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SUPPORTING INFORMATION

Tris(pyrazolyl)methane ^{99m}Tc tricarbonyl complexes for myocardial imaging

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General. All chemicals were of reagent grade and were used without purification. The syntheses of ligands and respective Re complexes were performed under a nitrogen atmosphere using dry and freshly distilled solvents, while the work-up was done in air. ^{1}H and ^{11}C NMR spectra were recorded on a Varian Unity 300 MHz spectrometer; ^{1}H and ^{11}C chemical shifts were referenced with the residual solvent resonances relative to tetramethylsilane. IR spectra were recorded as KBr pellets on a Bruker 27 Tensor spectrometer. C, H and N analyses were performed on an EA 110 CE Instruments automatic analyser. The starting material $[Re(H_2O)_3(CO)_3]Br$[1] was prepared according to published methods. The radioactive precursor *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ was prepared using a IsoLink® kit (Tyco Healthcare) following described procedures. Cardiolite kits for the preparation of ^{99m}Tc-Sestamibi were obtained from Bristol-Myers Squibb. $Na[^{99m}TcO_4]$ was eluted from a Elumatic ^{99}Mo/^{99m}Tc generator (CisBio) with 0.9% saline. HPLC analysis of the Re and ^{99m}Tc complexes was performed on a Perkin-Elmer

[1] Lazarova, N.; James, S.; Babich, J.; Zubieta, J. *Inorg. Chem. Commun.* **2004**, *7*, 1023-1026.

LC pump 200 coupled to a LC 290 tunable UV/Vis detector and to a Berthold LB-507A radiometric detector. Separations were achieved on a Nucleosil column (10μm, 250mm × 4mm), using a flow rate of 1mL/min; UV detection, 254 nm; eluents, A - aqueous 0.1% CF_3COOH solution, B- acetonitrile; method, t = 0-3 min, 0% B; 3-3.1 min, 0-25% B; 3.1-9 min, 25% B; 9-9.1 min, 25-34% B; 9.1-20 min, 34-100% B; 20-22 min, 100% B; 22-22.1 min, 100-0% B; 22.1-30 min, 0 % B.

Synthesis of [Na{HC[3,5-$(CH_3OCH_2)_2$pz]$_3$}$_2$]I (1) and [Na{HC[3,4,5-$(CH_3OCH_2)_3$pz]$_3$}$_2$]I (2)

Compounds **1** and **2** were synthesized by reacting tris[3,5-(methoxymethyl)pyrazolyl]methane **(L1)** or tris[3,4,5-(methoxymethyl)pyrazolyl]methane **(L2)** with NaI in THF, during 4 h at room temperature. After precipitation from a saturated THF solution (**1**) or recrystallization from THF/hexane (**2**), the solids obtained were vacuum dried yielding white microcrystalline solids. Crystals of **1** and **2** suitable for X-ray crystallographic analysis were obtained by recrystallization of **1** and **2** from THF and THF/hexane, respectively.

Compound 1. Starting from 89 mg (0.186 mmol) of **L1** and 14 mg (0.093 mmol) of NaI in 3mL THF, compound **1** was obtained in 83% yield (85 mg, 0.077 mmol).

^{1}H NMR ($CDCl_3$, δ (ppm)): 8.71 (2H, s, C<u>H</u>), 6.27 (2H, s, H(4) (pz)), 4.47 (12H, s, C<u>H</u>$_2$), 4.39 (12H, s, C<u>H</u>$_2$), 3.35 (18H, s, C<u>H</u>$_3$), 3.17 (18H, s, C<u>H</u>$_3$).^{13}C NMR ($CDCl_3$, δ (ppm)): 151.5 (C-3/5(pz)), 140.9 (C-3/5(pz)), 107.0 (C-4(pz)), 74.1 (C-H), 68.0 (<u>C</u>H$_2$), 64.2 (<u>C</u>H$_2$), 58.7 (C<u>H</u>$_3$), 57.6 (<u>C</u>H$_3$). ESI-MS *m/z*: 501.0 ([M-L$_1$]$^+$, calcd for $C_{22}H_{34}N_6O_6Na$, 501.2). Anal. Calcd. for $C_{44}H_{68}N_{12}O_{12}NaI$: C, 47.74; H, 6.19; N, 15.14%. Found: C, 48.35; H, 5.97; N, 15.34%.

