Supporting information

for

Solid-phase synthesis of peptide Mn(I)–carbonyl bioconjugates and their CO release upon visible light activation

Yi Zhou, Yonglu Chen and Chunmao He*

School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou 510640, China.

E-mail: hecm@scut.edu.cn

1. NMR-spectra of SAAC

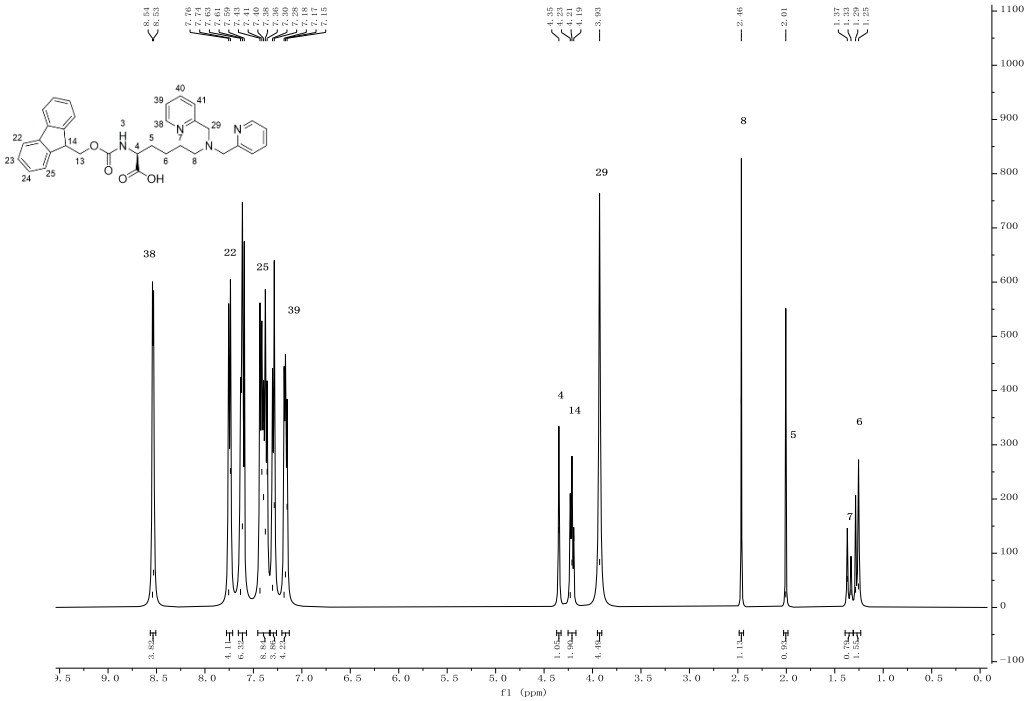
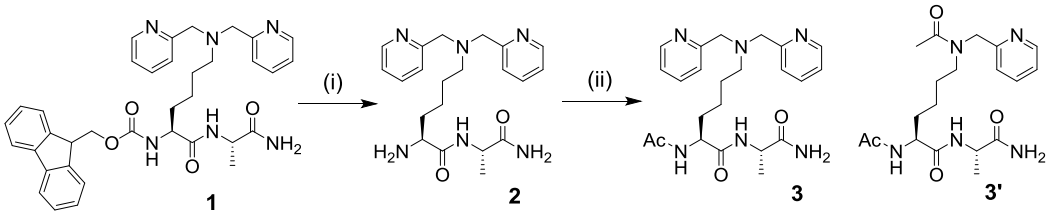


Figure S1 ^{1}H NMR spectrum of SAAC: Fmoc-Lys(dpa)-OH.

2. Side reaction of the SAAC approach



Model dipeptide **1**: Fmoc-Lys(dpa)-Ala-NH_2

compound **2**: Lys(dpa)-Ala-NH_2

compound **3**: Ac-Lys(dpa)-Ala-NH_2

compound **3'**: Ac-Lys(Ac-dpa)-Ala-NH_2

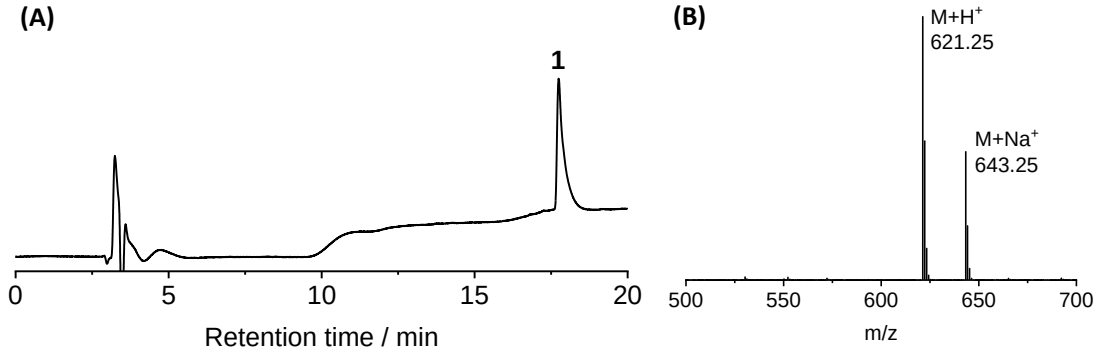


Figure S2. HPLC and ESI-MS trace of the purified **1**; (A) HPLC: t_R =17.7 min (15-65 % solvent B in solvent A over 15 min, λ = 214 nm, C18 column); (B) ESI-MS calculated for **1**: $[M + H]^+$ m/z = 621.32, $[M + Na]^+$ m/z = 643.30, found: 621.25, 643.25.

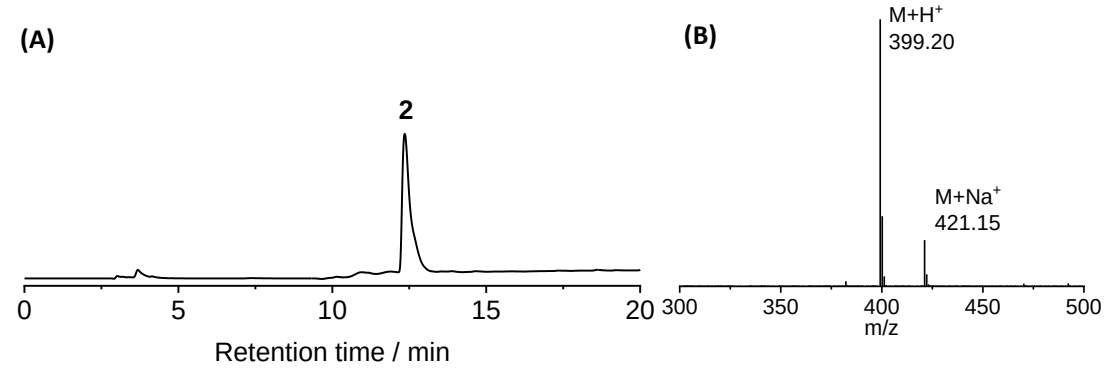


Figure S3. HPLC and ESI-MS trace of **2**; (A) HPLC: t_R =12.35 min (0-45 % solvent B in solvent A over 15 min, λ = 214 nm, C18 column); (B) ESI-MS calculated for **2**: $[M + H]^+$ m/z = 399.24, $[M + Na]^+$ m/z = 421.23, found: 399.20, 421.15.

