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A Novel Method to Synthesize Versatile Multiple-Branched DNA (MB-DNA) by Reversible Photochemical Ligation

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DNA has potential use both as a generic and a genetic material. Branched DNA molecules have various uses in a signal-amplification technology[1] and in several types of nanotechnology, such as DNA computing,[2] DNA nanostructures from self-assembled branched units,[3] DNA sensors,[4] and nanoelectronic devices.[5] The past 10 years have produced remarkable success in the construction of DNA nanoarchitectures. For example, one- and two-dimensional DNA lattices[6] and dendrimer-like DNA[3a,7] have been constructed from a rich set of branched DNA. Various autonomous DNA walker devices, based on DNA cleavage and ligation of branched DNA by using various enzymes, have also been explored theoretically and experimentally.[8]

However, most branched DNA has been chemically constructed by standard phosphoramidite chemistry on solid supports by using phosphoroamidites containing a branched structural unit or self-assembled addressed DNA. Such branched DNA has several practical disadvantages, such as the limited symmetry of the branched sequences and the low thermal stability of the DNA architecture due to noncovalent bonds. However, these methods cannot be use to create multiple-branched, single-stranded DNA, although the starburst-DNA oligomer has been readily synthesized. There is a need to find a new method to synthesize thermally stable and asymmetrically sequenced branched DNA that can be widely used as a platform for building nanodevices and machines.

Herein we report a novel method to construct a multiply branched DNA in which the multiple branches are connected with a longer single strand (base DNA) via 5-carboxyvinyldeoxyuridine (^{cv}U; Scheme 1). A highly efficient and reversible template-directed DNA photoligation with ^{cv}U contained at the 5′-terminal has been reported previously.[9] In this previous method, an oligonucleotide (ODN) containing ^{cv}U at the 5′-end and an ODN containing a pyrimidine ring were irradiated at 366 nm in the presence of a template to produce a linear combination (L-DNA) between the two ODNs with $>95\%$ yield.

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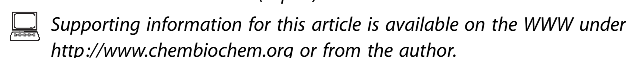
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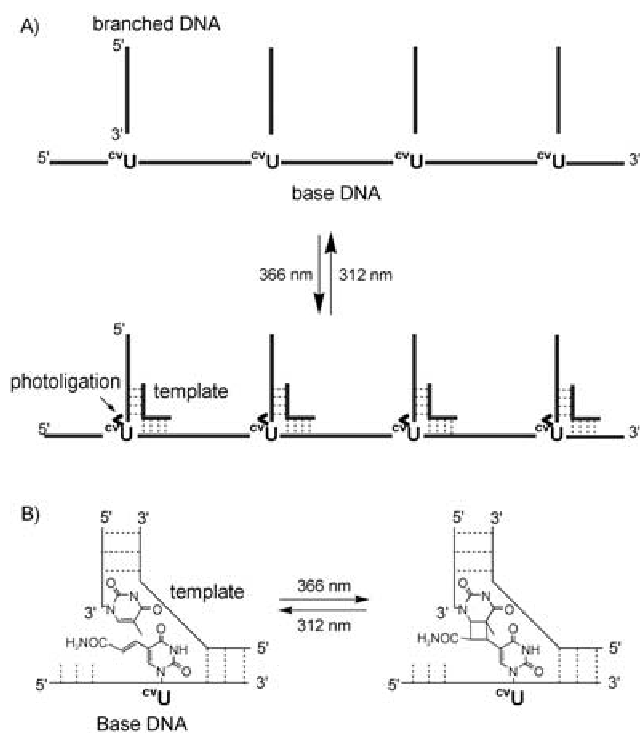


