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新型 2,2'-缩水-*L*-苏糖嘧啶膦酸核苷的合成

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摘要 首先通过九步反应合成了一系列 *L*-苏糖嘧啶核苷膦酸酯. 用二乙胺基三氟化硫(DAST)处理 *L*-苏糖嘧啶核苷膦酸酯, 分别以中高收率得到相应的 2,2'-缩水-1-(*L*-苏糖)尿嘧啶核苷膦酸酯类似物. 对所得到的 *L*-苏糖嘧啶核苷膦酸酯和 2,2'-缩水-*L*-苏糖嘧啶膦酸核苷进行了初步的生物学评价, 未发现任何活性.

关键词 2,2'-缩水核苷; 二乙胺基三氟化硫(DAST); *L*-苏糖嘧啶膦酸酯

Synthesis of Novel 2,2'-Anhydro-*L*-threosylpyrimidine Phosphonates

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Abstract A series of *L*-threoylnucleoside phosphonates were synthesized via a nine-step procedure firstly. When the *L*-threoylpyrimidine nucleoside phosphonate was treated by diethylaminosulphur trifluoride (DAST), all the tried pyrimidine *L*-threosylnucleoside phosphonate analogues produced corresponding 2,2'-anhydro-1-(*L*-threoyl)pyrimidine phosphonates in medium-high yields. The obtained *L*-threoylpyrimidine nucleoside phosphonates and 2,2'-anhydro-*L*-threosylpyrimidine phosphonic acids have not shown any activity by primary biological evaluations.

Keywords 2,2'-anhydronucleoside; diethylaminosulphur trifluoride (DAST); *L*-threosylpyrimidine phosphonate

1 Introduction

The viral infection has often posed a serious threat to global human health. Nucleoside drugs play a very important role in the treatment of viral infections. One of the most representative drugs is Remdesivir, developed by Gilead Sciences,[1] which was used as first-line drug in treatment of COVID-19 in the early days of the coronavirus outbreak in several countries. But one clinical report showed that remdesivir did not respond well in patients with the advance of the disease.[2] Scientists need to make efforts to develop new drugs for treatment of new emergences and mutations of viruses.

Anhydronucleosides are a class of nucleosides in which the sugar ring and the base are connected not only by the glycosidic bond but also by the C—O—C, C—O—N or C—S—C bonds. 2,2'-Anhydrouridine and 2,2'-anhydrocytidine are representatives of this kind of compounds. Because of their special structure, anhydronucleosides can be used as a class of drugs with potential biological activity, such as 2,2'-anhydrocytidine, which releases potent antitumor cytarabine in the body under the action of enzymes.[3] Because 2,2'-anhydronucleoside can be opened by various nucleophilic agents to introduce different groups, it is also used as an important class of intermediates in nucleoside modifications. For example, the nucleophilic attacking can introduce various potential active groups at C-2' of the sugar ring such as azide group[4] or fluorine atom.[5] 2,2'-Anhydronucleoside can be eliminated by telluride-mediated elimination[6] to form olefinic bond on the sugar ring to synthesize unsaturated nucleosides. Different methods for constructing 2,2'-anhydronucleosides have been reported in the literatures. The classical method is treatment of uridine or thymidine with diphenyl carbonate and sodium bicarbonate in *N*,*N*-dimethylformamide (DMF).[7-8] Robles *et al*.[9] found that 2,2'-anhydro-nucleosides can be obtained smoothly by reductive dehalogenation of 2'-deoxy-2'-iodinethimidine with tributyltin hydride. The 2,2'-anhydro linkage

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can also be directly formed by selective trifluoromethanesulfonylation of 2'-hydroxyl group of uridine,[10] or formed by a two-step procedure including selective methanesulfonylation of 2'-hydroxyl of uridine and following base treatment.[11] Diethylaminosulphur trifluorid (DAST)[12-13] is often used as fluorinating reagent for direct conversion of hydroxyl or carbonyl groups. It can also be used to form 2,3'-anhydropyrimidines[14-15] and 2,2'-anhydropyrimidines.[16] Among these methods, DAST is a facile and simple method to form anhydro-linkage in pyrimidine nucleosides with a satisfactory yield.