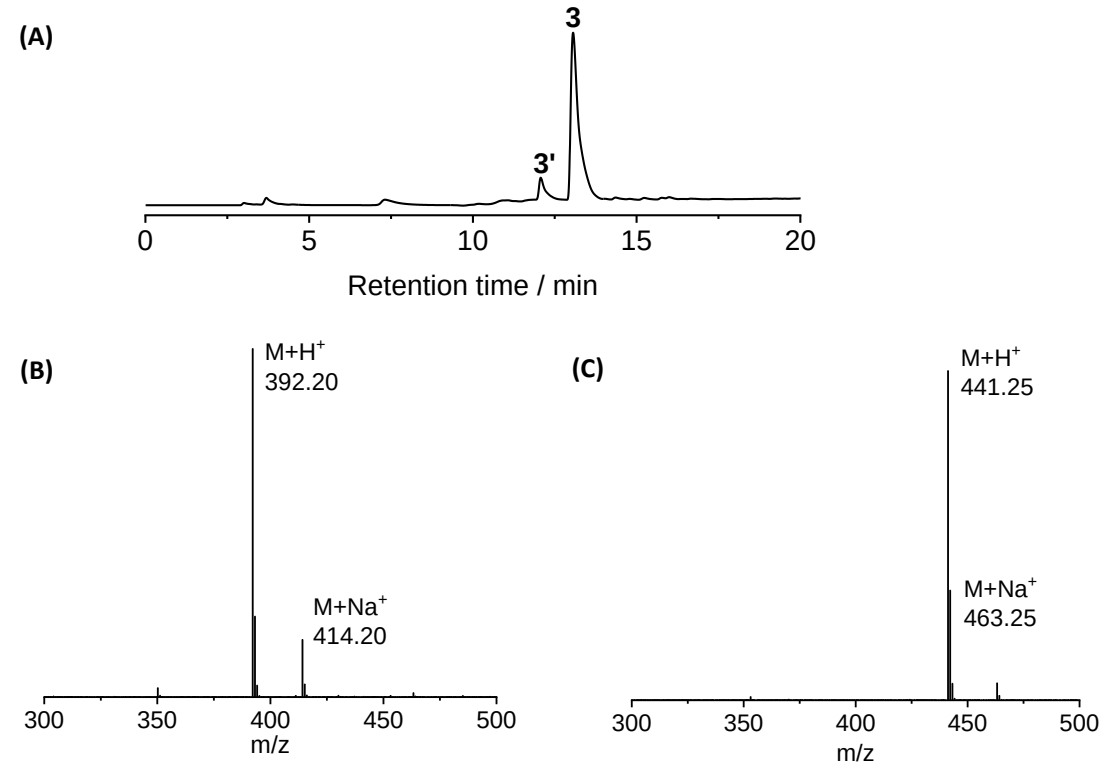
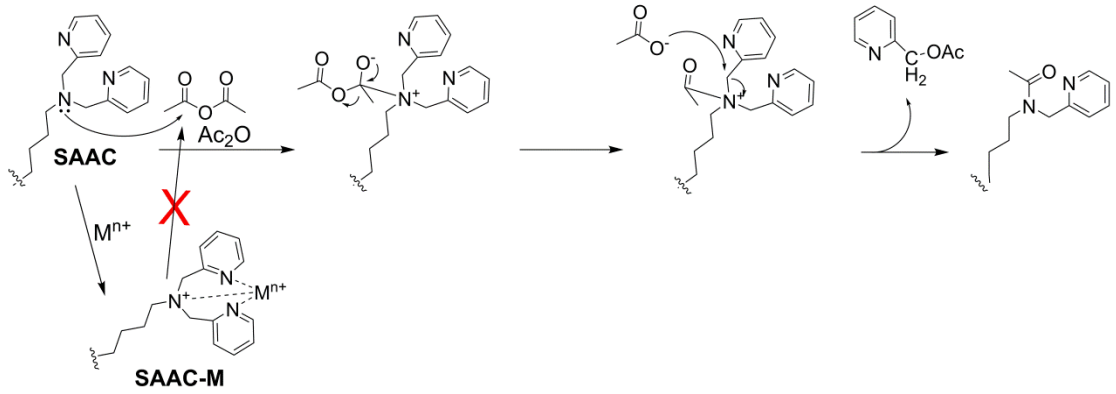


Figure S4. HPLC and ESI-MS trace of **3** and **3'**; (A) HPLC: t_{R1} =12.07 min, t_{R2} =13.06 min (0-45 % solvent B in solvent A over 15 min, λ = 214 nm, C18 column); (B) ESI-MS calculated for **3'**: $[M + H]^+$ m/z = 392.51, $[M + Na]^+$ m/z = 414.21, found: 392.20, 414.20. (C) ESI-MS calculated for **3**: $[M + H]^+$ m/z = 441.55, $[M + Na]^+$ m/z = 463.24, found: 441.25, 463.25.



Scheme S1. Proposed mechanism of the Ac_2O caused side-reaction in the SAAC strategy.

3. Synthesis of TAT-MnCO bioconjugates

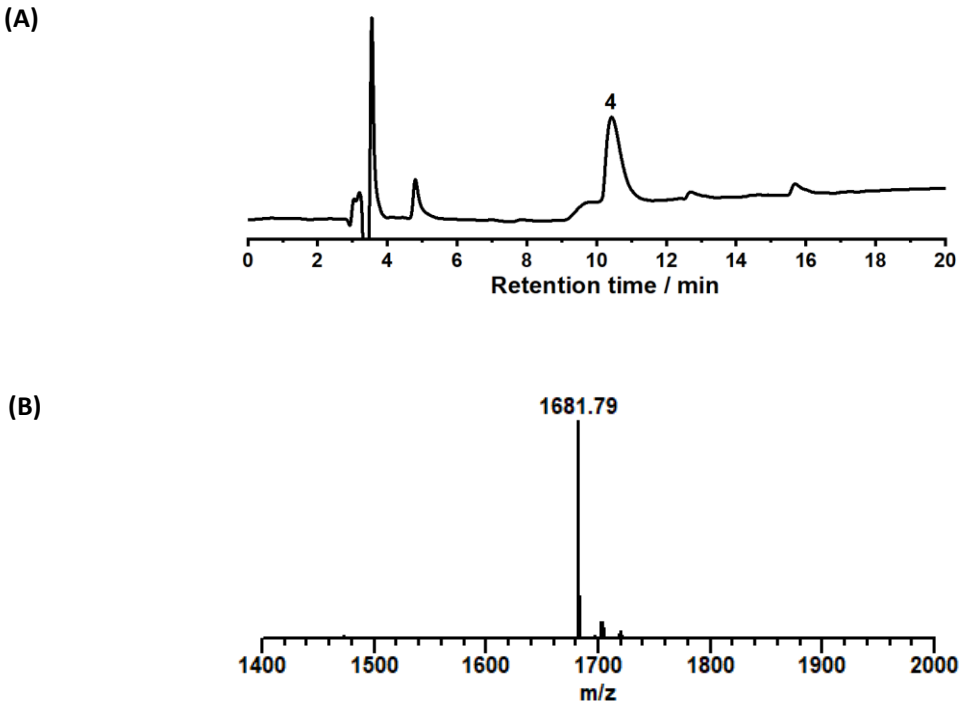


Figure S5. Analytical HPLC trace and MALDI-TOF-MS of the cleaved peptide from resin **4**. (A) Analytical HPLC of **4**: t_R=10.43 min (15-65 % solvent B in solvent A over 15 min); (B) MALDI-TOF-MS found 1681.79 Da of **4**, calculated: 1684.99 Da.

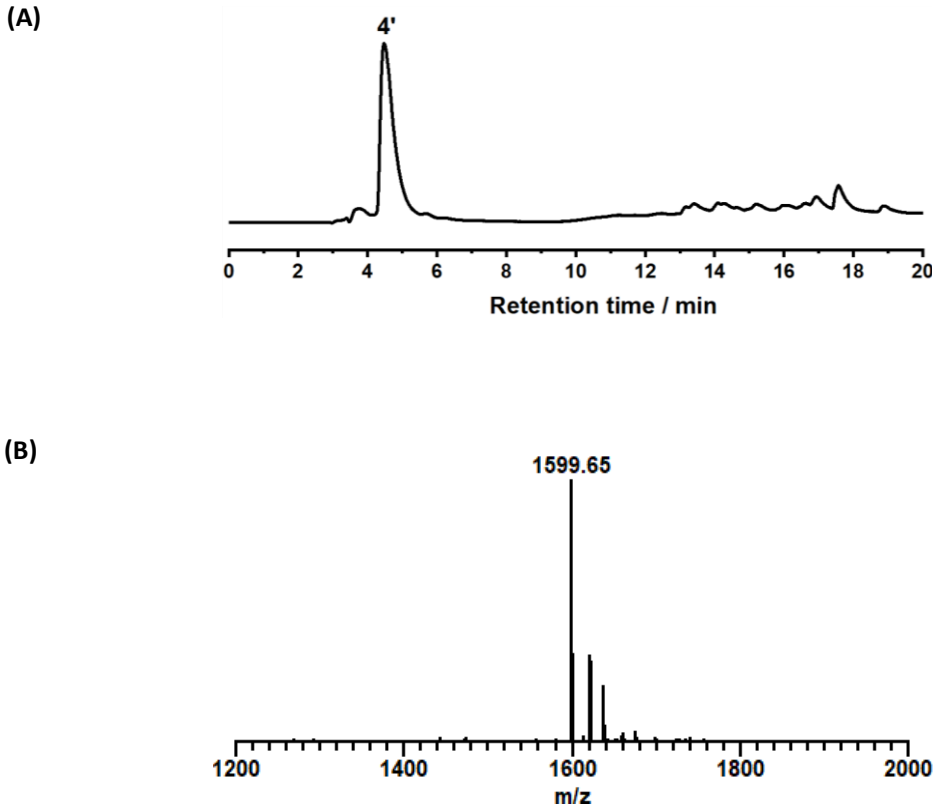


Figure S6. Analytical HPLC trace and MALDI-TOF-MS of the cleaved peptide from resin **4'**. (A) Analytical HPLC of **4'**: t_R=4.47 min (15-65 % solvent B in solvent A over 15min); (B) MALDI-TOF-MS found 1599.65 Da of **4'**, calculated: 1600.91 Da.

