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Synthesis of aminodisaccharide–nucleoside conjugates for RNA binding

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Abstract—Two types of aminodisaccharide–nucleoside conjugates were synthesized by the condensation of azidodisaccharide and nucleoside using aliphatic diamine as a linker. The corresponding azidodisaccharides could be yielded from neamine in good yield. The binding properties to 16S RNA of these conjugates were evaluated by SPR. It was found that the nucleobase played a significant role in the binding of these conjugates to 16S RNA and a shorter linker between the aminodisaccharide and nucleoside was favorable for 16S RNA binding.

1. Introduction

Aminoglycosides have long been used as highly potent broad-spectrum antibiotics against infectious diseases.[1–4] These drugs and their derivatives or mimics in the latest development could bind to a variety of RNA targets, such as rRNA, tRNA, mRNA, and so forth.[5] However, aminoglycosides are far from being ideal antibiotics because of the toxicity and adverse effects caused by non-specific binding to the RNA targets of interest. There are some examples of designing small molecules that specifically recognize certain RNA secondary structures.[6,7] However, different from DNA, RNA can adopt various intricate secondary structures. There is still no reliable rule to design specific RNA binders. Among the RNA–aminoglycoside interactions, the binding of neomycin type aminoglycosides to the A-site of 16S rRNA[8] is the most notable, because it is directly related to the biological function of these antibiotics. Recently, structures of several aminoglycoside/RNA complexes have been determined by X-ray or NMR.[9–12] Thus, based on the adenine base rich character of the binding pocket of A-site rRNA, a series of neamine–nucleoside conjugates were synthesized. It was proposed that the neamine moiety of such conjugates could bind to the groove of RNA and nucleobase moiety would bind specifically to the sequence of *Escherichia coli* rRNA A-site fragment (16S RNA). As a matter of fact, it was found that the designed compound **A** could bind specifically to 16S RNA and computer simulation indicated that the thymine moiety of compound **A** could form hydrogen bonding with adenine residue of 16S RNA.[13] The glycosidic bond between the ring II and ribose ring III of naturally occurring aminoglycosides, such as neomycin B and paromomycin, has an equatorial configuration (Fig. 1). To elucidate the relationship between the structure and binding activity to 16S RNA, we designed and synthesized the derivatives of neamine–nucleoside conjugates, **type I** and **type II**, which contain the connection between neamine and nucleoside with different configurations as shown in Figure 1. We reported here the synthesis of two types of aminodisaccharide–nucleoside conjugates and their binding properties to 16S RNA.

2. Results and discussion

2.1. Synthesis of aminodisaccharide–nucleosides

Azidodisaccharide **1** was obtained by the known procedures in good yield[14] and the configuration of the 5-hydroxyl in compound **1** can be inverted by treatment with trifluoromethanesulfonic anhydride followed by $NaNO_2$ to give compound **2**[15] (Scheme 1). The structures of compounds **1** and **2** were identified by ^1H NMR and compounds **type I** and **type II** were synthesized from compounds **2** and **1**, respectively. Aminodisaccharide–nucleosides **5a–e**, **7a–c** were synthesized according to the strategy shown in Scheme 2. Compound **1** was reacted with trifluoromethanesulfonic anhydride and the resulting triflate was converted to **3a–c** by appropriate lengths of aliphatic diamines via S_N2 substitution. Previously, we have found that ethylene diamine as a linker in compound **A** made the molecule fit to bind 16S RNA.[13] In this report, 1,3-propyldiamine and 1,4-butyldiamine were used to construct the compounds **type II** and ethylene diamine, 1,3-propyldiamine, and 1,4-butyldiamine were used as linkers in the compounds **type I**. Compounds **3b**,**c** were condensed with nucleoside-5′-carboxylic acids **13a** and **14**, respectively, to yield the corresponding compounds **4a**,**b** and **6a**,**b**. After reduction by H_2S, compounds **5a**,**b**

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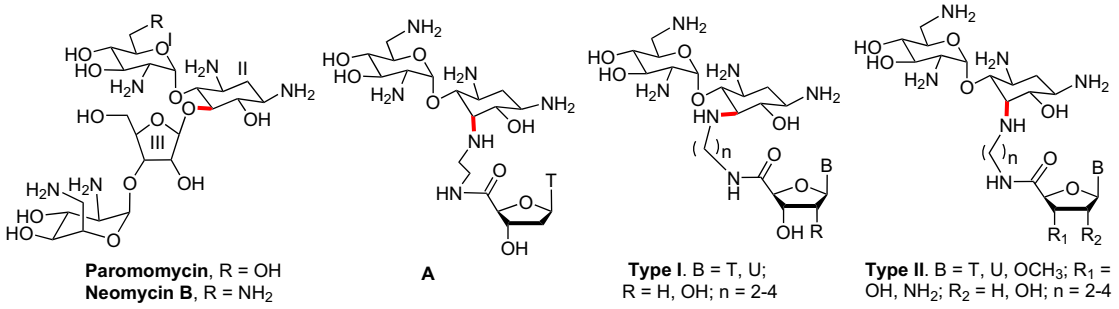
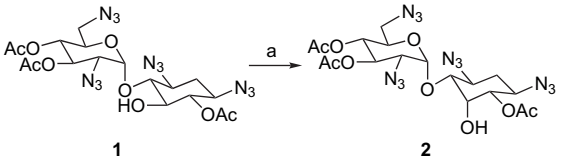


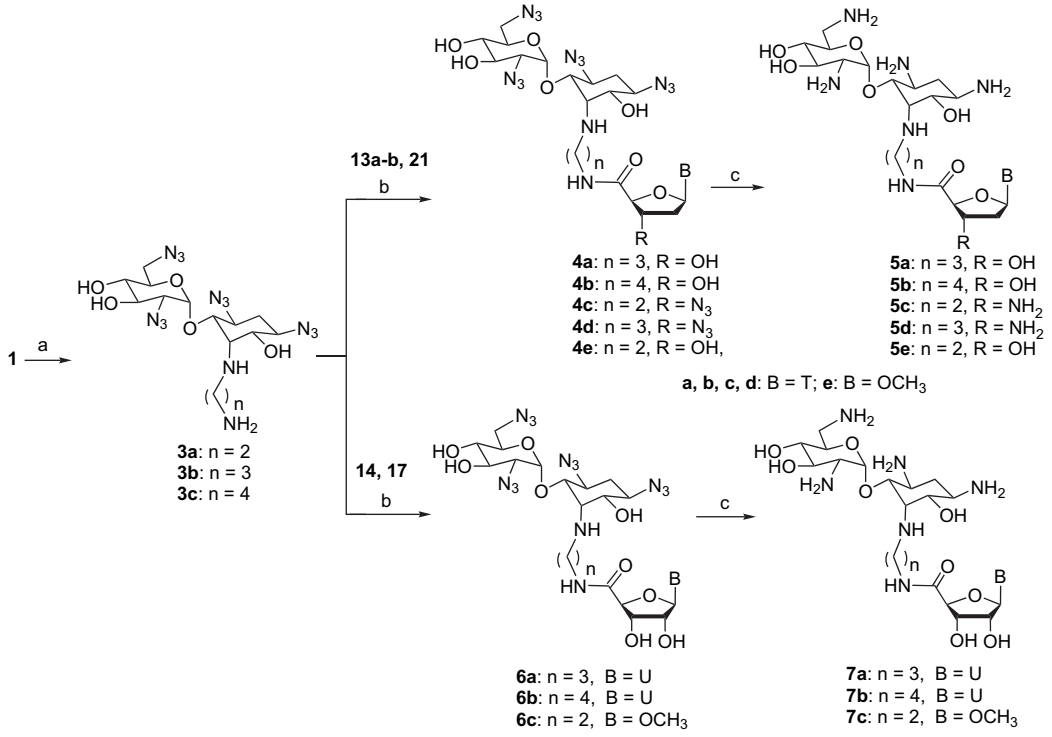
Figure 1. Structure of natural aminoglycosides, aminodisaccharide–nucleoside and the designed compounds **type I** and **type II**.

and **7a**,**b** were afforded. The designed stereoisomers **10a**–**c** and **12** were synthesized through the same strategy as described in Scheme 2. However, compound **2** was reacted with trifluoromethanesulfonic anhydride and the resulting triflate was treated with different aliphatic diamines to give only about 25% yield of products **8a**–**c**. The trifluoromethanesulfonylation of compound **2** was unfavorable due to strong intramolecular hydrogen bonding between 5-hydroxyl and the neighboring acetyl group (see ^{1}H NMR data of compound **2**) and steric hindrance (Scheme 3).

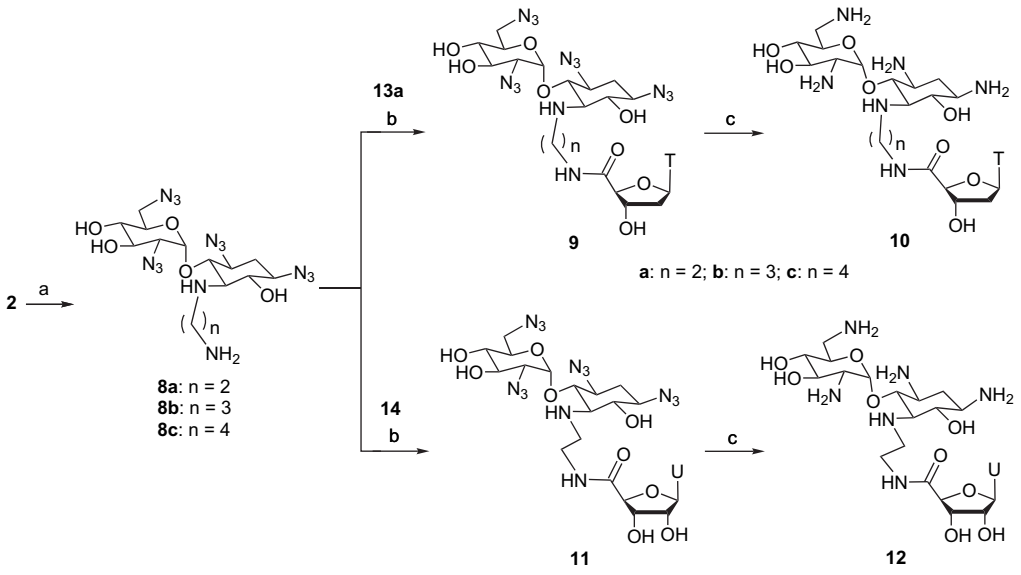


Scheme 1. Synthesis of azidodisaccharide **2**. Reagents and conditions: (a) (i) trifluoromethanesulfonic anhydride (Tf_2O), pyridine, CH_2Cl_2; (ii) $NaNO_2$, 15-crown-5, DMF, rt.

