SUPPORTING INFORMATION

Mössbauer effect using ^{57}Fe-ferrabisdicarbollide ([*o*-^{57}FESAN]$^{-}$): a glance into the potential of a low-dose approach for glioblastoma radiotherapy

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Synthesis and characterization of Na[3,3'-^{57}Fe(1,2-$C_2B_9H_{10}$)$_2$], Na[*o*-^{57}FESAN].

Experimental section:

Materials.

Instrumentation and Measurements.

Figure S1. ^{11}B-NMR of Na[*o*-^{57}FESAN].

Figure S2. ^{1}H-NMR of Na[*o*-^{57}FESAN]

Figure S3. a) MALDI-TOF-MS of Na[*o*-^{57}FESAN]. b) Comparition between Na[*o*-FESAN] and Na[*o*-^{57}FESAN].

Table S1. Average elemental concentrations and standard deviations (x ±sd, in ng/10^6 cells) determined by PIXE in U87 and RA whole-cells (T) and respective nuclear fractions (N), for controls (CTR) and after treatment with [*o*-^{57}FESAN]$^-$.

Table S2 - Estimated parameters from the Mössbauer spectra.

Experimental section:

Materials

The NaCl was purchased from Sigma–Aldrich, whereas the cationic exchanging resin used (Amberlite IR120, H form) was purchased from Acros Organics and the hydrochloric acid (37%) was purchased from Carlo Erba Reagents. Solvents used were from Carlo Erba SDS and purified by distillation from sodium and benzophenone under a nitrogen atmosphere before used. Na[3,3'-^{57}Fe(1,2-$C_2B_9H_{10}$)$_2$], Na[*o*-^{57}FESAN] was synthesized from [$HNMe_3$][7,8-$C_2B_9H_{12}$] by a modification method[1] of the original synthesis by Hawthorne *et al.*.[2]

[$HNMe_3$][7,8-$C_2B_9H_{12}$] was obtained from neutral *closo* 1,2-$C_2B_{10}H_{12}$ as described in the literature.[3] *closo* 1,2-$C_2B_{10}H_{12}$ was purchased from Katchem Spol.sr.o. $^{57}FeCl_2$ was a gift from https://www.porphyrin-laboratories.com/.

Instrumentation and Measurements:

The ^{1}H NMR (400.13 MHz) and ^{11}B NMR (128.37 MHz) spectra were recorded on a Bruker ARX 400 instrument equipped with the appropriate decoupling accessories. All NMR spectra were performed in deuterated acetone at 22ºC. The ^{11}B NMR chemical shift values were referenced to external $BF_3 \cdot OEt_2$, while the ^{1}H NMR chemical shift values were referenced to $SiMe_4$. Chemical shifts are reported in units of parts per million downfield from reference. The mass spectra were recorded in the negative ion mode using a Bruker Biflex MALDI-TOF-MS [N_2 laser; λ_{exc} 337 nm (0.5 ns pulses); voltage ion source 20.00 kV (Uis1) and 17.50 kV (Uis2)].

Synthesis of [NMe_4][3,3'-^{57}Fe(1,2-$C_2B_9H_{10}$)$_2$], Cs[*o*-^{57}FESAN]

In a two-necked flask, [NMe_4][7,8-$C_2B_9H_{12}$] (100 mg, 0.52 mmol) was dissolved in anhydrous THF (10 mL) under dinitrogen with stirring. The solution was cooled at 0ºC before adding K[*t*-BuO] (583 mg, 5.2 mmol), and left to react at room temperature for 1

h. Then, a magenta solution of anhydrous $^{57}FeCl_2$ (150 mg, 1.17 mmol) in THF (4 mL) was added under inert conditions. The reaction mixture was refluxed for 2.5 h, and once at room temperature, the solvent was evaporated, and the resulting solid was re-dissolved in THF (7.5 mL). Upon addition of 0.1M HCl (5 mL) and an excess of NaCl (236 mg, 1.4 mmol), the organic solution was isolated and washed with water. The THF was evaporated and the solid re-dissolved in water once again. After adding $[NMe_4]Cl$ to the aqueous solution, a dark violet precipitated was obtained, which was filtered and washed with water and hexane. $[NMe_4][3,3'\text{-}^{57}Fe(1,2\text{-}C_2B_9H_{11})_2]$, $[NMe_4]$[*o*-^{57}FESAN] (yield 310 mg, 0.78 mmol; 67%) was isolated.

