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Synthesis of aminoglycoside derivatives on a Cbz-type heavy fluorous tag

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Abstract—Four aminoglycoside derivatives containing a 2,6-diamino-2,6-dideoxy-D-glucopyranose disaccharide structure were successfully prepared by using a Cbz-type heavy fluorous tag in a fluorous synthesis. A Cbz-type heavy fluorous tag was prepared using the hexakis(fluorous chain)-type alcohol **11**, and the fluorous alcohol **11** was recovered in good yield after the synthesis of aminoglycoside derivatives.

Targeting RNA with small organic molecules is a promising approach for controlling gene expression and viral replication and for identifying new antibiotics. Aminoglycosides are well-known RNA-binding molecules, and some are important natural products with antibacterial activity against both gram-positive and gram-negative bacteria.[1,2] Furthermore, some types of aminoglycoside, such as the neamines, neomycin B and kanamycin B (Fig. 1), bind to the trans-activator responsive region (TAR) and retroviral protein responsive element (RRE) of HIV RNA, and inhibit the binding of regulatory proteins of HIV (Tat for TAR RNA, and Rev for REE RNA).[3–5]

Neomycin and kanamycin have a pseudodisaccharide neamine structure. Neamine is a glycoconjugate structure in which the anomeric position of 2,6-diamino-2,6-dideoxy-D-glucopyranose is connected by an α-linkage to the hydroxyl group at the 4-position of 2-deoxystreptamine.

We synthesized aminoglycoside derivatives containing a 2,6-diamino-2,6-dideoxy-D-glucopyranose disaccharide structure to study if the geometry of the glycosidic bond of neamine influences the binding to RNA. A fluorous synthesis method[6–9] was used to prepare the aminoglycoside disaccharide derivatives. Fluorous synthesis (the fluorous tag method) is a strategic alternative to solid-phase synthesis. This strategy is very efficient because, unlike the solid-phase method, it does not rely on chromatography to perform separations. Syntheses of oligosaccharides and peptides by using various fluorous tags have been achieved.[10–16] Recently, the concept of a novel recyclable fluorous tag system to realize a practical fluorous synthesis was described.[17] In this study, we prepared a benzyloxycarbonyl (Cbz)-type heavy fluorous tag and used it in the synthesis of aminoglycoside derivatives with the recyclable system.

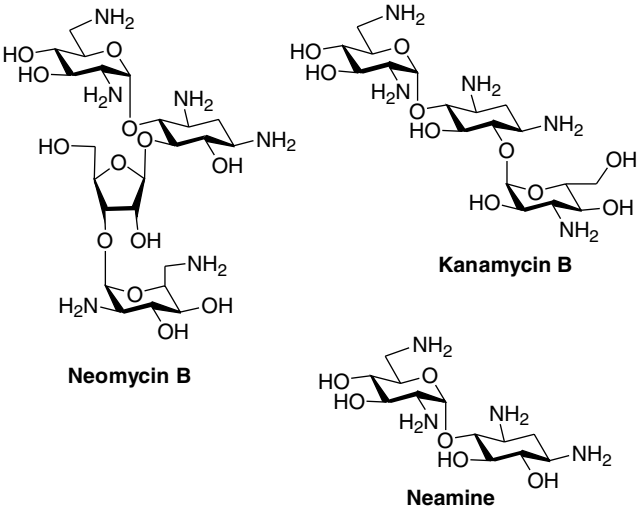

Figure 1. Structures of neomycin B, kanamycin B, and neamine.

