Supplementary Information

Conjugation of doxorubicin to siRNA through disulfide-based self-immolative linkers

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^{1}H-NMR and ^{13}C-NMR spectra

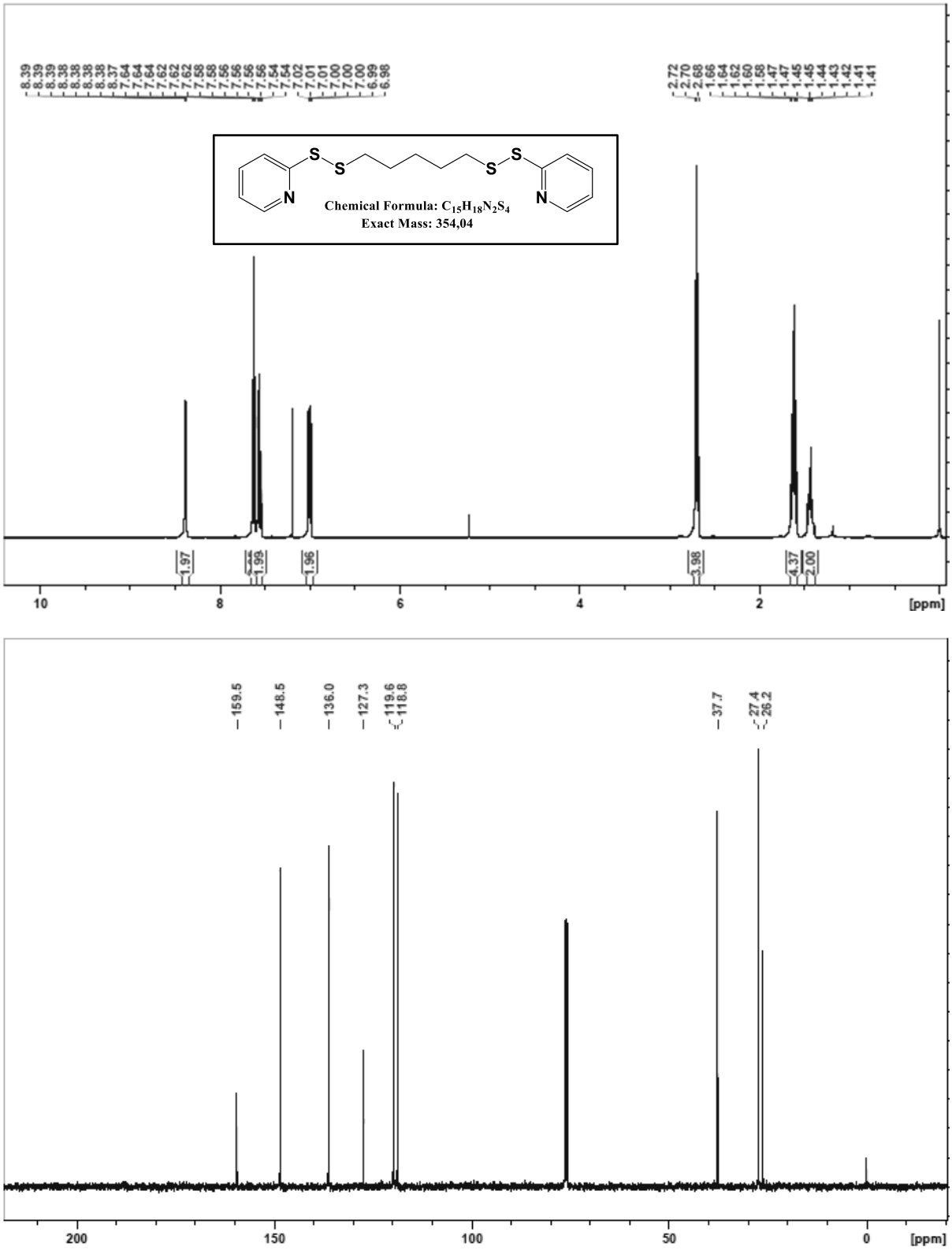


Figure 1. 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR spectra ($CDCl_3$) of **1.**

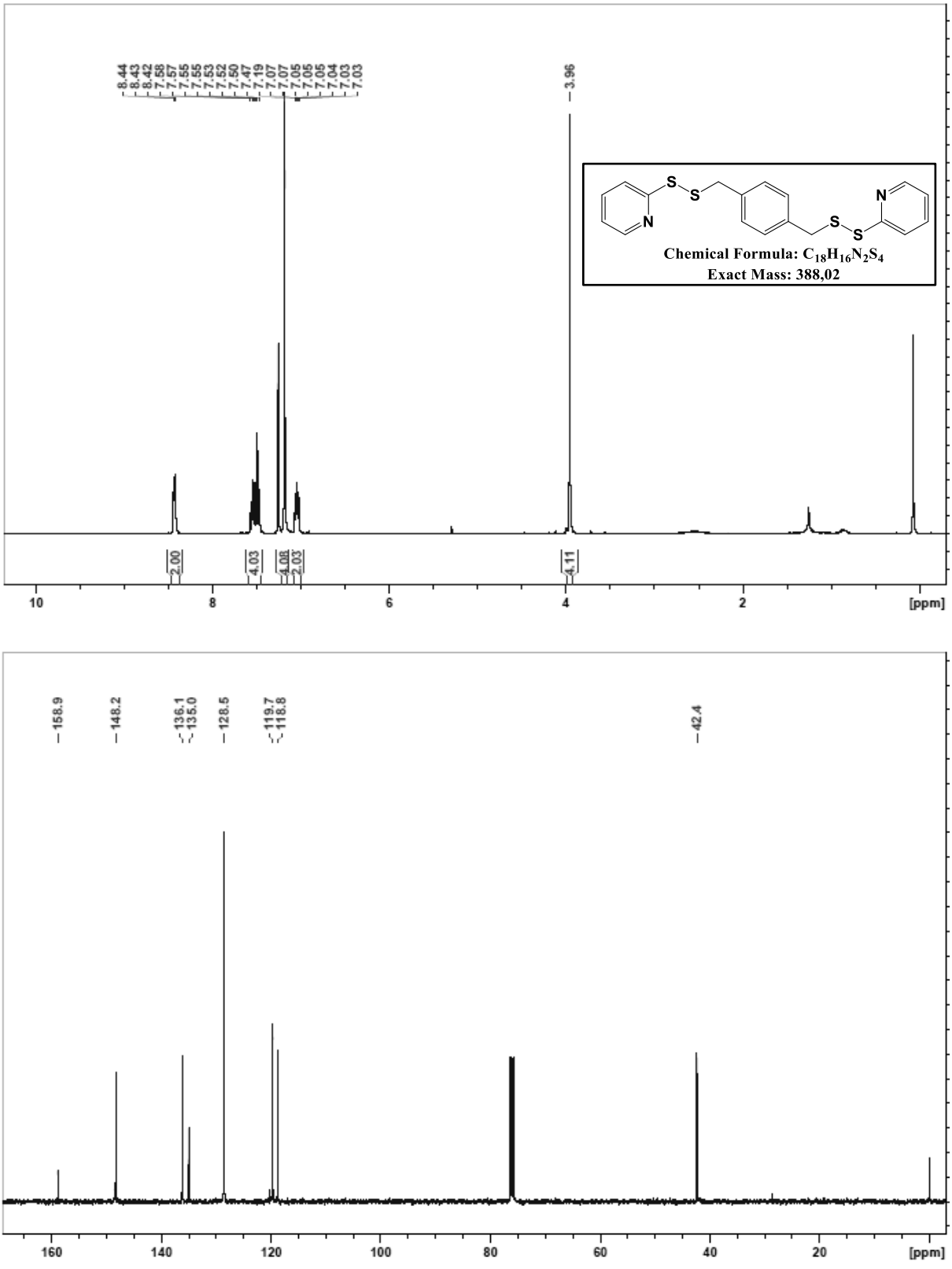
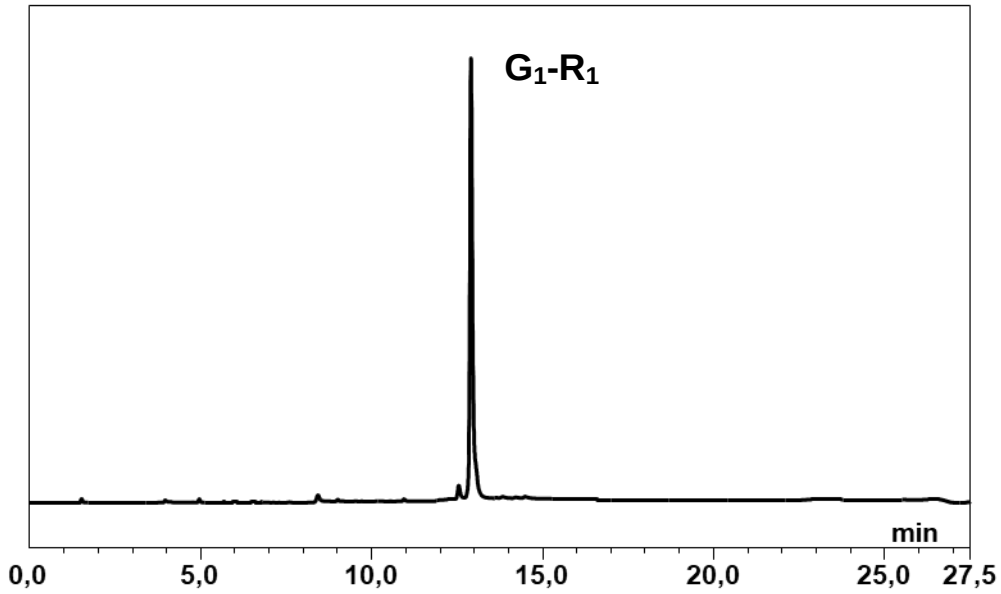


