Supporting information

Novel Chlorin with a HYNIC: Synthesis, ^{99m}Tc-Radiolabeling, and Initial Preclinical Evaluation

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NMR spectra of compounds 4-7

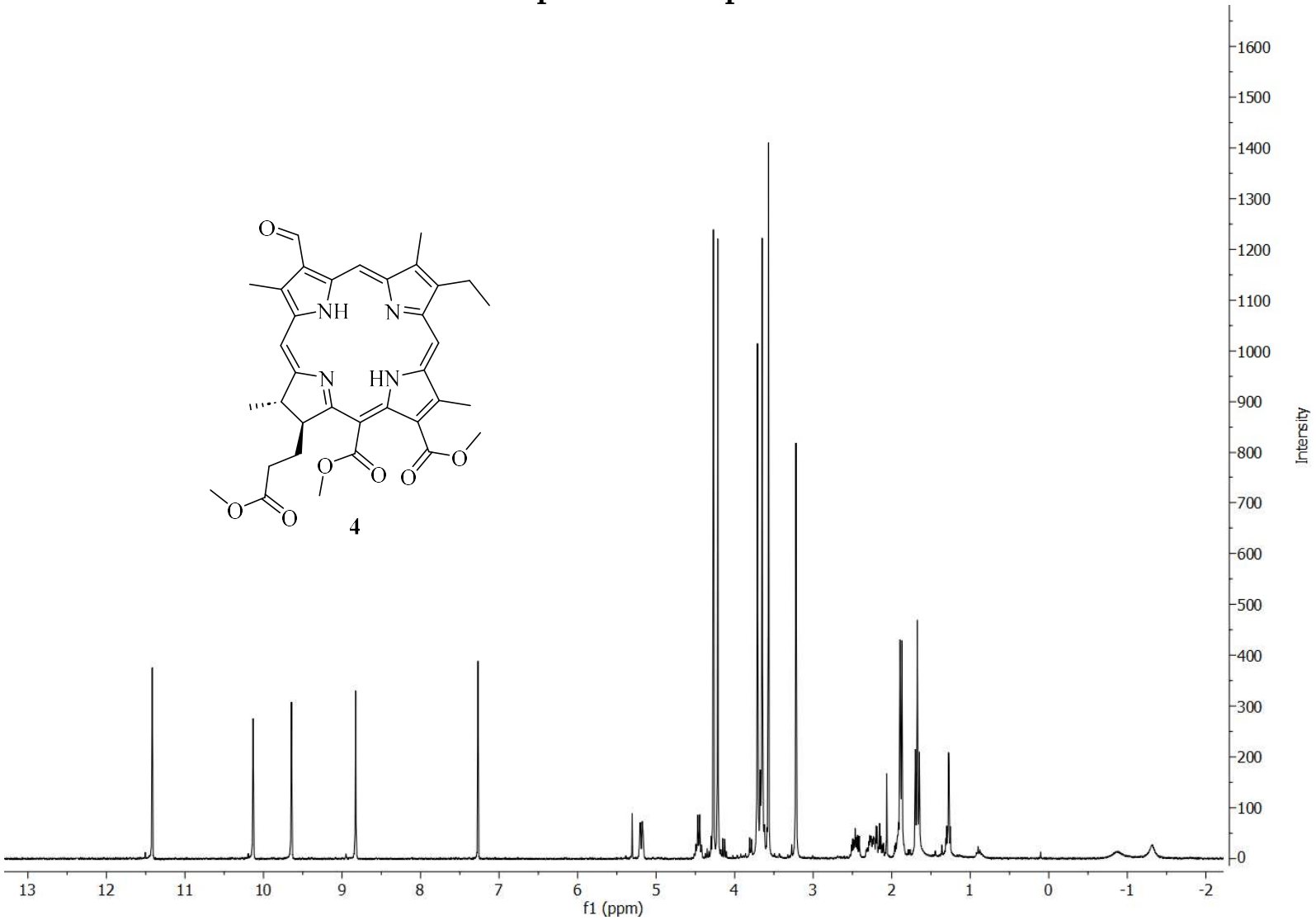

Figure S1: ^{1}H NMR spectrum of compound **4** (solvent: $CDCl_3$)

