

Supporting Information for

Double-stranded-RNA-binding artificial cationic oligosaccharides stabilizing siRNAs with low N/P ratio

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Compound 2

Glycosyl donor **1** (436 mg, 0.60 mmol) was coevaporated with toluene and dissolved in dichloromethane-diethyl ether (1:1, v/v, 3.0 mL). Methanol (36 μ L, 0.90 mmol) and *N*-iodosuccinimide (339 mg, 1.5 mmol) were added to the solution which was cooled to 0 °C. A solution of trifluoromethanesulfonic acid (6 μ L) in dichloromethane-diethyl ether (1:1, v/v, 3.0 mL) was subsequently added, and the solution was stirred for 20 min at 0 °C. The reaction was quenched by addition of an aqueous saturated NaHCO₃ solution (2 mL), and the mixture was diluted with dichloromethane (30 mL). The solution was washed with an aqueous saturated NaHCO₃ (30 mL), and 10% aqueous Na₂S₂O₃ (30 mL) solution. The organic layer was dried over Na₂SO₄, filtered and concentrated to dryness. The crude product was dissolved in dichloromethane (9.0 mL) and the resultant solution was cooled to 0 °C. To the solution, sodium methoxide (14.6 mg, 270 μ mol) in methanol (18.0 mL) was added and the resultant solution stirred at 0 °C. After 8 h, Dowex 50WX8 (2 g) was added to the mixture which was stirred for 10 min. The solution was filtered, concentrated, and the crude product purified by silica-gel column chromatography (ethyl acetate–toluene (1:3 to 3:7, v/v)) to afford **2** as a colorless foam (307 mg, 537 μ mol, 89%).

¹H NMR (CDCl₃, 300 MHz) δ 7.91–7.64 (m, 8H, *NPhth*), 6.94 (d, *J* = 8.4 Hz, 2H, PMB), 6.44 (d, *J* = 8.4 Hz, 2H, PMB), 4.98 (d, *J* = 8.4 Hz, 1H, H-1), 4.57–4.44 (m, 2H), 4.30–4.22 (m, 3H), 4.06–3.97 (m, 3H), 3.64 (s, 3H, PMB), 3.25 (s, 3H, anomeric-OMe), 3.02 (s, 1H, 4-OH)

¹³C NMR (CDCl₃, 75.5 MHz) δ 168.1, 167.6, 158.9, 134.0, 133.6, 131.7, 131.5, 129.3, 129.1, 123.3, 122.8, 113.4, 98.8, 74.4, 71.5, 71.0, 65.6, 55.8, 54.8, 51.8, 38.4.

MALDI-TOF MS: calcd for C₃₈H₂₈N₂NaO₉ *m/z* [M+Na]⁺: 595.17 Found: 595.03.

Compound 3

A mixture of glycosyl donor **1** (303 mg, 417 μ mol) and **2** (170.6 mg, 298 μ mol) was coevaporated with toluene (2 mL \times 4), and then dissolved in dichloromethane-diethyl ether (1:2, v/v, 1.0 mL). *N*-Iodosuccinimide was added to the solution, and the resultant mixture was cooled to 0 °C. A solution of trifluoromethanesulfonic acid (1 μ L) in dichloromethane-diethyl ether (1:2, v/v, 1.0 mL) was subsequently added to the solution and stirred for 1 h at 0 °C. The reaction was quenched by adding an aqueous saturated NaHCO₃ solution (2 mL), and the mixture was diluted with chloroform (30 mL). The solution was washed with aqueous saturated NaHCO₃ (20 mL) and 10% aqueous Na₂S₂O₃ (20 mL) solutions. The organic layer was dried over Na₂SO₄, filtered and concentrated to dryness. The crude product was dissolved in dichloromethane (6.0 mL) and the resultant

solution was cooled to 0 °C. To the solution, a 0.03 M solution of sodium methoxide in methanol (12.0 mL) was added and stirred at 0 °C. After 4 h, Dowex 50WX8 (2 g) was added to the mixture which was stirred for 30 min. The solution was filtered, concentrated, and the crude product purified by silica–gel column chromatography (ethyl acetate-toluene (7:13 to 2:3, v/v)) to afford **3** as a colorless foam (265 mg, 238 μmol, 80%).

¹H NMR (CDCl₃, 300 MHz) δ 8.09 (d, 1H, *J* = 7.2 Hz), 7.87–7.33 (m, 15H), 7.02 (d, *J* = 8.7 Hz, 2H), 6.49 (d, *J* = 8.7 Hz, 2H), 6.37 (d, *J* = 8.7 Hz, 2H), 6.18 (d, *J* = 8.7 Hz, 2H), 5.23 (d, *J* = 8.4 Hz, 1H), 4.75–4.59 (m, 3H), 4.46–4.23 (m, 4H), 4.14–3.58 (m, 16H), 3.08 (s, 3H), 2.79 (br, 1H).

¹³C NMR (CDCl₃, 75.5 MHz) δ 168.8, 168.1, 167.8, 166.2, 159.1, 158.6, 134.0, 133.8, 133.3, 133.1, 132.9, 132.8, 132.2, 131.8, 131.6, 131.5, 129.5, 129.4, 128.6, 127.4, 124.7, 123.4, 123.2, 122.6, 122.0, 113.6, 113.1, 99.7, 97.8, 74.8, 74.5, 73.8, 72.0, 71.6, 71.0, 66.2, 55.0, 54.8, 54.5, 52.1, 51.1, 40.0, 39.3, 29.7.

MALDI-TOF MS: calcd for C₆₁H₅₂N₄NaO₁₇ m/z [M+Na]⁺: 1135.32 Found: 1135.57.

Compound 4

A mixture of glycosyl donor **1** (135.2 mg, 186 μmol) and **3** (130.8 mg, 117 μmol) was coevaporated with toluene (1 mL × 4), and then dissolved in dichloromethane-diethyl ether (1:2, v/v, 4.0 mL). *N*-Iodosuccinimide (104.8 mg, 466 μmol) was added to the solution, and the mixture was cooled to 0 °C. A solution of trifluoromethane sulfonic acid (3 μL) in dichloromethane-diethyl ether (1:2, v/v, 2.0 mL) was subsequently added to the solution which was stirred for 1 h at 0 °C. The reaction was quenched by adding an aqueous saturated NaHCO₃ solution (2 mL), and the resultant mixture was diluted with chloroform (30 mL). The solution was washed with aqueous saturated NaHCO₃ (20 mL) and 10% aqueous Na₂S₂O₃ (20 mL) solutions. The organic layer was back extracted with chloroform (5 mL × 3). The combined organic layer was dried over Na₂SO₄, filtered and concentrated to dryness. The crude product was dissolved in dichloromethane (5.0 mL) and the resulting solution was cooled to 0 °C. To the solution, a 0.03 M solution of sodium methoxide in methanol (10.0 mL) was added and stirred at 0 °C. After 3 h, Dowex 50WX8 (2 g) was added to the mixture which was stirred for 30 min. The solution was filtered, concentrated, and the crude product purified by silica gel column chromatography (ethyl acetate-hexane (6:1, v/v)) to afford **4** as a colorless foam (135 mg, 75 μmol, 64%).

¹H NMR (CDCl₃, 300 MHz) δ 8.39 (d, 1H, J = 8.4 Hz), 7.90–7.07 (m, 25H), 6.57 (d, J = 8.4 Hz, 2H), 6.45 (d, J = 8.4 Hz, 2H), 6.29–6.21 (m, 4H), 6.08 (d, J = 8.4 Hz, 2H), 5.27 (d, J = 8.1 Hz, 1H), 4.94 (d, J = 8.1 Hz, 1H), 4.75–3.47 (m, 34H), 3.09 (s, 4H).

¹³C NMR (CDCl₃, 75.5 MHz) δ 168.5, 168.1, 168.0, 167.6, 166.1, 165.8, 159.0, 158.6, 133.9, 133.7, 133.6, 133.1, 132.4, 132.2, 132.0, 131.8, 131.7, 131.5, 129.7, 129.4, 129.1, 123.9, 123.3, 123.2, 123.1, 122.5, 122.4, 121.7, 99.4, 99.2, 97.7, 74.4, 73.9, 72.9, 71.8, 71.5, 71.0, 70.6, 66.6, 55.0, 54.8, 52.2, 51.8, 51.0, 40.4, 39.4, 29.7.

MALDI-TOF MS: calcd for C₉₁H₇₆N₆NaO₂₅ m/z [M+Na]⁺: 1676.48 Found: 1676.75.

Compound 5

A mixture of glycosyl donor **1** (80.1 mg, 110 μ mol) and **4** (110.3 mg, 68 μ mol) was coevaporated with toluene (1 mL \times 4), and subsequently dissolved in dichloromethane-diethyl ether (1:2, v/v, 4.0 mL). *N*-Iodosuccinimide (61.5 mg, 272 μ mol) was added to the solution, and the resulting mixture was cooled to 0 °C. A solution of trifluoromethane sulfonic acid (3 μ L) in dichloromethane-diethyl ether (1:2, v/v, 2.0 mL) was subsequently added to the solution which was stirred for 1 h at 0 °C. The reaction was quenched by adding an aqueous saturated NaHCO₃ solution (1 mL), and the mixture was diluted with chloroform (30 mL). The solution was washed with aqueous saturated NaHCO₃ (20 mL) and 10% aqueous Na₂S₂O₃ (20 mL) solutions. The organic layer was back extracted with chloroform (5 mL \times 3). The combined organic layer was dried over Na₂SO₄, filtered and concentrated to dryness. The crude product was dissolved in dichloromethane (5.0 mL) the resulting solution was cooled to 0 °C. To the solution, a 0.015 M solution of sodium methoxide in methanol (10.0 mL) was added and the mixture stirred at 0 °C. After 7 h, Dowex 50WX8 (2 g) was added to the mixture and stirred for 30 min. The solution was filtered, concentrated, and the crude product purified by silica-gel column chromatography (ethyl acetate–hexane (9:1, v/v)) to afford **5** as colorless foam (14.5 mg, 6.6 μ mol, 10%).

¹H NMR (CDCl₃, 400 MHz) δ 8.46 (d, 1H, J = 7.2 Hz), 8.16 (d, 1H, J = 7.5 Hz), 7.99–7.07 (m, 32H), 6.59–6.47 (m, 4H), 6.38–6.30 (m, 4H), 6.21–6.15 (m, 4H), 6.05 (d, J = 8.4 Hz, 2H), 5.25 (d, J = 7.8 Hz, 1H), 4.98 (d, J = 8.4 Hz, 1H), 4.92 (d, J = 7.8 Hz, 1H), 4.67–3.50 (m, 46H), 3.13 (s, 3H).

¹³C NMR (CDCl₃, 75.5 MHz) δ 168.9, 168.3, 168.2, 168.1, 168.0, 167.8, 167.6, 166.2, 166.0, 165.9, 159.0, 158.7, 158.6, 158.5, 158.4, 157.4, 138.8, 134.3, 134.0, 133.7, 133.4, 133.1, 133.0, 132.3, 132.2, 132.1, 132.0, 131.9, 131.8, 131.7, 131.6, 131.4, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.1, 129.0, 128.9, 128.5, 128.4, 124.7, 123.7, 123.3, 123.1,

122.6, 122.4, 122.1, 121.8, 113.6, 113.2, 113.0, 112.9, 110.5, 99.5, 99.4, 99.2, 98.9, 97.9, 85.5, 74.9, 74.6, 74.5, 74.2, 73.9, 73.6, 73.4, 73.1, 72.7, 72.6, 72.4, 71.9, 71.8, 71.4, 71.3, 71.1, 70.7, 70.6, 70.4, 70.3, 66.9, 66.7, 66.6, 56.1, 55.0, 54.9, 54.8, 54.7, 52.2, 52.1, 51.8, 51.2, 40.8, 40.2, 40.1, 40.0, 39.3, 29.6.

