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Phosphoramidite solid-phase synthesis of site-specifically glycosylated oligodeoxynucleotides

Kazunori Matsuura,[a] Miki Hibino,[a] Masanori Kataoka,[b] Yoshihiro Hayakawa[b] and Kazukiyo Kobayashi[a,*]

[a]*Department of Molecular Design, Graduate School of Engineering, Nagoya University, Chikusa, Nagoya 464-8603, Japan*

[b]*Graduate School of Human Informatics, Nagoya University, Chikusa, Nagoya 464-8601, Japan*

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Abstract

A galactose-modified deoxyuridine phosphoramidite was synthesized via the Heck reaction and applied to solid-phase synthesis to provide a new type of oligo DNA–galactose conjugates, which maintained stringent base-pairing fidelity for unique DNA sequences. © 2000 Elsevier Science Ltd. All rights reserved.

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Glycosylated nucleobases are found in the DNA of some bacteriophages[1] and *Trypanosoma brucei*,[2] and in the RNA of rabbit and rat livers.[3] These glycosylated bases may be involved in the protection of the phages from nucleases in infected host cells and also in the regulation of gene expression, although their detailed biological roles remain an intriguing question to be answered.[4] We are interested in constructing artificial covalent conjugates of DNA and carbohydrates. If the artificial DNA–carbohydrate conjugates maintain the physiological stability and duplex formation ability of DNA and also the recognition ability of carbohydrates, the conjugates will be useful not only as a mimic of naturally occurring glycosylated DNA, but also as a biomedical and clinical tool to apply the characteristic functions of both DNA and carbohydrates. Recently, we reported a facile synthesis of DNA–carbohydrate conjugates by diazo coupling onto fragmented salmon testes DNA and plasmid DNAs.[5] The conjugates were found to acquire resistance to nucleases and recognition by lectins. However, the relative spatial arrangement of the carbohydrates along the DNA sequences was not controlled and the attempted derivatization onto single-stranded oligodeoxynucleotides was unsuccessful.

* Corresponding author. Tel: +81 52 789 2488; fax: +81 52 789 2528; e-mail: kobayash@mol.nagoya-u.ac.jp

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This paper reports the synthesis and characterization of a new type of site-specifically galactosylated oligodeoxynucleotides (Fig. 1) via phosphoramidite solid-phase synthesis using a galactose-modified deoxyuridine derivative **14**. According to Scheme 1, peracetylated galactosyl trichloroacetimidate **9** derived from D-galactose in three steps was coupled with 5-hexyne-1-ol in the presence of TMSOTf. The β-selectively galactosylated derivative **10** was allowed to react with 3′,5′-TBDMS-protected 5-iododeoxyuridine in the presence of $Pd(PPh_3)_4$, CuI, and Et_3N in THF under nitrogen atmosphere at room temperature[6] to afford the coupling product **11** in 58% yield. Deprotection of TBDMS, followed by dimethoxytritylation in pyridine at 50°C, afforded **13** in 83% yield, although the reactivity of **12** was low at room temperature, probably owing to steric hindrance of the galactose moiety. Compound **13** was converted to the phosphoramidite **14** as a mixture of the diastereomers.

