



Synthesis and biological evaluation of novel neamine–nucleoside conjugates potentially targeting to RNAs

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ABSTRACT

Eighteen novel neamine–nucleoside conjugates with ethylenediamine–lysine or ethylenediamine–arginine as the linker were synthesized and their potential binding to A site of 16S RNA and TAR RNA was evaluated using SPR (surface plasmon resonance). Compared with neamine, compounds **10i** and **10q** show 6.3 and 4.8 times potential in binding to A site of 16S RNA and eight and six times potential in binding to TAR RNA, respectively. According to the data of SPR, it indicates that amino acid residue and nucleobase moieties of the designed neamine–nucleosides conjugates exhibit the important contributions for the binding to A site of 16S RNA and TAR RNA. The molecular docking study on the interaction between the ligands and A site of 16S RNA is in agreement with the experimental data. The novel type of modification may provide a promising way for the development of neamine derivatives effectively targeting to RNAs.

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1. Introduction

With the research advances in the clearance of RNA structure and its important function in the life cycle of pathogenic microorganisms and the processing of diseases, RNA has been viewed as an important small molecular drug target for finding potential drugs.^{1,2} Aminoglycoside antibiotic family including neomycin, kanamycin, paromomycin, and so on has been known to bind potentially to many important RNAs such as bacterial A site of 16S rRNA,^{3,4} HIV-1 trans-activation response element (TAR) RNA,⁵ HIV-1 Rev responsive element (RRE) RNA,⁶ group I introns,⁷ and the hammerhead ribozyme.⁸ But the high toxicity, which mainly resulted from the nonspecific electrostatic interactions limited their clinic use at a large degree,⁹ and also, aminoglycosides are prone to lead to drug resistance because of their own structural instability and the modification of aminoglycoside-modifying enzymes.¹⁰ The detailed study has shown that neamine (Fig. 1), as the common part of neomycin-class aminoglycosides, is the minimum motif that can specifically bind to the A site of 16S

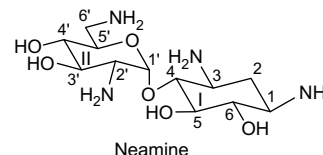


Figure 1. Structure of neamine.

RNA and affect the translation procedure in protein synthesis.⁴ It seems a promising way to keep the neamine core and develop novel potential neamine derivatives. Up to now, many efforts have been made to optimize the core structure neamine, among which neamine–peptide or nucleobase conjugates have been proved to be a convenient and effective strategy to improve the binding to RNAs. The neamine conjugates with arginine/lysine,^{11,12} arginine peptide,¹³ nucleobase,¹⁴ aromatic rings,¹⁵ peptide nucleic acid,¹⁶ and alkylamines¹⁷ can enhance its binding affinity and some of them have been showed certain binding selectivity. Until now there are no general rules for the design of the specific as well as potential RNA-binding small molecules, and all of the reported aminoglycoside conjugates were designed by connecting neamine with peptide or nucleobase directly to one of amino groups on the aminosugar moiety.

Previously, two types of neamine–nucleoside conjugates were synthesized from our group by the condensation of azidodisaccharide and nucleoside using ethylenediamine as a linker. Results from the data of SPR evaluation suggested that the nucleobase played a significant role to bind to A site of 16S RNA and an ethylenediamine

Abbreviations: Ac, acetyl; Arg, arginine; BAIB, [bis(acetoxy)-iodo]benzene; BOC, *tert*-butoxycarbonyl; DCC, dicyclohexylcarbodiimide; DMF, *N,N*-dimethyl formamide; DMSO, dimethyl sulfoxide; Eda, ethylenediamine; Et, ethyl; Fmoc, 9-fluorenylmethoxycarbonyl; HOBT, 1-hydroxybenzotriazole; Lys, lysine; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; Py, pyridine; TEMPO, 2,2,4,6-tetramethyl-1-piperidinyloxy; rt, room temperature; THF, tetrahydrofuran; Tf₂O, trifluoromethanesulfonic anhydride.

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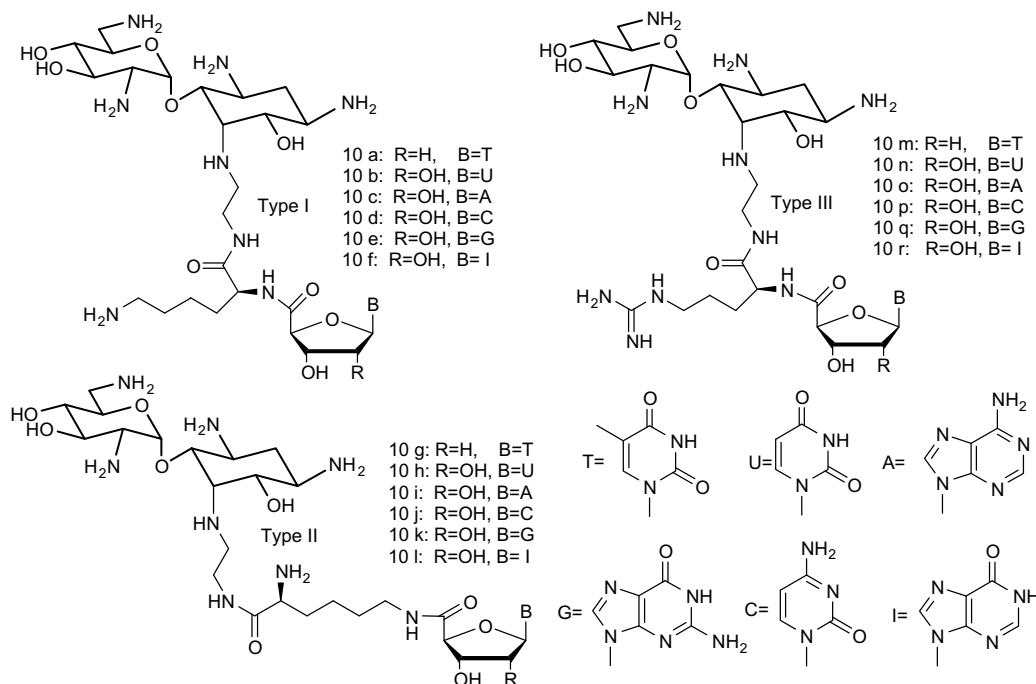


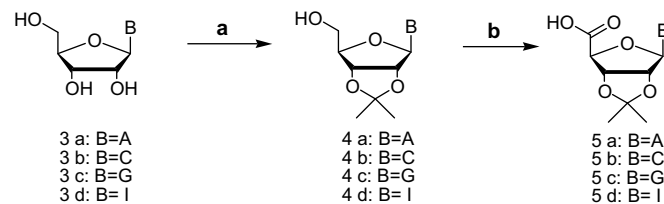
Figure 2. Structures of neamine–nucleoside conjugates.

linker between the neamine and nucleoside was favorable for this binding.¹⁸ In this report we prepared a new class of neamine–nucleoside conjugates (Fig. 2), in which an ethylenediamine–amino acid was used as linker for the modification of the 5-hydroxyl group of neamine by nucleosides. The amino groups on neamine play an important role in their binding to the target RNAs. Keeping the amino group free in the neamine molecule and modification of other positions of neamine may lead the derivatives contributing a various interaction to RNA. Compounds **10a–r** were designed using a flexible side chain to connect the neamine and nucleosides, which were expected to recognize the specific site of RNA. Moreover, lysines and arginines are often present in the RNA-binding proteins (for example, HIV-1 transactivating (Tat) proteins and regulator of expression of virion (Rev) proteins),^{19,20} neamine–arginine and neamine–lysine conjugates both enhance the inhibition of HIV-1 TAR–Tat interaction compared with their mother compound neamine.¹¹ Based on these backgrounds, three types of neamine–nucleoside conjugates were synthesized: *type I* (compounds **10a–f**) consists in neamine derivatives with ethylenediamine–lysine as the linker and nucleosides connecting at the α -NH₂ group of lysine; *type II* (compounds **10g–l**) is the similar structure as type II but the nucleosides are condensed with the ϵ -NH₂ group of lysine; *type III* (compounds **10m–r**) is formed by a number of neamine derivatives with ethylenediamine–arginine as the linker and nucleoside connecting at the α -NH₂ group of arginine. The interactions between compounds **10a–r** with the A site of 16S RNA and TAR RNA were evaluated by dissociation constants (K_D values in μ M) using SPR method. The dissociation constants clearly indicated that three types of neamine–nucleoside conjugates showed the better binding properties to 16S RNA and TAR RNA than the neamine derivatives reported previously.¹⁸

2. Results and discussion

2.1. Synthesis of neamine–nucleoside conjugates **10a–r**

Six protected 5'-carboxylic acid-nucleosides **2a**, **b**, **5a–d** were synthesized (Scheme 1, Fig. 3), among which **2a** and **2b** were



Scheme 1. Synthesis of protected 5'-carboxylic acid-nucleosides **5a**, **5b**, **5c**, and **5d**. Reagents and conditions: (a) CH₃COCH₃, 70% HClO₄, rt; (b) BAIB, TEMPO, CH₃CN, H₂O, rt.

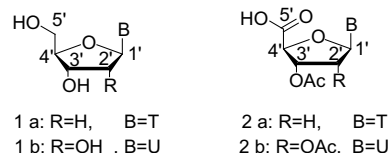
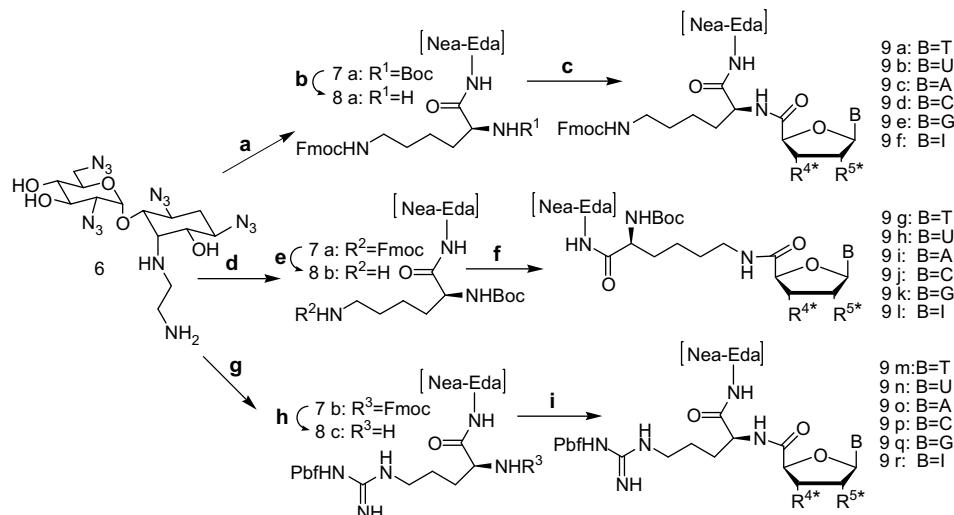


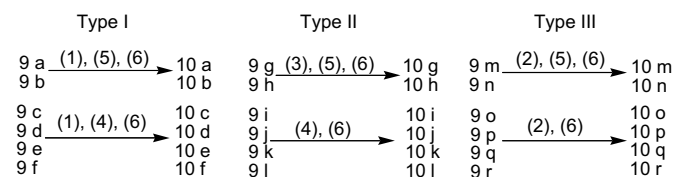
Figure 3. Structures of protected 5'-carboxylic acid-nucleosides **2a** and **2b**.

prepared from compounds **1a** and **1b** according to the published procedures.¹⁸ Compounds **5a**, **5b**, **5c**, and **5d** were synthesized from the nucleosides **3a–d**. After the protection of 2'- and 3'-hydroxyl group by isopropylidene group, the nucleoside intermediates **4a**, **4b**, **4c**, and **4d** were obtained in good yield. Compounds **4a–d** were oxidized by BAIB and TEMPO in mild conditions to afford the desired 5'-carboxylic acid-nucleosides **5a**, **5b**, **5c**, and **5d** in 75–82% yields.

For the synthesis of compounds of types I–III (Schemes 2 and 3), compound **6** was prepared by the reported procedure and used as the starting material.¹⁸ Compounds **8a** and **8b** were synthesized by the condensation reaction of compound **6** with Boc-Lys(Fmoc)-OH to give compound **7a** first. For compound **8a**, the Boc-protection group on α -NH₂ group of lysine was selectively removed by CF₃COOH in CH₂Cl₂ (1:4) and compound **8b** was obtained by the removal of Fmoc-protection group on ϵ -NH₂ group of lysine moiety using Et₂NH in DMF (1:9). Compound **6** was condensed with Fmoc-Arg(Pbf)-OH to yield **7b**, after the removal of the Fmoc group on the α -NH₂ group of arginine using the same procedure as the



Scheme 2. Synthesis of protected neamine–nucleoside conjugates. Reagents and conditions: (a) Boc-Lys(Fmoc)-OH, HOBT, DCC, DMF, 0 °C to rt; (b) CF₃COOH/CH₂Cl₂ (1:4), rt; (c) 5'-carboxylic acid-nucleoside (**2a**, **2b**, **5a**, **5b**, **5c**, and **5d**), HOBT, DCC, DMF, 0 °C to rt; (d) Boc-Lys(Fmoc)-OH, HOBT, DCC, DMF, 0 °C to rt; (e) Et₂NH/DMF (1:9), rt; (f) 5'-carboxylic acid-nucleoside (**2a**, **2b**, **5a**, **5b**, **5c**, and **5d**), HOBT, DCC, DMF, 0 °C to rt; (g) Fmoc-Arg(Pbf)-OH, HOBT, DCC, DMF, 0 °C to rt; (h) Et₂NH/DMF (1:9), rt; (i) 5'-carboxylic acid-nucleoside (**2a**, **2b**, **5a**, **5b**, **5c**, and **5d**), HOBT, DCC, DMF, 0 °C to rt. Note: (1) [Nea-Eda] represents the structure of compound **6**; (2) for the nucleoside part, when B=T, R^{4*}=OAc, R^{5*}=H; when B=U, R^{4*}=R^{5*}=OAc, and when B=A, C, G, and I, R^{4*} and R^{5*} represents the isopropylidene protection of nucleoside 2'- and 3'-hydroxyl group.



Scheme 3. Synthesis of neamine–nucleoside conjugates. Reagents and conditions: (1) Et₂NH/DMF (1:9), rt; (2) CF₃COOH/H₂O/PhSCH₃ (94:3:3), rt; (3) CF₃COOH/CH₂Cl₂ (1:4), rt; (4) CF₃COOH/H₂O (9:1), 0 °C; (5) NH₃/CH₃OH, 0 °C. (6) H₂S, pyridine/Et₃N/H₂O (4:3:2), rt.

preparation of compound **8b**, compound **8c** was obtained in 85% yield. Compounds **8a**, **8b**, and **8c** were condensed with the protected 5'-carboxylic acid-nucleosides **2a**, **b** and **5a–d**, respectively, to provide compounds **9a–r** in 70–82% yields.

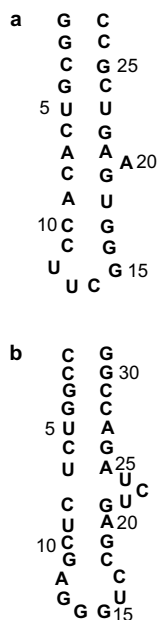


Figure 4. (a) Structure of A site of 16S RNA, (b) structure of TAR RNA.

Three types of desired products **10a–r** were obtained in good yields after removal of all the protection groups (Scheme 3). The target molecules **10a–r** were purified by reversed-phase HPLC (1% CF₃COOH in H₂O and CH₃OH) and obtained as the appropriate trifluoroacetic salts. The intermediates and final products were characterized by ¹H NMR, ¹³C NMR, HRMS, and optical rotations.