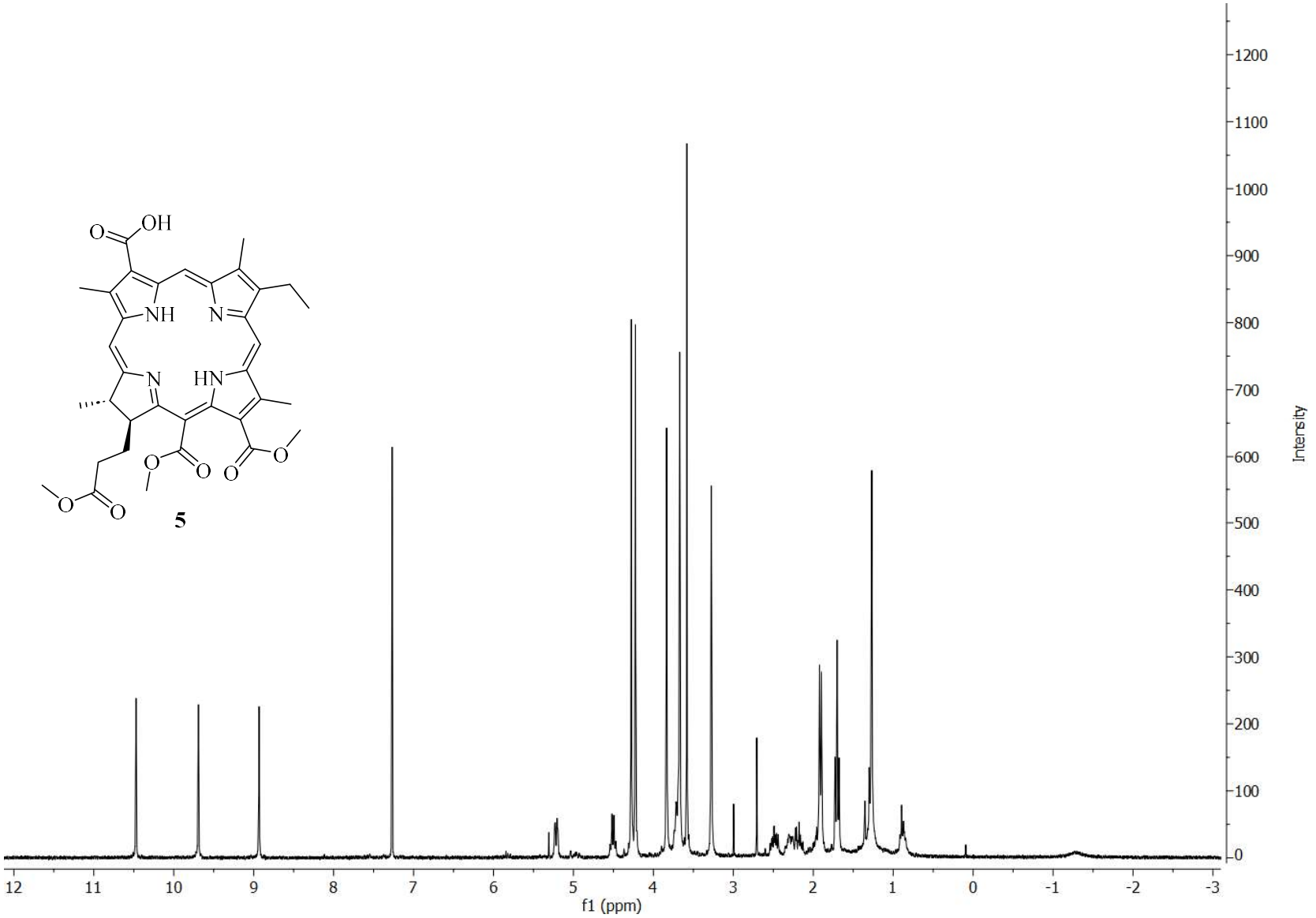

Figure S2: ^{1}H NMR spectrum of compound **5** (solvent: $CDCl_3$)