Synthesis of Na$[3,3'\text{-}^{57}Fe(1,2\text{-}C_2B_9H_{10})_2]$, Na[*o*-^{57}FESAN]

Na[*o*-^{57}FESAN] was obtained by means of cationic exchange resin from $[NMe_4]$[*o*-^{57}FESAN]. Approximately 2/3 of the volume of the column (30 cm) was filled by the strongly acidic cationic exchange resin (Amberlite IR120, H form). Before starting, the cationic resin was kept 24 h in HCl 3 M to hydrate it. Then, 150 mL solution of HCl 3M was slowly passed through the column to load it with H^+. To remove the excess of HCl, distilled water flows down the column fast until neutral pH. When the desired cation was sodium, a solution of NaCl 3M was passed through the column slowly to exchange H^+ by Na^+ till neutral pH. Distilled water was used to rinse the excess of NaCl through the column. To know if NaCl was removed, 3 drops of a solution of $AgNO_3$ 100 mM was added to a small fraction of solution coming out of the column until a clear solution is observed. Then, 30 mL of acetonitrile/water (50:50) mixture was flown through the column to set the column's liquid composition. Approximately 200 mg of $[NMe_4]$[*o*-^{57}FESAN] was dissolved in a minimum volume of acetonitrile/water (50:50) and let to go repeatedly (4 times) through the cationic resin. Before collecting the solution containing the metallacarborane, 50 mL of fresh acetonitrile/water (50:50) was added to the column.

50 mL were collected in a flask, the solvent was evaporated and the compound was dried in vacuum. ^{1}H NMR (400 MHz, CD_3COCD_3) δ: 69.55 (br s), 45.92 (s, C–H), 38.96 (br s), 1.32 (br s, B–H), -7.8 (br s, B–H). ^{11}B NMR (128.37 MHz, CD_3COCD_3) δ: 102.1, 21.1, -0.6, -10.8, -16.8, -22.3, -33.2, -37.9, -39.0, -401.5 (s, B-H). MALDI-TOF MS: m/z (%) M of [*o*-^{57}FESAN]$^-$ theoretical 321.81, experimental 322.01 [M, 100%].

Characterization of Na[*o*-^{57}FESAN]

Figure S1. ^{11}B-NMR of Na[*o*-^{57}FESAN]

