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Synthesis and in vitro anti-hepatitis B virus activity of six-membered azanucleoside analogues

Dan Wang [a], Yu-Huan Li [b], Yu-Ping Wang [b], Rong-Mei Gao [b], Li-He Zhang [a], Xin-Shan Ye [a,*]

[a] State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Xue Yuan Rd. No. 38, Beijing 100191, China
[b] Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

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ABSTRACT

Fifteen novel six-membered azanucleoside derivatives were prepared and evaluated for their anti-hepatitis B virus (HBV) activity and cytotoxicity in human hepatoblastoma-derived liver Hep-G2 cells. The most potent compound **16b** with an IC_{50} value of 2.74 μg/mL (lower than 3TC) and a SI value of 13.5 was disclosed. The key synthetic steps involved the rearrangement of lactones (which were readily obtained from monosaccharides) and the Lewis acid-catalyzed condensation of nucleobases with azasugar donors. Using the versatile acetylated azasugar donors, azanucleosides covering three types of azasugars and four types of natural nucleobases were successfully obtained. The experimental results showed that some six-membered azanucleosides may find applications in the discovery of new anti-viral agents.

1. Introduction

Considerable interests have been focused on the synthesis of new types of nucleosides since the appearance of many compounds such as acyclovir, ribavirine, AZT, showdomycin as antitumor, antibacterial and antiviral drugs.[1] Traditional modifications of nucleosides include changes on nucleobases and changes on sugar moiety.[2] Modifications of nucleosides have given a plethora of biologically active compounds,[3] and in this way many modified nucleosides exhibiting remarkable antiviral and anticancer properties have been discovered.[4] Recently, many sugar modified nucleosides,[2,5] including thionucleosides,[6] carbocyclic nucleosides[7] and azanucleosides,[8] have received a great interest for their biological and medical applications.

Azanucleosides, in which the oxygen atom of furanose or pyranose ring is replaced by nitrogen atom, represent an important class of modified nucleosides. D- and L-nucleoside analogues with a pyrrolidine,[9] pyridine,[10] piperidine,[11,12] or five-, six-, seven-membered lactam[13–17] ring have been synthesized and biologically evaluated. Among all types of azanucleosides, five-membered pyrrolidine-type nucleosides have been most investigated because of their high comparability with naturally occurring nucleosides. However, no pyrrolidine nucleosides with outstanding bioactivity have been reported to date. Six-membered azanucleosides, which are also called piperidine nucleosides, are the type of nucleosides with one more carbon atom in azasugar ring than pyrrolidine nucleosides. Few attempts have been reported so far to synthesize this type of nucleosides. We reasoned that piperidine nucleosides are likely to mimic the naturally occurring nucleosides because of the increasing flexibility in sugar rings.

For this purpose, we report herein the synthesis of fifteen novel six-membered azanucleosides covering three types of azasugars and four types of nucleobases. In previous studies, very limited types of nucleobases were introduced to any types of azasugars due to difficult synthetic access. In our work, more types of nucleobases (both pyrimidine type and purine type) were introduced to azasugar rings to further increase the structural diversity. Furthermore, the anti-hepatitis B virus (HBV) activity of these synthetic azanucleoside analogues was evaluated.

2. Results and discussion

2.1. Chemistry

The synthesis of thymine-containing azanucleosides was shown in Scheme 1. The glucose alkene **1a** was easily obtained from D-glucose through three steps[18] or five steps in high overall yield (77%),[19] and the mannose and galactose alkenes, **1b** and **1c**, respectively, were prepared in the similar way. Following the one-pot tandem procedure with lactone as intermediate, which was reported by our group,[20] the alkenes **1a–c** were smoothly converted to *N*-*p*-methoxybenzyl (PMB) substituted δ-lactams **2a–c**. To facilitate the subsequent reduction, the substituent group in nitrogen

* Corresponding author. Tel.: +86 10 62014949; fax: +86 10 82802724.
E-mail address: xinshan@bjmu.edu.cn (X.-S. Ye).

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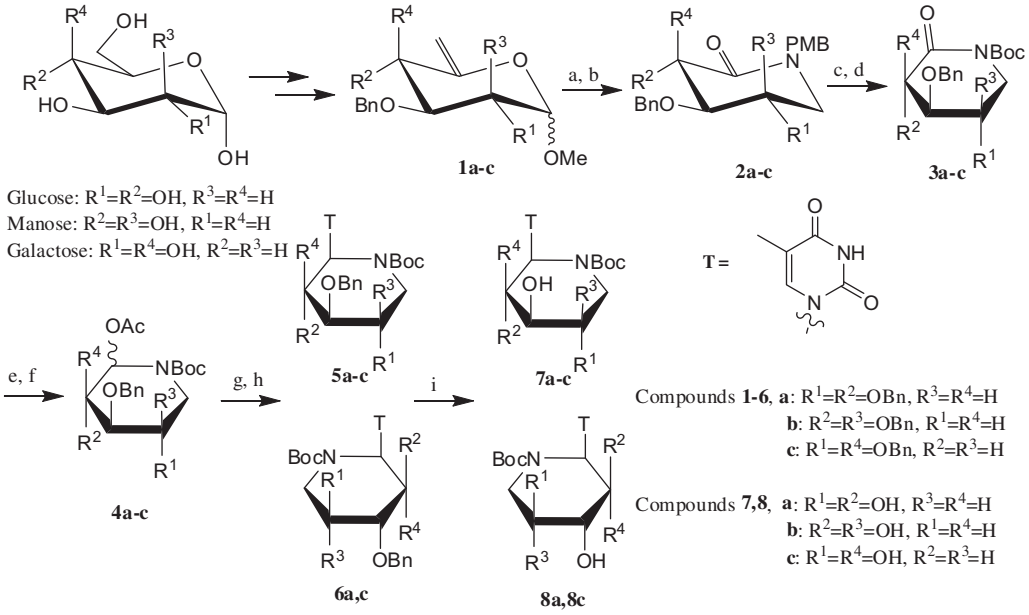


Scheme 1. Reagents and conditions: (a) O_3, Me_2S, MeOH; (b) $ZnCl_2$, $NaBH_3(CN)$, $PMBNH_2$, MeOH, 87%–99% for two steps; (c) CAN, H_2O/CH_3CN = 1:2, 68%–95%; (d) $(Boc)_2O$, pyridine, DMAP, 91%–92%; (e) $NaBH_4$, MeOH, 68%–91%; (f) Ac_2O, CH_2Cl_2, pyridine, 73%–99%; (g) Thymine (T), BSA, CH_3CN, reflux; (h) TMSOTf, CH_3CN, 81%–88% for two steps; (i) Pd/C, H_2, HCl in MeOH, 74%–94%.

atom was manipulated from PMB group, an electron donating group to *t*-butyl carbamate (Boc), an electron withdrawing group. After the introduction of Boc group, we calculated each dihedral angle from Karplus equation based on their ^3J-coupling constants in the ^1H NMR and we got enough proof that the conformation of the resulting six-membered azasugar ring was neither chair form nor other usual conformations of six-membered rings (such as boat or twisted boat). Instead, the ring seemed to be quite flat and unusual (that's why we used Haworth presentation to show the structures). For example, in compound **3a**, the *J* value between H_4 and H_3 (Fig. 1) was 4.2 Hz, which means the dihedral angle between these two C–H bonds was about 55° or 110° (according to Karplus equation); one of the two *J* values between $H_6/H_{6'}$ and H_5 was nearly 0, which indicated a dihedral angle of about 80°, while the other *J* value was 5.4, which corresponded to a dihedral angle of 50° or 115°. Since benzyl groups are very bulky compared with hydrogens, we assumed that the possibility that all three benzyl groups were in axial bonds was very low, which means the dihedral angles of H_5–H_6 and H_3–H_4 shouldn't be less than 60°. Considering the ring restriction, $\Phi_{H6'-C6-C5-H5}$ should be smaller than 60°. Based on all the information and reasonable assumptions listed above, the only possible conformation of compound **3a** was the flat conformation we mentioned, with $\Phi_{H6'-C6-C5-H5} = 50°$, $\Phi_{H6-C6-C5-H5} = 80°$ and $\Phi_{H3-C3-C4-H4} = 110°$ (as shown in Fig. 1). Similar phenomena were also observed in compounds **3b** and **3c**.

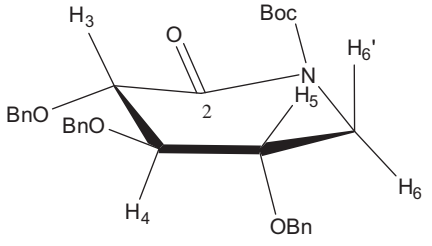
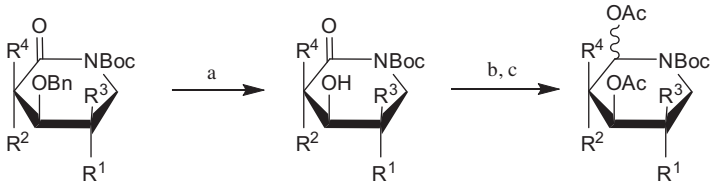


Figure 1. The assumed conformation of compound **3a**.

Using $NaBH_4$ as reducing agent, the lactam group in compounds **3a–c** were selectively reduced to the corresponding unstable *N,O*-hemiacetal intermediates, which were subjected to acetylation to afford compounds **4a–c**, the azasugar donors of the following coupling reaction. It should be noted that compounds **4a–c** are not so stable either, all of which cannot be stored more than a week even at very low temperature. With the glucose type (**4a**), mannose type (**4b**), and galactose type (**4c**) azasugar donors in hands, the coupling reactions were carried out. Coupling of **4a–c** and silylated thymine under Vorbrueggen's conditions (glycosylation reaction)[21] afforded the protected azanucleosides **5a–c**, **6a**, and **6c**. It is noteworthy that the coupling reactions of both the glucose and galactose type sugar donors resulted in two anomeric isomers, but the ratios of two anomers were different, which was verified by analyses of their H–H COSY and NOESY NMR spectra. In contrast, the glycosylation of mannose type sugar donor **4b** provided only one coupling product **5b**. Finally, global debenzylation by hydrogenolysis under acidic conditions led to target molecules **7a**, **7b**, **7c**, **8a**, and **8c**.

Encouraged by the success of preparation of five thymidine compounds, we tried the coupling of the benzylated azasugar donors with other nucleobases such as uracil, cytosine, and adenine. The coupling reaction proceeded smoothly. However, the following deprotection (cleavage of the benzyl groups) turned out to be extremely difficult. The base moieties of some nucleosides such as uracil and cytosine would be reduced in the hydrogenolysis conditions that cleaved the benzyl groups. Meanwhile, the glycosidic bonds in the synthesized adenosine compounds were very vulnerable under the acidic conditions we used in hydrogenolysis reactions. Realizing the restrictions benzyl groups brought to us, we decided to alter the benzyl groups before coupling reactions. After several attempts, an effective and convenient route that avoided the use of benzyl groups in azanucleosides was achieved.