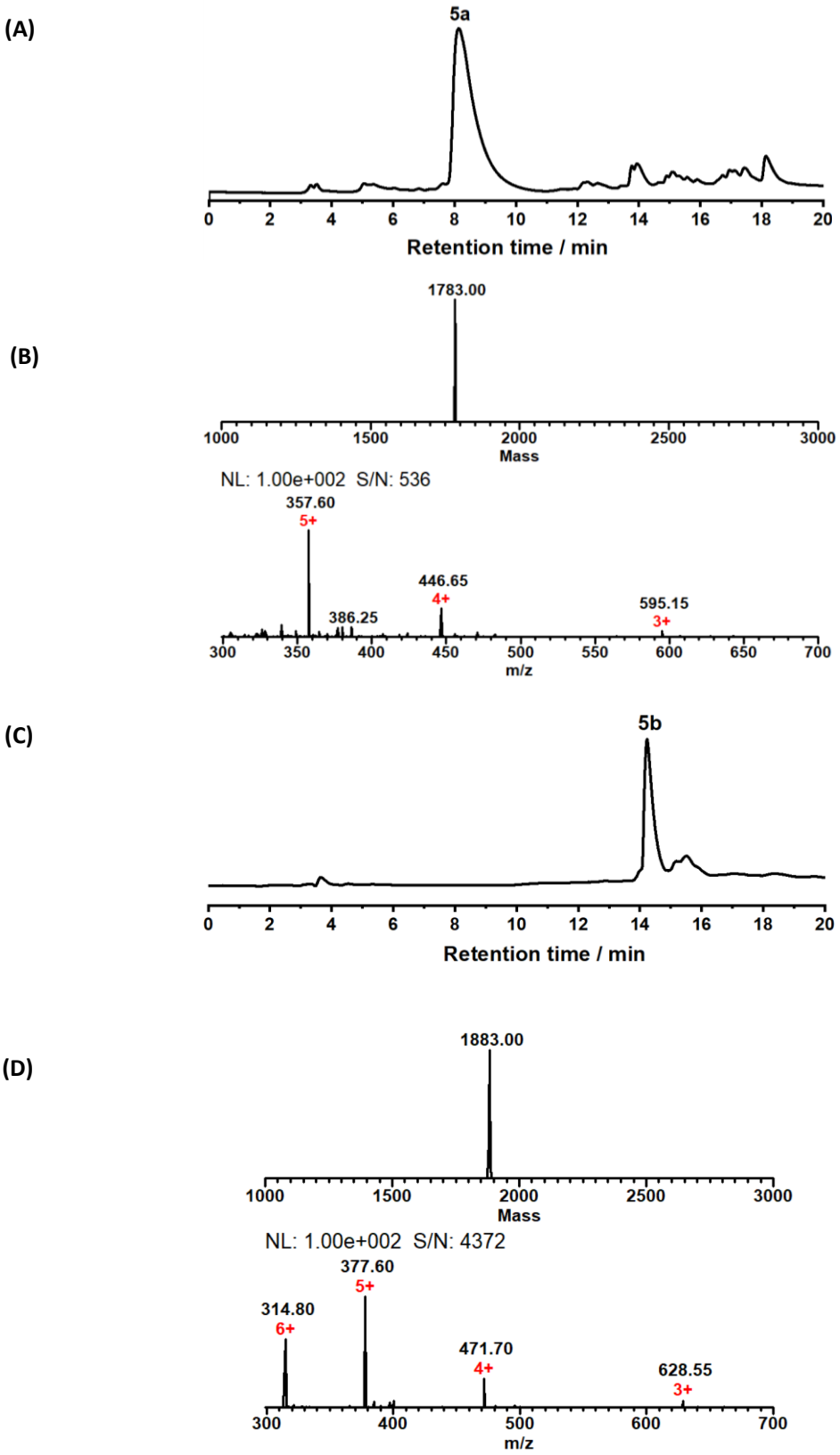


Figure S7. Analytical HPLC trace and LCMS of the cleaved peptide from resin **5a** and **5b**. (A) Analytical HPLC of **5a**: t_R=8.70 min (15-65 % solvent B in solvent A over 15min); (B) ESI-MS found of **5a**, calculated: 1783.14 Da; (C) Analytical HPLC of **5b**: t_R=14.23 min (15-65 % solvent B in solvent A over 15 min); (D) ESI-MS found 1883.00 Da of **5b**, calculated: 1883.14 Da.

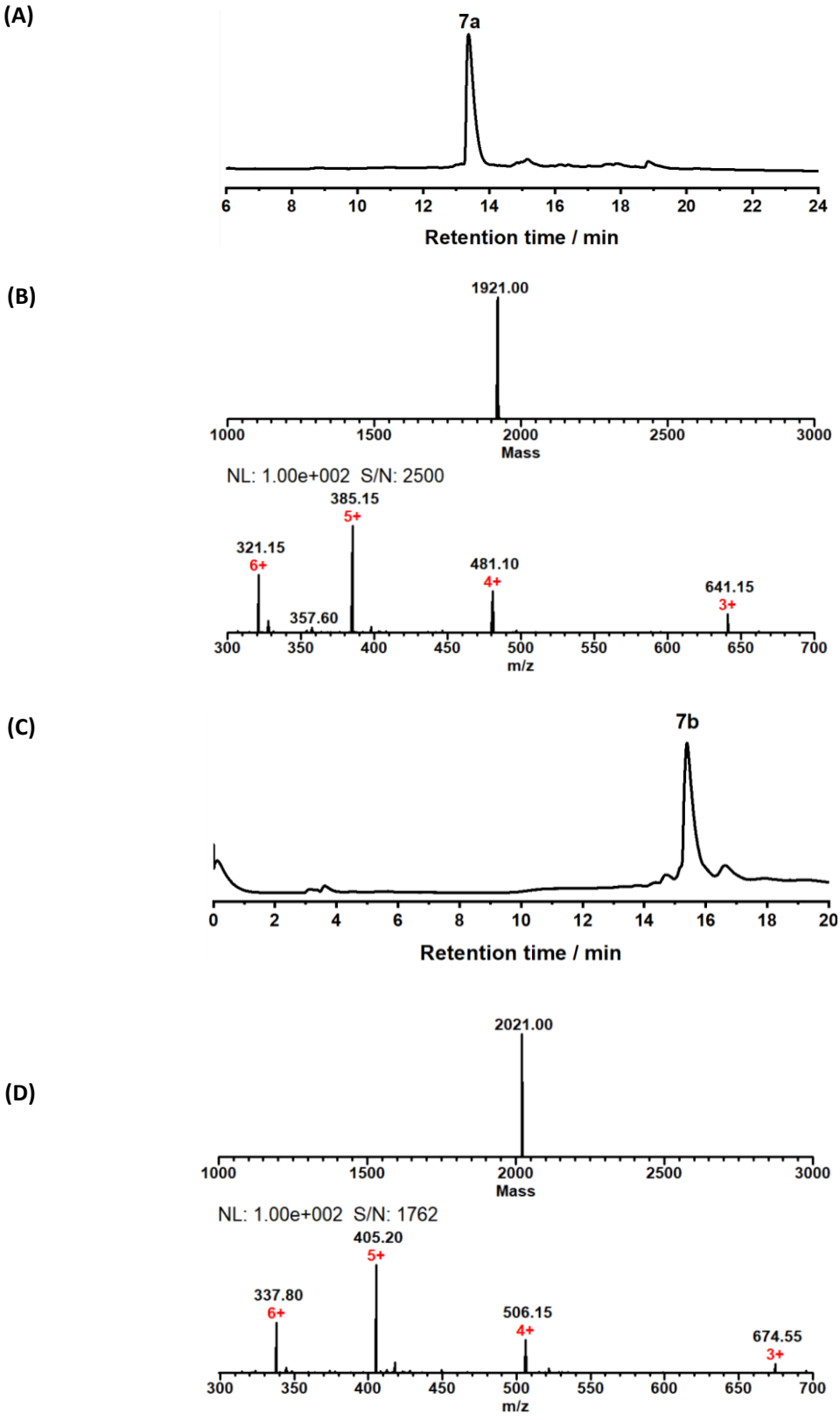


Figure S8. Analytical HPLC trace and LCMS of **7a** and **7b**. (A) Analytical HPLC of **7a**: t_R=13.37 min (15-65 % solvent B in solvent A over 15min); (B) ESI-MS found 1921.00 Da of **7a**, calculated: 1922.10 Da; (C) Analytical HPLC of **7b**: t_R= 15.39 min (15-65 % solvent B in solvent A over 15min); (D) ESI-MS found 2021.00 Da of **7b**, calculated:2022.23 Da.

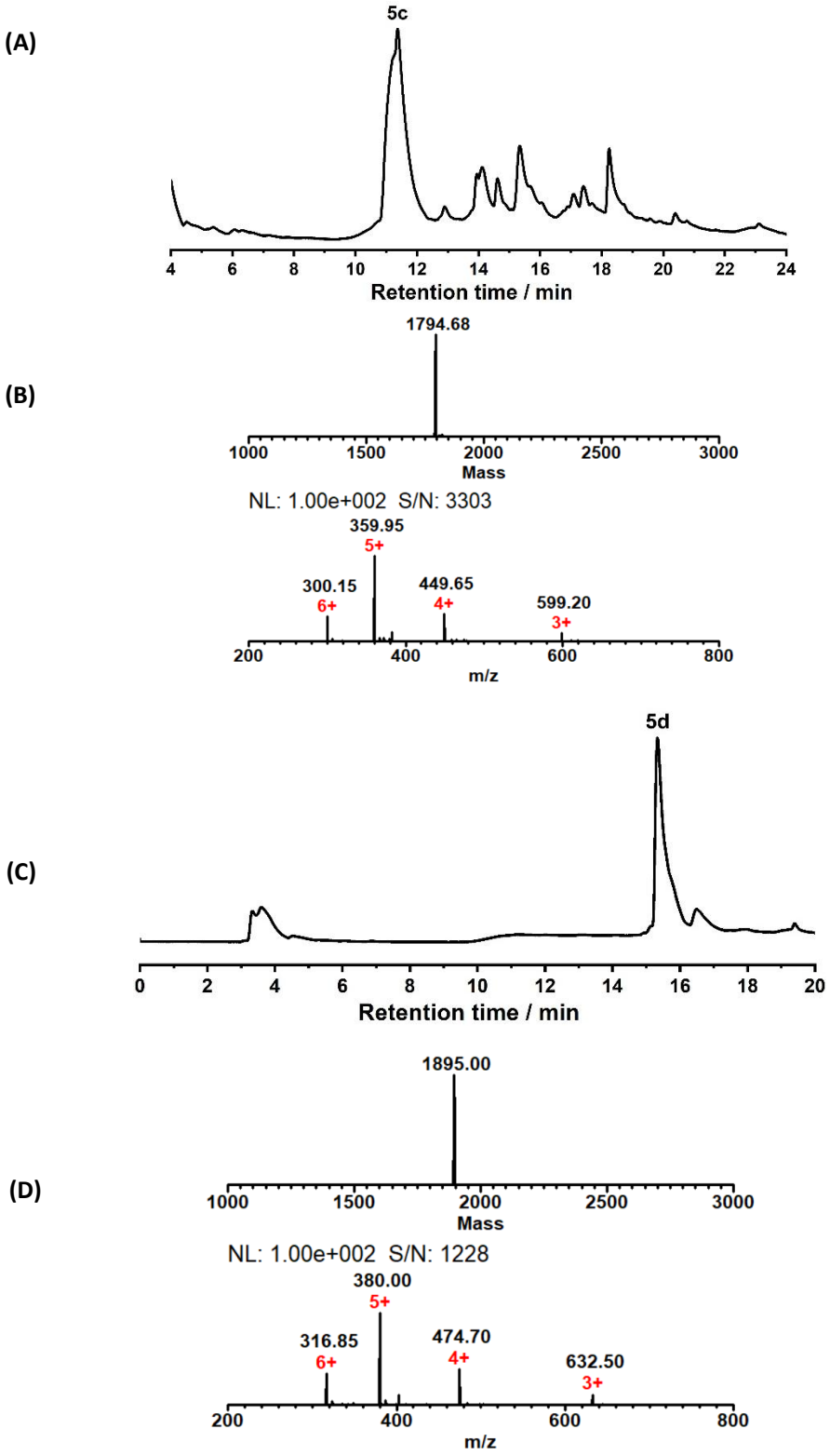


Figure S9. Analytical HPLC trace and LCMS of **5c** and **5d**. (A) Analytical HPLC of **5c**: t_R=11.37 min (15-65 % solvent B in solvent A over 15min); (B) ESI-MS found 1794.68 Da of **5c**, calculated: 1795.18 Da; (C) Analytical HPLC of **5d**: t_R= 15.33 min (15-65 % solvent B in solvent A over 15min); (D) ESI-MS found 1895.00 Da of **5d**, calculated:1895.30 Da.

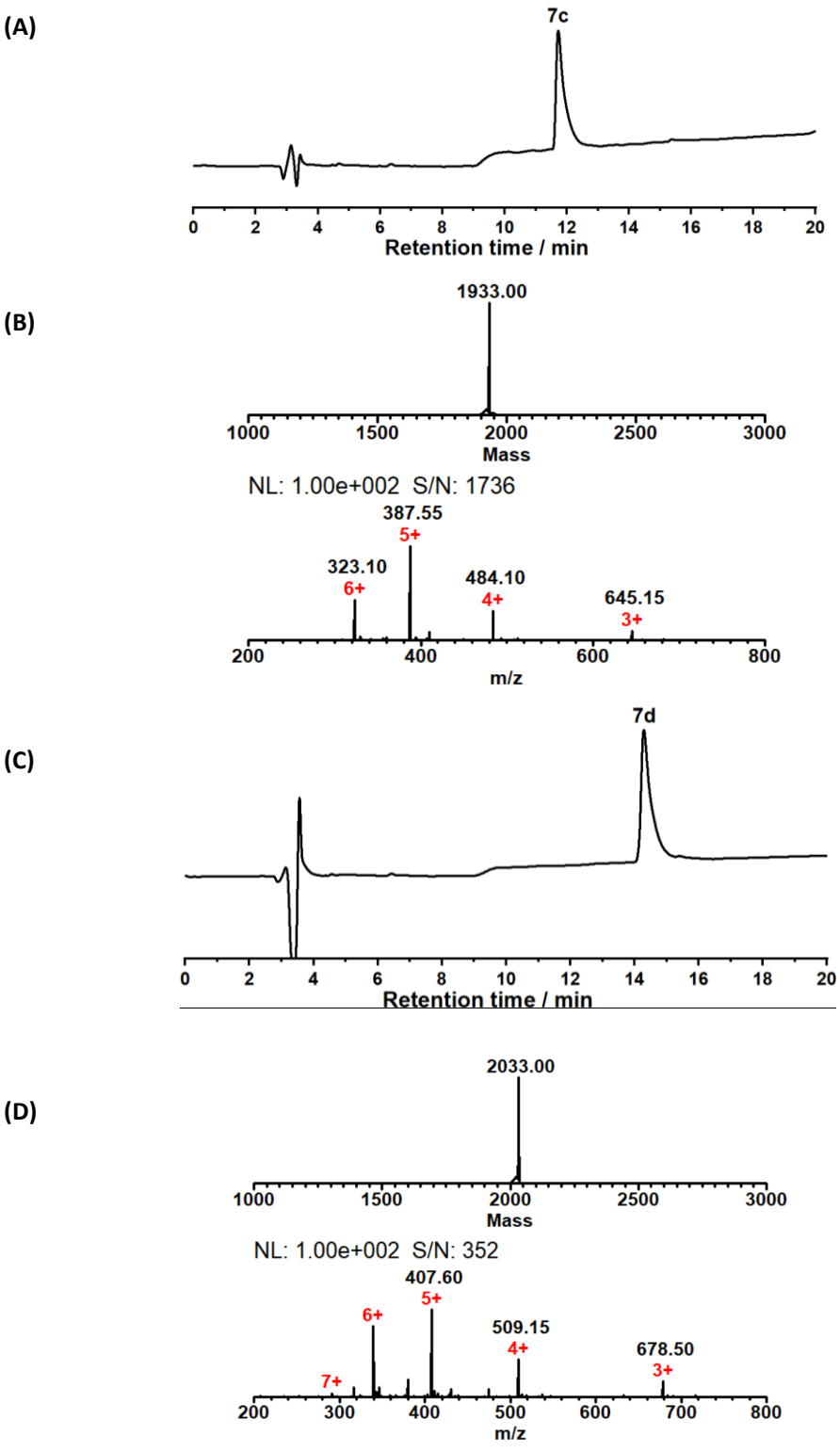


Figure S10. Analytical HPLC trace and LCMS of **7c** and **7d**. (A) Analytical HPLC of **7c**: t_R=11.72 min (15-65 % solvent B in solvent A over 15min); (B) ESI-MS found 1933.00 Da of **7c**, calculated: 1932.90 Da; (C) Analytical HPLC of **7d**: t_R= 14.29 min (15-65 % solvent B in solvent A over 15min); (D) ESI-MS found 2033.00 Da of **7d**, calculated:2032.23 Da.