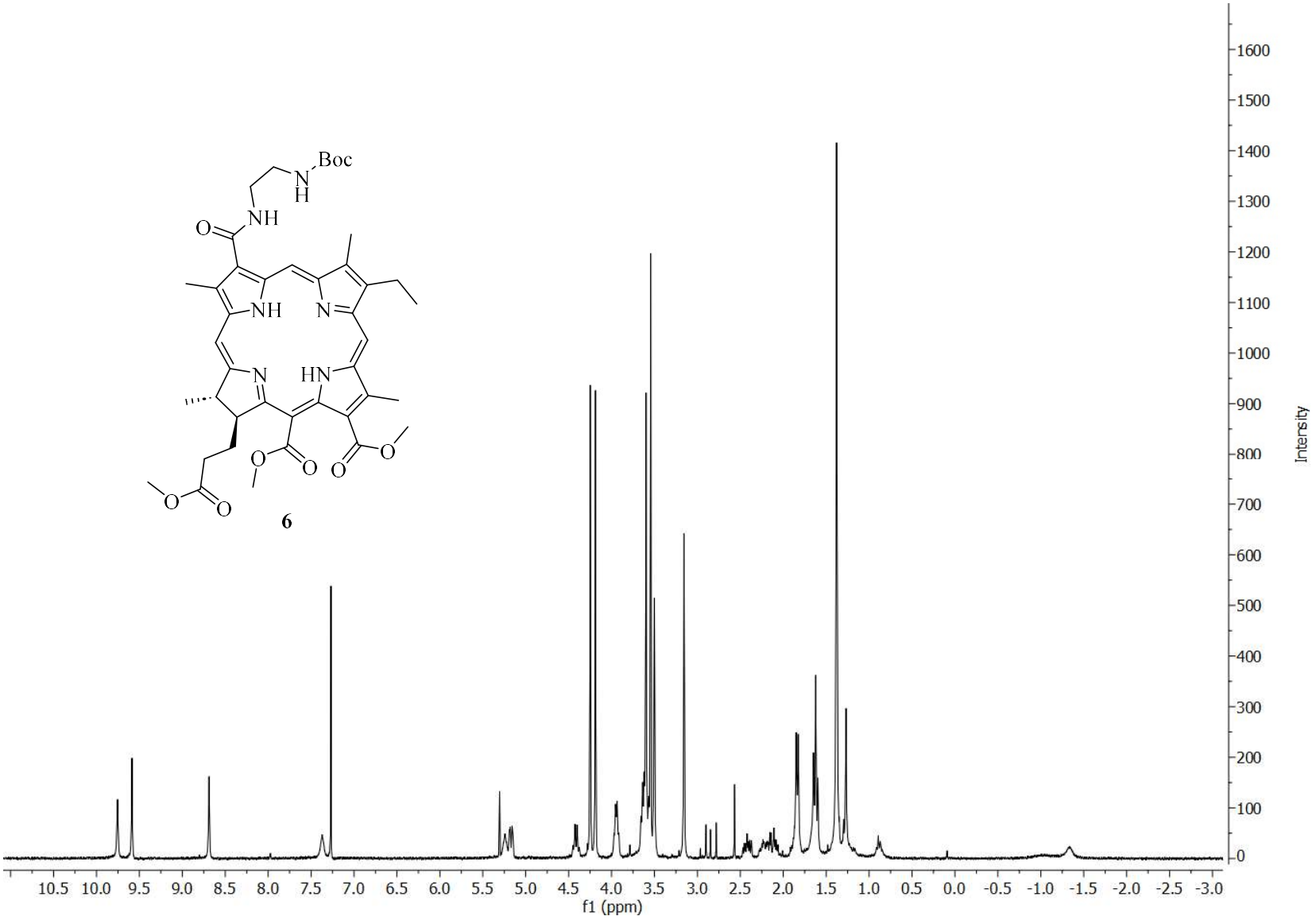

Figure S3: ^{1}H NMR spectrum of compound **6** (solvent: $CDCl_3$)