2.2. RNA-binding assay and molecular docking study

The binding properties of the neamine–nucleoside conjugates **10a–r** to the A site of 16S RNA and TAR RNA (Fig. 4a and b) were evaluated by the determination of dissociation constants using SPR Biacore 3000 instrument,^{21,22} and the dissociation constants (*K_D* values in μM) were listed in Table 1. The dissociation constants clearly indicate that (1) three types of neamine–nucleoside conjugates show the better binding properties to 16S RNA and TAR RNA than the neamine derivatives reported previously.¹⁸ It indicates that the amino acid–ethylenediamine linker in these compounds

Table 1

The *K_D* values of neamine–nucleoside conjugates binding to A site of 16S and TAR RNA

Comp	<i>K_D</i> (16S) μM	<i>K_D</i> (TAR) μM
Neamine	19	24
10a	35	46
10b	39	49
10c	14	12
10d	16	29
10e	18	21
10f	23	27
10g	13	19
10h	13	27
10i	3	3
10j	18	21
10k	9	15
10l	9	11
10m	14	13
10n	14	12
10o	7	9
10p	12	10
10q	4	4
10r	13	5

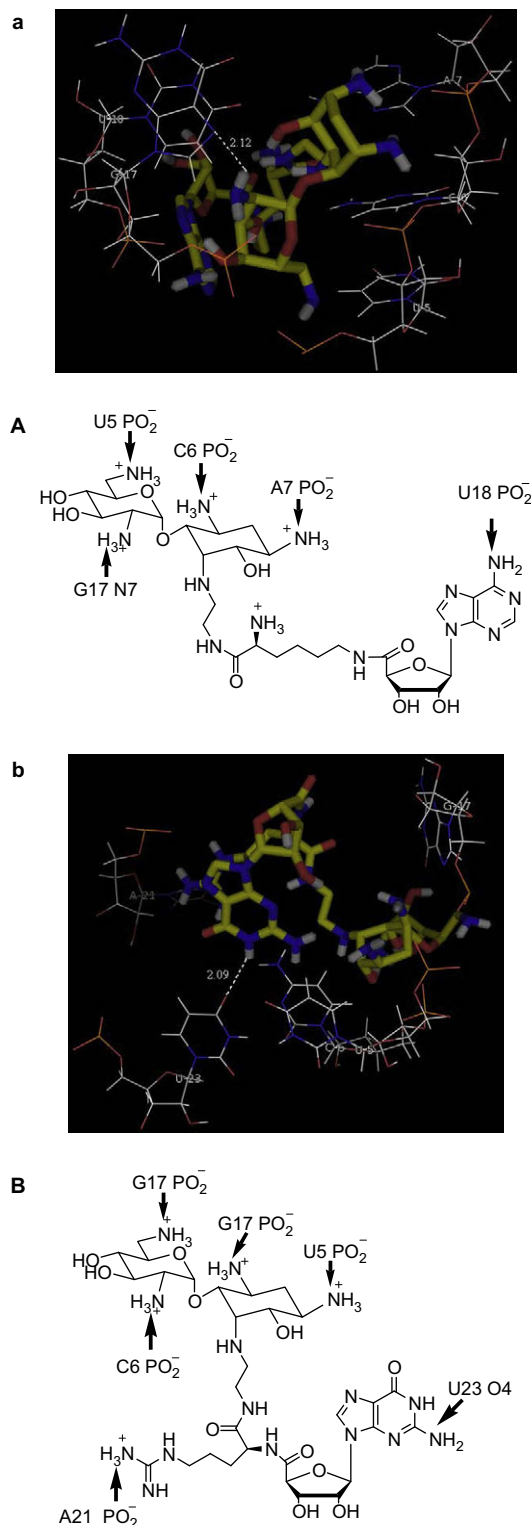


Figure 5. Molecular modeling for the interactions between **10i** (a, A), **10q** (b, B) and A site of 16S RNA. Panel (a) and (b) shows the hydrogen bonds only and panel (A) and (B) indicates the interactions including all the hydrogen bonds and electrostatic interactions.

could increase the binding affinities. (2) Compounds of types II and III show the binding properties with equal to or better than the parent neamine (for A site of 16S RNA, the dissociation constants K_D of compounds of type II are 3–18 μM and type III are 4–14 μM and for TAR RNA compounds of type II, K_D =3–27 μM and type III K_D =4–

13 μM , respectively). It seems that the ϵ -amino side chain of lysine in the structure of the compound of type I does not exhibit the contribution for the interaction with RNA and the guanidino group of arginine side chain exhibits more contributions in this interaction. (3) Interestingly, all of the compounds consisting of adenine or guanine (**10c**, **10g**, **10i**, **10k** and **10o**, **10q**) indicate the better K_D values than the others, among which **10i** and **10q** showed the K_D values of 6.3 and 4.8 times potential in binding to 16S RNA and eight and six times potential in binding to TAR RNA, respectively (3 and 4 μM to A site of 16S RNA and TAR RNA, respectively). It exhibits that the nucleobase could play an important role in the recognition of the designed compounds and target RNA.

To obtain more information about the interaction between the neamine–nucleoside conjugates and the A site of 16S RNA, molecular docking study of **10i** and **10q** was investigated.²³ The structure of A site of 16S RNA fragment was extracted from the protein databank (PDB code 1PBR) and the software AutoDock 3.0 was used for the molecular docking calculation of two neamine derivatives. By computer calculation, the final configuration (Fig. 5a and b) with the lowest docking energy was obtained and used for the analysis. Figure 5 shows the interaction between the two compounds and A site of 16S RNA. The electrostatic interactions and hydrogen bonds were considered as the major interactions, which were responsible for the ligands binding to the pocket of A site of 16S RNA. For compound **10i**, in addition to the four amino groups of neamine, nucleoside base adenine can form electrostatic interaction with U18 residue of A site of 16S RNA, and for compound **10q**, besides the four amino groups of neamine, the NH₂ on the guanidino group can form electrostatic interaction with A21 residue of A site of 16S RNA and the nucleobase guanine can form hydrogen bond with U23 residue of A site of 16S RNA. Figure 5a and b indicates that the amino acid–ethylenediamine linker could lead the neamine–nucleoside conjugates to the binding pocket of RNA and help improving the binding affinities to the target RNA. The molecular docking study is in agreement with the experimental data.

2.3. Conclusion

Eighteen novel neamine–nucleoside conjugates **10a–r** with ethylenediamine–lysine or ethylenediamine–arginine as the linker were synthesized and their potential binding to 16S RNA and TAR RNA was evaluated using SPR (surface plasmon resonance). Compared with neamine, compounds **10i** and **10q** show 6.3 and 4.8 times potential in binding to A site of 16S RNA and eight and six times potential in binding to TAR RNA, respectively. According to the data of SPR, it indicate that amino acid residue and nucleobase exhibit the important contributions for the binding of the designed neamine–nucleosides conjugates to A site of 16S RNA and TAR RNA. The molecular docking study on the interaction between the ligands and A site of 16S RNA is in agreement with the experimental data. The novel type of modification may provide a promising way for the development of neamine derivatives effectively targeting to RNAs.

3. Experimental section

3.1. Synthesis of neamine–nucleosides

3.1.1. General procedures for the synthesis of neamine–nucleoside conjugates **10a–r**

All chemical reagents and solvents were purchased from commercial suppliers. DMF was distilled from P₂O₅ below 40 °C. Silica gel 60H (200–300 mesh) manufactured by Qing-dao Haiyang Chemical Company (China) was used for column chromatography. ¹H and ¹³C NMR spectra were recorded on Bruker 400 MHz instrument with TMS as an internal standard. HRMS was obtained at MDS SCIEX QSTAR and Bruker DALTONICS APEX IV 70e

instruments, and the data were reported in *m/e* (intensity to 100%). UV absorption spectra were recorded on Cary 300 and optical rotations were obtained by Rudolph Research Analytical Autopol III Automatic Polarimeter.

3.1.2. General procedure for the synthesis of **4a–d**

To a solution of nucleosides **3a–d** (2.0 g) in acetone (120 mL) was added HClO₄ (70%, 0.9 mL), the reaction mixture was kept stirring for 1.5 h at room temperature before aqueous ammonia (28%, 1.5 mL) was added. After standing by 3 h, the reaction mixture was filtered and compounds **4a–d** were obtained as white powder.

3.1.2.1. 2',3'-O-Isopropylidene-adenosine (4a). Yield: 78% from **3a**. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.34 (s, 1H, H-8), 8.15 (s, 1H, H-2), 7.34 (s, 2H, –NH₂), 6.12 (d, *J*=3.0 Hz, H-1'), 5.35 (dd, 1H, *J*=6.0, 3.0 Hz, H-2'), 5.23 (t, 1H, *J*=5.5 Hz, –OH), 4.96 (dd, 1H, *J*=4.8, 2.5 Hz, H-3'), 4.20–4.22 (m, 1H, H-4'), 3.52–3.55 (m, 2H, H-5'), 1.55 (s, 3H, –CH₃), 1.33 (s, 3H, –CH₃).

3.1.2.2. 2',3'-O-Isopropylidene-cytidine (4b). Yield: 74% from **3b**. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.72 (d, 1H, *J*=7.4 Hz, H-6), 7.27 (d, *J*=33.0 Hz, 2H, –NH₂), 5.77 (d, *J*=2.0 Hz, 1H, H-1'), 5.71 (d, 1H, *J*=7.4 Hz, H-5), 4.99 (t, 1H, *J*=5.1 Hz, –OH), 4.85 (dd, 1H, *J*=6.3, 2.1 Hz, H-2'), 4.74 (dd, 1H, *J*=6.2, 3.8 Hz, H-3'), 4.03 (dd, 1H, *J*=8.3, 4.3 Hz, H-4'), 3.55–3.59 (m, 2H, H-5'), 1.48 (s, 3H, –CH₃), 1.28 (s, 3H, –CH₃).

3.1.2.3. 2',3'-O-Isopropylidene-guanosine (4c). Yield: 79% from **3c**. ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.66 (br, 1H, H-1), 7.91 (s, 1H, H-8), 6.50 (s, 2H, –NH₂), 5.92 (d, *J*=3.0 Hz, H-1'), 5.19 (dd, 1H, *J*=6.5, 3.0 Hz, H-2'), 5.03 (t, 1H, *J*=5.5 Hz, –OH), 4.97 (dd, 1H, *J*=6.5, 3.0 Hz, H-3'), 4.10–4.13 (m, 1H, H-4'), 3.51–3.54 (m, 2H, H-5'), 1.51 (s, 3H, –CH₃), 1.32 (s, 3H, –CH₃).

3.1.2.4. 2',3'-O-Isopropylidene-inosine (4d). Yield: 75% from **3d**. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.42 (s, 1H, H-1), 8.31 (s, 1H, H-2), 8.09 (s, 1H, H-8), 6.10 (d, 1H, *J*=3.0 Hz, H-1'), 5.26 (dd, 1H, *J*=6.0, 3.0 Hz, H-2'), 5.11 (t, 1H, *J*=5.0 Hz, –OH), 4.93 (dd, 1H, *J*=6.5, 2.5 Hz, H-3'), 4.20–4.24 (m, 1H, H-4'), 3.52–3.55 (m, 2H, H-5'), 1.54 (s, 3H, –CH₃), 1.32 (s, 3H, –CH₃).

3.1.3. General procedure for the synthesis of **5a–d**¹⁸

Compounds **4a–d** (3 mmol), BAIB (6 mmol), and TEMPO (0.6 mmol) were mixed and to this mixture was added 2 mL of a 1:1 CH₃CN/H₂O solution. The reaction mixture was stirred for 4 h at room temperature. The solvent was removed and the residue was washed with diethyl ether and acetone to afford compounds **5a–d** as white foam.

3.1.3.1. 5'-Carboxylic acid-2',3'-O-isopropylidene-adenosine (5a). Yield: 82% from **4a**. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.42 (s, 1H, H-8), 8.01 (s, 1H, H-2), 7.25 (s, 2H, –NH₂), 6.28 (s, 1H, H-1'), 5.34–5.38 (m, 2H, H-2' and 3'), 4.60 (s, 1H, H-4'), 1.52 (s, 3H, –CH₃), 1.34 (s, 3H, –CH₃).

3.1.3.2. 5'-Carboxylic acid-2',3'-O-isopropylidene-cytidine (5b). Yield: 77% from **4b**. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.51 (br, 1H, –COOH), 7.72 (d, 1H, *J*=6.0 Hz, H-6), 7.22 (d, 2H, *J*=26.0 Hz, –NH₂), 5.67 (d, *J*=7.0 Hz, 1H, H-5), 5.63 (s, 1H, H-1'), 5.52 (dd, 1H, *J*=6.0, 2.0 Hz, H-3'), 5.06 (d, 1H, *J*=6.0 Hz, H-2'), 4.47 (d, 1H, *J*=2.0 Hz, H-4'), 1.45 (s, 3H, –CH₃), 1.29 (s, 3H, –CH₃).

3.1.3.3. 5'-Carboxylic acid-2',3'-O-isopropylidene-guanosine (5c). Yield: 79% from **4c**. ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.80 (s, 1H, H-1), 8.03 (s, 1H, H-8), 6.55 (s, 2H, –NH₂), 6.03 (d, 1H, *J*=1.0 Hz, H-1'), 5.32 (s, 1H, H-2'), 5.15 (d, 1H, *J*=5.5 Hz, H-3'), 4.48 (s, 1H, H-4'), 1.51 (s, 3H, –CH₃), 1.36 (s, 3H, –CH₃).

3.1.3.4. 5'-Carboxylic acid-2',3'-O-isopropylidene-inosine (5d). Yield: 75% from **4d**. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.23 (s, 1H, H-2), 8.01 (s, 1H, H-8), 6.31 (s, 1H, H-1'), 5.45 (d, 1H, *J*=5.5 Hz, H-2'), 5.40 (d, 1H, *J*=5.5 Hz, H-3'), 4.70 (s, 1H, H-4'), 1.52 (s, 3H, –CH₃), 1.34 (s, 3H, –CH₃).

3.1.4. General procedure for the synthesis of **7a** and **7b**

DCC (29 mg, 0.14 mmol), HOBt (19 mg, 0.14 mmol), and Fmoc-Arg(Pbf)-OH or Boc-Lys(Fmoc)-OH (0.14 mmol) were dissolved in anhydrous DMF (1.5 mL). The mixture was stirred in an ice bath for 0.5 h then compound **6** (55 mg, 0.12 mmol) was added. After 6 h, the reaction mixture was filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH₂Cl₂/CH₃OH=100:4) to afford a white foam.

3.1.4.1. epi-5-N[[[¹⁵N-(tert-Butoxycarbonyl)-^εN-(9-fluorenylmethyloxycarbonyl)]-lysyl]aminoethyl]-1,3,2',6'-tetraazido neamine (7a). Yield: 74% from **6**, white foam. ¹H NMR (400 MHz, CD₃OD): δ 7.79 (d, 2H, *J*=7.5 Hz, 2×Fmoc-H), 7.65 (d, 2H, *J*=7.4 Hz, 2×Fmoc-H), 7.39 (t, 2H, *J*=7.4 Hz, 2×Fmoc-H), 7.31 (t, 2H, *J*=7.4 Hz, 2×Fmoc-H), 5.11 (d, 1H, *J*=3.5 Hz, H-1'), 4.35 (d, 2H, *J*=6.8 Hz, Fmoc-CH₂–), 4.20 (t, 1H, *J*=6.8 Hz, Fmoc-CH–), 3.98–4.02 (m, 2H, H-5' and Lys-α-CH–), 3.76–3.92 (m, 3H, H-3', 1 and 3), 3.59–3.62 (dd, 1H, *J*=10.2, 3.2 Hz, H-4), 3.50–3.54 (dd, 1H, *J*=13.3, 2.2 Hz, H-6'a), 3.27–3.41 (m, 7H, H-2', 4', 5, 6, 6'b and Lys-CH₂–), 2.98–3.07 (m, 2H, Eda-CH₂–), 3.10–3.14 (m, 2H, Eda-CH₂–), 2.18–2.22 (m, 1H, H_{eq}-2), 1.58–1.76 (m, 2H, Lys-CH₂–), 1.48–1.52 (m, 2H, Lys-CH₂–), 1.32–1.48 (m, 2H, Lys-CH₂–), 1.44 (s, 9H, –CH₃×3), 1.13–1.23 (q, 1H, *J*=12.5 Hz, H_{ax}-2). ¹³C NMR (100 MHz, CD₃OD): δ 178.00 (–C=O), 158.99 (–C=O), 157.90 (–C=O), 145.39 (2×Fmoc-C), 142.62 (2×Fmoc-C), 128.78 (2×Fmoc-C), 128.16 (2×Fmoc-C), 126.19 (2×Fmoc-C), 120.94 (2×Fmoc-C), 95.62 (C-1'), 80.66 (Boc-C), 78.97 (C-4), 75.79 (C-4'), 73.53 (C-6), 73.13 (C-3'), 72.59 (C-5), 67.64 (Fmoc-CH₂–), 64.91 (C-5'), 60.69 (C-2'), 59.72 (C-1), 59.44 (C-3), 56.50 (Lys-α-C), 52.53 (C-6'), 50.42 (Eda-CH₂– and Fmoc-CH–), 41.29 (Eda-CH₂– and Lys-CH₂–), 33.68 (C-2), 33.09 (Lys-CH₂–), 30.52 (Lys-CH₂–), 28.75 (3×Boc-CH₃), 24.09 (Lys-CH₂–). HRMS calcd for C₄₀H₅₄N₁₆O₁₀ [(M+H)⁺]: 919.4282, found: 919.4278. [α]_D²⁰ 0.545 (CH₃OH, c 0.099).