Considering the contribution of amino group in the molecule to the interaction with RNA, compounds **5c**,**d** were also synthesized by the condensation of **3a**,**b** and AZT-5′-carboxylic acid derivative **13b**. Nucleoside-5′-carboxylic acids **13a**,**b** and **14** can be synthesized by known procedure from the corresponding protected nucleosides (Scheme 4).[16] It seemed that thymine moiety of these conjugates played an important role for the interaction with RNA. To understand the relationship between the structure and binding activity, compounds **5e** and **7c** were also designed and synthesized by the strategy shown in Scheme 2. To obtain the corresponding glycosides **17** and **21**, compounds **15**[17] and **18**[18] were synthesized through the known protocols respectively. Selective deacetylation of **15** using iodine–methanol[19] afforded

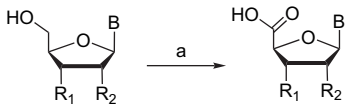


Scheme 2. Synthesis of aminodisaccharide–nucleoside conjugates **5a**–**e** and **7a**–**c**. Reagents and conditions: (a) (i) Tf_2O, pyridine, CH_2Cl_2; (ii) $H_2N(CH_2)_nNH_2$ (n=2, 3, or 4), THF, rt; (b) (i) dicyclohexylcarbodiimide, 1-hydroxybenzotriazole, DMF, 0 °C to rt; (ii) K_2CO_3, CH_3OH, rt; (c) (i) H_2S, pyridine/Et_3N/H_2O, rt; (ii) 1 N HCl.



Scheme 3. Synthesis of aminodisaccharide–nucleoside conjugates **10a–c** and **12**. Reagents and conditions: (a) (i) Tf_2O, pyridine, CH_2Cl_2; (ii) $H_2N(CH_2)_nNH_2$ (n=2, 3, or 4), THF or CH_3CN, rt; (b) (i) dicyclohexylcarbodiimide, 1-hydroxybenzotriazole, DMF, 0 °C to rt; (ii) K_2CO_3, CH_3OH, rt; (c) (i) H_2S, pyridine/Et_3N/H_2O, rt; (ii) 1 N HCl.

alcohol **16**, which was then oxidized to give carboxylic acid **17**. Acetylation of **18** led to **19**, which was subjected to detritylation using iodine–alcohol[20] to afford intermediate alcohol **20** as well as its α isomer. The β isomer was separated by silica gel column chromatography. Carboxylic acid **21** was furnished by similar oxidation (Scheme 5).



13a: R_1 = OAc, R_2 = H, B = T
13b: R_1 = N_3, R_2 = H, B = T
14: R_1 = OAc, R_2 = OAc, B = U

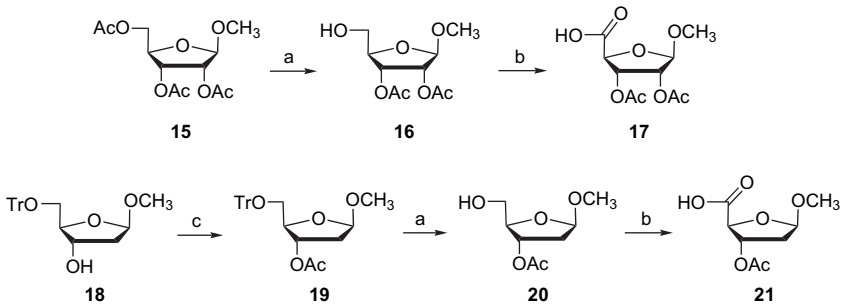
Scheme 4. Synthesis of nucleoside-5′-carboxylic acids **13a**,**b** and **14**. Reagents and conditions: (a) [bis(acetoxy)-iodo]benzene, 2,2,6,6-tetramethyl-1-piperidinyloxyl, CH_3CN/H_2O, rt.

2.2. Binding of the modified aminodisaccharide–nucleoside conjugates to 16S RNA

Surface Plasmon Resonance (SPR) has been successfully applied to observe the interactions between aminoglycoside and RNA.[21] The dissociation constants (K_d values in μM) were calculated from the slope of the *Scatchard* plot.[22] The resulting values are listed in Table 1.

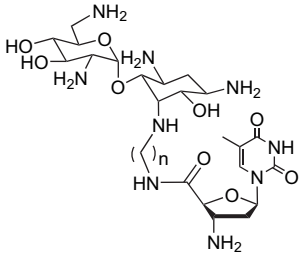
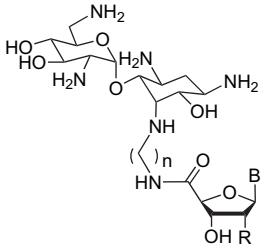
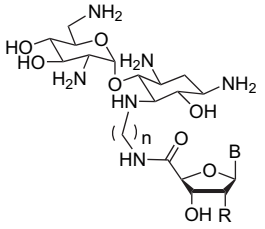
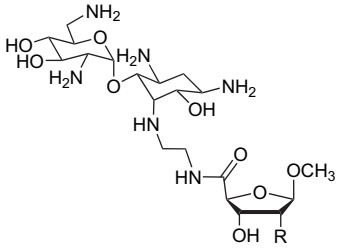
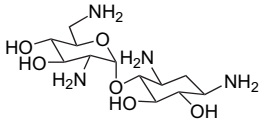
The data above indicated clearly that (1) the nucleobase made an important contribution to the binding of the designed aminodisaccharide–nucleoside conjugates to 16S RNA: compounds **5e** and **7c** displayed a sharp decrease of binding affinity to 16S RNA (Table 1, K_d **5e**, **7c**). (2) The conversion of 3′-hydroxyl of thymidine into amino group (**5c**,**d**) did not lead to significant increase of affinity as expected. (3) Compounds **10a** and **12** containing the ethylene diamine as linker had stronger binding interaction with 16S RNA compared to **10b**,**c** (Table 1, K_d **10a**, **12**).

In conclusion, two types of aminodisaccharide–nucleoside conjugates were synthesized by the condensation of azidodisaccharide and nucleoside using aliphatic diamine as a linker. Results from the SPR evaluation suggested that the nucleobase played a significant role to bind 16S RNA and a shorter linker between the aminodisaccharide and nucleoside was favorable for 16S RNA binding.



Scheme 5. Reagents and conditions: (a) I_2/CH_3OH, 60–80 °C; (b) 2,2,6,6-tetramethyl-1-piperidinyloxyl, [bis(acetoxy)-iodo]benzene, CH_3CN/H_2O, rt; (c) Ac_2O, pyridine, 4-dimethylamino pyridine, rt.

Table 1. The interaction between aminodisaccharide–nucleoside conjugates and 16S RNA

Structure		Entry	$K_{d(16S)}$ (μM)
	n=2	**5c**	39
	n=3	**5d**	35
	n=3, R=H, B=thymine	**5a**	41
	n=4, R=H, B=thymine	**5b**	139
	n=3, R=OH, B=uracil	**7a**	120
	n=4, R=OH, B=uracil	**7b**	196
	n=2, R=H, B=thymine	**10a**	25
	n=3, R=H, B=thymine	**10b**	49
	n=4, R=H, B=thymine	**10c**	56
	n=2, R=OH, B=uracil	**12**	29
	R=H	**5e**	476
	R=OH	**7c**	232
		**Neamine**	19

3. Experimental section

3.1. General

Commercial reagents were purchased from Acros and Aldrich Chemical Co., and were used without further purification. Dry dichloromethane, *N*,*N*-dimethyl formamide (DMF), and pyridine were distilled from CaH_2 when necessary. CH_3CN was distilled from P_2O_5. Tetrahydrofuran (THF) was dried over metallic Na. Silica gel 60H (200–300 mesh) manufactured by Qing-dao Haiyang Chemical Company (China) was used for column chromatography. ^1H and ^{13}C NMR spectra were recorded on JEOL JNM-AL300 or Varian INOVA-500 instrument with TMS as an internal standard. ^{13}C NMR spectra were calibrated relative to dioxane (d=66.66 ppm) in a separate NMR tube. Chemical shifts (δ values) and coupling constants (J values) were given in parts per million and hertz, respectively. Elemental analyses were recorded by Vario EL III instrument. High-resolution ESI mass spectra were obtained at MDS SCIEX QSTAR and Bruker DALTONICS APEX IV 70e instruments, and the data are reported in *m*/*e* (intensity to 100%). Optical rotations were recorded on Perkin–Elmer 243B Polarimeter.

3.2. *epi*-5-Hydroxyl-1,3,2′,6′-tetraazido-6,3′,4′-tri-*O*-acetyl neamine (2)

To a solution of **1** (1.10 g, 1.99 mmol) in anhydrous pyridine (11 mL) was added a solution of trifluoromethanesulfonic anhydride (2 mL, 11.6 mmol, 5.8 equiv) in dry CH_2Cl_2 (8 mL) at 0 °C, then the ice bath was removed, and the mixture was kept stirring for 1.5 h at room temperature. The reaction was quenched with ice and extracted with CH_2Cl_2 (2×150 mL), and the combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc=10/1) to provide a yellow syrup (1.05 g, 1.53 mmol), which was immediately dissolved in DMF (15 mL), and to the solution were consecutively added $NaNO_2$ (1.08 g, 15.6 mmol) and 15-crown-5 (0.6 mL, 3 mmol) at room temperature. After 24 h, the mixture was diluted with EtOAc (300 mL) and washed with H_2O (50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc=5/1) to afford **2** as a white foam (0.57 g, 1.03 mmol, 52% from **1**). ^1H NMR (500 MHz, $CDCl_3$): δ 5.48 (dd, 1H, J=10.0, 9.5 Hz, 3′-H), 5.05 (t, 1H, J=10.0 Hz, 4′-H), 5.02 (d, 1H, J=3.5 Hz, 1′-H), 4.66 (dd, 1H, J=10.5, 2.5 Hz, 6-H), 4.34 (dd, 1H, J=5.0, 3.0 Hz, 5′-H), 4.31 (d, 1H, J=2.5 Hz, 5-H), 4.02–4.08 (m, 1H, 1-H), 3.83–3.88 (m, 1H, 3-H), 3.75 (dd, 1H, J=10.0, 3.5 Hz, 2′-H), 3.54 (dd, 1H, J=10.0, 2.5 Hz, 4-H), 3.41 (dd, 1H, J=13.5, 2.5 Hz, 6′-Hb), 3.30 (dd, 1H, J=13.5, 5.0 Hz, 6′-Ha), 2.94 (s, 1H, –OH), 2.36 (dt, 1H, J=13.0, 4.5 Hz, 2-H_{eq}), 2.20 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 1.38 (q, 1H, J=12.5 Hz, 2-H_{ax}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 170.0, 169.7 (three C=O), 94.3 (C-1′), 78.8, 74.4, 71.6, 69.9, 69.0, 66.9, 61.5, 56.8, 55.6, 50.6 (C-6′), 31.9 (C-2), 20.9, 20.7, 20.6 (three CH_3). Anal. Calcd for $C_{18}H_{24}N_{12}O_9$: C, 39.13; H, 4.38; N, 30.42. Found: C, 39.27; H, 4.503; N, 30.19. HRESI-MS Calcd for $C_{18}H_{28}N_{13}O_9$ ($[M+NH_4]^+$): 570.2127, found: 570.2121.

3.3. *epi*-5-(2-Aminoethyl)-amino-1,3,2′,6′-tetraazido neamine (3a)

The compound was synthesized through same procedure as described for compound **3b**. A white foam was afforded in 41% yield from **1**. For ^1H NMR data, see Ref. 13.

