



**Synthesis of oligodiaminomannoses and analysis of their
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ARTICLE

Synthesis of oligodiaminomannoses and analysis of their RNA duplex binding properties and their potential application as siRNA-based drugs

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The synthesis of artificial cationic oligodiaminosaccharides, α -(1 \rightarrow 4)-linked-2,6-diamino-2,6-dideoxy-D-mannopyranose oligomers (ODAMans), and their interactions with RNA duplexes are described. The monomer through the pentamer, all bearing unnatural 2,6-diaminomannose moieties were successfully prepared. UV melting and fluorescence anisotropy analysis revealed that the ODAMan bound and thermodynamically stabilized both 12mer RNA duplexes and an siRNA. Furthermore, it was clearly shown the siRNA acquired substantial RNase A resistance due to the binding of the ODAMan 4mer.

Introduction

In recent years, RNA–RNA duplexes have attracted attention due to their variety of functions and unique higher-order structures. In particular, the discovery of RNA interference (RNAi) has made a considerable impact on nucleic acid-related research.¹ RNAi is triggered by small interfering RNAs (siRNAs) that consist of RNA duplexes. An siRNA targets a specific sequence of messenger RNA (mRNA) and suppresses its expression via a duplex formation between the siRNA guide strand and the target mRNA followed by its cleavage by the RNAi mechanism. Although this specificity for the target molecule is quite attractive to therapeutics,^{2–4} the use of siRNAs as drug substances presents many obstacles, such as their instability *in vivo* and the lack of effective drug delivery systems (DDSs). To increase the stability of siRNAs *in vivo*, various covalent chemical modifications have been proposed.^{5–7} The use of molecules that noncovalently bind to siRNAs has also been investigated^{8–9}. Note that both strategies are applicable to the conjugation of functional molecules to siRNAs for drug delivery.

In the latter strategy, cationic molecules, such as cationic liposomes, cationic polymers, aminoglycosides, and other cationic compounds, have been shown to be siRNA conjugatable reagents.^{8–12} Some can also protect siRNAs from nucleases. However, in many cases the cationic molecules exhibit cytotoxicity resulting from their nonspecific binding to other biomolecules, such as DNAs, RNAs, and proteins.^{13–14} Therefore, molecules that specifically bind to RNA duplexes

and do not require excess quantities for conjugation are needed as suitable carriers for siRNA.

We previously reported RNA-duplex-bindable “oligodiaminoglucoses (ODAGlcs),” which have amino groups at the 2- and 6-positions of α -(1 \rightarrow 4)-linked oligo-D-glucose.¹⁵ In that report, we hypothesized that the curved shape and electrostatic potential surface of these molecules might fit RNA duplexes via efficient interactions between the cationic ammonium groups of the ODAGlcs and the ambilateral anionic phosphate groups at the major groove of RNA. The experimental results were consistent with the above hypothesis, and the ODAGlcs selectively bound to A-type duplexes, such as RNA duplexes. Conversely, the α -linked glucose frameworks were somewhat cumbersome to synthesize and required separation from their β anomers.

Herein, we describe a facile synthesis of α -(1 \rightarrow 4) linked-

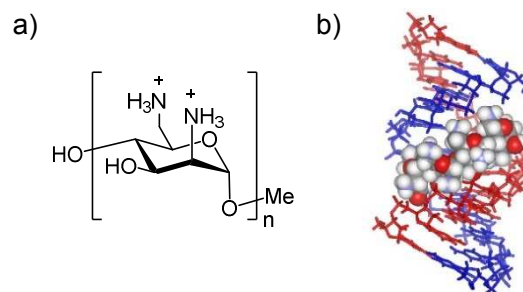


Figure 1. a) α -1-4 linked 2,6-diamino, 2,6-dideoxy D-mannose oligomers (ODAMans). b) Molecular model of ODAMan 4mer binding to an A-type RNA duplex (12mer).

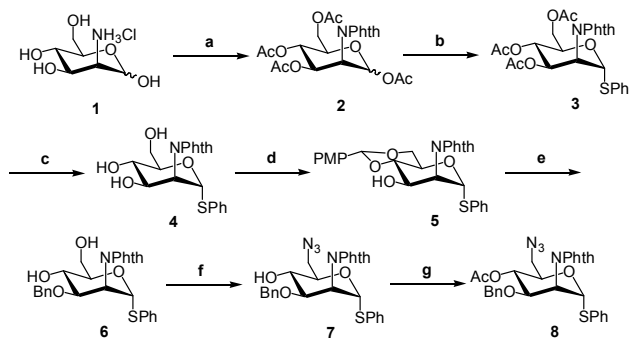
2,6-diamino-2,6-dideoxy oligo-D-mannose oligomers (ODAMans) as novel RNA-duplex-bindable oligosaccharides (Figure 1a). These oligosaccharides differ from ODAGlcs only in their C2 configuration which has an axial amino group. Based on molecular mechanics calculations using a generalized Born/surface area (GB/SA) water solvation model,¹⁶ it was determined that the 4mer of ODAMan should bind to the major groove of an RNA duplex with electrostatic interactions as efficient as those of the ODAGlcs (Figure. 1b).

Results and discussion

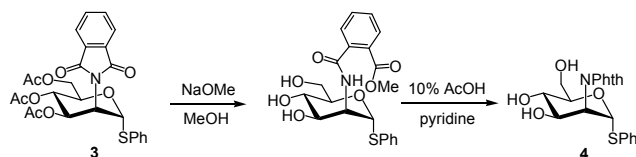
Synthesis of monomer building blocks

For the synthesis of α -linked ODAMan derivatives via glycosylation, the glycosyl donor **8** was designed and synthesized from commercially available D-mannosamine hydrochloride **1** (Scheme 1).

The hydroxyl groups and the 2-amino group of D-mannosamine hydrochloride **1** were protected by acetyl groups and a phthaloyl group, respectively to afford **2**.¹⁷ Compound **2** was then converted to **3** via treatment with boron trifluoride and benzenethiol, followed by the deacetylation of the thioglycoside. During the deacetylation of **3** using sodium methoxide in methanol, ring opening of the phthalimide group was observed as a side reaction (Scheme 2). This ring-opening reaction was also observed under anhydrous acidic conditions using acetyl chloride, and other side reactions (likely hydrolysis of the glycosyl bond) occurred with hydrochloric acid–acetone (data

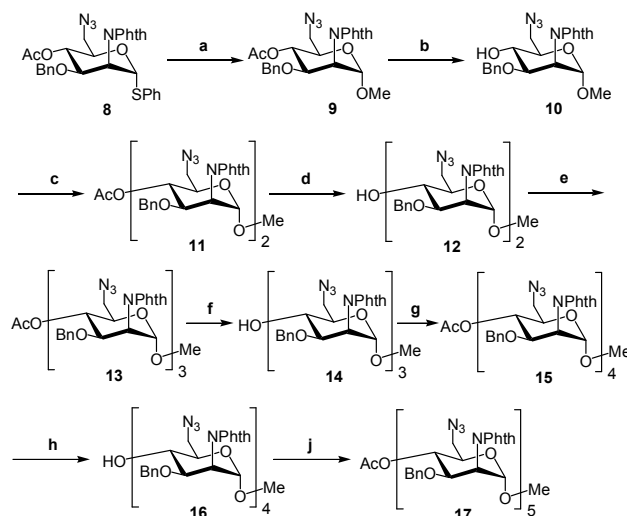


Scheme 1 Synthesis of the glycosyl donor **8**. Reagents and conditions: (a) (i) NaOMe, MeOH, rt, 40 min (ii) phthalic anhydride, TEA, MeOH, rt, 4 h (iii) Ac₂O, DMAP, pyridine, rt, 12 h, 83% over three steps. (b) PhSH, BF₃·OEt₂, CH₂Cl₂, rt, 5 h, 90% (c) (i) NaOMe, MeOH, rt, 1.5 h (ii) AcOH, pyridine, reflux, 14 h, 86% over two steps. (d) anisaldehyde dimethyl acetal, TsOH·H₂O, CH₃CN, 0 °C, 5 h, 96% (e) (i) BnBr, NaH, DMF, 0 °C to rt, 2 h, then quenched with MeOH (ii) AcOH, pyridine, reflux, 6 h (iii) AcOH, H₂O–EtOH, 50 °C, 27 h, 87% over three steps. (f) (i) MsCl, pyridine, -15 °C, 12 h (ii) NaN₃, DMF, 80 °C, 16 h, 79% over two steps (g) Ac₂O, DMAP, pyridine, rt, 12 h, 89%



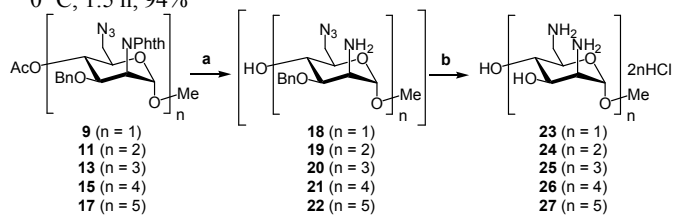
Scheme 2 Ring-opening and regeneration of the 2-phthalimide group

not shown). It was therefore difficult to synthesize **4** from **3** via a one-step process. Hence, the crude mixture including the ring-opened product was heated in acetic acid–pyridine to regenerate the phthalimide group, and the diacetylated compound **4** was obtained (Scheme 2). The 4- and 6-hydroxyl groups of **4** were then protected using a methoxybenzylidene group to give **5**,¹⁵ followed by the protection of the 3-OH via benzylation. Unfortunately, the ring-opening of the phthaloyl group was also observed during quenching with excess sodium hydride in methanol. This side reaction (resulting in the formation of a benzyl ester) could not be completely suppressed



Scheme 3 Elongation of the sugar chains by glycosylation.

