

Fluorogenic and bioorthogonal modification of RNA using photoclick chemistry

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Supplementary Information

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1. $^1\text{H}/^{13}\text{C}$ NMR spectra and MS analyses

Compound 2

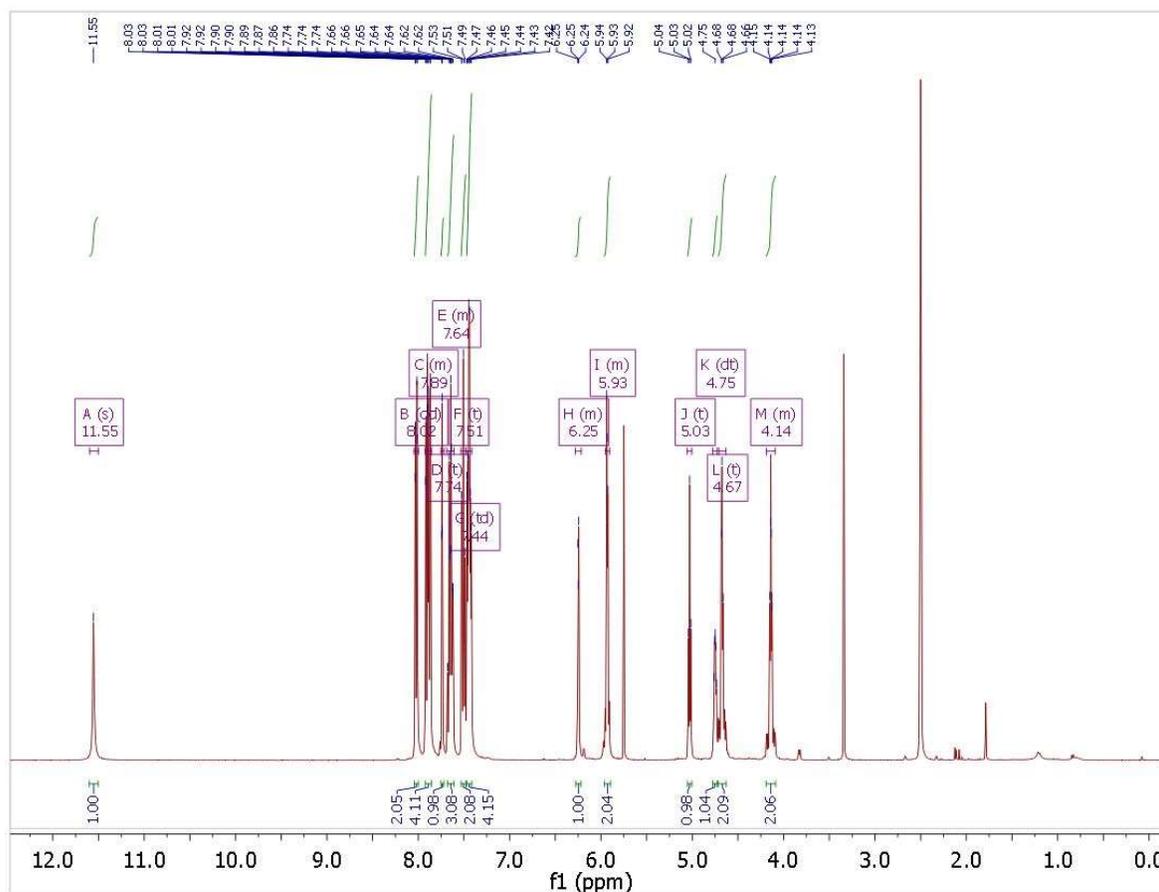


Figure S1. ^1H NMR spectrum (400 MHz) of **2**. Spectrum contains traces of dichloromethane ($\delta=5.76$ ppm).

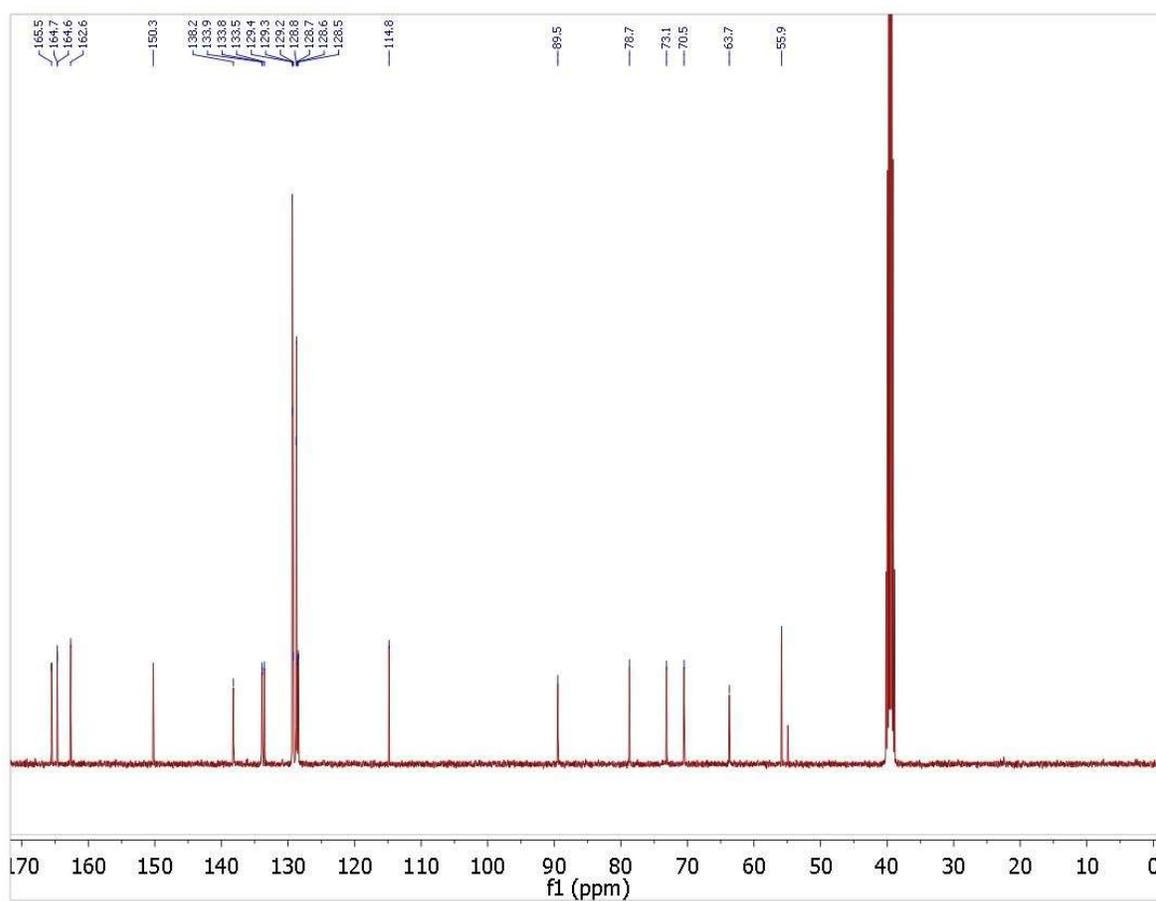


Figure S2. ^{13}C NMR spectrum (101 MHz) of **2**. Spectrum contains traces of dichloromethane ($\delta=54.8$ ppm).

MAT 95,+FAB 1/30/2020 10:47:03 AM KK-148,3-NBA
 moe K. Krell AK Wagenknecht
 kk-148 #22-28 RT: 4.58-5.15 AV: 7 NL: 8.51E4
 T: + c EI Full ms [84.50-1100.50]

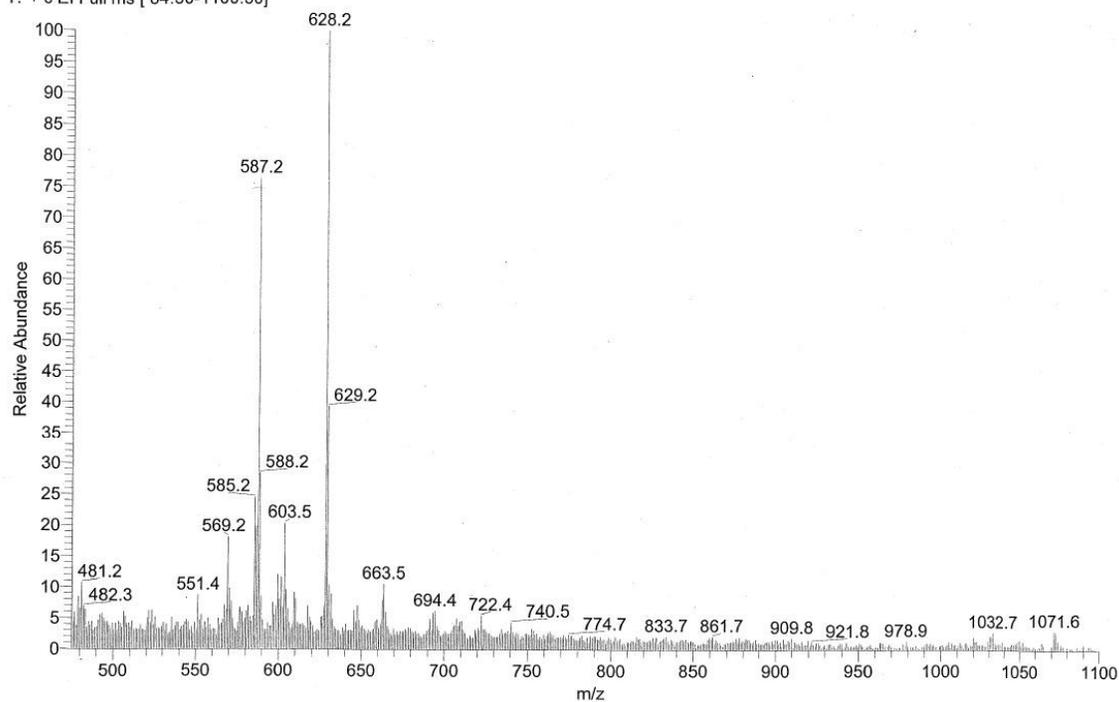


Figure S3. MS (FAB) analysis of **2**.

1/30/2020 10:56:44 AM File recalibrated by CMass.

kk-148-c4#26 RT: 4.96
 T: + c EI Full ms [84.50-1100.50]
 m/z= 587.0035-587.4138

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
587.1667	66998.0	100.00	587.1666	0.18	C ₃₁ H ₂₇ O ₁₀ N ₂

Figure S4. HR-MS (FAB) analysis of **2**.

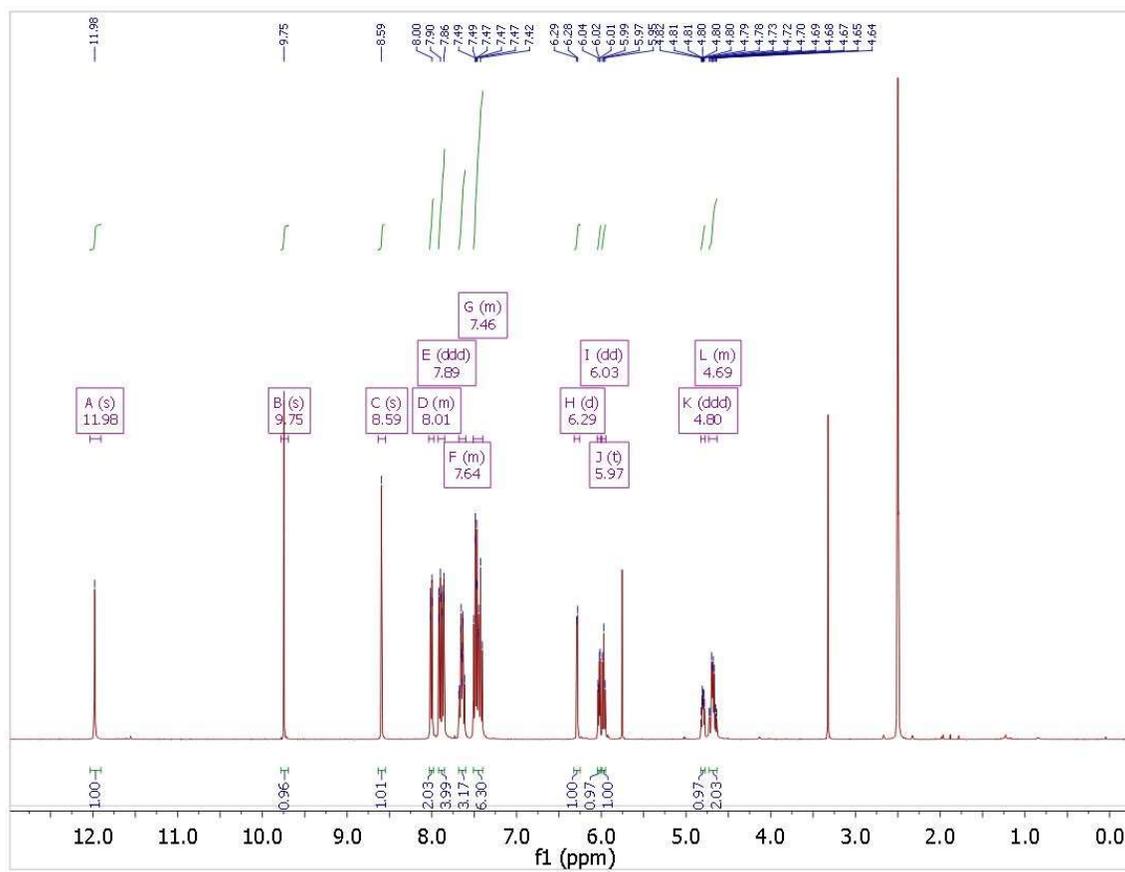
Compound 3


Figure S5. ^1H NMR spectrum (400 MHz) of **3**. Spectrum contains traces of dichloromethane ($\delta=5.76$ ppm).

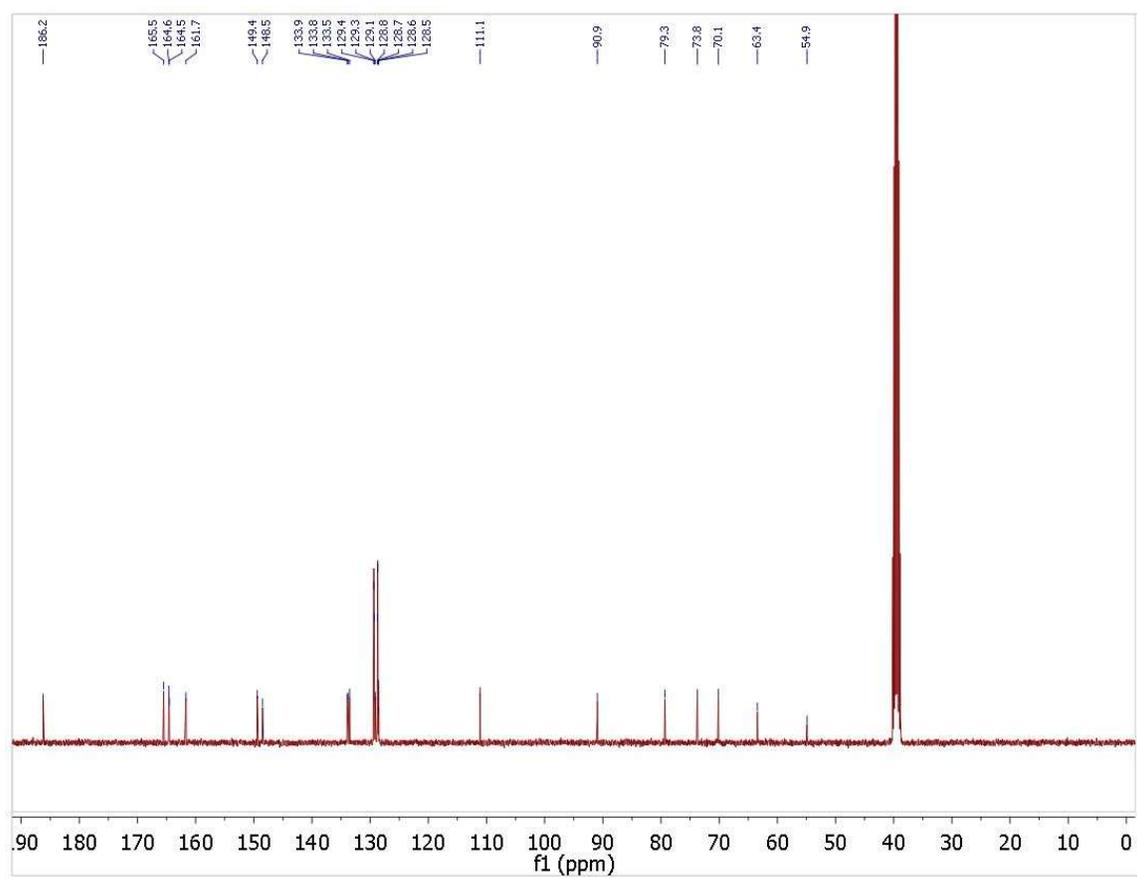


Figure S6. ^{13}C NMR spectrum (101 MHz) of **3**. Spectrum contains traces of dichloromethane ($\delta=54.8$ ppm).