3.4. *epi*-5-(3-Aminopropyl)-amino-1,3,2′,6′-tetraazido neamine (3b)

To a solution of starting material **1** (390 mg, 0.71 mmol) in anhydrous pyridine (4 mL) was added a solution of trifluoromethanesulfonic anhydride (0.73 mL, 4.24 mmol, 6 equiv) in dry CH_2Cl_2 (3 mL) at 0 °C, then the ice bath was removed,

and the mixture was kept stirring for 1.5 h at room temperature. The reaction was quenched with ice and extracted with CH_2Cl_2 (2×50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc =10/1) to provide a yellow syrup (400 mg, 0.58 mmol), which was unidentified by ^{1}H NMR. It was immediately dissolved in THF (4 mL), and to this solution was added $NH_2(CH_2)_3NH_2$ (0.5 mL, 10 equi.) at room temperature. After 24 h, the solvent was removed and the residue was purified by column chromatography on silica gel ($CH_2Cl_2/CH_3OH/NH_3\cdot H_2O$= 200/25/1.5) to afford **3b** (205 mg, 60% from **1**) as a white foam. ^{1}H NMR (500 MHz, CD_3OD): δ 5.14 (d, 1H, J=3.5 Hz, H-1′), 3.98–4.02 (m, 1H, H-5′), 3.78–3.91 (m, 3H, H-3′, 1 and 3), 3.63 (dd, 1H, J=10.0, 3.0 Hz, H-4), 3.52 (dd, 1H, J=13.5, 2.5 Hz, H-6′a), 3.31–3.46 (m, 5H, H-6′b, 2′, 4′, 5, and 6), 2.74–3.00 (m, 4H, –CH_2–), 2.21 (ddd, 1H, J=12.5, 5.0, 5.0 Hz, H_{eq}-2), 1.67–1.70 (m, 2H, –CH_2–), 1.19 (q, 1H, J=12.0 Hz, H_{ax}-2); ^{13}C NMR (125 MHz, CD_3OD): δ 95.4 (C-1′), 78.6 (C-4), 76.0 (C-4′), 73.6 (C-6), 73.1 (C-3′), 72.6 (C-5), 64.9 (C-5′), 60.9 (C-2′), 59.9 (C-1), 59.6 (C-3), 52.5 (C-6′), 49.5 (–CH_2–), 40.5 (–CH_2–), 33.7 (–CH_2–), 33.6 (C-2); HRESI-MS Calcd for $C_{15}H_{27}N_{14}O_5$ ([M+H]$^+$): 483.2283, found: 483.2271.

3.5. *epi*-5-(4-Aminobutyl)-amino-1,3,2′,6′-tetraazido neamine (3c)

The compound was synthesized through same procedure as described for compound **3b**. A white foam was afforded in 57% yield from **1**. ^{1}H NMR (500 MHz, CD_3OD): δ 5.12 (d, 1H, J=4.0 Hz, H-1′), 3.98–4.01 (m, 1H, H-5′), 3.79–3.92 (m, 3H, H-3′, 1 and 3), 3.63 (dd, 1H, J=10.5, 3.5 Hz, H-4), 3.52 (d, 1H, J=13.5, 2.5 Hz, H-6′a), 3.34–3.46 (m, 5H, H-6′b, 2′, 4′, 5, and 6), 2.81–2.96 (m, 2H, –CH_2–), 2.67–2.70 (m, 2H, –CH_2–), 2.21 (ddd, 1H, J=13.0, 5.0, 5.0 Hz, H_{eq}-2), 1.53–1.59 (m, 4H, –CH_2–), 1.19 (q, 1H, J= 12.0 Hz, H_{ax}-2); ^{13}C NMR (125 MHz, CD_3OD): δ 95.5 (C-1′), 78.7 (C-4), 75.9 (C-4′), 73.6 (C-6), 73.1 (C-3′), 72.6 (C-5), 64.9 (C-5′), 60.8 (C-2′), 59.9 (C-1), 59.7 (C-3), 52.5 (C-6), 51.9 (–CH_2–), 42.5 (–CH_2–), 33.7 (C-2), 31.4 (–CH_2–), 29.1 (–CH_2–); HRESI-MS Calcd for $C_{16}H_{29}N_{14}O_5$ ([M+H]$^+$): 497.2439, found: 497.2419.

3.6. 5-(2-Aminoethyl)-amino-1,3,2′,6′-tetraazido neamine (8a)

Compound **8a** was synthesized through same procedure as described for compound **3b**. A yellow syrup was afforded in 22% yield from **2**. ^{1}H NMR (500 MHz, CD_3OD): δ 5.50 (d, 1H, J=3.5 Hz, H-1′), 4.07–4.11 (m, 1H, H-5′), 3.82 (dd, 1H, J=10.5, 9.0 Hz, H-3′), 3.55 (dd, 1H, J=13.5, 2.5 Hz, H-6′a), 3.28–3.45 (m, 7H, H-1, 3, 4, 6, 6′b, 4′, and 2′), 2.91–2.95 (m, 1H, –CH_2–), 2.77–2.84 (m, 3H, –CH_2–), 2.58 (t, 1H, 10.0 Hz, H-5), 2.24 (dt, 1H, J=13.0, 4.0 Hz, H_{eq}-2), 1.40 (q, 1H, J=12.5 Hz, H_{ax}-2); ^{13}C NMR (125 MHz, CD_3OD): δ 100.1 (C-1′), 80.7 (C-4), 76.1 (C-6), 73.4 (C-5′), 73.1 (C-3′), 72.4 (C-4′), 65.8 (C-5), 65.5 (C-2′), 63.3 (C-1), 62.4 (C-3), 52.5 (C-6′), 42.7 (–CH_2–), 33.1 (C-2); DEPT: 48.5 (–CH_2–) (in solvent). HRESI-MS Calcd for $C_{14}H_{25}N_{14}O_5$ ([M+H]$^+$): 469.2126, found: 469.2106.

3.7. 5-(3-Aminopropyl)-amino-1,3,2′,6′-tetraazido neamine (8b)

Compound **8b** was synthesized through same procedure as described for compound **3b**. A yellow syrup was afforded in 23% yield from **2**. ^{1}H NMR (500 MHz, CD_3OD): δ 5.48 (d, 1H, J=4.0 Hz, H-1′), 4.05–4.08 (m, 1H, H-5′), 3.81 (dd, 1H, J=10.0, 8.5 Hz, H-3′), 3.56 (dd, 1H, J=13.0, 2.5 Hz, H-6′a), 3.33–3.45 (m, 7H, H-1, 3, 4, 6, 6′b, 4′, and 2′), 2.87–2.92 (m, 1H, –CH_2–), 2.74–2.79 (m, 3H, –CH_2–), 2.57 (t, 1H, J=9.5 Hz, H-5), 2.22–2.26 (ddd, 1H, J=13.0, 4.0, 4.0 Hz, H_{eq}-2), 1.66–1.71 (m, 2H, –CH_2–), 1.39 (q, 1H, J=13.0 Hz, H_{ax}-2); ^{13}C NMR (125 MHz, CD_3OD): δ 100.0 (C-1′), 80.5 (C-4), 75.9 (C-6), 73.5 (C-5′), 73.3 (C-3′), 72.4 (C-4′), 65.8 (C-5), 65.6 (C-2′), 63.4 (C-1), 62.5 (C-3), 52.5 (C-6′), 45.3 (–CH_2–), 40.6 (–CH_2–), 33.4 (CH_2), 33.2 (C-2). HRESI-MS Calcd for $C_{15}H_{27}N_{14}O_5$ ([M+H]$^+$): 483.2283, found: 483.2264.

3.8. 5-(4-Aminobutyl)-amino-1,3,2′,6′-tetraazido neamine (8c)

Compound **8c** was synthesized through same procedure as described for compound **3b**, while the solvent for diamine substitution was CH_3CN. A yellow syrup was afforded in 15% yield from **2**. ^{1}H NMR (500 MHz, CD_3OD): δ 5.46 (d, 1H, J=4.0 Hz, H-1′), 4.04–4.07 (m, 1H, H-5′), 3.81 (dd, 1H, J=10.0, 8.5 Hz, H-3′), 3.56 (dd, 1H, J=13.5, 2.5 Hz, H-6′a), 3.32–3.46 (m, 9H, H-1, 3, 4, 6, 4′, 6′b, and 2′), 2.85–2.90 (m, 1H, –CH_2–), 2.70–2.74 (m, 3H, –CH_2–), 2.57 (t, 1H, J=9.5 Hz, H-5), 2.24 (ddd, 1H, J=13.0, 4.0, 4.0 Hz, H_{eq}-2), 1.53–1.60 (m, 4H, –CH_2–CH_2–), 1.39 (q, 1H, J=12.0 Hz, H_{ax}-2); ^{13}C NMR (125 MHz, CD_3OD): δ 100.1 (C-1′), 80.7 (C-4), 76.0 (C-6), 73.5 (C-5′), 73.3 (C-3′), 72.4 (C-4′), 65.7 (C-5 and C-2′), 63.4 (C-1), 62.5 (C-3), 52.5 (C-6′), 47.7 (–CH_2–), 42.1 (–CH_2–), 33.2 (C-2), 30.6 (–CH_2–), 28.8 (–CH_2–). HRESI-MS Calcd for $C_{16}H_{29}N_{14}O_5$ ([M+H]$^+$): 497.2451, found: 497.2461.

3.9. General procedure for the syntheses of 4a–e, 6a–c, 9a–c, and 11

Dicyclohexylcarbodiimide (0.15 mmol, 1.2 equiv), 1-hydroxybenzotriazole (0.15 mmol, 1.2 equiv), and nucleoside-5-carboxylic acid (0.15 mmol, 1.2 equiv) were added in a reaction vessel and dissolved in 1.5 mL anhydrous DMF. The mixture was stirred in an ice bath for 0.5 h, at which point the solution of **8a–c** or **3a–c** (0.124 mmol, 1.0 equiv) in anhydrous DMF (2 mL) was added. After 20 h, the reaction mixture was filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH=20/1) to afford a white foam, which was then dissolved in CH_3OH (8 mL) and then K_2CO_3 (3 equiv) was added. After 2 h, the solvent was removed and the residue was purified by column chromatography on silica gel ($CH_2Cl_2/CH_3OH/NH_3\cdot H_2O$=150/17/2.5).

3.9.1. Compound 4a. Yield: 81% from **3b**, white foam. ^{1}H NMR (500 MHz, CD_3OD): δ 7.90 (d, 1H, J=1.0 Hz, H-6), 6.32 (dd, 1H, J=8.5, 5.5 Hz, H-1′), 5.13 (d, 1H, J=3.5 Hz, H-1‴), 4.54 (ddd, 1H, J=5.5, 2.0, 2.0 Hz, H-3′), 4.29 (d, 1H, J=2.0 Hz, H-4′), 3.79–4.01 (m, 4H, H-5‴, 3‴, 1″ and

3″), 3.63 (dd, 1H, *J*=10.0, 3.5 Hz, H-4″), 3.52 (dd, 1H, *J*=13.5, 2.5 Hz, H-6‴a), 3.33–3.46 (m, 8H, H-6‴b, 2‴, 4‴, 5″, 6″, and –CH_2–), 2.86–3.02 (m, 2H, –CH_2–), 2.39–2.45 (m, 1H, H-2′), 2.17–2.22 (m, 2H, H_{eq}-2″ and H-2′), 1.91 (d, 3H, *J*=1.0 Hz, 5-CH_3), 1.72–1.77 (m, 2H, –CH_2–), 1.18 (q, 1H, *J*=12.0 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, CD_3OD): *δ* 172.7 (–CO), 166.4 (C-2), 152.5 (C-4), 139.4 (C-6), 111.9 (C-5), 95.5 (C-1‴), 88.9 (C-1′), 87.5 (C-4′), 78.6 (C-3′), 76.0 (C-4″), 75.5 (C-4‴), 73.6 (C-6″), 73.1 (C-3‴), 72.6 (C-5″), 64.9 (C-5‴), 60.7 (C-2‴), 59.9 (C-1″), 59.7 (C-3″), 52.5 (C-6‴), 49.5 (–CH_2–), 39.3 (C-2′), 38.5 (–CH_2–), 33.6 (C-2″), 31.2 (–CH_2–), 12.6 (–CH_3). HRESI-MS Calcd for $C_{25}H_{37}N_{16}O_{10}$ ([M+H]$^+$): 721.2873, found: 721.2865.

