a Spex Fluorolog II spectrofluorimeter. Details of the correction of emission spectra and the determination of luminescence quantum yields were as reported previously.[14] Luminescence lifetimes on the nanosecond timescale were determined with an IBH single photon counting apparatus ($\lambda_{exc} = 337$ nm) or a single-shot Nd:YAG laser apparatus ($\lambda_{exc} = 532$ nm). Transient absorption spectra and lifetimes with picosecond and nanosecond resolution were obtained with two pump and probe systems based on Nd:YAG lasers; excitation with the second (532 nm) or third harmonic (355 nm) was used. Details of this time-resolved spectroscopy equipment were reported earlier.[14] Experimental uncertainties were estimated to be $\pm 8\%$ for lifetime determination, $\pm 20\%$ for quantum yields, and ± 3 nm for emission and absorption peaks.

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2-Phenylquinoline – Carbohydrate Hybrids: Molecular Design, Chemical Synthesis, and Evaluation of a New Family of Light-Activatable DNA-Cleaving Agents**

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The development of photochemical DNA-cleaving agents, which selectively cleave DNA by irradiation with light with a specific wavelength under mild conditions and without any additives such as metals and reducing agents, is very interest-

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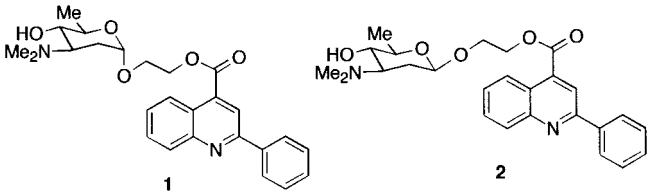
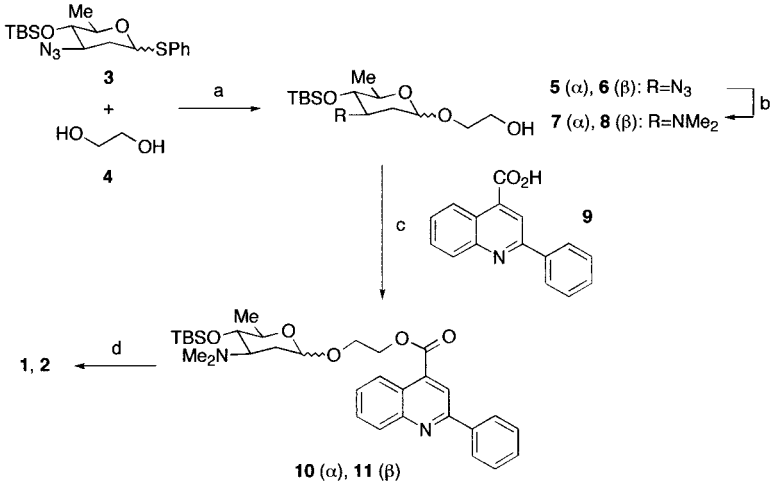
ing from both chemical and biological standpoints and offers considerable potential in medicine.[1] Here, we discuss the molecular design, chemical synthesis, DNA-photocleaving properties, and cytotoxicity of two novel and artificial light-activatable DNA-cleaving agents, namely, the 2-phenylquinoline–carbohydrate hybrids **1** and **2**.



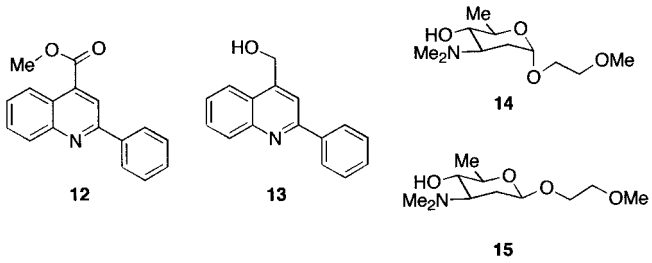
In our approach to create novel DNA-cleaving molecules, we designed artificial intercalator–carbohydrate hybrid systems,[2–4] because many clinically useful antitumor antibiotics such as anthracyclines[5] and aureolic acids,[6] which interact with DNA, were commonly found to contain both aromatic and carbohydrate domains. Since Denny et al.[7] demonstrated the efficacy of 2+1 unfused tricyclic aromatic systems such as phenylquinolines as "minimal intercalators", 2-phenylquinoline[8, 9] was selected as the DNA intercalator. The conjugated C=N bond in the 2-phenylquinoline unit was also expected to generate the photoexcited $^3(n \rightarrow \pi^*)$ state upon photoirradiation, which may have a radical character and could be capable of cleaving DNA. On the other hand, certain 2,6-dideoxy amino sugars seemed appropriate as the carbohydrate source, since they are DNA groove binders in some DNA-binding antitumor antibiotics[10] and our previously reported artificial DNA-interactive intercalator–carbohydate molecules.[4] Therefore, we designed novel, artificial intercalator–carbohydrate hybrids that consist of 2-phenylquinoline and a 2,6-dideoxy amino sugar, which are connected by an ethylene glycol linker.

