Supporting Information

Catalyst-Proximity Protein Chemical Labelling on Affinity Beads Targeting Endogenous Lectins.

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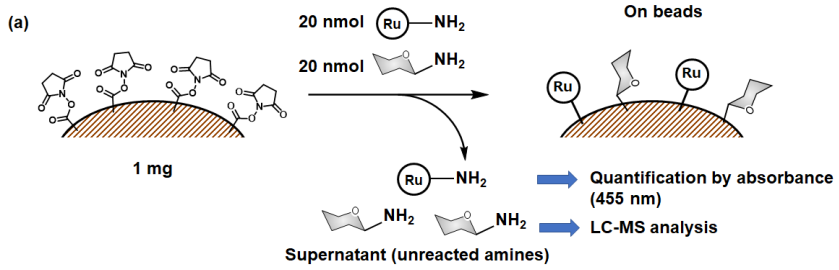
1. Supporting Data



(b) Ru/dcbpy and carbohydrate immobilized on beads

		Total (supernatant+on beads) [nmol]	Abs. intensity* (455 nm) or MS intensity (pre-reaction)	Abs. intensity* (455 nm) or MS intensity (after reaction)	unreacted substrate [%]	Supernatant [nmol]	on beads [nmol]
beads **2**	Ru/dcbpy	20	24397	276	1.1	0.23	19.8
	18	20	281727	228215	81	16.2	3.8
beads **23**	Ru/dcbpy	20	24397	0	0	0.0	20.0
	21	20	248359	83651	34	6.7	13.3

(c) Extracted chromatogram of compounds **18** and **21**

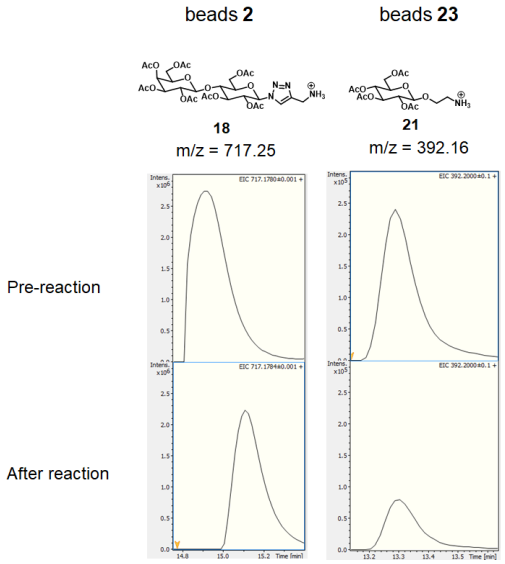


Fig S1. Calculation of the amounts of immobilized compounds on beads. (a) Reaction between NHS-functionalized beads and amine compounds. Unreacted amine compounds after conjugation reaction with NHS-functionalized beads were quantified by absorbance (455 nm) or were analyzed by LC-MS analysis. The acetyl groups in saccharides were removed on beads (See experimental section 2-9.) (b) Calculated amount of immobilized compounds on beads. (c) MS intensities of each compound before reaction and after reaction. See Figure S2 for the evaluation of beads functionalized with Ru/dcbpy and amine-glucose (**21**) (beads **23**).

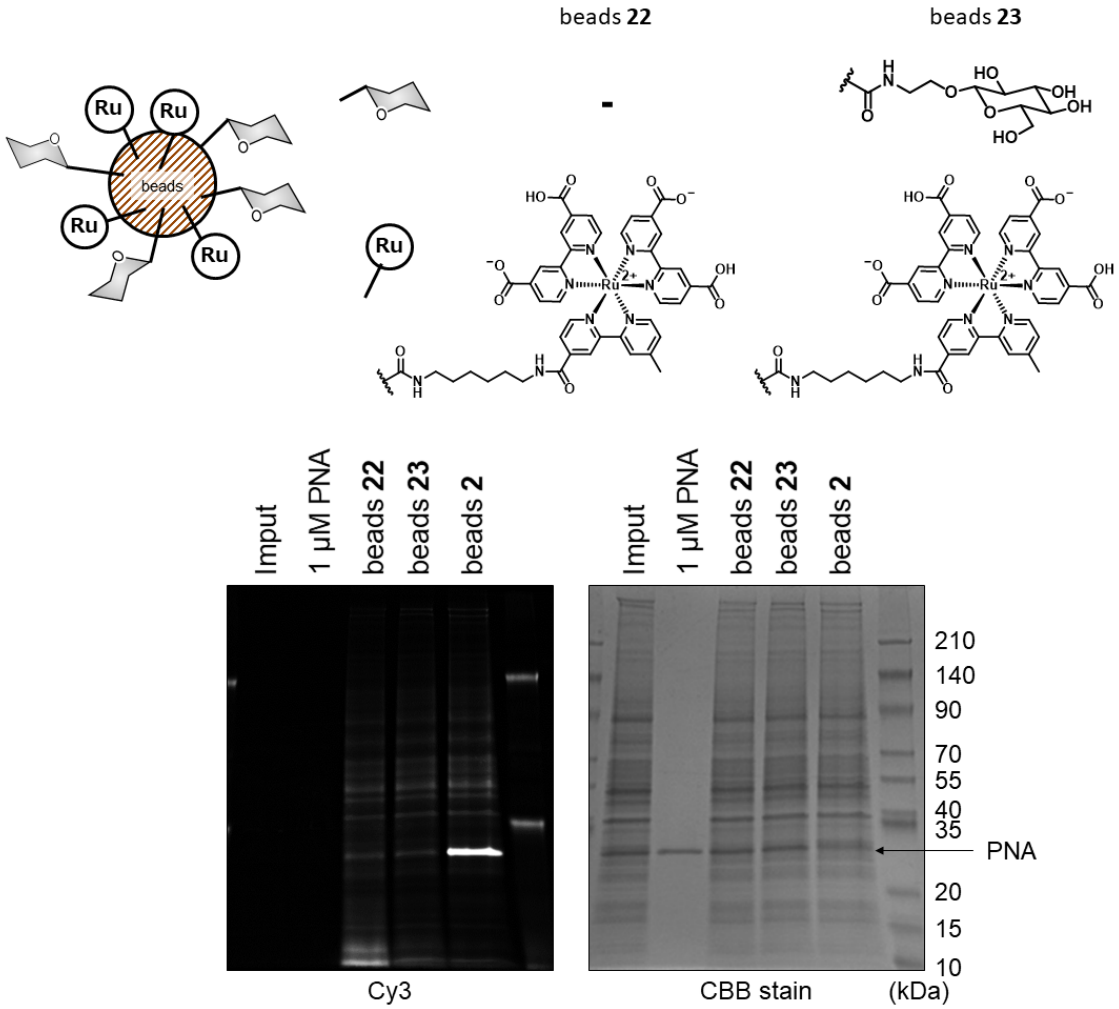


Fig S2. PNA labelling in HeLa cell lysate using beads **22**, **23** and **2**. Labelling reaction was operated according to protocol of section 2-11. PNA was labelled selectively only using beads **2,** and lactose-deficient beads (**22**), and glucose-functionalized beads instead of lactose (**23**) did not labeled PNA. These results suggested that the PNA-labelling was lactose-dependent.

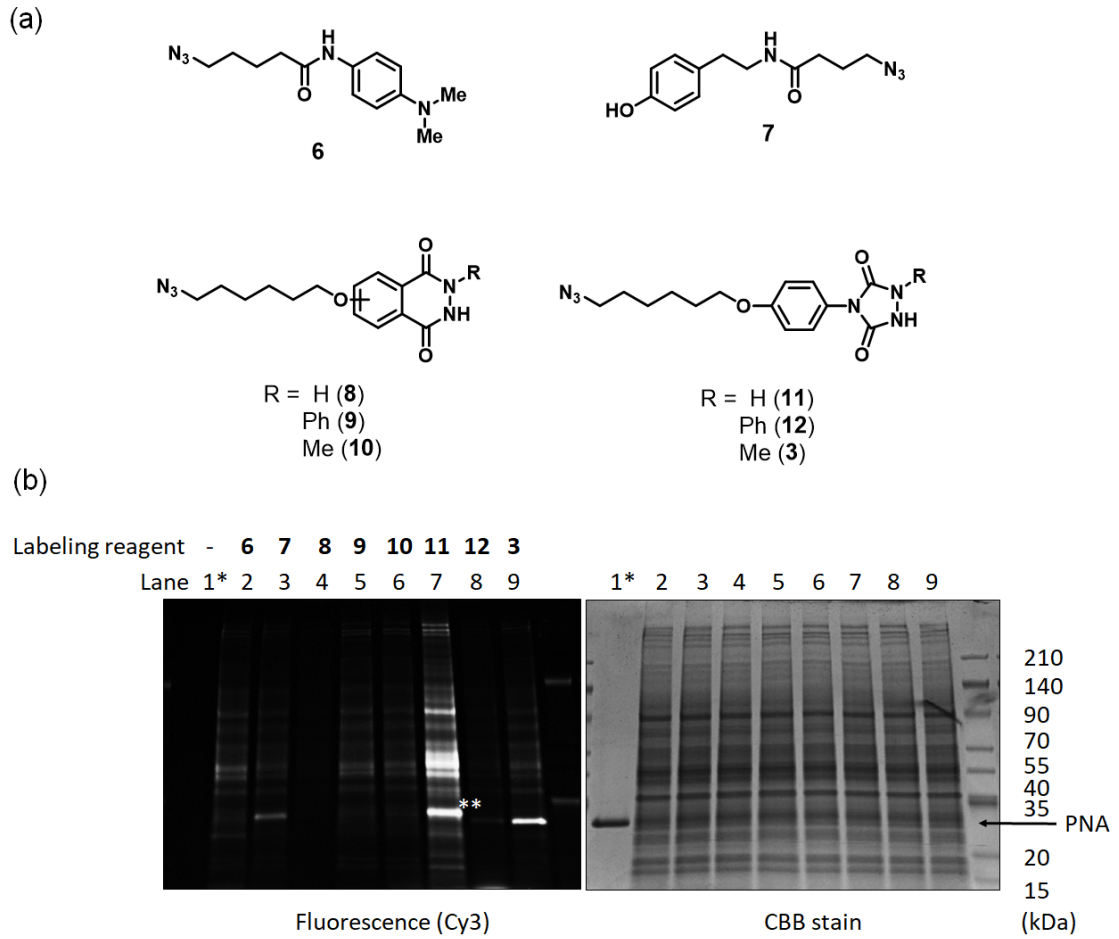


Fig S3. PNA-selective labelling using different labelling reagents. (a) Structure of ligand, photocatalyst and labelling reagents. (b) PNA-selective labelling on beads **2**. We selected azide-conjugated phenylenediamine derivative **6**, tyramide derivative **7**, luminol derivatives (**8**, **9**, **10**) and urazole derivatives (**11**, **12**, **3**) as labelling reagents. The mixture of PNA (1 μM) and HeLa cell lysate (1.0 mg/mL protein) in Lysis buffer (pH 7.4) was photo-irradiated (455 nm LED) in the presence of labelling reagent (500 μM) at 0 °C for 5 min. Azide-labelled proteins were visualized by copper-free click reaction with DBCO-Cy3. *PNA control (1 μM). **labelled PNA with **11** (this band was not detected in control experiment using cell lysate without PNA).

