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Target-selective photodegradation of oligosaccharides by a fullerene–boronic acid hybrid upon visible light irradiation†

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A fullerene derivative was found to be capable of photodegrading oligosaccharides under irradiation with not only UV but also visible light. Furthermore, target-selective photodegradation of oligosaccharides (β-D-galactofuranosides) was achieved by a designed and synthesized fullerene–boronic acid hybrid upon irradiation with visible light in the absence of any additives under neutral conditions.

Carbohydrates in living cells play important roles in many biological events, including bacterial cell wall recognition, viral and bacterial infection, cell signaling, tumor cell metastasis and fertilization.[1] Despite its importance, however, progress in this field has not been particularly rapid, because there has been comparatively little research on the development of smart technology for selective control of specific oligosaccharide functions. Therefore, the possibility of developing a chemical agent which can selectively and directly degrade target oligosaccharides under mild conditions has attracted much attention.[2,3] Herein, we report an innovative method for target-selective degradation of oligosaccharides induced by a light-activated fullerene derivative without additives and under neutral conditions. To the best of our knowledge, this is the first successful example of target-selective degradation of oligosaccharides by visible light switching.

Certain fullerene derivatives have been found to be efficient agents for photocleavage of DNA[4] and proteins.[5] However, there are no reports on the use of light-activated fullerenes for oligosaccharide degradation. In this context, we expected that if a fullerene derivative could be made to produce a radical or a reactive oxygen species (ROS) by photo-excitation, this molecule could be used for degradation of oligosaccharides.

To investigate this hypothesis, we selected a commercially available and water-soluble fullerene derivative **1** as an oligosaccharide photodegrading agent, and γ-cyclodextrin (γ-CD) (**3**) as the target oligosaccharide (Fig. 1). It was previously reported that a fullerene derivative bound with high affinity to **3** due to its ability to form an inclusion complex with the fullerene moiety through hydrophobic interaction.[6] We first

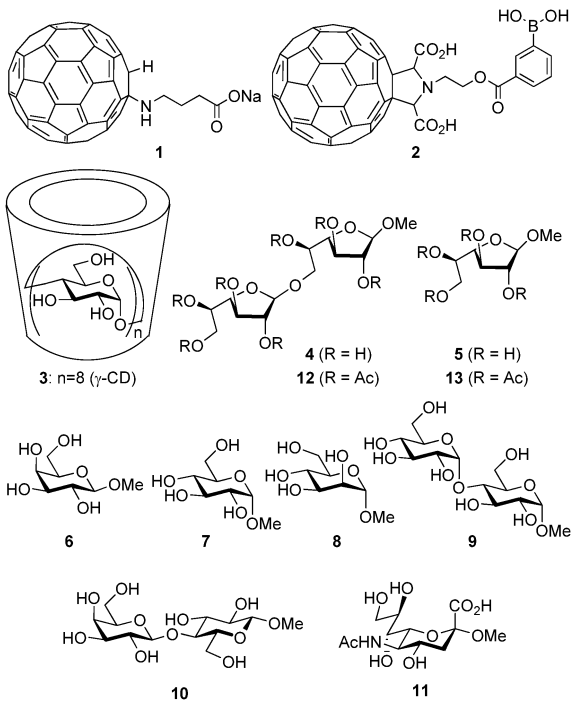

Fig. 1 Chemical structures of fullerene derivatives, γ-CD and several glycosides.

