Molecular design, chemical synthesis, and biological evaluation of anthracene-carbohydrate hybrids as novel DNA photocleaving and photoselective cytotoxic agents

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Dedicated to Professor Karsten Krohn on the occasion of his 60th birthday
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Abstract
The anthracene-carbohydrate hybrids were designed and synthesized via an effective glycosidation. The hybrids cleaved double stranded DNA at the guanine site upon irradiation with UV light of a long wavelength and without any additive. The anthracene-carbohydrate hybrid system was very effective for the DNA cleavage. Furthermore, the anthracene-carbohydrate hybrids exhibited photoselective and strong cytotoxicity against cancer cells with photoirradiation.

Keywords: Anthracene, carbohydrate, DNA cleavage, cytotoxicity, photoirradiation

Introduction

The 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 for 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 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 report 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 anthracene-carbohydrate hybrids.

Results and Discussion

In our first approach to create such novel DNA photocleaving molecules, we noted anthracene, because anthracene possesses an electron rich and planar structure, which is suitable as a DNA intercalator. Furthermore, anthracene is known to generate a photo-excited state by photo-irradiation.[4] Therefore, we expected that the photo-excited species coming from anthracene would be capable of cleaving DNA. To confirm our hypothesis, we first examined the photo-induced DNA cleaving activities of anthracene **1** and its derivative **2** along with a naphthalene derivative **3** (Figure 1) using supercoiled ΦX174 DNA (Form I). As is evident from Figure 1, the anthracene derivatives **1** (100 μM) and **2** (100 μM) caused a weak 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 4 in (a) and (b) in Figure 1). On the other hand, the naphthalene derivative **3** showed no DNA cleaving activity under similar conditions (lane 4 in (c) in Figure 1).

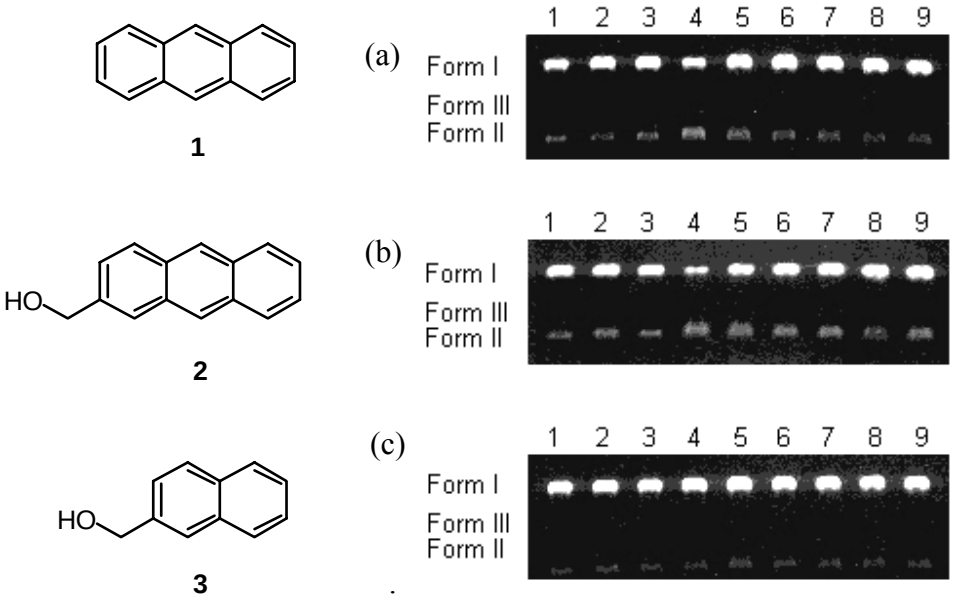
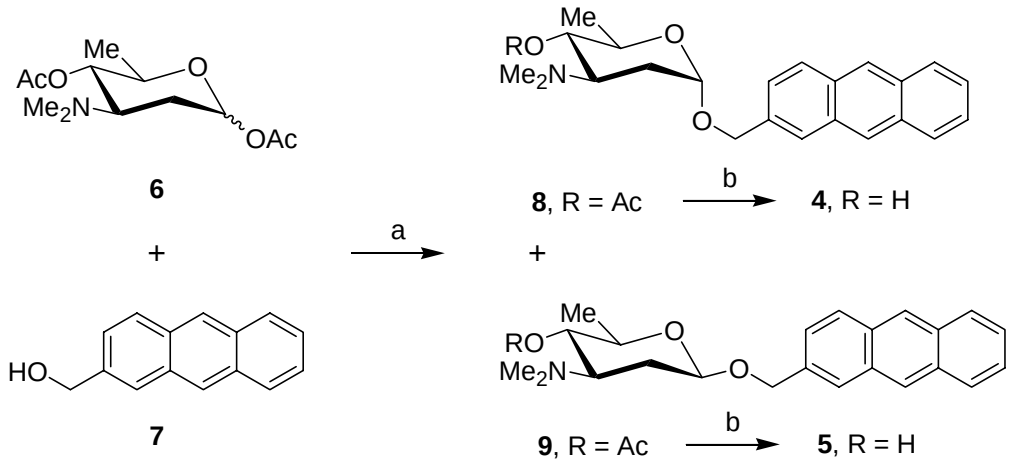