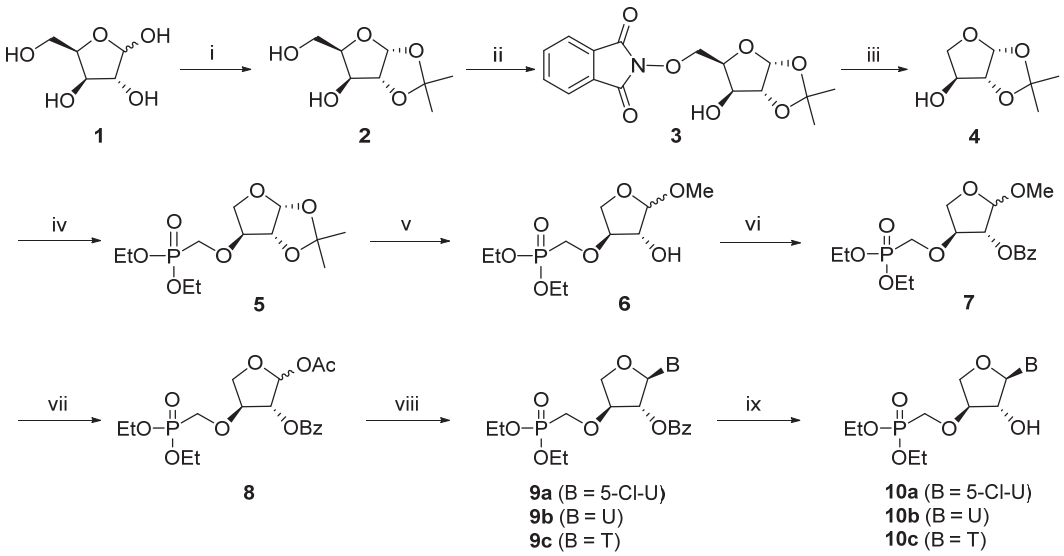
Based on the superior antiviral properties of *L*-threosphosphonate nucleosides, we attempted to synthesize a series of 2,2'-anhydro-*L*-threosphosphonate nucleosides to find some potential bioactive compounds. When 3'-*O*-phosphonate *L*-threosyluridine was treated with DAST, the corresponding novel 3-*O*-phosphonate 2,2'-anhydrouridine analogues were obtained. Herein we describe the research work which has not been reported in literatures described.

2 Results and discussion

In order to obtain the target compounds, the intermediates **10a**～**10c** were synthesized through a multistep procedure (Scheme 1) according to literatures.[17-21] The procedure mainly involves the construction of 1,2-*O*-isopropylidene *L*-threose from *D*-xylose, introduction of 3'-*O*-phosphonate, conversion of 1,2-*O*-isopropylidene to 1,2-di-*O*-acyl groups, glycosylation and the removal of protective groups. *D*-xylose (**1**) was treated with acetone in the presence of concentrated H_2SO_4 to give 1,2-*O*-iso-propylidene *D*-xylose (**2**).[17-18] Condensation of compound **2** with *N*-hydroxyphthalimide under Mitsunobu reaction conditions allowed 5-*O*-phthalimide intermediate **3**. Under classical tin conditions (Bu_3SnH/AIBN), *β*-fragmentation of intermediate **3** produced 1,2-*O*-isopropylidene-*L*-threose (**4**).[19] Reaction of **4** with diethyl *p*-tosyloxymethylphosphonate and sodium hydride in anhydrous tetrahydrofuran[20] provided 1,2-*O*-isopropylidene-*L*-threose-3-*O*-phosphonate (**5**). Protected sugar **5** was converted to 1-*O*-acetyl-2-*O*-benzoyl-3-*O*-phosphonate-*L*-threose (**8**) by a previously reported three-step procedure.[21] Then, different bases were introduced by Vorbruggen glycosylation to give the protected phosphonate nucleoside analogues **9a**～**9c**, subsequent debenzoylation of **9a**～**9c** with NH_3/MeOH produced **10a**～**10c**.

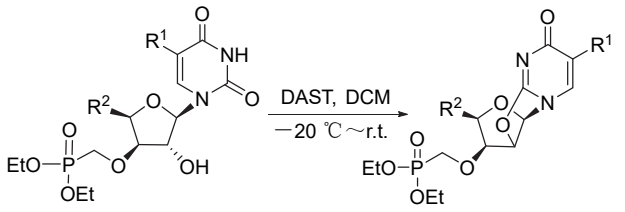
Compound **10a** was dissolved in dry dichloromethane (DCM) and treated with DAST at －20～0 ℃. Subsequent workup gave a new product **11a** as a colourless oil. Compound **11a** was analyzed by high resolution mass spectrum (HRMS) (*m*/*z* 381.0598 $[M+H]^+$). In comparison with compound **10a**, it can be confirmed that **11a** is an anhydro analogue of **10a** rather than the 2'-hydroxyl of **10a** substituted by fluorine atom for the molecular weight being reduced by 18. NMR spectral analysis was used for further structural elucidation of the compound. Comparing the ^1H NMR spectra of compounds **10a** and **11a**, we found that both signal of H-3 (δ 10.71) on the pyrimidine and 2'-OH (δ 5.37) on sugar moiety of compound **10a** disappeared in compound **11a**. Moreover, in the ^1H NMR spectrum of **10a** the signal of H-2' was observed at δ 4.36, the same H signal was shifted to δ 5.51 in compound **11a**. Therefore, we concluded that **11a** is a pyrimidine 2,2'-anhydro-*L*-threonucleoside phosphonate analogue as shown in Scheme 2.

The generality of this method was investigated by utilization of three other pyrimidine *L*-threonucleoside phosphonates in the reaction including above-obtained



Reagents and conditions: (i) acetone, H_2SO_4, 0 ℃～r.t.; (ii) *N*-hydroxyphthalimide, PPh_3, DEAD, THF; (iii) Bu_3SnH, AIBN, toluene, 80 ℃; (iv) diethyl *p*-tosyloxymethylphosphonate, NaH, THF, 0 ℃～r.t.; (v) MeOH, H_2SO_4, 0 ℃～r.t.; (vi) BzCl, pyridine, 0 ℃～r.t.; (vii) AcOH, Ac_2O, H_2SO_4; (viii) base, BSA, CH_3CN, TMSOTf; (ix) NH_3/MeOH, r.t.

