Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 3273–3278

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis of 2-Amino-2-deoxy-β-glycosyl-(1→5)-nucleosides and the Interaction with RNA

Guisheng Zhang, Zhu Guan, Liangren Zhang,* Jimei Min and Lihe Zhang

School of Pharmaceutical Sciences, Peking University, Beijing 100083, China

Received 9 January 2003; accepted 21 April 2003

Abstract—1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose and 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranose reacted with protected nucleosides in the presence of BF_3 as promoter at room temperature to give selectively 2-amino-2-deoxy-β-glycosyl (1→5)nucleosides in good yields. CD spectra and thermal melting studies showed that 2-amino-2-deoxy-β-D-glucopyranosyl-(1→5)-nucleosides could interact with RNA in solution and 2-deoxy-2-amino-β-D-galactopyranosyl-(1→5)-nucleosides (**17**–**19**) exhibit higher affinity to RNA than 2-deoxy-2-amino-β-D-glucopyranosyl-(1→5)-nucleosides (**14**–**16**). It indicated that the majority of interactions are established between the polar group of glycosylnucleosides and the sugar-phosphate backbone of RNA helices and weak stacking interaction is observed. The different configuration of hydroxyl group on the glycosyl moiety may affect the glycosyl-nucleoside binding to RNA by induced fit.

Introduction

Nucleoside analogues play an important role in the antiviral and anticancer chemotherapy. It has been found that many natural antibiotics possessing significant antitumor and antiviral activities have the structure of a nucleoside connected to oligosaccharides.[1–3] Aminoglycoside antibiotics have long been used as very efficient drugs against Gram-positive and Gram-negative bacteria, and against mycobacterial infection. Recently, it was found that the flexibility of the aminoglycosides facilitated accommodation into a binding pocket within internal loops of RNA helices or into ribozyme cores for making specific contacts. The positive charges of aminoglycosides are attracted to the negatively charged RNA backbone.[4] Aminoglycoside–RNA interactions could be designed and could potentially be used to modify gene expression.[5–7] It would be interesting to study whether the aminoglycosyl nucleosides could be used as a site-directing moiety towards RNA. In this way some aminoglycosyl nucleoside analogues might be expected to have a higher therapeutic index together with lower side effects and toxicity. To develop this work, we present here the synthesis of 2-amino-2-deoxy-glycosyl-(1→5)-nucleosides and their binding abilities with RNA.

Stimulated by the biological background, the method for the synthesis of aminosugar is becoming more and more important. From a survey of the current advances in methodology, the most of syntheses were performed by the azidonitration or cycloaddition or sulfonamidoglycosylation from glycal.[8–11] An aminoglycosyl nucleoside, tunicaminyl uracil, was synthesized via the same strategy.[12,13] In this paper, we report a concise method for the synthesis of aminoglycosyl nucleosides via the condensation of protected nucleoside and 2-deoxy-2-phthalimido-glycosyl acetate. The reaction is stereoselective and we found that 2-amino-2-deoxy-β-D-glucopyranosyl-(1→5)-nucleosides and 2-amino-2-deoxy-β-D-galactopyranosyl-(1→5)-nucleosides could interact with RNA in solution.

Results and Discussion

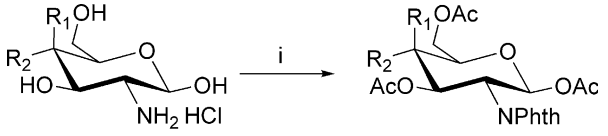
1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**3**) and 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranose (**4**) were synthesized from D-glucosamine (**1**) and D-galactosamine (**2**) respectively by one-pot reaction. For the synthesis of compound **3**, D-glucosamine hydrochloride **1** was treated first with sodium methoxide and then reacted with

*Corresponding author. Tel.: +86-10-6209-1570; fax: +86-10-6209-2724; e-mail: liangren@bjmu.edu.cn

0968-0896/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0968-0896(03)00278-5

phthalic anhydride in methanol. The reactant mixture was not further purified but treated with acetic anhydride-pyridine after methanol was removed under reduced pressure. Acetate **3** was obtained by chromatography on silica gel in 42% yield. The compound **4** was obtained in 30% yield from D-galactosamine hydrochloride **2** via the same procedure described above (Scheme 1). The ^{1}H NMR of **3** and **4** showed that the values of $^3J_{1,2}$ (**3**: 8.88 Hz; **4**: 9.00 Hz) were in accord with β-anomers.

The phthaloyl group is widely used as the 2-position amino protecting group for selectively synthesizing β-glycoside in the presence of Lewis acid.[14] The glycosyl donor **3** was condensed with protected nucleosides (**5**, **6** and **7**)[15] using boron trifluoride as promoter in dichloromethane at room temperature. After chromatography on silica gel, the compounds **8**, **9** and **10** were obtained in 87, 66 and 65% yield, respectively, exclusively as β-anomers (Scheme 2). It was found that the reaction in dichloromethane was much better than in acetone. Acetone reduced the yield of this condensation. The acetate **4** was condensed with nucleosides (**5**, **6** and **7**) as described above. After chromatography on silica gel, the compounds **11**, **12** and **13** were obtained in 90, 65 and 87% yield, respectively, exclusively as β-anomers. The ^{1}H NMR spectra of the condensation compounds **8**–**13** showed that the values of $^3J_{1'',2''}$ (**8**: 9.0 Hz; **9**: 8.5 Hz; **10**: 8.0 Hz; **11**: 8.0 Hz; **13**: 8.5 Hz) were in accord with β-type products. It was presumed that the condensation was carried out via the mechanism of unimolecular nucleophilic substitution. The stereoselective substitution was controlled by the hindrance of the neighboring 2-phthalimido group. The transition state of oxazocarbonium ion **3b** leds to form exclusively the β-glycosides (Scheme 3).



