Towards a comprehensive understanding of RNA deamination – Synthesis and properties of xanthosine-modified RNA

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1.1. 2′-O-(tert-Butyldimethylsilyl)-3′,5′-O-(di-tert-butylsilanediyl)guanosine 1

Guanosine (2.40 g, 8.47 mmol) was coevaporated three times each with pyridine, toluene and dichloromethane and dried overnight on the high vacuum. Subsequently the compound was suspended in $N,N$-dimethylformamide (30 mL) and di-tert-butylsilanediyl bis(trifluoro-methanesulfonate) (4.11 g, 9.32 mmol, 1.1 eq) was added over the course of 15 min at 0 °C. The suspension was then stirred for 45 min upon which a clear solution is formed. Imidazole (2.88 g, 42.4 mmol, 5 eq) was added and the solution was first stirred at 0 °C for 5 min and then at room temperature for 25 min. The reaction mixture was treated with tert-butyldimethylsilyl chloride (1.53 g, 10.2 mmol, 1.2 eq) and then heated to 60 °C for 2 h. The product precipitated upon cooling to room temperature, filtrated and washed with cold methanol.

Yield: 3.23 g of compound 1 as a white solid (71%). TLC: 0.11 (4/96 methanol/dichloromethane). HR-ESI-MS (m/z): [M+H]$^+$ (calc): 538.2875; [M+H]$^+$ (meas): 538.2860. $^1$H NMR (400 MHz, DMSO): δ 0.07 (s, 3H, H$_3$CSi), 0.09 (s, 3H, H$_3$CSi), 0.86 (s, 9H, (H$_3$C)$_3$CSi(TBDMS)), 1.00 (s, 9H, (H$_3$C)$_3$CSi), 1.06 (s, 9H, (H$_3$C)$_3$CSi), 1.07-1.09 (s, 9H, (H$_3$C)$_3$CSi), 3.93-3.97 (m, 2H, H(5′a) and H(4′)), 4.27-4.35 (m, 2H, H(5′a) and H(4′)), 5.72 (d, 1H, H(2′)), 5.72 (s, 1H, HC(1′)), 6.35 (b.r.s, 2H, H$_2$NC(2)), 7.91 (s, 1H, HC(8)), 10.65 (s, 1H, HNC(1)), ppm.

$^{13}$C NMR (100 MHz, CDCl$_3$): δ -5.15 (s, 1C, CH$_3$Si(TBDMS)), -4.61 (s, 1C, CH$_3$Si(TBDMS)), 18.01 (s, 1C, (CH$_3$)$_3$), 19.96 (s, 1C, C(CH$_3$)$_3$), 22.21 (s, 1C, (CH$_3$)$_3$), 25.68 (s, 3C, (CH$_3$)$_3$CSi), 26.82 (s, 3C, (CH$_3$)$_3$CSi), 27.29 (s, 3C, (CH$_3$)$_3$CSi, 66.94 (s, 1C, C(5′)), 73.88 (s, 1C, C(4′)), 74.70 (s, 1C, C(2′)), 75.65 (s, 1C, C(3′)), 90.05 (s, 1C, C(1′)), 116.55 (s, 1C, C(5)), 135.56 (s, 1C, C(8)), 150.75 (s, 1C, C(4)), 153.76 (s, 1C, C(2)), 156.66 (s, 1C, C(6)), ppm.
$^1$H-NMR (400 MHz, DMSO-$d_6$, 25 °C) of compound 1

- $-H-N(1)$ 10.65 ppm
- $-H-C(8)$ 7.91 ppm
- $-H_2N-C(2)$ 6.35 ppm
- $-H-C(1')$ 5.72 ppm
- $-H-C(2')$ 4.57 ppm
- $-H-C(3')$ and $-H-C(5'b)$ 4.35 ppm
- $-H-C(4')$ and $-H-C(5'a)$ 3.31 ppm
- $-H_2O$ 1.98 ppm

DMSO

$2x (H_3)Si$ 1.00 ppm

$(H_3)Si$ 0.96 ppm

$2x H_3CSi$ 0.07 ppm
\(^{13}\)C-NMR (100 Mhz, DMSO-d_6, 25 °C) of compound 1
1.2. $N^2$-(4,4'-Dimethoxytrityl)-2'-O-(tert-butyldimethylsilyl)-3',5'-O-(di-tert-butyldimethylsilyl)guanosine 2

Compound 1 (3.23 g, 6.01 mmol) and 4,4'-dimethoxytrityl chloride (2.85 g, 8.41 mmol, 1.4 eq) were dissolved in anhydrous pyridine (50 mL) and the solution was stirred for 20 h at room temperature. The solvent was evaporated and the brown oil was coevaporated in ethyl acetate until a light yellow solid was obtained. The residue was taken up in ethyl acetate (50 mL) and extracted with 5% aqueous citric acid, water and brine. The organic phase was dried over MgSO$_4$ and the solvent was removed on the rotavapor. The crude product was further purified via column chromatography (SiO$_2$, 1-4% methanol in dichloromethane with 0.5% triethylamine). Yield: 4.80 g of compound 2 as a white foam (95%). TLC: 0.33 (4/96 methanol/dichloromethane). HR-ESI-MS (m/z): [M+H]$^+$ (calc): 840.4182; [M+H]$^+$ (meas): 840.4150. $^1$H NMR (400 MHz, CDCl$_3$): δ 0.13 (s, 3H, H$_3$CSi), 0.13 (s, 3H, H$_3$CSi), 0.92 (s, 9H, (H$_3$C)$_3$Si(TBDMS)), 1.02 (s, 9H, (H$_3$C)$_3$Si), 1.03 (s, 9H, (H$_3$C)$_3$Si), 3.80 (s, 6H, DMTr), 3.99 (t, 1H, HC(5'a), $^2$J$_{HH}$ = 10 Hz), 4.13-4.17 (m, 1H, HC(4')), 4.21-4.24 (m, 1H H(3')), 4.38 (d, 1H, HC(2'), $^3$J$_{HH}$ = 5 Hz), 4.45-4.48 (m, 1H, HC(5'b)), 5.68 (s, 1H, H(1')), 6.22 (brs, 1H, HN(1)), 6.83-6.87 (m, 4H, HC(ar)), 7.19-7.34 (m, 9H, HC(ar)), 7.43 (s, 1H, H(8)), ppm. $^{13}$C NMR (100 MHz, CDCl$_3$): δ -4.83 (s, 1C, CH$_3$Si(TBDMS)), -4.08 (s, 1C, CH$_3$Si(TBDMS)), 18.51 (s, 1C, C(CH$_3$)$_3$), 20.45 (s, 1C, C(CH$_3$)$_3$), 22.87 (s, 1C, C(CH$_3$)$_3$), 26.05 (s, 3C, (CH$_3$)$_3$Si), 27.13 (s, 3C, (CH$_3$)$_3$Si), 27.59 (s, 3C, (CH$_3$)$_3$Si), 55.41 (s, 2C, CH$_3$O(DMTr)), 67.99 (s, 1C, C(5')), 70.30 (s, 1C, C(central, DMTr)), 74.52 (s, 1C, C(4')), 75.83 (s, 1C, C(2')), 76.27 (s, 1C, C(3')), 91.70 (s, 1C, C(1')), 114.2 (s, 4C, CH(ar)), 118.50 (s, 1C, C(5)), 127.98-129.79 (m, 5C, CH(ar)), 135.16-135.24 (m, 2C, C(8) and C(ar)), 144.05 (s, 1C, C(ar)), 149.91 (s, 1C, C(4)), 151.98 (s, 1C, C(2)), 156.08 (s, 1C, C(6)), 159.21 (s, 1C, COCH$_3$(ar)), ppm.
$^{1}$H-NMR (400 MHz, CDCl$_3$, 25 °C) of compound 2
$^{13}$C-NMR (100 MHz, CDCl$_3$, 25°C) of compound 2