The debenzylation of compounds **3a–c** over Pd–C under neutral hydrogenolysis conditions proceeded very efficiently, affording products **9a–c** in high yields (92%–99%). Compounds **9a–c** were reduced to *N,O*-hemiacetals followed by acetylation to produce **10a–c** in the similar manner as described in the preparation of **4a–c** (Scheme 2). Comparing with the former donors **4a–c**, the



3a: R^1=R^2=OBn, R^3=R^4=H **9a**: R^1=R^2=OH, R^3=R^4=H **10a**: R^1=R^2=OAc, R^3=R^4=H
3b: R^2=R^3=OBn, R^1=R^4=H **9b**: R^2=R^3=OH, R^1=R^4=H **10b**: R^2=R^3=OAc, R^1=R^4=H
3c: R^2=R^3=H, R^1=R^4=OBn **9c**: R^2=R^3=H, R^1=R^4=OH **10c**: R^2=R^3=H, R^1=R^4=OAc

Scheme 2. Reagents and conditions: (a) Pd/C, H_2, EtOAc, 92%–99%; (b) $NaBH_4$, MeOH; (c) Ac_2O, pyridine, DMAP, 75%–87% for two steps.

new glycosyl donors **10a–c** had many advantages. They were more stable (could be stored under 0 °C for more than one month) and the cleavage of protective groups affected neither the glycosidic bond nor the base groups, which meant they were universal donors that could be coupled with almost all kinds of nucleobases. As shown in Scheme 3, coupling reactions of the new donors **10a–c** with nucleobases such as N^6-benzoyladenine, uracil, 4-acetamido-cytosine, and 5-fluoro-uracil proceeded uneventfully. In most cases, only one anomeric isomer was obtained as the coupling product via the neighboring group participation of the acetyl group.[22] But in some cases, the reaction did not provide a preponderant anomer. Instead, the desired nucleosides were obtained as a mixture of anomeric isomers (**11b** and **11b′**; **11c** and **11c′**). Finally, the acyl groups were removed by using saturated ammonia in methanol[23] to yield the target molecules **15–18**. All structures of synthetic azanucleosides were identified by analyses of their 1D and/or 2D NMR spectra.

2.2. Anti-HBV activity

Stably HBV-transfected Hep-G2 2.2.15 cell line was derived from hepatoblastoma Hep-G2 cells and was used as in vitro model for evaluation of anti-HBV agents.[24,25] Azanucleosides **7a–c**, **8a**, **8c**, **15a–c**, **15b′**, **15c′**, **16a–c**, **17a**, and **18a**, along with the reference antiviral drug Lamivudine (3TC) as the positive control, were evaluated in vitro against the Hep-G2 2.2.15 cells. The levels of HBV DNA and the Hepatitis B surface antigen (HBsAg and HBeAg) in the culture supernatants were detected to determine the inhibitory effect of each compound. Toxic effects of the tested compounds were quantified using TC_{50} values. And the experimental results were shown in Table 1.

The present studies investigated the anti-HBV activity of azanucleoside analogues with different sugar types and different nucleobases. None of the compounds including 3TC showed any HBsAg or HBeAg inhibitory effects. However, some synthetic azanucleosides such as compounds **7c**, **8c**, **15c**, **15c′**, **15b** and **16b** inhibited the replication of viral DNA to different extent. Among all the compounds we synthesized, the 5-fluoro-uridine-containing mannose type azasugar (compound **16b**), with an IC_{50} value of 2.74 μg/mL and selective index value of 13.5, was found to be the most potent one (Table 1). The IC_{50} value of compound **16b** was even lower than the widely used anti-HBV drug 3TC, and the moderate selectivity of **16b** could potentially be increased by further structural modifications. Furthermore, based on the experimental results, some pilot conclusions of the structure–activity relationship of synthetic azanucleosides could be drawn. It appeared that the antiviral activities of mannose type and galactose type azanucleosides are better than those of glucose type, which might result from their different conformations of sugar moieties. Based on the NMR data (see Supplementary data for details), it is easy to conclude that the conformations of most mannose type and galactose type azanucleosides are quite flat. Instead of the traditional chair- or boat-shaped conformation, the six atoms in the rings of these azasugars are almost in a plane. This factor may lead to the dramatic increase of their activity. In addition, the antiviral activities of nucleosides with pyrimidine nucleobases are slightly better than those with purine nucleobases.

3. Conclusion

In summary, to increase the structural diversity and study the structure–activity relationships, a versatile method for synthesizing azanucleoside analogues was developed and a series of novel six-membered azanucleosides were prepared. The main difficulties of the synthetic work lied in the glycosyl coupling reactions and the deprotection procedure. In our approach, suitable donors (compounds **10a–c**) with high reactivity were adopted to overcome the problems. The anti-HBV activities of azanucleosides were tested in vitro. Among all the compounds being tested, the most potent one was compound **16b** (IC_{50} 2.74 μg/mL), which was even more potent than Lamivudine (IC_{50} 4.51 μg/mL). These findings provide a starting point for the discovery of new anti-HBV therapeutic agents and may warrant further investigation on the mechanism of action as well as other biological evaluation of these analogues.

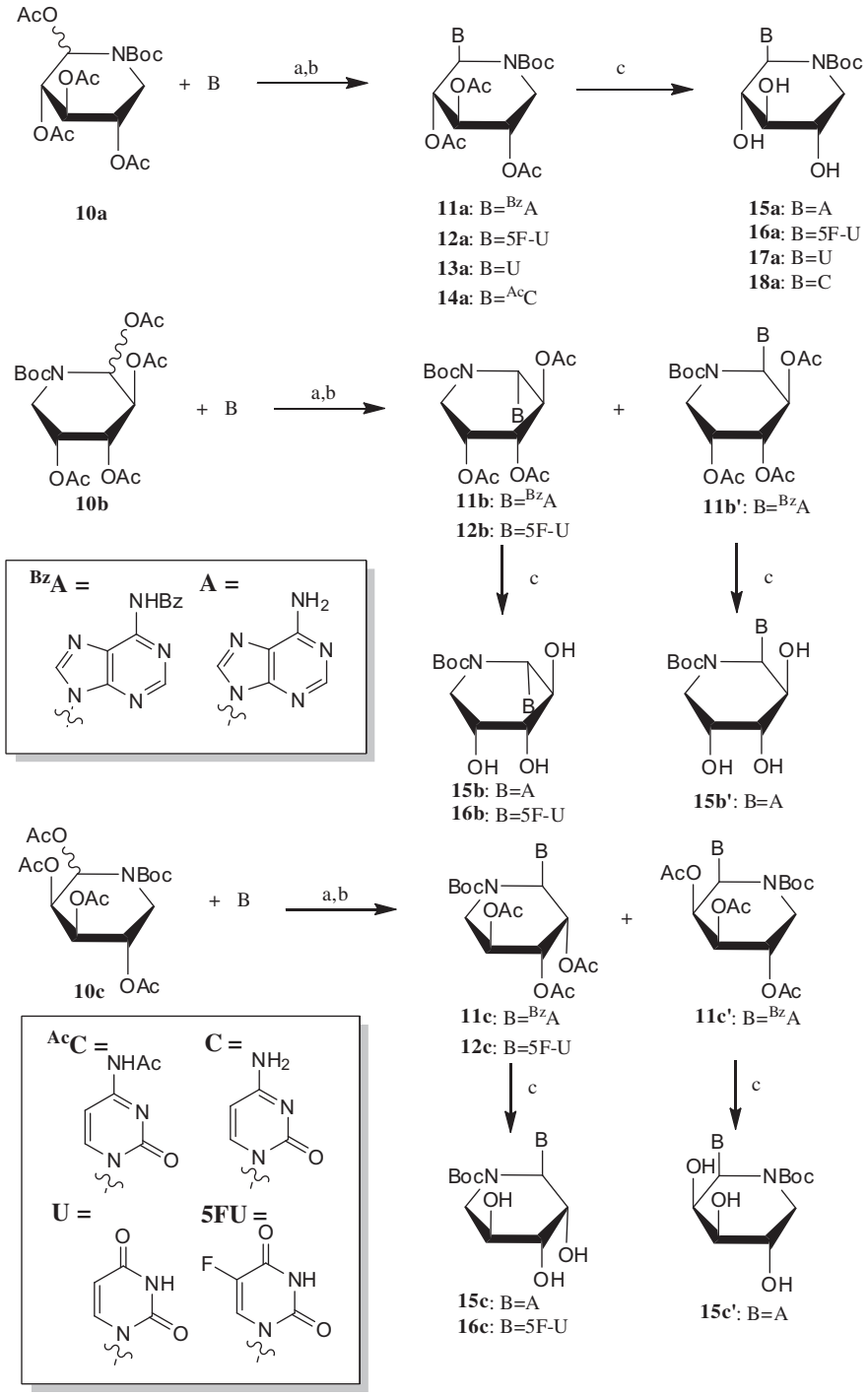
4. Experimental

4.1. General

All chemicals were purchased and used without further purification. Tetrahydrofuran (THF), toluene, and diethyl ether (Et_2O) were distilled over sodium/benzophenone, methylene chloride (CH_2Cl_2) and acetonitrile (CH_3CN) were distilled over calcium hydride. All reactions were carried out under an argon atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Reactions were monitored with analytical TLC on Silica Gel 60-F_{254} precoated on aluminum plates and visualized under UV (254 nm) and/or by staining with acidic ceric ammonium molybdate. Column chromatography was performed on silica gel (35–75 μm). ^1H NMR spectra were recorded on a Varian VXR-300 M, Bruker 400 M or Varian INOVA-500 M spectrometer at 20 °C. Chemical shifts (in ppm) were referenced to tetramethylsilane (δ = 0 ppm) in deuterated chloroform or residual proton solvent as internal standard. ^{13}C NMR spectra were obtained by using the same NMR spectrometers and were calibrated with $CDCl_3$ (δ = 77.00 ppm). Mass spectra were recorded using a PE SCLEX QSTAR spectrometer. Elemental analysis data were recorded on PE-2400C elemental analyzer.