Reagents and conditions: (a) NIS, TfOH, MeOH, CH₂Cl₂–Et₂O, 0 °C, 40 min, 92% (b) (i) NaOMe, MeOH, rt, 1 h (ii) AcOH, pyridine, reflux, 41 h, 75% over two steps. (c) **8**, NIS, TfOH, CH₂Cl₂–Et₂O, 0 °C, 1.5 h, 85% (d) (i) NaOMe, MeOH–CH₂Cl₂, rt, 2 h (ii) AcOH, pyridine, reflux, 30 h, 73% over two steps. (e) **8**, NIS, TfOH, CH₂Cl₂–Et₂O, 0 °C, 1.5 h, 90% (f) (i) NaOMe, MeOH–CH₂Cl₂, rt, 3.5 h (ii) AcOH, pyridine, reflux, 60 h, 78% over two steps. (g) **8**, NIS, TfOH, CH₂Cl₂–Et₂O, 0 °C, 1.5 h, 89% (h) (i) NaOMe, MeOH–CH₂Cl₂, rt, 5 h (ii) AcOH, pyridine, reflux, 26 h, 70% over two steps. (j) **8**, NIS, TfOH, CH₂Cl₂–Et₂O, 0 °C, 1.5 h, 94%



Scheme 4 Deprotection and reduction. Reagents and conditions:

(a) NH₂NH₂·H₂O, EtOH, reflux, 12–32 h (b-1) (i) 5%Pd/C, H₂, 0.01 M HCl, H₂O–MeOH, 4 h (ii) 10%Pd/C, H₂, 0.01 M HCl, H₂O–MeOH, 2 h, 8% from **9** (**23**, n = 1) (b-2) (i) PPh₃ or PMePh₂, dioxane–MeOH, reflux, 4–18 h (ii) NH₄OH, dioxane–MeOH, rt, 5–55 h (iii) 0.1 M HCl (iv) 10%Pd/C, H₂, 0.01–0.05 M HCl, H₂O or H₂O–MeOH, 6 h–3 d, 18% from **11** (**24**, n = 2), 37% from **13** (**25**, n = 3), 53% from **15** (**26**, n = 4), 30% from **17** (**27**, n = 5)

even when water or acetic acid was used. Hence, the regeneration of the phthalimide group was also performed at this stage to afford the 3-*O*-benzyl 2-phthalimide mannose derivative **6**. The phthalimide group at the 2-axial position in D-mannosamine derivatives may be less stable than the equatorial 2-phthalimide groups in glucosamine and galactosamine derivatives. Finally, the cleavage of the 4,6-*O*-*p*-methoxybenzylidene group of **5** under acidic conditions, followed by the azidation of the 6-position and acetylation of the 4-hydroxyl group afforded the glycosyl donor **8**.

Elongation of the sugar chains and deprotection

Elongation of the sugar chains was performed via a standard glycosylation procedure using *N*-iodosuccinimide and trifluoromethanesulfonic acid to activate the glycosyl donor **8**, and an α -mannoside was selectively obtained. When compound **11** was synthesized in dichloromethane or a dichloromethane-rich mixed solvent, the yield of the product was relatively low (39–79%). Although by-products could not be identified, side reactions of the azide groups under acidic conditions may have occurred; the yield was increased to 85% using a diethyl ether-rich mixed solvent with higher Lewis basicity. After glycosylation, deacetylation was performed under weak acidic conditions. Because ring-opening of the phthalimide groups also occurred in these reactions, the crude products were heated in acetic acid-pyridine to regenerate them. Repeating the synthetic cycle including glycosylation in the diethyl ether-rich mixed solvent, deacetylation, and regeneration of the phthalimide groups, as shown in Scheme 3, the protected 1-5 mers (**9**, **11**, **13**, **15**, and **17**, respectively) were obtained.

The acetyl and phthaloyl groups of these protected ODAMans were simultaneously removed via treatment with hydrazine followed by the reduction of the azide groups using the Staudinger reaction. The resulting 3-*O*-benzyl ODAMans **19–22** were purified via reversed phase HPLC, and finally **23**, **24**, **25**, **26**, and **27** were successfully obtained following reductive cleavage of the benzyl groups using a palladium on carbon catalyst (Scheme 4). Note that when attempting the simultaneous reduction of the azide groups and the removal of the benzyl groups in **20** via treatment with H₂–Pd/C, the benzyl groups were scarcely removed even under acidic conditions. Therefore, it was necessary for these conversions to be conducted separately, as shown in Scheme 4.

UV melting analysis

The sequences of RNA-I and RNA-II are rA₁₂/rU₁₂ and (5'-rCGCGAAUUCGCG-3')₂, respectively. All the experiments were conducted in a 10 mM phosphate buffer containing 100 mM NaCl at pH 7.0.

The UV melting curves and melting temperature (T_m) values for RNA-I in the absence and presence of different equivalents of ODAMan 4mer **26** are shown in Figure 2 and listed in Table 1, respectively. When 1 equivalent of **26** was added to the duplex, UV melting curve noticeably changed and

the T_m value of RNA-I increased by 5.1 °C. This result clearly indicated that ODAMan 4mer thermodynamically stabilized the RNA duplex. Furthermore, addition of two and three equivalents of ODAMan resulted in further elevation of T_m values. Relatively minor changes were then observed when more than three equivalents of the sugar were added to the RNA duplex.

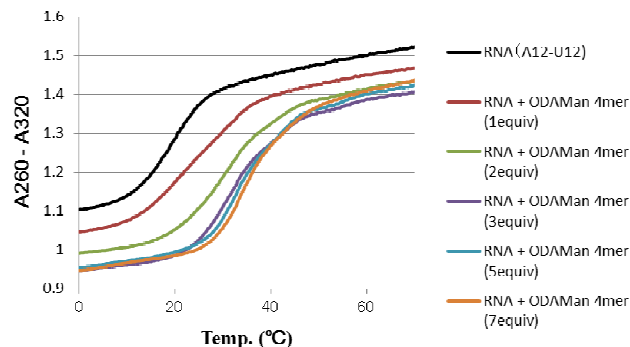


Figure 2. UV melting curves of RNA-I (5 μ M) in the absence and presence of a different equivalents of ODAMan 4mer.

Table 1 T_m values of RNA-I in the absence and presence of a different equivalents of the ODAMan 4mer.

Entry	ODAMan 4mer 26	$T_m / ^\circ\text{C}$	$\Delta T_m / ^\circ\text{C}$
1	0 equiv	19.2	-
2	1 equiv	24.3	5.1
3	2 equiv	30.4	11.2
4	3 equiv	32.7	13.5
5	5 equiv	34.1	14.9
6	7 equiv	35.4	16.2

Table 2 Melting temperature of RNA-I and RNA-II in the absence and presence of ODAMan 1-5mer.

Entry	ODAMan	RNA-I		RNA-II	
		$T_m / ^\circ\text{C}$	$\Delta T_m / ^\circ\text{C}$	$T_m / ^\circ\text{C}$	$\Delta T_m / ^\circ\text{C}$
1	RNA only	19.2	-	64.3	-
2	1mer 23 (12 equiv)	19.7	0.5	64.0	-0.3
3	2mer 24 (6 equiv)	22.5	3.2	64.7	0.4
4	3mer 25 (4 equiv)	29.0	9.7	66.8	2.5
5	4mer 26 (3 equiv)	32.5	13.2	67.7	3.4
6	5mer 27 (2.4 equiv)	36.2	17.0	70.3	6.0

Next, RNA-I and RNA-II were evaluated in the presence and absence of different ODAMans with the quantity of each

sugar added based on the number of phosphate groups in the duplex. The T_m values are presented in Table 2. These results also clearly revealed that the 3mer, 4mer and 5mer of ODAMans thermodynamically stabilized both RNA duplexes. Specifically, when the 3mer, 4mer and 5mer of ODAMans (**25-27**) were each added, the T_m values of RNA-I increased by 9.7 °C, 13.2 °C, respectively and 17.0 °C, while those of RNA-II increased by 2.5 °C, 3.4 °C and 6.0 °C, respectively. Conversely, the addition of ODAMan 2mer **24** scarcely stabilized RNA-II, and ODAMan 1mer **23** did not stabilize either RNA-I or II. The greater increases in the T_m values for the longer sugar chains is not unexpected, because the longer ODAMans have more functional groups that can interact with the RNA duplexes. In addition, these results also suggest that the ODAMans preferentially stabilize less thermodynamically stable RNA duplexes. ODAGlcs have been reported to exhibit a similar tendency.¹⁵

Based on the results of these experiments, it can be concluded that ODAMans with lengths greater than 3mer, have a high ability to thermodynamically stabilize the RNA duplexes.

Fluorescence anisotropy analysis

An evaluation of the binding affinity of the 4mer ODAMan **26** for RNA duplexes was then attempted using fluorescence anisotropy. ODAMan 4mer **26** was added in small steps to a 100 nM solution of a fluorophore labelled RNA duplex (5'-FAM-rCGCGAAUUCGCG-3')₂, and the respective fluorescence anisotropy values were recorded¹⁸. It can be clearly seen in Figure 3 that the ODAMan bound to the RNA duplex with high affinity. The K_d value was 1.6×10^{-7} ($\pm 0.5 \times 10^{-7}$) M, and the molar binding ratio was 1.5–1.6 moles of ODAMan 4mer **26** per mole of RNA duplex.

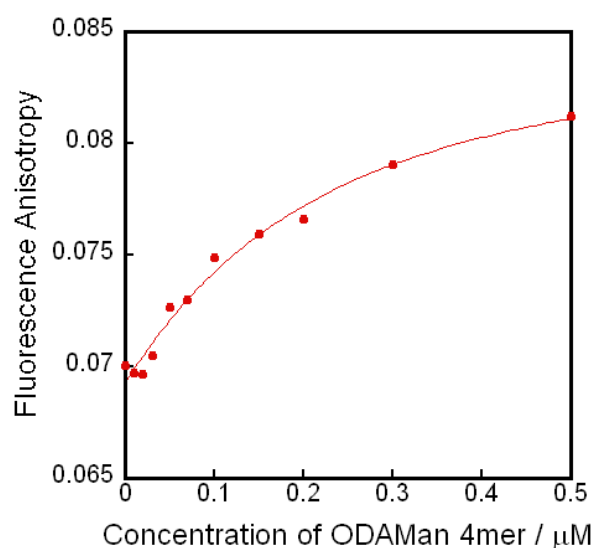


Figure 3. Fluorescence anisotropy of 100 nM of 5'-FAM-labeled 12mer RNA duplex was titrated by increasing concentration of ODAMan 4mer **26** at 20 °C in 10 mM phosphate buffer containing 100 mM NaCl, pH 7.0. The formation of the **26**-RNA complex is reflected an increase in the observed anisotropy values.

