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Synthesis of Oligodiaminosaccharides Having α-Glycoside Bonds and Their Interactions with Oligonucleotide Duplexes

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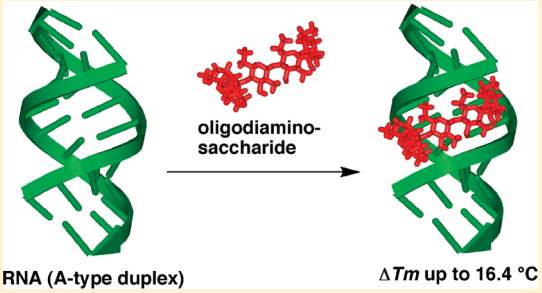
Supporting Information

ABSTRACT: Syntheses of the novel oligodiaminosaccharides, α-(1→4)-linked-2,6-diamino-2,6-dideoxy-D-glucopyranose oligomers, and their interactions with nucleic acid duplexes DNA−DNA, RNA−RNA, and DNA−RNA are described. Monomers to tetramers of oligodiaminoglucose derivatives having α-glycosyl bonds were successfully synthesized using a chain elongation cycle including glycosylation reactions of a 6-phthalimide glycosyl donor. UV melting experiments for a variety of nucleic acid duplexes in the absence and presence of the oligodiaminosaccharides were performed. The synthesized oligodiaminosaccharides exhibited notable thermodynamic stabilization effects on A-type RNA−RNA and DNA−RNA duplexes, whereas B-type DNA−DNA duplexes were not stabilized by the synthesized oligodiaminosaccharides. Among the oligodiaminosaccharides, the tetramer exhibited the highest ability to stabilize A-type duplexes, and the increase in T_m values induced by the tetramer were higher than those induced by neomycin B and tobramycin, which are known aminoglycosides having ability to bind and stabilize a variety of RNA molecules. CD spectrometry experiments revealed that the oligodiaminosaccharides caused small structural changes in RNA−RNA duplexes, whereas no appreciable changes were observed in the structure of DNA−DNA duplexes. ITC (isothermal titration calorimetry) experiments demonstrated that the amount of heat generated by the interaction between RNA−RNA duplexes and the tetradiaminosaccharides was approximately double that generated by that between DNA−DNA duplexes and the tetradiaminosaccharides. These results strongly suggested the existence of an A-type nucleic acid specific-binding mode of the oligodiaminosaccharides, which bind to these duplexes and cause small structural changes.



INTRODUCTION

Nucleic acids are vital biomolecules that can form a wide variety of higher order structures.[1,2] Numerous applications of nucleic acids as disease-specific drugs, highly functional devices, and components for molecular architecture have been reported.[3−6] Moreover, a wide variety of nucleic acid binding molecules such as intercalators[7] and groove binders[8] have been known. Among these nucleic-acid-binding molecules, naturally occurring aminoglycosides are known as RNA-binding molecules and they thermodynamically stabilize the higher order structures of RNA.[9−13] Concerning these cationic molecules, it has been reported that interactions between the protonated amino groups of these molecules and phosphate anions of nucleic acids are crucial to the stability of the RNA duplexes.[12] Among a variety of higher order structures of RNA, double-stranded RNA molecules recently have received attention because of the discovery of "RNA interference (RNAi)".[14] RNAi is a gene-regulating system that is controlled by external double-stranded RNAs (siRNAs). These siRNAs have been widely studied for therapeutic applications. However, such RNA molecules are not sufficiently stable under physiological conditions,[15,16] it is difficult to administer these RNAi-based drugs orally or intravenously.[17] To increase the stability of siRNAs, a number of chemical modifications have been proposed.[18] Another strategy to stabilize siRNAs is the use of molecules that can noncovalently bind to RNA and protect them from nucleases. For example, cationic liposomes, such as Lipofectamine, were broadly used as transfection reagents of nucleic acids.[19] An excess usage of cationic molecules including cationic liposomes and aminoglycosides, in many cases, causes cytotoxicity.[13,20] In contrast, if an RNA-interactive molecule can bind to the target in a stoichiometric manner, it would be useful as a less toxic carrier of RNA-drugs in vivo. On the basis of these considerations, we have designed "α-(1→4)-linked-2,6-diamino-2,6-dideoxy-D-glucopyranose oligomers (Figure 1, **1**)" which are expected to efficiently interact with double stranded RNA. Compound **1**, which possesses the properly arranged amino groups at the 2- and 6-positions in the repeating glucose units, can interact with ambilateral phosphate groups at the major groove of RNA duplexes because the major groove width of an A-type RNA−RNA duplex (7−9 Å[21,22]) is similar to the distance between 2-*N* and 6-*N* nitrogen atoms of **1** (approximately 6 Å). Moreover, because the α-(1→4)-linked glucose derivative is expected to form a curved structure similar to that of amylose,

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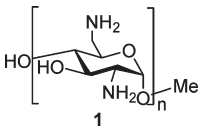

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Figure 1. α-(1→4)-Linked 2,6-diamino-2,6-dideoxy-D-glucopyranose oligomer.

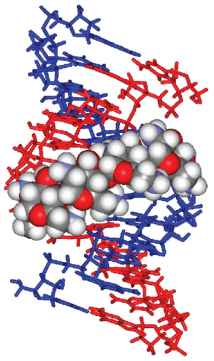


Figure 2. Molecular model of 4mer of **1** binding to A-type RNA duplex (12mer).

1 is entropically favored to bind to the nucleic acid duplexes. On the basis of molecular mechanics calculations with a GB/SA water solution model,[23] a 4mer of **1** can bind to the major groove of an A-type RNA−RNA duplex in which all the protonated amino groups of **1** form hydrogen bonds to the phosphate anions of the duplex (Figure 2). In a similar fashion, DNA−RNA hybrids that have an A-like duplex structure, can also be target molecules. To the best of our knowledge, only a few molecules are known to have an ability to bind and stabilize DNA-RNA duplexes.[24,25]

RESULT AND DISCUSSION

Synthesis of Monomer Building Blocks. Toward the syntheses of the α-linked oligodiaminoglucoses, we planned a synthetic cycle by recursively using a properly functionalized glycosyl donor recursively (Scheme 1). To make this strategy possible, the glycosyl donor should have a nonparticipating azide group at the 2-position, and the 4- and 6-hydroxyl groups should be protected by orthogonal protecting groups.

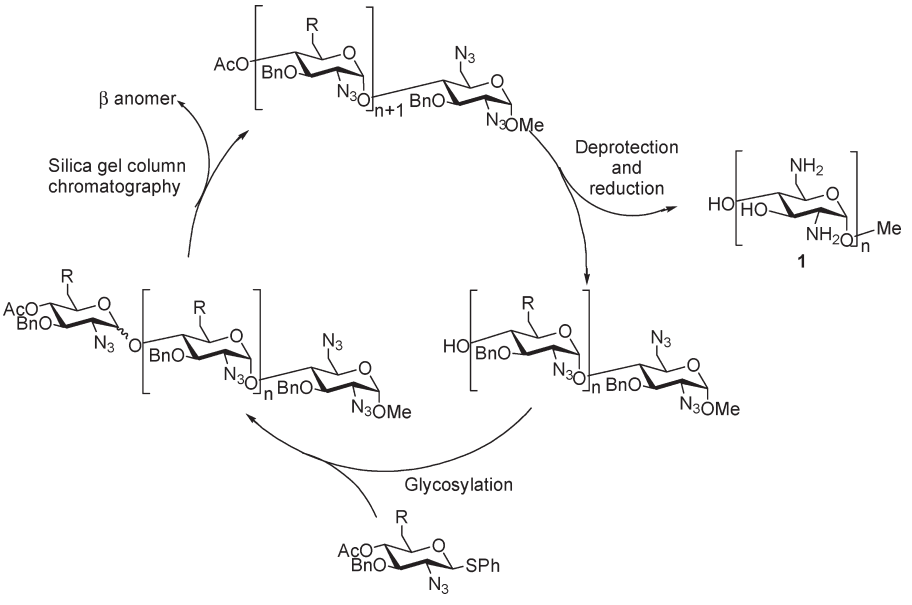
The glycosyl donors **4** and **6** were synthesized from a known 2-azidoglucose derivative **2** according to the procedure described in the literature.[26] Compound **2** was converted to **4** and **6** through a regioselective azidation[27] or phthalimidation[28] at the 6-position followed by acetylation of their 4-hydroxyl groups (Scheme 2). The starting glycosyl acceptor **12** was separately synthesized from a glucosamine derivative **7** that was synthesized following previously published procedures.[29] The 2-amino group of **7** was converted to an azido group,[30,31] and the 3-hydroxy group was protected by a benzyl group to produce **9**.[32] After acidic cleavage of 4,6-*O*-benzylidene acetal[33] of **9**, a 6-hydroxyl selective mesylation followed by a azidation afforded the glycosyl acceptor **12** (Scheme 3).

Extension of Sugar Chains. In the glycosylation reaction, commonly used *N*-iodosuccinimide and trifluoromethanesulfonic acid were used for activation of the thiophenyl glycoside **4** and **6**[34] (Table 1). The glycosylation reactions of both the glycosyl donors **4** and **6** with the glycosyl acceptor **12** were performed to obtain the disaccharides **13** and **14** in a similar stereoselectively. In the case of **14**, the α-isomer was easily separated from the α/β mixture by using silica gel column chromatography and pure $\mathbf{14}_{\alpha}$ was isolated with 65% yield (53% from the glycosyl acceptor **12**). In contrast, the α-isomer of **13** could not be isolated from the anomeric isomers.