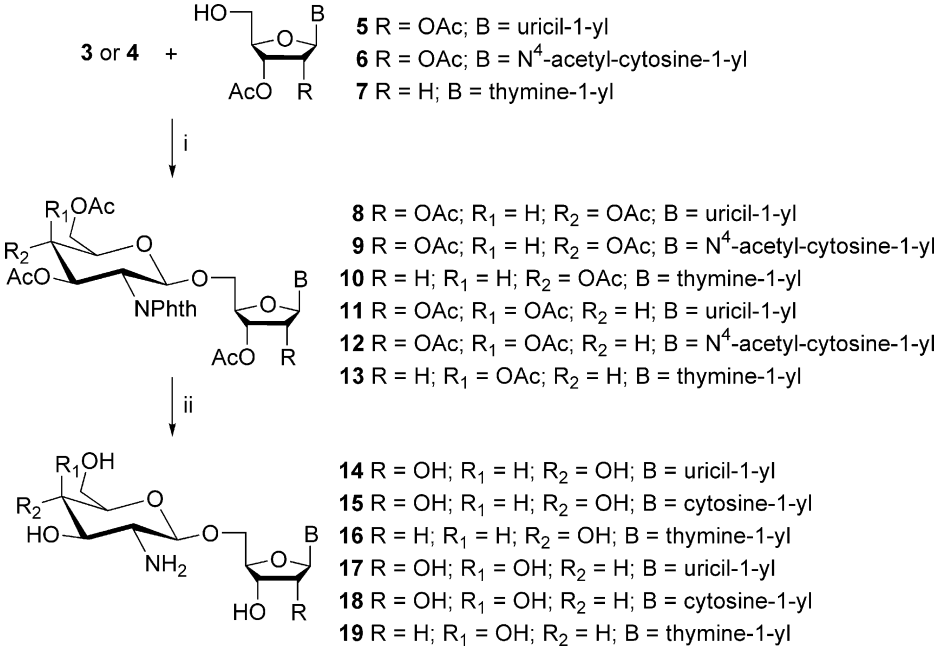
1 R_1 = H; R_2 = OH **3** R_1 = H; R_2 = OAc

2 R_1 = OH; R_2 = H **4** R_1 = OAc; R_2 = H

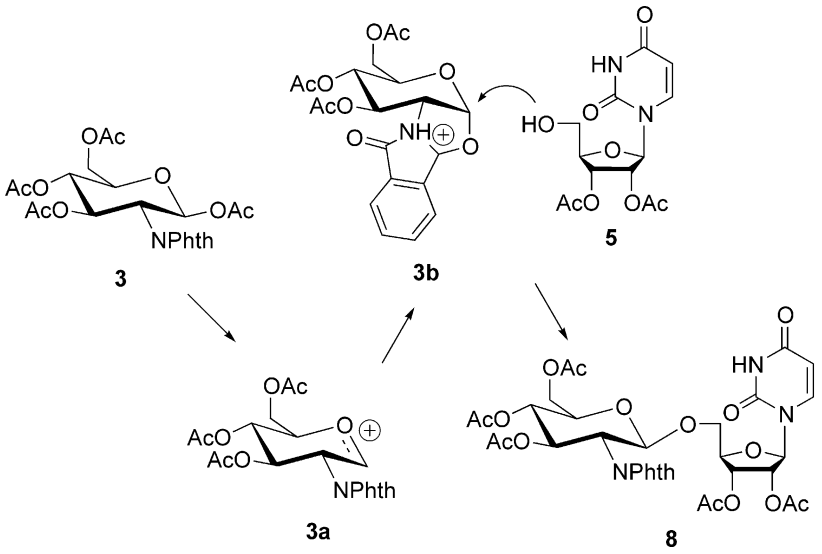
Scheme 1. Reagents and conditions: (i) (a) NaOMe, HOAc, rt, 30 min; (b) phthalic anhydride, rt, 15 h; (c) Ac_2O, pyridine, rt, 24 h.

Treatment of compounds **8**–**13** with 25–30% methylamine in absolute ethanol at room temperature, and then at reflux temperature resulted in completed deprotection of both the hydroxyl and the amino groups to give the designed compounds **14**–**19**.

The interactions of synthetic glycosyl nucleosides with polyA/polyU duplex were studied by CD spectra and thermal denaturation (Figs 1 and 2). The stability of RNA duplex was affected by the integrated effects of hydrogen bonding, stacking and electrostatic interactions between RNA and small molecule.[16–18] Melting curves showed that compounds **14**, **15**, **16**, and **18** resulted in an increase of melting temperature of RNA duplex with an extent of 0.5 to 2.4 °C, it indicated that the binding of these compounds could enhance the duplex stabilities (Table 1). No obvious melting behavior of duplexes was observed in the presence of compounds **17** or **19**, it implied that the stable complex of RNA single strand with compound **17** or **19** was formed in this condition, which hindered the formation of stable RNA duplex. CD spectra showed that the A-form conformation of RNA duplex was maintained in the presence of glycosyl nucleoside, but the changes of intensity suggested a slight rearrangement of the duplex,[19] and the rearrangements were more obvious in the case of galactopyranosyl nucleoside especially **17**, **19** in which slight increases of ellipticity were also observed. The decreases of hyperchromicity were also observed in all cases. The insertion of the heterocyclic



Scheme 2. Reagents and conditions: (i) BF_3/Et_2O, CH_2Cl_2, rt; (ii) (a) $MeNH_2$/EtOH, rt, 8 h; (b) reflux, 6 h.



