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Supporting Information

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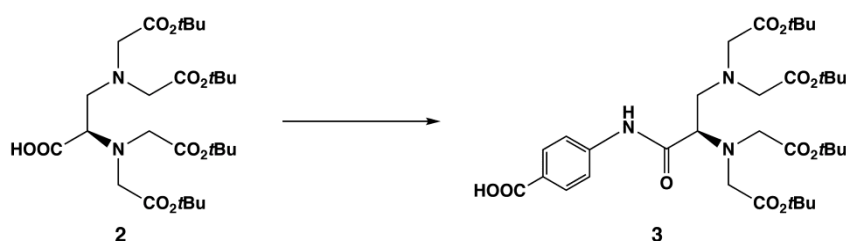
Paramagnetic Lanthanide Tagging for NMR Conformational Analyses of N-Linked Oligosaccharides

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General: Reagents and solvents were commercially available and used without any further purification unless otherwise noted. Column chromatography was performed on Silica Gel 60N purchased from Kanto Chemical Co., Inc., Wakosil 40C18 from Wako Pure Chemical Industries, Ltd. or Waters Sep-Pak C18. Elemental analysis (EA) and high resolution MS measurement were performed on Yanaco MT-6 and JEOL JMS-777V respectively at Instrument Center, IMS. NMR spectra were recorded on JEOL JNM ECA-600 spectrometer equipped with a 5-mm FG/HCN probe. TMS (in CDCl₃ and CD₃OD) and DSS (in D₂O) served as internal standard for ¹H and ¹³C NMR measurements. For PCS observation, ¹H-¹³C HSQC spectra were recorded at 300 K with 512 (*t*₁) and 1024 (*t*₂) complex points. NMR spectra were processed and analyzed with the program NMRPipe (F. Delaglio *et al. J. Biomol. NMR* 1995, **6**, 277.) and Sparky (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco).

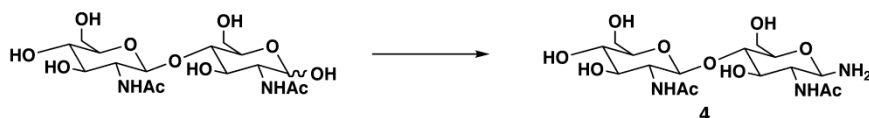
Synthesis of 4-[(R)-2',3'-bis[di(*tert*-butoxycarbonylmethyl)amino]-1'-oxopropyl]aminobenzoic acid (**3**).



A mixture of diaminopropionic acid derivative **2** (659 mg, 1.2 mmol, see ref. [A. Leonov *et al. Chem. Eur. J.* 2005, **11**, 3342.]), HATU (488 mg, 1.3 mmol) and DIPEA (200 μ l, 1.2 mmol) in DMSO (5 ml) was stirred for 10 min at room temperature. To this solution, 4-aminobenzoic acid (176 mg, 1.3 mmol) was added and the stirring was continued for 17 h. The reaction mixture was diluted with ethyl acetate (50 ml), washed with 0.1 M aqueous phosphoric acid (3 x 20 ml), dried over anhydrous Na₂SO₄ and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, CHCl₃) to give **3** as white solid (289 mg, 35%). ¹H-NMR (600 MHz, CDCl₃, 300 K): δ = 10.9 (s, 1H), 8.04 (AA' part of AA'XX', 2H), 7.78 (XX' part of AA'XX', 2H), 3.74 (dd, *J* = 7.8, 5.8 Hz, 1H), 3.58 (d, *J* = 17.9 Hz, 2H), 3.57 (s, 4H), 3.42 (d, *J* = 17.8 Hz, 2H), 3.35 (dd, *J* = 14.0, 6.48 Hz, 1H), 2.99 (dd, *J* = 15.0, 7.3 Hz, 1H), 1.44 (s, 36H); ¹³C-NMR (150 MHz, CDCl₃, 300 K): δ = 172.0, 171.8,

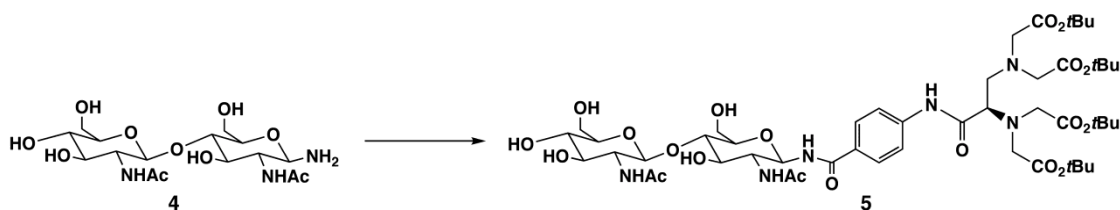
170.8, 170.4, 143.8, 131.4, 123.8, 118.8, 81.6, 81.3, 65.2, 56.7, 54.5, 54.3, 28.2, 28.1; EA: Calcd for C₃₄H₅₃N₃O₁₁•H₂O: C, 58.52; H, 7.94; N, 6.02. Found: C, 58.65; H, 7.67; N, 6.08.