- COCH$_3$(ar)
- C(6)
- C(4), C(2)
- C(ar)
- C(8) and C(ar)
- C(ar)
- C(5)
- C(ar)
- C(1’)

CDCl$_3$

- 159.21
- 156.08
- 151.98
- 149.91
- 144.05
- 135.24
- 135.16
- 129.79
- 128.87
- 128.19
- 127.98
- 118.50
- 114.21
- 91.70
- 77.47
- 77.16
- 76.84
- 76.37
- 75.83
- 74.62
- 70.30
- 67.99
- 55.41
- 46.38

2x CH$_3$O(DMTr)

- Et$_3$N

2x (CH$_3$)$_3$CSi (di-tert-butyldiethylsilyl)
2x (CH$_3$)$_3$CSi (TBDMS)

- 27.59
- 27.13
- 26.05
- 22.87
- 20.45
- 18.51
- 11.57

- Et$_3$N

2x CH$_3$(TBDMS)

- 4.08
- 4.83
1.3. 6-[(4-Nitrophenyl)ethyl-N'-{(4,4′-dimethoxytrityl)}-2'-O-(tert-butyldimethylsilyl)-3',5'-O-(di-tert-butyldimethylsiloxyethyldi)guanosine 3

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\begin{align*}
\text{Triphenylphosphane} \ (5.25 \text{ g, 20.0 mmol, 3.5 eq}) \ &\text{and 2-(4-nitrophenyl)ethan-1-ol (3.34 g, 20.0 mmol, 3.5 eq) were dissolved in dioxane (50 mL). Compound 2 (4.80 g, 5.71 mmol) was added and the mixture was stirred for 10 minutes. Subsequently diisopropyl azodicarboxylate (4.04 g, 3.93 mL, 20.0 mmol, 3.5 eq) was added over the course of 5 min and the solution was stirred for 18 h at 60 °C. The solvent was evaporated and the crude product was purified via column chromatography (SiO2, 0-50% dichloromethane in toluene). Yield: 5.65 g of compound 3 as a white foam (quantitative). TLC: 0.42 (3/7 ethyl acetate/cyclohexane). HR-ESI-MS (m/z): [M+H]+ (calc): 989.4659; [M+H]+ (meas): 989.4618.}
\end{align*}
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\begin{align*}
\text{1H NMR (400 MHz, CDCl3):} &\quad \delta \ 0.13 \ (s, 6H, 2\times H_3CSi), 0.91 \ (s, 9H, (H_3C)_3CSi(TBDMS)), 0.99 \ (s, 9H, (H_3C)_3CSi), 1.01 \ (s, 9H, (H_3C)_3CSi), 2.81 \ (t, 2H, H_2CH_2CO (NPE), ^3J_{HH} = 7 \text{ Hz}), 3.73 \ (s, 6H, H_2CO(DMTr)), 3.89 \ (brs, 2H, H_2CO (NPE)), 3.99 \ (t, 1H, H(4'), ^3J_{HH} = 10 \text{ Hz}), 4.12-4.18 \ (m, 1H, H(5'a)), 4.43-4.49 \ (m, 3H, H(2'), H(3') and H(5'b)), 5.74 \ (s, 1H, HNC(3)), 6.12 \ (s, 1H, HC(1')), 6.72-6.74 \ (m, 4H, HC(ar, DMTr)), 7.04 \ (d, 2H, HC(ar, NPE), ^3J_{HH} = 8 \text{ Hz}), 7.14-7.28 \ (m, 9H, HC(ar, DMTr)), 7.55 \ (s, 1H, HC(8)), 8.05 \ (d, 2H, HC(ar, NPE), ^3J_{HH} = 9 \text{ Hz}), ppm. \text{13C NMR (100 MHz, CDCl3):} &\quad \delta \ -4.686 \ (s, 1C, CH_3Si(TBDMS)), -4.10 \ (s, 1C, CH_3Si(TBDMS)), 18.46 \ (s, 1C, C(CH_3)_3), 20.38 \ (s, 1C, C(CH_3)_3), 22.80 \ (s, 1C, C(CH_3)_3), 26.01 \ (s, 3C, (CH_3)_3CSi), 27.08 \ (s, 3C, (CH_3)_3CSi), 27.55 \ (s, 3C, (CH_3)_3CSi), 34.81 \ (s, 1C, CH_2CH_2O(NPE)), 55.26 \ (s, 2C, 2x CH_2O (DMTr)), 65.75 \ (s, 1C, CH_2O (NPE)), 67.95 \ (s, 1C, C(5')), 70.25 \ (s, 1C, C(central DMTr)), 74.55 \ (s, 1C, C(4')), 75.52 \ (s, 1C, C(2')), 75.94 \ (s, 1C, C(3')), 92.32 \ (s, 1C, C(1')), 113.04 \ (s, 4C, C(ar)), 115.79 \ (s, 1C, C(5)), 123.56-130.10 \ (m, 12C, CH(ar)), 137.65 \ (s, 1C, C(8)), 138.31 \ (s, 2C, C(ar)), 146.03-146.70 \ (m, 4C, C(ar)), 153.01 \ (s, 1C, C(4)), 158.11-158.27 \ (m, 3C, C(2) and C(ar)), 159.56 \ (s, 1C, C(6)), ppm.}
\end{align*}
\]
$^1$H-NMR (400 Mhz, CDCl$_3$, 25 °C) of compound 3
$^{13}$C-NMR (100 MHz, CDCl$_3$, 25 °C) of compound 3

- C(6) C(2) and C(ar, DMTr)
- C(4)
- C(ar)
- C(8) and C(ar)

$\{ C(ar) $
- C(5)  
- C(ar) $\}$

-C(1')

CDCl$_3$

- C(4') - C(2') and C(3')
- C(central, DMTr)
- C(5') - CH$_2$O(NPE)

2x CH$_3$O(DMTr)  55.26

- CH$_2$CH$_2$O(NPE)  34.81

2x (CH$_3$)$_3$CSi (di-tert-butyldimethylsilyl)

- C(CH$_3$)$_3$(di-tert-butyldimethylsilyl)
- C(CH$_3$)$_3$(di-tert-butyldimethylsilyl)
- C(CH$_3$)$_3$(TBDMS)

(CH$_3$)$_3$CSi (TBDMS)

2x CH$_3$(TBDMS)  4.10

4.88
1.4. O6-(4-Nitrophenyl)ethyl-2′-O-(tert-butyldimethylsilyl)-3′,5′-O-(di-tert-butylsilanediyl)guanosine 4

Compound 3 (5.65 g, 5.71 mmol) was dissolved in dichloromethane/methanol (9/1, 75 mL) at 0 °C and benzenesulfonic acid (3.00 g in dichloromethane methanol (9/1, 75 mL) was added. The solution went from colourless to orange immediately and the solution was stirred at 0 °C for 10 min. The solution was neutralized with sat. aqueous NaHCO3 (100 mL) solution and stirred for 10 min. The layers were separated and the aqueous solution was extracted twice with dichloromethane (80 mL each). The combined organic phases were washed with sat. aqueous NaHCO3 solution and brine and dried over MgSO4. The solvent was evaporated and the crude product was purified via column chromatography (SiO2, 12-25% ethyl acetate in toluene).