examined the photoinduced oligosaccharide-degrading activity of **1** at concentrations of 15, 30, 90, and 300 μM against 30 μM **3** in 0.1 M phosphate buffer (pH 7.4) at 25 °C for 2 hours under irradiation with a long-wavelength UV light (365 nm, 100 W) placed 10 cm from the sample. The progress of the photodegradation reaction was monitored by HPLC/RI analysis[2,3] (Fig. 2a). It was found that the integrated HPLC peak area corresponding to **3** clearly decreased only after exposure of **1** to **3** with photo-irradiation, which indicated that degradation of **3** by photo-activated **1** did take place. MALDI/TOF-MS analysis of the degradation products confirmed the production of a mixture of oxidative products at the C-6 hydroxy group(s) and oxidative cleavage products at the C-1 glycosidic bond. The result was quite similar to that using an anthraquinone derivative which was previously reported by us.[2] In addition, it was found that the photodegradation activity did not increase simply in a concentration-dependent manner. Moreover, degradation activity was found to be

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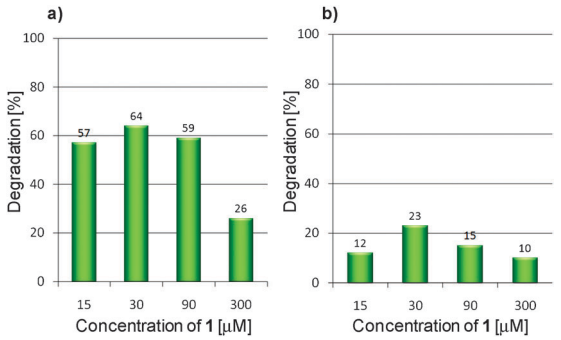
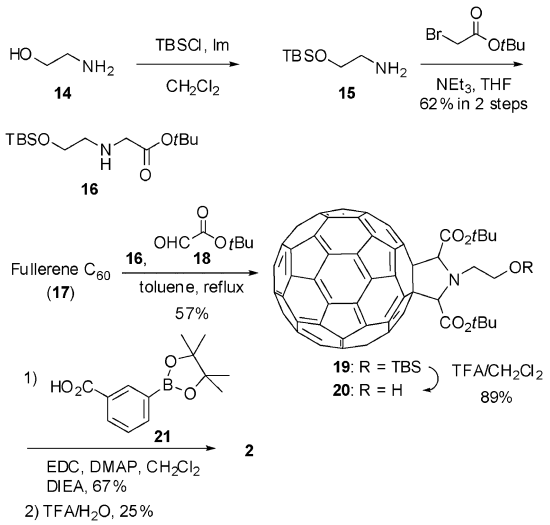


Fig. 2 Photodegradation of γ-CD (**3**) by fullerene derivative **1**. γ-CD (**3**) (30 μM) was incubated with **1** at different concentrations (15, 30, 90, and 300 μM) in 0.1 M phosphate buffer (30 μL, pH 7.4) at 25 °C for 2 h under irradiation with (a) a long-wavelength UV lamp (365 nm, 100 W), and (b) a visible-light lamp (diffuse sunlight, 100 W) placed 10 cm from the mixture, and analyzed by HPLC (TSK-GEL amide-80, 4.6 × 250 mm; 40 °C; detection by RI).

highest when equal amounts of **1** and **3** were used with photoirradiation. These results suggested that excess **1** which did not form a complex with **3** absorbed UV light and inhibited the absorbability of **1** complexed with **3**, which caused the photodegradation. Next, in order to examine the possibility of photodegradation of **3** by **1** upon irradiation using visible light, we measured the UV/vis spectrum of **1** (see ESI†). The results showed that **1** absorbs in both the UV and visible regions. To investigate this, photodegradation of **3** by **1** was performed under visible light (diffuse sunlight, 100 W xenon lamp) irradiation conditions. The results are shown in Fig. 2b. Although the photodegradation activity of **1** against **3** under visible light was found to be lower than under UV light, a similar tendency in the relationship between the degree of degradation and the dose equivalent of **1** was observed. These results clearly show that the fullerene derivative **1** is capable of degrading an oligosaccharide, γ-CD (**3**), upon irradiation not only with long-wavelength UV light but also with mild visible light in the absence of further additives.

