0.442/-0.263 |

**S4. Table S2.  Optimization of reaction conditions**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Entry | Reagent [equiv] | Solvent | Temp.[ᴼC ] | Time [h] | Yield [%] |
| 1 | TMSCHN2(3eq), HBF4(42%)(1eq) | DCM | r.t. | 20 | 70 |
| 2 | TMSCHN2(3eq), SiO2(3eq) | DCM | 0 | 20 | 42 |
| 3 | TMSCHN2(3eq),BF3OEt2(40%)(1.1eq),  TBAF·AcOH·THF(48%)(2.2eq) | DCM | r.t. | 20 | 25 |
| 4 | TMSCHN2(2eq), HBF4(42%)(1eq) | DCM | r.t. | 20 | 70 |
| 5 | CH3I(5eq), Ag2O(2.5eq) | DMF | 0 | 6 | 53 |
| 6 | CH3I(5eq), Ag2O(2.5eq) | DMF | r.t. | 5 | 58 |

|  |  |
| --- | --- |
| **S5. Table S3 Crystal data and structure refinement for 12.** | |
|  | |
| Identification code | 1 |
| Empirical formula | C18H20N4O10 |
| Formula weight | 452.38 |
| Temperature/K | 296(2) |
| Crystal system | orthorhombic |
| Space group | P2(1)2(1)2(1) |
| a/Å | 7.4296(7) |
| b/Å | 13.6558(12) |
| c/Å | 18.1898(16) |
| α/° | 90 |
| β/° | 90 |
| γ/° | 90 |
| Volume/Å3 | 1845.5(3) |
| Z | 4 |
| ρcalcg/cm3 | 1.628 |
| μ/mm‑1 | 0.135 |
| F(000) | 944 |
| Crystal size/mm3 | 0.260 x 0.200 x 0.170 |
| Radiation | MoKα (λ = 0.71073) |
| 2Θ range for data collection/° | 5.382 to 51.99 |
| Index ranges | -8 ≤ h ≤ 9, -16 ≤ k ≤ 15, -22 ≤ l ≤ 19 |
| Reflections collected | 10220 |
| Independent reflections | 3616 [R(int) = 0.0213] |
| Data/restraints/parameters | 3616/0/298 |
| Goodness-of-fit on F2 | 0.930 |
| Final R indexes [I>=2σ (I)] | R1 = 0.0278, wR2 = 0.0693 |
| Final R indexes [all data] | R1 = 0.0311, wR2 = 0.0719 |
| Largest diff. peak/hole / e Å-3 | 0.198/-0.144 |

**S6. References**

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., 1996. Structure− Activity Relationships of 1-(2-Deoxy-2-fluoro-β-l-arabino-furanosyl) pyrimidine Nucleosides as Anti-Hepatitis B Virus Agents. J. Med. Chem. 39, 2835-2843.

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