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SUPPORTING INFORMATION

COSAN as molecular imaging platform: Synthesis and "*in vivo*" radiopharmaceutical assays.

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Experimental Section

Chemistry:

Instrumentation. Elemental analyses were performed using a Carlo Erba EA1108 microanalyzer. ATR-IR spectra (ν, cm^{-1}) were obtained on a Shimadzu FTIR-8300 spectrophotometer. The ^{1}H- and $^{1}H\{^{11}B\}$-NMR (300.13 MHz), $^{13}C\{^{1}H\}$-NMR (75.47 MHz) and ^{11}B- and $^{11}B\{^{1}H\}$-NMR (96.29 MHz) spectra were recorded on a Bruker ARX 300 instrument equipped with the appropriate decoupling accessories. All NMR spectra were performed in acetone deuterated solvent at 22ºC. The ^{11}B- and $^{11}B\{^{1}H\}$-NMR shifts were referenced to external $BF_3 \cdot OEt_2$, while the ^{1}H, $^{1}H\{^{11}B\}$ and $^{13}C\{^{1}H\}$-NMR shifts were referenced to $SiMe_4$. Chemical shifts are reported in units of parts per million downfield from the reference, and all coupling constants are reported in Hertz. 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)).

Materials. Experiments were carried out, except when noted, under a dry, oxygen-free dinitrogen atmosphere using standard Schlenk techniques, with some subsequent manipulation in the open laboratory. Nicotinic acid, palmitic acid, I_2 and K_2CO_3 and Na_2SO_3 were purchased from Sigma-Aldrich. Acetone was reagent grade and was obtained by distillation from appropriate drying agent before using. Dichloromethane, diethyl ether, acetonitrile were purchased from Carlo Erba Reagents. Silica gel for preparative layer chromatography (containing a 13% of calcium sulphate) was purchased from Fluka Analytical. [3,3'-Co(8-$(OCH_2CH_2)_2$-1,2-$C_2B_9H_{10}$)(1',2'-$C_2B_9H_{11}$)] [1] and Na[3,3'-Co(8-$(OCH_2CH_2)_2COOC_6H_5$-1,2-$C_2B_9H_{11}$)(1,2-$C_2B_9H_{11}$)], Na[**2**], were prepared according to the literature.[2]

Synthesis of [$Na^{\cdot}CH_3CH_2OCH_2CH_3$][3,3'-Co(8-$(OCH_2CH_2)_2COOC_6H_5$-1,2-$C_2B_9H_{10}$)(8'-I-1',2'-$C_2B_9H_{10}$)]. [$Na^{\cdot}CH_3CH_2OCH_2CH_3$][3].

Iodine (256mg, 1.0 mmol) was added to a stirred solution of Na[**2**] (280 mg, 0.5 mmol) in 10 mL of CH_2Cl_2. The reaction mixture was heated under reflux 1.5h and stirred overnight at room temperature. A solution of Na_2SO_3 in water was added to the organic solution to eliminate the excess of iodine. Then, the solvent was evaporated and the orange solid extracted with diethyl ether (3x10mL). After separation the organic layer, it