MALDI-TOF MS: calcd for $C_{121}H_{100}N_8NaO_{33}$ m/z $[M+Na]^+$: 2216.63 Found: 2217.00.

Compound 6

Compound **3** (7.6 mg, 6.9 μ mol) was dissolved in ethanol (2.0 mL) and hydrazine monohydrate (60 μ L) was subsequently added to the solution. The mixture was sealed, heated to 90 °C, and stirred for 4 h. The solution was cooled to room temperature and concentrated. The crude product was dissolved in 0.1 M hydrochloric acid and concentrated, and this manipulation was repeated 6 times at 35 °C for complete removal of PMB groups. The crude product was dissolved in a little volume of methanol, reprecipitated from acetone, and washed with acetone. The precipitate was coevaporated with water (1.5 mL \times 6), and lyophilized to afford **6** as a colorless solid (2.4 μ mol, 36%).

1H NMR (D_2O , 300 MHz) δ 4.95 (d, J = 8.1 Hz, 1H, H-1'), 4.58 (d, J = 8.4, 1H, H-1'), 4.30 (s, 1H), 4.08-3.94 (m, 4H), 3.86 (dd, J = 3.3, 10.8 Hz, 1H), 3.61 (s, 3H), 3.50–3.19 (m, 6H).

ESI-MS: calcd for $C_{13}H_{29}N_4O_7$ m/z $[M+H]^+$: 353.2031 Found: 353.2035.

Compound 7

Compound **4** (8.1 mg, 4.9 μ mol) was dissolved in ethanol (2.0 mL) and hydrazine monohydrate (60 μ L) was subsequently added to the solution. The mixture was sealed, heated to 90 °C, and stirred for 5 h. The solution was cooled to room temperature and concentrated. The crude product was purified via C18 reversed-phase HPLC (0.05% TFA, water–acetonitrile). After the HPLC purification, the amino saccharide was dissolved in 0.1 M hydrochloric acid and concentrated, and this manipulation was repeated 6 times at 35 °C for complete removal of PMB groups. The crude product was dissolved in a little volume of methanol, reprecipitated from acetone, and washed with acetone. The precipitate was coevaporated with water (1.5 mL \times 6), and lyophilized to afford **7** as a colorless solid (2.0 μ mol, 40%).

1H NMR (D_2O , 300 MHz) δ 5.01 (d, J = 8.7 Hz, 2H), 4.65 (d, J = 8.7, 1H), 4.33 (s, 1H), 4.11–3.90 (m, 7H), 3.62 (s, 3H), 3.62–3.24 (m, 9H).

ESI-MS: calcd for C₁₉H₄₁N₆O₁₀ m/z [M+H]⁺: 513.2879 Found: 513.2883.

Compound 8

Compound **5** (14.5 mg, 6.6 μmol) was dissolved in ethanol (2.0 mL) and hydrazine monohydrate (60 μL) was subsequently added to the solution. The mixture was sealed, heated to 90 °C, and stirred for 5 h. The solution was cooled to room temperature and concentrated. The crude product was purified via C18 reversed-phase HPLC (0.05% TFA, water–acetonitrile). After the HPLC purification, the amino saccharide was dissolved in 0.1 M hydrochloric acid and concentrated, and this manipulation was repeated 4 times at 35 °C. The crude product was dissolved in a little volume of methanol, reprecipitated from acetone, and washed with acetone. The precipitate was coevaporated with water (1.5 mL × 6), and lyophilized to a **8** as a colorless solid (3.1 μmol, 47%).

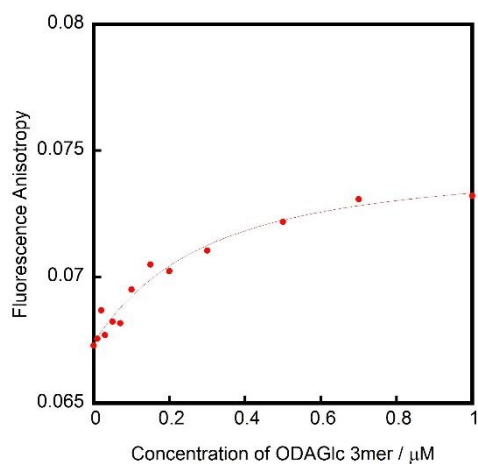
¹H NMR (D₂O, 300 MHz) δ 5.11–4.86 (m, 3H), 4.70 (d, *J* = 8.4 Hz), 4.38 (s, 3H), 4.22–3.96 (m, 9H), 3.70–3.63 (m, 5H), 3.52–3.32 (m, 10H)

¹³C NMR (D₂O, 75.5 MHz) δ 104.2, 101.2, 100.9, 100.8, 76.8, 76.4, 72.2, 71.4, 71.3, 71.0, 69.4, 69.1, 68.9, 68.6, 58.2, 53.9, 53.7, 53.5, 40.8, 40.7.

ESI-MS: calcd for C₂₅H₅₃N₈O₁₃ m/z [M+H]⁺: 673.3727 Found: 673.3731.

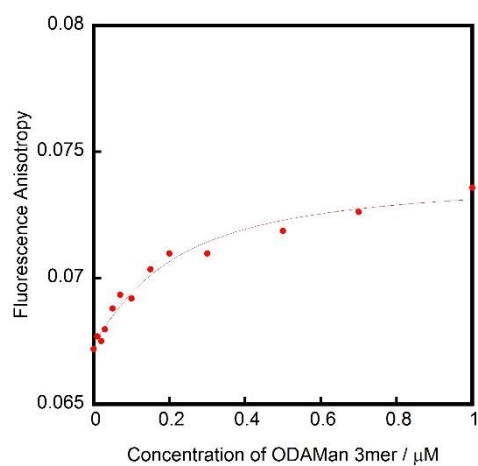
Fig. S1-S33: Fluorescence anisotropy measurements

All the experiments were conducted under at 20 °C in 10 mM phosphate buffer containing 100 mM NaCl and 0.02% Tween 20, pH 7.0.



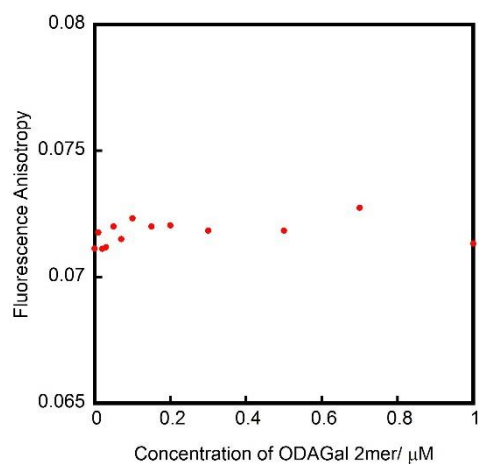
$$K_d = 0.23 \pm 0.08 \mu\text{M}$$

Fig. S1 Fluorescence anisotropy of 100 nM of (FAM-rCGCGAAUUCGCG)₂ (seq 1') was titrated by increasing concentration of ODAGlc 3mer.



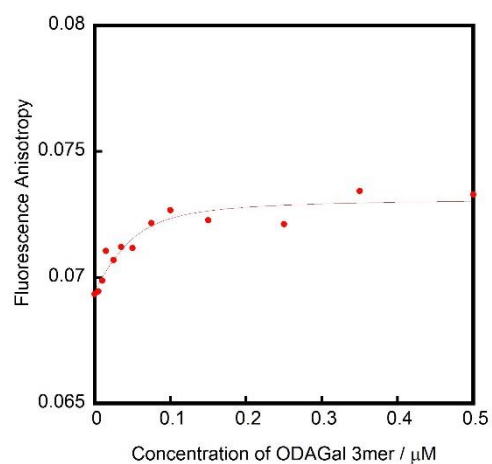
$$K_d = 0.15 \pm 0.05 \mu\text{M}$$

Fig. S2 Fluorescence anisotropy of 100 nM of (FAM-rCGCGAAUUCGCG)₂ (seq 1') was titrated by increasing concentration of ODAMan 3mer.



K_d value was not detected

Fig. S3 Fluorescence anisotropy of 100 nM of (5'-FAM-rCGCGAAUUCGCG)₂ (seq 1') was titrated by increasing concentration of ODAGal 2mer **6**.



$K_d = 0.016 \pm 0.012 \mu\text{M}$

Fig. S4 Fluorescence anisotropy of 100 nM of (FAM-rCGCGAAUUCGCG)₂ (seq 1') was titrated by increasing concentration of ODAGal 3mer **7**.

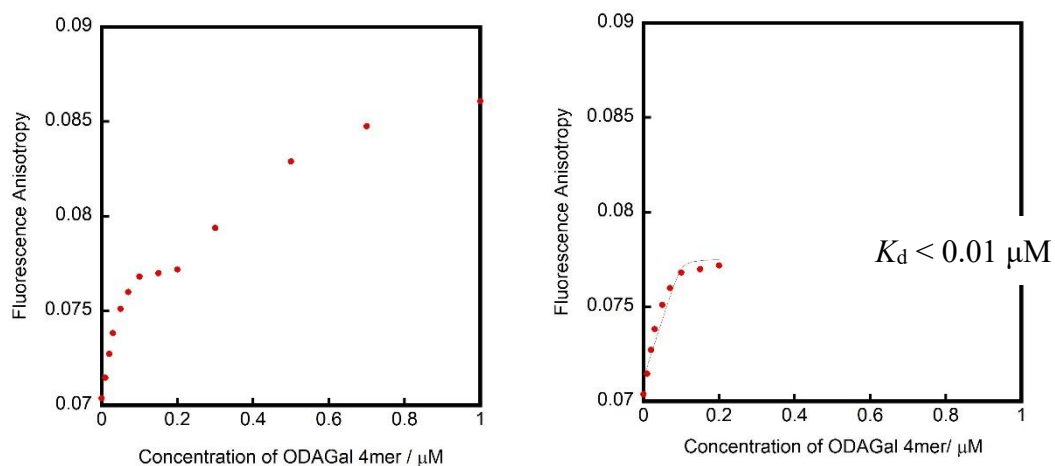


Fig. S5 Fluorescence anisotropy of 100 nM of (FAM-rCGCGAAUUCGCG)₂ (seq 1') was titrated by increasing concentration of ODAGal 4mer **8**. In this chart, a flexion point was observed, therefore the K_d value was calculated using data in 0.2 μM or less of ODAGal 4mer **8**.