Interactions between siRNA and ODAMan 4mer

Next, further experiments were conducted to evaluate the effects of ODAMan 4mer **26** on siRNAs. An siRNA for luciferase¹⁹, with the sequence 5'-rCUUACGCUGAGUACUUCGAdTdT-3'/5'-rUCGAAGUACUCAGCGUAAGdTdT-3', was selected for these experiments. The results of the UV melting analyses in the absence and presence of ODAMan 4mer **26** are shown in Table 3. As was observed for the 12mer duplexes (RNA-I and RNA-II), the T_m value of the siRNA increased following the addition of **26**, despite its original higher melting temperature. Fluorescence anisotropy was then performed by using FAM-siRNA, i.e. (5'-FAM-rCUUACGCUGAGUACUUCGAdTdT-3'/5'-rUCGAAGUACUCAGCGUAAGdTdT-3'), and the K_d value and molar binding ratio were determined to be 2.8×10^{-7} ($\pm 0.5 \times 10^{-7}$) M and 1.2 moles of 4mer **26** per mole of siRNA, respectively. Although FAM-siRNAs have more phosphate groups and longer major grooves than the 12mers, both the binding affinity and molar binding ratio for the siRNA were quite similar to the results obtained for the 12mer RNAs.

Table 3 Melting temperature of siRNA in the absence and presence of ODAMan 4mer.

Entry	siRNA		
	ODAMan 4mer 26	T_m / °C	ΔT_m / °C
1	RNA only	72.2	-
2	1 equiv	74.6	2.4
3	3 equiv	77.6	5.4

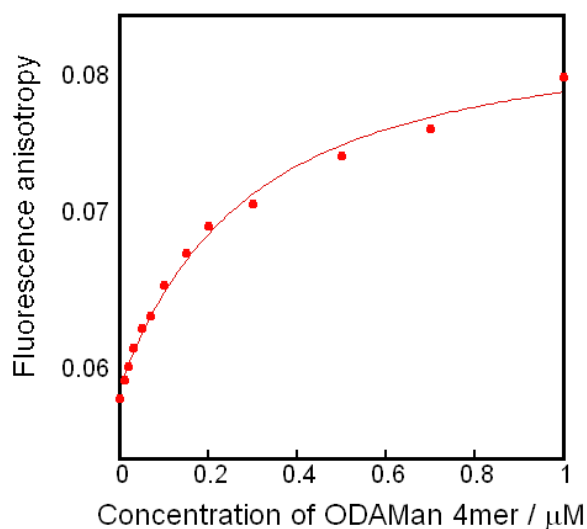


Figure 4. Fluorescence anisotropy of 100 nM of 5'-FAM-labeled siRNA was titrated by increasing concentration of ODAMan 4mer **26** at 20 °C in 10 mM phosphate buffer containing 100 mM NaCl, pH 7.0.

Inhibition of siRNA cleavage from RNase A

Finally, we aimed to evaluate the ability of the ODAMans to protect siRNAs from cleavage by nucleases was evaluated. It has been reported that the lability of siRNAs in serum is mainly due to cleavage by RNase A family enzymes.^{20, 21} Based on

these reports, the lability of an siRNA in the presence of calf bovine RNase A was monitored using the FRET system.²² 100 nM of fluorophore-labelled siRNA, 5'-FAM-rCUUACGCUGAGUACUUCGAdTdT-3'/5'-rUCGAAGUACUCAGCGUAAGdTdT-Dabcyl-3' was exposed to 0.33 $\mu\text{g}/\text{mL}$ of RNase A at 30 $^{\circ}\text{C}$. An increase in the fluorescence intensity due to the production of free fluorescein residues following siRNA cleavage, was recorded over time (Figure 5a, black). Next, the same experiments were conducted in the presence of 1, 3, and 10 equivalents (i.e., 0.1, 0.3 and 1.0 μM) of ODAMan 4mer **26** (Figure 5a; red, orange, and yellow, respectively). In addition, the relative initial rates of cleavage were approximately calculated the changes in the fluorescence

intensity during the initial 100 s of each experiment and are listed in Table 4 (left column). It can be seen in the table that the rate of increase in the fluorescence intensity was suppressed by 18%, 46%, 76% in the presence of 1, 3, and 10 equivalents of **26**, respectively. These results clearly demonstrated that ODAMan 4mer **26** inhibited the cleavage of siRNA by RNase A. It has been previously reported that siRNAs are labile in serum largely due to their thermodynamic instability, especially at the end of the duplex.²⁰ Therefore, it is possible that the ODAMan protected the siRNA from RNase A not only by sterically blocking the access of RNase A to the siRNA, but also by thermodynamically stabilizing it.

Figure 5b and Table 4 (right column) present the results for similar experiments conducted in the presence of 0.5 $\mu\text{g}/\text{mL}$ of the RNase A at 37 $^{\circ}\text{C}$, which is the optimal temperature for the nucleases. It can be clearly seen that 1 equivalent of ODAMan 4mer **26** scarcely protected the siRNA from RNase A under these conditions. These results are attributed to the higher activity of the nuclease and the weakening of the binding affinity at higher temperature. Upon the addition of more than three equivalents of ODAMan 4mer **26**, however, cleavage was suppressed to a level similar to that observed at 30 $^{\circ}\text{C}$. These results clearly indicated that only a small quantity of ODAMan 4mer **26** can effectively protect an siRNA from RNase A, even at low concentration (< 1 μM) at body temperature. Note that the amino groups-phosphate groups ratio is only 1:1 when 5 equivalents of ODAMan are used. Therefore, RNase A resistance of the siRNA was effectively increased without using an excess of cations.

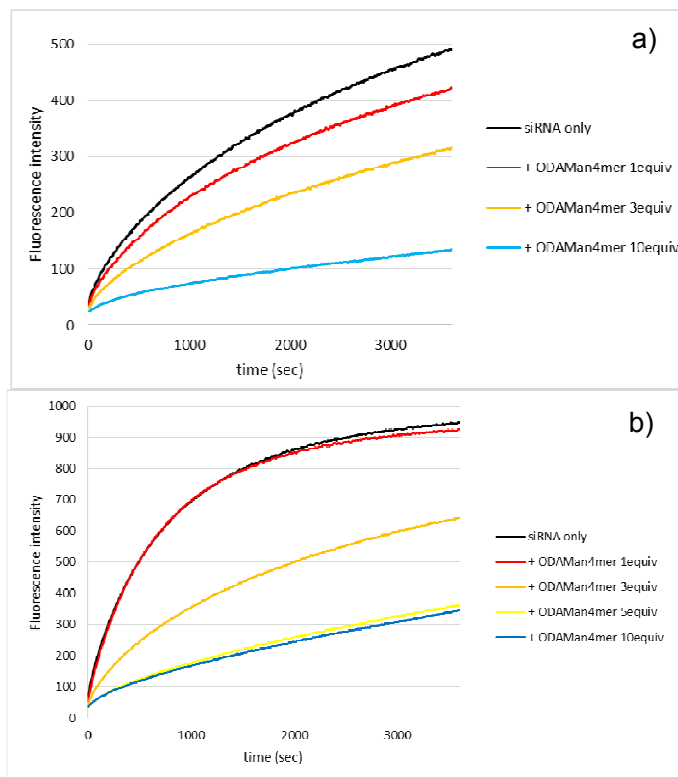


Figure 5 Time course of fluorescence signal due to fluorescein free from the duplex following to cleavage of the siRNA by RNase A. The initial concentration of the siRNA was 100nM in 10 mM Tris-HCl buffer containing 100 mM NaCl. a) at 30 $^{\circ}\text{C}$ in the presence of 0.33 $\mu\text{g}/\text{mL}$ of RNase A, pH 7.3; b) at 37 $^{\circ}\text{C}$ in the presence of 0.50 $\mu\text{g}/\text{mL}$ of RNase A, pH 7.2.

Table 4 Inhibition rate of ODAMan 4mer **26** at cleavage of siRNA by RNase A

ODAMan 4mer 26	30 $^{\circ}\text{C}$, 0.33 $\mu\text{g}/\text{mL}$ RNase A		37 $^{\circ}\text{C}$, 0.5 $\mu\text{g}/\text{mL}$ RNase A	
	increase in fluorescence intensity per initial 100 seconds	inhibition rate	increase in fluorescence intensity per initial 100 seconds	inhibition rate
siRNA only	47.1		137.2	
1 equiv	38.4	18%	135.1	2%
3 equiv	25.3	46%	63.0	54%
5 equiv	nd	-	29.2	79%
10 equiv	11.2	76%	28.7	79%

Conclusions

The design and facile synthesis of α -(1 \rightarrow 4)-linked 2,6-diamino-2,6-dideoxy oligo-D-mannose oligomers (oligodiaminomannoses, ODAMans) was achieved. The results of UV melting and fluorescence anisotropy analyses indicated that the ODAMans efficiently bind to RNA duplexes and thermodynamically stabilize them. In particular, ODAMans with lengths greater than 3mer exhibited good properties. Furthermore, ODAMan 4mer **26** was also shown to bind to an siRNA, composed of an RNA duplex, and the results of cleavage studies strongly indicated that the siRNA could be stabilized both thermodynamically and biologically via the formation of an siRNA-ODAMan conjugate. Further experiments, including their application as siRNA carriers, are now in progress.

