

# Supporting Information

## Fragment-based identification of ligands for bromodomain-containing factor 3 of *Trypanosoma cruzi*

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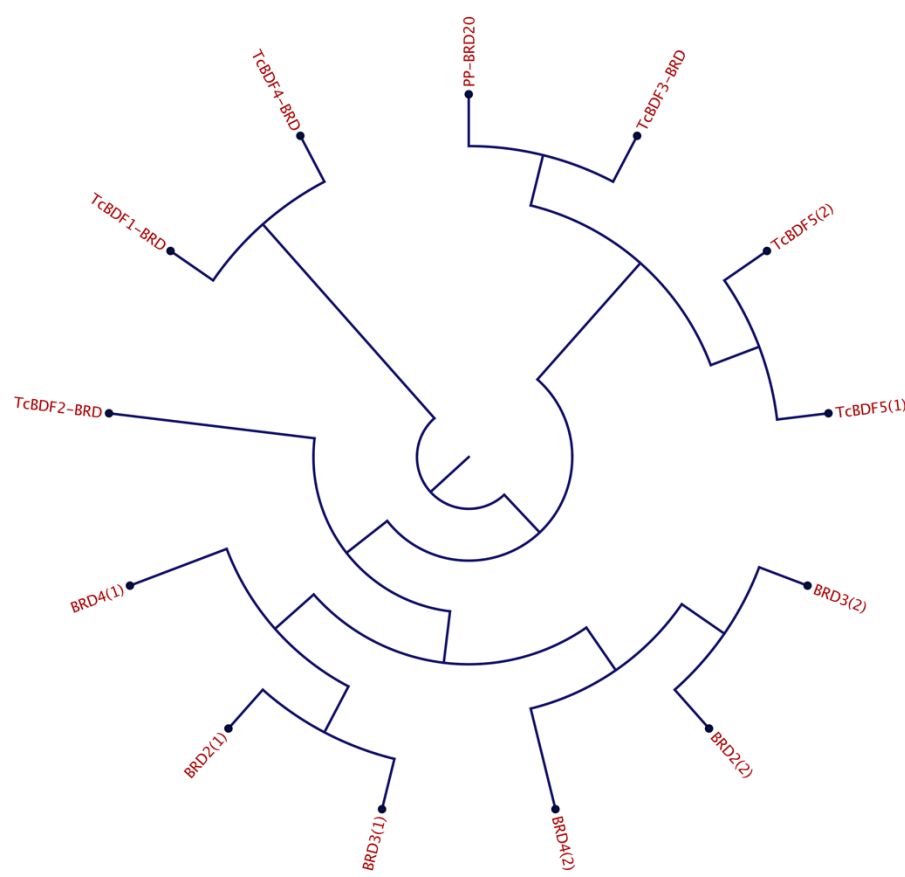
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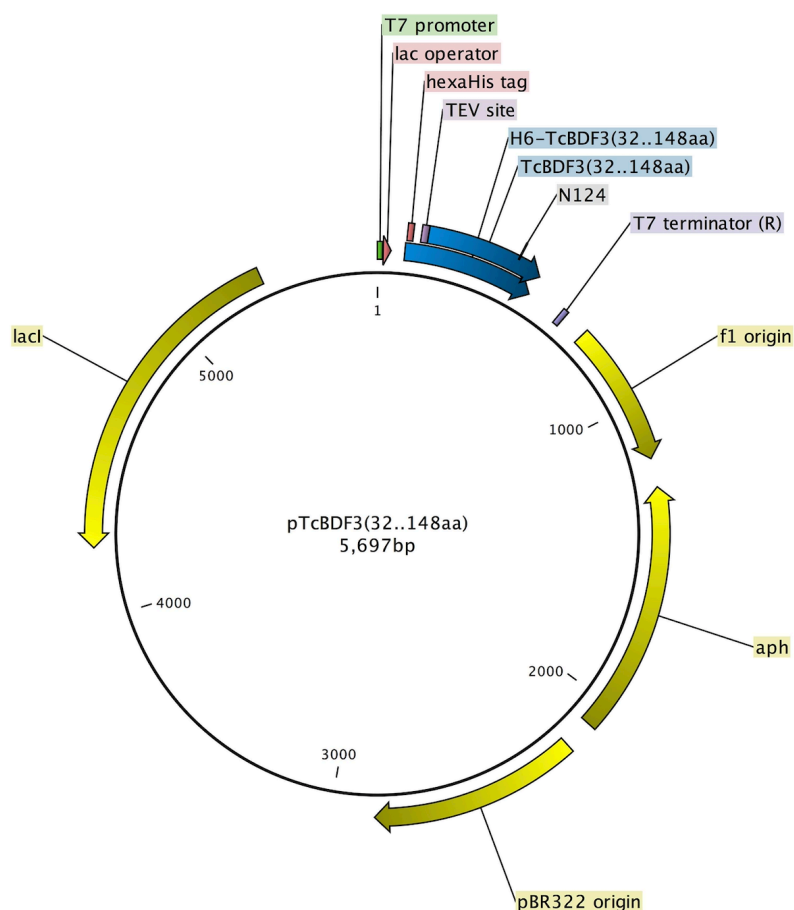
SI-A: SEQUENCE ANALYSIS & PROTEIN EXPRESSION

**Table S1:** Pairwise sequence identity percentages comparing the *T. cruzi* bromodomains (TcBDF1-5) protein to the human BET bromodomains (BRD2-4) and PP-BRD20, which is a bromodomain from *Trypanosoma brucei gambiense* (strain MHOM/CI/86/DAL972).

		1	2	3	4	5	6	7	8	9	10	11	12	13
TcBDF3-BRD	1		17.31	18.60	17.83	22.48	23.65	25.20	55.15	17.89	17.60	12.71	12.88	12.80
BRD2(1)	2	17.31		67.97	62.09	34.19	41.67	29.68	17.20	17.42	15.38	12.90	9.32	8.21
BRD3(1)	3	18.60	67.97		76.38	39.53	37.41	37.60	17.14	22.05	14.96	15.20	10.53	8.98
BRD4(1)	4	17.83	62.09	76.38		37.98	33.33	32.81	17.14	20.77	18.60	14.84	14.81	8.88
BRD4(2)	5	22.48	34.19	39.53	37.98		65.31	69.53	20.00	16.92	19.38	21.09	11.11	7.69
BRD2(2)	6	23.65	41.67	37.41	33.33	65.31		64.63	23.33	16.33	15.54	16.33	11.04	7.98
BRD3(2)	7	25.20	29.68	37.60	32.81	69.53	64.63		20.71	19.49	21.01	24.78	12.70	9.55
PP-BRD20	8	55.15	17.20	17.14	17.14	20.00	23.33	20.71		15.22	14.29	13.97	14.48	11.86
TcBDF2-BRD	9	17.89	17.42	22.05	20.77	16.92	16.33	19.49	15.22		16.53	11.30	10.32	7.50
TcBDF4-BRD	10	17.60	15.38	14.96	18.60	19.38	15.54	21.01	14.29	16.53		16.81	16.67	9.38
TcBDF1-BRD	11	12.71	12.90	15.20	14.84	21.09	16.33	24.78	13.97	11.30	16.81		13.49	8.11
TcBDF5(1)	12	12.88	9.32	10.53	14.81	11.11	11.04	12.70	14.48	10.32	16.67	13.49		25.16
TcBDF5(2)	13	12.80	8.21	8.98	8.88	7.69	7.98	9.55	11.86	7.50	9.38	8.11	25.16	

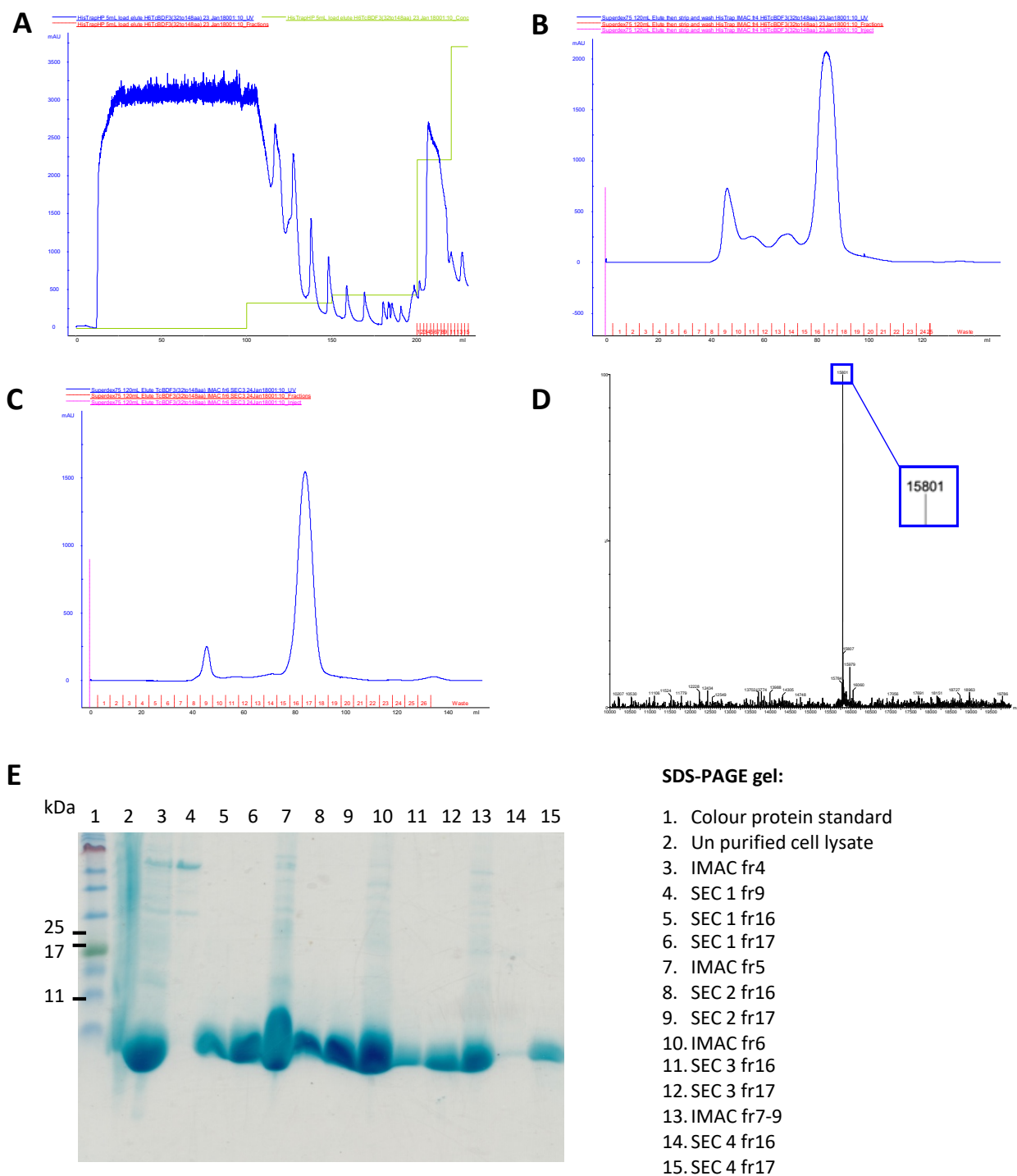


**Figure S1:** Cladogram summarizing the sequence identities similarities between the *T. cruzi* bromodomains (TcBDF1-5), the human BET bromodomains (BRD2-4), and PP-BRD20.

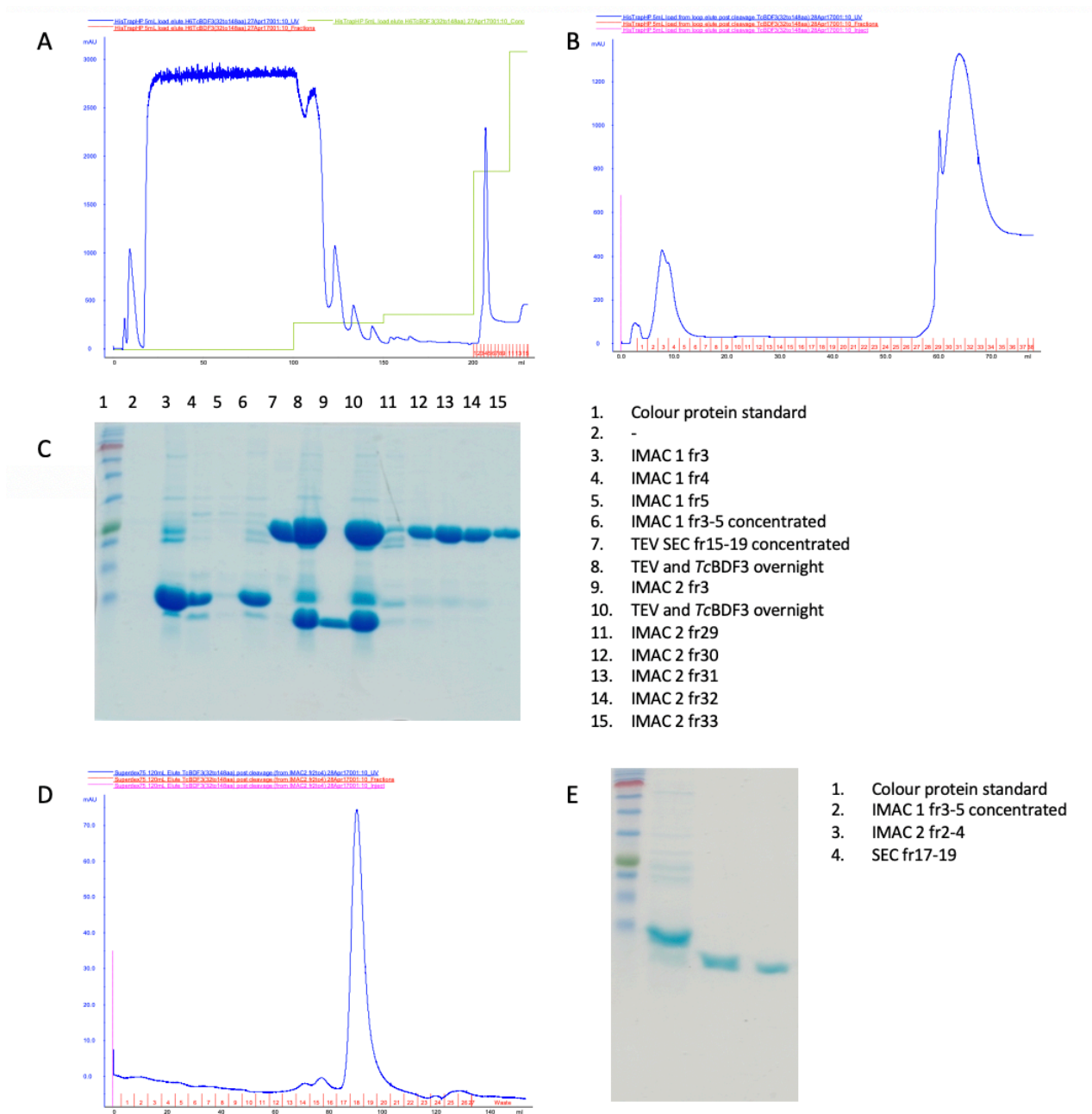


**Figure S2:** Plasmid map for pTcBDF3(32..148aa), encoding for the soluble protein construct: 32<sup>nd</sup> to 148<sup>th</sup> amino acid sequence of the full length *TcBDF3* protein (GenBank ID: XP\_812334.1). The *TcBDF3* protein sequence encoded in pDEST17-*TcBDF3* (a gift from Prof. Esteban Carlos Serra). The pDEST17-*TcBDF3* plasmid was used as the DNA template to amplify the full-length *TcBDF3* gene by PCR. The pTcBDF3\_FL plasmid was used as the DNA template to amplify the region encoding for amino acids 32 to 148. This PCR product was assembled into the NcoI-HF and BamHI-HF restricted pNIC28-Bsa4 vector to clone for the new pTcBDF3(32..148aa) plasmid. All of the sequence analysis, editing, and annotation were performed in CLC Main Workbench v7.9.1 (QIAGEN). The pTcBDF3(32..148aa) plasmid encodes for the H<sub>6</sub>-*TcBDF3*(32..148aa) protein with the following amino acid sequence:

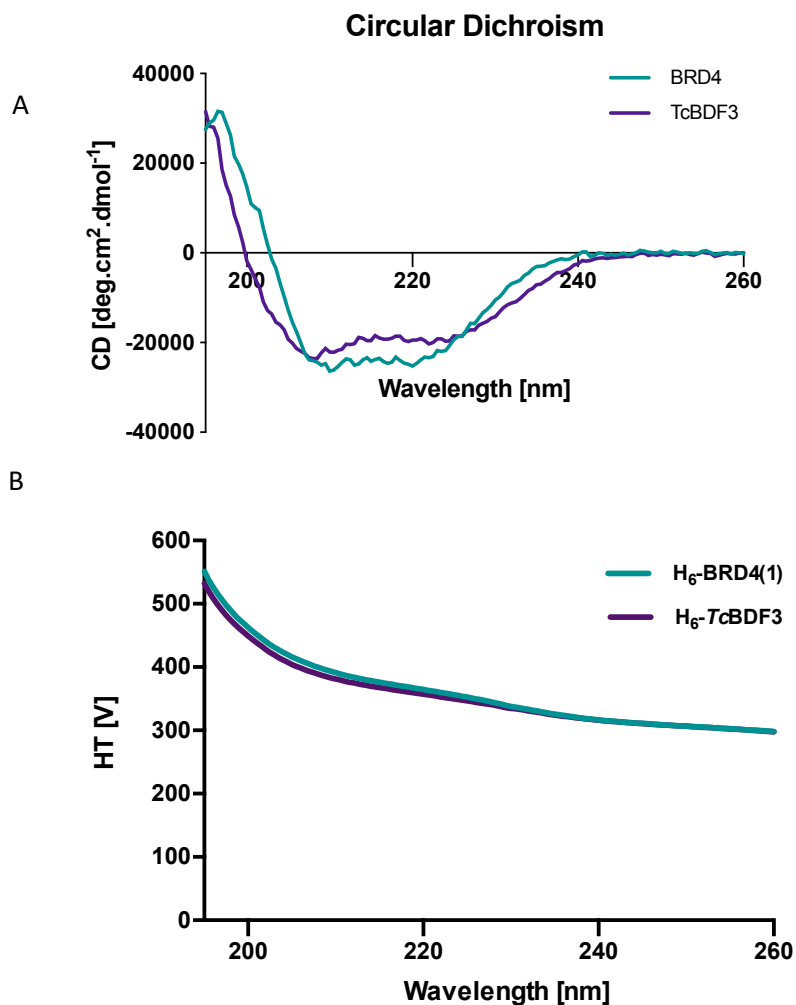
MHHHHHHSSGVDLG TENLYFQSGKHVFRSDLSTVPEVRDIYDCLYKLYAEESASASFREPV  
NALELGVFNYEYEWTEPMSLR TVLDRIAEGGHYSQASQVLADVEKIWSNCEKYNGADSALVK  
EAKKCQGILTRLRERL (MW: 15803.7 Da,  $\epsilon_{280} = 17545 \text{ M}^{-1}\text{cm}^{-1}$ ).



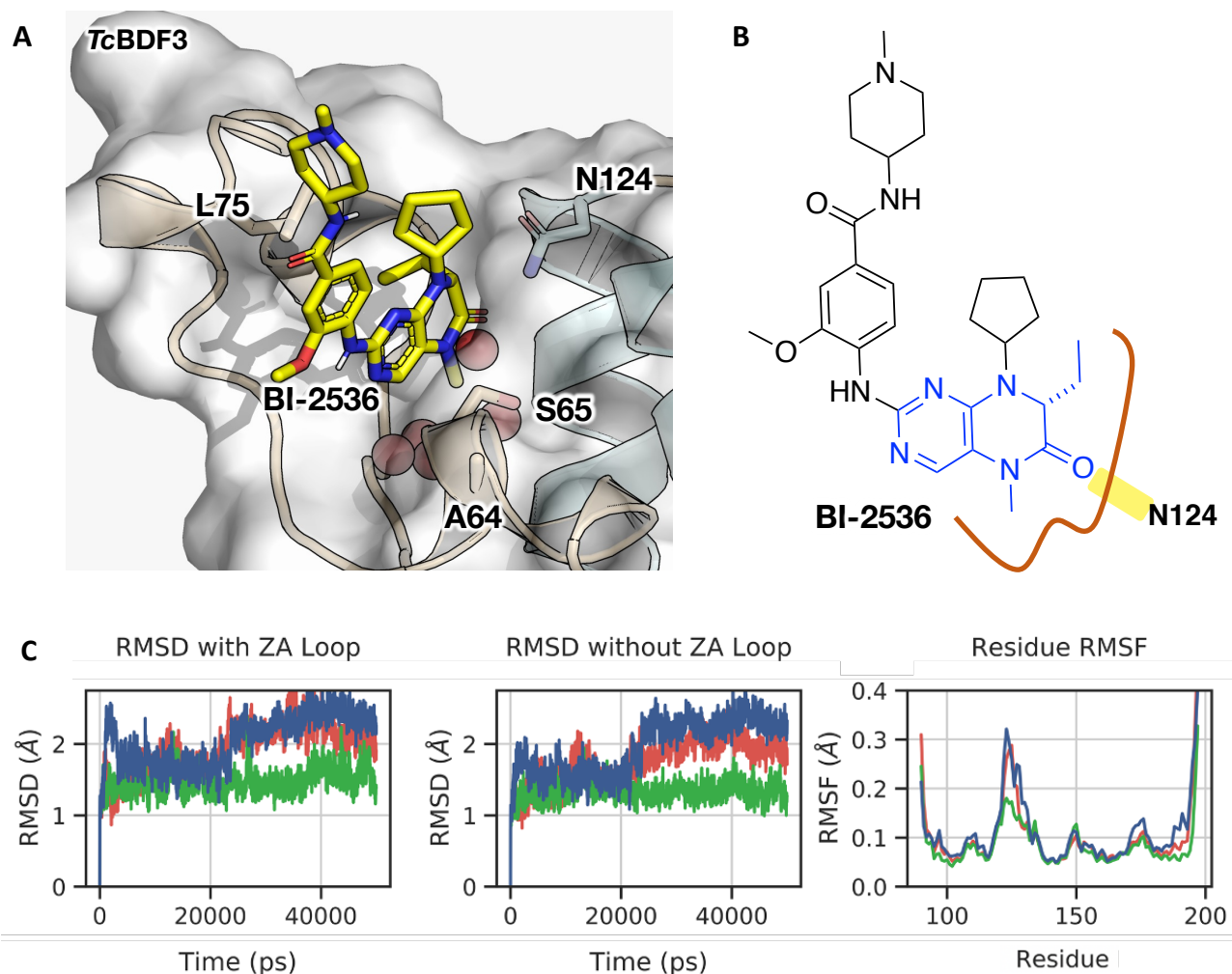
**Figure S3.** Purification of H<sub>6</sub>-TcBDF3(32..148aa). **A** IMAC purification profile. **B** SEC profile of IMAC fraction 4 (SEC1). **C** SEC profile of IMAC fraction 5 (SEC2) **D** Protein LCMS mass spectrometry spectrum, expected mass = 15803.7 Da. **E** SDS-PAGE (16% Tris/Gly/SDS gel) analysis of purified H<sub>6</sub>-TcBDF3(32..148aa); Lane 1: Colour protein standard, broad range (New England Biolabs, Inc.), lane 2: unpurified cell lysate, lane 3, 7, 10 and 13: IMAC fraction 4 to 9, lane 4-6, 8-9, 11-12, 14-15: SEC fractions from SEC1-4.



**Figure S4:** Purification and cleavage of H<sub>6</sub>-TcBDF3(32..148aa) using a 4 to 1 protein to TEV protease ratio: **A** IMAC profile of H<sub>6</sub>-TcBDF3(32..148aa). **B** IMAC 2 profile of TcBDF3(32..148aa) (post cleavage) showing cleaved protein eluting in the solvent front. **C** SDS-PAGE gel analysis of purified H<sub>6</sub>-TcBDF3(32..148aa) and cleaved TcBDF3(32..148aa); Lane 1: Colour protein standard, lane 3, to 6: IMAC 1 fraction 3 to 5, lane 7: H<sub>6</sub>-rTEV SEC fr15-19 (pTH26), lane 8 and 10: H<sub>6</sub>-TcBDF3(32..148aa) and H<sub>6</sub>-rTEV incubated for 18 h, lane 9: Post cleavage IMAC 2 fr3 showing cleaved TcBDF3(32..148aa), lane 11-15: Post cleavage IMAC 2 fr29 to 33 showing H<sub>6</sub>-rTEV. **D** SEC profile of the cleaved protein. **E** SDS-PAGE gel analysis of purified and cleaved TcBDF3(32..148aa); Lane 1: Colour protein standard, lane 2: IMAC 1 fraction 3 to 5, lane 3: Post cleavage IMAC 2 fr2-4, lane 4: post cleavage SEC fr 17-19.



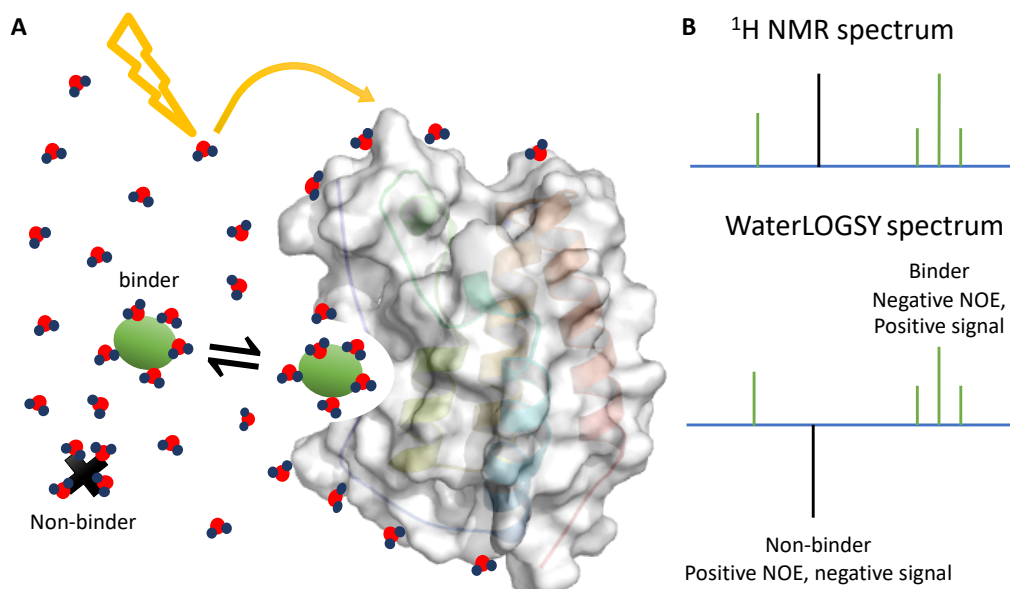
**Figure S5: A** Overlay of the CD spectra of H<sub>6</sub>-TcBDF3 and H<sub>6</sub>-HmBRD4(1). **B** Overlay of the photomultiplier tube voltage spectra of H<sub>6</sub>-TcBDF3 with H<sub>6</sub>-HmBRD4(1) showing HT ≤ 500 V. The CD of H<sub>6</sub>-TcBDF3 and H<sub>6</sub>-HmBRD4(1) was measured at 100 µg/mL, which corresponds to concentrations of 5.7 µM and 6.3 µM, respectively. The CD spectrum of the purified H<sub>6</sub>-TcBDF3 (32..148aa) protein was compared to H<sub>6</sub>-HmBRD4(1) (Figure SI-4). H<sub>6</sub>-TcBDF3(32..148aa) has a signature α-helical structure which is expected for bromodomains. These data suggest that the newly purified H<sub>6</sub>-TcBDF3 bromodomain is folded correctly and has a similar secondary structure to H<sub>6</sub>-HmBRD4(1)



**Figure S6:** **A** Docking of BI-2536 (**1**) into the homology model of *TcBDF3*. Image generated using PyMol. **B** The predicted mode of binding with the hydrogen bonding to N124 represented in yellow. The homology model suggests that the piperidine solubilising group of **1** is solvent exposed. Using this binding mode model, it was hypothesised that modifications of the tail of the ligand could be made without significant loss of binding affinity. **C**. MD assessment of homology model stability. The stability of the *TcBDF3* homology models was assessed using backbone atom RMSD of the whole protein, the protein backbone without the dynamic ZA loop and root mean squared fluctuation (RMSF). Simulations were performed in triplicate, with the different replicas shown in red, green and blue.

## SI-B: WATERLOGSY RESULTS

Water-ligand observed *via* gradient spectroscopy (waterLOGSY) is a NMR-based assay used to detect weak binders of a protein by observing changes in the sign of the nuclear Overhauser effect (nOe).<sup>1</sup> nOe is the change of nuclear spin population in non-irradiated nuclei caused by  $T_1$  relaxation of irradiated neighbouring nuclei by dipole-dipole mechanism. The waterLOGSY pulse sequence excites the bulk water molecules, suppresses the water peak and measures the changes in  $T_1$  relaxation. This pulse sequence allows for detection of binding through the change of nOe signs between free and bound ligands. nOe transferred directly from water protons to ligands is positive (due to fast molecular motion), and freely rotating compound appear as negative peaks in the waterLOGSY spectrum (**Error! Reference source not found.** S7). The nOe is negative when it is transferred through the protein (due to slow molecular motion), and the binding compound appears as positive peaks in the waterLOGSY spectrum. Therefore, binders can be identified by their change of nOe sign in the waterLOGSY assay. This technique relies on fast ligand-protein exchange, for the nOe to be transferred to a high enough portion of the ligand population to be measurable.<sup>1,2</sup>

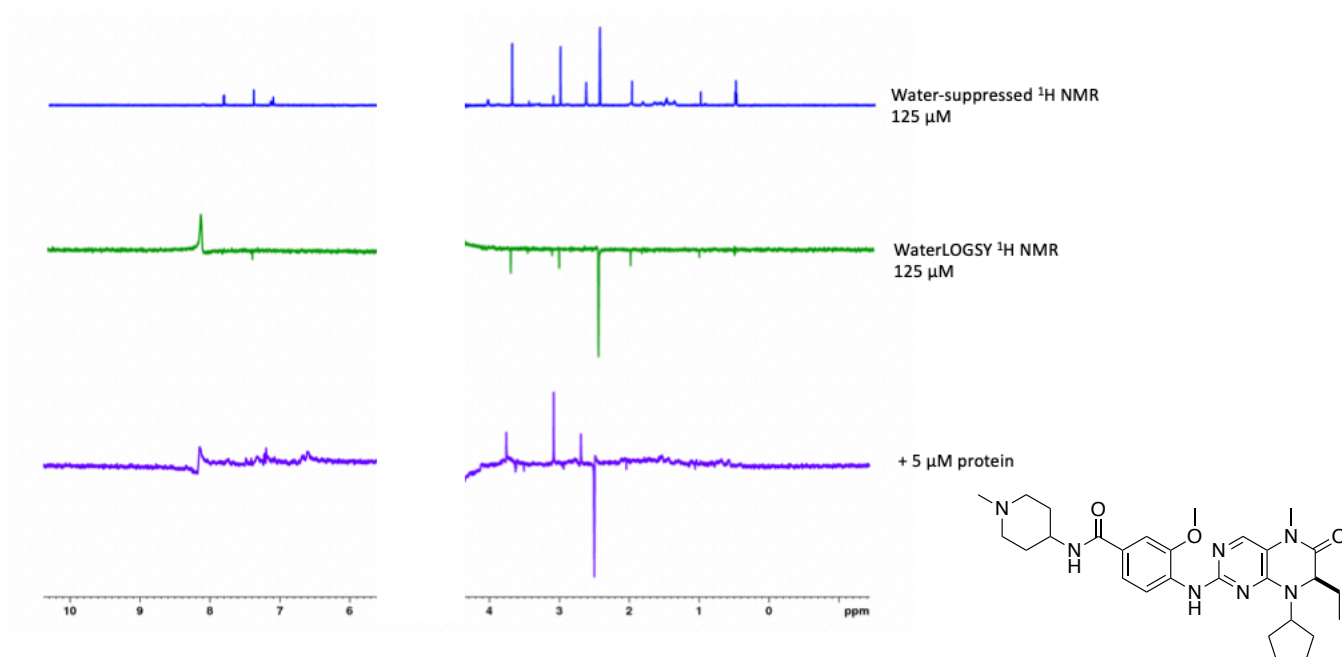


**Figure S7:** Schematic representation of WaterLOGSY assay. **A** The bulk water is excited by the pulse sequence (yellow). nOe is transferred from the water protons to the ligand results either directly (positive nOe) or via the protein bound (negative nOe). **B** The  $^1\text{H}$  NMR (top) and the corresponding waterLOGSY spectra (bottom) for a binding compound (green) in the presence of a non-binding reference (black).

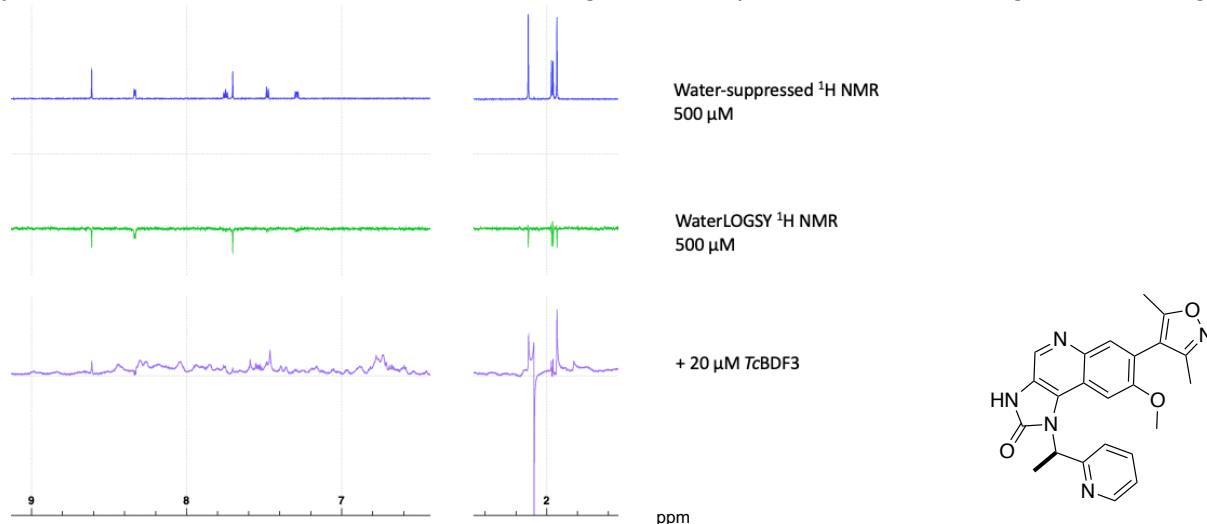
WaterLOGSY results were manually analyzed by comparison of the  $^1\text{H}$  NMR spectrum to the WaterLOGSY spectrum of the compound only sample (soluble samples display positive NOE, negative signals) and then to the WaterLOGSY spectrum of the compound in the presence of protein (binding compounds display negative NOE, positive signals, whereas non-binding compounds would appear as positive NOE and negative peaks). As the DMSO peak is always negative, compounds that bind to the protein will have resonances that possess peaks of the opposite sign in the waterLOGSY spectrum, in the presence of protein. As the amount by which peak invert is not directly correlated to the binding affinity, we chose to assign binding/non-binding based on the inversion or not all the majority of the critical peaks. For ambiguous cases, the peak sign of singlets resonances of protons near the acetyl-lysine mimic were used. Additionally, a few cases with partial inversion or peak disappearance were marked as “maybe”. It should be noted

that exchangeable protons do not behave as like other protons in waterLOGSY experiments. They usually appear as large positive peaks independent of the behaviour of the rest of the compound resonances.

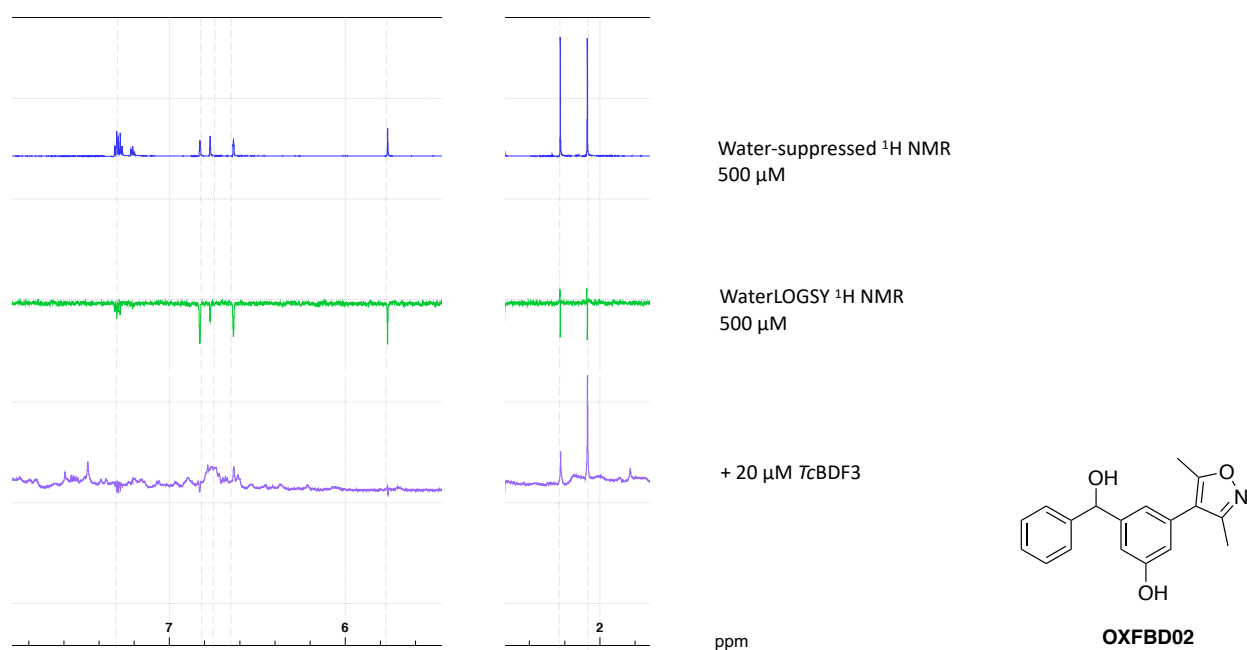
Soluble compounds were screened in the presence of a large excess of the protein, for example: 500  $\mu\text{M}$  compound with 20  $\mu\text{M}$  protein, 2500  $\mu\text{M}$  compound with 10  $\mu\text{M}$  protein, 125  $\mu\text{M}$  compound with 5  $\mu\text{M}$  protein, or 62.5  $\mu\text{M}$  compound with 5  $\mu\text{M}$  protein.



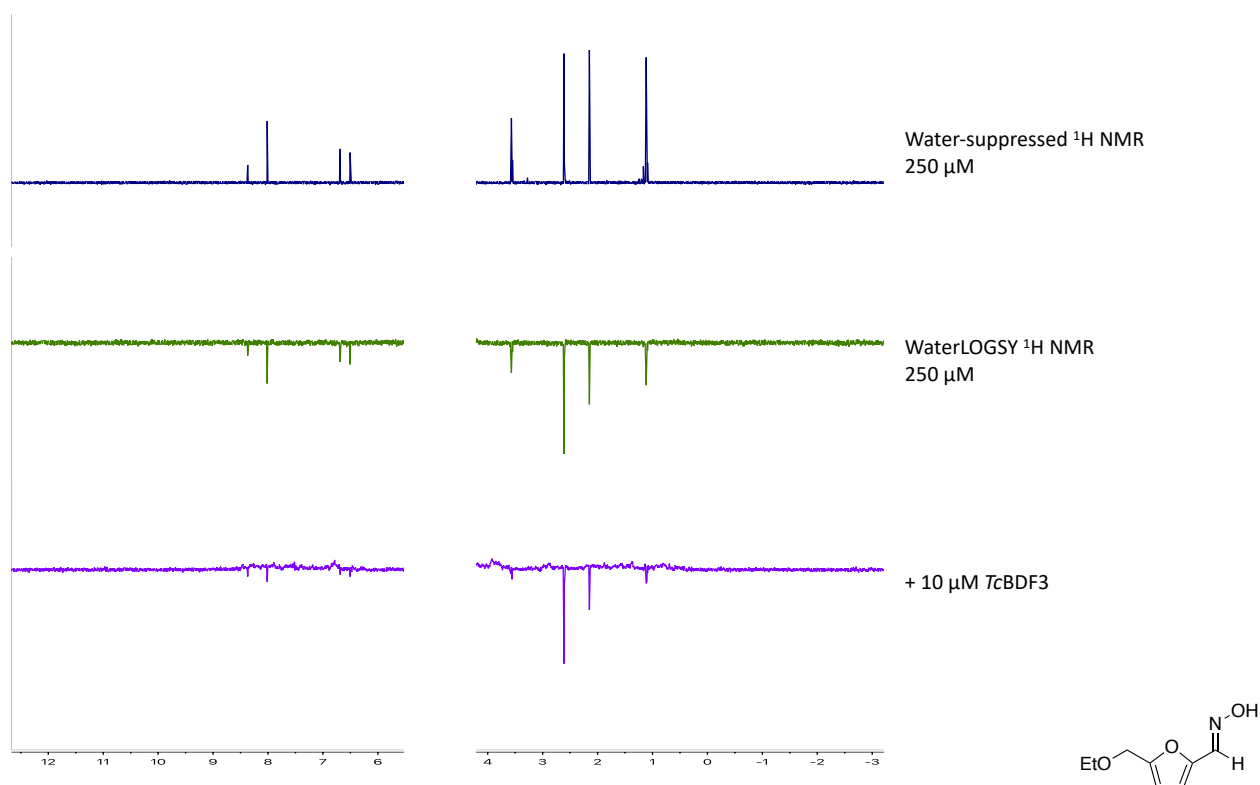
**Figure S8.** Partial waterLOGSY spectra of BI-2536 (1). The water suppressed  $^1\text{H}$  spectrum is shown in blue, the waterLOGSY NMR of the compound only (green) shows inversion of the compound peaks indicating suitable solubilization at 125  $\mu\text{M}$ . The waterLOGSY spectrum in the presence of 5  $\mu\text{M}$  of TcBDF3-BRD (purple) displays inversion of the ligand peaks, indicating binding.



**Figure S9.** Partial waterLOGSY spectra of I-BET151 (2). The water suppressed  $^1\text{H}$  spectrum is shown in blue, the waterLOGSY NMR of the compound only (green) shows inversion of the compound peaks indicating suitable solubilization at 500  $\mu\text{M}$ . The waterLOGSY spectrum in the presence of TcBDF3-BRD (purple) displays inversion of the ligand peaks, indicating binding to the protein.

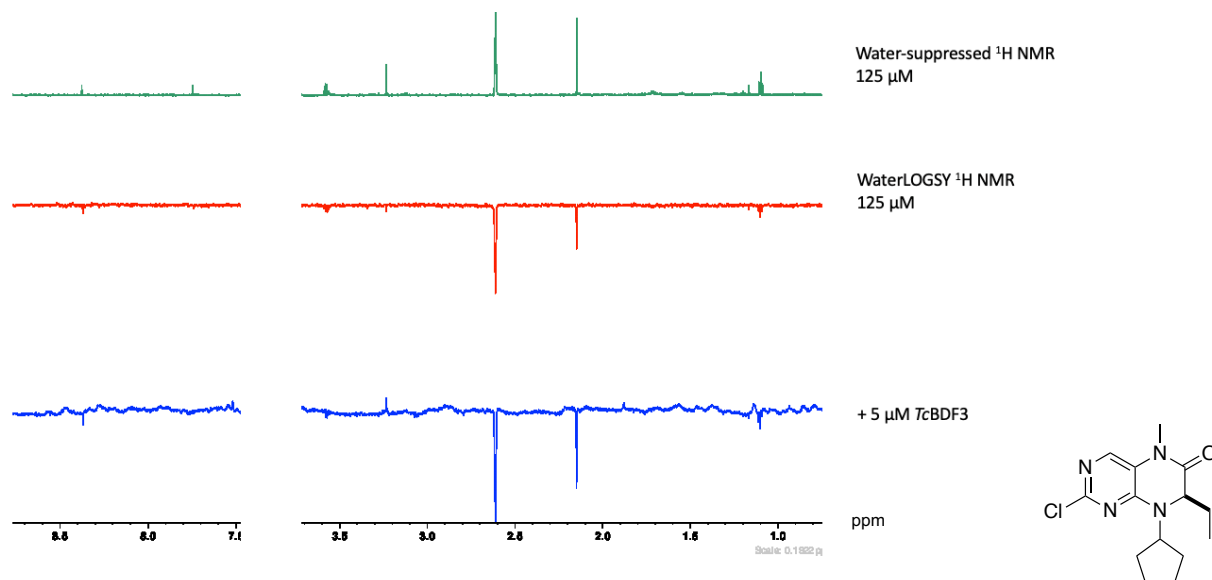


**Figure S10.** Partial waterLOGSY spectra of OXFBD02 (**3**). The water suppressed  $^1\text{H}$  spectrum is shown in blue, the waterLOGSY NMR of the compound only (green) shows inversion of the compound peaks indicating suitable solubilization at 500  $\mu\text{M}$ . The waterLOGSY spectrum in the presence of 20  $\mu\text{M}$  of TcBDF3-BRD (purple) displays inversion of the ligand peaks, indicating binding to TcBDF3-BRD.

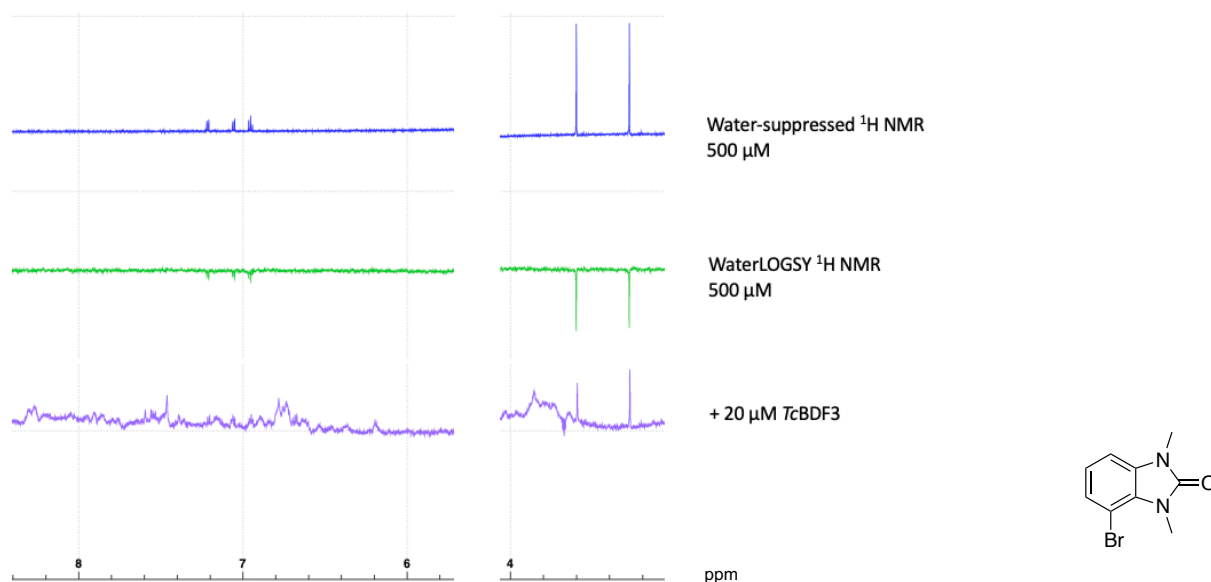


**Figure S11.** Partial waterLOGSY spectra of **6**. The water suppressed  $^1\text{H}$  spectrum is shown in blue, the waterLOGSY NMR of the compound only (green) shows inversion of the compound peaks indicating suitable solubilization at 250  $\mu\text{M}$ . The waterLOGSY spectrum in the presence of 10  $\mu\text{M}$  of TcBDF3-BRD (purple) displays inversion of the ligand peaks, indicating binding to TcBDF3-BRD.

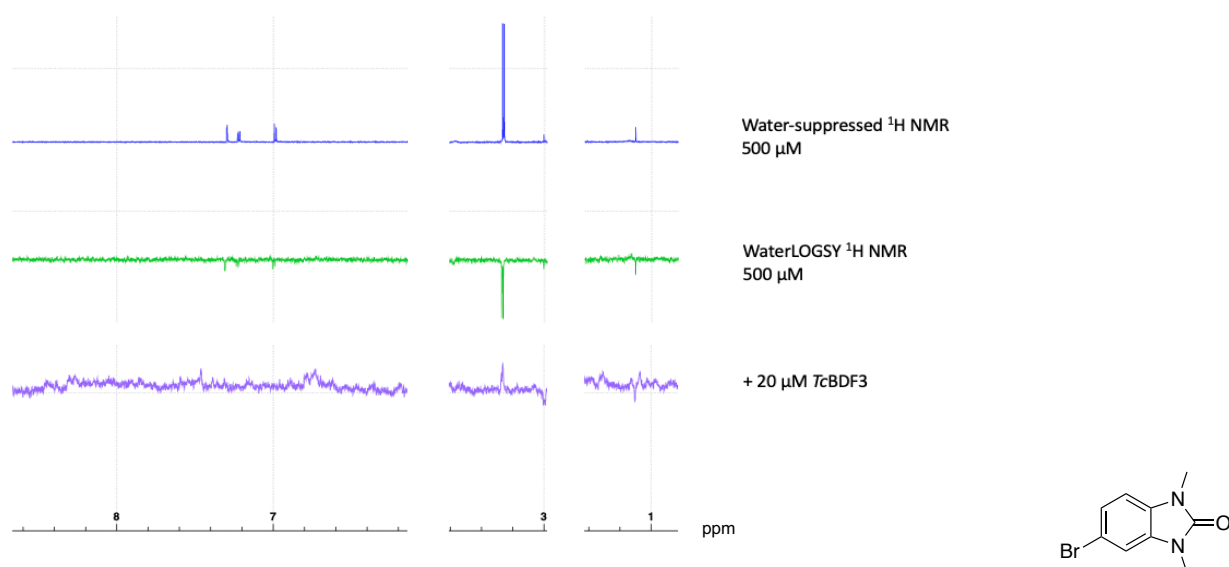
Note that compound **7** was visibly insoluble (despite heating and sonication) at concentrations of 500, 250 and 125  $\mu\text{M}$ , consequently, no spectra were recorded for this compound.



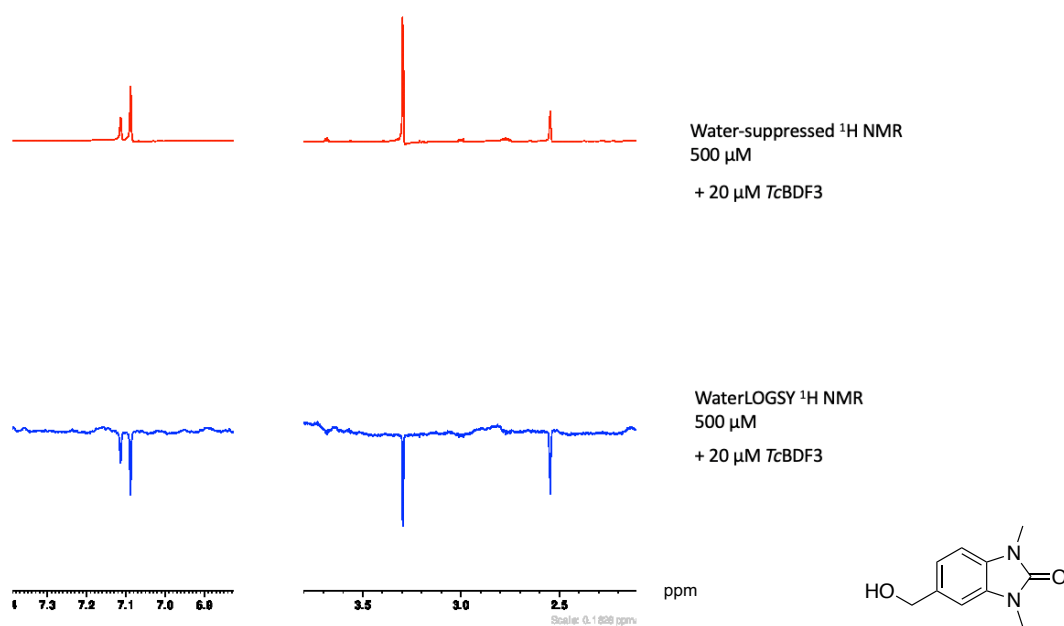
**Figure S12:** Partial waterLOGSY spectra of **8**. The water suppressed  $^1\text{H}$  spectrum is shown in green, the waterLOGSY NMR of the compound only (red) shows inversion of the compound peaks indicating suitable solubilization at 125  $\mu\text{M}$ . The waterLOGSY spectrum in the presence of 5  $\mu\text{M}$  of TcBDF3-BRD (blue) displays inversion of the ligand peaks, indicating binding to TcBDF3-BRD.



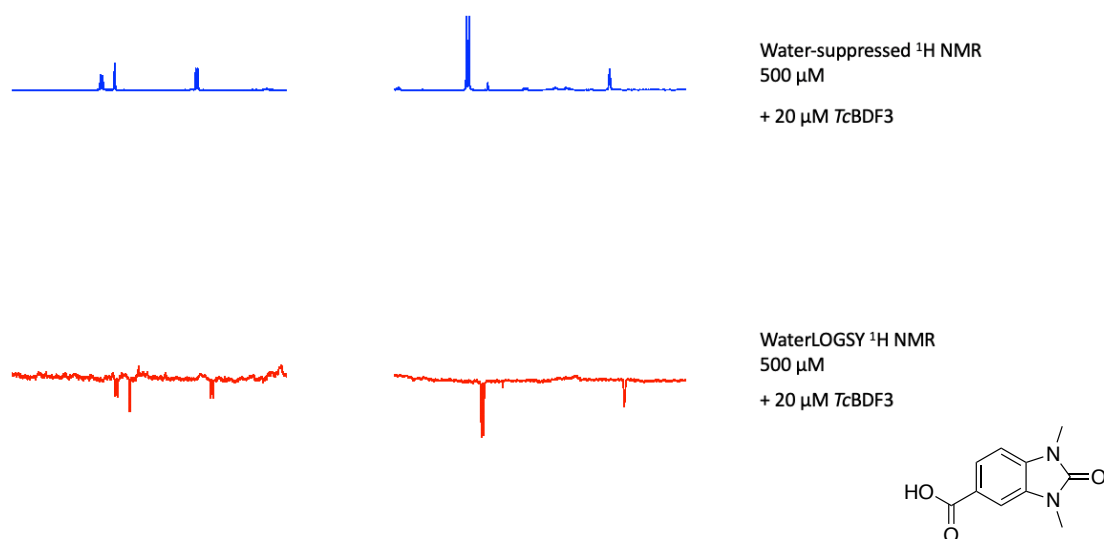
**Figure S13.** Partial waterLOGSY spectra of **9**. The water suppressed  $^1\text{H}$  spectrum is shown in blue, the waterLOGSY NMR of the compound only (green) shows inversion of the compound peaks indicating suitable solubilization at 500  $\mu\text{M}$ . The waterLOGSY spectrum in the presence 20  $\mu\text{M}$  of TcBDF3-BRD (purple) displays inversion of the ligand peaks, indicating binding to to TcBDF3-BRD .



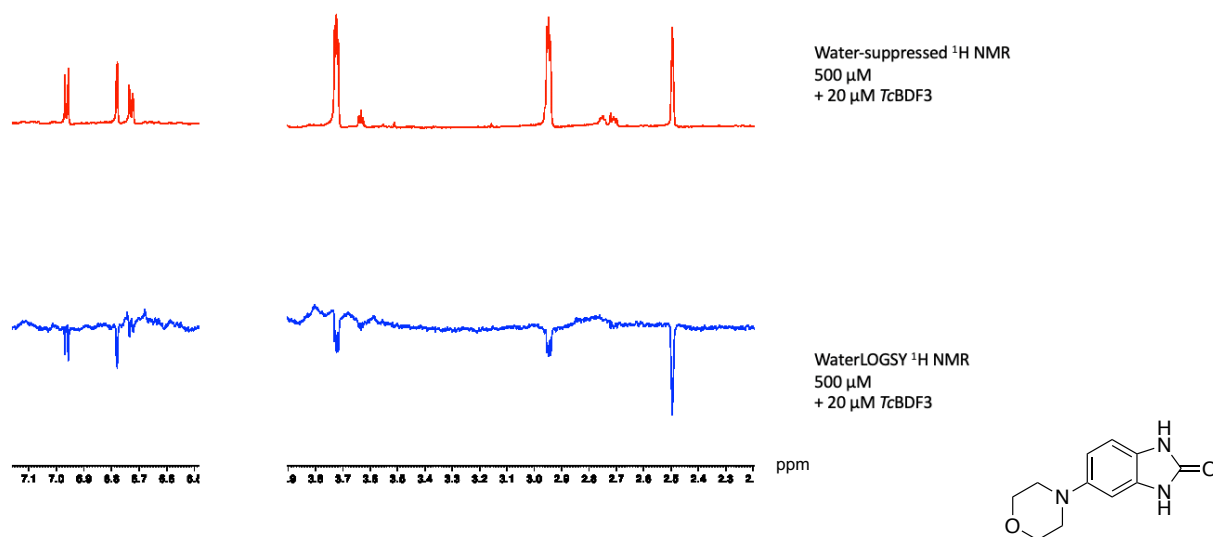
**Figure S14.** Partial waterLOGSY spectra of **10**. The water suppressed  $^1\text{H}$  spectrum is shown in blue, the waterLOGSY NMR of the compound only (green) shows inversion of the compound peaks indicating suitable solubilization at 500  $\mu\text{M}$ . The waterLOGSY spectrum in the presence of 20  $\mu\text{M}$  TcBDF3-BRD (purple) displays partial inversion of the ligand peaks, indicating potential binding to TcBDF3-BRD.



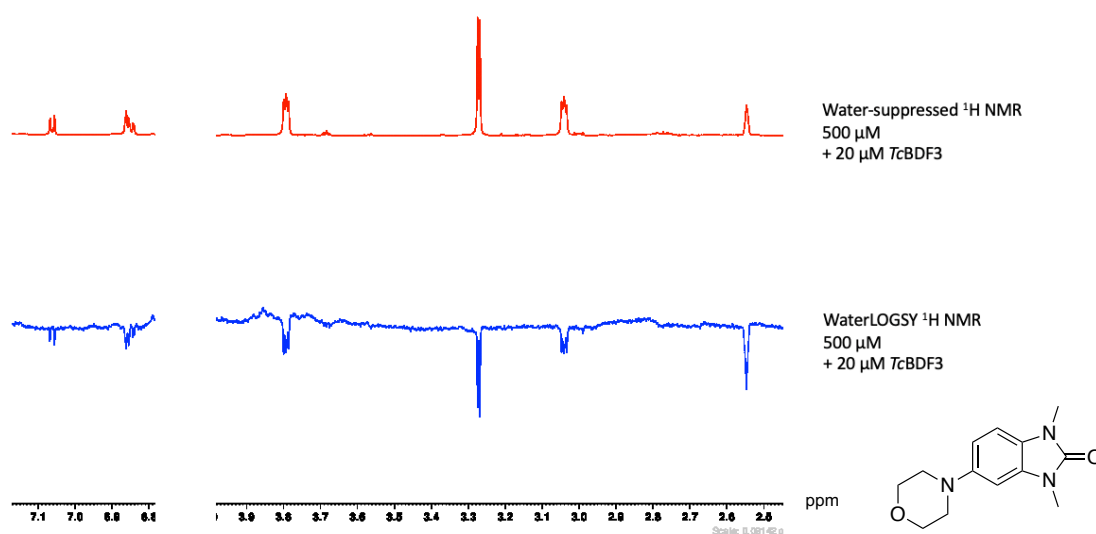
**Figure S15:** Partial waterLOGSY spectra of **11**. No clear inversion of the ligand peak is detected in the presence of 20  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The water suppressed  $^1\text{H}$  spectrum of 500  $\mu\text{M}$  ligand with 20  $\mu\text{M}$  protein (red) is given as a reference.



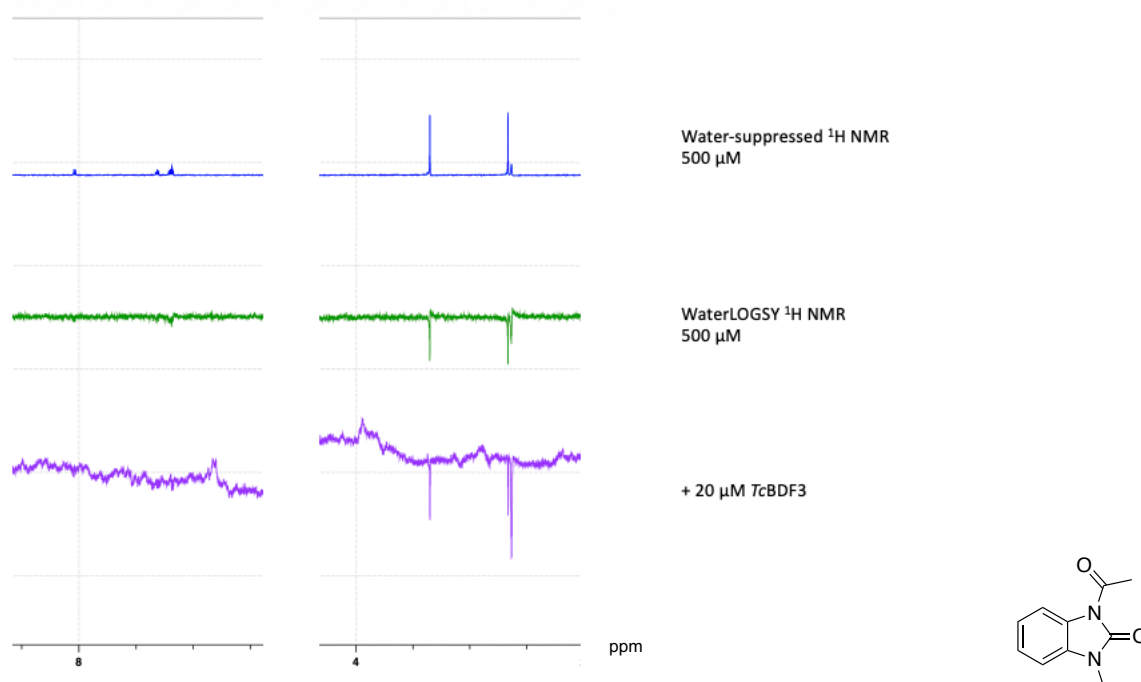
**Figure S16:** Partial waterLOGSY spectra of **12**. No clear inversion of the ligand peak is detected in the presence of 20  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The water suppressed  $^1\text{H}$  spectrum of 500  $\mu\text{M}$  ligand with 20  $\mu\text{M}$  protein (red) is given as a reference.



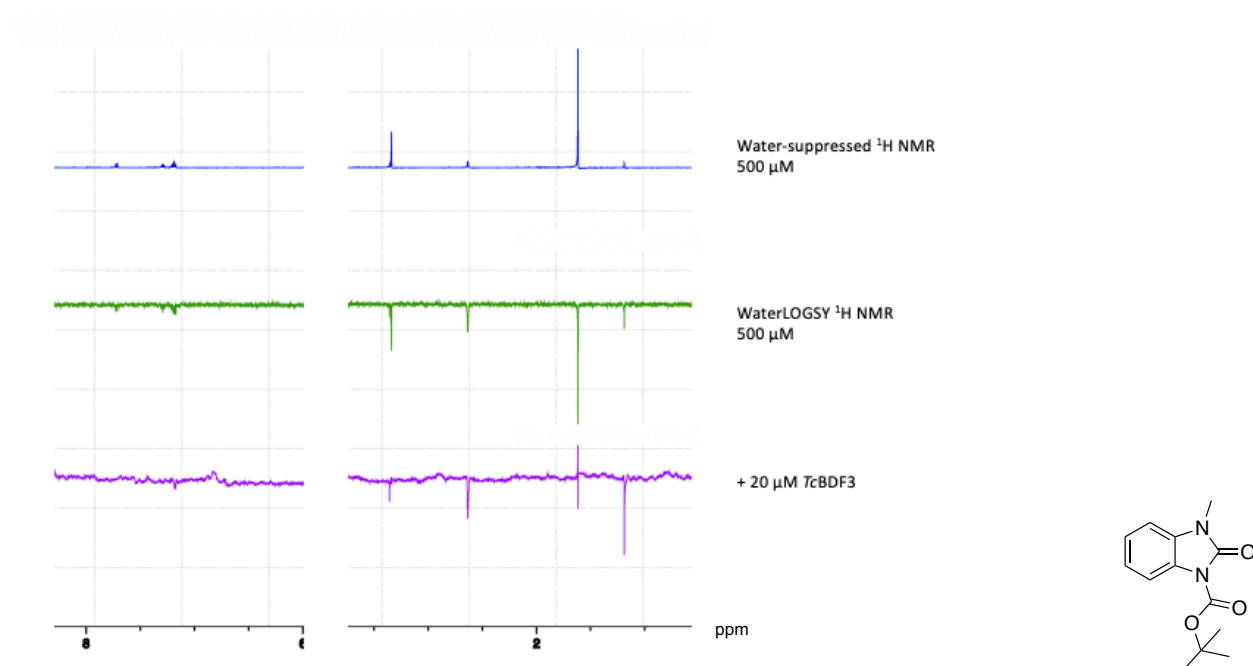
**Figure S17:** Partial waterLOGSY spectra of **13**. No clear inversion of the ligand peak is detected in the presence of 20  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The water suppressed  $^1\text{H}$  spectrum of 500  $\mu\text{M}$  ligand with 20  $\mu\text{M}$  protein (red) is given as a reference.



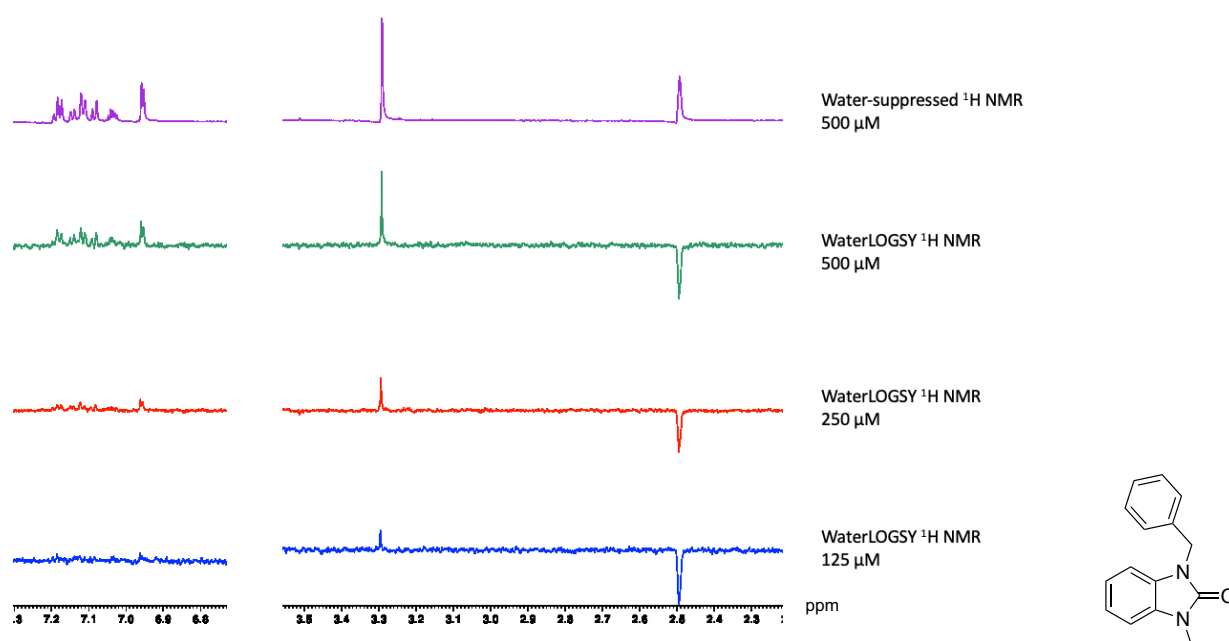
**Figure S18:** Partial waterLOGSY spectra of **14**. No clear inversion of the ligand peak is detected in the presence of 20  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The water suppressed  $^1\text{H}$  spectrum of 500  $\mu\text{M}$  ligand with 20  $\mu\text{M}$  protein (red) is given as a reference.



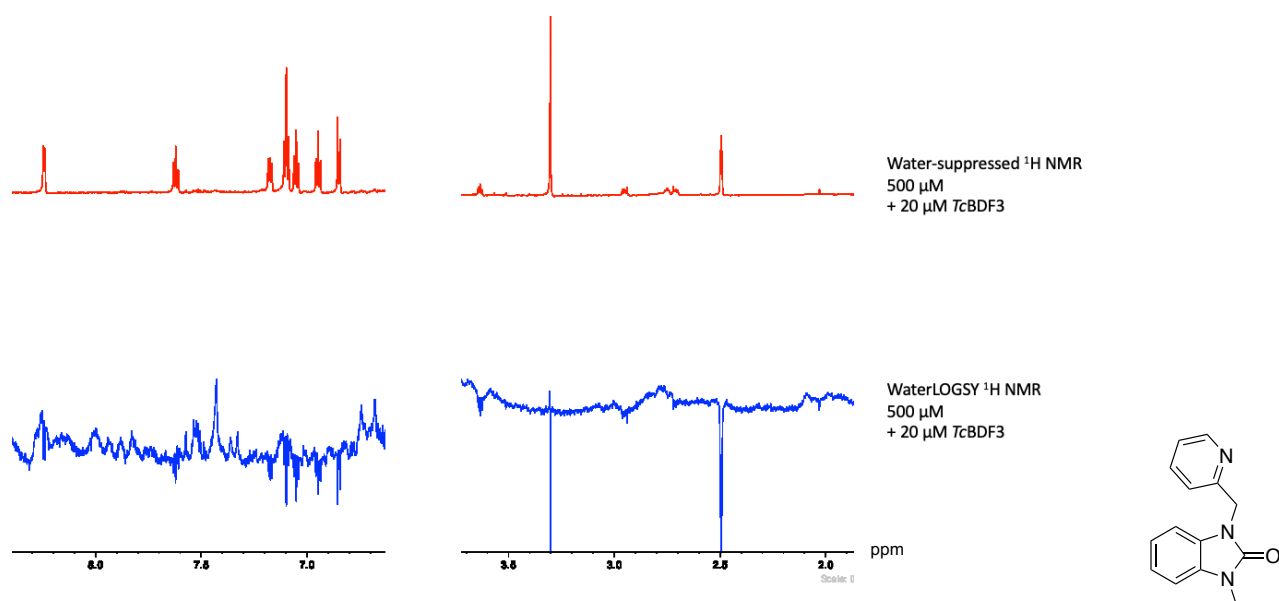
**Figure S19:** Partial waterLOGSY spectra of **15**. The water suppressed  $^1\text{H}$  spectrum is shown in green, the waterLOGSY NMR of the compound only (green) shows inversion of the compound peaks indicating suitable solubilization at 500  $\mu\text{M}$ . No inversion of the ligand peak is detected in the presence of 20  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD.



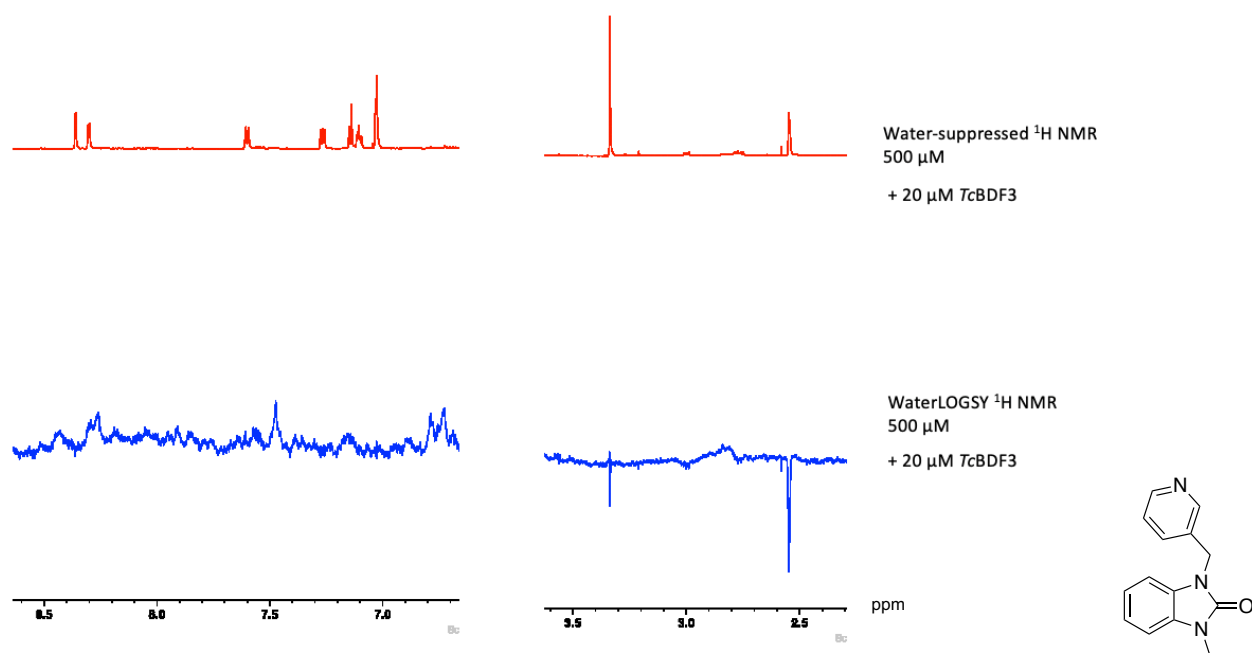
**Figure S20:** Partial waterLOGSY spectra of **16**. The water suppressed  $^1\text{H}$  spectrum is shown in blue, the waterLOGSY NMR of the compound only (green) shows inversion of the compound peaks indicating suitable solubilization at 500  $\mu\text{M}$ . No clear inversion of the ligand peak is detected in the presence of 20  $\mu\text{M}$  of TcBDF3-BRD (purple) indicating the ligand does not bind to TcBDF3-BRD.



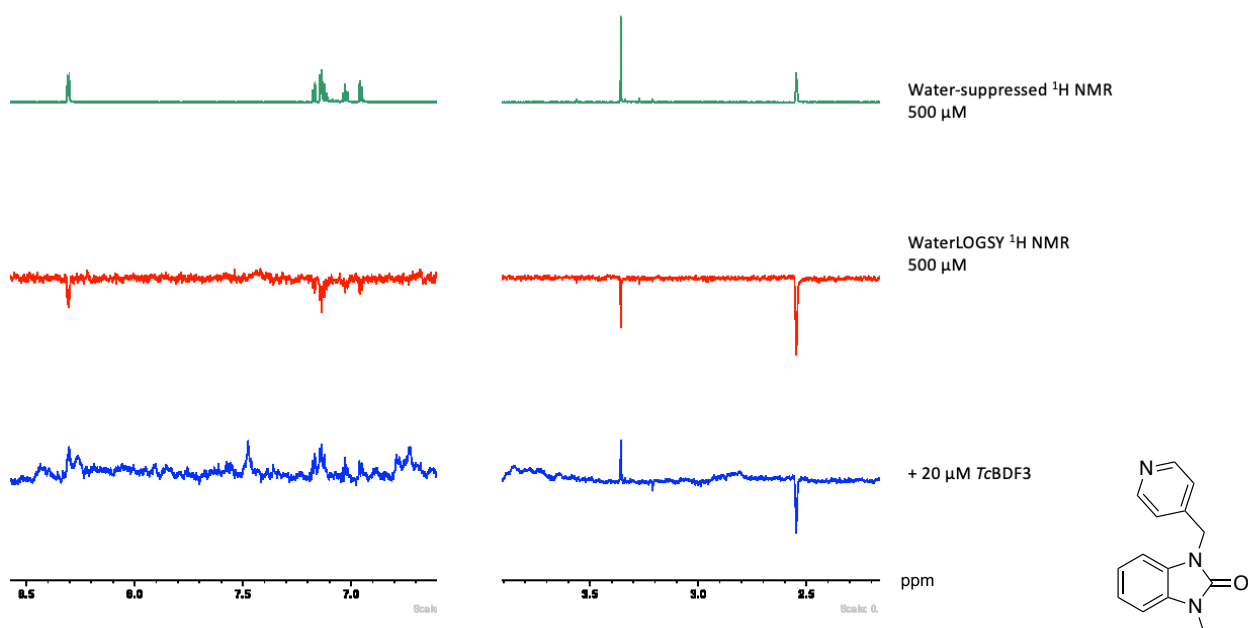
**Figure S21:** Partial waterLOGSY spectra of **17**. Positive ligand peaks in the waterLOGSY spectrum indicates aggregation at 500  $\mu\text{M}$  (green), 250  $\mu\text{M}$  (red) and 125  $\mu\text{M}$  (blue). The water-suppressed  $^1\text{H}$  NMR spectrum (purple) is given as reference.



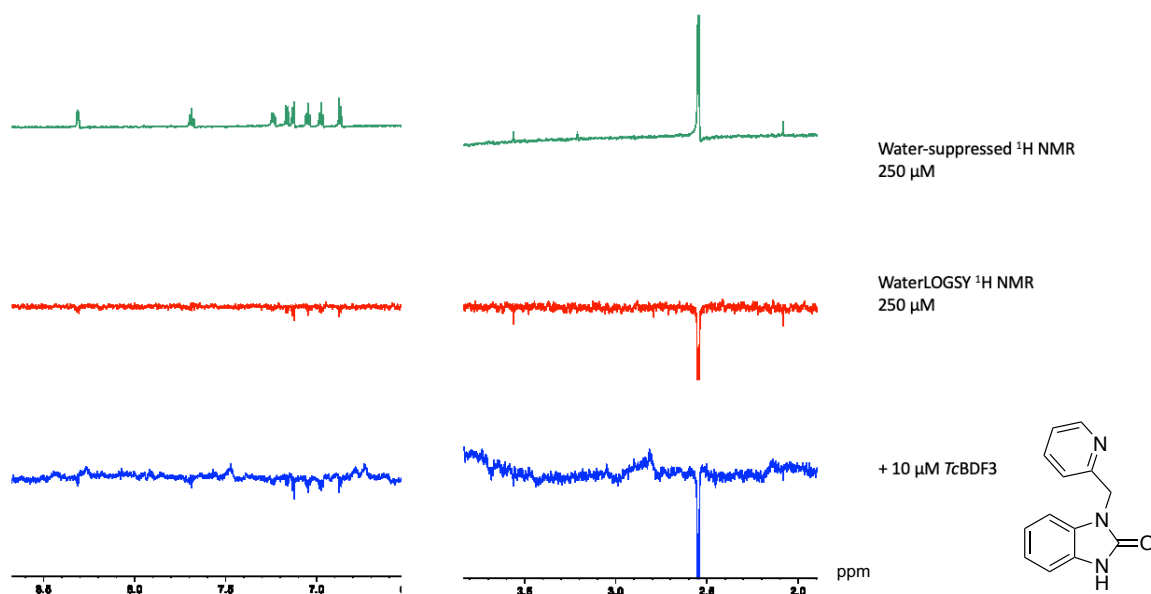
**Figure S22:** Partial waterLOGSY spectra of **18**. No clear inversion of the ligand peak is detected in the presence of 20  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The water suppressed  $^1\text{H}$  spectrum of 500  $\mu\text{M}$  ligand with 20  $\mu\text{M}$  protein (red) is given as a reference.



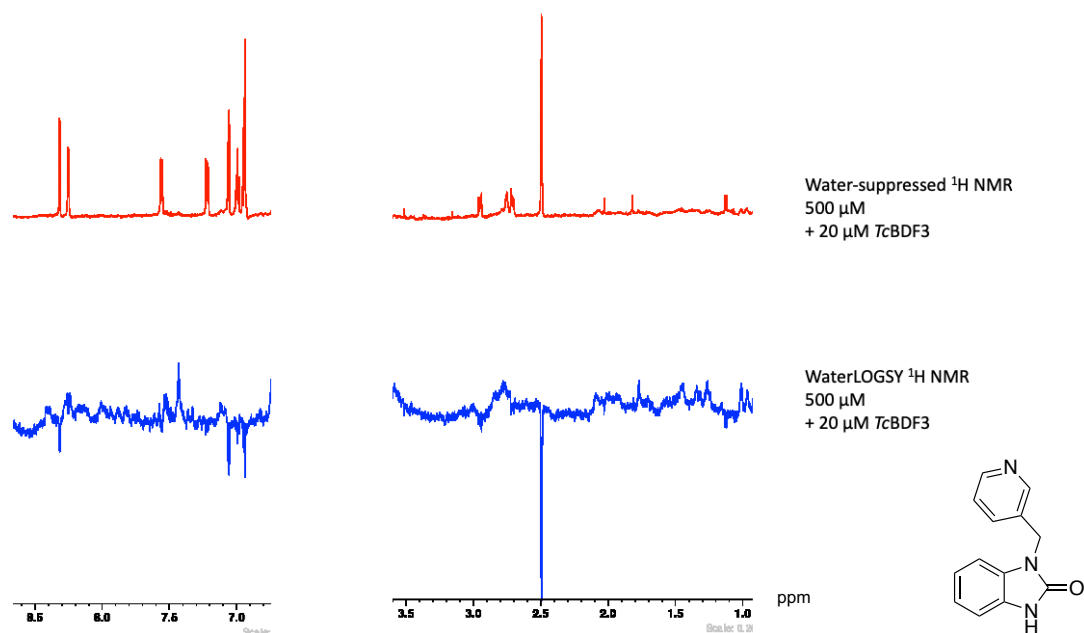
**Figure S23:** Partial waterLOGSY spectra of **19**. No clear inversion of the ligand peak is detected in the presence of 20  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The water suppressed  $^1\text{H}$  spectrum of 500  $\mu\text{M}$  ligand with 20  $\mu\text{M}$  protein (red) is given as a reference.



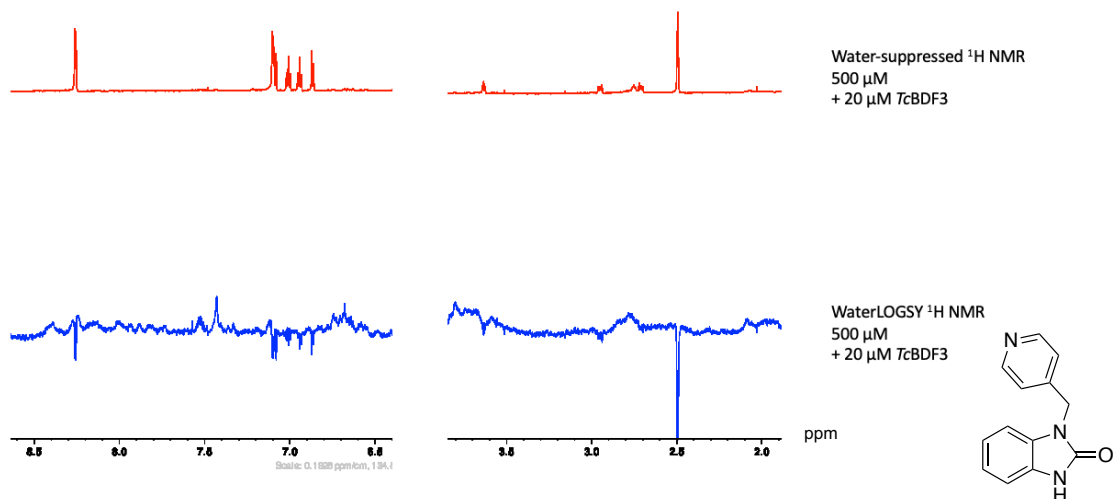
**Figure S24:** Partial waterLOGSY spectra of **20**. The water suppressed  $^1\text{H}$  spectrum is shown in green, the waterLOGSY NMR of the compound only (red) shows inversion of the compound peaks indicating suitable solubilization at 500  $\mu\text{M}$ . Some inversion of the ligand peaks is detected in the presence of 20  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating potential binding of the ligand to TcBDF3-BRD.



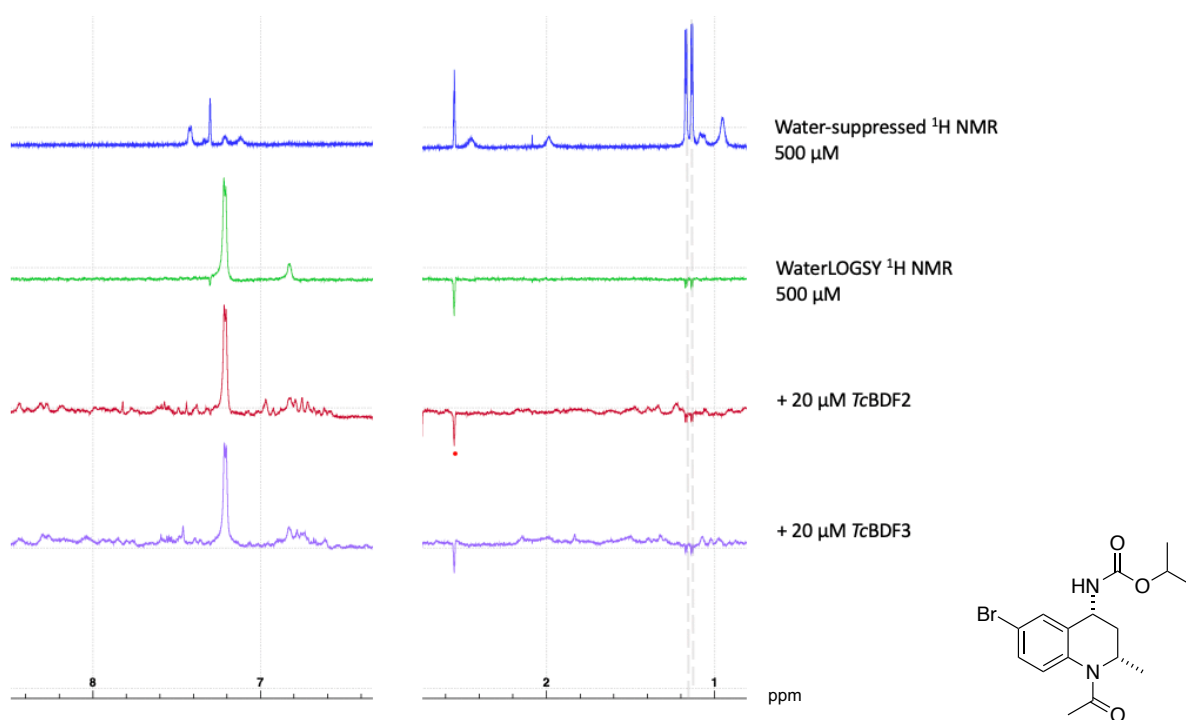
**Figure S25:** Partial waterLOGSY spectra of **21**. The water suppressed  $^1\text{H}$  spectrum is shown in green, the waterLOGSY NMR of the compound only (red) shows inversion of the compound peaks indicating suitable solubilization at 250  $\mu\text{M}$ . No clear inversion of the ligand peak is detected in the presence of 10  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD.



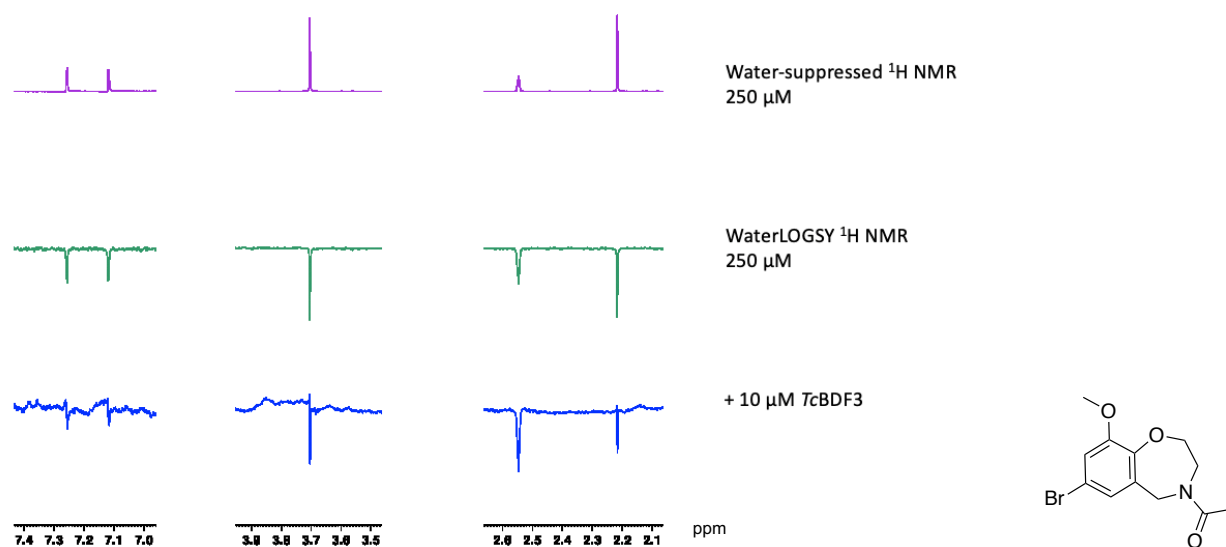
**Figure S26:** Partial waterLOGSY spectra of **22**. No clear inversion of the ligand peak is detected in the presence of 20  $\mu\text{M}$  of *TcBDF3*-BRD (blue) indicating the ligand does not bind to *TcBDF3*-BRD. The water suppressed  $^1\text{H}$  spectrum of 500  $\mu\text{M}$  ligand with 20  $\mu\text{M}$  protein (red) is given as a reference.



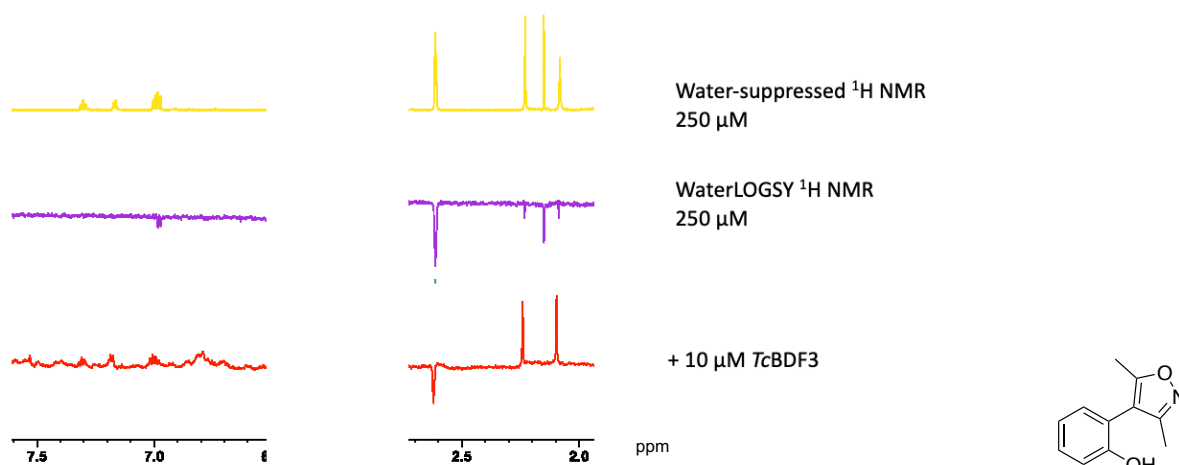
**Figure S27:** Partial waterLOGSY spectra of **23**. No clear inversion of the ligand peak is detected in the presence of 20  $\mu\text{M}$  of *TcBDF3*-BRD (blue) indicating the ligand does not bind to *TcBDF3*-BRD. The water suppressed  $^1\text{H}$  spectrum of 500  $\mu\text{M}$  ligand with 20  $\mu\text{M}$  protein (red) is given as a reference.



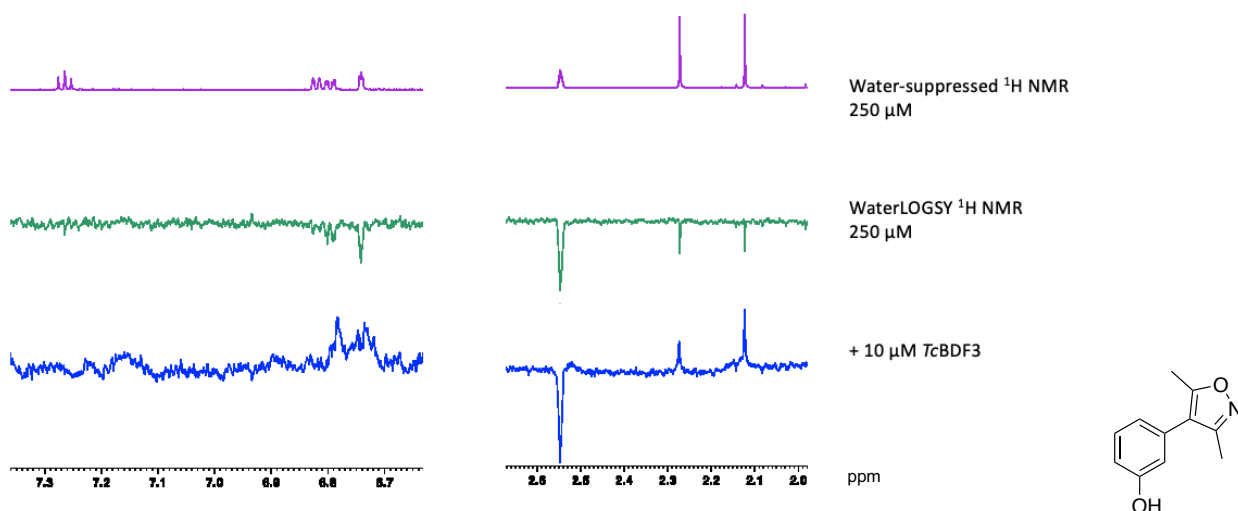
**Figure S28:** Partial waterLOGSY spectra of **24**. The water suppressed  $^1\text{H}$  spectrum is shown in green, the waterLOGSY NMR of the compound only (red) shows inversion of the compound peaks indicating suitable solubilization at 125  $\mu\text{M}$ . No inversion of the ligand peak is detected in the presence of 5  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD.



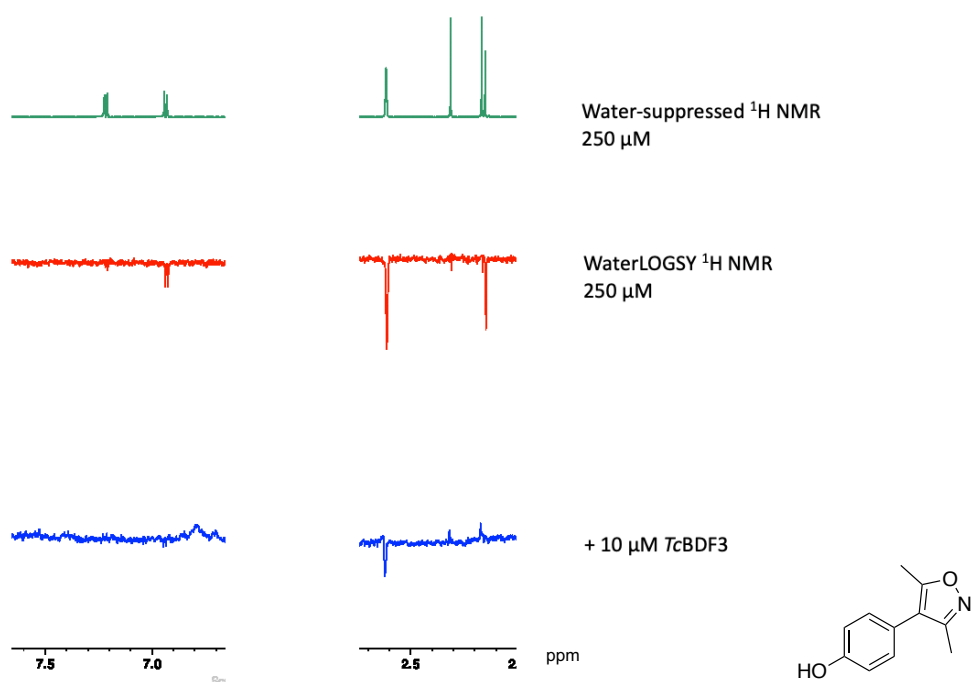
**Figure S29:** Partial waterLOGSY spectra of **25**. The water suppressed  $^1\text{H}$  spectrum is shown in purple, the waterLOGSY NMR of the compound only (green) shows inversion of the compound peaks indicating suitable solubilization at 125  $\mu\text{M}$ . No inversion of the ligand peak is detected in the presence of 5  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD.



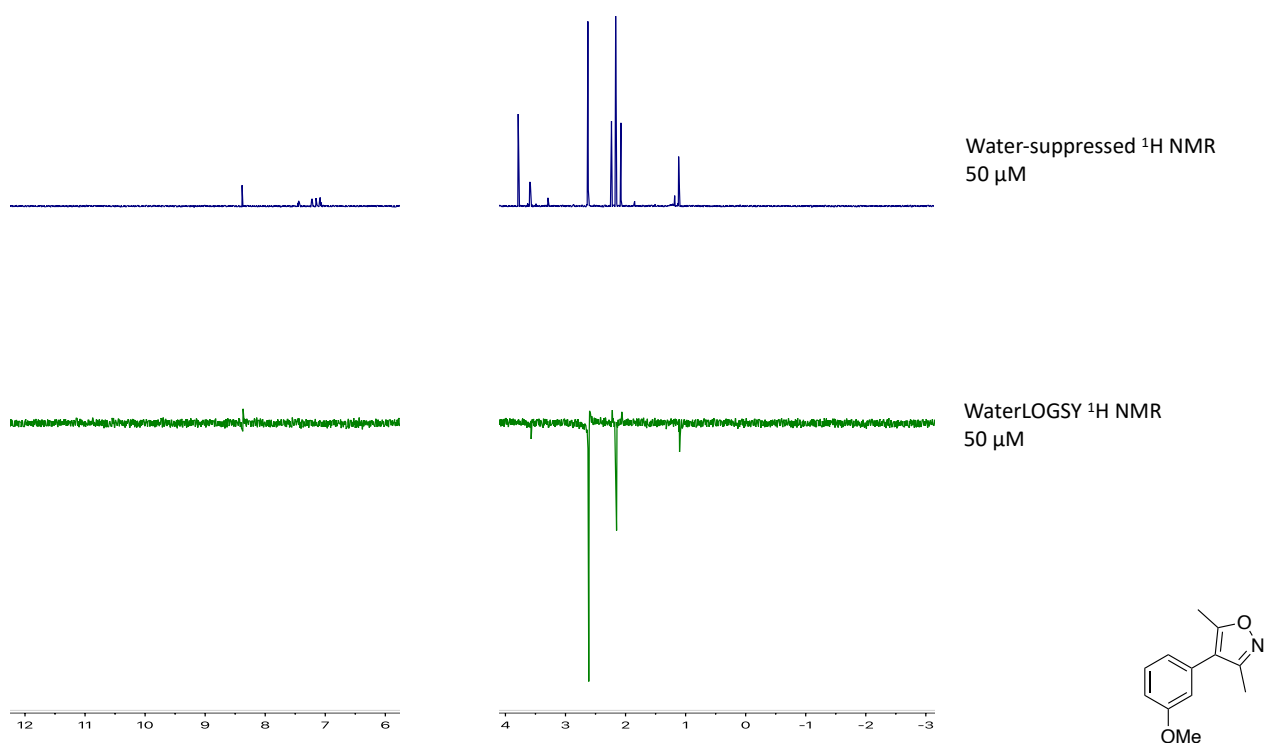
**Figure S30.** Partial waterLOGSY spectra of **26**. The water suppressed  $^1\text{H}$  spectrum is shown in yellow, the waterLOGSY NMR of the compound only (purple) shows inversion of the compound peaks indicating suitable solubilization at 250  $\mu\text{M}$ . The waterLOGSY spectrum in the presence of 10  $\mu\text{M}$  of TcBDF3-BRD (red) displays inversion of the ligand peaks, indicating binding to TcBDF3-BRD.



