Electronic Supplementary Material (ESI) for Chemical Science.

Electronic Supplementary Information

Shedding Light on the Mitochondrial Matrix Through a Functional Membrane Transporter

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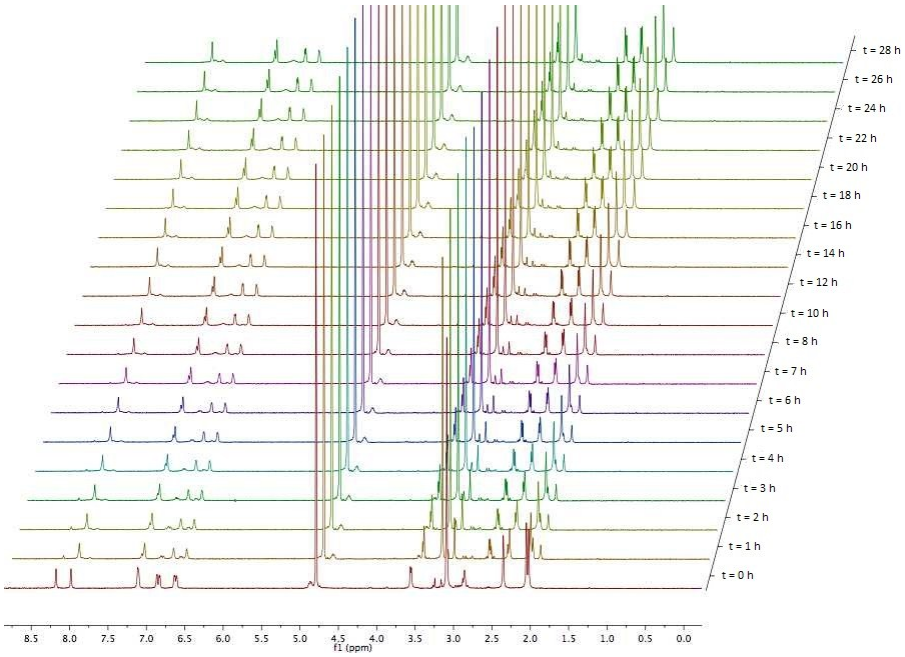
(a) Temporal evolution of the ^{1}H NMR spectra of the probe (*R*)-**BCT-1** dissolved in D_2O (15 mM) at 25 ºC.



(b) Temporal evolution of the ^{1}H NMR spectra of the probe (*R*)-**BCT-2** dissolved in D_2O (15 mM) at 25 ºC.

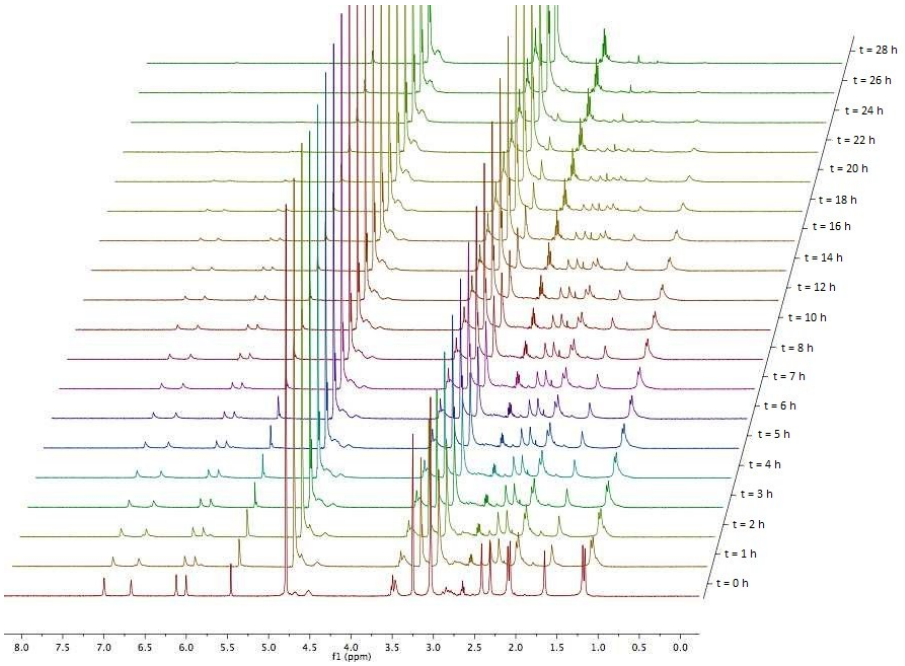


Figure S1. NMR study of the stability of the **BCT** probes in D_2O solution.

Table S1. Photophysical[a] properties of *(R)*-**BCT-1** in water and *(R)*-**BCT-2** in different solvents.

Probe	**Solvent**	λ_{ab} **(nm)**	$\varepsilon_{max} \times 10^4$ **(M^{-1} cm^{-1})**	λ_{fl} **(nm)**	$\Delta\nu$ **(cm^{-1})**	ϕ_f	τ **(ns)**	k_{fl} **(10^8 s^{-1})**	k_{nr} **(10^8 s^{-1})**
BCT-1	H_2O	501	4.1	515	542	0.40	6.00	0.66	1.00
BCT-2	H_2O	499	3.8	511	470	0.83	7.30	1.14	0.23
	EtOH	502	5.1	513	427	0.87	7.10	1.22	0.18
	MeCN	500	4.9	512	469	0.86	7.12	1.21	0.20
	Acetone	501	5.1	514	504	0.89	7.00	1.27	0.16
	$CHCl_3$	504	4.8	514	424	0.86	6.32	1.36	0.22

[a]λ_{ab}: Absorption peak wavelength; ε: Molar extinction coefficient at peak; λ_{fl}: Fluorescence peak wavelength, upon excitation at 490 nm; $\Delta\nu$: Stokes shift; ϕ: Quantum yield using an ethanolic solution of the commercial BODIPY called PM546 as reference.; τ: Fluorescence lifetime; k_{fl}: Radiative decay rate; k_{nr}: Non-radiative decay rate

Table S2. Temporal evolution of the photophysical properties of (*R*)-**BCT-2** in water.

Time (h:min)	λ_{ab} **(nm)**	ε_{max} **(10^4 $M^{-1}cm^{-1}$)**	λ_{fl} **(nm)**	ϕ_f	τ **(ns)**	k_{fl} **(10^8 s^{-1})**	k_{nr} **(10^8 s^{-1})**
00:00	499	3.8	511	0.83	7.30	1.14	0.23
00:10	498	3.8	511	0.82	7.25	1.14	0.24
00:25	498	3.8	511	0.82	7.20	1.14	0.25
00:50	498	3.8	509	0.83	7.15	1.16	0.24
01:20	498	3.8	509	0.83	7.05	1.17	0.25
02:00	497	3.8	509	0.83	7.00	1.18	0.25
03:00	497	3.8	509	0.82	6.95	1.18	0.26
04:00	497	3.8	509	0.82	6.90	1.19	0.26
05:00	497	3.8	508	0.82	6.80	1.22	0.26
06:00	496	3.8	508	0.83	6.70	1.23	0.26
07:00	496	3.8	508	0.83	6.65	1.24	0.26
24:00	496	3.7	507	0.65	6.50	1.03	0.54