Figure S2: 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR spectra ($CDCl_3$) of **2**

HPLC chromatograms & MALDI-TOF spectra of purified oligonucleotides



IEX-HPLC analysis conditions: DNAPac® PA100, 4 x 250 mm, elution with a 20 min linear gradient of 0 to 75% of B in eluent A. Column temperature 30 °C. Flow rate 1.5 mL.min^{-1}. λ 260 nm.

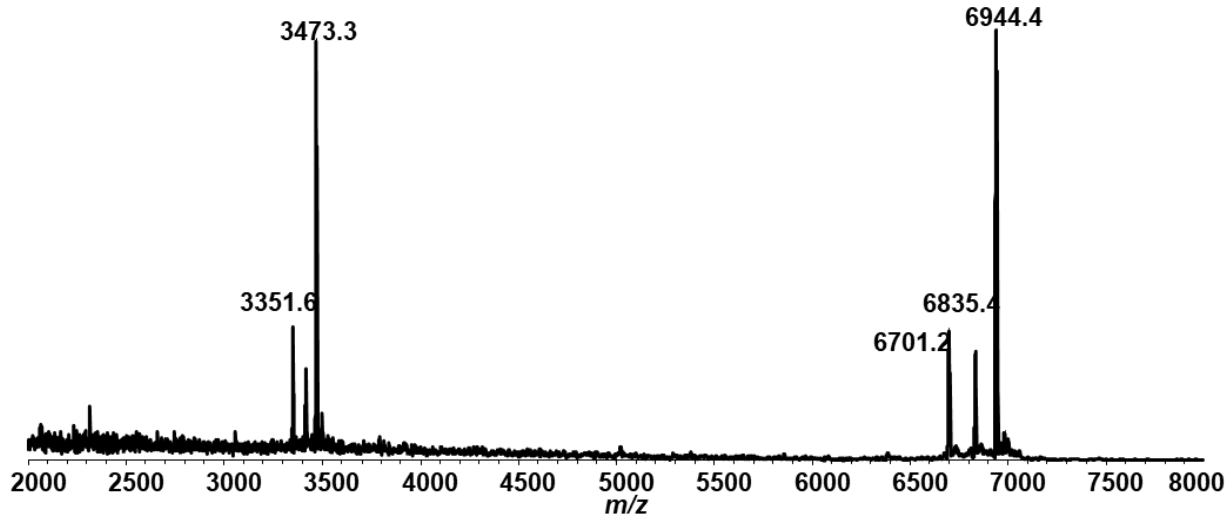
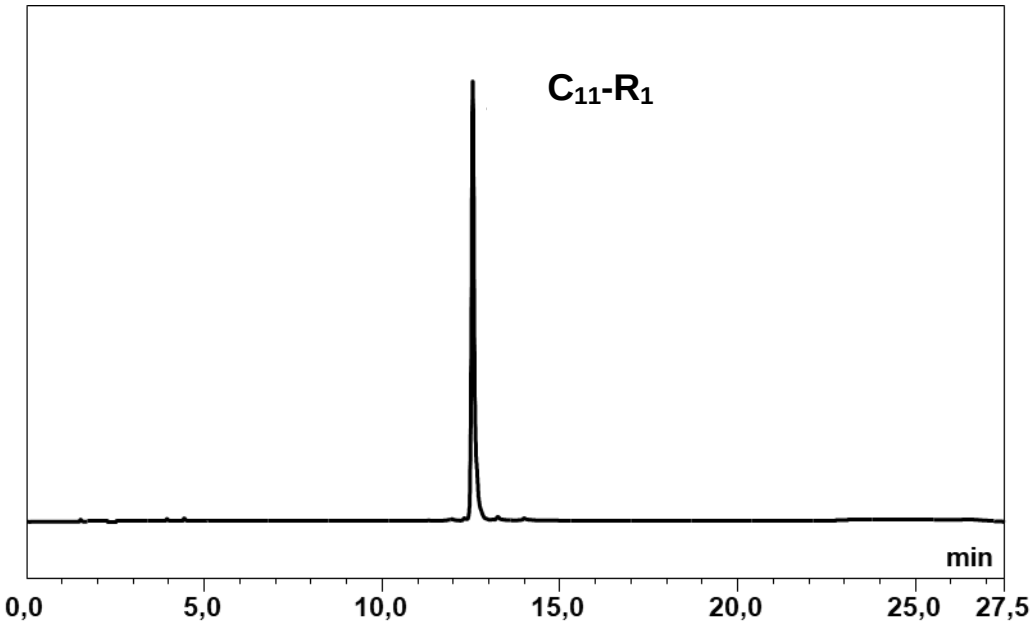


