SYNTHESIS, ENZYME ASSAYS AND MOLECULAR DOCKING STUDIES OF FLUORINATED BIOISOSTERES OF SANTACRUZAMATE A AS POTENTIAL HDAC TRACERS

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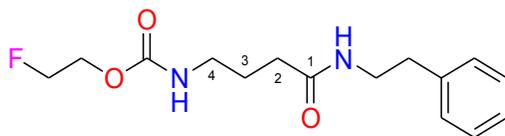## ORGANIC SYNTHESIS

General methods

Reagents and solvents were purchased from major commercial suppliers and were used without further purification. Melting points were measured with a Büchi-535 instrument and the data are uncorrected. Column chromatography was performed on Kieselgel 60 Merck 1.09385 (0.040-0.063 mm). TLC was accomplished on Macherey-Nagel Alugram® Sil G/UV$_{254}$ 40x80 mm aluminum sheets [0.25 mm silica gel with fluorescent indicator] with the following eluent systems (each v/v): [A]: dichloromethane-methanol 9:1, [B]: ethyl acetate-methanol 8:2, [C]: ethyl acetate-chloroform 7:3, [D]: chloroform-methanol 9:1, [E]: ethyl acetate-hexane 8:2. The spots were visualized with a 254 nm UV lamp or with 5% phosphomolybdic acid in ethanol.

^{1}H-, ^{13}C- and ^{19}F-NMR spectra were obtained with a Bruker AV 500 (Avance 500 MHz) spectrometer at 298 K, using BBO probehead. For ^{1}H- and ^{19}F-NMR experiments 10 mg of the appropriate derivative was dissolved in 500 μL of the corresponding deuterated solvent (CDCl$_3$ and CD$_3$OD). For measuring ^{13}C-NMR- and 2D-spectra: 20 mg of the synthesized compound was dissolved in 500 μL solvent. Chemical shifts (δ) are in parts per million (ppm), and coupling constants (J) reported in Hertz. ^{1}H and ^{13}C-NMR chemical shifts were referenced to the residual peak of CDCl$_3$ at 7.26 and 77.16 ppm, for proton and carbon respectively (CD$_3$OD: 3.31 and 49.00 ppm). Observation frequency: 500.130 MHz (^{1}H-NMR), 125.758 MHz (^{13}C-NMR), and 470.592 MHz (for ^{19}F-NMR spectra).

Homonuclear ^{1}H-^{1}H-COSY (Bruker Pulprog cosygpqf): observation frequency: 500.130 Mz, AQ = 0.239 sec, spectral with = 4280.82 Hz, D1 = 1.4 sec, NS = 16, DS = 8. Long-range ^{1}H-^{13}C correlations (HMBC) spectra (Bruker Pulprog hmbcgpndqf): observation frequency: 500.130 MHz (^{1}H), 125.758 MHz (^{13}C), AQ = 0.526 sec, spectral with = 7.78 ppm (f1), and 220 ppm (f2); D1 = 1.28 sec, NS = 32, DS = 16.

NMR Analysis

Table S1-A. NMR Data for Fluoroethyl-Santacruzamate A (**9**) in CDCl$_3$

Position	δ_H mult. (*J* in Hz)	δ_C mult .	COSY	HMBC
1	-	172.4	-	
2	2.17 t (7.0)	33.6	3	C-1, C-3, C-4
3	1.80 pent, (6.9)	25.8	2, 4	C-1, C-2, C-4
4	3.18 q (6.4)	40.3	3, COO*NH*CH$_2$	C-3, C-2, *COO*NHCH$_2$
Ph*CH$_2$*CH$_2$	2.81 t (7.0)	35.6	PhCH$_2$*CH$_2$	PhCH$_2$*CH$_2$, Ph-*C1*, Ph-*C2,6*
PhCH$_2$*CH$_2$*	3.51 dd, (7.0 and 13.0)	40.5	Ph*CH$_2$*CH$_2$, CO*NH*CH$_2$	C-1, Ph-*C1*, PhCH$_2$*CH$_2$
*Ph*CH$_2$CH$_2$	7.17-7.31 m	-	-	-
Ph-*C1*		138.8	-	Ph*CH$_2$CH$_2$*, Ph*CH$_2$*CH$_2$
Ph-*C2,6*		128.6		Ph*CH$_2$*CH$_2$
Ph-*C3,5*		128.7		
Ph-*C4*		126.4		
CO*NH*CH$_2$	5.89 br s	-	Ph*CH$_2$CH$_2$*	
OCO*NH*CH$_2$	5.21 br s	-	4	
O*CO*NHCH$_2$	-	156.4	-	
*CH$_2$*CH$_2$F	4.21–4.31 m	63.7 d ($^2J_{C,F}$ = 20.1)	CH$_2$*CH$_2$F	CH$_2$*CH$_2$F, FCH$_2$CH$_2$*OOC*NH
CH$_2$*CH$_2$F	4.50–4.61 m	81.8 d ($^1J_{C,F}$ = 169.5)	*CH$_2$*CH$_2$F	*CH$_2$*CH$_2$F

Table S1-B. Heteronuclear multiple bond correlations of Fluoroethyl-Santacruzamate-A (**9**)

		HMBC			
Position	δ^{1}H [ppm]	C-1	C-3	C-4	
2-CH$_2$	2.17 t				
δ^{13}C [ppm]		172.4	25.8	40.3	
Distance [bond]		2	2	3	

Position	δ^{1}H [ppm]	C-1	C-2	C-4	
3-CH$_2$	1.80 pent				
δ^{13}C [ppm]		145.3	137.1	128.5	
Distance [bond]		3	2	2	

Position	δ^{1}H [ppm]	C-3	C-2	COONH	
4-CH$_2$	3.18 q				
δ^{13}C [ppm]		25.8	33.6	156.4	
Distance [bond]		2	3	3	

Position	δ^{1}H [ppm]	PhCH$_2$CH$_2$	Ph-C-1	Ph-C-2,6	
PhCH$_2$CH$_2$	2.81 t				
δ^{13}C [ppm]		40.5	138.8	128.6	
Distance [bond]		2	2	3	

Position	δ^{1}H [ppm]	PhCH$_2$CH$_2$	Ph-C-1	C-1	
PhCH$_2$CH$_2$	3.51 dd				
δ^{13}C [ppm]		35.6	138.8	172.4	
Distance [bond]		2	3	3	

Position	δ^{1}H [ppm]	CH$_2$CH$_2$F		FCH$_2$CH$_2$OOCNH	
CH$_2$CH$_2$F	4.21-4.31				
δ^{13}C [ppm]		81.8		156.4	
Distance [bond]		2		3	

Position	δ^{1}H [ppm]	CH$_2$CH$_2$F			
CH$_2$CH$_2$F	4.50-4.61				
δ^{13}C [ppm]		63.7			
Distance [bond]		2			

Table S1-C NMR Data for Santacruzamate A (**6,** CAS RN: 1477949-42-0]) in CDCl$_3$
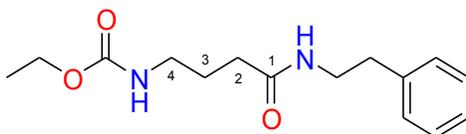
Position	1. ABX*		Pavlik *et al. J. Nat. Prod.* **2013**, 76, 2026-2033.		2. Leuven**	
	δ$_H$ mult. (*J* in Hz)	δ$_C$	δ$_H$ mult. (*J* in Hz)	δ$_C$	δ$_H$ mult. (*J* in Hz)	δ$_C$
1	-	172.5	-	172.5	-	172.7
2	2.16 t (7.0)	33.6	2.17 (6.9)	33.7	2.17 t (7.0)	33.8
3	1.79 pent, (6.8)	26.1	1.80 pent (6.8)	26.1	1.78-1.83 m	26.3
4	3.16 q (6.1)	40.1	3.18 q (5.9)	40.2	3.17 t (6.2)	40.3
Ph*CH₂*CH₂	2.82 t (7.1)	35.6	2.83 t (6.8)	35.7	2.82 t (7.0)	35.8
PhCH₂*CH₂*	3.51 dd, (7.0 and 13.0)	40.6	3.53 q (6.8)	40.6	3.51 q (6.2)	40.8
*Ph*CH₂CH₂	7.18-7.31 m	-	7.22-7.30 m	-	7.18-7.32	
Ph-*C1*		138.8		138.9		139.1
Ph-*C2,6*		128.6		128.6		128.9
Ph-*C3,5*		128.7		128.8		128.8
Ph-*C4*		126.4		126.5		126.6
CO*NH*CH₂	5.93 br s	-	5.92 br s	-	6.02 br s	-
OCO*NH*CH₂	4.93 br s	-	4.92 br s	-	5.0 br s	-
O*CO*NHCH₂	-	157.1	-	157.1	-	157.3
COO*CH₂*CH₃	4.08 q (7.1)	60.8	4.1 q (6.9)	60.8	4.10 (7.0)	60.9
COOCH₂*CH₃*	1.22 t (7.1)	14.6	1.23 t (7.3)	14.7	1.23 t (7.1)	14.8

Colourless Powder, mp. 114-115 °C, Lit. [Pavlik et al. 2013] mp. 112-113 °C.

HRMS (ESI) Calcd. For C$_{15}$H$_{23}$N$_2$O$_3$ [M+H]$^+$: 279.1703. Found: 279.1846.

*500 MHz, CDCl$_3$ [Data not shown]

400 MHz CDCl$_3$ [Figure S10** and **Figure S11**]

Hydrogen bonding contacts

Table S2 Predicted H-bond interactions, with the distance between the corresponding heteroatoms in Å, π-π interactions, distance of Zn^{2+} atom with the carbonyl oxygen (unless otherwise stated) for compounds 1-8. H-bonding and hydrophobic interactions are predicted by poseview or Ligplot analysis.

| Cpd	Hydrogen Bonds (Heteroatom distance Å)	π-π interaction (Distance in Å)	Distance of Zn^{2+} in Å	Hydrophobic interactions
1	O-His145A (2.72) NH-His146A (2.76) O-Tyr308A (2.58) H-His183A (2.75) O-Tyr209A (2.90	Tyr209A (3.36)	1.79 (OH) 2.06 (CO)	Phe155A Phe210A His183A Leu276A Tyr209A
2	O-His145A (3.50) O-His146A (3.24) NH-Tyr308A (2.73) O-Asp181A (2.50) NH-Asp104A (2.58)	Phe155A (3.47) Phe210A (4.04)	1.78 (CO) 4.23 (OH)	Asp186A Glu151A Phe210A Phe155A Lys149A
3	OH-His145A (3.14) OH-Gly143A (3.48) NH-Gly154A (3.14)	Phe155A (3.14) Phe210A (3.57)	1.72 (CO) 3.79 (OH)	Phe210A Asp104 Phe155A Gly154A
4	O-His145A (3.48) O-His146A (3.20) O-Asp181A (3.32) NH-Tyr308A (3.59)	Phe155A (2.89) Phe210A (3.30)	1.75 (CO) 3.54 (OH)	Phe155A Asp104A Leu276A Pro34A
5	CO-His145A (3.92) NH-His146A (3.35)	None	1.80 (CO)	Ser153A Phe210A Gly154A Asp104A His210A Pro106A
6	CO-His145A (2.58) NH-His146A (2.73) O-Tyr308A (2.66) H-His183A (2.87)	None	1.96 (CO) 1.91 (OH)	Leu276A Phe210A
7	O-His145A (3.76)	Phe155A (3.72)	1.90 (OMe)	Phe210A His183A His146A
8	NH-Gly154A (2.73) NMe$_2$-Glu103A (2.90) NMe$_2$-Asp104A(2.71)	None	2.39 (CO)	Phe155A Phe210A His33A His146A Leu276A

Pan-HDAC and HDAC2 inhibition assays
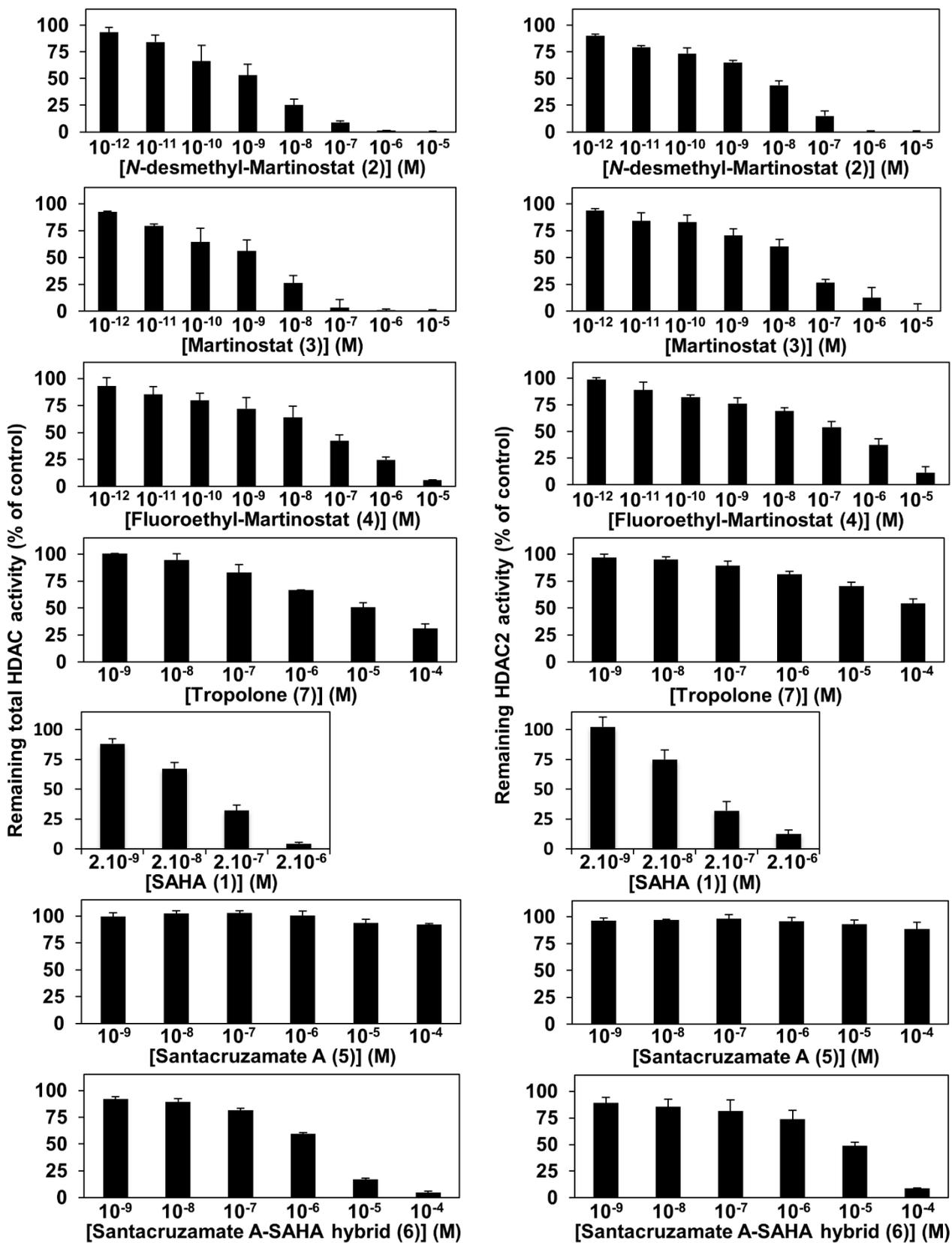
Figure S1 Effect of compounds **1**-**7** on total HDAC or HDAC2 activities between 1 nM and 100 µM.
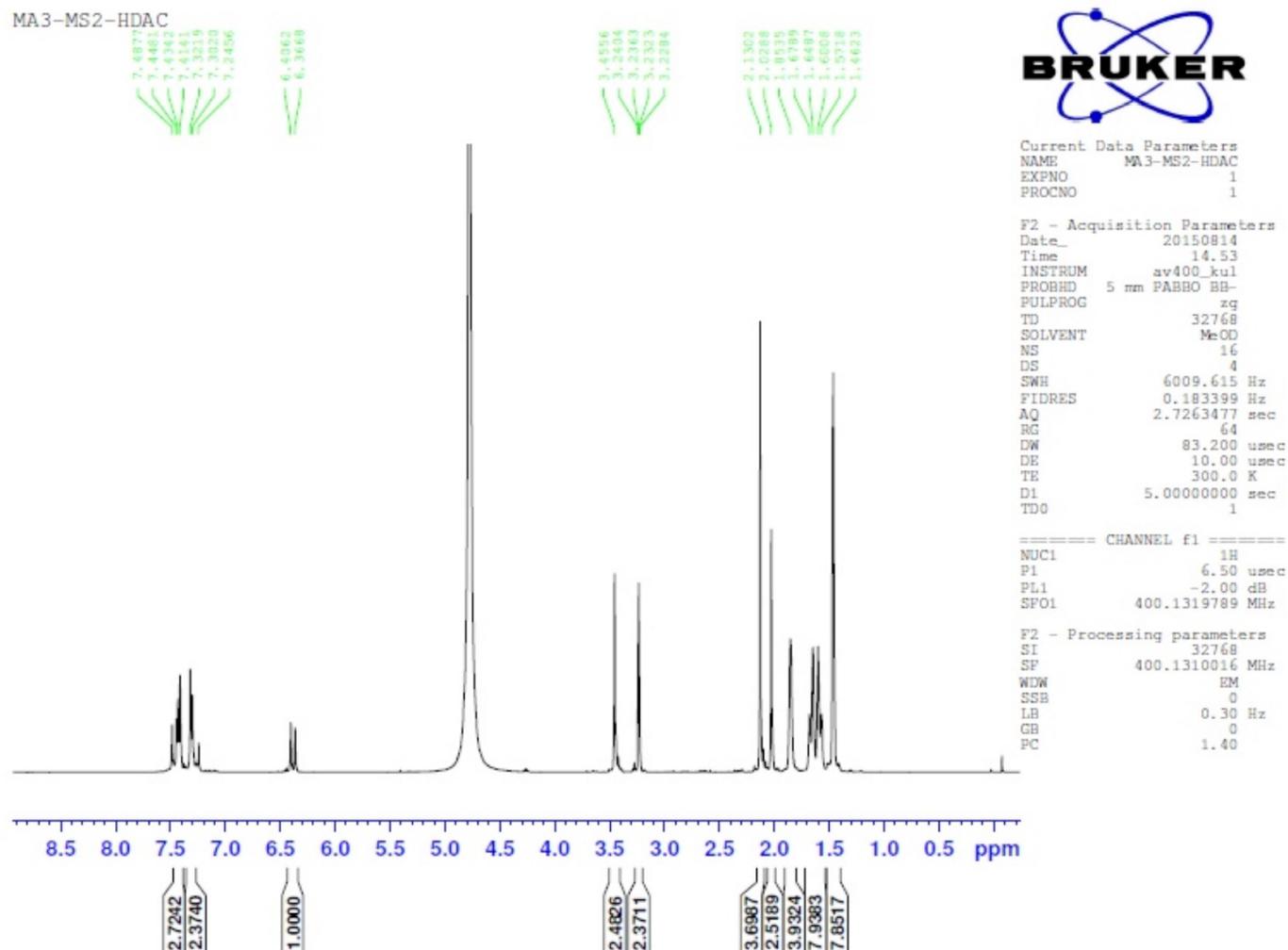
NMR spectral data for all final compounds