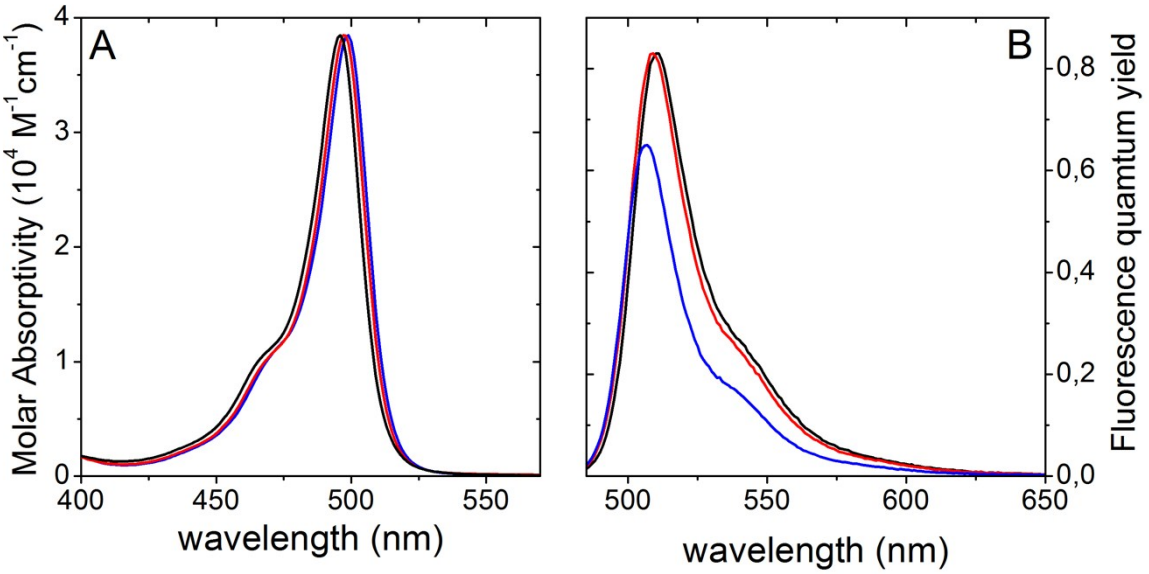


Figure S2. Temporal evolution of the absorption and fluorescence spectra of probe (*R*)-**BCT-2** dissolved in water: time zero (black), 6 hours (red), and 24 hours (blue).

Table S3. Temporal evolution of the photophysical properties of probe (*R*)-**BCT-2** in PBS (at pH 6.0, 7.4, and 8.0) and in culture medium (pH 7.4).

Solvent		λ_{ab} **(nm)**	ε_{max} **(10^4 M^{-1} cm^{-1})**	λ_{fl} **(nm)**	ϕ_f	τ **(ns)**	k_{fl} **(10^8 s^{-1})**	k_{nr} **(10^8 s^{-1})**
PBS (pH 6.0)	5 min	498.0	3.4	508.0	0.75	6.74	1.11	0.38
	1h	497.0	3.3	507.0	0.75	6.60	1.14	0.37
PBS (pH 7.4)	5 min	498.0	3.2	508.0	0.78	6.74	1.16	0.33
	1h	497.0	3.4	507.0	0.77	6.60	1.16	0.35
PBS (pH 8.0)	5 min	498.0	2.7	508.0	0.75	6.80	1.10	0.37
	1h	499.0	2.8	507.0	0.72	6.59	1.10	0.42
Culture medium	5 min	498.0	3.5	507.0	0.67	6.65	1.00	0.50
	1h	497.0	3.7	507.0	0.67	6.54	1.02	0.50

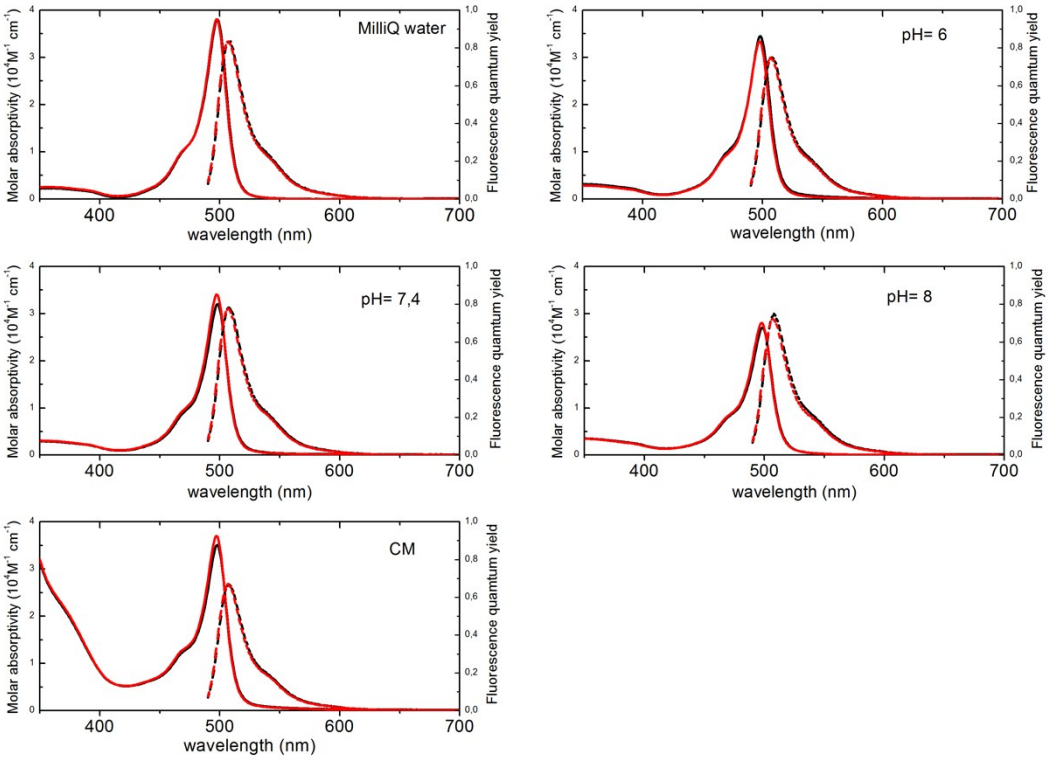


Figure S3. Absorption (height-normalized to the molar absorptivity) and emission (height-normalized to fluorescence quantum yield) spectra of (*R*)-**BCT2** in water (for comparison), in PBS (at pH 6.0, 7.4, and 8.0), and in culture medium (CM: Gibco™ DMEM, low glucose, pyruvate, no glutamine, no phenol red culture medium, supplemented with 10% Fetal Bovine Serum, pH 7.4), measured immediately after preparation of the sample (black curves) and after 1h (red curves).

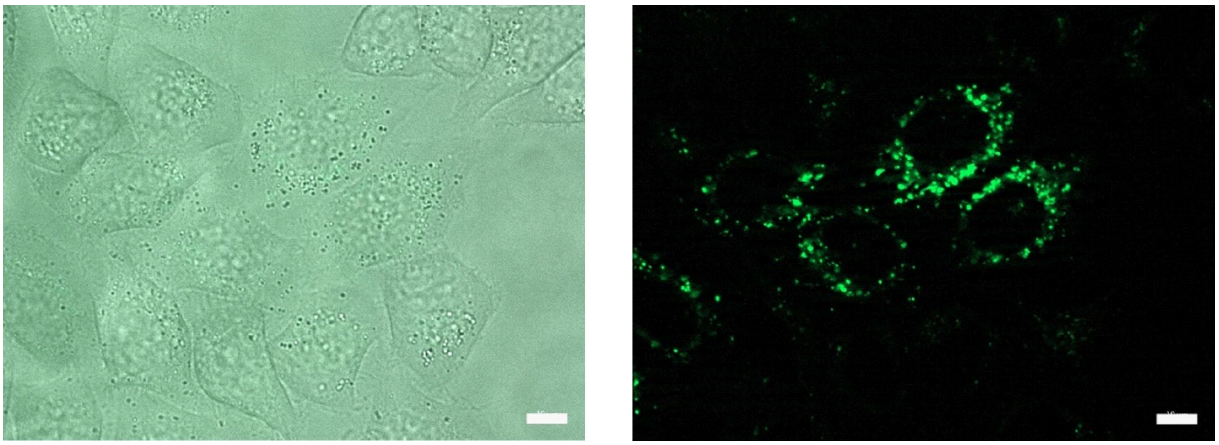


Figure S4. Representative bright field (left) and fluorescence (right) images of HeLa cells stained with (*R*)-**BCT-2**. Cells were incubated with 50 nM (*R*)-**BCT-2** for 30 min before cell washing and microscopy analysis. Scale bars, 10 μm.

