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Degradation of Target Oligosaccharides by Anthraquinone–Lectin Hybrids with Light Switching

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Abstract: Anthraquinone–lectin hybrids were effectively synthesized using water-soluble anthraquinone derivative **11** with concanavalin A (ConA) and hygrophorus russula lectin (HRL) to give anthraquinone–ConA (**16**) and anthraquinone–HRL (**17**) hybrids, respectively. These anthraquinone–lectin hybrids effectively and selectively degraded oligosaccharides containing a mannose residue as a non-reducing terminal sugar, which has affinity for ConA and HRL, under photo-irradiation with long-wavelength UV light without additives and under neutral conditions. In addition, anthraquinone–HRL (**17**) selectively photo-degraded only Man(α1,6)Man, which has a high affinity for HRL, among several mannosides by recognition of both the type and glycosidic linkage profile of the sugar in an oligosaccharide.

Keywords: anthraquinone · lectin · oligosaccharides · photochemistry · selective-degradation

Introduction

Oligosaccharides play important roles in numerous biological events, including protein folding, cell signaling, fertilization, pathogens binding to host tissues, leukocyte trafficking and the associated inflammatory response, tumor-cell metastasis, and regulation of hormone and enzyme activity.[1] Therefore, the development of innovative methods for selectively controlling specific functions of certain oligosaccharides has attracted much attention in the fields of chemistry, biology, and medicine. In this context, we recently found that certain anthraquinone derivatives could degrade oligosaccharides upon photo-irradiation in the absence of any additives and under neutral conditions.[2,3] In addition, a designed anthraquinone–lectin (peanut agglutinin; PNA) hybrid was found to selectively degrade an oligosaccharide, Gal(β1,3)GalNAc, with high affinity for PNA upon photo-irradiation.[2] Herein, we report the application and improvement of this fundamental study: a water-soluble anthraquinone derivative with high oligosaccharide photo-degrading ability was designed and synthesized to enhance the efficiency of the synthesis of anthraquinone–lectin hybrids. In addition, we found that an anthraquinone–hygrophorus-russula-lectin (HRL) hybrid selectively degraded an oligosaccharide by recognition of both type and glycosidic linkage profile of the sugar in an oligosaccharide.

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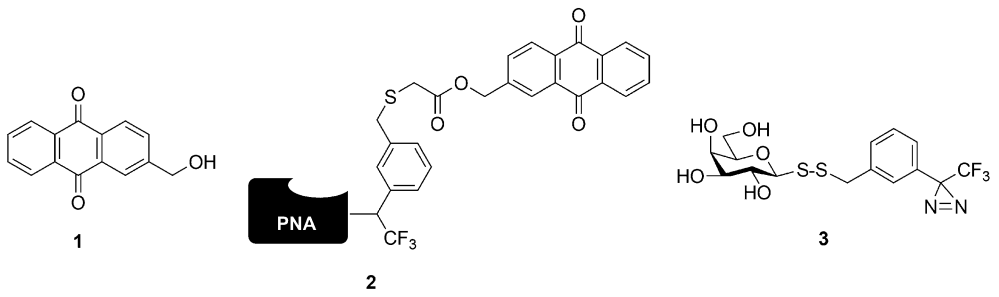
Results and Discussion

Design and Synthesis of Water-Soluble Anthraquinone Derivative with Oligosaccharide Photo-Degrading Activity

In our previous study, we found that anthraquinone derivative **1** could degrade oligosaccharides upon photo-irradiation, in the absence of any additives and under neutral conditions (Scheme 1). Furthermore, a designed and synthesized anthraquinone–lectin (PNA) hybrid (**2**) was found to selectively degrade an oligosaccharide, Gal(β1,3)GalNAc, with high affinity for PNA under photo-irradiation conditions.[2] However, it was also revealed that anthraquinone–lectin hybrid **2** was not effectively synthesized, owing to the low efficiency of the photoreaction[4] using a photo-reactive and ligand-tethered diazirine substrate (**3**) to attach the anthraquinone moiety to the sugar pocket of PNA.[2,5] Therefore, we planned to synthesize anthraquinone–lectin hybrids using an acyl-transfer reaction using a ligand-tethered dimethylaminopyridine (DMAP) substrate reported by Hamachi et al.[6] instead of the photoreaction of diazirine with lectin.

First, we synthesized anthraquinone-tethered acyl donors **4** and **5** and ligand (mannose)-tethered dimethylaminopyridine (DMAP) **6**[6] (for the synthesis, see the Supporting Information), and then carried out an acyl-transfer reaction using these substrates and concanavalin A (ConA). However, the acyl-transfer reaction in 50 mM 4-(2-hydroxyethyl)-1-



Scheme 1. Anthraquinone derivative **1**, anthraquinone–PNA hybrid **2**, and ligand-tethered diazirine **3**.

piperazineethanesulfonic acid (HEPES) buffer (pH 8.0) at 25 °C for 3 hours did not afford the desired anthraquinone–ConA hybrid **7**. At this stage, based on acyl donor **8**, reported by Hamachi and co-workers which contained a water-soluble fluorescein moiety,[6] we believed that the problem was the low water solubility of the anthraquinone-tethered acyl donor **4** or **5**.