Preparation of *N,N'*-diacetylchitobiosamine **4**.



N,N'-Diacetylchitobiose (50 mg, 0.12 mmol) was converted to glycosylamine **4** by treating with an excess amount of NH₄HCO₃ in water. The solvent and NH₄HCO₃ were removed by evaporation under reduced pressure after stirring for 35 h at room temperature to give colorless solid. This was applied into subsequent reaction immediately without any other purification.

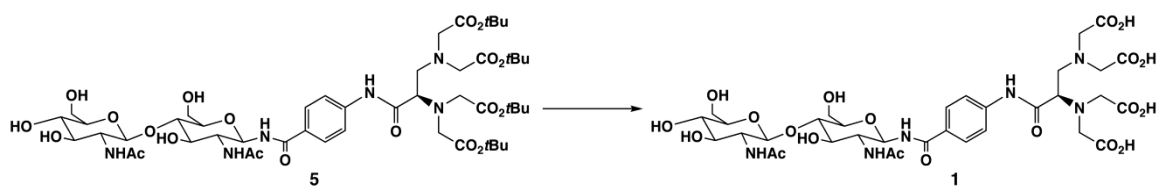
Synthesis of compound **5**.



Compound **3** (94 mg, 0.14 mmol), HATU (60.4 mg, 0.15 mmol) and DIPEA (28 μ l, 0.14 mmol) were dissolved in DMSO (1.5 ml) and the mixture was stirred at room temperature. Glycosylamine **4** and DIPEA (56 μ l, 0.28 mmol) were added after 10 min, and the reaction was continued. After 19 h, the reaction mixture was diluted with excess amount of water and purified by column chromatography (ODS, gradient elution from H₂O:ACN = 100:0 to 0:80) to give **5** as white solid (83 mg, 66%).

¹H-NMR (600 MHz, CD₃OD, 300 K): δ = 7.77 (AA' part of AA'XX', 2H), 7.74 (XX' part of AA'XX', 2H), 4.08 (d, *J* = 9.5 Hz, 1H), 4.51 (d, *J* = 8.9 Hz, 1H), 3.95 (t, *J* = 9.6 Hz, 1H), 3.90 (dd, *J* = 11.7, 2.1 Hz, 1H), 3.85 (dd, *J* = 12.2, 1.5 Hz, 1H), 3.65-3.56 (m, 16H), 3.52 (s, 1H), 3.50 (s, 2H), 3.47-3.15 (m, 4H), 3.04 (dd, 7.49, 7.33 Hz, 1H), 1.99 (s, 3H), 1.92 (s, 3H), 1.43 (s, 36H); ¹³C-NMR (150 MHz, CD₃OD, 300 K): δ = 173.1 (2 peaks overlap), 172.4, 171.6, 168.2, 142.9, 128.2 (2 peaks overlap), 118.9, 80.3, 79.9, 76.8, 74.3, 73.1, 70.7, 65.1, 61.4, 61.3, 60.3, 60.2, 56.1, 56.0, 54.4, 54.2, 27.2, 21.8, 21.2; HRMS (FAB): Calcd for C₅₀H₈₀N₆O₂₀[M+H⁺]: 1085.5461; Found: 1085.5514.

Synthesis of modified disaccharide **1**.



Precursor **5** (30 mg, 0.028 mmol) was deprotected by treating with TFA (3.8 ml) and water (0.2 ml) for 12 h at room temperature. The solvent was removed by evaporation under reduced pressure, and the residue was purified by column chromatography (ODS, gradient elution from H₂O:ACN = 100:0 to 20:80). The combined fractions were lyophilized to give **1** as white powder (22 mg, 93%).

