**DT-ART-03-2019-001338**

# Electronic Supplementary Information

## On the interaction of N-heterocyclic carbene $Ir^{+I}$ complexes with His and Cys containing peptides

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## 1. General experimental information

### 1.1 Chemicals and materials

All chemicals were purchased from the commercial suppliers Alfa Aesar, Sigma Aldrich, TCI or Iris Biotech and used without further purification unless otherwise stated. Air or moisture sensitive reactions were performed under a dry and inert nitrogen atmosphere using Schlenk technique with application of heavy silicon grease to prevent oxygen or moisture leakages. Solvents were purchased in analytical reagent grade or HPLC grade and used without further purification. Solvents for air and moisture sensitive reactions were dried prior use with an MBRAUN MB SPS-800. After initial distillation, the solvents were dried over 3Å molecular sieve, finally dried over an AlOx column and stored under inert nitrogen atmosphere. Methanol was dried over Mg and distilled as well as stored under inert gas.

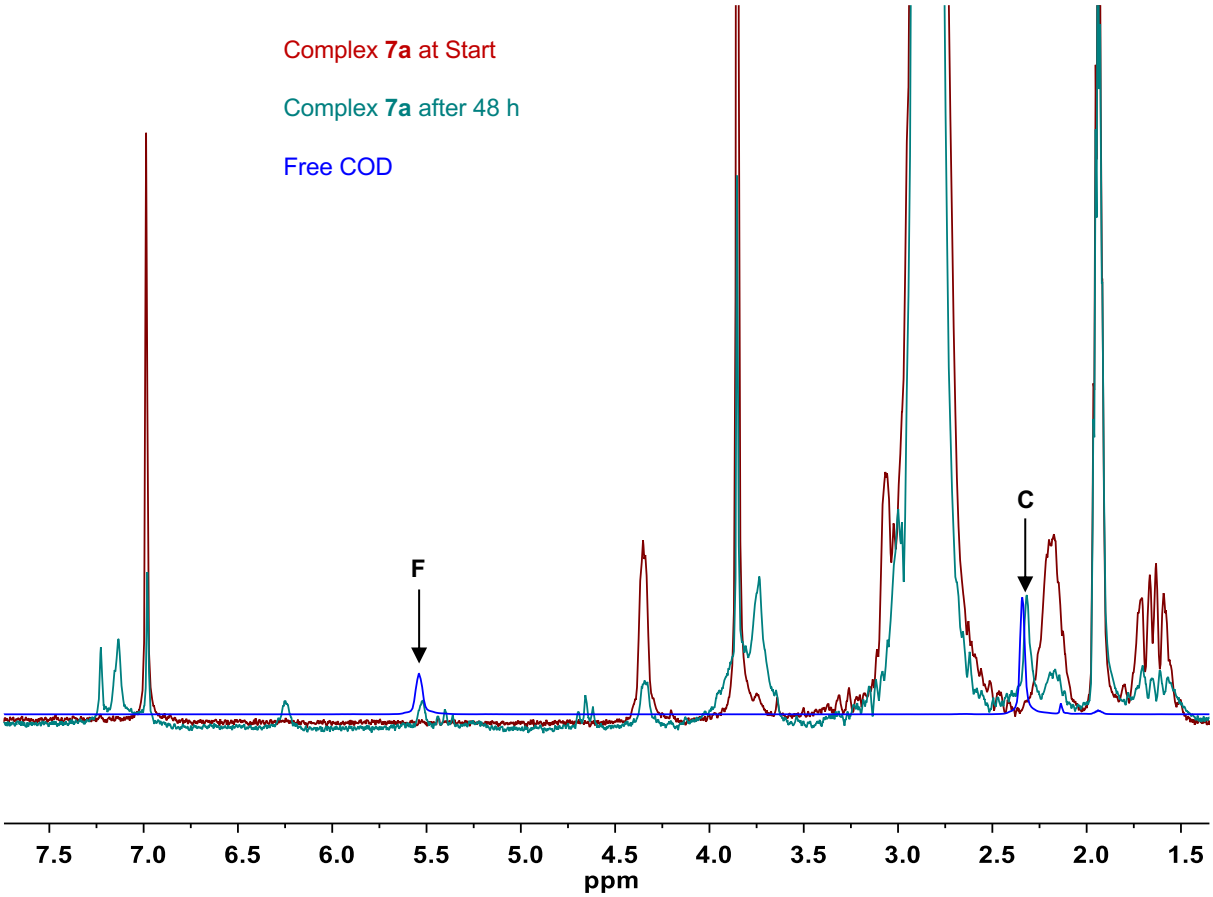
### 1.2 Technical equipment

Normal phase chromatographic purification of the crude products was carried out with the Combi Flash Rf chromatography system with Chromabond SiOH columns purchased from Macherey Nagel by solid load technique. Reverse phase purification of the peptides the preparative HPLC column LiChrospher 100 RP-18e 5 µm Hibar 250-25 column was used on a Varian ProStar HPLC system. Semi-preparative HPLC analysis and purifications were performed on a Knauer HPLC system using either one of the following columns: Dr. Maisch Reprosil pur C18 or C8 250 x 4.6 mm, EC 125/4 Nucleodur C4 Gravity 5 µm, EC 125/4 Nucleodur C18 Pyramid 5µm for analysis and the Dr. Maisch Reprosil pur C18 250 x 10 mm, VP 125/10 Nucleodur C4 Gravity 5 µm or VP 125/10 Nucleodur 100-5 C18ec for purification.

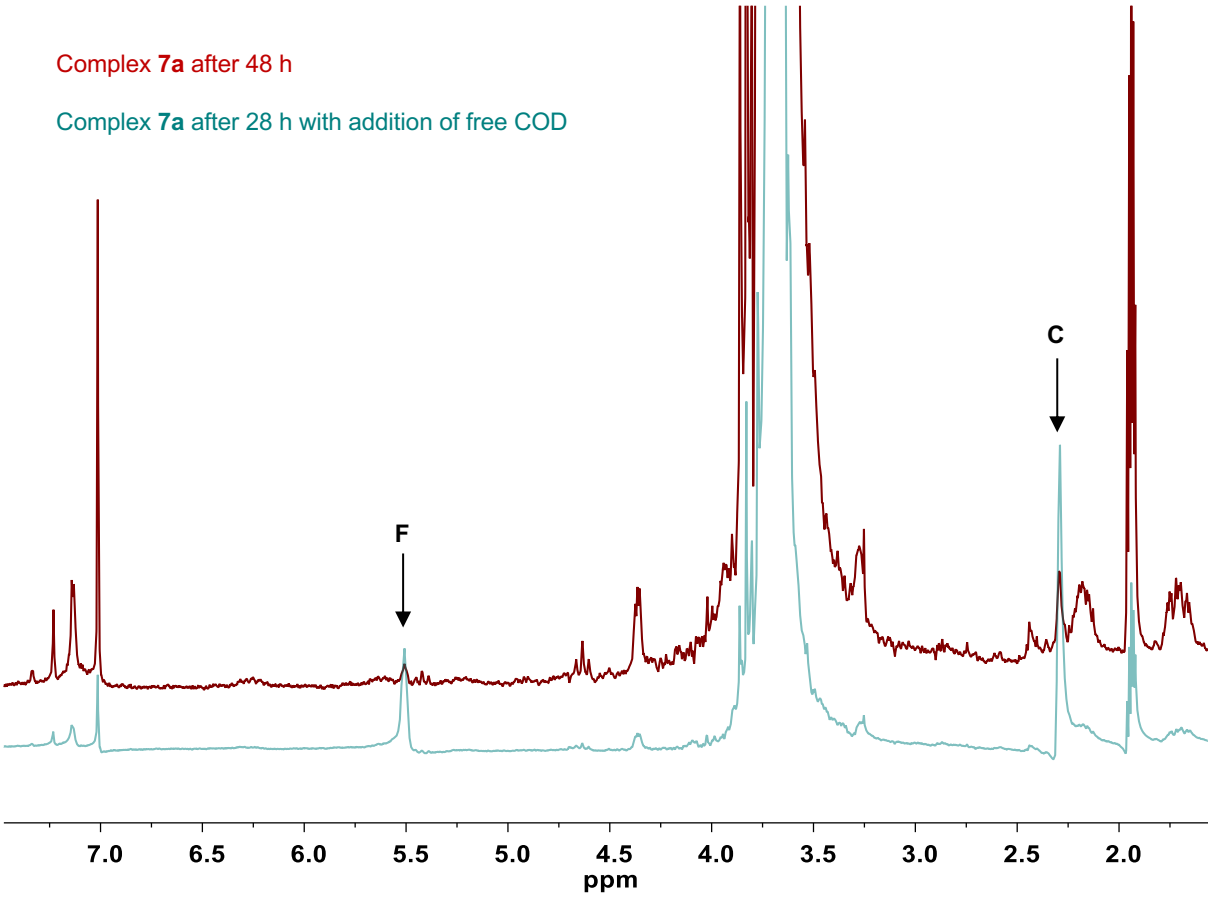
All samples for nuclear magnetic resonance analysis were prepared with deuterated solvents from commercial suppliers, which were also used as internal reference. $^{1}$H-NMR and $^{13}$C-NMR were recorded with either one of the following spectrometers: Bruker Advance DPX-200, Bruker DPX-250, Bruker AV III 300 Nanobay or Bruker DRX 400.

2. Supplementary Figures

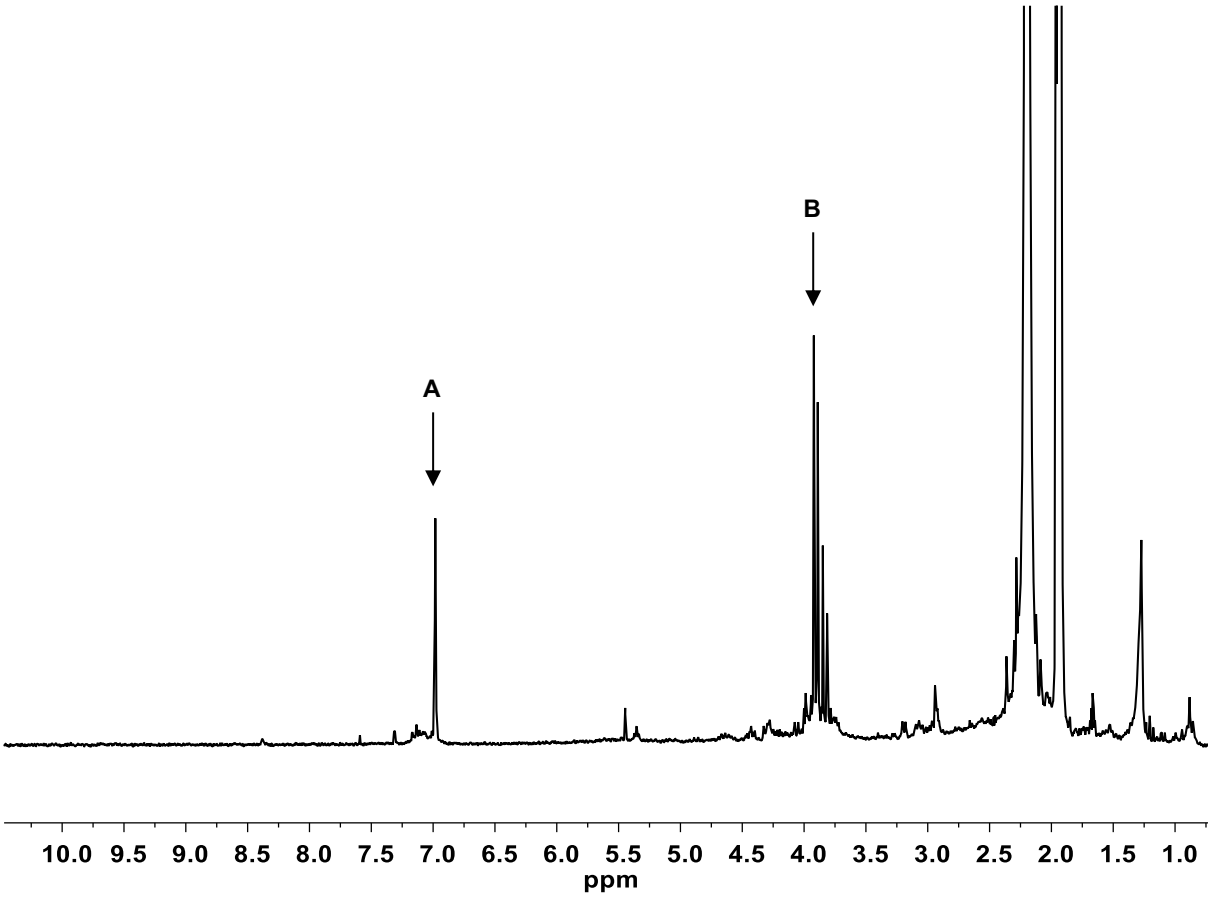
2.1 NMR Experiments



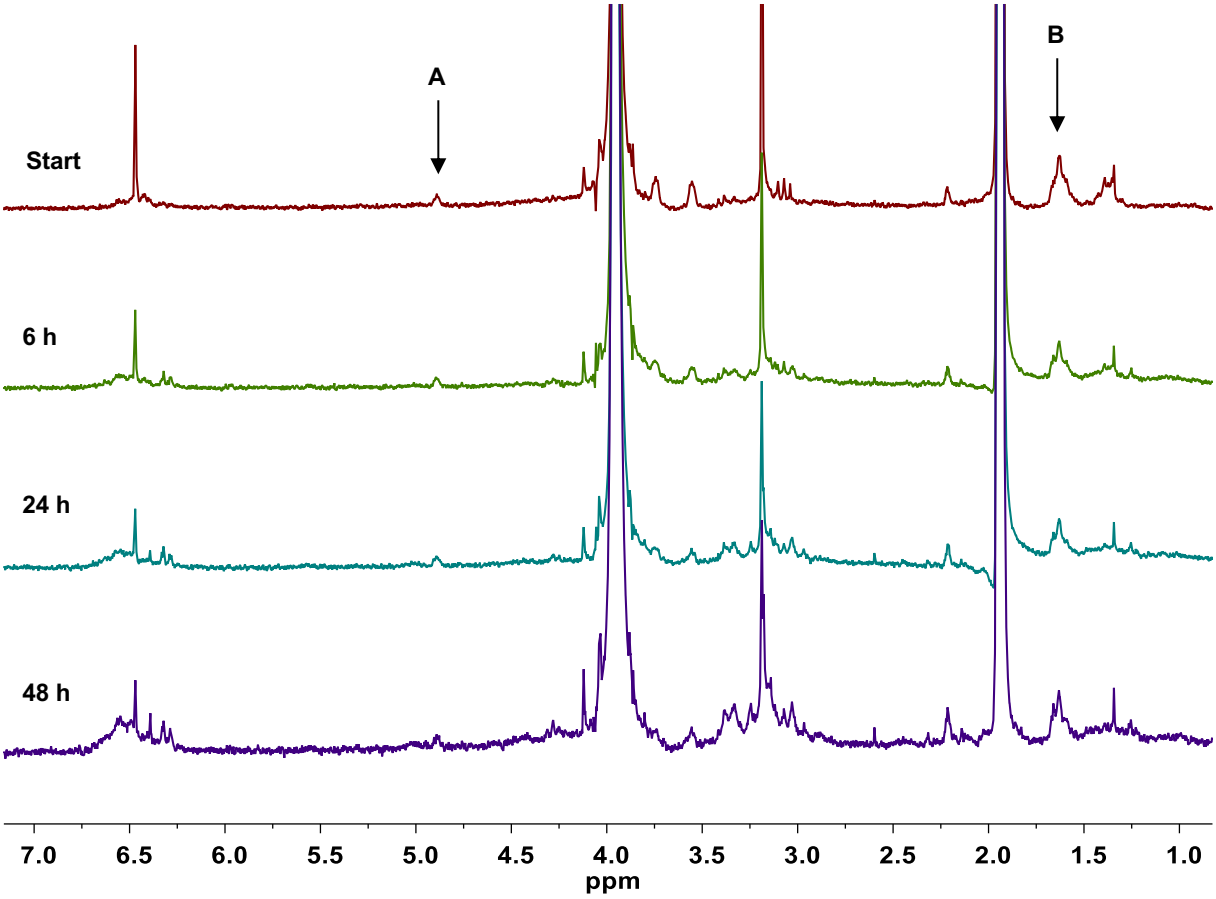

**Supplementary Figure S1:** Overlay of the $^{1}$H NMR spectra of complex **7a** at the beginning of the experiment (red trace), after 48 h (green trace) and of free COD (blue trace) in deuterated acetonitrile. The red and green traces nicely illustrate the overall decrease in peak intensity of the initial complex over time. The newly arising signals F and C (marked with an arrow) are closest to the signals of free COD



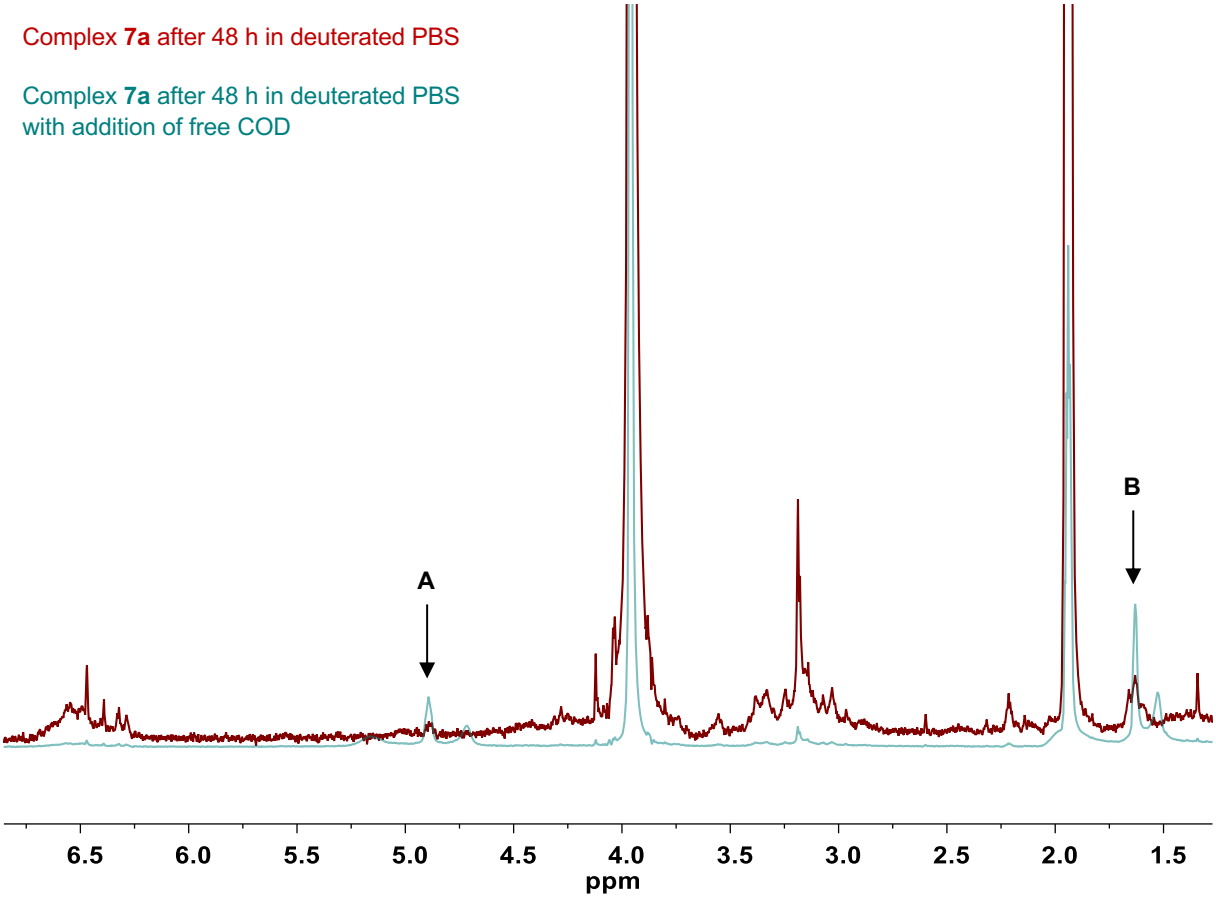

**Supplementary Figure S2:** Overlay of the $^{1}$H NMR spectra of complex **7a** after 48 h (red trace) and of the same sample after addition of free COD (green trace). Upon addition of COD the green trace shows an increase in peak intensity of the signals F and C (marked with an arrow) thus clearly identifying them as the COD ligand of the initial complex getting detached in solution.