was dried over anhydrous $MgSO_4$. The solvent was evaporated under vacuum giving an orange solid compound, with a yield of 84.5% *(290mg)*. FTIR: ν= 3055, 3037 (C_c-H), 2926, 2869 (C-H)$_{alkyl}$, 2584, 2549 (B-H), 1707 (C=O), 1662 (H_2O), 1176, 1098 (C-O-C). ^{1}H NMR (CD_3COCD_3): δ= 8.06 (d, ^{3}J(H,H)= 6, 2H, C_6H_5), 7.64 (t, ^{3}J(H,H)= 6, 1H, C_6H_5), 7.52 (dd, ^{3}J(H,H)= 6, 2H, C_6H_5), 4.52 (br s, 2H, C_c-H), 4.44 (t, ^{3}J(H,H)= 6, 2H, OCH_2CH_2), 4.22 (br s, 2H, C_c-H), 3.80 (t, ^{3}J(H,H)= 6, 2H, OCH_2CH_2), 3.58 (m, 4H, OCH_2CH_2), 3.19-1.68 (br m,16H, B-H). ^{1}H{^{11}B} NMR (CD_3COCD_3) δ= 8.06 (d, ^{3}J(H,H) = 6, 2H, C_6H_5), 7.64 (t, ^{3}J(H,H)= 6, 1H, C_6H_5), 7.52 (dd, ^{3}J(H,H)= 6, 2H, C_6H_5), 4.52 (br s, 2H, C_c-H), 4.44 (t, ^{3}J(H,H)= 6, 2H, OCH_2CH_2), 4.22 (br s, 2H, C_c-H), 3.80 (t, ^{3}J(H,H)= 6, 2H, OCH_2CH_2), 3.58 (m, 4H, OCH_2CH_2), 3.19 (s, 2H, B-H), 3.02 (s, 2H, B-H), 2.86 (s, 1H, B-H), 2.82 (s, 1H, B-H), 2.38 (s, 2H, B-H), 1.99 (s, 2H, B-H), 1.74 (s, 2H, B-H), 1.68 (s, 4H, B-H). ^{13}C{^{1}H} NMR (CD_3COCD_3): δ= 165.91 (s, COO), 132.94 (s, C_6H_5), 130.39 (s, C_6H_5), 129.40 (s, C_6H_5), 128.46 (s, C_6H_5), 71.84 (s, OCH_2), 68.75 (s, OCH_2), 68.29 (s, OCH_2), 64.18 (s, OCH_2), 56.48 (s, C_c-H), 54.64 (s, C_c-H). ^{11}B NMR (CD_3COCD_3): δ= 23.0 (s, 1B, B(8)), 0.9 (d, ^{1}J(B-H)=129, 2B), -4.2 (d, ^{1}J(B-H)=135, 6B), -5.8 (d, ^{1}J(B-H)=116, 3B), -16.5 (d, ^{1}J(B-H)=170, 2B), -18.6 (d, ^{1}J(B-H)=183, 2B), -21.8 (d, ^{1}J(B-H)= 151, 1B), -25.8 (d, ^{1}J(B-H)=153, 1B). MALDI-TOF-MS: m/z= 657.46 (M, 100%). Elem. Anal. calc'd (found) $C_{19}B_{18}H_{43}IO_5Na$: C, 30.51 (30.09); H, 6.18 (5.71).

Radiochemistry:

General

Radiolabelling reactions on compounds [**1**]$^-$ ([Na.2$H_2$0][3,3'-Co(8-I-1',2'-$C_2B_9H_{10}$)(1,2-$C_2B_9H_{10}$)]) and [**3**]$^-$ ([Na.2$H_2$0][3,3'-Co(8-$(OCH_2CH_2)_2COOC_6H_5$-1,2-$C_2B_9H_{10}$)(8'-I-1',2'-$C_2B_9H_{10}$)] were performed by adapting the palladium catalyzed iodine exchange reaction previously reported for iodinated 1,2-dicarba-*closo*-dodecaborane (*o*-carborane).[3] Incorporation of both ^{125}I (*ex vivo* experiments) and ^{124}I (*in vivo* experiments) was carried out.

Na[^{124}I]I (solution in 0.02M aqueous NaOH) and Na[^{125}I]I (solution in 0.1M aqueous NaOH) were obtained from Perkin Elmer and used without further purification. All solvents were HPLC grade and were degassed for 10 minutes before use.

The manipulation of radioactive materials was performed in lead-shielded cabinets (75 mm of lead) and all procedures were performed according to the current regulation on radioprotection and internal protocols.