The disaccharide **14** can be used as a glycosyl acceptor toward the synthesis of trisaccharides after deacetylation of the 4-hydroxyl group at the nonreducing terminus. However, deacetylation under basic conditions by using sodium methoxide or potassium carbonate, simultaneously caused the decomposition of phthaloyl groups. Meanwhile, deacetylation of **14** was successful under the anhydrous and acidic conditions using acetyl chloride[35] (Scheme 4).

The glycosylation and deacetylation established above were performed repeatedly to generate a protected trisaccharide and tetrasaccharide (Scheme 5). At these glycosylation steps, surprisingly,

Scheme 1. Synthetic Cycle of α-Linked Oligodiaminoglucose 1



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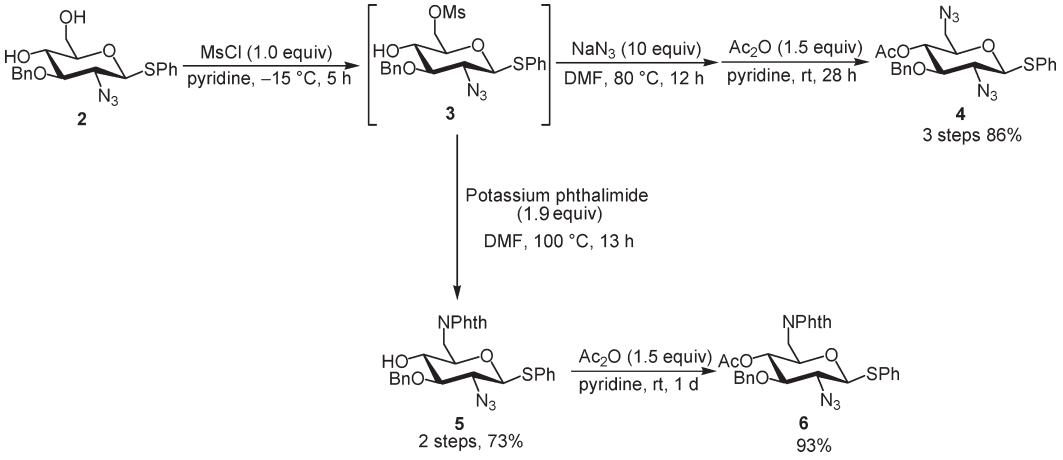
Scheme 2. Syntheses of the Glycosyl Donors



Scheme 3. Synthesis of the Glycosyl Acceptor

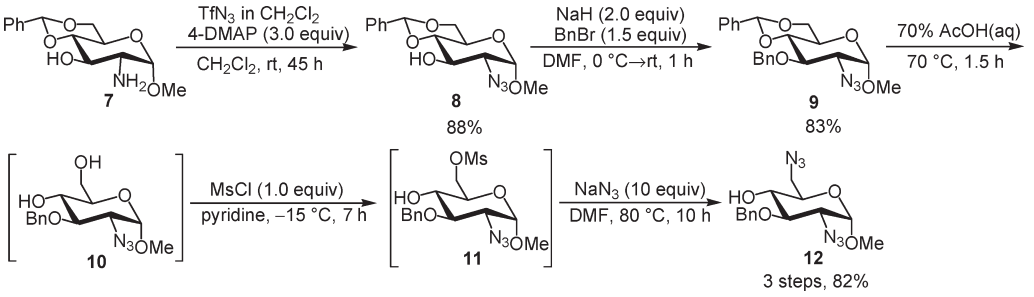
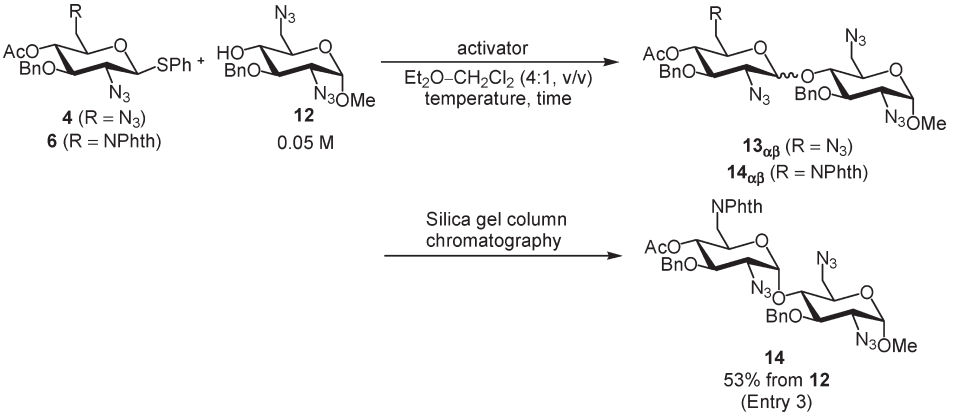


Table 1. Synthesis of α-Linked Disaccharides



entry	glycosyl donor	equiv of **4** or **6**	activator	temp (°C)	yield (%)	α:β[a]	time (h)
1	**6**	1.5	NIS (2.5 equiv)/TfOH (0.5 equiv)	rt	52	81:19	1
2	**4**	2.0	NIS (5.0 equiv)/TfOH (1.0 equiv)	rt	n.d.	79:21	1
3	**6**	2.0	NIS (5.0 equiv)/TfOH (1.0 equiv)	0	82	86:14	1
4	**6**	2.0	NIS (5.0 equiv)/TfOH (1.0 equiv)	−35	80	85:15	2
5	**6**	2.0	NIS (5.0 equiv)/TfOH (1.0 equiv)	−78	n.r.		

[a] Anomeric ratio was determined by ^{1}H NMR.

the α-isomers could be separated more easily than in the synthesis of the disaccharide.

Next, the simultaneous reduction of benzyl groups and azide groups was performed using a palladium carbon or palladium

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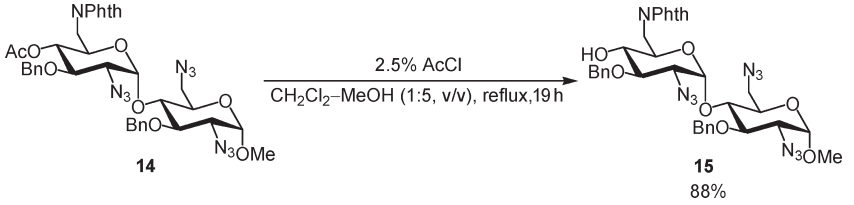
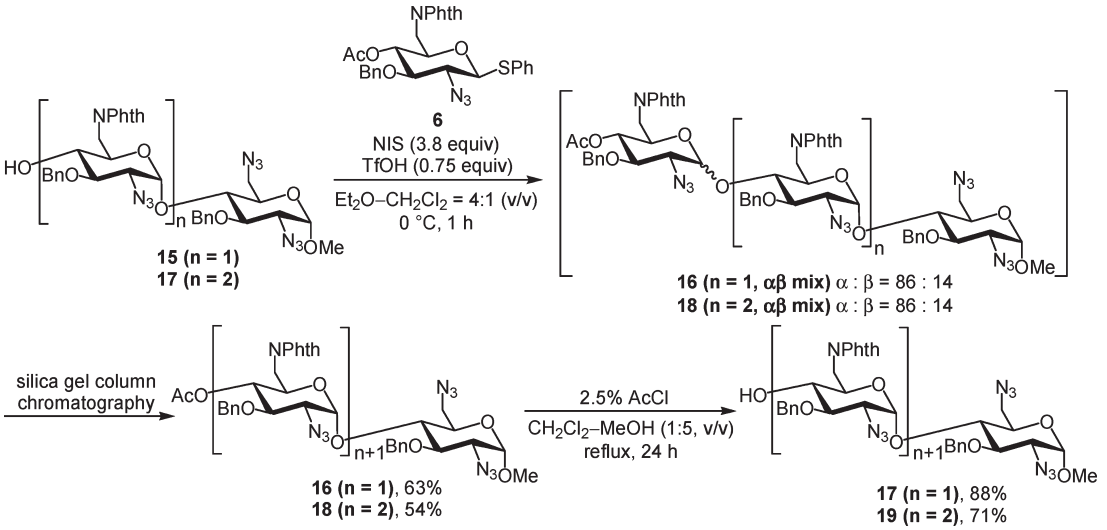
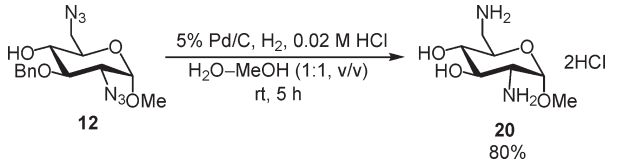
Scheme 4. Deacetylation under Anhydrous Acidic Conditions



Scheme 5. Syntheses of the Protected Oligodiaminosaccharides



Scheme 6. Synthesis of Mono-diaminosaccharide 20



hydroxide catalyst after the dephthaloylation. However, in this case, multiple products were produced (Scheme 6), while **12** was converted into **20** via a palladium-catalyzed reduction.

