The First Chemical Synthesis of Boronic Acid-modified DNA through Copper-free Click Reaction

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Experimental Section

General methods and materials
Solvents and reagents were purchased from VWR, or Aldrich and used without purification unless specified otherwise. When necessary, solid reagents were dried under high vacuum. Reactions with compounds sensitive to air or moisture were performed under argon. Solvent mixtures are indicated as volume/volume ratios. Thin layer chromatography (TLC) was run on Sorbtech W/UV254 plates (0.25 mm thick), and visualized under UV-light or by a Ce-Mo staining solution (phosphomolybdate, 25 g; Ce(SO$_4$)$_2$·4H$_2$O, 10 g; H$_2$SO$_4$, 60 mL, conc.; H$_2$O, 940 mL) with heating. Flash chromatography was performed using Fluka silica gel 60 (mesh size 0.040-0.063 mm) using a weight ratio of ca. 30:1 for silica gel over crude compound. $^1$H, $^{13}$C and $^{31}$P NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer in deuterated chloroform (CDCl$_3$), methanol-$d_4$ (CD$_3$OD), and DMSO-$d_6$ with either tetramethylsilane (TMS) (0.00 ppm) or the NMR solvent as the internal reference. HPLC purification was carried out using a Shimadzu LC-10AT VP system with a Zobax C18 reversed-phase column (9.4 mm × 25 cm). The sample was eluted (6 mL/min) with a linear gradient from buffer A (20 mM triethylammonium acetate, pH 6.9-7.1) to buffer B (50% acetonitrile, 20 mM triethyl ammonium acetate) in 20 min. HPLC analytical studies were carried out using a Shimadzu LC-10AT VP system with a Pinnacle C18 reverse phase column (4.6 mm × 25 cm). The sample was eluted (1 mL/min) with the same gradient as above. Water used for HPLC studies was doubly distilled and further purified with a Milli-Q filtration system.

Figure S1. Structures of monomer 5 and 6
Synthesis
DIFO 7 was prepared according to the Bertozzi method.1

5-(3-Amino-1-propyn-1-yl)-2’-deoxyuridine 9

\[
\begin{align*}
&\text{H2N} \\
&\text{\equiv} \\
&\text{NH} \\
&\text{HO} \\
&\text{OH}
\end{align*}
\]

Synthesized by following literature procedure.2
White needles Mp (180-183 °C, decomp.); IR \( \nu_{\text{max}} \): 3499, 3362, 2923, 2844, 1706, 1682, 1626, 1470, 1428, 1282, 1031 cm\(^{-1}\); \( ^1\text{H} \) NMR (CD\(_3\)OD): \( \delta \) 8.44 (s, 1H), 6.24 (t, \( J = 6.5 \) Hz, 1H), 4.41 (ddd, \( J = 3.5, 6.0, 6.5 \) Hz, 1H), 2.92 (s, 4H), 4.00 (s, 1H), 3.98 (dd, \( J = 3.3, 6.5 \) Hz, 2H), 3.82 (dd, \( J = 3.0, 12.1 \) Hz, 1H ), 3.75 (dd, \( J = 3.4, 12.1 \) Hz, 1H ), 2.33 (ddd, \( J = 3.6, 6.1, 13.5 \) Hz, 1H), 2.21 (ddd, \( J = 6.5, 6.5, 13.5 \) Hz, 1H); \( ^{13}\text{C} \) NMR (DMSO-\(d_6\)): \( \delta \) 162.2, 150.0, 143.4, 99.2, 95.0, 88.0, 85.1, 74.4, 70.7, 61.5, 31.9 ppm; MS (ESI, \( m/z \)): 280.1 [M – H]; HRMS (ESI) calcd for C\(_{12}\)H\(_{14}\)N\(_3\)O\(_5\)\(-\) [M – H]- 280.0933; found, 280.0921.

DIFO- succinimide activated ester 8

DIFO (480 mg, 1.7 mmol) and \( \text{N}-\)hydoxy-succinimide (240 mg, 2.1 mmol) were dissolved in DCM 20 mL at room temperature. EDCI (400 mg, 2.1 mmol) was added in one portion. The reaction mixture was allowed to stir at the same temperature until TLC showed complete disappearance of the starting material. The reaction mixture was diluted with DCM (200 mL), washed with water and brine, dried over Na\(_2\)SO\(_4\), concentrated in vacuum, purified by flash chromatography (EA/Hexane 1/2) to give compound 8 (605 mg, 95%) as a white solid. Mp: 117-119 °C; IR \( \nu_{\text{max}} \): 2940, 2215, 1764, 1734, 1609,1238, 1203, 1069, 1025 cm\(^{-1}\); \( ^1\text{H} \) NMR (CDCl\(_3\)): \( \delta \) 8.08 (d, \( J = 8.2 \) Hz, 2H), 7.35 (d, \( J = 8.2 \) Hz, 2H), 3.20 (m, 1 H), 2.92 (s, 4 H), 2.55 (m, 2H), 2.32 (m, 2H), 2.10-1.90 (m, 2H), 1.87-1.67 (m, 2H), 1.52 (dd, \( J = 7.4, 13.2 \) Hz 1H), 1.20-1.10 (m, 1H); \( ^{13}\text{C} \) NMR (CDCl\(_3\)): \( \delta \) 169.4, 161.7, 147.9, 130.9, 129.7, 123.1, 110.0 (t, \( J = 11.2 \) Hz), 84.9 (dd, \( J = 41.9, 46.4 \) Hz), 58.0 (t, \( J = 24.1 \) Hz), 34.6, 32.4, 30.7, 27.9, 25.7, 20.3 ppm; MS (ESI, \( m/z \)): 398.2 [M + Na]\(^+\); HRMS (ESI) calcd for C\(_{20}\)H\(_{19}\)F\(_2\)NNaO\(_4\)\(^+\) [M + Na]\(^+\) 398.1180; found, 398.1172.
Compound 10

