Electronic Supplementary Material (ESI) for RSC Advances.

Identification of the first surrogate agonists for the G protein-coupled receptor GPR132

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Supplementary Figures and Tables

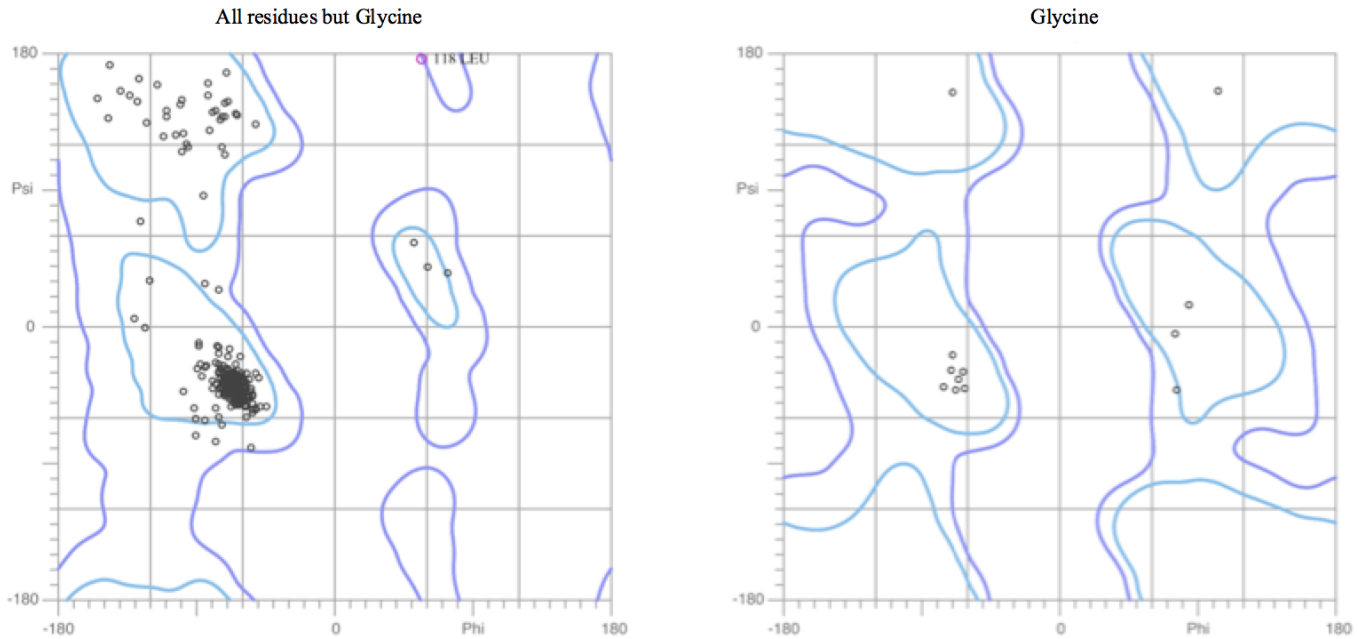


Figure SI1. Ramachandran plots (the right is specific for glycine, which lacks a sidechain) of the GPR132 model produced with PROCHECK[41] where 93.5%, 6.5% and 0.0% of the residues are falling within the favorable, allowed and disallowed Phi (φ) and Psi (Ψ) angle regions respectively.