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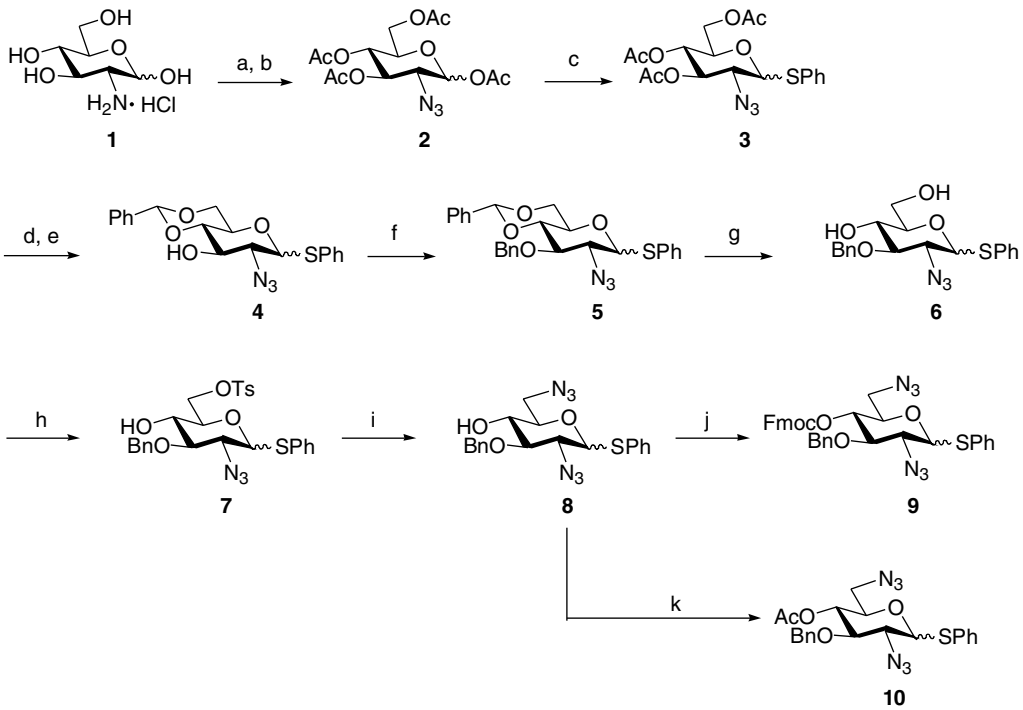
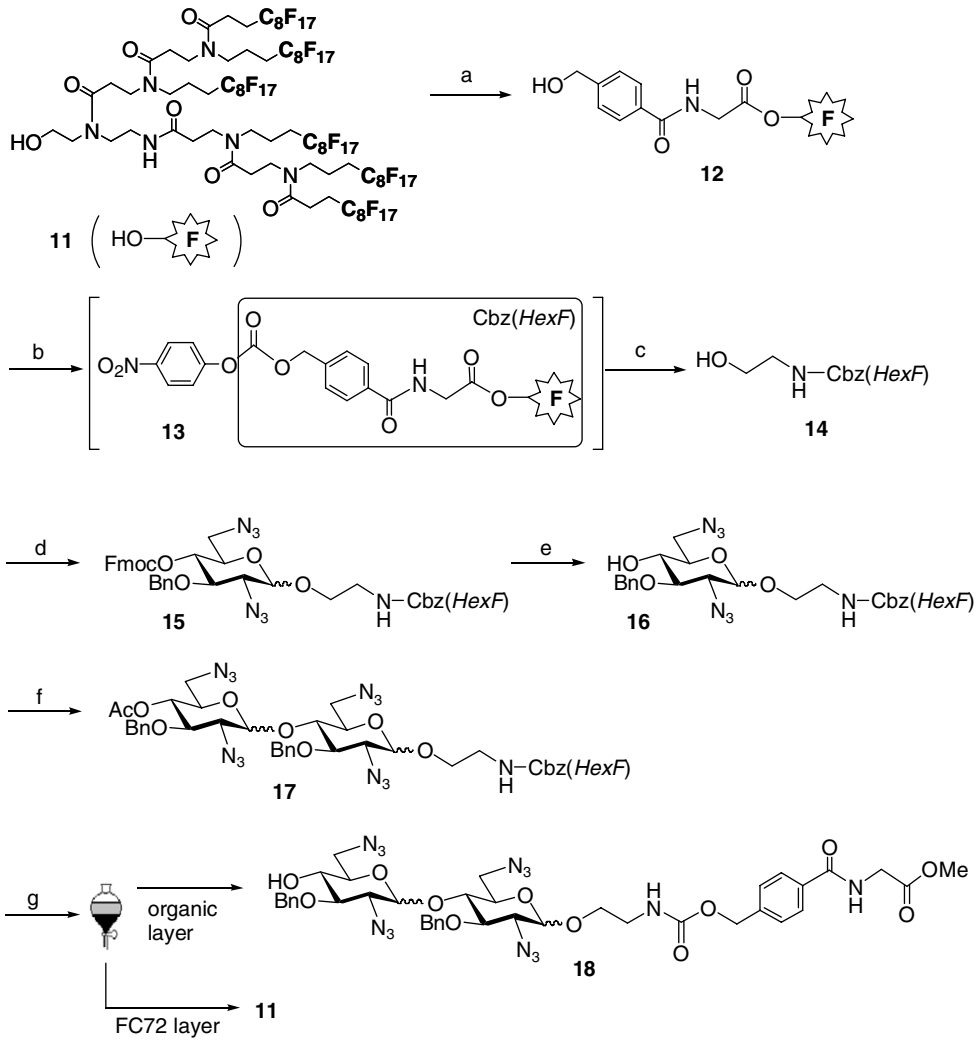
The 2,6-diazido-glucose derivatives **9** and **10** were first prepared as building blocks. The peracetylated 2-azido-2-deoxyglucose **2** was obtained by conventional amino–azido conversion from glucosamine **1**, followed by acetylation.[18] Treatment of **2** with benzenethiol and boron trifluoride–ethyl ether complex gave the thioglycoside **3**. Deacetylation followed by treatment with benzaldehyde dimethyl acetal afforded the 4,6-di-*O*-benzylidene derivative **4**. Benzylation of **4** followed by removal of the benzylidene group of **5** provided the monobenzyl derivative **6**. Selective tosylation of **6** in the 6-position gave the monotosylate **7**. Treatment of **7** with sodium azide in the presence of tetrabutylammonium hydrogen sulfate (TBAHS) at room temperature gave the 2,6-diazido-2,6-dideoxy derivative **8**. The hydroxyl group of **8** was protected by a 9-fluorenylmethoxycarbonyl (Fmoc) group or an acetyl group to afford the derivatives **9** and **10**, respectively (Scheme 1).