4.2. (3*S*,4*R*,5*R*)-3,4,5-Tris(benzyloxy)-1-(4-methoxybenzyl)piperidin-2-one (2a)

A solution of glucose alkene **1a**[20] (40 mg, 0.089 mmol) in MeOH (10 mL) at −78 °C was bubbled with O_3 until the solution became



Scheme 3. Reagents and conditions: (a) BSA, CH_3CN, reflux, 30 min; (b) TMSOTf, 0 °C to rt, 1–3.5 h, 57%–87%; (c) NH_3 in MeOH, rt, 5 h, 41%–93%.

pale blue. The solution was stirred at −78 °C for 3 min and then bubbled with N_2 until the solution became colorless. $PMBNH_2$ (24 μL, 0.178 mmol), $NaCNBH_3$ (10 mg, 0.134 mmol) and $ZnCl_2$ (2.5 mg, 0.0178 mmol) were added, and the mixture was heated under reflux for 1 h, followed by quenching with saturated $NaHCO_3$ aqueous solution. After removal of the solvent, the product mixture was dissolved in ethyl acetate (80 mL) and washed by saturated brine (20 mL × 2). The organic phase was dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 6:1) to provide **2a** (51 mg, 99%) as a colorless oil. R_f = 0.45 (petroleum ether/ethyl acetate, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.20 (dd, J = 7.2 Hz, J = 12.9 Hz, 1H), 3.29 (dd, J = 4.5 Hz, J = 12.9 Hz, 1H), 3.67 (dt, J = 4.8 Hz, J = 6.0 Hz, 1H), 3.77 (s, 3H), 3.83 (t, J = 6.6 Hz, 1H), 4.04 (d, J = 7.2 Hz, 1H), 4.39 (d, J = 11.7 Hz, 1H), 4.44–4.56 (m, 3H), 4.65 (d, J = 11.4 Hz, 1H), 4.72–4.79 (m, 2H), 5.15 (d, J = 11.4 Hz, 1H), 6.80–6.84 (m, 2H), 7.16–7.46 (m, 17H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 46.53, 49.36, 55.22, 71.90, 73.63, 74.22, 75.95, 79.34, 82.05, 113.99, 127.56, 127.73, 127.90, 128.35, 129.66, 137.66, 137.95,

Table 1
Anti-HBV activity and toxicity of the target compounds

Compound	Type of sugar	Type of base	TC_{50}[a] (μg/mL)	DNA replication	
				IC_{50}[b] (μg/mL)	SI[c]
7a	glu	T	1000	–[d]	–
8a	glu	T	1000	–	–
15a	glu	A	>1000	–	–
17a	glu	U	>1000	–	–
16a	glu	FU	192.45	–	–
18a	glu	C	>1000	–	–
7c	gal	T	429.52	93.00	4.6
8c	gal	T	577.35	33.03	17.5
15c	gal	A	693.36	280.99	3.6
15c′	gal	A	1000	315.90	3.2
16c	gal	FU	29.01	–	–
7b	man	T	>1000	–	–
15b	man	A	1000	247.42	4.0
15b′	man	A	1000	–	–
16b	man	FU	37.04	2.74	13.5
3TC			1000	4.51	221.9

[a] TC_{50} is 50% cytotoxic concentration in HepG2.2.15 cells.
[b] IC_{50} is 50% inhibitory concentration.
[c] Selectivity index (SI: TC_{50}/IC_{50}).
[d] That means no antiviral activity at the concentration lower than its TC_{50}.

137.97, 159.07, 168.80. Anal. Calcd for $C_{34}H_{35}NO_5$: C, 75.95; H, 6.56; N, 2.61. Found: C, 75.83; H, 6.26; N, 2.56; ESI-MS: 474 $[M+H]^+$.

4.3. (3*S*,4*R*,5*S*)-3,4,5-Tris(benzyloxy)-1-(4-methoxybenzyl)piperidin-2-one (2b)

A solution of mannose alkene **1b**[20] (502 mg, 1.12 mmol) in MeOH (20 mL) at −78 °C was bubbled with O_3 until the solution became pale blue. The solution was stirred at −78 °C for 3 min and then bubbled with N_2 until the solution became colorless. $PMBNH_2$ (0.436 mL, 3.36 mmol), $NaCNBH_3$ (150 mg, 2.24 mmol) and $ZnCl_2$ (32 mg, 0.224 mmol) were added, and the mixture was heated under reflux for 1.5 h, followed by quenching with saturated $NaHCO_3$ aqueous solution. After removal of the solvent, the product mixture was dissolved in ethyl acetate (80 mL) and washed by saturated brine (20 mL × 2). The organic phase was dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 6:1) to provide **2b** (536 mg, 89%) as a colorless oil. R_f = 0.51 (petroleum ether/ethyl acetate, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.14 (dd, J = 3.9 Hz, J = 12.0 Hz, 1H), 3.36 (dd, J = 6.3 Hz, J = 12.6 Hz, 1H), 3.77 (s, 3H), 3.85 (dd, J = 2.1 Hz, J = 6.6 Hz, 1H), 4.01 (m, 1H), 4.26 (d, J = 6.6 Hz, 1H), 4.35 (d, J = 14.4 Hz, 1H), 4.43–4.54 (m, 2H), 4.60–4.69 (m, 3H), 4.79 (d, J = 11.1 Hz, 1H), 5.14 (d, J = 11.1 Hz, 1H), 6.81 (m, 2H), 7.15–7.42 (m, 17H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 46.68, 49.09, 55.21, 71.53, 71.73, 72.38, 74.64, 77.12, 77.24, 113.94, 127.56, 127.69, 128.18, 128.30, 128.35, 129.30, 137.78, 138.06, 138.11, 158.92, 168.14. HRMS (ESI) Anal. Calcd for $C_{34}H_{36}NO_5$ $[M+H]^+$: 538.2588; found 538.2587.

4.4. (3*R*,4*R*,5*R*)-3,4,5-Tris(benzyloxy)-1-(4-methoxybenzyl)piperidin-2-one (2c)

A solution of galactose alkene **1c**[20] (175 mg, 0.39 mmol) in MeOH (20 mL) at −78 °C was bubbled with O_3 until the solution became pale blue. The solution was stirred at −78 °C for 3 min and then bubbled with N_2 until the solution became colorless. $PMBNH_2$ (59 μL, 0.78 mmol), $NaCNBH_3$ (31 mg, 0.47 mmol) and $ZnCl_2$ (7 mg, 0.047 mmol) were added, and the mixture was heated under reflux for 1.5 h, followed by quenching with saturated $NaHCO_3$ aqueous solution. After removal of the solvent, the product mixture was dissolved in ethyl acetate (80 mL) and washed by saturated brine (20 mL × 2). The organic phase was dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 6:1) to provide **2c** (183 mg, 87%) as a colorless oil. R_f = 0.40 (petroleum ether/ethyl acetate, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.11 (dd, J = 2.4 Hz, J = 12.9 Hz, 1H), 3.51 (dd, J = 4.2 Hz, J = 13.2 Hz, 1H), 3.77–3.80 (m, 4H), 3.97–3.99 (m, 1H), 4.28–4.37 (m, 4H), 4.59 (d, J = 12.0 Hz, 1H), 4.71–4.78 (m, 2H), 4.85 (d, J = 12.0 Hz, 1H), 5.16 (d, J = 12.3 Hz, 1H), 6.76–6.79 (m, 2H), 7.09–7.45 (m, 17H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 47.78, 48.95, 55.21, 71.42, 72.95, 73.30, 73.92, 75.24, 77.22, 113.88, 127.51, 127.59, 127.72, 127.85, 128.03, 128.30, 128.41, 128.57, 129.21, 137.40, 138.23, 138.33, 158.83, 169.24. HRMS (ESI) Anal. Calcd for $C_{34}H_{36}NO_5$ $[M+H]^+$: 538.2588; found 538.2578.

4.5. (3*S*,4*R*,5*R*)-*tert*-Butyl 3,4,5-tris(benzyloxy)-2-oxopiperidine-1-carboxylate (3a)

To a solution of **2a** (101 mg, 0.19 mmol) in CH_3CN/H_2O (2:1, 9 mL) was added cerium (IV) ammonium nitrate (312 mg, 0.57 mmol). The mixture was stirred for 3 h at rt and then diluted with water (5 mL). The aqueous layer was extracted with ethyl acetate (20 mL × 2). The combined organic layers were washed successively with saturated sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. Flash column chromatography (EtOAc/petroleum ether = 1:1) of the residue afforded the N-deprotected product (74 mg, 0.18 mmol, 95%) as a colorless oil. A mixture of the N-deprotected product, di-*tert*-butyl dicarbonate (136 mg, 0.72 mmol) and DMAP (cat.) in pyridine was stirred for 3 h under N_2. The residue obtained after removal of the solvent was purified by column chromatography (EtOAc/petroleum ether = 1:10) to afford **3a** (84 mg, 92%) as a colorless oil. R_f = 0.37 (petroleum ether/ethyl acetate, 3:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.54 (s, 9H), 3.54 (d, J = 13.8 Hz, 1H), 3.75–3.81 (m, 2H), 4.05 (d, J = 6.9 Hz, 1H), 4.34 (dd, J = 4.2 Hz, J = 14.1 Hz, 1H), 4.47 (d, J = 12.0 Hz, 1H), 4.55–4.69 (m, 4H), 5.04 (d, J = 11.7 Hz, 1H), 7.29–7.44 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 27.99, 42.81, 70.53, 72.85, 73.37, 75.30, 80.98, 82.09, 83.62, 127.62, 127.77, 127.84, 127.91, 128.14, 128.32, 128.40, 137.35, 137.59, 137.63, 151.69, 168.37; HRMS (ESI) Anal. Calcd for $C_{31}H_{35}NO_6Na$ $[M+Na]^+$: 540.2357; found 540.2373.

4.6. (3*S*,4*R*,5*S*)-*tert*-Butyl 3,4,5-tris(benzyloxy)-2-oxopiperidine-1-carboxylate (3b)

To a solution of **2b** (0.99 g, 1.86 mmol) in CH_3CN/H_2O (2:1, 30 mL) was added cerium (IV) ammonium nitrate (3.06 g, 5.59 mmol). The mixture was stirred for 3 h at rt and then diluted with water (10 mL). The aqueous layer was extracted with ethyl acetate (60 mL × 2). The combined organic layers were washed successively with saturated sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. Flash column chromatography (EtOAc/petroleum ether = 1:1) of the residue afforded the N-deprotected product (606 mg, 1.45 mmol, 77%) as a colorless oil. A mixture of the N-deprotected product, di-*tert*-butyl dicarbonate (1.57 g, 7.2 mmol) and DMAP (cat.) in pyridine was stirred for 5 h under N_2. The residue obtained after removal of the solvent was purified by column chromatography (EtOAc/petroleum ether = 1:10) to afford **3b** (690 mg, 92%) as a colorless oil. R_f = 0.42 (petroleum ether/ethyl acetate, 3:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.52 (s, 9H), 3.47 (dd, J = 3.6 Hz, J = 13.2 Hz, 1H), 3.80 (dd, J = 2.1 Hz, J = 7.2 Hz, 1H), 3.88 (dd, J = 6.3 Hz, J = 12.9 Hz, 1H), 4.02–4.06 (m, 1H), 4.26 (d, J = 6.9 Hz, 1H), 4.61–4.71 (m, 5H), 5.06 (d, J = 11.1 Hz, 1H), 7.21–7.45 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 27.97, 45.53, 71.31, 71.80, 72.57, 74.48, 79.14, 83.38, 127.70, 127.88, 128.23, 128.33, 128.37, 128.47, 137.72, 137.66, 137.86, 152.32, 169.48; HRMS (ESI) Anal. Calcd for $C_{31}H_{35}NO_6Na$ $[M+Na]^+$: 540.2357; found 540.2375.

