Supplementary Material

Copper-free click bioconjugation of technetium-99m complexes using strained cyclononyne derivatives

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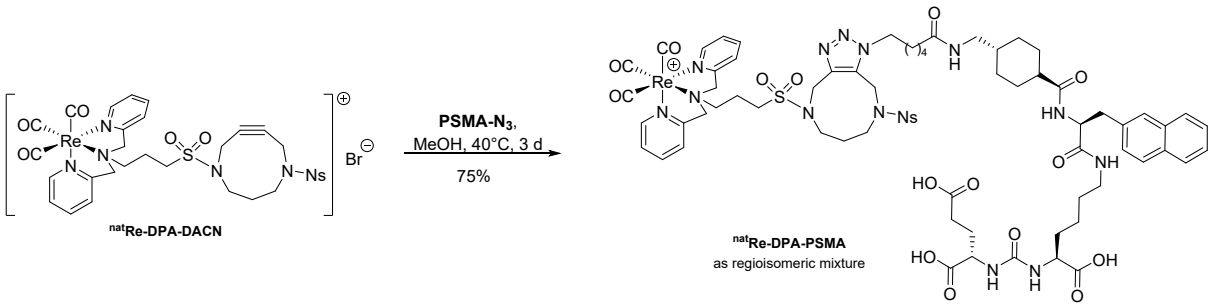
TOC

Preparation of the nonradioactive Re reference compounds

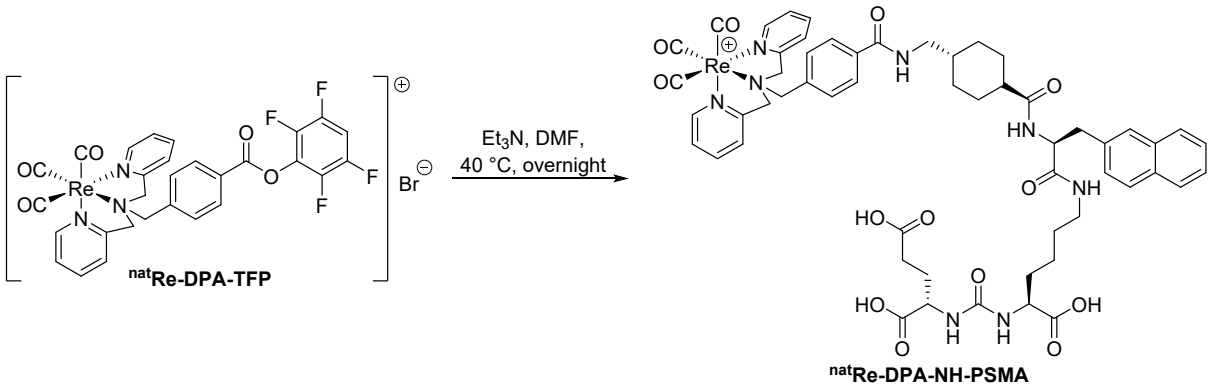
^{nat}Re-DPA-PSMA



PSMA-N_3 (11.49 mg, 14.5 µmol, 1.01 eq.) and **^{nat}Re-DPA-DACN** (12.74 mg, 14.4 µmol, 1.00 eq.) were dissolved in MeOH (5 mL) and the reaction was stirred at 40 °C for 3 d. After the consumption of **^{nat}Re-DPA-DACN** was confirmed by analytical HPLC analysis, the solvent was removed and the crude product was purified by preparative HPLC (Zorbax 300SB-C18 semi-preparative column, solvent A: water + 0.1% TFA, solvent B: acetonitrile + 0.1% TFA, flow rate: 6 mL/min, gradient: A/B 70/30 → 30/70 in 35 min, t_R = 13.0 min). The product was dried by lyophilization to give **^{nat}Re-DPA-PSMA** as colourless solid (18 mg, 75%).

HPLC: t_R = 11.3 min (Agilent C18 column, solvent A: water + 0.1% TFA, solvent B: acetonitrile + 0.1% TFA, flow rate: 1 mL/min, gradient: A/B 90/10 → 5/95 in 14 min), t_R = 17.6 min (Phenomenex C12 column, solvent A: water + 0.1% TFA, solvent B: acetonitrile + 0.1% TFA, flow rate: 1 mL/min, gradient: A/B 95/5 → 5/95 in 20 min).
MS (ESI+): *m/z* = 838 $[M\text{-}Br+H]^{2+}$ (^{185}Re), 839 $[M\text{-}Br+H]^{2+}$ (^{187}Re) / $C_{70}H_{86}BrN_{14}O_{19}ReS_2$ (1757.77).

^{nat}Re-DPA-NH-PSMA



PSMA-NH$_2$ (8.52 mg, 13.0 µmol, 1.1 eq.), **^{nat}Re-DPA-TFP** (8.88 mg, 11.8 µmol, 1.00 eq.), and Et_3N (20 mL) were dissolved in anhydrous DMF (3 mL) and the reaction was stirred at 40 °C overnight. After the consumption of **^{nat}Re-DPA-TFP** was confirmed by analytical HPLC analysis, the solvent was removed and the crude product was purified by preparative HPLC (Zorbax 300SB-C18 semi-preparative column, solvent A: water + 0.1% TFA, solvent B: acetonitrile + 0.1% TFA, flow rate: 6 mL/min, gradient: A/B 70/30 → 30/70 in 35 min, t_R = 10.0 min). The product was dried by lyophilization to give **^{nat}Re-DPA-NH-PSMA** as colourless solid (9.8 mg, 67%).

HPLC: t_R = 15.7 min (Phenomenex C12 column, solvent A: water + 0.1% TFA, solvent B: acetonitrile + 0.1% TFA, flow rate: 1 mL/min, gradient: A/B 95/5 → 5/95 in 20 min).
MS (ESI+): *m/z* = 621 for $[M+H]^{2+}$ (^{185}Re), 622 for $[M+H]^{2+}$ (^{187}Re) / $C_{56}H_{62}N_8O_{13}Re$ (1241.40).

NMR spectra of compounds

N-(3-((3-bromopropyl)sulfonamido)propyl)-2-nitrobenzenesulfonamide (**3**)

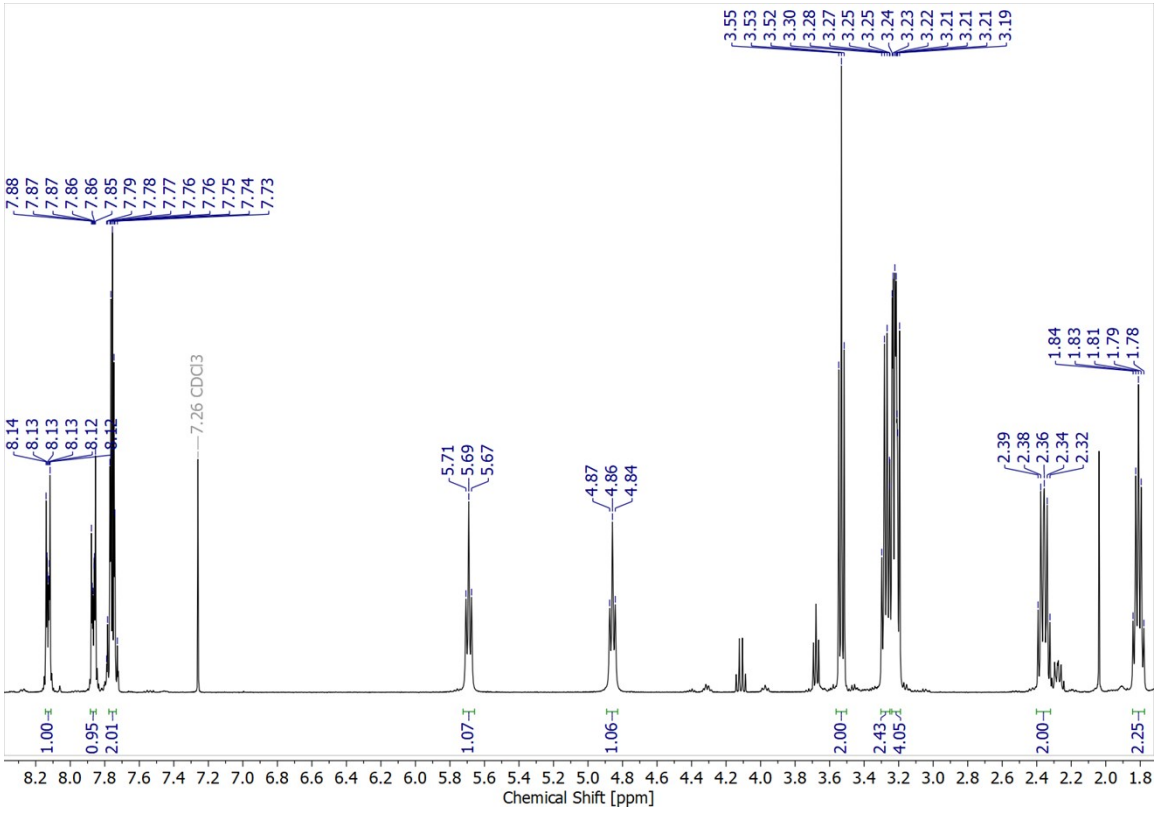


Figure S1. *^{1}H NMR spectrum of compound **3** in $CDCl_3$.*

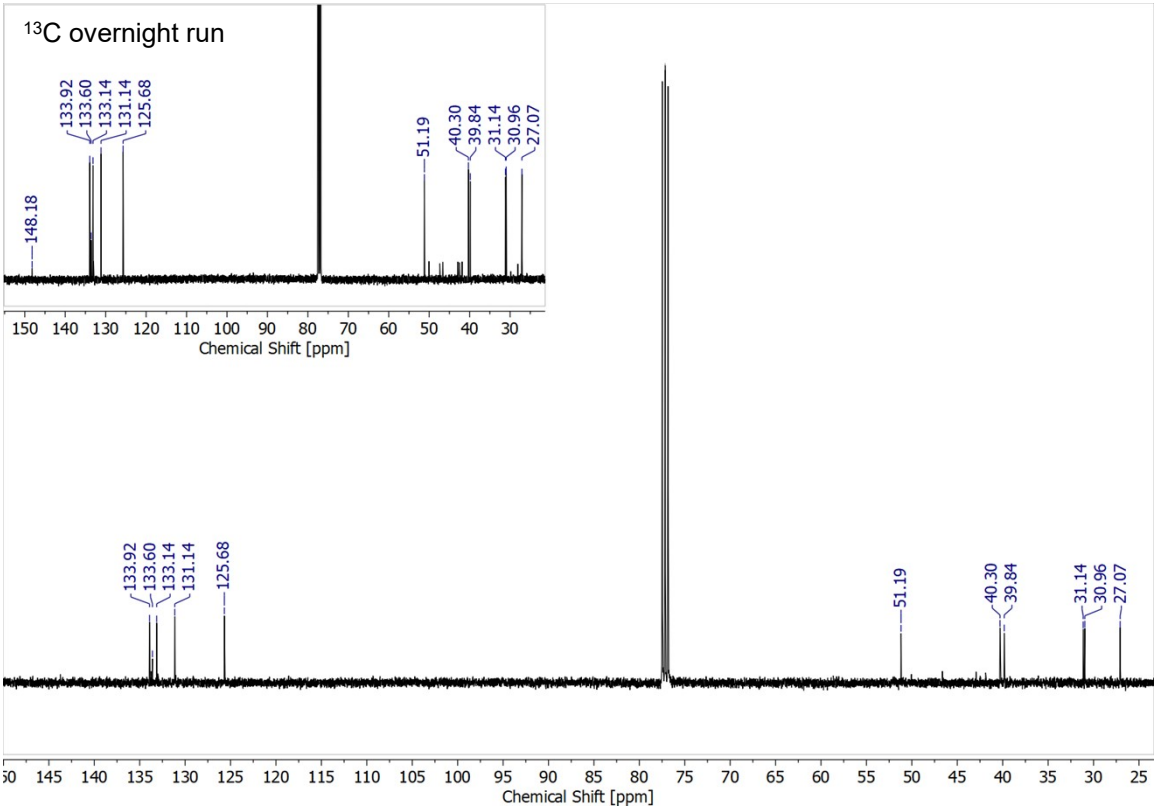


Figure S2. *^{13}C NMR spectrum of compound **3** in $CDCl_3$.*

8-(3-Bromopropanesulfonyl)-4-nosyl-4,8-diazacyclononyne (**5**)