Compound 2. Starting with 13 mg (0.085 mmol) of NaI in THF (1mL) and 103 mg (0.169 mmol) of **L2** dissolved in THF (3 mL) compound **2** was obtained in 81% yield

(94 mg, 0.069 mmol).

^{1}H NMR ($CDCl_3$, δ (ppm)): 8.80 (2H, s, C<u>H</u>), 4.53 (12H, s, C<u>H$_2$</u>), 4.41 (12H, s, C<u>H$_2$</u>), 4.32 (12H, s, C<u>H$_2$</u>), 3.32 (18H, s, C<u>H$_3$</u>), 3.27 (18H, s, C<u>H$_3$</u>), 3.16 (18H, s, C<u>H$_3$</u>).^{13}C NMR ($CDCl_3$, δ (ppm)): 149.9 (C-3/5(pz)), 139.0 (C-3/5(pz)), 116.5 (C-4(pz)), 73.5 (C-H), 67.0 (CH_2), 63.4 (CH_2), 62.0 (CH_2), 59.2 (CH_3), 58.0 (CH_3), 57.7 (CH_3). ESI-MS *m/z*: 633.1 ($[M-L_2]^+$, calcd for $C_{28}H_{46}N_6O_9Na$, 633.3). Anal. Calcd. for $C_{56}H_{92}N_{12}O_{18}NaI$: C, 49.00; H, 6.71; N, 12.25%. Found: C, 49.30; H, 6.50; N, 12.15%.

Synthesis of tris[3,5-(methoxymethyl)pyrazolyl]methane (L1) and tris[3,4,5-(methoxymethyl)pyrazolyl]methane (L2)

The tris(pyrazolyl)methane ligands, **L1** and **L2**, were synthesized by treatment of $CHCl_3$ with 3,5-bis(methoxymethyl)pyrazole (**a**) or 3,4,5-tris(methoxymethyl)pyrazole (**b**), using a phase transfer reaction[2] in the presence of $[NBu_4]Br$, under alkaline conditions. After reflux for 3 days, the organic phase was separated, washed with water and dried over $MgSO_4$. Evaporation of the solvent under vacuum yielded brown oils, which were purified by silica gel column chromatography to afford **L1** and **L2** as pale yellow oils.

Compound L1. Yield: 586 mg (1.22 mmol) 56 %, starting from 1.03g (6.61 mmol) of 3,5-bis(methoxymethyl)pyrazole (**a**).

^{1}H NMR ($CDCl_3$, δ ppm): 8.71 (1H, s, C<u>H</u>), 6.33 (3H, s, H-4 (pz)), 4.38 (6H, s, C<u>H$_2$</u>), 4.30 (6H, s, C<u>H$_2$</u>) 3.31 (9H, s, C<u>H$_3$</u>) 3.18(9H, s, C<u>H$_3$</u>). ^{13}C NMR ($CDCl_3$, δ ppm): 149.7 (C-3/5(pz)), 141.1 (C-3/5(pz)), 107.4 (C-4(pz)), 78.7 (C-H), 68.2 (<u>C</u>H$_2$), 64.6 (<u>C</u>H$_2$), 57.9, 57.8 (O<u>C</u>H$_3$). FTICR/MS *m/z*: 479.2616 (MH^+, calcd for $C_{22}H_{34}N_6O_6$, 479.2613).

Compound L2. Yield: 339 mg (0.56 mmol) 44%, starting from 3,4,5-

[2] Reger, D. L.; Grattan, T. C.; Brown, K. J.; Little, C. A.; Lamba, J. J. S.; Rheingold, A. L; Sommer, R. D. *J. Organomet. Chem.* **2000**, *607*, 120-128.

tris(methoxymethyl)pyrazole (**b**) (752 mg, 3.75 mmol).