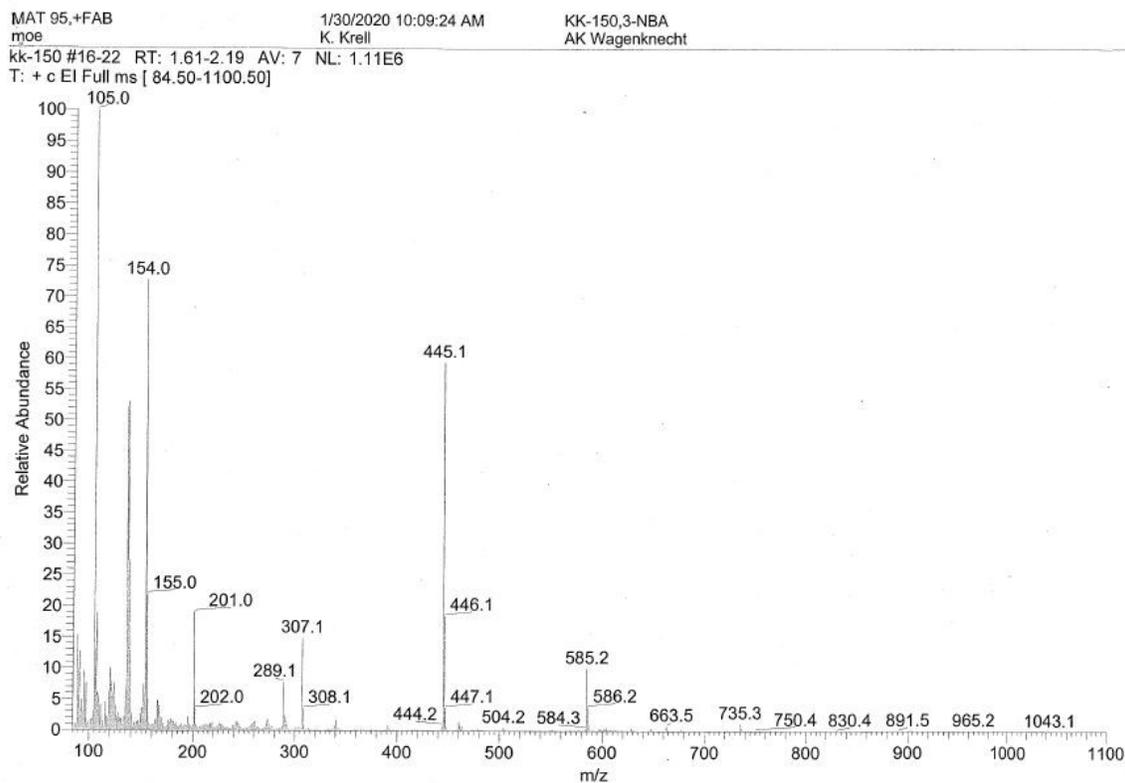


Figure S7. MS (FAB) analysis of **3**.

1/30/2020 10:19:33 AM File recalibrated by CMass.

kk-150-c1#14 RT: 1.42
T: + c EI Full ms [84.48-1100.48]
m/z= 585.1000-585.2084

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
585.1508	86929.0	100.00	585.1509	-0.16	C ₃₁ H ₂₅ O ₁₀ N ₂

Figure S8. HR-MS (FAB) analysis of **3**.

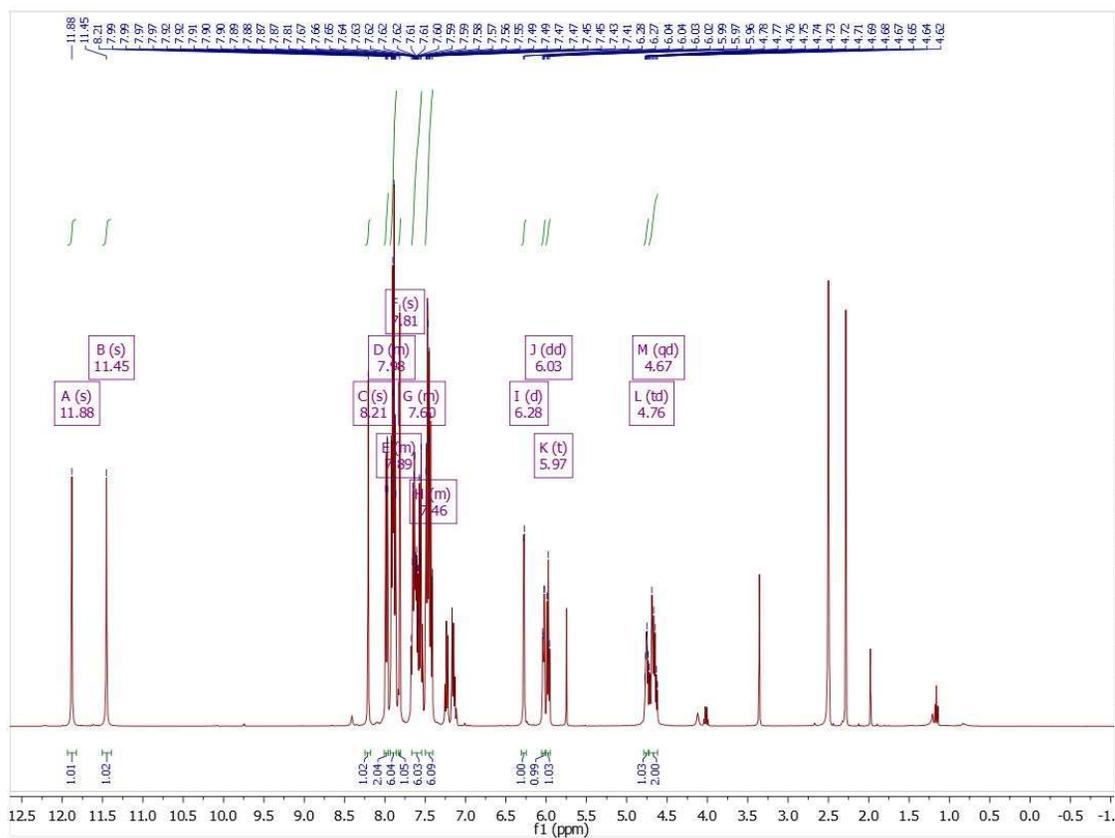
Compound 4


Figure S9. ^1H 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).

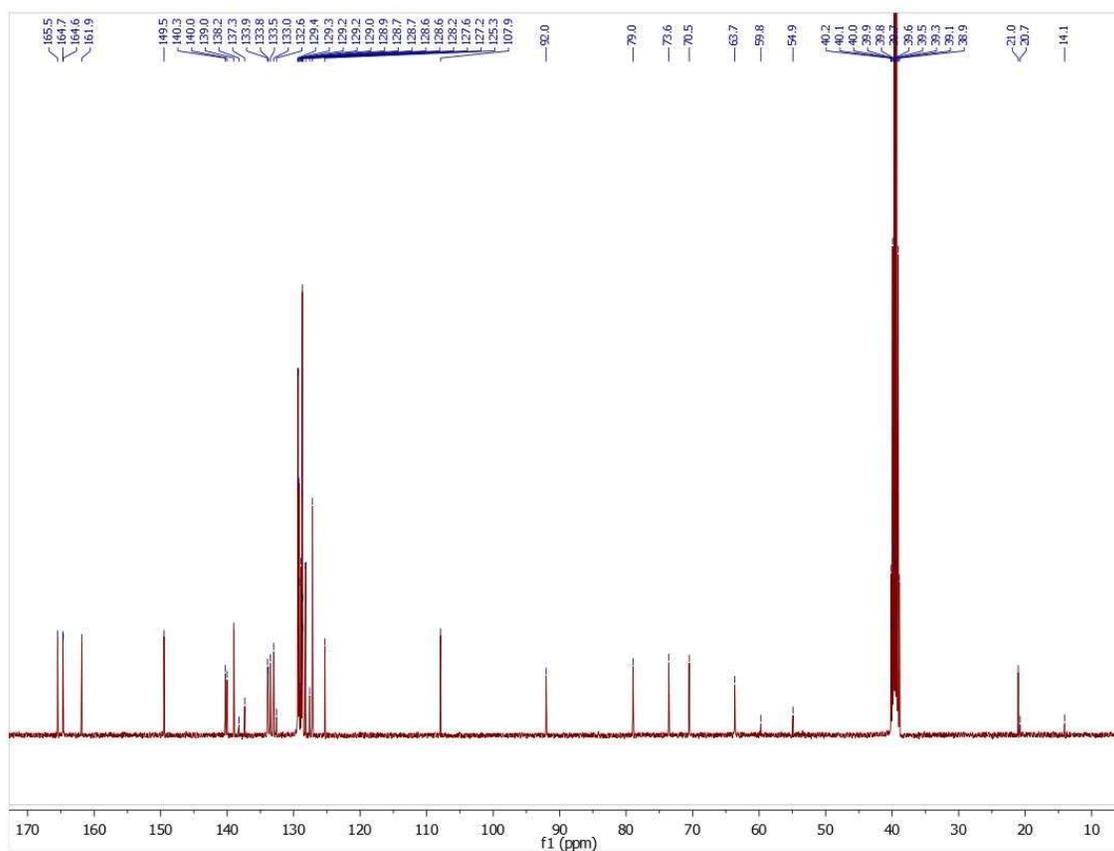


Figure S10. ^{13}C NMR spectrum (101 MHz) of **4**. Spectrum contains traces of toluene ($\delta = 137.4$ ppm, 128.9 ppm, 128.2 ppm, 125.3 ppm, 21.0 ppm), dichloromethane ($\delta = 54.9$ ppm) and ethyl acetate ($\delta = 170.3$ ppm, 59.8 ppm, 20.7 ppm, 14.1 ppm).

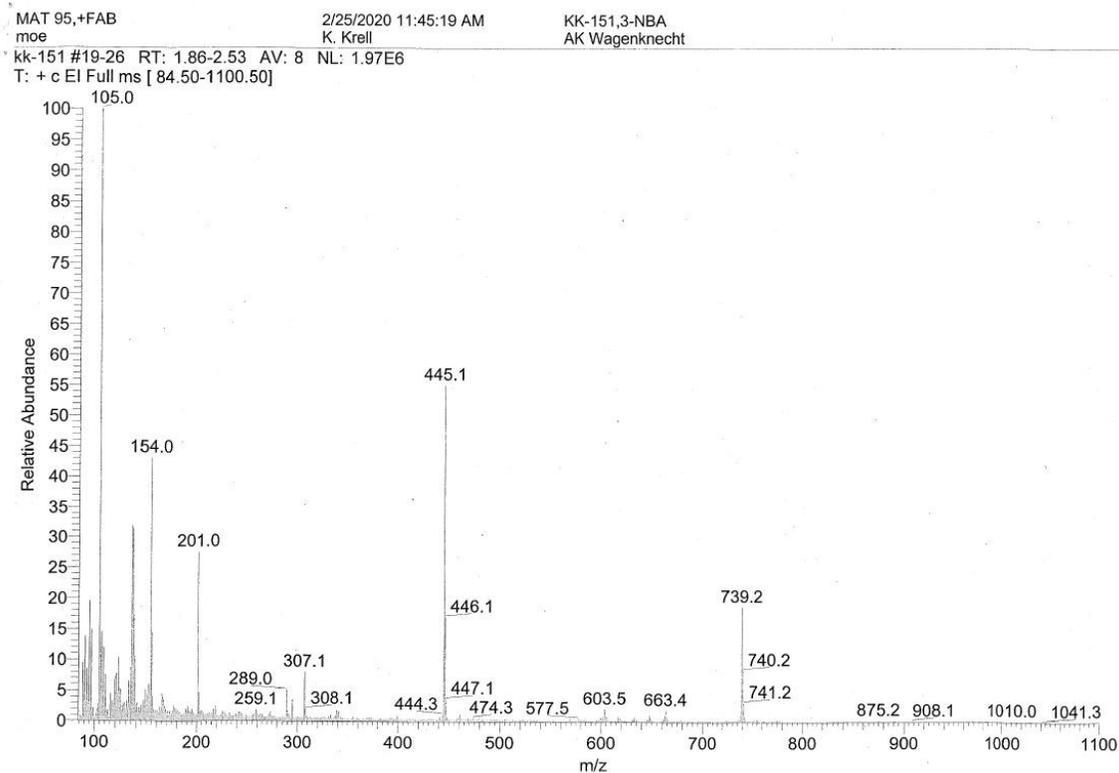


Figure S11. MS (FAB) analysis of **4**.

2/25/2020 11:54:52 AM File recalibrated by CMass.

kk-151-c5#27 RT: 2.63
T: + c EI Full ms [84.48-1100.47]
m/z= 738.8030-739.8474

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
739.1709	425007.0	100.00	739.1710	-0.07	C ₃₇ H ₃₁ O ₁₁ N ₄ ³² S ₁

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

Compound 5

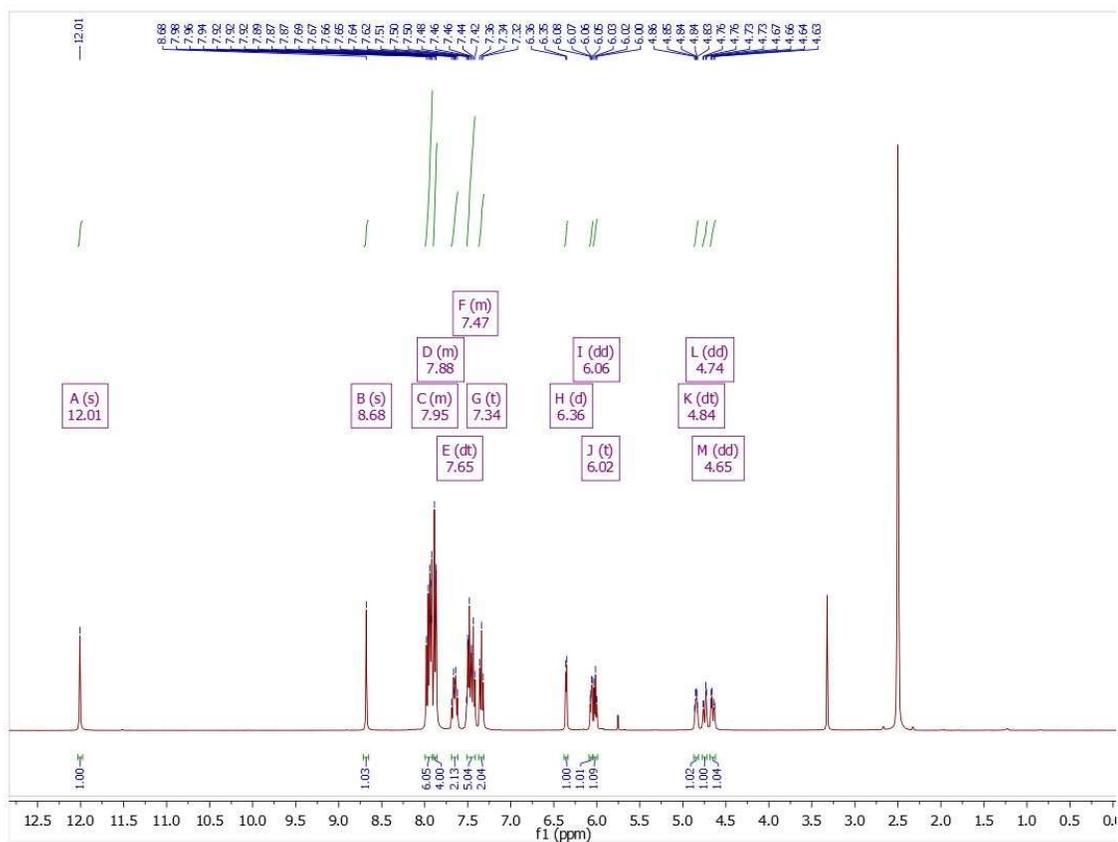


Figure S13. ^1H NMR spectrum (400 MHz) of **5**. Spectrum contains traces of dichloromethane ($\delta=5.76$ ppm).

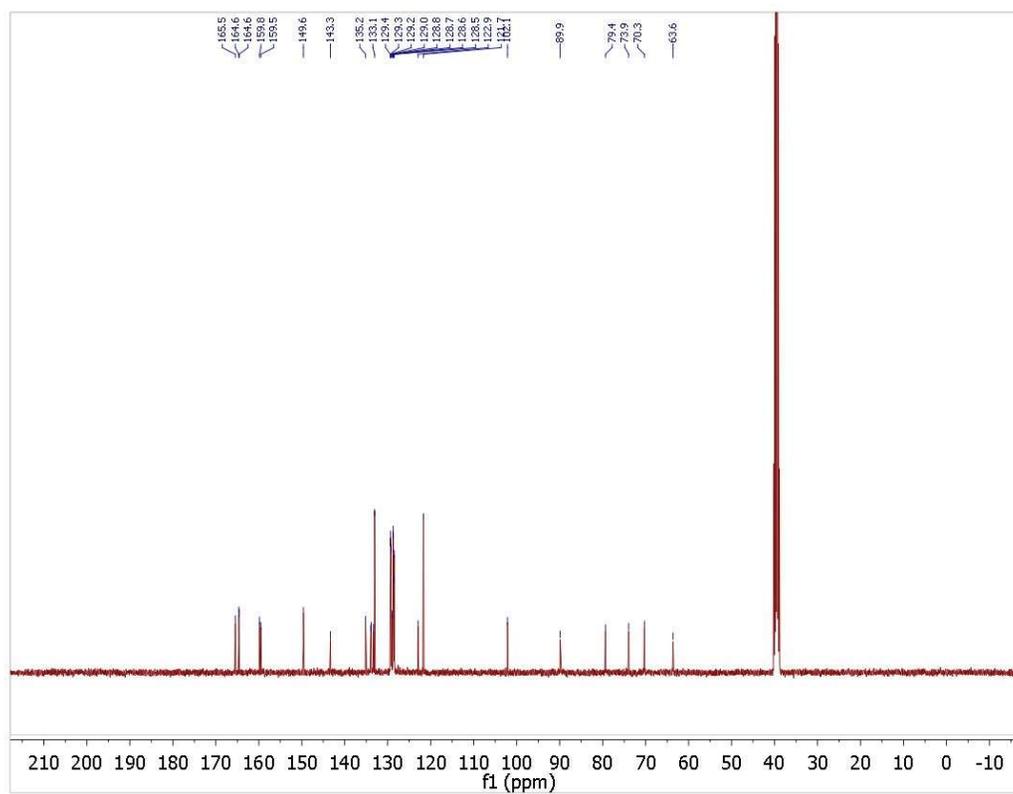


Figure S14. ^{13}C NMR spectrum (101 MHz) of **5**.