Scheme 1 Synthesis of 3'-*O*-phosphonate *L*-threosyluridines from *D*-xylose



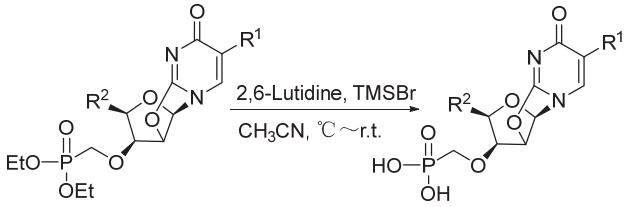
10a: R^1 = Cl, R^2 = H
10b: R^1 = H, R^2 = H
10c: R^1 = Me, R^2 = H
10d: R^1 = H, R^2 = Me

11a: R^1 = Cl, R^2 = H
11b: R^1 = H, R^2 = H
11c: R^1 = Me, R^2 = H
11d: R^1 = H, R^2 = Me

Scheme 2 Formation of 2,2'-anhydro-*L*-threonucleoside analogues

10b, **10c**, and previously reported 4(*R*)-methyl analogue **10d**.[21] All the reactions furnished the corresponding 2,2'-anhydro-*L*-threonucleoside phosphonate analogues (Scheme 2).

Finally, following the classical procedures for hydrolysis of nucleoside phosphonate, compounds **11a**～**11d** were smoothly hydrolyzed by treatment with TMSBr/lutidine to produce corresponding phosphonic acids **12a**～**12d** without disclosure of 2,2'-anhydro ring (Scheme 3).



11a: R^1 = Cl, R^2 = H
11b: R^1 = H, R^2 = H
11c: R^1 = Me, R^2 = H
11d: R^1 = H, R^2 = Me

12a: R^1 = Cl, R^2 = H
12b: R^1 = H, R^2 = H
12c: R^1 = Me, R^2 = H
12d: R^1 = H, R^2 = Me

Scheme 3 Synthesis of compounds **12**

3 Conclusions

In summary, the obtained cyclic nucleoside phosphonates were elucidated by spectral analysis. Unfortunately, all the obtained cyclic threonucleoside phosphonates have not shown any biological activity in some primary *in vitro* antiviral and antitumour investigations.

4 Experimental section

4.1 Instruments and reagents

All reagents and solvents were used as received without further purification unless otherwise noted. Anhydrous pyridine, dichloromethane, acetonitrile were treated with calcium hydride. Anhydrous tetrahydrofuran was treated with sodium. The reaction sensitive to moisture was carried out under nitrogen atmosphere. ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra were recorded on a Bruker AVANCE III 400 spectrometer. High-resolution mass spectra (HRMS) were recorded under electrospray ion conditions using a Q-Tof Micromass spectrometer. Thin-layer chromatography (TLC) was performed on silica gel GF254 with detection by charring with 5% phosphomolybdic acid in alcohol or by UV light. Column chromatography was conducted on a column of silica gel (200～300 mesh). All phosphonates were purified by silica gel chromatography eluting with dichloromethane (DCM)/MeOH or by C-18 column chromatography eluting with H_2O/MeOH or H_2O/MeCN.

4.2 Experimental method

4.2.1 Synthesis of (3a*R*,5*R*,6*S*,6a*R*)-5-(hydroxy-methyl)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-6-ol (**2**)

D-Xylose (50 g, 333 mmol) was dissolved in 500 mL of acetone and 98% sulfuric acid (40 mL, 747 mmol) was added dropwise under ice cooling. After dropping, the reaction mixture was moved to room temperature and stirred for 5 h. The system was light yellow and clear. The reaction mixture was then moved to an ice water bath and 30% sodium hydroxide solution was slowly added until pH＝1～2. Then the mixture was stirred for an additional 2 h at room temperature. Sodium hydroxide solid was added until pH＝7～8. The mixture was then filtered and the cake was washed with acetone. Filtrates and washings were combined and concentrated. The residue was purified by silica column chromatography [petroleum ether (PE)/EtOAc, *V*∶*V*＝3∶1～1∶1] to obtain **2** as a yellow oil (50 g, 79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.80 (d, *J*＝3.7 Hz, 1H), 5.14 (d, *J*＝4.8 Hz, 1H), 4.62 (t, *J*＝5.7 Hz, 1H), 4.37 (d, *J*＝3.7 Hz, 1H), 4.02～3.93 (m, 2H), 3.60 (dd, *J*＝11.3, 5.6 Hz, 1H), 3.51 (dd, *J*＝11.2, 5.6 Hz, 1H), 1.38 (s, 3H), 1.23 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 110.2, 104.2, 85.0, 81.3, 73.4, 58.8, 26.6, 26.1; HRMS (ESI-TOF) calcd for $C_8H_{15}O_5$ $[M+H]^+$ 191.0914, found 191.0919.

