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Oligonucleotides Containing 7-Vinyl-7-deazaguanine as a Facile Strategy for Expanding the Functional Diversity of DNA

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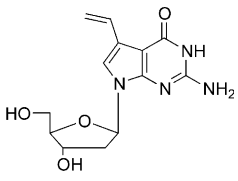
Abstract—A modified nucleobase 7-vinyl-7-deazaguanine (^{V}G) produced adducts with maleimides through Diels–Alder cycloaddition under very mild conditions. By this method, post-synthetic modification to oligonucleotides with diverse functionality (carboxylic acid, pyrene, benzophenone, succinimidyl ester, nitroxide and biotin) was accomplished.

Assembling of functionalized oligonucleotides (ODN) into complementary nucleic acids with target sequences via the formation of Watson–Crick base pairs has led to a rich variety of technologies exploiting new functionalities of ODN.[1–5] Thus, the development of a new synthetic method for ODN possessing diverse functionalities is of great interest. A number of methods for incorporating functionalities to ODN by means of post-synthetic modification have been described.[6–9] Commonly employed methods for the preparation of ODN bioconjugates include the introduction of the chemical modifier during solid-phase ODN synthesis or by post-synthetic modification using reactive handles already incorporated during ODN synthesis. These methods are often accompanied by undesirable operational complexity such as protection/deprotection and site-specific chemical activation.

The Diels–Alder reaction is a very attractive approach for bioconjugation due to the remarkable acceleration of the reaction in aqueous systems.[10,11] Only a few examples of nucleic acid modifications utilizing the Diels–Alder reaction have been reported so far.[12–15] However, most of these methods required long reaction times and/or a specific sequence that may catalyze the reaction. Furthermore, currently available Diels–Alder bioconjugation methods are restricted to only strand ends.

Herein, we report a facile method for the incorporation of functionalized groups into ODN via the Diels–Alder reaction using a novel nucleobase 7-vinyl-7-deazaguanine (^{V}G, **1** in Fig. 1). We found that the reaction of ^{V}G with *N*-substituted maleimides proceeded exceedingly rapidly. By this technique, post-synthetic modification of ODNs was achieved under very mild aqueous conditions.

Prior to post-synthetic modification of ^{V}G-containing ODNs, we first investigated the reaction of protected ^{V}G nucleoside **2** with *N*-methylmaleimide. The preparation of **2** was accomplished by the method reported earlier.[16] The reaction mixture of **2** and *N*-methylmaleimide in methanol was incubated at room temperature (Scheme 1). Within 5 min, **2** was completely converted to maleimide-adduct **3** [calcd 459.1992 for $(M+H)^+$, found 459.2004] via Diels–Alder cycloaddition and subsequent [1,3] H-shift. This experiment indicates that an exocyclic vinyl group at C7 and an endocyclic C7-C8 double bond of **2** serve as a very effective acceptor for the dienophile in the Diels–Alder reaction.



1 (^{V}G)

Figure 1. 7-Vinyl-7-deazaguanine (**1**, ^{V}G) used in this study.

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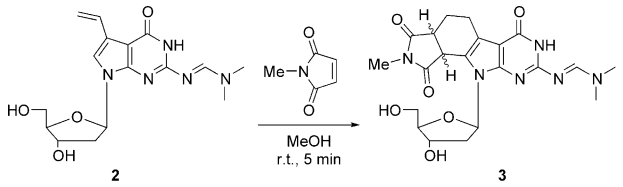
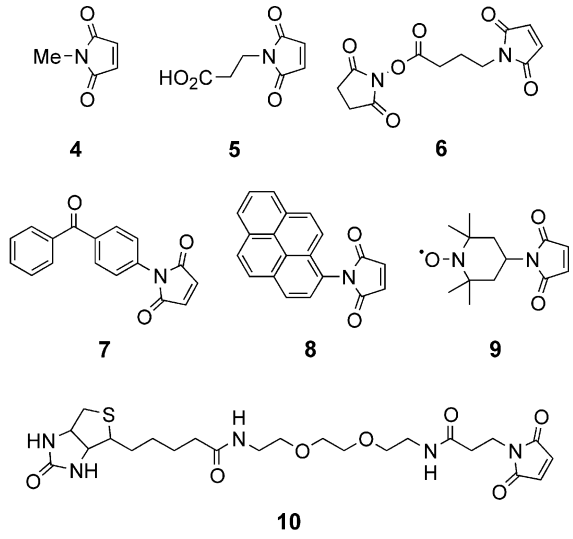