The designed 2-phenylquinoline–carbohydrate hybrids **1** and **2** were synthesized by a short reaction sequence (Scheme 1). Thus, the glycosidation of the phenylthio sugar **3**[11] (1.0 equiv) with ethylene glycol (**4**, 5.0 equiv) using *N*-bromosuccinimide (NBS)[12] in MeCN gave a mixture of the α-glycoside **5** and the β-glycoside **6** in 79% yield in a ratio of 1.4:1. The azide groups in **5** and **6** were next converted into *N*,*N*-dimethylamino groups by treatment with 35% HCHO (aq) and a catalytic amount of Pd/C in MeOH under a hydrogen atmosphere to afford **7** and **8** in 81% yield. After their separation by column chromatography, **7** (1.0 equiv) and **8** (1.0 equiv) were esterified with 2-phenylquinoline-4-carboxylic acid (**9**, 1.5 equiv) by using *N*,*N*-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (4-DMAP) in CH_2Cl_2 to give the hybrids **10** and **11** in 93 and 90% yields, respectively. Finally, removal of the silyl groups in **10** and **11** with HF/pyridine (Py) furnished the desired 2-phenylquinoline–carbohydrate hybrids **1** and **2**, respectively, in high yields.



Scheme 1. Synthesis of **1** and **2**. a) NBS (1.5 equiv with respect to **3**), 4-Å molecular sieves, MeCN, 0 °C, 1 h, 79%, **5**:**6** = 1.4:1; b) H_2, cat. Pd/C, 35% HCHO (aq). MeOH, 25 °C, 18 h, 81%; c) **9**, DCC (1.0 equiv), cat. 4-DMAP, CH_2Cl_2, 0 °C, 4.5 h, 93% for **10**, 90% for **11**; d) HF/Py, Py, 25 °C, 15 h, 99% for **1**, 94% for **2**. TBS = *tert*-butyldimethylsilyl.

The photoinduced DNA-cleaving activities of the hybrids **1** and **2** along with the components of these hybrids, **12**–**15**,[11] were assayed with supercoiled ΦX174 DNA. As apparent



from Figure 1, the 2-phenylquinoline–carbohydrate hybrids **1** (500 μM) and **2** (500 μM) caused significant cleavage of DNA, leading to small fragments upon photoirradiation with long-wavelength UV light (365 nm), while **12**–**15** did not show

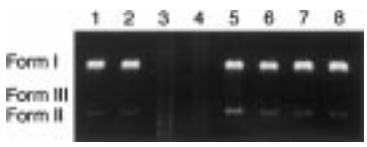


Figure 1. Photocleavage of supercoiled ΦX174DNA. ΦX174DNA (50 μM per base pair) was incubated with various compounds in 20% acetonitrile in Tris–HCl buffer (Tris = tris(hydroxymethyl)aminomethane, pH 7.5, 50 mM) at 25 °C for 1 h under UV irradiation (365 nm, 15 W) from a lamp placed 10 cm from the mixture, and analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain). Lane 1: DNA alone; lane 2: DNA with irradiation; lanes 3–8: compounds **1**, **2**, **12**, **13**, **14**, and **15** (500 μM), respectively. Form I = covalently closed supercoiled DNA, Form II = open circular DNA, and Form III = linear DNA.