3.1.4.2. epi-5-N[[[¹⁵N-(9-Fluorenylmethyloxycarbonyl)-^GN-(2,2,4,6,7-pentamethyl dihydrobenzofuran-5-sulfonyl)]arganyl]aminoethyl]-1,3,2',6'-tetraazido neamine (7b). Yield: 79% from **6**, white foam. ¹H NMR (400 MHz, CD₃OD): δ 7.78 (d, 2H, *J*=7.5 Hz, 2×Fmoc-H), 7.66 (t, 2H, *J*=7.7 Hz, 2×Fmoc-H), 7.38 (t, 2H, *J*=7.5 Hz, 2×Fmoc-H), 7.30 (t, 2H, *J*=7.3 Hz, 2×Fmoc-H), 5.12 (d, 1H, *J*=3.0 Hz, H-1'), 4.37–4.47 (m, 2H, Fmoc-CH₂–), 4.21 (t, 1H, *J*=6.8 Hz, Fmoc-CH–), 4.06–4.13 (m, 1H, Arg-α-CH–), 3.98–4.01 (m, 1H, H-5'), 3.75–3.94 (3H, H-1, 3 and 3'), 3.58–3.61 (dd, 1H, *J*=9.2, 2.7 Hz, H-4), 3.50 (dd, 1H, *J*=11.3, 2.1 Hz, H-6'a), 3.27–3.44 (m, 7H, H-6'b, 2', 4', 5, 6, and Arg-CH₂–), 3.15–3.18 (m, 2H, Eda-CH₂–), 2.96–3.08 (m, 2H, Eda-CH₂–), 2.98 (s, 2H, Pbf-CH₂–), 2.59 (s, 3H, Pbf-CH₃), 2.52 (s, 3H, Pbf-CH₃), 2.14–2.17 (m, 1H, H_{eq}-2), 2.07 (s, 3H, Pbf-CH₃), 1.52–1.79 (m, 4H, 2×Arg-CH₂–), 1.42 (s, 6H, 2×Pbf-CH₃), 1.11–1.20 (m, 1H, H_{ax}-2). ¹³C NMR (100 MHz, CD₃OD): δ 174.81 (–C=O), 159.90 (Pbf-C), 158.46 (–C=O), 158.17 (–C=NH), 145.39 (2×Fmoc-C), 142.63 (2×Fmoc-C), 139.43 (Pbf-C), 134.32 (Pbf-C), 133.55 (Pbf-C), 128.80 (2×Fmoc-C), 128.19 (2×Fmoc-C), 126.22 (2×Fmoc-C), 126.05 (Pbf-C), 120.95 (2×Fmoc-C), 118.47 (Pbf-C), 95.72 (C-1'), 87.65 (Pbf-C), 79.50 (C-4), 75.67 (C-4'), 73.53 (C-6), 73.14 (C-3'), 72.58 (C-5), 67.85 (Fmoc-CH₂–), 64.90 (C-5'), 60.66 (C-2'), 59.69 (C-1), 59.47 (C-3), 56.50 (Arg-α-CH–), 52.52 (C-6'), 50.45 (Eda-CH₂– and Fmoc-CH–), 43.95 (Pbf-CH₂–), 41.30 (Eda-CH₂– and Arg-CH₂–), 33.50 (C-2 and Arg-CH₂–), 28.68 (2×Pbf-CH₃ and Arg-CH₂–), 19.63 (Pbf-CH₃), 18.43 (Pbf-CH₃), 12.52 (Pbf-CH₃). HRMS calcd for C₄₈H₆₂N₁₈O₁₁S [(M+H)⁺]: 1099.4639, found: 1099.4643. [α]_D²⁰ 0.446 (H₂O, c 0.101).

3.1.5. *epi-5-N[[[¹⁵N-(9-Fluorenylmethoxycarbonyl)]-lysiny]aminoethyl]-1,3,2',6'-tetraazido neamine (8a)*

Compound **7a** (50 mg, 0.05 mmol) was dissolved in 20% CF₃COOH in CH₂Cl₂ (2 mL) and kept stirring for about 1.5 h at room temperature then Et₂O (2 mL) was added and the white solid precipitated was purified by column chromatography on silica gel with gradient elution (CH₂Cl₂/CH₃OH=100:5) and the final pure product **8a** was obtained as a white foam (34 mg, yield 76%). ¹H NMR (400 MHz, CD₃OD): δ 7.79 (d, 2H, J=7.5 Hz, 2×Fmoc-H), 7.65 (d, 2H, J=7.4 Hz, 2×Fmoc-H), 7.39 (t, 2H, J=7.4 Hz, 2×Fmoc-H), 7.32 (t, 2H, J=7.4 Hz, 2×Fmoc-H), 5.10 (d, 1H, J=3.5 Hz, H-1'), 4.35 (d, 2H, J=6.8 Hz, Fmoc-CH₂-), 4.20 (t, 1H, J=6.8 Hz, Fmoc-CH-), 3.98–4.03 (m, 1H, H-5'), 3.78–3.93 (m, 3H, H-1, 3 and 3'), 3.60 (dd, 1H, J=10.2, 3.2 Hz, H-4), 3.52 (dd, 1H, J=13.3, 2.2 Hz, H-6'a), 3.31–3.44 (m, 8H, H-6'b, 2', 4, 5, 6, Lys-α-CH- and Lys-CH₂-), 3.11–3.14 (m, 2H, Eda-CH₂-), 2.94–3.06 (m, 2H, Eda-CH₂-), 2.17–2.22 (m, 1H, H_{eq}-2), 1.36–1.45 (m, 2H, Lys-CH₂-), 1.48–1.74 (m, 4H, 2×Lys-CH₂-), 1.13–1.22 (q, 1H, J=12.5 Hz, H_{ax}-2). ¹³C NMR (100 MHz, CD₃OD): δ 177.74 (–C=O), 158.94 (–C=O), 145.38 (2×Fmoc-C), 142.62 (2×Fmoc-C), 128.78 (2×Fmoc-C), 128.16 (2×Fmoc-C), 126.18 (2×Fmoc-C), 120.94 (2×Fmoc-C), 95.61 (C-1'), 78.94 (C-4), 75.83 (C-4'), 73.55 (C-6), 73.12 (C-3'), 72.59 (C-5), 67.67 (Fmoc-CH₂-), 64.92 (C-5'), 60.78 (C-2'), 59.79 (C-1), 59.37 (C-3), 56.21 (Lys-α-CH-), 52.53 (C-6'), 50.53 (Eda-CH₂- and Fmoc-CH-), 41.15 (Eda-CH₂- and Lys-CH₂-), 36.01 (Lys-CH₂-), 33.67 (C-2), 30.72 (Lys-CH₂-), 23.92 (Lys-CH₂-). HRMS calcd for C₃₅H₄₆N₁₆O₈ ([M+H]⁺): 819.3757, found: 819.3759. [α]_D²⁰ 0.707 (CH₃OH, c 0.075).

3.1.6. *epi-5-N[[[¹⁵N-(tert-Butoxycarbonyl)]lysiny]aminoethyl]-1,3,2',6'-tetraazido neamine (8b)*

Compound **7a** (30 mg, 0.03 mmol) was dissolved in 10% Et₂NH in DMF (1 mL), after stirring for 10 min at room temperature, the resulting mixture was concentrated and the residue was purified by column chromatography on silica gel (CH₂Cl₂/CH₃OH=100:15) to afford a white foam (19 mg, yield 84%). ¹H NMR (400 MHz, CD₃OD): δ 5.15 (d, 1H, J=3.5 Hz, H-1'), 3.78–4.03 (m, 4H, H-5', 1, 3 and 3'), 3.61 (dd, 1H, J=10.2, 3.2 Hz, H-4), 3.52 (dd, 1H, J=13.3, 2.2 Hz, H-6'a), 3.31–3.65 (m, 8H, H-6'b, 2', 4, 5, 6, Lys-α-CH- and Lys-CH₂-), 2.98–3.08 (m, 2H, Eda-CH₂-), 2.85–2.89 (m, 2H, Eda-CH₂-), 2.18–2.24 (m, 1H, H_{eq}-2), 1.62–1.80 (m, 4H, 2×Lys-CH₂-), 1.46 (s, 9H, 3×Boc-CH₃), 1.29–1.59 (m, 2H, Lys-CH₂-), 1.19 (m, 1H, H_{ax}-2). ¹³C NMR (100 MHz, CD₃OD): δ 175.06 (–C=O), 157.91 (–C=O), 95.62 (C-1'), 80.80 (Boc-C), 78.98 (C-4), 75.76 (C-4'), 73.56 (C-6), 73.14 (C-3'), 72.59 (C-5), 64.92 (C-5'), 60.69 (C-2'), 59.75 (C-1), 59.46 (C-3), 55.93 (Lys-α-CH-), 52.53 (C-6'), 50.41 (Eda-CH₂-), 41.43 (Eda-CH₂-), 40.79 (Lys-CH₂-), 33.69 (C-2), 32.92 (Lys-CH₂-), 30.75 (Lys-CH₂-), 28.75 (3×Boc-CH₃), 23.95 (Lys-CH₂-). HRMS calcd for C₂₅H₄₄N₁₆O₈ ([M+H]⁺): 697.3601, found: 697.3601. [α]_D²⁰ 0.370 (CH₃OH, c 0.081).

3.1.7. *epi-5-N[[[¹⁵N-(2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl)]arginyl]aminoethyl]-1,3,2',6'-tetraazido neamine (8c)*

Compound **7b** (68 mg, 0.06 mmol) was dissolved in 10% Et₂NH in DMF (1 mL), after stirring for 10 min at room temperature, the resulting mixture was concentrated and the residue was purified by column chromatography on silica gel (CH₂Cl₂/CH₃OH=100:5) to afford a white foam (46 mg, yield 85%). ¹H NMR (400 MHz, CD₃OD): δ 5.15 (d, 1H, J=3.5 Hz, H-1'), 4.00–4.04 (m, 1H, H-5'), 3.80–3.96 (m, 3H, H-1, 3 and 3'), 3.61 (dd, 1H, J=10.2, 3.2 Hz, H-4), 3.52 (dd, 1H, J=13.3, 2.2 Hz, H-6'a), 3.27–3.46 (m, 8H, H-6'b, 2', 4', 5, 6, Arg-CH₂- and Arg-α-CH-), 3.10–3.25 (m, 2H, Eda-CH₂-), 2.96–3.08 (m, 2H, Eda-CH₂-), 3.00 (s, 2H, PbF-CH₂-), 2.58 (s, 3H, PbF-CH₃), 2.52 (s, 3H, PbF-CH₃), 2.17–2.22 (m, 1H, H_{eq}-2), 2.07 (s, 3H, PbF-CH₃), 1.56–1.72 (m, 4H, 2×Arg-CH₂-), 1.45 (s, 6H, 2×PbF-CH₃), 1.15 (q, 1H, J=12.5 Hz, H_{ax}-2). ¹³C NMR (100 MHz, CD₃OD): δ 177.26 (–C=O), 159.89 (PbF-C), 158.14 (–C=NH), 139.40 (PbF-C), 134.35 (PbF-C), 133.53 (PbF-C), 126.04

(PbF-C), 118.46 (PbF-C), 95.67 (C-1'), 87.69 (PbF-C), 79.03 (C-4), 75.81 (C-4'), 73.55 (C-6), 73.12 (C-3'), 72.58 (C-5), 64.92 (C-5'), 60.76 (C-2'), 59.80 (C-1), 59.42 (C-3), 55.75 (Arg-α-CH-), 52.54 (C-6'), 50.58 (Eda-CH₂-), 43.98 (PbF-CH₂-), 41.18 (Eda-CH₂- and Arg-CH₂-), 33.66 (C-2), 33.24 (Arg-CH₂-), 28.72 (2×PbF-CH₃ and Arg-CH₂-), 19.59 (PbF-CH₃), 18.40 (PbF-CH₃), 12.51 (PbF-CH₃). HRMS calcd for C₃₃H₅₂N₁₈O₉S ([M+H]⁺): 877.3958, found: 877.3955. [α]_D²⁰ 0.599 (CH₃OH, c 0.089).

3.1.8. General procedure for the synthesis of **9a–r**

DCC (0.13 mmol), HOBT (0.13 mmol), and compounds **2a**, **2b**, **5a**, **5b**, **5c** or **5d** (0.13 mmol) were dissolved in 1.5 mL anhydrous DMF. The mixture was stirred in an ice bath for 0.5 h then compound **8a**, **8b** or **8c** (0.10 mmol) was added. After 6 h, the reaction mixture was filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH₂Cl₂/CH₃OH=100:3) to afford a white foam.

3.1.8.1. Compound 9a. Yield: 80% from **8a**, white foam. ¹H NMR (400 MHz, CD₃OD): δ 7.84 (s, 1H, H-6), 7.78 (d, 2H, J=7.6 Hz, 2×Fmoc-H), 7.63 (d, 2H, J=7.4 Hz, 2×Fmoc-H), 7.38 (t, 2H, J=7.4 Hz, 2×Fmoc-H), 7.30 (t, 2H, J=7.4 Hz, 2×Fmoc-H), 6.20 (dd, 1H, J=8.0, 6.1 Hz, H-1'), 5.46 (d, 1H, J=6.1 Hz, H-3'), 5.11 (d, 1H, J=3.6 Hz, H-1'''), 4.51 (d, 1H, J=1.5 Hz, H-4'), 4.34–4.37 (m, 3H, Fmoc-CH₂- and Lys-α-CH-), 4.19 (t, 1H, J=6.8 Hz, Fmoc-CH-), 4.00–4.03 (m, 1H, H-5'''), 3.75–3.94 (m, 3H, H-3'', 1'' and 3''), 3.59–3.63 (dd, 1H, J=10.2, 3.3 Hz, H-4''), 3.50–3.54 (dd, 1H, J=13.2, 2.2 Hz, H-6'' a), 3.30–3.48 (m, 7H, H-6'' b, 2'', 4'', 5'', 6'' and Lys-CH₂-), 2.98–3.14 (m, 4H, 2×Eda-CH₂-), 2.56–2.60 (m, 1H, H-2'a), 2.33–2.37 (m, 1H, H-2'b), 2.15–2.23 (m, 1H, H_{eq}-2''), 2.10 (s, 3H, –CH₃), 1.87 (s, 3H, –CH₃), 1.72–1.87 (m, 2H, Lys-CH₂-), 1.49–1.54 (m, 2H, Lys-CH₂-), 1.40–1.46 (m, 2H, Lys-CH₂-), 1.14–1.20 (q, 1H, J=12.5 Hz, H_{ax}-2''). HRMS calcd for C₄₇H₅₈N₁₈O₁₄ ([M+H]⁺): 1099.4453, found: 1099.4419. [α]_D²⁰ 0.422 (CH₃OH, c 0.133). λ_{max}=210 nm, ε=26,356, λ_{max}=265 nm, ε=15,585.