Based on these favorable findings, we attempted to develop a hybrid molecule, consisting of a fullerene moiety and an artificial receptor that selectively binds to a target oligosaccharide, for selective photodegradation of target oligosaccharides under visible light irradiation. For this purpose, we focused on β-D-galactofuranosides (Gal*f*s) as target oligosaccharides, and designed a hybrid molecule **2** consisting of a fullerene derivative with one tertiary amino group and two carboxylic acids to increase water solubility, and a phenylboronic acid moiety, which has high affinity with Gal*f*s.[3] The hybrid **2** was synthesized as shown in Scheme 1. 1,3-Dipolar cycloaddition of an azomethine ylide (generated from a known aldehyde, **18**,[7] and a secondary amine, **16**, which was prepared from ethanolamine (**14**) in two steps) to C_{60} (**17**) under reflux conditions provided fullerene derivative **19** in 57% yield. Subsequent removal of the TBS protecting group with TFA/CH_2Cl_2, followed by esterification of the resulting alcohol **20** with a carboxylic acid, **21**, in the presence of EDC and DMAP in CH_2Cl_2, gave an ester in 67% yield. Finally, removal of the *tert*-butyl and pinacol groups of the resulting ester under aqueous acidic conditions provided the desired hybrid molecule **2**.



Scheme 1 Synthetic scheme for fullerene–boronic acid hybrid **2**.

In order to elucidate the complexing ability of **2** with various glycosides, including methyl 6-*O*-(β-D-galactofuranosyl)-β-D-galactofuranoside (**4**), methyl β-D-galactofuranoside (**5**), methyl β-D-galactopyranoside (**6**), methyl α-D-glucopyranoside (**7**), methyl α-D-mannopyranoside (**8**), methyl α-D-maltoside (**9**), methyl β-D-lactoside (**10**) and *N*-acetylneuraminic acid α-methylglycoside (Neu5Acα2Me) (**11**), we used a ^{11}B NMR titration binding assay[8] in 30% DMSO-d_6/0.1 M phosphate buffer (pH 7.4). The results are shown in Table 1. When β-D-galactofuranoside **4** or **5** was used, a change in the ^{11}B NMR spectrum of **2** was observed, and the peak area corresponding to the cyclic boronate was found to increase in a manner dependent on the concentration of the Gal*f* (see ESI†). Furthermore, the K_a values obtained for **2** with respect to **4** and **5** were almost the same; these values were almost 60 times greater than those for **2** with glycosides **6–11**, including Neu5Acα2Me (**11**), which possesses an extended glycerol side chain.

Next, we examined the photodegradation activity of **2** against various glycosides in 10% DMF/0.1 M phosphate buffer (pH 7.4) at 25 °C for 2 hour under visible light (diffuse sunlight) using a 100 W xenon lamp placed 10 cm from the sample. The progress of the photodegradation reaction was monitored by HPLC-UV (210 nm or 254 nm) analysis after total acetylation with Ac_2O in Py (for **4–10**) or primary alcohol *t*-butyldiphenylsilylation with TBDPSCl/imidazole in

Table 1 Association constants (K_a) for the hybrid molecule **2** with several glycosides

Entry	Glycoside	K_a[a]/M^{-1}
1	Methyl 6-*O*-(β-D-galactofuranosyl)β-D-galactofuranoside (**4**)	74
2	Methyl β-D-galactofuranoside (**5**)	68
3	Methyl β-D-galactopyranoside (**6**)	<1
4	Methyl α-D-glucopyranoside (**7**)	<1
5	Methyl α-D-mannopyranoside (**8**)	<1
6	Methyl α-D-maltoside (**9**)	<1
7	Methyl β-D-lactoside (**10**)	<1
8	*N*-Acetylneuraminic acid α-methylglycoside (Neu5Acα2Me) (**11**)	<1

[a] Determined by ^{11}B NMR titration in 30% DMSO-d_6/0.1 M phosphate buffer (pH 7.4); see ESI.†

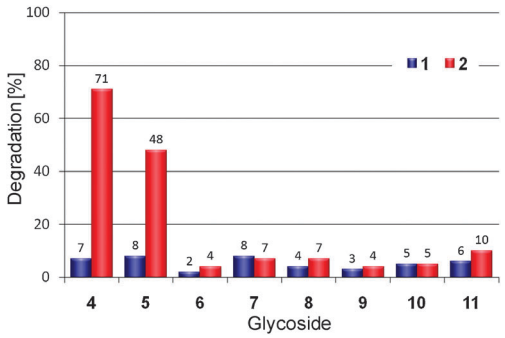


Fig. 3 Photodegradation of glycosides by fullerene derivatives. Each glycoside (1.0 mM) was incubated with fullerene derivative **1** or **2** (1.0 mM) in 10% DMF/0.1 M phosphate buffer (100 μL, pH 7.4) at 25 °C for 2 h under irradiation with a visible-light lamp placed 10 cm from the mixture, and analyzed by HPLC (Mightysil RP-18 GP 5 mm, 4.6 × 150 mm; 40 °C; detection by UV (215 nm or 254 nm) after acetylation (for **4–10**) or primary-alcohol silylation (for **11**) of the photodegradation products).

DMF (for **11**) of the resulting photodegradation products. The percentage degradation was calculated based on the peak area corresponding to each peracetylated glycoside or 9-*O*-TBDPS-Neu5Acα2Me, and the results are summarized in Fig. 3. When the fullerene derivative **1** was used as a control, less than 10% degradation of the glycosides took place, owing to the low affinity of **1** for glycosides. However, when the hybrid **2** was exposed to glycoside **4** or **5**, significant degradation took place. In addition, the degradation of **4** by **2** was found to be more effective than that of **5**. These results suggest that the binding affinity between the hybrid **2** and the target glycoside, and the number of protons in the glycoside that can react with the photo-activated fullerene moiety, are influential factors in the target-selective degradation of oligosaccharides by **2**.

These photodegradation phenomena were confirmed by ESI/TOF MS analysis after the photoreaction and subsequent acetylation of the resulting products. The MS peak corresponding to the acetylated monosaccharide **13**, along with **12**, was detected as one of the major peaks (see ESI†) only after incubation of **4** with **2** under visible-light irradiation. These results suggest that oxidative cleavage of the glycosidic linkage was caused by ROS generated by the photo-excited fullerene moiety and O_2.[2]

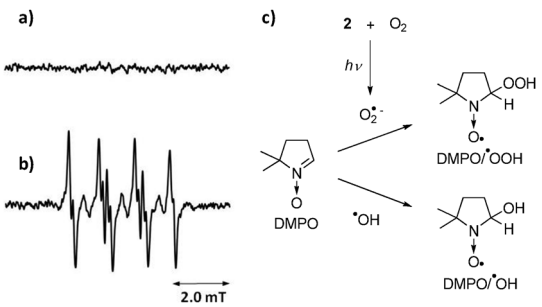


Fig. 4 EPR spectrum obtained during photo-irradiation of the hybrid **2** in the presence of DMPO. **2** (200 μM) and DMPO (500 mM) were incubated in 30% DMF/0.1 M phosphate buffer (pH 7.4) containing 1.0 mM DETAPAC and 10 mM NADH under irradiation with a visible-light lamp placed 40 cm from a flat cell. (a) Before irradiation; (b) after 2 min irradiation. (c) Possible pathways for the formation of DMPO/•OH and DMPO/•OOH. DETAPAC = diethylenetriaminepentaacetic acid, NADH = nicotinamide adenine dinucleotide.

