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Molecular design, chemical synthesis and biological evaluation of quinoxaline–carbohydrate hybrids as novel and selective photo-induced DNA cleaving and cytotoxic agents

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This paper is dedicated to Professor K. C. Nicolaou on the occasion of his receiving the Tetrahedron Prize

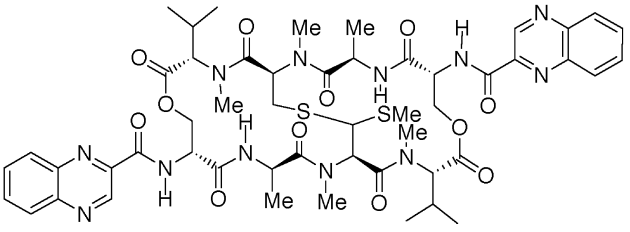
Abstract—The quinoxaline moiety in antitumor quinoxaline antibiotics cleaved double stranded DNA at the 5′ side guanine of the 5′-GG-3′ site upon irradiation with UV light with a long wavelength and without any additive. The quinoxaline–carbohydrate hybrid system was very effective for the DNA cleavage. Furthermore, the quinoxaline–carbohydrate hybrids exhibited strong and selective cytotoxicity against cancer cells with photoirradiation.

1. Introduction

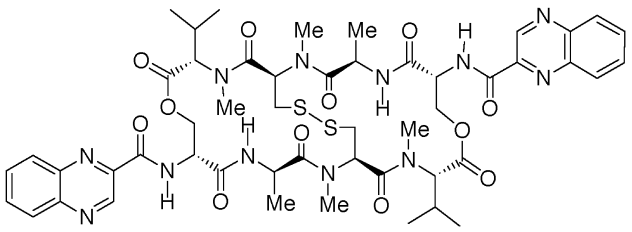
Studies of the interaction between the small molecules and DNA, especially the effects of the structural characteristics of the small molecules on the DNA interaction, are very important for the design of DNA targeting new antitumor drugs.[1] In this context, the development of photochemical DNA cleaving 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 considerable potential in medicine especially in the post-genome era.[2] Furthermore, photodynamic therapy using a photosensitizing drug has recently emerged as a promising modality against cancer and allied diseases.[3] In this paper, we discuss the molecular design, chemical synthesis, DNA photocleaving property and cytotoxicity of such novel and artificial light activatable DNA cleaving and cytotoxic agents, that include the quinoxaline–carbohydrate hybrids.[4]

2. Results and discussion

In our first approach to create such novel DNA photocleaving molecules, we noted the quinoxaline structure, because quinoxaline was found in DNA interactive and antitumor quinoxaline antibiotics[5] such as echinomycin and triostin A (Fig. 1). Although quinoxaline is known only as a DNA intercalator,[6] we expected that quinoxaline would be a novel DNA photocleaving agent, because the conjugated C=N bond in quinoxaline was expected to generate the photo-excited[3] ($n-\pi^*$) and/or[3] ($\pi-\pi^*$) state(s) by



Echinomycin



Triostin A

Figure 1. Molecular structures of quinoxaline antibiotics, echinomycin and triostin A.

Keywords: quinoxaline; carbohydrate; DNA photocleavage; cytotoxicity.
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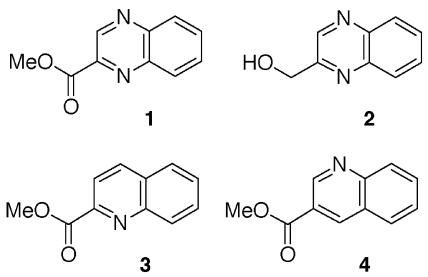


Figure 2. Quinoxaline and quinoline derivatives.

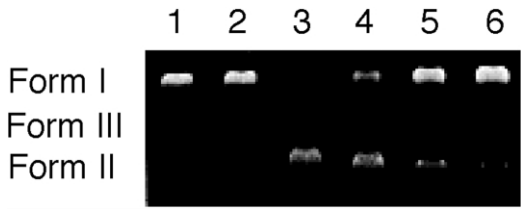


Figure 3. Photocleavage of supercoiled ΦX174 DNA. ΦX174 DNA (50 μM per base pair) was incubated with various compounds in 20% acetonitrile in Tris-HCl buffer (pH 7.5, 50 mM) at 25°C for 1 h under irradiation of the UV lamp (365 nm, 15 W) placed at 10 cm from the mixture, and analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain): lane 1, DNA alone; lane 2, DNA with UV; lanes 3–6, compounds **1**–**4** (500 μM), respectively, following UV irradiation. Form I: covalently closed supercoiled DNA, Form II: open circular DNA, and Form III: linear DNA.

photoirradiation which could be capable of cleaving DNA by H-abstraction, electron-transfer, and/or singlet oxygen oxidation pathway(s).[2] Therefore, we first examined the photo-induced DNA cleaving activities of the quinoxaline derivatives and the related compounds **1**–**4** (Fig. 2) using supercoiled ΦX174 DNA (Form I). As is evident from Figure 3, the quinoxaline derivatives **1** (500 μM) and **2** (500 μM) caused a significant single-strand scission of DNA by photoirradiation using long wavelength UV light (365 nm) without any further additives, leading to the nicked open circular DNA (Form II) (lanes 3 and 4 in Figure 3). On the other hand, the quinoline derivatives **3** and **4** showed little DNA cleaving activity under similar conditions (lanes 5 and 6 in Figure 3). These results clearly demonstrate, for the first time, that the quinoxaline moiety, present in certain antitumor antibiotics, is able to cleave DNA upon irradiation with UV light with a long wavelength without any additive and that the two nitrogen atoms in the quinoxaline are essential for the DNA cleavage. Furthermore, it was found that the ester function at the C-2 position of quinoxaline improved the DNA cleaving activity. To further improve the DNA cleaving ability of the quinoxaline derivatives, we designed the quinoxaline–carbohydrate hybrids **5**–**9** (Fig. 4), because we have previously demonstrated that a suitably deoxygenated amino sugar showed a high affinity to DNA, and significantly enhances the intercalating ability of certain intercalators.[7] The quinoxaline–carbohydrate hybrids **5** and **6** are anomers of each other, and **7**–**9** are bis(quinoxaline–carbohydrate) hybrids. The syntheses of the representative quinoxaline–carbohydrate hybrid **5** and bis(quinoxaline–carbohydrate) hybrid **7** are summarized in Schemes 1 and 2, respectively. The synthesis of **5** commenced with the conversion of the known methyl glycoside **10**[8] into the phenyl thioglycoside **11** using PhSH in the presence of $BF_3{\cdot}Et_2O$. The protection of **11** with a *t*-butyldimethylsilyl (TBS) group gave the glycosyl donor **12**, which was subjected to glycosidation with ethylene glycol (**13**) by Nicolaou's method[9] using *N*-bromosuccinimide (NBS) to afford the glycosides **14** as a mixture of the α- and β-anomers. After the conversion of the azide group of **14** into the *N*,*N*′-dimethylamino group using H_2 and Pd–C in the presence of 35% HCHO, the resultant anomers were easily separated by column chromatography at this stage to isolate the α-anomer **15** and its β-anomer. Esterification of the α-glycoside **15** with 2-quinoxaloyl chloride (**16**) in the presence of Et_3N furnished the hybrid **17**. Finally, deprotection of the TBS group of **17** employing HF/Py yielded the desired quinoxaline–carbohydrate