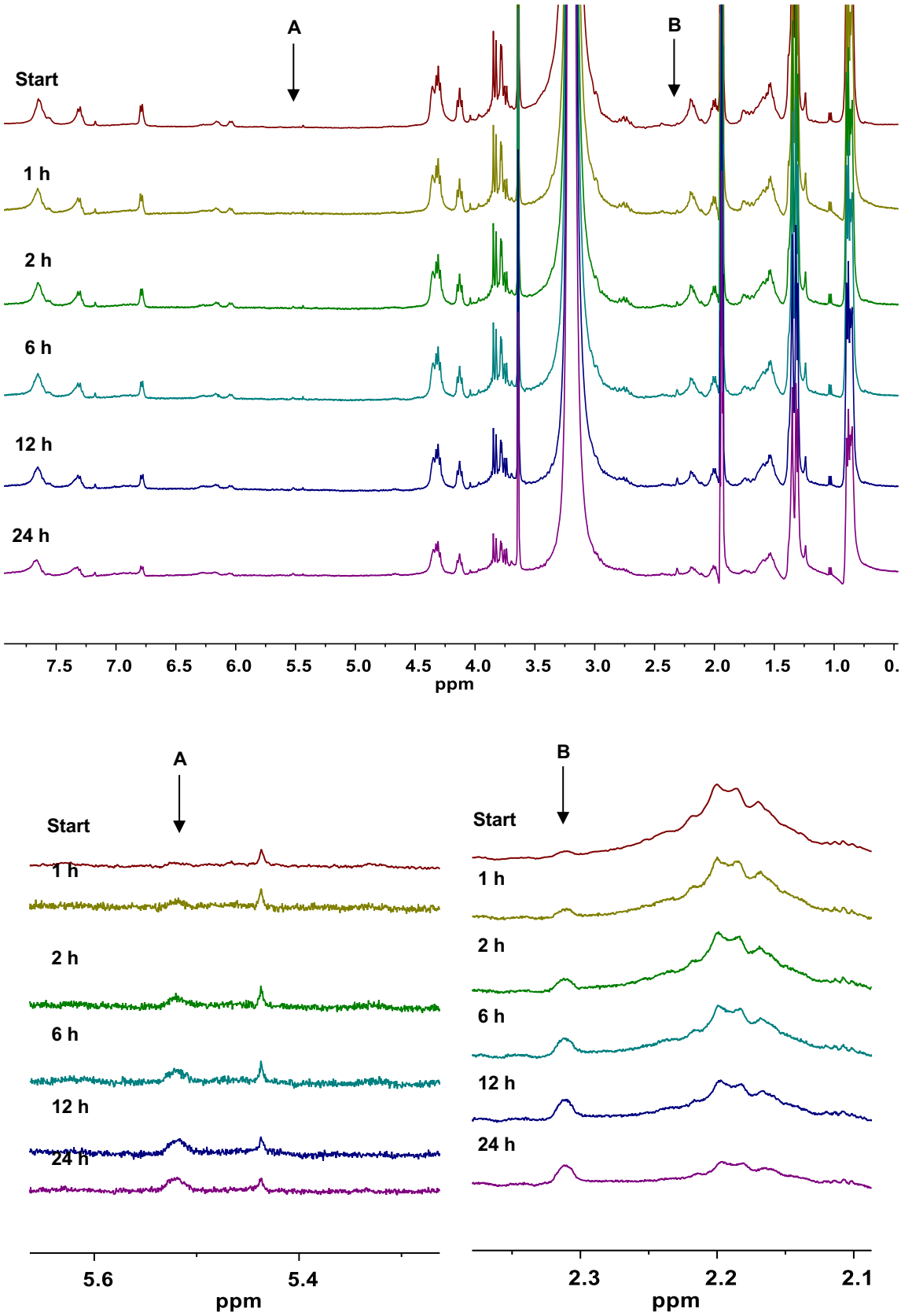

**Supplementary Figure S3:** $^{1}$H NMR spectra of the new spot isolated after incubation of complex **7a** for 48 h in deuterated Acetonitrile with addition of water. The signals A and B (marked with an arrow) can be assigned to the NHC ligand. The presence of several distinct singlets for signal B indicates the presence of more than one species. The absence of any signal in the area between 10 ppm and 9 ppm further indicates, that the NHC ligand is still bound to iridium. Furthermore, no COD signals are visible, thus proving displacement of the COD ligand going along with formation of a new Ir-NHC species.




**Supplementary Figure S4:** Time dependent $^{1}$H NMR spectra of complex **7a** in deuterated PBS with addition of 10% of DMSO. The spectrum shows the signals expected for the compound and the additional signals A and B (marked with an arrow). Over time an overall decrease in peak intensity can be observed for the signals representing complex **7a** while the intensity of the additional signals shows only a minor decrease.

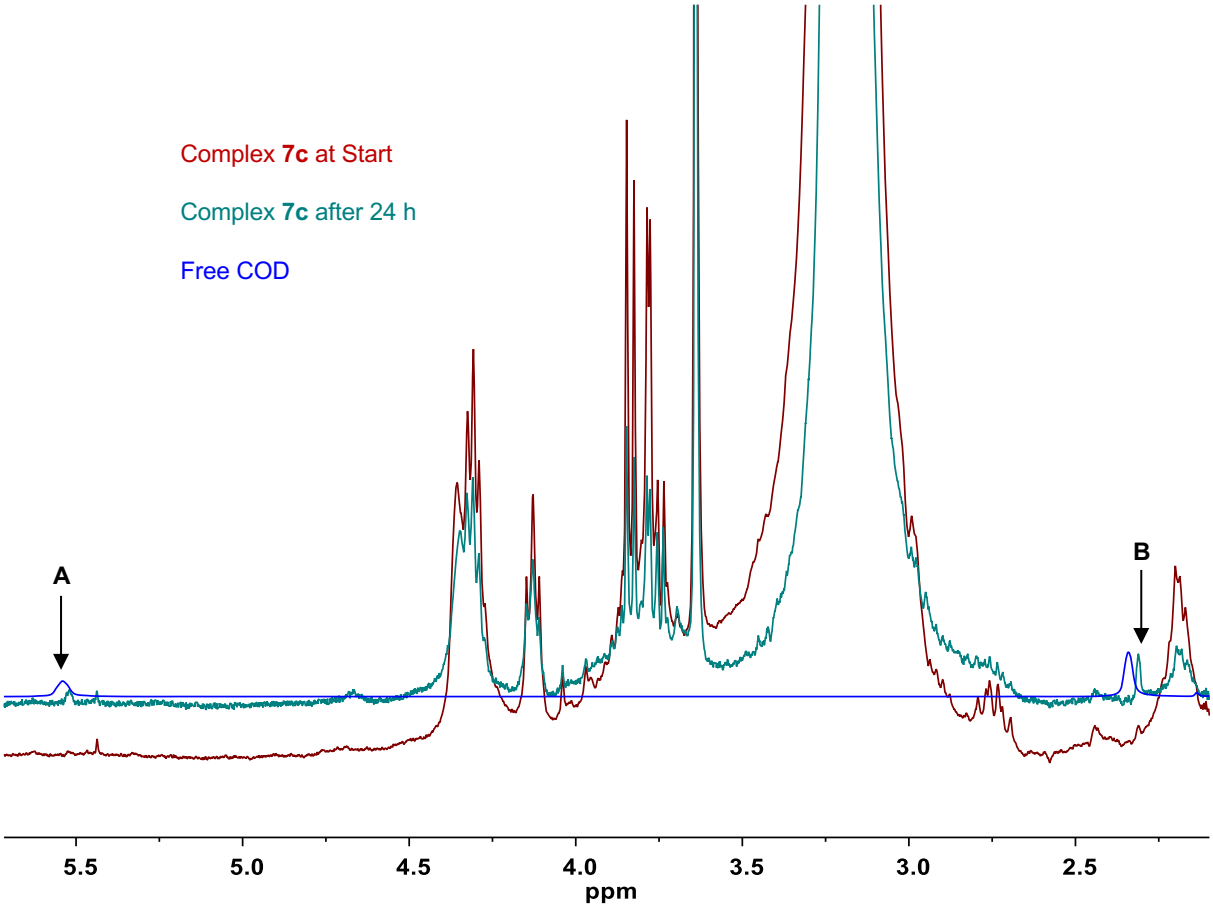



**Supplementary Figure S5:** Overlay of the $^{1}$H NMR spectra of complex **7a** after 48 h in deuterated PBS with addition of 10% of DMSO (red trace) and of the same sample after addition of free COD (green trace). Upon addition of COD the green trace shows an increase in peak intensity of the signals A and B (marked with an arrow) thus clearly identifying them as the COD ligand of the initial complex getting detached in solution.

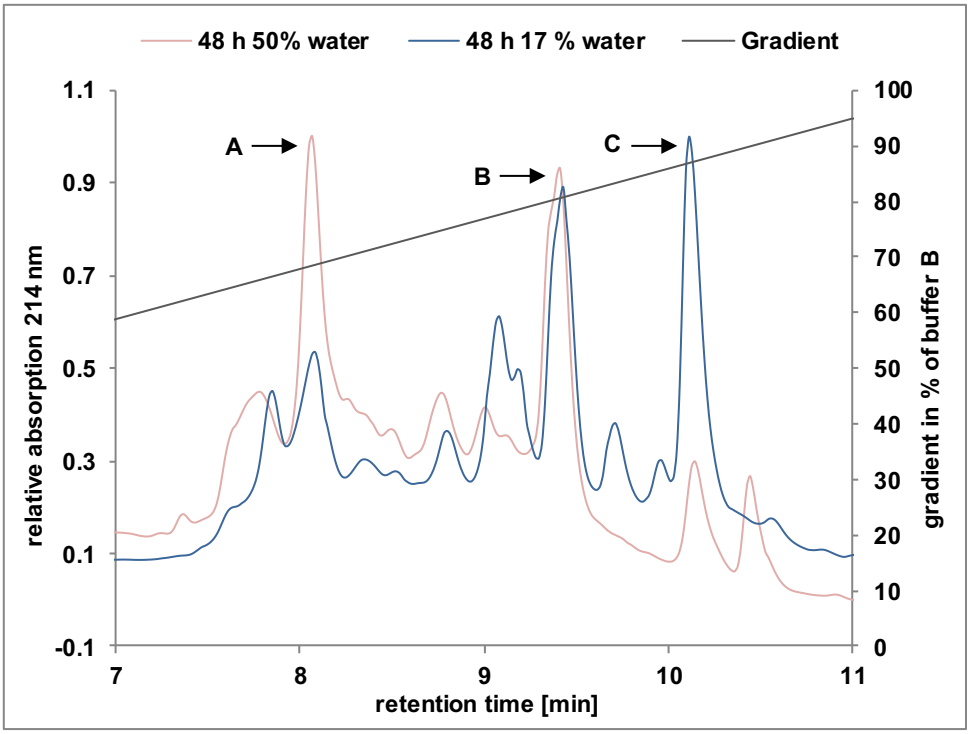



**Supplementary Figure S6:** Upper Part: Change in the overall $^{1}$H NMR of complex **7c** over time in deuterated ACN, which was used as an internal standard, after addition of water. The new signals A and

B are marked with an arrow as they represent the signals of the detached COD ligand. Lower part: Zoom into the area of signals A and B showing the rise of the new signals over time

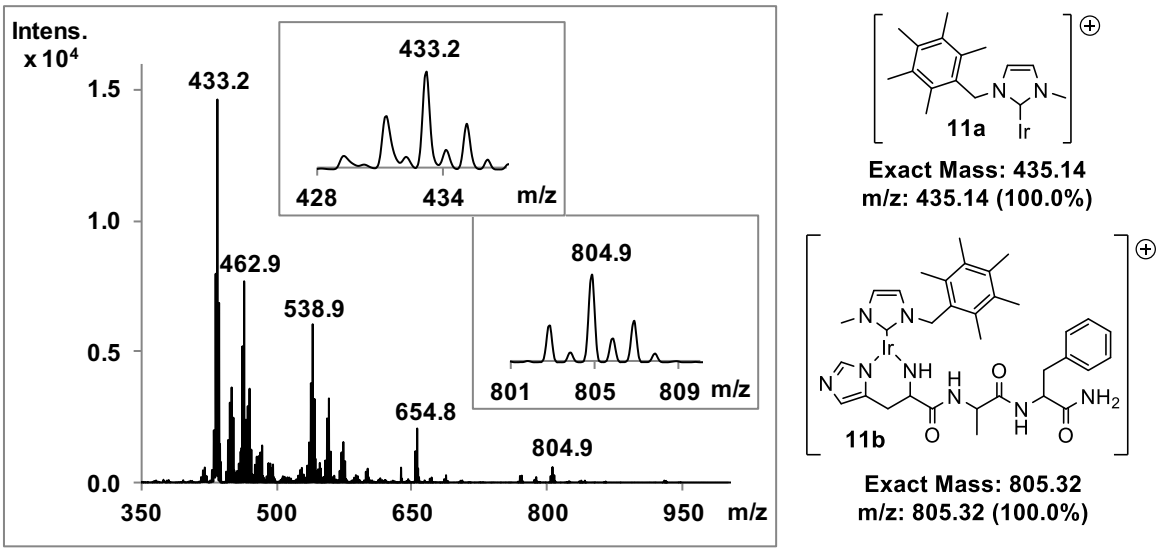



**Supplementary Figure S7:** Overlay of the $^{1}$H NMR spectra of complex **7c** at the beginning of the experiment (red trace), after 24 h (green trace) and of free COD in deuterated acetonitrile (blue trace). The newly arising signals A and B (marked with an arrow) show a nearly perfect overlay with free COD, identifying them as the COD ligand of the initial complex getting detached in solution.

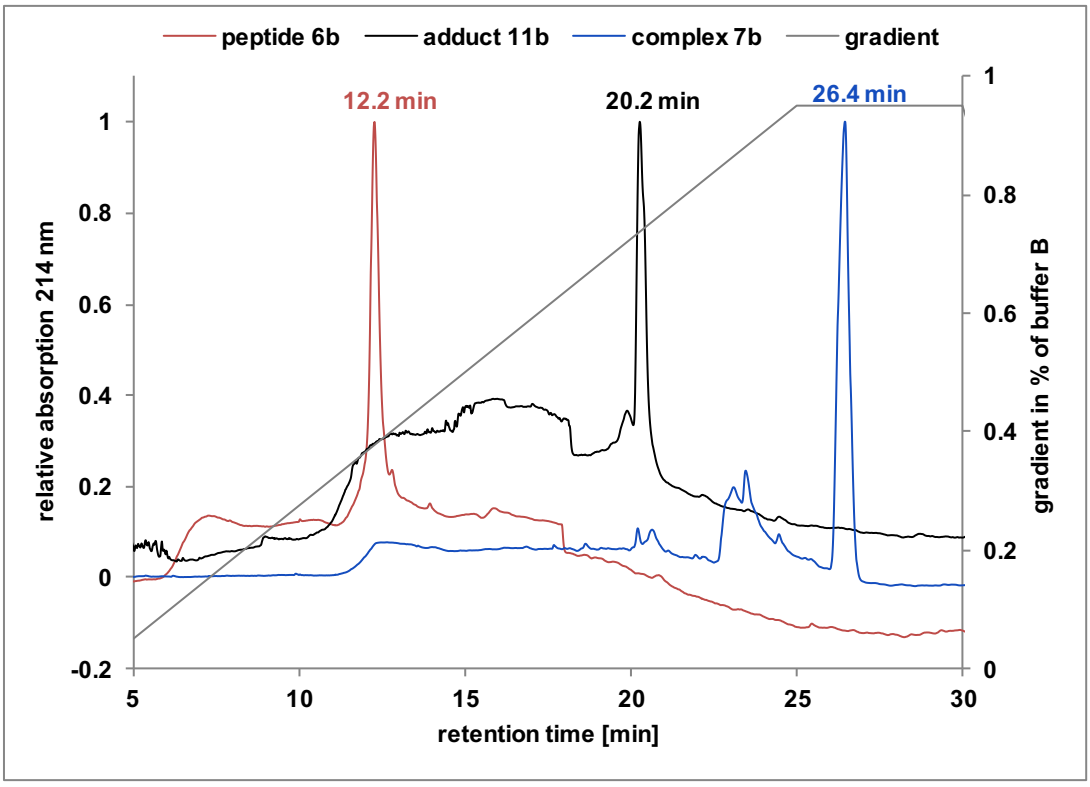



**Supplementary Figure S8:** Analytical HPLC chromatogram of complex **7c** after incubation in a solution of water and acetonitrile for 48 h showing the relative absorption at 214 nm (red trace: 50% of water, blue trace: 17% of water). The comparative chromatograms show that after 48 h, peak C which represents compound **7c** is still clearly visible as the main component in the sample with 17% of water, while it has almost completely disappeared in the sample with 50% of water, leading to the conclusion that the water concentration is a key component of the samples decomposition rate.

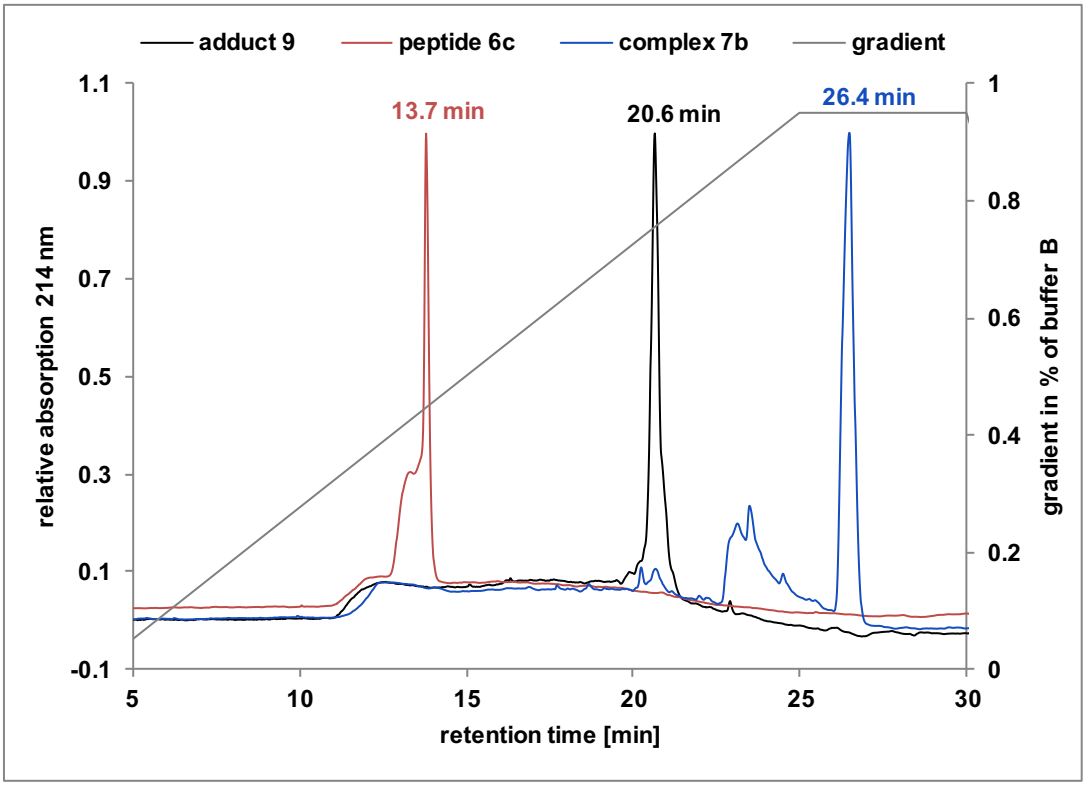
## 2.2 Peptide interaction studies



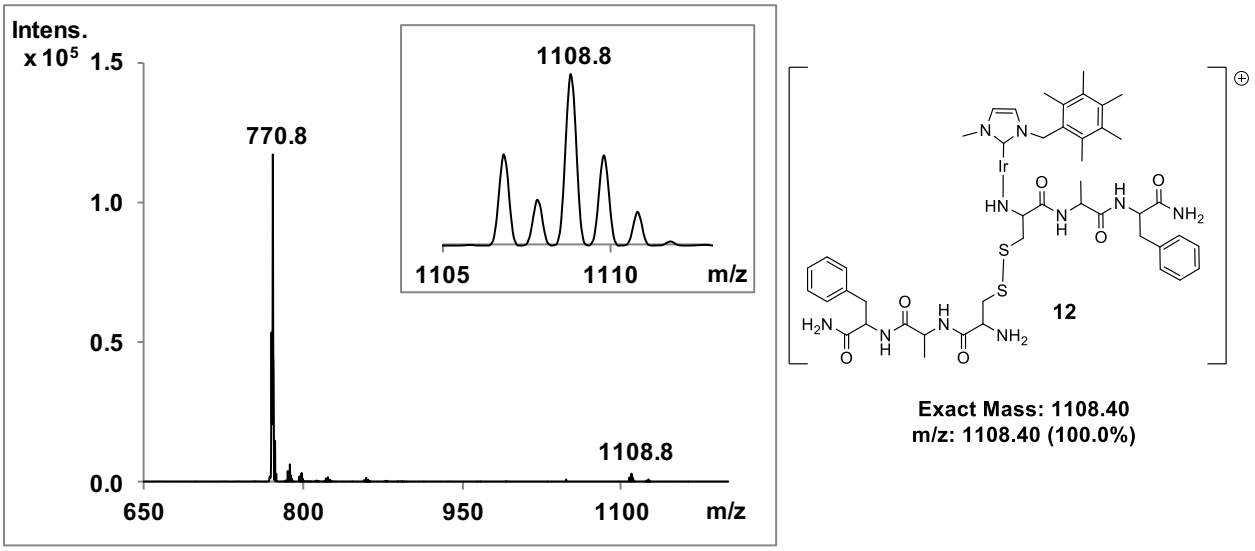

**Supplementary Figure S9:** ESI MS spectrum of the adduct between peptide **6b** and complex **7b** with the respective fragments **11a** and **11b** belonging to the suggested Iridium adduct. In contrast to the adducts of **6**c and **7b**, several other fragments with undefined structures can be seen.