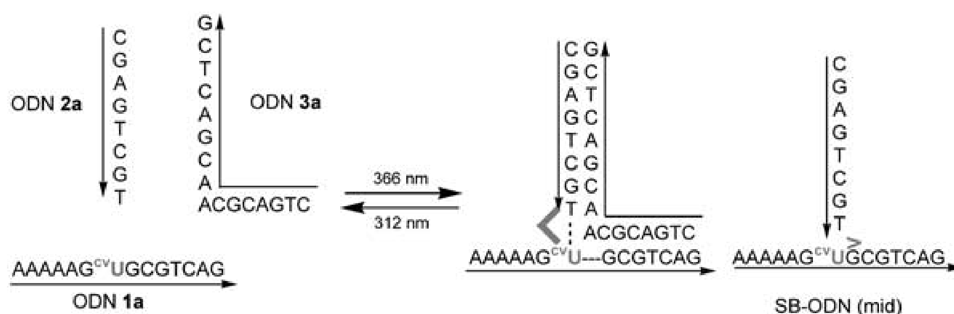
Scheme 1. A) Idealized drawing of our novel method of constructing the MB-DNA based on efficient template-directed photoreversible ligation. Base DNA containing multiple ^{cv}U at the midstream and branched DNA was photoligated by irradiation at 366 nm in the presence of the template. Irradiation at 312 nm caused the ligated product to revert to the original DNA. B) Schematic illustration of the junction site at which irradiation at 366 nm formed a cyclobutane ring between the vinyl group of ^{cv}U and the pyrimidine ring at 3′-end in branched DNA. Conversely, photoirradiation at 312 nm cleaved the cyclobutane ring.

The singly branched ODN [SB-ODN (5′-end)] can also be synthesized in a similar way. We have recently demonstrated a high signal-to-noise (S/N) ratio of a DNA computer by using this system.[10] However, the previous method was restricted by the need to have the ^{cv}U positioned at the 5′-end of the DNA. To overcome this restriction, we have examined a more useful, highly reactive novel method to synthesize branched ODNs based on template-directed[11] DNA photoligation in which ^{cv}U is contained in the middle of the ODN.

To evaluate the new method for synthesizing branched ODNs, we first demonstrated the construction of an SB-ODN (mid) (Scheme 2). When ODNs **1a** and **2a** were irradiated at 366 nm in the absence of template ODN **3a**, no photoligation product was observed; however, in the presence of the template, we observed rapid appearance of the peak of SB-ODN (mid), with a 96% yield after 3 h, as determined by capillary gel electrophoresis (CGE), and the concomitant disappearance of ODNs **1a** and **2a** (Figure 1A). MALDI-TOF MS indicated that the SB-ODN (mid) isolated by HPLC purification was a ligated product of ODNs **1a** and **2a**. Enzymatic digestion of isolated SB-ODN (mid) indicated the formation of dC, dA, dG, and dT in a ratio of 7:4:4:2 together with a new product (identified by MALDI-TOF MS), a dT–^{cv}U adduct[12] (see Supporting Information). The structure of dT–^{cv}U was designated *cis–syn*[2+2] based on the spectroscopic data including ^{1}H,^{1}H COSY and NOESY, as reported previously.[9]

The thermodynamic parameters of SB-ODN (mid) were less than those of a natural ODN; this demonstrates that the duplex formed between SB-ODN (mid) and ODN **3a** has a more deformative structure in the photoadducted region (Table 1). The thermodynamic parameters indicated that the stability of the duplex for other ligated products produced by the previous photoligation method (i.e., linear ODN (L-ODN) and SB-ODN (5′-end) was similar to that of SB-ODN (mid) (see Supporting Information).

To confirm the photoreversibility of the ligated product, we irradiated the SB-ODN (mid) at 312 nm. As shown in Figure 1C, irradiation at 312 nm produced a rapid disappearance of SB-ODN (mid) and reversion to two ODNs. CGE analysis of the mixture of SB-ODN (mid) photoligated at 312 nm confirmed the clean formation of ODNs **1a** and **2a** from SB-ODN (mid), as shown by the migration time. The MALDI-TOF MS analysis of the newly formed ODNs also indicated that these ODNs were **1a** and **2a** (see Supporting Information).



Scheme 2. Schematic drawing of the construction of SB-ODN (mid). ODN **1a** (base-ODN) containing ^{cv}U at the midstream and ODN **2a** were photoligated by irradiation at 366 nm in the presence of template ODN **3a**. Irradiation at 312 nm caused the ligated product to revert to ODN **1a** and ODN **2a**.

Table 1. Thermodynamic parameters for duplex formation.[a]

	T_m [°C]	$-\Delta H°$ [kcal mol^{-1}]	$-\Delta S°$ [cal K^{-1} mol^{-1}]	$-\Delta G°_{298\,K}$ [kcal mol^{-1}]
Nature	63.2	157	440	25.9
SB (mid)	48.2	102	290	15.6

[a] Determined in 50 mM sodium cacodylate and 100 mM NaCl, pH 7.0.