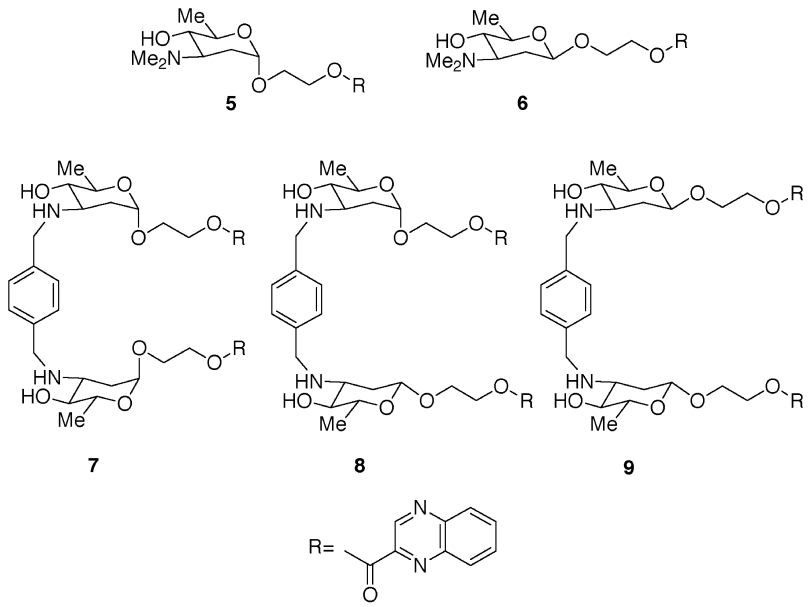
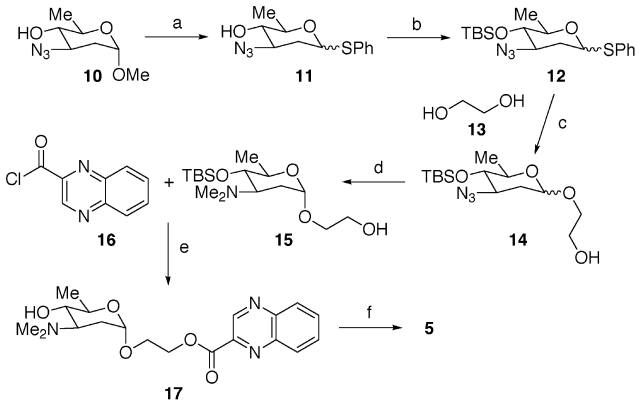
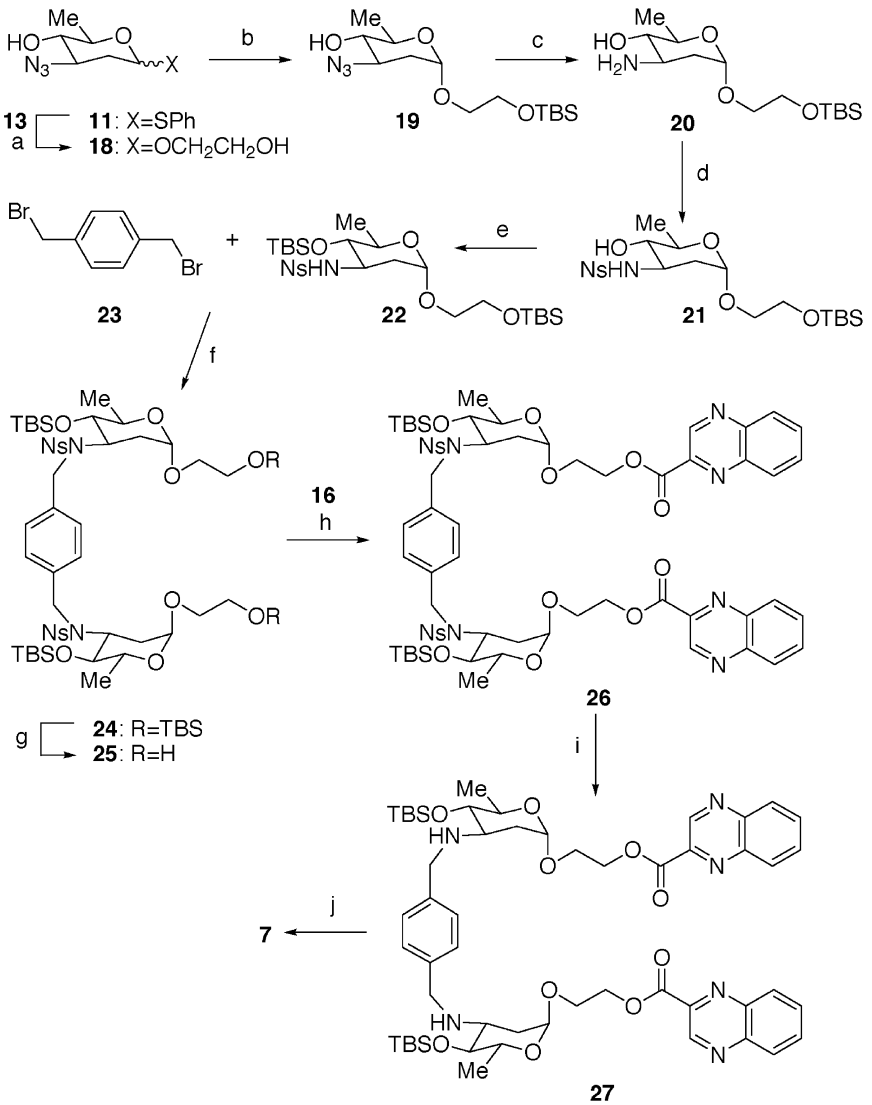


Figure 4. Quinoxaline–carbohydrate hybrids.



Scheme 1. Synthesis of **5**. *Reagents and conditions*: (a) PhSH, $BF_3{\cdot}Et_2O$, 0°C, 50 min, 99%; (b) TBSCl, imidazole, CH_2Cl_2, 40°C, 24 h, 94%; (c) NBS, MS 4A, MeCN, 0°C, 1 h, 79% (α/β=1.4/1); (d) H_2, Pd–C, 35% HCHO aq. MeOH, 25°C, 12 h, 81%; (e) Et_3N, CH_2Cl_2, 25°C, 15 h, 94%; (f) HF/Py, Py, 25°C, 12 h, 79%.

hybrid **5**. The β-anomer **6** was also obtained from the β-anomer of **15** using a similar procedure. On the other hand, the synthesis of **7** began with the glycosidation of **11** and ethylene glycol (**13**). Glycosidation of **11** and **13** using NBS smoothly proceeded to give the glycoside **18** as a mixture of the α- and β-anomers. The primary alcohol of **18** was regioselectively protected with a TBS group to afford the α-anomer **19** and its β-anomer, which were easily separated by column chromatography. The reduction of the azide group of the α-glycoside **19**, followed by protection of the resultant amine **20** with *p*-nitrobenzenesulfonyl (Ns)[10] and TBS groups, furnished **22** via **21**. The coupling reaction between **22** and *p*-dibromoxylene (**23**) in the presence of K_2CO_3 smoothly proceeded to yield the dimer **24**, the TBS groups of which at the primary position were regioselectively deprotected to give the alcohol **25**. Esterification of **25** with 2-quinoxaloyl chloride (**16**), followed by the deprotections of the Ns and TBS groups of the resultant **26**, gave the targeted bis(quinoxaline–carbohydrate) hybrid **7**. The other isomers **8** and **9** were also prepared using the α- and β-anomers of **19** and the β-anomer of **19**, respectively,



Scheme 2. Synthesis of **7**. *Reagents and conditions*: (a) NBS, MS 4A, THF, 0°C, 15 min, 80%; (b) TBSCl, imidazole, CH_2Cl_2, 25°C, 10 min, 84% (α/β=1/1.1); (c) H_2, $Pd/CaCO_3$, EtOH, 25°C, 16 h, 99%; (d) NsCl, K_2CO_3, CH_2Cl_2, 25°C, 2 h, 72%; (e) TBSOTf, 2,6-lutidine, CH_2Cl_2, 40°C, 16 h, 100%; (f) K_2CO_3, DMF, 25°C, 3 h, 41%; (g) HF/Py, Py, 25°C, 2 h, 95%; (h) Et_3N, 25°C, 20 min, 93%; (i) PhSH, K_2CO_3, DMF, 25°C, 4 h, 54%; (j) HF/Py, Py, 25°C, 16 h, 89%.