3.1.8.2. Compound 9b. Yield: 77% from **8a**, white foam. ¹H NMR (400 MHz, CD₃OD): δ 7.95 (d, 1H, J=8.1 Hz, H-6), 7.78 (d, 2H, J=7.5 Hz, 2×Fmoc-H), 7.63 (d, 2H, J=7.5 Hz, 2×Fmoc-H), 7.39 (t, 2H, J=7.5 Hz, 2×Fmoc-H), 7.30 (t, 2H, J=7.5 Hz, 2×Fmoc-H), 5.94 (d, 1H, J=2.6 Hz, H-1'), 5.73 (d, 1H, J=8.0 Hz, H-5), 5.63–5.65 (m, 2H, H-2' and H-3'), 5.11 (d, 1H, J=3.5 Hz, H-1'''), 4.61 (d, 1H, J=2.6 Hz, H-4'), 4.34–4.38 (m, 3H, Fmoc-CH₂- and Lys-α-CH-), 4.20 (t, 1H, J=7.5 Hz, Fmoc-CH-), 3.99–4.02 (m, 1H, H-5'''), 3.77–3.92 (m, 3H, H-3'', 1'' and 3''), 3.62 (dd, 1H, J=10.2, 3.2 Hz, H-4''), 3.53 (dd, 1H, J=13.3, 2.2 Hz, H-6'' a), 3.30–3.48 (m, 7H, H-6'' b, 2'', 4'', 5'', 6'' and Lys-CH₂-), 2.99–3.16 (m, 4H, 2×Eda-CH₂-), 2.18–2.23 (m, 1H, H_{eq}-2''), 2.13 (s, 3H, –COCH₃), 2.05 (s, 3H, –COCH₃), 1.72–1.86 (m, 2H, Lys-CH₂-), 1.47–1.54 (m, 2H, Lys-CH₂-), 1.33–1.46 (m, 2H, Lys-CH₂-), 1.21 (q, 1H, J=12.5 Hz, H_{ax}-2''). HRMS calcd for C₄₈H₅₈N₁₈O₁₆ ([M+H]⁺): 1143.4351, found: 1143.4321. [α]_D²⁰ 0.217 (CH₃OH, c 0.216). λ_{max}=210 nm, ε=23,203, λ_{max}=263 nm, ε=13,280.

3.1.8.3. Compound 9c. Yield: 73% from **8a**, white foam. ¹H NMR (400 MHz, CD₃OD): δ 8.16 (s, 1H, H-8), 8.14 (s, 1H, H-2), 7.79 (d, 2H, J=7.5 Hz, 2×Fmoc-H), 7.64 (d, 2H, J=7.5 Hz, 2×Fmoc-H), 7.39 (t, 2H, J=7.5 Hz, 2×Fmoc-H), 7.31 (t, 2H, J=7.5 Hz, 2×Fmoc-H), 6.34 (s, 1H, H-1'), 5.67 (dd, 1H, H-2'), 5.46 (d, 1H, H-3'), 5.08 (d, 1H, J=3.5 Hz, H-1'''), 4.75 (d, 1H, J=2.6 Hz, H-4'), 4.37 (d, 2H, J=6.8 Hz, Fmoc-CH₂-), 4.21 (t, 1H, J=6.6 Hz, Fmoc-CH-), 4.00–4.03 (m, 1H, H-5'''), 3.80–3.93 (m, 3H, H-3'', 1'' and Lys-α-CH-), 3.68–3.73 (m, 1H, H-3''), 3.58 (dd, 1H, J=10.2, 3.2 Hz, H-4''), 3.53 (dd, 1H, J=13.3, 2.2 Hz, H-6'' a), 3.27–3.41 (m, 7H, H-6'' b, 2'', 4'', 5'', 6'' and Lys-CH₂-), 2.86–3.23 (m, 4H, 2×Eda-CH₂-), 2.20 (m, 1H, H_{eq}-2''), 1.58 (s, 3H, –CH₃), 1.39 (s, 3H, –CH₃), 1.11–1.34 (m, 6H, 3×Lys-CH₂-), 1.21 (q, 1H, J=12.5 Hz, H_{ax}-2''). HRMS calcd for C₄₈H₅₉N₂₁O₁₂ ([M+H]⁺): 1122.4725, found: 1122.4681. [α]_D²⁰ 0.241 (CH₃OH, c 0.207). λ_{max}=210 nm, ε=21,240, λ_{max}=263 nm, ε=12,708.

3.1.8.4. Compound 9d. Yield: 75% from **8a**, white foam. ^1H NMR (400 MHz, CD_3OD): δ 7.79 (d, 2H, $J=7.5$ Hz, $2\times\text{Fmoc-H}$), 7.64 (d, 2H, $J=7.5$ Hz, $2\times\text{Fmoc-H}$), 7.59 (d, 1H, $J=7.4$ Hz, H-6), 7.38 (t, 2H, $J=7.5$ Hz, $2\times\text{Fmoc-H}$), 7.30 (t, 2H, $J=7.5$ Hz, $2\times\text{Fmoc-H}$), 5.84 (d, 1H, $J=7.3$ Hz, H-5), 5.60 (s, 1H, H-1'), 5.36 (dd, 1H, $J=6.2$, 2.0 Hz, H-2'), 5.20 (d, 1H, $J=6.3$ Hz, H-3'), 5.10 (d, 1H, $J=3.5$ Hz, H-1''), 4.59 (d, 1H, $J=2.6$ Hz, H-4'), 4.34 (d, 2H, $J=6.3$ Hz, Fmoc-CH_2), 4.18–4.23 (m, 2H, Fmoc-CH and $\text{Lys-}\alpha\text{-CH}$), 3.74–4.04 (m, 4H, H-5''', 3'', 1'' and 3''), 3.59 (dd, 1H, $J=10.2$, 3.2 Hz, H-4''), 3.53 (dd, 1H, $J=13.3$, 2.2 Hz, H-6''a), 3.27–3.44 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Lys-CH_2), 2.96–3.14 (m, 4H, $2\times\text{Eda-CH}_2$), 2.20 (m, 1H, $\text{H}_{\text{eq-2''}}$), 1.68–1.73 (m, 2H, Lys-CH_2), 1.50 (s, 3H, $-\text{CH}_3$), 1.40–1.58 (m, 2H, Lys-CH_2), 1.32 (s, 3H, $-\text{CH}_3$), 1.17–1.32 (m, 2H, Lys-CH_2), 1.16 (q, 1H, $J=12.5$ Hz, $\text{H}_{\text{ax-2''}}$). HRMS calcd for $\text{C}_{47}\text{H}_{59}\text{N}_{19}\text{O}_{13}$ ($[\text{M}+\text{H}]^+$): 1098.4613, found: 1098.4602. $[\alpha]_{\text{D}}^{20}$ 0.251 (CH_3OH , c 0.187). $\lambda_{\text{max}}=210$ nm, $\epsilon=26,875$, $\lambda_{\text{max}}=265$ nm, $\epsilon=13,988$.

3.1.8.5. Compound 9e. Yield: 70% from **8a**, white foam. ^1H NMR (400 MHz, CD_3OD): δ 7.80 (d, 2H, $J=7.5$ Hz, $2\times\text{Fmoc-H}$), 7.77 (s, 1H, H-8), 7.66 (d, 2H, $J=7.5$ Hz, $2\times\text{Fmoc-H}$), 7.40 (t, 2H, $J=7.5$ Hz, $2\times\text{Fmoc-H}$), 7.32 (t, 2H, $J=7.5$ Hz, $2\times\text{Fmoc-H}$), 6.21 (s, 1H, H-1'), 5.73 (dd, 1H, $J=6.0$, 1.8 Hz, H-2'), 5.33 (d, 1H, $J=6.0$ Hz, H-3'), 5.10 (d, 1H, $J=3.5$ Hz, H-1''), 4.74 (d, 1H, $J=1.5$ Hz, H-4'), 4.36 (d, 2H, $J=6.8$ Hz, Fmoc-CH_2), 4.22 (t, 1H, $J=6.8$ Hz, Fmoc-CH), 3.70–4.02 (m, 5H, H-5''', 3'', 1'', $\text{Lys-}\alpha\text{-CH}$ and 3''), 3.58 (dd, 1H, $J=10.2$, 3.2 Hz, H-4''), 3.52 (dd, 1H, $J=13.3$, 2.2 Hz, H-6''a), 3.22–3.44 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Lys-CH_2), 2.90–3.03 (m, 4H, $2\times\text{Eda-CH}_2$), 2.18–2.22 (m, 1H, $\text{H}_{\text{eq-2''}}$), 1.56 (s, 3H, $-\text{CH}_3$), 1.40 (s, 3H, $-\text{CH}_3$), 1.29–1.42 (m, 6H, $3\times\text{Lys-CH}_2$), 1.16 (q, 1H, $J=12.5$ Hz, $\text{H}_{\text{ax-2''}}$). HRMS calcd for $\text{C}_{48}\text{H}_{59}\text{N}_{21}\text{O}_{13}$ ($[\text{M}+\text{H}]^+$): 1138.4674, found: 1138.4605. $[\alpha]_{\text{D}}^{20}$ 0.377 (CH_3OH , c 0.159). $\lambda_{\text{max}}=211$ nm, $\epsilon=28,154$, $\lambda_{\text{max}}=262$ nm, $\epsilon=22,941$.

3.1.8.6. Compound 9f. Yield: 78% from **8a**, white foam. ^1H NMR (400 MHz, CD_3OD): δ 8.14 (s, 1H, H-2), 8.01 (s, 1H, H-8), 7.79 (d, 2H, $J=7.5$ Hz, $2\times\text{Fmoc-H}$), 7.65 (d, 2H, $J=7.5$ Hz, $2\times\text{Fmoc-H}$), 7.38 (t, 2H, $J=7.5$ Hz, $2\times\text{Fmoc-H}$), 7.29 (t, 2H, $J=7.5$ Hz, $2\times\text{Fmoc-H}$), 6.35 (s, 1H, H-1'), 5.61 (dd, 1H, $J=6.0$, 1.7 Hz, H-2'), 5.41 (d, 1H, $J=6.0$ Hz, H-3'), 5.08 (d, 1H, $J=3.5$ Hz, H-1''), 4.77 (d, 1H, $J=1.6$ Hz, H-4'), 4.35 (d, 2H, $J=6.9$ Hz, Fmoc-CH_2), 4.22 (t, 1H, $J=6.8$ Hz, Fmoc-CH), 3.73–4.03 (m, 5H, H-5''', 3'', 1'', $\text{Lys-}\alpha\text{-CH}$ and 3''), 3.58 (dd, 1H, $J=10.2$, 3.2 Hz, H-4''), 3.52 (dd, 1H, $J=13.3$, 2.2 Hz, H-6''a), 3.26–3.44 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Lys-CH_2), 2.86–3.24 (m, 4H, $2\times\text{Eda-CH}_2$), 2.18–2.22 (m, 1H, $\text{H}_{\text{eq-2''}}$), 1.57 (s, 3H, $-\text{CH}_3$), 1.39 (s, 3H, $-\text{CH}_3$), 1.21 (q, 1H, $J=12.5$ Hz, $\text{H}_{\text{ax-2''}}$), 1.11–1.40 (m, 6H, $3\times\text{Lys-CH}_2$). HRMS calcd for $\text{C}_{48}\text{H}_{58}\text{N}_{20}\text{O}_{13}$ ($[\text{M}+\text{H}]^+$): 1123.4565, found: 1123.4522. $[\alpha]_{\text{D}}^{20}$ 0.242 (CH_3OH , c 0.174). $\lambda_{\text{max}}=211$ nm, $\epsilon=23,650$, $\lambda_{\text{max}}=255$ nm, $\epsilon=12,639$.

3.1.8.7. Compound 9g. Yield: 82% from **8b**, white foam. ^1H NMR (400 MHz, CD_3OD): δ 7.95 (s, 1H, H-6), 6.30 (dd, 1H, $J=8.0$, 6.0 Hz, H-1'), 5.41 (d, 1H, $J=5.9$ Hz, H-3'), 5.15 (d, 1H, $J=3.5$ Hz, H-1''), 4.44 (d, 1H, $J=1.5$ Hz, H-4'), 3.79–4.04 (m, 5H, H-5''', 3'', 1'', 3'' and $\text{Lys-}\alpha\text{-CH}$), 3.62 (dd, 1H, $J=10.2$, 3.2 Hz, H-4''), 3.53 (dd, 1H, $J=13.3$, 2.2 Hz, H-6''a), 3.25–3.47 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Lys-CH_2), 3.24–3.28 (m, 2H, Eda-CH_2), 2.97–3.07 (m, 2H, Eda-CH_2), 2.53–2.58 (m, 1H, H-2'a), 2.36–2.41 (m, 1H, H-2'b), 2.20–2.24 (m, 1H, $\text{H}_{\text{eq-2''}}$), 2.12 (s, 3H, $-\text{COCH}_3$), 1.91 (s, 3H, $-\text{CH}_3$), 1.57–1.79 (m, 4H, $2\times\text{Lys-CH}_2$), 1.45 (s, 9H, $3\times\text{Boc-CH}_3$), 1.38–1.45 (m, 2H, Lys-CH_2), 1.21 (q, 1H, $J=12.5$ Hz, $\text{H}_{\text{ax-2''}}$). HRMS calcd for $\text{C}_{37}\text{H}_{56}\text{N}_{18}\text{O}_{14}$ ($[\text{M}+\text{H}]^+$): 977.4296, found: 977.4315. $[\alpha]_{\text{D}}^{20}$ 0.497 (CH_3OH , c 0.187). $\lambda_{\text{max}}=208$ nm, $\epsilon=11,903$, $\lambda_{\text{max}}=264$ nm, $\epsilon=7338$.

3.1.8.8. Compound 9h. Yield: 80% from **8b**, white foam. ^1H NMR (400 MHz, CD_3OD): δ 8.03 (d, 1H, $J=8.0$ Hz, H-6), 6.00 (d, 1H, $J=5.3$ Hz, H-1'), 5.76 (d, 1H, $J=8.0$ Hz, H-5), 5.65 (t, 1H, $J=5.6$ Hz, H-2'), 5.58 (m, 1H, H-3'), 5.15 (d, 1H, $J=3.5$ Hz, H-1''), 4.53 (d, 1H, $J=3.7$ Hz, H-4'), 3.78–4.04 (m, 5H, H-5''', 3'', 1'', 3'' and $\text{Lys-}\alpha\text{-CH}$), 3.62 (dd, 1H, $J=10.2$, 3.2 Hz, H-4''), 3.53 (dd, 1H, $J=13.3$, 2.2 Hz, H-

6''a), 3.25–3.47 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Lys-CH_2), 3.24–3.28 (m, 2H, Eda-CH_2), 2.97–3.07 (m, 2H, Eda-CH_2), 2.20–2.24 (m, 1H, $\text{H}_{\text{eq-2''}}$), 2.12 (s, 3H, $-\text{CH}_3$), 1.91 (s, 3H, $-\text{CH}_3$), 1.50–1.79 (m, 4H, $2\times\text{Lys-CH}_2$), 1.45 (s, 9H, $3\times\text{Boc-CH}_3$), 1.33–1.50 (m, 2H, Lys-CH_2), 1.21 (q, 1H, $J=12.5$ Hz, $\text{H}_{\text{ax-2''}}$). HRMS calcd for $\text{C}_{38}\text{H}_{56}\text{N}_{18}\text{O}_{16}$ ($[\text{M}+\text{H}]^+$): 1021.4194, found: 1021.4189. $[\alpha]_{\text{D}}^{20}$ 0.454 (CH_3OH , c 0.183). $\lambda_{\text{max}}=207$ nm, $\epsilon=11,253$, $\lambda_{\text{max}}=258$ nm, $\epsilon=6062$.