Experimental section

General methods and materials

All reactions were carried out under an atmosphere of argon except for the synthesis of **23-27**. ^1H NMR spectra were obtained at 300 MHz on a Varian MERCURY 300 spectrometer with tetramethylsilane (TMS) as an internal standard in CDCl_3 and with acetonitrile as an internal or external standard (d 2.06) in D_2O . ^{13}C NMR spectra were obtained at 75.45 MHz on a Varian MERCURY 300 spectrometer with CDCl_3 as an internal standard (d 77.0) in CDCl_3 with acetonitrile as an internal or external standard (d 1.47) in D_2O . Mass spectra were recorded on a Voyager System 4327 (Applied Biosystem) and a 910-MS FTMS system (Varian). Fluorescence anisotropies were recorded on a FP-6500 spectrofluorometer (JASCO). Analytical TLC was performed on

Merck Kieselgel 60-F254 plates. Melting curves of nucleic acid duplexes were recorded on a UV-1650PC UV-Visible spectrophotometer (Shimadzu). Silica gel column chromatography was carried out using Silica gel 60N (63-210 μm or 40-50 μm). Reversed phase HPLC was carried out using a $\mu\text{Bondasphere}$ 5- μm C18, 100 \AA , 19 mm \times 150 mm (Waters) with a gradient of 0-80% acetonitrile in water at 25 $^{\circ}\text{C}$ at a rate of 0.5 mL/min and acetonitrile and water is buffered by TFA (trifluoroacetic acid) with 0.05% v/v. Organic solvents were purified and dried by the appropriate procedure. RNA oligomers were purchased from Hokkaido System Science Co., Ltd. and Japan Bio Services Co., Ltd. The yields of compounds were calculated from their dry weights except for **23-27**. The yields of **23-27** were estimated from the values of integral in the NMR spectra compared with that of the internal standard of acetonitrile.

Conditions for the UV melting analyses.

Absorbance versus temperature profile measurements were carried out with eight-sample cell changer, in quartz cells of 1 cm pathlength. For all the experiments, the duplex concentration was 5 μM in a 10 mM phosphate buffer containing 100 mM NaCl at pH 7.0. The variation of the difference of UV absorbance at between 260 nm and 320 nm with temperature was monitored. RNAs were annealed after addition of amino sugars. The samples containing RNAs and ODAMans were first rapidly heated to 95 $^{\circ}\text{C}$, left for 20 min, and then allowed to cool slowly to room temperature. These samples were furthermore cooled to 0 $^{\circ}\text{C}$ and left for 1 h, and then the dissociation was recorded by heating to 95 $^{\circ}\text{C}$ at a rate of 0.5 $^{\circ}\text{C}/\text{min}$.

Fluorescence anisotropy measurement

The all titrations were measured at 20 $^{\circ}\text{C}$ and the average values of three-time measurements were recorded. The duplex concentration was 100 nM in a 10 mM phosphate buffer containing 100 mM NaCl and 0.02% Tween 20, pH 7.0. The following instrument settings were used: Ex/Em = 490 nm/520 nm; Response, 2 sec; Band width (Ex), 5 nm; Band width (Em), 5 nm; PMT voltage, 450 V; No. of cycle, 4;

Evaluation of RNase A inhibitory properties of ODAMan 4mer.

The all experiments were conducted using 100 nM of the siRNA and 0-1000 nM of ODAMan 4mer in a 10 mM Tris buffer containing 100 mM NaCl, pH 7.3 (at 30 $^{\circ}\text{C}$) or pH 7.2 (at 37 $^{\circ}\text{C}$). To the solution of the siRNA with or without the ODMan, 100 $\mu\text{g}/\text{mL}$ of RNase A from bovine pancreas in Tris was added to give final concentration of 0.33 or 0.50 $\mu\text{g}/\text{mL}$. 10 seconds after addition of the RNase A, the fluorescence intensity was recorded every 10 seconds over 3600 seconds. The following instrument settings were used: Ex/Em = 490 nm/520 nm; Response, 1 sec; Band width (Ex), 1 nm; Band width (Em), 3 nm;

1.1. Acetyl O-(3,4,6-O-triacetyl-2-deoxy-2-phthalimide-D-mannopyranoside) (2)

α -D-Mannosamine **1** (5.00 g, 23.0 mmol) was dissolved in methanol (115 mL) and sodium methoxide (1.24 g, 23.0 mmol, 1.0 equiv) was added to the solution. After the mixture was stirred over 1 h at rt, triethylamine (12.8 mL, 92.0 mmol, 4.0 equiv) and phthalic anhydride (6.81 g, 46 mmol, 2.0 equiv) were added at 0 $^{\circ}\text{C}$. After 4 h, the solvent was evaporated and the crude product was coevaporated with pyridine. The residue was dissolved in pyridine (230 mL) and acetic anhydride (34 mL) and *N,N*-dimethyl-4-aminopyridine (0.28 g, 2.3 mmol, 0.1 equiv) was added at 0 $^{\circ}\text{C}$. After 30 min, the mixture was warmed to rt and stirred over 17 h. The mixture was concentrated to dryness and then dissolved in dichloromethane (400 mL). The solution was washed with a saturated aqueous solution of NaHCO_3 (400 mL \times 2) and the aqueous layer was back extracted with dichloromethane (100 mL \times 2, 20 mL \times 3). The combined organic layer was dried over Na_2SO_4 , filtered and concentrated. The crude product was purified by silica gel column chromatography (ethyl acetate-hexane (1:1, v/v)) and **2** was obtained as a colorless solid (9.08 g, 18.9 mmol, 83%).

^1H NMR (CDCl_3) δ 7.91-7.86 (m, 2H, *NPhth*), 7.80- 7.76 (m, 2H, *NPhth*), 6.59 (d, $J = 3.9$ Hz, 0.65H, H-1 α), 6.09-5.95 (m, 0.7H), 5.59-5.44 (m, 1.65H), 5.08-5.05 (m, 0.35H, H-2 β), 4.91-4.88 (m, 0.65H, H-2 α), 4.51-4.20 (m, 2.65H), 3.97-3.91 (m, 0.35H), 2.17-1.91 (m, 12H, Ac).

^{13}C NMR (CDCl_3) δ 170.76, 169.89, 169.73, 169.66, 169.52, 168.50, 168.04, 167.60, 134.47, 134.35, 131.35, 131.23, 123.71, 123.68, 90.36, 74.01, 71.06, 69.88, 68.96, 67.67, 66.71, 62.54, 62.40, 50.46, 49.95, 20.94, 20.76, 20.65, 20.51.

1.2. Phenyl 3,4,6-O-triacetyl-2-deoxy-2-phthalimide-1-thio- α -D-mannopyranoside (3)

Compound **2** (9.08 g, 18.9 mmol) was coevaporated with toluene and dissolved in dichloromethane (68 mL). Boron trifluoride diethyl ether complex (7.0 mL, 56.7 mmol, 3.0 equiv) and benzenethiol (2.4 mL, 23.0 mmol, 1.2 equiv) were added to the solution and it was stirred over 5 h. A saturated aqueous solution of NaHCO_3 (50 mL) was added to the mixture and it was diluted with dichloromethane (200 mL). The solution was washed with a saturated aqueous solution of NaHCO_3 (200 mL) and the aqueous layer was back extracted with dichloromethane (50 mL \times 3). The combined organic layer was dried over Na_2SO_4 , filtered, and concentrated. The crude product was purified by silica gel column chromatography (ethyl acetate-hexane (2:3, v/v)) to afford **3** as a colorless solid (8.96 g, 17.0 mmol, 90%).

^1H NMR (CDCl_3) δ 7.87-7.84 (m, 2H, *NPhth*), 7.78-7.74 (m, 2H, *NPhth*), 7.51-7.48 (m, 2H, *SPh*), 7.30-7.27 (m, 3H, *SPh*), 6.21 (d, $J = 7.2$ Hz, 1H, H-1), 5.40 (t, $J = 4.5$ Hz, 1H, H-3), 5.24 (dd, $J = 4.8$ Hz, 6.0 Hz, 1H, H-4), 4.90-4.86 (m, 1H, H-2), 4.52-4.26 (m, 2H, H-5, H-6), 4.30 (d, $J = 8.4$ Hz, 1H, H-6), 2.14 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.01 (s, 3H, Ac).

^{13}C NMR (CDCl_3) δ 170.62, 169.91, 169.64, 167.63, 134.41, 132.68, 132.25, 131.35, 128.97, 128.12, 123.65, 81.04, 70.94, 69.71, 68.60, 62.06, 51.11, 20.88, 20.79, 20.72.

1.3. Phenyl 2-deoxy-2-phthalimide-1-thio- α -D-mannopyranoside (4)

Compound **3** (8.92 g, 16.9 mmol) was coevaporated with toluene and dissolved in methanol (150 mL). Sodium methoxide (9.7 mg, 1.7 mmol, 0.1 equiv) was added to the solution and stirred over 1.5 h. The crude product was concentrated to dryness and dissolved in dichloromethane (200 mL). The solution was washed with a saturated aqueous solution of NH_4Cl (200 mL \times 2) and the aqueous layer was back extracted with dichloromethane (100 mL \times 15, 50 mL \times 12). The combined organic layer was dried over Na_2SO_4 , filtered and concentrated. The crude product was dissolved in pyridine (169 mL), and then acetic acid (16.9 mL) is added to the solution. The mixture was heated to reflux and stirred over 14 h. The solvent was evaporated and the residue was dissolved in dichloromethane (200 mL). The solution was washed with a saturated aqueous solution of NaHCO_3 (400 mL \times 2) and the aqueous layer was back extracted with dichloromethane (50 mL \times 12). The combined organic layer was dried over Na_2SO_4 , filtered, and concentrated. The crude product was purified by silica gel column chromatography (ethyl acetate) to afford **4** as a yellow foam (5.85 g, 14.6 mmol, 86%).