4.7. (3*R*,4*R*,5*R*)-*tert*-Butyl 3,4,5-tris(benzyloxy)-2-oxopiperidine-1-carboxylate (3c)

To a solution of **2c** (464 mg, 0.864 mmol) in CH_3CN/H_2O (2:1, 30 mL) was added cerium (IV) ammonium nitrate (1.42 g, 2.59 mmol). The mixture was stirred for 3 h at rt and then diluted with water (10 mL). The aqueous layer was extracted with ethyl acetate (50 mL × 2). The combined organic layers were washed successively with saturated sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. Flash column chromatography (EtOAc/petroleum ether = 1:1) of the residue afforded the N-deprotected product (245 mg, 0.59 mmol, 68%) as a colorless oil. A solution of the N-deprotected product, di-*tert*-butyl dicarbonate (654 mg, 2.95 mmol) and DMAP (cat.) in pyridine was stirred for 3.5 h under N_2. The residue obtained after removal of the solvent was purified by column chromatography (EtOAc/petroleum ether = 1:9) to afford **3c** (278 mg, 91%) as a colorless oil. R_f = 0.32 (petroleum ether/ethyl acetate, 3:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.52 (s, 9H), 3.62 (dd, J = 3.9 Hz, J = 13.5 Hz, 1H), 3.78 (dd, J = 3.9 Hz, J = 7.8 Hz, 1H), 3.99–4.05 (m, 2H), 4.33 (d, J = 3.0 Hz, 1H), 4.44 (ABq, J = 11.7 Hz, 2H), 4.60 (d, J = 12.0 Hz, 1H), 4.61 (d, J = 12.0 Hz, 1H), 4.83 (d, J = 12.0 Hz, 1H), 5.10 (d, J = 12.0 Hz, 1H), 7.15–7.41 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.00, 46.02, 71.35, 73.04, 73.50, 73.76, 77.58, 83.21, 127.60, 127.75, 127.89, 127.93, 127.99, 128.33, 128.51, 137.22, 137.91, 137.94, 152.35, 170.01; HRMS (ESI) Anal. Calcd for $C_{31}H_{35}NO_6Na$ $[M+Na]^+$: 540.2357; found 540.2354.

4.8. (2*R*,3*S*,4*R*,5*R*)-*tert*-Butyl 2-acetoxy-3,4,5-tris(benzyloxy)piperidine-1-carboxylate (4a)

To a solution of **3a** (75 mg, 0.15 mmol) in MeOH (3 mL) was added $NaBH_4$ (6 mg, 0.15 mmol) at 0 °C. After 15 min, another portion of $NaBH_4$ (3 mg, 0.075 mmol) was added. The mixture was stirred at 0 °C for another 30 min. After removal of the solvent, the residue was dissolved in ethyl acetate (20 mL) and washed by saturated brine (10 mL × 2). The organic phase was dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated and purified by column chromatography on silica gel (petroleum ether/ethyl acetate 9:1) to provide the unstable *N,O*-hemiacetal product (68 mg, 0.13 mmol, 91%) as a colorless oil. To the solution of *N,O*-hemiacetal product in dry dichloromethane (3 mL) were added anhydrous pyridine (0.22 mL, 2.6 mmol) and Ac_2O (0.14 mL, 1.3 mmol). The mixture was then stirred at rt overnight. The residue obtained after removal of the solvent was purified by column chromatography (EtOAc/petroleum ether = 1:9) to afford **4a** (73 mg, 99%) as a colorless oil. R_f = 0.35 (petroleum ether/ethyl acetate, 3:1). Compound **4a** was actually a pair of anomers (3:1) and the above name and the following analytical data were from the major product. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.37 (s, 9H), 2.07(s, 3H), 2.78 (t, J = 11.5 Hz, 1H), 3.48–3.53 (m, 1H), 3.57 (dd, J = 3.5 Hz, J = 9.5 Hz, 1H), 3.64 (t, J = 9.5 Hz, 1H), 4.00–4.04 (m, 1H), 4.57 (d, J = 11.5 Hz, 1H), 4.67–4.69 (m, 3H), 4.72–4.77 (m, 2H), 6.88 (br s, 1H), 7.24–7.33 (m, 15H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 20.92, 28.09, 41.33, 73.00, 73.13, 75.79, 77.50, 78.83, 81.45, 81.71, 127.54, 127.81, 127.91, 128.17, 128.29, 128.36, 128.45, 137.69, 138.05, 138.78, 153.55, 169.22. HRMS (ESI) Anal. Calcd for $C_{33}H_{39}NO_7Na$ $[M+Na]^+$: 584.2619; found 584.2608.

4.9. (2*R*,3*S*,4*R*,5*S*)-*tert*-Butyl 2-acetoxy-3,4,5-tris(benzyloxy)-piperidine-1-carboxylate (4b)

To a solution of **3b** (610 mg, 1.18 mmol) in MeOH (10 mL) was added $NaBH_4$ (190 mg, 5 mmol) at 0 °C. After 15 min, another portion of $NaBH_4$ (76 mg, 2 mmol) was added. The mixture was stirred at 0 °C for another 30 min. After removal of the solvent, the residue was dissolved in ethyl acetate (60 mL) and washed by saturated brine (15 mL × 2). The organic phase was dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated and purified by column chromatography on silica gel (petroleum ether/ethyl acetate 9:1) to provide the unstable *N,O*-hemiacetal product (545 mg, 1.05 mmol, 89%) as a colorless oil. To the solution of *N,O*-hemiacetal product in dry dichloromethane (10 mL) were added anhydrous pyridine (1.3 mL, 15.4 mmol) and Ac_2O (1 mL, 9.63 mmol). The mixture was then stirred at rt overnight. The residue obtained after removal of the solvent was purified by column chromatography (EtOAc/petroleum ether = 1:9) to afford **4b** (589 mg, 99%) as a colorless oil. R_f = 0.35 (petroleum ether/ethyl acetate, 3:1). Compound **4b** was actually a pair of anomers (3:1) and the above name and the following analytical data were from the major product. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.45 (s, 9H), 1.96 (s, 3H), 3.37 (br s, 1H), 3.80 (s, 3H), 3.92 (br s, 1H), 4.16 (br s, 1H), 4.49–4.54 (m, 3H), 4.63–4.71 (m, 3H), 6.61 (br s, 1H), 7.21–7.35 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.99, 28.21, 37.58, 71.12, 72.18, 72.34, 72.76, 73.80, 74.21, 81.22, 127.32, 127.46, 127.60, 127.90, 128.22, 128.36, 128.42, 137.55, 138.33, 138.45, 154.46, 170.12. Anal. Calcd for $C_{33}H_{39}NO_7$: C, 70.57; H, 7.00; N, 2.49. Found: C, 70.30; H, 6.86; N, 2.41; ESI-MS: 584 $[M+H]^+$.

4.10. (2*R*,3*R*,4*R*,5*R*)-*tert*-Butyl 2-acetoxy-3,4,5-tris(benzyloxy)-piperidine-1-carboxylate (4c)

To a solution of **3c** (218 mg, 0.422 mmol) in MeOH (10 mL) was added $NaBH_4$ (76 mg, 2 mmol) at 0 °C. After 15 min, another portion of $NaBH_4$ (76 mg, 2 mmol) was added. The mixture was stirred at 0 °C for another 30 min. After removal of the solvent, the residue was dissolved in ethyl acetate (40 mL) and washed by saturated brine (10 mL × 2). The organic phase was dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated and purified by column chromatography on silica gel (petroleum

ether/ethyl acetate 9:1) to provide the unstable *N,O*-hemiacetal product (200 mg, 0.385 mmol, 91%) as a colorless oil. To the solution of *N,O*-hemiacetal product in dry dichloromethane (10 mL) were added anhydrous pyridine (0.65 mL, 7.7 mmol) and Ac_2O (0.50 mL, 3.85 mmol). The mixture was then stirred at rt overnight. The residue obtained after removal of the solvent was purified by column chromatography (EtOAc/petroleum ether = 1:9) to afford **4c** (157 mg, 73%) as a colorless oil. R_f = 0.30 (petroleum ether/ethyl acetate, 3:1). Compound **4c** was actually a pair of anomers (4:1) and the above name and the following analytical data were from the major product. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.46 (s, 9H), 2.03 (s, 3H), 3.38–3.42 (m, 1H), 3.71 (br s, 1H), 3.84 (t, *J* = 3.0 Hz, 1H), 3.90 (dd, *J* = 3.0 Hz, *J* = 4.0 Hz, 1H), 4.14 (br s, 1H), 4.38 (d, *J* = 11.5 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 4.58–4.62 (m, 2H), 4.78 (br s, 1H), 4.86 (d, *J* = 12.0 Hz, 1H), 7.03 (br s, 1H), 7.22–7.34 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.11, 28.19, 37.28, 70.57, 72.02, 72.94, 74.17, 74.68, 75.34, 77.19, 81.14, 127.20, 127.31, 127.58, 127.70, 128.16, 128.30, 128.36, 137.77, 138.15, 138.97, 154.37, 169.82. Anal. Calcd for $C_{32}H_{39}NO_7$: C, 70.57; H, 7.00; N, 2.49. Found: C, 70.29; H, 7.17; N, 2.45; ESI-MS: 402 $[M+H]^+$.

4.11. General procedure for the synthesis of aza-thymidines

To a stirred solution of thymine (50 mg, 0.39 mmol) in anhydrous acetonitrile (2 mL) was added *N,O*-bis(trimethylsilyl)acetamide (0.24 mL, 0.975 mmol) under argon. The reaction mixture was stirred under reflux for 15 min. After cooled to 0 °C, donors (compounds **4a–c**) (20 mg, 0.036 mmol) in anhydrous acetonitrile (5 mL) was added and TMSOTf (13 μL, 0.072 mmol) was added dropwise. The mixture was stirred at 0 °C for further 3 h. The reaction was quenched with cold saturated sodium bicarbonate aqueous solution (6 mL) and the resulting mixture was extracted with CH_2Cl_2 (40 mL × 3). The combined organic phases were washed with brine and dried over anhydrous sodium sulfate. After removal of the solvent, the resulting residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 3:1→petroleum ether/acetone, 4:1) to give the products **5a–c**, **6a**, **6c**.

4.12. (2*R*,3*R*,4*R*,5*R*)-*tert*-Butyl 3,4,5-tris(benzyloxy)-2-(thymidine-1-yl)piperidine-1-carboxylate (5a) and (2*S*,3*R*,4*R*,5*R*)-*tert*-butyl 3,4,5-tris(benzyloxy)-2-(thymidine-1-yl)piperidine-1-carboxylate (6a)

These two compounds were products of the coupling reaction of **4a** with thymine. The less polar one was confirmed by NMR to be compound **5a** (70% yield, colorless foam). R_f = 0.30 (petroleum ether/ethyl acetate, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.38 (s, 9H), 1.83 (s, 3H), 3.63–3.67 (m, 2H), 3.79 (d, *J* = 1.0 Hz, 1H), 4.28–4.32 (m, 1H), 4.38–4.42 (m, 2H), 4.52 (d, *J* = 12.0 Hz, 1H), 4.68–4.71 (m, 3H), 4.80 (d, *J* = 12.0 Hz, 1H), 5.02 (br s, 1H), 6.90 (br s, 1H), 7.12–7.14 (m, 2H), 7.23–7.37 (m, 12H), 7.93 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.13, 28.20, 41.26, 70.17, 72.87, 74.51, 74.91, 80.10, 81.59, 84.77, 108.76, 127.78, 127.85, 127.95, 128.14, 128.39, 128.42, 128.80, 137.17, 137.61, 137.95, 141.97, 149.69, 154.60, 163.68. HRMS (ESI) Anal. Calcd for $C_{36}H_{42}NO_7$ $[M+H]^+$: 628.3017; found 628.3017. The more polar compound was confirmed by NMR to be compound **6a** (11% yield, colorless foam). R_f = 0.29 (petroleum ether/ethyl acetate, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.35 (s, 9H), 1.82 (s, 3H), 3.42 (dd, *J* = 10.0 Hz, *J* = 12.5 Hz, 1H), 3.73 (br s, 1H), 3.83 (br s, 1H), 4.05 (br s, 1H), 4.31 (d, *J* = 11.5 Hz, 1H), 4.44 (br s, 1H), 4.49–4.60 (m, 5H), 6.24 (d, *J* = 4.5 Hz, 1H), 7.05 (s, 1H), 7.12–7.14 (m, 2H), 7.27–7.37 (m, 12H), 7.93 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.33, 28.06, 41.98, 63.99, 71.42, 71.94, 73.32, 75.15, 81.97, 109.16, 127.7 4, 127.94, 128.04, 128.30, 128.46, 128.50, 128.53, 136.50, 137.35, 137.71, 137.98, 149.75, 154.30, 163.46. HRMS (ESI) Anal. Calcd for $C_{36}H_{42}NO_7$ $[M+H]^+$: 628.3017; found 628.3023.