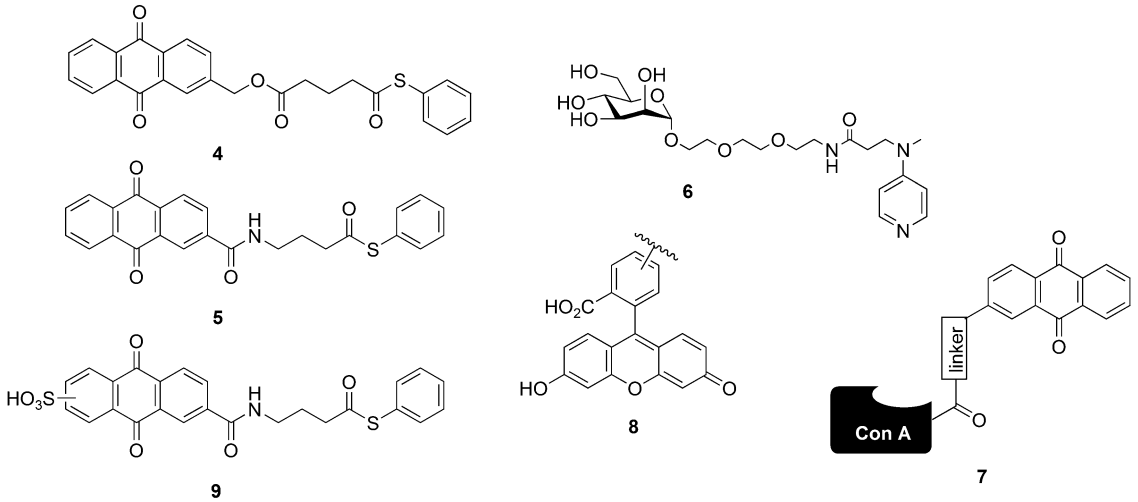
In order to overcome this problem, we designed water-soluble anthraquinone derivative **9**, which possessed a sulfonic acid group (Scheme 2). Thus, a water-soluble anthraquinone-tethered acyl donor **9** was synthesized as summarized in Scheme 3. Commercially available anthraquinone 2-carboxylic acid **10** was exposed to 30 % SO_3/H_2SO_4 with microwave irradiation to give **11** in 90 % yield in a 1:1 mixture of 2,6- and 2,7-disubstituted isomers. Thus obtained **11** was amidated by **12** in the presence of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) and (*N*-ethylmorpholine) NEM to afford **13**, whose *t*Bu ester was then removed using TFA to give **14** in 94 % overall yield. Finally, thioesterification of **14** using PhSH (**15**) in the presence of DMT-MM and NEM furnished the desired anthraquinone-tethered acyl donor **9** with high water solubility in 40 % yield.

At this stage, the oligosaccharide photo-degrading ability of **11** possessing a sulfonic acid group was examined using β-cyclodextrin (CD) as an oligosaccharide.[2,7] As shown in Figure 1, both regioisomers of **11** showed a high photo-degrading ability against β-CD, and no significant difference between the 2,6- and 2,7-isomers of **11** was observed. Therefore, **9** was used as a mixture of isomers for further steps.

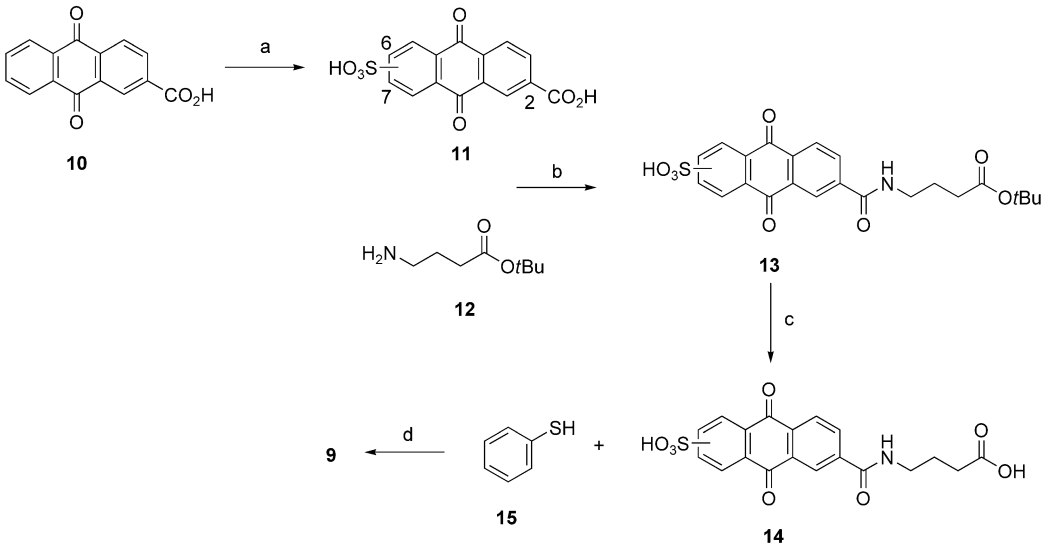
Design and Synthesis of Anthraquinone–ConA and Anthraquinone–HRL Hybrids

Using the water-soluble anthraquinone-tethered acyl donor **9**, we next conducted an acyl-transfer reaction with ConA, and mannose (Man)-tethered DMAP **6** (Scheme 4a). Thus, ConA (20 μM), Man-tethered DMAP **6** (100 μM), and anthraquinone-tethered acyl-donor **9** (100 μM) were mixed in 50 mM HEPES buffer (pH 8.0) at 25 °C for 3 hours to give the desired anthraquinone–ConA hybrid (**16**) in 85 % yield. The formation of hybrid **16** was clearly confirmed by both MALDI-TOF MS and UV spectroscopy (see the Supporting Information), and purification of **16** was successfully performed by affinity chromatography using Sephadex-100 with D-glucose.

In contrast, anthraquinone–HRL hybrid **17** was not obtained by a similar procedure using HRL, Man-tethered DMAP **6**, and anthraquinone-tethered acyl-donor **9**. At this stage, considering the affinity of HRL to several mannosides



Scheme 2. Anthraquinone or fluorescein-tethered acyl donors **4**, **5**, **8**, and **9**, ligand-tethered dimethylaminopyridine (DMAP) **6**, and anthraquinone–concanavalin A (ConA) hybrid **7**.



Scheme 3. Synthesis of anthraquinone-tethered acyl donor **9**. a) 30% SO_3/H_2SO_4, microwave (300 W), 170 °C, 15 min, 90%; b) DMT-MM, NEM, MeOH/H_2O 3:1, RT, 19 h, 94%; c) TFA, CH_2Cl_2, RT, 3 h, 100%; d) DMT-MM, NEM, MeCN, RT, 5 h, 40%. TFA = trifluoroacetic acid.