4.2.2 Synthesis of 2-(((3a*R*,5*R*,6*S*,6a*R*)-6-hydroxy-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)methoxy) isoindoline-1,3-dione (**3**)

Compound **2** (20 g, 105 mmol) was dissolved in toluene and evaporated to dryness, then dissolved in tetrahydrofuran (THF) (300 mL). *N*-Hydroxyphthalimide (18.9 g, 115.7 mmol) and triphenylphosphine (38.6 g, 147.2 mmol) were added. The system was then cooled in an ice bath. Diethyl azodicarboxylate (23.2 mL, 147.2 mmol) was dissolved in 100 mL of anhydrous THF and slowly added to the reaction system. The reaction was continued under an ice water bath for 6 h and quenched in water (5 mL). The solvent was removed by evaporation under reduced pressure to give a residue. The residue was added to a PE/EtOAc mixed solution (*V*∶*V*＝2∶3, 200 mL). The resulting mixture was stirred under cooling in an ice bath to precipitate triphenylphosphine oxide and then filtered to remove the precipitate. The filtrate was concentrated under reduced pressure. The residue was purified by silica column chromatography (PE/EtOAc, *V*∶*V*＝8∶1～2∶1) to produce **3** as a white solid (31.7 g, 90%). m.p. 101～103 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.90～7.82 (m, 2H), 7.82～7.75 (m, 2H), 5.93 (d, *J*＝3.5 Hz, 1H), 4.70～4.64 (m, 1H), 4.62 (d, *J*＝3.6 Hz, 1H), 4.54～4.46 (m, 2H), 4.32 (t, *J*＝8.5 Hz,

1H), 3.85 (s, 1H), 1.51 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 163.7, 134.8, 128.7, 123.9, 112.0, 105.2, 85.1, 77.2, 75.2, 74.8, 27.0, 26.3. These data are consistent with that in literature.[19]

4.2.3 Synthesis of (3a*R*,6*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-6-ol (**4**)

Azodiisobutyronitrile (6.2 g, 37.6 mmol) and tri-*n*-butyltin hydride (32.5 mL, 120.8 mmol) were dissolved in anhydrous toluene (200 mL). The resultant solution was added to a solution of compound **3** (30 g, 89 mmol) in anhydrous toluene (200 mL) at 80 ℃ under N_2. The reaction mixture was stirred for 4 h. When the reaction was completed, the resultant solution was cooled to room temperature and evaporated under reduced pressure. The residue was added to PE/EtOAc mixed solvent (V∶V=1∶1, 150 mL) and washed with water (60 mL×6). The organic layer was dried over anhydrous sodium sulfate, and concentrated *in vacuo*. The residue was purified by silica column chromatography (PE/EtOAc, V∶V=5∶1～1∶1) to produce **4** as a white solid (7.1 g, 50%). m.p. 83～84 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.95 (d, J=3.7 Hz, 1H), 4.49 (d, J=3.6 Hz, 1H), 4.26 (q, J=4.0 Hz, 1H), 4.09 (dd, J=10.2, 2.7 Hz, 1H), 3.87 (dd, J=10.2, 0.9 Hz, 1H), 2.10 (d, J=5.7 Hz, 1H), 1.47 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 111.8, 105.2, 85.0, 75.3, 72.9, 26.7, 26.2. These data are consistent with that in literature.[19]

4.2.4 Synthesis of diethyl ((((3a*R*,6*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-6-yl)oxy)methyl)-phosphonate (**5**)

To a solution of compound **4** (6 g, 37.5 mmol) in dry THF (100 mL) was added NaH (60% in mineral oil, 2.3 g, 56.3 mmol) and diethyl *p*-toluenesulfonyloxymethylphosphonate (18.1 g, 56.2 mmol) under a nitrogen atmosphere at 0 ℃. The resulting mixture was stirred for 10 min at 0 ℃ and then for 4 h at room temperature. After the disappearance of **4** as monitored by TLC, the reaction was quenched with water and concentrated under reduced pressure. Saturated sodium chloride solution (50 mL) was added, the mixture was extracted with EtOAc (30 mL×5). Then the organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Residue was purified by silica column chromatography (PE/EtOAc, V∶V=3∶1～1∶2) to give **5** as an oil (10.15 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.90 (d, J=3.8 Hz, 1H), 4.58 (d, J=3.7 Hz, 1H), 4.19～4.12 (m, 4H), 4.05 (d, J=5.4 Hz, 1H), 4.00 (t, J=6.5 Hz, 2H), 3.85～3.74 (m, 2H), 1.46 (s, 3H), 1.35～1.29 (m, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 111.7, 105.5, 84.9 (d, $J_{P,C}$=10.6 Hz), 82.5, 70.0, 63.4 (d, $J_{P,C}$=167.6 Hz), 62.66 (d, $J_{P,C}$=6.6 Hz), 62.59 (d, $J_{P,C}$=5.9 Hz), 26.8, 26.2, 16.5 (d, $J_{P,C}$=5.7 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ: 20.1; HRMS (ESI-TOF) calcd for $C_{12}H_{24}O_7P$ $[M+H]^+$ 311.1254, found 311.1260.

