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Synthesis of neamine–carboline conjugates for RNA binding and their antibacterial activities

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ABSTRACT

Three types of neamine-β-carboline conjugates were synthesized in good yields by the coupling of neamine and β-carboline-3-carboxylic acids using aliphatic diamine as a linker. The binding properties of these conjugates to 16S rRNA and 18S rRNA were evaluated by surface plasmon resonance (SPR), showing that some conjugates had stronger binding affinities than neamine. In vitro antimicrobial activities were also evaluated and the results showed that some synthetic compounds exhibited better antibacterial activities than neamine. The preliminary structure–activity relationship was discussed. The present experimental data demonstrated that synthetic neamine–carboline conjugates might hold the potential as new antibiotics.

1. Introduction

Aminoglycosides have been long used as potent broad-spectrum antibiotics for the treatment of infections caused by aerobic Gram-negative and Gram-positive bacteria. Previous studies showed that aminoglycosides could bind to various RNA targets[1–6] such as rRNA, tRNA and mRNA. However, aminoglycosides are not ideal antibiotics due to their toxicities and adverse effects caused by the lack of selectivity towards different RNA targets. That caused sharply increasing interests in understanding the interactions between RNA and ligands, resulting in the escalating number of research works such as studying the three dimensional structures of RNA–ligand complexes.[7] Therefore, many efforts[8–12] have been made to design and synthesize specific RNA binders, which may have potential activities against bacteria or virus infections. However, there is not a solid way to design molecules targeting RNA because RNA can form intricate structures. Previous work disclosed that neamine is the minimal consensus unit[7,13] of aminoglycoside antibiotics bound to A-site, TAR RNA, or RRE IIB RNA. Since aminoglycosides possess amino and hydroxyl functionalities and are positively charged around neutral pH condition, which contribute to the binding with RNA by hydrogen bonding and electrostatic interactions, most of the previous efforts tended to strengthen the RNA binding affinities by increasing the number of hydrogen bonding or electrostatic interactions. It turned out to have limited improvements. On the other hand, some aromatic compounds[14] can act as stackers or intercalators to interact with either double-stranded RNA or DNA. But polyaromatic hydrocarbons may have potential carcinogenic activity. The β-carboline[15,16] ring system is present in many naturally occurring alkaloids, among which β-carboline-3-carboxylic acid occurs in *Symplocos serchuensis*, a food plant indigenous to the south of China. We assumed that the combination of aminoglycoside and β-carboline might enhance both the affinity and specificity to RNA. Furthermore, it can also decrease the polarity of aminoglycoside derivatives and improve absorption characteristics of the designed compounds. We hereby report the synthesis of some neamine-β-carboline conjugates, their RNA binding affinities, and their antibacterial activities.

2. Results and discussion

2.1. Chemistry

The neamine structure and carboline moiety were connected by a flexible tether. Neamine (**1**), β-carboline-3-carboxylic acid derivatives **4a**, **4b** and **5** were obtained according to the known procedure.[17–19] As shown in Scheme 1, neamine was treated with triflyl azide[20] in the presence of $CuSO_4$ and triethylamine to produce tetraazidoneamine, which was followed by benzoylation, providing compound **2** in 54% isolated yield. Compound **2** was reacted with trifluoromethanesulfonic anhydride, providing the triflate intermediate, which was subsequently converted to compounds

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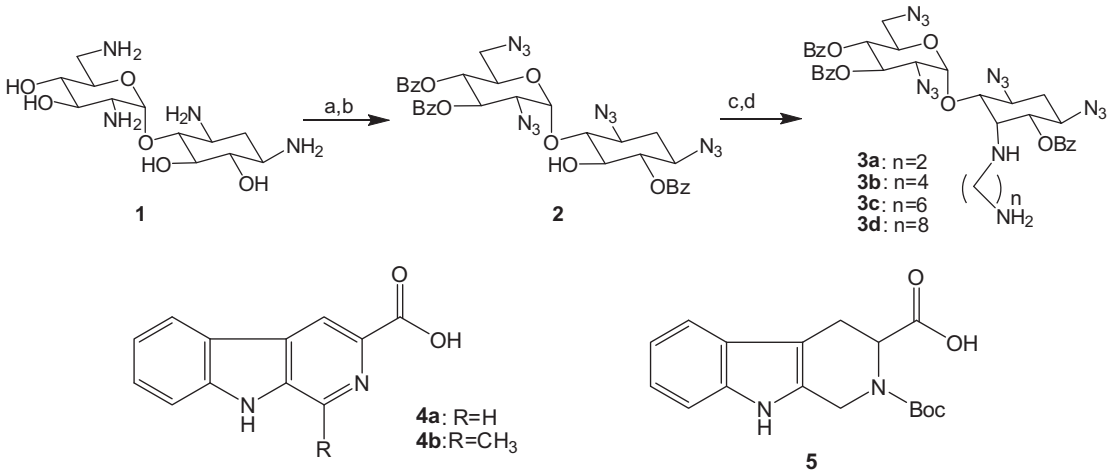


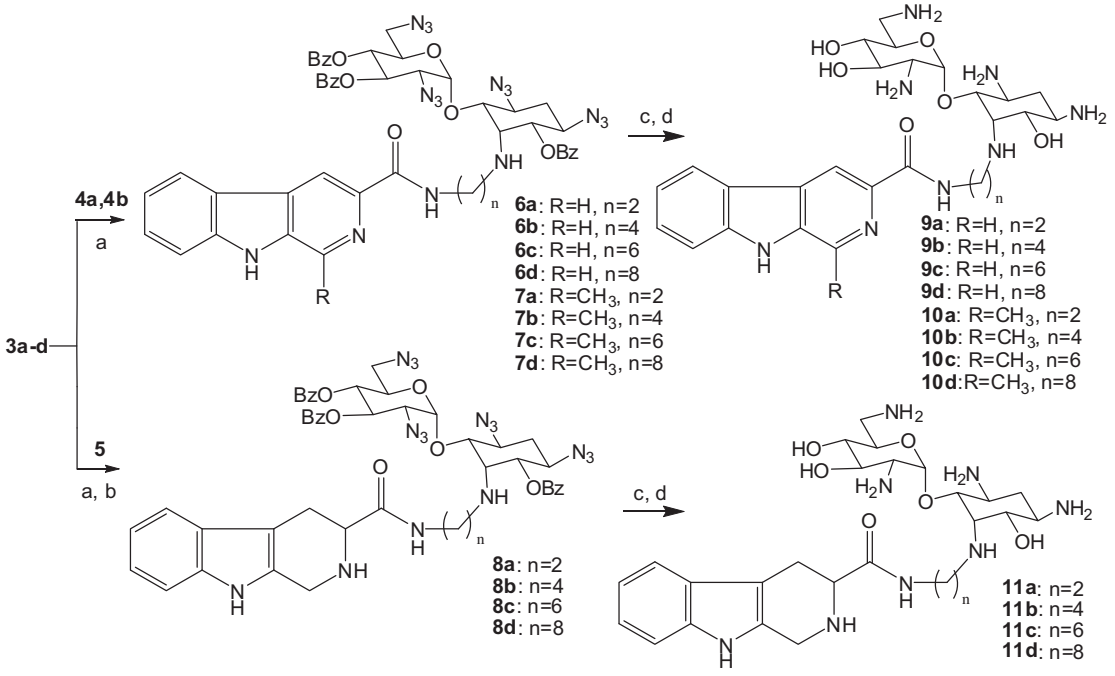
Scheme 1. Synthesis of compounds **3a–d** and the structures of compounds **4a**, **4b**, **5**. Reagents and conditions: (a) TfN_3, $CuSO_4$, Et_3N, CH_3CN; (b) benzoyl chloride, pyridine, 54%; (c) Tf_2O, pyridine, CH_2Cl_2; (d) $NH_2(CH_2)_nNH_2$, n=2,4,6,8, CH_3CN, 40% for **3a**, 48% for **3b**, 47% for **3c**, 47% for **3d**.

3a–d via amination using the corresponding aliphatic diamines. It is noteworthy that the high regioselectivity was achieved when the hydroxyl groups of neamine were protected with benzoate instead of acetate that was previously used in the literatures, leading to the 5-hydroxyl group of compound **2** exposed. Moreover, with more robust protective group (benzoyl vs acetyl), the side reaction between esters and diamines was alleviated, resulting in the increased overall yield of compounds **3a–d**.

