Supporting information for

Inhibition of off-target cleavage by RNase H using an artificial cationic oligosaccharide

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Figure S1 Putative ODAGal4 binding site on the duplex of a 9mer DNA (D1) and a 24mer RNA (R1). It should be noted that one molecule of ODAGal4 binds to the duplex in each case. It looks two molecules are in each figure just because the duplex structures are depicted in plane and therefore two sides of single ODAGal4 were inevitably depicted separately. (a) When RNase H cleaves the RNA to generate the 10mer p-RNA, RNase H would compete with ODAGal4. (b) When RNase H cleaves the RNA to generate the 7mer p-RNA, RNase H would compete with ODAGal4 for the phosphate groups of the RNA strand.



Figure S2 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R1). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S3 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S4 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R1) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S5 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R2). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S6 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R2) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S7 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R2) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S8 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R3). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S9 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R3) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S10 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R3) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S11 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S12 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R4) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%-12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S13 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R4) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S14 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S15 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R5) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%-10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S16 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R5) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S17 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R6). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S18 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R6) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S19 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R6) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S20 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M ammonium acetate (AA) buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S21 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R7) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%-10% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S22 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R7) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%-10% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S23 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R8). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S24 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R8) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S25 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R8) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S26 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R9). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S27 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R9) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%-12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S28 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R9) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S29 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R10). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S30 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R10) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S31 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R10) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S32 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R11). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S33 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R11) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S34 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R11) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.


Figure S35 RP-HPLC profile of the mixture of a 9mer DNA (D1) and 24mer RNA (R12). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S36 RP-HPLC profile of the mixture of a 9mer DNA (D1) and 24mer RNA (R12) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S37 RP-HPLC profile of the mixture of a 9mer DNA (D1) and a 24mer RNA (R12) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S38 RP-HPLC profile of the mixture of a 9mer DNA (D2) and a 24mer RNA (R1), which was used for the calculation of the amount of the cleaved RNA in Figure S40 and S41. The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S39 RP-HPLC profile of the mixture of a 9mer DNA (D2) and a 24mer RNA (R1), which was used for the calculation of the amount of the cleaved RNA in Figure S42 and S43. The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S40 RP-HPLC profile of the mixture of a 9mer DNA (D2) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%-10% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S41 RP-HPLC profile of the mixture of a 9mer DNA (D2) and a 24mer RNA (R1) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S42 RP-HPLC profile of the mixture of a 9mer DNA (D2) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 1 h (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S43 RP-HPLC profile of the mixture of a 9mer DNA (D2) and a 24mer RNA (R1) in the presence of ODAGal4 after treatment with RNase H for 1 h (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S44 RP-HPLC profile of the mixture of a 9mer DNA (D2) and a 24mer RNA (R3) in the presence of ODAGal4 after treatment with RNase H for 1 h (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S45 RP-HPLC profile of the mixture of a 9mer DNA (D2) and a 24mer RNA (R3) in the absence of ODAGal4 after treatment with RNase H for 1 h (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S46 RP-HPLC profile of the mixture of a 9mer DNA (D2) and a 24mer RNA (R3) in the presence of ODAGal4 after treatment with RNase H for 1 h (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S47 RP-HPLC profile of the mixture of a 9mer DNA (D3) and a 24mer RNA (R1). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S48 RP-HPLC profile of the mixture of a 9mer DNA (D3) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S49 RP-HPLC profile of the mixture of a 9mer DNA (D3) and a 24mer RNA (R1) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S50 RP-HPLC profile of the mixture of a 9mer DNA (D3) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min. The peak at 40 min is the intact RNA and the peak at 51 min is the DNA.