4.2.5 Synthesis of diethyl ((((3*S*,4*R*)-4-hydroxy-5-methoxytetrahydrofuran-3-yl)oxy)methyl)phosphonate (**6**)

Compound **5** (5 g, 16.1 mmol) was dissolved in anhydrous methanol (50 mL) in an ice bath. To the solution sulfuric acid (1 mL, 19 mmol) was added dropwise under ice cooling. The resulting solution was stirred for 30 min at 0 ℃ and reacted at room temperature for 10 h. After the completion of the reaction, sodium bicarbonate solid was added until pH=7～8. The solvent was removed *in vacuo* and the residue was dispersed with EtOAc, then the filtrate was concentrated and purified by silica gel chromatography (PE/EtOAc, V∶V=3∶1～1∶3) to provide **6** as a colorless liquid (3.5 g, 76%). The product was a mixture of α and β isomers. HRMS (ESI-TOF) calcd for $C_{10}H_{22}O_7P$ $[M+H]^+$ 285.1103, found 285.1125.

4.2.6 Synthesis of (3*R*,4*S*)-4-((diethoxyphosphoryl)-methoxy)-2-methoxytetrahydrofuran-3-yl benzoate (**7**)

Compound **6** (20 g, 0.1 mmol) was added to anhydrous pyridine, evaporated three times, dissolved in dry pyridine (300 mL), and followed by dropwise addition of benzoyl chloride (2.9 mL, 24.6 mmol) at 0 ℃ under N_2. The resulting solution was stirred for 30 min at 0 ℃ and then for an additional 4 h at room temperature. After the completion of the reaction, the reaction was quenched with saturated aqueous sodium bicarbonate. The pyridine was removed under reduced pressure and the residue was dissolved in saturated aqueous sodium chloride (30 mL), which was extracted with EtOAc (30 mL×4). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (PE/EtOAc, V∶V=3∶1～1∶3) to obtain **7** as a colorless oil (4.5 g, 94%). The product was a mixture of α and β isomers. HRMS (ESI-TOF) calcd for $C_{17}H_{26}O_8P$ $[M+H]^+$ 389.1365, found 389.1379.

4.2.7 Synthesis of (3*R*,4*S*)-2-acetoxy-4-((diethoxyphosphoryl)methoxy)tetrahydrofuran-3-yl benzoate (**8**)

To the ice-cold solution of **7** (4.5 g, 11.6 mmol) in acetic acid (38.4 mL, 672 mmol) acetic anhydride (4.9 mL, 52.1 mmol) and sulfuric acid (0.3 mL, 5.8 mmol) were added under N_2. The resultant solution was stirred for 10 min at 0 ℃ and then for an additional 8 h at room temperature. After the completion of the reaction, saturated aqueous sodium bicarbonate was added until pH=7～8 and then partitioned between EtOAc and water. Then the organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (PE/EtOAc, V∶V=3∶1～1∶3) to give **8** as a yellow oil (3.8 g, 80%). The product was a mixture of α and β isomers. HRMS (ESI-TOF) calcd for $C_{18}H_{26}O_9P$ $[M+H]^+$ 417.1314, found 417.1331.

4.2.8 Synthesis of compound **9**

Compound **8** (200 mg, 0.48 mmol) was evaporated three times with anhydrous toluene and dissolved in dry acetonitrile (5 mL) under N_2, then 5-chlorouracil (106 mg, 0.72 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide (BSA) (0.36 mL, 1.44 mmol) were added. The system was heated to 65 ℃ and then refluxed. When the mixture was clarified, the protection of the bases completed. The reaction mixture

was moved to room temperature and then trimethylsilyl trifluoromethanesulfonate (0.3 mL, 1.44 mmol) was added under ice cooling. After 10 min, the system was moved to room temperature and stirred for 6 h, and then quenched with water. The resultant solution was concentrated under reduced pressure. The residue was added to EtOAc (10 mL) and stirred at room temperature. The insoluble substance was filtered off. The filtrate was concentrated under reduced pressure and purified by silica gel chromatography (DCM/MeOH, V∶V=100∶1～70∶1) to furnish **9a** as a colourless oil (193 mg, 80%). Following the same method as described for compound **9a**, starting from compound **8** and uracil or thymine, compounds **9b** and **9c** were obtained, respectively. Among them, 1-(uracil-1-yl)-2-*O*-benzoyl-3-*O*-(diethoxyphosphonomethyl)-*α*-*L*-threose (**9b**) was obtained as a colorless oil, which was used directly in the next synthesis without further purification.

1-(5-Chlorouracil-1-yl)-2-*O*-ben-zoyl-3-*O*-(diethoxyphosphonomethyl)-*α*-*L*-threose (**9a**): ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.54 (s, 1H), 8.03～7.98 (m, 2H), 7.74 (s, 1H), 7.59 (t, J=7.4 Hz, 1H), 7.45 (t, J=7.8 Hz, 2H), 6.15 (s, 1H), 5.47 (s, 1H), 4.44 (d, J=10.6 Hz, 1H), 4.25～4.13 (m, 6H), 4.10～3.94 (m, 2H), 1.35～1.30 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.2, 159.1, 149.3, 137.1, 133.9, 129.9, 128.6, 128.5, 109.0, 90.1, 83.4 ($J_{P,C}$=10.1 Hz), 79.7, 74.0, 64.0 ($J_{P,C}$=168.7 Hz), 62.9 ($J_{P,C}$=7.1 Hz), 62.8 ($J_{P,C}$=7.1 Hz), 16.5 ($J_{P,C}$=6.1 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ: 19.8; HRMS (ESI-TOF) calcd for $C_{20}H_{25}ClN_2O_9P$ $[M+H]^+$ 503.0981, found 503.0982.