3.1.8.9. Compound 9i. Yield: 75% from **8b**, white foam. ^1H NMR (400 MHz, CD_3OD): δ 8.24 (s, 1H, H-8), 8.18 (s, 1H, H-2), 6.36 (d, 1H, $J=0.9$ Hz, H-1'), 5.63 (dd, 1H, $J=6.0$, 2.3 Hz, H-2'), 5.52 (d, 1H, $J=5.6$ Hz, H-3'), 5.15 (d, 1H, $J=3.5$ Hz, H-1''), 4.64 (d, 1H, $J=1.8$ Hz, H-4'), 3.79–4.04 (m, 5H, H-5''', 3'', 1'', 3'' and $\text{Lys-}\alpha\text{-CH}$), 3.62 (dd, 1H, $J=10.2$, 3.2 Hz, H-4''), 3.53 (dd, 1H, $J=13.3$, 2.2 Hz, H-6''a), 3.30–3.48 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Lys-CH_2), 2.96–3.08 (m, 2H, Eda-CH_2), 2.76–2.87 (m, 2H, Eda-CH_2), 2.20–2.24 (m, 1H, $\text{H}_{\text{eq-2''}}$), 1.59 (s, 3H, $-\text{CH}_3$), 1.47–1.63 (m, 2H, Lys-CH_2), 1.45 (s, 9H, $3\times\text{Boc-CH}_3$), 1.41 (s, 3H, $-\text{CH}_3$), 1.21 (q, 1H, $J=12.5$ Hz, $\text{H}_{\text{ax-2''}}$), 1.00–1.13 (m, 4H, $2\times\text{Lys-CH}_2$). HRMS calcd for $\text{C}_{38}\text{H}_{57}\text{N}_{21}\text{O}_{12}$ ($[\text{M}+\text{H}]^+$): 1000.4568, found: 1000.4552. $[\alpha]_{\text{D}}^{20}$ 0.334 (CH_3OH , c 0.111). $\lambda_{\text{max}}=209$ nm, $\epsilon=10,295$, $\lambda_{\text{max}}=260$ nm, $\epsilon=7107$.

3.1.8.10. Compound 9j. Yield: 72% from **8b**, white foam. ^1H NMR (400 MHz, CD_3OD): δ 7.65 (d, 1H, $J=7.4$ Hz, H-6), 5.87 (d, 1H, $J=7.4$ Hz, H-5), 5.64 (s, 1H, H-1'), 5.26 (d, 1H, H-2'), 5.19 (d, 1H, $J=6.0$ Hz, H-3'), 5.15 (d, 1H, $J=3.5$ Hz, H-1''), 4.49 (d, 1H, $J=2.4$ Hz, H-4'), 3.81–4.04 (m, 5H, H-5''', 3'', 1'', 3'' and $\text{Lys-}\alpha\text{-CH}$), 3.62 (dd, 1H, $J=10.2$, 3.2 Hz, H-4''), 3.53 (dd, 1H, $J=13.3$, 2.2 Hz, H-6''a), 3.30–3.48 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Lys-CH_2), 2.86–3.30 (m, 4H, $2\times\text{Eda-CH}_2$), 2.20–2.24 (m, 1H, $\text{H}_{\text{eq-2''}}$), 1.60–1.74 (m, 2H, Lys-CH_2), 1.53 (s, 3H, $-\text{CH}_3$), 1.45 (s, 9H, $3\times\text{Boc-CH}_3$), 1.36 (s, 3H, $-\text{CH}_3$), 1.21 (q, 1H, $J=12.5$ Hz, $\text{H}_{\text{ax-2''}}$), 1.14–1.50 (m, 4H, $2\times\text{Lys-CH}_2$). HRMS calcd for $\text{C}_{37}\text{H}_{57}\text{N}_{19}\text{O}_{13}$ ($[\text{M}+\text{H}]^+$): 976.4456, found: 976.4451. $[\alpha]_{\text{D}}^{20}$ 0.424 (CH_3OH , c 0.203). $\lambda_{\text{max}}=209$ nm, $\epsilon=14,298$, $\lambda_{\text{max}}=268$ nm, $\epsilon=4978$.

3.1.8.11. Compound 9k. Yield: 74% from **8b**, white foam. ^1H NMR (400 MHz, CD_3OD): δ 7.83 (s, 1H, H-8), 6.21 (s, 1H, H-1'), 5.72 (dd, 1H, $J=6.0$, 1.5 Hz, H-2'), 5.41 (d, 1H, $J=6.0$ Hz, H-3'), 5.16 (d, 1H, $J=3.5$ Hz, H-1''), 4.61 (d, 1H, $J=1.6$ Hz, H-4'), 3.80–4.05 (m, 5H, H-5''', 3'', 1'', 3'' and $\text{Lys-}\alpha\text{-CH}$), 3.62 (dd, 1H, $J=10.2$, 3.2 Hz, H-4''), 3.53 (dd, 1H, $J=13.3$, 2.2 Hz, H-6''a), 3.30–3.48 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Lys-CH_2), 2.78–3.09 (m, 4H, $2\times\text{Eda-CH}_2$), 2.22 (m, 1H, $\text{H}_{\text{eq-2''}}$), 1.56 (s, 3H, $-\text{CH}_3$), 1.46 (s, 9H, $3\times\text{Boc-CH}_3$), 1.41 (s, 3H, $-\text{CH}_3$), 1.36–1.64 (m, 4H, $2\times\text{Lys-CH}_2$), 1.21 (q, 1H, $J=12.5$ Hz, $\text{H}_{\text{ax-2''}}$), 1.14–1.24 (m, 2H, Lys-CH_2). HRMS calcd for $\text{C}_{38}\text{H}_{57}\text{N}_{21}\text{O}_{13}$ ($[\text{M}+\text{H}]^+$): 1016.4518, found: 1016.4517. $[\alpha]_{\text{D}}^{20}$ 0.490 (CH_3OH , c 0.165). $\lambda_{\text{max}}=206$ nm, $\epsilon=10,380$, $\lambda_{\text{max}}=256$ nm, $\epsilon=8490$.

3.1.8.12. Compound 9l. Yield: 78% from **8b**, white foam. ^1H NMR (400 MHz, CD_3OD): δ 8.22 (s, 1H, H-2), 8.02 (s, 1H, H-8), 6.39 (s, 1H, H-1'), 5.62 (d, 1H, $J=6.0$, 1.6 Hz, H-2'), 5.47–5.51 (m, 1H, H-3'), 5.16 (d, 1H, $J=3.5$ Hz, H-1''), 4.66 (d, 1H, $J=1.8$ Hz, H-4'), 3.84–4.04 (m, 5H, H-5''', 3'', 1'', 3'' and $\text{Lys-}\alpha\text{-CH}$), 3.62 (dd, 1H, $J=10.2$, 3.2 Hz, H-4''), 3.53 (dd, 1H, $J=13.3$, 2.2 Hz, H-6''a), 3.30–3.48 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Lys-CH_2), 2.78–3.09 (m, 4H, $2\times\text{Eda-CH}_2$), 2.20–2.24 (m, 1H, $\text{H}_{\text{eq-2''}}$), 1.61 (s, 3H, $-\text{CH}_3$), 1.46 (s, 9H, $3\times\text{Boc-CH}_3$), 1.41 (s, 3H, $-\text{CH}_3$), 1.40–1.64 (m, 4H, $2\times\text{Lys-CH}_2$), 1.21 (q, 1H, $J=12.5$ Hz, $\text{H}_{\text{ax-2''}}$), 1.12–1.20 (m, 2H, Lys-CH_2). HRMS calcd for $\text{C}_{38}\text{H}_{56}\text{N}_{20}\text{O}_{13}$ ($[\text{M}+\text{H}]^+$): 1001.4409, found: 1001.4382. $[\alpha]_{\text{D}}^{20}$ 0.413 (CH_3OH , c 0.196). $\lambda_{\text{max}}=207$ nm, $\epsilon=11,977$, $\lambda_{\text{max}}=245$ nm, $\epsilon=7761$.

3.1.8.13. Compound 9m. Yield: 78% from **8c**, white foam. ^1H NMR (400 MHz, CD_3OD): δ 7.88 (s, 1H, H-6), 6.26 (t, 1H, $J=7.0$ Hz, H-1'), 5.47 (d, 1H, $J=6.0$ Hz, H-4'), 5.14 (d, 1H, $J=3.5$ Hz, H-1''), 4.52–4.56 (m, 1H, H-3'), 4.44 (dd, 1H, $\text{Arg-}\alpha\text{-CH}$), 3.76–4.07 (m, 4H, H-5''', H-1'', 3'' and 3''), 3.61 (dd, 1H, $J=10.2$, 3.2 Hz, H-4''), 3.52 (dd, 1H,

$J=13.3, 2.2$ Hz, H-6''a), 3.30–3.46 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Arg-CH₂-), 3.14–3.20 (m, 2H, Eda-CH₂-), 2.96–3.10 (m, 2H, Eda-CH₂-), 3.00 (s, 2H, PbF-CH₂-), 2.63 (m, 1H, H-2'a), 2.58 (s, 3H, -CH₃), 2.52 (s, 3H, -CH₃), 2.37–2.41 (m, 1H, H-2'b), 2.17–2.21 (m, 1H, H_{eq}-2''), 2.13 (s, 3H, -COCH₃), 2.07 (s, 3H, -CH₃), 1.88 (s, 3H, -CH₃), 1.65–1.88 (m, 2H, Arg-CH₂-), 1.50–1.65 (m, 2H, Arg-CH₂-), 1.45 (s, 6H, 2×-CH₃), 1.15 (q, 1H, $J=12.5$ Hz, H_{ax}-2''). HRMS calcd for C₄₅H₆₄N₂₀O₁₅S ([M+H]⁺): 1157.4654, found: 1157.4641. $[\alpha]_D^{20}$ 0.446 (CH₃OH, c 0.182). $\lambda_{\max}=218$ nm, $\epsilon=35,050$, $\lambda_{\max}=257$ nm, $\epsilon=13,363$.

3.1.8.14. Compound 9n. Yield: 76% from **8c**, white foam. ¹H NMR (400 MHz, CD₃OD): δ 7.96 (d, 1H, $J=8.0$ Hz, H-6), 5.97 (d, 1H, $J=5.0$ Hz, H-1'), 5.73 (d, 1H, $J=8.0$ Hz, H-5), 5.64–5.68 (m, 2H, H-2' and 3'), 5.15 (d, 1H, $J=3.5$ Hz, H-1'''), 4.62 (d, 1H, $J=1.7$ Hz, H-4'), 4.45 (dd, 1H, $J=8.5, 5.0$ Hz, Arg- α -CH-), 3.76–4.03 (m, 4H, H-5'', 1'', 3'' and 3'''), 3.61 (dd, 1H, $J=10.2, 3.2$ Hz, H-4''), 3.52 (dd, 1H, $J=13.3, 2.2$ Hz, H-6''a), 3.28–3.46 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Arg-CH₂-), 3.00 (s, 2H, PbF-CH₂-), 2.95–3.19 (m, 4H, 2×Eda-CH₂-), 2.59 (s, 3H, PbF-CH₃), 2.52 (s, 3H, PbF-CH₃), 2.08–2.12 (m, 1H, H_{eq}-2''), 2.15 (s, 3H, -COCH₃), 2.08 (s, 3H, -COCH₃), 2.07 (s, 3H, PbF-CH₃), 1.50–1.90 (m, 4H, 2×Arg-CH₂-), 1.46 (s, 6H, 2×PbF-CH₃), 1.15 (q, 1H, $J=12.5$ Hz, H_{ax}-2''). HRMS calcd for C₄₆H₆₄N₂₀O₁₇S ([M+H]⁺): 1201.4552, found: 1201.4576. $[\alpha]_D^{20}$ 0.483 (CH₃OH, c 0.126). $\lambda_{\max}=218$ nm, $\epsilon=46,608$, $\lambda_{\max}=255$ nm, $\epsilon=20,341$.

3.1.8.15. Compound 9o. Yield: 72% from **8c**, white foam. ¹H NMR (400 MHz, CD₃OD): δ 8.26 (s, 1H, H-8), 8.15 (s, 1H, H-2), 6.38 (s, 1H, H-1'), 5.65 (dd, 1H, $J=6.0, 1.8$ Hz, H-2'), 5.51 (d, $J=5.6$ Hz, H-3'), 5.12 (d, 1H, $J=3.5$ Hz, H-1'''), 4.73 (d, 1H, $J=1.7$ Hz, H-4'), 3.71–4.02 (m, 5H, H-5'', 1'', 3'', 3''' and Arg- α -CH-), 3.61 (dd, 1H, $J=10.2, 3.2$ Hz, H-4''), 3.52 (dd, 1H, $J=13.3, 2.2$ Hz, H-6''a), 3.19–3.46 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Arg-CH₂-), 3.00 (s, 2H, PbF-CH₂-), 2.86–3.06 (m, 4H, 2×Eda-CH₂-), 2.59 (s, 3H, -CH₃), 2.52 (s, 3H, -CH₃), 2.08–2.12 (m, 1H, H_{eq}-2''), 2.09 (s, 3H, -CH₃), 1.60 (s, 3H, -CH₃), 1.45 (s, 6H, 2×-CH₃), 1.41 (s, 3H, -CH₃), 1.16 (q, $J=12.5$ Hz, H_{ax}-2''), 0.93–1.36 (m, 4H, 2×Arg-CH₂-). HRMS calcd for C₄₆H₆₅N₂₃O₁₃S ([M+H]⁺): 1180.4926, found: 1180.4910. $[\alpha]_D^{20}$ 0.316 (CH₃OH, c 0.105). $\lambda_{\max}=215$ nm, $\epsilon=45,876$, $\lambda_{\max}=257$ nm, $\epsilon=25,712$.

3.1.8.16. Compound 9p. Yield: 70% from **8c**, white foam. ¹H NMR (400 MHz, CD₃OD): δ 7.64 (d, 1H, $J=7.5$ Hz, H-6), 5.87 (d, 1H, $J=7.4$ Hz, H-5), 5.58 (s, 1H, H-1'), 5.22–5.24 (m, 2H, H-2' and H-3'), 5.12 (d, 1H, $J=3.6$ Hz, H-1'''), 4.56 (s, 1H, H-4'), 4.32–4.35 (m, 1H, H-5'''), 3.74–4.03 (m, 4H, H-1'', 3'', 3''' and Arg- α -CH-), 3.61 (dd, 1H, $J=10.2, 3.2$ Hz, H-4''), 3.52 (dd, 1H, $J=13.3, 2.2$ Hz, H-6''a), 3.18–3.46 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Arg-CH₂-), 2.95–3.24 (m, 4H, 2×Eda-CH₂-), 3.00 (s, 2H, PbF-CH₂-), 2.58 (s, 3H, -CH₃), 2.52 (s, 3H, -CH₃), 2.08–2.12 (m, 1H, H_{eq}-2''), 2.09 (s, 3H, -CH₃), 1.50–1.80 (m, 4H, 2×Arg-CH₂-), 1.53 (s, 3H, -CH₃), 1.45 (s, 6H, 2×-CH₃), 1.35 (s, 3H, -CH₃), 1.16 (q, 1H, $J=12.5$ Hz, H_{ax}-2''). HRMS calcd for C₄₅H₆₅N₂₁O₁₄S ([M+H]⁺): 1156.4813, found: 1156.4816. $[\alpha]_D^{20}$ 0.313 (CH₃OH, c 0.163). $\lambda_{\max}=218$ nm, $\epsilon=42,416$, $\lambda_{\max}=255$ nm, $\epsilon=15,459$.

3.1.8.17. Compound 9q. Yield: 71% from **8c**, white foam. ¹H NMR (400 MHz, CD₃OD): δ 7.84 (s, 1H, H-8), 6.24 (s, 1H, H-1'), 5.74 (dd, 2H, $J=6.0, 1.8$ Hz, H-2'), 5.40 (d, 1H, $J=6.0$ Hz, H-3'), 5.12 (d, 1H, $J=3.6$ Hz, H-1'''), 4.73 (d, 1H, $J=1.8$ Hz, H-4'), 3.75–4.02 (m, 5H, H-5'', 1'', 3'', 3''' and Arg- α -CH-), 3.59 (dd, 1H, $J=10.2, 3.2$ Hz, H-4''), 3.52 (dd, 1H, $J=13.3, 2.2$ Hz, H-6''a), 3.25–3.46 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Arg-CH₂-), 2.92–3.20 (m, 4H, 2×Eda-CH₂-), 3.01 (s, PbF-CH₂-), 2.58 (s, 3H, -CH₃), 2.52 (s, 3H, -CH₃), 2.18–2.22 (m, 1H, H_{eq}-2''), 2.09 (s, 3H, -CH₃), 1.57 (s, 3H, -CH₃), 1.45 (s, 6H, 2×-CH₃), 1.41 (s, 3H, -CH₃), 1.11–1.41 (m, 4H, 2×Arg-CH₂-), 1.16 (q, 1H, $J=12.5$ Hz, H_{ax}-2''). HRMS calcd for C₄₆H₆₅N₂₃O₁₄S ([M+H]⁺): 1196.4875, found: 1196.4874. $[\alpha]_D^{20}$ 0.344 (CH₃OH, c 0.131). $\lambda_{\max}=219$ nm, $\epsilon=20,832$, $\lambda_{\max}=256$ nm, $\epsilon=10,768$.