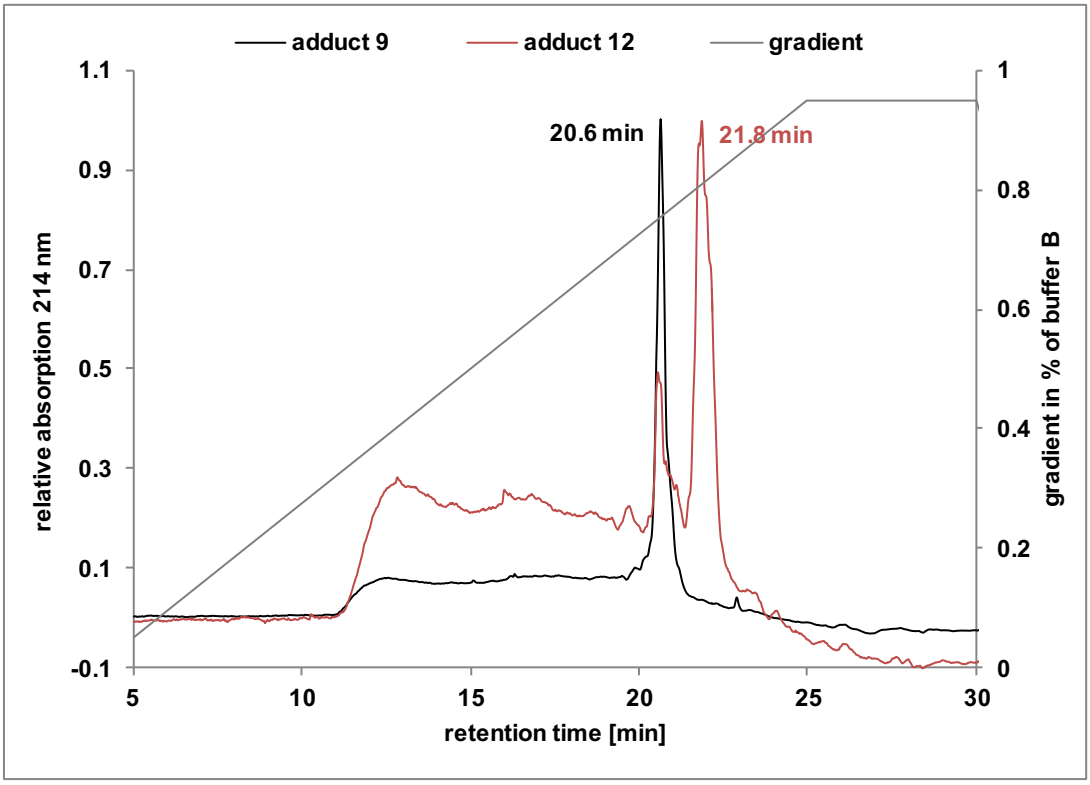

**Supplementary Figure S10:** Analytical HPLC trace of adduct **11b** in comparison to peptide **6b** and complex **7b** (Buffer A: $H_2O$ with 0.1% TFA, Buffer B: ACN with 0.1% of TFA, Column: Dr. Maisch Reprosil pur C8 250 x 4.6 mm)



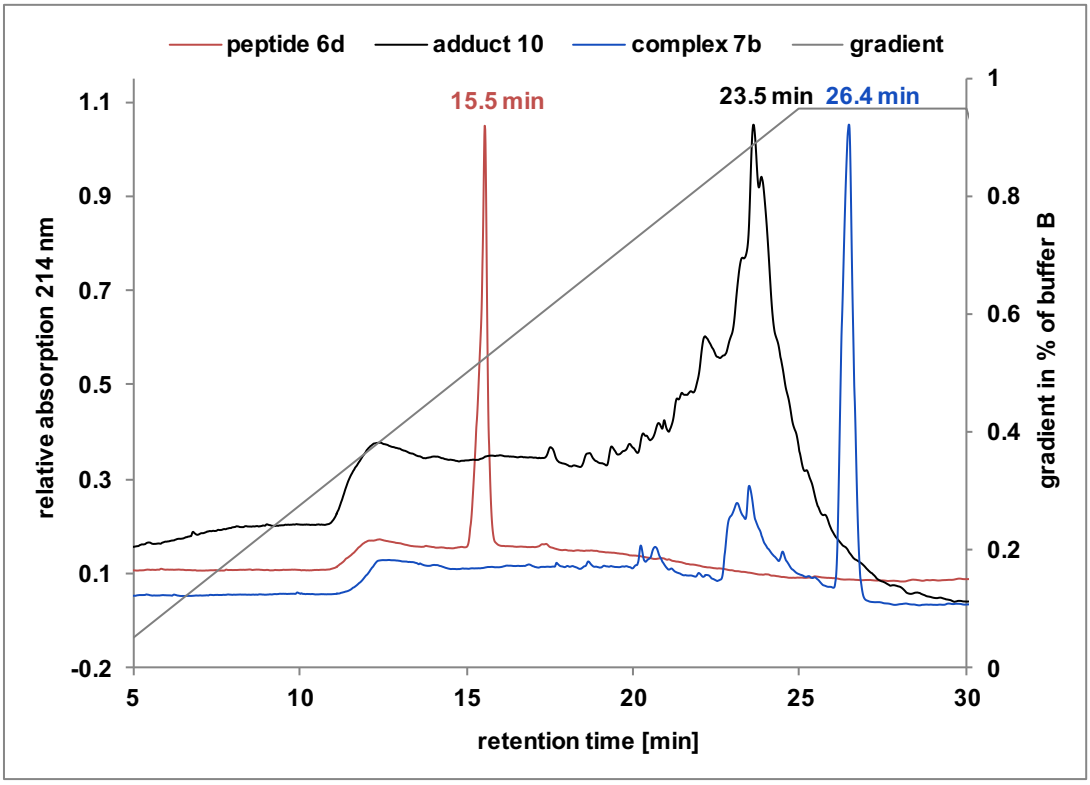

**Supplementary Figure S11:** Analytical HPLC trace of adduct **9a** or **b** in comparison to peptide **6c** and complex **7b** (Buffer A: $H_2O$ with 0.1% TFA, Buffer B: ACN with 0.1% of TFA, Column: Dr. Maisch Reprosil pur C8 250 x 4.6 mm)




**Supplementary Figure S12:** ESI MS spectrum of the adduct between dimerized peptide **6c**$_2$ and complex **7b** with the respective fragment **12** belonging to the suggested Iridium adduct.



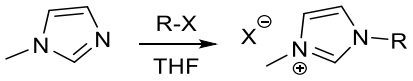

**Supplementary Figure S13:** Analytical HPLC trace of adduct **9a** or **b** in comparison to adduct **12** (Buffer A: $H_2O$ with 0.1% TFA, Buffer B: ACN with 0.1% of TFA, Column: Dr. Maisch Reprosil pur C8 250 x 4.6 mm)




**Supplementary Figure S14:** Analytical HPLC trace of adduct **10a, b, c** or **d** in comparison to peptide **6d** and complex **7b** (Buffer A: $H_2O$ with 0.1% TFA, Buffer B: ACN with 0.1% of TFA, Column: Dr. Maisch Reprosil pur C8 250 x 4.6 mm)

3. Experimental section

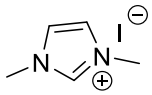
3.1 Synthesis of imidazolium salts




General procedure

In a heated Schlenk flask under $N_2$ atmosphere, N-methylimidazole was dissolved in THF. The respective alkyl halide was added and the reaction was left to stir for at least 24 h until precipitation of a white solid was observed. The solid was filtered off under $N_2$ atmosphere, washed with cold THF and dried to yield the respective imidazolium salt.

*3.1.1 1,3-dimethyl-1H-imidazol-3-ium iodide (1a)*

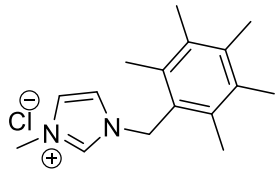



$C_5H_9IN_2$
224.05 g/mol

Yield: 3.98 g (17.8 mmol, 51 %)

Analytical data: **$^1$H NMR** (400 MHz, Chloroform-*d*) δ 10.00 (s, 1H, N-C**H**-N), 7.37 (d, *J* = 1.6 Hz, 2H, C**H**=C**H**), 4.09 (s, 6H, 2x N-C**H$_3$**) **$^{13}$C NMR** (101 MHz, Chloroform-*d*) δ 136.9 (N-**C**H-N), 122.5 (**C**H=**C**H), 37.2 (N-**C**H$_3$) **ESI-MS** m/z 97.0 $[M\text{-}Cl]^+$

*3.1.2 3-methyl-1-(2,3,4,5,6-pentamethylbenzyl)-1H-imidazol-3-ium chloride (**1b**)*




$C_{16}H_{23}ClN_2$
278.82 g/mol

Yield: 244 mg, (0.88mmol, 44 %)

Analytical data: **$^1$H NMR** (300 MHz, Chloroform-*d*) δ 10.74 (s, 1H, N-C**H**-N), 7.18 (t, *J* = 1.8 Hz, 1H, C**H**=CH), 6.79 (t, *J* = 1.8 Hz, 1H, CH=C**H**), 5.63 (s, 2H, N-C**H$_2$**), 4.12 (s, 3H, N-C**H$_3$**), 2.23 (s, 15H, 5x arom. C**H$_3$**) **$^{13}$C NMR** (101 MHz, Chloroform-*d*) δ 138.5 (N-**C**H-N), 137.6 (**C** arom.), 134.1 (**C** arom.), 133.8 (**C** arom.), 125.4, 122.7 (**C**H=CH), 120.8 (CH=**C**H), 49.4 (**C**H$_2$),

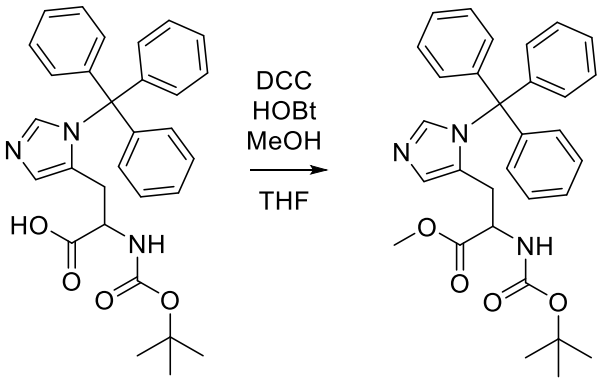
37.1 (N-**C**$H_3$), 17.5 (arom. **C**$H_3$), 17.1 (arom. **C**$H_3$), 17.0 (arom. **C**$H_3$) **ESI-MS** m/z 160.9 $[C_{12}H_{17}]^+$, 242.9 $[M\text{-}Cl]^+$

3.2 Synthesis of Boc-His($[Me_2]^+$)($I^-$)-OMe (**5**)

**General remark**

Compounds **3**-**5** were synthesized several times. The presented yield is therefore labeled as overall yield and represents the average yield calculated from the yields of the times the synthesis was performed.

*3.2.1 Boc-His(Trt)-OMe (**3**)*




$C_{30}H_{31}N_3O_4$
497.60 g/mol

$C_{31}H_{33}N_3O_4$
511.62 g/mol

General procedure

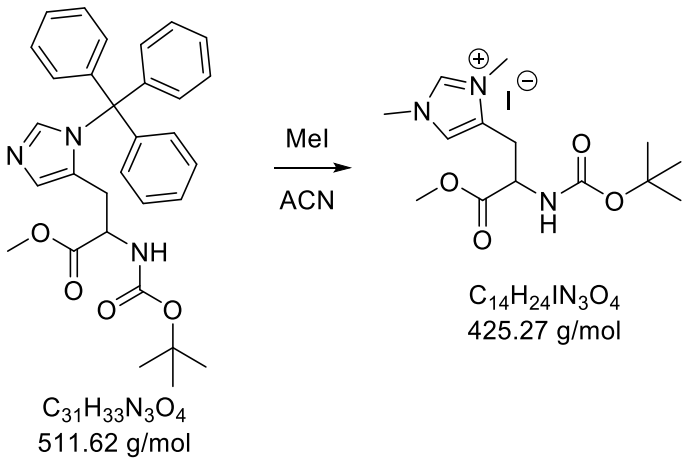
In a heated Schlenk flask under $N_2$ atmosphere, 1 eq of Boc-His(Trt)-OH **2** was dissolved in 5 ml of dry THF per mmol of starting material. 1.5 eq of HOBt were the added and the solution was cooled to -10°C. Afterwards, a solution of 1 eq of DCC in 5 ml of dry THF per mmol of DCC was added dropwise and the solution was stirred in the cold for 15 min. In the following, 35 eq of dry MeOH were added dropwise, the solution was then allowed to warm to room temperature and stirred over night. After filtration to remove the resulting dicyclohexylurea, the filtrate was evaporated under reduced pressure. The remaining residue was redissolved in 15 ml of DCM per mmol of starting material and extracted 3x with sat. $NaHCO_3$ and 3x with water. The organic phase was then dried over $Na_2SO_4$ and the solvent was evaporated under reduced pressure. Purification of the remaining oily residue by column chromatography (DCM/MeOH 99:1) yielded the product **3** as a white solid.

Overall yield: 70%

Analytical data:

**$^1$H NMR** (200 MHz, Chloroform-*d*) δ 7.34 (m, 9H, 9 x Trityl **H**), 7.10 (m, 6H, 6 x Trityl **H**), 6.52 (d, *J* = 1.4 Hz, 1H, His backbone C=**H**), 5.98 (s, 1H, α-N**H**), 4.53 (dt, *J* = 8.9, 4.8 Hz, 1H, α-C**H**), 3.60 (s, 3H, O-C**H$_3$**), 2.99 (m, 2H, His C**H$_2$**), 1.42 (s, 3x Boc C**H$_3$**) **ESI-MS** m/z 420.0 $[(M\text{-Boc})+Na]^+$ 533.9 $[M+Na]^+$ **$R_f$** (DCM/MeOH 99:1) 0.31

*3.2.2 Boc-His($[Me_2]^+$)($I^-$)-OMe (**4**)*




General procedure

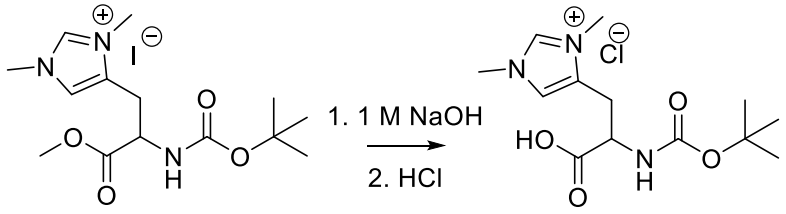
In a heated Schlenk flask 1 eq of Boc-His(Trt)-OMe **3** was dissolved in 25 ml of dry ACN per mmol of starting material under $N_2$ atmosphere. After dropwise addition of 10 eq of MeI, the resulting solution was refluxed over night during which time the reaction mixture turned brown. After evaporation of the solvent under reduced pressure, the residue was purified by flash column chromatography over silica. After elution of high amounts of $I_2$ in pure DCM, the product **4** could be obtained as a yellowish solid after addition of 20% of MeOH to the eluent.

Overall yield: 85%

Analytical data: $^1$H NMR (200 MHz, $CDCl_3$) δ 9.82 (s, 1H, N-C**H**-N), 7.13 (s, 1H, His backbone **H**), 5.57 (d, *J* = 6.7 Hz, 1H, α-N**H**), 4.49 (m, 1H, α-C**H**), 3.94 (s, 3H, N-$CH_3$), 3.90 (s, 3H, N-$CH_3$), 3.75 (s, 3H, O-C**H$_3$**), 3.21 (m, 2H, His C**H$_2$**), 1.35 (s, 9H, 3x Boc C**H$_3$**) **$^{13}$C NMR** (75 MHz, $CDCl_3$) δ 170.8 (**C**$OOCH_3$), 137.5 (N-**C**H-N), 131.8 (His backbone **C**=CH), 121.7 (His

backbone **C**H=C), 80.9 (Boc quat. **C**), 53.4 (α-**C**H), 52.3 (O-**C**$H_3$), 37.0 (N-**C**$H_3$), 34.5 (N-**C**$H_3$), 28.4 (Boc **C**$H_3$), 26.7 (**C**$H_2$) **ESI-MS** m/z 420.0 $[M-I]^+$, **$R_f$** (DCM/MeOH 9:1) 0.09

*3.2.3 Boc-His($[Me_2]^+$)($I^-$)-OH (**5**)*




$C_{14}H_{24}IN_3O_4$
425.27 g/mol

$C_{13}H_{22}ClN_3O_4$
319.79 g/mol

After dissolving 1 eq of Boc-His$(Me_2)^+$-$I^-$-OMe **4** in 7.5 ml of 1 M NaOH (approx. 7 eq), the solution was stirred at room temperature for 1 h. Afterwards, the solution was acidified to pH 4 with 6 M HCl and the solvent was evaporated to dryness under reduced pressure. The remaining white solid was extracted three times with approx. 30 ml of ACN per mmol of starting material and the combined organic solutions were evaporated to dryness, yielding the product **5** as a white, foamy solid.