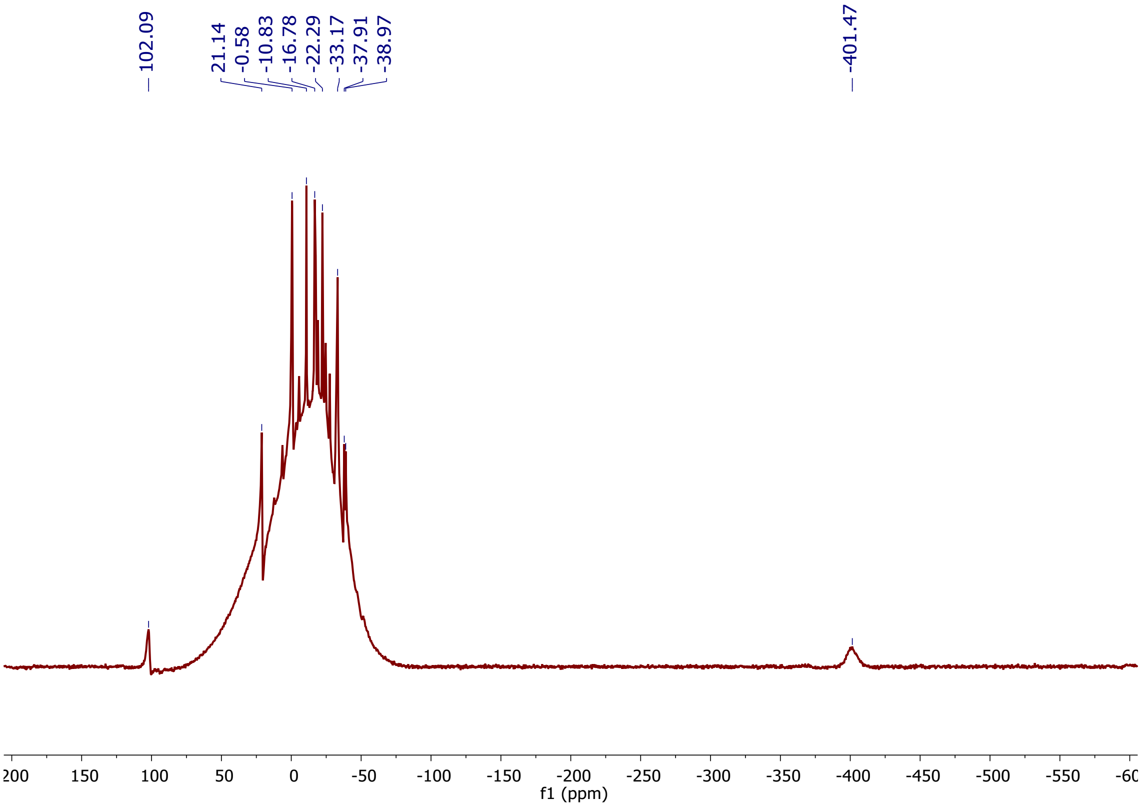

Figure S2. ^{11}H-NMR of Na[*o*-^{57}FESAN]