^1H NMR (CDCl_3) δ 7.74-7.70 (m, 2H, *NPhth*), 7.64-7.62 (m, 2H, *NPhth*), 7.45-7.42 (m, 2H, *SPh*), 7.32-7.26 (m, 3H, *SPh*), 5.68 (s, 1H, H-1), 5.04 (dd, $J = 3.0$ Hz, 5.7 Hz, 1H, H-2), 4.42-4.20 (m, 3H, H-3, H-4, H-5), 4.03-3.81 (m, 3H), 3.65-3.55 (br, 1H), 2.84-2.76 (br, 1H).

^{13}C NMR (CDCl_3) δ 168.71, 134.37, 133.21, 132.20, 131.13, 129.07, 127.90, 123.61, 85.20, 73.73, 71.44, 68.10, 61.89, 53.97.

1.4. Phenyl 2-deoxy-2-phthalimide-4,6-*O*-*p*-methoxybenzylidene-1-thio- α -D-mannopyranoside (5)

Compound **4** (5.85 g, 14.6 mmol) was coevaporated with acetonitrile and dissolved in acetonitrile (290 mL). The solution was stirred and cooled to 0 °C, and *p*-anisaldehyde dimethyl acetal (3.0 mL, 17.7 mmol, 1.2 equiv) and *p*-toluenesulfonic acid monohydrate (0.28 g, 1.47 mmol, 0.1 equiv) were added. After 3 h, another *p*-anisaldehyde dimethyl acetal (3.0 mL) was added and stirred over 2 h. Triethylamine (2 mL) was added to the mixture to stop the reaction and then a saturated aqueous solution of NaHCO_3 (50 mL) was added. The solution was diluted with ethyl acetate (200 mL) and washed with a saturated aqueous solution of NaHCO_3 (200 mL \times 2). The aqueous layer was back extracted with ethyl acetate (50 mL \times 3) and the combined organic layer was dried over Na_2SO_4 , filtered and concentrated. The crude product was purified by silica gel column chromatography (dichloromethane-ethyl acetate (1:0 to 1:1, v/v)) to afford **5** as a yellow solid (7.40 g, 14.2 mmol, 97%).

^1H NMR (CDCl_3) δ 7.89-7.83 (m, 2H, *NPhth*), 7.76-7.70 (m, 2H, *NPhth*), 7.50-7.42 (m, 4H, *SPh*, *p*-MeOPh), 7.34-7.26 (m, 3H, *SPh*), 6.91 (d, $J = 8.7$ Hz, 2H, *p*-MeOPh), 5.68 (s, 1H, H-1), 5.61 (s, 1H, benzylidene-CH), 5.14 (d, $J = 5.4$ Hz, 1H, H-2), 4.45-4.42 (m, 3H, H-3, H-4, H-5), 4.24-4.20 (m, 10.5 Hz, 1H, H-6), 4.01-3.95 (m, 1H, H-6), 3.81 (s, 3H, *OCH}_3*), 2.64-2.60 (br, 1H, 3-OH).

^{13}C NMR (CDCl_3) δ 168.44, 160.23, 134.32, 132.87, 132.59, 131.45, 129.64, 129.64, 129.06, 128.06, 127.61, 123.50, 113.73, 102.10, 86.32, 79.24, 68.54, 67.80, 64.43, 55.29, 53.78.

1.5. Phenyl 3-*O*-benzyl-2-deoxy-2-phthalimide-1-thio- α -D-mannopyranoside (6)

Compound **5** (7.40 g, 14.2 mmol) was coevaporated with pyridine and toluene and dissolved in *N,N*-dimethylformamide (142 mL). To the solution, sodium hydride (60% oil dispersion, 1.21 g, 30 mmol, 2.1 equiv) was added. Then benzyl bromide (3.4 mL, 28.7 mmol, 2.0 equiv) was added at 0 °C and the mixture was warmed to rt. After 23 h, methanol (20 mL) was added to the solution and the solvent was evaporated. The residue was dissolved in dichloromethane (200 mL) and the solution was washed with a saturated aqueous solution of NH_4Cl (150 mL \times 2). The aqueous layer was back extracted with dichloromethane (50 mL \times 4) and the combined organic layer was dried over Na_2SO_4 , filtered and concentrated. The crude product was dissolved in pyridine (142 mL), and then acetic acid (14.2 mL) is added to the solution. The mixture was heated to reflux and stirred over 6 h. The solvent was evaporated and the residue was dissolved in dichloromethane (200 mL). The solution was washed with a saturated aqueous solution of NaHCO_3 (300 mL \times 2) and the aqueous layer was back extracted with dichloromethane (50 mL \times 2). The combined organic layer was dried over Na_2SO_4 , filtered and concentrated. The crude product was dissolved in ethanol- H_2O (2:1, v/v, 300 mL), and then acetic acid (75 mL) was added. The solution was heated to 50 °C and stirred over 27 h. The mixture was cooled to rt, concentrated to dryness, and dissolved in ethyl acetate (300 mL). The solution was washed with a saturated aqueous solution of NaHCO_3 (300 mL \times 1, 600 mL \times 1) and the aqueous layer was back extracted with ethyl acetate (100 mL \times 3, 50 mL \times 5). The combined organic layer was dried over Na_2SO_4 , filtered, and concentrated. The crude product was purified by silica gel column chromatography (ethyl acetate-hexane (1:1 to 3:1, v/v)) to afford **6** as a colorless foam (6.88 g, 14.0 mmol, 99%).

^1H NMR (CDCl_3) δ 7.78-7.66 (m, 2H, *NPhth*), 7.69-7.66 (m, 2H, *NPhth*), 7.50-7.46 (m, 2H, *SPh*), 7.34-7.23 (m, 8H, *SPh*, *PhCH}_2*), 5.73 (s, 1H, H-1), 5.22 (d, $J = 6.3$ Hz, 1H, H-2), 4.70 (d, $J = 11.4$ Hz, 1H, *PhCH}_2*), 4.53 (d, $J = 11.7$ Hz, 1H, *PhCH}_2*), 4.41 (t, $J = 9.0$ Hz, 1H, H-4), 4.29-4.24 (m, 1H, H-5), 4.08-3.98 (m, 2H, H-3, H-6), 3.88-3.84 (m, 1H, H-6), 2.80-2.70 (br, 1H, OH), 2.70-2.60 (br, 1H, OH).

^{13}C NMR (CDCl_3) δ 168.12, 137.24, 134.28, 132.93, 132.58, 131.40, 129.12, 128.47, 128.08, 127.98, 123.45, 85.60, 78.03, 73.75, 72.49, 66.82, 62.08, 51.37.

1.6. Phenyl 6-azido-6-deoxy-3-*O*-benzyl-2-deoxy-2-phthalimide-1-thio- α -D-mannopyranoside (7)

Compound **6** (6.75 g, 14.0 mmol) was coevaporated with pyridine and dissolved in pyridine (350 mL). The solution was stirred and cooled to -15 °C, and methanesulfonyl chloride (1.2 mL, 15.9 mmol, 1.1 equiv) was added. After 12 h, the reaction was quenched by addition of methanol (5 mL), and the mixture was concentrated to dryness and coevaporated with toluene. The residue

was dissolved in ethyl acetate (200 mL) and washed with a saturated aqueous solution of NaHCO₃ (200 mL × 2) and the aqueous layer was back extracted with ethyl acetate (20 mL × 2). The combined organic layer was dried over MgSO₄, filtered and concentrated. The crude mesylate was coevaporated with *N,N*-dimethylformamide and dissolved in *N,N*-dimethylformamide (200 mL). Sodium azide (9.00 g, 139 mmol, 10.0 equiv) was added to the solution, and the mixture was stirred and heated to 80 °C. After 13 h, the solution was cooled to rt and concentrated. The crude product was extracted with ethyl acetate-water (1:1, v/v, 400 mL), the organic layer was washed with water (200 mL), and the water layer was back extracted with ethyl acetate (20 mL × 2). The combined organic layer was dried over MgSO₄, filtered, and concentrated to dryness. The crude product was purified by silica gel column chromatography (ethyl acetate-hexane (1:1, v/v)) to afford **7** as colorless foam (5.69 g, 11.0 mmol, 79%).

¹H NMR (CDCl₃) δ 7.84-7.80 (m, 2H, *NPhth*), 7.75-7.70 (m, 2H, *NPhth*), 7.53-7.49 (m, 2H, *SPh*), 7.34-7.15 (m, 8H, *SPh*, *PhCH*₂), 5.75 (d, *J* = 2.1 Hz, 1H, H-1), 5.19 (dd, *J* = 1.8 Hz, 6.3 Hz, 1H, H-2), 4.62 (d, *J* = 11.4 Hz, 1H, *PhCH*₂), 4.47 (d, *J* = 11.4 Hz, 1H, *PhCH*₂), 4.38-4.33 (m, 2H, H-4, H-5), 4.00 (t, *J* = 7.2 Hz, 1H, H-3), 3.71-3.54 (m, 2H, H-6).

¹³C NMR (CDCl₃) δ 167.98, 136.97, 134.26, 132.76, 132.35, 131.43, 129.08, 128.50, 128.06, 127.98, 127.94, 123.43, 85.42, 77.81, 73.21, 72.25, 68.21, 51.79, 51.07.

1.7. Phenyl 4-*O*-acetyl-6-azido-6-deoxy-3-*O*-benzyl-2-deoxy-2-phthalimide-1-thio- α -D-mannopyranoside (**8**)

Compound **7** (5.69 g, 11.0 mmol) was dissolved in pyridine (280 mL) and the solution was cooled to 0 °C. Acetic anhydride (1.75 mL, 18.7 mmol, 1.7 equiv) and *N,N*-dimethyl-4-aminopyridine (0.13 g, 0.1 equiv) were added to the solution, and the mixture was warmed to rt. After 12 h, the solvent was evaporated, and the residue was coevaporated with toluene and dissolved in dichloromethane (300 mL). The solution was washed with a saturated aqueous solution of NaHCO₃ (400 mL × 2) and the aqueous layer was back extracted with dichloromethane (50 mL × 4). The combined organic layer was dried over Na₂SO₄, filtered, and concentrated. The crude product was purified by silica gel column chromatography (chloroform including 1% methanol) to afford **8** as colorless foam (5.44 g, 9.7 mmol, 89%).

