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β-Carboline–Carbohydrate Hybrids: Molecular Design, Chemical Synthesis and Evaluation of Novel DNA Photocleavers

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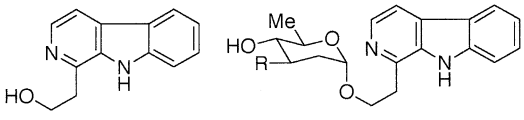
Abstract—β-Carboline present in β-carboline alkaloids from marine organisms was found, for the first time, to cleave DNA at the guanine site upon irradiation with UV light with a long wavelength without any additive, and β-carboline–carbohydrate hybrid system was effective for DNA cleavage.

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 DNA targeting new antitumor drugs. 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 a chemical and biological standpoint and offers a significant potential in medicine especially in the post-genome era.[1] Furthermore, photodynamic therapy using a photosensitizing drug has recently emerged as a promising modality against cancer and allied diseases.[2] In our approach to create such novel DNA cleaving molecules, we noted the β-carboline structure, which was found in β-carboline alkaloids such as the eudistomidins and the manzamines. Although β-carboline is known only as a DNA intercalator,[3] we expected that β-carboline would be a novel DNA photocleaving agent, because the conjugated C=N bond in β-carboline was expected to generate the photo-excited $^3(n-\pi^*)$ and/or $^3(\pi-\pi^*)$ state(s) by photoirradiation which could be capable of cleaving DNA by H-abstraction and/or electron-transfer pathway(s).[1] In this communication, we discuss the molecular design, chemical synthesis and DNA photocleaving properties of such novel and artificial light activatable DNA cleaving agents, that include the β-carboline–carbohydrate hybrids **2**–**6**.

To confirm our hypothesis, we first examined the photoinduced DNA cleaving activity of the β-carboline derivative **1** (Fig. 1), which was prepared by Torisawa's procedure,[4] using supercoiled ΦX174 DNA (form I). As obvious from (a) in Figure 2, **1** caused the single-strand scission of DNA by the photoirradiation using a long wavelength UV light (365 nm) without any additive, leading to the nicked open circular DNA (form II). This result clearly demonstrates, for the first time, that β-carboline found in β-carboline alkaloids cleaves DNA upon irradiation with UV light having a long wavelength without any additive. However, since the DNA cleavage ability of **1** was not very high, we next designed and synthesized the carboline–carbohydrate hybrids **2**–**6** to improve the DNA cleaving ability. We selected several types of 2,6-dideoxy sugars as the carbohydrate part of the artificial hybrids because several kinds of 2,6-dideoxy sugars also exist in many naturally occurring anticancer antibiotics which bind to DNA,[5] and the hydrophobicity of the 2,6-dideoxy sugar[6] would be suitable for the DNA groove binding.[7] Furthermore, based on this concept, we have previously demonstrated that a



1

2: R=NMe_2
3: R=NH_2
4: R=$OCONH_2$
5: R=O
6: R=OH

Figure 1. β-Carboline derivatives.

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suitably deoxylated amino sugar showed a high affinity to DNA, and significantly enhances the intercalating ability of certain intercalators.[7] The synthesis of the representative β-carboline–carbohydrate hybrid **2**[8] is summarized in Scheme 1.[9]

The key step in this synthesis was the highly effective glycosidation of **1** and **7**[10] using TMSOTf. The photo-induced DNA cleaving activities of the hybrids **2**–**6** were assayed using supercoiled ΦX174 DNA in concentrations of 1000–30 μM. Based on the results summarized as (b)–(f) in Figure 2, the DNA photocleaving abilities of the β-carboline–carbohydrate hybrids **2**–**6** were higher than that of **1** and the β-carboline–carbohydrate hybrids **2** and **3**, both of which had an amino group in the sugar moiety, produced most