To a solution of activated ester 8 (300 mg, 0.8 mmol) and compound 9 (269 mg, 0.96 mmol) in DMF (5 mL) was added triethylamine (0.22 mL, 1.6 mmol) at room temperature. After being stirred overnight, DMF was removed under vacuum. The residue was purified by flash chromatography (MeOH/DCM = 1/10) to give compound 10 (359 mg, 83%) as a pale yellow solid. Mp (130-132 °C, decomp.); IR νmax : 3375, 1679, 1539, 1458, 1277, 1027 cm⁻¹; ¹H NMR (CD3OD): δ 8.33 (s, 1H), 7.81 (d, J = 7.9 Hz, 2H), 7.34 (d, J = 7.9 Hz, 2H), 6.24 (t, J = 6.6 Hz, 1H), 4.41 (ddd, J = 3.2, 6.4, 6.4 Hz, 1H), 3.96 (dd, J = 3.2, 3.2 Hz, 1H), 3.82 (dd, J = 3.2, 8.1 Hz, 1H), 3.74 (dd, J = 3.6, 12.0 Hz, 2H), 3.12 (m, 1H), 2.55-2.51 (m, 2H), 2.40-2.20 (m, 3H), 2.02-1.10 (m, 5H), 1.53-1.40 (m, 1H); ¹³C NMR (DMSO-d₆): δ 166.1, 162.1, 149.9, 144.1, 143.4, 132.2, 129.5, 128.0, 120.4 (t, J = 235.4 Hz), 112.3 (t, J = 11.4 Hz), 98.7, 90.3, 88.1, 85.2, 74.6, 70.7, 61.5, 57.2 (t, J = 23.5 Hz), 52.2, 33.9, 32.6, 30.8, 29.7, 27.3, 25.5, 23.0, 20.0, 19.8, ppm; MS (ESI, m/z): 540.2 [M – H]; HRMS (ESI) calcd for C₂₈H₂₈F₂N₃O₆ [M – H]⁻ 540.1946; found, 540.1931.

Compound 11

Compound 10 (260 mg, 0.48 mmol) was dried by co-evaporation with anhydrous pyridine (3 mL × 2) and then dissolved in anhydrous pyridine (6 mL). 4,4’-Dimethoxytrityl chloride (224 mg, 0.57 mmol) was added in three portions to the remaining solution at r.t. with stirring for 5 h. Then MeOH (2 mL) was added, and the mixture was stirred for another 30 min. The mixture was dissolved in CH₂Cl₂ (100 mL) and extracted with 5% aq. NaHCO₃ soln. (100 mL) followed by H₂O (80 mL), dried over Na₂SO₄, and then concentrated under reduced pressure. The crude product
was purified by flash chromatography (silica gel, CH$_2$Cl$_2$/acetone 4:1) to yield compound 11 (263 mg, 65%) as a pale yellow solid. Mp (120-123 °C, decomp.); IR $\nu_{\text{max}}$: 3680, 2937, 1687, 1609, 1507, 1462, 1280, 1247, 1057, 1029, cm$^{-1}$; $^1$H NMR (CDCl$_3$): $\delta$ 8.18 (s, 1H), 7.58 (d, $J$ = 7.8 Hz, 2H), 7.42 (d, $J$ = 7.8 Hz, 2H), 7.33 (d, $J$ = 8.4 Hz, 4H), 7.27 (dd, $J$ = 5.6, 3.6 Hz, 2H), 7.17 (dd, $J$ = 5.6, 3.6 Hz, 2H), 6.83 (d, $J$ = 8.4 Hz, 4H) 6.33 (t, $J$ = 6.0 Hz, 1H), 4.57 (brs, 1H), 4.10-4.05 (m, 3H), 3.73 (s, 6H), 3.36 (m, 2H), 3.09 (d, $J$ = 10.2 Hz, 1H), 2.57-2.30 (m, 6H), 2.05-1.69 (m, 4H), 1.49 (m, 1H), 1.10 (m, 1H); $^{13}$C NMR (CDCl$_3$): $\delta$ 166.8, 162.5, 158.6, 149.4, 144.6, 143.8, 143.3, 135.6, 131.7, 130.0, 129.2, 128.1, 127.9, 127.4, 127.0, 119.4 (t, $J$ = 238.2 Hz), 113.4, 109.8, 99.4, 89.6, 87.1, 86.8, 86.0, 85.0 (t, $J$ = 44.2 Hz), 74.4, 72.2, 63.6, , 58.0 (t, $J$ = 23.5 Hz), 57.8, 53.5, 41.5, 34.2, 32.5, 30.7, 30.5, 27.8, 20.3 ppm; MS (ESI, m/z): 842.3 [M – H]; HRMS (ESI) calcd for C$_{49}$H$_{46}$F$_{2}$N$_{3}$O$_{8}$ [M – H]$^-$ 842.3253; found, 842.3268.

