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# The significant effect of the carbohydrate structures on the DNA photocleavage of the quinoxaline–carbohydrate hybrids

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**Abstract**—The novel DNA interactive quinoxaline–carbohydrate hybrids possessing disaccharides as the carbohydrate moieties were designed and synthesized, and their DNA photocleaving abilities were evaluated in order to examine the effect of the disaccharide structures. The configurations of the glycosidic bonds in the disaccharide strongly affected the DNA photocleaving ability, and two β-glycosidic bond linkages were very effective for the DNA photocleavage. Furthermore, the quinoxaline–disaccharide hybrids exhibited selective cytotoxicity against cancer cells with photoirradiation.


Studies of the interaction between small molecules and DNA, especially the effects of the structural characteristics of the small molecules on the DNA interaction, are very important in the design of new DNA targeting drugs.[1] A number of DNA interactive small molecules including DNA oligomers and polyamides have already been evaluated. In contrast, although carbohydrates play a key role in various biological processes, the interaction between carbohydrates and DNA, however, are still not yet well understood. Only limited studies on the interaction between certain carbohydrates present in DNA interactive antibiotics and DNA have been reported.[2] On the other hand, the development of photochemical DNA cleaving agents, which selectively cleave DNA by irradiation with light of a specific wavelength under mild conditions and without any additives such as metals and reducing agents, is very interesting from chemical and biological standpoints and offers a significant potential in medicine especially in the post-genome era.[3] In this context, we have previously reported several novel DNA interactive and photocleaving small molecules possessing carbohydrates.[4] Among them, it has been found that a quinoxaline–carbohydrate hybrid possessing a suitably deoxygenated monosaccharide showed a high binding affinity to DNA and cleaved the double-stranded DNA upon irradiation with UV light with a long wavelength and without any additive.[4e,f] Furthermore, it has been confirmed that the quinoxaline–carbohydrate hybrid system was very effective for the DNA cleavage. Based on our previous results, we had additional questions on the DNA cleaving activity–carbohydrate structure relationship. First, how do oligosaccharides work in the DNA binding and DNA photocleaving events? Second, which structure of the oligosaccharide is effective in such events? Therefore, in this study, several quinoxaline–carbohydrate hybrids possessing disaccharides were newly designed and synthesized, and their DNA photocleaving abilities were evaluated in order to examine the effect of the disaccharide structures. We now report the molecular design, chemical synthesis, DNA photocleaving, and cytotoxic properties of novel and artificial DNA interactive agents, that include the quinoxaline–disaccharide hybrids **1**–**4** (Fig. 1).

In this preliminary study, we designed the quinoxaline–disaccharide hybrids **1**–**4**. They were commonly constructed of a quinoxaline as the DNA intercalative photocleaving moiety and a disaccharide consisting of a 2,3,6-trideoxy-3-amino sugar linked 2,6-dideoxy sugar as a DNA groove binder. We selected two types of 2,6-dideoxy sugars as the carbohydrate part because several kinds of 2,6-dideoxy sugars are present in many naturally occurring anticancer antibiotics, which bind to DNA,[5] and the hydrophobicity of the 2,6-dideoxy sugar would be suitable for the DNA groove binding.[6]