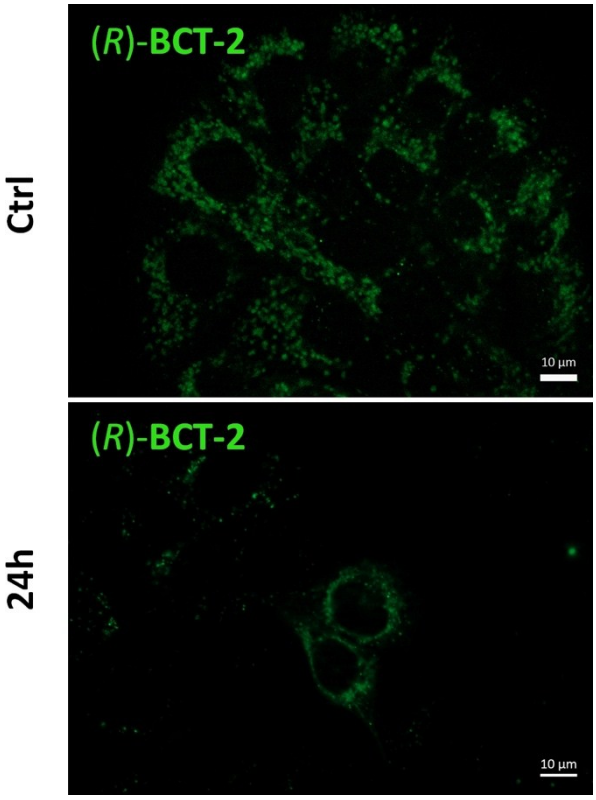
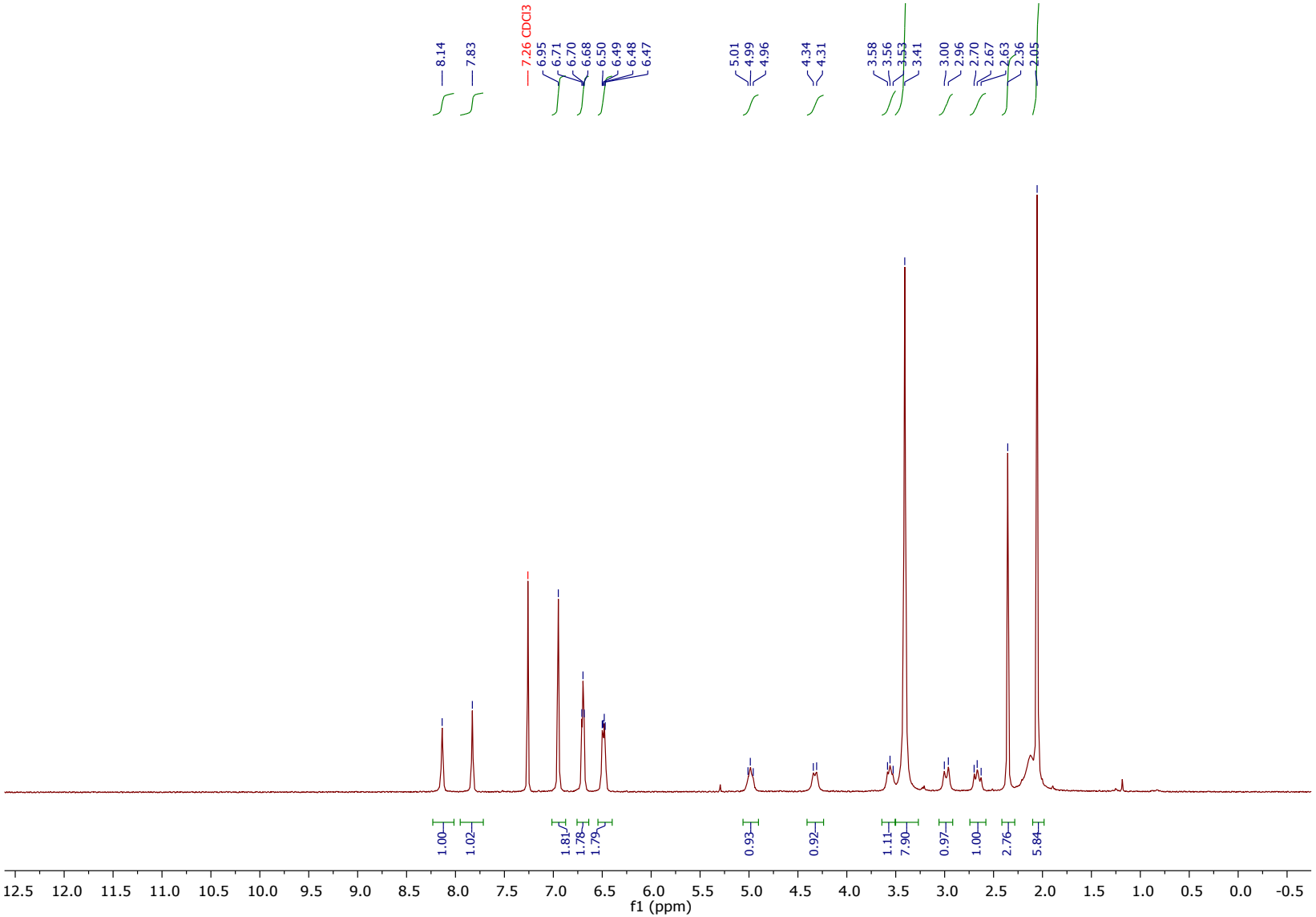
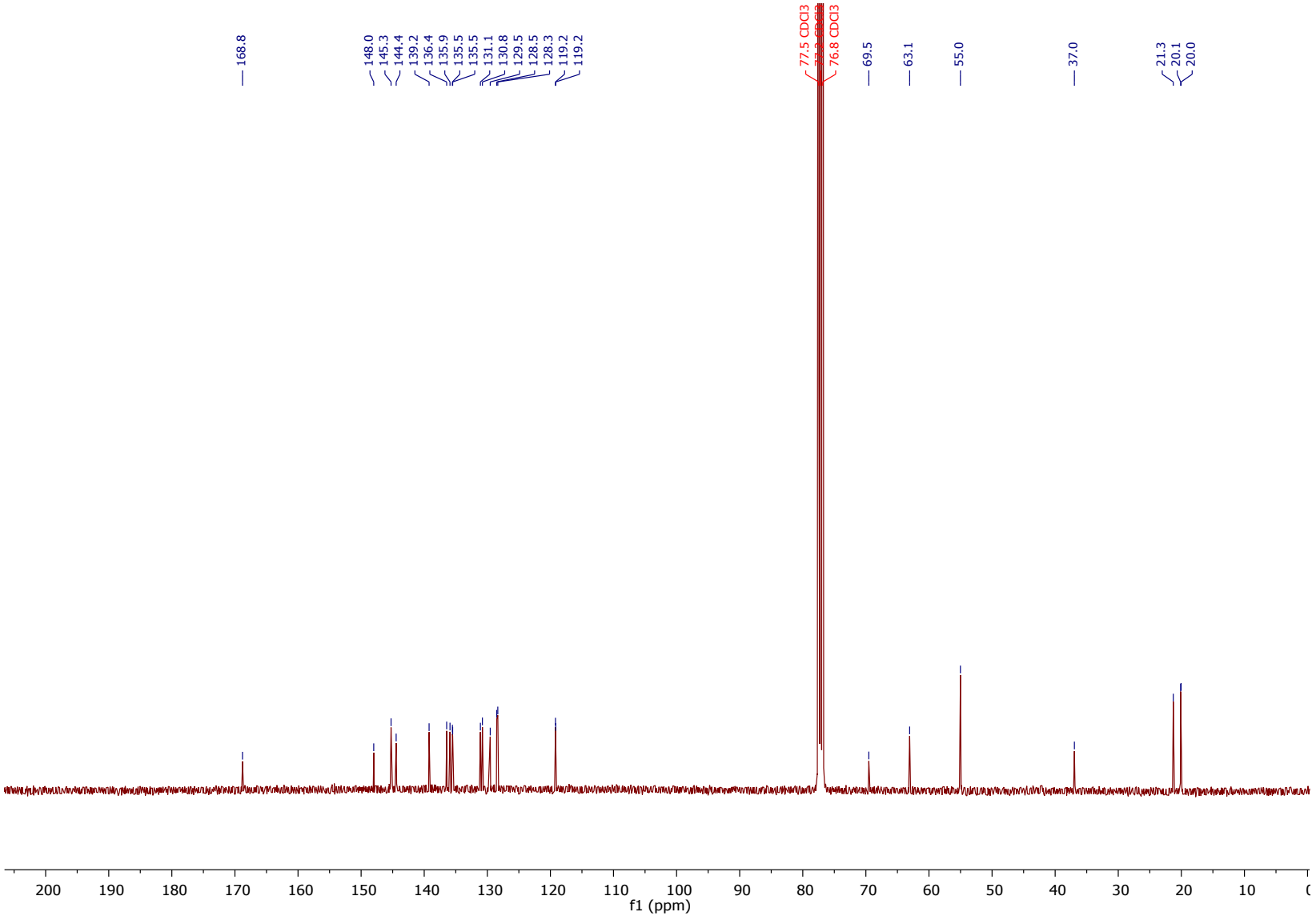