Next, a Cbz-type heavy fluorous tag was prepared. The synthesis and application of a tris(fluorous chain)-type fluorous Cbz tag was reported.[19] In this study, we decided to use a hexakis(fluorous chain)-type Cbz tag [Cbz(HexF)] to keep the high partition coefficient of fluorous aminoglycoside intermediates in a fluorous solvent. The hexakis(fluorous chain)-type benzyl alcohol **12** was prepared from the fluorous alcohol **11**.[17] The fluorous benzyl alcohol **12** was coupled with 4-nitrophenyl chloroformate in a homogeneous mixture of CH_2Cl_2 and $EtOC_4F_9$[20] to afford the fluorous-type benzyl-4-nitrophenyl carbonate **13**. Compound **13** could be obtained in a straightforward manner simply by partitioning between the fluorocarbon solvent FC72[21] and MeOH. Compound **13**, including the fluorous tag, was extracted into the FC72 layer, whereas the other reagents remained in the MeOH layer. No further purification, such as silica gel column chromatography, was necessary. The crude **13** was treated with 2-aminoethanol in THF, and fluorous compound **14**[22] was obtained in an 80% yield from **12** (two steps) after purification by silica gel column chromatography. Glycosylationof **14** with a 2-fold excess of the glycosyl donor **9** in the mixed homogeneous solvents CH_2Cl_2 and $EtOC_4F_9$ gave glycoside **15**. This was deprotected by removal of the Fmoc group by using the FC72–10% piperidine/DMF (1:1) immiscible system to give **16**. This was treated with a 2-fold excess of glycosyl donor **10** under similar glycosylation conditions to those described above to give the fluorous disaccharide **17**. Each of the fluorous intermediates **15**, **16**, and **17** was isolated by simple partitioning between FC72 and an organic solvent such as MeOH or MeCN, without purification by column chromatography. The fluorous disaccharide **17** was treated with NaOMe in the mixed homogeneous solvents MeOH and $EtOC_4F_9$ to give the crude disaccharide **18**, which was extracted into a MeOH layer by partitioning the mixture between FC72 and MeOH. After purification by silica gel column chromatography, the aminoglycoside disaccharide derivative **18** was obtained in a 32% yield as an anomeric mixture. The fluorous alcohol **11** was recovered from the FC72 layer in a 93% yield (Scheme 2).