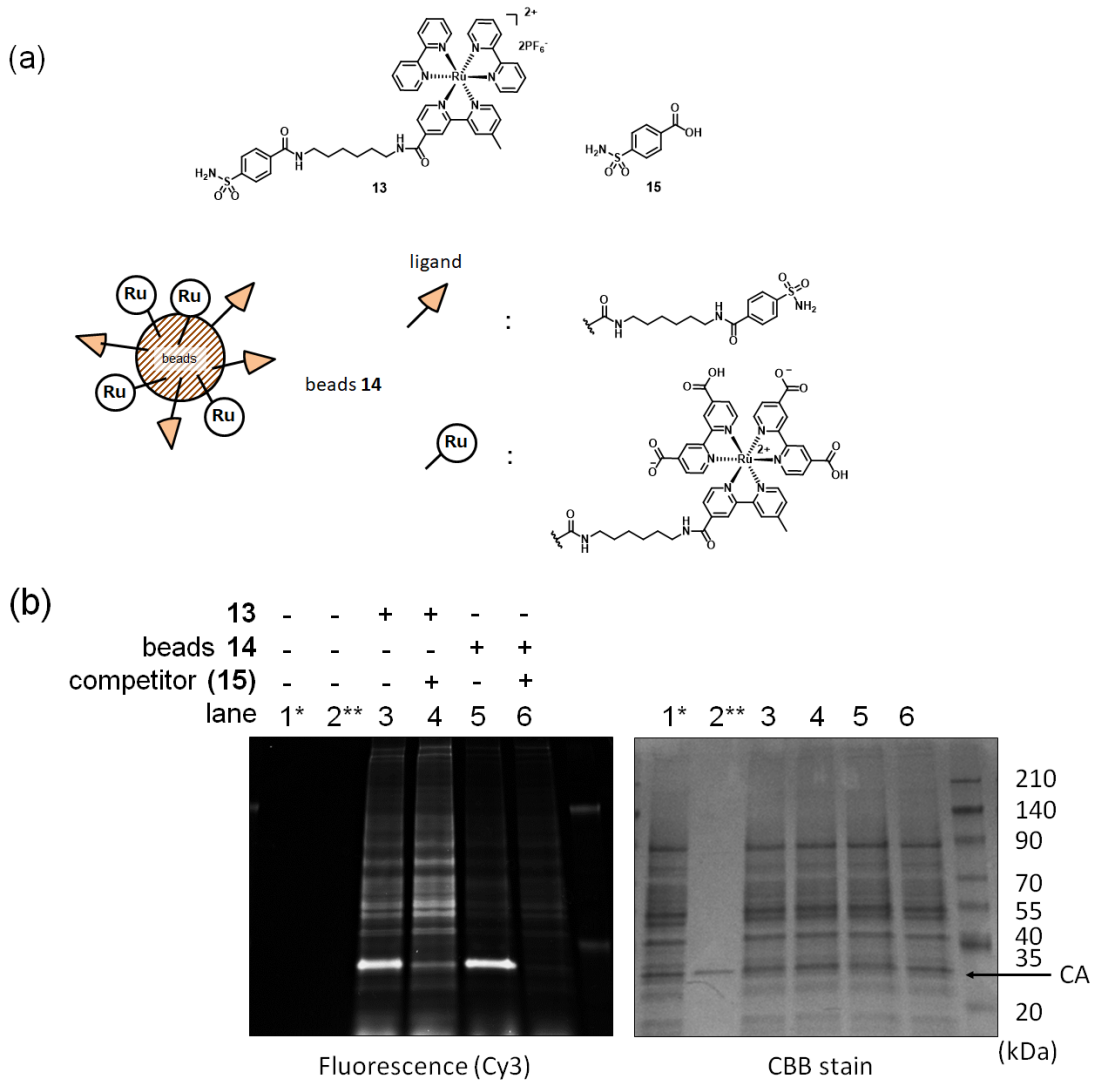


Figure S4. Carbonic anhydrase (CA)-selective labelling in CA containing HeLa cell lysate using ligand conjugated photocatalyst **13** or beads **14**. (a) Structure of ligand and photocatalyst. (b) CA-selective labelling using ligand conjugated photocatalyst **13** or beads **14**. The mixture of CA (1 μM) and HeLa cell lysate (1.0 mg/mL protein) in lysis buffer (pH 7.4) was photo-irradiated (455 nm LED) in the presence of **3** (500 μM) at 0 °C for 5 min. Azide-labelled proteins were visualized by copper-free click reaction with DBCO-Cy3. Competitive inhibition was carried out by incubating for 4 h at 4 °C in the presence of 1 mM **15** (free CA ligand). *Input: CA (1 μM) containing HeLa cell lysate (1.0 mg/mL protein). **CA control (1 μM).

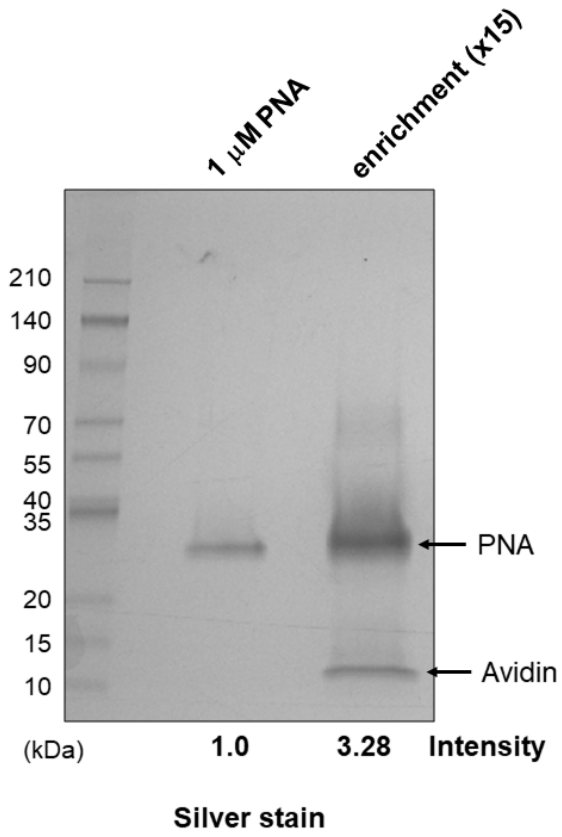


Figure S5. Evaluation of labelling efficiency using MAUra-DTB (**5**). Desthiobiotin labelled PNA purified and enriched by avidin beads (according to protocol of section 2-12). MAUra-DTB (**5**, 100 mM stock solution in DMSO, final concentration 500 μM) and beads **2** (final beads concentration: 5.0 mg/mL) were added to 1 μM PNA solution (50 μL x 15 vials, in MES buffer pH 7.4) and the mixture was incubated at 4 °C for 4 h. The light irradiation was operated according to section 2-11, and proteins were precipitated by 2D clean up kit (BioRad). The residue was rehydrated with lysis buffer, Dynabeads™MyOne™Streptavidin C1 (Invitrogen) (0.5 mg) was added and shaken at room temperature for 60 min. The beads were washed with lysis buffer three times. For the elution of desthiobiotin labelled proteins, beads was added DTB elution buffer (2 mM biotin, 200 mM DTT, 1 x NuPAGE buffer (Thermo)) (50 μL) and incubated at 95 °C for 5 min. After SDS-PAGE of enriched sample, signal intensities obtained by silver stain were quantified by ImageJ.

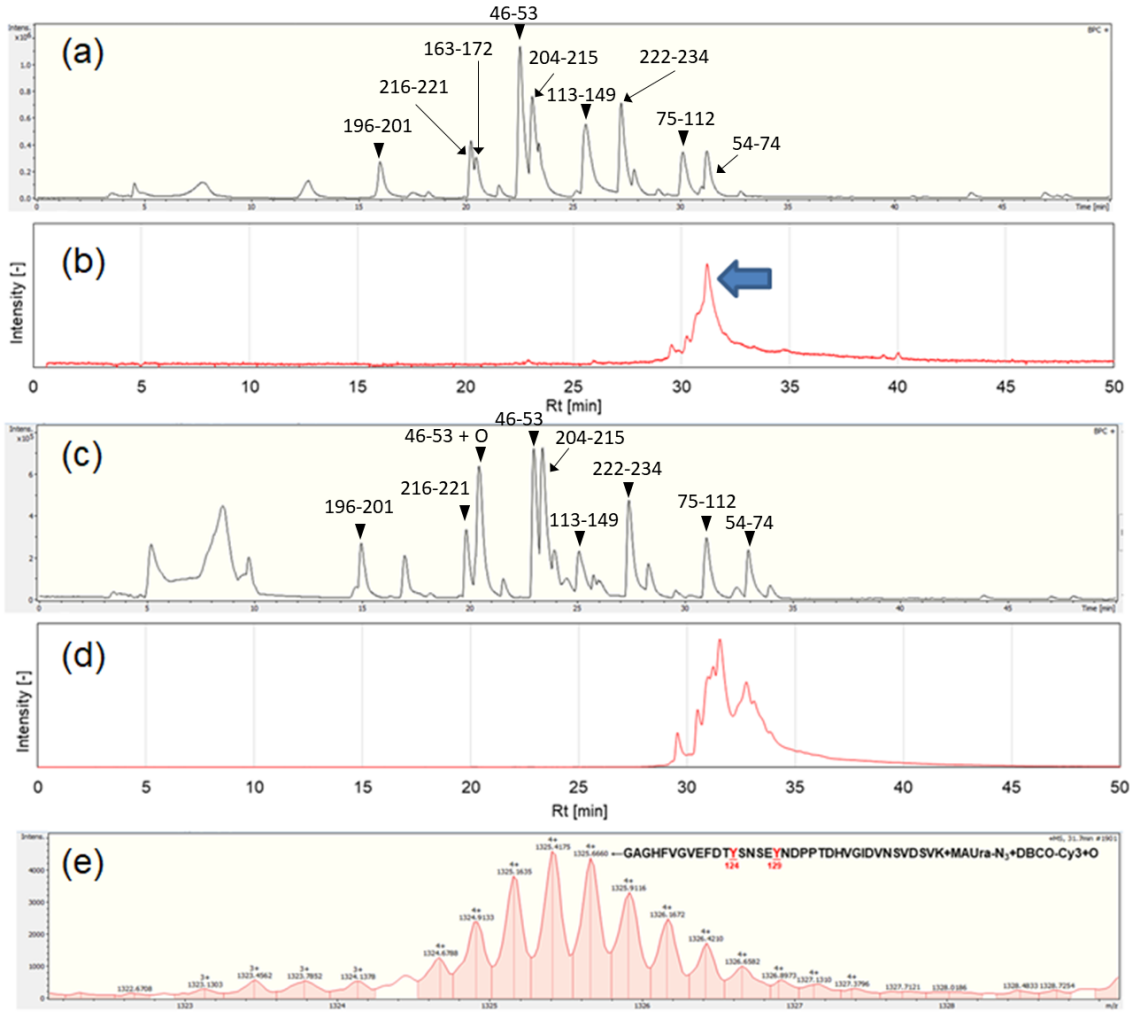


Figure S6. LC-MS analysis of tryptic digested PNA labelled with Cy3. PNA was labelled using **3** (500 µM) and beads **2** or $Ru(bpy)_3Cl_2$. (a)Total ion chromatogram of peptides obtained from PNA labelled using the beads **2**. (b) Detected fluorescence in the chromatography of (a). (c) Total ion chromatogram of peptides obtained from PNA labelled using $Ru(bpy)_3Cl_2$. (d) Detected fluorescence in the chromatography of (c). In Figure S6(a) and (c), peptide fragments that could be assigned from the detected m/z are shown. In Figure 6 (c), an oxidized peptide fragment was detected as a major peak (46-53 + O), but not in Figure 6(a). (e) MS spectrum of major peak in the retention time indicated by blue allows (b). Detected peak (m/z: 1325.6 (4+)) corresponds to the labelled peptide fragment containing Y124 and Y129.

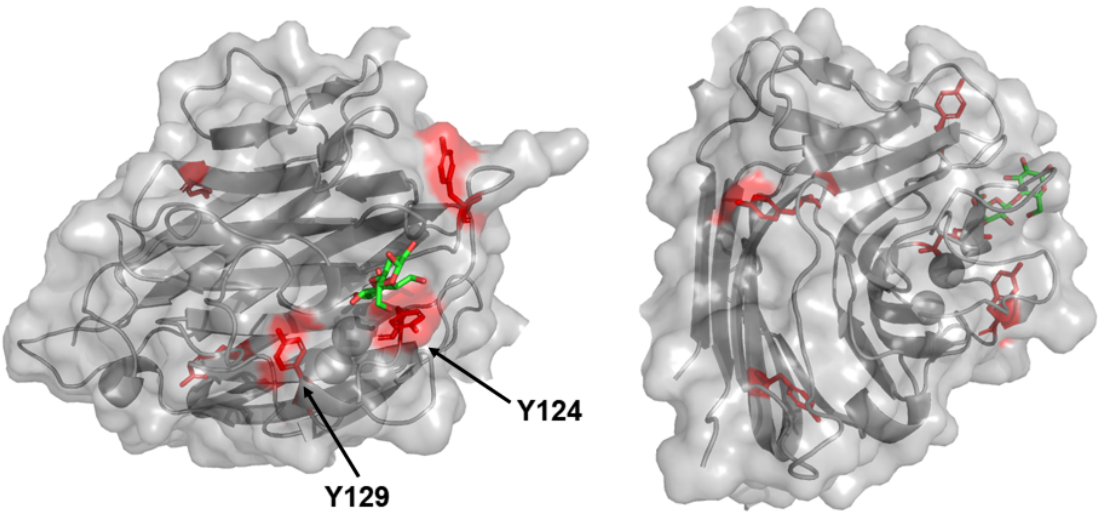


Figure S7. The X-ray structure of PNA (PDB: 2pel). Tyrosine residues: Red. Lactose: green sticks. The positions of Y124 and Y129 were shown.

