

Novel Anthraquinone-Carbohydrate Hybrids: The Significant Improvement of the DNA Photocleaving Activity and the Cytotoxic Selectivity

Kazunobu Toshima*, Yoko Nakajima, Yutaka Maeda and Shuichi Matsumura

Department of Applied Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan

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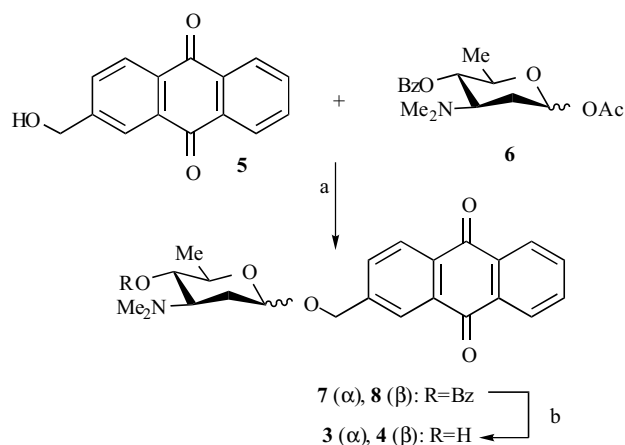
Abstract: Novel anthraquinone-carbohydrate hybrids were designed and synthesized as artificial DNA photocleavers. They were found to show high DNA cleaving activity under irradiation with a long wavelength UV light without any additives and also exhibited selective cytotoxicity against HeLa S3 cancer cells with the photoirradiation.

Key words: Anthraquinone, Carbohydrate, Photoirradiation, DNA cleavage, Cancer cells, Cytotoxicity.

The 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 in the design of new DNA targeting antitumor drugs [1]. In this context, the development of photochemical DNA cleaving agents, which selectively cleave DNA by irradiation with a specific light under mild conditions 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 [2]. Schuster and co-workers elegantly demonstrated the efficacy of the suitably substituted anthraquinones as DNA photocleavers [3]. On the other hand, in our previous studies, we reported that the artificial glycosyl anthraquinones **1** and **2** caused DNA photocleavage, and demonstrated the significance of the hybrid structure consisting of the 1-hydroxyanthraquinone and the deoxyamino sugar for the DNA cleavage [4]. In this communication, we disclose the molecular design, chemical synthesis, DNA photocleaving property and cytotoxicity of the newly designed anthraquinone-carbohydrate hybrids **3** and **4**, and the significant improvement of the DNA cleaving activity and the cytotoxic selectivity against cancer cells with photoirradiation (Fig. (1)).

In our novel approach to create effective DNA photocleaving agents, we designed novel anthraquinone-carbohydrate hybrids **3** and **4**, which are anomers of each other. These new hybrids lack the aromatic hydroxy group in the previously reported anthraquinone-carbohydrate hybrids **1** and **2**. We anticipated that the aromatic hydroxy group in **1** and **2**, which forms the hydrogen bond with one of the quinone carbonyl oxygens, would significantly prevent the photoexcitation of the anthraquinone. Therefore, the newly designed anthraquinone-carbohydrate hybrids **3** and **4** would have stronger DNA photocleaving activities than **1** and **2**. The anthraquinone-carbohydrate hybrids **3** and **4** were

synthesized by a short reaction sequence *via* the effective glycosidation reaction of the commercially available 2-hydroxymethylanthraquinone **5** and the 1-OAc sugars **6** [4] (Scheme 1). Thus, the glycosidation of **5** and **6** using TMSOTf as an activator in the presence of MS 4A in THF at 0 °C for 2 h smoothly proceeded to give the α -glycoside **7** and the β -glycoside **8** in 91% yield in a ratio of 2.3:1. After the separation and isolation of each anomer by column chromatography, the deprotection of the benzoyl group in **7** and **8** using NaOMe in MeOH at 60 °C for 2.5 h afforded the desired hybrids **3** and **4** in 76% and 88% yields, respectively.



Scheme 1. Synthesis of **3** and **4**. a) TMSOTf, MS 4A, THF, 0 °C, 2 h, 91% ($\alpha/\beta=2.3/1$); b) NaOMe, MeOH, 60 °C, 2.5 h, 76% for **3**, 88% for **4**.

Footnote: $^1\text{H-NMR}$ (270 Hz, CDCl_3) (δ , SiM₄; $J=\text{Hz}$); **3**: δ 1.32 (3H, d, $J=6.2$), 1.67 (1H, ddd, $J=12.8, 12.8$ and 4.0), 1.98 (1H, ddd, $J=12.8, 4.0$ and 1.2), 2.31 (6H, s), 2.98 (1H, ddd, $J=12.8, 10.0$ and 4.0), 3.16 (1H, dd, $J=10.0$ and 10.0), 3.72 (1H, dq, $J=10.0$ and 6.2), 4.65 and 4.84 (each 1H, ABq, $J=13.6$), 5.06 (1H, dd, $J=4.0$ and 1.2), 7.75-7.85 (3H, m), 8.25-8.36 (4H, m); **4**: δ 1.40 (3H, d, $J=6.0$), 1.61 (1H, ddd, $J=12.6, 12.6$ and 9.8), 2.07 (1H, ddd, $J=12.6, 3.9$ and 2.0), 2.32 (6H, s), 2.53 (1H, ddd, $J=12.6, 9.6$ and 9.6), 3.12 (1H, dd, $J=9.6$ and 9.6), 3.35 (1H, dq, $J=9.6$ and 2.0), 4.64 (1H, dd, $J=9.8$ and 2.0), 4.74 and 5.08 (each 1H, ABq, $J=13.2$), 7.76-7.84 (3H, m), 8.26-8.36 (4H, m).