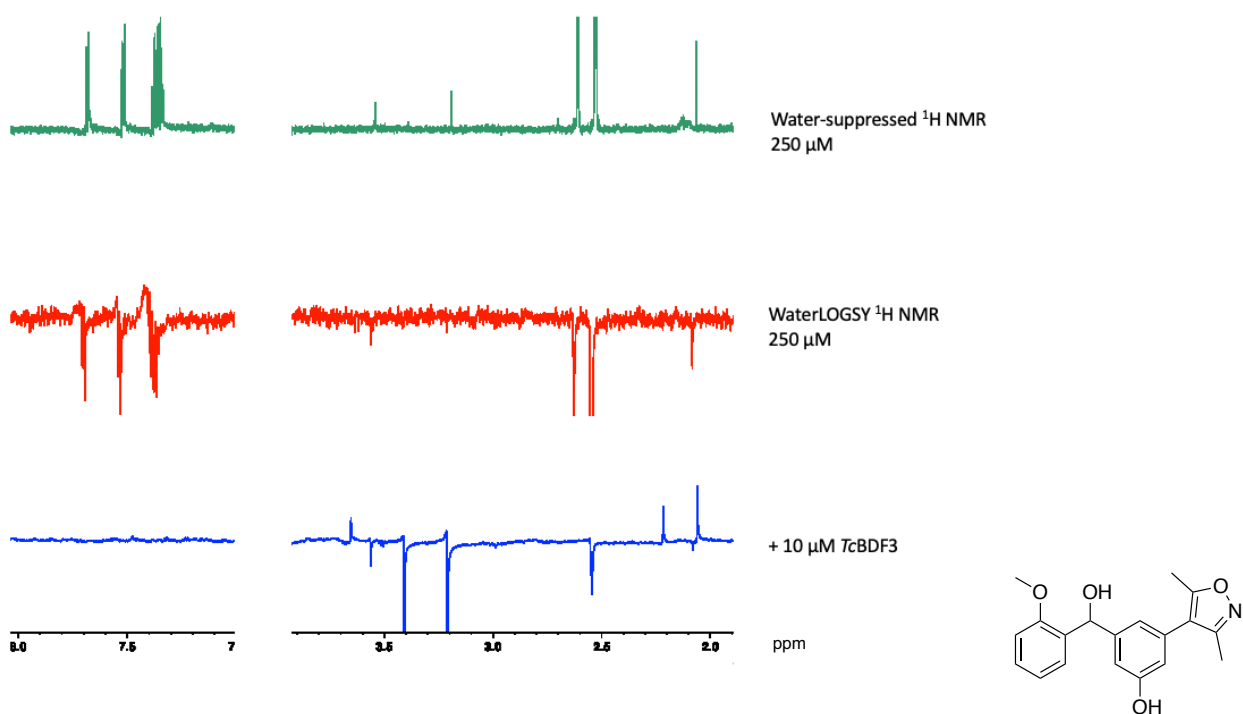
**Figure S31.** Partial waterLOGSY spectra of **27**. The water suppressed  $^1\text{H}$  spectrum is shown in purple, the waterLOGSY NMR of the compound only (green) shows inversion of the compound peaks indicating suitable solubilization at 250  $\mu\text{M}$ . The waterLOGSY spectrum in the presence of 10  $\mu\text{M}$  of TcBDF3-BRD (blue) displays inversion of the ligand peaks, indicating binding to TcBDF3-BRD.



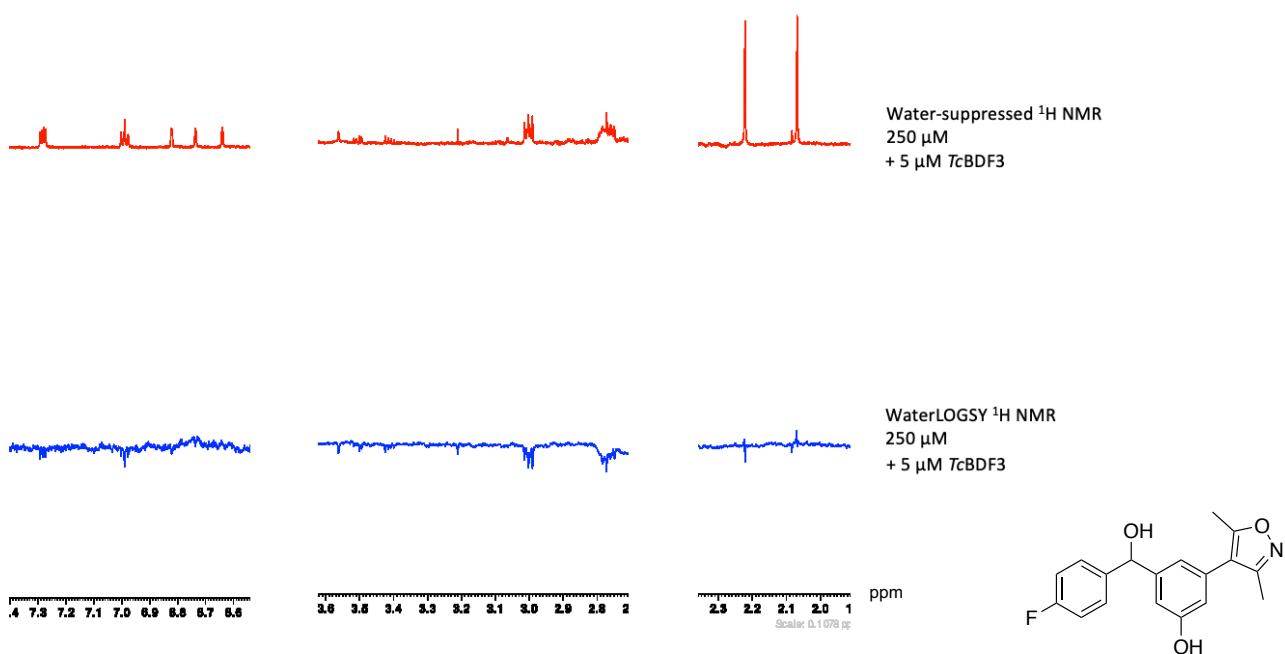
**Figure S32:** Partial waterLOGSY spectra of **28**. The water suppressed  $^1\text{H}$  spectrum is shown in green, the waterLOGSY NMR of the compound only (red) shows inversion of the compound peaks indicating suitable solubilization at 250  $\mu\text{M}$ . The waterLOGSY spectrum in the presence of 10  $\mu\text{M}$  of TcBDF3-BRD (blue) displays inversion of the ligand peaks, indicating binding to TcBDF3-BRD.



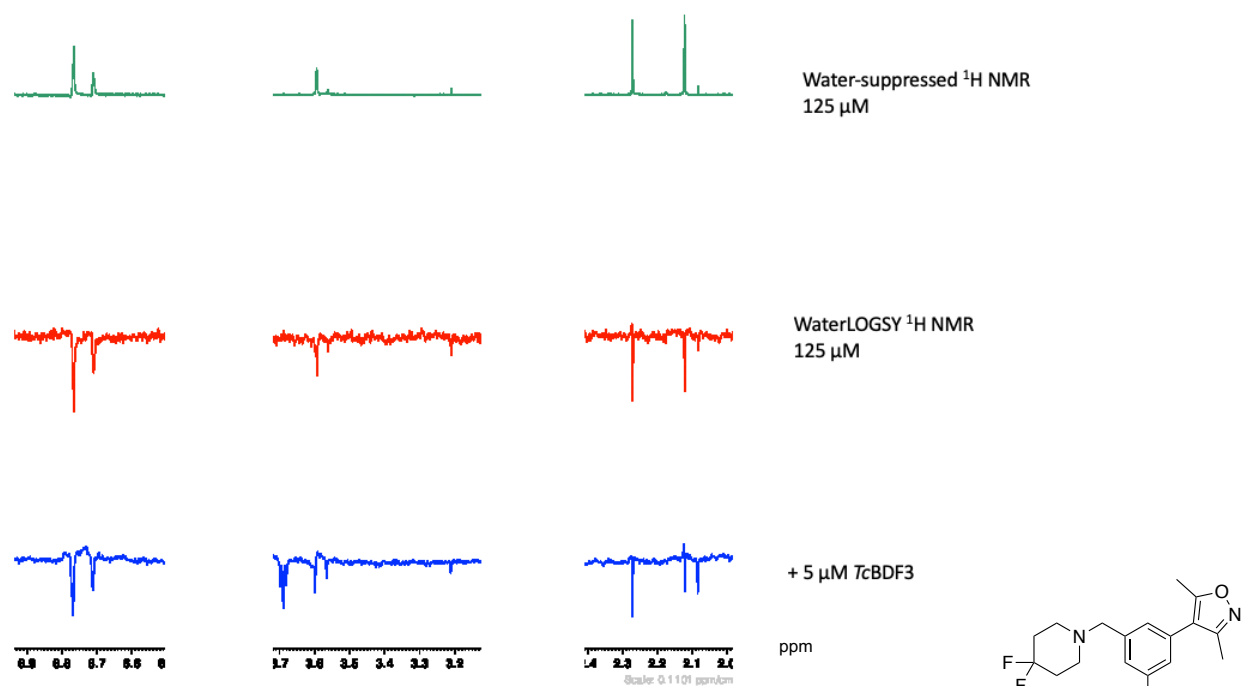
**Figure S33:** Partial waterLOGSY spectra of **29**. Positive ligand peaks in the waterLOGSY spectrum indicates aggregation at 500  $\mu\text{M}$  (green). The water-suppressed  $^1\text{H}$  NMR spectrum (blue) is given as reference.



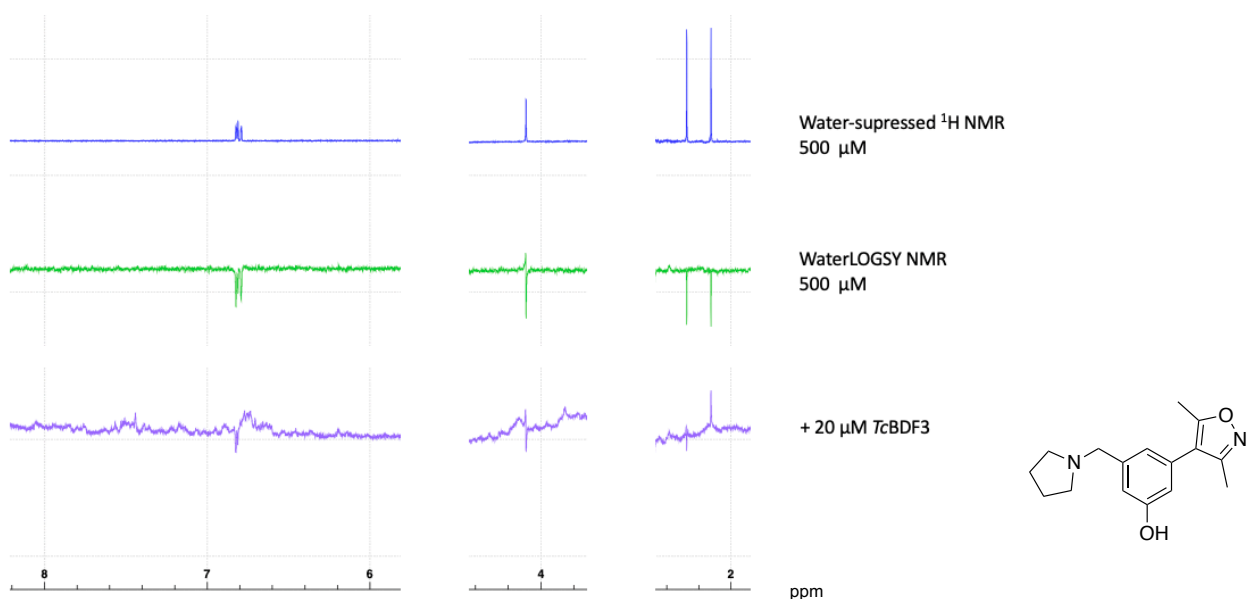
**Figure S34:** Partial waterLOGSY spectra of **30**. Partial inversion of the ligand aliphatic peaks is detected in the presence of 20  $\mu\text{M}$  of TcBDF3-BRD (blue), however, the aromatics peaks are not inverted. This ambiguous result might indicate binding. The water suppressed  $^1\text{H}$  spectrum of 500  $\mu\text{M}$  ligand with 20  $\mu\text{M}$  TcBDF3-BRD (red) is given as a reference.



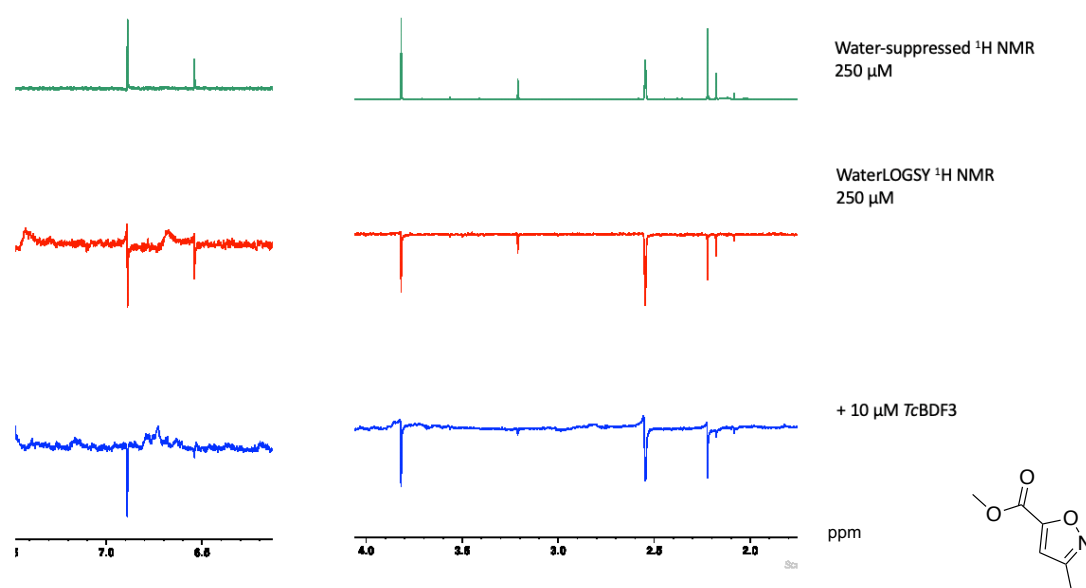
**Figure S35:** Partial waterLOGSY spectra of **31**. No clear inversion of the ligand peak is detected in the presence of 5  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The water suppressed  $^1\text{H}$  spectrum of 250  $\mu\text{M}$  ligand with 5  $\mu\text{M}$  protein (red) is given as a reference.



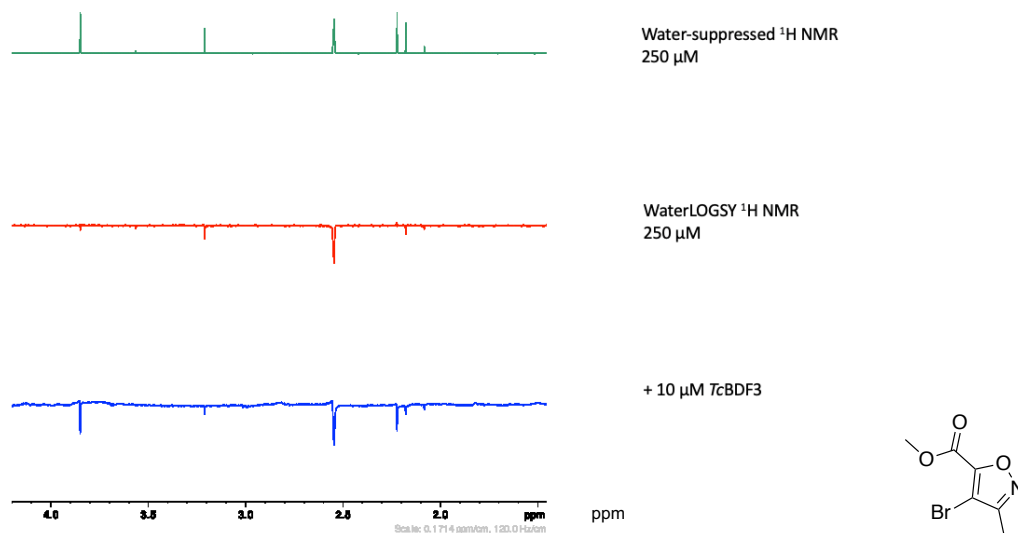
**Figure S36:** Partial waterLOGSY spectra of **32**. The water suppressed  $^1\text{H}$  spectrum is shown in green, the waterLOGSY NMR of the compound only (red) shows inversion of the compound peaks indicating suitable solubilization at 125  $\mu\text{M}$ . No inversion of the ligand peak is detected in the presence of 5  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRDs.



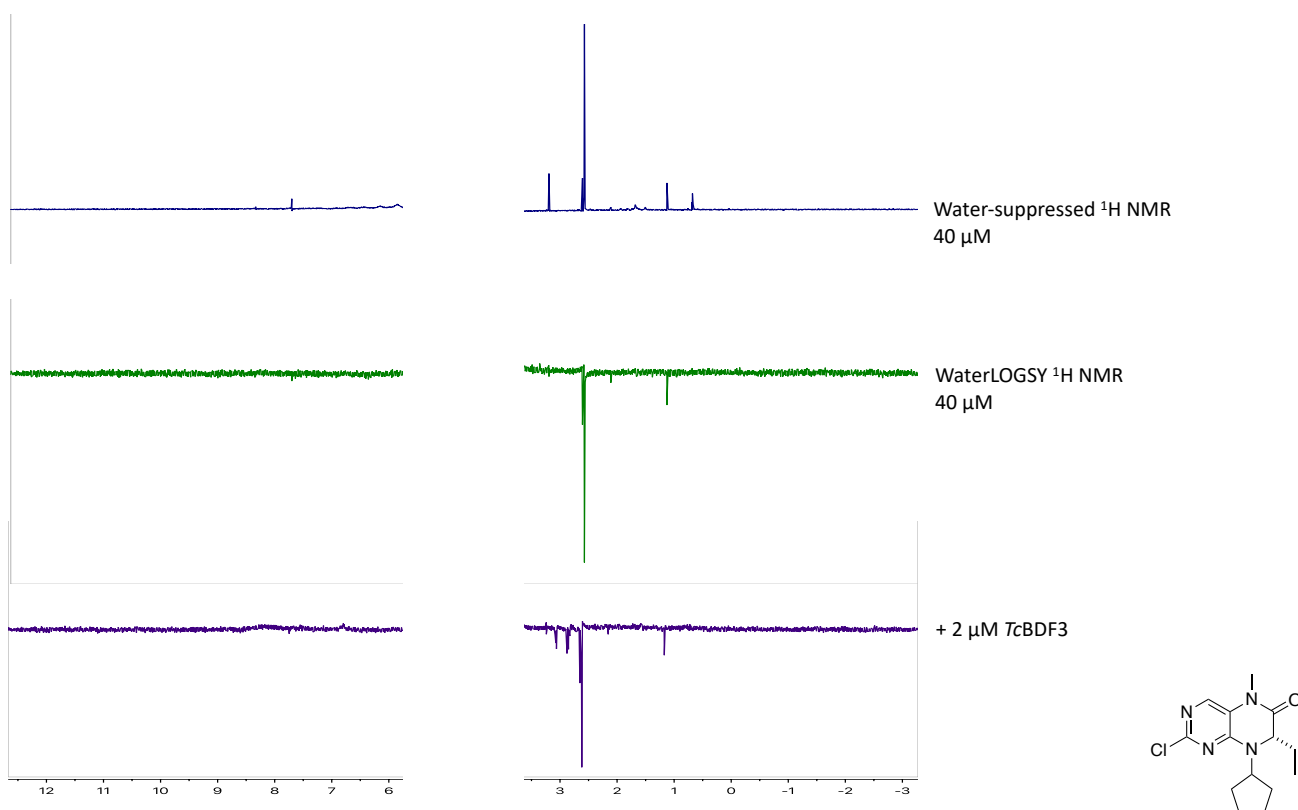
**Figure S37:** Partial waterLOGSY spectra of **33**. The water suppressed  $^1\text{H}$  spectrum is shown in blue, the waterLOGSY NMR of the compound only (green) shows inversion of the compound peaks indicating suitable solubilization at 500  $\mu\text{M}$ . The waterLOGSY spectrum in the presence 20  $\mu\text{M}$  TcBDF3-BRD (purple) display partial inversion of the ligand peaks, indicating binding to TcBDF3-BRD.



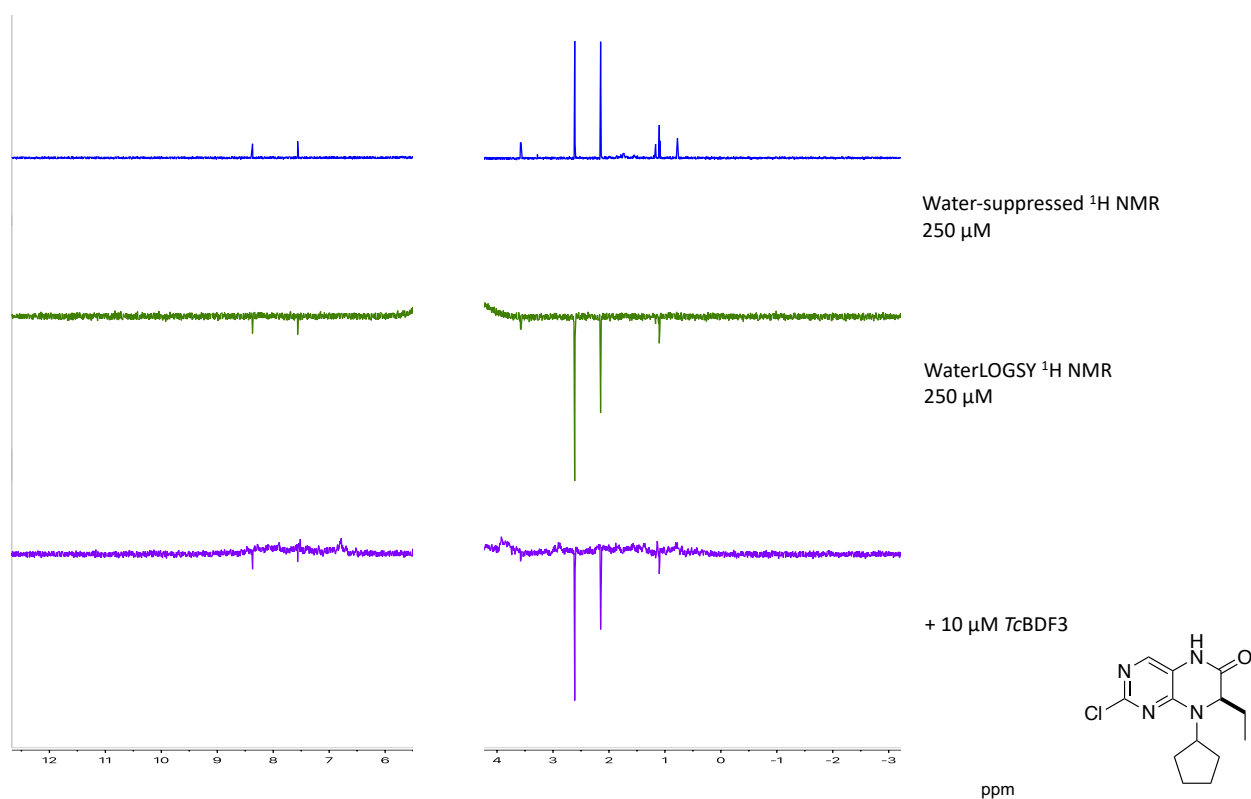
**Figure S38:** Partial waterLOGSY spectra of **34**. The water suppressed  $^1\text{H}$  spectrum is shown in green, the waterLOGSY NMR of the compound only (red) shows inversion of the compound peaks indicating suitable solubilization at 250  $\mu\text{M}$ . No clear inversion of the ligand peak is detected in the presence of 10  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD.



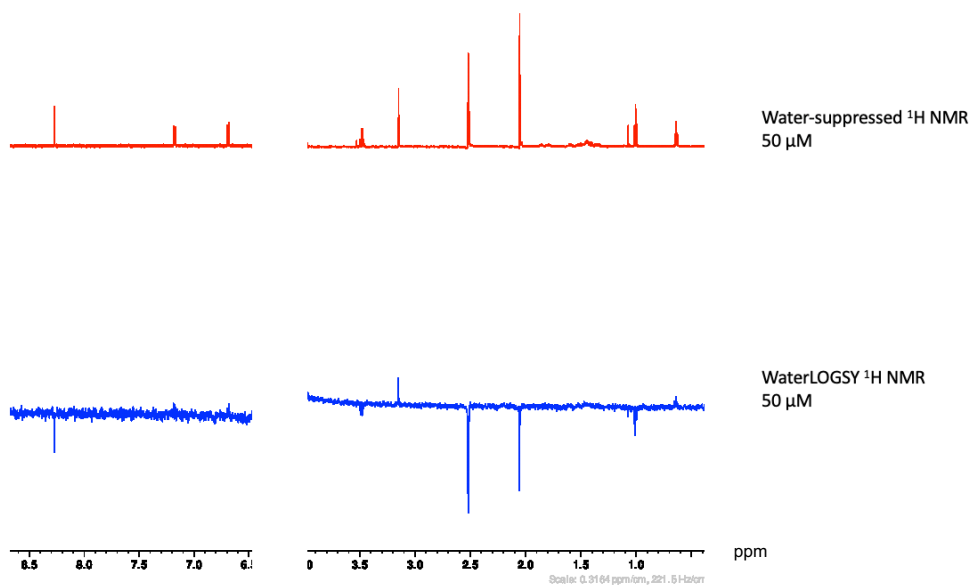
**Figure S39:** Partial waterLOGSY spectra of **35**. The water suppressed  $^1\text{H}$  spectrum is shown in green, the waterLOGSY NMR of the compound only (red) shows inversion of the compound peaks indicating suitable solubilization at 250  $\mu\text{M}$ . No clear inversion of the ligand peak is detected in the presence of 10  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD.



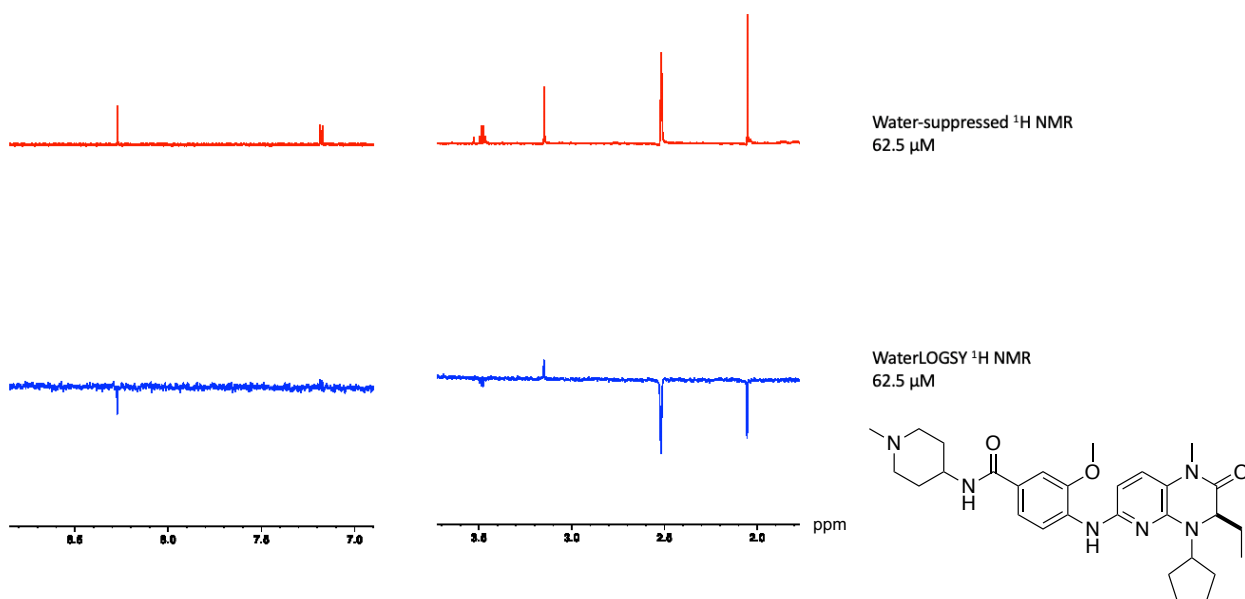
**Figure S40:** Partial waterLOGSY spectra of **36**. The water suppressed  $^1\text{H}$  spectrum is shown in blue, the waterLOGSY NMR of the compound only (green) shows inversion of the compound peaks indicating suitable solubilization at 40  $\mu\text{M}$ . No clear inversion of the ligand peak is detected in the presence of 2  $\mu\text{M}$  of TcBDF3-BRD (purple) indicating the ligand does not bind to TcBDF3-BRD.



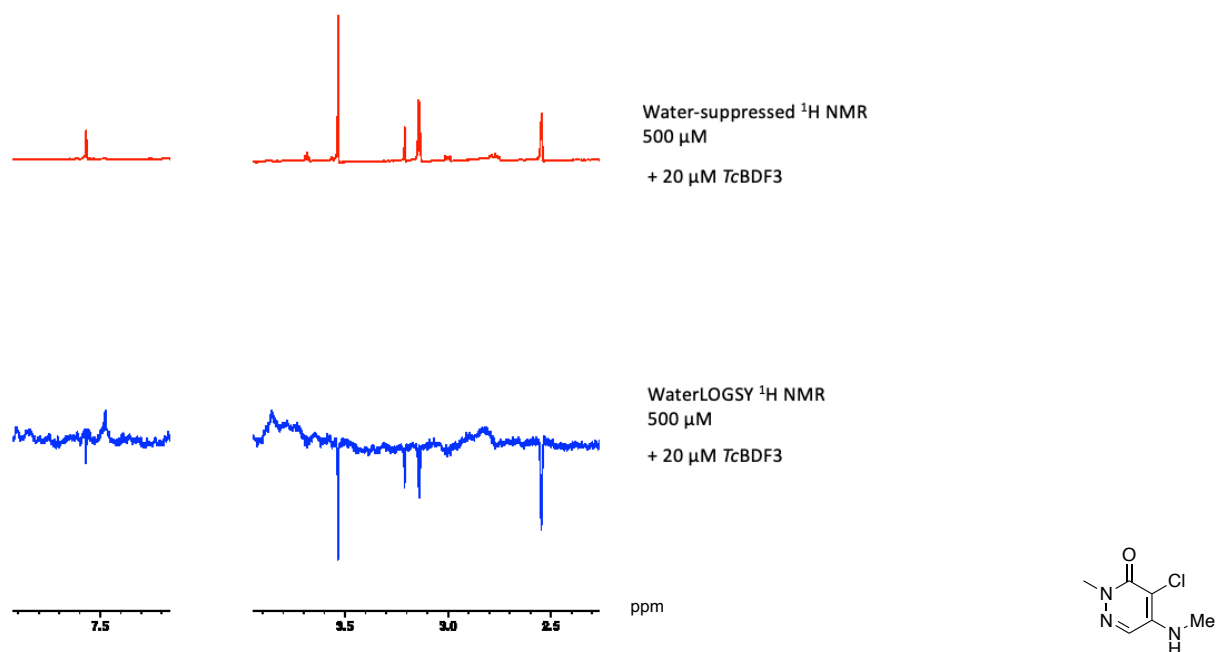
**Figure S41:** Partial waterLOGSY spectra of **37**. The water suppressed  $^1\text{H}$  spectrum is shown in blue, the waterLOGSY NMR of the compound only (green) shows inversion of the compound peaks indicating suitable solubilization at 250  $\mu\text{M}$ . No clear inversion of the ligand peak is detected in the presence of 10  $\mu\text{M}$  of TcBDF3-BRD (purple) indicating the ligand does not bind to TcBDF3-BRD.



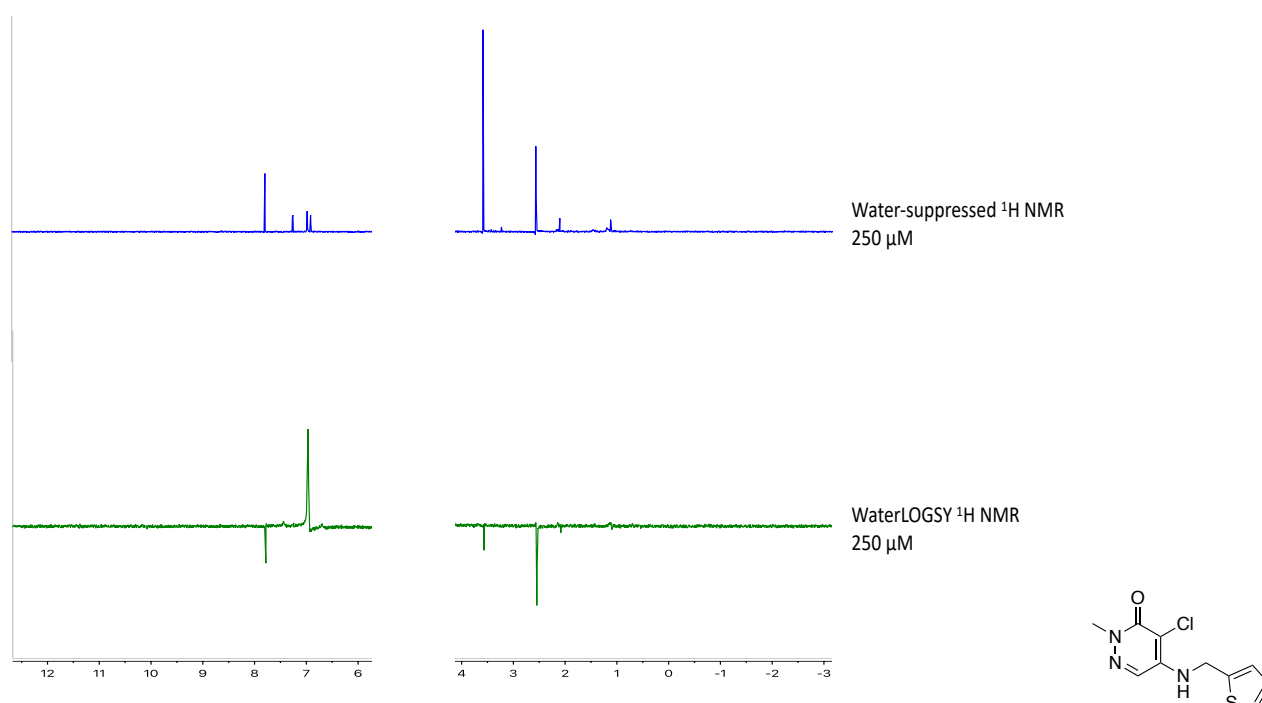
**Figure S42:** Partial waterLOGSY spectra of **38**. Positive ligand peaks in the waterLOGSY spectrum indicates aggregation at 62.5  $\mu\text{M}$  (blue). The water-suppressed  $^1\text{H}$  NMR spectrum (red) is given as reference.



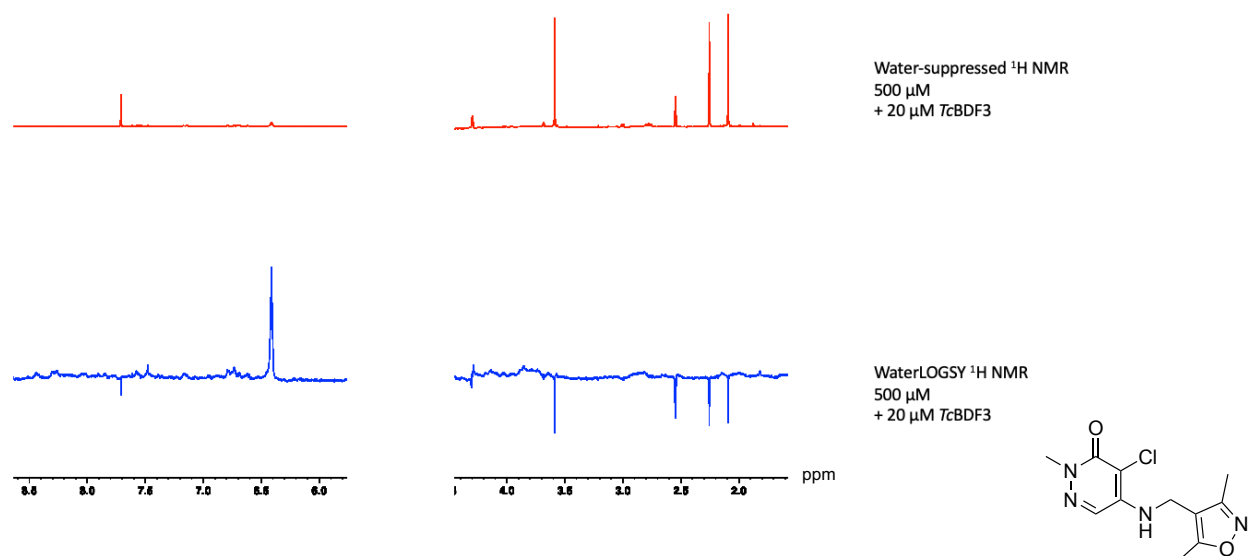
**Figure S43:** Partial waterLOGSY spectra of **39**. Positive ligand peaks in the waterLOGSY spectrum indicates aggregation at 62.5  $\mu\text{M}$  (blue). The water-suppressed  $^1\text{H}$  NMR spectrum (red) is given as reference.



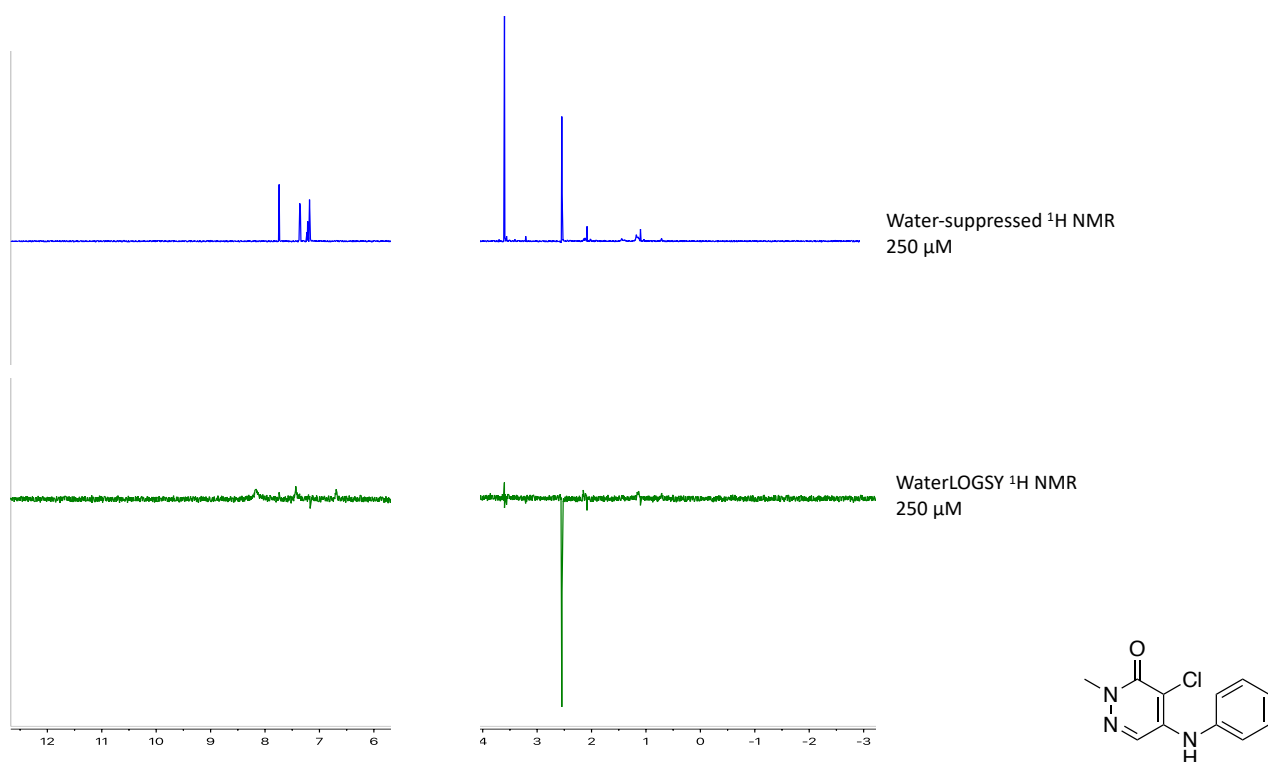
**Figure S44:** Partial waterLOGSY spectra of **40**. No clear inversion of the ligand peak is detected in the presence of 10  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The water suppressed  $^1\text{H}$  spectrum of 250  $\mu\text{M}$  ligand with 10  $\mu\text{M}$  protein (red) is given as a reference.



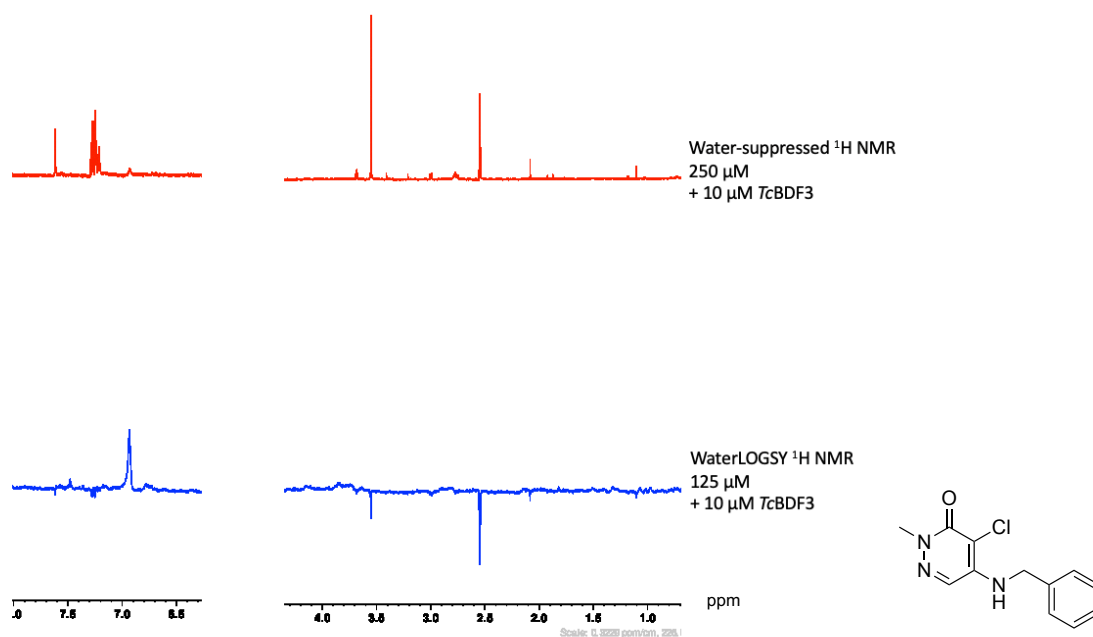
**Figure S45:** Partial waterLOGSY spectra of **41**. The water suppressed  $^1\text{H}$  spectrum is shown in blue, the waterLOGSY NMR of the compound only (green) does not show inversion of the compound peaks indicating that the compound is not soluble at 250  $\mu\text{M}$ .



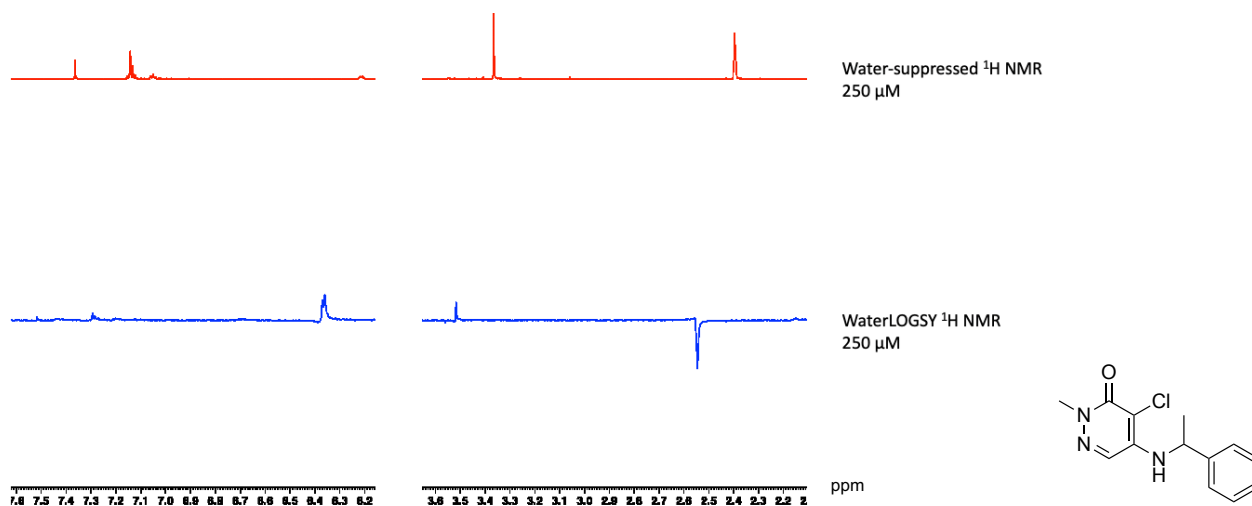
**Figure S46:** Partial waterLOGSY spectra of **42**. No clear inversion of the ligand peak is detected in the presence of 20  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The water suppressed  $^1\text{H}$  spectrum of 500  $\mu\text{M}$  ligand with 20  $\mu\text{M}$  protein (red) is given as a reference.



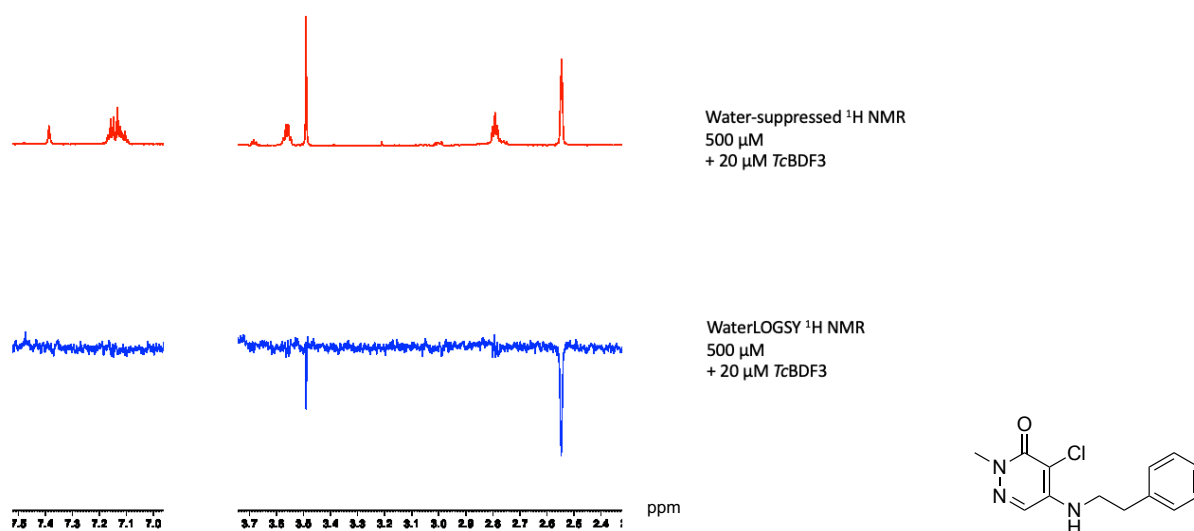
**Figure S47:** Partial waterLOGSY spectra of **43**. The water suppressed  $^1\text{H}$  spectrum is shown in blue, the waterLOGSY NMR of the compound only (green) does not show inversion of the compound peaks indicating that the compound is not soluble at 250  $\mu\text{M}$ .



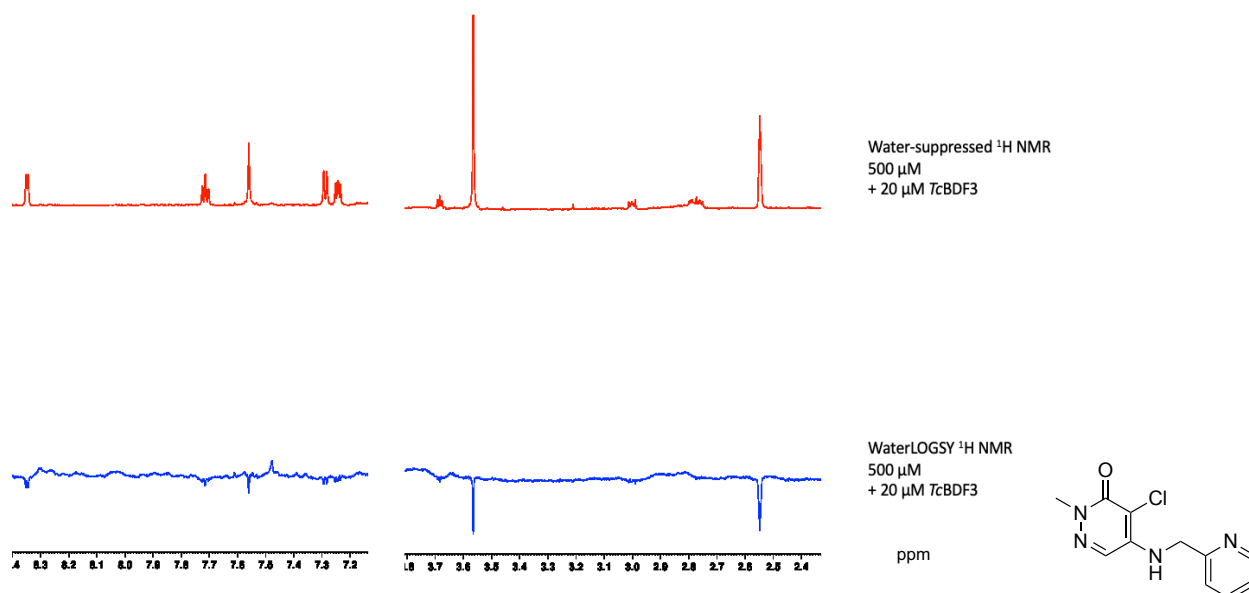
**Figure S48:** Partial waterLOGSY spectra of **44**. No clear inversion of the ligand peak is detected in the presence of 10  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The water suppressed  $^1\text{H}$  spectrum of 250  $\mu\text{M}$  ligand with 10  $\mu\text{M}$  protein (red) is given as a reference.



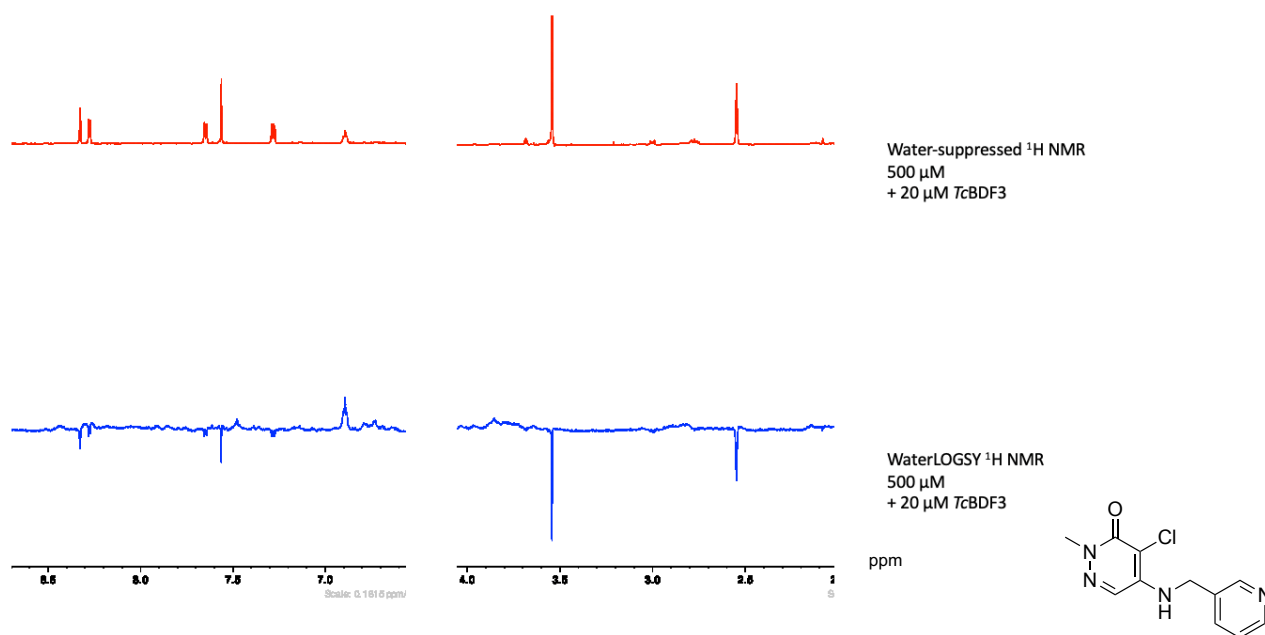
**Figure S49:** Partial waterLOGSY spectra of **45**. Positive ligand peaks in the waterLOGSY spectrum indicates aggregation at 250  $\mu\text{M}$  (blue). The water-suppressed  $^1\text{H}$  NMR spectrum (red) is given as reference.



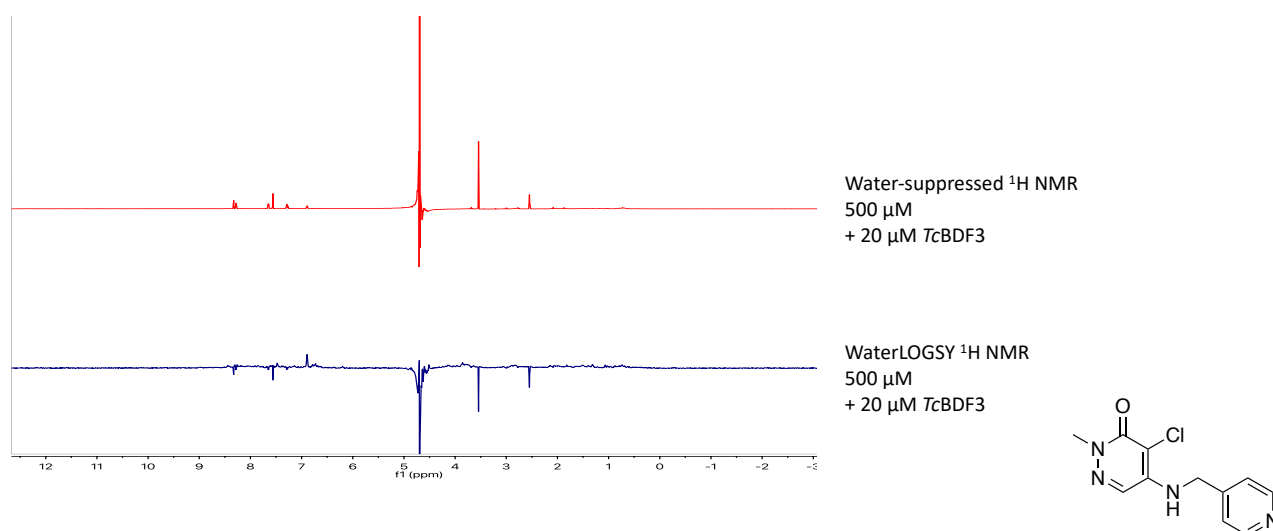
**Figure S50:** Partial waterLOGSY spectra of **46**. No clear inversion of the ligand peak is detected in the presence of 20  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The water suppressed  $^1\text{H}$  spectrum of 500  $\mu\text{M}$  ligand with 20  $\mu\text{M}$  protein (red) is given as a reference.



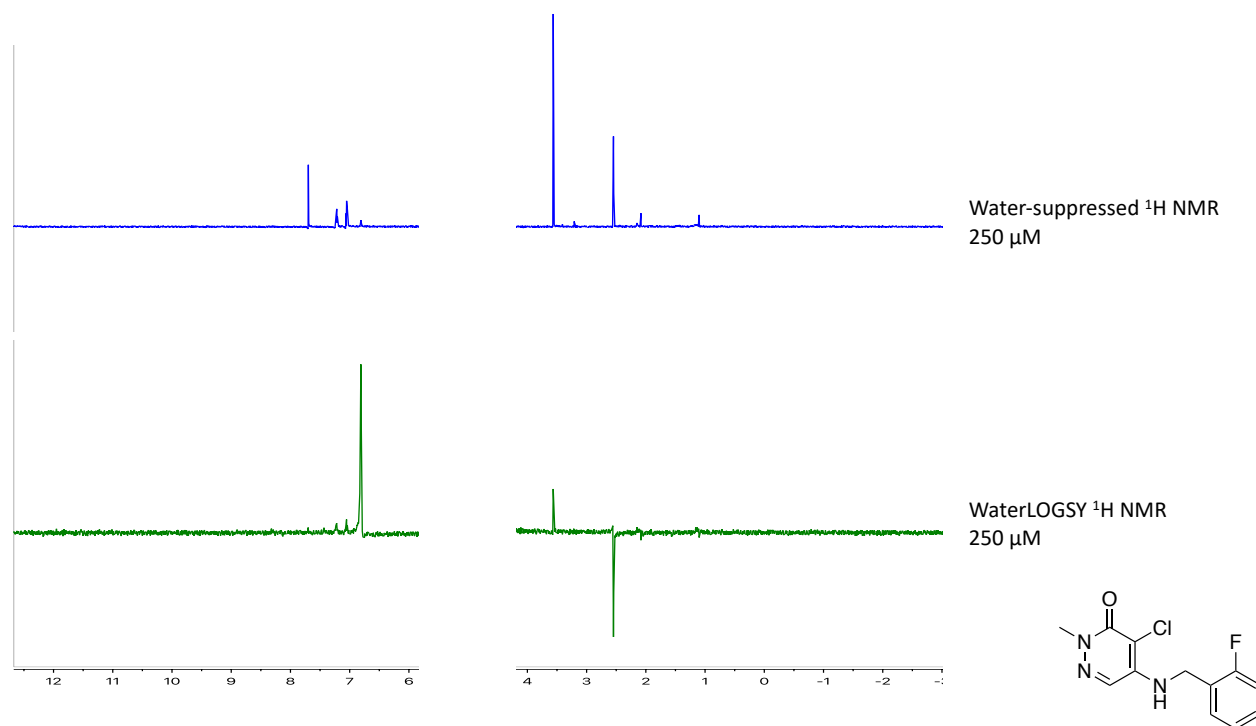
**Figure S51:** Partial waterLOGSY spectra of **47**. No clear inversion of the ligand peak is detected in the presence of 20  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The water suppressed  $^1\text{H}$  spectrum of 500  $\mu\text{M}$  ligand with 20  $\mu\text{M}$  protein (red) is given as a reference.



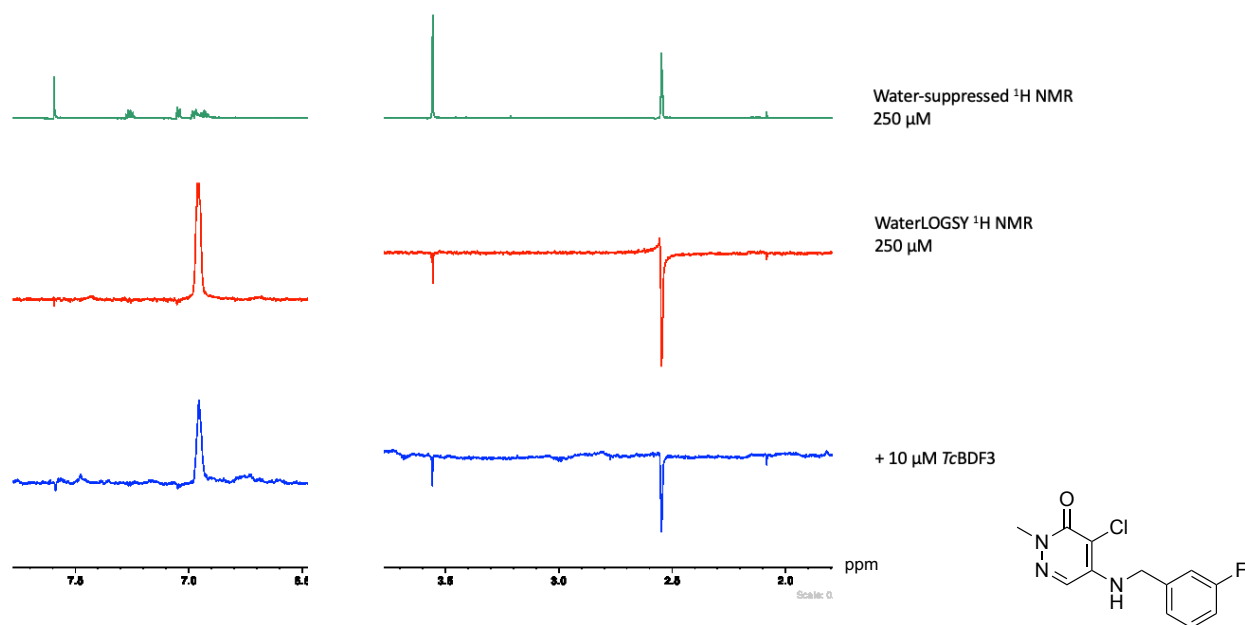
**Figure S52:** Partial waterLOGSY spectra of **48**. No clear inversion of the ligand peak is detected in the presence of 20  $\mu$ M of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The water suppressed  $^1\text{H}$  spectrum of 500  $\mu$ M ligand with 20  $\mu$ M protein (red) is given as a reference.



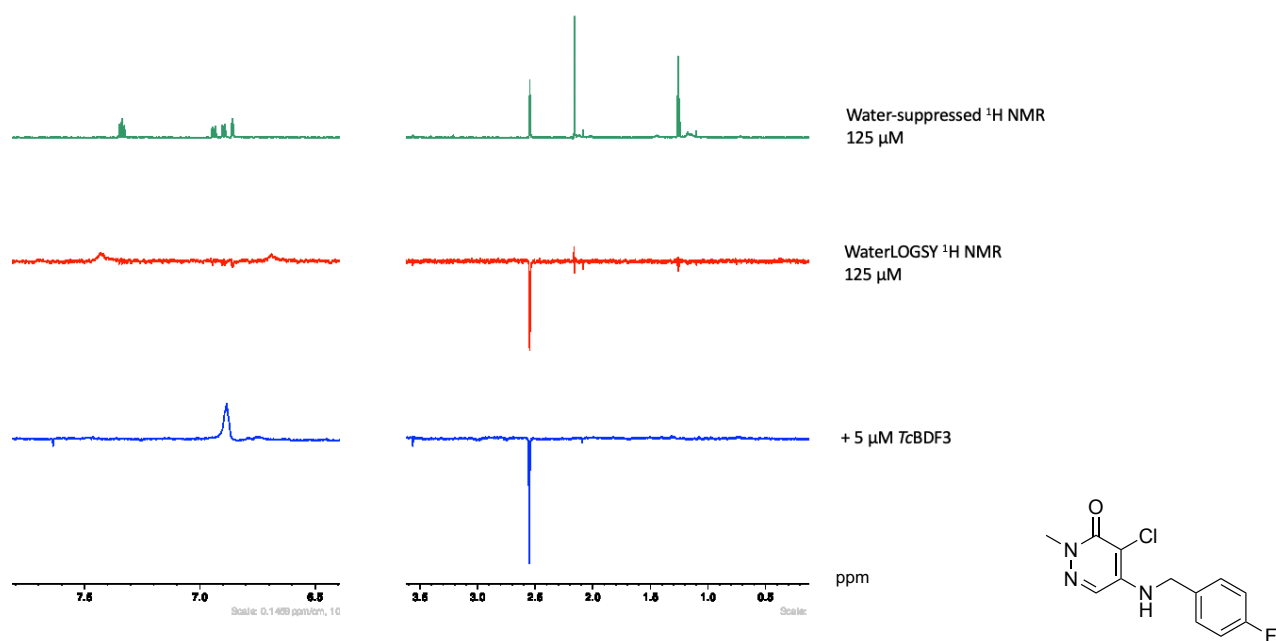
**Figure S53:** Partial waterLOGSY spectra of **49**. No clear inversion of the ligand peak is detected in the presence of 20  $\mu$ M of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The water suppressed  $^1$ H spectrum of 500  $\mu$ M ligand with 20  $\mu$ M protein (red) is given as a reference.



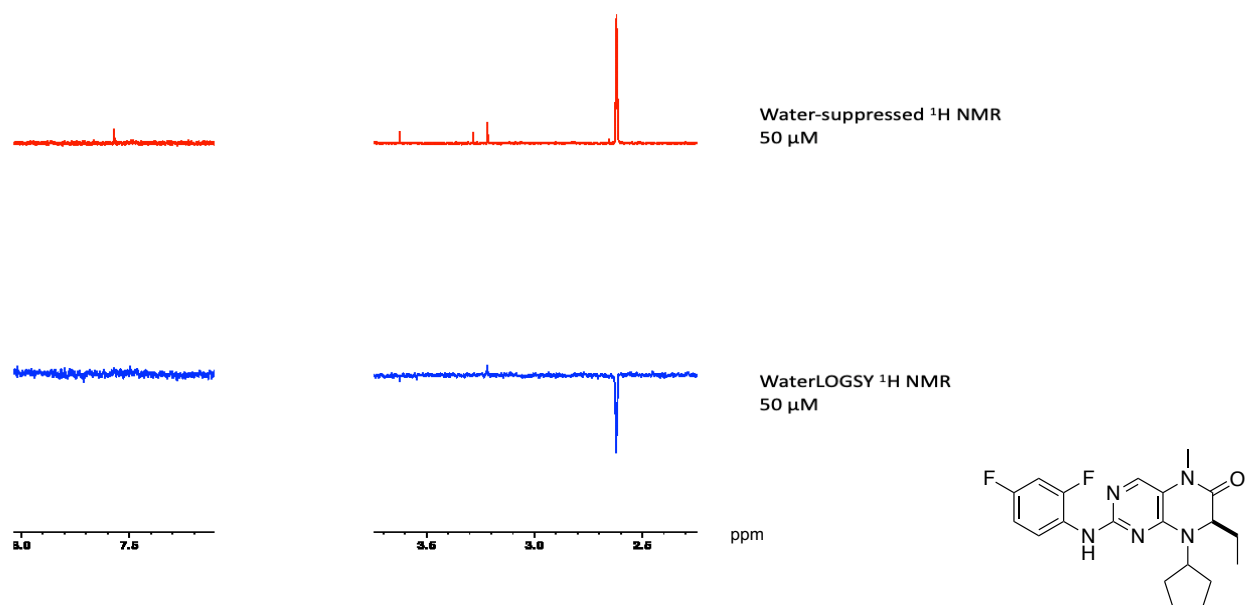
**Figure S54:** Partial waterLOGSY spectra of **50**. The water suppressed  $^1\text{H}$  spectrum is shown in blue, the waterLOGSY NMR of the compound only (green) does not show inversion of the compound peaks indicating that the compound is not soluble at 250  $\mu\text{M}$ .



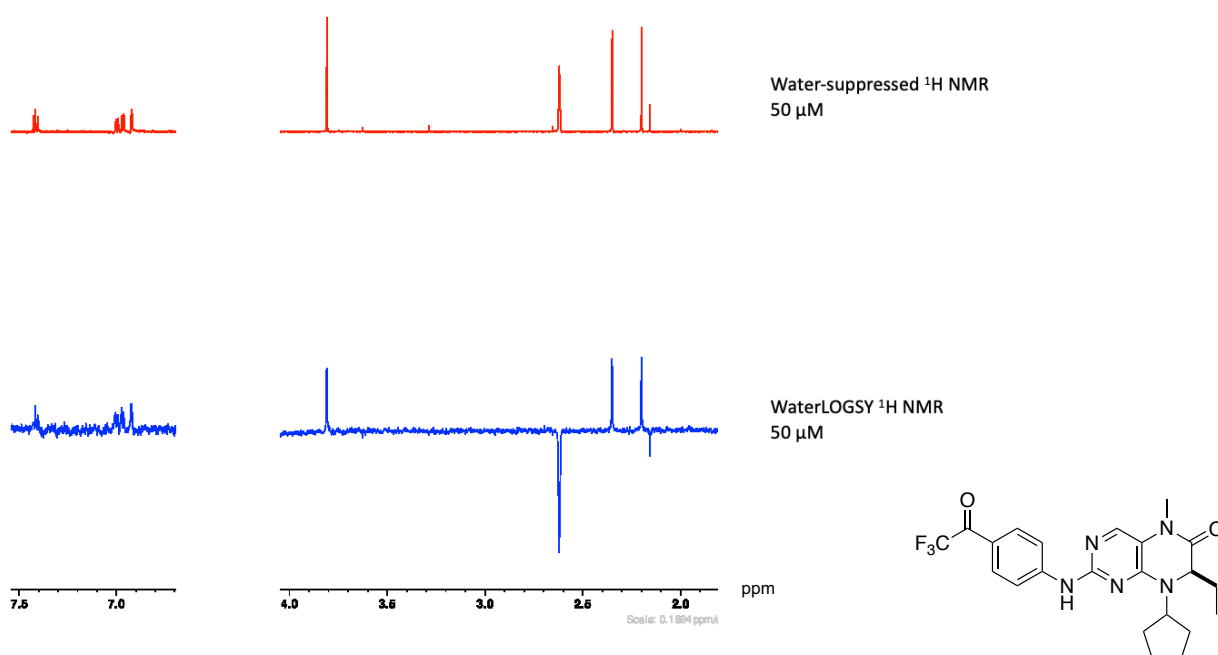
**Figure S55:** Partial waterLOGSY spectra of **51**. The water suppressed  $^1\text{H}$  spectrum is shown in green, the waterLOGSY NMR of the compound only (red) shows inversion of the compound peaks indicating suitable solubilization at 250  $\mu\text{M}$ . No clear inversion of the ligand peak is detected in the presence of 5  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD. The large positive signal in the waterLOGSY spectra corresponds to the exchangeable proton.



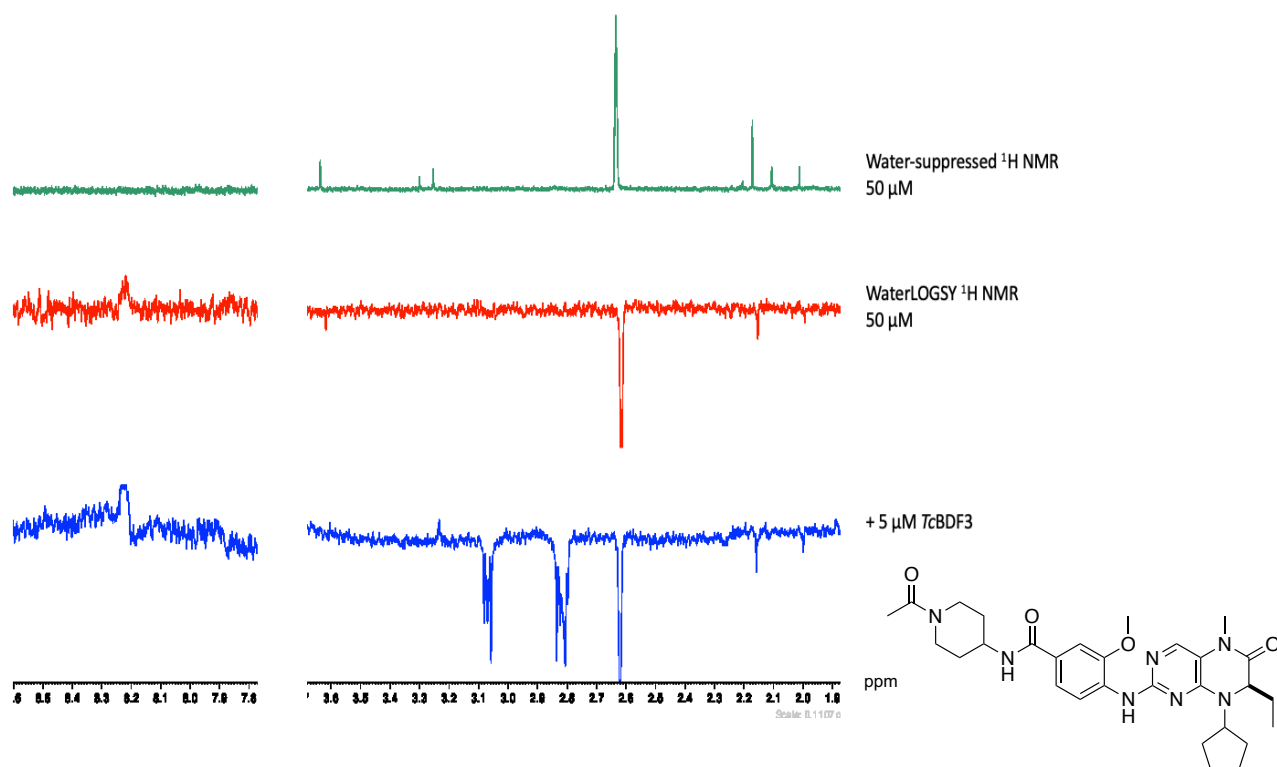
**Figure S56:** Partial waterLOGSY spectra of **52**. The water suppressed  $^1\text{H}$  spectrum is shown in green, the waterLOGSY NMR of the compound only (red) shows inversion of the compound peaks indicating suitable solubilization at 125  $\mu\text{M}$ . No inversion of the ligand peak is detected in the presence of 5  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD.



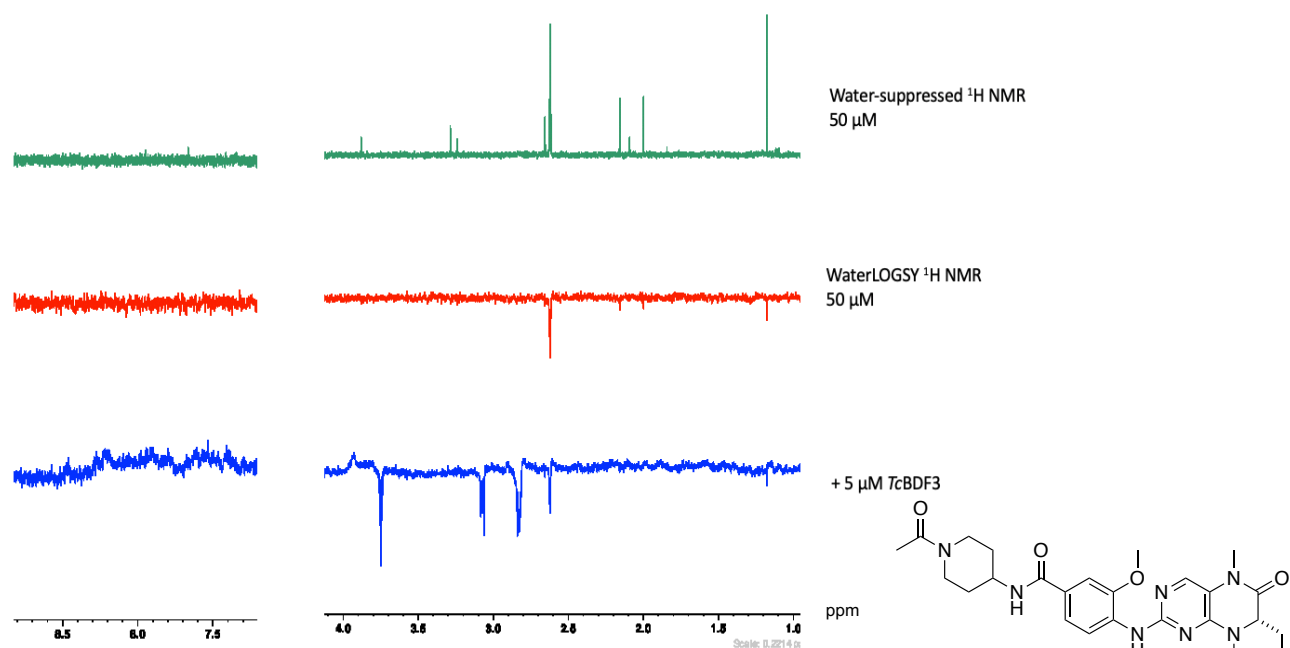
**Figure S57:** Partial waterLOGSY spectra of **54**. Positive ligand peaks in the waterLOGSY spectrum indicates aggregation at 50  $\mu\text{M}$  (blue). The water-suppressed  $^1\text{H}$  NMR spectrum (red) is given as reference.



**Figure S58:** Partial waterLOGSY spectra of **55**. Positive ligand peaks in the waterLOGSY spectrum indicates aggregation at 50  $\mu\text{M}$  (blue). The water-suppressed  $^1\text{H}$  NMR spectrum (red) is given as reference.

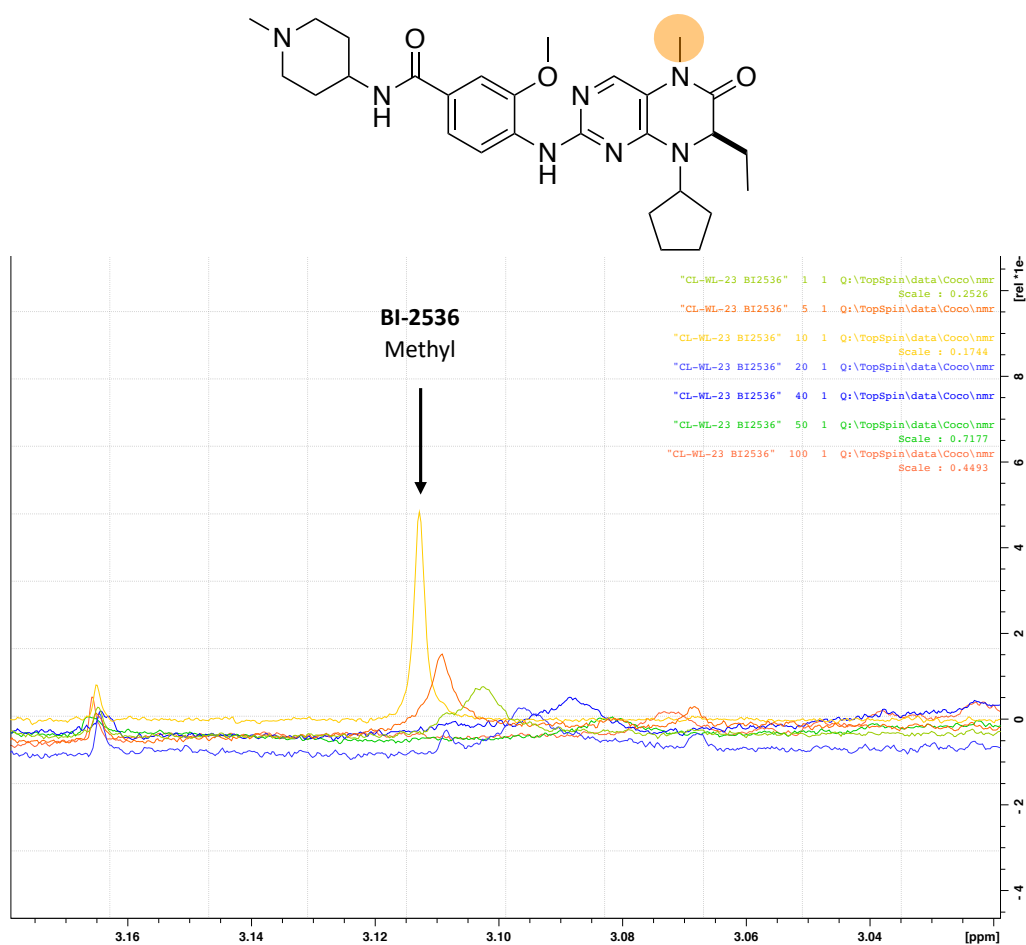


**Figure S59:** Partial waterLOGSY spectra of **59**. The water suppressed  $^1\text{H}$  spectrum is shown in green, the waterLOGSY NMR of the compound only (red) shows inversion of the compound peaks indicating suitable solubilization at 50  $\mu\text{M}$ . The waterLOGSY spectrum in the presence of 5  $\mu\text{M}$  of TcBDF3-BRD (blue) displays inversion of the ligand peaks, indicating binding to TcBDF3-BRD.

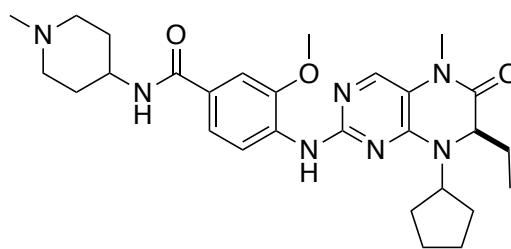


**Figure S60:** Partial waterLOGSY spectra of **60**. The water suppressed  $^1\text{H}$  spectrum is shown in green, the waterLOGSY NMR of the compound only (red) shows inversion of the compound peaks indicating suitable solubilization at 50  $\mu\text{M}$ . No clear inversion of the ligand peak is detected in the presence of 5  $\mu\text{M}$  of TcBDF3-BRD (blue) indicating the ligand does not bind to TcBDF3-BRD.

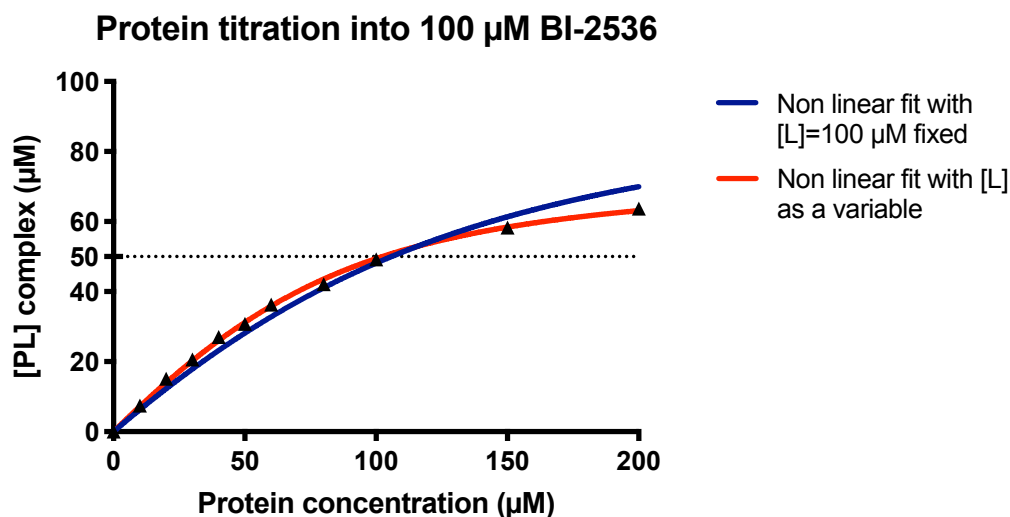
## SI-C: PROTEIN TITRATION EXPERIMENTS



**Figure S61:** Partial spectra overlay for the ligand-observed proton NMR of 0–200 μM *Tc*BDF3-BRD into 100 μM BI-2536 (1) ligand. *Tc*BDF3-BRD concentrations: 0 μM (yellow), 5 μM (orange), 10 μM (green), 20 μM (blue), 40 μM (dark blue), 100 μM (dark orange).

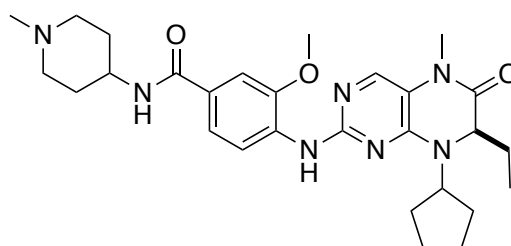


BI-2536



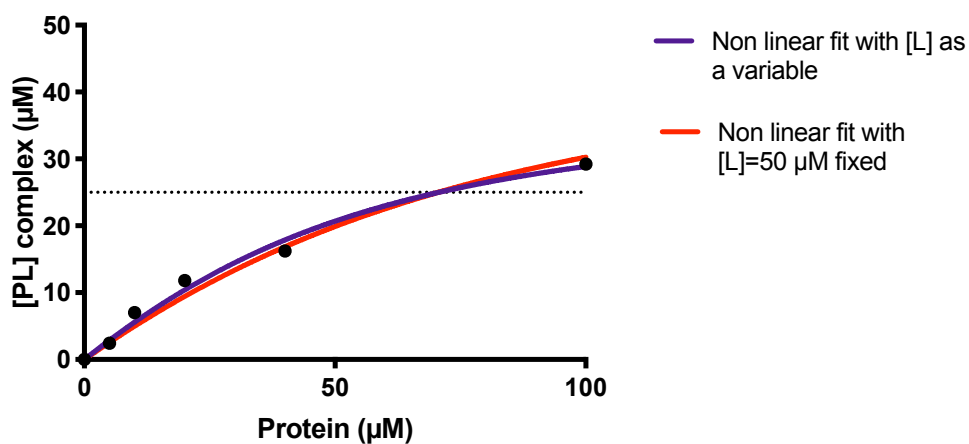
$\partial\text{obs}$		$\partial\text{obs}$	
NMR Titration (quadratic) [3]		NMR Titration (quadratic) [3]	
Best-fit values		Best-fit values	
Lt	= 100.0	Lt	75.42
Kd	55.81	Kd	26.64
Std. Error		Std. Error	
Kd	4.501	Lt	1.381
95% CI (asymptotic)		95% CI (asymptotic)	
Kd	45.79 to 65.84	Kd	1.604
Goodness of Fit		Goodness of Fit	
Degrees of Freedom	10	Lt	72.29 to 78.54
R squared	0.9753	Kd	23.02 to 30.27
Sum of Squares	103.2	Goodness of Fit	
Sy.x	3.212	Degrees of Freedom	9
Constraints		R squared	0.9987
Lt	Lt = 100	Sum of Squares	5.420
Number of points		Sy.x	0.7760
# of X values	11	Number of points	
		# of X values	11

**Figure S62:** Ligand-observed proton NMR titration analysis for the titration of 0–200  $\mu\text{M}$  TcBDF3-BRD into 100  $\mu\text{M}$  BI-2536 (**1**) ligand using Prism v8. The non-linear data fit is shown with the ligand concentration set either as a variable or as a fixed value.



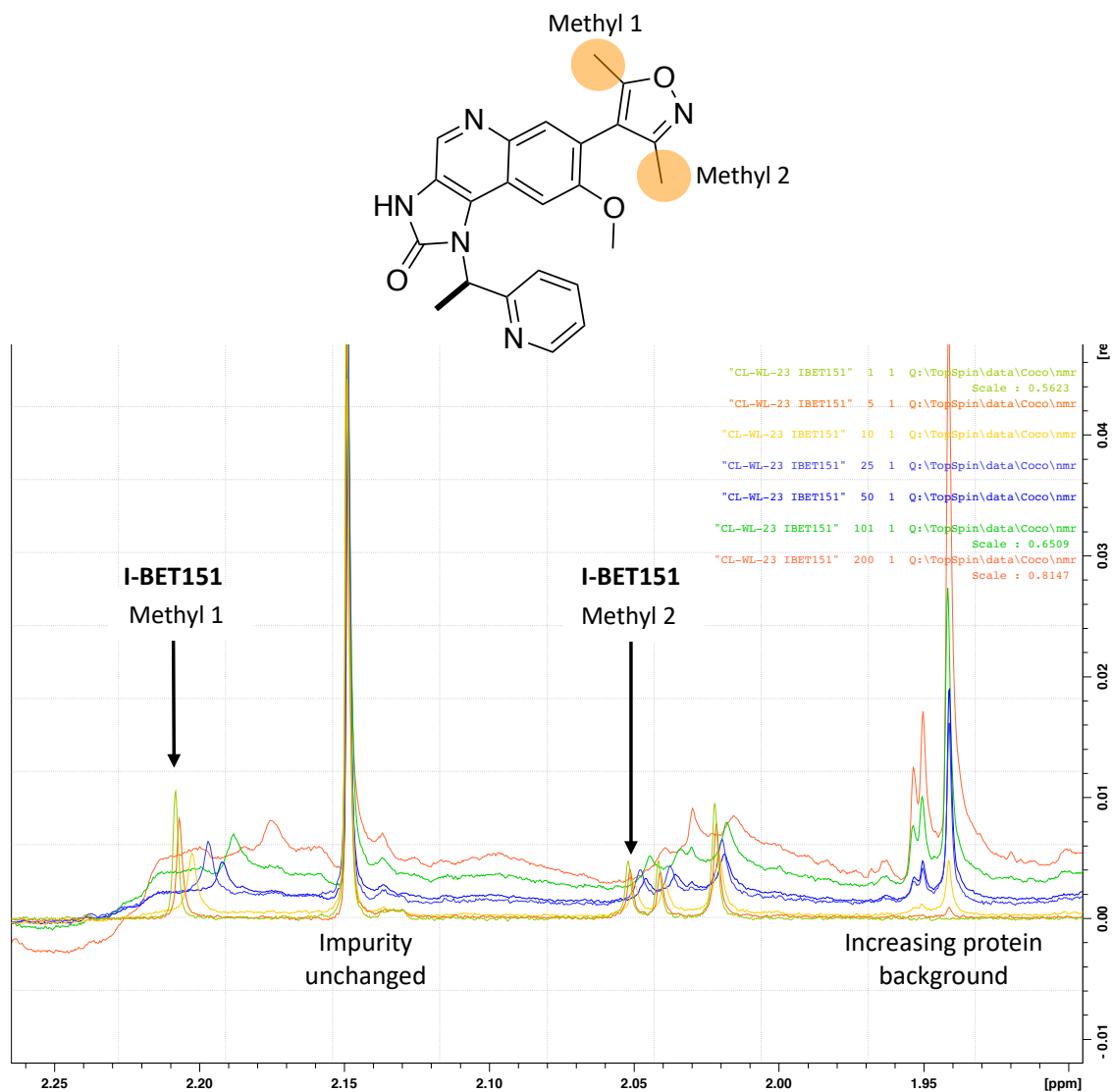
BI-2536

### Protein titration into 50 $\mu\text{M}$ BI-2536

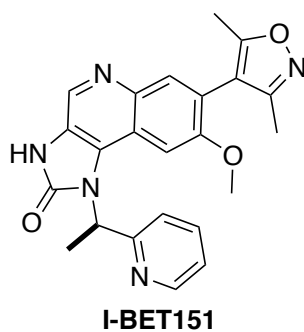


[PL] ( $\mu\text{M}$ )		[PL] ( $\mu\text{M}$ )	
NMR Titration (quadratic)		NMR Titration (quadratic)	
Best-fit values		Best-fit values	
Lt	= 50.00	Lt	39.96
Kd	45.54	Kd	27.33
Std. Error		Std. Error	
Kd	5.023	Lt	4.882
95% CI (asymptotic)		Kd	8.852
Kd	32.62 to 58.45	95% CI (asymptotic)	
Goodness of Fit		Lt	26.41 to 53.52
Degrees of Freedom	5	Kd	2.750 to 51.91
R squared	0.9800	Goodness of Fit	
Sum of Squares	11.42	Degrees of Freedom	4
Sy.x	1.511	R squared	0.9873
Constraints		Sum of Squares	7.252
Lt	Lt = 50	Sy.x	1.346
Number of points		Number of points	
# of X values	6	# of X values	6

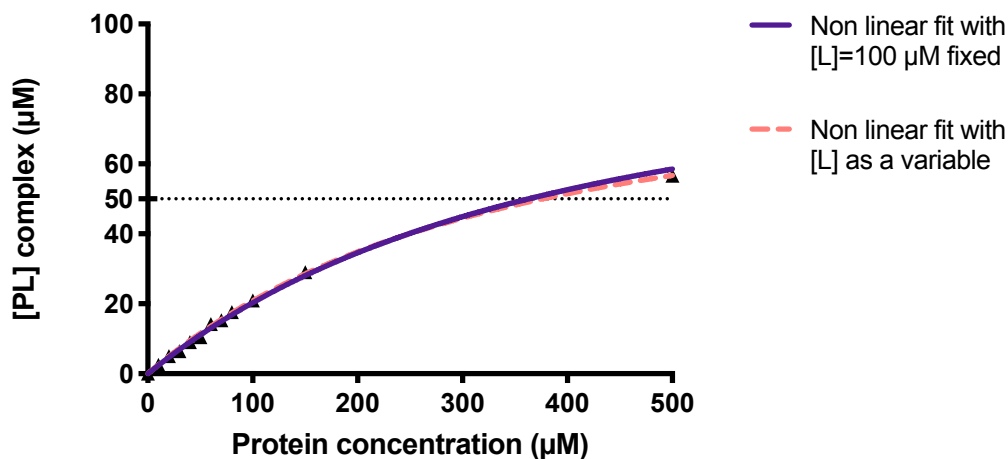
**Figure S63:** Ligand-observed proton NMR titration analysis for the titration of 0–100  $\mu\text{M}$  TcBDF3-BRD into 50  $\mu\text{M}$  BI-2536 (**1**) ligand using Prism v8. The non-linear data fit is shown with the ligand concentration set either as a variable or as a fixed value.



**Figure S64:** Partial spectra overlay for the ligand-observed proton NMR of 0–500  $\mu\text{M}$  TcBDF3-BRD into 100  $\mu\text{M}$  I-BET151 (**2**) ligand. TcBDF3-BRD concentrations: 0  $\mu\text{M}$  (light green), 5  $\mu\text{M}$  (orange), 10  $\mu\text{M}$  (yellow), 25  $\mu\text{M}$  (blue), 50  $\mu\text{M}$  (dark blue), 100  $\mu\text{M}$  (dark green), 200  $\mu\text{M}$  (dark orange).

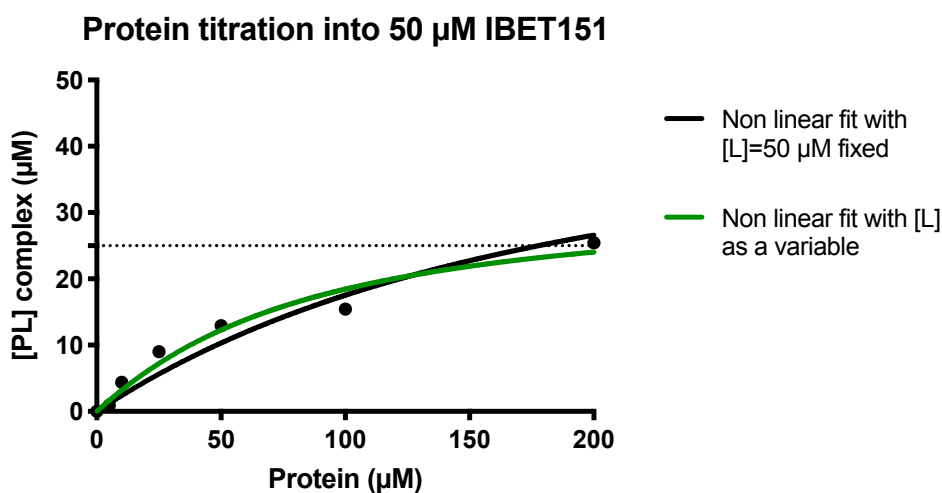
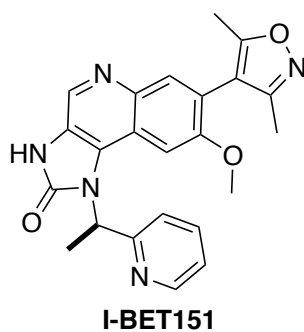


**Protein titration curve into 100  $\mu$ M I-BET151**



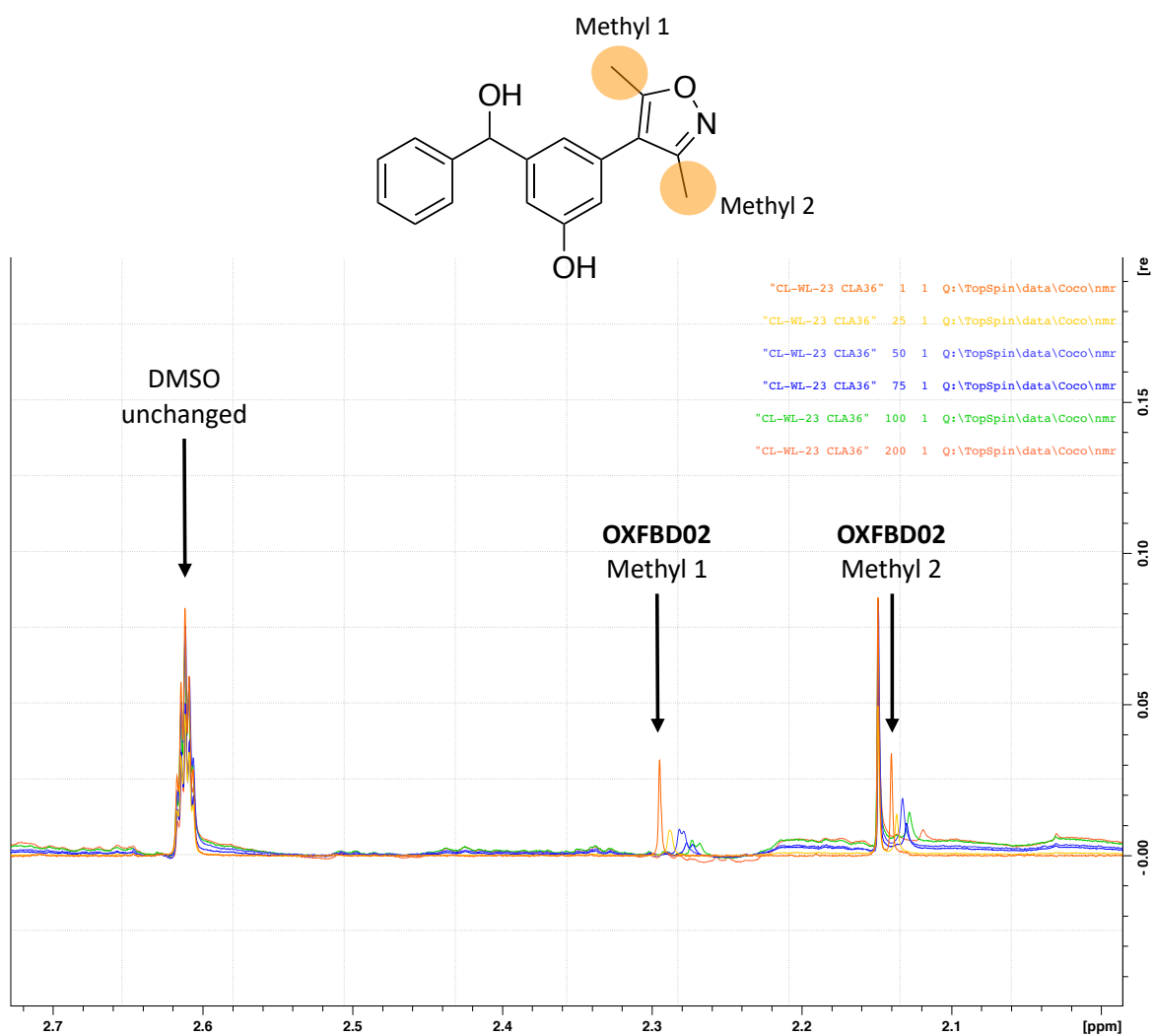
$\partial$ obs		$\partial$ obs	
NMR Titration (quadratic) [3]		NMR Titration (quadratic) [3]	
Best-fit values		Best-fit values	
Lt	= 100.0	Lt	90.39
Kd	312.9	Kd	263.3
Std. Error		Std. Error	
Kd	6.948	Lt	1.965
95% CI (asymptotic)		95% CI (asymptotic)	
Kd	297.6 to 328.2	Kd	10.48
Goodness of Fit		Goodness of Fit	
Degrees of Freedom	11	Lt	86.01 to 94.77
R squared	0.9969	Kd	240.0 to 286.7
Sum of Squares	8.002	Goodness of Fit	
Sy.x	0.8529	Degrees of Freedom	10
Constraints		R squared	0.9990
Lt	Lt = 100	Sum of Squares	2.677
Number of points		Sy.x	0.5174
# of X values	12	Number of points	
		# of X values	12

**Figure S65:** Ligand-observed proton NMR titration analysis for the titration of 0–500  $\mu$ M *TcBDF3*-BRD into 100  $\mu$ M I-BET151 (**2**) ligand using Prism v8. The non-linear data fit is shown with the ligand concentration set either as a variable or as a fixed value.

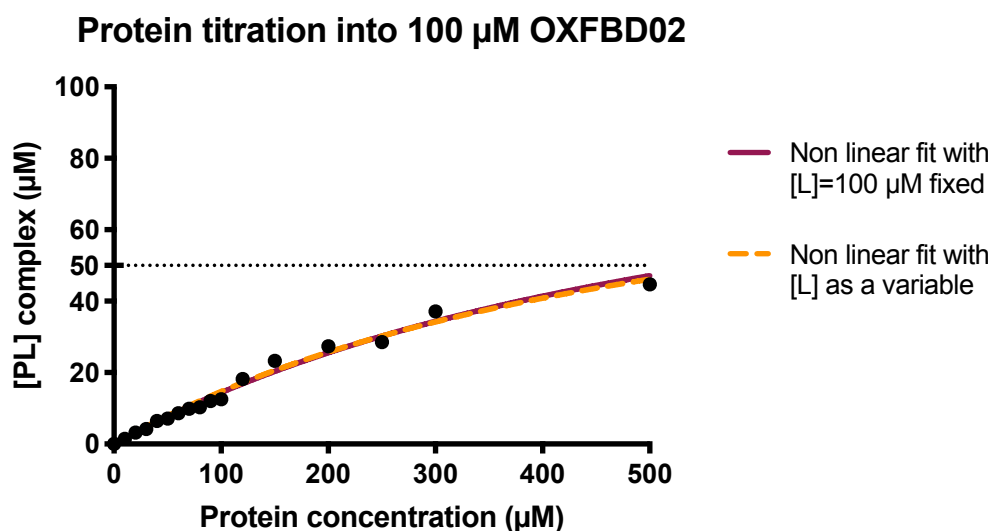
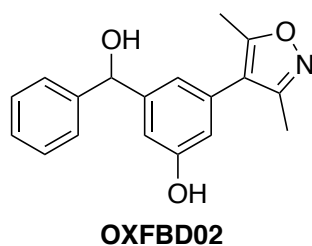


[PL] ( $\mu\text{M}$ )		[PL] ( $\mu\text{M}$ )	
NMR Titration (quadratic) [3]		NMR Titration (quadratic) [3]	
Best-fit values		Best-fit values	
Lt	= 50.00	Lt	32.54
Kd	152.6	Kd	62.22
Std. Error		Std. Error	
Kd	19.13	Lt	5.174
95% CI (asymptotic)		Kd	
Kd	105.8 to 199.4		24.76
Goodness of Fit		95% CI (asymptotic)	
Degrees of Freedom	6	Lt	19.24 to 45.84
R squared	0.9421	Kd	-1.439 to 125.9
Sum of Squares	28.34	Goodness of Fit	
Sy.x	2.173	Degrees of Freedom	5
Constraints		R squared	0.9660
Lt	Lt = 50	Sum of Squares	16.64
		Sy.x	1.824
Number of points		Number of points	
# of X values	7	# of X values	7

**Figure S66:** Ligand-observed proton NMR titration analysis for the titration of 0–500  $\mu\text{M}$  TcBDF3-BRD into 50  $\mu\text{M}$  I-BET151 (**2**) ligand using Prism v8. The non-linear data fit is shown with the ligand concentration set either as a variable or as a fixed value.