¹H-NMR (600 MHz, D₂O, 300 K): δ = 7.71 (AA' part of AA'XX', 2H), 7.64 (XX' part of AA'XX', 2H), 5.19 (d, J = 9.7 Hz, 1H), 4.54 (d, J = 8.7 Hz, 1H), 4.27 (dd, J = 5.2, 5.4 Hz, 1H), 4.07-3.38 (m, 26H), 3.36 (s, 1H), 3.33 (s, 1H); ¹³C-NMR (150 MHz, D₂O, 300 K): δ = 176.6, 176.2, 176.0, 175.3, 171.3, 142.0, 139.9, 129.3, 121.5, 101.5, 79.4, 79.0, 76.4, 76.0, 73.6, 72.7, 69.8, 63.0, 60.7, 60.6, 60.0, 55.8, 55.7, 54.4, 54.0, 22.5, 21.8; EA. Calcd for C₃₄H₄₈N₆O₂₀•6H₂O: C, 42.15; H, 6.24; N, 8.67. Found: C, 42.03; H, 5.94; N, 8.70.

Preparation of complex **1**•M

Compound **1** (1.5 μ mol) was dissolved in D₂O (0.5 ml) and the pH was increased to 8.0 by adding NaOD solution. D₂O solution of MCl₃ (150 mM, 10 μ l; M = Tm³⁺, Ho³⁺, Er³⁺, Yb³⁺ or La³⁺) was titrated and NMR measurements of this solution were subsequently performed.

Table S1. ^1H and ^{13}C chemical shifts of **1** complexed with La^{3+} and Tm^{3+} .^[a]

	La^{3+}		Tm^{3+}	
	$\delta^1\text{H/ppm}$	$\delta^{13}\text{C/ppm}$	$\delta^1\text{H/ppm}$	$\delta^{13}\text{C/ppm}$
1'	5.18	79.39	6.20	80.48
2'	3.93	54.04	4.88	55.00
3'	3.76	72.73	4.42	73.42
4'	3.64	78.98	4.27	79.61
5'	3.59	76.42	4.27	77.13
6'	3.78	60.05	4.39	60.68
	3.60	60.05	4.16	60.70
1	4.54	101.50	4.97	101.93
2	3.69	55.70	4.03	56.03
3	3.50	73.55	3.78	73.83
4	3.40	69.82	3.67	70.09
5	3.43	76.01	3.76	76.33
6	3.85	60.65	4.15	60.94
	3.69	60.64	3.97	60.94

[a]The measurement was repeated 3 times for **1**• La^{3+} and **1**• Tm^{3+} , respectively. Average chemical shifts are shown. The maximum deviation was less than 0.01 ppm.

Table S2. ^1H and ^{13}C chemical shifts of **1** complexed with Ho^{3+} , Er^{3+} and Yb^{3+} .

	Ho^{3+}		Er^{3+}		Yb^{3+}	
	$\delta^1\text{H/ppm}$	$\delta^{13}\text{C/ppm}$	$\delta^1\text{H/ppm}$	$\delta^{13}\text{C/ppm}$	$\delta^1\text{H/ppm}$	$\delta^{13}\text{C/ppm}$
1'	3.83	77.93	5.67	79.92	5.45	79.69
2'	2.68	52.84	4.38	54.49	4.19	54.31
3'	2.93	71.87	4.09	73.07	3.94	72.91
4'	2.87	78.20	3.94	79.30	3.79	79.16
5'	2.70	75.51	3.93	76.78	3.77	76.62
6'	3.03	59.25	4.08	60.37	3.94	60.23
	2.90	59.25	3.88	60.37	3.76	60.21
1	4.03	101.02	4.75	71.72	4.65	101.63
2	3.28	55.30	3.87	55.87	3.78	55.77
3	3.18	73.20	3.65	73.68	3.58	73.61
4	3.09	69.49	3.54	69.96	3.48	69.88
5	3.05	75.62	3.60	76.17	3.52	76.09
6	3.50	60.27	4.00	60.81	3.93	60.72
	3.35	60.27	3.83	60.79	3.76	60.72

