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The first chemical synthesis of boronic acid-modified DNA through a copper-free click reaction†

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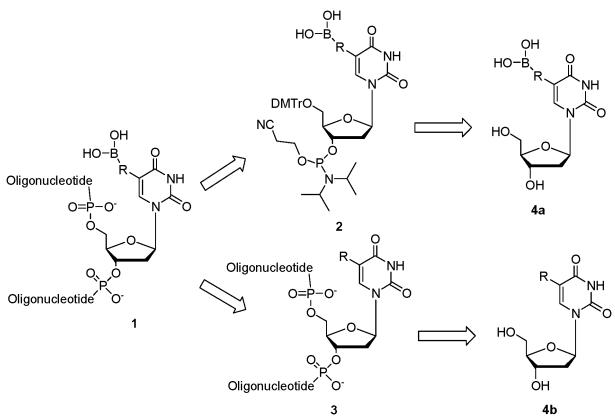
The first chemical incorporation of the boronic acid group into DNA using a copper-free click reagent was reported. Compared with the PCR-based method, this approach allows for site-specific incorporation and synthesis on a larger scale.

Nucleic acids have a wide range of applications in materials, sensing, therapeutics, and computing.[1,2] Side chain functionalization brings in a diverse range of new properties to nucleic acids for broadened applications.[3,4] Among the different functional groups that can be used to modify nucleic acids, we are especially interested in the introduction of boronic acids to DNA because of the ability of this group to function as a Lewis acid, bind to carbohydrate or compounds with other nucleophiles, engage in reversible interactions in assembly, emit alpha particles under neutron radiation, and undergo a wide range of highly efficient organic reactions for further derivatization.[5–8] However, thus far boronic acid introduction into DNA is limited to polymerase-mediated incorporation of modified thymidylate.[9,10] This enzymatic approach requires the synthesis of boronic acid-modified thymidine–triphosphate, which is cumbersome, and imposes a limit on the quantity of materials that can be reasonably produced. Furthermore, the PCR-based method does not allow for site-specific incorporations of the boronic acid group. In addition, the polymerase-based approach would require the boronic acid used to be stable at 90 °C for an extended period of time (PCR time scale). Therefore, we desire to develop a chemical method for synthesis of DNA with the boronic acid functional group.

Conceivably, the introduction of the boronic acid functional group can be accomplished by either using boronic acid-modified building blocks (phosphoramidite **2**) for DNA synthesis or post-synthesis attachment of the boronic acid functional group (Scheme 1). In the first approach, we would need monomer **4a** to contain the boronic acid unit. However, the preparation procedures of the monomeric unit are very moisture-sensitive and boronic acid compounds are generally very hygroscopic. Therefore, preparing **4a** with the boronic acid moiety "pre-installed" would be very challenging. The second approach requires the synthesis of monomer **4b** with a pre-installed handle that allows for the post-DNA synthesis attachment of the boronic acid unit to the DNA. For this, we decided to use click chemistry[11–13] developed by Sharpless because of its compatibility with DNA functional groups.[14,15] Post-synthesis modifications of DNA using click chemistry have been extensively studied.[15] However, application of such chemistry to modification with boronic acids has not been reported.

We started the project with the synthesis of monomer **4b** with a terminal alkyne group side chain in the 5-position of deoxyuridine. Specifically, 5-(octa-1,7-diynyl)-deoxyuridine (**5**) and 5-[3-[(1-oxo-4-pentyn-1-yl)amino]-1-propyn-1-yl] deoxyuridine (**6**) (Fig. S1 in ESI†) were prepared and individually incorporated into oligonucleotides following literature procedures.[16] However, boronic acid was degraded during conjugation using CuAAC (Copper (I)-catalyzed Azide–Alkyne Cycloaddition).[12,13,17] Earlier we have shown that Cu(I) poses stability problems to arylboronic acids.[18] Though fluoride addition sometimes helps improve stability, boronic acid degradation still occurs to a degree that is not acceptable in DNA modification where purification is a major issue and high reaction yield at each individual site is critical. Thus we turned to an alkyne, developed by the Bertozzi lab for a copper-free cycloaddition, as a handle. A copper free click reagent DIFO **7** was prepared using a method described in the



Scheme 1 Retrosynthetic analysis of boronic acid modified DNA.