UnProt ID	Description	MW (kDa)	calculated pI	Sum PEP Score	Coverage	Number of Peptides	Number of Unique Peptides	Number of PSMs
A6NCN2|KR87P_HUMAN	Putative keratin-87 protein	29.1	5.8	101.6	58.4	22	4	112
P12270|TPR_HUMAN	Nucleoprotein TPR	267.1	5.0	58.1	11.4	21	21	48
P09382|LEG1_HUMAN	**Galectin-1**	**14.7**	**5.5**	**35.7**	**63.7**	**7**	**7**	**37**
Q9Y230|RUVB2_HUMAN	RuvB-like 2	51.1	5.6	35.4	27.2	11	11	33
Q13308|PTK7_HUMAN	Inactive tyrosine-protein kinase	118.3	7.1	29.9	13.7	11	11	26
P16144-5|ITB4_HUMAN	Isoform Beta-4E of Integrin beta-4	106.7	6.3	29.3	12.9	9	9	24
P32929-2|CGL_HUMAN	Isoform 2 of Cystathionine gamma-lyase	39.5	6.9	24.8	34.1	8	8	24
Q01469|FABP5_HUMAN	Fatty acid-binding protein, epidermal	15.2	7.0	31.2	64.4	9	9	23
P05783|K1C18_HUMAN	Keratin, type I cytoskeletal 18	48.0	5.5	13.2	10.0	6	3	22
P17931|LEG3_HUMAN	**Galectin-3**	**26.1**	**8.6**	**19.0**	**24.0**	**5**	**5**	**22**
P61978|HNRPK_HUMAN	Heterogeneous nuclear ribonucleoprotein K	50.9	5.5	21.2	25.1	8	8	22
P62333|PRS10_HUMAN	26S protease regulatory subunit 10B	44.1	7.5	21.3	22.6	7	7	21
O43399|TPD54_HUMAN	Tumor protein D54	22.2	5.4	26.2	47.1	8	8	20
P17301|ITA2_HUMAN	Integrin alpha-2	129.2	5.3	25.3	10.1	9	9	20
P30048|PRDX3_HUMAN	Thioredoxin-dependent peroxide reductase, mitochondrial	27.7	7.8	21.1	40.2	7	7	20
P42704|LPPRC_HUMAN	Leucine-rich PPR motif-containing protein, mitochondrial	157.8	6.1	17.1	6.0	7	7	20
Q9Y266|NUDC_HUMAN	Nuclear migration protein nudC	38.2	5.4	22.2	28.1	8	8	20

Table S1. Labelled protein detected by LC-MS/MS. Labelling of lactose-binding protein was carried out using beads **2**, A431 cell lysate (3.0 mg/mL), and MAUra-DTB (sample 1). As a control, labelling was carried out in the presence of excess lactose (sample 2). Proteins detected in the sample 1 but not in sample 2 were extracted. Only proteins were listed with Number of peptide $\geq$ 5 and Number of PSMs $\geq$ 20. Also see primary data of identified proteins in excel file.

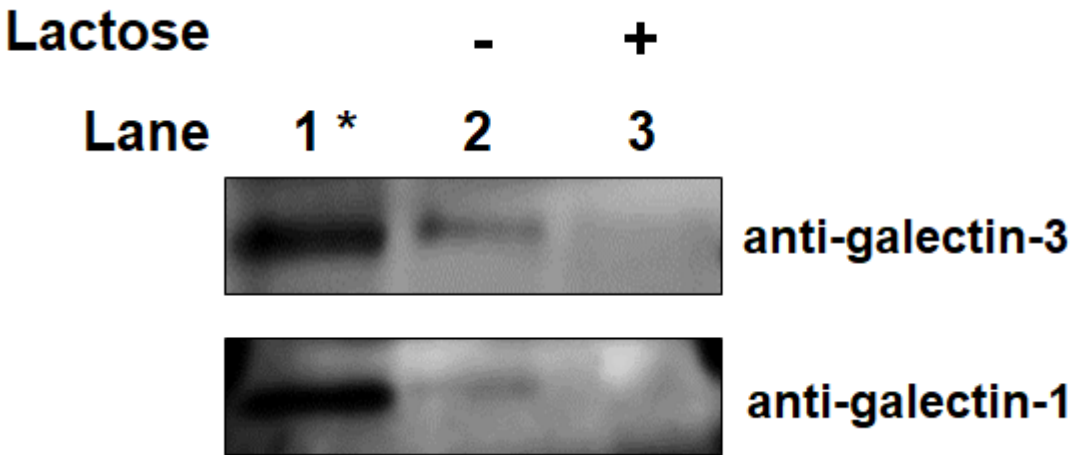


Figure S8. Western blot of labelled galectin-3 and galectin-1 after enrichment in each of the conditions shown in Figure 3b. *0.1 mg/mL A431 cell lysate.

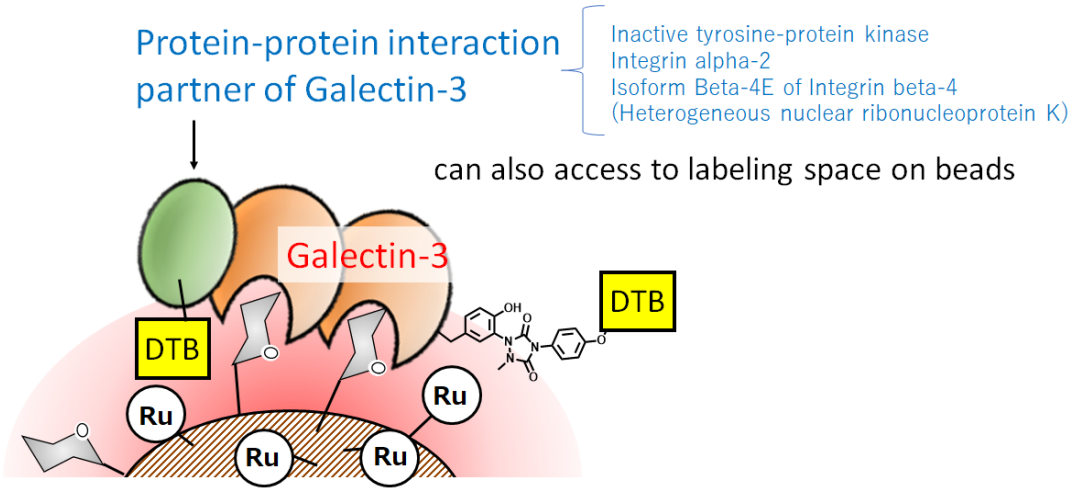


Figure S9. Image of protein-protein interaction (PPI) partners of galectin-3 (inactive tyrosine protein kinase, integrin alpha-2, isoform beta-4E of integrin beta-4 and heterogeneous nuclear ribonucleoprotein K). These PPI partners might access to labeling space via interaction with galectin-3 on beads.

The quantitative analysis of enriched protein by labeling with MAUra-DTB (**5**), revealed that not only galectin-1 and -3, but also PPI partners with galectin-3 was labeled with **5** and enriched in the absence of free lactose (See Figure S10).

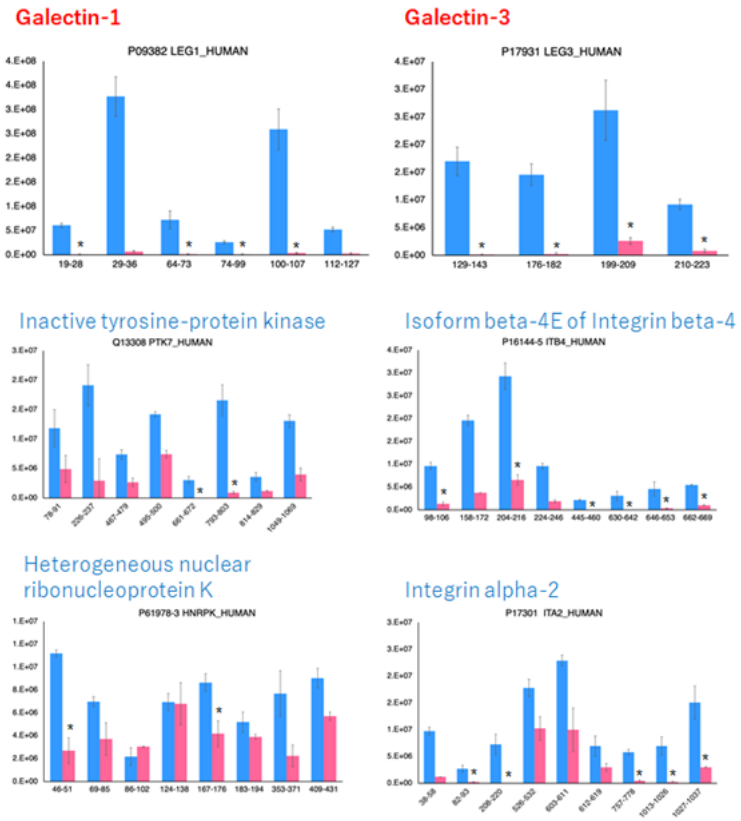


Figure S10. Quantification of the signal intensity of each peptide. The intensity of the ion chromatograph of each precursor ion was calculated by Skyline software[1]. Only the signals with a high isotope dot-product (>0.90) and independent peaks in were chosen. Asterisk (*) showed a poor isotope dot-product value (< 0.90).

Comparing the signals of the sample labeled in the absence of free lactose (blue, sample 1, Figure 3a left) and the sample in the presence of free lactose (red, sample 2, Figure 3a right), galectin-1 galectin-3, inactive tyrosine protein kinase, integrin alpha-2, isoform beta-4E of integrin beta-4 were clearly enriched in the former sample. The signal difference was slight in the quantitative analysis about heterogeneous nuclear ribonucleoprotein K.

Galectin-1 and -3 might be strongly labeled because they bound lactose on beads directly. These labeling was also detected in 2D-DIGE analysis (Figure 4). However, PPI partner with galectin-3 were not fully labeled due to their long binding distance to the beads surface.

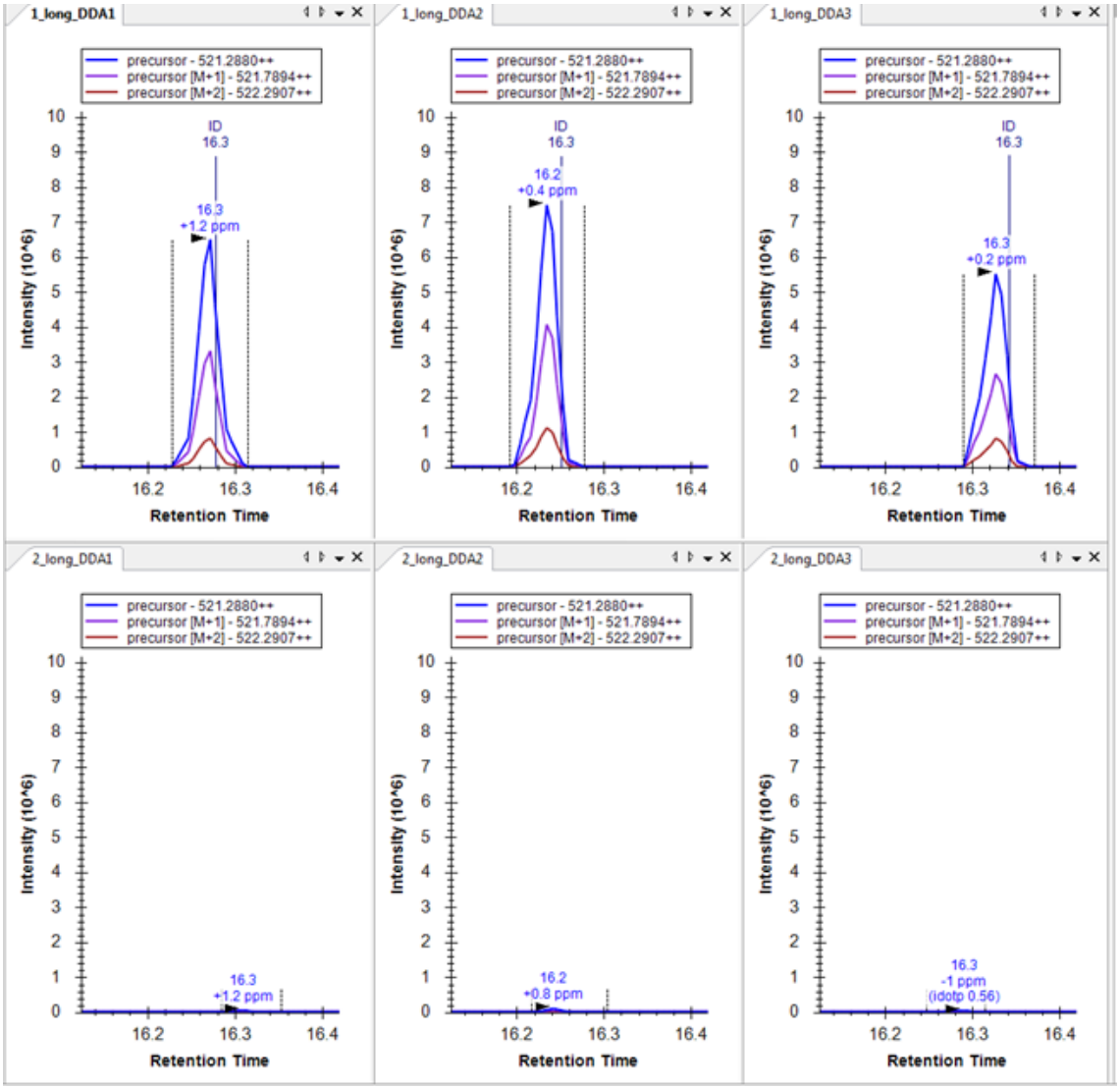


Figure S11. An example of extracted ion chromatography for single peptide (Galectin-1 V19-K28 (VRGEVAPDAK)). LC-MS/MS analysis was performed with triplicate (n = 3). The upper figures show the results of sample 1 (labelling in the absence of free lactose). The lower figures show the results of sample 2 (labelling in the presence of free lactose).