Figure S3: IEX-HPLC and MALDI-TOF MS analyses of purified RNA **G_1-R_1**



IEX-HPLC analysis conditions: DNAPac® PA100, 4 x 250 mm, elution with a 20 min linear gradient of 0 to 75% of B in eluent A. Column temperature 30 °C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

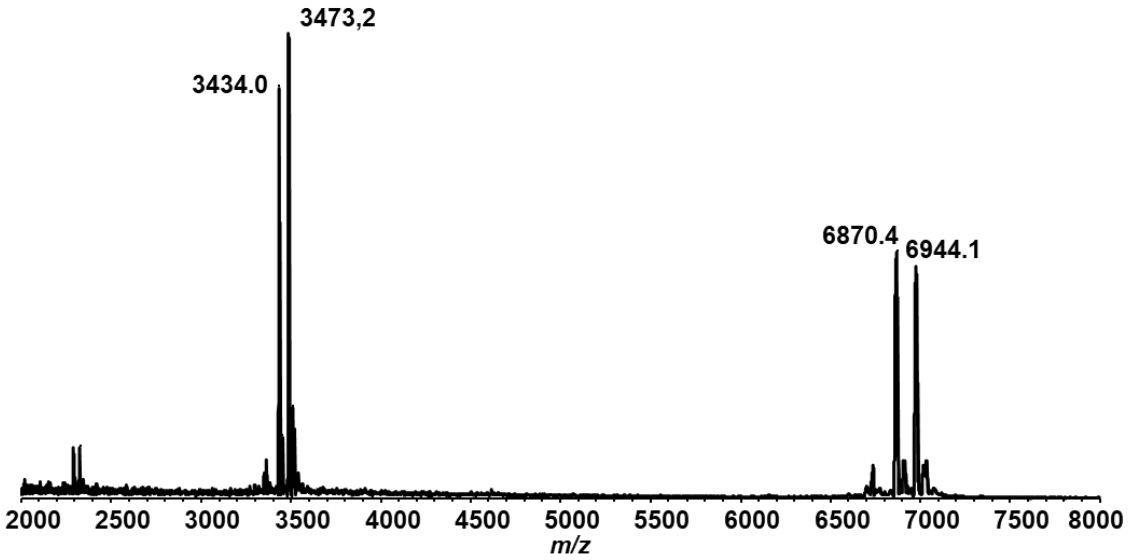
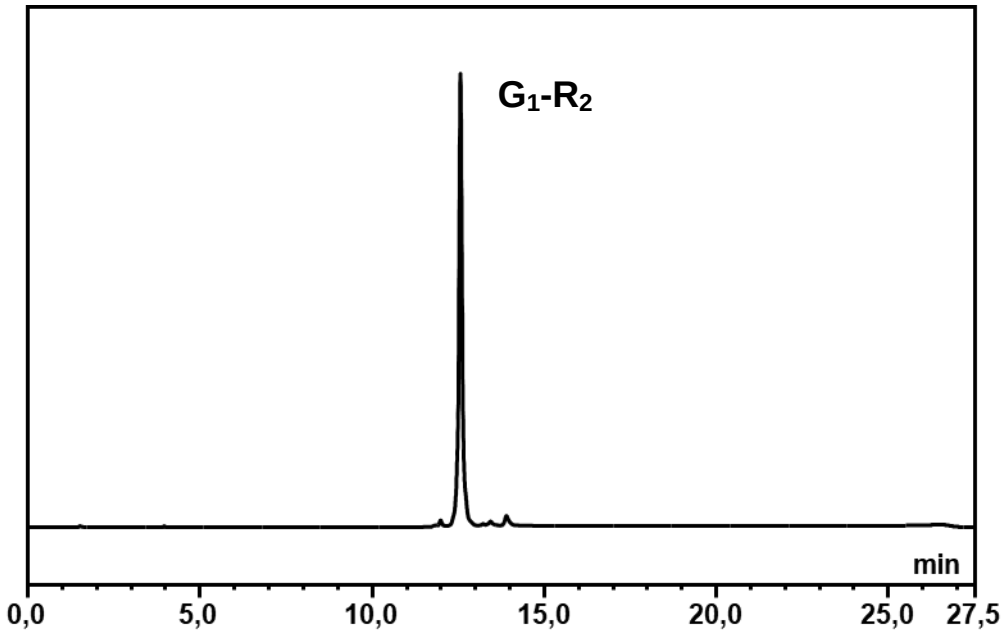


Figure S4: IEX-HPLC and MALDI-TOF MS analyses of purified RNA **C_{11}-R_1**



IEX-HPLC analysis conditions: DNAPac® PA100, 4 x 250 mm, elution with a 20 min linear gradient of 10 to 90% of B in eluent A. Column temperature 30 °C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

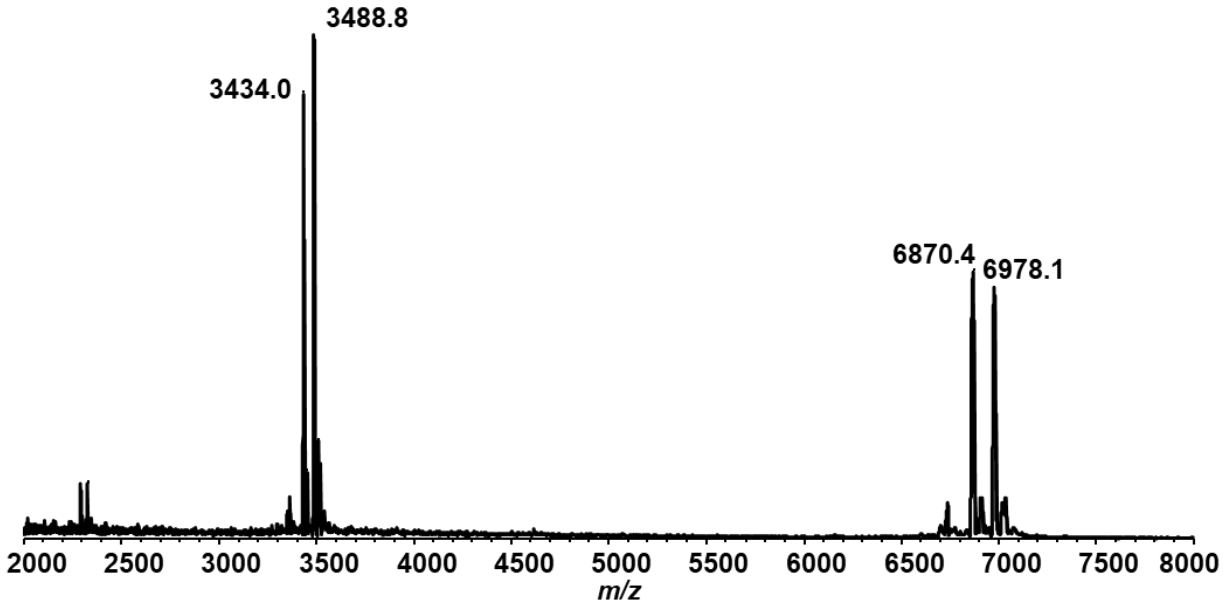
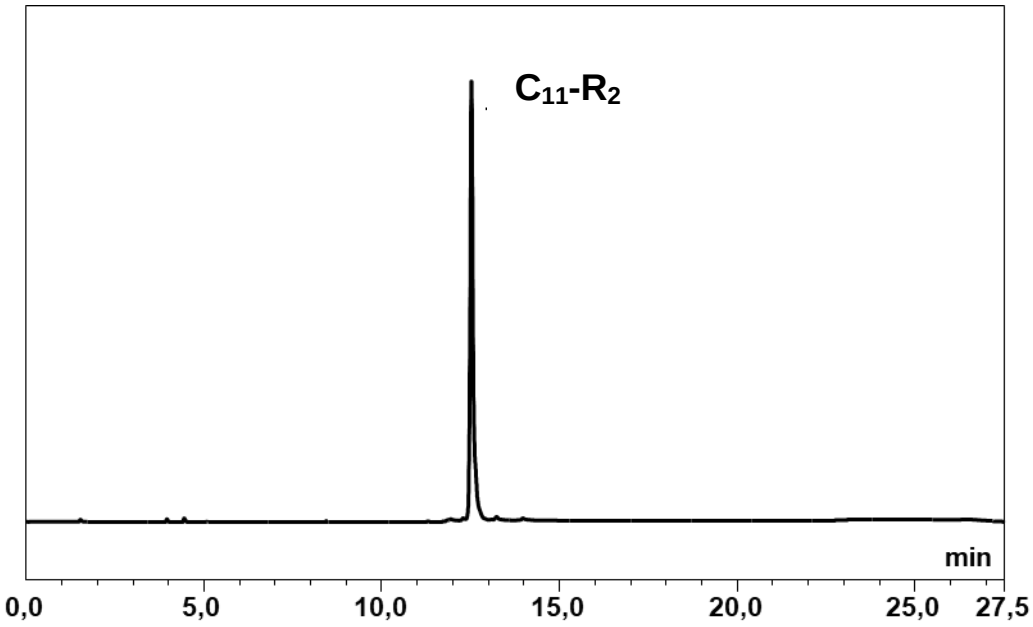


Figure S5: IEX-HPLC and MALDI-TOF MS analyses of purified RNA **G_1-R_2**



IEX-HPLC analysis conditions: DNAPac® PA100, 4 x 250 mm, elution with a 20 min linear gradient of 30 to 100% of B in eluent A. Column temperature 30 °C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

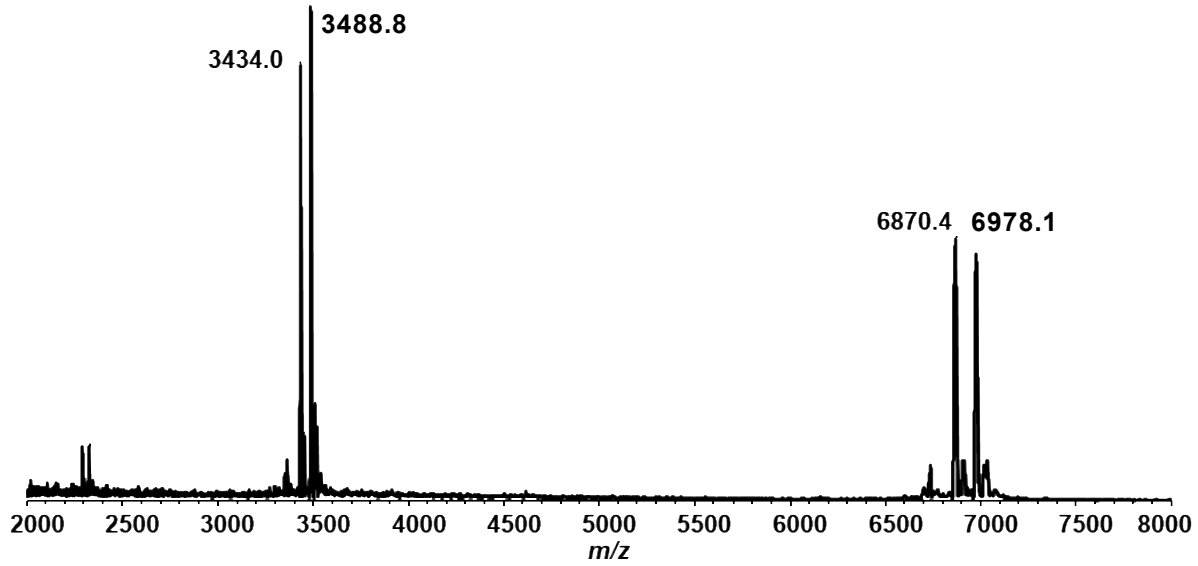
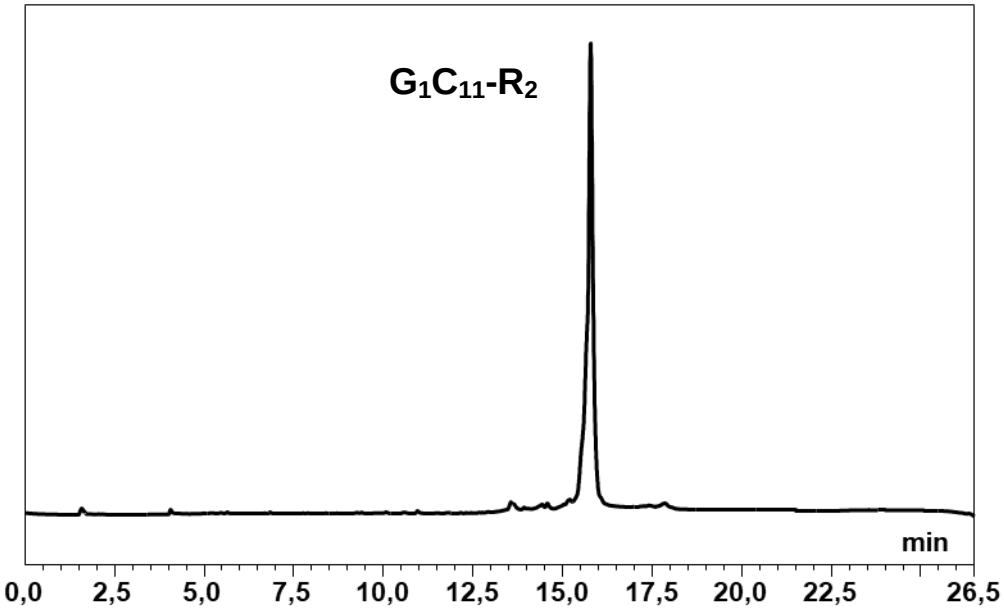


Figure S6: IEX-HPLC and MALDI-TOF MS analyses of purified RNA **C_{11}-R_2**



IEX-HPLC analysis conditions: DNAPac® PA100, 4 x 250 mm, elution with a 20 min linear gradient of 40 to 100% of B in eluent A. Column temperature 75 °C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

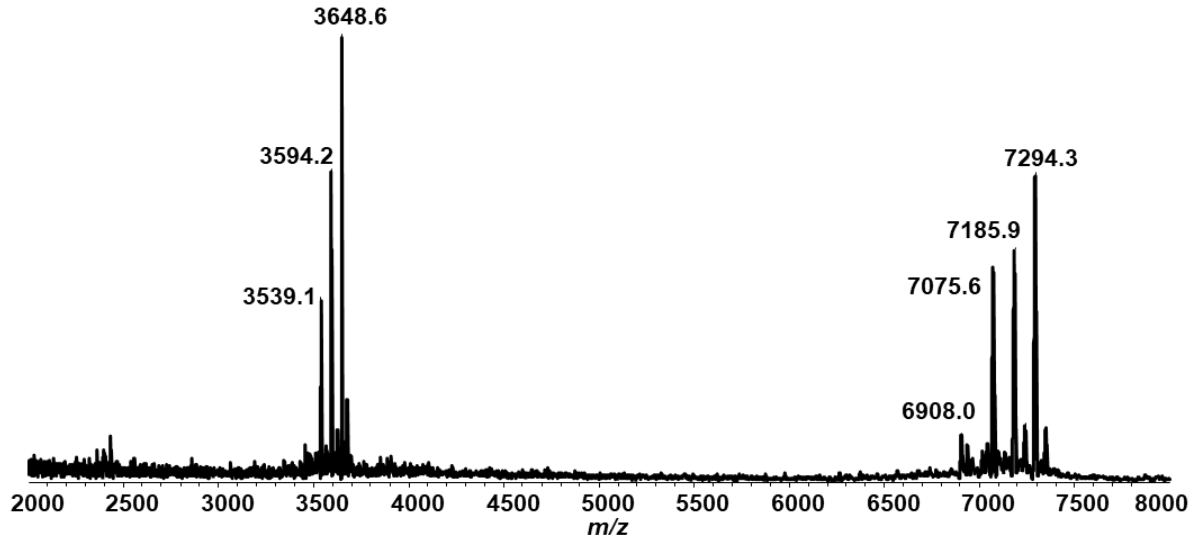
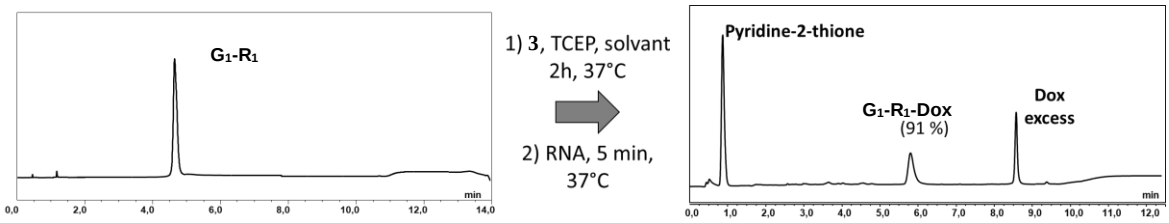


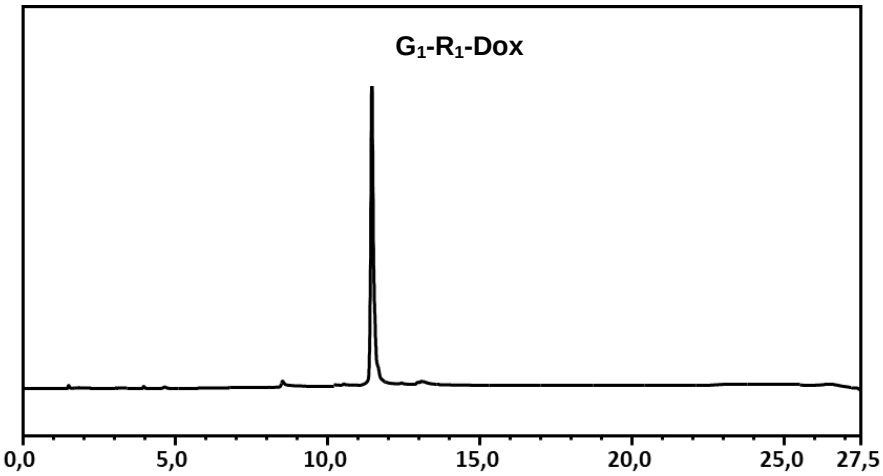
Figure S7: IEX-HPLC and MALDI-TOF MS analyses of purified RNA **G_1C_{11}-R_2**

A



RP-HPLC chromatograms of RNA **G_1-R_1** *(left), and of the crude conjugation mixture to* **Dox** *(right). Conditions: ThermoFisher Scientific LC Accucore® C18, 100Å, 2.1 x 50 mm, elution with a 10 min linear gradient of 0% to 50% of B (12.5 mM TEAAc in 1% ACN) in eluent A (12.5 mM TEAAc in 80% ACN). Column temperature 30°C. Flow rate 1.0 mL.min^{-1}. UV monitoring at 260 nm (percentage of peak area corresponding to the Dox-RNA conjugate in RNA crude material).*

B



IEX-HPLC analysis conditions: column DNAPac® PA100, 4 x 250 mm, elution with a 20 min linear gradient of 30 to 100% of B in eluent A. Column temperature 75 °C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

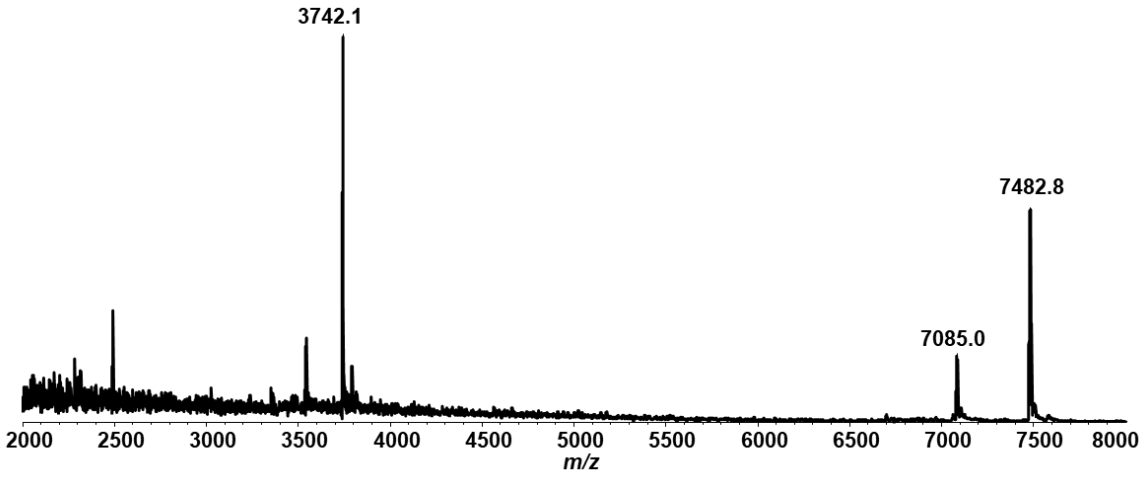


Figure S8:

A: HPLC profile of the conjugation reaction of RNA **G_1-R_1** to Dox-SS-Py **3**.

B: IEX-HPLC and MALDI-TOF MS analyses of purified **G_1-R_1-Dox** conjugate.

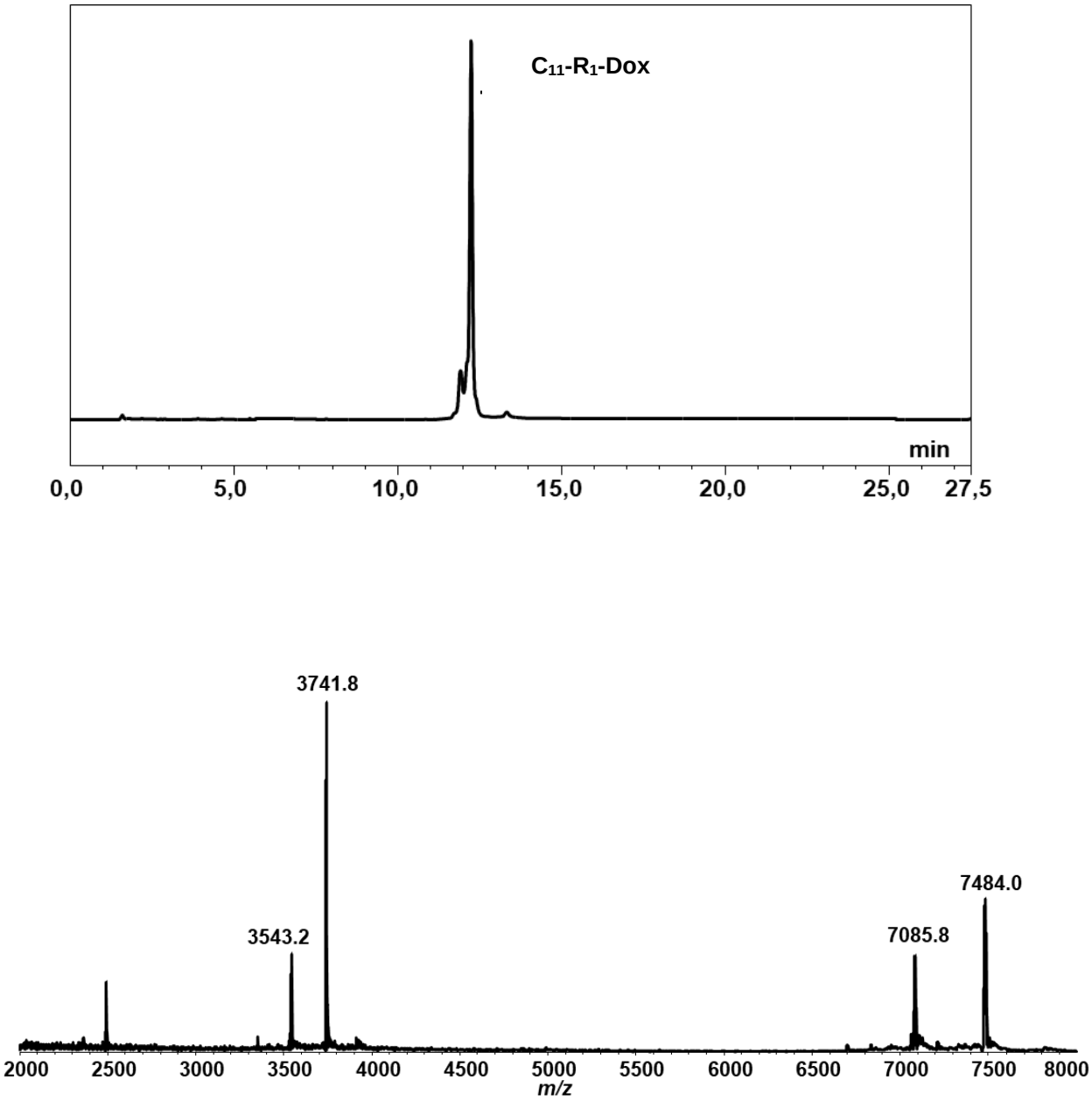


Figure S9: IEX-HPLC and MALDI-TOF MS analyses of purified **C_{11}-R_1-Dox**

IEX-HPLC analysis conditions: DNAPac® PA100, 4 x 250 mm, elution with a 20 min linear gradient of 40 to 100% of B in eluent A. Column temperature 75 °C. Flow rate 1.5 mL.min^{-1}. λ 260 nm.