**Compound 12**

To a stirred solution of compound 11 (180 mg, 0.21 mmol) in anhydrous CH$_2$Cl$_2$ (10 mL) was added $i$Pr$_2$EtN (65 $\mu$L, 0.38 mmol) under argon at room temperature, and then 2-cyanoethyl diisopropylphosphoramidochloridite (76 $\mu$L, 0.27 mmol). After being stirred for 1 h, the reaction mixture was diluted with CH$_2$Cl$_2$ (100 mL) and then washed with 5% sodium bicarbonate solution. The organic layer was dried over Na$_2$SO$_4$, and then concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, CH$_2$Cl$_2$/MeOH= 10:1 with 0.5 Et$_3$N) to yield a diastereomeric mixture of phosphoramidite 12 (95 mg, 43%) as a pale yellow foam. $^{31}$P NMR (CDCl$_3$): 149.7, 149.4. MS (ESI, m/z): 1145.7 [M + Et$_3$NH]$^+$; HRMS (ESI) calcd for C$_{64}$H$_{80}$F$_2$N$_6$O$_9$P$^+$ [M + Et$_3$NH]$^+$ 1145.5692; found, 1145.5708. $^1$H NMR (CDCl$_3$): $\delta$ 8.27-8.15 (m, 1H), 7.54-7.48(m, 4 H), 7.35-7.28 (m, 5H), 7.19-7.16 (m, 4 H), 6.83 (m, 4H), 6.32 (m, 1H), 4.30-4.05 (m, 4H), 3.75 (m, 6H), 3.65-3.35 (m, 4 H), 3.15 (m, 1H), 2.85-2.34 (m, 5H), 2.10-1.65 (m, 6H), 1.45 (m, 6H), 1.26 (m, 6H).
**Summary of Click Reactions of ODN1–4 and Azido Compounds a–d**

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* ODN1a: product of click reaction using ODN1 and azido compound a

MW: Molecular Weight; Calc. Calculated; Ob: Oberserved.

We assign [M+H-2×H2O]^+ based on our experimental observations that all the monoboronic acid-modified DNA show this peak and bisboronic acid modified DNA show [M+H-4×H2O]^2+.

Such results indicate that every boronic acid loses two H2O in the mass spectrometer. One possible explanation is the strong intramolecular interactions between the boronic acid group and phosphate backbone, which lead to the M-2H2O peak under ESI-MS conditions.

**References:**


[Chemical structure image]
Synthesis and purification of DIFO-modified DNA oligonucleotides (ODN 1-4).

All DNA oligonucleotides were synthesized by solid-phase synthesis using an ABI392 DNA/RNA synthesizer on a 1 μmol scale. The concentration of the DIFO-modified thymidine phosphoramidite (12) was identical to that of the standard phosphoramidite (0.1 M in acetonitrile). The coupling reaction was carried out using a 5-BMT solution (0.3 M) in acetonitrile with a coupling time of 25 seconds. All the oligonucleotides were prepared with DMTr-On, followed by the cleavage from the CPG solid support (Beads) and the deprotection with 0.05 M K₂CO₃ in MeOH for 5h at rt. After the DMTr-on purification by HPLC, the detritylation of the oligonucleotides was performed by the treatment of 3% trichloroacetic acid (aqueous solution) for five minutes, followed by neutralization to pH 7.0 with triethylamine and extraction with petroleum ether to remove the by-product DMTr-OH. The DMTr-off oligonucleotides were purified again by HPLC.

HPLC of ODN1  (5'-TXTTTTT-3')

HPLC of ODN2  (5'-ACTXACT-3')
HPLC of ODN3  (5'-TCGAXAGCT-3')

HPLC of ODN4  (5'-TCGXXAGCT-3')

HPLC of ODN1a

HPLC of ODN1b
HPLC of ODN2b-2

HPLC of ODN2c

HPLC of ODN2d-1

HPLC of ODN2d-2
MALDI of ODN1

ESI MS of ODN1a
ESI MS of ODN2a

ESI MS of ODN2b

Supplementary Material (ESI) for Chemical Communications
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Supplementary Material (ESI) for Chemical Communications
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ESI MS of ODN3

ESI MS of ODN3a
ESI MS of ODN3b

ESI MS of ODN3c
ESI MS of ODN4a

ESI MS of ODN4b