Figure 1. Photocleavage of supercoiled ΦX174 DNA. ΦX174 DNA (50 μM per base pair) was incubated with various compounds in 20% acetonitrile in Tris-HCl buffer (pH 7.5, 50 mM) at 25 °C for 2 h under irradiation of the UV lamp (365 nm, 15 W) placed at 10 cm from the mixture, and analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain): (a), (b) and (c) for the compounds **1**, **2** and **3**, respectively: lane 1, DNA alone; lane 2, DNA with UV; lane 3, DNA+compound (100 μM) without UV; lanes 4–9, compound (100, 30, 10, 3, 1, and 0.3 μM, respectively) following UV irradiation. Form I: covalently closed supercoiled DNA, Form II: open circular DNA, and Form III: linear DNA.

It was also confirmed that in the absence of light no DNA cleavage by **1** and **2** was observed (lanes 3 in (a) and (b) in Figure 1). Thus, UV light functioned as a trigger to initiate these anthracene derivatives to produce the DNA strand scission. These results clearly demonstrate, for the first time, that anthracene derivatives are able to cleave DNA upon irradiation with long wavelength UV light without any additive and that the aromatic rings system of anthracene is essential for the DNA cleavage.

To further improve the DNA cleaving ability of the anthracene derivatives, we designed the anthracene-carbohydrate hybrids **4** and **5** (Scheme 1), because we have previously demonstrated that a suitably deoxygenated amino sugar showed a high affinity to DNA and significantly enhanced the intercalating ability of certain intercalators.[5] The syntheses of the anomeric anthracene-carbohydrate hybrids **4** and **5** are summarized in Scheme 1. The glycosidation of the known 1-OAc sugar **6**[5e] and the commercially available anthracene derivative **7** using TMSOTf in THF smoothly proceeded to afford the α-glycoside **8** and its β-anomer **9** in a ratio of 1.2:1. The deprotection of the acetyl groups of **8** and **9** employing NaOMe in MeOH-$CHCl_3$ yielded the desired anthracene-carbohydrate hybrids **4** and **5**, respectively.



Scheme 1. Synthesis of anthracene-carbohydrate hybrids **4** and **5**.
Reagents and conditions: (a) TMSOTf, THF, 0 °C, 30 min, affording **8** (44%) and **9** (37%); (b) NaOMe, $CHCl_3$-MeOH, 25 °C, 6 h, yielding **4** (84%), **5** (62%).

With the designed anthracene-carbohydrate hybrids **4** and **5** in hand, the photo-induced DNA cleaving activities of these hybrids along with the sugar derivative **10** were next assayed using supercoiled ΦX174 DNA in concentrations of 100-0.3 μM. Based on the results shown in Figure 3, the anthracene-carbohydrate hybrids **4** and **5** caused effective DNA cleavage during irradiation using a long wavelength UV light. It was confirmed that the DNA cleaving abilities of the anthracene-carbohydrate hybrids **4** and **5** were found to be much stronger than those of **1** and **2**. Furthermore, it was found that the α-anomer **4** had a higher DNA cleaving ability than the β-anomer **5** [(a) and (b) in Figure 2]. Thus, the DNA cleaving activity was dependent on the

configuration of the glycosidic bond of the sugar moiety in the hybrids. On the other hand, the carbohydrate **10** had no DNA cleaving ability under similar conditions (c) in Figure 2). 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 anthracene. The DNA cleaving site specificity of the anthracene derivatives **1**, **2**, **4** and **5** was analyzed next 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]