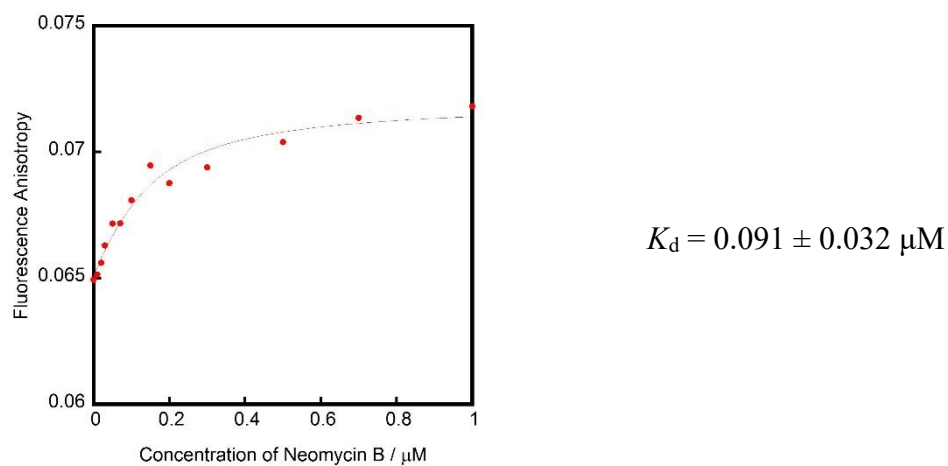
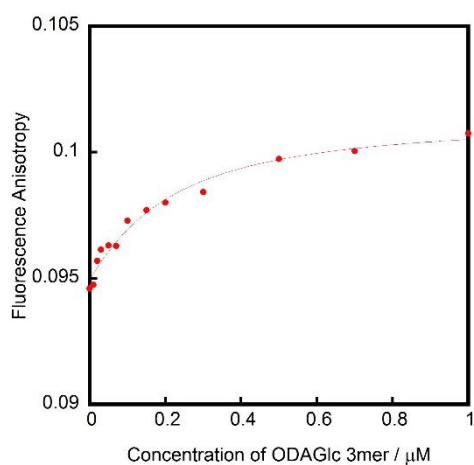
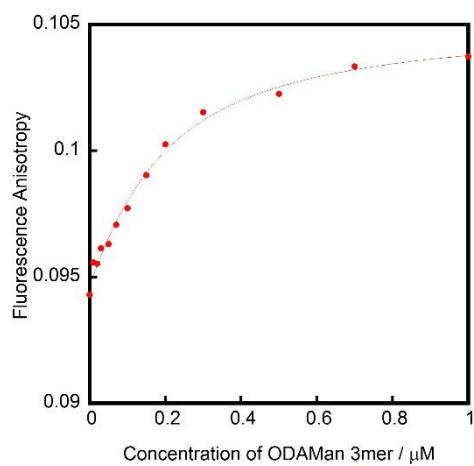


Fig. S6 Fluorescence anisotropy of 100 nM of (FAM-rCGCGAAUUCGCG)₂ (seq 1') was titrated by increasing concentration of neomycin B.



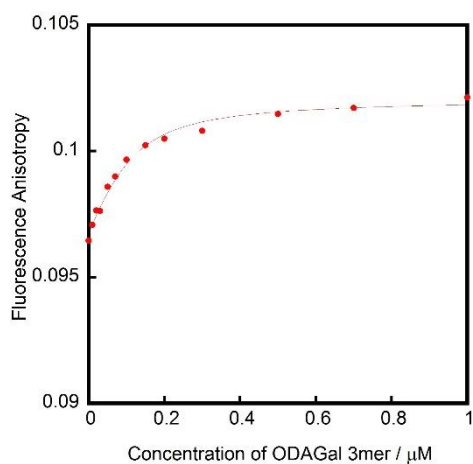
$$K_d = 0.16 \pm 0.05 \mu\text{M}$$

Fig. S7 Fluorescence anisotropy of 100 nM of FAM-r(ACUG)₃/r(CAGU)₃ (seq 2') was titrated by increasing concentration of ODAGlc 3mer.



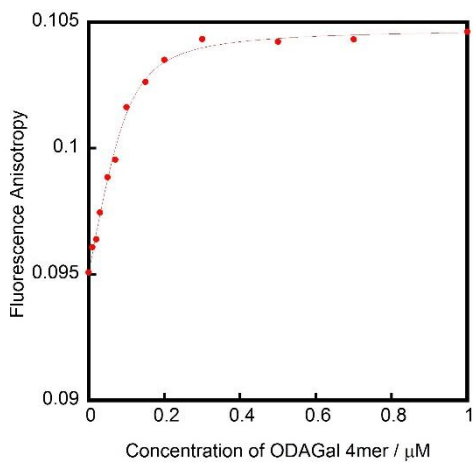
$$K_d = 0.15 \pm 0.02 \mu\text{M}$$

Fig. S8 Fluorescence anisotropy of 100 nM of FAM-r(ACUG)₃/r(CAGU)₃ (seq 2') was titrated by increasing concentration of ODAMan 3mer.



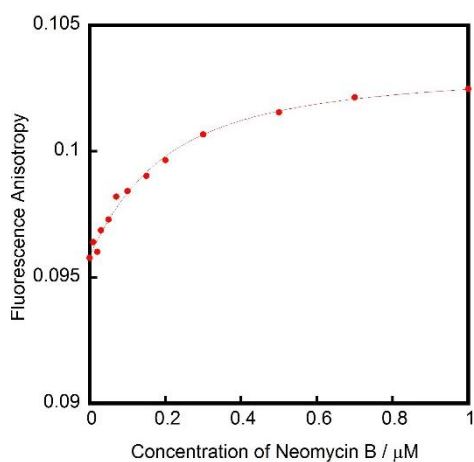
$$K_d = 0.046 \pm 0.010 \mu\text{M}$$

Fig. S9 Fluorescence anisotropy of 100 nM of FAM-r(ACUG)₃/r(CAGU)₃ (seq 2') was titrated by increasing concentration of ODAGal 3mer **7**.



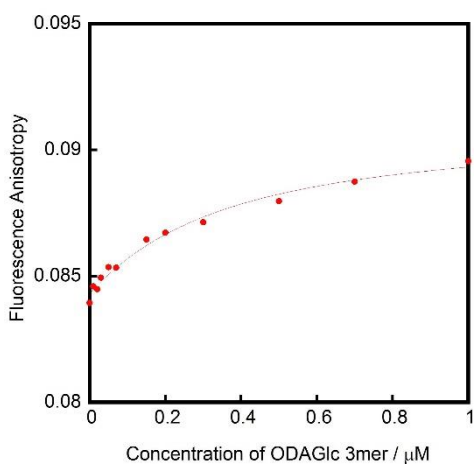
$$K_d = 0.019 \pm 0.003 \mu\text{M}$$

Fig. S10 Fluorescence anisotropy of 100 nM of FAM-r(ACUG)₃/r(CAGU)₃ (seq 2') was titrated by increasing concentration of ODAGal 4mer **8**.



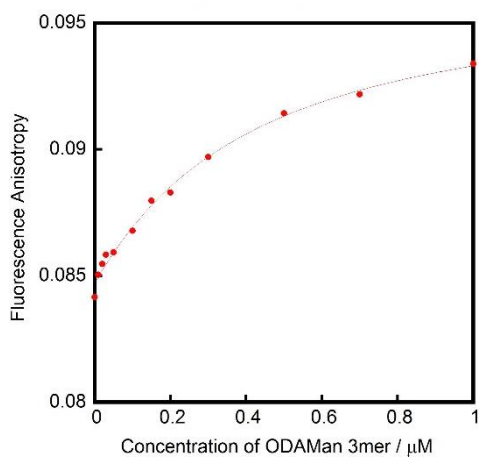
$$K_d = 0.14 \pm 0.02 \mu\text{M}$$

Fig. S11 Fluorescence anisotropy of 100 nM of FAM-r(ACUG)₃/r(CAGU)₃ (seq 2') was titrated by increasing concentration of neomycin B.



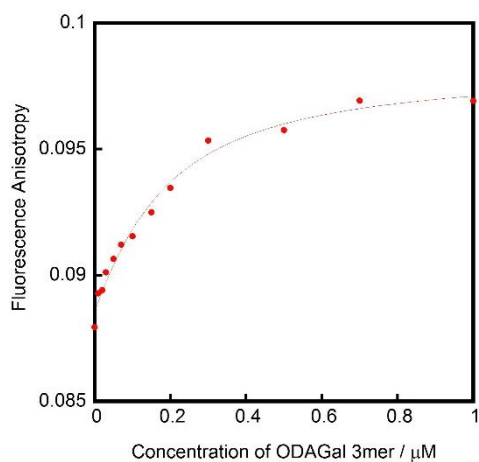
$$K_d = 0.31 \pm 0.08 \mu\text{M}$$

Fig. S12 Fluorescence anisotropy of 100 nM of (FAM-rAACCCGCGGGUU)₂ (seq 3') was titrated by increasing concentration of ODAGlc 3mer.



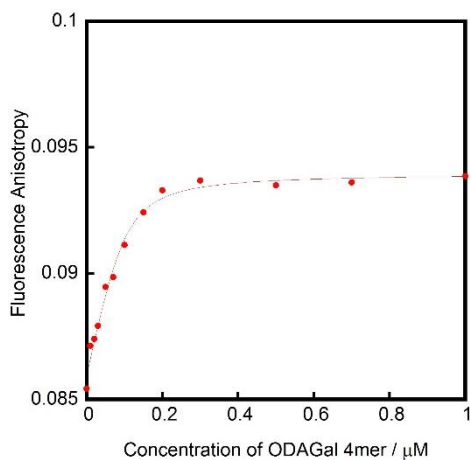
$$K_d = 0.36 \pm 0.06 \mu\text{M}$$

Fig. S13 Fluorescence anisotropy of 100 nM of (FAM-rAACCCGCGGGUU)₂ (seq 3') was titrated by increasing concentration of ODAMan 3mer.



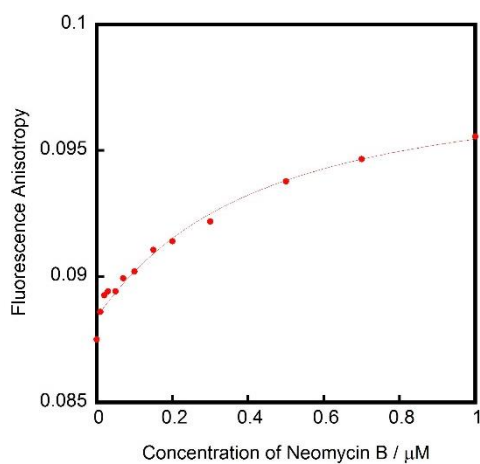
$$K_d = 0.13 \pm 0.03 \mu\text{M}$$

Fig. S14 Fluorescence anisotropy of 100 nM of (FAM-rAACCCGCGGGUU)₂ (seq 3') was titrated by increasing concentration of ODAGal 3mer 7.



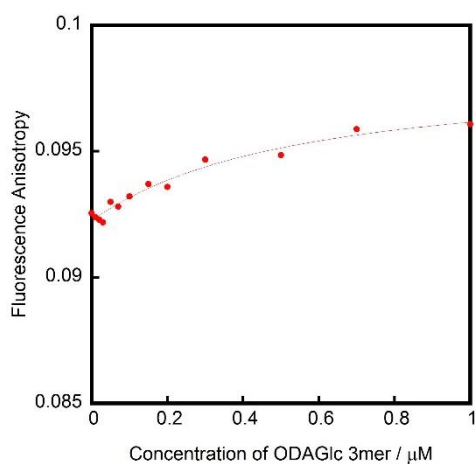
$$K_d = 0.016 \pm 0.004 \mu\text{M}$$

Fig. S15 Fluorescence anisotropy of 100 nM of (FAM-rAACCCGCGGGUU)₂ (seq 3') was titrated by increasing concentration of ODAGal 4mer **8**.