^{1}H NMR ($CDCl_3$, δ (ppm)): 8.83 (1H, s, C<u>H</u>), 4.41 (6H, s, C<u>H$_2$</u>), 4.38 (6H, s, C<u>H$_2$</u>), 4.37 (6H, s, C<u>H$_2$</u>), 3.25 (9H, s, C<u>H$_3$</u>), 3.24 (9H, s, C<u>H$_3$</u>), 3.13 (9H, s, C<u>H$_3$</u>). ^{13}C NMR ($CDCl_3$, δ (ppm)): 148.5 ((C-3/5(pz)), 139.6 (C-3/5(pz)), 117.9 (C-4(pz)), 78.7 (<u>C</u>-H), 67.1 (<u>C</u>H$_2$), 63.6 (<u>C</u>H$_2$), 62.6 (<u>C</u>H$_2$), 57.8 (<u>C</u>H$_3$), 57.4 (<u>C</u>H$_3$). FTICR/MS *m/z*: 611.3399 (MH^+, calcd for $C_{28}H_{47}N_6O_9$, 611.3396).

Synthesis of 3,5-bis(methoxymethyl)pyrazole (a) and 3,4,5-tris(methoxymethyl)pyrazole (b)

The preparation of the ether-containing pyrazoles **a** and **b** comprised a multi-step synthesis procedure which started with the ester derivatives dimethyl 1H-pyrazole-3,5-dicarboxylate or trimethyl 1H-pyrazole-3,4,5-tricarboxylate, respectively (Scheme S1).[3] The N-tritylation of these esters afforded protected pyrazoles that were reduced with $LiAlH_4$. The O-alkylation of the resulting di- or trialcohols with methyl iodide gave protected ether-containing pyrazoles which were converted to the final compounds (**a** and **b**) by removal of trityl with TFA (Scheme S1).

3,5-Bis(methoxymethyl)pyrazole (a). Overall yield: 589 mg (3.80 mmol) 50%, starting from 1.411 g (7.66 mmol) of dimethyl-3,5-pyrazoledicarboxylate.

^{1}H NMR ($CDCl_3$, δ ppm): 10.72 (1H, br, NH), 6.19 (1H, s, H-4 (pz)), 4.45 (4H, s, C<u>H$_2$</u>), 3.32 (6H, s, C<u>H$_3$</u>). ^{13}C NMR ($CDCl_3$, δ ppm): 145.2 (C-3/5 (pz)), 103.4 (C-4 (pz)), 66.5 (<u>C</u>H$_2$), 58.0 (<u>C</u>H$_3$).

3,4,5-tris(methoxymethyl)pyrazole (b). Overall yield: 752 mg (3.75 mmol) 83%, starting from 1.100 g (4.54 mmol) of trimethyl-3,5-pyrazoletricarboxylate.