Yield: 3.65 g of compound 4 as a white solid (93%). TLC: 0.60 (4/96 methanol/dichloromethane). HR-ESI-MS (m/z): [M+H]+ (calc): 687.3352; [M+H]+ (meas): 687.3346.

1H NMR (400 MHz, CDCl3): δ 0.14 (s, 3H, 2x H3CSi), 0.15 (s, 3H, 2x H3CSi), 0.93 (s, 9H, (H3C)3CSi(TBDMS)), 1.03 (s, 9H, (H3C)3CSi), 1.07 (s, 9H, (H3C)3CSi), 3.27 (t, 2H, H2CO (NPE)), 4.01 (t, 1H, H(5'a), 3JHH = 7 Hz), 4.15-4.21 (m, 1H, H(4')), 4.44-4.51 (m, 3H, H(2'), H(3') and H(5'b)), 4.70-4.74 (m, 2H, H2CO (NPE)), 5.78 (s, 1H, HC(1')), 7.48 (d, 2H, HC(ar, NPE), 9 Hz), 7.62 (s, 1H, HC(8)), 8.16 (d, 2H, HC(ar, NPE), 9 Hz), ppm. 13C NMR (100 MHz, CDCl3): δ -4.86 (s, 1C, CH3Si(TBDMS)), -4.14 (s, 1C, CH3Si(TBDMS)), 18.47 (s, 1C, C(CH3)3), 20.46 (s, 1C, C(CH3)3), 22.92 (s, 1C, C(CH3)3), 26.03 (s, 3C, (CH3)3CSi), 27.14 (s, 3C, (CH3)3CSi), 27.61 (s, 3C, (CH3)3CSi), 35.33 (s, 1C, CH2CH2O(NPE)), 66.34 (s, 1C, CH2O (NPE)), 68.01 (s, 1C, C(5')), 74.68 (s, 1C, C(4')), 75.55 (s, 1C, C(2')), 75.97 (s, 1C, C(3')), 92.33 (s, 1C, C(1')), 116.30 (s, 1C, C(5)), 123.87 (s, 2C, CH(ar)), 130.05 (s, 2C, CH(ar)), 137.74 (s, 1C, C(8)), 146.10 (s, 1C, C(ar)), 146.99 (s, 1C, C(ar)), 153.30 (s, 1C, C(4)), 159.21 (s, 1C, C(2)), 161.02 (s, 1C, C(6)), ppm.
$^1$H-NMR (400 MHz, CDCl$_3$, 25 °C) of compound 4

- **H-C(ar)**
  - 8.17
  - 8.15

- **H-C(8)**
  - 7.62
  - 7.49
  - 7.47
  - 7.26

- **H-C(1')**

- **H-C(2'), H-C(3') and H-C(5'b)**
  - 5.78
  - 4.99
  - 4.87
  - 4.79
  - 4.74
  - 4.72
  - 4.71
  - 4.61
  - 4.48
  - 4.45
  - 4.42
  - 4.21
  - 4.20
  - 4.19
  - 4.17
  - 4.16
  - 4.15
  - 4.03
  - 3.98
  - 3.28
  - 3.25

- **H$_2$CO(NPE)**

- **H-C(4')**
  - 2.02

- **H-C(5'a)**

- **H$_2$CH$_2$CO(NPE)**

- **2x (H$_3$C)$_3$Si**
  - 1.07
  - 1.03
  - 0.83

- **(H$_3$C)$_2$Si**
  - 0.15

- **2x H$_3$CSi**
  - 0.14
$^{13}$C-NMR (100 MHz, CDCl$_3$, 25 °C) of compound 4
1.5. O⁶-(4-Nitrophenyl)ethyl-2’-O-(tert-butyldimethylsilyl)-3’,5’-O-(di-tert-butyldimethyldisilyl)xanthosine 5

Compound 4 (3.65 g, 5.31 mmol) and sodium nitrite (12.0 g, 174 mmol, 33 eq) were dissolved in water/acetone (1/2, 60 mL). Acetic acid (18.4 g, 17.5 mL, 306 mmol, 58 eq) was added under vigorous stirring followed by intensive gas evolution. The solution was stirred for 24 h at room temperature. Subsequently the mixture was heated to 45 °C and stirred for another 2h. The mixture was neutralized with sat. NaHCO₃ solution (50 mL) and extracted three time with ethyl acetate (50 mL each). The combined organic phases were washed with sat. NaHCO₃ solution and brine and the solvent was removed. The crude product was further purified via column chromatography (SiO₂, 12-20% ethyl acetate in toluene).

Yield: 2.35 g of compound 5 as a white solid (64%). TLC: 0.36 (4/96 methanol/dichloromethane). HR-ESI-MS (m/z): [M+H]⁺ (calc): 688.3192; [M+H]⁺ (meas): 688.3187. ¹H NMR (400 MHz, CDCl₃): δ 0.13 (s, 3H, H₃CSi), 0.14 (s, 3H, H₃CSi), 0.91 (s, 9H, (H₃)₃CSi(TBDMS)), 1.04 (s, 9H, (H₃)₂CSi), 1.07 (s, 9H, (H₃)₂CSi), 3.30 (t, 2H, H₂CO (NPE), 3J_HH = 7 Hz), 4.01 (t, 1H, H(5'a), 3J_HH =10 Hz), 4.18-4.24 (m, 1H, H(4')), 4.28-4.31 (s, 1H, H(3')), 4.46-4.50 (m, 2H, H(2') and H(5'b)), 4.88 (t, 2H, H₂CO (NPE), 3J_HH = 7 Hz), 5.90 (s, 1H, HC(1')), 7.50 (d, 2H, HC(ar, NPE), 9 Hz), 7.70 (s, 1H, HC(8)), 8.16-8.18 (m, 2H, HC(ar, NPE)), ppm. ¹³C NMR (100 MHz, CDCl₃): δ -5.00 (s, 1C, CH₃Si(TBDMS)), -4.33 (s, 1C, CH₃Si(TBDMS)), 18.28 (s, 1C, C(CH₃)₃), 20.35 (s, 1C, C(CH₃)₃), 22.78 (s, 1C, C(CH₃)₃), 25.86 (s, 3C, (CH₃)₂CSi), 27.01 (s, 3C, (CH₃)₂CSi), 27.47 (s, 3C, (CH₃)₂CSi), 35.14 (s, 1C, CH₂CH₂O(NPE)), 67.77 (s, 1C, C(5')), 68.12 (s, 1C, CH₂O(NPE)), 74.61 (s, 1C, C(4')), 75.76 (s, 1C, C(2')), 76.21 (s, 1C, C(3')), 91.75 (s, 1C, C(1')), 116.00 (s, 1C, C(5)), 123.77 (s, 2C, CH(ar)), 130.03 (s, 2C, CH(ar)), 138.18 (s, 1C, C(8)), 145.38 (s, 1C, C(ar)), 146.95 (s, 1C, C(ar)), 152.08 (s, 1C, C(4)), 159.87 (s, 1C, C(2)), 161.52 (s, 1C, C(6)), ppm.
$^1$H-NMR (400 MHz, CDCl$_3$, 25 °C) of compound 5

- $H$-C(ar) 
- $H$-C(8)
- $H$-C(ar)
- CDCl$_3$
- $H$-C(1')
- $H_2$CO(NPE)
- $H$-C(2') and $H$-C(5'b)
- $H$-C(3')
- $H$-C(4')
- $H$-C(5'a)
- $H_2$CH$_2$CO(NPE)