4.13. (2*S*,3*R*,4*R*,5*S*)-*tert*-Butyl 3,4,5-tris(benzyloxy)-2-(thymine-1-yl)piperidine-1-carboxylate (5b)

The coupling reaction of **4b** with thymine afforded **5b** (85% yield, colorless foam). R_f = 0.35 (petroleum ether/ethyl acetate, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.38 (s, 9H), 1.55 (s, 3H), 3.33 (dd, *J* = 11.0 Hz, 12.5 Hz, 1H), 3.82 (dd, *J* = 2.0 Hz, *J* = 4.0 Hz, 1H), 3.91 (d, *J* = 3.0 Hz, 1H), 4.01 (ddd, *J* = 2.0 Hz, *J* = 6.0 Hz, *J* = 10.5, 1H), 4.31 (dd, *J* = 6.5 Hz, *J* = 13.0 Hz, 1H), 4.35 (d, *J* = 11.5 Hz, 1H), 4.58–4.70 (m, 4H), 4.85 (d, *J* = 12.0 Hz, 1H), 6.19 (d, *J* = 1.0 Hz, 1H), 6.91 (d, *J* = 1.0 Hz, 1H), 7.09–7.11 (m, 2H), 7.24–7.39 (m, 12H), 8.25 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.28, 28.08, 41.38, 65.50, 71.48, 71.55, 72.30, 73.23, 74.22, 74.86, 82.15, 109.46, 127.76, 127.83, 127.88, 127.97, 128.43, 128.49, 137.12, 137.60, 137.88, 150.31, 154.34, 163.79. HRMS (ESI) Anal. Calcd for $C_{36}H_{42}NO_7$ $[M+H]^+$: 628.3017; found 628.3028.

4.14. (2*S*,3*S*,4*R*,5*R*)-*tert*-Butyl 3,4,5-tris(benzyloxy)-2-(thymine-1-yl)piperidine-1-carboxylate (5c) and (2*R*,3*S*,4*R*,5*R*)-*tert*-butyl 3,4,5-tris(benzyloxy)-2-(thymine-1-yl)piperidine-1-carboxylate (6c)

These two compounds were products of the coupling reaction of **4c** with thymine. The less polar one was confirmed by NMR to be compound **6c** (53% yield, colorless foam). R_f = 0.28 (petroleum ether/ethyl acetate, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.38 (s, 9H), 1.86 (s, 3H), 3.54 (dd, *J* = 9.3 Hz, 13.2 Hz, 1H), 3.78–3.83 (m, 2H), 4.35 (d, *J* = 12.0 Hz, 2H), 4.41–4.54 (m, 3H), 4.57–4.60 (m, 3H), 5.42 (d, *J* = 8.4 Hz, 1H), 7.04 (br s, 1H), 7.17–7.36 (m, 14H), 9.10 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.10, 28.16, 43.01, 71.02, 71.70, 72.28, 72.93, 73.91, 75.00, 75.78, 81.47, 108.73, 127.58, 128.14, 128.25, 128.37, 137.08, 137.65, 137.84, 142.06, 150.13, 154.39, 164.47. HRMS (ESI) Anal. Calcd for $C_{36}H_{42}NO_7$ $[M+H]^+$: 628.3017; found 628.3012. The more polar compound was confirmed by NMR to be compound **5c** (35% yield, colorless foam). R_f = 0.26 (petroleum ether/ethyl acetate, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.36 (s, 9H), 1.80 (s, 3H), 3.62 (dd, *J* = 3.3 Hz, 14.7 Hz, 1H), 3.67 (dd, *J* = 2.1 Hz, *J* = 3.9 Hz, 1H), 3.93 (t, *J* = 3.6 Hz, 1H), 4.28–4.29 (m, 1H), 4.41–4.51 (m, 3H), 4.67–4.80 (m, 4H), 6.19 (d, *J* = 4.2 Hz, 1H), 6.92 (s, 1H), 7.11–7.14 (m, 2H), 7.26–7.34 (m, 12H), 9.12 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.34, 28.00, 42.98, 65.29, 70.54, 72.09, 73.66, 75.51, 78.71, 80.83, 81.74, 109.38, 127.45, 127.73, 127.83, 128.06, 128.20, 128.32, 128.39, 136.99, 137.21, 137.60, 137.73, 150.16, 154.13, 163.86. HRMS (ESI) Anal. Calcd for $C_{36}H_{42}NO_7$ $[M+H]^+$: 628.3017; found 628.3006.

4.15. (2*R*,3*R*,4*R*,5*R*)-*tert*-Butyl 3,4,5-trihydroxy-2-(thymidine-1-yl)piperidine-1-carboxylate (7a)

To the solution of **5a** (21 mg, 0.033 mmol) in MeOH (4 mL) were added hydrogen chloride solution (1.25 M in methanol, 0.2 mL) and Pd/C catalyst (10% wt, 8 mg). The reaction mixture was stirred under the atmosphere of hydrogen gas for 60 h. The reaction mixture was filtered through celite pad and the filtrate was concentrated. The residue was subjected to a C-18 reverse-phase column chromatography (H_2O/CH_3OH = 2:1) to give **7a** (12 mg, 94%) as a solid after lyophilization. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.40 (s, 9H), 1.87 (s, 3H), 3.40 (dd, *J* = 3.0 Hz, *J* = 9.9 Hz, 1H), 3.77–3.96 (m, 3H), 4.15 (t, *J* = 11.1 Hz, 1H), 5.20 (d, *J* = 8.4 Hz, 1H), 7.33 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ: 12.55, 28.56, 47.62, 70.42, 74.88, 76.28, 77.89, 82.53, 109.98, 143.24, 154.28, 156.45. HRMS (ESI) Anal. Calcd for $C_{15}H_{23}N_3O_7Na$ $[M+Na]^+$: 380.1428; found 380.1421.

4.16. (2*S*,3*R*,4*R*,5*S*)-*tert*-Butyl 3,4,5-trihydroxy-2-(thymine-1-yl) piperidine-1-carboxylate (7b)

Compound **7b** was prepared from **5b** as described in the preparation of **7a** from **5a**, yielding **7b** (89% yield) as colorless solid after lyophilization. ^{1}H NMR (500 MHz, C_5D_5N) δ: 1.39 (s, 9H), 1.95 (s, 3H), 4.15 (dd, J = 10.5 Hz, J = 12.5 Hz, 1H), 4.60 (t, J = 3.5 Hz, 1H), 4.80 (dd, J = 6.0 Hz, J = 12.5 Hz, 1H), 4.92–4.96 (m, 2H), 5.06 (m, 1H), 6.74 (d, J = 3.0 Hz, 1H), 6.89 (br s, 1H), 7.27 (br s, 1H), 7.84 (s, 1H), 8.08 (br s, 1H), 13.17 (br s, 1H). ^{13}C NMR (75 MHz, D_2O) δ: 12.21, 28.01, 45.21, 64.77, 69.85, 70.18, 71.17, 84.43, 111.49, 139.88, 152.96, 156.71, 167.98. HRMS (ESI) Anal. Calcd for $C_{15}H_{24}N_3O_7$ [M+H]$^+$: 358.1609; found 358.1604.

4.17. (2*S*,3*S*,4*R*,5*R*)-*tert*-Butyl 3,4,5-trihydroxy-2-(thymine-1-yl)piperidine-1-carboxylate (7c)

Compound **7c** was prepared from **5c** as described in the preparation of **7a** from **5a**, yielding **7c** (92% yield) as a solid after lyophilization. ^{1}H NMR (500 MHz, D_2O) δ: 1.36 (s, 9H), 1.89 (s, 3H), 3.68 (dd, J = 2.0 Hz, J = 5.0 Hz, 1H), 3.81 (dd, J = 4.5 Hz, J = 15.0 Hz, 1H), 4.01 (t, J = 4.5 Hz, 1H), 4.09 (d, J = 14.5 Hz, 1H), 4.27 (dd, J = 2.5 Hz, J = 4.5 Hz, 1H), 6.09 (d, J = 4.5 Hz, 1H), 7.31 (d, J = 1.5 Hz, 1H). ^{13}C NMR (75 MHz, D_2O) δ: 12.18, 27.85, 47.78, 66.92, 68.68, 72.01, 73.45, 83.94, 111.34, 138.89, 153.00, 156.30, 168.28; HRMS (ESI) Calcd for $C_{15}H_{23}N_3O_7Na$ [M+Na]$^+$: 380.1428; found 380.1434.

4.18. (2*S*,3*R*,4*R*,5*R*)-*tert*-Butyl 3,4,5-trihydroxy-2-(thymidine-1-yl) piperidine-1-carboxylate (8a)

Compound **8a** was prepared from **6a** as described in the preparation of **7a** from **5a**, yielding **8a** (74% yield) as a solid after lyophilization. ^{1}H NMR (500 MHz, CD_3OD) δ: 1.39 (s, 9H), 1.87 (s, 3H), 3.40 (dd, J = 10.5 Hz, J = 12.5 Hz, 1H), 3.63–3.67 (m, 1H), 3.85 (dd, J = 4.0 Hz, 5.5 Hz, 1H), 4.02 (t, J = 5.0 Hz, 1H), 4.20 (dd, J = 6.5 Hz, J = 13.0 Hz, 1H), 6.16 (d, J = 5.0 Hz, 1H), 7.34 (d, J = 1.0 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ: 12.44, 28.42, 45.84, 67.06, 71.59, 72.35, 76.01, 82.73, 110.09, 141.02, 152.61, 156.08, 166.79. HRMS (ESI) Anal. Calcd for $C_{15}H_{23}N_3O_7Na$ [M+Na]$^+$: 380.1428; found 380.1419.