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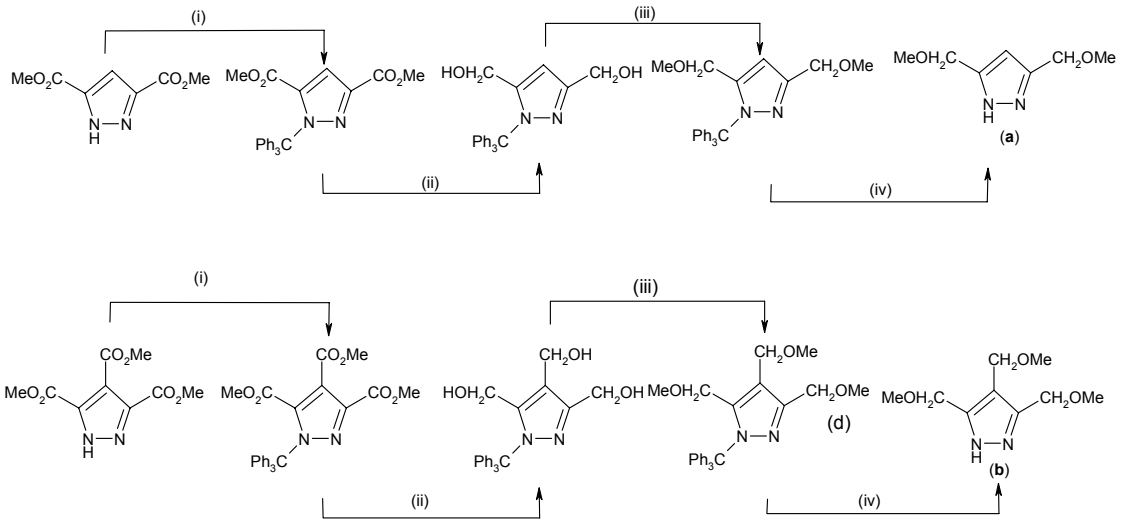
[3] (a) Schenck, T. G.; Downes ,J. M.; Milne, C. R. C.; Mackenzie, P. B.; Boucher, T. G., Whelan, J.; Bosnich, B. *Inorg. Chem.* **1985**, *24*, 2334-2337. (b) Chambers, D.; Denny, W. A.; Buckleton, J. S.; Clark, G. R. *J. Org. Chem.* **1985**, *50*, 4736-4738.

^{1}H NMR ($CDCl_3$, δ ppm): 10.85 (1H, br, NH), 4.51 (4H, s, C$\underline{H}_2$), 4.37 (2H, s, C$\underline{H}_2$), 3.35 (6H, s, C$\underline{H}_3$) 3.29 (3H, s, C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$, δ ppm): 144.1 (C-3/5 (pz)), 113.7 (C-4 (pz)), 65.5 (C$\underline{H}_2$), 63.7 (C$\underline{H}_2$), 58.1 (C$\underline{H}_3$), 57.7 (C$\underline{H}_3$).



Scheme S1. Synthesis of the ether-containing pyrazoles **a** and **b**.

(i) NaH, DMF, 30 min, rt; triphenylmethylchloride, overnight (o.n), rt; (ii) $LiAlH_4$, THF, o.n., rt; (iii) NaH, THF, 4 h, rt; CH_3I, 2h, rt; (iv) CF_3COOH, CH_2Cl_2/MeOH (1:1), 75-80 ºC, 24 h.

Synthesis of the Re complexes

***fac*-[Re(CO)$_3${HC[3,5-(CH$_3$OCH$_2$)$_2$pz]$_3$}]Br (3a).** A solution of [Re(CO)$_3$(H$_2$O)$_3$]Br (34 mg, 84 μmol) and **L1** (40 mg, 84 μmol) in methanol was refluxed overnight. The solvent was removed under vacuum and the residue was washed with diethyl ether. Compound **3a** was recovered as a beige solid after drying under vacuum. Yield: 63 mg (76 μmol) 91 %.

IR Data (KBr, ν/cm^{-1}): 1945s, 2037s (C≡O). ^{1}H NMR ($CDCl_3$, δ ppm): 9.47 (1H, s, C$\underline{H}$), 6.70 (3H, s, H-4 (pz)), 4.96 (6H, s, C$\underline{H}_2$), 4.63 (6H, s, C$\underline{H}_2$) 3.55 (9H, s, C$\underline{H}_3$) 3.50 (9H, s, C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$, δ ppm): 192.6 (br, CO), 157.0 (C-3/5(pz)), 144.3 (C-

3/5(pz)), 109.5 (C-4(pz)), 72.3 (CH), 67.9 (CH_2), 64.5 (CH_2), 59.3 (OCH_3). ESI-MS *m/z*: 748.9 (M^+, calcd for $C_{25}H_{34}N_6O_9Re$, 749.2).

***fac*-[Re(CO)$_3${HC[3,4,5-(CH_3OCH_2)$_3$pz]$_3$}]Br (4a)** Compound **4a** is a beige solid which was obtained according to the procedure described for **3a**, starting from $[Re(CO)_3(H_2O)_3]Br$ (12 mg, 30 μmol) and **L2** (19 mg, 31 μmol). Yield: 23 mg (23 μmol) 76 %.