The protected 2−4mers were dephthaloylated by treatment with hydrazine monohydrate[36,37] followed by reduction of azido groups by the Staudinger reaction.[38] Thus, the obtained oligodiamino-3-*O*-benzylated saccharides **22**, **25**, and **28** were purified by reversed-phase HPLC. Finally, **23**, **26**, and **29** were successfully obtained by the reductive cleavage of the benzyl groups by using a palladium carbon catalyst (Scheme 7). In addition, a trial to perform simultaneous deacetylation and dephthaloylation by hydrazine monohydrate was failed because of the rearrangement of the acetyl group to an amino group. These amido linkages are difficult to cleavage under mild conditions.[39]

Melting Temperature (T_m) Analysis. The ratio of an amino sugar to a duplex was based on the number of amino groups in the oligodiaminosaccharide and the number of phosphate groups in the nucleic acid duplex. All experiments were performed near the physiological conditions with a 10 mM phosphate buffer containing 100 mM NaCl at pH 6.91. Figure 3 shows the UV melting curves and Table 2 shows the T_m values for a self-complementary RNA 12mer $(5'\text{-rCGCGAAUUCGCG-}3')_2$ in the absence or presence of oligodiaminosaccharides. It is apparent that tri- and tetradiaminosaccharides markedly stabilize the RNA−RNA duplex by 3.4 and 7.9 °C, respectively. Conversely, mono- and di diaminosaccharides did not stabilize the RNA−RNA duplexes. Table 2 also shows the results obtained using different equivalents of tetradiaminosaccharides **29**. In these experiments, T_m values increased as the number of equivalents increased. Furthermore, the T_m values increased drastically until 2 equivalents of tetradiaminosaccharides were added. For comparison, Table 2 also shows the results of UV melting analyses of the duplexes in the absence and presence of the natural aminoglycosides neomycin B and tobramycin (Figure 4) that are widely studied and known to bind and stabilize a variety of RNA molecules including RNA−RNA duplexes. In particular, neomycin B is reported to have high ability to stabilize RNA−RNA duplexes among a variety of aminoglycosides.[40] In this study, it is clear that tetradiaminosaccharide **29** increased the melting temperature of RNA−RNA duplexes more strongly than the neomycin B.

The results of UV melting analyses for a variety of 12mer and 24mer nucleic acid duplexes are summarized in Table 3, and a typical T_m curve for the 24mer RNA−RNA duplex rA_{24}-rU_{24} is shown in Figure 5. In the cases of RNA−RNA duplexes, both the oligodiaminosaccharides and aminoglycosides increased the T_m values for a variety of duplexes. For $(5'\text{-rAACCCGCGGGUU-}3')_2$, its melting temperature (77.7 °C) was 14 °C higher than that

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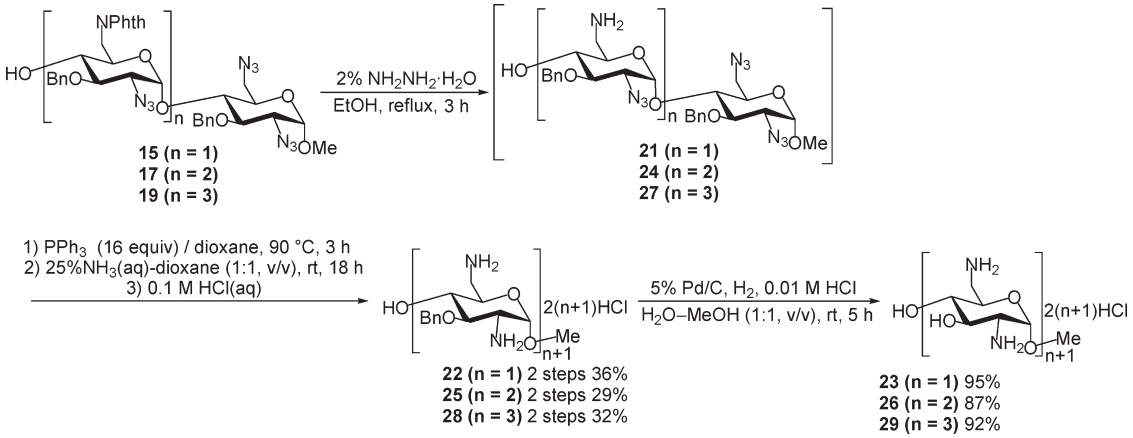
Scheme 7. Synthesis of Oligodiaminosaccharides 23, 26, and 29



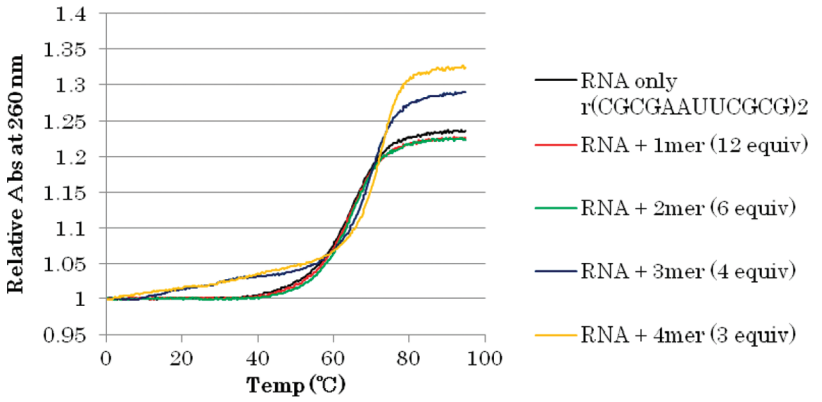


Figure 3. UV melting curves of $(5'\text{-rCGCGAAUUCGCG-}3')_2$.

of $(5'\text{-rCGCGAAUUCGCG-}3')_2$, which had the same nucleobase composition, but the T_m values were unaffected by the addition of mono- to tridiaminosaccharides. In this case, tetradiaminosaccharide **29** and aminoglycosides increased the T_m values, although these increases were smaller than those for $(5'\text{-rCGCGAAUUCGCG-}3')_2$. In contrast, for $rA_{12}\text{-}rU_{12}$, which has an innately low T_m of 18.8 °C, a prominent stabilization of the duplexes was observed by the addition of the amino sugars, even in the presence of didiaminosaccharides. These results suggest that the oligodiaminosaccharides and aminoglycosides stabilize thermodynamically unstable RNA duplexes. Notably, for almost all RNA duplexes that were used in the experiments, the ability of oligodiaminosaccharides to stabilize RNA duplexes increased as the length of the sugar chain increased, and tetradiaminosaccharides **29** exhibited the best ability to stabilize the duplexes among the amino sugars examined.

In clear contrast to the results of RNA duplex of the corresponding sequence (Figure 3, Table 2), all of the oligodiaminosaccharides did not stabilize DNA−DNA duplexes. Next, entries 5−7 show the results of DNA duplexes. In clear contrast to the results of RNA duplexes, none of the oligodiaminosaccharides and aminoglycosides induced substantial changes of T_m values for all of the DNA−DNA duplexes. These results suggest that the oligodiaminosaccharides and aminoglycosides do not increase the thermal stability of DNA−DNA duplexes. In some of the entries, however, oligodiaminosaccharides and aminoglycosides strengthened the relatively hypochromicity of DNA−DNA duplexes (see the Supporting Information). This may indicate the interactions between the sugars and the duplexes or single-stranded DNA at high temperature.

Table 2. Melting Temperatures of $(5'\text{-rCGCGAAUUCGCG-}3')_2$

entry	aminosaccharide	equiv[a]	T_m(°C)	Δ T_m (°C)
1	none		63.9	
2	1mer	12	63.9	0.0
3	2mer	6	64.3	0.4
4	3mer	4	69.0	5.1
5	4mer	1	68.0	4.1
6	4mer	2	70.9	7.0
7	4mer	3	71.8	7.9
8	4mer	4	72.7	8.8
9	neomycin B	4	69.4	5.5
10	tobramycin	4	66.2	2.3

[a] Equivalent is for the amount of nucleic acid duplex (not for the amount of nucleic acid molecule).

Entries 8−11 show the results of the UV melting analyses for the 24mer DNA−RNA hybrid duplexes, which have an A-type duplex structure, in the absence or presence of mono to tetradiaminosaccharides or aminoglycosides. For both $rA_{24}\text{-}dT_{24}$ and $dA_{24}\text{-}rU_{24}$, a certain degree of increase of the T_m values was observed. These results clearly suggest the oligodiaminosaccharides can also interact to A-like nucleic acid duplexes.

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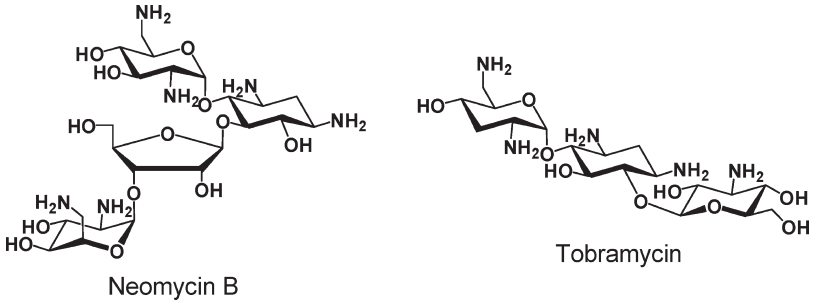

Figure 4. Chemical structures of aminoglycosides.

Table 3. Melting Temperatures of a Variety of Sequences of Duplexes[a]

			Δ T_m (°C)					
entry	duplex	T_m (°C)	1mer	2mer	3mer	4mer	neomycin	tobramycin
1	r(CGCGAAUUCGCG)$_2$	63.9	0.0	0.4	5.1	7.9	5.5	2.3
2	r(AACCCGCGGGUU)$_2$	77.7	−0.3	−0.2	0.3	1.9	2.3	0.6
3	rA$_{12}$-rU$_{12}$	18.8		3.4	11.7	16.4	14.5	6.4
4	rA$_{24}$-rU$_{24}$	39.3		2.5	7.5	15.2	10.4	5.2
5	d(CGCGAATTCGCG)$_2$	55.7	−0.3	−0.3	−0.4	−0.3	0.0	−1.2
6	d(AACCCGCGGGTT)$_2$	60.4	−0.4	−0.1	−0.3	0.0	−0.3	−0.3
7	d(AAAAAATTTTTT)$_2$	32.9		−0.1	0.1	0.2	0.4	0.1
8	dA$_{12}$-rU$_{12}$	11.9				8.1	6.3	
9	rA$_{12}$-dT$_{12}$	26.6				2.8	1.9	
10	dA$_{24}$-rU$_{24}$	24.9		7.1	14.7	21.8	20.8	14.8
11	rA$_{24}$-dT$_{24}$	45.2		0.0	1.3	4.0	2.7	0.6

[a] Equivalents of aminosaccharides are 12 (1mer **20**), 6 (2mer **23**), 4 (3mer **26**, neomycin, tobramycin), and 3 (4mer **29**) for 12mer duplexes and 12 (2mer **23**), 8 (3mer **26**, neomycin, tobramycin), and 6 (4mer **29**) for 24mer duplexes.

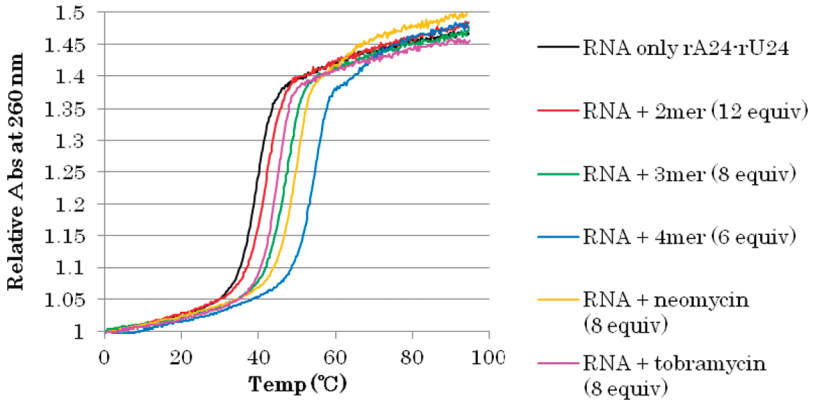

Figure 5. UV melting curves of rA$_{24}$-rU$_{24}$.

As an extension of this study, stereoselectively synthesized phosphorothioate DNA (R_P-PS-DNA and S_P-PS-DNA)-native RNA duplex was also used as substrates. Because the all-R_P-PS-DNA−RNA duplex has negatively charged sulfate atoms on the inner side of the major groove of the duplexes and the all-S_P-PS-DNA−RNA has these on the outer side,[41] we expected to observe differences of ΔT_m caused by electrostatic interactions with cationic oligodiaminosaccharides.

Table 4 shows the T_m values of PS-DNA-native RNA and PS-DNA-native DNA duplexes[42] in the absence or presence of the tetradiaminosaccharide **29**. Changes of the T_m values of PS-DNA-native RNA hybrid duplexes were observed, and these values varied with their stereochemistry. As shown in entries 1−3, the all R_P-PS-DNA−RNA duplexes, whose negatively charged sulfate atoms are located on the inner side of the major groove of the duplexes, were slightly more stabilized by the tetradiaminosaccharide. These results did not conflict with our hypothesis that oligodiaminosaccharides bind to the major groove of A-type nucleic acid duplexes. Conversely, regarding the PS-DNA-native DNA duplexes (entry 4−6), only slight

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Table 4. Melting Temperatures of PS-DNA−RNA and PS-DNA−DNA Duplexes in the Absence or Presence of Tetradiaminosaccharide

entry	duplex[a]	T_m (°C)[b]	Δ T_m (°C)[c]
1	native DNA−RNA	48.6	4.0
2	R_P-PS-DNA−RNA	42.1	6.9
3	S_P-PS-DNA−RNA	36.9	5.3
4	native DNA−DNA	49.7	0.0
5	R_P-PS-DNA−DNA	41.2	0.8
6	S_P-PS-DNA−DNA	43.3	0.4

[a] Base sequiences are 5′-dCpsApsGpsTpsCpsApsGpsTpsCpsApsGpsT-3′/5′-dACTGACTGACTG-3′ or 5′-dCpsApsGpsTpsCpsApsGpsTps-CpsApsGpsT-3′/5′-rACUGACUGACUG-3′. [b] T_m value in the absence of saccharide. [c] Δ T_m in the presence of 3 equiv of tetradiaminosaccharide **29**.

changes of the T_m values were observed and these results are similar to that for native DNA duplexes.

CD spectrometry. CD spectra of self-complementary RNA−RNA and DNA−DNA duplexes $(5'\text{-rCGCGAAUUCGCG-}3')_2$ and $(5'\text{-dCGCGAATTCGCG-}3')_2$ in the presence or absence of mono- to tetradiaminosaccharides were measured to detect their structural changes. Upon the addition of tri- or tetradiaminosaccharides, some changes of the spectra of RNA−RNA duplex were observed. The positive peak near 265 nm and the negative peak near 210 nm due to the Cotton effect slightly shifted 1−2 nm to the longer wavelength, and their peak intensities increased up to 24% at the positive peak of 265 nm on the addition of the tetradiaminosaccharide **29** (Figure 6). In contrast, appreciable spectral changes were not observed on adding the mono- or didiaminosaccharides. In the case of DNA−DNA duplexes, no spectral changes were observed under identical experimental conditions (Figure 7). Spectral changes of the RNA−RNA duplex caused by the addition of 1−4 equivalents of tetradiaminosaccharide **29** are shown in the Supporting Information. The spectral changes were nearly saturated with 2 equivalents of **29**. All these results correspond to those of T_m analyses described in the previous section. These facts strongly suggest **26** and **29** stabilize RNA−RNA duplexes by causing small structural changes and binding them to maintain the A-type duplex structure.

For comparison, CD spectra of RNA−RNA duplexes in the presence of neomycin B were measured (see the Supporting Information). The spectra of RNA−RNA duplexes changed with the peak shift to a longer wavelength and the increase of the peak intensity around 210 and 265 nm. These spectral changes were very similar to those for the tri- and tetradiaminosaccharides. These results suggest that the structural changes of the duplexes caused by neomycin B and oligodiaminosaccharides are quite similar.

Isothermal Titration Calorimetry (ITC) Experiments. In the ITC experiments, the duplex concentration was 2-fold higher than those in the UV melting and CD spectrometry analyses, because the amount of heat generated by binding between the nucleic acid duplexes and the tetradiaminosaccharide was expected not to be sufficient to calculate thermodynamic parameters. Figure 8 shows the results of ITC titration of the tetradiaminosaccharide with the self-complementary RNA−RNA duplex $(5'\text{-rCGCGAAUUCGCG-}3')_2$ and DNA−DNA

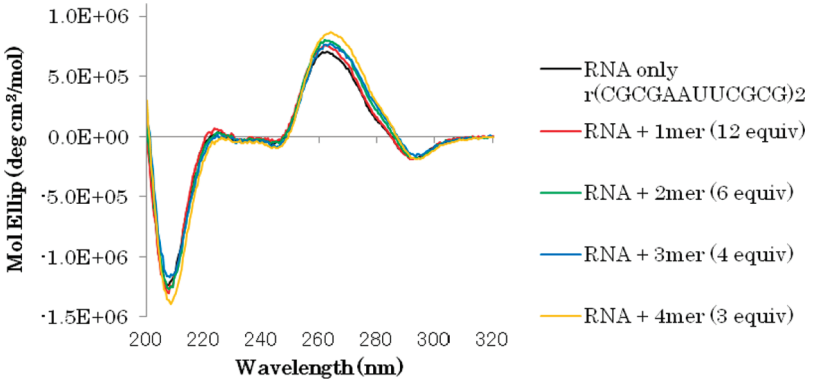

Figure 6. CD spectra of $(5'\text{-rCGCGAAUUCGCG-}3')_2$.

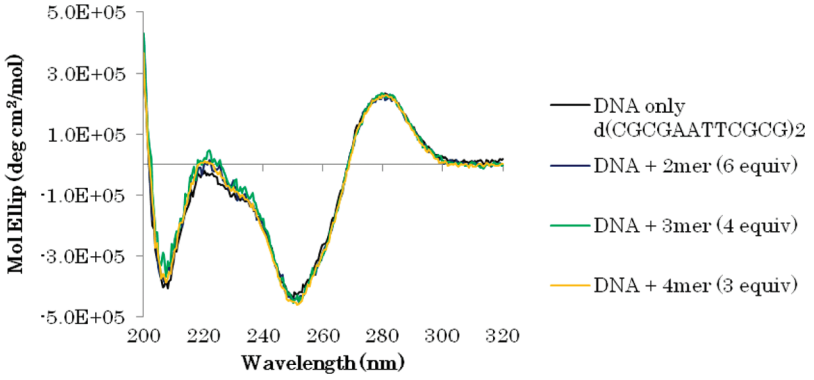

Figure 7. CD spectra of $(5'\text{-rCGCGAATTCGCG-}3')_2$.

