Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information

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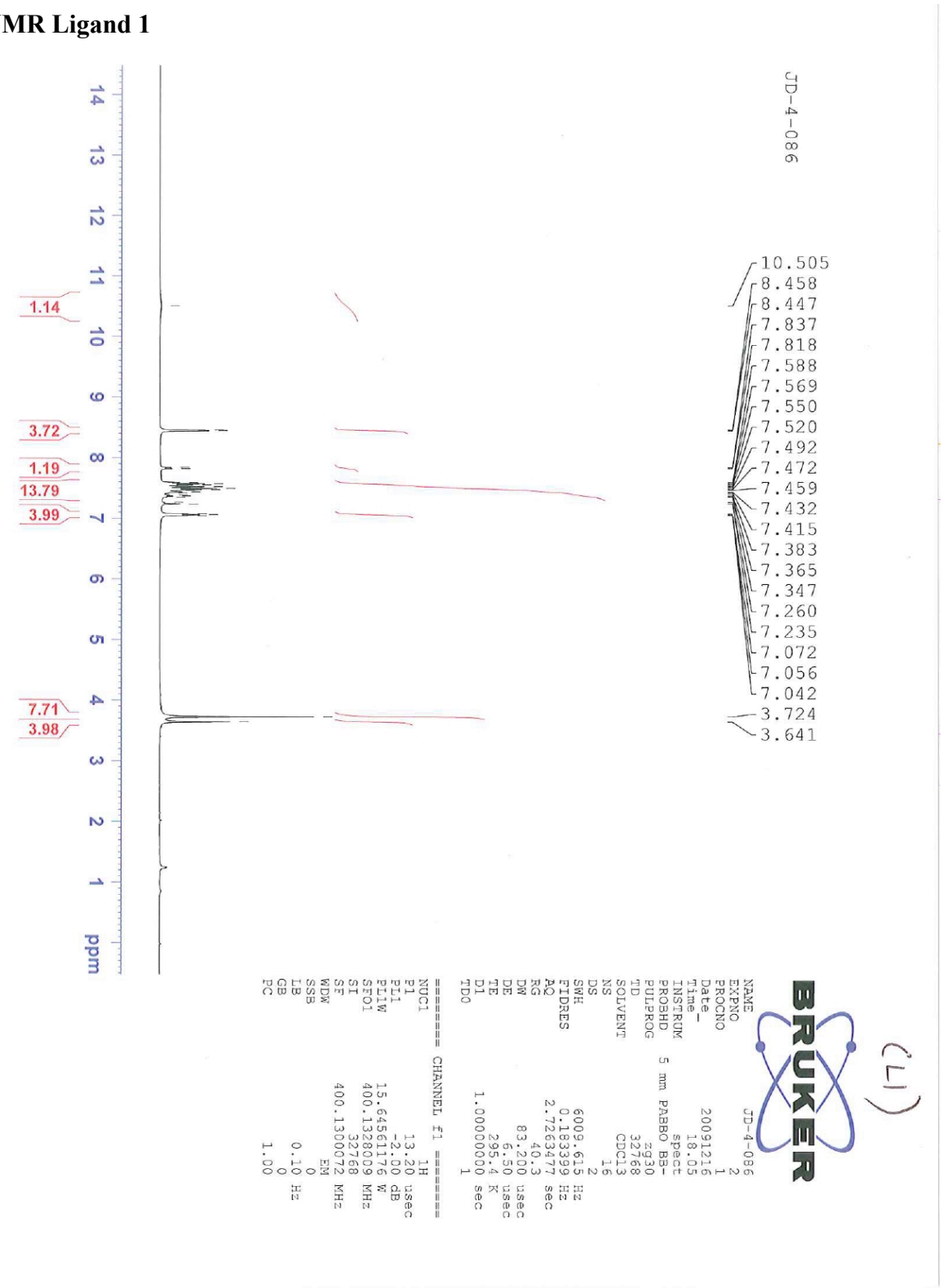
S1. Chemistry: General Methods. Anhydrous solvents methanol, DMSO, CH_2Cl_2, THF and DMF were purchased from Sigma Aldrich and used directly from Sure-Seal bottles. Molecular sieves were activated by heating to 300 °C under vacuum overnight. All reactions were performed under under atmospheric conditions in oven-dried glassware and were monitored for completeness by thin-layer chromatography (TLC) using silica gel (visualized by UV light, or developed by treatment with $KMnO_4$ stain or phosphomolybdic acid stain). All reactions with the exception of the final metallations were carried out in a Biotage Initiator microwave at temperatures and durations indicated in the representative synthesis shown in S3. ^1H and ^{13}C NMR spectra were recorded on Bruker 400 MHz and a Varian 500 MHz spectrometers in either $CDCl_3$, CD_3OD or d_6-DMSO. Chemical shifts (δ) are reported in parts per million after calibration to residual isotopic solvent. Coupling constants (*J*) are reported in Hz. Purification of scaffolds prior to metallation was achieved using flash chromatography (silcica). Ligand purity was confirmed by analytical rpHPLC using linear gradients from 100% 0.01M $NH_4OAc_{(aq)}$ (A) to 100% methanol (B), with changing solvent composition of either (I) 6.25% or (II) 9.34% per minute after an initial 2 minutes of 100% A.