IR Data (KBr, ν/cm^{-1}): 2039s, 2003m, 1939s, 1877s (C≡O). ^{1}H NMR ($CDCl_3$, δ ppm): 9.58 (1H, s, CH), 4.91 (6H, s, CH_2), 4.66 (6H, s, CH_2), 4.43 (6H, s, CH_2), 3.54 (9H, s, CH_3), 3.48 (9H, s, CH_3), 3.39 (9H, s, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 192.5 (br, CO), 154.8 (C-3/5(pz)), 143.3 (C-3/5(pz)), 119.7 (C-4(pz)), 72.7 (CH), 66.5 (CH_2), 63.0 (CH_2), 62.8 (CH_2), 59.7 (OCH_3), 59.3 (OCH_3), 58.9 (OCH_3). ESI-MS *m/z*: 881.0 (M^+, calcd for $C_{31}H_{46}N_6O_{12}Re$, 881.3).

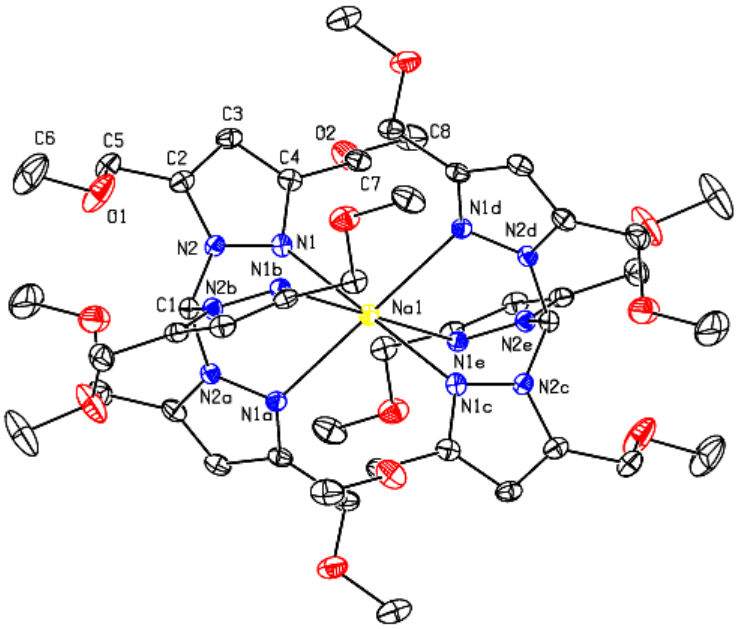


Figure S1. ORTEP diagram of compound **1**; ellipsoids are drawn at the 40% probability level.

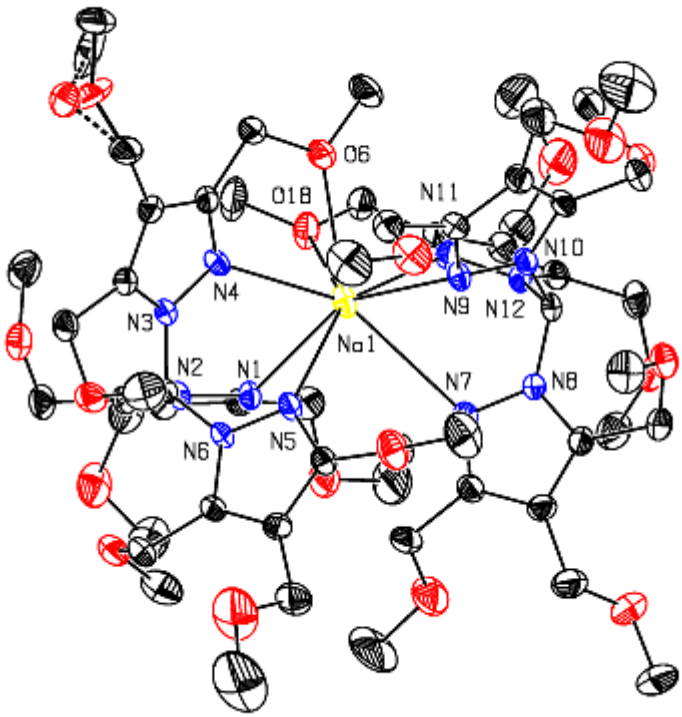


Figure S2. ORTEP diagram of compound **2**; ellipsoids are drawn at the 40% probability level.

General procedure for the synthesis of the ^{99m}Tc complexes. In a nitrogen-purged glass vial, 900 μL of the organometallic precursor *fac*-[^{99m}Tc$(OH_2)_3(CO)_3]^+$ were added to 100 μL of an ethanolic solution (10^{-2}-5.0x10^{-2} M) of **L1**/**L2** or to 100 μL of an aqueous solution (10^{-2}-5x10^{-2} M) of compounds **1** and **2**. The resulting mixtures were heated at 100ºC, for 30-60 min, yielding *fac*-[^{99m}Tc$(CO)_3${HC[3,5-$(CH_3OCH_2)_2$pz]$_3$}]$^+$ (**3**) and *fac*-[^{99m}Tc$(CO)_3${HC[3,4,5-$(CH_3OCH_2)_3$pz]$_3$}]$^+$ (**4**) , respectively. Complexes **3** and **4** have been obtained with a radiochemical yield ≥ 95 % (see Table S1), as checked by gradient HPLC analysis, and used in the biodistribution studies without further purification.

Table S1. Experimental conditions for the synthesis of complexes **3** and **4** and their radio-HPLC retention times and log *P* values

Complex	Yield (%)	[L]/M	Time (min)	T / ºC	t_R (min)[a]	log $P_{o/w}$
3	**96**	**10^{-3}**	**60**	**100**	**21.9 (21.4)**	**2.64 ± 0.002**
4	**≥ 98**	**5x10^{-3}**	**30**	**100**	**20.5 (19.8)**	**0.61 ± 0.04**

[a] Using a gradient of acetonitrile and aqueous 0.1 % CF_3COOH as the solvent. [b] The values in parentheses are for the Re congeners.

Animal Studies

Biodistribution Studies. The biodistribution of complexes **3** and **4** was evaluated in Sprague-Dawley rats (n=5) weighing approximately 125-165 g each. Rats were intravenously injected into the tail vein under light isofluorane anaesthesia with 100 μL (4.0-12.0 MBq) of each radioactive complex. The injected dose was assumed to be the difference between the measured radioactivity in the syringe before and after injection.

Rats maintained on normal diet *ad libitum* were sacrificed by excess anesthesia at 2, 5, 30 and 60 minutes. Blood was withdrawn from the heart with a syringe and main organs were excised, rinsed with saline, weighed and counted on a gamma counter. Studies were carried out according the EU guidelines for Animal Care and Ethic for Animal Experiments. Biodistribution results were expressed as percentage of the injected dose per gram tissue (%ID/g). ^{99m}Tc-Sestamibi was also evaluated in the same animal model, i.e. Sprague-Dawley rats, just for comparative purposes. The organ distribution (%ID/g) of **3**, **4** and ^{99m}Tc-Sestamibi in mice as a function of time is summarized in Table S2.

Imaging Studies. A separate set of Sprague-Dawley rats were anesthetized and intravenously injected with 37 MBq of **4.** Planar whole-body images were obtained at 5 and 30 min after injection with a gamma camera GE OPTIMA equipped with a LEGP collimator connected to a Starcam 400i computer. All the images were acquired in a 128 x 128 matrix.

Data and statistical analysis. The biodistribution data and heart to non-target organs ratios are expressed as an average value plus the standard deviation of results from 5 animals for each time point. Results were evaluated by an analysis of variance by using one-way ANOVA test. The level of significance was set at $p < 0.05$ (two-sided).

