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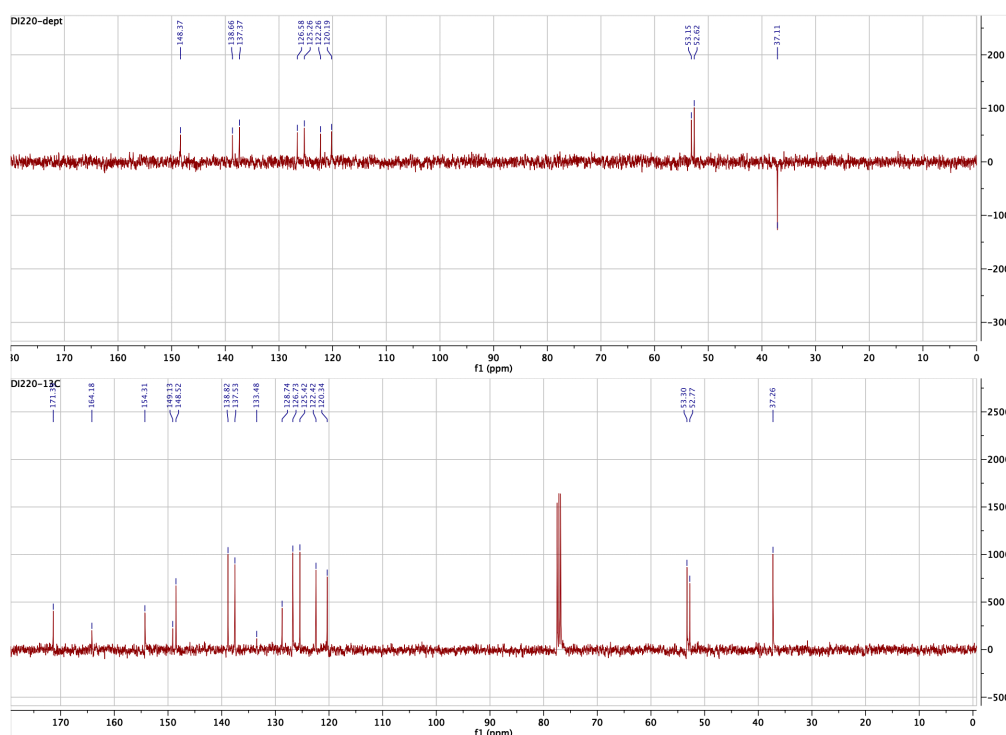
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1. ¹H and ¹³C NMR spectra

Compound 2:

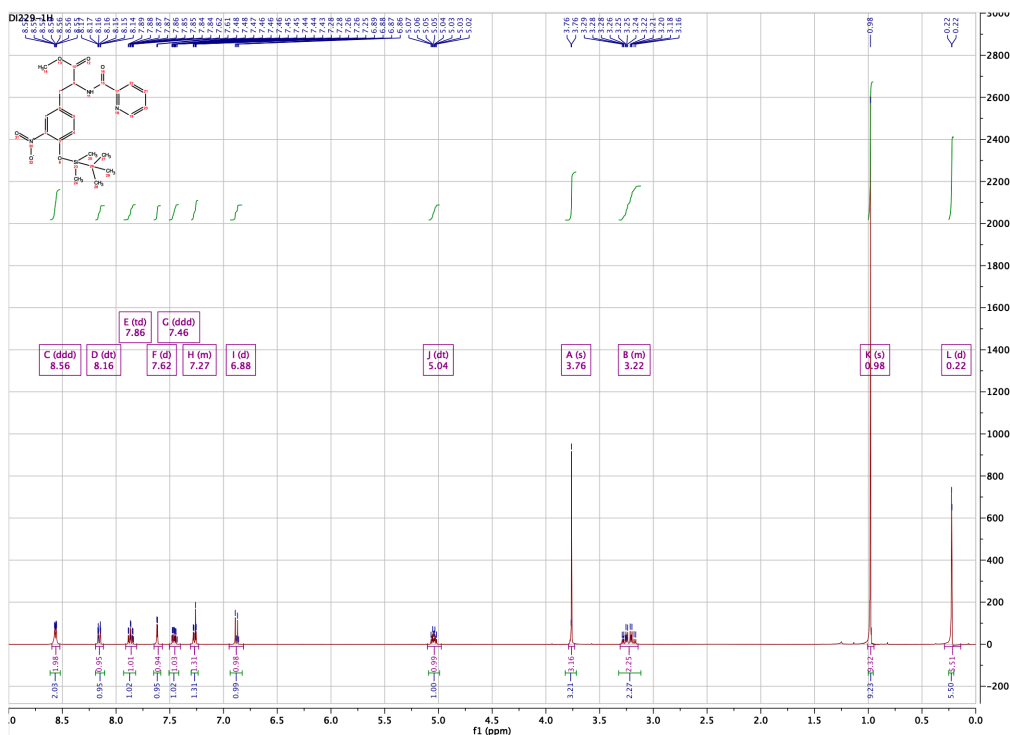
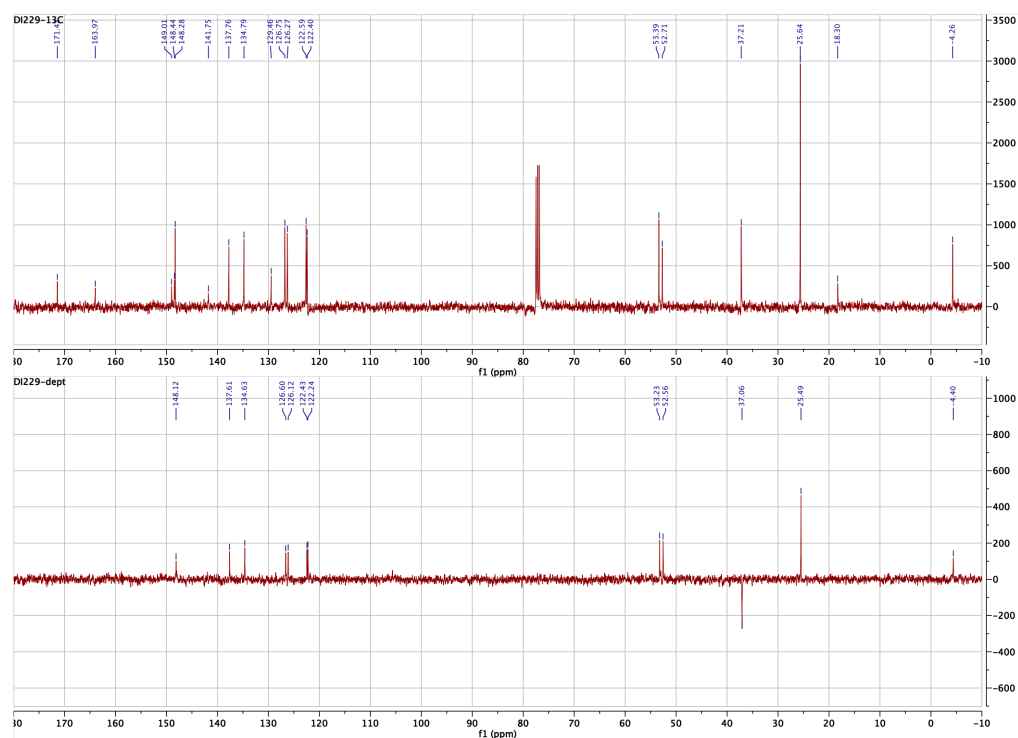


¹H NMR (400MHz, CDCl₃) compound 2



¹³C and DEPT NMR (101 MHz, CDCl₃) compound 2

Compound 3:

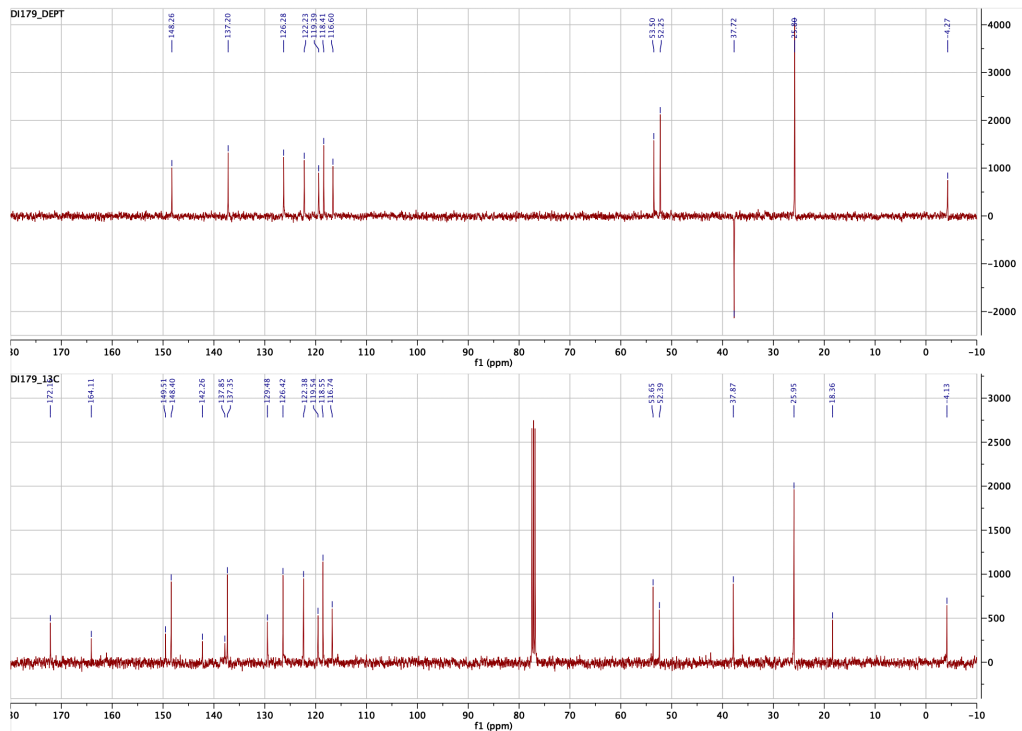
¹H NMR (400MHz, CDCl₃) compound **3**

¹³C and DEPT NMR (101 MHz, CDCl₃) compound **3**

Compound 4:

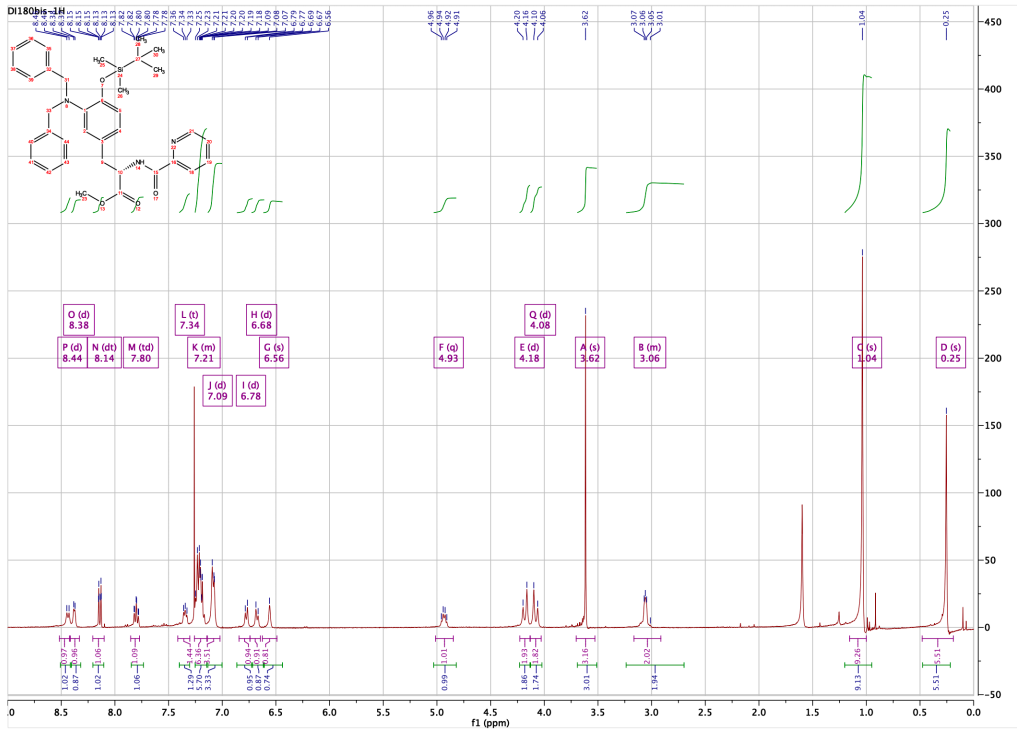
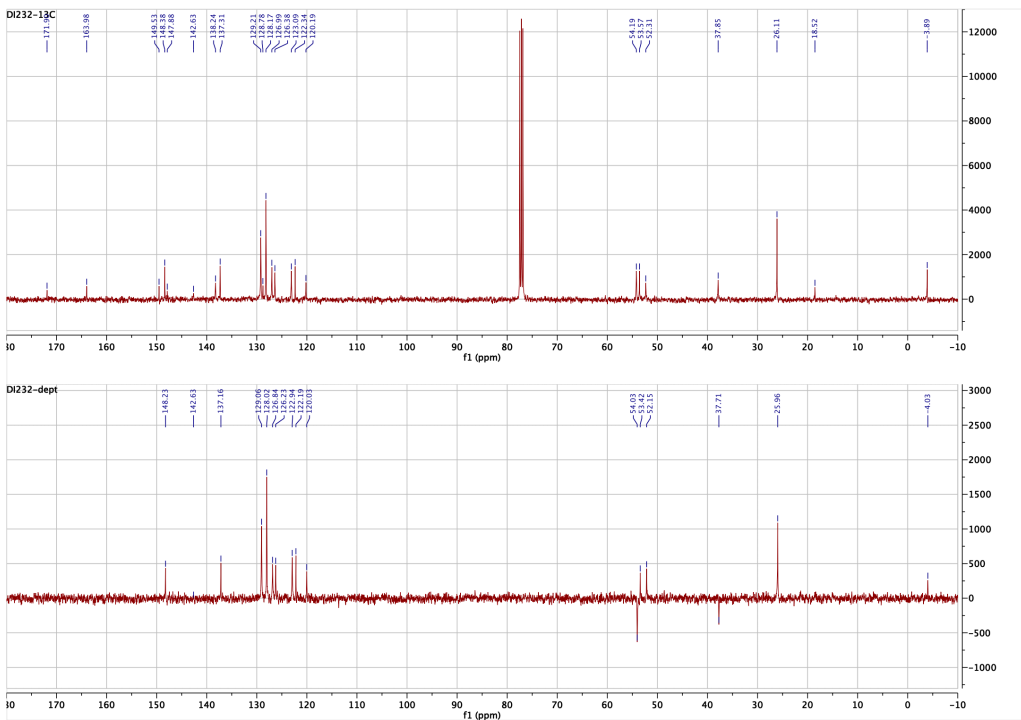