3.9.2. Compound 4b. Yield: 85% from **3c**, white foam. ^{1}H NMR (500 MHz, CD_3OD): *δ* 7.91 (d, 1H, *J*=1.0 Hz, H-6), 6.30 (dd, 1H, *J*=9.0, 6.0 Hz, H-1′), 5.12 (d, 1H, *J*=4.0 Hz, H-1‴), 4.50–4.52 (m, 1H, H-3′), 4.28 (d, 1H, *J*=2.0 Hz, H-4′), 3.80–4.01 (m, 4H, H-5‴, 3‴, 1″ and 3″), 3.63 (dd, 1H, *J*=10.0, 3.0 Hz, H-4″), 3.52 (dd, 1H, *J*=13.5, 2.5 Hz, H-6‴a), 3.25–3.46 (m, 8H, H-6‴b, 2‴, 4‴, 5″, 6″, and –CH_2–), 2.81–2.98 (m, 2H, –CH_2–), 2.39–2.44 (m, 1H, H-2′), 2.19–2.24 (m, 2H, H_{eq}-2″ and H-2′), 1.90 (d, 1H, *J*=1.0 Hz, –CH_3), 1.54–1.65 (m, 4H, –CH_2CH_2–), 1.19 (q, 1H, *J*=12.0 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, D_2O): *δ* 172.7 (–CO), 166.4 (C-2), 152.5 (C-4), 139.6 (C-6), 111.8 (C-5), 95.4 (C-1‴), 89.0 (C-1′), 87.4 (C-4′), 78.7 (C-3′), 75.9 (C-4″), 75.5 (C-4‴), 73.5 (C-6″), 73.0 (C-3‴), 72.6 (C-5″), 64.8 (C-5‴), 60.8 (C-2‴), 59.9 (C-1″), 59.6 (C-3″), 52.5 (C-6‴), 51.7 (–CH_2–), 40.3 (–CH_2–), 39.3 (C-2′), 33.6 (C-2″), 28.9 (–CH_2–), 28.2 (–CH_2–), 12.6 (–CH_3). HRESI-MS Calcd for $C_{26}H_{39}N_{16}O_{10}$ ([M+H]$^+$): 735.3029, found: 735.3051.

3.9.3. Compound 4c. Yield: 91% from **3a**, white foam. ^{1}H NMR (500 MHz, CD_3OD): *δ* 7.76 (d, 1H, *J*=1.0 Hz, H-6), 6.11 (t, 1H, *J*=6.5 Hz, H-1′), 5.14 (d, 1H, *J*=4.0 Hz, H-1‴), 4.57–4.60 (m, 1H, H-3′), 4.35 (d, 1H, *J*=4.0 Hz, H-4′), 3.99–4.02 (m, 1H, H-5‴), 3.77–3.93 (m, 3H, H-1″, 3‴, and 3″), 3.64 (dd, 1H, *J*=10.5, 3.5 Hz, H-6‴a), 3.34–3.54 (m, 8H, H-4″, 6‴b, 2‴, 4‴, 5″, 6″, and –CH_2–), 3.07–3.19 (m, 2H, –CH_2–), 2.63–2.68 (m, 1H, H-2′a), 2.30–2.34 (m, 1H, H-2′b), 2.20 (ddd, 1H, *J*=13.0, 4.5, 4.5 Hz, H_{eq}-2″), 1.89 (d, 3H, *J*=1.5 Hz, 5-CH_3), 1.21 (q, 1H, *J*=12.5 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, CD_3OD): *δ* 172.4 (–CO), 166.3 (C-2), 152.4 (C-4), 139.8 (C-6), 111.8 (C-5), 96.0 (C-1‴), 89.5 (C-1′), 84.5 (C-4′), 78.8 (C-4″), 75.0 (C-4‴), 73.6 (C-6″), 73.3 (C-3‴), 72.5 (C-5″), 65.2 (C-3′), 65.0 (C-5‴), 60.7 (C-2‴), 59.6 (C-1″ and 3″), 52.5 (C-6‴), 50.6 (–CH_2–), 40.5 (–CH_2–), 36.7 (C-2′), 33.4 (C-2″), 12.5 (–CH_3). HRESI-MS Calcd for $C_{24}H_{34}N_{19}O_9$ ([M+H]$^+$): 732.2781, found: 732.2753.

3.9.4. Compound 4d. Yield: 91% from **3b**, white foam. ^{1}H NMR (500 MHz, CD_3OD): *δ* 7.74 (d, 1H, *J*=1.0 Hz, H-6), 6.15 (t, 1H, *J*=6.5 Hz, H-1′), 5.14 (d, 1H, *J*=4.0 Hz, H-1‴), 4.59–4.62 (m, 1H, H-3′), 4.34 (d, 1H, *J*=4.0 Hz, H-4′), 3.99–4.02 (m, 1H, H-5‴), 3.81–3.91 (m, 3H, H-1″, 3‴, and 3″), 3.69 (dd, 1H, *J*=10.0, 3.5 Hz, H-6‴a), 3.34–3.55 (m, 8H, H-4″, 6‴b, 2‴, 4‴, 5″, 6″, and –CH_2–), 2.96–3.12 (m, 2H, –CH_2–), 2.59–2.64 (m, 1H, H-2′a), 2.31–2.36 (m, 1H, H-2′b), 2.22 (ddd, 1H, *J*=13.0, 5.0, 5.0 Hz, H_{eq}-2″), 1.90 (d, 3H, *J*=1.0 Hz, 5-CH_3), 1.76–1.82 (m, 2H, –CH_2–), 1.24 (q, 1H, *J*=13.0 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, CD_3OD): *δ* 172.1 (–CO), 166.4 (C-2), 152.4 (C-4), 139.6 (C-6), 111.8 (C-5), 96.0 (C-1‴), 89.2 (C-1′), 84.6 (C-4′), 78.2 (C-4″), 75.0 (C-4‴), 73.7 (C-6″), 73.3 (C-3‴), 72.5 (C-5″), 65.2 (C-3′), 65.0 (C-5‴), 60.6 (C-2‴), 60.1 (C-1″), 59.6 (C-3″), 52.5 (C-6‴), 38.2 (–CH_2–), 36.9 (C-2′), 33.1 (C-2″), 30.5 (–CH_2–), 12.5 (–CH_3). HRESI-MS Calcd for $C_{25}H_{36}N_{19}O_9$ ([M+H]$^+$): 746.2938, found: 746.2926.

3.9.5. Compound 4e. Yield: 88% from **3a**, white foam. ^{1}H NMR (500 MHz, CD_3OD): *δ* 5.30 (t, 1H, *J*=5.0 Hz, H-1′), 5.13 (d, 1H, *J*=4.0 Hz, H-1‴), 4.51–4.54 (m, 1H, H-3′), 4.28 (d, 1H, *J*=2.5 Hz, H-4′), 3.81–4.03 (m, 4H, H-5‴, 3‴, 1″, and 3″), 3.62 (dd, 1H, *J*=10.5, 3.5 Hz, H-4″), 3.51–3.54 (m, 4H, H-6‴a, OCH_3), 3.27–3.47 (m, 7H, H-6‴b, 2‴, 4‴, 5″, 6″, and –CH_2–), 2.98–3.07 (m, 2H, –CH_2–), 2.20 (ddd, 1H, *J*=13.0, 5.0, 5.0 Hz, H_{eq}-2″), 2.03–2.15 (m, 2H, H-2′), 1.19 (q, 1H, *J*=13.0 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, CD_3OD): *δ* 174.0 (–CO), 109.0 (C-1′), 95.6 (C-1‴), 87.5 (C-4′), 78.9 (C-3′), 75.7 (C-4″), 75.5 (C-4‴), 73.6 (C-6″), 73.2 (C-3‴), 72.6 (C-5″), 64.9 (C-5‴), 60.7 (C-2‴), 59.8 (C-1″), 59.3 (C-3″), 57.2 (–OCH_3), 52.5 (C-6‴), 50.4 (–CH_2–), 41.3 (C-2′), 40.8 (–CH_2–), 33.7 (C-2″). HRESI-MS Calcd for $C_{20}H_{33}N_{14}O_9$ ([M+H]$^+$): 613.2550, found: 613.2547.

3.9.6. Compound 6a. Yield: 68% from **3b**, white foam. ^{1}H NMR (500 MHz, CD_3OD): *δ* 8.10 (d, 1H, *J*=8.0 Hz, H-6), 5.85 (d, 1H, *J*=6.0 Hz, H-1′), 5.74 (d, 1H, *J*=8.5 Hz, H-5), 5.13 (d, 1H, *J*=3.5 Hz, H-1‴), 4.43 (dd, 1H, *J*=6.0, 5.0 Hz, H-2′), 4.37 (d, 1H, *J*=3.5 Hz, H-4′), 4.24 (dd, 1H, *J*=5.0, 3.5 Hz, H-3′), 3.80–4.02 (m, 4H, H-5‴, 3‴, 1″, and 3″), 3.65 (dd, 1H, *J*=10.0, 3.5 Hz, H-4″), 3.36–3.54 (m, 8H, H-6‴a, 6‴b, 2‴, 4‴, 5″, 6″, and –CH_2–), 2.89–3.04 (m, 2H, –CH_2–), 2.21 (ddd, 1H, *J*=13.0, 5.0, 5.0 Hz, H_{eq}-2″), 1.73–1.79 (m, 2H, –CH_2–), 1.21 (q, 1H, *J*=12.0 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, CD_3OD): *δ* 172.2 (–CO), 166.1 (C-2), 152.6 (C-4), 144.2 (C-6), 103.1 (C-5), 95.7 (C-1‴), 92.6 (C-1′), 85.2 (C-4′), 78.5 (C-3′), 75.6 (C-2′), 74.6 (C-4″), 73.9 (C-4‴), 73.6 (C-6″), 73.1 (C-3‴), 72.5 (C-5″), 64.9 (C-5‴), 60.7 (C-2‴), 59.8 (C-1″), 59.7 (C-3″), 52.5 (C-6‴), 38.4 (–CH_2–), 33.4 (C-2″), 30.9 (–CH_2–). HRESI-MS Calcd for $C_{24}H_{35}N_{16}O_{11}$ ([M+H]$^+$): 723.2665, found: 723.2670.