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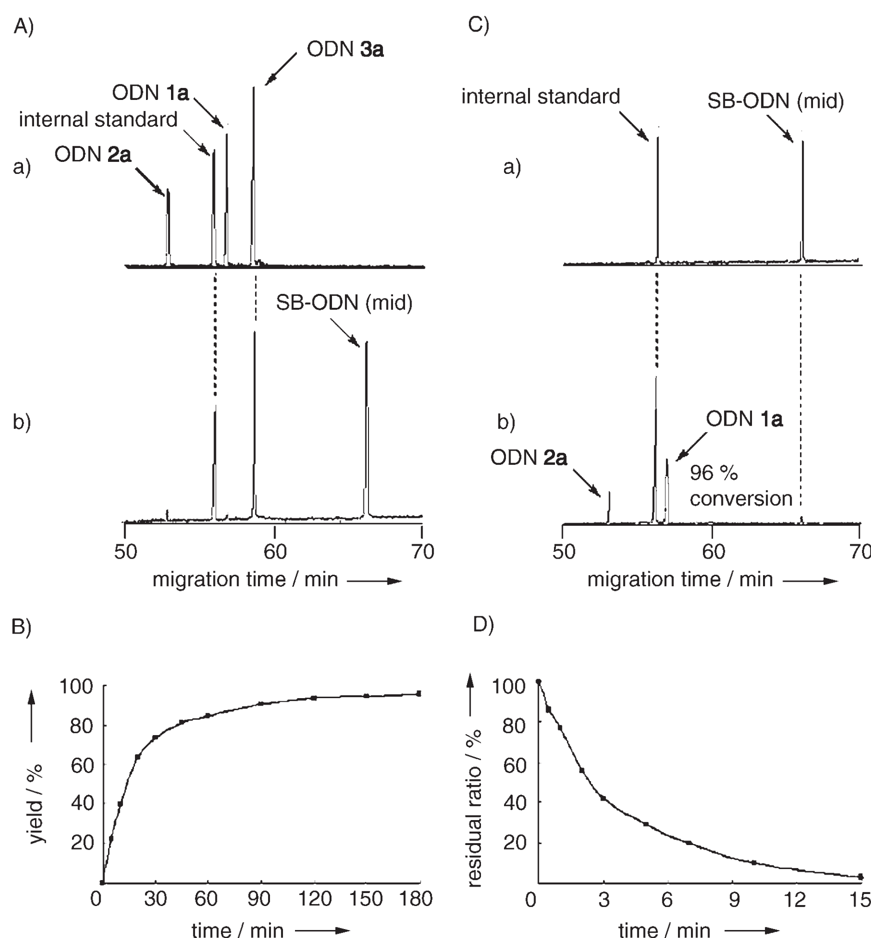


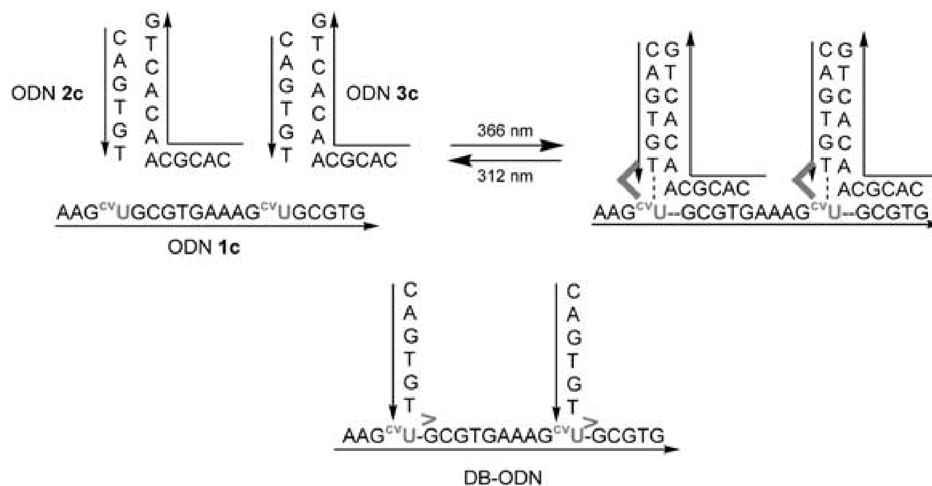
Figure 1. A) CGE analysis of ODNs **1a** and **2a** in the presence of template ODN **3** irradiated at 366 nm: a) before photoirradiation, b) irradiated at 366 nm for 3 h, 96% yield. The sequence of the internal standard was 5′-GTCGTA-GTGGCA-3′. B) Yield for SB-ODN (mid) during irradiation at 366 nm. C) CGE analysis of the SB-ODN (mid) irradiated at 312 nm. D) Residual ratio for SB-ODN (mid) during irradiation at 312 nm