*Address correspondence to this author at the 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

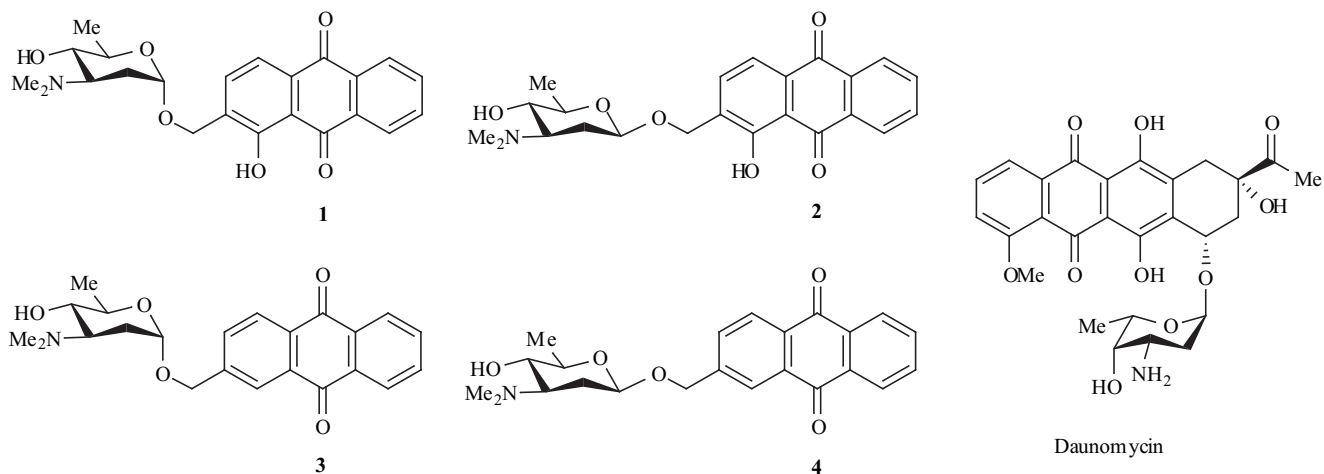


Fig. (1). The molecular structures of anthraquinone-carbohydrate hybrids and daunomycin.

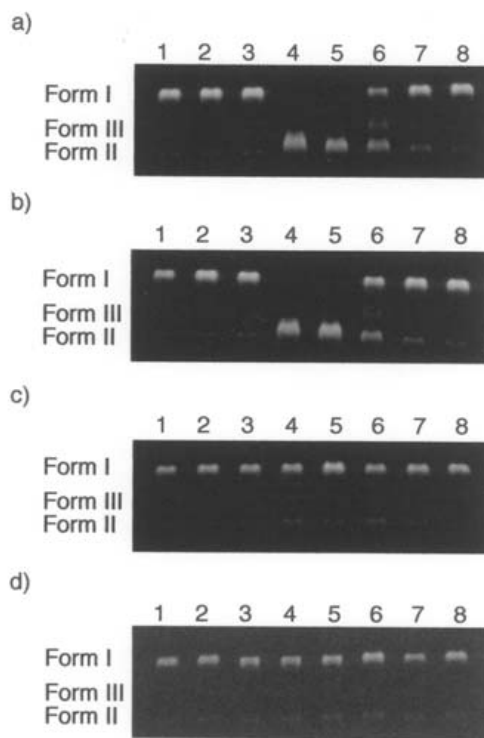


Fig. (2). Photocleavage of supercoiled Φ X174 DNA. Φ X174 DNA (50 μ M per base pair) was incubated with the compound in 20% acetonitrile in Tris-HCl buffer (pH 7.5, 50 mM) at 25 $^{\circ}$ C for 2 h under irradiation of the UV lamp (365 nm, 15 W) placed at 10 cm from the mixture, and analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain): a), b), c) and d) for the compounds **3**, **4**, **5**, and daunomycin, respectively: lane 1, DNA alone; lane 2, DNA with UV; lane 3, DNA+compound (10 μ M) without UV; lanes 4-8, compound (10), compound (3), compound (1), compound (0.3), and compound (0.1 μ M), respectively, following UV irradiation. Form I: covalently closed supercoiled DNA, Form II: open circular DNA, and Form III: linear DNA.

