Pergamon

SCIENCE DIRECT

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3281–3283

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Glycidol-Carbohydrate Hybrids: A New Family of DNA Alkylating Agents

Kazunobu Toshima,* Yukiko Okuno and Shuichi Matsumura

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

Received 21 May 2003; revised 19 June 2003; accepted 19 June 2003

Abstract—Novel and chiral glycidol–carbohydrate hybrids possessing an epoxy group as a DNA alkylating moiety were designed and synthesized. These artificial hybrids selectively alkylated DNA at the N-7 sites of the guanines and cleaved DNA without any additives. The binding ability of the glycidol was significantly enhanced by the attachment of the carbohydrate.

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 in the design of new DNA targeting drugs.[1] A number of DNA interactive small molecules including DNA oligomers and polyamides have been already evaluated. In contrast, although carbohydrates play a key role in various biological processes, the interaction between carbohydrates and DNA, however, are still not yet well understood. Only limited studies on the interaction between certain carbohydrates in DNA interactive antibiotics and DNA have been reported.[2] In our previous studies on the design of novel and artificial DNA interactive small molecules possessing carbohydrates, it has been found that a suitably deoxygenated amino sugar showed a high binding affinity to DNA, and significantly enhanced the intercalating ability of certain DNA intercalators.[3] Furthermore, we have also demonstrated that the carbohydrate-containing DNA interactive small molecules showed cell-permeable properties.[3a–c] DNA alkylating agents as well as DNA intercalators have attracted considerable attention as DNA interactive molecules.[4] Although many molecules causing alkylation of DNA have been evaluated, it is still required to create new molecules having a more potent biological activity. Therefore, based on our previous results,[3] we had a simple question whether the deoxygenated amino sugar, that appeared in our previously reported DNA interactive molecules, enhances the DNA binding ability of not only DNA intercalators but also DNA alkylating agents. In this paper, we report the molecular design, chemical synthesis, DNA alkylating and cleaving properties of novel and artificial DNA alkylating agents, that include the glycidol–carbohydrate hybrids possessing an epoxy function as a DNA alkylating site (Fig. 1). To the best of our knowledge, this is first example of DNA alkylating agents constructed from only an alkylating group and carbohydrate.

In this preliminary study, we noted chiral glycidols **1** and **2** as the DNA alkylating moiety, because the chiral epoxy structure was found in several DNA alkylating antitumor antibiotics such as the pluramycins[5] and also in DNA interactive carcinogens such as the aflatoxin B_1 oxide.[6] Some artificial DNA alkylating agents consisting of a DNA intercalator and epoxide have also been reported.[7] To examine the effect of the deoxygenated amino sugar on the DNA binding ability of the DNA alkylating agents, we newly designed the chiral glycidol–carbohydrate hybrids **3**–**6** (Fig. 1). Glycidols **1** and **2** are enantiomers of each other. Accordingly, the glycidol–carbohydrate hybrids **3** and **4** are the epimers of **5** and **6**, respectively, with respective to the epoxide structure. On the other hand, the α-glycosides **3** and **5** are the anomers of the β-glycosides **4** and **6**, respectively. The syntheses of the glycidol–carbohydrate hybrids **3** and **4** are summarized in Scheme 1. The syntheses of **3** and **4** commenced with the *t*-butyldiphenylsilyl (TPS) protected **8**[8] which was easily prepared from the commercially available chiral alcohol **7**. The primary hydroxy group in **8** was selectively protected with a pivaloyl (Pv) group and the remaining secondary hydroxy group was then

*Corresponding author. Tel.: +81-45-566-1576; fax: +81-45-566-1576; e-mail: toshima@applc.keio.ac.jp

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doi:10.1016/S0960-894X(03)00659-0

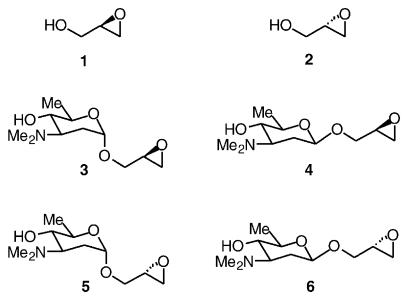
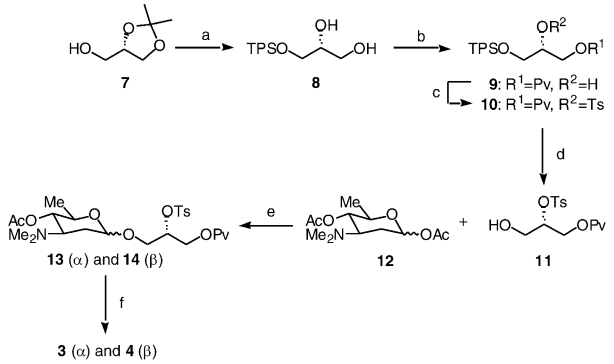


Figure 1.



Scheme 1. Syntheses of **3** and **4**. Reagents and conditions: (a) ref 8; (b) PvCl, Et_3N, CH_2Cl_2, 35 °C, 7 h, 96%; (c) TsCl, DMAP, Py, 60 °C, 14 h, 72%; (d) HCl, MeOH, 40 °C, 12 h, 72%; (e) TMSOTf, MS 4A, CH_2Cl_2, 0 °C, 0.5 h, 84% (13/14 = 4/1); (f) NaOMe, MeOH, 25 °C, 5 h, 52% for **3**, 59% for **4**.

masked with a *p*-toluenesulfonyl (Ts) group by standard procedures to afford **10** via **9**. After the removal of the TPS group under acidic conditions, the resultant alcohol **11** was subjected to glycosidation with the glycosyl acetate **12**[9] using TMSOTf as the activator to yield the α- and β-glycosides **13** and **14**. At this stage, these anomers were easily separated by silica-gel column chromatography. Finally, the treatment of the isolated glycosides **13** and **14** with NaOMe caused epoxide formation along with deprotection of the acetyl group to furnish the desired glycidol-carbohydrate hybrids **3** and **4**, respectively. The other hybrids **5** and **6** were also synthesized from the enantiomer of **7** by very similar protocols.

With all the designed glycidol–carbohydrate hybrids **3**–**6** in hand, the DNA cleaving activities of these hybrids along with the reference compounds **1** and **2** were next assayed using supercoiled ΦX174 DNA.[10] Based on the results shown in Figure 2, the glycidol–carbohydrate hybrids **3**–**6** caused significant DNA cleavage without any additives ((c)–f) in Fig. 2). In contrast, glycidols **1** and **2** showed little DNA cleaving activity under similar conditions ((a) and b) in Fig. 2). These results indicate that the suitably deoxygenated amino sugar in the hybrids significantly enhanced the DNA alkylating ability of the glycidols. Furthermore, these hybrids showed different DNA cleaving abilities. Thus, the DNA cleaving ability of the hybrid **6** was found to be stronger than those of the others, and the strongest **6** cleaved DNA in concentrations over 30 μM ((f) in Fig. 2). From these results, it was found that the DNA

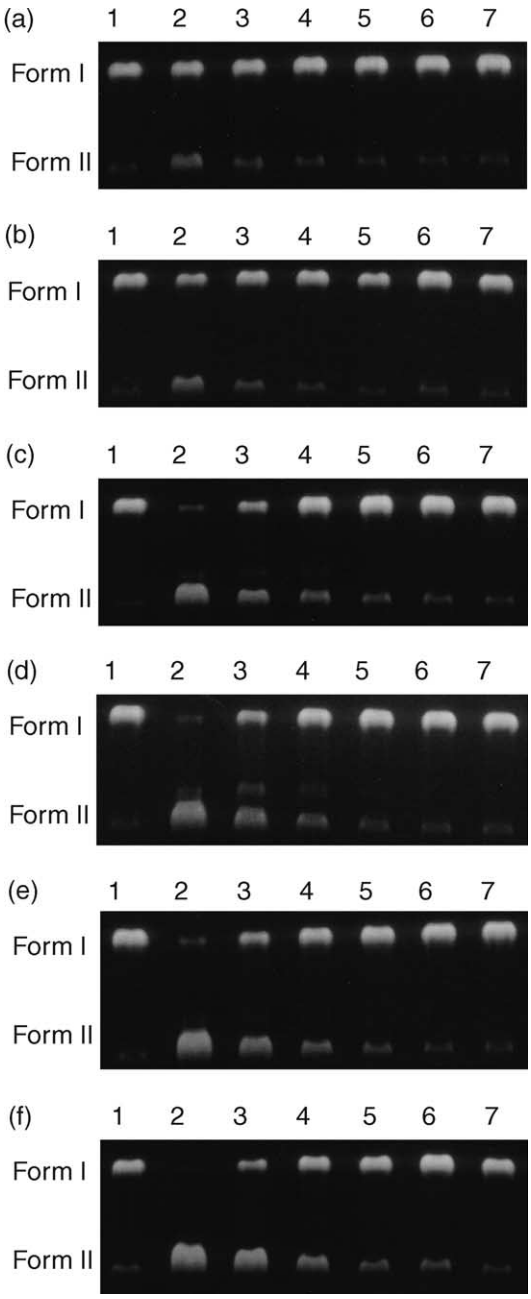


Figure 2. Cleavage of supercoiled ΦX174 DNA. ΦX174 DNA (50 μM per base pair) was incubated with various compounds in 20% acetonitrile in Tris–HCl buffer (pH 7.5, 50 mM) at 37 °C for 24 h, and analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain): (a), (b), (c), (d), (e) and (f) for the compounds **1**, **2**, **3**, **4**, **5** and **6**, respectively: lane 1, DNA alone; lanes 2–7, compound (1000), compound (300), compound (100), compound (30), compound (10) and compound (3 μM), respectively. Form I: covalently closed supercoiled DNA, Form II: open circular DNA.

cleaving activity was dependent on the configurations of both the glycosidic bond in the sugar moiety and the epoxide structure in the aglycon part in the hybrids.

The DNA cleaving site specificity of the glycidol derivatives **1**–**6** was next analyzed according to the Sanger protocol.[11] Since the Sanger sequencing reactions result in base incorporation, cleavage at nucleotide *N* (sequencing) represents a cleaving site by the agent or

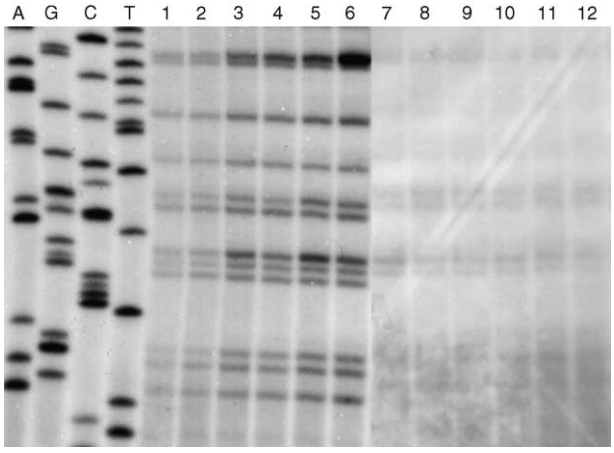


Figure 3. Autoradiogram of 12% polyacrylamide-8 M urea slab gel electrophoresis for sequence analysis. The 5′-end-labeled M13mp18 DNA and 7-deazaguanosine-substituted M13mp18 DNA were treated with various compounds at pH 7.5 and 37 °C for 24 h: lanes A, G, C and T; Sanger A, G, C and T reactions, respectively; lanes 1-6; the 5′-end-labeled M13mp18 DNA + compounds 1, 2, 3, 4, 5 and 6 (1000 μM), respectively: lanes 7–12; the 5′-end-labeled and 7-deazaguanosine-substituted M13mp18 DNA + compounds 1, 2, 3, 4, 5 and 6 (1000 μM), respectively. The DNAs for lanes 1–12 were treated with hot piperidine prior to the gel electrophoresis.

the Maxam-Gilbert reaction at $N+1$.[12] The results shown in Figure 3 clearly indicated that all the glycidol derivatives selectively cleaved DNA at the guanine sites, and the site-selective DNA cleavage was significantly enhanced upon treatment with hot piperidine[13] (lanes 1–6 in Fig. 3). Furthermore, we confirmed that when 7-deazaguanosin-substituted DNA was used in this assay, no DNA cleavage was observed even with hot piperidine treatment (lanes 7–12 in Fig. 3). These results strongly suggested that these hybrids selectively alkylated DNA at the N-7 sites of the guanines.[14] Moreover, it was observed that the intensities of the glycidol-carbohydrate hybrids induced DNA cleaving bands at the N-7 sites of the guanines were not always equal (lanes 1–6 in Fig. 3). These results showed that the hybrids had a moderate sequence selectivity in addition to the base selectivity during the DNA alkylation processes. These results suggest the further possibility of the creation of novel sequence selective DNA alkylating agents[15] based on carbohydrates including oligosaccharides.

In summary, the present work demonstrates not only the molecular design and chemical synthesis of novel glycidol–carbohydrate hybrids, but also their DNA alkylating and cleaving profiles. The effect of the carbohydrate on the DNA binding ability of the DNA alkylating agent was also demonstrated. The described chemistry and biological evaluation provided significant information about the molecular design of novel and selective DNA alkylating agents possessing carbohydrate(s).

Acknowledgements

This research was partially supported by Grant-in-Aid for the 21st Century COE program 'KEIO Life Conjugate Chemistry' and for General Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and Takeda Science Foundation.

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