2 3

Scheme 1. Diels–Alder reaction of *N*-protected ^{V}G (**2**) with *N*-methylmaleimide.



4 5 6 7 8 9 10

Figure 2. Functionalized maleimide derivatives incorporated into ^{V}G-containing ODN 5′-d(T^{V}GACGTCA)-3′ through Diels–Alder cycloaddition.

We next examined the post-synthetic modification of a ^{V}G-containing ODN 5′-d(T^{V}GACGTCA)-3′ by the Diels–Alder reaction. The preparation of d(T^{V}GACGTCA) was accomplished by conventional solid-phase DNA synthesis.[17,18] The ODN was incubated with *N*-methylmaleimide (**4**) in phosphate buffer (pH 7.0) at 0 °C. The progress of the reaction was monitored by MALDI-TOF mass spectrometry. The reaction proceeded up to 80% conversion in 10 min, and after 1 h the starting ODN had completely disappeared. The product was proved to be a 1:1 adduct of ^{V}G-containing ODN and *N*-methylmaleimide by MALDI-TOF mass [calcd 2544.76 for [(M−H)$^{-}$], found 2544.24].

Similarly, incorporation of other functionalized maleimides, into ^{V}G-containing ODN was examined in phosphate buffer (pH 7.0) at 0 °C. The corresponding adducts with ^{V}G-containing ODN were characterized by MALDI-TOF mass. The maleimide derivatives insoluble in phosphate buffer were added to the reaction mixture in a methanol solution. The 1 h incubation of the ^{V}G-containing ODN with various functionalized maleimides effectively afforded the corresponding adducts. These include maleimides containing carboxylic acid **5**, an activated ester **6**, benzophenone **7** for photoaffinity labelling, pyrene **8** for a fluorophore, TEMPO **9** as a nitroxide spin label, and biotin **10** (Fig. 2). In these reactions starting ODN completely disappeared and was converted to the corresponding adduct without any additive or protection at 0 °C.[19,20]

In conclusion, a facile method for the incorporation of functionalized groups into ODN was achieved by the Diels–Alder reaction. The present post-synthetic modification proceeded quantitatively under exceedingly mild conditions (pH 7.0, 0 °C). Furthermore, 7-vinyl-7-deazaguanine ^{V}G, possesses a high compatibility for the incorporation of diverse functionalities through Diels–Alder cycloaddition. Thus, the post-synthetic modification of oligonucleotides containing ^{V}G as a functionally diversifiable nucleotide is promising and applicable to site-selective DNA labelling and bioconjugation with inherently labile functionalized groups.

References and Notes

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17. MALDI-TOF MS for 5′-d(T^{V}GACGTCA)-3′, [(M−H)$^{-}$]: calcd 2433.66, found 2433.37.
18. ^{V}G forms a stable base pair with C in duplex DNA. For the stabilization of the duplex containing ^{V}G, see ref 16.
19. MALDI-TOF MS for d(T^{V}GACGTCA)–maleimide adducts, [(M−H)$^{-}$]: **5**, calcd 2602.80, found 2602.54; **6**, calcd 2713.89, found 2713.23; **7**, calcd 2710.93, found 2711.88; **8**, calcd 2730.97, found 2730.47; **9**, calcd 2684.96, found 2685.30; **10**, calcd 2959.28, found 2959.63.
20. Incubation of ^{V}G-containing ODN with succinimidyl ester **6** effectively affords the corresponding adduct, but the methyl ester produced by the solvolysis was also detected in low amounts in mass spectroscopy.