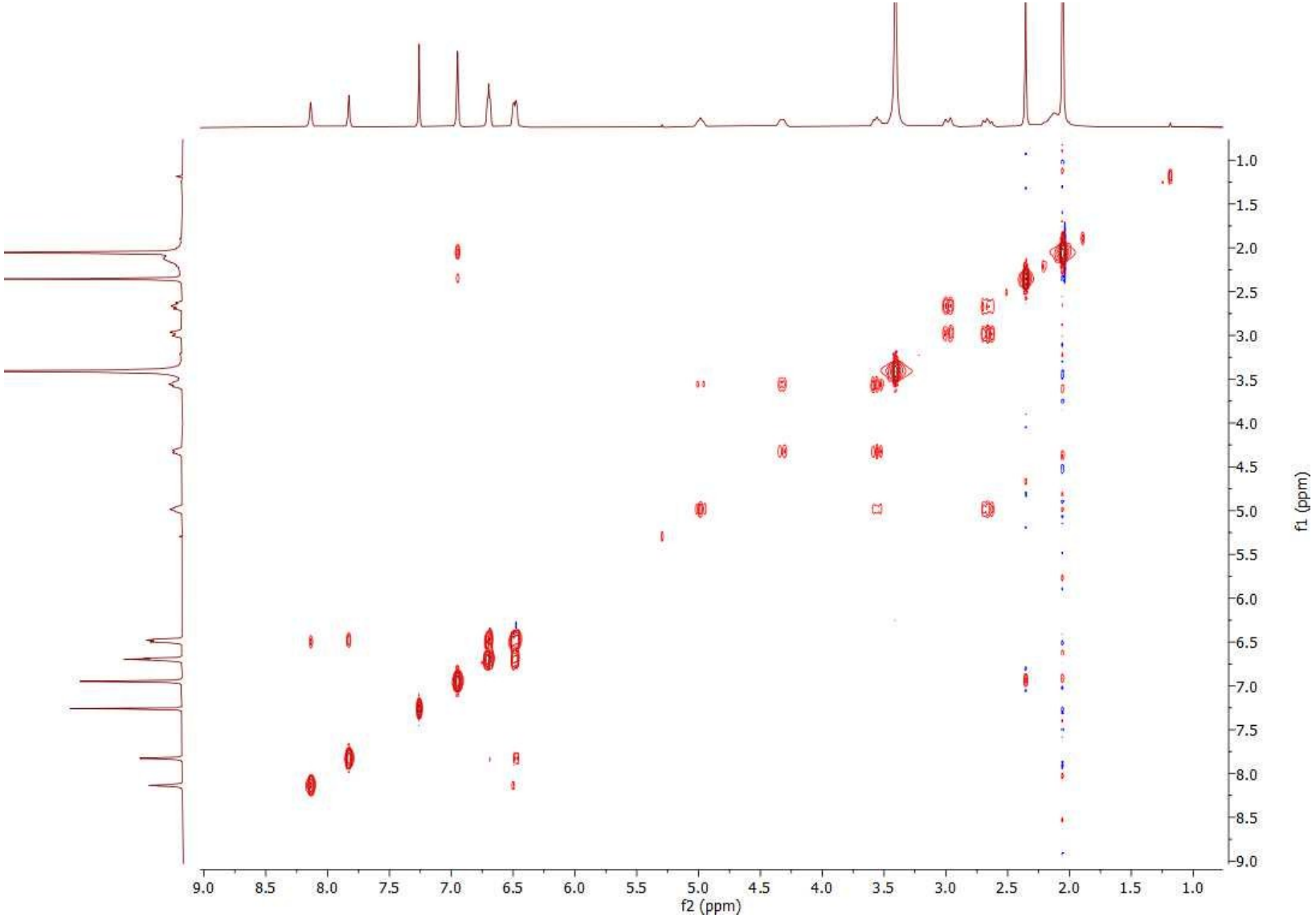
Figure S5. Staining of SCC38 cells incubated with just-prepared (Ctrl) or 24h-old aqueous solution of (*R*)-**BCT-2** (50 nM).



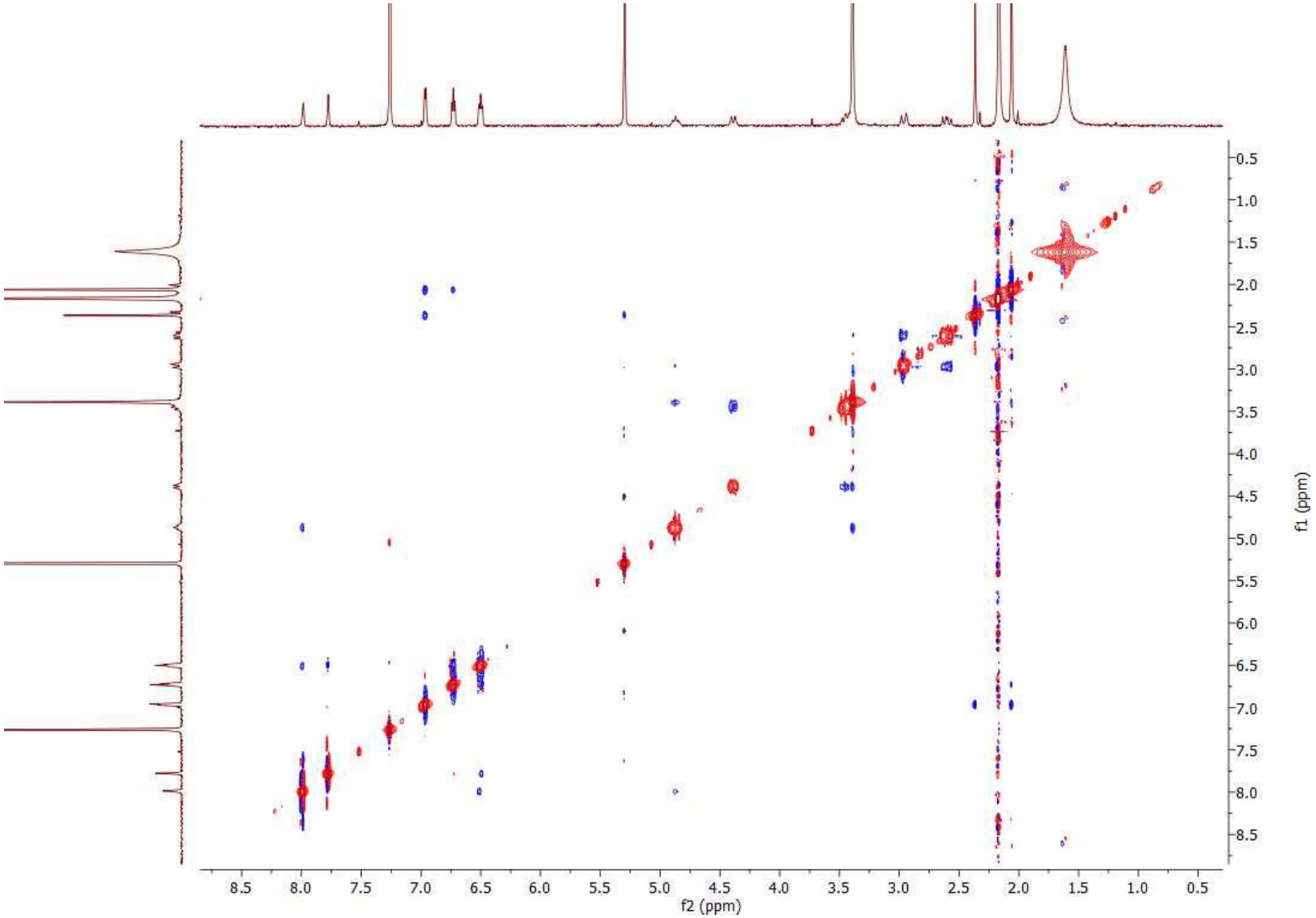
^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of (*R*)-**BCT-1**