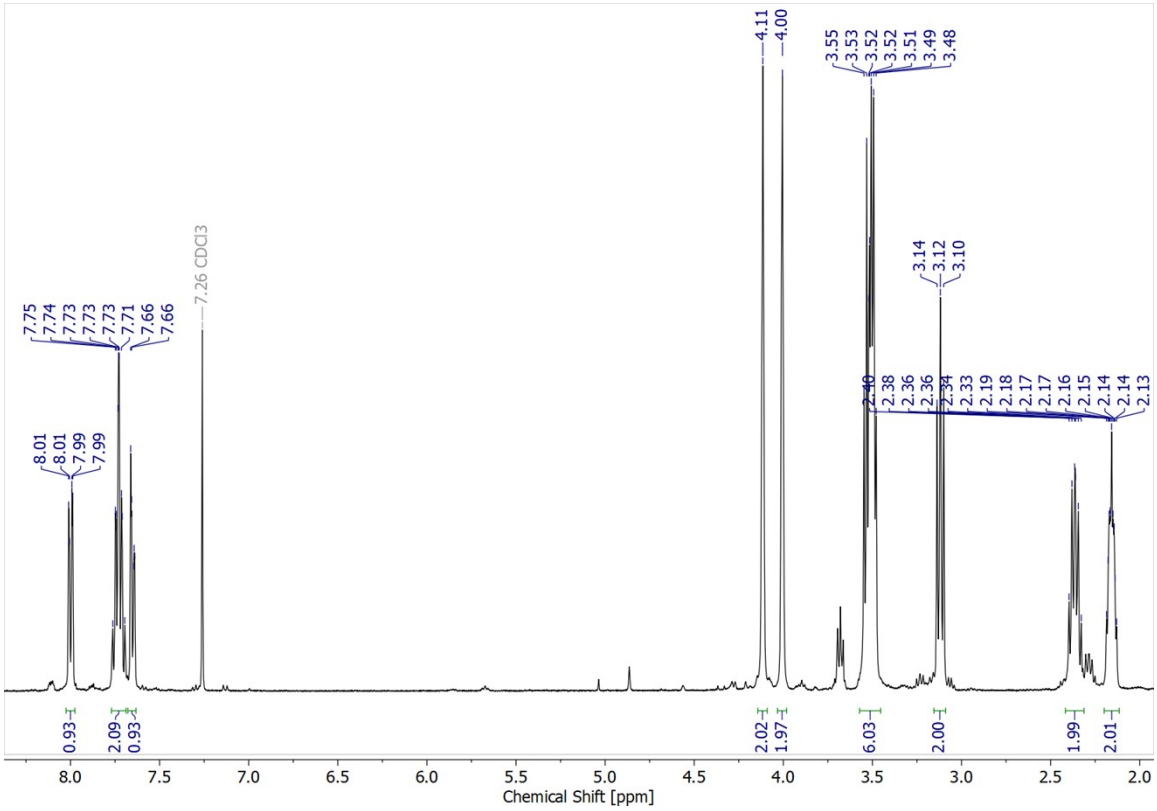


Figure S3. *^{1}H NMR spectrum of compound **5** in $CDCl_3$.*

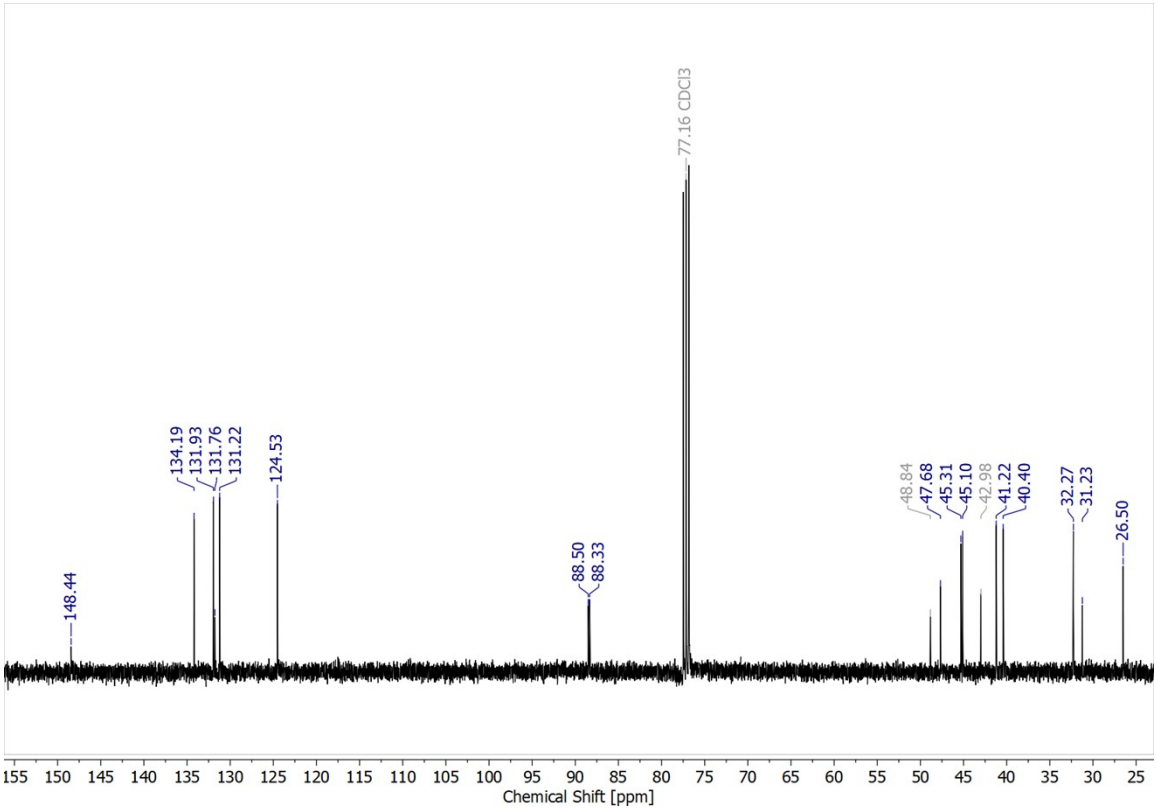


Figure S4. *^{13}C NMR spectrum of compound **5** in $CDCl_3$.*

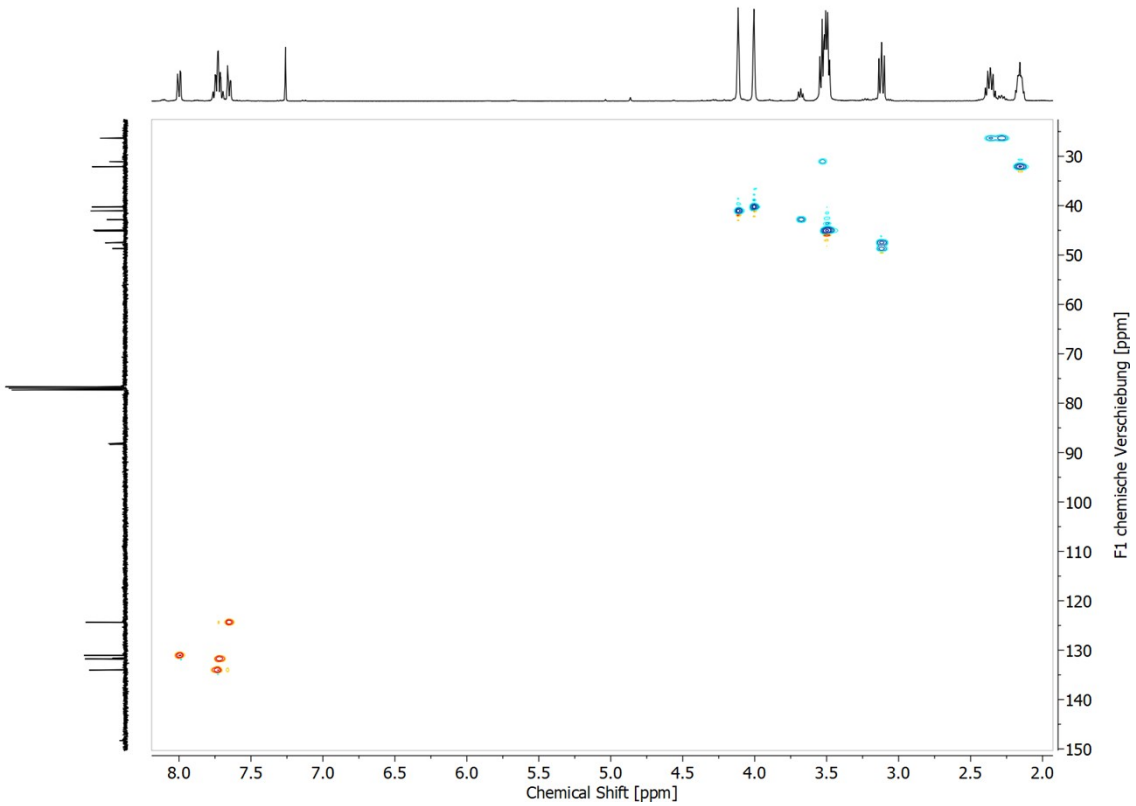


Figure S5. *HSQC spectrum of compound* ***5*** *in* $CDCl_3$.

8-(3-(Bis(pyridin-2-ylmethyl)amino)propanesulfonyl)-4-nosyl-4,8-diazacyclononyne (**6**)

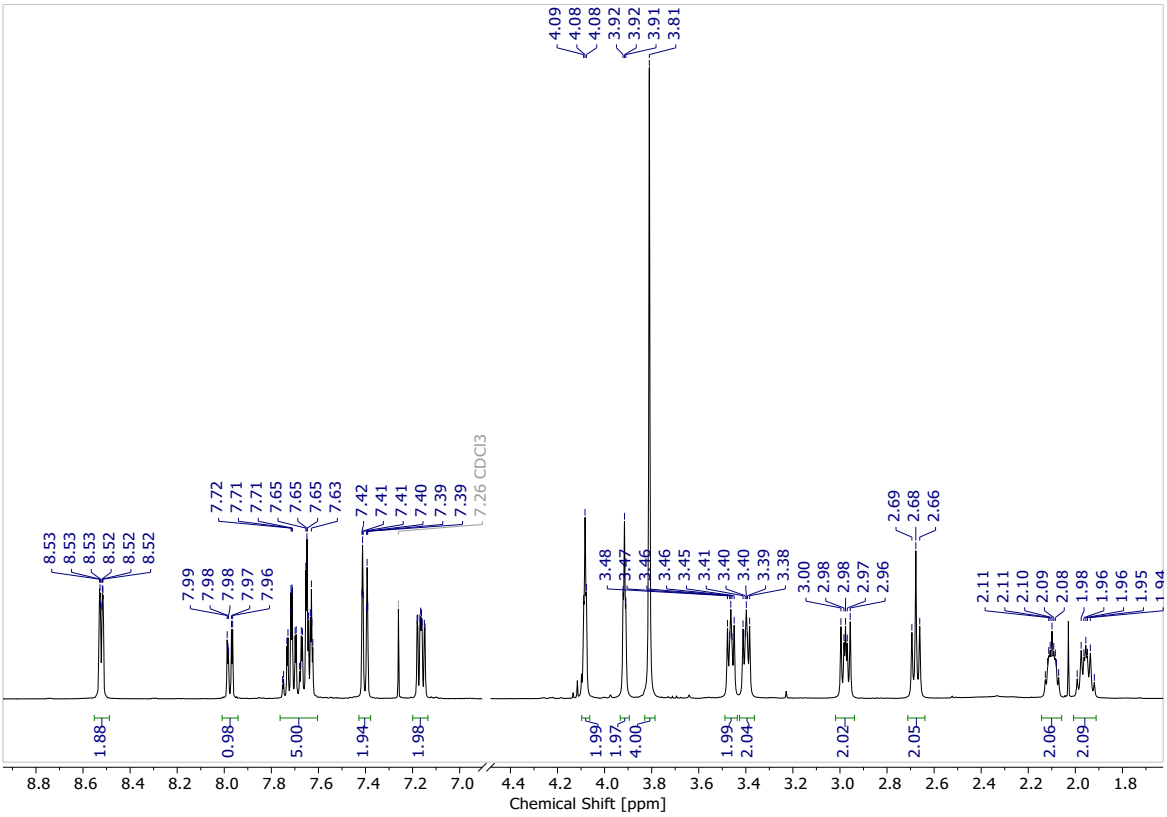


Figure S6. *^{1}H NMR spectrum of compound **6** in $CDCl_3$.*

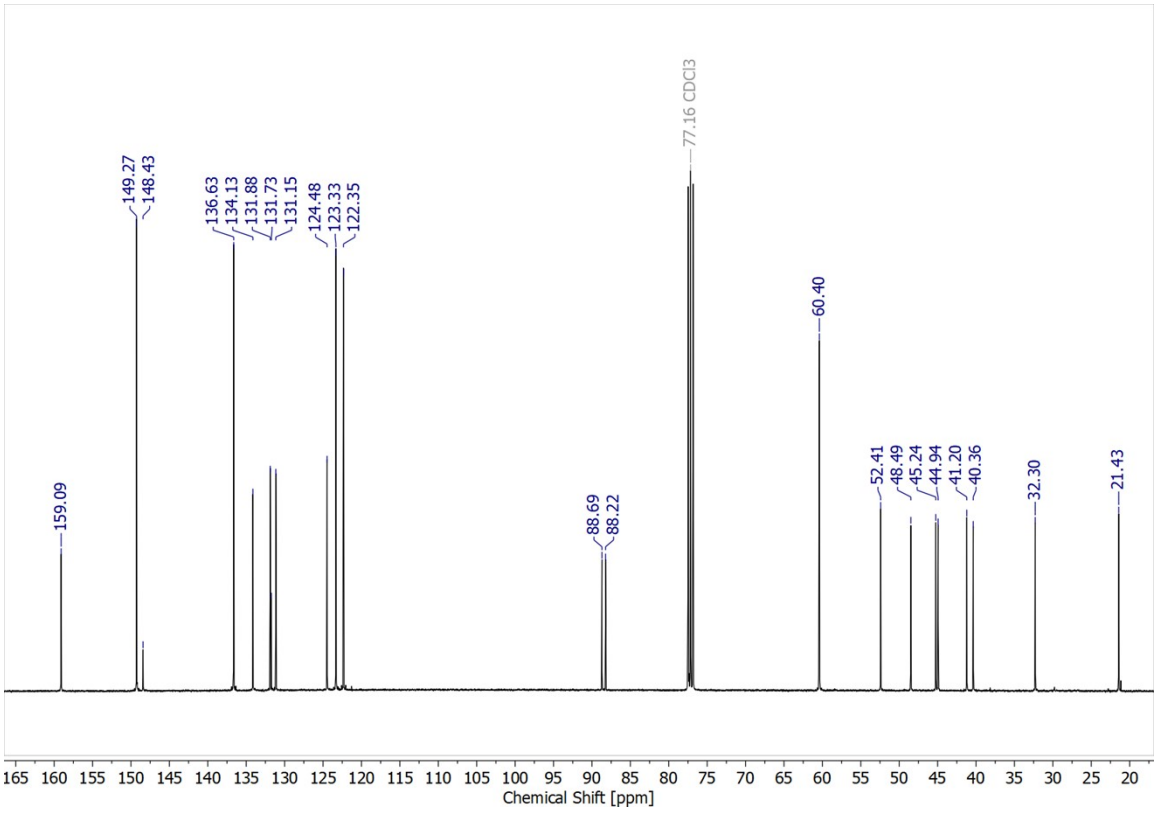


Figure S7. *^{13}C NMR spectrum of compound **6** in $CDCl_3$.*

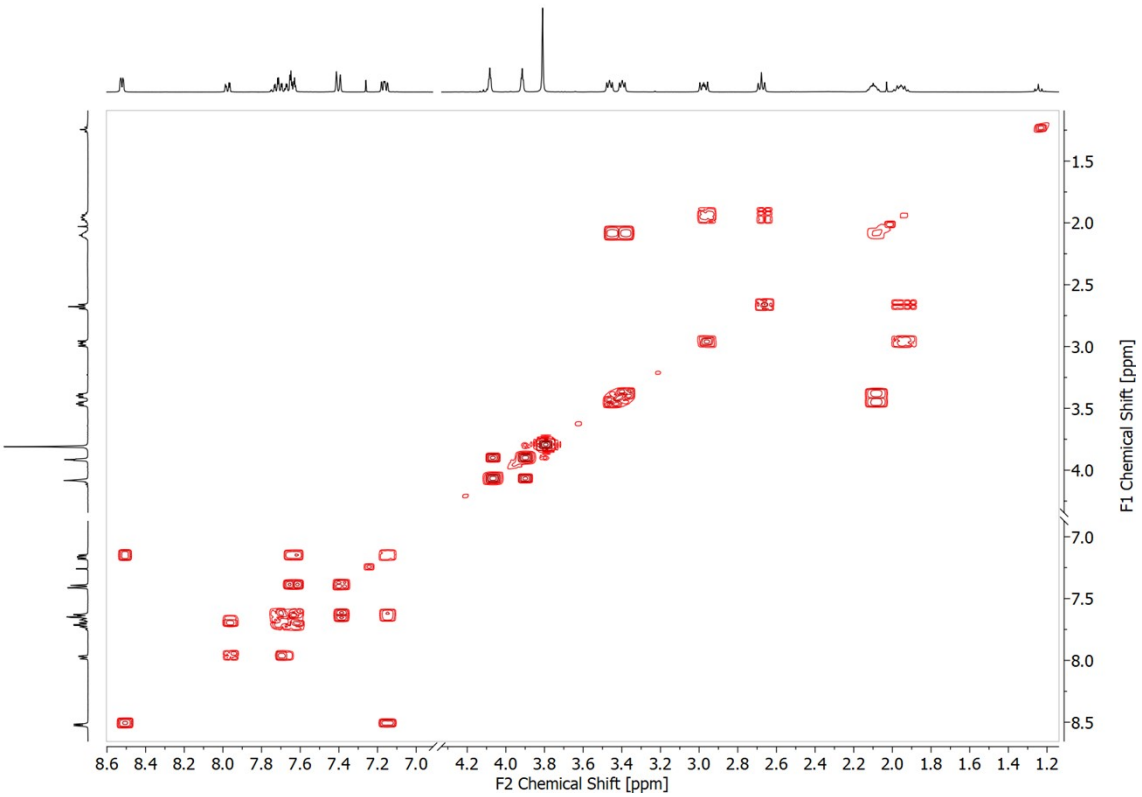


Figure S8. *COSY spectrum of compound* ***6*** *in* $CDCl_3$.

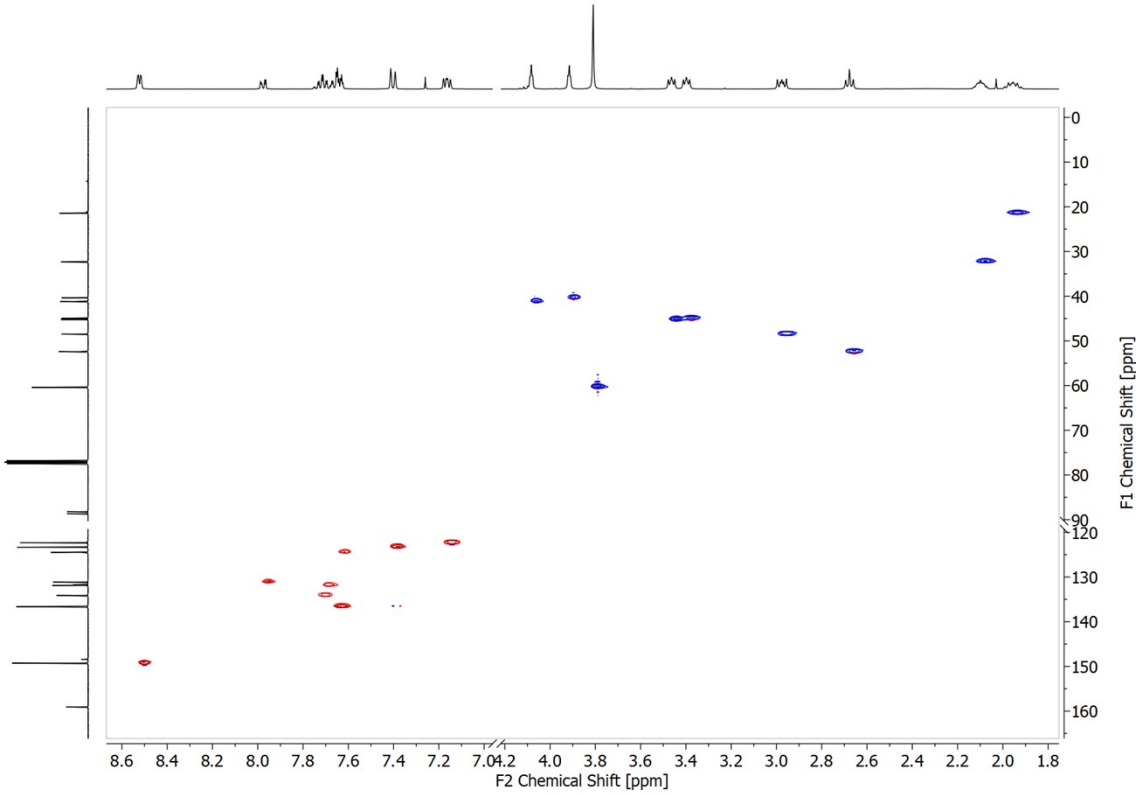


Figure S9. *HSQC spectrum of compound* ***6*** *in* $CDCl_3$.