4.19. (2*R*,3*S*,4*R*,5*R*)-*tert*-Butyl 3,4,5-trihydroxy-2-(thymine-1-yl)piperidine-1-carboxylate (8c)

Compound **8c** was prepared from **6c** as described in the preparation of **7a** from **5a**, yielding **8c** (89% yield) as a solid after lyophilization. ^{1}H NMR (300 MHz, D_2O) δ: 1.22 (s, 9H), 1.73 (s, 3H), 3.03–3.13 (m, 1H), 3.77–3.81 (m, 2H), 4.13 (dd, J = 7.2 Hz, J = 13.5 Hz, 1H), 4.28 (dd, J = 2.7 Hz, J = 8.1 Hz, 1H), 5.50 (d, J = 7.8 Hz, 1H), 7.25 (s, 1H); ^{13}C NMR (75 MHz, D_2O) δ: 12.08, 27.94, 44.88, 68.12, 69.71, 71.03, 73.79, 84.34, 111.42, 141.09, 153.37, 156.29, 168.65; HRMS (ESI) Calcd for $C_{15}H_{23}N_3O_7Na$ [M+Na]$^+$: 380.1428; found 380.1421.

4.20. (3*S*,4*R*,5*R*)-*tert*-Butyl 3,4,5-trihydroxy-2-oxopiperidine-1-carboxylate (9a)

To the solution of **3a** (205 mg, 0.397 mmol) in anhydrous EtOAc (5 mL) was added Pd/C catalyst (10% wt, 20 mg). The mixture was stirred under the atmosphere of hydrogen gas for 20 h. The reaction mixture was filtered through celite pad and the filtrate was concentrated to afford **9a** (97 mg, 99%) as a colorless oil. No further purification by chromatography was needed. ^{1}H NMR (300 MHz, D_2O) δ: 1.38 (s, 9H), 3.50 (dd, J = 4.5 Hz, J = 9.3 Hz, 1H), 3.67 (dd, J = 3.0 Hz, J = 13.5 Hz, 1H), 3.78–3.89 (m, 2H), 4.04 (d, J = 9.3 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 27.69, 47.71, 69.75, 73.89, 76.32, 86.31, 152.54, 173.63. HRMS (ESI) Anal. Calcd for $C_{10}H_{17}NO_6Na$ [M+Na]$^+$: 270.0948; found 270.0950.

4.21. (3*S*,4*R*,5*S*)-*tert*-Butyl 3,4,5-trihydroxy-2-oxopiperidine-1-carboxylate (9b)

Compound **9b** was prepared from **3b** as described in the preparation of **9a**, yielding **9b** (92% yield) as a colorless oil. ^{1}H NMR (300 MHz, D_2O) δ: 1.35 (s, 9H), 3.54 (d, J = 14.1 Hz, 1H), 3.76 (d, J = 11.7 Hz, 2H), 4.10 (s, 1H), 4.19 (d, J = 9.9 Hz, 1H). ^{13}C NMR (75 MHz, D_2O) δ: 27.63, 49.84, 66.73, 71.70, 71.82, 86.21, 153.40, 174.37. HRMS (ESI) Anal. Calcd for $C_{10}H_{17}NO_6K$ [M+K]$^+$: 286.0682; found 286.0693.

4.22. (3*R*,4*R*,5*R*)-*tert*-Butyl 3,4,5-trihydroxy-2-oxopiperidine-1-carboxylate (9c)

Compound **9c** was prepared from **3c** as described in the preparation of **9a**, yielding **9c** (98% yield) as a colorless oil. ^{1}H NMR (300 MHz, D_2O) δ: 1.38 (s, 9H), 3.47 (dd, J = 6.6 Hz, J = 15.3 Hz, 1H), 3.98–4.06 (m, 3H), 4.43 (d, J = 3.0 Hz, 1H). ^{13}C NMR (75 MHz, D_2O) δ: 27.66, 48.45, 67.80, 70.35, 72.78, 86.17, 152.91, 174.99. HRMS (ESI) Anal. Calcd for $C_{10}H_{17}NO_6Na$ [M+Na]$^+$: 270.0948; found 270.0946.

4.23. (2*R*,3*S*,4*R*,5*R*)-1-(*tert*-Butoxycarbonyl)piperidine-2,3,4,5-tetrayl tetraacetate (10a)

To the cooled solution of **9a** (33 mg, 0.13 mmol) in MeOH (2 mL) was added $NaBH_4$ (4 mg, 0.4 mmol). The reaction mixture was stirred at 0 °C for 10 min. After removal of the solvent, the residue was subjected to a C-18 reverse-phase column chromatography (H_2O/CH_3OH = 2:1) to give the intermediate product (32 mg, 96%) as a colorless oil. To the solution of the intermediate product in dry pyridine (5 mL) was added Ac_2O (1 mL). The mixture was then stirred at rt overnight. The residue obtained after removal of the solvent was purified by column chromatography (EtOAc/petroleum ether = 1:5) to afford **10a** (48 mg, 91%) as a colorless oil. R_f = 0.45 (petroleum ether/ethyl acetate, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47 (s, 9H), 2.01–2.09 (m, 12H), 3.52 (dd, J = 2.7 Hz, J = 14.1 Hz, 1H), 3.96 (d, J = 14.7 Hz, 1H), 5.00–5.06 (m, 3H), 6.62 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.61, 20.72, 20.79, 20.83, 28.13, 39.76, 68.36, 68.80, 69.09, 77.17, 81.71, 153.67, 168.66, 169.03, 169.11, 169.77. HRMS (ESI) Anal. Calcd for $C_{18}H_{27}NO_6K$ [M+K]$^+$: 456.1261; found 456.1258.

4.24. (2*R*,3*S*,4*R*,5*S*)-1-(*tert*-Butoxycarbonyl)piperidine-2,3,4,5-tetrayl tetraacetate (10b)

To the cooled solution of **9b** (31 mg, 0.13 mmol) in MeOH (2 mL) was added $NaBH_4$ (4 mg, 0.4 mmol). Then, at the 10th, 15th, and 20th min, $NaBH_4$ (4 mg, 0.4 mmol) was added respectively. After removal of the solvent at the 25th min, the residue was subjected to a C-18 reverse-phase column chromatography (H_2O/CH_3OH = 2:1) to give the intermediate product (30 mg, 95%) as a colorless oil. To the solution of the intermediate product in dry pyridine (5 mL) was added Ac_2O (1 mL). The mixture was then stirred at rt overnight. The residue obtained after removal of the solvent was purified by column chromatography (EtOAc/petroleum ether = 1:5) to afford **10b** (46 mg, 91%) as a colorless oil. R_f = 0.45 (petroleum ether/ethyl acetate, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47 (s, 9H), 2.04 (s, 3H), 2.08 (s, 3H), 2.12 (s, 3H), 2.13 (s, 3H), 3.28 (t, J = 11.7 Hz, 1H), 4.08 (br s, 1H), 5.05–5.15 (m, 2H), 5.25 (s, 1H), 6.57 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.15, 20.71, 28.10, 64.97, 66.63, 67.88, 75.66, 77.21, 81.95, 153.59,

168.44, 168.63, 169.38, 169.60. HRMS (ESI) Anal. Calcd for $C_{18}H_{27}NO_6K$ $[M+K]^+$: 456.1261; found 456.1256.

4.25. (2*R*,3*R*,4*R*,5*R*)-1-(*tert*-Butoxycarbonyl)piperidine-2,3,4,5-tetrayl tetraacetate (10c)

Compound **10c** was prepared from **9c** as described in the preparation of **10b**, yielding **10c** (75% yield for two steps) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.46 (s, 9H), 2.02 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H), 2.13 (s, 3H), 3.42 (m, 1H), 4.13 (d, J = 14.7 Hz, 1H), 4.99 (br s, 1H), 5.26–5.28 (m, 2H), 6.92 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.50, 20.69, 20.83, 28.02, 40.40, 61.79, 65.48, 66.29, 69.43, 81.93, 153.61, 169.00, 169.26, 169.35, 169.45. HRMS (ESI) Anal. Calcd for $C_{18}H_{27}NO_6Na$ $[M+Na]^+$: 440.1527; found 440.1527.

4.26. (2*S*,3*R*,4*R*,5*R*)-2-(N^6-Benzoyl-adenine-9-yl)-1-(*tert*-butoxycarbonyl)piperidine-3,4,5-triyl triacetate (11a)

To a stirred solution of N^6-benzoyladenine (75 mg, 0.317 mmol) in anhydrous acetonitrile (2 mL) under argon was added *N,O*-bis(trimethylsilyl)acetamide (0.12 mL, 0.48 mmol). The reaction mixture was stirred under reflux for 20 min. After cooled to 0 °C, compound **10a** (33 mg, 0.079 mmol) in anhydrous acetonitrile (5 mL) was added and TMSOTf (29 μL, 0.160 mmol) was added dropwise. The solution was stirred at 0 °C for 1 h and at rt for further 1.5 h. The reaction was then quenched with cold saturated sodium bicarbonate aqueous solution (6 mL) and the resulting mixture was extracted with CH_2Cl_2 (40 mL × 3). The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent, the resulting residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 5:1→CH_2Cl_2/MeOH, 70:1) to give **11a** (29 mg, 61%) as a colorless foam. R_f = 0.25 (CH_2Cl_2/MeOH, 25:1). Only crude product of **11a** was obtained because about 10% of its anomer and other impurity accompanied **11a** all the time, which was directly used for the next reaction.

4.27. (2*S*,3*R*,4*R*,5*R*)-2-(N^6-Benzoyl-adenine-9-yl)-1-(*tert*-butoxycarbonyl)piperidine-3,4,5-triyl triacetate (11b) and (2*R*-3*R*,4*R*,5*R*)-2-(N^6-benzoyl-adenine-9-yl)-1-(*tert*-butoxycarbonyl)piperidine-3,4,5-triyl triacetate (11b′)

Compounds **11b** and **11b′** were prepared from **10b** as described in the preparation of **11a**, yielding the inseparable mixture of **11b** and **11b′** (11:7, 68% yield in total) as a colorless oil, which was directly used for the next reaction. R_f = 0.28 (CH_2Cl_2/MeOH, 25:1).

