Fluorogenic and bioorthogonal modification of RNA using photoclick chemistry

Katja Krell and Hans-Achim Wagenknecht

Supplementary Information

Institute of Organic Chemistry
Karlsruhe Institute of Technology (KIT)
Fritz-Haber-Weg 6
76131 Karlsruhe, Germany

E-Mail: Wagenknecht@kit.edu

Table of Contents
1. $^1$H/$^{13}$C NMR spectra and MS analyses ................................................................. 2
2. Optical Spectroscopy ........................................................................................................ 25
3. MALDI spectra of RNA strands ........................................................................................ 31
4. Determination of yields ..................................................................................................... 40
5. Calculation of extinction coefficients .............................................................................. 44
6. References ......................................................................................................................... 44
1. $^1$H/$^{13}$C NMR spectra and MS analyses

**Compound 2**

---

**Figure S1.** $^1$H NMR spectrum (400 MHz) of 2. Spectrum contains traces of dichloromethane ($\delta=5.76$ ppm).
Figure S2. $^{13}$C NMR spectrum (101 MHz) of 2. Spectrum contains traces of dichloromethane ($\delta=54.8$ ppm).
**Figure S3.** MS (FAB) analysis of 2.

**Figure S4.** HR-MS (FAB) analysis of 2.
Figure S5. $^1$H NMR spectrum (400 MHz) of 3. Spectrum contains traces of dichloromethane ($\delta=5.76$ ppm).
Figure S6. $^{13}$C NMR spectrum (101 MHz) of 3. Spectrum contains traces of dichloromethane ($\delta$=54.8 ppm).
Figure S7. MS (FAB) analysis of 3.

Figure S8. HR-MS (FAB) analysis of 3.
Figure S9. $^1$H NMR spectrum (400 MHz) of 4. Spectrum contains traces of toluene ($\delta=7.25$ ppm, 7.18 ppm, 2.30 ppm), dichloromethane ($\delta = 5.76$ ppm) and ethyl acetate ($\delta = 1.17$ ppm, 4.03 ppm, 1.99 ppm).
Figure S10. $^{13}$C NMR spectrum (101 MHz) of 4. Spectrum contains traces of toluene ($\delta = 137.4$ ppm, 128.9 ppm, 128.2 ppm, 125.3 ppm, 21.0 ppm), dichloromethane ($\delta = 54.9$ ppm) and ethyl acetate ($\delta=170.3$ ppm, 59.8 ppm, 20.7 ppm, 14.1 ppm).
**Figure S11.** MS (FAB) analysis of 4.

**Figure S12.** HR-MS (FAB) analysis of 4.
Compound 5

Figure S13. $^1$H NMR spectrum (400 MHz) of 5. Spectrum contains traces of dichloromethane ($\delta=5.76$ ppm).
Figure S14. $^{13}$C NMR spectrum (101 MHz) of 5.
Figure S15. MS (FAB) analysis of 5.

Figure S16. HR-MS (FAB) analysis of 5.
Compound 6

Figure S17. $^1$H NMR spectrum (500 MHz) of 6. The spectrum contains traces of methanol ($\delta=4.01$ ppm, 3.16 ppm).
**Figure S18.** $^{13}$C NMR spectrum (126 MHz) of 6. The spectrum contains traces of methanol ($\delta=48.6$ ppm).
Figure S19. MS (FAB) analysis of 6.

Figure S20. HR-MS (FAB) analysis of 6.
Figure S21. $^1$H NMR spectrum (500 MHz) of 7. Spectrum contains traces of dichloromethane ($\delta=5.76$ ppm).
Figure S22. $^{13}$C NMR spectrum (126 MHz) of 7. Spectrum contains traces of dichloromethane ($\delta=54.9$ ppm).
Figure S23. MS (MALDI-TOF) analysis of 7.
Figure S24. $^1$H NMR spectrum (500 MHz) of 8. Spectrum contains traces of dichloromethane ($\delta=5.76$ ppm).
Figure S25. $^{13}$C NMR spectrum (126 MHz) of 8. Spectrum contains traces of dichloromethane ($\delta=54.9$ ppm).
**Figure S26.** MS (FAB) analysis of 8.

**Figure S27.** HR-MS (FAB) analysis of 8.
Compound 9

Figure S28. $^{31}$P NMR spectrum (202 MHz) of 9.
Figure S29. MS (MALDI-TOF) analysis of 9.
2. Optical Spectroscopy

![Graph showing UV/Vis absorbance of RNA1 and RNA2](image)

**Figure S30.** UV/Vis absorbance of RNA1 and RNA2 (2.5 μM) in 10 mM Na-P buffer, 250 mM NaCl, pH 7. The spectra were normalized to evaluate the relative tetrazole absorbances.

![Graph showing UV/Vis absorbance recorded during reaction](image)

**Figure S31.** UV/Vis absorbance recorded during reaction of RNA1 (2.5 μM) with Cy3-maleimide (3.75 μM, 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-P buffer, 250 mM NaCl, pH 7.
**Figure S32.** Fluorescence recorded during reaction of RNA1 (2.5 μM) with Cy3-maleimide (3.75 μM, (1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-Pi buffer, 250 mM NaCl, pH 7. Fluorescence excitation at 358 nm.

