Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 8393

www.rsc.org/obc

COMMUNICATION

Carbohydrate recognition and photodegradation by an anthracene–Kemp's acid hybrid†

Miyuki Nishibu, Daisuke Takahashi and Kazunobu Toshima*

Received 20th July 2012, Accepted 18th September 2012
DOI: 10.1039/c2ob26417j

Selective recognition and photodegradation of a monosaccharide, octyl β-D-glucopyranoside, was achieved without any additives under neutral conditions using an anthracene–Kemp's acid hybrid and long-wavelength UV irradiation.

Selective recognition of biomolecules, such as nucleic acids, proteins and carbohydrates, has attracted much attention in the areas of chemistry, biology, and medicine. Among all biomolecules, carbohydrates may be the most challenging substrates for host–guest chemistry due to the large number of stereoisomers, whose differences are often subtle.[1] In this context, lectins,[2] boronic acid derivatives,[3] and supramolecules[4] have been discovered and/or developed as carbohydrate recognition receptors to date. Artificial carbohydrate receptors show considerable potential due to their ease of design. Thus, the development of innovative methods using such artificial receptors for selective recognition of specific carbohydrate structures and control of specific carbohydrate functions poses a tremendous challenge that is of considerable interest. Herein we describe the molecular design, chemical synthesis, and functional evaluation of a novel artificial carbohydrate receptor that can selectively recognize and photodegrade a monosaccharide, octyl β-glucopyranoside, without the need for additives under neutral conditions. To the best of our knowledge, this is the first demonstrated example of target-selective recognition and degradation of a monosaccharide through non-covalent interactions using a small molecule by light switching under neutral conditions.

In designing a novel artificial carbohydrate receptor, we considered a hybrid structure consisting of Kemp's tricarboxylic acid[5] and an anthracene derivative. Previously, Rebek, Jr. and co-workers, in pioneering work, reported the molecular recognition of a nucleic acid base, 9-ethyladenine, using aromatic–Kemp's acid hybrids.[6] In their study, hydrogen bonding between the imide function in the hybrid and the nucleic acid base and aryl π–π stacking between the aromatic surface in the hybrid and the nucleic acid base significantly influenced molecular recognition. Based on these fruitful results, we expected that an anthracene–Kemp's acid hybrid would selectively recognize a particular monosaccharide through hydrogen bonding between the imide group in the hybrid and the hydroxyl group(s) of the carbohydrate, as well as CH/π interaction between the aromatic surface in the hybrid and the hydrogen atom(s) of the carbohydrate (Fig. 1).

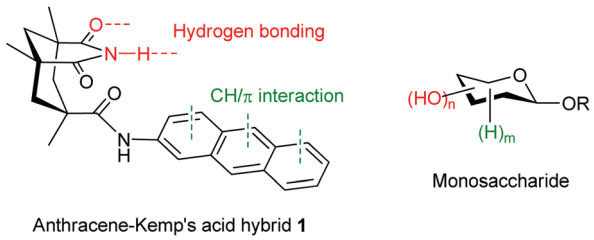

Fig. 1 Chemical structure of anthracene–Kemp's acid hybrid **1**, and its expected interaction with a sugar.

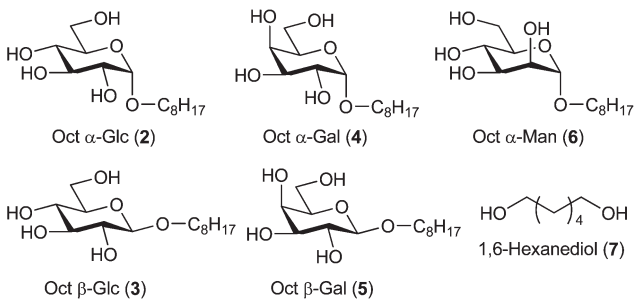

Fig. 2 Chemical structures of monosaccharides **2–5** and 1,6-hexanediol (**7**).

To investigate our hypothesis, we designed anthracene–Kemp's acid hybrid **1** (Fig. 1), which was synthesized using the procedure reported by Rebek, Jr. and co-workers.[6] After chemical synthesis of **1**, its binding affinity with several monosaccharides, including octyl α-Glc (**2**), octyl β-Glc (**3**), octyl α-Gal (**4**), octyl β-Gal (**5**), and octyl α-Man (**6**), was examined, along with 1,6-hexanediol (**7**) (Fig. 2), using a ^{1}H-NMR binding assay based on the chemical shift of the imide NH proton of **1** in $CDCl_3$ (see Fig. S1 and S3 in ESI†).[6,7] The results are summarized in Table 1.