Overall yield: 60%

Analytical data: **$^1$H NMR** (300 MHz, Methanol-$d_4$) δ 8.80 (s, 1H, N-C**H**-N), 7.32 (d, $J$ = 1.7 Hz, 1H, His backbone **H**), 4.27 (d, $J$ = 5.9 Hz, 1H, α-C**H**), 3.87 (s, 6H, N-C**H$_3$**), 3.12 (m, 2H, His C**H$_2$**), 1.40 (d, $J$ = 4.6 Hz, 9H, 3x Boc C**H$_3$**) **$^{13}$C NMR** (75 MHz, Methanol-$d_4$) δ 157.5 (**C**O-NH), 138.0 His (N-**C**H-N), 134.2 His backbone (**C**=CH), 123.0 His backbone (**C**H=C), 80.6 (Boc quat. **C**), 36.4 (N-**C**$H_3$), 34.2 (N-**C**$H_3$), 28.6 (Boc **C**$H_3$) **ESI-MS** m/z 227.9 $[M-tBu-Cl]^+$, 283.9 $[M-Cl]^+$

## 3.3 Peptide synthesis

*3.3.1 Synthesis of OMe-Ala-Val-Leu-His($Me_2$)-NH-Boc (**6a**)*

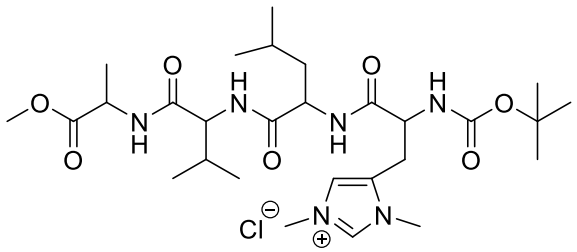
General procedure

Peptide chemistry was performed on the HMBA resin according to a literature known procedure with slight modifications. For the initial coupling of the first amino acid, the mixed anhydride

method was used. Therefore, 10 eq with respect to the loading of the resin of the first amino acid and 5 eq of DCC were suspended in 4 ml of DCM per mmol of amino acid and DMF was added dropwise until the solid was completely dissolved. The solution was left to stir at room temperature for an hour, then the solution was filtered to remove the resulting dicyclohexylurea. The filtrate was concentrated under reduced pressure until only the DMF was left. In the meantime, the resin was washed and swollen by shaking it 5x for 2 min in DCM and 5x for 2 min in DMF at room temperature. The solution containing the amino acid in DMF was then added to the resin. In a separate vial, 0.1 eq of DMAP were dissolved in little DMF (approx. 1 ml) and additionally added to the resin. The resin was then left to shake at room temperature for at least 3 h, but no longer than over night. Afterwards, the reaction mixture was filtered off and the resin was washed 3x for 1 min with DMF, DCM, IPA and $Et_2O$ respectively. Then, then resin was dried under reduced pressure over night, and the success of the coupling was monitored and the loading of the resin with the newly coupled amino acid was determined by weight gain. If a coupling efficiency of less than 75% was detected, the coupling procedure was repeated. After successful loading of the first amino acid, the resin was swollen in DCM and DMF by shaking it 5x for 2 min in each of the solvents and acetylation of potentially remaining free OH groups on the surface of the resin was performed by addition of 20 ml of a solution of 5% of acetic anhydride and 6% of DIPEA in DMF per mmol of resin loading. After shaking at room temperature for 1 h, the resin was washed by shaking it 3x for 1 min in DMF, NMP and DMF respectively. Coupling of the following amino acids was performed according to the following scheme: First, Fmoc deprotection was performed by shaking the resin in a solution of 20% of Piperidin in DMF 2x for 25 min at room temperature. After washing of the resin by shaking it 3x for 1 min with DMF, NMP and DMF respectively, a Kaiser Test was performed to determine successful deprotection. Therefore, some resin beads were suspended in three drops of DMF and sequentially two drops of Kaiser Test solution 1 (5 g ninhydrin in 100 ml ethanol), solution 2 (80 g phenol in 20 ml ethanol) and solution 3 (2 ml 0.001 M aqueous KCN in 98 ml pyridine) were added. The mixture was heated to 100 °C for 5 min and success of deprotection was detected by a color change to dark blue of the beads and supernatant. If positive, the procedure was followed by amino acid coupling. For this purpose, 3 eq of the respective Fmoc protected amino acid, 3 eq of TBTU, 3 eq of HOBt and 6 eq of DIPEA were dissolved in 20 ml of a mixture of NMP/DMF 3:1, which was stirred at room temperature for 5 min and then added to the resin. After shaking at room temperature for 45 min, the resin was washed three times with NMP and the coupling procedure was repeated. After the second coupling, the resin was washed by shaking it 3x for 1 min in DMF, NMP and DMF respectively

at room temperature and some of the resin beads were used to perform a Kaiser Test. If the coupling was successful, the beads and supernatant did not show any colour change and the coupling of the next amino acid was performed in a similar way after initial Fmoc deprotection. For the coupling of the imidazolium amino acids, base free coupling conditions were chosen. Therefore, 3 eq of the Boc protected imidazolium amino acid derivative **5** along with 3 eq of DCC and 3 eq of HOBt were dissolved in 20 ml of a mixture of NMP/DMF 1:1 and stirred at room temperature for 30 min, leading to precipitation of a white solid. The solid dicyclohexylurea was then filtered off and the filtrate was added to the resin, which was then shaken at room temperature over night. After washing of the resin3x for 1 min in DMF, NMP and DMF respectively, some of the resin beads were used for a Kaiser Test as described above. If unsuccessful coupling was detected by a color change to blue, the coupling procedure was repeated. In case of Fmoc protected imidazolium amino acids, the coupling procedure was followed by a final Fmoc deprotection as described above and afterwards, the N-terminus was acetylated similar to the coupling of the His derivative with 3 eq of acetic acid instead of the amino acid with a coupling time of 1 h at room temperature. Successful coupling was again monitored by the Kaiser Test.

After successful coupling of all amino acids and subsequent acetylation of the N-terminus if desired, the resin was washed 3x for 1 min with DMF, NMP and DMF respectively and afterwards subjected to peptide cleavage. For that purpose, the desired amount of resin was suspended in 20 ml of a mixture of DMF/MeOH/DIPEA 5:5:1 per mmol of resin loading and stirred at 50°C over night. The resin was then filtered off and washed three times with a mixture of DMF/MeOH 1:1. The combined filtrates were evaporated under reduced pressure and the remaining, oily residue was dissolved in as little MeOH as possible. The latter solution was dropped into cold $Et_2O$, leading to precipitation of the cleavage product, which was collected by centrifugation for 10 min at 8000 rpm and washed 2x with cold $Et_2O$. The crude product was dried under reduced pressure before being analyzed by analytical HPLC and ESI MS. If impurities could be found, the peptide was further purified by preparative HPLC (Acetonitrile/$H_2O$ 5:95 + 0.1 % TFA → Acetonitrile/$H_2O$ 95:5 + 0.1 % TFA) yielding the product as a white, slightly hygroscopic solid.



$C_{28}H_{49}ClN_6O_7$
617.19 g/mol

Yield: 191.3 mg (0.310 mmol, 48%)

Analytical data: **$^1$H NMR** (300 MHz, Methanol-$d_4$) δ 8.81 (s, 1H, His N-C**H**-N), 7.32 (m, 1H, His backbone **H**), 4.41 (m, 3H, 1x His, 1x Val, 1x Leu α-C**H**), 4.21 (d, $J$ = 7.3 Hz, 1H, Ala α-C**H**), 3.87 (d, 6H, 2x His N-C**H$_3$**), 3.70 (s, 3H, O-C**H$_3$**), 3.17 (dd, $J$ = 6.2, 0.9 Hz, 1H, 1x His C**H$_2$**), 2.99 (d, $J$ = 8.3 Hz, 1H, 1x His C**H$_2$**), 2.06 (dq, $J$ = 13.6, 6.7 Hz, 1H, Val C**H**), 1.69 (m, 1H, Leu C**H**), 1.59 (m, 2H, Leu C**H$_2$**), 1.39 (d, 12H, 3x Boc C**H$_3$**, 1x Ala C**H$_3$**), 0.96 (m, 12H, 2x Val C**H$_3$**, 2x Leu C**H$_3$**), **ESI-MS** m/z 581.2 $[M\text{-}Cl]^+$

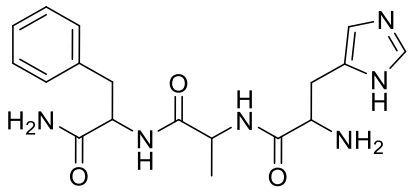
*3.3.2 Synthesis of peptides **6b-6c***

General procedure

Peptide synthesis was carried out using the Rink amide resin (loading of 0.71 mmol/g). After first swelling the resin by shaking it five times two minutes in DCM and afterwards five times two minutes in DMF at room temperature, Fmoc deprotection was carried out by shaking the resin for 2x 10 minutes in DMF with 20 % Piperidine at room temperature. After deprotection, the resin was washed by shaking it 3x 1 min in DMF, followed by 3x 1min with IPA and again 3x 1 min with DMF at room temperature. After washing, some beads of the resin were removed in order to perform the Kaiser Test as described for peptide **6a**. If positive, the procedure was followed by amino acid coupling, which was carried out by addition of 4 eq of the respective Fmoc protected amino acid, 4 eq of TBTU, and 8 eq of DIPEA or Collidine in case of Cys dissolved in 1 ml of DMF per mmol of resin loading. After shaking for 1,5 h at room temperature, the resin was again washed with DMF by shaking it 3x 1 min in DMF, followed by 3x 1 min with IPA and again 3x 1 min with DMF at room temperature. Afterwards, the Kaiser test was again performed to confirm successful coupling, which was revealed by no color change of the beads and the supernatant. If a blue color could be detected, the respective amino acid was recoupled following the same procedure as for the initial coupling with addition of 4 eq of HOBt followed by washing and another Kaiser Test. If the second Kaiser Test

revealed unsuccessful coupling, the free amino termini left were capped by acetylation. Therefore, a 10 fold molar excess of acetic anhydride and DIPEA were dissolved in 1 ml of DMF per mmol of resin loading, added to the resin and shaked for 30 min at room temperature. After washing, the Kaiser test was again performed to reveal success of the capping reaction, which usually showed no more color change. Following this scheme, all amino acids were coupled successively. After deprotection of the N-terminal amino acid, peptide **6d** was subjected to N-terminal acetylation, which was carried out identically to the capping procedure. Afterwards, the resin was again washed by shaking it 3x 1min in DMF, followed by 3x 1min with IPA and again 3x 1min in DMF at room temperature. Cleavage from the resin and deprotection of the protected side chains was carried out simultaneously by addition of a cleavage mixture of 2.5 ml per mmol of resin loading of TFA/TIS/$H_2O$ 95:2.5:2.5 respectively TFA/TIS/EDT 95:2.5:2.5 if Cys was present in the sequence to the resin and shaking it for 3 h at room temperature. Afterwards, the resin was filtered off and the filtrate was added dropwise to a cold solution of 20 ml of $Et_2O$/Hexane 1:1 per mmol of resin loading to precipitate the peptide, which was filtered off by centrifugation for 10 min at 8000 rpm and washed two times with 10 ml of cold $Et_2O$/Hexane 1:1 per mmol of resin loading, again using centrifugation to filter it off. The crude peptide was purified by preparative HPLC (Acetonitrile/$H_2O$ 5:95 + 0.1 % TFA → Acetonitrile/$H_2O$ 95:5 + 0.1 % TFA) yielding the peptide a white solid.

*3.3.2.1 $H_2$N-Phe-Ala-His-$NH_2$(**6b**)*



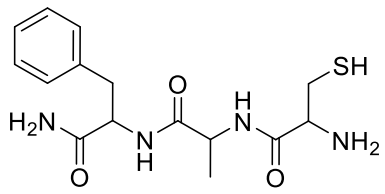

$C_{18}H_{24}N_6O_3$
372.43 g/mol

Yield: 173.0 mg (0.46 mmol, 84%)

Analytical data: **$^1$H NMR** (300 MHz, Methanol-$d_4$) δ 8.75 (s, 1H, His N-C**H**-N), 7.40 (s, 1H, His backbone **H**), 7.25 (m, 5H, Phe arom.), 4.59 (dd, *J* = 8.3, 6.0 Hz, 1H, Phe α-C**H**), 4.37 (q, *J* = 7.2 Hz, 1H, Ala α-C**H**), 4.24 (dd, *J* = 6.5, 5.0 Hz, 1H, His α-C**H**), 3.35 (m, 2H, Phe C**H$_2$**), 3.09 (m, 2H, His C**H$_2$**), 1.38 (d, *J* = 7.3 Hz, 3H, Ala C**H$_3$**) **$^{13}$C NMR** (75 MHz, Methanol-$d_4$) δ 175.6 (**C**O), 175.4 (**C**O), 168.6 (**C**O), 138.4 (Phe quat. **C**), 136.0 (His N-**C**H-N), 130.4 (Phe arom. **C**H), 129.5 (Phe arom. **C**H), 127.8 (d, Phe arom. **C**H), 120.1 (His backbone C=**C**H), 56.3

(Phe α-**C**), 52.9 (His α-**C**), 50.9 (Ala α-**C**), 38.5 (Phe **C**$H_2$), 27.7 (His **C**$H_2$), 17.7 (Ala **C**$H_2$), **ESI-MS** m/z 373.1 $[M+H]^+$

*3.3.2.1 $H_2$N-Phe-Ala-Cys-$NH_2$ (**6c**)*

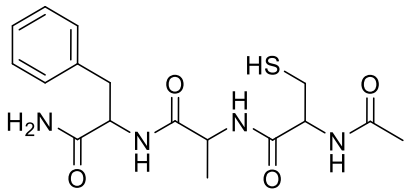



$C_{15}H_{22}N_4O_3S$
338.43 g/mol

Yield: 169.7 mg (0,50 mmol, 84 %)

Analytical data: **$^1$H NMR** (300 MHz, Methanol-$d_4$) δ 7.26 (m, 5H, Phe arom.), 4.58 (dd, $J$ = 8.2, 6.0 Hz, 1H, Phe α-C**H**), 4.35 (q, $J$ = 7.2 Hz, 1H, Ala α-C**H**), 4.02 (t, $J$ = 5.7 Hz, 1H, Cys α-C**H**), 3.01 (m, 4H, Phe C**H₂**, Cys C**H₂**), 1.33 (d, $J$ = 7.2 Hz, 3H, Ala C**H₃**) **$^{13}$C NMR** (75 MHz, Methanol-$d_4$) δ 175.6 (**C**O), 174.1 (**C**O), 168.3 (**C**O), 138.3 (Phe quat. **C**), 130.4 (Phe arom. **C**H), 129.4 (Phe arom. **C**H), 127.8 (Phe arom. **C**H), 55.7 (d, Phe α-**C**, Cys α-**C**), 50.8, (Ala α-**C**), 38.9 (Phe **C**$H_2$), 26.5 (Cys **C**$H_2$), 17.8 (Ala **C**$H_2$) **ESI-MS** m/z 360.9 $[M+Na]^+$

*3.3.2.1 $H_2$N-Phe-Ala-Cys-NH-Ac (**6d**)*




$C_{17}H_{24}N_4O_4S$
380.46 g/mol

Yield: 58.8 mg (0.16 mmol, 58%)

Analytical data: **$^1$H NMR** (300 MHz, DMSO-$d_6$) δ 8.19 (d, $J$ = 6.9 Hz, 1H, NH), 8.11 (d, $J$ = 7.9 Hz, 1H, NH), 7.78 (d, $J$ = 8.2 Hz, 1H, NH), 7.23 (m, 5H, Phe arom.), 4.38 (m, 2H, Phe α-C**H**), 4.17 (p, 1H, Ala α-C**H**), 3.02 (dd, $J$ = 13.8, 5.0 Hz, 1H, 1x Cys C**H₂**), 2.72 (m, 3H, 1x

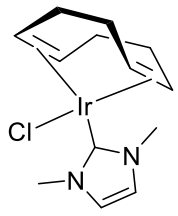
Cys C**H₂**, 1x Phe **CH₂**), 2.34 (t, 1H, S**H**), 1.88 (s, 3H, Ac C**H₃**), 1.15 (d, *J* = 7.1 Hz, 3H, Ala C**H₃**) **$^{13}$C NMR** (75 MHz, DMSO-$d_6$) δ 173.1 (**C**O), 172.2 (**C**O), 170.4 (**C**O), 170.0 (**C**O), 138.2 (Phe quat. **C**), 129.6 (Phe arom. **C**H), 128.5 (Phe arom. **C**H), 126.7 (Phe arom. **C**H), 55.5 (Phe α-**C**), 54.1 (Cys α-**C**), 49.2 (Ala α-**C**), 37.9 (Phe $CH_2$), 26.6 (Cys **C**$H_2$), 23.0 (Ac. **C**$H_3$), 18.2 (Ala **C**$H_2$) **ESI-MS** m/z 403.0 $[M+Na]^+$

3.4 Synthesis of [Ir(NHC)(COD)Cl] complexes

General procedure

In a heated Schlenk flask under $N_2$ atmosphere, 1 eq of the respective imidazolium salt was dissolved in 15 ml of dry DCM (in case of ligands **1a** and **1b**) or dry ACN (in case of peptide **6a**). The solution was degassed by three consecutive cycles of freeze-pump-thaw. After addition of 0.5 eq of $Ag_2O$ the solution was left to stir at room temperature for 1 h in the dark, during which disappearance of the black $Ag_2O$ could be seen, followed by addition of 0.5 eq of $[Ir(COD)Cl]_2$ leading to a color change to bright yellow. The solution was then left to stir over night at room temperature. Afterwards the reaction mixture was filtered through Celite (DCM/MeOH 9:1) and the filtrate was concentrated under reduced pressure. Purification of the product was carried out by flash column chromatography (Hexane/EtOAc 0:1→1:0) leading to the products as bright yellow solids.

*3.4.1 Complex **7a***

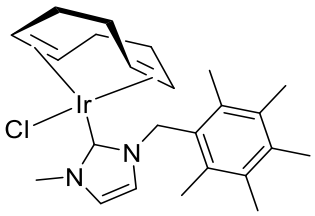



$C_{13}H_{20}ClIrN_2$
431.98 g/mol

Yield: 55.5 mg (0.12 mmol, 77%)

Analytical data: **$^1$H NMR** (200 MHz, Chloroform-*d*) δ 6.80 (s, 2H, C**H**=C**H**), 4.57 (m, 2H, COD C**H**), 3.94 (s, 6H, 2x N-C**H₃**), 2.94 (m, 2H, COD C**H**), 2.20 (m, 4H, COD C**H₂**), 1.66 (m, 4H, COD C**H₂**) $^{13}$C NMR (75 MHz, Chloroform-*d*) δ 180.7 (N-**C**-N), 121.7 (**C**H=**C**H), 84.6 (COD), 51.4 (COD), 37.5 (N-**C**$H_3$), 33.7 (COD), 29.7 (COD) **ESI-MS** m/z 392.8 $[M-Cl]^+$

*3.4.2 Complex **7b***

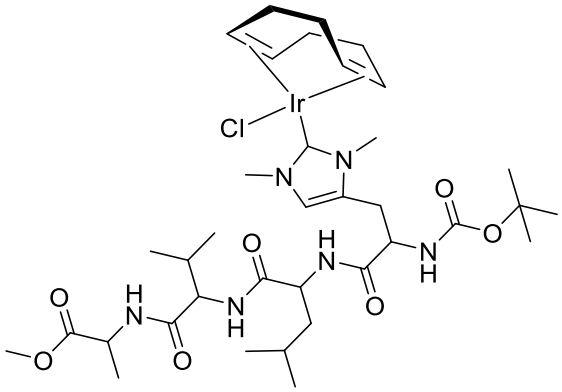



$C_{24}H_{34}ClIrN_2$
578.22 g/mol

Yield: 254.1 mg (0.44 mmol, 88%)

Analytical data: **$^1$H NMR** (200 MHz, Chloroform-*d*) δ 6.66 (d, *J* = 2.0 Hz, 1H, C**H**=CH), 6.26 (d, *J* = 2.0 Hz, 1H, CH=C**H**), 5.79 (d, 1H, C**H$_2$**), 5.33 (m, 1H, N-C**H$_2$**), 4.63 (s, 2H, 2x COD C**H**), 3.98 (s, 3H, N-C**H$_3$**), 3.11 (m, 2H, 2x COD C**H**), 2.25 (d, 17H, 5x arom C**H$_3$**, COD C**H$_2$**), 1.66 (m, 6H, 3x COD C**H$_2$**) **$^{13}$C NMR** (101 MHz, Chloroform-*d*) δ 180.4 (N-**C**H-N), 134.3 (**C** arom.), 133.2 (**C** arom.), 128.3 (**C** arom.), 120.6 (**C**H=CH), 119.2 (CH=**C**H), 84.5 (d, *J* = 19.1 Hz, COD), 51.6 (COD), 50.9 (COD), 49.7 (N-**C**H$_2$), 37.9 (N-**C**H$_3$), 33.8 (d, *J* = 10.0 Hz, COD), 29.8 (COD), 17.1 (arom. **C**H$_3$) **ESI-MS** m/z 432.0 $[Ir\text{-}NHC]^+$, 543.9 $[M\text{-}Cl]^+$

*3.4.3 Complex **7c***



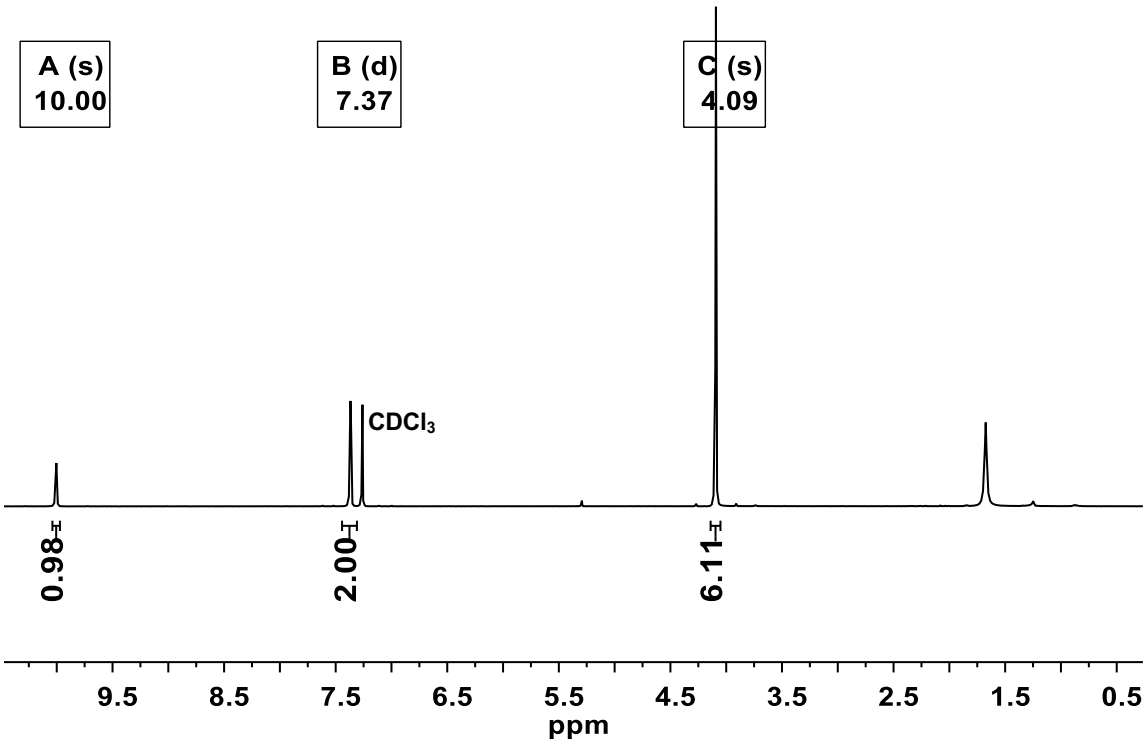

$C_{36}H_{60}ClIrN_6O_7$
916.58 g/mol

Yield: 11.9 mg (0.013 mmol, 25 %)