1-(Thymin-1-yl)-2-*O*-benzoyl-3-*O*-(diethoxyphosphonomethyl)-*α*-*L*-threose (**9c**): Colorless oil, 78% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 8.05～7.98 (m, 2H), 7.60 (t, J=7.4 Hz, 1H), 7.49～7.41 (m, 3H), 6.26 (d, J=1.6 Hz, 1H), 5.37 (s, 1H), 4.39 (d, J=10.8 Hz, 1H), 4.22 (d, J=3.3 Hz, 1H), 4.18～4.14 (m, 4H), 4.12 (d, J=4.5 Hz, 1H), 4.10～3.94 (m, 2H), 1.96 (d, J=0.9 Hz, 3H), 1.35～1.31 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 164.3, 162.7, 149.3, 135.1, 132.9, 128.9, 127.6, 110.3, 88.1, 82.8 (d, $J_{P,C}$=11.1 Hz), 79.2, 71.9, 62.8 (d, $J_{P,C}$=169.7 Hz), 61.7 (d, $J_{P,C}$=6.1 Hz), 61.6 (d, $J_{P,C}$=6.1 Hz), 15.5 (d, $J_{P,C}$=6.1 Hz), 11.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ: 20.0; HRMS (ESI-TOF) calcd for $C_{21}H_{28}N_2O_9P$ $[M+H]^+$ 483.1527, found 483.1533.

4.2.9 Synthesis of compound **10**

Compound **9a** (180 mg, 0.36 mmol) was dissolved in NH_3/MeOH (5 mL) and reacted at room temperature in a closed container for 4 h. Solvent was removed under reduced pressure after the reaction was complete as monitored by TLC. The residue was purified by silica gel chromatography (DCM/MeOH, V∶V=50∶1～20∶1) to give **10a** as a colorless oil (112 mg, 78%). Following the same method, starting from compound **9b** or **9c**, compounds **10b** and **10c** were obtained.

1-(5-Chlorouracil-1-yl)-3-*O*-(diethoxyphosphonomethyl)-*α*-*L*-threose (**10a**): ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.71 (s, 1H), 7.68 (s, 1H), 5.75 (s, 1H), 5.37 (s, 1H), 4.50 (s, 1H), 4.36～4.29 (m, 2H), 4.18～4.06 (m, 5H), 3.91～3.79 (m, 2H), 1.33～1.28 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.8, 150.2, 137.4, 108.4, 93.4, 85.3 (d, $J_{P,C}$=11.1 Hz), 77.9, 74.4, 64.5 (d, $J_{P,C}$=168.8 Hz), 62.9 (d, $J_{P,C}$=6.1 Hz), 62.8 (d, $J_{P,C}$=7.1 Hz) 16.5 (d, $J_{P,C}$=5.1 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ: 20.3; HRMS (ESI-TOF) calcd for $C_{13}H_{21}ClN_2O_8P$ $[M+H]^+$ 399.0719, found 399.0718.

1-(Uracil-1-yl)-3-*O*-(diethoxyphosphonomethyl)-*α*-*L*-threose (**10b**): Colorless oil, 60% yield in two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.47 (s, 1H), 7.55 (d, J=8.1 Hz, 1H), 5.79 (s, 1H), 5.69 (d, J=8.1 Hz, 1H), 5.45 (s, 1H), 4.42 (s, 1H), 4.33～4.29 (m, 2H), 4.18～4.04 (m, 5H), 3.80 (d, J=9.2 Hz, 2H), 1.32～1.28 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 164.1, 151.1, 140.6, 101.4, 93.3, 85.3 (d, $J_{P,C}$=11.1 Hz), 78.2, 74.0, 63.5 (d, $J_{P,C}$=168.7 Hz), 62.6 (d, $J_{P,C}$=6.1 Hz), 16.5 (d, $J_{P,C}$=5.1 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ: 20.3; HRMS (ESI-TOF) calcd for $C_{13}H_{22}N_2O_8P$ $[M+H]^+$ 365.1108, found 365.1115.

1-(Thymin-1-yl)-3-*O*-(diethoxyphosphonomethyl)-*α*-*L*-threose (**10c**): Colorless oil, 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.38 (s, 1H), 7.39 (d, J=1.0 Hz, 1H), 5.81 (s, 1H), 5.48 (d, J=2.5 Hz, 1H), 4.41 (s, 1H), 4.33～4.28 (m, 2H), 4.15～4.08 (m, 5H), 3.81 (d, J=9.2 Hz, 2H), 1.93 (d, J=0.6 Hz, 3H), 1.32 (t, J=7.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 164.6, 151.1, 136.3, 110.0, 93.2, 85.4 (d, $J_{P,C}$=11.1 Hz), 78.6, 73.7, 63.7 (d, $J_{P,C}$=168.7 Hz), 62.6 (d, $J_{P,C}$=6.1 Hz), 16.5 (d, $J_{P,C}$=5.1 Hz), 12.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ: 20.3; HRMS (ESI-TOF) calcd for $C_{14}H_{24}N_2O_8P$ $[M+H]^+$ 379.1265, found 379.1270.