Labelling procedure

Acetonitrile (200μL) was added to Na[^{124}I]I or Na[^{124}I]I (50 μL, 37 MBq) and the resulting solution was introduced in a 2.5 mL conic vial. The solvent was evaporated to dryness (100 ºC, 5 min, constant helium flow at 20 mL/min) and 1 mg of precursor ([**1**]$^-$ or [**3**]$^-$) dissolved in acetonitrile (100 μL) was added together with *trans-bis*(acetate)*bis*[o-(di-o-tolylphosphino)benzyl] dipalladium (II) (Herrmann's catalyst, HC, 0.1 mg, 0.101 μmol) dissolved in toluene (100 μL). The reaction mixture was heated (100 ºC, 3 min for [**1**]$^-$; 80 ºC, 8 min for [**3**]$^-$), the solvent was removed under a constant helium flow and the resulting solid was dissolved in 1 mL of 0.1M ammonium formate (AMF)/acetonitrile (1/1) and purified by semi-preparative high performance liquid chromatography (HPLC). The purification conditions were: Stationary phase: Mediterranea Sea18 column (10x250 mm, 5 μm particle size); mobile phase: 0.1M ammonium formate (AMF) buffer pH=5.9 / acetonitrile (20/80); flow rate: 2ml/min. The purified fraction (RT: 13.5 and 15.0 min for [**1**]$^-$ and [**3**]$^-$, respectively) was reformulated by dilution with water, retention on a C-18 cartridge (Sep-Pak® Light, Waters) and further elution with ethanol (500 μL, Sigma-Aldrich). The final solution was evaporated to a final volume of 100 μL and reconstituted with saline (total volume = 1000 μL). Quality control was performed by radio-HPLC (Figure S1 for compound ^{124}I-[**1**]$^-$). Analytical conditions were: Stationary phase: Mediterranea Sea18 column (4.6x150 mm, 5 μm particle size); mobile phase A: 0.1M ammonium formate (AMF) buffer pH= 5.9; B: acetonitrile; flow rate = 1mL/min; gradient: 0 min: 80% A-20% B; 2min: 80% A-20% B; 12min: 20% A- 80% B; 16min: 20% A- 80% B; 17min: 80% A-20% B; 20min: 80% A- 20% B. Retention times were 12 and 14.5 min for [**1**]$^-$ and [**3**]$^-$, respectively.

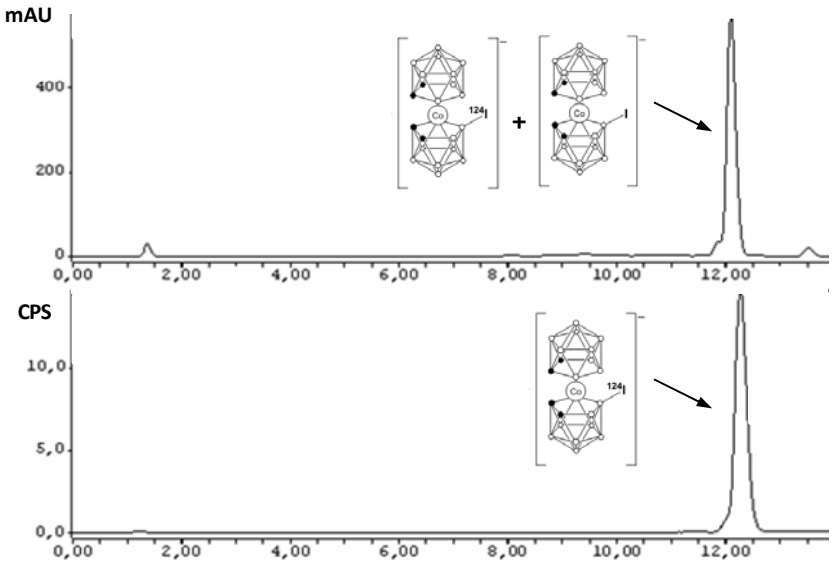

Figure S1. Chromatographic profiles corresponding to the quality control of ^{124}I-[**1**]$^{-}$; UV detector (top) and radiometric detector (bottom) profiles are shown. Connection of both detectors in series cause sligth difference in retention times in both profiles.