Figure S51 RP-HPLC profile of the mixture of a 9mer DNA (D3) and a 24mer RNA (R1) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S52 RP-HPLC profile of the mixture of a 9mer DNA (D3) and a 24mer RNA (R4). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S53 RP-HPLC profile of the mixture of a 9mer DNA (D3) and a 24mer RNA (R4) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S54 RP-HPLC profile of the mixture of a 9mer DNA (D3) and a 24mer RNA (R4) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S55 RP-HPLC profile of the mixture of a 9mer DNA (D3) and a 24mer RNA (R4) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S56 RP-HPLC profile of the mixture of a 9mer DNA (D3) and a 24mer RNA (R4) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 5). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S57 RP-HPLC profile of the mixture of a 9mer DNA (D4) and a 27mer RNA (R13). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S58 RP-HPLC profile of the mixture of a 9mer DNA (D4) and a 27mer RNA (R13) in the absence of ODAGal4 after treatment with RNase H for 1 h (Figure 6). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S59 RP-HPLC profile of the mixture of a 9mer DNA (D4) and a 27mer RNA (R13) in the presence of ODAGal4 after treatment with RNase H for 1 h (Figure 6). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–10% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 1.0 mL/min.



Figure S60 RP-HPLC profile of the mixture of a 9mer DNA (D4) and a 27mer RNA (R14). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–9% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S61 RP-HPLC profile of the mixture of a 9mer DNA (D4) and a 27mer RNA (R14) in the absence of ODAGal4 after treatment with RNase H for 1 h (Figure 6). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–9% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S62 RP-HPLC profile of the mixture of a 9mer DNA (D4) and a 27mer RNA (R14) in the presence of ODAGal4 after treatment with RNase H for 1 h (Figure 6). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–9% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S63 RP-HPLC profile of the mixture of an 8mer DNA (D5) and a 24mer RNA (R1). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S64 RP-HPLC profile of the mixture of an 8mer DNA (D5) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S65 RP-HPLC profile of the mixture of an 8mer DNA (D5) and a 24mer RNA (R1) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S66 RP-HPLC profile of the mixture of an 8mer DNA (D5) and a 24mer RNA (R2). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S67 RP-HPLC profile of the mixture of an 8mer DNA (D5) and a 24mer RNA (R2) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S68 RP-HPLC profile of the mixture of an 8mer DNA (D5) and a 24mer RNA (R2) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–12% CH₃CN in 0.1 M TEAA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S69 RP-HPLC profile of the mixture of a 10mer DNA (D6) and a 24mer RNA (R1). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S70 RP-HPLC profile of the mixture of a 10mer DNA (D6) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min. The peak at 46 min is the intact RNA and the peak at 57 min is the DNA.



Figure S71 RP-HPLC profile of the mixture of a 10mer DNA (D6) and a 24mer RNA (R1) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.


Figure S72 RP-HPLC profile of the mixture of a 10mer DNA (D6) and a 24mer RNA (R2). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S73 RP-HPLC profile of the mixture of a 10mer DNA (D6) and a 24mer RNA (R2) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S74 RP-HPLC profile of the mixture of a 10mer DNA (D6) and a 24mer RNA (R2) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S75 RP-HPLC profile of the mixture of a 11mer DNA (D7) and a 24mer RNA (R1). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S76 RP-HPLC profile of the mixture of a 11mer DNA (D7) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min. The peak at 43 min is the intact RNA and the peak at 53 min is the DNA.



Figure S77 RP-HPLC profile of the mixture of a 11mer DNA (D7) and a 24mer RNA (R1) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S78 RP-HPLC profile of the mixture of a 11mer DNA (D7) and a 24mer RNA (R2). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S79 RP-HPLC profile of the mixture of a 11mer DNA (D7) and a 24mer RNA (R2) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S80 RP-HPLC profile of the mixture of a 11mer DNA (D7) and a 24mer RNA (R2) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S81 RP-HPLC profile of the mixture of a 12mer DNA (D8) and a 24mer RNA (R1). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S82 RP-HPLC profile of the mixture of a 12mer DNA (D8) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S83 RP-HPLC profile of the mixture of a 12mer DNA (D8) and a 24mer RNA (R1) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S84 RP-HPLC profile of the mixture of a 12mer DNA (D8) and a 24mer RNA (R2). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S85 RP-HPLC profile of the mixture of a 12mer DNA (D8) and a 24mer RNA (R2) in the absence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S86 RP-HPLC profile of the mixture of a 12mer DNA (D8) and a 24mer RNA (R2) in the presence of ODAGal4 after treatment with RNase H for 5 min (Figure 7). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S87 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R1), which was used for the calculation of the amount of the cleaved RNA in Figure S88 and S89. The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S88 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R1), which was used for the calculation of the amount of the cleaved RNA in Figure S90–93. The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S89 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S90 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S91 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) at 50 °C with a flow rate of 0.5 mL/min.