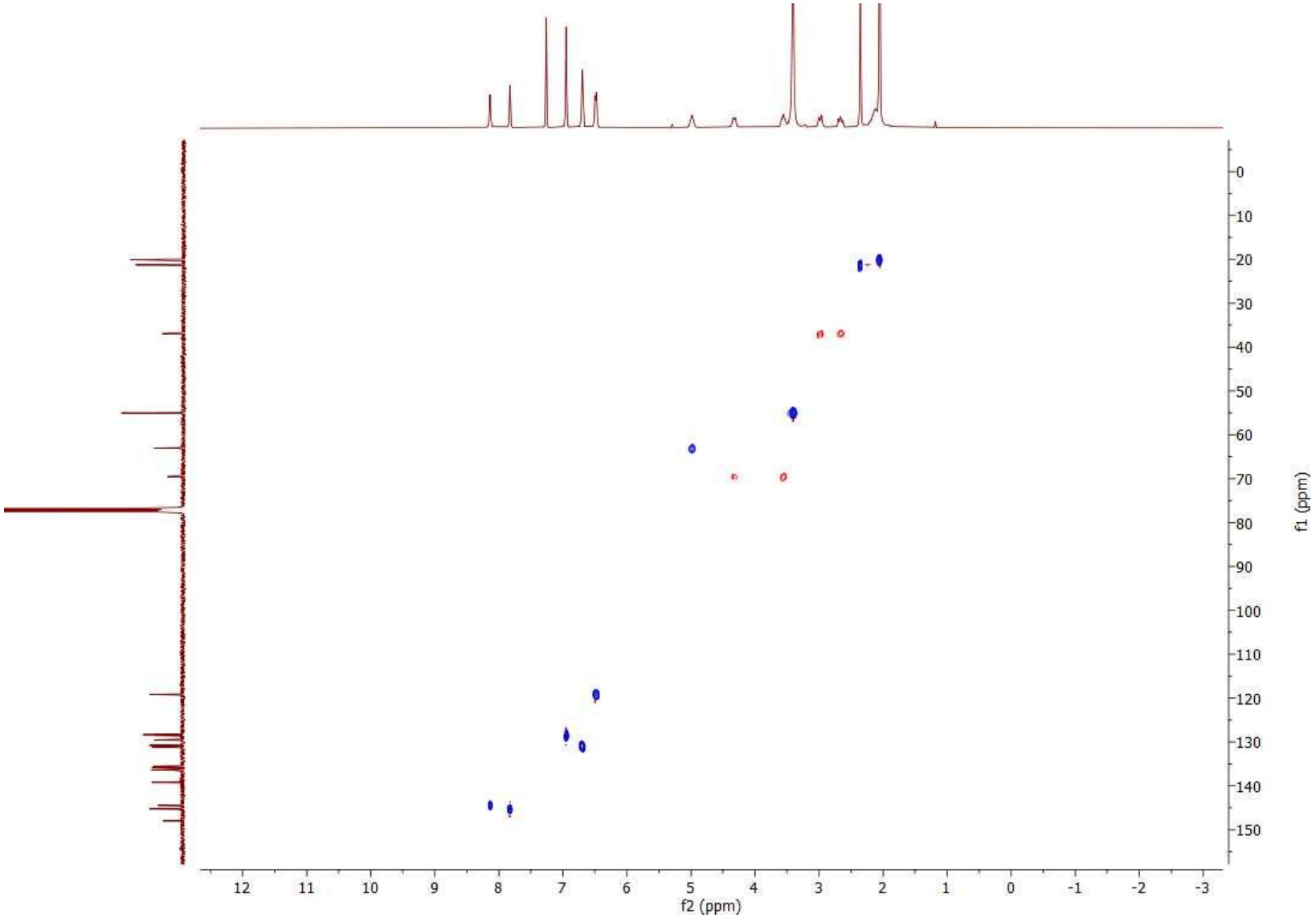
^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of (*R*)-**BCT-1**



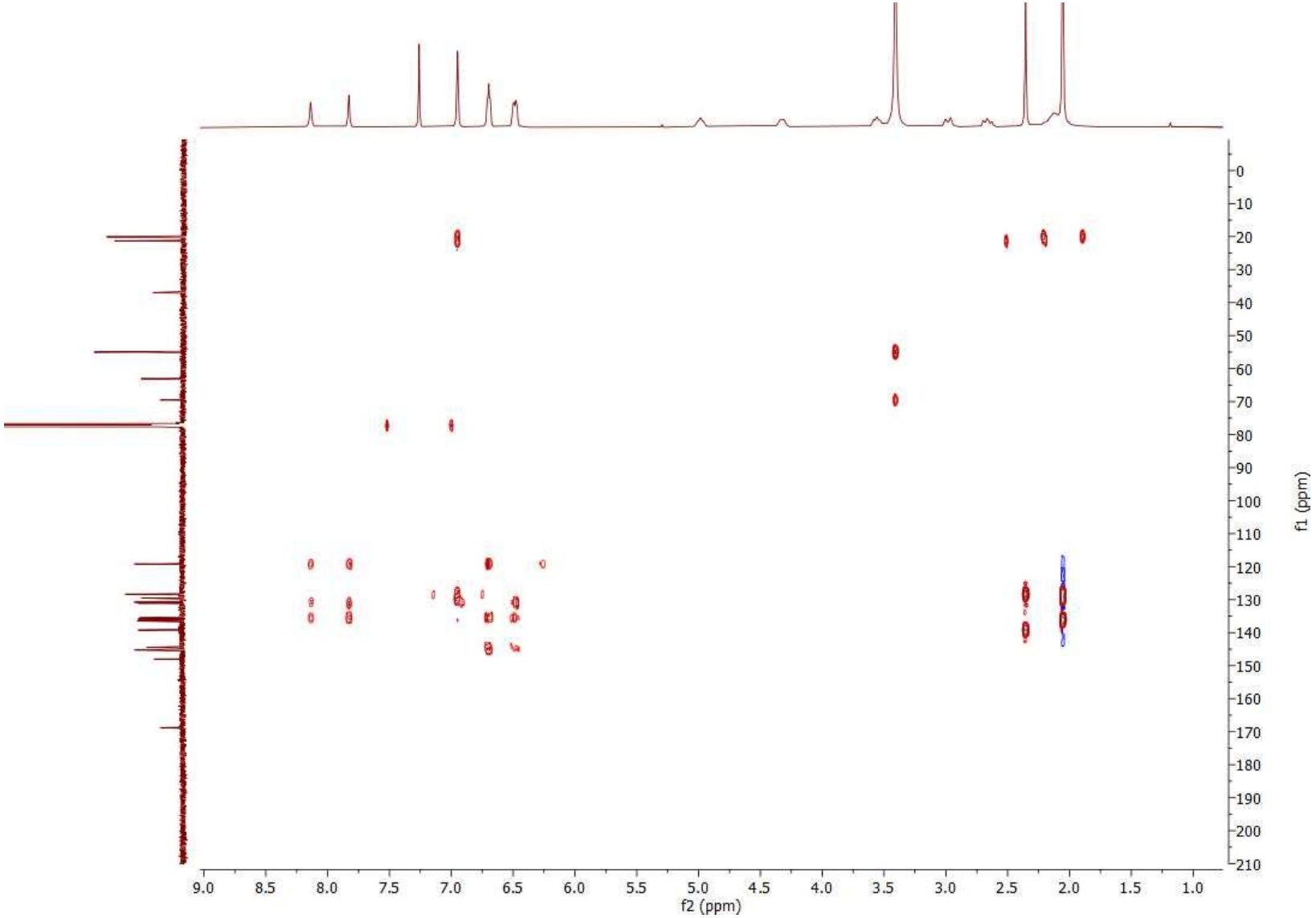
gCOSY ($CDCl_3$, 400 MHz) spectrum of (*R*)-**BCT-1**