MAT 95,+FAB 1/30/2020 10:28:27 AM KK-152,3-NBA
 moe K. Krell AK Wagenknecht
 kk-152 #11-18 RT: 1.14-1.81 AV: 8 NL: 3.60E4
 T: + c EI Full ms [84.50-1100.50]

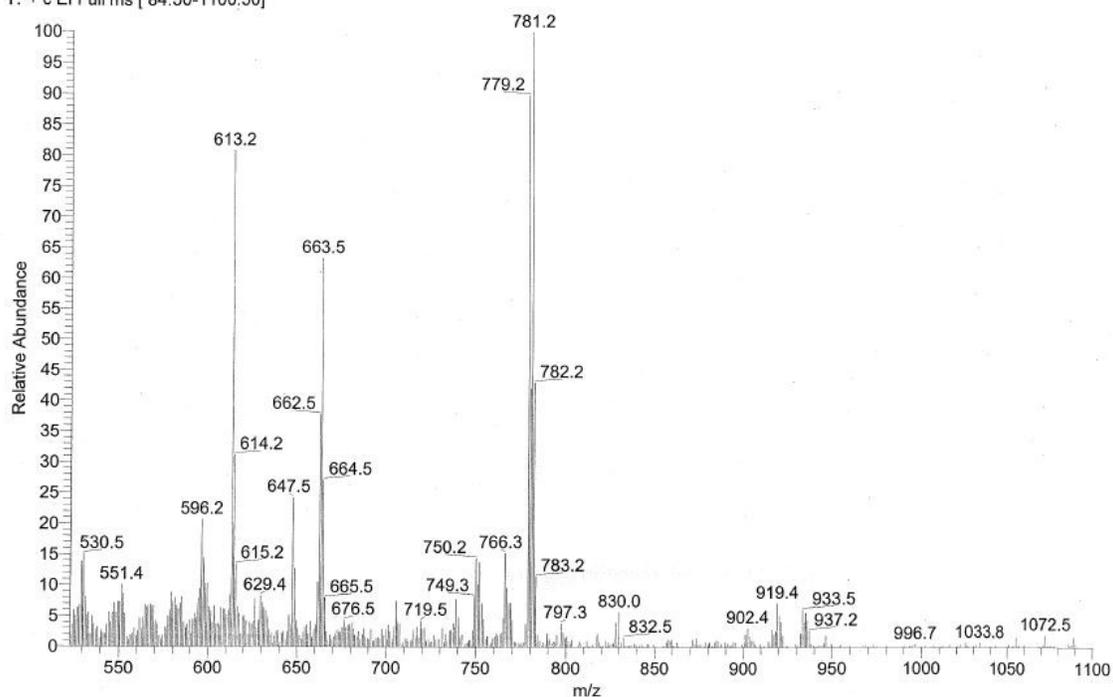


Figure S15. MS (FAB) analysis of **5**.

1/30/2020 10:41:36 AM File recalibrated by CMass.

kk-152-c9#10 RT: 1.04
 T: + c EI Full ms [84.42-1100.42]
 m/z= 778.9113-779.4618

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
779.1102	26609.0	100.00	779.1101	0.07	C ₃₇ H ₂₈ O ₉ N ₆ ⁷⁹ Br ₁

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

Compound 6

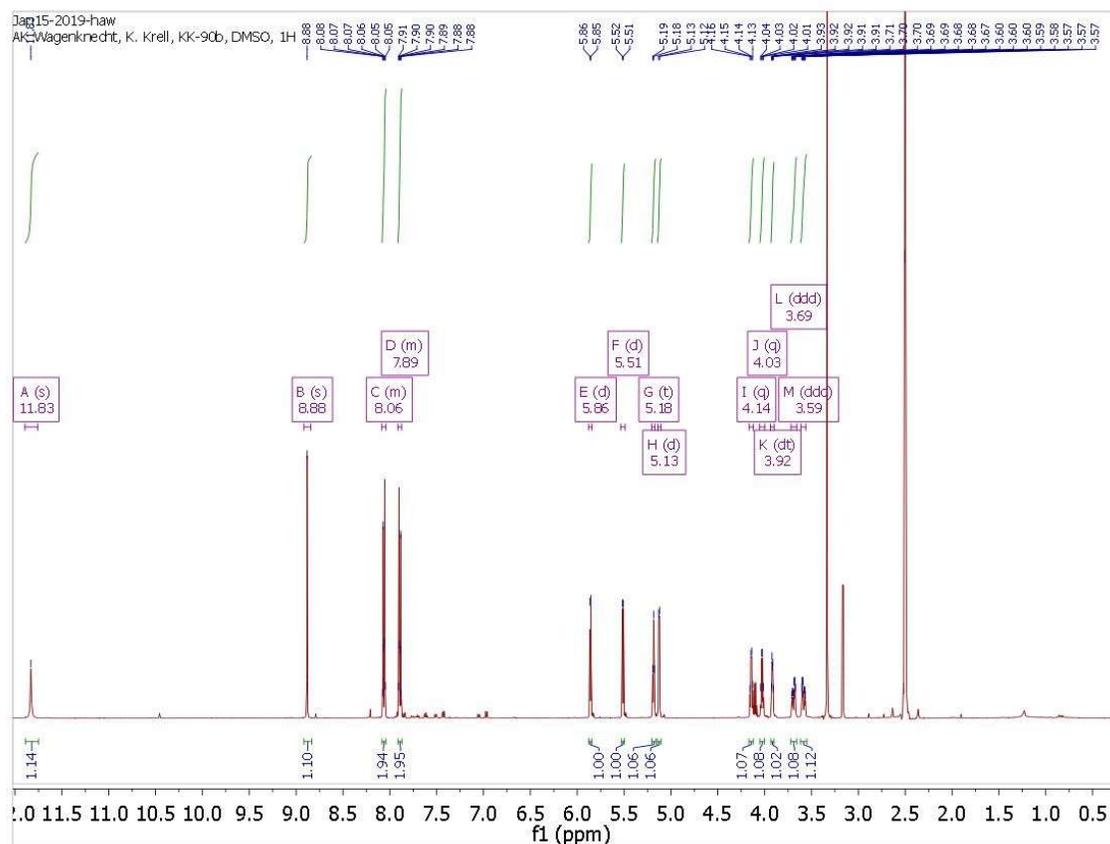


Figure S17. ^1H NMR spectrum (500 MHz) of **6**. The spectrum contains traces of methanol ($\delta=4.01$ ppm, 3.16 ppm).

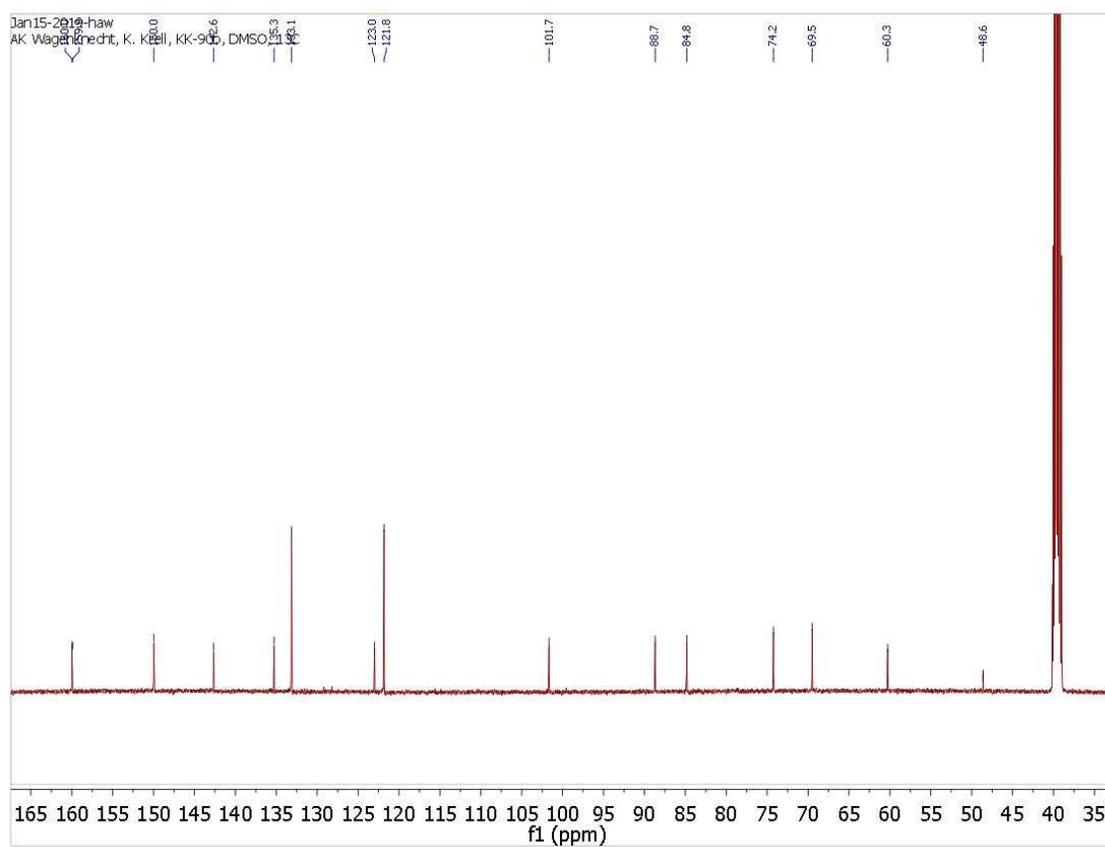


Figure S18. ^{13}C NMR spectrum (126 MHz) of **6**. The spectrum contains traces of methanol ($\delta=48.6$ ppm).

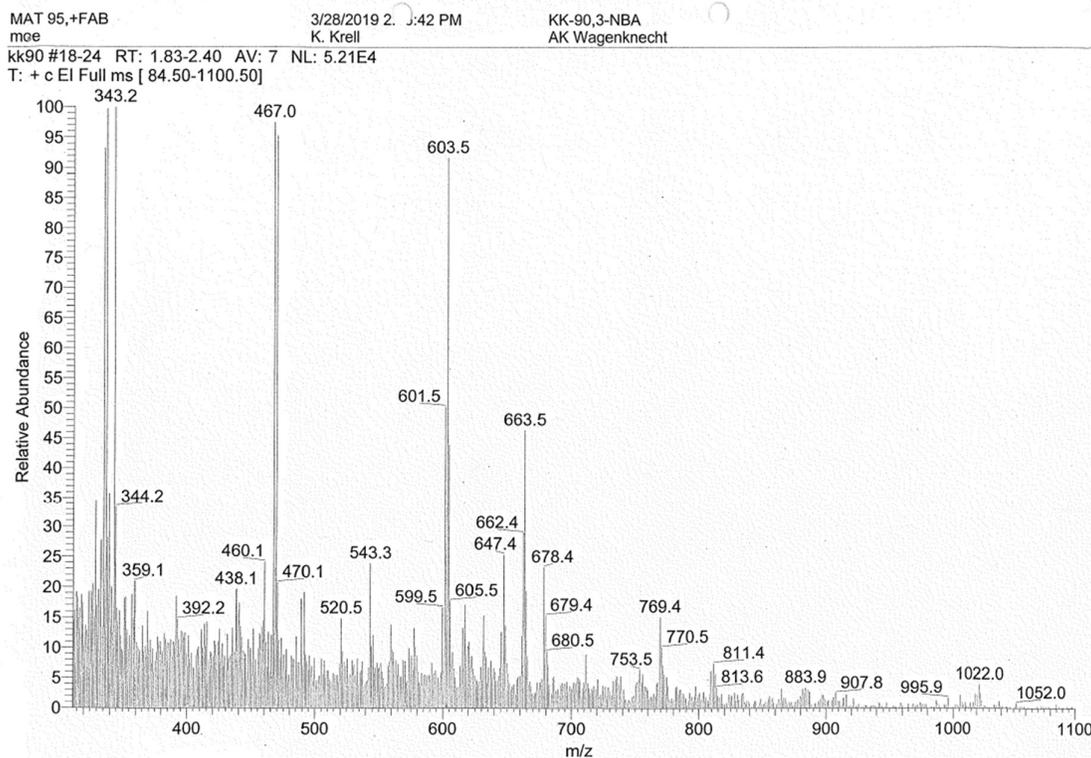


Figure S19. MS (FAB) analysis of **6**.

3/28/2019 2:31:16 PM File recalibrated by CMass.

kk90-c2#25 RT: 2.50
T: + c EI Full ms [84.52-1100.52]
m/z= 466.9708-467.0833

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
467.0314	53842.0	100.00	467.0315	-0.04	C ₁₆ H ₁₆ O ₆ N ₆ ⁷⁹ Br ₁

Figure S20. HR-MS (FAB) analysis of **6**.

Compound 7

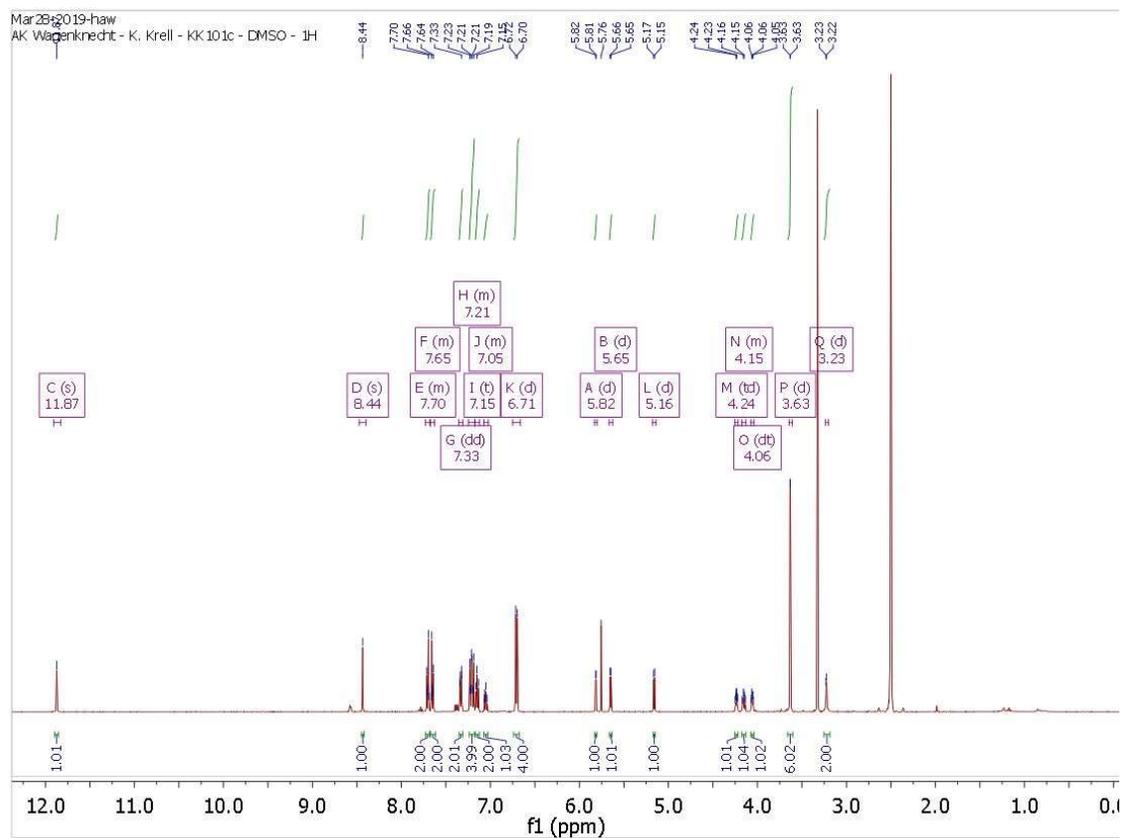


Figure S21. ^1H NMR spectrum (500 MHz) of **7**. Spectrum contains traces of dichloromethane ($\delta=5.76\text{ppm}$).

Confidence

Data: KK-1010001.J7[c] 10 Jan 2020 13:02 Cal: pepmix_refpos102018 4 Oct 2018 14:05

Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode 2019_Reflectron_new, Power: 90, Blanked, P.Ext. @ 550 (bin 59)

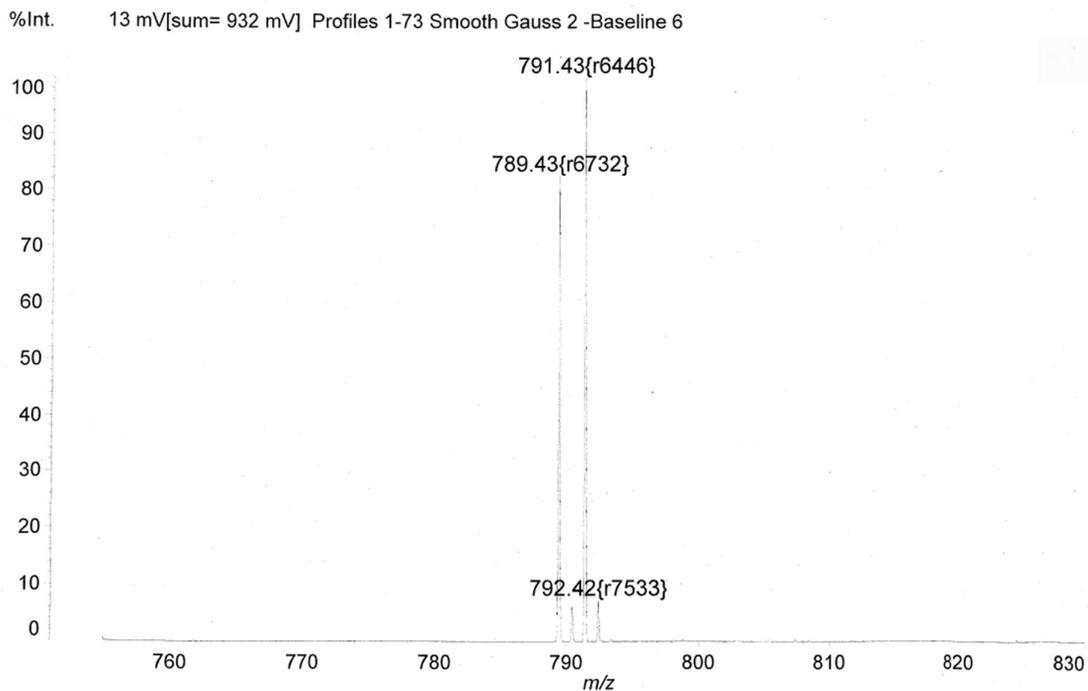


Figure S23. MS (MALDI-TOF) analysis of **7**.