Figure S92 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R1) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) at 50 °C with a flow rate of 0.5 mL/min.



Figure S93 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) at 50 °C with a flow rate of 0.5 mL/min.



Figure S94 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R1) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) at 50 °C with a flow rate of 0.5 mL/min.



Figure S95 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R2), which was used for the calculation of the amount of the cleaved RNA in Figure S97 and S98. The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S96 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R2), which was used for the calculation of the amount of the cleaved RNA in Figure S99–102. The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S97 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R2) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S98 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R2) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S99 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R2) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S100 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R2) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–8% CH₃CN in 0.1 M AA buffer (pH 7.0) at 50 °C for 60 min with a flow rate of 0.5 mL/min.



Figure S101 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R2) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) at 50 °C with a flow rate of 0.5 mL/min.



Figure S102 RP-HPLC profile of the mixture of a 12mer DNA (D9) and a 24mer RNA (R2) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) at 50 °C with a flow rate of 0.5 mL/min.



Figure S103 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S104 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S105 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R1) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S106 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S107 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R1) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S108 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R1) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S109 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R1) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.


Figure S110 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R2), which was used for the calculation of the amount of the cleaved RNA in Figure S112–S115. The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S111 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R2), which was used for the calculation of the amount of the cleaved RNA in Figure S116 and S117. The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S112 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R2) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S113 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R2) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S114 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R2) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S115 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R2) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S116 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R2) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S117 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R2) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S118 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R3), which was used for the calculation of the amount of the cleaved RNA in Figure S120 and S121. The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S119 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R3), which was used for the calculation of the amount of the cleaved RNA in Figure S122–S125. The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S120 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R3) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S121 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R3) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S122 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R3) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S123 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R3) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S124 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R3) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S125 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R3) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S126 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R5). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S127 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R5) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S128 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R5) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S129 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R5) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S130 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R5) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S131 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R5) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S132 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R5) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S133 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R7). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S134 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R7) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S135 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R7) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S136 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R7) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S137 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R7) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S138 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R7) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S139 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R7) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S140 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R9), which was used for the calculation of the amount of the cleaved RNA in Figure S142 and S143. The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S141 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R9), which was used for the calculation of the amount of the cleaved RNA in Figure S144–S147. The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S142 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R9) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S143 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R9) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S144 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R9) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S145 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R9) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S146 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R9) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S147 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R9) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.


Figure S148 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R11). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S149 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R11) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S150 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R11) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S151 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R11) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S152 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R11) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S153 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R11) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S154 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R11) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S155 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R12). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S156 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R12) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S157 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R12) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S158 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R12) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S159 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R12) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.



Figure S160 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R12) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S161 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R12) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S162 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R15). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S163 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R15) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S164 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R15) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #1). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S165 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R15) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S166 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R15) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #2). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S167 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R15) in the absence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S168 RP-HPLC profile of the mixture of a 12mer DNA (D10) and a 24mer RNA (R15) in the presence of ODAGal4 after treatment with RNase H for 2 min (Figure 9, Run #3). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–6% CH₃CN for 45 min, followed by a linear gradient of 6%–30% for 15 min in 0.1 M AA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S169 Amounts of cleaved RNA after RNase H treatment (1 U/200 μ L for 10 min at 37 °C) of each 27mer RNA (1 μ M) with 13mer gapmer (G1, 1 μ M) in the absence or presence of ODAGal4 (n = 1). Sequences are follows;

G1: G^{Lm}C^LattggtatT^{Lm}C^LA^L (all internucleotide linkages of G1 are phosphorothioated)

R13: CAUCACAC<u>UGAAUACCAAUGC</u>UGGACU

R14: CAGUAUUUUGGAUACCAAUGCAUAGGA

R20: UUUUUAUU<u>UGAAUACCAAAGC</u>GGUGUU

R21: UAAUGACUGGAAAACCAAUGCUGCUGG

x: DNA; X: RNA; X^L: LNA; ^mC^L; LNA-5-methyl-C The underlined bases in the RNA sequence indicate the complementary region to D1; The characters in bold indicate mismatch bases. The sequence of R20 was based on the mouse dpyd mRNA (GenBank accession GenBank accession No.: NM_170778), and the sequence of R21 was based on the mouse pcsk5 mRNA (GenBank accession GenBank accession No.: NM_001190483).

Figure S170 RP-HPLC profile of the mixture of a 13mer gapmer (G1) and 27mer RNA (R13). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–12.5% CH₃CN for 50 min, followed by a linear gradient of 12.5%–50% for 10 min in 0.1 M TEAA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S171 RP-HPLC profile of the mixture of a 13mer gapmer(G1) and 27mer RNA (R13) in the absence of ODAGal4 after treatment with RNase H for 10 min. The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–12.5% CH₃CN for 50 min, followed by a linear gradient of 12.5%–50% for 10 min in 0.1 M TEAA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S172 RP-HPLC profile of the mixture of a 13mer gapmer(G1) and 27mer RNA (R13) in the presence of ODAGal4 after treatment with RNase H for 10 min. The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–12.5% CH₃CN for 50 min, followed by a linear gradient of 12.5%–50% for 10 min in 0.1 M TEAA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S173 RP-HPLC profile of the mixture of a 13mer gapmer(G1) and 27mer RNA (R14). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–12.5% CH₃CN for 50 min, followed by a linear gradient of 12.5%–50% for 10 min in 0.1 M TEAA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S174 RP-HPLC profile of the mixture of a 13mer gapmer(G1) and 27mer RNA (R14) in the absence of ODAGal4 after treatment with RNase H for 10 min. The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–12.5% CH₃CN for 50 min, followed by a linear gradient of 12.5%–50% for 10 min in 0.1 M TEAA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S175 RP-HPLC profile of the mixture of a 13mer gapmer(G1) and 27mer RNA (R14) in the presence of ODAGal4 after treatment with RNase H for 10 min. The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–12.5% CH₃CN for 50 min, followed by a linear gradient of 12.5%–50% for 10 min in 0.1 M TEAA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S176 RP-HPLC profile of the mixture of a 13mer gapmer(G1) and 27mer RNA (R20). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–12.5% CH₃CN for 50 min, followed by a linear gradient of 12.5%–50% for 10 min in 0.1 M TEAA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S177 RP-HPLC profile of the mixture of a 13mer gapmer(G1) and 27mer RNA (R20) in the absence of ODAGal4 after treatment with RNase H for 10 min. The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–12.5% CH₃CN for 50 min, followed by a linear gradient of 12.5%–50% for 10 min in 0.1 M TEAA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S178 RP-HPLC profile of the mixture of a 13mer gapmer(G1) and 27mer RNA (R20) in the presence of ODAGal4 after treatment with RNase H for 10 min. The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–12.5% CH₃CN for 50 min, followed by a linear gradient of 12.5%–50% for 10 min in 0.1 M TEAA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S179 RP-HPLC profile of the mixture of a 13mer gapmer(G1) and 27mer RNA (R21, pcsk5). The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–12.5% CH₃CN for 50 min, followed by a linear gradient of 12.5%–50% for 10 min in 0.1 M TEAA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S180 RP-HPLC profile of the mixture of a 13mer gapmer(G1) and 27mer RNA (R21) in the absence of ODAGal4 after treatment with RNase H for 10 min. The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–12.5% CH₃CN for 50 min, followed by a linear gradient of 12.5%–50% for 10 min in 0.1 M TEAA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

Figure S181 RP-HPLC profile of the mixture of a 13mer gapmer(G1) and 27mer RNA (R21) in the presence of ODAGal4 after treatment with RNase H for 10 min. The RP-HPLC analysis (UV detection at 260 nm) was performed using a linear gradient of 0%–12.5% CH₃CN for 50 min, followed by a linear gradient of 12.5%–50% for 10 min in 0.1 M TEAA buffer (pH 7.0) for 60 min at 50 °C with a flow rate of 0.5 mL/min.

UV melting analysis

It should be noted that the melting temperatures (T_m values) of the DNA/RNA hybrids used in the manuscript were not correctly calculated in some cases because UV absorbance-versus-temperature curves of even single-stranded of 24mer RNAs have inflection points (Figure S182 and S183). Instead, we evaluated the effect of ODAGal4 on the thermal stability of fully-matched or mismatched DNA/RNA hybrids using 12mer DNA/12mer RNA hybrids (Figure S184 and Table S1).

General procedure

The absorbance versus temperature profile measurements were conducted with an eight-sample cell changer, in quarts cells of 1 cm pathlength. All the experiments were conducted in a 10 mM phosphate buffer containing 100 mM NaCl at pH = 7.0. The difference of the UV absorbances at 260 and 320 nm was monitored with the temperature. The samples containing oligonucleotides were first rapidly heated to 95 °C, left at this temperature for 10 min, and then cooled to room temperature at a rate of 0.5 °C/min. After annealing, an aqueous solution of ODAGal4 was added so that ODAGal4 concentration was equal to that of the hybrid or single strand in the case of adding ODAGal4. The final concentration of hybrid duplexes or single-stranded RNAs was 2 μ M (for Figure S182 and Figure S183) or 5 μ M (for Figure S184). These samples were additionally cooled to 0 °C at a rate of 0.5 °C/min, left at this temperature, and then the dissociation finally recorded by heating to 95 °C at a rate of 0.5 °C/min.

Figure S182 UV melting curves of the duplexes of a DNA (D1) and an RNA (R1–R12) or single-stranded RNAs (R1–R12) in the absence or presence of ODAGal4; black line: a DNA/RNA hybrid in the absence of ODAGal4; red line: a DNA/RNA hybrid in the presence of ODAGal4; green line: a single stranded RNA in the absence of ODAGal4; purple line: a single stranded RNA in the presence of ODAGal4.


Figure S183 UV melting curves of the duplexes of a DNA (D10) and an RNA (R1, R2, R3, R5, R7, and R9) or single-stranded RNAs in the absence or presence of ODAGal4; black line: a DNA/RNA hybrid in the absence of ODAGal4; red line: a DNA/RNA hybrid in the presence of ODAGal4; green line: a single stranded RNA in the absence of ODAGal4; purple line: a single stranded RNA in the presence of ODAGal4; purple line: a single stranded RNA in the presence of ODAGal4; green and purple lines) are same as those of Figure S182.



Figure S184 UV melting curves of the duplexes of DNA (D11: cagtcagtcagt) and an RNA (R16: ACUGACUGACUG, R17: AAUGACUGACUG, R18: ACUGAAUGACUG, R19: ACUGACUGAAUG, each bold character indicates a mismatch base) in the absence or presence of ODAGal4.

Entry	DNA	RNA	$T_{\rm m}$ value/°C ($\Delta T_{\rm m}$) ODAGal4	
_			(-)	(+)
1	D11: cagtcagtcagt	R16: ACUGACUGACUG	48.0	50.9 (+ 2.9)
2	D12: cagtcagtcagt	R17: A A UGACUGACUG	41.7	45.1 (+ 3.4)
3	D13: cagtcagtcagt	R18: ACUGA A UGACUG	31.3	36.3 (+ 5.0)
4	D14: cagtcagtcagt	R19: ACUGACUGA A UG	34.3	38.5 (+ 4.2)

Table S1 $T_{\rm m}$ values of DNA/RNA hybrids in the absence or presence of ODAGal4