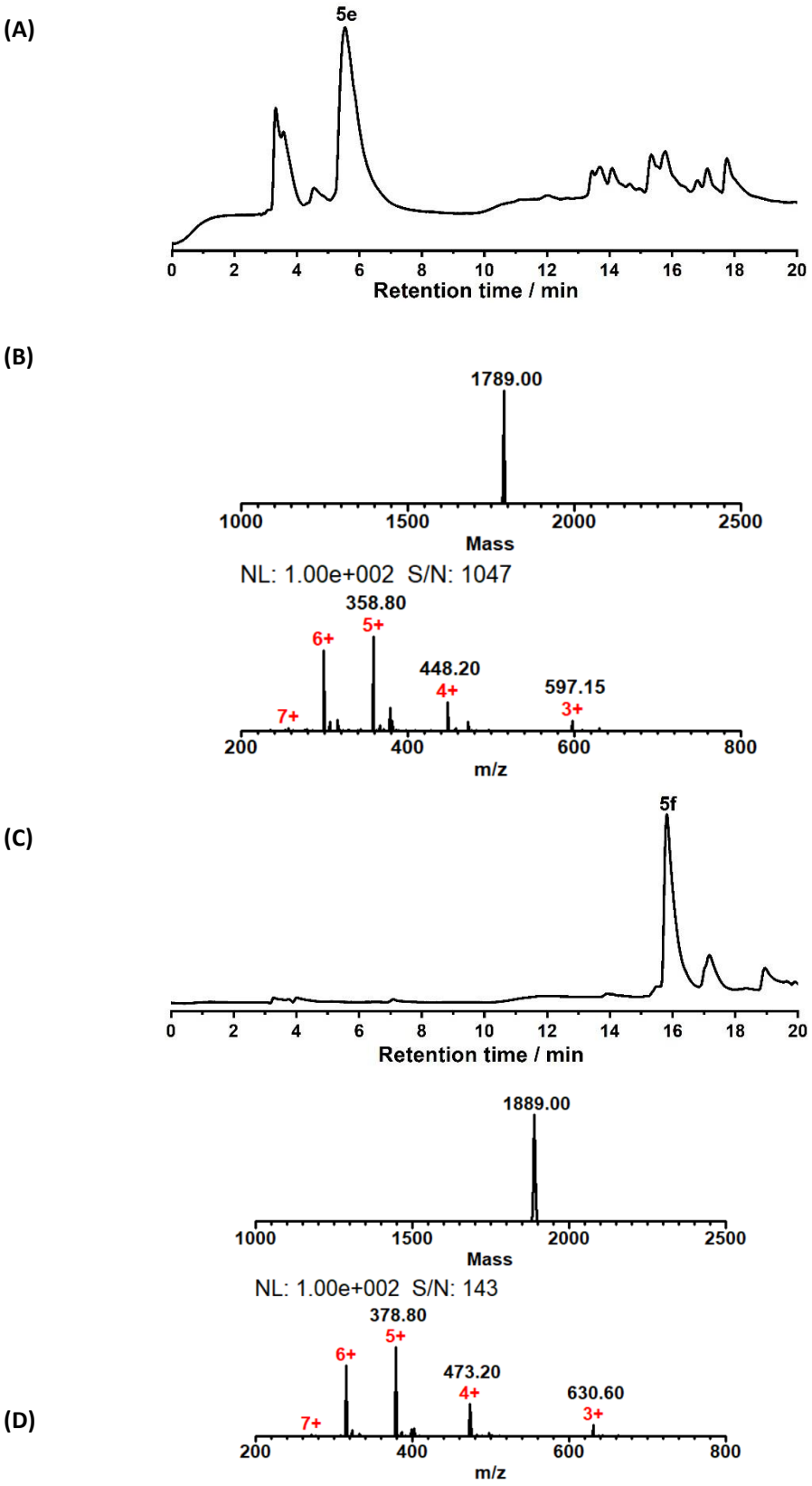


Figure S11. Analytical HPLC trace and LCMS of **5e** and **5f**. (A) Analytical HPLC of **5e**: t_R=5.53 min (15-65 % solvent B in solvent A over 15min); (B) ESI-MS found 1789.07 Da of **5e**, calculated: 1789.07 Da; (C) Analytical HPLC of **5f**: t_R= 15.81 min (15-65 % solvent B in solvent A over 15min); (D) ESI-MS found 1889.00 Da of **5f**, calculated:1889.10 Da.

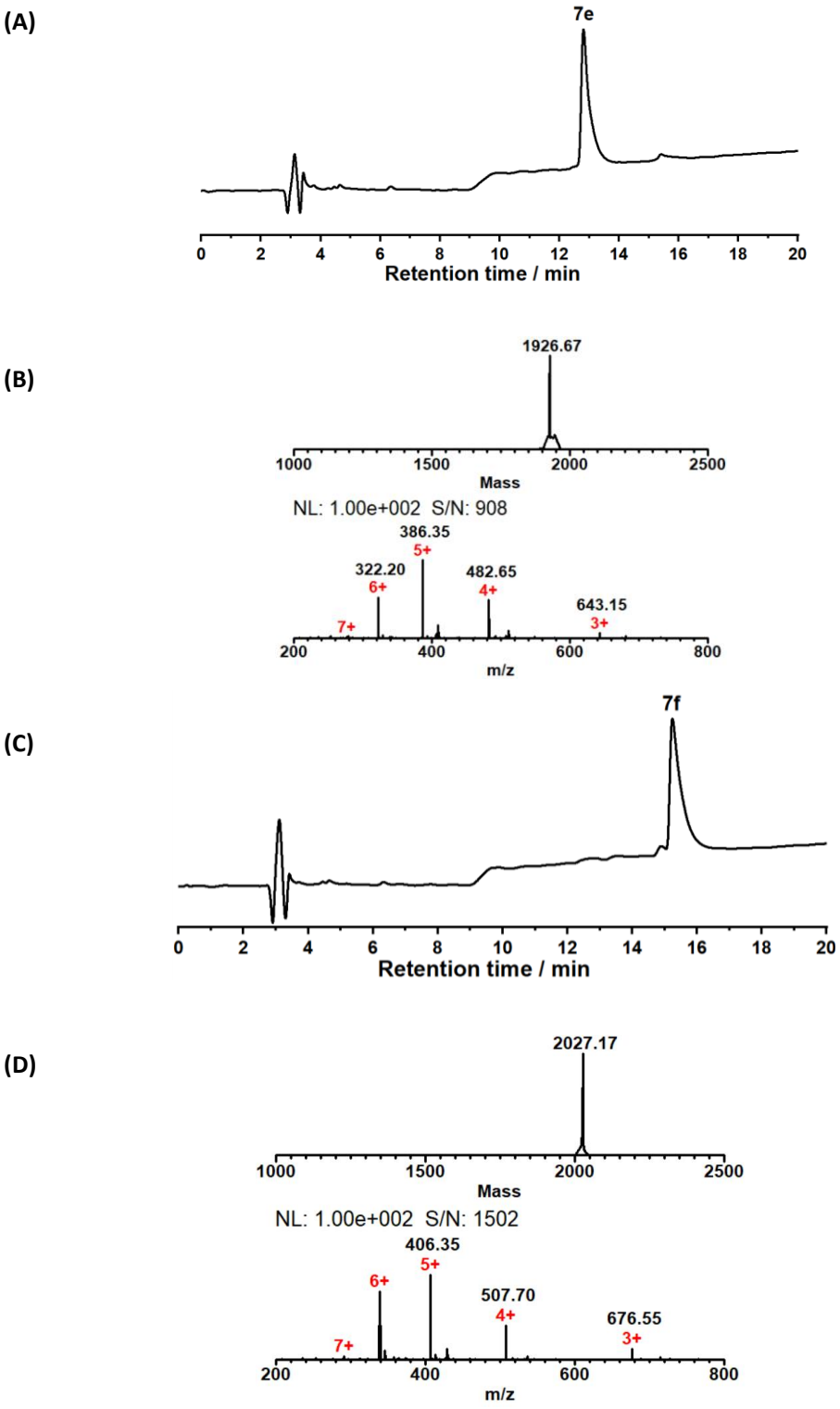


Figure S12. Analytical HPLC trace and LCMS of **7e** and **7f**. (A) Analytical HPLC of **7e**: t_R=12.8 min (15-65 % solvent B in solvent A over 15min); (B) ESI-MS found 1926.67 Da of **7e**, calculated: 1927.00 Da; (C) Analytical HPLC of **7f**: t_R= 15.34min (15-65 % solvent B in solvent A over 15min); (D) ESI-MS found 2027.17 Da of **7f**, calculated:2027.00 Da.