DNA-cleaving activity under the same conditions. These results clearly indicate the importance of the hybrid structure for DNA cleaving. These results also strongly suggest that the 2,6-dideoxy amino sugar works as the DNA groove binder and significantly enhances the intercalating ability of the 2-phenylquinoline. In the absence of light no DNA cleavage by **1** and **2** was observed. Furthermore, the DNA-cleaving ability of the β-anomer hybrid **2** was found to be stronger than that of the α-anomer hybrid **1** (Figure 2). This result demon-

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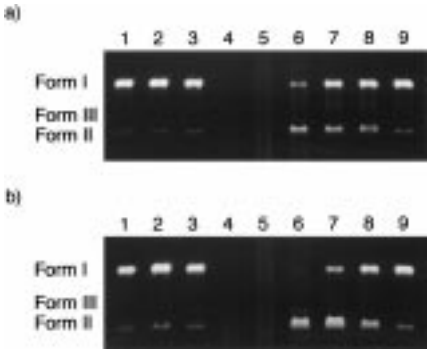

Figure 2. Photocleavage of supercoiled ΦX174DNA. ΦX174DNA (50 μM per base pair) was incubated with the hybrid in 20% acetonitrile in Tris–HCl buffer (pH 7.5, 50 mM) at 25 °C for 1 h under UV irradiation (365 nm, 15 W) from a lamp placed 10 cm from the mixture, and analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain). a) Lane 1: DNA alone; lane 2: DNA with irradiation; lane 3: DNA + **1** without irradiation; lanes 4–9: **1** (1000), **1** (500), **1** (300), **1** (100), **1** (30), and **1** (10 μM), respectively. b) Lane 1: DNA alone; lane 2: DNA with irradiation; lane 3: DNA + **2** without irradiation; lanes 4–9: **2** (1000), **2** (500), **2** (300), **2** (100), **2** (30), and **2** (10 μM), respectively.

strates that the DNA-cleaving activity is definitely dependent on the configuration of the sugar moiety in the hybrid. Since the DNA-cleaving activity of **1** and **2** significantly decreased in the presence of a radical scavenger, dimethyl sulfoxide, the DNA cleavage must arise from the photoexcited 2-phenylquinoline radical. The DNA-cleaving site specificity of the hybrids **1** and **2** was also analyzed according to the Sanger protocol.[13] The results in Figure 3 clearly show the identical high guanine selectivity.

The cytotoxicity of the DNA-cleaving hybrids **1** and **2** was next examined using HeLa S3 cells exposed to each agent for 72 h with or without 1 h of photoirradiation.[14] The IC_{50} values of **1** and **2** without photoirradiation were 12 and 18 μM, respectively, and those with photoirradiation were 0.44 and 0.25 μM, respectively. These results indicate that the cytotoxic activities of **1** and **2** with photoirradiation were much higher than those without, and correlated with their capacity to cleave DNA. Furthermore, we found that when the HeLa S3 cells were exposed to 1 μM of hybrid **1** or **2** without photoirradiation, practically all of the cells survived, while similar treatment combined with photoirradiation wiped out the cells. These results clearly show that the DNA-cleaving activity induced by photoirradiation significantly affects the cytotoxicity of the hybrids, and the life of the cancer cells can be controlled by treatment with an appropriate amount of the 2-phenylquinoline–carbohydrate hybrid with or without the photoirradiation.

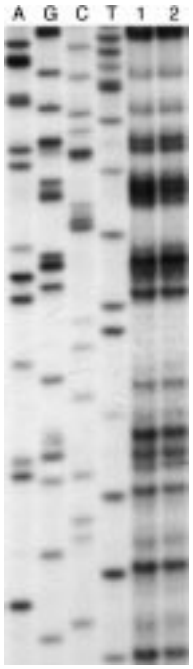

Figure 3. Autoradiogram of slab gel electrophoresis with 12% polyacrylamide/8 M urea for sequence analysis. The 5′-end-labeled M13mp18 DNA was cleaved by the hybrids at pH 7.5 and 25 °C for 1 h under UV irradiation (365 nm, 15 W) from a lamp placed 10 cm from the mixture (bases 49–105 are shown). Lanes A, G, C, and T: Sanger A, G, C, and T reactions, respectively; lanes 1 and 2: **1** and **2** (1000 μM), respectively.

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