by a similar protocol. With all the designed quinoxaline–carbohydrate hybrids **5**–**9** in hand, the photo-induced DNA cleaving activities of these hybrids along with the reference compound **1** were next assayed using supercoiled ΦX174 DNA in concentrations of 500–3 μM. Based on the results shown in Figure 5, the quinoxaline–carbohydrate hybrids **5**–**9** caused effective DNA cleavage during irradiation with a long wavelength UV light. It was confirmed that no DNA cleavage by **1** and **5**–**9** was observed in the absence of light (lanes 3 in Figure 5). Thus, the UV light functioned as a trigger to initiate these quinoxaline derivatives for the DNA strand scission. Furthermore, the DNA cleaving abilities of the quinoxaline–carbohydrate hybrids **5** and **6** were found to be stronger than that of **1**, and the α-anomer **5** had a higher DNA cleaving ability than the β-anomer **6** ((a)–(c) in Figure 5). Thus, the DNA cleaving activity was dependent on the configuration of the glycosidic bond of the sugar moiety in the hybrids. These results strongly suggest that the suitably deoxygenated amino sugar in these hybrids works well as the DNA groove binder and significantly enhances the intercalating ability of the quinoxaline. Moreover, the bis(quinoxaline–carbohydrate) hybrids **7**–**9** were the most effective photo-induced DNA cleaving agents among them, and the DNA cleaving activities of **9**, **8** and **7** increased in that order ((d)–(f) in Figure 5). Thus, the strongest DNA cleaving hybrid **7** cleaved DNA in concentrations over 3 μM, and caused a 100% DNA break at concentrations over 30 μM ((d) in Figure 5). These results clearly show that the dimerization of the quinoxaline–carbohydrate hybrids **5** and **6** via the *p*-xylene linker is very effective for the DNA cleavage as suggested from the observations by Chaires and co-workers,[11] and the configurations of the glycosidic bonds of the sugars in the bis(quinoxaline–carbohydrate) hybrids also influence their DNA cleaving activities. In addition, it was interesting to note that the DNA photocleaving abilities of these hybrids were much higher than that of the natural quinoxaline antibiotic, echinomycin ((g) in Figure 5). These results demonstrate that the artificial quinoxaline–carbohydrate hybrid is superior to the natural product possessing quinoxaline as a DNA photocleaving agent. The DNA cleaving site specificity of the representative quinoxaline derivatives **1**, **5**, **7** and **9** was next analyzed according to the Sanger protocol.[12] Since the Sanger sequencing reactions result in base incorporation, cleavage at the nucleotide *N* (sequencing) represents a cleaving site by the agent or the Maxam–Gilbert reaction at *N*+1.[13] The results shown in Figure 6 clearly indicated that all the quinoxaline derivatives selectively cleaved DNA at the 5′ side guanine of the 5′-GG-3′ sites and the site-selective DNA cleavage was enhanced upon treatment with hot piperidine. Since both the free radical and a singlet oxygen scavengers, dimethyl sulfoxide and 2,2,6,6-tetramethylpiperidine, did not inhibit the DNA cleavage, it is very likely that an electron transfer from the electron-rich 5′-GG-3′ site to the photo-excited quinoxaline is the initial step for the photo-induced destruction of the guanine base on the 5′ side of the 5′-GG-3′ sites.[2,14] In addition, confirmation that these quinoxaline derivatives bind to DNA with intercalation was obtained from a DNA unwinding assay using supercoiled pBR322 DNA and topoisomerase I.[15] With the favorable results on the interactive and photocleaving ability of the quinoxaline–carbohydrate hybrids against

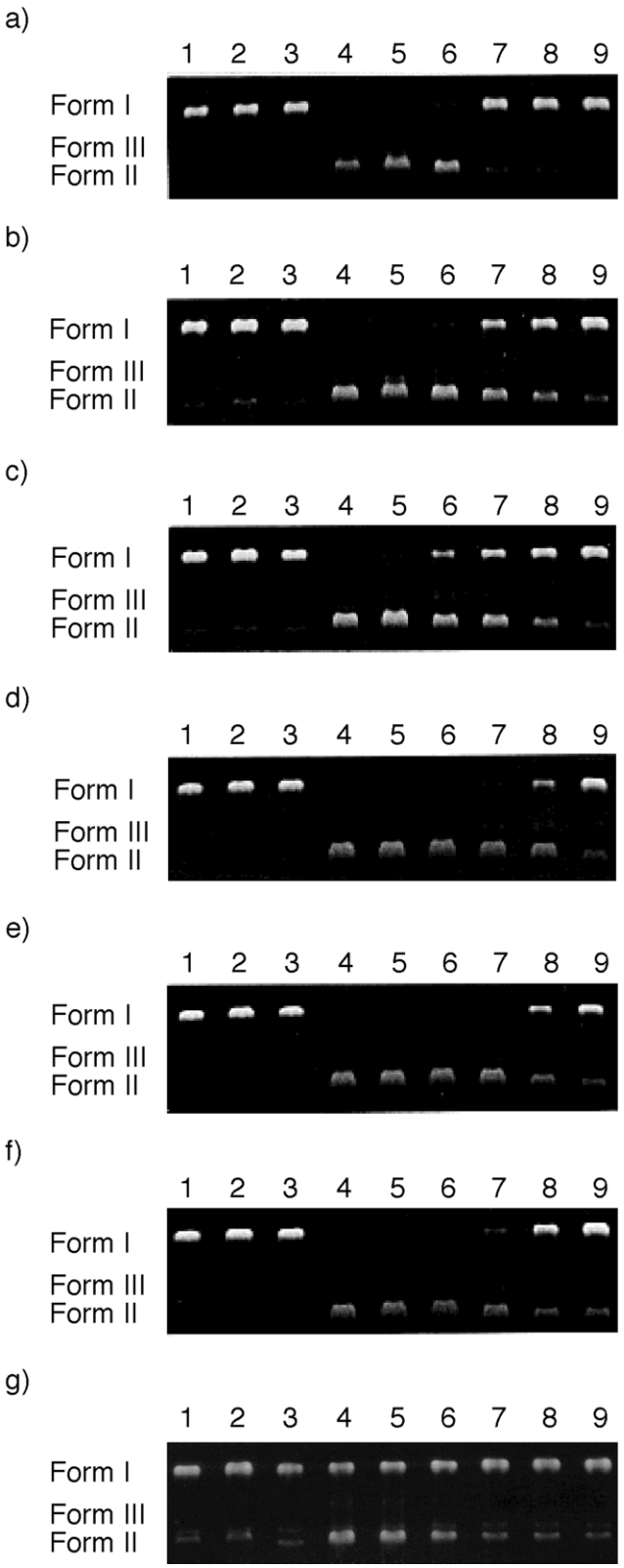


Figure 5. Photocleavage of supercoiled ΦX174 DNA. A same protocol as that mentioned in Figure 3 was carried out: (a), (b), (c), (d), (e), (f) and (g) for the compounds **1**, **5**, **6**, **7**, **8**, **9** and echinomycin, respectively: lane 1, DNA alone; lane 2, DNA with UV; lane 3, DNA+compound (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.

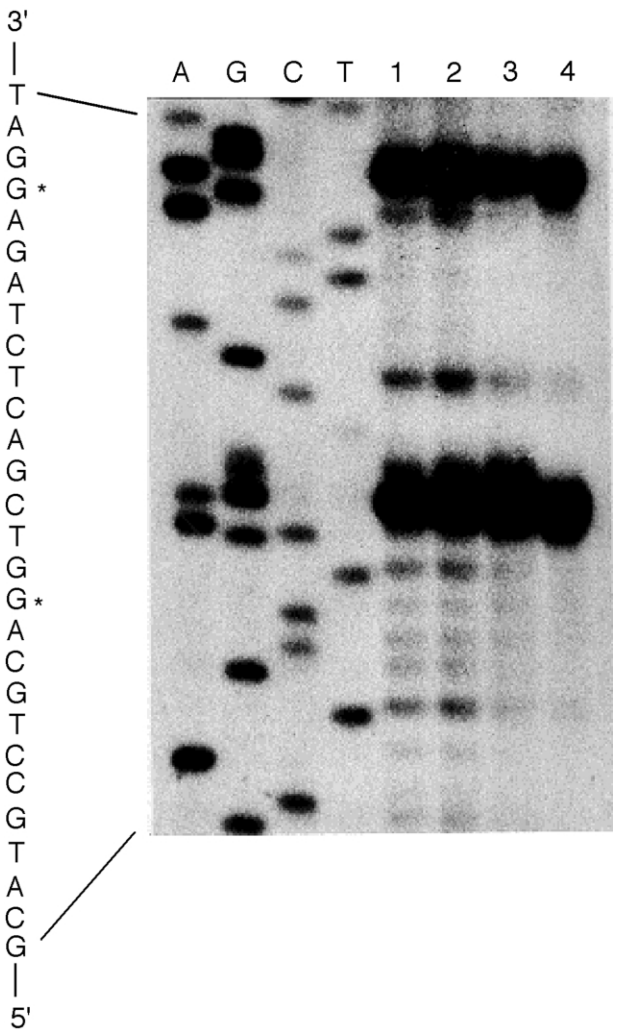


Figure 6. Autoradiogram of 12% polyacrylamide–8 M urea slab gel electrophoresis for sequence analysis. The 5′-end-labeled M13 mp18 DNA at the primer site was cleaved by the compound at pH 7.5 and 25°C for 1 h under irradiation of the UV lamp (365 nm, 15 W) placed at 10 cm from the mixture (bases 50–77 are shown): lanes A, G, C and T; Sanger A, G, C and T reactions, respectively; lanes 1, 2, 3 and 4; the compounds **7**, **9**, **5** and **1** (500 μM), respectively, following UV irradiation: DNAs for lanes 1~4 were treated with hot piperidine prior to gel electrophoresis.