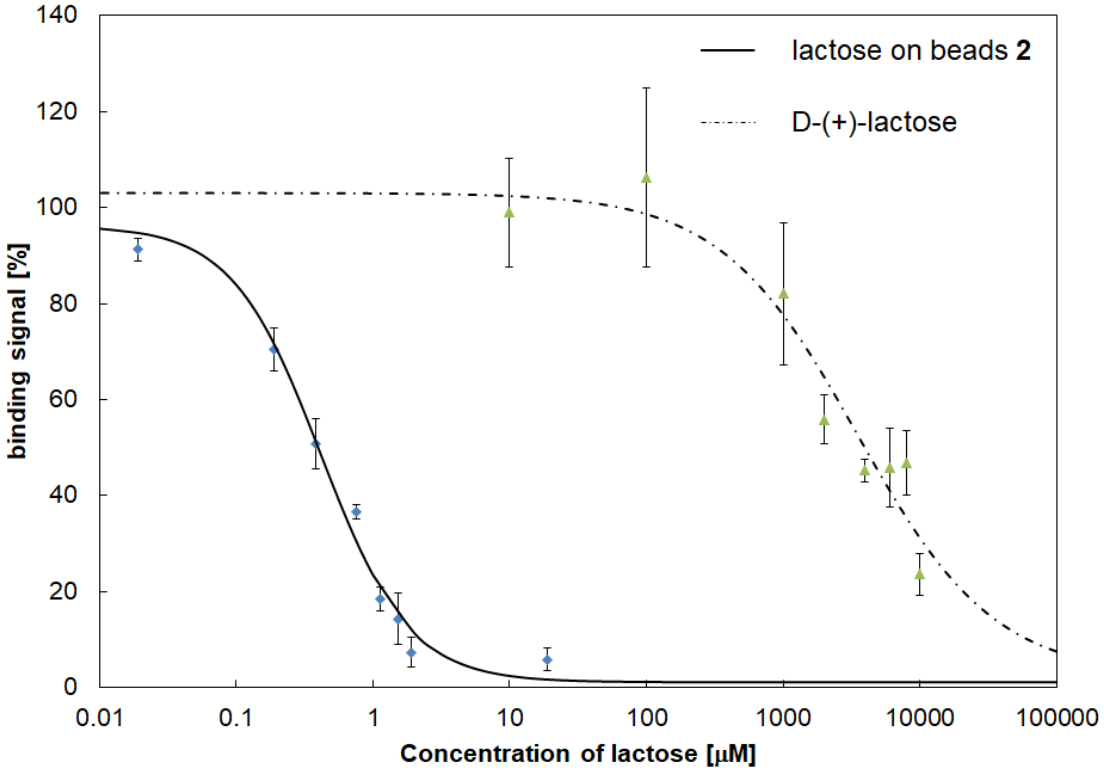


Figure S12. The inhibitory potency of beads **2** and lactose for the binding between ASF and galectin-3. Concentrations of lactose on beads **2** were normalized by calculation from lactose molecules immobilized on beads **2** (3.8 nmol/mg beads). The lactose on the beads showed higher inhibitory potency than lactose (lactose: IC_{50} = 3.87±1.43 mM, beads: IC_{50} = 0.39±0.03 μM).

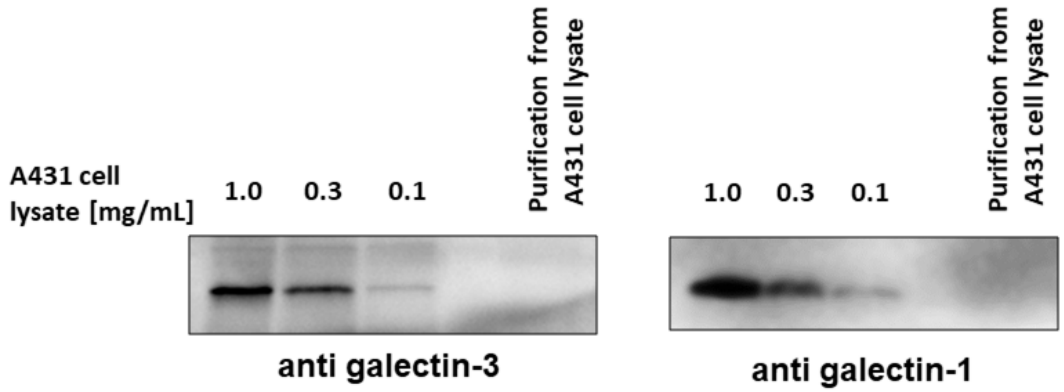


Figure S13. Affinity purification of galectin-1 and galectin-3 using lactose-functionalized affinity beads. Purification was carried out using A431 cell lysate (3.0 mg/mL protein) and β-D-lactose-functionalized affinity beads (5.0 mg/mL beads). Both galectin-1 and galectin-3 were not detected by affinity chromatography. Although the affinity of galectin-1 or -3 and lactose on the beads, it was not enough to purify by affinity chromatography.

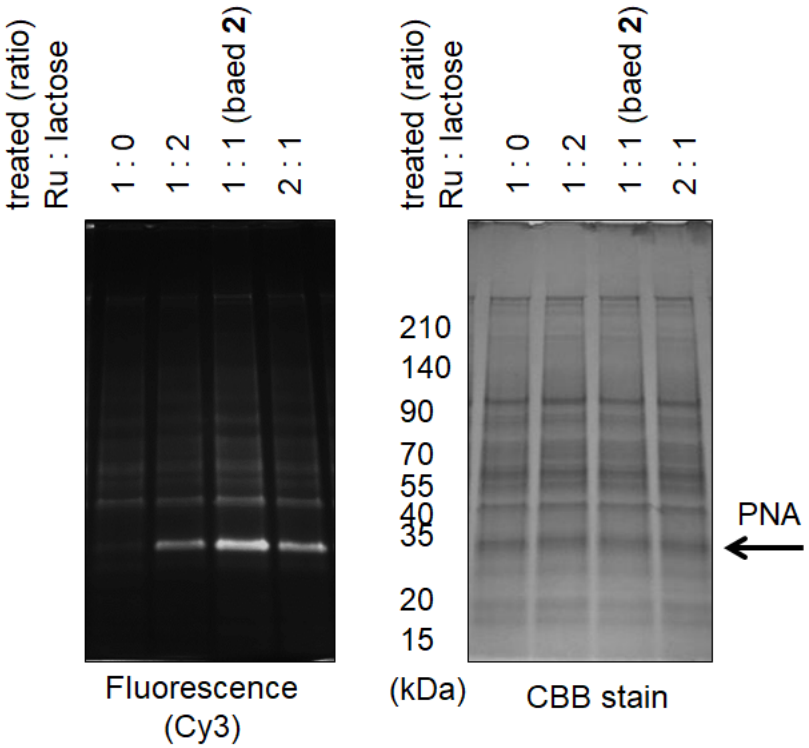
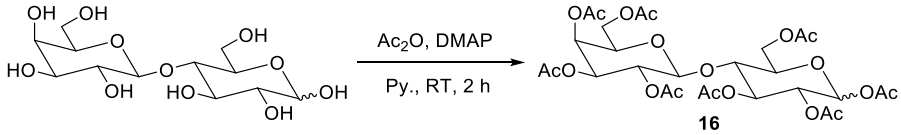


Figure S14. Optimizing the ratio of ruthenium photocatalyst and lactose on bead. The condition of bead **2** (ruthenium photocatalyst: lactose = 1: 1 (treatment ratio), 19.8 nmol mg^{-1}: 3.8 nmol mg^{-1} (immobilized concentration)) was suitable for PNA labelling in protein mixture.

2. Experimental section

2-1. General. NMR spectra were recorded on a Bruker biospin AVANCE III (500 MHz for ^{1}H, 125 MHz for ^{13}C) instrument in the indicated solvent. Chemical shifts are reported in units parts per million (ppm) relative to the signal (0.00 ppm) for internal tetramethylsilane for solutions in $CDCl_3$ (7.26 ppm for ^{1}H, 77.0 ppm for ^{13}C) or CD_3OD (3.34 ppm for ^{1}H, 49.00 ppm for ^{13}C). Multiplicities are reported using the following abbreviations: s; singlet, d; doublet, dd; doublet of doublets, t; triplet, q; quartet, m; multiplet, br; broad, *J*; coupling constants in Hertz. IR spectra were recorded on a JASCO FT/IR-4100 spectrometer. IR spectrum was recorded on a JASCO Corporation FT/IR-4100 FT-IR Spectrometer. ATR PRO ONE was attached to the FT/IR-4100 in measuring solid IR spectroscopy by single reflection attenuated total reflection. Only the strongest and/or structurally important peaks were reported as the IR data given in cm^{-1}. The absorption spectra were measured with JASCO V-670. High-resolution mass spectra (HRMS) were recorded on a Bruker ESI-TOF-MS (micrOTOF II). Analytical thin layer chromatography (TLC) was performed on a glass plate of silica gel 60 GF254 (Merck). Silica gel (Fuji Silysia, CHROMATOREX PSQ 60B, 50-200 μm) was used for column chromatography. Reverse phased column chromatography was performed with GL Science InterSep C18. Preparative high-performance liquid chromatography (HPLC) was performed with LC-forte/R (YMC) using a C18 reverse phase column (Kanto, Mightysil RP-18 250 × 20 mm, 5 μm). All chemicals and purified proteins for biological experiments were obtained from commercial sources and used without further purification.

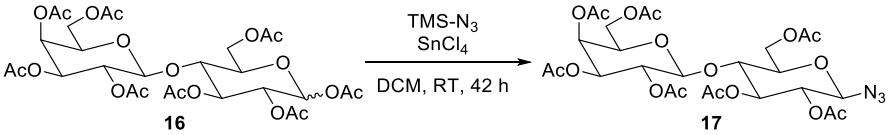
2-2. Synthesis of compounds. 5-azido-*N*-(4-(dimethylamino)phenyl)pentanamide (**6**), *N*-(6-aminohexyl)-4-sulfamoylbenzamide (component of beads **14**), sulfonamide conjugated ruthenium complex (**13**), Bis(2,2'-bipyridine)[4'-methyl-(2,2'-bipyridine)-4-carboxylicacid]ruthenium(II)bis(hexafluorophosphate) $(Ru(bpy)_2(mcbpy)(PF_6)_2$ (**19**)[2], Bis(2,2'-bipyridine)[*N*-(6-aminohexyl)-4'-methyl-(2,2'-bipyridine)-4-carboxamide]ruthenium(II)bis (hexafluorophosphate) (component of beads **1**), Bis(4,4'-dicarboxy-2,2'-bipyridine)[*N*-(6-aminohexyl)-4'-methyl-(2,2'-bipyridine)-4-carboxamide]ruthenium(II) (component of beads **2** and **14**)[3], urazole derivatives (**11**, **12**, **3**) and MAUra-DTB[4], luminol derivatives (**8**, **9**, **10**)[5] were synthesized according to previously reported procedure.



16

2-3. Synthesis of compound 16. Compound **16** was prepared by the similar procedure as that described in literature[6]. To a solution of D-(+)-lactose (1003.1 mg, 2.93 mmol) in 3.50 mL of pyridine was added Ac_2O (4.85 mL, 51.0 mmol) and DMAP (7.2 mg, 0.059 mmol) at room temperature. After stirring at room temperature for 2 h, the reaction mixture was quenched by addition of 10 mL of aqueous HCl solution (1 M). The mixture was extracted with CH_2Cl_2, the organic layer was washed with saturated aqueous NaCl solution, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give **16** as amorphous (1.7540 g, 2.64 mmol, 90% yield).