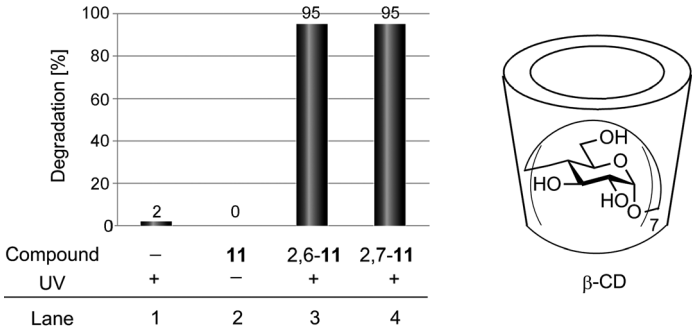
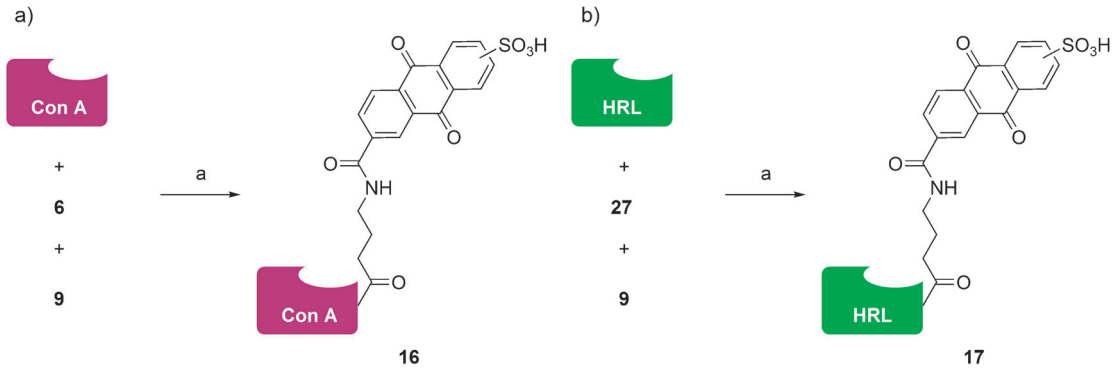


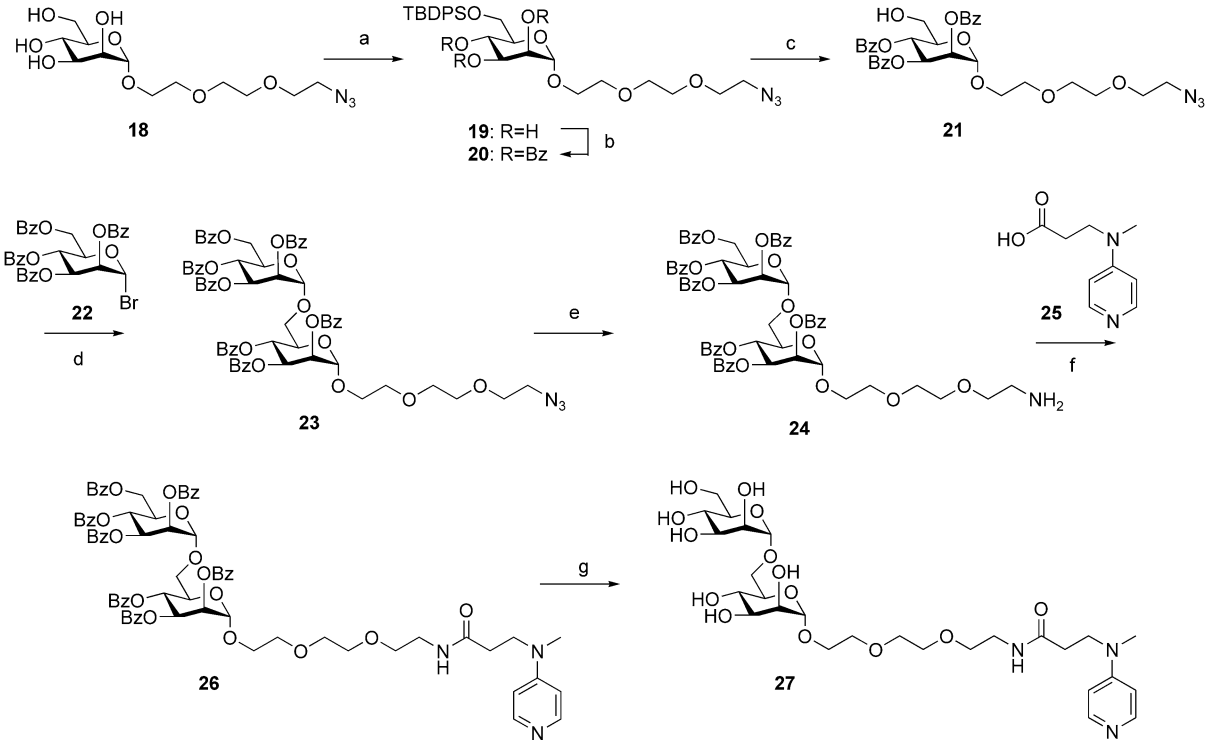
Figure 1. Photo-degradation of β-CD using **11**. β-CD (333 μM) was incubated with 2,6-disubstituted or 2,7-disubstituted **11** (100 μM) in H_2O (30 μL) at 25 °C for 2 h under irradiation with a UV lamp (365 nm, 100 W) placed at 10 cm from the mixture, and was analyzed by HPLC (TSKgel Amide-80, 4.6×250 mm; MeCN/H_2O = 3:2; flow rate = 1.0 mL min^{-1}; 30 °C; IR detection). The percentage degradation was determined based on the peak area of β-CD. Lane 1: β-CD with UV in the absence of **11**; lane 2: β-CD+**11** without UV irradiation; lane 3: β-CD+2,6-disubstituted **11** under UV irradiation; lane 4: β-CD+2,7-disubstituted **11** under UV irradiation.

reported by Kawagishi et al.,[8] we believed that the affinity of Man-tethered DMAP **6** was insufficient for effective recognition of HRL. Therefore, we decided to prepare Man(α1,6)Man-tethered DMAP **27** possessing a Man-(α1,6)Man residue, which exhibits higher affinity for HRL, and to use it in the acyl-transfer reaction instead of Man-tethered DMAP **6**.

The synthesis of Man(α1,6)Man-tethered DMAP **27** is summarized in Scheme 5. The primary alcohol in the known mannoside **18**[9] was protected with a *tert*-butyldiphenylsilyl (TBDPS) group to afford **19**. The remaining free hydroxy groups were protected with benzoyl groups to give **20**, which was subjected to desilylation using HF–Py to yield primary alcohol **21**. Glycosylation of **21** and mannosyl bromide **22** using AgOTf[10] proceeded smoothly to give disaccharide **23**. The azide moiety of **23** was reduced under H_2 in the presence of Pd/C to afford crude amine **24**. Amine **24** was coupled with carboxylic acid **25**[6] using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and diisopropylethylamine (DIEA) to give protected Man(α1,6)Man-



Scheme 4. Synthesis of anthraquinone-lectin hybrids **16** and **17**. a) 50 mM HEPES buffer (pH 8.0), 25 °C, 3 h, 85% for **16** and 35% for **17**.



