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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 15 (2005) 4332–4335

Molecular design, chemical synthesis, and evaluation of cytosine–carbohydrate hybrids for selective recognition of a single guanine bulged duplex DNA

Yusuke Idutsu, Ayaka Sasaki, Shuichi Matsumura and Kazunobu Toshima*

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

Received 17 May 2005; revised 11 June 2005; accepted 14 June 2005
Available online 2 August 2005

Abstract—The designed cytosine–carbohydrate hybrid molecule selectively recognized and stabilized the bulged duplex DNA possessing the complementary bulged DNA base, guanine, while the nucleotide base itself did not exhibit any such ability. It was also found that the assistance of the carbohydrate to stabilize the interaction between the nucleotide base and the complementally bulge DNA base is very helpful for the selective recognition and stabilization of the single-bulged duplex DNA.

A DNA bulge is the site where one or more extra nucleotide bases remain unpaired in the duplex DNA.[1] Single-base bulges arising from the slippage of DNA can result in mutation hotspots due to DNA strand misalignment during replication.[2] This irregular DNA site is considered to play an important role in the recognition by DNA repair proteins,[3] and shows a unique reactivity toward drug binding.[4] The ligands selectively binding to the bulged site have been acknowledged as novel reagents for the chemical-typing of genetic disorders, such as single nucleotide polymorphisms (SNPs). A successful representative is 2-amino-1,8-naphthyridine reported by Nakatani and Saito.[5] However, a chemical reagent or a device, which can selectively detect and discriminate four bulged DNA sites (A, C, G, and T-bulges), has never been reported.

In our previous studies on artificial DNA interactive small molecules, we have found that the artificial hybrid molecules consisting of an intercalator and a deoxyamino sugar strongly interacted with a duplex DNA,[6] because the deoxyamino sugar functioned as a DNA groove binder, and significantly enhanced the intercalating ability of the intercalator. In addition, it was found that the deoxyamino sugar exhibited an affinity to the duplex DNA with no site-specificity (Fig. 1). Based on these observations, we expected that although a nucleotide base itself could not stabilize a bulged duplex DNA by the interaction with the complementary bulged DNA base, a hybrid molecule constructed from a nucleotide base and the deoxyamino sugar would selectively interact with the complementary bulged DNA base, and stabilize the bulged duplex DNA. In this case, standard Watson–Crick type hydrogen bondings between the nucleotide base and the complementary bulged DNA base would induce the interaction with a base selectivity, and the deoxyamino sugar would cause a nonspecific affinity to the duplex DNA and assist in the selective interaction between the nucleotide base and the complementary bulged DNA base. These effects would be very useful for the selective recognition of a bulged duplex DNA with a specific interaction (Fig. 2). Furthermore,

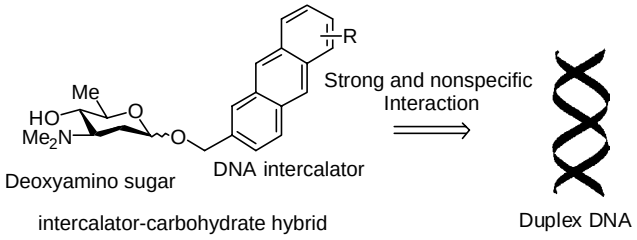

Figure 1. Interaction between intercalator–carbohydrate hybrid and duplex DNA.

Keywords: DNA; Bulge; Nucleotide base; Carbohydrate; Molecular recognition.
* 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/j.bmcl.2005.06.073

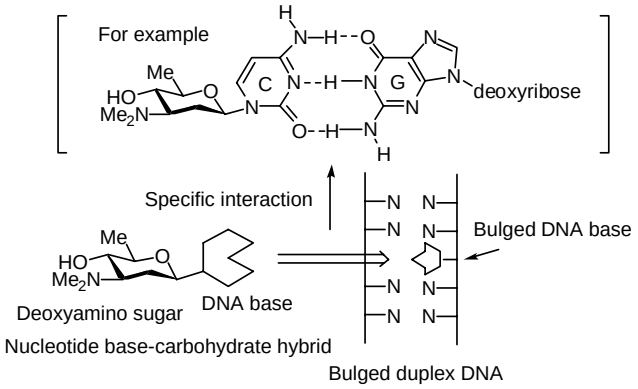


Figure 2. Interaction between nucleotide base–carbohydrate hybrid and bulged duplex DNA.

if this new concept that a hybrid molecule consisting of a nucleotide base and a nonspecific binder, such as a deoxyamino sugar, could selectively interact with the only complementary bulged DNA base and then stabilize the bulged duplex DNA, all four kinds of single bulged duplex DNAs would be selectively detected by the expanded methods using four nucleotide bases based on this concept. In this communication, we report that the designed cytosine–carbohydrate hybrid selectively recognized the guanine bulged DNA base and stabilized the guanine bulged duplex DNA.