Scheme 1. Synthesis of building blocks **9** and **10**. Reagents and conditions: (a) TfN_3, $CuSO_4$, K_2CO_3, MeOH, H_2O, CH_2Cl_2; (b) Ac_2O, DMAP, pyridine, 83% (two steps); (c) PhSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 95%; (d) NaOMe, MeOH; (e) $PhCH(OMe)_2$, CSA, MeCN, 75% (two steps); (f) NaH, BnBr, DMF; (g) 80% aq AcOH, CH_2Cl_2, 60 °C, 96% (two steps); (h) TsCl, pyridine, 95%; (i) NaN_3, TBAHS, DMF, 98%; (j) FmocCl, DMAP, pyridine, 50% and (k) Ac_2O, pyridine, quant DMAP = 4-dimethylaminopyridine, CSA = (+)-10-camphorsulfonic acid, Bn = benzyl, DMF = *N*,*N*-dimethylformamide, Ts = *p*-toluenesulfonyl, TBAHS = Bu_4N^+ HSO_4^-, Fmoc = 9-fluorenylmethoxycarbonyl.



Scheme 2. Synthesis of aminoglycoside disaccharide derivative **18**. Reagents and conditions: (a) see Ref. 18; (b) 4-nitrophenyl chloroformate, Et_3N, CH_2Cl_2, $EtOC_4F_9$; (c) 2-aminoethanol, THF, 80% (two steps); (d) **9**, NIS, TfOH, CH_2Cl_2, (e) 10% piperidine/DMF, FC72; (f) **10**, NIS, TfOH, CH_2Cl_2, $EtOC_4F_9$ and (g) NaOMe, MeOH, $EtOC_4F_9$. NIS = *N*-iodosuccinimide, Tf = trifluoromethylsulfonyl.

