Supporting Information to

Computational design of novel peptidomimetic inhibitors of cadherin homophilic interactions

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Crystallographic interfaces characterization

The pattern of interactions of the DWVI sequence in the E- and N-cadh X-ray dimer structures (3q2v.pdb and 3q2w.pdb[i], respectively) is nearly identical and it can be summarized as follows: (i) the formation of an intermolecular salt bridge between the charged N-terminal amino group of Asp1 and the side chain carboxylate of Glu89; (ii) the anchoring of the Trp2 side chain into a hydrophobic pocket and (iii) the formation of a hydrogen bond between the indole moiety and the carbonyl group of Asn90 (N-cadh) or Asp90 (E-cadh); (iv) the involvement of Val3-NH in a hydrogen bond with the carbonyl group of Arg25 (N-cadh) or Lys25 (E-cadh); (v) the formation of an hydrogen bond between the backbone carbonyl group of Asp1 and the Asp27-NH (N-cadh) or Asn27-NH (E-cadh) group. The main difference in the interaction pattern observed for the DWVI motif in the two systems is limited to a salt bridge formed between the Asp1-NH_3^+ group and the carboxyl group of Asp27 in N-cadh binding site that in E-cadh receptor is replaced by a hydrogen bond with the side chain of Asn27. Residues of the binding pocket that are in contact with the Trp2 side chain are mostly conserved, except for Ala78, Asn90 and Ile92 of N-cadh mutated into Ser78, Asp90 and Met92 in E-cadh (see Fig. S1 for a 2D representation of the interactions pattern of DWVI in the N-cadh receptor).

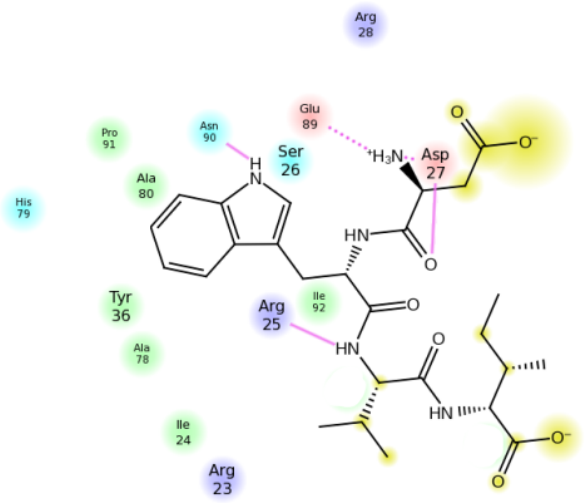


Figure S1. 2D representation of the DWVI interactions into the N-cadh binding site (residue within 4 Å from DWVI are shown).

Computational studies

Molecular Dynamics simulations

Proteins preparation. We built the EC1-EC2 dimer systems starting from the x-ray swap dimer structures of the E- and N-cadh. Each EC1-EC2 chain was truncated at residue number 218. Lys14 and Glu16 missing residues of E-cadh chain A and Lys30 CD, CE and NZ missing atoms of N-cadh chains were manually added. Three calcium ions Ca^{2+} were kept at the interface of EC1-EC2 domains ($Ca^{2+}_{601-603}$ for both E- and N-cadh) and one at the end of EC2 domain (Ca^{2+}_{605} for E-cadh and Ca^{2+}_{604} for N-cadh). All sugars and crystallographic waters were removed during the input preparation. In addition, for the E-cadh dimer, two manganese ions each coordinated to Glu13 side chain have been removed. The two systems were then prepared using the Protein Preparation Wizard of the Maestro graphical user interface (Schrödinger suite http://www.schrodinger.com) by optimizing the orientation of hydrogen bonds and charge interactions, and predicting the protonation state of histidine, aspartic and glutamic acid and the tautomeric state of histidine, followed by a restrained minimization of the whole system (0.30 Å of RMSD on heavy atoms) using the OPLSAA force field. The final refined structures were used to generate docking receptor grids and as input for Molecular Dynamic (MD) simulations.

Molecular Dynamics set up and calculations. MD simulations were performed using the AMBER11 package[ii] with the ff10 force field. Calcium ions were modeled on the basis of parameters reported by Bradbrook[iii] and histidine residue were set to HID (histidine with hydrogen on the delta nitrogen). The two systems were solvated in a cubic box with a 12 Å buffer by adding 48606 TIP3P waters[iv] for E-cadh and 41527 for N-cadh. Na+ counterions were added to ensure electroneutrality.

To allow the systems to relax and release the strain due to crystal-packing effects, the two dimers were minimized keeping the complex fixed (with an harmonic potential of force constant of k=10 kcal/mol $Å^2$) and just minimizing the positions of water and ions. Then the systems were energy minimized restraining the position of waters and ions (k=10 kcal/mol $Å^2$), and finally the entire systems were energy minimized unrestrained, always by performing 2000 steps of steepest descent algorithm and using the *sander* module of AMBER11. Afterward, the temperature of the system was slowly brought to the desired value of 300 K according to the following equilibration protocol. First the systems were heated at constant volume (NVT condition, time step of 0.5 fs) at 150 K for 50 ps restraining the protein positions (k=20 kcal/mol $Å^2$). Then, the systems were equilibrated at 300 K in NVT condition for 50 ps followed by 50 ps at constant pressure (NPT condition, p= 1 bar) using restraints on protein (k=10 kcal/mol $Å^2$). Finally, 10 ps of unrestricted NVT equilibration were performed. A cut-off of 9 Å was used to compute the non-bonded interactions and Particle Mesh Ewald summation method (PME)[v] was used to deal with long-range. The Berendsen's algorithm was used to control pressure with a relaxation time of 1.0 ps[vi] and the Langevin thermostat[vii] was employed with a collision frequency of 2 ps^{-1}. SHAKE[viii] was used to constrain all the bonds involving hydrogen atoms.

For the production step, five independent MD runs of 10 ns each were performed in NPT condition using a time step of 2 fs and the *pmemd* module of AMBER11. For each run temperatures were randomly chosen on the basis of a Maxwellian distribution at 300 K, while coordinates were taken for the first run from the structure of the equilibration step and for the following ones from the final structure of the previous 10 ns run. Structures for analysis were sampled every 10 ps and each 10 ns run concatenated resulting in a trajectory of 5,000 structures.

Molecular Dynamics results. The trajectories obtained from the MD simulations were analyzed using the *ptraj* module of Amber11 package.

Root Mean Square Displacement (RMSD). To assess the stability of the dimers and the folding of each single domain, we analyzed the RMSD of the backbone atoms C_α, C, N with respect to the input structure as a function of time. Either the EC1 (1-100 residues) and EC2 (101-218 residues) domains and the EC1-EC2 monomer forming the E- and N-cadh dimers showed little fluctuations

of the backbone RMSD compared to the corresponding x-ray structure (RMSD < 2 Å for single EC1 or EC2 domains and RMSD < 3 Å in the 93% of simulation time for E-cadh EC1-EC2 monomers and in the 99% of simulation time for the N-cadh EC1-EC2 monomers), i.e. the single domains seem to conserve the input folded structure and the monomer behaves like a rather rigid unit. Major RMSD fluctuations are observed for both E- and N-cadh dimers (Fig. S2), where the RMSD oscillated between 2 and 8 Å showing a similar evolution of the corresponding dimer gyration radii (Fig. S3). In fact, since compared to the x-ray structures we truncated our system to EC1-EC2 domains, some spatial rearrangements can occur. However, these movements seem not to interfere with the swap dimer interface interactions (see discussion below).

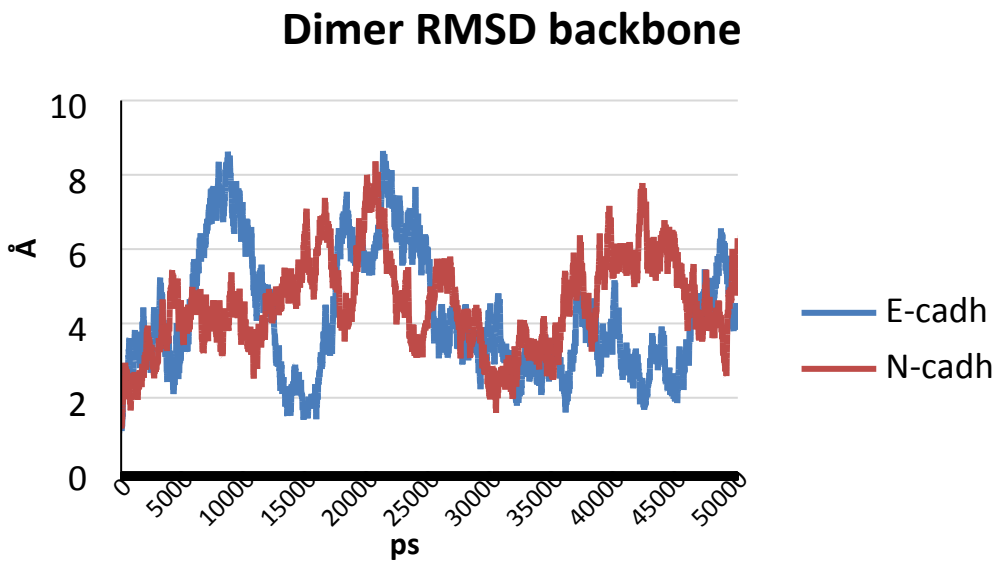


Figure S2. Time evolution of the dimer backbone (atoms C_α, C, N) RMSD for E- and N-cadh during 50ns of MD.

Radius of gyration (R_g). The input structures of E- and N-cadh have a R_g of 38.8 Å and 34.8 Å, respectively. During simulations both the R_g fluctuated (Fig. S3) showing a mean value of 37.9±0.7 Å and 37.5±0.8 Å for E- and N-cadh, respectively, in the last 20 ns of MD.

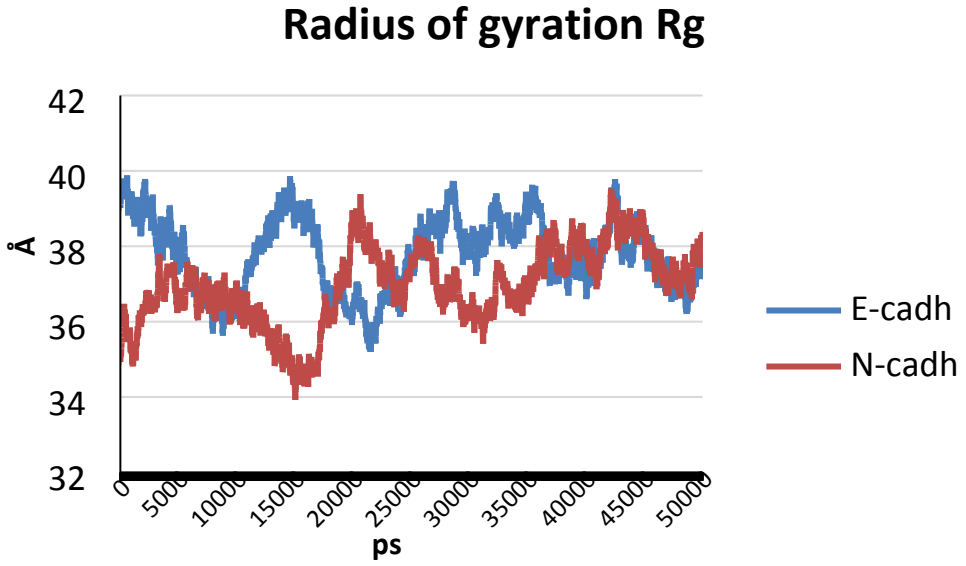


Figure S3. Time evolution of radius of gyration during the 50ns MD run of E-and N-cadh dimers.

Distance between EC1 centers of mass. In the input structure the distance between the EC1 centers of mass is about 23.0 Å and 21.5 Å for E- and N-cadh, respectively. During MD runs, the two partner molecules showed an average value of 22.5 Å and 23.4 Å for E- and N-cadh, respectively, confirming that the EC1 domains still interact each other during the simulations. These results are also supported by the analysis of the key EC1-EC1 binding features observed during simulations with respect to the crystallographic interactions (see below).
EC1-EC1 interactions. The crystallographic interactions of the EC1 swap-dimer interfaces, i.e. (i), (iii)-(v) interactions, the hydrogen bond between Asp1 and Asn27 in E-cadh and the charge-charge interaction between Asp1 and Asp27 in N-cadh described in the paper for the recognition sequence DWVI, have been monitored during the simulations. The results have been reported in Table S1 for both E- and N-cadh systems considering that each dimer has two EC1 domains interacting each other and acting as ligand, using the DWVI adhesive arm, and receptor at the same time.

Table S1. Percentage of MD structures forming the X-ray interactions of the DWVI sequence observed in E- and N-cadh swap dimers. L_A and L_B represent the DWVI sequence belonging to molecule A and B, respectively, while R_A and R_B the corresponding receptor pocket. To form the dimer, L_A interacts with R_B and L_B with R_A.

Interaction (Ligand-Receptor Residues)	N-cadh		E-cadh	
	L_A/R_B	L_B/R_A	L_A/R_B	L_B/R_A
$Asp^{1}NH_3^{+}/C^{\delta}OO^{-}Glu^{89*}$ (i)	88	98	100	100
$Trp^{2}N^{\varepsilon 1}H$--$COAsn_{N\text{-}cadh}^{90}/COAsp_{E\text{-}cadh}^{90**}$ (iii)	99	99	98	98
$Val^{3}NH$--$COArg_{N\text{-}cadh}^{25}/COLys_{E\text{-}cadh}^{25**}$ (iv)	98	96	99	99
$Asp^{1}CO$--$NHAsp_{N\text{-}cadh}^{27}/NHAsn_{E\text{-}cadh}^{27**}$ (v)	96	97	99	98
$Asp^{1}NH_3^{+}/C^{\gamma}OO^{-}Asp_{N\text{-}cadh}^{27}/C^{\gamma}OAsn_{E\text{-}cadh}^{27*}$	24	43	78	77

*distance between N and C < 4.0 Å,** distance between H and O < 2.5 Å

During MD simulations, both systems keep the input crystallographic interactions of the DWVI sequence. The charge-charge interaction $Asp^{1}NH_3^{+}/COO^{-}Asp^{27}$ of N-cadh and the corresponding hydrogen bond $Asp^{1}NH_3^{+}/COAsn^{27}$ of E-cadh showed less stability.

Docking studies
Models set up and validation. The automated docking calculations were performed using Glide (Grid-based Ligand Docking with Energetics) version 5.7109.[ix] The grids have been generated for E-cadh and N-cadh structures prepared as described in the **Proteins preparation** section and selecting as receptor the EC1 domain (1-103 residues) of one monomer and as ligand the DWVIPP esapeptide sequence of the partner monomer. The center of the grid enclosing box was defined by the center of the DWVIPP sequence. The enclosing box dimensions, which are automatically deduced from the ligand size, fit the entire active site. Docking calculations were performed using the standard precision mode (SP). The receptor was considered as a rigid body while the ligand sampling was set to 'Flexible' with the option 'Penalize non planar conformation' for amides. No Epik state penalities were used in the docking score calculations. The size of the bounding box for placing the ligand center was set to 14 Å. No further modifications were applied to the default settings. The GlideScore function was used to select 10 poses for each ligand. The Glide program was initially tested for its ability to reproduce the crystallized binding mode of fragments of the N-terminal native sequence (i.e. from the tripeptide DWV up to the decapeptide). The program was successful in reproducing the experimentally determined binding mode of these peptides, as it corresponds to the best-scored pose.

Screening of the tetrapeptide mimics. The library of DWVI peptidomimetics was evaluated in the E- and N-cadh models using the same protocol of the validation step. Ten poses for each compounds were saved and analyzed considering the Glide docking score and the x-ray reference interaction models of the DWVI sequence. In particular, we filtered the generated poses using the (i) and (ii) interactions criteria.
Among the members of the virtual library of general formula NH_3^+-Asp-scaffold-Ile-$NHCH_3$ built using the scaffolds reported in Fig. 2, peptidomimetics containing the diketopiperazine scaffolds (Fig. 2, type IV) showed the best results according to the Glide score and the number of poses reproducing the interactions (i) and (ii). Among them, we selected the most promising compounds **2** and **3** able to form the interaction (i) for at least 5 over 10 poses, and the interaction (ii) for all the poses in both E- and N-cadh models. With respect to the reference tetrapeptide sequence DWVI, **2** and **3** were also able to overlay to the backbone X-ray structure (Fig. S4).

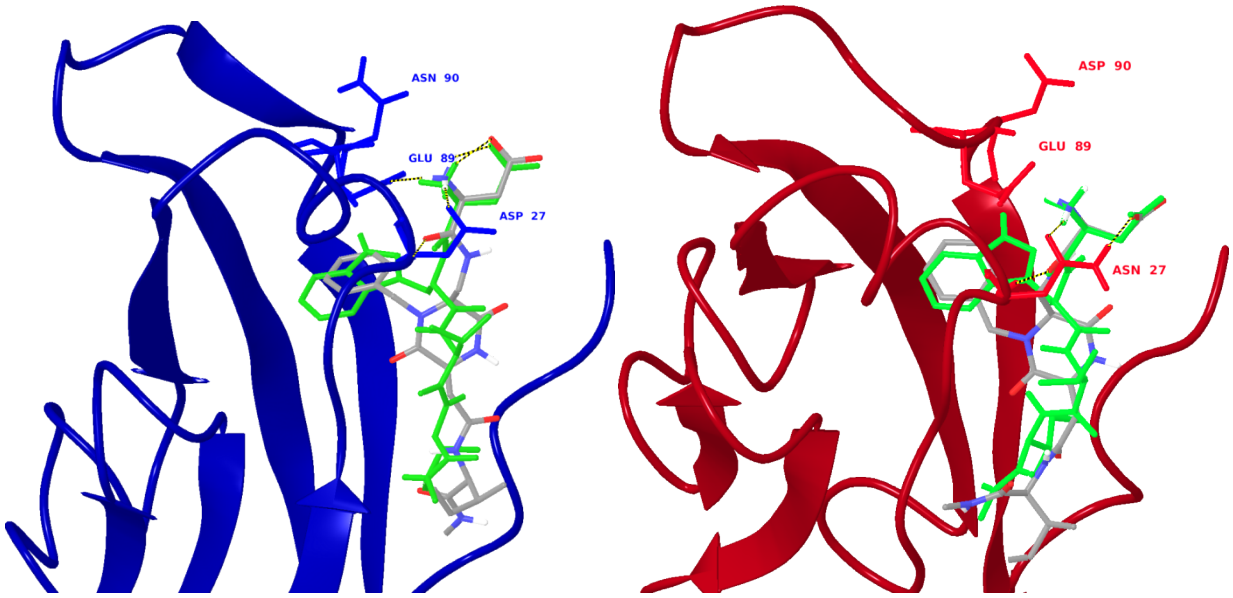


Figure S4. Best pose of **3** (tube representation, C in grey, N in blue and O in red) into the N- (left, blue ribbon representation) and E-cadh (right, red ribbon representation) models, overlaid to the DWVI sequence (green tube representation). Key receptor residues are labeled and highlighted in tube representation.

Most of the compounds including the other scaffold types failed in reproducing the interaction (i) and (ii) or they matched the pose filtering criteria just in the top-ranked pose. Only peptidomimetic **1** containing a type VI scaffold (Fig. 2) was able to form interaction (i) and (ii) for 3 poses over 10 and for 4 over 10 in the E- and N-cadh, respectively. The binding mode of **1** in both receptors (Fig. S5) showed a different disposition of the ligand compared to the DWVI sequence and no alignment to the backbone reference motif was observed.

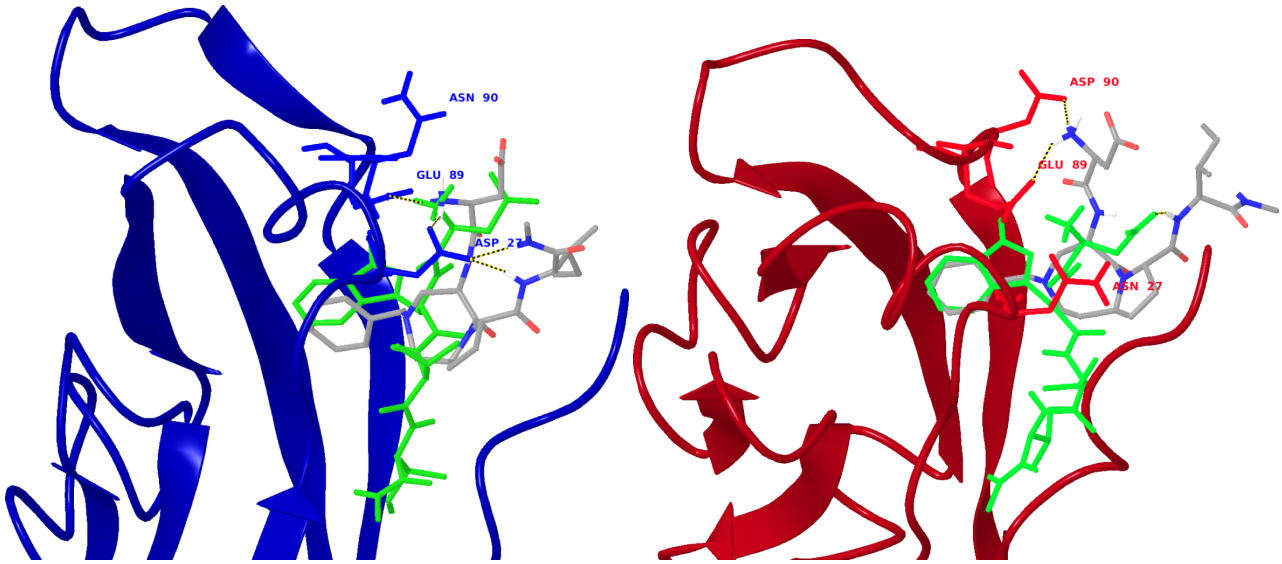
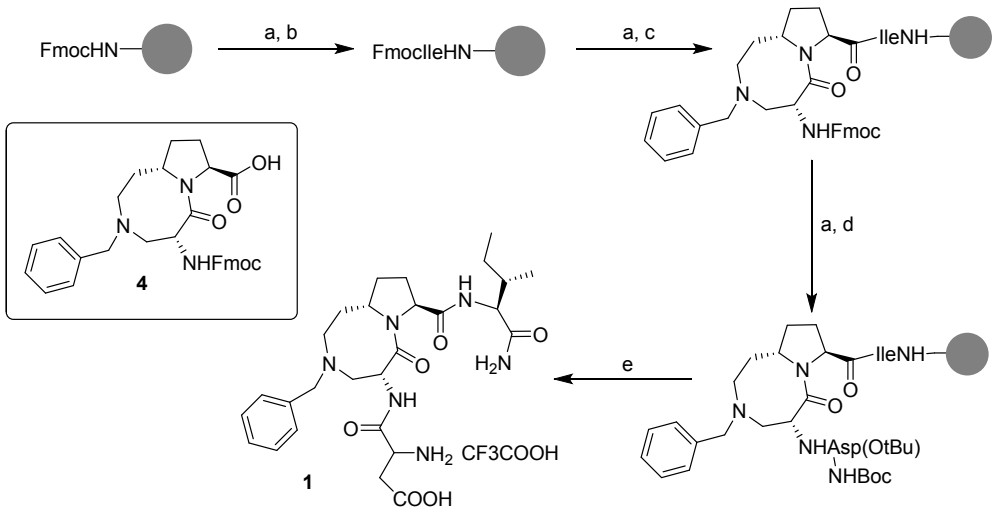


Figure S5. Best pose of **1** (tube representation, C in grey, N in blue and O in red) into the N- (left, blue ribbon representation) and E-cadh (right, red ribbon representation) models, overlaid to the DWVI sequence (green tube representation). Key receptor residues are labeled and highlighted in tube representation.