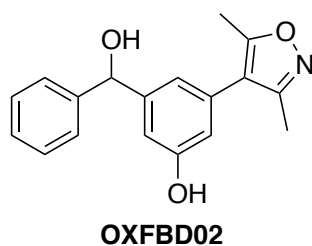


**Figure S67:** Partial spectra overlay for the ligand-observed proton NMR of 0–500  $\mu\text{M}$  *TcBDF3*-BRD into 100  $\mu\text{M}$  OXFBD02 (**3**) ligand. *TcBDF3*-BRD concentrations: 0  $\mu\text{M}$  (orange), 25  $\mu\text{M}$  (yellow), 50  $\mu\text{M}$  (blue), 75  $\mu\text{M}$  (dark blue), 100  $\mu\text{M}$  (green), 200  $\mu\text{M}$  (dark orange).

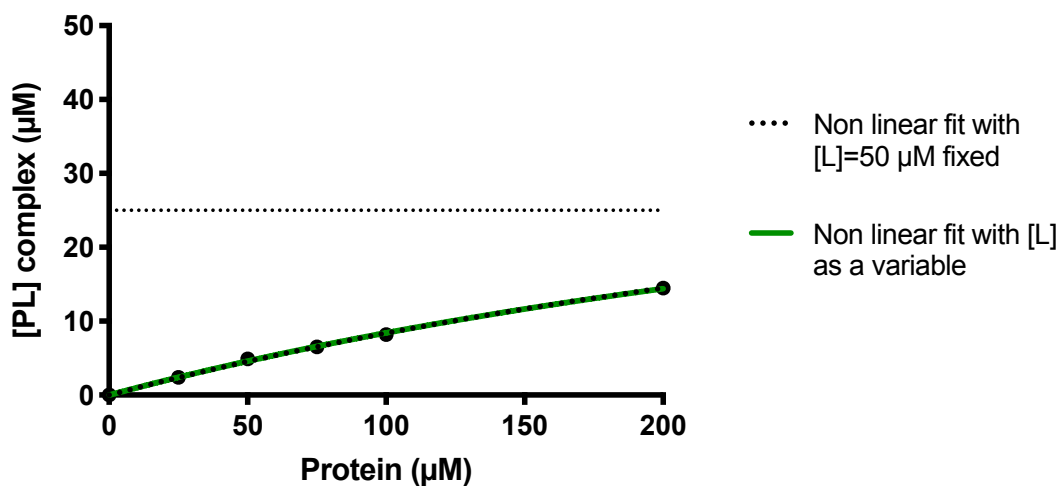


$\partial_{\text{obs}}$		$\partial_{\text{obs}}$	
NMR Titration (quadratic) [3]		NMR Titration (quadratic) [3]	
Best-fit values		Best-fit values	
Lt	= 100.0	Lt	91.34
Kd	507.9	Kd	444.4
Std. Error		Std. Error	
Kd	15.73	Lt	9.659
95% CI (asymptotic)		95% CI (asymptotic)	
Kd	474.5 to 541.2	Kd	71.77
Goodness of Fit		Goodness of Fit	
Degrees of Freedom	16	Lt	70.76 to 111.9
R squared	0.9857	Kd	291.4 to 597.4
Sum of Squares	38.55	Goodness of Fit	
Sy.x	1.552	Degrees of Freedom	15
Constraints		R squared	0.9863
Lt	Lt = 100	Sum of Squares	36.78
		Sy.x	1.566
Number of points		Number of points	
# of X values	17	# of X values	17

**Figure S68:** Ligand-observed proton NMR titration analysis for the titration of 0–500  $\mu\text{M}$  TcBDF3-BRD into 100  $\mu\text{M}$  OXFBD02 (**3**) ligand using Prism v8. The non-linear data fit is shown with the ligand concentration set either as a variable or as a fixed value.

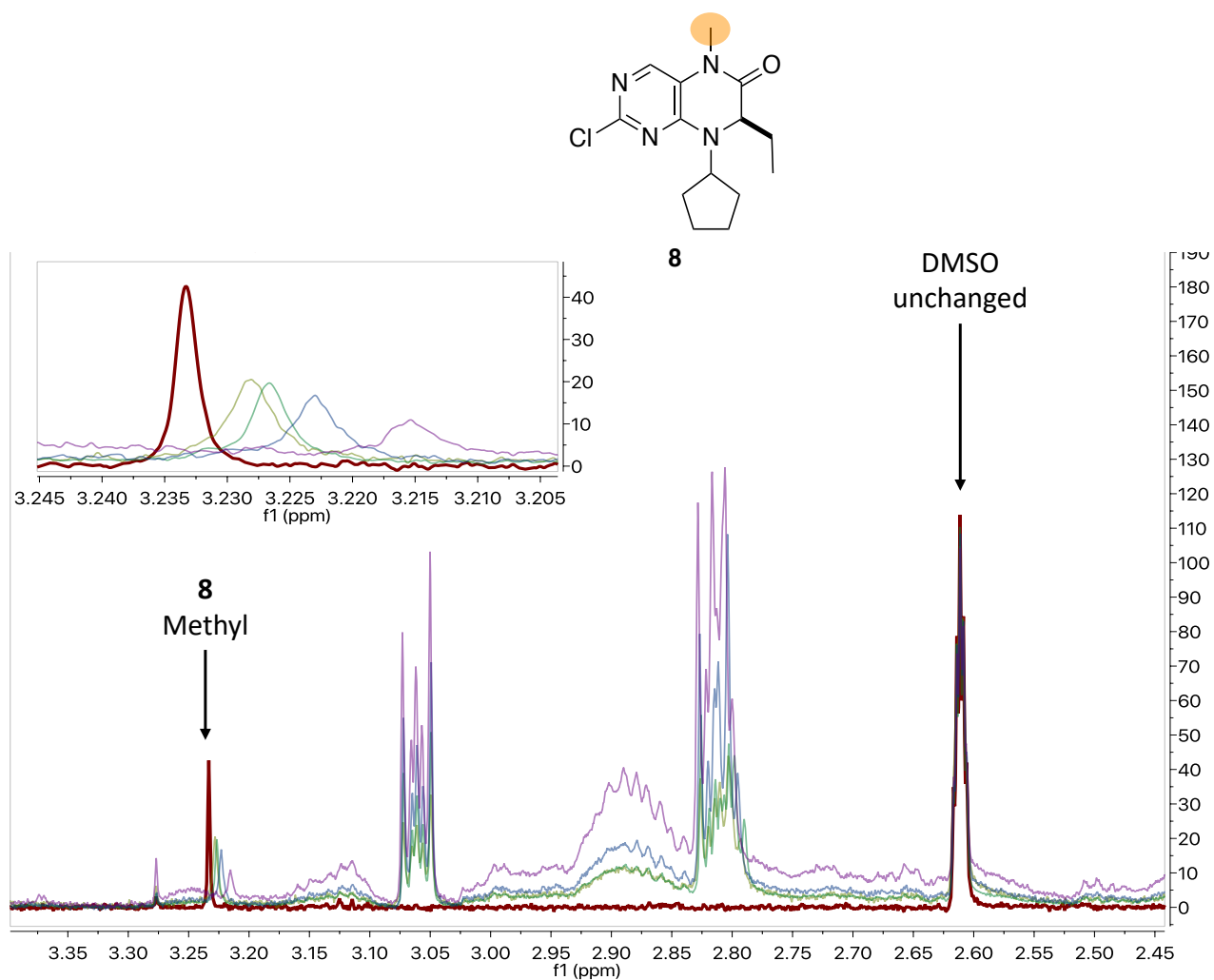


### Protein titration into 50 $\mu\text{M}$ OXFBD02

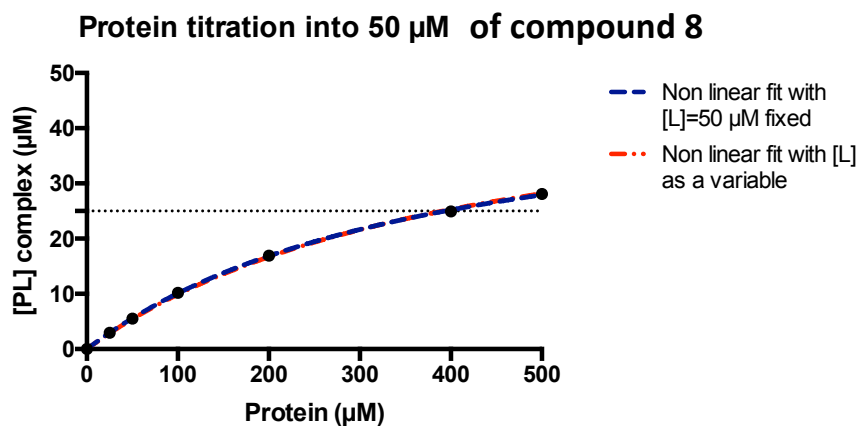
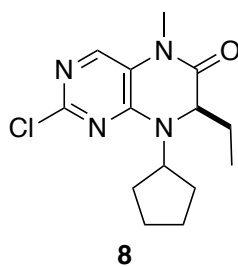


[PL] ( $\mu\text{M}$ )		[PL] ( $\mu\text{M}$ )	
NMR Titration (quadratic)		NMR Titration (quadratic)	
Best-fit values		Best-fit values	
Lt	= 50.00	Lt	46.89
Kd	455.1	Kd	418.7
Std. Error		Std. Error	
Kd	6.319	Lt	5.152
95% CI (asymptotic)		Kd	60.65
Kd	438.9 to 471.4	95% CI (asymptotic)	
Goodness of Fit		Lt	32.59 to 61.20
Degrees of Freedom	5	Kd	250.3 to 587.1
R squared	0.9986	Goodness of Fit	
Sum of Squares	0.1764	Degrees of Freedom	4
Sy.x	0.1879	R squared	0.9987
Constraints		Sum of Squares	0.1637
Lt	Lt = 50	Sy.x	0.2023
Number of points		Number of points	
# of X values	6	# of X values	6
		# Y values analyzed	6

**Figure S69:** Ligand-observed proton NMR titration analysis for the titration of 0–500  $\mu\text{M}$  TcBDF3-BRD into 50  $\mu\text{M}$  OXFBD02 (**3**) ligand using Prism v8. The non-linear data fit is shown with the ligand concentration set either as a variable or as a fixed value.



**Figure S70:** Partial spectra overlay for the ligand-observed proton NMR of 0–200  $\mu\text{M}$  TcBDF3-BRD into 50  $\mu\text{M}$  of **8**. TcBDF3-BRD concentrations: 0  $\mu\text{M}$  (brown), 50  $\mu\text{M}$  (green), 100  $\mu\text{M}$  (dark green), 150  $\mu\text{M}$  (blue), and 200  $\mu\text{M}$  (pink). The concentration dependent chemical shift changes of the N-Me resonance of **8** were used to estimate the  $K_d$  value for the binding of **8** to TcBDF3-BRD.

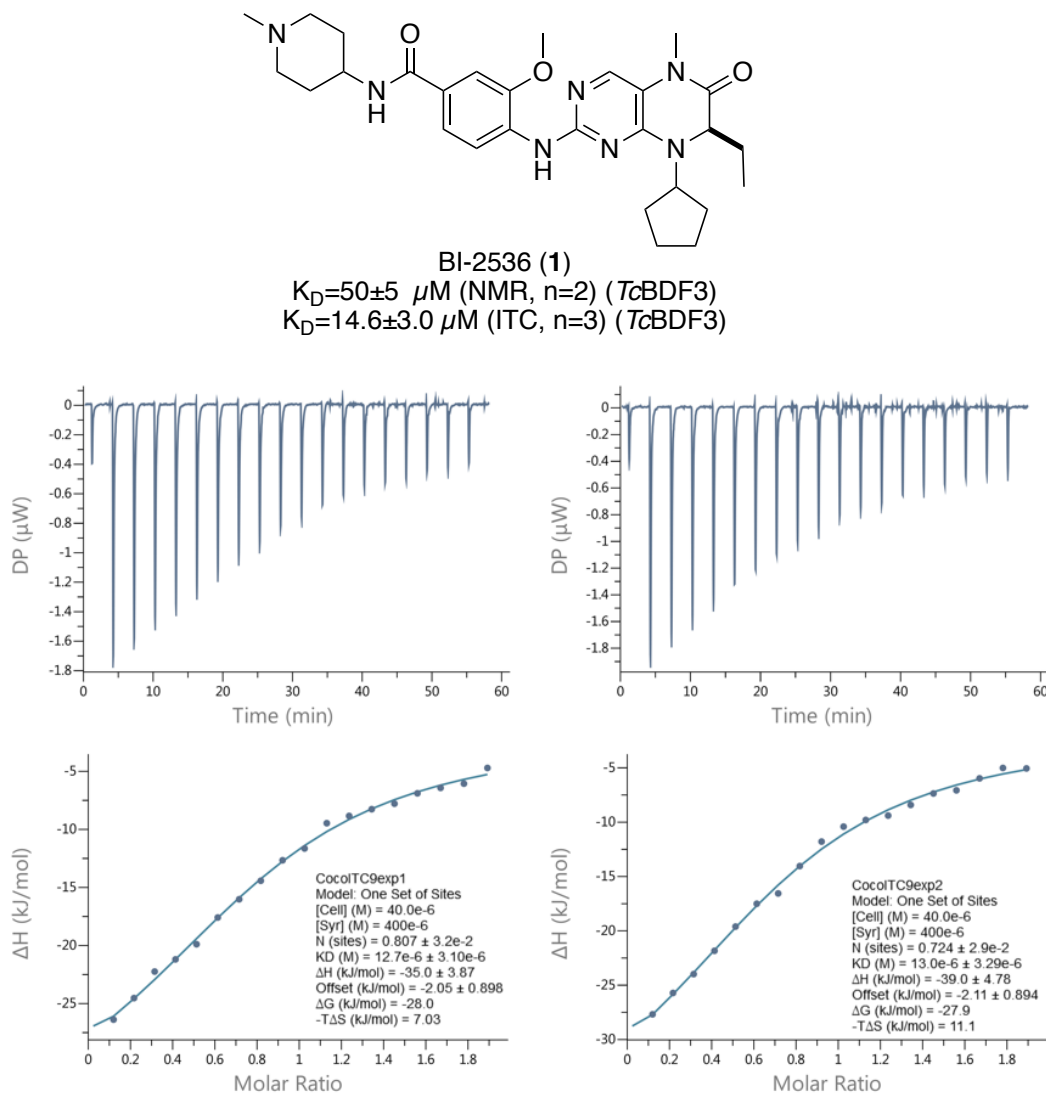


[PL] ( $\mu$ M)		[PL] ( $\mu$ M)	
NMR Titration (quadratic) [3]		NMR Titration (quadratic) [3]	
Best-fit values		Best-fit values	
Lt	47.74	Lt	= 50.00
Kd	335.1	Kd	365.7
Std. Error		Std. Error	
Lt	0.7443	Kd	3.670
Kd	10.23	95% Confidence Intervals	
95% Confidence Intervals		Kd	356.7 to 374.7
Lt	45.82 to 49.65	Goodness of Fit	
Kd	308.8 to 361.4	Degrees of Freedom	6
Goodness of Fit		R square	0.9996
Degrees of Freedom	5	Absolute Sum of Squares	0.2889
R square	0.9998	Sy.x	0.2194
Absolute Sum of Squares	0.1092	Constraints	
Sy.x	0.1478	Lt	Lt = 50.00
Number of points		Number of points	
Analyzed	7	Analyzed	7

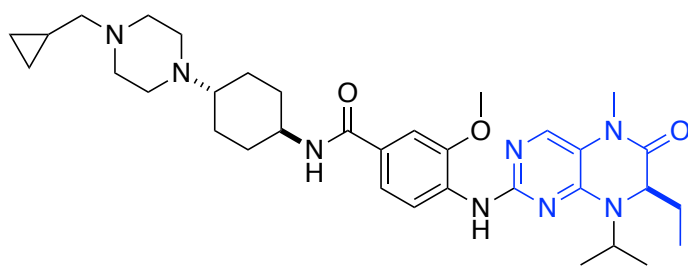
**Figure S71:** Ligand-observed proton NMR titration analysis for the titration of 0–100  $\mu$ M TcBDF3-BRD into 50  $\mu$ M **8** ligand using Prism v8. The non-linear data fit is shown with the ligand concentration set either as a variable or as a fixed value.

## SI-D: ISOTHERMAL CALORIMETRY

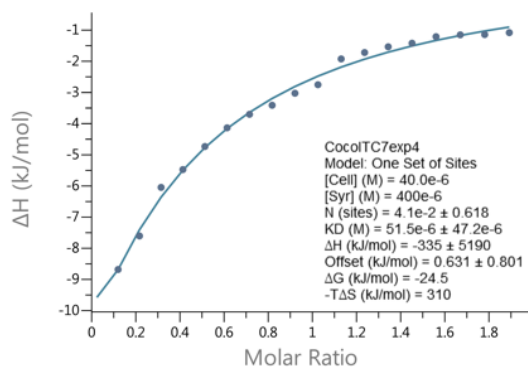
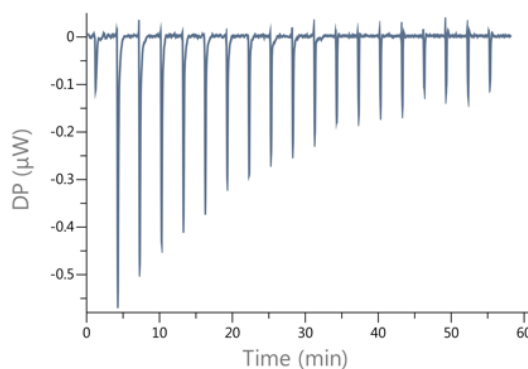
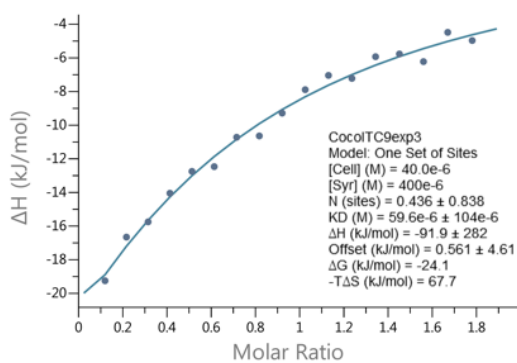
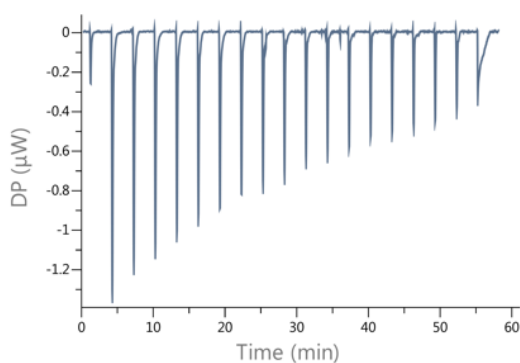
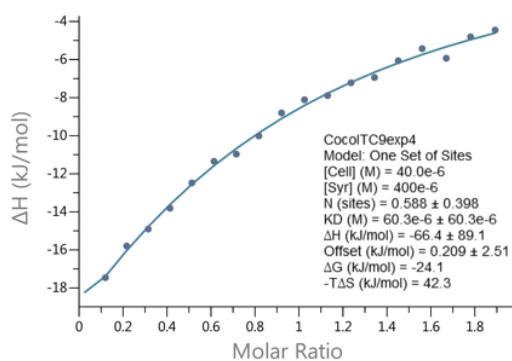
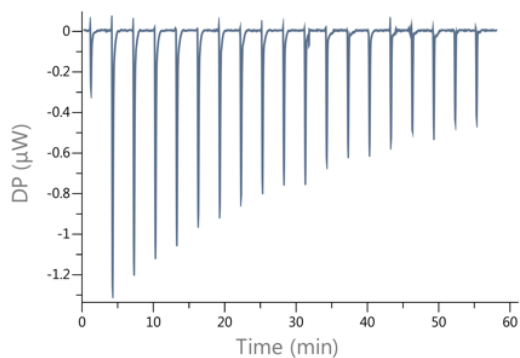
The isothermal titration calorimetry (ITC) data for the compounds shown, measured for the TcBDF3(32..148aa) bromodomain. Shown are heat effects for each injection (above) and the normalized binding isotherms (below) including the fitted function for compounds that showed binding (solid line).



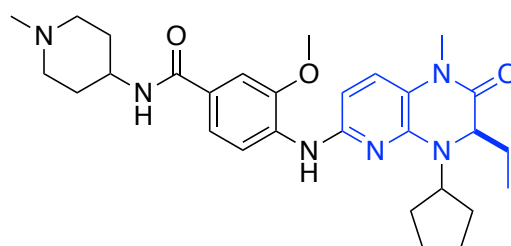
**Figure S72:** The binding of BI-2536 (**1**) to TcBDF3(32..148aa).



BI-6727 (**53**)  
 $K_D = 57.1 \pm 4.9 \mu\text{M}$  (ITC,  $n=3$ ) (TcBDF3)

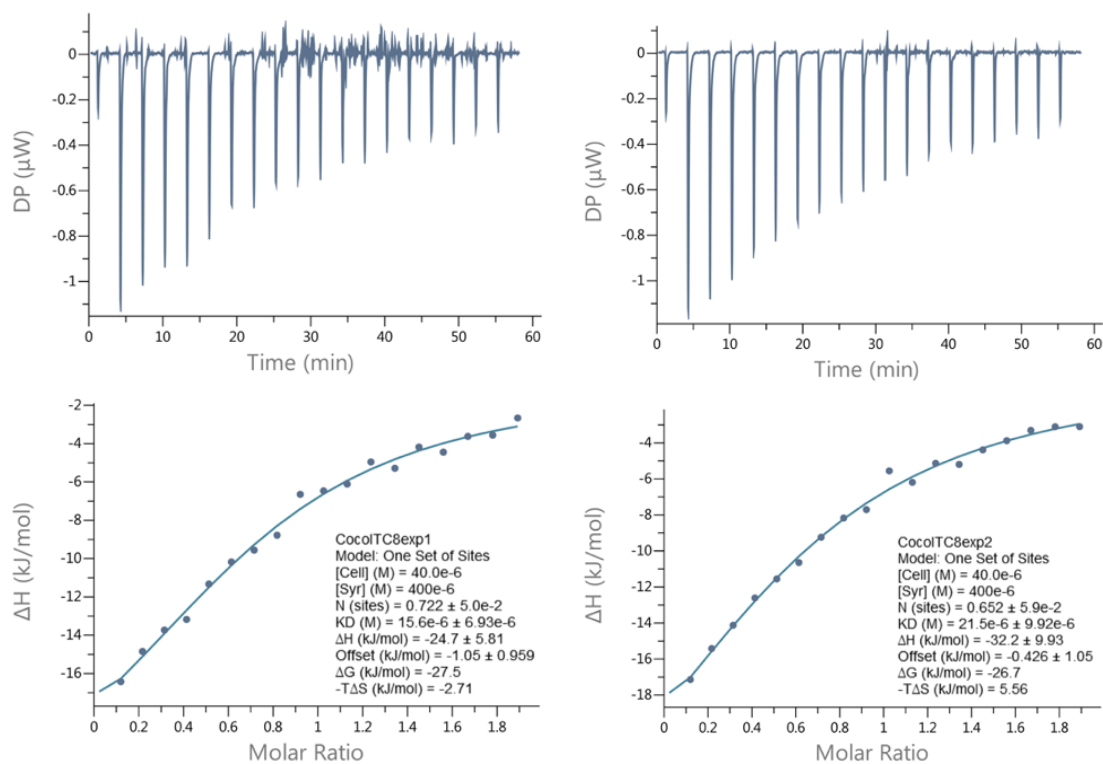


**Figure S73:** The binding of BI-6727 (**53**) to TcBDF3(32..148aa).

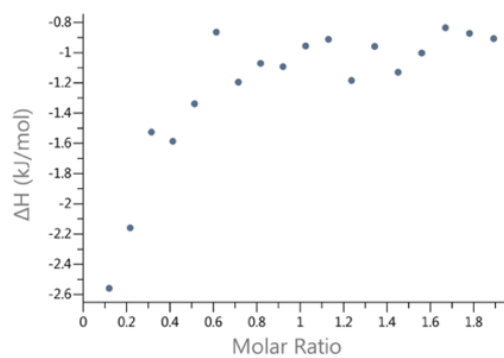
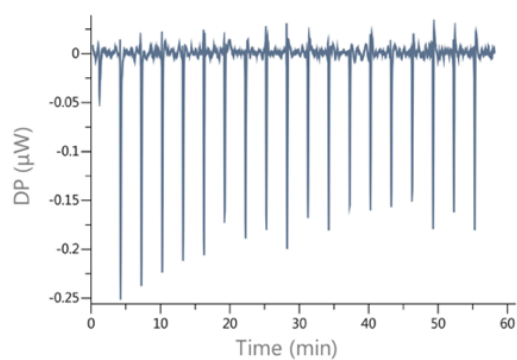
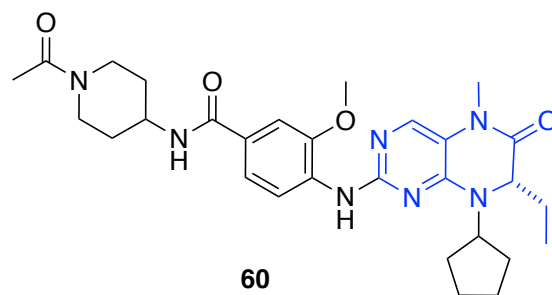
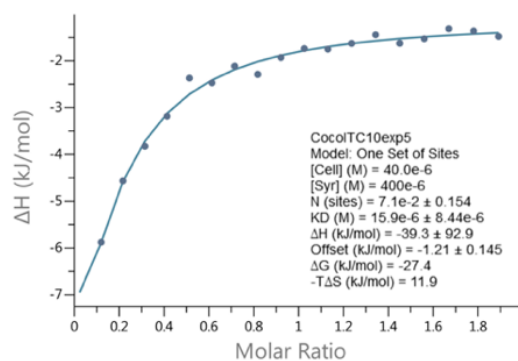
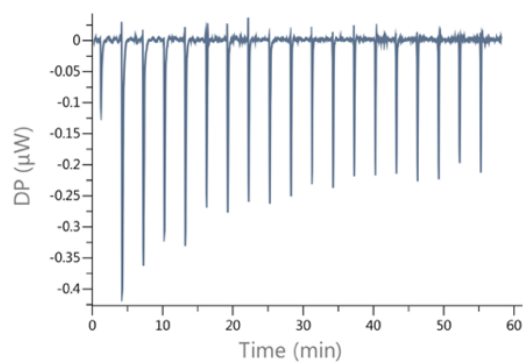
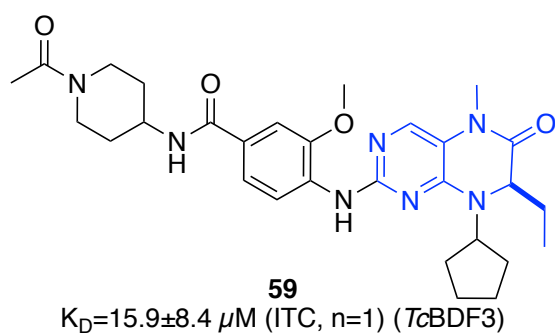


**39**

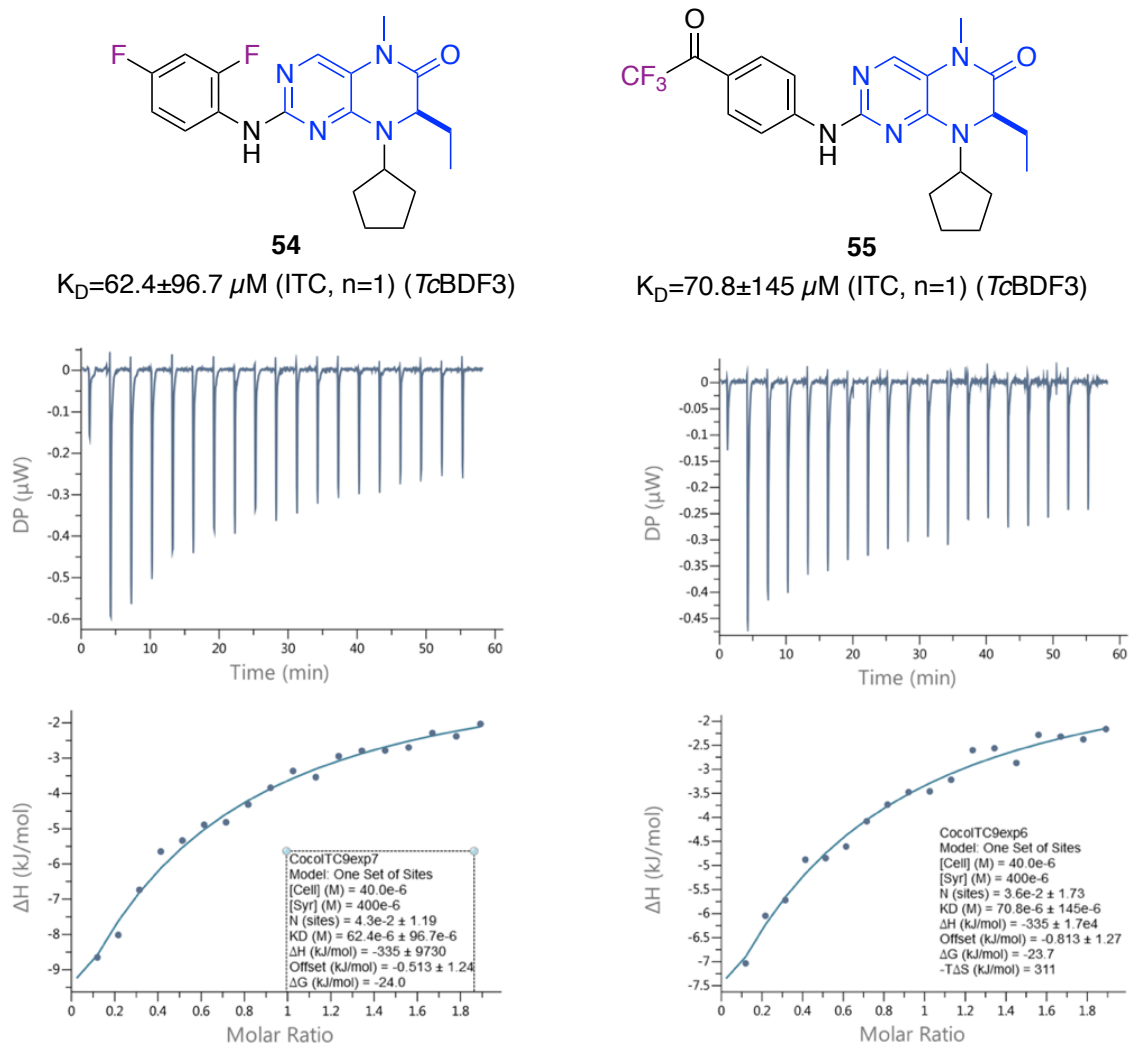
$K_D = 18.6 \pm 8.4 \mu\text{M}$  (ITC,  $n=2$ ) (*TcBDF3*)



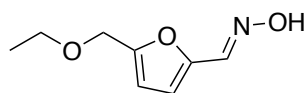
**Figure S74:** The binding of **39** to *TcBDF3*(32..148aa).



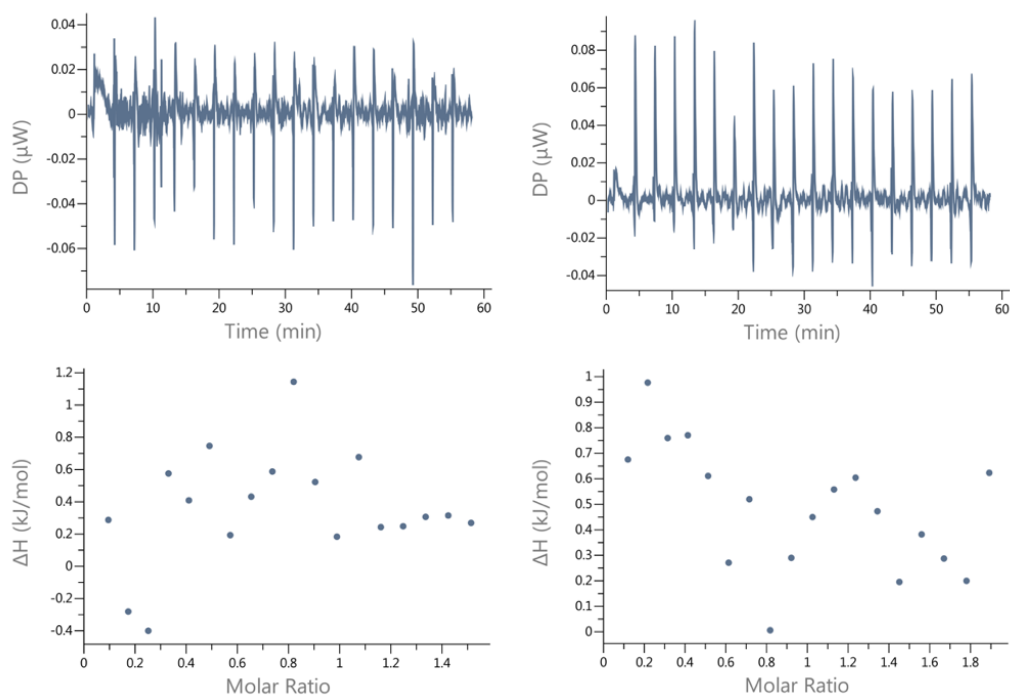
**Figure S75:** Binding to *TcBDF3*(32..148aa) is detected for **59** but not for **60**.



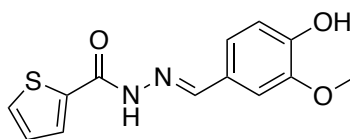
**Figure S76:** Binding of **54** and **55** to *TcBDF3*(32..148aa);



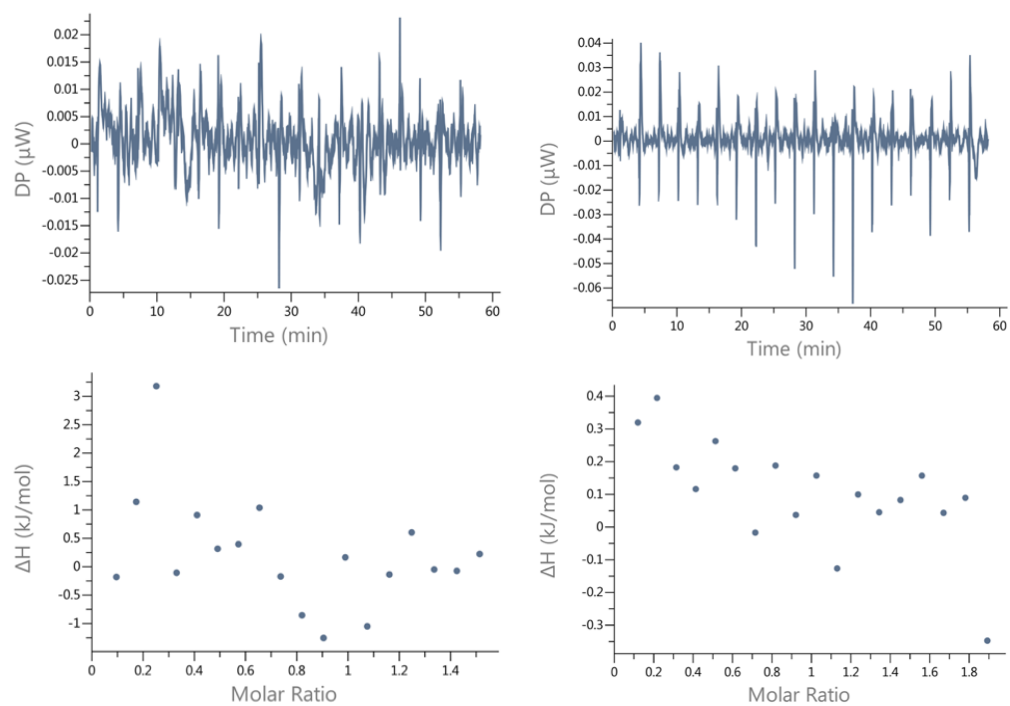
**6**



**Figure S77:** There no binding of **6** to TcBDF3(32..148aa).

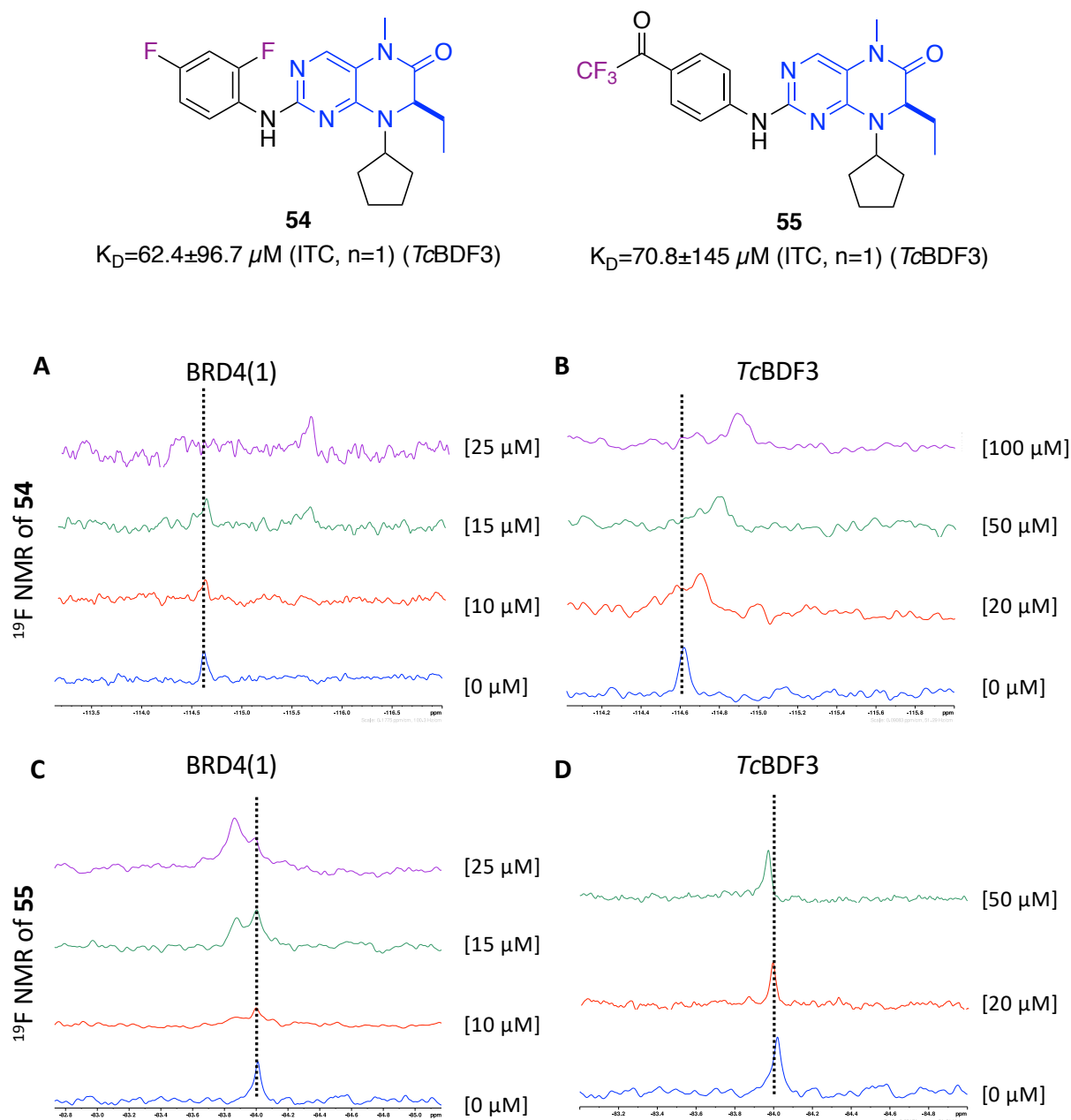


**7**



**Figure S78:** There no binding of **7** to TcBDF3(32..148aa).

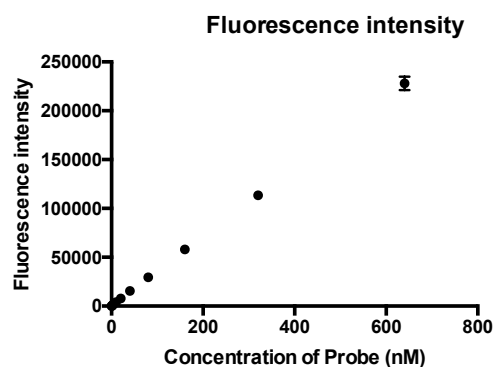
## SI-E: $^{19}\text{F}$ NMR EXPERIMENTS



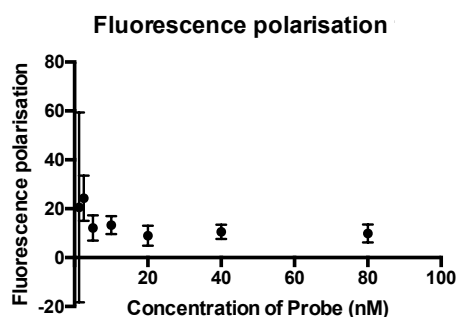
**Figure S79:** The overlay of 564 MHz  $^{19}\text{F}$  NMR spectra showing the changes of width and chemical shift of fluorine resonances of **50** and **51** were tested in the presence of BD proteins: *HmBRD4(1)* and *TcBDF3*-BRD, in HEPES buffer. The spectra have been calibrated to the resonances of 100  $\mu\text{M}$  of KF, the compound only spectra are shown in blue. (A) **50** titrated with *BRD4(1)* at 10  $\mu\text{M}$  (red), 15  $\mu\text{M}$  (green) and 25  $\mu\text{M}$  (purple) shows the appearance of a distinct new peak. (B) **51** titrated with *TcBDF3*-BRD at 20 (red), 50 (green) and 100  $\mu\text{M}$  (purple) shows a gradual change in chemical shift. (C) **50** titrated with *BRD4(1)* at 10 (red), 15 (green) and 25  $\mu\text{M}$  (purple) shows the appearance of an overlapping new peak. (D) **51** titrated with *TcBDF3*-BRD at 20  $\mu\text{M}$  (red), and 50  $\mu\text{M}$  (green) shows a gradual change in chemical shift.

## SI-F: FLUORESCENCE POLARIZATION

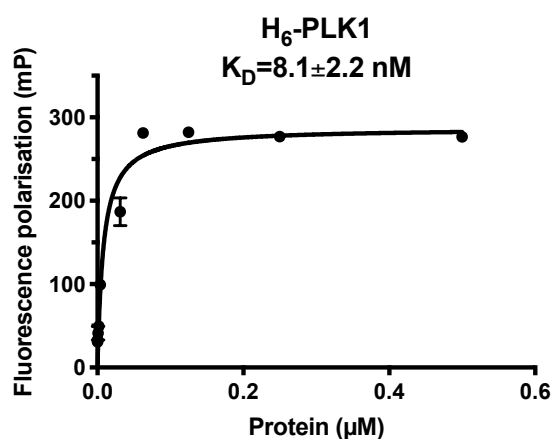
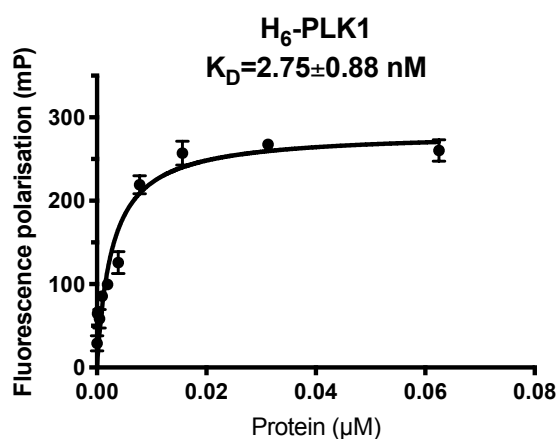
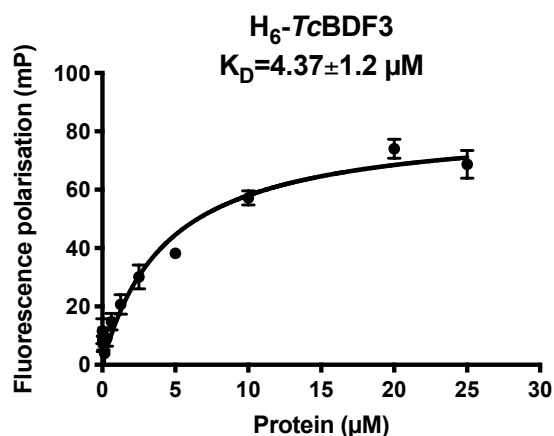
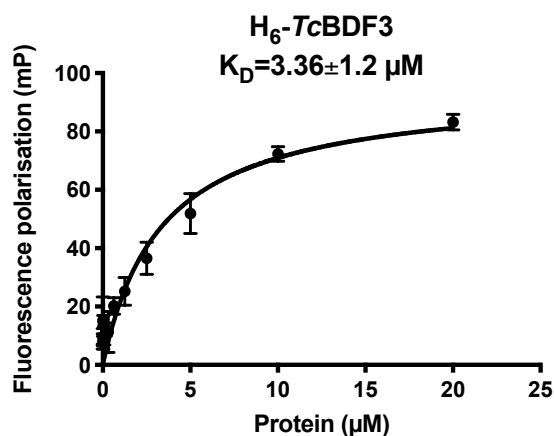
Concentration of Probe (nM)	Fluorescence intensity		
X	A:Y1	A:Y2	A:Y3
640.00	220662	229065	234194
320.00	113334	112051	114766
160.00	55276	57778	61181
80.00	28768	30072	29738
40.00	15006	15414	16119
20.00	7552	7975	7841
10.00	3748	4100	4198
5.00	2036	2101	2160
2.50	878	1035	992
1.25	440	443	451
0.63	124	166	238
0.31	15	30	18



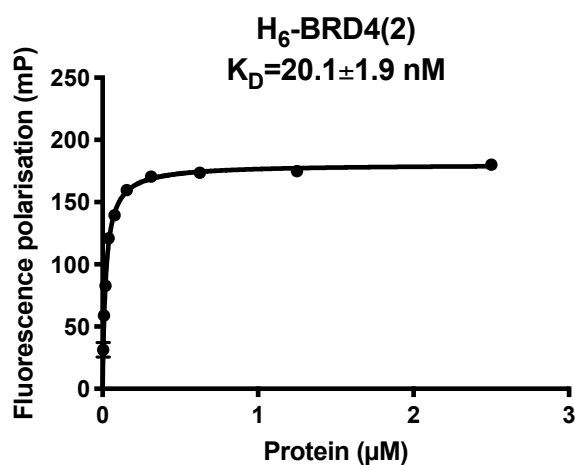
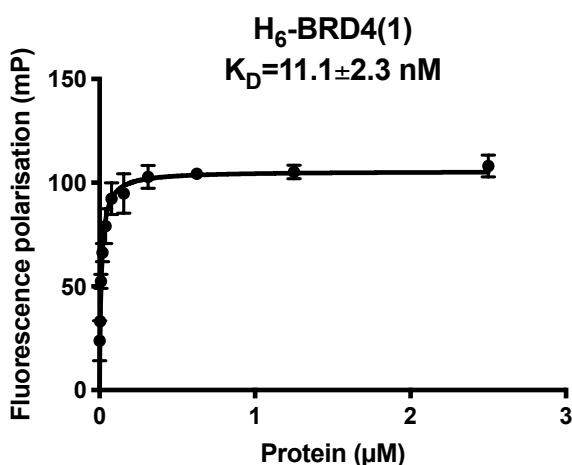
Concentration of Probe (nM)	Fluorescence polarisation		
X	A:Y1	A:Y2	A:Y3
80.00	13.0	5.8	10.8
40.00	13.2	7.4	10.9
20.00	11.4	4.2	11.2
10.00	13.2	9.7	17.0
5.00	17.5	7.1	11.7
2.50	21.5	34.6	16.7
1.25	43.6	42.3	-24.4



**Figure S80:** The fluorescent intensity and polarisation for the fluorescent probe **56**. Left-hand side show a linear FI vs probe concentration. Right-hand side show highly variable FP values at low probe concentration, and a stabilised FP at concentrations > 20 nM.



**Figure S81:** The fluorescence polarisation observed for the fluorescent probe **56** when titrating *TcBDF3*-BRD and *HmPLK1*. **54** shows binding to *TcBDF3*-BRD with  $K_D = 3.9 \pm 1.2 \mu\text{M}$  ( $n=2$ ), and to *HmPLK1* with  $K_D = 5.5 \pm 1.5 \text{ nM}$  ( $n=2$ ).



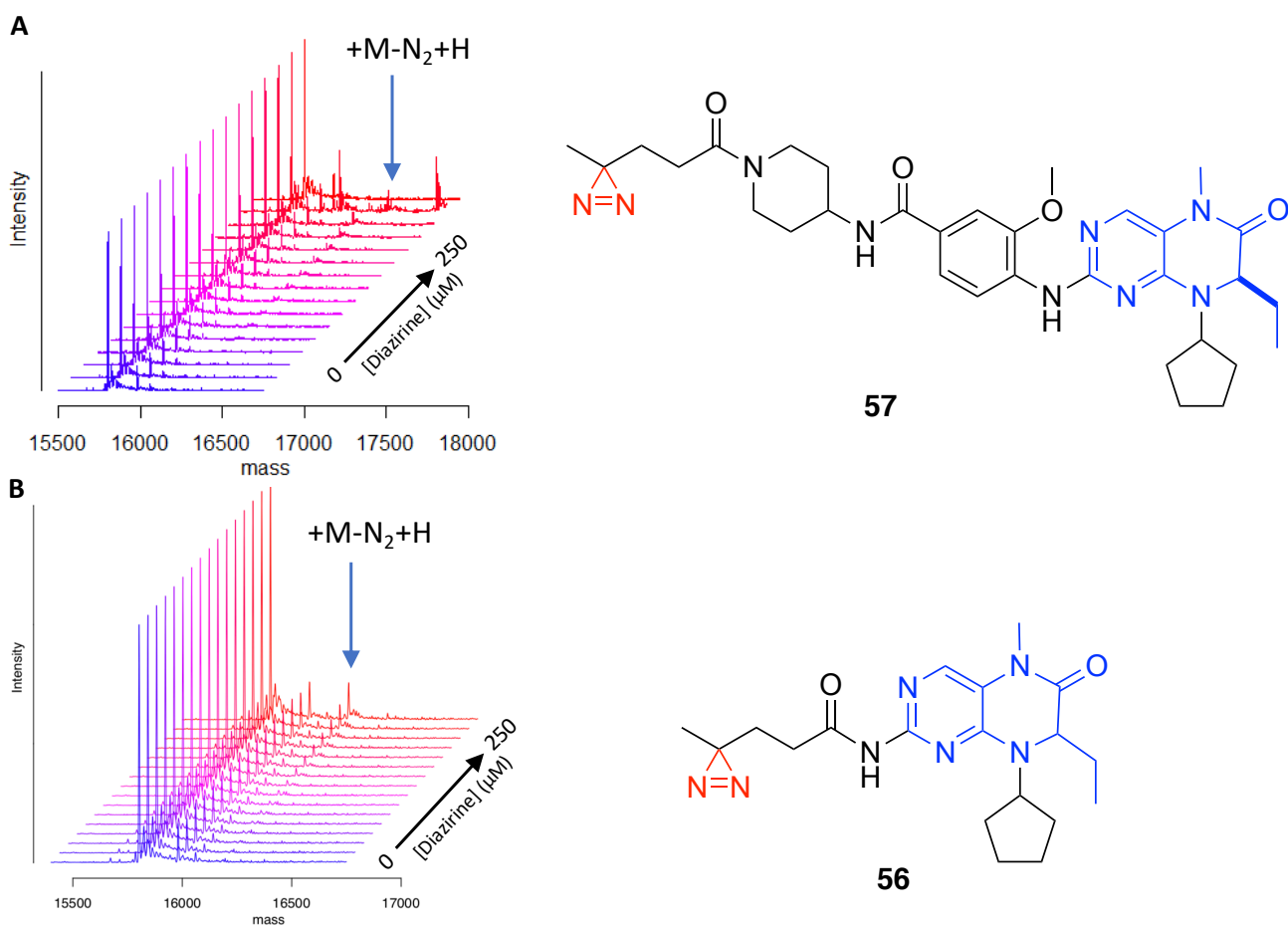
**Figure S82:** The fluorescence polarisation observed for the fluorescent probe **54** when titrating *HmBRD4*(1) and *HmBRD4*(2). **56** shows binding to both *HmBRD4*(1) and *HmBRD4*(2) with  $K_D = 11.1 \pm 2.3 \text{ nM}$  and  $20.1 \pm 1.9 \text{ nM}$ , respectively.

## SI-G: PHOTOCROSS-LINKING WITH DIAZIRINES

A diazirine probe must bind the target protein and the formed carbene must be in proximity of the protein surface to efficiently cross-link to the protein. The crosslinking yield is therefore a result of the binding affinity and the cross-linking efficiency, and is not a direct measure of either events. When the protein target is saturated with diazirine probes then the crosslinking yield should be at its maximum, provided that there is no unspecific binding and that the probe concentration is the only variable. To rank diazirine probes we propose to characterize them according to the concentration at which they reach 50% of their maximum cross-linking effect:  $XL_{50}$ . This calculation requires the probe to reach a cross-linking yield plateau from a single cross-linking event.

**Table S2:** The diazirine tool compound  $XL_{50}$  values for their binding and cross-linking to TcBDF3-BRD. This calculation assumes that the cross-linking ability of the diazirine probe is constant regardless at which concentration they are used. The  $XL_{50}$  values are given as the median and standard deviation for each triplicate experiment.

Diazirine probe	$XL_{50}$ from cross-linking titration	Maximum cross-linking yield
<b>56</b>	8.2±1.9 $\mu$ M	8%
<b>57</b>	14.5±3.9 $\mu$ M	17%



**Figure S83:** The photo-crosslinking of **56** and **57** to *TcBDF3*(32..148aa): The mass spectra stacks for the cross-linking titration of the diazirine probes to *TcBDF3*-BRD. The MS spectra corresponding to 0 to 250  $\mu M$  of the probe are stacked from low to high concentration into the page, as indicated by the grey arrow. The cross-linking peak, corresponding to  $M(\text{protein})+M(\text{probe})-M(\text{nitrogen})$ , is highlighted by a blue arrow. (A) The **57** probe shows low levels of cross-linking to *TcBDF3*-BRD (B) **56** shows cross-linking to *TcBDF3*-BRD.

## SI-H: METHODS

**Protein Expression and Purification.** The *TcBDF3* bromodomain (GenBank ID: XP\_812334.1) and BRD4(1) bromodomain (Addgene plasmid # 38942) constructs were transformed into *E. coli* BL21 (DE3) cells for expression.

*E. coli* BL21 (DE3) were cultured in 2× TY medium (16 g/L Oxoid™ Tryptone, 10 g/L Oxoid™ Yeast Extract, 5 g/L NaCl) at 37 °C and 180 rpm. When solid medium was required, Bacto agar was added to a final concentration of 1.5% (w/v). Standard sterile practices were followed using an open flame, on sterilised surfaced using aq. EtOH (70% v/v). Media and equipment were sterilised by autoclaving at 121 °C for 15 min. Solutions of IPTG, antibiotics and other labile compounds were sterilised by filtering through 0.22 µm filters (Minisart®, Sartorius Stedim). The final concentration of kanamycin was 50 µg/mL.

Large-scale recombinant protein expression was performed using 2 L Turnair® flasks containing 1 L of 2× TY medium containing kanamycin. Flasks were inoculated with 1% of the bacterial starter culture and incubated at 37 °C at 180 rpm. When the cultures reached an optical density OD<sub>600</sub> of 0.6 – 1.0, the shaker temperature was equilibrated to 16 °C. The cultures were induced with IPTG (0.1 – 0.5 mM) then incubated for 16 – 20 h. After this time, cultures were harvested by centrifugation, and the resulting bacterial pellet was stored at –80 °C. Cell pellets were resuspended in Extraction buffer (50 mM HEPES, 500 mM NaCl, 5 mM imidazole, 1 EDTA free Sigma protease inhibitor tablet) (100 mL per 20 g cell mass). 25 Units (U) of Benzoase was added, then the cells were lysed by sonication using thirty 5 sec bursts interrupted by 5 to 10 sec pauses. Polyethyleneimine (PEI, 0.15% v/v) was added, and the cells were incubated on ice for 15 mins. The lysate was clarified by centrifugation and the supernatant decanted, filtered through a 0.45 µm filter, and applied to a purification column.

Immobilized metal affinity chromatography (IMAC) was performed with a HisTrap™ (6 mL) column (GE Healthcare) charged with 4 CV of 100 mM NiSO<sub>4</sub> and washed with 10 CV of binding buffer prior to loading the cell lysate at 1 mL/min. The column was then washed with approximately 20 CV binding buffer (50 mM HEPES, 500 mM NaCl, 5 mM imidazole). When all residual products of bacterial fermentation were eluted from the column by binding buffer, the protein of interest was batch-eluted with 15 CV elution buffer (50 mM HEPES, 500 mM NaCl, 500 mM imidazole). Eluted protein was collected and fractionated; fractions containing the highest levels of pure protein (as determined by SDS-PAGE gels and UV trace) were concentrated using 5 or 10k concentrators (GE Healthcare) in a Beckmann Allegra™ 21R centrifuge. Further purification was achieved by gel filtration chromatography. Concentrated fractions (< 2 mL) were loaded on a 120 mL Superdex 75 size exclusion chromatography (SEC) column (Amersham). Eluted protein was collected then fractions containing the highest levels of pure protein (as determined by SDS-PAGE gels) were concentrated (to 50 – 1500 µM) as described before. Protein concentration was estimated by measuring the absorbance at 280 nm using a Nanodrop® ND-1000 spectrophotometer (Nanodrop® Technologies Inc.) with the 'Protein A280' program module according to the manufacturer's instructions. Protein molecular weight and molar extinction coefficient were calculated using ProtParam on the ExPASy Bioinformatics Resource Portal.

**Homology Modelling.** The sequence of *TcBDF3* (GenBankID XP\_812334.1) was used to identify the *TbBDF2* template (PDB accession: 5C8G). The section of the sequence corresponding to the bromodomain was isolated, and aligned to the template sequence using Clustal Omega.<sup>3</sup> The homology model was constructed using Modeller (v. 9.2),<sup>4</sup> where 100 models were generated and evaluated based on their DOPE score.<sup>5</sup>

**Molecular Docking.** The water molecules found within the binding pocket of the *TbBDF2* template were transferred to the homology model. Analysis of *apo* MD simulations showed these were good representations of the binding site water molecule positions. The homology model and water molecules were protonated at pH 7.4 using the MOE (v. 2018.2) software package. The ligand protonation states were calculated using the Marvin Suite 16.16.6.0 from ChemAxon (<https://www.chemaxon.com>). Molecular docking was performed using AutoDock4,<sup>6</sup> where 100 poses were generated using a 0.375 Å grid spacing and clustered using a 2 Å cut-off.

**Molecular Dynamics.** Docking models required no further preparation before MD studies. For all MD simulations, the AMBER99SB-IDLN forcefield was used for the protein.<sup>7</sup> Ligand parameters were derived from the General Amber forcefield (v. 1.8) from AmberTools16,<sup>8</sup> All binding site waters were retained from the protein structures, with all water modelled using the TIP3P model.<sup>9</sup> The systems were solvated in a dodecahedral box, where the minimum distance between the protein and box edge was 1.2 nm. System charges were neutralized using sodium ions, with additional NaCl ions added to form an overall salt concentration of 150 nM. All systems then underwent initial energy minimization using the steepest decent algorithm to remove poor contacts, which had a maximum force cut off of 1000 kJ mol<sup>-1</sup>. The systems then underwent further equilibration in the isothermal-isobaric ensemble for 200 ps. The temperature was coupled using a Langevin thermostat, with a target temperature of 298.15 K, and the pressure was coupled using the Berendsen weak coupling algorithm to a target pressure of 1 atm.<sup>10-11</sup> Simulations were performed using GROMACS 2018.2.<sup>12</sup> All simulations underwent 50 ns of production MD, in triplicate.

**WaterLOGSY.** Typical procedure for NMR WaterLOGSY: Protein in HEPES containing storage buffers were exchanged into Invitrogen 10 mM Na/K phosphate buffer (0.9% NaCl, pH 7.6) using GE Healthcare PD spintrap G-25 columns, eluting on two pre-equilibrated columns successively for each protein. Protein concentrations in the phosphate buffer were calculated from by measuring the absorbance at 280 nm using a Nanodrop® ND-1000 spectrophotometer (Nanodrop® Technologies Inc.) with the 'Protein A280' program module according to the manufacturer's instructions. Samples were prepared to a total sample volume of 160 µL phosphate buffer, with 500 µM compound (unless otherwise stated) from 50 mM D<sub>6</sub>-DMSO stock solutions, 10% D<sub>2</sub>O for signal locking and 20 µM protein (unless otherwise stated) for protein samples. After loading into 3 mm Hildenberg NMR tubes for Bruker Match™ system, samples were briefly centrifuged in a manual benchtop rotor. Spectra were recorded on a Bruker AVIII 700 MHz spectrometer with inverse TCI cryoprobe using a standard waterLOGSY pulse sequence. For each compound, a water suppressed <sup>1</sup>H NMR spectra was recorded as a reference, followed by a waterLOGSY spectra. When the WaterLOGSY of the compound showed suitable solubility, WaterLOGSY spectra in the presence of the studied protein were recorded [*H*<sub>6</sub>-*TcBDF3*(32..148aa)].

**Ligand-Observed Proton NMR.** TcBDF3 in HEPES-containing storage buffers were exchanged into Invitrogen 10 mM Na/K phosphate buffer (0.9% NaCl, pH 7.6) using dialysis. TcBDF3 bromodomain protein concentrations in the phosphate buffer were calculated from by measuring the absorbance at 280 nm using a NanoDrop Lite spectrophotometer (Nanodrop® Technologies Inc.) by using the predicted protein absorbance (MW: 15803.7 Da,  $\epsilon_{280} = 17545 \text{ M}^{-1}\text{cm}^{-1}$ ). Samples were prepared to a total sample volume of 160  $\mu\text{L}$  phosphate buffer, with 50  $\mu\text{M}$  compound from 5 mM D<sub>6</sub>-DMSO stock solutions, 10% D<sub>2</sub>O for signal locking and the appropriate amount of protein. After loading into 3 mm Hildenberg NMR tubes for Bruker Match™ system, samples were briefly centrifuged in a manual benchtop rotor. Spectra were recorded on a Bruker AVIII 700 MHz spectrometer with a TXI probe using a standard excitation-sculpting water suppression pulse sequence (256 or 512 scans each). For each compound, a compound only sample was recorded as a reference, followed by samples with protein. Each spectrum was processed with Bruker Topspin™ 3.2, the data were recorded using Microsoft Excel®, then the  $K_d$  was calculated using the “one site – specific binding; non-linear regression” function using Graphpad Prism®. In Topspin, spectra were phased corrected and calibrated to the residual DMSO peak, then local baseline corrections were performed (“absf n” function).). The chemical shift of the studied peaks and the DMSO reference peaks were measured. Using the evolution of the  $|\partial_{peak} - \partial_{DMSO}|$  distance over the titration, the change of studied peak chemical shift with increasing protein concentration was plotted for each compound and analysed. Briefly, The chemical shift change was plotted vs the protein concentration, and GraphPad Prism’s “one site - specific binding” equation [equation (1), below] was used to calculate the  $B_{max}$  value (here this value describes the maximum chemical shift) for the plateauing behaviour observed. The chemical shift change was used along  $B_{max}$  and the known protein and ligand concentrations to calculate the proportion of bound complex as reviewed by Fielding (*Curr. Top. Med. Chem.* **2003**, 3, 39). The calculated [P-L] complex was plotted against the protein concentration, and GraphPad Prism was used to calculate the  $K_d$  value using a one to one binding equation following the analysis described by Fielding (*Curr. Top. Med. Chem.* **2003**, 3, 39; *Prog Nucl Magn Reson Spectrosc* **2007**, 51 (4), 219-242)<sup>13</sup>,<sup>14</sup> [equation (2), below]:

$$Y = \frac{B_{max} \times X}{K_d + X} \quad (1)$$

X = concentration of the protein

Y = change in chemical shift

$B_{max}$  = maximum binding in the same units as Y, *ie.* the maximum chemical shift change.

$K_d$  = dissociation constant in the same units as X

$$PL = \frac{(X + Lt + K_d) - \sqrt{(X + Lt + K_d)^2 - 4(X \times Lt)}}{2} \quad (2)$$

PL = Concentration of protein-ligand complex, [PL], at equilibrium, here [PL]=Y/ $B_{max}$

X = Total protein concentration, [Pt].

Lt = Total ligand concentration, [Lt], which is held constant in NMR titration experiments.

$K_d$  = Equilibrium dissociation constant.

**Binding assay based on <sup>19</sup>F NMR.** Typical procedure for <sup>19</sup>F NMR binding assay: The protein samples were thawed on ice and centrifuged. The protein concentration was determined by measuring the absorbance at

280 nm using a Nanodrop® ND-1000 spectrophotometer (Nanodrop® Technologies Inc.) with the 'Protein A280' program module according to the manufacturer's instructions. Samples were prepared to a total sample volume of either 500 or 160 µL phosphate buffer, with 100 µM compound (unless otherwise stated) from 10–20 mM DMSO-D<sub>6</sub> stock solutions, 10% D<sub>2</sub>O for signal locking, 5 µL of a fluorinated compound reference in buffer, and protein (0 – 150 µM) for protein samples. The <sup>19</sup>F reference solution in buffer was either (a) 0.01% (v/v) solution of 1,1,1-trifluoroacetone or (b) 10 mM KF. After loading into either 5 or 3 mm Hildenberg NMR tubes for Bruker Match™ system, samples were briefly centrifuged in a manual benchtop rotor. All samples were incubated at room temperature for a minimum of 10 min prior to their measurement. Spectra were recorded using a Bruker AVIII 600 MHz spectrometer with Prodigy N2 broadband cryoprobe (128–384 scans).

**Isothermal Titration Calorimetry.** All calorimetric experiments were performed on a MicroCal iTC200 or MicroCal PEAQ-ITC Automated (Malvern) and analyzed with the MicroCal PEAQ-ITC Analysis software (Malvern 1.1.0.1262) using a single binding site model, with the N number set to 1. The first data point was excluded from the analysis. *HmBRD4*(1) and *TcBDF3* bromodomains were dialyzed at 4 °C overnight in a Slide-A-Lyzer® MINI Dialysis Device (2000 MWCO; Thermo Scientific Life Technologies) into 50 mM HEPES, 150 mM NaCl containing 0.2% DMSO; pH 7.4. Proteins were centrifuged to remove aggregates (3 min, 3000 rpm, 25 °C). Protein concentration were determined by measuring the absorbance at 280 nm using a NanoDrop Lite spectrophotometer (Nanodrop® Technologies Inc.) by using the predicted protein absorbance (*TcBDF3*:  $\epsilon_{280} = 17545 \text{ M}^{-1}\text{cm}^{-1}$  and *BRD4*(1):  $\epsilon_{280} = 28420 \text{ M}^{-1}\text{cm}^{-1}$ ). Small molecules ligands were dissolved as 10 to 50 mM DMSO stock solution and diluted to the required concentration using dialysis buffer. The cell was stirred at 750 rpm, reference power set to 5 µcal/sec and temperature held at 25 °C. After an initial delay of 60 sec, 20 × 2 µL injections (first injection 0.4 µL) were performed with a spacing of 150 sec. Heated dilutions were measured under the same conditions and subtracted for analysis. Small molecule solutions in the calorimetric cell were titrated with the protein solutions in the syringe.

**Fluorescence polarisation (FP).** All fluorescent polarisation experiments were performed using the BMG LABTECH CLARIOstar® or PHERAstar® FS microplate readers, processed using the BMG LABTECH Mars software and analysed using Graphpad Prism®. BSA (0.1% (w/w)) was added freshly to the FP assay buffer: 25 mM HEPES, 100 mM NaCl, CHAPS (0.05%), pH = 7.6, before each experiment. The fluorescent intensity and polarization measurements were performed using black 384-well non-binding microplate (CORNING) in triplicate (20 or 40 µL).

*Total fluorescence intensity:* Serial dilutions of the fluorescently labelled probe were made in assay buffer covering a range of 1.25 to 640 nM and dispensed in triplicate (20 µL). Blank controls contained only buffer (20 µL). Fluorescence intensities were recorded with a PHERAstar FS plate reader (BMG LABTECH). For the fluorescein-labelled probes we used the following settings: excitation filter – FP 485 nm, emission filter 1 – FP p-pol 520 nm, emission filter 2 – FP s-pol 520 nm. Total fluorescence intensities were calculated as  $2 \times I_p + I_s$ . The resulting values were plotted against the respective probe concentrations with GraphPad Prism and fitted with linear regression.

**Binding curves and  $K_D$  determination:** A 2× concentrated serial dilution series of the respective protein in buffer was made covering a total concentration range of 0.0048 to 10 μM (final concentrations). 10 μL of the 2× protein dilutions and 10 μL of a 20 – 80 nM solution of the fluorescence-labelled probe (final assay concentration is 10 – 40 nM, according to the probe used) were dispensed on a black 384-well non-binding microplate (CORNING) resulting in a final assay volume of 20 μL per well. Fluorescence polarisation (FP) was measured from these wells after incubation for 30 min at 25 °C with an PHERAstar FS plate reader (BMG LABTECH) using following settings: excitation filter – FP 485 nm, emission filter 1 – FP p-pol 520 nm, emission filter 2 – FP s-pol 520 nm. In general, fluorescence polarization (P) values were determined as  $\frac{(I_s - G \times I_p)}{(I_s + G \times I_p)} \times 1000 (mP)$ , where  $I_s$  is the fluorescence intensity measured in the s-plane and  $I_p$  is the fluorescence intensity measured in the p-plane, both blank corrected. G is the grating factor, which is an instrument specific constant. Finally, P was plotted to a binding curve with GraphPad Prism by using a one-site binding (hyperbola) equation:

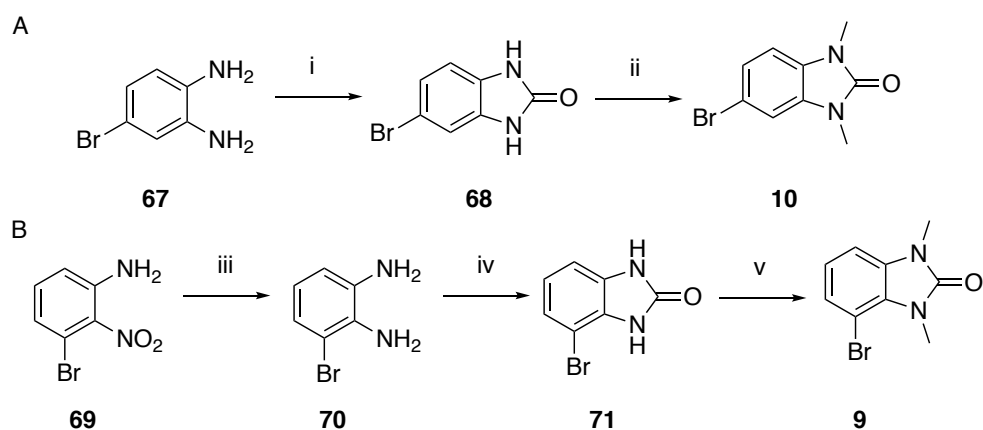
$$Y = \frac{B_{max} \times [R]}{K_D + [R]}$$

where  $B_{max}$  is the maximal binding.

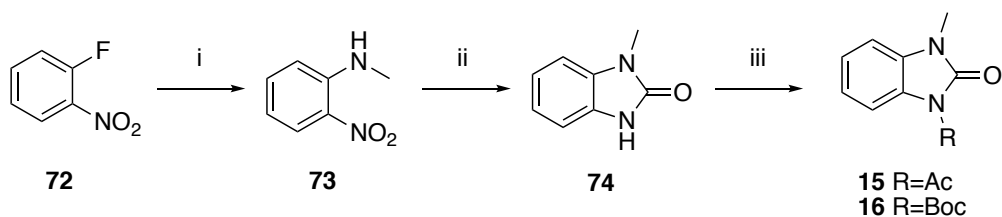
**Photo-crosslinking mass spectrometry assay.** Protein aliquots were thawed at 0 °C and the protein of interest was diluted to 10 mL at 1 μM in 10% MQ PBS. Using an Labcyte Echo® 555 Liquid Handler 150 nL of each well in the parent plate (containing the PhaBits library at 20 mM DMSO stock solutions) was transferred into a Greiner 384 low volume plate (white) to prepare the probe plate. The probe plate was placed on ice. 15 μL of protein stock solution (1 μM) was dispensed into wells A1–14 through to P1–14. The plate was left on ice for 15 min for incubation. The plate was then irradiated at 302 nm for 10 mins. The plate was centrifuged (1000 rpm, 1 min) to remove any bubbles. The plate was then sampled by LCMS-TOF mass spectrometry (Agilent, A: 0.2% formic acid H<sub>2</sub>O, B: 0.2% formic acid acetonitrile, 0.5 mL/min,  $\lambda$ = 200–350 nm), using the following gradient: 0-0.5 mins: 20% B, 0.5-2.5 mins: 40 to 80% B gradient, 2.5-4 mins: 100% then 4-4.5 mins: 20% B.

**Data Manipulation:** LCMS-TOF intact mass (ESI) spectrometry afforded a peak for the unlabelled protein, and a peak for the labelled protein (corresponding to  $M_{\text{protein+probe-N}_2}$ ). The .csv files of deconvoluted spectra results were analysed using R Studio software to generate pdf files of the spectra.

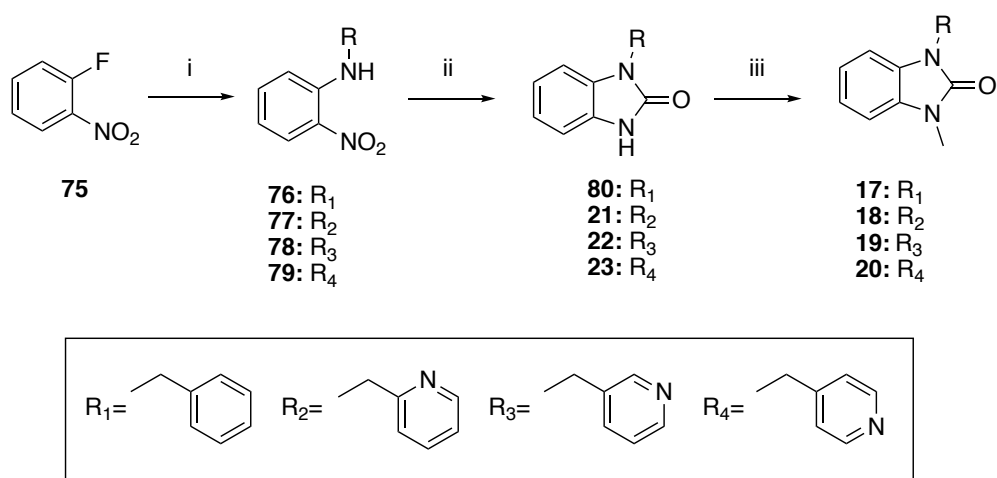
*Synthesis of the fragments, and intermediates:*



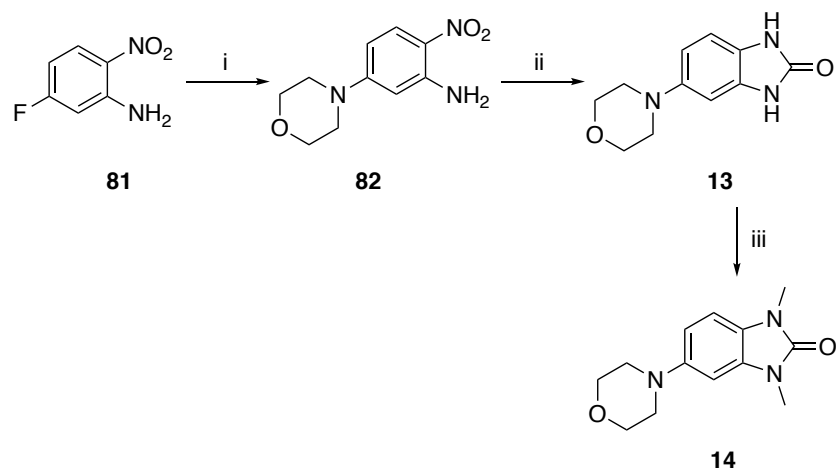
**Scheme S1:** Synthesis of the **9** and **10** fragments. *Reagents and conditions:* (A) (i) CDI, 1,4-dioxane, 40 °C, 1 h, 83%. (ii) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, RT, 6 h, 97%. (B) (iii) Fe, NH<sub>4</sub>Cl, EtOH:H<sub>2</sub>O 3:1, 90 °C, 16 h, 74%. (iv) CDI, 1,4-dioxane, 60 °C, 2 h, 91%. (v) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, RT, 2 h, 90%.



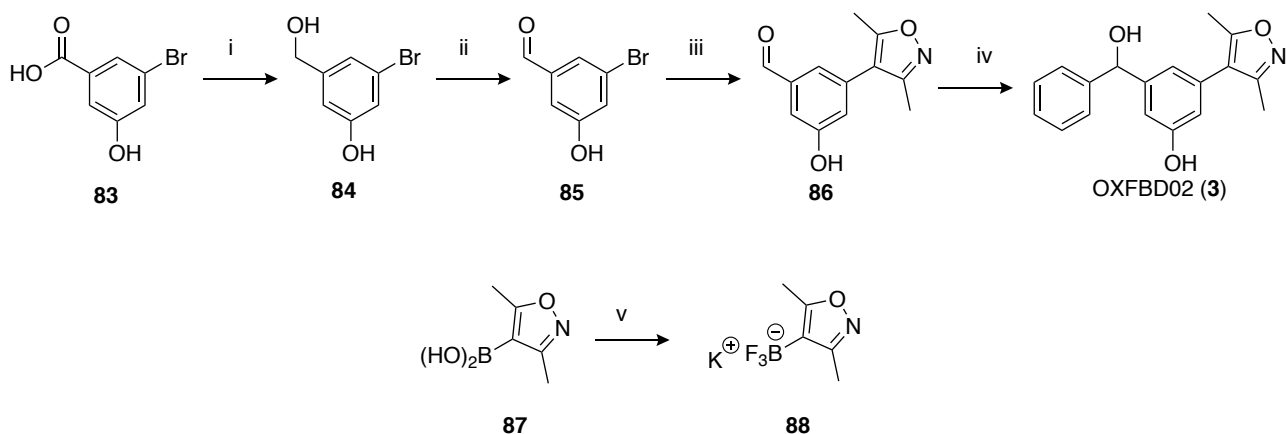
**Scheme S2:** Synthesis of benzimidazole fragments with different N-substituents. *Reagents and conditions:* (i) MeNH<sub>2</sub>·HCl, DIPEA, NMP, 80 °C, 18 h, 26%. (ii) (a) H<sub>2</sub>, Pd/C, EtOH, 1-3 h (b) Tripshogene, Et<sub>3</sub>N, THF, 0 °C then RT, 16 h, 36%. (iii) R<sub>2</sub>O, DMAP, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, RT, 3-5 h, R=Ac **15**: 82%, R=Boc **16**: 96%.



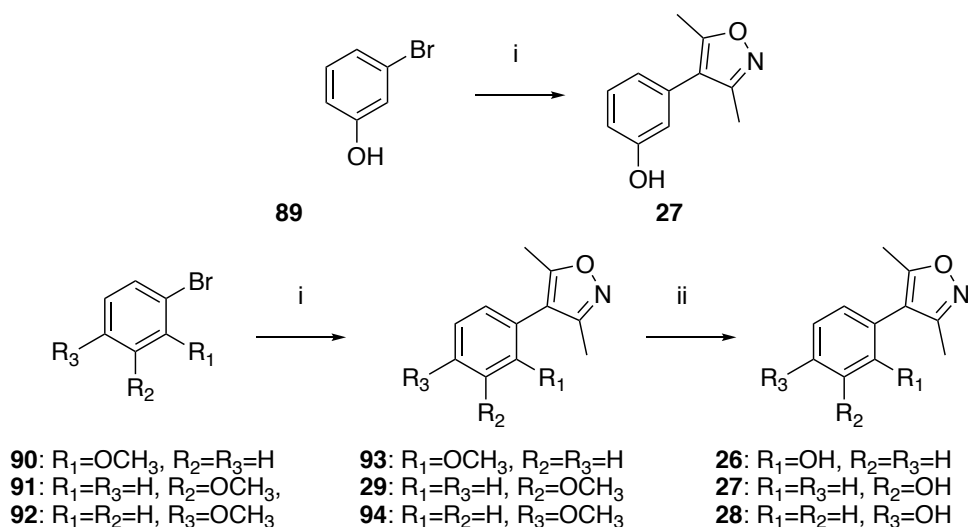
**Scheme S3:** Synthesis of benzyl and picolyl substituted benzimidazolones. *Reagents and conditions:* (i) RNH<sub>2</sub>, NMP, 80 °C, 2–16 h; **76**: 99%, **77**: 98%, **78**: 75%, and **79**: 30%. (ii) (a) H<sub>2</sub>, Pd/C, EtOH, 1–3 h (b) Triphosgene, Et<sub>3</sub>N, THF, 0 °C then RT, 16 h; **80**: 46%, **21**: 76%, **22**: 25%, and **23**: 22%. (iii) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, RT, 18 h; **17**: 98%, **18**: 28%, **19**: 28%, **20**: 11%.



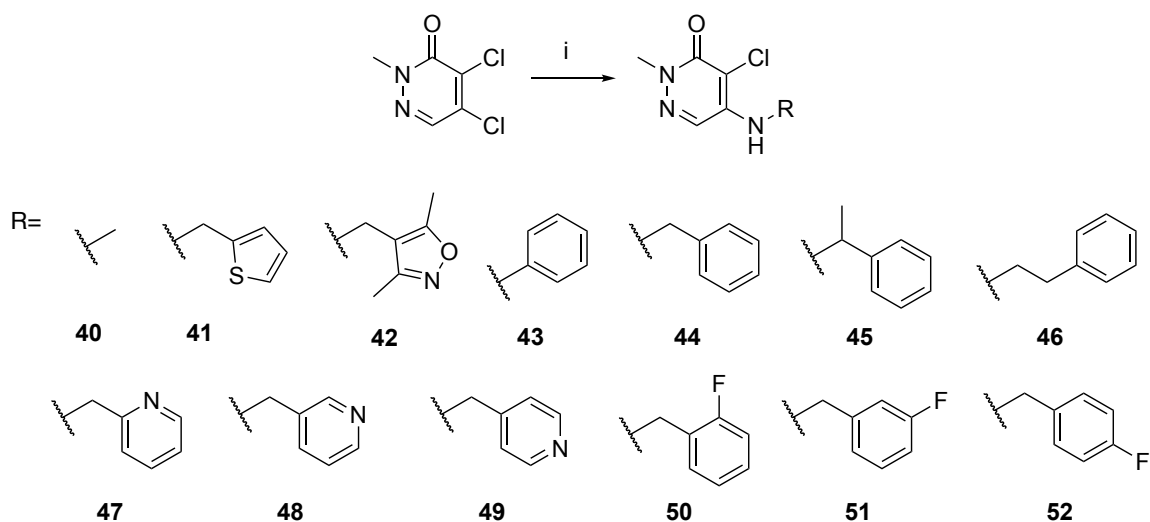
**Scheme S4:** Synthesis of morpholine substituted benzimidazolone fragment **14**. *Reagents and conditions:* (i) Morpholine, K<sub>2</sub>CO<sub>3</sub>, NMP, 100 °C, 3 h, 82% (ii) (a) H<sub>2</sub>, Pd/C, EtOH, 3 h (b) CDI, CH<sub>2</sub>Cl<sub>2</sub>, RT, 69% (iii) MeI, NaH, DMF, 0 °C, 2 h, 72%.



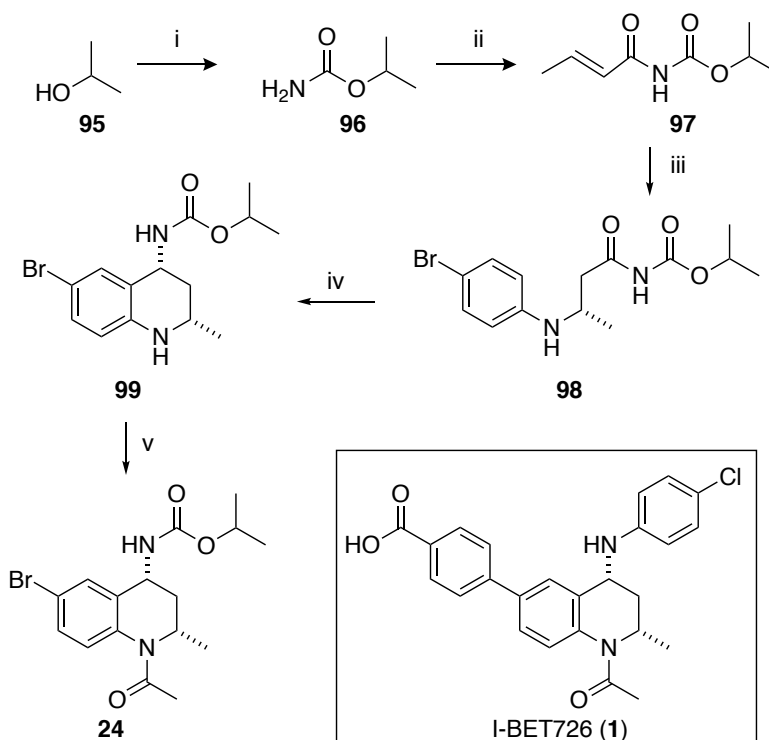
**Scheme S5:** Synthesis of the intermediate **86**. *Reagents and conditions:* (i)  $\text{BH}_3$  (1M in THF), THF, 0 °C then RT, 20 h, 82% (ii)  $\text{MnO}_2$ ,  $\text{CH}_2\text{Cl}_2$ , 35 °C, 2 d, 84% (iii) **53**,  $\text{Pd}(\text{OAc})_2$ , RuPhos,  $\text{Na}_2\text{CO}_3$ , EtOH, reflux, 3.5 h, 65% (iv)  $\text{PhMgBr}$  (1 M in THF), THF, 0 °C then RT, 2 h, 48% (v)  $\text{KHF}_2$ , MeOH,  $\text{H}_2\text{O}$ , 0 °C then RT, 15 mins, 80%. The compounds **27-30** were synthesized from **86** as previously described.<sup>15-17</sup>



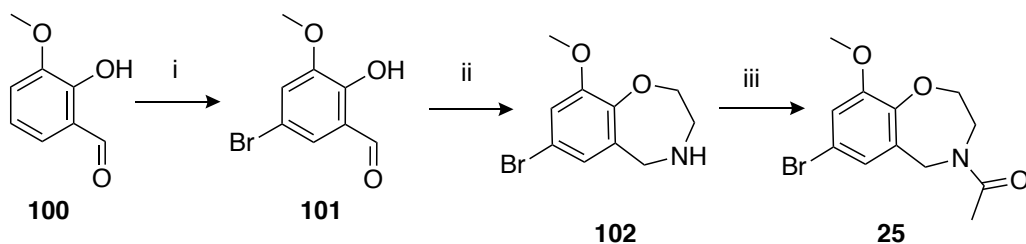
**Scheme S6:** Synthesis of phenolic dimethylisoxazole analogues *via* cross coupling and subsequent demethylation. *Reagents and conditions:* (i) **88**,  $\text{Pd}(\text{OAc})_2$ , RuPhos,  $\text{Na}_2\text{CO}_3$ , EtOH, 80 °C: **27**: 48 h, 58%; **93**: 16 h, 42%; **29**: 16 h, 89%; **94**: 16 h, 72%. (ii)  $\text{BBr}_3$  (1 M in  $\text{CH}_2\text{Cl}_2$ ),  $\text{CH}_2\text{Cl}_2$ , 0 °C to RT, 30 mins: **26**: 80%; **27**: 98%; **28**: 99%.



**Scheme S7:**  $S_NAr$  reactions to synthesise the pyridazolone analogues. *Reagents and conditions:* (i)  $RNH_2$ , NMP, 80 °C, 7–18 h. **40**: 16%, **41**: 63%, **42**: 55%, **43**: 60%, **44**: 80%, **45**: 67%, **46**: 84%, **47**: 46%, **48**: 23%, **49**: 46%, **50**: 75%, **51**: 70%, **52**: 62%.

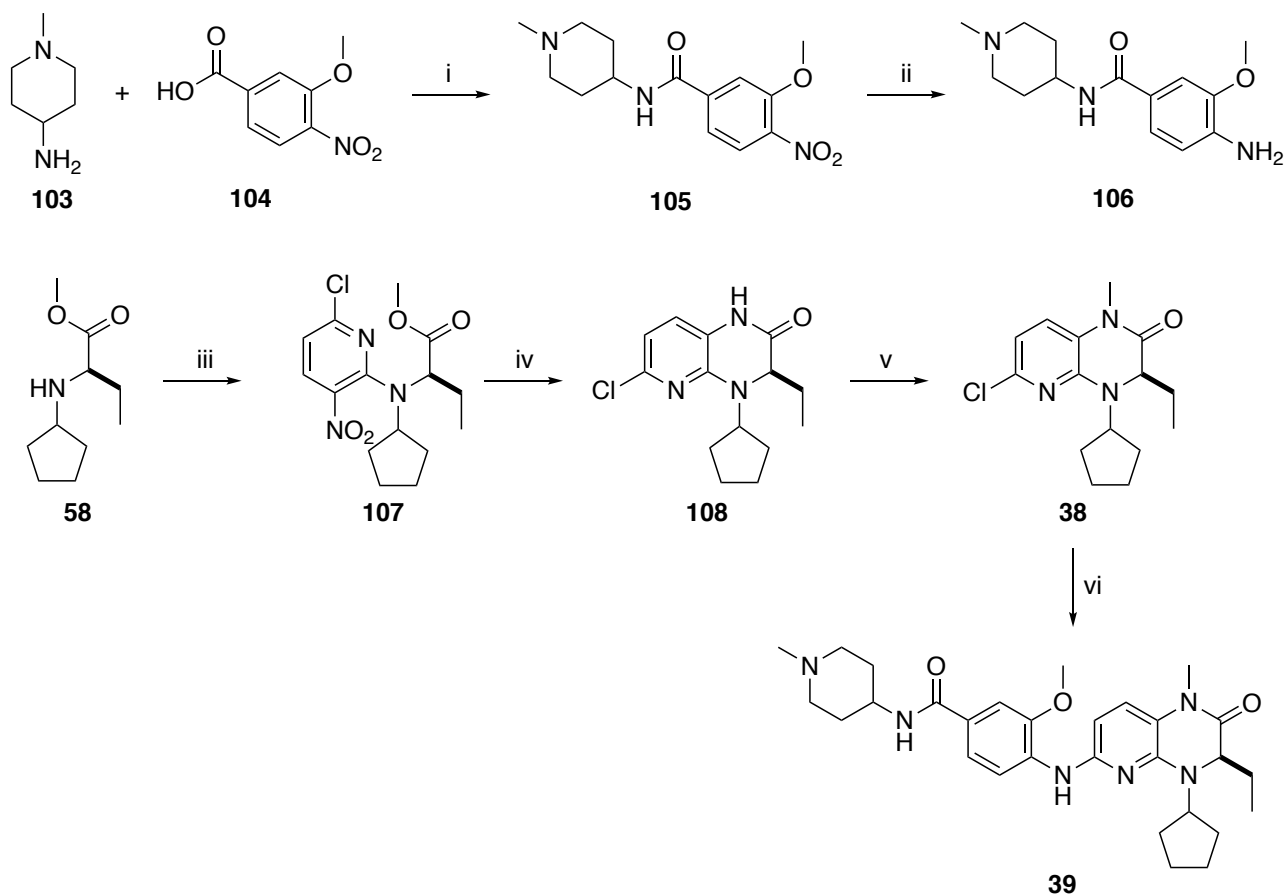


**Scheme S8:** Synthesis of **24**. *Reagents and conditions:* (i) NaOCN, TFA, THF, toluene, 25 °C, 4 h, 89% (ii) Chrotonyl chloride,  $LiO^tBu$  (1 M in THF), THF, -20 °C, 4 h, 39% (iii) 4-Bromoaniline, (*R*)-BINAP( $TfO$ ) $_2Pd(H_2O)_2$ , toluene, RT, 20 h, 80%. (iv)  $NaBH_4$ ,  $MgCl_2 \cdot 6H_2O$ , EtOH, -5 to 0 °C, 1 h, 80% (v) AcCl, pyridine,  $CH_2Cl_2$ , 88%.



**Scheme S9:** The synthetic route to the acetylated dihydrobenzooxazepine core **25**. *Reagents and conditions:*

(i) KBr, Br<sub>2</sub>, acetic acid, 120 °C then RT, 16 h, 37% (ii) 2-Chloroethyl amine, DCE, 3 Å molecular sieves, RT, 3 h then NEt<sub>3</sub>, RT, 1 h, then NaBH(OAc)<sub>3</sub>, RT, 22 h, 55%. (iii) Ac<sub>2</sub>O, K<sub>2</sub>CO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, RT, 4.5 h, 60%. (v) Ac<sub>2</sub>O, K<sub>2</sub>CO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, RT.



**Scheme S10:** Synthesis of **39**: (i) HBTU, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, rt, 20 h, 88%, n = 1; (ii) Activated iron powder, glacial AcOH, 70 °C, 4 h, 90%, n = 1. (iii) 2,4-dichloro-5-nitropyridine, NaHCO<sub>3</sub>, cyclooctane, 150 °C, 4 d, 31–48%, n = 2; (iv) Activated iron powder, glacial AcOH, 100 °C, 4–5 h, 94%, n = 2; (v) K<sub>2</sub>CO<sub>3</sub>, MeI, anhydrous DMF, 0 °C to rt, 19 h, 90%, n = 1. (vi) **106**, Pd<sub>2</sub>dba<sub>3</sub>, XPhos, K<sub>2</sub>CO<sub>3</sub>, <sup>t</sup>BuOH, 100 °C, 21 h, 25%, n = 1.

**Table S3.** Optimisation of S<sub>N</sub>Ar reaction to the key intermediate **63**.

Conditions	Temperature, time	Mechanism	Full reagent consumption (TLC)?	Isolated yield
K <sub>2</sub> CO <sub>3</sub> , acetone	0 °C then RT, 16 h to 7 d	S <sub>N</sub> Ar	No	19%
K <sub>2</sub> CO <sub>3</sub> , acetone	0 °C then 80 or 120 °C, 8 h to 2 d	S <sub>N</sub> Ar	No	< 15%
Pd(OAc) <sub>2</sub> , XPhos, Cs <sub>2</sub> CO <sub>3</sub> , dioxane	80 °C, 18 h	Buchwald Hartwig coupling	Yes	29%
NaHCO <sub>3</sub> , cyclohexane <sup>18</sup>	RT then 60 °C, 3 d	S <sub>N</sub> Ar	No	51–80%

### Synthetic chemistry experimental section

**Chemistry general experimental.** NMR data was processed using MestReNova® 10.0 or Bruker TopSpin® v3.5 or v4.0 software. Spectra were assigned using COSY, HSQC and HMBC experiments as necessary. <sup>1</sup>H NMR spectra were recorded on Bruker AVIIIHD 400 nanobay (400 MHz), Bruker AVII 500 (500 MHz) with dual <sup>13</sup>C(<sup>1</sup>H) cryoprobe, or Bruker AVIIIHD 500 (500 MHz) spectrometer in the stated solvents as a reference for the internal deuterium lock. The chemical shift data for each signal are given as δ<sub>H</sub> in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ<sub>H</sub> (TMS) = 0.00 ppm. The spectra are calibrated using the solvent peak with the data provided by Fulmer *et al.*<sup>23</sup> The multiplicity of each signal is indicated by s (singlet); br s (broad singlet); d (doublet); dd (doublet of doublets), ddd (doublet of doublet of doublets), t (triplet), q (quartet), qn (quintet), dq (double of quartet) or m (multiplet). The number of protons (n) for a given resonance signal is indicated by nH. Where appropriate, coupling constants (J) are quoted in Hz and are recorded to the nearest 0.1 Hz. Identical proton coupling constants (J) are averaged in each spectrum and reported to the nearest 0.1 Hz. <sup>13</sup>C NMR spectra were recorded on a Bruker AVIIIHD 400 nanobay (101 MHz), or Bruker AVII 500 (126 MHz) spectrometer, with dual <sup>13</sup>C(<sup>1</sup>H) cryoprobe, in the stated solvents, with broadband proton decoupling and an internal deuterium lock. The chemical shift data for each signal are given as δ<sub>C</sub> in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ<sub>C</sub> (TMS) = 0.00 ppm. The spectra are calibrated using the solvent peak with the data provided by Fulmer *et al.* The shift values of resonances are quoted to 1 decimal place unless peaks have similar chemical shifts, in which case 2 decimal places are used. Coupling constants (J) are quoted in Hz and are recorded to the nearest 0.1 Hz. <sup>19</sup>F NMR spectra were recorded on a Bruker AVIIIHD 400 nanobay (376 MHz) using a deuterium internal lock. The chemical shift data for each signal are given as δ<sub>F</sub> in units of parts per million (ppm). The multiplicity of each signal is indicated by: s (singlet); d (doublet); t (triplet); q (quartet) or m (multiplet). Coupling constants (J) are quoted in Hz and are recorded to the nearest 0.1 Hz. <sup>11</sup>B NMR spectra were recorded on a Bruker AVIIIHD 500 (160 MHz). The chemical shift data for each signal are given as δ<sub>B</sub> in units of parts per million (ppm). Coupling constants (J) are quoted in Hz and are recorded to the nearest 1 Hz. Identical coupling constants (J) are averaged in each spectrum and reported to the nearest 1 Hz. Low-resolution mass spectra (LRMS) were

recorded on a Waters LCT Premier spectrometer or Agilent 6120 Quadrupole LC/MS spectrometer (ESI).  $m/z$  values are reported in Daltons and followed by their percentage abundance in parentheses. High resolution mass spectra (HRMS) were recorded on a Bruker MicroTOF spectrometer, operating in positive or negative mode, as indicated, from solutions of MeOH, MeCN or H<sub>2</sub>O.  $m/z$  values are reported in Daltons and followed by their percentage abundance in parentheses. Electron ionization/field ionization (EI/FI) was carried out on a Waters GCT with a temperature programmed solids probe inlet. When a compound was not observed by LRMS, only HRMS is quoted. Melting points on crystallized samples were determined using either a) a Leica Galen III hot stage microscope or b) a Griffin capillary tube melting point apparatus and are uncorrected. The solvents of crystallization are shown in parentheses. Infrared spectra were obtained from thin films using a diamond attenuated total reflectance module. The spectra were recorded on a Bruker Tensor 27 spectrometer. Absorption maxima ( $\bar{\nu}_{\text{max}}$ ) are reported in wavenumbers (cm<sup>-1</sup>) and are classified as broad (br), strong (s), medium (m) or weak (w).

Analytical HPLC was carried out on a PerkinElmer Flexar system with a Binary LC Pump and UV/VIS LC Detector. For determination of compound purity following methods were applied. Method A: a Dionex Acclaim® 120 column (C18, 5  $\mu\text{m}$ , 120 Å, 4.6  $\times$  150 mm) was used and the solvents employed were A = H<sub>2</sub>O; B = MeCN. Linear gradient conditions (0–10 min, linear increase from 5% to 95% of B; 10–15 min, B = 95%) with a flow rate of 1.5 mL/min and detection at 254 nm. Method B: a Dionex Acclaim® 120 column (C18, 5  $\mu\text{m}$ , 120 Å, 4.6  $\times$  150 mm) was used and the solvents employed were A = 0.1% (v/v) solution of TFA in 95% H<sub>2</sub>O/5% MeCN; B = 0.1% (v/v) solution of TFA in 95% MeCN/5% H<sub>2</sub>O, and the gradient (A:B). A 10-min linear gradient of 0–100% B was run with a flow rate of 1 mL/min and detection at 254 nm. Method C: An Atlantis T3 (3  $\mu\text{m}$ , 120 Å, 4.6  $\times$  150 mm) column was used on an Agilent 1260 Infinity II system, and the solvents employed were A = H<sub>2</sub>O; B = MeCN. Linear gradient conditions (0–10 min, linear increase from 0% to 95% of B; 10–15 min, B = 95%) with a flow rate of 1.0 – 1.5 mL/min and detection at 220 and 254 nm. Samples were injected in DMSO, MeOH, DMSO/MeOH or DMSO/CHCl<sub>3</sub>. Samples were injected in DMSO, MeOH, DMSO/MeOH or DMSO/CHCl<sub>3</sub>. For determination of enantio-purity - Method 3 (M3): Daicel ChiralPak® (AD-H, 5  $\mu\text{m}$ , 120 Å, 4.6  $\times$  250 mm) was used with gradient elution of isopropanol and hexane (50:50). Samples were injected in isopropanol. Chromera software was used to determine purity and enantiomeric excess from relative peak areas of UV/VIS absorbance at 254 nm. All novel compounds that were biologically tested had purity of  $\geq 95\%$  as determined by HPLC analysis (representative HPLC traces are included below).

## Liquid chromatography mass spectrometry (LCMS)

For determination of compound purity and mass by LCMS, an Acquity UPLC CSH C18 column (50 mm × 2.1 mm, i.d. 1.7 µm packing diameter) was employed at 40 °C. The UV detection was a summed signal from wavelength of 210 nm to 350 nm, and the MS detection was conducted using Waters QDA spectrometer with alternate-scan positive and negative electrospray (100 to 1000 AMU; scan frequency: 5 Hz). *m/z* values are reported in Daltons and followed by the corresponding purity determined by the LC peak intensity percentage abundance in parentheses. The 2 mins methods for LCMS were either “high pH” or “formic acid” as describe below:

“high pH”

A = 10 mM ammonium bicarbonate in H<sub>2</sub>O adjusted to pH 10 with ammonia solution.