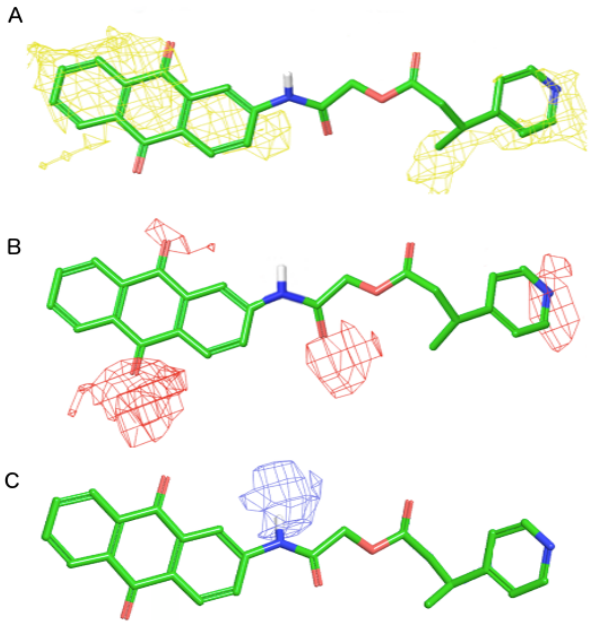


Figure SI2. (A) Hydrophobic, hydrogen bond **(B)** donor and **(C)** -acceptor sites within the GPR132 binding pocket identified by SiteMap[47]. The agonist **1** (shown for the enantiomer (*R*)-**1** here) involves 5 of 7 polar atoms in receptor interactions and it has a tight fit into the binding pocket with a good match to the hydrophobic and polar areas. Residue positions are indexed with the GPCRdb scheme[48] to correct for bulges and constrictions.

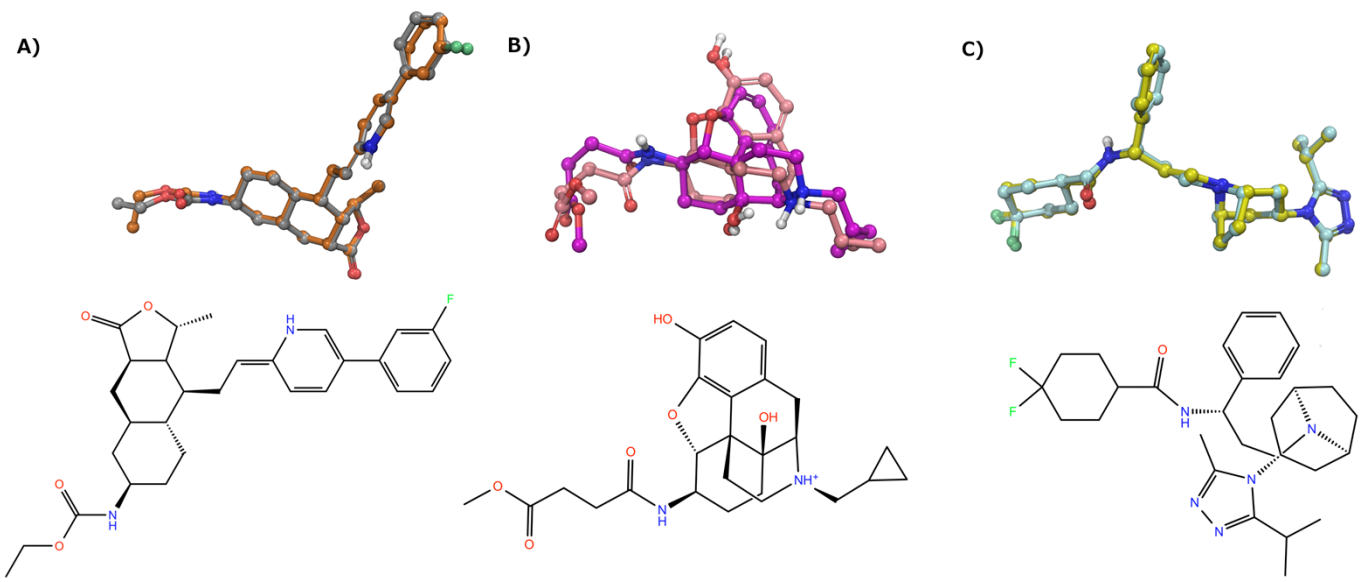


Figure SI3. Comparison between the observed docking poses and the respective bioactive conformation of the corresponding ligands co-crystallized with the three most homologous GPCRs to GPR132. The ligands of (**A**) the protease–activated 1, (**B**) the μ-opioid, and (**C**) the chemokine type 5 receptors, respectively were re-docked using Glide. Each ligand showed very similar docking pose to its corresponding bioactive conformation. Superimposition of the 3D conformations is shown on top, where grey, pink, and yellow colors represent the bioactive conformation compared to orang, purple, and lime-green in the re-docked ligands in the three receptors, respectively. The 2D structures of the compounds are shown below each ligand for convenience.