4-(Bis(pyridin-2-ylmethyl)amino)methyl)benzoic acid ethyl ester (**8**)

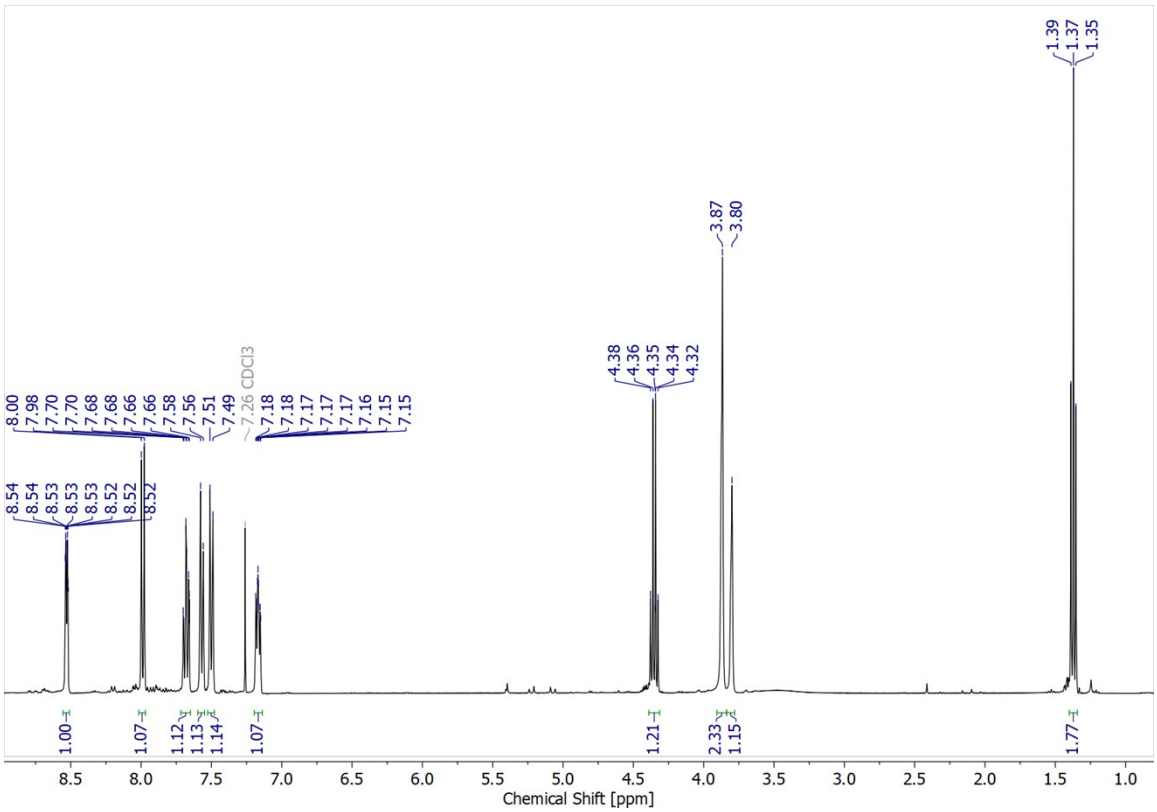


Figure S10. *^{1}H NMR spectrum of compound **8** in $CDCl_3$.*

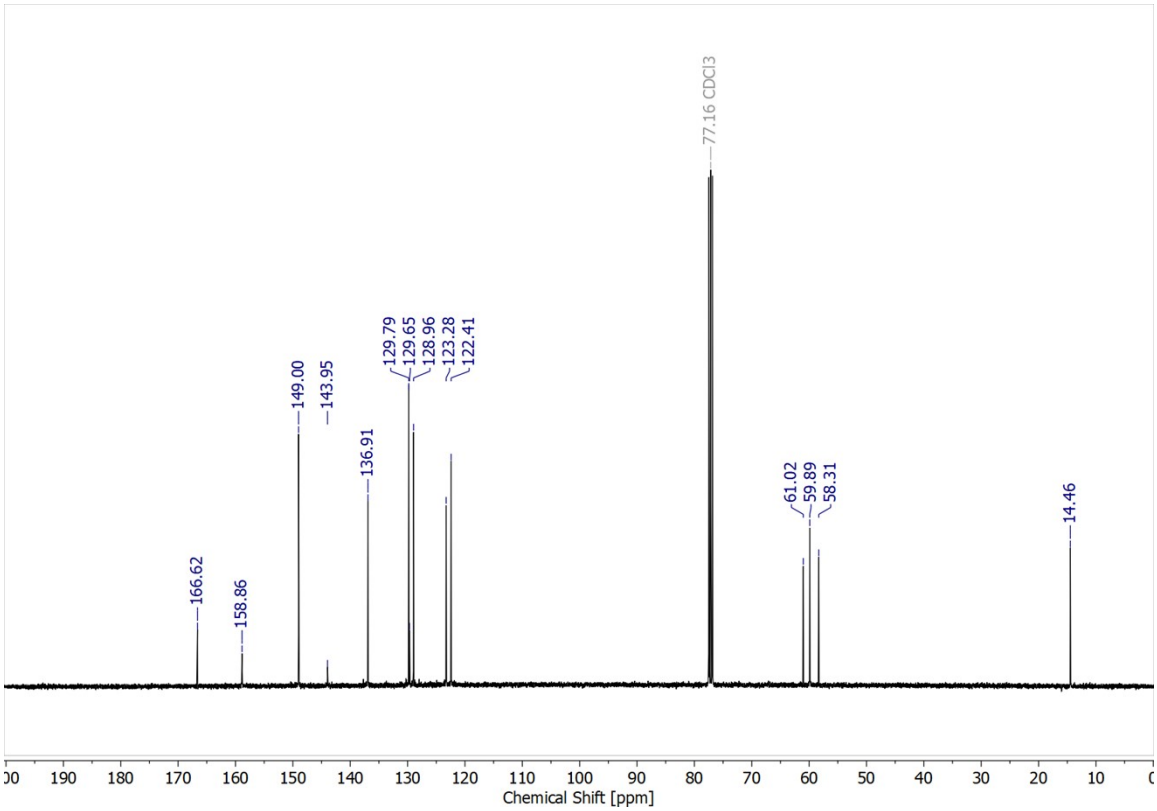


Figure S11. *^{13}C NMR spectrum of compound **8** in $CDCl_3$.*

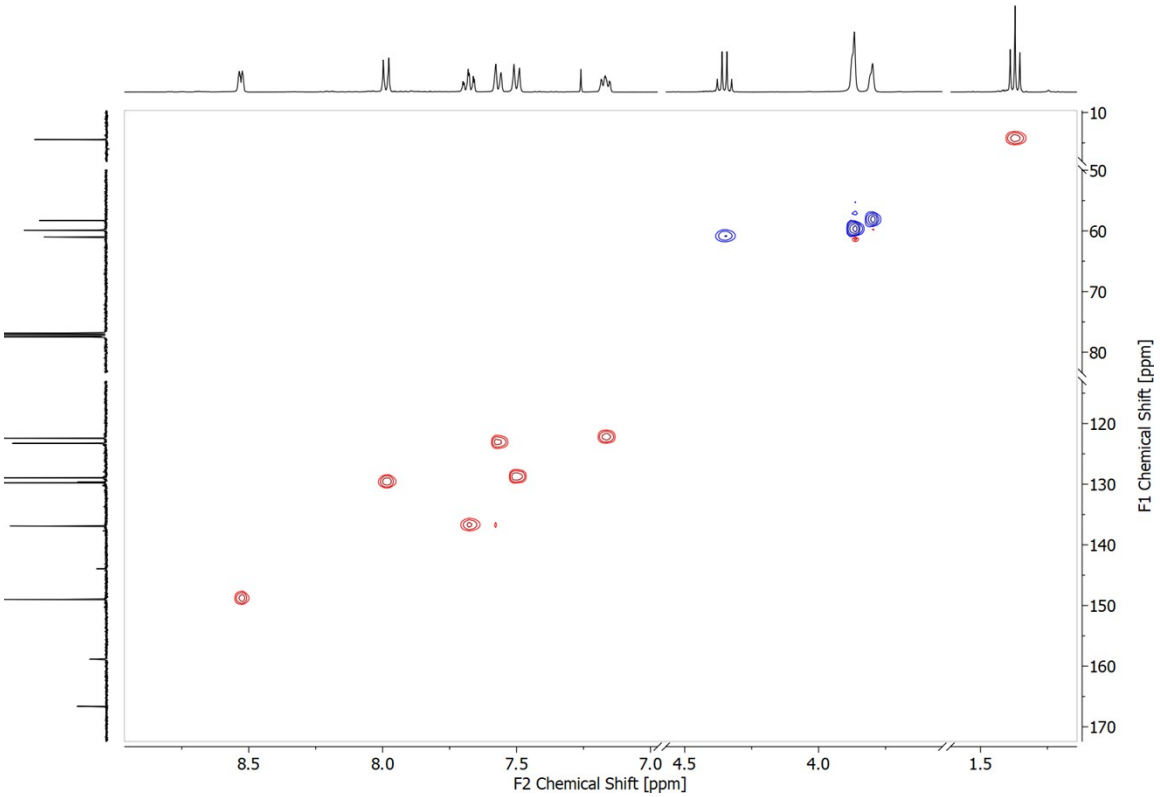


Figure S12. *HSQC NMR spectrum of compound* ***8*** *in* $CDCl_3$.

4-(Bis(pyridin-2-ylmethyl)amino)methyl)benzoic acid (**9**)