With the newly designed anthraquinone-carbohydrate hybrids in hand, the photoinduced DNA cleaving activities of these hybrids **3** and **4** along with the reference compound, 2-hydroxymethylanthraquinone **5**, were assayed using supercoiled Φ X174 DNA in concentrations of 10-0.1 μ M. As clear from Fig. (2), the anthraquinone-carbohydrate hybrids **3** and **4** showed a very high DNA cleaving activity under the photoirradiation with a long wavelength UV light (365 nm) (a) and b) in Fig. (2)), while **5** showed little DNA cleaving activity under the same conditions (c) in Fig. (2)). It was confirmed that no DNA cleavage by **3-5** was observed in the absence of light (lanes 3 in Fig. (2)). Thus, the UV light functioned as a trigger to initiate these anthraquinone derivatives for the DNA strand scission. Surprisingly, the DNA cleaving abilities of **3** and **4** were found to be about 300 times higher than those of **1** and **2** [4], cleaved DNA in concentrations over 0.3 μ M, and caused a 100% DNA break at concentrations over 3 μ M. These results clearly indicate the importance of the lack of the hydroxy group at the C1 position of the anthraquinone moiety for the DNA cleavage as we expected. Furthermore, it was confirmed that the hybrid structure constructed from the anthraquinone and the deoxyamino sugar was very effective for the DNA cleavage. In addition, the DNA cleaving ability was dependent on the configuration of the anomeric position of the hybrid, and the DNA cleaving activity of the α -anomer **3** is higher than that of the β -anomer **4**. At this stage, our attention turned to the comparison of the designed compounds with natural product in terms of their efficacy as DNA photocleavers. It was interesting to note that the DNA photocleaving abilities of these hybrids were much higher than that of the natural anthraquinone antibiotic, daunomycin [5] (a) and b) vs. d) in Fig. (2)). These results demonstrate that the artificial anthraquinone-carbohydrate hybrid is superior to the natural product possessing the anthraquinone structure as a DNA photocleaving agent.

The DNA cleaving site specificity of hybrids **3** and **4** was also analyzed according to the Sanger protocol [6]. 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 [7]. The results shown in Fig. (3) clearly indicated that

hybrids **3** and **4** selectively cleaved DNA at the guanine site of the DNA, and the site-selective DNA cleavage was enhanced upon treatment with hot piperidine. Since the free radical scavenger, dimethyl sulfoxide, did not inhibit the DNA cleavage, while the singlet oxygen scavenger, 2,2,6,6-tetramethylpiperidine, significantly prevented this event, it is very likely that the oxidation of the guanine by singlet oxygen generated from the photoexcited anthraquinone is the initial step for the photo-induced destruction of the guanine base [2].

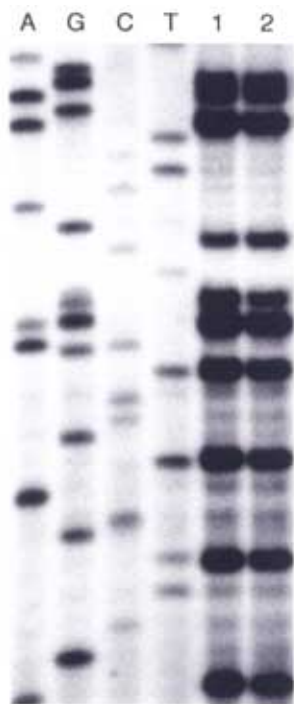


Fig. (3). Autoradiogram of 12% polyacrylamide-8 M urea slab gel electrophoresis for sequence analysis. The 5'-end-labeled M13mp18 DNA at the primer site was cleaved by the compound at pH 7.5 and 25 °C for 2 h under irradiation of the UV lamp (365 nm, 15 W) placed at 10 cm from the mixture: lanes A, G, C and T; Sanger A, G, C and T reactions, respectively; lanes 1 and 2; the compounds **3** and **4** (10 μ M), respectively, following UV irradiation: DNAs for lanes 1 and 2 were treated with hot piperidine prior to gel electrophoresis.

The cytotoxicity of the DNA cleaving hybrids **1-4** was next examined using HeLa S3 cancer cells exposed to each agent for 72 h with or without 1 h of photoirradiation [8]. It was confirmed that when the HeLa S3 cells were exposed to

1 μ M of the hybrids **1-4** without photoirradiation, practically all of the cells survived. In addition, when the HeLa S3 cells were treated with 1 μ M of **1** and **2** combined with photoirradiation, 70-80% of the cells survived. In drastic contrast, similar treatment of **3** and **4** wiped out the cells. These results indicate that the cytotoxic activities of **3** and **4** with photoirradiation are significantly higher than those of **1** and **2**, and the DNA cleaving activity by the photoirradiation strongly affects the cytotoxicity of the hybrids. Furthermore, these results also demonstrate that the life of the cancer cells can be controlled by treatment with the appropriate amount of the novel anthraquinone-carbohydrate hybrids.

In summary, the present work demonstrates not only the molecular design and chemical synthesis of novel anthraquinone-carbohydrate hybrids, but also their DNA photocleavage profiles and cytotoxic activities. The described chemistry and biological evaluation provided significant information about the molecular design of novel and artificial DNA photocleaving and cytotoxic agents based on DNA intercalator-carbohydrate hybrid system.

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