¹H NMR (400MHz, CDCl₃) compound 4



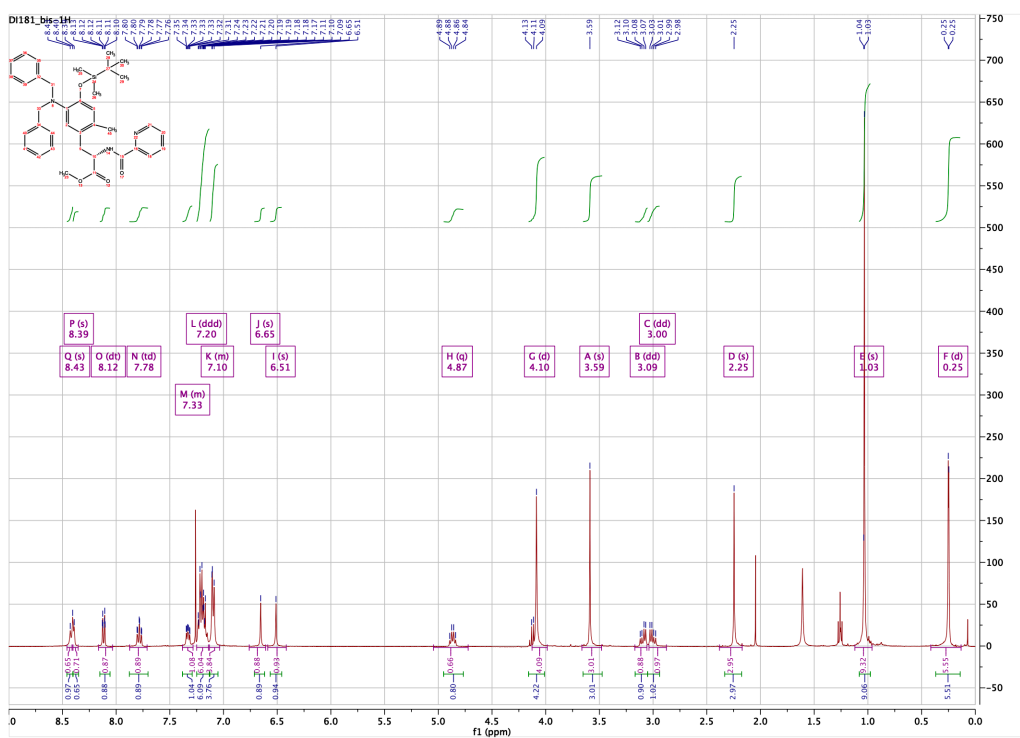
¹³C and DEPT NMR (101 MHz, CDCl₃) compound 4

Compound 5:

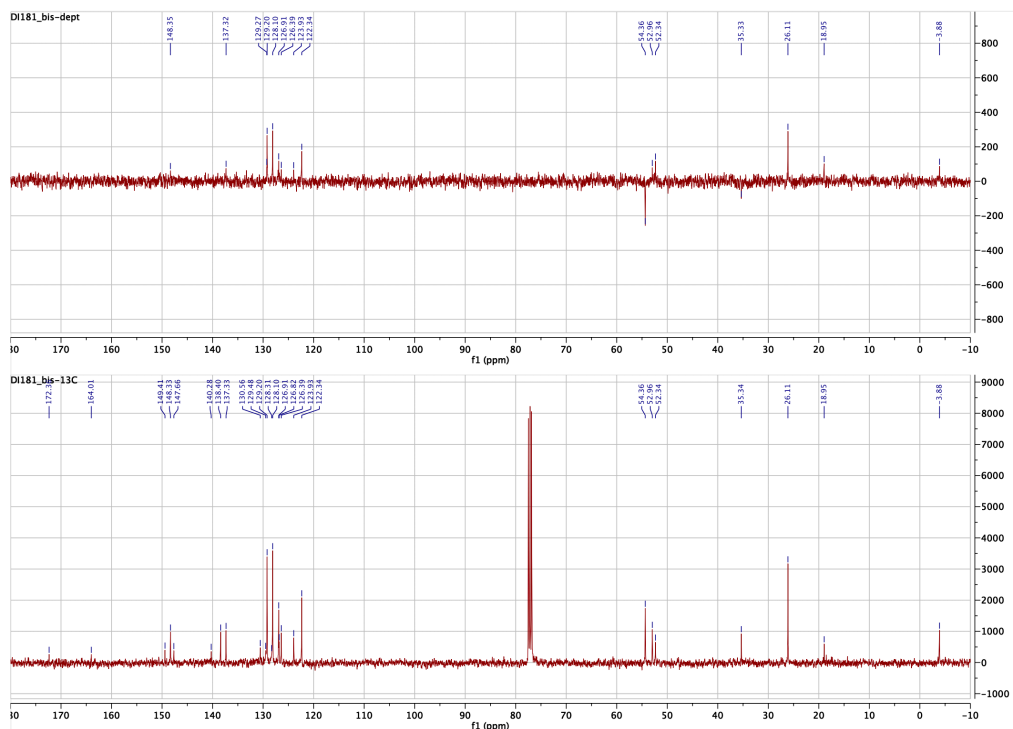
¹H NMR (400MHz, CDCl₃) compound **5**

¹³C and DEPT NMR (101 MHz, CDCl₃) compound **5**

Compound 6:

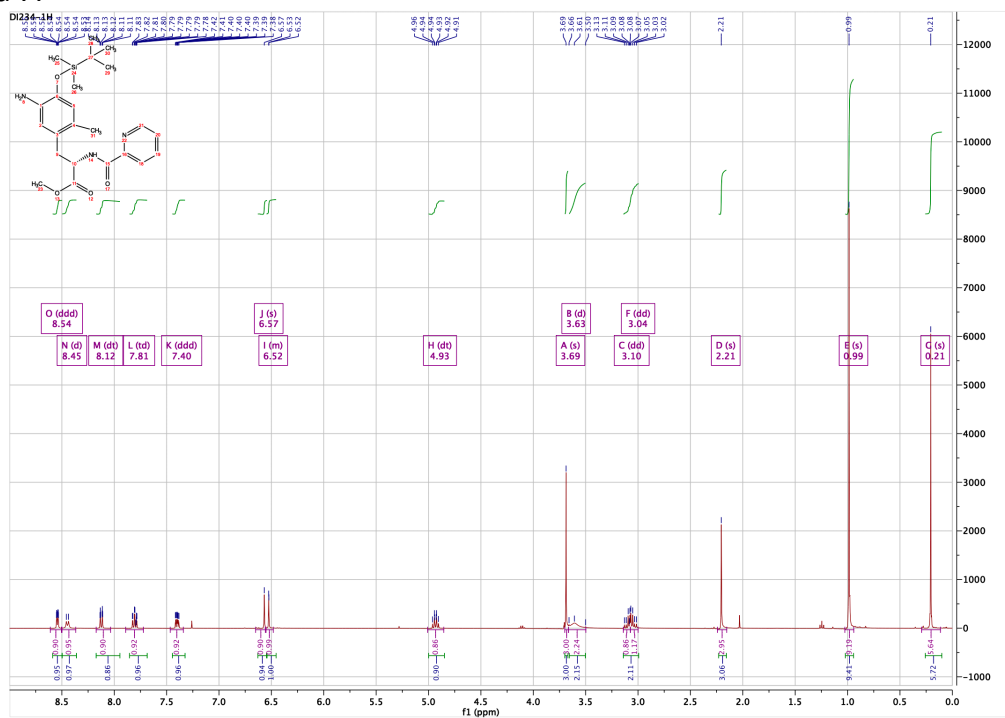


¹H NMR (400MHz, CDCl₃) compound **6**

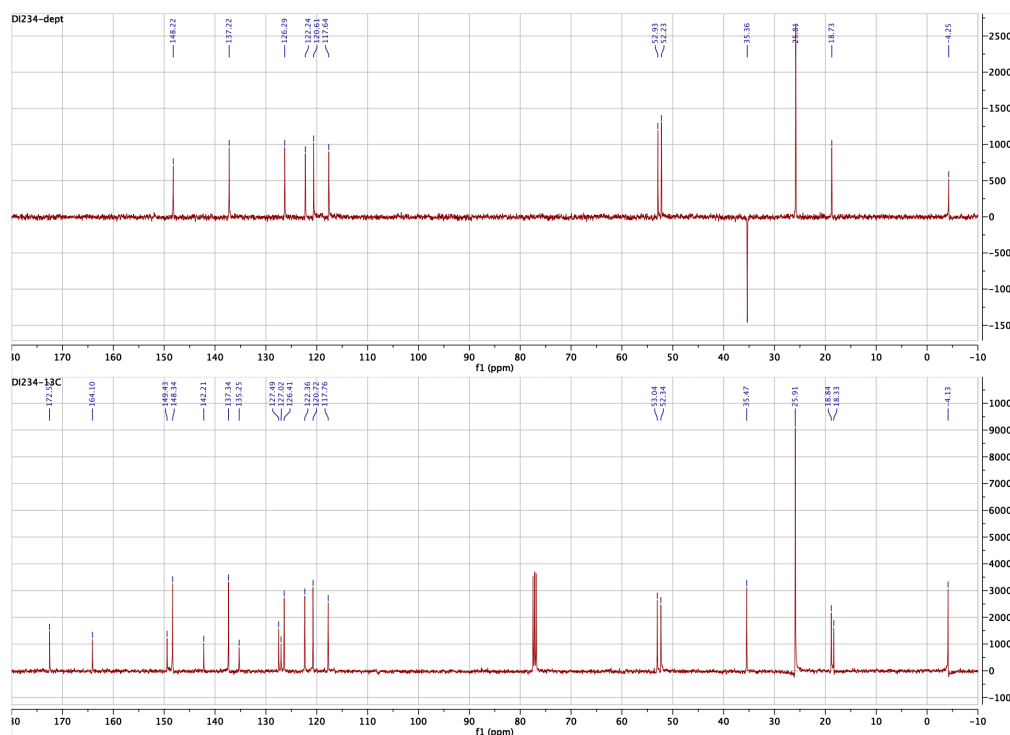


¹³C and DEPT NMR (101 MHz, CDCl₃) compound **6**

Compound 7:

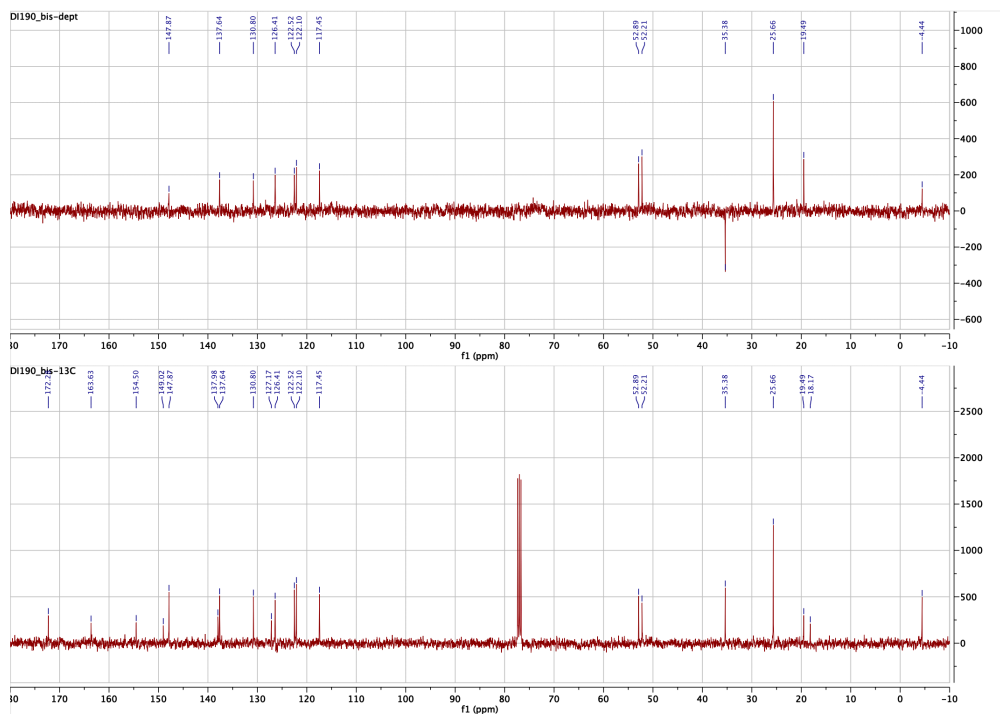
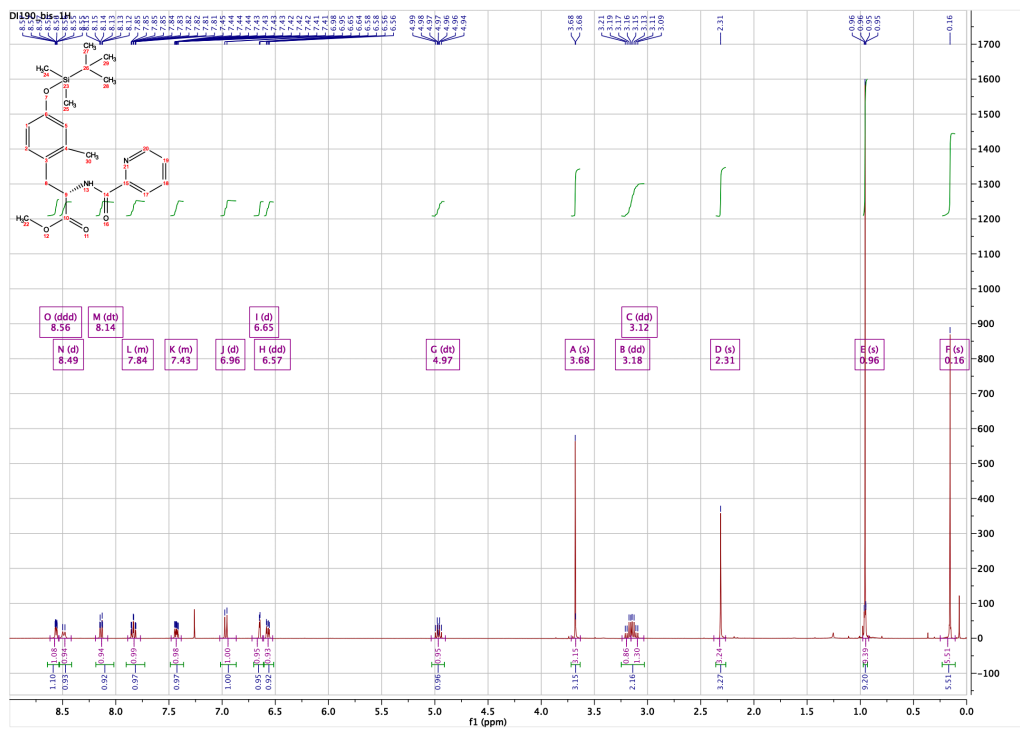