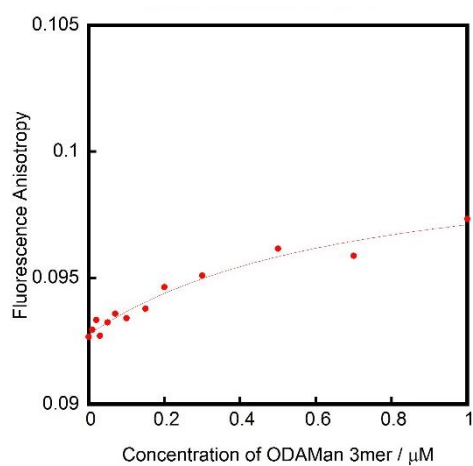
$$K_d = 0.36 \pm 0.10 \mu\text{M}$$

Fig. S16 Fluorescence anisotropy of 100 nM of (FAM-rAACCCGCGGGUU)₂ (seq 3') was titrated by increasing concentration of neomycin B.



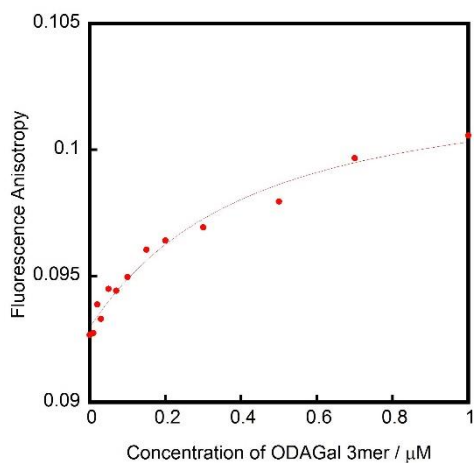
$$K_d = 0.51 \pm 0.18 \mu\text{M}$$

Fig. S17 Fluorescence anisotropy of 50 nM of FAM-r(ACUG)₃/d(CAGT)₃ (seq 4') was titrated by increasing concentration of ODAGlc 3mer.



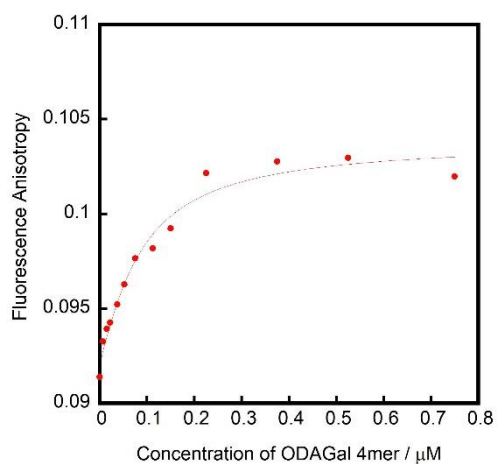
$$K_d = 0.59 \pm 0.27 \mu\text{M}$$

Fig. S18 Fluorescence anisotropy of 100 nM of FAM-r(ACUG)₃/d(CAGT)₃ (seq 4') was titrated by increasing concentration of ODAMan 3mer.



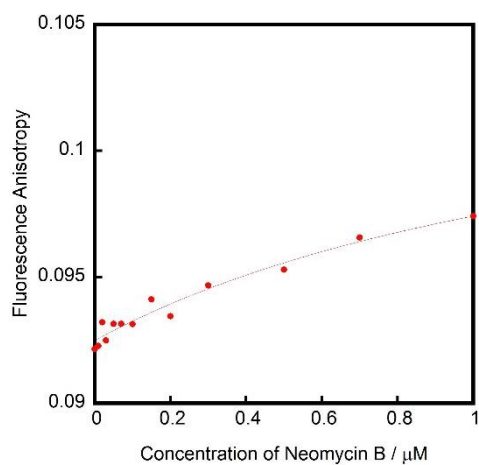
$$K_d = 0.35 \pm 0.11 \mu\text{M}$$

Fig. S19 Fluorescence anisotropy of 100 nM of FAM-r(ACUG)₃/d(CAGT)₃ (seq 4') was titrated by increasing concentration of ODAGal 3mer **7**.



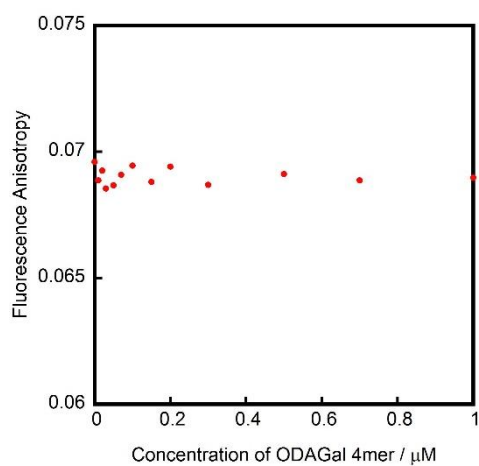
$$K_d = 0.061 \pm 0.016 \mu\text{M}$$

Fig. S20 Fluorescence anisotropy of 50 nM of FAM-r(ACUG)₃/d(CAGT)₃ (seq 4') was titrated by increasing concentration of ODAGal 4mer **8**.



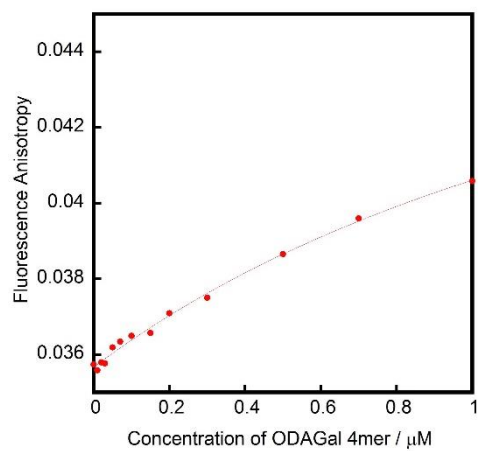
$$K_d = 1.36 \pm 0.91 \mu\text{M}$$

Fig. S21 Fluorescence anisotropy of 100 nM of FAM-r(ACUG)₃/d(CAGT)₃ (seq 4') was titrated by increasing concentration of neomycin B.



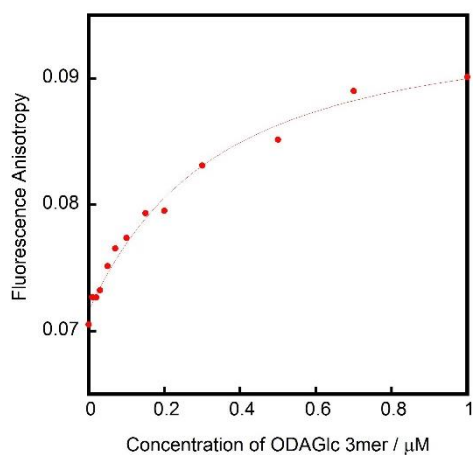
K_d was not detected

Fig. S22 Fluorescence anisotropy of 100 nM of (FAM-dCGCGAATTCGCG)₂ (dsDNA) was titrated by increasing concentration of ODAGal 4mer **8**.



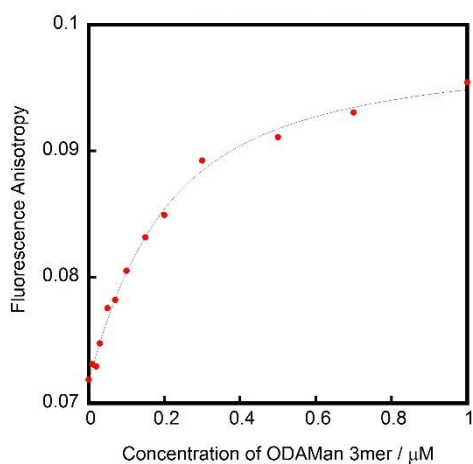
$$K_d = 1.75 \pm 0.47 \mu\text{M}$$

Fig. S23 Fluorescence anisotropy of 100 nM of 5'-FAM-r(ACUG)₃ (ssRNA) was titrated by increasing concentration of ODAGal 4mer **8**.



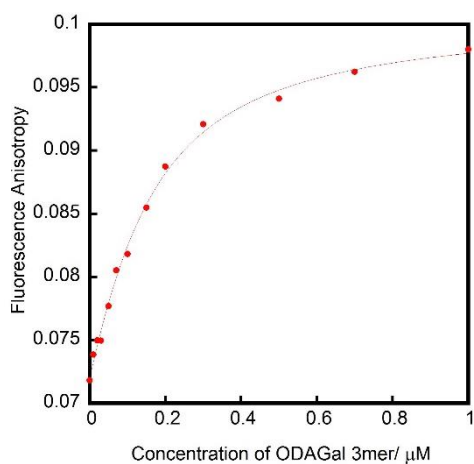
$$K_d = 0.27 \pm 0.06 \mu\text{M}$$

Fig. S24 Fluorescence anisotropy of 100 nM of 5'-FAM-TAR RNA was titrated by increasing concentration of ODAGlc 3mer.



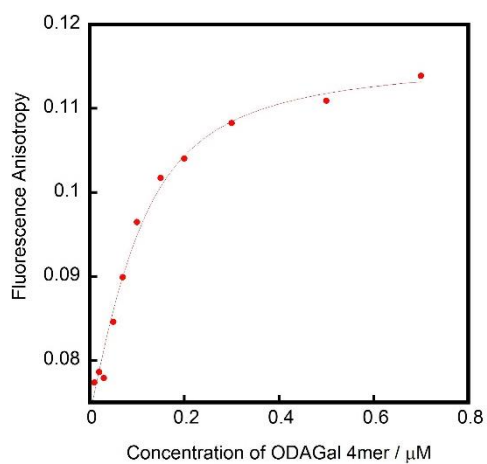
$$K_d = 0.14 \pm 0.02 \mu\text{M}$$

Fig. S25 Fluorescence anisotropy of 100 nM of 5'-FAM-TAR RNA was titrated by increasing concentration of ODAMan 3mer.



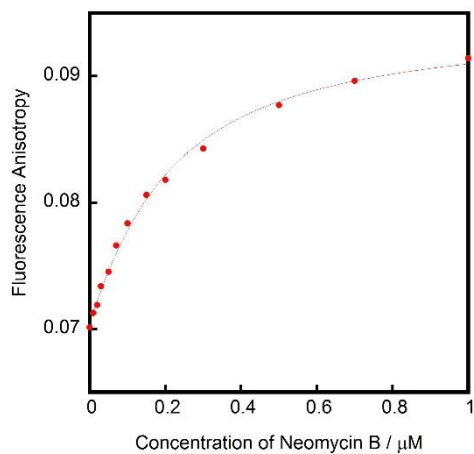
$$K_d = 0.11 \pm 0.01 \mu\text{M}$$

Fig. S26 Fluorescence anisotropy of 100 nM of 5'-FAM-TAR RNA was titrated by increasing concentration of ODAGal 3mer **7**.



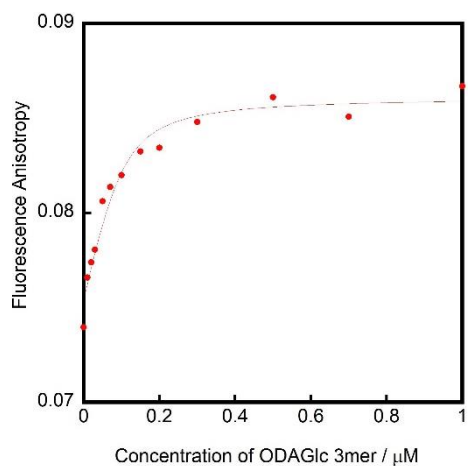
$$K_d = 0.050 \pm 0.011 \mu\text{M}$$

Fig. S27 Fluorescence anisotropy of 100 nM of 5'-FAM-TAR RNA was titrated by increasing concentration of ODAGal 3mer **8**.