mined by MALDI-TOF MS (see Supporting Information).

Figure 2B shoes that rising time for the yield of DB-ODN was delayed compared with the decline of ODN **1c** because DB-ODN was synthesized through the SB-ODN at the upstream ^{cv}U or downstream ^{cv}U. We observed these peaks clearly at the beginning of the irradiation at 366 nm (see Supporting Information). The afterglow of these peaks is confirmed in Figure 2A(b) as a broad peak at around 80 min. We observed a similar pattern for irradiation at 312 nm. The DB-ODN reverted to the original ODN through the SB-ODN as shown in Figure 3B, in which re-formation of ODN **1c** from DB-ODN irradiated at 312 nm occurred after conversion of DB-ODN.

In conclusion, we have demonstrated that ODN containing ^{cv}U at midstream can be used to synthesize branched ODNs in the presence of a template ODN by irradiation at 366 nm with high efficiency and without any side reaction. Irradiation at 312 nm caused the photoligated branched ODN to revert to the original ODNs. We have also shown the feasibility of constructing MB-DNA by creating a DB-ODN. This demonstration indicates that we can conceptual-

To demonstrate the feasibility of this novel synthetic method of using branched ODN to construct MB-ODN, we examined the effects of the construction of doubly branched ODN (DB-ODN) on base DNA (ODN **1c**) containing two ^{cv}U (Scheme 3). Figure 2A(b) presents a CGE profile of the photoirradiated mixture of ODNs **1c** and **2c** in the presence of template ODN **3c**, and shows the clean and efficient formation of the expected ligated 32-mer DB-ODN and the complete disappearance of ODNs **1c** and **2c**. The isolated new product was characterized by MALDI-TOF MS and enzymatic digestion (see Supporting Information). Further irradiation of DB-DNA at 312 nm resulted in a complete reversion to ODNs **1c** and **2c**, as deter-



Scheme 3. Schematic drawing of DB-ODN. ODN **1c**, which contains two ^{cv}U at midstream, and ODN **2c** were photoligated by irradiation at 366 nm in the presence of template ODN **3c**. Irradiation at 312 nm caused the ligated product to revert to ODNs **1c** (Base-ODN) and **2c**.

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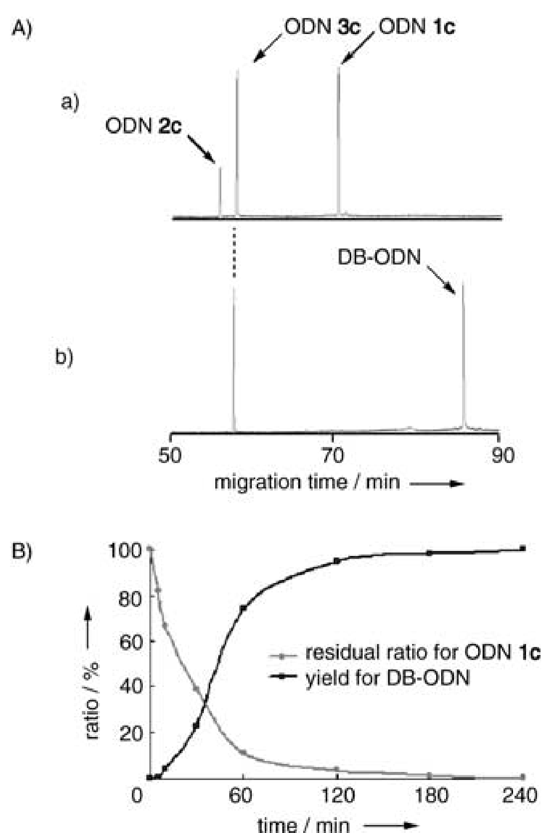

Figure 2. A) CGE analysis of ODNs **1 c** and **2 c** in the presence of template ODN **3 c** irradiated at 366 nm: a) before photoirradiation, b) irradiated at 366 nm for 4 h; >99% yield. B) Yield for DB-ODN and the residual ratio for ODN **1 c** as determined by CGE.