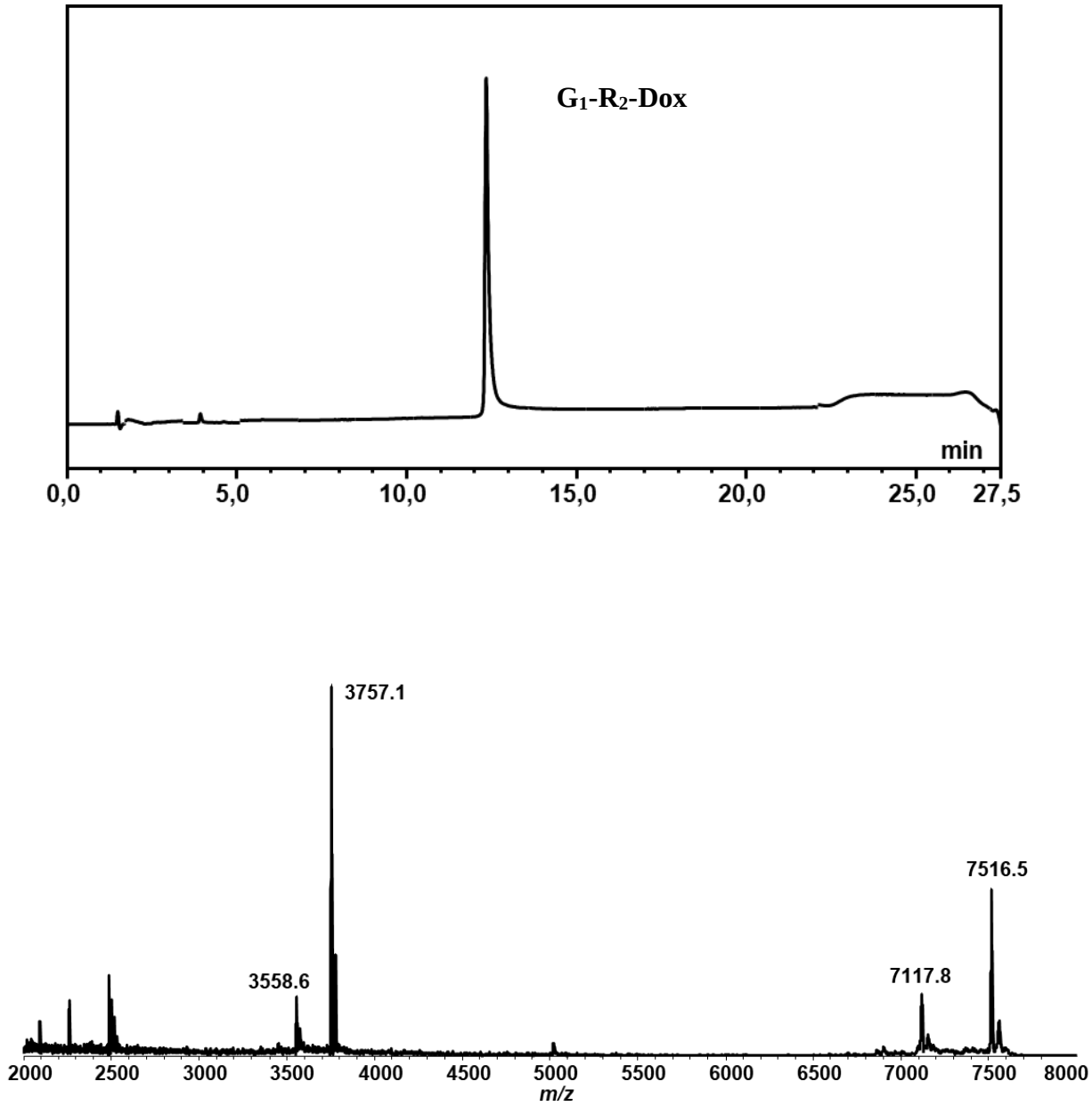


Figure S10: IEX-HPLC and MALDI-TOF MS analyses of purified G_1-R_2-Dox conjugate

IEX-HPLC analysis conditions: DNAPac® PA100, 4 x 250 mm, elution with a 20 min linear gradient of 30 to 90% of B in eluent A. Column temperature 75 °C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

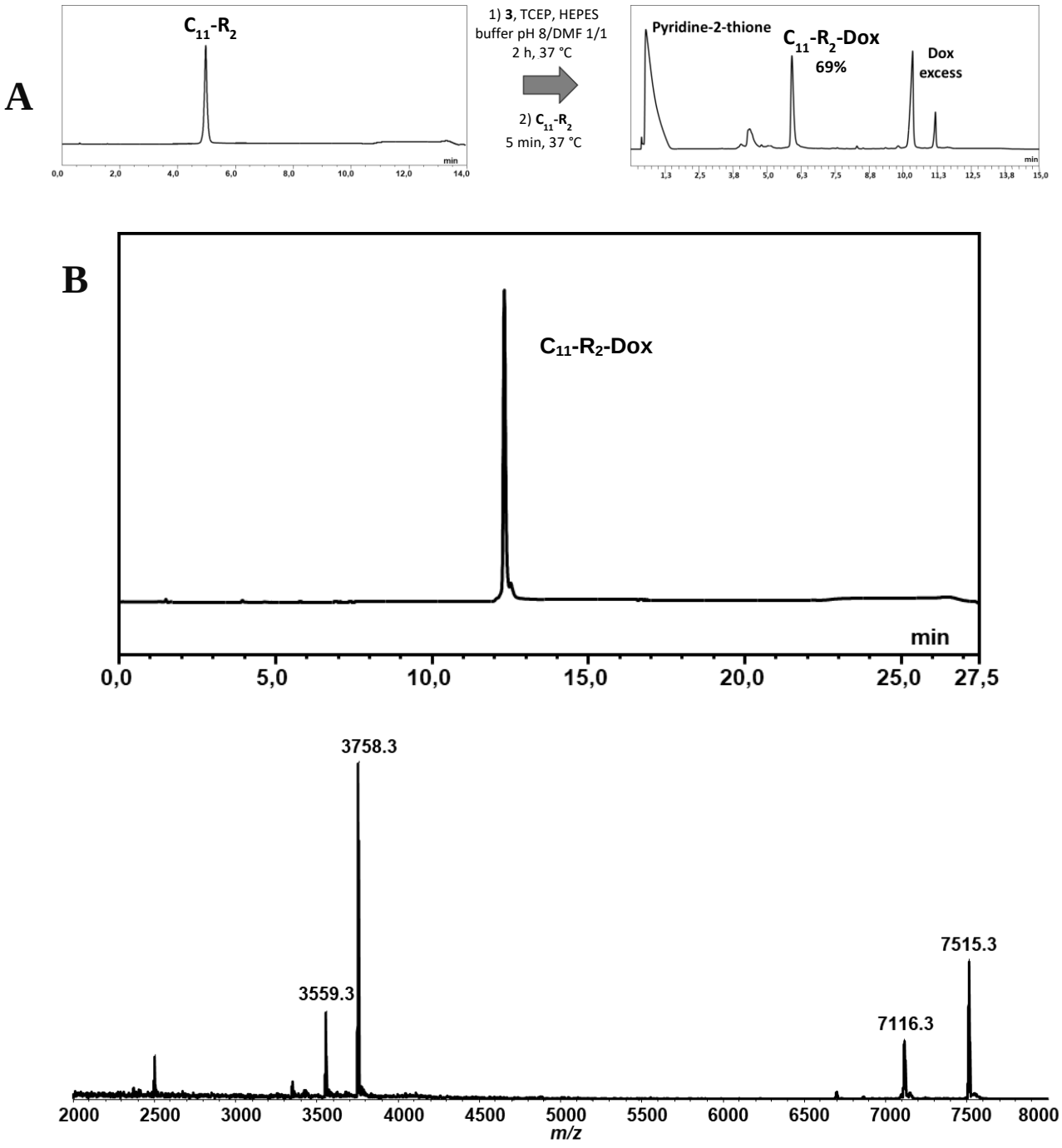


Figure S11:

A: HPLC profile of solution phase conjugation of **C_{11}-R_2** with Dox-SS-Py derivative **3**.

*RP-chromatogram of **C_{11}-R_2** (left), and of crude conjugation mixture **C_{11}-R_2-Dox** (right). Conditions: ThermoFisher Scientific LC Accucore® C18, 100Å, 2.1 x 50 mm, elution with a 10 min linear gradient of 0% to 50% of B (12.5 mM TEAAc in 1% ACN) in eluent A (12.5 mM TEAAc in 80% ACN). Column temperature 30°C. Flow rate 1.0 mL.min^{-1}. UV monitoring at 260 nm (percentage of peak area corresponding to the Dox-RNA conjugate in RNA crude material)*

B: IEX-HPLC and MALDI-TOF MS analyses of purified **C_{11}-R_2-Dox**

IEX-HPLC analysis conditions: DNAPac® PA100, 4 x 250 mm, elution with a 20 min linear gradient of 30 to 100% of B in eluent A. Column temperature 75 °C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