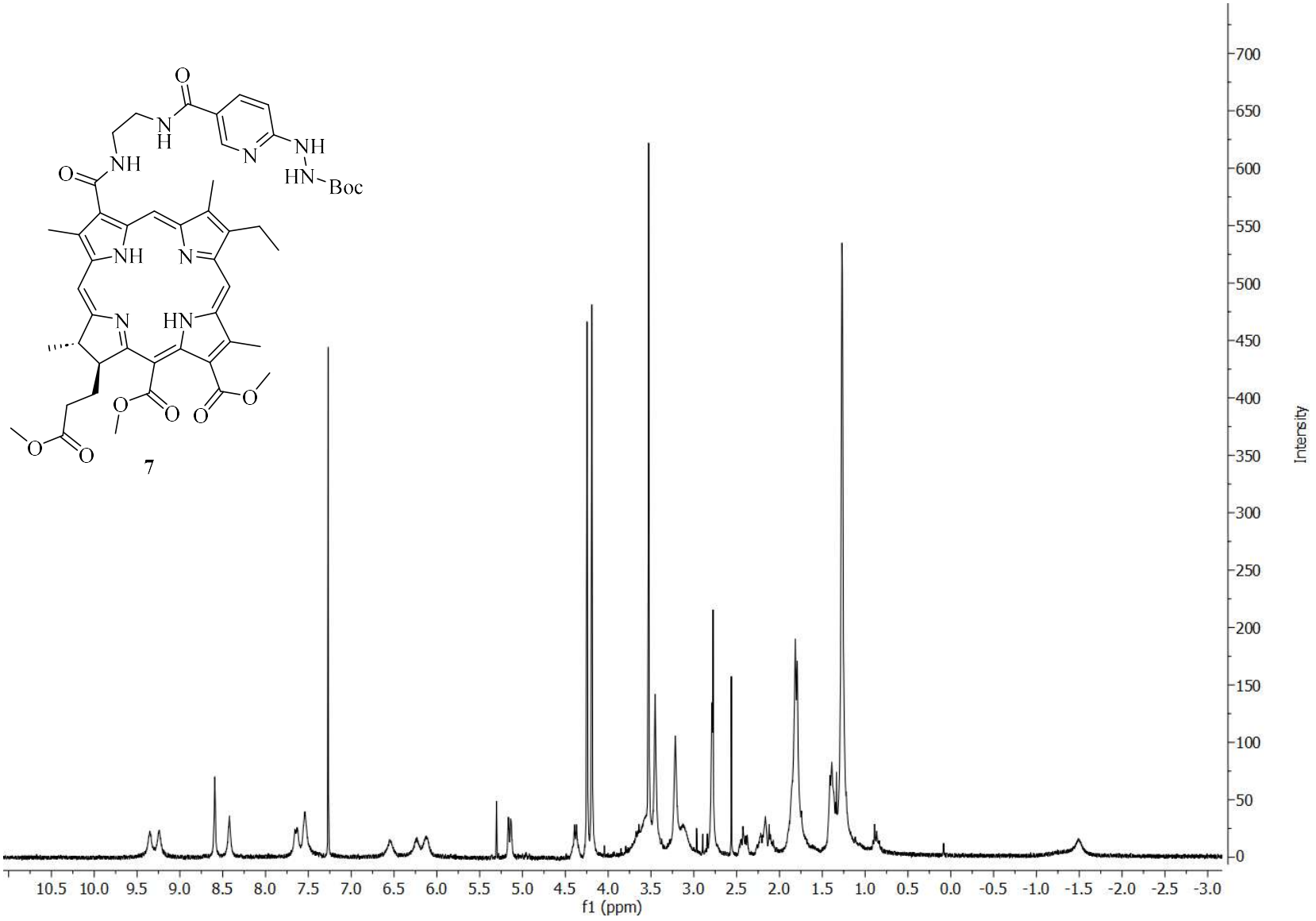

Figure S4: ^{1}H NMR spectrum of compound **7** (solvent: $CDCl_3$)