Scheme 3. Presumed mechanism of the formation of β-glycosyl nucleoside.

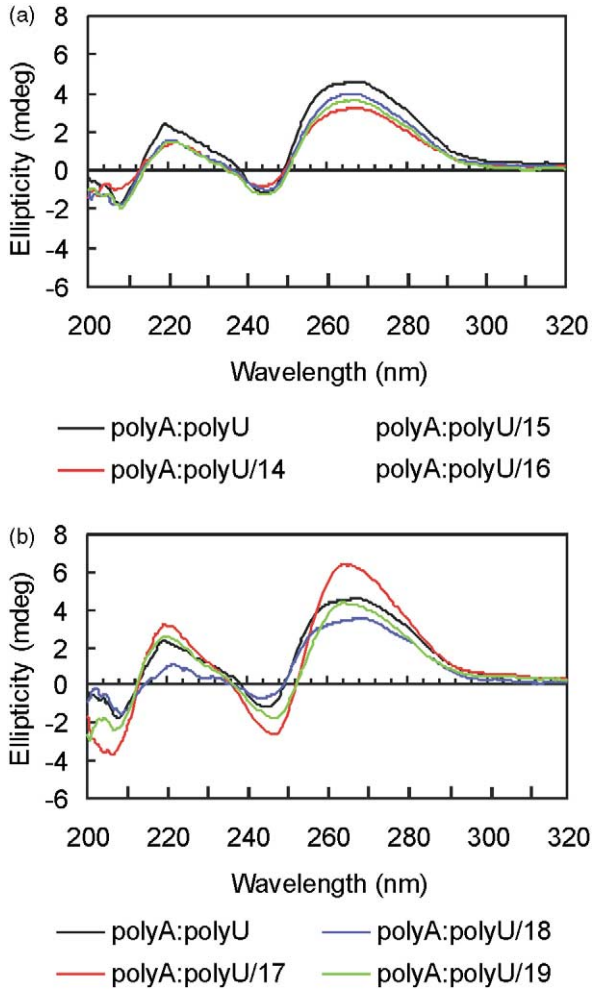


Figure 1. CD spectra of polyA/polyU in the absence or presence of synthetic glycosyl nucleoside.

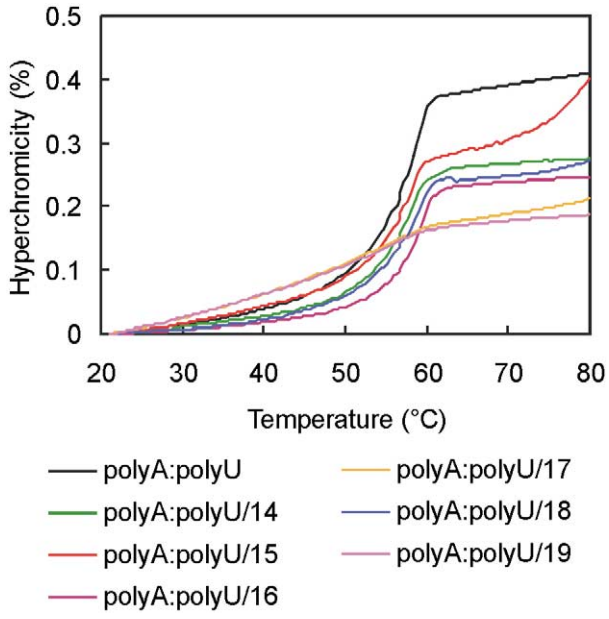


Figure 2. Thermal melting curves of polyA/polyU in the absence or presence of synthetic glycosyl nucleoside.

Table 1. Melting temperatures of polyA/polyU duplex in the absence or presence of glycosyl nucleoside

PolyA/polyU/compd	T_m (°C)	ΔT_m (x° C)
polyA/polyU	57.3	
polyA/polyU/**14**	58.6	1.3
polyA/polyU/**15**	57.8	0.5
polyA/polyU/**16**	59.7	2.4
polyA/polyU/**17**	—	—
polyA/polyU/**18**	59.1	1.8
polyA/polyU/**19**	—	—

base of the glycosyl nucleosides **14**–**19** to the RNA duplex leaded to the changes of stacking interaction and also resulted in the obvious decrease of hyperchromicity. Since RNA molecules are negatively charged, electrostatic interactions are critical for binding, the 2-deoxy-2-amino glycosyl moiety in the designed compounds **14**–**19** may play an important role in the molecular recognition. Considering the minor structural difference at C-4″ positon between glucopyranosyl (**14**–**16**) and galactopyranosyl nucleosides (**17**–**19**), it could be

conferred that the configuration of hydroxyl group at C-4″ affects the formation of hydrogen bonding between nucleosides and the phosphate backbone of RNA. It has known that drug binding to RNA occurs frequently by induced fit, therefore, these results are encouraging because they demonstrate that besides the electrostatic interaction and base pair stacking, the sugar rings also provide scaffold for inducing small molecules to fit the RNA recognition, and it is possible to design a new molecule with the specificity for the recognition of target RNA.