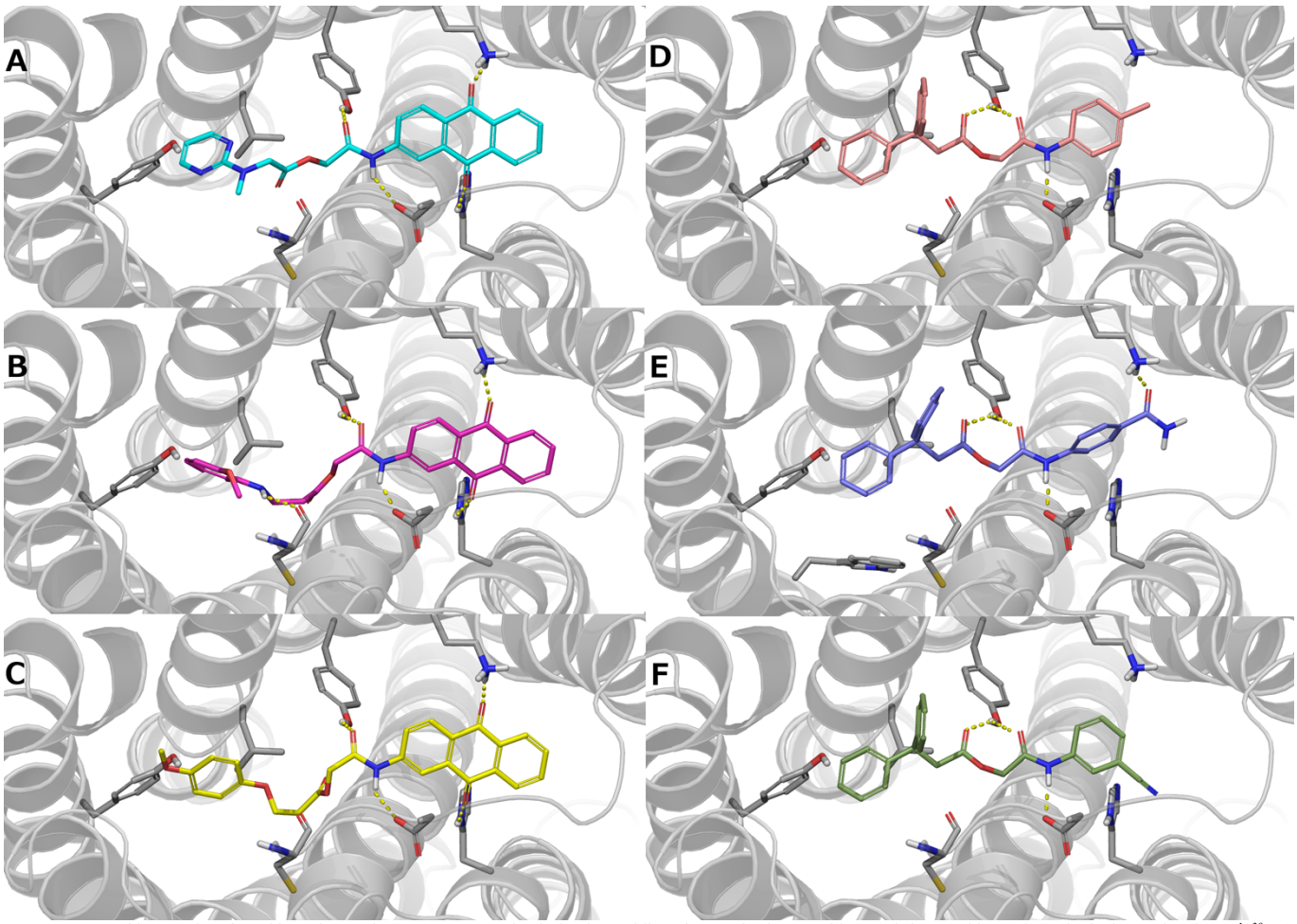
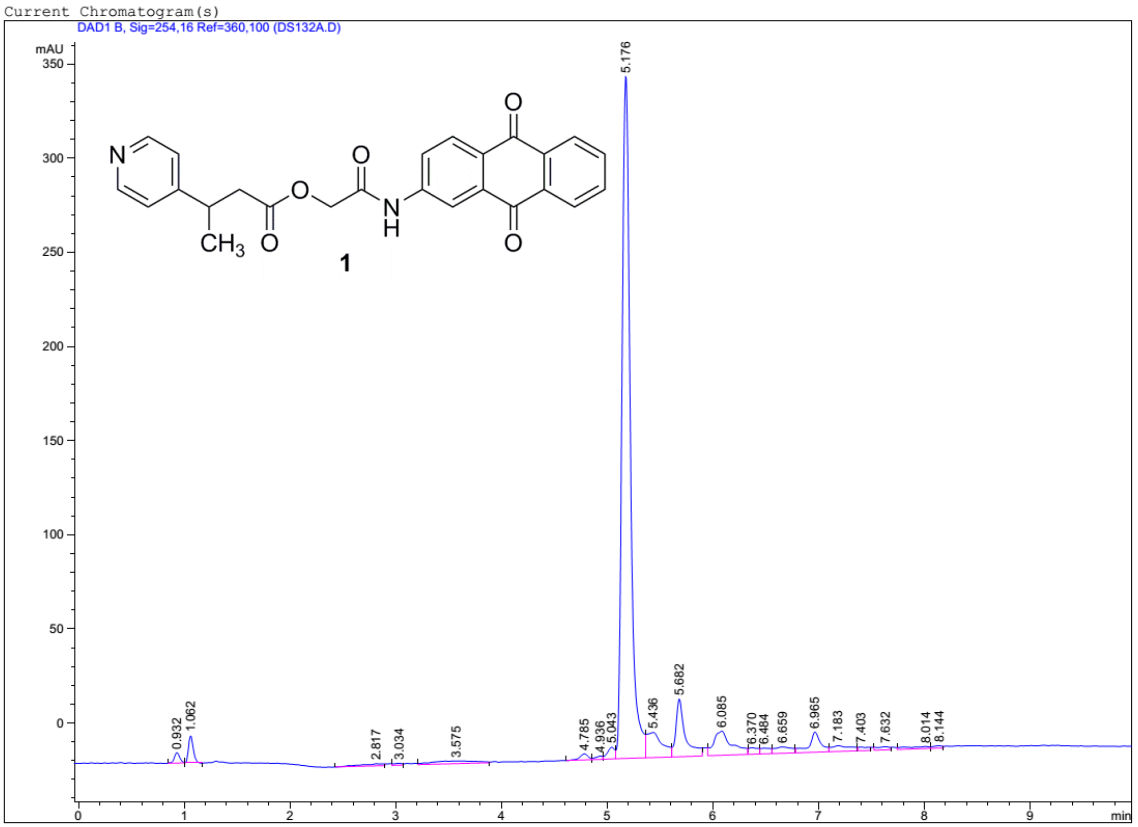