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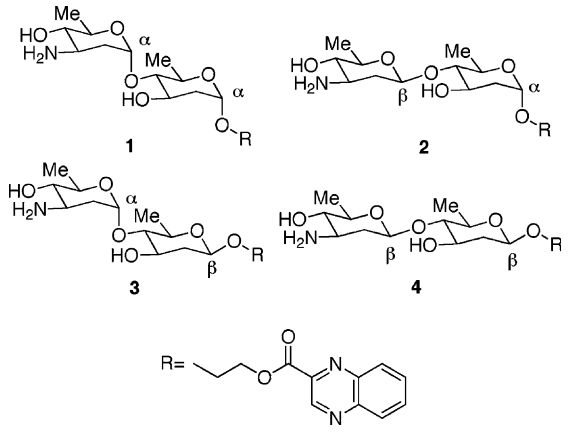
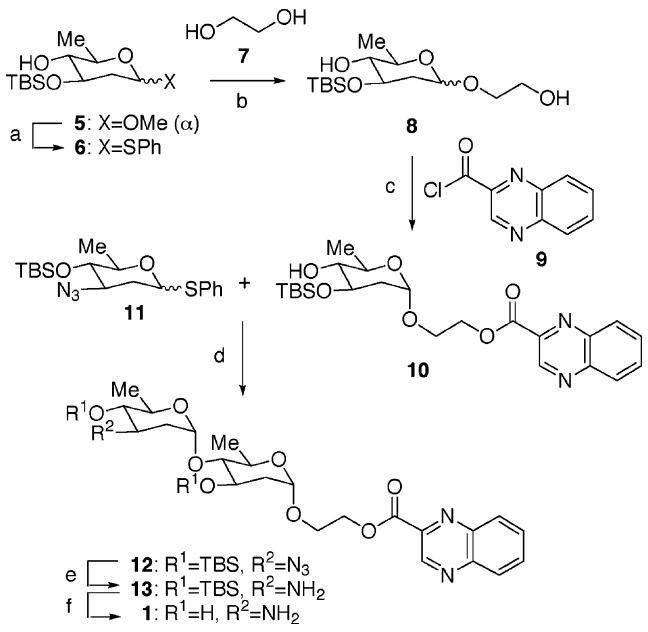



**Figure 1.** Molecular structures of quinoxaline–disaccharide hybrids **1**–**4**.

Actually, based on this concept, we have previously demonstrated that a suitably deoxygenated monosaccharide showed a high affinity to DNA, and significantly enhanced the intercalating ability of certain intercalators.[4,7] These hybrids, however, had different glycosidic bonds to each other in terms of their stereochemistry. Thus, the hybrids **1**–**4** possessed α,α-, α,β-, β,α-, and β,β-glycosidic linkages, respectively.

The synthesis of the representative quinoxaline–disaccharide hybrid **1** is summarized in Scheme 1. The synthesis of **1** commenced with the conversion of the known methyl glycoside **5**[8] into the phenyl thioglycoside **6** using $BF_3{\cdot}Et_2O$ in the presence of PhSH. The glycosidation of **6** and ethylene glycol (**7**) by Nicolaou et al.'s method[9] using NBS proceeded smoothly to give the glycoside **8** as a mixture of α- and β-anomers. The esterification of **8** with 2-quinoxaloyl chloride (**9**) in the presence of $Et_3N$ yielded the α-hybrid **10** after separation from the β-anomer. In these three steps, it was found that no protection of the C3-hydroxy group in the sugar moiety was necessary to carry out these regioselective reactions. The second glycosidation of **10** and the another phenyl thioglycoside **11**[4a] was effectively performed using NBS and TfOH[10] in EtCN at low temperature to furnish the α-disaccharide **12** after separation from the β-anomer. After the azide group in **12** was converted into the free amino group using $Ph_3P$, the TBS groups were removed employing HF·Py to afford the targeted quinoxaline–disaccharide hybrid **1**. The other hybrids **2**–**4** were also synthesized using the isomers produced in the glycosidation steps by very similar protocols.




**Scheme 1.** Synthesis of **1**. Reagents and conditions: (a) PhSH, $BF_3{\cdot}Et_2O$, −10 °C, 30 min, 80%; (b) NBS, MS 4A, THF, 0 °C, 10 min, 74% (α/β = 1.1/1); (c) $Et_3N$, $CH_2Cl_2$, rt, 10 min, 80%; (d) NBS, TfOH, MS 4A, EtCN, −78 °C, 20 min, 84% (α/β = 2.2/1); (e) $Ph_3P$, $H_2O$, THF, 60 °C, 13 h, 79%; (f) HF·Py, Py, 40 °C, 14 h, 71%.