With the amino-containing neamine derivatives **3a–d** and carboline carboxylic acid derivatives **4a**, **4b**, **5** in hand, the coupling reaction was carried out (Scheme 2). Compounds **6a–d** and **7a–d** were obtained by the coupling of amines **3a–d** and acids **4a**, **4b**. In the same way, compound **5** was condensed with **3a–d** to yield the corresponding amide products, which were followed by Boc-deprotection under acidic conditions at room temperature to produce **8a–d**. Due to the poor solubility of carboline carboxylic acids, the coupling reaction was performed in DMF solution. The amide-bond formation was efficient when 1-hydroxybenzotriazole (HOBT) and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (TBTU) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) were used, and the reaction was completed very soon after the addition of DIPEA was finished, showing a single product spot on TLC, which greatly facilitated the product purification. Finally, compounds **6a–d**, **7a–d** and **8a–d** were saponificated with sodium methoxide in methanol, and followed by reduction with H_2S to provide the target compounds **9a–d**, **10a–d** and **11a–d**, respectively. The structures of all of the final products and intermediates were identified by their ^1H NMR, ^{13}C NMR and high resolution mass spectra.

2.2. Bioassay

2.2.1. RNA binding affinities of the neamine–carboline conjugates. The interactions between the synthetic compounds and RNA were evaluated by surface plasmon resonance (SPR) assay[21]



Scheme 2. Synthesis of neamine–carboline conjugates **9a–d**, **10a–d**, **11a–d**. Reagents and conditions: (a) TBTU, HOBT, DIPEA, DMF; 65% for **6a**, 70% for **6b**, 50% for **6c**, 74% for **6d**, 67% for **7a**, 60% for **7b**, 66% for **7c**, 79% for **7d**; (b) HCl/ethyl acetate, 37% for **8a**, 44% for **8b**, 75% for **8c**, 83% for **8d**; (c) CH_3OH/CH_3ONa; (d) H_2S, $Py/Et_3N/H_2O$, 70%.

with *Escherichia coli* 16S rRNA, which is implicative of the antibiotic activity, and human 18S rRNA, which may reflect the toxicity to mammals. Their RNA binding properties were analyzed and characterized by the dissociation constants (K_d values in μM), which were calculated from the slope of the Scatchard plot,[22] and the resulting values are outlined in Table 1.

Table 1
The interaction between compounds **9a–d**, **10a–d**, **11a–d** and 16S/18S rRNA

Compound	K_d (16S rRNA) (μM)	K_d (18S rRNA) (μM)
9a	1.9	3.2
9b	N/A	N/A
9c	N/A	N/A
9d	1.9	3.4
10a	3.6	6.9
10b	3.5	7.3
10c	3.8	9.6
10d	3.5	6.8
11a	N/A	N/A
11b	N/A	N/A
11c	N/A	N/A
11d	N/A	N/A
Neamine	22	34

N/A: not available.

As shown in Table 1, the neamine–carboline conjugates **9a**, **9d** and **10a–d** showed significantly stronger binding affinities to 16S rRNA and 18S rRNA than that of neamine. The results indicated that the total aromatic carboline ring made an important contribution to the RNA binding affinities. Compounds **11a–d**, in which the aromatic carboline ring was replaced by the tetrahydrocarboline ring, displayed a sharp decrease of the binding abilities and they all showed no binding activities. When the methyl group was introduced to the aromatic ring (compounds **10a–d**), the binding affinities to 16S rRNA and 18S rRNA were decreased slightly. It seemed that the length of the aliphatic amine linkers also influenced the binding activities. It is interesting that compounds **9a** and **9d**, with the length of 2- or 8-carbon chain, have shown the strongest binding affinities, presumably due to the insertion of the aromatic ring into different base pairs of RNA.

2.2.2. Functional inhibition of bacterial growth. The antibacterial activities of the synthetic neamine–carboline conjugates were further evaluated in vitro against *Pseudomonas aeruginosa* standard strain with neamine as a positive control.[23] The results are outlined in Table 2. The rate of inhibition for each compound was tested at the concentration of 500 μg/mL. For those compounds showing good inhibitory activities such as **9a**, **9d** and **10d**, the corresponding IC_{50} values were measured. The data obtained in the test indicated that among all of the synthetic compounds, **9d** displayed the strongest antibacterial activity. From the result that compounds **9d** and **10d** had stronger activities than compounds **9a** and **10a**, we might reason the longer flexible linker could also play an important role for the antibacterial activity. Compounds **10a–c** did not show significant antibacterial activities as supposed to have from their RNA binding data, indicating that the antibacterial activity is not always correlative with the binding affinity.

Table 2
The inhibition effects on *P. aeruginosa* strain in vitro

Compound	IC_{50} (μg/mL)	Rate of inhibition[a] (%)
9a	312.5	
9b		29
9c		29
9d	85.4	
10a		30
10b		31
10c		31
10d	145.8	
11a		30
11b		31
11c		30
11d		18
Neamine	343.8	

[a] Rate of inhibition was measured at the concentration of 500 μg/mL.

3. Conclusions

In summary, some neamine-β-carboline conjugates **9a–d**, **10a–d** and **11a–d** were synthesized using aliphatic diamine as the linker. Their binding affinities to *E. coli* 16S rRNA and human 18S rRNA were tested using SPR method, suggesting that the synthetic conjugates **9a**, **9d**, **10a–d** had stronger RNA binding affinities than neamine. Their antibacterial activities were further evaluated in vitro against *P. aeruginosa* standard strain. The results showed that compounds **9a**, **9d**, **10d** displayed better antibacterial activities than neamine, and especially compound **9d** displayed the strongest antibacterial effect. The disclosed approach may lead to the discovery of new antibiotic entities with improved biological activities.

4. Experimental section

4.1. Chemistry

4.1.1. General information. All chemicals were purchased as reagent grade and used without further purification, unless otherwise noted. Dichloromethane (CH_2Cl_2), pyridine and acetonitrile (CH_3CN) were distilled over calcium hydride (CaH_2). Methanol was distilled from magnesium. DMF was stirred with CaH_2 and distilled under reduced pressure. Reactions were monitored with analytical TLC on silica gel 60-F_{254} precoated aluminium 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). ^{1}H NMR spectra were recorded on a JEOL AL-300 or Varian INOVA-500 spectrometer at 25 °C. Chemical shifts (in ppm) were referenced to tetramethylsilane (δ=0 ppm) in deuterated chloroform. ^{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.

*4.1.2. Preparation of compound **2**.* To a stirred suspension of NaN_3 (2.0 g, 30.8 mmol) in acetonitrile (20 mL) was added Tf_2O (4.3 mL, 7.3 g, 25.8 mmol) by syringe slowly at 0 °C. The mixture was stirred for another 2 h at this temperature. The insoluble solids were removed through filtration. At 0 °C, the filtrate was added dropwise into the mixture of neamine (2.0 g, 6.2 mmol), $CuSO_4$ (29 mg), H_2O (6 mL) and Et_3N (3.6 mL). The reaction mixture was stirred for 6 h at room temperature. The mixture was diluted with EtOAc and washed with brine. The organic layer was dried over Na_2SO_4 and evaporated to yield brown solids. To the solution of these solids in pyridine (5 mL), benzoyl chloride (3.0 mL, 3.63 g, 25.9 mmol) was added at 0 °C. The reaction mixture was stirred for 5 h at room temperature. The solvent was removed under reduced pressure. The residue was subjected to column chromatography on silica gel (petroleum ether/ethyl acetate 4:1) to afford compound **2** as a white foam (2.5 g, 54% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.09–8.11 (m, 2H), 7.91–7.93 (m, 4H), 7.58–7.61 (m, 1H), 7.46–7.54 (m, 4H), 7.35–7.39 (m, 4H), 5.97 (dd, J=9.5, 10.5 Hz, 1H), 5.58 (d, J=4.0 Hz, 1H, H1′), 5.48 (t, J=9.8 Hz, 1H), 5.22 (t, J=9.8 Hz, 1H), 4.55 (m, 1H), 3.92 (td, J=3.5, 9.3 Hz, 1H), 3.80 (dd, J=3.5, 10.5 Hz, 1H), 3.71–3.76 (m, 1H), 3.61–3.65 (m, 2H), 3.53–3.58 (m, 1H), 3.49 (dd, J=3.0, 13.5 Hz, 1H), 3.42 (dd, J=5.5, 9.0 Hz, 1H), 2.51 (dt, J_1=13.0 Hz, J_2=J_3=5.0 Hz, 1H, $H2_{eq}$), 1.75 (q, J=13.0 Hz, 1H, $H2_{ax}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.15, 165.67, 165.28, 133.61, 133.54, 129.99, 129.87, 129.80, 128.98, 128.63, 128.52,

128.46, 128.44, 98.94, 83.23, 75.63, 74.83, 71.01, 69.99, 69.67, 62.12, 58.49, 58.26, 50.96, 32.08. HRMS (ESI) for $C_{33}H_{30}N_{12}O_9$ calcd 761.2151 [$(M+Na)^+$]; found: 761.2166.