Scheme 5. Synthesis of Man(α1,6)Man-tethered dimethylaminopyridine **27**. a) TBDPSCl, imidazole, CH_2Cl_2, RT, 18 h, 84%; b) BzCl, Py, 60 °C, 17 h, 99%; c) HF-Py, Py, 0 °C to RT, 7 h, 100%; d) AgOTf, 4 Å M.S., CH_2Cl_2, 0 °C to RT, 4 h, 83%; e) H_2, Pd/C, MeOH/EtOAc 5:1, RT, 3 h; f) EDC, DIEA, DMF, RT to 60 °C, 38 h, 36% (2 steps); g) NaOMe, MeOH, RT, 2 h, 74%. DMF = *N*,*N*-dimethyl formamide.

tethered DMAP **26**. Finally, deprotection of the benzoyl groups in **26** using NaOMe yielded the desired Man(α1,6)Man-tethered DMAP **27**.

Next, an acyl-transfer reaction using HRL, Man(α1,6)Man-tethered DMAP **27**, and anthraquinone-tethered acyl-donor **9** was conducted (Scheme 4b). Thus, HRL (20 μM), Man(α1,6)Man-tethered DMAP **27** (100 μM), and anthraquinone-tethered acyl donor **9** (100 μM) were mixed in 50 mM HEPES buffer (pH 8.0) at 25 °C for 3 hours to give the desired anthraquinone–HRL hybrid **17** in 35% yield. Again, the formation of hybrid **17** was clearly confirmed by both MALDI-TOF MS and UV spectroscopy (see the Supporting Information), and purification of **17** was successfully performed by affinity chromatography using Sephadex-100 with D-glucose. Although the yield of hybrid formation in the synthesis of **17** was lower than that of **16**, both synthetic efficiencies were much higher than that of the previous method using a photoreaction and diazirine substrate **3**.[2]

Target-Selective Photo-Degradation of Oligosaccharides by Anthraquinone–ConA and Anthraquinone–HRL Hybrids

We examined the application of both anthraquinone–ConA (**16**) and anthraquinone–HRL (**17**) in the target-selective photo-degradation of oligosaccharides. Photo-induced degradation of five types of oligosaccharide (60 μM each), Man(α1,6)Man (**28**), Man(α1,2)Man (**29**), Man(α1,3)Man (**30**), Glc(α1,4)Glc (**31**), and Gal(β1,4)Glc (**32**) was first carried out using **16** (18 μM). The progress of the photo-degradation reaction was monitored by HPLC, and the percentage of degradation was calculated based on the peak area corresponding to each oligosaccharide. The results are summarized in Figure 2.

When anthraquinone derivative **11** (without lectin) and **28** were used in the reaction, no degradation was detected owing to the low concentration of **11** and its low affinity for the oligosaccharide (Figure 2a). However, when the anthraquinone–ConA hybrid **16** was exposed to oligosaccharides Man(α1,6)Man (**28**), Man(α1,2)Man (**29**), and Man(α1,3)Man (**30**) under photo-irradiation, significant degradation took place (degradation yields = 65%–69%) (Figure 2b). This result was in sharp contrast to those for the oligosaccharides Glc(α1,4)Glc (**31**) and Gal(β1,4)Glc (**32**), which showed less degradation (degradation yields < 25%) under photo-irradiation with **16**. These results indicate that anthraquinone–ConA hybrid **16** selectively degraded the oligosaccharides containing an α-mannoside residue at the non-reducible end of the oligosaccharides upon photo-irradiation without any additives under neutral conditions. Furthermore, the photo-degrading efficiency for these oligosaccharides (Man(α1,6)Man (**28**), Man(α1,2)Man (**29**), and Man(α1,3)Man (**30**) > Glc(α1,4)Glc (**31**) > Gal(β1,4)Glc (**32**)) by **16** correlated well with the affinity of ConA for these oligosaccharides.[11]

On the other hand, when the anthraquinone–HRL hybrid **17** was exposed to the oligosaccharide Man(α1,6)Man (**28**) under photo-irradiation, significant degradation took place (degradation yield: 20%; Figure 2c). This result was in