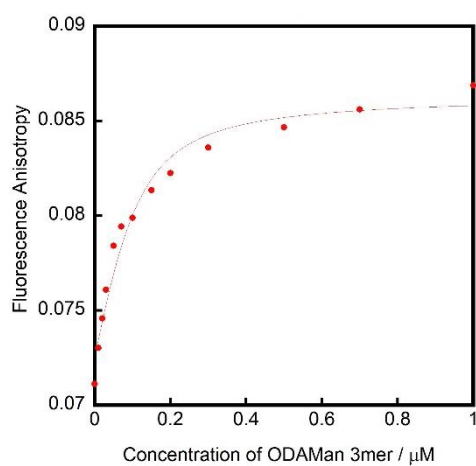
$$K_d = 0.16 \pm 0.02 \mu\text{M}$$

Fig. S28 Fluorescence anisotropy of 100 nM of 5'-FAM-TAR RNA was titrated by increasing concentration of neomycin B.



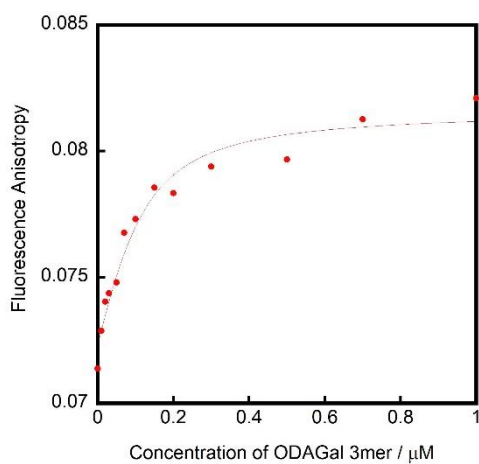
$$K_d = 0.022 \pm 0.011 \mu\text{M}$$

Fig. S29 Fluorescence anisotropy of 100 nM of 5'-FAM-16s rRNA was titrated by increasing concentration of ODAGlc 3mer.



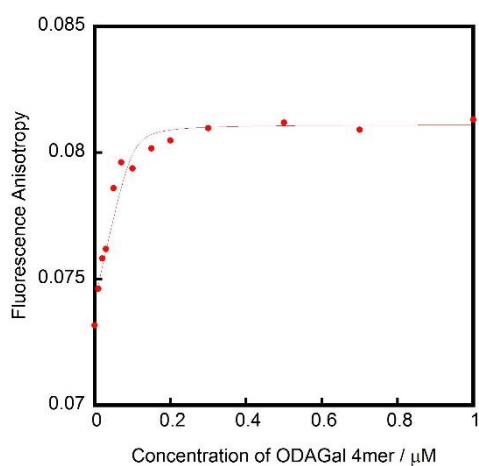
$$K_d = 0.039 \pm 0.015 \mu\text{M}$$

Fig. S30 Fluorescence anisotropy of 100 nM of 5'-FAM-16s rRNA was titrated by increasing concentration of ODAMan 3mer.



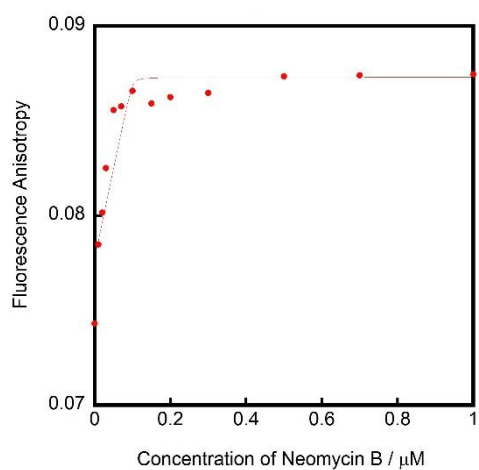
$$K_d = 0.049 \pm 0.020 \mu\text{M}$$

Fig. S31 Fluorescence anisotropy of 100 nM of 5'-FAM-16s rRNA was titrated by increasing concentration of ODAGal 3mer 7.



$K_d < 0.01 \mu\text{M}$

Fig. S32 Fluorescence anisotropy of 100 nM of 5'-FAM-16s rRNA was titrated by increasing concentration of ODAGal 4mer **8**.



$K_d < 0.01 \mu\text{M}$

Fig. S33 Fluorescence anisotropy of 100 nM of 5'-FAM-16s rRNA was titrated by increasing concentration of neomycin B.

Fig. S34-S38: UV melting analysis

All the experiments were conducted in 10 mM phosphate buffer containing 100 mM NaCl, pH 7.0. [duplex] = 5 μ M

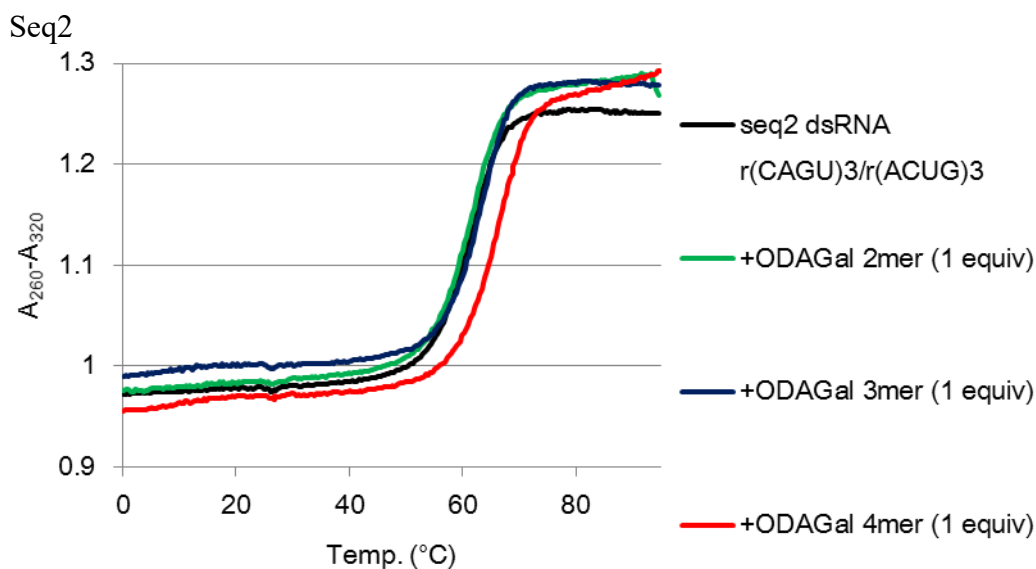


Figure S34. UV melting curves of r(ACUG)₃/r(CAGU)₃ (seq 2) in the absence and presence of 1 equivalent of ODAGal 2mer **6**, 3mer **7**, and 4mer **8**.

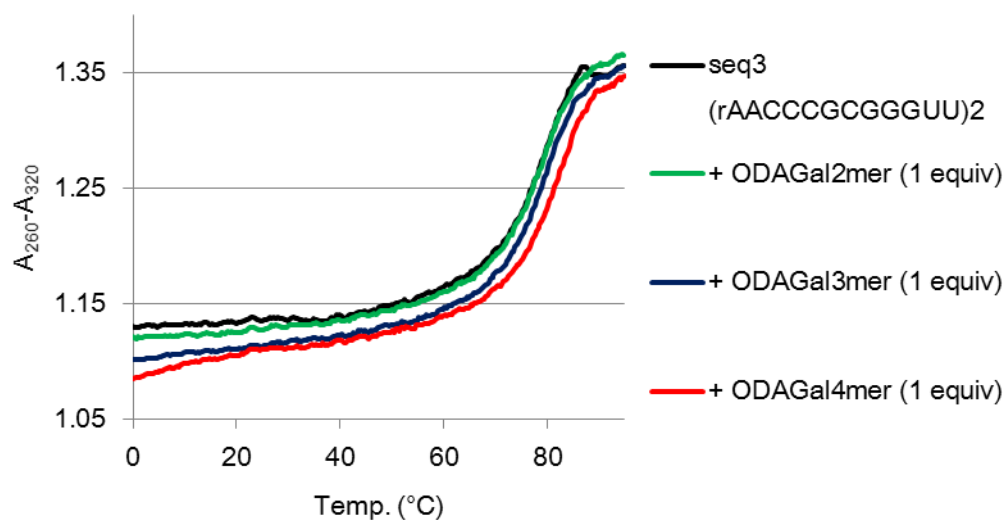


Figure S35. UV melting curves of (rAACCCGCGGGUU)₂ (seq 3) in the absence and presence of 1 equivalent of ODAGal 2mer **6**, 3mer **7**, and 4mer **8**.

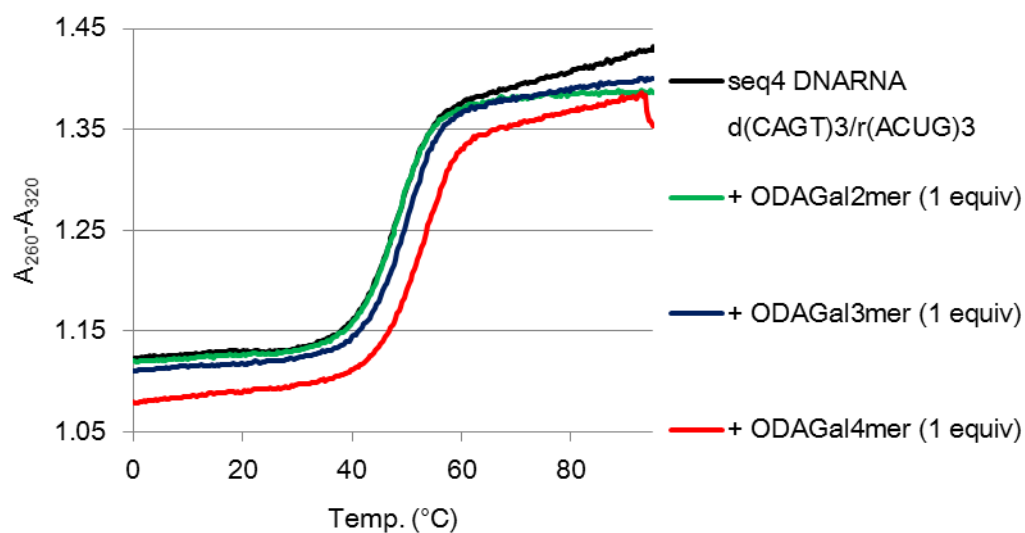


Figure S36. UV melting curves of $r(ACUG)_3/d(CAGT)_3$ (seq 4) in the absence and presence of 1 equivalent of ODAGal 2mer **6**, 3mer **7**, and 4mer **8**.