3.1.8.18. Compound 9r. Yield: 75% from **8c**, white foam. ¹H NMR (400 MHz, CD₃OD): δ 8.22 (s, 1H, H-2), 8.04 (s, 1H, H-8), 6.40 (s, 1H, H-1'), 5.60 (dd, 1H, $J=6.0, 2.4$ Hz, H-2'), 5.48 (d, $J=6.0$ Hz, H-3'), 5.12 (d, 1H, $J=3.5$ Hz, H-1'''), 4.75 (d, 1H, $J=2.5$ Hz, H-4'), 3.72–4.03 (m, 5H, H-5'', 1'', 3'', 3''' and Arg- α -CH-), 3.59 (dd, 1H, $J=10.2, 3.2$ Hz, H-4''), 3.52 (dd, 1H, $J=13.3, 2.2$ Hz, H-6''a), 3.20–3.46 (m, 7H, H-6''b, 2'', 4'', 5'', 6'' and Arg-CH₂-), 3.01 (s, PbF-CH₂-), 2.92–3.20 (m, 4H, 2×Eda-CH₂-), 2.58 (s, 3H, -CH₃), 2.52 (s, 3H, -CH₃), 2.18–2.22 (m, 1H, H_{eq}-2''), 2.09 (s, 3H, -CH₃), 1.59 (s, 3H, -CH₃), 1.45 (s, 6H, 2×-CH₃), 1.41 (s, 3H, -CH₃), 1.11–1.21 (m, 4H, 2×Arg-CH₂-), 1.16 (q, 1H, $J=12.5$ Hz, H_{ax}-2''). HRMS calcd for C₄₆H₆₄N₂₂O₁₄S ([M+H]⁺): 1181.4766, found: 1181.4763. $[\alpha]_D^{20}$ 0.302 (CH₃OH, c 0.155). $\lambda_{\max}=218$ nm, $\epsilon=36,739$, $\lambda_{\max}=252$ nm, $\epsilon=17,367$.

3.1.9. General procedure for the synthesis of **10a** and **10b**

Compound **9a** or **9b** (0.1 mmol) was dissolved in a solution of 10% Et₂NH in DMF (1 mL), after stirring for 10 min at room temperature, the resulting mixture was concentrated then the residue was added NH₃/CH₃OH (2 mL) and kept stirring for 4 h in an ice bath. The solvent was removed and the residue was dissolved in Py/Et₃N/H₂O (5 mL, 4:3:2) and slowly bubbled by hydrogen sulfide for 1 h at room temperature then the reaction mixture was concentrated in vacuum and the residue was purified by HPLC (1% CF₃COOH in H₂O and CH₃OH) to afford compounds **10a** and **10b** as a salt of trifluoroacetic acid.

3.1.9.1. Compound 10a. Yield: 58% from **9a**, white foam. ¹H NMR (400 MHz, D₂O): δ 7.63 (s, 1H, H-6), 6.23 (t, 1H, $J=6.6$ Hz, H-1'), 5.66 (d, 1H, $J=3.4$ Hz, H-1'''), 4.77–4.81 (m, 1H, H-3'), 4.46 (d, 1H, $J=3.4$ Hz, H-4'), 4.42–4.45 (m, 1H, H-5''), 4.30–4.33 (m, 3H, H-3'', 4'' and 6''), 4.09–4.14 (t, 1H, $J=9.3$ Hz, Lys- α -CH-), 3.47–4.00 (m, 11H, H-2'', 4'', 6''a, 1'', 6''b, 3'', 5'' and 2×Eda-CH₂-), 2.99 (t, 2H, $J=8.0$ Hz, Lys-CH₂-), 2.64–2.75 (m, 2H, H_{eq}-2'' and H-2'a), 2.40–2.47 (m, 1H, H-2'b), 1.92–2.05 (q, 1H, $J=12.5$ Hz, H_{ax}-2''), 1.92 (s, 3H, -CH₃), 1.67–1.88 (m, 4H, 2×Lys-CH₂-), 1.40–1.44 (m, 2H, Lys-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 175.26 (C=O), 172.79 (C=O), 166.48 (C-4), 151.51 (C-2), 139.92 (C-6), 110.92 (C-5), 95.00 (C-1''), 89.57 (C-1'), 85.19 (C-4'), 73.58 (C-3' and 4'), 70.28 (C-4''), 69.56 (C-3'' and 6''), 68.21 (C-5''), 57.50 (C-2''), 53.81 (Eda-CH₂-), 52.75 (C-1'' and Lys- α -CH-), 47.50 (C-3''), 46.72 (C-5''), 39.54 (C-6''), 39.00 (Eda-CH₂-), 37.69 (Lys-CH₂-), 37.34 (C-2'), 30.09 (Lys-CH₂-), 27.93 (C-2''), 26.23 (Lys-CH₂-), 22.30 (Lys-CH₂-), 11.33 (-CH₃). HRMS calcd for C₃₀H₅₄N₁₀O₁₁ ([M+H]⁺): 731.4046, found: 731.4062. $[\alpha]_D^{28.6}$ 1.063 (H₂O, c 0.073). $\lambda_{\max}=265$ nm, $\epsilon=14,750$.

3.1.9.2. Compound 10b. Yield: 55% from **9b**, white foam. ¹H NMR (400 MHz, D₂O): δ 7.79 (d, 1H, $J=8.0$ Hz, H-6), 5.86 (d, 1H, $J=8.0$ Hz, H-5), 5.73 (d, 1H, $J=4.0$ Hz, H-1'), 5.53 (d, 1H, $J=3.6$ Hz, H-1'''), 4.61 (m, 1H, H-2'), 4.47–4.50 (m, 2H, H-3' and 4'), 3.19–4.29 (m, 16H, H-4'', 5'', 6'', Lys- α -CH-, 4'', 5'', 1'', 3'', 2'', 3'', 6''a, 6''b and 2×Eda-CH₂-), 2.96 (t, 2H, $J=8.0$ Hz, Lys-CH₂-), 2.55–2.59 (m, 1H, H_{eq}-2''), 1.74–1.86 (m, 3H, H_{ax}-2'' and Lys-CH₂-), 1.62–1.73 (m, 2H, Lys-CH₂-), 1.39–1.43 (m, 2H, Lys-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 177.26 (C=O), 174.83 (C=O), 168.91 (C-4), 154.08 (C-2), 147.00 (C-6), 104.78 (C-5), 96.84 (C-1''), 93.97 (C-1'), 85.38 (C-4'), 75.20 (C-3'), 74.92 (C-2' and 4''), 72.66 (C-4''), 72.44 (C-3'' and 6''), 71.05 (C-5''), 59.33 (C-2''), 56.67 (Eda-CH₂-), 55.62 (Lys- α -CH-), 52.04 (C-1''), 50.65 (C-3''), 49.55 (C-5''), 42.33 (C-6''), 41.77 (Eda-CH₂-), 41.33 (Lys-CH₂-), 32.84 (Lys-CH₂-), 30.96 (C-2'), 28.96 (Lys-CH₂-), 25.01 (Lys-CH₂-). HRMS calcd for C₂₉H₅₂N₁₀O₁₂ ([M+H]⁺): 733.3839, found: 733.3854. $[\alpha]_D^{28.7}$ 1.148 (H₂O, c 0.073). $\lambda_{\max}=259$ nm, $\epsilon=8950$.

3.1.10. General procedure for the synthesis of **10c–f**

Compounds **9c–f** (0.1 mmol) were dissolved in a solution of 10% Et₂NH in DMF (2 mL), after stirring for 10 min at room temperature, the mixture was concentrated then the residue was added 90%

CF₃COOH in H₂O (2 mL) and stirred for 3 h in an ice bath. The solvent was removed and the residue was dissolved in Py/Et₃N/H₂O (5 mL, 4:3:2) and slowly bubbled by hydrogen sulfide for 1 h at room temperature, the reaction mixture was concentrated in vacuum and the residue was purified by HPLC (1% CF₃COOH in H₂O and CH₃OH) to afford compounds **10c–f** as a salt of trifluoroacetic acid.

3.1.10.1. Compound 10c. Yield: 52% from **9c**, white foam. ¹H NMR (400 MHz, D₂O): δ 8.58 (s, 1H, H-8), 8.46 (s, 1H, H-2), 6.27 (d, 1H, J=5.0 Hz, H-1'), 5.60 (d, 1H, J=3.4 Hz, H-1''), 4.67–4.72 (3H, H-2', 3' and 4'), 4.34–4.38 (m, 2H, H-4'' and 3''), 4.15–4.25 (m, 2H, H-6'' and 5''), 4.06 (t, 1H, J=9.0 Hz, Lys-α-CH-), 3.36–3.98 (m, 11H, H-4''', 5'', 1'', 3'', 2'', 6''a, 6''b and 2×Eda-CH₂-), 2.96 (t, 2H, J=7.6 Hz, Lys-CH₂-), 2.61–2.64 (m, 1H, H_{eq}-2''), 1.89–1.95 (q, 1H, J=12.5 Hz, H_{ax}-2''), 1.66–1.89 (m, 4H, 2×Lys-CH₂-), 1.35–1.40 (m, 2H, Lys-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 176.91 (–C=O), 174.47 (–C=O), 153.59 (C-6), 151.05 (C-2), 148.50 (C-4), 145.70 (C-8), 121.91 (C-5), 95.58 (C-1'''), 91.84 (C-1'), 85.86 (C-4'), 76.03 (C-3'), 75.51 (C-2'), 75.33 (C-4''), 72.82 (C-4'''), 72.52 (C-6''), 71.12 (C-3'''), 65.21 (C-5''), 59.43 (C-2'''), 56.55 (Eda-CH₂-), 55.74 (Lys-α-CH-), 51.94 (C-1''), 50.81 (C-3''), 49.69 (C-5''), 42.48 (C-6''), 41.70 (Eda-CH₂-), 41.45 (Lys-CH₂-), 33.32 (Lys-CH₂-), 31.05 (C-2''), 29.02 (Lys-CH₂-), 25.00 (Lys-CH₂-). HRMS calcd for C₃₀H₅₃N₁₃O₁₀ ([M+H]⁺): 756.4111, found: 756.4117. [α]_D^{28.7} 1.123 (H₂O, c 0.076). λ_{max}=266 nm, ε=10,800.

3.1.10.2. Compound 10d. Yield: 50% from **9d**, white foam. ¹H NMR (400 MHz, D₂O): δ 8.22 (d, 1H, J=8.0 Hz, H-6), 6.28 (d, 1H, J=8.0 Hz, H-5), 5.86 (d, 1H, J=3.7 Hz, H-1'), 5.67 (d, 1H, J=3.5 Hz, H-1''), 4.55–4.58 (m, 2H, H-2' and 3'), 4.33–4.49 (m, 5H, H-4', 3'', 5'', 6'' and 4''), 4.14 (t, 1H, J=9.5 Hz, Lys-α-CH-), 3.35–4.06 (m, 11H, H-4''', 5'', 1'', 3'', 2'', 6''a, 6''b and 2×Eda-CH₂-), 3.03 (t, 2H, J=7.5 Hz, Lys-CH₂-), 2.64–2.68 (m, 1H, H_{eq}-2''), 2.07 (q, 1H, J=12.5 Hz, H_{ax}-2''), 1.83–1.93 (m, 2H, Lys-CH₂-), 1.69–1.81 (m, 2H, Lys-CH₂-), 1.45–1.53 (m, 2H, Lys-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 177.32 (–C=O), 174.42 (–C=O), 162.32 (C-4), 151.29 (C-2), 148.83 (C-6), 97.65 (C-5), 96.67 (C-1'''), 91.00 (C-1'), 85.13 (C-4'), 75.36 (C-3'), 75.11 (C-2'), 75.01 (C-4''), 72.70 (C-4'''), 72.28 (C-6''), 71.12 (C-3'''), 65.26 (C-5''), 59.68 (C-2'''), 56.57 (Eda-CH₂-), 55.70 (Lys-α-CH-), 52.35 (C-1''), 50.81 (C-3''), 49.98 (C-5''), 42.44 (C-6''), 41.88 (Eda-CH₂-), 41.45 (Lys-CH₂-), 33.06 (Lys-CH₂-), 30.97 (C-2''), 29.00 (Lys-CH₂-), 24.97 (Lys-CH₂-). HRMS calcd for C₂₉H₅₃N₁₁O₁₁ ([M+H]⁺): 732.3999, found: 732.3998. [α]_D^{28.8} 1.226 (H₂O, c 0.073). λ_{max}=267 nm, ε=13,200.

3.1.10.3. Compound 10e. Yield: 48% from **9e**, white foam. ¹H NMR (400 MHz, D₂O): δ 8.34 (s, 1H, H-8), 6.12 (d, 1H, J=3.0 Hz, H-1'), 5.60 (d, 1H, J=3.3 Hz, H-1''), 4.94 (t, 1H, J=5.2 Hz, H-2'), 4.86 (m, 1H, H-3'), 4.62 (d, 1H, J=1.3 Hz, H-4'), 4.35 (m, 1H, H-3''), 4.18–4.36 (m, 3H, H-4'', 5'' and 6''), 4.07 (t, 1H, J=9.8 Hz, Lys-α-CH-), 3.34–3.99 (m, 11H, H-4''', 5'', 1'', 3'', 2'', 6''a, 6''b and 2×Eda-CH₂-), 2.90 (t, 2H, J=7.8 Hz, Lys-CH₂-), 2.62 (m, 1H, H_{eq}-2''), 1.91 (q, 1H, J=12.5 Hz, H_{ax}-2''), 1.73–1.76 (m, 2H, Lys-CH₂-), 1.57–1.65 (m, 2H, Lys-CH₂-), 1.16–1.29 (m, 2H, Lys-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 177.94 (–C=O), 175.10 (–C=O), 159.98 (C-6), 157.06 (C-2), 153.35 (C-4), 140.27 (C-8), 115.77 (C-5), 92.79 (C-1'' and 1'), 85.49 (C-4'), 76.00 (C-3'), 75.50 (C-2'), 73.88 (C-4''), 73.09 (C-4'''), 72.33 (C-6''), 70.91 (C-3'''), 70.59 (C-5''), 60.38 (C-2'''), 56.69 (Eda-CH₂-), 55.43 (Lys-α-CH-), 53.34 (C-1''), 50.59 (C-3''), 49.27 (C-5''), 42.23 (C-6''), 41.66 (Eda-CH₂-), 40.14 (Lys-CH₂-), 32.79 (Lys-CH₂-), 30.64 (C-2''), 28.99 (Lys-CH₂-), 24.98 (Lys-CH₂-). HRMS calcd for C₃₀H₅₃N₁₃O₁₁ ([M+H]⁺): 772.4060, found: 772.4045. [α]_D^{29.0} 4.254 (H₂O, c 0.015). λ_{max}=263 nm, ε=62,250.