2x (H$_3$C)$_3$Si
(H$_3$C)$_3$Si
2x H$_3$CSi
$^{13}$C-NMR (100 Mhz, CDCl$_3$, 25 °C) of compound 5

- C(6) - C(2)
- C(4)
C(ar)
- C(8)
- C(ar)
- C(ar)
- C(5)
- C(1')

- C(2') and C(3')
C(5') and CH$_2$O(NPE)

- CH$_2$CH$_2$O(NPE)

2x (CH$_3$)$_3$CSi (di-tert-butyldimethylsilyl)

- C(CH$_3$)$_3$ (di-tert-butyldimethylsilyl)
- C(CH$_3$)$_3$ (di-tert-butyldimethylsilyl)
- C(CH$_3$)$_3$ (TBDMS)

(CH$_3$)$_3$CSi (TBDMS)

2x CH$_3$ (TBDMS)
1.6. $O^2,O^6$-Bis(4-nitrophenyl)ethyl-2'-O-\{(tert-butyldimethylsilyl)-3',5'-O-\{(di-tert-butyldimethylsilyl)xanthosine 6

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\text{Compound 5 (2.35 mg, 3.42 mmol) was dissolved in anhydrous benzene (120 mL) and silver carbonate (1.41 g, 5.12 mmol, 1.5 eq) was added in one portion. The mixture was heated to reflux for one hour and subsequently $p$-nitrophenylethyl iodide (1.89 g, 6.83 mmol, 2 eq) was added. The reaction solution was stirred at 50 °C for 15 h and filtrated. The solvent was evaporated and the crude product purified via column chromatography (SiO$_2$, 10-20% ethyl acetate in toluene). Yield: 2.46 g of compound 6 as a white foam (86%). TLC: 0.26 (3/7 ethyl acetate/cyclohexane). HR-ESI-MS (m/z): [M+H]$^+$ (calc): 837.3669; [M+H]$^+$ (meas): 837.3639.}

\[
\text{^1H NMR (400 MHz, CDCl}_3\text{): $\delta$ 0.08 (s, 3H, H$_3$CSi), 0.11 (s, 3H, H$_3$CSi), 0.87 (s, 9H, (H$_3$C)$_3$CSi(TBDMS)), 1.04 (s, 9H, (H$_3$C)$_3$CSi), 1.04 (s, 9H, (H$_3$C)$_3$CSi), 3.23 (t, 2H, H$_2$CH$_2$CO (NPE), 3J$_{HH}$ = 7 Hz), 3.29 (t, 2H, H$_2$CH$_2$CO (NPE), 7 Hz), 3.99 (t, 1H, H(5'a), 10 Hz), 4.16-4.21 (m, 1H, H(4')), 4.28-4.31 (m, 1H, H(3')), 4.46-4.50 (m, 1H, H(5'b)), 4.52-4.55 (m, 1H, H(2')), 4.59-4.64 (m, 2H, H&CO (NPE), 3J$_{HH}$ = 7 Hz), 4.79 (t, 2H, H&CO (NPE), 3J$_{HH}$ = 7 Hz), 5.89 (s, 1H, H(1')), 7.47 (d, 2H, H(ar, NPE), 3J$_{HH}$ = 8 Hz), 7.76 (s, 1H, H(8)), 8.16 (t, 2H, H(ar, NPE), 3J$_{HH}$ = 9 Hz), ppm. ^13C NMR (100 MHz, CDCl$_3$: $\delta$ -4.90 (s, 1C, CH$_3$Si(TBDMS)), -4.13 (s, 1C, CH$_3$Si(TBDMS)), 18.43 (s, 1C, C(CH$_3$)$_3$), 20.49 (s, 1C, C(CH$_3$)$_3$), 22.82 (s, 1C, C(CH$_3$)$_3$), 25.95 (s, 3C, (CH$_3$)$_3$Si), 27.13 (s, 3C, (CH$_3$)$_3$Si), 27.56 (s, 3C, (CH$_3$)$_3$Si), 35.21 (s, 1C, CH$_2$CH$_2$O(NPE)), 35.28 (s, 1C, CH$_2$CH$_2$O(NPE)), 66.96 (s, 1C, CH$_2$O(NPE), 67.55 (s, 1C, C(5')), 67.83 (s, 1C, CH$_2$O(NPE), 74.58 (s, 1C, C(4')), 75.55 (s, 1C, C(2')), 76.41 (s, 1C, C(3')), 92.02 (s, 1C, C(1')), 118.02 (s, 1C, C(5)), 123.88 (s, 4C, C(ar)), 129.99 (s, 2C, C(ar)), 130.08 (s, 2C, C(ar)), 139.30 (s, 1C, C(8)), 145.77-147.04 (m, 4C, C(ar)), 152.68 (s, 1C, C(4)), 161.09 (s, 1C, C(2)), 161.68 (s, 1C, C(6))), ppm;}


$^1$H-NMR (400 MHz, CDCl$_3$, 25 °C) of compound 6

- $H$-C(ar)
- $H$-C(8i)
- $H$-C(ar)
- $H$-C(1')
- $H_2$CO(NPE)
- $H$-C(2')
- $H$-C(5'b)
- $H$-C(3')
- $H$-C(4')
- $H$-C(5'a)
- $H_2$CH$_2$CO(NPE)

Toluene
Water

2x (H$_3$C)$_2$Si
(H$_3$C)$_3$Si
2x H$_3$CSi
$^{13}$C-NMR (100 MHz, CDCl$_3$, 25 °C) of compound 6
1.7. O², O⁶-Bis(4-nitrophenyl)ethyl-2′-O- (tert-butyldimethylsilyl) xanthosine 7

Compound 6 (2.46 g, 2.90 mmol) was dissolved in dichloromethane (15 mL) and cooled to 0 °C. HF pyridine (0.726 mL of a 8 M solution diluted with 5 mL pyridine) was added and the mixture was stirred for 2 h at 0 °C. Subsequently the reaction solution was quenched with sat. NaHCO₃ solution (20 mL) and diluted with dichloromethane (20 mL). The organic layer was washed with brine, dried over MgSO₄ and evaporated to dryness. The crude product was purified via column chromatography (SiO₂, 0-3% methanol in dichloromethane). Yield: 1.84 g of compound 7 as a white solid (91%). TLC: 0.22 (4/96 methanol/dichloromethane). HR-ESI-MS (m/z): [M+H]+ (calc): 697.2648; [M+H]+ (meas): 697.2618. 

1H NMR (400 MHz, DMSO): \( \delta \) -0.22 (s, 3H, H₃CSi), -0.09 (s, 3H, H₃CSi), 0.69 (s, 9H, (H₃C)₃Si(TBDMS)), 3.22 (t, 2H, H₂CO (NPE), \( J_{HH} = 7 \) Hz), 3.27 (t, 2H, H₂CO (NPE), \( J_{HH} = 7 \) Hz), 3.57-3.72 (m, 2H, H(5')), 3.94-3.97 (m, 1H, H(4')), 4.11-4.15 (m, 1H, H(3')), 4.56-4.59 (m, 3H, H(2') and H₂CO(NPE)), 4.77 (t, 2H, H₂CO(NPE), \( J_{HH} = 7 \) Hz), 5.11-5.12 (m, 2H, HOC(3') and HOC(5')), 5.88 (d, 1H, HC(1'), \( J_{HH} = 6 \) Hz), 7.59-7.62 (m, 4H, HC(ar, NPE)), 8.15-8.18 (m, 4H, HC(ar, NPE)), 8.42 (s, 1H, HC(8)), ppm. 