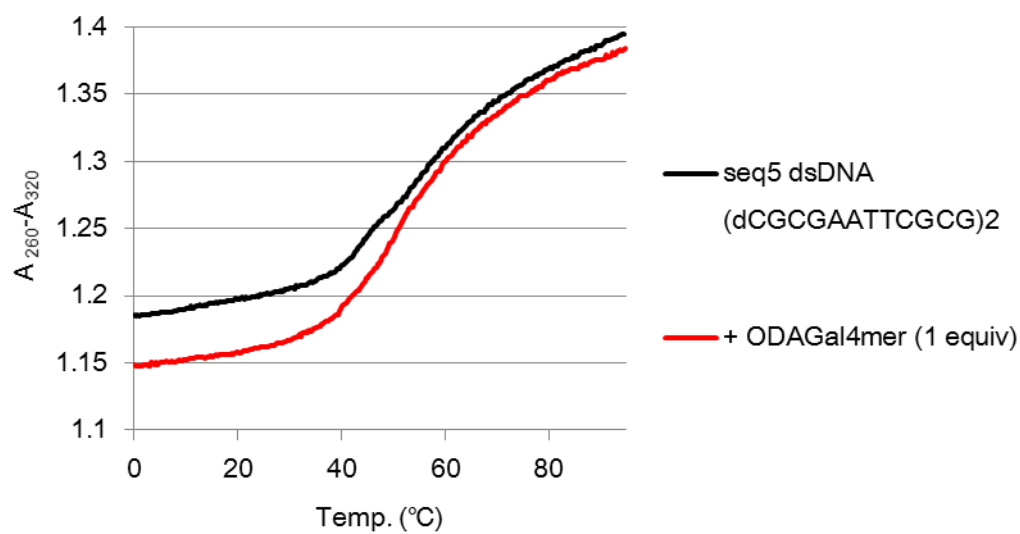


Figure S37. UV melting curves of $(dCGCGAATTCGCG)_2$ (seq 5) in the absence and presence of 1 equivalent of ODAGal 4mer **8**.

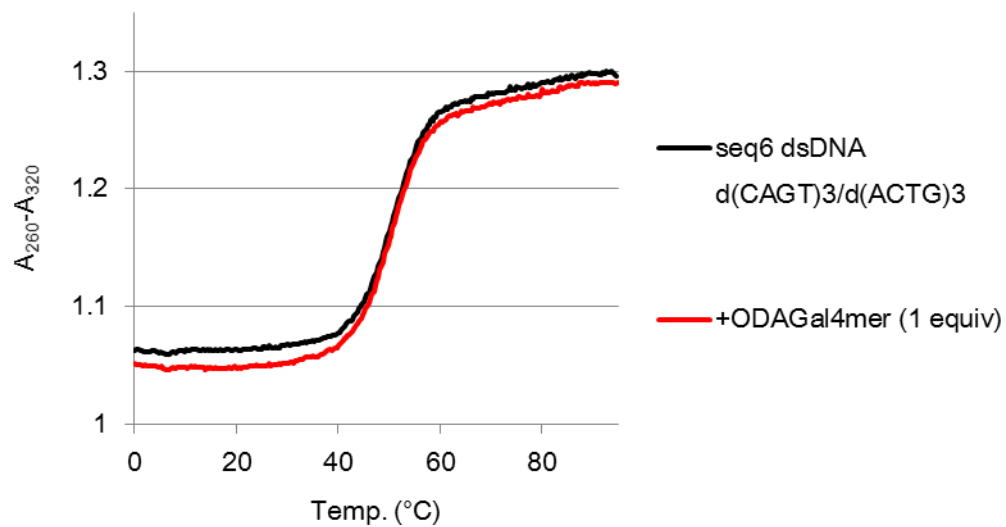


Figure S38. UV melting curves of d(ACTG)₃/d(CAGT)₃ (seq 6) in the absence and presence of 1 equivalent of ODAGal 4mer **8**.

Fig. S39-S42: UV melting analysis

All the experiments were conducted using 5 μ M of (rCGCGAAUUCGCG)₂ (seq 1).

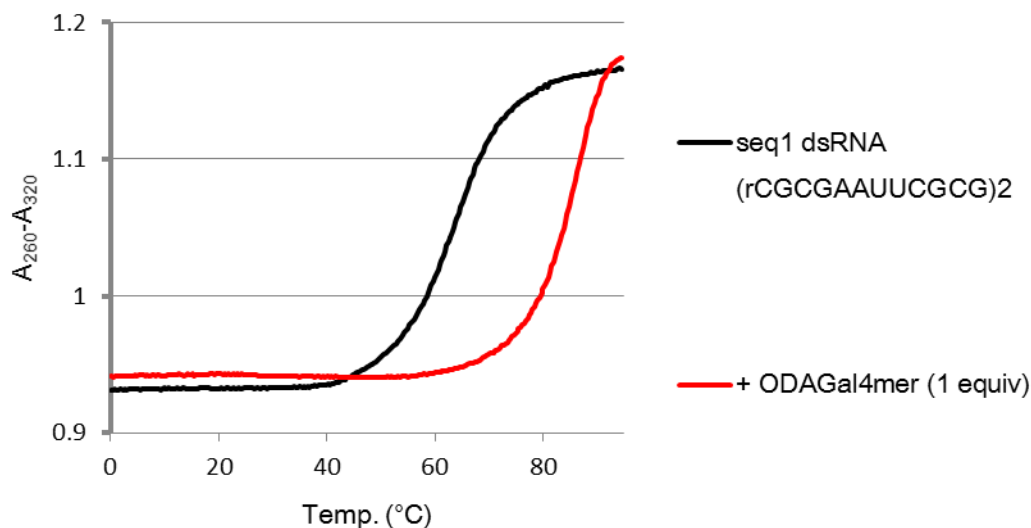


Figure S39. UV melting curves of (rCGCGAAUUCGCG)₂ (seq 1) in the absence and presence of 1 equivalent of ODAGal 4mer **8** in 10 mM phosphate buffer containing 100 mM NaCl, pH 5.7.

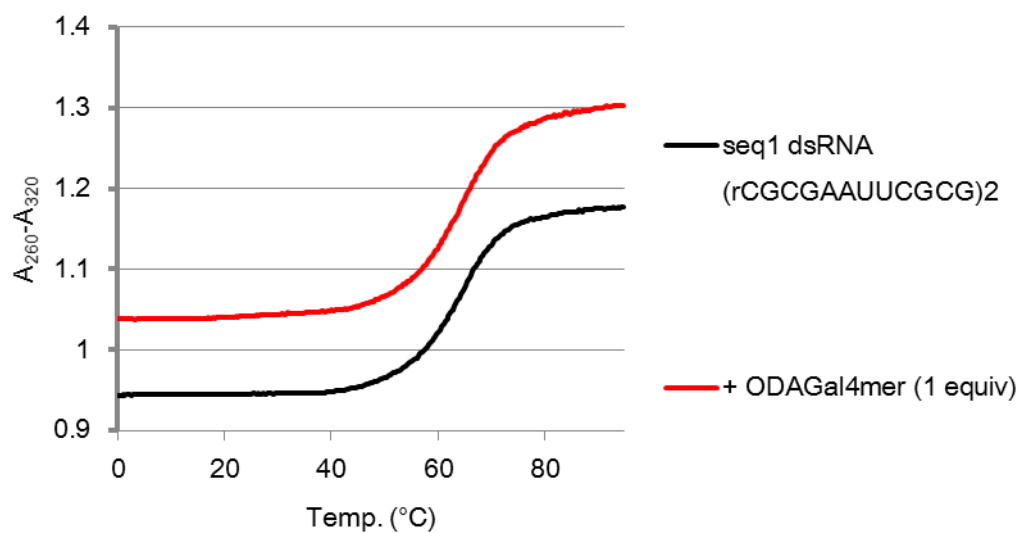


Figure S40. UV melting curves of (rCGCGAAUUCGCG)₂ (seq 1) in the absence and presence of 1 equivalent of ODAGal 4mer **8** in 10 mM phosphate buffer containing 100 mM NaCl, pH 7.8.

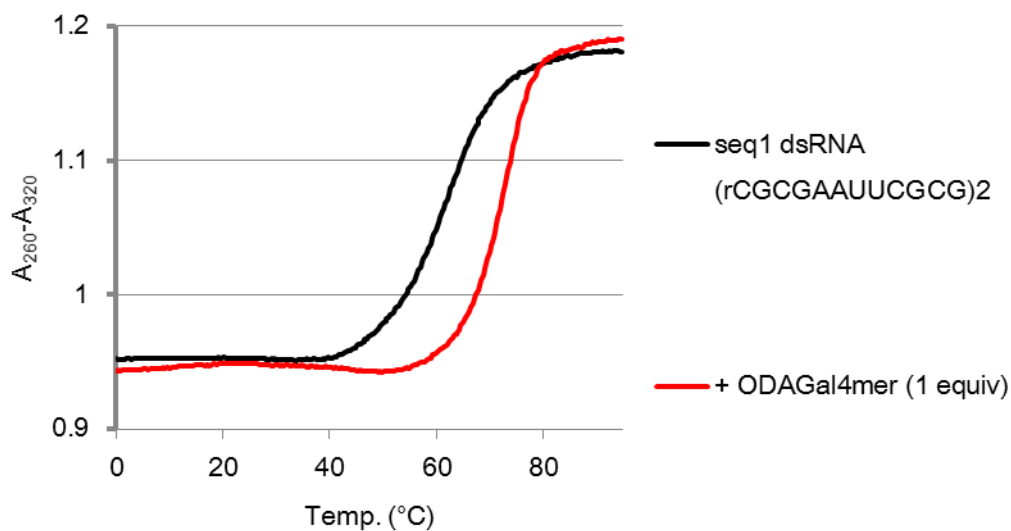


Figure S41. UV melting curves of (rCGCGAAUUCGCG)₂ (seq 1) in the absence and presence of 1 equivalent of ODAGal 4mer **8** in 10 mM phosphate buffer containing 50 mM NaCl, pH 7.0.

1 M

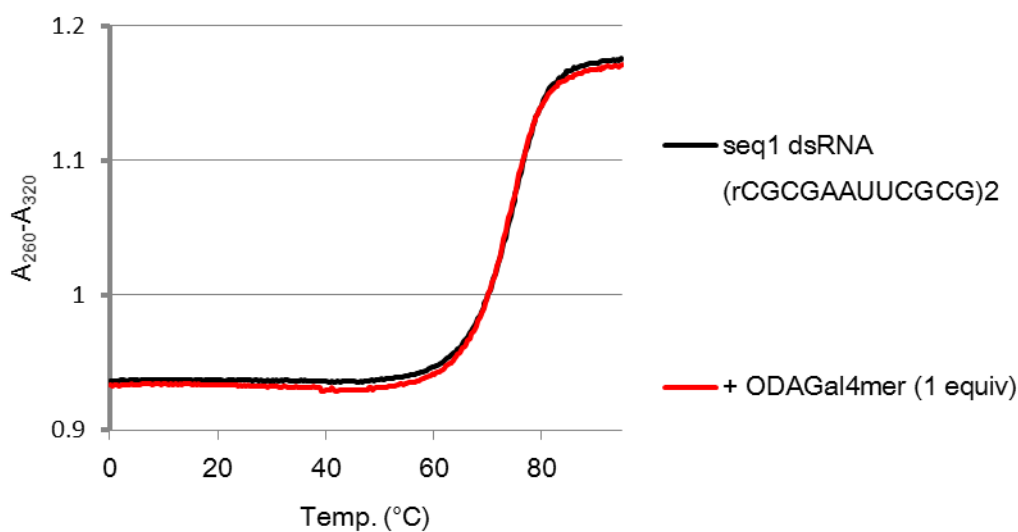


Figure S42. UV melting curves of (rCGCGAAUUCGCG)₂ (seq 1) in the absence and presence of 1 equivalent of ODAGal 4mer **8** in 10 mM phosphate buffer containing 1 M NaCl, pH 7.0.

