Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2163–2165

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Carbohydrate-Modulated DNA Photocleavage: Design, Synthesis, and Evaluation of Novel Glycosyl Anthraquinones

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Received 2 June 2000; accepted 6 July 2000

Abstract—Novel and artificial anthraquinone-carbohydrate hybrids were designed and synthesized, and found to effectively cleave DNA under irradiation with a long wavelength UV light and also exhibit cytotoxicity against HeLa S3 cells.

The development of photochemical DNA cleaving agents, which selectively cleave DNA by irradiation with a specific light under mild conditions and without any additives such as metals and reducing agents, is very interesting from a chemical and biological standpoint and offers considerable potential in medicine.[1] Furthermore, photodynamic therapy using photosensitizing drugs has recently emerged as a promising modality against cancer and allied diseases.[2] In this communication, we report the molecular design, chemical synthesis, DNA photocleaving properties, and cytotoxicity of the novel and artificial light activatable DNA cleaving agents, that include the anthraquinone-carbohydrate hybrids **1**,[3] **2** and **3** (Fig. 1).

In our approach to create such novel DNA cleaving molecules, we designed artificial intercalator–carbohydrate hybrid systems,[3–6] because many clinically useful antitumor antibiotics such as anthracyclines[7] and aureolic acids,[8] which interact with DNA, were commonly found to contain aromatic and carbohydrate domains. Since Schuster et al.[9] elegantly demonstrated the efficacy of the suitably substituted anthraquinones that have DNA intercalating and DNA photocleaving abilities, anthraquinone[10] was selected as the DNA intercalating and DNA photocleaving function. On the other hand, certain 2,6-dideoxy amino sugars seemed appropriate as the carbohydrate source, since they are found as the DNA groove binder in some naturally occurring DNA-binding antitumor antibiotics[11] and our previously reported artificial DNA interactive intercalator-carbohydate molecules.[3,4] Therefore, we designed novel and artificial intercalator-carbohydrate hybrids that consist of anthraquinone and a 2,6-dideoxy amino sugar. The newly designed and synthesized hybrids **2** and **3** are the anomers of each other.

The designed anthraquinone-carbohydrate hybrids **2** and **3** were synthesized by a short reaction sequence via the effective glycosylation reaction of the 1-OAc sugar **4**[12] with 2-(hydroxymethyl)-9,10-dimethoxy-9,10-dihydroanthracen-1-ol (**7**) which was easily obtained from 9,10-dimethoxy-9,10-dihydroanthracen-1-ol (**5**) via the regioselective hydroxymethylation by Kobayashi's method[13] using 35% HCHO (aq) and $Sc(OTf)_3$, and subsequent oxidation of the resultant diol **6** using CAN (Scheme 1). The glycosidation of the 1-OAc sugar **4** (1.0 equiv) with **7** (1.2 equiv) using TMSOTf[14] in the presence of MS 4A in THF gave both the α-glycoside **8** and the β-glycoside **9** in 89% yield in a ratio of 2.7:1. After their separation by column chromatography, the deprotection of the benzoyl groups in **8** and **9** using NaOMe afforded the designed hybrids **2**[15] and **3**[15] in 72% and 90% yields, respectively.

The photoinduced DNA cleaving activities of the anthraquinone-carbohydrate hybrids **1**–**3** along with the components of these hybrids, **10**, **11** and **12**[3,4] were assayed using supercoiled ΦX174 DNA at pH 7.5 under aerobic conditions. The dimethylamino groups of the carbohydrate moieties in **1**–**3** and **12** were protonated

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PII: S0960-894X(00)00410-8

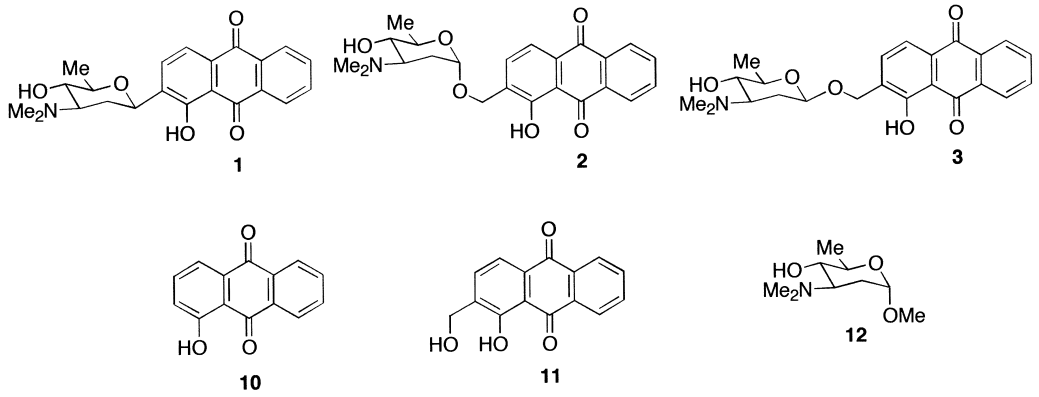
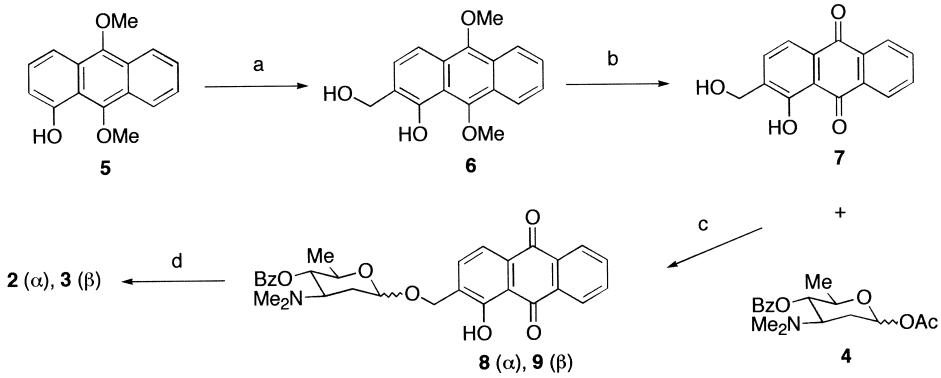


Figure 1. Artificial anthraquinone–carbohydrate hybrids and their components.



Scheme 1. Synthesis of **2** and **3**. (a) 35% HCHO (aq), $Sc(OTf)_3$ (0.2 equiv), THF, 25 °C, 7 h, 98%; (b) CAN (2.5 equiv), MeCN–H_2O, −17 °C, 15 min, 59%; (c) TMSOTf (1.2 equiv), MS 4A, THF, 0 °C, 40 min, 65% for **8**, 24% for **9**; (d) NaOMe (3.0 equiv), MeOH, 60 °C, 2.5 h, 72% for **2**, 90% for **3**.

under these conditions. As is clear from Figure 2, the anthraquinone–carbohydrate hybrids **1**–**3** (1000 μM) caused cleavage of DNA by the photoirradiation with a long wavelength UV light (365 nm), while **10**–**12** did not show DNA cleaving activity under the same conditions. These results clearly indicate the importance of the hybrid structure constructed from anthraquinone and the 2,6-dideoxy amino sugar 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 anthraquinone. It was confirmed that no DNA cleavage by **1**–**3** was observed in the absence of light. The DNA cleaving

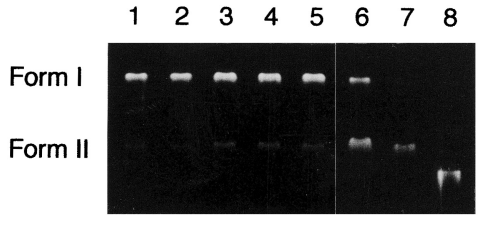


Figure 2. Photocleavage of supercoiled ΦX174DNA. ΦX174DNA (50 μM per base pair) was incubated with various compounds in 20% acetonitrile in Tris–HCl buffer (pH 7.5, 50 mM) at 25 °C for 2 h under irradiation of the UV lamp (365 nm, 15 W) placed at 10 cm from the mixture, and analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain): lane 1, DNA alone; lane 2, DNA with UV; lanes 3–8 compounds **10**, **11**, **12**, **1**, **2** and **3** (1000 μM), respectively.

ability of the hybrid **2** was stronger than that of the hybrid **1**. Furthermore, the hybrid **3** was interestingly found to form the complex with DNA after DNA cleaving as shown as the lower mobility DNA than Form II DNA. It was confirmed that the lower mobility DNA was transformed into Form II DNA by the removal of the hybrid **3** from the lower mobility DNA by the extraction with chloroform (data not shown). These results demonstrate that the DNA cleaving and binding abilities are definitely dependent on the structure of the sugar moiety in the hybrid.

The cytotoxicity of the DNA cleaving hybrids **1**–**3** was next examined by using HeLa S3 cells exposed to each agent for 72 h with or without 1 h of photoirradiation.[16] The IC_{50} values of **1**–**3** without the photoirradiation were 9.0, 13 and 8.8 μM, respectively, and those with the photoirradiation were 4.8, 4.3 and 1.9 μM, respectively. These results indicate that the cytotoxic activities of **1**–**3** with the photoirradiation were higher than those without the photoirradiation and the DNA cleaving activity by the photoirradiation affected the cytotoxicity of the hybrids.

In summary, the present work demonstrates not only the molecular design and chemical synthesis of novel intercalator–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 agents based on the intercalator–carbohydrate hybrid system. The DNA base selectivity of the hybrids **1**–**3** is under investigation and will be reported in detail elsewhere.

Acknowledgements

This research was partially supported by a Grant-in-Aid for Encouragement of Young Scientists from the Ministry of Education, Science, Sports and Culture, Japan, and a research grant of Keio University Special Grant-in-Aid for Innovative Collaborative Research Projects.

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15. Selected and significant ^{1}H NMR spectra (270 MHz, $CDCl_3$, δ (TMS), *J* (Hz)) are the following. **2**: 1.32 (3H, d, $J=6.4$), 1.69 (1H, ddd, $J=12.2$, 10.0 and 3.8), 1.99 (1H, ddd, $J=12.2$, 4.0 and 0.6), 2.00–2.15 (1H, br), 2.32 (3H×2, s), 3.01 (1H, ddd, $J=10.0$, 10.0 and 4.0). 3.17 (1H, dd, $J=10.0$), 3.76 (1H, dq, $J=10.0$ and 6.4), 4.68 and 4.87 (each 1H, ABq, $J=14.2$), 5.12 (1H, dd, $J=3.8$ and 0.6), 7.75–7.90 (4H, m), 8.26–8.37 (2H, m), 12.95 (1H, br s). **3**: 1.40 (3H, d, $J=6.4$), 1.61 (1H, ddd, $J=12.4$, 12.4, 9.6), 1.96–2.35 (1H, br), 2.07 (1H, ddd, $J=12.4$, 4.0 and 2.0), 2.30 (3H×2, s), 2.51 (1H, ddd, $J=12.4$, 9.8 and 4.0), 3.11 (1H, dd, $J=9.8$), 3.36 (1H, dq, $J=9.8$ and 6.4), 4.69 (1H, dd, $J=9.6$ and 2.0), 4.81 and 5.06 (each 1H, ABq, $J=14.4$), 7.77–7.88 (4H, m), 8.26–8.35 (2H, m), 12.96 (1H, br s).
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