*4.1.3. General procedure for the preparation of compounds **3a–d**.* Compound **2** (1.0 g, 1.35 mmol) was dissolved in dichloromethane (5 mL), followed by the addition of pyridine (1 mL). Tf_2O (0.5 mL, 0.84 g, 3.00 mmol) was slowly added for 1.5 h with stirring at 0 °C. The mixture was stirred for another 2.5 h. The reaction mixture was diluted with ethyl acetate and washed with brine. The organic layer was collected and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 8:1) to afford a yellow foam. This yellow foam was dissolved in acetonitrile (6 mL). Diamine (4.05 mmol) was added and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with dichloromethane and washed with brine. The organic layer was concentrated and the residue was subjected to column chromatography on silica gel to give products.

*4.1.3.1. Compound **3a**.* White foam, 40% yield, chloroform/methanol 30:1 as eluent: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16–8.19 (m, 2H), 7.87–7.92 (m, 4H), 7.43–7.52 (m, 5H), 7.26–7.36 (m, 4H), 5.99 (t, J=9.9 Hz, 1H), 5.52 (t, J=9.8 Hz, 1H), 5.21 (d, J=3.6 Hz, 1H, H1′), 5.02 (dd, J=2.1, 10.5 Hz, 1H), 4.70–4.76 (m, 1H), 4.48–4.54 (m, 1H), 4.27–4.36 (m, 1H), 3.86 (dd, J=3.3, 10.8 Hz, 2H), 3.72 (t, 1H, H5), 3.54 (dd, J=2.7, 13.5 Hz, 1H), 3.40 (dd, J=5.1, 13.5 Hz, 1H), 2.99–3.11 (m, 3H), 2.82–2.96 (m, 1H), 2.51 (dt, J=4.8, 12.9 Hz, 1H, $H2_{eq}$), 1.47 (q, J=12.3 Hz, 1H, $H2_{ax}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.53, 165.38, 165.24, 133.60, 133.41, 130.05, 129.86, 129.75, 128.79, 128.54, 128.44, 128.35, 94.46, 78.10, 70.93, 70.08, 69.33, 61.75, 57.55, 56.36, 56.07, 50.73, 41.12, 32.62. HRMS (ESI) for $C_{35}H_{36}N_{14}O_8$ calcd 781.2913 [$(M+H)^+$]; found: 781.2899.

*4.1.3.2. Compound **3b**.* White foam, 48% yield, dichloromethane/methanol 25:1 as eluent: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08–8.09 (m, 2H), 7.91–7.94 (m, 4H), 7.60–7.62 (m, 1H), 7.48–7.53 (m, 4H), 7.35–7.39 (m, 4H), 5.97 (dd, J=9.5, 10.5 Hz, 1H), 5.54 (t, J=9.8 Hz, 1H), 5.20 (d, J=4.0 Hz, 1H, H1′), 4.94 (dd, J=2.5, 10.5 Hz, 1H), 4.49–4.52 (m, 1H), 4.37–4.42 (m, 1H), 4.08–4.14 (m, 1H), 3.81–3.87 (m, 2H), 3.67 (t, J=2.8 Hz, 1H, H5), 3.54 (dd, J=2.5, 10.5 Hz, 1H), 3.41 (dd, J=5.5, 14.0 Hz, 1H), 2.84–2.89 (m, 1H), 2.69–2.71 (m, 2H), 2.44–2.55 (m, 4H), 1.40–1.56 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.65, 165.49, 165.23, 133.63, 133.55, 129.87, 129.78, 129.00, 128.65, 128.46, 128.42, 94.20, 77.72, 77.25, 70.91, 70.07, 69.27, 61.76, 57.68, 56.58, 56.10, 50.74, 50.52, 41.58, 32.55, 30.53, 27.90. HRMS (ESI) for $C_{37}H_{40}N_{14}O_8$ calcd 809.3226 [$(M+H)^+$]; found: 809.3233.

*4.1.3.3. Compound **3c**.* White foam, 47% yield, dichloromethane/methanol 15:1 as eluent: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05–8.07 (m, 2H), 7.90–7.92 (m, 4H), 7.56–7.60 (m, 1H), 7.44–7.52 (m, 4H), 7.32–7.38 (m, 4H), 5.98 (dd, J=9.0, 10.0 Hz, 1H), 5.52 (t, J=9.8 Hz, 1H), 5.19 (d, J=4.0 Hz, 1H, H1′), 4.91 (dd, J=2.5, 10.5 Hz, 1H), 4.45–4.49 (m, 1H), 4.33–4.38 (m, 1H), 4.05–4.10 (m, 1H), 3.80–3.85 (m, 2H), 3.68 (t, J=3.0 Hz, 1H, H5), 3.53 (dd, J=2.5, 10.5 Hz, 1H), 3.41 (dd, J=5.0, 10.5 Hz, 1H), 2.85 (t, J=7.8 Hz, 2H), 2.74–2.79 (m, 1H), 2.48–2.53 (m, 1H), 2.43 (dt, J=5.0, 13.5 Hz, 1H), 1.53–1.57 (m, 2H), 1.36–1.45 (m, 3H), 1.19–1.26 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.68, 165.55, 165.26, 133.72, 133.68, 133.57, 129.89, 129.79, 129.09, 128.66, 128.60, 128.51, 128.46, 93.93, 77.42, 77.32, 70.91, 69.98, 69.44, 61.53, 57.85, 56.67, 55.66, 50.81, 50.37, 40.41, 32.59, 30.16, 27.25, 26.50, 25.90. HRMS (ESI) for $C_{39}H_{44}N_{14}O_8$ calcd 837.3539 [$(M+H)^+$]; found: 837.3545.

*4.1.3.4. Compound **3d**.* White foam, 47% yield, chloroform/methanol 15:1 as eluent: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06–8.08 (m, 2H), 7.90–7.92 (m, 4H), 7.53–7.60 (m, 1H), 7.44–7.52 (m, 4H), 7.33–7.38 (m, 4H), 6.9–7.20 (br, 2H), 6.00 (t, J=9.5 Hz, 1H), 5.52 (t, J=9.5 Hz, 1H), 5.20 (d, J=3.5 Hz, 1H, H1′), 4.93 (dd, J=2.5, 10.5 Hz, 1H, H6), 4.45–4.49 (m, 1H), 4.38 (dt, J=5.0, 12.0 Hz, 1H), 4.09 (dt, J=5.0, 11.5 Hz, 1H), 3.82–3.86 (m, 2H), 3.68–3.70 (m, 1H, H5), 3.53 (dd, J=2.5, 13.5 Hz, 1H), 3.41 (dd, J=5.0, 8.5 Hz, 1H), 2.91 (t, J=7.8 Hz, 2H), 2.79–2.81 (m, 1H), 2.52 (br, 1H), 2.43 (dt, J=5.0, 13.5 Hz, 1H), 1.56–1.60 (m, 2H), 1.40–1.47 (m, 3H), 0.92–1.30 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.53, 165.28, 133.66, 133.53, 129.90, 129.83, 129.78, 129.10, 128.62, 128.49, 128.44, 93.94, 77.47, 70.91, 70.01, 69.47, 61.58, 57.86, 56.67, 55.78, 50.80, 50.64, 40.38, 32.61, 30.37, 29.02, 28.70, 27.20, 27.00, 26.00. HRMS (ESI) for $C_{41}H_{48}N_{14}O_8$ calcd 865.3852 [$(M+H)^+$]; found: 865.3902.

*4.1.4. General procedure for the preparation of compounds **6a–d**, **7a–d** and **8a–d**.* To a mixture of carboline carboxylic acid (**4a**, **4b**, or **5**, 0.15 mmol), amine (**3a**, **3b**, **3c**, or **3d**, 0.14 mmol), HBTU (0.18 mmol) and HOBT (0.18 mmol) in DMF (5 mL) was added DIPEA (0.3 mL), and the mixture was stirred. The reaction process was monitored by TLC. The solvent was removed and the residue was dissolved in dichloromethane. The solution was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (dichloromethane/chloroform as eluent) to yield products **6a–d** and **7a–d**. For the preparation of **8a–d**, a Boc-deprotection operation was followed, using 1 N HCl/ethyl acetate solution.