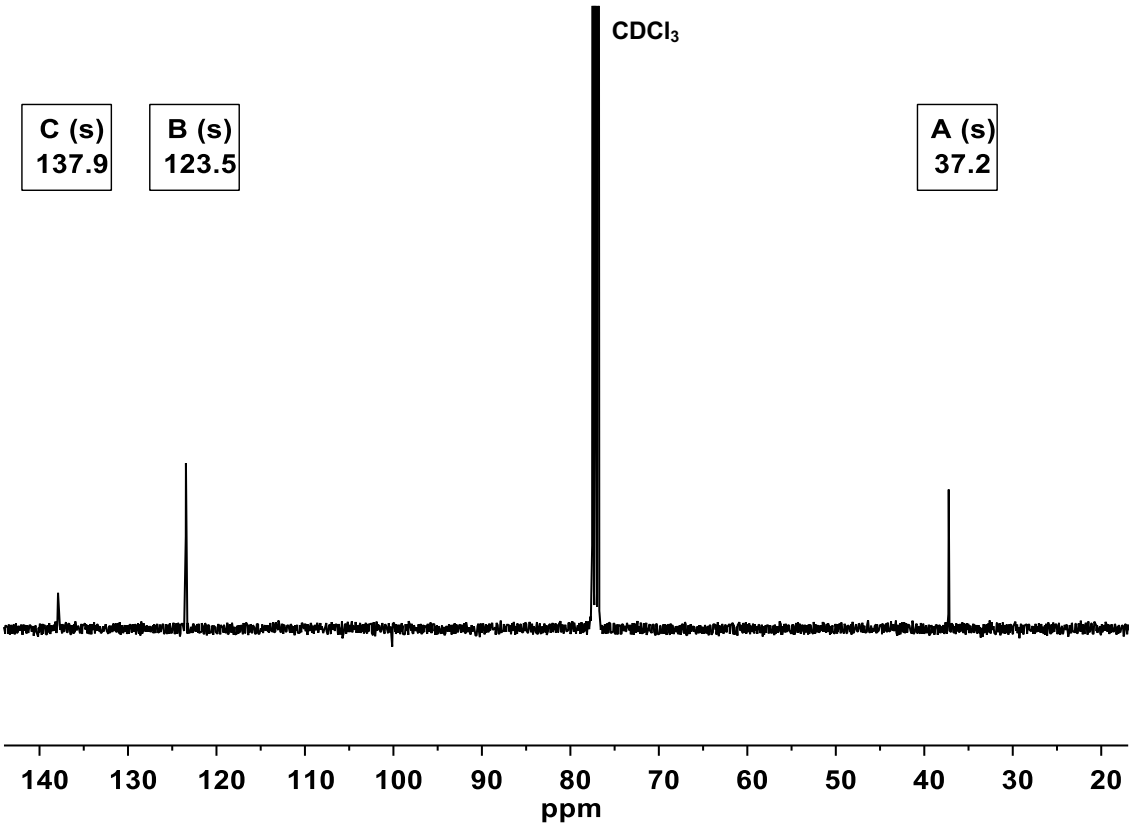
Analytical data: **$^1$H NMR** (300 MHz, Methanol-$d_4$) δ 6.86 (s, 1H, His backbone C**H**), 4.42 (m, 5H, 2x COD C**H**, 1x Leu α-C**H**, 1x Val α-C**H**, 1x His α-C**H**), 4.23 (d, 1H, Ala α-C**H**), 3.91 (m, 6H, 2x N-C**H$_3$**), 3.73 (s, 3H, O-C**H$_3$**), 3.07 (m, 4H, COD C**H$_2$**, His C**H$_2$**), 2.11 (m, 5H, 2x COD

C**H**$_2$, Val C**H**), 1.66 (m, 6H, COD C**H**$_2$, Leu C**H**, Leu C**H**$_2$), 1.43 (m, 12H, 3x Boc C**H**$_3$, 1x Ala C**H**$_3$), 0.97 (m, 12H, 2x Val C**H**$_3$, 2x Leu C**H**$_3$), **ESI-MS** m/z 882.3 $[M\text{-}Cl]^+$, 941.9 $[M+Na]^+$