6.FT-IR spectra characterization (with Gaussian fittings)

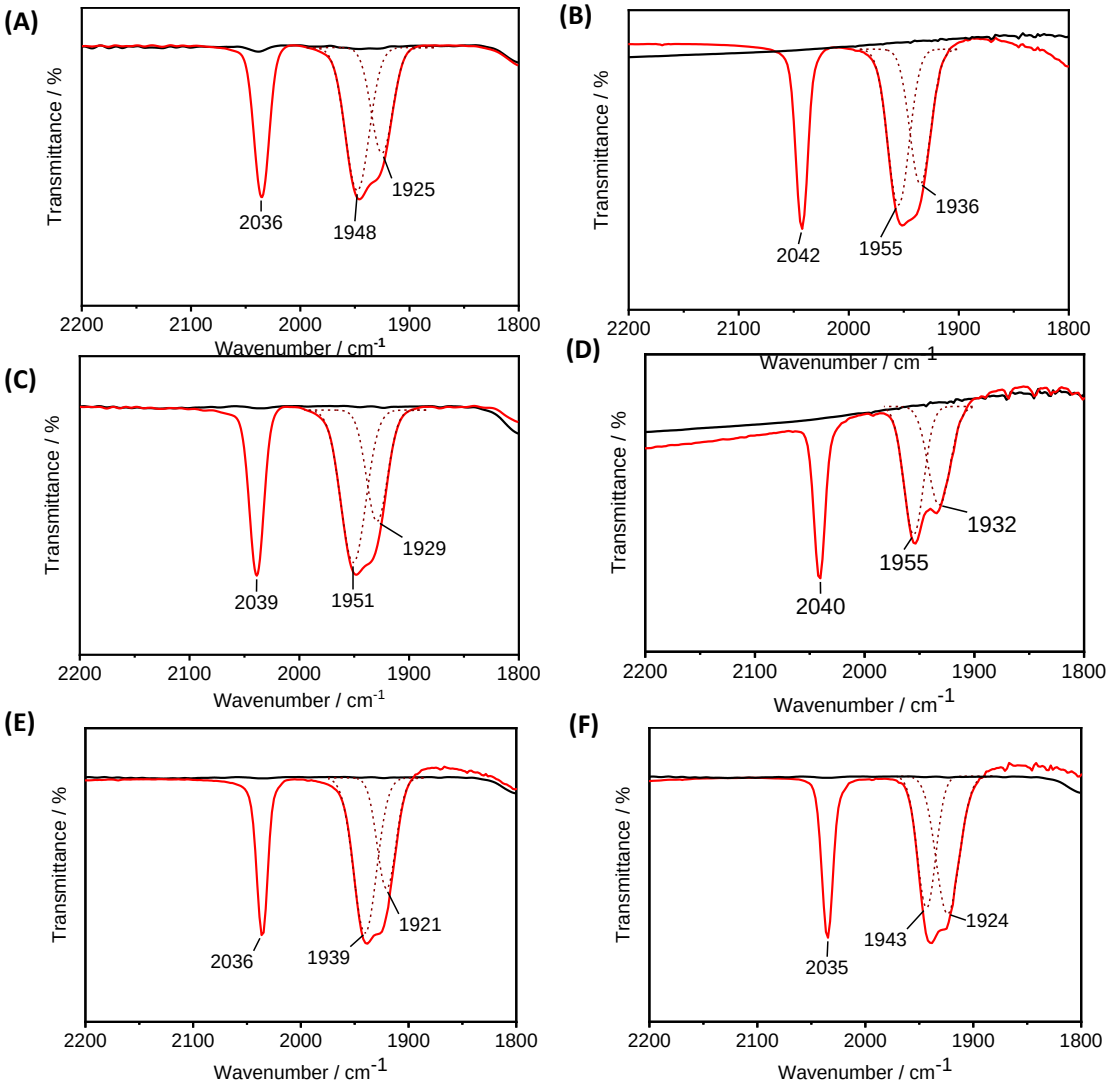


Figure S13. (A)-(F): FT-IR spectra of**7a-7f** in KBr pellets. The Gaussian fittings allowed for the assignment of the two un-resolved ν_{CO} bands are shown as dashed lines.

7. Stability test

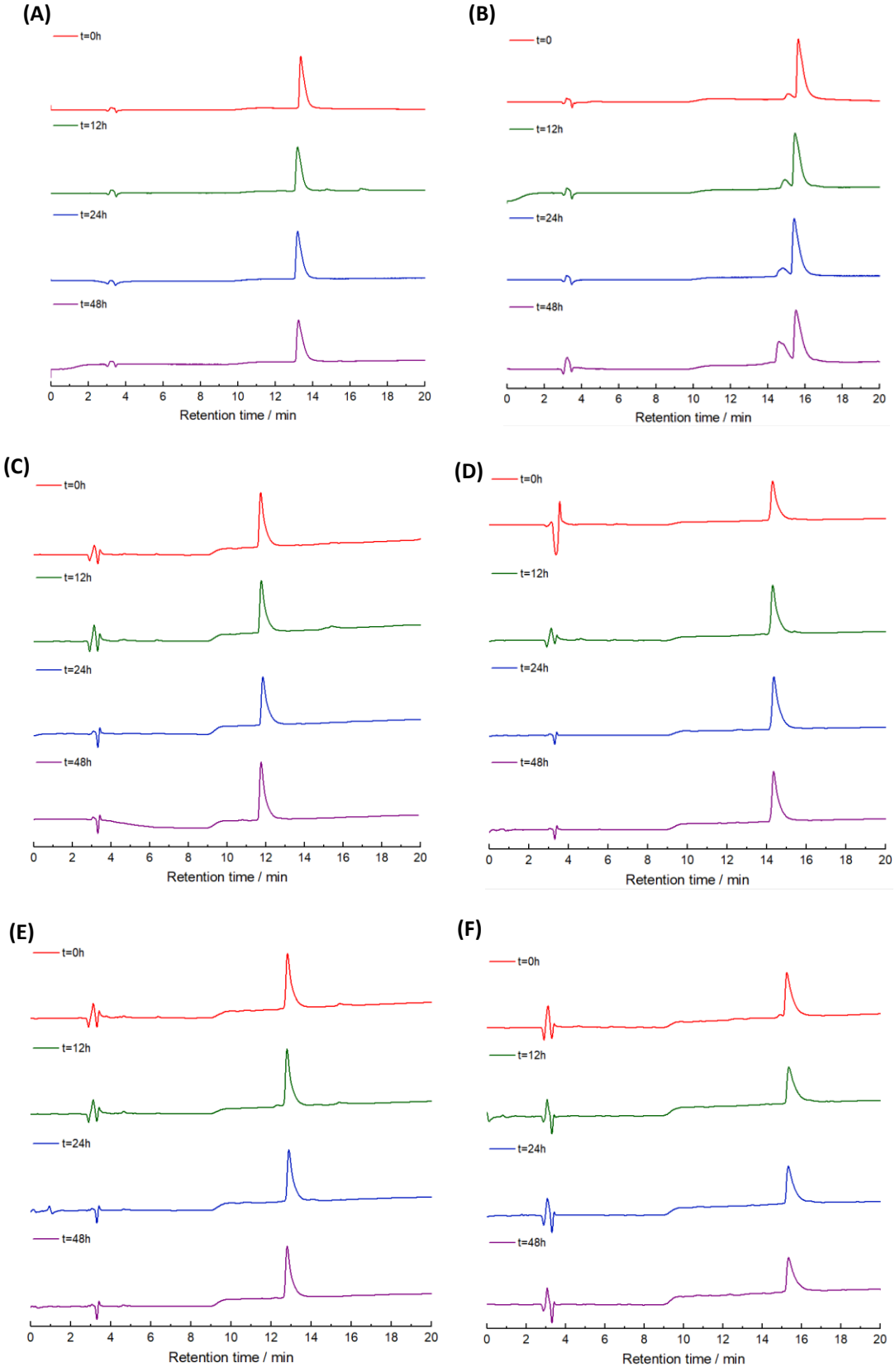


Figure S14. (A)-(F) HPLC traces of **7a**-**7f** incubation in 10mM PBS (pH 7.4) in the dark.