B = Acetonitrile.

The gradient employed was:

Time (min)	Flow rate (mL/min)	%A	%B
0	1	100	0
0.05	1	100	0
1.5	1	3	97
1.9	1	3	97
2	1	100	0

“Formic acid”

A = 0.1% v/v Solution of formic acid in H<sub>2</sub>O.

B = 0.1% v/v Solution of formic acid in acetonitrile.

The gradient employed was:

Time (min)	Flow rate (mL/min)	%A	%B
0	1	97	3
1.5	1	3	97
1.9	1	3	97
2.0	1	98	2

### Mass-directed assisted purification (MDAP)

For the purification of small amounts of compounds, MDAP HPLC was conducted on a Xselect CSH C18 column (150 mm × 30 mm i.d. 5 µm packing diameter) at ambient temperature. The UV detection was a summed signal from wavelength of 210 nm to 350 nm, and the MS detection was conducted using a Waters QDA spectrometer with alternate scan positive/negative electrospray (scan range : 100 to 1000 AMU; scan frequency : 4.3 Hz). The samples (up to 100 mg) were injected in 1 mL of DMSO. The 15 mins methods for MDAP PLC were either “MDAP method A”, “MDAP method B” or “MDAP method C” as described below:

A = 0.1% v/v solution of formic acid in H<sub>2</sub>O. B = 0.1% v/v solution of formic acid in acetonitrile.

“MDAP Method A”; formic acid focused preparative open access LC/MS

The gradient employed was:

Time (min)	Flow rate (mL/min)	%A	%B
0	40	100	0
1	40	100	0
10	40	75	25
10.5	40	1	99
15	40	1	99

“MDAP Method B”; formic acid focused preparative open access LC/MS gradient

The gradient employed was:

Time (min)	Flow rate (mL/min)	%A	%B
0	40	85	15
1	40	85	15
10	40	45	55
10.5	40	1	99
15	40	1	99

“MDAP Method C”; formic acid focused preparative open access LC/MS gradient

The gradient employed was:

Time (min)	Flow rate (mL/min)	%A	%B
0	40	70	30
1	40	70	30
10	40	15	85
10.5	40	1	99
15	40	1	99

### High resolution liquid chromatography mass spectrometry (HR-LCMS)

For the determination of high-resolution mass spectra by LCMS, an Acquity UPLC CSH C18 column (100 mm × 2.1 mm i.d. 1.7 µm packing diameter) at 50 °C was employed. The UV detection was a summed signal from wavelength of 210 nm to 350 nm, and the MS detection was acquired using a Waters XEVO G2-XS QToF

spectrometer with positive electrospray ionisation. The HRMS are reported by their retention times (rt) in mins, followed by the corresponding purity determined by the LC peak intensity percentage abundance in parentheses, then the detected *m/z* values in Daltons, followed by the relative *m/z* peak intensity in parentheses.

A = 0.1% v/v solution of Formic Acid in H<sub>2</sub>O.

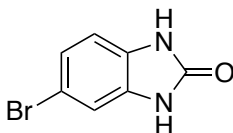
B = 0.1% v/v solution of Formic Acid in Acetonitrile.

The gradient employed was:

Time (min)	Flow Rate (mL/min)	%A	%B
0	0.8	97	3
8.5	0.8	0	100
9.0	0.8	0	100
9.5	0.8	97	3
10	0.8	97	3

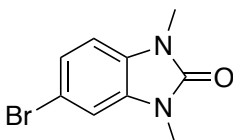
Anhydrous solvents were obtained under the following conditions: Et<sub>2</sub>O, toluene and CH<sub>2</sub>Cl<sub>2</sub> were dried by passing through a column of activated basic alumina according to the Grubbs' procedure.<sup>24</sup> Anhydrous 1,2-dichloroethane, DMF, DMSO, MeOH and MeCN were purchased from Sigma Aldrich UK in SureSeal™ bottles and used without further purification. All other solvents were used as supplied (analytical or HPLC grade) without purification. Solvents used for cross coupling reactions were degassed with nitrogen for 30 min. Chemicals were purchased from Acros UK, Apollo Scientific, Enamine, Sigma Aldrich UK, Alfa Aesar UK, Fisher Scientific UK, Fluka UK, Fluorochem, Merck, Argo International Limited and TCI-Europe. All reagents were purified, when necessary, by standard techniques.<sup>25</sup> Zinc and iron metals were activated according to the Kishi's procedure.<sup>26</sup> Where appropriate and if not otherwise stated, all non-aqueous reactions were performed in a flame dried flask under an inert atmosphere.

### 5-Bromo-1,3-dihydro-2H-benzo[d]imidazol-2-one (68)



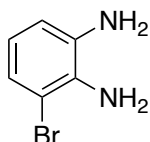
Carbonyldiimidazole (1.95 g, 12.1 mmol, 1.5 eq.) was added to a solution of 5-bromobenzene-1,2-diamine (1.50 g, 8.02 mmol, 1.0 eq.) in 1,4-dioxane (15 mL). The solution was stirred at 40 °C for 1 h. H<sub>2</sub>O (10 mL) was added, the precipitate was collected by filtration and washed with H<sub>2</sub>O (20 mL), then CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and petroleum ether (20 mL). Further precipitate from the aqueous washes was collected by filtration, the solids were combined, and dried *in vacuo* to afford 5-bromo-1,3-dihydro-2H-benzo[d]imidazol-2-one (**68**) (1.42 g, 83%) as a brown solid: *R*<sub>f</sub> 0.12 (EtOH); m.p. > 300 °C (H<sub>2</sub>O); <sup>1</sup>H (400 MHz, DMSO-*D*<sub>6</sub>) δ 10.77 (2 H, s), 7.08 (1 H, dd, *J* 8.2, 1.9), 7.05 (1 H, d, *J* 1.9), 6.87 (1 H, d, *J* 8.2). LRMS *m/z* (ES<sup>+</sup>) 215 [M<sup>81</sup>Br+H]<sup>+</sup>, 100%), 213 [M<sup>79</sup>Br+H]<sup>+</sup>, 70%). The data are in good agreement with the literature.<sup>19</sup>

### 5-Bromo-1,3-dimethyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (10)



Mel (400 μL, 6.37 mmol, 3.00 eq.) was added to a solution of 5-bromo-1,3-dihydro-2H-benzo[d]imidazol-2-one (450 mg, 2.12 mmol, 1.00 eq.) and K<sub>2</sub>CO<sub>3</sub> (1.76 g, 12.7 mmol, 6.00 eq.) in DMF (4 mL), and the reaction mixture was stirred at room temperature for 6 h. H<sub>2</sub>O (30 mL) was added and the mixture was extracted with EtOAc (3 × 50 mL). The organic components were washed with LiCl (10% w/v, 6 × 100 mL) then dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated *in vacuo* to afford 5-bromo-1,3-dimethyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (**10**) (497 mg, 97%) as a light brown solid: *R*<sub>f</sub> 0.85 (CH<sub>2</sub>Cl<sub>2</sub>:MeOH = 9:1); m.p. > 300 °C (EtOAc);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 3065 (w), 2980 (w), 1697 (s) (C=O), 1505 (m), 1446 (m), 1240 (w), 1134 (w), 942 (w), 857 (w), 788, 680 (w); <sup>1</sup>H NMR (400 MHz, DMSO-*D*<sub>6</sub>) 7.22 (1 H, dd, *J* 8.2, 1.8), 7.11 (1 H, d, *J* 1.8), 6.83 (1 H, d, *J* 8.2), 3.405 (3 H, s) 3.395 (3 H, s); <sup>13</sup>C NMR (101 MHz, DMSO-*D*<sub>6</sub>) δ 154.2, 131.6, 129.4, 123.7, 113.2, 111.0, 109.7, 27.58, 27.52; LRMS *m/z* (ES<sup>+</sup>) 241 ([M<sup>81</sup>Br+H]<sup>+</sup>, 100%), 243 ([M<sup>79</sup>Br+H]<sup>+</sup>, 70%); HRMS *m/z* (ES<sup>+</sup>) found 242.99514, C<sub>9</sub>H<sub>9</sub><sup>81</sup>BrN<sub>2</sub>O requires 242.99506. The data are in good agreement with the literature.<sup>20</sup>

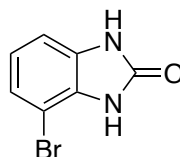
### 3-Bromobenzene-1,2-diamine (70)



Iron(0) powder (2.3 g, 41.5 mol, 3.0 eq.), which had been activated with concentrated HCl for 30 mins, then filtered, and washed with H<sub>2</sub>O and Et<sub>2</sub>O, was added to a solution of 3-bromo-2-nitroaniline (3.0 g, 13.8 mol, 1.0 eq.) in EtOH:H<sub>2</sub>O (3:1, 40 mL). NH<sub>4</sub>Cl (4.43 g, 82.9 mol, 6.0 eq.) was added portion wise at 0 °C, and the reaction mixture stirred at 90 °C for 16 h. The mixture was cooled to room temperature, and filtered through Celite®, washed with H<sub>2</sub>O (160 mL) and EtOH (80 mL). The volatile components were removed *in vacuo* and the mixture extracted with EtOAc (2 × 250 mL). The combined organic components were washed with brine

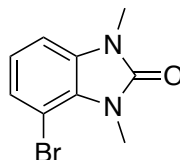
(2 × 150 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification using 25 g SNAP column on Biotage chromatography system, eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (1:0 to 9:1), afforded 3-bromobenzene-1,2-diamine (**70**) (1.89 g, 74%) as a light brown solid: *R<sub>f</sub>* 0.61 (CH<sub>2</sub>Cl<sub>2</sub>:MeOH = 9:1); m.p. 44–46 °C (MeOH) [lit.<sup>21</sup> 52–54 °C]; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 6.97 (1 H, dd, *J* 7.9, 1.4), 6.65 (1 H, dd, *J* 7.7, 1.4), 6.56 (1 H, dd, *J* 7.9, 7.9), 3.81 (2 H, s, NH<sub>2</sub>), 3.46 (2 H, NH<sub>2</sub>); LRMS *m/z* (ES<sup>+</sup>) 189 ([M<sup>79</sup>Br+H]<sup>+</sup>, 100%), 187 ([M<sup>81</sup>Br+H]<sup>+</sup>, 90%). The data are in good agreement with the literature.<sup>22</sup>

#### 4-Bromo-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one (**71**)



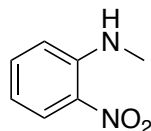
Carbonyldiimidazole (156 mg, 0.96 mmol, 1.2 eq.) was added to a solution of 3-bromobenzene-1,2-diamine (150 mg, 0.80 mmol, 1.0 eq.) in dioxane (3 mL). The solution was stirred at 60 °C for 2 h. H<sub>2</sub>O (10 mL) was added, the precipitate was collected by filtration and washed with H<sub>2</sub>O (20 mL) to afford 4-bromo-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one (**71**) (154 mg, 91%) as a pale yellow solid: *R<sub>f</sub>* 0.22 (EtOH); m.p. > 300 °C (H<sub>2</sub>O); <sup>1</sup>H δ (400 MHz, DMSO-*D*<sub>6</sub>) 11.04 (1 H, s), 10.92 (1 H, s), 7.10 (1 H, dd, *J* 7.8, 1.3), 6.96–6.80 (2 H, m). LRMS *m/z* (ES<sup>−</sup>) 214 [M<sup>81</sup>Br−H]<sup>−</sup>, 100%), 212 [M<sup>79</sup>Br−H]<sup>−</sup>, 60%). The data are in good agreement with the literature.<sup>23</sup>

#### 4-Bromo-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one (**9**)



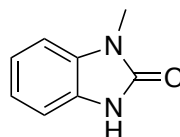
Mel (705 μL, 11.3 mol, 3.0 eq.) was added dropwise to a solution of 4-bromo-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one (1.0 g, 3.77 mmol, 1.0 eq.) and K<sub>2</sub>CO<sub>3</sub> (3.13 g, 22.7 mol, 6.0 eq.) in DMF (1.5 mL). The reaction mixture was stirred at room temperature for 2 h. H<sub>2</sub>O (30 mL) was added, the precipitate was collected by filtration and dried under vacuum, affording 4-bromo-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one (**9**) (825 mg, 90%) as brown solid: *R<sub>f</sub>* 0.85 (CH<sub>2</sub>Cl<sub>2</sub>:MeOH = 9:1); m.p. > 300 °C (H<sub>2</sub>O);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>−1</sup> 2946 (w) (CH), 1698 (s) (C=O), 1491 (m), 1461 (m), 1331 (w), 1256 (w), 1087 (m), 1029 (m), 758 (m), 740 (m), 723 (m); <sup>1</sup>H NMR (400 MHz, DMSO-*D*<sub>6</sub>) 7.23–7.15 (2 H, m), 7.00 (1 H, dd, *J* 7.9, 7.9), 3.61 (3 H, s), 3.33 (3 H, s); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 154.4, 132.3, 127.4, 125.9, 122.8, 107.8, 100.9, 29.9, 27.7; LRMS *m/z* (ES<sup>+</sup>) 242 ([M<sup>81</sup>Br+H]<sup>+</sup>, 100%), 244 ([M<sup>79</sup>Br+H]<sup>+</sup>, 70%), 263 [M<sup>79</sup>Br+Na]<sup>+</sup>, 20%), 265 [M<sup>81</sup>Br+Na]<sup>+</sup>, 40%); Analytical HPLC (RP, method A) *rt* 8.67 mins, purity 99%. The data are in good agreement with the literature.<sup>23</sup>

### ***N*-Methyl-2-nitroaniline (73)**



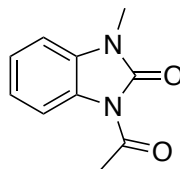
2-Fluoronitroaniline (1.00 g, 7.08 mol, 1.00 eq.) and MeNH<sub>2</sub>·HCl (1.43 g, 21.3 mol, 3.00 eq.) were dissolved in NMP (12 mL). DIPEA (6.1 mL, 35.4 mol, 5.00 eq.) was added, the vial was sealed then stirred at 80 °C for 17 h. H<sub>2</sub>O (50 mL) was added, and the mixture was extracted with EtOAc (2 × 200 mL). The combined organic components were washed with LiCl (10% w/v, 6 × 80 mL) and brine (2 × 100 mL) then dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification of the oil by silica gel chromatography (Biotage; SNAP 10 g), eluting with petroleum ether and CH<sub>2</sub>Cl<sub>2</sub> (1:0 to 3:1), afforded *N*-methyl-2-nitroaniline (**73**) (281 mg, 26% yield) as an orange solid: *R*<sub>f</sub> 0.34 (petroleum ether:CH<sub>2</sub>Cl<sub>2</sub> = 4:1); m.p. 26–28 °C (CH<sub>2</sub>Cl<sub>2</sub>) [lit.<sup>24</sup> 34–35 °C (MeCN)]; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.18 (1 H, dd, *J* 8.5, 1.3), 8.05 (1 H, s), 7.47 (1 H, ddd, *J* 8.7, 7.4, 1.3), 6.84 (1 H, dd, *J* 8.7, 1.3), 6.66 (1 H, ddd, *J* 8.5, 7.4, 1.3), 3.03 (3 H, d, *J* 5.1); The compound did not ionise under ESI or ES. The data are in good agreement with the literature.<sup>23, 24</sup>

### **1-Methyl-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one (74)**



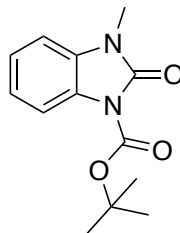
A solution of *N*-methyl-2-nitroaniline (**73**) (500 mg, 3.28 mmol, 1.0 eq.) and 10% Pd/C (175 mg, 0.164 mmol, 0.050 eq.) in EtOH (8.0 mL) was stirred under H<sub>2</sub>-atmosphere for 2 h at room temperature. The reaction mixture was filtered through Celite<sup>®</sup> and concentrated *in vacuo* to afford *N*-methylbenzene-1,2-diamine. The intermediate was dissolved in dry THF (4 mL), and Et<sub>3</sub>N (920 μL, 6.56 mmol, 2.0 eq.) was added. Triphosgene (777 mg, 2.62 mmol, 0.80 eq.) in THF (2 mL) was added dropwise to the reaction mixture at 0 °C. Precipitate appeared and the reaction mixture was diluted with THF (2 mL). The mixture was left to warm slowly to room temperature and stirred for 18 h. Saturated aq. NaHCO<sub>3</sub> (50 mL) was added dropwise, then EtOAc (30 mL) was added and the mixture was stirred for 20 mins. The layers were separated then the aqueous was extracted with EtOAc (2 × 20 mL). The organic components were washed with sat. aq. NaHCO<sub>3</sub> (100 mL), brine (100 mL), and H<sub>2</sub>O (100 mL), then dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. Recrystallisation from the minimum amount of boiling THF afforded 1-methyl-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one (**74**) (177 mg, 36% yield) as light brown solid: *R*<sub>f</sub> 0.59 (EtOH); m.p. 166–168 °C (THF) [lit.<sup>25</sup> 187–190 °C (EtOH)]; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 10.80 (1 H, s), 7.09–7.05 (1 H, m), 7.03–6.94 (3 H, m), 3.27 (3 H, s); LRMS *m/z* (ES<sup>−</sup>) 248 ([M−H]<sup>−</sup>, 100 %). The data are in good agreement with the literature.<sup>26</sup>

### 1-Acetyl-3-methyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (15)



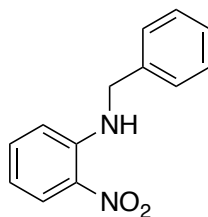
1-Methyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (**74**) (50 mg, 0.34 mmol, 1.0 eq.) and DMAP (10 mg, 0.07 mmol, 0.20 eq.) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (1 mL). Et<sub>3</sub>N (60  $\mu$ L, 0.40 mmol, 1.2 eq.) and Ac<sub>2</sub>O (90  $\mu$ L, 0.37 mmol, 1.1 eq.) were added, then the reaction mixture was stirred at room temperature for 1 h. The reaction was diluted with CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and washed with H<sub>2</sub>O (5 mL), 1 M aq. HCl (5 mL), H<sub>2</sub>O (5 mL), sat. aq. NaHCO<sub>3</sub> (5 mL), then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Filtration followed by concentration *in vacuo*. afforded 1-acetyl-3-methyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (**15**) (81 mg, 96%) as a colourless solid: *R<sub>f</sub>* 0.90 (CH<sub>2</sub>Cl<sub>2</sub>:MeOH = 20:1); m.p. 118–119 °C (EtOAc) [lit.<sup>27</sup> 122–123 °C (IPA)]; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.22–8.14 (1 H, m), 7.26 (1 H, ddd, *J* 7.9, 7.8, 1.3), 7.15 (1 H, ddd, *J* 7.9, 7.8, 1.3), 7.01–6.88 (1 H, m), 3.39 (3 H, s), 2.75 (3 H, s); LRMS *m/z* (ES<sup>+</sup>) 271 ([M+Na]<sup>+</sup>, 100 %); Analytical HPLC (RP, method A) *rt* 6.11 mins, purity 96%. The data are in good agreement with the literature.<sup>27</sup>

### *tert*-Butyl 3-methyl-2-oxo-2,3-dihydro-1H-benzo[d]imidazole-1-carboxylate (16)



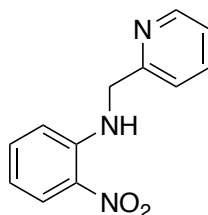
1-Methyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (**74**) (50 mg, 0.34 mmol, 1.0 eq.) and DMAP (2.0 mg, 0.020 mmol, 0.050 eq.) were dissolved in THF (1 mL). Et<sub>3</sub>N (50  $\mu$ L, 0.37 eq, 1.1 eq.) was added then the reaction mixture was cooled to 0 °C. Boc<sub>2</sub>O (90  $\mu$ L, 0.37 mmol, 1.1 eq.) was added and the reaction mixture was stirred at room temperature for 3 h. The volatile components were removed *in vacuo* then H<sub>2</sub>O (2.0 mL) was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2  $\times$  5 mL). The combined organic components were washed with 1 M aq. HCl (5.0 mL) and H<sub>2</sub>O (5.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification of the oil by silica gel chromatography, eluting with CH<sub>2</sub>Cl<sub>2</sub> and MeOH (1:0 to 9:1), afforded *tert*-butyl 3-methyl-2-oxo-2,3-dihydro-1H-benzo[d]imidazole-1-carboxylate (**16**) (81 mg, 96%) as a colourless solid: *R<sub>f</sub>* 0.73 (EtOAc); m.p. 62–64 °C (MeOH);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 2934 (br, w), 2360 (w) (N-H), 1789 (s) (C=O), 1749 (s) (C=O), 1494 (m), 1327 (s), 1156 (m), 1088 (w), 747 (m); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.85–7.79 (1 H, m), 7.21 (1 H, ddd, *J* 7.8, 7.7, 1.2), 7.12 (1 H, ddd, *J* 7.8, 7.7, 1.2), 6.98–6.91 (1 H, m), 3.38 (3 H, s), 1.67 (9 H, s); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  148.9, 130.0, 126.2, 123.9, 122.1, 114.3, 107.3, 84.6, 28.1, 27.0; LRMS *m/z* (ES<sup>+</sup>) 271 ([M+Na]<sup>+</sup>, 100 %); HRMS *m/z* (ESI<sup>+</sup>) found 271.1051, C<sub>13</sub>H<sub>15</sub>N<sub>2</sub>O<sub>3</sub> requires [M+H]<sup>+</sup> 271.1053; Analytical HPLC (RP, method A) *rt* 9.41 mins, purity > 99%.

### ***N*-Benzyl-2-nitroaniline (76)**



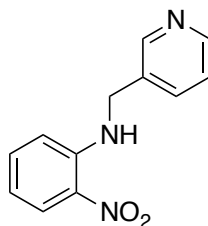
2-Fluoronitroaniline (**75**) (1.00 g, 7.08 mol, 1.00 eq.) was dissolved in NMP (8 mL). Benzyl amine (1.54 mL, 14.2 mol, 2.00 eq.) was added, and the reaction mixture was stirred at 80 °C for 3 h. H<sub>2</sub>O (15 mL) was added, and the mixture was extracted with EtOAc (2 × 40 mL). The combined organic components were washed with aq. LiCl (10% w/v, 6 × 60 mL) then dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated *in vacuo* to afford *N*-benzyl-2-nitroaniline (**76**) (1.62 g, > 99%) as an orange solid: *R<sub>f</sub>* 0.28 (petroleum ether/CH<sub>2</sub>Cl<sub>2</sub> = 3:1); m.p. 70–72 °C (EtOAc) [lit.<sup>28</sup> 72 °C (EtOAc)]; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.42 (1 H, s), 8.20 (1 H, dd, *J* 8.5, 1.6), 7.45–7.27 (6 H, m), 6.82 (1 H, dd, *J* 8.7, 1.2), 6.67 (1 H, ddd, *J* 8.5, 6.9, 1.2), 4.56 (2 H, d, *J* 5.7); HRMS *m/z* (ESI<sup>+</sup>) found 229.0975, C<sub>13</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub> requires [M+H]<sup>+</sup> 229.0972. The data are in good agreement with the literature.<sup>28,29</sup>

### **2-Nitro-*N*-(pyridin-2-ylmethyl)aniline (77)**



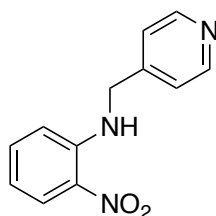
2-Fluoronitroaniline (**75**) (1.00 g, 7.08 mol, 1.00 eq.) was dissolved in NMP (8 mL). 2-(Aminomethyl)pyridine (2.92 mL, 28.32 mol, 4.00 eq.) was added, and the reaction mixture was stirred at 80 °C for 2 h. Sat. aq. NaHCO<sub>3</sub> (20 mL) was added, and the mixture was extracted with EtOAc (2 × 50 mL). The combined organic components were washed with LiCl (10% v/w, 5 × 60 mL) then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo* to afford *N*-pyridin-2'-yl-2-nitroaniline (**77**) (1.66 g, 98%) as a yellow solid: *R<sub>f</sub>* 0.06 (petroleum ether:EtOAc = 5:1); m.p. 86–88 °C (EtOAc) [lit.<sup>30</sup> 92–93 °C (EtOH)]; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 8.88 (1 H, s), 8.64 (1 H, dt, *J* 4.9, 1.2), 8.21 (1 H, dd, *J* 8.5, 1.6), 7.68 (1 H, ddd, *J* 7.5, 1.8), 7.40 (1 H, ddd, *J* 8.5, 7.0, 1.4), 7.32 (1 H, d, *J* 7.8), 7.24 (1 H, dd, *J* 7.5, 4.9), 6.82 (1 H, dd, *J* 8.7, 1.2), 6.68 (1 H, ddd, *J* 8.5, 7.0, 1.4), 4.68 (2 H, d, *J* 5.5); Analytical HPLC (RP, method A) rt 9.20 mins, purity >99%. The compound could not be detected by mass spectrometry. The data are in good agreement with the literature.<sup>30</sup>

### 2-Nitro-*N*-(pyridin-3-ylmethyl)aniline (**78**)



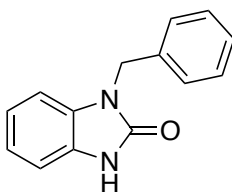
2-Fluoronitroaniline (**75**) (1.0 g, 7.1 mol, 1.0 eq.) was dissolved in NMP (6.0 mL). 3-(Aminomethyl)pyridine (2.9 mL, 28.32 mol, 4.0 eq.) was added, and the reaction mixture was stirred at room temperature for 5 h. H<sub>2</sub>O (20 mL) was added, and the mixture was extracted with EtOAc (3 × 150 mL). The combined organic components were washed with LiCl (10% v/w, 5 × 60 mL) then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude mixture (1.6 g) was filtered through silica, washing with EtOAc, then crystallised with minimum amounts of boiling CHCl<sub>3</sub> to afford 2-nitro-*N*-(pyridin-3-ylmethyl)aniline (**78**) (1.2 g, 75%) as bright orange crystals: *R*<sub>f</sub> 0.51 (petroleum ether:EtOAc = 3:1); m.p. 80–82 °C (CHCl<sub>3</sub>);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 3331 (w), 1568 (w), 1507 (m), 1233 (m), 1156 (w), 1045 (w), 774 (w), 744 (w); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) 8.64 (1 H, dd, *J* 2.1, 0.8), 8.61 (1 H, dd, *J* 4.8, 1.6), 8.40 (1 H, t, *J* 5.8), 8.20 (1 H, dd, *J* 8.7, 1.6), 7.67 (1 H, dd, *J* 7.9, 2.1), 7.40 (1 H, ddd, *J* 8.5, 7.0, 1.6), 7.30 (1 H, ddd, *J* 7.9, 4.8, 0.8), 6.78 (1 H, dd, *J* 8.7, 1.2), 6.70 (1 H, ddd, *J* 8.5, 7.0, 1.2), 4.58 (2 H, d, *J* 5.8). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  149.4, 149.0, 144.9, 136.5, 134.8, 133.1, 132.7, 127.1, 123.9, 116.4, 114.0, 44.8; LRMS *m/z* (ES<sup>+</sup>) 230 ([M+H]<sup>+</sup>, 100%); HRMS *m/z* (ESI<sup>+</sup>) found 230.0925, C<sub>12</sub>H<sub>11</sub>N<sub>2</sub>O<sub>2</sub> requires [M+H]<sup>+</sup> 230.0924; Analytical HPLC (RP, method A) rt 8.57 mins, purity > 99%.

### 2-Nitro-*N*-(pyridin-4-ylmethyl)aniline (**779**)



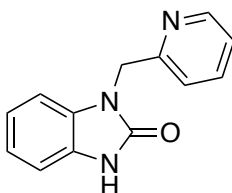
2-Fluoronitroaniline (**75**) (1.00 g, 7.08 mol, 1.00 eq.) was dissolved in NMP (6 mL). 4-(Aminomethyl)pyridine (1.45 mL, 14.2 mol, 2.00 eq.) was added, and the reaction mixture was stirred at 80 °C for 1 h. H<sub>2</sub>O (80 mL) was added, and the mixture was extracted with EtOAc (2 × 100 mL). The combined organic components were washed with aq. LiCl (10% w/v, 3 × 80 mL) then dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated *in vacuo* to afford 2-nitro-*N*-(pyridin-4-ylmethyl)aniline (**79**) (1.19 g, 74%) as an orange solid: *R*<sub>f</sub> 0.30 (EtOAc); m.p. 66–68 °C (EtOAc);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 3387 (w), 2360 (m), 1615 (m), 1503 (m), 1416 (m), 1354 (m), 1282 (m), 1249 (m), 1037 (m), 984 (m), 861 (m); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.61–8.57 (2 H, m), 8.49 (1 H, br t, *J* 6.0), 8.22 (1 H, dd, *J* 8.5, 1.6), 7.37 (1 H, ddd, *J* 8.5, 7.0, 1.6), 7.28–7.24 (2 H, m), 6.72 (1 H, ddd, *J* 8.5, 7.0, 1.2), 6.65 (1 H, dd, *J* 8.5, 1.2), 4.60 (2 H, d, *J* 6.0); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  150.5, 146.9, 144.9, 136.5, 132.8, 127.1, 121.8, 116.5, 114.1, 46.1; LRMS *m/z* (ES<sup>+</sup>) 230 ([M+H]<sup>+</sup>, 100%); HRMS *m/z* (ESI<sup>+</sup>) found 230.0925 (100%), C<sub>12</sub>H<sub>11</sub>N<sub>2</sub>O<sub>2</sub> requires [M+H]<sup>+</sup>, 230.0924; Analytical HPLC (RP, method A) rt 6.05 mins, purity 97%.

### 1-Benzyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (80)

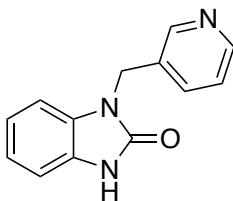


A solution of *N*-benzyl-2-nitroaniline (**76**) (500 mg, 2.52 mmol, 1.0 eq.) and 10% Pd/C (134 mg, 0.13 mmol, 0.050 eq.) in EtOH (25.0 mL) was stirred under H<sub>2</sub>-atmosphere for 30 mins at room temperature. The reaction mixture was filtered through Celite® and concentrated *in vacuo*. The intermediate was dissolved in dry THF (5 mL), then Et<sub>3</sub>N (700 µL, 6.56 mmol, 2.0 eq.) was added. Triphosgene (260 mg, 0.88 mmol, 0.35 eq.) was added portionwise to the reaction mixture at 0 °C, then the reaction mixture was stirred at 0 °C for 1.5 h. Sat. aq. NaHCO<sub>3</sub> (5 mL) then 1 M NaOH (15 mL) were added dropwise at 0 °C. The THF was removed *in vacuo*, the precipitate was collected by filtration and washed with H<sub>2</sub>O (2 × 30 mL) to afford 1-benzyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (**80**) (259 mg, 24%) as an off-white solid: *R*<sub>f</sub> 0.37 (petroleum ether:EtOAc = 5:1); m.p. 190–192 °C (THF) [lit.<sup>31</sup> 198–200 °C (EtOH)]; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 10.96 (1 H, s), 7.37–7.23 (5 H, m), 7.04–6.91 (4 H, m), 5.00 (2 H, s); LRMS *m/z* (ES<sup>−</sup>) 223 ([*M*−H]<sup>−</sup>, 100%); Analytical HPLC (RP, method A) rt 8.35 mins, purity >99%. The data are in good agreement with the literature.<sup>31</sup>

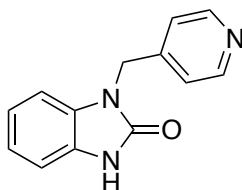
### 1-(Pyridin-2-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (21)



A solution of 2-nitro-*N*-(pyridin-2-ylmethyl)aniline (**77**) (500 mg, 2.18 mmol, 1.0 eq.) and 10% Pd/C (116 mg, 0.11 mmol, 0.050 eq.) in EtOH (25.0 mL) was stirred under an H<sub>2</sub>-atmosphere for 30 mins at room temperature. The reaction mixture was filtered through Celite® and concentrated *in vacuo*. The intermediate was dissolved in dry THF (10 mL), then Et<sub>3</sub>N (600 µL, 4.36 mmol, 2.0 eq.) was added. Triphosgene (230 mg, 0.76 mmol, 0.35 eq.) was added portion wise to the reaction mixture at 0 °C, then the reaction mixture was stirred at 0 °C for 1.5 h. Saturated aq. NaHCO<sub>3</sub> (5 mL) then 1 M NaOH (15 mL) were added dropwise at 0 °C and stirred for 20 mins. The THF was removed *in vacuo*, the precipitate was collected by filtration and washed with H<sub>2</sub>O (2 × 30 mL). The filtrate was concentrated *in vacuo* and purified by silica gel chromatography, eluting with petroleum ether:EtOAc (1:0 to 0:1). The purified solid and the filtered solid were combined and dried *in vacuo* to afford 1-(pyridin-2-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (**21**) (372 mg, 76%) as an off-white solid: *R*<sub>f</sub> 0.26 (EtOAc); m.p. 178–179 °C (EtOAc);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>−1</sup> 3145 (w), 1687 (s) (C=O), 1395 (m), 1150 (w), 748 (m), 686 (m), 641 (m); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) 9.12 (1 H, s), 8.59 (1 H, ddd, *J* 4.9, 1.8, 1.0), 7.62 (1 H, ddd, *J* 7.7, 7.7, 1.8), 7.24–7.16 (2 H, m), 7.11–7.08 (1 H, m), 7.06 (1 H, ddd, *J* 7.6, 7.5 1.4), 7.02 (1 H, ddd, *J* 7.6, 7.5, 1.5), 6.95 (1 H, dd, *J* 7.6, 1.2), 5.23 (2 H, s); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 156.1, 155.2, 149.5, 137.1, 130.2, 127.7, 122.7, 121.9, 121.62, 121.55, 109.5, 108.8, 46.5; LRMS *m/z* (ES<sup>−</sup>) 224 ([*M*−H]<sup>−</sup>, 100%); HRMS *m/z* (ESI<sup>−</sup>) found 224.0829, C<sub>13</sub>H<sub>11</sub>N<sub>3</sub>O requires [*M*−H]<sup>−</sup> 224.08294; Analytical HPLC (RP, method A) rt 6.72 mins, purity 99%.

**1-(Pyridin-3-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (22)**

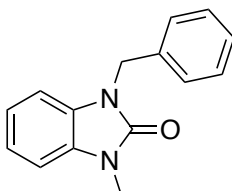
A solution of 2-Nitro-*N*-(pyridin-3-ylmethyl)aniline (**78**) (500 mg, 2.18 mmol, 1.0 eq.) and 10% Pd/C (116 mg, 0.11 mmol, 0.050 eq.) in EtOH (20.0 mL) was stirred under H<sub>2</sub>-atmosphere for 40 mins at room temperature. The reaction mixture was filtered through Celite® and concentrated *in vacuo*. The intermediate was dissolved in dry THF (15 mL), then pyridine (450 µL, 4.4 mmol, 2.0 eq.) was added. Triphosgene (230 mg, 0.76 mmol, 0.35 eq.) was added dropwise portion wise to the reaction mixture at 0 °C, then the reaction mixture was stirred at 0 °C for 3 h. Saturated aq. NaHCO<sub>3</sub> (2 mL) then 1 M NaOH (4 mL) were added dropwise at 0 °C, then the reaction mixture was warmed to room temperature and stirred for 2 h. The THF was removed *in vacuo*, and the precipitate was removed by filtration. The filtrate was lyophilised and triturated with MeCN. Purification by silica gel chromatography, using a 10 g SNAP column on a Biotage purification system, eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (1:0 to 20:1) afforded 1-(pyridin-3-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (**22**) (125 mg, 25%) as an off-white solid: *R*<sub>f</sub> 0.20 (EtOAc); m.p. 140–144 °C (MeOH);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 3016 (w), 2970 (w), 1738 (s) (C=O), 1366 (w), 1228 (w), 1216 (w), 869 (w), 639 (w); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  10.58 (1 H, s), 8.64–8.53 (2 H, m), 7.25–7.20 (2 H, m), 7.18–7.14 (1 H, m), 7.10 (1 H, ddd, *J* 7.7, 1.4, 1.1), 7.04 (1 H, ddd, *J* 7.7, 7.7, 1.4), 6.81 (1 H, ddd, *J* 7.7, 7.7, 1.1), 5.13 (2 H, s); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  155.6, 149.3, 148.9, 135.3, 132.0, 129.7, 128.0, 123.8, 122.1, 121.6, 109.8, 108.2, 42.1; LRMS *m/z* (ES<sup>+</sup>) 226 ([M+H]<sup>+</sup>, 100%); HRMS *m/z* (ESI<sup>+</sup>) found 226.0975, C<sub>13</sub>H<sub>11</sub>N<sub>3</sub>O requires [M+H]<sup>+</sup> 226.0975; Analytical HPLC (RP, method A) *rt* 6.52 mins, purity 99%.

**1-(Pyridin-4-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (23)**

A solution of 2-nitro-*N*-(pyridin-4-ylmethyl)aniline (**79**) (0.5 g, 2.2 mmol, 1.0 eq.) and 10% Pd/C (0.12 mg, 0.11 mmol, 0.050 eq.) in EtOH (25 mL) was stirred under H<sub>2</sub>-atmosphere for 40 mins at room temperature. The reaction mixture was filtered through Celite® and concentrated *in vacuo*. The intermediate was dissolved in dry THF (10 mL), then pyridine (0.45 mL, 4.4 mmol, 2.0 eq.) was added. Triphosgene (0.24 g, 0.78 mmol, 0.35 eq.) was added portion wise to the reaction mixture at 0 °C, then the reaction mixture was stirred at 0 °C for 3 h. Saturated aq. NaHCO<sub>3</sub> (2 mL) then 1 M NaOH (4 mL) were added dropwise at 0 °C, and the warmed to room temperature and stirred for 2 h. The THF was removed *in vacuo*, and the precipitate was removed by filtration. The filtrate was lyophilised and purified by silica gel chromatography, using a 10 g SNAP column on a Biotage purification system, eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (1:0 to 20:1). The product was collected and purified further by column chromatography, eluting with petroleum ether/EtOAc (1:0 to 0:1) to afford 1-(pyridin-4-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (**23**) (94 mg, 22%) as a colourless solid: *R*<sub>f</sub> 0.22

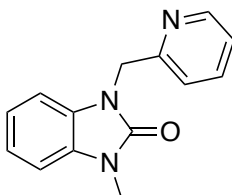
(EtOAc); m.p. 140–142 °C (EtOAc);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 2980 (w), 1690 (C=O), 1484 (m), 1395 (w), 1156 (m), 1004 (m), 750 (m), 727 (m); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  10.58 (1 H, s), 8.64–8.53 (2 H, m), 7.25–7.20 (2 H, m), 7.18–7.12 (1 H, m), 7.10 (1 H, ddd, *J* 7.7, 1.4, 1.1), 7.04 (1 H, ddd, *J* 7.7, 7.7, 1.4), 6.81 (1 H, ddd, *J* 7.7, 7.7, 1.1), 5.13 (2 H, s); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  155.5, 150.3, 145.1, 129.8, 127.9, 122.2, 122.0, 121.7, 109.9, 108.2, 43.4; LRMS *m/z* (ES<sup>+</sup>) 226 ([M+H]<sup>+</sup>, 100%); HRMS *m/z* (ESI<sup>-</sup>) found 224.08284, C<sub>13</sub>H<sub>11</sub>N<sub>3</sub>O requires [M-H]<sup>-</sup> 224.08294; Analytical HPLC (RP, method A) rt 6.51 mins, purity >99%;

### 1-Benzyl-3-methyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (17)



Mel (140  $\mu$ L, 2.22 mmol, 10.0 eq.) was added dropwise to a solution of 1-benzyl-3-methyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (**80**) (50 mg, 0.22 mmol, 1.0 eq.) and K<sub>2</sub>CO<sub>3</sub> (125 mg, 0.89 mol, 4.0 eq.) in DMF (1.0 mL). The reaction mixture was stirred at room temperature for 16 h. H<sub>2</sub>O (30 mL) was added, and the mixture was extracted with EtOAc (2  $\times$  50 mL). The combined organic components were washed with aq. LiCl (10% w/v, 2  $\times$  150 mL), then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo* to afford 1-benzyl-3-methyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (**17**) (52 mg, 98%) as colourless amorphous solid: *R*<sub>f</sub> 0.35 (petroleum ether:EtOAc = 5:1); m.p. 72–74 °C (CH<sub>2</sub>Cl<sub>2</sub>);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 2931 (w) (CH), 1702 (s) (C=O), 1497 (m), 1401 (w), 1250 (w), 1020 (w), 798 (m), 698 (m); <sup>1</sup>H NMR (700 MHz, DMSO-*d*<sub>6</sub>) 7.31 (4 H, m), 7.25 (1 H, m), 7.16 (1 H, dd, *J* 7.7, 1.2), 7.09 (1 H, dd, *J* 7.7, 1.2), 7.05 (1 H, ddd, *J* 7.7, 7.6, 1.2), 7.00 (1 H, ddd, *J* 7.7, 7.6, 1.2), 5.04 (2 H, s), 3.36 (3 H, s); <sup>13</sup>C NMR (176 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  154.2, 137.5, 130.2, 129.11, 129.08, 127.9, 127.8, 121.6, 121.4, 108.6, 108.4, 44.14, 27.5; LRMS *m/z* (ES<sup>+</sup>) 239 ([M+H]<sup>+</sup>, 60%), 261 ([M+Na]<sup>+</sup>, 100%); HRMS *m/z* (ESI<sup>+</sup>) found 239.11795, C<sub>15</sub>H<sub>14</sub>N<sub>2</sub>O requires ([M+H]<sup>+</sup>, 239.11789; Analytical HPLC (RP, method A) rt 9.23 mins, purity 99%.

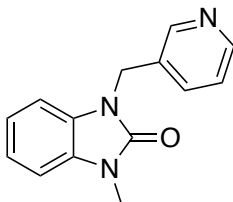
### 1-Methyl-3-(pyridin-2-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (18)



Mel (140  $\mu$ L, 2.22 mmol, 10 eq.) was added dropwise to a solution of 1-(pyridin-2-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (**21**) (50.0 mg, 0.221 mmol, 1.0 eq.) and K<sub>2</sub>CO<sub>3</sub> (123 mg, 0.81 mol, 4.0 eq.) in DMF (1.0 mL). The reaction mixture was stirred at room temperature for 16 h. H<sub>2</sub>O (5 mL) was added, and the mixture was extracted with EtOAc (5  $\times$  50 mL). The combined organic components were washed with LiCl (10% w/v, 2  $\times$  50 mL), then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification by column chromatography, eluting with petroleum ether:EtOAc (1:1 to 0:1) afforded 1-methyl-3-(pyridin-2-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (**18**) (14 mg, 28%) as an amorphous brown residue: *R*<sub>f</sub> 0.52 (EtOAc);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 3478 (w), 2957 (w), 1684 (s) (C=O), 1508 (w), 1448 (m), 1220 (w), 1118 (w), 969 (w);

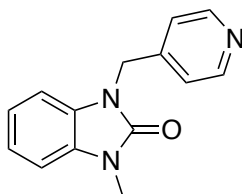
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.57 (1 H, ddd,  $J$  4.8, 1.9, 1.0), 7.60 (1 H, dd,  $J$  7.7, 7.7, 1.8), 7.22–7.15 (2 H, m), 7.09 (1 H, ddd,  $J$  7.6, 7.5, 1.3), 7.05–6.93 (3 H, m), 5.21 (2 H, s), 3.48 (3 H, s);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  156.3, 155.5, 149.4, 136.9, 130.1, 129.2, 122.6, 121.7, 121.5, 121.4, 108.5, 107.4, 46.9, 27.3; LRMS  $m/z$  ( $\text{ES}^+$ ) 240 ( $[\text{M}+\text{H}]^+$ , 100%); HRMS  $m/z$  ( $\text{ESI}^+$ ) found 240.11304,  $\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}$  requires  $[\text{M}+\text{H}]^+$  240.11314; Analytical HPLC (RP, method A)  $r_t$  7.41, purity 98%.

#### 1-Methyl-3-(pyridin-3-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (19)



Mel (20  $\mu\text{L}$ , 0.26 mmol, 3.0 eq.) was added dropwise to a solution of 1-(pyridin-3-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (**22**) (20 mg, 0.087 mmol, 1.0 eq.) and  $\text{K}_2\text{CO}_3$  (72 mg, 0.56 mol, 6.0 eq.) in DMF (1.0 mL). The reaction mixture was stirred at room temperature for 20 h.  $\text{H}_2\text{O}$  (10 mL) was added, and the mixture was extracted with EtOAc ( $3 \times 20$  mL). The combined organic components were washed with LiCl (10% w/v,  $5 \times 50$  mL), then dried over anhydrous  $\text{MgSO}_4$ , filtered, and concentrated *in vacuo*. Purification by silica gel chromatography, elution with  $\text{CH}_2\text{Cl}_2$ :MeOH (slow gradient from 1:0 to 20:1) afforded 1-methyl-3-(pyridin-3-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (**19**) (6 mg, 28%) as colourless solid:  $R_f$  0.52 ( $\text{CH}_2\text{Cl}_2$ :MeOH = 20:1); m.p. 104–105°C (MeOH);  $\tilde{\nu}_{\text{max}}$  (neat)/ $\text{cm}^{-1}$  3467 (w), 2980 (w), 1701 (s) (C=O), 1498 (m), 1401 (w), 1289 (w), 1127 (w), 751 (m), 633 (m);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ) 8.65 (1 H, s), 8.56–8.50 (1 H, m), 7.69–7.62 (1 H, m), 7.24 (1 H, d,  $J$  7.7), 7.10 (1 H, ddd,  $J$  7.7, 7.7, 1.2), 7.04 (1 H, ddd,  $J$  7.7, 7.7, 1.2), 7.00 (1 H, dd,  $J$  7.7, 1.2), 6.92–6.86 (1 H, m), 5.10 (2 H, s), 3.47 (3 H, s);  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  154.5, 149.3, 149.0, 135.4, 132.1, 130.1, 128.7, 123.7, 121.7, 121.5, 107.9, 107.7, 42.4, 27.3; LRMS  $m/z$  ( $\text{ES}^+$ ) 240 ( $[\text{M}+\text{H}]^+$ , 100%); HRMS  $m/z$  ( $\text{ESI}^+$ ) found 240.11316,  $\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}$  requires  $[\text{M}+\text{H}]^+$  240.11314; Analytical HPLC (RP, method A)  $r_t$  7.16 mins, purity >99%.

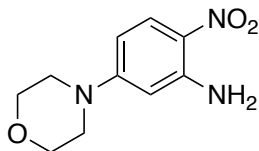
#### 1-Methyl-3-(pyridin-4'-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (20)



Mel (20  $\mu\text{L}$ , 0.26 mmol, 3.0 eq.) was added dropwise to a solution of 1-(pyridin-4-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (**23**) (20 mg, 0.087 mmol, 1.0 eq.) and  $\text{K}_2\text{CO}_3$  (72 mg, 0.56 mol, 6.0 eq.). The reaction mixture was stirred at room temperature for 20 h.  $\text{H}_2\text{O}$  (10 mL) was added, and the mixture was extracted with EtOAc ( $3 \times 20$  mL). The combined organic components were washed with aq. LiCl (10% w/v,  $5 \times 50$  mL), then dried over anhydrous  $\text{MgSO}_4$ , filtered, and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with  $\text{CH}_2\text{Cl}_2$ :MeOH (1:0 to 20:1) afforded 1-methyl-3-(pyridin-3-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (**20**) (3 mg, 11%) as colourless oil:  $R_f$  0.58 ( $\text{CH}_2\text{Cl}_2$ :MeOH = 20:1);  $\tilde{\nu}_{\text{max}}$  (neat)/ $\text{cm}^{-1}$  3015 (w), 2970 (w), 1738 (s) (C=O), 1435 (w), 1366 (m), 1228 (m), 1216 (m);  $^1\text{H}$  NMR (500 MHz,

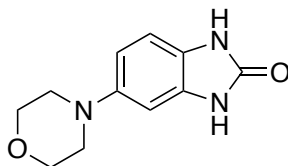
CDCl<sub>3</sub>) δ 8.62–8.48 (2 H, m), 7.20 (2 H, d, *J* 5.0), 7.13 (1 H, dd, *J* 7.6, 1.3), 7.04 (2 H, 2 × t, *J* 7.6, 7.5), 6.80 (1 H, dd, *J* 7.6, 1.3), 5.09 (2 H, s), 3.49 (3 H, s). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 154.5, 150.1, 145.5, 130.1, 128.8, 122.1, 121.9, 121.5, 107.9, 107.8, 43.8, 27.4. LRMS *m/z* (ES<sup>+</sup>) 240 ([M+H]<sup>+</sup>, 100%); HRMS *m/z* (ESI<sup>+</sup>) found 240.11327, C<sub>14</sub>H<sub>13</sub>N<sub>3</sub>O requires [M+H]<sup>+</sup> 240.11314; Analytical HPLC (RP, method A) rt 6.22 mins, purity 94%.

### 5-Morpholino-2-nitroaniline (**82**)



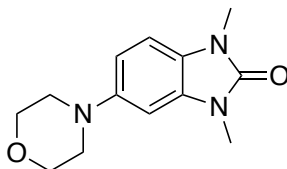
2-Amino-4-fluoronitrobenzene (**81**) (507 mg, 2.98 mmol, 1.0 eq.) was dissolved in dry NMP (4 mL). Morpholine (400 μL, 4.57 mmol, 1.58 eq.) was added, and the reaction mixture was stirred at 100 °C for 3 h. H<sub>2</sub>O (20 mL) and Et<sub>2</sub>O (50 mL) were added. The precipitate was collected by filtration, and dried under vacuum. The filtrate was extracted with EtOAc (100 mL). The organic components were washed with aq. LiCl (10% w/v, 5 × 60 mL), dried over MgSO<sub>4</sub>, and concentrated *in vacuo* then crystallised from EtOAc/Et<sub>2</sub>O 1:1. The combined solids were dried under vacuum to afford 5-morpholino-2-nitroaniline (**82**) as an off-white solid (530 mg, 82%); *R<sub>f</sub>* 0.16 (petroleum ether:EtOAc = 2:1); m.p. 183–184 °C (EtOAc:Et<sub>2</sub>O = 1:1) [lit.<sup>32</sup> 184–186 °C (MeOH)]; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.04 (1 H, d, *J* 9.7), 6.27 (1 H, dd, *J* 9.7, 2.6), 6.14 (2 H, s), 5.95 (1 H, d, *J* 2.6), 3.88–3.76 (4 H, m), 3.34–3.26 (4 H, m). LRMS *m/z* (ES<sup>+</sup>) 246 ([M+Na]<sup>+</sup>, 30%). The data are in good agreement with the literature.<sup>32</sup>

### 5-Morpholino-1,3-dihydro-2*H*-benzo[d]imidazol-2-one (**13**)



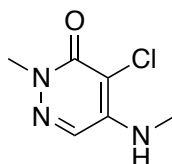
A solution of 5-morpholino-2-nitroaniline (**82**) (0.50 g, 2.2 mmol, 1.0 eq.) and 10% Pd/C (0.13 g, 0.12 mmol, 0.050 eq.) in EtOH (50 mL) was stirred under H<sub>2</sub>-atmosphere for 2 h at room temperature. The reaction mixture was filtered through Celite® and concentrated *in vacuo* to afford 4-morpholinobenzene-1,2-diamine. The intermediate was dissolved in dry 1,4-dioxane (10 mL), then Et<sub>3</sub>N (0.600 mL, 4.6 mmol, 2.0 eq.) was added. Triphosgene (0.27 mg, 0.89 mmol, 0.40 eq.) was added to the solution at 0 °C, then the reaction mixture was stirred at 0 °C for 2 h. Saturated aq. NaHCO<sub>3</sub> (5 mL) then 1 M NaOH (15 mL) were added dropwise at 0 °C, then the mixture was left to warm to room temperature with vigorous stirring for 15 mins. The 1,4-dioxane was removed *in vacuo*, and the precipitate was collected by filtration. Crystallisation from the minimum amount of EtOH:H<sub>2</sub>O 1:1 yielded 5-morpholino-1,3-dihydro-2*H*-benzo[d]imidazol-2-one (**13**) (380 mg, 69%) as a brown solid; *R<sub>f</sub>* 0.75 (EtOH); m.p.: 268–272 °C (dec.) (EtOAc);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 2957 (br) (NH), 2846, 1709 (s) (C=O), 1511, 1337 (w), 1263, 1127, 858, 743; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) 10.42 (1 H, br s), δ 10.29 (1 H, br s), 6.68 (1 H, d, *J* 8.4), 6.55 (1 H, dd, *J* 8.4, 2.3), 6.52 (1 H, d, *J* 2.3), 3.74–3.70 (4 H, m), 2.98–2.94 (4 H, m); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 156.1, 146.8, 130.9, 123.9, 109.5, 109.1, 98.0, 66.7, 50.8; LRMS *m/z* (ES<sup>-</sup>) 218 ([M-H]<sup>-</sup>, 100%); HRMS *m/z* (ES<sup>+</sup>) found 220.1079, C<sub>11</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub> requires [M+H]<sup>+</sup> 220.1080; Analytical HPLC (RP, method A) rt 5.18 mins, purity 98%.

### 1,3-Dimethyl-5-morpholino-1,3-dihydro-2H-benzo[d]imidazol-2-one (14)



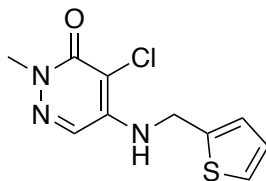
To a solution of 5-morpholino-1,3-dihydro-2H-benzo[d]imidazol-2-one (**13**) (100 mg, 0.45 mmol, 1.0 eq.) in DMF (2 mL), NaH (60% in mineral oil) (38 mg, 0.88 mmol, 2.2 eq.) was added at 0 °C. The cloudy solution was stirred for 40 mins at 0 °C, followed by addition of MeI (55  $\mu$ L, 0.88 mmol, 2.2 eq.). The reaction mixture was stirred for a further 1.5 h until TLC analysis show full consumption of the starting material. Brine (20 mL) and sat. NaHCO<sub>3</sub> (10 mL) were added, and the mixture was extracted with EtOAc (3  $\times$  60 mL). The combined organic components were washed with sat. NaHCO<sub>3</sub> (20 mL) and aq. LiCl (10% w/v, 3  $\times$  60 mL), then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with CH<sub>2</sub>Cl<sub>2</sub> and MeOH (slow gradient from 1:0 to 98:2), afforded 1,3-dimethyl-5-morpholino-1,3-dihydro-2H-benzo[d]imidazol-2-one (**14**) (82 mg, 72%) as a colourless solid: *R*<sub>f</sub> 0.61 (CH<sub>2</sub>Cl<sub>2</sub>:MeOH = 20:1); m.p. 120–122 °C (MeOH);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 2961 (w), 2857 (w), 1683 (s) (C=O), 1509 (m), 1326 (m), 1147 (m), 1068 (w), 969 (w), 789 (w), 626 (w); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  6.98 (1 H, d, *J* 8.5), 6.81 (1 H, d, *J* 2.3), 6.66 (1 H, dd, *J* 8.5, 2.3), 3.77–3.68 (4 H, m), 3.29 (3 H, s), 3.27 (3 H, s), 3.09–3.00 (4 H, m); <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  154.1, 147.0, 130.4, 123.5, 108.8, 107.7, 97.0, 66.2, 50.2, 26.9, 26.8; LRMS *m/z* (ES<sup>+</sup>) 248 ([M+H]<sup>+</sup>, 100%); HRMS *m/z* (ES<sup>+</sup>) 248.13935 found, C<sub>13</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub> requires [M+H]<sup>+</sup> 248.13935; Analytical HPLC (RP, method A) rt 6.39 mins, purity >99%.

### 4-Chloro-2-methyl-5-(methylamino)pyridazin-3(2H)-one (40)



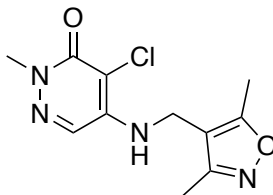
A solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (90 mg, 0.50 mmol, 1.0 eq.), MeNH<sub>2</sub>·HCl (101 mg, 1.5 mmol, 3.0 eq.) and DIPEA (44  $\mu$ L, 2.5 mmol, 5.0 eq.) in NMP (1 mL) was stirred at 60 °C for 16 h. TLC analysis showed the presence of remaining starting material. Additional MeNH<sub>2</sub>·HCl (101 mg, 1.5 mmol, 3.0 eq.) and DIPEA (44  $\mu$ L, 2.5 mmol, 5.0 eq.) were added and the solution was stirred at 80 °C for 3 h. The reaction mixture was cooled to room temperature and diluted with H<sub>2</sub>O (2 mL), then extracted with EtOAc (2  $\times$  60 mL). The combined organic components were washed with LiCl (10% w/v, 4  $\times$  60 mL), then dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 10:7), recovered a portion of the starting material (20 mg, 23% recovery) and afforded 4-chloro-2-methyl-5-(methylamino)pyridazin-3(2H)-one (**40**) (14 mg, 16%) as a colourless solid: *R*<sub>f</sub> 0.29 (petroleum ether:EtOAc = 1:1); m.p. 140–142 °C (EtOAc);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 3307 (w), 2922 (w), 2851 (w), 1605 (s) (C=O), 1562 (w), 1421 (w), 1036 (w), 962 (w), 773 (m), 665; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  7.48 (1 H, s), 5.88 (1 H, s), 3.72 (3 H, s), 2.17 (3 H, s); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  156.4, 140.3, 139.6, 106.2, 40.0, 31.6; LRMS *m/z* (ES<sup>+</sup>) 174 ([M+H]<sup>+</sup>, 100%); HRMS *m/z* (ES<sup>+</sup>) found 174.0430 (100%), C<sub>6</sub>H<sub>8</sub>ClN<sub>3</sub>O requires [M<sup>35</sup>Cl+H]<sup>+</sup> 174.0429; Analytical HPLC (RP, method A) rt 7.01 mins, purity 98%.

#### 4-Chloro-2-methyl-5-((thiophen-2-ylmethyl)amino)pyridazin-3(2H)-one (41)



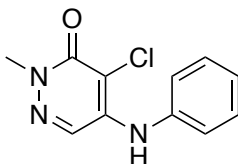
A solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (90 mg, 0.50 mmol, 1.0 eq.), thiophen-2-ylmethanamine (77  $\mu$ L, 0.75 mmol, 1.5 eq.) and DIPEA (0.25 mL, 1.5 mmol, 3.0 eq.) in NMP (0.75 mL) was stirred at 80 °C for 18 h. The reaction mixture was cooled to room temperature and diluted with H<sub>2</sub>O (20 mL), then extracted with EtOAc (2  $\times$  40 mL). The combined organic components were washed with aq. LiCl (10% w/v, 6  $\times$  30 mL), dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 0:1) afforded 4-chloro-2-methyl-5-((thiophen-2-ylmethyl)amino)pyridazin-3(2H)-one (**41**) (82 mg, 63%) as an off-white solid: *R*<sub>f</sub> 0.42 (petroleum ether:EtOAc = 1:1); m.p. 138–140 °C (EtOAc);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 32926 (w), 3081 (w), 1588 (s) (C=O), 1513 (m), 1451 (w), 1212 (w), 997 (m), 867 (m), 772 (m), 659 (m); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.59 (1 H, s), 7.28 (1 H, dd, *J* 5.1, 1.2), 7.03 – 6.97 (2 H, m), 5.16 (1 H, s), 4.71 (2 H, d, *J* 6.5), 3.75 (3 H, s); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  157.5, 142.8, 139.0, 129.9, 128.1, 125.4, 124.0, 109.0, 42.5, 40.1; LRMS *m/z* (ES<sup>-</sup>) 254 ([M-H]<sup>-</sup>, 100%); HRMS *m/z* (ES<sup>-</sup>) found 254.01618 (100%), C<sub>10</sub>H<sub>10</sub>ON<sub>3</sub><sup>35</sup>Cl<sup>32</sup>S requires [M<sup>35</sup>C-H]<sup>-</sup> 254.01603; Analytical HPLC (RP, method A) rt 7.44 mins, purity 97%.

#### 4-Chloro-5-(((3',5'-dimethylisoxazol-4'-yl)methyl)amino)-2-methylpyridazin-3(2H)-one (42)



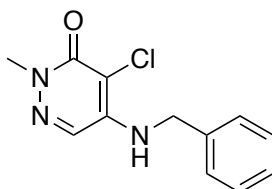
A solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (180 mg, 1.00 mmol, 1.0 eq.), and (3,5-dimethylisoxazol-4-yl)methanamine (380 mg, 3.0 mmol, 3.0 eq.) in NMP (1.5 mL) was stirred at 80 °C for 6 h. The reaction mixture was cooled to room temperature and diluted with H<sub>2</sub>O (30 mL), then extracted with EtOAc (2  $\times$  50 mL). The combined organic components were washed with aq. LiCl (10% w/v, 4  $\times$  50 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude was first purified using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 0:1). Then, purification by silica gel chromatography, eluting with petroleum ether:EtOAc (1:0 to 0:1), afforded 4-chloro-5-(((3',5'-dimethylisoxazol-4'-yl)methyl)amino)-2-methylpyridazin-3(2H)-one (**42**) (148 mg, 55%) as a colourless amorphous residue: *R*<sub>f</sub> 0.40 (EtOAc);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 3291 (w), 2360 (w), 1607 (s) (C=O), 1446 (m), 997 (m), 872 (m); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.58 (1 H, s), 4.52 (1 H, br s), 4.25 (2 H, d, *J* 5.1), 3.78 (3 H, s), 2.42 (3 H, s), 2.28 (3 H, s); LRMS *m/z* (ES<sup>+</sup>) 269 ([M<sup>35</sup>Cl+H]<sup>+</sup>, 100%), HRMS *m/z* (ES<sup>+</sup>) found 269.0800 (100%), C<sub>11</sub>H<sub>13</sub>ClO<sub>2</sub>N<sub>4</sub> requires [M<sup>35</sup>Cl + H]<sup>+</sup> 269.0800; Analytical HPLC (RP, method A) rt 6.27 mins, purity >99%.

#### 4-Chloro-2-methyl-5-(phenylamino)pyridazin-3(2H)-one (43)



A solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (180 mg, 1.00 mmol, 1.0 eq.), aniline (455  $\mu$ L, 5.00 mmol, 5.00 eq.) in NMP (0.50 mL) was stirred at 120 °C for 16 h. Aniline (455  $\mu$ L, 5.00 mmol, 5.00 eq.) was added and the reaction mixture heated to 120 °C under microwave irradiation for 2 h. Aniline (455  $\mu$ L, 5.00 mmol, 5.00 eq.) was added and the reaction mixture heated to 120 °C under microwave irradiation for 1 h. The reaction mixture was cooled to room temperature and diluted with H<sub>2</sub>O (20 mL) and 1 M HCl (20 mL), then extracted with EtOAc (2  $\times$  60 mL). The combined organic components were washed with 1 M HCl (80 mL) and aq. LiCl (10% w/v, 6  $\times$  80 mL), dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 0:1) afforded 4-chloro-2-methyl-5-(phenylamino)pyridazin-3(2H)-one (143 mg, 60%) as a purple solid. A portion of the product (16 mg) was stirred with decolourising charcoal in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) for 2 h at room temperature, filtered through Celite<sup>®</sup> and concentrated *in vacuo* to give 4-chloro-2-methyl-5-(phenylamino)pyridazin-3(2H)-one (**43**) (14 mg, 93% recovery) as a colourless solid: *R*<sub>f</sub> 0.35 (petroleum ether:EtOAc = 1:1); m.p. 154–156 °C (EtOAc);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 3255 (w), 1632 (w), 1678 (C=O), 1526 (m), 1494 (m), 1345 (w), 875 (m), 749 (m); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.75 (1 H, s), 7.65 (1 H, s), 7.45–7.36 (2 H, m), 7.29–7.24 (2 H, m), 7.24–7.15 (2 H, m); <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  157.5, 142.8, 139.0, 129.8, 128.1, 125.4, 124.0, 109.0, 40.1; LRMS *m/z* (ES<sup>-</sup>) 234 ([M-H]<sup>-</sup>, 100%); HRMS *m/z* (ES<sup>-</sup>) found 234.04393 (100%), C<sub>11</sub>H<sub>10</sub>ON<sub>3</sub>Cl requires [M<sup>35</sup>Cl-H]<sup>-</sup> 234.04396; Analytical HPLC (RP, method A) *rt* 7.67 mins, purity 99%.

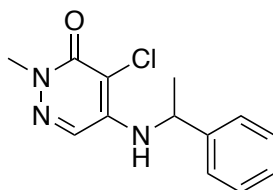
#### 5-(Benzylamino)-4-chloro-2-methylpyridazin-3(2H)-one (44)



A solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (90 mg, 0.50 mmol, 1.0 eq.) and BnNH<sub>2</sub> (110  $\mu$ L, 1.0 mmol, 2.0 eq.) in NMP (1 mL) was stirred at room temperature for 16 h. TLC analysis showed the presence of remaining starting material. Additional BnNH<sub>2</sub> (110  $\mu$ L, 1.0 mmol, 2.0 eq.) was added and the solution was stirred at 80 °C for 3 h. The reaction mixture was cooled to room temperature and diluted with H<sub>2</sub>O (2 mL), then extracted with EtOAc (2  $\times$  60 mL). The combined organic components were washed with aq. LiCl (10% w/v, 4  $\times$  60 mL), then dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 1:4), afforded 5-(benzylamino)-4-chloro-2-methylpyridazin-3(2H)-one (**44**) (98.8 mg, 80%) as a colourless solid: *R*<sub>f</sub> 0.34 (petroleum ether:EtOAc = 1:1); m.p. 164–166 °C (EtOAc);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 3283 (w), 1644 (w), 1604 (s) (C=O), 1519 (m), 1313 (w), 1005 (w), 875 (m), 764 (w), 695 (w); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  7.50 (1 H, s), 7.41–7.36 (2 H, m), 7.36–7.32 (1 H, m), 7.32–7.28 (2 H, m), 5.10 (1 H, s), 4.55 (2 H, d, *J* 6.0), 3.75 (3 H, s); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  157.9, 144.1, 136.9, 129.3, 128.3, 127.0, 125.5, 108.3, 47.3, 40.4; LRMS *m/z* (ES<sup>-</sup>) 248

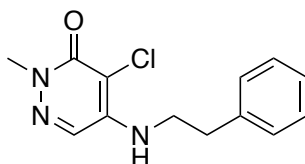
([M-H]<sup>-</sup>, 100%); HRMS *m/z* (ES<sup>+</sup>) found 250.07393 (100%), C<sub>12</sub>H<sub>12</sub>ClN<sub>3</sub>O requires [M<sup>35</sup>Cl+H]<sup>+</sup> 250.07417; Analytical HPLC (RP, method A) rt 7.75 mins, purity >99%.

#### 4-Chloro-2-methyl-5-((1-phenylethyl)amino)pyridazin-3(2H)-one (45)



A solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (180 mg, 1.00 mmol, 1.0 eq.), 1-phenylethylamine (200 μL, 1.50 mmol, 1.5 eq.) and pyridine (160 μL, 2.00 mmol, 2.0 eq.) in NMP (1.5 mL) was stirred at 80 °C for 4 h. 1-Phenylethylamine (200 μL, 1.50 mmol, 1.5 eq.) and pyridine (160 μL, 2.00 mmol, 2.0 eq.) were added and the solution was stirred at 40 °C for 16 h. The reaction mixture was cooled to room temperature and diluted with H<sub>2</sub>O (20 mL), then extracted with EtOAc (3 × 30 mL). The combined organic components were washed with aq. LiCl (10% w/v, 4 × 60 mL), then dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 1:1), afforded 4-chloro-2-methyl-5-((1-phenylethyl)amino)pyridazin-3(2H)-one (**45**) (175 mg, 67%) as a colourless solid: *R<sub>f</sub>* 0.35 (petroleum ether:EtOAc = 1:1); m.p. 106–108 °C (EtOAc);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 3264 (w), 1633 (s) (C=O), 1434 (w), 1347 (w), 849 (m), 705 (m); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.27–7.22 (2 H, m), 7.19 (1 H, s), 7.19–7.14 (3 H, m), 4.84 (1 H, d, *J* 6.2), 4.58 (1 H, dq, *J* 6.2, 6.7), 3.57 (3 H, s), 1.51 (3 H, d, *J* 6.7); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 157.8, 143.4, 142.8, 129.3, 127.9, 126.0, 125.4, 108.2, 53.3, 40.2, 25.0; LRMS *m/z* (ES<sup>+</sup>) 264 ([M+H]<sup>+</sup>, 100%), 286 ([M+Na]<sup>+</sup>, 70%); HRMS *m/z* (ES<sup>+</sup>) found 264.08975 (100%), C<sub>13</sub>H<sub>15</sub>ClN<sub>3</sub>O requires [M<sup>35</sup>Cl+H]<sup>+</sup> 264.08982; Analytical HPLC (RP, method A) rt 8.33 mins, purity >99%.

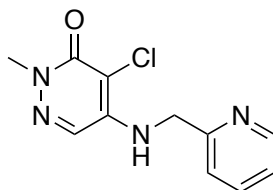
#### 4-Chloro-2-methyl-5-(phenethylamino)pyridazin-3(2H)-one (46)



A solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (0.18 g, 1.0 mmol, 1.0 eq.) and 2-phenylethan-1-amine (0.40 mL, 2.0 mmol, 2.0 eq.) in NMP (1.5 mL) was stirred at 80 °C for 6 h. The reaction mixture was cooled to room temperature and diluted with H<sub>2</sub>O (25 mL), then extracted with EtOAc (2 × 40 mL). The combined organic components were washed with aq. LiCl (10% w/v, 4 × 30 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 0:1), afforded 4-chloro-2-methyl-5-(phenethylamino)pyridazin-3(2H)-one (**46**) (224 mg, 84%) as a colourless solid: *R<sub>f</sub>* 0.46 (EtOAc); m.p. 140–142 °C (CH<sub>2</sub>Cl<sub>2</sub>);  $\nu_{\text{max}}$  (neat)/cm<sup>-1</sup> 3301 (w), 2360 (w), 1601 (s) (C=O), 1521 (m), 1449 (m), 1321 (w), 873 (m), 737 (m), 694 (m); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.50 (1 H, s), 7.37–7.31 (2 H, m), 7.31–7.26 (1 H, m), 7.22–7.17 (2 H, m), 4.69 (1 H, s), 3.75 (3 H, s), 3.58 (3 H, td, *J* 6.9, 6.0), 2.94 (2 H, t, *J* 6.9). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 157.8, 144.0, 137.45, 129.1, 128.8, 127.2, 125.2, 107.9, 44.5, 40.3, 36.3; LRMS *m/z* (ES<sup>+</sup>) 286 ([M<sup>35</sup>Cl+Na]<sup>+</sup>,

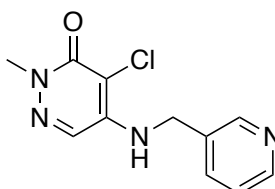
100%), 288 ( $[M^{37}\text{Cl}+\text{Na}]^+$ , 30%); HRMS  $m/z$  ( $\text{ES}^+$ ) found 264.08972 (100%),  $\text{C}_{13}\text{H}_{14}\text{ClN}_3\text{O}$  requires  $[M^{35}\text{Cl}+\text{H}]^+$  264.08982; Analytical HPLC (RP, method A) rt 8.13 mins, purity 99%.

#### 4-Chloro-2-methyl-5-((pyridin-2-ylmethyl)amino)pyridazin-3(2H)-one (47)



A solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (180 mg, 1.00 mmol, 1.0 eq.) and 2-(aminomethyl)pyridine (515  $\mu\text{L}$ , 5.00 mmol, 5.0 eq.) in NMP (1.5 mL) was stirred at 80 °C for 7 h. The reaction mixture was cooled to room temperature and diluted with  $\text{H}_2\text{O}$  (20 mL), then extracted with EtOAc ( $2 \times 30$  mL). The combined organic components were washed with aq. LiCl (10% w/v,  $4 \times 40$  mL), then dried over anhydrous  $\text{MgSO}_4$ , filtered, and concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 0:1), afforded 4-chloro-2-methyl-5-((pyridin-2-ylmethyl)amino)pyridazin-3(2H)-one (**47**) (116 mg, 46%) as a colourless solid:  $R_f$  0.22 (EtOAc); m.p. 130–138 °C (EtOAc);  $\tilde{\nu}_{\text{max}}$  (neat)/ $\text{cm}^{-1}$  3328 (w), 2060 (w), 2160 (w), 1615 (C=O), 1521 (m), 1442 (m), 996 (m), 872 (m), 837 (w), 776 (m);  $^1\text{H}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.63 (1 H, ddd,  $J$  5.0, 1.8, 1.0), 7.73 (1 H, ddd,  $J$  7.7, 7.7, 1.8), 7.59 (1 H, s), 7.32–7.26 (2 H, m), 6.13 (1 H, br t,  $J$  5.3), 4.65 (2 H, d,  $J$  5.3), 3.77 (3 H, s);  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  157.9, 155.2, 149.5, 143.8, 137.1, 125.7, 122.9, 121.4, 108.2, 47.5, 40.3; LRMS  $m/z$  ( $\text{ES}^+$ ) 273 ( $[M+\text{Na}]^+$ , 100%); HRMS  $m/z$  ( $\text{ES}^+$ ) found 273.05127 (100%),  $\text{C}_{11}\text{H}_{11}\text{ON}_4\text{Cl}$  requires  $[M^{35}\text{Cl} + \text{Na}]^+$  273.05136; Analytical HPLC (RP, method A) rt 5.97 mins, purity 99%.

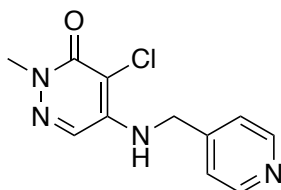
#### 4-Chloro-2-methyl-5-((pyridin-3-ylmethyl)amino)pyridazin-3(2H)-one (48)



A solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (180 mg, 1.00 mmol, 1.0 eq.) and 3-(aminomethyl)pyridine (509  $\mu\text{L}$ , 5.00 mmol, 5.0 eq.) in NMP (1.5 mL) was stirred at 80 °C for 7 h. The reaction mixture was cooled to room temperature and diluted with  $\text{H}_2\text{O}$  (20 mL), then extracted with EtOAc ( $2 \times 35$  mL). The combined organic components were washed with aq. LiCl (10% w/v,  $4 \times 40$  mL), then dried over anhydrous  $\text{MgSO}_4$ , filtered, and concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with EtOAc:EtOH (1:0 to 10:2), afforded 4-chloro-2-methyl-5-((pyridin-3-ylmethyl)amino)pyridazin-3(2H)-one (**48**) (58 mg, 23%) as a colourless solid:  $R_f$  0.28 (EtOAc); m.p. 86–88 °C (EtOH);  $\tilde{\nu}_{\text{max}}$  (neat)/ $\text{cm}^{-1}$  3292 (CH), 2980 (w), 2160 (w), 1592 (C=O), 1542 (m), 1479 (m), 1424 (m), 933 (m), 897 (m), 712 (m);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.59 (1 H, dd,  $J$  2.3, 1.6), 8.57 (1 H, dd,  $J$  4.8, 1.6), 7.65 (1 H, dddd,  $J$  7.9, 2.3, 1.6, 0.8), 7.51 (1 H, s), 7.31 (1 H, ddd,  $J$  7.9, 4.8, 0.8), 6.20 (1 H, br s), 4.98 (2 H, d,  $J$  6.8), 3.76 (3 H, s);  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  156.3, 149.2, 148.9, 139.1, 134.8, 134.3, 123.7, 107.7, 45.3, 40.1;

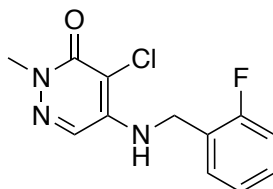
LRMS  $m/z$  ( $ES^-$ ) 249 ( $[M-H]^-$ , 100%); HRMS  $m/z$  ( $ESI^+$ ) found 251.0696,  $C_{11}H_{11}N_4OCl$  requires  $[M^{35}Cl+H]^+$  251.0694; Analytical HPLC (RP, method A) rt 6.97 mins, purity 99%.

#### 4-Chloro-2-methyl-5-((pyridin-4-ylmethyl)amino)pyridazin-3(2H)-one (49)



A solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (180 mg, 1.00 mmol, 1.0 eq.), 4-(aminomethyl)pyridine (508  $\mu$ L, 5.00 mmol, 5.0 eq.) in NMP (1.5 mL) were stirred at 80 °C for 7 h. The reaction mixture was cooled to room temperature and diluted with  $H_2O$  (20 mL), then extracted with EtOAc (2  $\times$  35 mL). The combined organic components were washed with aq. LiCl (10% w/v, 4  $\times$  40 mL), then dried over anhydrous  $MgSO_4$ , filtered, and concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with EtOAc:EtOH (1:0 to 10:3), afforded 4-chloro-2-methyl-5-((pyridin-4-ylmethyl)amino)pyridazin-3(2H)-one (**49**) (33 mg, 13%) as a colourless solid:  $R_f$  0.10 (EtOAc); m.p. 202–204 °C (EtOAc);  $\tilde{\nu}_{max}$  (neat)/ $cm^{-1}$  3216 (w), 1598 (C=O), 1519 (m), 1402 (m), 1231 (w), 972 (m), 874 (m), 720 (m);  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  8.61–8.50 (2 H, m), 7.48 (1 H, s), 7.22–7.14 (2 H, m), 6.30 (1 H, br t,  $J$  7.3), 4.94 (2 H, d,  $J$  7.3), 3.75 (3 H, s);  $^{13}C$  NMR (126 MHz,  $CDCl_3$ )  $\delta$  156.3, 150.3, 148.3, 139.20, 139.19, 121.6, 107.9, 46.5, 40.3; LRMS  $m/z$  ( $ES^+$ ) 251 ( $[M+H]^+$ , 100%); HRMS  $m/z$  ( $ES^+$ ) found 251.0696 (100%),  $C_{11}H_{11}ON_4Cl$  requires  $[M^{35}Cl+H]^+$  251.0694; Analytical HPLC (RP, method B) rt 5.56 mins, purity 95%.

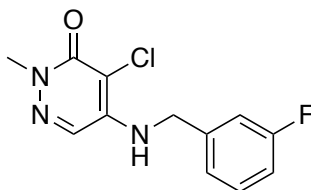
#### 4-Chloro-5-((2-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (50)



A solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (90 mg, 0.50 mmol, 1.0 eq.), 2-(fluorophenyl)methanamine (85  $\mu$ L, 0.75 mmol, 1.5 eq.) and DIPEA (0.25 mL, 1.5 mmol, 3.0 eq.) in NMP (0.75 mL) was stirred at 80 °C for 18 h. The reaction mixture was cooled to room temperature and diluted with  $H_2O$  (20 mL), then extracted with EtOAc (2  $\times$  35 mL). The combined organic components were washed with aq. LiCl (10% w/v, 5  $\times$  30 mL), dried over anhydrous  $MgSO_4$ , filtered, and concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 0:1) afforded 4-chloro-5-((2-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (**50**) (101 mg, 75%) as an off-white solid:  $R_f$  0.21 (petroleum ether:EtOAc = 1:1); m.p. 182–184 °C (EtOAc);  $\tilde{\nu}_{max}$  (neat)/ $cm^{-1}$  3282 (CH), 1632 (w), 1606 (C=O), 1455 (w), 1223 (w), 872, 759, 742;  $^1H$  NMR (500 MHz, acetone- $D_6$ )  $\delta$  7.70 (1 H, s), 7.46–7.38 (1 H, m), 7.38–7.32 (1 H, m), 7.23–7.12 (2 H, m), 6.45 (1 H, br s), 4.77 (2 H, d,  $J$  6.3), 3.60 (3 H, s);  $^{13}C$  NMR (126 MHz, acetone- $D_6$ )  $\delta$  161.3 (d,  $J_{CF}$  239), 157.7, 145.3, 130.34 (d,  $J_{CF}$  8.2), 129.8 (d,  $J_{CF}$  4.4), 126.5 (d,  $J_{CF}$  14.7), 126.3, 125.5, 116.3 (d,  $J_{CF}$  21.4), 107.4, 40.9 (d,  $J_{CF}$  4.8), 39.9;  $^{19}F$  NMR (377 MHz, acetone- $D_6$ )  $\delta$  -120.0 – -120.1 (1 F, m); LRMS  $m/z$  ( $ES^-$ ) 266 ( $[M-H]^-$ , 100%); HRMS  $m/z$  ( $ES^+$ )

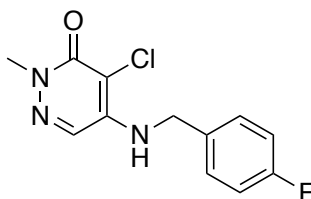
found 268.06469 (100%),  $C_{12}H_{11}ClFN_3O$  requires  $[M^{35}Cl+H]^+$  268.06474; Analytical HPLC (RP, method A) rt 7.89 mins, purity >99%.

#### 4-Chloro-5-((3-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (51)



A solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (90 mg, 0.50 mmol, 1.0 eq.), 3-(fluorophenyl)methanamine (85  $\mu$ L, 0.75 mmol, 1.5 eq.) and DIPEA (0.25 mL, 1.5 mmol, 3.0 eq.) in NMP (0.75 mL) was stirred at 80 °C for 18 h. The reaction mixture was cooled to room temperature and diluted with  $H_2O$  (20 mL), then extracted with EtOAc (2  $\times$  35 mL). The combined organic components were washed with aq. LiCl (10% w/v, 5  $\times$  30 mL), dried over anhydrous  $MgSO_4$ , filtered, and concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 0:1) afforded 4-chloro-5-((3-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (**51**) (98 mg, 70%) as a colourless solid:  $R_f$  0.15 (petroleum ether:EtOAc = 1:1); m.p. 130–132 °C (EtOAc);  $\tilde{\nu}_{max}$  (neat)/ $cm^{-1}$  3299 (CH), 2979 (w), 1631 (w), 1606 (s) (C=O), 1449 (w), 1249 (w), 872 (m), 768 (m), 768 (m);  $^1H$  NMR (500 MHz, acetone- $D_6$ )  $\delta$  7.63 (1 H, s), 7.41 (1 H, td,  $J$  8.0, 6.0), 7.27–7.24 (1 H, m), 7.17 (1 H, ddd,  $J$  9.9, 2.1, 2.1), 7.08–7.01 (1 H, m), 6.62 (1 H, br s), 4.75 (2 H, d,  $J$  6.3), 3.59 (3 H, s);  $^{13}C$  NMR (126 MHz, acetone- $D_6$ )  $\delta$  164.4–163.2 (m), 156.8, 144.5, 141.98 (d,  $J_{CF}$  6.4), 130.61 (d,  $J_{CF}$  8.2), 125.5, 122.63 (d,  $J_{CF}$  2.8), 114.02 (d,  $J_{CF}$  21.4), 113.55 (d,  $J_{CF}$  22.2), 106.6, 45.4, 39.0;  $^{19}F$  NMR (377 MHz, acetone- $D_6$ )  $\delta$  –114.4 – –114.6 (1 F, m); LRMS  $m/z$  ( $ES^-$ ) 266 ( $[M-H]^-$ , 100%); HRMS  $m/z$  ( $ES^+$ ) found 268.06462 (100%),  $C_{12}H_{11}ClFN_3O$  requires  $[M^{35}Cl+H]^+$  268.06474; Analytical HPLC (RP, method A, 300 nm) rt 7.91 mins, purity >99%.

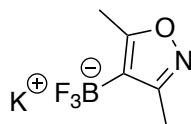
#### 4-Chloro-5-((4-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (52)



A solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (180 mg, 1.00 mmol, 1.0 eq.), 4-(fluorobenzyl)methanamine (172  $\mu$ L, 1.50 mmol, 1.5 eq.) and pyridine (160  $\mu$ L, 2.00 mmol, 2.0 eq.) in NMP (1.5 mL) was stirred at 80 °C for 18 h. 4-Fluorobenzylamine (172  $\mu$ L, 1.50 mmol, 1.5 eq.) and pyridine (160  $\mu$ L, 2.00 mmol, 2.0 eq.) were added and the solution was stirred at 80 °C for 16 h. The reaction mixture was cooled to room temperature and diluted with  $H_2O$  (25 mL), then extracted with EtOAc (3  $\times$  25 mL). The combined organic components were washed with aq. LiCl (10% w/v, 4  $\times$  40 mL), then dried over anhydrous  $MgSO_4$ , filtered, and concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 10:8), afforded 4-chloro-5-((4-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (**48**) (165 mg, 62%) as a colourless solid:  $R_f$  0.28 (petroleum ether:EtOAc = 1:1); m.p. 200–202 °C (EtOAc);  $\tilde{\nu}_{max}$  (neat)/ $cm^{-1}$  3282 (w), 1605 (s) (C=O), 1505 (m), 1159 (m),

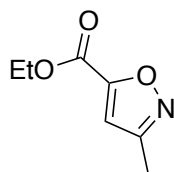
876 (m), 815 (m), 656 (m);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.47 (1 H, s), 7.31–7.26 (2 H, m), 7.12–7.05 (2 H, m), 5.06 (1 H, br t,  $J$  5.2), 4.52 (2 H, dd,  $J$  6.0, 0.9), 3.75 (3 H, s);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ ) 162.5 (d,  $J_{\text{CF}}$  247), 157.7, 143.8, 132.51 (d,  $J_{\text{CF}}$  3.3), 128.60 (d,  $J_{\text{CF}}$  8.4), 125.2, 116.2 (d,  $J_{\text{CF}}$  21.5), 108.4, 46.5, 40.3;  $^{19}\text{F}$  NMR (377 MHz,  $\text{CDCl}_3$ )  $\delta$  –113.6 – –113.8 (1 F, m); LRMS  $m/z$  ( $\text{ES}^-$ ) 266 ( $[\text{M}-\text{H}]^-$ , 100%); HRMS  $m/z$  ( $\text{ES}^+$ ) found 268.06476 (100%),  $\text{C}_{12}\text{H}_{11}\text{ClFN}_3\text{O}$  requires  $[\text{M}^{35}\text{Cl}+\text{H}]^+$  268.06474; Analytical HPLC (RP, method A) rt 7.95 mins, purity 99%.

### Potassium (3,5-dimethylisoxazol-4-yl)trifluoroborate (**88**)



$\text{KHF}_2$  (4.2 g, 53 mmol, 3.0 eq.) was added to a suspension of (3,5-dimethylisoxazol-4-yl)boronic acid (2.5 g, 18 mmol, 1.0 eq.) in MeOH (5 mL) at  $0^\circ\text{C}$ .  $\text{H}_2\text{O}$  (12 mL) was then added dropwise. The reaction mixture was then warmed to room temperature and stirred for 10 mins. The solvents were removed *in vacuo*. The crude solid was purified by Soxhlet extraction for 18 h with acetone (200 mL). The extraction solvent was concentrated *in vacuo*, and the residue was dissolved in a minimal amount of boiling acetone (250 mL). Precipitation was achieved by addition of cold  $\text{Et}_2\text{O}$  (200 mL). The colourless solid was collected by filtration. The precipitation protocol was repeated for the filtrate concentrated *in vacuo* (using 100 mL of cold  $\text{Et}_2\text{O}$ ). The combined solids were dried under vacuum to give the potassium (3,5-dimethylisoxazol-4-yl)trifluoroborate (**88**) (2.9 g, 81%) as a powdery colourless solid: m.p.  $342\text{--}345^\circ\text{C}$  ( $\text{Et}_2\text{O}$ ) [lit.<sup>33</sup>  $>200^\circ\text{C}$ ];  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  2.05 (3 H, s.), 2.15 (3 H, s);  $^{11}\text{B}$  NMR (160 MHz,  $\text{DMSO}-d_6$ )  $\delta$  2.32 (1 B, q,  $J_{\text{BF}}$  48.1);  $^{19}\text{F}$  NMR (470 MHz,  $\text{DMSO}-d_6$ )  $\delta$  –134.51 (3 F, 1:1:1:1 q,  $J_{\text{BF}}$  47.0); LRMS:  $m/z$  ( $\text{ES}^+$ ) 204 ( $[\text{M}+\text{H}]^+$ , 100%). The data are in good agreement with the literature.<sup>33</sup>

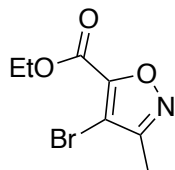
### Ethyl 3-methylisoxazole-5-carboxylate (**34**)



To a solution of acetaldoxime (1.0 mL, 17 mmol, 1.0 eq.) in DMF (18 mL) at  $0^\circ\text{C}$  was added *N*-chlorosuccinimide (2.94 g, 22.0 mmol, 1.3 eq.). The solution was warmed to room temperature and stirred for 3 h, then diluted with brine (120 mL) and extracted with  $\text{Et}_2\text{O}$  ( $5 \times 100$  mL). The combined organic layers were washed with  $\text{H}_2\text{O}$  (200 mL) and brine (200 mL), dried ( $\text{MgSO}_4$ ), filtered, and concentrated *in vacuo*. The residues were dissolved in  $\text{CH}_2\text{Cl}_2$  (10 mL) and added to a suspension of ethyl propiolate (1.9 mL, 19 mmol, 1.1 eq.),  $\text{KHCO}_3$  (4.40 g, 43.9 mmol, 2.6 eq.) and  $\text{H}_2\text{O}$  (170  $\mu\text{L}$ ) in  $\text{CH}_2\text{Cl}_2$  (70 mL). After 75 min,  $\text{H}_2\text{O}$  (13 mL) was added and the mixture stirred vigorously to dissolve the  $\text{KHCO}_3$ . The phases were separated, and the organic components was washed with  $\text{H}_2\text{O}$  ( $3 \times 50$  mL) and brine (50 mL), dried ( $\text{MgSO}_4$ ), filtered, and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 5 to 40%  $\text{Et}_2\text{O}$ /petroleum ether) gave **34** as a pale yellow oil which crystallized slowly at  $4^\circ\text{C}$  (1.37 g, 52%):  $R_f$  0.14 (10%  $\text{Et}_2\text{O}$ /40–60  $^\circ\text{C}$  petroleum ether); m.p.  $26\text{--}27^\circ\text{C}$  [lit.<sup>34</sup>  $27\text{--}28^\circ\text{C}$ ];  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.40 (3H, t,

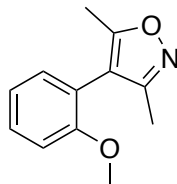
*J* 7.1), 2.37 (3H, s), 4.42 (2H, q, *J* 7.1), 6.78 (1H, s); LRMS *m/z* (ES<sup>+</sup>) 156 ([M+H]<sup>+</sup>, 45), 178 ([M+Na]<sup>+</sup>, 100). These data are in good agreement with the literature values.<sup>34</sup>

#### Ethyl 4-bromo-3-methylisoxazole-5-carboxylate (**35**)



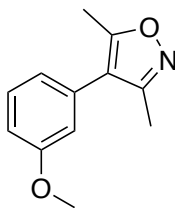
Following the procedure of Li *et al.*,<sup>35</sup> **34** (2.08 g, 13.4 mmol, 1.0 eq), NBS (2.87 mg, 16.1 mmol, 1.2 eq) and TFA (60 mL) were heated with microwave irradiation in a sealed vial at 150 °C for 50 min. The acidic solvent was removed by azeotropic distillation with toluene, and the residue was filtered through Celite® (eluent Et<sub>2</sub>O). Purification by silica gel column chromatography (gradient elution, 1 to 10% Et<sub>2</sub>O/petroleum ether) gave **35** as a pale yellow oil which solidified at 4 °C (2.67 g, 85%); *R<sub>f</sub>* 0.20 (5% Et<sub>2</sub>O/40-60 °C petroleum ether);  $\tilde{\nu}_{\text{max}}$  (solid)/cm<sup>-1</sup>: 2986, 2939 (C-H) (w), 1737 (C=O) (s), 1278 (s), 1078 (s), 1022 (m); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  1.44 (3H, t, *J* 7.1), 2.37 (3H, s), 4.46 (2H, q, *J* 7.1); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  10.7, 14.1, 62.4, 101.3, 155.8, 156.2, 161.2; HRMS *m/z* (ES<sup>+</sup>) found [M+Na]<sup>+</sup> 255.9587, 257.9568; C<sub>7</sub>H<sub>8</sub><sup>79</sup>BrNNaO<sub>3</sub> requires M<sup>+</sup> 255.9580, C<sub>7</sub>H<sub>8</sub><sup>81</sup>BrNNaO<sub>3</sub> requires M<sup>+</sup> 257.9560; LRMS *m/z* (ES<sup>+</sup>) 234 ([<sup>79</sup>M+H]<sup>+</sup>, 19), 236 ([<sup>81</sup>M+H]<sup>+</sup>, 19), 256 ([<sup>79</sup>M+Na]<sup>+</sup>, 94), 258 ([<sup>81</sup>M+Na]<sup>+</sup>, 100), 288 ([<sup>79</sup>M+Na+MeOH]<sup>+</sup>, 57), 290 ([<sup>81</sup>M+Na+MeOH]<sup>+</sup>, 55). Anal. Calcd for C<sub>7</sub>H<sub>8</sub>BrNO<sub>3</sub>: C, 35.9; H, 3.5; N, 6.0. Found: C, 35.8; H, 3.3; N, 5.8.

#### 4-(2-Methoxyphenyl)-3,5-dimethylisoxazole (**93**)



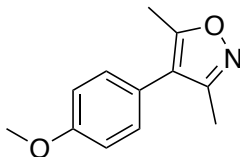
Potassium (3,5-dimethylisoxazol-4-yl)trifluoroborate (**88**) (179 mg, 0.882 mmol, 1.10 eq.), Na<sub>2</sub>CO<sub>3</sub> (255 mg, 2.41 mmol, 3.00 eq.), RuPhos (75 mg, 0.16 mmol, 0.20 eq.), and Pd(OAc)<sub>2</sub> (18 mg, 0.080 mmol, 0.10 eq.) were placed in a microwave vial. The vial was evacuated under vacuum for 5 mins then refilled with argon. 2-Bromoanisole (**90**) (100  $\mu$ L, 0.802 mmol, 1.00 eq.) in degassed EtOH (4 mL) was added, the vial was sealed and stirred at 80 °C for 16 h. RuPhos (75 mg, 0.16 mmol, 0.20 eq.), and Pd(OAc)<sub>2</sub> (18 mg, 0.080 mmol, 0.10 eq.) were added, and the reaction mixture was stirred at 80 °C for 3 h. The mixture was filtered over a plug of silica, washing with EtOAc (4  $\times$  40 mL) then concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 1:1), afforded 4-(2-methoxyphenyl)-3,5-dimethylisoxazole (**93**) (68 mg, 42%) as a colourless oil: *R<sub>f</sub>* 0.52 (petroleum ether:EtOAc = 5:1); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.36 (1 H, ddd, *J* 8.3, 7.5, 1.8), 7.12 (1 H, dd, *J* 7.6, 1.8), 7.01 (1 H, ddd, *J* 7.6, 7.5, 1.1), 6.98 (1 H, dd, *J* 8.3, 1.1), 3.80 (3 H, s), 2.30 (3 H, s), 2.17 (3 H, s); LRMS *m/z* (ES<sup>+</sup>) 203 ([M+H]<sup>+</sup>, 100%); Analytical HPLC (RP, method A) *rt* 14.4 mins, purity >99%. The data are in good agreement with the literature.<sup>36</sup>

#### 4-(3-Methoxyphenyl)-3,5-dimethylisoxazole (29)



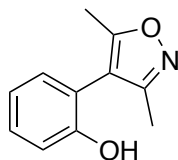
Potassium (3,5-dimethylisoxazol-4-yl)trifluoroborate (**88**) (179 mg, 0.882 mmol, 1.10 eq.), Na<sub>2</sub>CO<sub>3</sub> (255 mg, 2.406 mmol, 3.00 eq.), RuPhos (75 mg, 0.16 mmol, 0.20 eq.), and Pd(OAc)<sub>2</sub> (18 mg, 0.080 mmol, 0.10 eq.) were placed in a microwave vial. The vial was evacuated under vacuum for 5 mins then refilled with argon. 3-Bromoanisole (**91**) (101  $\mu$ L, 0.802 mmol, 1.00 eq.) and EtOH (4.0 mL) were added, the vial was sealed and stirred at 80 °C for 16 h. RuPhos (75 mg, 0.16 mmol, 0.20 eq.), and Pd(OAc)<sub>2</sub> (18 mg, 0.080 mmol, 0.10 eq.) were added, and the reaction mixture was stirred at 80 °C for 2 h. The mixture was filtered over a plug of silica, washing with EtOAc (4  $\times$  40 mL) then concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 1:1), afforded 4-(3-methoxyphenyl)-3,5-dimethylisoxazole (**29**) (145 mg, 89%) as a light yellow oil: *R*<sub>f</sub> 0.43 (petroleum ether:EtOAc = 5:1); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.35 (1 H, ddd, *J* 8.2, 7.6, 0.4), 6.90 (1 H, ddd, *J* 8.2, 2.6, 1.0), 6.84 (1 H, ddd, *J* 7.6, 1.6, 1.0), 6.78 (1 H, ddd, *J* 2.6, 1.6, 0.4), 3.84 (3 H, s), 2.41 (3 H, s), 2.28 (3 H, s); LRMS *m/z* (ES<sup>+</sup>) 204 ([M+Na]<sup>+</sup>, 100%); Analytical HPLC (RP, method A) *rt* 14.4 mins, purity >99%. The data are in good agreement with the literature.<sup>37</sup>

#### 4-(4-Methoxyphenyl)-3,5-dimethylisoxazole (94)



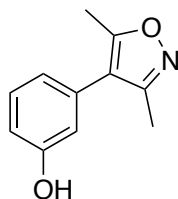
Potassium (3,5-dimethylisoxazol-4-yl)trifluoroborate (**88**) (180 mg, 0.88 mmol, 1.1 eq.), Na<sub>2</sub>CO<sub>3</sub> (255 mg, 2.4 mmol, 3.0 eq.), RuPhos (75 mg, 0.16 mmol, 0.20 eq.), and Pd(OAc)<sub>2</sub> (18 mg, 0.080 mmol, 0.10 eq.) were placed in a microwave vial. The vial was evacuated *in vacuo* for 5 mins then refilled with argon. 4-Bromoanisole (**92**) (100  $\mu$ L, 0.80 mmol, 1.0 eq.) and EtOH (4 mL) were added, the vial was sealed and stirred at 80 °C for 16 h. The mixture was filtered over a plug of silica, washing with EtOAc (5  $\times$  20 mL) then concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 1:1), afforded 4-(4-methoxyphenyl)-3,5-dimethylisoxazole (**94**) (120 mg, 72%) as a colourless solid: *R*<sub>f</sub> 0.70 (petroleum ether:EtOAc = 5:2); m.p. 63–65 °C (CH<sub>2</sub>Cl<sub>2</sub>) [lit.<sup>38</sup> 66–66 °C (EtOAc)]; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.21–7.15 (2 H, m), 7.01–6.89 (2 H, m), 3.85 (3 H, s), 2.38 (3 H, s), 2.25 (3 H, s); LRMS *m/z* (ES<sup>+</sup>) 204 ([M+H]<sup>+</sup>, 40%); Analytical HPLC (RP, method A) *rt* 2.96 mins, purity >99%. The data are in good agreement with the literature.<sup>38,39</sup>

## 2-(3,5-Dimethylisoxazol-4-yl)phenol (**26**)



BBr<sub>3</sub> (1 M in CH<sub>2</sub>Cl<sub>2</sub>, 0.75 mL, 0.75 mmol, 6.0 eq.) was added dropwise, over 15 min under vigorous stirring, to a solution of 4-(2-methoxyphenyl)-3,5-dimethylisoxazole (**93**) (25 mg, 0.12 mmol, 1.0 eq.) in dry CH<sub>2</sub>Cl<sub>2</sub> (1 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for an additional 18 h. The reaction mixture was poured into a cooled sat. aq. NaHCO<sub>3</sub> solution (20 mL) then extracted with EtOAc (3 × 20 mL). The combined organic components were washed with sat. aq. NaHCO<sub>3</sub> (50 mL), then dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. The residue was dissolved in MeOH and concentrated *in vacuo*, three successive times. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 1:4), afforded 2-(3,5-dimethylisoxazol-4-yl)phenol (**26**) (18 mg, 80%) as an off-white solid: *R*<sub>f</sub> 0.20 (petroleum ether:EtOAc = 5:1); m.p. 120–122 °C (EtOAc) [lit.<sup>40</sup> 61–62 °C (petroleum ether)]; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.30 (1 H, ddd, *J* 8.2, 7.3, 1.8), 7.07 (1 H, dd, *J* 7.6, 1.7), 7.02–6.94 (2 H, m), 5.71 (1 H, s), 2.33 (3 H, s), 2.17 (3 H, s); LRMS *m/z* (ES<sup>+</sup>) 190 ([M+H]<sup>+</sup>, 100%). The <sup>1</sup>H NMR data are in good agreement with the literature.<sup>40</sup>

## 4-(3-Hydroxyphenyl)-3,5-dimethylisoxazole (**27**)

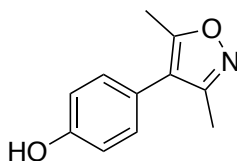


**Method 1:** BBr<sub>3</sub> (1 M in CH<sub>2</sub>Cl<sub>2</sub>, 750 μL, 0.75 mmol, 6.0 eq.) was added dropwise, over 15 min under strong stirring, to 4-(3-Methoxyphenyl)-3,5-dimethylisoxazole (**29**) (25 mg, 0.12 mmol, 1.0 eq.) in dry CH<sub>2</sub>Cl<sub>2</sub> (1 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for an additional 30 mins. The reaction mixture was poured into a cooled sat. aq. NaHCO<sub>3</sub> solution (25 mL) then extracted with EtOAc (2 × 20 mL). The combined organic components were washed with sat. aq. NaHCO<sub>3</sub> (50 mL), then dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. The residue was dissolved in MeOH and concentrated *in vacuo* three successive times, to afford 4-(3-hydroxyphenyl)-3,5-dimethylisoxazole (**27**) (23 mg, 98%) as a colourless solid: *R*<sub>f</sub> 0.73 (petroleum ether:EtOAc = 1:1); m.p. 114–116 °C (MeOH) [lit.<sup>16</sup> 102–106 °C (EtOAc)]; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.3–7.27 (1 H, m), 6.88 (1 H, ddd, *J* 8.2, 2.5, 1.0), 6.82–6.76 (2 H, m), 2.41 (3 H, s), 2.28 (3 H, s); LRMS *m/z* (ES<sup>-</sup>) 188 ([M-Na]<sup>-</sup>, 100%). The data are in good agreement with the literature.<sup>16</sup>

**Method 2:** Potassium (3,5-dimethylisoxazol-4-yl)trifluoroborate (**88**) (129 mg, 0.64 mmol, 1.1 eq.), Na<sub>2</sub>CO<sub>3</sub> (129 mg, 1.2 mmol, 2.1 eq.), RuPhos (33 mg, 0.069 mmol, 0.20 eq.), and Pd(OAc)<sub>2</sub> (7.8 mg, 0.080 mmol, 0.10 eq.) were placed in a microwave vial. The vial was evacuated under vacuum for 5 mins then refilled with argon. 3-Bromophenol (**89**) (100 mg, 0.58 mmol, 1.0 eq.) and EtOH (3 mL) were added, the vial was sealed and stirred at 80 °C for 48 h. The mixture was filtered over a plug of silica, washing with EtOAc (3 × 30 mL) then concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system,

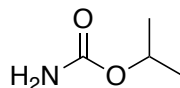
eluting with petroleum ether:EtOAc (1:0 to 1:1), afforded 4-(3-hydroxyphenyl)-3,5-dimethylisoxazole (**27**) (63 mg, 58%) as a colourless solid. The data are identical to those obtained using method 1.