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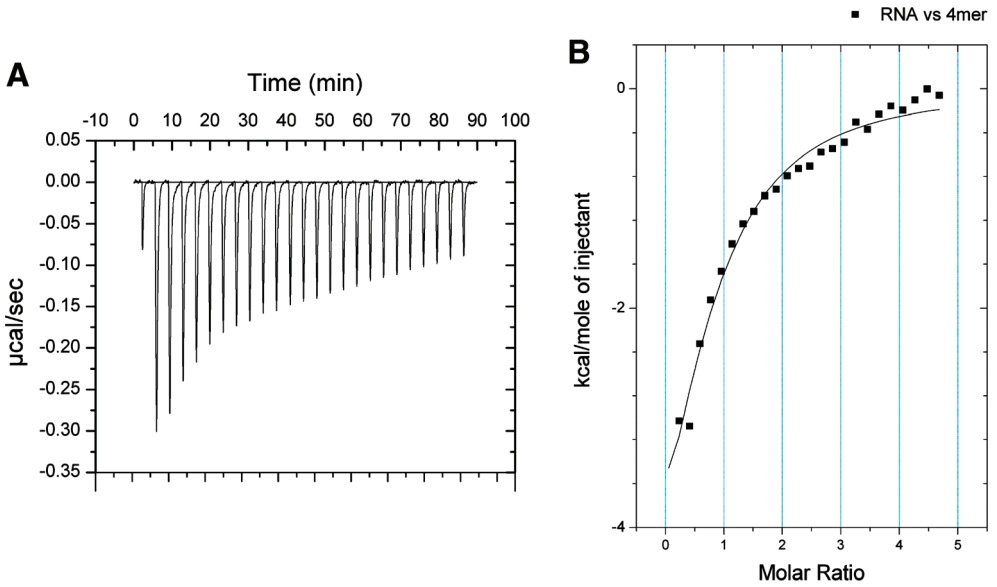

Figure 8. (A) ITC profiles at 25 °C for the titration of tetradiaminosaccaride **29** into a solution of RNA−RNA duplex; Each curve is the result of a 10 μL injection of 250 μM saccharide. The duplex concentration was 10 μM in a 10 mM phosphate buffer with 100 mM NaCl at pH 6.91; (B) Corrected injection heats in the case of RNA−RNA were plotted.

duplex $(5'\text{-dCGCGAATTCGCG-}3')_2$. These results indicated that the tetradiaminosaccharide bound to both the RNA−RNA and DNA−DNA duplexes. However, the binding modes were clearly different. The amount of heat generated by the instillation of the saccharide solution to the RNA solution is approximately double that for DNA (See the Supporting Information). These results indicate that the binding of the saccharide to RNA−RNA is stronger than to DNA−DNA, and strongly suggest the existence of an A-type duplex specific binding mode of the oligodiaminosaccharides. In the case of tetradiaminosaccharide-RNA, binding ratio N was 0.73. ΔH and ΔS were estimated to be −7705 (kcal/mol) and 2.67 (kcal/mol·K), respectively. In contrast, in the case of DNA, precise parameters could not be calculated because of the large margin of error in the same experimental conditions. These errors may be attributable to nonspecific interactions between the tetradiaminosaccharide and the DNA at the present concentration. With a higher concentration of nucleic acid duplexes, more than two state binding modes were observed (data not shown). Under this condition, thermodynamic parameters corresponding to the UV melting analysis and CD spectrometry experiments were not calculated.

CONCLUSION

We have developed a method to synthesize oligodiaminoglucoses, and we successfully synthesized 1- to 4mers of α-(1→4)-linked-2,6-diamino-2,6-dideoxy-D-glucopyranose oligomers. Based on the UV melting analysis, CD spectrometry findings, and ITC measurements, these oligodiaminosaccharides, particularly the 3mer and 4mer, stabilized only A-type nucleic acid duplexes such as RNA−RNA and DNA-RNA, by causing small structural changes. The experimental results suggested that the existence of an A-type duplex specific binding mode of the oligodiaminosaccharides. In particular, tetradiaminosaccharide **29** had the largest stabilization effect on these duplexes among the saccharides used in this study, including neomycin B and tobramycin, which are naturally occurring aminoglycosides known to bind and stabilize a variety of RNA molecules. Conversely, all of the oligodiaminosaccharides did not stabilize DNA−DNA duplexes or change their structures. This is probably because the major groove width of well-known DNA−DNA duplexes is 14−16 Å in solution, which is too wide to be efficiently bound by the amino groups of the saccharides. On the basis of these unique properties, **26** and **29** will be useful as stabilizers of siRNA- and miRNA-based drugs or new materials for their DDS.

EXPERIMENTAL SECTION

Conditions for Measurement of T_m Analysis. Absorbance versus temperature profile measurements were carried out with eight-sample cell changer, in quarts cells of 1 cm path length. The variation of UV absorbance with temperature was monitored at λ = 260 nm. The temperature was scanned between 0 and 95 °C, and rate of temperature increase was 0.2 °C/min. Oligonucleotides were annealed after addition of amino sugars. The samples containing oligonucleotides and amino sugars were first rapidly heated to 95 °C, left for 20 min, and then allowed to cool slowly to room temperature. These samples were furthermore cooled to 0 °C and left for 1 h, and then the dissociation was recorded by heating to 95 °C at rate of 0.25 °C/min.

Conditions for Measurement of CD Spectra. All CD spectra were recorded at 25 °C. The following instrument settings were used: resolution, 0.1 nm; sensitivity, 10 mdeg; response, 4 s; speed, 10 nm/min; accumulation, 6.

Conditions for ITC Experiments. The tetradiaminosaccharide **29** and the nucleic acid duplexes were in a 10 mM phosphate buffer with 100 mM NaCl at pH 6.91. The tetradiaminosaccharide solution (250 μM) was titrated into the nucleic acid duplex solutions (10 μM) at 25 °C. Each titration of the tetradiaminosaccharide solution consisted of a preliminary 3 μL injection followed by 24 subsequent 10 μL additions, which were performed over 20 s periods at 210 s intervals.

*Phenyl 4-O-Acetyl-2,6-dazido-2,6-dideoxy-3-O-benzyl-1-thio-β-D-glucopyranoside (**4**).* Under argon atmosphere, **2** (0.194 g, 0.5 mmol) was coevaporated with dry pyridine (5 mL × 3) and dissolved in dry pyridine (10 mL). The solution was stirred and cooled to −15 °C, and methanesulfonyl chloride (43 μL, 0.53 mmol, 1.06 equiv) was added.

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After 11 h, the reaction was quenched by the addition of methanol (2 mL), concentrated, and coevaporated with toluene (7 mL × 2). The mixture was dissolved in chloroform, washed with saturated aqueous solution of $NaHCO_3$ (15 mL × 3), dried over Na_2SO_4, and evaporated. The crude **3** was coevaporated with dry *N*,*N*-dimethylformamide (8 mL × 3) and dissolved in dry *N*,*N*-dimethylformamide (5 mL). Sodium azide (0.324 g, 5.0 mmol, 10 equiv) was added to the solution, and it was stirred and heated to 80 °C. After 12 h, the mixture was cooled to rt and concentrated to dryness. The crude product was extracted with ethyl acetate−water (1:1, v/v, 20 mL), the organic layer was washed with water (10 mL × 2), and the aqueous layer was back extracted with ethyl acetate (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude azidosugar was dissolved in dry pyridine (10 mL) under argon atmosphere. The solution was stirred and acetic anhydride (75 μL, 0.79 mmol, 1.58 equiv) was added. After 28 h, the mixture was concentrated to dryness and dissolved in chloroform. The solution was washed with saturated aqueous solution of $NaHCO_3$ (15 mL × 2), dried over Na_2SO_4, filtered, and evaporated. The crude product was purified with silica gel column chromatography (chloroform) and **4** was obtained as colorless oil (0.196 g, 0.43 mmol, 86%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28−7.62 (m, 10H, S*Ph*, *Ph*-CH_2), 4.80−4.90 (m, 2H, H-4, Ph-CH_2a), 4.62 (d, *J* = 11.3 Hz, 1H, Ph-CH_2b), 4.43 (d, *J* = 9.9 Hz, 1H, H-1), 3.47−3.54 (m, 2H, H-3, H-5), 3.22−3.39 (m, 3H, H-2, H-6), 1.96 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.5, 137.2, 134.5, 129.8, 129.1, 129.0, 128.5, 128.1, 128.0, 85.9, 82.3, 75.4, 70.5, 64.5, 51.3, 20.7; ESI-HRMS *m*/*z* calcd for $C_{21}H_{22}KN_6O_4S^+$ (M + K^+) 493.1060, found 493.1075.