*4.1.4.1. Compound **6a**.* Yield 65%: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.94–8.96 (m, 2H), 8.81 (m, 1H), 8.65–8.68 (m, 1H), 8.18 (d, J=7.8 Hz, 1H), 8.02 (d, J=7.2 Hz, 2H), 7.86–7.97 (m, 4H), 7.44–7.60 (m, 5H), 7.21–7.42 (m, 8H), 6.01 (t, J=9.9 Hz, 1H), 5.50 (t, J=9.8 Hz, 1H), 5.24 (d, J=3.6 Hz, 1H, H1′), 4.97 (dd, J=2.4, 10.2 Hz, 1H), 4.47–4.56 (m, 2H), 4.04–4.09 (m, 1H), 3.74–3.89 (m, 4H), 3.37–3.55 (m, 3H), 3.06–3.09 (m, 1H), 2.89–2.94 (m, 1H), 2.44 (dt, J=5.0, 12.9 Hz, 1H, $H2_{eq}$), 1.43 (q, J=12.9 Hz, 1H, $H2_{ax}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.51, 165.42, 165.29, 140.67, 140.44, 137.07, 133.65, 133.48, 131.91, 129.89, 129.75, 129.50, 128.89, 128.63, 128.54, 128.49, 128.40, 122.17, 121.81, 120.84, 114.38, 111, 71, 94.19, 70.86, 70.05, 69.52, 61.56, 57.68, 56.91, 55.39, 50.81, 49.86, 39.92, 32.58. HRMS (ESI) for $C_{47}H_{42}N_{16}O_9$ calcd 975.3393 [$(M+H)^+$]; found: 975.3411.

*4.1.4.2. Compound **6b**.* Yield 70%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.90 (s, 1H), 8.81 (s, 1H), 8.73 (d, J=1.0 Hz, 1H), 8.24 (t, J=6.0 Hz, 1H), 8.17 (d, J=8.0 Hz, 1H), 8.07–8.09 (m, 2H), 7.90–7.93 (m, 4H), 7.56–7.59 (m, 2H), 7.45–7.53 (m, 5H), 7.31–7.38 (m, 5H), 6.03 (dd, J=9.0, 10.5 Hz, 1H), 5.52 (t, J=10.0 Hz, 1H), 5.21 (d, J=4.0 Hz, 1H, H1′), 4.94 (dd, J=2.5, 10.5 Hz, 1H), 4.46–4.49 (m, 1H), 4.36–4.42 (m, 1H), 4.07–4.13 (m, 1H), 3.81–3.86 (m, 2H), 3.69 (t, J=2.8 Hz, 1H, H5), 3.48–3.55 (m, 3H), 3.41 (dd, J=5.0, 14.0 Hz, 1H), 2.85–2.90 (m, 1H), 2.61–2.66 (m, 1H), 2.43 (dt, J=13.0, 5.0 Hz, 1H, $H2_{eq}$), 1.56–1.72 (m, 5H), 1.43 (q, J=13.0 Hz, 1H, $H2_{ax}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.54, 165.46, 165.28, 140.68, 140.51, 137.06, 133.67, 133.64, 133.46, 131.67, 129.90, 129.80, 129.54, 129.04, 128.86, 128.70, 128.49, 128.42, 122.17, 121.83, 120.81, 114.35, 111.67, 93.92, 77.48, 77.25, 70.94, 70.00, 69.49, 61.57, 57.89, 56.66, 55.93, 50.81, 50.33, 39.16, 32.67, 27.95, 27.58. HRMS (ESI) for $C_{49}H_{46}N_{16}O_9$ calcd 1003.3706 [$(M+H)^+$]; found: 1003.3708.

*4.1.4.3. Compound **6c**.* Yield 50%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.02 (d, J=3.0 Hz, 1H), 8.92 (s, 1H), 8.76 (d, J=1.0 Hz, 1H), 8.13–8.18 (m, 2H), 8.07–8.09 (m, 2H), 7.91–7.92 (m, 4H), 7.46–7.60 (m, 7H), 7.31–7.38 (m, 5H), 6.01 (dd, J=9.5, 10.5 Hz, 1H), 5.51 (t, J=10.0 Hz, 1H), 5.18 (d, J=4.0 Hz, 1H, H1′), 4.93 (dd, J=2.0, 10.0 Hz, 1H), 4.45–4.48 (m, 1H), 4.35–4.41 (m, 1H), 4.05–4.10 (m, 1H), 3.79–3.84 (m, 2H), 3.67 (t, J=3.0 Hz, 1H, H5), 3.52 (dd, J=2.5, 13.5 Hz, 1H), 3.46 (q, J=7.0 Hz, 2H), 3.40 (dd, J=5.5, 14.0 Hz, 1H), 2.79–2.84 (m, 1H), 2.50–2.55 (m, 1H), 2.43 (dt, J=13.5, 5.0 Hz, 1H, $H2_{eq}$), 1.73 (br, 1H), 1.56–1.61 (m, 2H), 1.38–1.50 (m, 3H), 1.26–1.32 (m, 4H). ^{13}C NMR

(75 MHz, $CDCl_3$) δ 165.53, 165.42, 165.30, 140.74, 140.50, 137.09, 133.68, 133.64, 133.47, 131.67, 129.89, 129.78, 129.56, 129.09, 128.87, 128.65, 128.48, 128.41, 122.15, 121.83, 120.80, 114.36, 111.73, 93.91, 77.48, 77.26, 70.91, 69.97, 69.50, 61.54, 57.86, 56.66, 55.80, 50.81, 50.61, 39.39, 32.66, 30.41, 29.62, 26.93, 26.80. HRMS (ESI) for $C_{51}H_{50}N_{16}O_9$ calcd 1031.4019 [$(M+H)^+$]; found: 1031.4028.

4.1.4.4. Compound ***6d***. Yield 74%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.93 (s, 1H), 8.77 (d, J=1.0 Hz, 1H), 8.14–8.18 (m, 2H), 8.07–8.09 (m, 2H), 7.91–7.93 (m, 4H), 7.45–7.60 (m, 7H), 7.31–7.38 (m, 5H), 6.01 (dd, J=9.5, 10.5 Hz, 1H), 5.51 (t, J=10.0 Hz, 1H), 5.17 (d, J=3.5 Hz, 1H, H1′), 4.93 (dd, J=2.5, 10.5 Hz, 1H), 4.45–4.48 (m, 1H), 4.36–4.41 (m, 1H), 4.05–4.10 (m, 1H), 3.79–3.84 (m, 2H), 3.67 (t, J=3.0 Hz, 1H, H5), 3.47–3.54 (m, 3H), 3.40 (dd, J=5.0, 13.5 Hz, 1H), 2.79–2.84 (m, 1H), 2.41–2.51 (m, 2H), 1.66 (br, 1H), 1.57–1.62 (m, 2H), 1.16–1.47 (m, 11H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.57, 165.53, 165.44, 165.31, 140.75, 140.51, 137.09, 133.64, 133.49, 131.66, 129.89, 129.81, 129.77, 129.57, 129.09, 128.86, 128.62, 128.48, 128.42, 122.15, 121.83, 120.78, 114.37, 111.73, 93.89, 77.49, 77.31, 70.92, 69.98, 69.50, 61.56, 57.86, 56.67, 55.82, 50.80, 50.70, 39.48, 32.65, 30.47, 29.63, 29.21, 29.17, 27.12, 26.89. HRMS (ESI) for $C_{53}H_{54}N_{16}O_9$ calcd 1059.4332 [$(M+H)^+$]; found: 1059.4344.

4.1.4.5. Compound ***7a***. Yield 67%: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.23 (br, 1H), 8.82 (s, 1H), 8.70–8.74 (m, 1H), 8.11 (d, J=7.8 Hz, 1H), 8.01 (d, J=7.2 Hz, 2H), 7.85–8.00 (m, 4H), 7.22–7.53 (m, 12H), 6.02 (t, J=9.9 Hz, 1H), 5.52 (t, J=9.9 Hz, 1H), 5.25 (d, J=3.6 Hz, 1H, H1′), 5.00 (dd, J=2.7, 10.2 Hz, 1H), 4.47–4.55 (m, 2H), 4.08–4.15 (m, 1H), 3.76–3.91 (m, 4H), 3.38–3.56 (m, 3H), 2.99–3.08 (m, 2H), 2.82 (s, 3H, CH_3), 2.44 (dt, J=5.1, 13.2 Hz, 1H, $H2_{eq}$), 1.85 (br, 1H), 1.46 (q, J=12.6 Hz, 1H, $H2_{ax}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.86, 165.37, 165.22, 140.50, 139.27, 136.05, 133.55, 133.37, 129.76, 129.66, 128.56, 128.44, 128.27, 122.15, 121.96, 121.88, 120.47, 112.58, 111, 82, 94.02, 77.42, 76.57, 70.72, 69.93, 69.40, 61.40, 57.60, 56.46, 55.30, 50.69, 49.80, 39.77, 32.37, 20.31. HRMS (ESI) for $C_{48}H_{44}N_{16}O_9$ calcd 989.3550 [$(M+H)^+$]; found: 989.3582.