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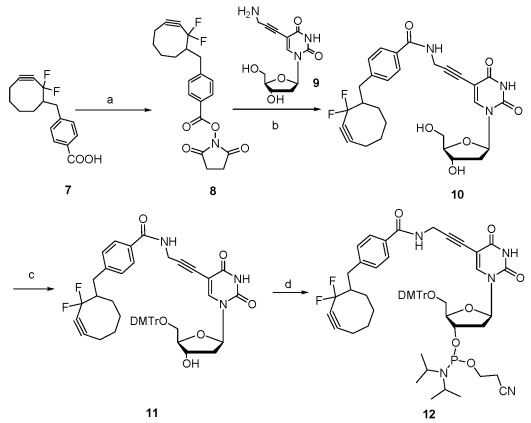


Reagents and conditions: a).EDCI, SuOH, rt, 2h, 95%; b).**9**, Et_3N, DMF, rt, overnight, 83%; c) DMTrCl, Py, rt, 5h, 65%; d). 2-cyanoethyl *N,N*-diiso-propyl chlorophos phoramitide, DIPEA, CH_2Cl_2, 2h, 43%

Scheme 2 Synthesis of DIFO bearing phosphoramidite for solid phase DNA synthesis.

literature.[19] This was followed by succinimide activation and attachment to the thymidine 5-position to give **10**, which was converted to the phosphoramidite building block **12** for DNA synthesis (Scheme 2).[20]

Incorporation of the DIFO bearing phosphoramidite **12** into oligonucleotides *via* solid phase synthesis proceeded smoothly using an ultra mild DNA synthesis protocol.[21] Four oligonucleotides were designed. The first three have the modification sites at different positions, and the fourth one has two modifications next to each other (Fig. 1). Thus, **ODN1–4** were synthesized, purified by HPLC, characterized by MS (see ESI† for details) and then treated with benzyl azide (**a**, Fig. 2) as a model reaction to optimize reaction conditions. This was followed by reactions with azido boronic acids (**b**, **c**, **d**, Fig. 2) individually. The click reactions were carried out at 10 μM concentration of DNA with 20 equivalents of the azide compound at room temperature for 30 min.

Fig. 3 shows a typical set of HPLC chromatograms of the starting materials azido boronic acid **b** (i), **ODN2** (ii) and the reaction mixture (iii) after mixing at room temperature for 30 min. Under such conditions, the oligonucleotide (**ODN2**) disappeared with the formation of two new peaks corresponding to the two regioisomers of the desired products. These two isomers were isolated (iv and v) for mass spectrometric characterizations. At this point it is important to note that

ODN1 5'-TXTTTTTT-3'
ODN2 5'-ACTXACT-3'
ODN3 5'-TCGAXAGCT-3'
ODN4 5'-TCGXXAGCT-3'

Fig. 1 A list of the ODN series containing **10** (X = compound **10**).

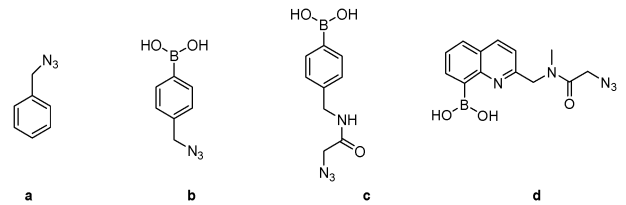


Fig. 2 Structures of the azido compounds used.