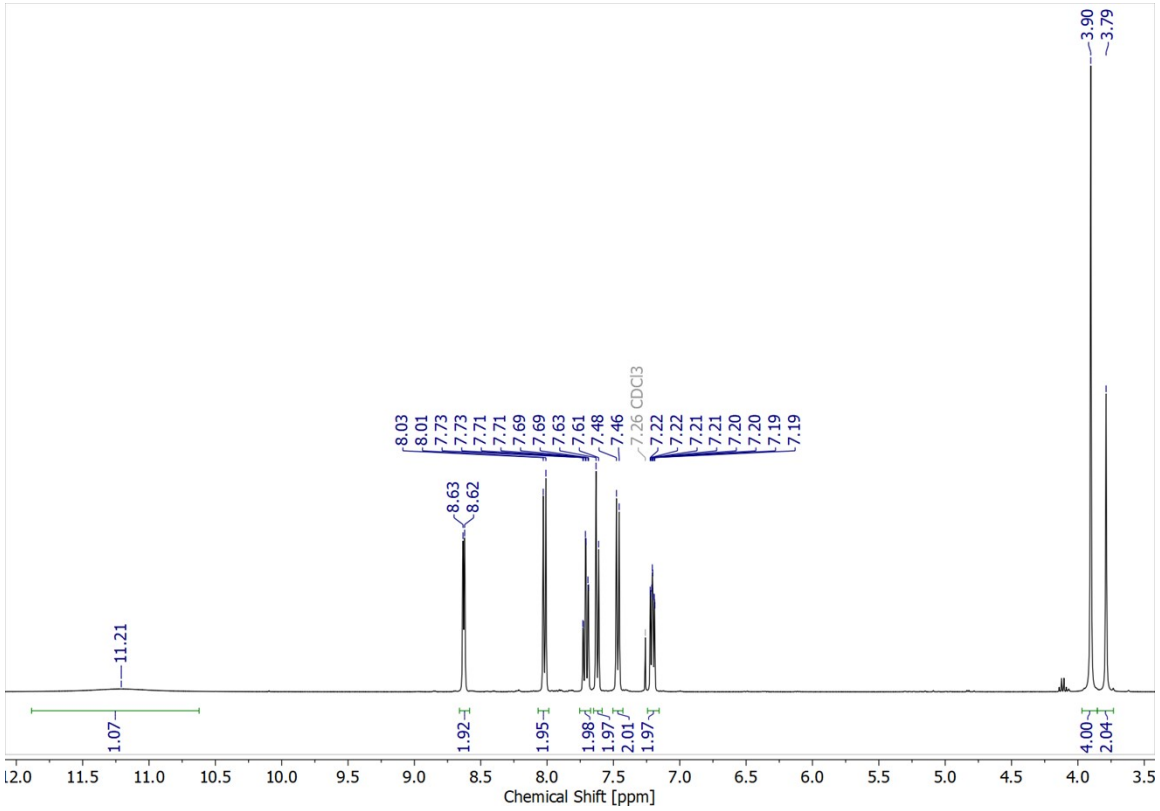


Figure S13. *^{1}H NMR spectrum of compound **9** in $CDCl_3$.*

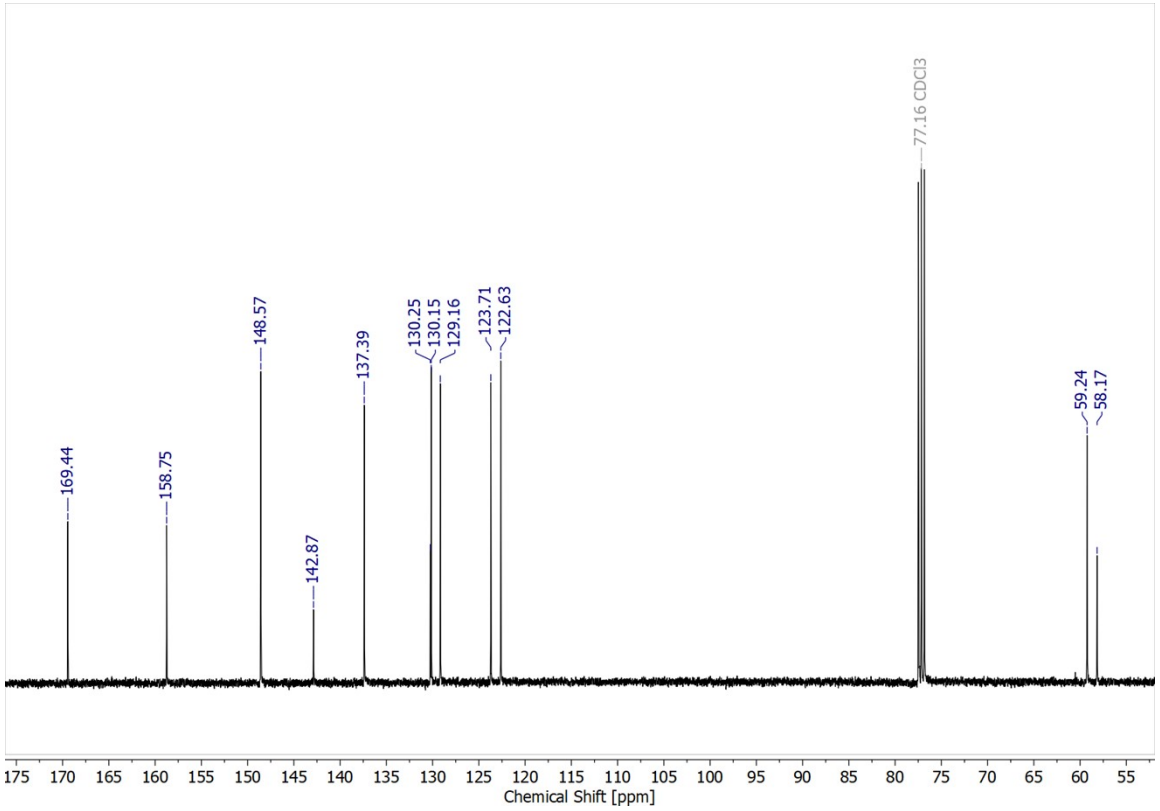


Figure S14. *^{13}C NMR spectrum of compound **9** in $CDCl_3$.*

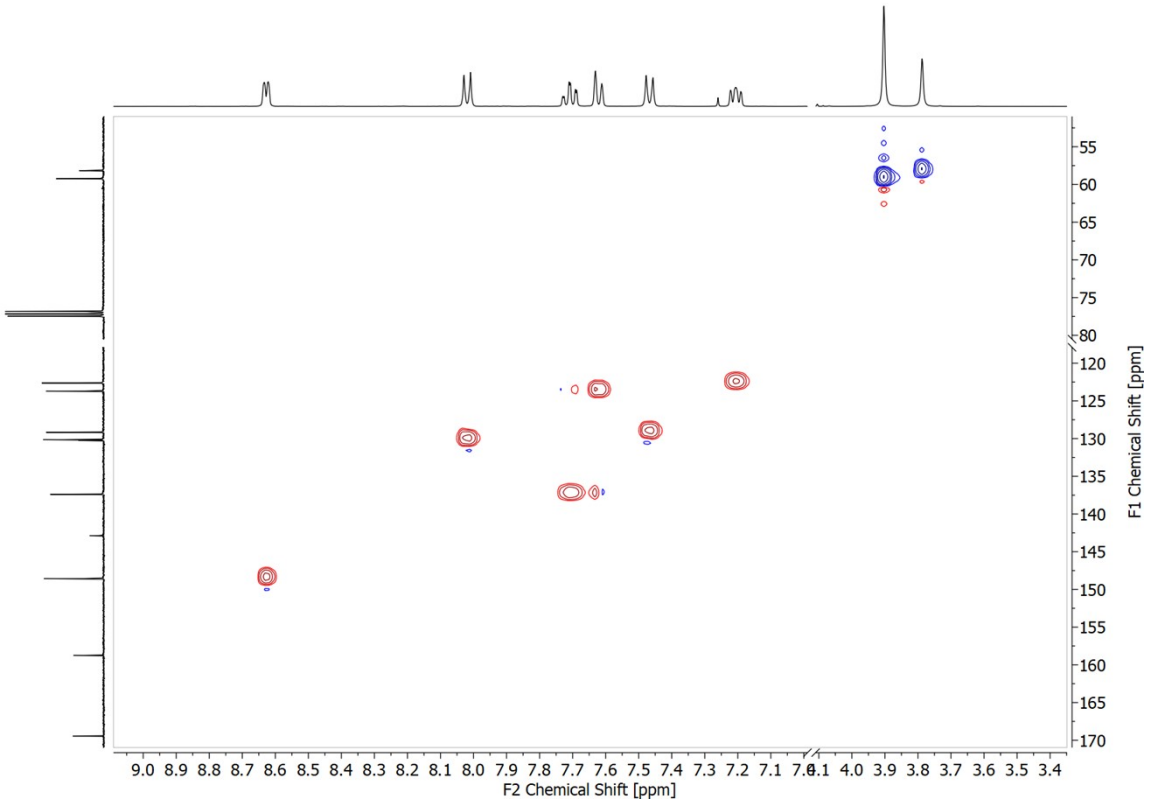


Figure S15. *HSQC NMR spectrum of compound* ***9*** *in* $CDCl_3$.

4-(Bis(pyridin-2-ylmethyl)amino)methyl)benzoic acid 2,3,5,6-tetrafluorophenyl ester (**10**)

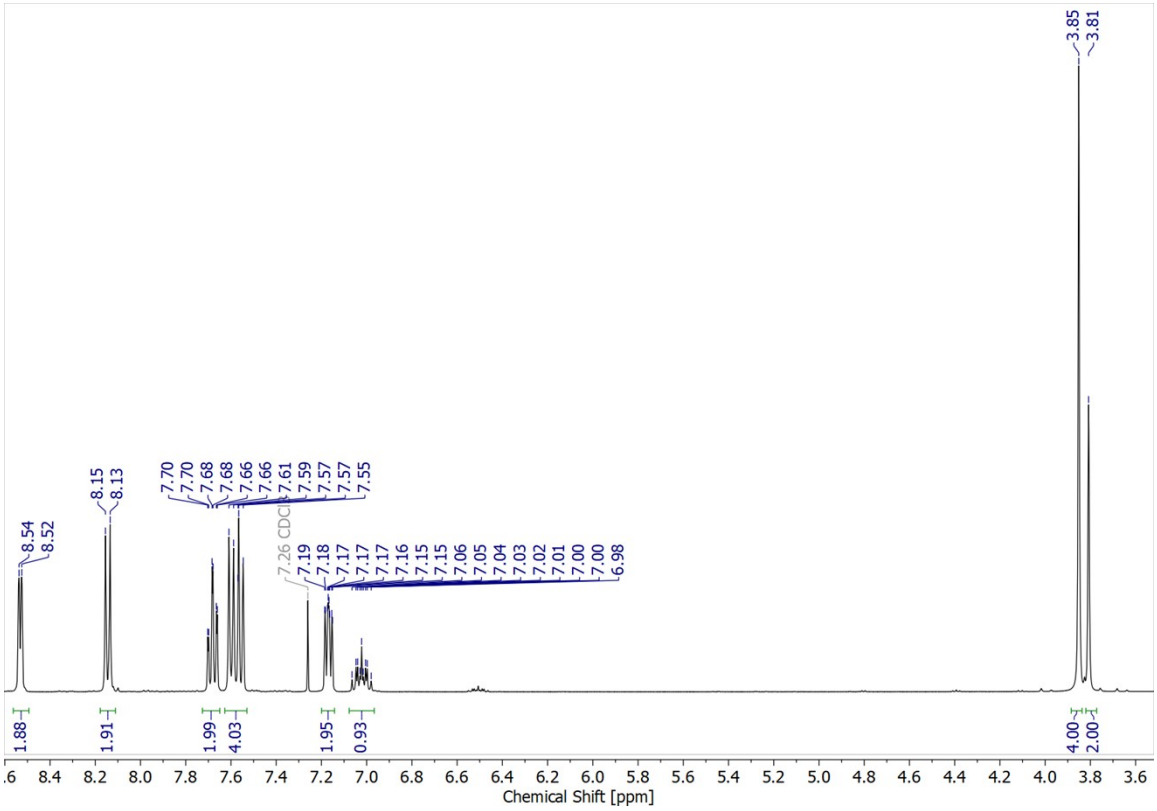


Figure S16. *^{1}H NMR spectrum of compound **10** in $CDCl_3$.*

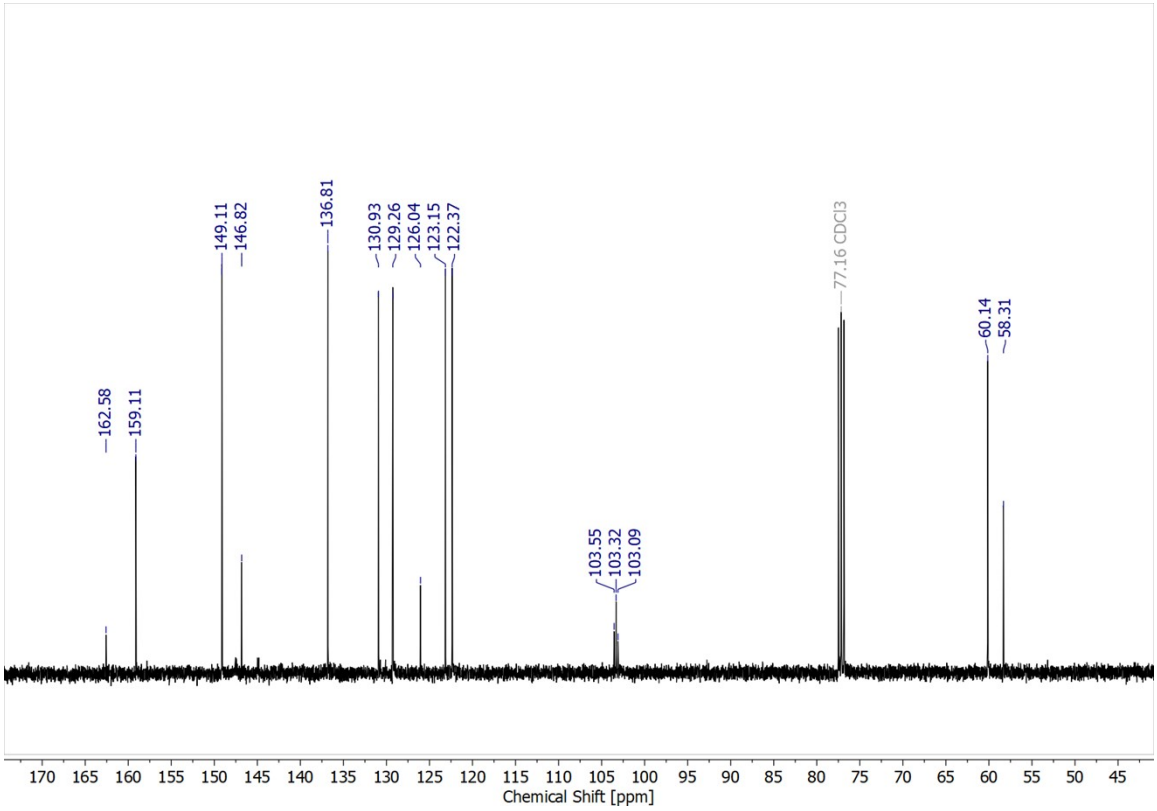


Figure S17. *^{13}C NMR spectrum of compound **10** in $CDCl_3$.*

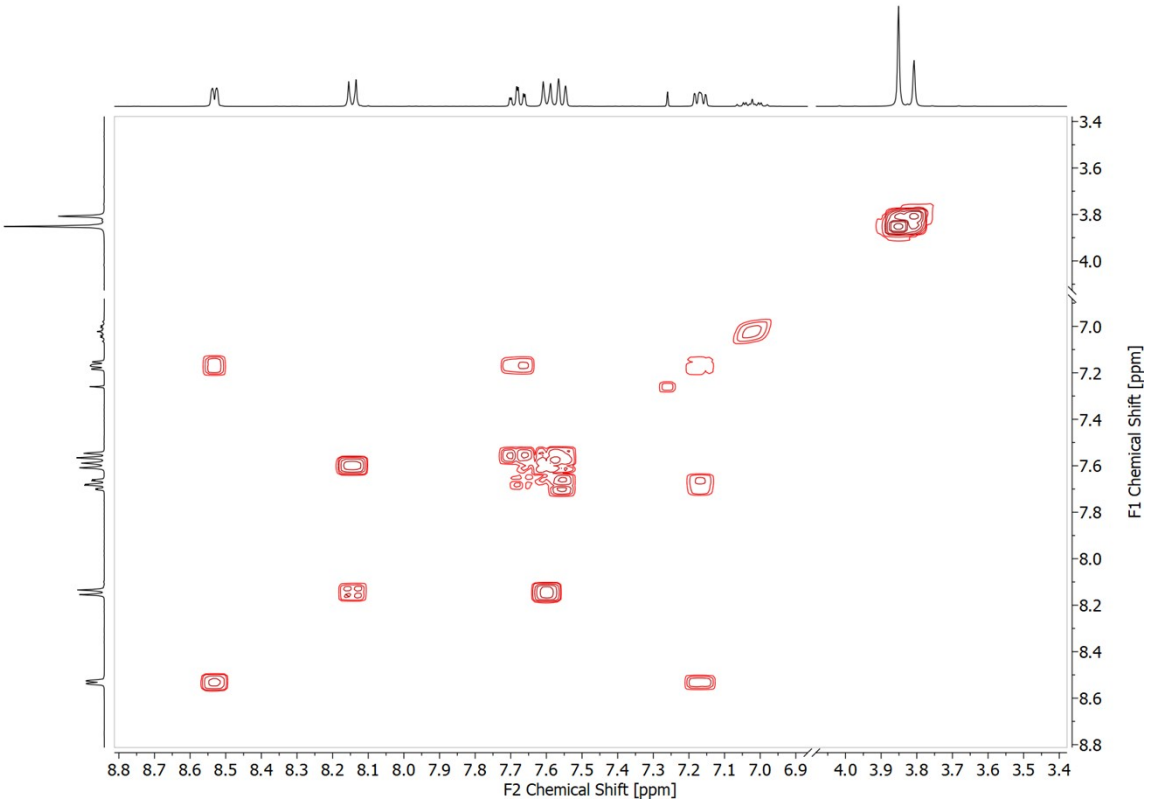


Figure S18. *COSY NMR spectrum of compound* ***10*** *in* $CDCl_3$.

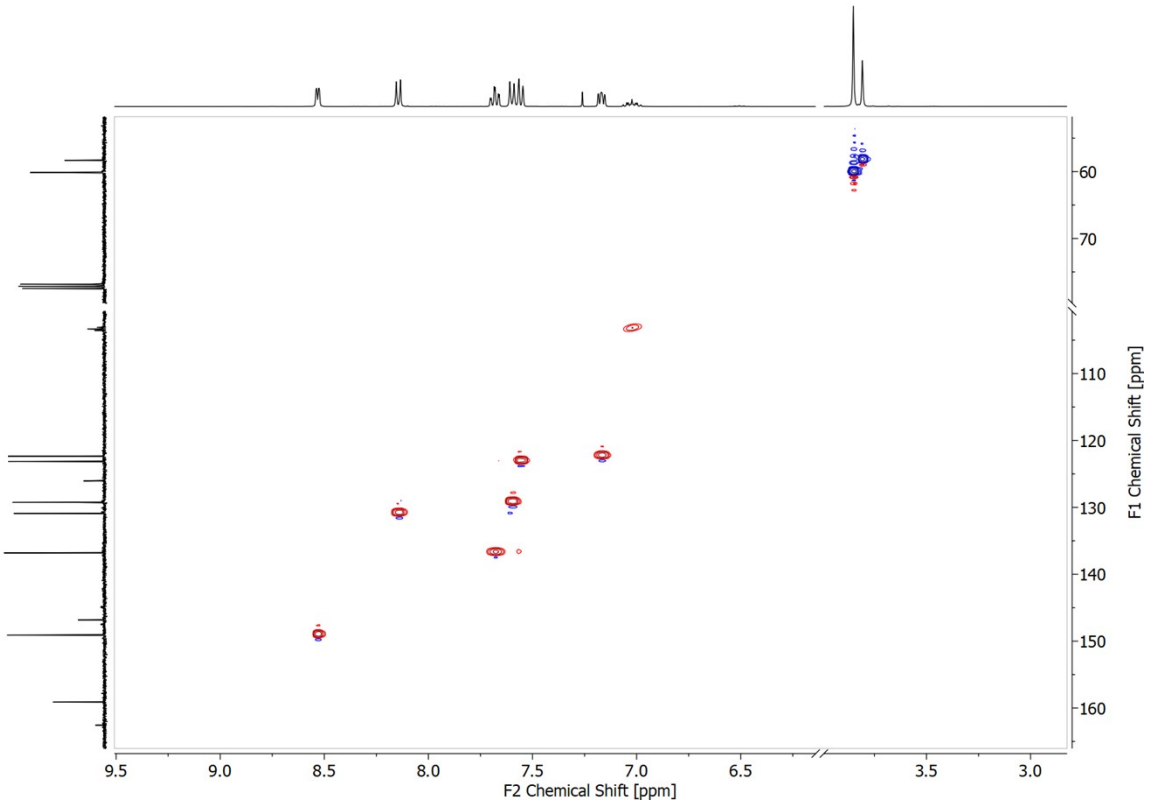


Figure S19. *HSQC spectrum of compound* ***10*** *in* $CDCl_3$.