#### 4-(3,5-Dimethylisoxazol-4-yl)phenol (**28**)



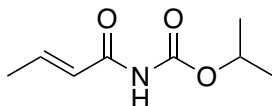
BBr<sub>3</sub> (1 M in CH<sub>2</sub>Cl<sub>2</sub>, 0.75 mL, 0.75 mmol, 6.0 eq.) was added dropwise, over 15 min under vigorous stirring, to a solution of 4-(4-methoxyphenyl)-3,5-dimethylisoxazole (**94**) (25 mg, 0.12 mmol, 1.0 eq.) in dry CH<sub>2</sub>Cl<sub>2</sub> (1 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for an additional 30 mins. The reaction mixture was poured into a cooled sat. aq. NaHCO<sub>3</sub> solution (15 mL) then extracted with EtOAc (2 × 20 mL). The combined organic components were washed with sat. aq. NaHCO<sub>3</sub> (2 × 40 mL), then dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. The residue was dissolved in MeOH and concentrated *in vacuo*, three successive times, to afford 4-(3,5-dimethylisoxazol-4-yl)phenol (**28**) (23 mg, 98%) as a colourless solid: *R*<sub>f</sub> 0.25 (petroleum ether:EtOAc = 5:1); m.p. 148–150 °C (MeOH); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.18–7.07 (2 H, m, C<sup>2</sup>-H), 6.97–6.88 (2 H, m), 5.27 (1 H, s), 2.38 (3 H, s), 2.25 (3 H, s); LRMS *m/z* (ES<sup>-</sup>) 288 ([M-H]<sup>-</sup>, 100%); Analytical HPLC (RP, method B) rt 6.94 mins, purity >99%. The data are in good agreement with the literature.<sup>41</sup>

#### Isopropyl carbamate (**96**)



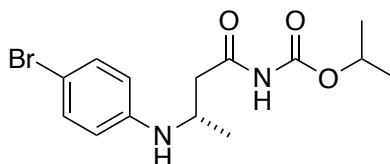
TFA (20 mL, 30 g, 0.3 mol, 2.0 eq.) was added dropwise to a solution of <sup>i</sup>PrOH (10 mL, 7.8 g, 0.13 mol, 1.0 eq.) and NaOCN (12.6 g, 0.26 mol, 1.5 eq.) in toluene (40 mL) at 0 °C. The biphasic reaction mixture was stirred for 4 h. H<sub>2</sub>O (100 mL) was added and the mixture was extracted with EtOAc (2 × 150 mL). The combined organic components were washed with brine (75 mL), dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material (10.7 g) was dissolved in hot EtOAc (15 mL) and hexane (200 mL, added in small portion) over a water bath. When the solids were completely dissolved in the hot solvents the mixture was cooled to room temperature then placed in the freezer overnight. The needle-like crystals were washed with successive addition and removal of hexane by pipetting, then the crystals were collected by filtration to afford isopropyl carbamate (**96**) as colourless crystals (8.25 g, 60%): m.p. 90–92 °C (EtOAc and hexane) [lit.<sup>42</sup> 88–90 °C (Et<sub>2</sub>O)]; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.89 (1 H, hept, *J* 6.3, C<sup>1</sup>-H), 4.67 (s, 2H, NH<sub>2</sub>), 1.24 (6 H, d, *J* 6.3, C<sup>2</sup>-H<sub>3</sub>); LRMS *m/z* (ES<sup>+</sup>) 126 ([M+Na]<sup>+</sup>, 100%). The data are in good agreement with literature.<sup>42</sup>

### Isopropyl (*E*)-but-2-enoylcarbamate (**97**)



In a flame-dried pear-shaped 2-neck flask, fitted with a thermometer, isopropylcarbamate (500 mg, 4.84 mmol, 1.00 eq.) was dissolved in distilled THF (5 mL). Crotonyl chloride (520  $\mu$ L, 570 mg, 5.43 mmol, 1.12 eq.) was added. The reaction mixture was cooled to approximately  $-40\text{ }^{\circ}\text{C}$  then LiO<sup>t</sup>Bu (1 M in THF, 10.9 mL, 10.9 mmol, 2.25 eq.) was added dropwise over an hour. During the addition the reaction mixture was kept below  $-30\text{ }^{\circ}\text{C}$  using an unsaturated dry ice/acetone bath. The reaction mixture was left to warm to  $0\text{ }^{\circ}\text{C}$  and stirred for 30 mins. A mixture of Et<sub>2</sub>O and H<sub>2</sub>O (25 mL and 20 mL, respectively) was added followed by vigorous stirring until the solution reached room temperature. The layers were separated, then the aqueous layer was extracted with Et<sub>2</sub>O (15 mL). The combined organic components were washed with brine (15 mL) and H<sub>2</sub>O (15 mL) then dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with petroleum ether and EtOAc (gradient from 1:0 to 1:1) with 0.5% of Et<sub>3</sub>N, afforded isopropyl (*E*)-but-2-enoylcarbamate (**97**) (322 mg, 39%) as a colourless solid: *R*<sub>f</sub> 0.70 (petroleum ether:EtOAc = 2:1); mp:  $72\text{--}74\text{ }^{\circ}\text{C}$  (EtOAc);  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 3270 (w), 2982 (w), 1752 (s) (C=O), 1649 (m), 1506 (s), 1102 (s), 938 (s); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.27 (1 H, br s), 7.13 (1 H, dq, *J* 15.3, 6.9), 6.87 (1 H, dq, *J* 15.3, 1.7), 4.98 (1 H, hept, *J* 6.2), 1.94 (3 H, dd, *J* 6.9, 1.7), 1.29 (6 H, d, *J* 6.3); <sup>13</sup>C NMR (126 MHz, DMSO-*D*<sub>6</sub>)  $\delta$  166.2, 151.5, 146.4, 123.1, 70.4, 21.9, 18.5. LRMS: *m/z* (ES<sup>+</sup>) 172 ([M+H]<sup>+</sup>, 35%), 194 ([M+Na]<sup>+</sup>, 40%), 365 ([2M+Na]<sup>+</sup>, 100%); HRMS *m/z* (ESI<sup>+</sup>) found 172.0968, C<sub>8</sub>H<sub>14</sub>NO<sub>3</sub> requires [M+H]<sup>+</sup> 172.0968. The <sup>1</sup>H NMR data are in good agreement with literature.<sup>43</sup>

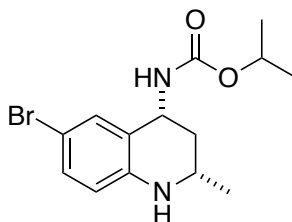
### Isopropyl (*S*)-(3-((4'-bromophenyl)amino)butanoyl)carbamate (**98**)



((*R*)-BINAP)ditriflatebis(acetonitrile)palladium(II) (93 mg, 0.088 mmol, 0.05 eq.) was added to a solution of isopropyl (*E*)-but-2-enoylcarbamate (300 mg, 1.75 mmol, 1.00 eq.) in dry toluene (8.0 mL). A red gummy ball formed, and the colourless solution turned yellow. The mixture was stirred for 30 min prior to the addition of 4-bromoaniline (453 mg, 2.63 mmol, 1.50 eq.). After this, solids dissolved completely, and the mixture was stirred for 20 h. The solvents were removed *in vacuo*. Purification of the yellow oil by silica gel chromatography, eluting with CH<sub>2</sub>Cl<sub>2</sub>, afforded isopropyl (*S*)-(3-((4'-bromophenyl)amino)butanoyl)carbamate (**98**) (530 mg, 88%): *R*<sub>f</sub> 0.59 (CH<sub>2</sub>Cl<sub>2</sub>); [ $\alpha$ ]<sub>D</sub><sup>25</sup>:  $-16.14$  (c 1.0, CHCl<sub>3</sub>); m.p.  $128\text{--}130\text{ }^{\circ}\text{C}$  (EtOAc) [lit.<sup>44</sup>  $131\text{ }^{\circ}\text{C}$  (EtOAc)];  $\tilde{\nu}_{\text{max}}$  (neat)/cm<sup>-1</sup> 3346 (m), 3262 (w), 2980 (s), 1747 (s) (C=O), 1504 (s), 1260 (m), 1103 (m), 825 (m); <sup>1</sup>H NMR (500 MHz, DMSO-*D*<sub>6</sub>)  $\delta$  10.48 (1 H, s), 7.27–7.06 (2 H, m), 6.61–6.34 (2 H, m), 5.67 (1 H, d, *J* 8.7), 4.85 (1 H, hept, *J* 6.3), 3.86–3.65 (1 H, m), 2.68 (1 H, dd, *J* 16.0, 5.6), 2.45 (1 H, dd, *J* 16.0, 5.9), 1.22 (6 H, d, *J* 6.3), 1.12 (3 H, d, *J* 6.4); <sup>13</sup>C NMR (126 MHz, DMSO-*D*<sub>6</sub>)  $\delta$  171.1, 150.9, 146.9, 131.4, 114.3, 106.1, 68.5, 44.5, 42.7, 21.6, 20.1; LRMS: *m/z* (ESI<sup>+</sup>) 345 ([M<sup>81</sup>Br+H]<sup>+</sup>, 100%), 344 ([M<sup>80</sup>Br+H]<sup>+</sup>, 80%). HRMS *m/z* (ESI<sup>+</sup>) found 343.0651, C<sub>14</sub>H<sub>19</sub><sup>79</sup>BrN<sub>2</sub>O<sub>3</sub> requires [M+H]<sup>+</sup> 343.0652; Analytical HPLC (RP, method A) rt 11.96 mins,

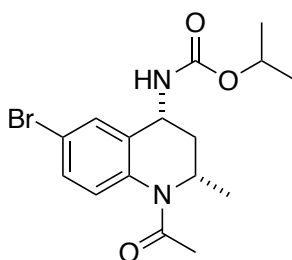
97%. Chiral HPLC (heptane:IPA = 80:20, 0.5 mL/min) rt 4.70 mins, purity >99%. The  $^1\text{H}$  NMR data are in good agreement with literature.<sup>43</sup>

**Isopropyl ((2*S*,4*R*)-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (**99**)**



Isopropyl (S)-(3-((4-bromophenyl)amino)butanoyl)carbamate (**98**) (25.0 mg, 0.07 mmol, 1.00 eq.) was dissolved in EtOH (1 mL, denatured with 0.5 % toluene). The reaction mixture was cooled to  $-10\text{ }^{\circ}\text{C}$ ,  $\text{NaBH}_4$  (3.0 mg, 0.05 mmol, 0.75 eq.) was added and the reaction mixture was stirred for 30 mins.  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  (16.3 mg, 0.08 mmol, 1.10 eq.) in 0.1 mL of  $\text{H}_2\text{O}$  was added dropwise, keeping the temperature below  $-10\text{ }^{\circ}\text{C}$ . The reaction mixture was stirred at  $-10$  to  $-5\text{ }^{\circ}\text{C}$  for 1 h. The reaction mixture was quenched by the dropwise addition of citric acid (69 mg) in 1 M aq. HCl (1.5 mL), then was diluted with  $\text{CH}_2\text{Cl}_2$  (1.5 mL). The biphasic mixture was stirred vigorously for 1 hour. The phases were separated, and the aqueous phase was extracted with  $\text{CH}_2\text{Cl}_2$  ( $2 \times 2\text{ mL}$ ). The organic components were washed with  $\text{H}_2\text{O}$  (5 mL) and brine (5 mL), dried over  $\text{MgSO}_4$ , filtered, and concentrated *in vacuo*. Purification of the oil by silica gel chromatography, eluting with petroleum ether:EtOAc (1:0 to 1:1), afforded isopropyl ((2*S*,4*R*)-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (**99**) as a colourless solid (16 mg, 68%):  $R_f$  0.57 (petroleum ether:EtOAc 1:1);  $[\alpha]_D^{25}$ :  $-14.9$  (c 1.00,  $\text{CHCl}_3$ ); mp:  $148\text{--}150\text{ }^{\circ}\text{C}$  (EtOAc) [lit.<sup>44</sup>  $167\text{ }^{\circ}\text{C}$  (EtOAc)];  $\tilde{\nu}_{\text{max}}$  (neat)/ $\text{cm}^{-1}$  3276 (w), 2979 (m), 1682 (s) (C=O), 1537 (s), 1487 (s), 1250 (m), 1156 (m), 1060 (m), 798 (m);  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO-}d_6$ )  $\delta$  7.39 (1 H, d,  $J$  9.1, 1H), 7.03 (1 H, dd,  $J$  8.6, 2.4), 6.95 (1 H, d,  $J$  2.4), 6.42 (1 H, d,  $J$  8.6), 5.89 (1 H, s), 4.83 (1 H, hept,  $J$  6.3), 4.74 (1 H, ddd,  $J$  12.0, 9.1, 5.5), 3.44 (1 H, dqd,  $J$  12.0, 6.2, 2.2), 1.91 (1 H, ddd,  $J$  12.0, 5.5, 2.2), 1.44 (1 H, ddd,  $J$  12.0, 12.0, 12.0), 1.24 (3 H, d,  $J$  6.3), 1.21 (3 H, d,  $J$  6.3), 1.13 (3 H, d,  $J$  6.2);  $^{13}\text{C}$  NMR (126 MHz,  $\text{DMSO-}d_6$ )  $\delta$  156.30, 144.7, 129.8, 128.5, 124.0, 115.2, 105.8, 66.9, 47.2, 46.0, 36.6, 22.1, 21.7; LRMS  $m/z$  ( $\text{ES}^+$ ) 224 ( $[\text{M}^{79}\text{Br}+3\text{CH}_3\text{CN}+2\text{H}]^{2+}$ , 100%), 226 ( $[\text{M}^{81}\text{Br}+3\text{CH}_3\text{CN}+2\text{H}]^{2+}$ , 90%), 349 ( $[\text{M}^{79}\text{Br}+\text{Na}]^+$ , 45%), 351 ( $[\text{M}^{81}\text{Br}+\text{Na}]^+$ , 41%); Analytical HPLC (RP, method A) rt 12.28 mins, purity 96%. The  $^1\text{H}$  NMR data are in good agreement with literature.<sup>43</sup>

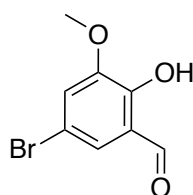
**Isopropyl ((2*S*,4*R*)-1-acetyl-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (**24**)**



In a flame-dried flask under argon, isopropyl ((2*S*,4*R*)-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (**99**) (38.2 mg, 0.12 mmol, 1.00 eq.) was dissolved in dry  $\text{CH}_2\text{Cl}_2$  (1 mL). Pyridine (28  $\mu\text{L}$ , 0.35 mmol, 3.00 eq.) in dry  $\text{CH}_2\text{Cl}_2$  (0.1 mL) then  $\text{AcCl}$  (13  $\mu\text{L}$ , 0.18 mmol, 1.50 eq.) in dry  $\text{CH}_2\text{Cl}_2$  (0.1 mL) were

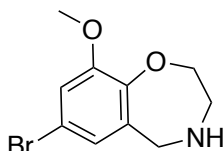
added. The solution was stirred at room temperature for 4 h. H<sub>2</sub>O (5 mL) was added and the mixture was extracted with EtOAc (2 × 8 mL). The organic components were washed with sat. aq NaHCO<sub>3</sub> (5 mL), followed by H<sub>2</sub>O (5 mL), dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification of the crude solid by silica gel chromatography, eluting with petroleum ether and EtOAc (1:0 to 1:1), afforded isopropyl ((2*S*,4*R*)-1-acetyl-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (**24**) as a colourless solid (38 mg, 88%): *R*<sub>f</sub> 0.17 (petroleum ether:EtOAc = 2:1);  $[\alpha]_D^{25}$ : +298.4 (c 1.00, CHCl<sub>3</sub>) [lit.<sup>44</sup>  $[\alpha]_D^{23.1}$ : +251.1 (c 0.61, EtOH), lit.<sup>43</sup>  $[\alpha]_D^{20}$ : +281.1 (c 0.508, EtOH),]; m.p. 141–143 °C (EtOAc) [lit.<sup>44</sup> 163 °C (EtOAc)];  $\tilde{\nu}_{\max}$  (neat)/cm<sup>-1</sup> 3303 (w), 2979 (w), 2361 (m), 1704 (s) (C=O), 1644 (s) (C=O), 1533 (m), 1255 (m), 964 (m); <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.64 (1 H, d, *J* 8.6), 7.47 (1 H, dd, *J* 8.4, 2.2), 7.30 (1 H, d, *J* 8.4), 7.21 (1 H, d, *J* 2.2), 4.83 (1 H, hept, *J* 6.3), 4.63–4.58 (1 H, m), 4.37 (1 H, ddd, *J* 12.2, 8.6, 4.3), 2.44 (1 H, ddd, *J* 12.2, 8.4, 4.3), 2.05 (3 H, s), 1.25 (3 H, d, *J* 6.3), 1.22 (3 H, d, *J* 6.3), 1.21–1.16 (1 H, m), 1.01 (3 H, d, *J* 6.3 Hz); <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  168.4, 155.7, 139.2, 135.5, 129.6, 128.2, 125.5, 117.9, 67.3, 47.0, 46.7, 22.6, 22.0, 21.3; LRMS *m/z* (ES<sup>+</sup>) 369 ([M<sup>79</sup>Br+H]<sup>+</sup>, 90%), 371 ([M<sup>81</sup>Br+H]<sup>+</sup>, 100%), 391 ([M<sup>79</sup>Br+Na]<sup>+</sup>, 40%), 393 ([M<sup>81</sup>Br+Na]<sup>+</sup>, 47%); HRMS *m/z* (ESI<sup>+</sup>) found 391.0624, C<sub>16</sub>H<sub>21</sub>BrN<sub>2</sub>O<sub>3</sub> requires [M<sup>79</sup>Br+Na]<sup>+</sup> 391.0628; Analytical HPLC (RP, method A) *rt* 12.23 mins, purity 99%. The <sup>1</sup>H NMR data are in good agreement with literature.<sup>43</sup>

#### 5-Bromo-2-hydroxy-3-methoxybenzaldehyde (**101**)



2-Hydroxy-3-methoxybenzaldehyde (**100**) (1.00 g, 6.57 mol, 1.00 eq.) and KBr (1.56 g, 13.2 mol, 2.00 eq.) were dissolved in 80% aq. acetic acid (30 mL). Br<sub>2</sub> (0.36 mL, 6.93 mol, 1.05 eq.) in glacial acetic acid (9 mL) was added dropwise with a Pasteur pipette over 2 h. Precipitation was observed at the end of the addition. The reaction mixture was heated at 115 °C for 15 mins, and stirred at room temperature for 16 h. The precipitate was collected by filtration and crystallised from hot aq. EtOH (50% v/v, 100 mL) to afford 5-bromo-2-hydroxy-3-methoxybenzaldehyde (**101**) (55.7 mg, 37%) as a yellow needles: *R*<sub>f</sub> 0.63 (petroleum ether:EtOAc = 5:1); m.p. 120–122 °C (H<sub>2</sub>O:EtOH = 1:1) [lit.<sup>45</sup> 119–121 °C (H<sub>2</sub>O:EtOH = 3:2)]; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  11.03 (1 H, s), 9.88 (1 H, s), 7.34 (1 H, d, *J* 2.2), 7.20 (1 H, d, *J* 2.2), 3.94 (3 H, s); LRMS *m/z* (ES<sup>-</sup>) 229 ([M<sup>81</sup>Br-H]<sup>-</sup>, 80 %), 231 ([M<sup>79</sup>Br-H]<sup>-</sup>, 100 %). The data are in good agreement with literature.<sup>45</sup>

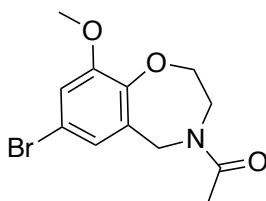
#### 7-Bromo-9-methoxy-2,3,4,5-tetrahydrobenzo[*f*][1,4]oxazepane (**102**)



5-Bromo-2-hydroxy-3-methoxybenzaldehyde (**101**) (360 mg, 1.29 mmol, 1.00 eq.) was dissolved in dry 1,2-dichloroethane (8.0 mL) in a flame dried flask with 3 Å molecular sieves. 2-Chloroethyl amine hydrochloride (180 mg, 1.55 mmol, 1.20 eq.) was added and the reaction mixture was stirred at room temperature for 3 h. Et<sub>3</sub>N (360  $\mu$ L, 2.58 mmol, 2.00 eq.) was added and the reaction mixture was added for 1 h. NaBH(OAc)<sub>3</sub>

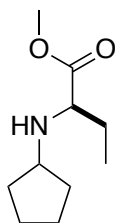
(920 mg, 4.32 mmol, 4.00 eq.) was added, and the mixture was stirred at room temperature for 22 h. The mixture was diluted with aq. sat.  $\text{NaHCO}_3$  (50 mL) and extracted with EtOAc ( $2 \times 100$  mL). The combined organic components were washed with  $\text{H}_2\text{O}$  (50 mL) and sat. aq.  $\text{NaHCO}_3$  (50 mL), dried over anhydrous  $\text{MgSO}_4$ , filtered, and concentrated *in vacuo*, giving 7-bromo-9-methoxy-2,3,4,5-tetrahydrobenzo[f][1,4]oxazepane (**102**) (284 mg, 75%) as a purple solid:  $R_f$  0.48 (petroleum ether:EtOAc = 3:1); m.p. 156–158 °C (EtOAc) [lit.<sup>46</sup> 119–120 °C ( $\text{CH}_2\text{Cl}_2$ )];  $\tilde{\nu}_{\text{max}}$  (neat)/ $\text{cm}^{-1}$  3160 (w), 2933 (w), 2829 (w), 1480 (s), 1245 (m), 1227 (s), 1084 (m);  $^1\text{H}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.00 (1 H, d,  $J$  2.2), 6.94 (1 H, d,  $J$  2.2), 6.19 (1 H, s), 4.43 (2 H, s), 4.37–4.26 (2 H, m), 3.89 (3 H, s), 3.57–3.46 (2 H, m). LRMS  $m/z$  ( $\text{ES}^+$ ) 302 ( $[\text{M}^{79}\text{Br}+2\text{Na}-\text{H}]^+$ , 100%), 304 ( $[\text{M}^{81}\text{Br}+2\text{Na}-\text{H}]^+$ , 90%); Analytical HPLC (RP, method A) rt 9.87 mins, purity 98%. The  $^1\text{H}$  NMR data are in good agreement with the literature.<sup>46</sup>

### 1-(7-Bromo-9-methoxy-2,3-dihydrobenzo[f][1,4]oxazepin-4(5H)-yl)ethan-1-one (**25**)



$\text{Ac}_2\text{O}$  (36  $\mu\text{L}$ , 0.38 mmol, 2.0 eq.) was added to a solution of 7-bromo-9-methoxy-2,3,4,5-tetrahydrobenzo[f][1,4]oxazepane (**102**) (50 mg, 0.19 mmol, 1.0 eq.) and  $\text{K}_2\text{CO}_3$  (105 mg, 0.76 mmol, 4.0 eq.) in  $\text{CH}_2\text{Cl}_2$  (2.0 mL). The reaction mixture was stirred at room temperature for 4.5 h, then diluted with  $\text{H}_2\text{O}$  (10 mL) and extracted with EtOAc ( $2 \times 20$  mL). The combined organic components were washed with sat. aq.  $\text{NaHCO}_3$  (20 mL),  $\text{H}_2\text{O}$  (20 mL), dried over  $\text{MgSO}_4$ , filtered, and concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (95:5 to 3:7), afforded 1-(7-bromo-9-methoxy-2,3-dihydrobenzo[f][1,4]oxazepin-4(5H)-yl)ethan-1-one (**25**) (35 mg, 60%) as a colourless oil:  $R_f$  0.41 (petroleum ether:EtOAc = 2:1);  $\tilde{\nu}_{\text{max}}$  (neat)/ $\text{cm}^{-1}$  2980 (w), 1744 (s) ( $\text{C}=\text{O}$ ), 1482 (m), 1169 (s), 1035 (m), 851 (m), 760 (m);  $^1\text{H}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.07 (1 H, d,  $J$  2.2), 7.04 (1 H, d,  $J$  2.2), 4.34 (2 H, s), 4.30–4.19 (2 H, m), 3.82 (3 H, s), 3.41–3.29 (2 H, m), 2.32 (3 H, s);  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  169.1, 158.1, 152.4, 138.1, 124.6, 119.5, 116.0, 62.0, 56.5, 43.8, 43.6, 20.6; LRMS  $m/z$  ( $\text{ES}^+$ ) 301 ( $[\text{M}^{79}\text{Br}+\text{H}]^+$ , 100%), 303 ( $[\text{M}^{81}\text{Br}+\text{H}]^+$ , 85%); Analytical HPLC (RP, method A) rt 8.33 mins, purity > 99%. The data are in good agreement with the literature.<sup>46</sup>

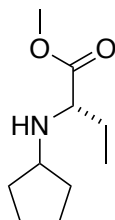
### Methyl (*R*)-2-(cyclopentylamino)butanoate (**62**)



Methyl (*R*)-2-aminobutanoate hydrochloride (1.25 g, 8.14 mol, 1.2 eq.) and  $\text{Ga}(\text{OTf})_2$  (175 mg, 0.339 mol, 0.05 eq.) were dissolved in dry 1,2-DCE (20 mL) in the presence of 3 Å molecular sieves.  $\text{Et}_3\text{N}$  (950  $\mu\text{L}$ , 6.78 mol, 1.0 eq.) and cyclopentanone (600  $\mu\text{L}$ , 6.78 mol, 1.0 eq.) were added and the reaction mixture was

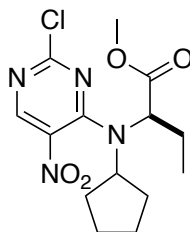
stirred at room temperature for 2 h. NaHB(OAc)<sub>3</sub> (2.87 g, 13.6 mol, 2.0 eq.) was added and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was basified with Et<sub>3</sub>N (3 mL), then filtered through a pad of Celite®, and washed with CH<sub>2</sub>Cl<sub>2</sub> (250 mL, with 1% Et<sub>3</sub>N). Sat. aq. K<sub>2</sub>CO<sub>3</sub> (250 mL) was added to the filtrate and the layers were separated. The aqueous layer was further extracted with CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The organic components were combined, washed with sat. aq. K<sub>2</sub>CO<sub>3</sub> (2 × 150 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. MeOH (200 mL) was added and removed *in vacuo* to remove the excess borane species. The mixture was suspended in CH<sub>3</sub>Cl (50 mL) and the insoluble impurities were removed by filtration to afford methyl (R)-2-(cyclopentylamino)butanoate (**62**) (977 mg, 78%) as light yellow oil: *R*<sub>f</sub> 0.08 (petroleum ether:EtOAc = 20:1) (not UV active, monitored with ninhydrin stain); [ $\alpha$ ]<sub>D</sub><sup>25</sup>: +25.3 (c 1.0, MeOH) [lit.<sup>18</sup> [ $\alpha$ ]<sub>D</sub><sup>21.4</sup>: +11.8 (c 1.0, MeOH)];  $\tilde{\nu}_{\text{max}}$  (thin film)/cm<sup>-1</sup> 2951 (br), 1737 (s, C=O), 1460 (w), 1098 (w), 801; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  3.72 (3 H, s), 3.21 (1 H, dd, *J* 6.6, 6.6), 2.97 (1 H, dddd, *J* 6.7, 6.7, 6.6, 6.6), 2.07 (1 H, br s), 1.94–1.71 (2 H, m), 1.71–1.58 (4 H, m), 1.57–1.39 (2 H, m), 1.36–1.22 (2 H, m), 0.85 (3 H, dd, *J* 7.5, 7.5); LRMS *m/z* (ES<sup>+</sup>) 186 ([M<sup>35</sup>Cl+H]<sup>+</sup>, 100%). The data are in good agreement with the literature.<sup>47</sup>

#### Methyl (S)-2-(cyclopentylamino)butanoate ((S)-62)



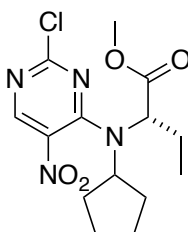
Methyl (S)-2-aminobutanoate hydrochloride (1.25 g, 8.14 mol, 1.2 eq.) and Ga(OTf)<sub>2</sub> (175 mg, 0.339 mol, 0.05 eq.) were dissolved in dry 1,2-DCE (25 mL) in the presence of 3 Å molecular sieves. Et<sub>3</sub>N (950  $\mu$ L, 6.78 mol, 1.0 eq.) and cyclopentanone (600  $\mu$ L, 6.78 mol, 1.0 eq.) were added and the reaction mixture was stirred at room temperature for 2 h. NaHB(OAc)<sub>3</sub> (2.87 g, 13.6 mol, 2.0 eq.) was added and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was basified with Et<sub>3</sub>N (3 mL) then filtered through a pad of Celite®, and washed with CH<sub>2</sub>Cl<sub>2</sub> (200 mL, with 1% Et<sub>3</sub>N) and CH<sub>2</sub>Cl<sub>2</sub> (100 mL). Sat. aq. K<sub>2</sub>CO<sub>3</sub> (200 mL) was added to the filtrate and the layers were separated. The aqueous layer was further extracted with CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The organic components were combined, washed with sat. aq. K<sub>2</sub>CO<sub>3</sub> (2 × 150 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. MeOH (200 mL) was added and removed *in vacuo* to remove the excess borane species. The mixture was suspended in CHCl<sub>3</sub> (50 mL) and the insoluble impurities were removed by filtration, washing with CHCl<sub>3</sub> (3 × 50 mL). The solvents were removed *in vacuo* to afford methyl (S)-2-(cyclopentylamino)butanoate ((S)-62) (1.16 g, 92%) as light yellow oil: *R*<sub>f</sub> 0.08 (petroleum ether:EtOAc = 20:1) (not UV active, monitored with ninhydrin stain); [ $\alpha$ ]<sub>D</sub><sup>25</sup>: -14.2 (c 1.0, MeOH) [lit.<sup>18</sup> [ $\alpha$ ]<sub>D</sub><sup>21.4</sup>: -12.0 (c 1.0, MeOH)]; LRMS *m/z* (ES<sup>+</sup>) 186 ([M<sup>35</sup>Cl+H]<sup>+</sup>, 100%). The spectrometry data are identical to (**R**)-**62**, and are in good agreement with the literature.<sup>18,47</sup>

### Methyl (R)-2-(cyclopentylamino)butanoate (**63**)



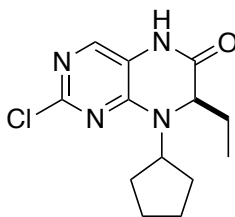
Methyl (R)-2-(cyclopentylamino)butanoate (**62**) (124 mg, 0.667 mmol, 1.0 eq.) was dissolved in cyclohexane (7 mL).  $\text{NaHCO}_3$  (224 mg, 2.67 mmol, 4.0 eq.) was added, then the reaction mixture was stirred at room temperature for 30 mins. 2,4-Dichloro-5-nitropyrimidine (150 mg, 0.773 mmol, 1.1 eq.) was added and the reaction mixture was stirred at 60 °C for 3 days.  $\text{H}_2\text{O}$  (30 mL) was added, and the mixture was extracted with EtOAc (2  $\times$  40 mL). The organic components were combined, washed with  $\text{H}_2\text{O}$  (20 mL), dried over  $\text{MgSO}_4$ , filtered, and concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (1:0 to 9:1, slow gradient; then 9:1 to 5:1, fast gradient), afforded methyl (R)-2-(cyclopentylamino)butanoate (**63**) (117 mg, 51%) as a light orange solid:  $R_f$  0.75 (petroleum ether:EtOAc = 1:1;  $[\alpha]_D^{25}$ : +492 (c 1.0,  $\text{CHCl}_3$ ) [lit.<sup>18</sup>  $[\alpha]_D^{21.4}$ : +228.3 (c 1.0, MeOH)]; m.p. 96–98 °C (EtOAc) [lit.<sup>47</sup> 111 °C];  $\tilde{\nu}_{\text{max}}$  (thin film)/ $\text{cm}^{-1}$  2971 (w), 1740 (m), 1579 (s), 1347 (m), 1201 (m);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) 8.66 (1 H, s), 3.75 (4 H, s), 3.57 (1 H, dd,  $J$  8.2, 8.4), 2.40 (1 H, dqd,  $J$  14.9, 7.5, 6.1), 2.26–2.14 (1 H, m), 2.06 (1 H, ddd,  $J$  15.5, 14.4, 7.7), 1.97–1.85 (1 H, m), 1.88–1.43 (6 H, m), 1.04 (3 H, dd,  $J$  7.5, 7.5); LRMS  $m/z$  ( $\text{ES}^+$ ) 343 ( $[\text{M}^{35}\text{Cl}+\text{H}]^+$ , 100%). The data are in good agreement with the literature.<sup>47</sup>

### Methyl (S)-2-((2-chloro-5-nitropyrimidin-4-yl)(cyclopentyl)amino)butanoate ((S)-**63**)



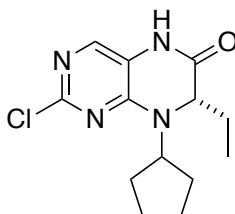
Methyl (S)-2-(cyclopentylamino)butanoate (2.1 g, 10 mmol, 1.0 eq.) was dissolved in cyclohexane (65 mL).  $\text{NaHCO}_3$  (8.2 g, 97 mmol, 9.5 eq.) was added, then the reaction mixture was stirred at room temperature for 30 mins. 2,4-Dichloro-5-nitropyrimidine (2.2 g, 11 mmol, 1.1 eq.) was added and the reaction mixture was stirred at 60 °C for 3 days.  $\text{H}_2\text{O}$  (200 mL) was added, and the mixture was extracted with EtOAc (2  $\times$  200 mL). The organic components were combined, washed with  $\text{H}_2\text{O}$  (20 mL), dried over  $\text{MgSO}_4$ , filtered, and concentrated *in vacuo*. Purification by silica gel chromatography (Combiflash  $Rf$ +, 80 g column), eluting with cyclohexane:EtOAc (1:0 to 5:1), afforded methyl (S)-2-((2-chloro-5-nitropyrimidin-4-yl)(cyclopentyl)amino)butanoate ((S)-**63**) (2.8 g, 74%) as a light orange solid:  $R_f$  0.75 (petroleum ether:EtOAc = 1:1; lit.<sup>18</sup>  $[\alpha]_D^{21.4}$ : –229.9 (c 1.0, MeOH); m.p. 96–98 °C (EtOAc). LCMS (formic acid) rt 1.28 min (94%),  $m/z$  ( $\text{ES}^+$ ) 343 ( $[\text{M}^{35}\text{Cl}+\text{H}]^+$ , 100%). The spectroscopic data are identical to the enantiomer (**R**)-**63** and in good agreement with the literature.<sup>18,47</sup>

**(R)-2-Chloro-8-cyclopentyl-7-ethyl-7,8-dihydropteridin-6(5H)-one (37)**



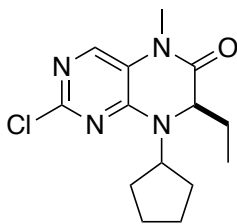
Methyl (*R*)-2-((2-chloro-5-nitropyrimidin-4-yl)(cyclopentyl)amino)butanoate (**63**) (60 mg, 0.18 mmol, 1.0 eq.) was dissolved in glacial AcOH (0.7 mL) and heated to 70 °C. Fe (50 mg, 0.87 mmol, 2.0 eq.) was added in 2 portions, and the solution was stirred at 70 °C for 2 h. The temperature was increased to 100 °C and the reaction mixture was stirred for a further 3 h. The mixture was allowed to cool to room temperature, then was filtered over Celite®, washing with MeOH (4 × 50 mL). The filtrate was concentrated *in vacuo*. Purification using a SNAP 10 g column on the Biotage chromatography system, eluting with petroleum ether:EtOAc (20:1 to 10:7), afforded (*R*)-2-chloro-8-cyclopentyl-7-ethyl-7,8-dihydropteridin-6(5H)-one (**64**) (31 mg, 62%) as a colourless solid: *R*<sub>f</sub> 0.45 (petroleum ether:EtOAc = 1:1); [ $\alpha$ ]<sub>D</sub><sup>25</sup>: –111 (c 1.0, MeOH) [lit.<sup>18</sup> [ $\alpha$ ]<sub>D</sub><sup>21.4</sup>: –96.2 (c 1.0, MeOH)];  $\tilde{\nu}_{\text{max}}$  (thin film)/cm<sup>–1</sup> 2968 (br), 1692 (s, C=O), 1603, 1363, 1193, 767 (w); m.p. 168–170 °C (EtOAc) [lit.<sup>47</sup> 173 °C]; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.42 (1 H, s), 7.61 (1 H, s), 4.33 (1 H, dddd, *J* 8.2, 8.2, 8.2, 8.2), 4.20 (1 H, dd, *J* 7.6, 3.6), 2.12–2.03 (1 H, m), 2.00–1.70 (7 H, m), 1.71–1.60 (2 H, m), 0.93 (3 H, dd, *J* 7.5, 7.5). LRMS *m/z* (ES<sup>+</sup>) 279 ([M<sup>35</sup>Cl–H]<sup>+</sup>, 100%). Chiral HPLC (AD-H, Hexane:IPA = 97:3, 1.8 mL/min, 20 mins) major rt 11.24 mins, >99%, minor peak not seen. The data are in good agreement with the literature.<sup>18,47</sup>

**(S)-2-Chloro-8-cyclopentyl-7-ethyl-7,8-dihydropteridin-6(5H)-one ((S)-37)**



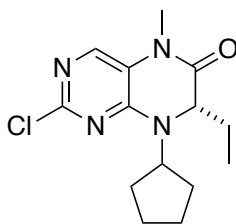
Methyl (*S*)-2-((2-chloro-5-nitropyrimidin-4-yl)(cyclopentyl)amino)butanoate ((**S**)-**63**) (2.26 g, 6.59 mmol, 1.0 eq.) was dissolved in glacial AcOH (35 mL) and heated to 70 °C. Fe (1.84 g, 33.0 mmol, 5.0 eq.) was added, and the solution was stirred at 70 °C for 1 h. The temperature was increased to 100 °C and the reaction mixture was stirred for a further 2 h. The mixture was allowed to cool to room temperature, then was filtered over Celite®, washing with MeOH (3 × 150 mL). The filtrate was concentrated *in vacuo*. Purification by silica gel chromatography (Combiflash Rf+, 120 g column), eluting with cyclohexane:EtOAc (1:0 to 0:1), afforded (*S*)-2-chloro-8-cyclopentyl-7-ethyl-7,8-dihydropteridin-6(5H)-one ((**S**)-**64**) (1.4 g, 72%) as a colourless solid: *R*<sub>f</sub> 0.45 (petroleum ether:EtOAc = 1:1); lit.<sup>18</sup> [ $\alpha$ ]<sub>D</sub><sup>21.4</sup>: +95.3 (c 1.0, MeOH);  $\tilde{\nu}_{\text{max}}$  (thin film)/cm<sup>–1</sup> 2968 (br), 1692 (s, C=O), 1603, 1363, 1193, 767 (w); m.p. 168–170 °C (EtOAc); LCMS (formic acid) rt 1.02 min (>99%), *m/z* (ES<sup>+</sup>) 281 ([M<sup>35</sup>Cl+H]<sup>+</sup>, 100%); Chiral HPLC (AD-H, Hexane:IPA = 97:3, 1.8 mL/min, 20 mins) major rt 9.26 mins, purity >99%, minor peak not seen. The spectroscopic data are identical to the enantiomer (*R*)-**96** and in good agreement with the literature.<sup>18,47</sup>

**(R)-2-Chloro-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5H)-one (8)**



Mel (10  $\mu$ L, 0.14 mmol, 2.0 eq.) was added to a solution of (R)-2-chloro-8-cyclopentyl-7-ethyl-7,8-dihydropteridin-6(5H)-one (**37**) (20 mg, 0.071 mmol, 1.0 eq.) in dry DMF (0.5 mL). The reaction mixture was cooled to  $-10\text{ }^{\circ}\text{C}$  using a NaCl and ice bath, then NaH (3.5 mg, 0.14 mmol, 2.0 eq.) was added. The reaction mixture was stirred at  $-10\text{ }^{\circ}\text{C}$  for 1 h, then was warmed to room temperature and stirred for 1 h. The mixture was quenched with the addition of crushed ice, then was diluted with  $\text{H}_2\text{O}$  (20 mL) and extracted with EtOAc (2  $\times$  35 mL). The combined organic components were washed with aq. LiCl (10% w/v, 6  $\times$  25 mL), dried over  $\text{MgSO}_4$ , filtered, and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with petroleum ether:EtOAc (1:0 to 0:1), afforded (R)-2-chloro-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5H)-one (**8**) (21 mg, 98%) as a colourless solid:  $R_f$  0.56 (petroleum ether:EtOAc = 1:1);  $[\alpha]_D^{25}$ :  $-80.0$  (c 1.0, MeOH) [lit.<sup>18</sup>  $[\alpha]_D^{21.4}$ :  $-88.3$  (c 1.0, MeOH)];  $\tilde{\nu}_{\text{max}}$  (thin film)/ $\text{cm}^{-1}$  2968 (w), 1682 (s) (C=O), 1578 (m), 1409 (m), 1185 (m), 1058 (m); m.p.  $82\text{--}84\text{ }^{\circ}\text{C}$  (EtOAc) [lit.<sup>47</sup>  $94\text{ }^{\circ}\text{C}$  (EtOAc)];  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.66 (1 H, s), 4.41–4.28 (1 H, m), 4.24 (1 H, dd,  $J$  7.5, 3.6), 3.32 (3 H, s), 2.13–2.02 (1 H, m), 2.02–1.80 (4 H, m), 1.80–1.60 (5 H, m), 0.86 (3 H, dd,  $J$  7.5, 7.5);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  163.9, 154.1, 152.5, 138.1, 121.4, 60.9, 59.6, 29.6, 29.4, 28.4, 27.5, 24.2, 23.9, 9.2; LRMS  $m/z$  ( $\text{ES}^+$ ) 295 ( $[\text{M}^{35}\text{Cl}+\text{H}]^+$ , 100%); Analytical HPLC (RP, method A) rt 10.17 mins, purity >99%. Chiral HPLC (AD-H, Hexane:IPA = 90:10, 1.0 mL/min, 20 mins) minor rt 7.74 mins, 2.5%, major rt 10.18 mins, 97.5%. The  $^1\text{H}$  spectroscopic data are in good agreement with the literature.<sup>18,47</sup>

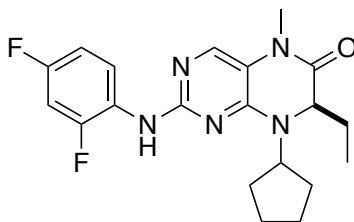
**(S)-2-Chloro-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5H)-one ((S)-36)**



Mel (540  $\mu$ L, 8.55 mmol, 2.0 eq.) was added to a solution of (S)-2-chloro-8-cyclopentyl-7-ethyl-7,8-dihydropteridin-6(5H)-one ((S)-**37**) (1.20 g, 4.27 mmol, 1.0 eq.) in dry DMF (15 mL). The reaction mixture was cooled to  $0\text{ }^{\circ}\text{C}$ , then NaH (350 mg, 8.55 mmol, 2.0 eq.) was added. The reaction mixture was stirred at  $0\text{ }^{\circ}\text{C}$  for 30 mins, then was warmed to room temperature and stirred for 1 h. The mixture was quenched with the addition of crushed ice, then was diluted with brine (150 mL) and extracted with EtOAc (2  $\times$  150 mL). The combined organic components were washed with sat. aq. LiCl (5  $\times$  100 mL), dried over an hydrophobic frit, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with cyclohexane:EtOAc (1:0 to 0:1), afforded (S)-2-chloro-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5H)-one ((S)-**36**) (1.3 g, 90%) as a colourless solid:  $R_f$  0.56 (petroleum ether:EtOAc = 1:1);  $[\alpha]_D^{25}$ :  $+28.9$  (c 1.0, MeOH) [lit.<sup>18</sup>  $[\alpha]_D^{21.4}$ :  $+93.5$  (c 1.0, MeOH)]; m.p.  $82\text{--}84\text{ }^{\circ}\text{C}$  (EtOAc); LCMS (formic acid) rt 1.02 min (99%),  $m/z$  ( $\text{ES}^+$ ) 295 ( $[\text{M}^{35}\text{Cl}+\text{H}]^+$ , 100%); Chiral HPLC (AD-H, Hexane:IPA = 90:10, 1.0 mL/min, 20 mins) major rt 7.27 mins, 92.2%,

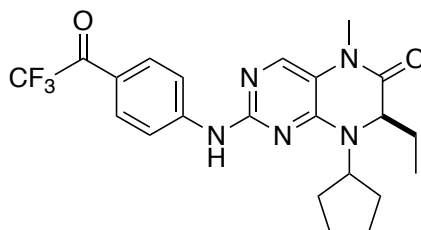
minor rt 9.74 mins, 6.8%. The spectroscopic data are identical to the enantiomer **8** and in good agreement with the literature.<sup>18,47</sup>

**(7R)-8-Cyclopentyl-2-((2',4'-difluorophenyl)amino)-7-ethyl-5-methyl-7,8-dihydropteridin-6(5H)-one (54)**



(*R*)-2-Chloro-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5*H*)-one (**8**) (20 mg, 0.068 mmol, 1.0 eq.) was suspended in H<sub>2</sub>O (0.8 mL) and EtOH (0.2 mL). 2,4-difluoroniline (12  $\mu$ L, 0.14 mmol, 2.0 eq.) and 20  $\mu$ L conc. HCl were added. The resulting solution was stirred under reflux conditions for 18 h, then sat. aq. NaHCO<sub>3</sub> (20 mL) was added. The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2  $\times$  40 mL). The combined organic components were washed with sat. aq. NaHCO<sub>3</sub> (20 mL), dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with petroleum ether and EtOAc (1:0 to 0:1), afforded (7*R*)-8-cyclopentyl-2-((2',4'-difluorophenyl)amino)-7-ethyl-5-methyl-7,8-dihydropteridin-6(5*H*)-one (**54**) as an amorphous brown solid (26 mg, > 99%): *R*<sub>f</sub> 0.79 (petroleum ether:EtOAc = 1:1); [ $\alpha$ ]<sub>D</sub><sup>25</sup>: -57.8 (c 1.0, CHCl<sub>3</sub>);  $\tilde{\nu}_{\text{max}}$  (thin film)/cm<sup>-1</sup> 3053 (w), 1673 (s, C=O), 1974 (w), 1506 (m), 1428 (s), 1279 (m), 845 (m), 778 (m); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.37–8.24 (1 H, m), 7.63 (1 H, s), 6.93–6.82 (2 H, m), 6.82–6.71 (1 H, m), 4.47–4.32 (1 H, m), 4.20 (1 H, dd, *J* 7.8, 3.7), 3.31 (3 H, s), 2.13–2.02 (1 H, m), 2.02–1.89 (1 H, m), 1.90–1.74 (4 H, m), 1.74–1.54 (4 H, m), 0.87 (3 H, dd, *J* 7.5, 7.5); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  163.7, 157.0 (dd, *J*<sub>CF</sub> 244.3, 11.6), 152.4 (dd, *J*<sub>CF</sub> 244.3, 11.6), 155.3, 152.3, 137.8, 125.1, 121.3 (dd, *J*<sub>CF</sub> 8.6, 2.4), 116.4, 110.5 (dd, *J*<sub>CF</sub> 21.4, 3.6), 103.4 (dd, *J*<sub>CF</sub> 26.6, 26.6), 60.2, 58.6, 29.6, 29.2, 28.2, 27.2, 23.5, 23.2, 9.2; <sup>19</sup>F NMR (471 MHz, CDCl<sub>3</sub>)  $\delta$  -126.8 (1 F, br d, *J*<sub>CF</sub> 2.9), -119.0 (1 F, br s); LRMS *m/z* (ES<sup>+</sup>) 388 ([*M*+*H*]<sup>+</sup>, 100%); HRMS *m/z* (ES<sup>+</sup>) found 388.19406 (100%), C<sub>20</sub>H<sub>23</sub>ON<sub>5</sub>F<sub>2</sub> requires [*M*+*H*]<sup>+</sup> 388.19434; Analytical HPLC (RP, method B) rt 7.53 mins, purity 99%.

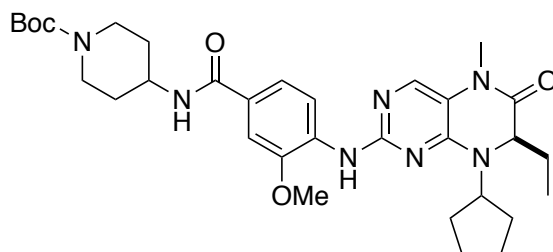
**(7R)-8-Cyclopentyl-7-ethyl-5-methyl-2-((4'-(2,2,2-trifluoroacetyl)phenyl)amino)-7,8-dihydropteridin-6(5H)-one (55)**



(Trifluoroacetyl)aniline (77 mg, 0.41 mmol, 2.0 eq.) and conc. HCl (60  $\mu$ L) were added to a suspension of (*R*)-2-chloro-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5*H*)-one (**8**) (60 mg, 0.21 mmol, 1.0 eq.) in H<sub>2</sub>O (2.4 mL) and EtOH (0.6 mL). The resulting solution was stirred under reflux for 50 h. The precipitated mixture was suspended in H<sub>2</sub>O (10 mL), the pH was adjusted to pH 5–6 using sat. K<sub>2</sub>CO<sub>3</sub>, then was fully solubilized using 0.5 M citric acid (30 mL) and H<sub>2</sub>O (10 mL). The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2  $\times$  125 mL). The combined organic components were washed with 0.5 M citric acid (2  $\times$  50 mL), dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with petroleum

ether and EtOAc (slow gradient from 1:0 to 1:1, then fast gradient from 1:1 to 0:1), afforded (7*R*)-8-cyclopentyl-7-ethyl-5-methyl-2-((4-(2,2,2-trifluoroacetyl)phenyl)amino)-7,8-dihydropteridin-6(5*H*)-one (**55**) (26 mg, 28%) as bright yellow solid:  $R_f$  0.31 (petroleum ether:EtOAc = 1:1);  $[\alpha]_D^{25}$ : -66.0 (c 1.0, CHCl<sub>3</sub>); m.p. 183–185 °C (EtOAc);  $\tilde{\nu}_{\max}$  (thin film)/cm<sup>-1</sup> 3267 (w), 2966 (w), 1656 (s) (C=O), 1601 (s) (C=O), 1584 (s), 1424 (s), 1340 (m), 1238 (m), 1162 (w), 1141 (m), 935 (m), 765 (m); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.08–7.97 (2 H, m), 7.82–7.73 (2 H, m), 7.69 (1 H, s), 7.30 (1 H, br s), 4.55–4.39 (1 H, m), 4.25 (1 H, dd,  $J$  7.8, 3.7), 3.34 (3 H, s), 2.23–2.08 (1 H, m), 2.08–1.95 (1 H, m), 1.95–1.52 (8 H, m), 0.88 (3 H, dd,  $J$  7.6, 7.6); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  178.8 (q,  $J_{CF}$  34.2), 163.6, 154.3, 152.39, 147.2, 137.54, 132.2, 122.6, 117.26, 117.17 (q,  $J_{CF}$  290.0, CF<sub>3</sub>), 60.2, 58.8, 29.8, 29.3, 28.4, 27.4, 23.6, 23.3, 9.3; <sup>19</sup>F NMR (471 MHz, CDCl<sub>3</sub>)  $\delta$  -70.8 (3 F, br s, CF<sub>3</sub>); LRMS  $m/z$  (ES<sup>+</sup>) 448 ([M+H]<sup>+</sup>, 100%); HRMS  $m/z$  (ES<sup>+</sup>) found 448.19522 (100%), C<sub>22</sub>H<sub>24</sub>F<sub>3</sub>N<sub>5</sub>O<sub>2</sub> requires [M+H]<sup>+</sup> 448.19549; Analytical HPLC (RP, method B) rt 6.47 mins, purity 98%.

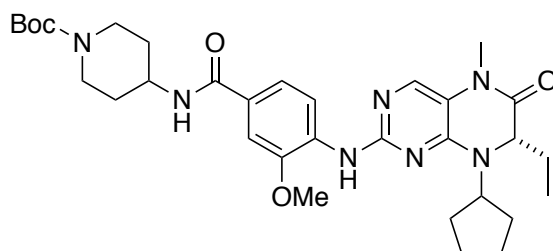
***tert*-Butyl (7*R*)-4-(4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzamido)piperidine-1-carboxylate (**64**)**



Conc. HCl (0.51 mL, 16.6 mmol, 10.0 eq.) was added to a suspension of **8** (700 mg, 1.66 mmol, 1.00 eq.) and 4-amino-3-methoxybenzoic acid (420 mg, 2.49 mmol, 1.50 eq.) in H<sub>2</sub>O (4.8 mL) and EtOH (1.2 mL). The mixture was stirred at 100 °C for 20 h, then the reaction mixture was concentrated *in vacuo*. The residue was dissolved in DMF (12 mL), then diisopropylamine (0.47 mL, 3.32 mmol, 2.00 eq.) and HBTU (1.01 g, 2.66 mmol, 1.60 eq.) were added. The reaction was stirred at room temperature for 2 h, then *tert*-butyl 4-aminopiperidine-1-carboxylate (566 mg, 2.83 mmol, 1.70 eq.) was added. After 2 h, no conversion was observed. HBTU (316 mg, 0.831 mmol, 0.50 eq.) was added, and the mixture was stirred at room temperature for 18 h. Diisopropylamine (1.19 mL, 8.31 mmol, 5.00 eq.) and *tert*-butyl 4-aminopiperidine-1-carboxylate (333 mg, 1.66 mmol, 1.00 eq.) were added, and the reaction mixture was stirred for 2 h, then diluted with H<sub>2</sub>O (100 mL) and extracted with EtOAc (2 × 150 mL). The combined organic components were washed with sat. aq. LiCl (4 × 100 mL), filtered over an hydrophobic frit and concentrated *in vacuo*. Purification by silica gel chromatography (CombiflashRf+, 80 g column), eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (1:0 to 10:1) afforded *tert*-butyl (7*R*)-4'-((4'-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3'-methoxybenzamido)piperidine-1''''-carboxylate (**64**) (736 mg, 64%) as a pale brown oil. A small amount of **64** (50 mg) was further purified by MDAP (method D), while the remainder was used as such in the following reactions. The mass-directed assisted purification (MDAP) preparative HPLC fractions were collected and concentrated *in vacuo* to give *tert*-butyl (7*R*)-4-(4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzamido)piperidine-1-carboxylate (**64**) (26 mg, 51% recovery) as a colourless solid:  $R_f$  0.55 (CH<sub>2</sub>Cl<sub>2</sub>:MeOH:NH<sub>4</sub>OH = 90:9:1);  $[\alpha]_D^{25}$ : -44.9 (c 1.0, MeOH) [lit.<sup>48</sup>  $[\alpha]_D^{22}$ : -112 (c 0.2, CHCl<sub>3</sub>)]; m.p. 88–90 °C (CH<sub>2</sub>Cl<sub>2</sub>) [lit.<sup>48</sup> 132 °C (EtOAc)]; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.51 (1 H, d,  $J$  8.4), 7.66

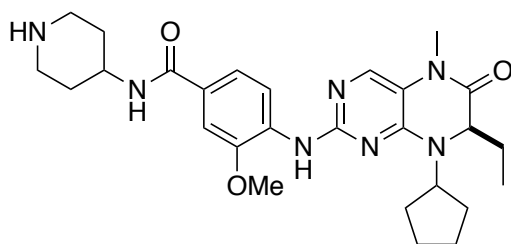
(1 H, s), 7.41 (1 H, d, *J* 1.9), 7.23 (1 H, dd, *J* 8.5, 1.9), 6.00 (1 H, d, *J* 7.8), 4.56–4.40 (1 H, m), 4.21 (1 H, dd, *J* 7.9, 3.7), 4.16–4.02 (1 H, m), 3.96 (3 H, s), 3.31 (3 H, s), 2.92 (2 H, t, *J* 12.6), 2.21–2.07 (3 H, m), 2.08–1.92 (3 H, m), 1.92–1.60 (8 H, m), 1.46 (11 H, s), 0.87 (3 H, dd, *J* 7.5, 7.5); LCMS (high pH) *rt* 1.32 mins (97%), *m/z* ( $\text{ES}^+$ ) 609 ( $[\text{M}+\text{H}]^+$ , 100%); Chiral HPLC (IB-N5 (250 × 4.6 mm, 5  $\mu\text{m}$ ), Hexane:EtOH = 75:25 with 0.2% *v/v* isopropylamine, 1.0 mL/min, 25 mins) major *rt* 14.9 mins, 92.9%, minor *rt* 17.9 mins, 7.1%. The data are in good agreement with the literature.<sup>48</sup>

***tert*-Butyl (7*S*)-4-(4'-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3'-methoxybenzamido)piperidine-1-carboxylate ((*S*)-64)**



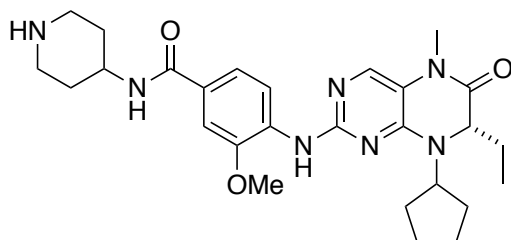
Conc. HCl (0.51 mL, 16.6 mmol, 10.0 eq.) was added to a suspension of (S)-2-chloro-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5*H*)-one ((*S*)-37) (700 mg, 1.66 mmol, 1.00 eq.) and 4-amino-3-methoxybenzoic acid (420 mg, 2.49 mmol, 1.50 eq.) in H<sub>2</sub>O (4.8 mL) and EtOH (1.2 mL). The mixture was stirred at 100 °C for 20 h, then the reaction mixture was concentrated *in vacuo*. The residue was dissolved in DMF (12 mL), then diisopropylamine (0.47 mL, 3.32 mmol, 2.00 eq.) and HBTU (1.01 g, 2.66 mmol, 1.60 eq.) were added. The reaction was stirred at room temperature for 2 h, then *tert*-butyl 4-aminopiperidine-1-carboxylate (566 mg, 2.83 mmol, 1.70 eq.) was added. After 2 h, no conversion was observed. HBTU (316 mg, 0.831 mmol, 0.50 eq.) was added, and the mixture was stirred at room temperature for 18 h. Diisopropylamine (1.19 mL, 8.31 mmol, 5.00 eq.) and *tert*-butyl 4-aminopiperidine-1-carboxylate (334 mg, 1.66 mmol, 1.00 eq.) were added, and the reaction mixture was stirred for 2 h, then diluted with H<sub>2</sub>O (100 mL) and extracted with EtOAc (2 × 150 mL). The combined organic components were washed with sat. aq. LiCl (4 × 100 mL), filtered over an hydrophobic frit and concentrated *in vacuo*. Purification by silica gel chromatography (CombiflashRf+, 80 g column), eluting with CH<sub>2</sub>Cl<sub>2</sub> and MeOH (gradient from 1:0 to 10:1) afforded *tert*-butyl (7*S*)-4-(4'-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3'-methoxybenzamido)piperidine-1-carboxylate ((*S*)-64) (1.10 g, 72%) as a pale brown oil. A small amount of *tert*-butyl (7*S*)-4-(4'-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3'-methoxybenzamido)piperidine-1-carboxylate (*S*)-64 (50 mg) was further purified by MDAP (method D), while the remainder was used as such in the following reactions. The MDAP fractions were collected and concentrated *in vacuo* to give *tert*-butyl (7*S*)-4-(4'-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3'-methoxybenzamido)piperidine-1-carboxylate ((*S*)-64) (22 mg, 44% recovery) as a colourless solid: *R<sub>f</sub>* 0.55 (CH<sub>2</sub>Cl<sub>2</sub>:MeOH:NH<sub>4</sub>OH = 90:9:1);  $[\alpha]_{\text{D}}^{25}$ : +72.2 (c 1.0, MeOH); LCMS (high pH) *rt* 1.32 mins (97%), *m/z* ( $\text{ES}^+$ ) 609 ( $[\text{M}+\text{H}]^+$ , 100%); Chiral HPLC (IB-N5 (250 × 4.6 mm, 5  $\mu\text{m}$ ), Hexane:EtOH = 75:25 with 0.2% *v/v* isopropylamine, 1.0 mL/min, 25 mins) minor *rt* 14.9 mins, 12.0%, major *rt* 17.9 mins, 88.0%. The spectroscopic data are identical to the enantiomer (*R*)-64 and are in good agreement with the literature.<sup>48</sup>

**(7R)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(piperidin-4-yl)benzamide (65)**



Trifluoroacetic acid (1.52 ml, 19.7 mmol, 20.0 eq.) was added to a solution of **64** (600 mg, 0.987 mmol, 1.00 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (6.0 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C, then the reaction mixture was warmed to room temperature, and stirred for 1 h. The reaction mixture was concentrated *in vacuo*, then the residue was dissolved in MeOH (10 mL) and K<sub>2</sub>CO<sub>3</sub> (ca. 2 g) was added at 0 °C. The mixture was stirred vigorously for 10 mins, then diluted with H<sub>2</sub>O (50 mL) and extracted with EtOAc (2 × 50 mL). The combined organic components were washed with H<sub>2</sub>O (25 mL), then filtered over an hydrophobic frit and concentrated *in vacuo*. The residue was taken in minimal amount of MeOH, then loaded onto a 10 g SCX column, washed with MeOH (5 × 20 mL), then eluted with 2 M NH<sub>3</sub> in MeOH (5 × 10 mL) using a fish tank. The collected fractions were concentrated *in vacuo*, then purified by silica gel chromatography (CombiflashRf+, 40 g column), eluting with CH<sub>2</sub>Cl<sub>2</sub>:2 M NH<sub>3</sub> in MeOH (1:0 to 8:2) to afford (7R)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(piperidin-4-yl)benzamide (**65**) (354 mg, 70%) as a beige solid *R*<sub>f</sub> 0.17 (CH<sub>2</sub>Cl<sub>2</sub>:MeOH:NH<sub>4</sub>OH = 90:9:1); [ $\alpha$ ]<sub>D</sub><sup>25</sup>: -92.1 (c 1.0, MeOH) [lit.<sup>48</sup> [ $\alpha$ ]<sub>D</sub><sup>22</sup>: -86 (c 0.2, CHCl<sub>3</sub>)]; m.p. 164–166 °C (MeOH); [lit.<sup>48</sup> 138 °C (EtOAc)] <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.54 (1 H, d, *J* 8.4), 7.68 (1 H, s, C<sup>4</sup>-H), 7.59 (1 H, s), 7.42 (1 H, d, *J* 2.0), 7.23 (1 H, dd, *J* 8.4, 2.0), 5.95 (1 H, d, *J* 8.0), 4.52 (1 H, p, *J* 8.0), 4.21 (1 H, dd, *J* 7.9, 3.8), 4.16–4.04 (1 H, m), 3.97 (3 H, s), 3.33 (3 H, s), 3.11 (2 H, dt, *J* 12.8, 3.8), 2.77 (2 H, td, *J* 12.8, 2.5), 2.23–1.92 (3 H, m), 1.92–1.58 (8 H, m), 1.52–1.35 (2 H, m), 0.88 (3 H, dd, *J* 7.5, 7.5); LCMS (high pH) rt 1.15 mins (>99%), *m/z* (ES<sup>+</sup>) 508 ([M+H]<sup>+</sup>, 100%); HR-LCMS (formic acid) rt 1.92 mins (95%), *m/z* (ES<sup>+</sup>) found 508.3037 (100%), C<sub>27</sub>H<sub>37</sub>N<sub>7</sub>O<sub>3</sub> requires [M+H]<sup>+</sup> 508.3031. Chiral HPLC (IE5 (250 × 4.6 mm, 5  $\mu$ m), Hexane:IPA = 50:50 with 0.2% *v/v* isopropylamine, 1.0 mL/min, 30 mins) minor rt 19.0 mins, 9.0%, major rt 21.4 mins, 90.0%. The data are in good agreement with the literature.<sup>48</sup>

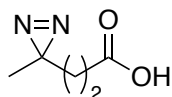
**(7S)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3'-methoxy-N-(piperidin-4-yl)benzamide ((S)-65)**



Trifluoroacetic acid (1.52 ml, 19.7 mmol, 20.0 eq.) was added to a solution of (S)-2-chloro-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5*H*)-one (**(S)-64**) (600 mg, 0.987 mmol, 1.00 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (6.0 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C, then the reaction mixture was warmed to room temperature, and stirred for 1 h. The reaction mixture was concentrated *in vacuo*, then the residue was

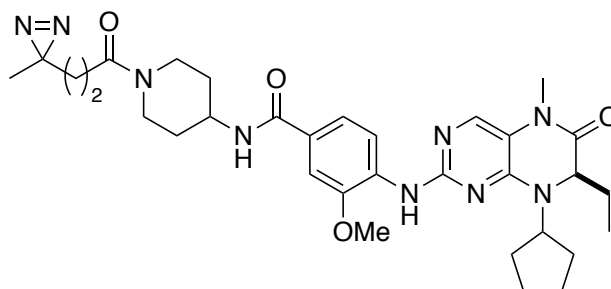
dissolved in MeOH (10 mL) and  $K_2CO_3$  (ca. 2 g) was added at 0 °C. The mixture was stirred vigorously for 10 mins, then diluted with  $H_2O$  (50 mL) and extracted with EtOAc (2 × 50 mL). The combined organic components were washed with  $H_2O$  (25 mL), then filtered over an hydrophobic frit, and concentrated *in vacuo*. The residue was taken in minimal amount of MeOH, then loaded onto a 10 g SCX column, washed with MeOH (5 × 20 mL), then eluted with 2 M  $NH_3$  in MeOH (5 × 10 mL) using a fish tank. The collected fractions were concentrated *in vacuo*, then purified by silica gel chromatography (CombiflashRf+, 40 g column), eluting with  $CH_2Cl_2$ :2 M  $NH_3$  in MeOH (1:0 to 8:2) to afford (7*S*)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3'-methoxy-*N*-(piperidin-4-yl)benzamide ((**S**)-**65**) (299 mg, 57%) as a pale beige solid:  $R_f$  0.17 ( $CH_2Cl_2$ :MeOH: $NH_4OH$  = 90:9:1);  $[\alpha]_D^{25}$ : +91.1 (c 1.0, MeOH); m.p. 165–166 °C (MeOH); LCMS (high pH) rt 1.01 mins (98%),  $m/z$  ( $ES^+$ ) 509 ( $[M+H]^+$ , 100%); Chiral HPLC (IE5 (250 × 4.6 mm, 5  $\mu$ m), Hexane:IPA = 50:50 with 0.2% v/v isopropylamine, 1.0 mL/min, 30 mins) major rt 19.0 mins, 90.4%, minor rt 21.4 mins, 9.6%. The spectroscopic data are identical to the enantiomer (**R**)-**65** and are in good agreement with the literature.<sup>48</sup>

### 3-(3*H*-Diazirin-3-yl)pentanoic acid (**67**)



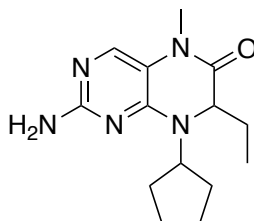
Levulinic acid (4.0 g, 34 mmol, 1.0 eq.) and 7 M  $NH_3$  in MeOH (45 mL, 3.2 mmol, 9.1 eq.) were stirred at 0 °C for 3 h, then hydroxylamine-O-sulfonic acid (5.8 g, 51 mmol, 1.5 eq.) was added portionwise and the mixture was left to warm to room temperature then stirred for 42 h. Nitrogen was bubbled through the reaction mixture for 1 h and the mixture was filtered. The retentate was washed with MeOH (2 × 20 mL) and the filtrate was concentrated *in vacuo*. The residue was dissolved in MeOH (25 mL), triethylamine (14 mL, 1.0 mol, 3.0 eq.) was added and the reaction mixture was cooled to 0 °C. Iodine (8.7 g, 34 mmol, 1.0 eq.) was added portionwise at 0 °C under nitrogen in the dark with vigorous stirring until the dark colour remained. 2 M aq. HCl (ca. 30 mL) and aq. sodium thiosulfate (10% w/v, 150 mL) were added, and the mixture was extracted with EtOAc (3 × 60 mL). The combined organic components were washed with aq. sodium thiosulfate (10% w/v, 2 × 100 mL), then filtered over an hydrophobic frit and concentrated *in vacuo*. The crude was filtered over silica, flushing with  $CH_2Cl_2$ :MeOH (9:1, ca. 4 CV), then purified by silica gel chromatography (CombiflashRf+, 80 g column), eluting with  $CH_2Cl_2$ :MeOH (1:0 to 9:1) to afford 3-(3-methyl-3*H*-diazirin-3-yl)propanoic acid (**67**) (1.02 g, 22%) as a colourless liquid.  $R_f$  0.05 ( $CH_2Cl_2$ :MeOH = 9:1);  $\tilde{\nu}_{max}$  (neat)/ $cm^{-1}$  2980 (w), 2666 (w), 1708 (s) (C=O), 1437 (m), 1387 (m), 1223 (m), 918 (w);  $^1H$  (600 MHz,  $CDCl_3$ ) 2.25 (2 H, t,  $J$  6.5), 1.73 (2 H, t,  $J$  6.5), 1.06 (3 H, s);  $^{13}C$  (176 MHz,  $CDCl_3$ )  $\delta$  178.0, 29.4, 28.4, 25.0, 19.7; LCMS (formic acid) rt 0.36 mins (20%),  $m/z$  ( $ES^-$ ) 127 ( $[M-H]^-$ , 100%). The compound is not UV active, so LCMS or HPLC could not be used to determine purity. Purity by  $^1H$  NMR is estimated at >90%. The data are in good agreement with the literature.<sup>49</sup>

**(*R*)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-*N*-(1-(3-(3-methyl-3*H*-diazirin-3-yl)propanoyl)piperidin-4-yl)benzamide (57)**



HATU (17.8 mg, 0.047 mmol, 1.2 eq.) and DIPEA (14  $\mu$ L, 0.078 mmol, 2.0 eq.) were added to a solution of 3-(3-methyl-3*H*-diazirin-3-yl)propanoic acid (**68**) (5.0 mg, 0.039 mmol, 1.0 eq.) in  $\text{CH}_2\text{Cl}_2$  (0.4 mL). The reaction was stirred for 1 h at room temperature, then (*R*)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-*N*-(piperidin-4-yl)benzamide (**66**) (24 mg, 0.047 mmol, 1.2 eq.) was added, and the mixture was stirred for 1.5 h. The volatile components were removed under a flow of nitrogen, then the residue was dissolved in DMSO (0.9 mL) and purified by MDAP (method C). The solvents were removed under a flow of nitrogen to afford (*R*)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-*N*-(1-(3-(3-methyl-3*H*-diazirin-3-yl)propanoyl)piperidin-4-yl)benzamide (**57**) (14 mg, 53%) as an amorphous colorless solid:  $R_f$  0.46 (EtOAc);  $[\alpha]_D^{25}$ :  $-48.2$  (c 0.41, MeOH);  $\tilde{\nu}_{\text{max}}$  (thin film)/ $\text{cm}^{-1}$  3339 (w), 2967 (w), 1674 (br, s) (C=O) 1630 (s) (C=O), 1502 (s), 1381 (m), 1322 (m), 1245 (m), 1035 (m), 953 (w), 757 (w);  $^1\text{H}$  NMR (700 MHz,  $\text{CDCl}_3$ ) 8.54 (1 H, d,  $J$  8.4), 7.68 (1 H, s), 7.65 (1 H, s), 7.42 (1 H, d,  $J$  2.0), 7.23 (1 H, dd,  $J$  8.5, 2.0), 5.95 (1 H, d,  $J$  7.7), 4.60 (1 H, m), 4.51 (1 H, tt,  $J$  8.0, 8.0), 4.25–4.16 (2 H, m), 3.98 (3 H, s), 3.80 (1 H, m), 3.33 (3 H, s), 3.22–3.14 (1 H, m), 2.81–2.72 (1 H, m), 2.23–1.92 (6 H, m), 1.91–1.75 (6 H, m), 1.75–1.65 (4 H, m), 1.51–1.37 (2 H, m), 1.06 (3 H, s), 0.88 (3 H, dd,  $J$  7.5, 7.5);  $^{13}\text{C}$  NMR (176 MHz,  $\text{CDCl}_3$ )  $\delta$  169.6, 166.7, 163.7, 155.0, 152.3, 147.3, 137.7, 133.4, 126.1, 118.9, 116.5, 116.0, 109.1, 59.9, 58.4, 56.0, 47.1, 44.4, 41.0, 32.9, 31.9, 29.7, 29.6, 29.3, 27.1, 25.5, 23.6, 23.2, 20.1, 9.2; LCMS (formic acid)  $r_t$  0.82 mins (95%),  $m/z$  ( $\text{ES}^+$ ) 618 ( $[\text{M}+\text{H}]^+$ , 100%); HRMS  $m/z$  ( $\text{ES}^+$ ) found 618.3486 (100%),  $\text{C}_{32}\text{H}_{43}\text{N}_9\text{O}_4$   $[\text{M}+\text{H}]^+$  requires 618.3511.

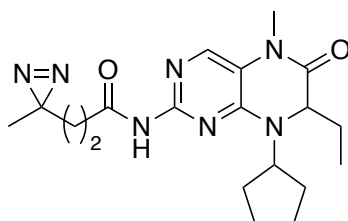
**2-Amino-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5*H*)-one (66)**



(*R*)-2-Chloro-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5*H*)-one (**8**) (0.15 g, 0.51 mmol, 1.0 eq.) was dissolved in isopropanol (1.2 mL) and ammonium hydroxide (2.6 mL, 70 mmol, 250 eq.), then submitted to microwave irradiation for 6 h at 170  $^{\circ}\text{C}$ , 6 h at 160  $^{\circ}\text{C}$  and then 4 h at 170  $^{\circ}\text{C}$ .  $\text{H}_2\text{O}$  (150 mL) was added and the mixture was extracted with EtOAc (150 mL). The organic components were filtered over an hydrophobic frit. Purification by silica gel chromatography (Combiflash  $R_f$ +, 24 g column), eluting with cyclohexane:EtOAc (1:0 to 0:1) and then with EtOH:Et<sub>3</sub>N (9:1, 2 CV), afforded 2-amino-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5*H*)-one (**66**) (112 mg, 80%) as a colourless oil:  $R_f$  0.30 (EtOAc);  $\tilde{\nu}_{\text{max}}$  (thin film)/ $\text{cm}^{-1}$  3339

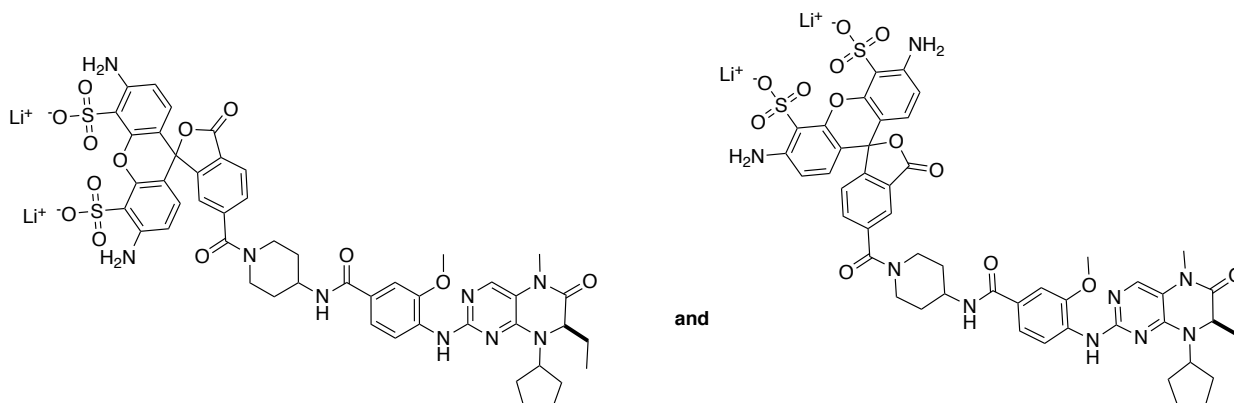
(w), 2970 (w), 2873 (w), 1665 (s) (C=O), 1459 (s), 1247 (m), 954 (m);  $^1\text{H}$  NMR (600 MHz, DMSO- $\text{D}_6$ )  $\delta$  7.58 (1 H, s), 5.87 (2 H, s), 4.36–4.27 (1 H, m), 4.07 (1 H, dd,  $J$  7.9, 3.7), 3.17 (3 H, s), 1.93–1.83 (2 H, m), 1.79–1.65 (5 H, m), 1.62–1.48 (3 H, m), 0.75 (3 H, dd,  $J$  7.5, 7.5);  $^{13}\text{C}$  NMR (151 MHz, DMSO- $\text{D}_6$ )  $\delta$  162.7, 159.1, 151.7, 139.2, 113.8, 59.6, 57.5, 28.9, 28.7, 27.6, 26.1, 23.4, 23.1, 9.0. LCMS (formic acid) rt 0.52 mins (96%),  $m/z$  ( $\text{ES}^+$ ) 277 ( $[\text{M}+\text{H}]^+$ , 100%).

***N*-(8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)-3-(3-methyl-3*H*-diazirin-3-yl)propenamide (57)**



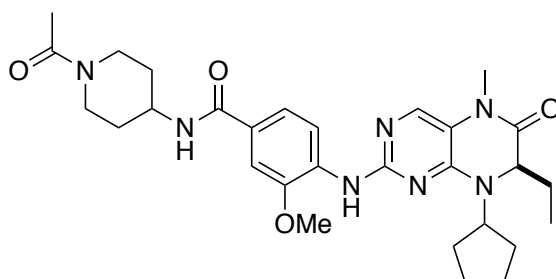
HATU (72 mg, 0.19 mmol, 1.2 eq.) and DIPEA (0.055 mL, 0.31 mmol, 2.0 eq.) were added to a solution of 3-(3-methyl-3*H*-diazirin-3-yl)propanoic acid (**67**) (20 mg, 0.16 mmol, 1.2 eq.) in  $\text{CH}_2\text{Cl}_2$  (1.0 mL). The reaction mixture was stirred for 1.5 h at room temperature, then 2-amino-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5*H*)-one (**66**) (52 mg, 0.19 mmol, 1.2 eq.) in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) was added. The reaction mixture was stirred for 2.5 h, then HATU (72 mg, 0.19 mmol, 1.2 eq.) and DIPEA (0.055 mL, 0.31 mmol, 2.0 eq.) were added. The reaction was stirred for 2 h, then left to stand for 48 h. Evaporation was observed, hence  $\text{CH}_2\text{Cl}_2$  (0.5 mL) and DMF (0.25 mL) were added to re-solubilise the mixture. HATU (72 mg, 0.19 mmol, 1.2 eq.) and DIPEA (0.055 mL, 0.31 mmol, 2.0 eq.) were added and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with  $\text{H}_2\text{O}$  (30 mL), then extracted with EtOAc (2  $\times$  30 mL). The combined organic components were washed with sat. aq. LiCl (50 mL), then concentrated *in vacuo*. The residue was dissolved in MeOH:DMSO (1:1, 1 mL) and purified by MDAP (method C). The fractions were combined and concentrated under a flow of nitrogen in the dark to afford *N*-(8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)-3-(3-methyl-3*H*-diazirin-3-yl)propenamide (**56**) (36 mg, 57%) as an off-white powdery solid:  $R_f$  0.20 (petroleum ether:EtOAc = 1:1);  $\tilde{\nu}_{\text{max}}$  (thin film)/ $\text{cm}^{-1}$  3339 (w), 2970 (w), 2873 (w), 1665 (s) (C=O), 1459 (s), 1247 (m), 954 (m);  $^1\text{H}$  NMR (600 MHz, DMSO- $\text{D}_6$ )  $\delta$  7.58 (1 H, s), 5.87 (2 H, s), 4.36–4.27 (1 H, m), 4.07 (1 H, dd,  $J$  7.9, 3.7), 3.17 (3 H, s), 1.93–1.83 (2 H, m), 1.79–1.65 (5 H, m), 1.62–1.48 (3 H, m), 0.75 (3 H, dd,  $J$  7.5, 7.5);  $^{13}\text{C}$  NMR (151 MHz, DMSO- $\text{D}_6$ )  $\delta$  162.7, 159.1, 151.7, 139.2, 113.8, 59.6, 57.5, 28.9, 28.7, 27.6, 26.1, 23.4, 23.1, 9.0. LCMS (high pH) rt 1.08 mins (100%),  $m/z$  ( $\text{ES}^+$ ) 387 ( $[\text{M}+\text{H}]^+$ , 100%); HR-LCMS (formic acid) rt 2.91 mins (100%),  $m/z$  ( $\text{ES}^+$ ) found 386.2311 (100%),  $\text{C}_{19}\text{H}_{27}\text{N}_7\text{O}_2$  requires  $[\text{M}+\text{H}]^+$  386.2299.

**(*R*)-3',6'-Diamino-[5/6]-(4-(4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzamido)piperidine-1-carbonyl)-3-oxo-3*H*-spiro[isobenzofuran-1,9'-xanthene]-4',5'-disulfonate, dilithium salt (58)**



(*R*)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-*N*-(piperidin-4-yl)benzamide (**65**) (2.9 mg, 5.8  $\mu$ mol, 1.5 eq.) in DMSO (0.4 mL) was combined with Alexa Fluor™ 488 NHS Ester (3',6'-diamino-[5/6]-(((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)-3-oxo-3*H*-spiro[isobenzofuran-1,9'-xanthene]-4',5'-disulfonate) (2.5 mg, 3.9  $\mu$ mol, 1.0 eq.) in 0.1 mL DMSO. DIPEA (4.1  $\mu$ L, 23  $\mu$ mol, 6.0 eq.) was added and the mixture was stirred in the dark for 18 h. PyBOP (2.0 mg, 3.9  $\mu$ mol, 1.0 eq.) was added and the reaction mixture was stirred at room temperature for 30 mins in the dark. The crude material was diluted with DMSO (1 mL) and purified by MDAP (method B). Collected fractions were frozen in tared vials and lyophilized. The fractions were combined to afford (*R*)-3',6'-diamino-6-(4-(4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzamido)piperidine-1-carbonyl)-3-oxo-3*H*-spiro[isobenzofuran-1,9'-xanthene]-4',5'-disulfonate, dilithium salt (**58**) (1.5 mg, 34%) as an amorphous bright yellow solid: LCMS (high pH) rt: 0.74 mins (100%),  $m/z$  ( $ES^+$ ) 513.3 ( $[M+2H]^{2+}$ , 45%), 521.8 ( $[M+H+Na]^{2+}$ , 100%); HR-LCMS (formic acid) rt: 3.54 mins (70%),  $m/z$  ( $ES^+$ ) 1024.2938,  $C_{48}H_{49}N_9O_{13}S_3$  requires  $[M+H]^+$  1024.2970; Two peaks seen in the LCMS due to partial separation of the 5- and 6-regioisomers in the 10 mins LC method. LCMS consistent with product with purity >90%.

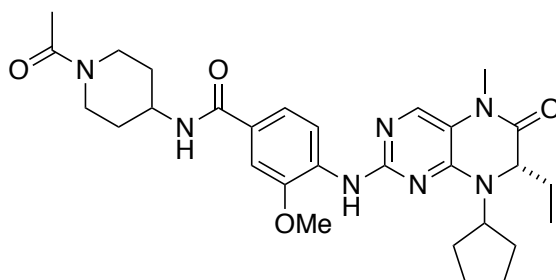
**(*R*)-*N*-(1-Acetylpiperidin-4-yl)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzamide (59)**



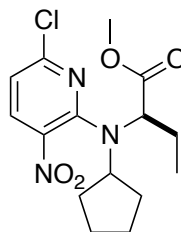
AcCl (5  $\mu$ L, 0.032 mmol, 1.1 eq.) and  $NEt_3$  (8  $\mu$ L, 0.059 mmol, 2.0 eq.) were added to a solution of (*R*)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-*N*-(piperidin-4-yl)benzamide (**65**) (15 mg, 0.029 mmol, 1.0 eq.) in  $CH_2Cl_2$  (300  $\mu$ L) at 0 °C. The reaction mixture was stirred for 1 h, then diluted with  $H_2O$  (15 mL), and extracted with  $CH_2Cl_2$  (3  $\times$  10 mL). The combined organic components were washed with sat. aq.  $NaHCO_3$  (30 mL) and brine (30 mL), then dried over  $MgSO_4$ , filtered,

and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with CHCl<sub>3</sub>:MeOH:Et<sub>3</sub>N (100:0:1 to 95:5:1), was followed by a MeCN:hexane grease wash to afford (*R*)-*N*-(1-acetylpiperidin-4-yl)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzamide (**59**) (6 mg, 40%) as a colourless solid: *R*<sub>f</sub> 0.28 (CHCl<sub>3</sub>:MeOH:Et<sub>3</sub>N = 95:5:1); [ $\alpha$ ]<sub>D</sub><sup>25</sup>: -46.3 (c 0.68, MeOH); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.54 (1 H, d, *J* 8.4), 7.75–7.58 (2 H, m), 7.42 (1 H, d, *J* 1.9), 7.24–7.20 (1 H, m), 5.99 (1 H, d, *J* 7.8), 4.68–4.56 (1 H, m), 4.51 (1 H, dddd, *J* 8.0, 8.0, 8.0, 8.0), 4.26–4.13 (2 H, m), 3.98 (3 H, s), 3.91–3.78 (1 H, m), 3.32 (3 H, s), 3.23 (1 H, ddd, *J* 14.1, 12.0, 2.8), 2.87–2.77 (1 H, m), 2.25–1.92 (8 H, m), 1.91–1.64 (8 H, m), 1.49–1.35 (2 H, m), 0.87 (3 H, dd, *J* 7.5, 7.5); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  168.9, 166.7, 163.7, 155.0, 152.3, 147.2, 137.7, 133.4, 126.1, 118.9, 116.4, 116.0, 109.1, 59.9, 58.4, 56.0, 47.1, 45.4, 40.6, 32.9, 31.9, 29.8, 29.3, 28.2, 27.2, 23.6, 23.2, 21.5, 9.2, 8.6; LRMS *m/z* (ES<sup>+</sup>) 550 ([M+H]<sup>+</sup>, 40%); HRMS *m/z* (ES<sup>+</sup>) found 550.31395 (100%), C<sub>29</sub>H<sub>39</sub>O<sub>4</sub>N<sub>7</sub> requires [M+H]<sup>+</sup> 550.31363; Analytical HPLC (RP, method A) rt 2.05 mins, purity >99%.

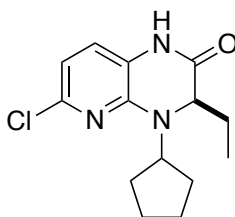
**(*S*)-*N*-(1-acetylpiperidin-4-yl)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzamide (**60**)**



AcCl (5  $\mu$ L, 0.032 mmol, 1.1 eq.) and NEt<sub>3</sub> (8  $\mu$ L, 0.059 mmol, 2.0 eq.) were added to a solution of (*S*)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-*N*-(piperidin-4-yl)benzamide (**(S)-65**) (15 mg, 0.029 mmol, 1.0 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (300  $\mu$ L) at 0 °C. The reaction mixture was stirred for 1 h, then diluted with H<sub>2</sub>O (15 mL), and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3  $\times$  10 mL). The combined organic components were washed with sat. aq. NaHCO<sub>3</sub> (30 mL) and brine (30 mL), then dried on MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with CHCl<sub>3</sub>:MeOH (1:0 to 95:5), was followed by a MeCN:hexane grease wash to afford (*S*)-*N*-(1-acetylpiperidin-4-yl)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzamide (**60**) (4 mg, 25%) as colourless solid: *R*<sub>f</sub> 0.28 (CHCl<sub>3</sub>:MeOH:Et<sub>3</sub>N = 95:5:1); [ $\alpha$ ]<sub>D</sub><sup>25</sup>: +50.6 (c 0.38, MeOH); LRMS *m/z* (ES<sup>+</sup>) 550 ([M+H]<sup>+</sup>, 40%); HRMS *m/z* (ES<sup>+</sup>) found 550.31273 (100%), C<sub>29</sub>H<sub>39</sub>O<sub>4</sub>N<sub>7</sub> requires [M+H]<sup>+</sup> 550.31363; Analytical HPLC (RP, method A) rt 2.40 mins, purity 99%. The spectroscopic data are identical to the enantiomer, **59**.

**Methyl (2'*R*)-2-[(6-chloro-3-nitropyridin-2-yl)(cyclopentyl)amino]butanoate (107)****107**

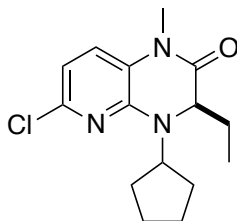
2,6-Dichloro-3-nitropyridine (127 mg, 0.648 mmol, 1.2 eq) was added to a solution of methyl (2*R*)-2-(cyclopentylamino)butanoate (**61**) (112 mg, 0.540 mmol, 1.0 eq) in degassed cyclooctane (0.65 mL). Finely ground NaHCO<sub>3</sub> (182 mg, 2.16 mmol, 4.0 eq) was added and the reaction mixture was stirred for 94 h at 150 °C. The reaction mixture was diluted with H<sub>2</sub>O (75 mL) and extracted with EtOAc (4 × 50 mL). The combined organic components were washed with brine (3 × 50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated *in vacuo*. The crude was purified by flash column chromatography (elution with 0–45% CH<sub>2</sub>Cl<sub>2</sub> / cyclohexane, 22 CV) to give methyl (2*R*)-2-[(6-chloro-3-nitropyridin-2-yl)(cyclopentyl)amino]butanoate (**107**) (89.0 mg, 48%) as a yellow solid: *R*<sub>f</sub> 0.48 (60:40 CH<sub>2</sub>Cl<sub>2</sub>:cyclohexane); [ $\alpha$ ]<sub>D</sub><sup>25</sup> +907.7 (*c* 0.1, MeOH); m.p. 103–105 °C (from MeOH);  $\tilde{\nu}_{\text{max}}$  (thin film)/cm<sup>-1</sup> 2949 (C-H, w), 2360 (s), 2340 (s), 1736 (C=O, s), 1590 (N-O, s), 1549 (m), 1339 (N-O, m); <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>)  $\delta_{\text{H}}$  7.98 (1H, d, *J* 8.3, H-4), 6.63 (1H, d, *J* 8.3), 3.74 (3H, s), 3.69 (1H, dd, *J* 8.8, 5.5), 3.56–3.46 (1H, m), 2.35–2.15 (2H, m), 2.08–1.95 (1H, m), 1.90–1.81 (1H, m), 1.79–1.64 (3H, m), 1.63–1.51 (2H, m), 1.50–1.38 (1H, m), 1.02 (3H, t, *J* 7.5); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>)  $\delta_{\text{C}}$  172.5, 150.7, 150.6, 138.0, 131.6, 112.8, 64.6, 59.8, 52.3, 31.1, 27.1, 23.0, 22.9, 11.6; HRMS *m/z* (ESI<sup>+</sup>) [Found: 364.1034, 366.1006, C<sub>15</sub>H<sub>20</sub>O<sub>4</sub>N<sub>3</sub><sup>35</sup>ClNa requires [M(<sup>35</sup>Cl)+Na]<sup>+</sup> 364.1035, C<sub>15</sub>H<sub>20</sub>O<sub>4</sub>N<sub>3</sub><sup>37</sup>ClNa requires [M(<sup>37</sup>Cl)+Na]<sup>+</sup> 366.1006]; Analytical HPLC (RP, method B) rt 11.5 mins, purity >99%.

**(3*R*)-6-Chloro-4-cyclopentyl-3-ethyl-3,4-dihydropyrido[2,3-*b*]pyrazin-2(1*H*)-one (108)**

Methyl (2*R*)-2-[(6-chloro-3-nitropyridin-2-yl)(cyclopentyl)amino]butanoate (**107**) (193 mg, 0.56 mmol, 1.0 eq) was dissolved in glacial acetic acid (3.00 mL). Activated iron powder (162 mg, 2.82 mmol, 5.0 eq) was added and the reaction mixture was stirred at 100 °C for 5 h. The reaction mixture was left to cool to rt and diluted with H<sub>2</sub>O (25 mL), and sat. aq. NaHCO<sub>3</sub> (~200 mL) was added to obtain pH 8. The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 100 mL, then 200 mL). The combined organic components were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The crude was purified by flash chromatography (elution with 0–50% EtOAc / petroleum ether 40–60 °C, 9 CV) to give (3*R*)-6-chloro-4-cyclopentyl-3-ethyl-3,4-dihydropyrido[2,3-*b*]pyrazin-2(1*H*)-one (**108**) (148 mg, 94%) as a yellow oily solid: *R*<sub>f</sub> 0.41 (40% EtOAc / petroleum ether 40–60 °C); [ $\alpha$ ]<sub>D</sub><sup>25</sup> –171.0 (*c* 0.5, CHCl<sub>3</sub>);  $\tilde{\nu}_{\text{max}}$  (thin film)/cm<sup>-1</sup> 3207 (N-H, w, br), 3070 (C-H, w), 2942 (C-H, w), 2879 (C-H, w), 1684 (C=O, s), 1473 (s); <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>)  $\delta_{\text{H}}$  9.25 (1H, s), 6.85 (1H, d, *J* 7.8), 6.57 (1H, d, *J* 7.8), 4.39 (1H, dddd, *J* 8.37, 8.37, 8.20, 8.20), 4.07 (1H, dd, *J* 8.3, 4.3), 2.10–1.94 (2H, m), 1.88–1.73 (3H, m), 1.73–1.55 (5H, m),

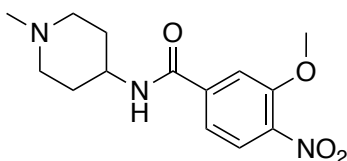
0.94 (3H, t, *J* 7.5); HRMS *m/z* (ESI<sup>+</sup>) [Found: 280.1211, 282.2790, C<sub>14</sub>H<sub>19</sub>ON<sub>3</sub><sup>35</sup>Cl requires [M(<sup>35</sup>Cl)+H]<sup>+</sup> 280.1211, C<sub>14</sub>H<sub>19</sub>ON<sub>3</sub><sup>37</sup>Cl requires [M(<sup>37</sup>Cl)+H]<sup>+</sup> 282.1182]; LRMS *m/z* (ESI<sup>+</sup>) 280.2 ([M(<sup>35</sup>Cl)+H]<sup>+</sup>, 37%), 282.2 ([M(<sup>37</sup>Cl)+H]<sup>+</sup>, 25%); Analytical HPLC (RP, method A) *rt* 9.4 mins, >99%. The spectroscopic data are in good agreement with the literature values.<sup>50</sup>

**(3*R*)-6-Chloro-4-cyclopentyl-3-ethyl-1-methyl-3,4-dihydropyrido[2,3-*b*]pyrazin-2(1*H*)-one (38)**



K<sub>2</sub>CO<sub>3</sub> (267 mg, 1.19 mmol, 4.0 eq) was added to a solution of (3*R*)-6-chloro-4-cyclopentyl-3-ethyl-3,4-dihydropyrido[2,3-*b*]pyrazin-2(1*H*)-one (**108**) (134 mg, 0.48 mmol, 1.0 eq) in anhydrous DMF (4.5 mL). The suspension was cooled to 0 °C, and MeI (119 μL, 1.91 mmol, 4.0 eq) was added dropwise. The reaction mixture was left to warm to *rt* and then stirred at *rt* for 19 h. The reaction mixture was diluted with H<sub>2</sub>O (100 mL) and extracted with EtOAc (5 × 75 mL). The combined organic components were washed with aq. LiCl solution (10% w/v) (5 × 100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated. The crude was purified by flash chromatography (elution with 0–12.5% EtOAc / petroleum ether 40–60 °C, 13 CV) to give (3*R*)-6-chloro-4-cyclopentyl-3-ethyl-1-methyl-3,4-dihydropyrido[2,3-*b*]pyrazin-2(1*H*)-one (**38**) (132 mg, 94%) a beige oil: *R*<sub>f</sub> 0.22 (15% EtOAc / petroleum ether 40–60 °C); [*α*]<sub>D</sub><sup>25</sup> –129.7 (*c* 1.0, CHCl<sub>3</sub>); *ν*<sub>max</sub> (thin film)/cm<sup>–1</sup> 2980 (C–H, s), 2888 (C–H, m), 1679 (C=O, s), 1473 (C–H, m), 1382 (C–H, m), 1157 (C–C, m); <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ<sub>H</sub> 6.93 (1H, d, *J* 8.0), 6.63 (1H, d, *J* 8.0), 4.43–4.33 (1H, m), 4.12 (1H, dd, *J* 8.5, 4.4), 3.31 (3H, s), 2.09–1.98 (2H, m), 1.86–1.74 (2H, m), 1.74–1.60 (4H, m), 1.60–1.51 (2H, m), 0.86 (3H, t, *J* 7.5); HRMS *m/z* (ESI<sup>+</sup>) [Found: 294.1369, 296.1341, C<sub>15</sub>H<sub>21</sub>ON<sub>3</sub><sup>35</sup>Cl requires [M+H]<sup>+</sup> 294.1368, C<sub>15</sub>H<sub>21</sub>ON<sub>3</sub><sup>37</sup>Cl requires [M+H]<sup>+</sup> 296.1338]; Analytical HPLC (RP, method A) *rt* 11.2 min, purity 98.7%. The spectroscopic data are in good agreement with the literature values.<sup>50</sup>

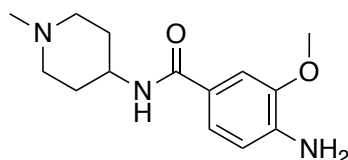
**3-Methoxy-*N*-(1'-methylpiperidin-4'-yl)-4-nitrobenzamide (105)**



HBTU (769 mg, 2.03 mmol, 1.6 eq) and anhydrous Et<sub>3</sub>N (0.360 mL, 2.54 mmol, 2.0 eq) were added to a solution of 3-methoxy-4-nitrobenzoic acid (249 mg, 1.27 mmol, 1.0 eq) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (12.7 mL), and the reaction mixture was left to stir at *rt* for 30 min. 1-Methylpiperidin-4-amine (160 μL, 1.27 mmol, 1.0 eq) was added, and the reaction mixture was left to stir at *rt* for 20 h. The reaction mixture was diluted with H<sub>2</sub>O (100 mL) and extracted with EtOAc (5 × 60 mL). The combined organic components were washed with 0.1 M NaOH (3 × 130 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The crude was purified by flash chromatography (elution with 0–10% polar eluent (MeOH with 10% NH<sub>4</sub>OH solution) / CH<sub>2</sub>Cl<sub>2</sub>, 14 CV) to give the title compound as a yellow solid (326 mg, 88%): *R*<sub>f</sub> 0.22 (10% polar eluent (MeOH with 10% NH<sub>4</sub>OH

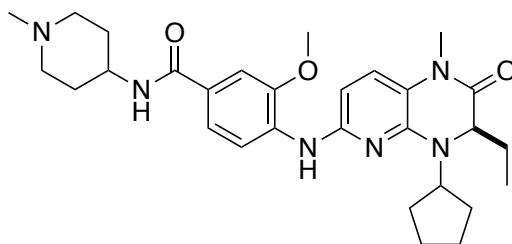
solution) / CH<sub>2</sub>Cl<sub>2</sub>); 170 °C (dec., from MeOH);  $\tilde{\nu}_{\text{max}}$  (thin film)/cm<sup>-1</sup> 2980 (s), 2889 (m), 1382 (m), 1251 (m); <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>)  $\delta_{\text{H}}$  8.58 (1H, d, *J* 7.6), 7.95 (1H, d, *J* 8.4), 7.70 (1H, d, *J* 1.7), 7.55 (1H, dd, *J* 8.4, 1.7), 3.99 (3H, s), 3.86–3.72 (1H, m), 2.89 (2H, m), 2.26 (3H, s), 2.20–2.09 (2H, m), 1.86–1.77 (2H, m), 1.70–1.57 (2H, m); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>)  $\delta_{\text{C}}$  164.0, 151.6, 140.7, 139.7, 124.8, 119.4, 113.1, 56.9, 54.0, 46.5, 45.3, 30.8; HRMS *m/z* (ESI<sup>+</sup>) [Found: 294.1449, C<sub>14</sub>H<sub>20</sub>O<sub>4</sub>N<sub>3</sub> requires [M+H]<sup>+</sup> 294.1448]; Analytical HPLC (RP, method C) rt 9.75 mins, purity >99%. The spectroscopic data do not agree with the literature values.<sup>47</sup> However, 2D NMR analysis indicates that this is the desired compound.