The hybrid molecule **1** bound with high affinity to **3**, in which all substituents were at equatorial orientations. In addition, monosaccharides **2** and **6** were moderately recognized by **1**.

Department of Applied Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama, 223-8522, Japan. E-mail: toshima@applc.keio.ac.jp; Fax: +81 45-566-1576

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c2ob26417j

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

Entry	Substrate	K_a [a] [M^{-1}] Hybrid **1**
1	Octyl α-Glc (**2**)	313
2	Octyl β-Glc (**3**)	539
3	Octyl α-Gal (**4**)	43
4	Octyl β-Gal (**5**)	86
5	Octyl α-Man (**6**)	323
6	1,6-Hexanediol (**7**)	6

[a] The K_a values were determined by a ^{1}H-NMR binding assay based on the chemical shift of the imide NH proton of the hybrid **1** in $CDCl_3$, and are the average of at least two reproducible measurements; see ESI.†

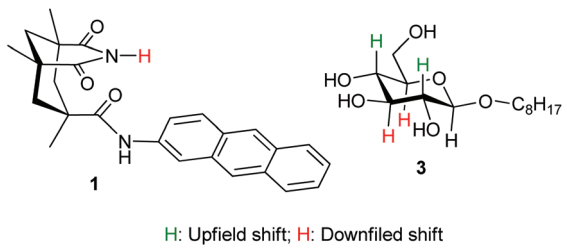

Fig. 3 Representative chemical shifts of the protons in **1** and **3** in complexation between anthracene–Kemp's acid hybrid **1** and octyl β-Glc (**3**).

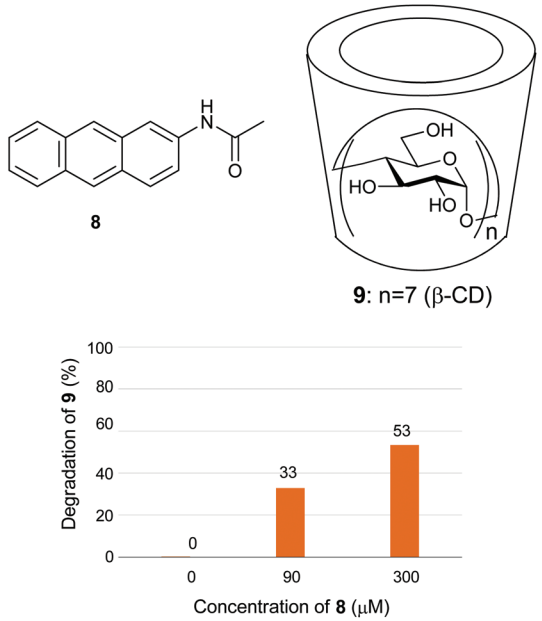

Fig. 4 Photodegradation of β-CD (**9**) by anthracene derivative **8**. β-CD (**9**) (30 μM) was incubated with **8** at different concentrations (0, 90, and 300 μM) in 1% DMSO/MeCN–H_2O (9 : 1) at 25 °C for 2 h under irradiation with a long-wavelength UV lamp (365 nm, 100 W) placed 10 cm from the mixture, and analyzed by HPLC/RI.

However, the K_a values obtained for **4** and **5** were low, and **1** did not bind to **7**. These results clearly indicated that our designed hybrid molecule **1** selectively bound only to monosaccharide **3** rather than to the other monosaccharides **2** and **4–6** and diol **7**. A comparison of the results for **2** with those for **3** showed that β-orientation of the relatively bulky C1 substituent, rather than the α-orientation for the present binding, was favorable. Based on the results for **2** and **6**, it was concluded that the orientation of the C2 hydroxy group was not important in the molecular recognition process. In addition, based on a comparison of **2** and **6** with **4** and **5**, the α-configuration of the C4 hydroxy group was significant for binding with **1**. From a comparison of **3** with **7**, it was suggested that hydrogen bonding between the imide group of **1** and the hydroxy group(s) was not sufficient for significant molecular recognition between **1** and **3**, and CH/π interaction between the anthracene moiety in **1** and the hydrogen atom(s) on the pyran ring of **3** was also an important factor in binding. Furthermore, it was confirmed by a ^{1}H-NMR assay in $CDCl_3$ (see Fig. S2 in ESI†) that the C3 and C5 protons of **3** shifted at low field while the C2 and C4 protons of **3** shifted at high field upon addition of **1** (Fig. 3). Although the exact binding mode of **1** with **3** is still not clear, considering all these results, the anthracene–Kemp's acid hybrid **1** must recognize monosaccharide **3** selectively through hydrogen bonding between the imide group of **1** and the hydroxyl group(s) of **3** and CH/π interaction between the anthracene surface in **1** and the axially oriented hydrogen atom(s) of **3**.