Synthesis and Characterization of Compounds

General. All chemicals and solvents were of reagent grade and were used without further purification. Solvents were dried by standard procedures and reactions requiring anhydrous conditions were performed under nitrogen atmospheres. The Fmoc protected scaffold **4** (needed for the synthesis of **1)** was prepared as shown in Scheme S1. The Cbz protected diketopiperazine scaffolds **5** and **6**[x] (needed for the synthesis of **3** and **2**, respectively) were prepared according to literature procedures and their analytical data were in agreement with those already published. Reactions were monitored by analytical thin layer chromatography using 0.25 mm pre-coated silica gel glass plates (Fluka, UV254) and compounds visualized using UV fluorescence, aqueous potassium permanganate or ninhydrin. Flash column chromatography was performed according to the method of Still and co-workers[xi] using Chromagel 60 ACC (40-63 μm) silica gel. Semi-preparative HPLC was carried out on a Waters Atlantis Prep T3 OBD 5μm, 19 x 100 mm, column; solvents: A) H_2O + 0.1% TFA, B) CH_3CN + 0.1% TFA. ^1H and ^{13}C NMR spectra were recorded at 300 K on a Bruker AVANCE-400 spectrometer. Chemical shifts δ are expressed in ppm relative to internal Me_4Si as standard. The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal, dd = doublet of doublet. High resolution mass spectra (HRMS) were performed on a Fourier Transform Ion Cyclotron Resonance (FT-ICR) Mass Spectrometer APEX II & Xmass software (Bruker Daltonics) – 4.7 T Magnet (Magnex) equipped with ESI source, available at CIGA (Centro Interdipartimentale Grandi Apparecchiature) of the Università degli Studi di Milano. HPLC-MS data were collected with an Agilent 1100 HPLC connected to a Bruker Esquire 3000+ ion trap mass spectrometer through an ES interface. All described compounds showed a purity > 98%, as determined by HPLC (UV and MS detectors). Method A: Column: Waters Atlantis 50x4.6 mm, 3 μm; phase A: Milli-Q water containing 0.05 % (v/v) TFA; phase B: Acetonitrile (LC-MS grade) containing 0.05 % TFA; flow: 1 mL/min, partitioned after UV detector (50 % to MS ESI); Temperature: 40°C; UV Detection at 206 and 220 nm with reference at 500 nm (40 nm bandwith); gradient: from 0 % B to 30 % B in 6 min; ESI+ detection in the 50-2400 m/z range with alternating MS/MS. Method B: Column: Waters Atlantis 50x4.6 mm, 3 μm; phase A: Milli-Q water containing 0.05 % (v/v) TFA; phase B: Acetonitrile (LC-MS grade) containing 0.05 % TFA; flow: 1 mL/min, partitioned after UV detector (50 % to MS ESI); Temperature: 40°C; UV Detection at 220 and 254 nm with reference at 500 nm (40 nm bandwith); gradient: from 10 % B to 90 % B in 6 min ESI+ detection in the 50-2400 m/z range with alternating MS/MS.

Synthesis of compound 1



Reagents and conditions: a) 25% Piperidine, DMF; b) Fmoc-Ile-OH, HBTU, HOBt, collidine, DMF; c) **4**, HATU, HOAt, collidine DMF; d) Boc-Asp(OtBu)-OH, HATU, HOAt, collidine DMF; e) TFA/H_2O/TES 95:2.5:2.5.

General procedure A: Fmoc deprotection

The resin was suspended in a solution of 25% piperidine in DMF (v/v) and shaken for 15 min at room temperature. The solution was removed and the resin was washed thoroughly with DMF (3 mL x 8 times)

General procedure B: Capping

A solution of Ac_2O in DMF 1:4 (v/v) (3 ml) was added to the resin. The suspension was shaken for 30 min at room temperature, then the solution was removed and the resin was washed thoroughly with DMF. Kaiser test was performed before proceeding to the next step.

General procedure C: Kaiser Test

Phenol (80% solution in ethanol) (two drops), ninhydrin (6% solution in ethanol) (two drops) and pyridine (two drops) were added to a small sample of the resin (some beads) and then heated in a boiling water bath for 1 min. If the color of the resin maintained yellow, quantitative coupling was achieved. In the case of a slight blue color of the resin, the coupling step was not fully completed and had to be repeated.

Synthesis of Fmoc-Ile-Rink Amide Resin

Rink-Amide-HBHA-Fmoc resin (210 mg, 0.1 mmol, 1 mol eq.) was de-protected according to general procedure A.

Fmoc-Ile-OH (106 mg, 0.3 mmol, 3 mol eq.), HBTU (114 mg, 0.3 mmol, 3 mol eq.), HOBt (41 mg, 0.3 mmol, 3 mol eq.) were dissolved in 1.5 mL of DMF at 0°C and collidine (80 µL, 0,6 mmol, 6 mol eq.) was added. After 10 min, the solution was added to the de-protected resin. The suspension was shaken overnight at room temperature, then the solution was removed and the resin was washed thoroughly with DMF (3 mL x 6 times).

A capping step was performed following general procedure B.

Synthesis of Fmoc-Daba-Ile-Rink Amide Resin

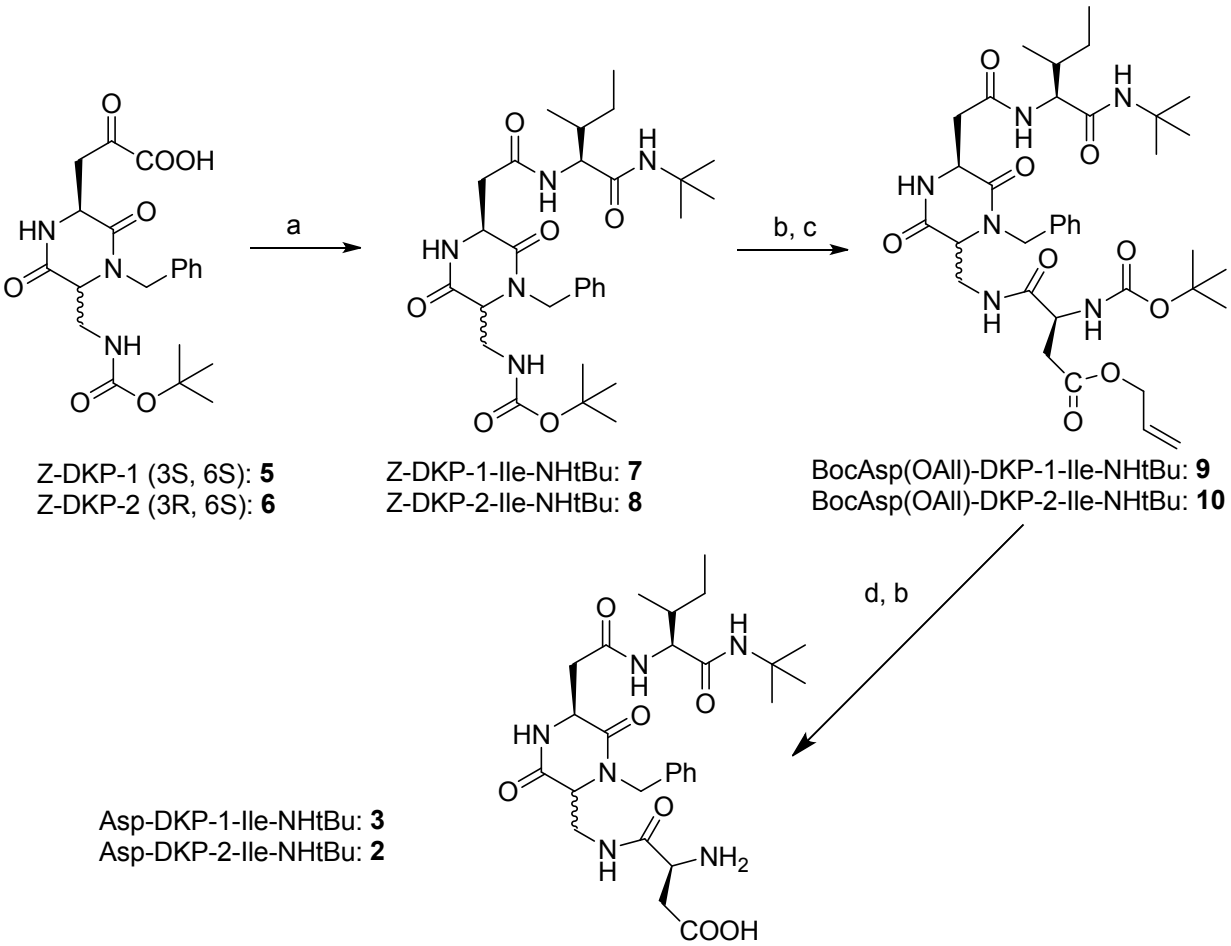
The Fmoc-Ile-Rink Amide Resin (0.1 mmol, 1 mol eq.) was deprotected according to general procedure A.

To Fmoc protected diazabicycloalkane amino acid (Daba) **4** (65 mg, 0.12 mmol, 1.2 mol eq.), HATU (76 mg, 0.2 mmol, 2 mol eq.), HOAt (27 mg, 0.2 mmol, 2 mol eq.) were dissolved in DMF (1.5 mL) at 0°C and collidine (26 µL, 0.2 mmol, 2 mol eq.) was added. After 10 min, the solution was added to the resin and the suspension was shaken overnight at room temperature. After this time the solution was removed and the resin was washed thoroughly with DMF (3 mL x 6 times).
A capping step was performed following general procedure B.

Synthesis of Asp-Daba-Ile: compound 1

The Fmoc-Daba-Ile-Rink Amide Resin (0.1 mmol, 1 mol eq.) was deprotected according to general procedure A.
To Boc-Asp(OtBu)-OH (87 mg, 0.3 mmol, 3 mol eq.), HATU (114 mg, 0.3 mmol, 3 mol eq.), HOAt (41 mg, 0.3 mmol, 3 mol eq.) were dissolved in DMF (1.5 mL) at 0°C. Collidine (52 µL, 0.4 mmol, 4 mol eq.) was added and the solution was stirred for 10 min at 0°C. The solution was added to the resin and the suspension was shaken overnight at room temperature. After this time the solution was removed (drained) and the resin was washed thoroughly with DMF (3 mL x 6 times). The resin was then washed with DCM (3mL) and MeOH (3mL). This sequence of washing was repeated 3 times. Then the resin was washed with DCM (3 mL x 5 times) and dried under vacuum.
Finally the dried resin was suspended in a TFA/H_2O/TES 95:2.5:2.5 mixture (4 mL) and shaken for 3 h at room temperature. After this time, the solvent was collected and partially evaporated under reduced pressure, then Et_2O was added and the crude product was collected after centrifugation. The desired compound was obtained after HPLC purification (Waters Atlantis Prep T3 OBD 5µm, 19 x 100 mm, column; solvents: A) H_2O + 0.1% TFA, B) CH_3CN + 0.1% TFA gradient from 100%A-0%B to 70%A-30%B over 15 min; flow rate 15 mL/min, λ = 210 nm (50 mg, 76%).
R_t=4.5 min (HPLC-MS, Method A). ^{1}H-NMR (400 MHz, D_2O) δ: 7.58 (m, 5H), 5.44 (dd, 1H, J = 11.5 Hz, J = 4.5 Hz), 4.65 (m, 1H), 4.62 (d, 1H, J = 9.1 Hz), 4.50 (m, 2H), 4.42 (t, 1H, J = 6.2 Hz), 4.10 (d, 1H, J = 8.0 Hz), 3.77 (m, 1H), 3.65 (m, 1H), 3.52 (dd, 1H, J = 12.5 Hz, J = 4.6 Hz), 3.41 (t, 1H, J = 12.2 Hz), 3.07 (m, 2H), 2.41 (m, 1H), 2.29 (m, 1H), 2.13-1.98 (m, 3H), 1.93-1.79 (m, 2H), 1.65 (m, 1H), 1.24 (m, 1H), 0.97 (d, 3H, J = 6.8 Hz), 0.91 (t, 3H, J = 7.4 Hz). ^{13}C-NMR (100.6 MHz, D_2O) δ: 176.3, 173.4, 172.7, 168.3, 168.0, 131.7, 130.7, 129.5, 128.2, 63.4, 61.0, 58.4, 58.1, 55.4, 52.9, 49.6, 47.9, 35.8, 35.0, 33.3, 31.0, 27.2, 24.8, 14.9, 10.5. HRMS (ESI) *m/z* calc. for $[C_{27}H_{41}N_6O_6]^+$: 545.3082; found: 545.30786 $[M+H]^+$.

Synthesis of compounds 2 and 3



Reagents and conditions: a) Ile-CONHtBu, HOAt, HATU, DIPEA, CH_2Cl_2, 63-82%; b) TFA/CH_2Cl_2 1:2; c) Boc-Asp(OAll)-OH, HOAt, HATU, DIPEA, CH_2Cl_2, 78%; d) pyrrolidine,PPh_3, [$Pd(PPh_3)_4$], CH_2Cl_2, (**2**, 74%; **3**, 99%).

General procedure D: deprotection reaction. To a solution of the N-Boc-protected amino acid or peptide in CH_2Cl_2 (0.13 M) was added half volume of TFA and the reaction was stirred at room temperature for 2 h. The solvent was evaporated, toluene (2×) was added followed by evaporation, and then ether was added and evaporated to afford the corresponding TFA salt.

Synthesis of BocIle-CONHtBu

To a solution of N-Boc-L-isoleucine (450 mg, 1.95 mmol, 1 eq.) in DMF (15 ml) at 0°C, HOBt (277 mg, 2.05 mmol, 1.05 eq.) and EDC·HCl (397 mg, 2.05 mmol, 1.05 eq.) were added in one portion. After stirring the mixture for 30 min, *tert* butylamine was added and the mixture stirred at 0°C for 1 h, then warmed up to room temperature and stirred overnight. DMF was removed under reduced pressure and the resulting mixture was diluted with EtOAc (60 ml) and washed with 1 M $KHSO_4$ (2x30 ml), aqueous $NaHCO_3$ (2x30 ml) and brine (2x30 ml), dried over Na_2SO_4, and volatiles were removed under reduced pressure. The residue was purified by flash chromatography on silica gel (Hexane/EtOAc, 8:2) affording the desired product (503 mg, 90%) as a white solid.
R_f=0.34 (Hexane/EtOAc 80:20); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.14 (br s, 1H), 5.31 (d, J = 6.8 Hz, 1H), 3.78–3.74 (m, 1H), 1.79–1.65 (m, 1H), 1.54–1.40 (m, 1H), 1.38 (s, 9H), 1.28 (s, 9H), 1.15–0.97 (m, 1H), 0.86 (m, 6H). ^{13}C NMR (101 MHz, CDCl3) δ 171.1, 155.9, 79.4, 59.6, 51.2, 37.1, 28.6, 28.2, 24.8, 15.4, 11.1. IR (KBr): ν_{max} 3080, 2968, 2935, 2878, 1688, 1655, 1454, 1364, 1178. MS (ESI) *m/z* calcd for $[C_{15}H_{30}N_2NaO_3]^+$: 309.2; found: 309.2 $[M+Na]^+$.

Synthesis of compound 8

Compound **6** (76 mg, 0.195 mmol, 1 eq.) was dissolved in DMF (2.5 ml) under nitrogen atmosphere, and at 0 ºC, HATU (82 mg, 0.215 mmol, 1.1 eq.), HOAt (30 mg, 0.215 mmol, 1.1 eq.) and DIPEA (0.134 ml, 0.78 mmol, 4 eq.) were added. After 30 min, a solution in DMF of the TFA salt of Ile-CONHtBu prepared according to general procedure D, was added and the reaction mixture was stirred at 0 ºC for 1 h and at room temperature overnight. The mixture was then diluted with EtOAc (30 ml) and washed with 1 M $KHSO_4$ (2x10 ml), aqueous $NaHCO_3$ (2x10 ml) and brine (2x10 ml), dried over Na_2SO_4. Volatiles were removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 97:3) affording the desired product (89 mg, 82%) as a white solid. R_f=0.34 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.38 – 7.28 (m, 6H), 6.69 (d, J = 7.8 Hz, 1H), 5.99 (br s, 1H), 5.40 (d, J = 15.3 Hz, 1H), 4.47 (dd, J = 8.6, 4.0 Hz, 1H), 4.18 (t, J = 7.9 Hz, 1H), 4.09 (d, J = 14.9 Hz, 1H), 3.79 (br s, 1H), 3.76–3.69 (m, 1H), 3.53–3.47 (m, 1H), 3.13 (dd, J = 15.3, 2.3 Hz, 1H), 2.69 (dd, J = 15.7, 8.5 Hz, 1H), 1.82 (br s, 1H), 1.54–1.46 (m, 4H), 1.43 (s, 9H), 1.35 (s, 9H), 1.19–1.07 (m, 1H), 0.92–0.89 (m, 6H). ^{13}C NMR (101 MHz, CD_2Cl_2) δ 169.9, 166.9, 165.8, 155.9, 135.9, 128.8, 128.1, 127.7, 79.8, 59.7, 58.1, 51.4, 51.3, 47.2, 40.7, 38.4, 37.6, 29.6, 28.4, 28.0, 25.0, 15.1, 11.1. IR (neat): ν_{max} 3054, 2685, 2291, 1684, 1653, 1367. MS (ESI) *m/z* calcd for $[C_{29}H_{45}N_5NaO_6]^+$: 582.33; found: 582.6 $[M+Na]^+$.