¹H NMR (400MHz, CDCl₃) compound 7

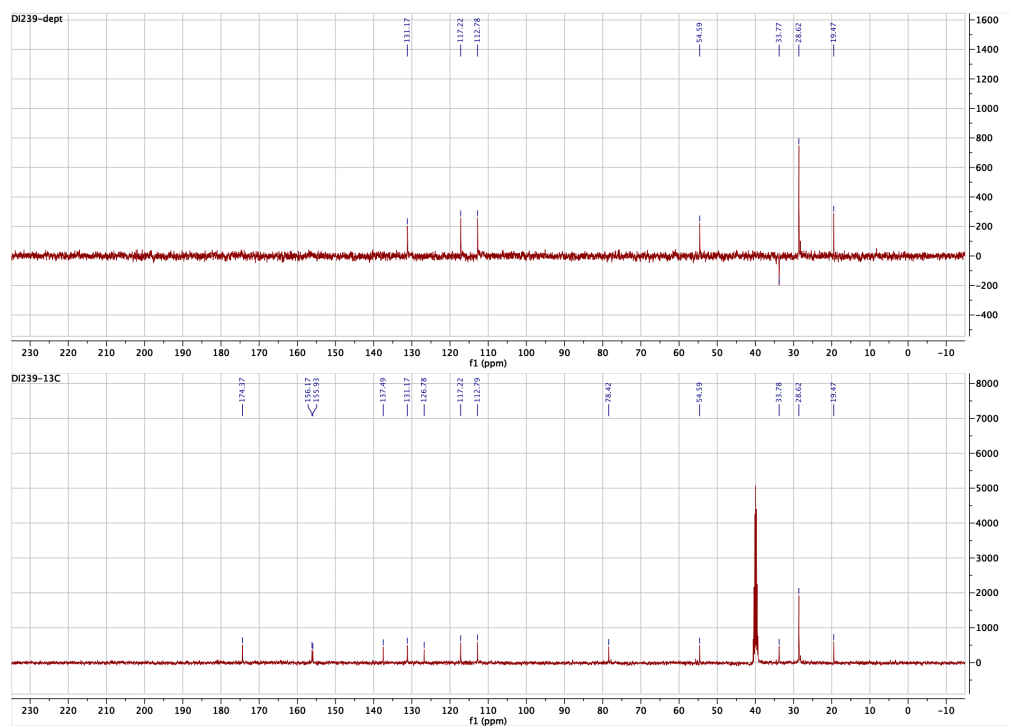
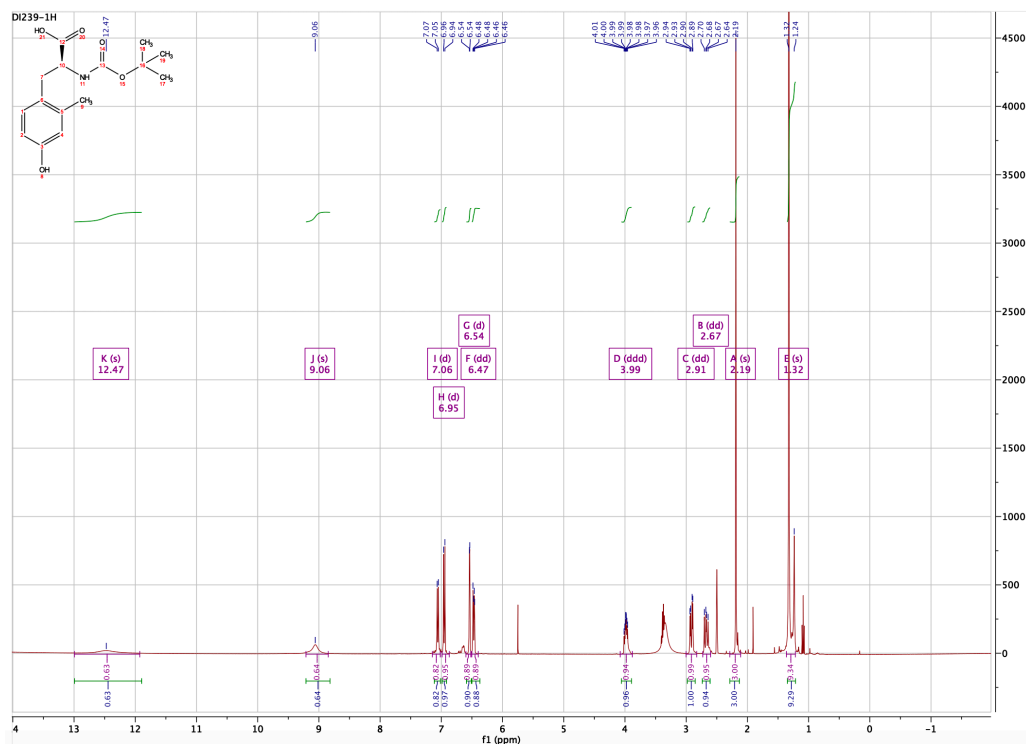


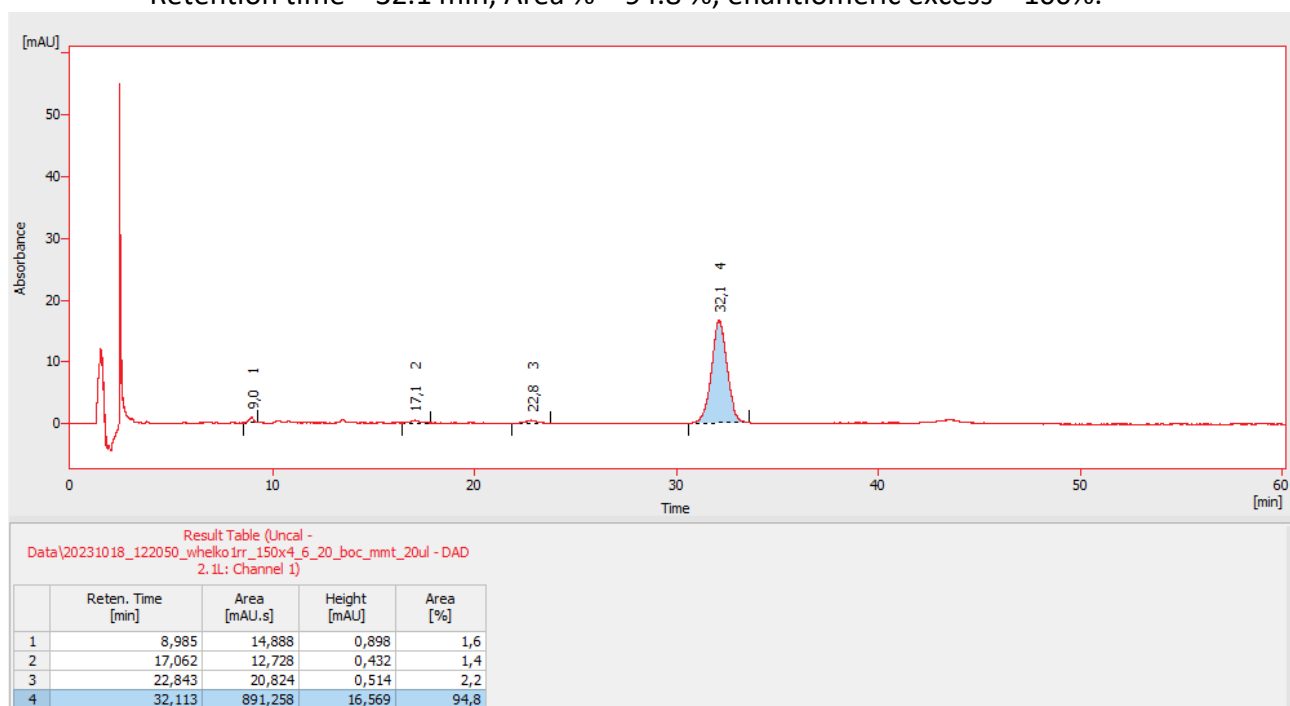
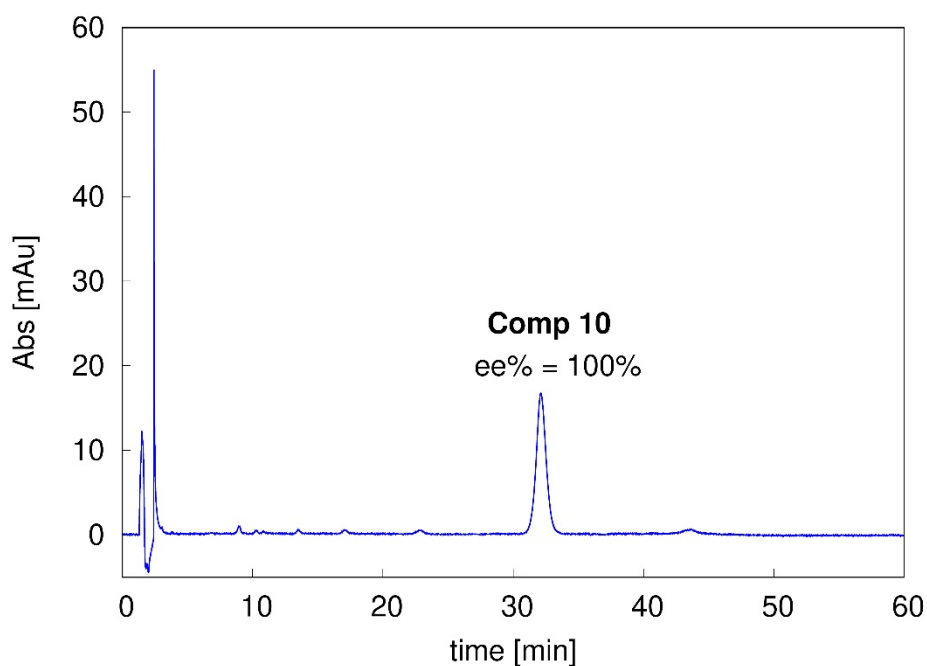
¹³C and DEPT NMR (101 MHz, CDCl₃) compound **7**

Compound 9:

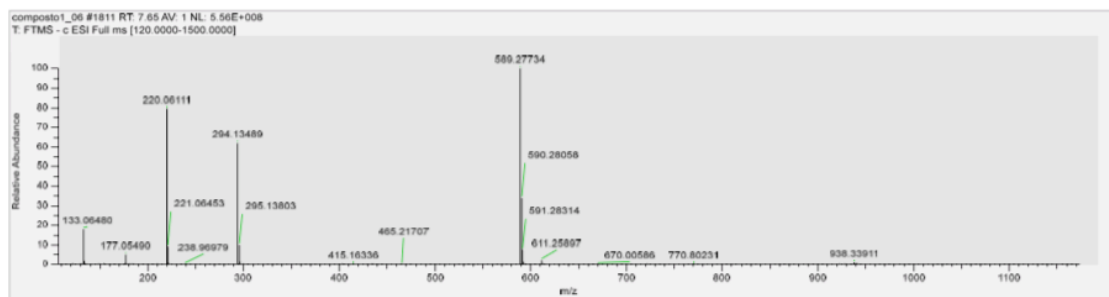
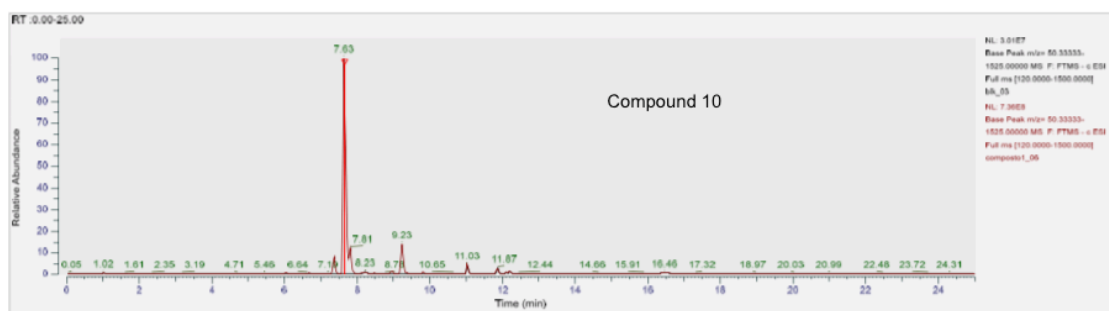


Compound 10:





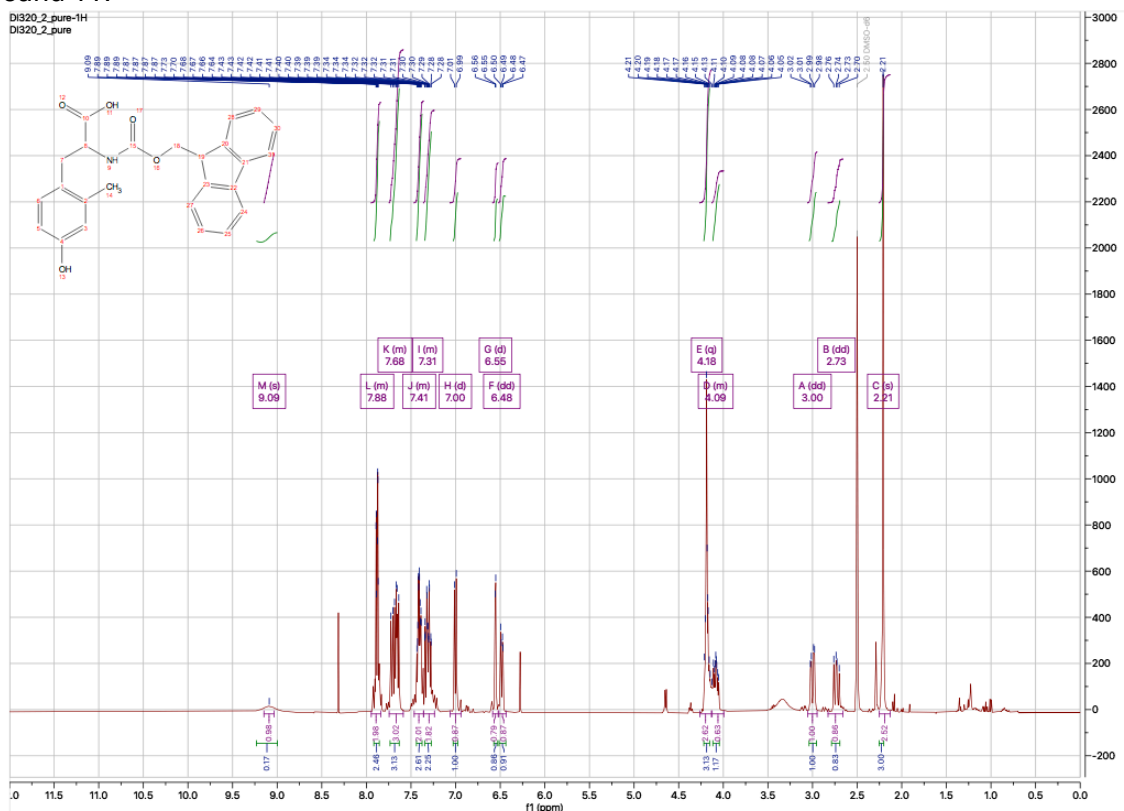
Chiral HPLC compound **10**



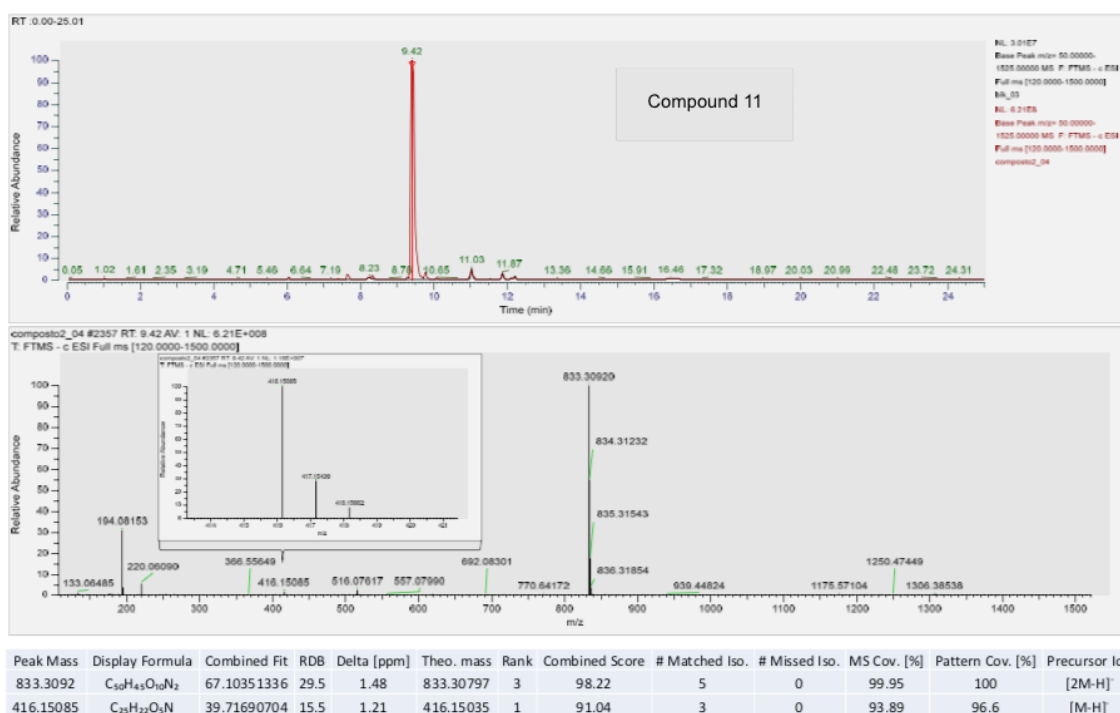
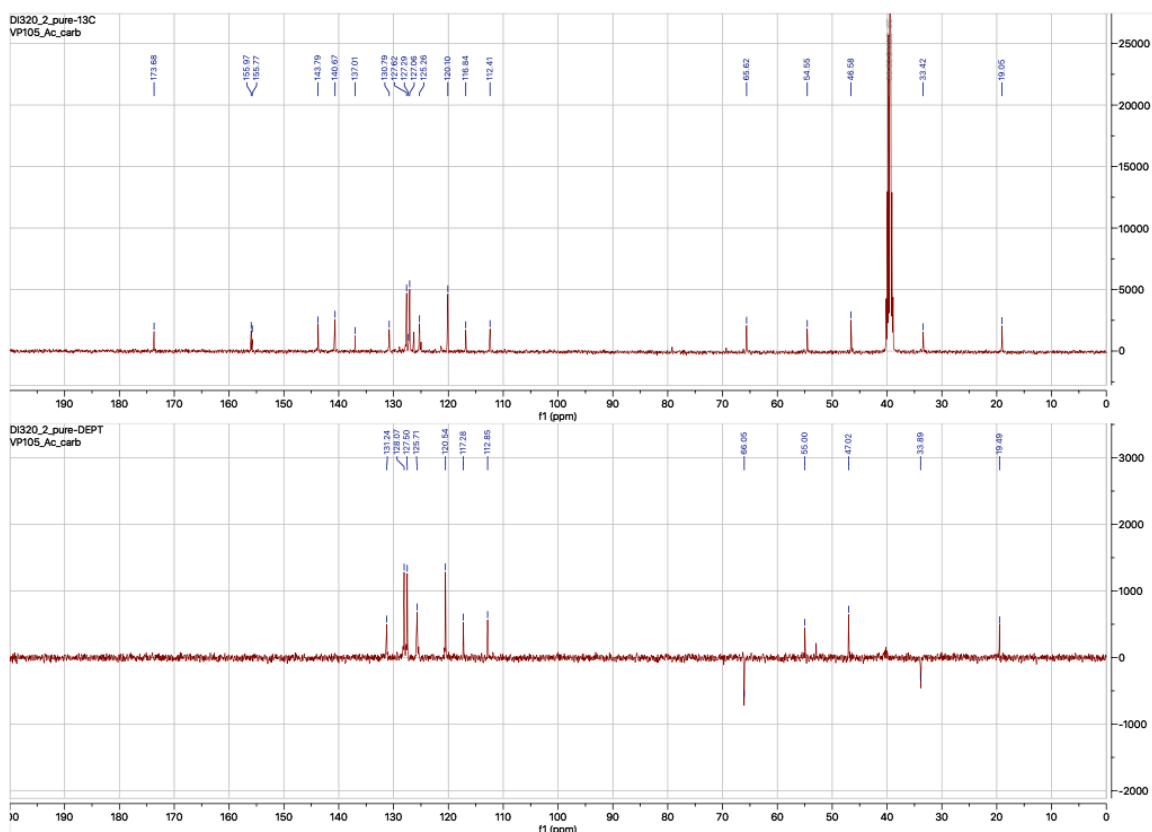
Peak Mass	Display Formula	Combined Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined Score	# Matched Iso.	# Missed Iso.	MS Cov. [%]	Pattern Cov. [%]	Precursor Ion
294.13489	C ₁₅ H ₂₀ O ₃ N	49.6407589	6.5	0.65	294.1347	1	97.35	3	1	100	99.82	[M-H] ⁺
589.27734	C ₃₀ H ₄₁ O ₁₀ N ₂	85.67083182	11.5	1.15	589.27667	1	99.25	5	0	100	100	[2M-H] ⁺

HRMS compound 10

Compound 11:



¹H NMR (400MHz, DMSO-d₆) compound 11



HRMS compound **11**

2. Application on SPPS

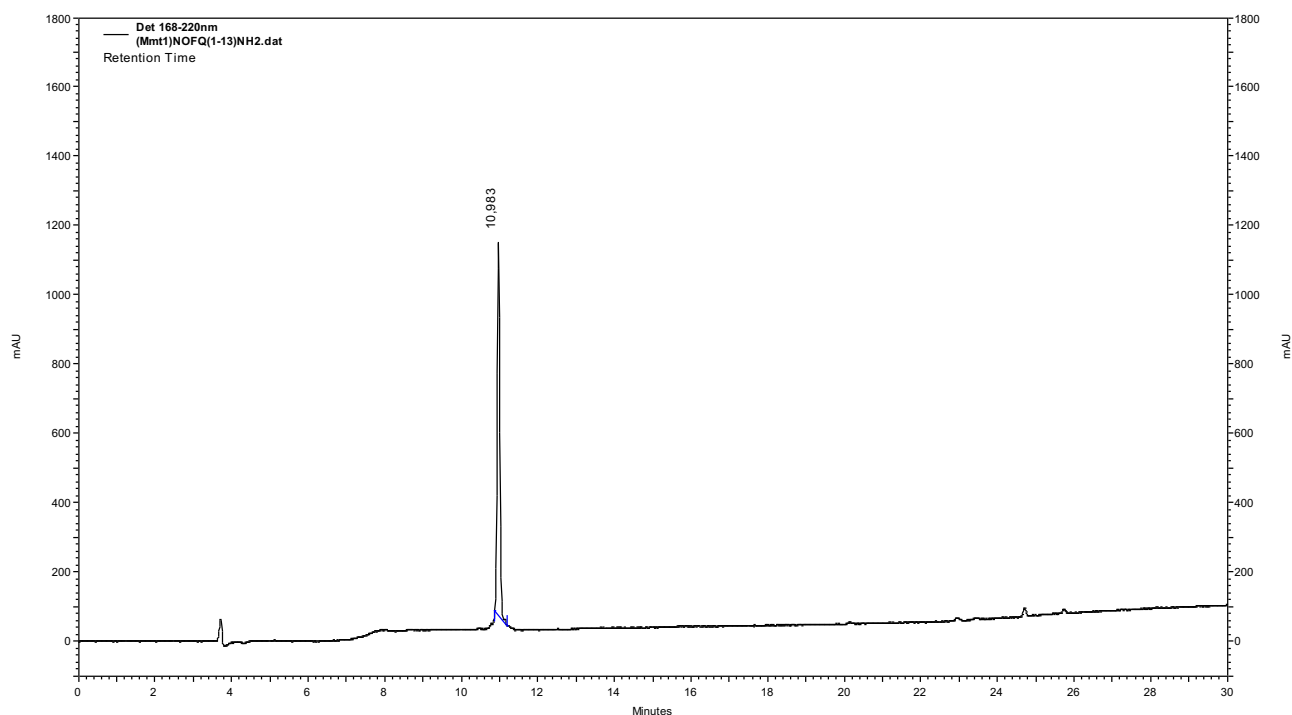
The compound **11** described was applied in SPPS for the replacement of Phe¹ in the N/OFQ(1-13)-NH₂ peptide sequence. All the peptides were synthesized by taking advantage of the Fmoc/tBu strategy, using an automatic Synthesizer (XP Syro, Biotage Sweden).

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-pentamethylchromane-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 glycole) was used as starting material. The resin is weighted into an empty syringe, then it is swelled in DMF, at room temperature.

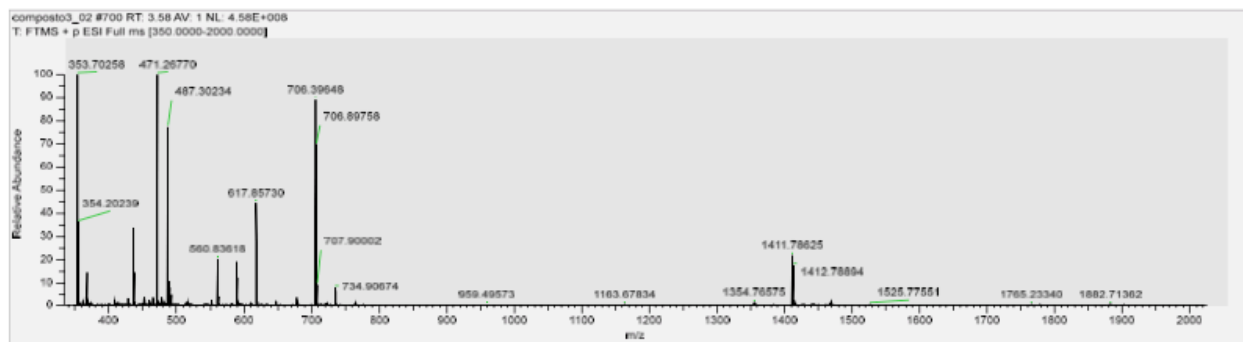
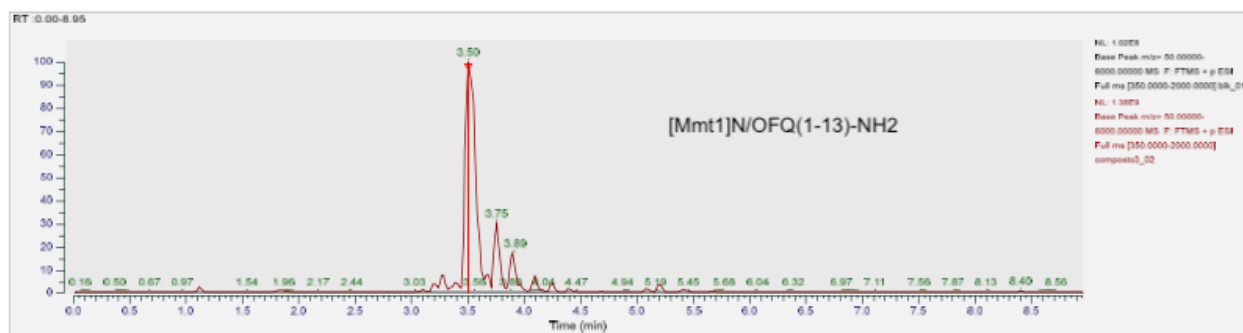
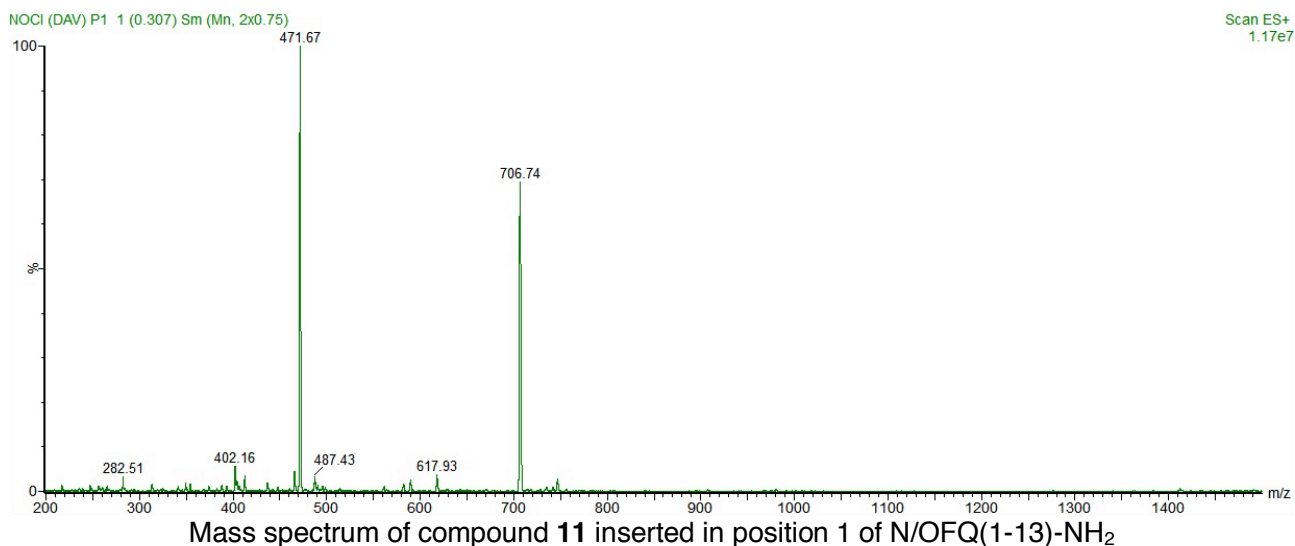
Once the last α -amino acid is inserted, the N-terminal Fmoc protection is removed, then a single acidic treatment is performed, allowing the removal of side chain protecting groups and the cleavage of the neo-synthesized peptide from the resin. Specifically, the 'cleavage cocktail' used to this aim is composed of trifluoroacetic acid (TFA), water and triethyl silane (Et₃SiH) 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 (Et₂O), 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 (H₂O + 0.1% TFA) and B (CH₃CN + 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: [(Mmt¹)](N/OFQ(1-13)-NH₂) and it was compared to the [Dmt¹](N/OFQ(1-13)-NH₂), N/OFQ(1-13)-NH₂ and N/OFQ.



HPLC of compound **11** inserted in position 1 of N/OFQ(1-13)-NH₂



Peak Mass	Display Formula	Combined Fit	RDB	Delta (ppm)	Theo. mass	Rank	Combined Score	# Matched Iso.	# Missed Iso.	MS Cov. [%]	Pattern Cov. [%]	Precursor Ion
1411.78625	C ₆₂ H ₁₀₃ O ₁₆ N ₂₂	62.23344667	22.5	-3.85	1411.79169	1	93.76	6	0	95.51	100	[M+H] ⁺
706.39648	C ₆₂ H ₁₀₄ O ₁₆ N ₂₂	64.01266112	22	-4.25	706.39948	2	98.11	6	0	100	100	[M+2H] ⁺
471.2677	C ₆₂ H ₁₀₅ O ₁₆ N ₂₂	59.58519634	21.5	-2.22	471.26875	1	97.74	6	0	99.86	100	[M+3H] ⁺

HRMS of compound **11** inserted in position 1 of N/OFQ(1-13)-NH₂