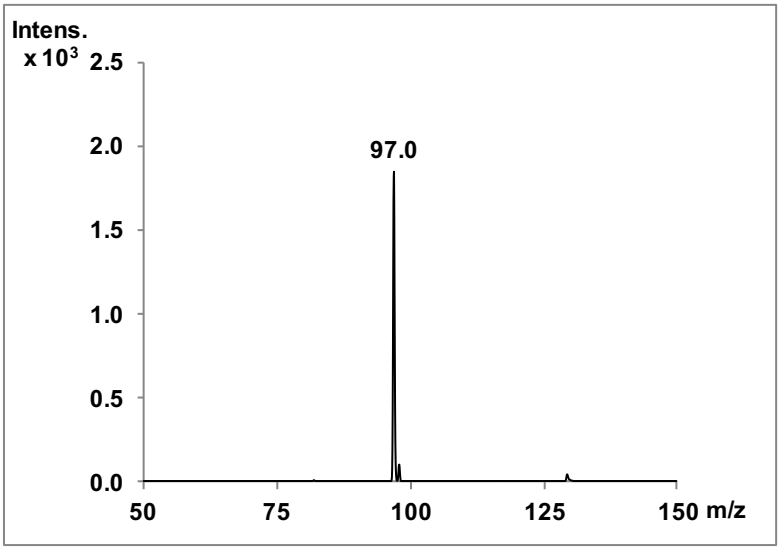
## 4. Analytical data



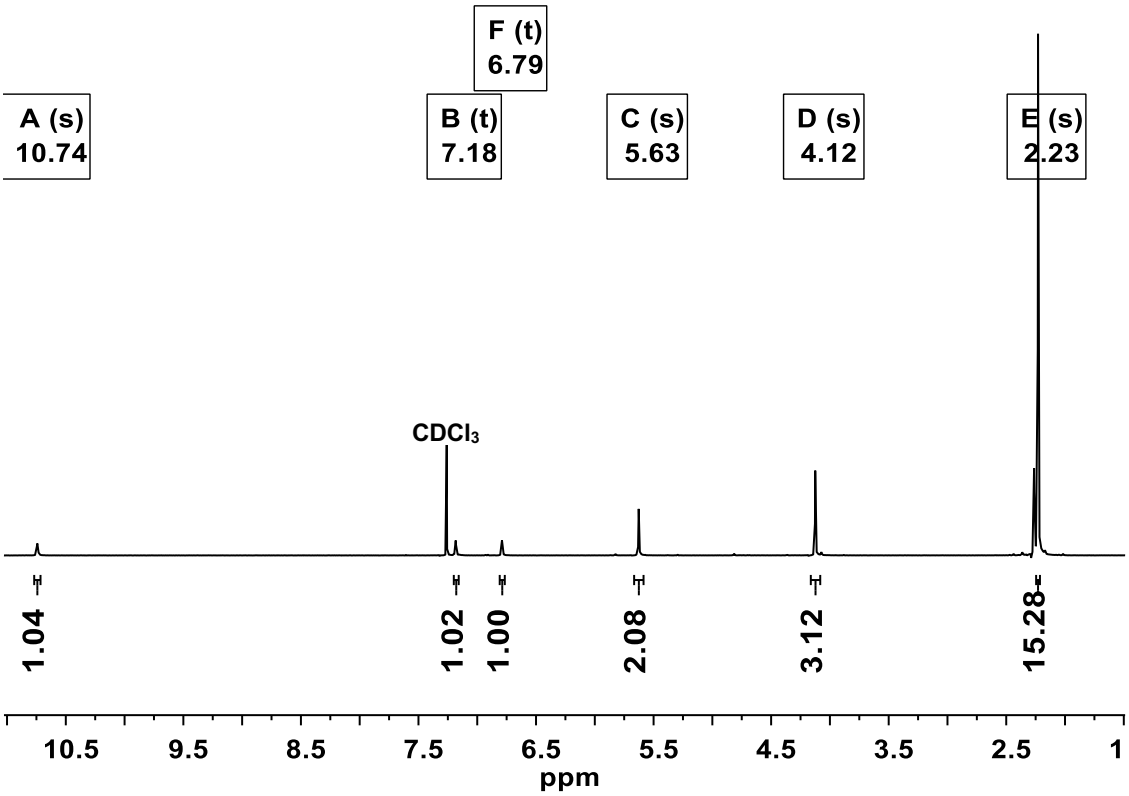

**Supplementary Figure S15:** $^1$H NMR of compound **1a**



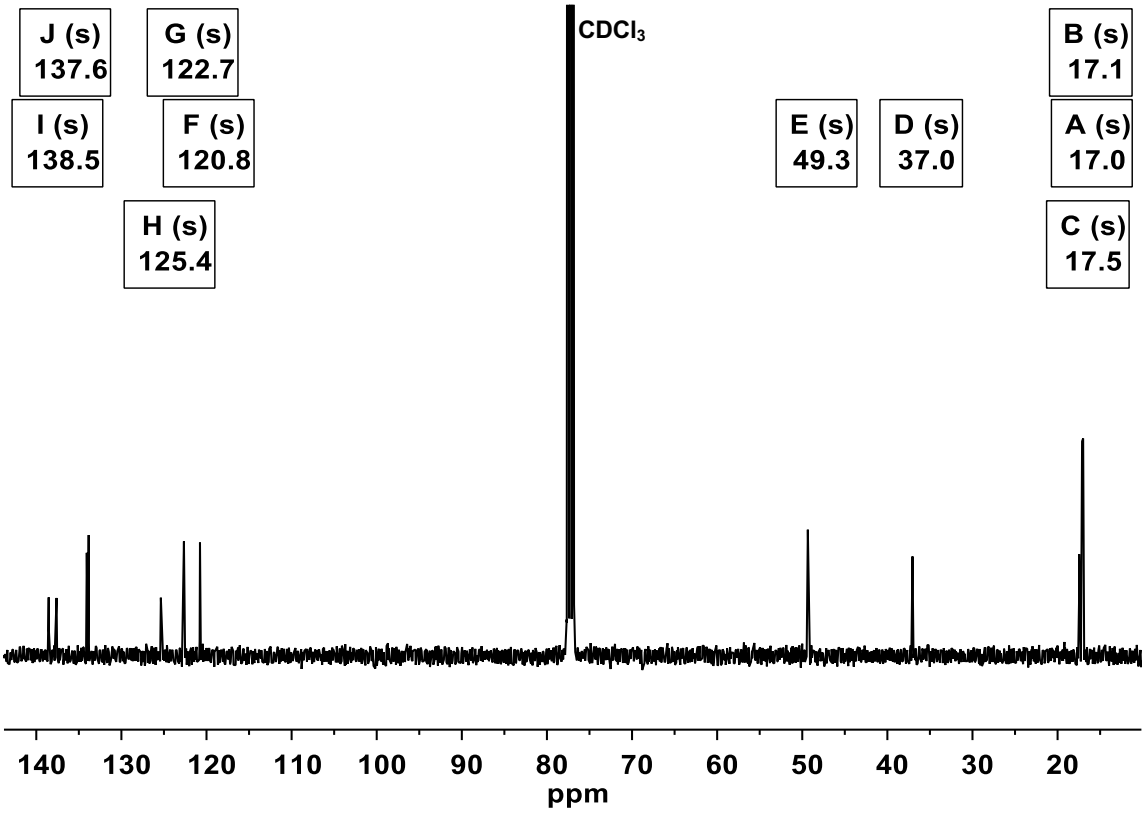

**Supplementary Figure S16:** $^{13}C$ NMR of compound **1a**



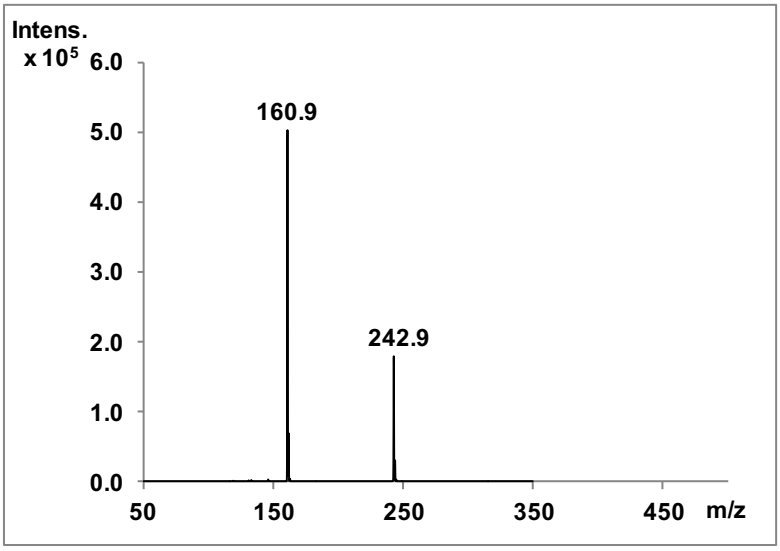

**Supplementary Figure S17:** ESI-MS spectrum of compound **1a**



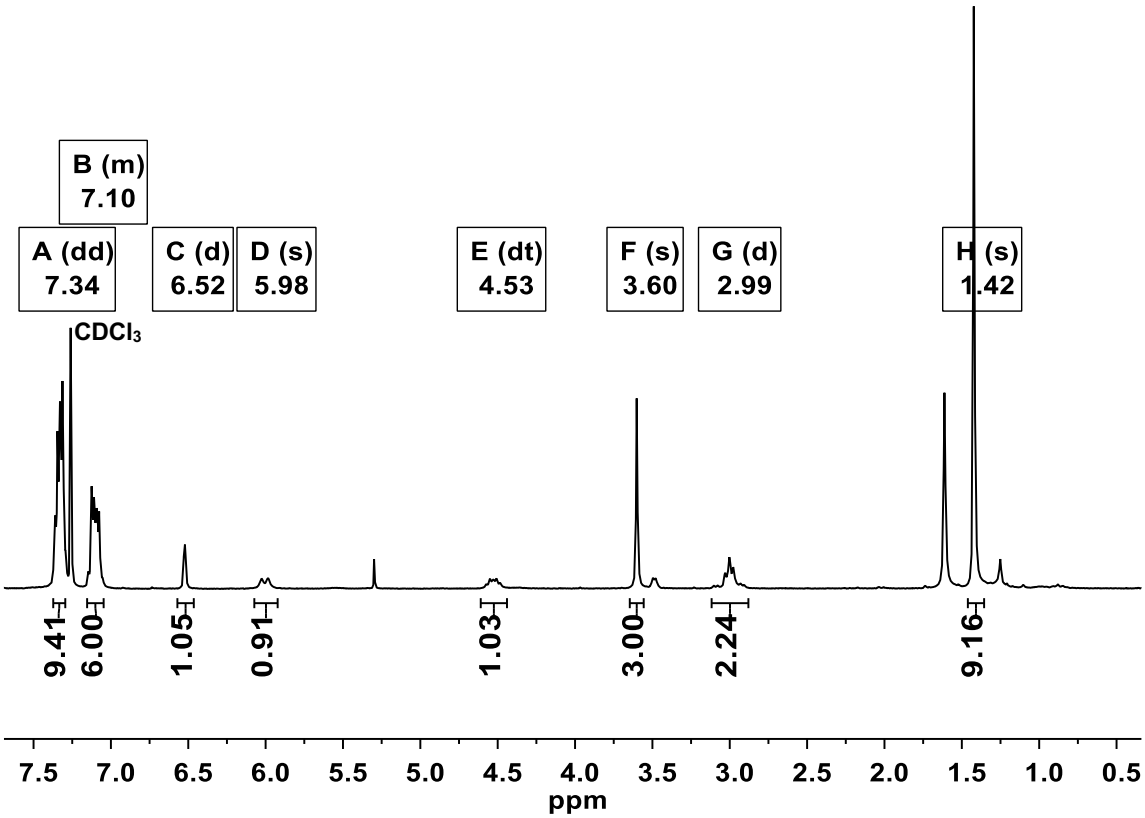

**Supplementary Figure S18:** $^{1}$H NMR of compound **1b**



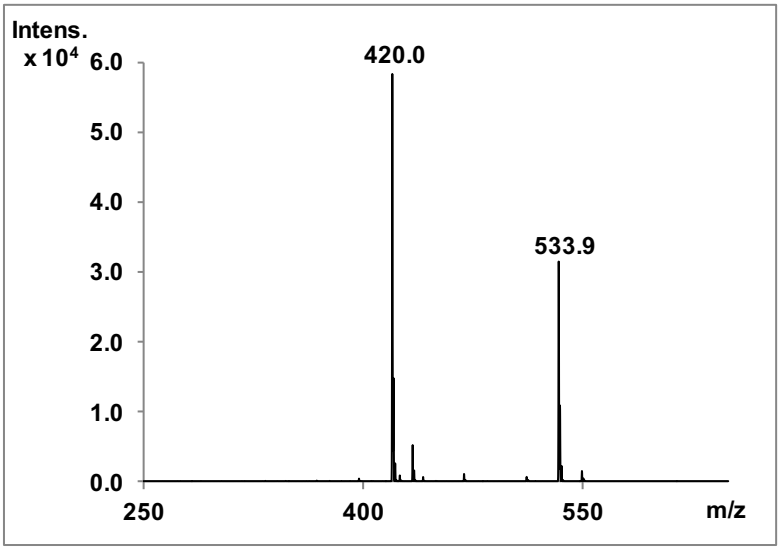

**Supplementary Figure S19:** $^{13}$C NMR of compound **1b**



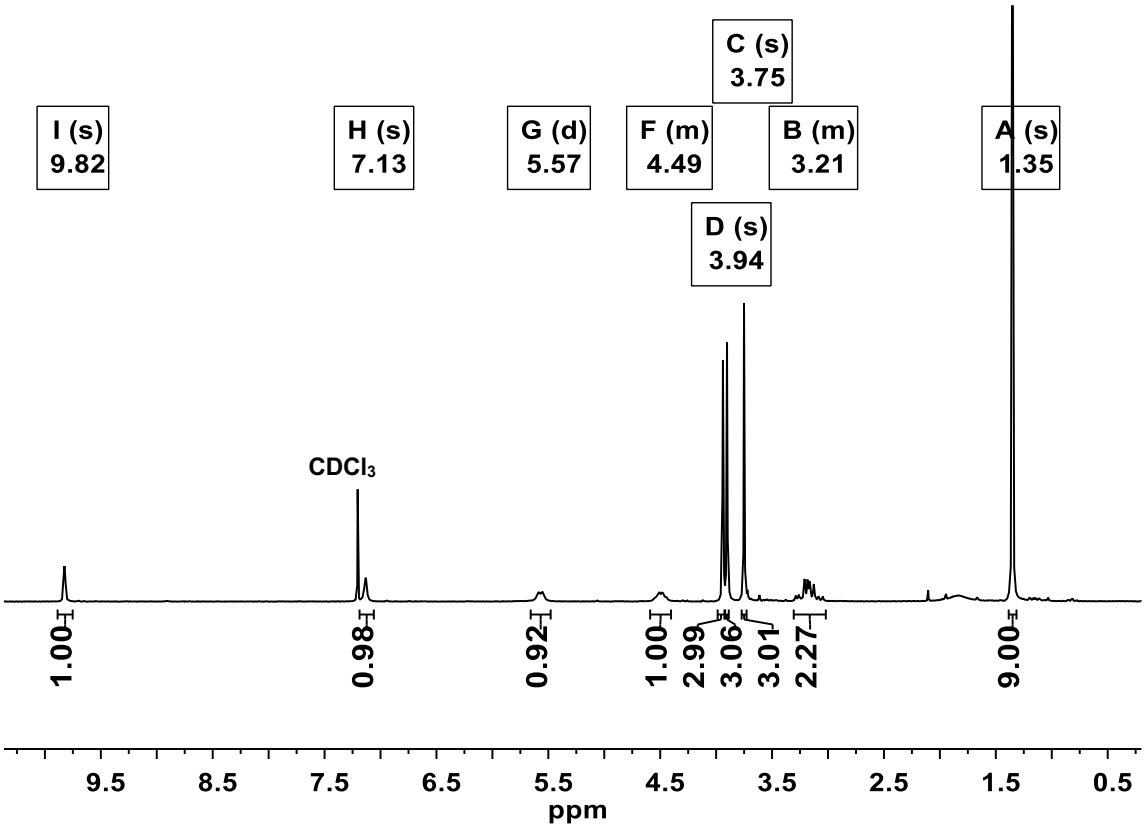

**Supplementary Figure S20:** ESI-MS spectrum of compound **1b**



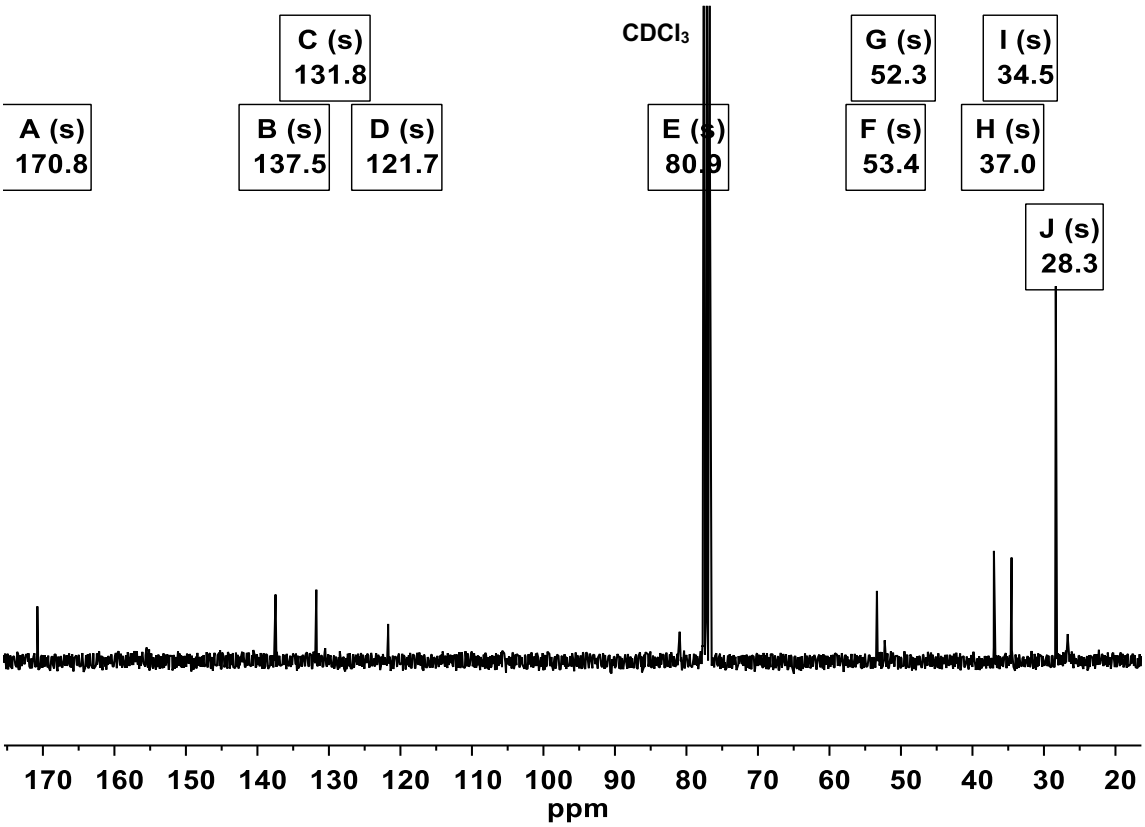

**Supplementary Figure S21:** $^{1}$H NMR of compound **3**



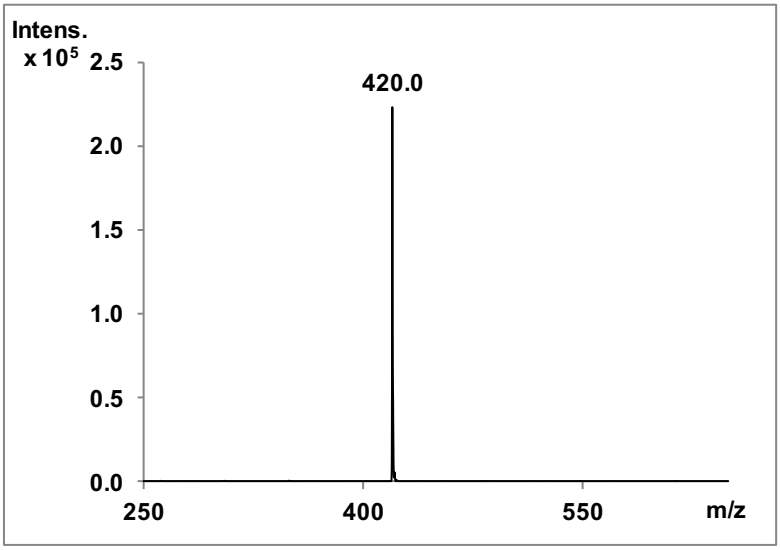

**Supplementary Figure S22:** ESI-MS spectrum of compound **3**



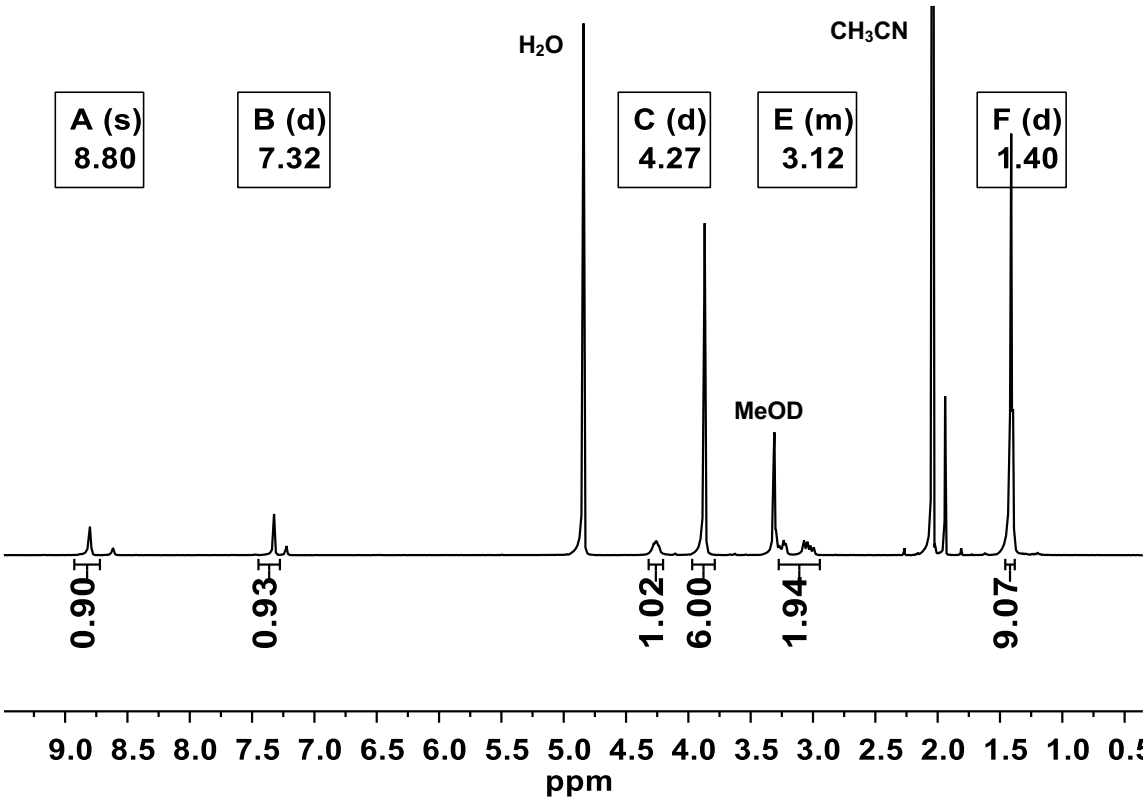