*Phenyl 2-Azido-2-deoxy-3-O-benzyl-6-deoxy-6-phthalimide-1-thio-β-D-glucopyranoside (**5**).* Under argon atmosphere, **2** (5.417 g, 14.0 mmol) was coevaporated with dry pyridine (10 mL × 3) and dissolved in dry pyridine (200 mL). The solution was stirred and cooled to −15 °C, and methanesulfonyl chloride (1.1 mL, 14.3 mmol, 1.02 equiv) was added. After 5 h, the reaction was quenched by addition of methanol (10 mL), concentrated and coevaporated with toluene (10 mL × 2). The mixture was dissolved in chloroform (100 mL), washed with saturated aqueous solution of $NaHCO_3$ (100 mL × 3), dried over Na_2SO_4 and evaporated. The crude mesylate **3** was coevaporated with dry *N*,*N*-dimethylformamide (15 mL × 3) and dissolved in dry *N*,*N*-dimethylformamide (140 mL). Potassium phthalimide (4.89 g, 26.4 mmol, 1.9 equiv) was added to it, and the solution was stirred and heated to 100 °C. After 13 h, the mixture was cooled to rt and concentrated. The solution containing the crude product was diluted with chloroform (100 mL) and washed with 10% brine (100 mL). After further washing of the organic layer with 10% brine (100 mL), the organic layer was dried over Na_2SO_4, filtered, and evaporated. The crude product was recrystallized from ethanol (400 mL), and **5** was obtained as a colorless solid (5.28 g, 10.2 mmol, 73%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74−7.88 (m, 4H, N*Phth*), 7.02−7.51 (m, 10H, S*Ph*, *Ph*-CH_2), 4.81−4.86 (dd, *J* = 10.7 and 16.5 Hz, 2H, Ph−CH_2), 4.32 (d, *J* = 10.2 Hz, 1H, H-1), 4.16 (dd, *J* = 3.3 and 14.6 Hz, 1H, H-6a), 3.97 (dd, *J* = 4.1 and 14.6 Hz, 1H, H-6b), 3.48−3.54 (m, 1H, 5-H), 3.38 (t, *J* = 9.0 Hz, 1H, 3-H), 3.25 (m, 1H, 4H), 3.10 (dd, *J* = 9.1 and 10.2 Hz, 1H, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.7, 137.6, 135.3, 134.4, 131.7, 129.4, 128.8, 128.6, 128.3, 128.1, 123.6, 85.3, 83.4, 75.7, 71.4, 64.2, 38.1; ESI-HRMS *m*/*z* calcd for $C_{27}H_{24}KN_4O_5S^+$ (M + K^+) 555.1099, found 555.1106.

*Phenyl 4-O-Acetyl-2-azido-2-deoxy-3-O-benzyl-6-deoxy-6-phthalimide-1-thio-β-D-glucopyranoside (**6**).* Under argon atmosphere, **5** (5.28 g, 10.2 mmol) was dissolved in dry pyridine (100 mL). The solution was stirred, and acetic anhydride (1.5 mL, 15.9 mmol, 1.56 equiv) was added. After 1 d, the mixture was concentrated and dissolved in chloroform. The solution was washed with a saturated aqueous solution of $NaHCO_3$ (30 mL × 3), dried over Na_2SO_4, filtered, and evaporated. The crude product was recrystallized from ethanol (50 mL), and **6** was obtained as a colorless solid (5.29 g, 9.5 mmol, 93%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72−7.86(m, 4H, N*Phth*), 6.98−7.42 (m, 10H, S*Ph*, *Ph*-CH_2), 4.79−4.90 (m, 2H, H-4, Ph-CH_2a), 4.63 (d, *J* = 11.3 Hz, 1H, Ph-CH_2b), 4.36 (d, *J* = 9.9 Hz, 1H, H-1), 4.00 (dd, *J* = 9.6 and 14.0 Hz, 1H, H-6a), 3.72 (m, 1H, H-5), 3.57 (dd, *J* = 2.5 and 14.0 Hz, 1H, H-6b), 3.49 (t, *J* = 9.3 Hz, 1H, 3-H), 3.35 (t, *J* = 9.3 Hz, 1H, H-2), 2.00 (s, 3H, Ac); ^{13}C NMR (300 MHz, $CDCl_3$) δ 169.9, 167.8, 137.2, 134.3, 134.1, 131.2, 129.9, 128.7, 128.5, 128.1, 128.0, 123.4, 85.7, 82.2, 75.4, 75.0, 71.8, 69.6, 64.6, 39.2, 20.8; ESI-HRMS *m*/*z* calcd for $C_{29}H_{26}KN_4O_6S^+$ (M + K^+) 597.1205, found 597.1237.

*Methyl O-(2,6-Diazido-2,6-dideoxy-3-O-benzyl-α-D-glucopyranoside) (**12**).* Under argon atmosphere, **9** (2.53 g, 6.4 mmol) was dissolved to 70% aqueous solution of acetic acid (300 mL) and heated to 70 °C. After 1.5 h, the solution was cooled to rt and concentrated. The solution of crude product was diluted to ethyl acetate (200 mL) and washed with saturated aqueous solution of $NaHCO_3$ (100 mL × 2). The aqueous layer was back-extracted with ethyl acetate (100 mL). The organic layer was dried over Mg_2SO_4, filtered, and evapolated to dryness. The mixture was purified by silica gel column chromatography (Ethyl acetate/hexane, 1:2, v/v and then ethyl acetate), and crude **10** was obtained. Under argon atmosphere, the crude **10** was coevaporated with dry pyridine (15 mL × 3) and dissolved in dry pyridine (130 mL). The solution was stirred and cooled to −15 °C, and methanesulfonyl chloride (0.50 mL, 6.46 mmol, 1.02 equiv) was added. After 7 h, the reaction was quenched by addition of methanol (5 mL) and saturated aqueous solution of $NaHCO_3$ (3 mL) and the mixture was concentrated to dryness. The mixture was dissolved in ethyl acetate (100 mL) and washed with saturated aqueous solution of $NaHCO_3$ (50 mL × 3), and the water phase was back extracted with ethyl acetate (50 mL), dried over $MgSO_4$, and evaporated. The crude mesylate **11** was coevaporated with dry *N*,*N*-dimethylformamide (10 mL × 3) and dissolved to dry *N*,*N*-dimethylformamide (64 mL). Sodium azide (4.16 g, 64 mmol, 10 equiv) was added to the solution, and the solution was stirred and heated to 80 °C. After 10.5 h, the mixture was cooled to rt and concentrated. The crude product was extracted with ethyl acetate−water (2:1, v/v, 150 mL), the organic layer was washed with water (50 mL × 2), and the water layer was back extracted with ethyl acetate (50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. The crude product was purified with silica gel column chromatography (dichloromethane−hexane = 2:1 to 1:0, v/v) and **12** was obtained as pale yellow oil (1.76 g, 5.3 mmol, 82%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31−7.43 (m, 5H, *Ph*-CH_2), 4.99 (d, *J* = 11.2 Hz, 1H, Ph-CH_2a), 4.83 (d, *J* = 3.6 Hz, 1H, H-1), 4.67 (d, *J* = 11.6 Hz, 1H, Ph−CH_2b), 3.73−3.80 (m, 2H, H-3, H-5 or H-6), 3.36−3.55 (m, 6H, H-4, OCH_3, H-5 or H-6), 2.08 (d, *J* = 2.8 Hz, 1H, OH-4), 3.35 (t, 1H, H-2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.8, 128.6, 128.4, 128.1, 98.7, 80.1, 75.1, 71.0, 70.6, 63.2, 55.5, 51.3; ESI-HRMS *m*/*z* calcd for $C_{14}H_{18}KN_6O_4^+$ (M + K^+) 373.1021, found 373.1025.

*Methyl O-((4-O-Acetyl-2-azido-2-deoxy-3-O-benzyl-6-deoxy-6-phthalimide-α-D-glucopyranosyl)-(1→4)-2,6-dazido-2,6-dideoxy-3-O-benzyl-α-D-glucopyranoside) (**14**).* Under argon atmosphere, *N*-iodosuccinimide (0.0562 g, 250 μmol) was added to a mixture of **6** (56.1 mg, 100 μmol, 2.0 equiv) and **12** (50 μmol) after coevaporation with dry toluene (1 mL × 6). The mixture was dissolved in a mixed solvent (1 mL, Et_2O−CH_2Cl_2, 4:1, v/v), cooled to 0 °C, and stirred, and then trifluoromethanesulfonic acid (4.4 μL, 50 μmol) was added to the solution. After 1 h, water (2 μL) was added and the mixture warmed to rt. The solution was diluted with chloroform (20 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (15 mL) and 10% $Na_2S_2O_3$aq (15 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product purified by silica gel column chromatography (ethyl acetate−hexane (3:7, v/v)) to afford the pure α-glycoside **14** as a pale yellow oil (20.4 mg, 26.5 μmol, 53%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72−7.88 (m, 4H, N*Phth*), 7.28−7.38 (m, 10H, *Ph*-CH_2), 5.61 (d, *J* = 3.9 Hz, 1H, H-1′), 4.95 (m, 2H, H-4′, Ph-CH_2a), 4.80−4.86 (m, 2H, Ph-CH_2b, Ph-CH_2c), 4.74 (d, *J* = 3.3 Hz, H-1), 4.65 (d, *J* = 11.0 Hz, 1H, Ph-CH_2d), 3.89−4.00 (m, 4H, H-3′, H-6a′, H-6b′,

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H-3), 3.75 (m, 1H, H-5), 3.60−3.66 (m, 2H, H-5′, H-4), 3.27−3.39 (m, 6H, H-2, H-6a, H-2′, OCH_3), 3.01 (dd, J = 4.4 and 13.2 Hz, 1H, H-6b), 2.05 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9, 168.0, 137.4, 137.2, 134.0, 131.9, 128.5, 128.0, 127.9, 127.6, 123.4, 123.2, 98.4, 97.4, 80.1, 74.9, 74.5, 74.4, 72.1, 69.8, 69.6, 63.8, 62.4, 55.5, 51.0, 39.1, 20.9; ESI-HRMS m/z calcd for $C_{37}H_{38}KN_{10}O_{10}^+$ (M + K^+) 821.2404, found 821.2413.