**Figure S33.** Fluorescence recorded during reaction of RNA1 (2.5 μM) with AF555-maleimide (3.75 μM, (1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-Pi buffer, 250 mM NaCl, pH 7. Fluorescence excitation at 358 nm.
Figure S34. UV/Vis absorbance recorded during reaction of RNA1 (2.5 μM) with AF647-maleimide (3.75 μM, 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-Pi buffer, 250 mM NaCl, pH 7.

Figure S35. Fluorescence recorded during reaction of RNA1 (2.5 μM) with AF647-maleimide (3.75 μM, 1.50 eq), irradiated at 300 nm (LED) in 10 mM Na-Pi buffer, 250 mM NaCl, pH 7. Fluorescence excitation at 358 nm.
Figure S36. UV/vis absorbance recorded during reaction of RNA2 (2.5 μM) with Cy3-maleimide (3.75 μM, 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-Pi buffer, 250 mM NaCl, pH 7.

Figure S37. Fluorescence recorded during reaction of RNA2 (2.5 μM) with Cy3-maleimide (3.75 μM, 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-Pi buffer, 250 mM NaCl, pH 7. Fluorescence excitation at 358 nm.
Figure S38. UV/Vis absorbance recorded during reaction of RNA2 (2.5 μM) with AF555-maleimide (3.75 μM, 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-Pi buffer, 250 mM NaCl, pH 7.

Figure S39. Fluorescence recorded during reaction of RNA2 (2.5 μM) with AF555-maleimide (3.75 μM, 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-Pi buffer, 250 mM NaCl, pH 7. Fluorescence excitation at 358 nm.
Figure S40. UV/Vis absorbance recorded during reaction of RNA2 (2.5 μM) with AF647-maleimide (3.75 μM, 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-Pi buffer, 250 mM NaCl, pH 7.

Figure S41. Fluorescence recorded during reaction of RNA2 (2.5 μM) with AF647-maleimide (3.75 μM, 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-Pi buffer, 250 mM NaCl at pH 7. Fluorescence excitation at 358 nm.
3. MALDI spectra of RNA strands

**RNA1**

*Figure S42.* MS (MALDI-TOF) analysis of RNA1. Calculated mass [M⁺]: 5544.6; m/z=5546.99 [M⁺], 5584.98 [M+K⁺].
Figure S43. MS (MALDI-TOF) analysis of RNA1-Cy3 adduct. Calculated mass [M⁺]: 6253.9; m/z=5523.65 [RNA1-N₂⁺], 5541.82 [RNA1-N₂+H₂O⁺], 6261.44 [M⁺].
Figure S44. MS (MALDI-TOF) analysis of RNA1-AF555 adduct. Calculated mass: 6485.9 [M$^+$]; m/z=5518.14 [RNA1-N$_2$$^+$], 5536.19 [RNA1-N$_2$+H$_2$O$^+$], 6486.22 [M$^+$]. The molecular mass of AF555-maleimide was reported in literature and verified by MS (MALDI-TOF) analysis.$^1$
Figure S45. Zoomed area of MS (MALDI-TOF) analysis (Figure S44) of RNA1-AF555 adduct.
**Figure S46.** MS (MALDI-TOF) analysis of RNA1-AF647 adduct. Calculated Mass [M⁺]: 6497.9; m/z=5519.49 [RNA1-N₂⁺], 5537.50 [RNA1-N₂+H₂O⁺], 6499.90 [M⁺]. The molecular mass of AF647-maleimide was reported in literature and verified by MS (MALDI-TOF) analysis.¹
Figure S47. Zoomed area of MS (MALDI-TOF) analysis (Figure S46) of RNA1-AF647 adduct.
Figure S48. MS (MALDI-TOF) analysis of RNA2. Calculated mass [M⁺]: 5544.6; m/z= 5546.50 [M⁺], 5584.48 [M+K⁺], 5622.49 [M+2K⁺], 5659.92 [M+3K⁺].
Figure S49. MS (MALDI-TOF) analysis of RNA2-Cy3 adduct. Calculated mass [M⁺]: 6253.9; m/z=5516.65 [RNA2-N₂⁺], 5534.67 [RNA2-N₂+H₂O⁺], 6254.67 [M⁺].

Figure S50. MS (MALDI-TOF) analysis of RNA2-AF555 adduct. Calculated Mass [M⁺]: 6485.9; m/z=5519.45 [RNA2-N₂⁺], 5537.50 [RNA2-N₂+H₂O⁺], 6485.06 [M⁺]. The molecular mass AF555-maleimide was reported in literature and verified by MS (MALDI-TOF) analysis.¹
Figure S51. Zoomed area of MS (MALDI-TOF) analysis (Figure S50) of RNA2-AF555 adduct.
Figure S52. MS (MALDI-TOF) analysis of RNA2-AF647 adduct. Calculated mass [M⁺]: 6497.9; m/z=5519.01 [RNA2-N₂⁺], 5537.02 [RNA2-N₂+H₂O⁺], 6499.74 [M⁺]. The molecular mass AF647-maleimide was reported in literature and verified by MS (MALDI-TOF) analysis.¹

Figure S53. Zoomed area of MS (MALDI-TOF) analysis (Figure S53) of RNA2-AF647 adduct.