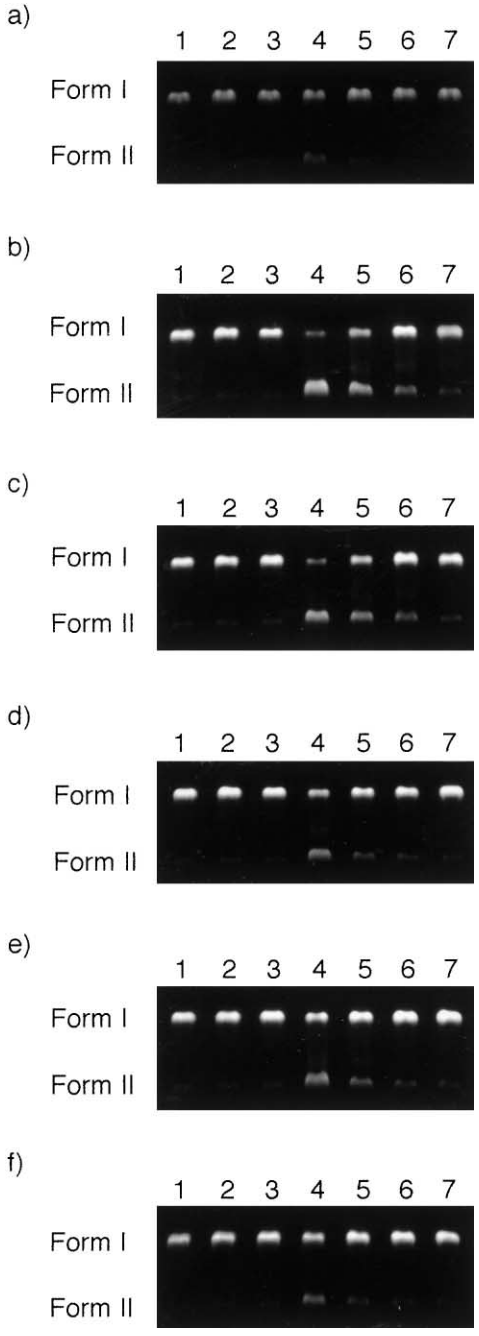
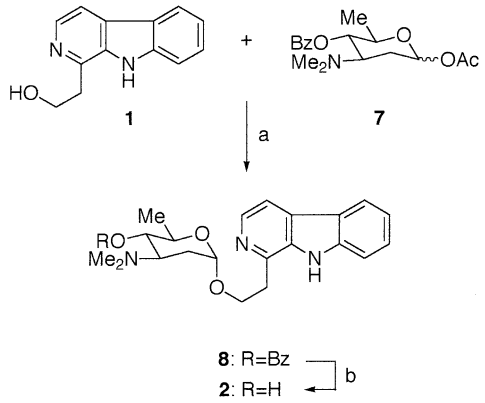


Figure 2. Photocleavage of supercoiled ΦX174 DNA. ΦX174 DNA (50 μM per base pair) was incubated with the compound in 20% acetonitrile in Tris–HCl buffer (pH 7.5, 50 mM) at 25 °C for 2 h under irradiation of the UV lamp (365 nm, 15 W) placed at 5 cm from the mixture, and analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain): (a), (b), (c), (d), (e) and (f) for the compounds **1**, **2**, **3**, **4**, **5** and **6**, respectively: lane 1, DNA alone; lane 2, DNA with UV; lane 3, DNA + compound (1000 μM) without UV; lanes 4–7, DNA + compound with UV, the concentrations are 1000, 300, 100 and 30 (μM), respectively.



Scheme 1. Synthesis of **2**: (a) TMSOTf, MS 4A, THF–CH_2Cl_2, 25 °C, 3 h, 91% (α/β = 3.5/1); (b) NaOMe, MeOH, 60 °C, 1.5 h, 84%.

