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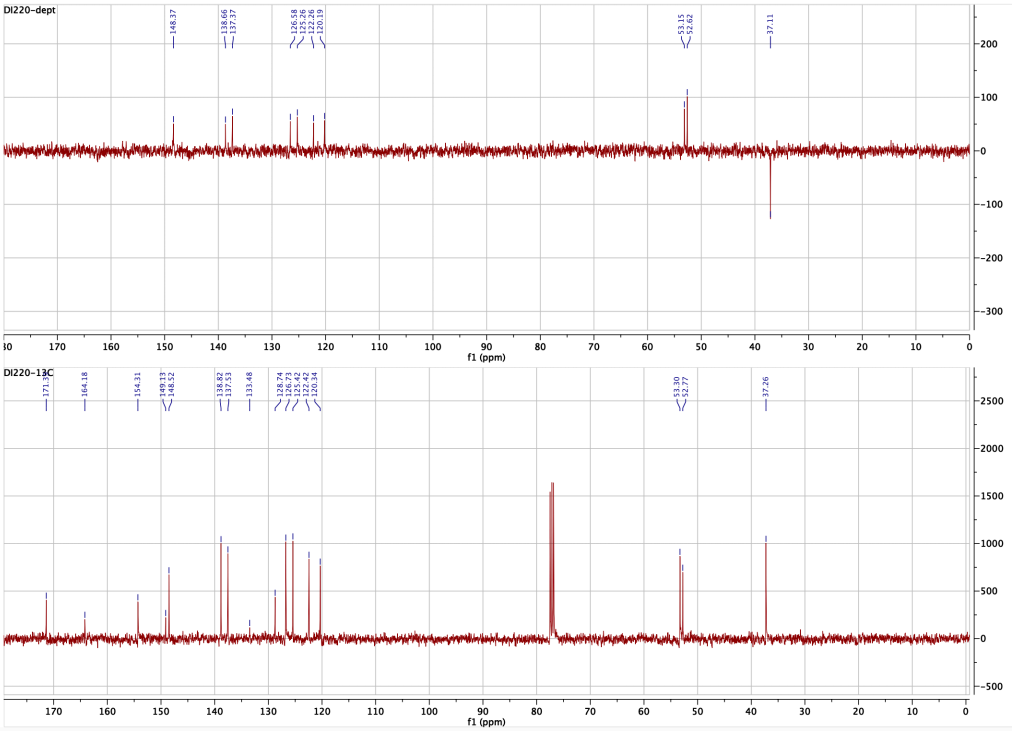
1. ^{1}H and ^{13}C NMR spectra

Compound 2:

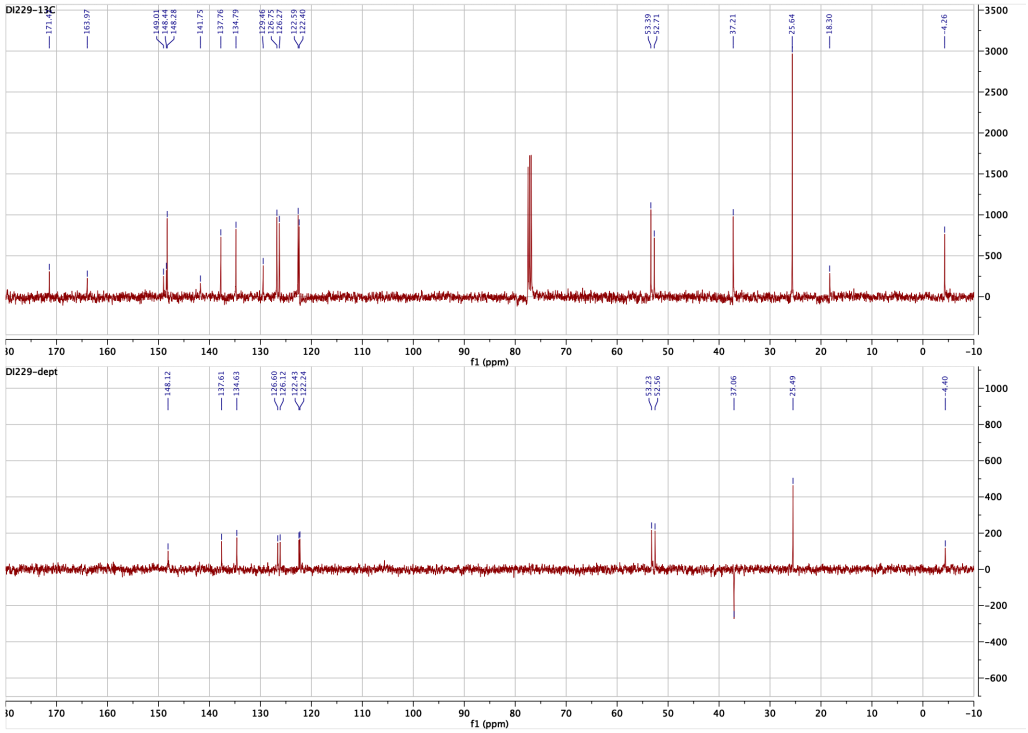
^{1}H NMR (400MHz, $CDCl_3$) compound **2**



^{13}C and DEPT NMR (101 MHz, $CDCl_3$) compound **2**

Compound 3:

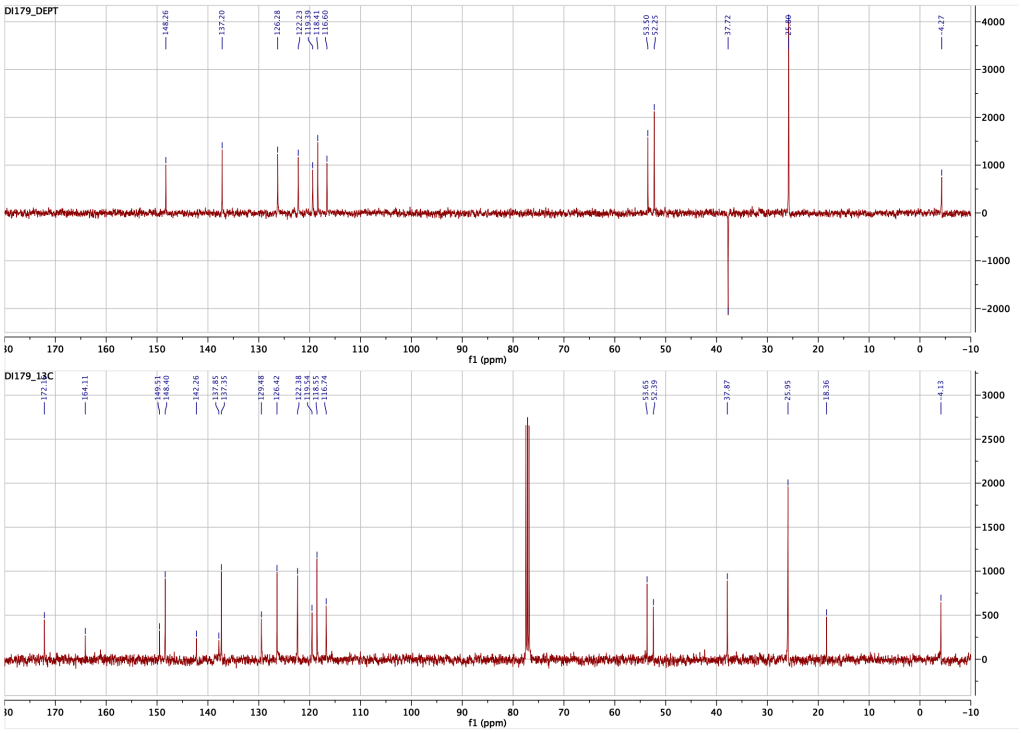
^{1}H NMR (400MHz, $CDCl_3$) compound **3**



^{13}C and DEPT NMR (101 MHz, $CDCl_3$) compound **3**

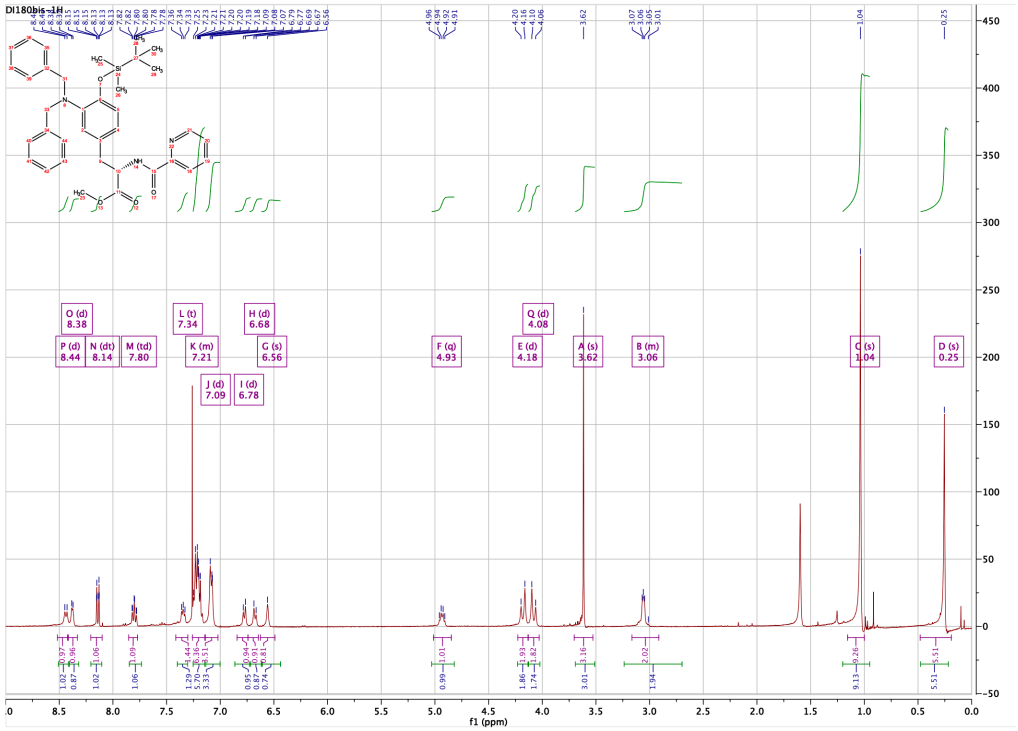
Compound 4:

^{1}H NMR (400MHz, $CDCl_3$) compound **4**

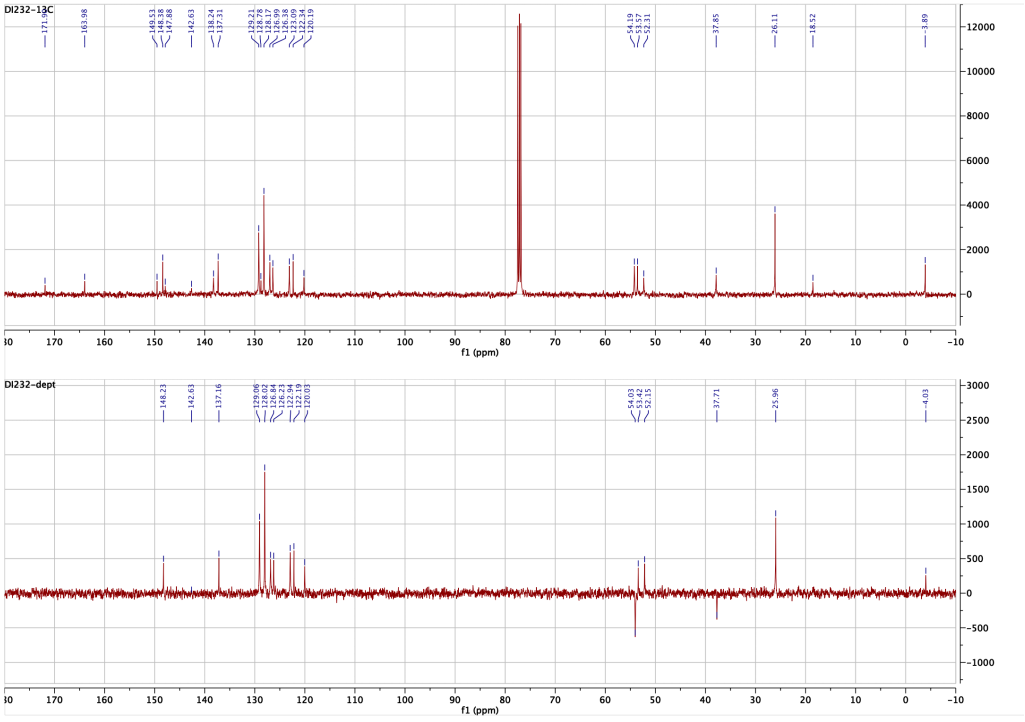


^{13}C and DEPT NMR (101 MHz, $CDCl_3$) compound **4**

Compound 5:



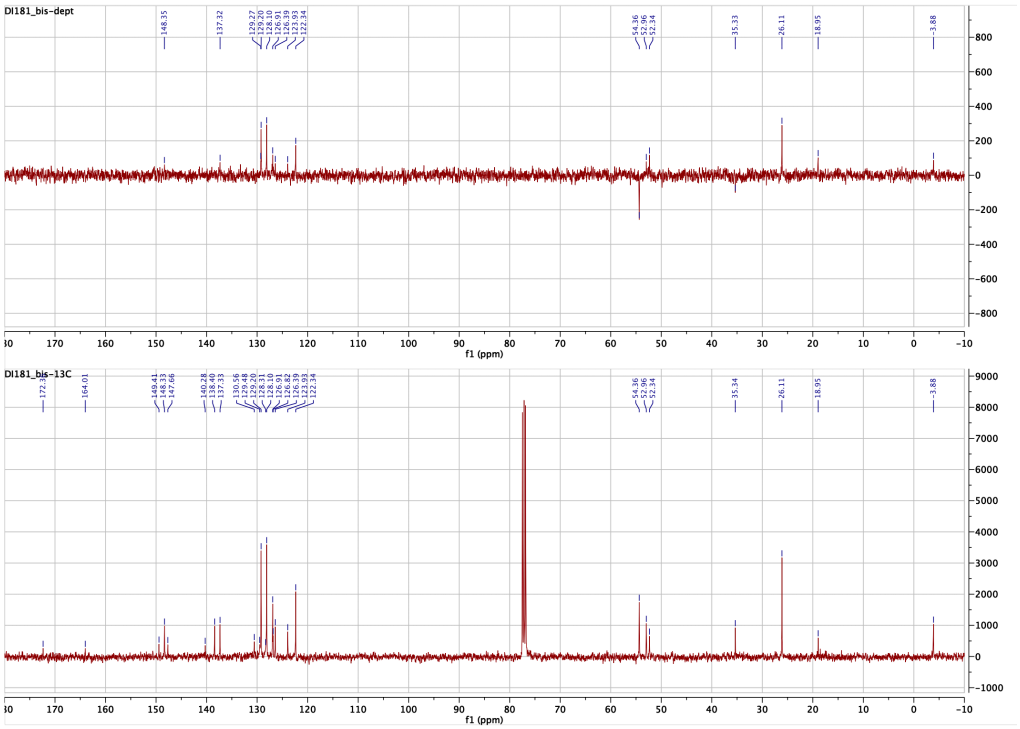
^{1}H NMR (400MHz, $CDCl_3$) compound **5**