Chromatograms and high resolution mass spectra of HYNIC-containing compounds

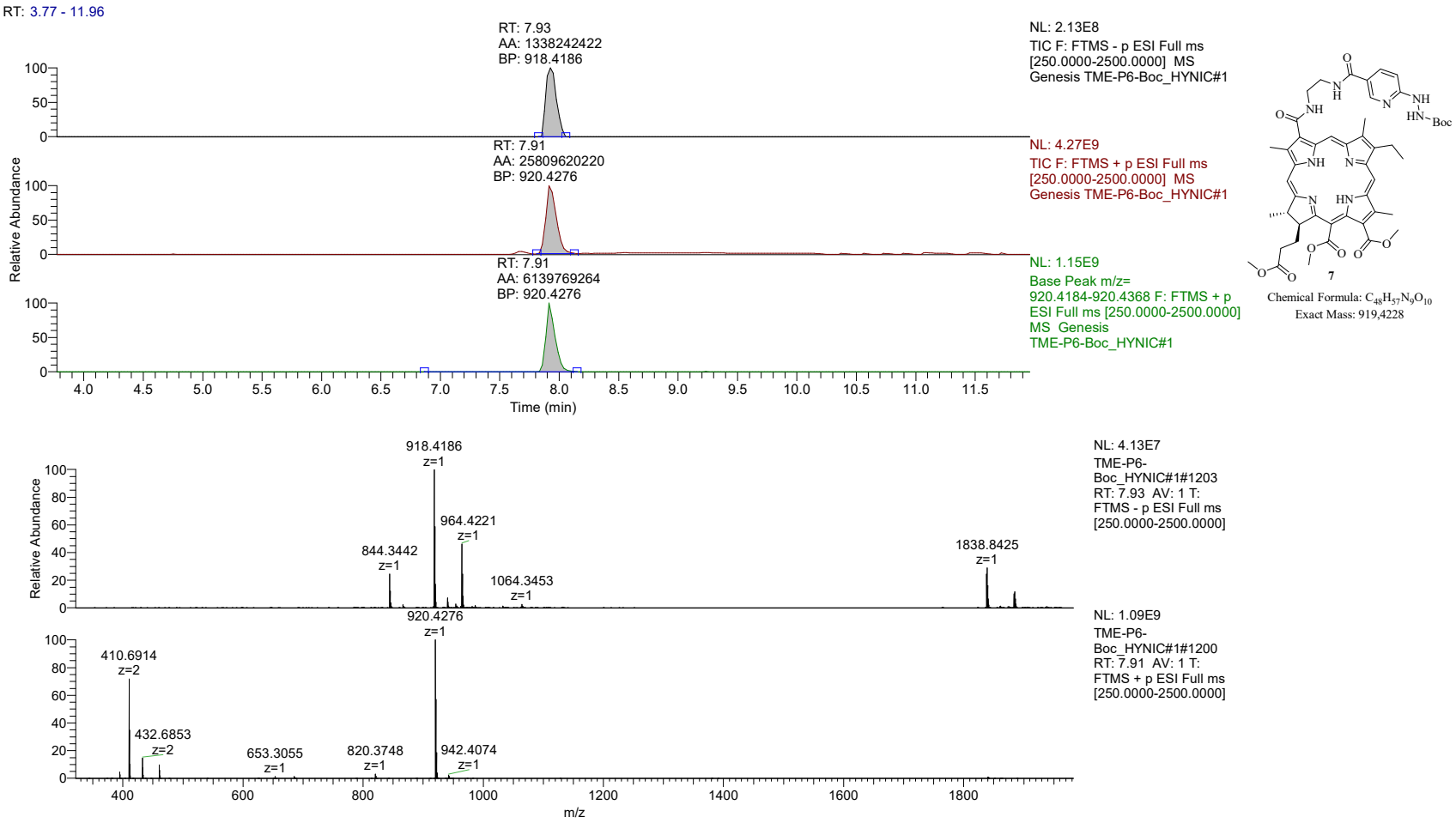

Figure S5: Chromatogram and high-resolution mass spectrum of compound **7**

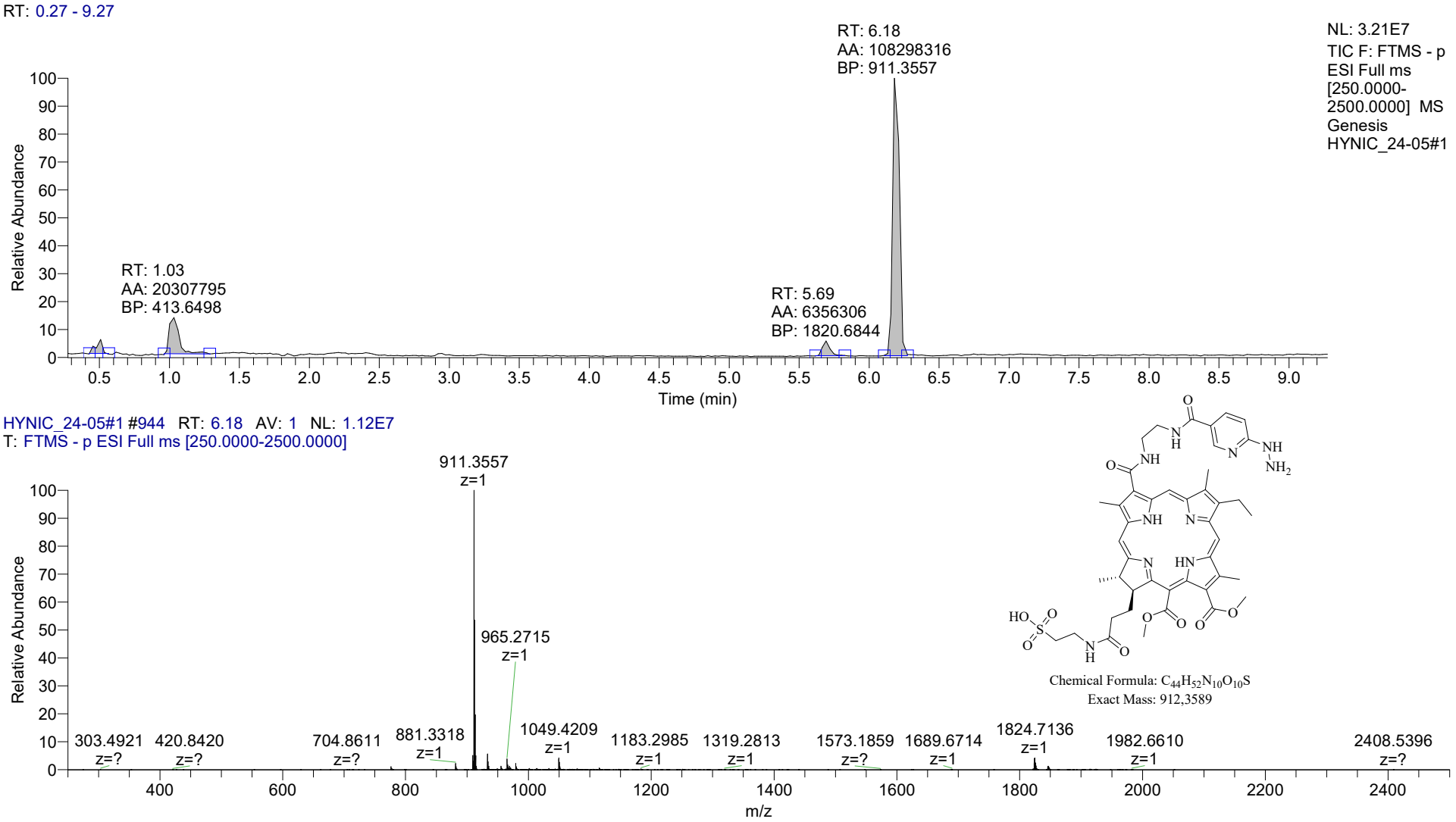

Figure S6: Chromatogram and high-resolution mass spectrum of **HYNIC-Chl**

UV-vis absorbance spectra of compounds 3-6

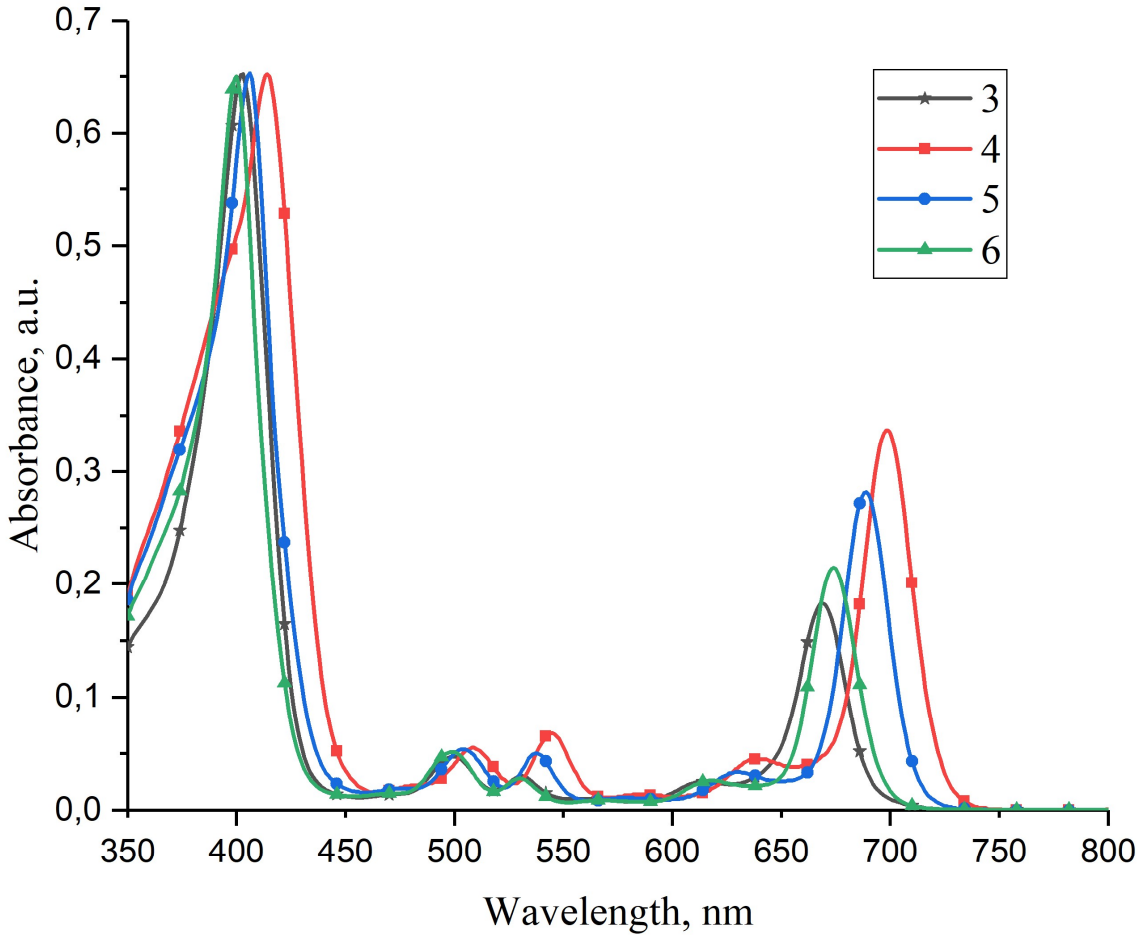