the naked DNA, we further examined their DNA photocleaving ability in cancer cells using the hybrids **5** and **6**. These results are summarized in Figure 7. After the HeLa S3 cells were exposed to each hybrid **5** (10 μM) or **6** (10 μM) for 72 h with or without 1 h of photoirradiation, the DNA in the cells was extracted and analyzed by gel electrophoresis. It was found that the DNA was effectively cleaved by the hybrids **5** and **6** under photoirradiation while **5** and **6** without photoirradiation caused no DNA cleavage. These results indicate that the quinoxaline–carbohydrate hybrid cleaves DNA only with photoirradiation even in the cells. These phenomena are quite consistent with those observed using naked DNA. The cytotoxicity of the hybrids **5**–**7** was next examined using HeLa S3 and MDA-MB-231 cells exposed to each agent for 72 h with or without 1 h of photoirradiation (Table 1).[16] The IC_{50} values of **5**–**9** against the HeLa S3 cells without photoirradiation were >100 μM, and those with photoirradiation were 1.5, 1.3, 1.0, 4.0 and 4.0 μM, respectively. In addition, the IC_{50} values of **5**–**9** against the

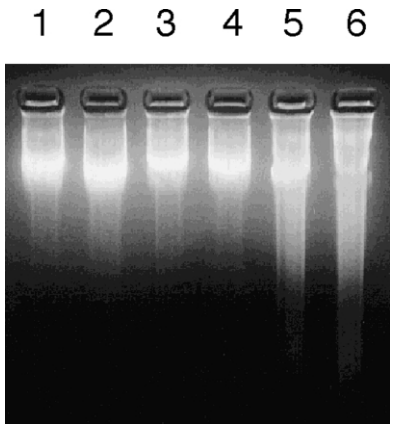


Figure 7. DNA ladders in HeLa S3 cells. HeLa S3 cells were treated with **5** (10 μM) and **6** (10 μM) with or without photoirradiation at 25°C for 1 h with a UV lamp (365 nm, 30 W) placed at 25 cm from the samples. After cells were cultured for 72 h at 37°C, the DNA was extracted from cells and analyzed by gel electrohoresis in 1.5% agarose gel. The gel was stained with ethidium bromide and photographed to observe fluoresence. lane 1, DNA from HeLa S3 cells; lane 2, DNA from HeLa S3 cells with UV; lane 3, DNA from HeLa S3 cells+**5** without UV; lane 4, DNA from HeLa S3 cells+**6** without UV; lane 5, DNA from HeLa S3 cells+**5** with UV; lane 6, DNA from HeLa S3 cells+**6** with UV.

MDA-MB-231 cells without photoirradiation were also >100 μM, and those with photoirradiation were 6.0, 7.0, 7.0, 6.5 and 5.0 μM, respectively. These results indicate that the quinoxaline–carbohydrate hybrids **5**–**9** are themselves non-toxic while they show high cytotoxic activity with photoirradiation. Furthermore, these results also demonstrate that the DNA-cleaving activity induced by photoirradiation significantly affects the cytotoxicity of the hybrids, and the life of cancer cells can be controlled by treatment with an appropriate amount of the quinoxaline–carbohydrate hybrid with or without the photoirradiation.

Table 1. Cytotoxicity of quinoxaline–carbohydrate hybrids against HeLa S3 and MDA-MB-231 cells

Compounds		**5**	**6**	**7**	**8**	**9**
IC_{50}(μM)	Without UV	>100	>100	>100	>100	>100
Hela S3	With UV	1.5	1.3	1.0	4.0	4.0
IC_{50}(μM)	Without UV	>100	>100	>100	>100	>100
MDA-MB-231	With UV	6.0	7.0	7.0	6.0	5.0

3. Conclusions

In summary, the present study demonstrates not only the molecular design and chemical synthesis of novel quinoxaline–carbohydrate hybrids, but also their DNA photocleavage and cytotoxic profiles. The described chemistry and biological evaluation provided significant information about the molecular design of novel and selective DNA photocleaving and cytotoxic agents.

4. Experimental

4.1. General synthetic methods

Melting points are uncorrected. ^{1}H NMR spectra were

measured in $CDCl_3$ using TMS as internal standard unless otherwise noted. Silica gel TLC and column chromatography were performed on Merck TLC 60F-254 (0.25 mm) and Merck Kieselgel 60 or Fuji-Davison BW-820MH, respectively. Air- and/or moisture-sensitive reactions were carried out under an atmosphere of argon with oven-dried glassware. In general, organic solvents were purified and dried by the appropriate procedure, and evaporation and concentration were carried out under reduced pressure below 30°C, unless otherwise noted.

4.1.1. Phenyl 3-Azide-2,3,6-trideoxy-1-thio-D-*arabino*-hexopyranoside (11). To a stirred solution of **10** (0.120 g, 0.641 mmol) in dry PhSH (0.36 mL) was added dropwise $BF_3{\cdot}Et_2O$ (0.122 mL, 0.961 mmol) at 0°C. The reaction mixture was stirred at 0°C for 50 min before the addition of Et_3N (1.15 mL). The resultant mixture was concentrated in vacuo. Purification of the residue by column chromatography (58 g of silica gel, 5/2 *n*-hexane/EtOAc) gave **11** (0.168 g, 99%) as a pale yellow syrup. R_f 0.35 (3/1 *n*-hexane/EtOAc); ^{1}H NMR δ 1.31 (4/5×3H, d, J=6.0 Hz), 1.37 (1/5×3H, d, J=6.0 Hz), 1.81 (1/5×1H, ddd, J=12.0, 12.0, 12.0 Hz), 2.12 (4/5×1H, ddd, J=13.8, 12.6, 6.0 Hz), 2.21 (1H, d, J=4.0 Hz), 2.37 (1/5×1H, ddd, J=12.0, 5.2, 2.0 Hz), 2.38 (4/5×1H, ddd, J=13.8, 5.0, 1.0 Hz), 3.16 (1/5×1H, ddd, J=9.0, 9.0, 4.0 Hz), 3.19 (4/5×1H, ddd, J=9.0, 9.0, 4.0 Hz), 3.38 (1/5×1H, dq, J=9.0, 6.0 Hz), 3.48 (1/5×1H, ddd, J=12.0, 9.0, 5.2 Hz), 3.77 (4/5×1H, ddd, J=12.6, 9.0, 5.0 Hz), 4.20 (4/5×1H, dq, J=9.0, 6.0 Hz), 4.48 (1/5×1H, dd, J=12.0, 2,0 Hz), 5.57 (4/5×1H, dd, J=6.0, 1.0 Hz), 7.24–7.34 (3H, m), 7.42–7.52 (2H, m); HRMS (EI) *m/z* 265.0883 (265.0885 calcd for $C_{12}H_{15}N_3O_2S$, M^+).

4.1.2. Phenyl 3-Azide-4-*O*-*tert*-butyldimethylsilyl-2,3,6-trideoxy-1-thio-D-*arabino*-hexopyranoside (12). To a stirred solution of **11** (1.12 g, 4.22 mmol) in dry CH_2Cl_2 (22.4 mL) were added imidazole (1.43 g, 21.1 mmol) followed by TBSCl (2.11 g, 14.0 mmol) at 0°C. The reaction mixture was stirred at 40°C for 24 h before the addition of cold saturated aqueous NH_4Cl (30 mL). The resultant mixture was extracted with EtOAc (30 mL×3) and the extracts were washed with brine (30 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (64 g of silica gel, 10/1 *n*-hexane/EtOAc) gave **12** (1.51 g, 94%) as a pale yellow syrup. R_f 0.60 (4/1 *n*-hexane/EtOAc); ^{1}H NMR δ 0.10 (1/5×3H, s), 0.12 (4/5×3H, s), 0.18 (1/5×3H, s), 0.21 (4/5×3H, s), 0.90 (1/5×9H, s), 0.94 (4/5×9H, s), 1.27 (4/5×3H, d, J=6.0 Hz), 1.31 (1/5×3H, d, J=6.0 Hz), 1.82 (1/5×1H, ddd, J=12.0, 12.0, 12.0 Hz), 2.13 (4/5×1H, ddd, J=13.8, 12.6, 5.9 Hz), 2.33–2.44 (1H, m), 3.07 (1/5×1H, dd, J=9.0, 9.0 Hz), 3.11 (4/5×1H, dd, J=9.0, 9.0 Hz), 3.25–3.42 (1/5×2H, m), 3.64 (4/5×1H, ddd, J=12.6, 9.0, 5.0 Hz), 4.14 (4/5×1H, dq, J=9.0, 6.0 Hz), 4.78 (1/4×1H, dd, J=12.0, 2.0 Hz), 5.55 (4/5×1H, dd, J=5.9, 1.0 Hz), 7.23–7.35 (3H, m), 7.40–7.52 (2H, m); HRMS (EI) *m/z* 379.1773 (379.1750 calcd for $C_{18}H_{29}N_3O_2SSi$, M^+).