^{13}C and DEPT NMR (101 MHz, $CDCl_3$) compound **5**

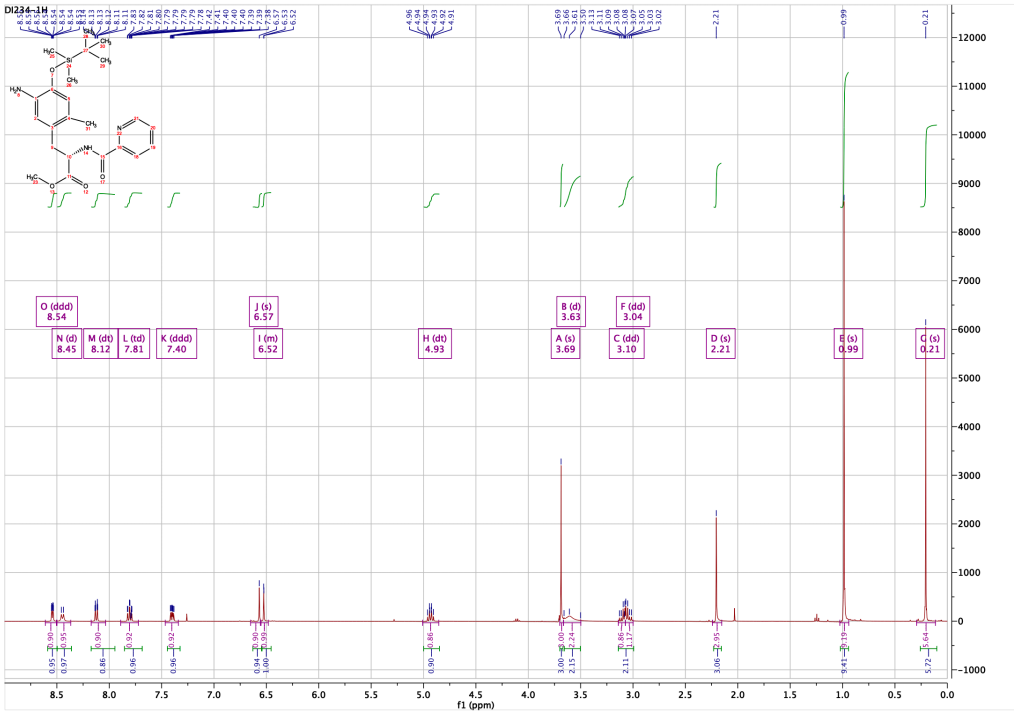
Compound 6:

^{1}H NMR (400MHz, $CDCl_3$) compound **6**

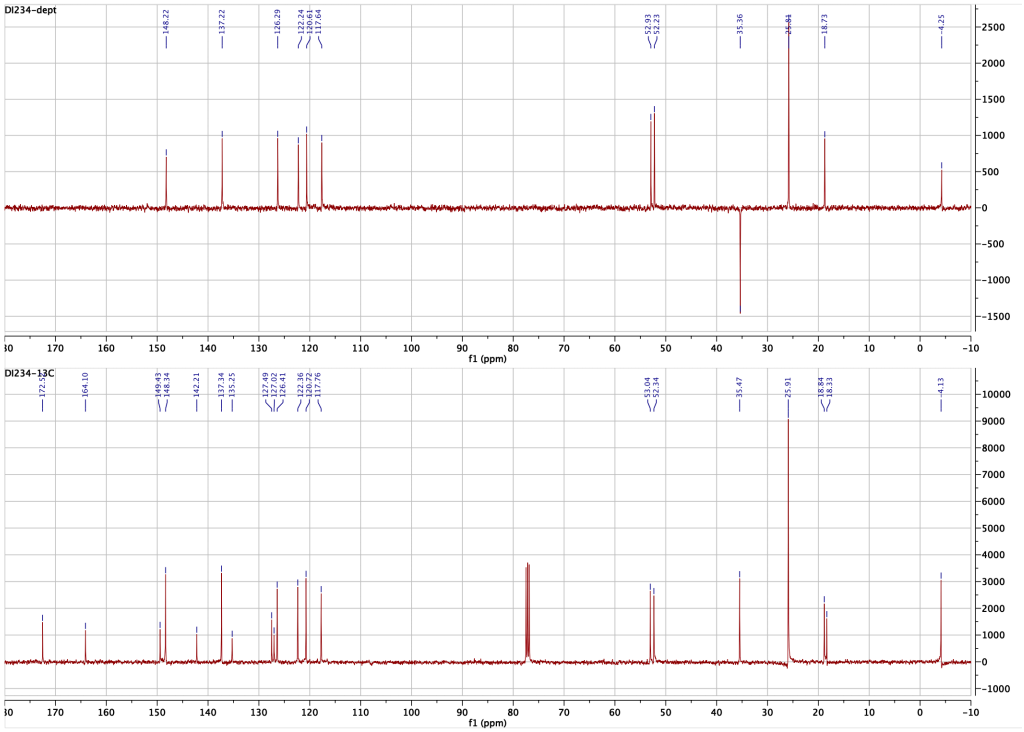


^{13}C and DEPT NMR (101 MHz, $CDCl_3$) compound **6**

Compound 7:



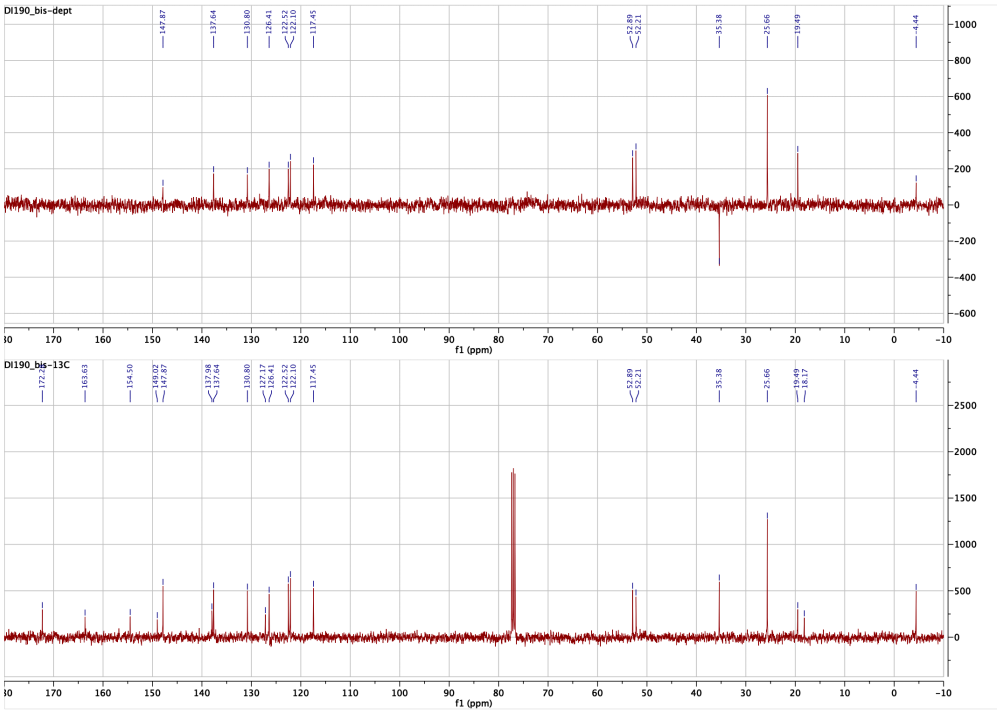
^{1}H NMR (400MHz, $CDCl_3$) compound **7**



^{13}C and DEPT NMR (101 MHz, $CDCl_3$) compound **7**

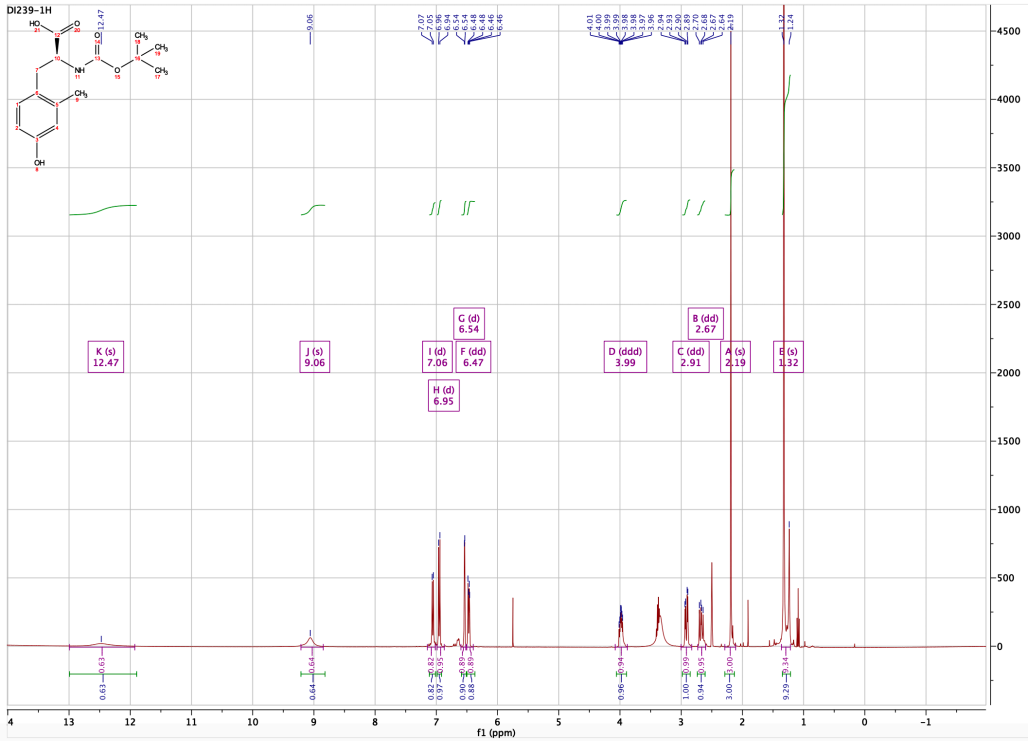
Compound 9:

^{1}H NMR (400MHz, $CDCl_3$) compound **9**

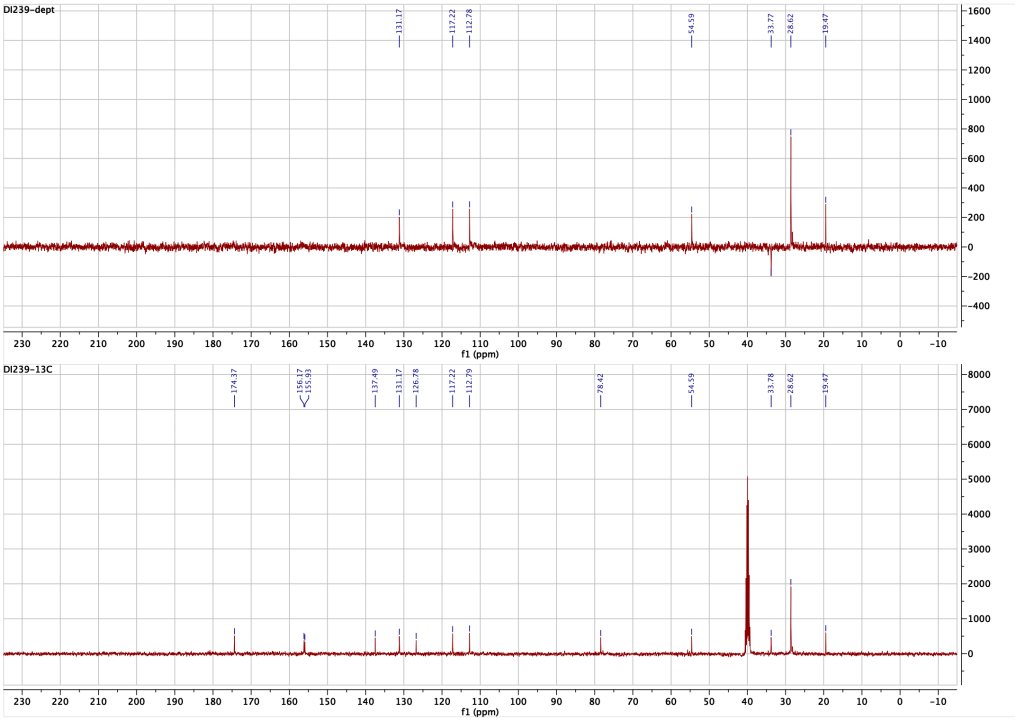


^{13}C and DEPT NMR (101 MHz, $CDCl_3$) compound **9**

Compound 10:

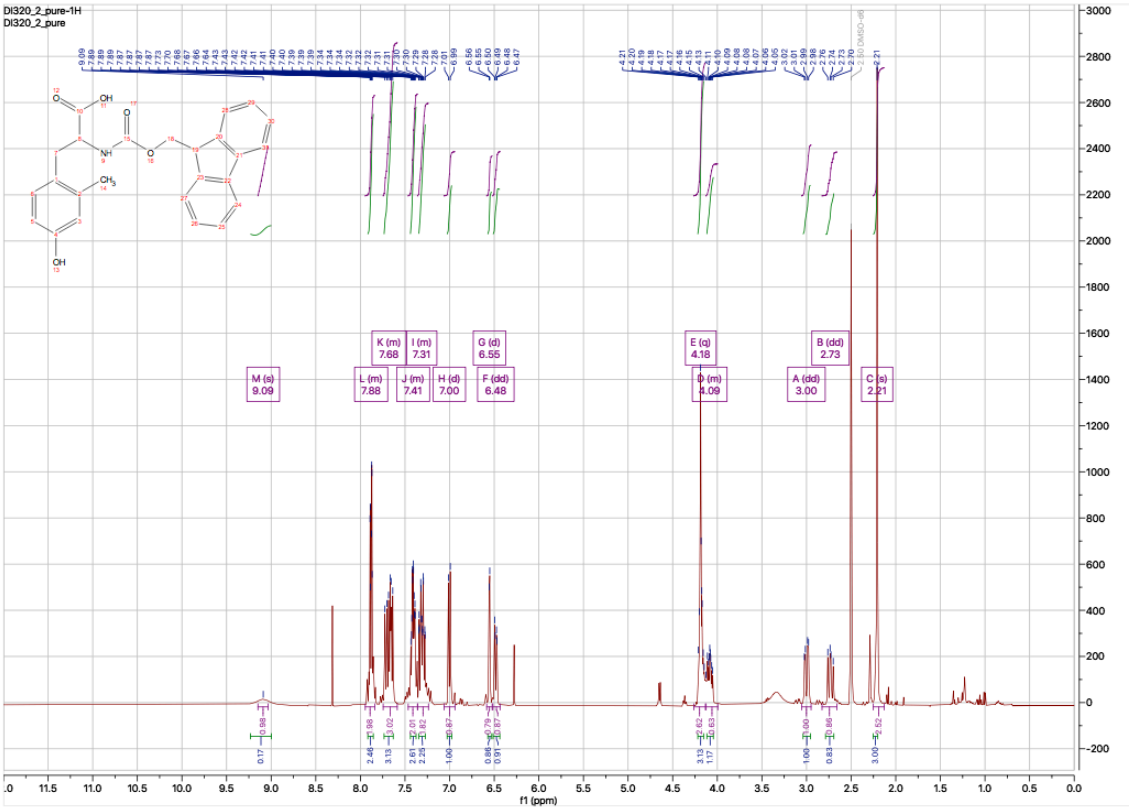


^{1}H NMR (400MHz, DMSO-d_6) compound **10**

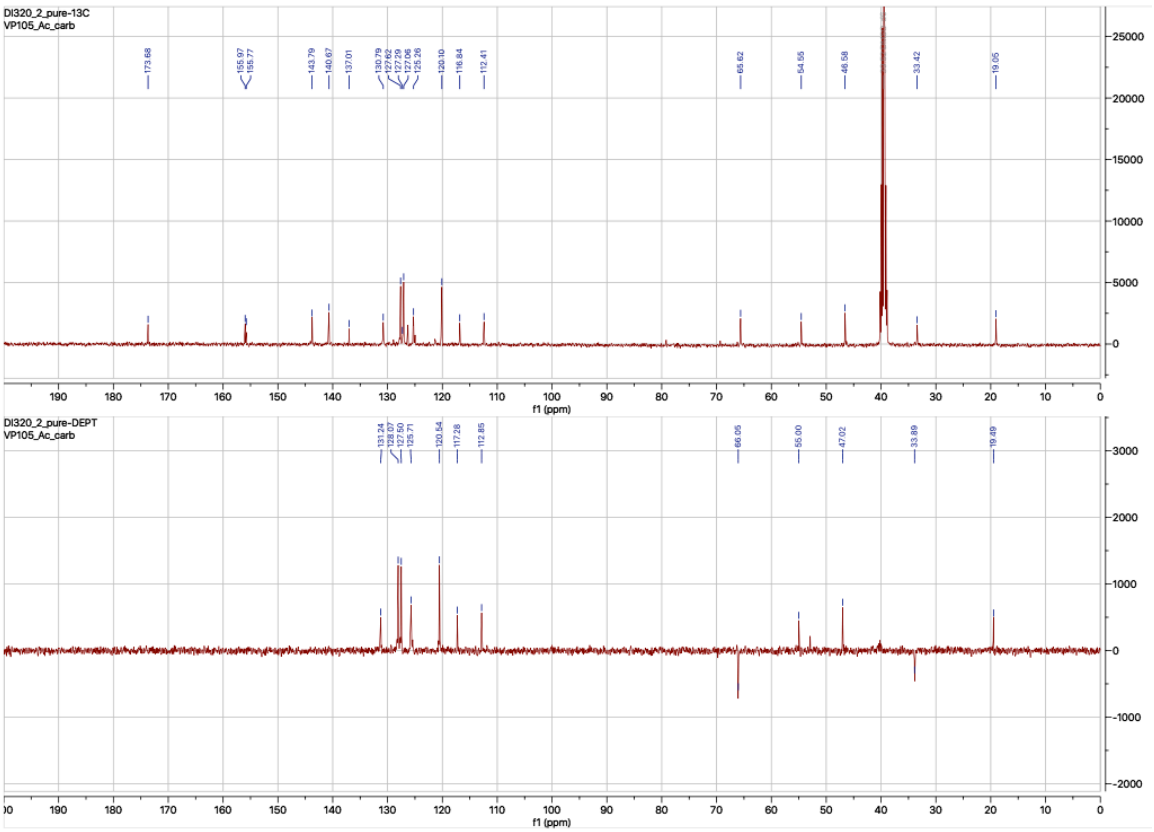


^{13}C and DEPT NMR (101 MHz, DMSO-d_6) compound **10**

Compound 11:



^{1}H NMR (400MHz, DMSO-d_6) compound **11**



^{13}C and DEPT NMR (101 MHz, DMSO-d_6) compound **11**

2. Application on SPPS

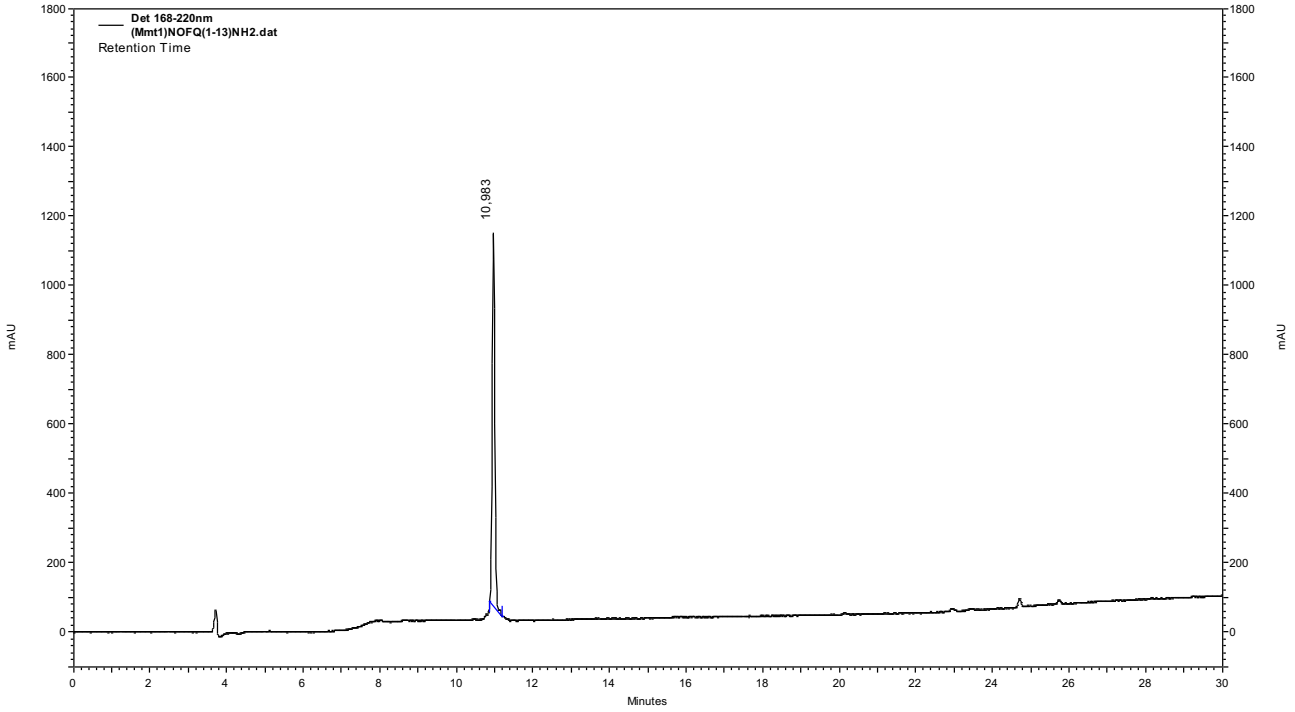
The compound **11** described was applied in SPPS for the replacement of Phe[1] in the N/OFQ(1-13)-NH_2 peptide sequence. All the peptides were synthesized by taking advantage of the Fmoc/tBu strategy, using an automatic Synthesizer (XP Syro, Biotage Sweden).

Briefly, in SPPS the peptide chain is built up from the *C*-terminal amino acid, anchored to the resin, to the *N*-terminal one, through repeated cycles of alternate *N*-terminal deprotection and coupling reactions with activated *N*-Fmoc-*α*-amino acids. All the amino acids (aa) are orthogonally protected: *N*-Fmoc in the aliphatic α amine and/or Boc, *tert*-butyl (tBu), trityl (Trt), 2,2,5,7,8-pentamethylcromane-6-sulfonyl (Pmc), benzyloxy carbonyl (Z or Cbz) in eventually side chain functional groups. The polystyrene resin Amispheres 20 RAM (20% of its weights is made of polyethylene glicole) was used as starting material. The resin is weighted into an empty syringe, then it is swelled in DMF, at room temperature.

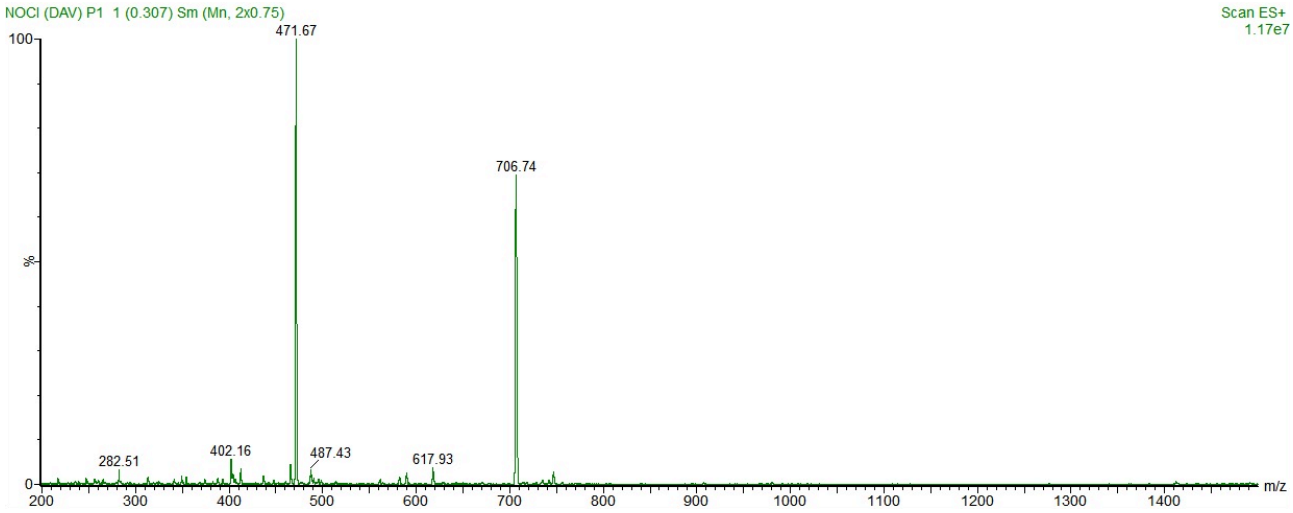
Once the last *α*-amino acid is inserted, the *N*-terminal Fmoc protection is removed, then a single acidic treatment is performed, allowing the removal of side chain protecting groups and the cleavage of the neo-synthesized peptide from the resin. Specifically, the 'cleavage cocktail' used to this aim is composed of trifluoroacetic acid (TFA), water and triethyl silane (Et_3SiH) in 9:0,5:0,5 proportion. The treatment is carried out at room temperature for 3 hours.

The exhausted resin is filtered off, TFA is removed from the filtrate under vacuum, and the neo-synthesized peptide is then precipitated in diethyl ether (Et_2O), isolated through centrifugation and finally purified via reverse phase preparative high-performance liquid chromatography (RP-HPLC) then dried over lyophilization. RP-HPLC analytical gradients were run using a solvent system consisting of A (H_2O + 0.1% TFA) and B (CH_3CN + 0.1% TFA. The conditions used to characterize pure peptides consisted of a linear gradient from 0% to 100% of B solution over 25 min).

The Mmt **11** was incorporated in peptide built by solid-phase peptide synthesis: [(Mmt[1])](N/OFQ(1-13)-NH_2) and it was compared to the [Dmt[1]](N/OFQ(1-13)-NH_2), N/OFQ(1-13)-NH_2 and N/OFQ.



HPLC of compound **11** inserted in position 1 of N/OFQ(1-13)-NH_2



Mass spectrum of compound **11** inserted in position 1 of N/OFQ(1-13)-NH_2