Next, our attention turned to selective degradation of the target monosaccharide **3** using **1** under photoirradiation. In previous studies, we found that certain anthracene derivatives efficiently photodegraded DNA.[8] Based on these findings, we hypothesized that if an anthracene derivative could be made to produce a radical or reactive oxygen species (ROS) upon photoexcitation, this compound could also degrade carbohydrate molecules. To investigate this hypothesis, we selected anthracene derivative **8** as a model saccharide photodegrading agent, and β-cyclodextrin (β-CD) (**9**) as the model saccharide (Fig. 4). It was previously reported that an anthracene derivative bound with high affinity to **9** due to its ability to form an inclusion complex with the anthracene moiety through hydrophobic interactions.[9] We first examined the photoinduced saccharide-degrading activity of **8** at concentrations of 0, 90, and 300 μM against 30 μM **9** in H_2O–MeCN (4 : 1) at 25 °C for 2 h under irradiation with a long-wavelength UV lamp (365 nm, 100 W) placed 10 cm from the sample. The progress of the photodegradation reaction was monitored by HPLC/RI analysis[10] (Fig. 4). It was found that the integrated HPLC peak area corresponding to **9** clearly decreased only after exposure of **8** to **9** with photoirradiation in a dose-dependent manner, which indicated that degradation of **9** by photo-activated **8** had taken place. The result was quite similar to our previous report using an anthraquinone derivative.[10] These results clearly show that the anthracene derivative **8** is capable of degrading a saccharide, β-CD (**9**), upon irradiation with long-wavelength UV light in the absence of further additives.

Next, in order to confirm ROS generation, we conducted EPR studies using **8** and DMPO with and without UV irradiation. It was found that photoirradiation of **8** in the presence of DMPO gave the DMPO–hydroxy radical spin adduct DMPO/˙OH (see Fig. S4 in ESI†).[11] This result indicated that the ˙OH species generated by the photo-excited anthracene moiety and O_2 play an important role in oxidative damage of the saccharide.[12]

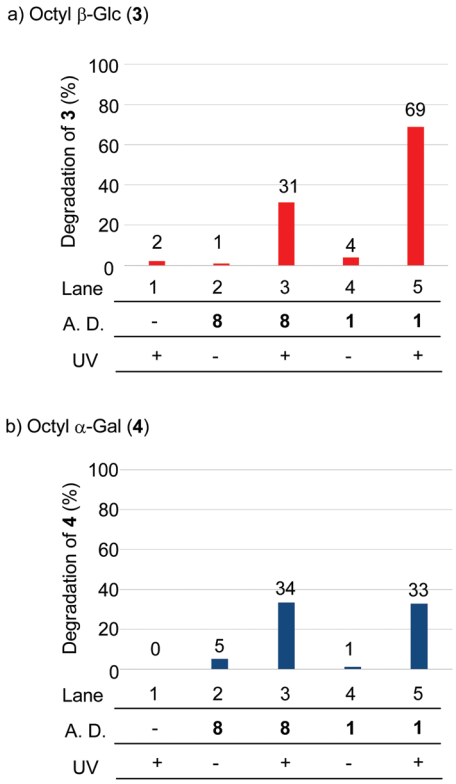

Fig. 5 Photodegradation of monosaccharides by anthracene derivatives. Each monosaccharide (1.0 mM) was incubated with anthracene derivative **1** or **8** (1.0 mM) in 1% DMF/MeCN at 25 °C for 2 h under irradiation with a long-wavelength UV lamp (365 nm, 100 W) placed 10 cm from the mixture, and analyzed by HPLC/UV after acetylation of the photodegradation products. (a) Octyl β-Glc (**3**) and (b) octyl α-Gal (**4**). Lane 1, UV irradiation alone; lane 2, **8** without UV irradiation; lane 3, **8** upon UV irradiation; lane 4, **1** without UV irradiation; lane 5, **1** upon UV irradiation. A.D. = anthracene derivative.

With these favorable results in hand, we next examined the photodegradation activity of **1** against two typical monosaccharides, **3** and **4**, which possess high and low affinity, respectively, with **1**. Photoirradiation was conducted in 1% DMF/MeCN at 25 °C for 2 h using a long-wavelength UV lamp (365 nm, 100 W) placed 10 cm from the sample. Since the use of a halogenated solvent such as $CHCl_3$ for the photoreaction was not suitable, considering solvent stability and the substrates solubility, we conducted the photodegradation assay in the different media. The progress of the photodegradation reaction was monitored by HPLC-UV analysis after total acetylation of the resulting photodegradation products with Ac_2O in Py. The percentage degradation was calculated based on the peak area corresponding to each peracetylated monosaccharide, and the results are summarized in Fig. 5. When the anthracene derivative **8** (without the Kemp's acid moiety) was used as a control, less than 35% degradation of **3** and **4** took place (lane 3 in (a) and (b)) owing to the low affinity of **8** for these monosaccharides. However, when **1** was exposed to glycoside **3**, which shows high affinity with **1**, significant degradation took place (lane 5 in (a)). In contrast, the degradation of **4**, which has low affinity with **1**, by **1** was found to be much less effective than that of **3** (lane 5 in (b)). These results indicate that the binding affinity between the hybrid **1** and the target monosaccharide is a significant factor in the target-selective degradation of the monosaccharide. It was also confirmed that hybrid **1** could be reused as a carbohydrate recognition agent, but not as a photodegradation one due to the self-photodegradation.

In conclusion, it was found that the anthracene–Kemp's acid hybrid **1** selectively recognized a particular monosaccharide through hydrogen-bonding and CH/π interactions. In addition, an anthracene derivative was found to cause effective degradation of a carbohydrate upon irradiation with UV light, without additives under neutral conditions. In a combination of these new findings, we demonstrated that hybrid **1** can selectively and effectively degrade a target monosaccharide by light switching under neutral conditions. Although selective binding and degradation cannot be achieved in water, we hope these preliminary results will provide a novel strategy for recognition and degradation of target carbohydrates using small molecules and light irradiation through non-covalent interactions. The development of more specific and more tightly binding hybrid molecules that can be used in water is now under investigation in our laboratory.

This research was supported in part by the MEXT-supported Program for the Strategic Research Foundation at Private Universities, 2012–2016, Scientific Research (B) (No. 23310153) and Scientific Research on Innovative Areas "Chemical Biology of Natural Products" from MEXT.

Notes and references

1 *Carbohydrates 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 H. Lis and N. Sharon, *Chem. Rev.*, 1998, **98**, 637; T. K. Dam and C. F. Brewer, *Chem. Rev.*, 2002, **102**, 387; N. Sharon and H. Lis, *Glycobiology*, 2004, **14**, 53R.
3 T. D. James, M. D. Phillips and S. Shinkai, *Boronic Acids in Saccharide Recognition*, Royal Society of Chemistry, Cambridge, 2006.
4 D. B. Walker, G. Joshi and A. P. Davis, *Cell. Mol. Life Sci.*, 2009, **66**, 3177; M. Mazik, *RSC Adv.*, 2012, **2**, 2630.
5 D. S. Kemp and K. S. Petrakis, *J. Org. Chem.*, 1981, **46**, 5140.
6 B. Askew, P. Ballester, C. Buhr, K. S. Jeong, S. Jones, K. Parris, K. Williams and J. Rebek Jr., *J. Am. Chem. Soc.*, 1989, **111**, 1082; K. Williams, B. Askew, P. Ballester, C. Buhr, K. D. Jeong, S. Jones and J. Rebek Jr., *J. Am. Chem. Soc.*, 1989, **111**, 1090.
7 N. Hayashi, T. Ujihara and K. Kohata, *Biosci., Biotechnol., Biochem.*, 2004, **68**, 2512; K. Hirose, *J. Inclusion Phenom. Macrocyclic Chem.*, 2001, **39**, 193.
8 K. Toshima, M. Hasegawa, J. Shimizu and S. Matsumura, *ARKIVOC*, 2004, 28.
9 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer-Verlag, New York, 1978.
10 M. Ishii, S. Matsumura and K. Toshima, *Angew. Chem., Int. Ed.*, 2007, **46**, 8396.
11 J. E. Wertz and J. R. Bolton, *Electron Spin Resonance*, McGraw-Hill, New York, 1972; H. M. Swartz, J. R. Bolton and D. C. Borg, *Biological Application of Electron Spin Resonance*, Wiley-Interscience, New York, 1972.
12 B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford, 1985; K. J. A. Davies, *J. Biol. Chem.*, 1987, **262**, 9895; M. J. Davies, *Biochem. Biophys. Res. Commun.*, 2003, **305**, 761.