3.1.10.4. Compound 10f. Yield: 53% from **9f**, white foam. ¹H NMR (400 MHz, D₂O): δ 8.39 (s, 1H, H-2), 8.21 (s, 1H, H-8), 6.21 (d, 1H, J=4.0 Hz, H-1'), 5.56 (d, 1H, J=4.0 Hz, H-1''), 4.78–4.83 (m, 2H, H-2'

and 3'), 4.62 (d, 1H, J=4.4 Hz, H-4'), 4.23–4.37 (m, 4H, H-3''', 5'', 6'' and 4''), 4.00 (t, 1H, J=8.8 Hz, Lys-α-CH-), 3.32–3.95 (m, 11H, H-4''', 5'', 1'', 3'', 2'', 6''a, 6''b and 2×Eda-CH₂-), 2.89 (t, 2H, J=8.0 Hz, Lys-CH₂-), 2.58–2.63 (m, 1H, H_{eq}-2''), 1.88 (q, 1H, J=12.5 Hz, H_{ax}-2''), 1.52–1.79 (m, 4H, 2×Lys-CH₂-), 1.19–1.26 (m, 2H, Lys-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 177.91 (C=O), 174.90 (C=O), 160.99 (C-6), 151.21 (C-4), 149.08 (C-2), 143.24 (C-8), 126.59 (C-5), 94.61 (C-1'''), 92.07 (C-1'), 85.82 (C-4'), 78.27 (C-3'), 76.03 (C-2'), 75.69 (C-4''), 73.33 (C-4'''), 72.35 (C-6''), 70.98 (C-3'''), 70.52 (C-5''), 60.65 (C-2'''), 56.56 (Eda-CH₂-), 55.51 (Lys-α-CH-), 53.42 (C-1''), 50.83 (C-3''), 49.51 (C-5''), 42.31 (C-6''), 41.82 (Eda-CH₂-), 40.11 (Lys-CH₂-), 32.97 (Lys-CH₂-), 30.70 (C-2''), 29.01 (Lys-CH₂-), 25.07 (Lys-CH₂-). HRMS calcd for C₃₀H₅₂N₁₂O₁₁ ([M+H]⁺): 757.3051, found: 757.3970. [α]_D^{28.9} 1.058 (H₂O, c 0.076). λ_{max}=251 nm, ε=13,150.

3.1.11. General procedure for the synthesis of **10g** and **10h**

Compound **9g** or **9h** (0.1 mmol) was dissolved in 20% CF₃COOH in CH₂Cl₂ (2 mL), after stirring for 1.5 h at room temperature, Et₂O (2 mL) was added and the white solid precipitated was added NH₃/CH₃OH (2 mL) and stirred for 4 h in an ice bath. The solvent was removed and the residue was dissolved in Py/Et₃N/H₂O (5 mL, 4:3:2) and slowly bubbled by hydrogen sulfide for 1 h at room temperature, the reaction mixture was concentrated in vacuum and the residue was purified by HPLC (1% CF₃COOH in H₂O and CH₃OH) to afford compounds **10g** and **10h** as a salt of trifluoroacetic acid.

3.1.11.1. Compound 10g. Yield: 55% from **9g**, white foam. ¹H NMR (400 MHz, D₂O): δ 7.67 (s, 1H, H-6), 6.25 (t, 1H, J=6.8 Hz, H-1'), 5.58 (d, 1H, J=3.6 Hz, H-1''), 4.64 (m, 1H, H-3'), 4.38 (d, 1H, J=3.0 Hz, H-4'), 3.21–4.31 (m, 18H, H-5''', H-3''', 4'', 6'', Lys-α-CH-, 2'', 4'', 6''a, 1'', 6''b, 3'', 5'', Lys-CH₂- and 2×Eda-CH₂-), 2.57–2.60 (m, 1H, H_{eq}-2''), 2.45–2.52 (m, 1H, H-2'a), 2.29–2.35 (m, 1H, H-2'b), 1.85 (s, 3H, –CH₃), 1.79–1.92 (m, 3H, H_{ax}-2'' and Lys-CH₂-), 1.47–1.54 (m, 2H, Lys-CH₂-), 1.32–1.38 (m, 2H, Lys-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 174.50 (–C=O), 173.82 (–C=O), 169.13 (C-4), 154.39 (C-2), 141.48 (C-6), 113.95 (C-5), 94.59 (C-1'''), 90.32 (C-1'), 87.76 (C-4'), 76.35 (C-3'), 74.09 (C-4''), 73.22 (C-4'''), 72.42 (C-6''), 70.75 (C-3'''), 70.44 (C-5''), 61.05 (C-2'''), 55.68 (Eda-CH₂-), 55.45 (C-1''), 53.10 (Lys-α-CH-), 50.81 (C-3''), 49.43 (C-5''), 42.27 (C-6''), 41.38 (Eda-CH₂-), 41.29 (C-2'), 39.88 (Lys-CH₂-), 39.78 (Lys-CH₂-), 33.06 (C-2''), 30.61 (Lys-CH₂-), 24.25 (Lys-CH₂-), 14.20 (–CH₃). HRMS calcd for C₃₀H₅₄N₁₀O₁₁ ([M+H]⁺): 730.3974, found: 730.4062. [α]_D^{28.3} 1.227 (H₂O, c 0.073). λ_{max}=264 nm, ε=18,350.

3.1.11.2. Compound 10h. Yield: 54% from **9h**, white foam. ¹H NMR (400 MHz, D₂O): δ 7.92 (d, 1H, J=8.0 Hz, H-6), 5.86 (d, 1H, J=8.0 Hz, H-5), 5.83 (d, 1H, J=4.4 Hz, H-1'), 5.58 (d, 1H, J=3.5 Hz, H-1''), 4.53 (t, 1H, J=5.0 Hz, H-2'), 4.41 (d, 1H, J=4.8 Hz, H-3'), 4.38 (d, 1H, J=5.2 Hz, H-4'), 3.17–4.36 (m, 18H, H-5''', H-3''', 4'', 6'', Lys-α-CH-, 2'', 4'', 6''a, 1'', 6''b, 3'', 5'', Lys-CH₂- and 2×Eda-CH₂-), 2.56–2.62 (m, 1H, H_{eq}-2''), 1.83–1.89 (m, 3H, H_{ax}-2'' and Lys-CH₂-), 1.51–1.58 (m, 2H, Lys-CH₂-), 1.32–1.40 (m, 2H, Lys-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 173.93 (–C=O), 173.82 (–C=O), 168.85 (C-4), 154.27 (C-2), 145.85 (C-6), 105.00 (C-5), 94.37 (C-1'' and 1'), 85.24 (C-4'), 75.16 (C-3'), 75.07 (C-2'), 74.25 (C-4''), 73.22 (C-4'''), 72.39 (C-6'' and 3'''), 70.76 (C-5''), 61.03 (C-2'''), 55.69 (Eda-CH₂-), 55.44 (Lys-α-CH-), 52.94 (C-1''), 50.83 (C-3''), 49.33 (C-5''), 42.25 (C-6''), 41.31 (Eda-CH₂-), 39.83 (Lys-CH₂-), 33.05 (Lys-CH₂-), 30.53 (C-2''), 24.43 (Lys-CH₂-), 24.20 (Lys-CH₂-). HRMS calcd for C₂₉H₅₂N₁₀O₁₂ ([M+H]⁺): 733.3839, found: 733.3834. [α]_D^{28.4} 1.279 (H₂O, c 0.073). λ_{max}=262 nm, ε=14,050.

3.1.12. General procedure for the synthesis of **10i–l**

Compounds **9i–l** were added a solution of 90% CF₃COOH in H₂O (2 mL), and stirred for 3 h in an ice bath. The solvent was removed

and the residue was dissolved in Py/Et₃N/H₂O (5 mL, 4:3:2) and slowly bubbled by hydrogen sulfide for 1 h at room temperature, the reaction mixture was concentrated in vacuum and the residue was purified by HPLC (1% CF₃COOH in H₂O and CH₃OH) to afford compounds **10i–l** as a salt of trifluoroacetic acid.

3.1.12.1. Compound 10i. Yield: 50% from **9i**, white foam. ¹H NMR (400 MHz, D₂O): δ 8.57 (s, 1H, H-8), 8.43 (s, 1H, H-2), 6.21 (d, 1H, J=5.6 Hz, H-1'), 5.57 (d, 1H, J=3.6 Hz, H-1''), 4.57–4.59 (m, 2H, H-2' and 3'), 3.14–4.34 (m, 19H, H-4', 5'', H-3'', 4'', 6'', Lys-α-CH-, 2'', 4'', 6''a, 1'', 6''b, 3'', 5'', Lys-CH₂- and 2×Eda-CH₂-), 2.56–2.61 (m, 1H, H_{eq}-2''), 1.81–1.93 (m, 3H, H_{ax}-2'' and Lys-CH₂-), 1.50–1.57 (m, 2H, Lys-CH₂-), 1.32–1.40 (m, 2H, Lys-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 173.74 (–C=O), 173.65 (–C=O), 152.79 (C-6), 150.93 (C-2), 147.29 (C-4), 145.97 (C-8), 121.81 (C-5), 94.42 (C-1'), 91.43 (C-1''), 86.13 (C-4'), 75.85 (C-3'), 75.50 (C-2'), 74.37 (C-4''), 73.07 (C-4''), 72.49 (C-6''), 70.80 (C-3''), 65.18 (C-5''), 60.78 (C-2''), 55.72 (Eda-CH₂-), 55.49 (Lys-α-CH-), 52.82 (C-1''), 50.79 (C-3''), 49.46 (C-5''), 42.30 (C-6''), 41.34 (Eda-CH₂-), 40.18 (Lys-CH₂-), 33.05 (Lys-CH₂-), 30.65 (C-2'' and Lys-CH₂-), 24.26 (Lys-CH₂-). HRMS calcd for C₃₀H₅₃N₁₃O₁₀ ([M+H]⁺): 756.4111, found: 756.4130. [α]_D^{28.5} 1.219 (H₂O, c 0.076). λ_{max}=260 nm, ε=19,200.

3.1.12.2. Compound 10j. Yield: 52% from **9j**, white foam. ¹H NMR (400 MHz, D₂O): δ 8.28 (d, 1H, J=8.0 Hz, H-6), 6.18 (d, 1H, J=3.8 Hz, H-1'), 5.81 (d, 1H, J=8.0 Hz, H-5), 5.56 (d, 1H, J=3.5 Hz, H-1''), 4.37–4.41 (m, 3H, H-2', 3' and 4'), 3.08–4.30 (m, 18H, H-5'', H-3'', 4'', 6'', Lys-α-CH-, 2'', 4'', 6''a, 1'', 6''b, 3'', 5'', Lys-CH₂- and 2×Eda-CH₂-), 2.56–2.64 (m, 1H, H_{eq}-2''), 1.77–1.93 (m, 3H, H_{ax}-2'' and Lys-CH₂-), 1.47–1.52 (m, 2H, Lys-CH₂-), 1.31–1.37 (m, 2H, Lys-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 174.03 (–C=O), 173.65 (–C=O), 161.83 (C-4), 150.87 (C-2), 147.93 (C-6), 97.72 (C-5), 94.68 (C-1' and 1''), 85.13 (C-4'), 75.73 (C-3'), 75.04 (C-2'), 73.37 (C-4''), 72.34 (C-4''), 70.67 (C-3'' and 6''), 69.82 (C-5''), 61.38 (C-2''), 55.66 (Eda-CH₂-), 55.39 (Lys-α-CH-), 53.46 (C-1''), 50.78 (C-3''), 49.28 (C-5''), 42.24 (C-6''), 41.41 (Eda-CH₂-), 39.30 (Lys-CH₂-), 37.21 (Lys-CH₂-), 33.03 (C-2''), 30.43 (Lys-CH₂-), 24.25 (Lys-CH₂-). HRMS calcd for C₂₉H₅₄N₁₁O₁₁ ([M+H]⁺): 732.4004, found: 732.3984. [α]_D^{28.5} 1.248 (H₂O, c 0.073). λ_{max}=265 nm, ε=25,900.

3.1.12.3. Compound 10k. Yield: 48% from **9k**, white foam. ¹H NMR (400 MHz, D₂O): δ 8.92 (s, 1H, H-8), 6.11 (d, 1H, J=4.0 Hz, H-1'), 5.61 (d, 1H, J=3.4 Hz, H-1''), 4.63 (t, 1H, J=5.0 Hz, H-2'), 4.56 (d, 1H, J=6.0 Hz, H-4'), 3.00–4.16 (m, 19H, H-3', 5'', H-3'', 4'', 6'', Lys-α-CH-, 2'', 4'', 6''a, 1'', 6''b, 3'', 5'', Lys-CH₂- and 2×Eda-CH₂-), 2.59–2.64 (m, 1H, H_{eq}-2''), 1.82–1.95 (m, 3H, H_{ax}-2'' and Lys-CH₂-), 1.46–1.54 (m, 2H, Lys-CH₂-), 1.24–1.40 (m, 2H, Lys-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 173.86 (2×C=O), 158.80 (C-6), 157.69 (C-2), 152.84 (C-4), 139.59 (C-8), 114.70 (C-5), 94.62 (C-1'), 92.63 (C-1''), 85.95 (C-4'), 75.92 (C-3'), 75.42 (C-2'), 74.72 (C-4''), 73.29 (C-4''), 72.40 (C-6''), 70.74 (C-3''), 65.15 (C-5''), 61.20 (C-2''), 55.69 (Eda-CH₂-), 55.44 (Lys-α-CH-), 53.11 (C-1''), 50.84 (C-3''), 49.43 (C-5''), 49.34 (C-6''), 42.27 (Eda-CH₂-), 41.42 (Lys-CH₂-), 39.64 (Lys-CH₂-), 33.08 (C-2''), 30.56 (Lys-CH₂-), 24.28 (Lys-CH₂-). HRMS calcd for C₃₀H₅₃N₁₃O₁₁ ([M+H]⁺): 772.4021, found: 772.4038. [α]_D^{28.8} 1.089 (H₂O, c 0.077). λ_{max}=257 nm, ε=16,500.

3.1.12.4. Compound 10l. Yield: 54% from **9l**, white foam. ¹H NMR (400 MHz, D₂O): δ 8.50 (s, 1H, H-2), 8.23 (s, 1H, H-8), 6.18 (d, 1H, J=5.4 Hz, H-1'), 5.59 (d, 1H, J=3.6 Hz, H-1''), 4.78 (m, 1H, H-2'), 4.57–4.58 (m, 2H, H-3' and 4'), 3.13–4.39 (m, 18H, H-5'', H-3'', 4'', 6'', Lys-α-CH-, 2'', 4'', 6''a, 1'', 6''b, 3'', 5'', Lys-CH₂- and 2×Eda-CH₂-), 2.57–2.62 (m, 1H, H_{eq}-2''), 1.80–1.93 (m, 3H, H_{ax}-2'' and Lys-CH₂-), 1.44–1.49 (m, 2H, Lys-CH₂-), 1.26–1.34 (m, 2H, Lys-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 173.97 (–C=O), 173.83 (–C=O), 160.75 (C-6), 151.17 (C-4), 149.31 (C-2), 143.12 (C-8), 126.11 (C-5), 94.89 (C-1''),

91.65 (C-1'), 86.30 (C-4'), 75.81 (C-3'), 75.67 (C-2'), 74.45 (C-4''), 74.15 (C-4''), 73.35 (C-6''), 72.45 (C-3''), 70.83 (C-5''), 61.11 (C-2''), 55.75 (Eda-CH₂-), 55.51 (Lys-α-CH-), 53.03 (C-1''), 50.94 (C-3''), 49.53 (C-5''), 42.33 (C-6''), 41.32 (Eda-CH₂-), 39.88 (Lys-CH₂-), 33.12 (Lys-CH₂-), 30.72 (C-2''), 28.33 (Lys-CH₂-), 24.31 (Lys-CH₂-). HRMS calcd for C₃₀H₅₂N₁₂O₁₁ ([M+H]⁺): 757.3951, found: 757.3981. [α]_D^{28.8} 1.206 (H₂O, c 0.076). λ_{max}=250 nm, ε=10,500.

3.1.13. General procedure for the synthesis of **10m** and **10n**

Compound **9m** or **9n** (0.1 mmol) was dissolved in CF₃COOH/PhSCH₃/H₂O (2 mL, 94:3:3), after stirring for 1 h, the resulting mixture was concentrated and the residue was added NH₃/CH₃OH (2 mL) and kept stirring for 4 h in an ice bath. The solvent was removed and the residue was dissolved in Py/Et₃N/H₂O (5 mL, 4:3:2) and slowly bubbled by hydrogen sulfide for 1 h at room temperature, the reaction mixture was concentrated in vacuum and the residue was purified by HPLC (1% CF₃COOH in H₂O and CH₃OH) to afford compounds **10m** and **10n** as a salt of trifluoroacetic acid.