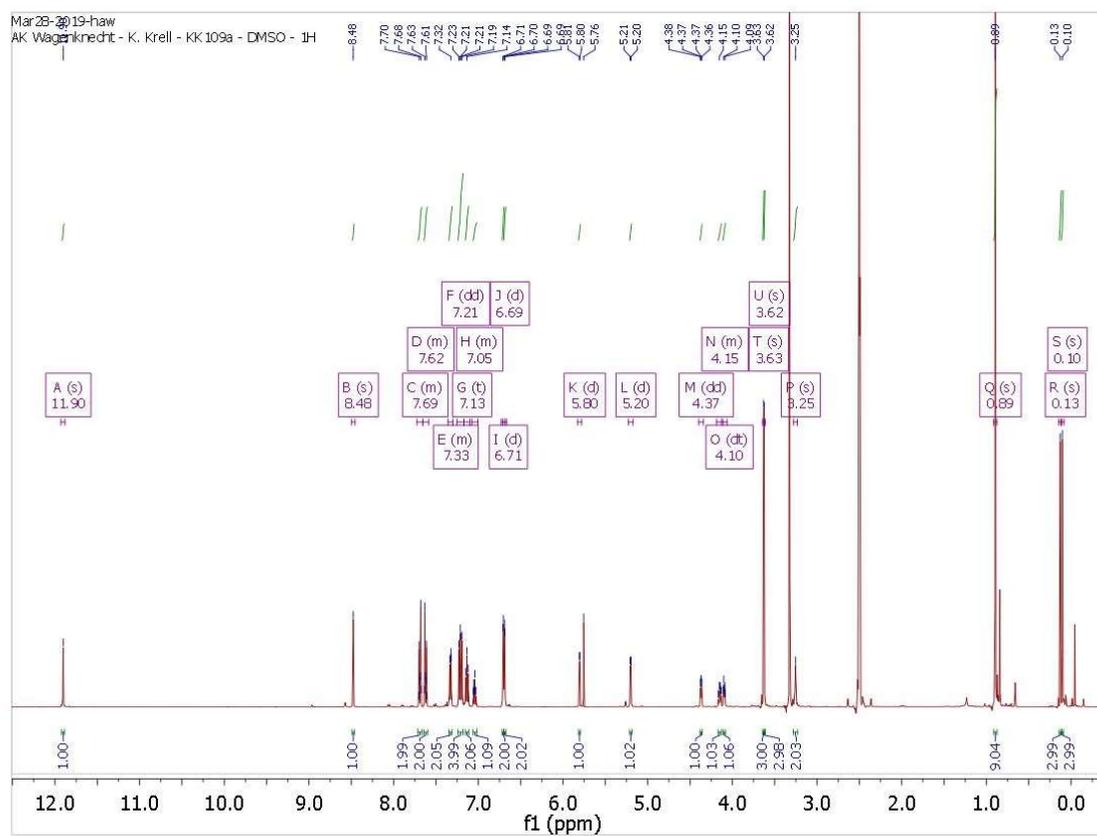
Compound 8


Figure S24. ^1H NMR spectrum (500 MHz) of **8**. Spectrum contains traces of dichloromethane ($\delta=5.76$ ppm).

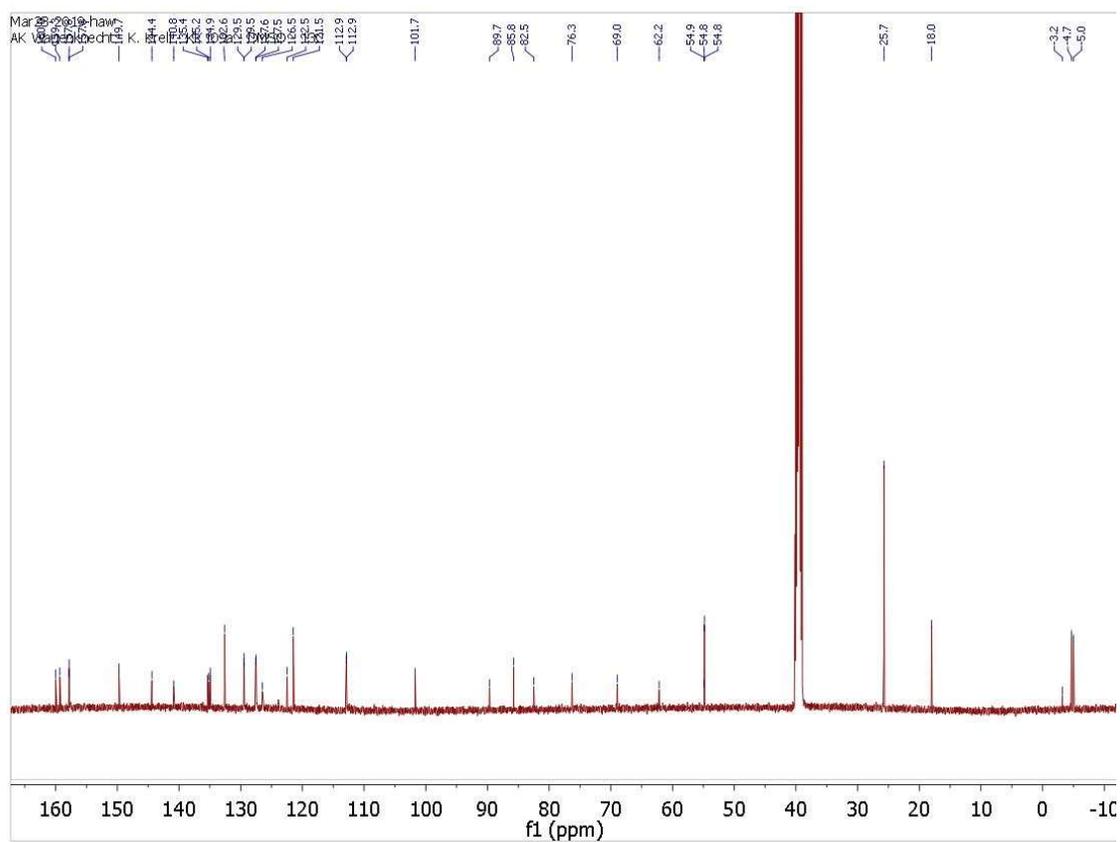


Figure S25. ^{13}C NMR spectrum (126 MHz) of **8**. Spectrum contains traces of dichloromethane ($\delta=54.9$ ppm).

MAT 95,+FAB 3/27/2019 4:48 PM KK-109,3-NBA
 moe K. Krell AK Wagenknecht
 kk109#14-23 RT: 1.37-2.24 AV: 10 NL: 6.11E4
 T: + c EI Full ms [84.50-1100.50]

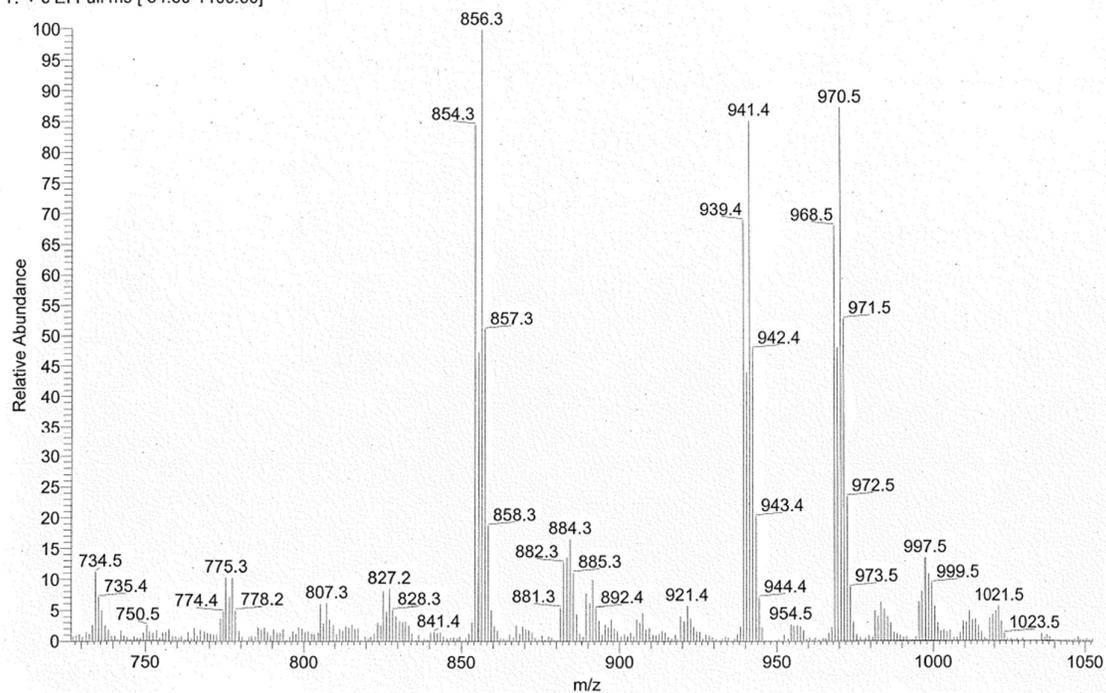


Figure S26. MS (FAB) analysis of **8**.

3/27/2019 4:29:11 PM File recalibrated by CMass.

kk109-c1#15 RT: 1.47
 T: + c EI Full ms [84.42-1100.42]
 m/z= 881.9756-882.6086

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
882.2411	6889.0	100.00	882.2408	0.30	C ₄₃ H ₄₇ O ₈ N ₆ ⁷⁹ Br ₁ ²⁸ Si ₁

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

Compound 9

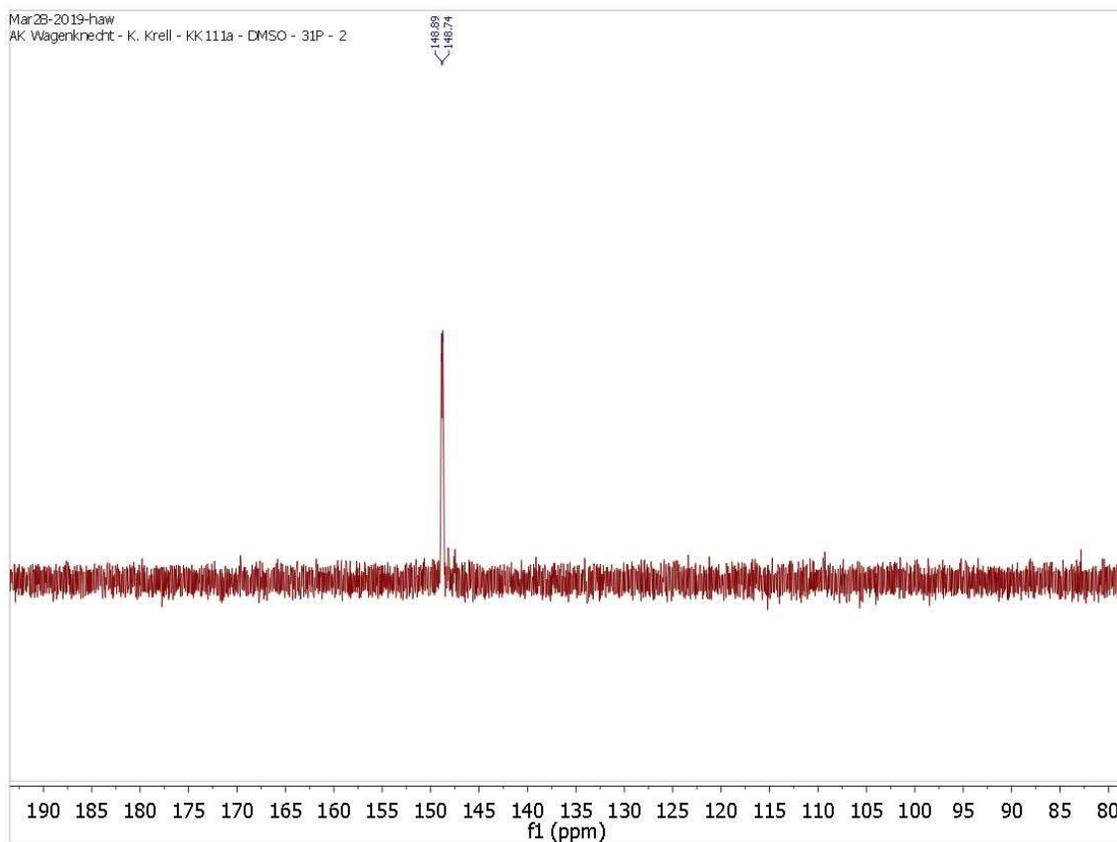


Figure S28. ³¹P NMR spectrum (202 MHz) of **9**.

Confidence

Data: KK_111_ATT_0002.D13[c] 28 Mar 2019 8:44 Cal: small_Molc_07032019 ; Mar 2019 11:27

Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode Reflectron_new, Power: 95, Blanked, P.Ext. @ 800 (bin 65)

%Int. 1.4 mV[sum= 184 mV] Profiles 1-128 Smooth Gauss 5 -Baseline 15

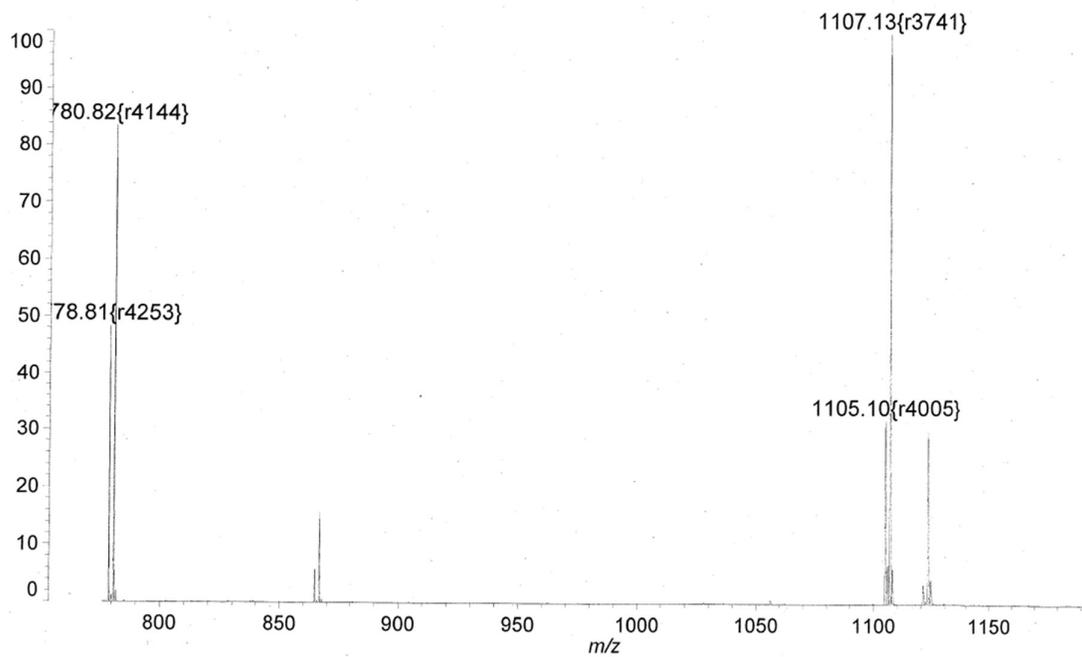


Figure S29. MS (MALDI-TOF) analysis of **9**.

2. Optical Spectroscopy

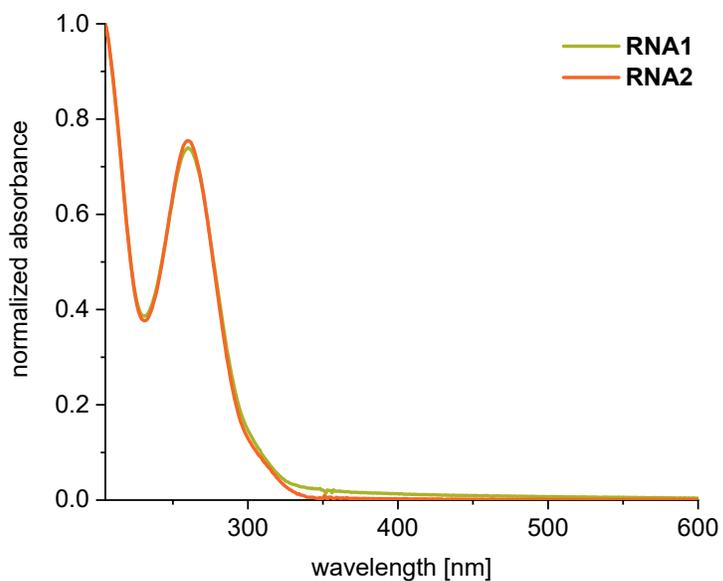


Figure S30. UV/Vis absorbance of **RNA1** and **RNA2** (2.5 μM) in 10 mM Na-P_i buffer, 250 mM NaCl, pH 7. The spectra were normalized to evaluate the relative tetrazole absorbances.