4.1.3. 2-Hydroxyethyl 3-Azide-4-*O*-*tert*-butyldimethylsilyl-2,3,6-trideoxy-D-*arabino*-hexopyranoside (14). To a stirred mixture of **12** (0.480 g, 1.26 mmol), ethylene glycol (**13**) (0.352 mL, 6.32 mmol) and MS 4A (0.480 g) in dry MeCN (12.6 mL) was added NBS (0.336 g, 1.89 mmol) at 0°C. The reaction mixture was stirred at 0°C for 1 h and poured into ice-cold 10% $NaHSO_4$ aq. (20 mL). The resultant mixture was extracted with EtOAc (20 mL×3) and the extracts were washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (20 g of silica gel, 1/1 *n*-hexane/EtOAc) gave **14** (0.325 g, 79%, α/β=1.4/1) as a colorless syrup. R_f 0.46 and 0.47 (4/1 *n*-hexane/EtOAc); ^{1}H NMR δ 0.11 (3/5×6H, s), 0.20 (2/5×6H, s), 0.91 (2/5×9H, s), 0.92 (3/5×9H, s), 1.23 (3/5×3H, d, J=6.2 Hz), 1.28 (2/5×3H, d, J=6.0 Hz), 1.62–1.80 (1H, m), 2.14–2.33 (8/5H, m), 2.74 (2/5×1H, br t, J=6.0 Hz), 3.07 (3/5×1H, dd, J=9.0, 9.0 Hz), 3.08 (2/5×1H, dd, J=9.0, 9.0 Hz), 3.27–3.38 (2/5×2H, m), 3.53–3.85 (26/5H, m), 4.52 (2/5×1H, dd, J=9.6, 1.8 Hz), 4.86 (3/5×1H, dd, J=3.6, 0.4 Hz); HRMS (EI) *m/z* 331.1949 (331.1927 calcd for $C_{14}H_{29}N_3O_4Si$, M^+).

4.1.4. 2-Hydroxyethyl 4-*O*-*tert*-Butyldimethylsilyl-3-*N*,*N*′-dimethylamino-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (15). To a solution of **14** (0.303 g, 0.914 mol) and 35% HCHO aq. (1.57 mL, 18.3 mmol) in MeOH (30.3 mL) was added 10% Pd–C (0.152 g). After the mixture was vigorously stirred at 25°C for 12 h under H_2, the mixture was filtered and the catalyst was washed with MeOH. The combined filtrate and washings were concentrated in vacuo. Purification of the residue by column chromatography (40 g of silica gel, 1/1 *n*-hexane/EtOAc) gave **15** (0.145 g, 47%) as a colorless syrup and its β-anomer (0.103 g, 34%) as a colorless syrup.

Compound **15**. R_f 0.45 (1/1 *n*-hexane/EtOAc); $[\alpha]_D^{28}$=+49.3° (*c* 1.24, $CHCl_3$); ^{1}H NMR δ 0.04 (3H, s), 0.07 (3H, s), 0.86 (9H, s), 1.25 (3H, d, J=6.0 Hz), 1.59 (1H, ddd, J=13.0, 13.0, 3.9 Hz), 1.90 (1H, br dd, J=13.0, 4.4 Hz), 2.04 (1H, 6H, s), 2.76 (1H, ddd, J=13.0, 9.0, 4.4 Hz), 3.14 (1H, dd, J=9.0, 9.0 Hz), 3.55–3.75 (5H, m), 4.88 (1H, br d, J=3.9 Hz); HRMS (EI) *m/z* 333.2319 (333.2335 calcd for $C_{16}H_{35}NO_4Si$, M^+). The β-anomer of **15**: R_f 0.50 (1/1 *n*-hexane/EtOAc); $[\alpha]_D^{28}$=−19.1° (*c* 3.15, $CHCl_3$); ^{1}H NMR δ 0.04 (3H, s), 0.06 (3H, s), 0.86 (9H, s), 1.32 (3H, d, J=6.0 Hz), 1.49 (1H, ddd, J=12.6, 12.6, 9.8 Hz), 1.99 (1H, ddd, J=12.6, 4.4, 2.0 Hz), 2.20 (6H, s), 2.45 (1H, ddd, J=12.6, 9.0, 4.4 Hz), 3.00 (1H, br t), 3.10 (1H, dd, J=9.0, 9.0 Hz), 3.27 (1H, dq, J=9.0, 6.0 Hz), 3.60–3.90 (4H, m), 4.45 (1H, dd, J=9.8, 2.0 Hz); HRMS (EI) *m/z* 333.2339 (333.2335 calcd for $C_{16}H_{35}NO_4Si$, M^+).

4.1.5. [2-(2-Quinoxaloyloxy)ethyl] 4-*O*-*tert*-Butyldimethylsilyl-3-*N*,*N*′-dimethylamino-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (17). To a stirred solution of **15** (0.193 g, 0.579 mmol) in dry CH_2Cl_2 (7.7 mL) were added Et_3N (0.323 mL, 2.32 mmol) and 2-quinoxaloyl chloride (0.334 g, 1.74 mmol) at 0°C. The reaction mixture was stirred at 25°C for 15 h before the addition of ice-cold saturated aqueous $NaHCO_3$ (15 mL). The resultant mixture was extracted with Et_2O (15 mL×3) and the extracts were washed with brine (15 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (30 g of silica gel, 2/1 *n*-hexane/EtOAc) gave **17** (0.270 g, 94%) as a colorless syrup. R_f 0.58

(1/1 *n*-hexane/EtOAc); $[\alpha]_D^{28}$=+36.1° (*c* 1.10, $CHCl_3$); ^1H NMR δ 0.01 (3H, s), 0.03 (3H, s), 0.78 (9H, s), 1.24 (3H, d, *J*=6.0 Hz), 1.48–1.56 (1H, m), 1.88 (1H, br dd, *J*=12.4, 4.0 Hz), 2.17 (3H, br s), 2.70–2.84 (1H, m), 3.10 (1H, dd, *J*=9.0, 9.0 Hz), 3.63 (1H, dq, *J*=9.0, 6.0 Hz), 3.80–3.90 (1H, m), 3.98–4.08 (1H, m), 4.56–4.70 (1H, m), 4.72–4.82 (1H, m), 4.97 (1H, br d, *J*=2.8 Hz), 7.86 (1H, ddd, *J*=8.0, 8.0, 1.6 Hz), 7.92 (1H, ddd, *J*=8.0, 8.0, 1.6 Hz), 8.20 (1H, dd, *J*=8.0, 1.6 Hz), 8.32 (1H, dd, *J*=8.0, 1.6 Hz), 9.54 (1H, s); HRMS (EI) *m/z* 489.2657 (489.2659 calcd for $C_{25}H_{39}N_3O_5Si$, M^+).

4.1.6. [2-(2-Quinoxaloyloxy)ethyl] 3-*N*,*N*′-Dimethylamino-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (5). To a stirred solution of **17** (27. 0 mg, 0.0547 mmol) in dry pyridine (1.35 mL) was added 70% HF/Py (0.243 mL) dropwise at 0°C. The reaction mixture was stirred at 25°C for 12 h and poured into ice-cold saturated aqueous $NaHCO_3$ (6 mL). The resultant mixture was extracted with EtOAc (5 mL×3) and the extracts were washed with brine (5 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (2 g of silica gel, 5/1 $CHCl_3$/MeOH) gave **5** (16.2 mg, 79%) as a colorless syrup. R_f 0.25 (5/1 $CHCl_3$/MeOH); $[\alpha]_D^{28}$=+38.4° (*c* 0.94, $CHCl_3$); ^1H NMR δ 1.29 (3H, d, *J*=6.0 Hz), 1.64 (1H, ddd, *J*=12.4, 12.4, 3.8 Hz), 1.92 (1H, ddd, *J*=12.4, 4.0, 1.0 Hz), 2.32 (6H, s), 2.98 (1H, ddd, *J*=12.4, 9.0, 4.0 Hz), 3.15 (1H, dd, *J*=9.0, 9.0 Hz), 3.78 (1H, dq, *J*=9.0, 6.0 Hz), 3.82–3.94 (1H, m), 4.00–4.10 (1H, m), 4.61–4.79 (2H, m), 5.05 (1H, br d, *J*=3.8 Hz), 7.85–7.97 (2H, m), 8.17–8.23 (1H, m), 8.30–8.36 (1H, m), 9.55 (1H, s); HRMS (EI) *m/z* 375.1820 (375.1794 calcd for $C_{19}H_{25}N_3O_5$, M^+).