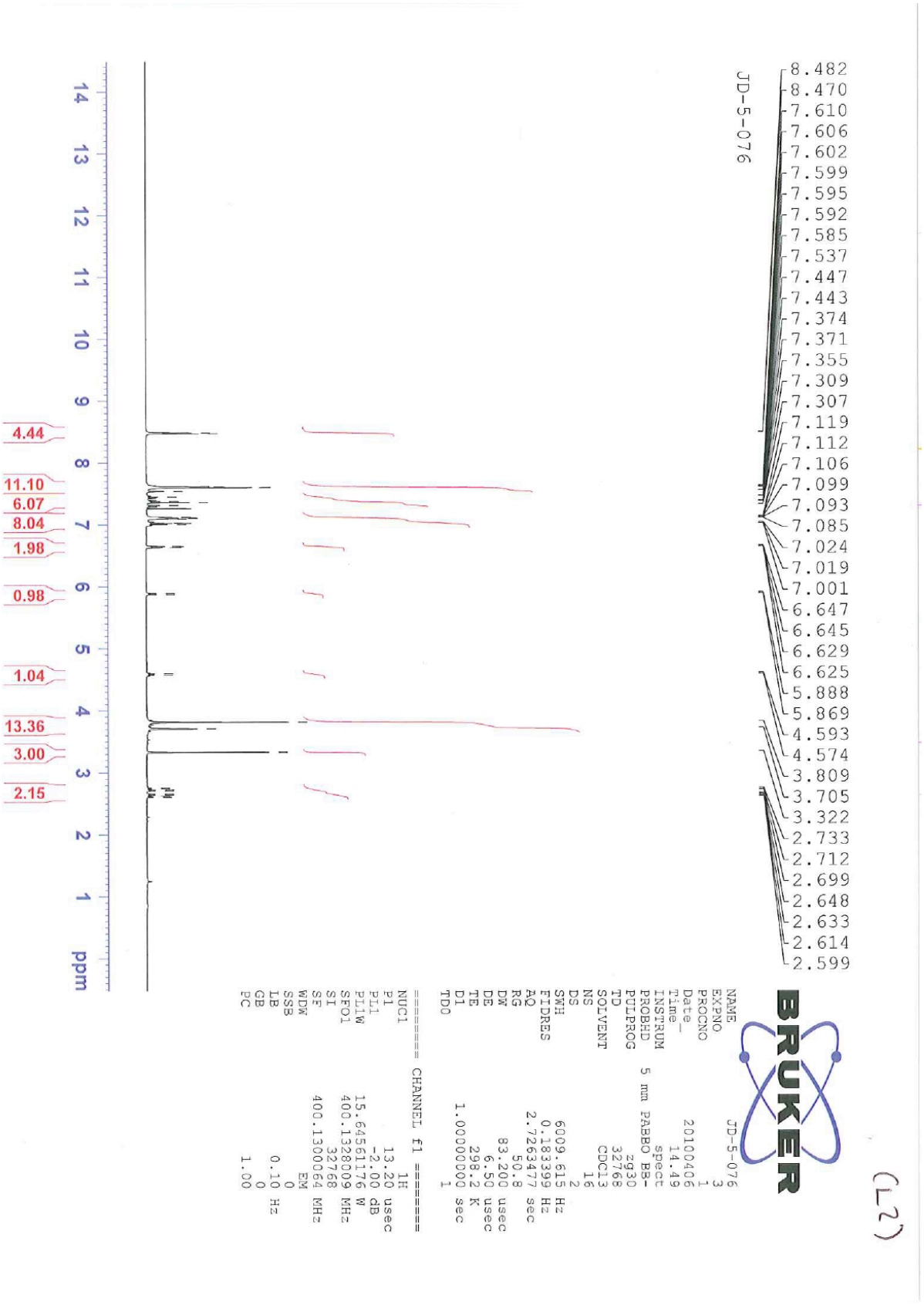
S2. Compound Characterization

S2.1 Analytical HPLC Traces and NMR (^{1}H, ^{13}C) Spectra of Final Scaffolds

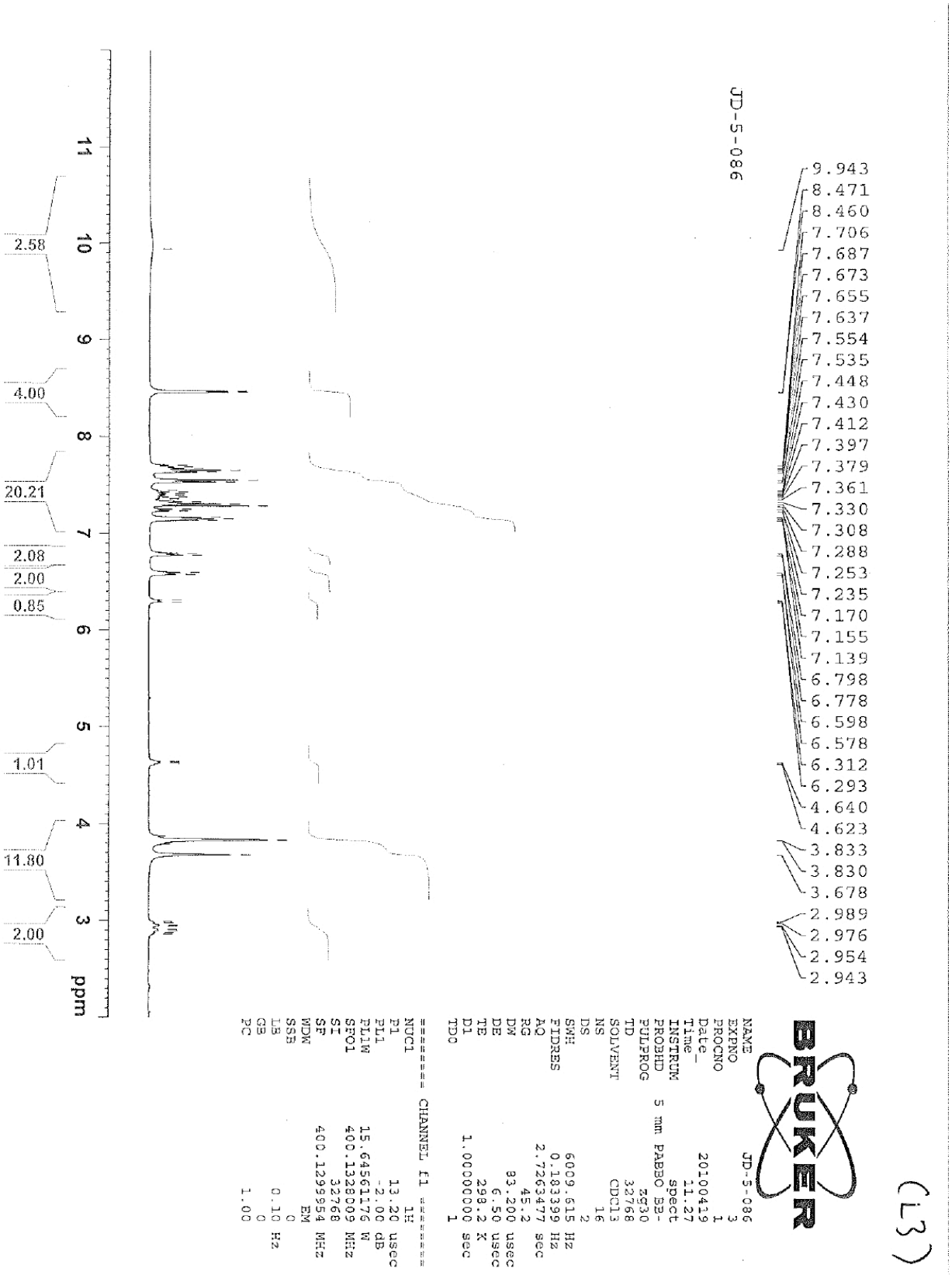