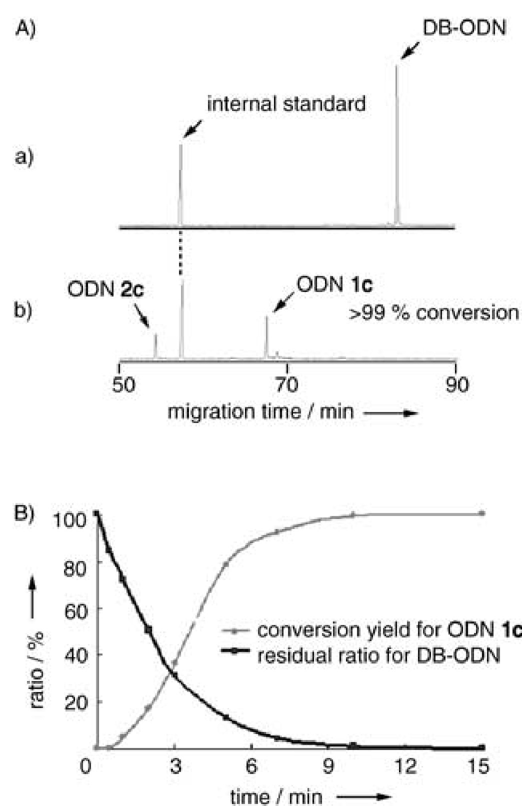

Figure 3. A) CGE analysis of DB-ODN irradiated at 312 nm: a) before photoirradiation, b) irradiated at 312 nm for 15 min; >99% conversion. B) Residual ratio for DB-ODN and conversion yield for ODN **1 c** as determined by CGE.

ly construct the multiply branched structure from a long unnatural base DNA that contains ^{cv}U at the target site, which could not be created by previous methods. We can synthesize a more than 200-mer base DNA by introducing ^{cv}U to DNA using an enzymatic PCR method. This novel method is well suited to DNA nanodevices such as the DNA walker, DNA computer, and DNA architecture, as well as in signal amplification.

Experimental Section

^{cv}U was synthesized from 5-iodo-2′-deoxyuridine; the scheme has been reported previously.[9] ^{cv}U-containing oligonucleotides were prepared by the β-cyanoethylphosphoramidite method on controlled-pore glass supports by using an Applied Biosystems 3400 DNA synthesizer and the standard method. After automated synthesis, the oligomers were deprotected with concentrated aqueous ammonia at 65 °C for 5 h, and purified by HPLC. The oligonucleotides were determined by MALDI-TOF MS.

The reaction mixture containing **1 a**, **2 a** (each 20 μM), and **3 a** (25 μM) in sodium cacodylate buffer (50 mM, pH 7.0) and NaCl (100 mM) was irradiated with a transilluminator at 366 nm at 0 °C for 3 h. CGE analysis was performed by using a Beckman Coulter, P/ACE MDQ capillary electrophoresis system. The denaturing gel (eCAP ssDNA 100-R Kit) purchased from Beckman Coulter contained Tris-borate buffer and urea, and was prepared according to the instruction manual. The enzymatic digestion was carried out with alkaline phosphatase, P1 nuclease and phosphodiesterase in NaCl (100 mM), Tris-HCl (100 mM, pH 8.9), 50% glycerol (5 μL), and $MgCl_2$ (15 mM) at 37 °C for 4 h.

To construct the DB-ODN, **1 c** (20 μM), **2 c** (40 μM), and **3 c** (50 μM) in sodium cacodylate buffer (50 mM, pH 7.0) and NaCl (100 mM) were irradiated with a transilluminator at 366 nm at 0 °C for 4 h.

Keywords: DNA structures · nanostructures · photochemistry

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[12] The UV/Vis spectra were recoded on a Beckman Coulter, DU800 spectrophotometer. dT-^{cv}U adduct: UV (H_2O) λ_{max} (ε) 270 nm (8830 $M^{-1}cm^{-1}$).

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