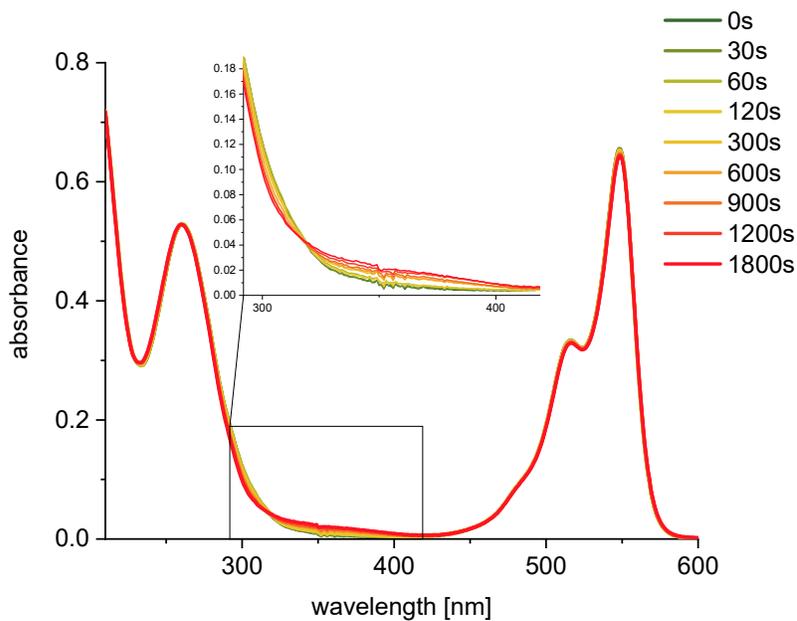


Figure S31. UV/Vis absorbance recorded during reaction of **RNA1** (2.5 μM) with Cy3-maleimide (3.75 μM , 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-P_i buffer, 250 mM NaCl, pH 7.

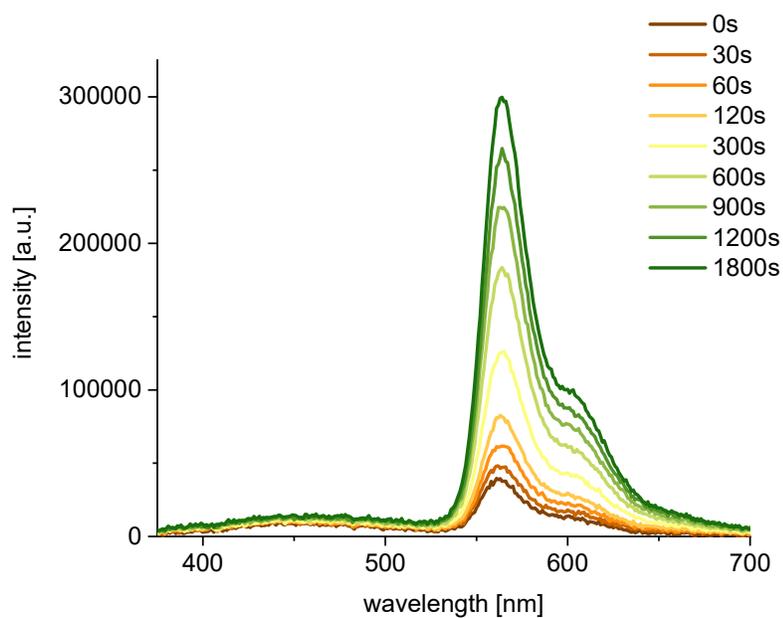


Figure S32. Fluorescence recorded during reaction of **RNA1** (2.5 μM) with Cy3-maleimide (3.75 μM , (1.50 equiv.)), irradiated at 300 nm (LED) in 10 mM Na- P_i buffer, 250 mM NaCl, pH 7. Fluorescence excitation at 358 nm.

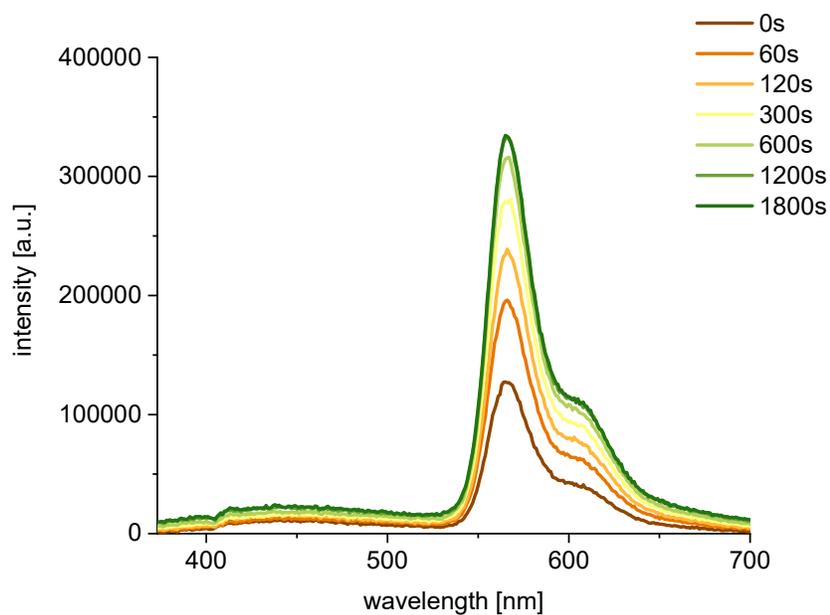


Figure S33. Fluorescence recorded during reaction of **RNA1** (2.5 μM) with AF555-maleimide (3.75 μM , (1.50 equiv.)), irradiated at 300 nm (LED) in 10 mM Na- P_i buffer, 250 mM NaCl, pH 7. Fluorescence excitation at 358 nm.

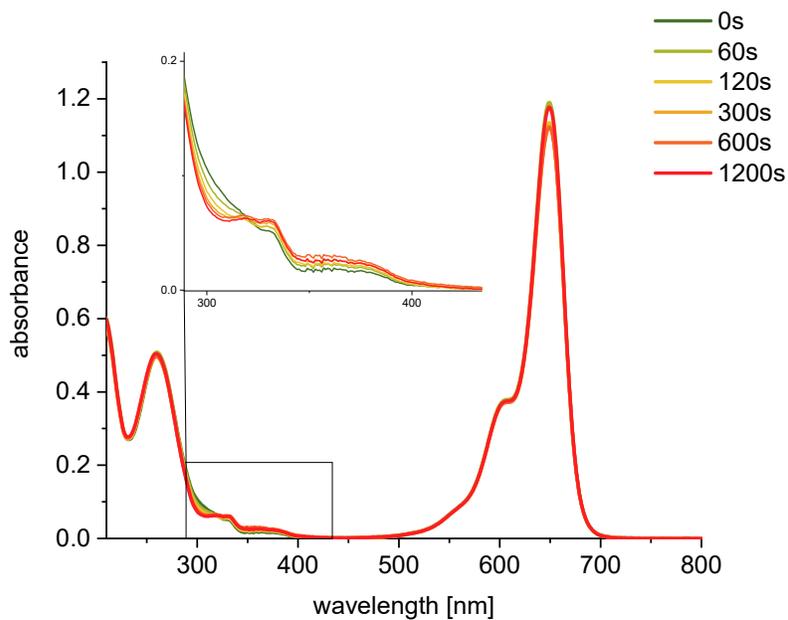


Figure S34. UV/Vis absorbance recorded during reaction of **RNA1** (2.5 μM) with AF647-maleimide (3.75 μM , 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-P_i buffer, 250 mM NaCl, pH 7.

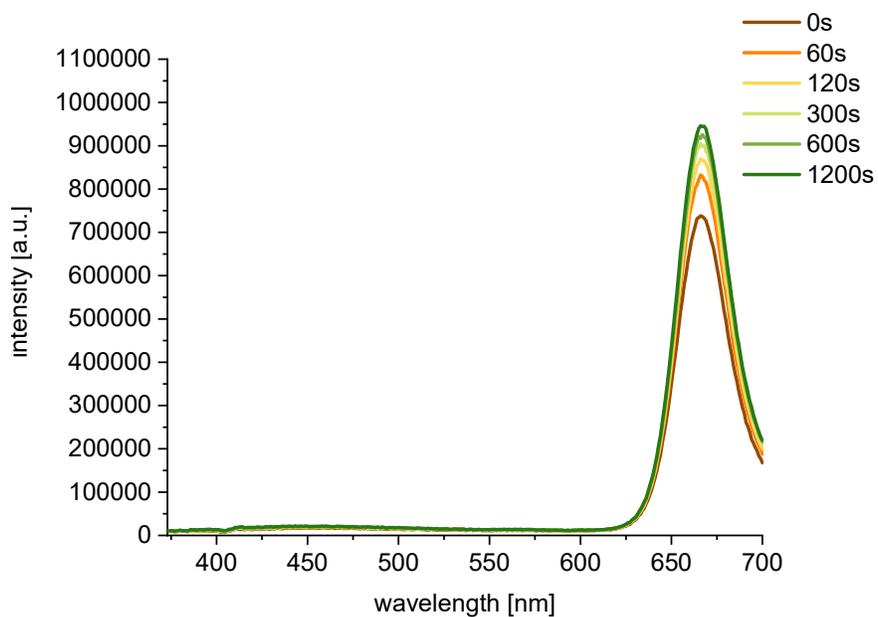


Figure S35. Fluorescence recorded during reaction of **RNA1** (2.5 μM) with AF647-maleimide (3.75 μM , 1.50 eq), irradiated at 300 nm (LED) in 10 mM Na-P_i buffer, 250 mM NaCl, pH 7. Fluorescence excitation at 358 nm.

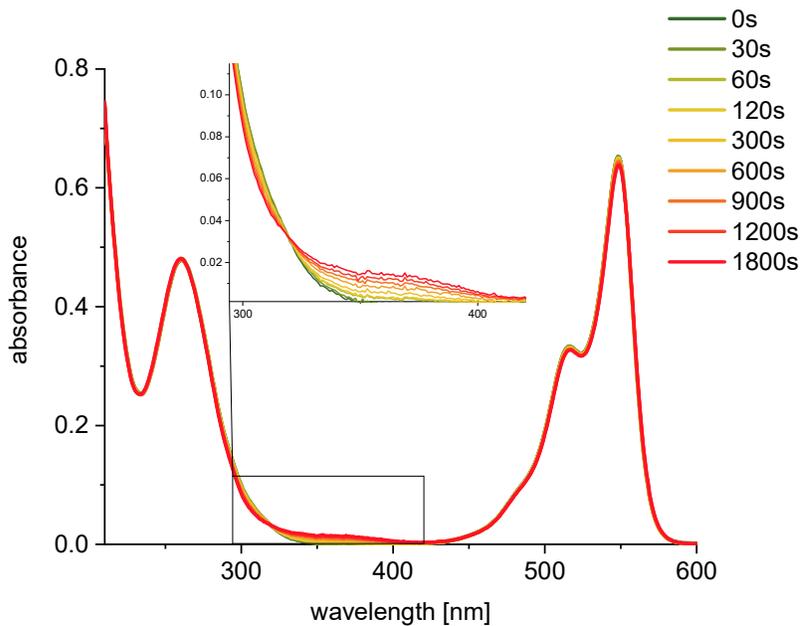


Figure S36. UV/vis absorbance recorded during reaction of **RNA2** (2.5 μM) with Cy3-maleimide (3.75 μM , 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-P_i buffer, 250 mM NaCl, pH 7.

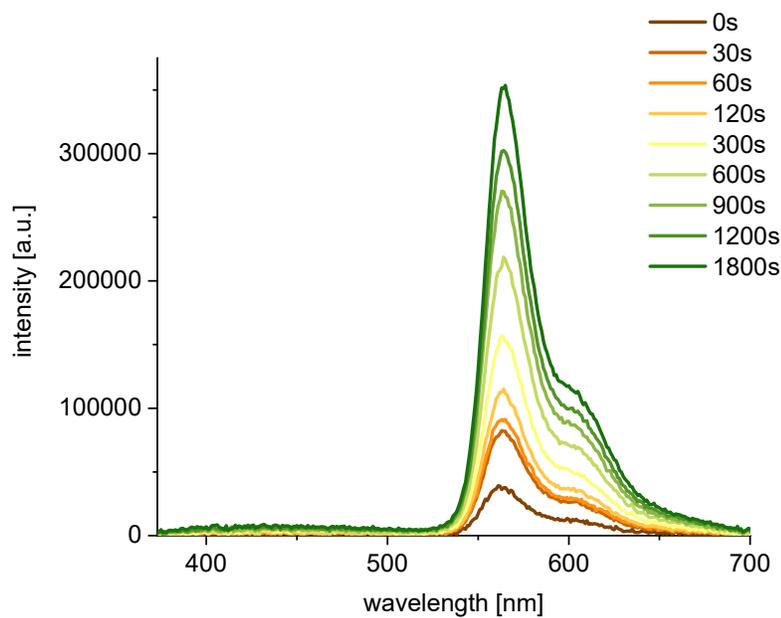


Figure S37. Fluorescence recorded during reaction of **RNA2** (2.5 μM) with Cy3-maleimide (3.75 μM , 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-P_i buffer, 250 mM NaCl, pH 7. Fluorescence excitation at 358 nm.

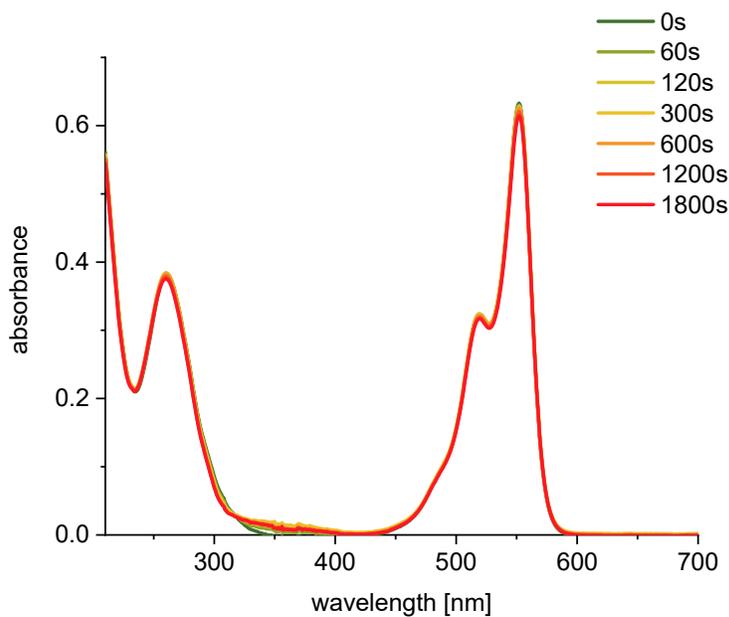


Figure S38. UV/Vis absorbance recorded during reaction of **RNA2** (2.5 μM) with AF555-maleimide (3.75 μM , 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-P_i buffer, 250 mM NaCl, pH 7.

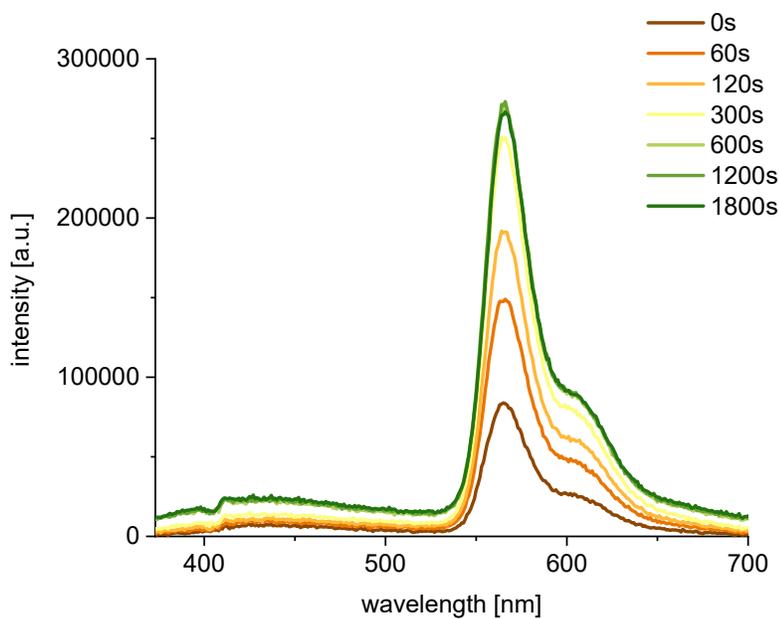


Figure S39. Fluorescence recorded during reaction of **RNA2** (2.5 μM) with AF555-maleimide (3.75 μM , 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-P_i buffer, 250 mM NaCl, pH 7. Fluorescence excitation at 358 nm.