4.2.10 Synthesis of compound **11**

To a solution of **10a** (112 mg, 0.28 mmol) in anhydrous DCM (5 mL) was added diethylaminosulfur trifluoride (0.3 mL, 2.2 mmol) at −20 ℃. The mixture was stirred for 0.5 h at the same temperature and then warmed to room temperature to stir for 4 h. The reaction was quenched with saturated sodium bicarbonate solution (5 mL) after the completion of the reaction, which was extracted with DCM (10 mL×3). Then the organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was collected and concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM/MeOH, V∶V=50∶1～10∶1) to give **11a** as a colorless oil (86 mg, 80%). Following the same method, starting from compounds **10b**～**10d**, compounds **11b**～**11d** were obtained.

2,2'-Anhydro-5-chloro-1-(3-*O*-(diethoxyphosphonomethyl)-*α*-*L*-threopentofuranosyl)uracil (**11a**): ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 (s, 1H), 6.28 (d, J=5.4 Hz, 1H), 5.51 (t, J=5.4 Hz, 1H), 4.52～4.47 (m, 1H), 4.30～4.25 (m, 1H), 4.24～4.13 (m, 4H), 4.07～3.89 (m, 2H), 3.60 (t, J=9.3 Hz, 1H), 1.38～1.33 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.4, 159.6, 131.7, 118.8, 90.6, 79.5, 78.9 (d, $J_{P,C}$=8.1 Hz), 67.8, 65.0 (d, $J_{P,C}$=166.7 Hz), 62.9 (d, $J_{P,C}$=7.1 Hz), 16.5 (d, $J_{P,C}$=5.1 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ: 19.0; HRMS (ESI-TOF) calcd for $C_{13}H_{19}ClN_2O_7P$ $[M+H]^+$ 381.0613, found 381.0598.

2,2'-Anhydro-1-(3-*O*-(diethoxy-phosphonomethyl)-*α*-*L*-

threo-pentofuranosyl)uracil (**11b**): Colorless oil, 64% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.37 (d, J=7.5 Hz, 1H), 6.18 (d, J=5.3 Hz, 1H), 6.06 (d, J=7.5 Hz, 1H), 5.42 (t, J=5.3 Hz, 1H), 4.47～4.42 (m, 1H), 4.26～4.12 (m, 5H), 4.06～3.89 (m, 2H), 3.56 (t, J=9.3 Hz, 1H), 1.37～1.32 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 171.8, 160.6, 134.6, 110.7, 90.2, 79.0 (d, $J_{P,C}$=9.1 Hz), 78.6, 67.5, 65.4 (d, $J_{P,C}$=166.7 Hz), 63.0 (d, $J_{P,C}$=5.1 Hz), 16.5 (d, $J_{P,C}$=5.1 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ: 19.0; HRMS (ESI-TOF) calcd for $C_{13}H_{20}N_2O_7P$ $[M+H]^+$ 347.1003, found 347.1011.

2,2'-Anhydro-1-(3-*O*-(diethoxy-phosphonomethyl)-*α*-*L*-threo-pentofuranosyl)thymine (**11c**): Colorless oil, 59% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.21 (d, J=1.1 Hz, 1H), 6.14 (d, J=5.4 Hz, 1H), 5.36 (t, J=5.4 Hz, 1H), 4.46～4.29 (m, 1H), 4.24～4.11 (m, 5H), 4.05～3.87 (m, 2H), 3.53 (t, J=9.3 Hz, 1H), 1.95 (d, J=0.9 Hz, 3H), 1.36～1.31 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 171.3, 159.3, 129.0, 118.5, 89.3, 78.0 (d, $J_{P,C}$=9.1 Hz), 77.3, 66.9, 63.0 (d, $J_{P,C}$=166.7 Hz), 61.9 (d, $J_{P,C}$=3.3 Hz), 61.9 (d, $J_{P,C}$=3.3 Hz), 15.5 (d, $J_{P,C}$=6.1 Hz), 13.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ: 19.0; HRMS (ESI-TOF) calcd for $C_{14}H_{22}N_2O_7P$ $[M+H]^+$ 361.1159, found 361.1166.

2,2'-Anhydro-1-(3-*O*-(diethoxy-phosphonomethyl)-4*R*-methyl-*α*-*L*-threo-pentofuranosyl)uracil (**11d**): Colorless oil, 63% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.88 (d, J=7.4 Hz, 1H), 6.15 (d, J=5.9 Hz, 1H), 5.88 (d, J=7.4 Hz, 1H), 5.54 (t, J=5.6 Hz, 1H), 4.44～4.34 (m, 2H), 4.15～3.95 (m, 7H), 1.24 (t, J=7.0 Hz, 6H), 1.01 (d, J=6.6 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 163.2, 150.4, 140.8, 101.3, 90.6, 86.1(d, $J_{P,C}$=11.1 Hz), 77.7, 77.6, 62.9 (d, $J_{P,C}$=163.6 Hz), 61.8 (d, $J_{P,C}$=7.1 Hz), 16.3 (d, $J_{P,C}$=5.1 Hz), 13.3; HRMS (ESI-TOF) calcd for $C_{14}H_{22}N_2O_7P$ $[M+H]^+$ 361.1159, found 361.1161.