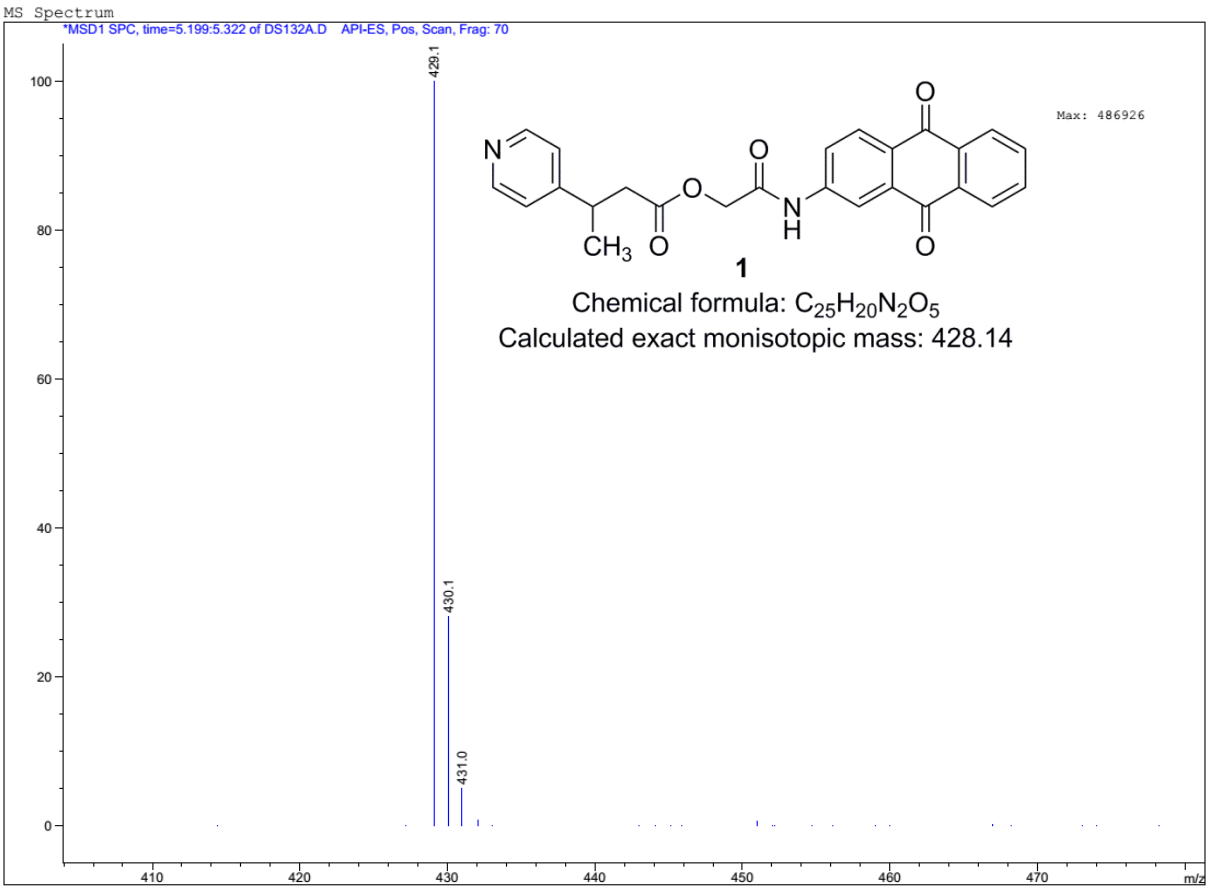


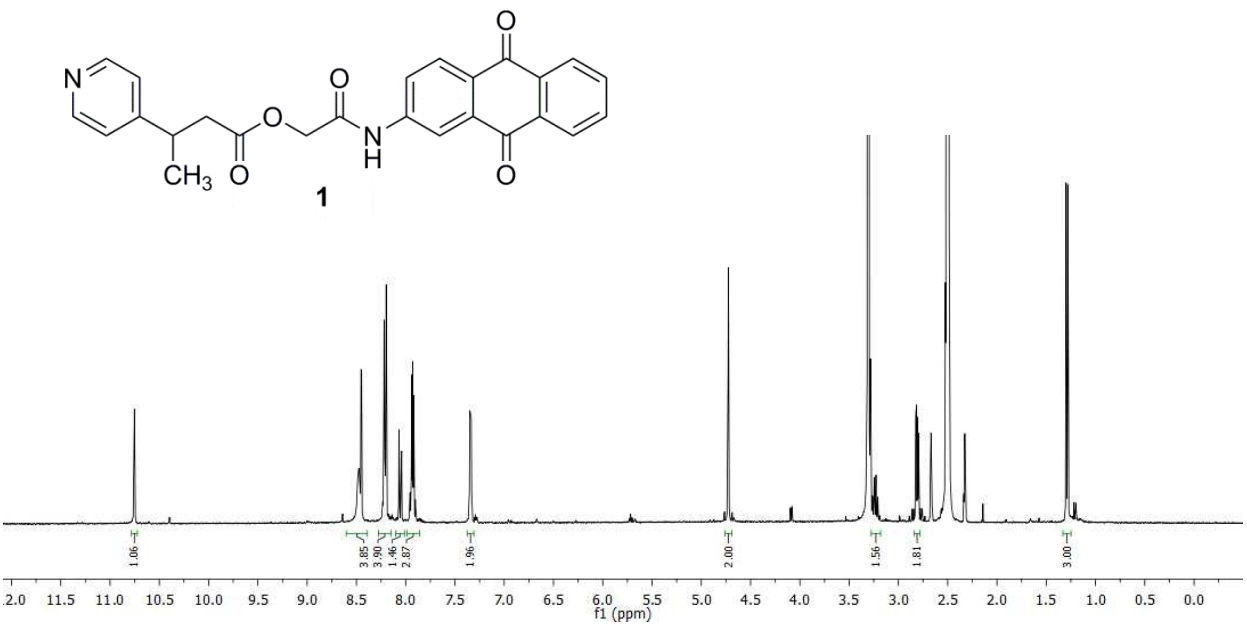
Figure SI4. Docking results of weakly active analogs. **A**) Compound **2** lacks the pyridine nitrogen in the 4-position, and thus could not share the hydrogen bond to $Y^{1.x39}$. **B**) Compound **9** showed that a nitrogen atom could possibly form hydrogen bond interaction to the backbone carbonyl of C^{45x50} (the ECL2 residue forming a disulfide bridge to the top of TM3). **C**) The oxygen in **10** displayed no such interaction. **D-F**) In analogs **31**-**33**, the 1,1-diphenyl group docked into the same site as the pyridine moiety of compound **1**, consequently, the linker displayed same interactions while ligands lost hydrogen bond interactions with Y^{1x39} and K^{6x58}. An extra hydrogen bond by the propionate linker was also observed to Y^{6x51}.