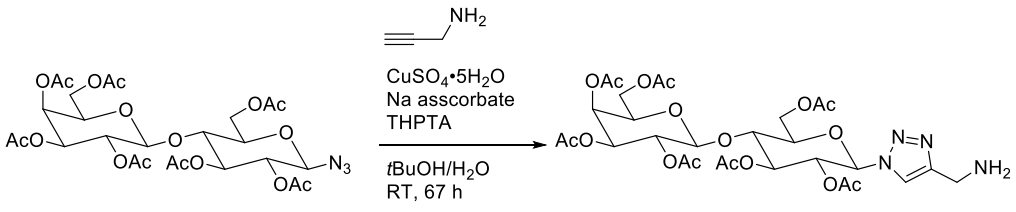
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.25 (d, J = 4.0 Hz, 1H), 5.46 (t, J = 9.2 Hz, 1H), 5.36 (dd, J = 1.0, 3.4 Hz, 1H), 5.15-5.10 (m, 1H), 5.03-4.95 (m, 2H), 4.51-4.43 (m, 2H), 4.18-4.05 (m, 4H), 4.03-3.99 (m, 1H), 3.91-3.87 (m, 1 H), 3.85-3.80 (m, 1H), 2.18 (s, 3H), 2.16 (s, 3H), 2.13 (s, 3H), 2.07-2.06 (m, 9H), 2.01 (s, 3H), 1.97 (s, 3H).



16 17

2-4. Synthesis of compound 17. Compound **17** was prepared by the similar procedure as that described in literature[6]. To a solution of **16** (1007.5 mg, 1.52 mmol) in 3.0 mL of CH_2Cl_2 was added $SnCl_4$ (0.055 mL, 0.47 mmol) and TMS-N_3 (0.275 mL, 2.1 mmol) at room temperature under Ar atmosphere. After stirring at room temperature for 67 h, the reaction mixture was quenched by addition of aqueous KHF_2 solution, extracted with CH_2Cl_2, the organic layer was washed with saturated aqueous NaCl solution, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified with silica gel chromatography with Hex:AcOEt=1:1 to give **17** as amorphous (612.1 mg, 0.925 mmol, 63% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (d, J = 2.8 Hz, 1H), 5.16 (t, J = 9.2 Hz, 1H), 5.08-5.03 (m, 1H), 4.93-4.90 (m, 1H), 4.81 (t, J = 9.0 Hz, 1H), 4.59 (d, J = 8.8 Hz,, 1H) 4.48-4.44 (m, 2H), 4.10-4.01 (m, 4H), 3.86-3.76 (m, 2H), 3.69-3.65 (m, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.03-2.00 (m, 12H), 1.92 (s, 3H)..

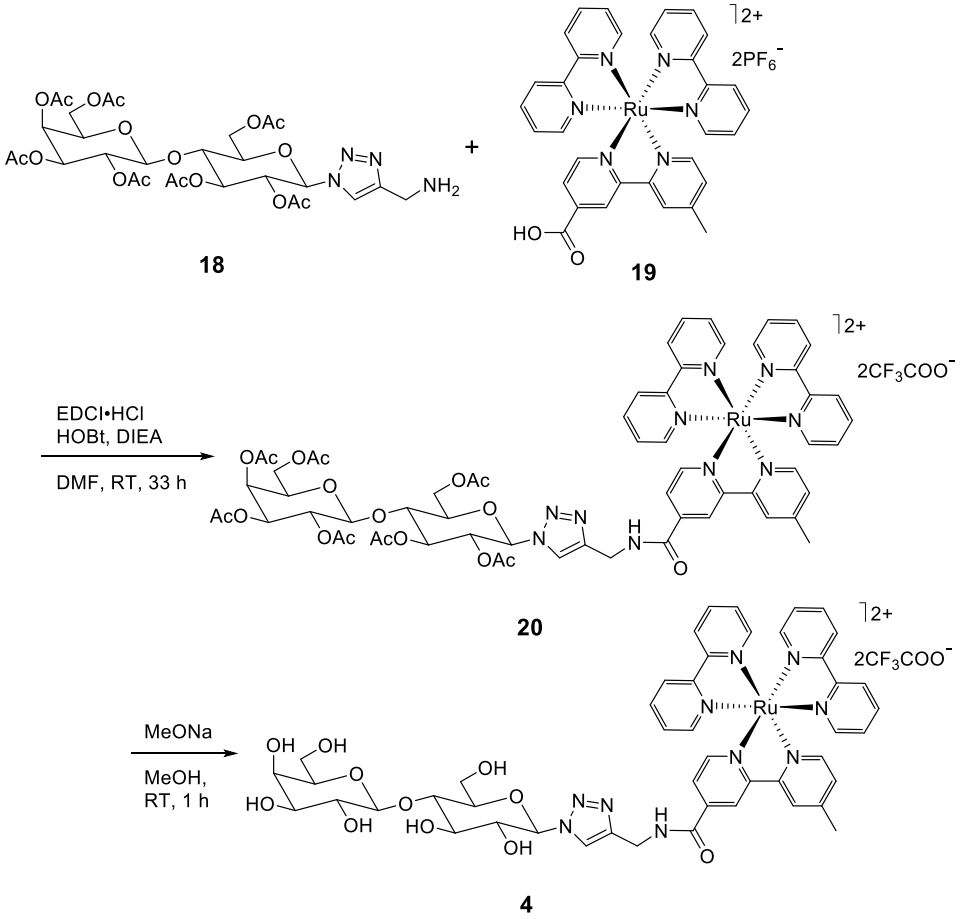


17 18

2-5. Synthesis of compound 18. To a solution of **17** (203.1 mg, 0.307 mmol) and THPTA (26.0 mg, 0.0599 mmol) in 2.5 mL of *t*BuOH was added aqueous $CuSO_4$ solution (65.0 mM, 1.25 mL,

0.0812 mmol) and aqueous sodium ascorbate (78.3 mM, 1.25 mL, 0.0980 mmol) at room temperature under Ar atmosphere. After stirring at room temperature for 67 h, the reaction mixture was extracted with CH_2Cl_2, the organic layer was washed with saturated aqueous NaCl solution, dried over Na_2SO_4, filtered and concentrated in *vacuo*. The residue was purified with silica gel chromatography with CH_2Cl_2:MeOH=10:1 to give **18** as amorphous (48.0 mg, 0.067 mmol, 23% yield) .

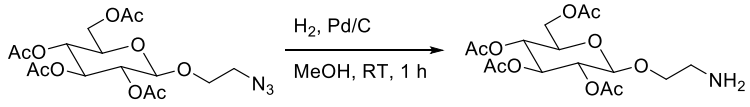
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (s, 1H), 5.83-5.81 (m, 1H), 5.41-5.39 (m, 2H), 5.37 (d, J = 3.0 Hz, 1H), 5.16-5.12 (m, 1H), 4.99-4.97 (m, 1H), 4.53 (d, J = 7.9 Hz, 1H), 4.48 (d, J = 11.0 Hz, 1H), 4.18-4.08 (m, 4H), 3.98-3.89 (m, 4H), 2.17 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 1.98 (s, 3H), 1.88 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 170.3, 170.2, 170.1, 169.6, 169.3, 169.2, 119.3, 101.2, 85.6, 76.0, 75.7, 72.7, 71.0, 70.9, 70.6, 69.1, 66.7, 61.8, 60.9, 20.9, 20.8, 20.7, 20.6, 20.4; FT-IR (neat) 3382, 3142, 2919, 2851, 1738, 1651, 1557, 1433, 1368, 1213, 1041 cm^{-1}; HRMS (ESI, Positive): *m/z* calced. for $C_{29}H_{41}N_4O_{17}$ $[M+H]^+$: 717.2461, found 717.2460.



2-6. Synthesis of lactose conjugated Ru photocatalyst 4. To a solution of **19** (10.0 mg, 10.9 μmol) in 1.0 mL of DMF was added EDCI･HCl (2.3 mg, 12.0 μmol), HOBt･H_2O (1.6 mg, 18.0

μmol), **18** (8.6 mg, 12.0 μmol) and 10 μL of DIEA at room temperature. After stirring for 33 h, the reaction mixture was concentrated *in vacuo*. The residues were purified by reverse-phase column chromatography (10-100% MeCN/H_2O) and HPLC (40-100% MeOH/0.1%TFA aq.) to obtain the semi-pure product **20** as orange oil. **20** was dissolved in 2.0 mL of methanol and 100 μL of NaOMe (28% MeOH solution) and stirred at room temperature for 1 h. The reaction was quenched by addition of 1.0 mL of 4 M HCl solution in dioxane and concentrated *in vacuo*. The residues were purified by reverse-phase column chromatography (0-100% MeCN/H_2O) and HPLC (10-100% MeOH/0.1%TFA aq.) to obtain **4** as red amorphous (1.9 mg, 12%).

^{1}H NMR (500 MHz, CD_3OD) δ 9.08 (s, 1H), 8.71 (d, *J* = 7.0 Hz, 4H), 8.66 (s, 1H), 8.19 (s, 1H), 8.18-8.10 (m, 4H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.85-7.80 (m, 4H), 7.64 (d, *J* = 6.0 Hz, 1H), 7.52-7.47 (m, 4H), 7.38 (d, *J* = 5.0 Hz), 5.64 (d, *J* = 8.5 Hz, 1H), 4.73(s, 2H), 4.41(d, *J* = 7.0 Hz, 1H), 3.97-3.50 (m, 21H), 2.61(s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 158.6, 153.4, 152.7, 151.8, 139.4, 130.2, 129.0, 126.9, 126.2, 125.7, 125.6, 123.7, 123.0, 105.1, 89.3, 79.7, 79.5, 77.2, 76.8, 74.8, 73.7, 72.5, 70.3, 69.0, 62.6, 61.5, 61.3, 60.9, 36.3, 30.8, 21.8; FT-IR (neat) 3353, 3123, 3054, 2941, 2854, 1681, 1611, 1541, 1444, 1372, 1266, 1137 cm^{-1}; HRMS (ESI, positive): *m/z* calced. for $C_{47}H_{50}N_{10}O_{11}Ru$ $[M]^{2+}$: 516.1353, found 516.1356.



21

2-7. Synthesis of compound 21. Compound **21** was prepared by the similar procedure as that described in literature[7]. To a solution of 2-azidoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (45.1 mg, 0.108 mmol) in 5.0 mL of MeOH was added Pd/C (1.0 mg). After stirring under H_2 for 1 h at room temperature, Pd/C was removed by Celite filtration and the filtrate was concentrated under the reduced pressure to give **21** as amorphous (42.1 mg, quant.).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.21 (t, *J* = 9.4 Hz,1H), 5.09 (t, *J* = 9.8 Hz,1H), 5.03-4.99 (m, 1H), 4.54 (d, *J* = 8.0 Hz, 1H), 4.26 (dd, *J* = 4.8, 12.3 Hz, 1H), 4.15 (dd, *J* = 2.4, 12.3 Hz, 1H), 3.91-3.86 (m, 1H), 3.73-3.69 (m, 1H), 3.61-3.55 (m, 1H), 2.94-2.80 (m, 2H), 2.09 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H).

2-8. Preparation of NHS ester-functionalized affinity beads. The 4.0 mg of Dynabeads (Dynabeads® MyOne™ carboxylic acid, 400-800 nmol mg^{-1} carboxylate, Invitrogen)[8] was washed with DMF and dissolved in 800 μL of 200 mM NHS/DMF solution. Then, the mixture was added EDCI･HCl (final concentration ; 200 mM), stirred for 2 h at room temperature and the beads was washed with DMF to give NHS ester functionalized Dynabeads. The NHS ester-functionalized affinity beads applied to section 2-9 (For storing the beads, stored in isopropyl alcohol at -20 °C).

2-9. Preparation of ruthenium photocatalyst- and lactose-functionalized affinity beads. 0.5 mg of NHS ester-functionalized affinity beads (prepared according to section 2-8) was washed three times with 100 μL of DMF. To the beads in DMF was added Bis(4,4'-dicarboxy-2,2'-bipyridine)[*N*-(6-aminohexyl)-4'-methyl-(2,2'-bipyridine)-4-carboxamide]ruthenium(II)[2] (from 10 mM stock solution in DMF, final concentration 0.1 mM), **18** (from 10 mM stock solution in DMF, final concentration 0.1 mM) and Et_3N (from 100 mM stock solution in DMF, final concentration 1 mM) (final concentration of beads: 5.0 mg/mL). After stirring for 1 h at room temperature, the supernatant was collected by magnetic separation. The amount of Ru/dcbpy complex and **18** immobilized on beads was determined by LC-MS analysis of this supernatant (**18**: 3.8 nmol/mg beads, Ru/dcbpy complex: 19.3 nmol/mg beads, see Figure S1). Then, to the beads was added 1 M 4-amino-1-butanol solution in DMF and stirred at room temperature for capping of unreacted NHS ester. The supernatant was removed by using magnetic separation, the beads was washed with water three times. To the beads was added aqueous 0.1 M NaOH solution for the purpose of deprotecting *O*-acetyl group in lactose structure and stirred at room temperature for 1 h. The resulted beads were washed with 10 mM MES buffer (pH 7.4) and applied to section 2-11, 2-12, 2-13 and 2-14 (For storing the beads, stored in aqueous 50% MeOH solution at -20 °C).