4.1.4.6. Compound ***7b***. Yield 60%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.41 (s, 1H), 8.23 (t, J=6.3 Hz, 1H), 8.15 (d, J=8.0 Hz, 1H), 8.08–8.10 (m, 2H), 7.89–7.92 (m, 4H), 7.46–7.60 (m, 7H), 7.31–7.38 (m, 5H), 6.00 (dd, J=9.5, 10.5 Hz, 1H), 5.50 (t, J=10.0 Hz, 1H), 5.21 (d, J=4.0 Hz, 1H, H1′), 4.94 (dd, J=2.5, 10.5 Hz, 1H), 4.45–4.49 (m, 1H), 4.37–4.43 (m, 1H), 4.06–4.12 (m, 1H), 3.80–3.86 (m, 2H), 3.69 (t, J=3.0 Hz, 1H, H5), 3.47–3.54 (m, 3H), 3.40 (dd, J=5.0, 14.0 Hz, 1H), 2.87–2.92 (m, 1H), 2.76 (s, 3H, CH_3), 2.61–2.65 (m, 1H), 2.44 (dt, J=13.0, 5.0 Hz, 1H, $H2_{eq}$), 1.56–1.73 (m, 5H), 1.43 (q, J=12.5 Hz, 1H, $H2_{ax}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.56, 165.53, 165.44, 165.29, 140.25, 140.03, 139.97, 135.78, 133.67, 133.63, 133.43, 129.92, 129.83, 129.80, 129.07, 128.83, 128.70, 128.56, 128.49, 128.39, 122.48, 122.20, 120.85, 112.68, 111.64, 94.01, 77.58, 77.26, 70.88, 70.02, 69.52, 61.58, 57.89, 56.64, 55.95, 50.84, 50.37, 39.28, 32.69, 28.10, 27.75, 20.19. HRMS (ESI) for $C_{50}H_{48}N_{16}O_9$ calcd 1017.3863 [$(M+H)^+$]; found: 1017.3850.

4.1.4.7. Compound ***7c***. Yield 66%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.00–9.01 (m, 1H), 8.78 (s, 1H), 8.23 (t, J=5.5 Hz, 1H), 8.07–8.11 (m, 3H), 7.91–7.92 (m, 4H), 7.45–7.59 (m, 7H), 7.25–7.37 (m, 5H), 6.00 (dd, J=9.5, 10.0 Hz, 1H), 5.51 (t, J=10.0 Hz, 1H), 5.17 (d, J=3.5 Hz, 1H, H1′), 4.93 (dd, J=2.5, 10.0 Hz, 1H), 4.45–4.49 (m, 1H), 4.36–4.41 (m, 1H), 4.05–4.10 (m, 1H), 3.79–3.84 (m, 2H), 3.68 (t, J=2.8 Hz, 1H, H5), 3.38–3.54 (m, 4H), 2.79–2.84 (m, 4H), 2.50–2.55 (m, 1H), 2.43 (dt, J=13.5, 5.0 Hz, 1H, $H2_{eq}$), 1.58–1.61 (m, 3H), 1.39–1.47 (m, 3H), 1.26–1.31 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.58, 165.54, 165.47, 165.29, 140.38, 140.10, 139.84, 135.88, 133.66, 133.61, 133.44, 129.88, 129.77, 129.07, 128.74, 128.63, 128.47, 128.38, 122.38, 122.09, 120.70, 112.61, 111.72, 93.93, 77.51, 77.25, 70.87, 69.97, 69.49, 61.54, 57.85, 56.64, 55.79, 50.80, 50.63, 39.38, 32.63, 30.46, 29.76, 27.00, 26.87, 20.24. HRMS (ESI) for $C_{52}H_{52}N_{16}O_9$ calcd 1045.4176 [$(M+H)^+$]; found: 1045.4160.

4.1.4.8. Compound ***7d***. Yield 79%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.01 (s, 1H), 8.79 (s, 1H), 8.24 (t, J=6.0 Hz, 1H), 8.07–8.11 (m, 3H), 7.91–7.93 (m, 4H), 7.45–7.59 (m, 7H), 7.28–7.38 (m, 5H), 6.01 (dd, J=11.0, 10.0 Hz, 1H), 5.51 (t, J=10.0 Hz, 1H), 5.18 (d, J=4.0 Hz, 1H, H1′), 4.93 (dd, J=2.0, 10.0 Hz, 1H), 4.45–4.49 (m, 1H), 4.36–4.41 (m, 1H), 4.05–4.10 (m, 1H), 3.80–3.84 (m, 2H), 3.67 (t, J=3.0 Hz, 1H, H5), 3.47–3.54 (m, 3H), 3.40 (dd, J=5.5, 13.5 Hz, 1H), 2.79–2.84 (m, 4H), 2.47–2.52 (m, 1H), 2.43 (dt, J=13.5, 5.0 Hz, 1H, $H2_{eq}$), 1.58–1.64 (m, 2H), 1.30–1.50 (m, 6H), 1.17–1.28 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.61, 165.54, 165.49, 165.29, 140.42, 140.13, 139.84, 135.92, 133.62, 133.47, 129.88, 129.79, 129.76, 129.09, 128.74, 128.61, 128.46, 128.40, 122.38, 122.08, 120.67, 112.61, 111.74, 93.91, 77.51, 77.26, 70.91, 69.98, 69.49, 61.57, 57.85, 56.66, 55.83, 50.80, 50.67, 39.48, 32.64, 30.51, 29.76, 29.28, 29.24, 27.19, 26.96, 20.29. HRMS (ESI) for $C_{54}H_{56}N_{16}O_9$ calcd 1073.4489 [$(M+H)^+$]; found: 1073.4484.

4.1.4.9. Compound ***8a***. Yield 37%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (dd, J=1.0, 8.5 Hz, 2H), 7.90–7.91 (m, 2H), 7.83 (dd, J=1.0, 8.5 Hz, 2H), 7.40–7.55 (m, 6H), 7.35–7.38 (m, 2H), 7.26–7.30 (m, 4H), 7.14–7.17 (m, 1H), 7.09–7.12 (m, 1H), 5.99 (dd, J=9.5, 10.5 Hz, 1H), 5.51 (t, J=10.0 Hz, 1H), 5.21 (d, J=3.5 Hz, 1H, H1′), 4.96 (dd, J=2.0, 10.5 Hz, 1H), 4.48–4.52 (m, 1H), 4.33–4.38 (m, 1H), 4.00–4.11 (m, 3H), 3.81–3.85 (m, 2H), 3.69 (t, J=3.0 Hz, 1H, H5), 3.63 (q, J=4.7 Hz, 1H), 3.51–3.58 (m, 2H), 3.41 (dd, J=5.5, 13.5 Hz, 1H), 3.26–3.32 (m, 1H), 3.21 (dd, J=4.8, 15.5 Hz, 1H), 2.84–2.95 (m, 3H), 2.41 (dt, J=13.5, 5.0 Hz, 1H, $H2_{eq}$), 1.82 (br, 2H), 1.45 (q, J=12.5 Hz, 1H, $H2_{ax}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.63, 165.65, 165.47, 165.26, 135.96, 133.80, 133.67, 133.54, 129.88, 129.78, 129.75, 128.84, 128.75, 128.50, 128.42, 127.37, 121.84, 119.63, 118.13, 110.73, 94.41, 77.77, 70.92, 70.12, 69.42, 61.81, 57.62, 57.13, 56.73, 55.83, 50.83, 49.54, 42.72, 39.84, 32.46, 24.32. HRMS (ESI) for $C_{47}H_{46}N_{16}O_9$ calcd 979.3706 [$(M+H)^+$]; found: 979.3856.

4.1.4.10. Compound ***8b***. Yield 44%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07–8.09 (m, 2H), 7.89–7.92 (m, 3H), 7.77 (br, 1H), 7.60 (t, J=7.5 Hz, 1H), 7.47–7.53 (m, 5H), 7.26–7.38 (m, 5H), 7.08–7.17 (m, 3H), 5.99 (t, J=10.0 Hz, 1H), 5.51 (t, J=10.0 Hz, 1H), 5.18 (d, J=3.5 Hz, 1H, H1′), 4.93 (dd, J=2.5, 10.5 Hz, 1H), 4.46–4.49 (m, 1H), 4.34–4.39 (m, 1H), 4.04–4.10 (m, 1H), 3.94–4.02 (m, 2H), 3.82 (dt, J=4.0, 10.5 Hz, 2H), 3.67 (t, J=3.0 Hz, 1H, H5), 3.51–3.55 (m, 2H), 3.41 (dd, J=5.0, 13.5 Hz, 1H), 3.20–3.31 (m, 3H), 2.79–2.88 (m, 2H), 2.57–2.60 (m, 1H), 2.42 (dt, J=13.5, 5.0 Hz, 1H, $H2_{eq}$), 1.67 (br, 2H), 1.39–1.60 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.40, 165.56, 165.26, 135.95, 133.71, 133.66, 133.53, 132.11, 129.89, 129.81, 129.79, 129.04, 128.72, 128.62, 128.49, 128.46, 127.36, 121.81, 119.60, 118.12, 110.68, 108.66, 94.12, 77.69, 70.94, 70.01, 69.43, 61.62, 57.83, 57.25, 56.65, 55.92, 50.81, 50.15, 42.82, 38.93, 32.62, 27.87, 27.41, 24.49. HRMS (ESI) for $C_{49}H_{50}N_{16}O_9$ calcd 1007.4019 [$(M+H)^+$]; found: 1007.4050.