4.1.7. [2-(2-Quinoxaloyloxy)ethyl] 3-*N*,*N*′-Dimethylamino-2,3,6-trideoxy-β-D-*arabino*-hexopyranoside (6). The β-anomer **6** was prepared from the β-anomer of **15** in a way similar to that for **5** from **15**. R_f 0.38 (5/1 $CHCl_3$/MeOH); $[\alpha]_D^{28}$=−36.6° (*c* 1.33, $CHCl_3$); ^1H NMR δ 1.35 (3H, d, *J*=6.0 Hz), 1.50 (1H, ddd, *J*=12.2, 12.2, 9.8 Hz), 1.99 (1H, ddd, *J*=12.2, 4.0, 2.0 Hz), 2.25 (6H, s), 2.46 (1H, ddd, *J*=12.2, 9.0, 4.0 Hz), 3.05 (1H, dd, *J*=9.0, 9.0 Hz), 3.32 (1H, dq, *J*=9.0, 6.0 Hz), 3.92–4.02 (1H, m), 4.22–4.32 (1H, m), 4.62 (1H, dd, *J*=9.8, 2.0 Hz), 4.64–4.78 (2H, m), 7.84–7.96 (2H, m), 8.16–8.23 (1H, m), 8.29–8.34 (1H, m), 9.55 (1H, s); HRMS (EI) *m/z* 375.1803 (375.1794 calcd for $C_{19}H_{25}N_3O_5$, M^+).

4.1.8. 2-*tert*-Butyldimethysilyloxyethyl 3-Azide-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (19). To a stirred mixture of **11** (0.183 g, 0.688 mmol), MS 4A (0.183 g) and ethylene glycol (**13**) (0.192 mL, 3.44 mol) in dry MeCN (4.6 mL) was added NBS (0.184 g, 1.03 mmol) at 0°C. The reaction mixture was stirred at 0°C for 15 min and poured into ice-cold 10% $NaHSO_4$ aq. (15 mL). The resultant mixture was extracted with EtOAc (15 mL×3) and the extracts were washed with brine (15 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (10 g of silica gel, 1/5 *n*-hexane/EtOAc) gave **18** (0.120 g, 80%, α/β=1/1.1) as a mixture of the α- and β-anomers. To a stirred solution of **18** (0.120 g, 0.552 mmol) in dry CH_2Cl_2 (2.4 mL) were added imidazole (56.2 mg, 0.828 mmol) followed by TBSCl (99.6 mg, 0.662 mmol) at 0°C. The reaction mixture was stirred at 0°C for 10 min and poured into ice-cold water (5 mL). The resultant mixture was extracted with EtOAc (5 mL×3) and the extracts were washed with brine (5 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (10 g of silica gel, 3/1 *n*-hexane/EtOAc) gave **19** (73.2 mg, 40%) as a colorless syrup and the β-anomer (80.6 mg, 44%) as a colorless syrup. **19**: R_f 0.60 (3/1 *n*-hexane/EtOAc); $[\alpha]_D^{29}$=+82.2° (*c* 1.91, $CHCl_3$); ^1H NMR δ 0.07 (6H, s), 0.89 (9H, s), 1.28 (3H, d, *J*=6.0 Hz), 1.92 (1H, ddd, *J*=12.0, 12.0, 3.8 Hz), 2.18 (1H, ddd, *J*=12.0, 4.4, 1.0 Hz), 3.14 (1H, dd, *J*=9.0, 9.0 Hz), 3.48 (1H, ddd, *J*=10.4, 5.2, 5.2 Hz), 3.64–3.82 (5H, m), 4.88 (1H, br d, *J*=3.8 Hz); HRMS (EI) *m/z* 331.1949 (331.1927 calcd for $C_{14}H_{29}N_3O_4Si$, M^+). The β-anomer of **19**: R_f 0.51 (3/1 *n*-hexane/EtOAc); $[\alpha]_D^{29}$=−43.6° (*c* 1.48, $CHCl_3$); ^1H NMR δ 0.06 (6H, s), 0.89 (9H, s), 1.34 (3H, d, *J*=6.0 Hz), 1.64 (1H, ddd, *J*=12.4, 12.4, 9.8 Hz), 2.20 (1H, d, *J*=4.0 Hz), 2.25 (1H, ddd, *J*=12.4, 5.0, 2.0 Hz), 3.14 (1H, ddd, *J*=9.0, 9.0, 4.0 Hz), 3.24–3.46 (2H, m), 3.56–3.66 (1H, m), 3.70–3.92 (3H, m), 4.59 (1H, dd, *J*=9.8, 2.0 Hz); HRMS (EI) *m/z* 331.1932 (331.1927 calcd for $C_{14}H_{29}N_3O_4Si$, M^+).

4.1.9. 2-*tert*-Butyldimethysilyloxyethyl 3-Amino-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (20). To a solution of **19** (92.2 mg, 0.278 mmol) in EtOH (9.2 mL) was added $Pd/CaCO_3$ (46.1 mg). After the mixture was vigorously stirred at 25°C for 16 h under H_2, the mixture was filtered and the catalyst was washed with EtOH. The combined filtrate and washings were concentrated in vacuo. Purification of the residue by column chromatography (1 g of silica gel, 3/1 $CHCl_3$/MeOH) gave **20** (84.9 mg, 99%) as a colorless syrup. R_f 0.10 (2/1 $CHCl_3$/MeOH); $[\alpha]_D^{27}$=+50.2° (*c* 2.19, $CHCl_3$); ^1H NMR δ 0.06 (6H, s), 0.88 (9H, s), 1.26 (3H, ddd, *J*=12.4, 12.4, 3.6 Hz), 2.00 (1H, br dd, *J*=12.4, 4.4 Hz), 2.06 (3H, br s), 2.87 (1H, dd, *J*=9.0, 9.0 Hz), 3.02 (1H, ddd, *J*=12.4, 9.0, 4.4 Hz), 3.45 (1H, ddd, *J*=10.0, 5.6, 5.6 Hz), 3.60–3.78 (4H, m), 4.81 (1H, br d, *J*=3.6 Hz); HRMS (EI) *m/z* 305.2017 (305.2022 calcd for $C_{14}H_{31}NO_4Si$, M^+).

4.1.10. 2-*tert*-Butyldimethysilyloxyethyl 2,3,6-Trideoxy-3-*N*-*p*-nitrobenzenesulfonylamino-α-D-*arabino*-hexopyranoside (21). To a solution of **20** (0.417 g, 1.36 mmol) in dry CH_2Cl_2 (8.3 mL) were added K_2CO_3 (0.576 g, 4.17 mmol) and *p*-nitrobenzensulfonyl chloride (0.646 g, 3.41 mmol) at 0°C. The reaction mixture was stirred at 25°C for 2 h and poured into ice-cold water (15 mL). The resultant mixture was extracted with $CHCl_3$ (15 mL×3) and the extracts were washed with brine (15 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (25 g of silica gel, 3/1 $CHCl_3$/acetone) gave **5** (0.479 g, 72%) as a colorless syrup. R_f 0.90 (2/1 $CHCl_3$/MeOH); $[\alpha]_D^{28}$=+64.4° (*c* 1.33, $CHCl_3$); ^1H NMR δ 0.02 (3H, s), 0.04 (3H, s), 0.87 (9H, s), 1.24 (3H, d, *J*=6.0 Hz), 1.62 (1H, ddd, *J*=12.4, 12.4, 3.8 Hz), 1.93 (1H, br dd, *J*=12.4, 4.4 Hz), 2.51 (1H, br s), 3.07 (1H, br dd, *J*=9.0, 9.0 Hz), 3.42 (1H, ddd, *J*=10.0, 5.4, 5.4 Hz), 3.50–3.76 (5H, m), 4.75 (1H, br d, *J*=3.8 Hz), 5.10 (1H, br d, *J*=7.2 Hz), 8.07–8.13 (2H), 8.34–8.40 (2H); HRMS (EI) *m/z* 490.1824 (490.1805 calcd for $C_{20}H_{34}N_2O_8SSi$, M^+).

4.1.11. 2-*tert*-Butyldimethysilyloxyethyl 4-*O*-*tert*-Butyldimethylsilyl-2,3,6-trideoxy-3-*N*-*p*-nitrobenzenesulfonylamino-α-D-*arabino*-hexopyranoside (22). To a stirred solution of **21** (0.479 g, 0.975 mmol) in dry CH_2Cl_2 (9.6 mL) were added dropwise 2,6-lutidine (0.454 mL, 3.90 mmol) followed by TBSOTf (0.672 mL, 2.93 mmol) at 0°C. The reaction mixture was stirred at 40°C for 16 h and poured into ice-cold water (10 mL). The resultant mixture was extracted with $CHCl_3$ (10 mL×3) and the extracts were washed with brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (15 g of silica gel, 2/1 *n*-hexane/EtOAc) gave **22** (0.590 g, 100%) as a colorless syrup. R_f 0.80 (3/1 $CHCl_3$/acetone); $[\alpha]_D^{29}$=+53.6° (*c* 1.32, $CHCl_3$); ^{1}H NMR δ 0.05 (3H, s), 0.06 (3H, s), 0.10 (3H, s), 0.18 (3H, s), 0.85 (9H, s), 0.88 (9H, s), 1.18 (3H, d, *J*=6.0 Hz), 1.58 (1H, ddd, *J*=12.4, 12.4, 3.8 Hz), 2.02 (1H, ddd, *J*=12.4, 4.4, 1.0 Hz), 3.12 (1H, dd, *J*=9.0, 9.0 Hz), 3.40 (1H, ddd, *J*=10.0, 5.6, 5.4 Hz), 3.46–3.77 (5H, m), 4.56 (1H, d, *J*=6.2 Hz), 4.71 (1H, dd, *J*=3.8, 1.0 Hz), 8.02–8.10 (2H), 8.32–8.38 (2H); HRMS (EI) *m*/*z* 604.2650 (604.2670 calcd for $C_{26}H_{48}N_2O_8SSi_2$, M^+).