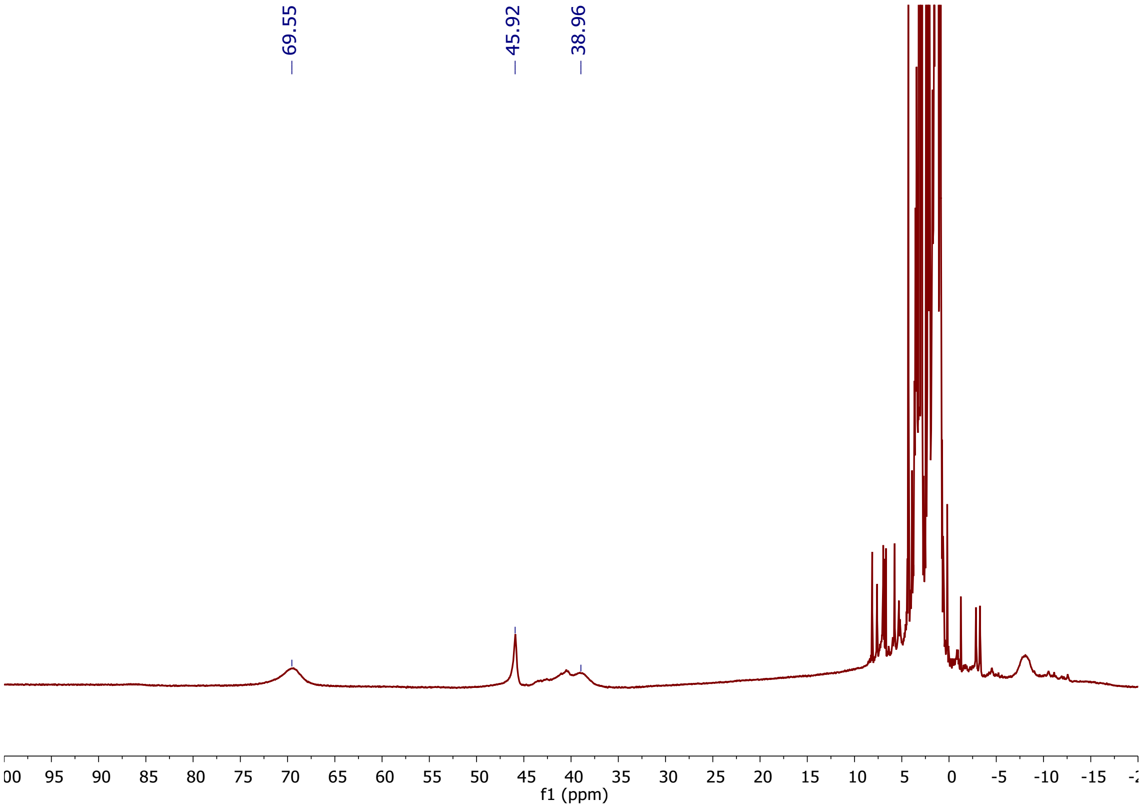

Figure S3. a) MALDI-TOF-MS of Na[*o*-^{57}FESAN]. b) Comparition between Na[*o*-FESAN] and Na[*o*-^{57}FESAN].