With all the designed quinoxaline–disaccharide hybrids **1**–**4** in hand, the photo-induced DNA cleaving activities of these hybrids were next assayed using supercoiled ΦX174 DNA in concentrations of 3–500 μM. Based on the results shown in Figure 2, the quinoxaline–disaccharide hybrids **1**–**4** caused DNA cleavage during irradiation with a long wavelength UV light (365 nm). It was confirmed that no DNA cleavage by **1**–**4** was observed in the absence of light (lanes 3 in Fig. 2). Thus, the UV light functioned as the trigger to initiate these quinoxaline–disaccharide hybrids for the DNA strand scission. Furthermore, interestingly, the DNA cleaving abilities of the quinoxaline–disaccharide hybrids **1**–**4** were quite different and significantly increased in that order. The

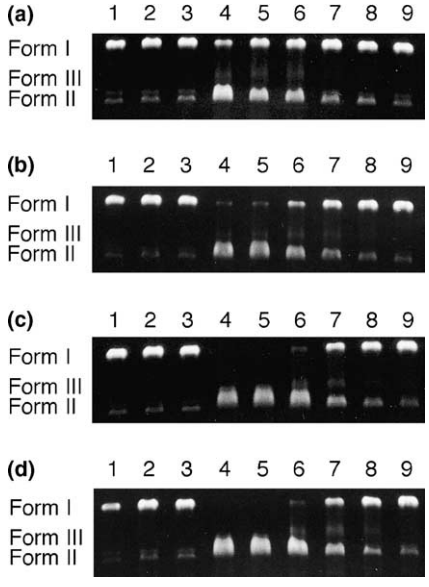



**Figure 2.** Photocleavage of supercoiled ΦX174 DNA. ΦX174 DNA (50 μM per base pair) was incubated with various compounds in 20% acetonitrile in Tris–HCl buffer (pH 7.5, 50 mM) at 25 °C for 1 h under irradiation of the UV lamp (365 nm, 15 W) placed at 10 cm from the mixture, and analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain): (a)–(d) for the compounds **1**, **2**, **3**, and **4**, respectively: lane 1, DNA alone; lane 2, DNA with UV; lane 3, DNA+compound (500 μM) without UV; lanes 4–9, compound (500), compound (300), compound (100), compound (30), compound (10), and compound (3 μM), respectively, following UV irradiation.

$CC_{50}$ values, which were the concentrations at which 50% Form I DNA was converted into Form II DNA, of **1**–**4** were 160, 65, 43, and 25 μM, respectively. The strongest DNA cleaving hybrid **4** possessing β, β-glycosidic linkages was more than six times stronger than the weakest hybrid **1** possessing α, α-glycosidic linkages, and cleaved DNA in concentrations over 3 μM, and caused a 100% DNA break at concentrations over 100 μM ((d) in Fig. 2). Since only the quinoxaline had the DNA photocleaving activity and the carbohydrates themselves showed no DNA photocleaving ability, the difference in the DNA photocleaving ability of these hybrids came from the structure difference of the disaccharide moieties in the hybrids. It was also confirmed that no difference was observed in the absorbance of each hybrid at 365 nm. Thus, it was noteworthy that the DNA cleaving activity was highly dependent on the structure of the carbohydrate, especially the configurations of the two glycosidic bonds, and the two β-glycosidic bond linkages were very effective for the DNA photocleavage.[11] Furthermore, these results also strongly suggest that the disaccharide containing the suitable glycosidic linkages works well as the DNA groove binder and significantly enhances the intercalating ability of the quinoxaline.