*Methyl O-(2-Azido-2-deoxy-3-O-benzyl-6-phthalimide-6-deoxy-α-D-glucopyranosyl)-(1→4)-2,6- dazido-2,6-dideoxy-3-O-benzyl-α-D-glucopyranoside) (**15**).* Under argon atmosphere, **14** (0.148 g, 0.19 mmol) was dissolved in a mixed solvent (18 mL, MeOH−CH_2Cl_2, 5:1, v/v) and stirred at rt. Acetyl chloride (450 μL) was added to the solution, and it was refluxed. After 19 h, the solution was cooled to rt, and a saturated aqueous solution of $NaHCO_3$ was added. The mixture was concentrated, dissolved in chloroform (20 mL), and washed with saturated aqueous solution of $NaHCO_3$ (15 mL × 3). The organic was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate−toluene (1:9, v/v)) to give **15** as a pale yellow oil (0.123 g, 0.165 mmol, 88%): ^{1}H NMR (300 MHz, $CDCl_3$) δ7.72−7.88 (m, 4H, N*Phth*), 7.24−7.42 (m, 10H, *Ph*-CH_2), 5.51 (d, J = 3.9 Hz, 1H, H-1′), 4.83−4.97 (m, 5H, H-1, Ph-CH_2), 4.10 (dd, J = 3.3 and 14.6 Hz, 1H, H-6a′), 3.85−4.04 (m, 4H, H-3′, H-5′, H-6′, H-3), 3.71−3.78 (m, 2H, H-3, H-5), 3.35−3.56 (m, 8H, H-4′, H-6b, H-2, OCH_3, H-6a, OH-4′), 3.21 (dd, J = 4.1 and 10.2 Hz, 1H, H-2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 137.7, 137.5, 134.3, 131.7, 128.6, 128.4, 128.2, 128.0, 127.8, 127.7, 123.6, 98.5, 97.9, 80.4, 78.8, 75.5, 74.6, 74.1, 72.4, 71.3, 69.8, 69.6, 63.9, 62.6, 55.5, 51.2, 38.2; ESI-HRMS m/z calcd for $C_{35}H_{36}KN_{10}O_9^+$ (M + K^+) 779.2298, found 779.2314.

*Compound **16**.* The same method for **14** was applied for the synthesis of **16**, except that **6** (0.117 g, 0.209 mmol, 1.5 equiv) and **15** (0.139 g, 0.103 mmol) were used as starting materials. Purification by silica gel column chromatography was performed with ethyl acetate−toluene (1:9, v/v) and ethyl acetate−hexane (7:13, v/v) as eluent. **16** was obtained as colorless oil (0.105 g, 88 μmol, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57−7.90 (m, 8H), 7.31−7.57 (m, 15H), 5.59 (d, J = 3.9 Hz, 1H), 5.49 (d, J = 3.6 Hz, 1H), 4.71−5.05 (m, 8H), 4.34 (t, J = 8.7 Hz, 1H), 4.09−4.17 (m, 3H), 3.87−3.98 (m, 3H), 3.54−3.75 (m, 5H), 3.23−3.43 (m, 8H), 3.14 (dd, J = 3.6 and 10.2 Hz, 1H), 2.98 (dd, J = 4.1 and 13.5 Hz, 1H), 2.07 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 167.8, 137.5, 137.3, 137.2, 134.2, 133.9, 132.0, 131.4, 128.6, 128.5, 128.0, 127.9, 127.7, 127.6, 127.5, 123.4, 123.2, 98.4, 98.3, 97.6, 80.1, 79.2, 77.3, 75.2, 74.4, 74.1, 74.0, 72.5, 69.7, 69.4, 63.8, 63.2, 62.5, 55.5, 50.9, 39.6, 39.1, 20.9; ESI-HRMS m/z calcd for $C_{58}H_{56}KN_{14}O_{15}^+$ (M + K^+) 1227.3681, found 1227.3706.

*Compound **17**.* The same method for **15** was applied for the synthesis of **17**, except for using **16** (81.8 mg, 69 μmol) as a starting material. Purification by silica gel column chromatography was performed with ethyl acetate-toluene (3:17, v/v) as an eluent. **17** was obtained as a colorless oil (69.2 mg, 60 μmol, 88%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65−7.91 (m, 8H), 7.29−7.46 (m, 15H), 5.57 (d, J = 3.3 Hz, 1H), 5.47 (d, J = 4.1 Hz, 1H), 4.79−5.07 (m, 6H), 4.80 (d, J = 10.2 Hz, 1H), 4.27 (m, 1H), 3.91−4.09 (m, 6H), 3.25−3.84 (m, 14H), 3.03 (J = 3.9 and 10.5 Hz, dd, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.7, 168.1, 137.9, 137.3, 134.3, 134.0, 132.0, 131.6, 128.5, 128.2, 128.0, 127.9, 127.6, 123.5, 123.3, 98.8, 98.4, 97.5, 80.2, 79.2, 78.9, 75.5, 74.5, 74.3, 73.9, 72.9, 71.2, 70.5, 69.7, 63.8, 63.1, 62.6, 55.5, 50.9, 39.8, 38.4; ESI-HRMS m/z calcd for $C_{56}H_{54}N_{14}NaO_{14}^+$ (M + Na^+) 1169.3836, found 1169.3860.

*Compound **18**.* The same method for **14** was applied for the synthesis of **18**, except for using **6** (50.3 mg, 90 μmol, 1.45 equiv) and **17** (71.0 mg, 62 μmol) as starting materials. Purification by silica gel column chromatography was performed with ethyl acetate−toluene (1:6, v/v) and ethyl acetate−hexane (2:3, v/v) as an eluent. Compound **16** was obtained as colorless oil (53.3 mg, 33.4 μmol, 54%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59−7.88 (m, 12H), 7.30−7.36 (m, 20H, *Ph*-CH_2), 5.55 (d, J = 3.9 Hz, 1H), 5.44−5.48 (2d, 3.6 Hz, 3.9 Hz, 2H), 4.73−5.03 (m, 10H), 4.08−5.03 (m, 4H), 3.71−3.96 (m, 7H), 3.15−3.98 (m, 13H), 3.09 (dd, J = 3.6 and 10.3 Hz, 1H, H-2′ or H-2″), 3.00 (dd, J = 4.4 and 13.5 Hz, 1H), 1.97 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 167.8, 167.6, 137.4, 137.3, 137.1, 134.1, 133.9, 132.0, 131.5, 131.4, 128.5, 127.9, 127.8, 127.5, 127.4, 127.3, 123.4, 123.3, 123.2, 98.3, 98.1, 97.4, 80.0, 79.2, 78.9, 77.7, 76.8, 75.1, 74.3, 74.1, 74.0, 72.3, 69.7, 69.5, 69.2, 68.9, 63.8, 63.2, 63.1, 62.6, 55.5, 50.1, 39.7, 39.4, 39.1, 20.8; ESI-HRMS m/z calcd for $C_{79}H_{74}N_{18}NaO_{20}^+$ (M + Na^+) 1617.5219, found 1617.5205.

*Compound **19**.* The same method for **15** was applied for the synthesis of **19**, except for using **18** (53.3 mg, 33 μmol) as a starting material. Purification by silica gel column chromatography was performed with ethyl acetate−toluene (1:6, v/v) as an eluent. Compound **19** was obtained as colorless oil (36.6 mg, 23.6 μmol, 71%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64−7.91 (m, 12H), 7.31−7.42 (m, 20H), 5.33−5.53 (3d, J = 3.9 Hz, J = 3.6 Hz, J = 3.9 Hz, 3H), 4.72−5.14 (m, 9H), 4.43 (t, 1H), 4.11−4.29 (m, 4H), 3.54−4.04 (m, 11H), 3.26−3.46 (m, 10H), 3.11 (dd, J = 1H, J = 3.6 and 10.2 Hz), 3.01 (dd, J = 3.9 and 13.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8, 168.0, 167.9, 137.9, 137.8, 137.4, 137.2, 134. 2, 133.9, 132.1, 131.6, 128.6, 128.5, 128.4, 128.2, 127.9, 127.8, 127.6, 127.5, 123.5, 123.4, 123.2, 98.9, 98.3, 97.6, 80.2, 79.8, 79.1, 78.6, 75.6, 74.7, 74.5, 74.1, 73.9, 72.6, 71.3, 70.0, 69.7, 69.6, 63.8, 63.2, 63.1, 63.0, 55.5, 50.9, 39.7, 38.0; ESI-HRMS m/z calcd for $C_{77}H_{72}N_{18}NaO_{19}^+$ (M + Na^+) 1575.5113, found 1575.5097.