8.Myoglobin assay

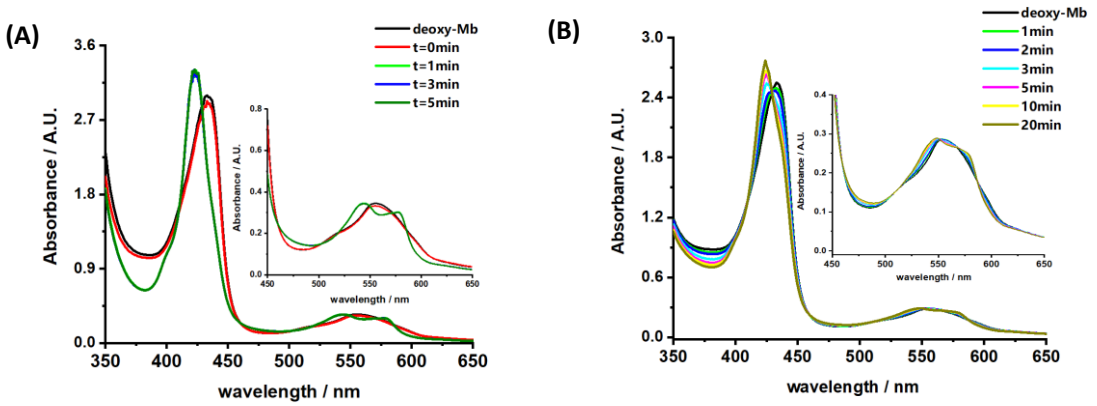


Figure S15. Time course of absorption spectral change of deoxy-Mb at RT (final concentration: 40 μM, in 10 mM PBS buffer (pH 7.4)) in a solution containing **7a** (final concentration 10 μM, in 10 mM PBS buffer (pH 7.4)) after Violet light (A) and Blue light (B) irradiation.

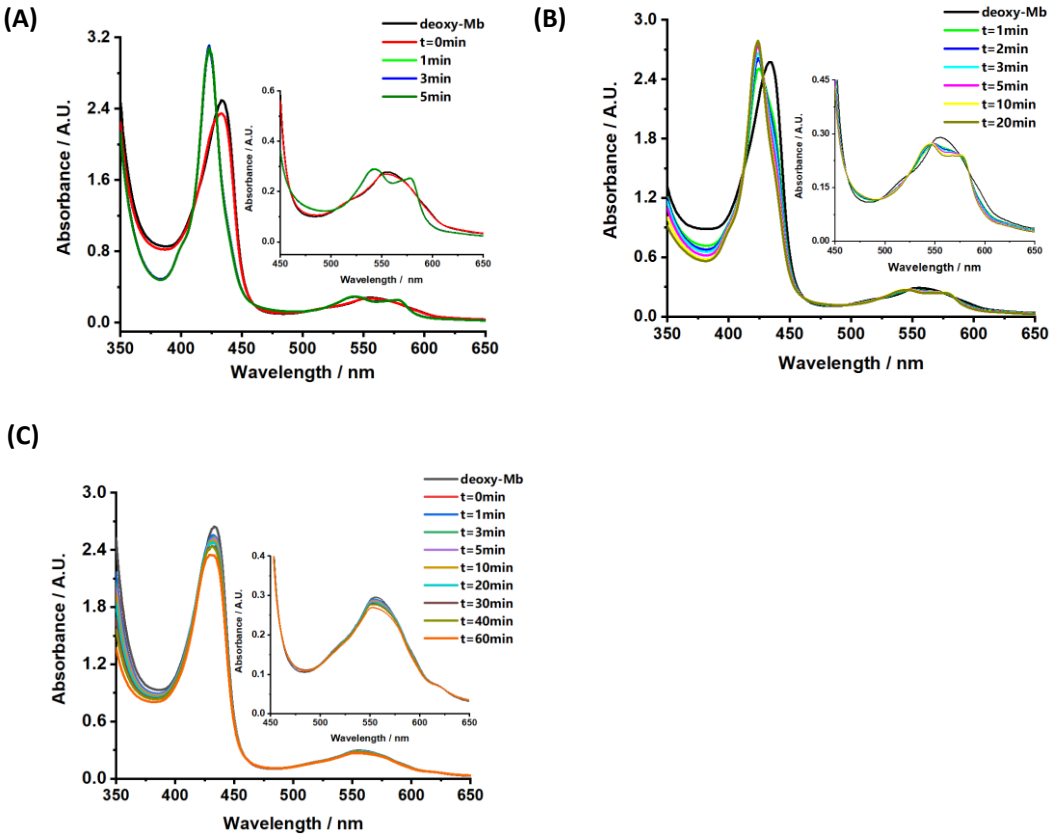


Figure S16. Time course of absorption spectral change of deoxy-Mb at r.t. (final concentration: 40 μM, in 10 mM PBS buffer (pH 7.4)) in a solution containing **7b** (final concentration 10 μM, in 10 mM PBS buffer (pH 7.4)) after violet light (A), blue light (B) and green light (C) irradiation.

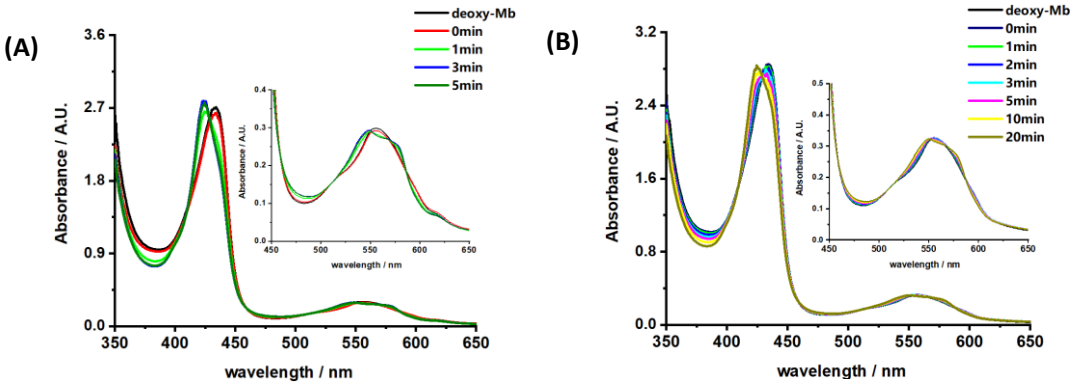


Figure S17. Time course of absorption spectral change of deoxy-Mb at RT (final concentration: 40 μM, in 10 mM PBS buffer (pH 7.4)) in a solution containing **7c** (final concentration 10 μM, in 10 mM PBS buffer (pH 7.4)) after Violet light (A) and Blue light (B) irradiation.

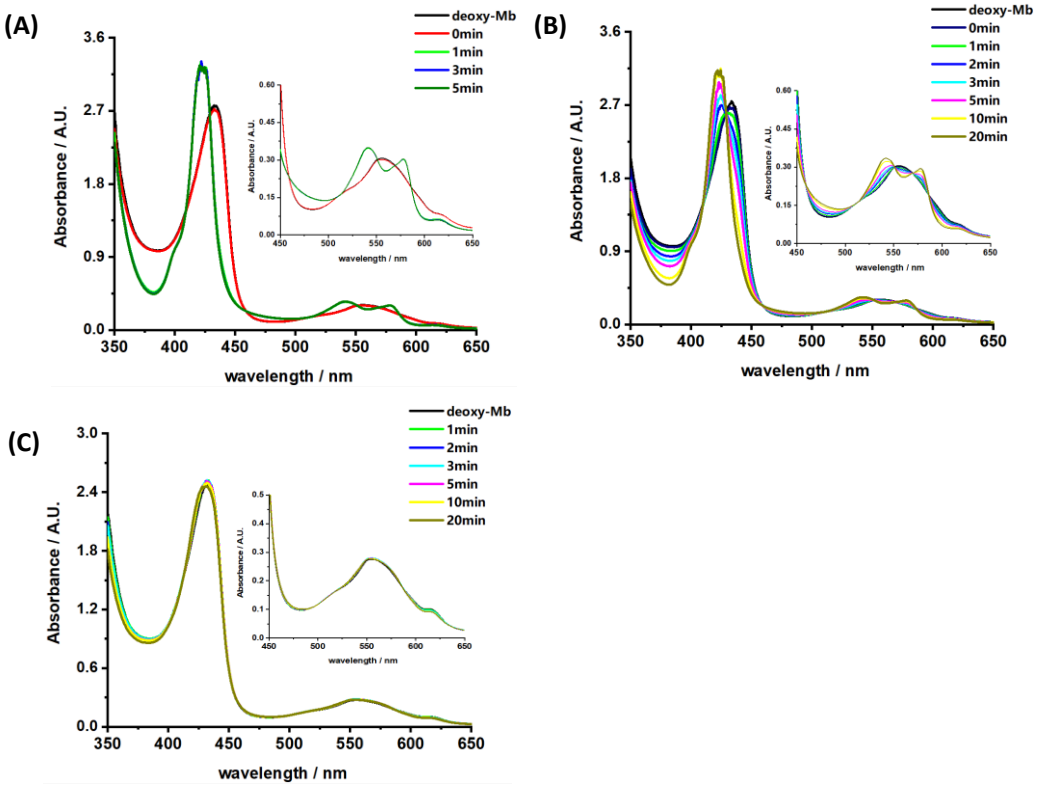


Figure S18. Time course of absorption spectral change of deoxy-Mb at r.t. (final concentration: 40 μM, in 10 mM PBS buffer (pH 7.4)) in a solution containing **7d** (final concentration 10 μM, in 10 mM PBS buffer (pH 7.4)) after violet light (A), blue light (B) and green light (C) irradiation.