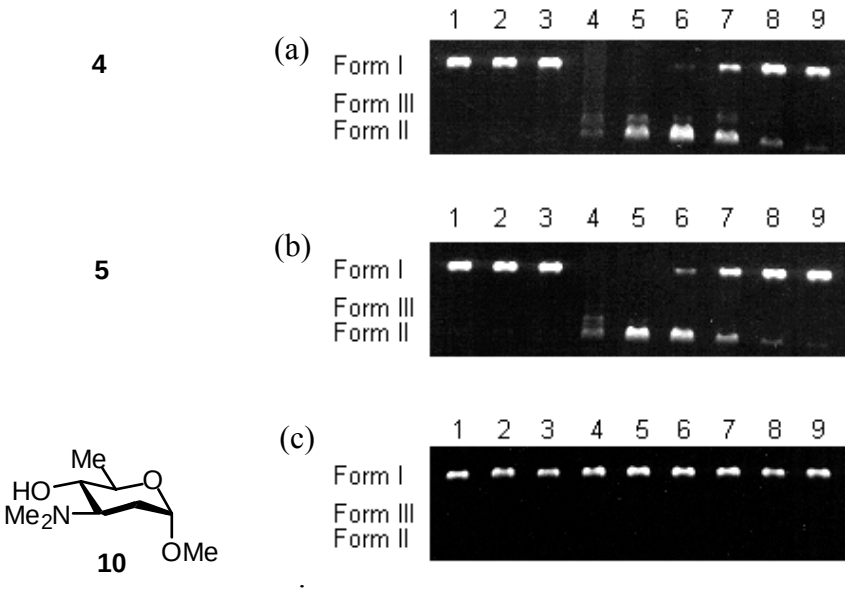


Figure 2. Photocleavage of supercoiled ΦX174 DNA. ΦX174 DNA (50 μM per base pair) was incubated with various compounds in 20% acetonitrile in Tris-HCl buffer (pH 7.5, 50 mM) at 25 °C for 2 h under irradiation of the UV lamp (365 nm, 15 W) placed at 10 cm from the mixture, and analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain): (a), (b) and (c) for the compounds **4**, **5** and **10**, respectively: lane 1, DNA alone; lane 2, DNA with UV; lane 3, DNA+compound (100 μM) without UV; lanes 4–9, compound (100, 30, 10, 3, 1, and 0.3 μM, respectively) following UV irradiation. Form I: covalently closed supercoiled DNA, Form II: open circular DNA, and Form III: linear DNA.

The results shown in Figure 3 clearly indicate that all the anthracene derivatives selectively cleaved DNA at the guanine sites and the site-selective DNA cleavage was enhanced upon treatment with hot piperidine. Since both the free radical and singlet oxygen scavengers, dimethyl sulfoxide and 2,2,6,6-tetramethylpiperidine, inhibited the DNA cleavage, it is very likely that the oxidation by •OH and $^1O_2$ is the initial step for the photo-induced destruction of the guanine bases.[2]

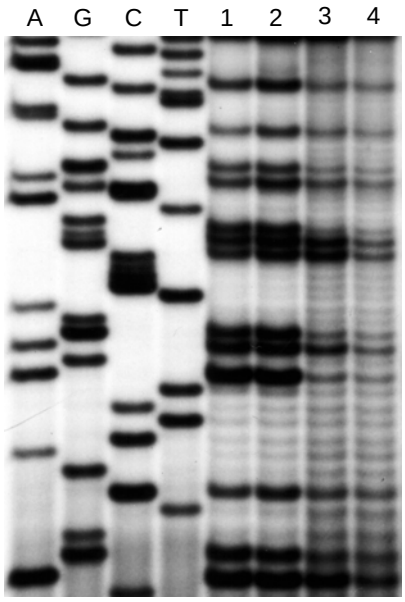


Figure 3. Autoradiogram of 12% polyacrylamide-8 M urea slab gel electrophoresis for sequence analysis. The 5'-end-labeled M13mp18 DNA at the primer site was cleaved by the compound at pH 7.5 and 25 °C for 1 h under irradiation of the UV lamp (365 nm, 15 W) placed at 10 cm from the mixture: lanes A, G, C and T; Sanger A, G, C and T reactions, respectively; lanes 1, 2, 3 and 4; the compounds **4**, **5**, **1** and **2** (30 μM), respectively, following UV irradiation: DNAs for lanes 1~4 were treated with hot piperidine prior to gel electrophoresis.

With the favorable results for the interactive and photocleaving ability of the anthracene-carbohydrate hybrids against the naked DNA, the cytotoxicities of the hybrids **4** and **5** were next examined using HeLa S3 cancer cells exposed to each agent for 72 h with or without 1 h of photoirradiation.[8] The IC_{50} values of **4** and **5** aginst the HeLa S3 cells without photoirradiation were 6.3 and 19 μM, respectively, while those with photoirradiation were 0.19 and 0.055 μM, respectively. These results indicate that the cytotoxicities of the anthracene-carbohydrate hybrids **4** and **5** with photoirradiation are much higher than those without 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 anthracene-carbohydrate hybrid with or without photoirradiation.

Conclusions

We demonstrated not only the molecular design and chemical synthesis of novel anthracene-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.

Experimental Section

General Procedures. Melting points were determined on a micro hot-stage Yanako MP-S3. Optical rotations were measured on a JASCO DIP-360 photoelectric polarimeter in chloroform unless otherwise noted. ^{1}H-NMR spectra were recorded on a Lambda 300 (300 MHz) 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 Kanto Chemical Co., Inc Silica Gel 60 N (spherical, neutral), 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.