Synthesis of compound 10

Compound **8** was deprotected according to general procedure D and used without further purification. To a solution of *β*-allyl (2*S*)-*N*-(tert-butoxycarbonyl) aspartate ester (51 mg, 0.186 mmol, 2 eq.) in DMF (1 ml) under nitrogen atmosphere and at 0 ºC, HATU (82 mg, 0.215 mmol, 1.1 eq.), HOAt (30 mg, 0.215 mmol, 1.1 eq.) and DIPEA (0.134 ml, 0.78 mmol, 4 eq.) were added. After 30 min, a solution in DMF of the TFA salt of compound **8** (54 mg, 0.093 mmol, 1 eq.), was added and the reaction mixture was stirred at 0 ºC for 1 h and at room temperature overnight. The mixture was then diluted with EtOAc (30 ml) and washed with 1 M $KHSO_4$ (2x10 ml), aqueous $NaHCO_3$ (2x10 ml) and brine (2x10 ml), dried over Na_2SO_4. Volatiles were removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 97:3) affording the desired product (52 mg, 78%) as a white solid. R_f=0.52 (CH_2Cl_2/MeOH 9:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (br s, 1H), 7.62 (d, J = 19.5 Hz, 1H), 7.35–7.23 (m, 5H), 7.06 (br s, 1H), 6.33 (br s, 1H), 5.92–5.87 (m, 2H), 5.39 (d, J = 15.2 Hz, 6H), 5.31–5.19 (m, 2H), 4.61–4.56 (m, 4H), 4.22–4.16 (m, 3H), 3.94–3.86 (m, 1H), 3.84 (br s, 1H), 3.60 (m, 1H), 3.08 (d, J = 14.3 Hz, 7H), 2.93–2.67 (m, 3H), 1.84 (br s, 1H), 1.58–1.42 (m, 1H), 1.40 (s, 9H), 1.34 (s, 9H), 1.16–1.06 (m, 1H), 0.95–0.85 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 170.9, 170.6, 167.3, 165.9, 155.6, 135.6, 131.8, 128.9, 128.4, 127.9, 118.5, 80.4, 65.7, 58.9, 58.5, 51.9, 51.2, 50.8, 47.3, 39.5, 38.4, 37.3, 36.5, 29.7, 28.6, 28.3, 25.1, 15.4, 11.3. IR (neat): ν_{max} 3426, 3052, 2982, 2930, 2682, 2304, 1726, 1678, 1513, 1447, 1260. MS (ESI) *m/z* calcd for $[C_{36}H_{54}N_6NaO_9]^+$: 737.4; found: 737.6 $[M+Na]^+$

Synthesis of compound 2

To a solution of compound **10** (50 mg, 0.07 mmol, 1 eq.) in CH_2Cl_2 (0.6 ml), under nitrogen atmosphere and at 0 ºC, pyrrolidine (7 µl, 0.084 mmol, 1.2 eq), PPh_3 (3.3 mg, 0.013 mmol, 0.18 eq) and then $[Pd(PPh_3)_4]$ (3.2 mg, 0.003 mmol, 0.04 eq) were added. After stirring for 1 h at 0 ºC, and 15 min at room temperature EtOAc (10 ml) was added and the solution was extracted with aqueous $NaHCO_3$ (4x 5 ml). The combined aqueous phases were acidified to pH 2 with a 1 M $KHSO_4$ solution and then extracted with CH_2Cl_2. The resulting organic phase was dried over Na_2SO_4 and the solvent evaporated to afford the desired product as a fluffy white solid (35 mg, 74%) that was deprotected according to general procedure D to give the crude compound that was purified by HPLC (Waters Atlantis Prep T3 OBD 5µm, 19 x 100 mm, column; solvents: A) H_2O + 0.1% TFA,

B) CH_3CN + 0.1% TFA gradient from 90%A-10%B to 30%A-70% B over 15 min; flow rate 15 mL/min, λ = 210 nm). (46 mg, quantitative yield).
R_t=3.5 min (HPLC-MS, Method B). ^{1}H NMR (400 MHz, CD_3OD) δ 8.04 (d, *J* = 9.2 Hz, 1H), 7.50 (br s, 1H), 7.32 (m, 5H), 5.31 (d, *J* = 15.2 Hz, 1H), 4.56 (t, *J* = 5.4 Hz, 1H), 4.22–4.13 (m, 3H), 3.89–3.83 (m, 2H), 3.68 (d, *J* = 11.9 Hz, 1H), 3.06–2.74 (m, 4H), 1.90 (m, 1H), 1.50 (m, 1H), 1.33 (s, 9H), 1.25–1.10 (m, 1H), 0.98–0.87 (m, 6H). ^{13}C NMR (101 MHz, CD_3OD) δ 171.6, 170.6, 168.5, 167.5, 167.4, 135.7, 128.5, 127.7, 127.5, 58.8, 58.2, 53.4, 50.9, 50.8, 39.0, 36.8, 36.5, 29.3, 27.5, 24.3, 14.6, 10.4; HRMS (ESI) *m/z* calc. for $[C_{28}H_{43}N_6O_7]^+$: 575.3188; found: 575.31830 $[M+H]^+$

Synthesis of compound 7
Compound **5** (145 mg, 0.37 mmol, 1 eq.) was dissolved in DMF (5 ml) under nitrogen atmosphere and at 0 ºC, HATU (155 mg, 0.407 mmol, 1.1 eq.), HOAt (56 mg, 0.407 mmol, 1.1 eq.) and DIPEA (0.254 ml, 1.48 mmol, 4 eq.) were added. After 30 min, a solution in DMF of the TFA salt of Ile-CONHtBu prepared according to general procedure D, was added and the reaction mixture was stirred at 0 ºC for 1 h and at room temperature overnight. The mixture was then diluted with EtOAc (30 ml) and washed with 1 M $KHSO_4$ (2x10 ml), aqueous $NaHCO_3$ (2x10 ml) and brine (2x10 ml), and dried over Na_2SO_4. Volatiles were removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 97:3) affording the desired product (130 mg, 63%) as a white solid. R_f=0.34 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.55 (s, 1H), 7.44–7.22 (m, 5H), 7.08 (s, 1H), 6.25 (s, 1H), 5.76 (s, 1H), 5.47 (d, *J* = 15.4 Hz, 1H), 5.35–5.34 (m, 1H), 4.47 (d, *J* = 9.1 Hz, 1H), 4.24 (t, *J* = 7.8 Hz, 1H), 4.10 (d, *J* = 14.4 Hz, 1H), 3.86–3.80 (m, 1H), 3.76–3.68 (m, 1H), 3.57 (s, 1H), 3.13 (d, *J* = 16.0 Hz, 1H), 2.81 (dd, *J* = 15.8, 9.5 Hz, 1H), 1.84–1.76 (m, 1H), 1.56–1.48 (m, 1H), 1.46 (s, 9H), 1.33 (s, 9H), 1.20–1.07 (m, 1H), 0.95–0.86 (m, 6H). ^{13}C NMR (101 MHz, CD_2Cl_2) δ 170.3, 170.1, 165.8, 165.3, 155.8, 135.7, 128.8, 128.3, 127.8, 79.7, 58.7, 58.2, 51.5, 46.9, 40.7, 37.4, 28.3, 28.2, 25.1, 15.1, 11.0; IR (neat): v_{max} 3054, 2986, 2312, 1690, 1663, 1265. MS (ESI) *m/z* calcd for $[C_{29}H_{45}N_5NaO_6]^+$: 582.33; found: 582.18 $[M+Na]^+$

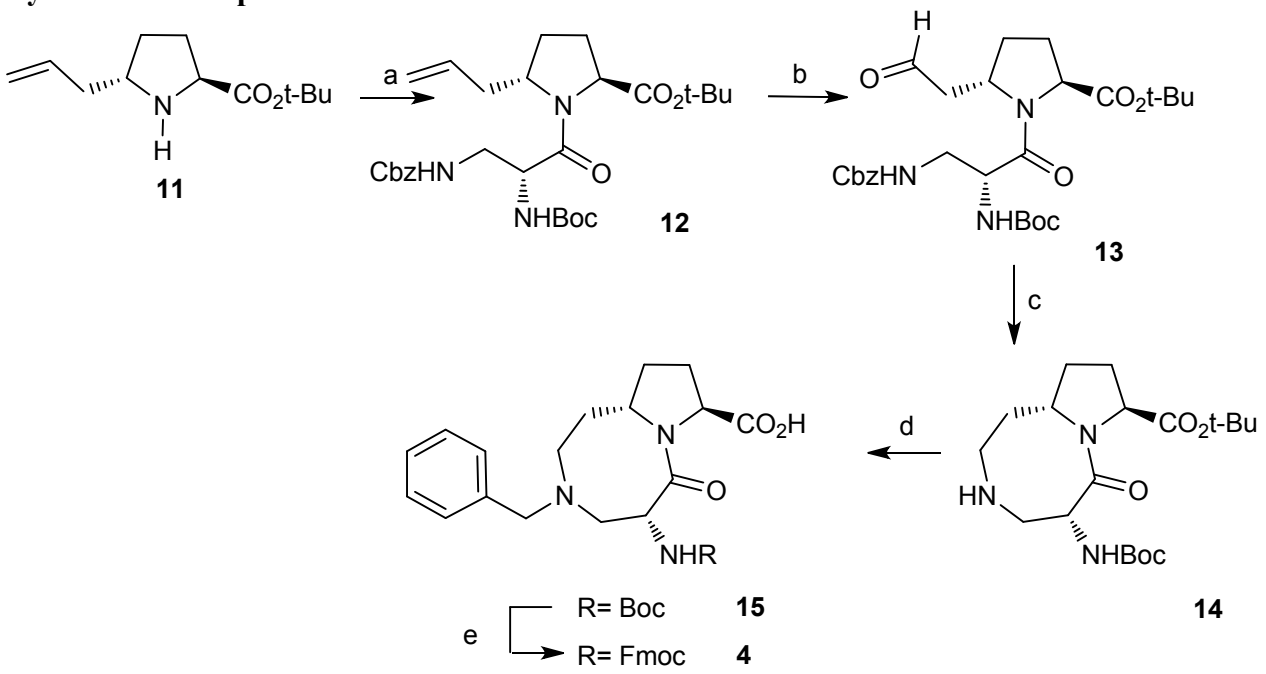
Synthesis of compound 9
Compound **7** was deprotected according to general procedure D and used without further purification. To a solution of *β*-allyl (2*S*)-*N*-(tert-butoxycarbonyl) aspartate ester (76.5 mg, 0.28 mmol, 2 eq.) in DMF (2 ml) under nitrogen atmosphere and at 0 ºC, HATU (112 mg, 0.294 mmol, 2.1 eq.), HOAt (40 mg, 0.294 mmol, 2.1 eq.) and DIPEA (72µl, 0.42 mmol, 3 eq.) were added. After 30 min, a solution in DMF of the TFA salt of **7** (80 mg, 0.143 mmol, 1 eq.), was added and the reaction mixture was stirred at 0 ºC for 1 h and at room temperature overnight. The mixture was then diluted with EtOAc (30 ml) and washed with 1 M $KHSO_4$ (2x10 ml), aqueous $NaHCO_3$ (2x10 ml) and brine (2x10 ml), and dried over Na_2SO_4. Volatiles were removed under reduced pressure. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 90:10) affording the desired product (80 mg, 78%) as a white solid.
R_f=0.47 (CH_2Cl_2/MeOH 9:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.85 (s, 1H), 7.66 (s, 1H), 7.36–7.14 (m, 5H), 6.97 (s, 1H), 5.95 – 5.85 (m, 1H), 5.79 (d, *J* = 8.8 Hz, 1H), 5.52 (d, *J* = 14.8 Hz, 1H), 5.31 (dd, *J* = 10.4, 1.2 Hz, 1H), 5.22 (dd, *J* = 10.4, 1.2 Hz, 1H), 4.85 (s, 1H), 4.59 (d, *J* = 5.6 Hz, 2H), 4.40–4.33 (m, 2H), 3.94–3.85 (m, 4H), 3.69 (d, *J* = 13.5 Hz, 1H), 3.20 (dd, *J* = 15.7, 3.7 Hz, 1H), 3.06 (dd, *J* = 14.4, 3.0 Hz, 1H), 2.89–2.67 (m, 2H), 1.86–1.72 (m, 1H), 1.63–1.47 (m, 1H), 1.42 (s, 9H), 1.31 (s, 9H), 1.20–1.11 (m, 1H), 0.95–0.85 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.44, 170.67, 170.36, 165.51, 165.39, 155.65, 135.08, 131.83, 128.92, 128.43, 128.06, 118.50, 80.23, 65.67, 58.23, 56.45, 52.33, 52.00, 50.75, 46.33, 39.13, 38.99, 38.03, 37.27, 28.53, 28.36, 25.14, 15.11, 10.88. IR (neat): v_{max} 3281, 3088, 2960, 2927, 2866, 2357, 1733, 1650, 1540, 1445, 1363. MS (ESI) *m/z* calcd for $[C_{36}H_{54}N_6NaO_9]^+$: 737.38; found: 737.8 $[M+Na]^+$

Synthesis of compound 3

To a solution of Compound **9** (50 mg, 0.07 mmol, 1 eq.) in CH_2Cl_2 (0.6 ml), under nitrogen atmosphere and at 0 ºC, pyrrolidine (7 µl, 0.084 mmol, 1.2 eq), PPh_3 (3.3 mg, 0.013 mmol, 0.18 eq) and then [$Pd(PPh_3)_4$] (3.2 mg, 0.003 mmol, 0.04 eq) were added. After stirring for 1 h at 0 ºC, and 15 min at room temperature EtOAc (10 ml) was added and the solution was extracted with aqueous $NaHCO_3$ (4x 5 ml). The combined aqueous phases were acidified to pH 2 with a 1 M $KHSO_4$ solution and then extracted with CH_2Cl_2. The resulting organic phase was dried over Na_2SO_4 and the solvent evaporated to afford the desired product as a white solid (45 mg, 95%) that was deprotected according to general procedure D to give the crude compound that was purified by HPLC (Waters Atlantis Prep T3 OBD 5µm, 19 x 100 mm, column; solvents: A) H_2O + 0.1% TFA, B) CH_3CN + 0.1% TFA gradient from 90%A-10%B to 30%A-70% B over 15 min; flow rate 15 mL/min, λ = 210 nm). (36 mg, quantitative yield).

R_t=3.0 min (HPLC-MS, Method B).^{1}H NMR (400 MHz, CD_3OD) δ 7.39–7.29 (m, 5H), 4.78 (d, *J* = 15.2 Hz, 1H), 4.67 (d, *J* = 15.2 Hz, 1H), 4.42 (t, *J* = 4.0 Hz, 1H), 4.23–4.19 (m, 2H), 4.08 (d, *J* = 8.1 Hz, 1H), 3.87 (dd, *J* = 14.2, 2.9 Hz, 1H), 3.63 (dd, *J* = 14.1, 5.6 Hz, 1H), 3.16 (dd, *J* = 16.6, 4.9 Hz, 1H), 2.87 (dd, *J* = 16.6, 4.4 Hz, 1H), 2.81 (dd, *J* = 18.2, 3.4 Hz, 1H), 2.66 (dd, *J* = 18.2, 10.1 Hz, 1H), 1.88–1.72 (m, 1H), 1.64–1.50 (m, 1H), 1.36 (s, 9H), 1.25–1.14 (m, 1H), 0.98–0.92 (m, 6H). ^{13}C NMR (101 MHz, CD_3OD) δ 171.9, 171.3, 170.2, 167.7, 167.1, 165.8, 136.7, 128.4, 127.8, 127.4, 58.5, 58.2, 51.6, 51.0, 49.5, 40.5, 37.6, 37.3, 34.9, 27.4, 24.6, 14.4, 10.1. HRMS (ESI) *m/z* calc. for $[C_{28}H_{43}N_6O_7]^+$: 575.3188; found: 575.31830 $[M+H]^+$

Synthesis of compound 4



Scheme S1. a) Boc-D-Dap(Z)-OH, isobutylchloroformate, 4-methylmorfoline, THF, -30 °C, 78%; b) $OsCl_3$, Me_3NO, DCM, then $NaIO_4$, diossane:H_2O 4:1, 96%; c) H_2, Pd/C, THF:H_2O 4:1, 66%; d) benzyl bromide, DIPEA, CH_3CN, 85%; e) TFA, DCM then Fmoc-OSu, Na_2CO_3 aq, THF, 97% over two steps.

Synthesis of compound 12. To a solution of Boc-D-Dap(Z)-OH (430 mg, 1.27 mmol) in dry THF (8 ml), at -30°C and under nitrogen atmosphere, 4-Methylmorpholine (140 µl, 1.27 mmol) was added. After 10 min isobutylchloroformate (160 µl, 1.27 mmol) was added and the reaction mixture was stirred for further 10 min. at -30°C, then a solution of the allylproline **11** (322 mg, 1.524 mmol) in dry THF was added by canula. The temperature was allowed to warm up gradually to room temperature and the reaction mixture was stirred for 12 h. After reaction completion (TLC DCM:MeOH 9:1) a 0.1 M HCl solution was added. The organic phase was washed with 0.1 M HCl, a saturated solution of $NaHCO_3$, and brine. The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude was purified by flash chromatography (hexane: EtOAc from 8:2 to 6:4) affording **12** (525 mg, 78%) as white foam.

^{1}H-NMR (400 MHz, $CDCl_3$) (1:1 mixture of conformers): δ 1.42-1.45 (m, 18H, Boc, tBu), 1.73 (m, 0.5H, H4 conformer A), 1.82 (m, 1H, H3 conformer B, H4 conformer A), 1.87 (m, 0.5 H, H4 conformer B), 2.07 (m, 1H, H3 conformer A, H3 conformer B), 2.14 (m, 0.5H, H6 conformer A), 2.17 (m, 1H, H6 conformer B, H4 conformer B), 2.31 (m, 0.5H, H3 conformer A), 2.35 (m, 0.5H, H6 conformer B), 2.48 (m, 0.5H, H6 conformer A), 3.40 (m, 1H, H_β conformer A, H_β conformer B), 3.55 (m, 1H, H_β conformer A, H_β conformer B), 4.15 (m, 0.5H, Hα one conformer), 4.24 (m, 0.5H, H5 conformer A), 4.26 (m, 0.5H, H2 conformer B), 4.38 (m, 0.5H, H5 conformer B), 4.69 (m, 0.5H, Hα other conformer), 4.86 (m, 0.5H, H2 conformer A), 5.05 (m, 1H, H8 conformer A), 5.09 (s, 2H, Cbz), 5.12 (m, 1H, H8 conformer B), 5.13 (m, 0.5H, NHBoc one conformer), 5.24 (bd, 0.5H, NHBoc other conformer), 5.48 (m, 0.5H, NHCbz one conformer), 5.66 (m, 0.5H, NHCbz other conformer), 5.68-5.79 (m, 1H, H7 conformer A, H7 conformer B), 7.35 (m, 5H, aromatics). ^{13}C-NMR (100.6 MHz, $CDCl_3$) (1:1 mixture of conformers): δ 172.0, 171.0, 170.7, 170.1, 156.9, 156.6, 155.6, 155.4, 136.7, 134.8, 133.8, 128.6, 128.2, 128.1, 118.9, 117.6, 82.7, 81.3, 80.3, 66.9, 66.8, 60.9, 60.5, 57.9, 57.8, 50.9, 50.7, 43.4, 42.1, 40.3, 36.6, 29.3, 28.4, 28.3, 28.1, 28.0, 26.8, 26.5. MS (ESI) found *m/z* 554.3 $[M + Na]^+$ (calcd. for $C_{28}H_{41}N_3O_7$: 531.29).

Synthesis of compound 13. To a solution of **12** (520 mg, 0.978 mmol) in aqueous DCM (10 ml), $OsCl_3$ (0.029 mg, 0.097 mmol), and trimethylamine N-oxide (217 mg, 1.956 mmol) were added.

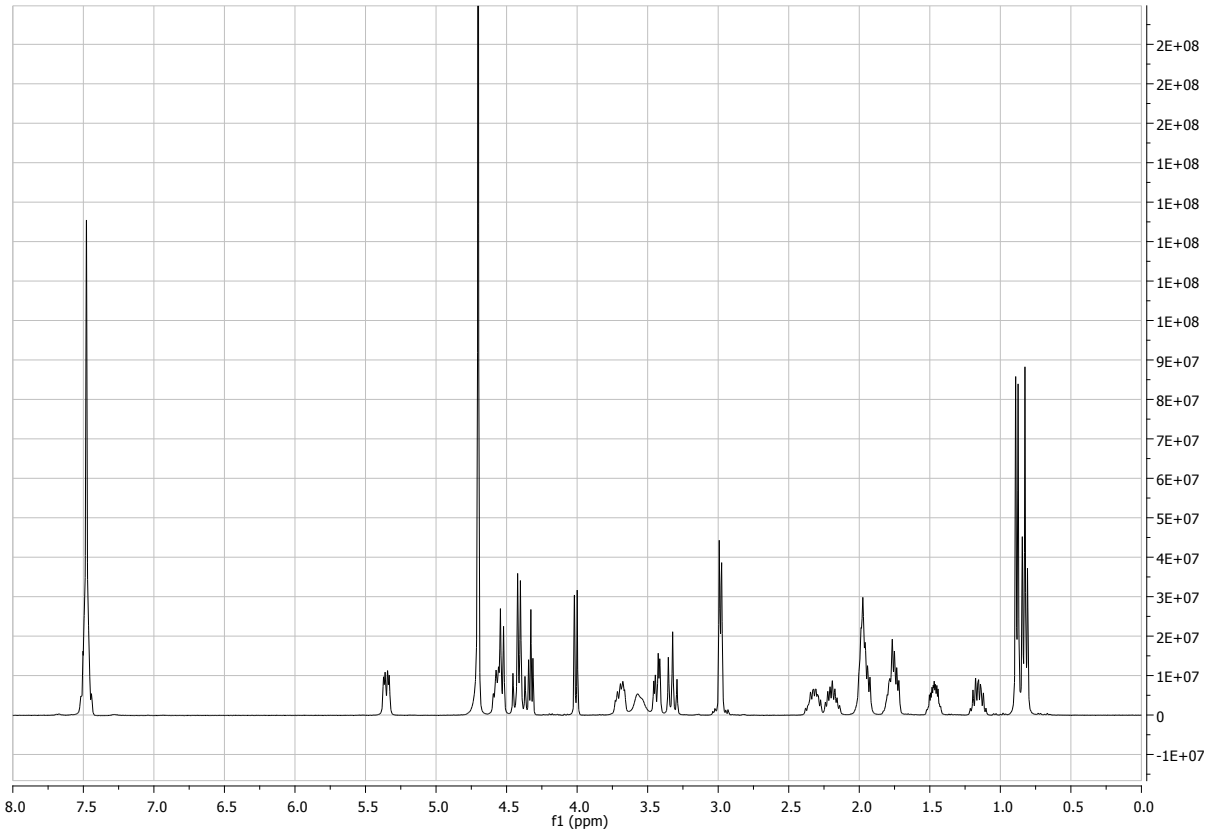
The reaction mixture was stirred for 12 h. After reaction completion (TLC DCM:MeOH 9:1) Na_2SO_3 and water were added. The organic phase was extracted with DCM, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude was dissolved in dioxane:water 4:1 (10 ml) and $NaIO_4$ (523 mg, 2.445 mmol) was added. After 2 h., the reaction was completed (TLC DCM:MeOH 9:1) and the solvent was evaporated under reduced pressure. The residue was rinsed with EtOAc and washed with brine. The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude was purified by flash chromatography ($CHCl_3$:MeOH 98:2) affording the pure product (502 mg, 96%) as white foam.
^{1}H-NMR (400 MHz, $CDCl_3$) (2.6:1 mixture of conformers): δ 1.37-1.47 (m, 18H, Boc, tBu), 1.65 (m, 0.72H, H4 conformer A), 1.73 (m, 0.56H, H4 conformer B), 1.94 (m, 0.28H, H3 conformer B), 2.02 (m, 0.72H, H4 conformer A), 2.11 (m, 0.28H, H3 conformer B), 2.16 (m, 0.72H, H3 conformer A), 2.28 (m, 0.72H, H3 conformer A), 2.40 (m, 0.72H, H6 conformer A), 2.51 (m, 0.28H, H6 conformer B), 2.92 (m, 1H, H6 conformer A, H6 conformer B), 3.34 (m, 0.28H, H7 conformer B), 3.41 (m, 0.72H, H7 conformer A), 3.48 (m, 0.28H, H7 conformer B), 3.55 (m, 0.72H, H7 conformer A), 4.12 (m, 0.72H, H8 conformer A), 4.24 (m, 0.28H, H2 conformer B), 4.60 (m, 0.72H, H5 conformer A), 4.67 (m, 0.28H, H8 conformer B), 4.80 (m, 0.28H, H5 conformer B), 4.87 (m, 0.72H, H2 conformer A), 5.06-5.11 (m, 2H, H9), 5.19 (m, 0.28H, NHBoc conformer B), 5.30 (m, 0.72H, NHBoc conformer A), 5.48 (m, 0.28H, NHCbz conformer B), 5.55 (m, 0.72H, NHCbz conformer A), 7.27-7.38 (m, 5H, aromatics), 9.70 (s, 1H, CHO). ^{13}C-NMR (75.5 MHz, $CDCl_3$) (2.6:1 mixture of conformers): δ 199.9, 199.2, 171.4, 171.1, 170.3, 169.7, 156.8, 155.6, 136.5, 128.5, 128.0, 82.9, 81.4, 80.3, 66.7, 60.5, 53.3, 52.7, 51.1, 49.1, 47.3, 43.3, 41.8, 29.7, 29.1, 28.2, 27.9, 26.4. MS (ESI) found *m/z* 556.4 $[M + Na]^+$ (calcd. for $C_{27}H_{39}N_3O_8$: 533.27).