^{1}H NMR Ligand 1



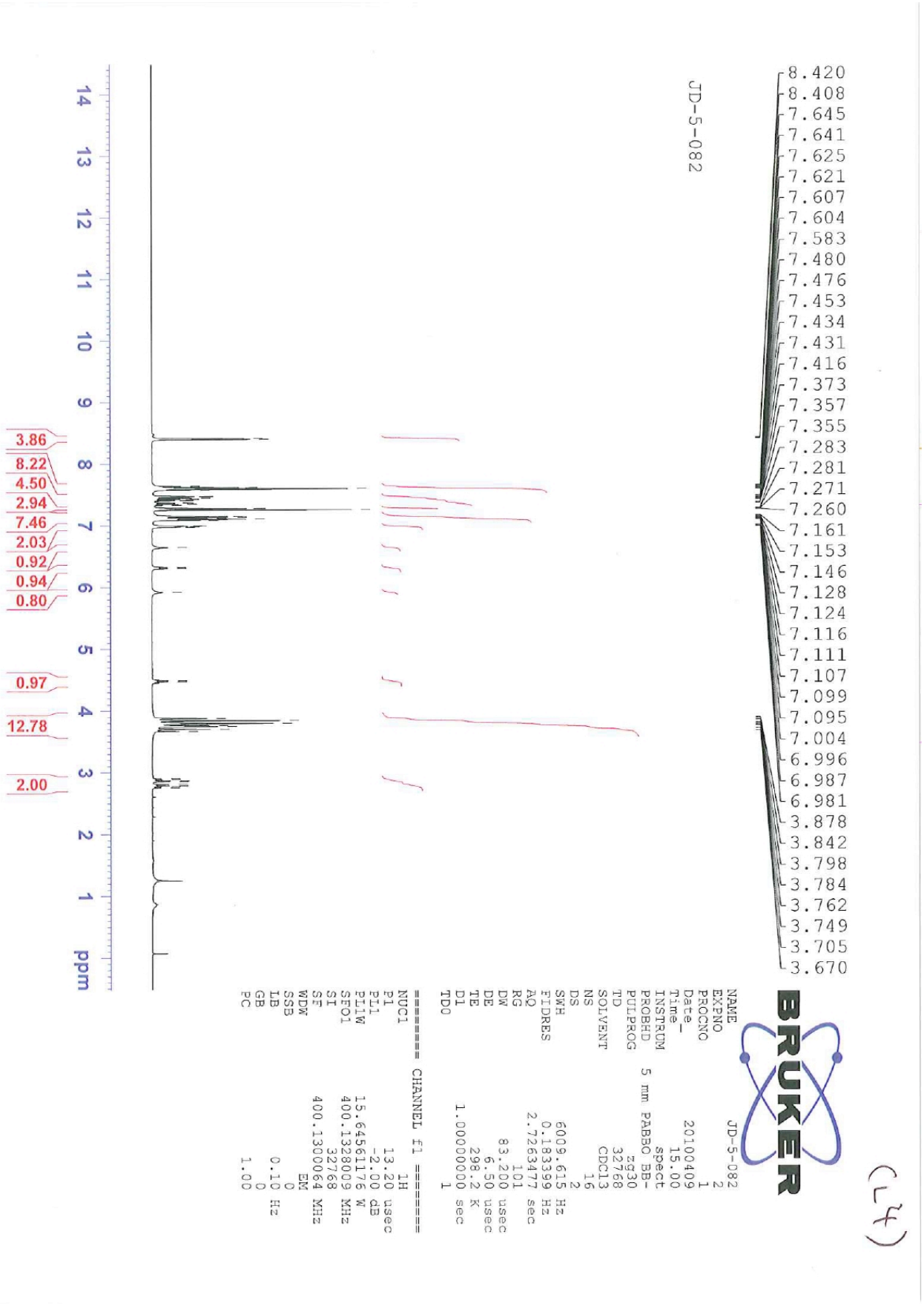
^{1}H NMR Ligand 2



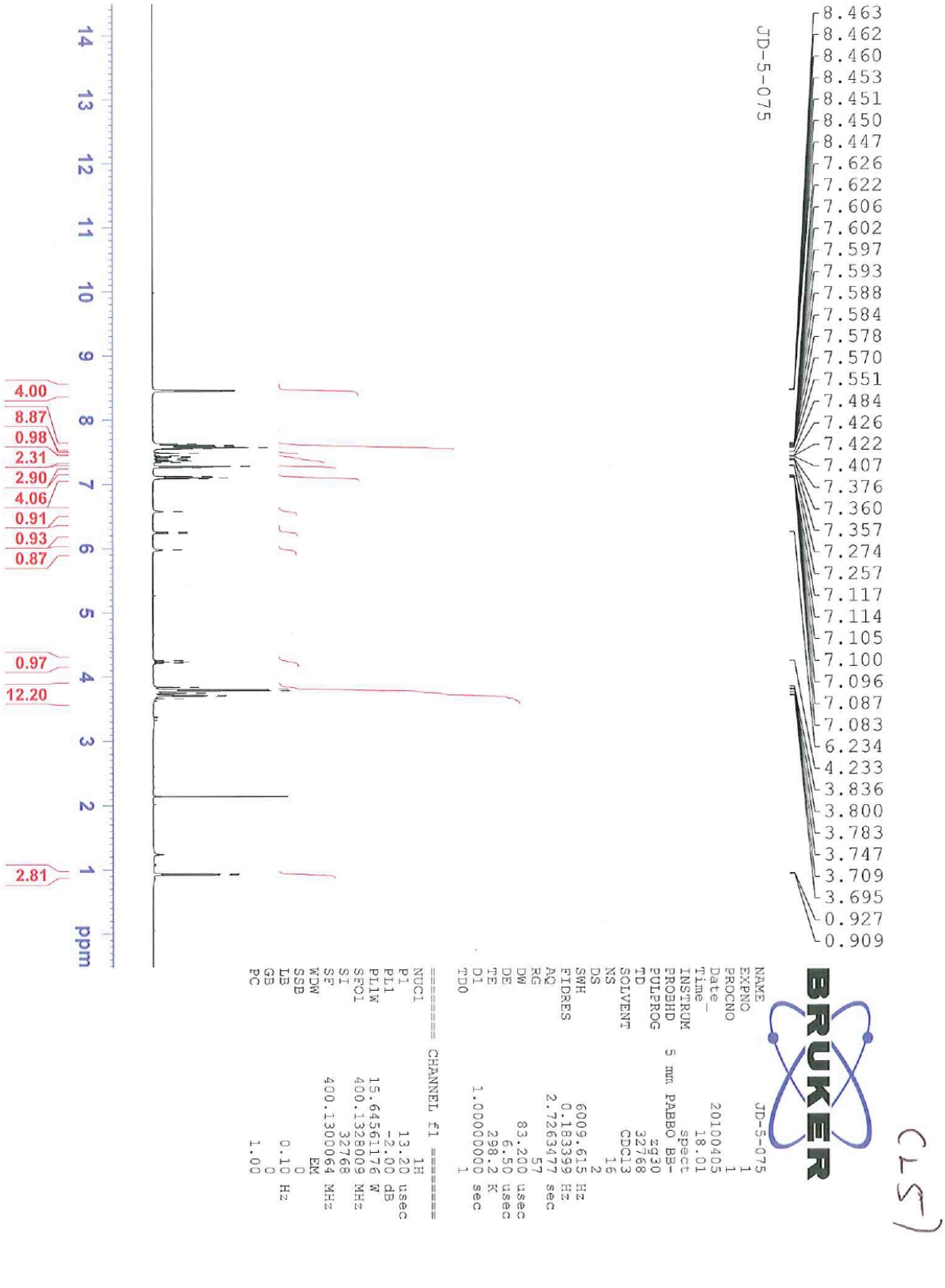
^{1}H NMR Ligand 3



^{1}H NMR Ligand 4



^{1}H NMR Ligand 5



S3. Computational Studies

Quantum mechanical calculations were done with Gaussian09 and all molecular mechanical (MM) calculations were done with the AMBER software package. Parameters for the amino acid sequence and derivatized ends were taken from the ff99SB force field. For the BDPA scaffold parameters were not available nor was there a crystal structure.