¹H NMR (CDCl₃) δ 7.81-7.71 (m, 4H, *NPhth*), 7.54-7.50 (m, 2H, *SPh*), 7.33-7.26 (m, 4H, *SPh*, *PhCH*₂), 7.14-7.03 (m, 4H, *PhCH*₂), 6.22 (d, *J* = 6.9 Hz, 1H, H-1), 5.30 (t, *J* = 5.7 Hz, 1H, H-4), 4.78-4.74 (m, 1H, H-2), 4.57 (d, *J* = 12.0 Hz, 1H, *PhCH*₂), 4.47 (d, *J* = 12.0 Hz, 1H, *PhCH*₂), 4.34-4.27 (m, 1H, H-5), 3.88 (t, *J* = 4.8 Hz, 1H, H-3), 3.76 (dd, *J* = 8.4 Hz, 13.2 Hz, 1H, H-6), 3.44 (dd, *J* = 4.5 Hz, 13.2 Hz, 1H, H-6), 2.10 (s, 1H, *Ac*).

¹³C NMR (CDCl₃) δ 170.07, 167.67, 136.50, 134.08, 132.68, 132.23, 131.53, 129.00, 128.37, 128.22, 128.09, 127.87, 123.37, 81.16, 74.20, 72.67, 71.78, 68.81, 51.50, 50.93, 21.01.

1.8. Compound **9**

Compound **8** (0.80 g, 1.4 mmol) was coevaporated with toluene and dissolved in dichloromethane-diethyl ether (1:1, v/v, 26 mL). Methanol (88 mL, 2.2 mmol, 1.6 equiv) and *N*-iodosuccinimide (0.64 g, 2.8 mmol, 2.0 equiv) were added to the solution, and it was cooled to 0 °C. Then a solution of trifluoromethanesulfonic acid (91 mL) in dichloromethane-diethyl ether (1:1, v/v, 2 mL) was added, and the solution was stirred over 40 min at 0 °C in dark. The reaction was quenched by addition of a saturated aqueous solution of NaHCO₃ (20 mL), and the mixture was diluted with dichloromethane (50 mL). The solution was washed with a saturated aqueous solution of NaHCO₃ (50 mL) and 10% aqueous Na₂S₂O₃ solution (50 mL), and the aqueous layer was back extracted with dichloromethane (10 mL × 4). The combined organic layer was dried over Na₂SO₄, filtered and concentrated to dryness. The crude product was purified by silica gel column chromatography (ethyl acetate-hexane (3:7, v/v)) to afford **9** as colorless foam (0.63 g, 1.3 mmol, 92%).

¹H NMR (CDCl₃) δ 7.82-7.77 (m, 2H, *NPhth*), 7.74-7.70 (m, 2H, *NPhth*), 7.16-7.09 (m, 5H, *PhCH*₂), 5.45 (t, *J* = 7.2 Hz, 1H, H-4), 5.32 (d, *J* = 3.9 Hz, 1H, H-1), 4.76 (t, *J* = 4.8 Hz, 1H, H-2), 4.48 (s, 2H, *PhCH*₂), 4.06-3.94 (m, 2H, H-3, H-5), 3.83-3.75 (m, 1H, H-6), 3.45 (s, 3H, *CH*₃O), 3.31 (dd, *J* = 3.0 Hz, 13.2 Hz, 1H, H-6), 2.05 (s, 3H, *Ac*).

¹³C NMR (CDCl₃) δ 170.02, 168.02, 136.89, 134.03, 131.54, 128.19, 128.08, 127.75, 123.30, 97.46, 73.98, 71.54, 71.48, 69.04, 55.49, 51.51, 51.37, 20.95.

1.9. Compound **10**

Compound **9** (1.89 g, 3.9 mmol) was coevaporated with toluene, and dissolved in methanol. Sodium methoxide (23 mg, 0.43 mmol, 0.1 equiv) was added to the solution and stirred over 3 h at rt. The solvent was evaporated and the residue was dissolved in dichloromethane (100 mL). The solution was washed with a saturated aqueous solution of NH₄Cl (50 mL × 2) and the aqueous layer was back extracted with dichloromethane (20 mL × 5). The combined organic layer was dried over Na₂SO₄, filtered and concentrated. The crude product was dissolved in pyridine (75 mL), and then acetic acid (7.5 mL) is added to the solution. The mixture was heated to reflux and stirred over 41 h. The solution was cooled to rt, and then the solvent was evaporated and the residue was dissolved in dichloromethane (20 mL). The solution was washed with a saturated aqueous solution of NaHCO₃ (100 mL × 2) and the aqueous layer was back extracted with dichloromethane (20 mL × 4). The combined organic layer was dried over Na₂SO₄, filtered and concentrated. The crude product was purified by silica gel column chromatography (ethyl acetate-hexane (1:1, v/v)) to afford **10** as yellow oil (1.29 g, 2.94 mmol, 75%).

¹H NMR (CDCl₃) δ 7.85-7.81 (m, 2H, *NPhth*), 7.75-7.71 (m, 2H, *NPhth*), 7.21-7.14 (m, 5H, *PhCH*₂), 5.01 (d, *J* = 6.9 Hz, 1H, H-4), 4.87 (s, 1H, H-1), 4.59 (d, *J* = 11.7 Hz, 1H, *PhCH*₂), 4.45-4.33 (m, 2H, H-2, *PhCH*₂), 3.99-3.94 (m, 1H, H-3), 3.88-3.83 (m, 1H, H-6), 3.72-3.57 (m, 2H, H-5, H-6), 3.40 (s, 3H, *CH*₃O), 2.43-2.42 (m, 1H, OH).

^{13}C NMR (CDCl_3) δ 168.24, 137.27, 134.20, 131.47, 128.44, 127.93, 127.93, 123.37, 100.03, 77.46, 71.96, 71.82, 67.89, 54.88, 51.94, 50.48.

1.10. Compound 11

A mixture of **10** (0.14 g, 318 μmol) and glycosyl donor **8** (0.27 g, 477 μmol , 1.5 equiv) were coevaporated with toluene, and then dissolved in dichloromethane-diethyl ether (3:14, v/v, 6.8 mL). *N*-Iodosuccinimide (0.23 g, 1.0 mmol, 3.1 equiv) was added to the solution, and the mixture was cooled to 0 °C. Then a solution of trifluoromethanesulfonic acid (28 mL) in dichloromethane (0.2 mL) was added, and the solution was stirred over 1.5 h at 0 °C in dark. The reaction was quenched by addition of a saturated aqueous solution of NaHCO_3 (5.0 mL), and the mixture was diluted with dichloromethane (30 mL). The solution was washed with a saturated aqueous solution of NaHCO_3 (30 mL) and 10% aqueous $\text{Na}_2\text{S}_2\text{O}_3$ solution (30 mL), and the aqueous layer was back extracted with dichloromethane (10 mL \times 2). The combined organic layer was dried over Na_2SO_4 , filtered and concentrated to dryness. The crude product was purified by silica gel column chromatography (ethyl acetate-hexane (3:7, v/v)) to afford **11** as colorless foam (0.24 g, 271 μmol , 85%).

^1H NMR (CDCl_3) δ 7.74-7.67 (m, 8H, *NPhth*), 7.11-7.04 (m, 7H, *PhCH}_2*), 6.89-6.85 (m, 3H, *PhCH}_2*), 6.00 (d, $J = 4.5$ Hz, 1H, H-1'), 5.27 (t, $J = 6.3$ Hz, 1H, H-4'), 5.17 (d, $J = 3.6$ Hz, 1H, H-1), 4.79-4.77 (m, 1H, H-2), 4.69 (t, $J = 4.8$ Hz, 1H, H-2'), 4.63-4.38 (m, 5H), 4.16-3.96 (m, 3H), 3.89-3.77 (m, 2H), 3.66-3.56 (m, 2H), 3.42-3.33 (m, 4H), 2.09 (s, 3H, *Ac*).

^{13}C NMR (CDCl_3) δ 170.08, 167.88, 137.14, 136.86, 133.93, 133.77, 131.71, 131.53, 128.21, 127.93, 127.74, 127.32, 123.25, 123.20, 98.02, 95.58, 74.63, 73.09, 72.22, 71.84, 71.80, 71.40, 68.89, 55.35, 52.34, 51.74, 51.54, 51.04, 21.02.

1.11. Compound 12

The same method for **10** was applied for the synthesis of **12**, except for using **11** (0.32 g, 365 μmol) as a starting material. Purification by silica gel column chromatography was conducted with ethyl acetate-hexane (2:3, v/v) as an eluent. Compound **12** was obtained as colorless oil (0.23 g, 272 μmol , 73%).

^1H NMR (CDCl_3) δ 7.79-7.65 (m, 8H, *NPhth*), 7.22-7.15 (m, 5H, *PhCH}_2*), 6.95-6.93 (m, 2H, *PhCH}_2*), 6.79-6.75 (m, 3H, *PhCH}_2*), 5.52 (s, 1H, H-1'), 5.03 (d, $J = 7.8$ Hz, 1H), 4.99 (s, 1H, H-1), 4.93 (m, 1H), 4.65-4.40 (m, 5H), 4.27-4.17 (m, 2H), 3.97-3.77 (m, 4H), 3.70-3.58 (m, 3H), 3.46 (s, 3H, CH_3O), 2.34 (d, 1H, 4-OH).

^{13}C NMR (CDCl_3) δ 167.99, 167.93, 137.22, 136.99, 134.07, 133.95, 131.43, 131.36, 128.46, 127.98, 127.84, 127.10, 126.79, 123.34, 123.27, 99.59, 99.00, 77.80, 73.79, 73.00, 72.31, 71.28, 67.60, 55.20, 52.10, 51.86, 50.99, 50.64.