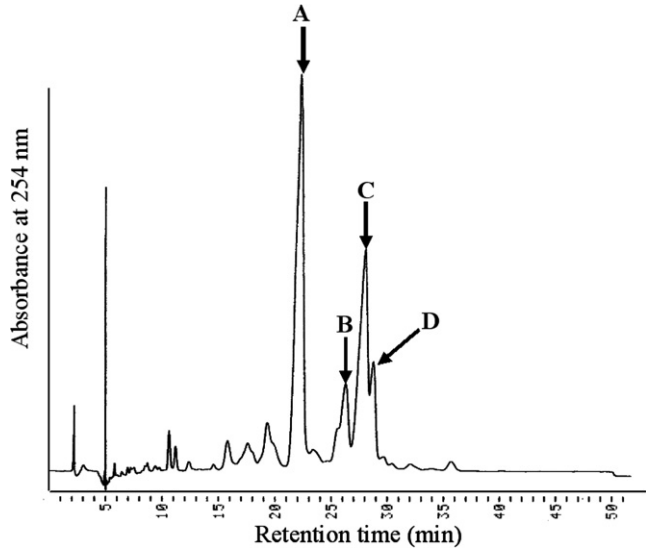
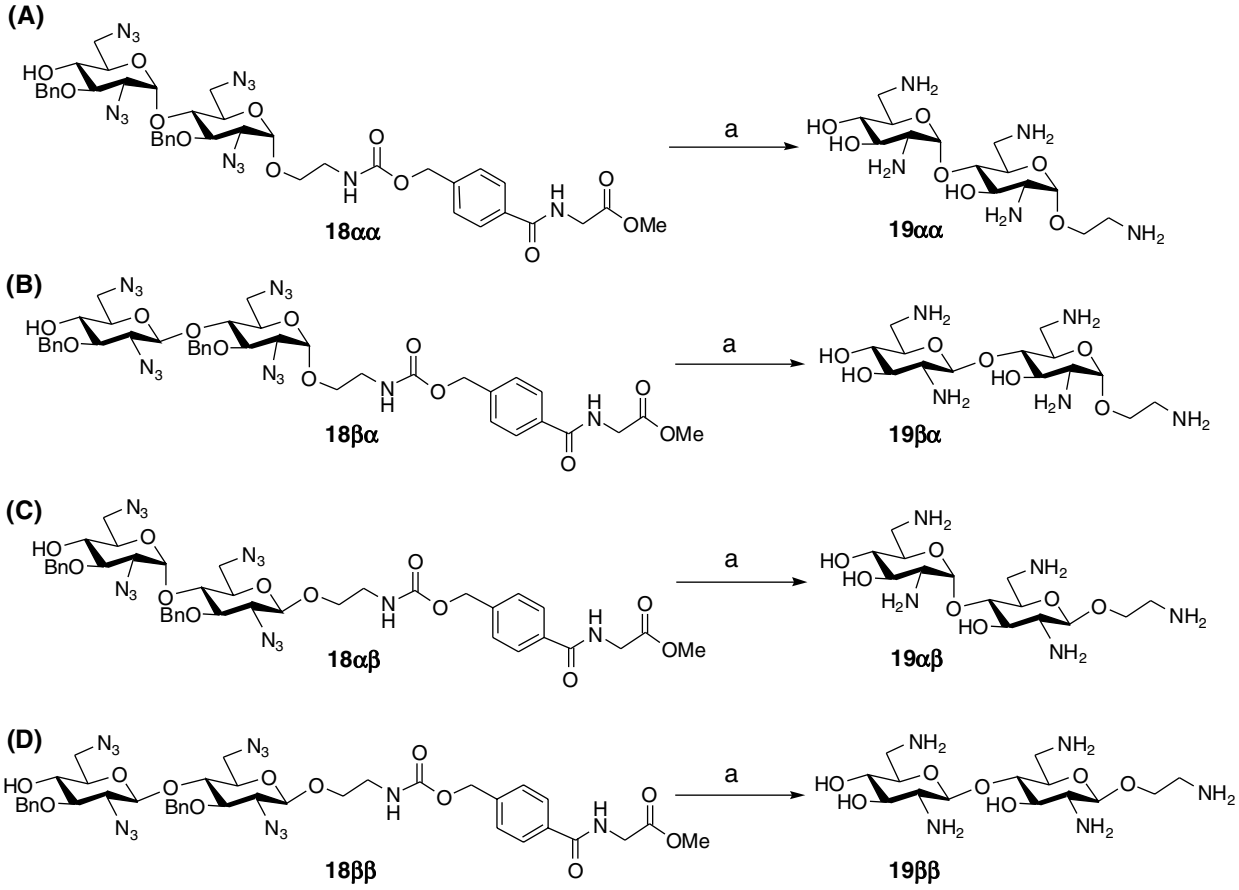


Figure 2. HPLC profile of the crude disaccharide **18** produced by the fluorous synthesis. Elution conditions: column, GL Sciences Inertsil SIL 100A (20 × 250 mm); eluent, 8–15% ethanol/*n*-hexane (v/v), 50 min; Flow rate, 30.0 mL/min.

HPLC analysis of crude **18** showed four major peaks (Fig. 2**A**–**D**). The product disaccharide was estimated as the mixture of the four anomeric isomers. These compounds associated with these peaks were isolated and then identified by ^1H NMR as a **18αα** (**A**, 13%), **18βα** (**B**, 4%), **18αβ** (**C**, 11%), **18ββ** (**D**, 4%), respectively.[23]

Finally, compounds **18αα**, **18βα** , **18αβ**, and **18ββ** were subjected to hydrogenation in the presence of $Pd(OH)_2$/C to reduce the azido groups and cleave the benzyl and Cbz groups, giving the corresponding aminoglycoside compounds **19αα**, **19βα**, **19αβ**, and **19ββ**[24] (Scheme 3).

In conclusion, four aminoglycoside derivatives containing a 2,6-diamino-2,6-dideoxy-D-glucopyranose disaccharide structure were successfully prepared by using a Cbz-type heavy fluorous tag. The recyclable fluorous tag system was applied to Cbz-type fluorous tag, and fluorous alcohol **11** was recovered in excellent yield. The mixture of anomers was separated by HPLC. The inhibition potentials of the resulting aminoglycoside compounds for HIV TAR–Tat interaction are now



Scheme 3. Reagents and conditions: (a) H_2 (8 atm), $Pd(OH)_2/C$ (20 wt%), AcOH, EtOH, 2–5 days. **19αα** (53%), **19βα** (75%), **19αβ** (77%), **19 ββ** (71%).

being examined, and the results will be reported in due course.