Determination of distribution coefficient (LogD):

1 MBq of the purified labelled compound was added to a premixed suspension containing 3 g of *n*-octanol and 3 g of 0.05 M phosphate buffer (PB) solution (pH=7.4) in a test tube. The tube was vortexed for 5 minutes at room temperature (RT) and centrifuged for 3 min at 5000 rpm. A weighted sample of each layer was measured in a gamma counter (2470 Wizard, Perkin Elmer). The partition coefficient was calculated as the logarithm of the ratio of the counts per gram from *n*-octanol versus PB.

***In vivo* studies:**

Biodistribution studies using Positron Emission Tomography-Computerized Tomography (PET-CT)

PET studies with ^{124}I-[**1**]$^{-}$ and ^{124}I-[**3**]$^{-}$ were carried out in mice (n=3 per compound) using an eXploreVista-CT small animal PET-CT system (GE Healthcare). Anesthesia was induced with 3% isoflurane and maintained by 1.5 to 2% of isoflurane in 100% O_2. For intravenous administration of the radiotracer, the tail vein was catheterized with a 24-gauge catheter and the radiotracer (250 ± 5 μCi, 100 μL) was injected concomitantly with the start of a PET dynamic acquisition.

Dynamic images (20 frames: 4x5s, 4x10s, 4x60s, 4X300s, 4x600s) were acquired in 2 bed positions in the 400-700 keV energetic window, with a total acquisition time of 130 minutes; after each PET scan, CT acquisitions were also performed, providing anatomical information as well as the attenuation map for the later image reconstruction. Mice were kept normothermic throughout the scans using a heating blanket (Homeothermic Blanket Control Unit; Bruker).

Dynamic acquisitions were reconstructed (decay and CT-based attenuation corrected) with filtered back projection (FBP) using a Ramp filter with a cut off frequency of 1 Hz. PET images were analyzed using PMOD image analysis software (PMOD Technologies Ltd, Zürich, Switzerland). Volumes of interest (VOIs) were manually drawn in the lungs, heart, kidneys, liver, small intestine, brain, bladder and stomach using the CT images as anatomical reference. VOIs were then transferred to the PET images and time activity curves (decay corrected) were obtained for each organ as cps/cm^3. Curves were transformed into real activity (Bq/cm^3) curves. Injected dose normalization was finally applied to data to get time activity curves as percentage of injected dose per cm^3 of tissue. Results at 3 selected time points are shown in Figures S2 and S3 for compounds ^{124}I-[**1**]$^-$ and ^{124}I-[**3**]$^-$, respectively.

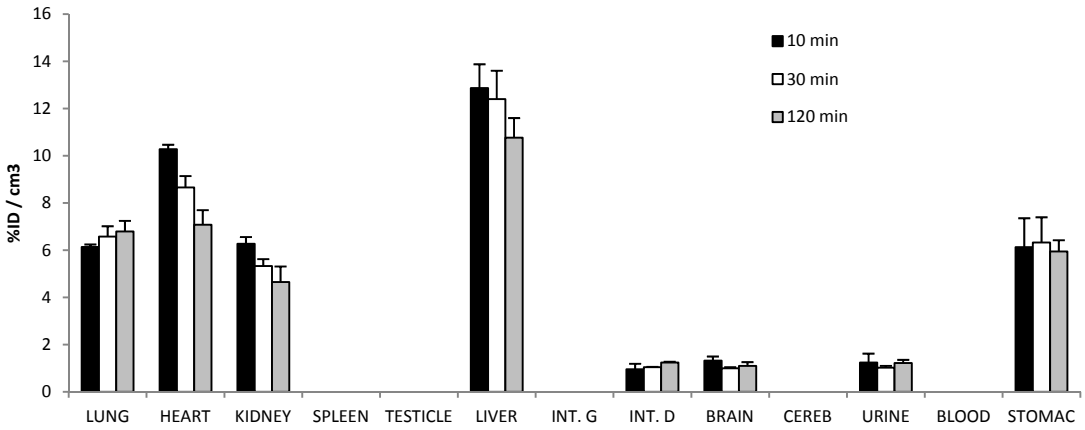

Figure S2: Accumulation of radioactivity in different organs for compound ^{124}I-[**1**]$^-$ determined by PET-CT at selected time points after administration; results are expressed as % of injected dose (%ID) per cm^3 of tissue. Mean ± standard deviation values are presented (n=3).