[Re(CO)$_3$6]Br (**11**)

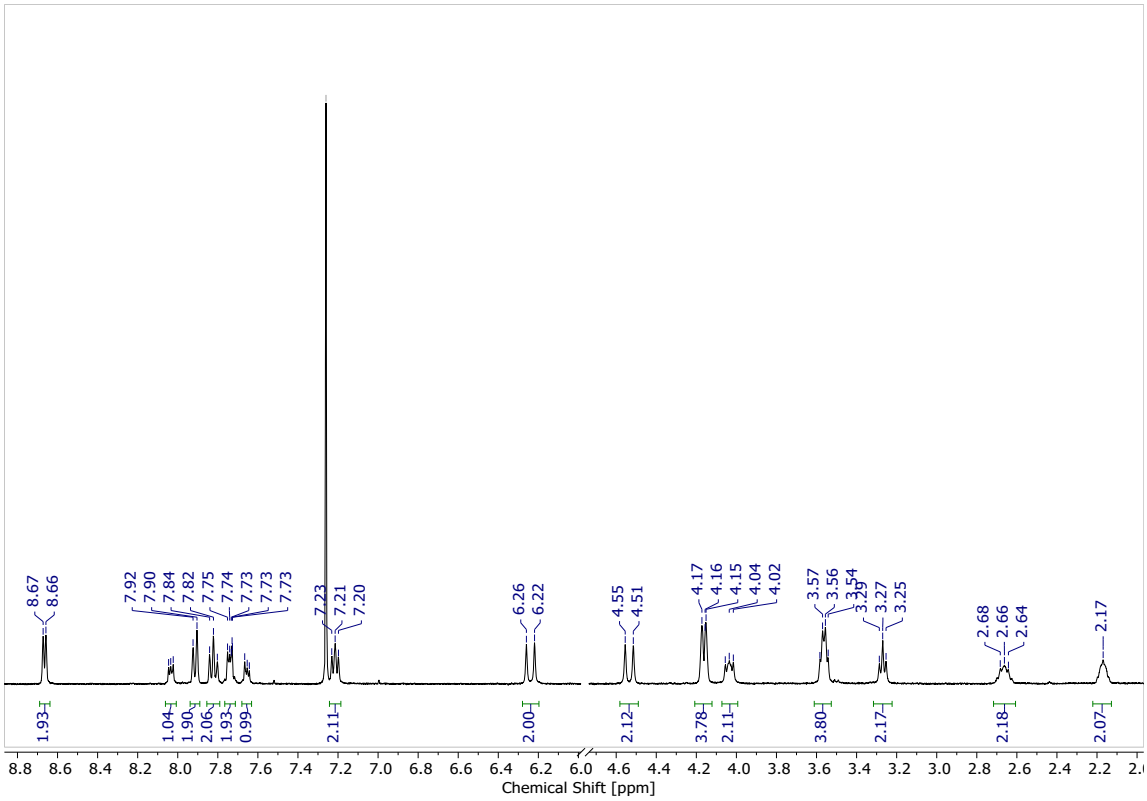


Figure S20. *^{1}H NMR spectrum of compound **11** in $CDCl_3$.*

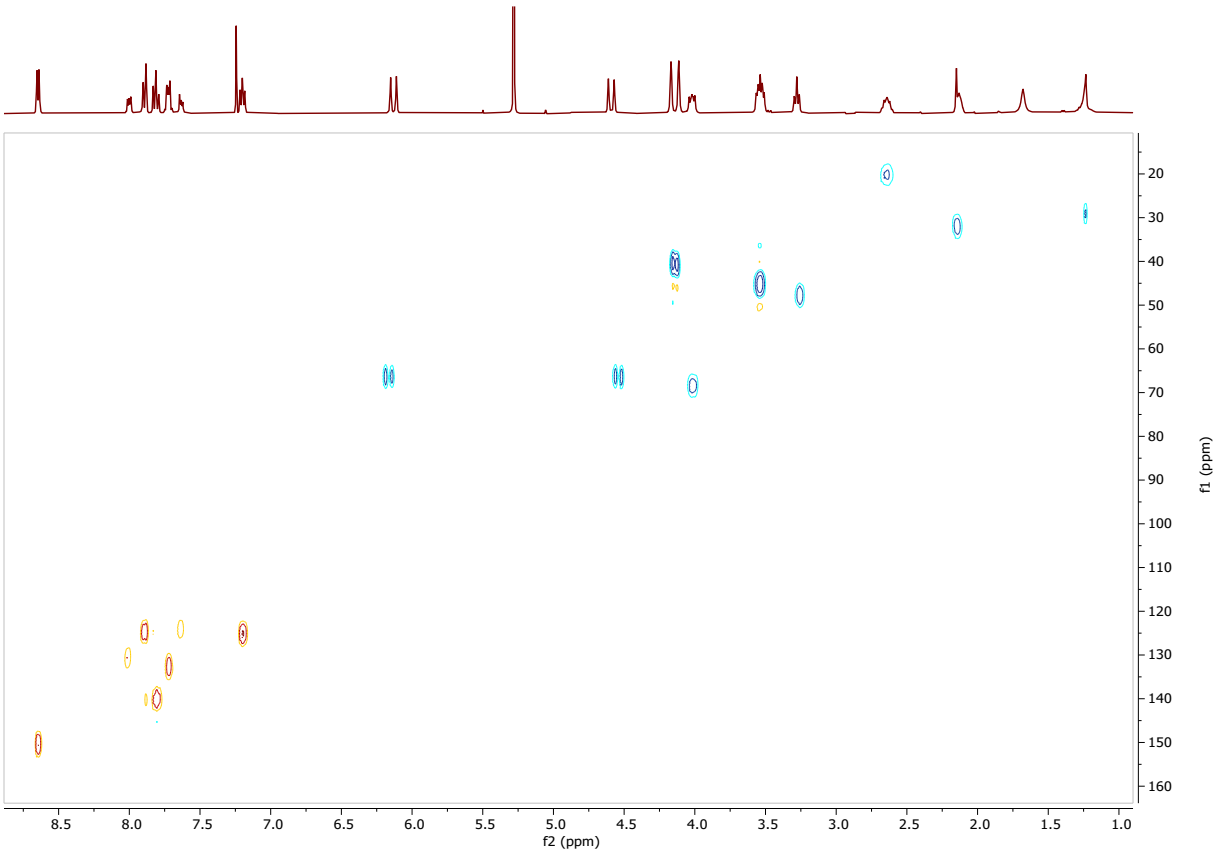


Figure S21. *HSQC spectrum of compound **11** in $CDCl_3$.*

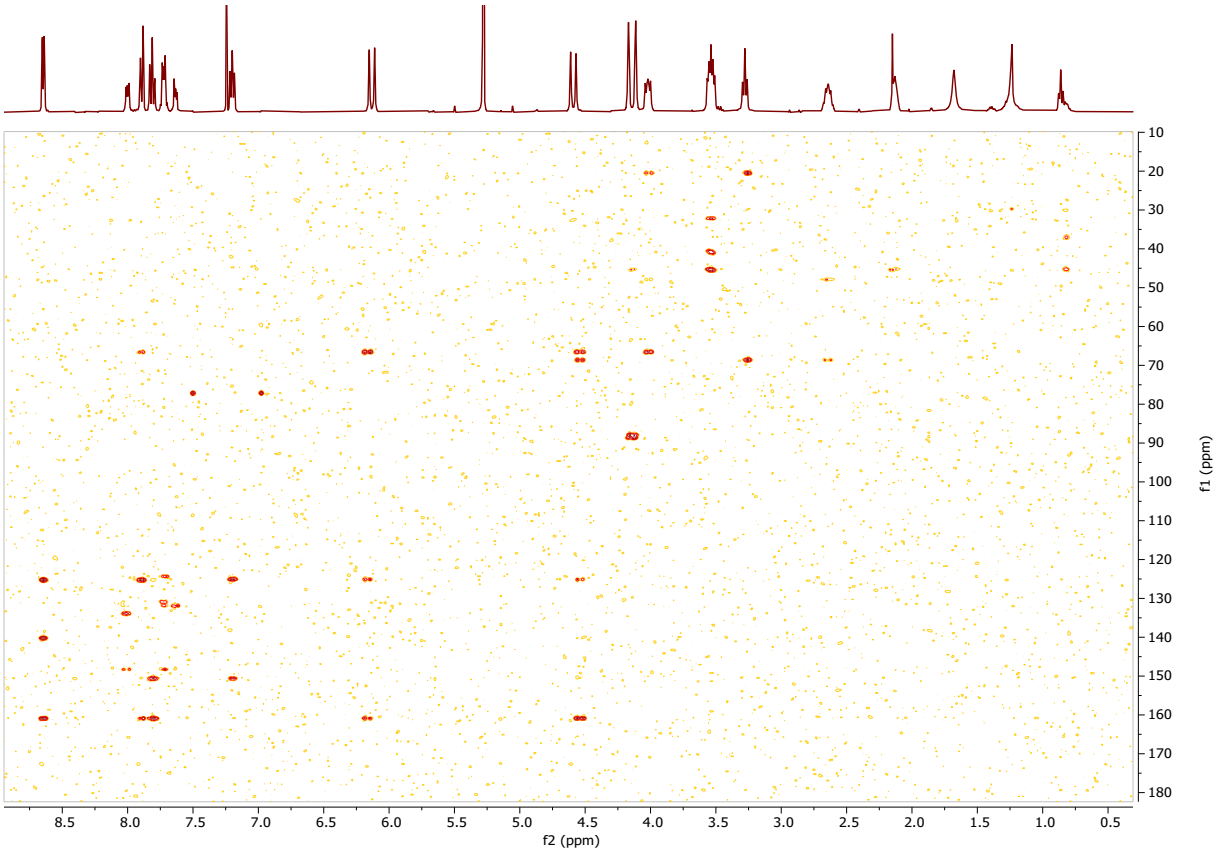


Figure S22. *HMBC spectrum of compound* ***11*** *in* $CDCl_3$.

[$Re(CO)_3$10]Br (**12**)

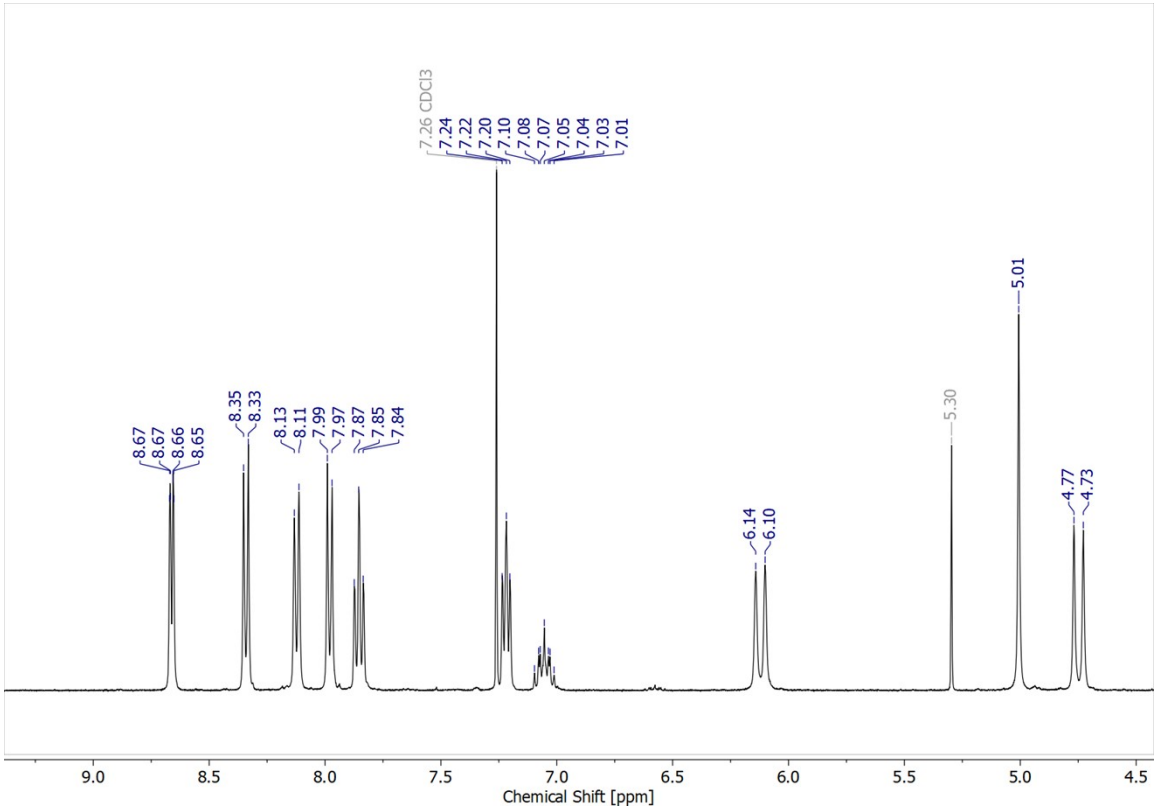


Figure S23. *^{1}H NMR spectrum of compound **12** in $CDCl_3$.*

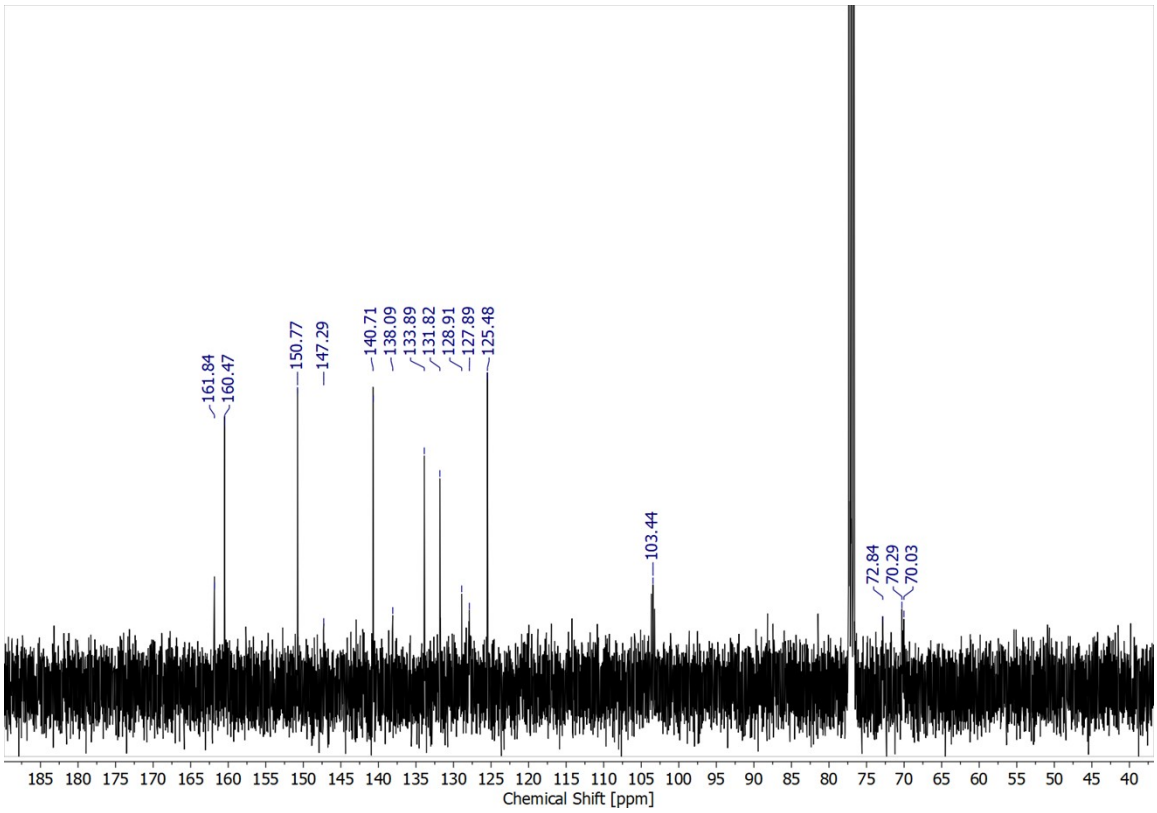


Figure S24. *^{13}C NMR spectrum of compound **12** in $CDCl_3$.*

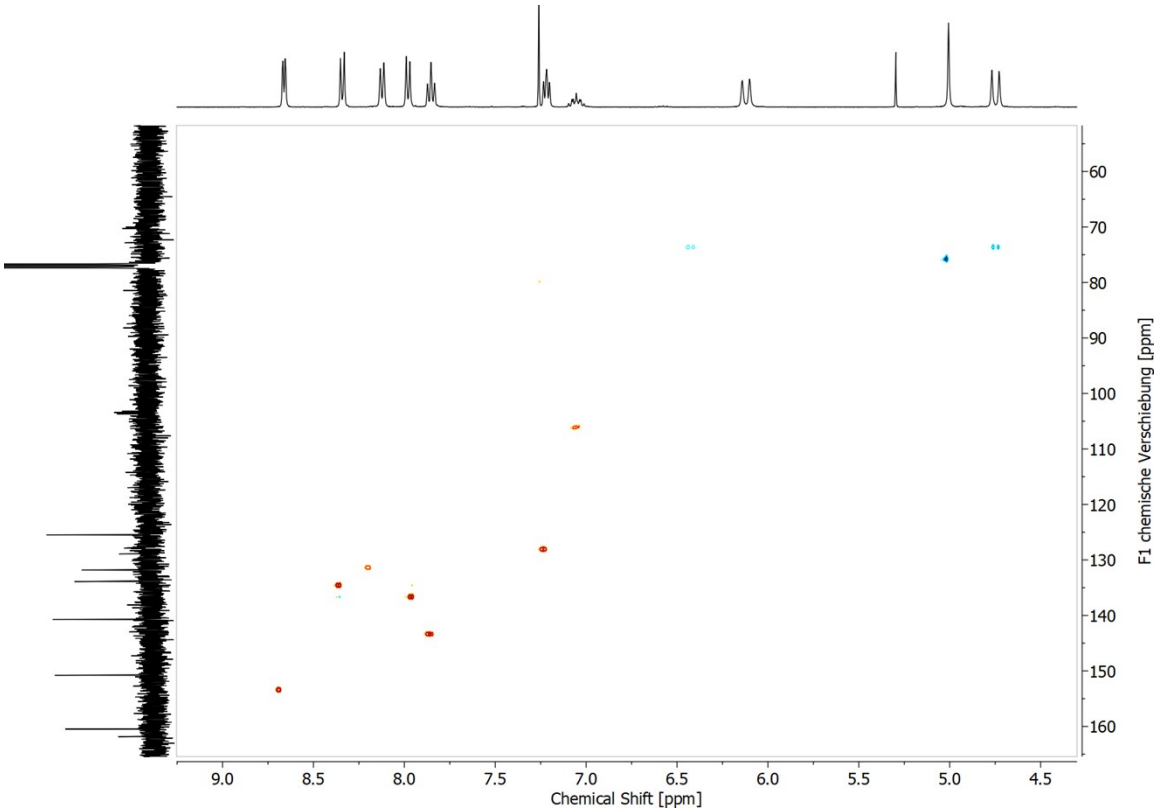


Figure S25. *HSQC spectrum of compound* ***12*** *in* $CDCl_3$.