Job plot (siRNA)

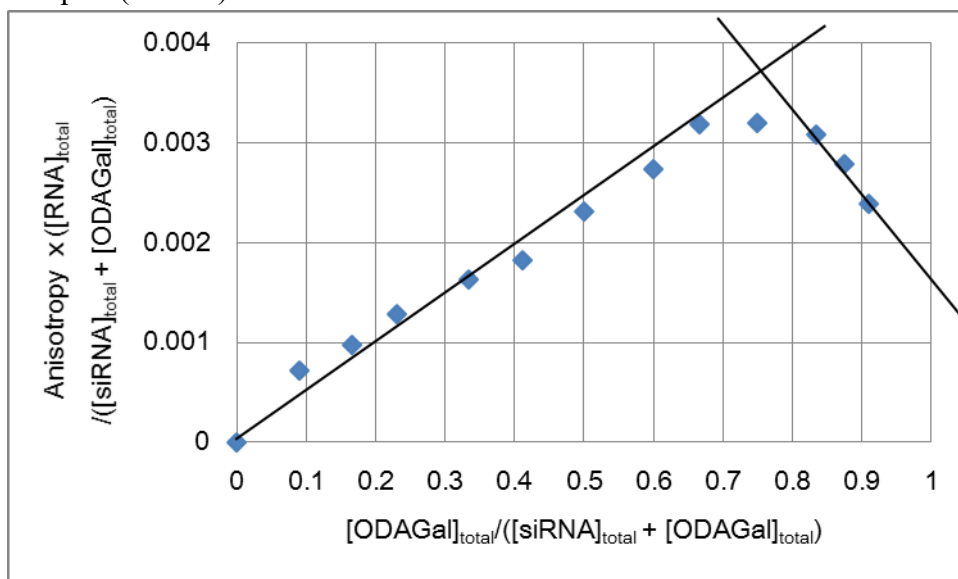


Figure S43. A job plot for estimating binding ratio of ODAGal 4mer-siRNA. $N = 3$.

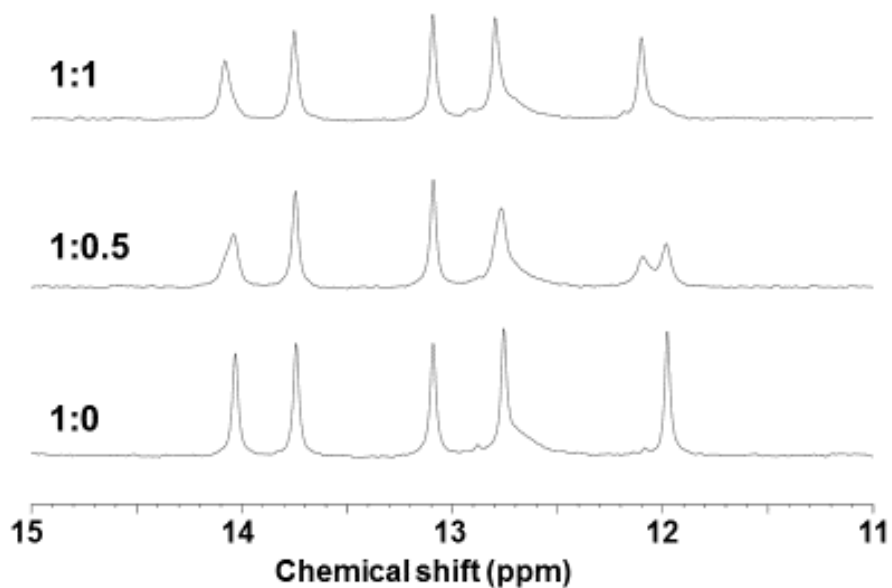


Fig. S44 NMR titration of the dsRNA with ODAGal. Imino proton spectra were measured at 25 °C. Molar ratio (dsRNA: ODAGal) and assignment are indicated.

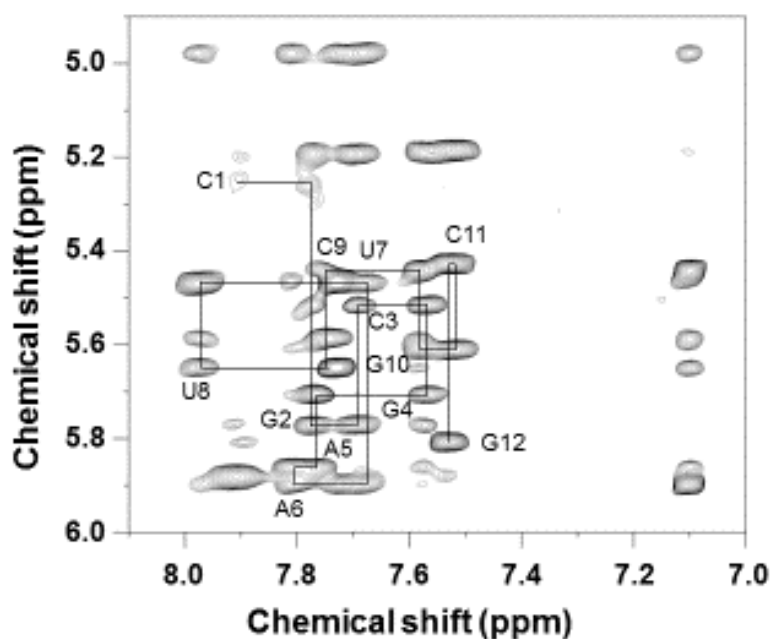


Fig. S45 2D NOESY spectrum of the dsRNA–ODAGal 4mer complex. The NOESY spectrum (mixing time 400 ms) was recorded at 10 °C and cross peaks between aromatic H6 or H8 protons and ribose H1' protons are shown. Sequential connectivities are indicated by lines and intra-residue NOEs are labelled.

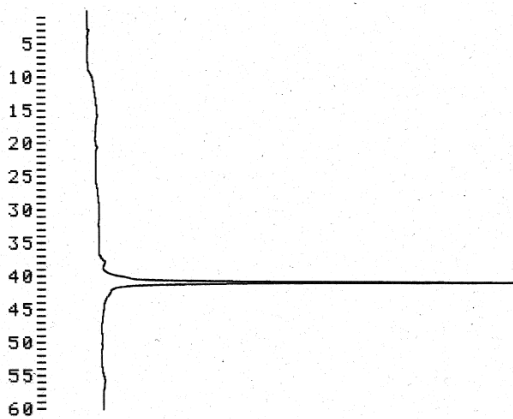


Fig. S46 HPLC profiles of the luciferase siRNA (= Fig. 5A).

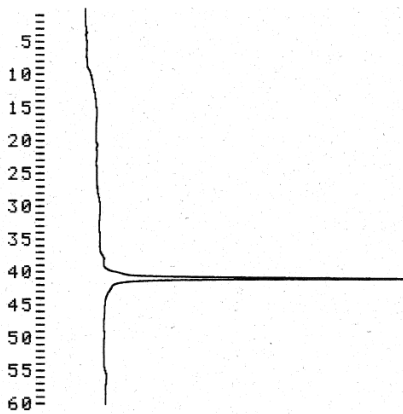


Fig. S47 HPLC profiles of the luciferase siRNA in the presence of 10 equivalents of ODAGal 4mer **8**.

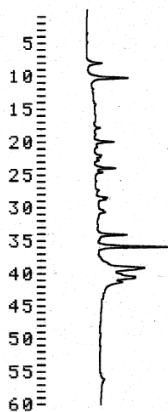


Fig. S48 HPLC profiles of the luciferase siRNA after treatment with 0.5 µg/mL RNase A over 12 hours at 37 °C (= Fig. 5B).

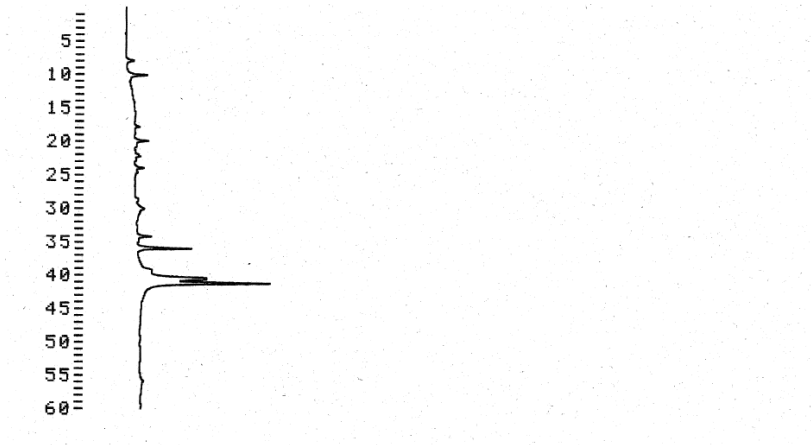


Fig. S49 HPLC profiles of the siRNA in the presence of 1 equivalent of ODAGal 4mer **8** after treatment with 0.5 $\mu\text{g}/\text{mL}$ RNase A over 12 hours at 37 $^{\circ}\text{C}$ (= Fig. 5C).

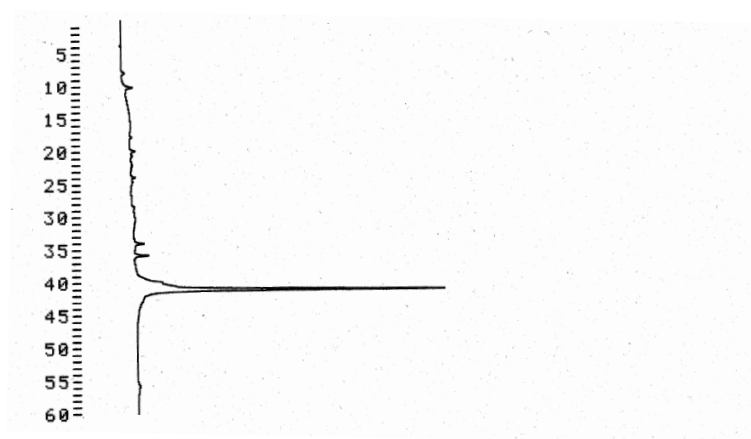


Fig. S50 HPLC profiles of the siRNA in the presence of 3 equivalents of ODAGal 4mer **8** after treatment with 0.5 $\mu\text{g}/\text{mL}$ RNase A over 12 hours at 37 $^{\circ}\text{C}$ (= Fig. 5D).

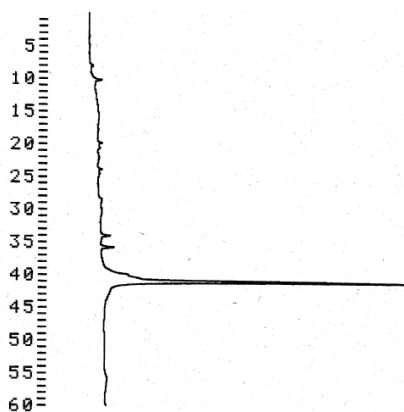


Fig. S51 HPLC profiles of the siRNA in the presence of 5 equivalents of ODAGal 4mer **8** after treatment with 0.5 $\mu\text{g}/\text{mL}$ RNase A over 12 hours at 37 $^{\circ}\text{C}$ (= Fig. 5D).

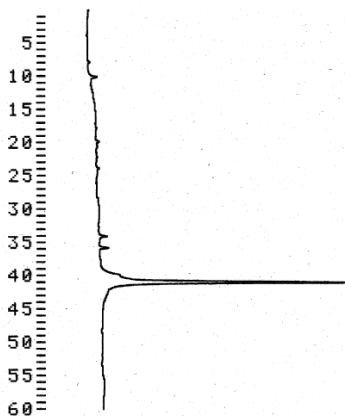


Fig. S52 HPLC profiles of the siRNA in the presence of 10 equivalents of ODAGal 4mer **8** after treatment with 0.5 $\mu\text{g}/\text{mL}$ RNase A over 12 hours at 37 $^{\circ}\text{C}$ (Fig. 5E).