Figure S7: UV-vis absorbance spectra of compounds **3-6** in CH_2Cl_2

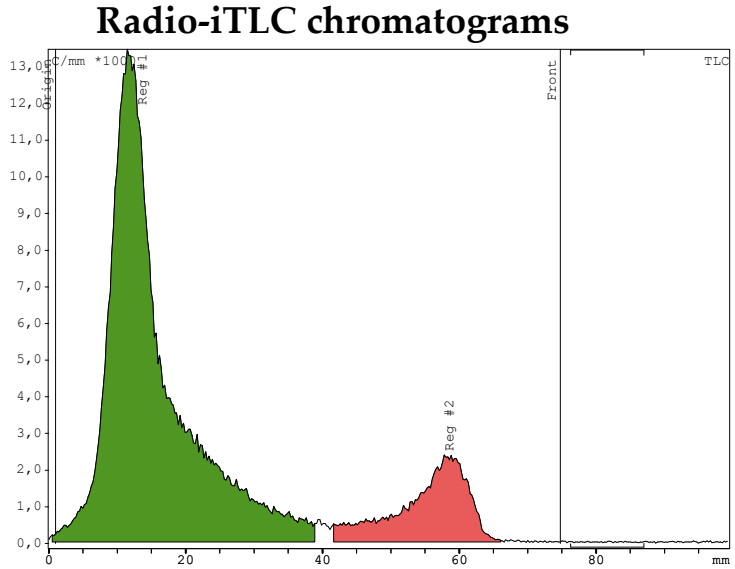

Figure S8: Radio-iTLC chromatogram of [^{99m}Tc]Tc-HYNIC-Chl yield after incubation at 85°C for 15 min (PBS as a mobile phase). The peak 1 corresponds to a mixture of [^{99m}Tc]Tc-HYNIC-Chl and [^{99m}Tc]TcO_2 impurity, the peak 2 corresponds to free $^{99m}TcO_4^-$ ions, [^{99m}Tc]Tc-EDDA and [^{99m}Tc]Tc-tricine.