*Methyl O-(2,6-Diamino-2,6-dideoxy-α-D-glucopyranoside) hydrochloride (**20**).* Compound **12** (8.0 mg, 24 μmol) was dissolved in a mixed solvent (1.5 mL, water−methanol, 1:1, v/v, 0.1 M HCl), 5% Pd/C (0.01 g) was added and stirred. After 5 h of hydrogen bubbling, Pd/C was removed by filtration with filter paper and membrane filter (0.45 μm), and the solution was concentrated and coevaporated with water (2 mL × 4). The crude product was dissolved in methanol (0.1 mL), reprecipitated from acetone (1.5 mL), and washed with acetone (1.5 mL × 2). The precipitate was dissolved in water and lyophilized from water to give **20** as colorless solid (19.1 μmol, 80%): ^{1}H NMR (300 MHz, D_2O) δ 5.07 (d, J = 3.9 Hz, 1H, H-1), 3.86−3.90 (m, 2H, H-3, H-5), 3.22−3.51 (m, 6H, H-2, H-4, OCH_3, H-6a), 3.17 (dd, J = 10.4 and 13.5 Hz, 1H, H-6b); ^{13}C NMR (75 MHz, D_2O) δ 96.91, 71.82, 70.11, 68.47, 56.19, 54.29, 40.88; ESI-HRMS m/z calcd for $C_7H_{17}N_2O_4^+$ (M + H^+) 193.1183, found 193.1187.

*Methyl O-((2,6-Diamino-2,6-dideoxy-3-O-benzyl-α-D-glucopyranosyl)-(1→4)-2,6-diamino-2,6-dideoxy-3-O-benzyl-α-D-glucopyranoside) hydrochloride (**22**).* Under argon atmosphere, **15** (13.9 mg, 18.8 μmol), ethanol (1 mL), and hydrazine monohydrate (25 μL) were added to a round-bottom flask, stirred, and refluxed. After 3 h, the mixture was cooled to rt and concentrated. The crude product was dissolved in chloroform (2 mL), and insoluble phthalyl hydrazide was removed by filtration. The chloroform solution was washed with water (10 mL × 3) and the organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude sugar was dissolved in a mixed solvent (2.4 mL, dioxane−methanol, 5:1, v/v) and stirred. Triphenylphosphin (0.0800 g, 305 μmol, 16.2 equiv) was added to the solution and refluxed over 3 h. After cooling to rt, 25% aqueous solution of ammonia (2.4 mL) was added to the solution and the flask was sealed. After the solutionw as stirred overnight, the mixture was concentrated. Then water (5 mL) and 0.1 M hydrochloric acid (1 mL) were added and the mixture sonicated for 1 min. The aqueous solution was filtered, washed with chloroform (10 mL × 3), concentrated, and coevaporated with water (3 mL × 3). The crude product was purified with C18 reversed-phase HPLC (0.05% TFA, water−acetonitrile). After HPLC purification, fraction was concentrated and 0.02 M hydrochloric acid (5 mL) was added. The solution was concentrated and coveaporated with water (1 mL × 4) to give a benzyl-protected **22** as colorless oil (6.8 μmol, 36%): ^{1}H NMR (300 MHz, D_2O) δ 7.38−7.45 (m, 10H, *Ph*-CH_2), 5.64 (d, J = 3.3 Hz, 1H, H-1′), 5.14

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(d, J = 3.9 Hz, 1H, H-1), 4.68−4.89 (m, 4H, Ph-CH_2), 4.38 (dd, J = 8.0 and 10.5 Hz, 1H), 4.13−4.19 (m, 2H), 3.78−3.94 (m, 3H), 3.70 (t, J = 8.0 Hz, 1H), 3.31−3.56 (m, 8H); ^{13}C NMR (75 MHz, D_2O) δ 137.4, 136.6, 129.7, 129.4, 129.2, 129.1, 128.4, 96.9, 93.8, 78.0, 76.1, 75.2, 72.7, 72.2, 71.4, 70.0, 66.9, 56.6, 52.4, 41.1, 40.0; ESI-HRMS m/z calcd for $C_{27}H_{41}N_4O_7^+$ (M + H$^+$) 533.2970, found 533.3015.

*Methyl O-((2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl)-(1→4)-2,6-diamino-2,6-dideoxy-α-D- glucopyranoside Hydrochloride (**23**).* The same method for **20** was applied for the synthesis of **23**, except that **22** (6.8 μmol) was used as a starting material. Compound **23** was obtained as a colorless solid (6.5 μmol, 95%): ^{1}H NMR (300 MHz, D_2O) δ 5.78 (d, J = 3.3 Hz, 1H, H-1′), 5.09 (d, J = 3.6 Hz, 1H, H-1), 4.03−4.18 (m, 2H, H-5, H-3), 3.76−3.88 (m, 3H, H-4, H-5′, H-3′), 3.38−3.53 (m, 8H, H-2′, H-6a, H-6a′, H-2, OCH_3, H-4′), 3.24−3.48 (m, 2H, H-6b, H-6b′); ^{13}C NMR (75 MHz, D_2O) δ 96.8, 96.6, 77.1, 71.1, 70.6, 69.9, 69.2, 66.7, 56.5, 54.6, 54.2, 41.1, 40.6; ESI-HRMS m/z calcd for $C_{13}H_{29}N_4O_7^+$ (M + H$^+$) 353.2031, found 353.2049.

*Compound **25***. The same method for **22** was applied for the synthesis of **25**, except **17** (10.7 mg, 9.3 μmol) was used as a starting material. Compound **25** was obtained as a colorless oil (2.79 μmol, 29%): ^{1}H NMR (300 MHz, D_2O) δ 7.20−7.42 (m, 15H), 5.48 (d, J = 1.9 Hz, 1H), 5.35 (d, J = 3.6 Hz, 1H), 5.10 (d, J = 3.6 Hz, 1H), 4.71−4.99 (m, 6H), 4.51−4.57 (m, 3H), 4.07−4.28 (m, 6H), 3.80−3.88 (m, 1H), 3.74 (t, J = 8.5 Hz, 1H), 3.36−3.60 (m, 10H), 3.15−3.22 (m, 2H); ^{13}C NMR (75 MHz, D_2O) δ 137.09, 136.98, 129.81, 129.71, 129.57, 129.49, 129.38, 128.87, 128.47, 97.10, 96.57, 91.81, 78.37, 76.93, 75.19, 75.80, 73.64, 72.93, 71.16, 70.94, 70.42, 67.94, 67.36, 56.57, 53.96, 53.01, 49.78, 41.06, 39.99, 38.82; ESI-HRMS m/z calcd for $C_{40}H_{59}N_6O_{10}^+$ (M + H$^+$) 783.4287, found 783.4271.

*Compound **26***. The same method for **20** was applied for the synthesis of **26**, except **25** (2.79 μmol) was used as a starting material. Compound **26** was obtained as a colorless solid (2.42 μmol, 87%): ^{1}H NMR (300 MHz, D_2O) δ5.78−5.83 (2d, J = 3.9 Hz, 3.9 Hz, 2H), 5.10 (d, J = 3.6 Hz, 1H), 4.03−4.24 (m, 4H), 3.80−3.94 (m, 4H), 3.28−3.55 (m, 13H); ^{13}C NMR (75 MHz, D_2O) δ 97.08, 96.74, 96.22, 77.45, 77.24, 70.92, 70.52, 69.93, 69.51, 69.12, 68.34, 66.61, 56.50, 54.57, 54.44, 54.27, 41.18, 40.45; ESI-HRMS m/z calcd for $C_{19}H_{41}N_6O_{10}^+$ (M + H$^+$) 513.2879, found 513.2883.

*Compound **28***. The same method for **22** was applied for the synthesis of **28**, except **19** (13.7 mg, 8.8 μmol) was used as a starting material. Compound **28** was obtained as a colorless oil (2.82 μmol, 32%): ^{1}H NMR (300 MHz, D_2O) δ 7.31−7.46 (m, 20H), 5.46−5.62 (3d, J = 2.8 Hz, J = 3.0 Hz, J = 2.5 Hz, 3H), 5.16 (d, J = 3.9 Hz, 1H), 4.69−4.94 (8H), 4.33−4.49 (m, 4H), 4.02−4.26 (m, 7H), 3.67−3.80 (m, 4H), 3.32−3.57 (m, 12H); ^{13}C NMR (75 MHz, D_2O) δ 137.46, 136.90, 136.76, 129.80, 129.68, 129.62, 129.49, 129.31, 129.22, 128.56, 128.43, 128.39, 96.97, 95.51, 93.76, 92.96, 78.27, 76.28, 75.51, 75.22, 74.46, 73.62, 72.99, 72.62, 71.91, 67.10, 56.57, 53.24, 52.53, 51.00 41.20, 39.82; ESI-HRMS m/z calcd for $C_{53}H_{77}N_8O_{13}^+$ (M + H$^+$) 1033.5605, found 1033.5637.

*Compound **29***. The same method for **20** was applied for the synthesis of **29**, except **28** (2.82 μmol) was used as a starting material. Compound **29** was obtained as a colorless solid (2.59 μmol, 92%): ^{1}H NMR (300 MHz, D_2O) δ 5.81−5.87 (m, 3H), 5.11 (d, J = 3.6 Hz, 1H), 4.10−4.34 (m, 6H), 3.82−3.96 (m, 5H), 3.31−3.60 (m, 16H); ^{13}C NMR (75 MHz, D_2O) δ 97.03, 96.72, 96.58, 96.16, 77.40, 77.27, 70.89, 70.51, 69.92, 69.38, 69.10, 68.43, 68.23, 66.59, 56.50, 54.57, 54.42, 54.28, 41.34, 41.25, 41.11, 40.42; ESI-HRMS m/z calcd for $C_{25}H_{53}N_8O_{13}^+$ (M + H$^+$) 673.3727, found 673.3705.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra of **4**−**6**, **12**, **14**−**20**, **22**, **23**, **25**, **26**, **28**, and **29**, methods of experiments, UV melting curves, and ITC profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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