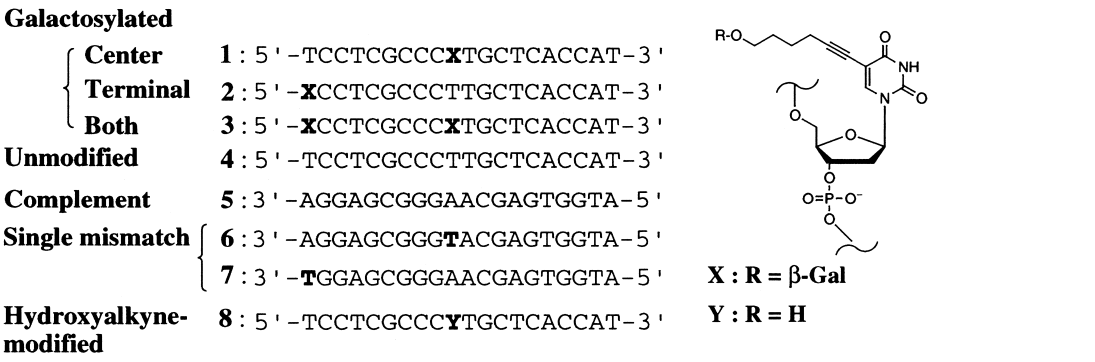
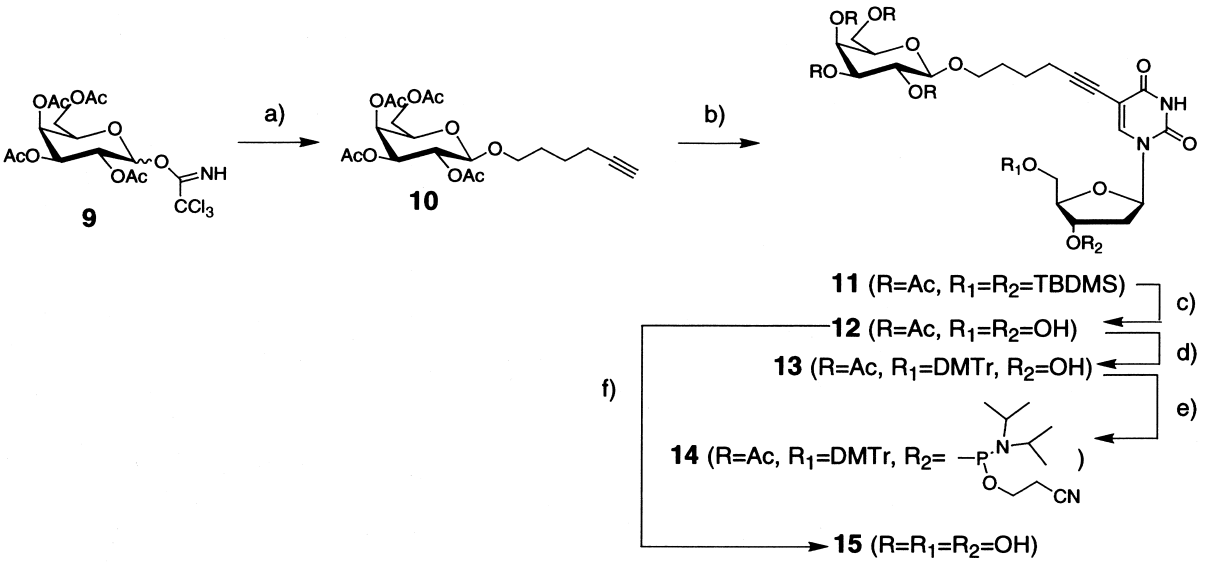


Figure 1. Sequence of oligo DNAs used in the present paper



Scheme 1. Synthesis of galactose-modified deoxyuridine derivatives. *Reagents and conditions*: (a) 5-hexyne-1-ol, TMSOTf, CH_2Cl_2, −40°C then 0°C; (b) 2′-deoxy-3′,5′-bis(*O*-*tert*-butyldimethylsilyl)-5-iodouridine, $Pd(PPh_3)_4$, CuI, Et_3N, THF, rt; (c) TBAF, THF, rt; (d) DMTrCl, Pyr, 50°C; (e) $(iso\text{Pr})_2NP(Cl)OCH_2CH_2CN$, $EtN(iso\text{Pr})_2$, THF, −78°C then 0°C; (f) NH_3, MeOH, rt

The conformational contribution of these galactose derivatives was analyzed by ^{1}H NMR spectroscopy using deprotected nucleoside **15** in CD_3OD.[7] The coupling constants between H1′ and H2′ ($J=6.5$ Hz) and between H3′ and H4′ ($J=3.5$ Hz) suggest that the puckering equilibrium (*C2′-endo*: *C3′-endo*) of the deoxyribose ring of **15** was 65:35, which is comparable to that of thymidine. A strong NOE correlation between H6 of the uridine base and H2′ of the deoxyribose ring and a weak one between H6 and H3′ were observed in the NOESY spectrum of **15**, indicating that the deoxyuridine derivative takes an *anti* conformation similar to the native pyrimidine nucleoside. The modification of the 5-position of deoxyuridine with the galactose derivative has been found to minimally affect the conformational characteristic of the nucleoside.

Solid-phase synthesis of oligonucleotides was performed on an automated Expedite Nucleic Acid Synthesis System from the 3′ to the 5′ end using a standard protocol (1.0 μmol scale). When *1H*-tetrazole was used as the activator, a complex mixture was obtained owing to the low reactivity due to steric hindrance of the galactose moiety of the phosphoramidite **14**. A more reactive activator, benzimidazolium triflate,[8] produced the site-specifically galactosylated oligonucleotides as the main product in about 60–80% coupling efficiency (condition: the amidite **14** = 31 equiv.; the activator = 62 equiv.; time = 45 s). The center galactosylated **1**, terminal galactosylated **2**, and both galactosylated **3** were prepared. The oligonucleotides were deprotected with aqueous ammonia at room temperature for 24 h and then purified by Sephadex G-25 cartridge column and reverse phase HPLC (a linear gradient of 0.1 M ammonium acetate containing 2–30% acetonitrile over 30 min). As summarized in Table 1, MALDI-TOF MS of each conjugate showed a single peak corresponding to the calculated molecular weight. Enzymatic digests of the conjugates with a mixture of DNase I, nuclease P1, and bacterium alkaliphosphatase were analyzed on HPLC to confirm that the conjugates consisted of the desired base-composition including the galactosyl deoxyuridine component. The conjugates hybridized with the complementary DNA showed a typical CD pattern of B-type duplex (Fig. 2), which is reasonably predicted from the *C2′-endo anti* conformation. Thus, the modification of the uridine base at the 5-position with galactose is demonstrated to minimally affect the secondary structure of the oligonucleotide duplexes.