4.2.11 Synthesis of compound **12**

To a solution of **11a** (76 mg, 0.2 mmol) in anhydrous acetonitrile (3 mL) was added 2,6-lutidine (140 μL, 1.2 mmol) and bromotrimethylsilane (320 μL, 2.4 mmol) under N_2. The resultant solution was stirred for 30 min at 0 ℃ and kept for an additional 4 h at room temperature. The reaction was complete as monitored by TLC and the reaction was quenched with water. Then the residue was concentrated by under reduced pressure and purified by reverse-phase silica gel column chromatography (H_2O/MeOH, V∶V=10∶1～5∶1) to give **12a** as a syrup (34 mg, 53%). Following the same method, starting from compounds **11b**～**11d** (69 mg, 0.2 mmol), compounds **12b**～**12d** were obtained.

2,2'-Anhydro-5-chloro-1-(3-*O*-(phosphonomethyl)-*α*-*L*-threo-pentofuranosyl)uracil (**12a**): ^{1}H NMR (400MHz, D_2O) δ: 8.16 (s, 1H), 6.39 (d, J=5.4 Hz, 1H), 5.61 (t, J=5.3 Hz, 1H), 4.46 (m, 1H), 4.31～4.27 (m, 1H), 3.84～3.67 (m, 2H), 3.56 (t, J=9.5 Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ:170.0, 160.6, 135.2, 117.2, 91.3, 81.0, 78.5 (d, $J_{P,C}$=11.1 Hz), 67.3 (d, $J_{P,C}$=157.6 Hz), 67.2; ^{31}P NMR (162 MHz, D_2O) δ:14.2; HRMS (ESI-TOF) calcd for $C_9H_9ClN_2O_7P$ $[M-H]^-$ 322.9836, found 322.9844.

2,2'-Anhydro-1-(3-*O*-(phosphonomethyl)-*α*-*L*-threo-pentofuranosyl)uracil (**12b**): Syrup, 59% yield. ^{1}H NMR (400 MHz, D_2O) δ: 7.86 (d, J=7.5 Hz, 1H), 6.39 (d, J=5.4 Hz, 1H), 6.18 (d, J=7.5 Hz, 1H), 5.60 (t, J=5.4 Hz, 1H), 4.51～4.46 (m, 1H), 4.32～4.28 (m, 1H), 3.88～3.70 (m, 2H), 3.57 (t, J=9.5 Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ: 175.7, 161.7, 138.4, 109.1, 90.8, 80.4, 78.6 (d, $J_{P,C}$=11.1 Hz), 81, 67.2 (d, $J_{P,C}$=156.6 Hz), 67.1; ^{31}P NMR (162 MHz, D_2O) δ: 16.7; HRMS (ESI-TOF) calcd for $C_9H_{10}N_2O_7P$ $[M-H]^-$ 289.0226, found 289.0233.

2,2'-Anhydro-1-(3-*O*-(phosphonomethyl)-*α*-*L*-threo-pentofuranosyl)thymine (**12c**): Syrup, 65% yield. ^{1}H NMR (400 MHz, D_2O) δ: 7.68 (d, J=1.1 Hz, 1H), 6.34 (d, J=5.4 Hz, 1H), 5.54 (t, J=5.3 Hz, 1H), 4.48～4.41 (m, 1H), 4.28～4.24 (m, 1H), 3.85～3.68 (m , 2H), 3.50 (t, J =9.5 Hz, 1H), 1.88 (s, 3H); ^{13}C NMR (101 MHz, D_2O) δ: 175.8, 161.1, 133.9, 118.7, 90.9, 80.1, 78.6 (d, $J_{P,C}$=12.1 Hz), 67.1 (d, $J_{P,C}$ = 155.5 Hz), 67.0, 13.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ: 16.8; HRMS (ESI-TOF) calcd for $C_{10}H_{12}N_2O_7P$ $[M-H]^-$ 303.0388, found 303.0390.

2,2'-Anhydro-1-(3-*O*-phosphonomethyl-4*R*-methyl-*α*-*L*-threo-pentofuranosyl)uracil (**12d**): Colorless oil, 63 yield. ^{1}H NMR (400 MHz, D_2O) δ: 7.90 (d, J=7.4 Hz, 1H), 6.31 (d, J=5.6 Hz, 1H), 6.18 (d, J=7.4 Hz, 1H), 5.66 (t, J=5.6 Hz, 1H), 4.63～4.51 (m, 2H), 3.73 (dd, J=13.0, 8.0 Hz, 1H), 3.64 (dd, J=13.0, 8.4 Hz, 1H), 1.07 (d, J=6.8 Hz, 3H); ^{13}C NMR (101 MHz, D_2O) δ: 178.1, 163.7, 141.0, 111.5, 91.9, 85.1, 81.8 (d, $J_{P,C}$=9.0 Hz), 80.0, 71.5 (d, $J_{P,C}$=150.0 Hz), 17.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ: 16.8; HRMS (ESI-TOF) calcd for $C_{10}H_{12}N_2O_7P$ $[M-H]^-$ 303.0388, found 303.0396.

Supporting Information NMR spectra of compounds **10a**～**10c**, **11a**～**11d** and **12a**～**12c**. The Supporting Information is available free of charge via the Internet at http://sioc-journal.cn.

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