Synthesis of compound 14. To a solution of the aldehyde (502 mg, 1.31 mmol) in THF:water 4:1 (195 ml), Pd/C 10% (10% w/w) was added. The reaction mixture was stirred under hydrogen atmosphere. After 4 h. the reaction was completed (TLC DCM:MeOH 98:2), and the mixture was filtered on a pad of celite. The solvent was evaporated under reduced pressure and the crude was purified by flash chromatography ($CHCl_3$:MeOH from 98:2 to 95:5) affording the pure product (238 mg, 66%) as white foam.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.38-1.45 (m, 18H, Boc, tBu), 1.59 (m, 2H, H7), 1.65 (m, 1H, H9), 1.93 (m, 1H, H10), 2.19-2.36 (m, 2H, H9, H10), 2.66 (m, 1H, H4), 2.90 (m, 2H, H6), 3.24 (m, 1H, H4), 4.31 (m, 1H, H11), 4.36 (m, 1H, H8), 4.64 (m, 1H, H3), 5.56 (m, 1H, NHBoc). ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 170.8, 155.0, 129.7, 123.2, 118.9, 81.2, 79.5, 60.0, 57.6, 54.5, 53.2, 46.4, 39.2, 31.1, 29.7, 28.4, 27.9, 27.1. MS (ESI) found *m/z* 406.2 $[M + Na]^+$ (calcd. for $C_{19}H_{33}N_3O_5$: 383.24)

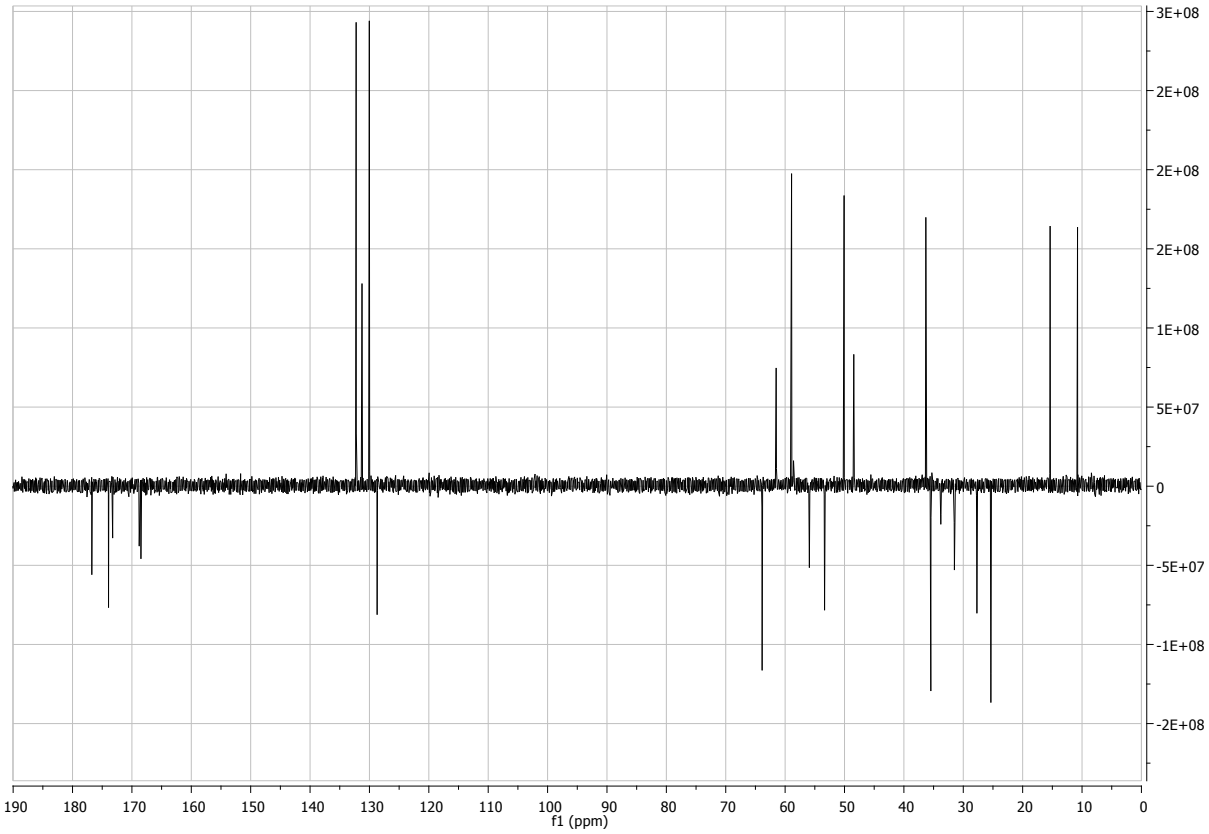
Synthesis of compound 15. To a solution of **14** (260 mg, 0.678 mmol) in dry CH_3CN (4.5 ml), under nitrogen atmosphere, DIPEA (0.746 mmol, 0.130 ml) and benzyl bromide (0.816 mmol, 0.097 ml) were added. The reaction mixture was stirred at room temperature. After 24 h. the reaction was completed (TLC CH_2Cl_2:MeOH 95:5), than EtOAc was added (4 ml) and the mixture was washed with water (2 x 5 ml) and brine (2 x 5 ml). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude was purified by flash chromatography (hexane:EtOAc 70:30) affording the pure product (274 mg, 85%) as white foam. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.23-1.39 (m, 2H, H7), 1.42, 1.45 (2s, 18H, Boc, tBu), 1.54 (m, 1H, H9), 1.91 (m, 1H, H10), 2.12-2.32 (m, 2H, H10, H9), 2.60-2.72 (m, 2H, H6, H4), 2.81 (m, 1H, H6), 3.04 (m, 1H, H4), 3.58 (d, 1H, J = 13.4 Hz, H12), 3.71 (d, 1H, J = 13.4 Hz, H12), 4.30 (m, 1H, H8), 4.37 (m, 1H, H11), 4.74 (m, 1H, H3), 5.53 (bd, 1H, NHBoc), 7.18-7.32 (m, 5H, aromatics). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 171.8, 171.5, 155.7, 140.0, 129.3, 128.6, 127.5, 81.3, 79.6, 62.3, 61.6, 60.0, 58.4, 52.1, 36.9, 31.3, 28.2, 27.8, 27.0. HRMS (ESI) found *m/z* 496.27810 $[M + Na]^+$ (calcd. for $C_{26}H_{39}N_3O_5Na^+$: 496.2782).

Synthesis of compound 4. To a solution of **15** (130 mg, 0.275 mmol) in DCM (2.9 ml) TFA (14.475 mmol, 1.1 ml) was added. The reaction mixture was stirred for 2h. After reaction completion (TLC hexane:EtOAc 7:3) the solvent was evaporated under reduced pressure. The crude was submitted to the next reaction without further purification.
The crude was dissolved in THF (2 ml) and an aqueous solution (0.9 ml) of Na_2CO_3 (0.093 g, 0.88 mmol) was added. After 15 min the reaction mixture was cooled at 0 °C and Fmoc-OSuc (0.102 g, 0.302 mmol) was added. The reaction was stirred 1 h at 0 °C, than the temperature was raised at 30 °C and stirred for other 1.5 h. After reaction completion (TLC $CHCl_3$:MeOH 9:1) the solvent was evaporated under reduced pressure. The crude was rinsed with water (2 ml), EtOAc (2 ml) and acidified with a 2M HCl solution until the pH was acid. The aqueous phase was extracted with EtOAc (3 x 4 ml). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude was purified by flash chromatography ($CHCl_3$:MeOH from 98:2 to 90:10) affording the pure product (145 mg, 97% over two steps) as white foam. ^{1}H-NMR (400 MHz, CD_3OD): δ (mixture of conformers): 1.43 (m, 1H, H7), 1.58 (m, 1H, H7), 1.68 (m, 1H, H9), 1.93-2.16 (m, 1H, H10), 2.16-2.38 (m, 2H, H9, H10), 2.76-2.95 (m, 4H, H6, H4), 3.54-3.76 (m, 2H, H12), 3.97-4.31 (m, 3H, H13, H8), 4.35 (m, 1H, H11), 4.50 (m, 1H, NHFmoc), 4.70-4.81 (m, 1H, H3), 6.85-7.47 (m, 13H, aromatics). ^{13}C-NMR (100.6 MHz, CD_3OD): δ 177.1, 176.5, 175.8, 173.6, 173.2, 158.4, 157.2, 146.3, 145.9, 145.7, 143.3, 143.1, 140.7, 140.5, 130.6, 130.4, 129.8, 129.7, 129.3, 129.2, 128.9, 128.8, 128.7, 126.7, 126.2, 126.1, 121.5, 121.4, 68.3, 66.7, 63.6, 63.5, 61.2, 61.0, 60.8, 60.3, 53.9, 53.6, 53.5, 49.9, 48.3, 37.5, 37.4, 32.3, 28.0, 27.9, 26.1. MS (ESI) found *m/z* 562.5 $[M + Na]^+$ (calcd. for $C_{32}H_{33}N_3O_5$: 539.2).

Compound 1. ^{1}H-NMR (D_2O, 400 MHz)



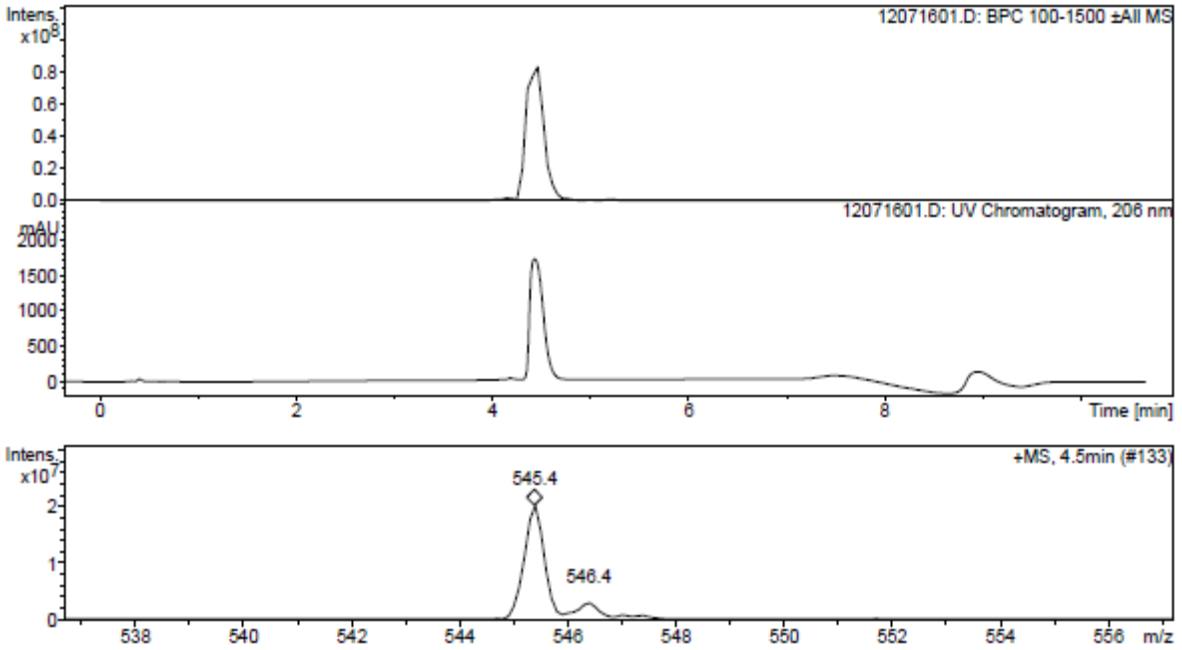
Compound 1. ^{13}C-NMR (D_2O, 100.6 MHz)



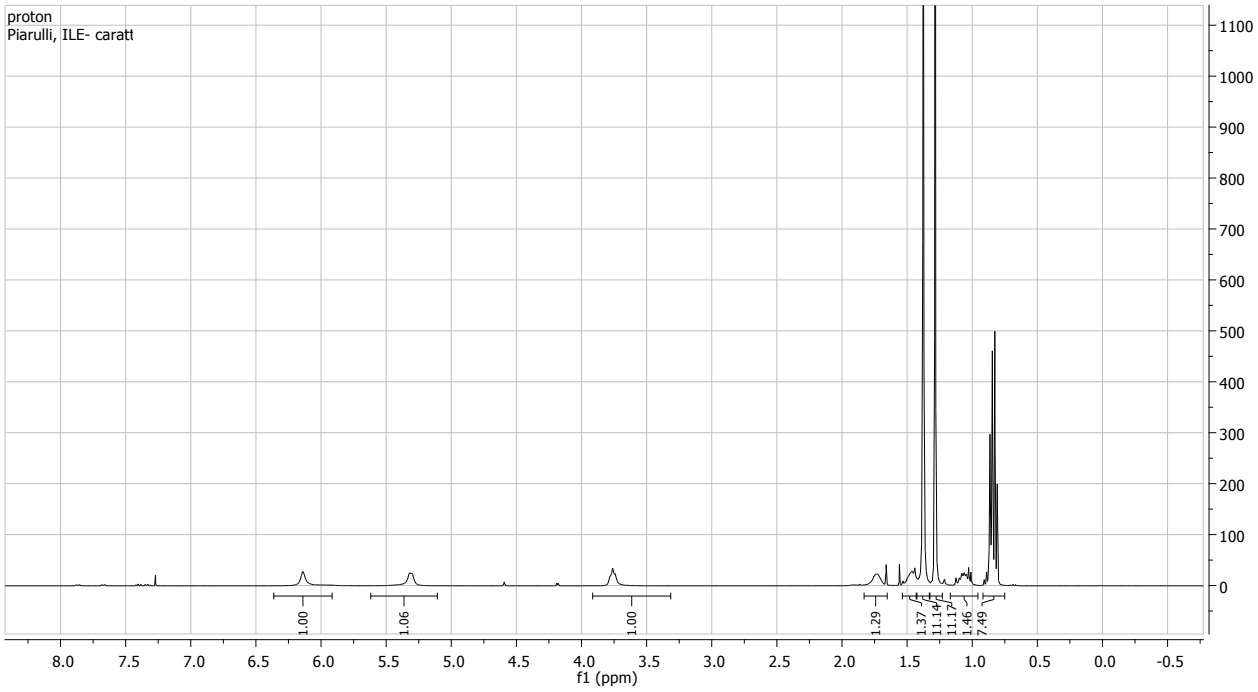
Compound 1. HPLC-MS, Method A

Acquisition Parameter

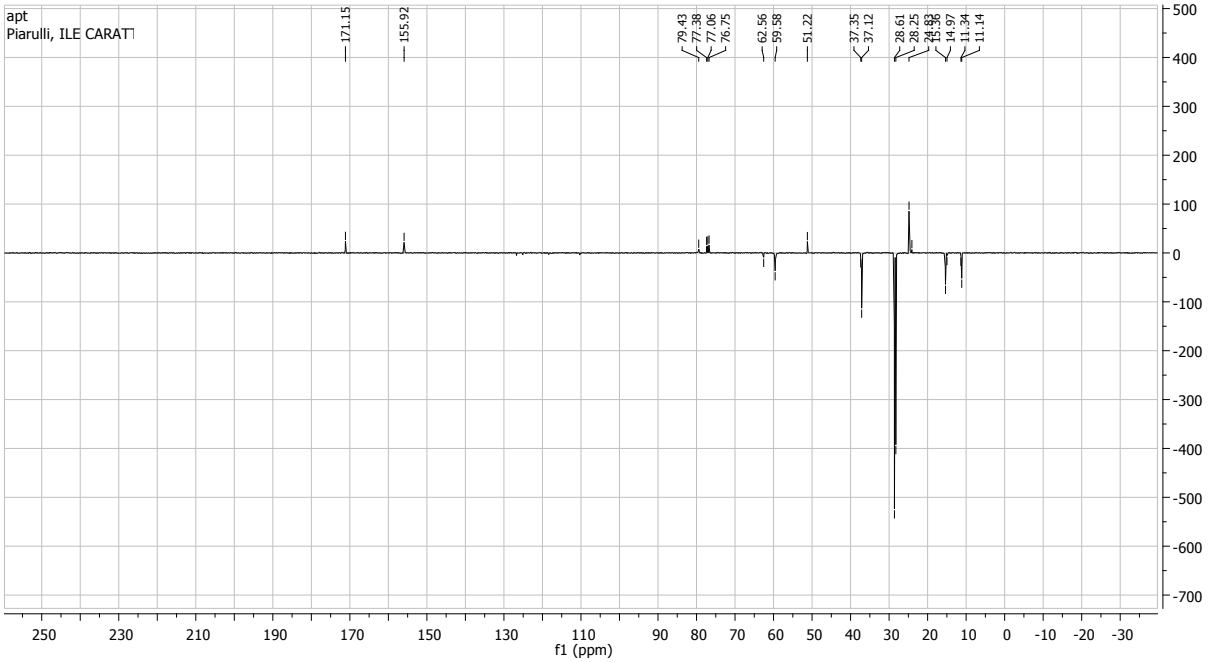
Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	Std/Normal	Scan Begin	100 m/z	Scan End	1500 m/z
Capillary Exit	143.5 Volt	Skim 1	40.0 Volt	Trap Drive	64.2
Accumulation Time	31767 µs	Averages	5 Spectra	Auto MS/MS	on



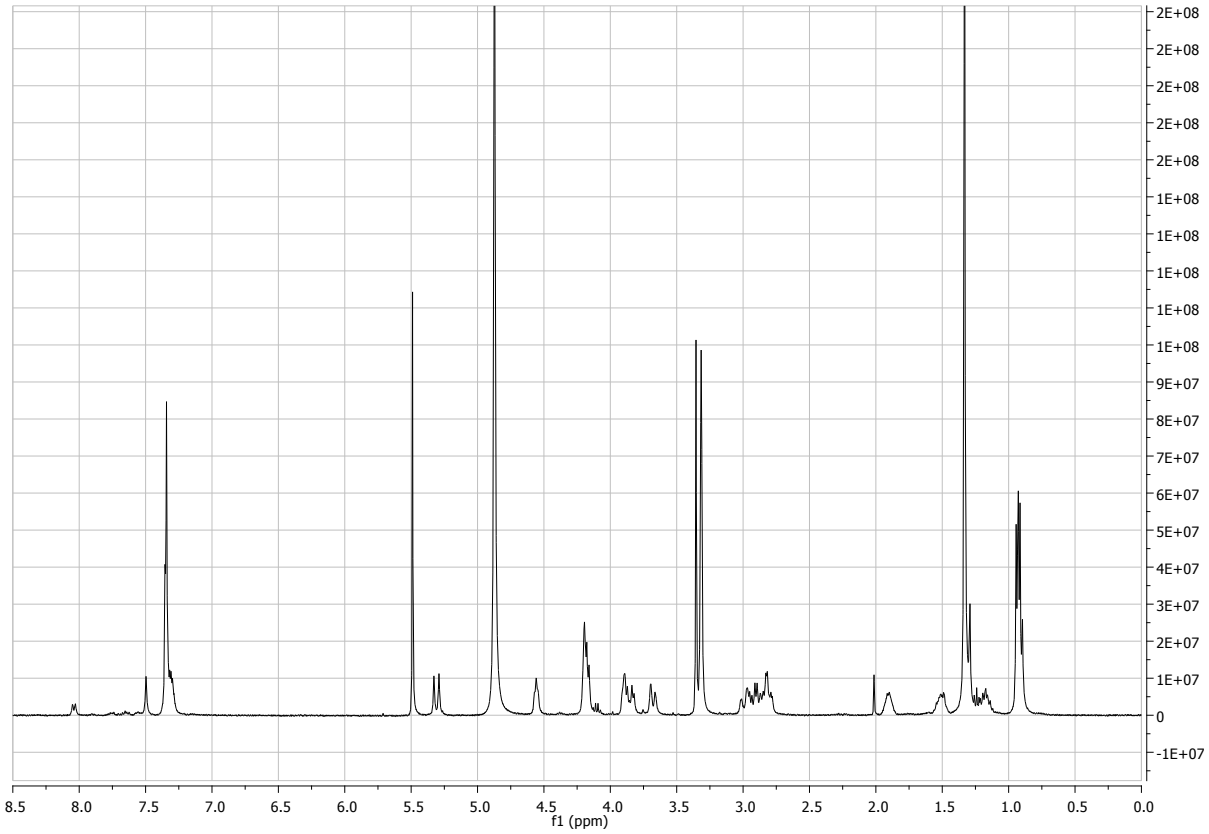
Compound BocIleCONHtBu. ^{1}H-NMR ($CDCl_3$, 400 MHz)