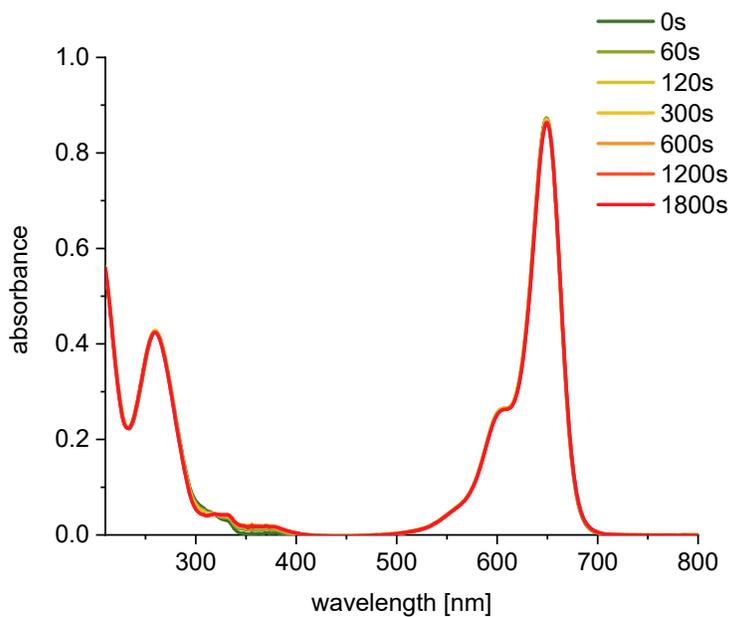


Figure S40. UV/Vis absorbance recorded during reaction of **RNA2** (2.5 μM) with AF647-maleimide (3.75 μM , 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-P_i buffer, 250 mM NaCl, pH 7.

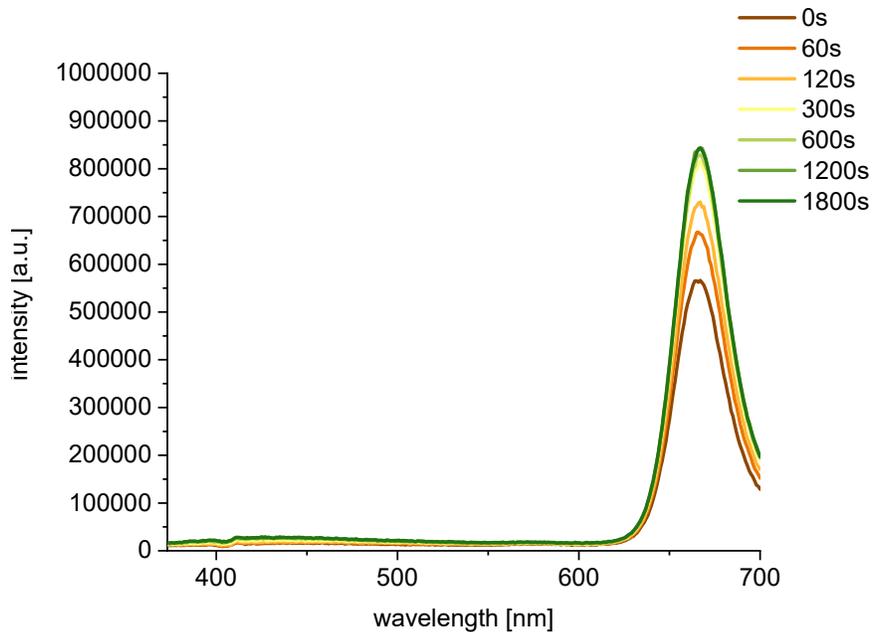


Figure S41. Fluorescence recorded during reaction of **RNA2** (2.5 μM) with AF647-maleimide (3.75 μM , 1.50 equiv.), irradiated at 300 nm (LED) in 10 mM Na-P_i buffer, 250 mM NaCl at pH 7. Fluorescence excitation at 358 nm.

3. MALDI spectra of RNA strands

RNA1

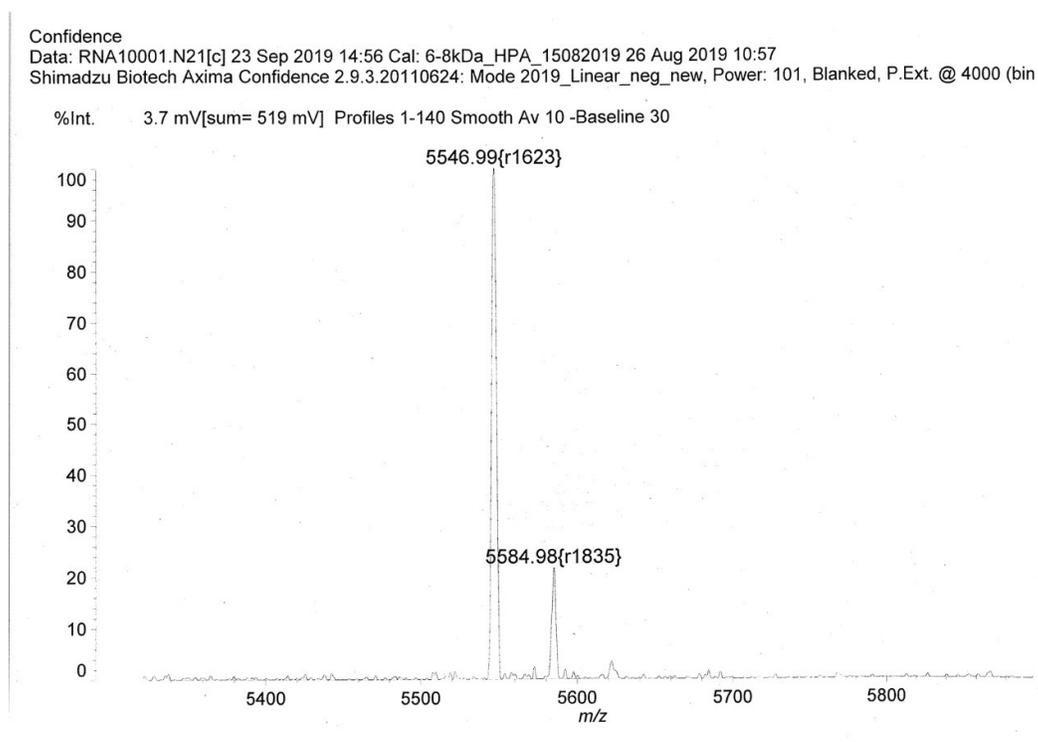


Figure S42. MS (MALDI-TOF) analysis of **RNA1**. Calculated mass [M^+]: 5544.6; $m/z=5546.99$ [M^+], 5584.98 [$M+K^+$].

Confidence
 Data: RNA1_Cy3_DOWEX0001.114[c] 23 Aug 2019 9:59 Cal: 6-8kDa_HPA_15082019 26 Aug 2019 10:57
 Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode 2019_Linear_neg_new, Power: 124, Blanked, P.Ext. @ 4130 (bin

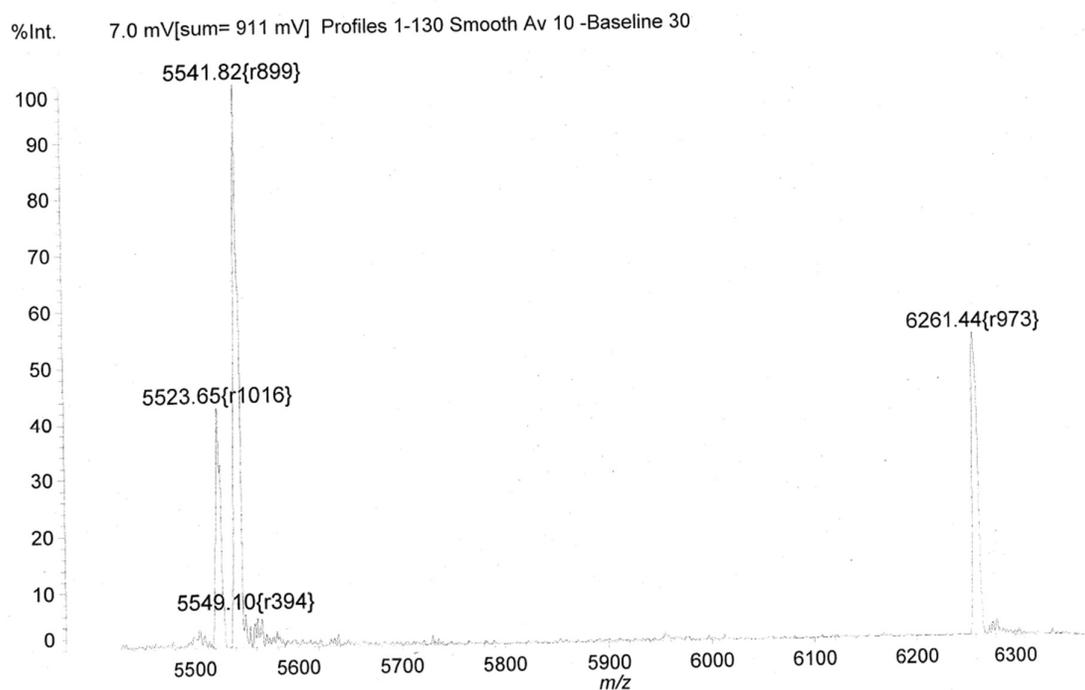


Figure S43. MS (MALDI-TOF) analysis of RNA1-Cy3 adduct. Calculated mass $[M^+]$: 6253.9; $m/z=5523.65$ $[RNA1-N_2^+]$, 5541.82 $[RNA1-N_2+H_2O^+]$, 6261.44 $[M^+]$.

Confidence
 Data: RNA1_AF5550001.C8[c] 16 Jan 2020 16:26 Cal: 6-8kDa_HPA_16102018 21 Nov 2018 11:26
 Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode 2019_Linear_neg_new, Power: 124, Blanked, P.Ext. @ 4000 (bin

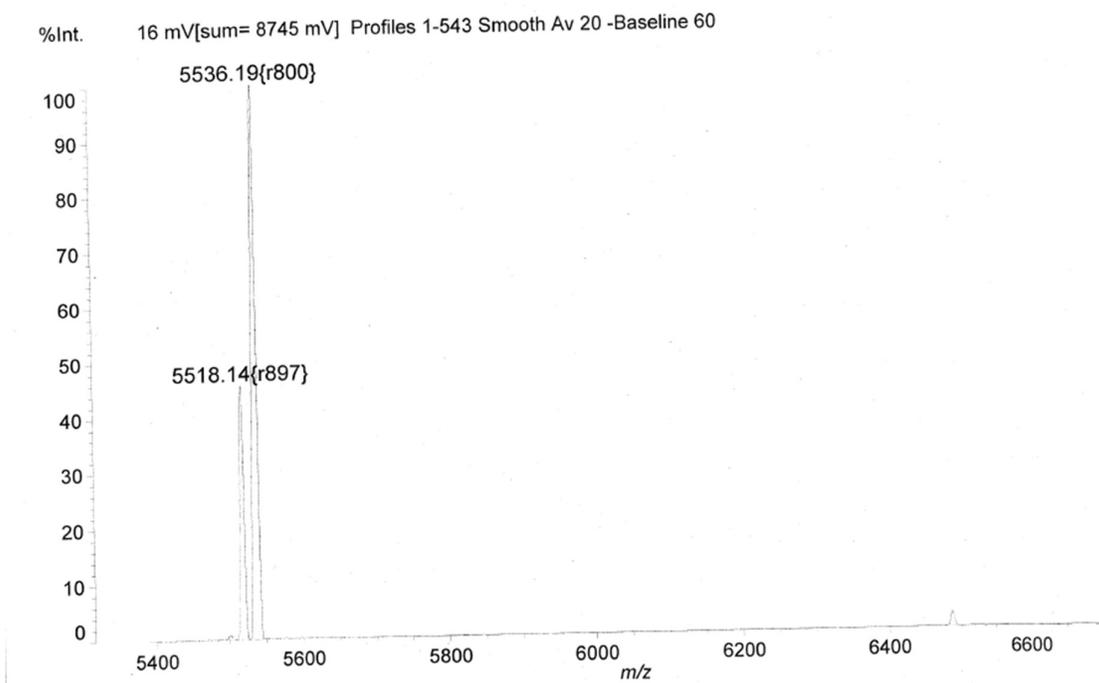


Figure S44. MS (MALDI-TOF) analysis of **RNA1-AF555** adduct. Calculated mass: 6485.9 [M⁺]; m/z=5518.14 [RNA1-N₂⁺], 5536.19 [RNA1-N₂+H₂O⁺], 6486.22 [M⁺]. The molecular mass of AF555-maleimide was reported in literature and verified by MS (MALDI-TOF) analysis.¹

Confidence
Data: RNA1_AF5550001.C8[c] 16 Jan 2020 16:26 Cal: 6-8kDa_HPA_16102018 21 Nov 2018 11:26
Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode 2019_Linear_neg_new, Power: 124, Blanked, P.Ext. @ 4000 (bin

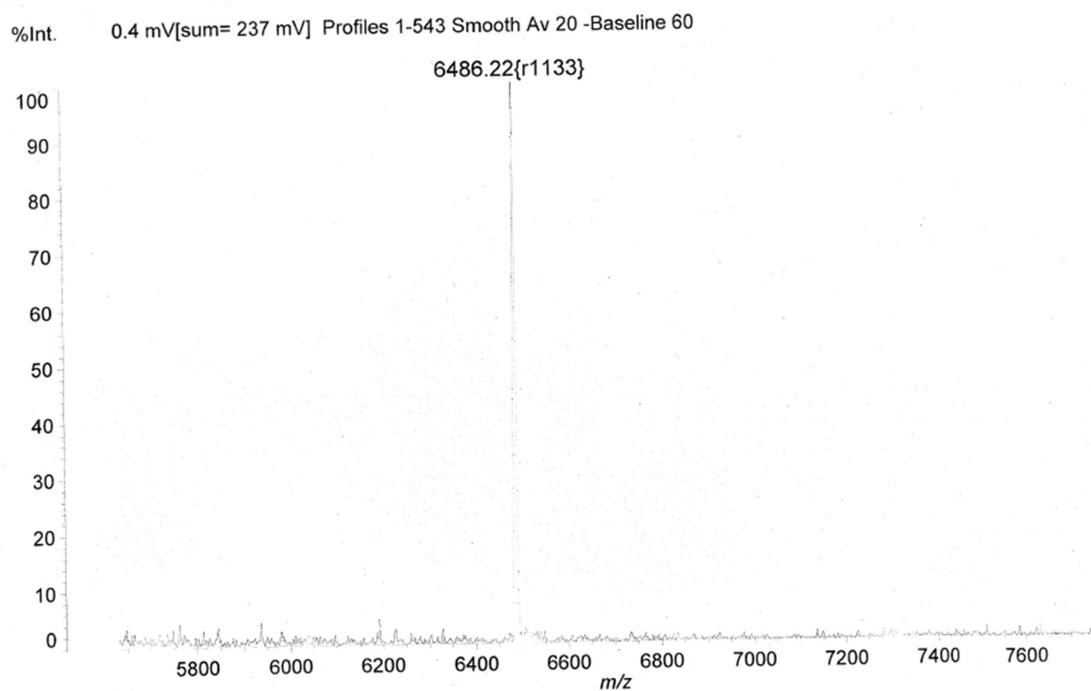


Figure S45. Zoomed area of MS (MALDI-TOF) analysis (Figure S44) of **RNA1-AF555** adduct.

Confidence
 Data: RNA1_AF647__30001.C11[c] 16 Jan 2020 17:29 Cal: 6-8kDa_HPA_16102018 21 Nov 2018 11:26
 Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode 2019_Linear_neg_new, Power: 127, Blanked, P.Ext. @ 4290 (bin

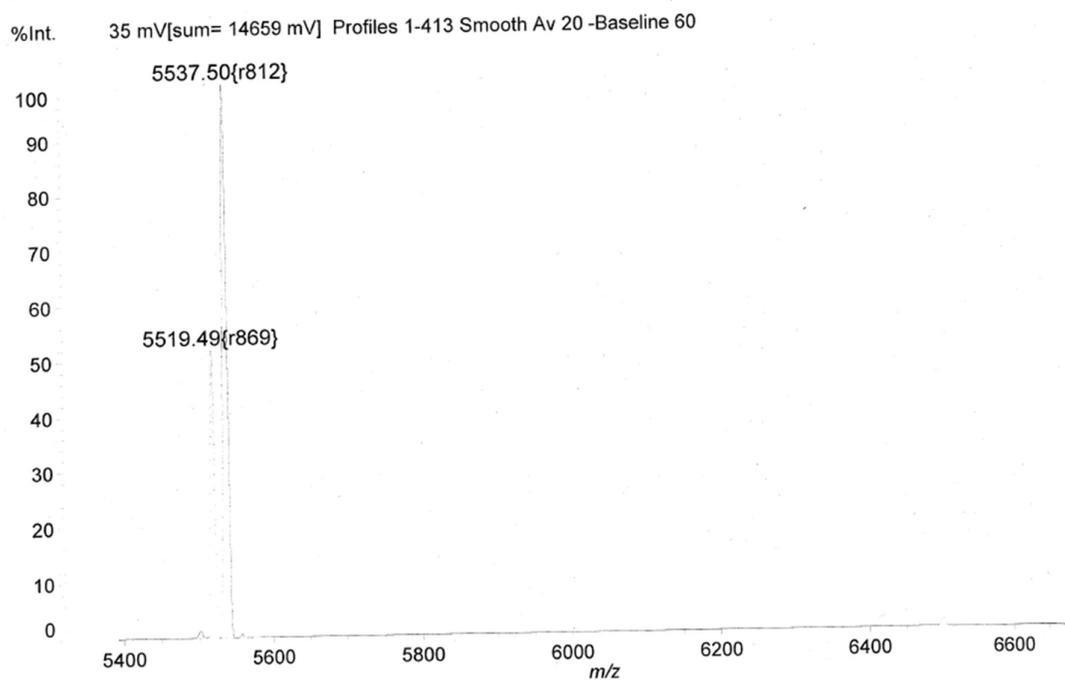


Figure S46. MS (MALDI-TOF) analysis of **RNA1-AF647** adduct. Calculated Mass [M^+]: 6497.9; $m/z=5519.49$ [$RNA1-N_2^+$], 5537.50 [$RNA1-N_2+H_2O^+$], 6499.90 [M^+]. The molecular mass of AF647-maleimide was reported in literature and verified by MS (MALDI-TOF) analysis.¹

