Supplementary Information

PARP1-targeted boron-10 delivery by an olaparib analogue induces DNA damage *via* neutron capture while enhancing radiosensitivity in glioma cells

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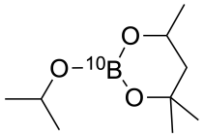
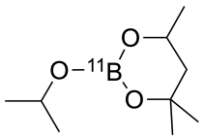
Synthesis of intermediates

*[^{10}B]2-Isopropoxy-4,4,6-trimethyl-1,3,2-dioxaborinane (**[^{10}B]4**)*



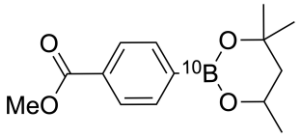
As per the method of *Demory et al.*[1], with slight modification; [^{10}B]-boric acid (673.3 mg, 11.0 mmol; > 95 % enrichment, Merck) and hexylene glycol (1.303 g, 11.0 mmol) were stirred in hexane at room temperature overnight. Upon completion, the precipitate was isolated by vacuum filtration and washed with hexane. The precipitate was then dissolved in 10 mL isopropanol and stirred at room temperature overnight. Upon completion, the reaction mixture was evaporated under reduced pressure, taken up in toluene, and dried under reduced pressure to give the title compound as a clear oil (1.80 g, 88 %) in sufficient purity for the following step. Spectral data matched that previously described[1].

*[^{11}B]2-Isopropoxy-4,4,6-trimethyl-1,3,2-dioxaborinane (**[^{11}B]4**)*



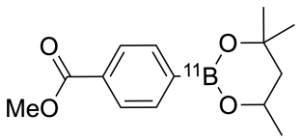
This compound was synthesised in an identical manner to its ^{10}B-enriched equivalent (**[^{10}B]4**), from [^{11}B]-boric acid (261.0 mg, 1.40 mmol; > 99 % enrichment, Merck), yielding the product in satisfactory purity for the next step (237.9 mg, 78 %). ^{1}H, and ^{13}C NMR data matched that reported for **[^{10}B]4**.

*[^{10}B]Methyl 4-(4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl-2-)benzoate (**[^{10}B]5**)*



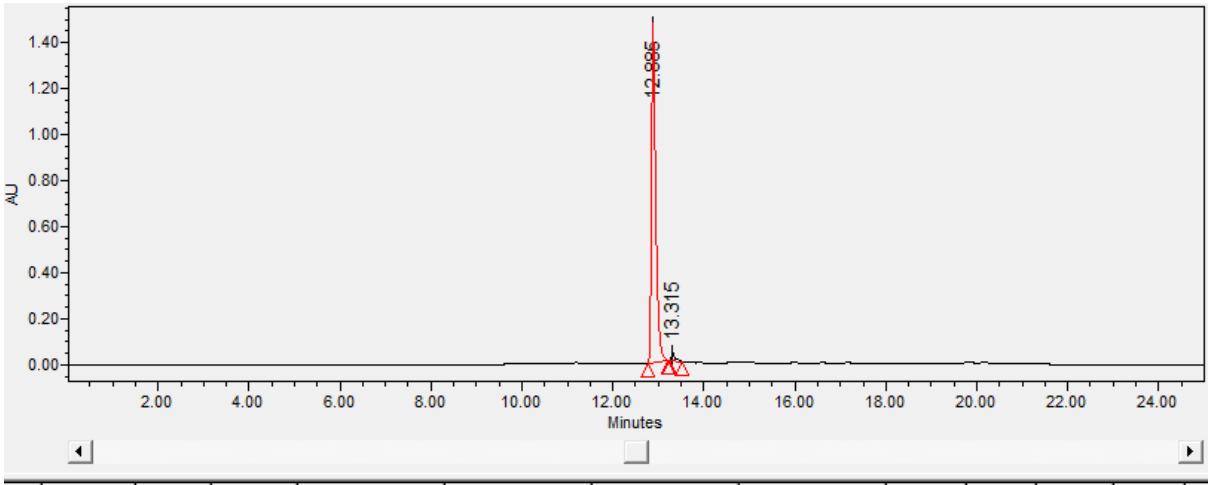
As per the method of Demory et al.[1], with slight modification; methyl 4-iodobenzoate (610.9 mg, 2.33 mmol, Merck) and **[^{10}B]4** (518.3 mg, 2.80 mmol) were weighed into a dry RBF, dissolved in 5 mL dry THF under Argon, and stirred while cooling to -78 °C. iPrMgCl.LiCl (1.3M in THF, 3.4 mL, 4.4 mmol) was then added dropwise, and the reaction mixture allowed to return to room temperature for 1 hour. Upon completion, the reaction was quenched by addition of 5 mL saturated $NH_4Cl_{(aq)}$, diluted with 20 mL Et_2O, and the two phases separated. The aqueous phase was further washed with 20 mL Et_2O, the combined organic extracts dried with $MgSO_4$, and concentrated under reduced pressure. The crude residue was then filtered through a thin pad of silica to remove borate impurities, and dried to give the product in satisfactory purity for the next step (524.7 mg, 86 %). Spectral data matched that previously described.[2]

*[^{11}B]Methyl 4-(4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl-2-)benzoate (**[^{11}B]5**)*



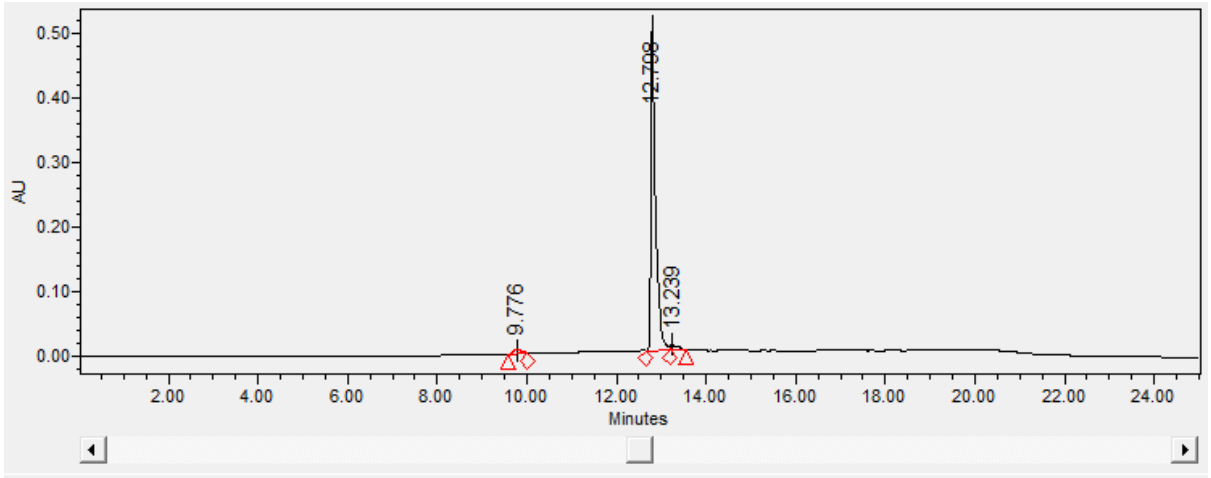
This compound was synthesised in an identical manner to its ^{10}B-enriched equivalent (**[^{10}B]5**), from **[^{11}B]4** (261.0 mg, 1.40 mmol), yielding the product in satisfactory purity for the next step (237.9 mg, 78 %). ^{1}H, and ^{13}C NMR data matched that reported for **[^{10}B]5**.

HPLC traces of tested compounds



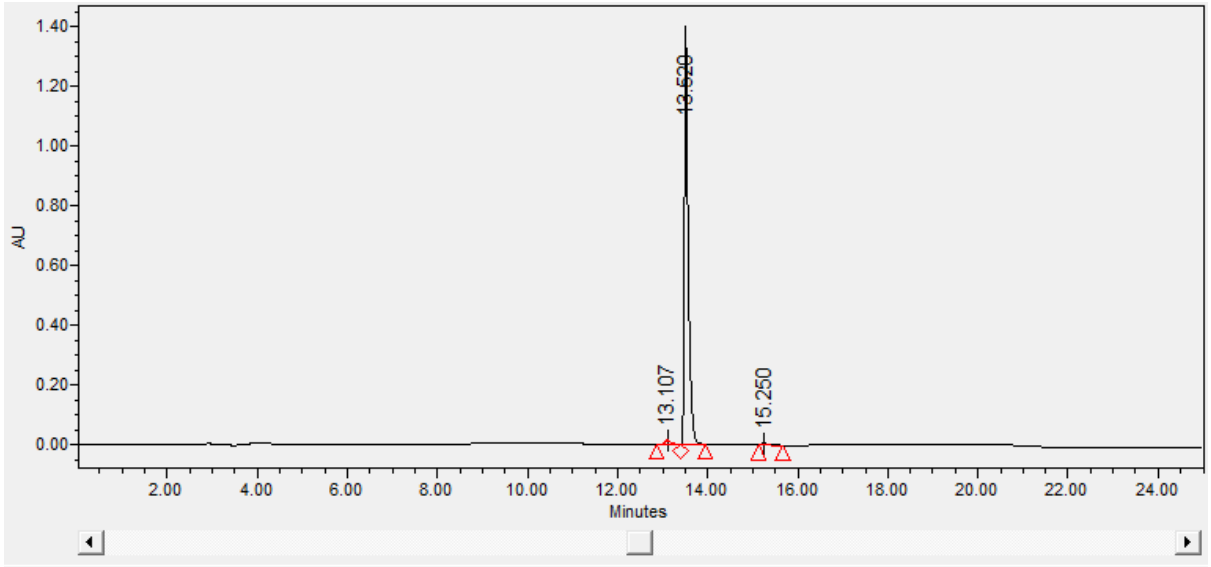
ame	Retention Time (min)	Purity1 Angle	Purity1 Threshold	PDA/FLR Match1 Spect. Name	PDA/FLR Match1 Angle	PDA/FLR Match1 Threshold	PDA/FLR Match1 Lib. Name	Area (µV*sec)	% Area	Height (µV)	Int Type	Ar
	12.885							9603655	98.22	1460710	bb	
	13.315							173960	1.78	29191	bb	

Figure S1: Analytical HPLC trace of [^{10}B]4-phenylboronic acid olaparib (**[^{10}B]2**)



lame	Retention Time (min)	Purity1 Angle	Purity1 Threshold	PDA/FLR Match1 Spect. Name	PDA/FLR Match1 Angle	PDA/FLR Match1 Threshold	PDA/FLR Match1 Lib. Name	Area (µV*sec)	% Area	Height (µV)
	9.776							88838	2.29	6611
	12.798							3687293	95.17	499931
	13.239							98407	2.54	10425

Figure S2: Analytical HPLC trace of [^{11}B]4-phenylboronic acid olaparib (**[^{11}B]2**)



lame	Retention Time (min)	Purity1 Angle	Purity1 Threshold	PDA/FLR Match1 Spect. Name	PDA/FLR Match1 Angle	PDA/FLR Match1 Threshold	PDA/FLR Match1 Lib. Name	Area (µV*sec)	% Area	Height (µV)	Int
	13.107							114325	1.45	13853	BV
	13.520							7741813	97.97	1373907	Vt
	15.250							45845	0.58	5615	BE

Figure S3: Analytical HPLC trace of 4-phenol olaparib (**8**)

Neutron irradiation conditions

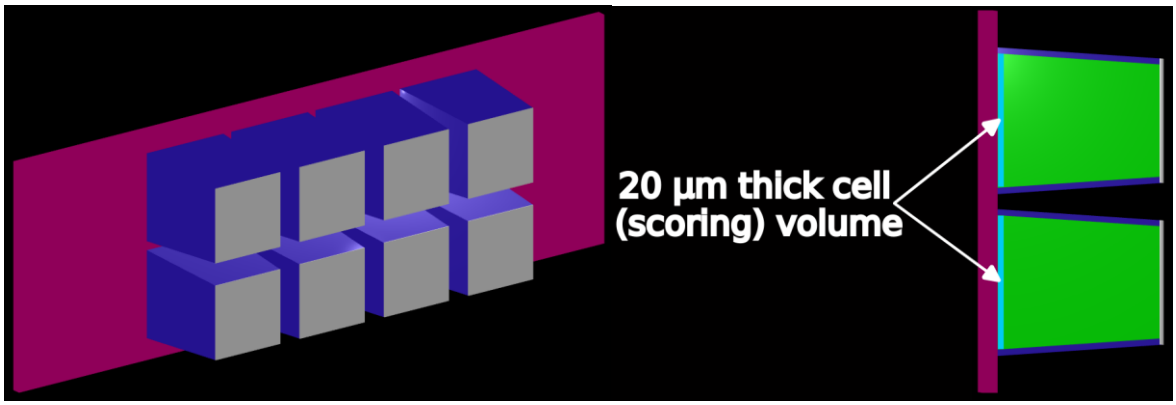


Figure S4: - Geant4 visualisation of the sample geometry, consisting of eight wells with PBS media adjacent to a polystyrene plate and enclosed with a 0.1 mm film, used to simulate the local (attenuated) neutron spectrum (a) at the 20 µm thick cell level highlighted in the sample's side view (b), where pink illustrates the polystyrene plate, green - volume of the media and teal - cell layer where the local neutron spectra were scored.

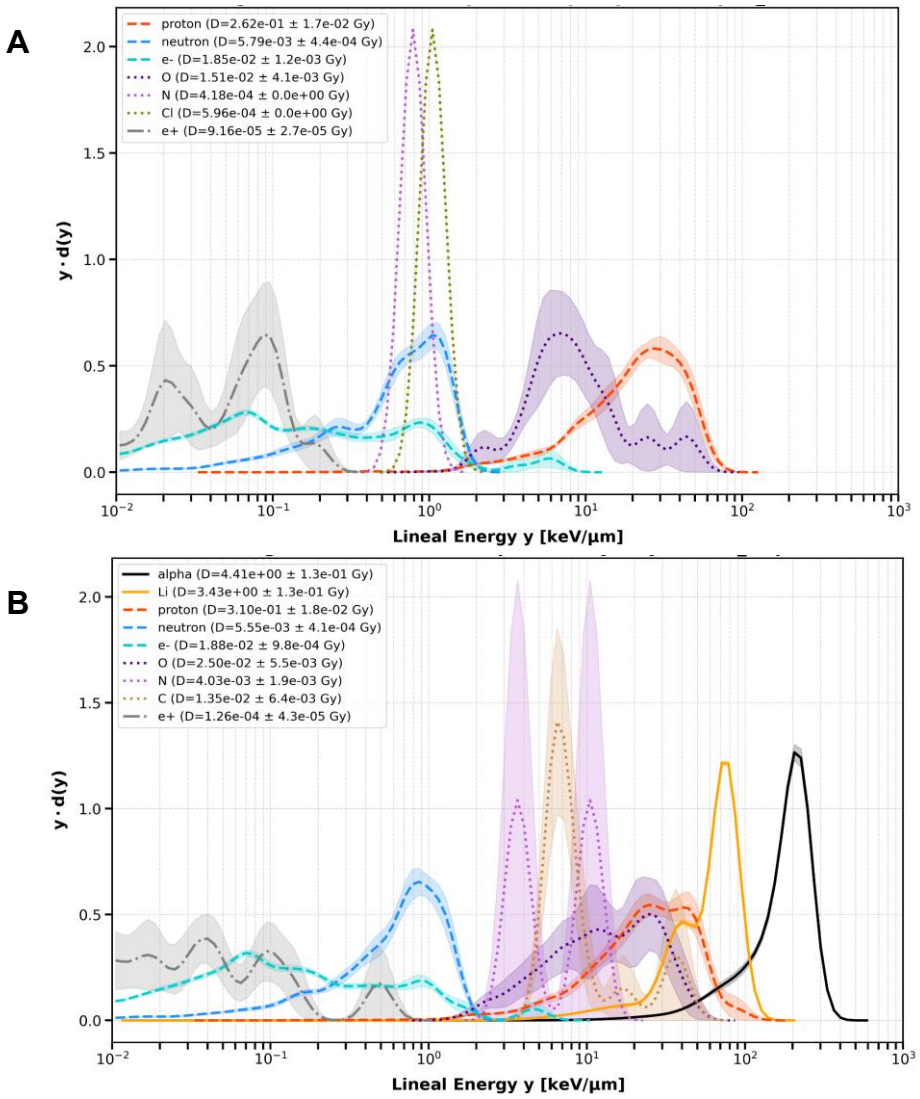


Figure S5: Microdosimetric spectra for the 25 µm x 25 µm x 6 µm cell with a) no NCA (control) and b) 2.88 pg of ^{10}B per cell. Shading illustrates the standard deviation of the mean.

Neutron capture degradation analysis

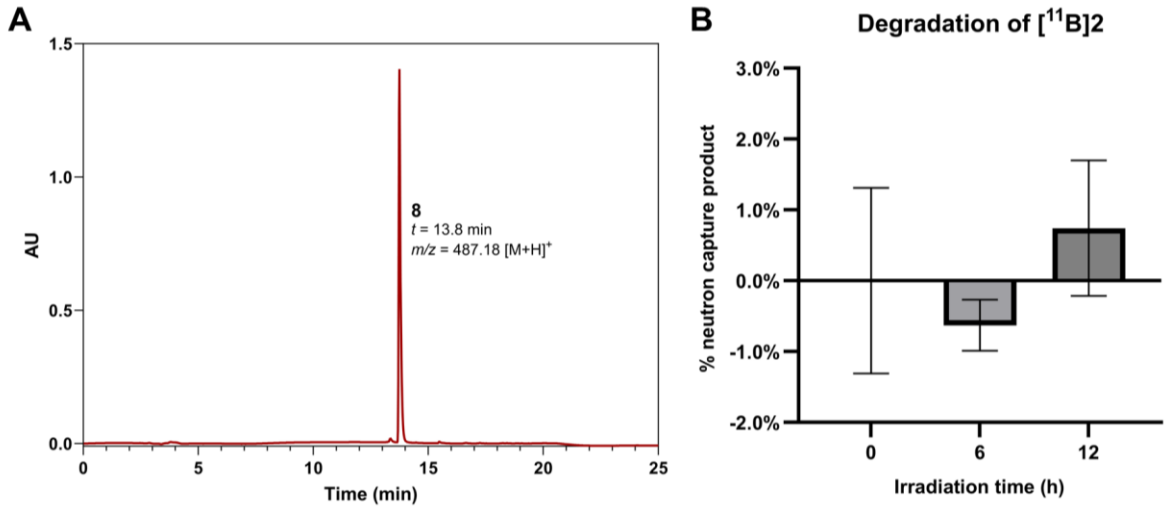


Figure S6: Additional results from neutron degradation analysis of 4-PBA olaparib (**2**). A) HPLC trace of 4-phenol olaparib (**8**), with mass observed by LC-MS, showing the compound has identical mass to the major neutron capture product seen in Figure 6C. B) percentage of neutron capture product 4-phenol olaparib (**8**) relative to $t = 0$ h, with increasing neutron irradiation time in the **[^{11}B]2** samples. There was no statistically significant change observed.

Cell uptake

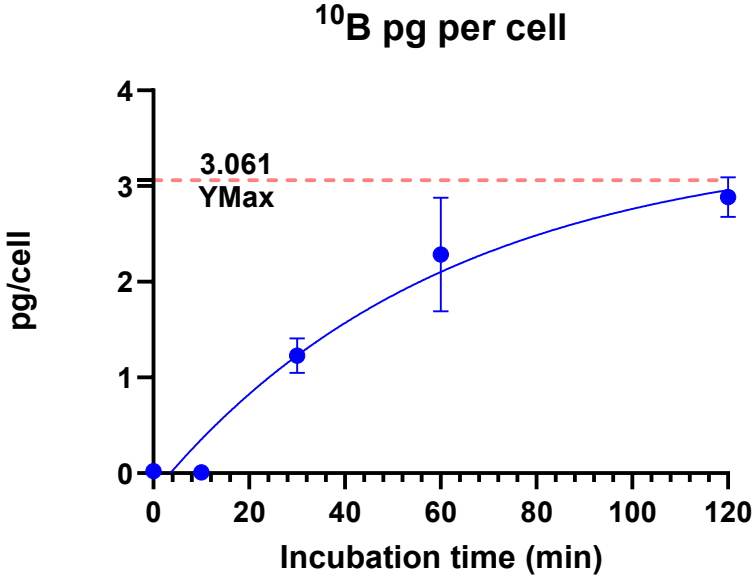
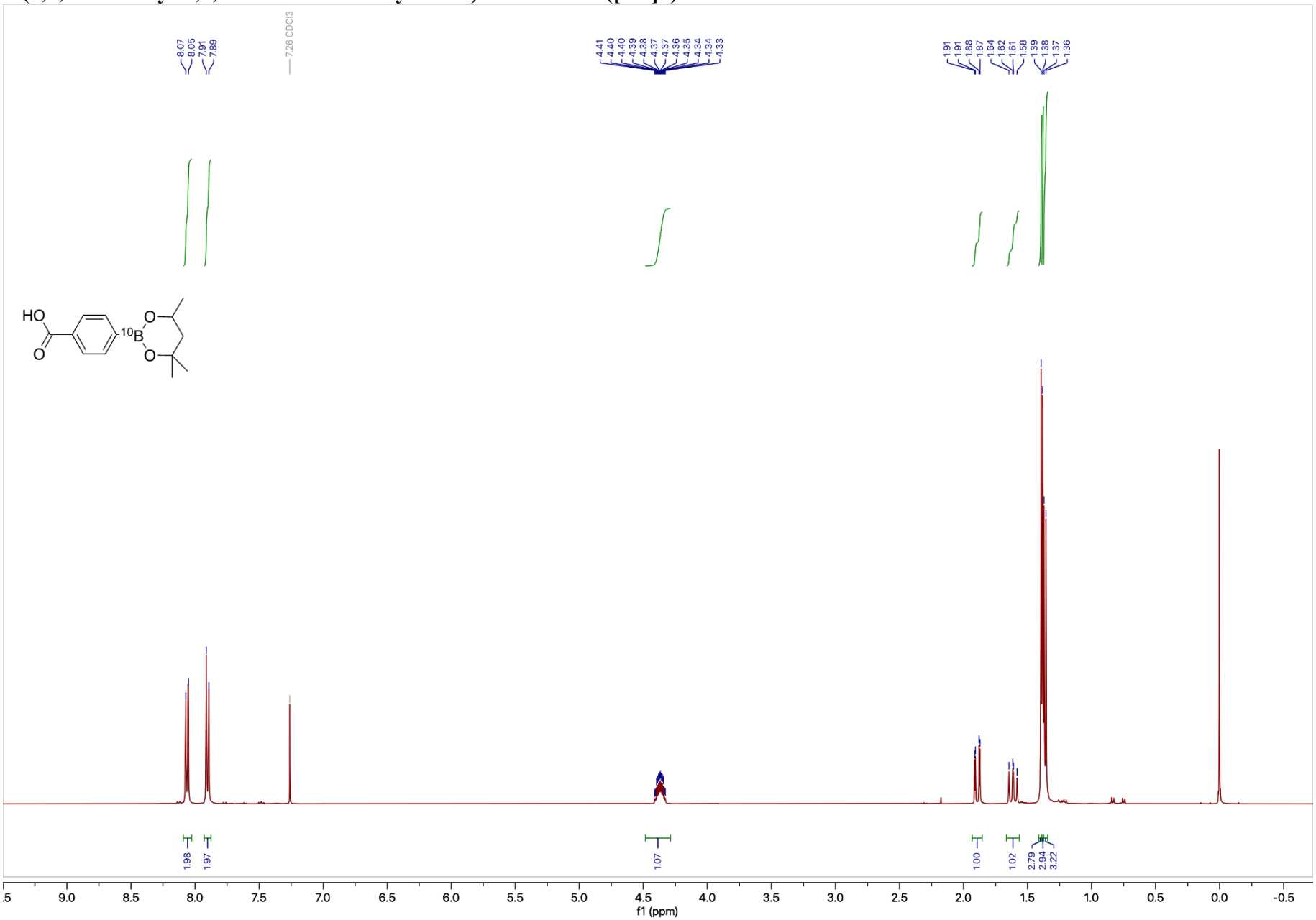


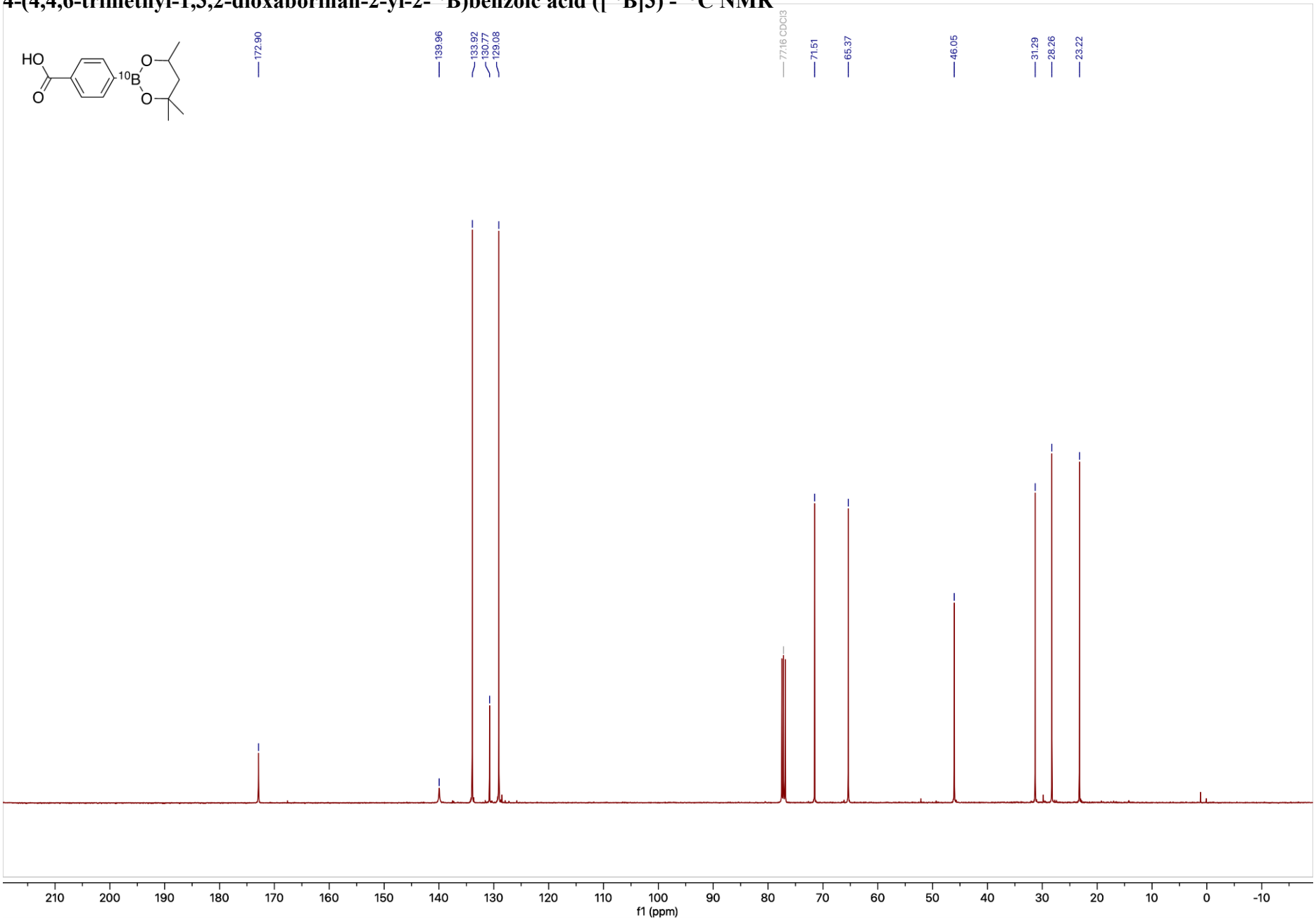
Figure S7: Uptake of [^{10}B]-4-PBA olaparib (**[^{10}B]2**) into T98G glioma cells measured by ICP-MS showing maximum uptake at 2 h of 2.88 ± 0.17 pg ^{10}B per cell with Y_{max} of 3.061 pg ^{10}B per cell.

NMR spectra of synthesised compounds

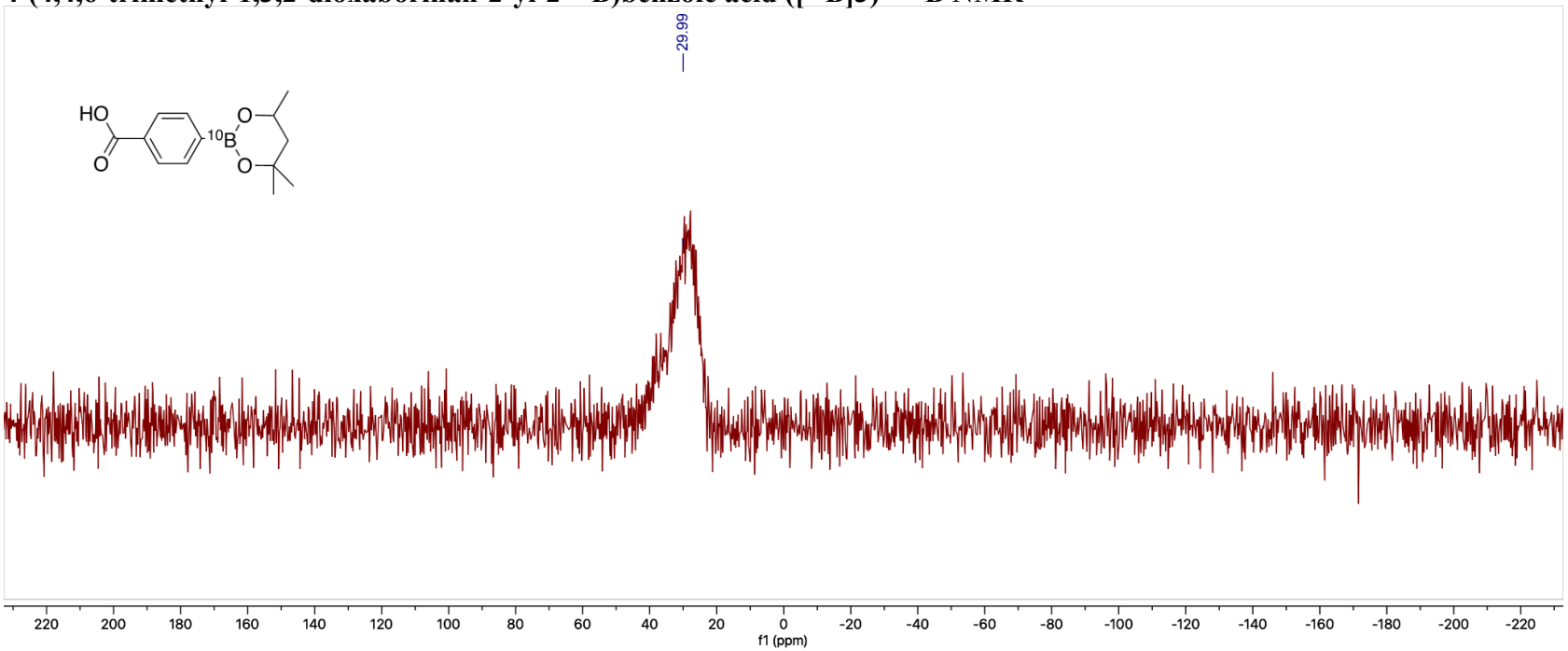
4-(4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl-2-^{10}B)benzoic acid ([^{10}B]3) - ^{1}H NMR



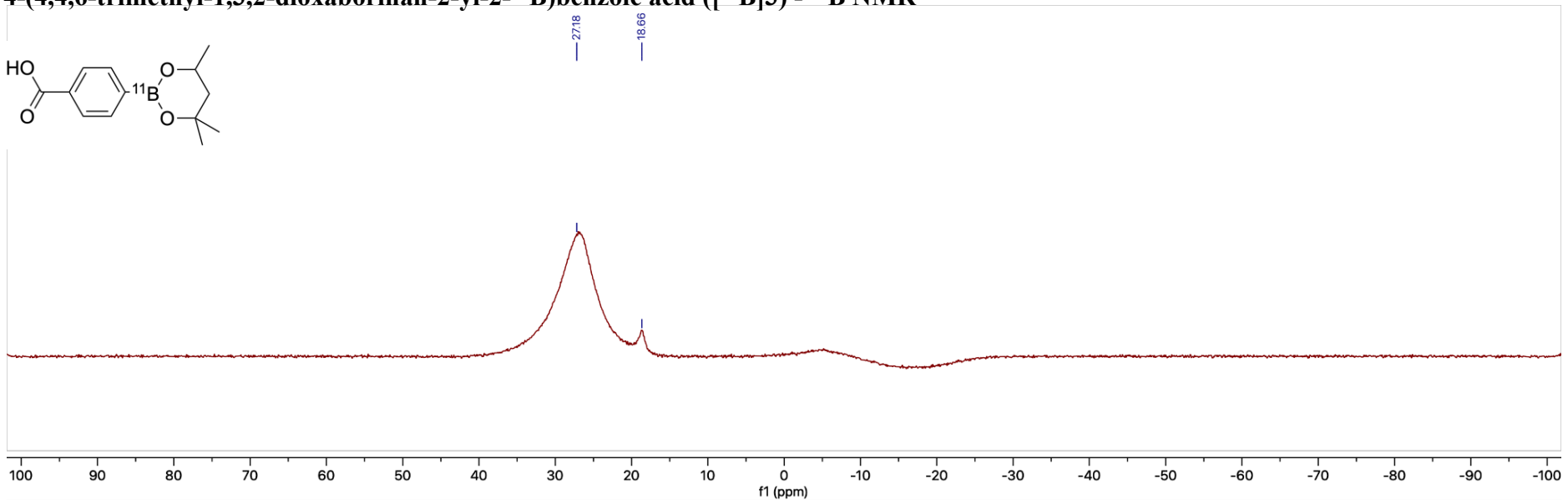
4-(4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl-2-^{10}B)benzoic acid ([^{10}B]3) - ^{13}C NMR



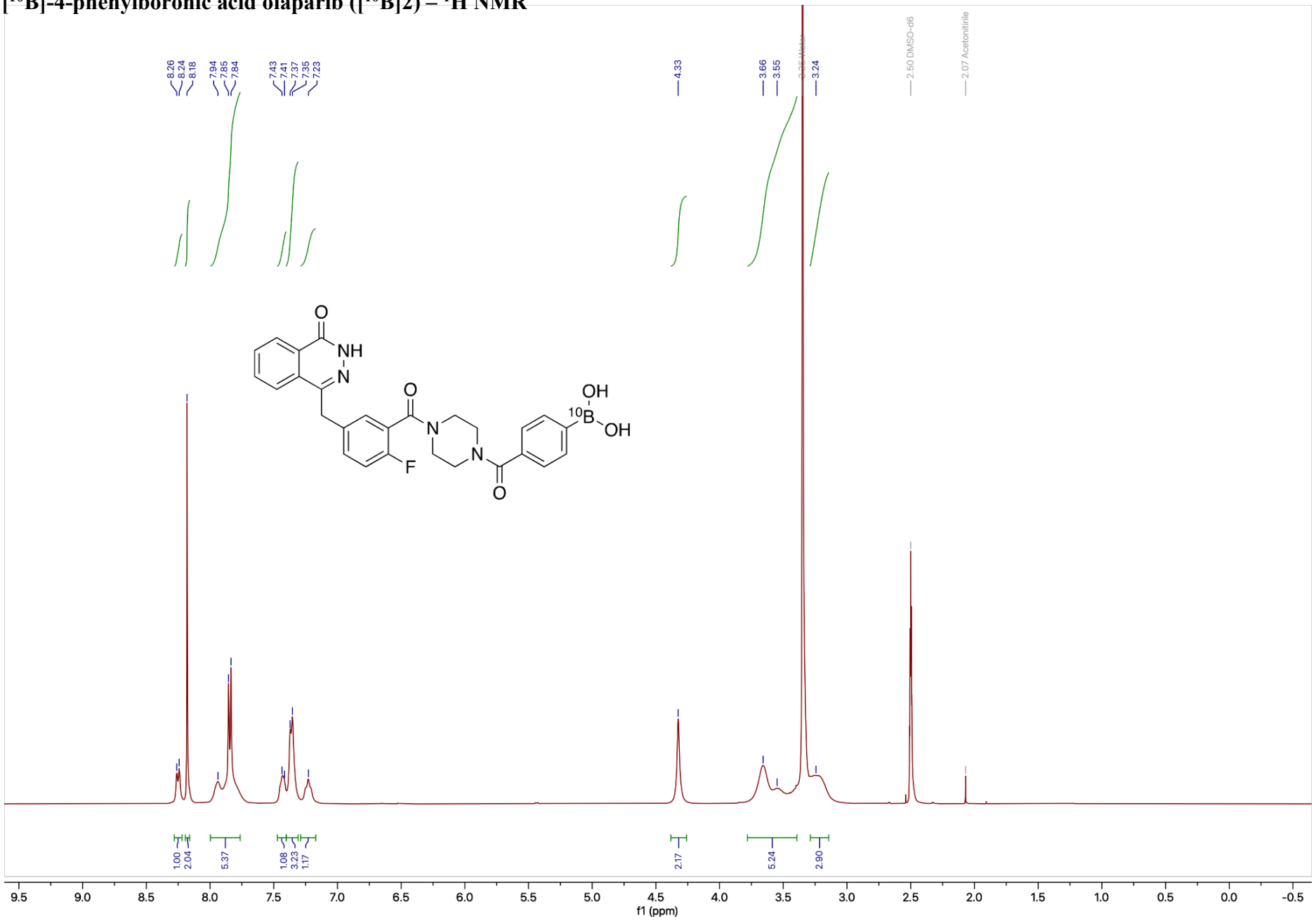
4-(4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl-2-^{10}B)benzoic acid ([^{10}B]3) - ^{10}B NMR



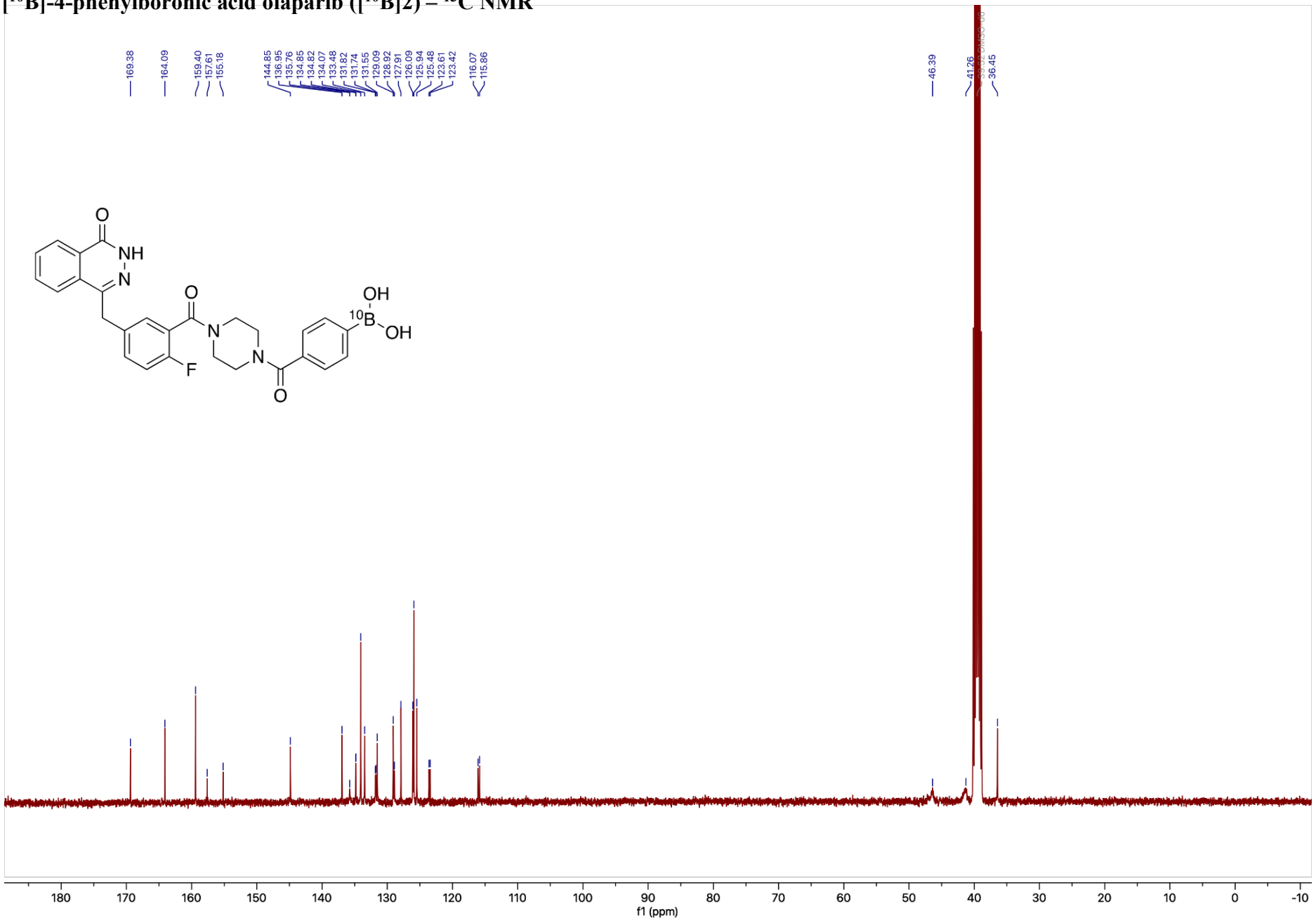
4-(4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl-2-^{11}B)benzoic acid ([^{11}B]3) - ^{11}B NMR



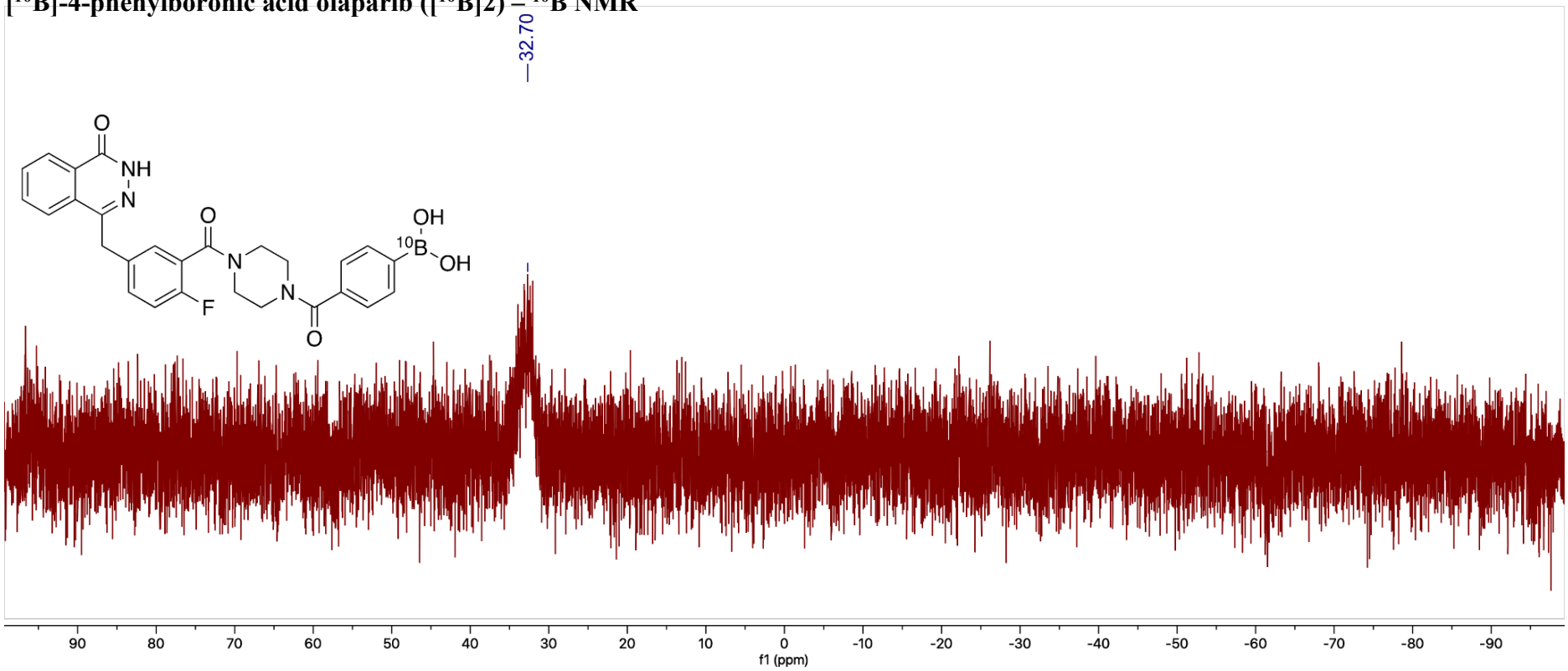
[^{10}B]-4-phenylboronic acid olaparib ([^{10}B]2) – ^{1}H NMR



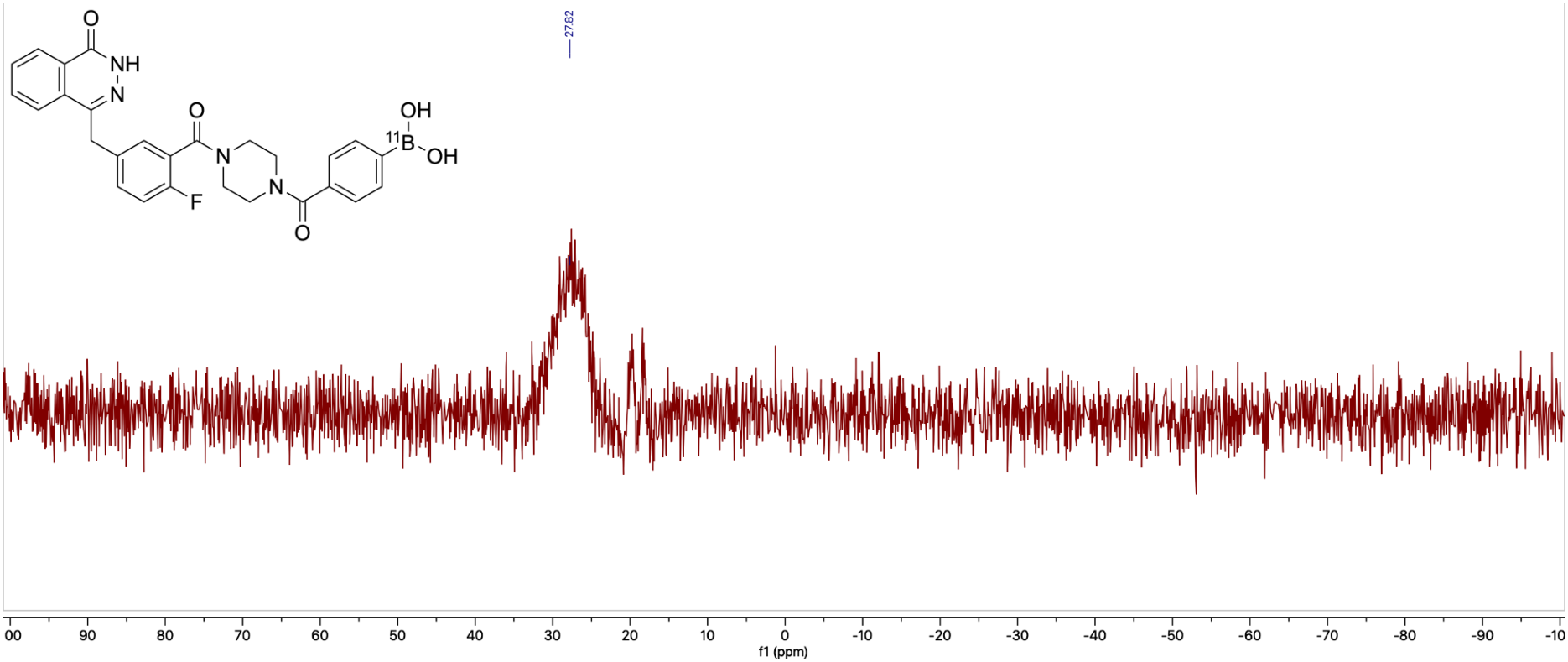
[^{10}B]-4-phenylboronic acid olaparib ([^{10}B]2) – ^{13}C NMR



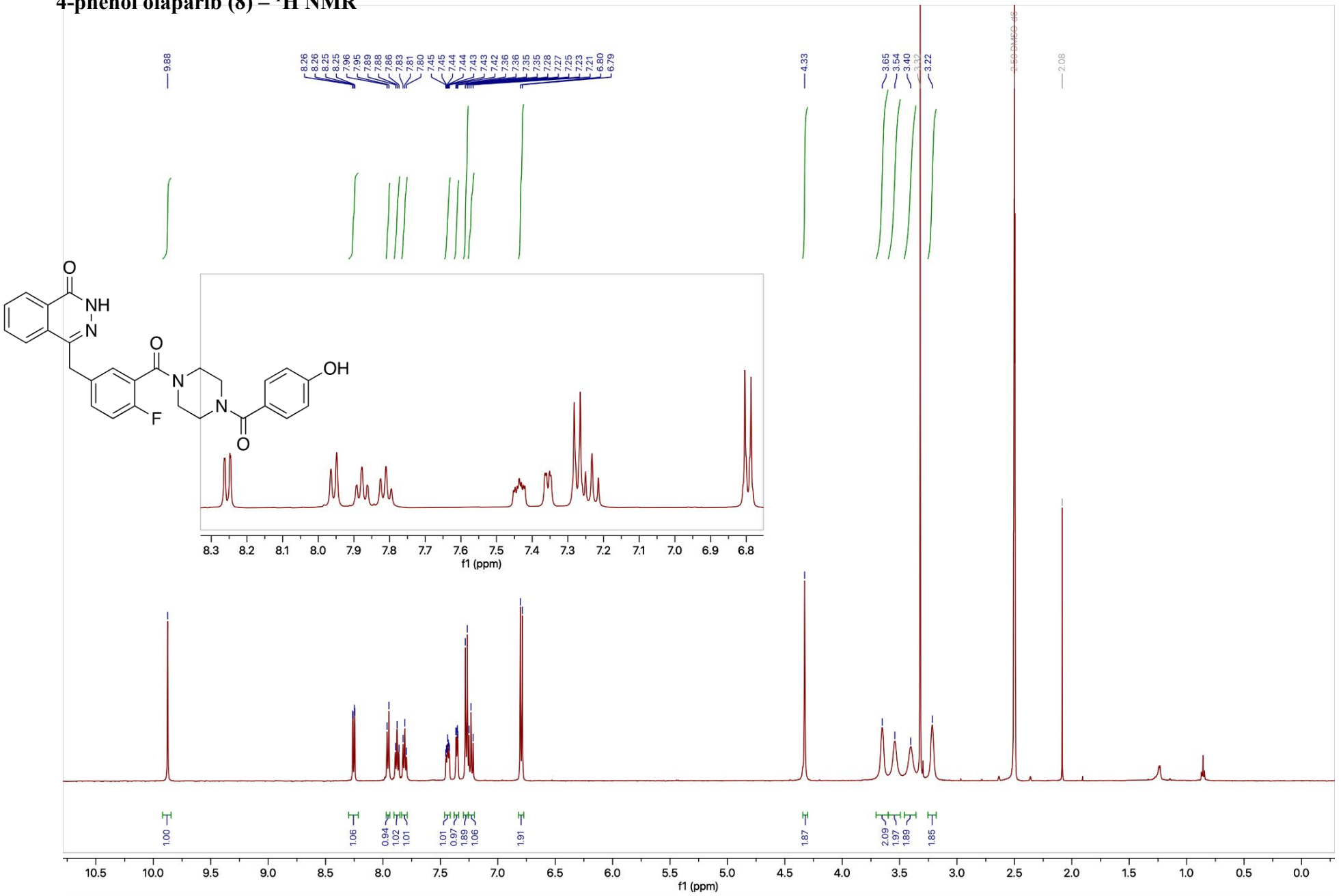
[^{10}B]-4-phenylboronic acid olaparib ([^{10}B]2) – ^{10}B NMR



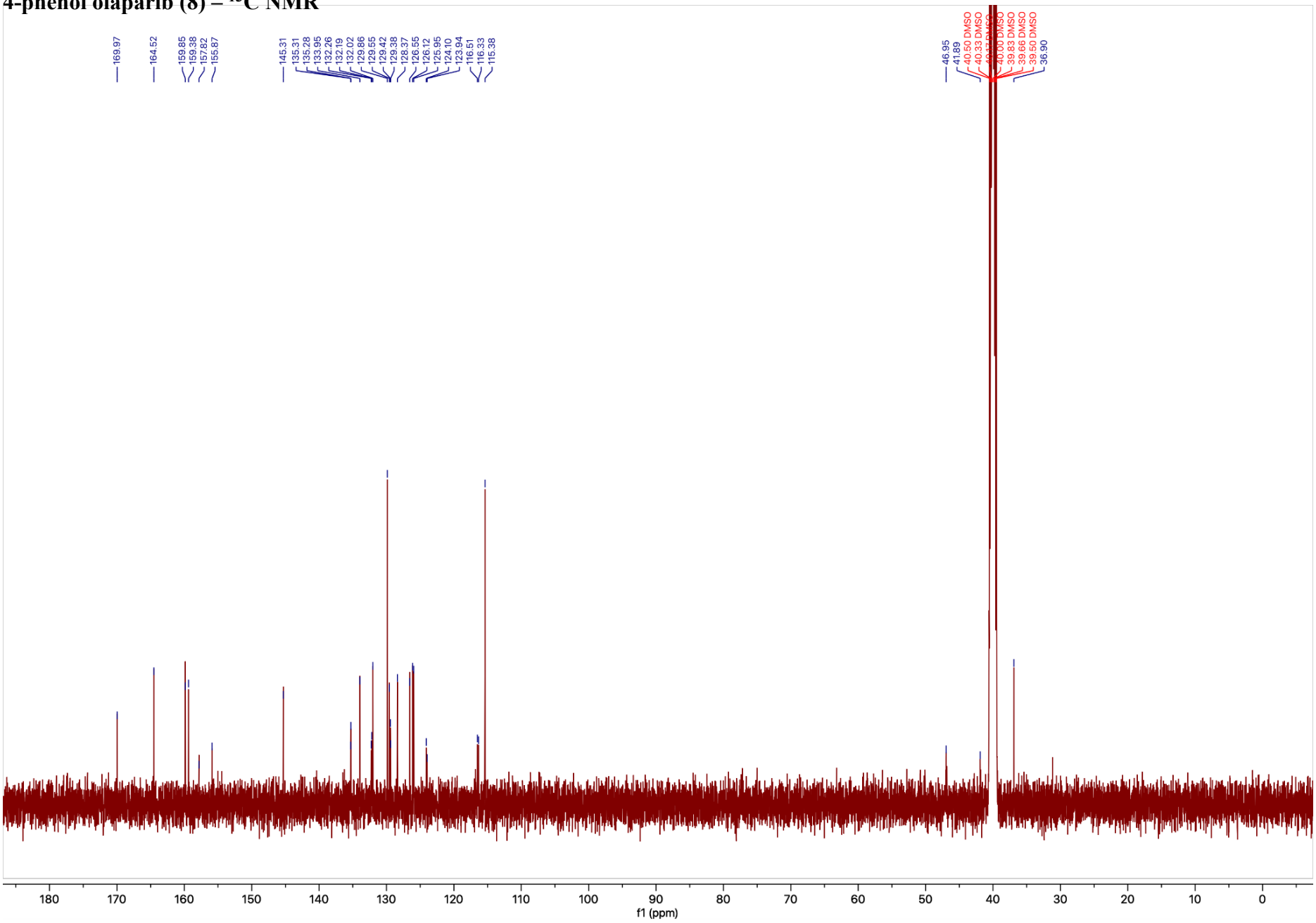
[^{11}B]-4-phenylboronic acid olaparib ([^{11}B]2) – ^{11}B NMR



4-phenol olaparib (8) – ^{1}H NMR



4-phenol olaparib (8) – ^{13}C NMR



References

1 Demory, E., Blandin, V., Einhorn, J. & Chavant, P. Y. Noncryogenic preparation of functionalized arylboronic esters through a magnesium−iodine exchange with in situ quench. *Org. Process Res. Dev.* **15**, 710–716; 10.1021/op2000089 (2011).

2 PraveenGanesh, N. & Chavant, P. Y. Improved preparation of 4,6,6-trimethyl-1,3,2-dioxaborinane and its use in a simple [$PdCl_2(TPP)_2$]-catalyzed borylation of aryl bromides and iodides. *Eur. J. Org. Chem.* **2008**, 4690–4696; 10.1002/ejoc.200800526 (2008).