2-Anthrylmethyl 3-*N,N'*-dimethylamino-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (4). To a stirred mixture of **6** (135 mg, 0.522 mmol), **7** (130 mg, 0.626 mmol) and MS 4A (95 mg) in dry THF (5.2 mL) was added TMSOTf (0.236 mL, 1.31 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and poured into ice-cold saturated $NaHCO_3$ aq. The resultant mixture was extracted with $CHCl_3$ and the extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in *vacuo*. Purification of the residue by column chromatography (60 g of silica gel, 1/10 *n*-hexane/EtOAc) gave the α-anomer **8** (94.0 mg, 44%) and β-anomer **9** (78.3 mg, 37%) as white solids. To a stirred solution of **8** (64.5 mg, 0.158 mmol) in dry $CHCl_3$-MeOH (1/1, 1.9 mL) was added 5N NaOMe (0.095 mL, 0.475 mmol) at 0 °C. After stirring at 25 °C for 6 h, the reaction mixture was neutralized with dryice and then poured into water. The resultant mixture was extracted with $CHCl_3$ and the extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in *vacuo*. Purification of the residue by column chromatography (20 g of silica gel, 1/3 $CHCl_3$/MeOH) gave **4** (48.6 mg, 84%) as white solids. R_f 0.24 (1/3 $CHCl_3$/MeOH); $[\alpha]^{28}_D$+19.9° (*c* 0.33, $CHCl_3$), mp 186.0–187.0 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.34 (3H, d, *J* = 6.0 Hz), 1.66 (1H, ddd, *J* = 12.8, 12.8 and 4.0 Hz), 1.94 (1H, ddd, *J* = 12.8, 4.0 and 1.0 Hz), 2.30 (6H, s), 3.02 (1H, ddd, *J* = 12.8, 9.8 and 4.0 Hz), 3.17 (1H, dd, *J* = 9.8 and 9.8 Hz), 3.83 (1H, dq, *J* = 9.8 and 6.0 Hz), 4.64 and 4.88 (each 2H, ABq, *J* = 13.0 Hz), 5.10 (1H, dd, *J* = 4.0 and 1.0 Hz), 7.42–7.50 (3H, m), 7.92–8.04 (4H, m), 8.41 (2H, s). Anal. Calcd for $C_{23}H_{27}NO_3$: C, 75.59; H, 7.45; N, 3.83. Found: C, 75.30; H, 7.36; N, 3.66.

2-Anthrylmethyl 3-*N,N'*-dimethylamino-2,3,6-trideoxy-β-D-*arabino*-hexopyranoside (5). To a stirred solution of **9** (61.6 mg, 0.150 mmol) in dry $CHCl_3$/MeOH (1:1, 1.2 mL) was added 5N NaOMe (0.090 mL, 0.451 mmol) at 0 °C. After stirring at 25 °C for 6 h, the reaction mixture was neutralized with dryice and then poured into water. The resultant mixture was extracted with $CHCl_3$ and the extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in *vacuo*. Purification of the residue by column chromatography (15 g of silica gel, $CHCl_3$/MeOH 1:3) gave **4** (34.0 mg, 62%) as white solids. R_f 0.44 ($CHCl_3$/MeOH 1:3); $[\alpha]^{28}_D$ -15.8° (*c* 0.21, $CHCl_3$), mp 209.0–210.0 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.44 (3H, d, *J* = 6.0 Hz), 1.60 (1H, ddd, *J* = 12.8, 12.8 and 10.0 Hz), 2.05 (1H, ddd, *J* = 12.8, 4.0 and 2.0 Hz),

2.27 (6H, s), 2.45 (1H, ddd, *J* = 12.8, 9.8 and 4.0 Hz), 3.11 (1H, dd, *J* = 9.8 and 9.8 Hz), 3.34 (1H, dq, *J* = 9.8 and 6.0 Hz), 4.64 (1H, dd, *J* = 9.8 and 2.0 Hz), 4.79 and 5.10 (each 2H, ABq, *J* = 13.0 Hz), 7.43–7.50 (3H, m), 7.93–8.04 (4H, m), 8.40 (2H, s). Anal. Calcd for $C_{23}H_{27}NO_3$: C, 75.59; H, 7.45; N, 3.83. Found: C, 75.41; H, 7.38; N, 3.65.

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.

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 2 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 1 and 2 show the pictures of the agarose gel electrophoresis results.

Identification of DNA cleavage sites. The reaction samples contained the compounds (30 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 4 shows a picture of the autoradiogram.

Cytotoxicity assay

The cells were treated with each compound at the concentrations of 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.[8]

Acknowledgements

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