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20. $EtOC_4F_9$ is a commercially available fluorocarbon solvent called Novec™ HFE-7200 (3M, Tokyo), that is, miscible in common organic solvents and fluorous solvents.
21. FC72 is a commercially available fluorocarbon solvent that consists mainly of perfluorohexane (C_6F_{14}) isomers and is called Fluorinert™ FC-72.
22. Compound **14**: ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 1.77$–1.95 (m, 9H), 2.00–2.16 (m, 8H), 2.40–2.75 (m, 13H), 3.28–3.49 (m, 15H), 3.51–3.63 (m, 8H), 3.69–3.74 (m, 2H), 4.12–4.35 (m, 5H), 5.13 (s, 2H), 7.42 (s, 2H), 7.81 (s, 2H). MALDI-TOF-MS: calcd for $C_{95}H_{72}F_{102}N_8O_{12}Na$ *m/z* $[M+Na]^+$: 3477.4. Found: 3480.2.
23. Compound **18αα**: ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 2.05$ (s, 1H), 3.24 (dd, $J = 6.2$, 9.7 Hz, 1H), 3.32 (dd, $J = 4.9$, 13.1 Hz, 1H), 3.39–3.44 (m, 2H), 3.45–3.66 (m, 4H), 3.72 (t, $J = 9.6$ Hz, 1H), 3.79 (s, 3H), 3.81–3.90 (m, 2H), 4.04–4.07 (t, $J = 8.9$ Hz, 1H), 4.16–4.21 (m, 2H), 4.72 (d, $J = 10.9$ Hz, 1H), 4.87 (d, $J = 11.0$ Hz, 1H), 4.90 (d, $J = 10.3$ Hz, 1H), 4.97 (d, $J = 3.4$ Hz, 1H), 5.00 (d, $J = 10.3$ Hz, 1H), 5.07 (d, $J = 13.0$ Hz, 1H), 5.27–5.33

(m, 2H), 5.67 (d, $J = 3.5$ Hz, 1H), 6.71 (s, 1H), 7.30–7.45 (m, 12H), 7.81–7.83 (m, 2H). MALDI-TOF-MS: calcd for $C_{40}H_{46}N_{14}O_{12}Na$ m/z $[M+Na]^+$: 937.3. Found: 937.9. Compound **18αβ**: ^1H NMR (600 MHz, $CDCl_3$): $\delta = 3.03$ (dd, $J = 6.2$, 13.0 Hz, 1H), 3.21–3.29 (m, 4H), 3.34–3.39 (m, 2H), 3.41–3.46 (m, 1H), 3.48–3.54 (m, 2H), 3.57–3.64 (m, 2H), 3.80–3.81 (m, 3H), 3.82–3.87 (m, 1H), 3.88–3.92 (m, 2H), 4.14–4.19 (m, 1H), 4.22–4.26 (m, 1H), 4.36 (d, $J = 8.2$ Hz, 1H), 4.69 (d, $J = 11.0$ Hz, 1H), 4.81 (d, $J = 11.6$ Hz, 1H), 4.90 (d, $J = 3.4$ Hz, 1H), 4.96 (d, $J = 11.6$ Hz, 1H), 5.16 (s, 2H), 5.30–5.33 (m, 1H), 7.26–7.45 (m, 12H), 7.80–7.81 (m, 2H). MALDI-TOF-MS: calcd for $C_{40}H_{46}N_{14}O_{12}Na$ m/z $[M+Na]^+$: 937.3. Found: 937.7. Compound **18βα**: ^1H NMR (600 MHz, $CDCl_3$): $\delta = 3.26$ (dd, $J = 3.4$, 9.6 Hz, 1H), 3.39 (dd, $J = 5.5$, 13.1 Hz, 1H), 3.43–3.55 (m, 8H), 3.59–3.63 (m, 1H), 3.67 (t, $J = 9.7$ Hz, 1H), 3.80–3.83 (m, 5H), 3.92–3.98 (m, 1H), 4.24 (d, $J = 4.8$ Hz, 1H), 4.39 (d, $J = 7.5$ Hz, 1H), 4.71 (d, $J = 11.7$ Hz, 1H), 4.83 (d, $J = 10.4$ Hz, 1H), 4.96 (d, $J = 11.0$ Hz, 1H), 5.06 (d, $J = 10.3$ Hz, 1H), 5.16 (s, 2H), 5.28–5.32 (m, 1H), 5.63 (d, $J = 3.4$ Hz, 1H), 6.65 (s, 1H), 7.29–7.45 (m, 12H), 7.80–7.81 (m, 2H). MALDI-TOF-MS: calcd for $C_{40}H_{46}N_{14}O_{12}Na$ m/z $[M+Na]^+$: 937.3. Found: 937.5. Compound **18ββ**: ^1H NMR (600 MHz, $CDCl_3$): $\delta = 2.08$ (s, 1H), 3.12 (dd, $J = 6.2$, 13.0 Hz, 1H), 3.19–3.31 (m, 4H), 3.34–3.53 (m, 8H), 3.64 (d, $J = 11.0$ Hz, 1H), 3.70–3.75 (m, 1H), 3.77–3.80 (m, 3H), 3.84 (t, $J = 7.6$ Hz, 1H), 3.89–3.93 (m, 1H), 4.23 (d,$J = 5.5$ Hz, 2H), 4.26–4.31 (m, 1H), 4.34 (d, $J = 8.4$ Hz, 1H), 4.66 (d, $J = 11.0$ Hz, 1H), 4.79 (d, $J = 11.7$ Hz, 1H), 4.87 (d, $J = 11.0$ Hz, 1H), 4.94 (d, $J = 11.7$ Hz, 1H), 5.14 (s, 2H), 5.26–5.30 (m, 1H), 6.60 (s, 1H), 7.24–7.42 (m, 12H), 7.78–7.79 (m, 2H). MALDI-TOF-MS: calcd for $C_{40}H_{46}N_{14}O_{12}Na$ m/z $[M+Na]^+$: 937.3. Found: 937.5.