13C NMR (100 MHz, CDCl₃): \( \delta \) -5.42 (s, 1C, CH₃Si(TBDMS)), -4.92 (s, 1C, CH₃Si(TBDMS)), -17.71 (s, 1C, C(CH₃)₃), 25.44 (s, 3C, (CH₃)₃Si), 34.20 (s, 1C, CH₂CH₂O(NPE)), 34.24 (s, 1C, CH₂CH₂O(NPE)), 60.94 (s, 1C, C(5')), 66.40 (s, 1C, CH₂O(NPE)), 67.08 (s, 1C, CH₂O(NPE)), 69.96 (s, 1C, C(3')), 75.74 (s, 1C, C(2')), 85.53 (s, 1C, C(4')), 87.51 (s, 1C, C(1')), 118.73 (s, 1C, C(5)), 123.39 (s, 2C, CH(ar)), 123.41 (s, 2C, CH(ar)), 130.25 (s, 2C, CH(ar)), 130.27 (s, 2C, CH(ar)), 140.62 (s, 1C, C(8)), 146.24-146.82 (m, 4C, C(ar)), 153.19 (s, 1C, C(4)), 160.16 (s, 1C, C(2)), 160.72 (s, 1C, C(6)), ppm;
$^1$H-NMR (400 MHz, DMSO-d$_6$, 25 °C) of compound 7

- H-C(8)
- H-C(ar)
- H-C(ar)
- H-C(1')
- HO-C(3') and HO-C(5')
- H$_2$CO(NPE)
- H$_2$CO(NPE) and H-C(2')
- H-C(3')
- H-C(4')
- H-C(5')

Water

H$_2$CH$_2$CO(NPE)

Toluene

DMSO

(H$_2$C)$_3$Si

2x H$_2$CSi
$^{13}$C-NMR (100 Mhz, DMSO-\(d_6\), 25 °C) of compound 7

- $-\text{C(6)}$
- $-\text{C(2)}$
- $-\text{C(4)}$
- $-\text{C(5)}$
- $-\text{C(8)}$
- $-\text{C(ar)}$
- $-\text{C(ar)}$
- $-\text{C(ar)}$
- $\text{CH}_2\text{O}(\text{NPE})$
- $\text{CH}_2\text{O}(\text{NPE})$
- $2\times \text{CH}_2\text{O}(\text{NPE})$
- $\text{C(CH}_3)_3\text{(TBDMS)}$
- $-(\text{CH}_3)_3\text{CSi (TBDMS)}$
- $2\times \text{CH}_3\text{(TBDMS)}$
1.8. \(O^2,O^6\)-Bis(4-nitrophenyl)ethyl-5’-O-(4,4’-dimethoxytrityl)-2’-O-(tert-butyldimethylsilyl)xanthosine 8

Compound 7 (1.84 g, 2.64 mmol) was dissolved in anhydrous pyridine (20 mL) and DMTrCl (1.16 g, 3.43 mmol, 1.3 eq) was added in two portions over the course of 30 min. The reaction solution was stirred at room temperature for 3 h. The solvent was evaporated and the residue was taken up in ethyl acetate. The solution was washed twice with 5% citric acid, sat. NaHCO₃, brine and then dried over MgSO₄. The solvent was removed and the crude product purified via column chromatography (SiO₂, 0-2% methanol in dichloromethane). Yield: 2.51 g of compound 8 as a white foam (95%). TLC: 0.75 (4/96 methanol/dichloromethane).

HR-ESI-MS (m/z): [M+H]+ (calc): 999.3955; [M+H]+ (meas): 999.3910.

\(^1\)H NMR (400 MHz, DMSO): \(\delta\) 0.19 (s, 3H, H₃CSi), 0.00 (s, 3H, H₃CSi), 0.83 (s, 9H, (H₃C)₃CSi(TBDMS)), 3.17 (t, 2H, H₂CH₂CO (NPE), \(^3\)Jₓₓ = 7 Hz), 3.31 (t, 2H, H₂CH₂CO (NPE), \(^3\)Jₓₓ =7 Hz), 3.37-3.50 (m, 2H, H(5’)), 3.78 (s, 3H, H₃CO(DMTr)), 3.79 (s, 3H, H₃CO(DMTr)), 4.22-4.24 (m, 1H, H(4’)), 4.35-4.38 (m, 1H, H(3’)), 4.47-4.58 (m, 2H, H₂CO(NPE)), 4.78-4.84 (m, 3H, H(2’) and H₂CO(NPE)), 6.02 (d, 1H, HC(1’), \(^3\)Jₓₓ =6 Hz), 6.79-6.82 (m, 4H, HC(ar)), 7.16-7.50 (m, 8H, HC(ar)), 8.01 (s, 1H, HC(8)), 8.14-8.18 (m, 8H, HC(ar)), 8.61-8.64 (m, 4H, HC(ar)), ppm.

\(^{13}\)C NMR (100 MHz, CDCl₃): \(\delta\) -5.00 (s, 1C, CH₃Si(TBDMS)), -4.84 (s, 1C, CH₃Si(TBDMS)), 18.00 (s, 1C, C(CH₃)₃), 25.64 (s, 3C, (CH₃)₂C(Si), 35.20 (s, 1C, CH₂CH₂O(NPE)), 35.25 (s, 1C, CH₂CH₂O(NPE)), 55.36 (s, 2C, CH₂O(DMTr)), 63.67 (s, 1C, C(5’)), 66.92 (s, 1C, CH₂O(NPE), 67.50 (s, 1C, CH₂O(NPE)), 71.80 (s, 1C, C(3’)), 76.40 (s, 1C, C(2’)), 84.20 (s, 1C, C(4’)), 86.89 (s, 1C, C(centeral, DMTr)), 87.43 (s, 1C, C(1’)), 113.31 (s, 2C, CH(ar)), 113.40 (s, 2C, CH(ar)), 117.65 (s, 1C, C(5’)), 123.87-136.06 (s, 19C, CH(ar)), 139.48 (s, 1C, C(8)), 144.57-150.02 (m, 5C, C(ar)), 153.67 (s, 1C, C(4’)), 158.77 (s, 2C, CH(ar)), 161.07 (s, 1C, C(2’)), 161.64 (s, 1C, C(6)), ppm;
$^1$H-NMR (400 MHz, CDCl$_3$, 25 °C) of compound 8

- H-C(ar)
- H-C(8)

H-C(ar)

CDCl$_3$

H$_2$CO(NPE) and H-C(2')

H$_2$CO(NPE)

-H-C(3')

-H-C(4')

H-C(5')

H$_2$CH$_2$CO(NPE)

2x H$_2$CO(DMTr)

(H$_3$C)$_3$Si

2x H$_3$CSi

ppm
$^{13}$C-NMR (100 Mhz, CDCl$_3$, 25 °C) of compound 8
1.9. \(O^2,O^6\)-Bis(4-nitrophenyl)ethyl-5'-O-(4,4'-dimethoxytrityl)-2'-O-( tert butylidimethylsilyl)xanthosine-3'-O-(2-cyanoethyl) \(N,N\)-diisopropylphosphoramidite

Compound 8 (1.00 g, 1.00 mmol) was coevaporated three times each with pyridine, toluene and dichloromethane. It was then dissolved in tetrahydrofuran (10 mL) and 1-methylimidazole (49 mg, 0.60 mmol, 0.6 eq) and 2,4,6-trimethylpyridine (849 mg, 7.01 mmol, 7 eq) was added. 2-cyanoethyl \(N,N\)-diisopropylchlorophosphoramidite (592 mg, 2.50 mmol, 2.5 eq) was added dropwise and the solution was stirred at room temperature for 2 h. The mixture was diluted with dichloromethane and washed with sat. NaHCO\(_3\) solution and brine and dried over MgSO\(_4\). The solvent was evaporated and the crude product was purified via column chromatography (SiO\(_2\), 2-5% methanol in dichloromethane). Yield: 850 mg of compound 9 as a white foam (71%). HR-ESI-MS (m/z): \([M+H]^+\) (calc): 1199.5033; \([M+H]^+\) (meas): 1199.4992.