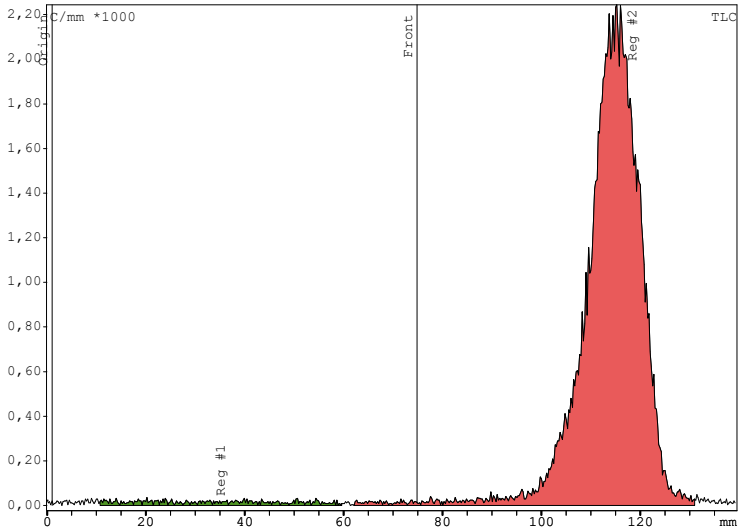

Figure S9: Radio-iTLC chromatograms of [^{99m}Tc]Tc-HYNIC-Chl yield after incubation at 85°C for 15 min (acetonitrile-water (1:1) as a mobile phase). The peak 1 corresponds to [^{99m}Tc]TcO_2 impurity, the peak 2 corresponds to [^{99m}Tc]Tc-HYNIC-Chl, free $^{99m}TcO_4^-$ ions, and other complexes.

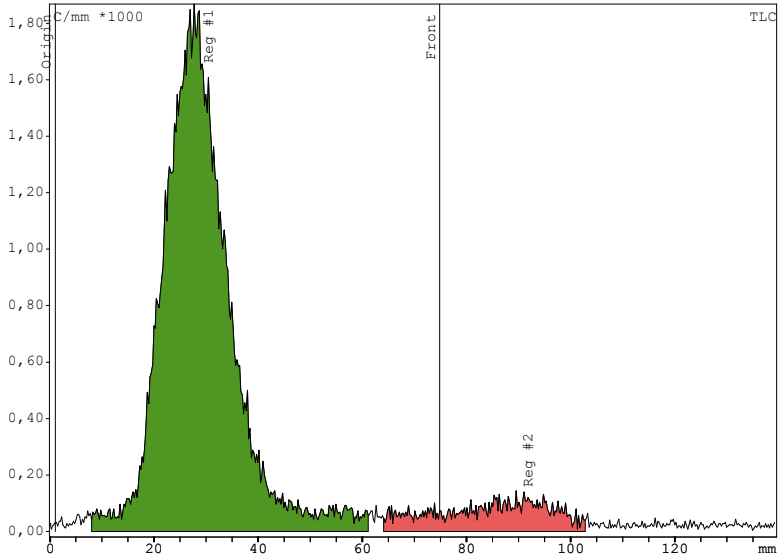

Figure S10: Radio-iTLC chromatograms of [^{99m}Tc]Tc-HYNIC-Chl purity after incubation at 85°C for 15 min and purification using C18 phase (PBS as mobile phase). The peak 1 corresponds to a mixture of [^{99m}Tc]Tc-HYNIC-Chl and [^{99m}Tc]TcO_2 impurity, the peak 2 corresponds to free $^{99m}TcO_4^-$ ions, [^{99m}Tc]Tc-EDDA and [^{99m}Tc]Tc-tricine.

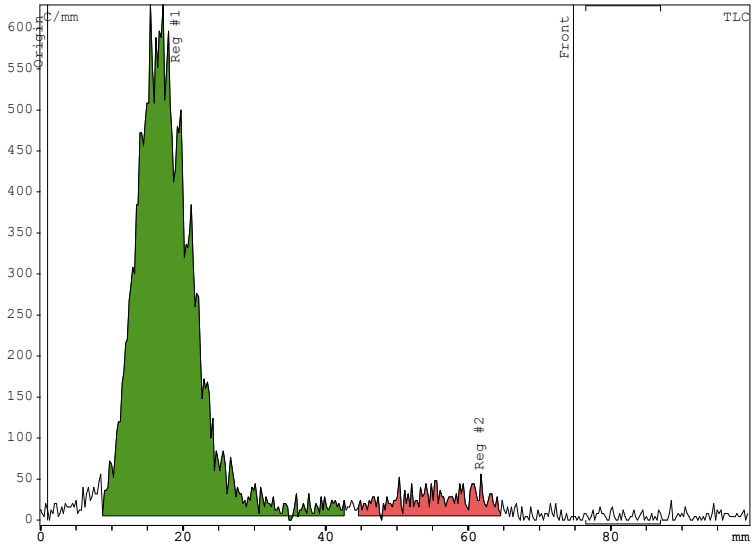

Figure S11: Radio-iTLC chromatograms of [^{99m}Tc]Tc-HYNIC-Chl purity after incubation during 4 h for stability test (PBS as mobile phase). The peak 1 corresponds to a mixture of [^{99m}Tc]Tc-HYNIC-Chl and [^{99m}Tc]TcO_2 impurity, the peak 2 corresponds to free $^{99m}TcO_4^-$ ions, [^{99m}Tc]Tc-EDDA and [^{99m}Tc]Tc-tricine.