4.1.4.11. Compound ***8c***. Yield 75%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07–8.09 (m, 2H), 7.90–7.92 (m, 5H), 7.61 (t, J=7.5 Hz, 1H), 7.46–7.53 (m, 5H), 7.29–7.38 (m, 5H), 7.08–7.17 (m, 2H), 7.02 (br, 1H), 6.00 (t, J=10.0 Hz, 1H), 5.51 (t, J=10.0 Hz, 1H), 5.18 (d, J=3.5 Hz, 1H, H1′), 4.93 (dd, J=2.5, 10.5 Hz, 1H), 4.45–4.49 (m, 1H), 4.35–4.40 (m, 1H), 4.01–4.10 (m, 3H), 3.79–3.85 (m, 2H), 3.68 (t, J=2.5 Hz, 1H, H5), 3.53 (dd, J=2.5, 13.5 Hz, 2H), 3.41 (dd, J=5.0, 13.5 Hz, 1H), 3.20–3.24 (m, 3H), 2.76–2.84 (m, 2H), 2.49–2.53 (m, 1H), 2.43 (dt, J=13.5, 5.0 Hz, 1H, $H2_{eq}$), 1.99 (br, 2H), 1.39–1.46 (m, 5H), 1.22–1.30 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.27, 165.53, 165.27, 135.98, 133.70, 133.65, 133.51, 131.94, 129.89, 129.81, 129.78, 129.10, 128.65, 128.49, 128.43, 127.27, 121.81, 119.58, 118.07, 110.76, 108.51, 93.96, 77.53, 70.91, 69.98, 69.46, 61.54, 57.85, 57.27, 56.67, 55.86, 50.81, 50.61,

42.85, 39.08, 32.62, 30.44, 29.40, 26.90, 26.74, 24.61. HRMS (ESI) for $C_{51}H_{54}N_{16}O_9$ calcd 1035.4332 [$(M+H)^+$]; found: 1035.4334.

4.1.4.12. Compound ***8d***. Yield 83%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06–8.09 (m, 2H), 7.90–7.93 (m, 5H), 7.59–7.63 (m, 1H), 7.47–7.53 (m, 5H), 7.33–7.38 (m, 4H), 7.29 (d, J=8.0 Hz, 1H), 7.08–7.17 (m, 2H), 7.01 (t, J=5.5 Hz, 1H), 6.00 (dd, J=9.5, 10.5 Hz, 1H), 5.50 (t, J=10.0 Hz, 1H), 5.16 (d, J=4.0 Hz, 1H, H1′), 4.93 (dd, J=2.5, 10.0 Hz, 1H), 4.45–4.48 (m, 1H), 4.35–4.41 (m, 1H), 3.98–4.10 (m, 3H), 3.78–3.84 (m, 2H), 3.67 (t, J=2.5 Hz, 1H, H5), 3.50–3.54 (m, 2H), 3.41 (dd, J=5.0, 13.5 Hz, 1H), 3.23–3.27 (m, 3H), 2.74–2.85 (m, 2H), 2.47–2.52 (m, 1H), 2.43 (dt, J=13.5, 5.0 Hz, 1H, $H2_{eq}$), 1.64 (br, 2H), 1.38–1.51 (m, 5H), 1.20–1.23 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.46, 165.53, 165.48, 165.27, 135.96, 133.64, 133.49, 132.34, 129.88, 129.81, 129.76, 129.10, 128.62, 128.47, 128.42, 127.34, 121.76, 119.56, 118.06, 110.72, 108.70, 93.91, 77.50, 77.30, 70.89, 69.97, 69.47, 61.54, 57.85, 57.42, 56.67, 55.85, 50.80, 50.68, 43.08, 39.11, 32.62, 30.50, 29.47, 29.25, 29.10, 27.14, 26.82, 24.75. HRMS (ESI) for $C_{53}H_{58}N_{16}O_9$ calcd 1063.4645 [$(M+H)^+$]; found: 1063.4676.

4.1.5. General procedure for the preparation of compounds ***9a–d***, ***10a–d*** *and* ***11a–d***. Compounds (**6a–d**, **7a–d**, or **8a–d**, 0.05 mmol) were suspended in methanol (15 mL), followed by the addition of 30% sodium methoxide (1 mL in methanol). The mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure to give white solids. These solids were dissolved in a mixed solvent (4 mL of pyridine, 2 mL of triethylamine and 1 mL of water). The mixture was stirred for 1 h when H_2S gas was introduced into the reaction flask. The gas was discontinued after the reaction solution turned dark green. The reaction mixture was stirred overnight. The solvent was removed under reduced pressure. The residue was subjected to column chromatography on silica gel (ammonia aqueous solution/methanol 1:5 as eluent) to give products. The resulting products were dissolved in water, and the pH values of solutions were adjusted to around 6.0 using diluted hydrochloride acid. Lyophilization yielded the products as white foams in 70% overall yield.

4.1.5.1. Compound ***9a***. ^{1}H NMR (500 MHz, D_2O) δ 8.68 (d, J=0.5 Hz, 1H), 8.50 (s, 1H), 8.03 (d, J=8.0 Hz, 1H), 7.66 (td, J=1.0, 8.5 Hz, 1H), 7.52 (d, J=8.0 Hz, 1H), 7.35 (td, J=1.0, 8.5 Hz, 1H), 5.65 (d, J=4.0 Hz, 1H, H1′), 4.25 (dd, J=3.5, 11.0 Hz, 1H), 4.11 (dd, J=9.0, 10.5 Hz, 1H), 4.06 (dd, J=3.0, 10.5 Hz, 1H), 3.95–4.00 (m, 2H), 3.78–3.90 (m, 2H), 3.72 (t, J=6.5 Hz, 2H), 3.51–3.60 (m, 3H), 3.43–3.48 (m, 1H), 3.35 (dd, J=6.5, 13.5 Hz, 1H), 3.25–3.30 (m, 1H), 2.57 (dt, J=12.5, 4.5 Hz, 1H, $H2_{eq}$), 1.88 (q, J=12.5 Hz, 1H, $H2_{ax}$). ^{13}C NMR (75 MHz, D_2O) δ 164.51, 143.26, 135.96, 132.94, 132.04, 131.56, 129.11, 122.92, 122.53, 120.36, 115.34, 113.32, 92.14, 73.54, 71.23, 71.05, 70.36, 69.15, 57.49, 53.90, 49.95, 48.93, 47.87, 40.73, 40.65, 29.15. HRMS (ESI) for $C_{26}H_{38}N_8O_6$ calcd 559.2987 [$(M+H)^+$]; found: 559.3047.

4.1.5.2. Compound ***9b***. ^{1}H NMR (500 MHz, D_2O) δ 8.39 (s, 1H), 8.11 (s, 1H), 7.86 (d, J=8.0 Hz, 1H), 7.53 (t, J=7.5 Hz, 1H), 7.37 (d, J=8.5 Hz, 1H), 7.23 (t, J=7.5 Hz, 1H), 5.56 (d, J=8.5 Hz, 1H, H1′), 4.24 (dd, J=2.5, 10.5 Hz, 1H), 4.11 (dd, J=3.0, 11.0 Hz, 1H), 4.04 (dd, J=9.0, 10.5 Hz, 1H), 3.95–3.99 (m, 2H), 3.71–3.78 (m, 2H), 3.45–3.54 (m, 5H), 3.28–3.32 (m, 2H), 3.07–3.09 (m, 1H), 2.55 (dt, J=12.5, 4.5 Hz, 1H, $H2_{eq}$), 1.86 (q, J=12.5 Hz, 1H, $H2_{ax}$), 1.74 (br, 4H). ^{13}C NMR (75 MHz, D_2O) δ 165.87, 142.16, 136.37, 135.55, 130.76, 129.87, 122.39, 121.72, 120.53, 114.65, 112.91, 92.69, 73.56, 71.09, 70.49, 69.27, 57.88, 53.86, 51.21, 49.04, 47.86, 40.68, 40.16, 29.18, 27.04, 26.43. HRMS (ESI) for $C_{28}H_{42}N_8O_6$ calcd 587.3300 [$(M+H)^+$]; found: 587.3314.