2-10. Preparation of crude cellular extract. HeLa cells or A431 cells (2.0×10^7 cell) were washed with phosphate buffered saline (PBS) three times and EDTA solution was added. After incubation for 5 min at 37 °C, the suspension was added to PBS and supernatant were removed after centrifugation (1500 rpm, r.t., 3 min). The cells were washed with PBS three times and 1.0 mL of Lysis buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 v/v% Triton X) was added. After incubation on the ice for 15 min, the sample was vortexed for 10 s and centrifuged (13200 rpm, 4 °C, 7 min) to give supernatant as HeLa or A431 cell lysate. The concentration of cell lysate was determined by protein BCA assay.

2-11. Target protein-selective labelling in HeLa cell lysate. MAUra-N_3 (**3**, from 100 mM solution in DMSO, final concentration 500 μM) and beads **1** or beads **2** (concentration of beads: 5.0 mg/mL) were added to the protein solution (PNA (Wako, 1 μM) containing HeLa cell lysate (1.0 mg/mL proteins) 50 μL or A431 cell lysate (3.0 mg/mL proteins) 50 μL), and the mixture was incubated at 4 °C for 4 h. The light irradiation (RELYON, Twin LED light, 455 nm, 230 mW/cm^2) was performed on ice, 0.5 cm from the light source for 5 min. The reaction mixture was added to 2-iodoacetamide (from 100 mM solution in H_2O, final concentration 2 mM), incubated at 37 °C for 30 min. Then, to the reaction mixture, D-(+)-lactose (from 100 mM solution in H_2O, final concentration 10 mM) was added. After shaking the tube for 1 h at room temperature, the

beads were removed by magnetic separation, low MW molecules was removed by gel filtration (GE Healthcare, Sephadex™ G-25 Medium), the filtrate was added DBCO-Cy3 (Aldrich) (from 10 mM solution in DMF, final concentration 50 μM) or DBCO-Cy5 (Aldrich) (from 10 mM solution in DMF, final concentration 50 μM) and incubated for 1 h at 37 °C (When performing 2D-DIGE analysis, refer to section 2-12 for subsequent operations.). The resulted samples were added 5×SDS-PAGE sample buffer (final concentration: 50 mM Tris–HCl pH 6.8, 125 mM 2-mercaptoethanol, 2% sodium dodecyl sulfate (SDS), 0.025% bromophenol blue, 10% glycerol) and heated 95 °C for 5 min. Proteins were separated by SDS-PAGE using 4-20% acrylamide gels (Biorad). Fluorescence of labelled proteins was detected with a Molecular Imager Fusion Solo S (VILBER LOURMAT). After obtaining of fluorescent image, the same gel was visualized with Coomassie brilliant blue (CBB) stain, and the image was obtained with a Molecular Imager ChemiDoc XRS system (Bio-Rad).

2-12. Two-dimension electrophoresis analysis of fluorescent-labelled lactose-binding protein. Lactose-binding proteins were labelled in A431 cell lysate (3.0 mg/mL proteins) with Cy3 or Cy5 according to protocol of section 2-10. Low MW molecules were removed with ReadyPrep™ 2-D cleanup kit (BioRad). The rehydration of IPG strips (Immobiline DryStrip, pH 3−10, 7 cm, Bio Rad) were performed for 12 h in rehydration buffer (8 M urea, 2% CHAPS, 50 mM DTT, 0.2%(w/v) Bio-Lyte® 3/10 ampholyte, trace of BPB) containing the probe-labelled protein (mixed with Cy3-labelled proteins and Cy5 labelled proteins). Isoelectric focusing was carried out using the Ettan IPGphor 3 (GE Healthcare). The IPG strips were incubated for 15 min in equilibration buffer I (0.375 M Tris-HCl (pH 8.8), 2% SDS, 6 M urea, 20% glycerol and 2% (w/v) DTT) followed by equilibration buffer II (0.375 M Tris-HCl (pH 8.8), 2% SDS, 6 M urea, 20% glycerol and 2.5% (w/v) iodoacetamide) for 15 min. The strips were then transferred to 4-20% polyacrylamide gradient gel and electrophoresis was performed. Fluorescence image was obtained with a Fusion Solo 4S (Vilber Lourmat). The gel was visualized with coomassie brilliant blue (CBB) stain or silver stain and obtained image with a Molecular Imager ChemiDoc XRS (Bio Rad).

2-13. Enrichment of desthiobiotin-labelled proteins. MAUra-DTB (**5**, 100 mM stock solution in DMSO, final concentration 500 μM) and beads **2** (final beads concentration: 5.0 mg/mL) were added to 1 μM PNA solution (50 μL x 15 vials, in MES buffer pH 7.4) or A431 cell lysate (3.0 mg/mL), and the mixture was incubated at 4 °C for 4 h. The light irradiation was operated according to section 2-11, and proteins were precipitated by 2D clean up kit (BioRad). The residue was rehydrated with lysis buffer, Dynabeads™MyOne™Streptavidin C1 (Invitrogen) (0.5 mg) was added and shaken at room temperature for 60 min. The beads were washed with lysis buffer

three times. For the elution of desthiobiotin labelled proteins, beads were added DTB elution buffer (2 mM biotin, 200 mM DTT, 1 x NuPAGE buffer (Thermo)) (50 μL) and incubated at 95 °C for 5 min. Then, the supernatant was collected by magnetic separation and applied to section 2-14 or 2-19.

2-14. Western-blotting analysis of labelled galectin-1 and galectin-3. Desthiobiotin labelled proteins in A431 cell lysate were enriched according to section 2-13. The protein mixture was separated by SDS-PAGE, transferred to polyvinylidene difluoride (PVDF) membrane (GE Healthcare), blocked with Immuno Block (DS Pharma), treated with anti-galectin-1 (rabbit, abcam) or anti-galectin-3 (rabbit, abcam) and anti-rabbit-HRP (Santa Cruz Biotechnology), a blot was treated with ImmunoStar LD (Wako Pure Chemical Industries, Ltd.) and chemical luminescence was detected with a Molecular Imager Fusion Solo S (VILBER LOURMAT).

2-15. In-gel tryptic digestion. Separated proteins by SDS-PAGE were cut out (ca. 1 mm pieces) and gel pieces were transferred into tube. Then, to remove low MW molecules, the gel pieces were incubated with 1 mL of water at 37 °C for 10 min and supernatant was removed (Repeat 3 times). The gel pieces were added Silver Stain KANTO Gel Washing Solution For MS and de-stained gel pieces were washed by water. Gel pieces were dehydrated by addition of MeCN. After removal of the supernatant, the gel pieces were added 100 mM DTT in 100 mM aqueous NH_4HCO_3 solution, the tubes were incubated at 37 °C for 30 min. After removal of the supernatant, the gel pieces were added 250 mM 2-iodoacetamide in 100 mM aqueous NH_4HCO_3 solution for carbamide methylation of cysteine residue, incubated at room temperature for 30 min in the dark. After the carbamide methylation, the gel pieces were washed with 100 mM aqueous NH_4HCO_3 solution and 50% MeCN in 100 mM aqueous NH_4HCO_3 solution and dehydrated by addition of MeCN. The dehydrated gels were added trypsin solution and incubated at 37 °C overnight. Resulted solution was quenched by aqueous TFA solution (final conc. 0.1%) and desalted using C18 pipette tips (Nikkyo Technos Co., Ltd.). Desalted solution was applied to LC-MS analysis.

2-16. LC-MS analysis of labelled peptide fragments. PNA-labelling was operated using **3** (500 μM) and $Ru(bpy)_3Cl_2$ (1 mM for 5 μM PNA monomer) or beads **2** (5.0 mg/mL for 1 μM PNA monomer), and DBCO-Cy3 according to section 2-11. After trypsin digestion, the peptides were detected by LC-MS detecting the fluorescence of Cy3 (ex. 550 nm, em. 570 nm). The micropump gradient method was used, as follows. Mobile phase A: 0.1% FA, mobile phase B: 100% acetonitrile. 0−5 min: 5% B, 5−40 min: 5−60% B, 40−45 min: 60−100% B, 45−51 min: 100% B, 51−53 min: 100−5% B, 53−60 min: 5% B.

2-17. Galectin-3 inhibitory assay. To the 96 well microplate (F-bottom, black, high binding, Greiner) was added ASF solution (20 μg/mL, final: 1 μg/well) and incubated at 4 °C overnight. Then, unoccupied binding sites were blocked by Immunoblock (DS pharma) at room temperature for 1 h. Recombinant galectin-3 conjugated His-tag (abcam) was pre-incubated with lactose or beads at room temperature for 30 min. Concentrations of lactose on beads **2** were normalized by calculation from lactose molecules immobilized on beads **2** (3.8 nmol/mg beads). After washing operation of the plate with TTBS, to the ASF-immobilized plate was added pre-incubated galectin-3 solution (1 μM, 50 μL) and the plate was incubated at 37 °C for 1 h. After washing operation, to the well was added anti-His-tag mAb-Alexa Fluor® 488 (Medical & Biological Laboratories Co., Ltd.) and the inhibitory activity was quantified by detecting fluorescence (ex. 485 nm, em. 535 nm) with plate reader (TECAN, infinite F200).

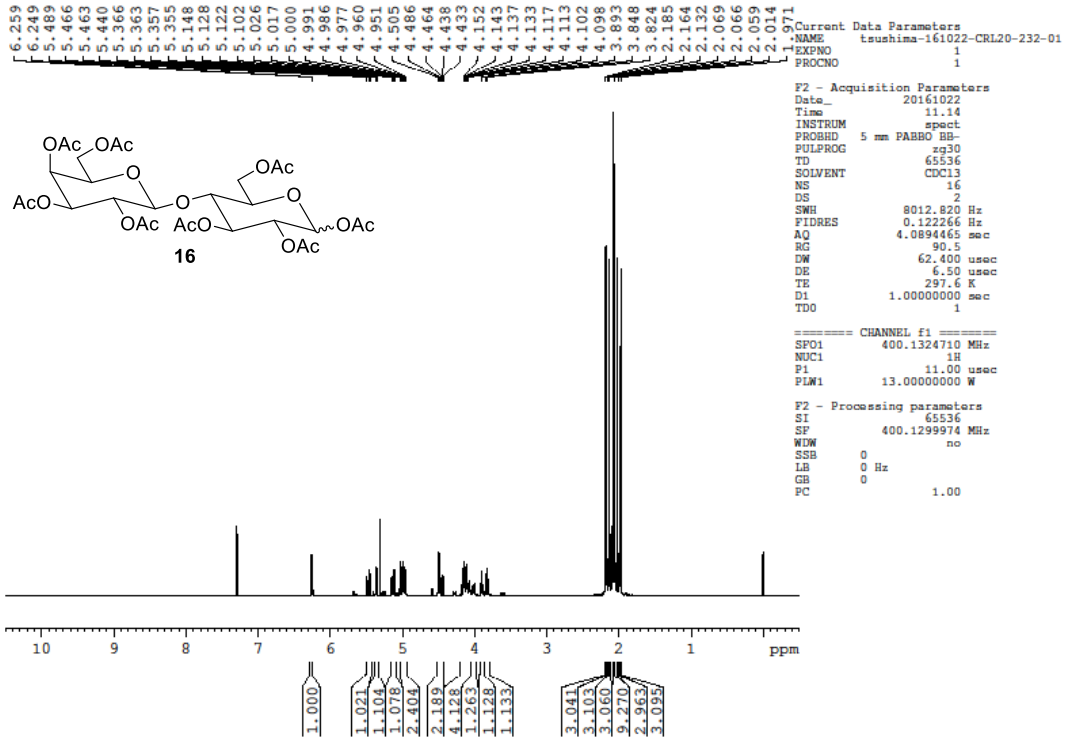
2-18. Affinity purification of lactose-binding proteins using lactose-functionalized affinity beads. Lactose-functionalized beads was prepared according to section 2-9. To the 0.25 mg beads functionalized with lactose was added 50 μL of A431 cell lysate (3.0 mg/mL proteins). After incubation for 4 h at 4 °C, the supernatant was removed by magnetic separation and the beads was washed by lysis buffer three times. Then, to the beads was added 1×SDS-PAGE sample buffer (50 mM Tris–HCl pH 6.8, 125 mM 2-mercaptoethanol, 2% sodium dodecyl sulfate (SDS), 0.025% bromophenol blue, 10% glycerol). The samples were heated at 95 °C for 5 min and the beads was removed by magnetic separation. The proteins were separated by SDS-PAGE with 4-20% acrylamide gradient gels (Bio Rad), transferred to polyvinylidene difluoride (PVDF) membrane (GE Healthcare), blocked with Immuno Block (DS Pharma), treated with anti-galectin-1 (rabbit, abcam) or anti-galectin-3 (rabbit, abcam) and anti-rabbit-HRP (Santa Cruz Biotechnology), a blot was treated with ImmunoStar LD (Wako Pure Chemical Industries, Ltd.) and chemical luminescence was detected with a Molecular Imager Fusion Solo S (VILBER LOURMAT).

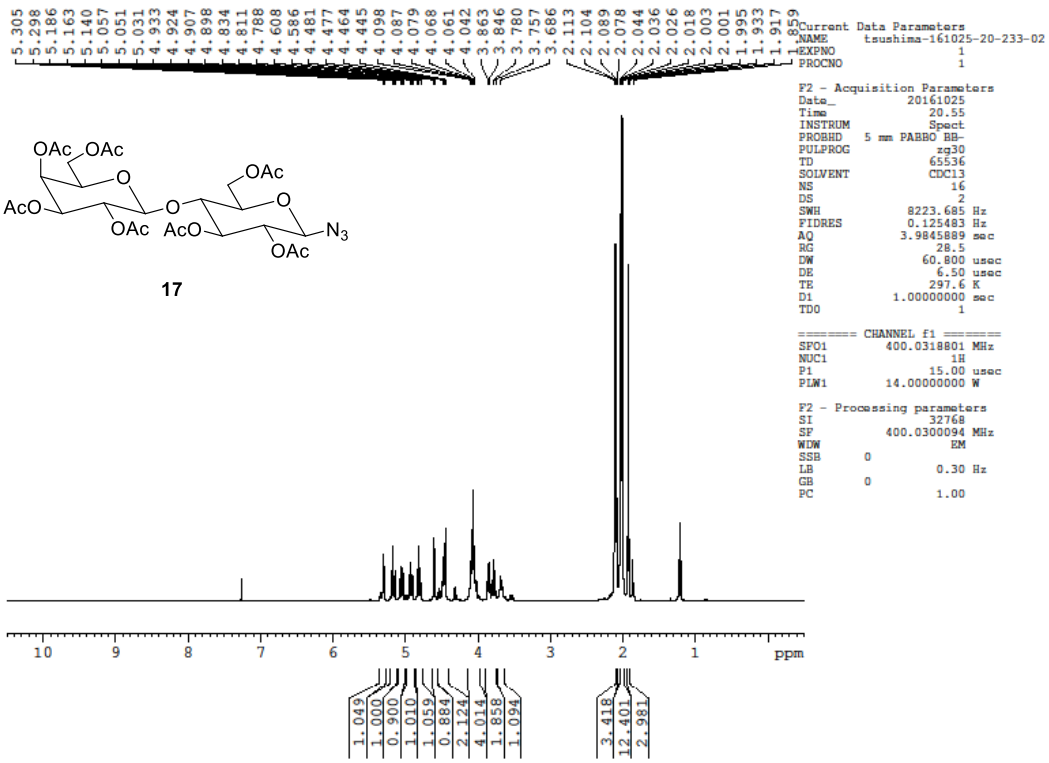
2-19. Preparation of digested peptides for nano LC-MS/MS analysis. Desthiobiotin labelled proteins were enriched according to section 2-13. Low MW molecules were removed with ReadyPrep™ 2-D cleanup kit (BioRad). Resulted proteins (25-50 μg) were denatured by addition of 14.25 μL of 8 M urea solution. To the solution was added DTT (from a 100 mM solution in 100 mM NH_4HCO_3, final 5 mM) and incubated at 37 °C for 30 min. Then, to the solution was added 2-iodoacetamide (from 220 mM solution in 100 mM NH_4HCO_3, final 55 mM) and incubated at 37 °C for 30 min. Resulted solution was diluted by addition of 60 μL of 100 mM

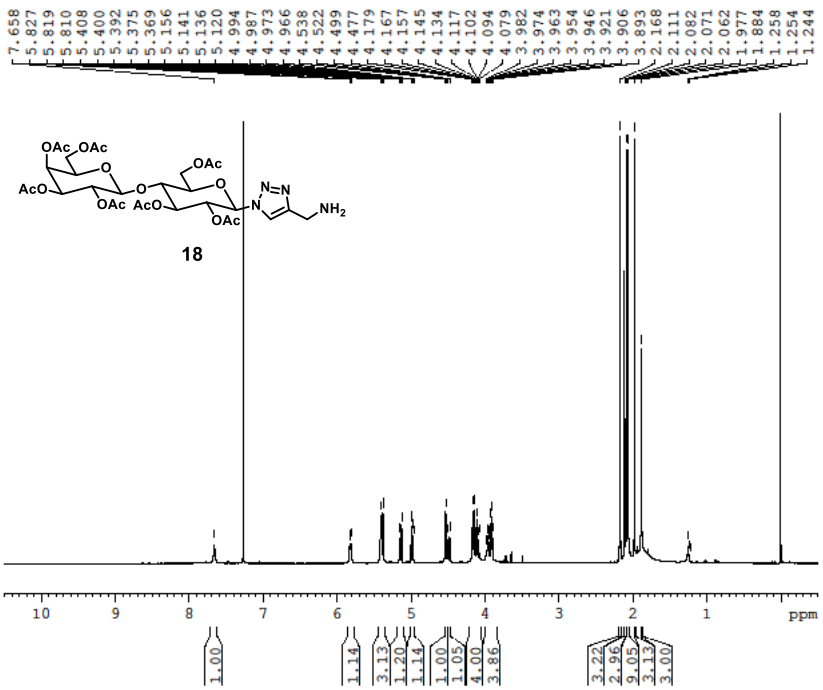
NH_4HCO_3. Trypsin solution (final 10 ng/μL) was added to the solution, and pH was adjusted by addition of 10% TFA solution (pH≒8). After incubation at 37 °C for 1 h, to the solution was added 10 μL of 100 mM NH_4HCO_3 and trypsin solution (final 20 ng/μL). The solution was incubated overnight, and quenched by aqueous TFA solution (final conc. 0.1%) and desalted using C18 pipette tips (Nikkyo Technos Co., Ltd.). Desalted solution was applied to LC-MS/MS analysis (section 2-20).

2-20. Nano LC-MS/MS analysis. LC-MS/MS analysis was performed by LC-nano-ESI-MS composed of a quadrupole-orbitrap hybrid mass spectrometer (Q-Exactive; Thermo Fisher Scientific) equipped with a nanospray ion source and a nano HPLC system (Easy-nLC 1000; Thermo Fisher Scientific). The trap column used for the nano HPLC was a 2 cm × 75 μm capillary column packed with 3 μm C18-silica particles (Thermo Fisher Scientific) and the separation column was a 12.5 cm × 75 μm capillary column packed with 3μm C18-silica particles (Nikkyo Technos Co., Ltd.). The flow rate of the nano HPLC was 300 nL/min. The separation was conducted using a 10−40% linear acetonitrile gradient at 70 min in the presence of 0.1% formic acid. The LC-MS/MS data were acquired in data-dependent acquisition mode controlled by Xcalibur 4.0 (Thermo Fisher Scientific). The settings of data-dependent acquisition were as follows: the resolution was 70,000 for a full MS scan and 17,500 for MS2 scan; the AGC target was 3.0E6 for a full MS scan and 5.0E5 for MS2 scan; the maximum IT was 60 ms for both a full MS scan and MS2 scan; the scan range was 310−1,500 m/z for a full MS scan and 200−2,000 m/z for MS2 scan; and the top 10 signals were selected for MS2 scan per one full MS scan. The MS/MS spectra were searched against the UniProt database of human (taxonomy 9606; downloaded on Feb. 29, 2016) using the Sequest algorithm within the Proteome Discoverer 2.1 (Thermo Fisher Scientific).

3. ^{1}H and ^{13}C NMR spectra of compounds





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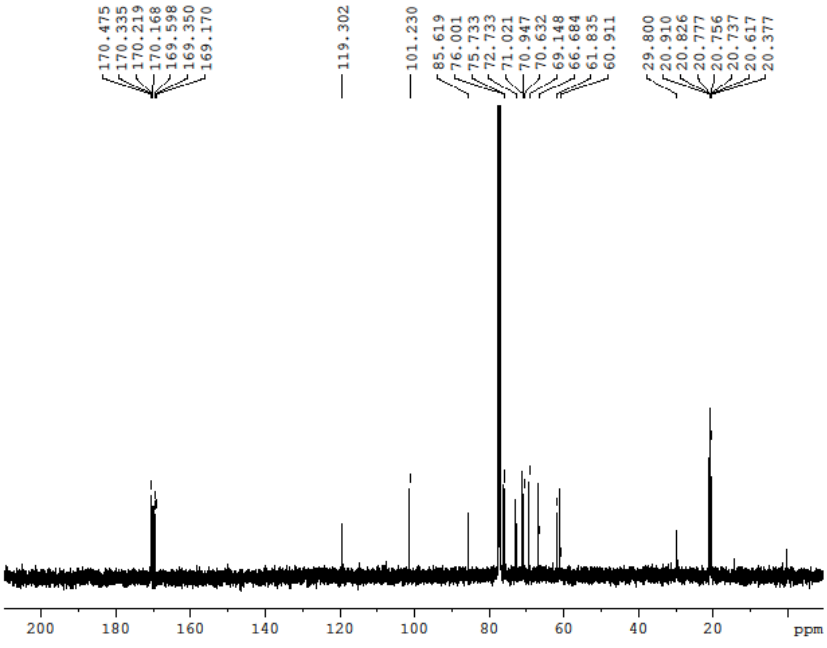


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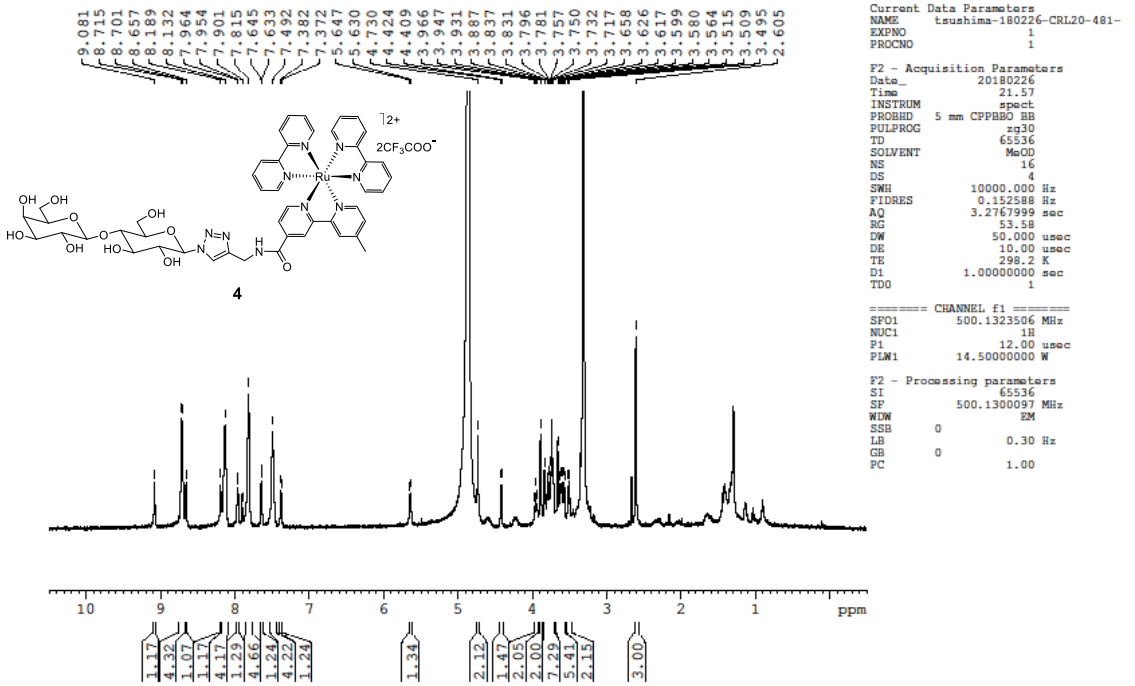