Biodistribution data of ^{99m}Tc-HYNIC-Chl

Table S1: The dose-dependent biodistribution of [^{99m}Tc]Tc-HYNIC-Chl (injected doses of 1.2 mg/kg, 6 mg/kg, 12 mg/kg) in Nu/j mice bearing A-431 (epidermoid cancer tumor) xenografts at 2 h post-injection (pi). Data are presented as mean %ID/g ± SD for four animals. Data for the rest of the gastrointestinal tract with contents (GI tract) and the rest of the body are presented as %ID per whole sample.

Organs	^{99m}Tc-HYNIC-Chl 1.2 mg/kg	^{99m}Tc-HYNIC-Chl 6 mg/kg	^{99m}Tc-HYNIC-Chl 12 mg/kg
Blood	2.6 ± 0.3	3.5 ± 0.4	3.3 ± 1.0
Salivary glands	0.9 ± 0.1[b]	1.1 ± 0.3	1.3 ± 0.1
Brain	0.1 ± 0.1	0.13 ± 0.02	0.11 ± 0.01
Heart	1.2 ± 0.2	1.6 ± 0.4	1.6 ± 0.2
Lung	2.7 ± 0.4	2.9 ± 0.4	2.8 ± 0.4
Liver	33.5 ± 5.8	31.6 ± 4.8	26.7 ± 2.5
Spleen	7.0 ± 0.8[a]	9.4 ± 0.8	8.1 ± 1.0
Pancreas	0.6 ± 0.1	1.0 ± 0.3	0.9 ± 0.3
Small intestine	1.6 ± 0.5	2.1 ± 0.8	2.7 ± 0.6
Large intestine	1.7 ± 1.1	3.5 ± 2.1	2.3 ± 1.8
Stomach	1.3 ± 0.2	1.6 ± 0.3	1.8 ± 0.3
Kidney	3.7 ± 0.5	4.5 ± 1.1	3.6 ± 0.3
Muscle	0.33 ± 0.04	0.4 ± 0.1	0.5 ± 0.1
Bone	2.4 ± 0.1	3.0 ± 0.7	2.8 ± 0.8
Skin	1.1 ± 0.2	1.4 ± 0.4	1.5 ± 0.8
Fat	0.3 ± 0.1	0.5 ± 0.2	0.6 ± 0.3
Tumor	0.7 ± 0.2	0.8 ± 0.2	0.8 ± 0.1
GI	8.6 ± 0.6[b]	7.2 ± 1.4	6.6 ± 0.5
BODY	10.6 ± 1.6	12.8 ± 2.4	12.2 ± 1.0

a: significant differences between ^{99m}Tc-HYNIC-Chl 1.2 mg/kg and ^{99m}Tc-HYNIC-Chl 6 mg/kg

b: significant differences between ^{99m}Tc-HYNIC-Chl 1.2 mg/kg and ^{99m}Tc-HYNIC-Chl 12 mg/kg

c: significant differences between ^{99m}Tc-HYNIC-Chl 6 mg/kg and ^{99m}Tc-HYNIC-Chl 12 mg/kg

Table S2: Dose-dependent tumor-to-organ ratios of [^{99m}Tc]Tc-HYNIC-Chl (injected doses of 1.2 mg/kg, 6 mg/kg, 12 mg/kg) in Nu/j mice bearing A-431 (epidermoid cancer tumor) xenografts at 2 h post-injection (pi). Data are presented as mean %ID/g ± SD for four animals. Data for the rest of the gastrointestinal tract with contents (GI tract) and the rest of the body are presented as %ID per whole sample.

Organs	**^{99m}Tc-HYNIC-Chl 1.2 mg/kg**	**^{99m}Tc-HYNIC-Chl 6 mg/kg**	**^{99m}Tc-HYNIC-Chl 12 mg/kg**
Blood	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Salivary glands	0.8 ± 0.2	0.7 ± 0.1	0.7 ± 0.1
Brain	9.3 ± 5.2	5.7 ± 1.5	8.2 ± 1.4
Heart	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Lung	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Liver	0.02 ± 0.01	0.023± 0.004	0.03 ± 0.01
Spleen	0.11 ± 0.03	0.08 ± 0.02	0.11 ± 0.02
Pancreas	1.1 ± 0.3	0.8 ± 0.2	1.0 ± 0.3
Small intestine	0.5 ± 0.2	0.4 ± 0.1	0.3 ± 0.1
Large intestine	0.5 ± 0.4	0.3 ± 0.3	0.5 ± 0.3
Stomach	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.1
Kidney	0.2 ± 0.1	0.16 ± 0.03	0.23 ± 0.03
Muscle	2.0 ± 0.4	1.8 ± 0.1	1.8 ± 0.3
Bone	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Skin	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.2
Fat	2.8 ± 0.7	1.8 ± 0.4	1.9 ± 1.2