To confirm our hypothesis, we newly synthesized two cytosine–carbohydrate hybrids **1** and **2**, and chose cytosine (**3**) and cytidine (**4**) as the reference materials in this preliminary study (Fig. 3). The designed hybrid **1** possesses a hybrid structure in which cytosine and the deoxyamino sugar are directly connected to each other, while the artificial **2** has another hybrid structure in which cytosine and the deoxyamino sugar are linked through a C2 linker, $-OCH_2CH_2-$. These hybrids are different in terms of the distance between cytosine and the deoxyamino sugar. Needless to say, cytosine (**3**) is a nucleotide base itself without possessing a carbohydrate, and cytidine (**4**) possesses another carbohydrate, ribose, instead of the deoxyamino sugar found in **1** and **2**.

The chemical synthesis of the hybrid **1** is outlined in Scheme 1. Thus, the known glycosyl acetate **5**[7] was

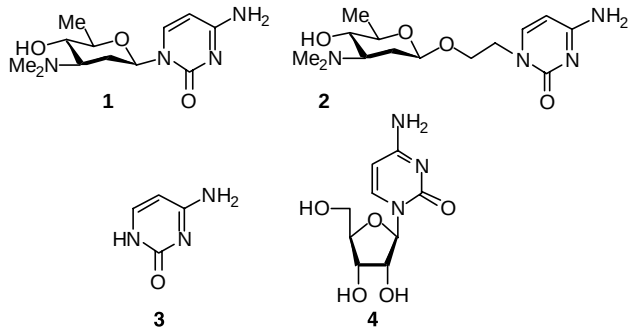
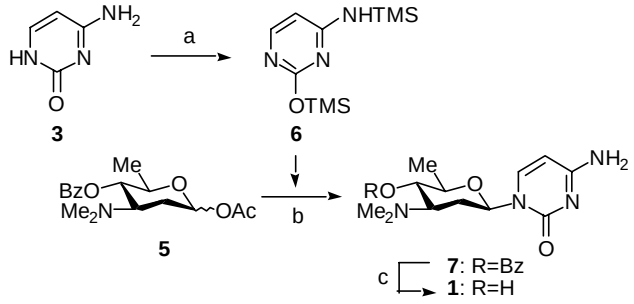


Figure 3. Nucleotide base–carbohydrate hybrids **1** and **2**, cytosine (**3**), and cytidine (**4**).



Scheme 1. Synthesis of **1**. Regents and conditions: (a) HMDS, TMSCl, reflux, 6 h, 93%; (b) TMSOTf, $ClCH_2CH_2Cl$, reflux, 2 h, 74%; (c) NaOMe, MeOH, 60 °C, 1.5 h, 87%.

smoothly coupled to the trimethylsilylated cytosine **6**,[8] prepared from cytosine (**3**), hexamethyldisilazane (HMDS), and trimethylsilyl chloride (TMSCl), in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in $ClCH_2CH_2Cl$ under reflux for 2 h to give the cytosine glycoside **7** in 74% yield with a very high β-stereoselectivity. Deprotection of the benzoyl group in **7** using NaOMe in MeOH at 60 °C for 1.5 h furnished the desired hybrid **1** in 87% yield. Another hybrid **2** was also obtained by a similar procedure involving a glycosidation reaction using ethyleneglycol.[9,10]

With the designed cytosine–carbohydrate hybrids **1** and **2** in hand, base selective stabilization of the bulged duplex DNA was examined by measuring the melting temperature (T_m) of Nakatani and Saito's four duplex DNAs (4.77 μM), d(TCCAGGCAAC)/d(GTTGC *X*CTGGA) involving a single nucleotide bulge (*X* = A, C, G, or T), in the presence of each molecule (5.00 μM) in sodium cacodylate buffer (10 mM, pH 7.0) containing NaCl (100 mM).[5a,b] The measurements were carried out at least three times and these results are summarized in Table 1. In the case of **1**, the T_m was significantly increased by 2.6 °C for the G bulged duplex DNA, d(TCCAGGCAAC)/d(GTTGC*G*CTGGA). However, in drastic contrast, no increase in the T_m was observed for the A, C, and T bulged duplex DNAs as well as for the fully complementary duplex DNA, d(TCCAGGCAAC)/d(GTTGCCTGGA). These results clearly indicated that the hybrid **1** selectively stabilized only the G bulged duplex DNA. Furthermore, the 1:1 ratio of hybrid **1** and the G bulged duplex DNA was sufficient to observe the detectable ΔT_m. To obtain more information on the structure and activity relationship for stabilizing the G bulged duplex DNA, more T_m measurements were conducted in the presence of the other compounds **2**–**4**. The ΔT_m values in the presence of these compounds were 0.9, 0.5, and 0.3 °C for **2**, **3**, and **4**, respectively, in sharp contrast to the ΔT_m of 2.6 °C in the presence of hybrid **1**. These results showed that the hybrid structure consisting of the cytosine and the deoxyamino sugar is indispensable for stabilizing the G bulged DNA, and the deoxyamino sugar could not be replaced by another sugar, ribose, for this purpose. Both the suitably hydrophobic nature[11] of the deoxyamino sugar and the *N*,*N*-dimethyl group positively charged under neutral conditions must be very helpful