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 0.23 and 0.21 (s, 3H, H\(_3\)CSi), 0.03 (s, 3H, H\(_3\)CSi), 0.73 and 0.75 (s, 9H, (H\(_3\)C\(_3\))CSi(TBDMS)), 1.14-1.16 (m, 12H, (H\(_3\)C\(_2\))CH(N,N-diisopropyl)), 2.26-2.28 and 2.60-2.61 (m, 2H, H\(_2\)C(cyanoethyl)), 3.11-3.16 (m, 2H, H\(_2\)CH\(_2\)CO (NPE)), 3.29-3.32 (m, 3H, H\(_2\)CH\(_2\)CO(NPE) and Hb-C(5')), 3.55-3.59 (m, 4H, Ha-C(5'), Hb-C(Cyanoethyl) and 2x HC(CH\(_3\))\(_2\)(N,N-diisopropyl)), 3.83-3.95 (m, 1H, Ha-C(cyanoethyl)), 4.30-4.59 (m, 4H, HC(3'), HC(4') and HbCO(NPE)), 4.79-4.80 (m, 3H, HC(2') and HbCO(NPE)), 5.99 - 6.01 and 6.06 - 6.07 (m, 1H HC(1')), 6.79-6.82 (m, 4H, HC(ar)), 7.20-7.50 (m, 8H, HC(ar)), 8.03 and 8.06 (m, 1H, HC(8)), 8.12-8.17 (m, 4H, HC(ar)), ppm. \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 13.87 (s, 2C, CH\(_3\)(DMTr)), 17.97 and 18.05 (s, 1C, C(CH\(_3\))\(_3\)), 20.17 and 20.24 (s, 1C, C(cyanoethyl)), 24.73 (s, 4C, 4x CH\(_3\)(N,N-diisopropyl) 25.66 and 25.70 (s, 3C, (CH\(_3\))\(_2\)CSi), 35.15 (s, 1C, CH\(_3\)CH\(_2\)O(NPE)), 35.25 (s, 1C, CH\(_3\)CH\(_2\)O(NPE)), 43.48 and 43.61 (s, 2C, 2x CH(CH\(_3\))(N,N-diisopropyl)), 55.37 (s, 2C, CH\(_2\)O(DMTr)), 55.51 and 55.71 (s, 1C, C(cyanoethyl)), 63.47 (s, 1C, C(5')), 66.85 and 67.35 (s, 1C, CH\(_3\)O(NPE), 67.50 (s, 1C, CH\(_3\)O(NPE), 73.68 and 73.78 (s, 1C, C(3')), 75.39 and 75.77 (s, 1C, C(2')), 84.26 (s, 1C, C(4'))), 86.80 (s, 1C, C(cen-tral, DMTr)), 87.18 (s, 1C, C(1'))), 113.38 (s, 2C, CH(ar)), 113.42 (s, 2C, CH(ar)), 117.36 and 117.74 (s, 1C, C(5)), 123.82-135.70 (s, 19C, CH(ar)), 139.59 (s, 1C, C(8)), 144.46-147.03 (m, 5C, C(ar)), 153.79 (s, 1C, C(4)), 158.77 (s, 2C, C(9)), 160.93 (s, 1C, C(2)), 161.59 (s, 1C, C(6)), ppm. \(^{31}\)P NMR (162 MHz, CDCl\(_3\)): \(\delta\) 149.29 (s, 1P), 151.13 (s, 1P), ppm;
$^1$H-NMR (400 MHz, CDCl$_3$, 25 °C) of compound 9

- H-C(ar)
- H-C(5')

H-C(ar)  CDCl$_3$

- H$_2$CO(NPE) and H-C(2')
H$_2$CO(NPE), H-C(3') and H-C(4')

Ha-C(cyanoethyl)  -2x H$_2$CO(DMTr)

Ha-C(5'), Hb-C(cyanoethyl) and 2x HC (N,N-diisopropyl)

-Hb-C(5') and H$_2$CH$_2$CO(NPE)

H$_2$CH$_2$CO(NPE)

H$_2$C(cyanoethyl)

4x H$_3$C(N,N-diisopropyl)

(H$_3$)$_3$Si

2x H$_3$CSi

ppm
$^{13}$C-NMR (100 MHz, CDCl$_3$, 25 °C) of compound 9
$^{31}$P-NMR (162 MHz, CDCl$_3$, 25 °C) of compound 1
1.10. 1-(2-Iodoethyl)-4-nitrobenzene

\[
\begin{align*}
\text{OH} & \quad \rightarrow \\
\text{I} & \\
\end{align*}
\]

Triphenylphosphane (2.09 g, 7.96 mmol, 1.33 eq) and imidazole (0.542 g, 7.96 mmol, 1.33 eq) were dissolved in anhydrous toluene. A solution of iodine (2.02 g, 7.96 mmol, 1.33 eq) in toluene (20 mL) was added dropwise and the solution was stirred for 30 minutes. 2-(4-Nitrophenyl)-ethanol (1.00 g, 5.98 mmol, 1 eq) in toluene (20 mL) was added and the reaction mixture was stirred for 3 hours at room temperature. Sat. aqu. sodium bicarbonate solution (20 mL) was added and stirring was continued for 10 minutes. The organic phase was separated and stirred with LiI (1.06 g, 7.96 mmol, 1.33 eq) for another 10 minutes. The solution was extracted once with sat. aqu. sodium thiosulfate and twice with water. The triphenylphosphinoxide can then be directly precipitated from the solution with petroleum ether and filtrated. The solvent is evaporated to dryness and the crude product is purified via column chromatography (SiO\textsubscript{2}, 10-15% ethyl acetate in cyclohexane). Yield: 1.40 g of compound 10 as a brown oil (85%). TLC: 0.62 (3/7 ethyl acetate/cyclohexane). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta\) 3.27 - 3.31 (m, 2H, H\textsubscript{2}C), 3.37-3.41 (m, 2H, H\textsubscript{2}C), 7.35-7.38 (m, 2H, HC(ar)), 8.16-8.19 (m, 2H, HC(ar)), ppm. \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): \(\delta\) 3.79 (s, 1C, CH\textsubscript{2}), 39.58 (s, 1C, CH\textsubscript{2}), 124.03 (s, 1C, C(ar)), 129.41 (s, 1C, C(ar)), 147.10 (s, 1C, C(ar)), 147.79 (s, 1C, C(ar)), ppm;
$^1$H-NMR (400 MHz, CDCl$_3$, 25 °C) of compound 10
$^{13}$C-NMR (100 Mhz, CDCl$_3$, 25 °C) of compound 10
### Supporting Table 1. Overview of synthesized RNAs and mass spectrometric analysis.