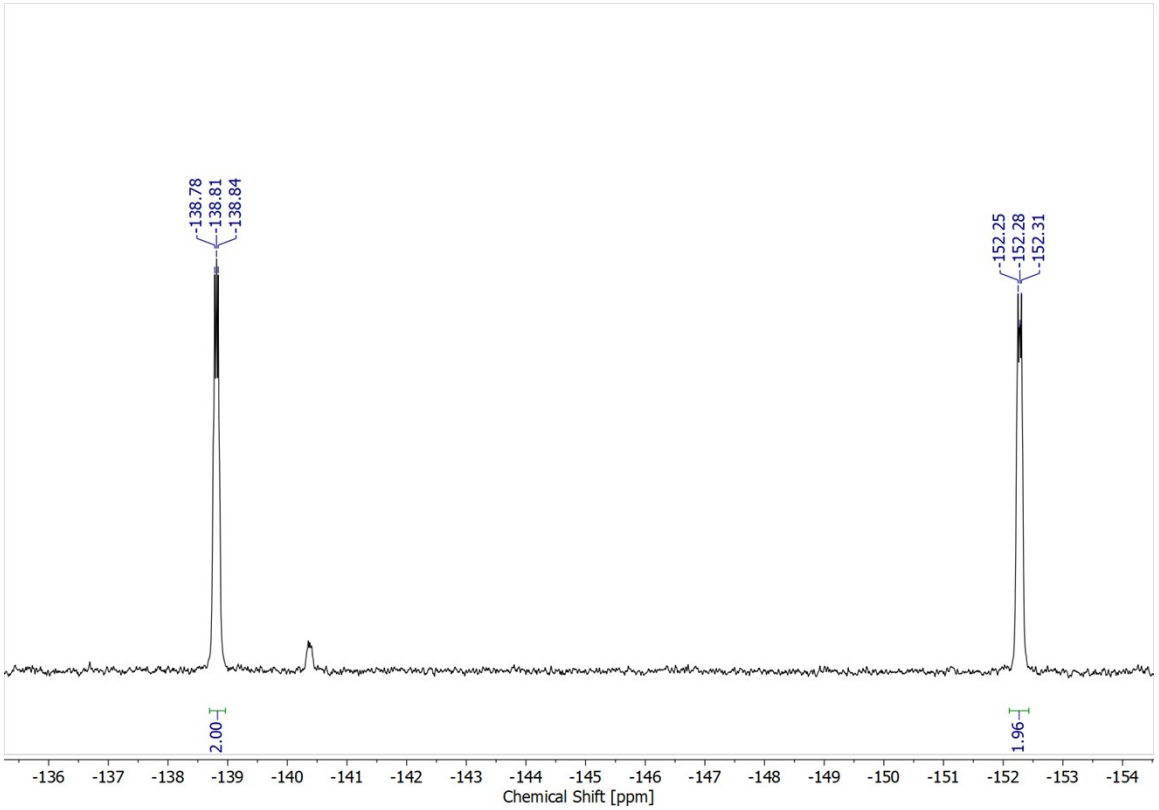
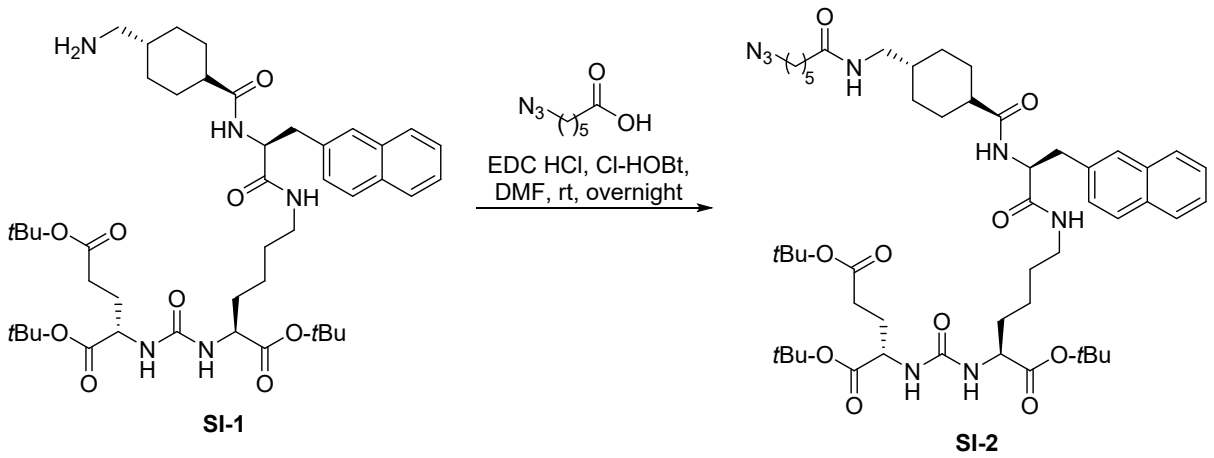


Figure S26. *^{19}F NMR spectrum of compound* ***12*** *in* $CDCl_3$.

Synthesis of the functionalized PSMA-derivatives PSMA-N_3 and PSMA-NH_2

(in accordance to ref: Cancers 2021, 13, 1974)

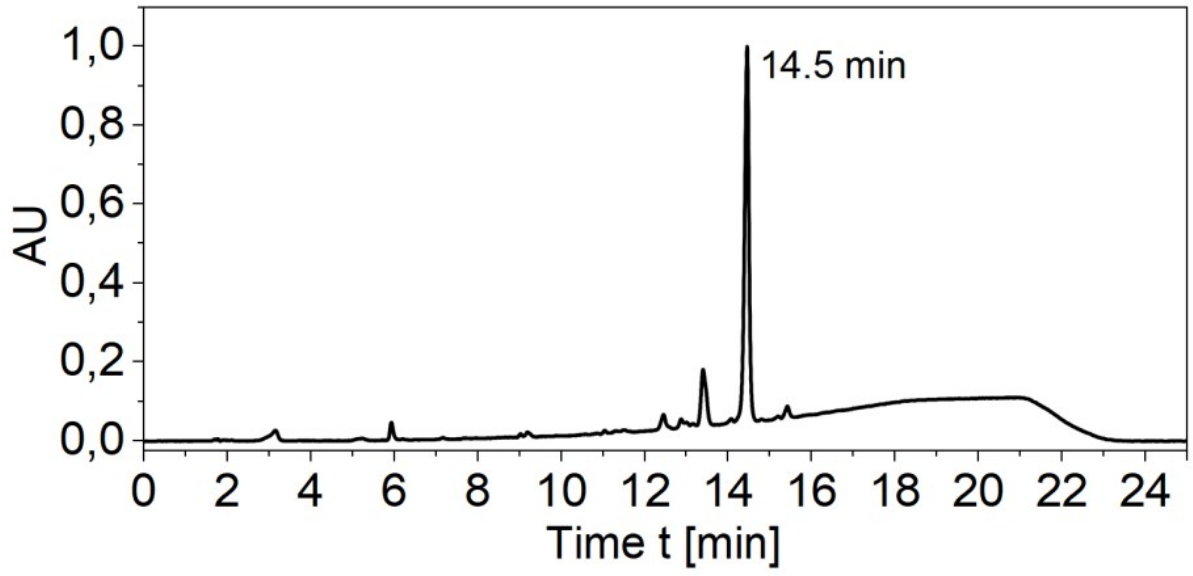
Di-tert-butyl (((S)-6-((S)-2-((1s,4R)-4-((2-azidoacetamido)methyl)cyclohexane-1-carboxamido)-3-(naphthalen-2-yl)propanamido)-1-(tert-butoxy)-1-oxohexan-2-yl)carbamoyl)-L-glutamate SI-2



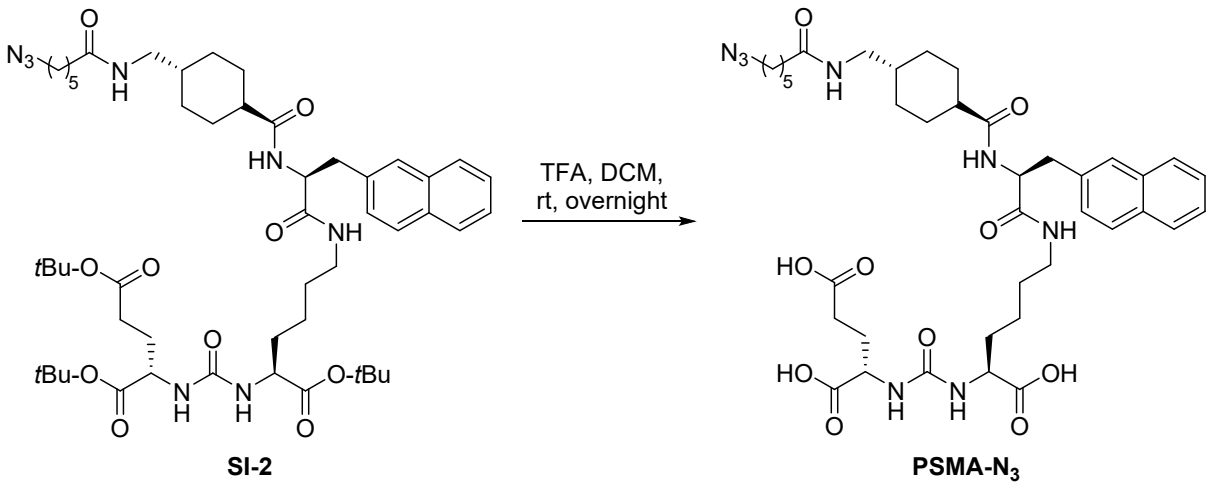
SI-1 **SI-2**

Compound **SI-1** (403 mg, 0.49 mmol, 1.00 eq.), 6-azido-hexanoic acid (92 mg, 0.59 mmol, 1.20 eq.), 6-Cl-HOBT (97 mg, 0.64 mmol, 1.20 eq.) and EDC·HCl (122 mg, 0.64 mmol, 1.20 eq.) were dissolved in anhydrous DMF (10 mL) and the resulting mixture was allowed to stir at rt overnight. After HPLC control, the solvent was removed, and the crude product was purified by automated flash column chromatography ($CHCl_3$/EtOH 10/0 → 9/1) to yield compound **SI-2** as yellowish solid (166 mg, 35%).

R_f (EtOAc v/v) = 0.34.
HPLC (Agilent C18 column, solvent A: water + 0.1% TFA, solvent B: acetonitrile + 0.1% TFA, flow rate: 1 mL/min, gradient: A/B 90/10 → 5/95 in 14 min): t_R = 14.5 min. MS (ESI+): m/z = 964 $[M+H]^+$. $C_{51}H_{78}N_8O_{10}$ (963.23).



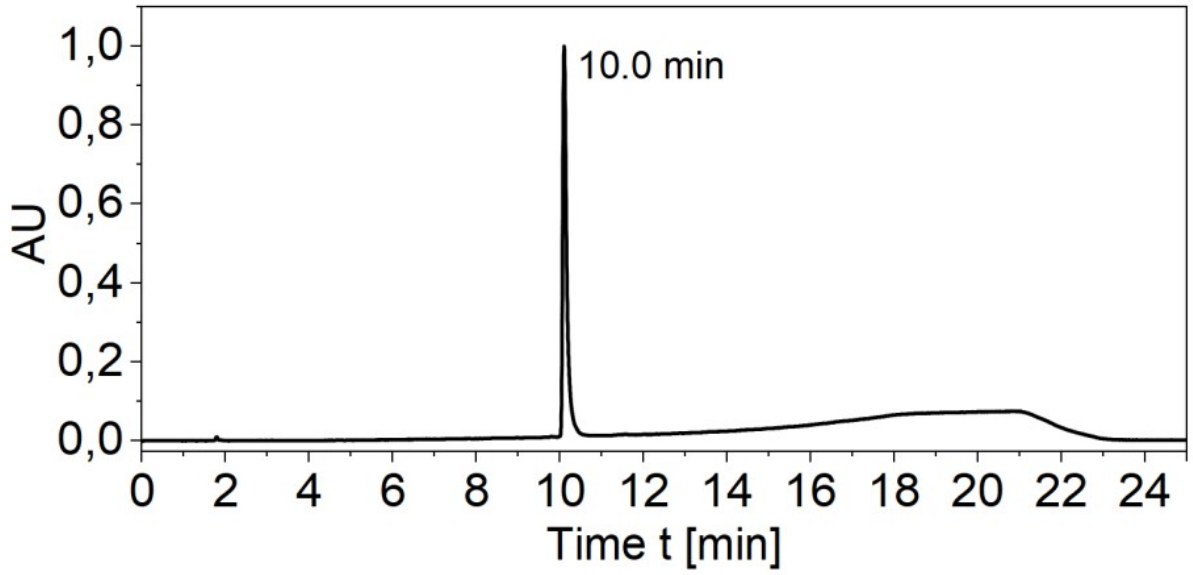
((((S)-5-((S)-2-((1s,4R)-4-((2-Azidoacetamido)methyl)cyclohexane-1-carboxamido)-3-(naphthalen-2-yl)propanamido)-1-carboxypentyl)carbamoyl)-L-glutamic acid ***PSMA-N$_3$***



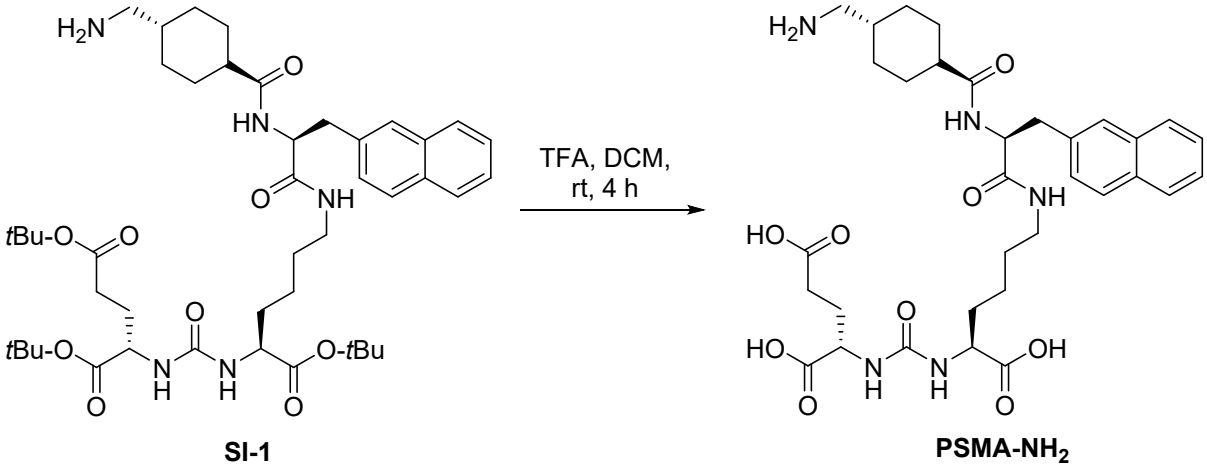
SI-2 **PSMA-N$_3$**

SI-2 (116 mg, 0.12 mmol, 1.00 eq.) was dissolved in anhydrous DCM (8 mL), TfOH (7 mL) was added and the mixture was allowed to stir at rt overnight. Afterwards, the solvent was removed in an argon stream, the crude product was precipitated with ice-cold diethyl ether, and washed with ice-cold n-hexane (2 x 10 mL) and chloroform (2 x 10 mL). The crude product was purified via preparative HPLC (Method (2) in Table 5.1, t R = 12.9 min) and dried via lyophilization to afford **PSMA-N$_3$** as white solid (22 mg, 23%).