Passenger strand (PS): 5'-rCUUACGCUGAGUACUUCGAdTdT-3'
Guide strand (GS): 5'-rUCGAAGUACUCAGCGUAAGdTdT-3'

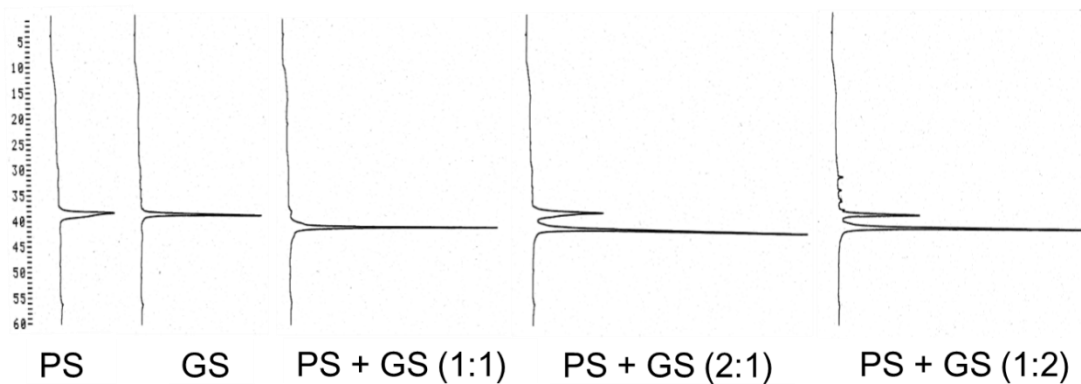


Fig. S53-56 HPLC profiles of the guide strand (GS) and the passenger strand (PS).

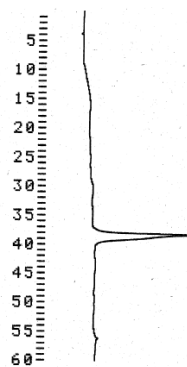


Fig. S53 HPLC profiles of the passenger strand.

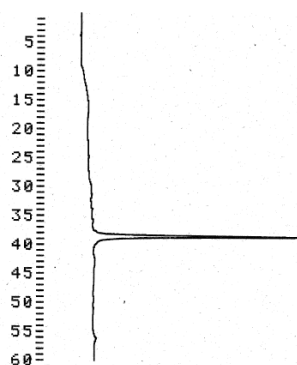


Fig. S54 HPLC profiles of the guide strand.

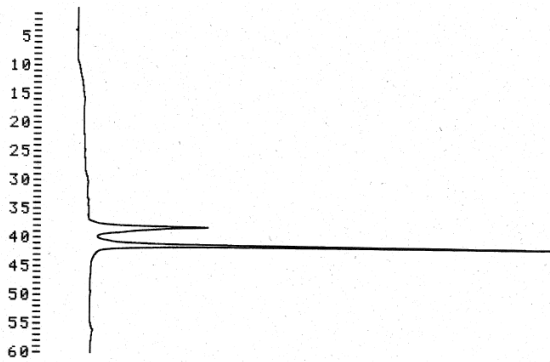


Fig. S55 HPLC profiles of the passenger strand and guide strand. (PS:GS = 2:1)

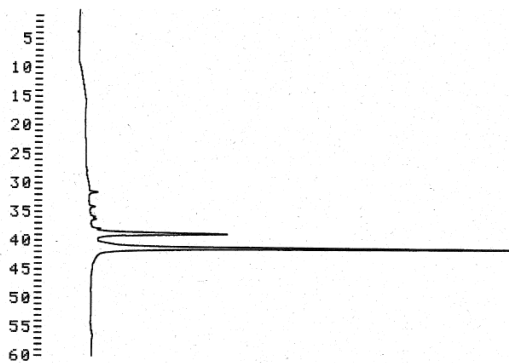


Fig. S56 HPLC profiles of the passenger strand and guide strand. (PS:GS = 1:2)

Compound 6

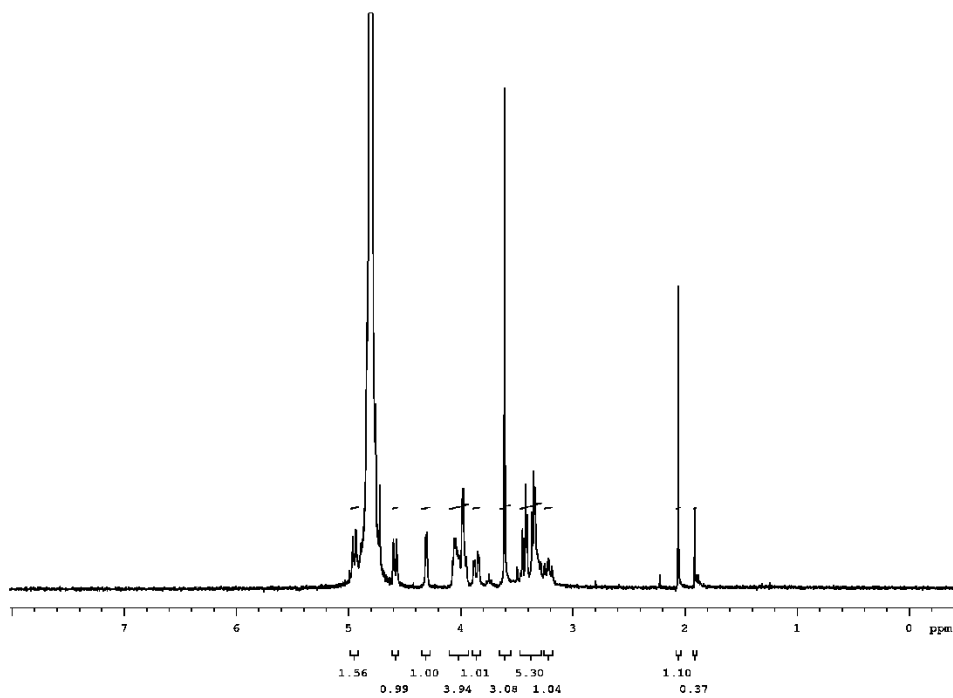
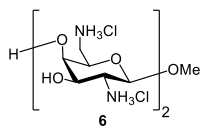


Fig. S57 ¹H NMR spectrum of **6** in D₂O (300 MHz)

Compound 7

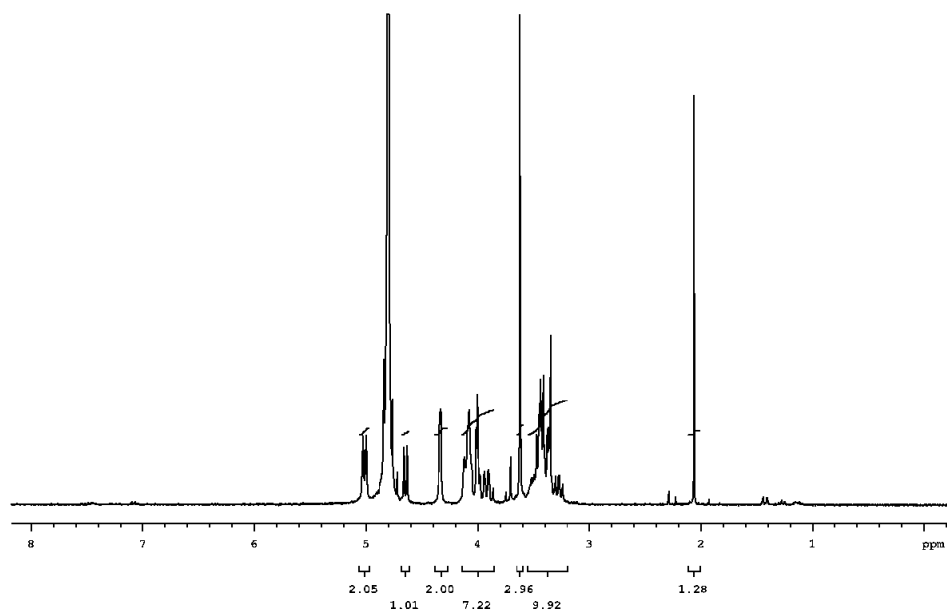
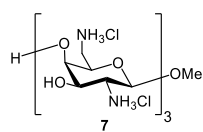


Fig. S58 ^1H NMR spectrum of 7 in D_2O (300 MHz)

Compound **8**

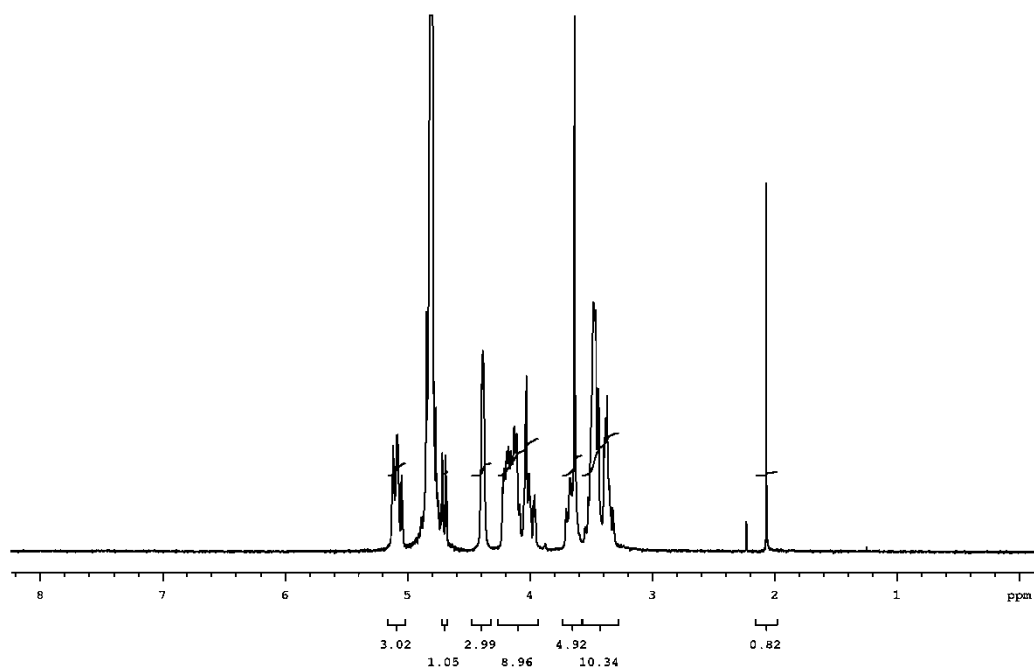
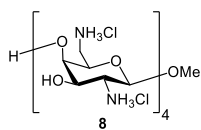


Fig. S59 ¹H NMR spectrum of **8** in D₂O (300 MHz)

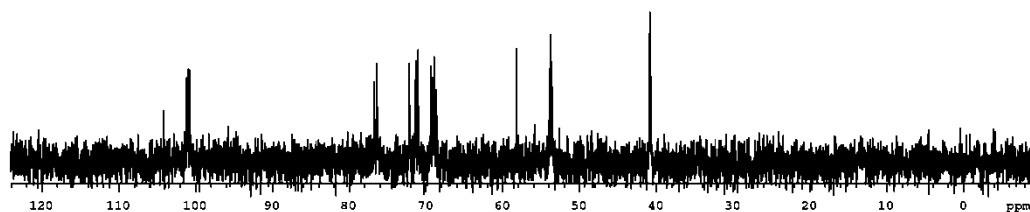


Fig. S60 ¹³C NMR spectrum of **8** in D₂O (75.5 MHz)