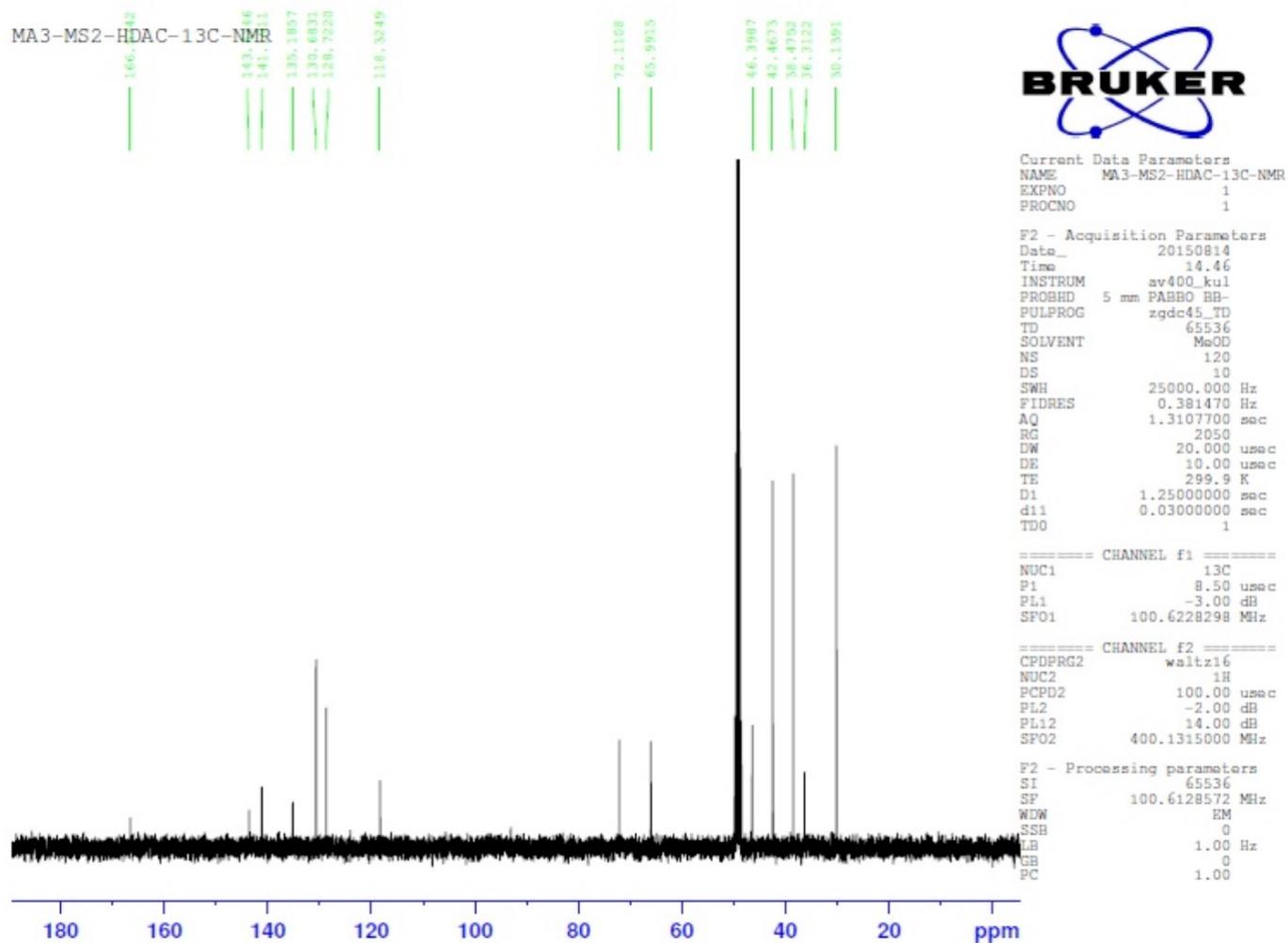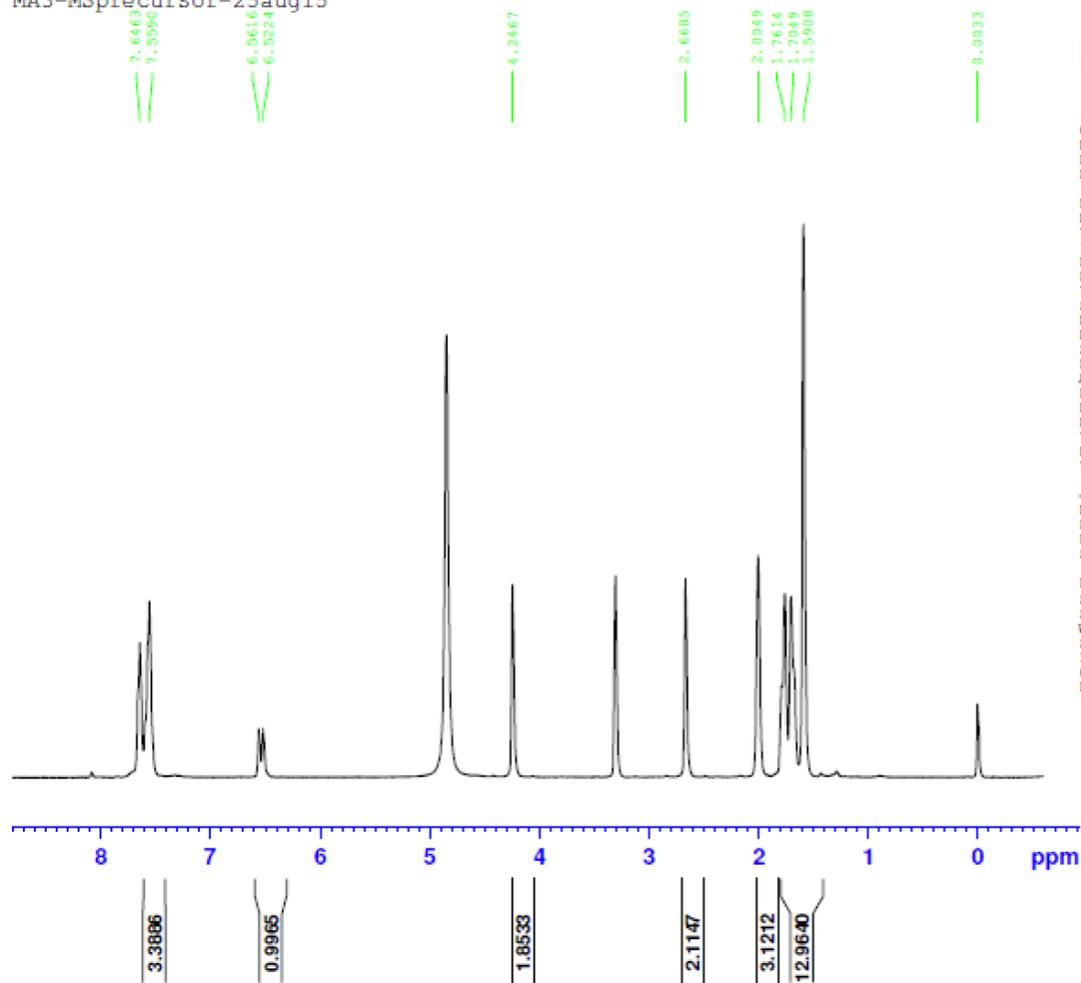**Figure S2:** ^{1}H-NMR spectrum of martinostat (**3**) in CD$_3$OD.

Figure S3: ^{13}C-NMR spectrum of martinostat (**3**) in CD$_3$OD.

Figure S4: ¹H-NMR spectrum of *N*-desmethyl-martinostat (**2**) in CDCl₃.
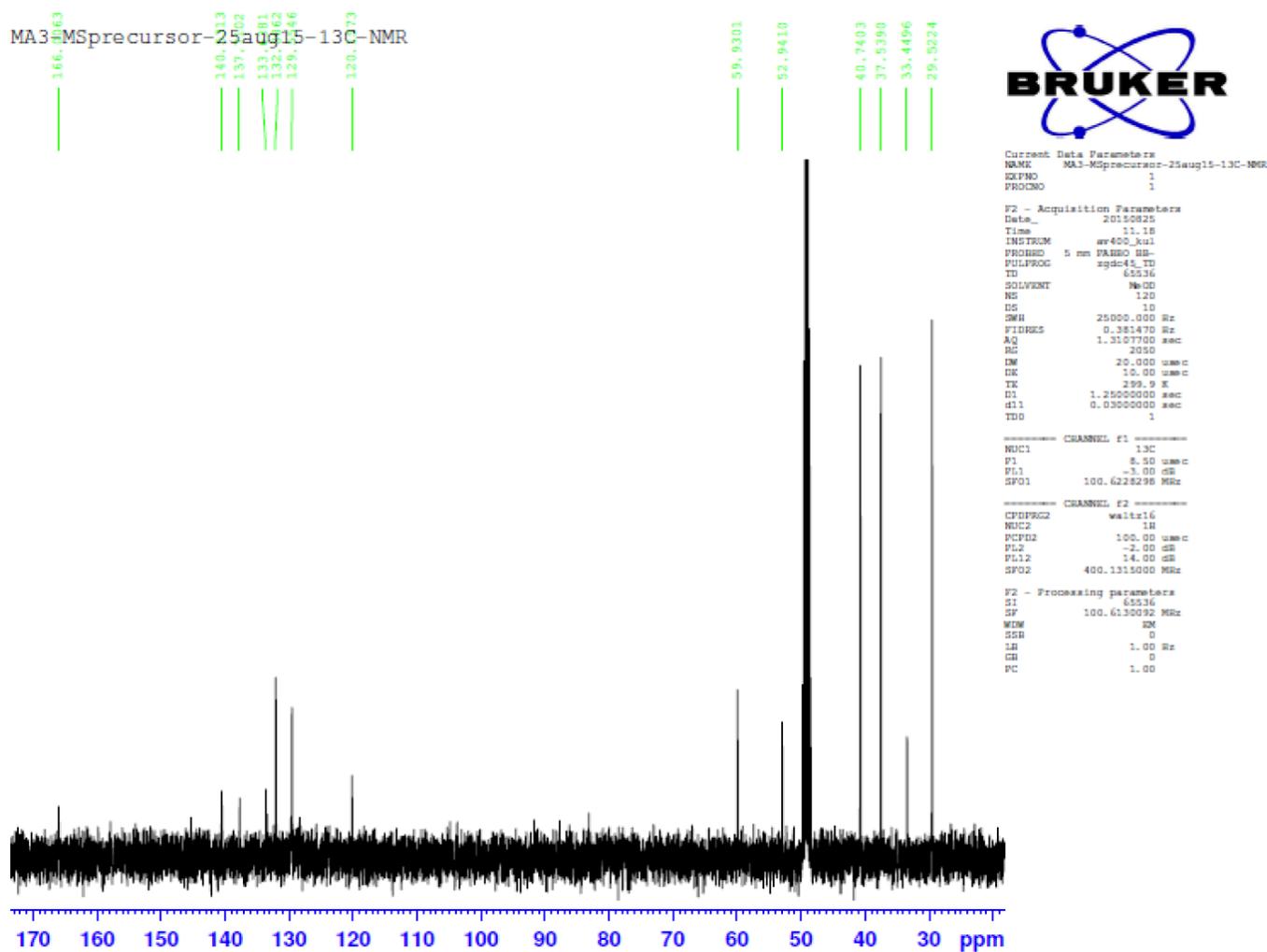
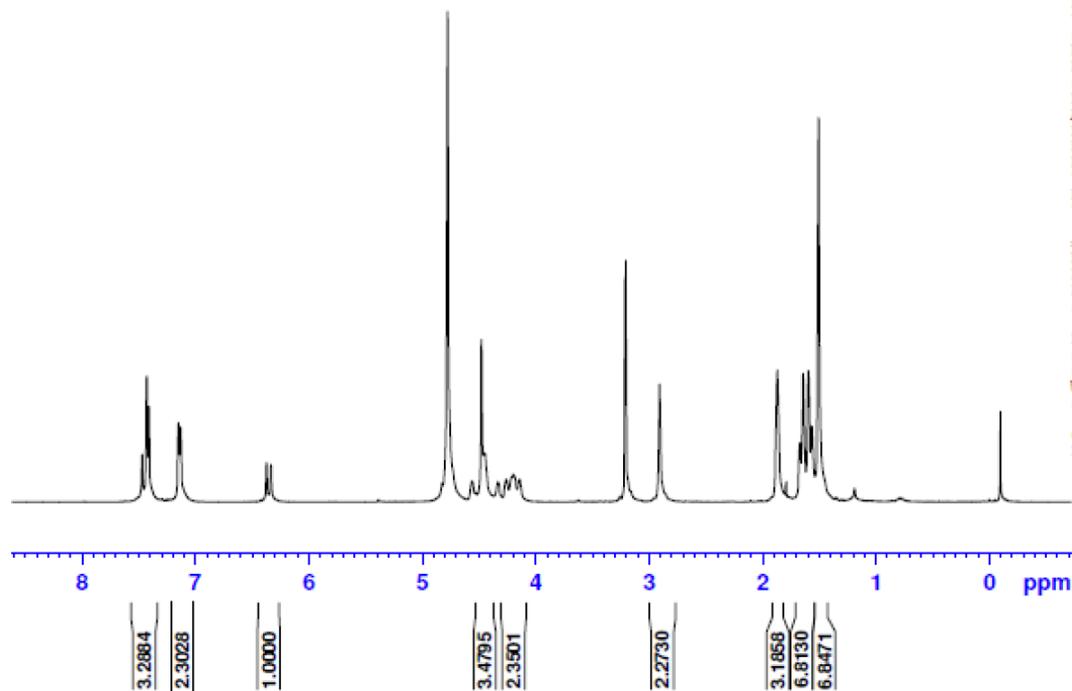**Figure S5:** ^{13}C-NMR spectrum of *N*-desmethyl-martinostat (**2**) in CDCl$_3$.

Figure S6: ^{1}H-NMR spectrum of fluoroethyl-martinostat (**4**) in CD$_3$OD.
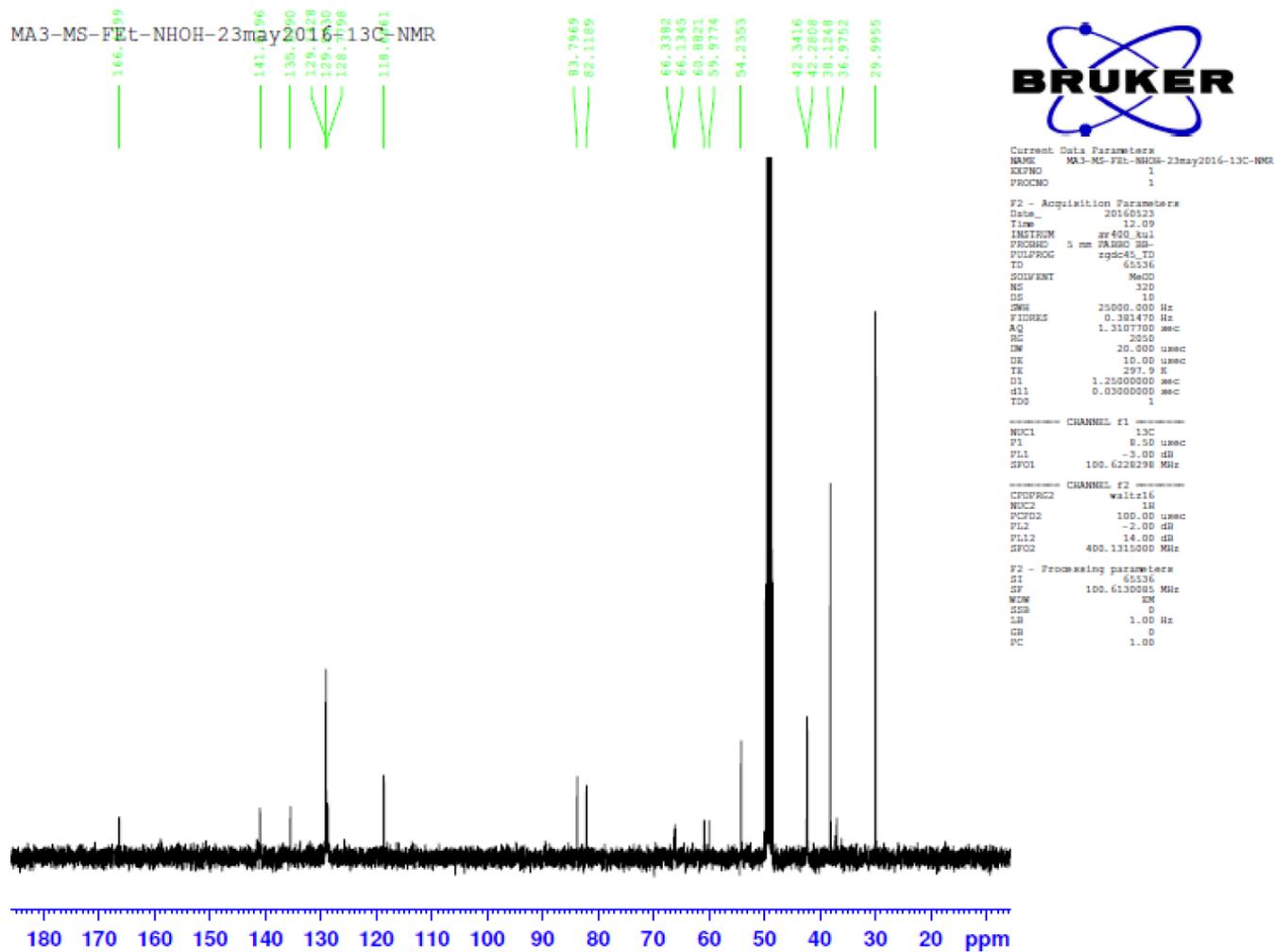
Figure S7: ^{13}C-NMR spectrum of fluoroethyl-martinostat (**4**) in CD$_3$OD.

Figure S8: ^{1}H-NMR spectrum of α-(4-methoxyphenyl) tropolone (**7**) in CDCl$_3$.
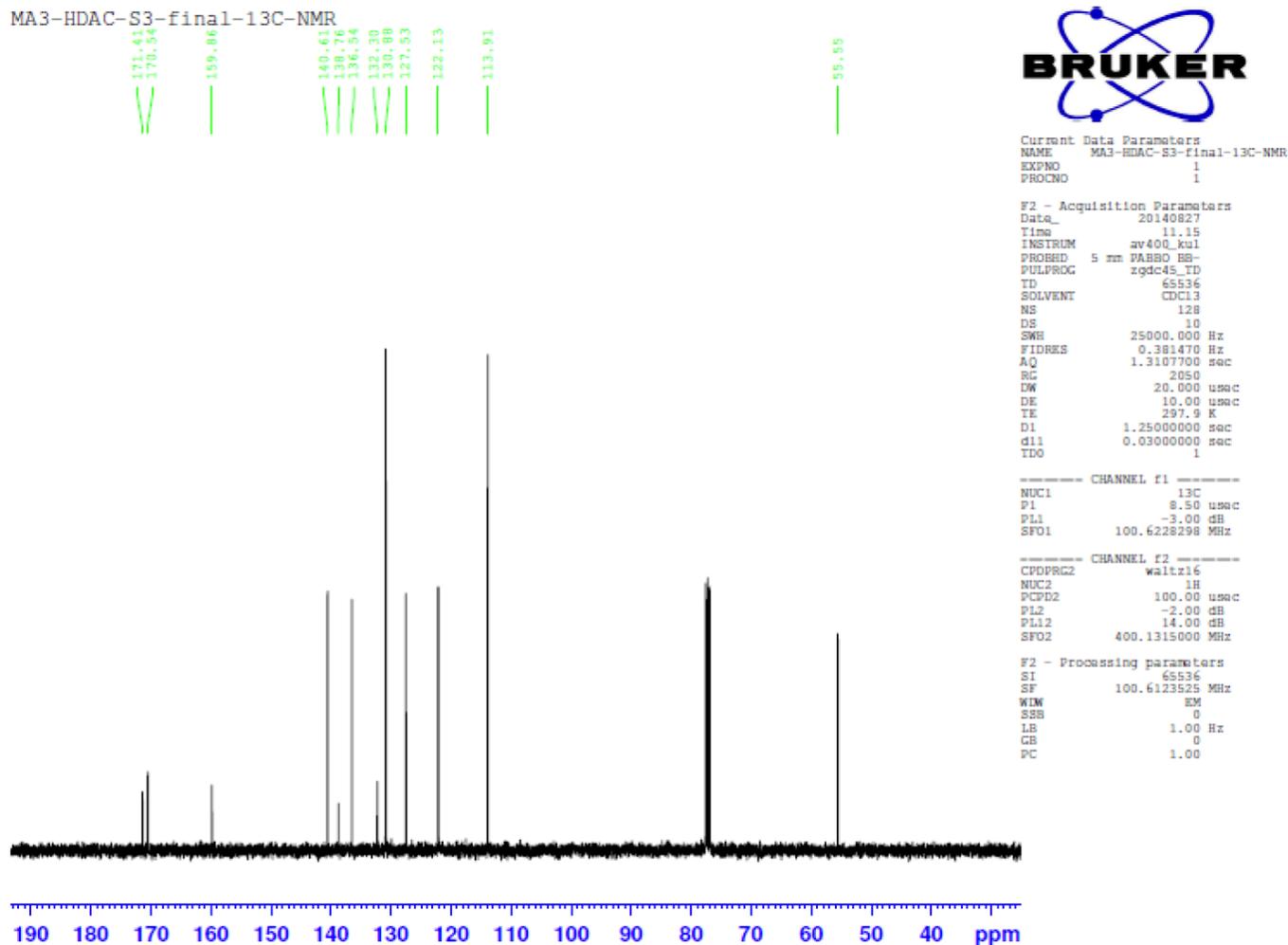
Figure S9: ^{13}C-NMR spectrum of α-(4-methoxyphenyl) tropolone (**7**) in CDCl$_3$.

Figure S10: ^{1}H-NMR spectrum of santacruzamate A (**5**) in CDCl$_3$.