To obtained a starting structure a number of different geometries were optimized using HF/6-31G* with extra diffuse functions placed on the Zn, O, P and N atoms. The coordination of the dizinc center, the number of waters present and the distance between the two zinc atoms were all varied. The lowest total solution free energy structure evaluated at the B3LYP level was used for further parametrization. To obtain charges a Restrained Electrostatic Potential (RESP) fit was done with AMBER using BDPA bound to pY with a methyl group used as the -R group. Force constants for most of the structure were obtained with antechamber. Parameters for the dizinc center were not available and so an in-house software package was used to obtain these through an iterative procedure of matching up the frequencies from a HF calculation with those from the MM parameters. When minimized, the resulting structure had a low root mean square deviation (RMSD) to the HF optimized one.

The parametrized residue was incorporated into a polypeptide sequence with the 'tleap' program provided with AMBER. Molecular dynamics (MD) using the Born implicit solvent model was then run on the resulting bound BDPA derivative after a short sequence of minimization and equilibration. The production MD was done at 300K using a Berendsen thermostat, using a time step of 2fs. Initial biasing was done by introducing a 10kcal/mol distance restraint between atoms and running 50ps of equilibration MD.

For the interactions between 3 and the Stat3 50ns MD trajectories were run with two different initial distance restraints to atoms on residues at the Y+3 and Y+4 position. The trajectories converged to similar structures. The trajectory initially biased to the Y+3 position was used for the hydrogen bond analysis. The potential energy of this run and the RMSD of the backbone are given in Figure 2 and 3:

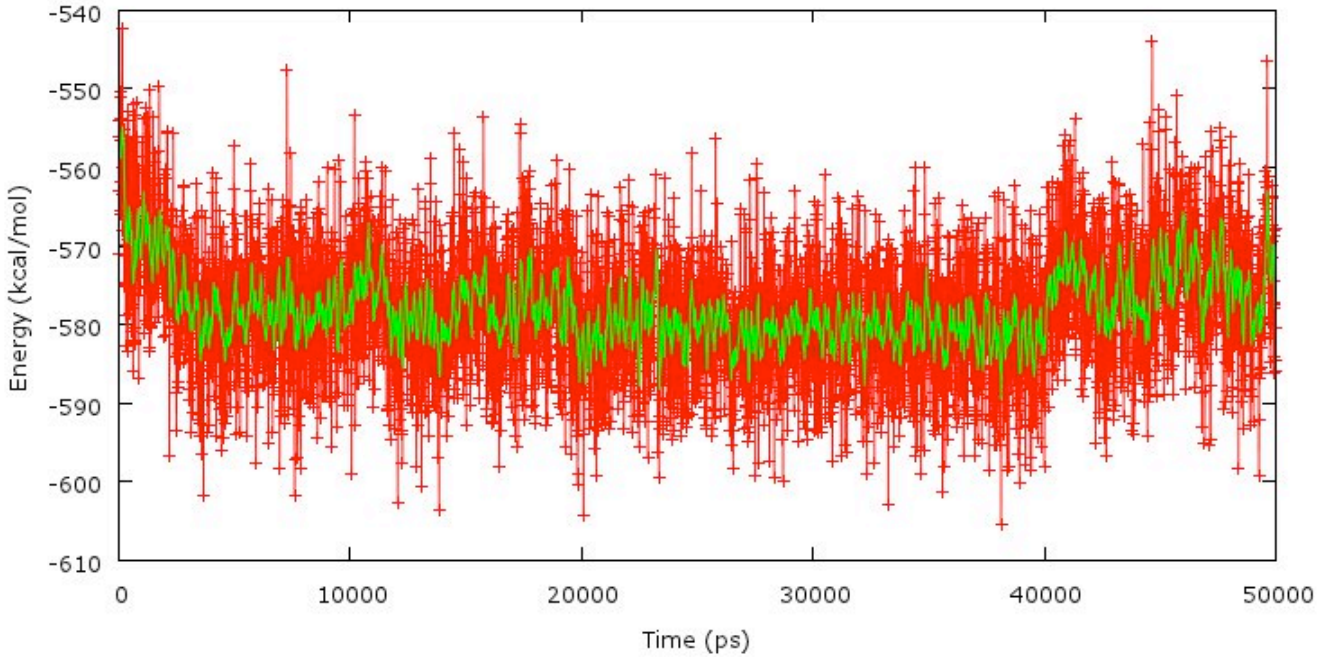


Figure 1. Potential energy of the interactions between 3 and the Stat3 from a 50ns MD trajectory with implicit solvent.