Confidence
Data: RNA1_AF647_30001.C11[c] 16 Jan 2020 17:29 Cal: 6-8kDa_HPA_16102018 21 Nov 2018 11:26
Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode 2019_Linear_neg_new, Power: 127, Blanked, P.Ext. @ 4290 (bin)

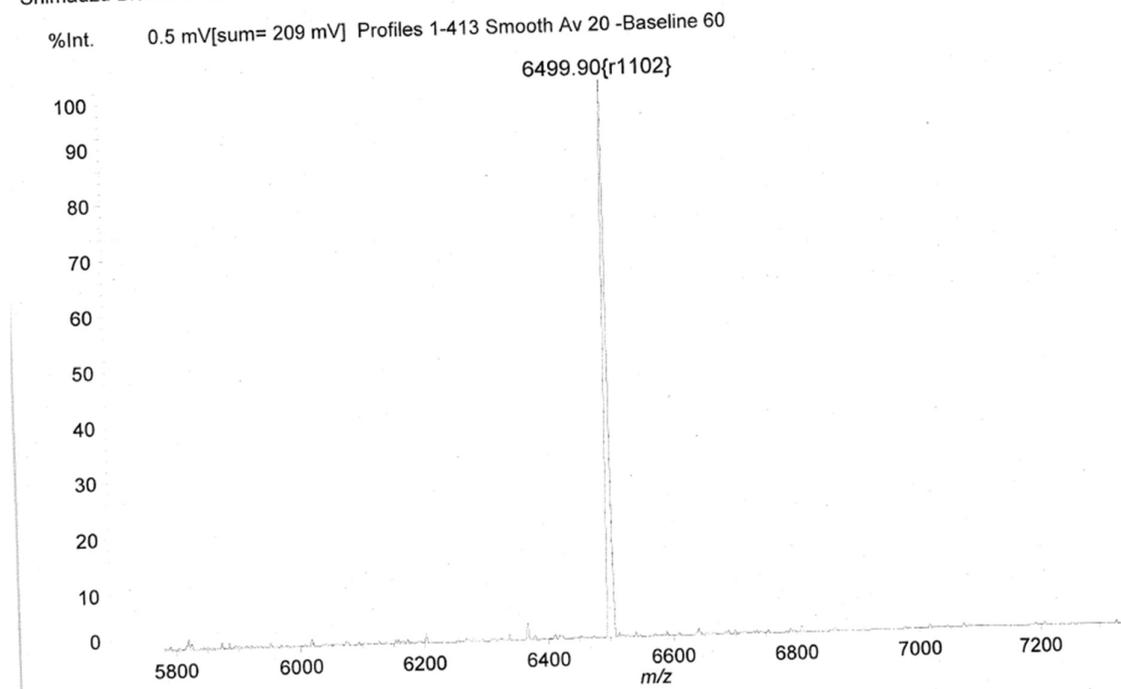


Figure S47. Zoomed area of MS (MALDI-TOF) analysis (Figure S46) of **RNA1**-AF647 adduct.

RNA2

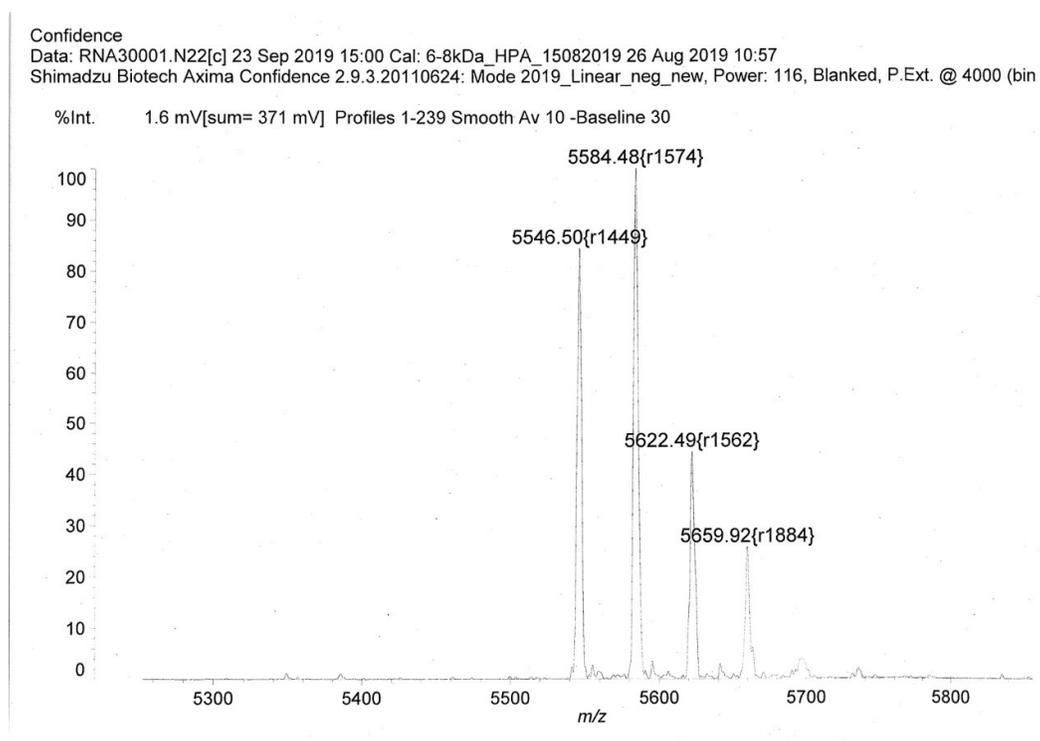


Figure S48. MS (MALDI-TOF) analysis of **RNA2**. Calculated mass [M^+]: 5544.6; m/z = 5546.50 [M^+], 5584.48 [$M+K^+$], 5622.49 [$M+2K^+$], 5659.92 [$M+3K^+$].

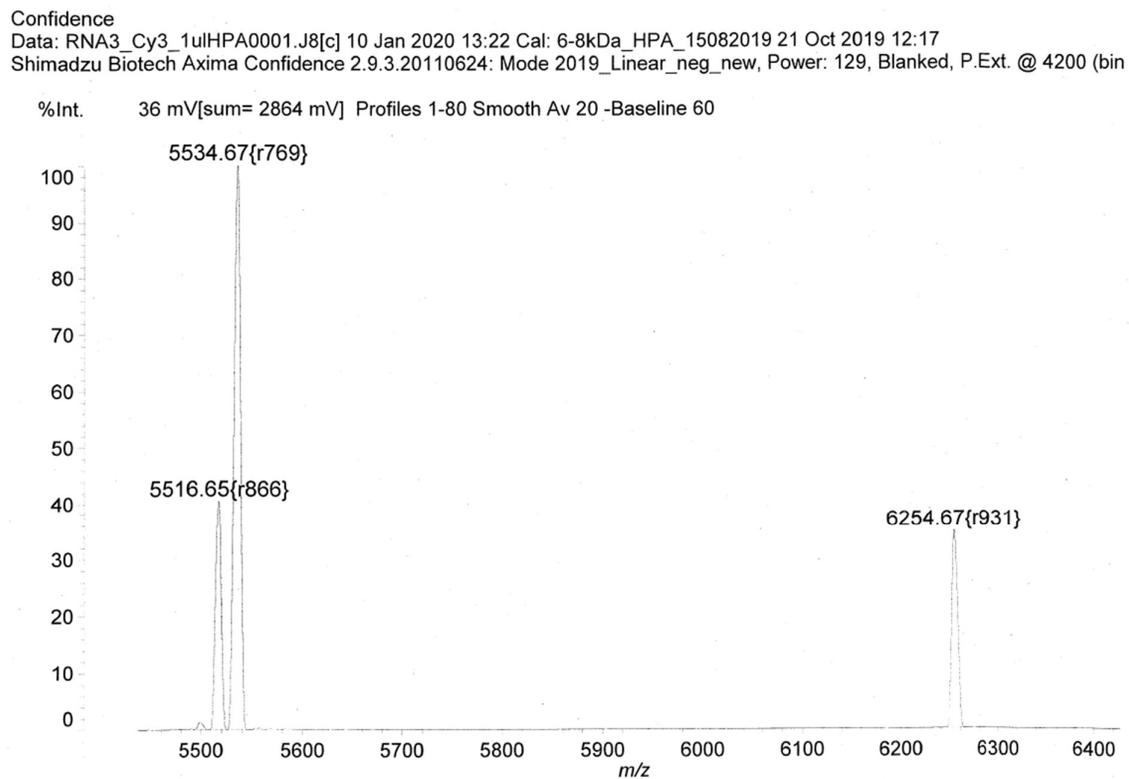


Figure S49. MS (MALDI-TOF) analysis of **RNA2-Cy3** adduct. Calculated mass [M^+]: 6253.9; $m/z=5516.65$ [$RNA2-N_2^+$], 5534.67 [$RNA2-N_2+H_2O^+$], 6254.67 [M^+].

Confidence
 Data: RNA3_AF5550001.J6[c] 10 Jan 2020 13:18 Cal: 6kDa_HPA_15082019 4 Nov 2019 10:42
 Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode 2019_Linear_neg_new, Power: 129, Blanked, P.Ext. @ 4200 (bin

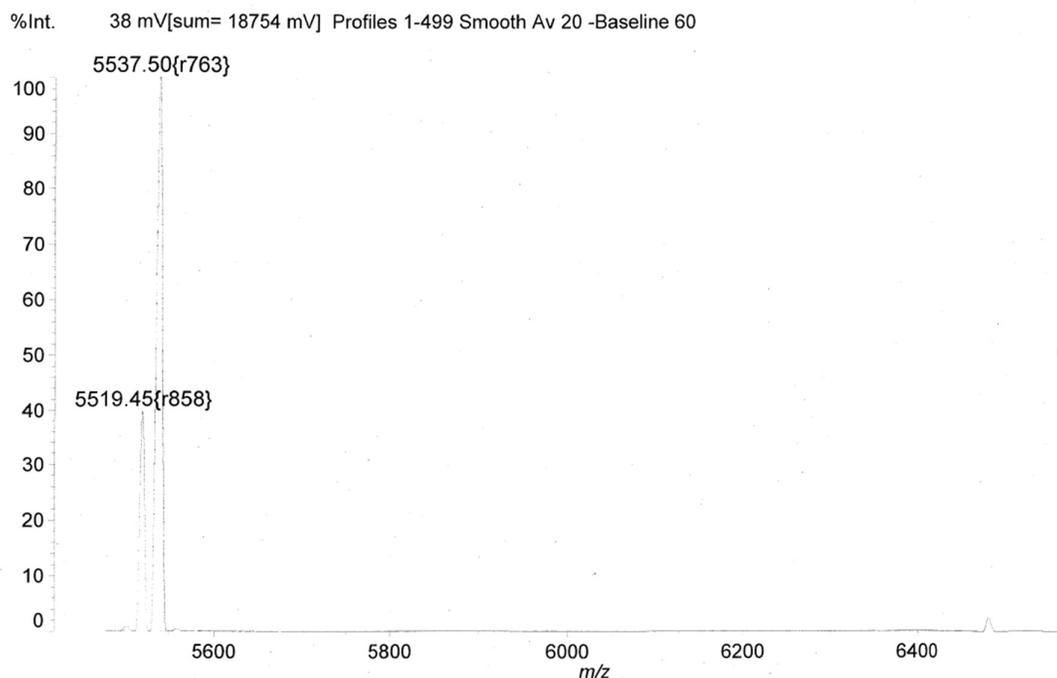


Figure S50. MS (MALDI-TOF) analysis of **RNA2-AF555** adduct. Calculated Mass [M^+]: 6485.9; $m/z=5519.45$ [$RNA2-N_2^+$], 5537.50 [$RNA2-N_2+H_2O^+$], 6485.06 [M^+]. The molecular mass AF555-maleimide was reported in literature and verified by MS (MALDI-TOF) analysis.¹

Confidence
Data: RNA3_AF5550001.J6[c] 10 Jan 2020 13:18 Cal: 6kDa_HPA_15082019 4 Nov 2019 10:42
Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode 2019_Linear_neg_new, Power: 129, Blanked, P.Ext. @ 4200 (bin

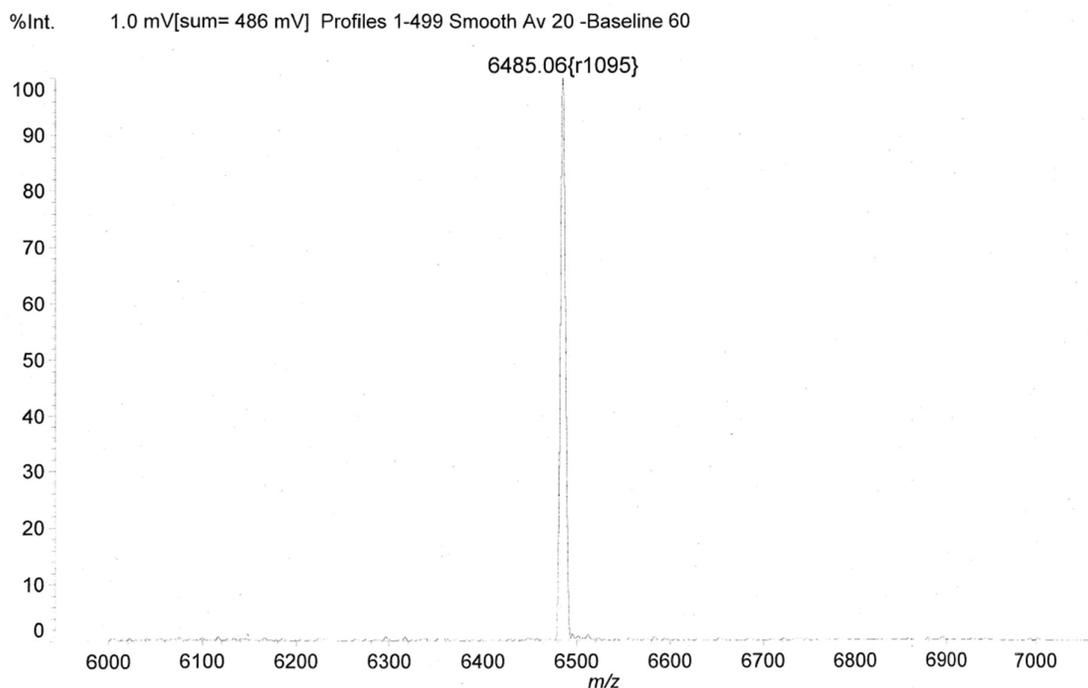


Figure S51. Zoomed area of MS (MALDI-TOF) analysis (Figure S50) of **RNA2-AF555** adduct.

Confidence
Data: RNA3_AF6470001.J5[c] 10 Jan 2020 13:10 Cal: 6-8kDa_HPA_16102018 21 Nov 2018 11:26
Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode 2019_Linear_neg_new, Power: 130, Blanked, P.Ext. @ 4200 (bin

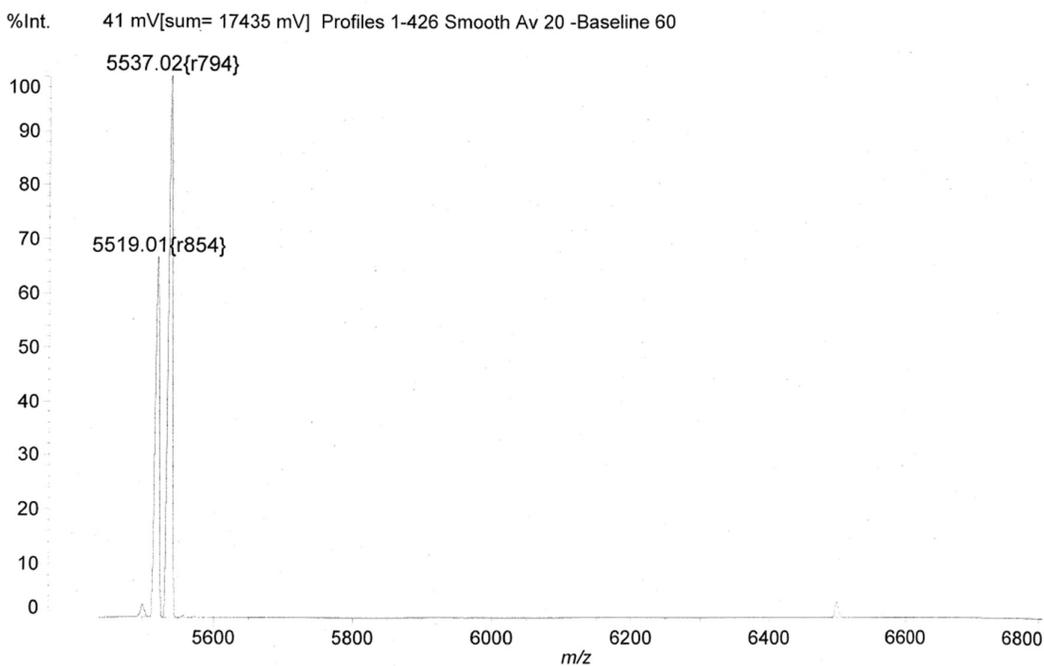


Figure S52. MS (MALDI-TOF) analysis of **RNA2-AF647** adduct. Calculated mass [M^+]: 6497.9; $m/z=5519.01$ [$RNA2-N_2^+$], 5537.02 [$RNA2-N_2+H_2O^+$], 6499.74 [M^+]. The molecular mass AF647-maleimide was reported in literature and verified by MS (MALDI-TOF) analysis.¹

Confidence

Data: RNA3_AF6470001.J5[c] 10 Jan 2020 13:10 Cal: 6-8kDa_HPA_16102018 21 Nov 2018 11:26

Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode 2019_Linear_neg_new, Power: 130, Blanked, P.Ext. @ 4200 (bin

%Int. 1.2 mV[sum= 520 mV] Profiles 1-426 Smooth Av 20 -Baseline 60

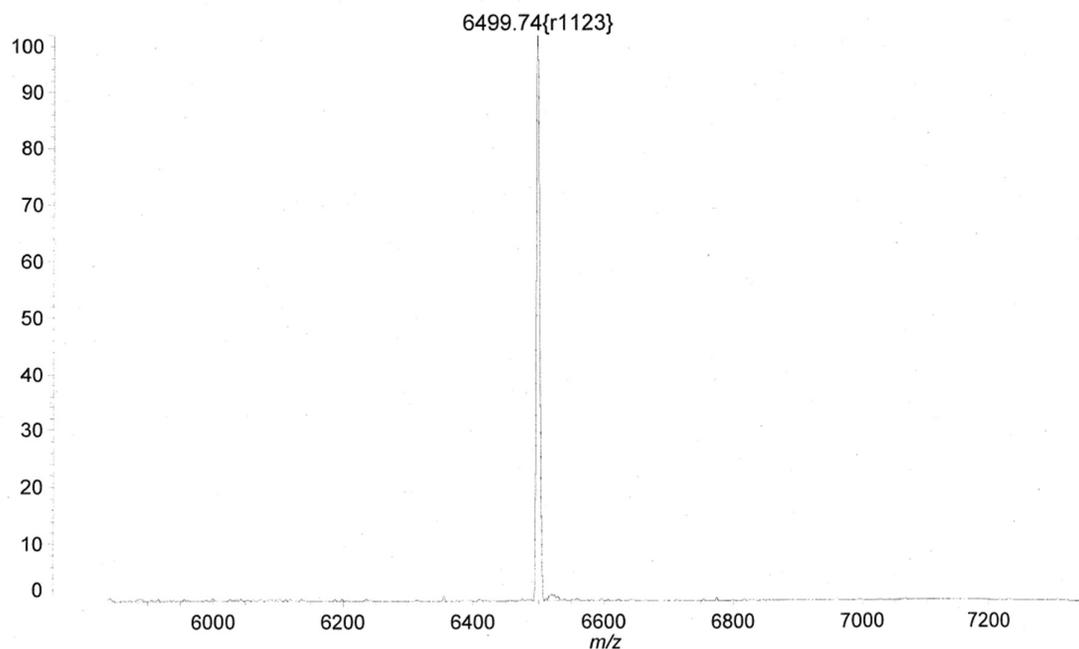


Figure S53. Zoomed area of MS (MALDI-TOF) analysis (Figure S53) of **RNA2-AF647** adduct.