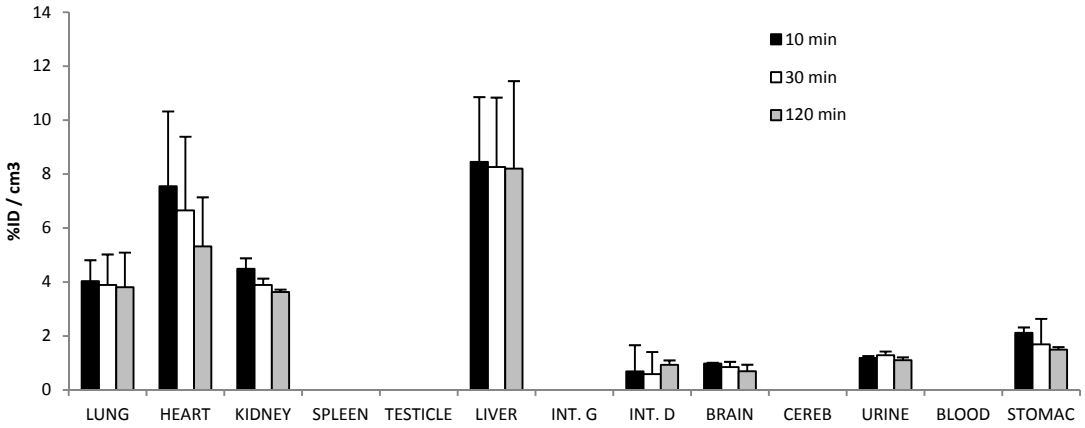

Figure S3: Accumulation of radioactivity in different organs for compound ^{124}I-[**3**]$^-$ determined by PET-CT at selected time points after administration; results are expressed as % of injected dose (%ID) per cm^3 of tissue. Mean ± standard deviation values are presented (n=3).

Biodistribution studies using dissection and gamma counting

Biodistribution studies using dissection and gamma counting were performed with ^{125}I-[**1**]$^-$ and ^{125}I-[**3**]$^-$. Three animals per compound and time point were used.

Anesthesia was induced with 3% isoflurane and maintained by 1.5 to 2% of isoflurane in 100% O_2. For intravenous administration of the radiotracer, the tail vein was catheterized with a 24-gauge catheter and the radiotracer (25 ± 5 μCi, 100 μL) was injected. The animals were kept under anesthesia throughout the duration of the study. At pre-selected time points (10, 30 and 120 minutes after administration) animals were sacrificed by exsanguination. The animals were perfused with saline solution, the organs were harvested, weighted and the amount of radioactivity was determined using a gamma counter. Comparison with a standard calibration curve enabled the determination of accumulated radiotracer as percentage of injected dose per gram of tissue.