1.12. Compound 13

The same method for **11** was applied for the synthesis of **13**, except for using **12** (54 mg, 64 μmol) and **8** (64 mg, 114 μmol , 1.8 equiv) as starting materials. Purification by silica gel column chromatography was conducted with ethyl acetate-hexane (2:3, v/v) as an eluent. Compound **13** was obtained as colorless oil (74 mg, 57 μmol , 90%).

^1H NMR (CDCl_3) δ 7.74-7.57 (m, 12H, *NPhth*), 7.11-7.05 (m, 9H, *PhCH}_2*), 6.86-6.82 (m, 6H, *PhCH}_2*), 6.02 (d, $J = 5.1$ Hz, 1H, H-1'), 5.88 (d, $J = 3.9$ Hz, 1H, H-1'), 5.25-5.21 (m, 2H, H-1 H-4'), 4.68-4.28 (m, 11H), 4.13-3.55 (m, 11H), 3.44-3.34 (m, 4H), 2.11 (s, 3H, *Ac*).

^{13}C NMR (CDCl_3) δ 170.12, 167.93, 167.79, 167.66, 137.08, 137.00, 136.82, 133.84, 133.71, 133.59, 131.76, 131.61, 131.51, 128.24, 128.19, 127.90, 127.81, 127.74, 127.60, 127.27, 127.22, 123.26, 123.14, 123.07, 97.64, 96.13, 95.13, 74.72, 73.27, 72.72, 72.59, 72.04, 71.97, 71.86, 71.66, 71.40, 68.99, 55.35, 52.41, 51.83, 51.66, 51.59, 51.24, 21.02.

1.13. Compound 14

Compound **13** (1.12 g, 865 μmol) was coevaporated with toluene, and dissolved in methanol-dichloromethane (3:1, v/v, 13 mL). Sodium methoxide (8.0 mg, 148 μmol , 0.2 equiv) was added to the solution and stirred over 3.5 h at rt. The solvent was evaporated and the residue was dissolved in dichloromethane (50 mL). The solution was washed with a saturated aqueous solution of NH_4Cl (60 mL \times 2) and the aqueous layer was back extracted with dichloromethane (5 mL \times 6). The combined organic layer was dried over Na_2SO_4 , filtered and concentrated. The crude product was dissolved in pyridine (18 mL), and then acetic acid (1.8 mL) is added to the solution. The mixture was heated to reflux and stirred over 60 h. The solution was cooled to rt, and then the solvent was evaporated and the residue was dissolved in dichloromethane (30 mL). The solution was washed with a saturated aqueous solution of NaHCO_3 (40 mL \times 2) and the aqueous layer was back extracted with dichloromethane (5 mL \times 5). The combined organic layer was dried over Na_2SO_4 , filtered, and concentrated. The crude product was purified by silica gel column chromatography (ethyl acetate-hexane (1:1, v/v)) to afford **14** as colorless oil (0.85 g, 680 μmol , 78%).

^1H NMR (CDCl_3) δ 7.75-7.62 (m, 12H, *NPhth*), 7.26-7.16 (m, 7H, *PhCH}_2*), 7.01-6.99 (m, 2H, *PhCH}_2*), 6.94-6.90 (m, 2H, *PhCH}_2*), 6.84-6.82 (m, 2H, *PhCH}_2*), 6.76-6.72 (m, 2H, *PhCH}_2*), 5.66 (d, $J = 2.4$ Hz, 1H, H-1'), 5.49 (s, 1H, H-1'), 5.13 (d, $J = 3.0$ Hz, 1H, H-1), 5.03 (d, $J = 6.6$ Hz, 1H, H-2'), 4.95-4.92 (m, 1H, H-2'), 4.86-4.83 (m, 1H, H-2), 4.67-4.40 (m, 7H), 4.22-3.62 (m, 14H), 3.46 (s, 3H, CH_3O), 2.37 (br, 1H, 4-OH).

^{13}C NMR (CDCl_3) δ 167.93, 167.76, 137.23, 136.99, 136.87, 133.98, 133.93, 133.82, 131.46, 131.39, 128.45, 128.28, 127.97, 127.90, 127.86, 127.79, 127.58, 127.28, 127.22, 127.07, 126.83, 123.26, 99.24, 98.35, 97.82, 77.89, 77.53, 77.09, 73.68, 73.07, 72.83, 72.41, 72.30, 71.68, 71.49, 71.35, 67.53, 55.30, 52.04, 51.98, 51.82, 51.32, 50.92.

1.14. Compound 15

A mixture of **14** (145 mg, 116 μmol) and glycosyl donor **8** (118 mg, 209 μmol , 1.8 equiv) were coevaporated with toluene, and then dissolved in dichloromethane-diethyl ether (3:24, v/v, 2.7 mL). *N*-Iodosuccinimide (78 mg, 348 μmol , 3.0 equiv) was added to the solution, and the mixture was cooled to 0 °C. Then a solution of trifluoromethanesulfonic acid (13 mL) in dichloromethane (0.3 mL) was added, and the solution was stirred over 1.5 h at 0 °C in dark. The reaction was quenched by addition of a saturated aqueous solution of NaHCO_3 (5.0 mL), and the mixture was diluted with dichloromethane (15 mL). The solution was washed with a saturated aqueous solution of NaHCO_3 (10 mL) and 10% aqueous $\text{Na}_2\text{S}_2\text{O}_3$ solution (10 mL), and the aqueous layer was back extracted with dichloromethane (2 mL \times 3). The combined organic layer was dried over Na_2SO_4 , filtered and concentrated to dryness. The crude product was purified by silica gel column chromatography (ethyl acetate-hexane (2:3 to 1:1, v/v)) to afford **15** as colorless foam (176 mg, 103 μmol , 89%).

^1H NMR (CDCl_3) δ 7.73-7.56 (m, 16H, *NPhth*), 7.13-7.03 (m, 11H, *PhCH}_3*), 6.91-6.81 (m, 9H, *PhCH}_3*), 6.06 (d, $J = 5.1$ Hz, 1H, H-1'''), 5.93 (d, $J = 4.8$ Hz, 2H, H-1', H-1''), 5.28 (d, $J = 3.9$ Hz, 1H, H-1), 5.22 (t, $J = 5.1$ Hz, 1H, H-4'''), 4.70-3.55 (m, 30H), 3.40 (m, 4H), 2.12 (s, 3H, *Ac*).

^{13}C NMR (CDCl_3) δ 170.15, 167.97, 167.74, 167.66, 167.58, 137.06, 136.97, 136.94, 136.77, 133.79, 133.69, 133.50, 131.77, 131.66, 131.49, 128.27, 128.18, 127.90, 127.86, 127.71, 127.66, 127.25, 127.18, 123.26, 123.07, 97.42, 95.55, 95.34, 94.86, 77.10, 74.72, 73.11, 72.92, 72.63, 72.51, 71.88, 71.81, 71.63, 71.38, 69.06, 55.32, 52.46, 51.97, 51.81, 51.63, 51.32, 21.03.

MALDI-TOF MS: calcd for $\text{C}_{87}\text{H}_{78}\text{N}_{216}\text{NaO}_{22}$ m/z $[\text{M}+\text{Na}]^+$: 1721.5 Found: 1721.7

1.15. Compound 16

The same method for **14** was applied for the synthesis of **16**, except for using **15** (0.10 g, 59 μmol) as a starting material. Purification by silica gel column chromatography was conducted with ethyl acetate-hexane (2:1, v/v) as an eluent. Compound **16** was obtained as colorless oil (68 mg, 41 μmol , 70%).

^1H NMR (CDCl_3) δ 7.72-7.64 (m, 16H, *NPhth*), 7.21-7.12 (m, 6H, *PhCH}_2*), 7.05-6.99 (m, 3H, *PhCH}_2*), 6.95-6.72 (m, 11H, *PhCH}_2*), 5.80 (d, $J = 3.6$ Hz, 1H, H-1'''), 5.65 (d, $J = 2.7$ Hz, 1H, H-1''), 5.48 (s, 1H, H-1'), 5.20 (d, $J = 3.6$ Hz, 1H, H-1), 5.05 (d, $J = 6.3$ Hz, 1H), 4.94-4.92 (m, 1H), 4.85-4.82 (m, 1H), 4.79-4.77 (m, 1H), 4.69-4.34 (m, 10H), 4.21-3.62 (m, 18H), 3.45 (s, 3H, CH_3O), 2.30 (br, 1H, 4-OH).

^{13}C NMR (CDCl_3) δ 167.88, 167.79, 167.74, 137.26, 137.06, 136.93, 133.93, 133.71, 131.51, 128.49, 127.91, 127.88, 127.80, 127.50, 127.33, 127.25, 126.87, 123.29, 123.21, 101.09, 99.23, 98.01, 96.91, 77.92, 73.50, 72.93, 72.81, 72.52, 72.27, 71.85, 71.58, 71.42, 67.55, 55.36, 52.02, 51.59, 51.11, 50.93.

1.16. Compound 17

The same method for **15** was applied for the synthesis of **17**, except for using **16** (68 mg, 41 μmol) and **8** (41 mg, 74 μmol , 1.8 equiv) as starting materials. Purification by silica gel column chromatography was conducted with ethyl acetate-hexane (1:1, v/v) as an eluent. Compound **17** was obtained as colorless foam (81 mg, 38 μmol , 94%).

^1H NMR (CDCl_3) δ 7.77-7.50 (m, 20H, *NPhth*), 7.20-7.03 (m, 13H, *PhCH}_2*), 6.91-6.81 (m, 12H, *PhCH}_2*), 6.11 (d, $J = 5.4$ Hz, 1H, H-1'''), 6.02-5.99 (m, 3H, H-1', H-1'', H-1'''), 5.33 (d, $J = 3.9$ Hz, 1H, H-1), 5.19 (m, 1H, H-4'''), 4.68-3.53 (m, 39H), 3.45-3.36 (m, 4H), 2.13 (s, 3H, *Ac*).