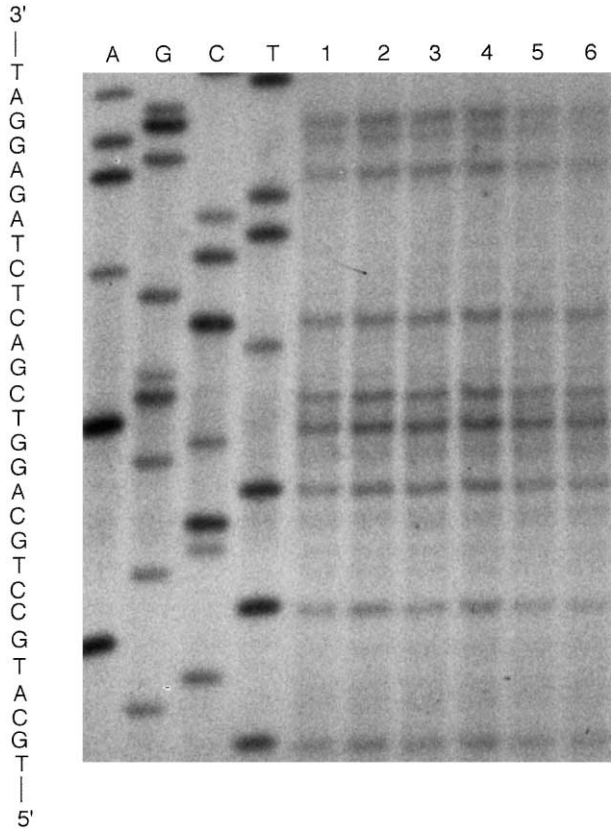


Figure 3. Autoradiogram of 12% polyacrylamide–8 M urea slab gel electrophoresis for sequence analysis. The 5′-end-labeled M13mp18 DNA was cleaved by the compound at pH 7.5 and 25 °C for 2 h under irradiation of the UV lamp (365 nm, 15 W) placed at 5 cm from the mixture (bases 49–77 are shown): lanes A, G, C and T; Sanger A, G, C and T reactions, respectively; lanes 1, 2, 3, 4, 5 and 6; the compounds **1**, **2**, **3**, **4**, **5** and **6** (1000 μM), respectively: DNAs for lanes 1–6 were treated with hot piperidine prior to gel electrophoresis.

effective DNA photocleavage [(b) and (c) in Fig. 2)]. Thus, the strongest DNA cleaving hybrids **2** and **3** cleaved DNA in concentrations over 30–100 μM [lanes 6 and 7 in (b) and (c) in Fig. 2]. It was confirmed that no DNA cleavage by **1**–**6** was observed in the absence of light [lanes 3 in (a)–(f) in Fig. 2]. Thus, the UV light functioned as a trigger to initiate these β-carboline derivatives for the DNA strand scission. These results clearly show that the β-carboline–carbohydrate hybrid system is effective for the DNA cleavage and that the DNA cleaving activity is dependent on the C3 substituent of the sugar moiety in the hybrids. Furthermore, it was confirmed that no change was observed between the UV–vis spectral of **1** around 365 nm and that of **2** (data not shown). These results strongly suggest that the suitably deoxylated amino sugar in these hybrids works as the DNA groove binder and/or external electrostatic binder, and significantly enhances the intercalating ability of the β-carboline.[7] The DNA cleaving site specificity of the β-carboline derivatives **1**–**6** was analyzed according to the Sanger protocol.[11] 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 $N+1$.[12] The results shown in Figure 3 clearly demonstrated that all the β-carboline derivatives selectively cleaved DNA at the guanine site and the site-selective DNA cleavage was enhanced upon treatment with hot piperidine (data without hot piperidine not shown). Furthermore, a singlet oxygen scavenger, 2,2,6,6-tetramethylpiperidine, significantly inhibited the DNA cleavage, while a free radical scavenger, dimethyl sulfoxide, did not inhibit the DNA cleavage. Therefore, it was found that singlet oxygen generated by the photo-excited β-carboline is essential for the present DNA photocleavage.[1]

The present work demonstrated the determination of the novel chemical nature of β-carboline as a DNA photocleaver, and also the molecular design and DNA photocleavage profile of the novel β-carboline–carbohydrate hybrids. The described chemistry and evaluation also provide significant information about the molecular design of novel and artificial DNA photocleaving agents.

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8. ^{1}H NMR (300 MHz, $CDCl_3$) (δ, $SiMe_4$; J = Hz), **2**: δ 1.24 (3H, d, J = 6.3), 1.56 (1H, ddd, J = 12.4, 12.4 and 4.0), 1.76 (1H, ddd, J = 12.4, 4.0 and 1.0), 2.20 (6H, s), 2.73 (1H, ddd, J = 12.4, 9.5 and 4.0), 3.06 (1H, dd, J = 9.5 and 9.5), 3.25–3.50 (m, 3H), 3.75–3.92 (1H, m), 4.14–4.25 (1H, m), 4.93 (1H, dd, J = 4.0 and 1.0), 7.27–8.38 (6H), 8.98 (1H, br s).
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