**Supplementary Figure S23:** $^{1}$H NMR of compound **4**



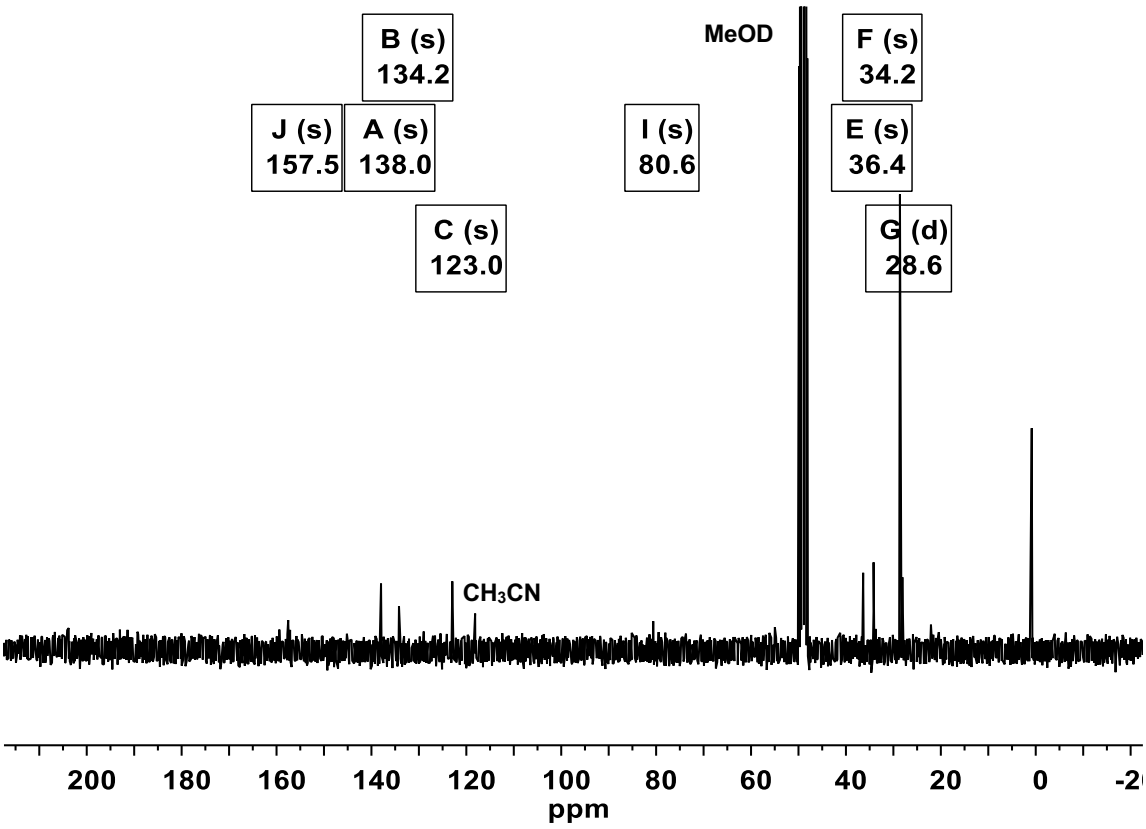

**Supplementary Figure S24:** $^{13}C$ NMR of compound **4**



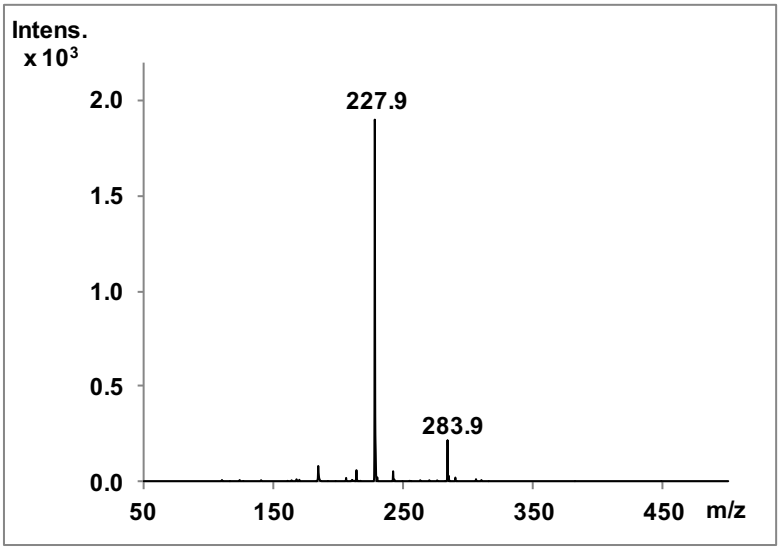

**Supplementary Figure S25:** ESI-MS spectrum of compound **4**



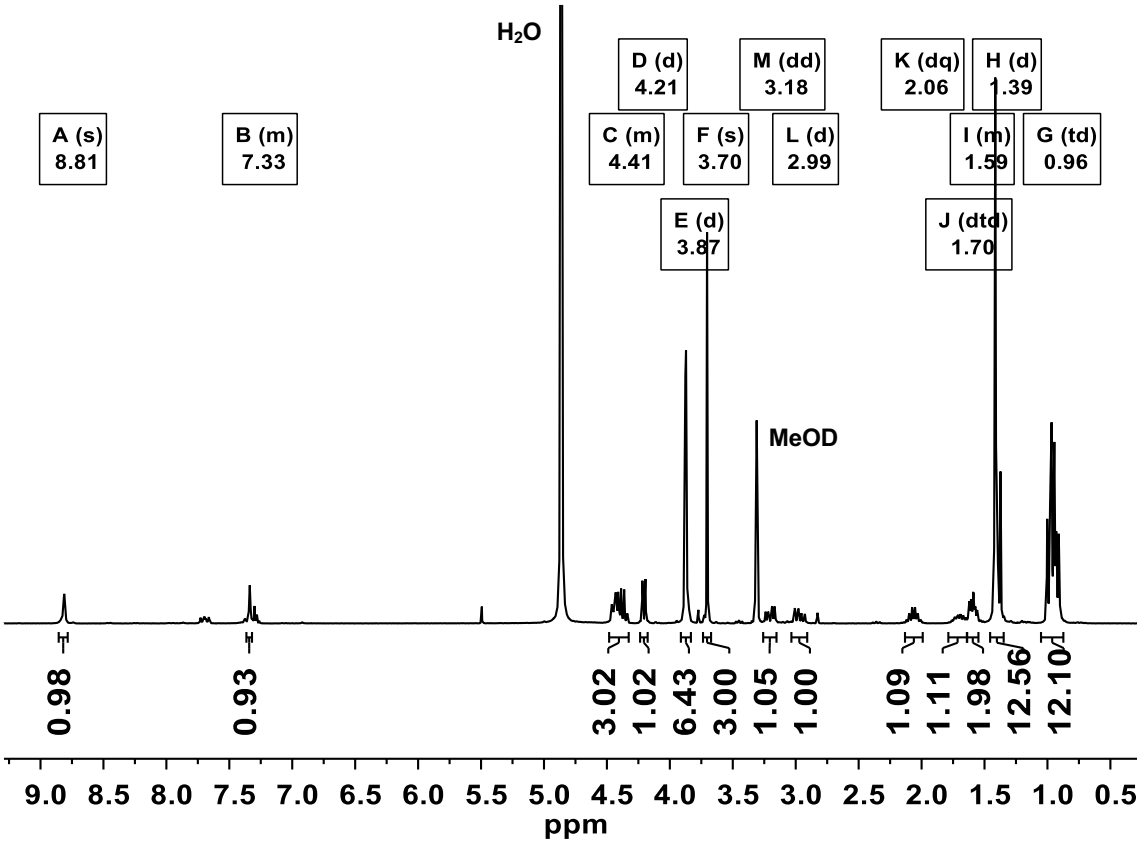

**Supplementary Figure S26:** $^{1}$H NMR of compound **5**



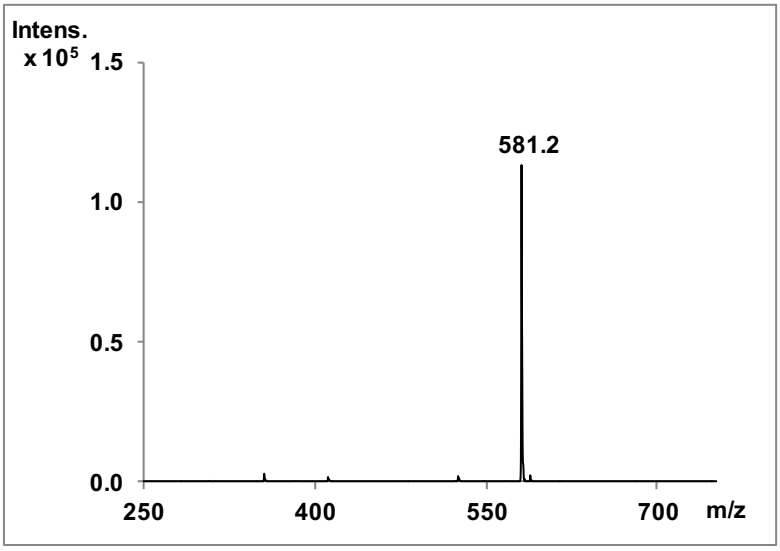

**Supplementary Figure S27**: $^{13}$C NMR of compound **5**



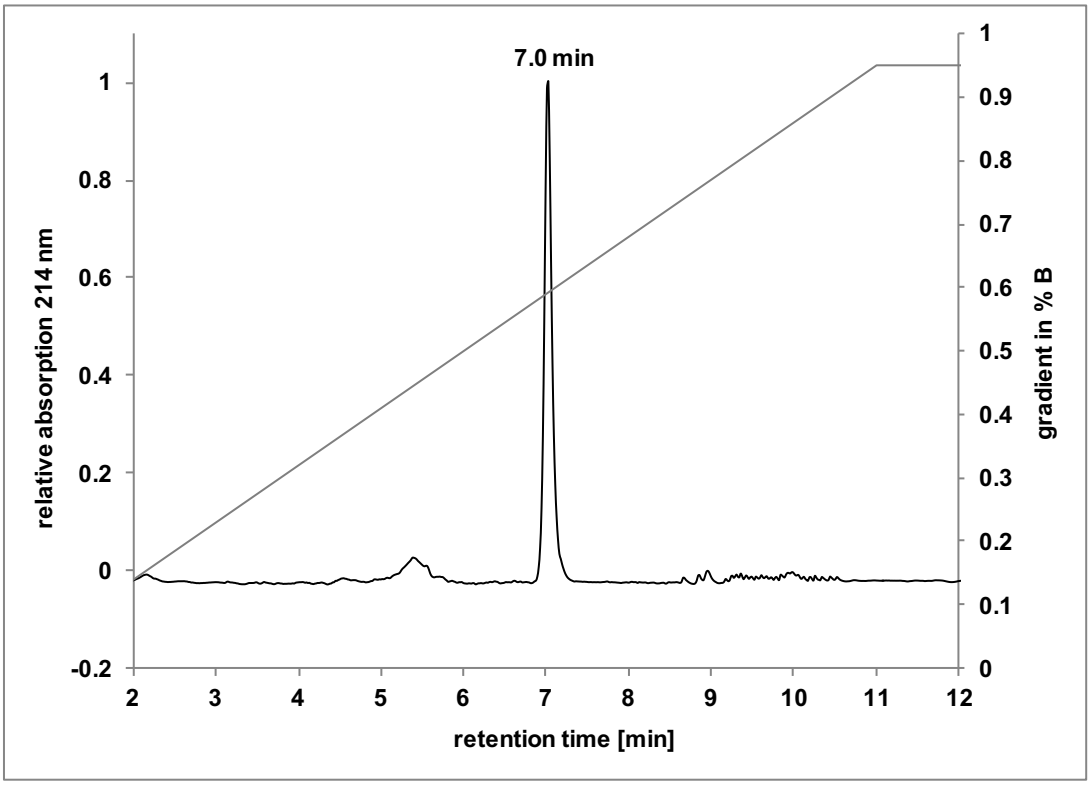

**Supplementary Figure S28:** ESI-MS spectrum of compound **5**



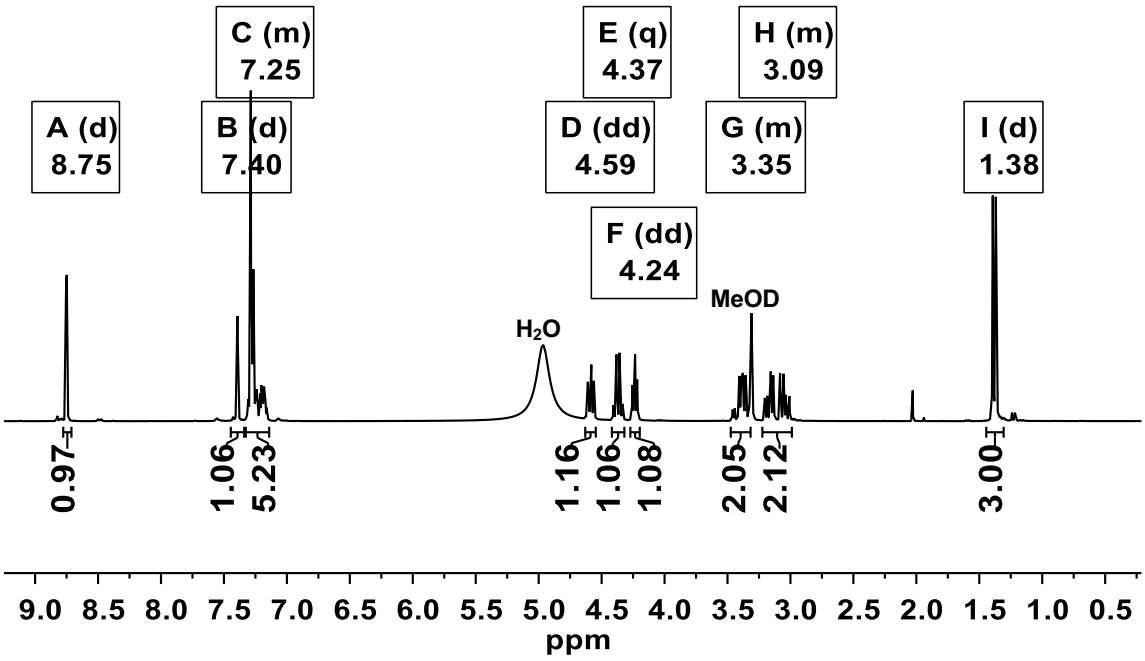

**Supplementary Figure S29:** $^{1}$H NMR of peptide **6a**



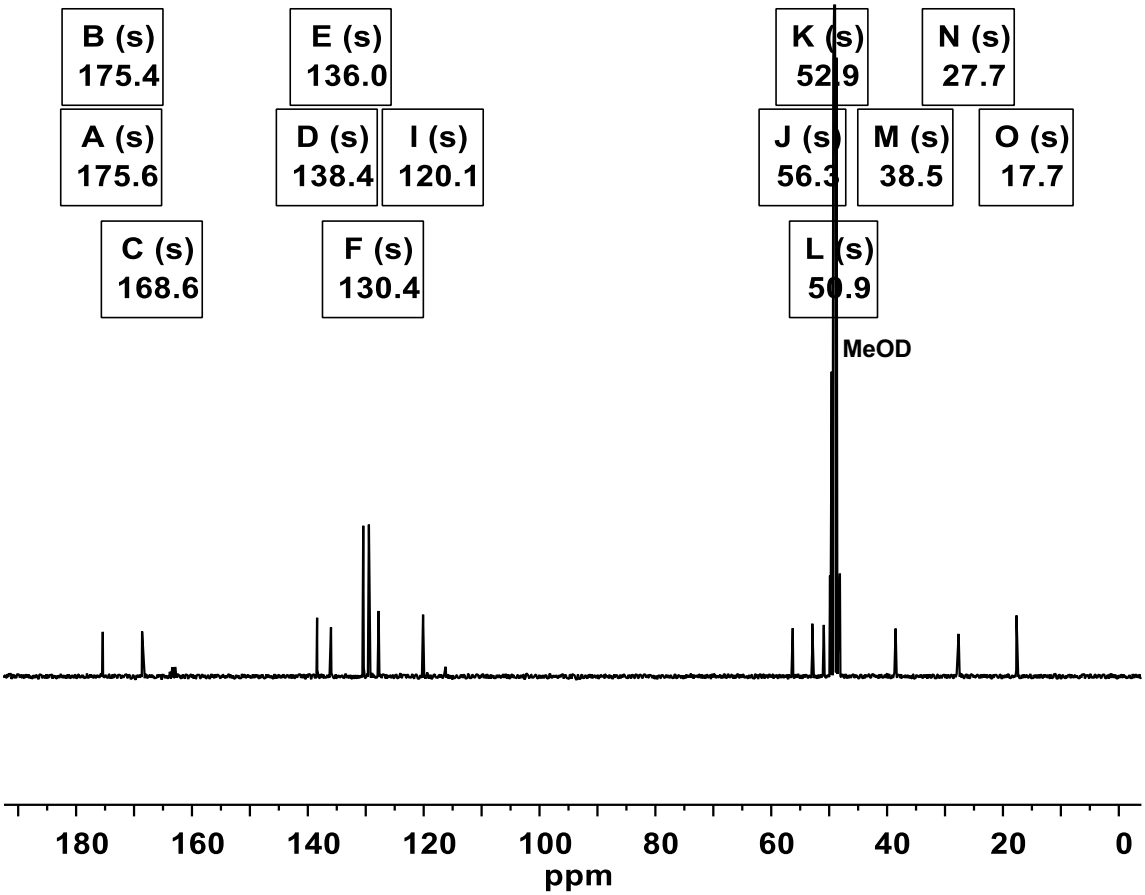

**Supplementary Figure S30:** ESI-MS spectrum of peptide **6a**



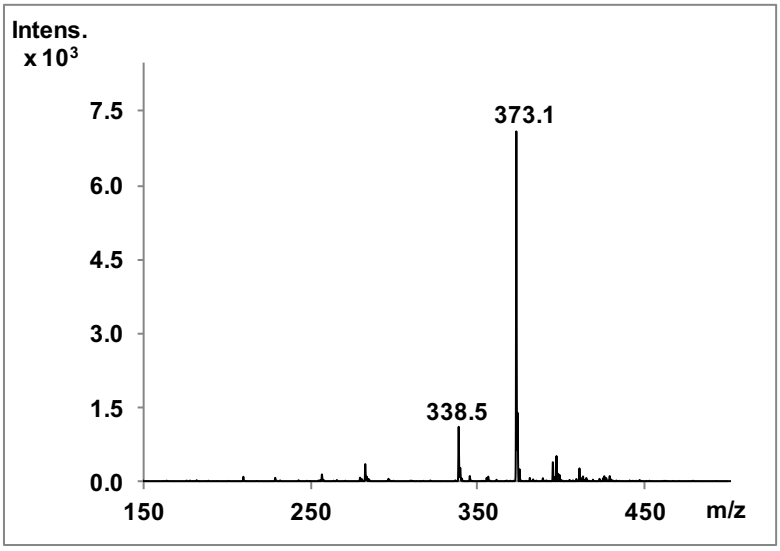

**Supplementary Figure S31:** Analytical HPLC trace of peptide **6a** (Buffer A: $H_2O$ with 0.1% TFA, Buffer B: ACN with 0.1% of TFA, Column: EC 125/4 Nucleodur C18 Pyramid 5µm)