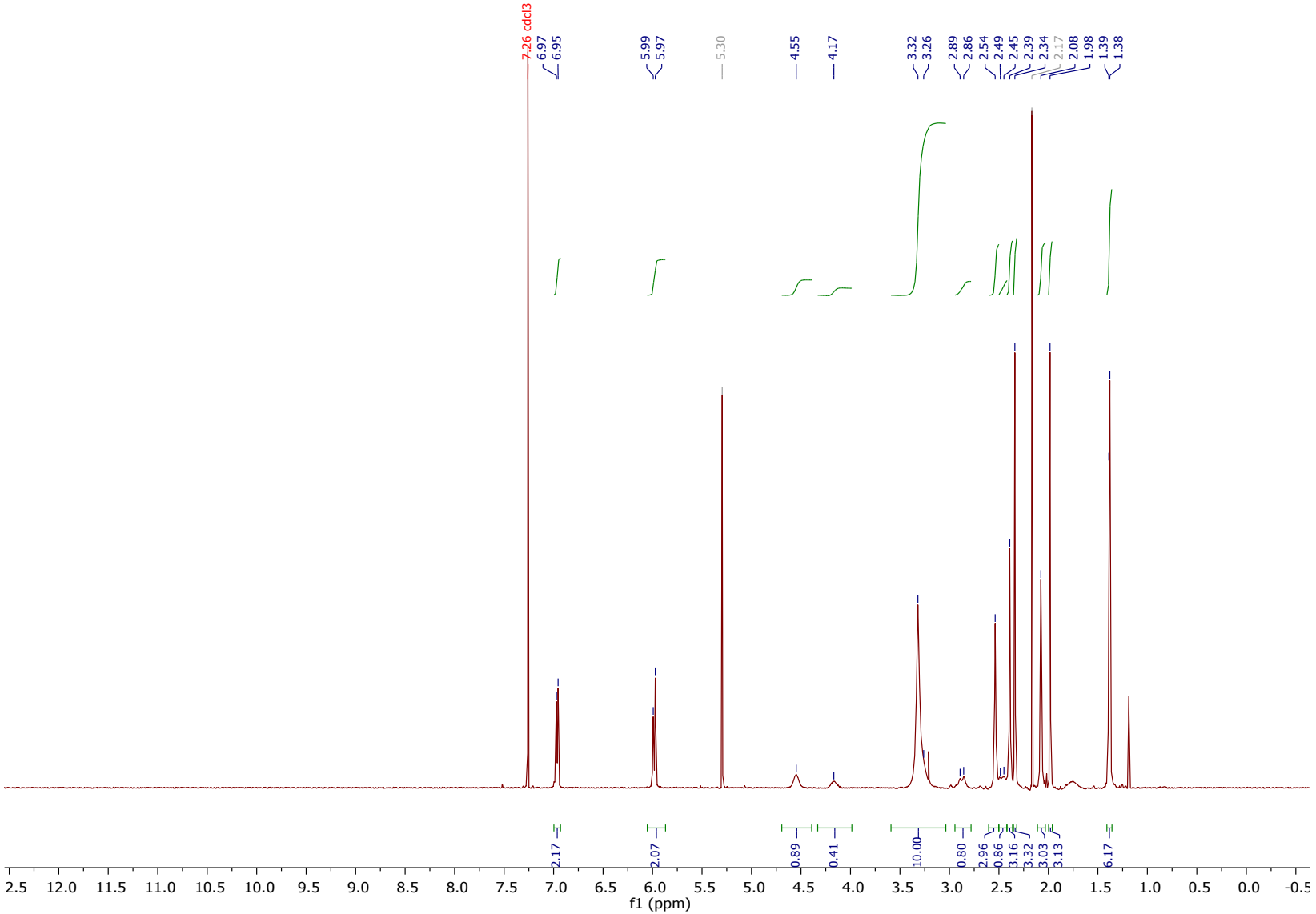
NOESY ($CDCl_3$, 400 MHz) spectrum of (*R*)-**BCT-1**



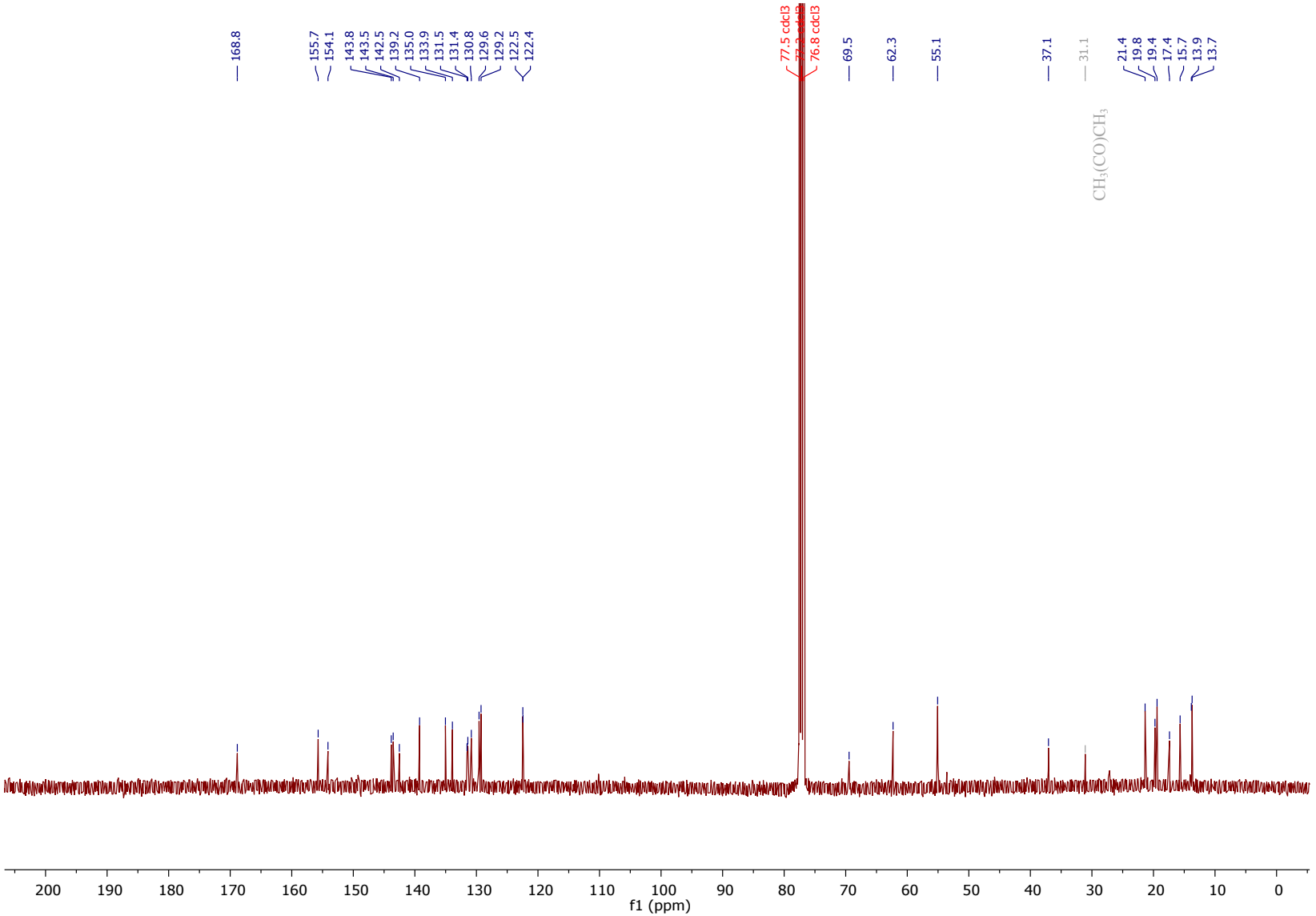
gHSQC ($CDCl_3$, 400 MHz) spectrum of (*R*)-**BCT-1**