3.9.7. Compound 6b. Yield: 82% from **3c**, white foam. ^{1}H NMR (500 MHz, CD_3OD): *δ* 8.10 (d, 1H, *J*=8.0 Hz, H-6), 5.83 (d, 1H, *J*=6.0 Hz, H-1′), 5.74 (d, 1H, *J*=8.0 Hz, H-5), 5.13 (d, 1H, *J*=4.0 Hz, H-1‴), 4.43 (dd, 1H, *J*=6.0, 5.0 Hz, H-2′), 4.35 (d, 1H, *J*=3.0 Hz, H-4′), 4.22 (dd, 1H, *J*=5.0, 3.5 Hz, H-3′), 3.80–4.02 (m, 4H, H-5‴, 3‴, 1″, and 3″), 3.66 (dd, 1H, *J*=10.0, 3.5 Hz, H-4″), 3.27–3.54 (m, 8H, H-6‴a, 6‴b, 2‴, 4‴, 5″, 6″, and –CH_2–), 2.88–3.04 (m, 2H, –CH_2–), 2.24 (ddd, 1H, *J*=13.0, 5.0, 5.0 Hz, H_{eq}-2″), 1.55–1.67 (m, 4H, –CH_2CH_2–), 1.23 (q, 1H, *J*=12.0 Hz, H_{ax}-2″); ^{13}C NMR(100 MHz, CD_3OD): *δ* 170.8 (–CO), 164.7 (C-2), 151.2 (C-4), 142.9 (C-6), 101.6 (C-5), 94.4 (C-1‴), 91.4 (C-1′), 83.8 (C-4′), 77.0 (C-3′), 73.8 (C-2′), 73.2 (C-4″), 72.6 (C-4‴), 72.2 (C-6″), 71.8 (C-3‴), 71.1 (C-5″), 63.5 (C-5‴), 59.3 (C-2‴), 58.4 (C-1″), 58.3 (C-3″), 51.1 (C-6‴), 50.3 (–CH_2–), 38.8 (–CH_2–), 31.9 (C-2″), 26.9 (–CH_2–), 26.7 (–CH_2–). HRESI-MS Calcd for $C_{25}H_{37}N_{16}O_{11}$ ([M+H]$^+$): 737.2822, found: 737.2821.

3.9.8. Compound 6c. Yield: 81% from **3a**, white foam. ^{1}H NMR (500 MHz, CD_3OD): δ 5.13 (d, 1H, J=3.5 Hz, H-1‴), 4.88 (d, 1H, J=2.0 Hz, H-1′), 4.27–4.31 (m, 2H, H-3′ and 2′), 3.79–4.03 (m, 5H, H-5‴, 3‴, 4′, 1″, and 3″), 3.62 (dd, 1H, J=10.5, 3.5 Hz, H-4″), 3.49–3.54 (m, 4H, H-6‴a, OCH_3), 3.32–3.46 (m, 7H, H-6‴b, 2‴, 4‴, 5″, 6″, and $-CH_2-$), 3.04 (t, 2H, J=6.5 Hz, $-CH_2-$), 2.21 (ddd, 1H, J=13.0, 5.0, 5.0 Hz, H_{eq}-2″), 1.19 (q, 1H, J=12.5 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, CD_3OD): δ 174.2 (–CO), 111.0 (C-1′), 95.7 (C-1‴), 83.6 (C-4′), 79.0 (C-3′), 76.2 (C-2′), 75.8 (C-4″), 75.4 (C-4‴), 73.6 (C-6″), 73.2 (C-3‴), 72.6 (C-5″), 64.9 (C-5‴), 60.7 (C-2‴), 59.8 (C-1″), 59.3 (C-3″), 56.9 ($-OCH_3$), 52.5 (C-6‴), 50.4 ($-CH_2-$), 40.8 ($-CH_2-$), 33.7 (C-2″). HRESI-MS Calcd for $C_{20}H_{33}N_{14}O_{10}$ ([M+H]$^+$): 629.2499, found: 629.2472.

3.9.9. Compound 9a. Yield: 73% from **8a**, white foam. ^{1}H NMR (500 MHz, CD_3OD): δ 7.89 (d, 1H, J=1.0 Hz, H-6), 6.27 (dd, 1H, J=9.0, 6.0 Hz, H-1′), 5.63 (d, 1H, J=4.0 Hz, H-1‴), 4.51–4.53 (m, 1H, H-3′), 4.29 (d, 1H, J=2.0 Hz, H-4′), 4.09–4.12 (m, 1H, H-5‴), 3.83 (dd, 1H, J=10.5, 9.0 Hz, H-3‴), 3.54 (dd, 1H, J=13.5, 2.5 Hz, H-6‴a), 3.26–3.46 (m, 9H, H-6‴b, 2‴, 4‴, 1″, 3″, 4″, 6″, and $-CH_2-$), 2.86–3.01 (m, 2H, $-CH_2-$), 2.59 (t, 1H, J=10.0 Hz, H-5″), 2.43–2.49 (m, 1H, H-2′), 2.17–2.25 (m, 2H, H-2′ and H_{eq}-2″), 1.90 (d, 3H, J=1.0 Hz, $-CH_3$), 1.38 (q, 1H, J= 12.5 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, CD_3OD): δ 172.9 (–CO), 166.4 (C-2), 152.6 (C-4), 139.8 (C-6), 111.8 (C-5), 99.8 (C-1‴), 89.4 (C-1′), 87.5 (C-4′), 79.8 (C-3′), 76.1 (C-4″), 75.5 (C-6″), 73.4 (C-5‴), 72.8 (C-3‴), 72.6 (C-4‴), 65.9 (C-5″), 65.1 (C-2‴), 63.3 (C-1″), 62.4 (C-3″), 52.6 (C-6‴), 46.8 ($-CH_2-$), 40.9 ($-CH_2-$), 39.1 (C-2′), 33.1 (C-2″), 12.5 ($-CH_3$). HRESI-MS Calcd for $C_{24}H_{35}N_{16}O_{10}$ ([M+H]$^+$): 707.2716, found: 707.2718.

3.9.10. Compound 9b. Yield: 75% from **8b**, white foam. ^{1}H NMR (500 MHz, CD_3OD): δ 7.91 (d, 1H, J=1.0 Hz, H-6), 6.27 (dd, 1H, J=9.0, 6.0 Hz, H-1′), 5.47 (d, 1H, J=4.0 Hz, H-1‴), 4.51–4.53 (m, 1H, H-3′), 4.28 (d, 1H, J=2.0 Hz, H-4′), 4.05–4.08 (m, 1H, H-5‴), 3.81 (dd, 1H, J=10.0, 8.5 Hz, H-3‴), 3.56 (dd, 1H, J=13.5, 2.5 Hz, H-6‴a), 3.32–3.46 (m, 9H, H-6‴b, 2‴, 4‴, 6″, 4″, 1″, 3″, and $-CH_2-$), 2.73–2.92 (m, 2H, $-CH_2-$), 2.59 (t, 1H, J=9.5 Hz, H-5″), 2.43–2.48 (m, 1H, H-2′), 2.18–2.26 (m, 2H, H_{eq}-2″ and H-2′), 1.90 (d, 3H, J=1.0 Hz, $-CH_3$), 1.72–1.77 (m, 2H, 1–CH_2-), 1.38 (q, 1H, J=12.5 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, CD_3OD): δ 172.9 (–CO), 166.4 (C-2), 152.6 (C-4), 139.8 (C-6), 111.8 (C-5), 100.1 (C-1‴), 89.4 (C-1′), 87.5 (C-4′), 80.4 (C-3′), 75.8 (C-4″), 75.6 (C-6″), 73.5 (C-5‴), 73.3 (C-3‴), 72.4 (C-4‴), 65.8 (C-5″), 65.6 (C-2‴), 63.3 (C-1″), 62.4 (C-3″), 52.5 (C-6‴), 45.1 ($-CH_2-$), 39.2 ($-CH_2-$), 38.3 (C-2′), 33.1 (C-2″), 31.0 ($-CH_2-$), 12.5 ($-CH_3$). HRESI-MS Calcd for $C_{25}H_{37}N_{16}O_{10}$ ([M+H]$^+$): 721.2873, found: 721.2853.

3.9.11. Compound 9c. Yield: 70% from **8c**, white foam. ^{1}H NMR (500 MHz, CD_3OD): δ 7.90 (d, 1H, J=1.5 Hz, H-6), 6.27 (dd, 1H, J=8.5, 5.5 Hz, H-1′), 5.46 (d, 1H, J=4.0 Hz, H-1‴), 4.49–4.51 (m, 1H, H-3′), 4.28 (d, 1H, J=2.0 Hz, H-4′), 4.03–4.07 (m, 1H, H-5‴), 3.80 (dd, 1H, J=10.5, 9.0 Hz, H-3‴), 3.56 (dd, 1H, J=13.0, 2.5 Hz, H-6‴a), 3.22–3.47 (m, 9H, H-6‴b, 2‴, 4‴, 6″, 4″, 1″, 3″, and $-CH_2-$), 2.73–2.92 (m, 2H, $-CH_2-$), 2.61 (t, 1H, J=9.5 Hz, H-5″), 2.41–2.47 (m, 1H, H-2′), 2.18–2.27 (m, 2H, H_{eq}-2″ and H-2′), 1.90 (d, 3H, J=1.5 Hz, $-CH_3$), 1.51–1.64 (m, 4H, $-CH_2CH_2-$), 1.39 (q, 1H, J=12.0 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, CD_3OD): δ 172.8 (–CO), 166.4 (C-2), 152.6 (C-4), 139.7 (C-6), 111.8 (C-5), 100.1 (C-1‴), 89.3 (C-1′), 87.5 (C-4′), 80.6 (C-3′), 75.8 (C-4″), 75.5 (C-6″), 73.5 (C-5‴), 73.4 (C-3‴), 72.4 (C-4‴), 65.7 (C-5″ and C-2‴″), 63.4 (C-1″), 62.4 (C-3″), 52.5 (C-6‴″), 47.4 ($-CH_2-$), 40.1 ($-CH_2-$), 39.3 (C-2′), 33.2 (C-2″), 28.5 ($-CH_2-$), 28.2 ($-CH_2-$), 12.5 ($-CH_3$). HRESI-MS Calcd for $C_{26}H_{39}N_{16}O_{10}$ ([M+H]$^+$): 735.3030, found: 735.3021.

3.9.12. Compound 11. Yield: 78% from **8a**, white foam. ^{1}H NMR (500 MHz, CD_3OD): δ 8.06 (d, 1H, J=8.0 Hz, H-6), 5.81 (d, 1H, J=6.5 Hz, H-1′), 5.73 (d, 1H, J=8.0 Hz, H-5), 5.62 (d, 1H, J=3.5 Hz, H-1‴), 4.46 (dd, 1H, J=6.0, 5.0 Hz, H-2′), 4.37 (d, 1H, J=3.5 Hz, H-4′), 4.24 (dd, 1H, J=5.0, 3.0 Hz, H-3′), 4.08–4.11 (m, 1H, H-5‴), 3.83 (dd, 1H, J=10.5, 9.0 Hz, H-3‴), 3.55 (dd, 1H, J=13.0, 2.5 Hz, H-6‴a), 3.32–3.46 (m, 9H, H-6‴b, 2‴, 4‴, 1″, 3″, 4″, 6″, and $-CH_2-$), 2.88–3.02 (m, 2H, $-CH_2-$), 2.62 (t, 1H, J= 10.0 Hz, H-5″), 2.22–2.26 (m, 1H, H_{eq}-2″), 1.39 (q, 1H, J=12.5 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, CD_3OD): δ 172.4 (–CO), 166.1 (C-2), 152.6 (C-4), 144.5 (C-6), 103.0 (C-5), 99.8 (C-1‴), 93.1 (C-1′), 85.2 (C-4′), 79.7 (C-3′), 75.9 (C-2′), 74.6 (C-4″), 73.6 (C-6″), 73.4 (C-5‴), 72.9 (C-3‴), 72.5 (C-4‴), 65.8 (C-5″), 65.1 (C-2‴), 63.3 (C-1″), 62.4 (C-3″), 52.6 (C-6‴), 46.7 ($-CH_2-$), 40.8 ($-CH_2-$), 33.1 (C-2″). HRESI-MS Calcd for $C_{23}H_{33}N_{16}O_{11}$ ([M+H]$^+$): 709.2509, found: 709.2516.

3.10. General procedure for the syntheses of 5a–e, 7a–c, 10a–c, and 12

To a solution of **4a–e**, **6a–c**, **9a–c**, or **11** from the previous step in 2:1.5:1 pyridine/triethylamine/water (9 mL) was slowly bubbled hydrogen sulfide for 1 h. The flask was then sealed and stirring was continued for 1 h. The resulting mixture was concentrated and the residue was purified by column chromatography on silica gel with gradient elution (CH_2Cl_2/CH_3OH/concd $NH_3\cdot H_2O$=from 50/40/5 to 50/40/10 to 50/40/25); the fractions containing the desired product were analyzed by TLC and collected. The solvents were removed and the product in water was adjusted to pH 6.0; then lyophilization from water gave the final product.

3.10.1. Compound 5a. Yield: 94%, white foam. $[\alpha]_D^{20}$ +42.0 (c 0.20, H_2O). ^{1}H NMR (500 MHz, D_2O): δ 7.61 (s, 1H, H-6), 6.23 (t, 1H, J=7.5 Hz, H-1′), 5.69 (d, 1H, J=4.0 Hz, H-1‴), 4.75–4.78 (m, 1H, H-3′), 4.48 (dd, 1H, J=10.5, 3.0 Hz, H-4″), 4.41 (d, 1H, J=4.5 Hz, H-4′), 4.32–4.37 (m, 2H, H-6″ and 5″), 4.03–4.11 (m, 2H, H-3‴ and 5‴), 3.82–3.89 (m, 2H, H-1″ and 3″), 3.59–3.65 (m, 3H, H-2‴, 4‴, and $-CH_2-$), 3.52 (dd, 1H, J=14.0, 3.5 Hz, H-6‴a), 3.40–3.45 (m, 2H, H-6‴b and $-CH_2-$), 3.31–3.37 (m, 2H, $-CH_2-$), 2.62–2.68 (m, 2H, H-2′ and 2″), 2.38–2.43 (m, 1H, H-2′), 2.00–2.10 (m, 3H, H-2″ and $-CH_2-$), 1.90 (s, 3H, $-CH_3$); ^{13}C NMR (125 MHz, D_2O): δ 174.2 (–CO), 167.3 (C-2), 152.4 (C-4), 140.3 (C-6), 111.9 (C-5), 92.6 (C-1‴), 89.4 (C-1′), 85.6 (C-4′), 74.5 (C-3′), 71.7 (C-4″), 71.4 (C-4‴), 70.4 (C-6″), 69.0 (C-3‴), 68.2 (C-5″), 58.8 (C-2‴), 53.5 ($-CH_2-$), 49.8 (C-1″), 48.9 (C-3″), 47.6 (C-5‴), 40.4 (C-6‴), 38.3 ($-CH_2-$), 36.4 (C-2′), 28.7 (C-2″), 27.1 ($-CH_2-$), 12.2 ($-CH_3$). HRESI-MS Calcd for $C_{25}H_{45}N_8O_{10}$ ([M+H]$^+$): 617.3253, found: 617.3257.

3.10.2. Compound 5b. Yield: 90%, white foam. $[\alpha]_D^{20}$ +21.4 (*c* 0.49, H_2O). ^1H NMR (500 MHz, D_2O): δ 7.68 (s, 1H, H-6), 6.29 (t, 1H, *J*=7.0 Hz, H-1′), 5.68 (d, 1H, *J*=3.5 Hz, H-1‴), 4.70–4.73 (m, 1H, H-3′), 4.53 (d, 1H, *J*=10.0 Hz, H-4″), 4.37–4.41 (m, 3H, H-4′, 5″, and 6″), 4.01–4.12 (m, 2H, H-3‴ and 5‴), 3.77–3.86 (m, 2H, H-1″ and 3″), 3.26–3.64 (m, 8H, H-2‴, 4‴, –CH_2–, 6‴a, 6‴b, and –CH_2–), 2.54–2.65 (m, 2H, H_{eq}-2″ and 2′), 2.34–2.39 (m, 1H, H-2′), 2.02 (q, 1H, *J*=12.5 Hz, H_{ax}-2″), 1.89 (s, 3H, –CH_3), 1.80–1.87 (m, 2H, –CH_2–), 1.57–1.63 (m, 2H, –CH_2–); ^{13}C NMR (125 MHz, D_2O): δ 172.9 (–CO), 167.3 (C-2), 152.5 (C-4), 139.7 (C-6), 112.0 (C-5), 92.7 (C-1‴), 88.5 (C-1′), 85.8 (C-4′), 74.4 (C-3′), 71.6 (C-4″), 71.6 (C-4‴), 70.4 (C-6″), 68.7 (C-3‴), 67.7 (C-5″), 59.3(C-2‴), 53.4 (–CH_2–), 49.0 (C-1″ and C-3″), 47.7 (C-5‴), 40.4 (C-6‴), 39.0 (–CH_2–), 38.0 (C-2′), 28.5 (C-2″), 26.5 (–CH_2–), 23.9 (–CH_2–), 12.3 (–CH_3). HRESI-MS Calcd for $C_{26}H_{47}N_8O_{10}$ ($[M+H]^+$): 631.3409, found: 631.3391.

3.10.3. Compound 5c. Yield: 88%, white foam. $[\alpha]_D^{20}$ +28.4 (*c* 0.23, H_2O). ^1H NMR (500 MHz, D_2O): δ 7.51 (s, 1H, H-6), 6.20 (dd, 1H, *J*=8.5, 4.0 Hz, H-1′), 5.52 (d, 1H, *J*=3.5 Hz, H-1‴), 4.70 (d, 1H, *J*=4.5 Hz, H-4′), 4.48–4.52 (m, 1H, H-3′), 4.08 (dd, 1H, *J*=10.5, 3.5 Hz, H-4″), 3.88–4.05 (m, 3H, H-3‴, 5‴, and 6″), 3.66–3.75 (m, 3H, H-1″, 3″, and 5″), 3.47–3.54 (m, 3H, H-2‴, 4‴, and 6‴a), 3.29–3.34 (m, 3H, H-6‴b and –CH_2–), 2.91–3.01 (m, 3H, –CH_2– and H-2′a), 2.61–2.67 (m, 1H, H2′b), 2.49 (ddd, 1H, *J*=12.5, 4.5, 4.5 Hz, H_{eq}-2″), 1.88 (s, 3H, –CH_3), 1.77 (q, 1H, *J*= 12.5 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, D_2O): δ 171.9 (–CO), 167.3 (C-2), 152.2 (C-4), 141.1 (C-6), 111.8 (C-5), 92.2 (C-1‴), 90.9 (C-1′), 82.9 (C-4′), 74.1 (C-4″), 72.1 (C-4‴), 71.3 (C-6″), 70.0 (C-3‴), 69.5 (C-5″), 56.9 (C-2‴), 54.1 (C-3′), 54.1 (–CH_2–), 49.0 (C-1″), 48.9 (C-3″), 47.8 (C-5‴), 40.8 (C-6‴), 40.4 (–CH_2–), 35.1 (C-2′), 29.4 (C-2″), 12.1 (–CH_3). HRESI-MS Calcd for $C_{24}H_{44}N_9O_9$ ($[M+H]^+$): 602.3257, found: 602.3256.

3.10.4. Compound 5d. Yield: 92%, white foam. $[\alpha]_D^{20}$ +16.2 (*c* 0.23, H_2O). ^1H NMR (500 MHz, D_2O): δ 7.52 (d, 1H, *J*=1.5 Hz, H-6), 6.16 (dd, 1H, *J*=8.0, 3.5 Hz, H-1′), 5.57 (d, 1H, *J*=3.5 Hz, H-1‴), 4.70 (d, 1H, *J*=4.5 Hz, H-4′), 4.51–4.55 (m, 1H, H-3′), 3.95–4.25 (m, 4H, H-4″, 3‴, 5‴, and 6″), 3.74–3.87 (m, 3H, H-1″, 3″, and 5″), 3.49–3.59 (m, 3H, H-2‴, 4‴, and 6‴a), 3.23–3.38 (m, 3H, H-6‴b and –CH_2–), 2.91–3.17 (m, 3H, –CH_2– and H-2′a), 2.62–2.68 (m, 1H, H-2′b), 2.56 (ddd, 1H, *J*=12.5, 4.5, 4.5 Hz, H_{eq}-2″), 1.85–1.89 (m, 4H, –CH_3 and H_{ax}-2″), 1.77 (m, 2H, –CH_2–); ^{13}C NMR (125 MHz, D_2O): δ 171.8 (–CO), 167.4 (C-2), 152.0 (C-4), 141.5 (C-6), 111.6 (C-5), 92.3 (C-1‴), 91.3 (C-1′), 82.9 (C-4′), 73.4 (C-4″), 71.0 (C-4‴ and 6″), 70.5 (C-3‴), 69.1 (C-5″), 57.7 (C-2‴), 54.1 (C-3′), 53.8 (–CH_2–), 49.0 (C-1″), 48.6 (C-3″), 47.8 (C-5‴), 40.6 (C-6‴), 37.4 (–CH_2–), 35.2 (C-2′), 29.0 (C-2″ and –CH_2–), 12.1 (–CH_3). HRESI-MS Calcd for $C_{25}H_{46}N_9O_9$ ($[M+H]^+$): 616.3413, found: 616.3417.

3.10.5. Compound 5e. Yield: 93%, white foam. $[\alpha]_D^{20}$ +23.3 (*c* 0.13, H_2O); ^1H NMR (500 MHz, D_2O): δ 5.52 (d, 1H, *J*=4.0 Hz, H-1‴), 5.38 (t, 1H, *J*=4.5 Hz, H-1′), 4.61 (dd, 1H, *J*=9.5, 5.5 Hz, H-3′), 4.39 (d, 1H, *J*=3.5 Hz, H-4′), 4.09 (dd, 1H, *J*=11.0, 3.5 Hz, H-4″), 4.04 (dd, 1H, *J*= 11.0, 9.0 Hz, H-3‴), 3.89–3.95 (m, 2H, H-5‴ and 6″), 3.65–3.76 (m, 3H, H-5″, 1″, and 3″), 3.30–3.55 (m, 9H, H-6‴a, 6‴b, 2‴, 4‴, –OCH_3, and –CH_2–), 2.93–3.11 (m, 2H, –CH_2–), 2.50 (ddd, 1H, *J*=12.5, 4.5, 4.5 Hz, H_{eq}-2″), 2.09–2.24 (m, 2H, H-2′), 1.79 (q, 1H, *J*=12.5 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, D_2O): δ 174.1 (–CO), 108.0 (C-1′), 92.1 (C-1‴), 85.2 (C-4′), 74.2 (C-3′), 74.1 (C-4″), 71.9 (C-4‴), 71.2 (C-6″), 70.0 (C-3‴), 69.4 (C-5″), 57.1 (C-2‴), 56.9 (–OCH_3), 54.0 (–CH_2–), 49.5 (C-1″), 48.9 (C-3″), 47.9 (C-5‴), 40.7 (C-6‴), 40.4 (–CH_2–), 40.2 (C-2′), 29.4 (C-2″). HRESI-MS Calcd for $C_{20}H_{41}N_6O_9$ ($[M+H]^+$): 509.2930, found: 509.2941.