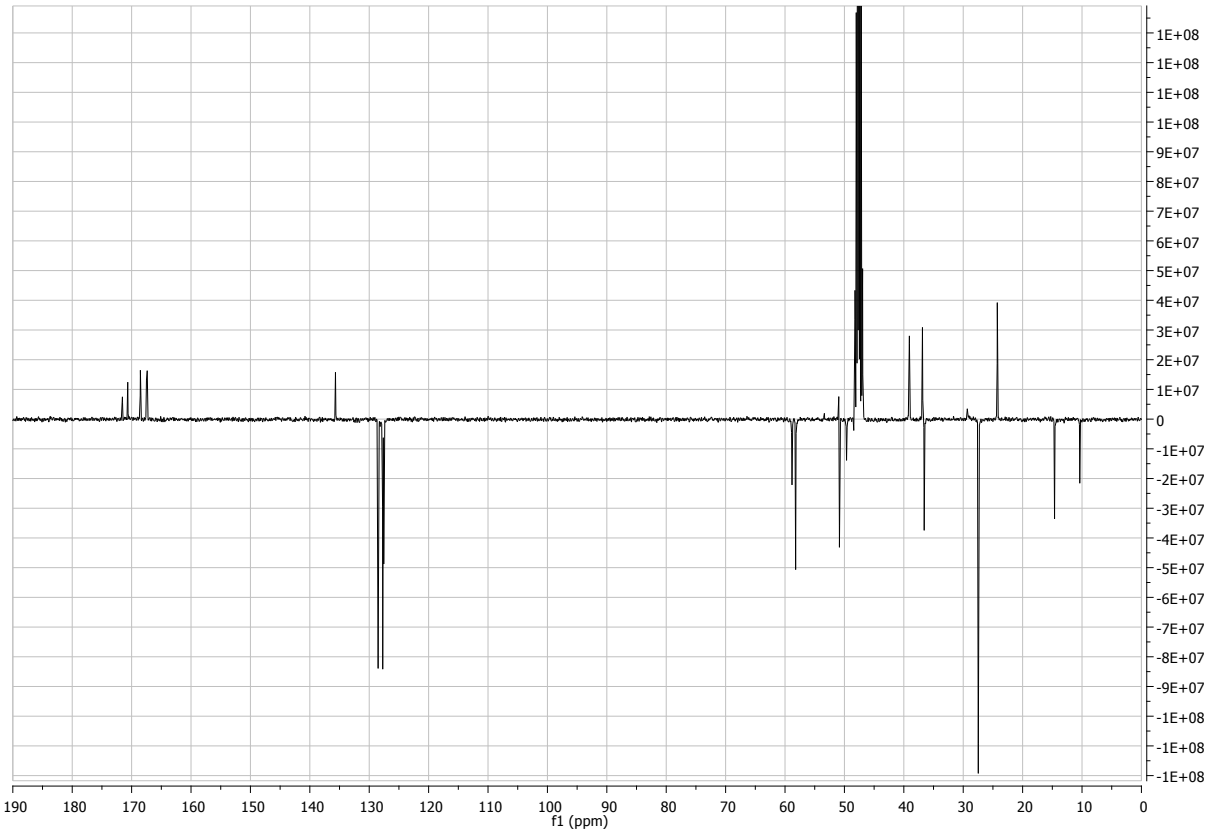
Compound BocIleCONHtBu. ^{13}C-NMR ($CDCl_3$, 100.6 MHz)



Compound 2. ^{1}H-NMR (CD_3OD, 400 MHz)



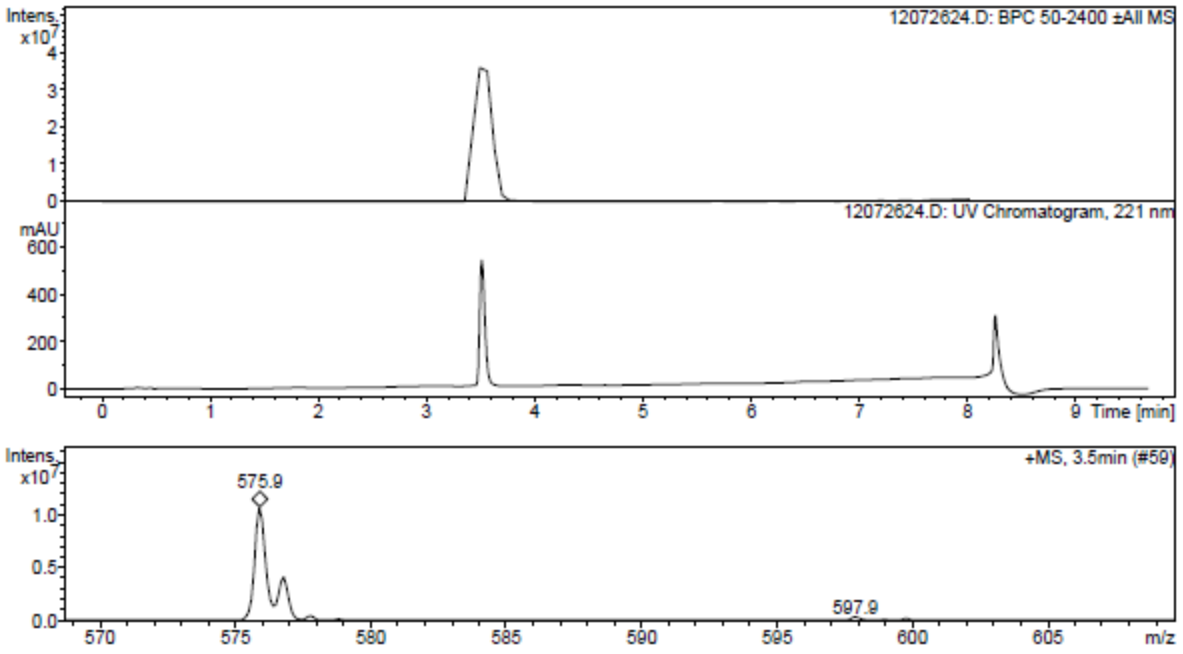
Compound 2. ^{13}C-NMR (CD_3OD, 100.6 MHz)



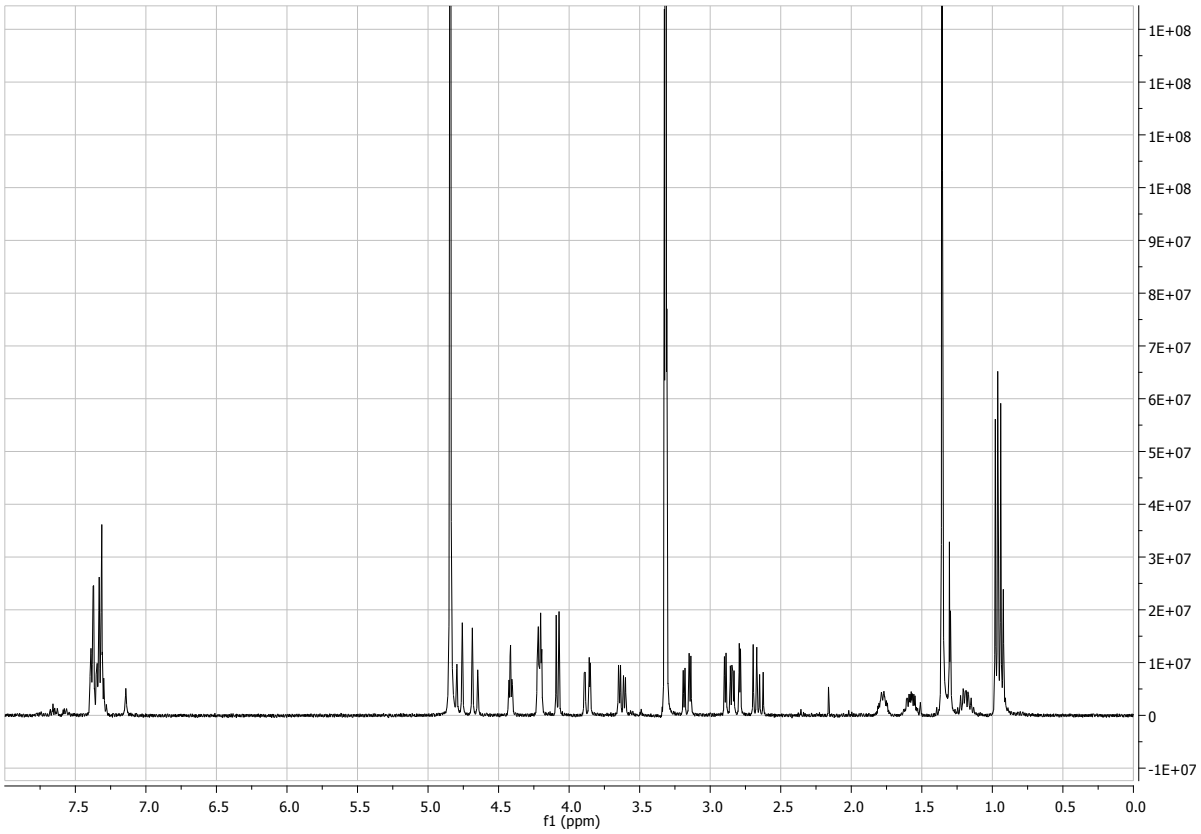
Compound 2. HPLC-MS, Method B

Acquisition Parameter

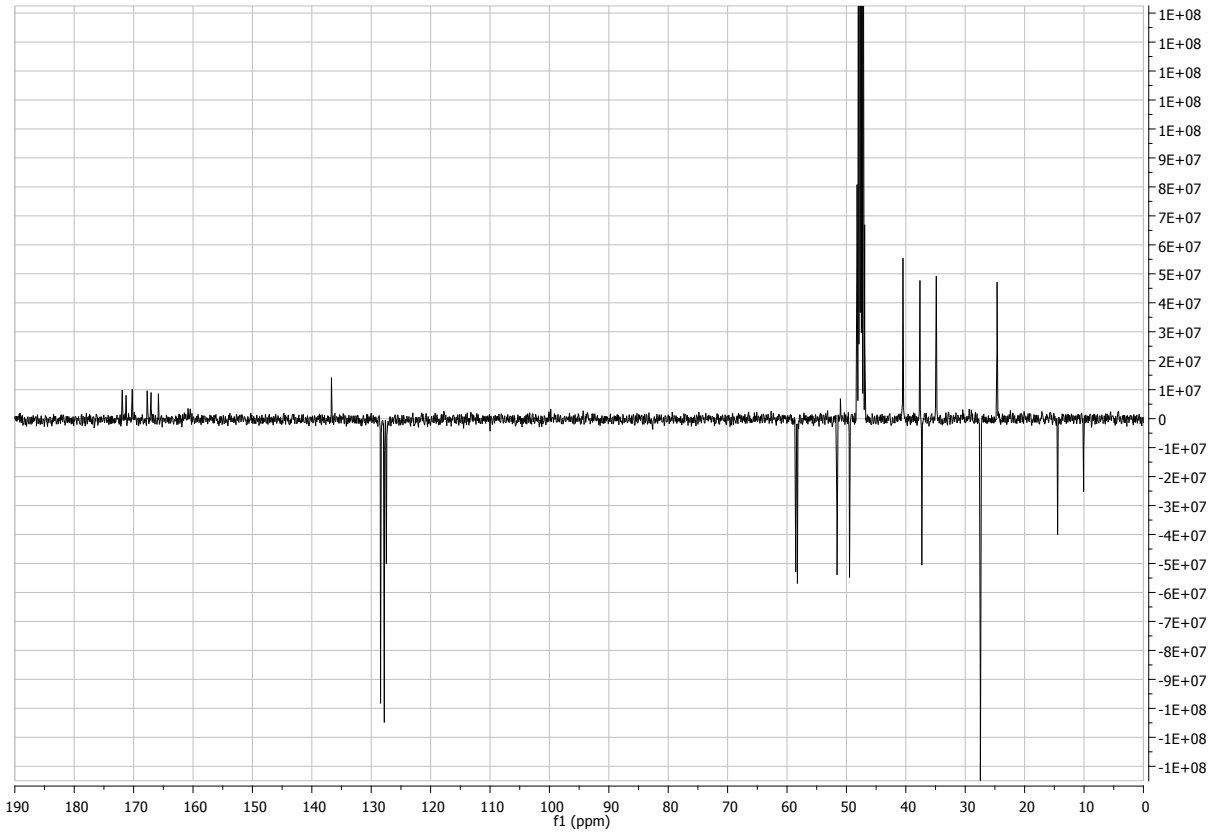
Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	Std/Normal	Scan Begin	50 m/z	Scan End	2400 m/z
Capillary Exit	158.5 Volt	Skim 1	40.0 Volt	Trap Drive	78.7
Accumulation Time	200000 µs	Averages	5 Spectra	Auto MS/MS	on



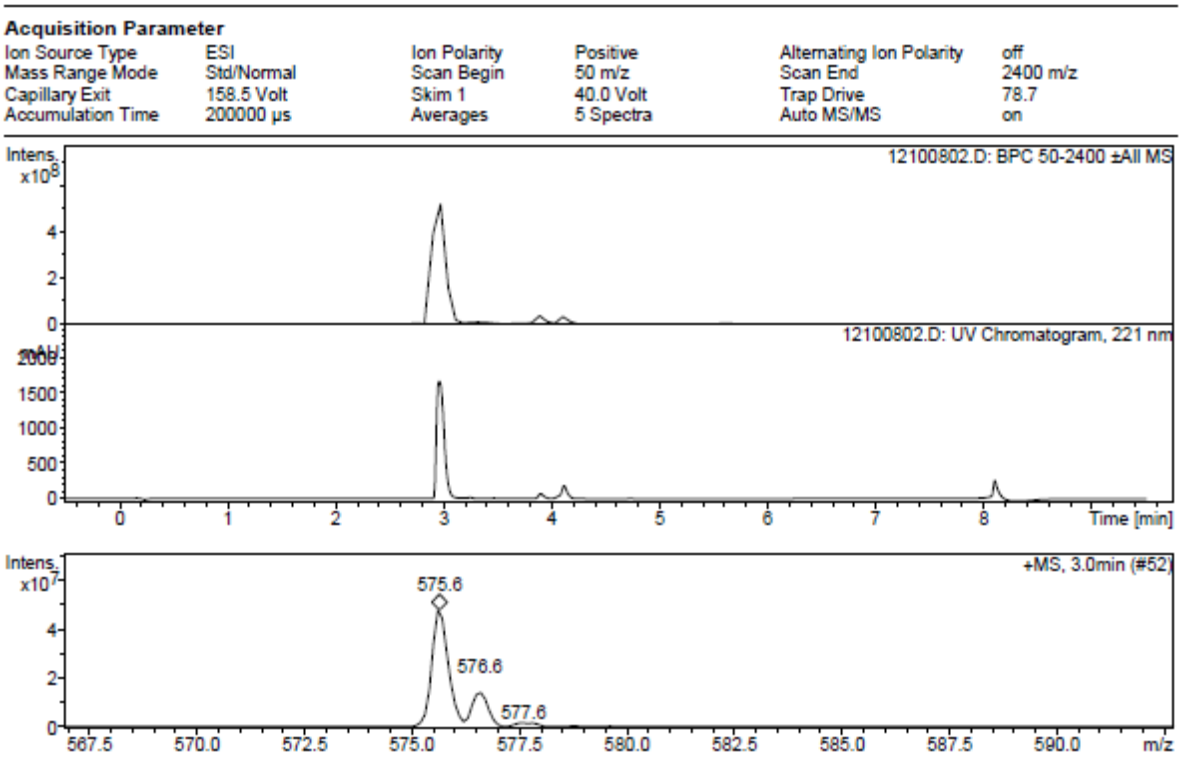
Compound 3. ^{1}H-NMR (CD_3OD, 400 MHz)



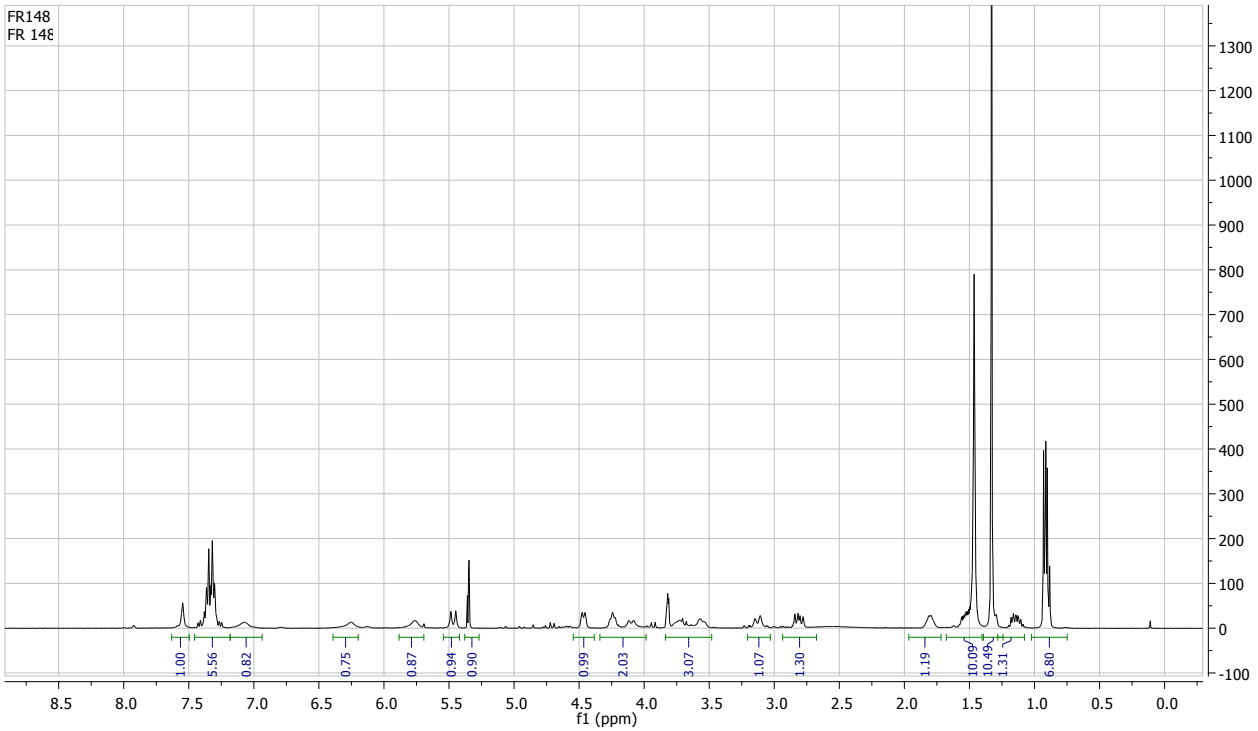
Compound 3. ^{13}C-NMR (CD_3OD, 100.6 MHz)



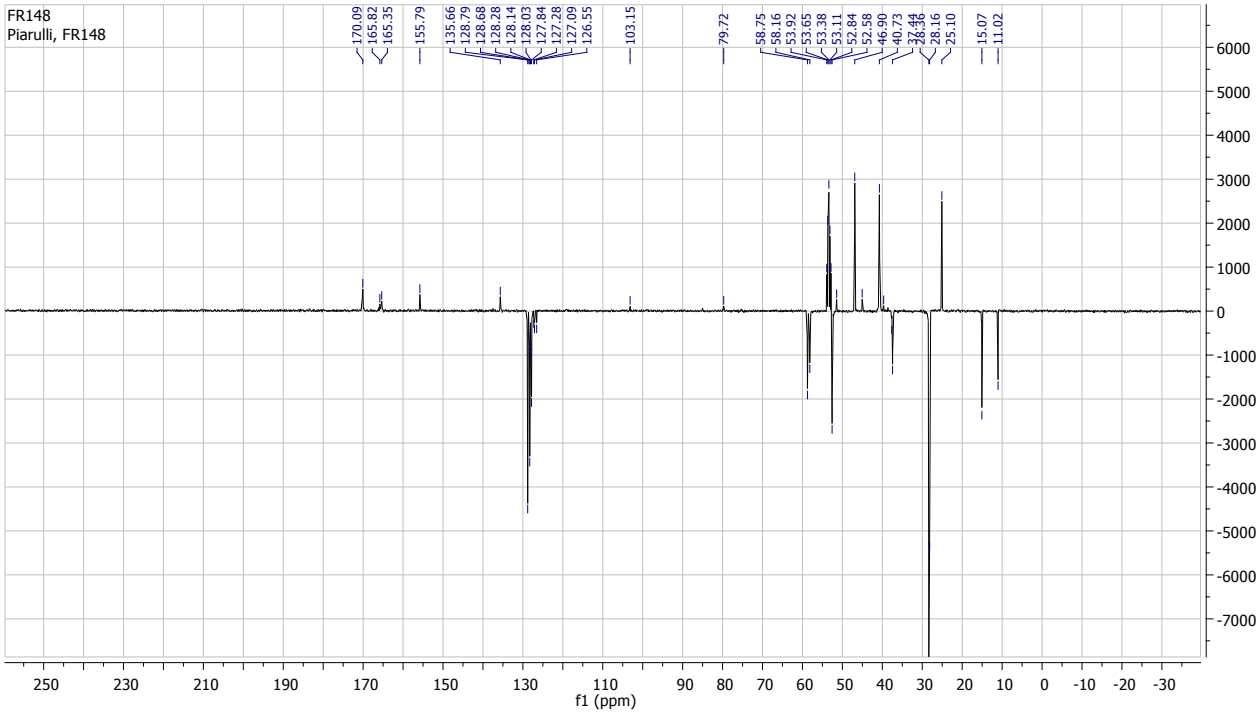
Compound 3. HPLC-MS, Method B



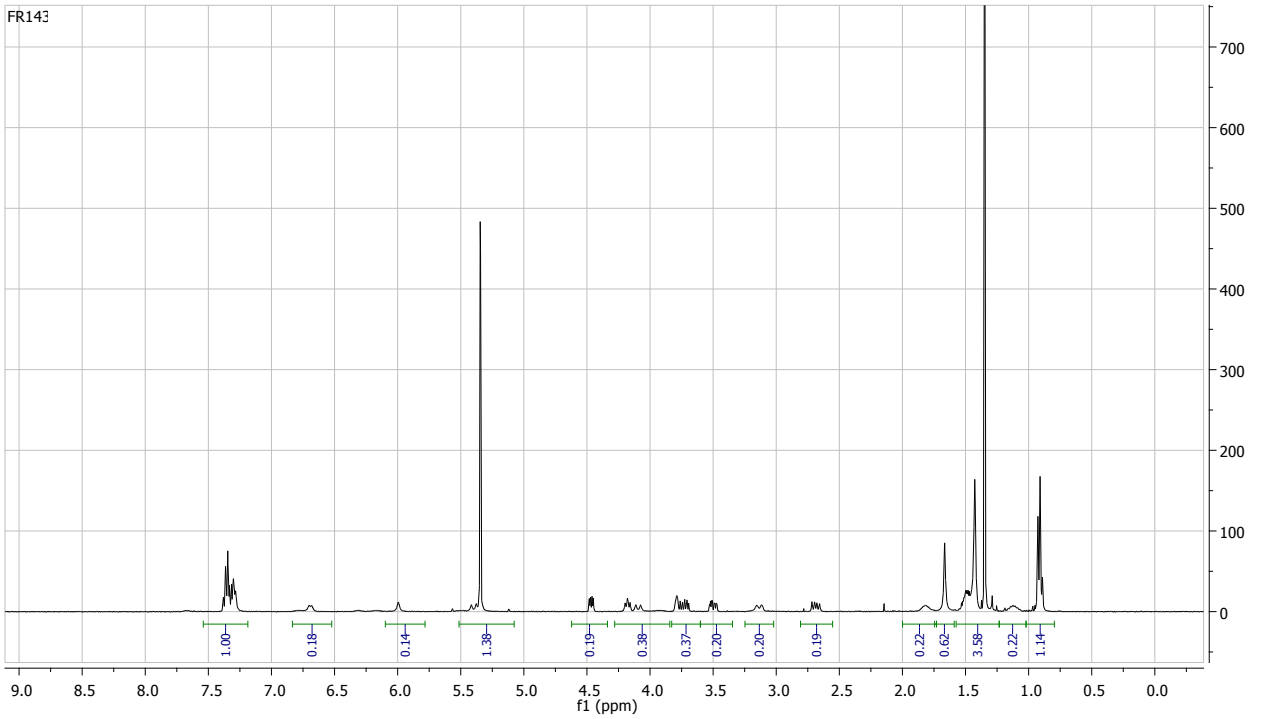
Compound 7. ^{1}H-NMR (CD_2Cl_2, 400 MHz)