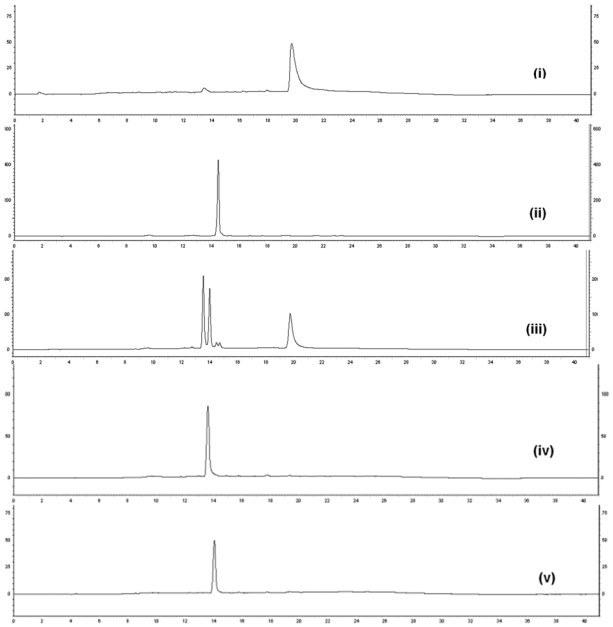


Fig. 3 A typical set of HPLC chromatograms (i) azido boronic acid **b**; (ii) **ODN2**; (iii) reaction mixture; (iv and v) isolated two isomers.

the reaction is very clean with a high conversion (>95%) of the starting material to products.

The isolated peaks corresponding to two regioisomers were further characterized with mass spectrometry. Both gave the same *m*/*z* (2497.3) as the major product peak (Fig. 4b) and a new peak of *m*/*z* 2505.4 after treatment with H_2O_2 (Fig. 4c). These peaks were assigned as the dehydrated

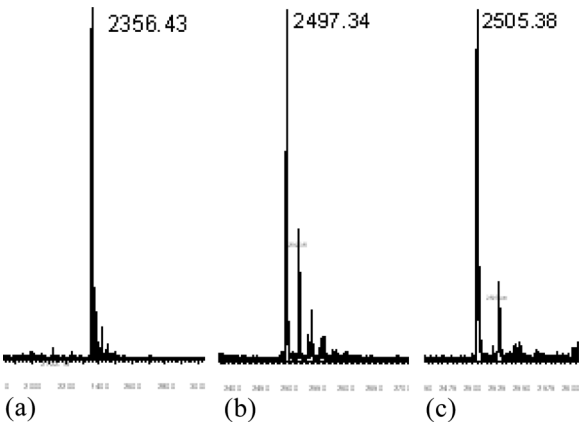
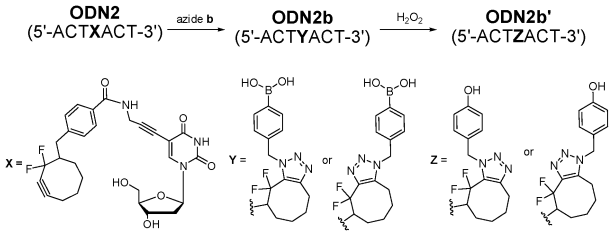


Fig. 4 ESI MS of **ODN2** (a); after click reaction with azido boronic acid **ODN2b** (b); and after being treated with H_2O_2 **ODN2b′** (c).



Scheme 3 Boronic acid modification by a copper free click reaction and further transformation by treating with H_2O_2.

(calc. $[M + H - 2 \times H_2O]^+$: 2497.5) and oxidative deborylated oligonucleotides (calc. $[M + H - HBO]^+$: 2505.5) product (**ODN2b′**, Scheme 3), respectively. Such results confirmed the formation of the desired products. The reaction products for the other azido boronic acids were similarly characterized (see ESI† for details), demonstrating general applicability.

In summary, we have prepared oligonucleotides bearing the DIFO group through solid-phase synthesis. The attachment of a boronic acid group was readily achieved by reaction with an azido boronic acid. HPLC and mass spectrometry studies confirmed the final products. This represents the very first example that boronic acid-modified DNA was synthesized chemically. This method will be very useful for the preparation of boronic acid-modified DNA for various applications.

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