4. Determination of yields

A solution of RNA (2.5 µM) and of the dye (3.75 µM) in 10 mM Na-Pi buffer, 250 mM NaCl, pH 7, with a total volume of 500 µL was irradiated at 300 nm (LED) in a 10 mm quartz glass cuvette for 30 minutes. To remove the excess dye, the solution was purified via illustra™ NAP-5 columns (GE Healthcare) using the standard protocol. The eluted sample was lyophilized and redissolved in water (500 µL). The concentration was calculated spectrophotometrically by Lambert-Beer-Law using the extinction coefficient provided by the manufacturers of the clicked dye: ε₅₄₈ (Cy3) = 162 000 L mol⁻¹ cm⁻¹ (Lumiprobe); ε₅₅₅ (AF555) = 158 000 L mol⁻¹ cm⁻¹ (JenaBioscience); ε₆₄₈ (AF647) = 270 000 L mol⁻¹ cm⁻¹ (JenaBioscience).
**Figure S54.** UV/vis absorbance of “photoclicked” RNA1 dye adducts (reaction with 1.50 equiv. dye-maleimide) strands after purification. \( c_{\text{AF647}} = 1.19 \, \mu \text{M} \equiv 48\% \text{ yield}, \ c_{\text{AF555}} = 1.96 \, \mu \text{M} \equiv 78\% \text{ yield}, \ c_{\text{Cy3}} = 0.67 \, \mu \text{M} \equiv 27\% \text{ yield}.

**Figure S55.** Fluorescence of “photoclicked” RNA1 dye adducts (reaction with 1.50 equiv. dye-maleimide) after purification. \( c_{\text{AF647}} = 1.19 \, \mu \text{M}, \ c_{\text{AF555}} = 1.96 \, \mu \text{M}, \ c_{\text{Cy3}} = 0.67 \, \mu \text{M} \).
Figure S56. UV/vis absorbance of “photoclicked” RNA2 dye adducts (reaction with 1.50 equiv. dye-maleimide) after purification. $c_{AF647}$=1.20 $\mu$M $\pm$ 48% yield, $c_{AF555}$=2.10 $\mu$M $\pm$ 84% yield, $c_{Cy3}$=0.77 $\mu$M $\pm$ 31% yield.

Figure S57. Fluorescence of “photoclicked” RNA2 dye adducts (after reaction with 1.50 equiv. dye-maleimide) after purification. $c_{AF647}$=1.20 $\mu$M, $c_{AF555}$=2.10 $\mu$M, $c_{Cy3}$=0.77 $\mu$M.
Figure S58. UV/vis absorbance of “photoclicked” RNA1-Cy3 adduct (reaction with 10.0 equiv. Cy3-maleimide) after purification. $c_{\text{Cy3}}=1.76 \, \mu M \pm 70\%$ yield.

Figure S59. Fluorescence spectrum of “photoclicked” RNA2-Cy3 adduct (reaction with 10.0 equiv. Cy3-maleimide) after purification. $c_{\text{Cy3}}=1.76 \, \mu M$. 
5. Calculation of extinction coefficients

![Graph showing absorbance vs. wavelength for adenosine, cytidine, guanosine, uridine, and an artificial nucleoside 6.]

**Figure S60.** UV/vis absorbance of A, C, G, U and 6 in comparison.

The molar extinction coefficient $\varepsilon_{300}$ were calculated for the natural nucleosides and the artificial building block 6 using the $\varepsilon_{260}$ values and the recorded UV/vis absorbances (Figure S60) using the Lambert-Beer-Law.

**Table S1.** Molar extinction coefficients of the natural bases and the artificial nucleoside 6.

| nucleoside   | $\varepsilon_{260}$ [L mol$^{-1}$ cm$^{-1}$] | concentration [µmol L$^{-1}$] | $\varepsilon_{300}$ [L mol$^{-1}$ cm$^{-1}$] |
|--------------|---------------------------------|-----------------|------------------|
| Adenosine    | 15,400                          | 52.6            | $\approx$60      |
| Cytidine     | 7,400                           | 70.9            | $\approx$260     |
| Guanosine    | 11 500                          | 42.8            | $\approx$110     |
| Uridine      | 8,700                           | 42.9            | $\approx$90      |
| 6            | 13,800                          | 18.4            | 20,300           |

6. References

1. Tridgett, M.; Moore-Kelly, C.; Duprey, J.-L. H. A.; Iturbe, L. O.; Tsang, Chi W.; Little, H. A.; Sandhu, S. K.; Hicks, M. R.; Dafforn, T. R.; Rodger, A., Linear dichroism of visible-region chromophores using M13 bacteriophage as an alignment scaffold. *RSC Adv.* **2018**, *8* (52), 29535-29543.