4.1.5.3. Compound ***9c***. ^{1}H NMR (500 MHz, D_2O) δ 8.29 (s, 1H), 8.00 (s, 1H), 7.80 (d, J=7.5 Hz, 1H), 7.48 (t, J=7.5 Hz, 1H), 7.31 (d, J=8.0 Hz, 1H), 7.19 (t, J=7.5 Hz, 1H), 5.52 (d, J=3.5 Hz, 1H, H1′), 4.22 (d, J=10.5 Hz, 1H), 4.12 (dd, J=3.0, 10.5 Hz, 1H), 3.95–4.03 (m, 3H), 3.67–3.76 (m, 2H), 3.44–3.53 (m, 3H), 3.38 (t, J=7.0 Hz, 2H), 3.25–3.31 (m, 2H), 3.00–3.06 (m, 1H), 2.54 (dt, J=13.0, 4.5 Hz, 1H, $H2_{eq}$), 1.85 (q, J=12.5 Hz, 1H, $H2_{ax}$), 1.68 (br, 4H), 1.45 (br, 4H). ^{13}C NMR (75 MHz, D_2O) δ 166.01, 141.91, 136.37, 135.94, 130.94, 130.47, 129.52, 122.25, 121.52, 120.54, 114.45, 112.78, 93.05, 73.69, 71.15, 70.48, 70.09, 69.41, 58.06, 53.89, 51.75, 49.08, 47.82, 40.70, 40.46, 29.24, 28.55, 26.74, 26.70. HRMS (ESI) for $C_{30}H_{46}N_8O_6$ calcd 615.3613 [$(M+H)^+$]; found: 615.3624.

4.1.5.4. Compound ***9d***. ^{1}H NMR (500 MHz, D_2O) δ 8.52 (s, 1H), 8.27 (s, 1H), 7.86 (d, J=8.0 Hz, 1H), 7.57 (t, J=7.5 Hz, 1H), 7.38 (d, J=8.5 Hz, 1H), 7.26 (t, J=7.5 Hz, 1H), 5.67 (d, J=3.5 Hz, 1H, H1′), 4.51 (d, J=10.0 Hz, 1H), 4.33–4.37 (m, 2H), 4.10 (t, J=9.3 Hz, 1H), 4.01–4.05 (m, 1H), 3.80–3.85 (m, 2H), 3.60–3.64 (m, 2H), 3.34–3.52 (m, 6H), 2.64 (dt, J=13.0, 4.5 Hz, 1H, $H2_{eq}$), 1.97–2.07 (m, 1H), 1.83 (br, 2H), 1.38–1.73 (m, 2H), 1.42 (br, 8H). ^{13}C NMR (75 MHz, D_2O) δ 162.38, 143.37, 135.22, 132.41, 132.03, 131.87, 127.73, 122.79, 122.71, 119.91, 114.72, 113.19, 92.70, 72.05, 71.59, 70.45, 68.72, 67.84, 59.10, 53.47, 49.07, 47.71, 41.07, 40.40, 29.23, 29.08, 29.05, 28.61, 26.98, 26.79, 26.58, 22.01. HRMS (ESI) for $C_{32}H_{50}N_8O_6$ calcd 643.3926 [$(M+H)^+$]; found: 643.3914.

4.1.5.5. Compound ***10a***. ^{1}H NMR (500 MHz, D_2O) δ 8.16 (s, 1H), 7.90 (d, J=7.5 Hz, 1H), 7.56 (t, J=7.5 Hz, 1H), 7.40 (d, J=8.5 Hz, 1H), 7.26 (t, J=7.5 Hz, 1H), 5.61 (d, J=4.0 Hz, 1H, H1′), 4.17 (dd, J=3.5, 11.0 Hz, 1H), 4.09 (dd, J=9.0, 10.5 Hz, 1H), 3.94–3.99 (m, 2H), 3.75–3.87 (m, 3H), 3.65 (t, J=6.5 Hz, 2H), 3.50–3.56 (m, 3H), 3.31–3.37 (m, 2H), 3.13–3.18 (m, 1H), 2.62 (s, 3H, CH_3), 2.54 (dt, J=12.5, 4.8 Hz, 1H, $H2_{eq}$), 1.83 (q, J=12.5 Hz, 1H, $H2_{ax}$). ^{13}C NMR (75 MHz, D_2O) δ 165.78, 141.06, 141.18, 135.34, 134.45, 130.83, 129.05, 122.39, 121.95, 120.86, 113.37, 112.82, 92.00, 73.96, 71.96, 71.20, 70.11, 69.23, 57.05, 53.98, 49.53, 48.92, 47.91, 41.00, 40.71, 29.28, 18.22. HRMS (ESI) for $C_{27}H_{40}N_8O_6$ calcd 573.3144 [$(M+H)^+$]; found: 573.3121.

4.1.5.6. Compound ***10b***. ^{1}H NMR (500 MHz, D_2O) δ 7.94 (s, 1H), 7.76 (d, J=8.0 Hz, 1H), 7.49 (t, J=7.5 Hz, 1H), 7.29 (d, J=8.5 Hz, 1H), 7.18 (t, J=7.5 Hz, 1H), 5.57 (d, J=3.0 Hz, 1H, H1′), 4.24 (d, J=10.0 Hz, 1H), 4.12 (dd, J=11.0, 3.5 Hz, 1H), 4.05 (dd, J=10.0, 9.0 Hz, 1H), 3.95–3.99 (m, 2H), 3.74–3.79 (m, 2H), 3.40–3.53 (m, 5H), 3.27–3.31 (m, 2H), 3.03–3.15 (m, 1H), 2.56 (dt, J=12.5, 4.5 Hz, 1H, $H2_{eq}$), 2.49 (s, 3H, CH_3), 1.88 (q, J=12.5 Hz, 1H, $H2_{ax}$), 1.76 (br, 4H). ^{13}C NMR (75 MHz, D_2O) δ 165.46, 141.75, 140.89, 135.07, 134.69, 130.59, 128.66, 122.16, 121.75, 120.72, 112.92, 112.64, 92.70, 73.60, 71.19, 70.41, 69.31, 57.78, 53.90, 51.14, 49.06, 47.86, 40.72, 40.25, 39.85, 29.20, 27.09, 26.56, 18.22. HRMS (ESI) for $C_{29}H_{44}N_8O_6$ calcd 601.3457 [$(M+H)^+$]; found: 601.3454.

4.1.5.7. Compound ***10c***. ^{1}H NMR (500 MHz, D_2O) δ 8.51 (s, 1H), 8.07 (d, J=8.0 Hz, 1H), 7.69 (td, J=1.0, 8.0 Hz, 1H), 7.54 (d, J=8.0 Hz, 1H), 7.38 (t, J=7.5 Hz, 1H), 5.69 (d, J=3.5 Hz, 1H, H1′), 4.54 (d, J=9.5 Hz, 1H), 4.37–4.39 (m, 2H), 4.11 (t, J=8.8 Hz, 1H), 4.02–4.06 (m, 1H), 3.81–3.86 (m, 2H), 3.62–3.65 (m, 2H), 3.49–3.53 (m, 4H), 3.39–3.45 (m, 2H), 2.89 (s, 3H, CH_3), 2.65 (dt, J=13.0, 4.5 Hz, 1H, $H2_{eq}$), 2.01 (q, J=12.5 Hz, 1H, $H2_{ax}$), 1.86–1.91 (m, 2H), 1.74–1.79 (m, 2H), 1.51 (br, 4H). ^{13}C NMR (75 MHz, D_2O) δ 162.15, 143.61, 140.34, 134.89, 132.60, 131.46, 131.16, 123.03, 120.50, 113.93, 113.28, 92.75, 71.80, 71.63, 70.38, 68.69, 67.78, 59.21, 53.44, 49.06, 47.70, 40.98, 40.36, 29.06, 28.52, 26.69, 26.59, 26.29, 16.33. HRMS (ESI) for $C_{31}H_{48}N_8O_6$ calcd 629.3770 [$(M+H)^+$]; found: 629.3773.

4.1.5.8. Compound ***10d***. ^{1}H NMR (500 MHz, D_2O) δ 7.83 (s, 1H), 7.66 (d, J=7.5 Hz, 1H), 7.44 (t, J=7.5 Hz, 1H), 7.21 (d, J=8.0 Hz, 1H), 7.14 (t, J=7.5 Hz, 1H), 5.55 (d, J=3.5 Hz, 1H, H1′), 4.28 (dd, J=3.0, 10.5 Hz, 1H), 4.17 (dd, J=3.5, 10.5 Hz, 1H), 3.97–4.05 (m, 3H), 3.67–3.77 (m, 2H), 3.54 (t, J=9.5 Hz, 1H), 3.47–3.51 (m, 2H), 3.38–3.41 (m, 2H), 3.27–3.35

(m, 2H), 3.05–3.10 (m, 1H), 2.56 (dt, J=12.5, 4.7 Hz, 1H, $H2_{eq}$), 2.44 (s, 3H, CH_3), 1.87 (q, J=12.5 Hz, 1H, $H2_{ax}$), 1.68–1.70 (m, 4H), 1.35–1.48 (m, 8H). ^{13}C NMR (75 MHz, D_2O) δ 164.68, 141.75, 140.45, 134.73, 134.25, 130.72, 128.69, 122.02, 121.80, 120.49, 112.64, 112.53, 93.13, 73.47, 71.04, 70.64, 69.67, 69.32, 58.23, 53.83, 52.04, 49.08, 47.79, 40.76, 40.66, 29.35, 29.27, 29.21, 28.29, 27.06, 26.95, 17.89. HRMS (ESI) for $C_{33}H_{52}N_8O_6$ calcd 657.4083 [$(M+H)^+$]; found: 657.4079.