| Sequence (5' to 3') | nt | m.w. [Da] (calculated) | m.w. [Da] (measured) |
|---------------------|----|------------------------|----------------------|
| Ia                  | 9  | 2932.87                | 2932.97              |
| Ib                  | 9  | 2916.87                | 2916.70              |
| Ic                  | 9  | 2933.85                | 2934.00              |
| Id                  | 9  | 2766.70                | 2766.78              |
| Ie                  | 9  | 2767.69                | 2767.95              |
| If                  | 9  | 2806.73                | 2806.89              |
| Ig                  | 9  | 2790.73                | 2791.02              |
| IIa                 | 10 | 3174.98                | 3174.79              |
| IIb                 | 10 | 3159.97                | 3159.98              |
| IIc                 | 10 | 3175.96                | 3175.89              |
| IIId                | 10 | 3176.95                | 3176.51              |
| IIe                 | 10 | 3175.97                | 3175.99              |
| IIIa                | 15 | 4813.99                | 4814.08              |
| IIIb                | 15 | 4814.97                | 4814.96              |
| IIIc                | 15 | 4830.97                | 4830.72              |
| IIIId               | 15 | 4829.99                | 4830.01              |
| IVa                 | 46 | 14828.01               | 14827.81             |
| IVb                 | 37 | 11809.09               | 11808.61             |
| IVc                 | 83 | 26618.07               | 26617.89             |
| V                   | 37 | 11728.10               | 11728.31             |
## Supporting Table 2. Complete set of thermodynamic data of xanthosine modified RNA.

| No.  | RNA sequences 5' to 3'                         | $T_m$ [°C][a] | $\Delta T_m$ [°C] vs GC/AU | $\Delta G^{298}$ [kcal mol$^{-1}$][b] | $\Delta H^{298}$ [kcal mol$^{-1}$][b] | $\Delta S^*$ [cal mol$^{-1}$ K$^{-1}$][b] |
|------|-----------------------------------------------|---------------|-----------------------------|----------------------------------------|----------------------------------------|---------------------------------------------|
| IaId | GGCGAGGC / GCCUCUGCC                          | 67.7 ± 0.2    | -                          | -17.2 ± 0.3                            | -84.0 ± 2.0                            | -224 ± 6                                    |
| IbIe | GGCAGAGGC / GCCUUUGCC                         | 59.5 ± 0.1    | -8.2                       | -15.4 ± 0.1                            | -84.0 ± 0.7                            | -230 ± 2                                    |
| IcIe | GGCAGAGGC / GCCUGUGCC                         | 45.7 ± 0.4    | -22.0 / -13.8              | -11.4 ± 0.1                            | -71.9 ± 1.0                            | -203 ± 4                                    |
| IcIg | GGCAGAGGC / GCCUGUGCC                         | 45.1 ± 0.3    | -22.6 / -14.4              | -11.3 ± 0.3                            | -72.4 ± 3.6                            | -205 ± 11                                   |
| IcId | GGCAGAGGC / GCCUCUGCC                         | 46.8 ± 0.2    | -20.9 / -12.7              | -12.2 ± 0.2                            | -80.9 ± 2.7                            | -230 ± 8                                    |
| IcIe | GGCAGAGGC / GCCUGUGCC                         | 55.1 ± 0.2    | -12.6 / -4.4               | -14.9 ± 0.2                            | -89.4 ± 1.7                            | -250 ± 5                                    |
| IbIe | GGCAGAGGC / GCCUUUGCC                         | 54.3 ± 0.1    | -13.4 / -5.2               | -14.0 ± 0.3                            | -81.3 ± 2.8                            | -226 ± 9                                    |
| lla pH 7 | GGACCGGUCC (Palindrome)                     | 74.5 ± 0.2    | -                          | -18.7 ± 0.2                            | -83.9 ± 1.9                            | -219 ± 6                                    |
| lla pH 6 |                                               | 73.5 ± 0.1    | -1.0                       | -19.6 ± 0.5                            | -91.9 ± 3.3                            | -243 ± 9                                    |
| lla pH 5 |                                               | 71.3 ± 0.1    | -3.2                       | -20.4 ± 0.3                            | -102.1 ± 1.9                           | -274 ± 5                                    |
| llb pH 7 | GGAUCGAUCC (Palindrome)                     | 59.7 ± 0.3    | -14.8                      | -15.8 ± 0.1                            | -87.0 ± 0.3                            | -239 ± 1                                    |
| llb pH 6 |                                               | 60.9 ± 0.1    | -13.6                      | -16.1 ± 0.1                            | -87.5 ± 0.2                            | -240 ± 1                                    |
| llb pH 5 |                                               | 58.9 ± 0.2    | -15.6                      | -15.6 ± 0.3                            | -86.5 ± 2.5                            | -238 ± 8                                    |
| llc pH 7 | GGACCGXUCC (Palindrome)                     | 42.0 ± 0.2    | -32.5 / -17.7              | -11.6 ± 0.1                            | -89.9 ± 1.7                            | -263 ± 5                                    |
| llc pH 6 |                                               | 48.9 ± 0.3    | -25.6 / -10.9              | -13.5 ± 0.3                            | -91.6 ± 4.4                            | -262 ± 14                                   |
| llc pH 5 |                                               | 51.1 ± 0.4    | -23.4 / -8.6               | -13.5 ± 0.2                            | -83.7 ± 2.9                            | -236 ± 9                                    |
| lld pH 7 | GGAUCGXUCC (Palindrome)                     | 55.5 ± 0.2    | -19.0 / -4.2               | -15.1 ± 0.1                            | -90.7 ± 1.2                            | -254 ± 4                                    |
| lld pH 6 |                                               | 54.5 ± 0.2    | -20.0 / -5.2               | -15.2 ± 0.1                            | -94.1 ± 0.1                            | -264 ± 61                                   |
| lld pH 5 |                                               | 51.2 ± 0.2    | -23.3 / -8.5               | -14.9 ± 0.2                            | -101.6 ± 1.7                           | -291 ± 5                                    |
| lle pH 7 | GGAUCGGUCC (Palindrome)                    | 56.0 ± 0.2    | -18.5 / -3.7               | -15.6 ± 0.1                            | -94.0 ± 0.7                            | -263 ± 2                                    |
| lle pH 6 |                                               | 55.6 ± 0.2    | -18.9 / -4.1               | -15.6 ± 0.5                            | -95.2 ± 4.8                            | -270 ± 11                                   |
| lle pH 5 |                                               | 53.5 ± 0.1    | -21.0 / -6.2               | -15.0 ± 0.3                            | -91.7 ± 1.5                            | -267 ± 69                                   |
| llla | GAAGGGCAACCUCUCC (Hairpin)                | 71.1 ± 0.1    | -                          | -7.8 ± 0.1                             | -57.7 ± 0.3                            | -168 ± 1                                    |
| llib | GAXGGCAACCUCUCC (Hairpin)                  | 39.4 ± 0.1    | -31.7                      | -1.8 ± 0.1                             | -37.2 ± 1.3                            | -120 ± 4                                    |
| llc | GAXGGCAACCUCUCC (Hairpin)                  | 65.8 ± 0.4    | -5.3                       | -6.1 ± 0.1                             | -51.6 ± 1.7                            | -153 ± 5                                    |
| llld | GAGGGCAACCUCUCC (Hairpin)                  | 64.2 ± 0.1    | -6.9                       | -4.9 ± 0.1                             | -43.7 ± 0.7                            | -130 ± 2                                    |

[a] Buffer: 10 mM Na$_2$HPO$_4$, 150 mM NaCl, pH 7.0. $\Delta H$ and $\Delta S$ values were obtained by van’t Hoff analysis or based on RNA concentration dependent measurements according to references 18 and 19. [b] The estimated errors of UV-spectroscopically determined $T_m$ values are ±0.3 °C. [c] Errors for $\Delta H$ and $\Delta S$ were determined from at least three independent measurements; in general, errors arising from noninfinite cooperativity of two-state transitions and from the assumption of a temperature-independent enthalpy, are typically 10–15%. Additional error is introduced when free energies are extrapolated far from melting transitions; errors for $\Delta G$ are typically 3–5%. 