Table S2. Biodistribution of **3**, **4** and ^{99m}Tc-Sestamibi (MIBI) in Sprague-Dawley rat (% I.D./ g organ)

Organ	^{99m}Tc-3,4,5-(CO)$_3$-TMEOP (3)				^{99m}Tc-3,5-(CO)$_3$-DMEOP (4)				^{99m}Tc-MIBI	
	2 min	**5 min**	**30 min**	**60 min**	**2 min**	**5 min**	**30 min**	**60 min**	**5 min**	**60 min**
Blood	0.46 ± 0.16	0.22 ±0.04	0.21 ±0.01	0.14 ± 0.05	0.25 ±0.05	0.31 ±0.05	0.28 ±0.07	0.11 ±0.04	0.22 ±0.01	0.14 ±0.04
Liver	0.9 ± 0.2	0.24 ±0.07	0.25 ±0.02	0.10 ± 0.03	0.8 ± 0.1	1.0 ± 0.3	0.4 ± 0.1	0.19 ±0.02	1.2 ± 0.3	0.4 ± 0.1
Intestine	2.2 ± 0.6	1.31 ±0.04	1.62 ±0.2	1.8 ± 0.4	1.5± 0.3	1.5 ± 0.2	1.7 ± 0.5	1.5 ± 0.3	1.5 ± 0.1	3.9 ± 2.1
Spleen	0.9 ± 0.2	0.29 ±0.04	0.6 ±0.2	0.32 ±0.01	0.7 ± 0.2	0.9 ± 0.3	0.8 ± 0.5	0.4 ± 0.1	1.75 ±0.03	0.9 ± 0.6
Heart	4.7 ± 0.7	3.0 ±0.3	5.3 ±1.3	2.9 ± 0.1	3.3 ± 0.7	3.5 ± 0.6	3.9 ± 2.3	3.8 ± 0.8	2.8 ± 0.1	2.8 ± 0.3
Lung	1.4 ± 0.1	0.6 ±0.1	0.97 ±0.01	0.7 ± 0.1	0.9 ± 0.4	0.9 ± 0.5	1.6 ± 0.7	1.1 ± 0.4	1.9 ± 0.6	0.70 ±0.01
Kidney	5.2 ± 0.7	2.6 ±0.3	3.2 ± 0.3	1.5 ± 0.2	9.1 ± 0.7	8.3 ± 1.2	3.5 ± 0.5	2.6 ± 0.3	6.8 ± 2.2	2.5 ± 0.2
Muscle	0.6 ± 0.1	0.71 ±0.01	0.6 ± 0.1	0.47 ±0.05	0.4 ± 0.1	0.48 ±0.08	0.5 ± 0.1	0.5 ± 0.2	0.6 ± 0.1	0.38 ±0.08
Bone	0.6 ± 0.1	0.50 ±0.05	0.68 ±0.08	0.6 ±0.04	0.4 ± 0.1	0.54 ±0.07	0.5 ± 0.1	0.44 ±0.08	0.8 ± 0.1	0.6 ± 0.1
Stomach	0.9 ± 0.2	0.7 ± 0.1	0.9 ± 0.2	0.37 ±0.03	0.17 ±0.07	0.15 ±0.01	0.19 ±0.06	0.13 ±0.03	0.4 ± 0.0	0.7 ± 0.1
Heart/ Blood	11.7 ± 0.2	13.8 ± 1.8	28.1 ± 1.2	20.5 ±0.9	12.2 ± 3.4	12.4 ± 4.1	12.2 ± 5.5	34.9 ± 6.4	12.4 ±0.1	21.2 ± 5.8
Heart/ Liver	5.6 ± 1.3	14.9 ± 3.5	21.2 ± 3.3	28.8 ±1.2	4.0± 0.6	4.1 ± 1.7	13.2 ± 1.1	17.8 ± 2.1	2.5 ± 0.5	8.3 ± 2.3
Heart/ Lung	3.4 ± 0.6	4.9 ± 0.6	6.1 ± 0.6	5.0 ± 1.4	4.1 ± 0.4	4.4 ± 1.3	4.3 ± 0.9	4.1 ± 0.3	1.6 ± 0.4	4.0 ± 0.2