Next, in order to confirm ROS generation, we conducted EPR studies using **2** and DMPO with or without visible-light irradiation. Interestingly, it was found that photo-irradiation of **2** in the presence of DMPO gave the DMPO-superoxide anion spin adduct DMPO/•OOH, not DMPO/•OH, as shown in Fig. 4b and c.[9] Furthermore, it was confirmed that peaks corresponding to DMPO/•OOH were not detected either after treatment of **2** without photo-irradiation or after photo-irradiation in the absence of **2** (Fig. 4a). These results indicate that $O_2^{\bullet-}$ species generated by the photo-excited fullerene moiety and O_2 play an important role in oxidative damage[10] of the glycosides (see ESI†), although $O_2^{\bullet-}$ is generally regarded as a rather unreactive radical species.[11]

In conclusion, it was found that the fullerene derivative **1** effectively degraded oligosaccharides upon irradiation not only with UV but also with visible light, without additives and under neutral conditions. Furthermore, we have developed a new chemical agent that can selectively and effectively degrade Gal*f* oligosaccharides by visible light switching under neutral conditions. Although the binding constant between our hybrid and target oligosaccharide is still relatively low, we hope this method will provide a means of controlling the specific functions of certain oligosaccharides. The development of more specific and tighter binding hybrid molecules is now under investigation in our laboratory.

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Notes and references

1 *Carbohydrate in Chemistry & Biology*, ed. B. Ernst, G. W. Hart and P. Sinaÿ, Wiley-VCH, Weinheim, 2000; *Comprehensive Glycoscience*, ed. J. P. Kamerling, G.-J. Boons, Y. C. Lee, A. Suzuki, N. Taniguchi and A. G. J. Voragen, Elsevier, Oxford, 2007.
2 M. Ishii, S. Matsumura and K. Toshima, *Angew. Chem., Int. Ed.*, 2007, **46**, 8396.
3 D. Takahashi, S. Hirono, C. Hayashi, M. Igarashi, Y. Nishimura and K. Toshima, *Angew. Chem., Int. Ed.*, 2010, **49**, 10096.
4 *Fullerenes: Principles and Applications*, ed. F. Langa De La Puente and J.-F. Nierengarten, Royal Society of Chemistry, Cambridge, UK, 2007; S. Bosi, T. D. Ros, G. Spalluto and M. Prato, *Eur. J. Med. Chem.*, 2003, **38**, 913; H. Tokuyama, S. Yamago, E. Nakamura, T. Shiraki and Y. Sugiura, *J. Am. Chem. Soc.*, 1993, **115**, 7918; F. Prat, C.-C. Hou and C. S. Foote, *J. Am. Chem. Soc.*, 1997, **119**, 5051; R. Bernstein, F. Prat and C. S. Foote, *J. Am. Chem. Soc.*, 1999, **121**, 464.
5 S. Tanimoto, S. Sakai, S. Matsumura, D. Takahashi and K. Toshima, *Chem. Commun.*, 2008, 5767; Y. Ishida, S. Tanimoto, D. Takahashi and K. Toshima, *MedChemComm*, 2010, **1**, 212.
6 Y. Nishibayashi, M. Saito, S. Uemura, S. Takemura, H. Takemura and Z. Yoshida, *Nature*, 2004, **428**, 279.
7 L. A. Carpino, *J. Org. Chem.*, 1964, **29**, 2830.
8 S. Chapelle and J. Verchere, *Tetrahedron*, 1988, **44**, 4469; H. Otsuka, E. Uchimura, H. Koshino, T. Okano and K. Kataoka, *J. Am. Chem. Soc.*, 2003, **125**, 3493.
9 E. Wertz and B. Bolton, in *Electron Spin Resonance*, McGraw-Hill, New York, 1972; H. M. Swartz, J. R. Bolton and D. C. Borg, in *Biological Application of Electron Spin Resonance*, Wiley-Interscience, New York, 1972.
10 T. Kawashima, K. Ohkubo and S. Fukuzumi, *Org. Biomol. Chem.*, 2010, **8**, 994.
11 D. T. Sawyer and J. S. Valentine, *Acc. Chem. Res.*, 1981, **14**, 393.