24. Compound **19αα**: ^1H NMR (600 MHz, D_2O): $\delta = 2.76$–2.82 (m, 1H), 2.88–2.92 (m, 1H), 2.96–3.06 (m, 1H), 3.15–3.23 (m, 4H), 3.26–3.35 (m, 3H), 3.41–3.50 (m, 1H), 3.53–3.62 (m, 2H), 3.69–3.73 (m, 1H), 3.81–3.87 (m, 1H), 3.88–3.93 (m, 1H), 4.96–5.04 (m, 1H), 5.29–5.34 (m, 1H). Compound **19βα**: ^1H NMR (600 MHz, D_2O): $\delta = 2.59$–2.64 (m, 1H), 2.99–3.12 (m, 3H), 3.14–3.19 (m, 2H), 3.22–3.31 (m, 2H), 3.31–3.37 (m, 2H), 3.49–3.54 (m, 1H), 3.54–3.58 (m, 1H), 3.63–3.63 (m, 1H), 3.67–3.77 (m, 1H), 3.85–3.95 (m, 2H), 4.67–4.42 (m, 1H), 4.93–4.98 (m, 1H). Compound **19αβ**: ^1H NMR (600 MHz, D_2O): $\delta = 2.62$–2.67 (m, 1H), 2.99–3.17 (m, 5H), 3.24–3.33 (m, 3H), 3.46–3.55 (m, 2H), 3.58–3.62 (m, 1H), 3.64–3.71 (m, 2H), 3.72–3.77 (m, 1H), 4.03–4.08 (m, 1H), 4.38–4.40 (m, 1H), 5.46 (d, $J = 3.4$ Hz, 1H). Compound **19ββ**: ^1H NMR (600 MHz, D_2O): $\delta = 2.61$–2.64 (m, 1H), 2.66–2.70 (m, 1H), 3.03–3.18 (m, 4H), 3.21–3.30 (m, 4H), 3.31–3.40 (m, 4H), 3.43–3.48 (m, 1H), 3.48–3.57 (m, 1H), 3.65–3.71 (m, 1H), 3.72–3.78 (m, 1H), 4.01–4.08 (m, 1H), 4.35–4.46 (m, 2H).