3.1.13.1. Compound 10m. Yield: 56% from **9m**, white foam. ¹H NMR (400 MHz, D₂O): δ 7.53 (s, 1H, H-6), 6.14 (t, 1H, J=6.7 Hz, H-1'), 5.56 (d, 1H, J=3.3 Hz, H-1''), 4.70–4.80 (m, 1H, H-3'), 4.39 (d, 1H, J=3.6 Hz, H-4'), 3.14–4.39 (m, 18H, H-5'', H-3'', 4'', 6'', Arg-α-CH-, 2'', 4'', 6''a, 1'', 6''b, 3'', 5'', Arg-CH₂- and 2×Eda-CH₂-), 2.59–2.63 (m, 2H, H-2'a and H_{eq}-2''), 2.32–2.40 (m, 1H, H-2'b), 1.84 (s, 3H, –CH₃), 1.83–1.95 (m, 1H, H_{ax}-2''), 1.65–1.85 (m, 2H, Arg-CH₂-), 1.50–1.60 (m, 2H, Arg-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 178.13 (–C=O), 175.49 (–C=O), 169.13 (C-4), 159.36 (–C=NH), 154.25 (C-2), 142.48 (C-6), 113.76 (C-5), 94.42 (C-1''), 92.24 (C-1'), 87.93 (C-4'), 76.26 (C-3' and 4'), 73.33 (C-4''), 72.24 (C-6''), 70.92 (C-3''), 69.99 (C-5''), 60.72 (C-2''), 56.28 (Eda-CH₂-), 55.42 (C-1''), 53.68 (Arg-α-CH-), 50.71 (C-3''), 49.38 (C-5''), 43.12 (C-6''), 42.23 (Eda-CH₂-), 40.04 (Arg-CH₂-), 39.79 (C-2'), 30.50 (C-2'' and Arg-CH₂-), 27.40 (Arg-CH₂-), 14.05 (–CH₃). HRMS calcd for C₃₀H₅₄N₁₂O₁₁ ([M+H]⁺): 759.4108, found: 759.4114. [α]_D^{27.6} 0.443 (H₂O, c 0.076). λ_{max}=265 nm, ε=10,600.

3.1.13.2. Compound 10n. Yield: 54% from **9n**, white foam. ¹H NMR (400 MHz, D₂O): δ 7.76 (d, 1H, J=8.0 Hz, H-6), 5.85 (d, 1H, J=8.0 Hz, H-5), 5.73 (d, 1H, J=4.4 Hz, H-1'), 5.56 (d, 1H, J=3.6 Hz, H-1''), 4.58 (t, 1H, J=4.6 Hz, H-2'), 4.48–4.52 (m, 2H, H-3' and 4'), 3.15–4.32 (m, 18H, H-5'', H-3'', 4'', 6'', Arg-α-CH-, 2'', 4'', 6''a, 1'', 6''b, 3'', 5'', Arg-CH₂- and 2×Eda-CH₂-), 2.57–2.61 (m, 1H, H_{eq}-2''), 1.71–1.90 (m, 3H, Arg-CH₂- and H_{ax}-2''), 1.57–1.66 (m, 2H, Arg-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 177.66 (C=O), 174.91 (C=O), 168.91 (C-4), 159.46 (–C=NH), 154.19 (C-2), 147.00 (C-6), 104.96 (C-5), 96.81 (C-1''), 94.41 (C-1'), 85.56 (C-4'), 75.24 (C-3'), 74.93 (C-2'), 74.45 (C-4''), 73.05 (C-4''), 72.51 (C-6'' and 3''), 71.06 (C-5''), 60.18 (C-2''), 56.45 (Eda-CH₂-), 55.60 (Arg-α-CH-), 53.04 (C-1''), 50.79 (C-3''), 49.58 (C-5''), 43.20 (C-6''), 42.37 (Eda-CH₂-), 40.72 (Arg-CH₂-), 30.82 (C-2''), 30.57 (Arg-CH₂-), 27.47 (Arg-CH₂-). HRMS calcd for C₂₉H₅₂N₁₂O₁₂ ([M+H]⁺): 761.3900, found: 761.3916. [α]_D^{27.9} 1.211 (H₂O, c 0.076). λ_{max}=261 nm, ε=13,300.

3.1.14. General procedure for the synthesis of **10o–r**

Compounds **9o–r** (0.1 mmol) were dissolved in CF₃COOH/PhSCH₃/H₂O (2 mL, 94:3:3), after stirring for 1 h, the resulting mixture was concentrated and the residue was dissolved in Py/Et₃N/H₂O (5 mL, 4:3:2) and slowly bubbled by hydrogen sulfide for 1 h at room temperature, the reaction mixture was concentrated in vacuum and the residue was purified by HPLC (1% CF₃COOH in H₂O and CH₃OH) to afford compounds **10o–r** as a salt of trifluoroacetic acid.

3.1.14.1. Compound 10o. Yield: 51% from **9o**, white foam. ¹H NMR (400 MHz, D₂O): δ 8.52 (s, 1H, H-8), 8.41 (s, 1H, H-2), 6.22 (d, 1H,

$J=5.2$ Hz, H-1'), 5.56 (d, 1H, $J=3.6$ Hz, H-1'''), 4.64–4.79 (m, 3H, H-2', 3' and 4'), 3.08–4.39 (m, 18H, H-5''', H-3''', 4'', 6'', Arg- α -CH-, 2'', 4''', 6'' a, 1'', 6'' b, 3'', 5'', Arg-CH₂- and 2 $\times$ Eda-CH₂-), 2.59–2.63 (m, 1H, H_{eq}-2''), 1.84–1.93 (m, 1H, H_{ax}-2''), 1.64–1.81 (m, 2H, Arg-CH₂-), 1.45–1.55 (m, 2H, Arg-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 177.57 (–C=O), 174.44 (–C=O), 159.35 (–C=NH), 152.72 (C-6), 150.80 (C-2), 147.21 (C-4), 146.09 (C-8), 121.85 (C-5), 96.00 (C-1'''), 91.96 (C-1'), 85.70 (C-4'), 76.07 (C-3'), 75.29 (C-2'), 73.28 (C-4''), 72.29 (C-4'''), 70.88 (C-6'' and 3'''), 70.28 (C-5''), 60.70 (C-2'''), 56.17 (Eda-CH₂-), 55.43 (Arg- α -CH-), 53.42 (C-1''), 50.72 (C-3''), 49.40 (C-5'''), 43.01 (C-6'''), 42.24 (Eda-CH₂-), 39.81 (Arg-CH₂-), 30.63 (C-2'' and Arg-CH₂-), 27.24 (Arg-CH₂-). HRMS calcd for C₃₀H₅₃N₁₅O₁₀ ([M+H]⁺): 784.4173, found: 784.4174. [α]_D^{28.1} 1.254 (H₂O, c 0.052). $\lambda_{\max}$ =261 nm, ϵ =16,392.

3.1.14.2. Compound 10p. Yield: 52% from **9p**, white foam. ¹H NMR (400 MHz, D₂O): δ 8.11 (d, 1H, $J=8.0$ Hz, H-6), 6.20 (d, 1H, $J=8.0$ Hz, H-5), 5.76 (d, 1H, $J=3.5$ Hz, H-1'), 5.57 (d, 1H, $J=2.8$ Hz, H-1'''), 4.50–4.53 (m, 2H, H-2' and 3'), 4.42 (t, 1H, $J=5.6$ Hz, H-4'), 3.14–4.37 (m, 18H, H-5''', H-3''', 4'', 6'', Arg- α -CH-, 2'', 4''', 6'' a, 1'', 6'' b, 3'', 5'', Arg-CH₂- and 2 $\times$ Eda-CH₂-), 2.57–2.63 (m, 1H, H_{eq}-2''), 1.70–1.93 (m, 3H, H_{ax}-2'' and Arg-CH₂-), 1.56–1.63 (m, 2H, Arg-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 177.74 (–C=O), 174.43 (–C=O), 162.04 (C-4), 159.41 (–C=NH), 150.94 (C-2), 148.88 (C-6), 97.74 (C-5), 96.75 (C-1'' and 1'), 85.14 (C-4'), 75.28 (C-3' and 2'), 75.02 (C-4''), 73.17 (C-4'''), 72.32 (C-6'' and 3'''), 70.94 (C-5''), 60.51 (C-2'''), 56.22 (Eda-CH₂-), 55.46 (Arg- α -CH- and C-1''), 50.71 (C-3''), 49.33 (C-5'''), 43.11 (C-6'''), 42.25 (Eda-CH₂-), 40.10 (Arg-CH₂-), 30.59 (C-2'' and Arg-CH₂-), 27.22 (Arg-CH₂-). HRMS calcd for C₂₉H₅₃N₁₃O₁₁ ([M+H]⁺): 760.4060, found: 760.4067. [α]_D^{28.2} 1.265 (H₂O, c 0.076). $\lambda_{\max}$ =265 nm, ϵ =15,500.

3.1.14.3. Compound 10q. Yield: 49% from **9q**, white foam. ¹H NMR (400 MHz, D₂O): δ 8.66 (s, 1H, H-8), 6.08 (d, 1H, $J=3.0$ Hz, H-1'), 5.56 (d, 1H, $J=4.0$ Hz, H-1'''), 4.78–4.87 (m, 2H, H-2' and 3'), 4.58 (d, 1H, $J=5.6$ Hz, H-4'), 3.03–4.38 (m, 18H, H-5''', H-3''', 4'', 6'', Arg- α -CH-, 2'', 4''', 6'' a, 1'', 6'' b, 3'', 5'', Arg-CH₂- and 2 $\times$ Eda-CH₂-), 2.58–2.63 (m, 1H, H_{eq}-2''), 1.88 (q, 1H, $J=12.5$ Hz, H_{ax}-2''), 1.57–1.79 (m, 2H, Arg-CH₂-), 1.38–1.50 (m, 2H, Arg-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 177.89 (–C=O), 175.00 (–C=O), 159.41 (–C=NH), 159.27 (C-6), 157.41 (C-2), 153.01 (C-4), 140.20 (C-5), 114.52 (C-8), 95.00 (C-1'''), 93.36 (C-1'), 85.60 (C-4'), 76.15 (C-3'), 75.36 (C-2'), 74.00 (C-4''), 73.37 (C-4'''), 72.37 (C-6''), 70.99 (C-3'''), 70.43 (C-5''), 60.71 (C-2'''), 56.52 (Eda-CH₂-), 55.52 (Arg- α -CH-), 53.50 (C-1''), 50.78 (C-3''), 49.42 (C-5'''), 43.19 (C-6'''), 42.33 (Eda-CH₂-), 40.04 (Arg-CH₂-), 30.54 (C-2'' and Arg-CH₂-), 27.35 (Arg-CH₂-). HRMS calcd for C₃₀H₅₃N₁₅O₁₁ ([M+H]⁺): 800.4122, found: 800.4121. [α]_D^{28.9} 0.991 (H₂O, c 0.080). $\lambda_{\max}$ =252 nm, ϵ =10,050.

3.1.14.4. Compound 10r. Yield: 54% from **9r**, white foam. ¹H NMR (400 MHz, D₂O): δ 8.35 (s, 1H, H-2), 8.19 (s, 1H, H-8), 6.18 (d, 1H, $J=5.0$ Hz, H-1'), 5.54 (d, 1H, $J=3.6$ Hz, H-1'''), 4.73–4.78 (m, 2H, H-2' and 3'), 4.63 (d, 1H, $J=4.0$ Hz, H-4'), 3.04–4.34 (m, 18H, H-5''', H-3''', 4'', 6'', Arg- α -CH-, 2'', 4''', 6'' a, 1'', 6'' b, 3'', 5'', Arg-CH₂- and 2 $\times$ Eda-CH₂-), 2.57–2.62 (m, 1H, H_{eq}-2''), 1.60–1.91 (m, 3H, H_{ax}-2'' and Arg-CH₂-), 1.41–1.50 (m, 2H, Arg-CH₂-). ¹³C NMR (100 MHz, D₂O): δ 177.42 (–C=O), 174.81 (–C=O), 161.06 (–C=NH), 159.42 (C-6), 151.15 (C-4), 148.91 (C-2), 143.38 (C-8), 126.92 (C-5), 94.55 (C-1'''), 92.04 (C-1'), 85.94 (C-4'), 75.95 (C-3'), 75.61 (C-2'), 74.31 (C-4''), 73.20 (C-4'''), 72.46 (C-6'' and 3'''), 71.00 (C-5''), 60.47 (C-2'''), 56.29 (Eda-CH₂-), 55.57 (Arg- α -CH-), 53.06 (C-1''), 50.84 (C-3''), 49.57 (C-5'''), 43.07 (C-6'''), 42.35 (Eda-CH₂-), 40.37 (Arg-CH₂-), 30.71 (C-2'' and Arg-CH₂-), 27.35 (Arg-CH₂-). HRMS calcd for C₃₀H₅₂N₁₄O₁₁ ([M+H]⁺): 785.4013, found: 785.4008. [α]_D^{29.0} 1.888 (H₂O, c 0.047). $\lambda_{\max}$ =250 nm, ϵ =12,000.

3.2. Determination of dissociation constants for the neamine–nucleoside conjugates binding to A site of 16S RNA and TAR RNA

The binding properties of neamine–nucleoside conjugates to A site of 16S RNA and TAR RNA were evaluated by SPR using Biacore 3000 instrument. Biotinylated A site of 16S and TAR RNA were purchased from Bioneer corporation of Korea. The biochip and the streptavidin were purchased from Biacore and Sigma, respectively. All the glass containers, pipette tips, eppendorf tubes and so on, which have been used in the experiment were pretreated to RNase free and keep not contaminated. All the solutions were prepared with RNase free water and filtered (pore size: 0.22 μ m) before use. For preparing the streptavidin-functionalized (SA) biochip used in the assay, streptavidin in an acetate buffer solution (200 μ g/mL, 60 μ L) flowed through the surface of the carboxy-methyl dextran (CM-5) sensor chip and was covalently attached to the surface through the ethanolamine linkers. Biotinylated A site of 16S RNA and TAR RNA in HBS (HEPES-buffered saline, 10 mM HEPES, 100 mM NaCl, 0.1 mM EDTA, pH 6.8) buffer (0.1 OD/mL, 6 μ L) was flowed through the surface and immobilized on the streptavidin-coated sensor chip and a solution with different concentrations of the neamine–nucleoside conjugates (160 μ M, 80 μ M, 40 μ M, 20 μ M, 10 μ M, 5 μ M, 2.5 μ M, 1.25 μ M, 0.625 μ M, 0.3125 μ M, ..., 0 μ M as blank control) in HBS buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, pH 7.4) was flowed through on the surface, the response units (RUs) for diverse concentrations were obtained and the dissociation constants (K_D) were determined by the curve fitting analysis according to the equation: $RU_{AG}=RU_{max}[AG]/K_D+[AG]$, among which $RU_{max}=RU_{RNA}(MW_{AG}/MW_{RNA})$. Where RU_{AG} is the response unit of the neamine–nucleoside conjugates, $[AG]$ is the concentration of the neamine–nucleoside conjugates, RU_{max} is the expected maximum response unit value of the neamine–nucleoside conjugates. RU_{RNA} is the response unit of the immobilized A site of 16S RNA and TAR RNA on the biochip, MW_{AG} and MW_{RNA} are the molecular weights of the neamine–nucleoside conjugates and A site of 16S RNA and TAR RNA.

3.3. Procedures for the molecular docking study

AutoDock 3.0 program was used for the molecular docking procedure. The target structure of A site of 16S RNA was extracted from the protein databank (PDB code 1PBR). The ligands were minimized with AMBER force field using the steepest decent and conjugated gradient methods consecutively. The obtained optimized structures were used for the following docking.

The molecular docking calculations were carried out using an empirical free energy function and Lamarckian Genetic Algorithm. Each dihedral angle of ligands was chosen to be flexible. The number of generations, energy evaluation, and docking runs were set to 370,000, 1,500,000, and 50, respectively, and the kinds of atomic charges were taken as Kollman-all-atom for 16S RNA and Gasteiger–Hucel for the ligands.

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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.2009.04.084.

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