Melting temperatures (T_m) of several galactosylated and unmodified duplexes in PBS buffer (pH 7.4, $[Na^+]=153$ mM, $[K^+]=4.2$ mM) at 45 μM-DNA concentration are summarized in Table 2. The duplex between the center-galactosylated **1** and the complementary **5** showed a 3°C

Table 1
MALDI-TOF MS analysis of oligonucleotide-galactose conjugates and HPLC analysis of their enzymatic digests[a]

DNA	Molecular mass (m/z)	Proportion of nucleoside/found (calcd)				
	Found (calcd)	Gal-dU(**12**)	dC	dG	T	dA
1	6179.73 (6183.24)	0.9 (1.0)	10.2 (10.0)	2.4 (2.0)	4.8 (5.0)	1.7 (2.0)
2	6186.58 (6183.24)	1.3 (1.0)	9.9 (10.0)	2.2 (2.0)	4.9 (5.0)	1.7 (2.0)
3	6428.33 (6427.48)	2.0 (2.0)	9.9 (10.0)	2.2 (2.0)	4.1 (4.0)	1.8 (2.0)

[a] Incubated with a mixture of DNase I, nuclease P1, and BAP in 0.1 M TES buffer (pH 7.4) containing 10 mM $MgCl_2$ and 50 mM $CaCl_2$ for 24 h at 37°C.

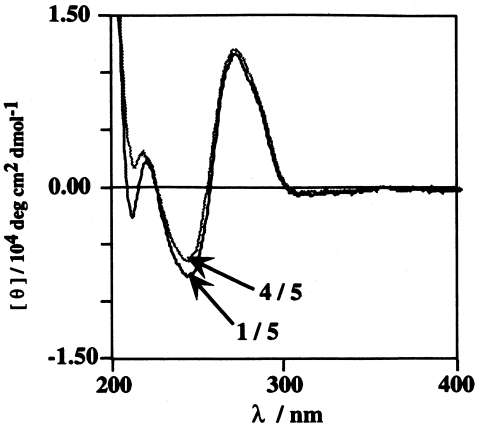


Figure 2. CD spectra of oligo DNA–galactose conjugates in PBS (pH 7.4) at 25°C. [DNA]=45 μM

lower T_m (65°C) than the unmodified duplex (**4**/**5**, T_m=68°C). On the other hand, the terminal galactosylated **2** resulted in a 3°C increase and galactosylated **3** resulted in a 1°C increase. It was reported[9] that the introduction of an alkyne function at the 5-position of the deoxyuridine base stabilizes the duplex, which may explain an increase of T_m of the center 5-hexyn-1-ol substituted **8**/**5** (69°C) and also of **3**/**5**. The stability may be decreased owing to steric hindrance of the bulky galactose moiety, particularly of the center-modified duplex (**1**/**5**). On the other hand, the duplexes of mismatched combinations of galactosylated oligonucleotides were destabilized by 2–4°C, which was similar to that of the unmodified one. It has been confirmed that the recognition ability of complementary sequences is maintained in the galactosylated oligonucleotides.

In conclusion, galactose-modified deoxyuridine phosphoramidite **14** was synthesized and incorporated into oligonucleotide strands (20-mer) at the center, terminal, and both positions on the automated solid-phase DNA synthesis to give the site-specifically galactosylated oligonucleotides. The introduction of the galactose moiety at the 5-position of deoxyuridine resulted in no decrease in both duplex stability and base-pairing fidelity of oligonucleotides. This is a new strategy in biological and supramolecular chemistry employing both DNA and carbohydrates. In short, the galactosylated oligodeoxynucleotides showed stronger nuclease resistance than the unmodified one and formed a periodic glycocluster by hybridizing with the half-sliding complementary oligodeoxynucleotide to bind strongly to lectin, which will be reported in due course elsewhere.

Table 2
Melting temperatures of oligonucleotide-galactose conjugates[a]

DNAs	T_m/°C	DNAs	T_m/°C
1/**5**	65	**1**/**6**	63
2/**5**	71	**2**/**7**	68
3/**5**	69	**3**/**6**	65
4/**5**	68	**4**/**6**	65
8/**5**	69	**4**/**7**	66

[a] T_m were measured in PBS buffer (pH 7.4) at 45 μM-DNA concentration.

Acknowledgements

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