HPLC: t_R = 10.0 min (Agilent C18 column, solvent A: water + 0.1% TFA, solvent B: acetonitrile + 0.1% TFA, flow rate: 1 mL/min, gradient: A/B 90/10 → 5/95 in 14 min), t_R = 16.6 min (Phenomenex C12 column, solvent A: water + 0.1% TFA, solvent B: acetonitrile + 0.1% TFA, flow rate: 1 mL/min, gradient: A/B 95/5 → 5/95 in 20 min). MS (ESI+): m/z = 796 $[M+H]^+$. $C_{39}H_{54}N_8O_{10}$ (794.91).



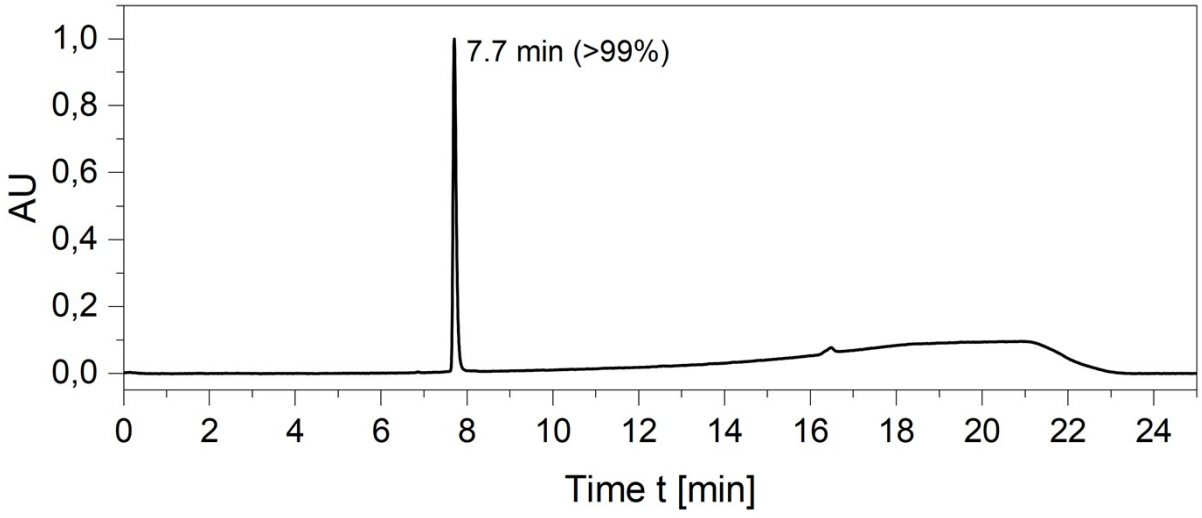
(((S)-5-((S)-2-((1s,4R)-4-(aminomethyl)cyclohexane-1-carboxamido)-3-(naphthalen-2-yl)propanamido)-1-carboxypentyl)carbamoyl)-L-glutamic acid ***PSMA-NH$_2$***

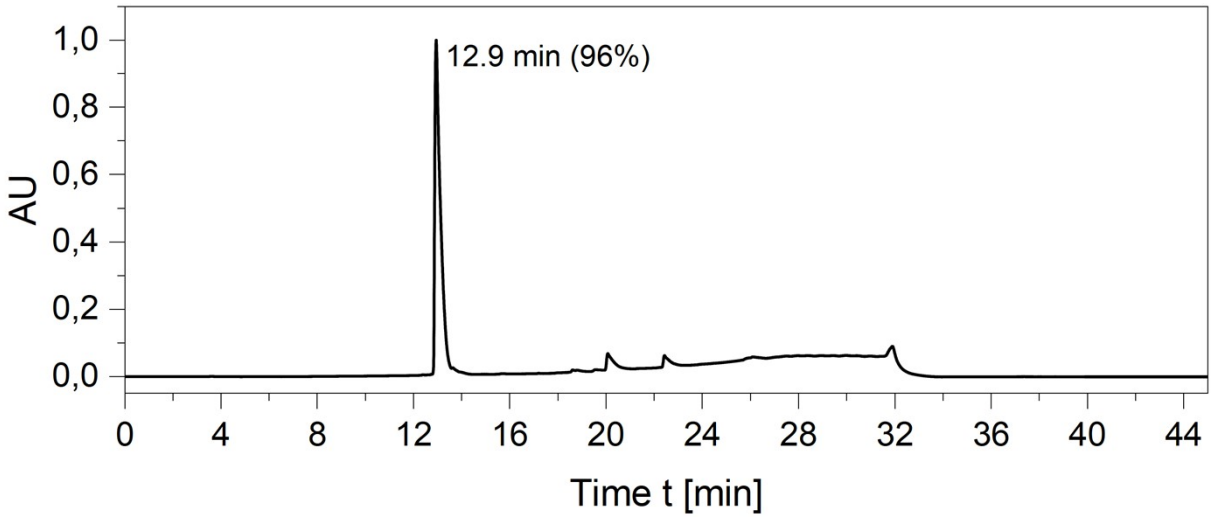


SI-1 **PSMA-NH$_2$**

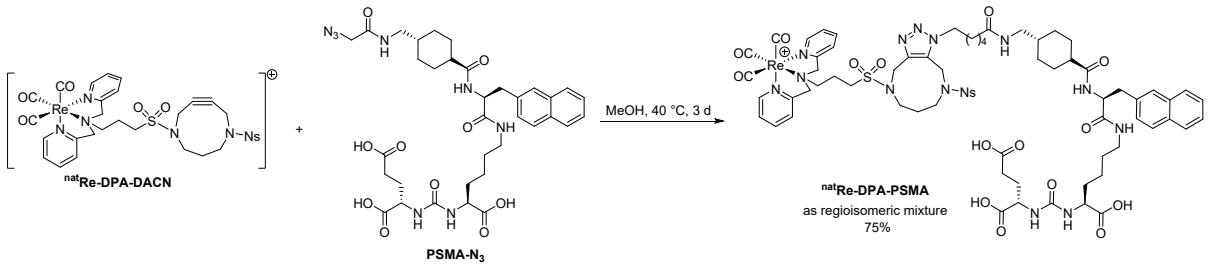
Compound **SI-1** (50 mg, 60.7 µmol, 1.00 eq.) was dissolved in anhydrous DCM (6mL), TfOH (4 mL) was added, and the mixture was stirred at room temperature for 4 h. Afterwards, the solvent and the remaining TFA were removed in an argon stream, and the crude product was purified via preparative HPLC (Agilent C18 column, solvent A: water + 0.1% TFA, solvent B: acetonitrile + 0.1% TFA, flow rate: 6 mL/min, gradient: 85/15 → 40/60 in 35 min, t_R = 6.1 min) and dried via lyophilization to afford **PSMA-NH$_2$** as white solid (34 mg, 85%).

HPLC: t_R = 7.7 min (Agilent C18 column, solvent A: water + 0.1% TFA, solvent B: acetonitrile + 0.1% TFA, flow rate: 1 mL/min, gradient: A/B 90/10 → 5/95 in 14 min); t_R = 12.9 min (Phenomenex C12 column, solvent A: water + 0.1% TFA, solvent B: acetonitrile + 0.1% TFA, flow rate: 1 mL/min, gradient: A/B 95/5 → 5/95 in 20 min). MS (ESI+): *m/z* = 657 $[M+H]^+$. $C_{33}H_{45}N_5O_9$ (655.75).



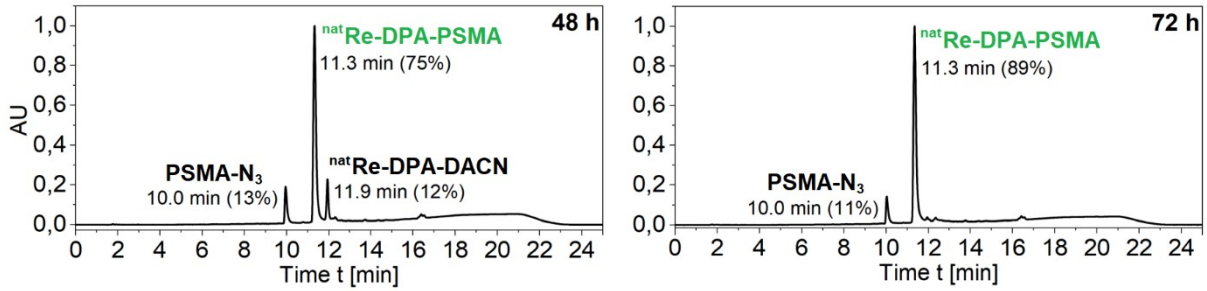

12.9 min (96%)
AU
1,0
0,8
0,6
0,4
0,2
0,0
0
4
8
12
16
20
24
28
32
36
40
44
Time t [min]

Copper-free strain-promoted cycloaddition between PSMA-N_3 and ^{nat}Re-DPA-DACN

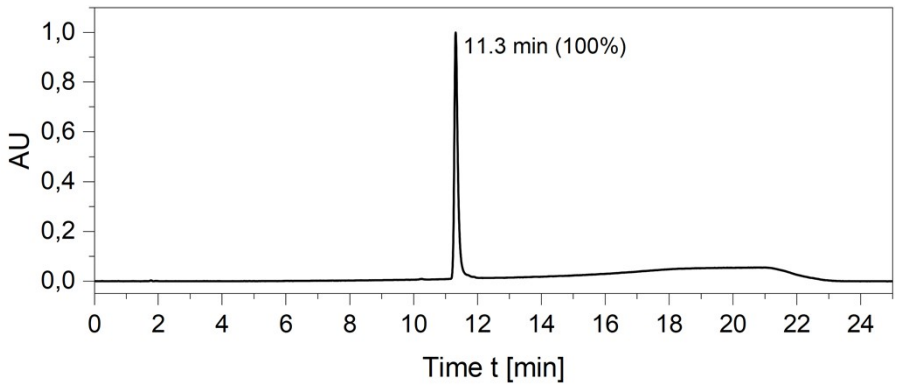


PSMA-N_3 (11.49 mg, 14.5 µmol, 1.01 eq.) and **^{nat}Re-DPA-DACN** (12.74 mg, 14.4 µmol, 1.00 eq.) were dissolved in anhydrous MeOH (5 mL) and the reaction was stirred at 40 °C for 3 d. After reaction control by analytical HPLC analysis, the solvent was removed and the crude product was purified by preparative HPLC (Agilent C18 column, solvent A: water + 0.1% TFA, solvent B: acetonitrile + 0.1% TFA, flow rate: 6 mL/min, gradient: 70/30 → 30/70 in 35 min, t_R = 13.0 min). The product was dried by lyophilization to give **^{nat}Re-DPA-PSMA** as white solid (18 mg, 75%).

HPLC: t_R = 11.3 min (Agilent C18 column, solvent A: water + 0.1% TFA, solvent B: acetonitrile + 0.1% TFA, flow rate: 1 mL/min, gradient: A/B 90/10 → 5/95 in 14 min), t_R = 17.6 min (Phenomenex C12 column, solvent A: water + 0.1% TFA, solvent B: acetonitrile + 0.1% TFA, flow rate: 1 mL/min, gradient: A/B 95/5 → 5/95 in 20 min).
MS (ESI+): *m/z* = 838 $[M\text{-}Br+H]^{2+}$ (^{185}Re), 839 $[M\text{-}Br+H]^{2+}$ (^{187}Re). $C_{70}H_{86}BrN_{14}O_{19}ReS_2$ (1757.77).

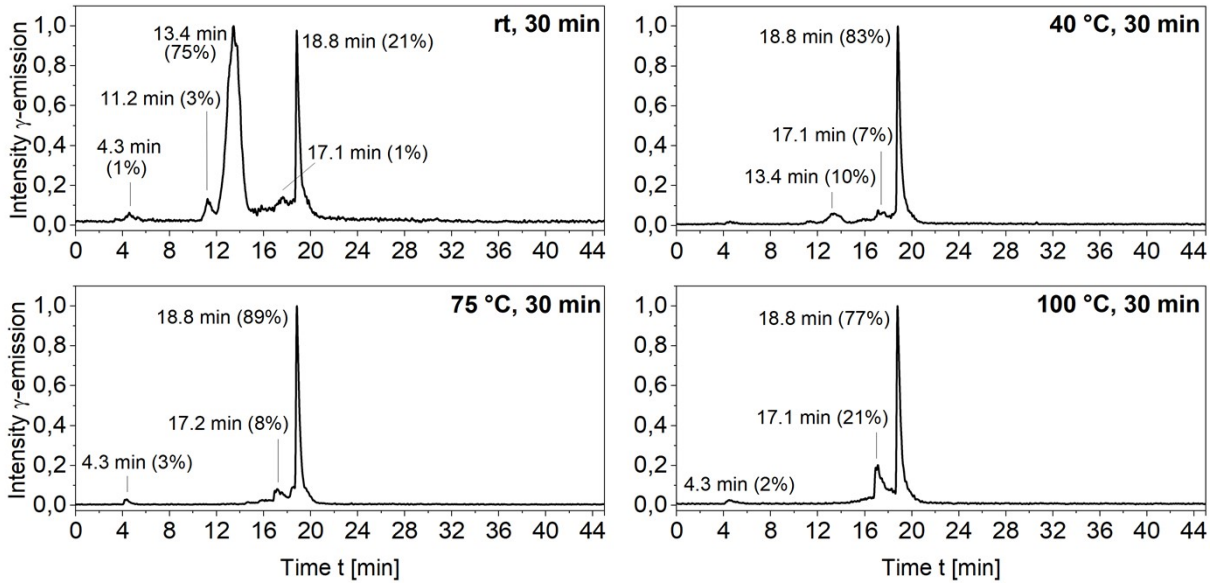


*Reaction progress of the strain-promoted click reaction between for **^{nat}Re-DPA-DACN** and **PSMA-N_3** after 48 h and 72 h, respectively.*