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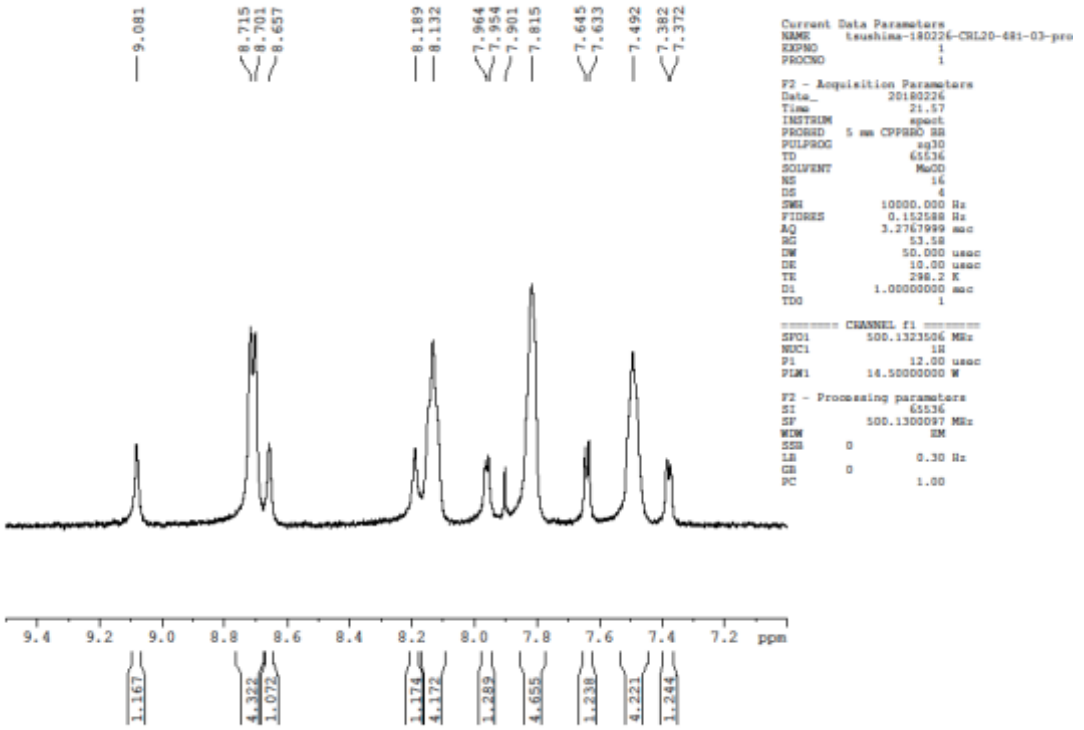

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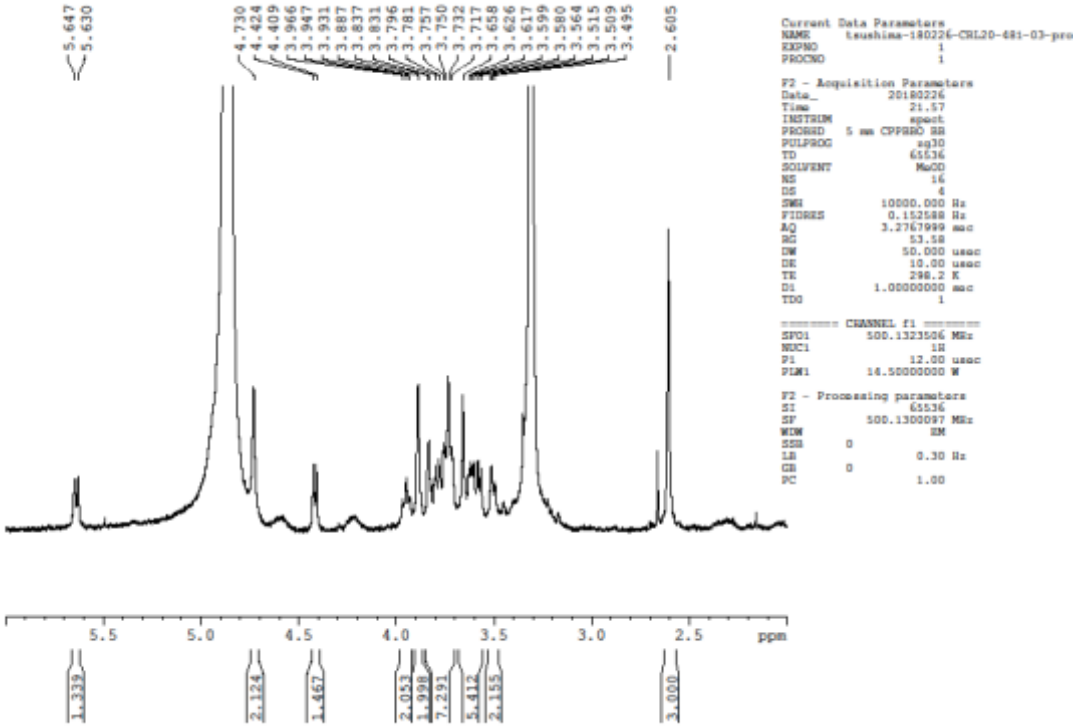

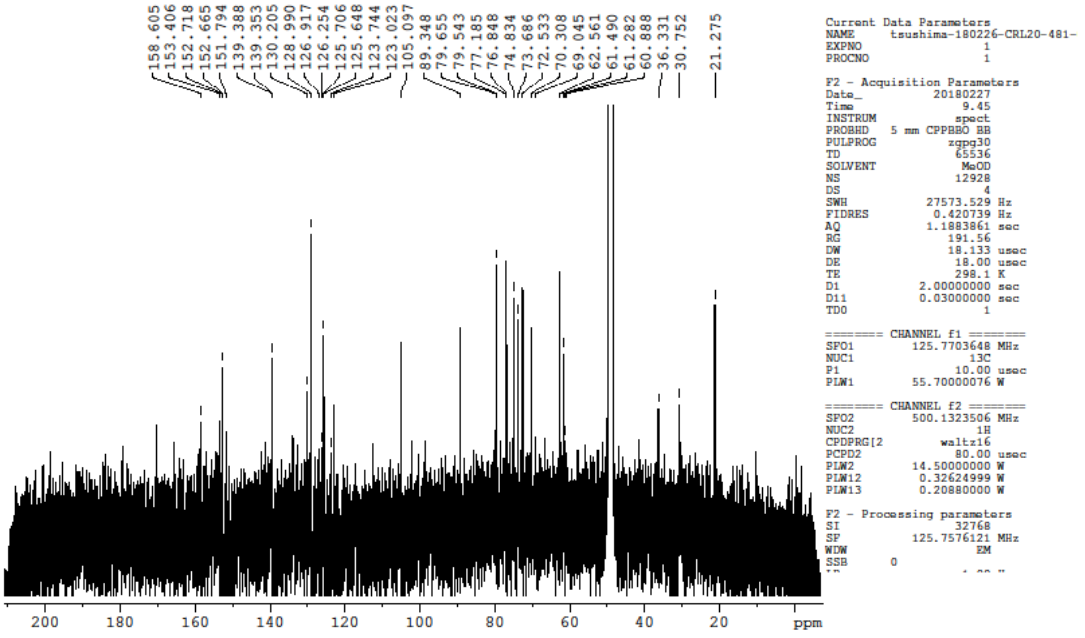

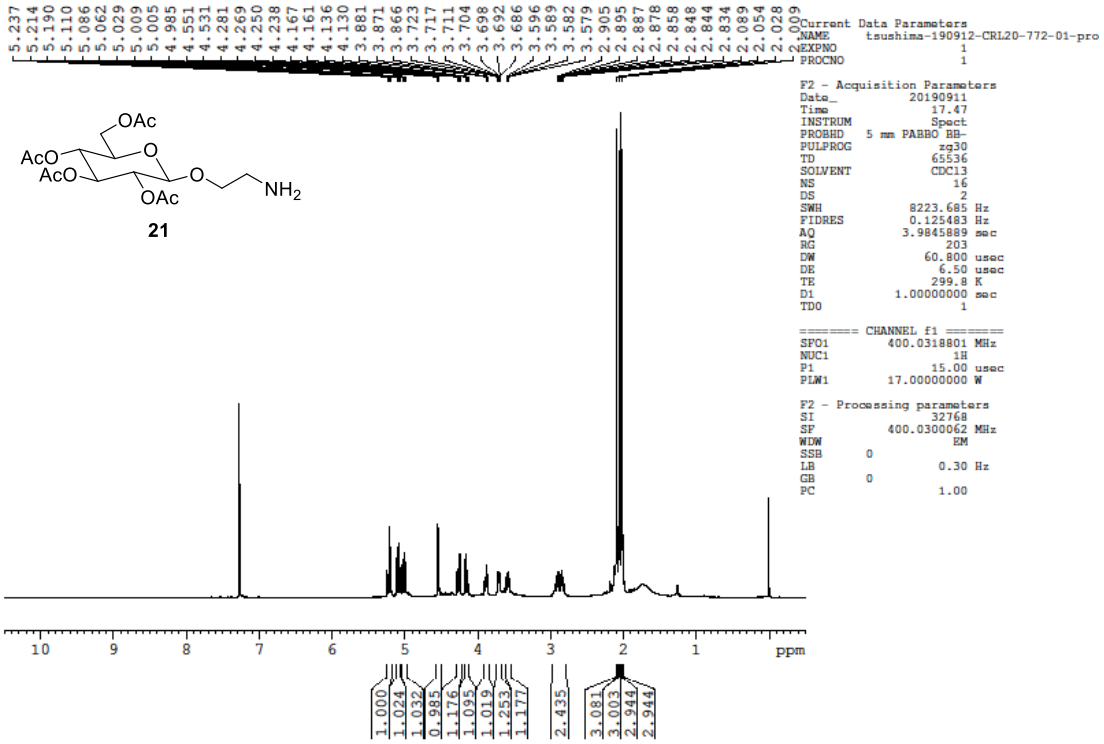
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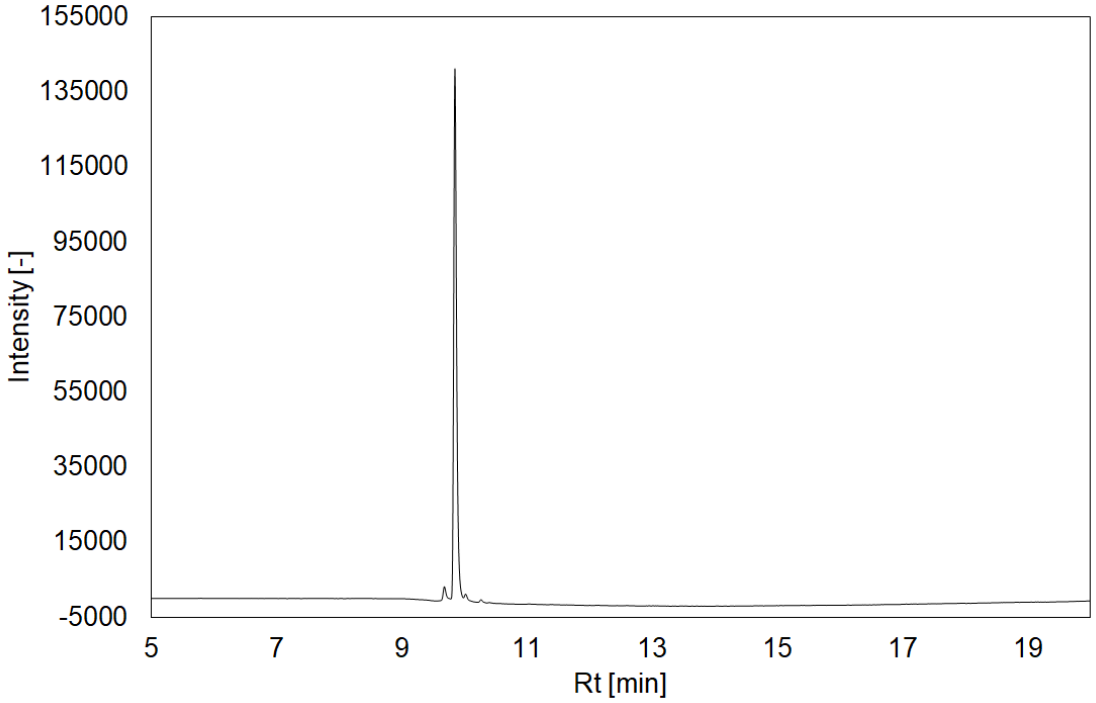
4. HPLC analysis of compound 4



Analysis conditions:

Absorbance 455 nm

Column: InertSustainSwift™ C18 4.6 x 250 mm (GL Science Inc.)

Mobile phase A: 0.1% FA in H2O, mobile phase B: 0.1% FA in acetonitrile. 0–5 min: 5% B, 5–17 min: 5–100% B, 17–22 min: 100% B, 22–23 min: 100–5% B, 23–25 min: 5% B.

5. Reference

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