MA3-santacruzamate-13C-NMR
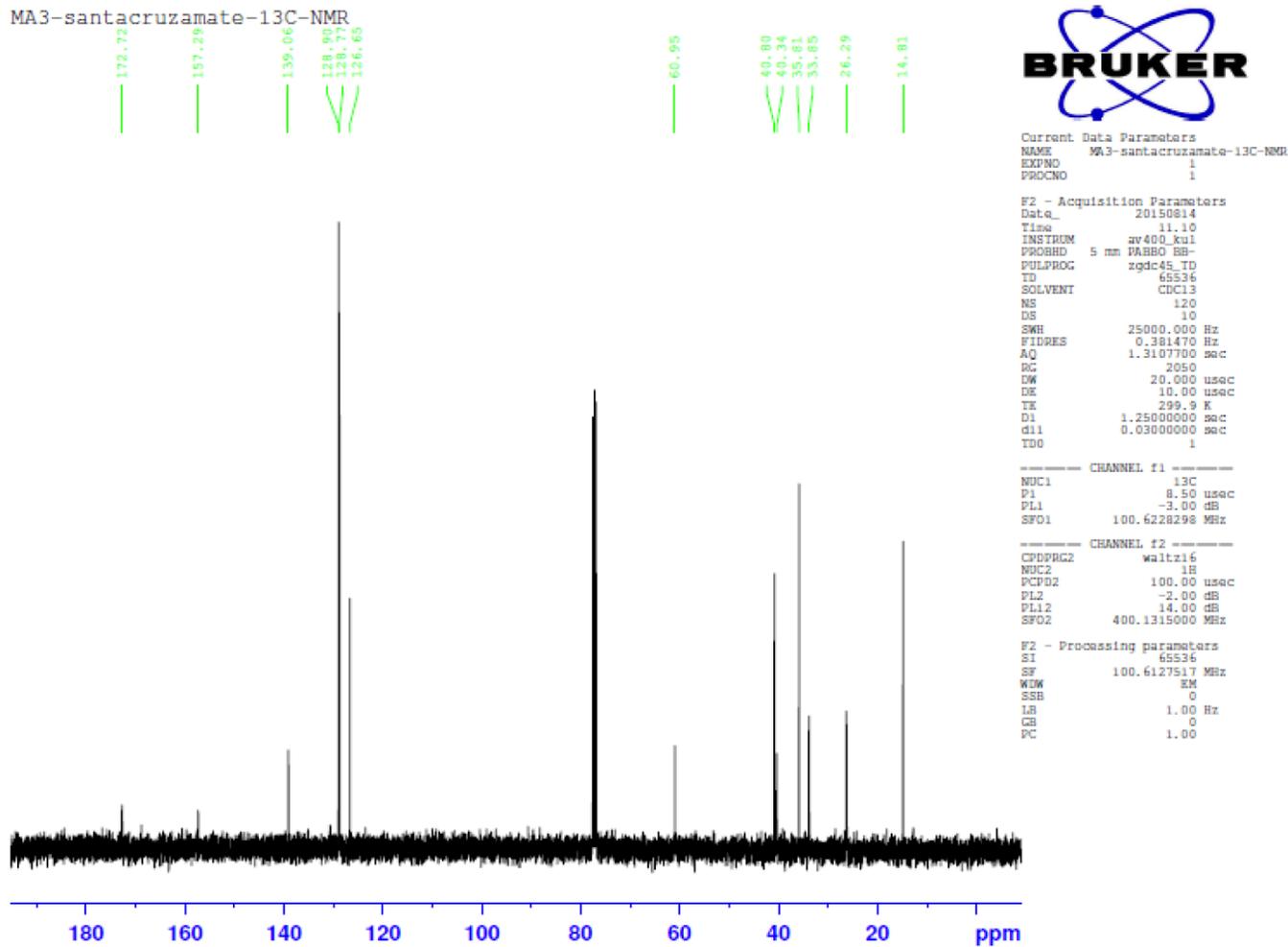
Figure S11: ^{13}C-NMR spectrum of santacruzamate A (**5**) in CDCl$_3$.
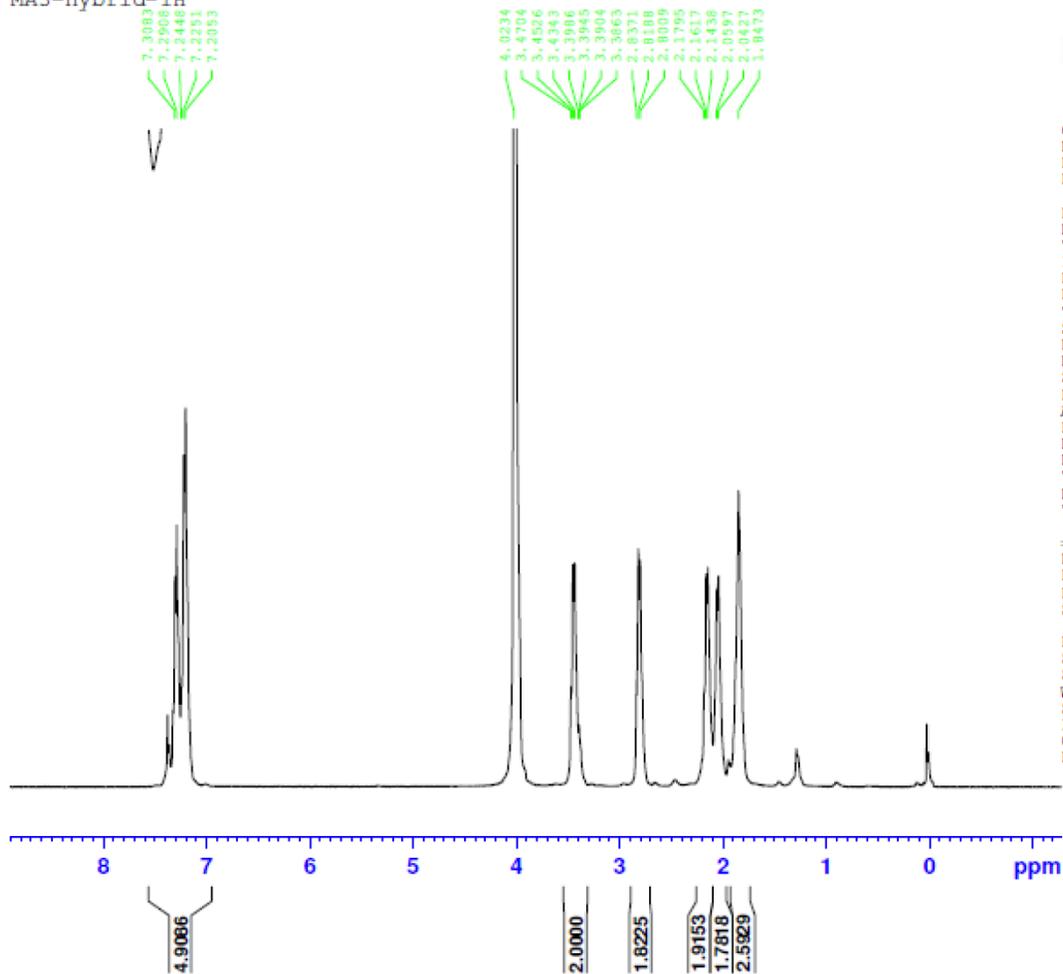
Figure S12: ^{1}H-NMR spectrum of santacruzamate A-SAHA hybrid (**6**) in CDCl$_3$ + CD$_3$OD.

MA3-hybrid-13C-NMR
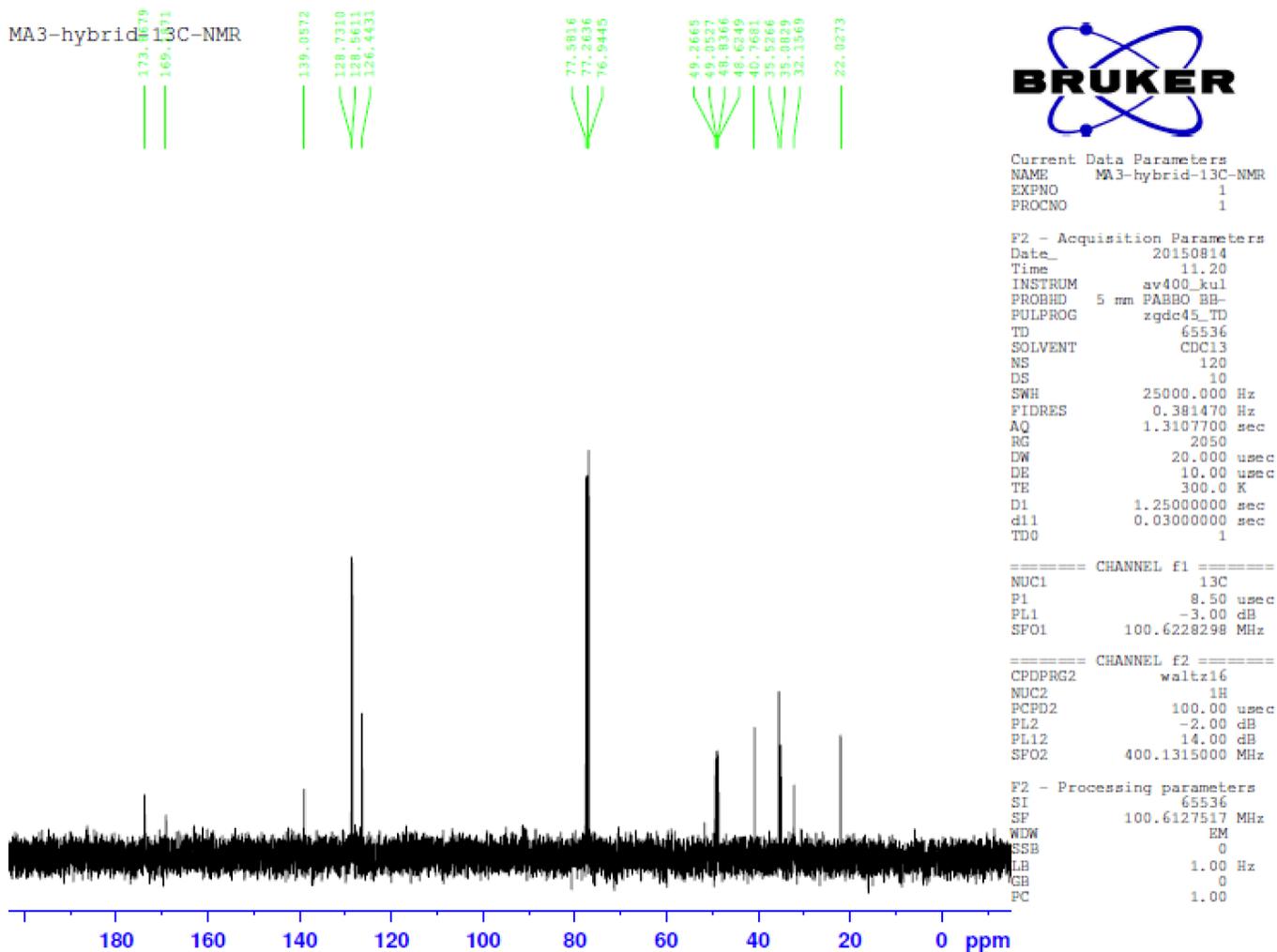
Figure S13: ^{13}C-NMR spectrum of santacruzamate A-SAHA hybrid (**6**) in CDCl$_3$ + CD$_3$OD.
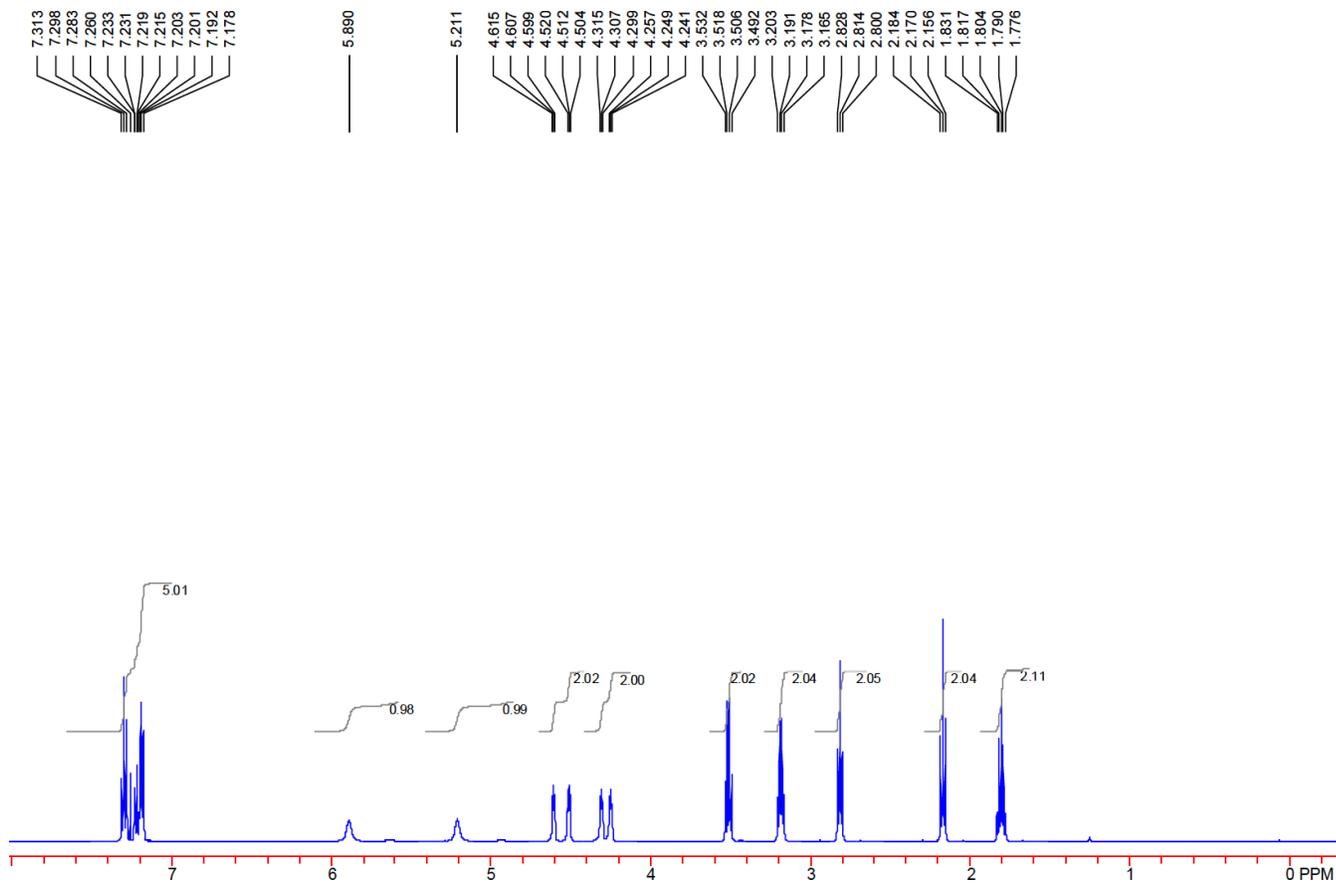
Figure S14: ^{1}H-NMR spectrum of fluoroethyl-santacruzamate A (**9**) in CDCl$_3$.
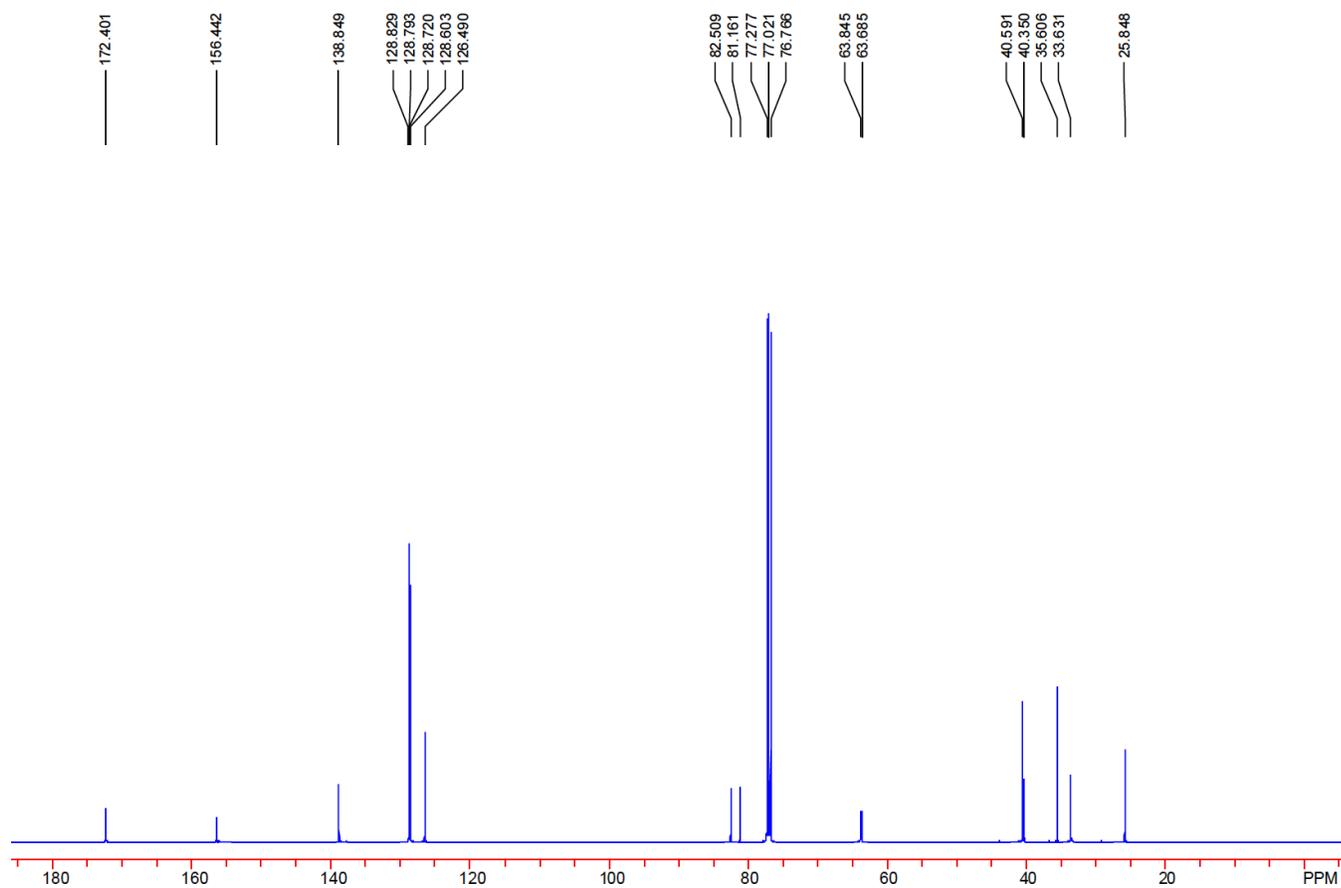
Figure S15: ^{13}C-NMR spectrum of fluoroethyl-santacruzamate (**9**) in CDCl$_3$.
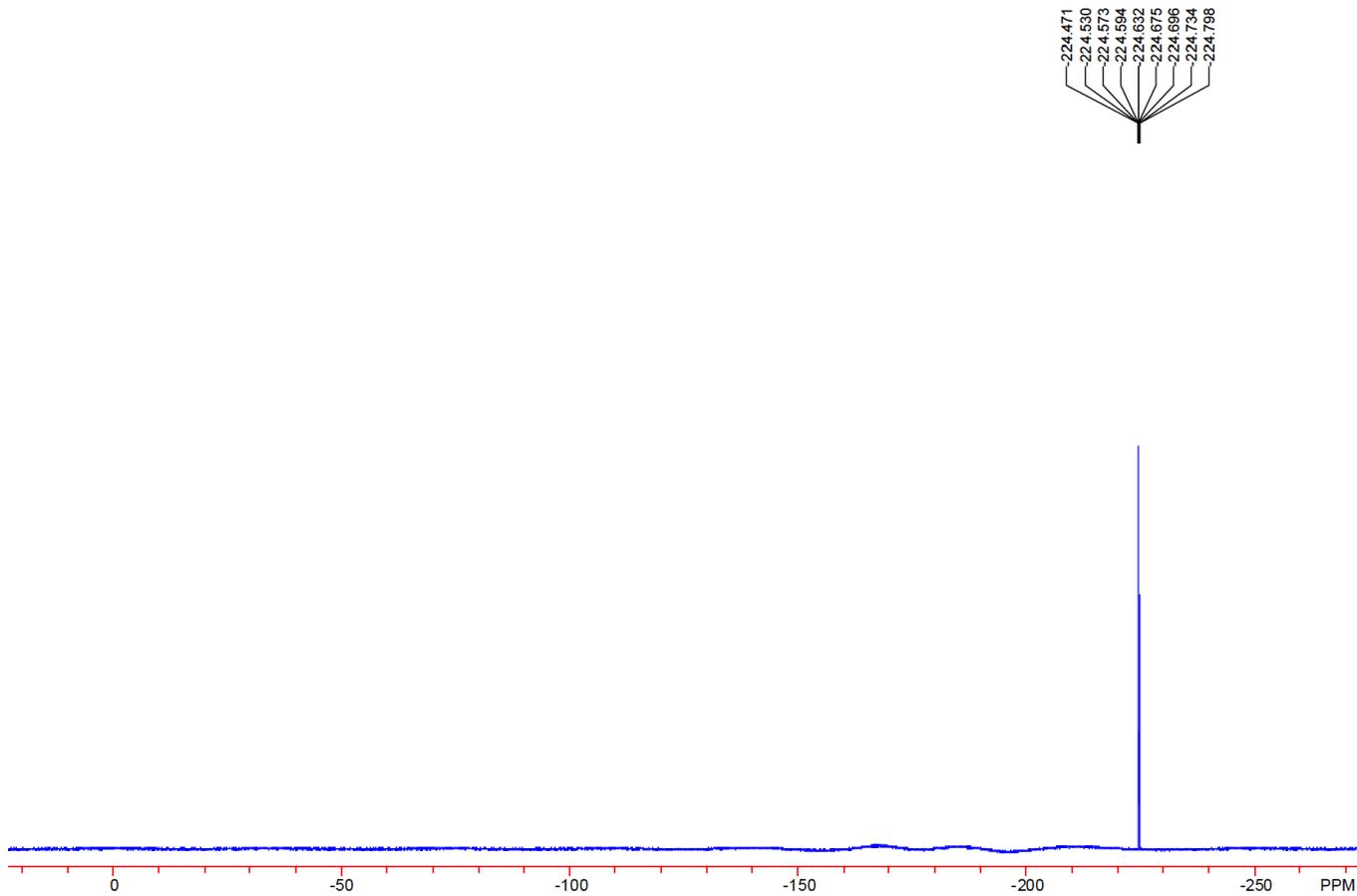
Figure S16: ^{19}F-NMR spectrum of fluoroethyl-santacruzamate (**9**) in CDCl$_3$.
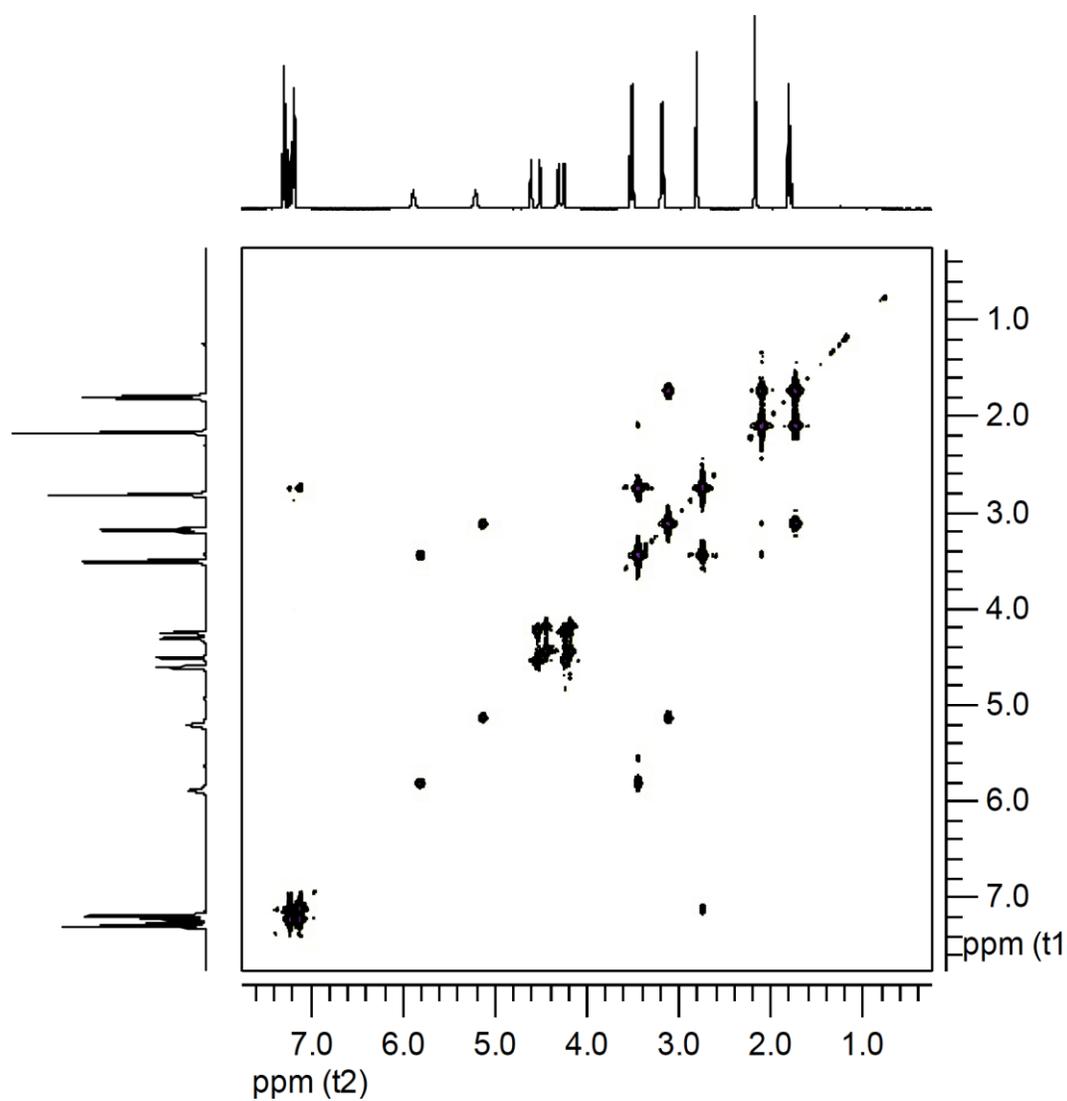
Figure S17: ^{1}H-^{1}H COSY spectrum of fluoroethyl-santacruzamate (**9**) in CDCl$_3$.
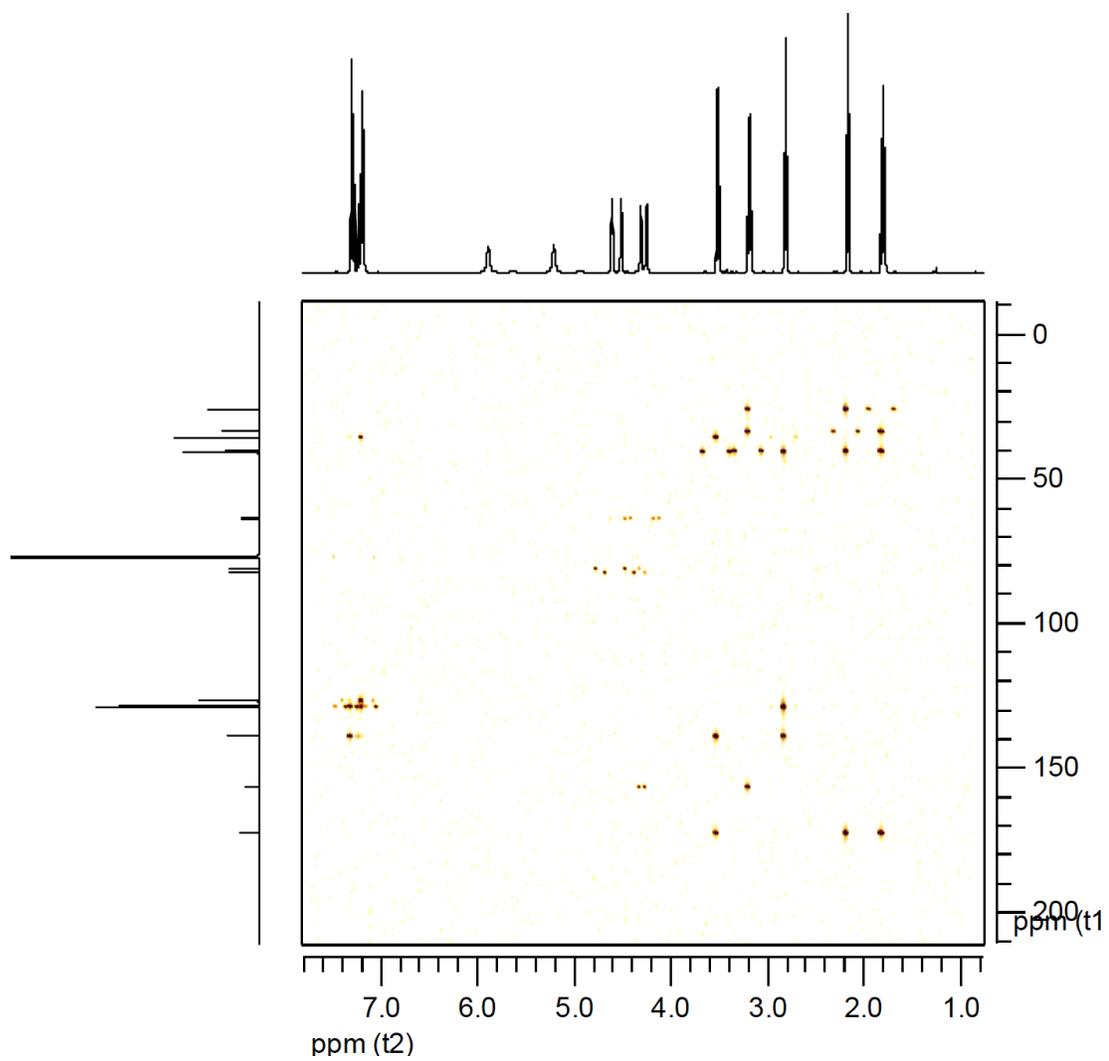
Figure S18: ^1H-^{13}C HMBC spectrum of fluoroethyl-santacruzamate (**9**) in CDCl$_3$.
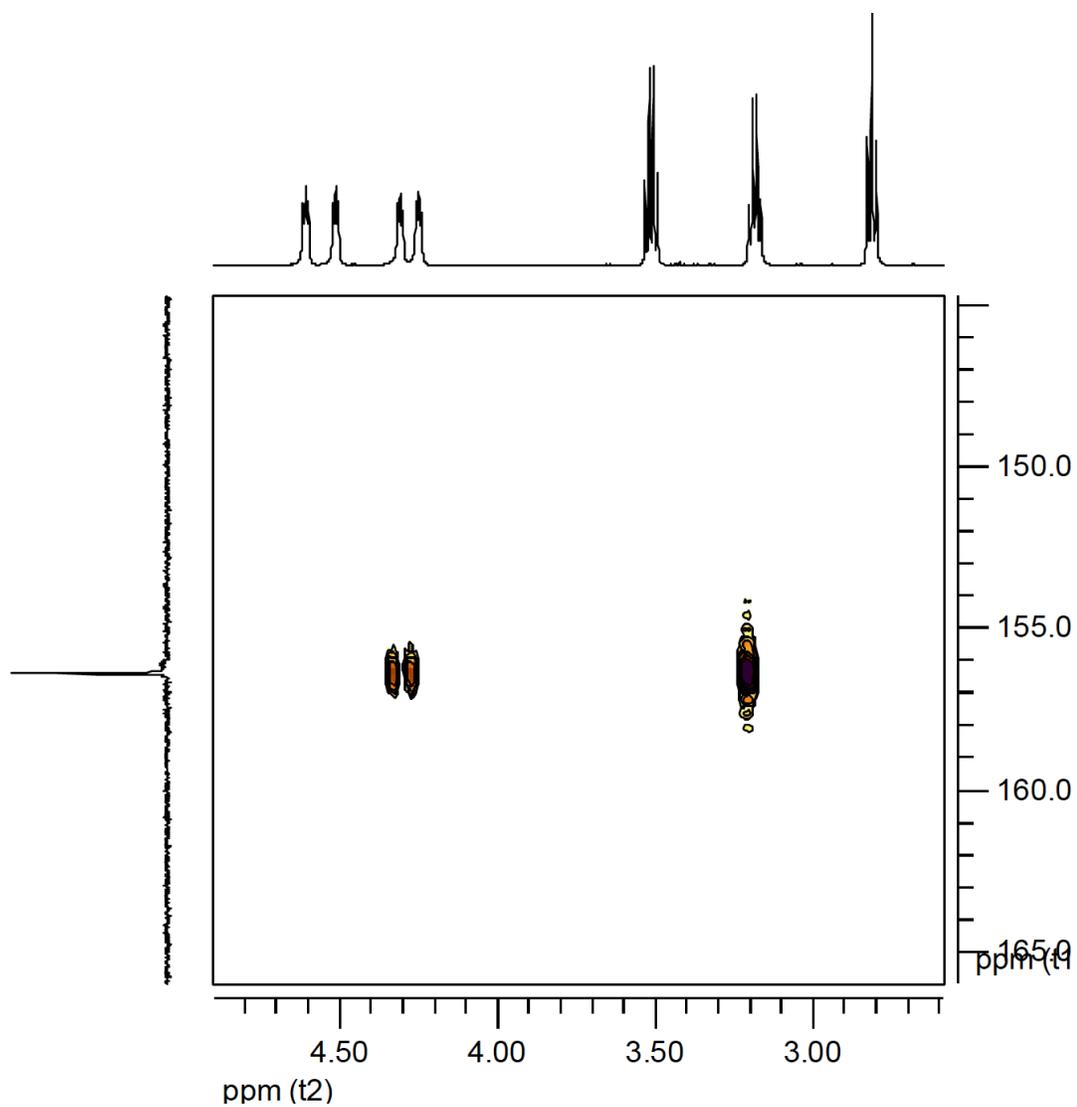
Figure S19: Part of the ^{1}H-^{13}C HMBC spectrum of fluoroethyl-santacruzamate-A (**9**) showing correlation to determine the position of the 2- fluoroethyl group and the incorporation of the COO group.
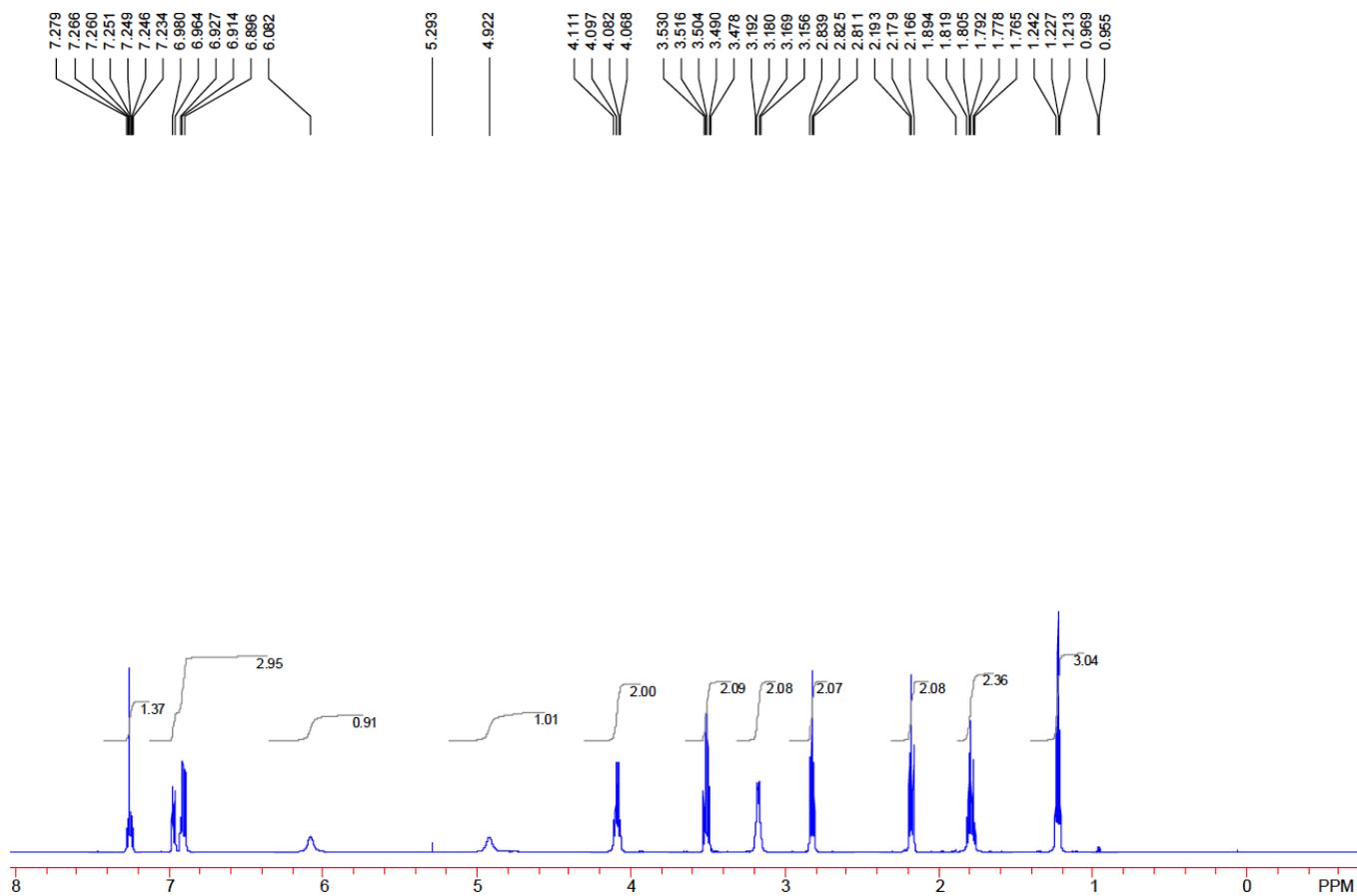
Figure S20: ^{1}H-NMR spectrum of 3-fluorophenethyl-santacruzamate A (**10**) in CDCl$_3$.
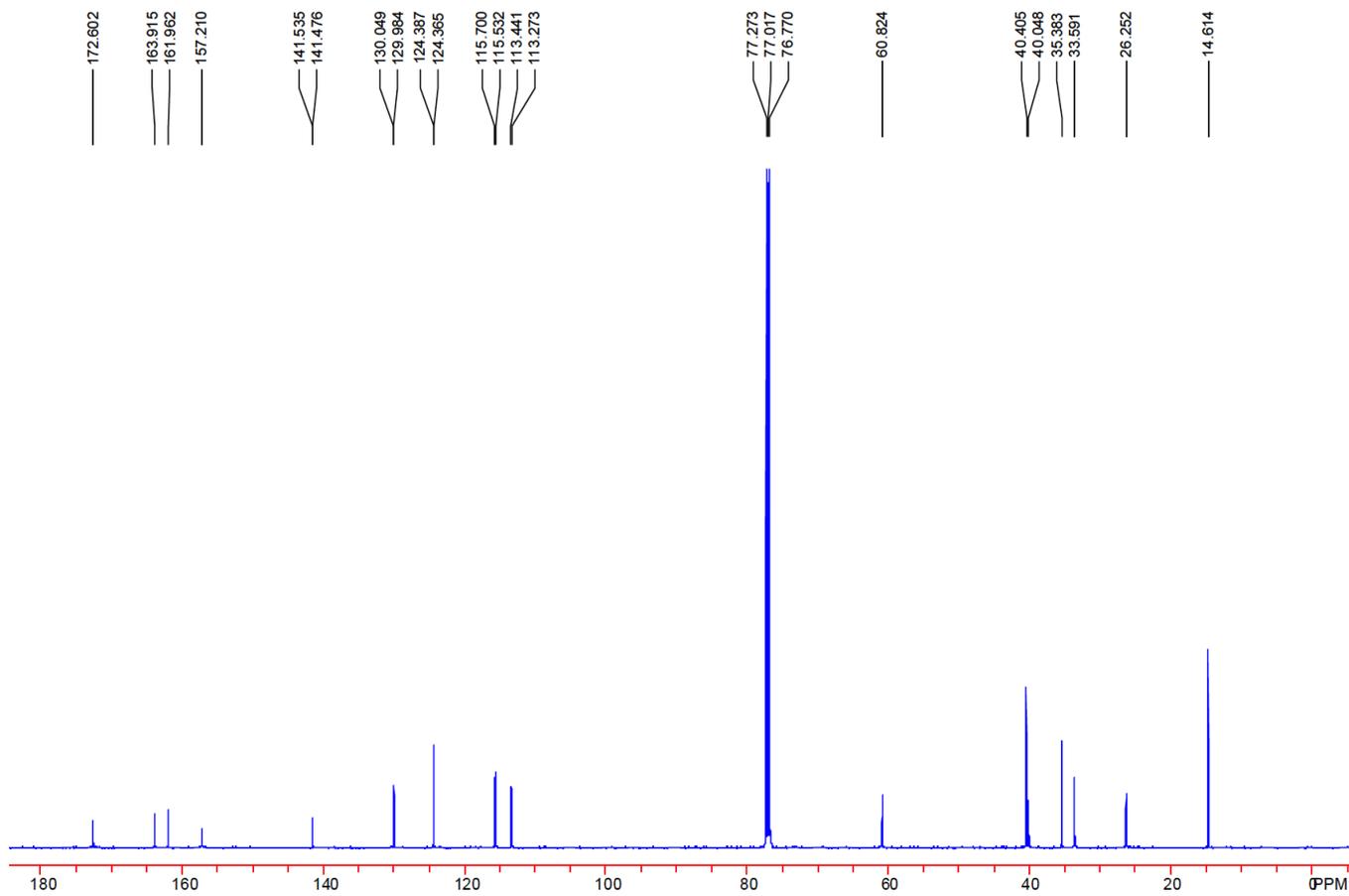
Figure S21: ^{13}C-NMR spectrum of 3-fluorophenethyl-santacruzamate A (**10**) in CDCl$_3$.
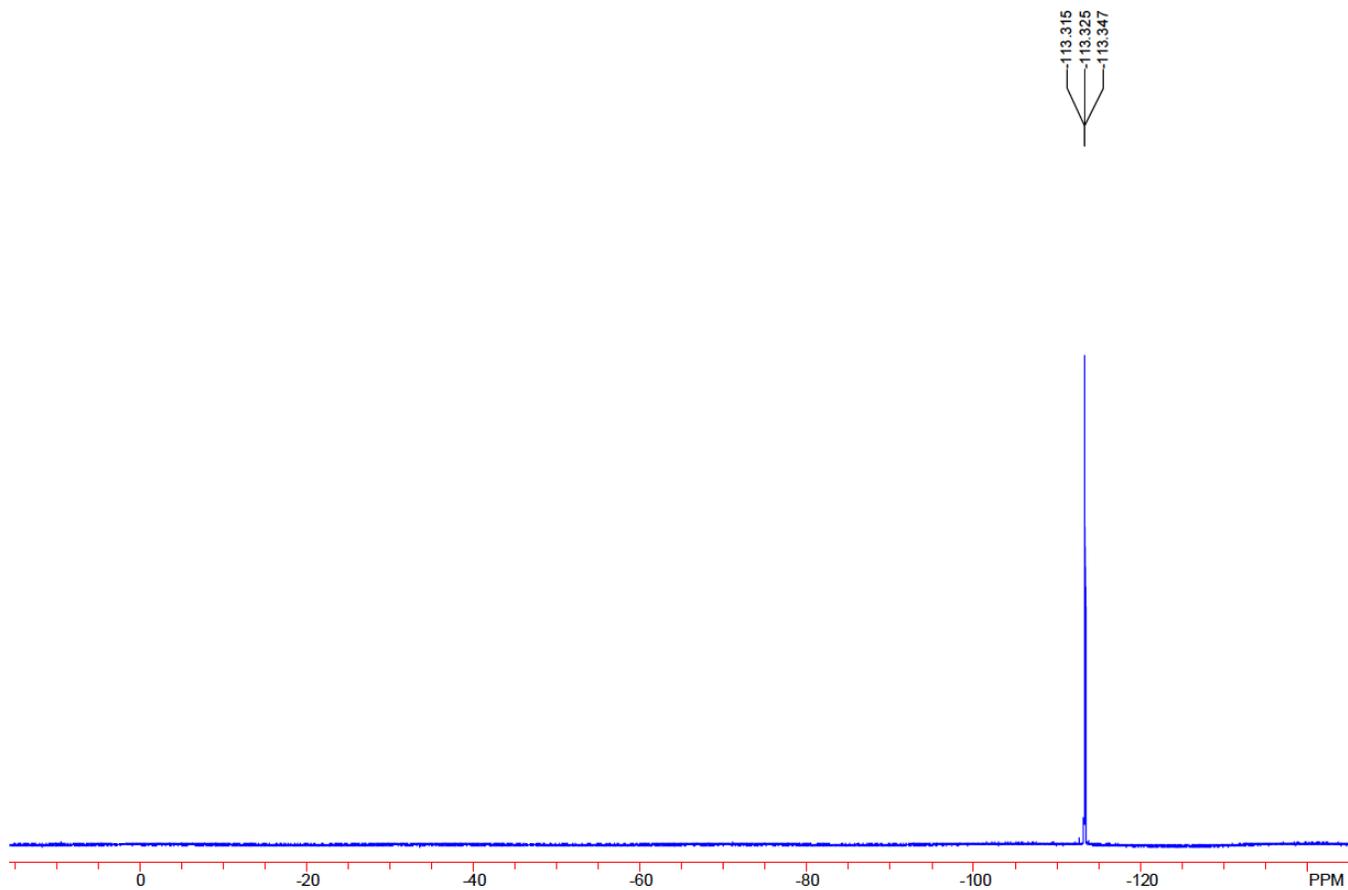
Figure S22: ^{19}F-NMR spectrum of 3-fluorophenethyl-santacruzamate A (**10**) in CDCl$_3$.
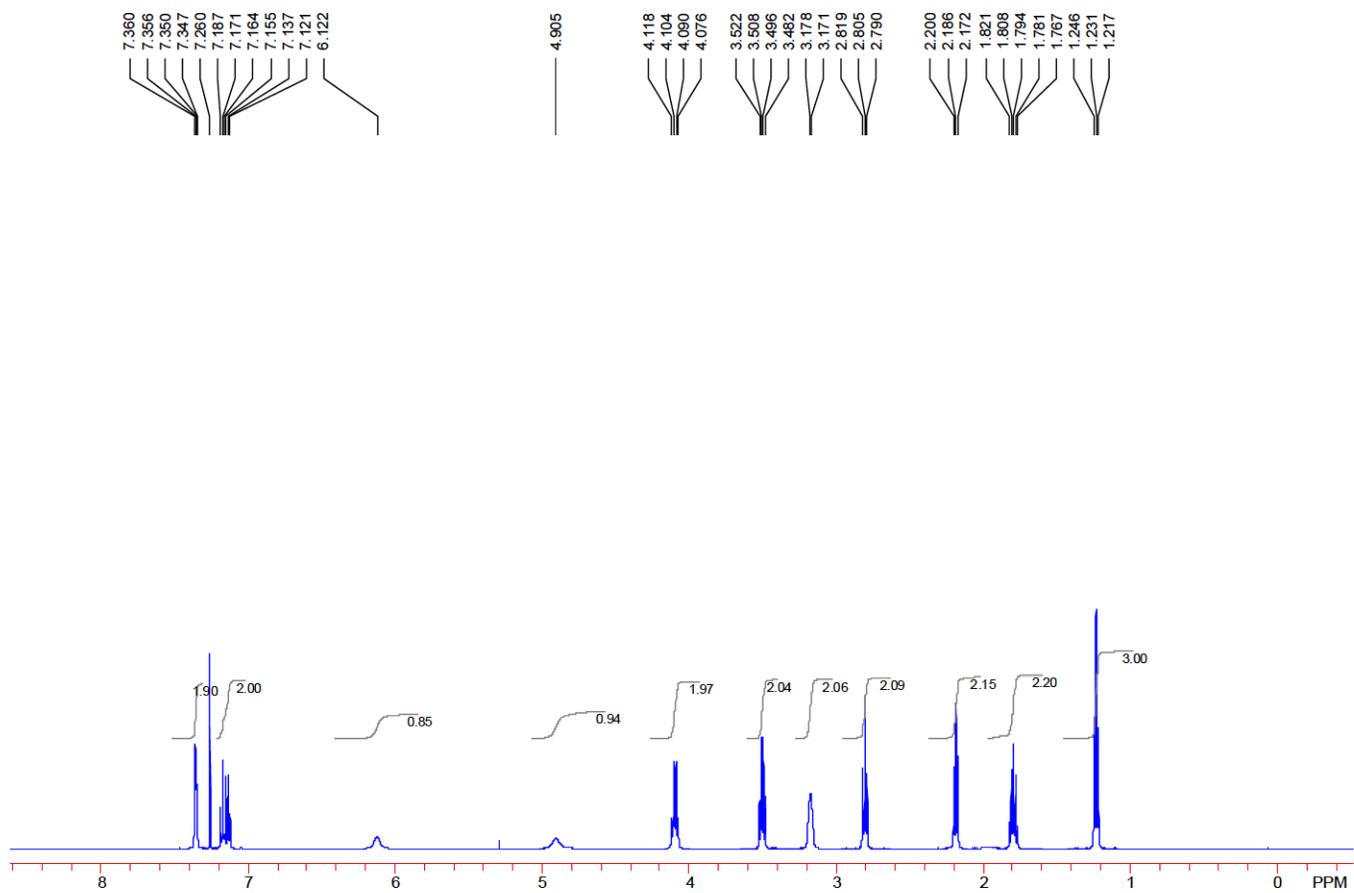
Figure S23: ^{1}H-NMR spectrum of 3-bromophenethyl-santacruzamate A (**14**) in CDCl$_3$.
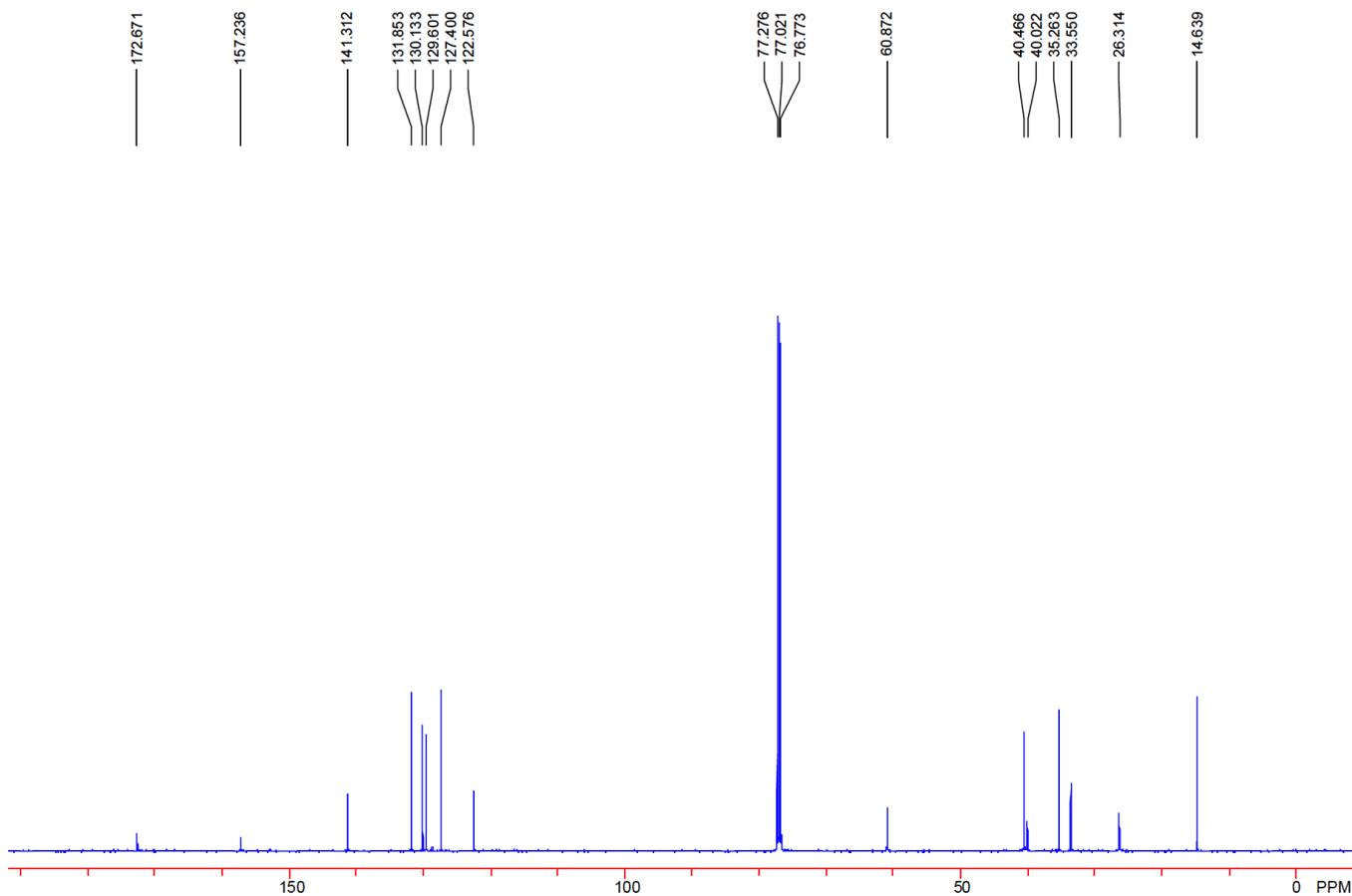
Figure S24: ^{13}C-NMR spectrum of 3-bromophenethyl-santacruzamate A (**14**) in CDCl$_3$.
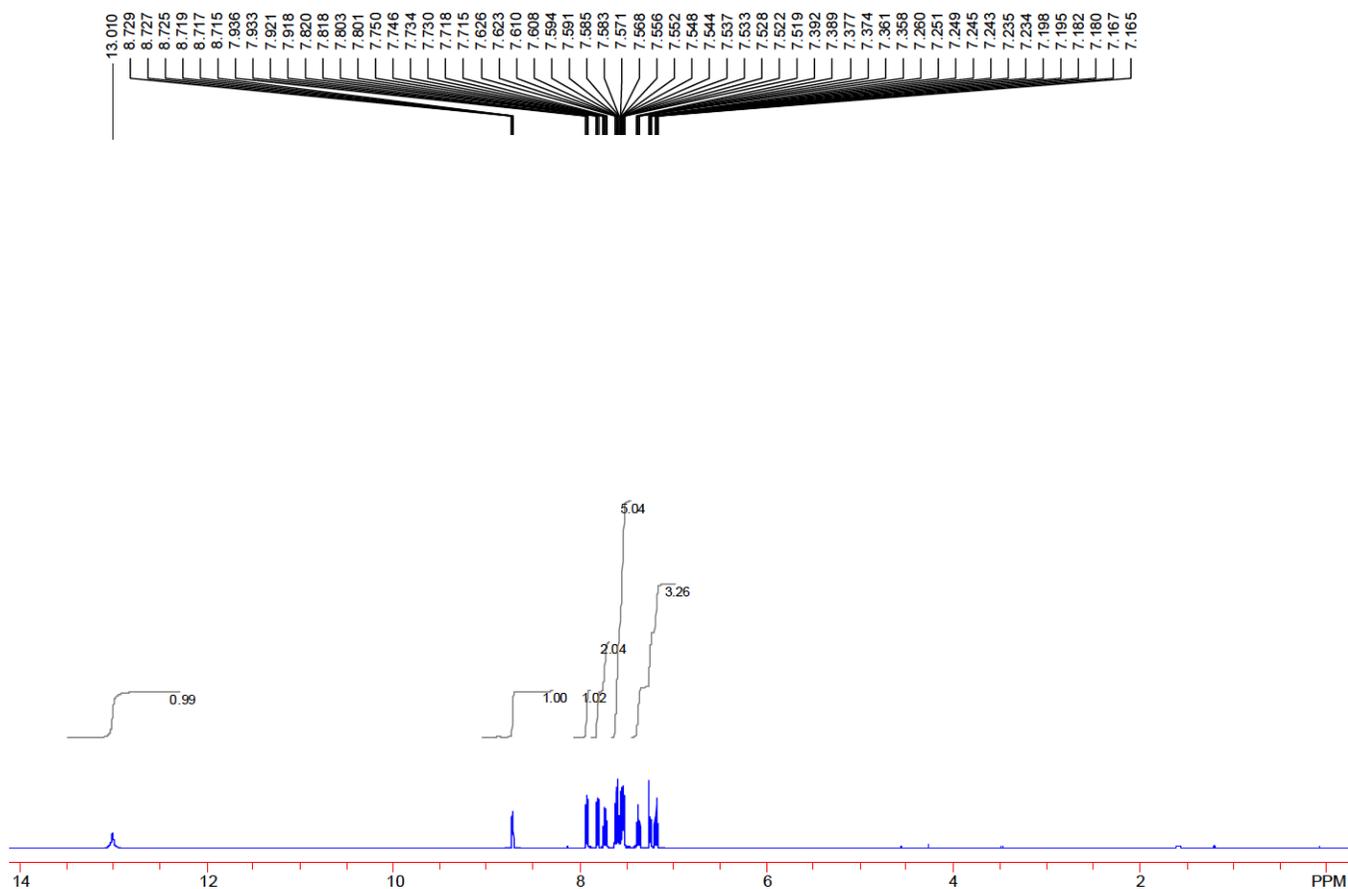
Figure S25: ^{1}H-NMR spectrum of 2-(2-pyridinyl)-2-nitrobenzenesulfonanilide in CDCl$_3$.
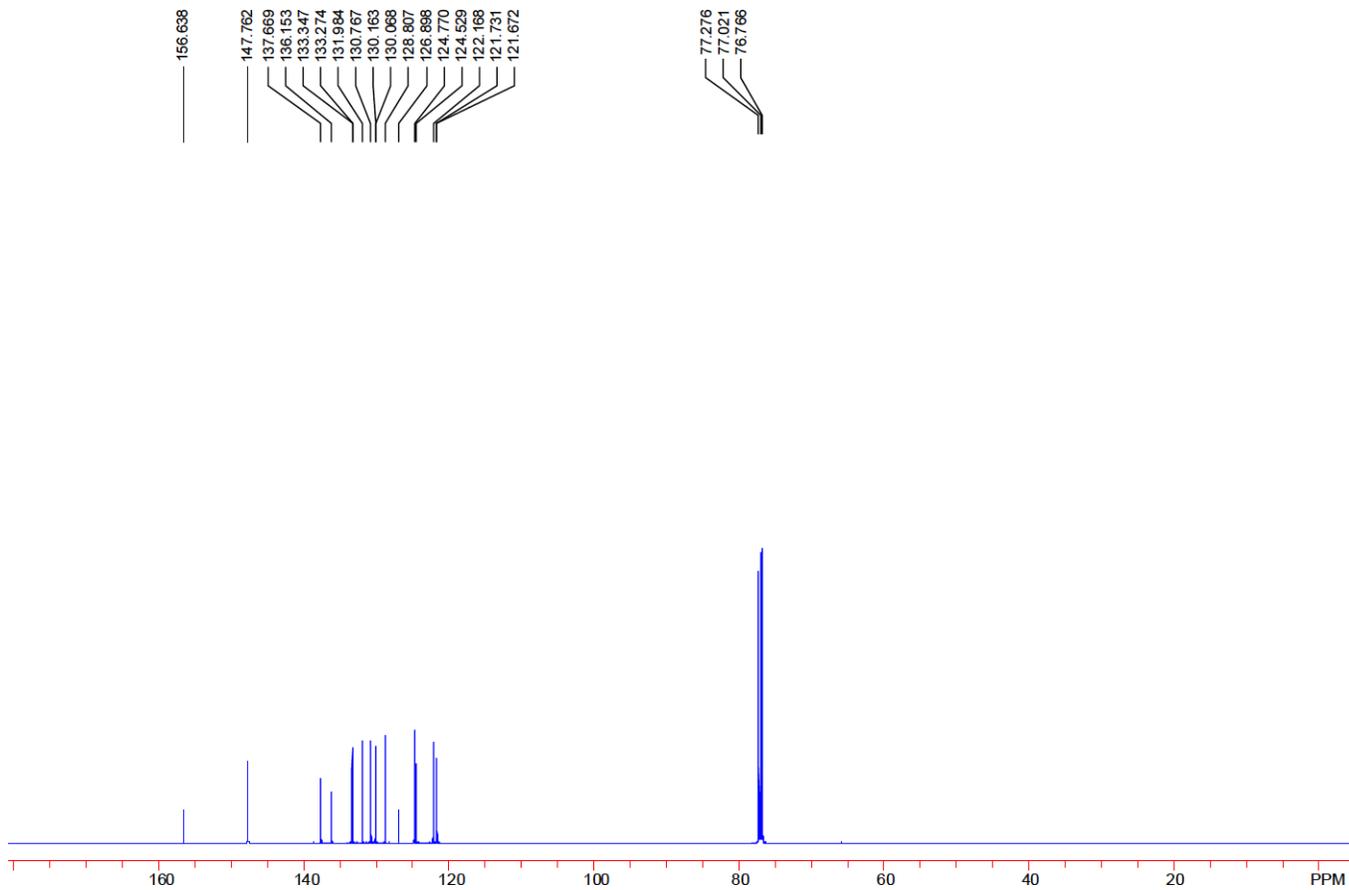
Figure S26: ^{13}C-NMR spectrum of 2-(2-pyridinyl)-2-nitrobenzenesulfonanilide in CDCl$_3$.
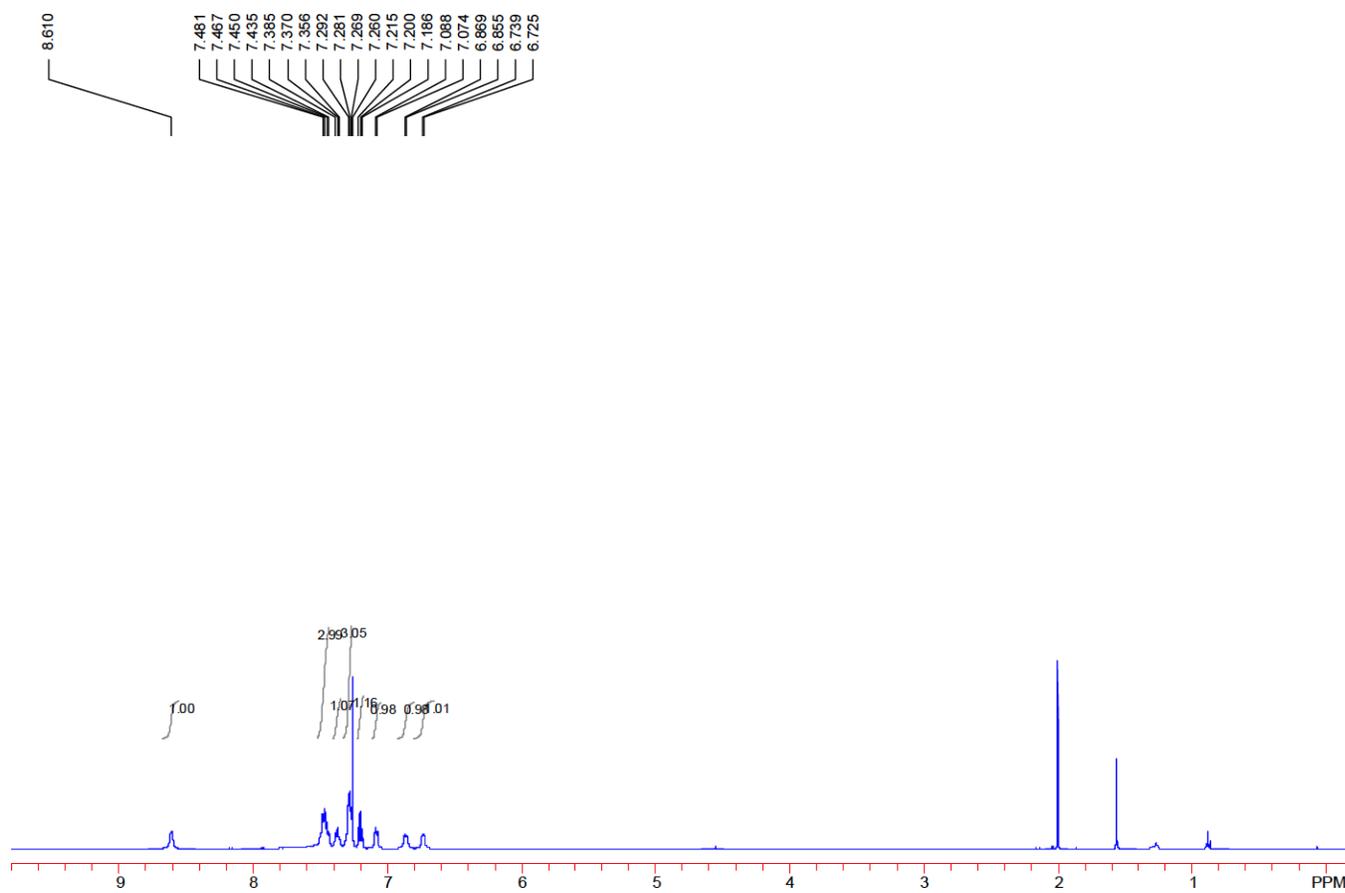
Figure S27: ^{1}H-NMR spectrum of [2-(2-pyridinyl)-2-nitrobenzenesulfonamide]silver(I) (**17**) in CDCl$_3$.
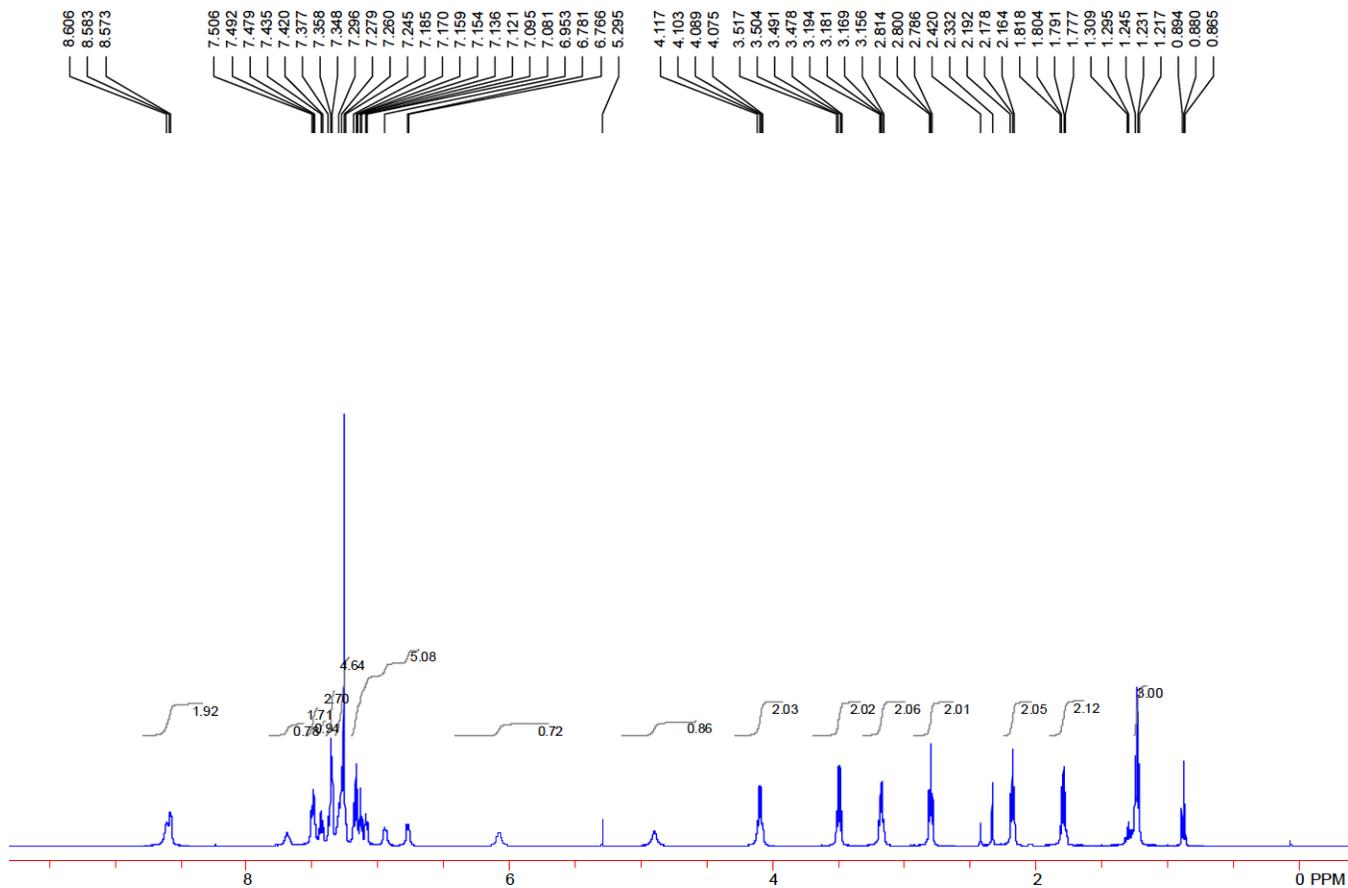
Figure S28: ^{1}H-NMR spectrum of 3-(phenethyl-Santacruzamate A)-nickel aryl complex (**16**) in CDCl$_3$.
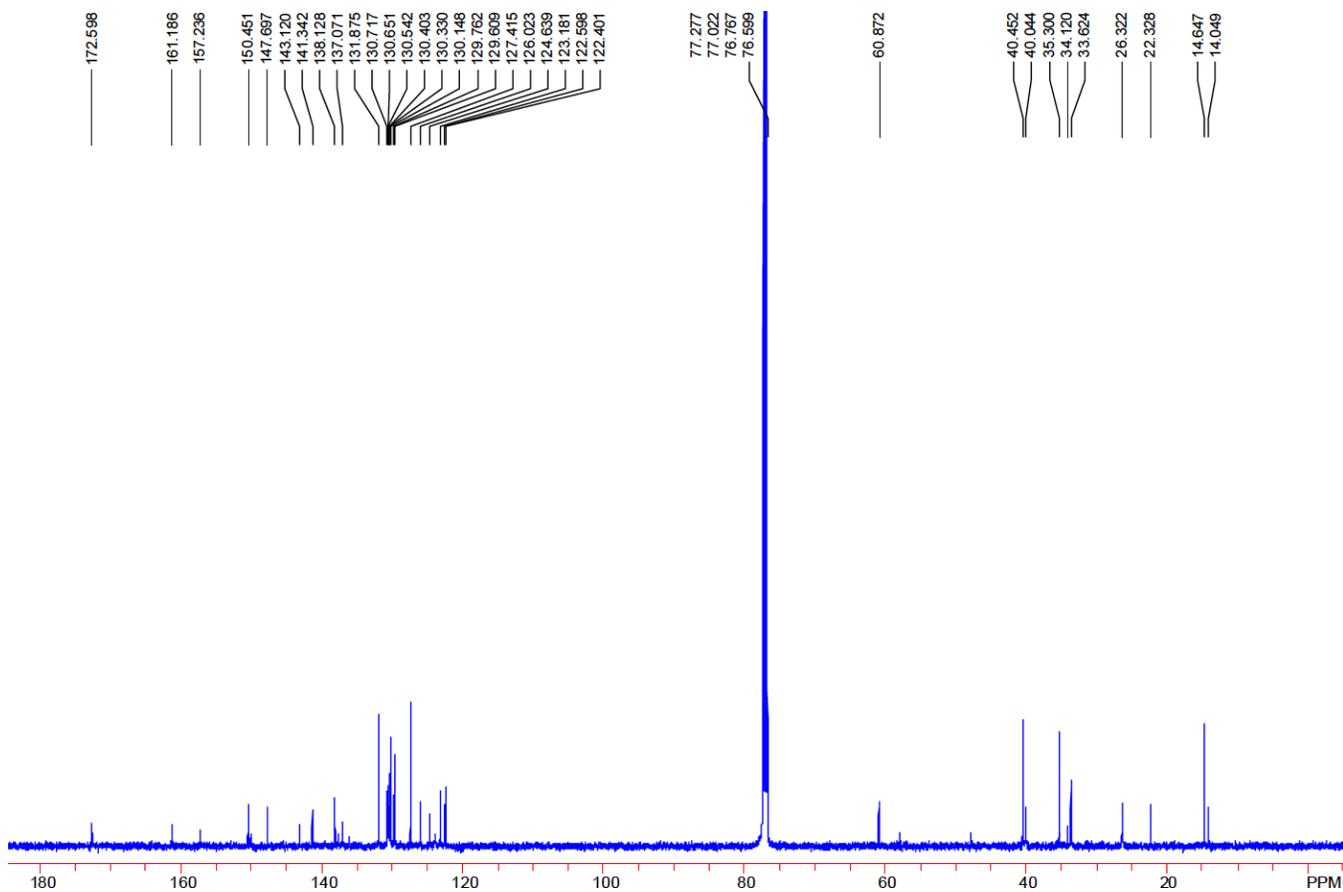
Figure S29: ^{13}C-NMR spectrum of 3-(phenethyl-Santacruzamate A)-nickel aryl complex (**16**) in CDCl$_3$.
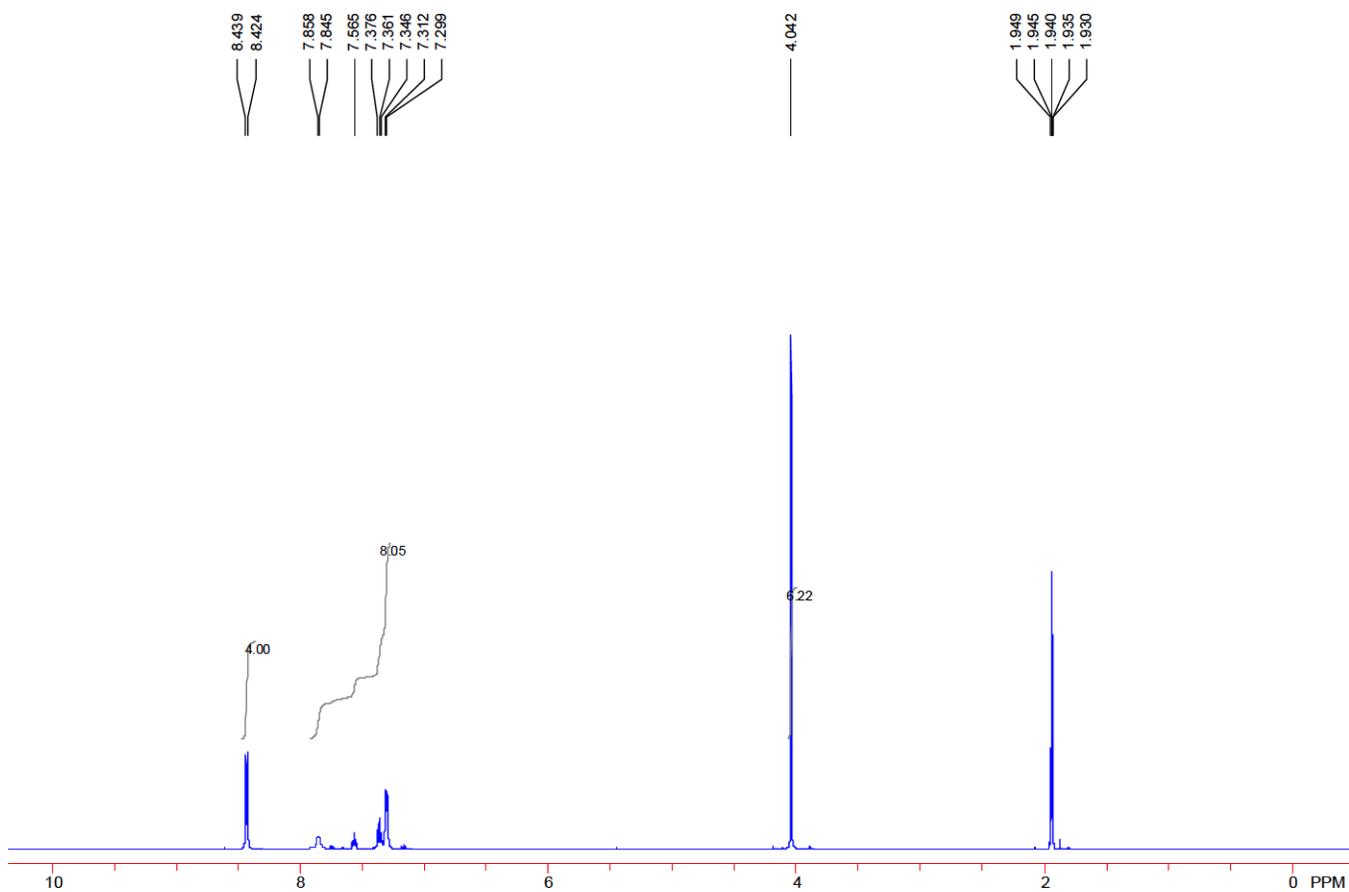
Figure S30: ^{1}H-NMR spectrum of 1,1'-(phenyl-λ^3-iodanediyl)-bis(4-methoxypyridinium)-bis(trifluoromethansulfonate) (**18**) in CD$_3$CN.
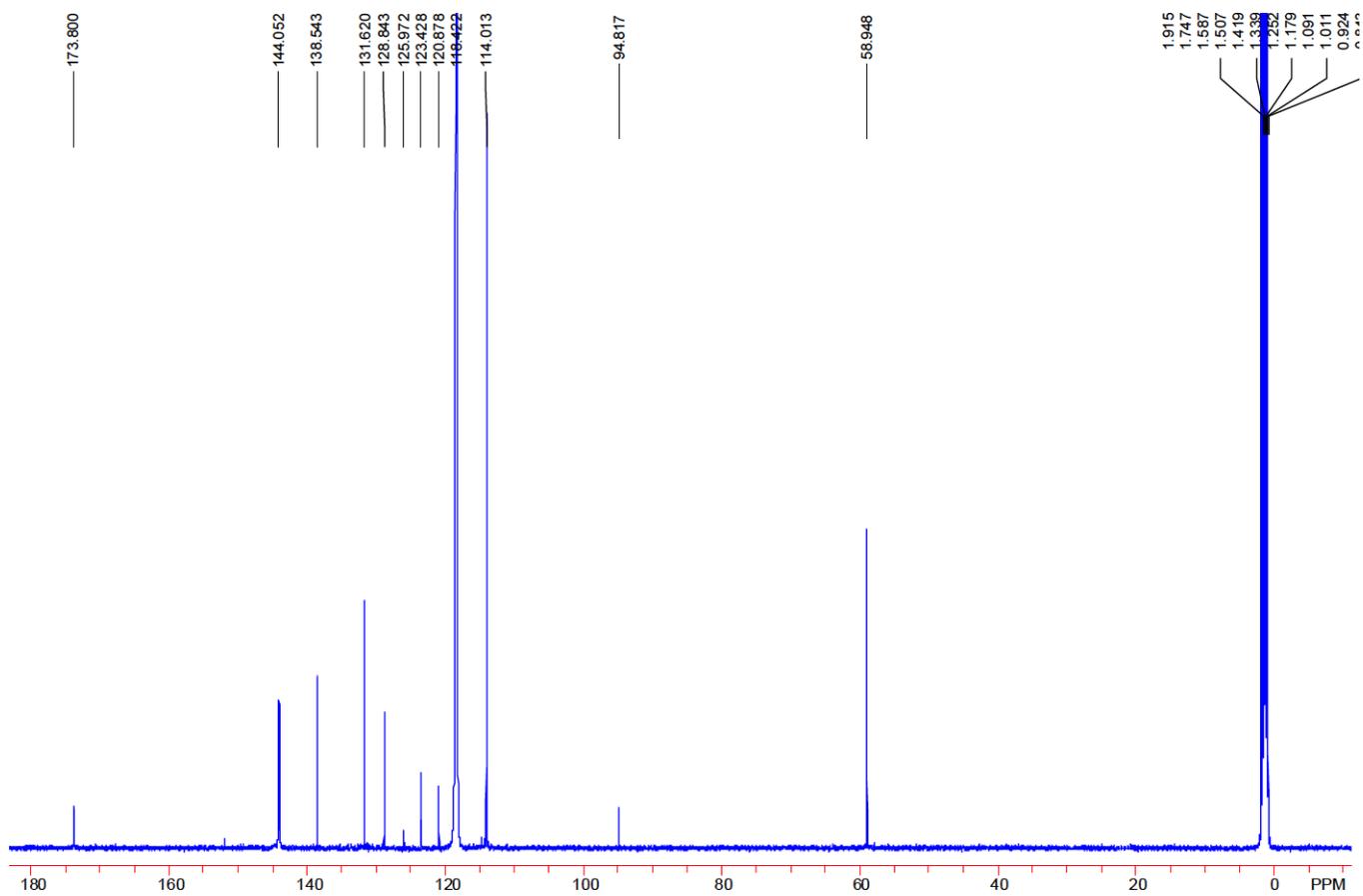
Figure S31: ^{13}C-NMR spectrum of 1,1'-(phenyl-λ^3-iodanediyl)-bis(4-methoxypyridinium)-bis(trifluoromethansulfonate) (**18**) in CD$_3$CN.
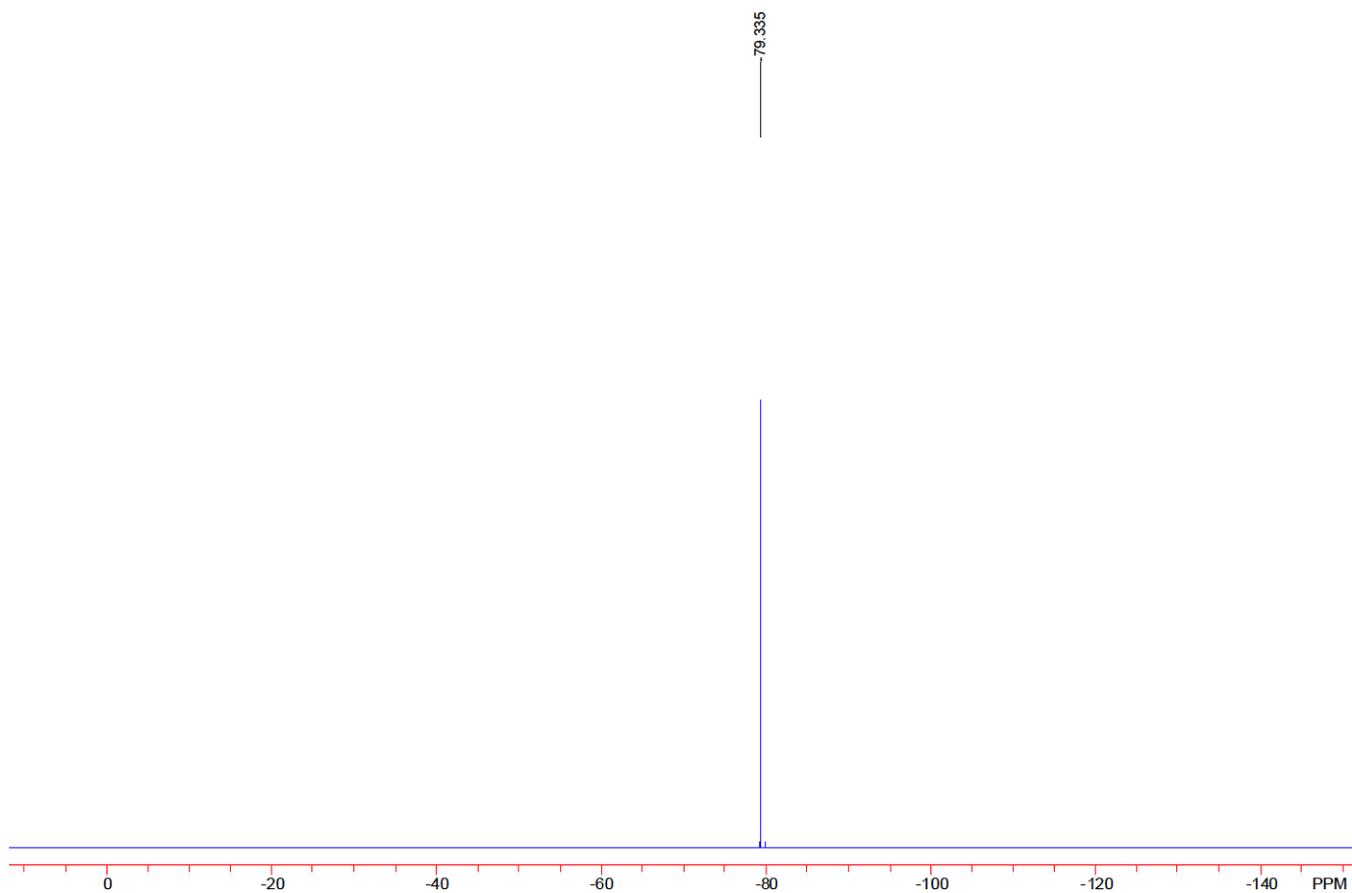
Figure S32: ^{19}F-NMR spectrum of 1,1'-(phenyl-λ^3-iodanediyl)-bis(4-methoxypyridinium)-bis(trifluoromethansulfonate) (**18**) in CD$_3$CN.

CHAPTER III: Supplementary information

EVALUATION OF [11C]KB631 AS A PET TRACER FOR *IN VIVO* VISUALISATION OF HDAC6 IN B16.F10 MELANOMA

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[3] Switch Laboratory, VIB-KU Leuven Center for Brain & Disease Research, KU Leuven, Leuven, Belgium
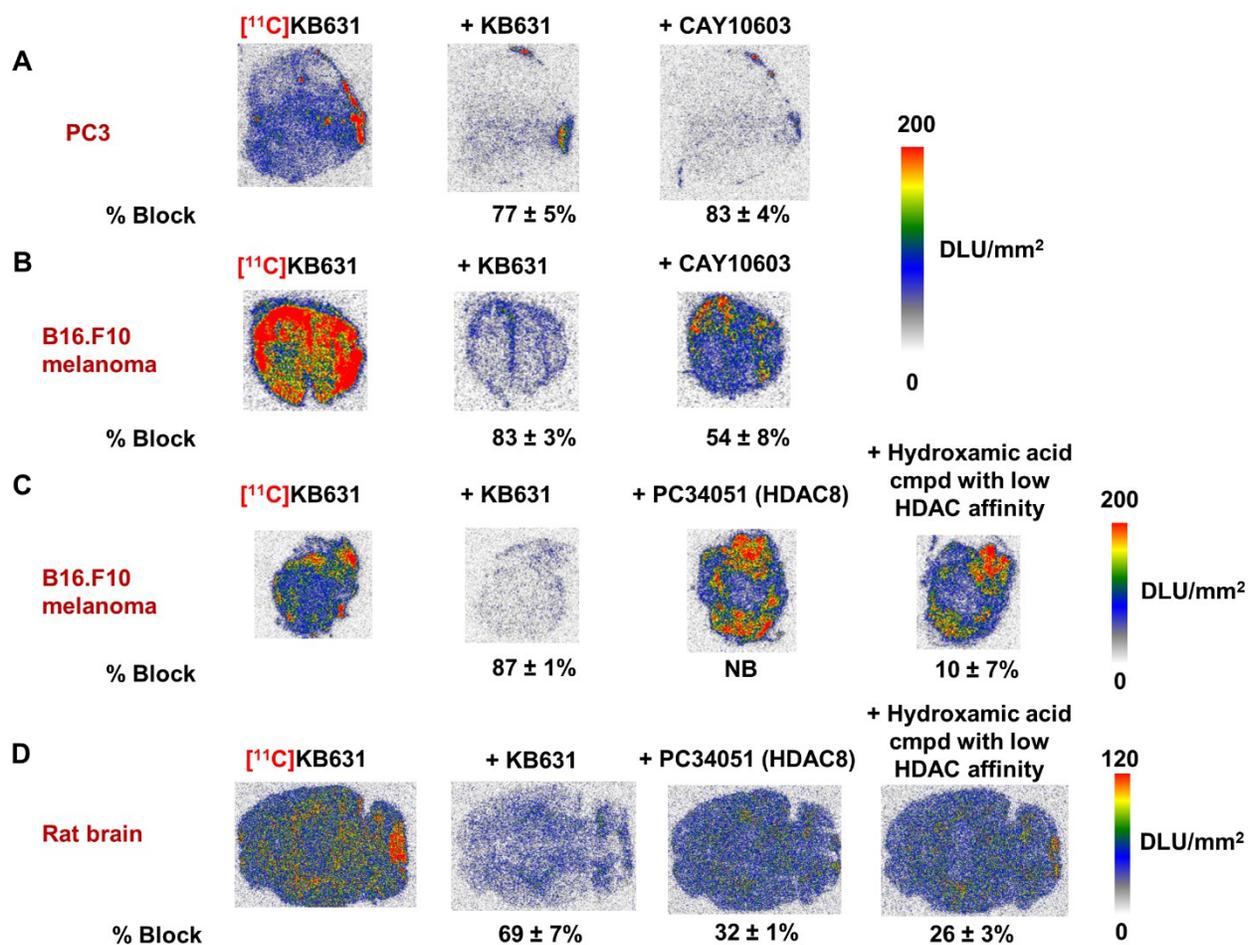
Figure S1: In vitro autoradiography study on different tissue sections. A) PC3 prostate carcinoma **B-C)** B16.F10 melanoma **D)** rat brain. Sections in A-B were incubated with [^{11}C]KB631 (74 kBq/mL) with/without KB631 or CAY10603 (100 µM of blocking agents). Sections in C-D were incubated with [^{11}C]KB631 (74 kBq/mL) with/without KB631, HDAC8 inhibitor PCI34051 or a hydroxamic acid compound with low HDAC affinity (100 µM of blocking agents). Intensity is depicted as DLU/mm². N = 3-4 sections per group. % Block was calculated as (average DLU/mm² in tissue slice in the presence of 100 µM blocker) / (average DLU/mm² in tissue slice, tracer only) and presented as mean ± SD. NB = no block

Table S1: Biodistribution data of [¹¹C]KB631 in male NMRI mice at 2, 10, 30 and 60 min after tracer injection, presented as %ID.

	%ID[a]			
	2 min	**10 min**	**30 min**	**60 min**
Blood	6.2 ± 0.3	3.7 ± 0.3	2.8 ± 0.45	1.9 ± 0.6
Bone	4.5 ± 0.4	5.1 ± 0.5	3.1 ± 0.3	1.8 ± 0.1
Brain	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Carcass	31.5 ± 2.5	38.2 ± 1.7	26.6 ± 1.4	17.0 ± 1.6
Heart	1.2 ± 0.0	0.6 ± 0.1	0.4 ± 0.1	0.1 ± 0.0
Intestines	8.9 ± 1.5	15.4 ± 0.7	31.1 ± 1.9	48.6 ± 2.5
Kidneys	17.4 ± 0.6	7.2 ± 1.2	5.6 ± 1.0	2.0 ± 0.3
Liver	30.1 ± 4.2	25.8 ± 1.2	20.2 ± 0.3	13.1 ± 2.9
Lungs	2.5 ± 0.1	1.7 ± 0.1	1.0 ± 0.1	0.7 ± 0.1
Muscle	27.5 ± 4.1	25.3 ± 5.3	15.2 ± 1.5	6.8 ± 1.3
Pancreas	1.2 ± 0.2	0.8 ± 0.1	0.7 ± 0.0	0.4 ± 0.1
Spleen	0.8 ± 0.2	0.5 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
Stomach	1.2 ± 0.0	1.2 ± 0.4	2.2 ± 1.2	1.2 ± 0.2
Urine	0.5 ± 0.1	5.0 ± 3.1	9.3 ± 3.3	15.1 ± 3.3

%ID = Percentage of injected dose. Data expressed as mean ± SD; n = 3 per time point.

Table S2: Biodistribution data of [^{11}C]KB631 in male NMRI mice 10 min after tracer injection. Mice were pretreated with vehicle, KB631 (10 mg/kg), SAHA (10-100 mg/kg) or Ricolinostat (10-50 mg/kg), presented as %ID.

	%ID					
	Control	**KB631**	**SAHA 10 mg/kg**	**SAHA 100 mg/kg**	**Ricolinostat 10 mg/kg**	**Ricolinostat 50 mg/kg**
Blood	1.7 ± 0.5	6.4 ± 0.4	3.9 ± 0.6	8.6 ± 2.1	4.3 ± 0.2	11.2 ± 2.1
Bone	4.6 ± 0.5	5.0 ± 0.5	4.8 ± 0.7	6.3 ± 2.0	5.1 ± 0.5	7.0 ± 1.0
Brain	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Carcass	31.1 ± 1.3	41.5 ± 1.4	36.0 ± 3.4	51.1 ± 17.2	33.8 ± 0.8	42.7 ± 2.2
Heart	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.8 ± 0.1
Intestines	16.5 ± 2.1	13.2 ± 0.8	14.1 ± 2.1	8.6 ± 3.2	14.6 ± 2.2	9.5 ± 0.3
Kidneys	6.9 ± 0.7	14.9 ± 1.6	17.2 ± 4.8	13.8 ± 5.7	6.7 ± 1.0	14.7 ± 5.1
Liver	25.0 ± 4.5	19.6 ± 3.1	21.0 ± 2.3	16.8 ± 6.0	26.7 ± 5.8	18.9 ± 2.1
Lungs	1.6 ± 0.0	1.6 ± 0.4	1.5 ± 0.2	1.4 ± 0.8	2.3 ± 0.6	1.7 ± 0.5
Muscle	21.7 ± 2.2	27.7 ± 4.0	29.1 ± 1.2	29.1 ± 10.5	22.6 ± 3.1	31.1 ± 3.6
Pancreas	1.1 ± 0.2	1.0 ± 0.2	1.1 ± 0.1	1.0 ± 0.4	0.9 ± 0.2	1.1 ± 0.1
Spleen	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1
Stomach	1.0 ± 0.0	1.1 ± 0.2	1.0 ± 0.1	0.9 ± 0.3	1.6 ± 0.4	1.2 ± 0.2
Urine	12.6 ± 1.6	2.3 ± 1.0	3.4 ± 4.3	0.2 ± 0.2	8.3 ± 1.4	3.6 ± 5.6

%ID = Percentage of injected dose. Data expressed as mean ± SD; n = 3 per pre-treatment.

Table S3: Biodistribution data of [^{11}C]KB631 in B16.F10 melanoma inoculated C57BL/6 mice 10 min after tracer injection. Mice were pretreated with vehicle or Ricolinostat (50 mg/kg), presented as %ID.

	%ID	
	Control	**Ricolinostat**
Blood	6.4 ± 0.8	13.9 ± 0.9
Bone	4.8 ± 0.1	7.3 ± 2.0
Brain	0.2 ± 0.0	0.2 ± 0.0
Carcass	36.3 ± 0.6	41.6 ± 5.3
Heart	0.8 ± 0.1	0.7 ± 0.1
Intestines	15.1 ± 0.3	12.3 ± 1.6
Kidneys	12.0 ± 0.7	8.4 ± 1.9
Liver	23.9 ± 1.9	19.2 ± 3.1
Lungs	1.8 ± 0.2	2.0 ± 0.3
Muscle	20.4 ± 1.2	27.3 ± 0.3
Pancreas	1.1 ± 0.1	1.2 ± 0.2
Spleen	0.5 ± 0.0	0.6 ± 0.0
Stomach	0.9 ± 0.2	1.0 ± 0.1
Tumour	1.7 ± 0.3	3.9 ± 1.1
Urine	1.9 ± 2.4	3.4 ± 5.6

%ID = Percentage of injected dose. Data expressed as mean ± SD; n = 3 per pre-treatment.

CHAPTER IV: Supplementary information

EVALUATION OF [11C]NMS-E973 AS A PET TRACER FOR *IN VIVO* VISUALISATION OF HSP90

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Chemical synthesis of precursor compound, 7
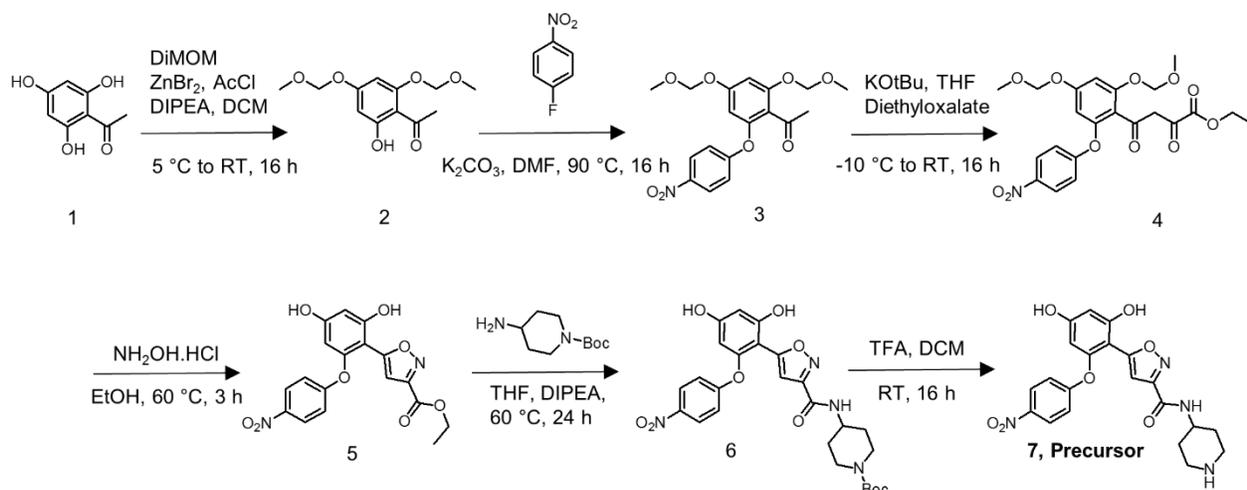
Figure S1: Synthesis of precursor compound **7**.

1-[2-hydroxy-4,6-*bis*(methoxymethoxy)phenyl]ethanone (2). To a stirred solution of dimethoxymethane (2.64 ml, 29.8 mmol) and zinc bromide (29.28 mg, 0.13 mmol) in DCM (23,2 mL), acetyl chloride (2.12 mL, 29.8 mmol) was added dropwise during 30 min maintaining the temperature below 30 °C. After stirring for 3 h at room temperature, the solution was diluted with DCM (48 mL), then cooled to 5 °C before the portion wise addition of 1-(2,4,6-trihydroxyphenyl)ethanone (**1**), 2.00 g, 11.89 mmol) followed by the dropwise addition of *N,N*-diisopropylethylamine (8.32 mL, 47.6 mmol). After 1 h the ice bath was removed and the temperature was allowed to rise to RT. The resulting solution was stirred for 16 h, and washed with NH_4Cl saturated solution, followed by washing with 10% citric acid solution. After drying over $MgSO_4$, the solvent was removed and purification was performed with column chromatography to yield (**2**) (Yield: 0.75 g, 37%) *Colorless oil*, ^1H NMR (CDCl$_3$-*d*): δ 2.66 (s, 3H, CH$_3$), 3.48 (s, 3H, CH$_3$), 3.52 (s, 3H, CH$_3$), 5.17 (s, 2H, CH$_2$), 5.26 (s, 2H, CH$_2$), 6.23-6.28 (m, 2H, Ar). ^{13}C NMR (CDCl$_3$): δ 33.2, 56.7, 56.9, 94.2 (2C), 94.7, 97.4, 107.2, 160.6, 163.7, 167.0, 200.4. HRMS (ESI) calculated for $C_{12}H_{17}O_6$ [M+H]$^+$: 257.1020. Found: 257.1023

1-[2,4-*Bis*(methoxymethoxy)-6-(4-nitrophenoxy-phenyl]ethanone (3). To a stirred solution of (**2**) (745 mg, 2.91 mmol) in DMF (6 mL) 4-nitro-1-fluorobenzene (0.34 mL, 3.20 mmol) was added, followed by K_2CO_3 (803 mg, 5.82 mmol). After stirring for 15 min at room temperature, the resulting suspension was heated for 16 h at 90 °C. After cooling, the dark solution was diluted with EtOAc (20 mL) and thoroughly washed with 10% citric acid solution and brine and dried over $MgSO_4$. The solvent was removed and the residue was purified by column chromatography to yield (**3**). (Yield: 0.43 g, 58%). *Yellow powder*, ^1H NMR (CDCl$_3$): δ 2.43 (s, 3H, CH$_3$), 3.44 (s, 3H, CH$_3$), 3.49 (s, 3H, CH$_3$),

5.11 (s, 2H, CH$_2$), 5.20 (s, 2H, CH$_2$), 6.35 (s, 1H, Ar), 6.73 (s, 1H, Ar), 7.00 (d, 2H, J = 9.2 Hz, Ar), 8.18 (d, 2H J = 9.2 Hz, Ar). ^{13}C NMR (CDCl$_3$-d): δ 33.0, 57.0, 57.2, 95.1, 95.6, 101.3, 102.9, 117.8, 120.0, 126.6, 143.6, 153.3, 157.1, 160.6, 163.6, 200.0. HRMS (ESI) calculated for C$_{18}$H$_{19}$NO$_8$ [M+H]$^+$: 378.1184. Found: 378.1179.

Ethyl 5-[2,4-*bis*(methoxymethoxy)-6-(4-nitrophenoxy)phenyl]-2,4-dioxobutanoate (4). To a stirred solution of sodium *tert*-butoxide (0.284 g, 2.51 mmol) in THF (8 mL) at -10 °C, was added diethyl oxalate (0.5 mL, 3.42 mmol) in 2 mL of precooled THF. After 30 min a solution of (**3**) (430 mg, 1.14 mmol) in THF (4 mL) was added drop wise. The reaction mixture was stirred for 1 h at -10 °C and then further 16 h at room temperature. The solution was poured into a 10% citric acid solution (30 mL) and thoroughly extracted with EtOAc. After washing with brine and drying over MgSO$_4$, the combined organic fractions were evaporated to provide a yellow crude residue. Purification using column chromatography yielded (**4**). (Yield: 98%, proceeded to the next step without further characterization)

Ethyl 5-[2,4-*bis*(methoxymethoxy)-6-(4-nitrophenoxy)phenyl]-isoxazole-3-carboxylate (**5**). To a stirred solution of (**4**) (0. 42 g, 0.88 mmol) in EtOH (12 mL), hydroxylamine hydrochloride (2.67 mg, 4.02 mmol) was added. After stirring for 3 h at 60 °C, the solvent was evaporated, saturated NaHCO$_3$ (30 mL) was added and the suspension was taken up in DCM (30 mL) and thoroughly washed with water and dried over MgSO$_4$. The solvent was removed by evaporation and purification using column chromatography was performed to afford (**5**). (Yield: 0.15 g, 35%) *Yellow oil*, ^{1}H NMR (MeOD-d_4): δ 1.30-1.37 (m, 3H, CH$_3$), 4.32-4.36 (m, 2H, CH$_2$), 6.11 (s, 1H, Ar), 6.38 (s, 1H, Ar), 6.87 (s, 1H, Ar), 7.04 (d, 2H J = 8.8 Hz, Ar), 8.16 (d, 2H J = 8.8 Hz, Ar). ^{13}C NMR (MeOD-d_4): δ 13.6, 62.3, 100.6, 101.2, 104.7, 117.2, 120.1, 126.2, 143.4, 154.8, 156.4, 159.2, 160.7, 162.2, 163.7, 167.4. HRMS (ESI) calculated for C$_{18}$H$_{15}$N$_2$O$_8$ [M+H]$^+$: 387.0822. Found: 387.0841.

Tert-butyl-4-[({5-[2,4-*bis*(methoxymethoxy)-6-(4-nitrophenoxy)phenyl]-isoxazole-3-yl}carbonyl)amino]piperidin-1-carboxylate (6). (**5**) (149 mg, 0.3859 mmol) and *tert*-butyl 4-aminopiperidine-1-carboxylate (386 mg, 1.93 mmol) were dissolved in THF (12 mL). *N,N*-diisopropylethylamine (1.34 mL, 0.77 mmol) was added to the mixture. The reaction mixture was stirred at 60 °C for 24 h. After cooling to room temperature, the solvent was removed. The residue was purified by column chromatography to afford (**6**). *Brownish oil*, ^{1}H NMR (MeOD-d_4): δ 1.13-1.20 (m, 4H, CH$_2$), 1.40 (s, 9H, 3CH$_3$), 3.93-4.01 (m, 5H, CH$_2$ & CH), 6.05 (s, 1H, Ar), 6.31 (s, 1H, Ar), 6.79 (s, 1H, Ar), 6.95 (d, 2H J = 8.6 Hz, Ar), 8.09 (d, 2H J = 8.6 Hz, Ar). ^{13}C NMR (MeOD-d_4): δ 13.8, 20.2, 28.0, 31.6, 80.5, 100.7, 100.9, 101.2, 103.7, 117.1, 126.2, 143.3, 154.7, 155.7, 158.7, 159.1, 160.3, 162.0, 163.8, 166.8, 172.3. HRMS (ESI) calculated for C$_{26}$H$_{29}$N$_4$O$_9$ [M+H]$^+$: 541.1929. Found: 541.1914.

5-[2,4-Dihydroxy-6-(4-nitrophenoxy) phenyl]-*N*-(piperidin-4-yl)-isoxazole-3-carboxamide (**7**). To a stirred solution of (**6**) (50 mg) in DCM (5 mL) was added TFA (3 eq), the reaction mixture was stirred for 16 h and monitored by LC-MS. After completion, the reaction mixture was diluted with DCM (10 mL) and washed with 0.1 M NaOH and saturated NaHCO$_3$. Purification over a short pad of silica gel yielded (**7**). *Pale yellow oil*, ^{1}H NMR (DMSO-*d$_6$*): δ 1.32-1.38 (m, 4H), 2.57-3.59 (m, 2H), 2.91-2.93 (m, 2H), 3.98-4.01 (m, 1H), 6.07 (s, 1H), 6.33 (s, 1H), 6.80 (s, 1H), 6.96-6.98 (m, 2H), 8.12-8.14 (m, 2H). ^{13}C NMR (DMSO-*d$_6$*): δ 27.8, 42.3, 44.0, 99.5, 100.4, 100.5, 103.3, 116.8, 126.1, 142.1, 153.4, 158.1, 158.3, 158.3, 161.2, 162.8, 165.4. HRMS (ESI) calculated for C$_{21}$H$_{21}$N$_4$O$_7$ [M + H$^+$]: 441.1404. Found: 441.1410.

Immunofluorescent staining of B16.F10 melanoma cells and high-content screening

Cells were plated in a 96-well plate (Greiner) at a density of 8000-10000 cells/well. At day 1, cells were treated with vehicle (DMSO; 0.001% final concentration), or with a compound (NMS-E973 or Ganetespib) at indicated final concentration (250 nM or 500 nM) for 16 hours. At day 2 cells were washed with PBS and fixed with 4% paraformaldehyde (Thermo Scientific, 16% PFA diluted in PBS) for 30 minutes and were either permeabilised or not by adding 0.2% Triton X100 to blocking buffer (1% Bovine Serum Albumine (BSA) in PBS) for 60 minutes. Primary antibodies were added to the cells in blocking buffer at a dilution of 1:500 (Anti-HSP90, AC88, Abcam) and incubated overnight at 4 °C while shaking gently. Cells were washed 3 times for 5 minutes in PBS and subsequently incubated with DAPI (0.1 µg/ml and/or secondary antibody (1:1000 goat-anti mouse alexa-594; PROMEGA) in blocking buffer for 60 minutes. Imaging was performed on the IN Cell Analyser 2000 (GE Healthcare). The IN Cell Developer package (v1.9.2) allows visualization, imaging, and quantification of staining intensity and quantification of inclusions in cells following immunofluorescent staining.
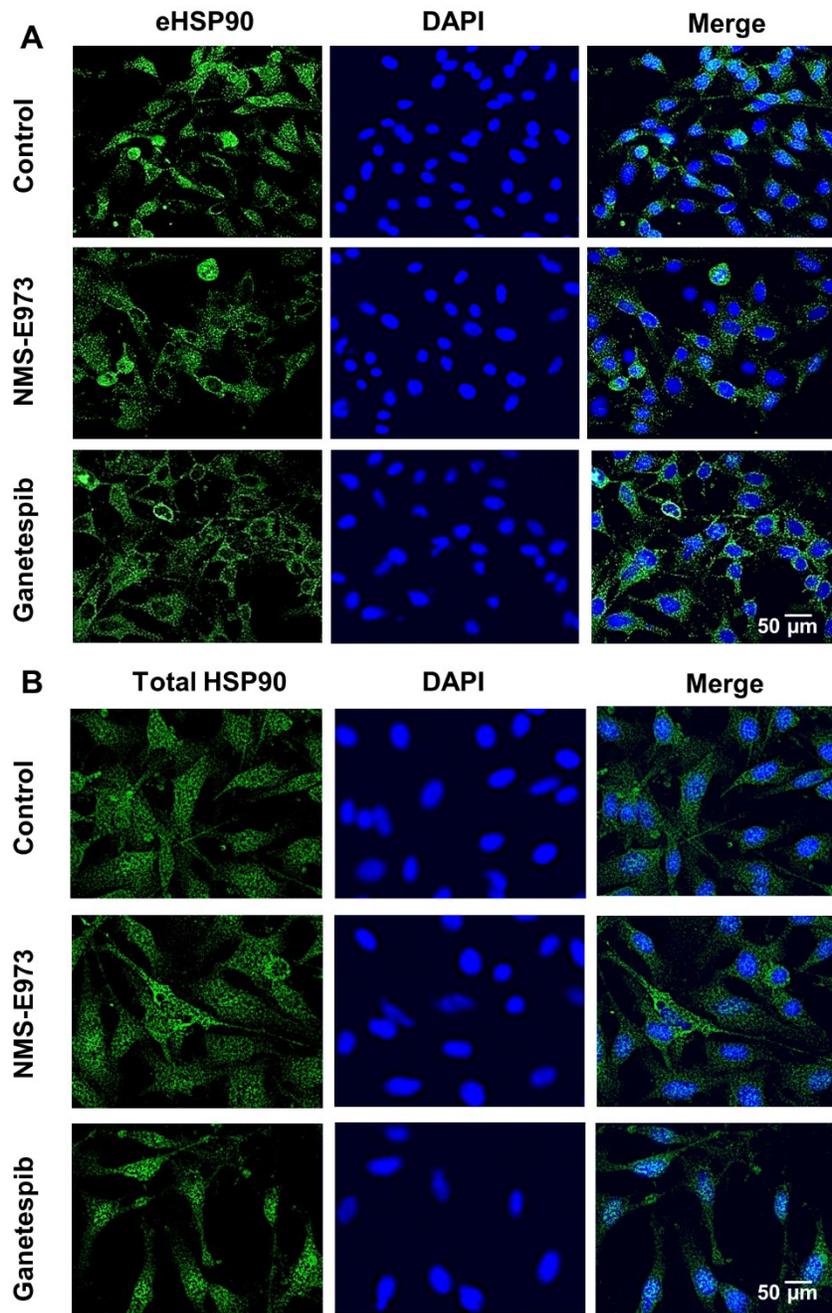
Figure S2: **Immunofluorescent staining of eHSP90 and HSP90 of the B16.F10 melanoma cells. (A)** Extracellular staining of HSP90 shows membrane staining that excludes the nucleus. FITC-Staining of HSP90 (left, Green), nuclear staining by DAPI (Middle, Blue) and the overlay (Right). Treatment with either Ganetespib or NMS-E973, both at 500 nM for 16 hours, shows no visual difference in phenotype (scale bar 50 μm). **(B)** Total staining of HSP90 after permeabilisation, shows homogeneous cellular staining. FITC-Staining of HSP90 (Left, Green), nuclear staining by DAPI (Middle, Blue) and the overlay (Right). Treatments with either Ganetespib or NMS-E973, both at 500 nM for 16 hours, shows no visual difference in phenotype (scale bar 50 μm).

SDS/Western blot analysis on B16.F10 melanoma cells

Analysis of protein expression was carried out on all cell lines plated at a density of 250.000 cells in a 6-well plate at day 0. At day 1, cells were treated with vehicle (DMSO; 0.001% final concentration), or with 250 nM or 500 nM of NMS-E973 or Ganetespib for 16 hours. At day 2, cells were washed with PBS and lysed in 200 µl NP40 lysis buffer (150 mM NaCl, 50 mM Tris-HCl pH 8, 1% IGEPAL(NP40), containing a 1X PBS dissolved complete protease inhibitor cocktail (Roche) and 1U/µl Universal Nuclease (Pierce) for 30 minutes on ice. Lysates were subjected to regular SDS/Western blot analysis. Antibodies for detection include anti-Cyclin-dependent kinase 1 (CDK1) (Santa Cruz Biotechnology) and anti-GAPDH (6C5; Santa Cruz Biotechnology). Secondary HRP-linked antibodies were used (PROMEGA). Quantification is done by densitometry using the ImageJ software package. Normalization is corrected to GAPDH levels of the input.
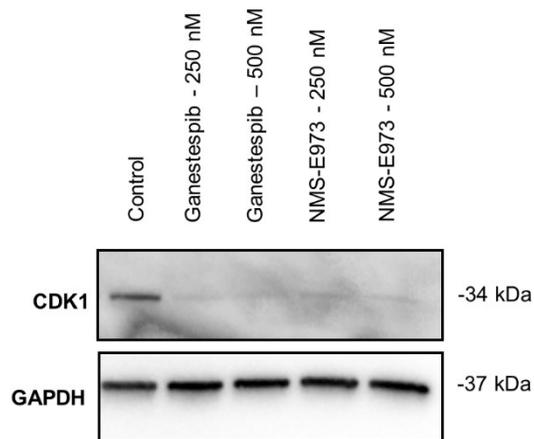
Figure S3: **Effect of NMS-E973 and Ganetespib treatment on client protein CDK1 expression.** Representative Western blot of decreased CDK1 expression after treatment shows that HSP90 is effectively inhibited by different concentrations of Ganetespib and NMS-E973.

QC chromatogram of [^{11}C]NMS-E973
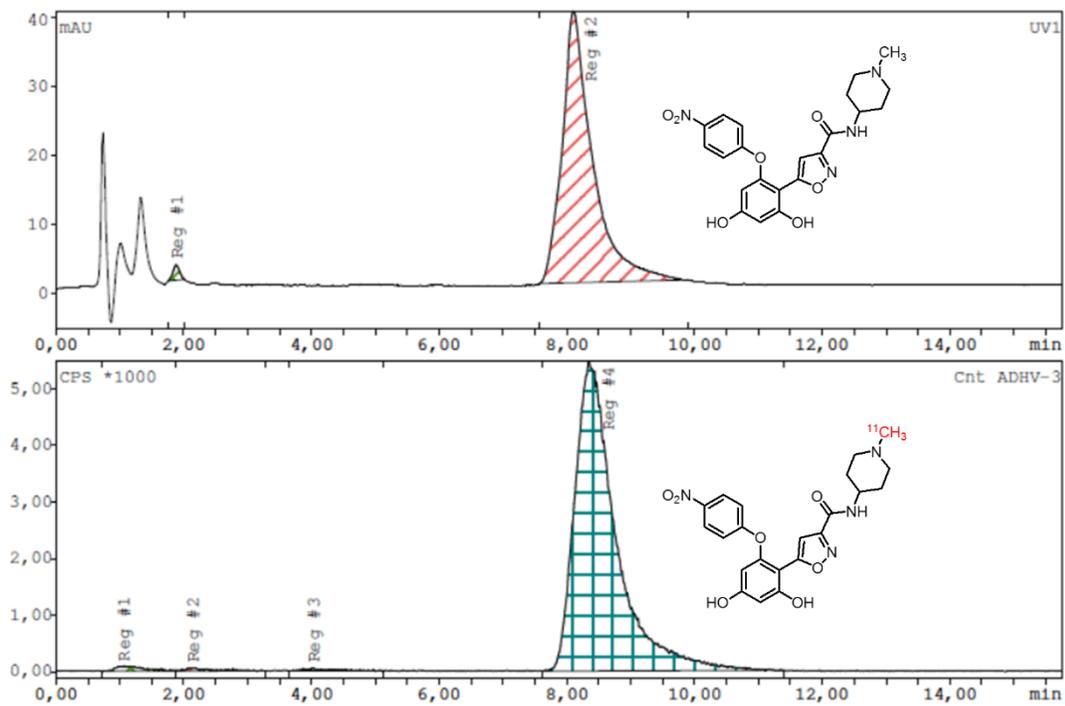
Figure S4: QC chromatogram of [^{11}C]NMS-E973 spiked with authentic reference compound NMS-E973 on an X-bridge RP-C$_{18}$ column (100 x 3 mm 3.5 μm) (upper channel UV 254 nm, lower channel radioactivity)

Confirmation of N-methylation
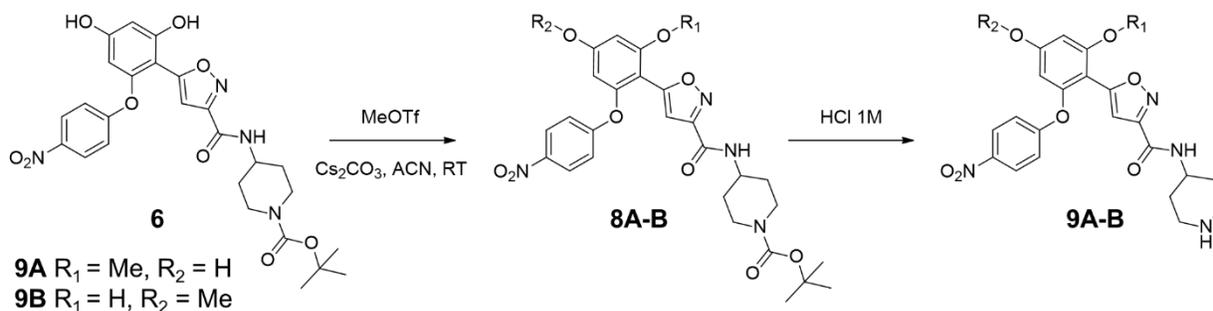
Figure S5: Synthesis of O-alkylated compound starting from (**6**) which is treated with Methyl triflate (MeOTf) under alkaline conditions to form **8A-B**, which is treated with 1 M HCl to remove the BOC-protection group to yield compounds **9A-B**.
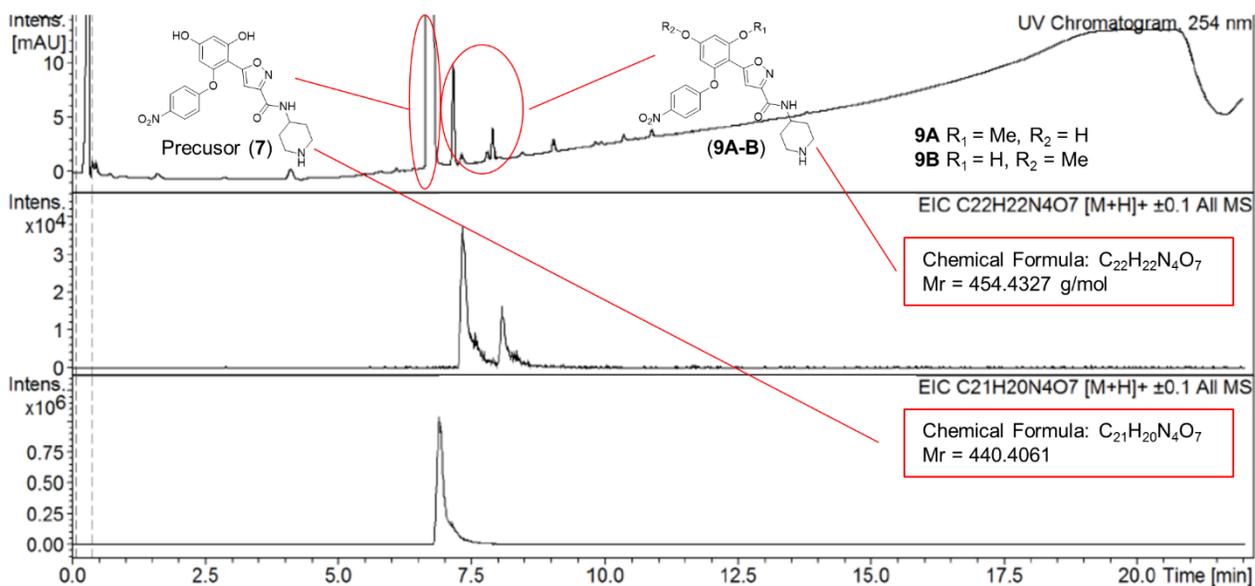
Figure S6: LC-MS-chromatograms of the reaction mixture yielding compound **9A-B**. Extracted ion chromatograms (EIC 454.4 ± 0.1 Da, Rt 7.7 min and 8.5 min) indicate formation of 2 O-methylated compounds **9A-B** that are present besides excess of deprotected precursor compound **7** (EIC 440.4 ± 0.1 Da, Rt 6.9 min). Samples were run on a LC/HRMS system, described in the materials and method section, over an Acquity UPLC BEH C_{18} column (1.7 μm, 2.1 mm x 150 mm, Waters), using a 22 min gradient containing H_2O + 0.1% HCOOH and ACN + 0.1% HCOOH (95/5 to 5/95) with a flow rate of 0.3 mL/min.

9A R$_1$ = Me, R$_2$ = H
9B R$_1$ = H, R$_2$ = Me
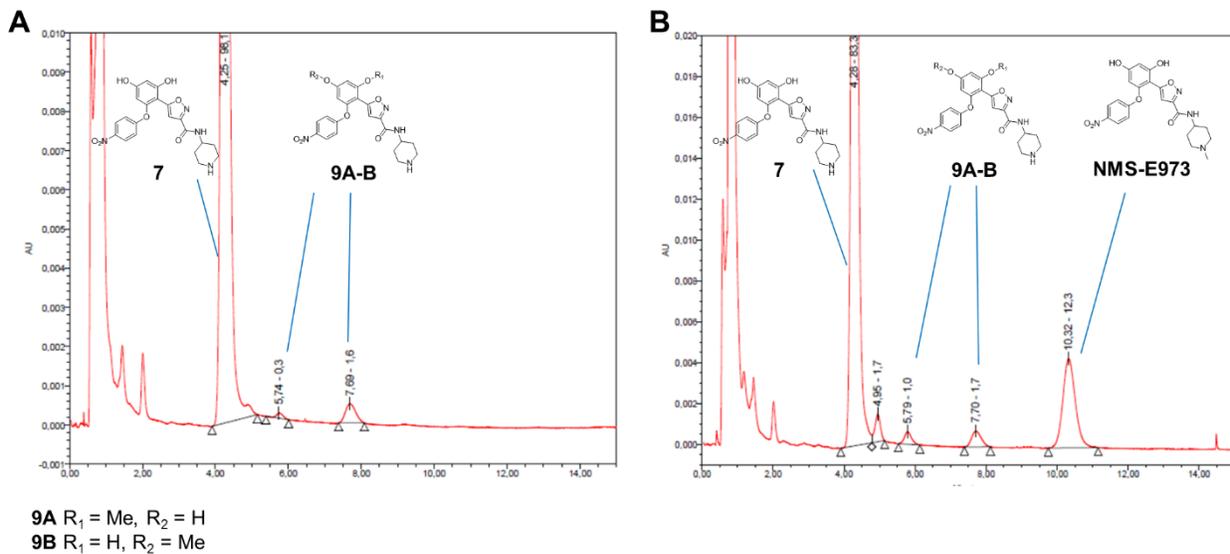
Figure S7: QC HPLC analysis (UV 254 nm) – chromatograms. **A)** Injection of crude reaction mixture, containing precursor compound (**7**) (Rt 4.2 min) and **O-methylated compounds 9A-B** (Rt 5.7 min and 7.7 min). **B)** Coinjection of crude reaction mixture with NMS-E973. Precursor (**7**) (Rt 4.2 min), O-methylated products **9A-B** (Rt 5.8 min and 7.7) and NMS-E973 (Rt 10.3 min) show a clear difference in retention time. UV detection was performed at 254 nm.

Plasma radiometabolite study and biodistribution studies

Table S1: Gradient mixture and flow rate used at given time points for radiometabolite study of [^{11}C]NMS-E973 with a Chromolith RP C$_{18}$ column.

Time (min)	A (ACN) (%)	B (NaOAc 0.05 M pH 5.5) (%)	Flow (mL/min)
0	1	99	0.5
4	1	99	0.5
4.1	1	99	1.0
9	90	10	1.0
12	90	10	1.0
12.1	90	10	0.5
15	1	99	0.5

Table S2: Biodistribution data of [^{11}C]NMS-E973 in female Wistar rats at 2, 10, 30 and 60 min after tracer injection.

		%ID[a]		
	2 min	**10 min**	**30 min**	**60 min**
Blood	17.6 ± 1.5	9.7 ± 1.1	5.3 ± 1.1	2.6 ± 0.8
Bone	5.7 ± 1.0	3.7 ± 0.3	3.8 ± 0.2	1.6 ± 0.1
Brain	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Carcass	27.3 ± 1.9	23.2 ± 0.7	23.7 ± 1.0	17.2 ± 2.6
Heart	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.0	0.3 ±0.0
Intestines	9.3 ± 1.0	25.8 ± 2.9	41.8 ± 7.6	40.6 ± 4.2
Kidneys	14.8 ± 1.2	9.6 ±1.4	5.2 ± 0.4	2.1 ± 0.4
Liver	33.8 ± 3.7	25.9 ± 3.4	12.5 ± 4.0	11.3 ± 0.7
Lungs	1.2 ± 0.3	1.0 ± 0.3	0.6 ± 0.1	0.6 ± 0.1
Muscle	13.4 ± 0.7	12.7 ± 0.2	14.5 ± 1.5	10.2 ± 0.8
Pancreas	0.5 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	0.6 ± 0.2
Spleen	0.6 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
Stomach	1.1 ± 1.1	3.9 ± 0.6	6.1 ± 5.1	14.0 ± 5.2
Urine	0.0 ± 0.0	2.7 ± 1.0	4.8 ± 1.2	11.3 ± 1.4

		SUV[b]		
	2 min	**10 min**	**30 min**	**60 min**
Blood	2.5 ± 0.2	1.4 ± 0.2	0.8 ± 0.2	0.4 ± 0.1
Bone	0.5 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.1 ± 0.0
Brain	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Heart	1.6 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.1 ± 0.1
Kidneys	17.3 ± 0.6	12.5 ± 1.6	5.8 ± 0.5	3.0 ± 0.7
Liver	9.3 ± 0.3	7.9 ± 1.1	3.3 ± 1.1	3.5 ± 0.5
Lungs	1.8 ± 0.3	1.4 ± 0.1	1.2 ± 0.1	1.0 ± 0.1
Muscle	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
Pancreas	1.6 ± 0.3	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.2
Spleen	2.3 ± 0.2	2.2 ± 0.1	1.4 ± 0.1	1.3 ± 0.1

[a] Percentage of injected dose calculated as cpm in organ/total cpm recovered) x 100. [b] SUV calculated as (radioactivity in cpm in organ/weight of organ in grams)/(total cpm recovered/body weight). Data expressed as mean ± SD; n = 3 per time point.

Table S3: Biodistribution data of [^{11}C]NMS-E973 in male NMRI-mice at 2, 10, 30 and 60 min after tracer injection.

%ID[a]	2 min	10 min	30 min	60 min
Blood	8.8 ± 0.6	4.7 ± 0.9	2.1 ± 0.6	1.3 ± 0.1
Bone	2.8 ± 0.3	2.0 ± 0.3	1.8 ± 0.2	1.2 ± 0.1
Brain	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
Carcass	15.6 ± 0.7	15.5 ± 1.1	14.8 ± 1.3	10.9 ± 2.3
Heart	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.2 ± 0.0
Intestines	12.2 ± 1.5	35.7 ± 0.4	47.0 ± 0.7	56.2 ± 6.2
Kidneys	14.4 ± 0.6	7.8 ± 1.1	5.2 ± 1.9	2.1 ± 0.4
Liver	48.5 ± 1.8	24.1 ± 3.9	11.0 ± 2.3	7.9 ± 1.5
Lungs	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.3 ± 0.1
Muscle	9.8 ± 0.5	8.6 ± 1.0	8.7 ± 1.2	5.3 ± 0.4
Pancreas	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.3 ± 0.0
Spleen	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
Stomach	0.4 ± 0.0	1.1 ± 1.1	0.7 ± 0.4	0.4 ± 0.2
Testes	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0
Urine	0.2 ± 0.2	10.6 ± 2.7	16.7 ± 2.4	20.3 ± 3.5

SUV[b]	2 min	10 min	30 min	60 min
Blood	1.3 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
Bone	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Brain	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Heart	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
Kidneys	7.7 ± 0.8	4.4 ± 0.2	2.7 ± 1.1	1.1 ± 0.2
Liver	8.6 ± 0.4	4.4 ± 0.7	2.1 ± 0.1	1.4 ± 0.3
Lungs	0.8 ± 0.1	0.8 ± 0.2	0.5 ± 0.1	0.5 ± 0.2
Muscle	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
Pancreas	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.0	0.5 ± 0.1
Spleen	0.6 ± 0.1	0.7 ± 0.2	0.4 ± 0.1	0.3 ± 0.1
Testes	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	± 0.0

[a] Percentage of injected dose calculated as (cpm in organ/total cpm recovered) x 100. [b] SUV calculated as (radioactivity in cpm in organ/weight of organ in grams)/(total cpm recovered/body weight). Data expressed as mean ± SD; n = 3 per time point.

Table S4: Biodistribution data of [^{11}C]NMS-E973 in male NMRI-mice pretreated with vehicle, NMS-E973 (25mg/kg) or PU-H71 (50 mg/kg) at 10 min after tracer injection.

|	%ID[a] 10 min p.i.		
	Control	**NMS-E973 pretreatment**	**PU-H71 pretreatment**
Blood	4.9 ± 0.8	2.5 ± 0.2	6.3 ± 0.2
Bone	2.6 ± 0.0	2.6 ± 0.5	3.1 ± 0.5
Brain	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
Carcass	20.0 ± 1.8	17.1 ± 2.2	28.0 ± 1.5
Heart	0.5 ± 0.2	0.3 ± 0.0	0.5 ± 0.1
Intestines	27.0 ± 0.5	33.3 ± 4.0	20.6 ± 0.6
Kidneys	12.8 ± 10.0	5.3 ± 0.4	17.2 ± 6.4
Liver	23.8 ± 2.3	24.6 ± 2.6	18.4 ± 2.6
Lungs	0.7 ± 0.1	0.5 ± 0.1	0.8 ± 0.2
Muscle	11.7 ± 0.4	10.2 ± 1.8	14.6 ± 3.1
Pancreas	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.1
Spleen	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.0
Stomach	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.0
Testes	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Urine	10.8 ± 15.1	15.4 ± 1.7	7.2 ± 7.4
	SUV[b] 10 min p.i.		
	Control	**NMS-E973 pretreatment**	**PU-H71 pretreatment**
Blood	0.7 ± 0.1	0.4 ± 0.0	0.9 ± 0.0
Bone	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
Brain	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
Heart	1.0 ± 0.2	0.8 ± 0.0	1.0 ± 0.1
Kidneys	7.0 ± 4.8	3.6 ± 0.2	9.2 ± 3.2
Liver	3.8 ± 0.2	4.3 ± 0.7	2.9 ± 0.4
Lungs	0.9 ± 0.0	0.6 ± 0.0	0.9 ± 0.3
Muscle	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1
Pancreas	0.7 ± 0.0	0.7 ± 0.1	0.8 ± 0.1
Spleen	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.1
Testes	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0

[a] Percentage of injected dose calculated as (cpm in organ/total cpm recovered) x 100. [b] SUV calculated as (radioactivity in cpm in organ/weight of organ in grams)/(total cpm recovered/body weight). Data expressed as mean ± SD; n = 2 (control) or 3 (NMS-E973 and PU-H71) per time point.

Table S5: Biodistribution data of [^{11}C]NMS-E973 in B16.F10 melanoma inoculated C57BL/6 mice at 60 min after tracer injection with pretreatment of vehicle, PU-H71 or Ganetespib.

%ID[a]		
	60 min control	PU-H71 pretreatment	Ganetespib pretreatment
Blood	2.6 ± 0.7	0.6 ± 0.2	0.6 ± 0.3
Bone	2.1 ± 0.0	0.8 ± 0.3	0.7 ± 0.6
Brain	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Carcass	15.2 ± 0.5	9.2 ± 5.3	14.5 ± 8.0
Heart	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
Intestines	43.4 ± 2.0	53.9 ± 7.3	49.4 ± 4.4
Kidneys	1.8 ± 0.2	0.3 ± 0.1	1.2 ± 0.3
Liver	6.8 ± 2.0	8.6 ± 3.1	5.8 ± 2.4
Lungs	0.5 ± 0.0	0.2 ± 0.1	0.3 ± 0.1
Muscle	8.2 ± 1.2	5.1 ± 1.0	6.4 ± 1.4
Pancreas	0.5 ± 0.1	0.5 ± 0.2	0.3 ± 0.3
Spleen	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Stomach	1.0 ± 0.3	0.3 ± 0.2	0.3 ± 0.1
Tumor	0.8 ± 0.3	1.0 ± 0.4	1.2 ± 0.5
Urine	27.4 ± 3.5	28.9 ± 11.2	25.7 ± 7.4

SUV[b]		
	60 min control	PU-H71 pretreatment	Ganetespib pretreatment
Blood	0.4 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
Bone	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Brain	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Heart	0.6 ± 0.0	0.3 ± 0.1	0.4 ± 0.1
Kidneys	1.4 ± 0.1	0.2 ± 0.0	0.9 ± 0.2
Liver	1.4 ± 0.3	1.6 ± 0.4	1.3 ± 0.6
Lungs	0.6 ± 0.1	0.3 ± 0.1	0.3 ± 0.0
Muscle	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
Pancreas	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.2
Spleen	0.4 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
Tumour	0.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0

[a] Percentage of injected dose calculated as (cpm in organ/total cpm recovered) x 100. [b] SUV calculated as (radioactivity in cpm in organ/weight of organ in grams)/(total cpm recovered/body weight). Data expressed as mean ± SD; n = 3 per time point.
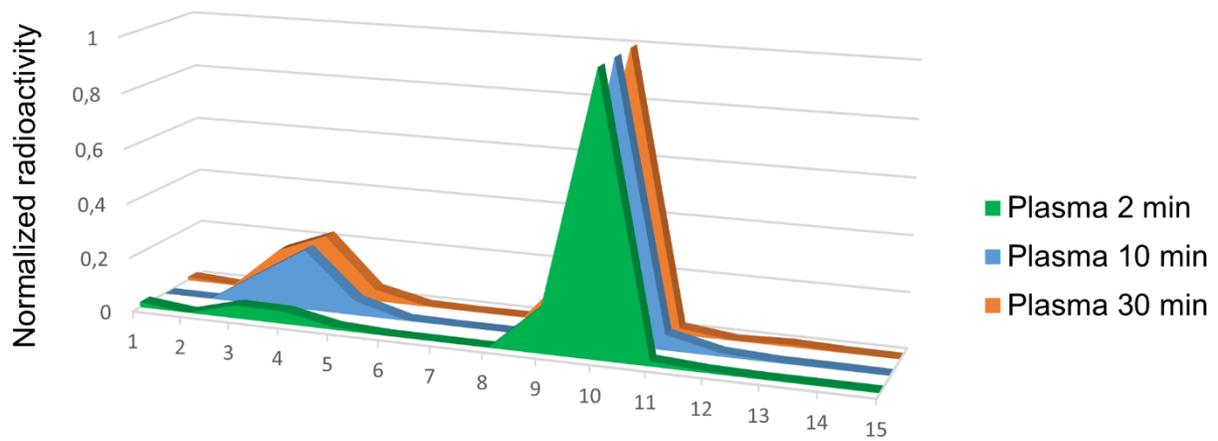
Figure S8: RadioHPLC analysis of plasma samples (radiometabolite study). Polar radiometabolite fractions for respectively 2, 10 and 30 min account for 11 ± 4%, 25 ± 6% and 28 ± 8% of total plasma activity.