#### 4-Amino-3-methoxy-*N*-(1'-methylpiperidin-4'-yl)benzamide (106)



3-Methoxy-*N*-(1-methylpiperidin-4-yl)-4-nitrobenzamide (192 mg, 0.656 mmol, 1.0 eq) was stirred in glacial AcOH (3.5 mL) for 5 min at rt. The reaction mixture was heated to 70 °C, and activated iron powder (185 mg, 3.28 mmol, 5.0 eq) was added. The reaction mixture was stirred at 70 °C for 4 h. The reaction mixture was left to cool to rt and then diluted with water (25 mL). Sat. aq. NaHCO<sub>3</sub> (~200 mL) was added slowly to obtain pH 9. The mixture was extracted with EtOAc (3 × 150 mL). The combined organic components were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The aqueous component contained some desired product by TLC analysis and was therefore basified with aq. 1M NaOH to give pH 11 and extracted with EtOAc (3 × 130 mL). Some NaCl was added to assist phase separation. The organic components were combined, and concentrated. The crude was purified by flash chromatography (elution with 0–10% polar eluent (MeOH with 10% NH<sub>4</sub>OH solution) / CH<sub>2</sub>Cl<sub>2</sub>, 16 CV) to give the title compound as a light yellow solid (156 mg, 90%): *R*<sub>f</sub> 0.12 (10% polar eluent (MeOH with 10% NH<sub>4</sub>OH solution) / CH<sub>2</sub>Cl<sub>2</sub>); m.p. 198–200 °C (dec.) (from CH<sub>2</sub>Cl<sub>2</sub>) [lit.<sup>51</sup> 215 °C (dec)];  $\tilde{\nu}_{\text{max}}$  (thin film)/cm<sup>-1</sup> 3335 (N-H, m, br), 2980 (C-H, s), 2889 (C-H, m), 1616 (C=O, m), 1509 (m), 1380 (m), 1151 (m); <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>)  $\delta_{\text{H}}$  7.79 (1H, d, *J* 7.8), 7.33–7.26 (2H, m), 6.59 (1H, d, *J* 8.6), 5.21 (2H, s), 3.80 (3H, s), 3.75–3.64 (1H, m), 2.82–2.70 (2H, m), 2.15 (3H, s), 1.92 (2H, td, *J* 11.8, 2.5), 1.78–1.67 (2H, m), 1.62–1.47 (2H, m); HRMS *m/z* (ESI<sup>+</sup>) [Found: 264.1704, C<sub>14</sub>H<sub>22</sub>O<sub>2</sub>N<sub>3</sub> requires [M+H]<sup>+</sup> 264.1707]; LRMS *m/z* (ESI<sup>+</sup>) 264.2 ([M+H]<sup>+</sup>, 55%); Analytical HPLC (RP, method C) rt 8.4 mins, 96%. The spectroscopic data are in good agreement with the literature values.<sup>47</sup>

#### 4-[[*(3R)*-4-Cyclopentyl-3-ethyl-1-methyl-2-oxo-1,2,3,4-tetrahydropyrido[2,3-*b*]pyrazin-6-yl]amino]-3-methoxy-*N*-(1-methylpiperidin-4-yl)benzamide (39)

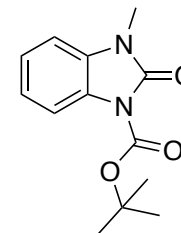


(*3R*)-6-Chloro-4-cyclopentyl-3-ethyl-1-methyl-3,4-dihydropyrido[2,3-*b*]pyrazin-2(*1H*)-one (**38**) (20.0 mg, 0.0682 mmol, 1.0 eq), and 4-amino-3-methoxy-*N*-(1-methylpiperidin-4-yl)benzamide (**106**) (20.3 mg),

0.0819 mmol, 1.2 eq) were dissolved in degassed <sup>t</sup>BuOH (0.68 mL). Pd<sub>2</sub>dba<sub>3</sub> (3.5 mg, 0.00341 mmol, 0.05 eq), XPhos (5.0 mg, 0.0102 mmol, 0.15 eq), and K<sub>2</sub>CO<sub>3</sub> (37.0 mg, 0.272 mmol, 4.0 eq) were added, and the reaction mixture was heated at 100 °C for 21 h. The reaction mixture was left to cool, and then filtered through celite, washing with CH<sub>2</sub>Cl<sub>2</sub> (150 mL) and EtOAc (100 mL). The crude was purified by flash chromatography (elution with 0–8% polar eluent (MeOH with 10% NH<sub>4</sub>OH solution) / CH<sub>2</sub>Cl<sub>2</sub>, 15 CV) to give 4-[(3*R*)-4-cyclopentyl-3-ethyl-1-methyl-2-oxo-1,2,3,4-tetrahydropyrido[2,3-*b*]pyrazin-6-yl]amino}-3-methoxy-*N*-(1-methylpiperidin-4-yl)benzamide (**39**) as a yellow solid (9 mg, 25%): *R*<sub>f</sub> 0.26 (10% polar eluent (MeOH with 10% NH<sub>4</sub>OH solution) / CH<sub>2</sub>Cl<sub>2</sub>); [ $\alpha$ ]<sub>D</sub><sup>25</sup> –131.2 (c 0.5, MeOH); m.p. 116–117 °C (from MeOH);  $\tilde{\nu}_{\text{max}}$  (thin film)/cm<sup>–1</sup> 3335 (N-H, w, br), 2980 (C-H, s), 2888 (C-H, m), 1653 (C=O, m), 1608 (C=O, m), 1474 (s), 1395 (m); <sup>1</sup>H NMR (500 MHz; CD<sub>3</sub>OD)  $\delta_{\text{H}}$  8.42 (1H, d, *J* 8.2), 7.49–7.22 (2H, m), 7.23 (1H, d, *J* 8.4), 6.43 (1H, d, *J* 8.4), 4.65–4.47 (1H, m), 4.09 (1H, dd, *J* 8.8, 4.7), 3.99 (3H, s), 3.94–3.87 (1H, m), 3.32 (3H, s), 3.00–2.86 (2H, m), 2.33 (3H, s), 2.26–2.16 (2H, m), 2.15–2.00 (2H, m), 1.98–1.92 (2H, m), 1.90–1.48 (10H, m), 0.88 (3H, t, *J* 7.5); <sup>13</sup>C NMR (126 MHz; CD<sub>3</sub>OD)  $\delta_{\text{C}}$  169.5, 167.1, 151.0, 148.5, 147.3, 136.0, 126.1, 124.8, 121.4, 118.2, 116.3, 110.1, 101.1, 60.9, 59.5, 56.4, 55.8, 48.2, 46.15, 32.4, 31.37, 31.35, 29.3, 25.9, 24.5, 23.9, 10.2; HRMS *m/z* (ESI<sup>+</sup>) [Found: 521.3232, C<sub>29</sub>H<sub>41</sub>O<sub>3</sub>N<sub>6</sub> requires [M+H]<sup>+</sup> 521.3235]; LRMS *m/z* (ESI<sup>+</sup>) 521.3 ([M+H]<sup>+</sup>, 100%); Analytical HPLC (RP, method C) rt 12.1 mins, purity >99%. The spectroscopic data are in good agreement with the literature values.<sup>50</sup>

# SI-G: NOVEL COMPOUNDS NMRS AND LCMS

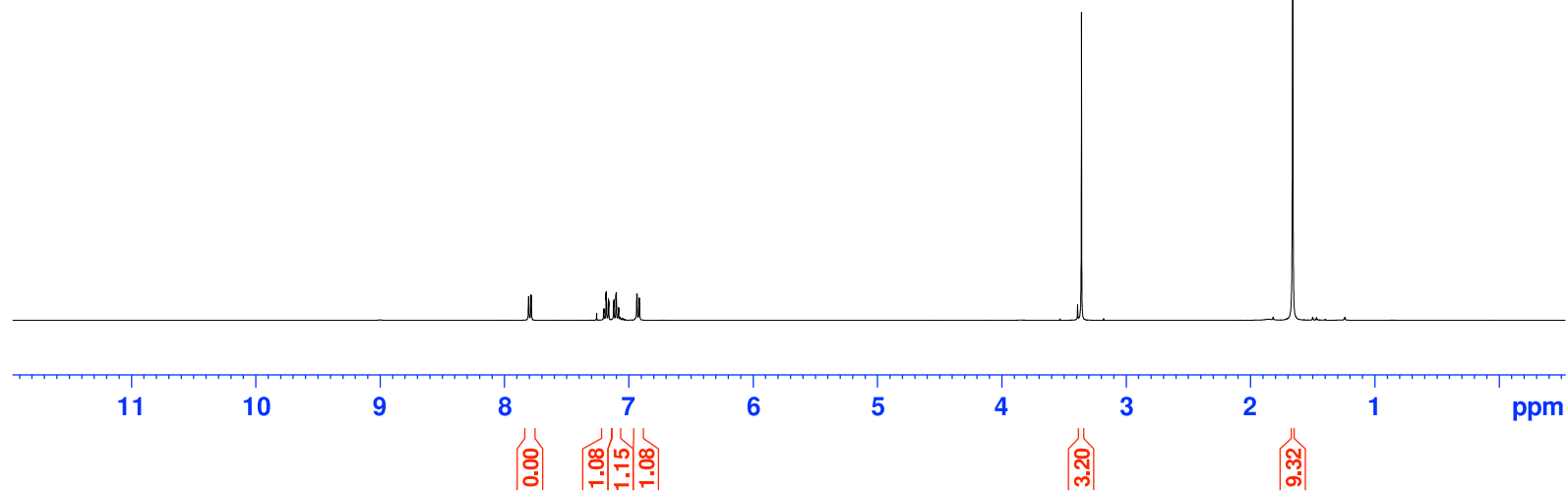
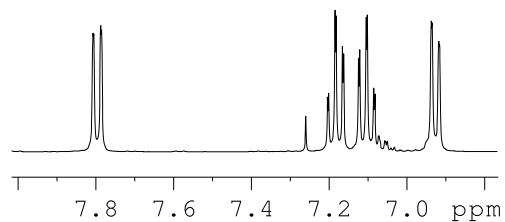
## 1-Methyl-3-(*tert*-butyloxycarbonyl)benzimidazol-2-one (16)



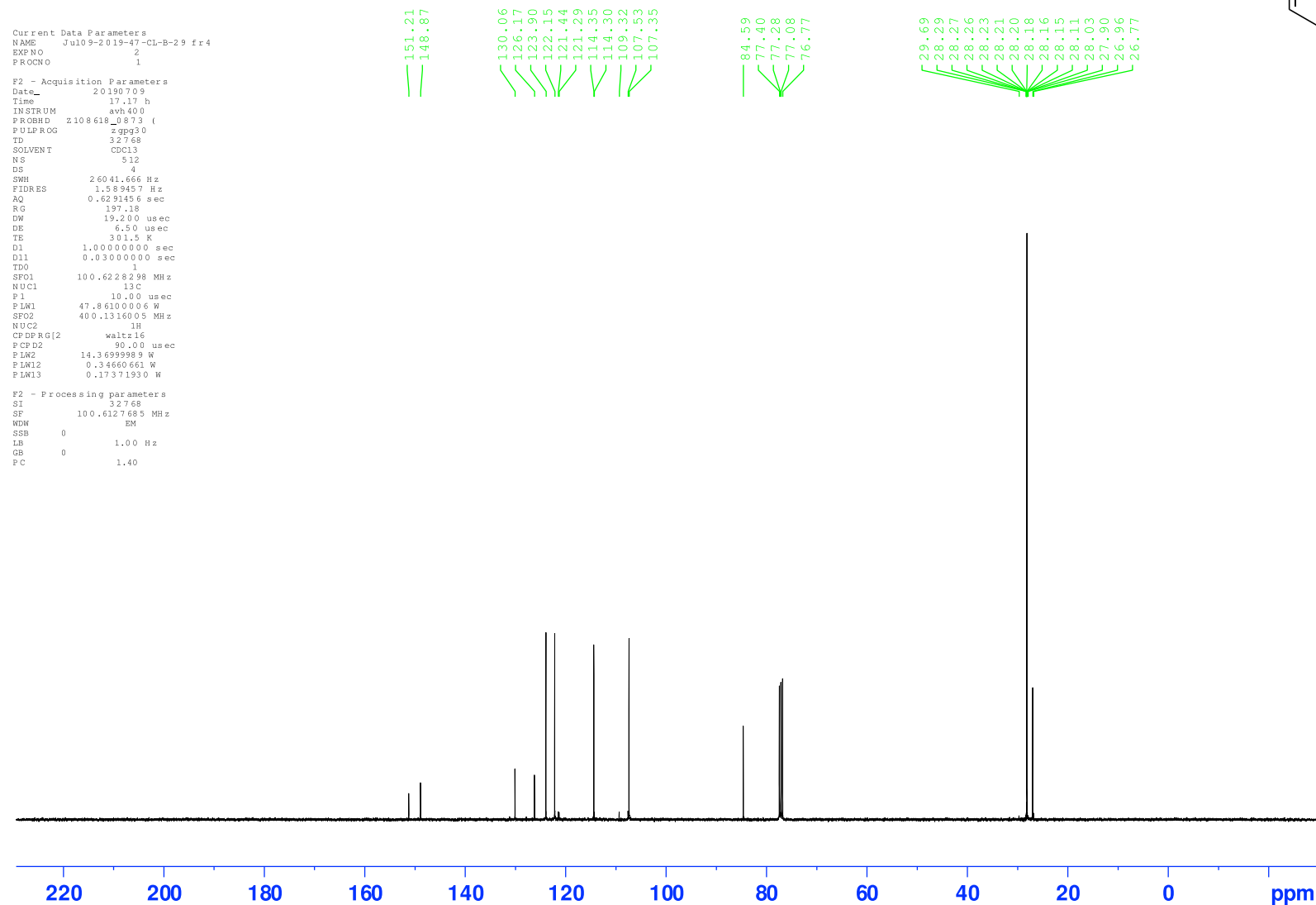
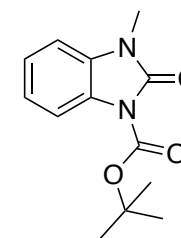
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FIDRES 0.244532 Hz  
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RG 34.15  
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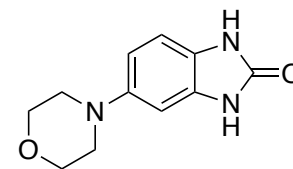
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PC 1.00



# Methyl-3-(*tert*-butoxycarbonyl)benzimidazol-2-one (16)



# 5-Morpholino-1,3-dihydro-2H-benzo[d]imidazol-2-one (13)

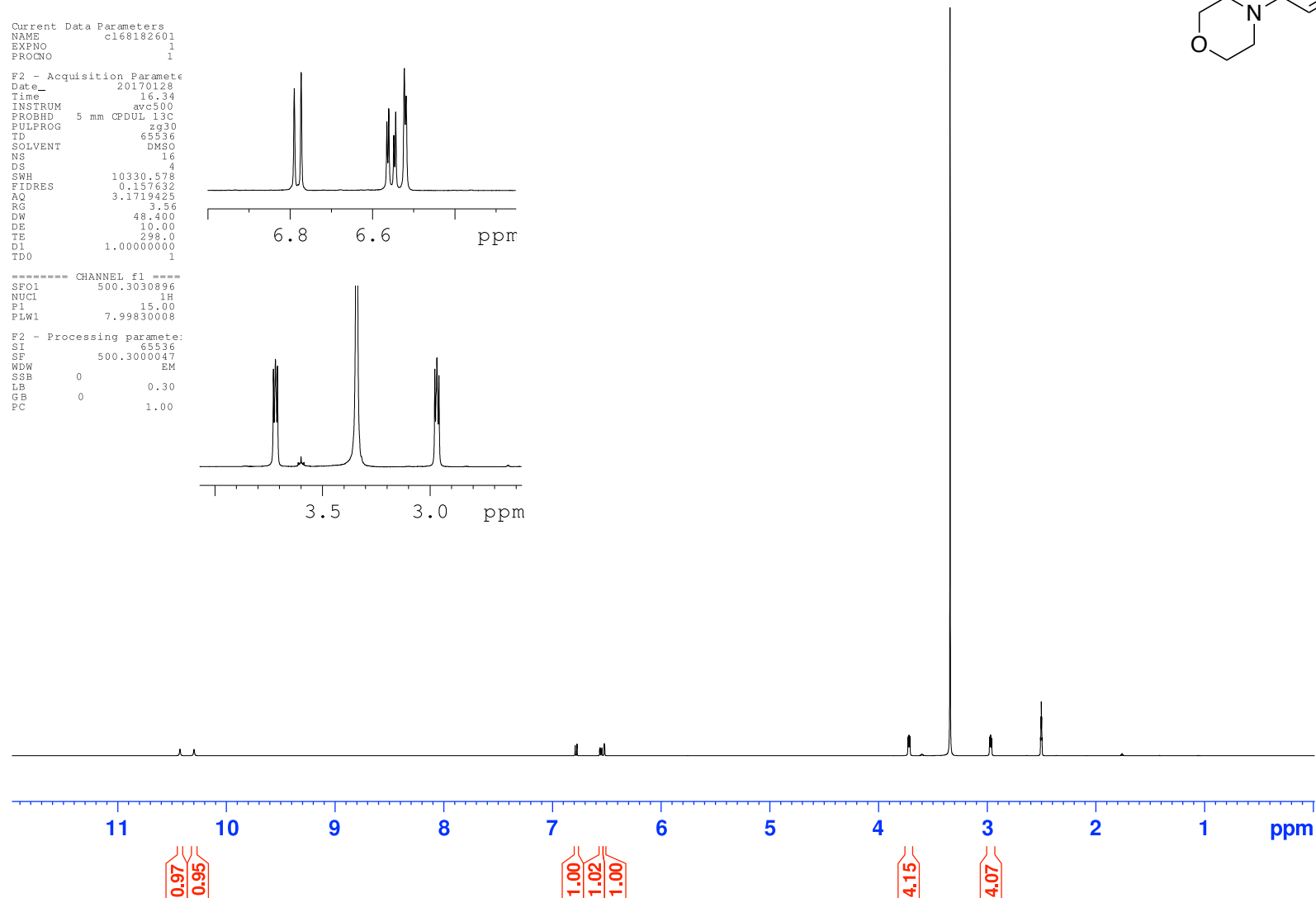
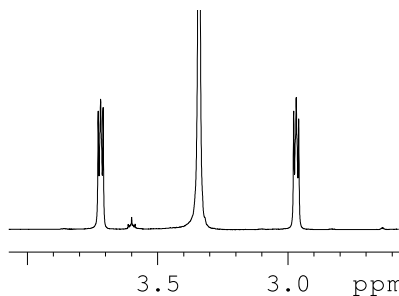
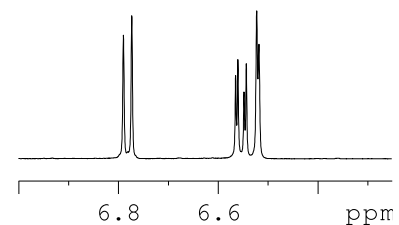


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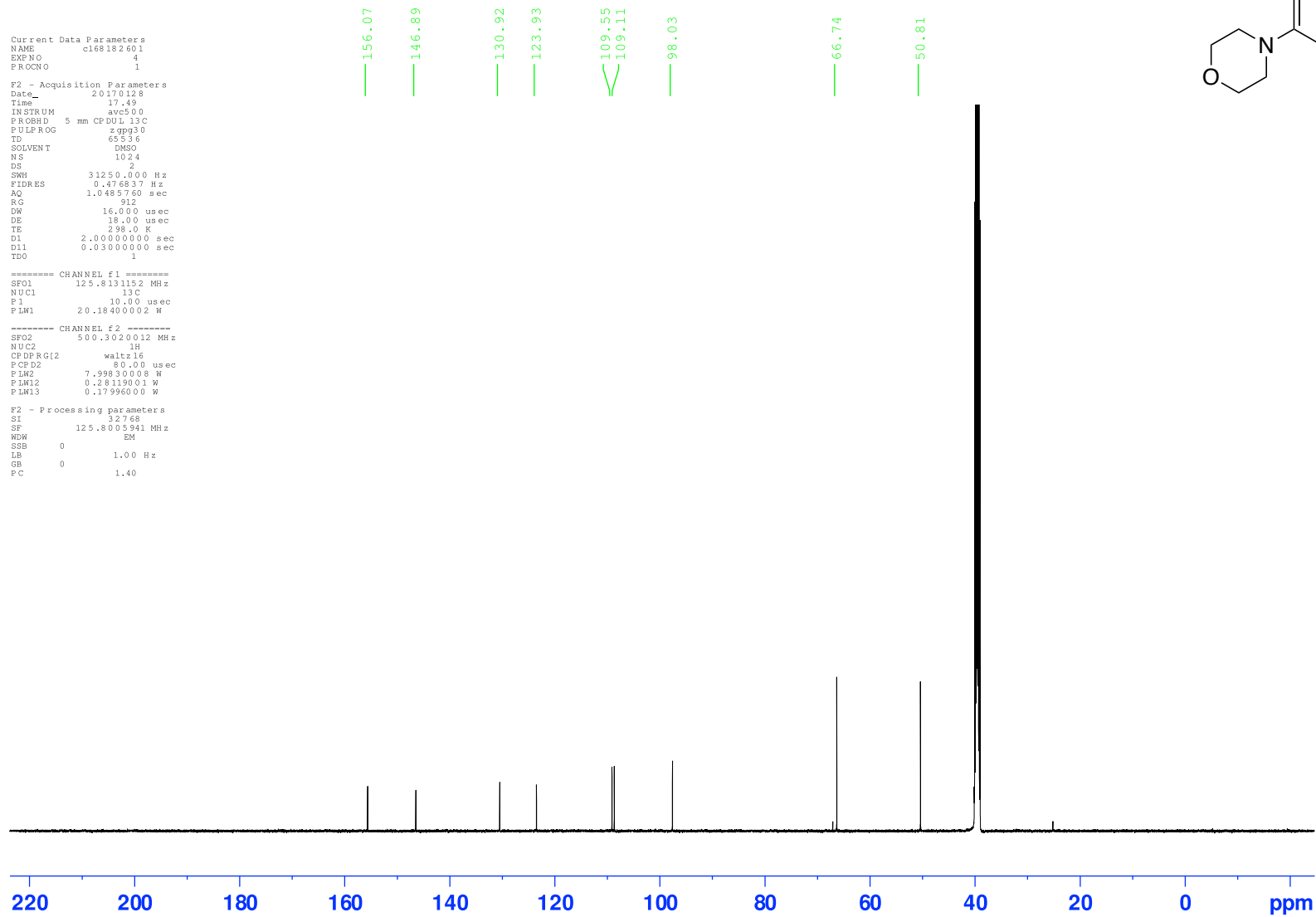
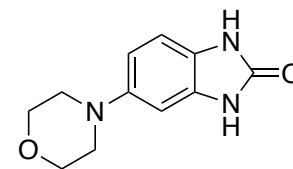
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TE 298.0  
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PLW1 7.99830008

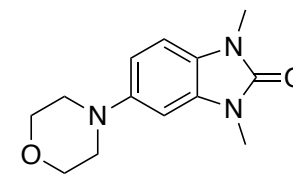
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# 5-Morpholino-1,3-dihydro-2H-benzo[d]imidazol-2-one (13)



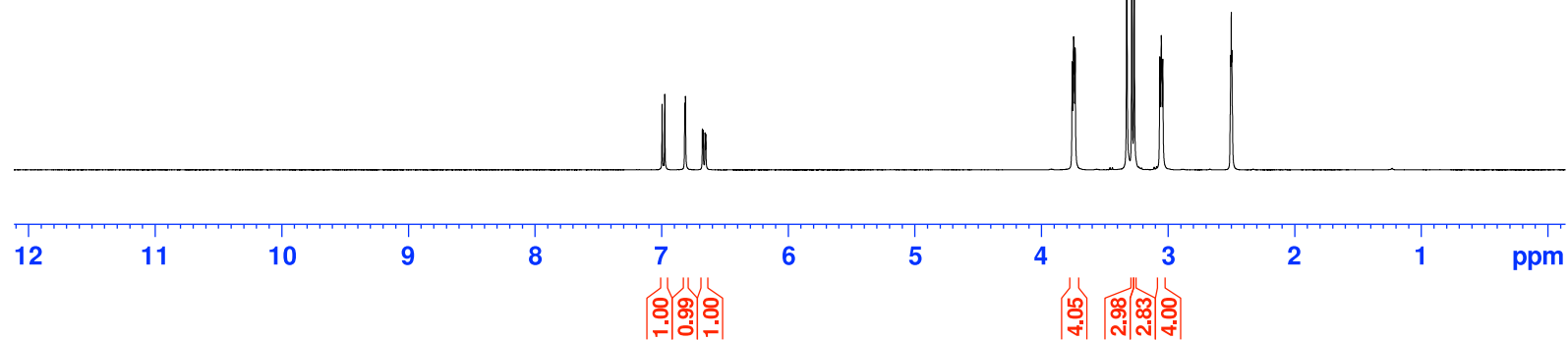
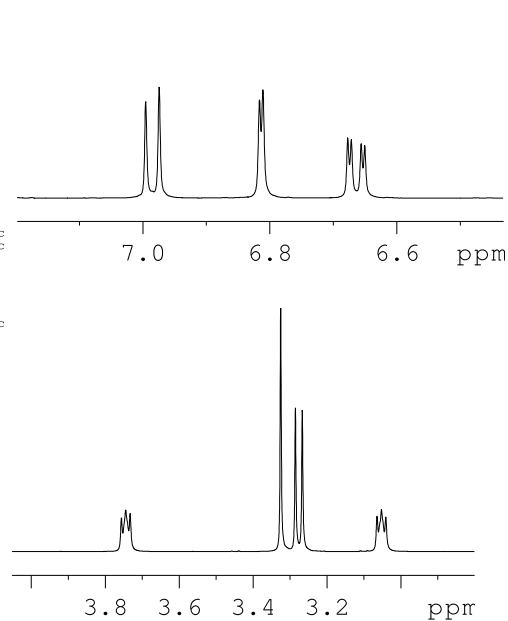
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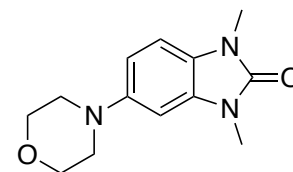
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FIDRES 0.244532 Hz  
AQ 4.0894465 sec  
RG 113.46  
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DE 6.50 usec  
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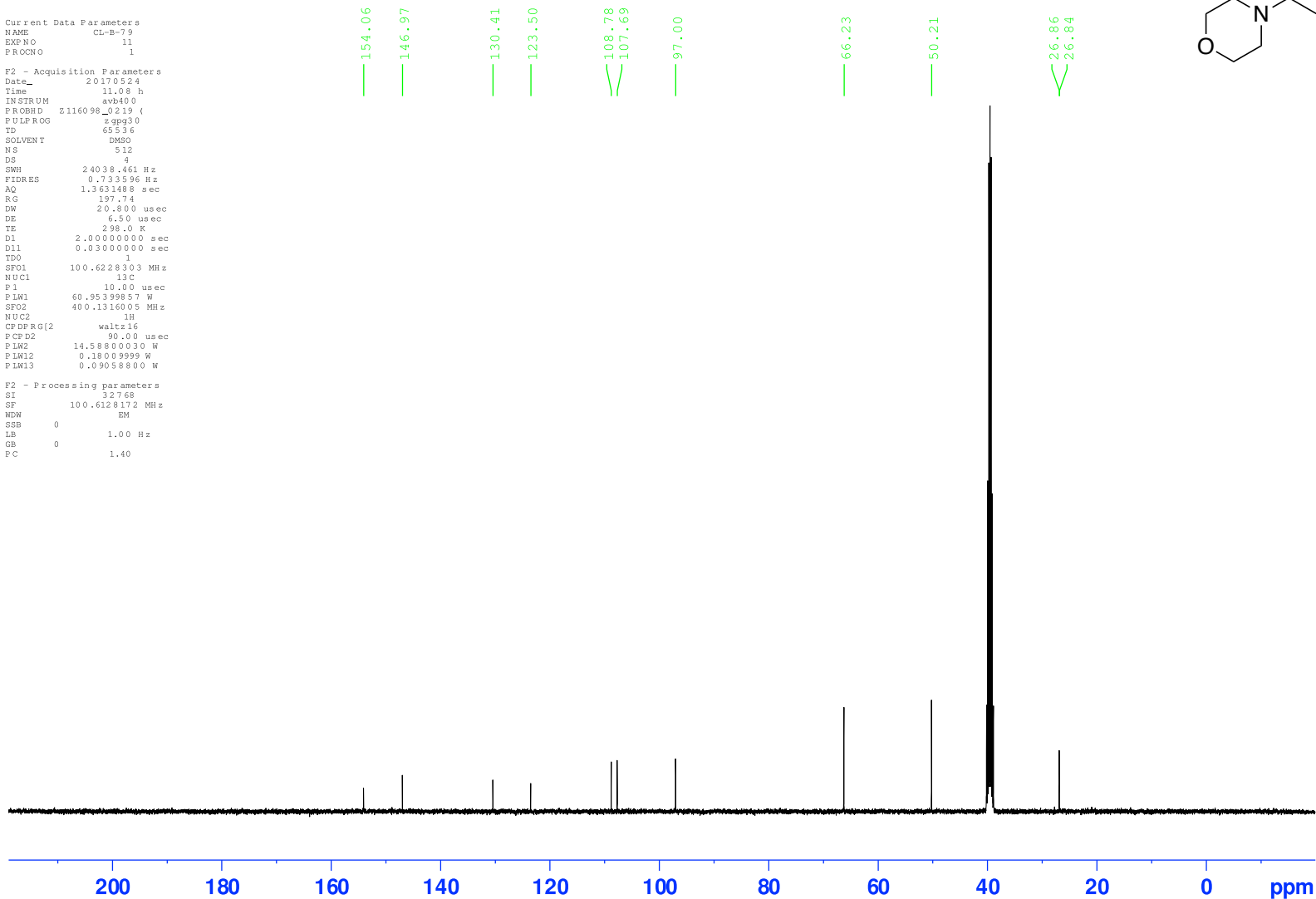
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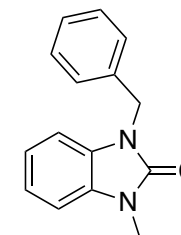
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CPDPRG2 waltz16  
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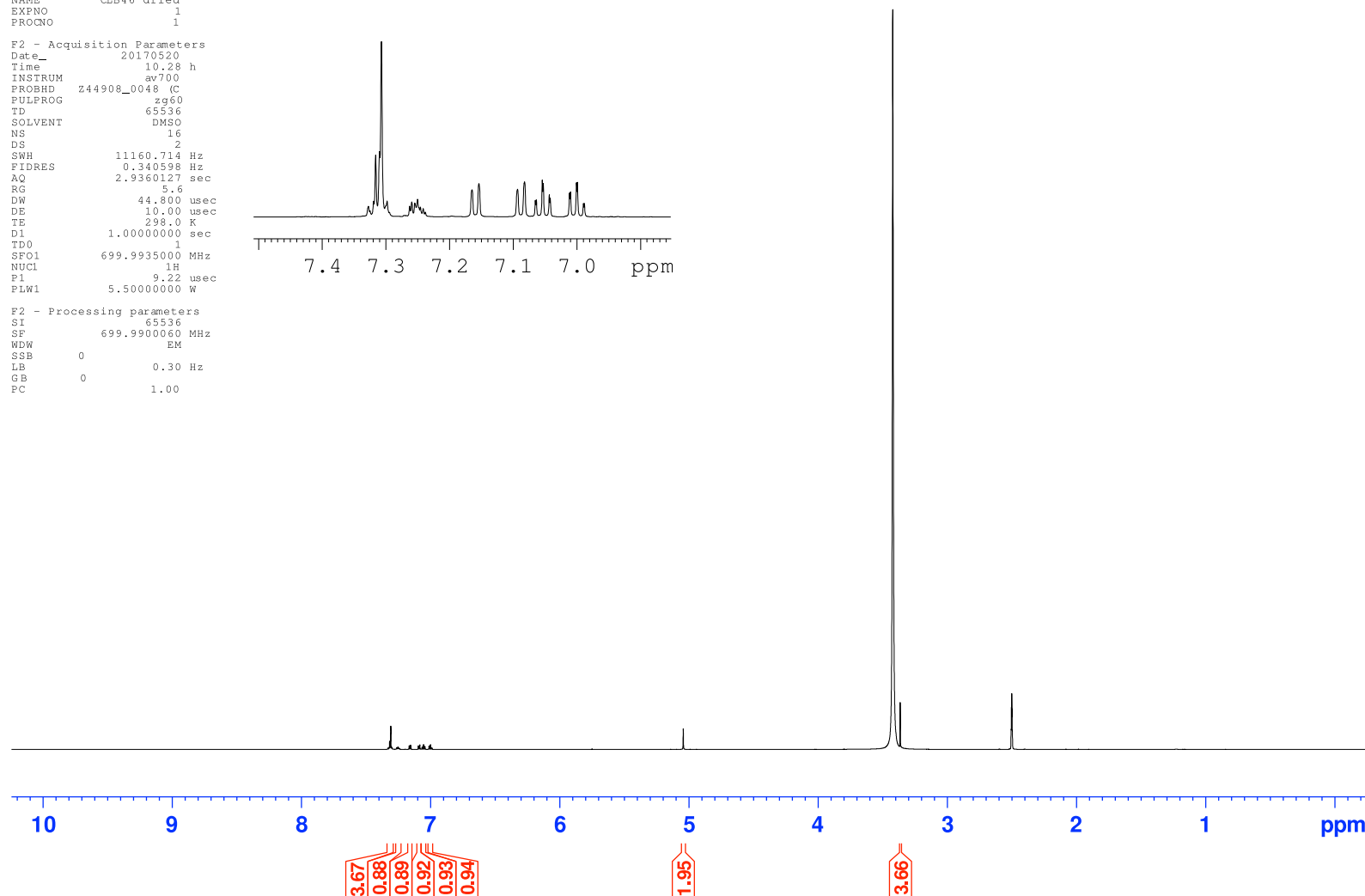
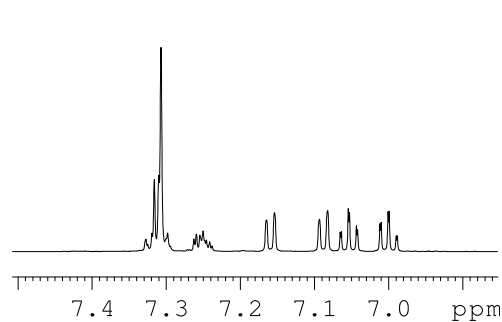
# 1-Benzyl-3-methyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (17)



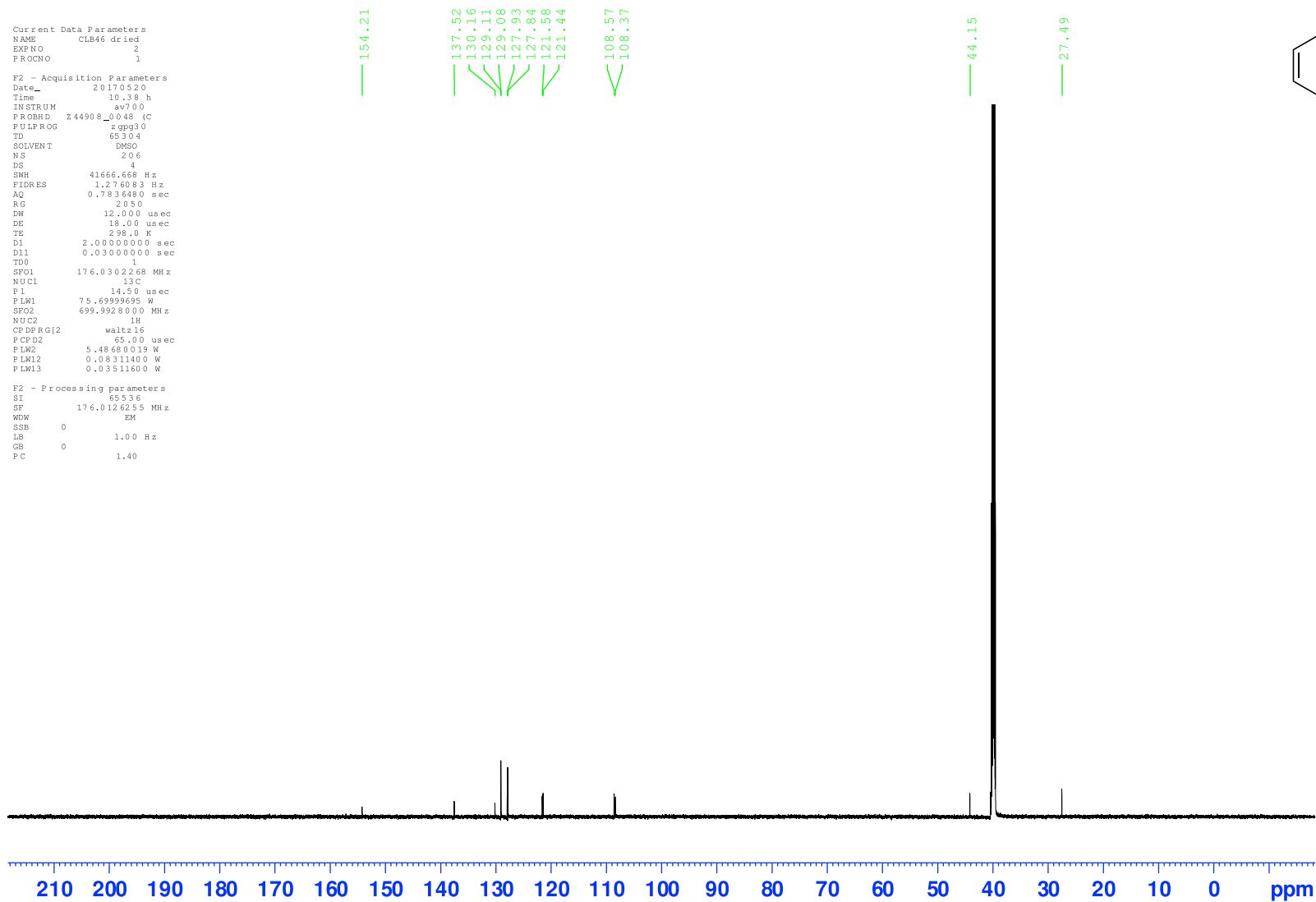
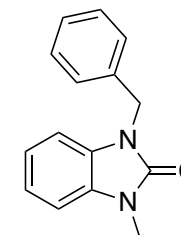
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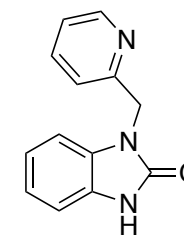
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# 1-Benzyl-3-methyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (17)



# 1'-(Pyridin-2'-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (21)

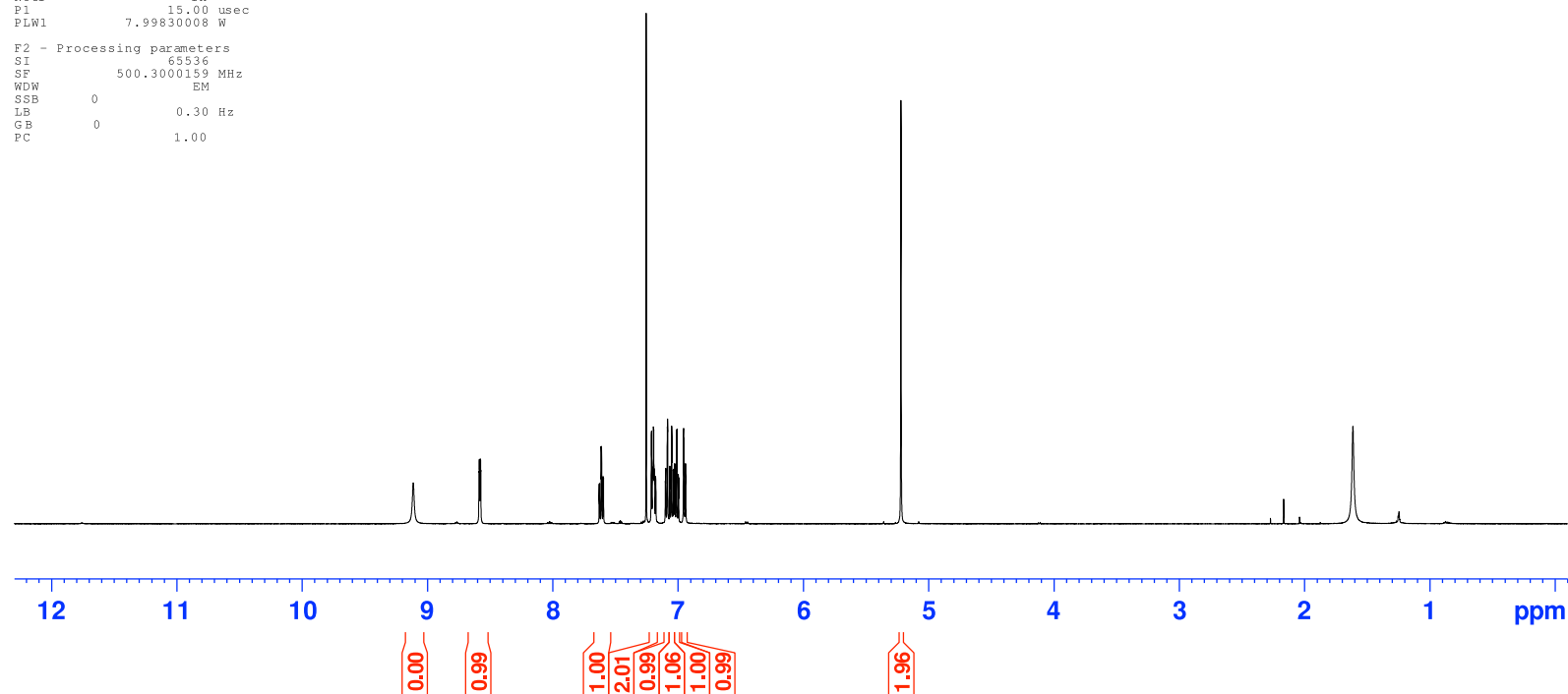
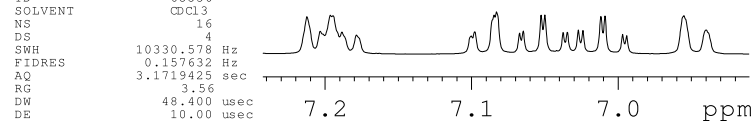


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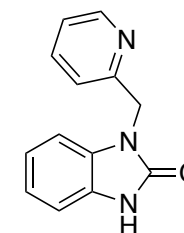
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# **1'-(Pyridin-2'-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (21)**



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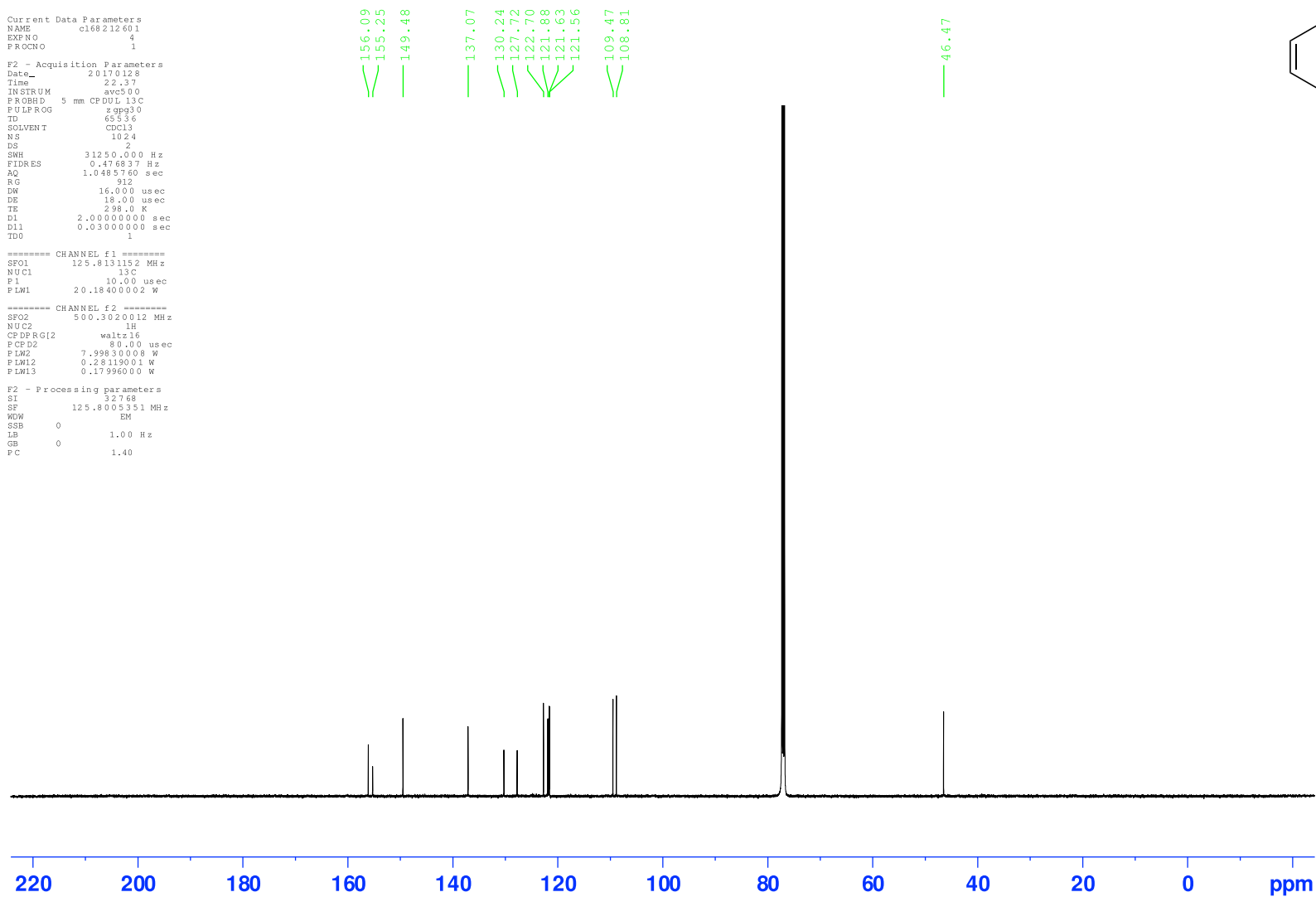
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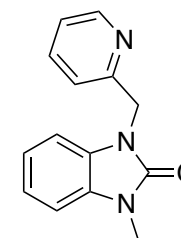
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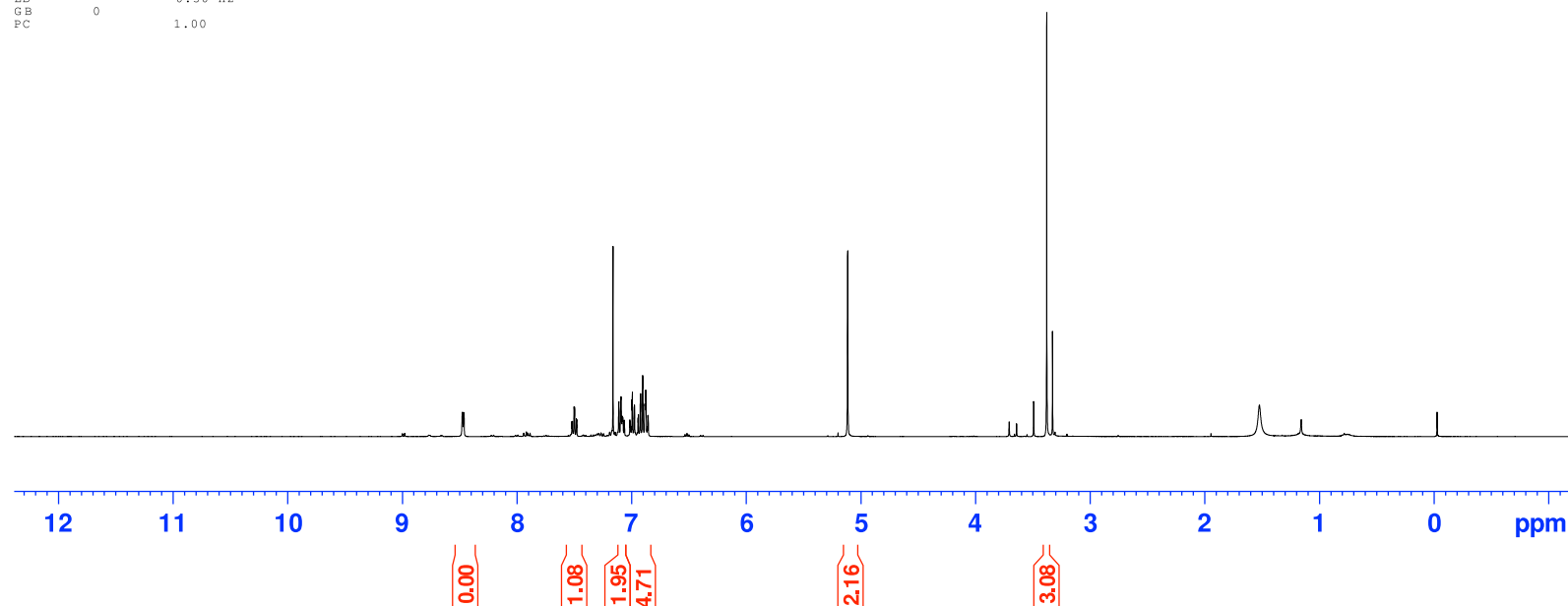
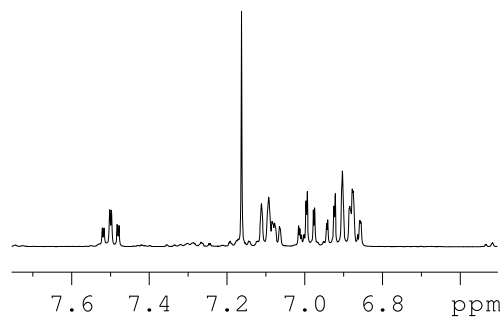
# 1-Methyl-3-(pyridin-2-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (18)



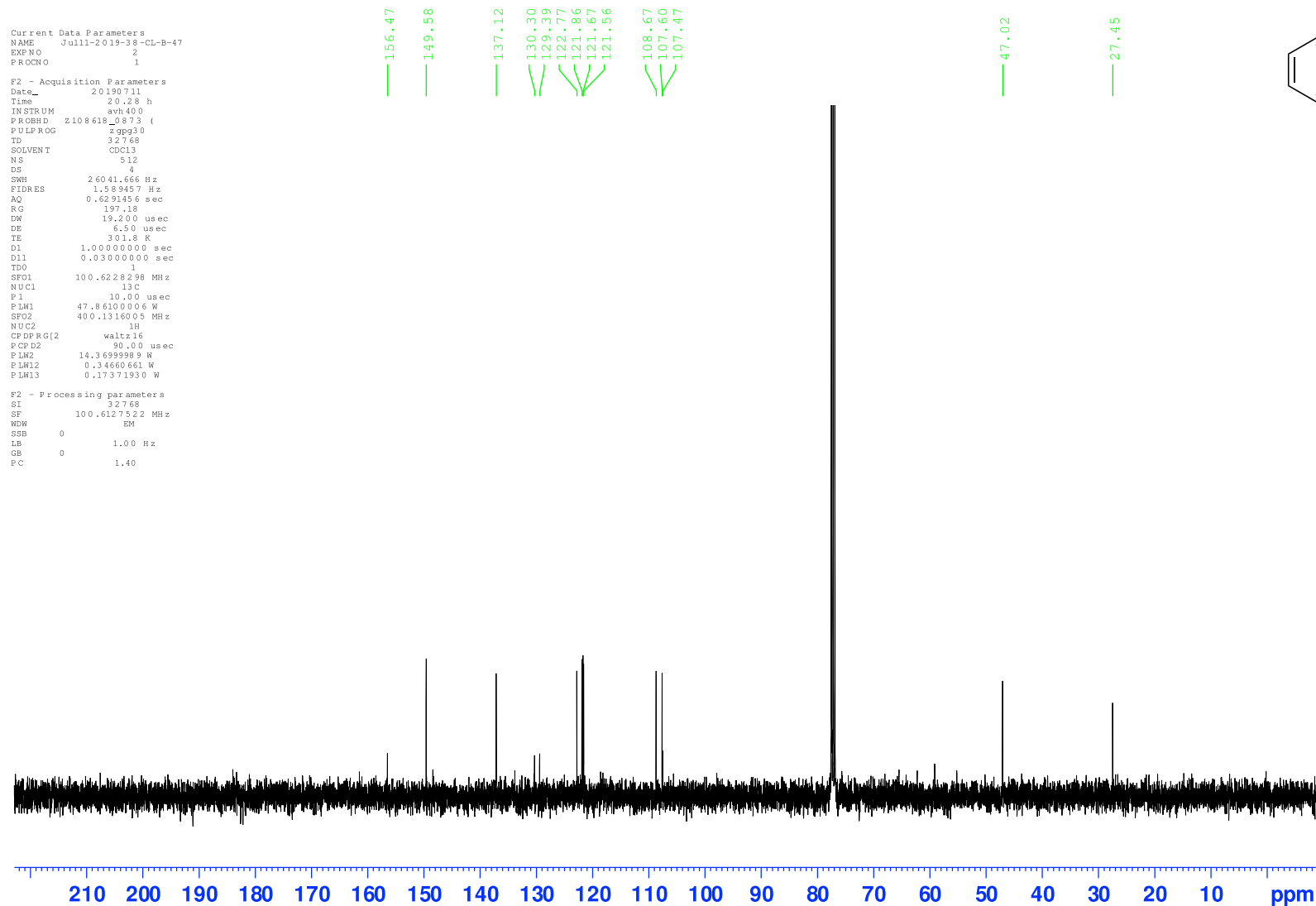
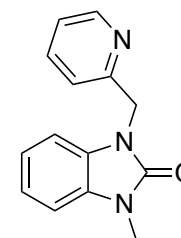
Current Data Parameters  
NAME Jul11-2019-38-CL-B-47  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190711  
Time 17.28 h  
INSTRUM avh400  
PROBHD Z108618\_0873 (   
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 2  
SWH 8012.820 Hz  
FIDRES 0.244532 Hz  
AQ 4.0894465 sec  
RG 88.17  
DW 62.400 usec  
DE 6.50 usec  
TE 300.6 K  
D1 1.00000000 sec  
TD0 1  
SFO1 400.1324008 MHz  
NUC1 1H  
P1 14.00 usec  
PLW1 14.36999989 W

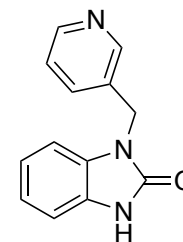
F2 - Processing parameters  
SI 32768  
SF 400.1300491 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



# 1-Methyl-3-(pyridin-2-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (18)



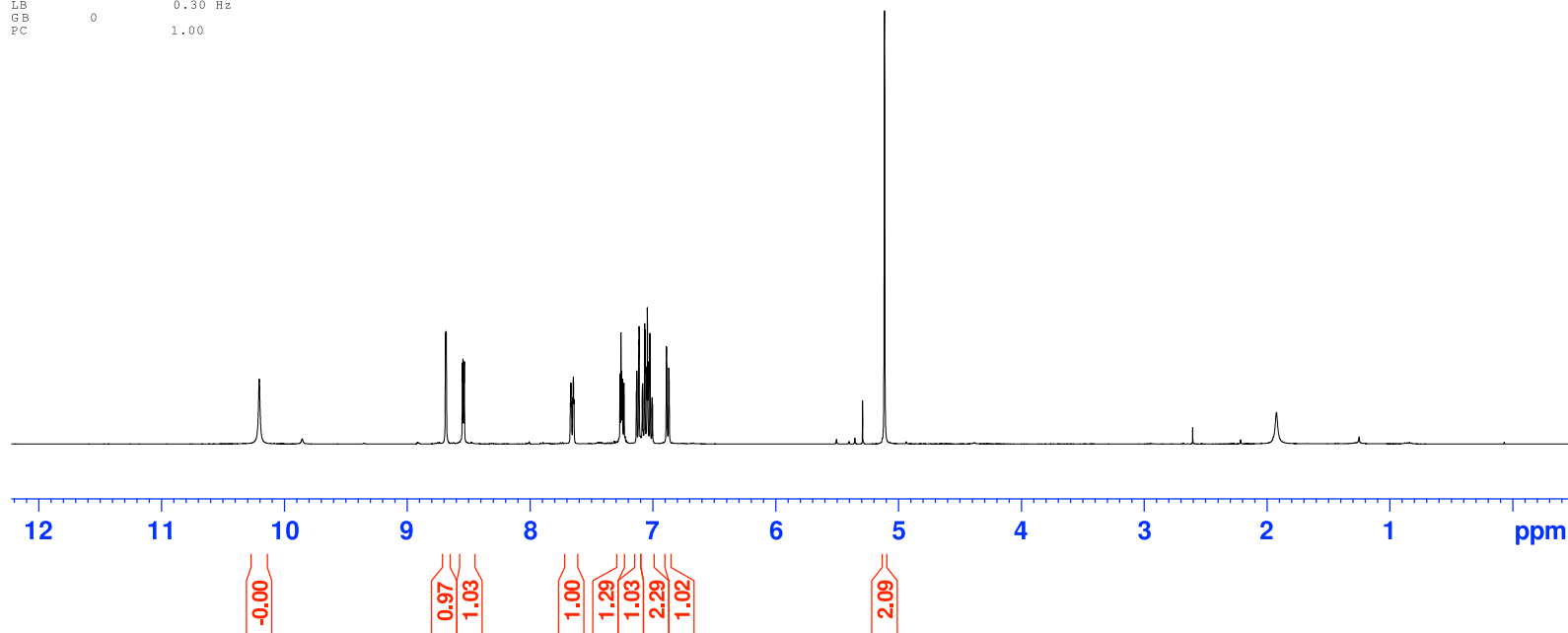
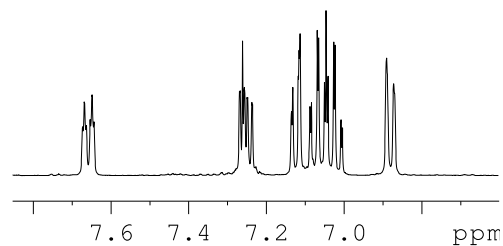
# 1-(Pyridin-3-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (22)



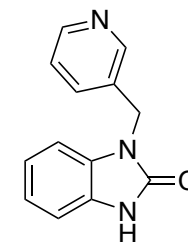
Current Data Parameters  
NAME Feb03-2017-40-CL-B-44 fr6-  
EXPNO 10  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20170203  
Time 23.59 h  
INSTRUM avh400  
PROBHD Z108618\_0873 (   
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 2  
SWH 8012.820 Hz  
FIDRES 0.244532 Hz  
AQ 4.0894465 sec  
RG 88.17  
DW 62.400 usec  
DE 6.50 usec  
TE 296.1 K  
D1 1.00000000 sec  
TD0 1  
SFO1 400.1324008 MHz  
NUC1 1H  
P1 14.00 usec  
PLW1 14.36999989 W

F2 - Processing parameters  
SI 32768  
SF 400.1300098 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



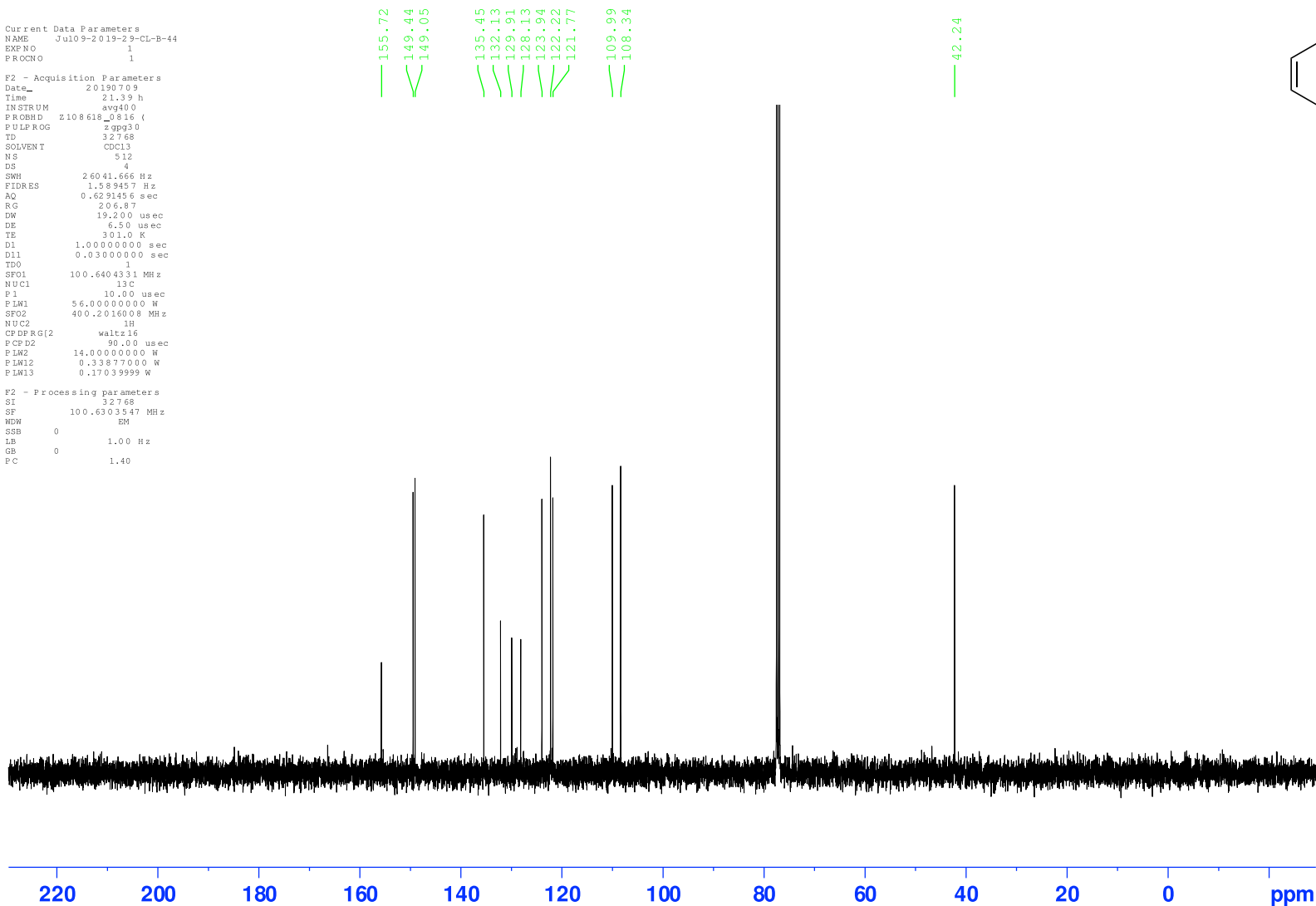
# 1-(Pyridin-3-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (22)



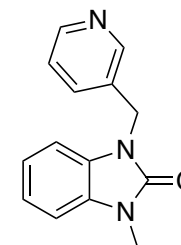
Current Data Parameters  
NAME Jul09-2019-29-CL-B-44  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190709  
Time 21:39 h  
INSTRUM avg400  
PROBHD Z108618\_0816 (   
PULPROG zgpg30  
TD 32768  
SOLVENT CDCl3  
NS 512  
DS 4  
SWH 26041.666 Hz  
FIDRES 1.589457 Hz  
AQ 0.6291456 sec  
RG 206.87  
DW 19.200 usec  
DE 6.50 usec  
TE 301.0 K  
D1 1.00000000 sec  
D11 0.03000000 sec  
TDO 1  
SFO1 100.6404331 MHz  
NUC1 13C  
P1 10.00 usec  
PLW1 56.00000000 W  
SFO2 400.2016008 MHz  
NUC2 1H  
CPDPRG2 waltz16  
PCPD2 90.00 usec  
PLW2 14.00000000 W  
PLW12 0.33877000 W  
PLW13 0.17039999 W

F2 - Processing parameters  
SI 32768  
SF 100.6303547 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40



# 1-Methyl-3-(pyridin-3-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (19)

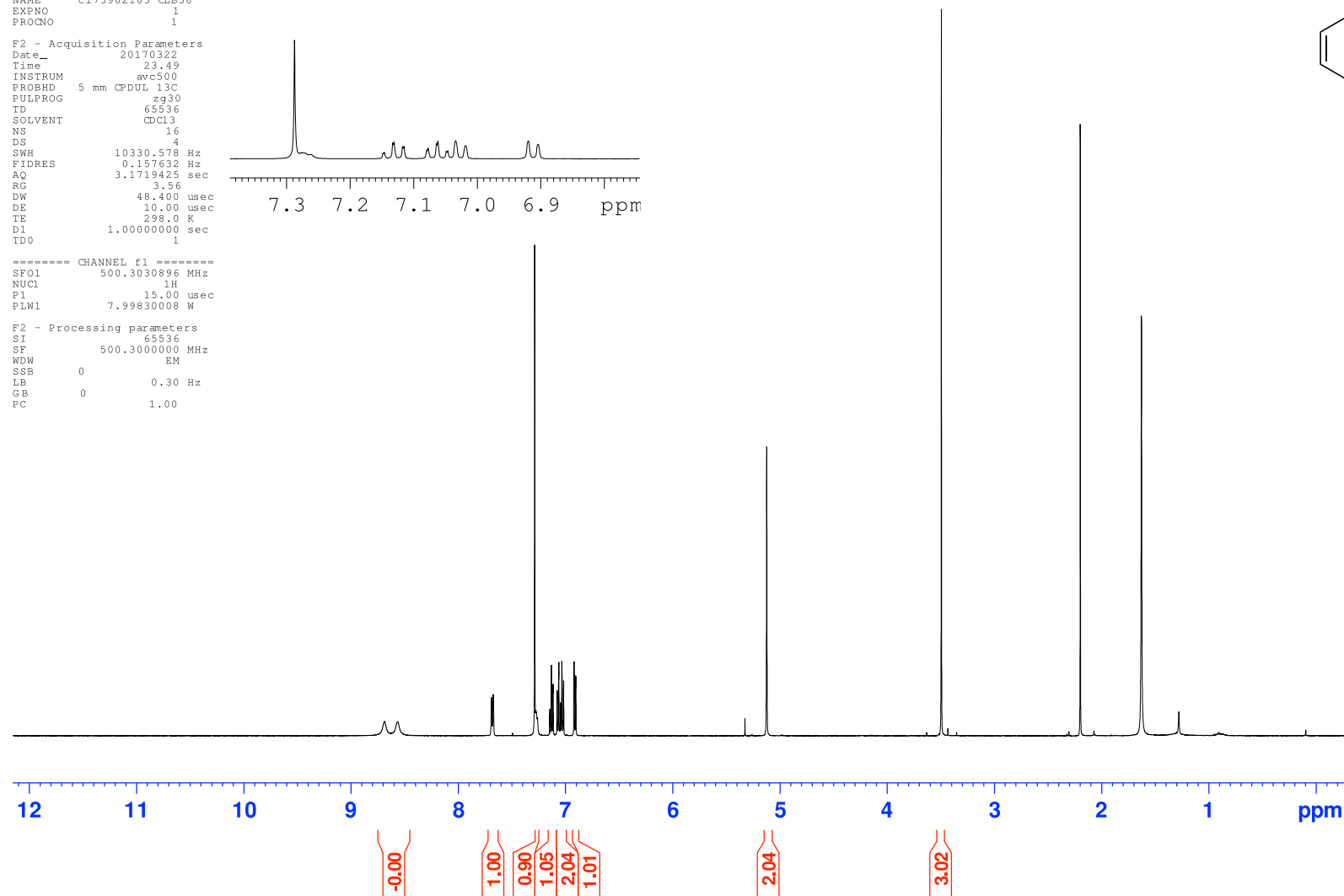


Current Data Parameters  
NAME c173902103 CLB58  
EXPNO 1  
PROCNO 1

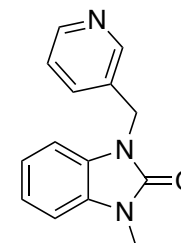
F2 - Acquisition Parameters  
Date\_ 20170322  
Time 23.49  
INSTRUM avc500  
PROBHD 5 mm CPDUL 13C  
PULPROG zg30  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 4  
SWH 10330.579 Hz  
FIDRES 0.157632 Hz  
AQ 3.1719425 sec  
RG 3.56  
DW 48.400 usec  
DE 10.00 usec  
TE 298.0 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
SF01 500.3030896 MHz  
NUC1 1H  
P1 15.00 usec  
PLW1 7.99830008 W

F2 - Processing parameters  
SI 65536  
SF 500.3000000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



# 1-Methyl-3-(pyridin-3-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (19)



```

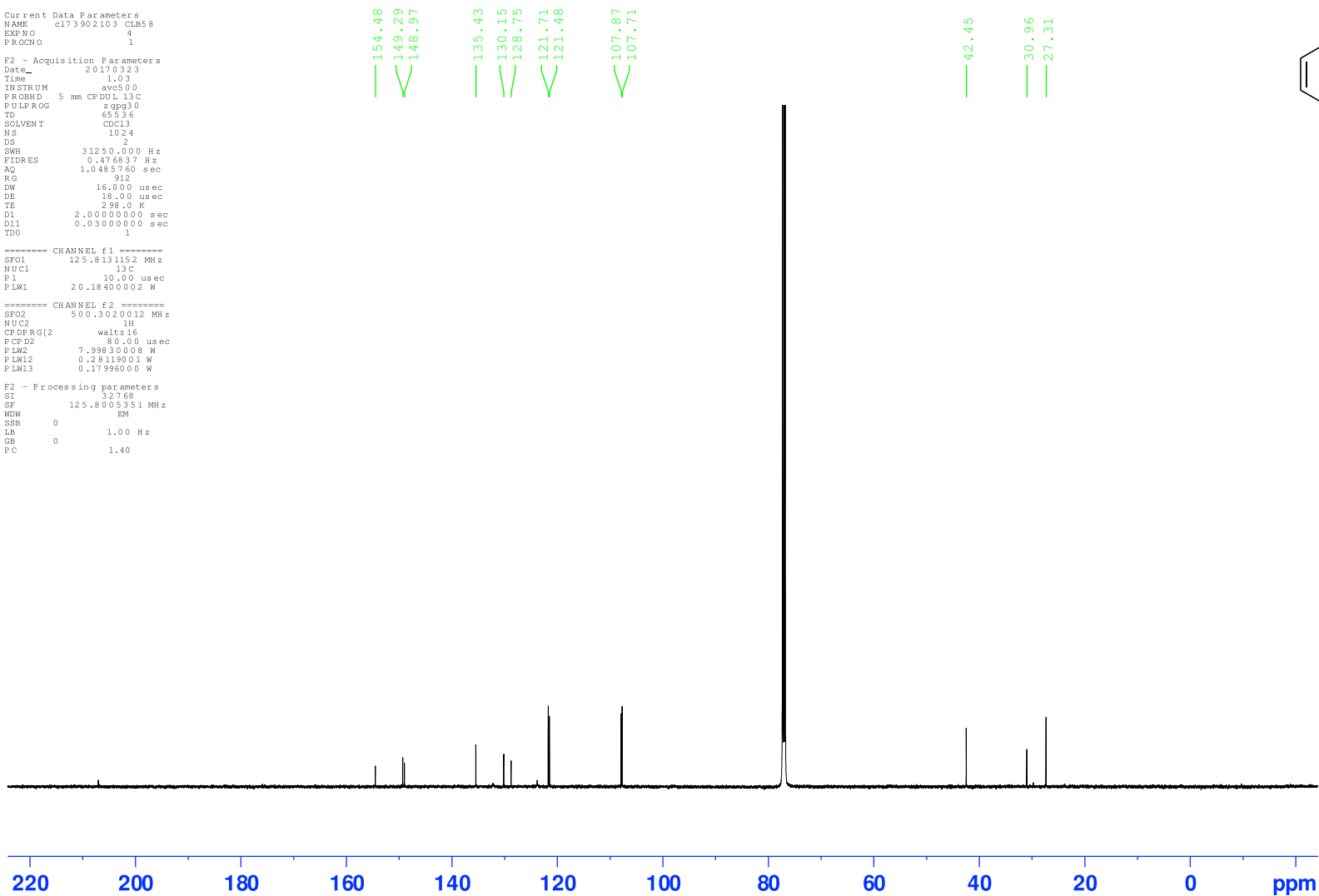
Current Data Parameters
NAME      c173902103 CLB58
EXP NO    4
PROCNO    1

F2 - Acquisition Parameters
Date_     20170323
Time      1.03
INSTRUM   ave500
PROBHD    5 mm CPD113C
PULPROG   zgpg30
TD        65536
SOLVENT   CDCl3
NS        1024
DS        2
SWH       31250.000 Hz
FIDRES    0.476837 Hz
AQ        1.0485760 sec
RG        912
DW        16.000 usec
DE        18.000 usec
TE        298.0 K
D1        2.00000000 sec
D11       0.03000000 sec
TDO       1

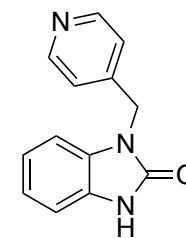
===== CHANNEL f1 =====
SFO1      125.8131152 MHz
NUC1      13C
P1        10.00 usec
PLW1      20.18400002 W

===== CHANNEL f2 =====
SFO2      500.3020012 MHz
NUC2      1H
CPDPRG2   waltz16
PCPD2     80.00 usec
PLM2      7.99830008 W
PLW12     0.28119001 W
PLW13     0.17996000 W

F2 - Processing parameters
SI        32768
SF        125.8005351 MHz
WDW       EM
SSB       0
LB        1.00 Hz
GB        0
PC        1.40
    
```



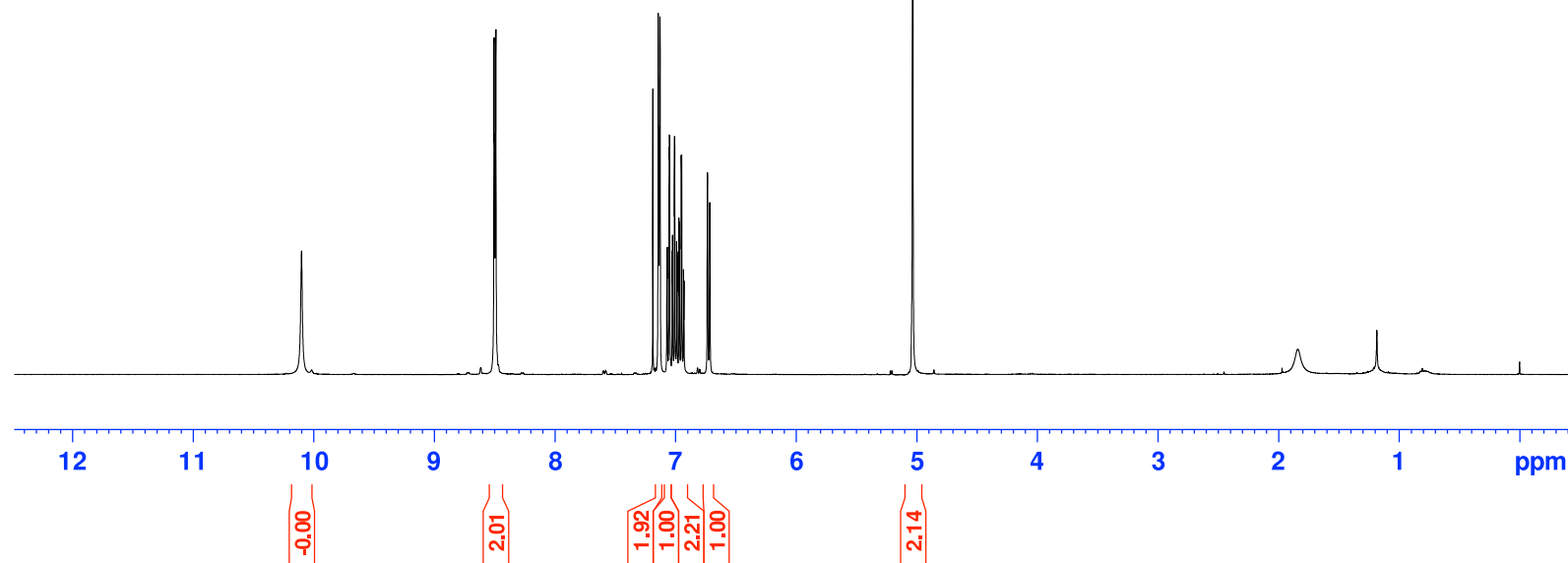
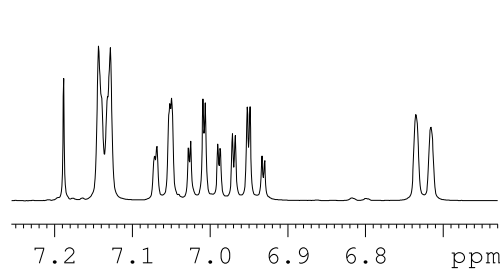
# 1-(Pyridin-4'-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (23)



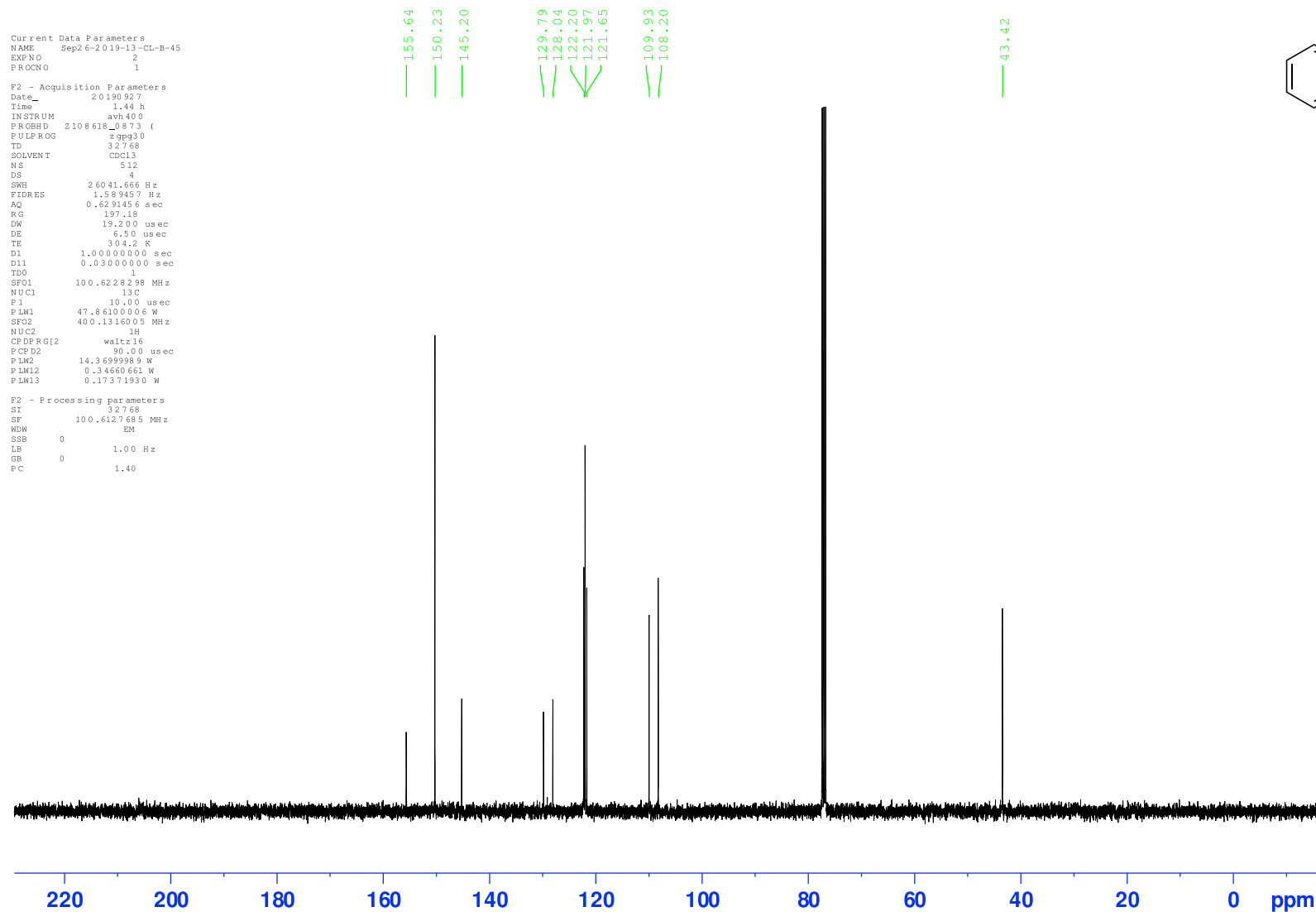
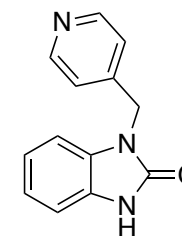
Current Data Parameters  
NAME Sep26-2019-13-CL-B-45  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190927  
Time 1.28 h  
INSTRUM avh400  
PROBHD Z108618\_0873 (   
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 2  
SWH 8012.820 Hz  
FIDRES 0.244532 Hz  
AQ 4.0894465 sec  
RG 88.17  
DW 62.400 usec  
DE 6.50 usec  
TE 303.2 K  
D1 1.00000000 sec  
TD0 1  
SFO1 400.1324008 MHz  
NUC1 1H  
P1 14.00 usec  
PLW1 14.36999989 W

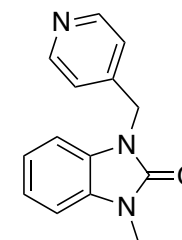
F2 - Processing parameters  
SI 32768  
SF 400.1300385 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



# 1-(Pyridin-4'-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (23)



# 1-Methyl-3-(pyridin-4'-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (20)

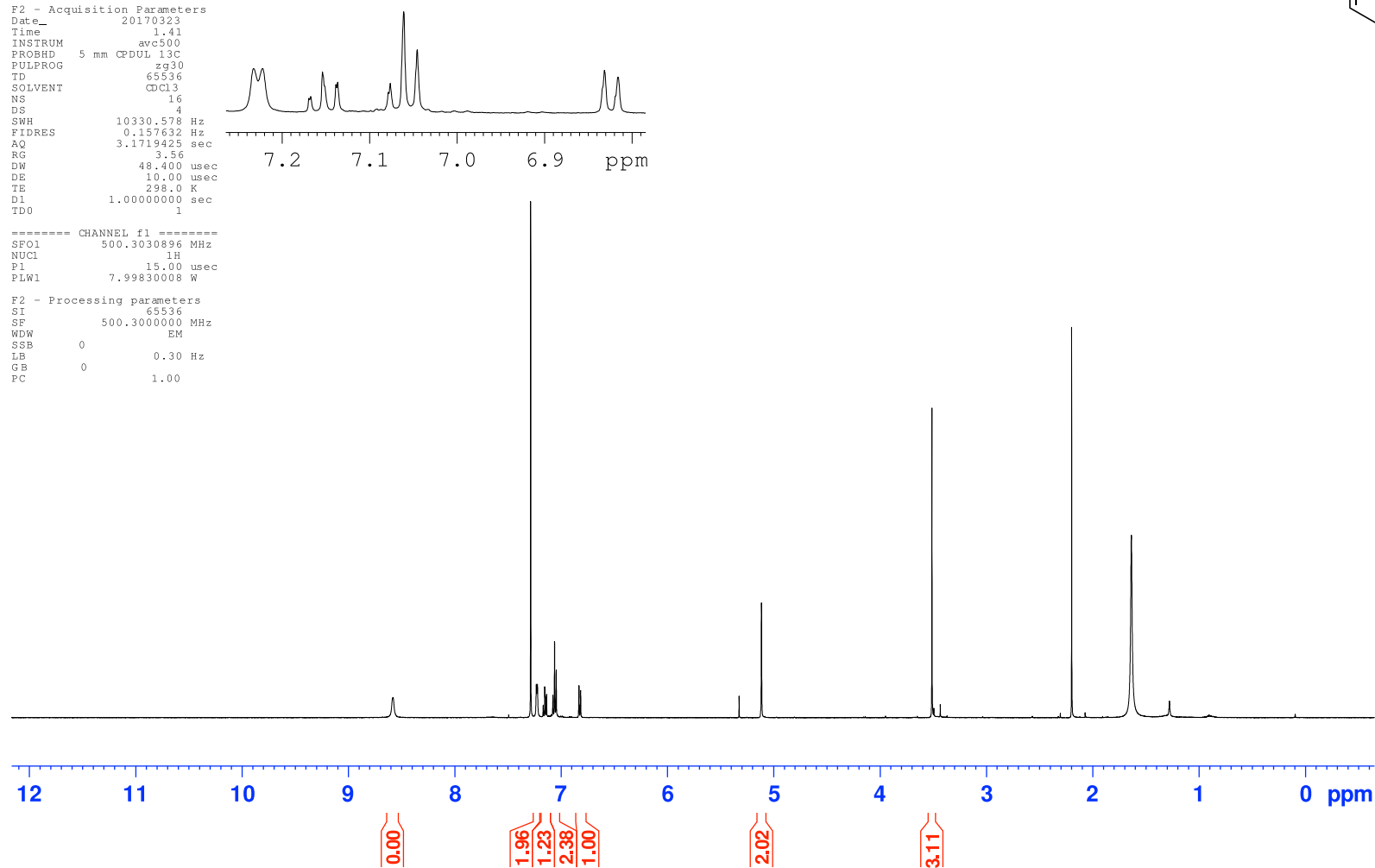


Current Data Parameters  
NAME c173912103 CLB59  
EXPNO 1  
PROCNO 1

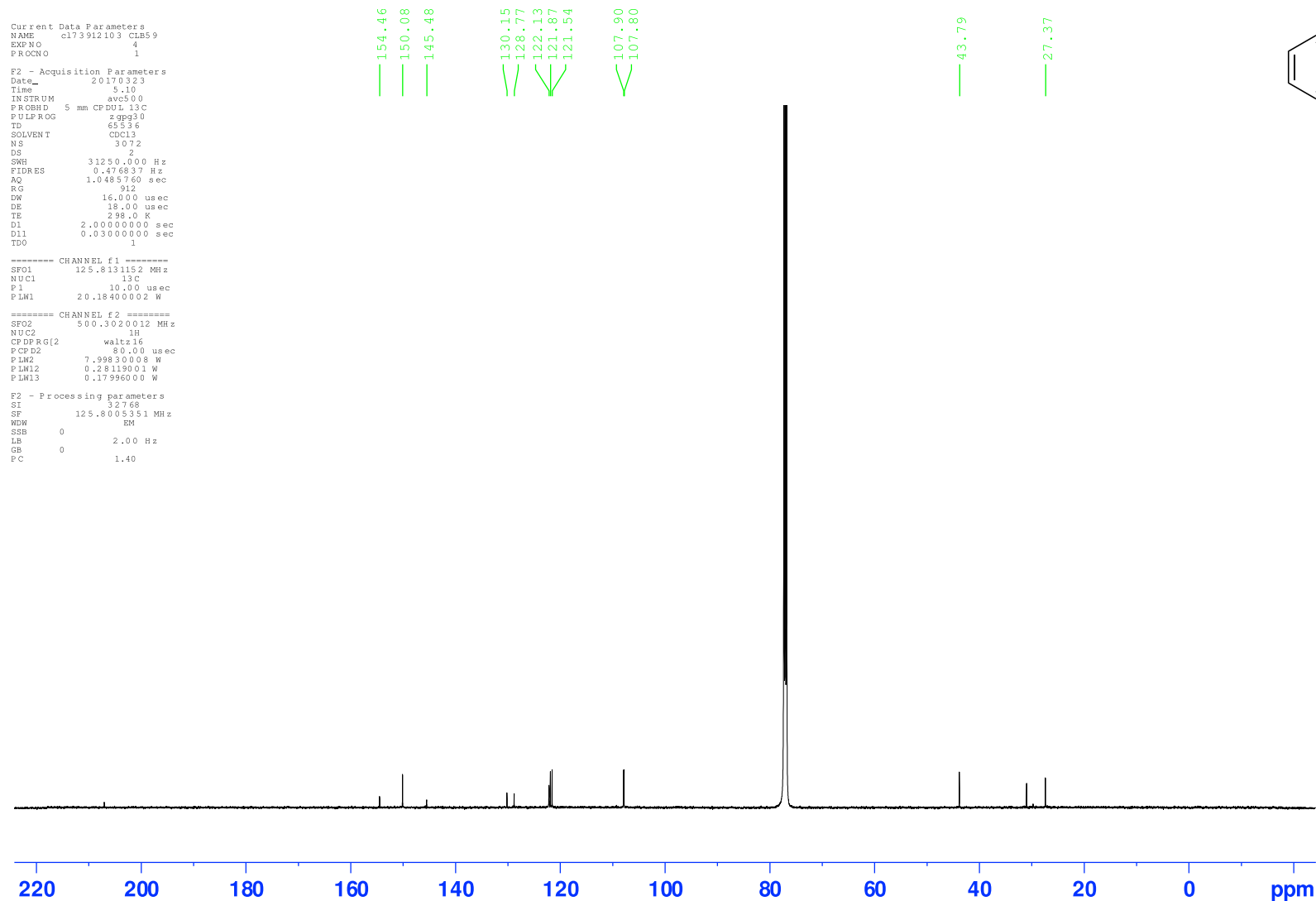
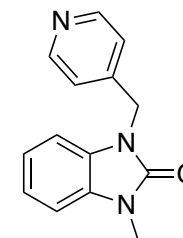
F2 - Acquisition Parameters  
Date\_ 20170323  
Time 1.41  
INSTRUM avc500  
PROBHD 5 mm CPDUL 13C  
PULPROG zg30  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 4  
SWH 10330.578 Hz  
FIDRES 0.157632 Hz  
AQ 3.1719425 sec  
RG 3.56  
DW 48.400 usec  
DE 10.00 usec  
TE 298.0 K  
D1 1.0000000 sec  
TD0 1

===== CHANNEL f1 =====  
SFO1 500.3030896 MHz  
NUC1 1H  
P1 15.00 usec  
PLW1 7.99830008 W

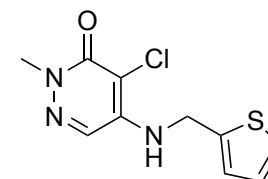
F2 - Processing parameters  
SI 65536  
SF 500.3000000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



# 1-Methyl-3-(pyridin-4'-ylmethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (20)



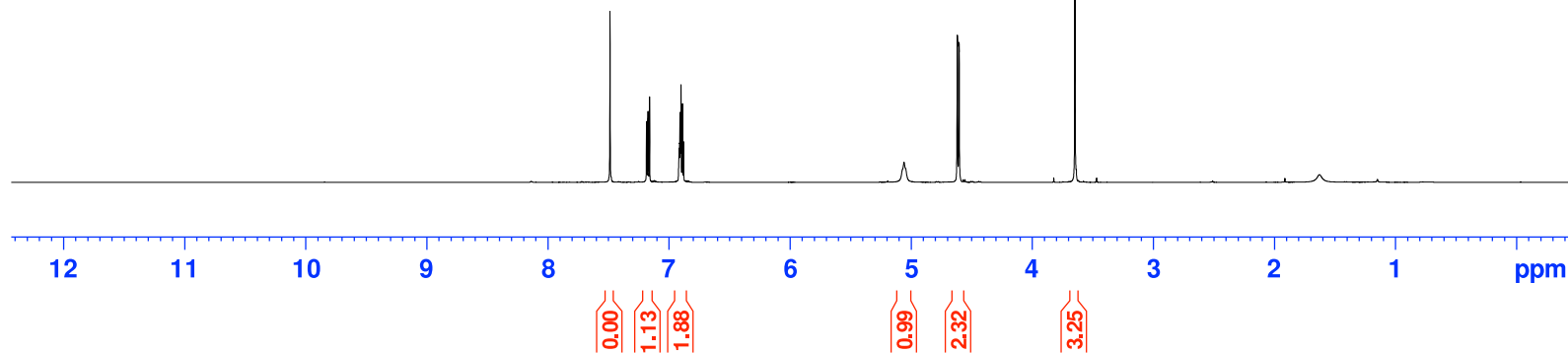
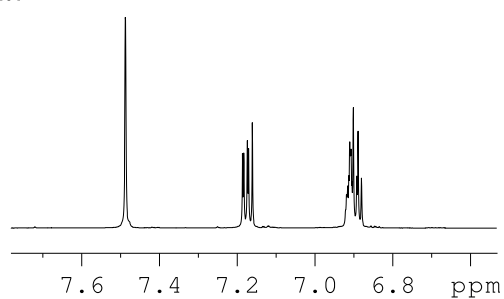
# 4-Chloro-2-methyl-5-((thiophen-2-ylmethyl)amino)pyridazin-3(2H)-one (41)



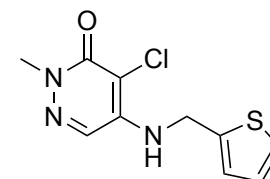
Current Data Parameters  
NAME Jun11-2019-4-CL-C-04 fr34-  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190611  
Time 18.24 h  
INSTRUM avg400  
PROBHD Z108618\_0816  
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 2  
SWH 8012.820 Hz  
FIDRES 0.244532 Hz  
AQ 4.0894465 sec  
RG 91.39  
DW 62.400 usec  
DE 6.50 usec  
TE 298.1 K  
D1 1.00000000 sec  
TD0 1  
SFO1 400.2024012 MHz  
NUC1 1H  
P1 14.00 usec  
PLW1 14.00000000 W

F2 - Processing parameters  
SI 32768  
SF 400.2000496 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



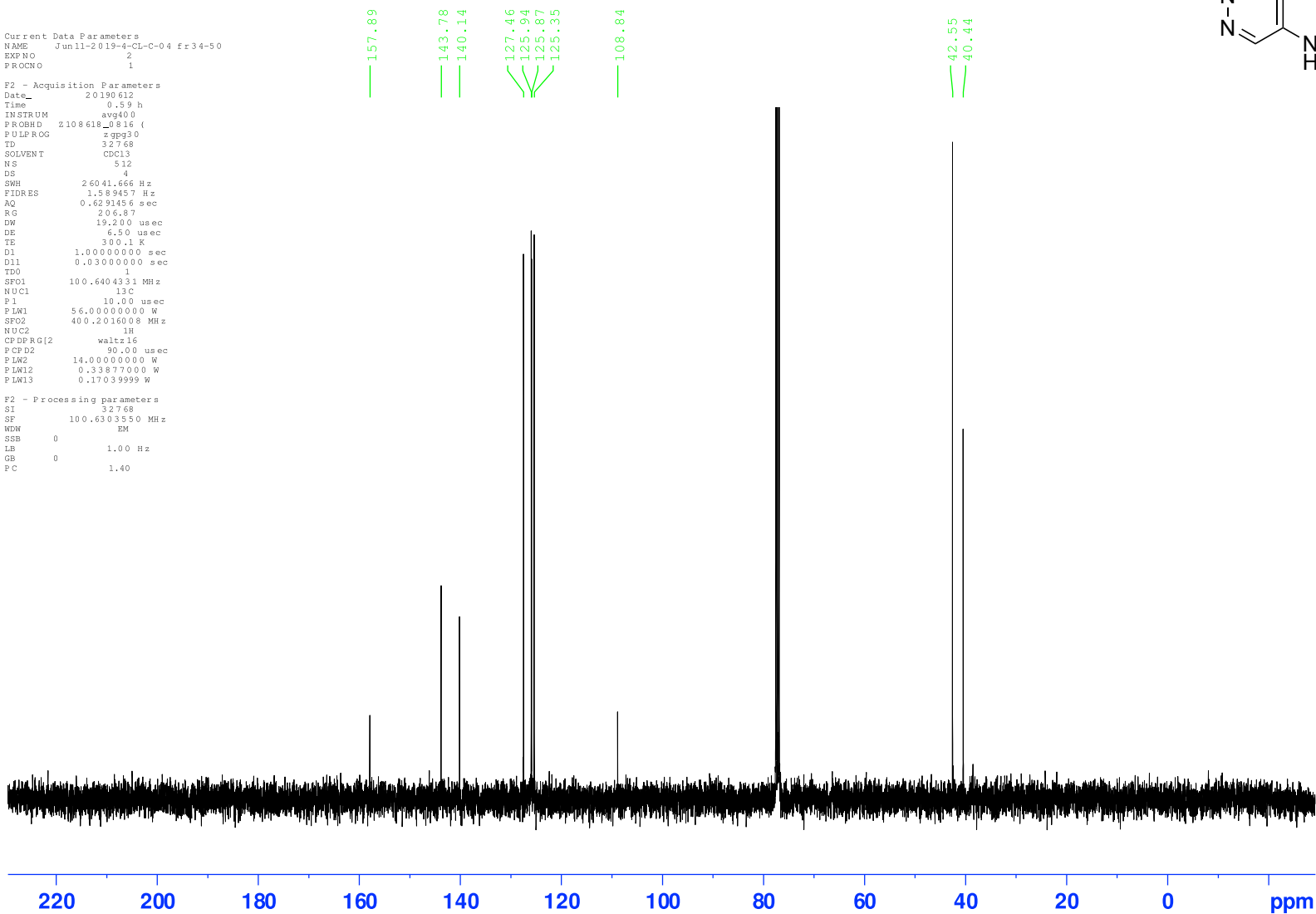
# 4-Chloro-2-methyl-5-((thiophen-2-ylmethyl)amino)pyridazin-3(2H)-one (41)



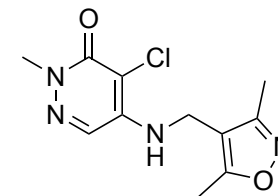
Current Data Parameters  
NAME Jun11-2019-4-CL-C-04 fr34-50  
EXPNO 2  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190612  
Time 0.59 h  
INSTRUM avq400  
PROBHD Z108618\_0816 ( 2 qnp30  
PULPROG zgpg30  
TD 32768  
SOLVENT CDCl3  
NS 512  
DS 4  
SWH 26041.666 Hz  
FIDRES 1.589457 Hz  
AQ 0.6291456 sec  
RG 206.87  
DW 19.200 usec  
DE 6.50 usec  
TE 300.1 K  
D1 1.00000000 sec  
D11 0.03000000 sec  
TD0 1  
SFO1 100.6404331 MHz  
NUC1 13C  
P1 10.00 usec  
PLW1 56.00000000 W  
SFO2 400.2016008 MHz  
NUC2 1H  
CPDPRG2 waltz16  
PCPD2 90.00 usec  
PLW2 14.00000000 W  
PLW12 0.33877000 W  
PLW13 0.17039999 W

F2 - Processing parameters  
SI 32768  
SF 100.6303550 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40



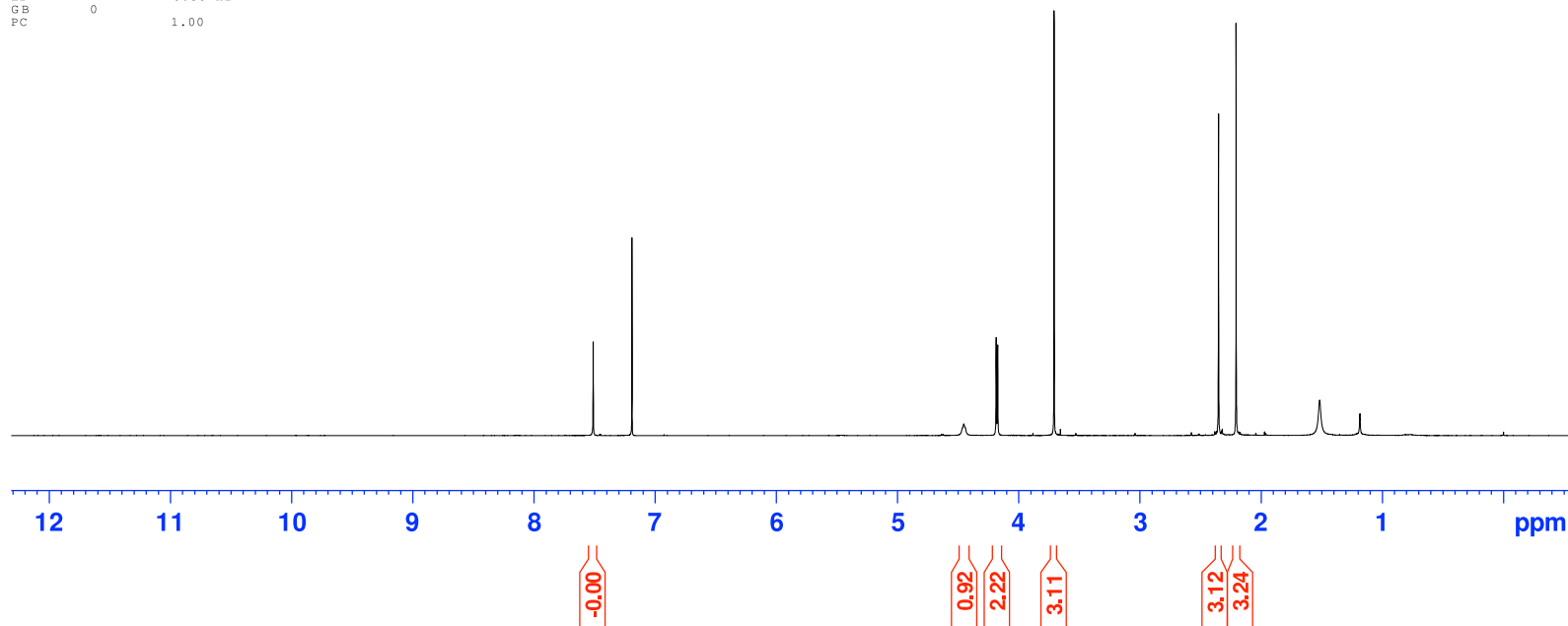
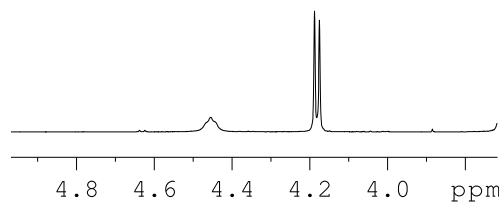
**4-Chloro-5-(((3',5'-dimethylisoxazol-4'-yl)methyl)amino)-2-methylpyridazin-3(2H)-one (42)**



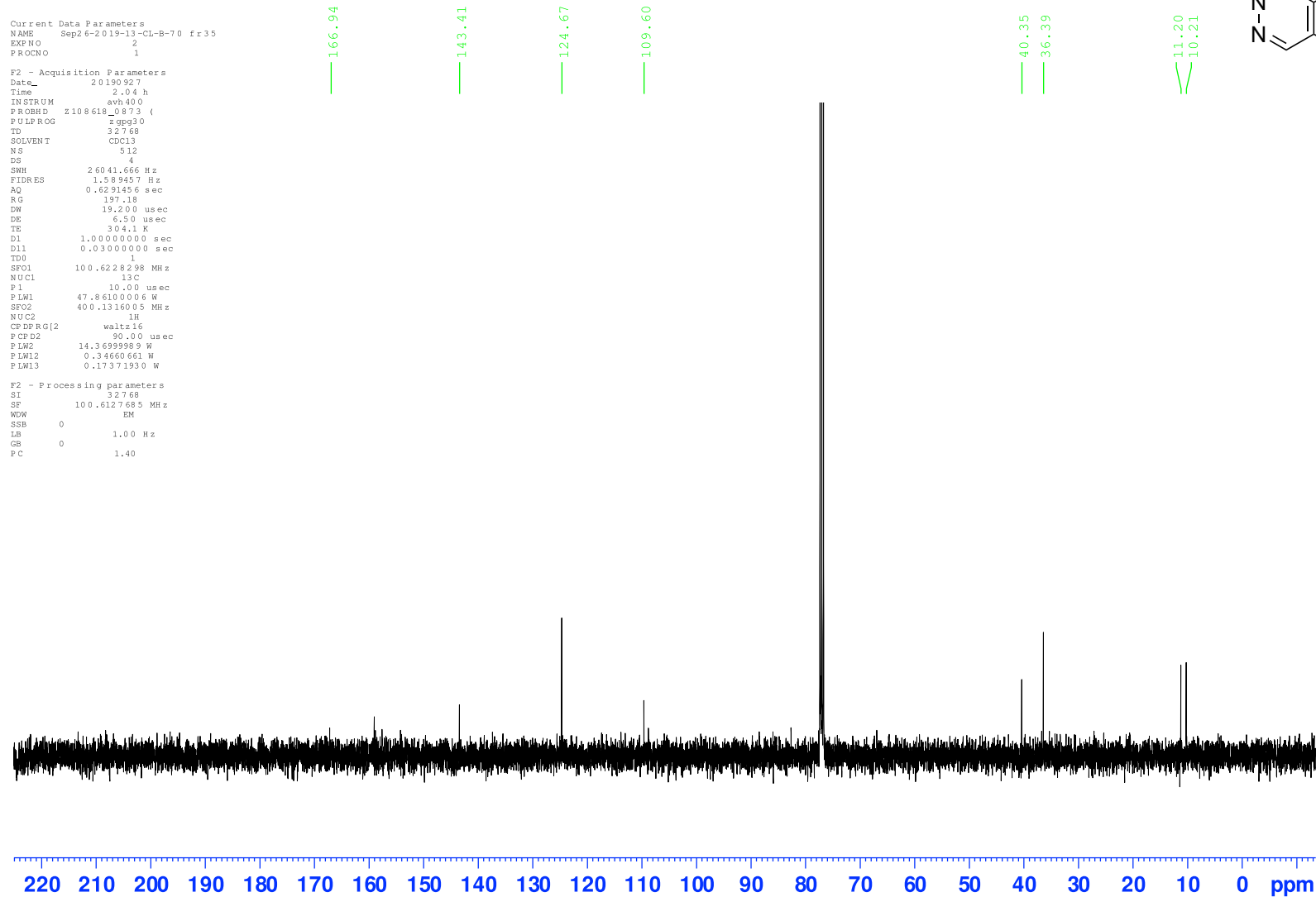
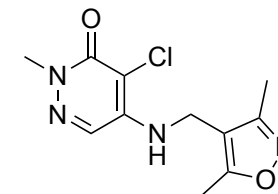
Current Data Parameters  
 NAME Sep26-2019-13-CL-B-70 fr35  
 EXPNO 1  
 PROCNO 1

F2 - Acquisition Parameters  
 Date\_ 20190927  
 Time 1.48 h  
 INSTRUM avh400  
 PROBHD Z108618\_0873 (   
 PULPROG zgpg30  
 TD 65536  
 SOLVENT CDCl3  
 NS 16  
 DS 2  
 SWH 8012.820 Hz  
 FIDRES 0.244532 Hz  
 AQ 4.0894465 sec  
 RG 88.17  
 DW 62.400 usec  
 DE 6.50 usec  
 TE 303.2 K  
 D1 1.00000000 sec  
 TD0 1  
 SFO1 400.1324008 MHz  
 NUC1 1H  
 P1 14.00 usec  
 PLW1 14.36999989 W

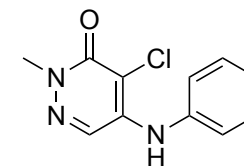
F2 - Processing parameters  
 SI 32768  
 SF 400.1300371 MHz  
 WDW EM  
 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 1.00



# 4-Chloro-5-(((3',5'-dimethylisoxazol-4'-yl)methyl)amino)-2-methylpyridazin-3(2H)-one (42)



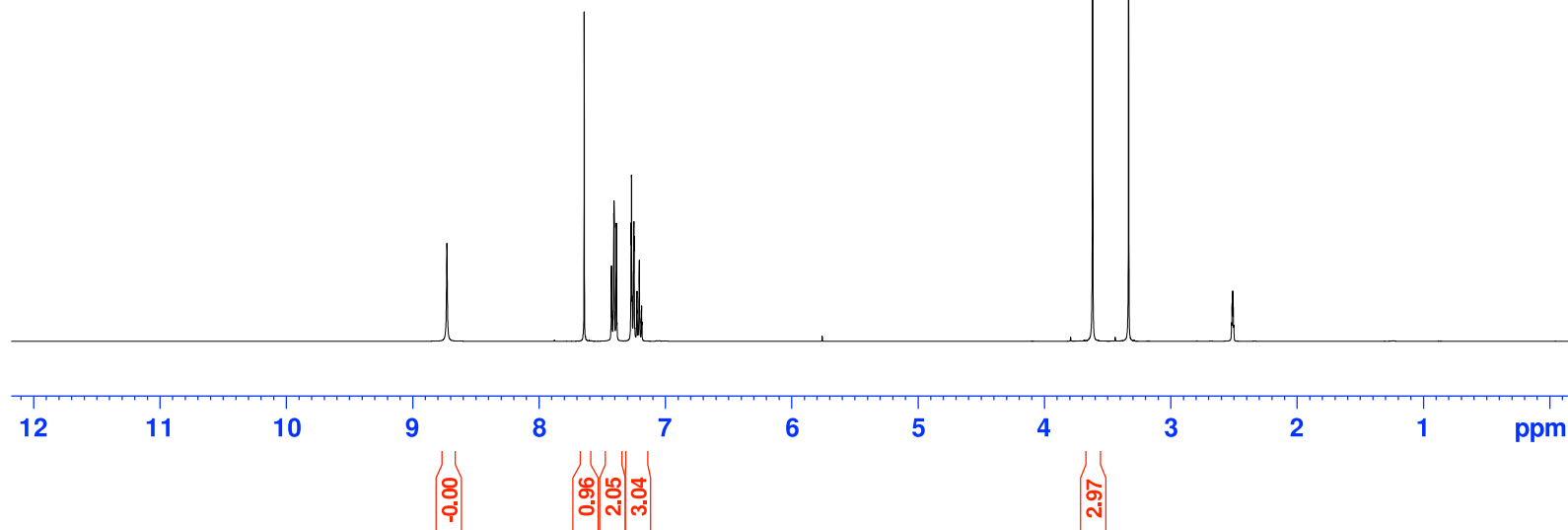
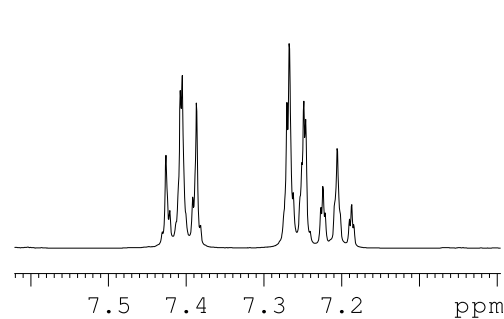
# 4-Chloro-2-methyl-5-(phenylamino)pyridazin-3(2H)-one (43)



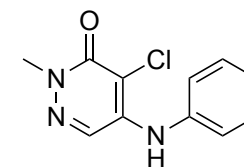
Current Data Parameters  
NAME Jul11-2019-38-CL-C-05  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190711  
Time 17.23 h  
INSTRUM avh400  
PROBHD Z108618\_0873 (PULPROG zgpg30)  
TD 65536  
SOLVENT DMSO  
NS 16  
DS 2  
SWH 8012.820 Hz  
FIDRES 0.244532 Hz  
AQ 4.0894465 sec  
RG 88.17  
DW 62.400 usec  
DE 6.50 usec  
TE 300.6 K  
D1 1.00000000 sec  
TD0 1  
SFO1 400.1324008 MHz  
NUC1 1H  
P1 14.00 usec  
PLW1 14.36999989 W

F2 - Processing parameters  
SI 32768  
SF 400.1300000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



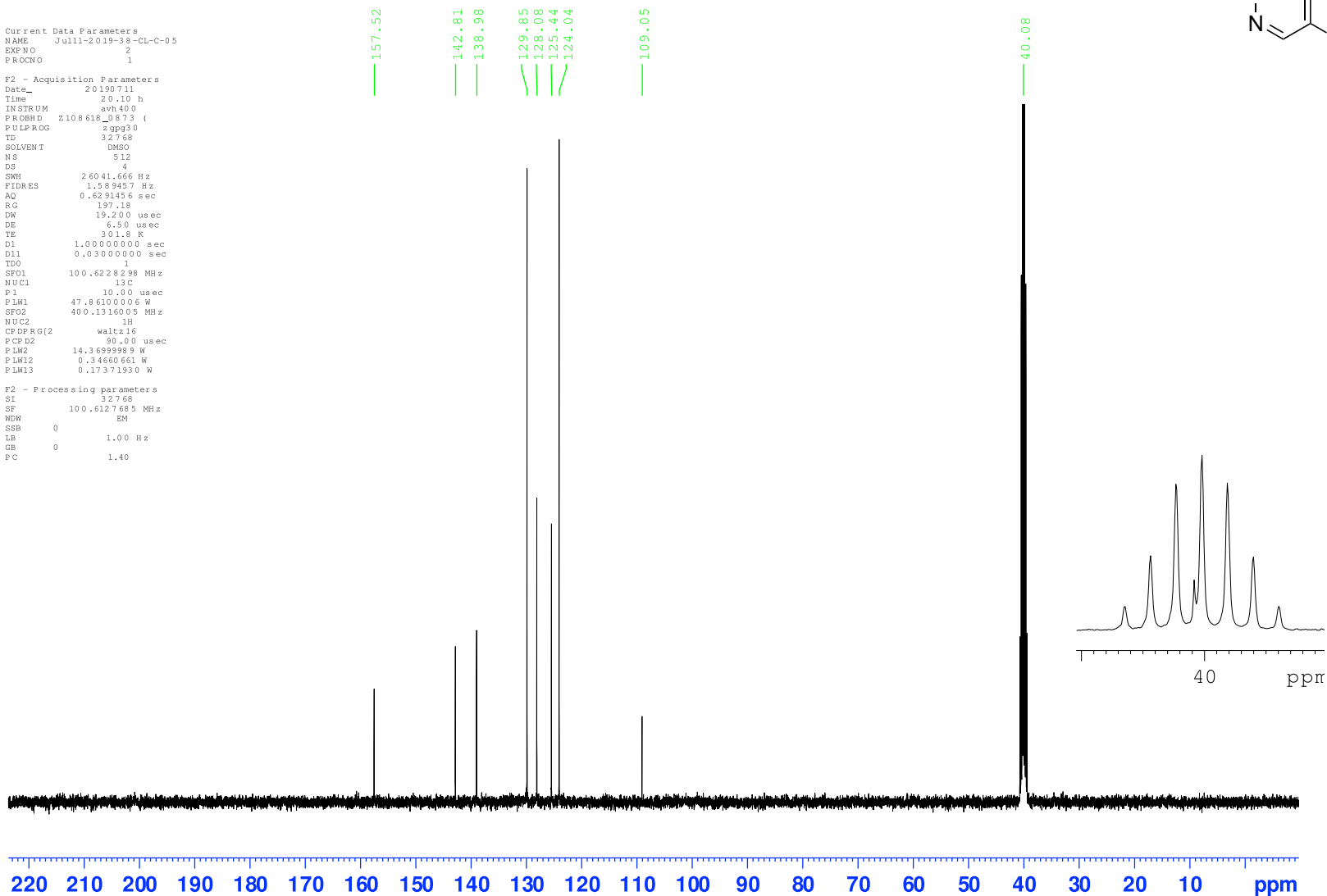
# 4-Chloro-2-methyl-5-(phenylamino)pyridazin-3(2H)-one (43)



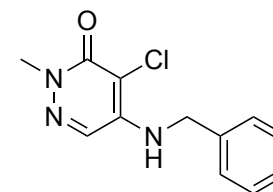
Current Data Parameters  
NAME Jul11-2019-38-CL-C-05  
EXP NO 2  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190711  
Time 20.10 h  
INSTRUM avh400  
PROBHD Z108618\_0873 (   
PULPROG zgpg30  
TD 32768  
SOLVENT DMSO  
NS 512  
DS 4  
SWH 26041.666 Hz  
FIDRES 1.589457 Hz  
AQ 0.6291456 sec  
RG 197.18  
DW 19.200 usec  
DE 6.50 usec  
TE 301.8 K  
D1 1.00000000 sec  
D11 0.03000000 sec  
TDO 1  
SFO1 100.6228298 MHz  
NUC1 13C  
P1 10.00 usec  
PLW1 47.86100006 W  
SFO2 400.1316005 MHz  
NUC2 1H  
CPDPRG2 waltz16  
PCPD2 90.00 usec  
PLW2 14.36999989 W  
PLW12 0.34660661 W  
PLW13 0.17371930 W

F2 - Processing parameters  
SI 32768  
SF 100.6127685 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40



# 5-(Benzylamino)-4-chloro-2-methylpyridazin-3(2H)-one (44)

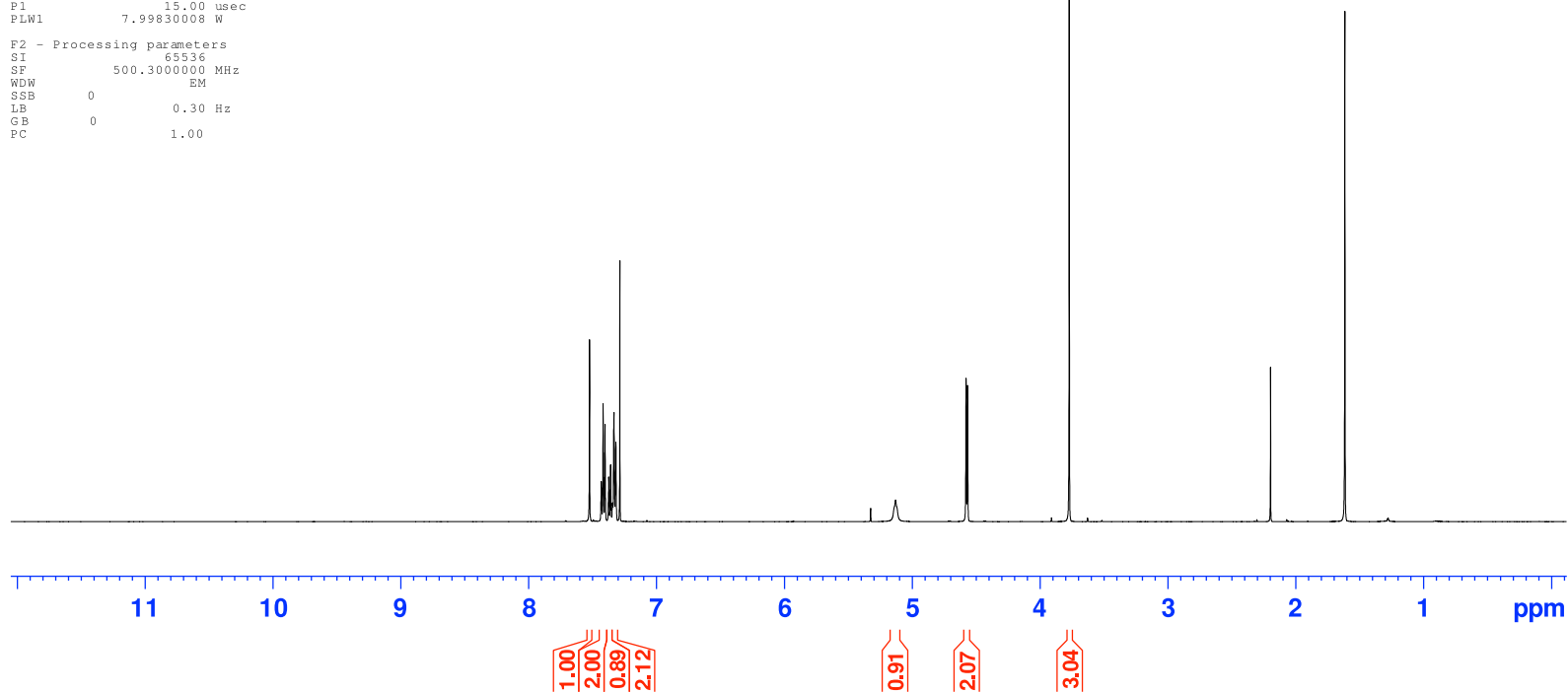
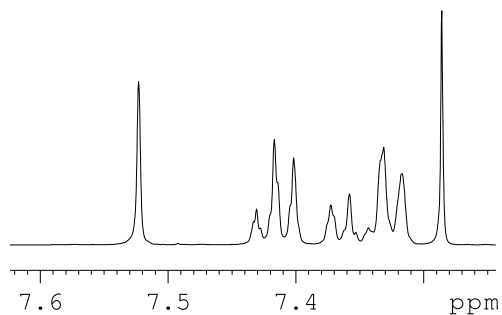


Current Data Parameters  
NAME c173932103 CLB64  
EXPNO 1  
PROCNO 1

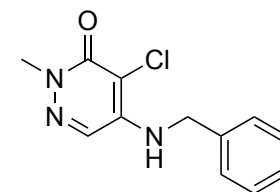
F2 - Acquisition Parameters  
Date\_ 20170323  
Time 8.01  
INSTRUM avc500  
PROBHD 5 mm CPDUL 13C  
PULPROG zg30  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 4  
SWH 10330.578 Hz  
FIDRES 0.157632 Hz  
AQ 3.1719425 sec  
RG 4  
DW 48.400 usec  
DE 10.00 usec  
TE 298.0 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
SF01 500.3030896 MHz  
NUC1 1H  
P1 15.00 usec  
PLW1 7.99830008 W

F2 - Processing parameters  
SI 65536  
SF 500.3000000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



# 5-(Benzylamino)-4-chloro-2-methylpyridazin-3(2H)-one (44)



```

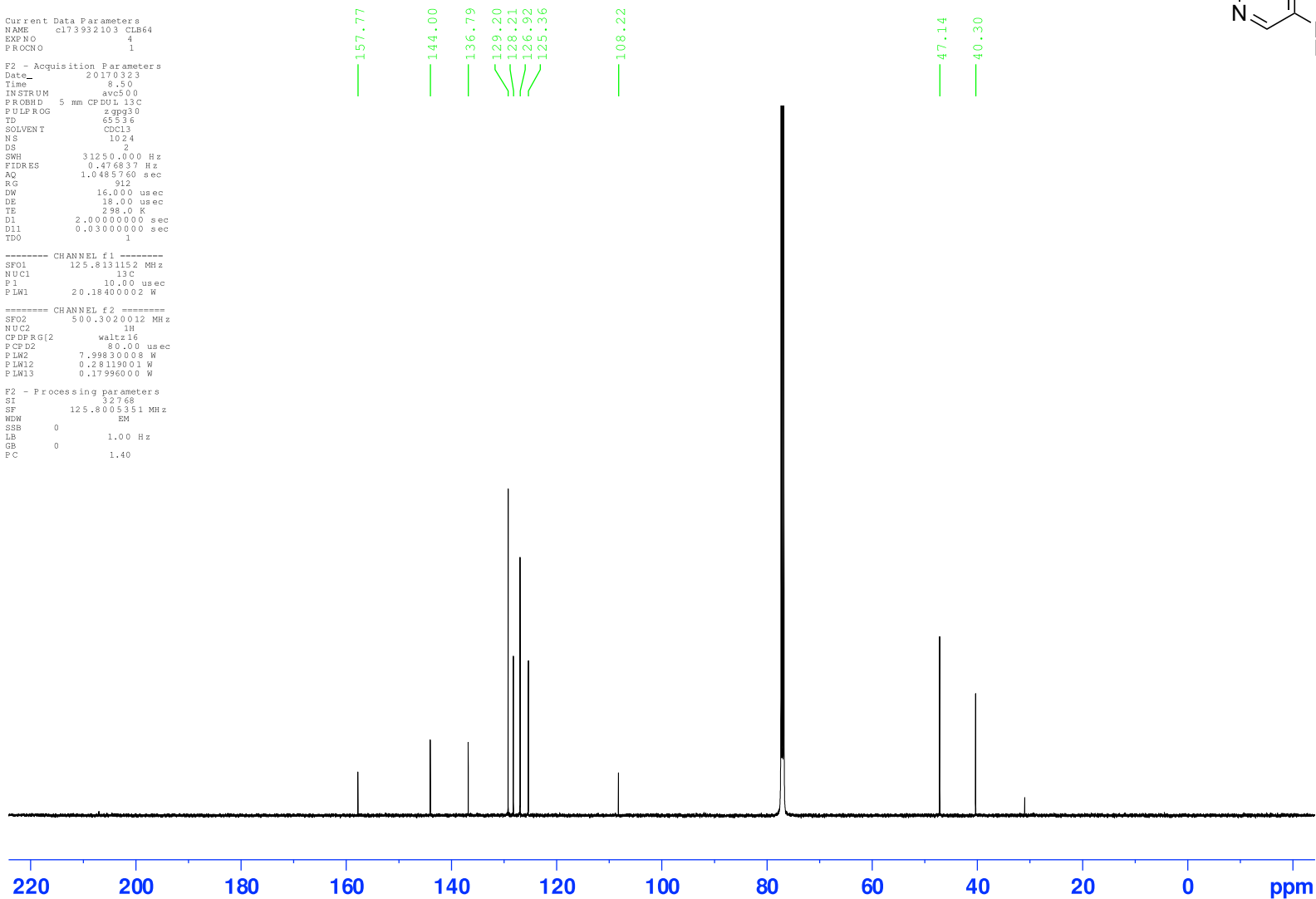
Current Data Parameters
NAME      cl73932103_CLE64
EXPNO     4
PROCNO    1

F2 - Acquisition Parameters
Date_     20170323
Time      8.50
INSTRUM   avc500
PROBHD    5 mm CPDUL13C
PULPROG   zgpg30
TD        65536
SOLVENT   CDCl3
NS         1024
DS         2
SWH        31250.000 Hz
FIDRES     0.476837 Hz
AQ         1.0485760 sec
RG         912
DW         16.000 usec
DE         18.00 usec
TE         298.0 K
D1         2.00000000 sec
D11        0.03000000 sec
TD0        1

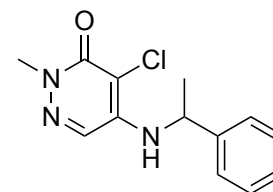
===== CHANNEL f1 =====
SFO1      125.8131152 MHz
NUC1       13C
P1         10.00 usec
PLW1       20.18400002 W

===== CHANNEL f2 =====
SFO2      500.13020012 MHz
NUC2       1H
CPDPRG2    waltz16
PCPD2      80.00 usec
PLM2       7.99830008 W
PLW12      0.28119001 W
PLW13      0.17996000 W

F2 - Processing parameters
SI         32768
SF         125.8005351 MHz
WDW        EM
SSB        0
LB         1.00 Hz
GB         0
PC         1.40
    
```



# 4-Chloro-2-methyl-5-((1-phenylethyl)amino)pyridazin-3(2H)-one (45)

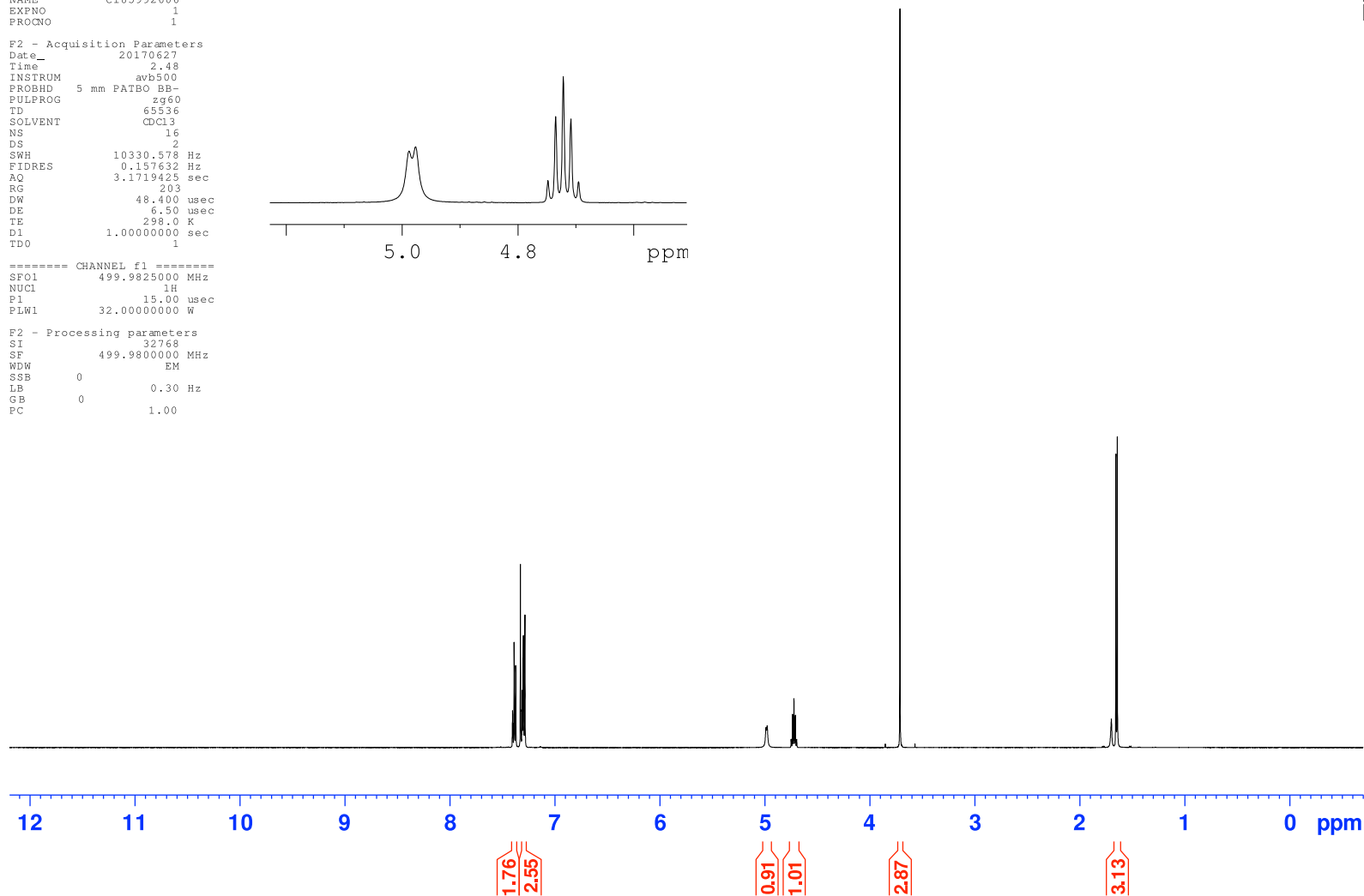


Current Data Parameters  
 NAME c185992606  
 EXPNO 1  
 PROCNO 1

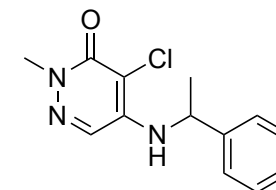
F2 - Acquisition Parameters  
 Date\_ 20170627  
 Time 2.48  
 INSTRUM avb500  
 PROBHD 5 mm PATBO BB-  
 PULPROG zgpg30  
 TD 65536  
 SOLVENT CDCl3  
 NS 16  
 DS 2  
 SWH 10330.578 Hz  
 FIDRES 0.157632 Hz  
 AQ 3.1719425 sec  
 RG 203  
 DW 48.400 usec  
 DE 6.50 usec  
 TE 298.0 K  
 D1 1.00000000 sec  
 TD0 1

===== CHANNEL f1 =====  
 SFO1 499.9825000 MHz  
 NUC1 1H  
 P1 15.00 usec  
 PLW1 32.00000000 W

F2 - Processing parameters  
 SI 32768  
 SF 499.9800000 MHz  
 WDW EM  
 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 1.00



# 4-Chloro-2-methyl-5-((1-phenylethyl)amino)pyridazin-3(2H)-one (45)



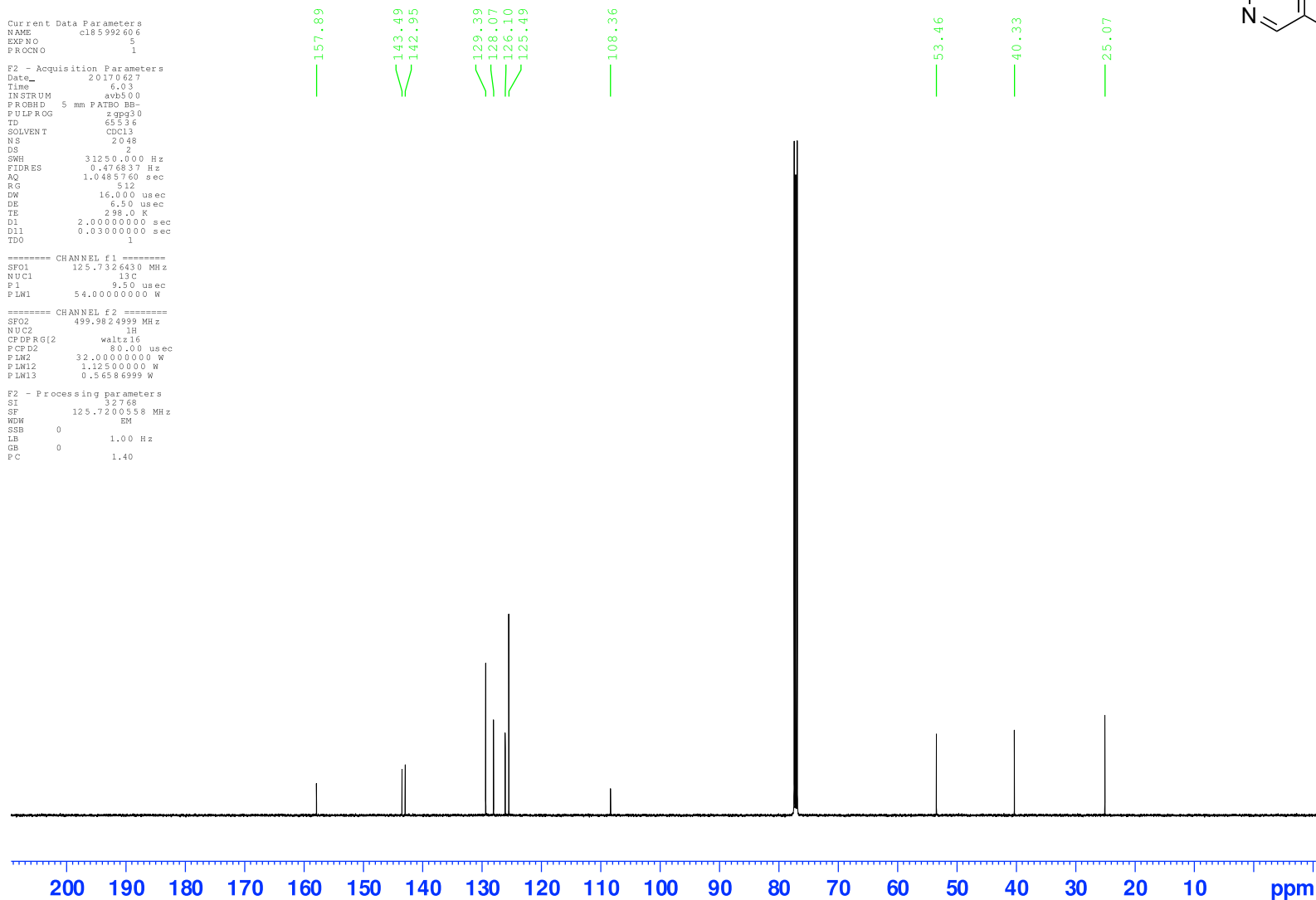
Current Data Parameters  
NAME c185992606  
EXPNO 5  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20170627  
Time 6.03  
INSTRUM avb500  
PROBHD 5 mm PABBO BB-  
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 2048  
DS 2  
SWH 31250.000 Hz  
FIDRES 0.476837 Hz  
AQ 1.0485760 sec  
RG 512  
DW 16.000 usec  
DE 6.50 usec  
TE 298.0 K  
D1 2.00000000 sec  
D11 0.03000000 sec  
TDO 1

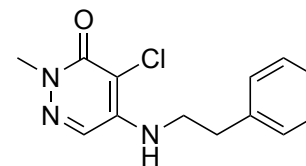
===== CHANNEL f1 =====  
SFO1 125.7326430 MHz  
NUC1 13C  
P1 9.50 usec  
PLW1 54.00000000 W

===== CHANNEL f2 =====  
SFO2 499.9824999 MHz  
NUC2 1H  
CPDPRG2 waltz16  
PCPD2 80.00 usec  
PLW2 32.00000000 W  
PLW12 1.12500000 W  
PLW13 0.56586999 W

F2 - Processing parameters  
SI 32768  
SF 125.7200558 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40



# 4-Chloro-2-methyl-5-(phenethylamino)pyridazin-3(2H)-one (46)

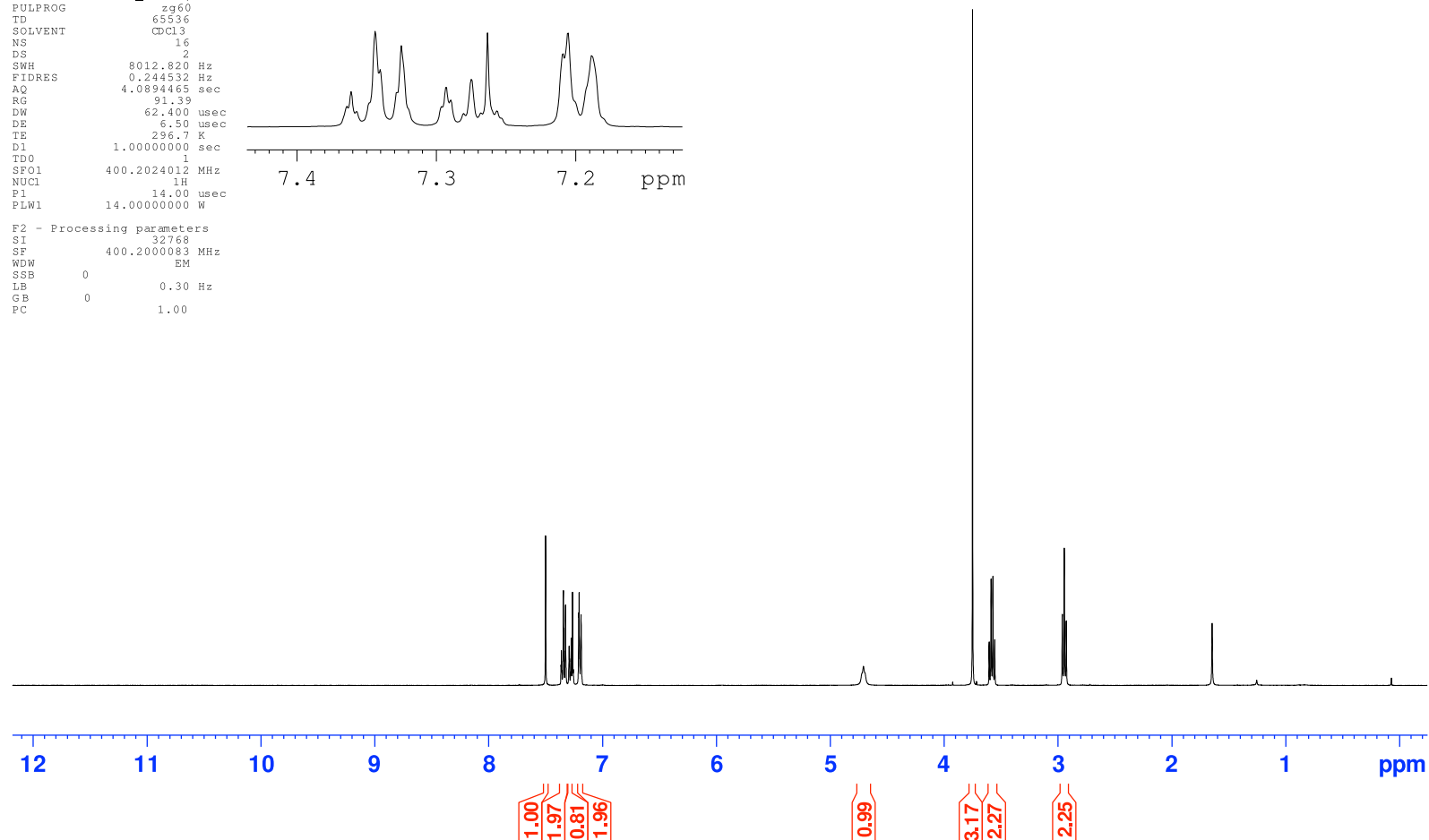
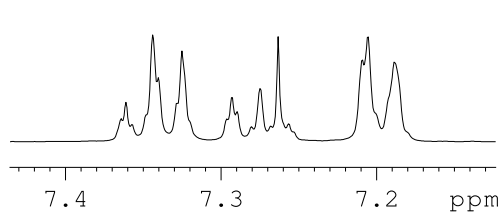


Current Data Parameters  
NAME Jan23-2019-34-CL-B-72  
EXPNO 1  
PROCNO 1

## F2 - Acquisition Parameters

Date\_ 20190124  
Time 6.40 h  
INSTRUM avg400  
PROBHD Z108618\_0816 (  
PULPROG zg60  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 2  
SWH 8012.820 Hz  
FIDRES 0.244532 Hz  
AQ 4.089465 sec  
RG 91.39  
DW 62.400 usec  
DE 6.50 usec  
TE 296.7 K  
D1 1.00000000 sec  
TD0 1  
SF01 400.2024012 MHz  
NUC1 1H  
P1 14.00 usec  
PLW1 14.00000000 W

F2 - Processing parameters  
SI 32768  
SF 400.2000083 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

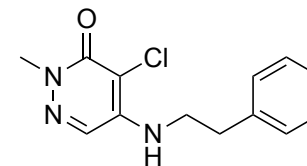
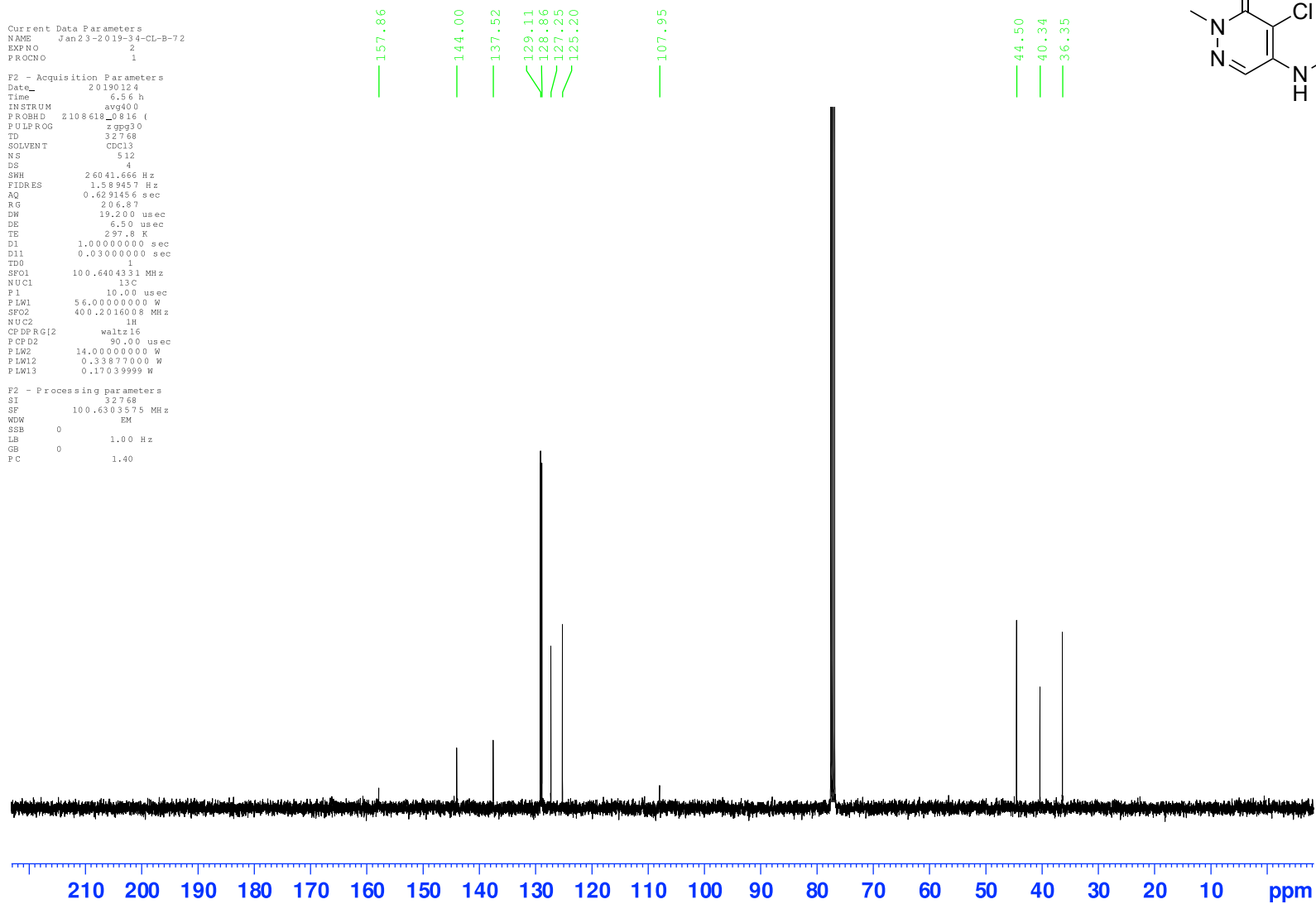


# 4-Chloro-2-methyl-5-(phenethylamino)pyridazin-3(2H)-one (46)

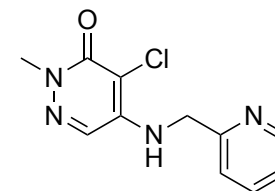
Current Data Parameters  
NAME Jan23-2019-34-CL-B-72  
EXPNO 2  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190124  
Time 6.56 h  
INSTRUM avq400  
PROBHD Z108618\_0816 (   
PULPROG zgpg30  
TD 32768  
SOLVENT CDCl3  
NS 512  
DS 4  
SWH 26041.666 Hz  
FIDRES 1.589457 Hz  
AQ 0.6291456 sec  
RG 206.87  
DM 19.200 usec  
DE 6.50 usec  
TE 297.8 K  
D1 1.00000000 sec  
D11 0.03000000 sec  
TD0 1  
SFO1 100.6404331 MHz  
NUC1 13C  
P1 10.00 usec  
PLW1 56.00000000 W  
SFO2 400.2016008 MHz  
NUC2 1H  
CPDPRG2 waltz16  
PCPD2 90.00 usec  
PLW2 14.00000000 W  
PLW12 0.33877000 W  
PLW13 0.17039999 W

F2 - Processing parameters  
SI 32768  
SF 100.6303575 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40



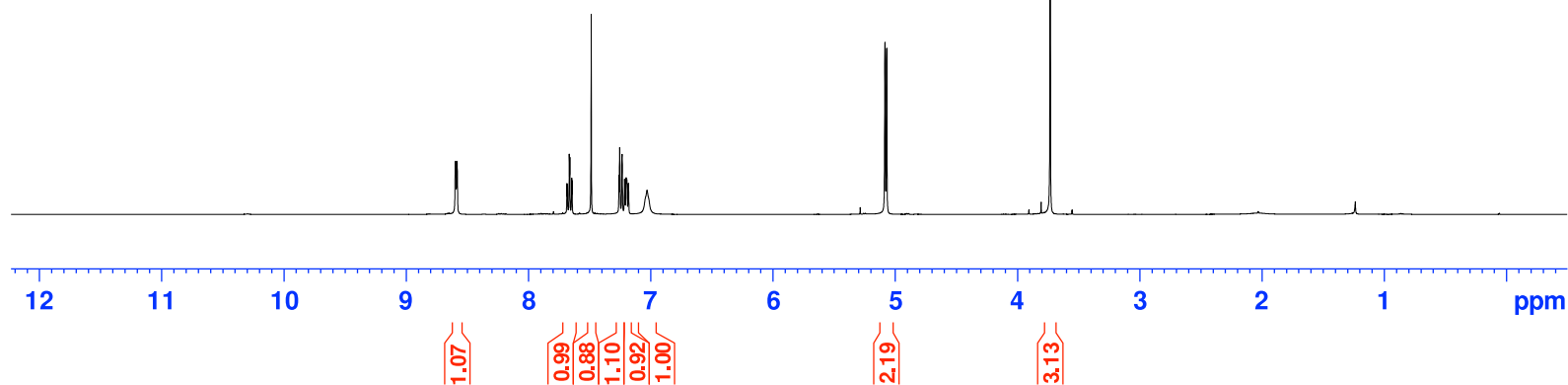
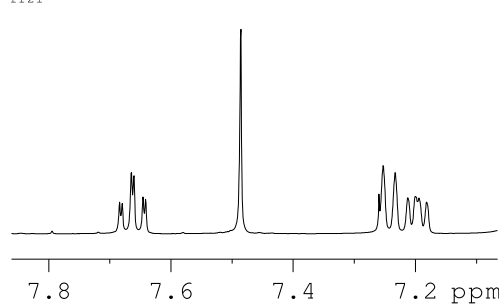
# 4-Chloro-2-methyl-5-((pyridin-2-ylmethyl)amino)pyridazin-3(2H)-one (47)



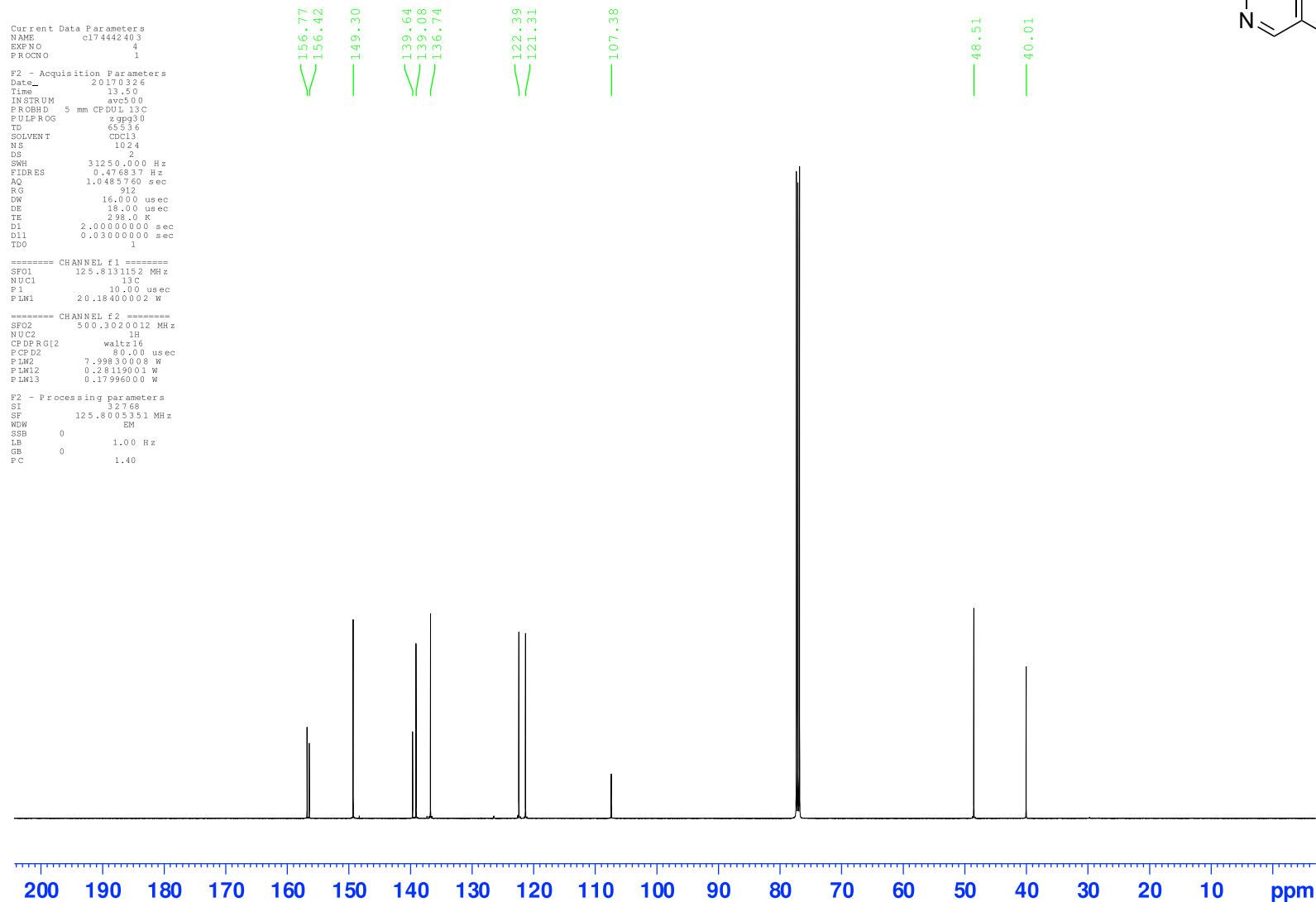
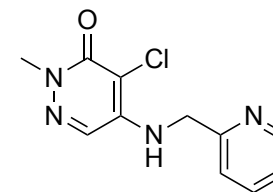
Current Data Parameters  
NAME Apr19-2017-54-CL-B-67 fr21  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20170419  
Time 10.54 h  
INSTRUM avq400  
PROBHD Z108618\_0816  
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 2  
SWH 8012.820 Hz  
FIDRES 0.244532 Hz  
AQ 4.0894465 sec  
RG 91.39  
DW 62.400 usec  
DE 6.50 usec  
TE 294.1 K  
D1 1.00000000 sec  
TD0 1  
SFO1 400.2024012 MHz  
NUC1 1H  
P1 14.00 usec  
PLW1 14.00000000 W

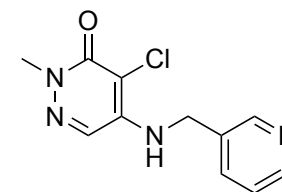
F2 - Processing parameters  
SI 32768  
SF 400.200098 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



# 4-Chloro-2-methyl-5-((pyridin-2-ylmethyl)amino)pyridazin-3(2H)-one (47)



# 4-Chloro-2-methyl-5-((pyridin-3-ylmethyl)amino)pyridazin-3(2H)-one (48)

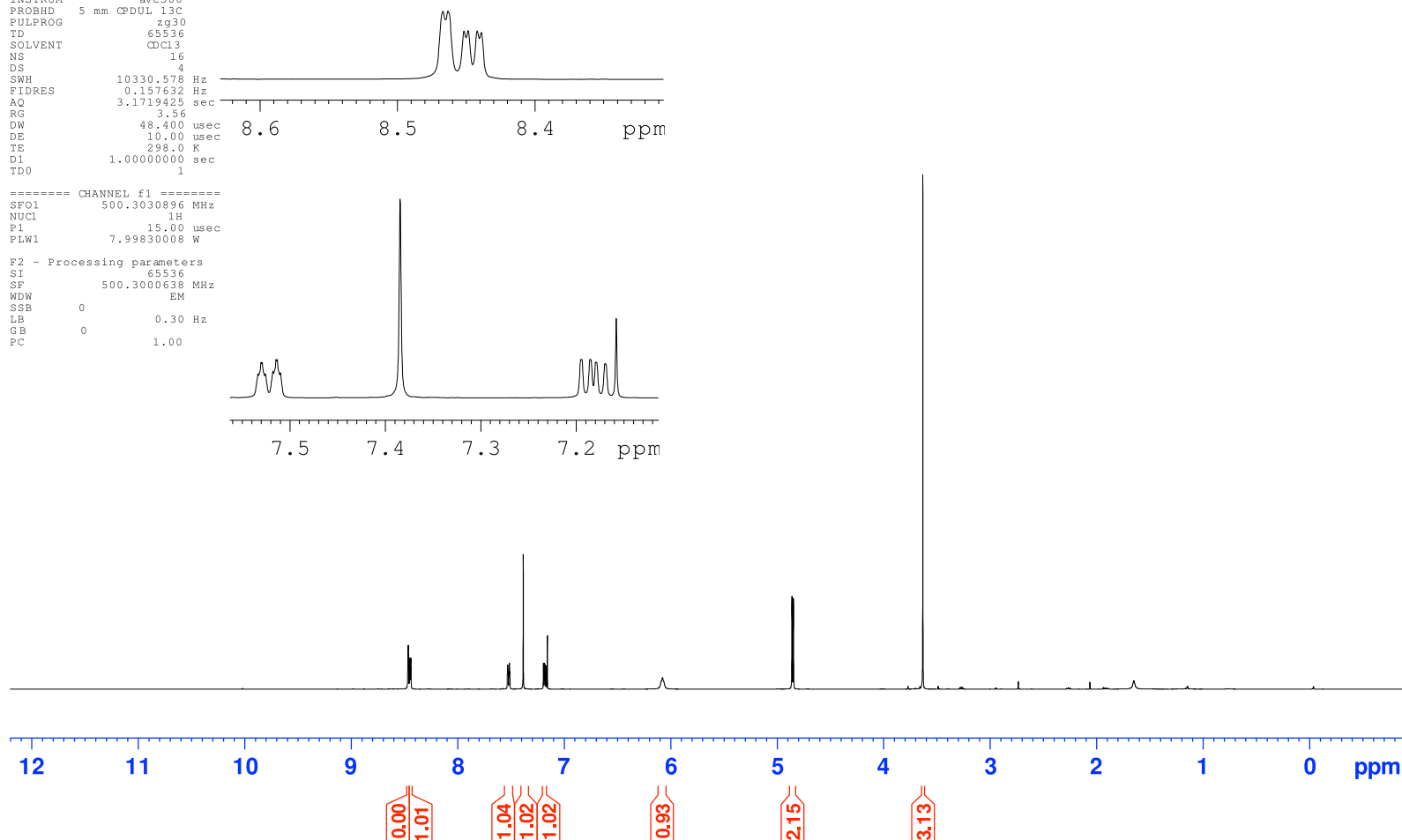


Current Data Parameters  
NAME c174462703  
EXPNO 1  
PROCNO 1

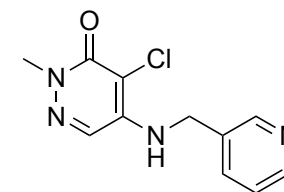
F2 - Acquisition Parameters  
Date\_ 20170328  
Time 15.41  
INSTRUM avc500  
PROBHD 5 mm CPDUL 13C  
PULPROG zg30  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 4  
SWH 10330.578 Hz  
FIDRES 0.157632 Hz  
AQ 3.1719425 sec  
RG 3.56  
DW 48.400 usec  
DE 10.00 usec  
TE 298.0 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
SFO1 500.3030896 MHz  
NUC1 1H  
P1 15.00 usec  
PLW1 7.99830008 W

F2 - Processing parameters  
SI 65536  
SF 500.3000638 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



# 4-Chloro-2-methyl-5-((pyridin-3-ylmethyl)amino)pyridazin-3(2H)-one (48)



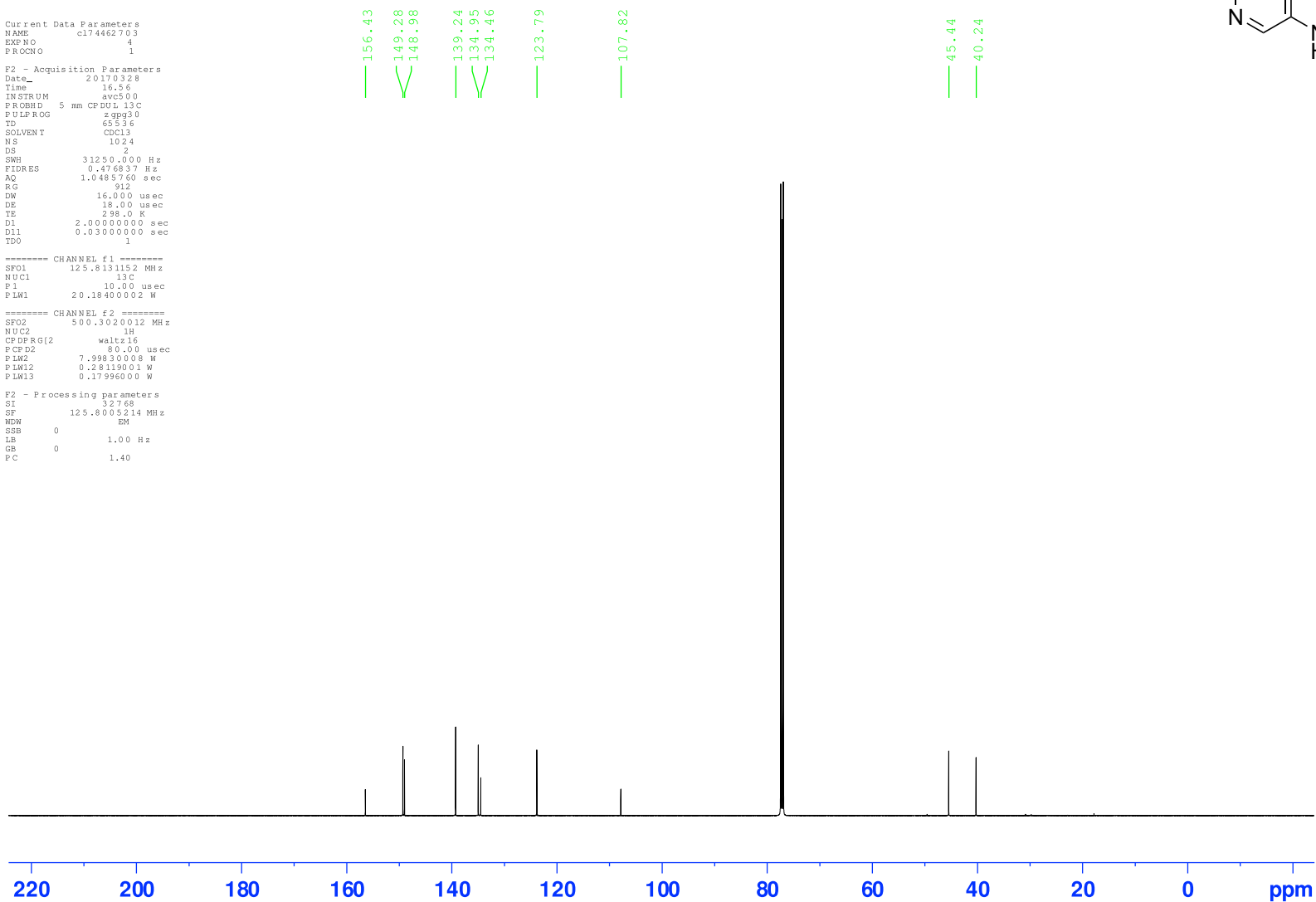
Current Data Parameters  
NAME c174462703  
EXP NO 4  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20170328  
Time 16.56  
INSTRUM avc500  
PROBHD 5 mm CPD L13C  
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 1024  
DS 2  
SWH 31250.000 Hz  
FIDRES 0.476837 Hz  
AQ 1.0485760 sec  
RG 912  
DW 16.000 usec  
DE 18.00 usec  
TE 298.0 K  
D1 2.00000000 sec  
D11 0.03000000 sec  
TD0 1

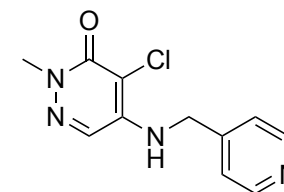
===== CHANNEL f1 =====  
SFO1 125.8131152 MHz  
NUC1 13C  
P1 10.00 usec  
PLW1 20.18400002 W

===== CHANNEL f2 =====  
SFO2 500.13020012 MHz  
NUC2 1H  
CPDPRG2 waltz16  
PCPD2 80.00 usec  
PLW2 7.99830000 W  
PLW12 0.28119001 W  
PLW13 0.17996000 W

F2 - Processing parameters  
SI 32768  
SF 125.8005214 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40



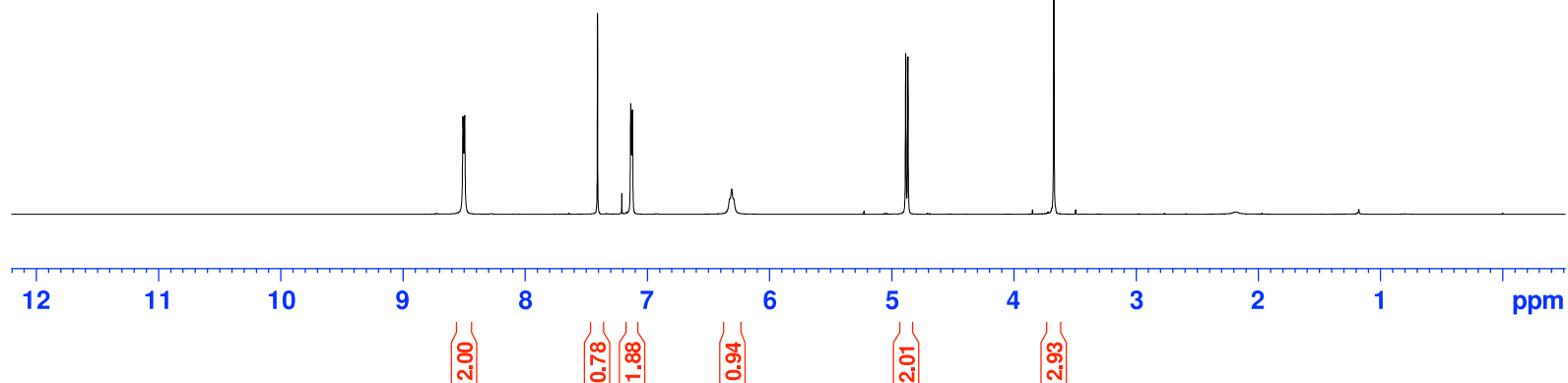
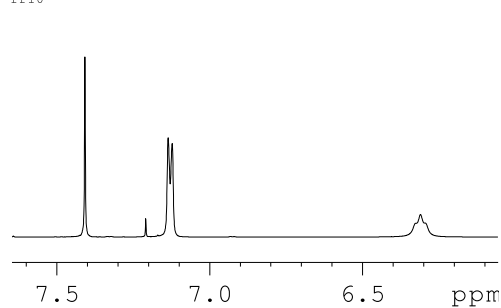
# 4-Chloro-2-methyl-5-((pyridin-4-ylmethyl)amino)pyridazin-3(2H)-one (49)



Current Data Parameters  
NAME Apr19-2017-54-CL-B-69 fr10  
EXPNO 1  
PROCNO 1

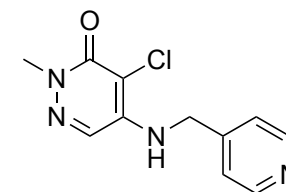
F2 - Acquisition Parameters  
Date\_ 20170419  
Time 11.04 h  
INSTRUM avq400  
PROBHD Z108618\_0816 (   
PULPROG zg60  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 2  
SWH 8012.820 Hz  
FIDRES 0.244532 Hz  
AQ 4.0894465 sec  
RG 91.39  
DW 62.400 usec  
DE 6.50 usec  
TE 293.7 K  
D1 1.00000000 sec  
TD0 1  
SFO1 400.2024012 MHz  
NUC1 1H  
P1 14.00 usec  
PLW1 14.00000000 W

F2 - Processing parameters  
SI 32768  
SF 400.2000301 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



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# 4-Chloro-2-methyl-5-((pyridin-4-ylmethyl)amino)pyridazin-3(2H)-one (49)



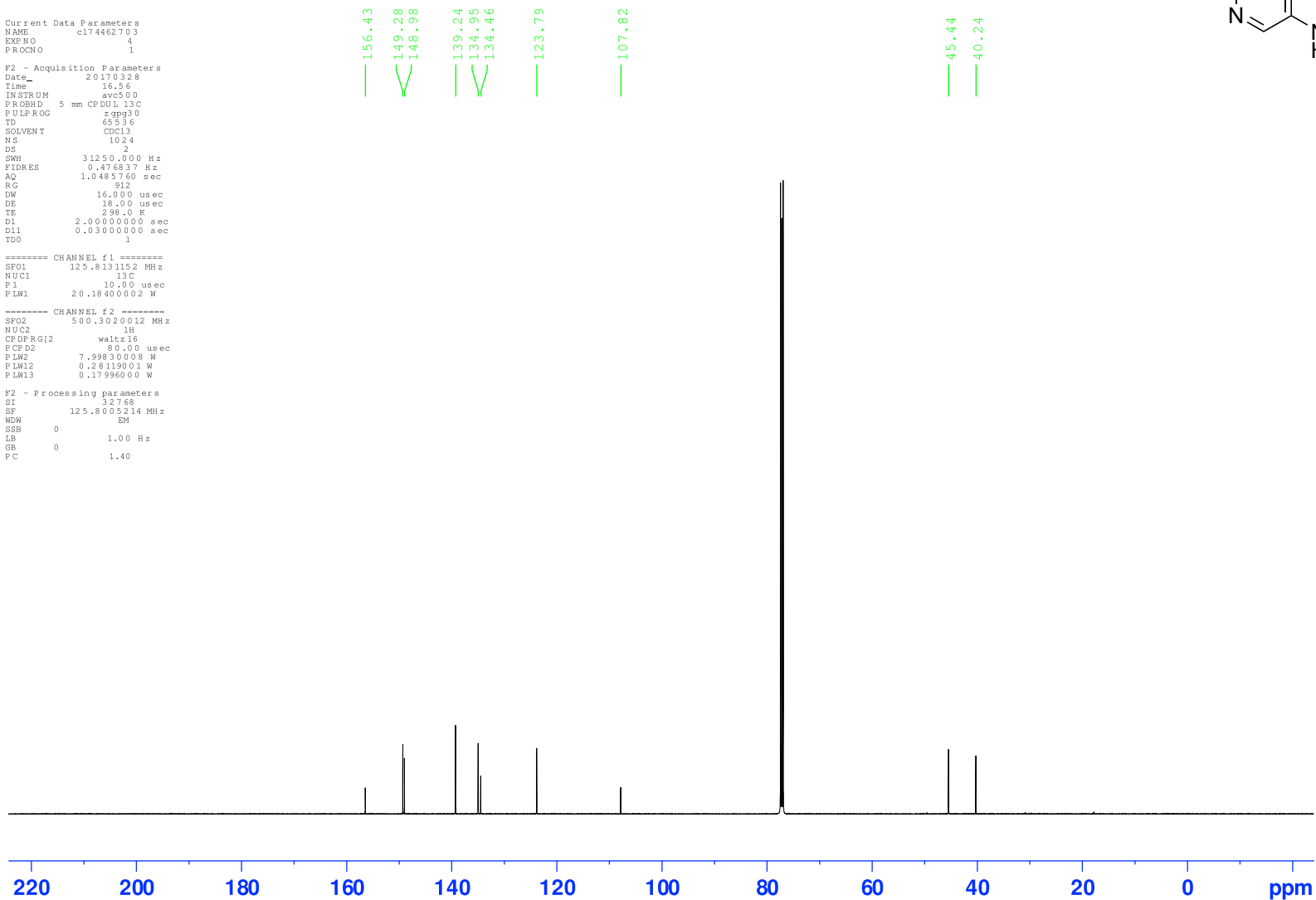
Current Data Parameters  
NAME c174462703  
EXPNO 4  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20170328  
Time 16.56  
INSTRUM avc500  
PROBHD 5 mm CPDUL13C  
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 1024  
DS 2  
SWH 31250.000 Hz  
FIDRES 0.476837 Hz  
AQ 1.0485760 sec  
RG 912  
DW 16.000 usec  
DE 18.00 usec  
TE 298.0 K  
D1 2.00000000 sec  
D11 0.03000000 sec  
TD0 1

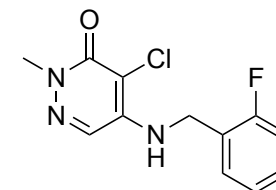
===== CHANNEL f1 =====  
SFO1 125.8131152 MHz  
NUC1 13C  
P1 10.00 usec  
PLW1 20.18400002 W

===== CHANNEL f2 =====  
SFO2 500.13020012 MHz  
NUC2 1H  
CPDPRG2 waltz16  
PCPD2 80.00 usec  
PLW2 7.99830008 W  
PLW12 0.28119001 W  
PLW13 0.17996000 W

F2 - Processing parameters  
SI 32768  
SF 125.8005214 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40



# 4-Chloro-5-((2-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (50)

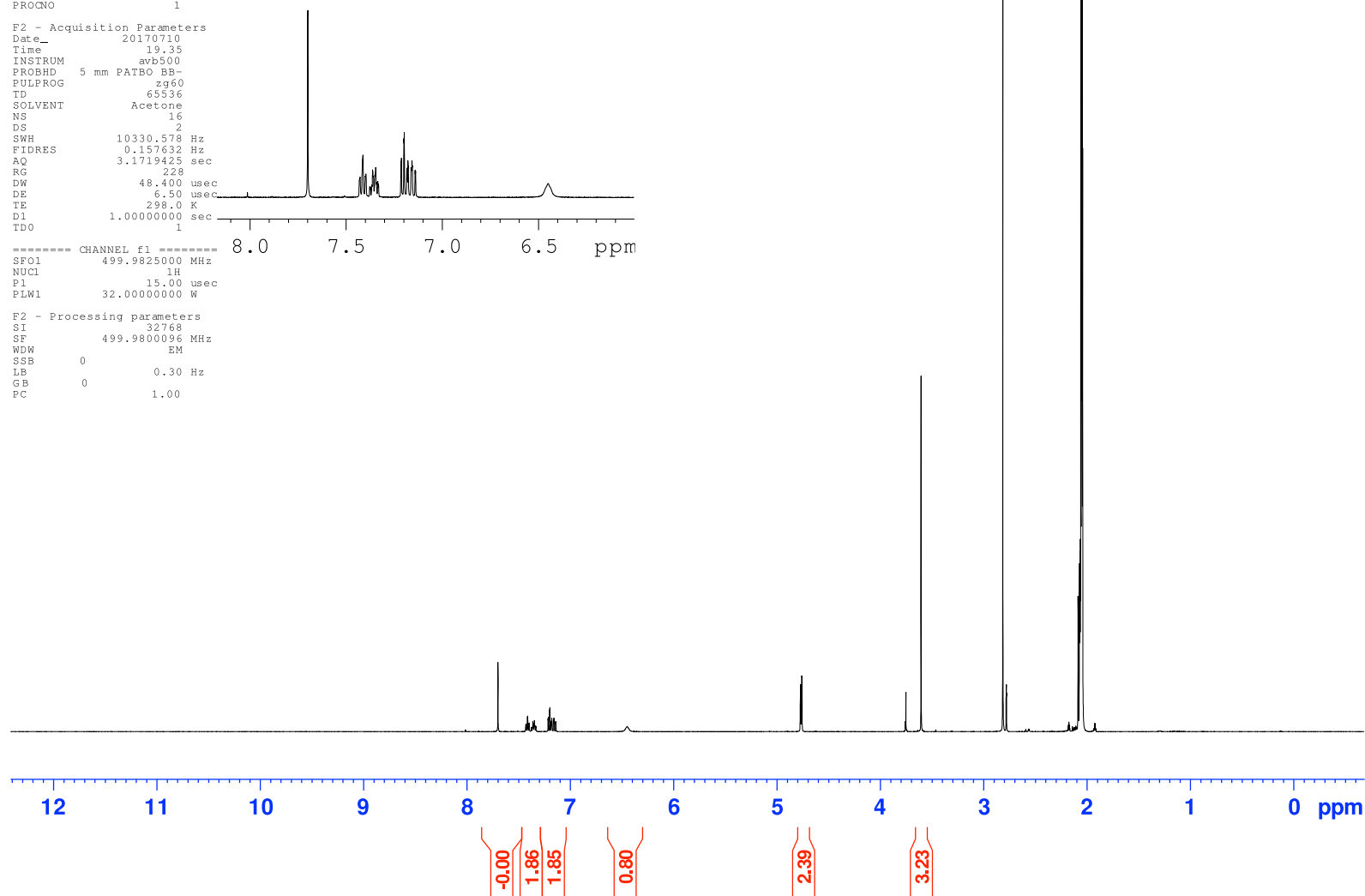


Current Data Parameters  
NAME c187161007 LCO1  
EXPNO 1  
PROCNO 1

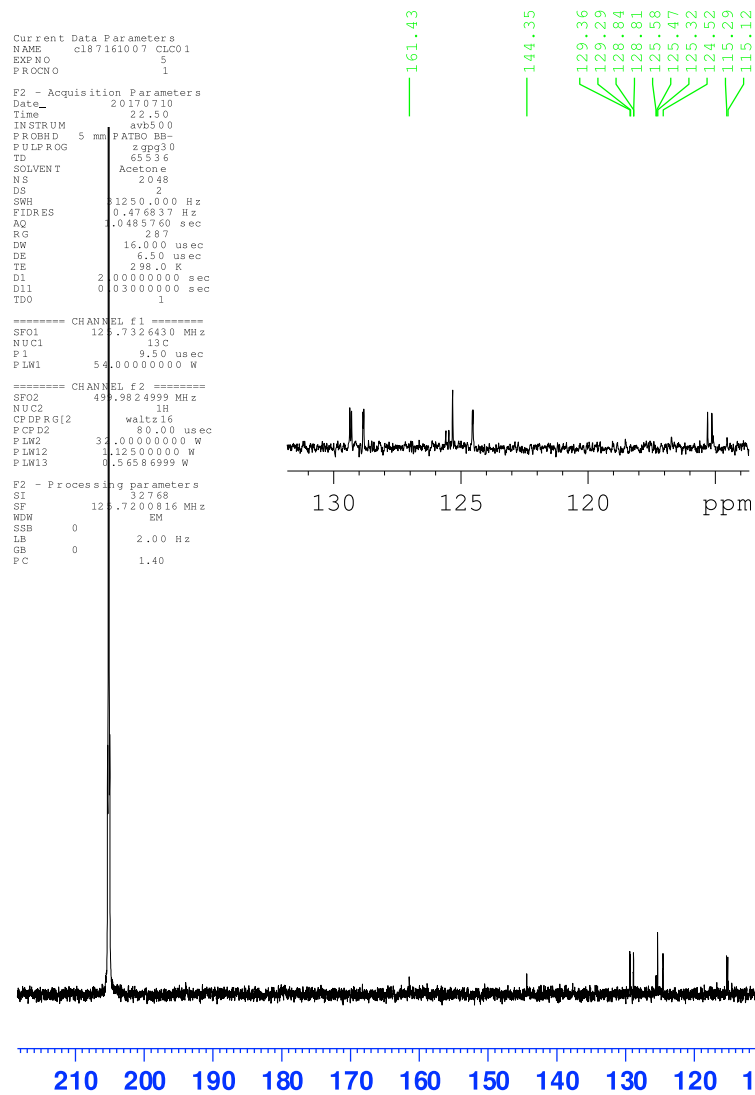
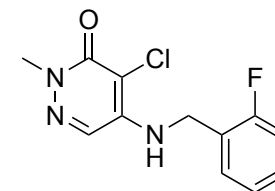
F2 - Acquisition Parameters  
Date\_ 20170710  
Time 19.35  
INSTRUM avb500  
PROBHD 5 mm PATBO BB-  
PULPROG zgpg30  
TD 65536  
SOLVENT Acetone  
NS 16  
DS 2  
SWH 10330.578 Hz  
FIDRES 0.157632 Hz  
AQ 3.1719425 sec  
RG 228  
DW 48.400 usec  
DE 6.50 usec  
TE 298.0 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
SFO1 499.9825000 MHz  
NUC1 1H  
P1 15.00 usec  
PLW1 32.00000000 W

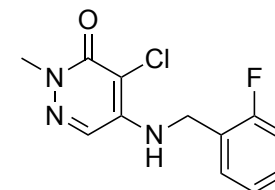
F2 - Processing parameters  
SI 32768  
SF 499.980096 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



# 4-Chloro-5-((2-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (50)



# 4-Chloro-5-((2-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (50)

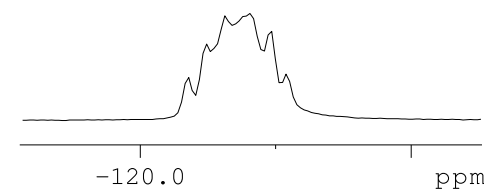


Current Data Parameters  
NAME Jul11-2019-36-CL-C-01  
EXPNO 3  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190712  
Time 2.58 h  
INSTRUM avg400  
PROBHD Z108618\_0816 (  
PULPROG zgpg30  
TD 261912  
SOLVENT Acetone  
NS 256  
DS 4  
SWH 131578.953 Hz  
FIDRES 1.004757 Hz  
AQ 0.9952656 sec  
RG 206.87  
DW 3.800 usec  
DE 6.50 usec  
TE 300.4 K  
D1 2.00000000 sec  
TD0 1  
SFO1 376.5359897 MHz  
NUC1 19F  
P1 15.00 usec  
PLW1 20.00000000 W

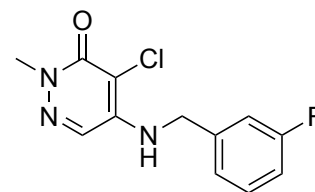
F2 - Processing parameters  
SI 131072  
SF 376.5642320 MHz  
WDW EM  
SSB 0  
LB 0.50 Hz  
GB 0  
PC 1.00

120.04  
120.05  
120.06  
120.08  
120.10  
120.11



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# 4-Chloro-5-((3-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (51)

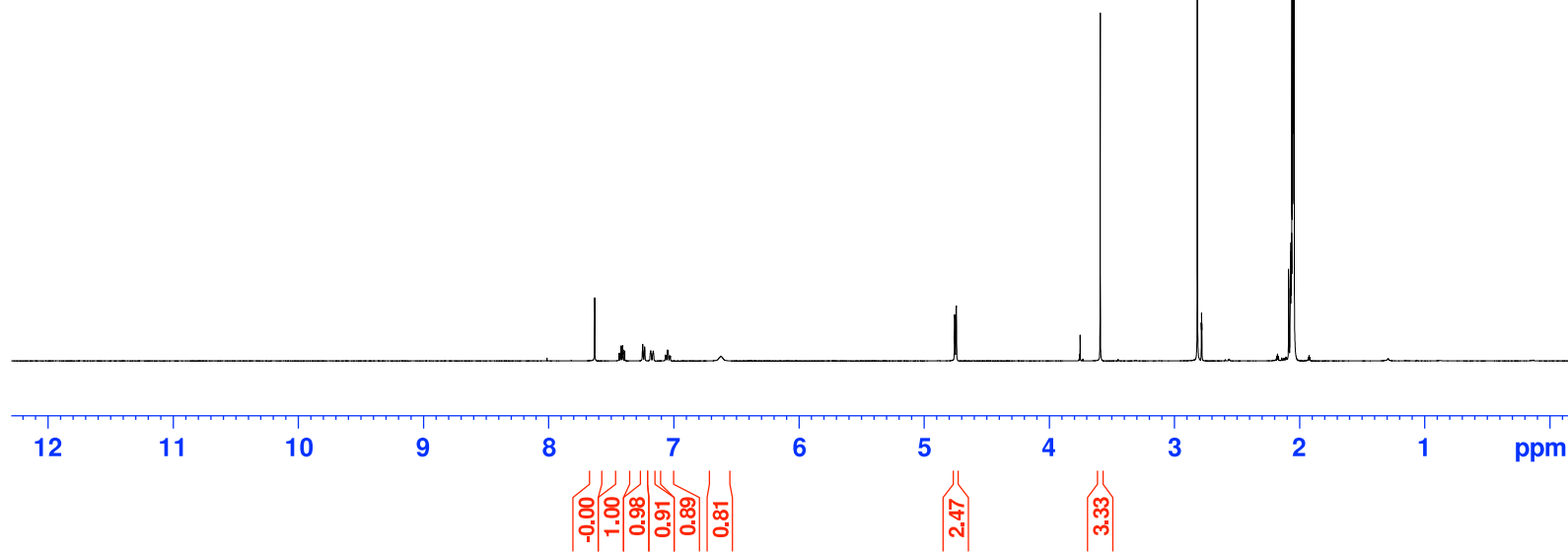
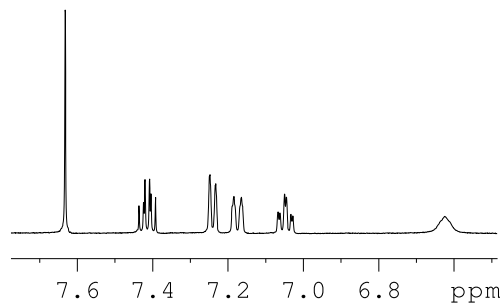


Current Data Parameters  
 NAME c187171007 CLC02  
 EXPNO 1  
 PROCNO 1

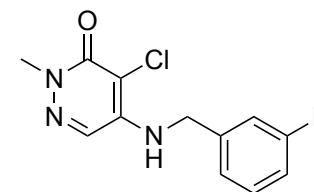
F2 - Acquisition Parameters  
 Date\_ 20170710  
 Time 22.56  
 INSTRUM avb500  
 PROBHD 5 mm PATBO BB-  
 PULPROG zgpg30  
 TD 65536  
 SOLVENT Acetone  
 NS 16  
 DS 2  
 SWH 10330.578 Hz  
 FIDRES 0.157632 Hz  
 AQ 3.1719425 sec  
 RG 228  
 DW 48.400 usec  
 DE 6.50 usec  
 TE 298.0 K  
 D1 1.00000000 sec  
 TD0 1

===== CHANNEL f1 =====  
 SFO1 499.9825000 MHz  
 NUCL 1H  
 P1 15.00 usec  
 PLW1 32.00000000 W

F2 - Processing parameters  
 SI 32768  
 SF 499.980092 MHz  
 WDW EM  
 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 1.00



# 4-Chloro-5-((3-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (51)



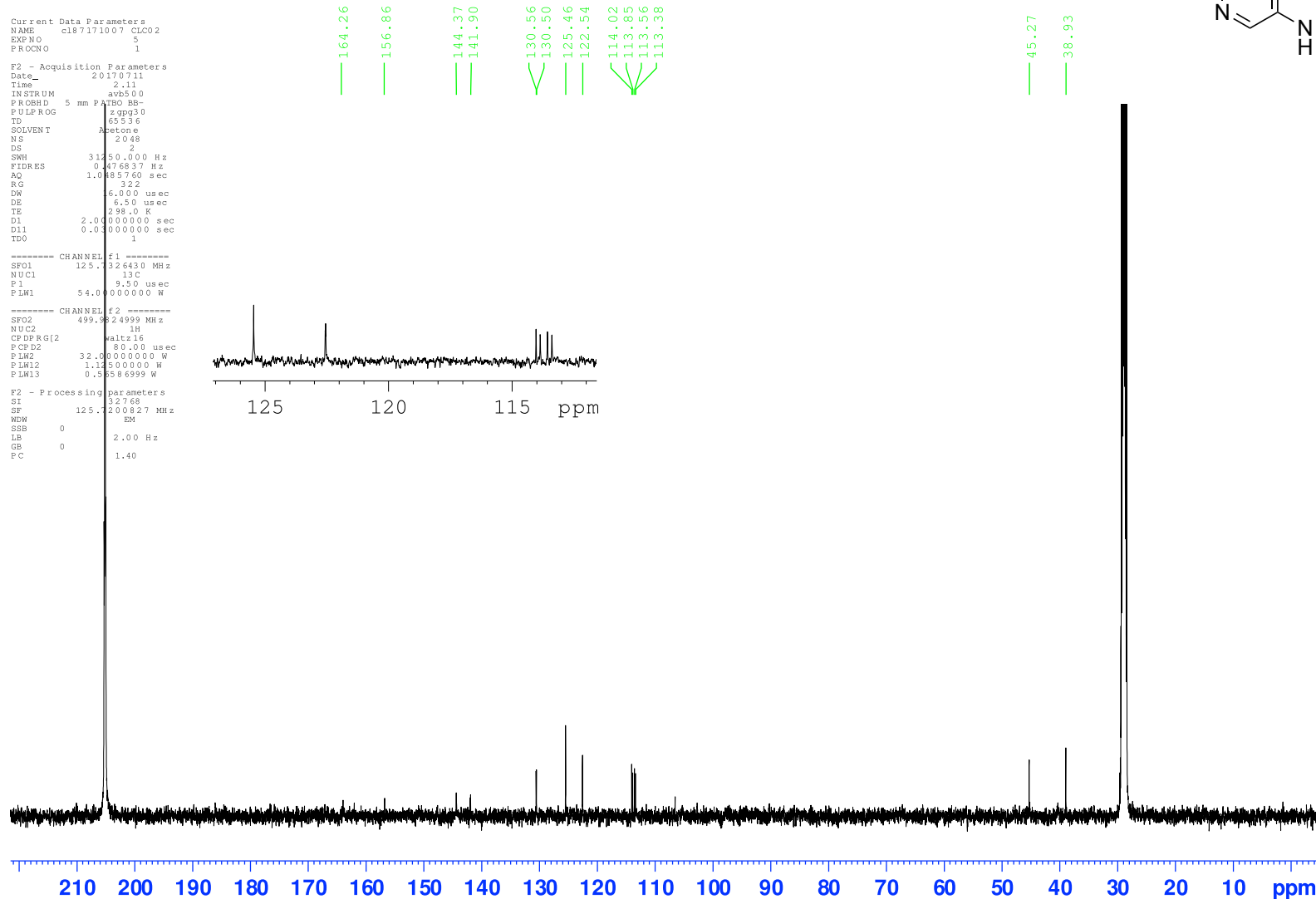
Current Data Parameters  
NAME c187171007 c1c02  
EXPNO 5  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20170711  
Time 2.11  
INSTRUM avb500  
PROBHD 5 mm PABBO BB-  
PULPROG zgpg30  
TD 65536  
SOLVENT Acetone  
NS 2048  
DS 2  
SWH 31250.000 Hz  
FIDRES 0.476837 Hz  
AQ 1.0485760 sec  
RG 322  
DW 6.000 usec  
DE 6.50 usec  
TE 298.0 K  
D1 2.0000000 sec  
D11 0.0000000 sec  
TD0 1

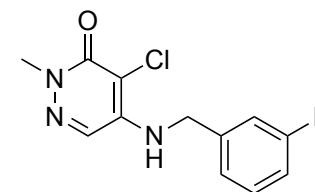
===== CHANNEL f1 =====  
SFO1 125.762430 MHz  
NUC1 13C  
P1 9.50 usec  
PLW1 54.0000000 W

===== CHANNEL f2 =====  
SFO2 499.824999 MHz  
NUC2 1H  
CPDPRG2 waltz16  
PCPD2 80.00 usec  
PLW2 32.0000000 W  
PLW12 1.12500000 W  
PLW13 0.56566999 W

F2 - Processing parameters  
SI 32768  
SF 125.7620827 MHz  
WDW EM  
SSB 0  
LB 2.00 Hz  
GB 0  
PC 1.40



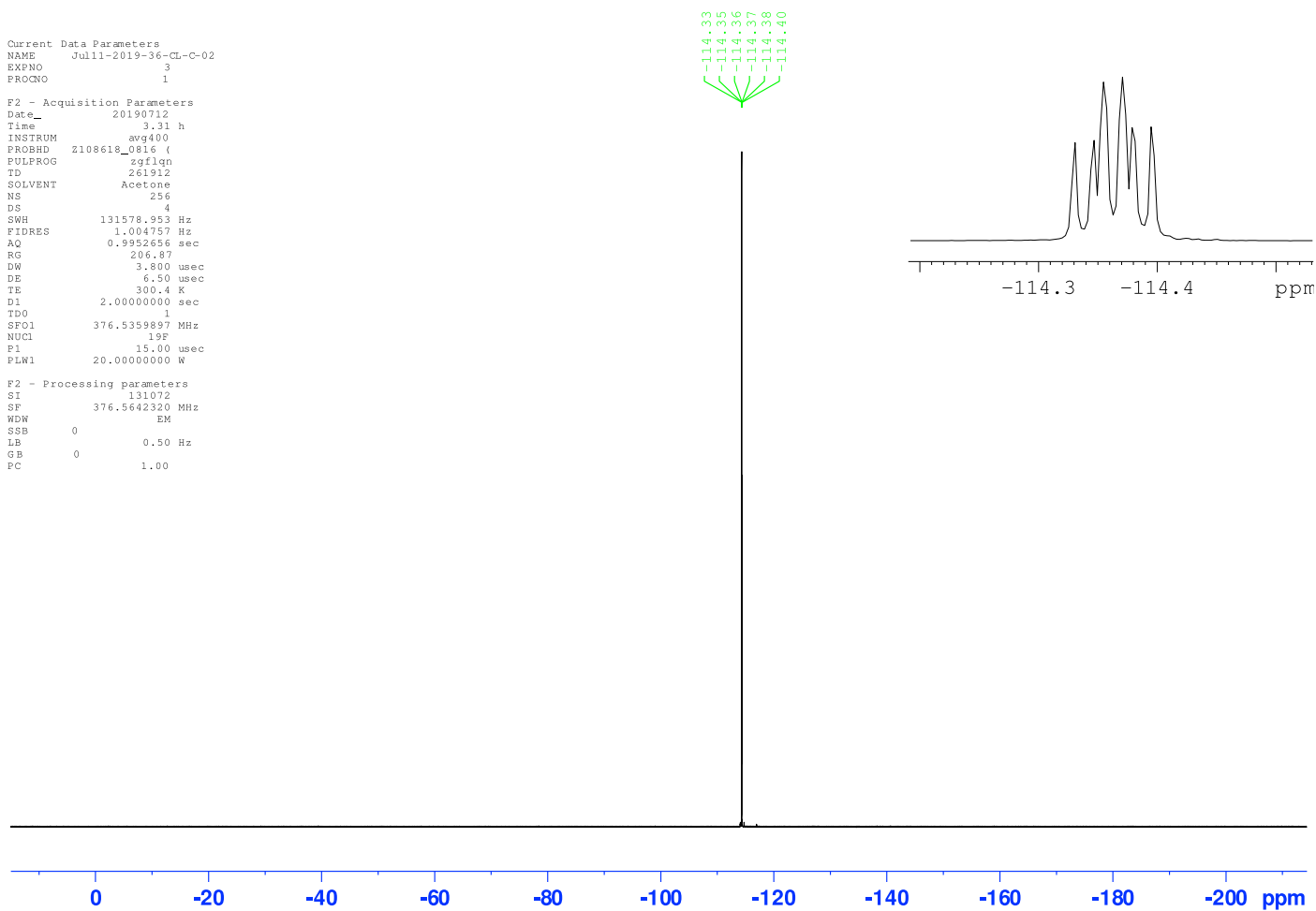
# 4-Chloro-5-((3-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (51)



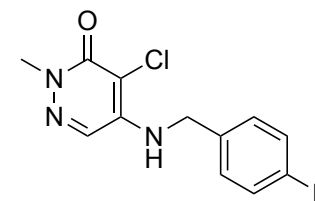
Current Data Parameters  
NAME Jul11-2019-36-CL-C-02  
EXPNO 3  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190712  
Time 3.31 h  
INSTRUM avq400  
PROBHD Z108618\_0816 (   
PULPROG zgpg30  
TD 261912  
SOLVENT Acetone  
NS 256  
DS 4  
SWH 131578.953 Hz  
FIDRES 1.004757 Hz  
AQ 0.9952656 sec  
RG 206.87  
DW 3.800 usec  
DE 6.50 usec  
TE 300.4 K  
D1 2.00000000 sec  
TD0 1  
SFO1 376.5359897 MHz  
NUC1 19F  
P1 15.00 usec  
PLW1 20.00000000 W

F2 - Processing parameters  
SI 131072  
SF 376.5642320 MHz  
WDW EM  
SSB 0  
LB 0.50 Hz  
GB 0  
PC 1.00



# 4-Chloro-5-((4-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (52)

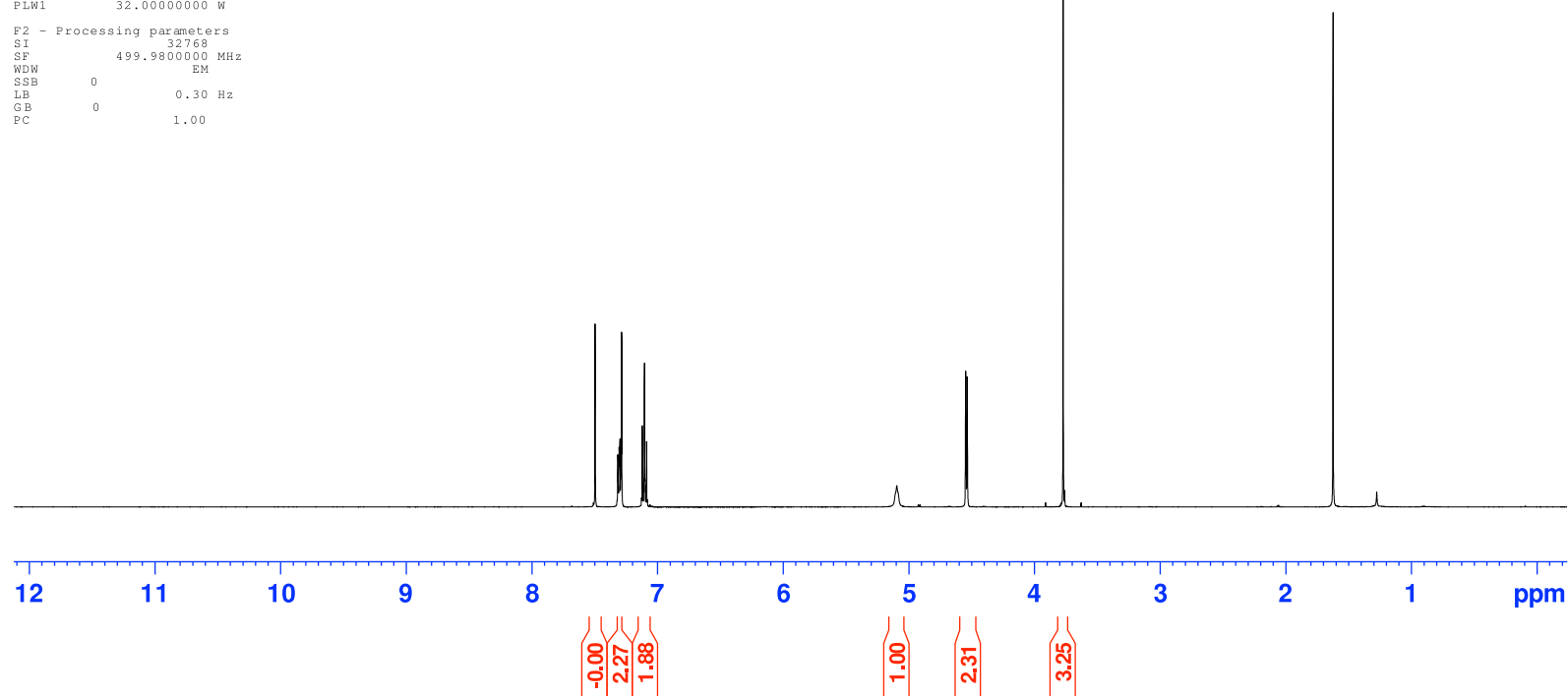
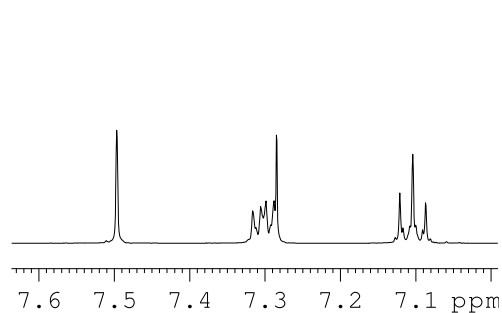


Current Data Parameters  
NAME c186002606  
EXPNO 1  
PROCNO 1

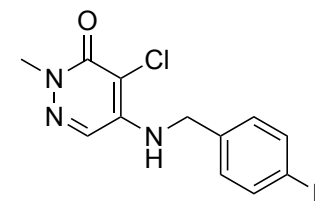
F2 - Acquisition Parameters  
Date\_ 20170626  
Time 11.43  
INSTRUM avb500  
PROBHD 5 mm PATBO BB-  
PULPROG zg60  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 2  
SWH 10330.578 Hz  
FIDRES 0.157632 Hz  
AQ 3.1719425 sec  
RG 287  
DW 48.400 usec  
DE 6.50 usec  
TE 298.0 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
SFO1 499.9825000 MHz  
NUC1 1H  
P1 15.00 usec  
PLW1 32.00000000 W

F2 - Processing parameters  
SI 32768  
SF 499.9800000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



# 4-Chloro-5-((4-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (52)



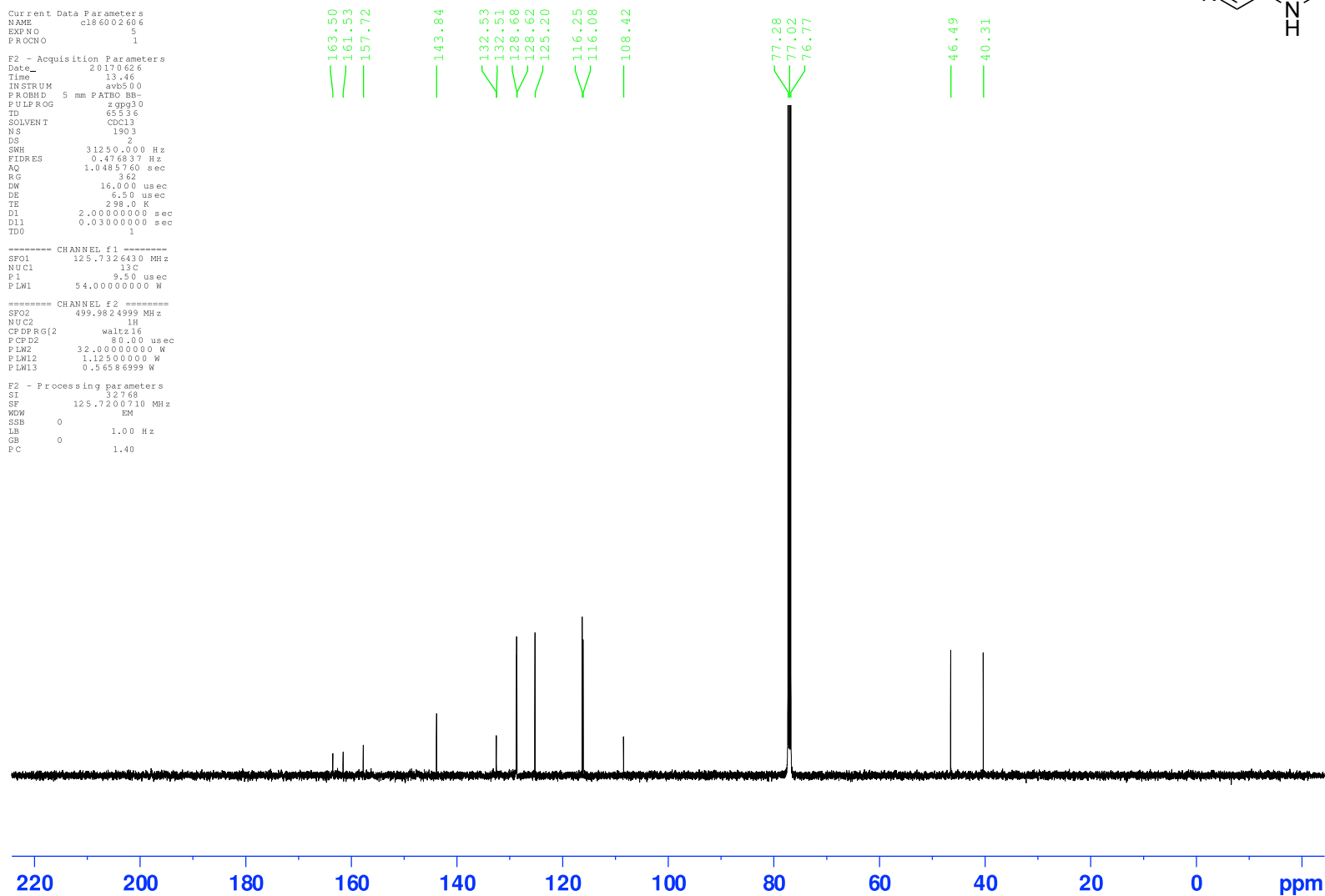
Current Data Parameters  
NAME c186002606  
EXPNO 5  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20170626  
Time 13.46  
INSTRUM av6500  
PROBHD 5 mm PABBO BB-  
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 1903  
DS 2  
SWH 31250.000 Hz  
FIDRES 0.476837 Hz  
AQ 1.0485760 sec  
RG 362  
DW 16.000 usec  
DE 6.50 usec  
TE 299.0 K  
D1 2.00000000 sec  
D11 0.03000000 sec  
TD0 1

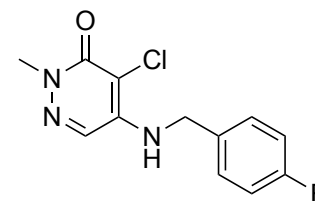
===== CHANNEL f1 =====  
SFO1 125.7326430 MHz  
NUC1 13C  
P1 9.50 usec  
PLW1 54.00000000 W

===== CHANNEL f2 =====  
SFO2 499.9824999 MHz  
NUC2 1H  
CPDPRG2 waltz16  
PCPD2 80.00 usec  
PLW2 32.00000000 W  
PLW12 1.12500000 W  
PLW13 0.56586999 W

F2 - Processing parameters  
SI 32768  
SF 125.7200710 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40



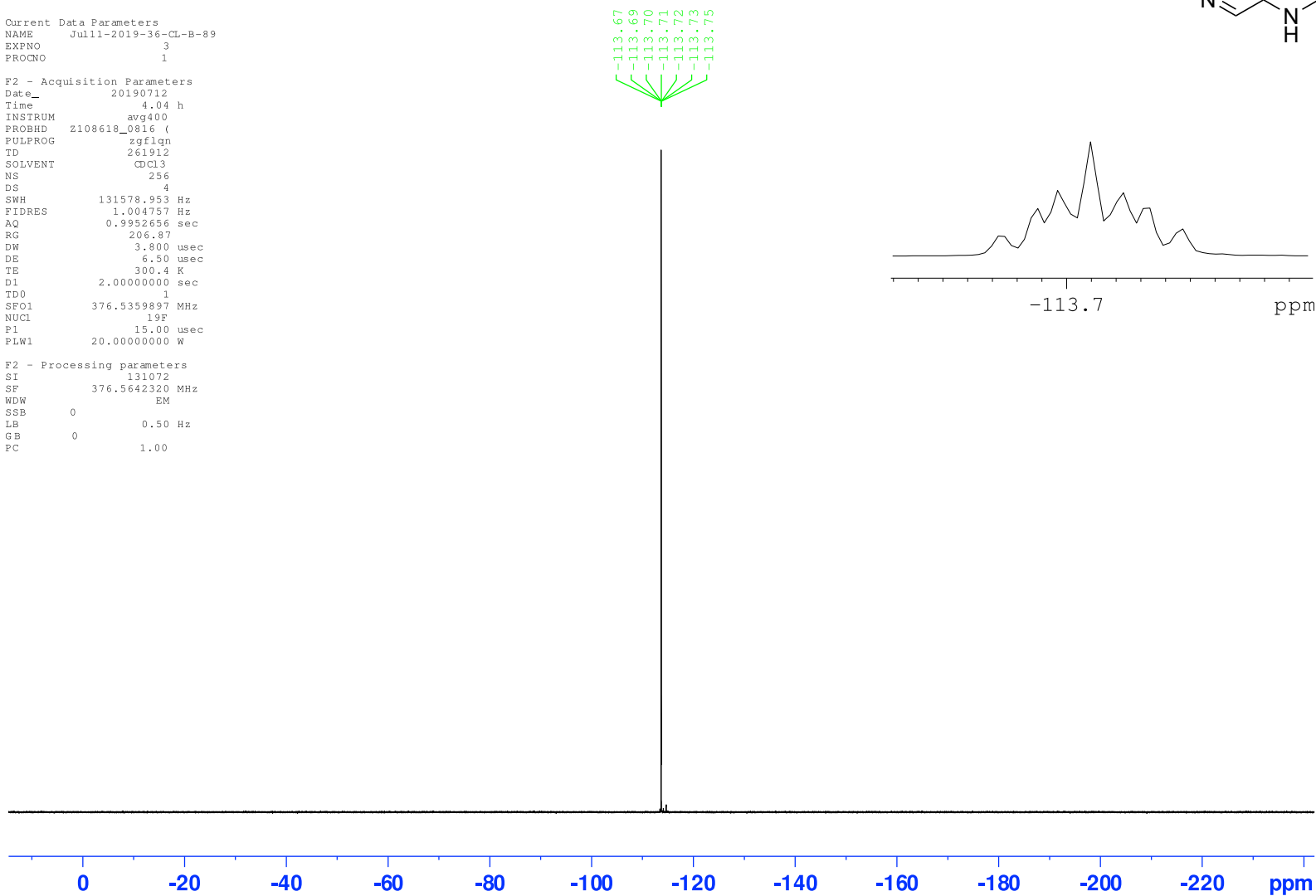
# 4-Chloro-5-((4-fluorobenzyl)amino)-2-methylpyridazin-3(2H)-one (52)



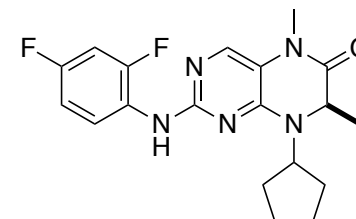
Current Data Parameters  
NAME Jull11-2019-36-CL-B-89  
EXPNO 3  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190712  
Time 4.04 h  
INSTRUM avg400  
PROBHD 2108618\_0816 (   
PULPROG zgpg30  
TD 261912  
SOLVENT CDCl3  
NS 256  
DS 4  
SWH 131578.953 Hz  
FIDRES 1.004757 Hz  
AQ 0.9952656 sec  
RG 206.87  
DW 3.800 usec  
DE 6.50 usec  
TE 300.4 K  
D1 2.00000000 sec  
TD0 1  
SFO1 376.5359897 MHz  
NUC1 19F  
P1 15.00 usec  
PLW1 20.00000000 W

F2 - Processing parameters  
SI 131072  
SF 376.5642320 MHz  
WDW EM  
SSB 0  
LB 0.50 Hz  
GB 0  
PC 1.00



**(7R)-8-Cyclopentyl-2-((2',4'-difluorophenyl)amino)-7-ethyl-5-methyl-7,8-dihydropteridin-6(5H)-one (54)**

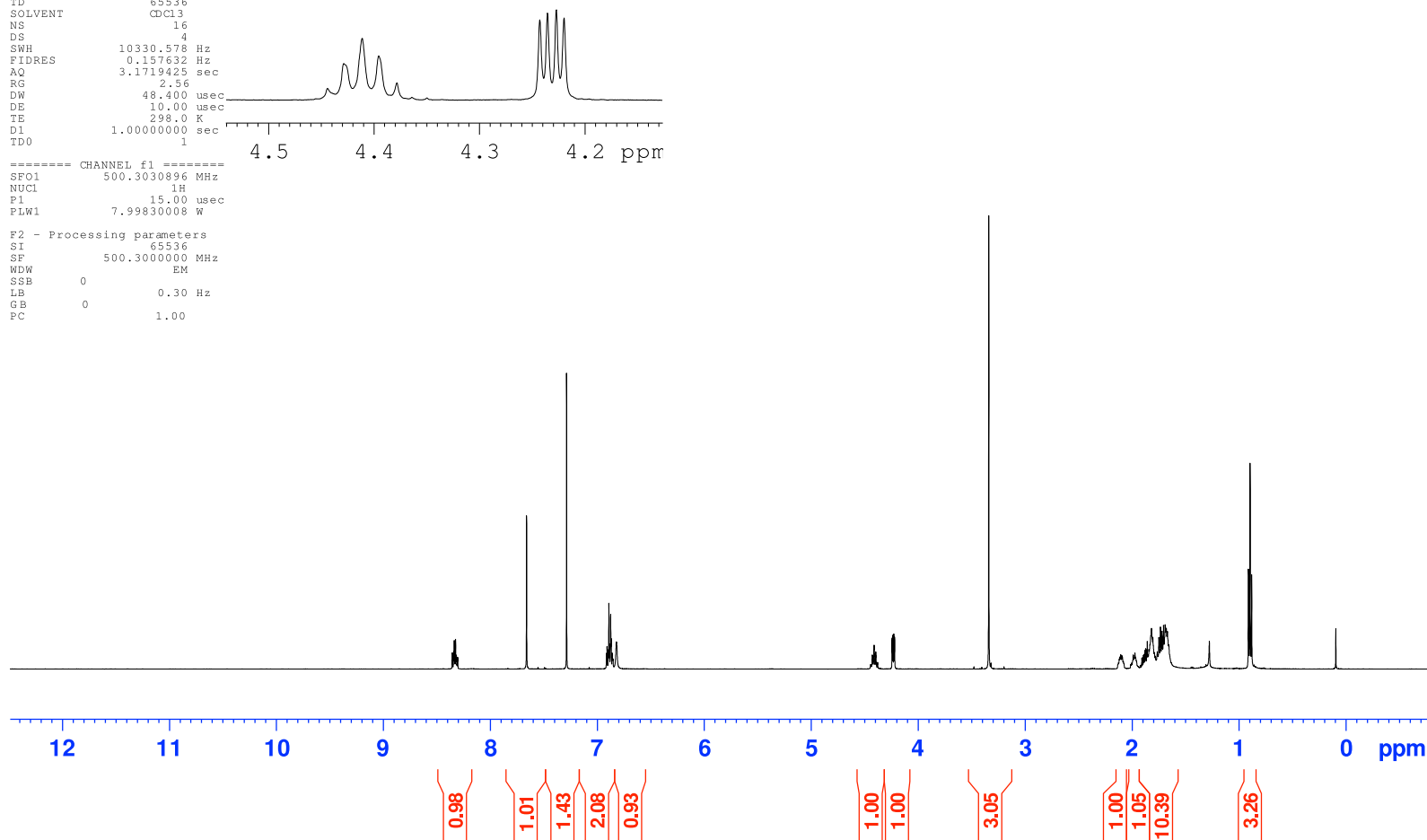


Current Data Parameters  
NAME c122680406 CLC65  
EXPNO 1  
PROCNO 1

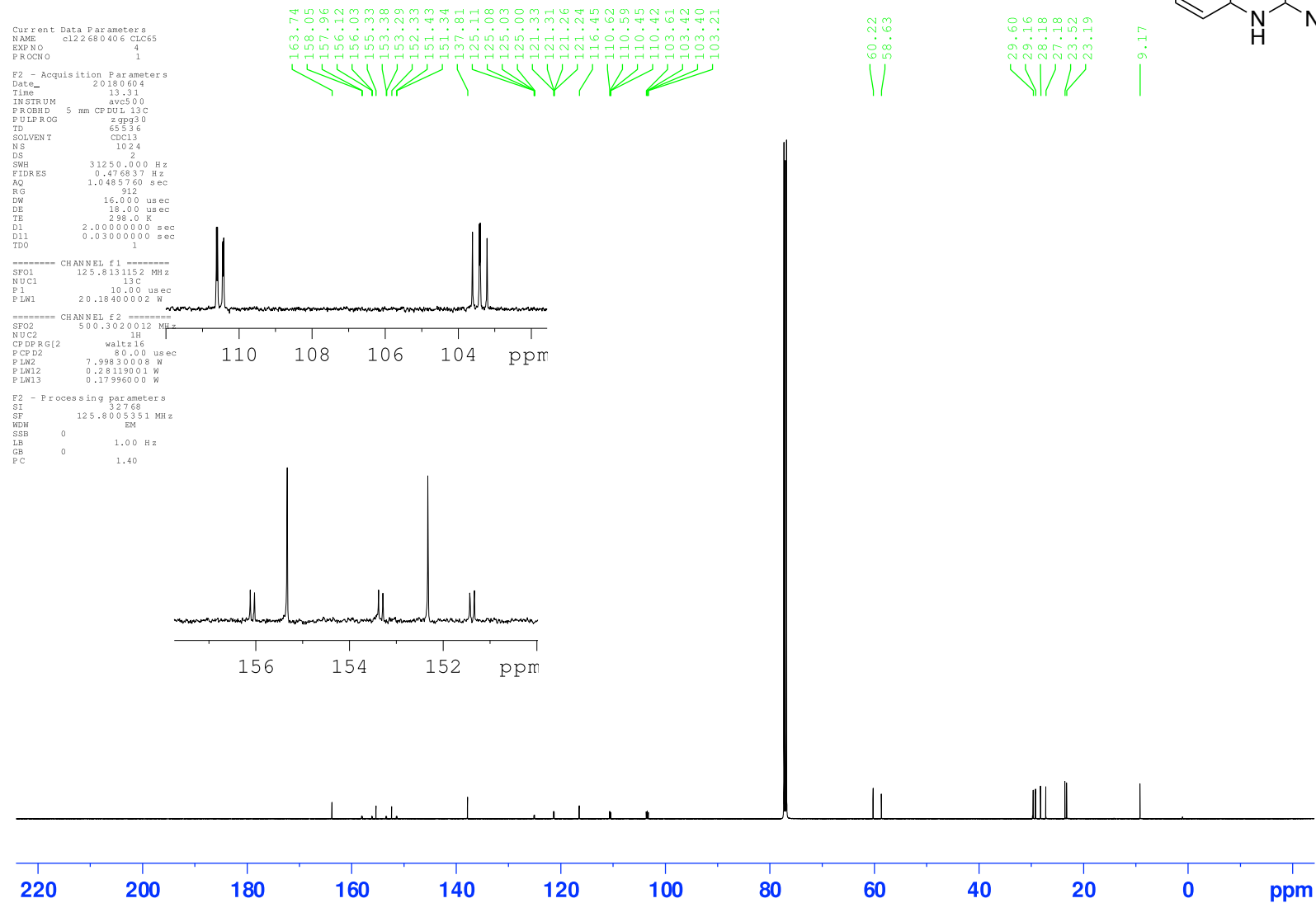
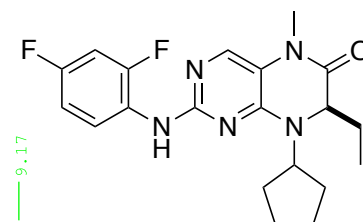
F2 - Acquisition Parameters  
Date\_ 20180604  
Time 12.16  
INSTRUM avc500  
PROBHD 5 mm CPDUL 13C  
PULPROG zg30  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 4  
SWH 10330.578 Hz  
FIDRES 0.157632 Hz  
AQ 3.1719425 sec  
RG 2.56  
DW 48.400 usec  
DE 10.00 usec  
TE 298.0 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
SFO1 500.3030896 MHz  
NUCL 1H  
P1 15.00 usec  
PLW1 7.99830008 W

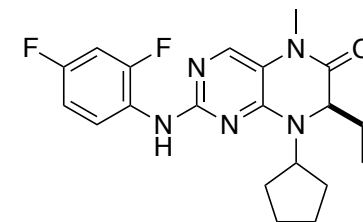
F2 - Processing parameters  
SI 65536  
SF 500.3000000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



**(7*R*)-8-Cyclopentyl-2-((2',4'-difluorophenyl)amino)-7-ethyl-5-methyl-7,8-dihydropteridin-6(5*H*)-one (54)**



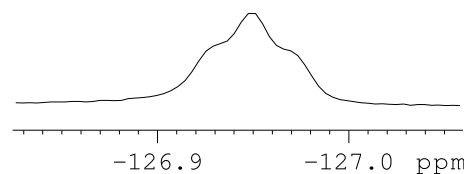
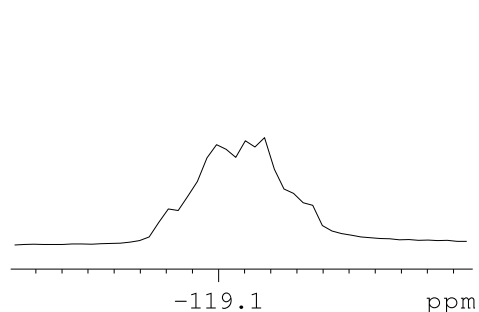
**(7*R*)-8-Cyclopentyl-2-((2',4'-difluorophenyl)amino)-7-ethyl-5-methyl-7,8-dihydropteridin-6(5*H*)-one (54)**



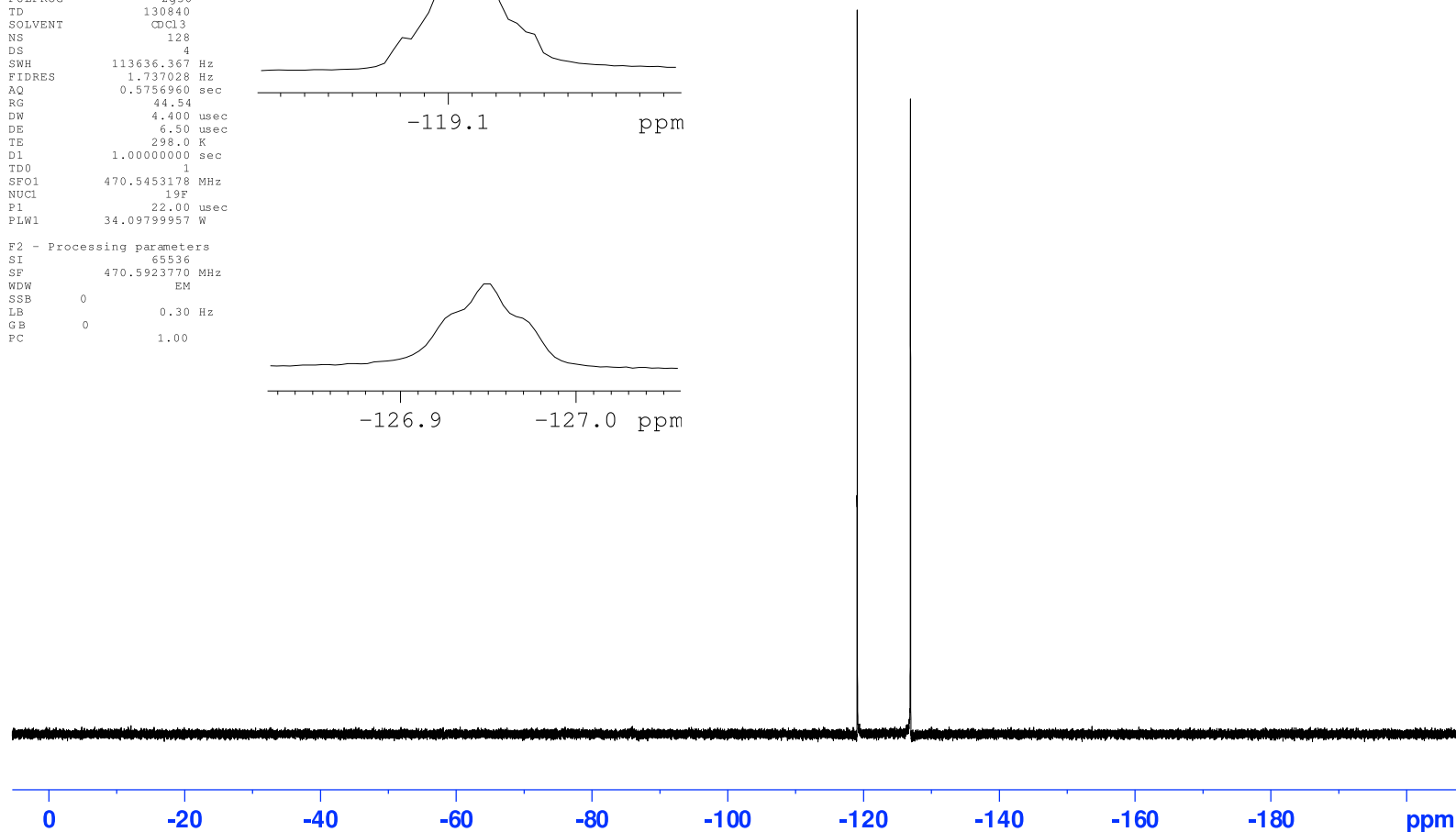
Current Data Parameters  
NAME c122683105  
EXPNO 2  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20180531  
Time 13.27 h  
INSTRUM avx500  
PROBHD Z119877\_0007 (   
PULPROG zg30  
TD 130840  
SOLVENT CDCl3  
NS 128  
DS 4  
SWH 113636.367 Hz  
FIDRES 1.737028 Hz  
AQ 0.5756960 sec  
RG 44.54  
DW 4.400 usec  
DE 6.50 usec  
TE 298.0 K  
D1 1.00000000 sec  
TD0 1  
SFO1 470.5453178 MHz  
NUC1 19F  
P1 22.00 usec  
PLW1 34.09799957 W

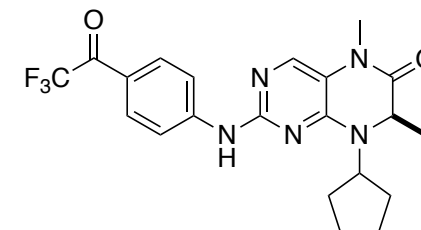
F2 - Processing parameters  
SI 65536  
SF 470.5923770 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



-119.08  
-119.10  
-119.11  
-119.12  
-126.93  
-126.95  
-126.97



**(7*R*)-8-Cyclopentyl-7-ethyl-5-methyl-2-((4'-(2,2,2-trifluoroacetyl)phenyl)amino)-7,8-dihydropteridin-6(5*H*)-one (55)**

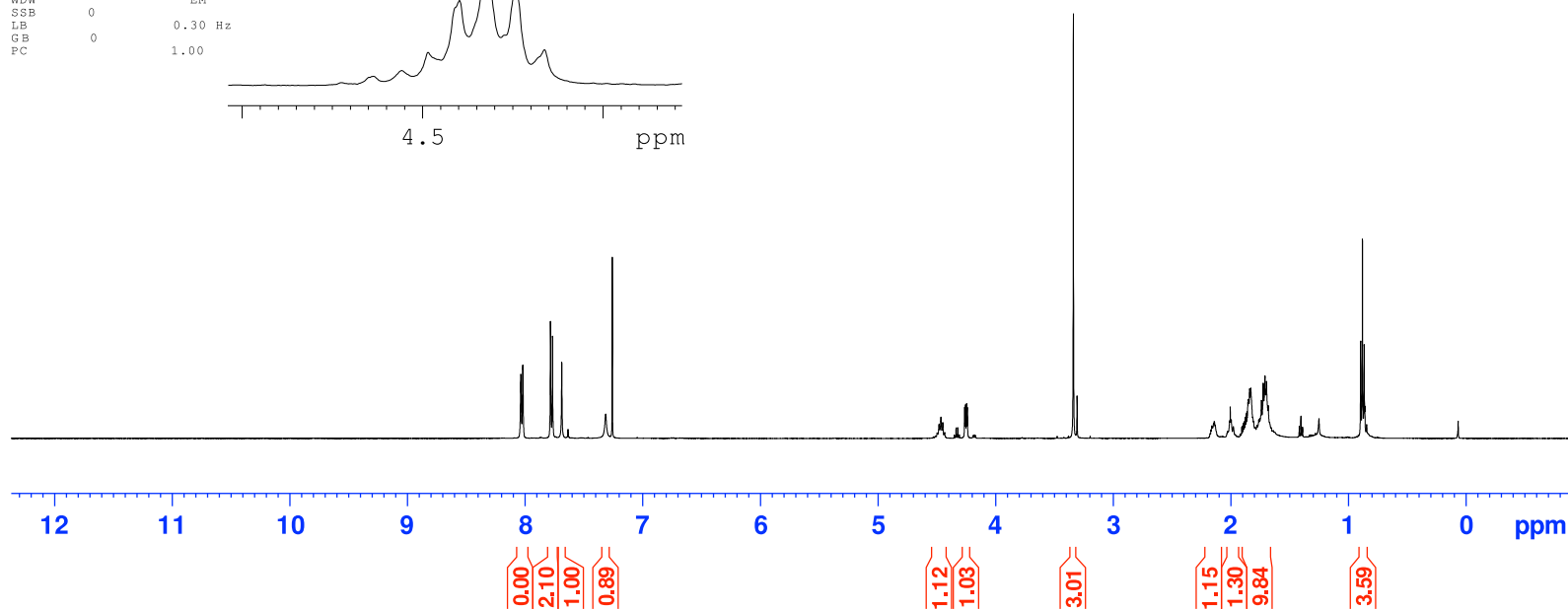
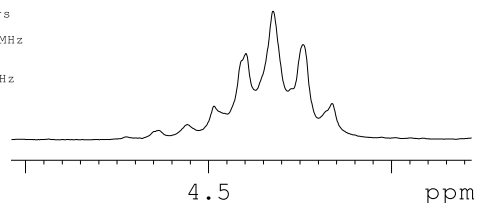
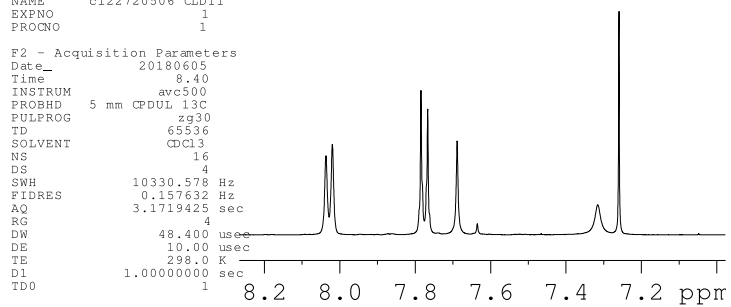


Current Data Parameters  
NAME cl22720506 CLD11  
EXPNO 1  
PROCNO 1

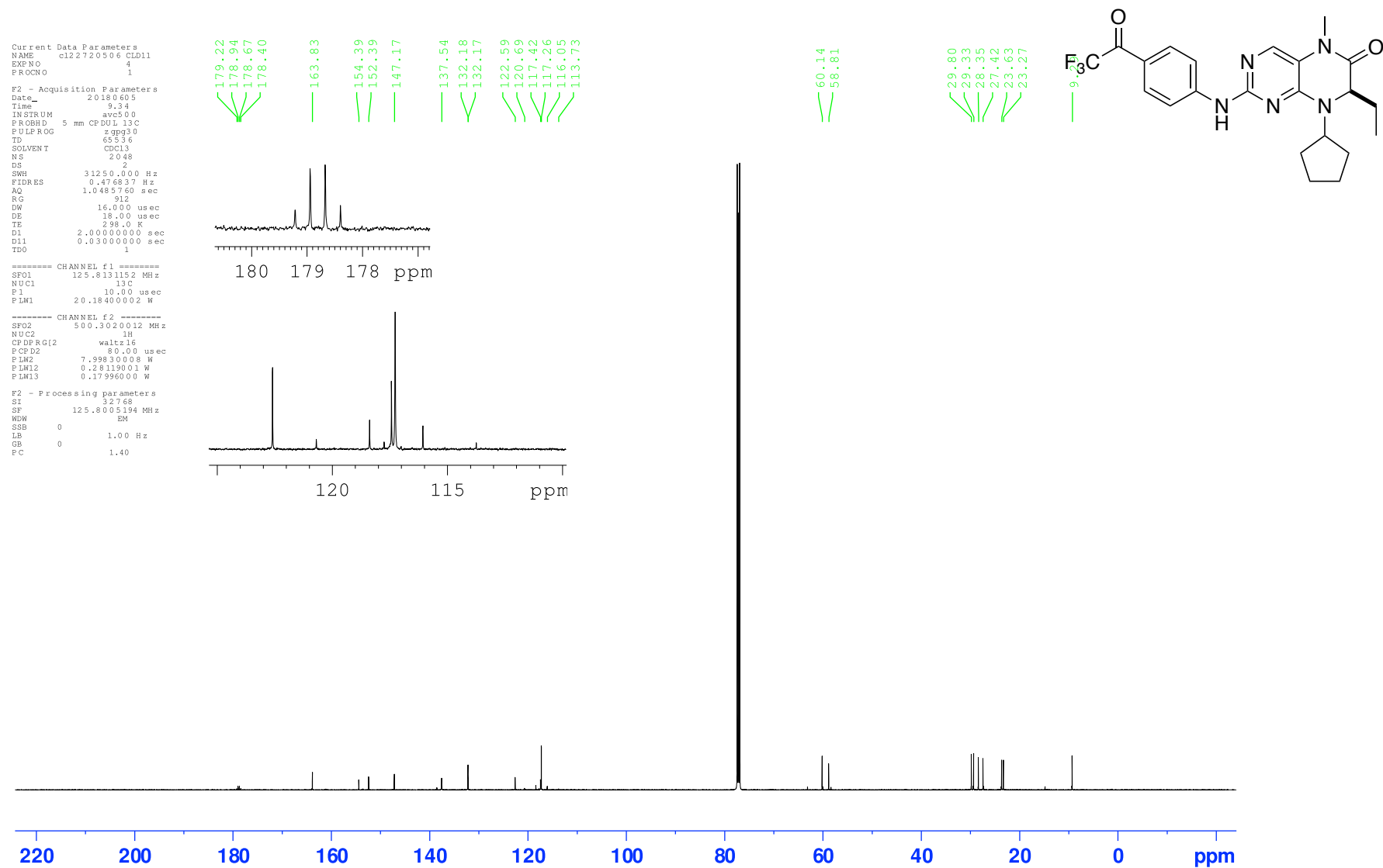
F2 - Acquisition Parameters  
Date\_ 20180605  
Time 8.40  
INSTRUM avc500  
PROBHD 5 mm CPDUL 13C  
PULPROG zg30  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 4  
SWH 10330.578 Hz  
FIDRES 0.157632 Hz  
AQ 3.1719425 sec  
RG 4  
DW 48.400 usec  
DE 10.00 usec  
TE 298.0 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
SFO1 500.3030896 MHz  
NUC1 1H  
P1 15.00 usec  
PLW1 7.99830008 W

F2 - Processing parameters  
SI 65536  
SF 500.3000137 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
FC 1.00



**(7*R*)-8-Cyclopentyl-7-ethyl-5-methyl-2-((4'-(2,2,2-trifluoroacetyl)phenyl)amino)-7,8-dihydropteridin-6(5*H*)-one (55)**

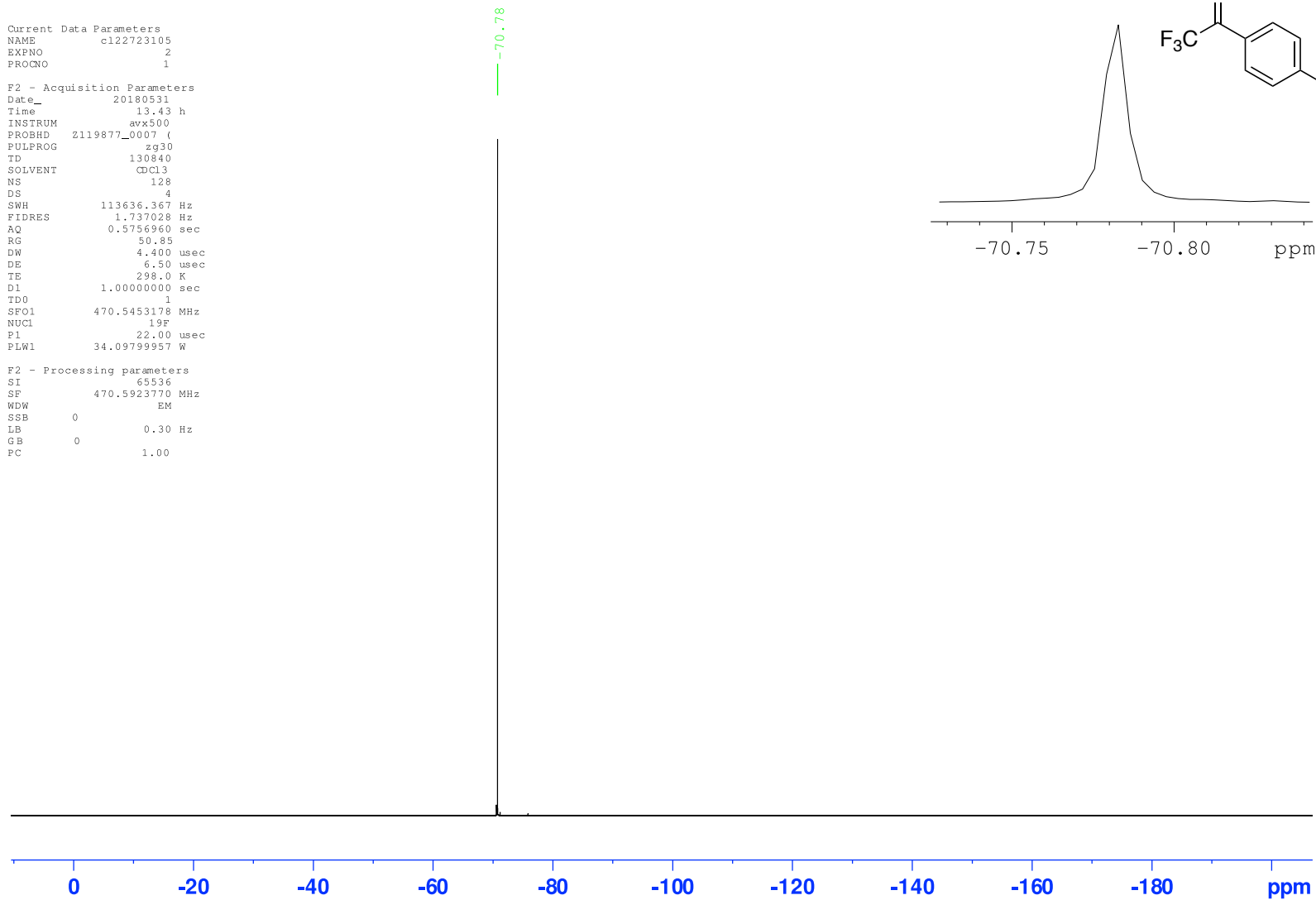


**(7R)-8-Cyclopentyl-7-ethyl-5-methyl-2-((4'-(2,2,2-trifluoroacetyl)phenyl)amino)-7,8-dihydropteridin-6(5H)-one (55)**

Current Data Parameters  
NAME c122723105  
EXPNO 2  
PROCNO 1

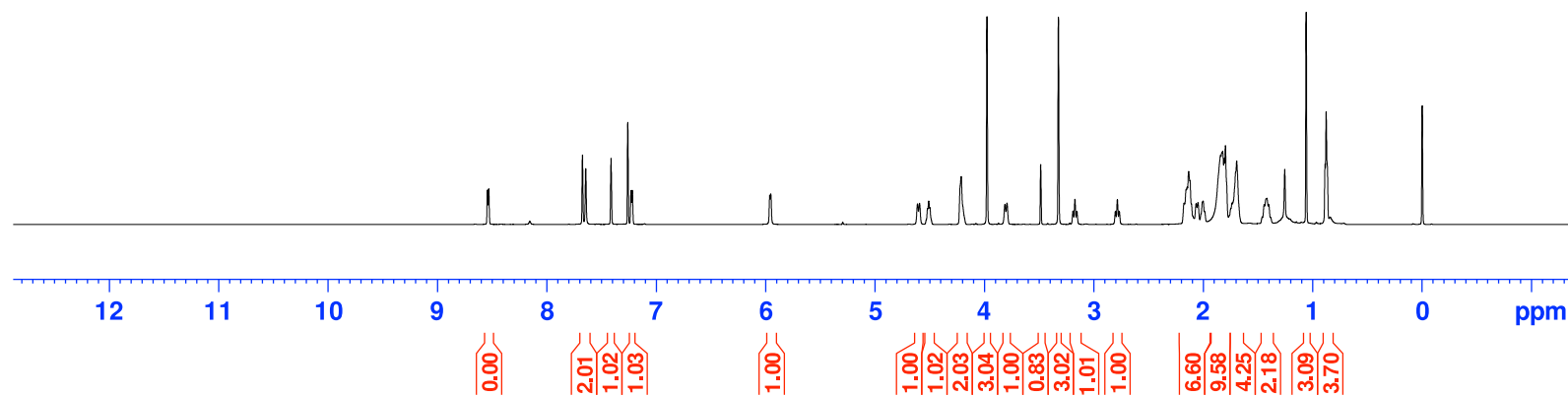
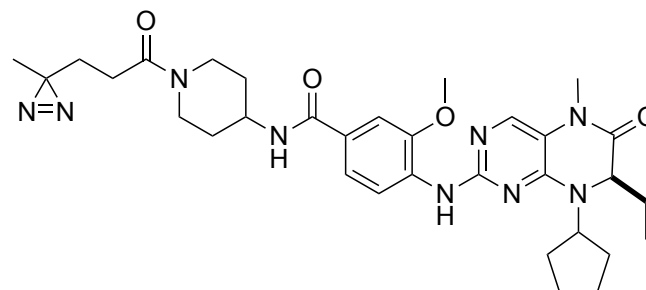
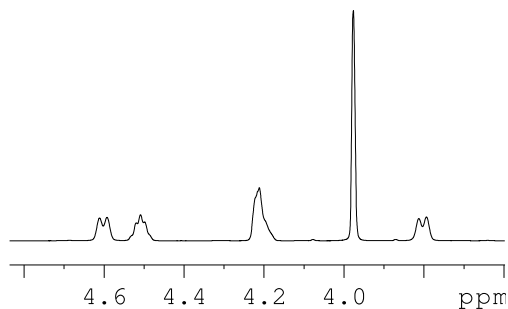
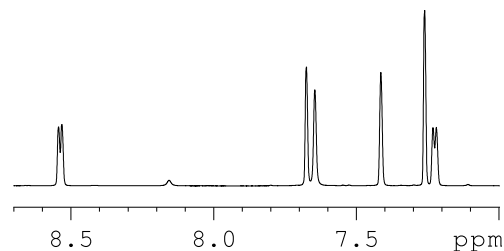
F2 - Acquisition Parameters  
Date\_ 20180531  
Time 13.43 h  
INSTRUM avx500  
PROBHD Z119877\_0007 (   
PULPROG zg30  
TD 130840  
SOLVENT CDCl3  
NS 128  
DS 4  
SWH 113636.367 Hz  
FIDRES 1.737028 Hz  
AQ 0.5756960 sec  
RG 50.85  
DW 4.400 usec  
DE 6.50 usec  
TE 298.0 K  
D1 1.0000000 sec  
TD0 1  
SFO1 470.5453178 MHz  
NUC1 19F  
P1 22.00 usec  
PLW1 34.09799957 W

F2 - Processing parameters  
SI 65536  
SF 470.5923770 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



**(R)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(1-(3-(3-methyl-3H-diazirin-3-yl)propanoyl)piperidin-4-yl)benzamide (57)**

Current Data Parameters  
NAME DS10987\_10\_1H  
EXPNO 10  
PROCNO 1  
F2 - Acquisition Parameters  
Date\_ 20180801  
Time 10.25 h  
INSTRUM GH002716  
PROBHD Z44908\_0026 (C  
PULPROG zg30  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 0  
SWH 13888.889 Hz  
FIDRES 0.423855 Hz  
AQ 2.3592961 sec  
RG 51.4984  
DW 36.000 usec  
DE 10.00 usec  
TE 303.0 K  
D1 2.0000000 sec  
TD0 1  
SFO1 700.2052515 MHz  
NUC1 1H  
P0 2.67 usec  
P1 8.00 usec  
PLW1 8.62209988 W  
F2 - Processing parameters  
SI 65536  
SF 700.2000202 MHz  
WDW EM  
SBB 0  
LB 0.20 Hz  
GB 0  
PC 1.50



**(R)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-N-(1-(3-(3-methyl-3H-diazirin-3-yl)propanoyl)piperidin-4-yl)benzamide (57)**

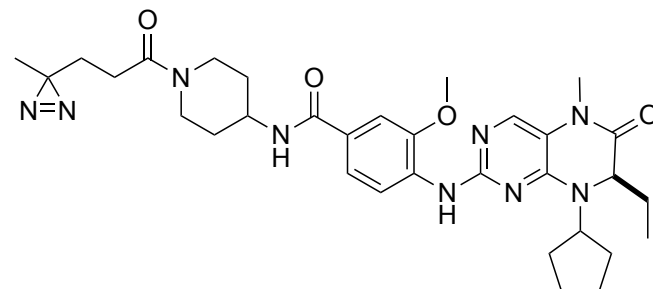
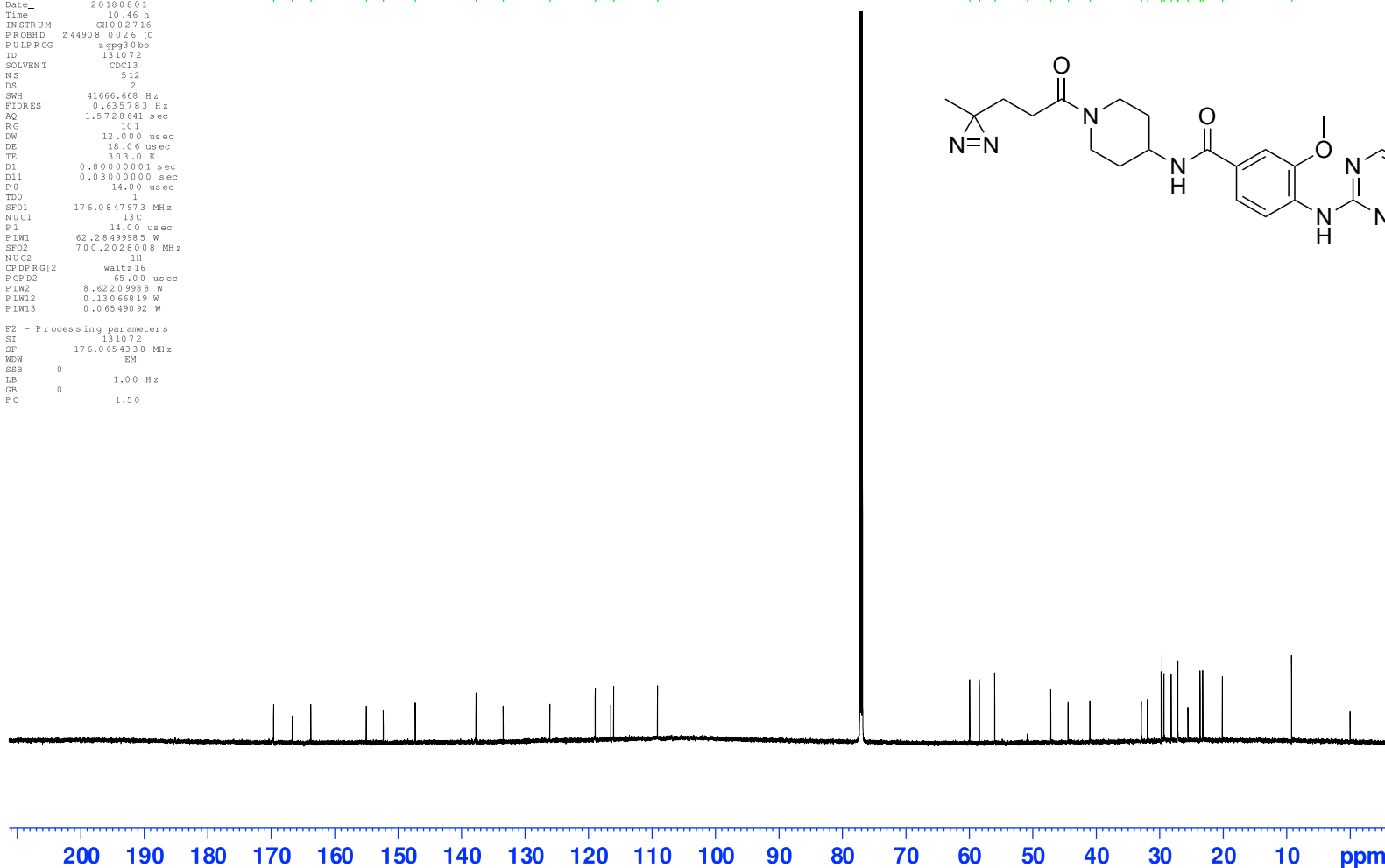
Current Data Parameters  
 NAME DS10987\_11\_13C  
 EXP NO 11  
 PROCNO 1

F2 - Acquisition Parameters  
 Date\_ 20180801  
 Time 10.46 h  
 INSTRUM GH002716  
 PROBD 2.44908\_0026 (C  
 PULPROG zgpg30bo  
 TD 131072  
 SOLVENT CDCl3  
 NS 512  
 DS 2  
 SWH 41666.668 Hz  
 FIDRES 0.635783 Hz  
 AQ 1.5728641 sec  
 RG 101  
 DW 12.000 usec  
 DE 18.06 usec  
 TE 303.0 K  
 D1 0.80000001 sec  
 D11 0.03000000 sec  
 PD 14.00 usec  
 TDO 1  
 SFO1 176.0847973 MHz  
 NUC1 13C  
 P1 14.00 usec  
 PLW1 62.2849985 W  
 SFO2 700.2028008 MHz  
 NUC2 1H  
 CPDPRG2 waltz16  
 PCPD2 65.00 usec  
 PLW2 8.62209988 W  
 PLW12 0.13066819 W  
 PLW13 0.06549092 W

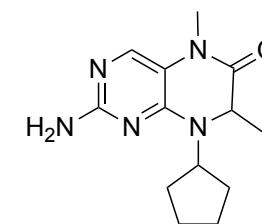
F2 - Processing parameters  
 SI 131072  
 SF 176.0654338 MHz  
 WDW EM  
 SSB 0  
 LB 1.00 Hz  
 GB 0  
 PC 1.50

169.6  
166.8  
163.7  
154.98  
152.5  
147.26  
137.70  
133.42  
126.07  
118.92  
116.45  
116.00  
109.10

59.92  
58.40  
55.97  
50.84  
47.14  
44.40  
40.97  
32.87  
31.92  
29.74  
29.68  
29.61  
29.30  
28.17  
27.17  
27.11  
25.52  
25.61  
23.20  
20.09  
9.19



## 2-Amino-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5H)-one (66)

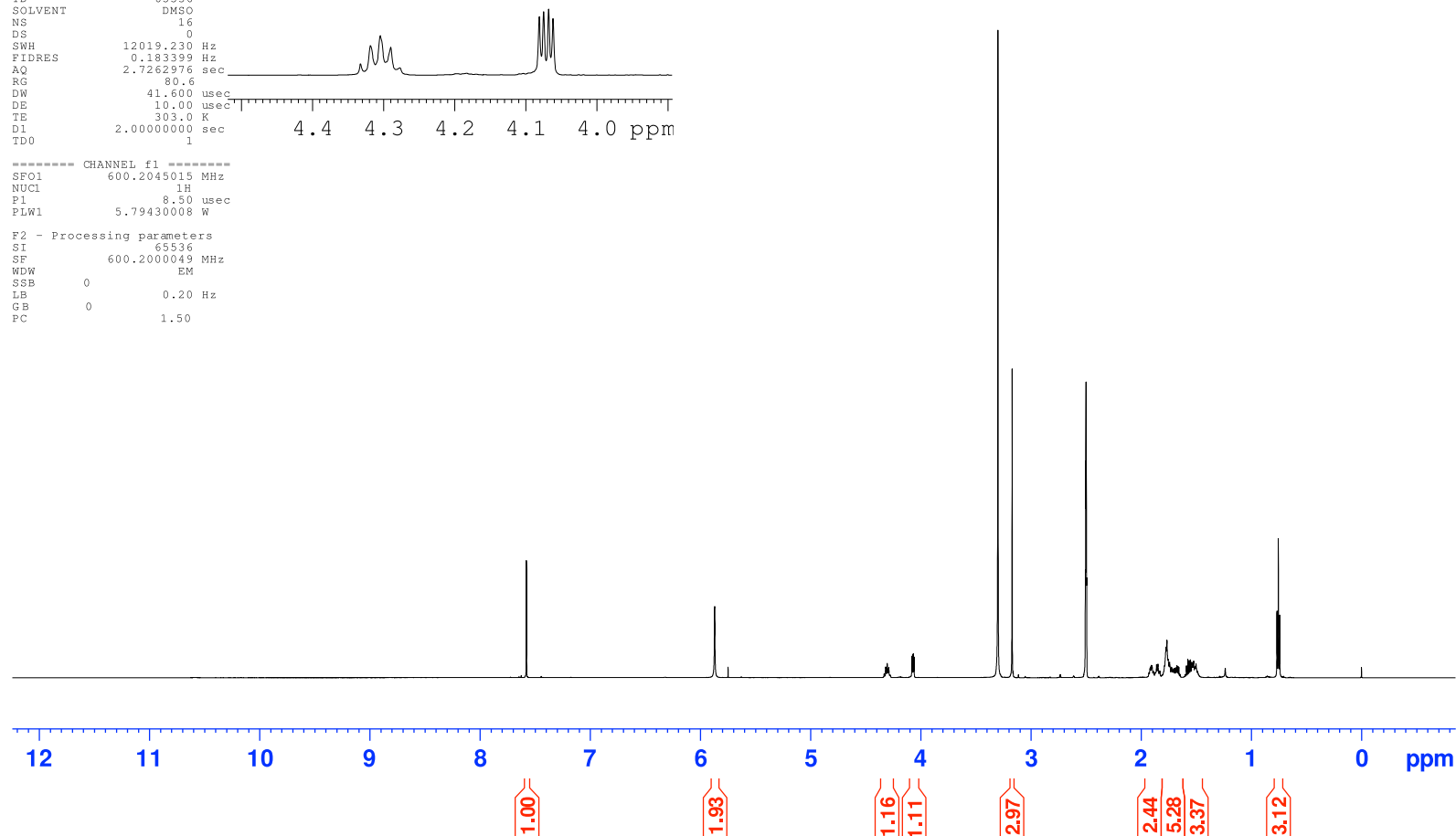


Current Data Parameters  
NAME PT18453\_11  
EXPNO 11  
PROCNO 1

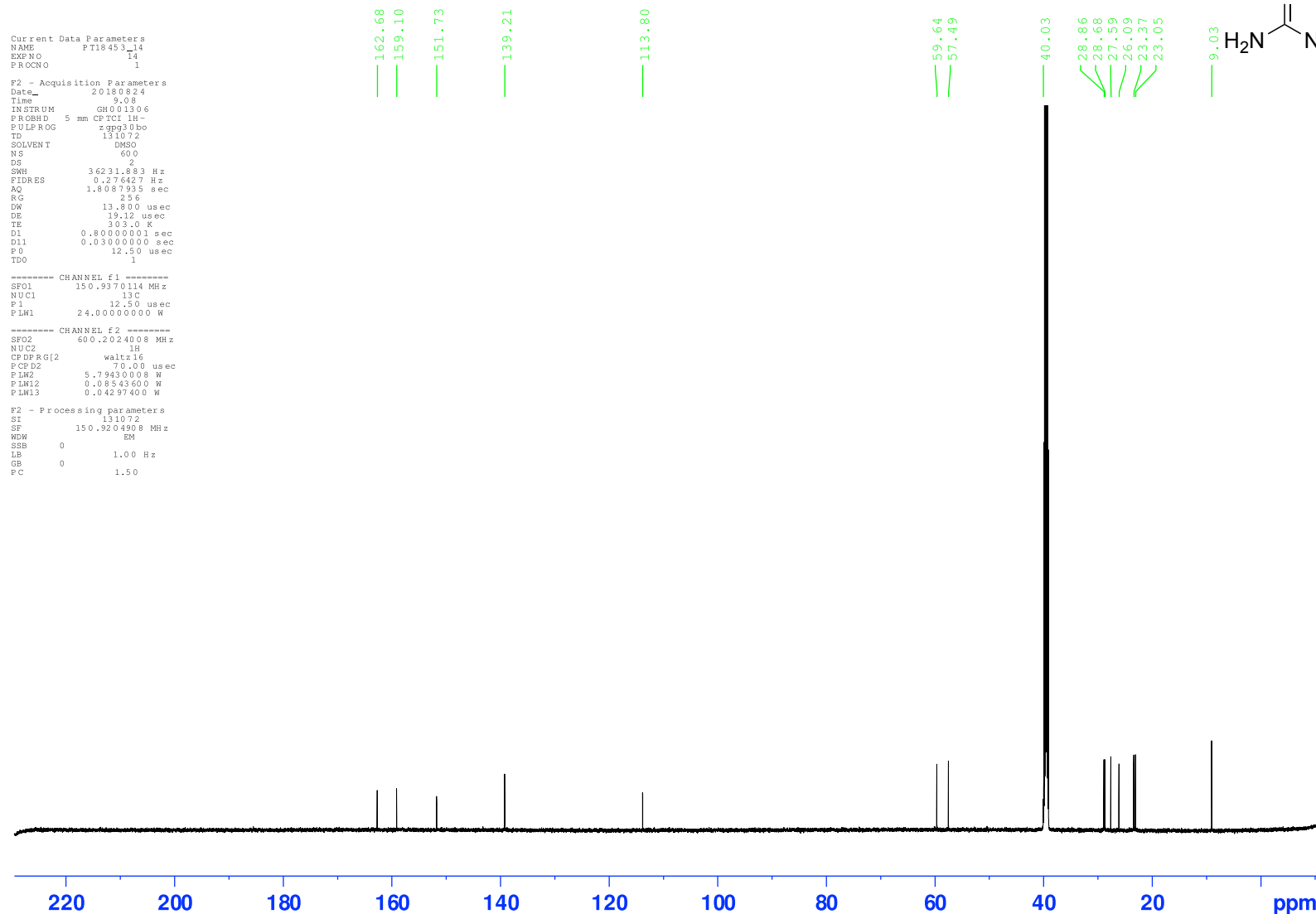
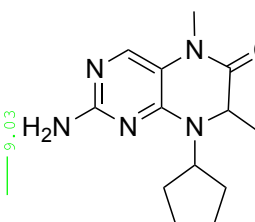
F2 - Acquisition Parameters  
Date\_ 20180824  
Time 8.01  
INSTRUM GH001306  
PROBHD 5 mm CPTCI 1H-  
PULPROG zg30  
TD 65536  
SOLVENT DMSO  
NS 16  
DS 0  
SWH 12019.230 Hz  
FIDRES 0.183399 Hz  
AQ 2.7262976 sec  
RG 80.6  
DW 41.600 usec  
DE 10.00 usec  
TE 303.0 K  
D1 2.0000000 sec  
TD0 1

----- CHANNEL f1 -----  
SFO1 600.2045015 MHz  
NUC1 1H  
P1 8.50 usec  
PLW1 5.79430008 W

F2 - Processing parameters  
SI 65536  
SF 600.2000049 MHz  
WDW EM  
SSB 0  
LB 0.20 Hz  
GB 0  
PC 1.50



# 2-Amino-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5H)-one (66)



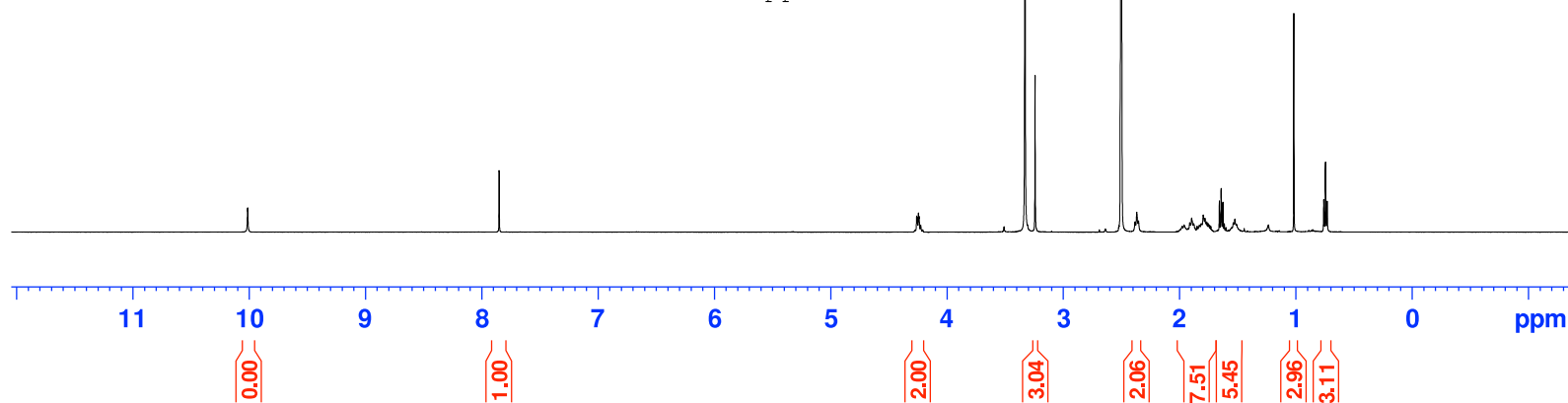
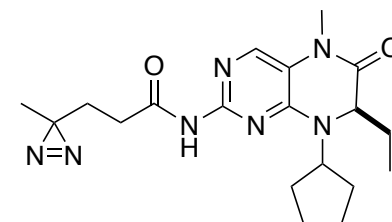
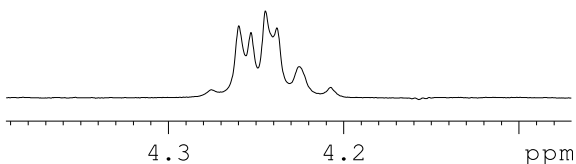
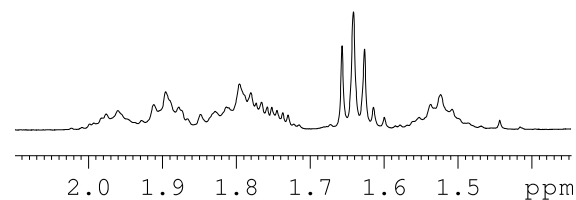
**(R)-N-(8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)-3-(3-methyl-3H-diazirin-3-yl)propenamide (56)**

Current Data Parameters  
NAME c159260107 CLD81  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190701  
Time 13.13  
INSTRUM avc500  
PROBHD 5 mm CPDUL 13C  
PULPROG zg30  
TD 65536  
SOLVENT DMSO  
NS 16  
DS 4  
SWH 10330.578 Hz  
FIDRES 0.157632 Hz  
AQ 3.1719425 sec  
RG 3.56  
DW 48.400 usec  
DE 10.00 usec  
TE 298.0 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
SFO1 500.3030896 MHz  
NUC1 1H  
P1 22.00 usec  
PLW1 7.99830008 W

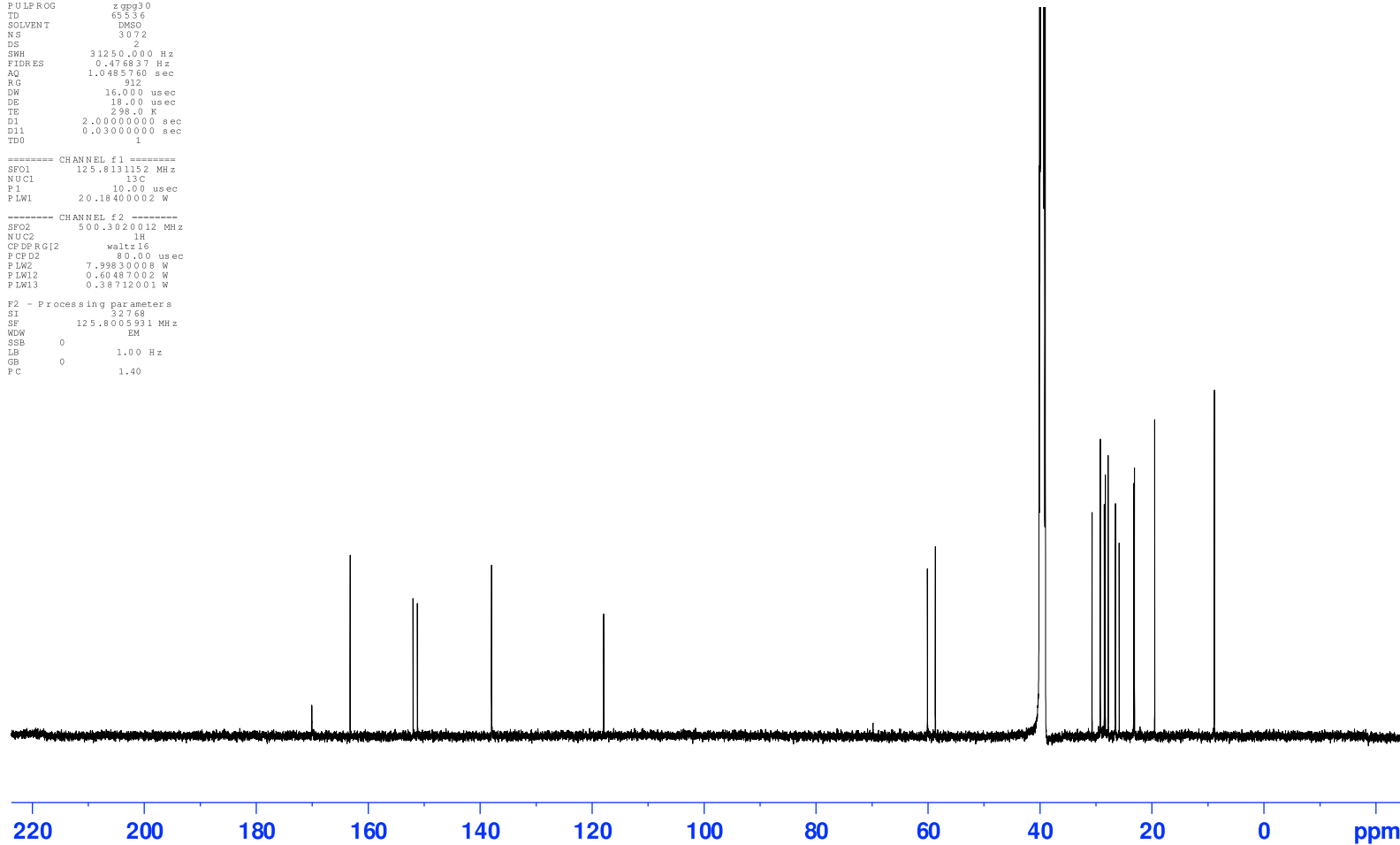
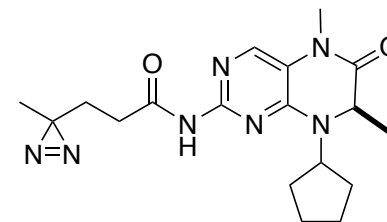
F2 - Processing parameters  
SI 65536  
SF 500.3000041 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



**(R)-N-(8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)-3-(3-methyl-3H-diazirin-3-yl)propenamide (56)**

Current Data Parameters  
 NAME c159260107 CLD81  
 EXPNO 4  
 PROCNO 1  
 F2 - Acquisition Parameters  
 Date\_ 20190701  
 Time 14:57  
 INSTRUM avc500  
 PROBHD 5 mm CPDUL 13C  
 PULPROG zgpg30  
 TD 65536  
 SOLVENT DMSO  
 NS 3072  
 DS 2  
 SWH 31250.000 Hz  
 FIDRES 0.476837 Hz  
 AQ 1.0485760 sec  
 RG 912  
 DW 16.000 usec  
 DE 18.00 usec  
 TE 298.0 K  
 D1 2.00000000 sec  
 D11 0.03000000 sec  
 TD0 1  
 ===== CHANNEL f1 =====  
 SFO1 125.8131152 MHz  
 NUC1 13C  
 P1 10.00 usec  
 PLW1 20.18400002 W  
 ===== CHANNEL f2 =====  
 SFO2 500.3020012 MHz  
 NUC2 1H  
 CPDPRG2 waltz16  
 PCPD2 80.00 usec  
 PLW2 7.99830008 W  
 PLW12 0.60487002 W  
 PLW13 0.38712001 W  
 F2 - Processing parameters  
 SI 32768  
 SF 125.8005931 MHz  
 WDW EM  
 SSB 0  
 LB 1.00 Hz  
 GB 0  
 PC 1.40

170.04  
 163.21  
 151.96  
 151.19  
 137.93  
 117.92  
 60.07  
 58.66  
 30.66  
 29.18  
 28.47  
 28.28  
 27.77  
 26.49  
 25.81  
 23.23  
 23.08  
 19.47  
 8.81



**(R)-N-(1-Acetylpiperidin-4-yl)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzamide**

**(59)**

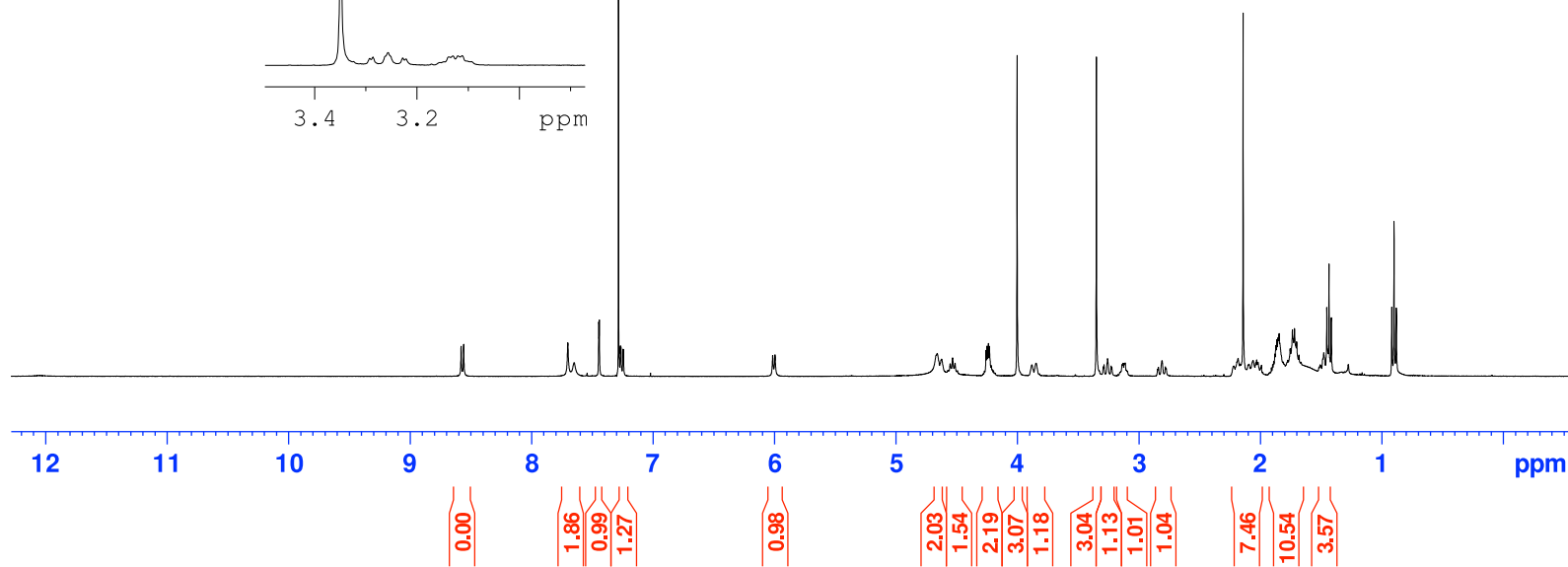
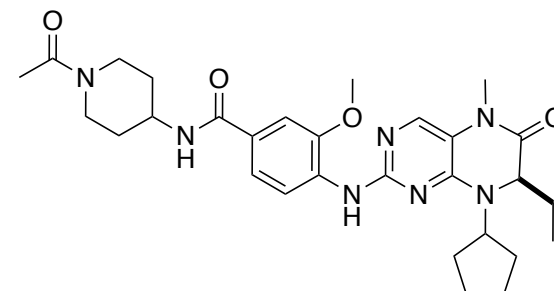
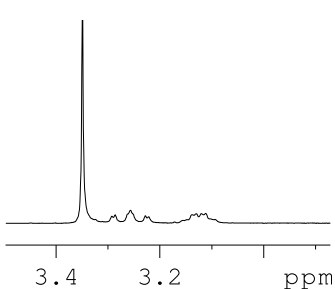
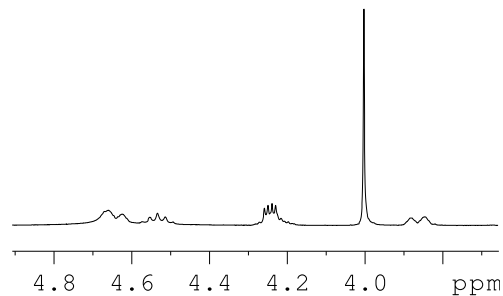
Current Data Parameters  
NAME Feb23-2019-17-CL-D-43 gw  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters

Date\_ 20190223  
Time 12.42 h  
INSTRUM avg400  
PROBHD Z108618\_0816 (   
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 2  
SWH 8012.820 Hz  
FIDRES 0.244532 Hz  
AQ 4.0894465 sec  
RG 162.47  
DW 62.400 usec  
DE 6.50 usec  
TE 296.7 K  
D1 1.00000000 sec  
TD0 1  
SFO1 400.2024012 MHz  
NUC1 1H  
P1 14.00 usec  
PLW1 14.00000000 W

F2 - Processing parameters

SI 32768  
SF 400.2000000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



**(R)-N-(1-Acetylpiperidin-4-yl)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzamide**

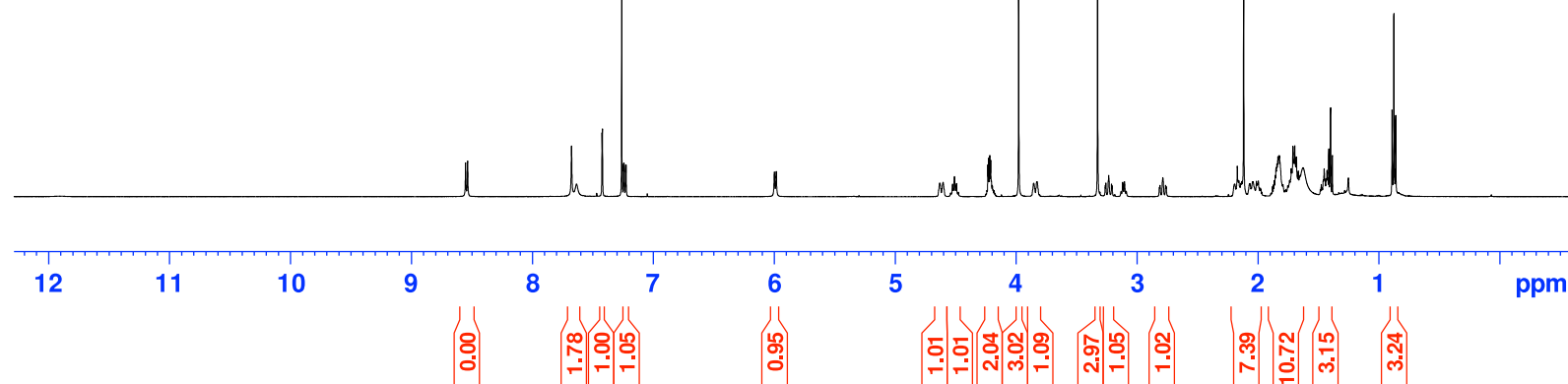
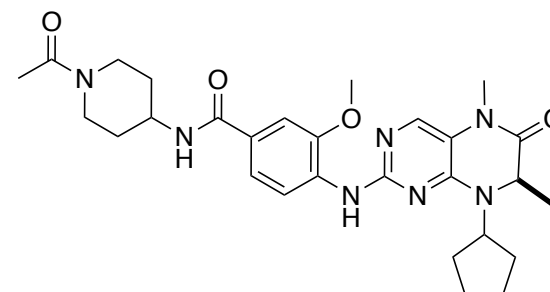
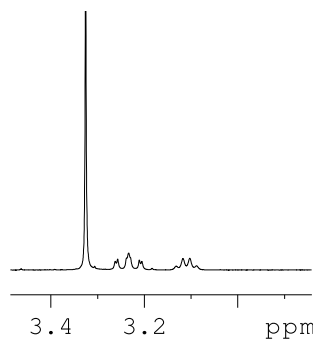
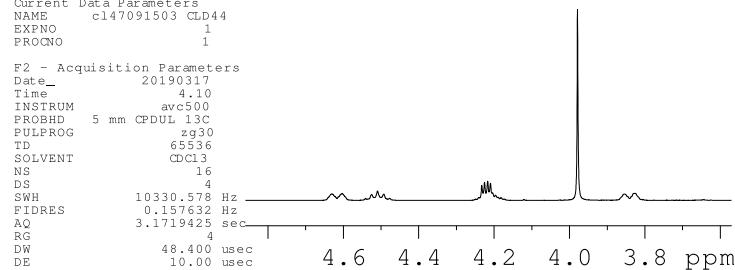
**(59)**

Current Data Parameters  
NAME cl47091503 CLD44  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190317  
Time 4.10  
INSTRUM avc500  
PROBHD 5 mm CPDUL 13C  
PULPROG zg30  
TD 65536  
SOLVENT CDCl3  
NS 16  
DS 4  
SWH 10330.578 Hz  
FIDRES 0.157632 Hz  
AQ 3.1719425 sec  
RG 4  
DW 48.400 usec  
DE 10.00 usec  
TE 298.0 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
SFO1 500.3030896 MHz  
NUC1 1H  
P1 22.00 usec  
PLW1 7.99830008 W

F2 - Processing parameters  
SI 65536  
SF 500.3000125 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



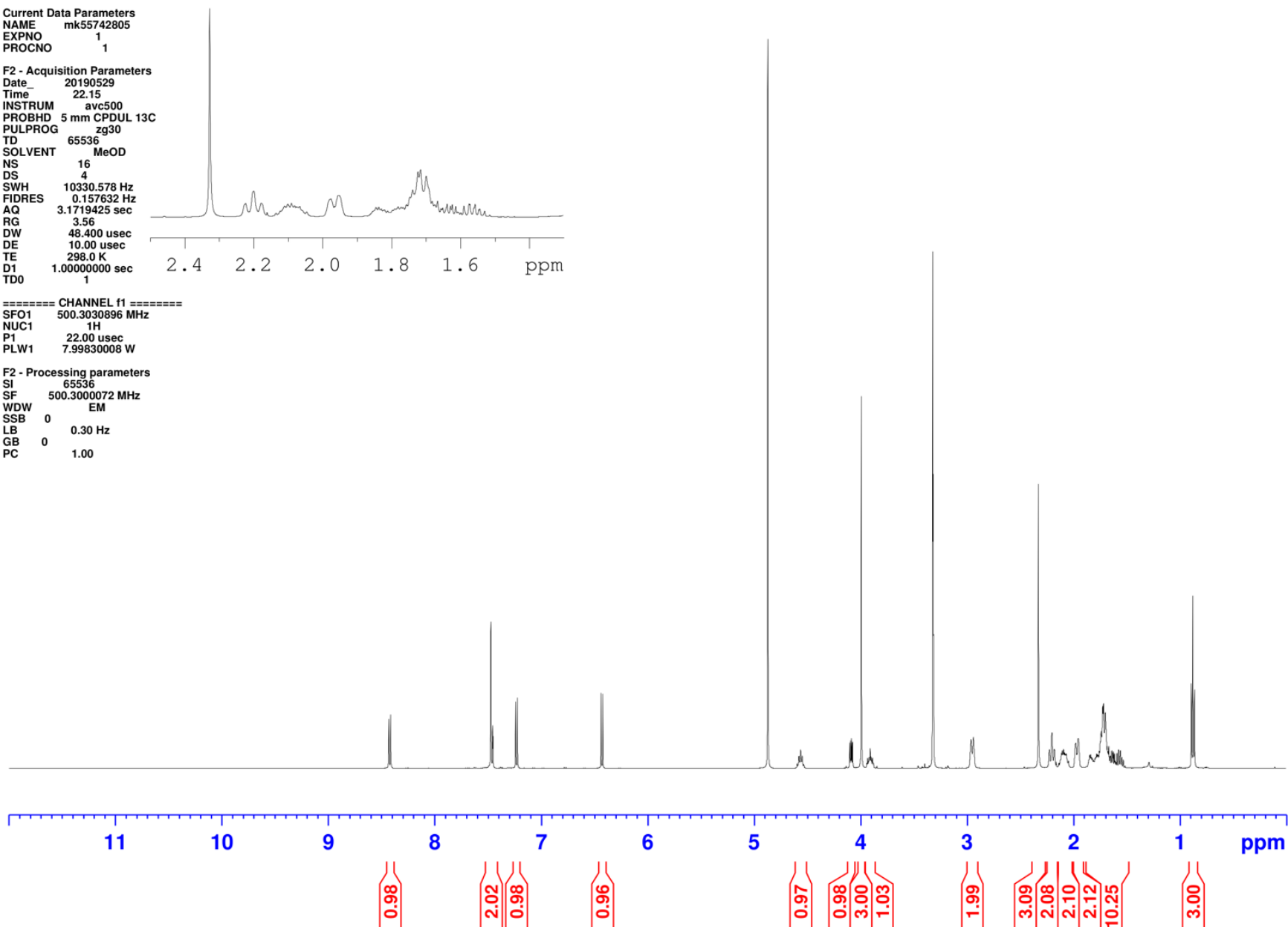
4-[[*(3R)*-4-Cyclopentyl-3-ethyl-1-methyl-2-oxo-1,2,3,4-tetrahydropyrido[2,3-*b*]pyrazin-6-yl]amino}-3-methoxy-*N*-(1-methylpiperidin-4-yl)benzamide (39)

Current Data Parameters  
NAME mk55742805  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20190529  
Time 22.15  
INSTRUM avc500  
PROBHD 5 mm CPDUL 13C  
PULPROG zg30  
TD 65536  
SOLVENT MeOD  
NS 16  
DS 4  
SWH 10330.578 Hz  
FIDRES 0.157632 Hz  
AQ 3.1719425 sec  
RG 3.56  
DW 48.400 usec  
DE 10.00 usec  
TE 298.0 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
SFO1 500.3030896 MHz  
NUC1 1H  
P1 22.00 usec  
PLW1 7.99630008 W

F2 - Processing parameters  
SI 65536  
SF 500.3000072 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00



4-[[*(3R)*-4-Cyclopentyl-3-ethyl-1-methyl-2-oxo-1,2,3,4-tetrahydropyrido[2,3-*b*]pyrazin-6-yl]amino}-3-methoxy-*N*-(1-methylpiperidin-4-yl)benzamide (39)

```

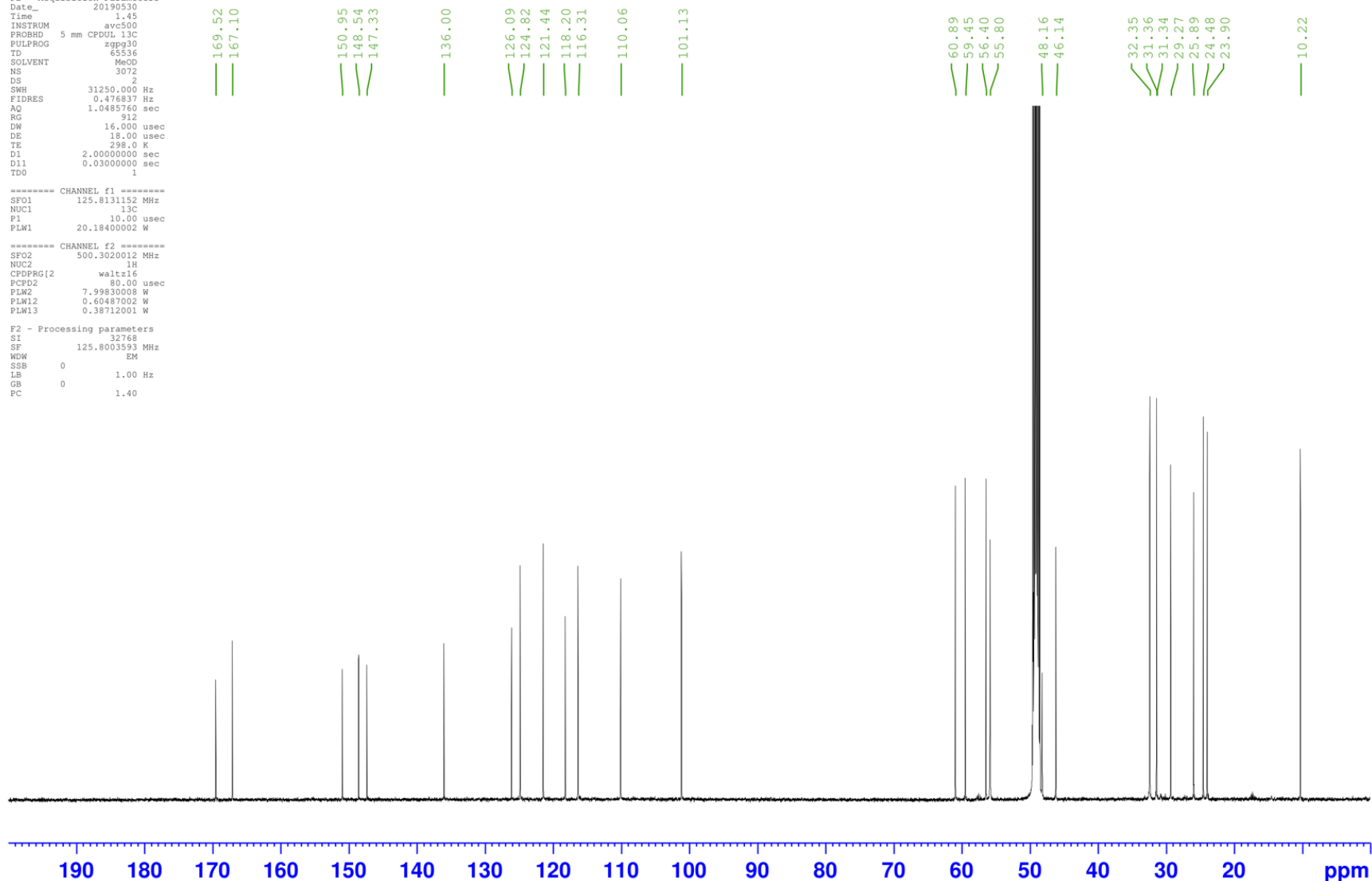
Current Data Parameters
NAME      mk55742805
EXPNO     4
PROCNO    1

F2 - Acquisition Parameters
Date_     20190530
Time      1.45
INSTRUM   avc500
PROBHD    5 mm CPDUL 13C
PULPROG   zgpg30
TD        65536
SOLVENT   MeOD
NS         3072
DS         2
SWH        31250.000 Hz
FIDRES     0.476837 Hz
AQ         1.0485760 sec
RG         912
DW         16.000 usec
DE         18.00 usec
TE         298.0 K
D1         2.00000000 sec
D11        0.03000000 sec
TD0        1

===== CHANNEL f1 =====
SFO1      125.8131152 MHz
NUC1       13C
P1         10.00 usec
PLW1       20.18400002 W

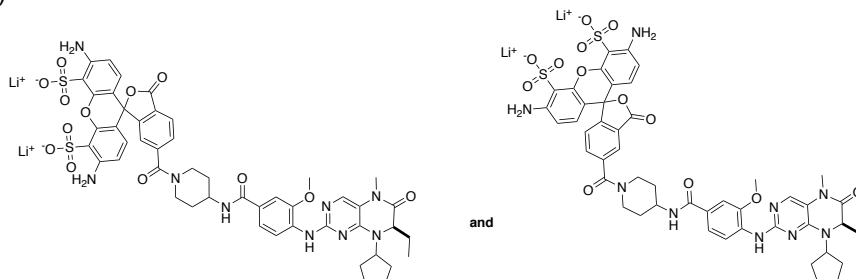
===== CHANNEL f2 =====
SFO2      500.3020012 MHz
NUC2       1H
CPDPRG[2] waltz16
PCPD2      80.00 usec
PLW2       7.99830008 W
PLW12      0.60487002 W
PLW13      0.38712001 W

F2 - Processing parameters
SI         32768
SF         125.8003593 MHz
WDW        EM
SSB        0
LB         1.00 Hz
GB         0
PC         1.40
    
```



## LCMS or HPLC for novel compounds

(*R*)-3',6'-Diamino-[5/6]-(4-(4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzamido)piperidine-1-carbonyl)-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-4',5'-disulfonate, dilithium salt (**58**)



INSTRUMENT: ORANGE

Page 1

Sample: 2  
File: VSR20559-2  
Submitter: Coco M Laurin (cml36793)

Vial: 2-26  
Date: 06-Aug-2018  
Method: E:\GSK.PRO\ICSH-2min\_HPH.olp

LNB ref: N68750-50-MF3

Time: 15:10:52

Comment:

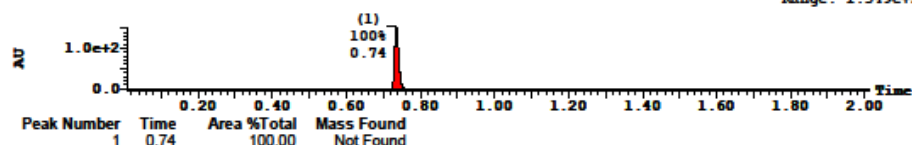
Printed: Mon Aug 06 15:18:35 2018

### Sample Report:

#### Sample: 2

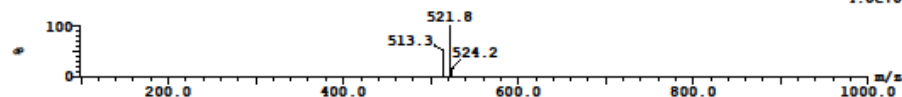
3: UV Detector: TAC :Wavelength Range: (210 - 350)

1.526e+2  
Range: 1.549e+2



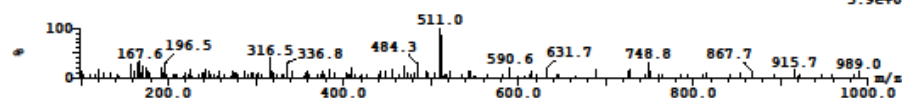
Peak Time  
1 0.74  
1: (Time: 0.74)

1: MS ES+  
4.0e+006



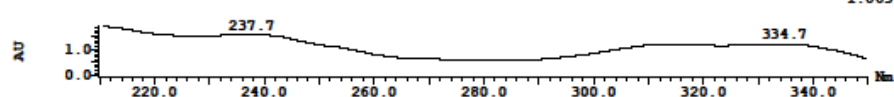
Peak Time  
1 0.74  
1: (Time: 0.74)

2: MS ES-  
3.9e+003

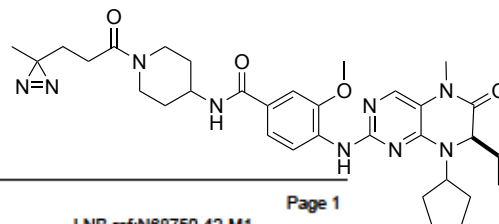


Peak Time  
1 0.74  
1: (Time: 0.74)

3: UV Detector  
1.863 AU



(*R*)-4-((8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxy-*N*-(1-(3-(3-methyl-3*H*-diazirin-3-yl)propanoyl)piperidin-4-yl)benzamide (**57**)



INSTRUMENT: GREEN

Page 1

Sample: 1  
File: V5106491-1  
Submitter: Coco M Laurin (cml36793)

Vial: 142  
Date: 30-Jul-2018  
Method: E:\GSK.PRO\CSH~2min\_For.olp

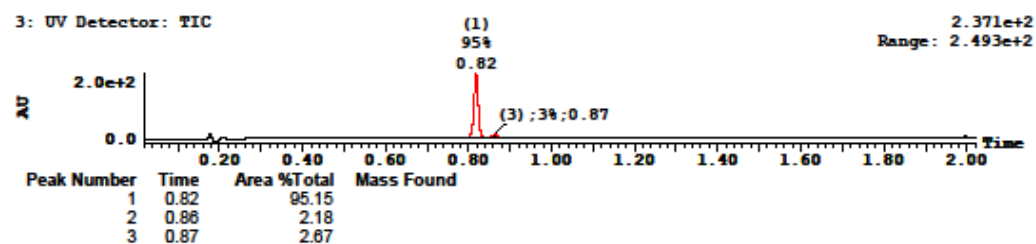
LNB ref: N88750-42-M1  
Time: 18:51:55  
Comment: N88750-42-M1

Printed: Mon Jul 30 16:55:56 2018

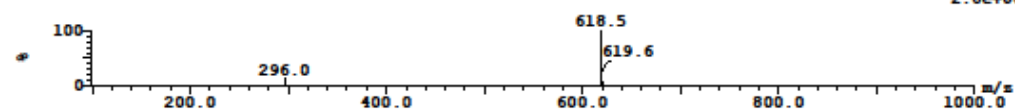
# Sample Report:

Sample: 1

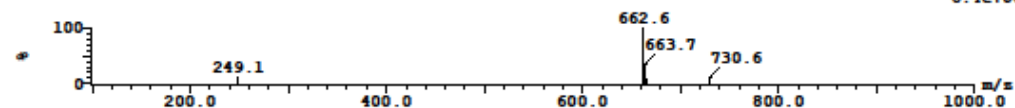
3: UV Detector: TIC



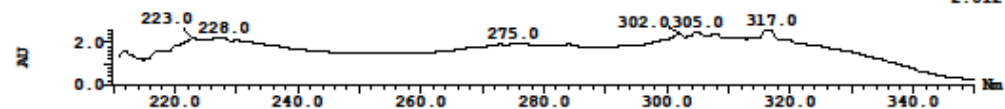
Peak Time  
1 0.82  
1: (Time: 0.82) Combine (209:222-(180:182+246:248)) 1:MS ES+  
2.6e+007



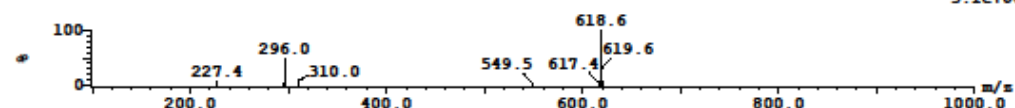
Peak Time  
1 0.82  
1: (Time: 0.82) Combine (208:222-(179:182+245:248)) 2:MS ES-  
6.4e+005



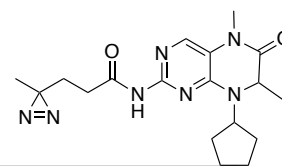
Peak Time  
1 0.82  
1: (Time: 0.82) Combine (1917) 3:UV Detector  
2.612 AU



Peak Time  
2 0.86  
2: (Time: 0.86) Combine (221:234-(193:196+252:255)) 1:MS ES+  
5.1e+005



*N*-(8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)-3-(3-methyl-3*H*-diazirin-3-yl)propenamide (**56**)



INSTRUMENT: ORANGE

Page 1

Sample: 1  
File: VSR21084-1  
Submitter: Coco M Laurin (cm136793)

Vial: 2:26  
Date: 16-Aug-2018  
Method: E:\GSK.PRO\ICSH-2min\_HPH.lolp

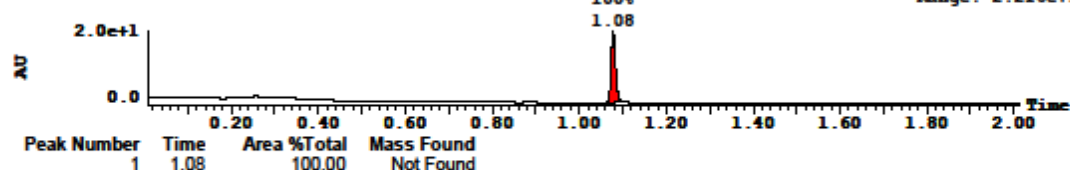
LNB ref: N68750-61-1  
Time: 09:17:33  
Comment:

Printed: Thu Aug 16 09:24:47 2018

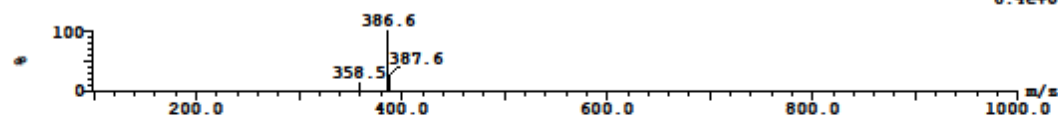
# Sample Report:

Sample: 1

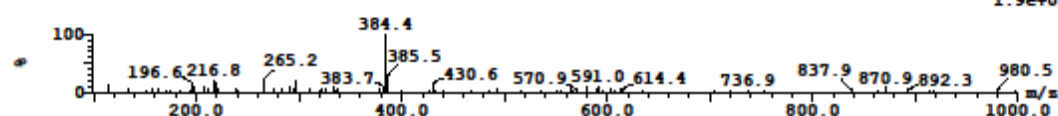
3: UV Detector: TAC :Wavelength Range: (210 - 350) (1) 100% 2.035e+1  
Range: 2.216e+1



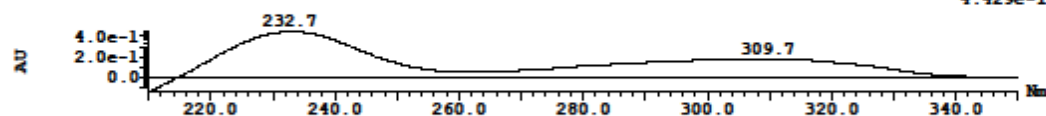
Peak Time  
1 1.08  
1: (Time: 1.08) 1:MS ES+ 6.4e+006



Peak Time  
1 1.08  
1: (Time: 1.08) 2:MS ES- 1.9e+004



Peak Time  
1 1.08  
1: (Time: 1.08) 3:UV Detector 4.429e-1 AU

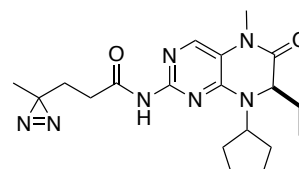


(*R*)-*N*-(8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)-3-(3-methyl-3*H*-diazirin-3-yl)propenamide (**56**)

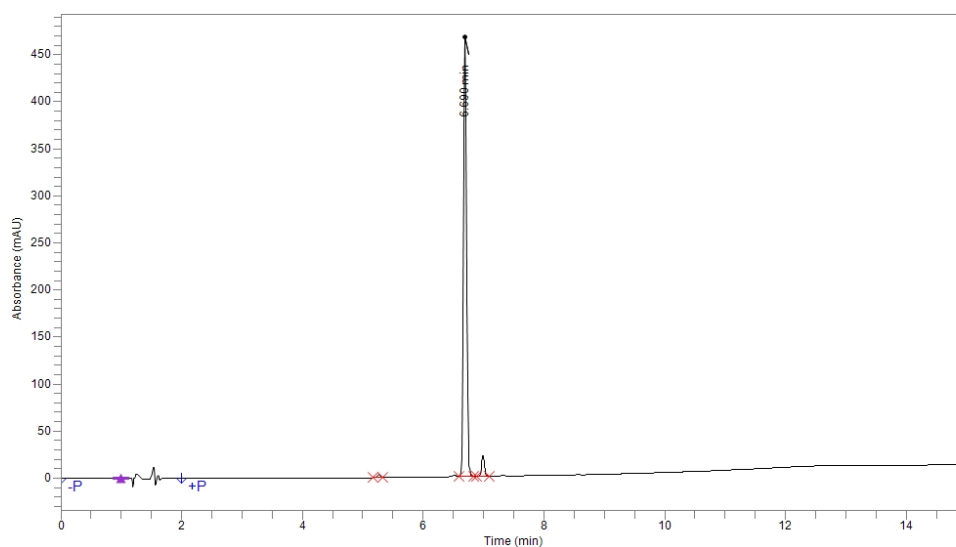
CLD81

7/8/2019

Acquisition Method Purity short run @254 nm  
 Acquisition Date/Time 7/8/2019 5:07 pm  
 Injection Volume 20  
 Sample Name CLD81  
 Sample Description  
 Batch Description CL Coco



CLD81 : Injection 1



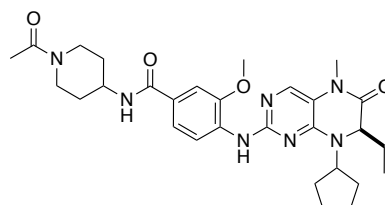
Time	Height	Area	Area %
5.266	3,068.4	9,908.0	0.61
6.690	467,411.6	1,552,087.7	94.90
6.989	22,046.1	73,453.5	4.49
<b>Total</b>		1,635,449.1	100.00

(*R*)-*N*-(1-Acetylpiperidin-4-yl)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzamide (**59**)

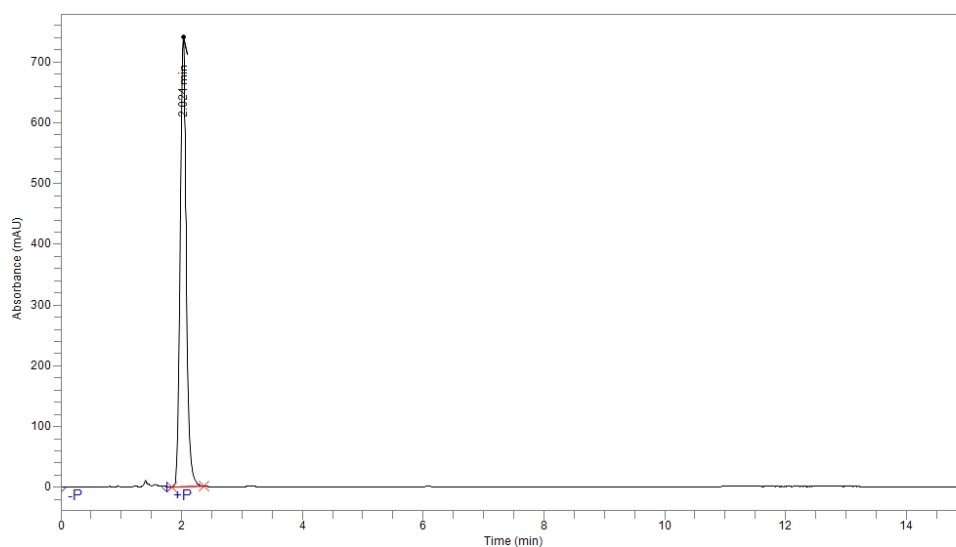
CLD43 254nm

3/5/2019

Acquisition Method Purity short run @254 nm  
 Acquisition Date/Time 3/5/2019 4:24 pm  
 Injection Volume 20  
 Sample Name CLD43 254nm  
 Sample Description  
 Batch Description CL OS



CLD43 254nm : Injection 1



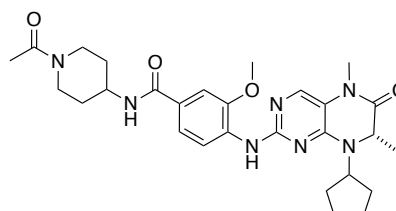
Time	Height	Area	Area %
2.024	741,018.3	5,275,646.1	100.00
<b>Total</b>		5,275,646.1	100.00

(S)-N-(1-Acetylpiperidin-4-yl)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzamide (**60**)

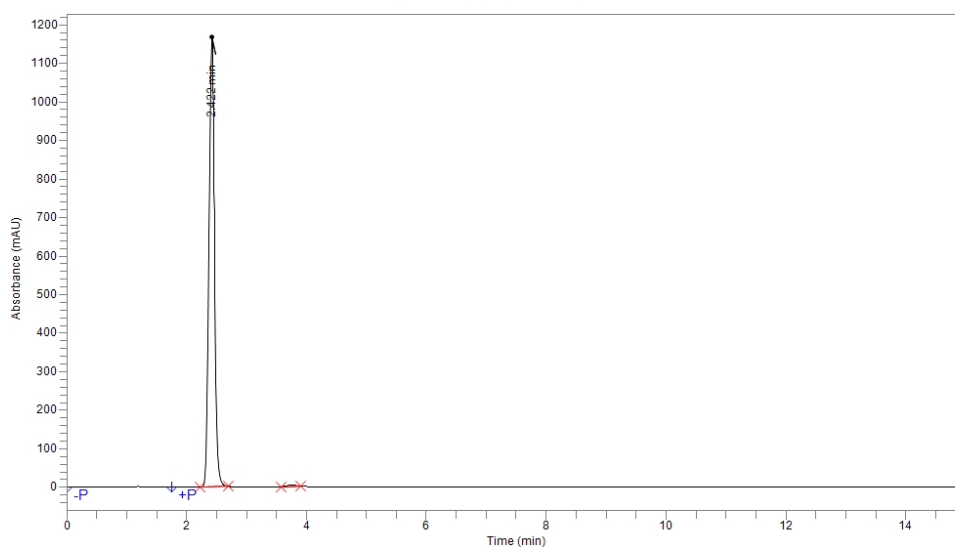
CLD44 254nm

3/5/2019

Acquisition Method Purity short run @254 nm  
 Acquisition Date/Time 3/5/2019 3:48 pm  
 Injection Volume 20  
 Sample Name CLD44 254nm  
 Sample Description  
 Batch Description CL OS



CLD44 254nm : Injection 1



Time	Height	Area	Area %
2.422	1,168,962.6	7,652,981.5	99.41
3.757	4,018.0	45,133.9	0.59
<b>Total</b>		7,698,115.3	100.00

## References

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