The cytotoxicity of the potent hybrids **3** and **4** was next examined using HeLa S3 cells exposed to each agent for 72 h with or without 1 h of photoirradiation (Table 1).[12] It was found that the $IC_{50}$ values of **3** and **4** against the HeLa S3 cells without photoirradiation were >100 μM, and those with photoirradiation were 6.0 and 5.6 μM, respectively. These results clearly indicate that the quinoxaline–disaccharide hybrids **3** and **4** are themselves nontoxic while they show high cytotoxic activities with photoirradiation. Furthermore, these results also demonstrate that the DNA-cleaving activity induced by photoirradiation significantly affects the cytotoxicity of the hybrid, and the life of cancer cells can be controlled by treatment with an appropriate amount of the quinoxaline–disaccharide hybrid with or without the photoirradiation.

**Table 1.** Cytotoxicity of **3** and **4** against HeLa S3 cells with or without photoirradiation

| Compounds | | **3** | **4** |
|---|---|---|---|
| $IC_{50}$ [μM] HeLa S3 | Without UV | >100 | >100 |
| | With UV | 6.0 | 5.6 |

In summary, the present study demonstrates not only the molecular design and chemical synthesis of novel quinoxaline–disaccharide hybrids, but also their DNA photocleavage and cytotoxic profiles. The effect of the carbohydrate structures on the DNA binding and cleaving abilities of the DNA photocleaving agents was also demonstrated. The described chemistry and biological evaluation provided significant information about the molecular design of novel and selective DNA photocleaving and cytotoxic agents including carbohydrate(s), especially oligosaccharide(s).

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## References and notes

1. Demeunynck, M.; Bailly, C.; Wilson, W. D. *DNA and RNA Binders*; Wiley-Vch: Weinheim, 2003.
2. Moser, H. E. In *Carbohydrates in Chemistry and Biology*; Ernst, B., Hart, G. W., Sinaÿ, P., Eds.; Wiley-Vch: Weinheim, 2000; Vol. 2, pp 1095–1124.
3. Armitage, B. *Chem. Rev.* **1998**, *98*, 1171.
4. (a) Toshima, K.; Takano, R.; Maeda, Y.; Suzuki, M.; Asai, A.; Matsumura, S. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 3733; (b) Toshima, K.; Maeda, Y.; Ouchi, H.; Asai, A.; Matsumura, S. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2163; (c) Toshima, K.; Takai, S.; Maeda, Y.; Takano, R.; Matsumura, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 3656; (d) Toshima, K.; Okuno, Y.; Nakajima, Y.; Matsumura, S. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 671; (e) Toshima, K.; Takano, R.; Ozawa, T.; Matsumura, S. *Chem. Commun.* **2002**, *23*, 212; (f) Toshima, K.; Kimura, T.; Takano, R.; Ozawa, T.; Ariga, A.; Shima, Y.; Umezawa, K.; Matsumura, S. *Tetrahedron* **2003**, *59*, 7057.
5. Jütten, P.; Greven, R. In *Polysaccharides in Medicinal Applications*; Dumitriu, S., Ed.; Marcel Dekker: New York, 1996; pp 339–410.
6. (a) Walker, S.; Valentine, K. G.; Kahne, D. *J. Am. Chem. Soc.* **1990**, *112*, 6428; (b) Ding, W.; Ellestad, G. A. *J. Am. Chem. Soc.* **1991**, *113*, 6617; (c) Uesugi, M.; Sugiura, Y. *Biochemistry* **1993**, *32*, 4622.
7. Toshima, K.; Ouchi, H.; Okazaki, Y.; Kano, T.; Moriguchi, M.; Asai, A.; Matsumura, S. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2748.
8. Juergen, D.; Alexander, K.; Jan, R.; Dieter, S. H. *Liebigs Ann. Chem.* **1985**, 1838.
9. Nicolaou, K. C.; Seitz, S. P.; Papahatjis, D. P. *J. Am. Chem. Soc.* **1983**, *105*, 2430.
10. (a) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331; (b) Konradsson, P.; Udodong, U. K.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313.
11. The site selectivities of the DNA cleavages using **1**–**4** were quite similar and identical with that of the corresponding monosaccharide derivative previously reported[4e,f] due to the electron transfer mechanism of the DNA photocleavage of quinoxaline.
12. Scudieero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827.