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**Supporting Table 3.** X-ray data collection and crystallographic refinement statistics.

|                         | Xan-2648-SRL | Xan-12-mer | Xan-14-mer |
|-------------------------|--------------|------------|------------|
| **PDB ID**              | 7QSH         | 7QUA       | 7QTN       |
| **Space group**         | P4_3         | C2         | P6_322     |
| a (Å)                   | 29.50        | 41.07      | 44.88      |
| b (Å)                   | 29.50        | 35.15      | 44.88      |
| c (Å)                   | 76.51        | 31.93      | 149.70     |
| β (°)                   | 90           | 129.05     | 90         |
| **Beamline**            | SLS PX III - X06DA | SLS PX III - X06DA | SLS PX III - X06DA |
| **Resolution range (Å)**| 50 – 0.86    | 50 – 1.00  | 50 – 1.20  |
| **Number of frames**    | 9000         | 3600       | 5400       |
| **Oscillation angle**   | 0.2°         | 0.2°       | 0.2°       |
| **Wavelength**          | 0.9          | 0.9        | 1.0        |
| **Average redundancy**  | 55.8         | 11.2       | 65.3       |
| **Completeness**        | 98.9% (86.9%)| 98.8% (81.8%)| 100.0% (100.0%) |
| **CC**_1/2 \(^1\)      | 100% (49.0%) | 100% (24.6%) | 100% (20.2%) |
| **Average I/σ**         | 56.0 (1.4)   | 18.9 (0.9) | 32.3 (0.7) |
| **ISa0**                | 22           | 20         | 22         |
| **R/R**_free \(^1\)    | 14.6 / 16.8  | 13.8 / 15.8 | 18.3 / 20.9 |
| **Coordinate error (Å)**| 0.10         | 0.18       | 0.23       |
| **Wilson B (Å\(^2\))** | 7.9          | 9.3        | 18.8       |
| **Number of molecules** |              |            |            |
| RNA strands             | 1            | 1          | 2          |
| Water                   | 147          | 108        | 122        |
| Glycerol                | 1            | 0          | 0          |
| Sulfate                 | 1            | 0          | 0          |
| Sulfate                 | 1            | 0          | 0          |
| Magnesium               | 0            | 2          | 0          |
| Sodium                  | 0            | 2          | 0          |

\(^1\) Values for last resolution shell are shown in parenthesis
Supporting Figure 1. UV-melting profile analysis of RNA duplexes la/ib and lb/le.
**Supporting Figure 2.** UV-melting profile analysis of RNA duplexes Ic/lf and Ic/lg.
Supporting Figure 3. UV-melting profile analysis of RNA duplexes Ic/Id and Ic/le.
Supporting Figure 4. UV-melting profile analysis of RNA duplex la/le.
Supporting Figure 5. UV-melting profile analysis of RNA duplexes IIa at different pH 7 and pH 6.
Supporting Figure 6. UV-melting profile analysis of RNA duplexes IIa at pH 5 and Ib and pH 7.
Supporting Figure 7. UV-melting profile analysis of RNA duplex Ib at pH 6 and pH 5.
Supporting Figure 8. UV-melting profile analysis of RNA duplex IIc at pH 7 and pH 6.
Supporting Figure 9. UV-melting profile analysis of RNA duplexes IIC at pH 5 and IID at pH 7.
Supporting Figure 10. UV-melting profile analysis of RNA duplex Ild at pH 6 and pH 5.
Supporting Figure 11. UV-melting profile analysis of RNA duplex Ile at pH 7 and pH 6.
Supporting Figure 12. UV-melting profile analysis of RNA duplex Ile at pH 5.
Supporting Figure 13. UV-melting profile analysis of RNA hairpin IIIa at pH 7.
Supporting Figure 14. UV-melting profile analysis of RNA hairpin IIIb at pH 7.
Supporting Figure 15. UV-melting profile analysis of RNA hairpin IIIc at pH 7.
Supporting Figure 16. UV-melting profile analysis of RNA hairpin IIId at pH 7; and overview of melting profiles of IIIa-d (bottom right).
Supporting Figures S17. $^1$H NMR imino proton spectra of 10 nt RNA palindromes llb, lld, lle, and llc, with (A) U-A, (B) U-X, (C) U-G, and (D) C-X base pairs in position 4-7’ and 4’-7. Conditions: c(RNA) = 0.1 mM, 25 mM NaCl, 15 mM sodium phosphate buffer, 10% D$_2$O, pH 6.5.
Supporting Figures S18. NMR spectroscopic analysis of the 10 nt RNA palindrome \textbf{IIC}. (A) RNA sequence and chemical structure C-X wobble pairs in position 4-7' and 4'-7. (B) $^1$H,$^{15}$N-HSQC NMR spectrum at pH 6.5. (C) $^1$H,$^{15}$N-HSQC NMR spectrum at pH 4.5. Conditions: c(RNA) = 1.0 mM, 25 mM NaCl, 15 mM sodium phosphate buffer, 10% D$_2$O, pH and temperature as indicated.
**Supporting Figures S19.** NMR spectroscopic analysis of the 10 nt RNA palindrome IIa. (A) RNA sequence and chemical structure C-G pairs in position 4-7' and 4'-7. (B) $^1$H,$^{15}$N-HSQC NMR spectrum at pH 6.5. c(RNA) = 0.2 mM, 25 mM NaCl, 15 mM sodium phosphate buffer, 10% D$_2$O, temperature as indicated.
Supporting Figures S20. NMR spectroscopic analysis of the 10 nt RNA palindrome IIc. RNA sequence and chemical structure C-X pairs in position 4-7' and 4'-7. $^1$H-NMR spectra at 10°C. c(RNA) = 1.0 mM, 25 mM NaCl, 15 mM sodium phosphate buffer, 10% D$_2$O, pH as indicated.
Supporting Figures S21. Crystal structure of a X-U modified 12 nt RNA palindrome. (A) RNA sequence and secondary structure. (B) Side view on the X5/U8' base pair and U7' highlighting the sodium ion binding site (PDB ID 7QUA).
Supporting Figures S22. NMR spectroscopic analysis of the 12 nt RNA palindrome used in the X-ray study. RNA sequence and potential secondary structures. $^1$H-NMR spectra at pH 6.5. c(RNA) = 0.1 mM, 25 mM NaCl, 15 mM sodium phosphate buffer, 10% D$_2$O, temperatures as indicated.
Supporting Figures S23. NMR spectroscopic analysis of the 14 nt RNA palindrome used in the X-ray study. (A) RNA sequence, potential secondary structures, and chemical structure U-G wobble pairs in position 5'-10’ and 5’-10; $^1$H-NMR spectra at pH 6.5; temperatures as indicated. (B) RNA sequence, potential secondary structures, and chemical structure C-X wobble pairs in position 5'-10’ and 5’-10; $^1$H-NMR spectra at pH 6.5; temperatures as indicated. (C) $^1$H, $^{15}$N-HSQC NMR spectrum of the C-X modified RNA palindrome, at pH 6.5, 10°C. Conditions: c(RNA) = 0.1 mM, 25 mM NaCl, 15 mM sodium phosphate buffer, 10% D$_2$O.