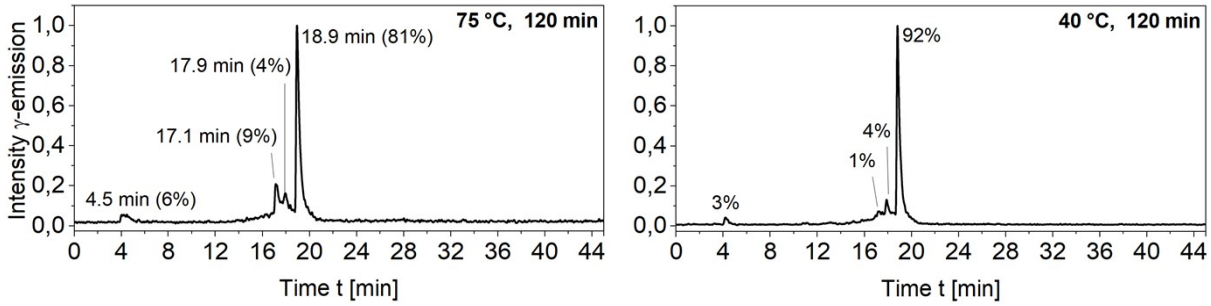


*HPLC chromatogram of the purified clicked product **^{nat}Re-DPA-PSMA**.*

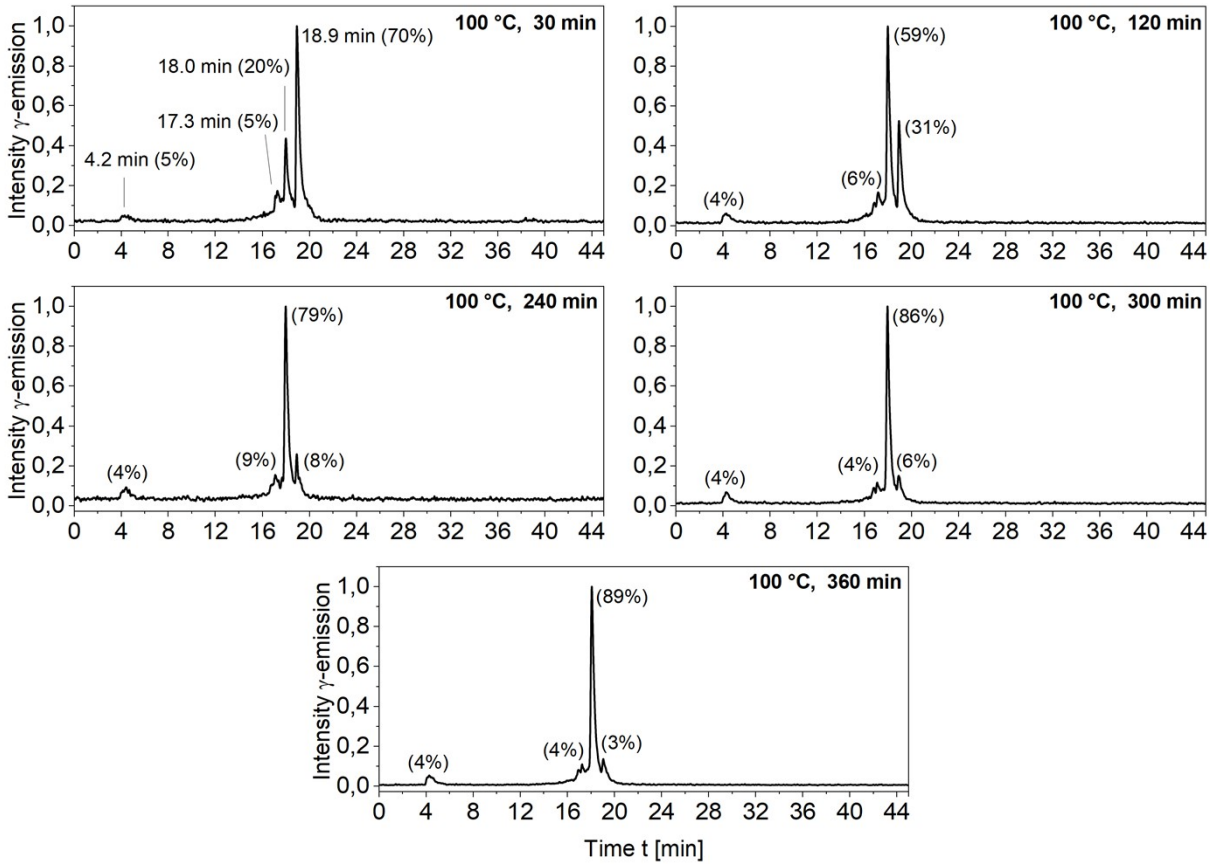
^{99m}Tc-Radiolabeling of DACN ligand 6 and SPAAC-labeling with PSMA-N_3



Radio-HPLC chromatograms of the reaction progress of the strain-promoted click reaction between ***[^{99m}Tc]Tc-DPA-DACN*** *and* ***PSMA-N_3*** *proceeding at rt (top left), 40 °C, 75 °C, and 100 °C.*

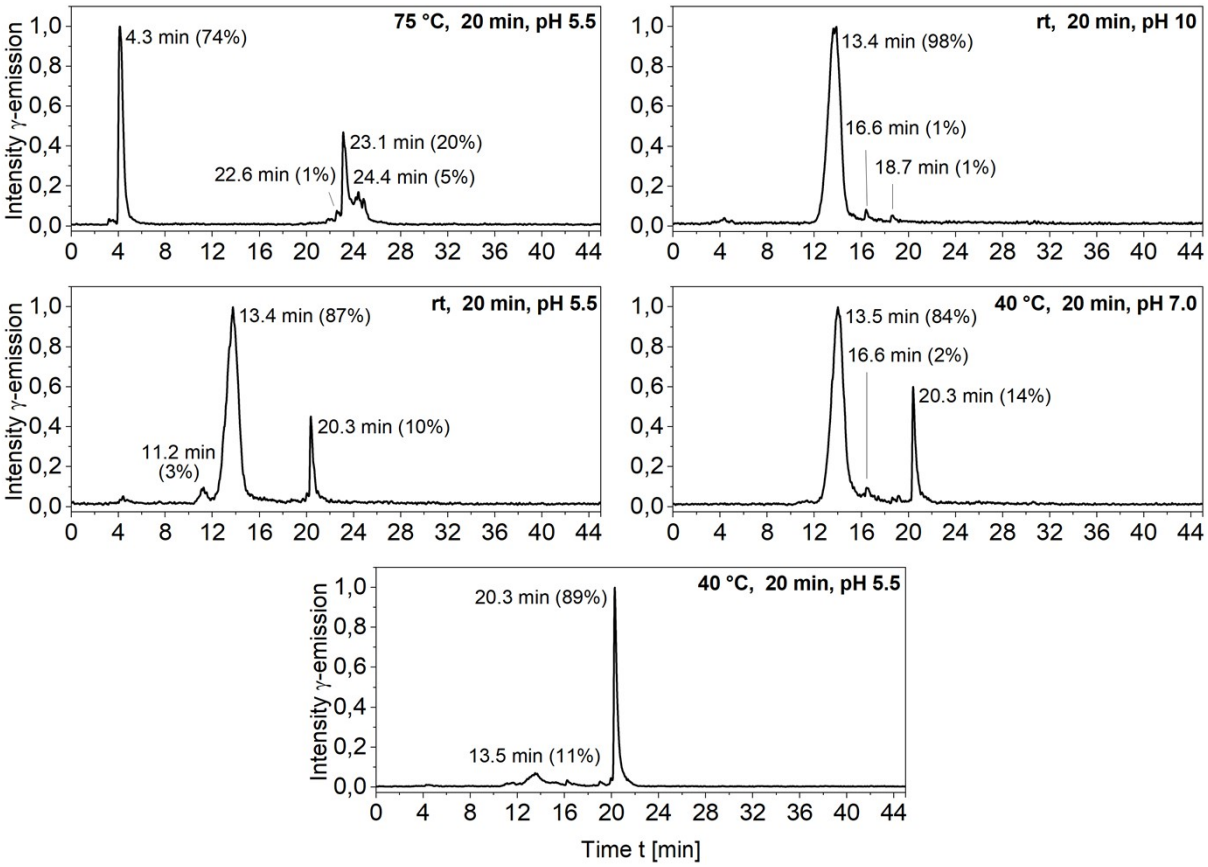


Radio-HPLC chromatograms of the reaction progress of the strain-promoted click reaction between ***[^{99m}Tc]Tc-DPA-DACN*** *and* ***PSMA-N_3*** *at 75 °C and 40 °C after 2 h. The clicked ^{99m}Tc-PSMA derivative* ***[^{99m}Tc]Tc-DPA-DACN-PSMA*** *has a retention time of t_R = 17.9 min.*

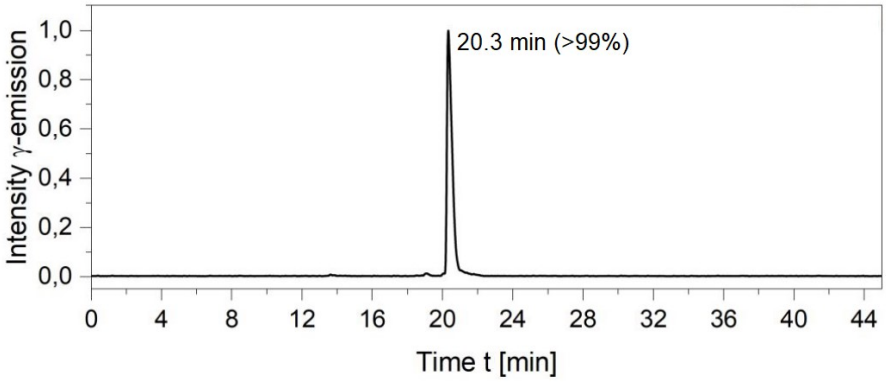


Radio-HPLC chromatograms of the reaction progress of the strain-promoted click reaction between ***[^{99m}Tc]Tc-DPA-DACN*** *and* ***PSMA-N$_3$*** *at 100 °C after 30 min, 120 min, 240 min, 300 min, and 360 min. The clicked ^{99m}Tc-PSMA derivative* ***[^{99m}Tc]Tc-DPA-PSMA*** *has a retention time of t_R = 18.0 min.*

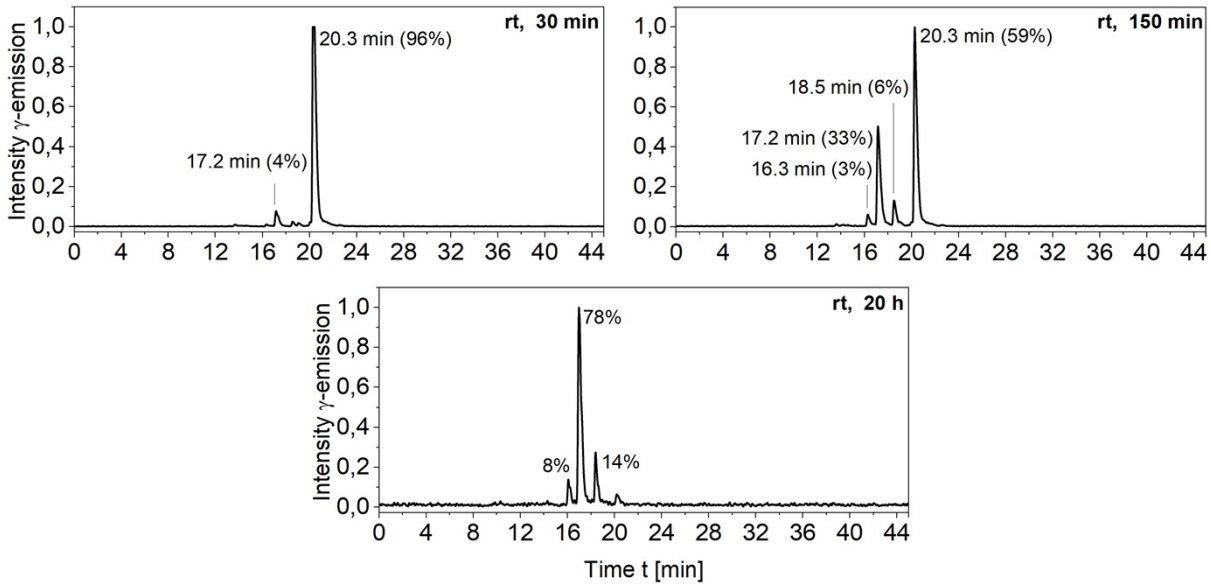
^{99m}Tc-Radiolabeling of TFP-ester 10 and conventional labeling of PSMA-NH_2



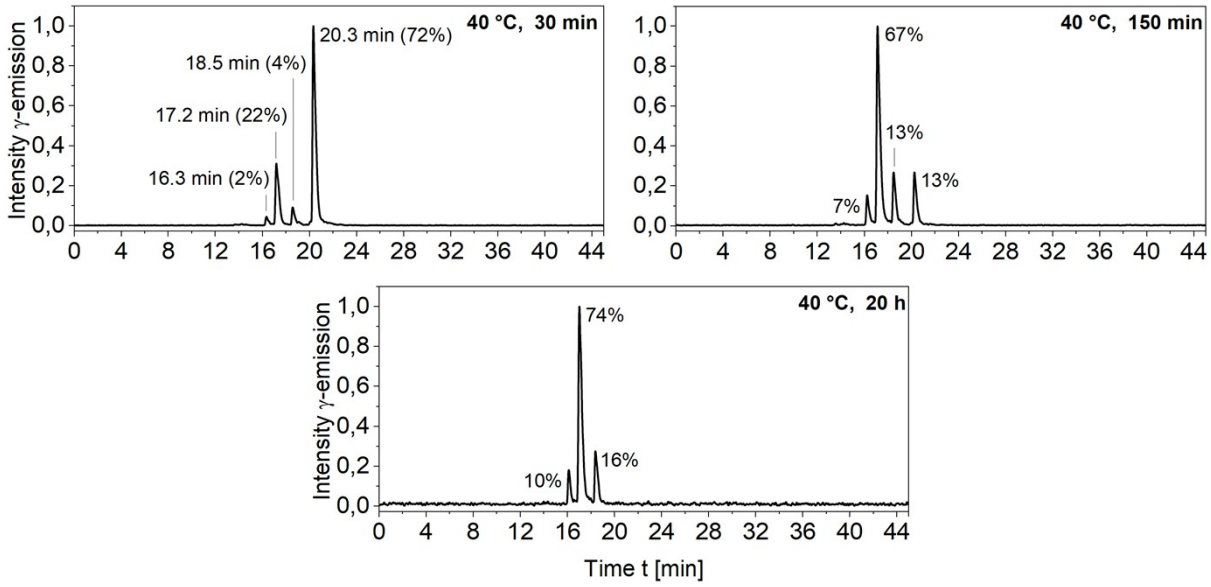
*Radio-HPLC chromatograms of ^{99m}Tc-radiolabeling of DPA-TFP ester **10** (t_R = 20.3 min) at different reaction conditions.*



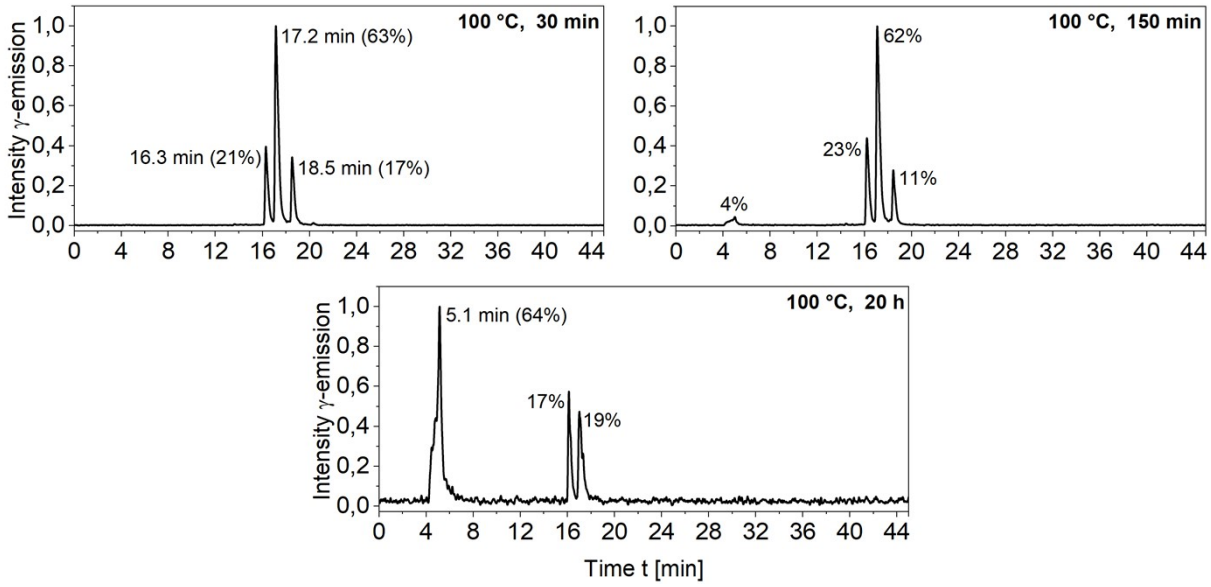
*Radio-HPLC chromatogram of purified complex **[^{99m}Tc]Tc-DPA-TFP** (t_R = 20.3 min) after separation using an RP18 cartridge.*



*Radio-HPLC chromatograms of the reaction progress of the reaction between **[^{99m}Tc]Tc-DPA-TFP*** (t_R = 20.3 min) *and **PSMA-NH$_2$** to **[^{99m}Tc]Tc-DPA-PSMA*** (t_R = 16.3 min) *at room temperature after 30 min, 150 min, and 20 h.*



*Radio-HPLC chromatograms of the reaction progress of the reaction between **[^{99m}Tc]Tc-DPA-TFP*** (t_R = 20.3 min) *and **PSMA-NH$_2$** to **[^{99m}Tc]Tc-DPA-PSMA*** (t_R = 16.3 min) *at 40 °C after 30 min, 150 min, and 20 h.*



*Radio-HPLC chromatograms of the reaction progress of the reaction between **[^{99m}Tc]Tc-DPA-TFP*** (t_R = 20.3 min) *and **PSMA-NH$_2$** to **[^{99m}Tc]Tc-DPA-PSMA*** (t_R = 16.3 min) *at 100 °C after 30 min, 150 min, and 20 h.*