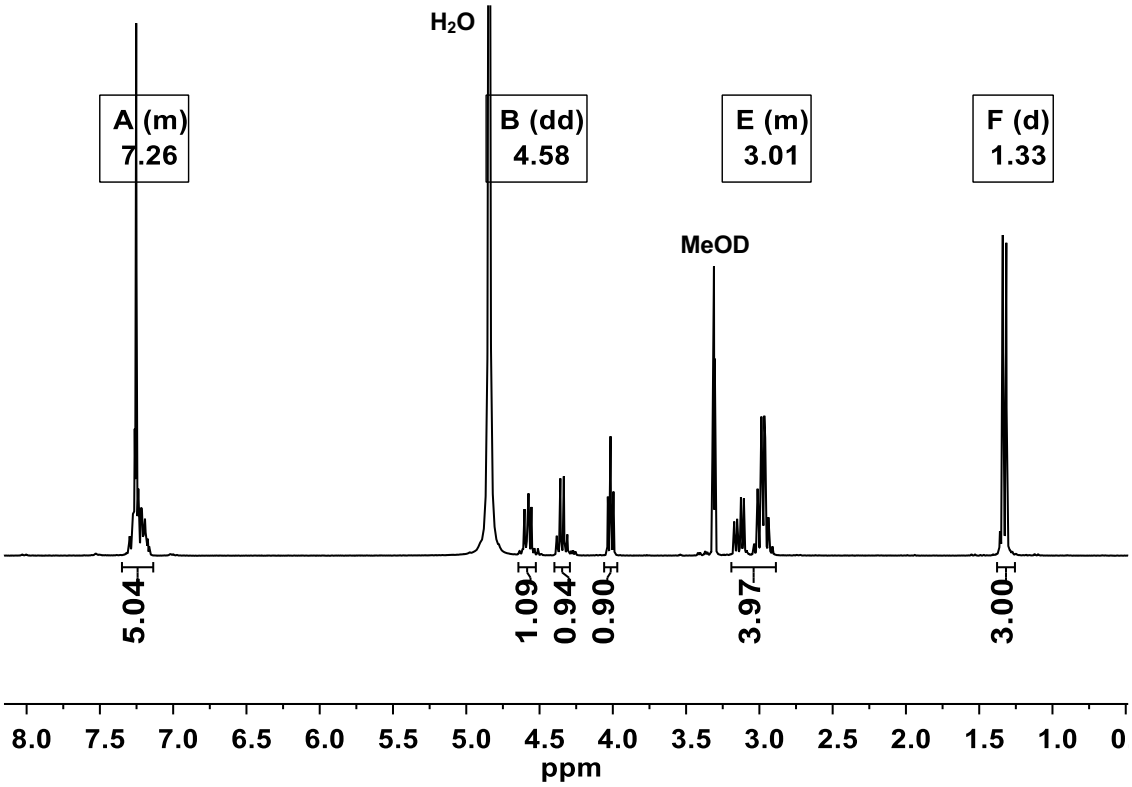

**Supplementary Figure S32:** $^{1}$H NMR of peptide **6b**



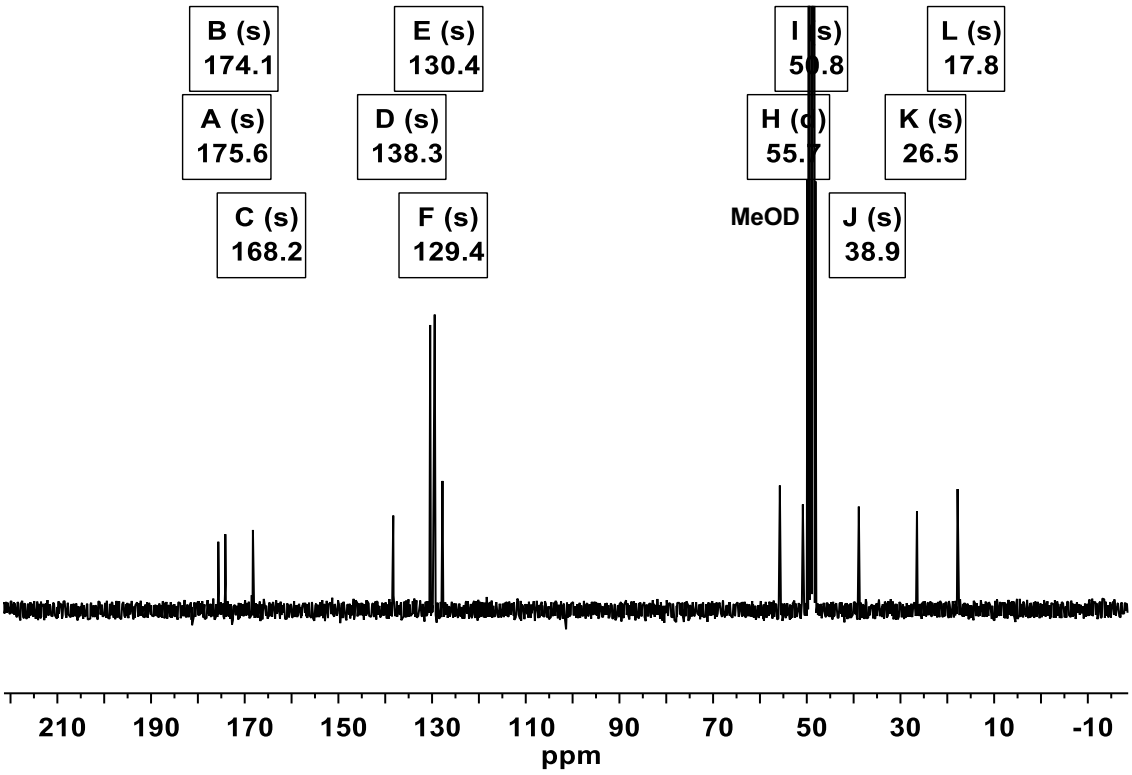

**Supplementary Figure S33:** $^{13}$C NMR of peptide **6b**



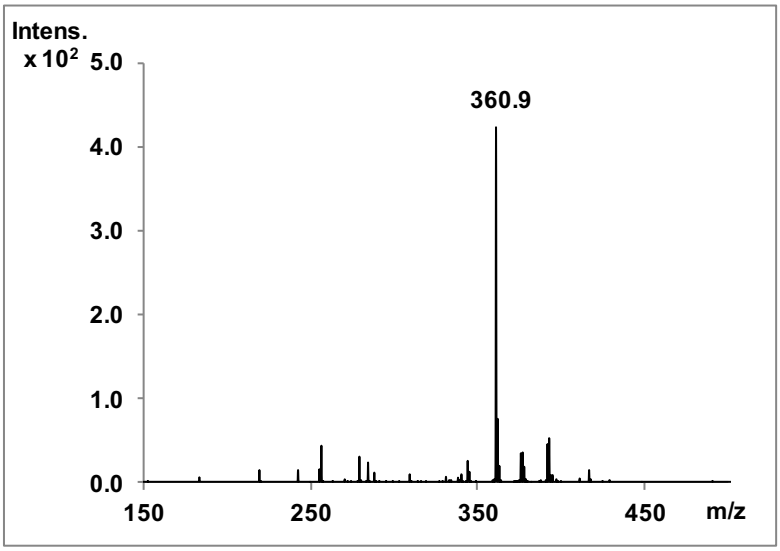

**Supplementary Figure S34:** ESI-MS spectrum of peptide **6b**



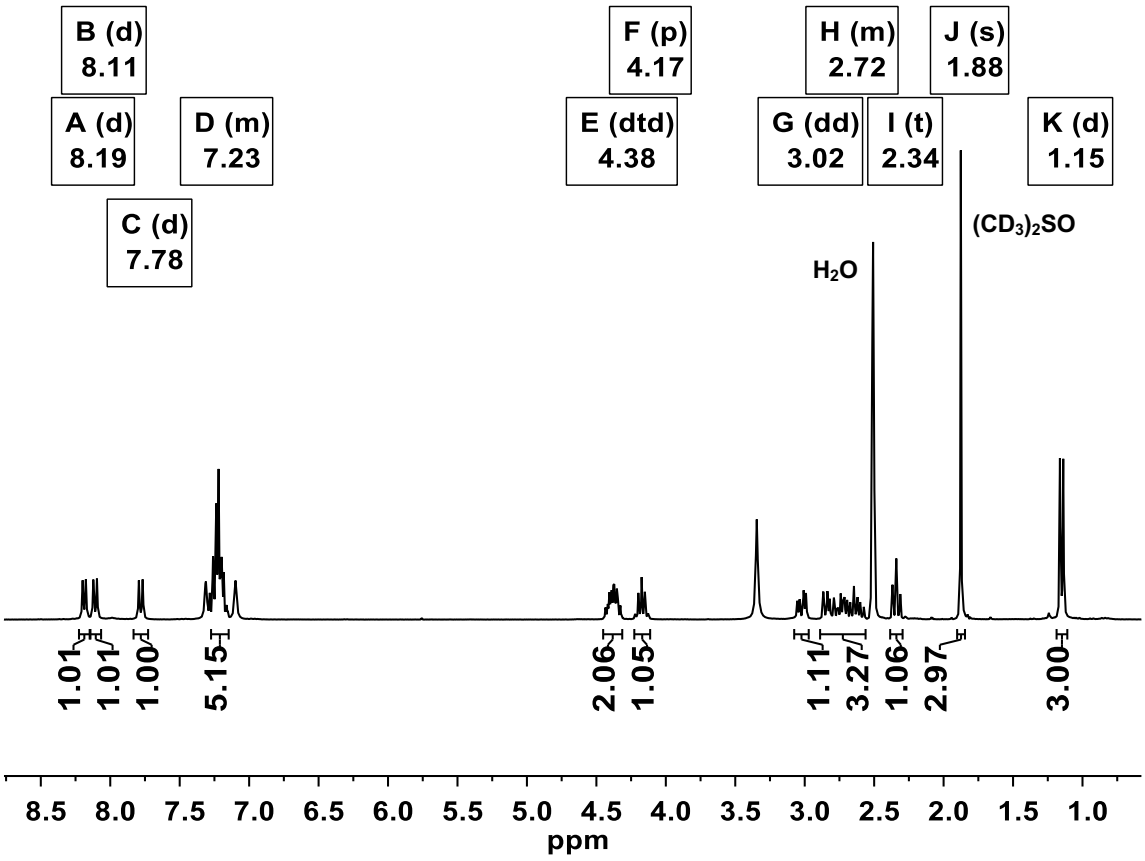

**Supplementary Figure S35:** $^{1}$H NMR of peptide **6c**



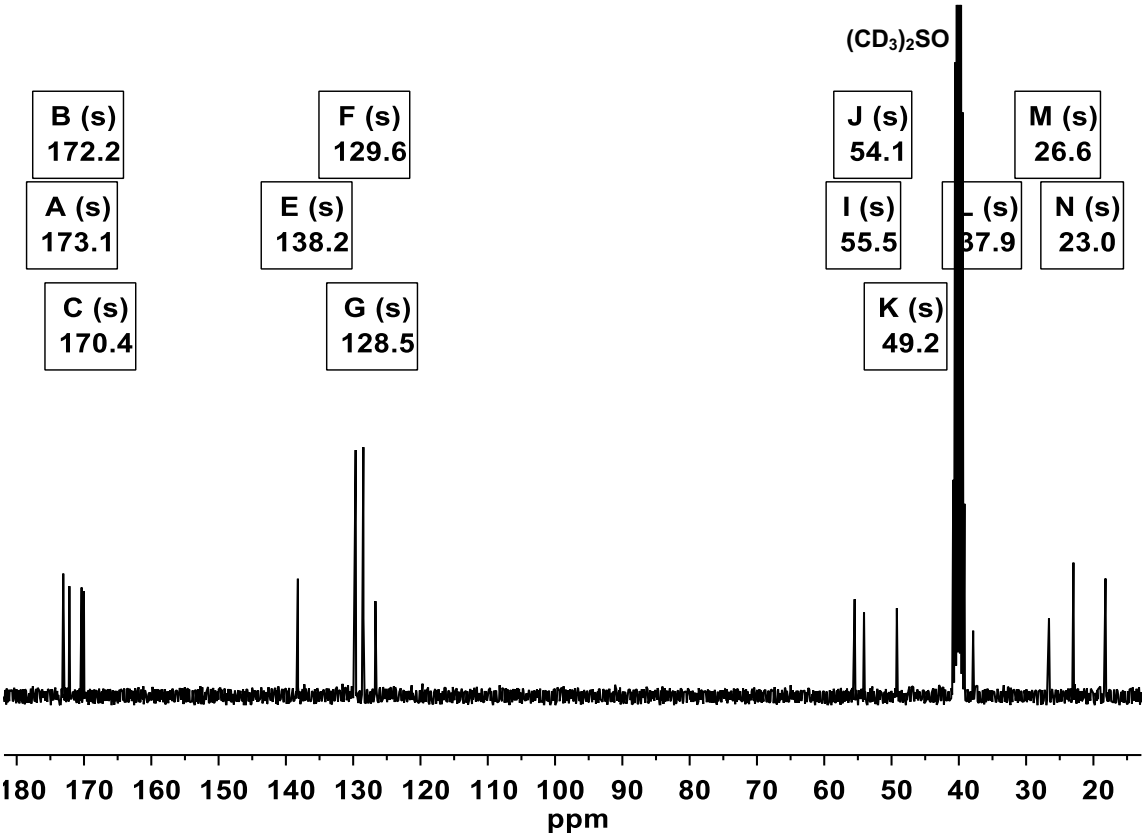

**Supplementary Figure S36:** $^{13}$C NMR of peptide **6c**



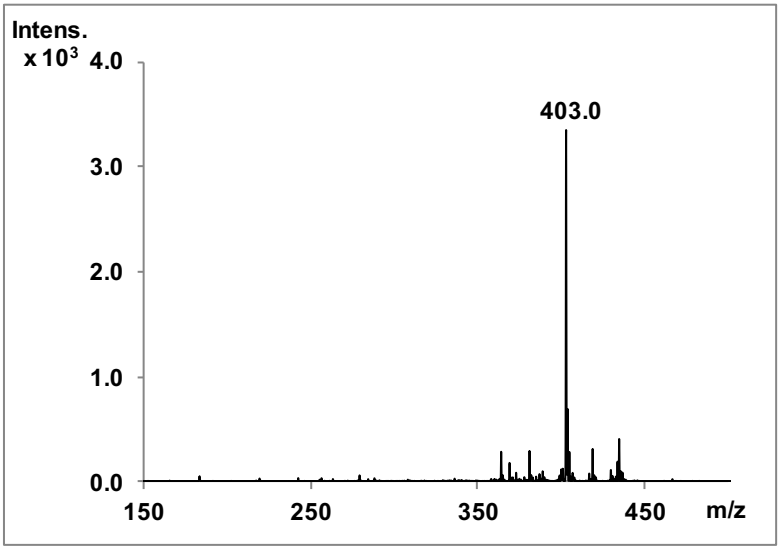

**Supplementary Figure S37:** ESI-MS spectrum of peptide **6c**



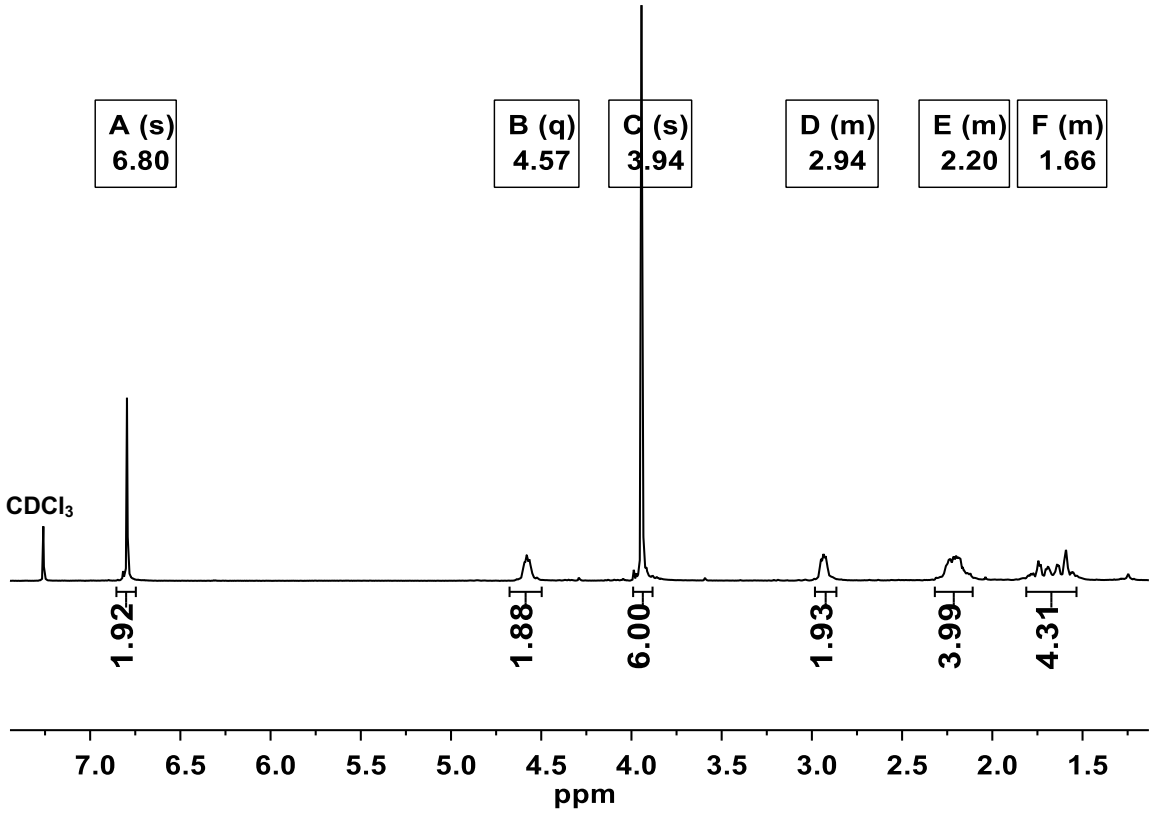

**Supplementary Figure S38:** $^{1}$H NMR of peptide **6d**



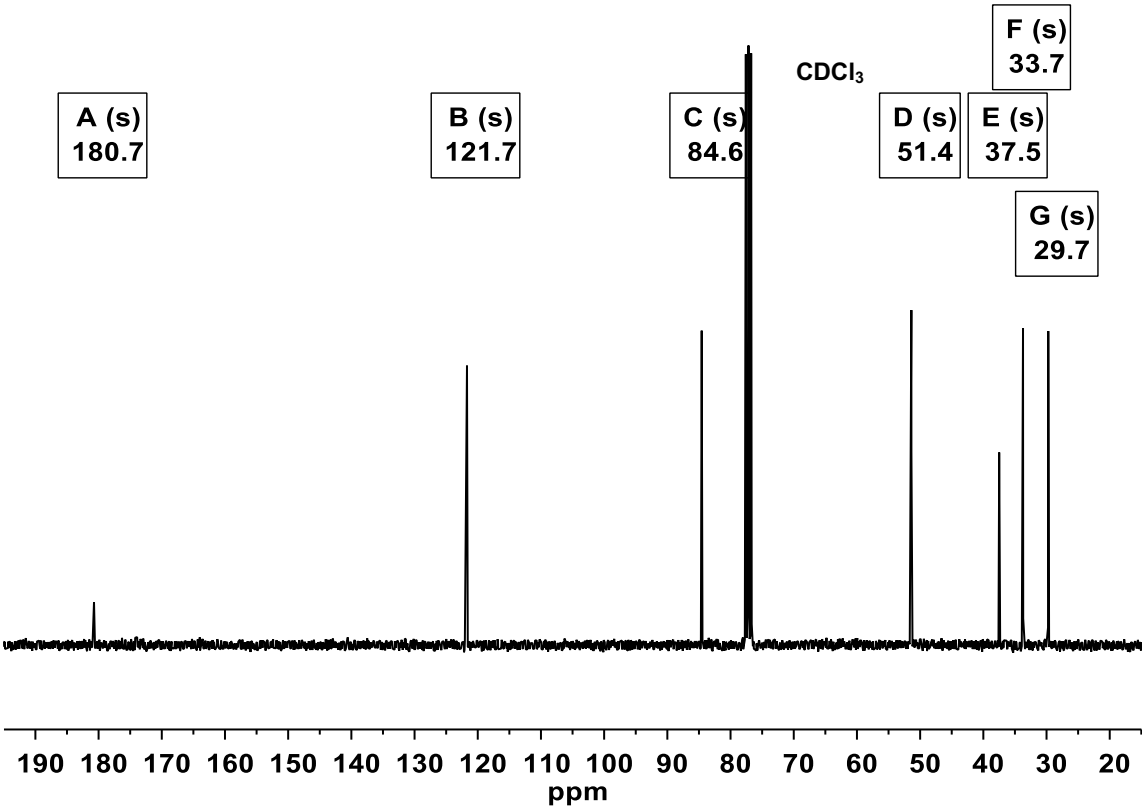

**Supplementary Figure S39:** $^{13}$C NMR of peptide **6d**



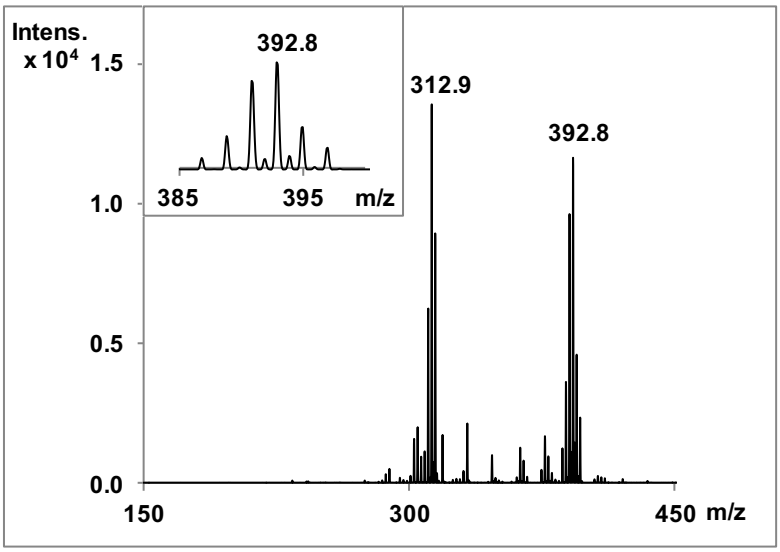

**Supplementary Figure S40:** ESI-MS spectrum of peptide **6d**



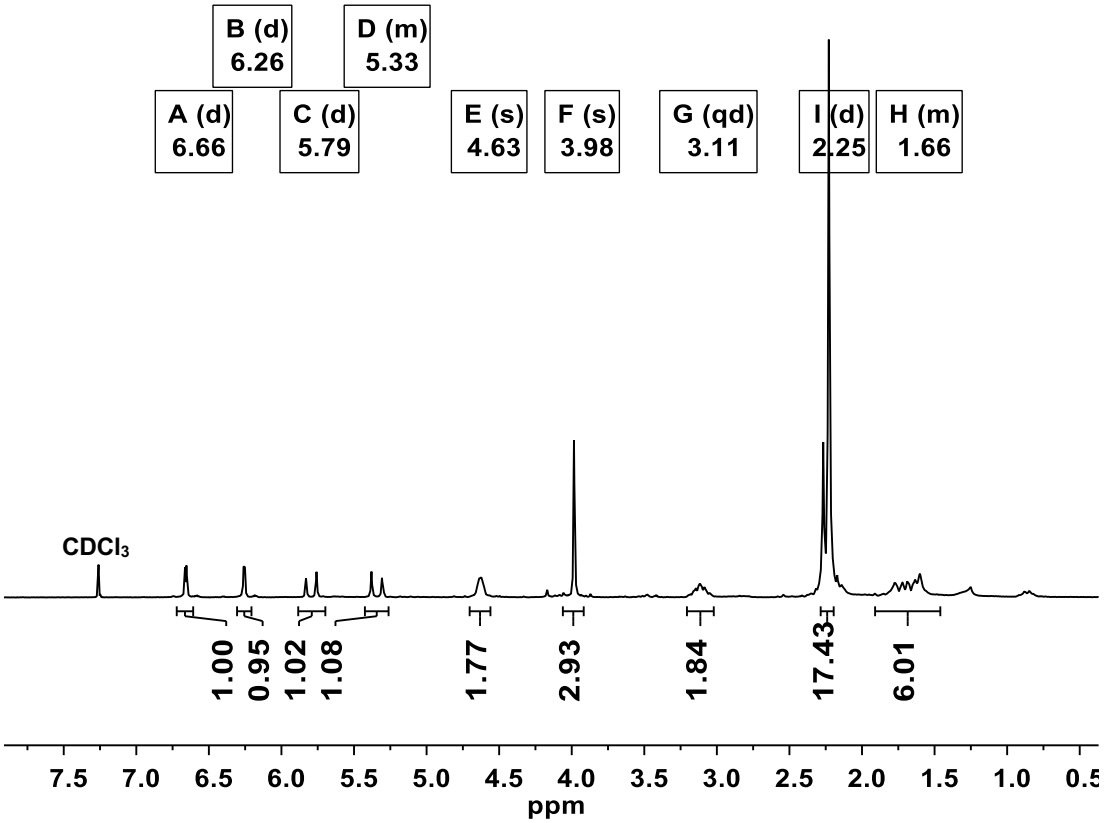

**Supplementary Figure S41:** $^{1}$H NMR of complex **7a**



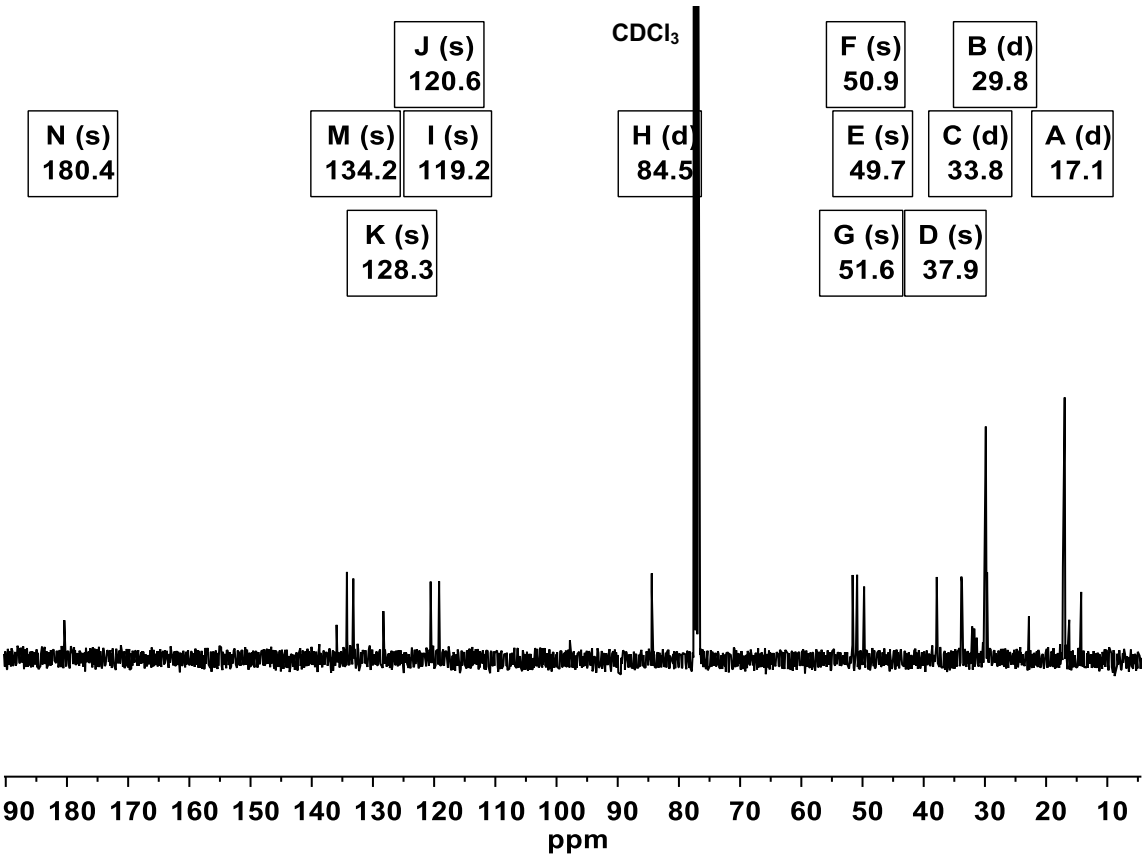

**Supplementary Figure S42:** $^{13}$C NMR of complex **7a**



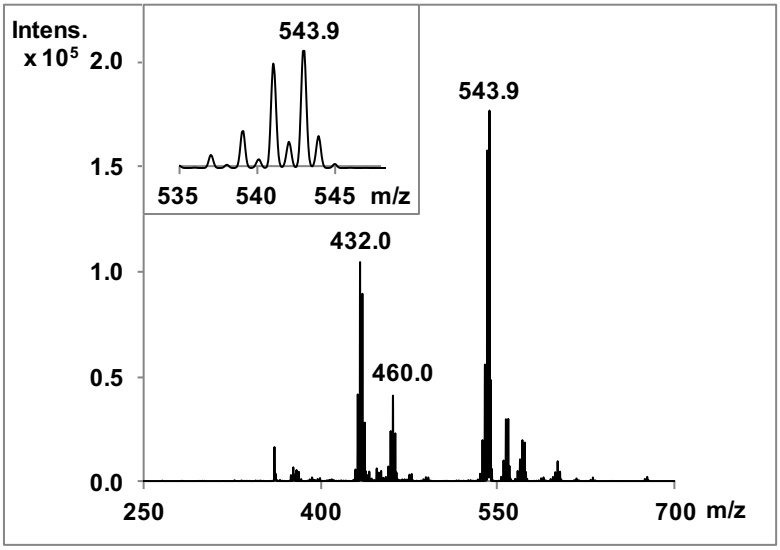

**Supplementary Figure 2:** ESI-MS spectrum of complex **7a**



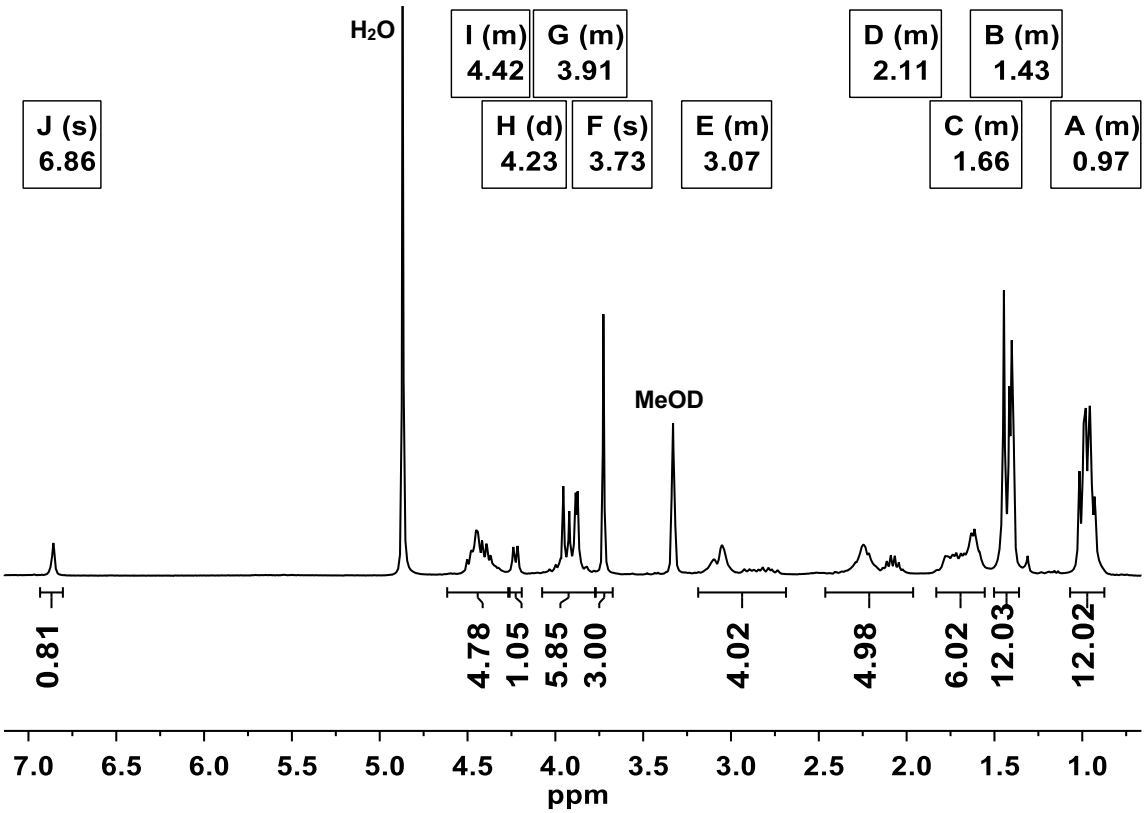

**Supplementary Figure S43:** $^{1}$H NMR of complex **7b**



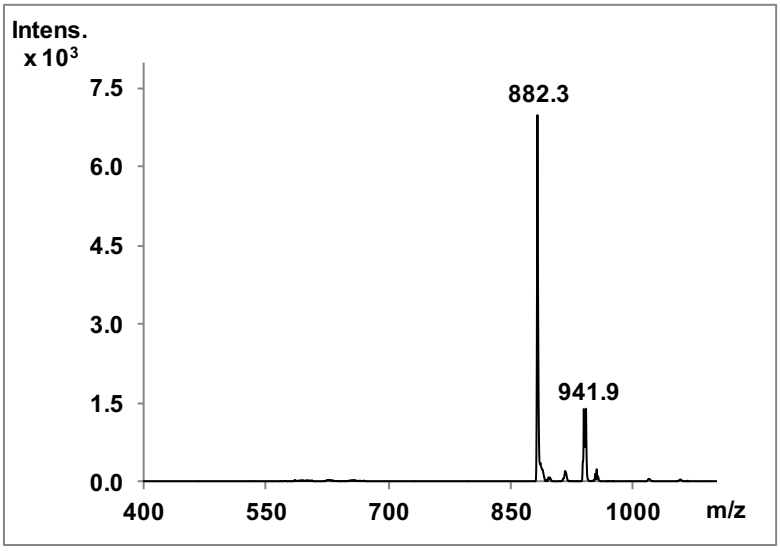

**Supplementary Figure S44:** $^{13}$C NMR of complex **7b**




**Supplementary Figure S45:** ESI-MS spectrum of complex **7b**




**Supplementary Figure S46:** $^{1}$H NMR of complex **7c**




**Supplementary Figure S48:** ESI-MS spectrum of complex **7c**