Experimental

General methods

Thin-layer chromatography was performed by using silica gel GF-254 (Qing Dao Chemical Company, China) plates with detection by UV, or charting with 5% ethanolic solution of phosphomolybdic acid hydrate. Optical rotations were recorded on a Perkin–Elmer 243B polarimeter. NMR spectra were recorded on Varian VXR-300 or Varian INOVA-500 instrument with TMS as an internal standard. PE SCLEX QSTAR and Autospec-Ultima ETOF spectraometers were used for mass spectra.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose 3 and 1,3,4,6- tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranose 4. To a solution of sodium methoxide (3.0 g, 55.6 mmol) in absolute methanol (60 mL) was added glucosamine hydrochloride **1** (10.0 g, 45.6 mmol). After vigorously stirring for 30 min at room temperature, phthalic anhydride (6.9 g, 46.6 mmol) was added and the mixture was kept stirring for 15 h. The solvent was removed under reduced pressure and the residue was treated with acetic anhydride (60 mL) and pyridine (50 mL) with stirring for 24 h at room temperature. The reactant mixture was poured into ice-water (250 mL). After stirred for 30 min, the mixture was extracted with CH_2Cl_2 and the extract was washed with 3 M H_2SO_4, H_2O, saturated aqueous solution of $NaHCO_3$ and water sequencially, then concentrated to dryness to give a syrup (21 g). After purification by chromatography on silica gel (petroleum ether/ethyl acetate/2:1), 9.05 g of **3** were obtained.[20] Yield: 42%; ^{1}H NMR: ($CDCl_3$) δ: 7.87–7.75 (m, 4H, Ar–H), 6.54 (d, 1H, H-1, $J_{1,2}$ = 8.88 Hz), 5.89 (t, 1H, H-4), 5.22 (t, 1H, H-3), 4.49 (t, 1H, H-2), 4.37, 4.16 (m, 2H, H-6a, H-6b), 4.03 (m, 1H, H-5), 2.13 (s, 3H, –OAc), 2.05 (s, 3H, –OAc), 2.01 (s, 3H, –OAc), 1.88 (s, 3H, –OAc).

4 was synthesized from **2** via the same procedure described above.[21] Yield: 30%; ^{1}H NMR: ($CDCl_3$) δ: 7.75–7.86 (m, 4H, Ar–H), 6.44 (d, 1H, H-1, $J_{1,2}$ = 9.0 Hz), 5.95 (dd, 1H, H-4), 4.91 (dd, 1H, H-3), 4.49 (m, 1H, H-2), 4.39, 4.22 (m, 2H, H-6a, H-6b), 4.1 5(m, 1H, H-5), 2.21 (s, 3H, –OAc), 2.10 (s, 3H, –OAc), 2.06 (s, 3H, –OAc), 1.88 (s, 3H, –OAc).

General procedure for the synthesis of compounds 8–13

Glycosyl acceptor (**5**–**7**) (0.027 mmol) and 2 equivalent of glycosyl donors **3** or **4** were dissolved in a solution of 2 mL of dry dichloromethane and 0.1 mL of boron trifluoride-ether. The mixture was stirred at room temperature till the disappearance of glycosyl acceptor via TLC monitoring. After cooling in ice bath and neutralization with saturated $NaHCO_3$, the solution was extracted with dichloromethane and the organic layer was dried over anhydrous Na_2SO_4, then purified with silica gel chromatography to give the products.

3″,4″,6″-Tri-*O*-acetyl-2″-deoxy-2″-phthalimido-β-D-glucopyranosyl (1→5)-2′, 3′-di-*O*-acetyl-uridine 8. Yield: 87%; $[\alpha]_D^{18}$ −6.3 (*c* 0.025, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 9.07 (s, 1H, HN), 7.83, 7.73 (2m, 4H, Ph), 7.56 (d, 1H, $J_{5,6}$ = 7.5 Hz, H-6), 6.10 (d, 1H, $J_{1',2'}$ = 7.0 Hz, H-1′), 5.91 (d, 1H, H-5), 5.86 (dd, 1H, $J_{2'',3''}$ = 11 Hz, $J_{3'',4''}$ = 9.0 Hz, H-3″), 5.46 (d, 1H, $J_{1'',2''}$ = 9.0 Hz, H-1″), 5.20 (t, 1H, H-4″), 5.05 (m, 1H, H-2′), 4.93 (t, 1H, H-3′), 4.39–4.31 (m, 2H, H-2″, H-6a″), 4.23–4.16 (m, 3H, H-4′,H-5a′, H-6b″), 3.93 (m, 1H, H-5″), 3.69 (m, 1H, H-5b′), 1.87, 1.95, 1.99, 2.05, 2.13 (5s, 15H, 5×OAc); ^{13}C NMR ($CDCl_3$) δ: 170.6–168.9 (5C, CO), 162.7 (PhCO), 150.4 (C-4), 139.4 (C-6), 134.3, 123.8 (2C, Ph), 131.8 (C-2), 103.6 (C-5), 97.9 (C-1″), 85.9 (C-1′), 81.2 (C-4′), 72.9 (C-3′), 72.1 (C-5″), 70.7 (C-3″), 70.3 (C-2′), 68.6 (C-4″), 68.0 (C-5′), 61.7 (C-6″), 54.4 (C-2″), 20.7–20.2 (5C, 5×CH_3CO); ESI-TOF MS: 746.1090 (M^+ + 1) 768.0907 (M^+ + Na).

3″,4″,6″-Tri-*O*-acetyl-2″-deoxy-2″-phthalimido-β-D-glucopyranosyl (1→5)-2′,3′-di-*O*-acetyl-N^4-acetyl-cytidine 9. Yield: 66%; $[\alpha]_D^{18}$ +13.5 (*c* 0.017, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 9.62 (br., 1H, HNAc), 7.99 (d, 1H, $J_{5,6}$ = 7.5 Hz, H-6), 7.83, 7.73 (2m, 4H, Ph), 7.58 (d, 1H, H-5), 6.13 (d, 1H, $J_{1',2'}$ = 4.0 Hz, H-1′), 5.86 (dd, 1H, $J_{2'',3''}$ = 11 Hz, $J_{3'',4''}$ = 9.5 Hz, H-3″), 5.45 (d, 1H, $J_{1'',2''}$ = 8.5 Hz, H-1″), 5.23 (t, 1H, H-4″), 5.03 (m, 2H, H-2′,H-3′), 4.40–4.33 (m, 2H, H-2″,H-6a″), 4.28 (m, 1H, H-5a′), 4.22–4.18 (m, 2H, H-4′, H-6b″), 3.92 (m, 1H, H-5″), 3.68 (m, 1H, H-5b′), 1.87, 1.93, 1.97, 2.05, 2.15, 2.31 (6s, 18H, 5×OAc, 1×NHAc); ^{13}C NMR ($CDCl_3$) δ: 170.8–168.9 (6C, CO), 144.7 (C-6), 131.8 (C-2), 134.3, 123.8 (Ph), 98.1 (C-1″), 97.3 (C-5), 87.7 (C-1′), 81.0 (C-4′), 74.0 (C-3′), 72.2 (C-5″), 70.3 (C-3″), 69.9 (C-2′), 68.7 (C-4″), 67.8 (C-5′), 61.8 (C-6″), 54.3 (C-2″), 25.0–20.2 (6C, 6×CH_3CO); ESI-TOF MS: 787.1522 (M^+ + 1).

3″,4″,6″-Tri-*O*-acetyl-2″-deoxy-2″-phthalimido-β-D-glucopyranosyl (1→5)-3′-*O*-acetyl-thymidine 10. Yield: 65%; $[\alpha]_D^{18}$ −1.77 (*c* 0.022, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 8.84 (s, 1H, HN), 7.88–7.77 (m, 4H, Ph), 7.49 (s, 1H, H-6), 6.28 (m, 1H, H-1′), 5.90 (dd, 1H, $J_{2'',3''}$ = 11 Hz, $J_{3'',4''}$ = 9.5 Hz, H-3″), 5.38 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1″), 5.18 (dd, 1H, $J_{4'',5''}$ = 10.5 Hz, H-4″), 4.75 (m, 1H, H-3′), 4.39 (m, 1H, H-2″), 4.30–4.23 (m, 2H, H-6a″, H-5a′), 4.17 (m, 1H, H-6b″), 4.07 (m, 1H, H-4′), 3.93 (m, 1H, H-5″), 3.69 (m, 1H, H-5b′), 2.11, 2.10, 2.05, 1.96, 1.89 (5s, 15H, 4×OAc, CH_3), 1.79 (m, 2H, H-2′); ^{13}C NMR ($CDCl_3$) δ: 170.6–169.4 (4C, CO), 163.6 (PhCO), 150.4 (C-4), 135.2 (C-6), 134.7, 123.8 (2C, Ph), 131.8 (C-2), 111.6 (C-5), 98.1 (C-1″), 84.6 (C-1′), 83.1 (C-4′), 75.0 (C-3′), 72.2 (C-5″), 70.1 (C-5′), 69.6 (C-3″), 68.7 (C-4″), 61.7

(C-6″), 54.4 (C-2″), 37.0 (C-2′), 29.6 (CH_3), 20.8–20.4 (4C, 4×CH_3CO); ESI-TOF MS: 702.1284 ($M^+ + 1$) 724.1027 ($M^+ + Na$).

3″,4″,6″-Tri-*O*-acetyl-2″-deoxy-2″-phthalimido-β-D-galactopyranosyl (1→5)-2′, 3′-di-*O*-acetyl-uridine 11. Yield: 90%; $[\alpha]_D^{18}$ −25.6 (*c* 0.027, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 9.08 (s, 1H, HN), 7.83, 7.75 (2m, 4H, Ph), 7.72 (d, 1H, $J_{5,6}$ = 8.5 Hz, H-6), 6.14 (d, 1H, $J_{1″,2′}$ = 6.0 Hz, H-1′), 5.91–5.88 (m, 2H, H-5, H-3″), 5.53 (m, 1H, H-4″), 5.31 (d, 1H, $J_{1″,2″}$ = 8.0 Hz, H-1″), 5.08 (m, 1H, H-2′), 5.00 (t, 1H, H-3′), 4.51 (dd, 1H, $J_{2″,3″}$ = 11.50 Hz, H-2″), 4.25–4.12 (m, 5H, H-6a″, H-4′, H-5a′, H-6b″, H-5″), 3.69 (m, 1H, H-5b′), 1.86, 1.98, 1.99, 2.08, 2.25 (5s, 15H, 5×OAc); ^{13}C NMR ($CDCl_3$) δ: 170.4–162.8 (8C, MeCO, PhCO), 150.4 (C-4), 139.8 (C-6), 134.4, 123.7 (2C, Ph), 131.8 (C-2), 103.2 (C-5), 98.0 (C-1″), 86.0 (C-1′), 81.4 (C-4′), 73.2 (C-3′), 71.0 (C-5″), 70.8 (C-3″), 67.7 (C-2′), 67.4 (C-4″), 66.6 (C-5′), 61.2 (C-6″), 51.1 (C-2″), 20.8–20.2 (5C, 5×CH_3CO); ESI-TOF MS: 746. 2148 ($M^+ + 1$).

3″,4″,6″-Tri-*O*-acetyl-2″-deoxy-2″-phthalimido-β-D-galactopyranosyl (1→5)-2′,3′-di-*O*-acetyl-N⁴-acetyl-cytidine 12. Yield: 65%; $[\alpha]_D^{18}$ −10.3 (*c* 0.017, $CHCl_3$); ^1H NMR (CDCl3) δ: 9.97 (s, 1H, HNAc), 8.17 (d, 1H, $J_{5,6}$ = 7.0 Hz, H-6), 7.85–7.71 (2m, 4H, Ph), 7.58 (d, 1H, H-5), 6.24 (d, 1H, $J_{1′,2′}$ = 4.0 Hz, H-1′), 5.68 (m, H-3″), 5.47-5.28 (m, 4H, H-1″;, H-4″, H-2′, H-3′), 5.07 (m, 1H, H-2″), 4.70 (m, 1H, H-6a″), 4.49–4.15 (m, 4H, H-4′, H-5a′, H-6b″, H-5″), 3.65 (m, 1H, H-5b′), 2.00, 2.06, 2.08, 2.09, 2.25, 2.30 (6s, 18H, 5×OAc, 1×NHAc); ^{13}C NMR ($CDCl_3$) δ: 170.5–167.4 (6C, CO), 131.6 (C-2), 134.2, 123.7 (Ph), 104.4 (C-5), 97.3 (C-1″), 88.0 (C-1′), 81.2 (C-4′), 78.4 (C-3′), 74.1 (C-5″), 72.6 (C-3″), 70.1 (C-2′), 68.7 (C-4″), 67.1 (C-5′), 62.2 (C-6″), 61.2 (C-2″), 24.9–20.3 (6C, 6×CH_3CO); ESI-TOF MS: 787.2454 ($M^+ + 1$).

3″,4″,6″-Tri-*O*-acetyl-2″-deoxy-2″-phthalimido-β-D-galactopyranosyl (1→5)-3′-*O*-acetyl-thymidine 13. Yield: 87%; $[\alpha]_D^{18}$ −37.8 (*c* 0.046, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 8.49 (s,1H, HN), 7.88–7.76 (2m, 4H, Ph), 7.62 (s, 1H, H-6), 6.34 (m, 1H, H-1′), 5.94 (dd, 1H, $J_{2″,3″}$ = 11 Hz, $J_{3″,4″}$ = 3.0 Hz, H-3″), 5.55 (m, 1H, H-4″), 5.29 (d, 1H, $J_{1″,2″}$ = 8.5 Hz, H-1″), 4.74 (m, 1H, H-3′), 4.53 (dd, 1H, H-2″), 4.27–4.14 (m, 4H, H-6a″, H-5a′, H-6b″, H-5″), 4.08 (m, 1H, H-4′), 3.67 (m, 1H, H-5b′), 2.17–1.95 (5s, 15H, 4×OAc, CH_3), 1.88 (m, 2H, H-2′); ^{13}C NMR($CDCl_3$) δ: 170.0, 170.1, 170.0, 169.6 (4C, CO), 168.0, 163.8 (PhCO), 150.6 (C-4), 135.6 (C-6), 134.4, 123.7 (2C, Ph), 130.9 (C-2), 111.5 (C-5), 98.5 (C-1″), 84.4 (C-1′), 83.0 (C-4′), 75.0 (C-3′), 71.2 (C-5″), 69.7 (C-5′), 67.4 (C-3″), 66.6 (C-4″), 61.2 (C-6″), 51.3 (C-2″), 37.0 (C-2′), 20.7–20.4 (4C, 4×CH_3CO); 12.6 (CH_3); ESI-TOF MS: 702.2216 ($M^+ + 1$), 724.2122 ($M^+ + Na$).

General procedure for deprotection

Compound (**8**–**13**) (50 mg) was added to 10 mL of 25–30% methylamine in absolute ethanol with stirring at room temperature. After 8 h, the clear solution obtained was refluxed for 6 h. The mixture was evaporated under reduced pressure. The residue was purified by chromatography on silica gel using dichloromethane/methanol (1:1) to elute the desired products. The product was purified again on the short C_{18} column (H_2O) to give the pure target compound (**14**–**19**).

2″-Deoxy-2″-amino-β-D-glucopyranosyl (1→5)-uridine 14. Yield: 77%; $[\alpha]_D^{18}$ +4.4 (*c* 0.020, H_2O); ^1H NMR (D_2O) δ: 7.65 (d, 1H, $J_{5,6}$ = 7.5 Hz, H-6), 5.72 (m, 2H, H-1′, H-5), 4.69 (1H, H-1″), 4.23 (t, 1H, $J_{1′,2′}$ = 4.2 Hz, H-2′), 4.12 (m, 3H, H-4′, H-3′, H-5a′), 3.82 (m, 2H, H-5b′, H-3″), 3.57 (m, 2H, H-6a″, H-4″), 3.35 (m, 2H, H-5″, H-6b″), 2.94 (t, 1H, $J_{1″,2″}$ = 8.7 Hz, H-2″); ^{13}C NMR (D_2O) δ: 168.9 (C-4), 154.3 (C-6), 145.0 (C-2), 105.0 (C-5), 101.4 (C-1″), 93.0 (C-1′), 85.2 (C-4′), 79.0 (C-3′), 76.0 (C-5″), 74.7 (C-3″), 72.6 (C-2′), 72.3 (C-4″), 71.9 (C-5′), 63.2 (C-6″), 58.6 (C-2″); HRFAB$^+$ ($C_{15}H_{24}O_{10}N_3 + H^+$) calcd 406.1462, found 406.1494.

2″-Deoxy-2″-amino-β-D-glucopyranosyl (1→5)-cytidine 15. Yield: 83%; $[\alpha]_D^{18}$ +10.1 (*c* 0.018, H_2O); ^1H NMR (D_2O) δ: 7.69 (d, 1H, $J_{5,6}$ = 7.5 Hz, H-6), 5.89 (d, 1H, H-5), 5.73 (d, $J_{1′,2′}$ = 3.9 Hz, H-1′), 4.50 (d,1H, $J_{1″,2″}$ = 8.4 Hz, H-1″), 4.21 (t, 1H, $J_{1′,2′}$ = 4.2 Hz, H-2′), 4.19–4.13 (m, 3H, H-4′, H-3′, H-5a′), 3.80 (m, 2H, H-6a″,H-3″), 3.40 (m, 1H, H-5b′), 3.43–3.26 (m, 3H, H-4″, H-5″, H-6b″), 2.74 (1H, H-2″); ^{13}C NMR (D_2O) δ: 168.9 (C-4), 160.1 (C-6), 145.0 (C-2), 101.5 (C-5), 98.9 (C-1″), 94.1 (C-1′), 84.8 (C-4′), 79.0 (C-3′), 76.2 (C-5″), 74.8 (C-3″), 72.6 (C-2′), 72.2 (C-4″), 71.9 (C-5′), 63.2 (C-6″), 58.6 (C-2″); HRFAB$^+$ ($C_{15}H_{25}O_9N_4 + H^+$) calcd 405.1622, found 405.1614.

2″-Deoxy-2″-amino-β-D-glucopyranosyl (1→5)-thymidine 16. Yield: 85%; $[\alpha]_D^{18}$ +0.5 (*c* 0.008, H_2O); ^1H NMR (D_2O) δ: 7.44 (s, 1H, H-6), 6.17 (t, $J_{1′,2′}$ = 6.7 Hz, H-1′), 4.68 (d, 1H, H-1″), 4.40 (dd, 1H, H-3′), 4.09–4.05 (m, 2H, H-5a′, H-4′), 3.84–3.79 (m, 2H, H-2″, H-6a″), 3.64–3.57 (m, 2H, H-4″, H-5b′), 3.39–3.33 (m, 2H, H-5″, H-6b″), 2.95 (dd, 1H, $J_{2″,3″}$ = 8.5 Hz, $J_{3″,4″}$ = 10.5 Hz, H-3″), 2.34–2.25 (m, 2H, H-2′), 1.79 (s, 3H, CH_3); ^{13}C NMR (D_2O) δ: 166.8 (C-4), 152.0 (C-6), 138.1 (C-2), 111.8 (C-5), 99.1 (C-1″), 85.9 (C-1′), 85.0 (C-4′), 76.7 (C-3′), 72.4 (C-5″), 71.0 (C-5′), 70.2 (C-3″), 70.0 (C-6″), 60.9 (C-6″), 56.2 (C-2″), 38.3 (C-2′), 26.3 (CH_3); HRFAB$^+$ ($C_{16}H_{26}O_9N_3 + H^+$) calcd 404.1669, found 404.1680.

2″-Deoxy-2″-amino-β-D-galactopyranosyl (1→5)-uridine 17. Yield: 83%; $[\alpha]_D^{18}$ +10.4 (*c* 0.020, H_2O); ^1H NMR (D_2O) δ: 7.78 (d, 1H, $J_{5,6}$ = 8.1 Hz, H-6), 5.72 (m, 2H, H-1′, H-5), 4.28 (d,1H, $J_{1″,2″}$ = 8.1 Hz, H-1″), 4.21 (m, 4H, H-2′, H-4′, H-3′, H-5a′), 3.76–3.43 (m, 6H, H-6a″, H-4″, H-3″, H-6b″, H-5″, H-5b′), 2.84-2.73 (m, 1H, H-2″); ^{13}C NMR (D_2O) δ: 169.5 (C-4), 154.7 (C-2), 144.8 (C-6), 105.7 (C-5), 104.9 (C-1″), 92.5 (C-1′), 85.5 (C-4′), 78.1 (C-3′), 76.4 (C-5″), 74.9 (C-3″), 72.2 (C-2′), 71.2 (C-4″), 70.6 (C-5′), 64.0 (C-6″), 55.7 (C-2″); HRFAB$^+$ ($C_{15}H_{24}O_{10}N_3 + H^+$) calcd 406.1462, found 406.1478.

2″-Deoxy-2″-amino-β-D-galactopyranosyl (1→5)-cytidine 18. Yield: 83%; $[\alpha]_D^{18}$ +5.7 (*c* 0.021, H_2O); ^1H NMR (D_2O) δ: 7.63 (d, 1H, $J_{5,6}$ = 7.5 Hz, H-6), 5.89 (d, 1H, H-5), 5.69 (d, 1H, $J_{1′,2′}$ = 3.6 Hz, H-1′), 4.66 (1H, H-1″), 4.21 (t, 1H, H-2′), 4.11 (m, 3H, H-4′, H-3′, H-5a′), 3.77 (m, 2H, H-6a″, H-3″), 3.61 (m, 2H, H-5b′, H-4″), 3.32

(m, 2H, H-5″, H-6b″), 2.80 (1H, H-2″); ^{13}C NMR (D_2O) δ: 167.0 (C-4), 158.2 (C-6), 142.9 (C-2), 100.2 (C-5), 96.8 (C-1″), 92.1 (C-1′), 82.8 (C-4′), 77.0 (C-3′), 74.3 (C-5″), 73.4 (C-3″), 70.6 (C-2′), 70.2 (C-4″), 69.8 (C-5′), 61.3 (C-6″), 56.7 (C-2″); HRFAB$^+$ ($C_{15}H_{25}O_9N_4+H^+$) calcd 405.1616, found 405.1612.

2″-Deoxy-2″-amino-β-D-galactopyranosyl (1→5)-thymidine 19. Yield: 83%; $[\alpha]_D^{18}$ +70 (*c* 0.008, H_2O); ^{1}H NMR (D_2O) δ: 7.47 (s, 1H, H-6), 6.16 (t, $J_{1',2'}=6.9$ Hz, H-1′), 4.39 (m, 1H, H-3′), 4.25 (d, 1H, $J_{1'',2''}=8.1$ Hz, H-1″), 4.09 (m, 1H, H-5a′), 4.02 (m, H, H-4′), 3.84 (m, 1H, H-4″), 3.77–3.59 (m, 5H, H-6a″, H-6b″, H-3″, H-5″, H-5b′), 2.95 (dd, 1H, $J_{2'',3''}=10.5$ Hz, H-2″), 2.35–2.24 (2H, H-2′), 1.79(s, 3H, CH_3); ^{13}C NMR (D_2O) δ: 169.5 (C-4), 154.6 (C-6), 140.2 (C-2), 114.2 (C-5), 106.2 (C-1″), 88.0 (C-1′), 87.6 (C-4′), 78.1 (C-3′), 75.2 (C-5′), 73.3 (C-3″), 71.9 (C-4″), 70.5 (C-6″), 63.9 (C-5″), 55.6 (C-2″), 41.0 (C-2′), 14.4 (CH_3); HRFAB$^+$ ($C_{16}H_{26}O_9N_3+H^+$) calcd 404.1663, found 404.1659.

Circular dichroism and thermal melting measurements

CD spectra were recorded by JASCO J-720 spectropolarimeter at 4 °C in thermostatically controlled 1-cm cuvette. Thermal denaturation studies of polyA/polyU were conducted on Varian Cray 300. CD spectra and T_m values in the absence and presence of synthetic nucleoside were determined in buffer containing 10 mM Na_2HPO_4, 0.14 M NaCl, 1.0 mM EDTA (pH 7.2). The solution containing synthetic nucleoside was mixed with equimolar per nucleotide amount of polyA and polyU. The concentration of synthetic nucleoside was 10 and 20 μM in the case of CD spectra and thermal melting measurements, respectively.

Acknowledgements

This work was supported by the National Natural Science Foundation of China.

References and Notes

1. Takahashi, M.; Kagasaki, T.; Hosoya, T.; Takahashi, S. *J. Antibiot*. **1993**, *46*, 1643.
2. Takahashi, S.; Kinoshita, T.; Takahashi, M. *J. Antibiot*. **1994**, *47*, 95.
3. Nagata, T.; WaKayama, T.; Asano, M.; Segawa, T. Jpn. Kokai Tokkyo Koho JP 66 234 646 (94236646) (1994).
4. Ye, X.-S.; Zhang, L.-H. *Curr. Med. Chem*. **2002**, *9*, 929.
5. Howard, M.; Frizell, R. A.; Bedwell, D. M. *Nat. Med*. **1996**, *2*, 467.
6. Werstuck, G.; Green, M. R. *Science* **1998**, *282*, 296.
7. Sucheck, S. J.; Greenberg, W. A.; Tolbert, T. J.; Wong, C.-H. *Angew. Chem., Int. Ed*. **2000**, *39*, 1080.
8. Leblanc, Y.; Fitzsimmons, B. J. *Tetrahedron Lett*. **1989**, *30*, 2889.
9. Griffith, D. A.; Danishefsky, S. J. *J. Am. Chem. Soc*. **1990**, *112*, 5811.
10. Griffith, D. A.; Denishefsky, S. J. *J. Am. Chem. Soc*. **1991**, *113*, 5863.
11. Lemieux, R. U.; Morgan, A. R. *Can. J. Chem*. **1979**, *57*, 1244.
12. Danishefsky, S. J.; DeNinno, S. L.; Chen, S. H.; Boisuert, L.; Barbachyn, M. *J. Am. Chem. Soc*. **1989**, *111*, 5810.
13. Denishefsky, S. J.; Barbachyn, M. A. *J. Am. Chem. Soc*. **1985**, *107*, 6647.
14. Banoub, J.; Boullanger, P.; Lafant, D. *Chem. Rev*. **1992**, *92*, 1167.
15. Smith, M.; Rammler, D. H.; Goldberg, I. H.; Khorana, H. G. *J. Am. Chem. Soc*. **1962**, *84*, 430.
16. Gallego, J.; Varani, G. *Acc. Chem. Res*. **2001**, *34*, 836.
17. Alper, P. B.; Hendrix, M.; Sears, P.; Wong, C.-H. *J. Am. Chem. Soc*. **1998**, *120*, 1965.
18. Wong, C.-H.; Hendrix, M.; Manning, D. D.; Rosenbohm, C.; Greenberg, W. A. *J. Am. Chem. Soc*. **1998**, *120*, 8319.
19. Li, K.; Fernandez-Saiz, M.; Rigl, C. T.; Kumar, A.; Ragunathan, K. G.; McConnaughie, A. W.; Boykin, D. W.; Schneider, H.-J.; Wilson, W. D. *Bioorg. Med. Chem*. **1997**, *5*, 1157.
20. Baker, B. R.; Joseph, J. P.; Schaub, R. E.; Williams, J. H. *J. Org. Chem*. **1954**, *19*, 1786.
21. Lemieux, R. U., Takeda, T., Chung, B. Y. In; *Synthetic Methods for Carbohydrates, ACS Symposium Series No. 39*; El Khadem, H. S., Ed.; American Chem. Soc.: Washington, DC, 1976; p 90.