4.1.5.9. Compound ***11a***. ^{1}H NMR (500 MHz, D_2O) δ 7.61 (d, J=8.0 Hz, 1H), 7.51 (d, J=8.0 Hz, 1H), 7.28 (t, J=7.5 Hz, 1H), 7.20 (t, J=7.5 Hz, 1H), 5.49 (d, J=4.0 Hz, 1H, H1′), 4.55 (d, J=16.0 Hz, 1H), 4.48 (d, J=16.0 Hz, 1H), 4.29 (dd, J=11.0, 5.0 Hz, 1H), 3.98–4.06 (m, 2H), 3.88–3.96 (m, 2H), 3.65–3.72 (m, 3H), 3.38–3.53 (m, 6H), 3.30 (dd, J=13.5, 7.5 Hz, 1H), 3.07–3.16 (m, 2H), 2.97–3.02 (m, 1H), 2.47 (dt, J=12.5, 4.5 Hz, 1H, $H2_{eq}$), 1.74 (q, J=12.5 Hz, 1H, $H2_{ax}$). ^{13}C NMR (75 MHz, D_2O) δ 170.79, 137.24, 126.86, 126.25, 123.38, 120.58, 118.81, 112.54, 105.54, 92.70, 74.64, 72.07, 71.45, 70.05, 69.89, 57.38, 56.61, 54.29, 49.69, 49.11, 47.79, 41.33, 40.86, 29.90, 23.80. HRMS (ESI) for $C_{26}H_{42}N_8O_6$ calcd 563.3300 [$(M+H)^+$]; found: 563.3296.

4.1.5.10. Compound ***11b***. ^{1}H NMR (500 MHz, D_2O) δ 7.61 (d, J=8.0 Hz, 1H), 7.51 (dd, J=1.0, 7.5 Hz, 1H), 7.29 (td, J=8.0, 1.0 Hz, 1H), 7.20 (td, J=8.0, 1.0 Hz, 1H), 5.55 (d, J=4.0 Hz, 1H, H1′), 4.60 (dd, J=1.0, 15.5 Hz, 1H), 4.54 (d, J=15.5 Hz, 1H), 4.34 (dd, J=11.0, 5.5 Hz, 1H), 4.19 (dd, J=10.8, 3.3 Hz, 1H), 4.02–4.08 (m, 2H), 3.95–3.99 (m, 1H), 3.88 (t, J=8.5 Hz, 1H), 3.70–3.79 (m, 2H), 3.42–3.56 (m, 4H), 3.29–3.40 (m, 3H), 3.13–3.20 (m, 2H), 2.91–2.95 (m, 1H), 2.55 (dt, J=12.5, 4.5 Hz, 1H, $H2_{eq}$), 1.85 (q, J=12.5 Hz, 1H, $H2_{ax}$), 1.61–1.67 (m, 4H). ^{13}C NMR (75 MHz, D_2O) δ 170.01, 137.31, 126.25, 126.16, 123.46, 120.63, 118.85, 112.56, 105.50, 92.69, 73.88, 71.22, 70.90, 70.31, 69.38, 57.70, 56.64, 53.95, 50.90, 49.05, 47.88, 41.32, 40.75, 39.94, 29.31, 26.83, 26.66, 23.79. HRMS (ESI) for $C_{28}H_{46}N_8O_6$ calcd 591.3613 [$(M+H)^+$]; found: 591.3637.

4.1.5.11. Compound ***11c***. ^{1}H NMR (500 MHz, D_2O) δ 7.59 (d, J=8.0 Hz, 1H), 7.51 (d, J=8.0 Hz, 1H), 7.28 (t, J=7.5 Hz, 1H), 7.19 (t, J=7.5 Hz, 1H), 5.36 (d, J=3.5 Hz, 1H, H1′), 4.50 (d, J=16.0 Hz, 1H), 4.42 (d, J=15.5 Hz, 1H), 4.15 (dd, J=11.0, 5.0 Hz, 1H), 3.93–4.01 (m, 3H), 3.86 (t, J=10.0 Hz, 1H), 3.78 (t, J=3.5 Hz, 1H, H5), 3.64 (dt, J=12.0, 4.5 Hz, 1H), 3.43–3.53 (m, 3H), 3.32–3.38 (m, 2H), 3.18–3.27 (m, 3H), 3.05–3.15 (m, 2H), 2.84–2.91 (m, 1H), 2.42 (dt, J=12.5, 4.5 Hz, 1H, $H2_{eq}$), 1.67 (q, J=12.5 Hz, 1H, $H2_{ax}$), 1.45–1.57 (m, 4H), 1.34 (br, 4H). ^{13}C NMR (75 MHz, D_2O) δ 170.56, 137.21, 127.21, 126.32, 123.30, 120.55, 118.77, 112.51, 105.63, 94.51, 75.36, 71.77, 71.19, 71.04, 69.82, 58.29, 56.64, 54.63, 51.81, 49.37, 47.73, 41.33, 41.06, 40.11, 30.67, 29.00, 28.91, 26.67, 26.50, 23.91. HRMS (ESI) for $C_{30}H_{50}N_8O_6$ calcd 619.3926 [$(M+H)^+$]; found: 619.3935.

4.1.5.12. Compound ***11d***. ^{1}H NMR (500 MHz, D_2O) δ 7.60 (d, J=8.0 Hz, 1H), 7.51(d, J=7.5 Hz, 1H), 7.28 (t, J=7.5 Hz, 1H), 7.20 (t, J=7.5 Hz, 1H), 5.46 (d, J=3.5 Hz, 1H, H1′), 4.58 (d, J=16.0 Hz, 1H), 4.50 (d, J=15.5 Hz, 1H), 4.27 (dd, J=10.0, 5.0 Hz, 1H), 4.12 (dd, J=10.5, 3.0 Hz, 1H), 4.05 (dd, J=10.5, 3.0 Hz, 1H), 3.94–3.98 (m, 2H), 3.87 (s, 1H, H5), 3.68 (td, J=12.0, 4.5 Hz, 1H), 3.61 (td, J=12.0, 4.0 Hz, 1H), 3.47–3.52 (m, 2H), 3.12–3.42 (m, 7H), 2.87–2.94 (m, 1H), 2.49 (dt, J=12.5, 4.5 Hz, 1H, $H2_{eq}$), 1.78 (q, J=12.5 Hz, 1H, $H2_{ax}$), 1.54–1.58 (m, 4H), 1.28 (br, 8H). ^{13}C NMR (75 MHz, D_2O) δ 169.81, 137.26, 126.34, 126.20, 123.40, 120.59, 118.82, 112.53, 105.45, 93.51, 74.37, 71.45, 70.56, 70.09, 69.98, 57.95, 56.46, 54.16, 51.62, 49.16, 47.82, 41.19, 40.88, 40.25, 29.73, 29.17, 28.97, 28.92, 27.00, 26.60, 23.63. HRMS (ESI) for $C_{32}H_{54}N_8O_6$ calcd 669.4058 [$(M+Na)^+$]; found: 669.4062.

4.2. Biological assay

4.2.1. SPR binding studies. Biotin-labelled RNA fragments were purchased at Bioneer (Korea). SPR measurements were conducted on a Biocore 3000 system from Biocore AB and performed as described in the literature.[9b] Streptavidin coated sensor-chip (SA-chip) were obtained from Biocore and loaded with RNA fragments to 591–648 RU. An empty cell was used as reference surface. Calculation of dissociation constants by fitting the steady state responses was performed by using the formulae as $R=R_{max}[c/(K_d+c)]$, where R=response, R_{max}=maximum response of one binding site occupied, c=concentration.

4.2.2. Antibacterial activity assay. The antibacterial activities were measured according to the published method.[24] The *P. aeruginosa* standard strain was grown in 1 mL tubes and Mueller-Hinton broth to an optical density OD_{600} of 0.5 units. The desired concentrations of antibiotic were added from stock solutions. The samples were incubated at 37 °C for 12 h, when the control culture (with no antibiotic) had an OD_{600} of 1.2–1.6. The OD at 600 nm of each sample was recorded.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.03.034.

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