4.1.12. 1,4-Bis(2-*tert*-butyldimethysilyloxyethyl 4-*O*-*tert*-butyldimethylsilyl-2,3,6-trideoxy-3-*N*-*p*-nitrobenzenesulfonylamino-α-D-*arabino*-hexopyranoside)-3-ylmethyl)benzene (24). To a stirred solution of **22** (0.376 g, 0.621 mmol) and *p*-dibromoxylene (82. 0 mg, 0.310 mmol) in dry DMF (7.5 mL) was added K_2CO_3 (0.343 g, 2.48 mmol) at 0°C. The reaction mixture was stirred at 25°C for 3 h and poured into ice-cold water (10 mL). The resultant mixture was extracted with a mixture of *n*-hexane and EtOAc (1/1) (10 mL×3) and the extracts were washed with brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (15 g of silica gel, 50/1 $CHCl_3$/EtOAc) gave **24** (0.165 g, 41%) as white solids. R_f 0.60 (5/1 *n*-hexane/EtOAc). Mp 178–180°C; $[\alpha]_D^{29}$=+28.5° (*c* 1.40, $CHCl_3$); ^{1}H NMR δ 0.04 (6H, s), 0.06 (6H, s), 0.17 (6H, s), 0.21 (6H, s), 0.88 (18H, s), 0.90 (18H, s), 1.25 (6H, d, *J*=6.0 Hz), 1.65 (2H, ddd, *J*=12.4, 4.0, 1.0 Hz), 1.87 (2H, ddd, *J*=12.4, 12.4, 3.6 Hz), 3.43 (2H, ddd, *J*=10.0, 5.2, 5.2 Hz), 3.58–3.76 (10H, m), 4.19 (2H, ddd, *J*=12.4, 9.0, 4.0 Hz), 4.48, 4.63 (each 2H, ABq, *J*=16.0 Hz), 4.72 (2H, br d, *J*=3.6 Hz), 7.10 (4H, s), 7.72–7.79 (4H), 8.12–8.19 (4H); HRMS (EI) *m*/*z* 1310.5825 (1310.5809 calcd for $C_{60}H_{102}N_4O_{16}S_2Si_4$, M^+).

4.1.13. 1,4-Bis(2-*tert*-butyldimethysilyloxyethyl 4-*O*-*tert*-butyldimethylsilyl-2,3,6-trideoxy-3-*N*-*p*-nitrobenzenesulfonylamino-α-D-*arabino*-hexopyranoside)-3-ylmethyl)benzene (25). To a stirred solution of **24** (87.5 mg, 0.0667 mmol) in dry pyridine (4.4 mL) was added 70% HF/Py (0.788 mL) dropwise at 0°C. The reaction mixture was stirred at 25°C for 2 h and poured into ice-cold saturated aqueous $NaHCO_3$ (6 mL). The resultant mixture was extracted with $CHCl_3$ (5 mL×3) and the extracts were washed with brine (5 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (5 g of silica gel, 1/1 *n*-hexane/acetone) gave **25** (68.6 mg, 95%) as a colorless syrup. R_f 0.20 (2/1 *n*-hexane/acetone); $[\alpha]_D^{29}$=+16.1° (*c* 1.18, $CHCl_3$); ^{1}H NMR δ 0.19 (6H, s), 0.25 (6H, s), 1.26 (6H, d, *J*=6.0 Hz), 1.51 (2H, br s), 1.62 (2H, ddd, *J*=12.4, 4.0, 0.8 Hz), 1.98 (2H, ddd, *J*=12.4, 12.4, 3.6 Hz), 3.50 (2H, dq, *J*=9.0, 6.0 Hz), 3.55–3.70 (8H, m), 3.75 (2H, dd, *J*=9.0, 9.0 Hz), 4.03 (2H, ddd, *J*=12.4, 9.0, 4.0 Hz), 4.58, 4.65 (each 2H, ABq, *J*=16.0 Hz), 4.72 (2H, br d, *J*=3.6 Hz), 7.08 (4H), 7.78–7.85 (2H), 8.17–8.24 (2H); HRMS (EI) *m*/*z* 1082.4087 (1082.4080 calcd for $C_{48}H_{74}N_4O_{16}S_2Si_2$, M^+).

4.1.14. 1,4-Bis[2-(2-quinoxaloyloxy)ethyl 4-*O*-*tert*-butyldimethylsilyl-2,3,6-trideoxy-3-*N*-*p*-nitrobenzenesulfonylamino-α-D-*arabino*-hexopyranoside-3-ylmethyl]-benzene (26). To a stirred solution of **25** (23.6 mg, 0.0218 mmol) in dry CH_2Cl_2 (0.472 mL) were added Et_3N (0.0152 mL, 0.109 mmol) and 2-quinoxaloyl chloride (16.8 mg, 0.0871 mmol) at 0°C. The reaction mixture was stirred at 25°C for 20 min and poured into ice-cold water (3 mL). The resultant mixture was extracted with $CHCl_3$ (3 mL×3) and the extracts were washed with brine (3 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (2 g of silica gel, 2/1 $CHCl_3$/EtOAc) gave **26** (28.3 mg, 93%) as pale yellow solids. R_f 0.75 (1/1 *n*-hexane/acetone); $[\alpha]_D^{29}$=+31.0° (*c* 0.91, $CHCl_3$); ^{1}H NMR δ 0.11 (6H, s), 0.16 (6H, s), 0.75 (18H, s), 1.23 (6H, d, *J*=6.0 Hz), 1.63 (2H, br dd, *J*=12.4, 4.0 Hz), 1.86 (2H, ddd, *J*=12.4, 12.4, 3.6 Hz), 3.55–3.70 (4H, m), 3.80 (2H, ddd, *J*=10.0, 6.0, 4.0 Hz), 3.99 (2H, ddd, *J*=10.0, 6.2, 3.9 Hz), 4.06–4.20 (2H, m), 4.41, 4.58 (each 2H, ABq, *J*=16.0 Hz), 4.55–4.65 (2H, m), 4.73 (2H, ddd, *J*=10.0, 6.2, 3.9 Hz), 4.78 (2H, d, *J*=3.6 Hz), 7.08 (4H, s), 7.75–7.93 (8H, m), 8.10–8.27 (8H, m), 9.46 (2H, s); HRMS (EI) *m*/*z* 1394.4710 (1394.4727 calcd for $C_{66}H_{82}N_8O_{18}S_2Si_2$, M^+).

4.1.15. 1,4-Bis[2-(2-quinoxaloyloxy)ethyl 3-amino-4-*O*-*tert*-butyldimethylsilyl-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside-3-ylmethyl]benzene (27). To a stirred solution of **26** (28.3 mg, 0.0203 mmol) in dry DMF (0.566 mL) were added K_2CO_3 (22.4 mg, 0.162 mmol) and PhSH (0.0125 mL, 0.122 mmol) at 0°C. The reaction mixture was stirred at 25°C for 4 h and poured into ice-cold saturated aqueous $NaHCO_3$ (3 mL). The resultant mixture was extracted with a mixture of *n*-hexane and EtOAc (1/1) (3 mL×3) and the extracts were washed with brine (3 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (2 g of silica gel, 1/1 $CHCl_3$/acetone) gave **27** (11.8 mg, 54%) as a colorless syrup. R_f 0.10 (2/5 $CHCl_3$/EtOAc); $[\alpha]_D^{29}$=+14.6° (*c* 1.17, $CHCl_3$); ^{1}H NMR δ −0.03 (6H, s), 0.03 (6H, s), 0.78 (18H, s), 1.18 (6H, d, *J*=6.0 Hz), 1.51 (2H, ddd, *J*=12.4, 12.4, 3.6 Hz), 2.23 (2H, br dd, *J*=12.4, 4.2 Hz), 2.98 (2H, ddd, *J*=12.4, 9.0, 4.2 Hz), 3.16 (2H, dd, *J*=9.0, 9.0 Hz), 3.54, 3.77 (each 2H, ABq, *J*=13.0 Hz), 3.69 (2H, dq, *J*=9.0, 6.0 Hz), 3,80–3.90 (2H, m), 3.98–4.10 (2H, m), 4.60–4.82 (4H, m), 4.94 (2H, d, *J*=3.6 Hz), 7.20 (4H, s), 7.82–7.95 (4H, m), 8.14–8.22 (2H), 8.26–8.34 (2H), 9.55 (2H, s); HRMS (EI) *m*/*z* 1024.5139 (1024.5161 calcd for $C_{54}H_{76}N_6O_{10}Si_2$, M^+).