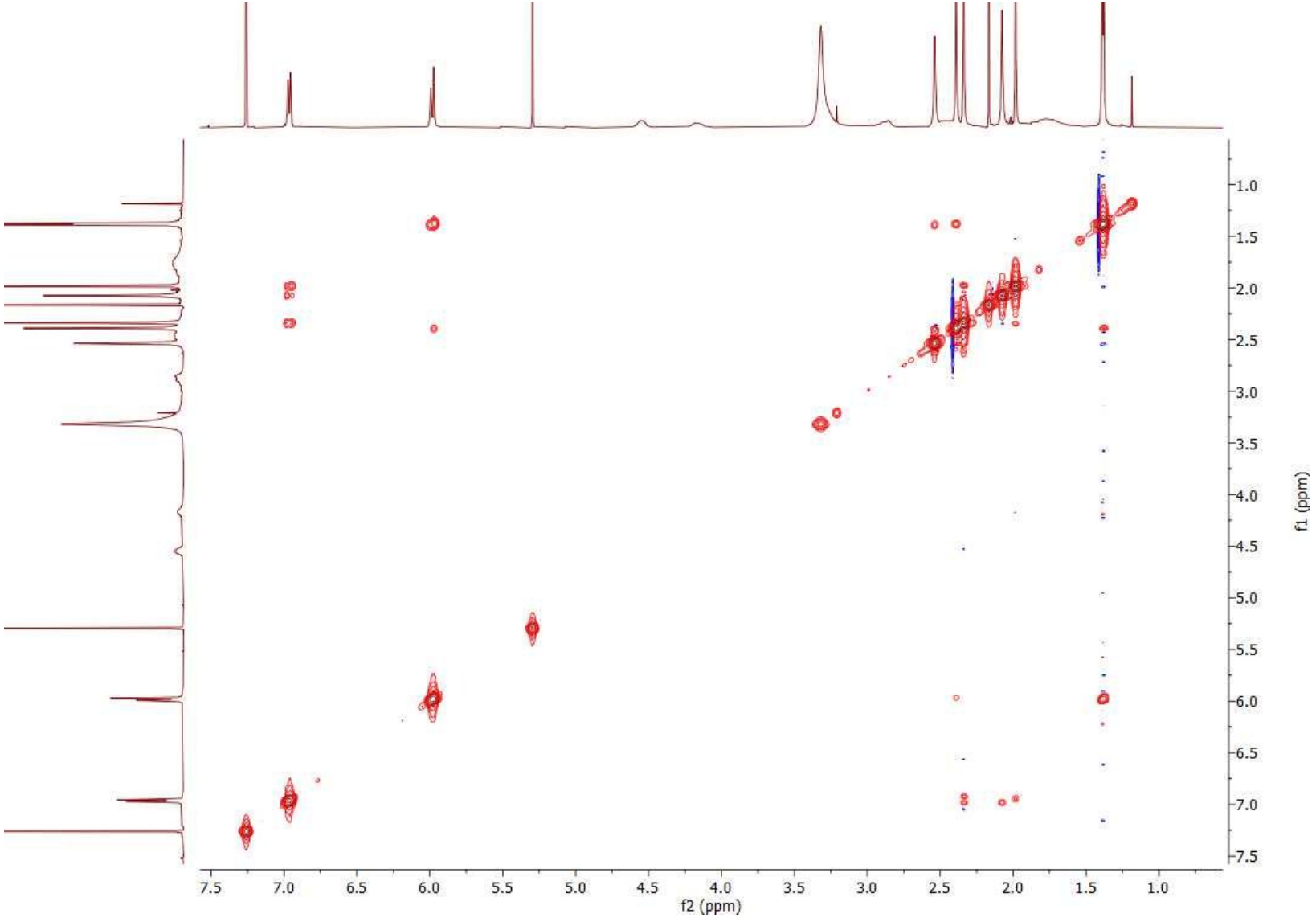
HMBC ($CDCl_3$, 400 MHz) spectrum of (*R*)-**BCT-1**



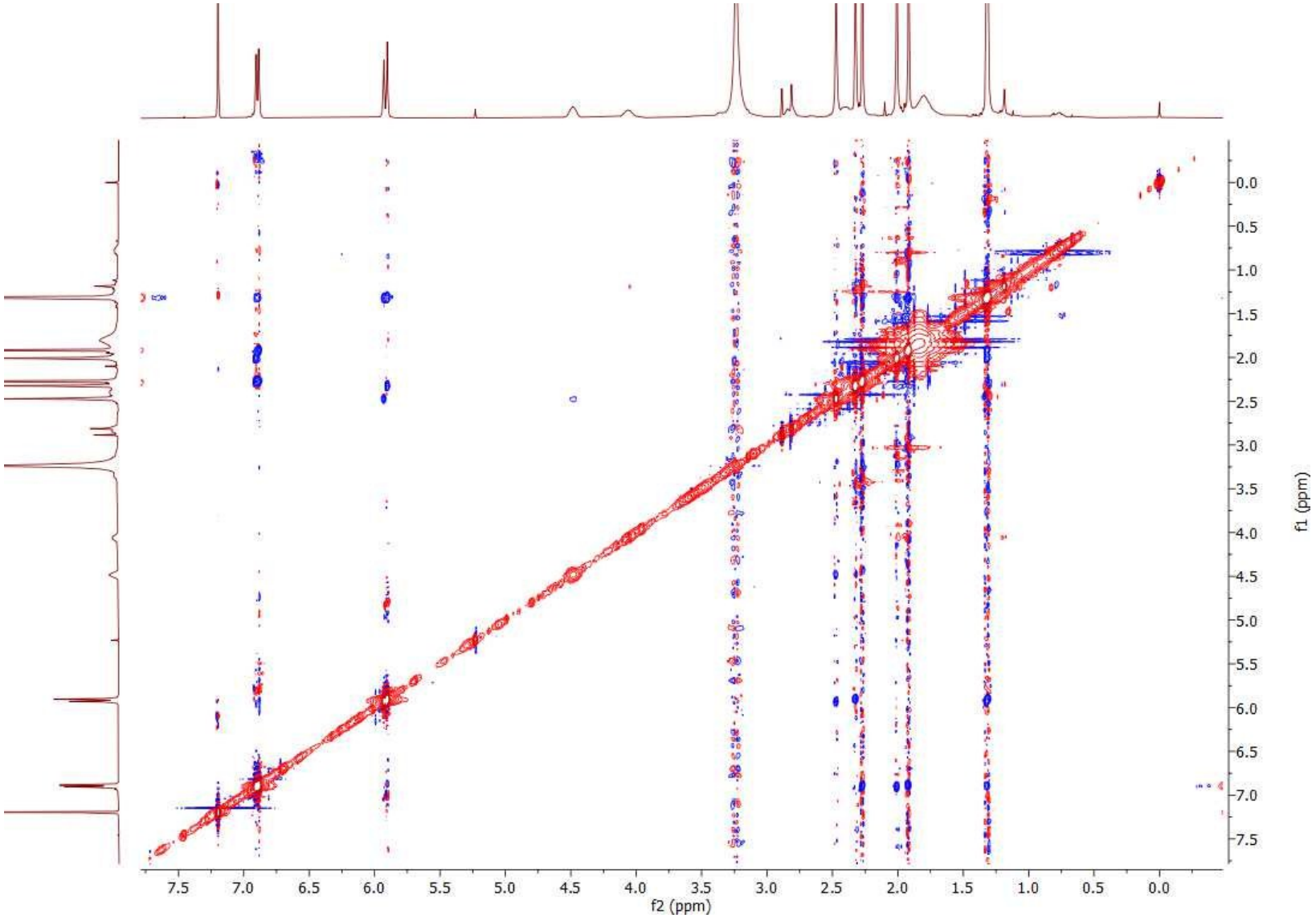
^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of (*R*)-**BCT-2**