3.10.6. Compound 7a. Yield: 90%, white foam. $[\alpha]_D^{20}$ +10.7 (*c* 0.15, H_2O). ^1H NMR (500 MHz, D_2O): δ 7.85 (d, 1H, *J*=8.0 Hz, H-6), 5.91 (d, 1H, *J*=8.0 Hz, H-5), 5.82 (d, 1H, *J*=4.0 Hz, H-1‴), 5.69 (d, 1H, *J*=3.0 Hz, H-1′), 4.51–4.57 (m, 2H, H-2′ and 4′), 4.48 (d, 1H, *J*=5.0 Hz, H-3′), 4.28–4.44 (m, 3H, H-4″, 6″, and 5″), 4.02–4.11 (m, 2H, H-3‴ and 5‴), 3.82–3.91 (m, 2H, H-1″ and 3″), 3.56–3.65 (m, 3H, H-2‴, 4‴, and –CH_2–), 3.53 (dd, 1H, *J*=14.0, 3.5 Hz, H-6‴a), 3.25–3.50 (m, 4H, H-6‴b and –CH_2–), 2.62–2.66 (ddd, 1H, *J*=12.5, 4.5, 4.5 Hz, H_{eq}-2″), 1.98–2.09 (m, 3H, H_{ax}-2″ and –CH_2–); ^{13}C NMR (125 MHz, D_2O): δ 173.3 (–CO), 167.0 (C-2), 152.3 (C-4), 144.5 (C-6), 103.1 (C-5), 93.7 (C-1‴), 92.6 (C-1′), 83.1 (C-4′), 73.3 (C-3′), 73.2 (C-2′), 72.0 (C-4″), 71.2 (C-4‴), 70.5 (C-6″), 69.0 (C-3‴), 68.8 (C-5″), 58.6 (C-2‴), 53.6 (–CH_2–), 49.5 (C-1″), 48.9 (C-3″), 47.7 (C-5‴), 40.5 (C-6‴), 36.7 (–CH_2–), 28.8 (C-2″), 27.5 (–CH_2–). HRESI-MS Calcd for $C_{24}H_{43}N_8O_{11}$ ($[M+H]^+$): 619.3045, found: 619.3020.

3.10.7. Compound 7b. Yield: 94%, white foam. $[\alpha]_D^{20}$ +40.4 (*c* 0.28, H_2O). ^1H NMR (500 MHz, D_2O): δ 7.93 (d, 1H, *J*=8.5 Hz, H-6), 5.91 (d, 1H, *J*=8.0 Hz, H-5), 5.86 (d, 1H, *J*=4.5 Hz, H-1‴), 5.63 (d, 1H, *J*=4.0 Hz, H-1′), 4.52 (t, 1H, *J*=5.0 Hz, H-2′), 4.39–4.48 (m, 3H, H-4′, 3′, and 4″), 4.17–4.27 (m, 2H, H-6″ and 5″), 3.99–4.10 (m, 2H, H-3‴ and 5‴), 3.73–3.82 (m, 2H, H-1″ and 3″), 3.57–3.61 (m, 2H, H-2‴ and 4‴), 3.51 (dd, 1H, *J*=14.0, 3.5 Hz, H-6‴a), 3.34–3.41 (m, 2H, H-6‴b and –CH_2–), 3.22–3.31 (m, 3H, –CH_2–), 2.60 (ddd, 1H, *J*=12.5, 4.5, 4.5 Hz, H_{eq}-2″), 1.95 (q, 1H, *J*=12.5 Hz, H_{ax}-2″), 1.72–1.80 (m, 2H, –CH_2–), 1.58–1.64 (m, 2H, –CH_2–); ^{13}C NMR (125 MHz, D_2O): δ 172.2 (–CO), 166.9 (C-2), 152.3 (C-4), 144.0 (C-6), 103.1 (C-5), 92.6 (C-1‴ and C-1′), 83.3 (C-4′), 73.2 (C-3′), 73.1 (C-2′), 72.5 (C-4″), 71.1 (C-4‴), 70.7 (C-6″), 68.9 (C-3‴ and C-5″), 58.7 (C-2‴), 53.6 (–CH_2–), 52.0 (C-1″), 49.0 (C-3″), 47.7 (C-5‴), 40.5 (C-6‴), 39.2 (–CH_2–), 28.8 (C-2″), 26.6 (–CH_2–), 24.9 (–CH_2–). HRESI-MS Calcd for $C_{25}H_{45}N_8O_{11}$ ($[M+H]^+$): 633.3202, found: 633.3190.

3.10.8. Compound 7c. Yield: 83%, white foam. $[\alpha]_D^{20}$ +33.3 (*c* 0.28, H_2O); ^1H NMR (500 MHz, D_2O): δ 5.63 (d, 1H, *J*=4.0 Hz, H-1‴), 5.02 (s, 1H, H-1′), 4.38–4.42 (m, 2H, H-2′ and 3′), 4.26 (dd, 1H, *J*=10.5, 3.5 Hz, H-4″), 4.05–4.13 (m, 4H, H-5″, 6″, 3‴, and 4′), 3.94–3.98 (m, 1H, H-5‴), 3.74–3.84 (m, 2H, H-1″ and 3″), 3.45–3.60 (m, 9H, H-6‴a, 6‴b, 2‴, 4‴, –OCH_3, and –CH_2–), 3.18–3.37 (m, 2H, –CH_2–), 2.59 (ddd, 1H, *J*=12.5, 4.5, 4.5 Hz, H_{eq}-2″), 1.92 (q, 1H, *J*=12.5 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, D_2O) δ 175.8 (–CO), 109.9 (C-1′), 91.9 (C-1‴), 81.0 (C-4′), 74.6 (C-3′), 74.2 (C-2′), 72.5 (C-4″), 70.8 (C-4‴), 70.4 (C-3‴ and 6″), 69.3 (C-5″), 57.3 (C-2‴), 57.0 (–OCH_3), 53.8

(–CH_2–), 50.7 (C-1″), 48.8 (C-3″), 47.7 (C-5‴), 40.6 (C-6‴), 39.7 (–CH_2–), 29.0 (C-2″). HRESI-MS Calcd for $C_{20}H_{41}N_6O_{10}$ ([M+H]$^+$): 525.2879, found: 525.2881.

3.10.9. Compound 10a. Yield: 85%, white foam. $[\alpha]_D^{20}$ +20.3 (*c* 0.25, H_2O). ^{1}H NMR (500 MHz, D_2O): δ 7.63 (s, 1H, H-6), 6.25 (t, 1H, *J*=7.0 Hz, H-1′), 5.63 (d, 1H, *J*=3.5 Hz, H-1‴), 4.72–4.74 (m, 1H, H-3′), 4.39 (d, 1H, *J*=3.5 Hz, H-4′), 4.06–4.10 (m, 1H, H-5‴), 3.90–3.96 (m, 2H, H-3‴ and 6″), 3.77 (t, 1H, *J*=10.5 Hz, H-4″), 3.41–3.52 (m, 5H, H-2‴, 4‴, 6‴a, 1″, and –CH_2–), 3.26–3.35 (m, 3H, H-6‴b, 3″, and –CH_2–), 2.84–2.93 (m, 3H, –CH_2– and H-5″), 2.60–2.65 (m, 1H, H-2′), 2.34–2.44 (m, 2H, H_{eq}-2″ and H-2′), 1.90 (s, 3H, CH_3), 1.75 (q, 1H, *J*=12.5 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, D_2O): δ 173.4 (–CO), 167.3 (C-2), 152.5 (C-4), 140.2 (C-6), 112.0 (C-5), 97.9 (C-1‴), 89.1 (C-1′), 85.8 (C-4′), 78.1 (C-6″), 74.3 (C-3′), 71.4 (C-4‴), 70.1 (C-3‴), 69.8 (C-5‴), 69.4 (C-4″), 63.3 (C-5″), 54.4 (C-2‴), 52.1 (C-3″), 50.7 (C-1″), 42.1 (–CH_2–), 40.9 (C-6‴), 39.5 (–CH_2–), 37.9 (C-2′), 29.7 (C-2″), 12.2 (–CH_3). HRESI-MS Calcd for $C_{24}H_{43}N_8O_{10}$ ([M+H]$^+$): 603.3096, found: 603.3093.

3.10.10. Compound 10b. Yield: 90%, white foam. $[\alpha]_D^{20}$ +21.1 (*c* 0.205, H_2O). ^{1}H NMR (500 MHz, D_2O): δ 7.66 (s, 1H, H-6), 6.26 (t, 1H, *J*=7.0 Hz, H-1′), 5.71 (d, 1H, *J*=3.0 Hz, H-1‴), 4.74–4.76 (m, 1H, H-3′), 4.43 (d, 1H, *J*=3.5 Hz, H-4′), 4.36–4.40 (m, 1H, H-5‴), 4.03–4.17 (m, 2H, H-3‴ and 6″), 3.61–3.79 (m, 5H, H-4″, 2‴, 4‴, 6‴a, and 1″), 3.49–3.56 (m, 1H, H-3″), 3.25–3.42 (m, 6H, H-6‴b, –CH_2–, –CH_2–, and 5″), 2.58–2.64 (m, 2H, H_{eq}-2″ and 2′), 2.35–2.40 (m, 1H, H-2′), 1.97–2.04 (m, 3H, H_{ax}-2″ and –CH_2–), 1.90 (s, 3H, –CH_3); ^{13}C NMR (125 MHz, D_2O): δ 173.5 (–CO), 167.3 (C-2), 152.5 (C-4), 140.1 (C-6), 112.0 (C-5), 94.6 (C-1‴), 89.0 (C-1′), 85.8 (C-4′), 76.1 (C-6″), 74.8 (C-3′), 74.4 (C-4‴), 69.0 (C-3‴), 68.5 (C-5‴), 67.8 (C-4″), 62.1 (C-5″), 52.6 (C-2‴), 51.1 (C-3″), 50.8 (C-1″), 43.2 (–CH_2–), 39.7 (C-6‴), 38.1 (–CH_2–), 36.9 (C-2′), 28.9 (C-2″), 27.0 (–CH_2–), 12.3 (–CH_3). HRESI-MS Calcd for $C_{25}H_{45}N_8O_{10}$ ([M+H]$^+$): 617.3253, found: 617.3238.