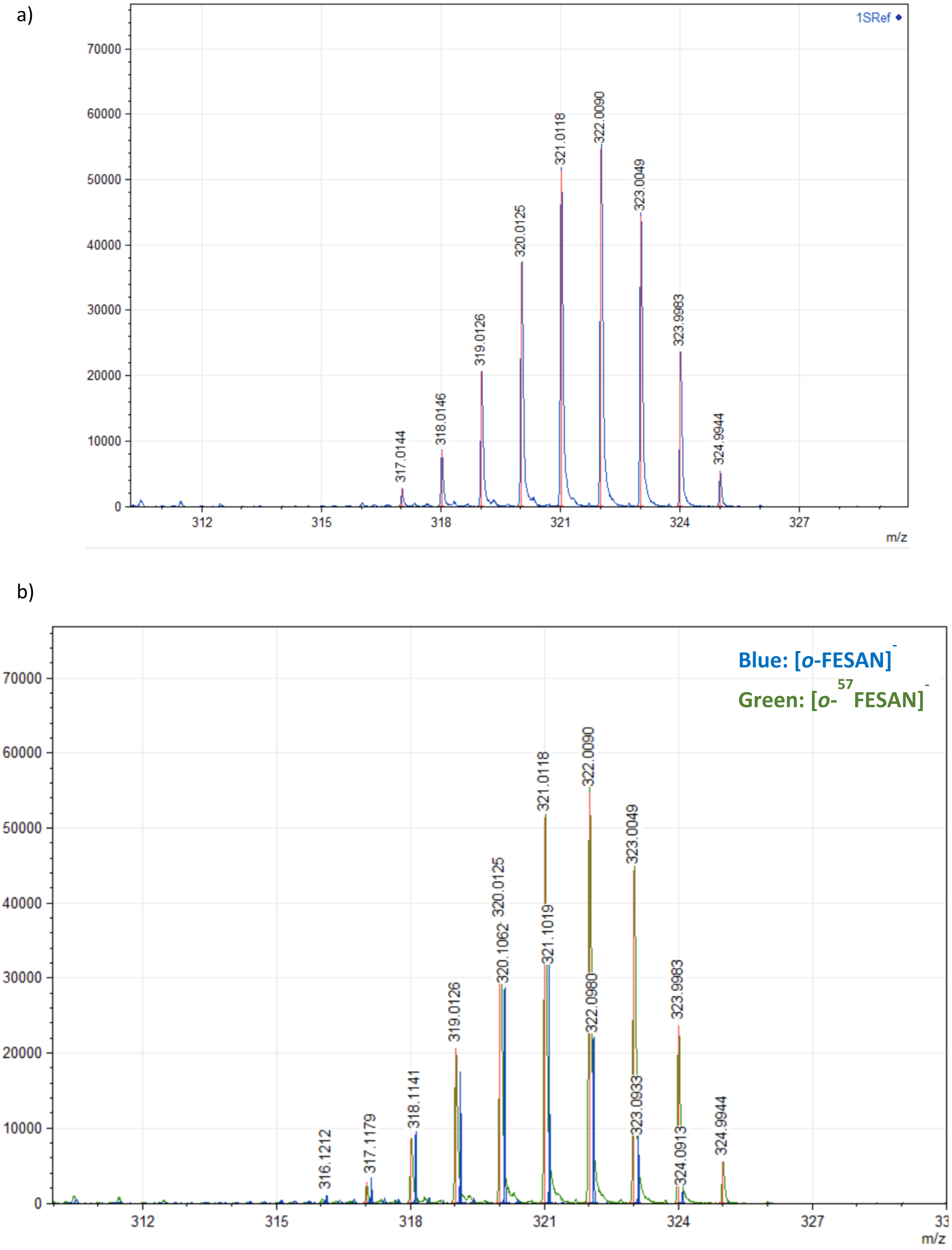

Table S1.- Average elemental concentrations and standard deviations (x ±sd, in ng/10^6 cells) determined by PIXE in U87 and RA whole-cells (T) and respective nuclear fractions (N), for controls (CTR) and after treatment with [*o*-^{57}FESAN]$^-$.

	K x±sd	**Ca** x±sd	**Fe** x±sd	**Cu** x±sd	**Zn** x±sd
U87 – CTR (T)	6090±66	670±21	75±4	11±3	99±7
U87 – CTR (N)	210±21	460±10	82±4	9±4	60±2
U87 - [*o*-^{57}FESAN]$^-$ (T)	7220±100	684±100	431±23	16±3	180±17
U87 - [*o*-^{57}FESAN]$^-$ (N)	336±8	180±4	112±3	8±4	20±2
RA – CTR (T)	2000±100	860±37	157±4	9±1	28±2
RA – CTR (N)	440±11	415±8	54±4	12±2	57±4
RA - [*o*-^{57}FESAN]$^-$ (T)	1125±37	736±17	195±4	14±2	48±3
RA - [*o*-^{57}FESAN]$^-$ (N)	215±6	237±4	49±2	8±1	26±2

Table S2 - Estimated parameters from Mössbauer spectra taken at 295 K and 80 K.

Sample	T	IS (mm/s)	QS (mm/s)
Na[*o*-^{57}FESAN]	295 K	0.15	0.55
Na[*o*-^{57}FESAN]	80 K	0.26	0.67
Ferrocene $[Fe(C_5H_5)_2]$ [7]	295 K	0.44*	2.36
Ferrocene $[Fe(C_5H_5)_2]$ [7]	80 K	0.53*	2.37

IS isomer shift relative to metallic α-Fe at 295 K; QS quadrupole splitting.

Estimated errors are ≤ 0.02 mm/s.

*Values for ferrocene are taken from ref [7]. In this reference the IS are given relative to a source of ^{57}Co diffused into metallic chromium. A value of 0.154 mm/s is therefore subtracted from the reported values to convert the IS for the metallic αFe standard.[4]

References

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[1] C. Viñas, J. Pedrajas, J. Bertran, F. Teixidor, R. Kivekäs and R. Sillanpää, *Inorg. Chem.*, 1997, 36, 2482.

[2] M. F. Hawthorne and T. D. Andrews, *J. Chem. Soc., Chem. Comm.*,1965, 443.

[3] M. F. Hawthorne, D. C. Young, P. M. Garrett, D. A. Owen, S. G. Schwerin, F. N. Tebbe and P. A. Wegner, *J. Am. Chem. Soc*. 1968, 90, 862-868.

[4] G.K. Shenoy and F.E.Wagner (eds.) "Mössbauer isomer shifts", North Holland Publ. Co., Amsterdam (1978).