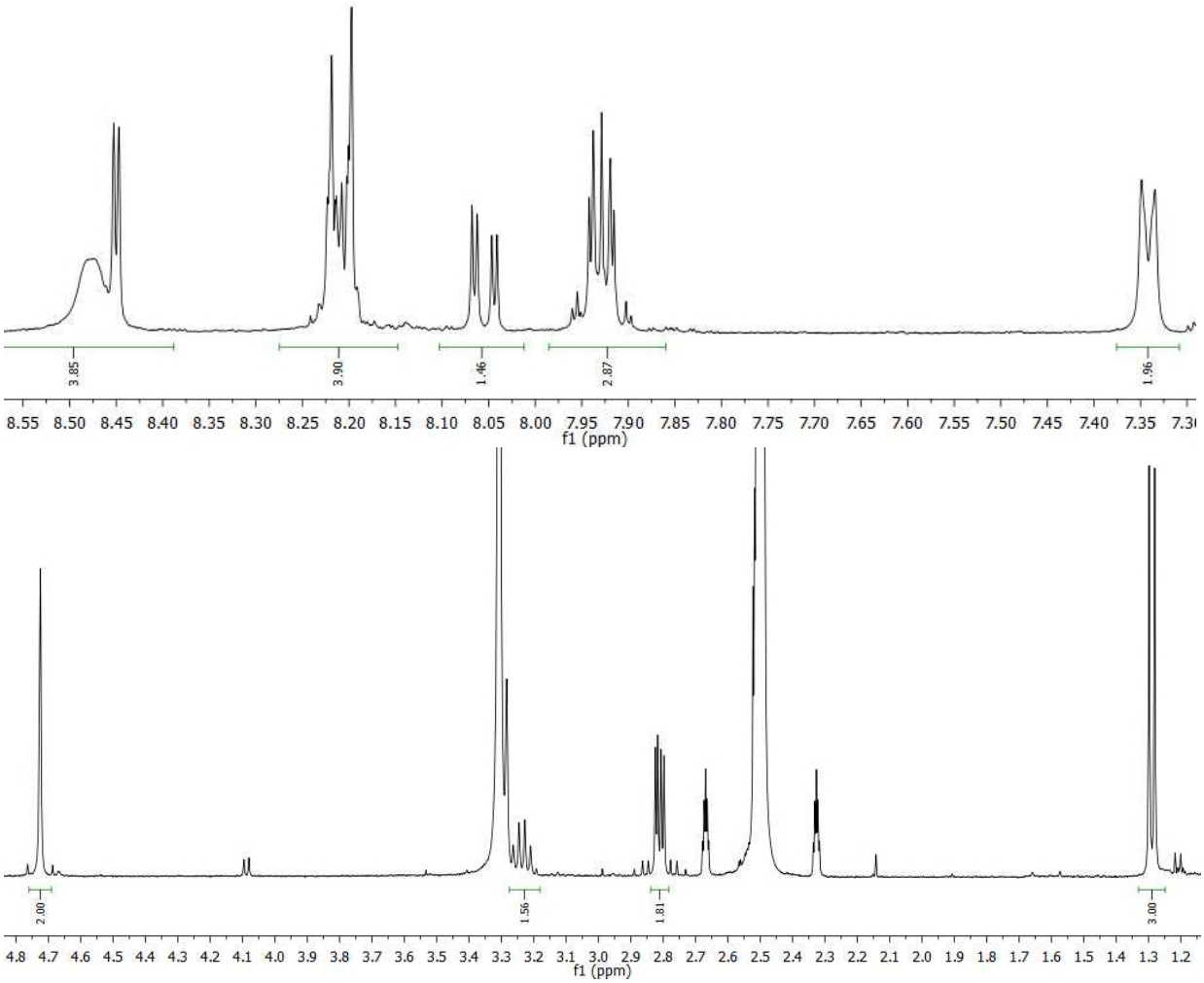
Chart SI2. HPLC chromatogram, MS and ^{1}H NMR spectrum for commercially acquired ligand **1**.