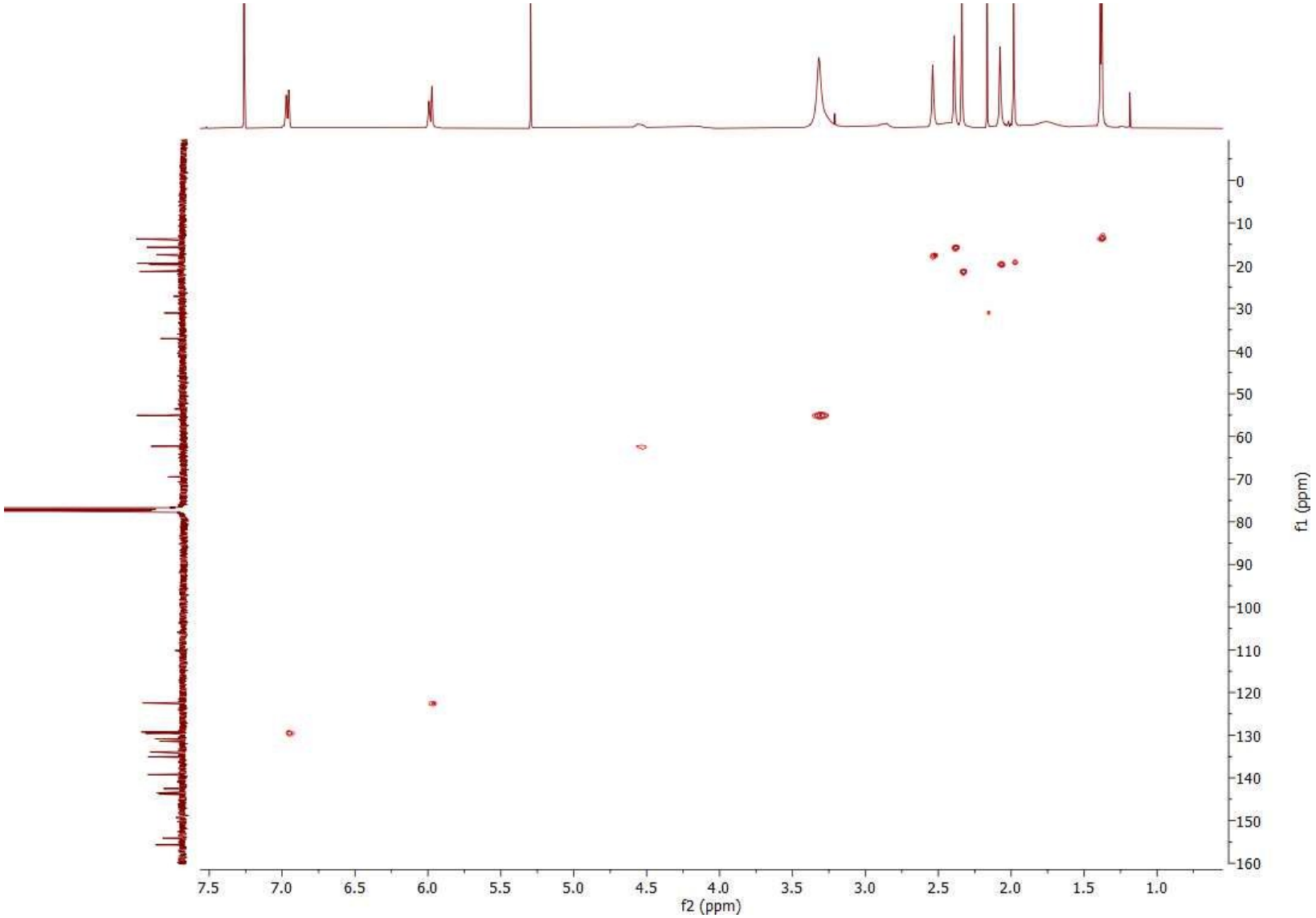
^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of (*R*)-**BCT-2**



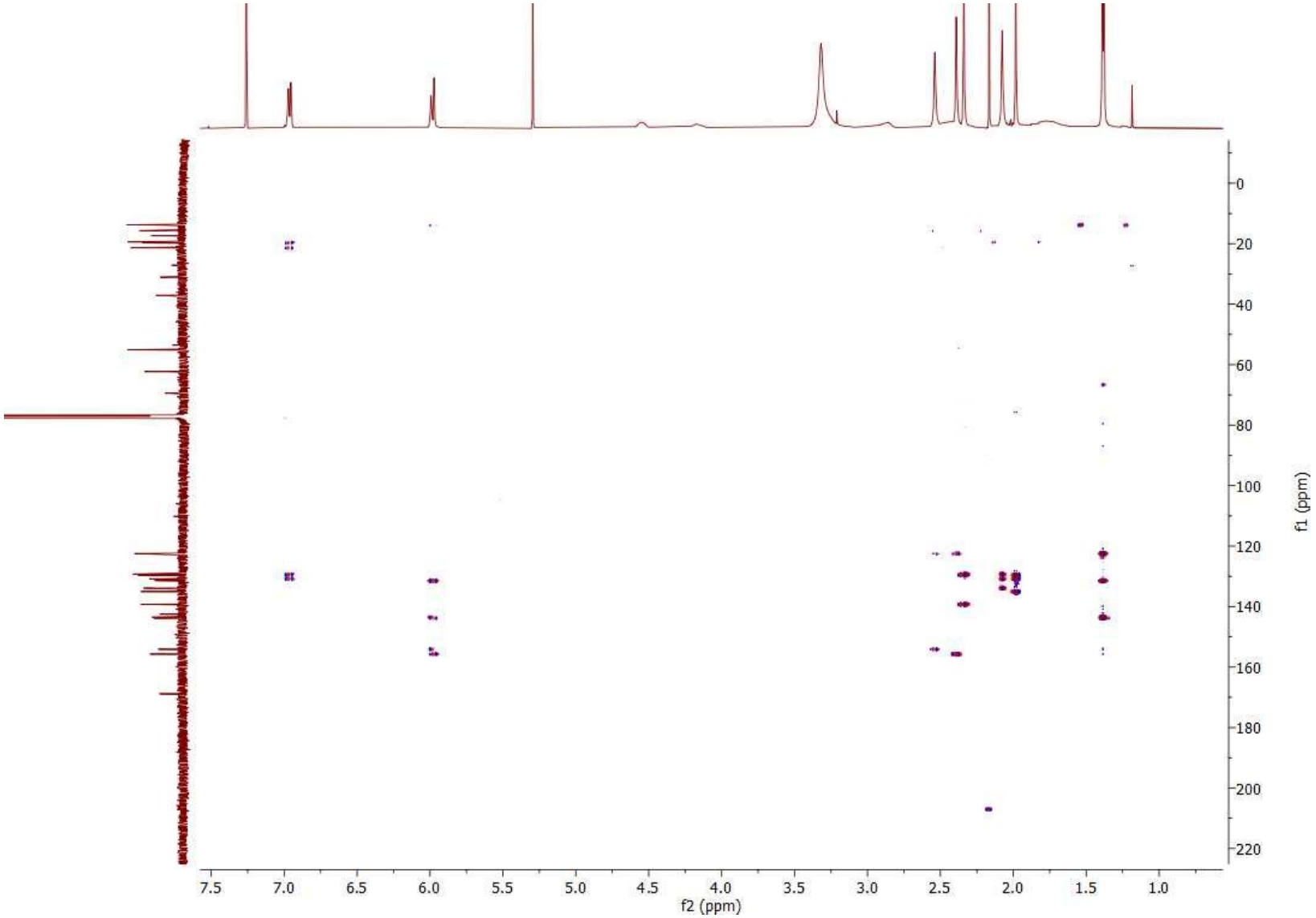
gCOSY ($CDCl_3$, 400 MHz) spectrum of (*R*)-**BCT-2**



NOESY ($CDCl_3$, 400 MHz) spectrum of (*R*)-**BCT-2**



gHSQC ($CDCl_3$, 400 MHz) spectrum of (*R*)-**BCT-2**



HMBC ($CDCl_3$, 400 MHz) spectrum of (*R*)-**BCT-2**