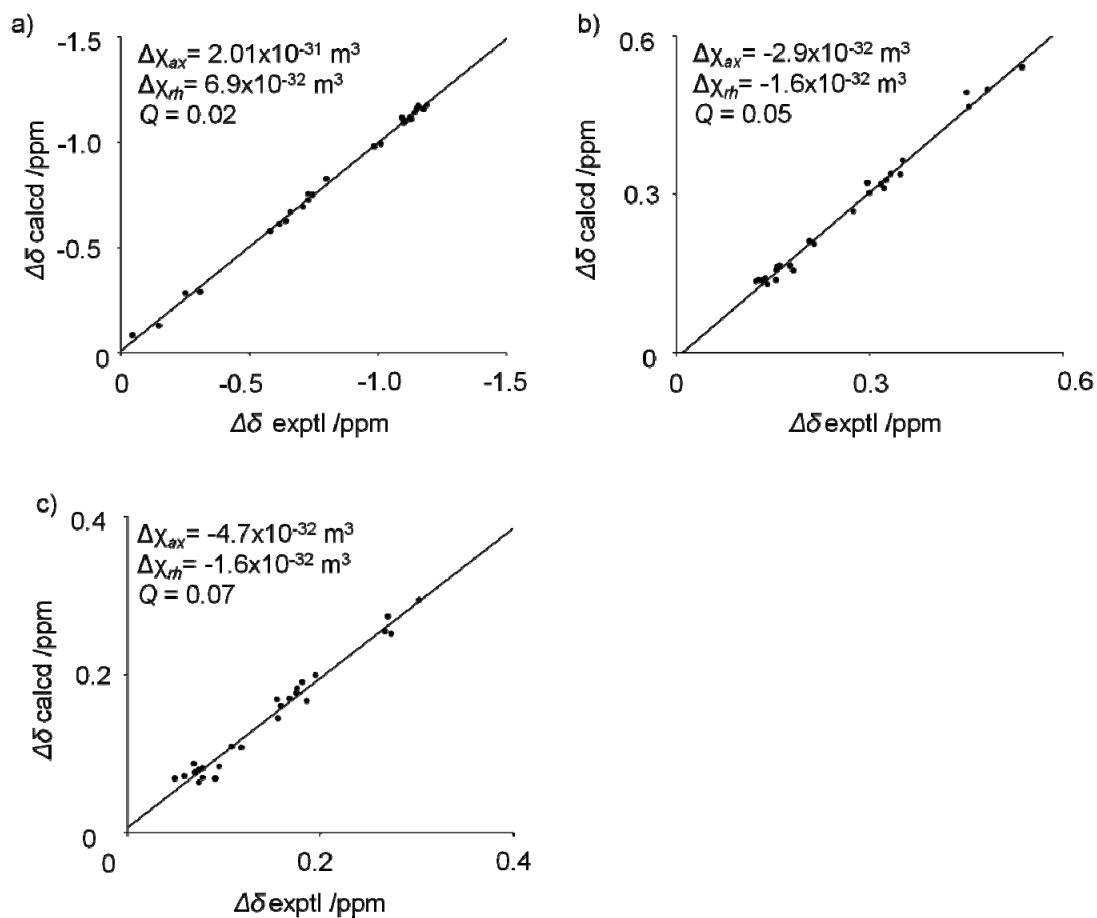


Figure S1. Correlation of observed and back-calculated PCSs induced by a) Ho^{3+} , b) Er^{3+} and c) Yb^{3+} . Q value is defined as follows.

$$Q = \frac{\sqrt{\sum (\text{PCS}_{\text{obs.}} - \text{PCS}_{\text{calc.}})^2}}{\sqrt{\sum (\text{PCS}_{\text{obs.}})^2}}$$

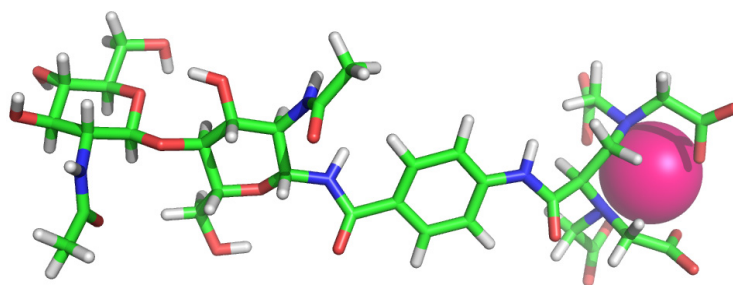


Figure S2. Structural model of modified disaccharide **1·M**.

The reported crystal structure of the ion as $[\text{LaEDTA} \cdot 3\text{H}_2\text{O}]^-$ (*Acta Crystallogr. Sect. C: Struct. Commun.* 1995, **51**, 1559) was applied to build the model of the tag. The structural model of the metal loaded tagged *N,N'*-diacetylchitobiose (**1·M**) was developed by performing molecular dynamics simulation using MacroModel (Schrödinger, LLC). The structure of disaccharide moiety found here agreed with the previously reported structure of methyl *N,N'*-diacetyl- β -chitobioside found by molecular dynamics simulation (*Int. J. Biol. Macromol.* 1995, **17**, 227).