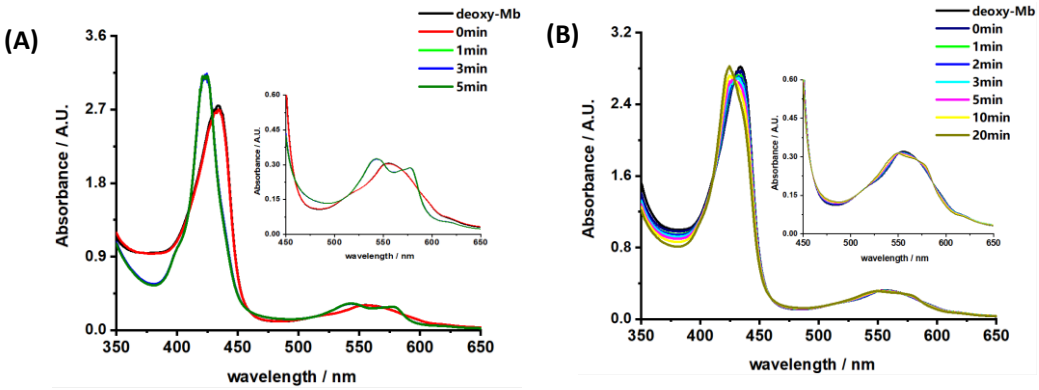


Figure S19. Time course of absorption spectral change of deoxy-Mb at RT (final concentration: 40 μM, in 10 mM PBS buffer (pH 7.4)) in a solution containing **7e** (final concentration 10 μM, in 10 mM PBS buffer (pH 7.4)) after Violet light (A) and Blue light (B) irradiation.

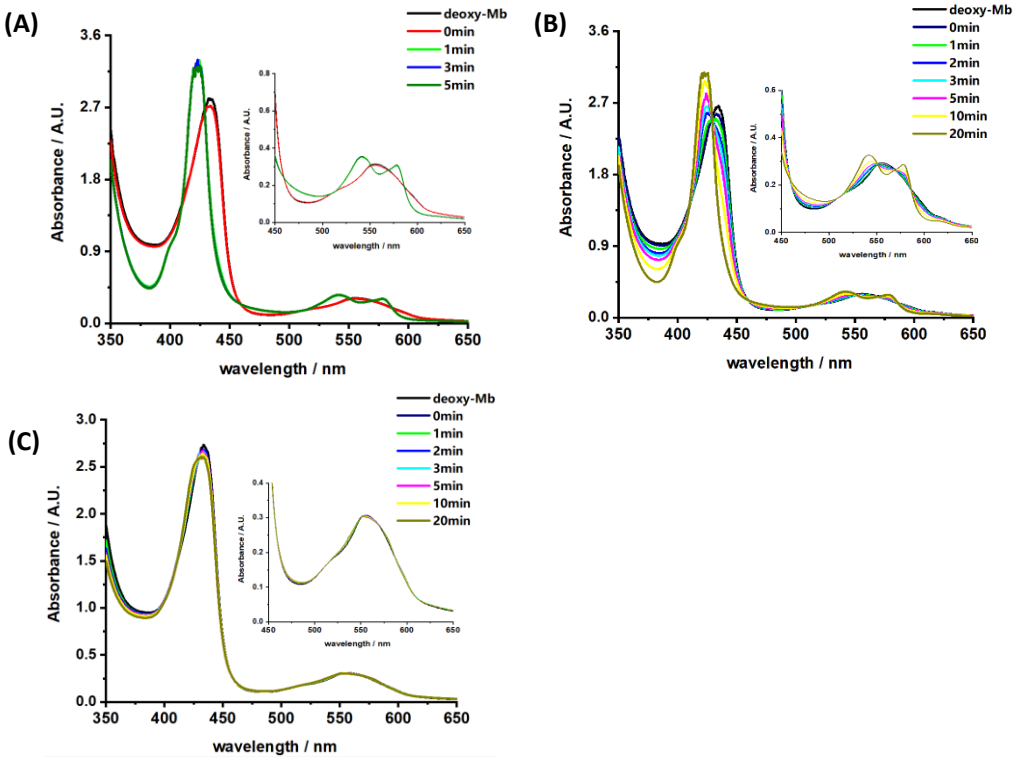


Figure S20. Time course of absorption spectral change of deoxy-Mb at r.t. (final concentration: 40 μM, in 10 mM PBS buffer (pH 7.4)) in a solution containing **7f** (final concentration 10 μM, in 10 mM PBS buffer (pH 7.4)) after violet light (A), blue light (B) and green light (C) irradiation.

9. EPR spectrum of TAT-CORM before and after irradiation

TAT-MnCO conjugates were prepared in dd-H_2O (final concentration: 5 mM). The samples were added to the capillary EPR tubes and measured. Violet light (~ 415 nm, LED, 5 W) was then irradiated to the capillary EPR tubes for 5 min, the resulting spectrum was recorded. All EPR spectra were recorded at 293 K.

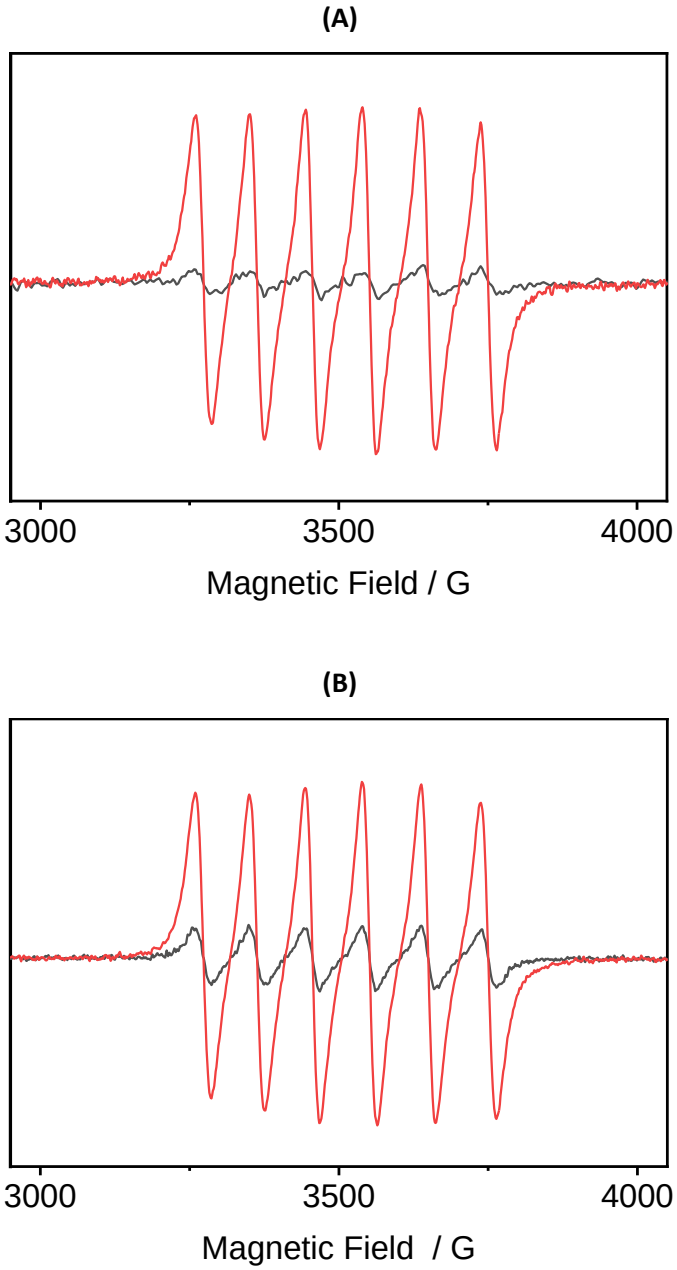


Figure S21. X-band EPR spectra (at 295 K) of **7a** (A) and **7b** (B) before (black trace) and after irradiated by violet light (5 min) in H_2O. Microwave frequency, 9.87 GHz; Modulation amplitude, 1.0 G; Modulation frequency, 100 kHz and power of the microwave source, 1.262 mW. Note that the background Mn^{II} signals in both cases (black traces) was probably caused by partial CO release during sample preparation, due to light exposure and further oxidation.