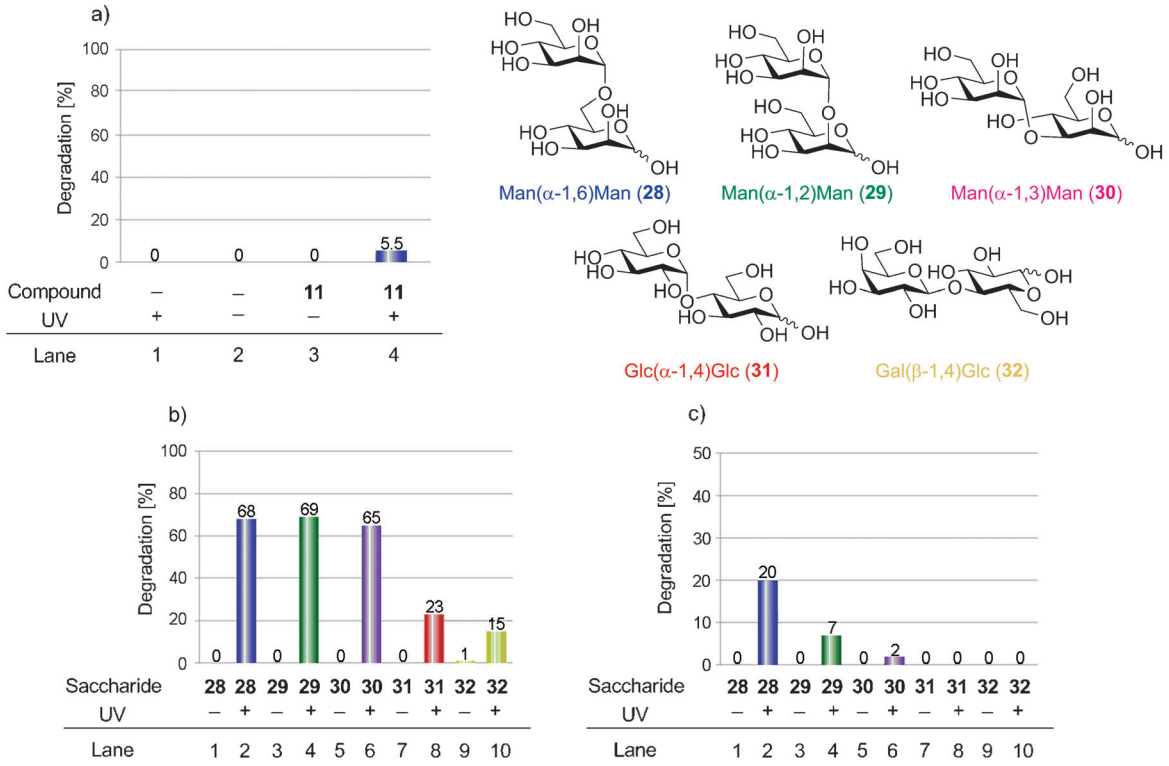


Figure 2. Photo-degradation of oligosaccharides. a) Man(α1,6)Man (**28**) (60 μM) was incubated with and without **11** (18 μM) in phosphate buffer (120 μL, 10 mM, pH 7.4) under the conditions described in Figure 1, and was analyzed by HPLC (Mightysil RP-18 GP 5 mm, 4.6×150 mm; MeOH/H_2O=1:2 (0.1% TFA); flow rate=1.0 mL min^{-1}; 40 °C; UV detection (210 nm)) after total acetylation of photo-degradation products. Percentage degradation was determined based on the peak area of **28**. Lane 1: **28** alone, lane 2: **28** with UV in the absence of **11**, lane 3: **28+11** without UV irradiation, lane 4: **28+11** under UV irradiation. b) Man(α1,6)Man (**28**), Man(α1,2)Man (**29**), Man(α1,3)Man (**30**), Glc(α1,4)Glc (**31**), or Gal(β1,4)Glc (**32**) (60 μM each) was incubated with **16** (18 μM) in phosphate buffer (120 μL, 10 mM, pH 7.4) under the conditions described in Figure 1, and was analyzed by the method indicated in Figure 2a). Percentage degradation was determined based on the peak area of each oligosaccharide. Lane 1: **28+16** without UV irradiation, lane 2: **28+16** with UV irradiation, lane 3, **29+16** without UV irradiation, lane 4: **29+16** with UV irradiation, lane 5: **30+16** without UV irradiation, lane 6: **30+16** with UV irradiation, lane 7: **31+16** without UV irradiation, lane 8: **31+16** with UV irradiation, lane 9: **32+16** without UV irradiation, lane 10: **32+16** with UV irradiation. c) Man(α1,6)Man (**28**), Man(α1,2)Man (**29**), Man(α1,3)Man (**30**), Glc(α1,4)Glc (**31**), or Gal(β1,4)Glc (**32**) (60 μM each) was incubated with **17** (18 μM) in phosphate buffer (120 μL, 10 mM, pH 7.4) under the conditions described in Figure 1, and was analyzed by the method indicated in Figure 2a). Percentage degradation was determined based on the peak area of each oligosaccharide. Lane 1: **28+17** without UV irradiation, lane 2: **28+17** with UV irradiation, lane 3: **29+17** without UV irradiation, lane 4: **29+17** with UV irradiation, lane 5: **30+17** without UV irradiation, lane 6: **30+17** with UV irradiation, lane 7: **31+17** without UV irradiation, lane 8: **31+17** with UV irradiation, lane 9: **32+17** without UV irradiation, lane 10: **32+17** with UV irradiation.

sharp contrast to those for the oligosaccharides Man(α1,2)Man (**29**), Man(α1,3)Man (**30**), Glc(α1,4)Glc (**31**), and Gal(β1,4)Glc (**32**), which showed much-less degradation (degradation yield<7%) under photo-irradiation with **17**. These results indicate that anthraquinone–HRL hybrid **17** selectively degraded the target oligosaccharide, Man(α1,6)Man (**28**), upon photo-irradiation without any additives and under neutral conditions. These results also indicate that anthraquinone–HRL **17** selectively photo-degraded Man(α1,6)Man, which has a high affinity for HRL, among several mannosides by recognition of both type and glycosidic linkage profile of the sugar in an oligosaccharide.

Conclusions

In the present study, we have developed an efficient and general method for the selective degradation of a target oligosaccharide by photo-irradiation using anthraquinone–lectin hybrids under neutral conditions. The target selectivity of the hybrids is dependent on the recognition profile of the lectin attached. The results presented here will contribute to the molecular design of novel and artificial oligosaccharide photo-degradation agents. We hope that this method will provide a means of controlling specific functions of certain oligosaccharides.

Experimental Section

General Methods

Concanavalin A (ConA) and anthraquinone 2-carboxylic acid (**10**) were purchased from Funakoshi Chem. Co. and Sigma–Aldrich Co., respectively, and used without further purification. Melting points were determined on a micro hot-stage (Yanako MP-S3) and were uncorrected. Optical rotations were measured on a JASCO DIP-370 photo-electric polarimeter. NMR spectra were recorded on a JEOL JNM-Lambda 300

(300 MHz for ^{1}H) or a JEOL ECA-500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) spectrometer using trimethylsilane as the internal standard, unless otherwise noted. ESI-TOF Mass spectra were measured on a Waters LCT premier XE. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was conducted using a Bruker Ultraflex mass spectrometer with detection in linear mode. Sinapinic acid was used as the matrix, with positive ionization mode. Silica gel TLC, column chromatography and reverse phase column chromatography were performed using Merck TLC 60F-254 (0.25 mm), Silica Gel 60 N (spherical, neutral) (Kanto Chemical Co., Inc.), and Wakosil 40C18 (Wako), respectively. High performance liquid chromatography (HPLC) was performed on a JASCO apparatus using a TSKgel Amide-80 or a Mightysil RP-18. Air- and/or moisture-sensitive reactions were carried out under an argon atmosphere using oven-dried glassware. In general, organic solvents were purified and dried using appropriate procedures, and evaporation and concentration were carried out under reduced pressure below 30 °C, unless otherwise noted.

*Synthesis of **11***

Anthraquinone 2-carboxylic acid (**10**; 205 mg, 0.814 mmol) was dissolved in 30% SO_3/H_2SO_4 (1.50 mL), and the resulting solution was stirred under microwave irradiation (300 W) for 15 min at 170 °C. After cooling, the reaction mixture was added dropwise to ice-cold water (40 mL), and then NaCl (6.0 g) was added to the resulting mixture. Then, the thus-formed precipitate was filtered, and the resulting solid was washed with 10% NaCl (aq.) and EtOH, and dried in vacuo to give **11** (244 mg, 90% yield, 2,6-disubstituted **11**/2,7-disubstituted **11** = 1/1) as a dark orange solid; TLC ($CHCl_3$/MeOH/AcOH, 3:1:0.1 v/v/v): $R_f = 0.13$; m.p. > 300 °C; HRMS (ESI-TOF) (*m*/*z*): $[M]^-$ calcd. for $C_{15}H_7O_7S$, 330.9912; found, 330.9910; 2,6-disubstituted **11**: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 8.69$ (1H, d, $J = 6.4$ Hz), 8.43 (1H, m), 8.41 (1H, dd, $J = 1.8$, 8.0 Hz), 8.34 (1H, d, $J = 8.0$ Hz), 8.24 (1H, d, $J = 8.1$ Hz), 8.13 ppm (1H, d, $J = 8.1$ Hz); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 182.50$, 182.13, 166.52, 154.23, 136.36, 136.22, 134.91, 133.93, 133.55, 133.46, 131.92, 127.99, 127.94, 127.73, 124.32 ppm. 2,7-disubstituted **11**: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 8.70$ (1H, d, $J = 1.5$ Hz), 8.42 (1H, d, $J = 2.0$ Hz), 8.41 (1H, m), 8.33 (1H, d, $J = 8.1$ Hz), 8.22 (1H, d, $J = 8.0$ Hz), 8.11 ppm (1H, dd, $J = 1.5$, 8.0 Hz); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 182.35$, 182.29, 166.52, 154.34, 136.36, 136.15, 134.95, 133.98, 133.53, 133.45, 131.89, 127.96, 127.71, 127.31, 124.31 ppm.

*Synthesis of **13***

To a solution of **11** (177 mg, 0.535 mmol) in MeOH (3.30 mL) and H_2O (1.10 mL) were added DMT-MM (231 mg, 0.834 mmol) and NEM (47.6 μL, 0.433 mmol) at room temperature. The reaction mixture was stirred for 15 min, and then **12** (53.6 mg, 0.337 mmol) was added to the mixture. After being stirred for 19 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was subjected to silica gel column chromatography (3:1:1:0.1 $CHCl_3$/acetone/MeOH/AcOH) to give **13** (150 mg, 94% yield) as a light-yellow solid; TLC ($CHCl_3$/MeOH/AcOH, 3:1:0.1 v/v/v): $R_f = 0.39$; m.p. > 300 °C; ^{1}H NMR (500 MHz, CD_3OD): $\delta = 8.91$ (1H, m), 8.69 (2H, m), 8.35 (m, 2H), 8.26 (m, 2H), 3.46 (2H, dt, $J = 6.6$, 12.5 Hz), 2.34 (2H, t, $J = 7.5$ Hz), 1.91 (2H, quint, $J = 7.2$ Hz), 1.44 ppm (9H, s); HRMS (ESI-TOF) (*m*/*z*): $[M]^-$ calcd. for $C_{23}H_{22}NO_8S$, 472.1048; found, 472.1066.

*Synthesis of **14***

To a solution of **13** (140 mg, 0.296 mmol) in CH_2Cl_2 (4.20 mL) was added TFA (4.20 mL) at room temperature. After being stirred for 3 h at the same temperature, the reaction mixture was concentrated in vacuo, and the residue was subjected to reverse-phase column chromatography on silica gel (H_2O/MeOH, 1:0 to 4:1) to give **14** (123 mg, quant.) as a pale-yellow solid; TLC ($CHCl_3$/MeOH/AcOH, 3:1:0.1 v/v/v): $R_f = 0.22$; m.p. > 300 °C; ^{1}H NMR (500 MHz, D_2O): $\delta = 8.14$ (1H, d, $J = 5.2$ Hz), 7.99–7.78 (5H, m), 3.30 (2H, dt, $J = 1.9$, 5.6 Hz), 2.34 (2H, t, $J = 7.2$ Hz), 1.82 ppm (2H, quint, $J = 6.4$ Hz); HRMS (ESI-TOF) (*m*/*z*): $[M]^-$ calcd. for $C_{19}H_{14}NO_8S$, 416.0440; found, 416.0441.

*Synthesis of **9***

To a solution of **14** (26.2 mg, 62.8 μmol) in MeCN (1.60 mL) were added DMT-MM (43.4 mg, 157 μmol) and NEM (13.8 μL, 126 μmol), and the reaction mixture was stirred for 15 min at room temperature. And then, thiophenol (**15**) (27.7 μL, 0.251 mmol) was added dropwise to the mixture at 0 °C. After being stirred for 5 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was subjected to column chromatography on silica gel (39:1:1 to 32:8:1 $CHCl_3$/MeOH/HCO_2H) to give **9** (12.9 mg, 40% yield) as a pale-yellow oil; TLC ($CHCl_3$:MeOH:AcOH, 3:1:0.1 v/v/v): $R_f = 0.50$; ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 9.03$ (1H, m), 8.69 (1H, m), 8.43–8.21 (4H, m), 8.12–8.09 (1H, m), 7.44 (5H, m), 3.38–3.34 (2H, m), 2.83 (2H, t, $J = 7.4$ Hz), 1.92 ppm (2H, quint, $J = 6.8$ Hz); HRMS (ESI-TOF) (*m*/*z*): $[M]^-$ calcd. for $C_{25}H_{18}NO_7S_2$, 508.0525; found, 508.0548.

*Synthesis of **19***

To a solution of **18** (91.3 mg, 0.271 mmol) in CH_2Cl_2 (4.50 mL) were added imidazole (55.3 mg, 0.812 mmol) and TBDPSCl (106 μL, 0.408 mmol) at 0 °C. After the reaction mixture was stirred for 18 h at room temperature, H_2O (5.0 mL) was added to the reaction mixture. The resulting solution was extracted with two portions of $CHCl_3$. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (toluene/acetone, 9:1 to 3:2) to give **19** (132 mg, 84% yield) as a colorless oil; TLC (toluene/EtOAc, 3:2 v/v): $R_f = 0.47$; $[\alpha]_D^{25} = -12.8°$ ($c = 0.48$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.69$–7.67 (4H, m), 7.46–7.38 (6H, m), 4.86 (1H, br-d), 3.95–3.80 (6H, m), 3.69–3.57 (10H, m), 3.37 (2H, t, $J = 4.9$ Hz), 2.97 (1H, m), 2.68 (1H, d, $J = 3.7$ Hz), 2.37 (1H, d, $J = 4.6$ Hz), 1.07 ppm (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 135.66$, 132.98, 132.91, 129.96, 127.87, 99.60, 71.67, 70.99, 70.76, 70.38, 70.29, 70.12, 66.58, 65.15, 50.72, 26.89, 19.26 ppm; HRMS (ESI-TOF) (*m*/*z*): $[M+Na]^+$ calcd. for $C_{28}H_{41}N_3O_8NaSi$, 598.2561; found, 598.2583.