4. Determination of yields

A solution of RNA (2.5 μM) and of the dye (3.75 μM) in 10 mM Na-P_i 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 spectroscopically by Lambert-Beer-Law using the extinction coefficient provided by the manufacturers of the clicked dye: ϵ_{548} (Cy3) = 162 000 L mol⁻¹ cm⁻¹ (Lumiprobe); ϵ_{555} (AF555) = 158 000 L mol⁻¹ cm⁻¹ (JenaBioscience); ϵ_{648} (AF647) = 270 000 L mol⁻¹ cm⁻¹ (JenaBioscience).

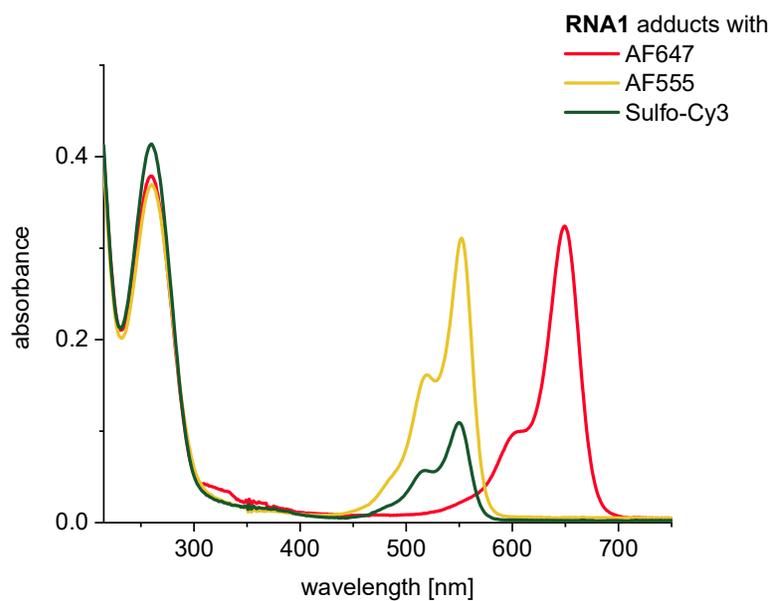


Figure S54. UV/vis absorbance of “photoclicked” **RNA1** dye adducts (reaction with 1.50 equiv. dye-maleimide) strands after purification. $c_{AF647}=1.19 \mu\text{M} \triangleq 48\%$ yield, $c_{AF555}=1.96 \mu\text{M} \triangleq 78\%$ yield, $c_{Cy3}=0.67 \mu\text{M} \triangleq 27\%$ yield.

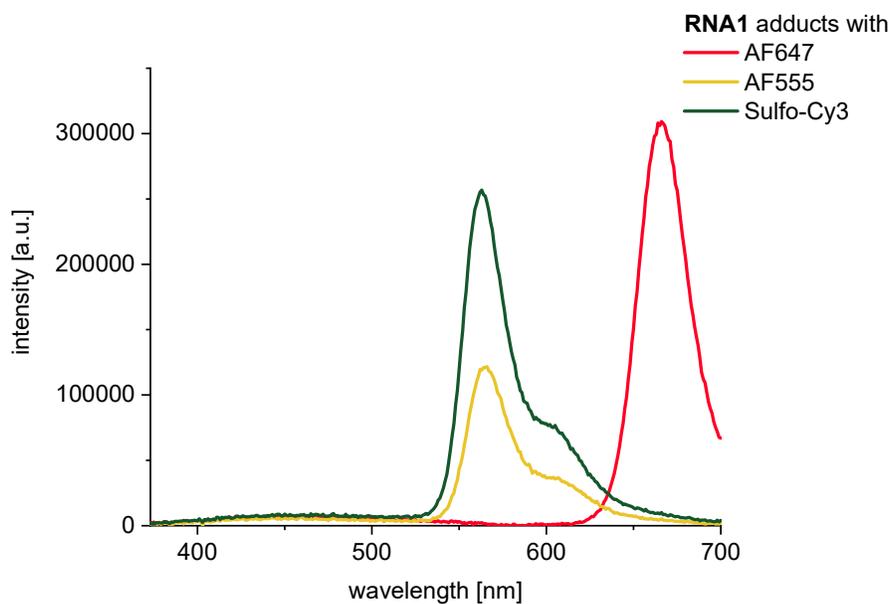


Figure S55. Fluorescence of “photoclicked” **RNA1** dye adducts (reaction with 1.50 equiv. dye-maleimide) after purification. $c_{AF647}=1.19 \mu\text{M}$, $c_{AF555}=1.96 \mu\text{M}$, $c_{Cy3}=0.67 \mu\text{M}$.

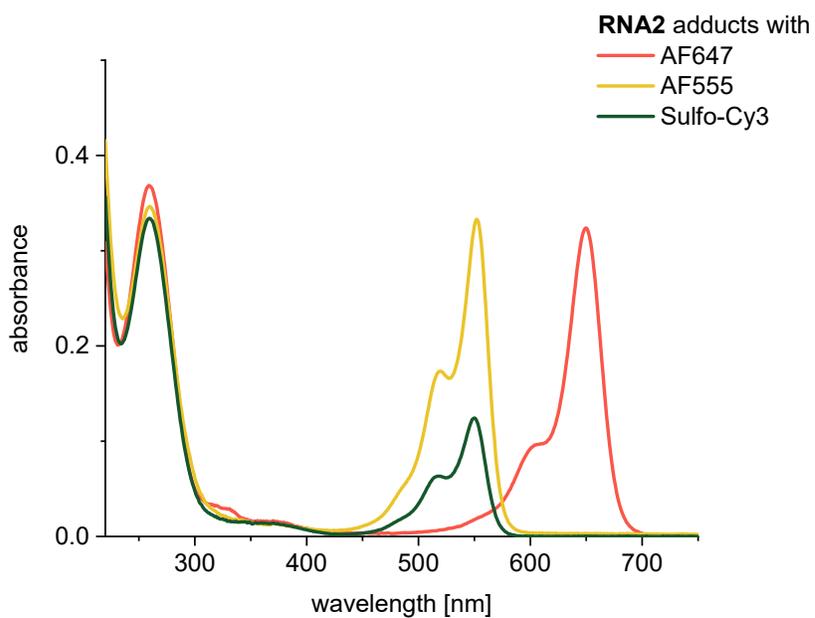


Figure S56. UV/vis absorbance of "photoclicked" RNA2 dye adducts (reaction with 1.50 equiv. dye-maleimide) after purification. $C_{AF647}=1.20 \mu\text{M} \cong 48\%$ yield, $C_{AF555}=2.10 \mu\text{M} \cong 84\%$ yield, $C_{Cy3}=0.77 \mu\text{M} \cong 31\%$ yield.

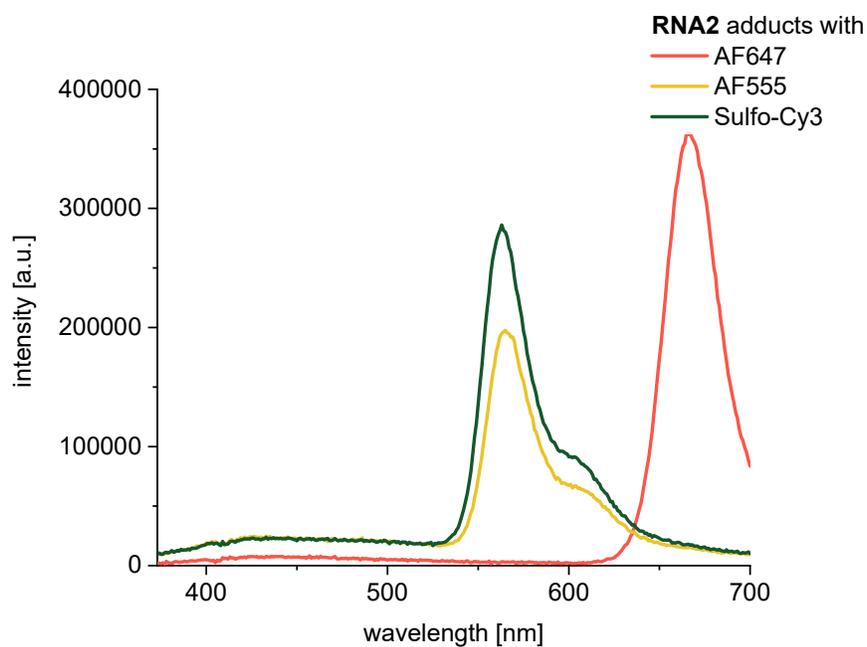


Figure S57. Fluorescence of "photoclicked" RNA2 dye adducts (after reaction with 1.50 equiv. dye-maleimide) after purification. $C_{AF647}=1.20 \mu\text{M}$, $C_{AF555}=2.10 \mu\text{M}$, $C_{Cy3}=0.77 \mu\text{M}$.

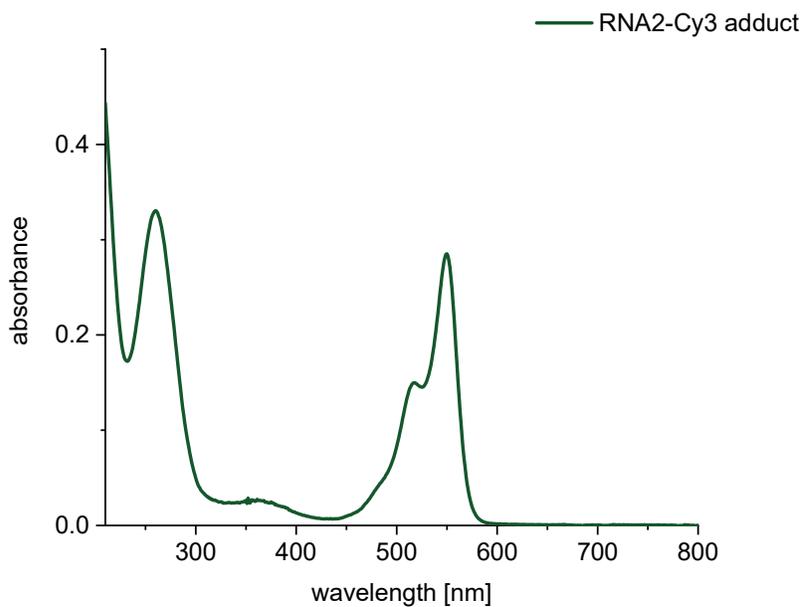


Figure S58. UV/vis absorbance of “photoclicked” **RNA1**-Cy3 adduct (reaction with 10.0 equiv. Cy3-maleimide) after purification. $c_{\text{Cy3}}=1.76 \mu\text{M} \cong 70\%$ yield.

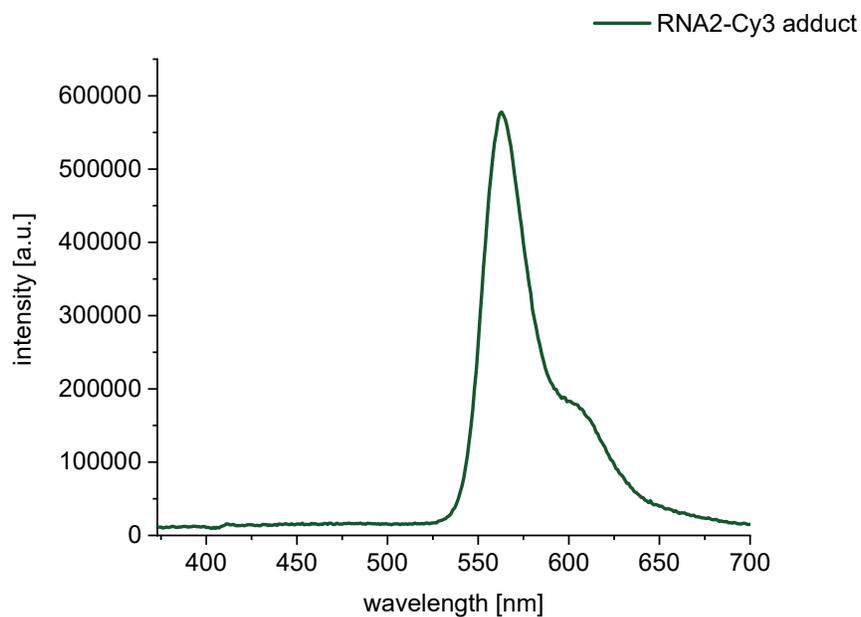


Figure S59. Fluorescence spectrum of “photoclicked” **RNA2**-Cy3 adduct (reaction with 10.0 equiv. Cy3-maleimide) after purification. $c_{\text{Cy3}}=1.76 \mu\text{M}$.

5. Calculation of extinction coefficients

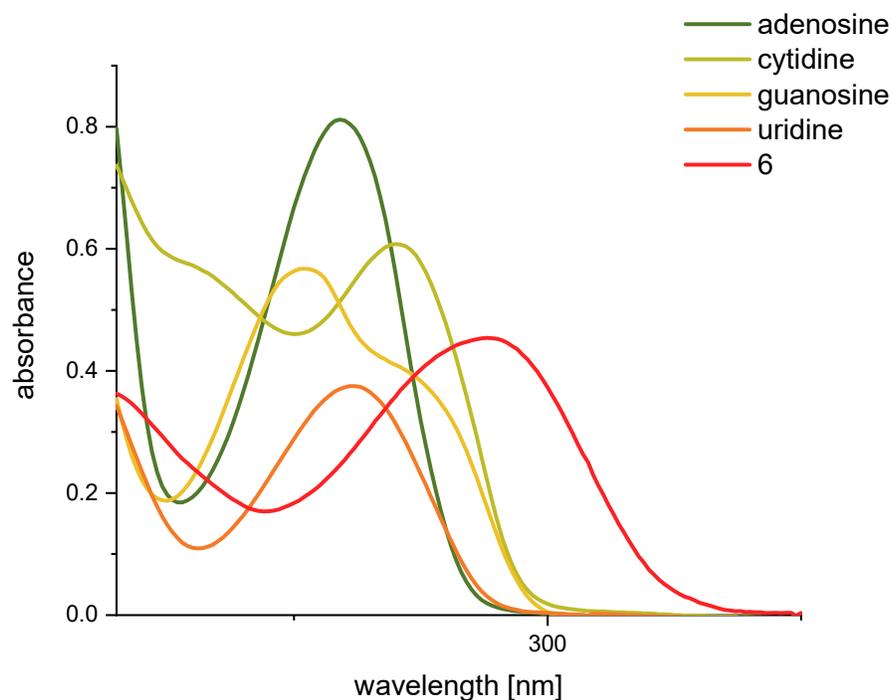


Figure S60. UV/vis absorbance of A, C, G, U and **6** in comparison.

The molar extinction coefficient ϵ_{300} were calculated for the natural nucleosides and the artificial building block **6** using the ϵ_{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	ϵ_{260} [L mol ⁻¹ cm ⁻¹]	concentration [μ mol L ⁻¹]	ϵ_{300} [L mol ⁻¹ cm ⁻¹]
Adenosine	15,400	52.6	≈60
Cytidine	7,400	70.9	≈260
Guanosine	11 500	42.8	≈110
Uridine	8,700	42.9	≈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.