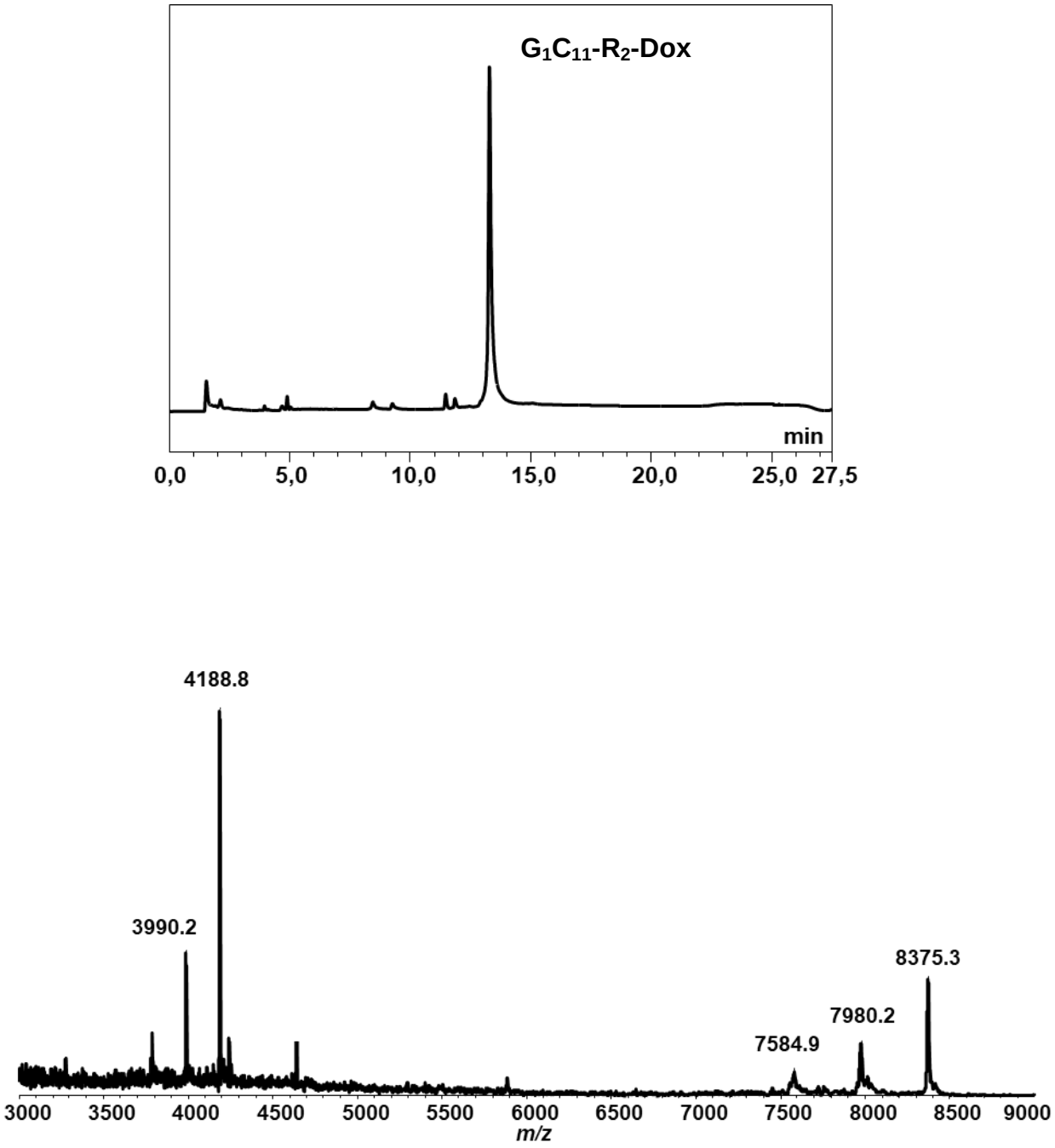


Figure S12: IEX-HPLC and MALDI-TOF MS analyses of purified **G_1C_{11}-R_2-Dox** conjugate

IEX-HPLC analysis conditions: DNAPac® PA100, 4 x 250 mm, elution with a 20 min linear gradient of 40 to 100% of B in eluent A. Column temperature 75 °C. Flow rate 1.0 mL.min^{-1}. λ 260 nm.

Enzymatic Stability of Dox-siRNAs in human serum

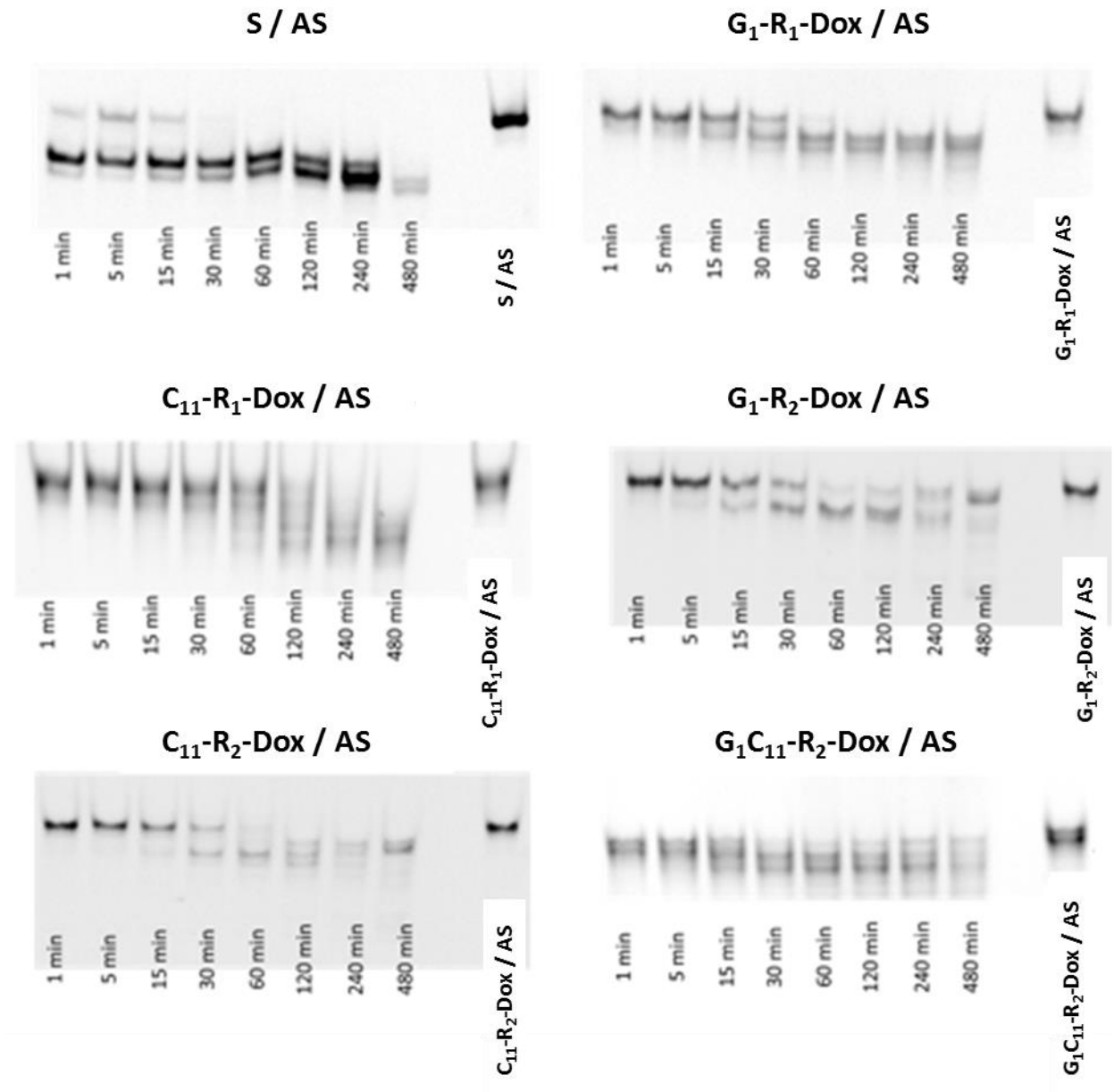


Figure S13: Gel electrophoresis of Dox-siRNA conjugates incubated in 10% human serum AB type over an 8 h period at 37 °C.