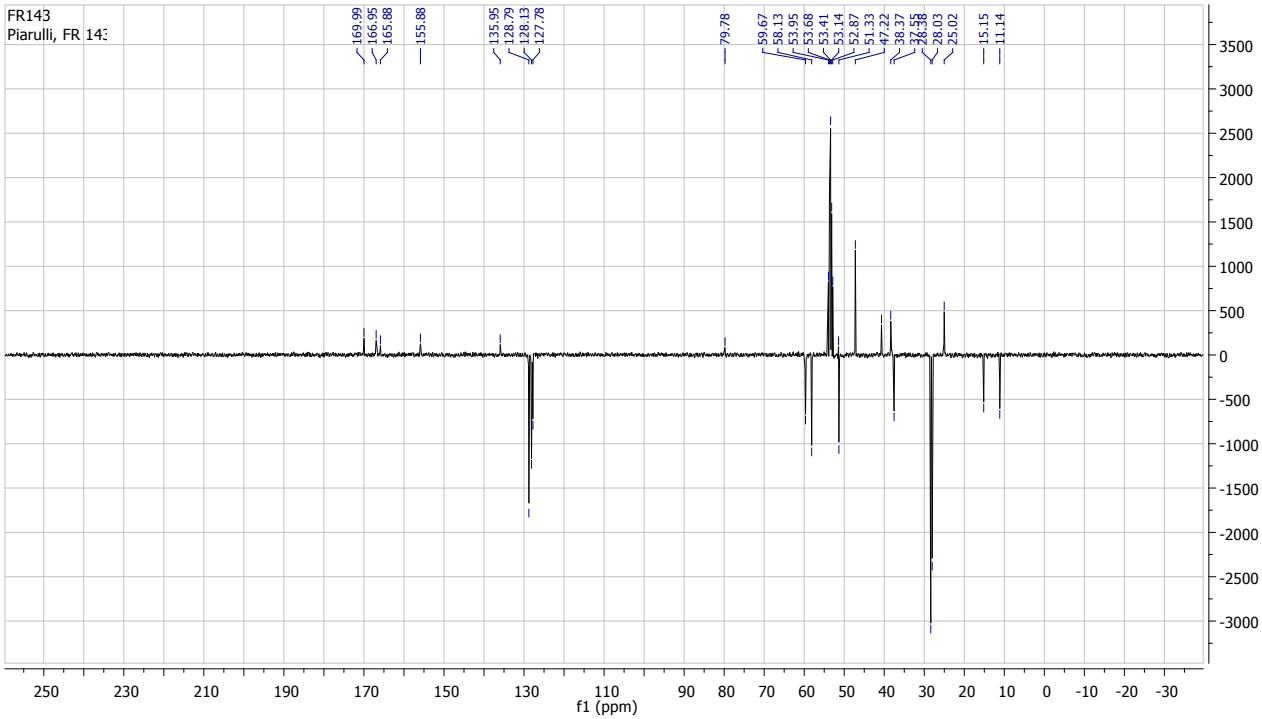
Compound 7. ^{13}C-NMR (CD_2Cl_2, 100.6 MHz)



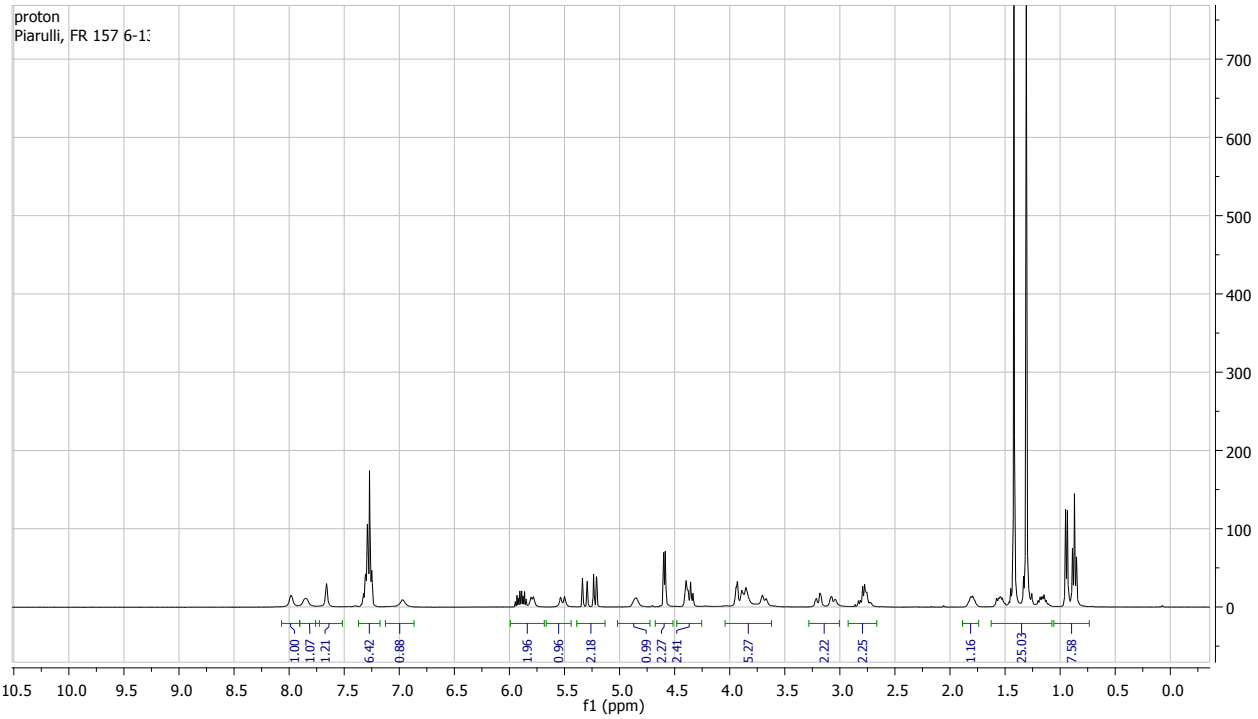
Compound 8. ^{1}H-NMR (CD_2Cl_2, 400 MHz)



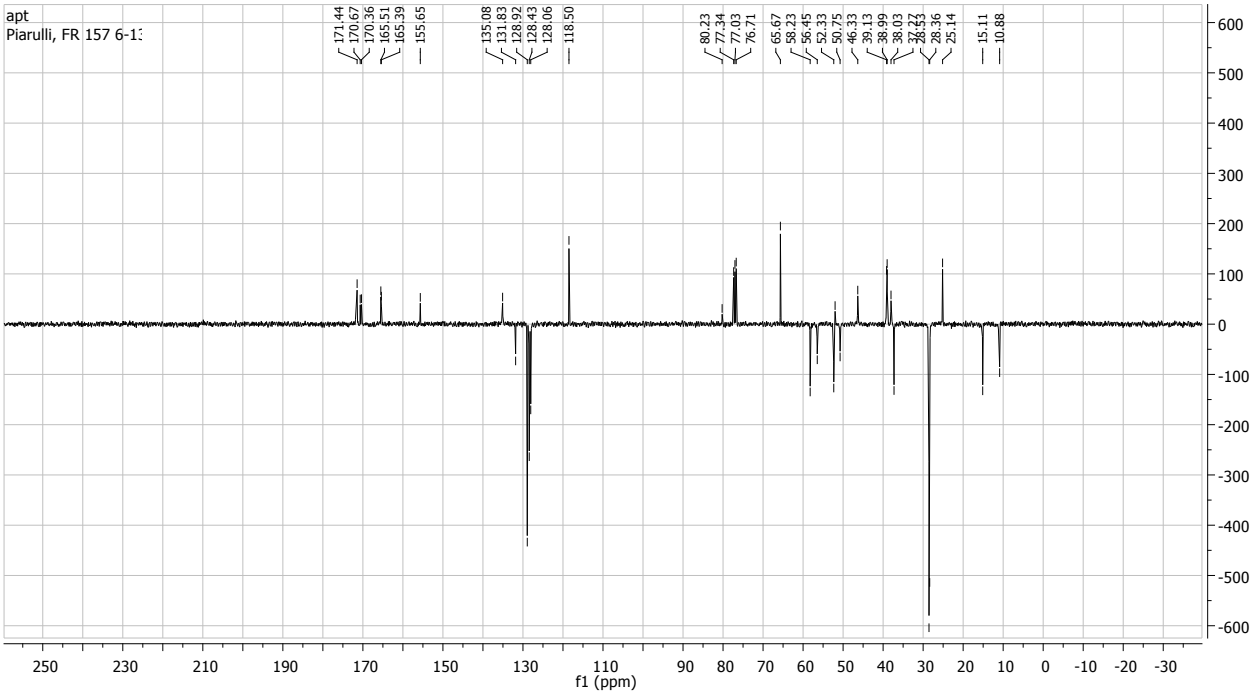
Compound 8. ^{13}C-NMR (CD_2Cl_2, 100.6 MHz)



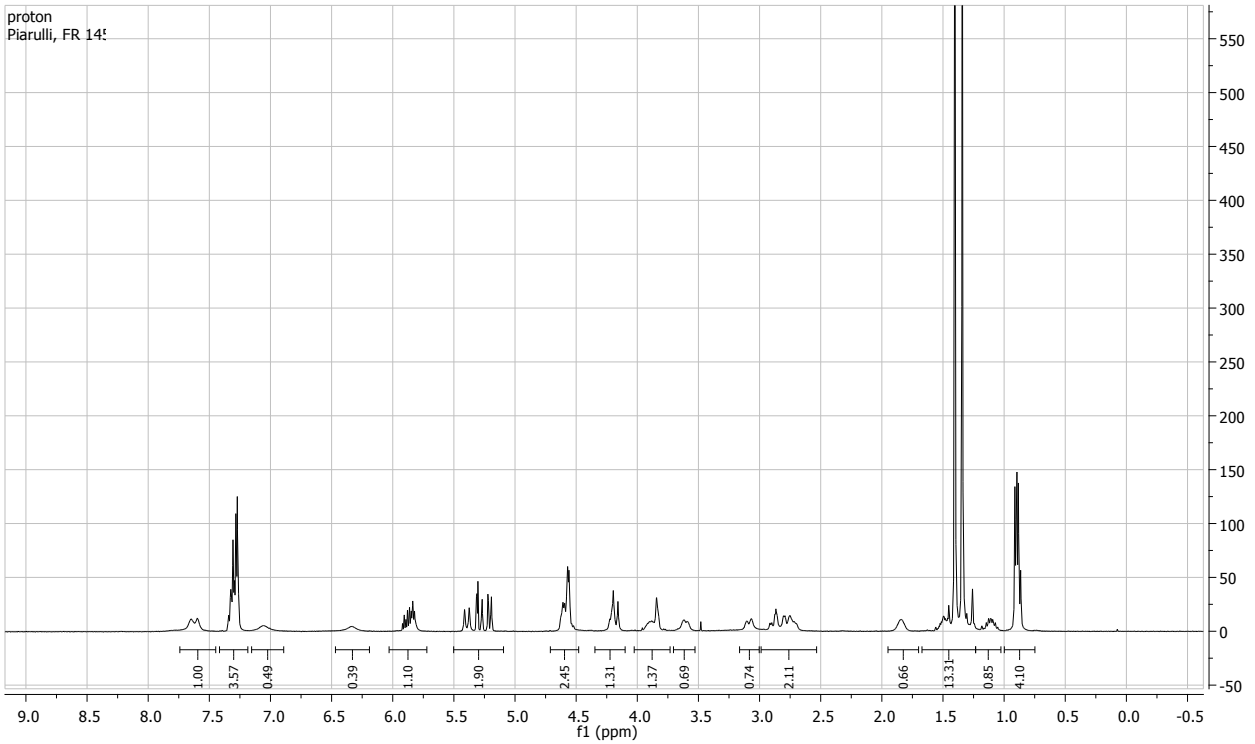
Compound 9. ^{1}H-NMR ($CDCl_3$, 400 MHz)



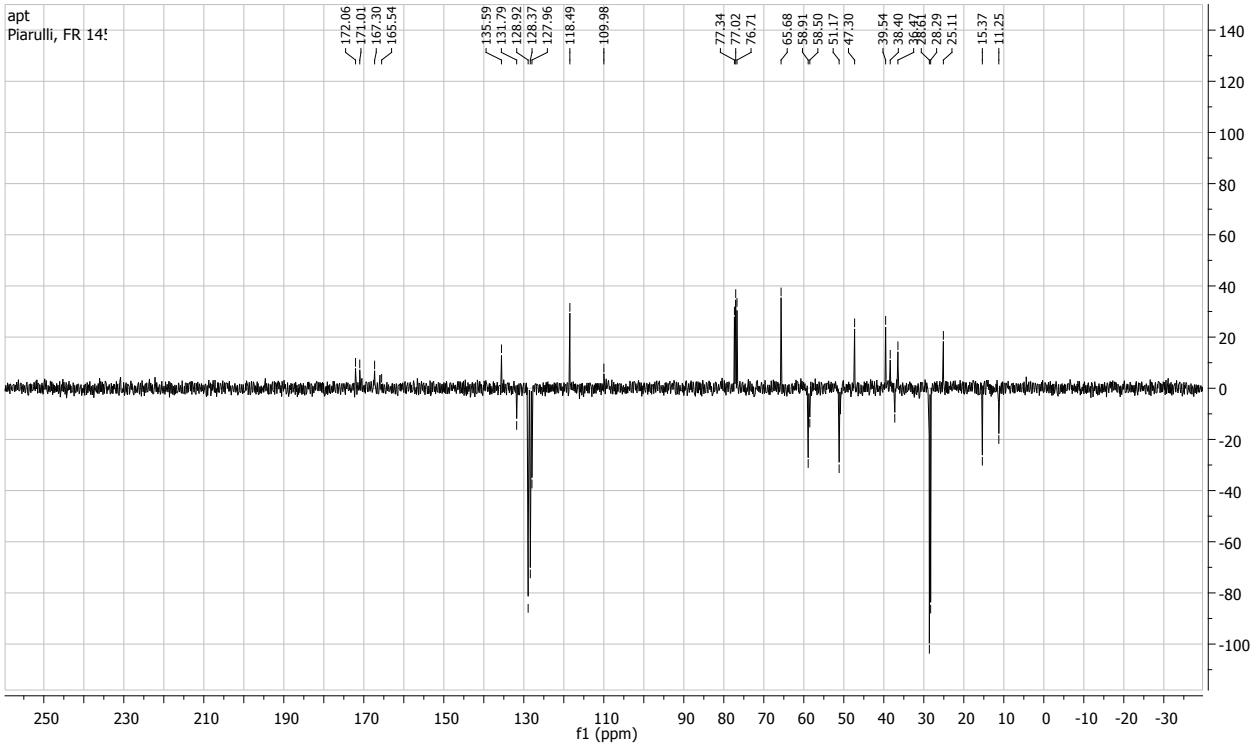
Compound 9. ^{13}C-NMR ($CDCl_3$, 100.6 MHz)



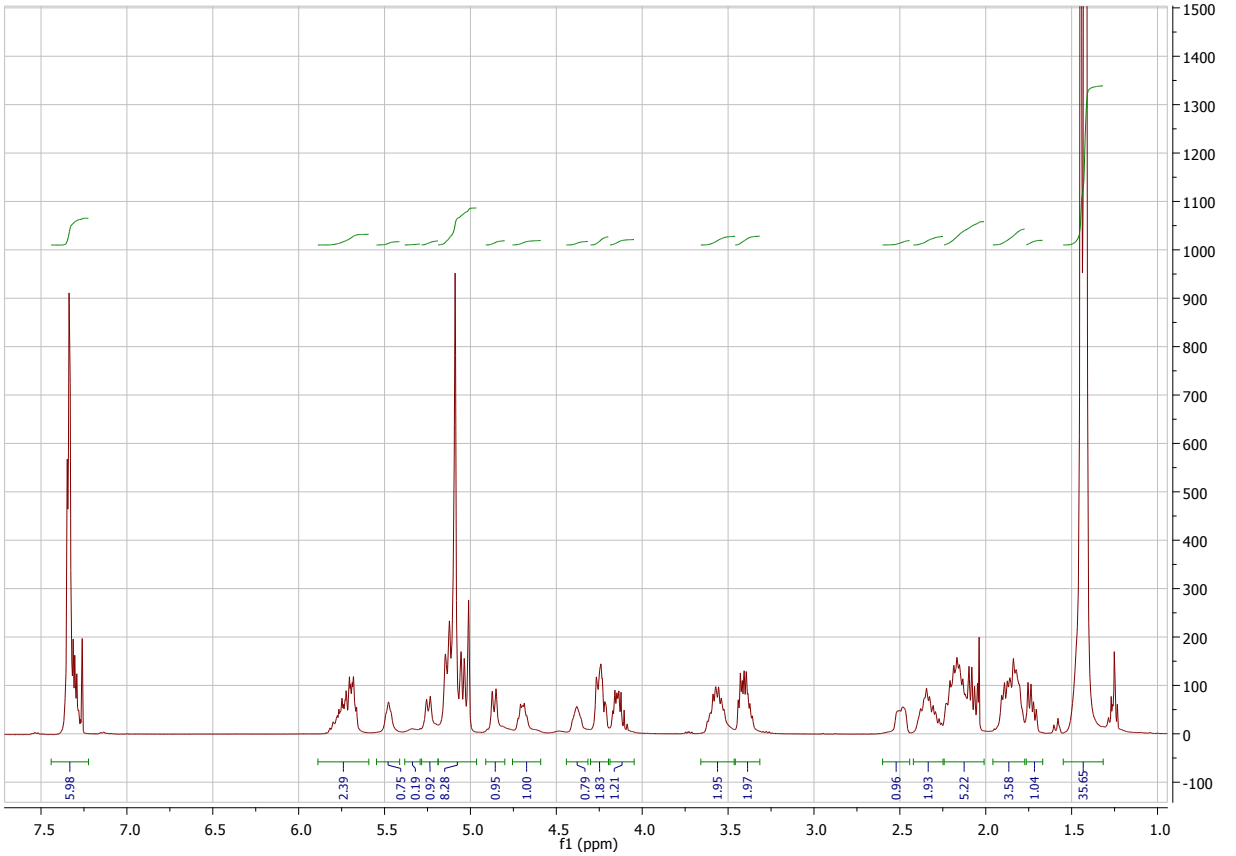
Compound 10. ^{1}H-NMR ($CDCl_3$, 400 MHz)



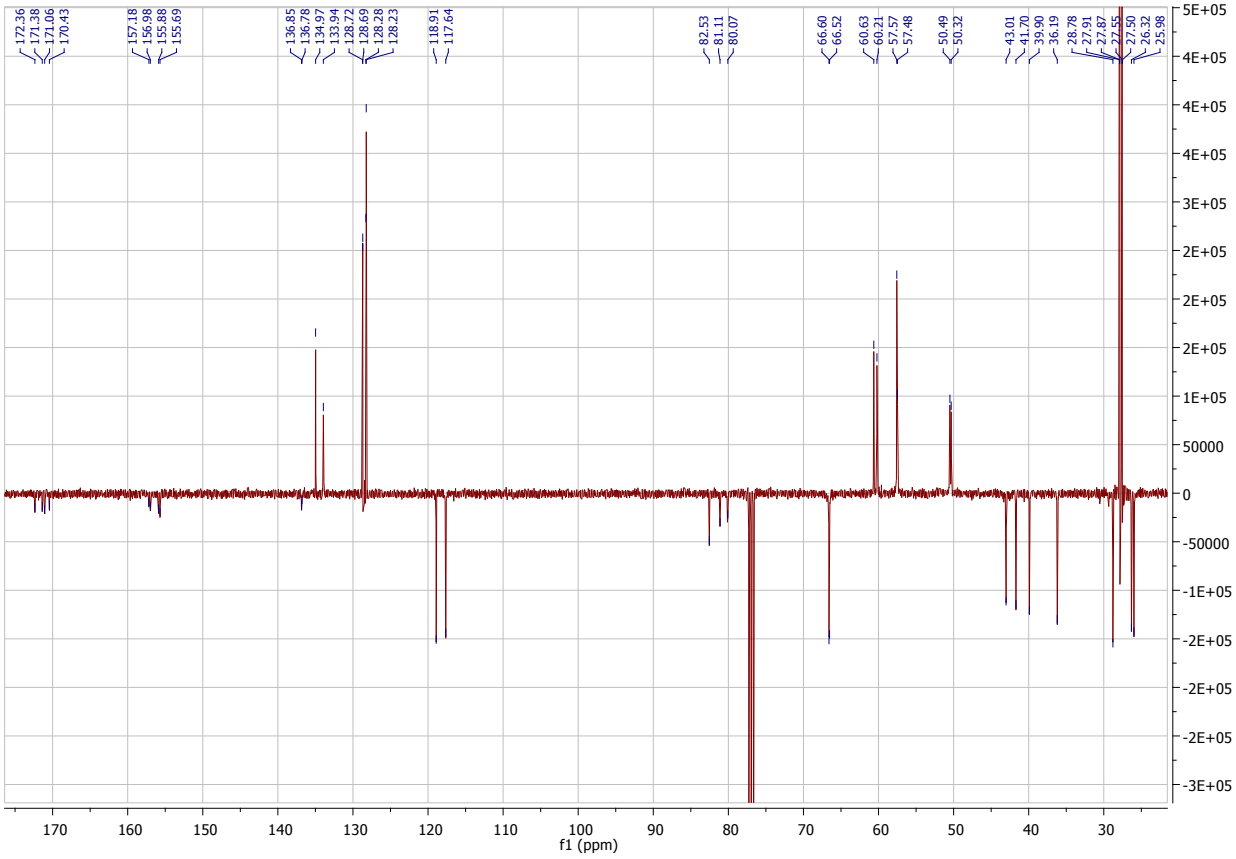
Compound 10. ^{13}C-NMR ($CDCl_3$, 100.6 MHz)



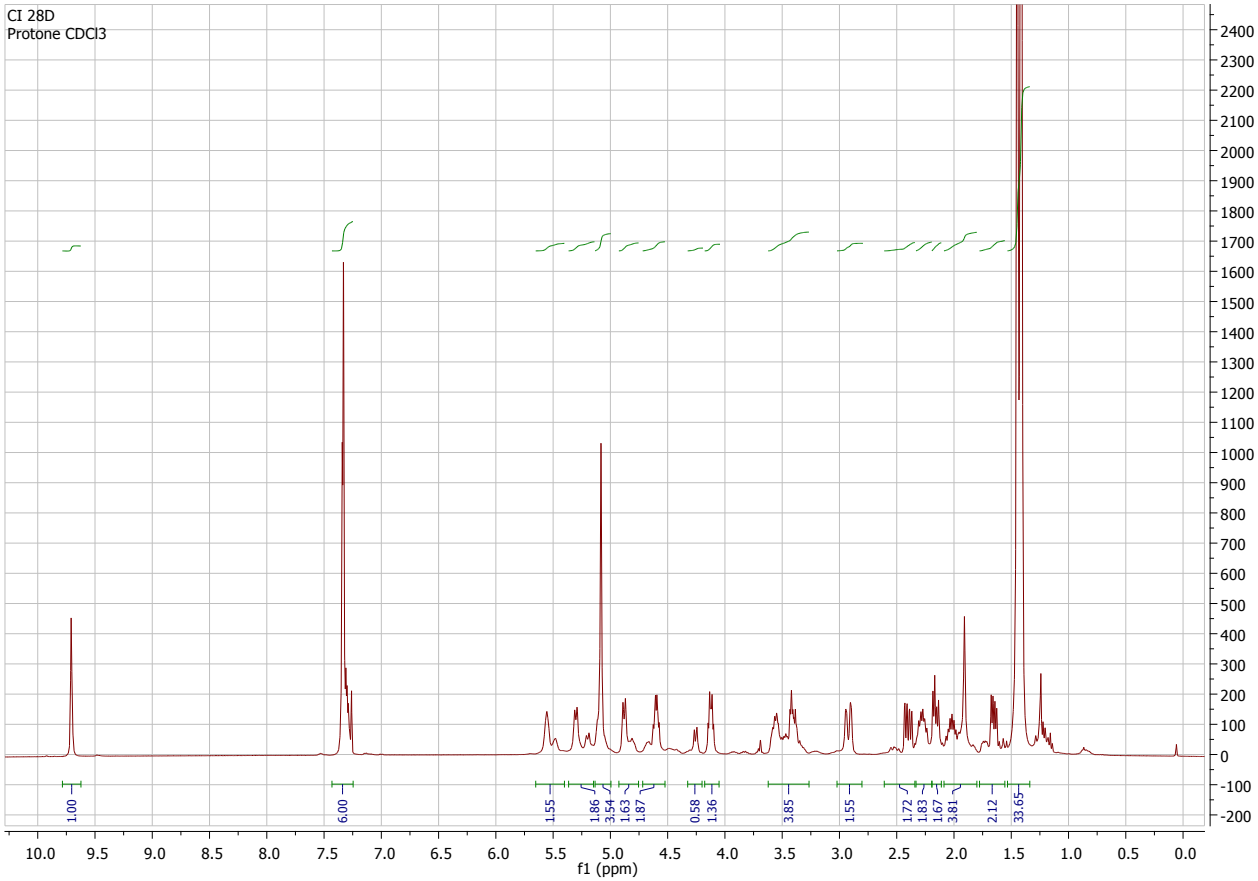
Compound 12. ^{1}H-NMR ($CDCl_3$, 400 MHz)



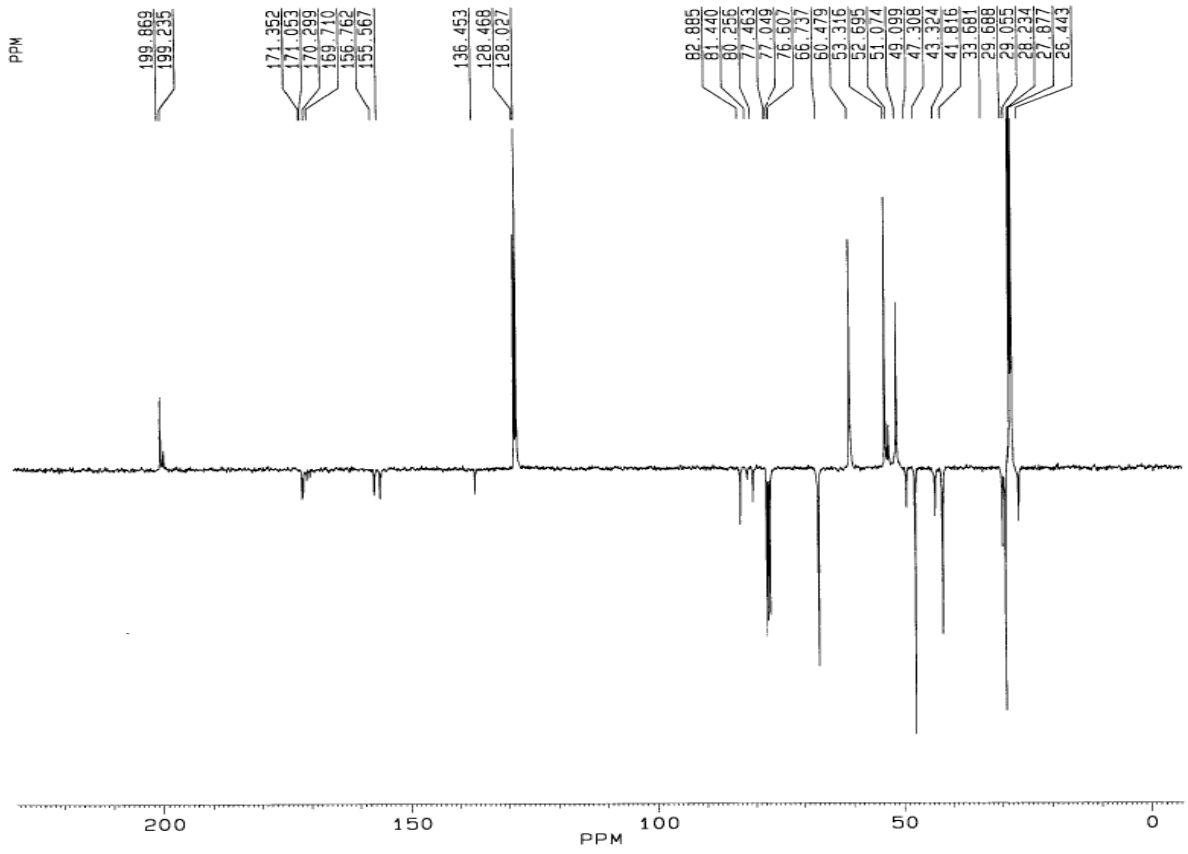
Compound 12. ^{13}C-NMR ($CDCl_3$, 100.6 MHz)



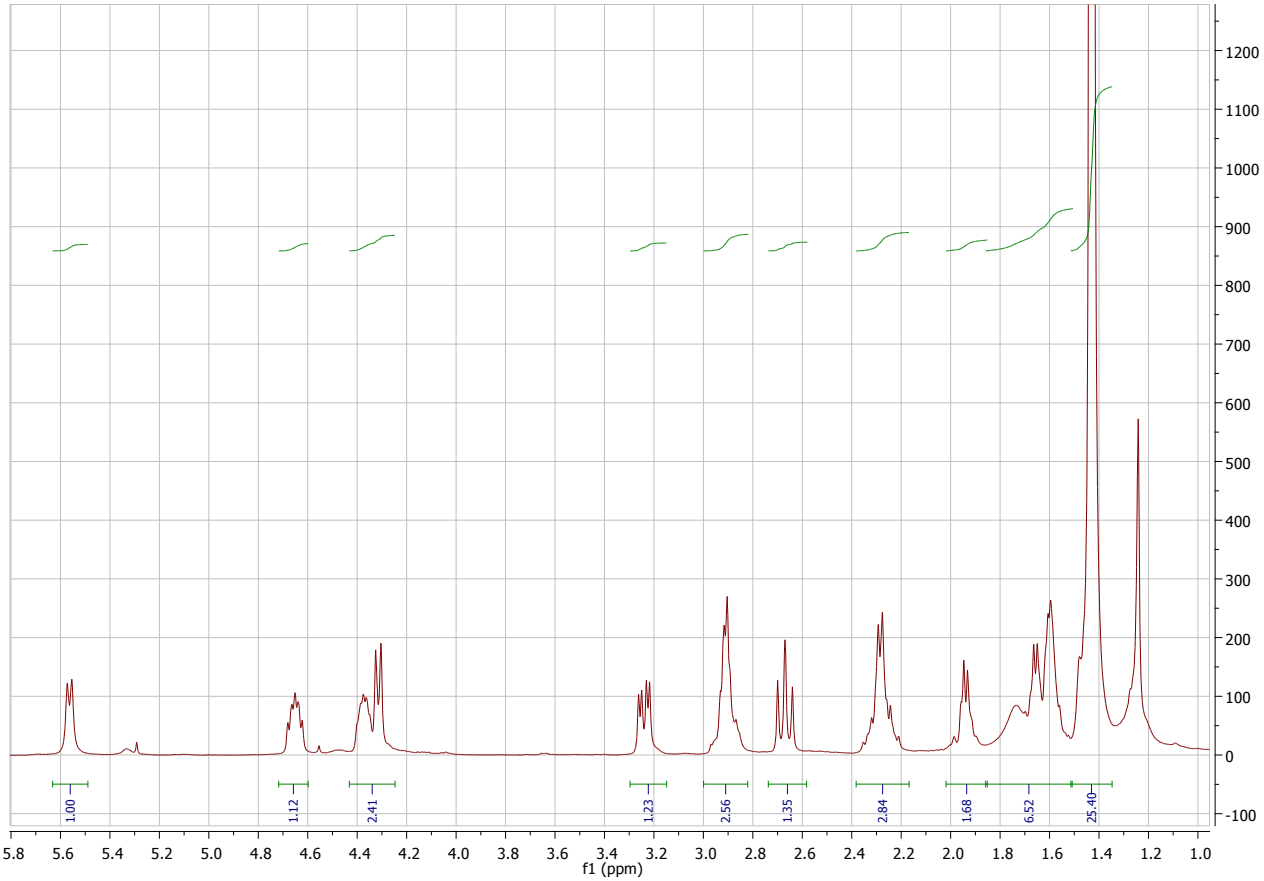
Compound 13. ^{1}H-NMR ($CDCl_3$, 400 MHz)



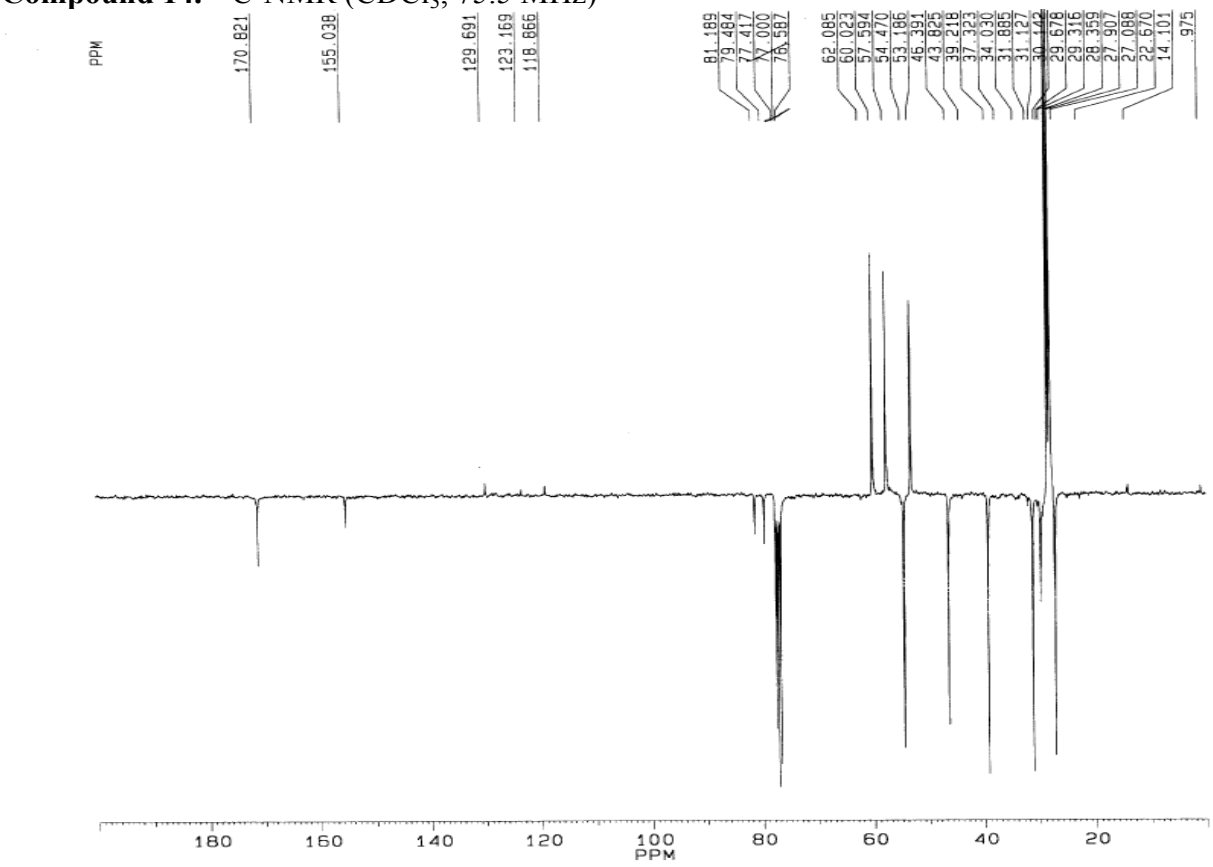
Compound 13. ^{13}C-NMR ($CDCl_3$, 75.5 MHz)



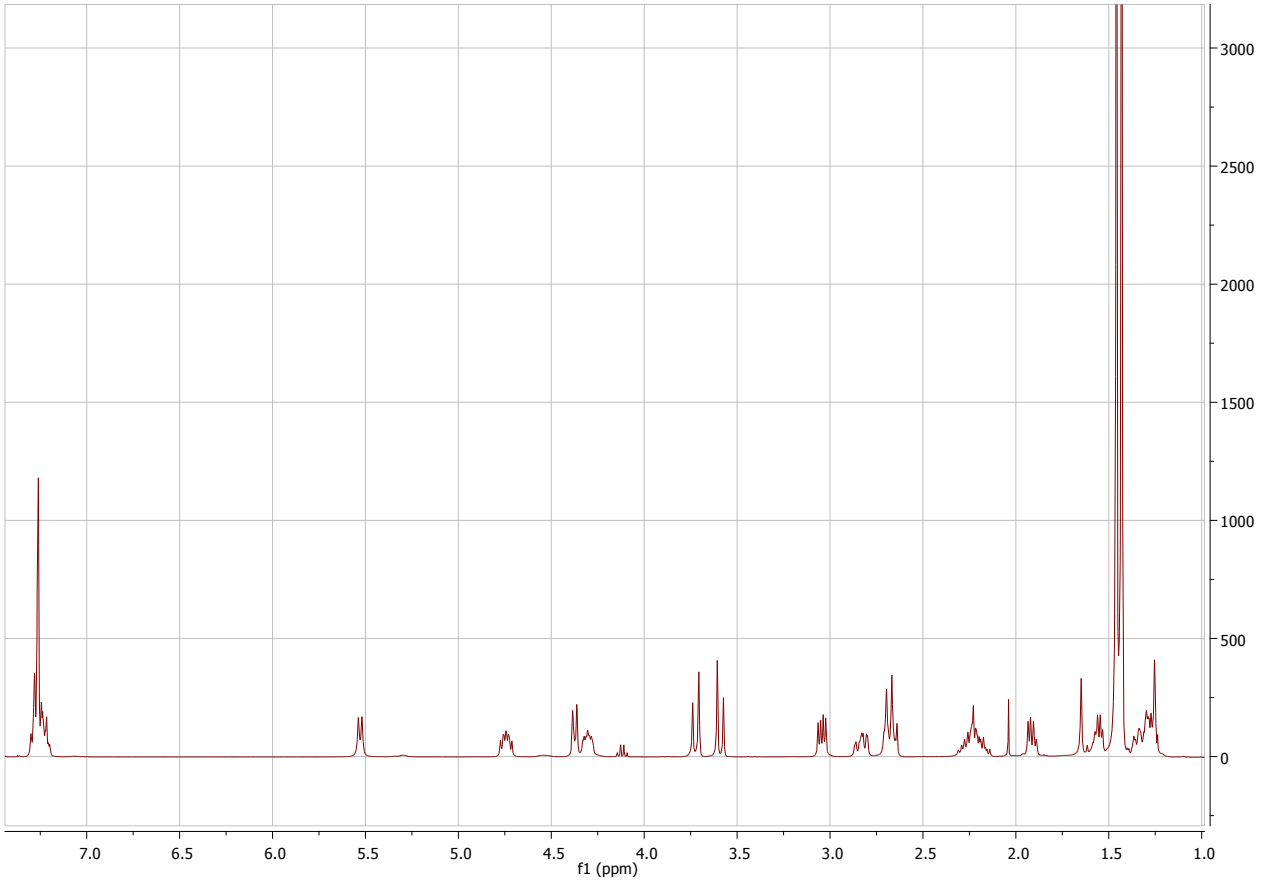
Compound 14. ^{1}H-NMR ($CDCl_3$, 400 MHz)



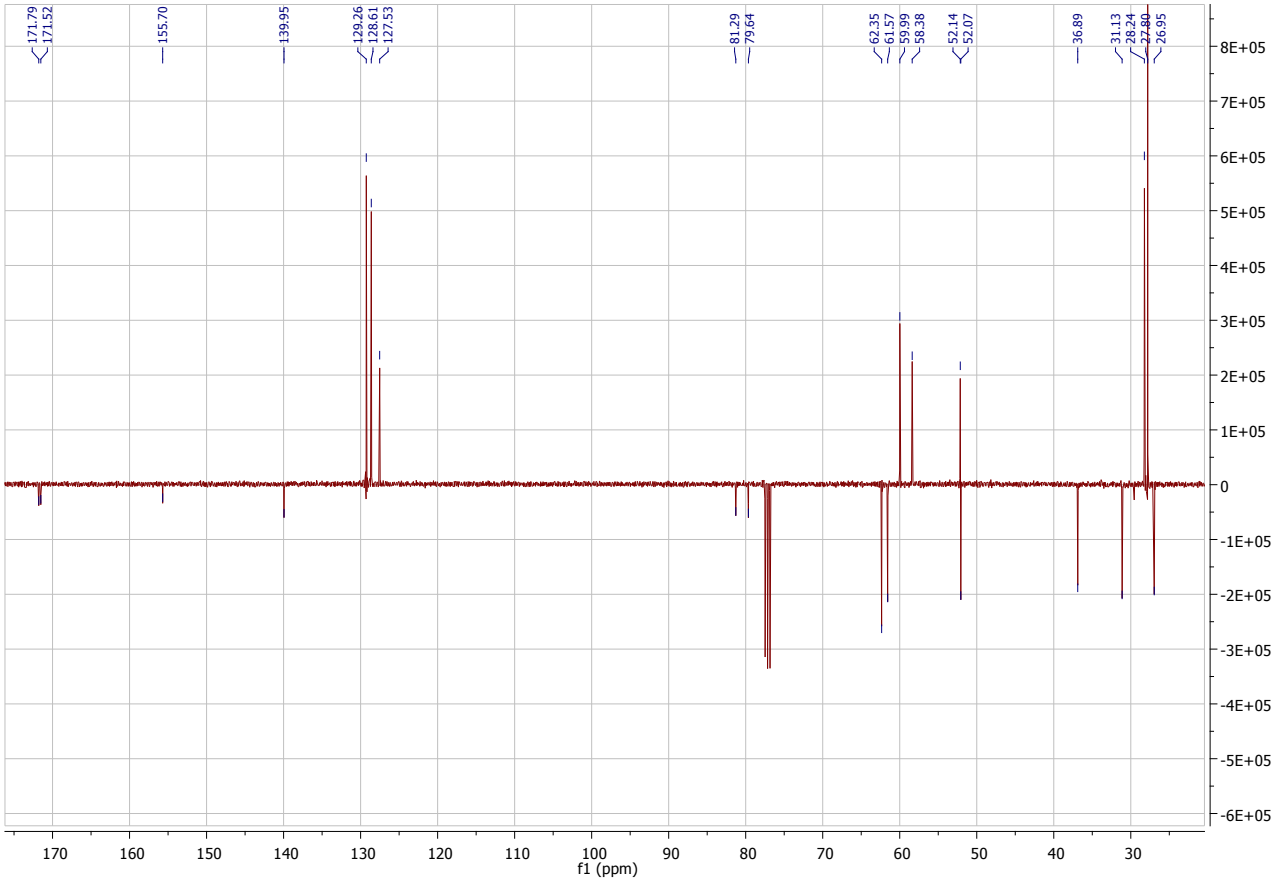
Compound 14. ^{13}C-NMR ($CDCl_3$, 75.5 MHz)