4.1.16. 1,4-Bis[2-(2-quinoxaloyloxy)ethyl 3-amino-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside-3-ylmethyl]benzene (7). To a stirred solution of **27** (11.8 mg, 0.0115 mmol) in dry pyridine (0.590 mL) was added 70% HF/Py (0.106 mL) dropwise at 0°C. The reaction mixture was

stirred at 25°C for 16 h and poured into ice-cold saturated aqueous $NaHCO_3$ (3 mL). The resultant mixture was extracted with $CHCl_3$ (3 mL×3) and the extracts were washed with brine (3 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (5 g of silica gel, 3/1 $CHCl_3$/MeOH) gave **7** (7.9 mg, 89%) as a colorless syrup. R_f 0.50 (3/1 $CHCl_3$/MeOH); $[\alpha]_D^{29}=+15.0°$ (*c* 1.55, $CHCl_3$); ^1H NMR δ 1.27 (6H, d, *J*=6.0 Hz), 1.48 (2H, ddd, *J*=12.8, 12.8, 4.0 Hz), 2.29 (2H, br dd, *J*=12.8, 3.4 Hz), 2.86–3.05 (4H, m), 3.62, 3.83 (each 2H, ABq, *J*=13.0 Hz), 3.68–3.92 (4H, m), 4.00–4.12 (2H, m), 4.59–4.80 (4H, m), 4.98 (2H, br d, *J*=3.4 Hz), 7.20 (4H, s), 7.83–7.97 (4H, m), 8.16–8.25 (2H), 8.28–8.37 (2H), 9.52 (2H, s); HRMS (EI) *m/z* 796.3450 (796.3432 calcd for $C_{42}H_{48}N_6O_{10}$, M^+).

4.1.17. 1-[2-(2-Quinoxaloyloxy)ethyl 3-amino-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside-3-ylmethyl]-4-[2-(2-quinoxaloyloxy)ethyl 3-amino-2,3,6-trideoxy-β-D-*arabino*-hexopyranoside-3-ylmethyl]benzene (8). 8 was prepared from **19** and the β-anomer in a way similar to that for **7** from **19**. R_f 0.45 (3/1 $CHCl_3$/MeOH); $[\alpha]_D^{28}=-17.4°$ (*c* 1.72, $CHCl_3$); ^1H NMR δ 1.27 (3H, d, *J*=6.0 Hz), 1.32 (3H, d, *J*=6.0 Hz), 1.39 (1H, ddd, *J*=12.2, 12.2, 9.4 Hz), 1.48 (1H, ddd, *J*=12.8, 12.8, 4.0 Hz), 2.29 (1H, br dd, *J*=12.8, 3.4 Hz), 2.36 (1H, ddd, *J*=12.2, 4.0, 2.0 Hz), 2.55 (1H, ddd, *J*=12.2, 9.0, 4.0 Hz), 2.86–3.05 (3H, m), 3.32 (1H, dq, *J*=9.0, 6.0 Hz), 3.62, 3.83 (each 1H, ABq, *J*=13.0 Hz), 3.64, 3.89 (each 1H, ABq, *J*=12.4 Hz), 3.68–4.12 (3H, m), 4.19–4.30 (1H, m), 4.59–4.80 (4H, m), 4.60 (1H, dd, *J*=9.4, 2.0 Hz), 4.98 (2H, br d, *J*=3.4 Hz), 7.22 (4H, s), 7.83–7.97 (4H), 8.16–8.25 (2H), 8.27–8.37 (2H), 9.52 (1H, s), 9.55 (1H, s); HRMS (EI) *m/z* 796.3411 (796.3432 calcd for $C_{42}H_{48}N_6O_{10}$, M^+).

4.1.18. 1,4-Bis-[2-(2-quinoxaloyloxy)ethyl 3-amino-2,3,6-trideoxy-β-D-*arabino*-hexopyranoside-3-ylmethyl]benzene (9). Compound **9** was prepared from the β-anomer of **19** in a way similar to that for **7** from **19**. R_f 0.50 (3/1 $CHCl_3$/MeOH); $[\alpha]_D^{30}=-59.5°$ (*c* 0.95, $CHCl_3$); ^1H NMR δ 1.32 (6H, d, *J*=6.0 Hz), 1.39 (2H, ddd, *J*=12.2, 12.2, 9.4 Hz), 2.36 (2H, ddd, *J*=12.2, 4.0, 2.0 Hz), 2.55 (2H, ddd, *J*=12.2, 9.0, 4.0 Hz), 2.95 (2H, dd, *J*=9.0, 9.0 Hz), 3.32 (2H, dq, *J*=9.0, 6.0 Hz), 3.64, 3.89 (each 2H, ABq, *J*=12.4 Hz), 3.85–4.03 (2H, m), 4.19–4.30 (2H, m), 4.60 (2H, dd, *J*=9.4, 2.0 Hz), 4.65–4.80 (4H, m), 7.25 (4H, s), 7.80–7.97 (4H, m), 8.17–8.23 (2H, m), 8.27–8.35 (2H, m), 9.55 (2H, s); HRMS (EI) *m/z* 796.3427 (796.3432 calcd for $C_{42}H_{48}N_6O_{10}$, M^+).

4.2. DNA cleavage studies

ΦX174 DNA and M13mp18 ss DNA were purchased from Nippon Gene Co., Ltd., and TaKaRa Bio Inc., respectively. XX-15 BLB (UVP Inc.) was used as a UV lamp (365 nm, 15 or 30 W) for photoirradiation.

4.2.1. Assay for damage to DNA. All the DNA cleavage experiments were performed with ΦX174 DNA (50 μM/base pair) in a volume of 6 μL containing 20% acetonitrile in 50 mM Tris–HCl buffer (pH 7.5) at 25°C for 1 h under irradiation of the UV lamp (365 nm, 15 W) placed 10 cm from the mixture. The DNA-sample levels were varied as indicated in the figure captions. The results were analyzed using 0.9% agarose gel electrophoresis and detection with ethidium bromide fluorescence. The electrophoresis gels were immediately visualized on a UV transilluminator and photographed using black and white instant film. Figures 3 and 5 show the pictures of the agarose gel electrophoresis results.

4.2.2. Identification of DNA cleavage sites. The reaction samples contained the compounds (500 μM) and the 5′-end-labelled M13mp18 DNA (40 ng) in a volume of 30 μL containing 20% acetonitrile in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.5). The cleavage reactions were allowed to proceed under the same conditions described above. To stop the reactions, each reaction sample was washed with a solution of TE buffer-saturated phenol–chloroform–isoamyl alcohol (25:24:1) and the resulting aqueous layer was lyophilized. After each lyophilized sample was dissolved in 1 M piperidine–water (20 μL) and then heated at 90°C for 30 min, each sample was again lyophilized. Each lyophilized sample was dissolved in a loading buffer containing distilled water, 95% deionized formamide, 10 mM EDTA, 0.05% xylene cyanole FF, and 0.05% bromophenol blue and then the mixture was loaded onto a 12% polyacrylamide gel containing 8 M urea in TBE buffer. DNA sequencing was carried out by the Sanger method. Figure 6 shows a picture of the autoradiogram.

4.2.3. Assay for DNA cleavage in cells. HeLa S3 cells were treated with the compounds (10 μM) with or without photoirradiation at 25°C for 1 h. The UV lamp (365 nm, 30 W) was placed at 25 cm from the mixture. After the cells were cultured for 72 h at 37°C, the DNA was extracted from cells and analyzed by gel electrophoresis in 1.5% agarose gel. The gel was stained with ethidium bromide, visualized on a UV transilluminator and photographed using black and white instant film. Figure 7 shows the pictures of the agarose gel electrophoresis results. In this experiment, it was confirmed that UV irradiation alone under these conditions did not influence the cell survival, and the DNA cleaving activity of each compound under these conditions is similar to that under the conditions mentioned in Section 4.2.1.

4.3. Cytotoxicity assay

The cells were treated with each compound at the concentrations of 300, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03 and 0.01 μM with photoirradiation at 25°C for 1 h with a UV lamp (365 nm, 30 W) placed 25 cm from the mixture, and incubated for 72 h at 37°C. The cell viability was determined using the XTT-tetrazolium assay described by Scudiero. Table 1 indicated the IC_{50} values of each compound against the HeLa S3 and MDA-MB-231 cells.

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