^{1}H NMR was recorded in DMSO-d_6 at 300K on a 400 MHz Bruker Avance instrument and the obtained FID-file processed with MNova 8 software. HPLC-MS was carried out on an Agilent 1100 series system using a Waters XBridge C18 column (3.5 µm, 100 ×4.6 mm) with UV detection at 254 nm. Mobile phase A: 0.5% HCOOH, 5% MeCN, 95% H_2O (v/v/v). Mobile phase B: 0.5% HCOOH, 5% H_2O, 95% MeCN (v/v/v). Flow rate: 1 ml/min. Gradient: 0-1 min: 100% A; 1-6 min: 0-100% B; 6-8 min: 100% B. MS were recorded on a Hewlett Packard Series 1100 MSD mass detection system that was connected to an Agilent 1100 HPLC system using atmospheric pressure ionization electrospray (API-ES).




MS Spectrum
*MSD1 SPC, time=5.199:5.322 of DS132A.D API-ES, Pos, Scan, Frag: 70
Max: 486926
429.1
430.1
431.0
100
80
60
40
20
0
410
420
430
440
450
460
470
m/z
1
Chemical formula: C25H20N2O5
Calculated exact monisotopic mass: 428.14


1
1.06
3.05
3.90
1.96
2.87
1.96
2.00
1.56
1.81
3.00
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


3.85
3.90
1.46
2.87
1.96
8.55 8.50 8.45 8.40 8.35 8.30 8.25 8.20 8.15 8.10 8.05 8.00 7.95 7.90 7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35
f1 (ppm)
2.00
1.56
1.81
3.00
4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2
f1 (ppm)