3.10.11. Compound 10c. Yield: 90%, white foam. $[\alpha]_D^{20}$ +25.6 (*c* 0.27, H_2O). ^{1}H NMR (500 MHz, D_2O): δ 7.70 (s, 1H, H-6), 6.31 (t, 1H, *J*=7.0 Hz, H-1′), 5.72 (s, 1H, H-1‴), 4.69–4.74 (m, 1H, H-3′), 4.38–4.41 (m, 2H, H-4′ and H-5‴), 4.16 (t, 1H, *J*=7.0 Hz, H-3‴), 4.06 (t, 1H, *J*=10.5 Hz, H-6″), 3.50–3.81 (m, 6H, H-4″, 2‴, 4‴, 6‴a 1″, and 3″), 3.38 (dd, 1H, *J*=14.5, 3.5 Hz, H-6‴b), 3.22–3.31 (m, 5H, –CH_2CH_2– and 5″), 2.54–2.63 (m, 2H, H_{eq}-2″ and 2′), 2.35–2.40 (m, 1H, H-2′), 2.03 (q, 1H, *J*=12.5 Hz, H_{ax}-2″), 1.91 (s, 3H, –CH_3), 1.62–1.82 (m, 4H, –CH_2CH_2–); ^{13}C NMR (125 MHz, D_2O): δ 172.8 (–CO), 167.3 (C-2), 152.5 (C-4), 139.7 (C-6), 112.0 (C-5), 94.6 (C-1‴), 88.5 (C-1′), 85.8 (C-4′), 76.1 (C-6″), 74.8 (C-3′), 74.4 (C-4‴), 69.0 (C-3‴), 68.6 (C-5‴), 67.6 (C-4″), 62.2 (C-5″), 52.6 (C-2‴), 51.1 (C-3″), 50.8 (C-1″), 44.9 (–CH_2–), 39.7 (C-6‴), 39.1 (–CH_2–), 38.0 (C-2′), 28.9 (C-2″), 26.5 (–CH_2–), 24.3 (–CH_2–), 12.3 (–CH_3). HRESI-MS Calcd for $C_{26}H_{46}N_8O_{10}$ ([M+H]$^+$): 631.3410, found: 631.3419.

3.10.12. Compound 12. Yield: 93%, white foam. $[\alpha]_D^{20}$ +28.4 (*c* 0.095, H_2O). ^{1}H NMR (500 MHz, D_2O): δ 7.87 (dd, 1H, *J*=8.0, 0.5 Hz, H-6), 5.91 (dd, 1H, *J*=8.0, 0.5 Hz, H-5), 5.84 (d, 1H, *J*=4.5 Hz, H-1‴), 5.71 (d, 1H, *J*=4.0 Hz, H-1′), 4.54–4.57 (m, 1H, H-2′), 4.48–4.49 (m, 2H, H-4′ and 3′), 4.24 (t, 1H, *J*=10.5 Hz, H-3‴), 4.17 (m, 1H, H-5‴), 4.01 (t, 1H, *J*=9.5 Hz, H-6″), 3.88 (t, 1H, *J*=10.5 Hz, H-4″), 3.37–3.63 (m, 8H, H-2‴, 4‴, 6‴a, 6‴b, 1″, 3″, and –CH_2–), 3.01–3.14 (m, 3H, –CH_2– and H-5″), 2.51–2.55 (m, 1H, H_{eq}-2″), 1.90 (q, 1H, *J*=12.5 Hz, H_{ax}-2″); ^{13}C NMR (125 MHz, D_2O) δ 173.0 (–CO), 166.9 (C-2), 152.4 (C-4), 144.4 (C-6), 103.2 (C-5), 97.0 (C-1‴), 93.1 (C-1′), 83.3 (C-4′), 76.4 (C-6″), 73.1 (C-3′), 72.8 (C-2′), 71.3 (C-4‴), 70.8 (C-3‴), 69.3 (C-5‴), 68.9 (C-4″), 63.1 (C-5″), 53.8 (C-2‴), 51.8 (C-3″), 50.7 (C-1″), 42.7 (–CH_2–), 40.6 (C-6‴), 39.2 (–CH_2–), 28.7 (C-2″). HRESI-MS Calcd for $C_{23}H_{41}N_8O_{11}$ ([M+H]$^+$): 605.2889, found: 605.2873.

3.11. 3′-Azido-3′-deoxy-thymidine-5′-carboxylic acid (13b)

[Bis(acetoxy)-iodo]benzene (1.78 mg, 5.52 mmol), 2,2,6,6-tetramethyl-1-piperidinyloxyl (90 mg, 0.58 mmol), and **AZT** (2′,3′-deoxy-3′-azido-thymidine) (0.74 g, 2.78 mmol) were combined in a reaction vessel, and to this mixture was added 5 mL of a 1:1 acetonitrile/water solution. The reaction mixtures were stirred for 24 h. Upon completion, the solvent was removed and the residue was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH=50/1) to afford a yellow foam, which was dissolved in CH_2Cl_2 to precipitate. The resulting white precipitate (**13b**) was filtered and dried in vacuo (600 mg, 76.6%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.52 (br s, 1H, –COOH), 11.36 (s, 1H, –NH), 7.85 (s, 1H, H-6), 6.18 (t, 1H, *J*=7.0 Hz, H-1′), 4.74–4.77 (m, 1H, H-3′), 4.43 (d, 1H, *J*=4.0 Hz, H-4′), 2.30–2.33 (m, 2H, H-2′), 1.78 (d, 3H, *J*=1.0 Hz, –CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.2 (–COOH), 163.7 (C-2), 150.4 (C-4), 136.4 (C-6), 109.5 (C-5), 85.0 (C-1′), 81.0 (C-4′), 63.0 (C-3′), 35.6 (C-2′), 12.3 (–CH_3). Anal. Calcd for $C_{10}H_{11}N_5O_5$: C, 42.71; H, 3.94; N, 24.90. Found: C, 42.71; H, 3.885; N 24.69.

3.12. Methyl 2,3-di-*O*-acetyl-β-D-ribofuranoside (16)

Compound **15** (1.60 g, 5.51 mmol) was dissolved into a solution of I_2 (1.00 g, 3.95 mmol) in methanol (100 mL). The solution was heated to 80 °C. Upon completion, a small quantity of sodium thiosulfate was added to quench the reaction. The solvent was removed and the residue was purified by column chromatography on silica gel (petroleum ether/EtOAc=2.5/1) to afford **16** as a yellow syrup (0.45 g, 33%). For ^{1}H NMR data, see Ref. 23.

3.13. Methyl 2,3-di-*O*-acetyl-β-D-ribofuranoside-5-carboxylic acid (17)

[Bis(acetoxy)-iodo]benzene (1.17 g, 3.62 mmol), 2,2,6,6-tetramethyl-1-piperidinyloxyl (57 mg, 0.36 mmol), and **16** (0.45 g, 1.81 mmol) were combined in a reaction vessel, and to this mixture was added 4 mL of a 1:1 acetonitrile/water solution. The reaction mixtures were stirred for 24 h and the solvent was removed. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc=2.5/1) to give **17** as a colorless syrup (380 mg, 80%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.24 (br s, 1H, –COOH), 5.45 (t, 1H, *J*=5.5 Hz, H-3), 5.05 (d, 1H, *J*=5.5 Hz, H-2), 5.00 (s, 1H, H-1), 4.49 (d, 1H, *J*=5.5 Hz, H-4), 3.33 (s, 3H, –OCH_3),

2.07 (s, 3H, –$COCH_3$), 2.04 (s, 3H, –$COCH_3$); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.4 (–COOH), 169.4 (2$COCH_3$), 105.8 (C-1), 78.1 (C-4), 73.8 (C-2), 72.9 (C-3), 54.7 (–OCH_3), 20.3 ($COCH_3$), 20.2 ($COCH_3$). HRESI-MS Calcd for $C_{10}H_{14}O_8K$ ($[M+K]^+$): 301.0320, found: 301.0317.

3.14. Methyl 2-deoxy-5-*O*-triphenylmethyl-3-*O*-acetyl-β-D-ribofuranoside (19)

To a solution of compound **18** (440 mg, 1.127 mmol) in anhydrous pyridine (15 mL) were added 4-dimethylamino pyridine (53 mg) and acetic anhydride (0.25 mL, 2.25 mmol, 2 equiv) in ice bath. After 3 h, the reaction was quenched with CH_3OH and concentrated in vacuo. The residue was diluted with CH_2Cl_2 (150 mL) and washed with saturated $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc=10/1) to afford **19** as a colorless syrup (485 mg, 1.12 mmol, 100%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.21–7.48 (m, 15H, arom), 5.21–5.24 (m, 1H, H-3), 5.12 (dd, 1H, J=5.0, 2.5 Hz, H-1), 4.15–4.18 (m, 1H, H-4), 3.29 (s, 3H, –OCH_3), 3.20–3.26 (m, 2H, H-5 and 5′), 2.27–2.32 (m, 1H, H-2), 2.06–2.10 (m, 1H, H-2′), 2.03 (s, 3H, –$COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4 (–$COCH_3$), 143.9, 128.7, 127.8, 127.0 (trityl), 105.5, 86.7, 82.9, 75.5, 64.7, 55.3, 38.9, 21.1 (–$COCH_3$). Anal. Calcd for $C_{27}H_{28}O_5$: C, 74.98; H, 6.53. Found: C, 74.71; H, 6.425.

3.15. Methyl 3-*O*-acetyl-2-deoxy-β-D-ribofuranoside (20)

Compound **19** (460 mg, 1.06 mmol) was dissolved into a solution of I_2 (100 mg, 0.39 mmol) in methanol (10 mL). The procedure was similar as compound **16** except the temperature of 60 °C. Compound **20** was given as a colorless syrup (70 mg, 34.6%) and its α isomer was given as a byproduct (90 mg, 44.5%). For ^1H NMR data, see Ref. 23.

3.16. Methyl 3-*O*-acetyl-2-deoxy-β-D-ribofuranoside-5-carboxylic acid (21)

[Bis(acetoxy)-iodo]benzene (237 mg, 0.736 mmol), 2,2,6,6-tetramethyl-1-piperidinyloxyl (11.5 mg, 0.074 mmol), and **20** (70 mg, 0.368 mmol) were combined in a reaction vessel, and the procedure was similar to that of compound **17**. Compound **21** was given as a colorless syrup (56.4 mg, 75%). ^1H NMR (500 MHz, DMSO-d_6): δ 12.90 (br s, 1H, –COOH), 5.48–5.51 (m, 1H, H-3), 5.16 (dd, 1H, J=5.0, 2.0 Hz, H-1), 4.47 (d, 1H, J=3.0 Hz, H-4), 3.27 (s, 3H, –OCH_3), 2.18–2.23 (m, 1H, H-2), 2.04–2.07 (m, 1H, H-2′), 2.02 (s, 3H, –$COCH_3$); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.4, 169.9 (CO), 105.3 (C-1), 80.4 (C-4), 75.3 (C-3), 54.7 (–OCH_3), 38.8 (C-2), 20.7 (C-2). Anal. Calcd for $C_8H_{12}O_6$: C, 47.06; H, 5.92. Found: C, 46.80; H, 5.95.

3.17. General for SPR evaluation

The results were obtained at BIAcore 3000 instrument. Streptavidin-functionalized BIAcore sensorchips were prepared from carboxymethylated sensorchips (CM5, BIAcore) by EDC activation followed by injection of streptavidin (Sigma, salt-free) in acetate buffer (10 mM, pH 4.5). Biotinylated 16S RNA was bought from GenePharma, Shanghai.

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