*Synthesis of **20***

To a solution of **19** (19.7 mg, 34.2 μmol) in pyridine (1.00 mL) was added BzCl (23.9 μL, 0.206 mmol) at 0 °C. After the reaction mixture was stirred for 17 h at 60 °C, 1 M HCl (aq.) was added to the reaction mixture at 0 °C to adjust the pH between 4 and 5. The resulting solution was extracted with two portions of $CHCl_3$. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (9:1 to 3:2 *n*-hexane/EtOAc) to give **20** (30.1 mg, 99% yield) as a colorless oil; TLC (*n*-hexane/EtOAc, 3:2 v/v): $R_f = 0.47$; $[\alpha]_D^{25} = -15.4°$ ($c = 0.50$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.13$–7.26 (25H, m), 6.16 (1H, t, $J = 10.1$ Hz), 5.85 (1H, dd, $J = 1.7$, 10.3 Hz), 5.72 (1H, m), 5.20 (1H, d, $J = 1.7$ Hz), 4.20–4.17 (1H, m), 3.94–3.87 (2H, m), 3.79–3.69 (10H, m), 3.37 (2H, t, $J = 5.4$ Hz), 1.05 ppm (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 171.95$, 165.69, 165.37, 135.81, 135.63, 133.87, 133.48, 133.25, 133.16, 133.04, 130.30, 130.09, 128.59, 128.46, 128.36, 127.69, 97.85, 71.46, 71.02, 70.82, 70.33, 70.21, 67.36, 66.72, 62.56, 50.78, 26.72, 19.30 ppm; HRMS (ESI-TOF) (*m*/*z*): $[M+Na]^+$ calcd. for $C_{49}H_{53}N_3O_{11}NaSi$, 910.3347; found, 910.3308.

*Synthesis of **21***

To a solution of **20** (139 mg, 0.157 mmol) in pyridine (4.20 mL) was added HF-Py (0.400 mL) at 0 °C. After the reaction mixture was stirred at room temperature for 7 h, saturated aq. $NaHCO_3$ (20 mL) was added to the reaction mixture to quench the reaction. The resulting mixture was extracted with two portions of $CHCl_3$. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (toluene/EtOAc = 1:1) to give **21** (101 mg, quant.) as a colorless oil; TLC (*n*-hexane/EtOAc, 1:4 v/v): $R_f = 0.54$; $[\alpha]_D^{28} = -109.9°$ ($c = 0.50$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.10$ (2H, m), 7.97 (2H, m), 7.81 (2H, m), 7.63–7.37 (7H, m), 7.27–7.23 (2H, m), 5.99 (1H, dd, $J = 3.4$, 10.2 Hz), 5.82 (1H, t, $J = 10.2$ Hz), 5.70 (1H, dd, $J = 1.7$, 3.4 Hz), 5.16 (1H, d, $J = 1.7$ Hz), 4.16 (1H, ddd, $J = 2.3$, $J = 3.9$, $J = 9.8$ Hz), 3.94 (1H, ddd, $J = 3.9$,

$J=5.1$, $J=10.8$ Hz), 3.85–3.68 (11H, m), 3.37 (2H, t, $J=5.1$ Hz), 2.67 ppm (1H, dd, $J=6.0$, $J=8.1$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=$ 166.58, 165.59, 165.55, 133.72, 133.61, 133.24, 130.00, 129.97, 129.77, 129.40, 129.24, 128.88, 128.71, 128.58, 128.36, 97.97, 71.06, 70.90, 70.84, 70.71, 70.36, 70.18, 69.73, 67.69, 67.52, 61.54, 50.76 ppm; HRMS (ESI-TOF) (m/z): $[M+Na]^+$ calcd. for $C_{33}H_{35}N_3O_{11}Na$, 672.2169; found, 672.2167.

Synthesis of ***23***

A suspension of **21** (73.1 mg, 0.113 mmol), **22** (222 mg, 0.338 mmol) and 4 Å M.S. (36.5 mg, 50 wt% to **21**) in CH_2Cl_2 (11.0 mL) was stirred for 30 min at 0°C, and then AgOTf (105 mg, 0.409 mmol) was added to the suspension. After the suspension was stirred at room temperature for 4 h, triethylamine (1.0 mL) was added to the reaction mixture to quench the reaction, and then saturated aq. $NaHCO_3$ was added to the resulting solution. The resulting mixture was extracted with two portions of $CHCl_3$. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (*n*-hexane/EtOAc = 4:1 to 3:2) to give disaccharide **23** (115 mg, 83% yield) as a colorless oil; TLC (*n*-hexane/EtOAc, 7:5 v/v): $R_f=0.48$; $[\alpha]_D^{25}=-20.3°$ ($c=0.46$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=$8.18–7.28 (35H, m), 6.15–5.94 (4H, m), 5.79 (2H, m), 5.19 (1H, br-d), 5.16 (1H, br-d), 4.52–4.12 (6H, m), 3.85–3.69 (10H, m), 3.38 ppm (2H, t, $J=4.9$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=$166.12, 165.74, 165.63, 165.57, 165.32, 133.60, 133.52, 133.22, 133.09, 130.12, 129.91, 129.79, 129.39, 129.24, 129.13, 129.05, 128.87, 128.65, 128.52, 128.38, 98.04, 97.79, 70.97, 70.83, 70.62, 70.36, 70.20, 69.50, 69.00, 67.67, 67.14, 66.74, 66.70, 50.78 ppm; HRMS (ESI-TOF) (m/z): $[M+Na]^+$ calcd. for $C_{67}H_{61}N_3O_{20}Na$, 1250.3746; found, 1250.3691.

Synthesis of ***26***

A suspension of **23** (57.4 mg, 46.7 μmol), 10% Pd/C (5.00 mg), and EtOAc (1.10 mL) in MeOH (5.70 mL) was stirred under H_2 atmosphere (balloon). After the suspension was stirred for 3 h at room temperature, it was filtered through a pad of celite. The filtrate was concentrated in vacuo to afford crude **24**. The residue was used in the next step without further purification. To a solution of the crude **24** and **25** (33.7 mg, 0.187 mmol) in DMF (5.60 mL) were added DIEA (24.0 mL, 0.140 mmol) and EDC (13.4 mg, 70.1 μmol) at 0°C. After the reaction mixture was stirred for 38 h at 60°C, H_2O (5.0 mL) was added to the reaction mixture. The resulting mixture was extracted with three portions of $CHCl_3$. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel ($CHCl_3$/MeOH, 4:1) to give **26** (22.9 mg, 36% yield) as a colorless oil; TLC ($CHCl_3$/MeOH, 4:1 v/v): $R_f=0.50$; $[\alpha]_D^{25}=-21.5°$ (c 0.51, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=$8.16–7.16 (37H, m), 6.50 (3H, m), 6.11–5.91 (4H, m), 5.78–5.75 (2H, m), 5.19 (1H, br d), 5.15 (1H, br d), 4.54–4.04 (6H, m), 3.83–3.63 (10H, m), 3.54 (2H, t, $J=4.9$ Hz), 3.46 (2H, t, $J=5.1$ Hz), 2.94 (3H, s), 2.44 ppm (2H, t, $J=6.8$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=$170.77, 166.14, 165.85, 165.78, 165.63, 165.52, 165.37, 153.44, 148.96, 133.68, 133.58, 133.69, 133.26, 133.14, 130.08, 129.90, 129.78, 128.91, 128.67, 128.54, 128.44, 106.69, 97.95, 97.73, 70.72, 70.56, 70.41, 70.19, 69.79, 69.59, 69.02, 62.59, 47.89, 39.49, 37.68, 33.72 ppm; HRMS (ESI-TOF) (m/z): $[M+H]^+$ calcd. for $C_{76}H_{74}N_3O_{21}$, 1364.4815; found, 1364.4786.

Synthesis of ***27***

To a solution of **26** (18.8 mg, 13.8 μmol) in MeOH (1.90 mL) was added 0.2 M NaOMe (96.6 μL, 19.3 μmol). After being stirred for 2 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was subjected to reverse-phase column chromatography on silica gel (H_2O/MeOH, 1:0 to 1:1) to give **27** (6.50 mg, 74% yield) as a colorless oil. Reverse-phase TLC (H_2O/MeOH, 1:1 v/v): $R_f=0.30$; $[\alpha]_D^{25}=-18.6°$ (c 0.38, MeOH); ^{1}H NMR (300 MHz, CD_3OD): $\delta=$8.06 (2H, d, $J=$ 6.6 Hz), 6.69 (2H, dd, $J=1.4$, 3.5 Hz), 4.81 (1H, d, $J=1.7$ Hz), 4.77 (1H, d, $J=1.5$ Hz), 3.84–3.57 (22H, m), 3.47 (2H, t, $J=4.9$ Hz), 3.34–3.29 (2H, m), 3.02 (3H, s), 2.49 ppm (2H, t, $J=6.8$ Hz); ^{13}C NMR (125 MHz, CD_3OD): $\delta=$172.56, 154.03, 148.08, 106.74, 100.49, 99.98, 73.07, 71.75, 71.40, 71.31, 70.79, 70.70, 70.23, 70.04, 69.93, 69.16, 67.28, 67.24, 66.34, 66.11, 61.57, 48.52, 39.18, 36.51, 33.25 ppm; HRMS (ESI-TOF) (m/z): $[M+H]^+$ calcd. for $C_{27}H_{46}N_3O_{14}$, 636.2980; found, 636.2968.

Preparation of Anthraquinone–ConA Hybrid ***16***

To a solution of 100 μM ConA in 50 mM HEPES buffer (pH 8.0, 5.90 mL) was slowly added 250 μM Man-tethered DMAP **6** in 50 mM HEPES buffer (pH 8.0, 11.8 mL), followed by addition of 250 μM acyl donor **9** in the same buffer (11.8 mL; final concentrations of ConA, **6** and **9** are 20 μM, 100 μM and 100 μM, respectively). After incubation for 3 h at 25°C in the dark, the reaction mixture was dialyzed against H_2O, and the resulting solution was lyophilized. The residue was subsequently purified by affinity chromatography (Sephadex G100, 2.6 cm × 12 cm), eluted with a linear gradient of 10 mM acetate buffer (pH 5.0) containing D-glucose (from 0 mM to 40 mM), followed by dialysis against H_2O, and the resulting solution was lyophilized to afford anthraquinone–ConA hybrid **16** in 85% yield.

Preparation of Anthraquinone–HRL Hybrid ***17***

To a solution of 100 μM HRL in 50 mM HEPES buffer (pH 8.0, 5.90 mL) was slowly added 250 μM Man(α1–6)Man-tethered DMAP **27** in 50 mM HEPES buffer (pH 8.0, 11.8 mL), followed by addition of 250 μM acyl donor **9** in the same buffer (11.8 mL; final concentrations of HRL, **27**, and **9** are 20 μM, 100 μM, and 100 μM, respectively). After incubation for 3 h at 25°C in the dark, the reaction mixture was dialyzed against H_2O, and the resulting solution was lyophilized. The residue was subsequently purified by affinity chromatography (Sephadex G100, 2.6 cm × 12 cm), eluted with a linear gradient of 10 mM phosphate buffer (pH 8.0) containing D-mannose (from 0 mM to 300 mM), followed by dialysis against H_2O, and the resulting solution was lyophilized to afford anthraquinone–HRL hybrid **17** in 35% yield.

Photo-Degradation of Oligosaccharides

Each oligosaccharide (60 μM) was incubated with the anthraquinone–lectin hybrid (18 μM) in phosphate buffer (120 μL, 10 mM, pH 7.4) at 25°C for 2 h under irradiation with a UV lamp (365 nm, 100 W, Blak-ray (B-100A), UVP, Inc.) placed at 10 cm from the mixture. After photo-irradiation, the mixture was dialyzed with a 30 kDa molecular cut off Amicon Ultradialysis column. The resulting solution was lyophilized, and the residue was acetylated using Ac_2O in pyridine at room temperature. After incubation for 22 h, the reaction mixture was concentrated, and then the residue was subjected to column chromatography on silica gel (7:3 to 0:1 *n*-hexane/EtOAc) and analyzed by HPLC (Mightysil RP-18 GP-5 μm, 4.6 × 150 mm; 40°C; UV detection (210 nm)).

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