^{13}C NMR (CDCl_3) δ 170.15, 167.98, 167.67, 167.58, 167.51, 137.06, 136.95, 136.92, 136.70, 133.71, 133.44, 133.39, 131.72, 131.64, 131.47, 128.29, 128.16, 127.95, 127.90, 127.84, 127.72, 127.26, 127.14, 123.25, 123.01, 98.32, 97.13, 95.00, 94.76, 94.59, 94.40, 76.87, 74.76, 73.05, 72.98, 72.83, 72.55, 71.87, 71.77, 71.64, 71.38, 69.12, 55.30, 52.56, 52.14, 51.82, 51.61, 51.45, 21.02.

MALDI-TOF MS: calcd for $\text{C}_{108}\text{H}_{96}\text{N}_{20}\text{NaO}_{27}$ m/z $[\text{M}+\text{Na}]^+$: 2127.7 Found: 2128.4

1.17. Compound 23

Compound **9** (25.4 mg, 52.9 μmol) was dissolved in ethanol (1.5 mL) and then hydrazine monohydrate (60 mL) was added to the solution. The mixture was refluxed and stirred over 25 h. The solution was cooled to rt and the solvent was evaporated. The crude product was dissolved in chloroform (3.0 mL), and an insoluble residue was removed by filtration. The solution was washed with water (10 mL \times 3) and the aqueous layer was back extracted with chloroform (5 mL \times 3). The combined organic layer was dried over Na_2SO_4 , filtered, and concentrated to dryness. The residue was dissolved in 0.01 M hydrochloric acid (2.0 mL), and 5% Pd/C (0.03 g) was added to the solution after 10 min of argon bubbling. Hydrogen gas was contentiously bubbled in the mixture over 4 h, and the Pd/C was removed by filtration. After the filtrate was concentrated and dissolved in 0.01 M hydrochloric acid (3.5 mL) again. 10% Pd/C (33 mg) was added to the solution after 10 min of argon bubbling. Hydrogen gas was contentiously bubbled in the mixture over 2 h, and the Pd/C was removed by filtration. The filtrate was concentrated and coevaporated with water (5 mL \times 4). The crude product was dissolved in a little volume of methanol, reprecipitated from acetone, and washed with acetone. The precipitate was dissolved in water and lyophilized from water to give **23** as a colorless solid (4.25 μmol , 8%).

^1H NMR (D_2O) δ 5.03 (s, 1H, H-1), d 4.13 (q, $J = 4.8$ Hz, 1H, H-3), 3.92 (m, 1H, H-5), 3.72 (dd, $J = 1.2$ Hz, 4.8 Hz, 1H, H-2), 3.59-3.48 (m, 5H, H-4, H-6, CH_3O), 3.25-3.17 (m, 1H, H-6).

^{13}C NMR (D_2O) δ 97.92, 96.43, 68.58, 68.33, 67.23, 56.18, 54.16, 40.84.

ESI -MS: calcd for $\text{C}_7\text{H}_{17}\text{N}_2\text{O}_4$ m/z $[\text{M}+\text{H}]^+$: 193.1183 Found: 193.1187.

1.18. Compound 24

Compound **11** (48.7 mg, 54.9 μmol) was dissolved in ethanol (3.0 mL) and then hydrazine monohydrate (0.12 mL) was added to the solution. The mixture was refluxed and stirred over 23 h. The solution was cooled to rt and the solvent was evaporated. The crude product was dissolved in chloroform (20 mL), and an insoluble residue was removed by filtration. The solution was washed with water (10 mL \times 3) and the aqueous layer was back extracted with chloroform (2 mL \times 3). The combined organic layer was dried over Na_2SO_4 , filtered, and concentrated to dryness. The residue was dissolved in 1,4-dioxane-methanol (5:1, v/v, 1.8 mL), and then triphenylphosphine (0.144 g, 547 μmol , 10 equiv) was added, and the mixture was refluxed over 5 h. After cooling to rt, 25% aqueous solution of ammonia (2.5 mL) was added to the solution and the flask was sealed. After the mixture was stirred overnight, the solvent was evaporated, and then the product was dissolved in 0.05 M hydrochloric acid (10 mL), and an insoluble residue was removed by filtration. The filtrate was washed with chloroform (10 mL \times 2) and concentrated. The crude product including **19** was purified with C18 reversed-phase HPLC (0.05% TFA, water-acetonitrile). After HPLC purification, fraction was concentrated and coevaporated with water. A half amount of the residue was dissolved in 0.01 M hydrochloric acid (1.0 mL). 10% Pd/C (35 mg) was added to the solution after 10 min of argon bubbling. Hydrogen gas was contentiously bubbled in the mixture over 6 h, and the Pd/C was removed by filtration. The filtrate was concentrated and coevaporated with water (2 mL \times 4). The crude product was dissolved in a little volume of methanol, reprecipitated from acetone, and washed with acetone. The precipitate was dissolved in water and lyophilized from water to give **24** as a colorless solid (5.06 μmol , 18%).

$^1\text{H NMR}$ (D_2O) δ 5.63 (s, 1H, H-1'), 5.06 (s, 1H, H-1), 4.40 (dd, $J = 4.5$ Hz, 8.4 Hz, 1H, H-3), 4.20-4.08 (m, 2H, H-3', H-5), 3.98-3.84 (m, 3H, H-2', H-4, H-5'), 3.75-3.58 (m, 2H, H-2, H-4'), 3.50-3.48 (m, 5H, H-6, H-6', CH_3O), 3.38-3.27 (m, 2H, H-6, H-6').

$^{13}\text{C NMR}$ (D_2O) δ 97.42, 97.21, 75.19, 70.34, 67.84, 67.20, 66.78, 56.49, 54.40, 54.06, 40.84, 40.64.

ESI-MS: calcd for $\text{C}_{13}\text{H}_{29}\text{N}_4\text{O}_7$ m/z $[\text{M}+\text{H}]^+$: 353.2031 Found: 353.2034.

1.19. Compound 25

The same method for **24** was applied for the synthesis of **25**, except for using **13** (78.2 mg, 60.5 μmol) as a starting material. Reaction time of the hydrolysis of iminophosphorane and the catalytic reduction of benzyl groups are 5 h and 30 h respectively. 63% of the Staudinger reaction product was used to the catalytic reduction after purification of **20** by HPLC. Compound **25** was obtained as colorless oil (14.0 μmol , 37%).

$^1\text{H NMR}$ (D_2O) δ 5.61 (d, $J = 3.9$ Hz, 1H, H-1''), 5.58 (s, 1H, H-1'), 5.06 (s, 1H, H-1), 4.48-4.39 (m, 2H), 4.29-4.19 (m, 2H), 4.11-4.02 (m, 1H), 3.99-3.93 (m, 3H), 3.87-3.70 (m, 3H), 3.65-3.27 (m, 10H).

$^{13}\text{C NMR}$ (D_2O) δ 97.53, 97.08, 96.04, 74.73, 70.73, 70.18, 67.87, 67.23, 66.82, 66.43, 56.49, 54.42, 54.11, 53.71, 40.86, 40.65, 40.29.

ESI-MS: calcd for $\text{C}_{19}\text{H}_{41}\text{N}_6\text{O}_{10}$ m/z $[\text{M}+\text{H}]^+$: 513.2879 Found: 513.2875.

1.20. Compound 26

The same method for **24** was applied for the synthesis of **26**, except for using **15** (21.5 mg, 12.7 μmol) as a starting material and methylidiphenylphosphine (35 mL, 191 μmol , 15 equiv) instead of triphenylphosphine. Reaction time of the hydrolysis of iminophosphorane and the catalytic reduction of benzyl groups are 55 h and 24 h respectively. All the Staudinger reaction product was used to the catalytic reduction after purification of **21** by HPLC. Compound **26** was obtained as colorless oil (6.68 μmol , 53%).

$^1\text{H NMR}$ (D_2O) δ 5.61-5.55 (m, 3H, H-1', H-1'', H-1'''), 5.05 (s, 1H, H-1), 4.49-4.38 (m, 3H), 4.27-3.79 (m, 11H), 3.71-3.31 (m, 13H).

$^{13}\text{C NMR}$ (D_2O) δ 97.61, 97.33, 96.31, 75.14, 74.51, 70.27, 67.93, 67.28, 66.91, 66.58, 56.52, 54.42, 54.14, 53.93, 40.92, 40.69, 40.42.

ESI-MS: calcd for $\text{C}_{25}\text{H}_{53}\text{N}_8\text{O}_{13}$ m/z $[\text{M}+\text{H}]^+$: 673.3727 Found: 673.3726.

1.21. Compound 27

The same method for **24** was applied for the synthesis of **27**, except for using **17** (80.0 mg, 38 μmol) as a starting material and methylidiphenylphosphine (0.14 mL, 760 μmol , 20 equiv) instead of triphenylphosphine. Reaction time of the hydrolysis of iminophosphorane is 52 h. The catalytic reduction of benzyl groups are 1 h of hydrogen gas bubbling and another 72 h under 1069 hPa of hydrogen gas. All the Staudinger reaction product was used to the catalytic reduction after purification of **22** by HPLC. Compound **27** was obtained as colorless oil (11.7 μmol , 30%).

$^1\text{H NMR}$ (D_2O) δ 5.61-5.58 (m, 4H, H-1', H-1'', H-1''', H-1''''), 5.05(s, 1H, H-1), 4.54-4.39 (m, 4H), 4.29-4.20 (m, 4H), 4.15-4.08-3.82 (m, 14H), 3.72-3.70 (m, 1H), 3.65-3.27 (m, 14H).

$^{13}\text{C NMR}$ (D_2O) δ 97.52, 97.17, 96.09, 75.05, 74.52, 70.22, 67.88, 67.23, 66.83, 66.43, 56.50, 54.41, 54.09, 53.87, 40.67, 40.42.

ESI-MS: calcd for $\text{C}_{31}\text{H}_{65}\text{N}_{10}\text{O}_{16}$ m/z $[\text{M}+\text{H}]^+$: 833.4575 Found: 833.4579.

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