4.28. (2*R*,3*S*,4*R*,5*R*)-2-(N^6-Benzoyl-adenine-9-yl)-1-(*tert*-butoxycarbonyl)piperidine-3,4,5-triyl triacetate (11c) and (2*S*,3*S*,4*R*,5*R*)-2-(N^6-benzoyl-adenine-9-yl)-1-(*tert*-butoxycarbonyl)piperidine-3,4,5-triyl triacetate (11c′)

Compounds **11c** and **11c′** were prepared from **10c** as described in the preparation of **11a**, yielding **11c** (51%, the less polar product) as a colorless oil. R_f = 0.25 (CH_2Cl_2/MeOH, 25:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.46 (s, 9H), 2.01 (s, 3H), 2.13 (s, 3H), 2.17 (s, 3H), 3.80 (dd, J = 10.5 Hz, J = 13.2 Hz, 1H), 4.51 (m, 1H), 5.09–5.16 (m, 1H), 5.61 (s, 1H), 6.31–6.32 (m, 2H), 7.51–7.65 (m, 3H), 8.02–8.12 (m, 3H), 8.83 (s, 1H), 9.03 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.56, 20.68, 20.89, 28.09, 65.99, 68.04, 68.76, 70.11, 77.20, 82.76, 123.64, 127.83, 128.89, 132.85, 133.45, 149.57, 151.54, 164.49, 169.31, 169.42, 169.83. HRMS (ESI) Anal. Calcd for $C_{28}H_{33}N_6O_9$ $[M+H]^+$: 597.2304; found 597.2306. The more polar product was **11c′** (30%, colorless oil). R_f = 0.23 (CH_2Cl_2/MeOH, 25:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.28 (s, 9H), 1.85 (s, 3H), 2.03 (s, 3H), 2.16 (s, 3H), 3.90 (dd, J = 2.4 Hz, J = 15.2 Hz, 1H), 4.47 (d, J = 15.2 Hz,1H), 5.20 (br s, 1H), 5.30 (dd, J = 3.2 Hz, J = 4.4 Hz, 1H), 5.78 (dd, J = 2.8 Hz, J = 5.6 Hz, 1H), 6.96 (d, J = 5.6 Hz, 1H), 7.52–7.55 (m, 2H), 7.60–7.62 (m, 1H), 8.03 (d, J = 7.2 Hz, 2H), 8.17 (s, 1H), 8.80 (s, 1H), 9.01 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.47, 20.67, 20.92, 27.94, 42.80, 62.18, 65.78, 68.09, 68.93, 82.86, 121.82, 127.81, 128.88, 132.82, 133.55, 141.31, 149.42, 152.32, 152.82, 153.59, 164.52, 168.98, 169.05, 169.66. HRMS (ESI) Anal. Calcd for $C_{28}H_{33}N_6O_9$ $[M+H]^+$: 597.2304; found 597.2306.

4.29. (2*S*,3*R*,4*R*,5*R*)-1-(*tert*-Butoxycarbonyl)-2-(5-fluoro-uracil-1-yl) piperidine-3,4,5-triyl triacetate (12a)

To a stirred solution of 5-fluoro-uracil (50 mg, 0.36 mmol) in anhydrous acetonitrile (2 mL) under argon was added *N,O*-bis(trimethylsilyl)acetamide (0.14 mL, 0.58 mmol). The reaction mixture was stirred under reflux for 20 min. After cooled to 0 °C, compound **10a** (30 mg, 0.072 mmol) in anhydrous acetonitrile (5 mL) was added and TMSOTf (26 μL, 0.144 mmol) was added dropwise. The solution was stirred at 0 °C for 1 h. The reaction was then quenched with cold saturated sodium bicarbonate aqueous solution (6 mL) and the resulting mixture was extracted with CH_2Cl_2 (40 mL × 3). The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent, the resulting residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 5:1→CH_2Cl_2/MeOH, 80:1) to give **12a** (31 mg, 87%) as a colorless foam. R_f = 0.30 (CH_2Cl_2/MeOH, 20:1). Only crude product of **12a** was obtained because some inseparable impurity accompanied **12a** all the time, which was directly used for the next reaction.

4.30. (2*S*,3*R*,4*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-2-(5-fluoro-uracil-1-yl)piperidine-3,4,5-triyl triacetate (12b)

Compound **12b** was prepared from **10b** as described in the preparation of **12a** from **10a**, yielding **12b** (83% yield) as a colorless foam. R_f = 0.32 (CH_2Cl_2/MeOH, 30:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.47 (s, 9H), 2.02 (s, 3H), 2.04 (s, 3H), 2.11 (s, 3H), 3.86 (d, J = 14.0 Hz, 1H), 4.24 (dd, J = 3.2 Hz, J = 14.4 Hz, 1H), 5.33 (dd, J = 8.4 Hz, J = 10.0 Hz, 1H), 5.45 (d, J = 1.6 Hz, 1H), 5.83 (dd, J = 3.2 Hz, J = 10.0 Hz, 1H), 6.13 (d, J = 8.4 Hz, 1H), 7.38 (d, J = 5.2 Hz, 1H), 9.26 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.39, 20.67, 20.82, 28.14, 45.14, 67.18, 67.24, 69.05, 70.19, 83.01, 130.60, 130.94, 138.00, 140.37, 149.65, 154.68, 156.88, 157.15, 169.63, 169.80, 170.69. HRMS (ESI) Anal. Calcd for $C_{20}H_{26}FN_3O_{10}Na$ $[M+Na]^+$: 510.1494; found 510.1501.

4.31. (2*R*,3*S*,4*R*,5*R*)-1-(*tert*-Butoxycarbonyl)-2-(5-fluoro-uracil-1-yl)piperidine-3,4,5-triyl triacetate (12c)

Compound **12c** was prepared from **10c** as described in the preparation of **12a** from **10a**, yielding **12c** (57% yield) as a colorless foam. R_f = 0.30 (CH_2Cl_2/MeOH, 30:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.49 (s, 9H), 2.06 (s, 3H), 2.12 (s, 6H), 3.59 (dd, J = 9.6 Hz, J = 14.0 Hz, 1H), 4.44 (dd, J = 7.6 Hz, J = 14.0 Hz,1H), 4.89–4.94 (m, 1H), 5.37–5.39 (m, 2H), 5.98 (dd, J = 2.8 Hz, J = 8.4 Hz, 1H), 7.37 (d, J = 5.6 Hz, 1H), 9.06 (d, J = 3.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.58, 20.64, 20.75, 28.13, 42.42, 67.02, 69.50, 70.65, 72.13, 82.91, 128.93, 129.26, 138.62, 140.99, 148.92, 154.12, 156.59, 156.86, 169.20, 169.48, 169.60. HRMS (ESI) Anal. Calcd for $C_{20}H_{26}FN_3O_{10}Na$ $[M+Na]^+$: 510.1494; found 510.1482.

4.32. (2*S*,3*R*,4*R*,5*R*)-*tert*-Butyl 2-(uracil-1-yl)-3,4,5-trihydroxypiperidine-1-carboxylate (13a)

To a stirred solution of uracil (40 mg, 0.36 mmol) in anhydrous acetonitrile (2 mL) under argon was added *N,O*-bis(trimethylsilyl)

acetamide (0.14 mL, 0.58 mmol). The reaction mixture was stirred under reflux for 15 min. After cooled to 0 °C, compound **10a** (30 mg, 0.072 mmol) in anhydrous acetonitrile (5 mL) was added and TMSOTf (26 μL, 0.144 mmol) was added dropwise. The solution was stirred at 0 °C for 1 h. The reaction was then quenched with cold saturated sodium bicarbonate aqueous solution (6 mL) and the resulting mixture was extracted with CH_2Cl_2 (40 mL × 3). The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent, the resulting residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 5:1→CH_2Cl_2/MeOH, 80:1) to give **13a** (35 mg ⩾ 100%) as a colorless foam. R_f = 0.35 (CH_2Cl_2/MeOH, 25:1). Only crude product of **13a** was obtained because some inseparable impurity accompanied **13a** all the time, which was directly used for the next reaction.

4.33. (2*S*,3*R*,4*R*,5*R*)-2-(4-Acetamido-cytosine-1-yl)-1-(*tert*-butoxycarbonyl)piperidine-3,4,5-triyl triacetate (14a)

To a stirred solution of 4-acetamido-cytosine (101 mg, 0.66 mmol) in anhydrous acetonitrile (2 mL) under argon was added *N*, *O*-bis(trimethylsilyl)acetamide (0.19 mL, 0.79 mmol). The reaction mixture was stirred under reflux for 15 min. After cooled to 0 °C, compound **10a** (55 mg, 0.132 mmol) in anhydrous acetonitrile (5 mL) was added and TMSOTf (48 μL, 0.264 mmol) was added dropwise. The mixture was stirred at 0 °C for 1.5 h. The reaction was then quenched with cold saturated sodium bicarbonate aqueous solution (6 mL) and the resulting mixture was extracted with CH_2Cl_2 (40 mL × 3). The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent, the resulting residue was purified by flash column chromatography (petroleum ether/ethyl acetate, 3:1→CH_2Cl_2/MeOH, 80:1) to give **14a** (51 mg, 76%) as a colorless foam. R_f = 0.32 (CH_2Cl_2/MeOH, 25:1). Only crude product of **14a** was obtained because some inseparable impurity accompanied **14a** all the time, which was directly used for the next reaction.

4.34. (2*S*,3*R*,4*R*,5*R*)-*tert*-Butyl 2-(adenine-9-yl)-3,4,5-trihydroxypiperidine-1-carboxylate (15a)

The crude product obtained in the previous reaction (compound **11a**, 29 mg, 0.049 mmol) was dissolved in MeOH saturated with ammonia (5 mL) and stirred at rt overnight. The reaction mixture was concentrated, and the residue was purified by C-18 reverse-phase column chromatography (H_2O/CH_3OH = 2:1). Further purification was carried out using preparative HPLC (C-18, 40% MeOH in water) to yield compound **15a** (15 mg, 82%, white solids). ^{1}H NMR (300 MHz, D_2O) δ: 1.00 (s, 9H), 3.51 (dd, J = 3.9 Hz, J = 10.5 Hz, 1H), 3.85–3.90 (m, 3H), 4.13 (dd, J = 9.0 Hz, J = 10.2 Hz, 1H), 5.71 (d, J = 9.0 Hz, 1H), 8.08 (s, 1H), 8.15 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ: 27.15, 45.70, 68.75, 70.77, 72.98, 75.76, 83.36, 118.33, 141.21, 149.03, 152.67, 155.52, 155.68. HRMS (ESI) Anal. Calcd for $C_{15}H_{22}N_6O_5Na$ [M+Na]$^+$: 389.1544; found 389.1549.

4.35. (2*S*,3*R*,4*R*,5*S*)-*tert*-Butyl 2-(adening-9-yl)-3,4,5-trihydroxy-piperidine-1-carboxylate (15b) and (2*R*,3*R*,4*R*,5*S*)-*tert*-butyl 2-(adening-9-yl)-3,4,5-trihydroxypiperidine-1-carboxylate (15b′)

The product obtained in the previous reaction (compounds **11b** and **11b′**, 29 mg, 0.049 mmol) was dissolved in MeOH saturated with ammonia (5 mL) and stirred at rt overnight. The reaction mixture was concentrated, and the residue was purified by C-18 reverse-phase column chromatography (H_2O/CH_3OH = 2:1). Further purification was carried out using column chromatography ($CHCl_3$/CH_3OH/$NH_3{\cdot}H_2O$ = 100:10:1). The less polar product was **15b** (9 mg, 51%, white solids after lyophilization). ^{1}H NMR (400 MHz, D_2O) δ: 1.10 (s, 9H), 3.48 (dd, J = 9.6 Hz, J = 13.2 Hz, 1H), 3.91 (dd, J = 3.6 Hz, J = 6.0 Hz, 1H), 4.05 (dd, J = 6.0 Hz, J = 13.2 Hz, 1H), 4.14–4.18 (m, 1H), 4.31 (dd, J = 4.0 Hz, J = 6.0 Hz, 1H), 6.08 (d, J = 4.0 Hz, 1H), 8.15 (s, 1H), 8.20 (s, 1H). ^{13}C NMR (75 MHz, D_2O) δ: 27.66, 43.56, 49.38, 64.50, 67.50, 70.00, 70.46, 84.23, 118.41, 141.31, 149.27, 153.10, 156.08, 156.60. HRMS (ESI) Anal. Calcd for $C_{15}H_{22}N_6O_5Na$ [M+Na]$^+$: 389.1544; found 389.1550. The more polar product was **15b′** (6 mg, 37%, white solids after lyophilization). ^{1}H NMR (400 MHz, D_2O) δ: 1.13 (s, 9H), 3.56 (dd, J = 1.6 Hz, J = 14.8 Hz, 1H), 4.02 (dd, J = 3.2 Hz, J = 10.8 Hz, 1H), 4.09 (dd, J = 2.4 Hz, J = 14.8 Hz, 1H), 4.17–4.18 (m, 1H), 4.26 (dd, J = 6.4 Hz, J = 10.4 Hz, 1H), 6.71 (d, J = 6.4 Hz, 1H), 8.13 (s, 1H), 8.19 (s, 1H). ^{13}C NMR (75 MHz, D_2O) δ: 27.76, 46.99, 65.96, 66.90, 67.81, 69.73, 84.19, 118.15, 141.33, 150.35, 153.09, 156.14, 156.52. HRMS (ESI) Anal. Calcd for $C_{15}H_{22}N_6O_5Na$ [M+Na]$^+$: 389.1544; found 389.1555.