Table 1. Melting temperature (T_m) of bulged-containing duplex DNAs in the presence and absence of compound[a]

Duplex DNA	$T_m(-)$	Compound	$T_m(+)$	^2T_m
5′-TCCAG_GCAAC-3′ 3′-AGGTCGCGTTG-5′	32.0	**1**	34.6	2.6
		2	32.9	0.9
		3	32.5	0.5
		4	32.3	0.3
5′-TCCAG_GCAAC-3′ 3′-AGGTCACGTTG-5′	32.2	**1**	31.9	−0.3
5′-TCCAG_GCAAC-3′ 3′-AGGTCCCGTTG-5′	33.6	**1**	33.5	−0.1
5′-TCCAG_GCAAC-3′ 3′-AGGTCTCGTTG-5′	31.1	**1**	30.8	−0.3
5′-TCCAGGCAAC-3′ 3′-AGGTCCGTTG-5′	45.3	**1**	44.9	−0.4

[a] Measurement of the melting temperature was conducted using bulged duplex DNA (4.77 μM, strand concentration) and compound (5.00 μM). The absorbance at 260 nm was measured in sodium cacodylate buffer (10 mM, 7.0) containing NaCl (100 mM). Temperature was increased at a rate of 1 °C/min from 15 to 60 °C. Measurements were carried out at least three times.

when interacting with the duplex DNA and assist in the selective interaction between the cytosine and the complementary bulged DNA base, guanine.[12] In addition, the length between the cytosine and the deoxyamino sugar was also found to be very important for inducing such an effect. The longer linker leading to the more flexible structure of **2** probably lost the suitable fitting form between **2** and the bulged DNA.

In summary, we demonstrated here, for the first time that the cytosine–carbohydrate hybrid **1** produced a stable complex with a G bulged duplex DNA. Although the ΔT_m value of the G bulged DNA duplex in the presence of hybrid **1** was not very high, the stabilization phenomenon was clearly detectable. The deoxyamino sugar was essential and the distance from the cytosine was important for the selective construction of such a thermodynamically stable complex. This new strategy using a hybrid molecule constructed from a nucleotide base and a nonspecific binder, such as a carbohydrate, will find wide application for the selective recognition of other single nucleotide bulged duplex DNAs. To further improve the ability of hybrid **1**, the replacement of the amino sugar in hybrid **1** with other types of amino sugars and the attachment of oligosaccharides to cytosine are now under investigation in our laboratories.

Acknowledgments

This research was partially supported by a Grant-in-Aid for the 21st Century COE Program 'KEIO Life Conjugate Chemistry' and a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

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9. The synthesis of **2** will be reported in detail elsewhere.
10. ^{1}H NMR (300 MHz, CD_3OD): (δ, $SiMe_4$; J Hz) data for **1** and **2**. Compound **1**: δ 1.33 (3H, d, J = 6.0), 1.62 (1H, ddd, J = 12.0, 12.0, and 10.0), 2.08 (1H, ddd, J = 12.0, 4.0, and 2.0), 2.37 (6H, s), 2.78 (1H, ddd, J = 12.0, 9.0, and 4.0), 3.21 (1H, dd, J = 9.8 and 9.8), 3.47 (1H, dq, J = 9.8 and 6.0), 5.72 (1H, dd, J = 10.0 and 2.0), 5.91 (1H, d, J = 8.0), 7.70 (1H, d, J = 8.0). Compound **2**: δ 1.29 (3H, d, J = 6.0), 1.66 (1H, ddd, J = 12.0, 12.0, and 9.0), 2.23 (1H, ddd, J = 12.0, 4.0, and 2.0), 2.75–2.90 (1H, m), 2.79 (3H, s), 2.86 (3H, s), 3.20–3.40 (3H, m), 3.67 (1H, dq, J = 9.8 and 6.0), 3.82–3.95 (1H, m), 4.0–4.10 (1H, m), 4.65 (1H, dd, J = 9.0 and 2.0), 6.01 (1H, d, J = 8.0), 7.85 (1H, d, J = 8.0).

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12. Nakatani and Saito's group also reported a cytosine derivative[5a] possessing an amino group, $-CH_2CH_2CH_2NH_2$. The ΔT_m value (0.7°) was very similar to those of **2** and **3** and lower than that of **1** under similar conditions.