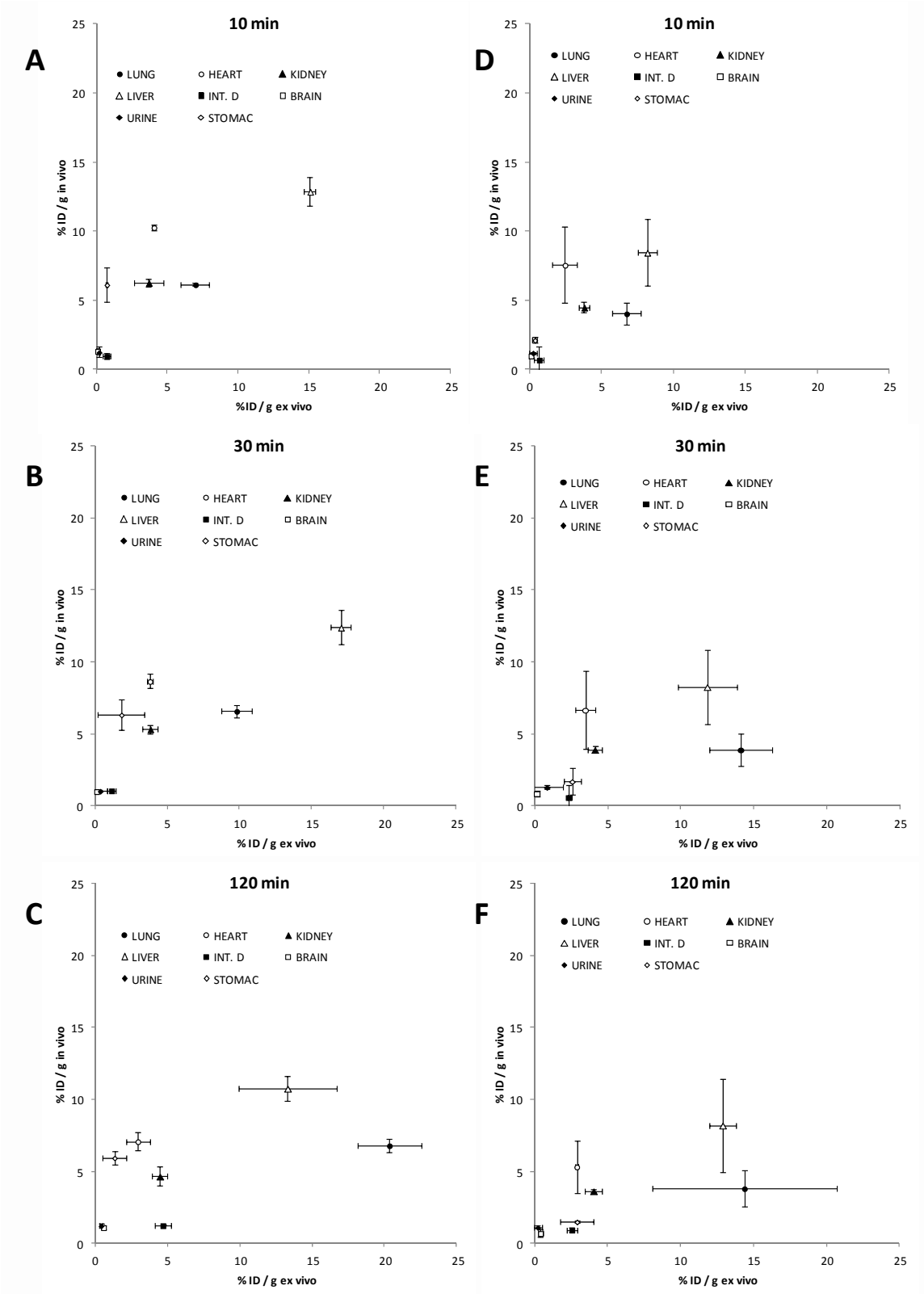

Figure S4: Correlation between results obtained using PET-CT (expressed as % of injected dose per cm^3 of tissue) and dissection and gamma counting (expressed as % of injected dose per gram of tissue) for compounds ^{125}I-[**1**]$^-$ (A-C) and ^{125}I-[**3**]$^-$ (D-F), at 10 (A, D), 30 (B, E) and 120 (C, F) minutes after administration.

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[2] P. Farràs, F. Teixidor, R. Kivekäs, R. Sillanpää, C. Viñas, B. Grüner and I. Cisarova, Inorg. Chem. 2008, 47(20), 9497.
[3] K. J. Winberg, G. Barberà, L. Eriksson, F. Teixidor, V. Tolmachev, C. Viñas, and S. Sjöberg, *J. Organomet. Chem.* 2003, *680*, 188.