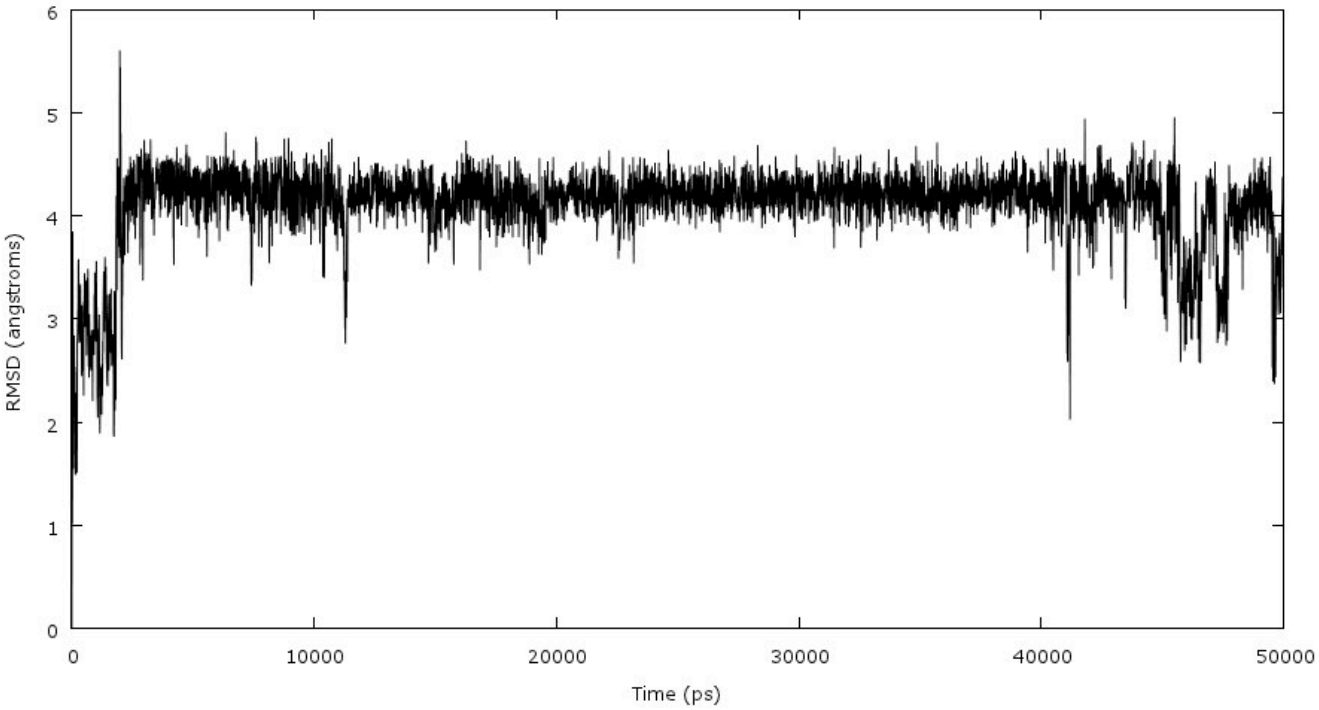


Figure 2. Root mean square deviation of the Tyr derivative bound to Stat3.

To analyze the Phe derivative bound to the GP130 sequence, cluster analysis was done to find similar structures since hydrogen bonding was not the predominant interaction governing binding. Four different initials biases were used in the equilibration stage to generate five different trajectories of length 20ns (one had no bias). The program 'kclust' which is part of the MMTSB package was then used to do the cluster analysis. Different cluster radii ranging from 3-6 were used. With a radius of 5, ~25% of the population was close to the centroid resembling the structure shown in Figure 3B (Main paper). Many of these structures came from the trajectory in which biasing was not introduced in the equilibration phase: