Oligosaccharides

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Target-Selective Degradation of Oligosaccharides by a Light-Activated Small-Molecule–Lectin Hybrid**

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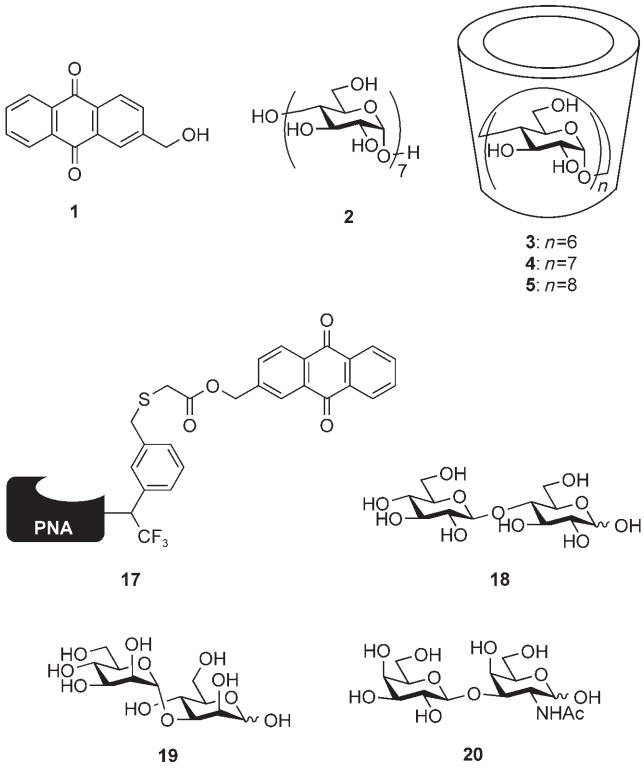
Oligosaccharides play important roles in many biological events, including protein folding, cell signalling, fertilization, pathogen binding to host tissue, leukocyte trafficking and associated inflammatory response, tumour cell metastasis, and regulation of hormone and enzyme activity.[1] The development of innovative methods for selectively controlling specific functions of certain oligosaccharides has attracted much attention in the fields of chemistry, biology, and medicine. However, there have as yet been no reports of methods involving specific inhibition of function by selective degradation of a target oligosaccharide. Herein we report the target-selective degradation of an oligosaccharide induced by a light-activated small-molecule–lectin hybrid. To the best of our knowledge, this is the first successful example of target-selective degradation of an oligosaccharide by light switching under neutral conditions.

With the aim of investigating a novel small molecule for degradation of oligosaccharides, we examined the anthraquinone molecule, which is found in several biologically important natural products, particularly antibiotics.[2] Certain anthraquinone derivatives have been found by Schuster and co-workers and by us to be efficient agents for DNA photocleavage.[3,4] In addition, photoinduced deterioration of cellulose by anthraquinone-related dyes has been reported, although the mechanism and the precise degradation products have not yet been elucidated.[5] On the basis of these previous findings, we expected that if an anthraquinone derivative could be made to produce a radical species by photoexcitation,[6] this species could be used for degradation not only of DNA, but also of oligosaccharide molecules.

To investigate this hypothesis, we selected the anthraquinone derivative 2-hydroxymethylanthraquinone (**1**) owing to its solubility in aqueous media. For the oligosaccharides, we chose maltoheptaose (**2**), α-cyclodextrin (α-CD, **3**), β-cyclodextrin (β-CD, **4**), and γ-cyclodextrin (γ-CD, **5**; Scheme 1). Although all of these oligosaccharides consist of α-D-glucose, they may be divided into two groups on the basis of their affinity for the anthraquinone derivative **1**. Thus, the members of the first group, maltoheptaose and α-CD, have no specific interaction with **1**, while those in the other group, β-CD and γ-CD, show strong interaction with **1** owing to their ability to form an inclusion complex with **1** through hydrophobic interaction.[7] Therefore, although a simple anthraquinone derivative was used, the oligosaccharides employed clearly have different affinities for this molecule.



Scheme 1. Anthraquinone derivatives and oligosaccharides. Ac = acetyl.

We first examined the photoinduced oligosaccharide-degrading activity of **1** at a concentration of 300 μM against 30 μM of β-CD (10:1 **1**/β-CD) in 20% acetonitrile/water solution, using a long-wavelength UV light source (365 nm, 100 W) for photoirradiation. The progress of the photodegradation reaction was monitored by HPLC. It was found that no change in the HPLC profile was obtained either by treatment of β-CD with **1** without photoirradiation or by photoirradiation of β-CD in the absence of **1**. In stark contrast, the HPLC peak corresponding to β-CD completely disappeared after exposure of β-CD to **1** with photoirradiation, which indicates that degradation of β-CD did take place. These results clearly show that this anthraquinone derivative is capable of degrading an oligosaccharide, β-CD, upon

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irradiation with long-wavelength UV light and without further additives. Because no degradation of β-CD by **1** took place in the absence of light, it was confirmed that UV light functioned as a trigger, initiating oligosaccharide degradation by the anthraquinone derivative. These phenomena were also confirmed by MALDI-TOF MS analysis. Again, the MS peak corresponding to β-CD completely disappeared only after incubation of β-CD with **1** under photoirradiation.

The degradation product(s) could not be specifically detected by either HPLC or MS analysis at this stage. We therefore attempted to convert the products into their acetylated derivatives, which would be more easily detected and analyzed. After the photoreaction, total acetylation of the resulting degradation products was carried out with Ac_2O and TFA.[8] Analysis of the acetylated products using MALDI-TOF MS (Figure 1) clearly indicated the presence of oxidation products including β-CD oxidized at the C-6 position (β-CD aldehyde),[9,10] and at the C-1 and C-6 positions, the latter of which results from oxidative cleavage of the glycosidic bond of β-CD. Thus, in Figure 1, peaks A1–A5 correspond to the molecular weights of compounds **6**–**10**, respectively, and peaks B1–B5 correspond to compounds **11**–**15**. Peak C1, representing compound **16**, which results from hydrolysis of β-CD and subsequent acetylation, was not detected. The ^{1}H NMR spectrum of a mixture of the degradation products also indicated the presence of an aldehyde function ($\delta =$ 10.2 ppm, br). Furthermore, it was confirmed that the degradation products could not be converted back into β-CD by reduction with $NaBH_4$.[9,10] These results show that photoirradiation of β-CD in the presence of the anthraquinone derivative **1** caused not only oxidation of the C-6 hydroxy group(s), but also oxidative cleavage of the C-1 glycosidic bond of β-CD.

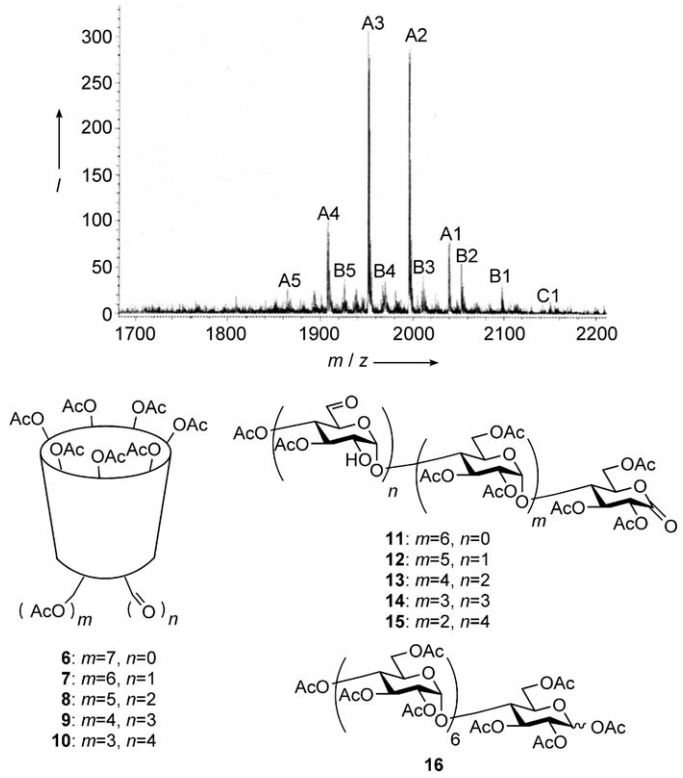
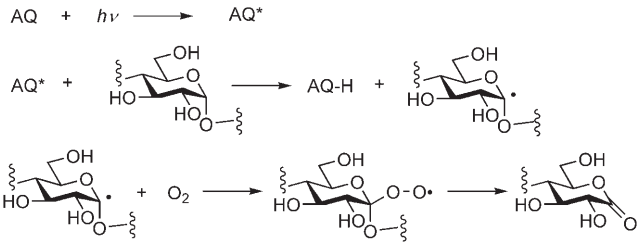


Figure 1. MALDI-TOF MS profile of acetylated products obtained by photodegradation of β-CD and subsequent acetylation. β-CD (30 μM) was incubated with **1** (100 μM) in 20% acetonitrile/H_2O (30 μL) at 25 °C for 2 h under irradiation with a UV lamp (365 nm, 100 W) placed 10 cm from the mixture, and then acetylated using Ac_2O and TFA at 25 °C for 15 h. The resulting products were analyzed by MALDI-TOF MS (matrix: 2,5-dihydroxybenzoic acid). The peaks A1–A5 and B1–B5 are observed at *m*/*z* 2039.17, 1995.15, 1951.12, 1907.10, 1863.12, 2097.17, 2053.15, 2009.13, 1965.19, and 1921.16, respectively; these signals correspond to the molecular weights of compounds **6**–**15**. TFA = trifluoroacetic acid.



Scheme 2. Presumed photodegradation pathway (oxidative cleavage of glycosidic bond) of oligosaccharide by **1** (shown here as AQ).

Next, we examined the effects of the concentration of **1** and its ability to interact with the oligosaccharide on the photodegradation reaction. For the former investigation, photoinduced β-CD (30 μM) degradation was conducted by using **1** at concentrations of 30, 90, and 300 μM. The progress of the photodegradation reaction was monitored by HPLC, and the percentage degradation was calculated on the basis of the area of the peak corresponding to β-CD. It was found that the degradation ability of **1** is dependent on its concentration, and its activity increased as the concentration increased (Figure 2a). Furthermore, the use of three equivalents of **1** in the reaction with β-CD was sufficient to cause almost 100% degradation. Next, we carried out photoinduced oligosaccharide degradation using **1** (90 μM) with oligosaccharides other than β-CD: maltoheptaose (**2**), α-CD (**3**), and γ-CD (**5**) at concentrations of 30 μM each. As shown in Figure 2b, maltoheptaose, α-CD, and γ-CD also underwent degradation by **1** with photoirradiation. These results demonstrate that the anthraquinone derivative **1** is capable of degrading not only β-CD, but a range of other oligosaccharides. However, it was noted that degradation of β- and γ-CDs by **1** was much more effective than that of maltoheptaose and α-CD. These results clearly show that the degradation ability of **1** is highly dependent on its interaction with the oligosaccharides.

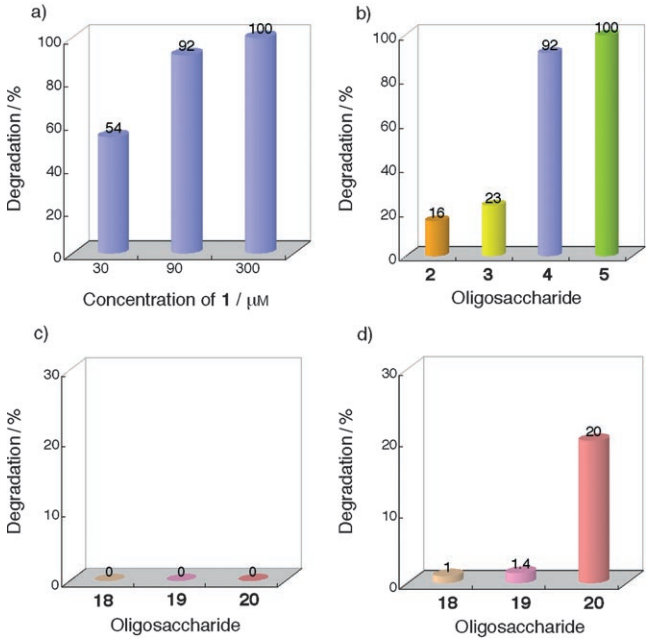
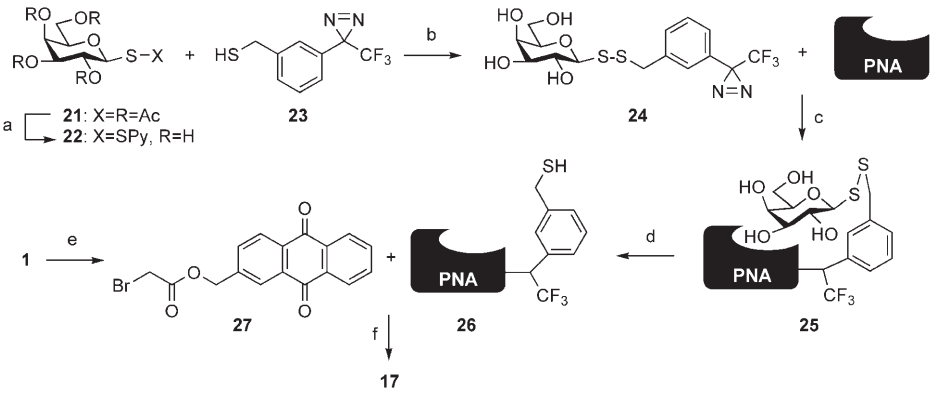


Figure 2. Photodegradation of oligosaccharides. a) β-CD (30 μM) was incubated with **1** (30, 90, and 300 μM) in 20% acetonitrile/H_2O (30 μL) under the conditions described in Figure 1 and analyzed by HPLC (TSKgel Amide-80, 4.6×250 mm; 3:2 MeCN/H_2O; flow rate 1.0 mL min^{-1}; 30 °C; detection by IR). b) Maltoheptaose (**2**), α-CD (**3**), β-CD (**4**), and γ-CD (**5**) at concentrations of 30 μM each were incubated with **1** (90 μM) in 20% acetonitrile/H_2O (30 μL) under the conditions described in Figure 2a and analyzed by the method indicated in Figure 2a. c) Glc(β-1,4)Glc (**18**), Man(α-1,3)Man (**19**), and Gal(β-1,3)GalNAc (**20**) at concentrations of 30 μM each were incubated with **1** (9 μM) in phosphate buffer (30 μL, 10 mM, pH 7.4) at 25 °C for 2 h under irradiation with a UV lamp (365 nm, 100 W) placed 10 cm from the mixture and analyzed by HPLC (TSKgel Amide-80, 4.6×250 mm; 3:2 MeCN/H_2O; flow rate 0.5 mL min^{-1}; 30 °C; detection by IR). d) Glc(β-1,4)Glc (**18**), Man(α-1,3)Man (**19**), and Gal(β-1,3)GalNAc (**20**) at concentrations of 30 μM each were incubated with **17** (9 μM) under the conditions described in Figure 2c and analyzed by the method indicated in Figure 2c.

On the basis of these favorable results, we turned to the investigation of target-selective degradation of oligosaccharides. For this purpose, we attempted to design a molecule in which the anthraquinone derivative **1** was attached to a lectin,[14] which has specific and strong affinity for sugars, to create a hybrid containing both degradation and recognition sites for a target oligosaccharide. For this purpose, we chose peanut agglutinin (PNA)[15,16] as a lectin, and the tumor-associated disaccharide Gal(β-1,3)GalNAc, generally known as T-antigen, as the target oligosaccharide.[17] Scheme 1 shows the designed hybrid molecule **17** (Scheme 3). This molecule, which possesses an anthraquinone unit at the sugar-binding pocket of PNA, was synthesized by a modified version of the procedure reported by Hamachi et al.[18] To carry out binding of the affinity ligand **24**, which was prepared from **21**[19] and **23**,[20] to the sugar pocket of PNA, a photoreaction was conducted with UV irradiation. Subsequent treatment of the labelled PNA **25** with DTT afforded a structure containing a mercaptobenzyl site (**26**), which was modified using 2-bromoacetoxymethylanthraquinone (**27**) to furnish the desired hybrid **17**.

With our designed anthraquinone–lectin hybrid in hand, we then examined its application in the target-selective photodegradation of oligosaccharides. Photoinduced degradation of three types of oligosaccharide (30 μM each)—Glc(β-1,4)Glc (**18**), Man(α-1,3)Man (**19**), and Gal(β-1,3)GalNAc (**20**)—was carried out using **17** (9 μM). The progress of the photodegradation reaction was monitored by HPLC, and the percentage degradation was calculated based on the peak area corresponding to each oligosaccharide. The results are summarized in Figure 2c,d. When 2-hydroxymethylanthraquinone (**1**) was used in the reaction, no degradation was detected owing to the low concentration of **1** and its low affinity for the oligosaccharides. However, when the hybrid **17** was exposed to the oligosaccharide Gal(β-1,3)GalNAc (**20**) under photoirradiation, significant degradation took place. This result is in sharp contrast to those of the other oligosaccharides **18** and **19**, which showed no degradation under photoirradiation with **17**. These results clearly indicate that the anthraquinone–lectin hybrid **17** selectively degraded only the target tumor-associated disaccharide Gal(β-1,3)GalNAc upon photoirradiation, without any additives and under neutral conditions.



Scheme 3. Synthesis of anthraquinone–lectin hybrid **17**. Reagents and conditions: a) PySSPy, LiOH, MeOH, 54%; b) AcOH, MeOH, H_2O, 84%; c) *hν*, phosphate buffer (10 mM, pH 7.4); d) DTT, phosphate buffer (10 mM, pH 8.1, 10 mM EDTA); e) $BrCH_2COCl$, 2,6-lutidine, CH_2Cl_2, 90%; f) DMF, phosphate buffer, (10 mM, pH 7.4). Py = pyridyl; DTT = 1,4-dithiothreitol.

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