4.36. (2*R*,3*S*,4*R*,5*R*)-*tert*-Butyl 2-(adenine-9-yl)-3,4,5-trihydroxypiperidine-1-carboxylate (15c)

Compound **11c** (14 mg, 0.023 mmol) was dissolved in MeOH saturated with ammonia (5 mL) and stirred at rt overnight. The reaction mixture was concentrated, and the residue was purified by C-18 reverse-phase column chromatography (H_2O/CH_3OH = 2:1) to afford **15c** (8 mg, 93%) as colorless solids after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ: 1.17 (s, 9H), 3.17 (dd, J = 10.0 Hz, J = 13.6 Hz, 1H), 3.81 (dd, J = 3.2 Hz, J = 6.0 Hz, 1H), 3.94 (td, J = 6.0 Hz, J = 10.0 Hz, 1H), 4.28 (dd, J = 6.4, J = 13.6 Hz, 1H), 4.57 (dd, J = 2.8 Hz, J = 5.2 Hz, 1H), 6.19 (d, J = 5.6 Hz, 1H), 8.14 (s, 2H). ^{13}C NMR (100 MHz, D_2O) δ: 27.19, 44.64, 67.42, 67.44, 69.10, 71.92, 83.76, 118.46, 140.44, 149.00, 152.76, 155.60, 155.72. HRMS (ESI) Anal. Calcd for $C_{15}H_{23}N_6O_5$ [M+H]$^+$: 367.1724; found 367.1725.

4.37. (2*S*,3*S*,4*R*,5*R*)-*tert*-Butyl 2-(adenine-9-yl)-3,4,5-trihydroxypiperidine- 1-carboxylate (15c′)

Compound **15c′** was prepared from **11c′** as described in the preparation of **15c** from **11c**, yielding **15c′** (90% yield) as colorless solids after lyophilization. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.22 (s, 9H), 3.75–3.84 (m, 2H), 4.02–4.11 (m, 2H), 4.31 (dd, J = 2.7 Hz, J = 5.7 Hz, 1H), 6.61 (d, J = 5.7 Hz, 1H), 8.19 (s, 1H), 8.34 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD)δ: 28.31, 45.43, 65.76, 67.66, 71.07, 72.64, 82.61, 118.89, 142.64, 151.35, 153.41, 156.35, 157.11; HRMS (ESI) Calcd for $C_{15}H_{23}N_6O_5$ [M+H]$^+$: 367.1724; found 367.1715.

4.38. (2*S*,3*R*,4*R*,5*R*)-*tert*-Butyl 2-(5-fluoro-uracil-1-yl)-3,4,5-trihydroxypiperidine-1-carboxylate (16a)

The crude product obtained in the previous reaction (compound **12a**, 31 mg, 0.064 mmol) was dissolved in MeOH saturated with ammonia (5 mL) and stirred at rt overnight. The reaction mixture was concentrated, and the residue was purified by C-18 reverse-phase column chromatography (H_2O/CH_3OH = 3:1). Further purification was carried out using preparative HPLC (C-18, 40% MeOH in water) to yield compound **16a** (20 mg, 85%, white solids). ^{1}H NMR (300 MHz, D_2O) δ: 1.20 (s, 9H), 3.34 (dd, J = 3.3 Hz, J = 10.2 Hz, 1H), 3.49 (dd, J = 2.7 Hz, J = 14.7 Hz, 1H), 3.76–3.92 (m, 3H), 5.32 (d, J = 9.3 Hz, 1H), 7.67 (d, J = 6.0 Hz, 1H). ^{13}C NMR (75 MHz, D_2O) δ: 28.41, 46.76, 70.36, 73.39, 74.22, 77.00, 84.71, 129.10, 129.53, 140.07, 143.16, 151.90, 156.87, 161.21, 161.54. HRMS (ESI) Anal. Calcd for $C_{14}H_{20}FN_3O_7Na$ [M+Na]$^+$: 384.1178; found 384.1170.

4.39. (2*S*,3*R*,4*R*,5*S*)-*tert*-Butyl 2-(5-fluoro-uracil-1-yl)-3,4,5-trihydroxy piperidine-1-carboxylate (16b)

Compound **16b** was prepared from **12b** as described in the preparation of **15c** from **11c**, yielding **16b** (41% yield) as white solids after

lyophilization. ^{1}H NMR (300 MHz, C_5D_5N) δ: 1.35 (s, 9H), 4.18–4.21 (m, 1H), 4.57 (br s, 1H), 4.65–4.74 (m, 2H), 5.05 (t, J = 6.0 Hz, 1H), 6.98 (d, J = 5.4 Hz, 1H), 8.23 (d, J = 6.9 Hz, 1H); ^{13}C NMR (75 MHz, C_5D_5N) δ: 16.12, 36.03, 54.76, 56.86, 57.09, 58.05, 68.75, 117.04, 117.48, 127.08, 130.14, 138.93, 143.78, 146.29, 146.63; HRMS (ESI) Calcd for $C_{14}H_{20}FN_3O_7Na$ [M+Na]$^+$: 384.1178; found 384.1178.

4.40. (2*R*,3*S*,4*R*,5*R*)-*tert*-Butyl 2-(5-fluoro-uracil-yl)-3,4,5-trihydroxy piperidine-1-carboxylate (16c)

Compound **16c** was prepared from **12c** as described in the preparation of **15c** from **11c**, yielding **16c** (81% yield) as white solids after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ: 1.30 (s, 9H), 3.07 (dd, J = 9.6 Hz, J = 14.0 Hz, 1H), 3.81–3.86 (m, 2H), 4.19 (dd, J = 7.6 Hz, J = 14.4 Hz, 1H), 4.30 (dd, J = 2.4 Hz, J = 7.6 Hz, 1H), 5.60 (d, J = 7.6 Hz, 1H), 7.72 (d, J = 6.4 Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ: 27.36, 44.32, 67.64, 68.93, 70.61, 73.08, 83.96, 128.15, 128.49, 139.34, 141.67, 150.43, 155.58, 159.64, 159.90. HRMS (ESI) Anal. Calcd for $C_{14}H_{20}FN_3O_7Na$ [M+Na]$^+$: 384.1178; found 384.1182.

4.41. (2*S*,3*R*,4*R*,5*R*)-*tert*-Butyl 2-(uracil-1-yl)-3,4,5-trihydroxypiperidine-1-carboxylate (17a)

The crude product obtained in the previous reaction (compound **13a**, 35 mg, 0.075 mmol) was dissolved in MeOH saturated with ammonia (5 mL) and stirred at rt overnight. The reaction mixture was concentrated, and the residue was purified by C-18 reverse-phase column chromatography (H_2O/CH_3OH = 3:1). Further purification was carried out using preparative HPLC (C-18, 40% MeOH in water) to yield compound **17a** (16 mg, 62%, white solids). ^{1}H NMR (300 MHz, D_2O) δ: 1.25 (s, 9H), 3.39 (d, J = 10.5 Hz, 1H), 3.56 (d, J = 13.8 Hz, 1H), 3.82–3.87 (m, 2H), 3.97 (t, J = 9.6 Hz, 1H), 5.36 (d, J = 9.3 Hz, 1H), 5.75 (d, J = 7.8 Hz, 1H), 7.53 (d, J = 8.1 Hz, 1H). ^{13}C NMR (75 MHz, D_2O) δ: 28.35, 46.78, 70.18, 73.66, 74.21, 77.00, 84.62, 102.83, 145.94, 152.72, 156.83, 167.56. HRMS (ESI) Anal. Calcd for $C_{14}H_{21}N_3O_7Na$ [M+Na]$^+$: 366.1272; found 366.1265.

4.42. (2*S*,3*R*,4*R*,5*R*)-*tert*-Butyl 2-(cytosine-1-yl)-3,4,5-trihydroxy piperidine-1-carboxylate (18a)

The crude product obtained in the previous reaction (compound **14a**, 51 mg, 0.10 mmol) was dissolved in MeOH saturated with ammonia (5 mL) and stirred at rt overnight. The reaction mixture was concentrated, and the residue was purified by C-18 reverse-phase column chromatography (H_2O/CH_3OH = 3:1). Further purification was carried out using preparative HPLC (C-18, 40% MeOH in water) to yield compound **18a** (24 mg, 71%, white solids). ^{1}H NMR (400 MHz, D_2O) δ: 1.24 (s, 9H), 3.41 (dd, J = 3.6 Hz, J = 10.0 Hz, 1H), 3.66 (dd, J = 3.6 Hz, J = 15.2 Hz, 1H), 3.82–3.85 (m, 2H), 4.05 (t, J = 9.6, 1H), 5.25 (d, J = 9.2 Hz, 1H), 5.91 (d, J = 7.6 Hz, 1H), 7.47 (d, J = 7.6 Hz, 1H). ^{13}C NMR (125 MHz, D_2O) δ: 28.21, 46.66, 69.92, 74.10, 74.99, 76.96, 84.16, 96.41, 145.96, 146.48, 156.78, 158.25, 166.95. HRMS (ESI) Anal. Calcd for $C_{14}H_{22}N_4O_6Na$ [M+Na]$^+$: 365.1432; found 365.1431.

4.43. HBV antiviral assay

Anti-HBV assays were conducted as described previously.[24,25] Briefly, confluent cultures of 2.2.15 cells were maintained on 96-well flat-bottomed tissue culture and treated with eight consecutive daily doses of tested compounds and lamivudine (purchased from Glaxo & Welcome Co.) in RPMI1640 medium with 2% fetal bovine serum. HBV nucleic acids and proteins were analyzed at the end of the treatment period (day 8). HBsAg and HBeAg were analyzed in culture medium by semi-quantitative EIA. Intracellular HBV DNA forms were extracted from culture media and analyzed by a slot blot hybridization technique.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.11.063.

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