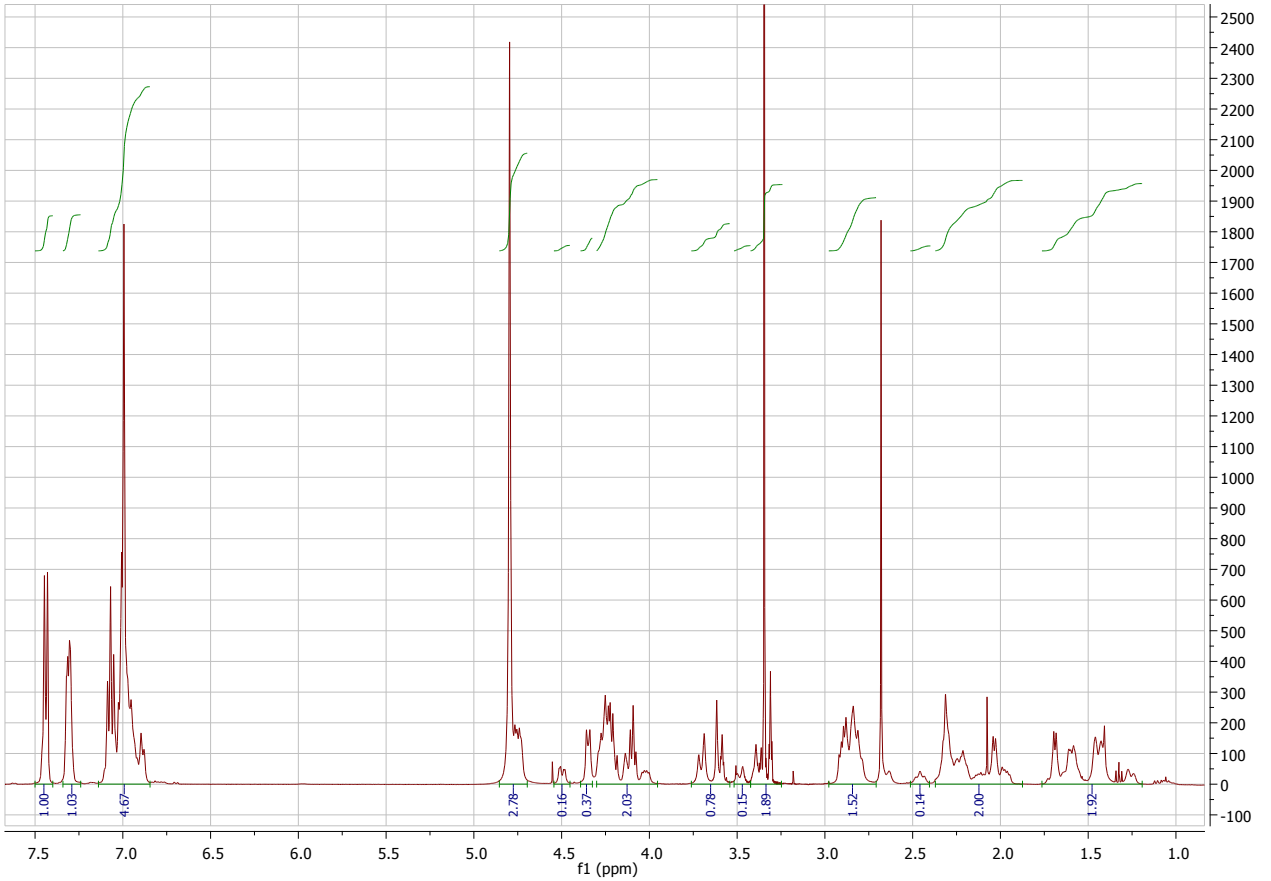
Compound 15. ^{1}H-NMR ($CDCl_3$, 400 MHz)



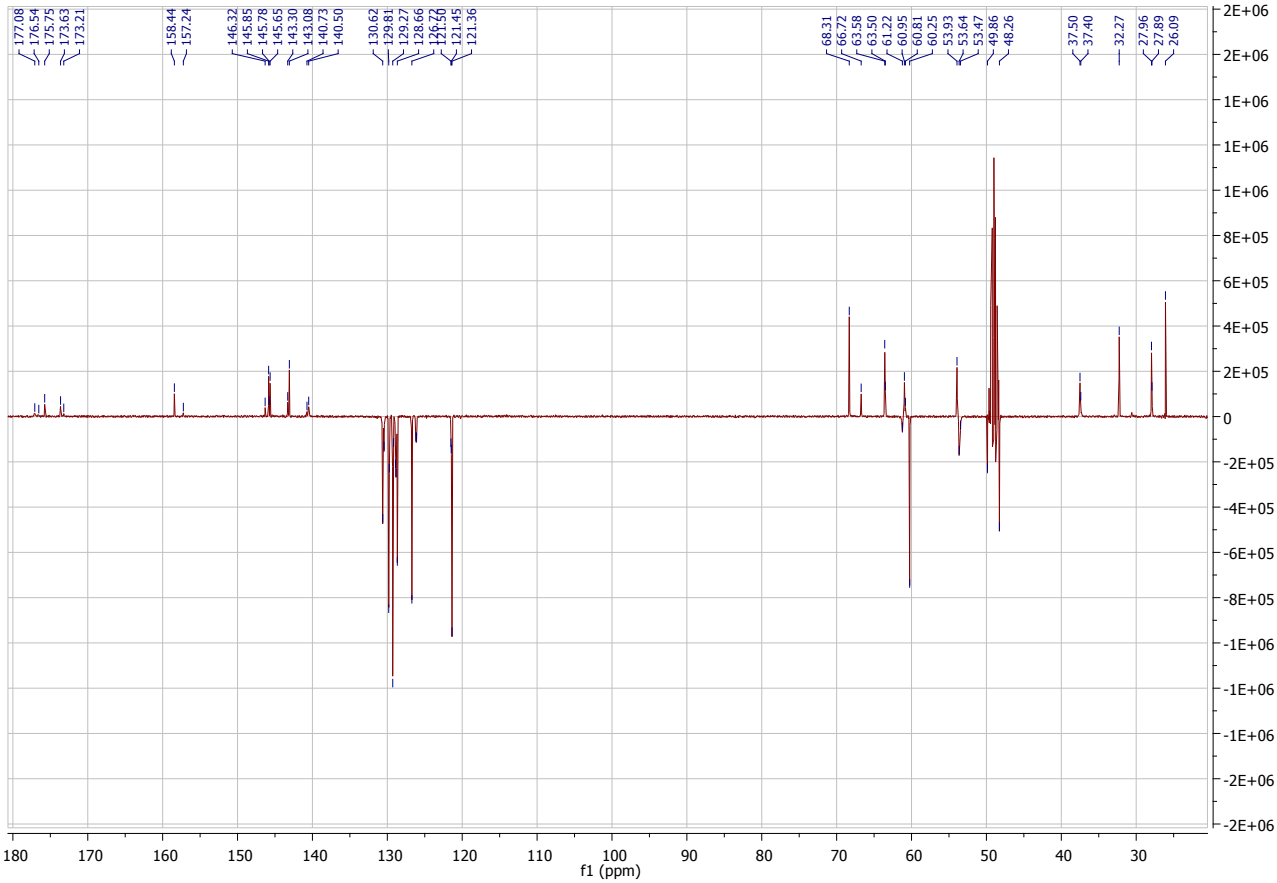
Compound 15. ^{13}C-NMR ($CDCl_3$, 100.6 MHz)



Compound 4. ^{1}H-NMR (CD_3OD, 400 MHz)



Compound 4. ^{13}C-NMR (CD_3OD, 100.6 MHz)



Biological assays

The N-cadh-expressing SKOV3 cells[xii] were obtained from ATCC (Rockville, MD) and maintained in RPMI 1640 medium (Sigma, Sant Louis, Missouri) supplemented with 10% fetal calf serum (FCS) (Hyclone, Logan, UT), 1% L-glutamine at 37°C in a humidified atmosphere of 5% CO_2 in air. The E-cadh-expressing OAW42 cells[xiii] were provided by Dr. A. Ullrich, (Max Planck Institute of Biochemistry, Martinsried, Germany) and maintained in DMEM supplemented with 10% FCS and 1% L-glutamine in a humidified atmosphere of 5% CO_2.

Prior adhesion assay or ELISA, confluent cells were detached using EDTA to protect cadherins from proteolysis and washed twice with PBS without calcium to prevent cell-cell adhesion. For adhesion assay cells were suspended in cell culture medium supplemented with 2% FCS containing varying concentrations of each of the peptidomimetic ligand or the solvent and left to form the monolayer in a humidified atmosphere of 5% CO_2. For ELISA cells were suspended in PBS containing 1.2 mM $CaCl_2$ and varying concentrations (2 and 1 mM) of each of the peptidomimetic ligand or the solvent as above. After 1 h incubation at 37°C cells were added to cadherin-coated plates and incubated for further 2h at 37°C. Ninty-six well plates were coated overnight with 30 ng/well of N-, E-cadh-Fc chimeric protein or human IgG1 Fc fragment (R&D Systems, Minneapolis, MN). After incubation, the wells were washed three times to remove unbound cells. The adherent cells were detected by crystal violet substrate. The intensity of the color was measured at 450 nM. Binding to human Fc fragment of IGg1 was evaluated to exclude unspecific cell binding and subtracted from those obtained with cadherin-Fc proteins.

ADH-1 and compound **1** were dissolved in water, compounds **2** and **3** in 10% DMSO.

SPR analysis

SPR experiments were performed using Biacore 2000 (GE Healthcare). Standard EDC\NHS coupling was used to covalently immobilized recombinant N-cadh-Fc on CM5 (GE Healthcare) sensor chip. Briefly, CM5 chip was activated with ECD\NHS for 7 min with excess activated carboxyl groups blocked with ethanolamine for 7 min following immobilization of N-cadh-Fc, resuspended as suggested by the manufacturer, was diluted to 10 µg\ml in 10 mM sodium acetate, pH 4.8. For all experiments with covalently immobilized N-cadh-Fc, one flow cell served as a reference surface following activation and blocking on each chip in the absence of N-cadh-Fc. One flow cell was immobilized with an uncorrelated recombinant protein (Axl-Fc, R&D) with a similar molecular weight (120 Kd). The sensor chip was equilibrated with PBS in absence of calcium ions, and N-cadh-Fc was dissociated from the chip using the same buffer and the response was recorded for at least 240 s.. N-cadh-Fc (10 nM), freshly diluted in PBS plus 1 mM $CaCl_2$, was applied to the surface of the N-cadh immobilized CM5 sensor chip for 1800 s. Since the compounds to be analyzed (**2** and **3**) are only 600 Dalton and their direct binding to N-cadh is not detectable by the Biacore 2000 instrumentation, an inhibition experiment was performed. For compound **2** and **3**, 10 nM of N-cadh-Fc were pre-incubated for 30 min at 25°C in the presence of each inhibitor at a concentration of 10 µM in PBS plus 1 mM $CaCl_2$ and then applied on the chip. N-cadh-Fc alone was injected after each injection of N-cadh/inhibitor to verify the functionality of the chip and the resulted RU (Resonance Units) max was considered as 100%. After each injection, the sensor chip was regenerated using 30-60 µl of 20 mM EGTA pH 7 to eliminate calcium ions from the system. A flow rate of 5µl\min was kept for all the experiments.

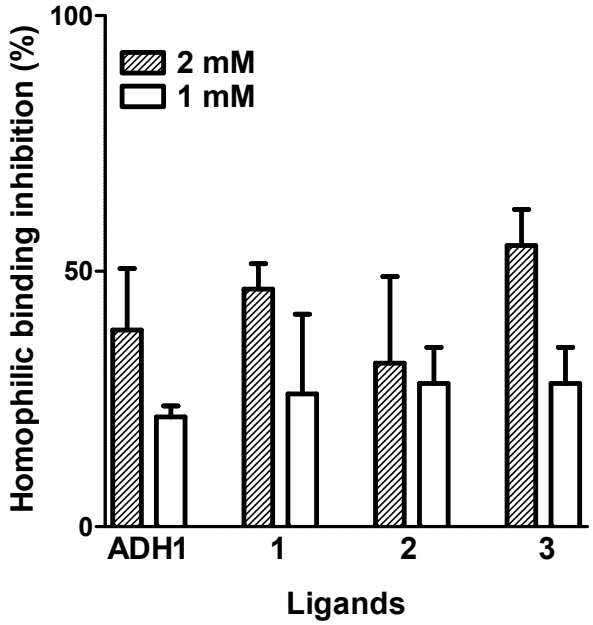


Figure S6. Inhibition of E-cadh homophilic binding by the small peptidomimetic ligands. The inhibition by ADH-1 is reported as control. E-cadh-expressing cells (OAW42) were harvested by EDTA treatment, incubated for 1 h with each ligand at 2 and 1 mM and the homophilic binding to the E-cadh-Fc chimeric protein was evaluated as described above. Binding to the Fc fragment of IGg1 was evaluated and subtracted to exclude unspecific cell binding. The graph reports the mean values±SD.

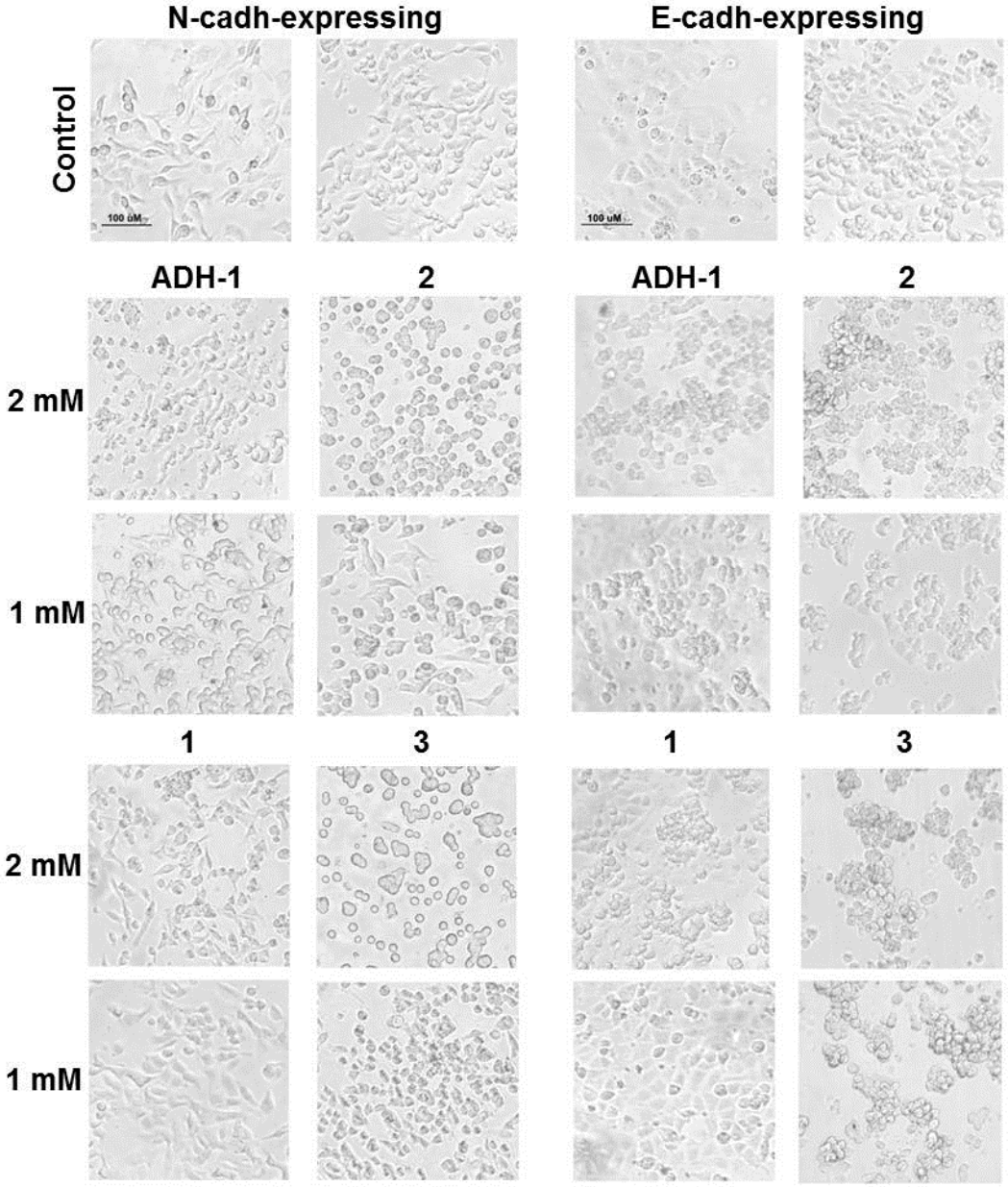


Figure S7. Adhesion assay to evaluate the inhibition of the formation of the cell monolayer by the small peptidomimetic ligands. EDTA-detached N-cadh- (SKOV3) or E-cadh-(OAW42) expressing cells were seeded in absence (Control) or in presence of the ligands at 2 and 1 mM. Micrographs were collected after 3 h seeding. For each cell line the controls without the ligand but with same amount of the solvent (water on the left, DMSO on the right) are reported. The micrographs were taken from three random fields for each experiment. Representative images from three independent experiments are shown.

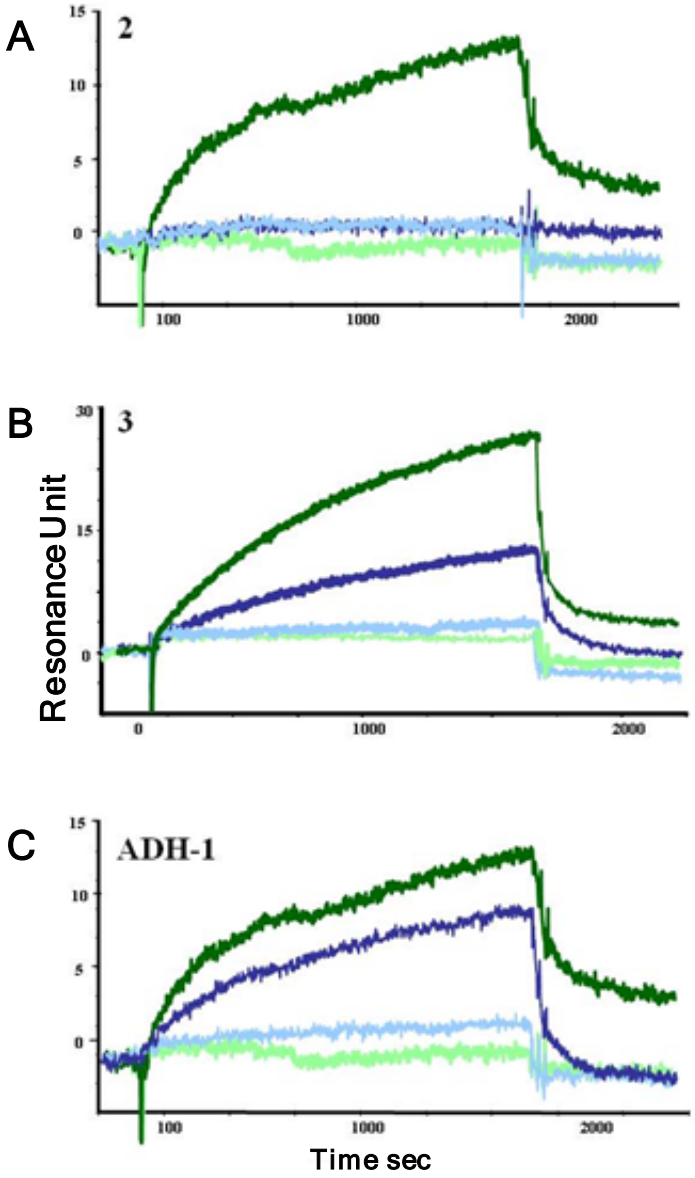


Figure S8. Biacore profiles of the homophilic binding of N-cadh in the presence or absence of inhibitor. A. Inhibition with compound **2**. B. Inhibition with compound **3**. C. Inhibition with ADH-1. Dark green and blue line represent the non-inhibited and inhibited N-cadh, respectively, of the different compounds on N-cadh-Fc-bound to the sensor chip; pale green and pale blue lines represent the binding to the uncorrelated protein-bound to the sensor chip. The percentage of inhibition has been calculated considering as 100% the resonance unit max obtained with the N-Cadh-Fc alone run immediately before the loading of each inhibitor/N-cadh-Fc complex.

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