Supporting Information for

^{68}Ga-NODAGA-Indole: An Allysine-Reactive Positron Emission Tomography Probe for Molecular Imaging of Pulmonary Fibrogenesis

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General

Chemicals were purchased from commercial suppliers and used as received unless stated otherwise. Polymer-bound piperazine: 200-400 mesh, extent of labeling: 1.0-2.0 mmol/g loading, 2 % cross-linked with divinylbenzene (Sigma Aldrich, USA). 2,2′-(7-(1-amino-19-carboxy-16-oxo-3,6,9,12-tetraoxa-15-azanonadecan-19-yl)-1,4,7-triazonane -1,4-diyl)diacetic acid (NODAGA-PEG$_4$-NH$_2$ (**5**)) was purchased from CheMatech (Dijon, France) and used as received. Sep-Pak C_{18} Plus Long Cartridge: 820 mg Sorbent per Cartridge, 55-105 µm Particle Size, 50/pk (Waters, USA); preconditioning: 12 mL ethanol, 36 mL 10 mM HCl. Sep-Pak C_{18} Plus Light Cartridge: 130 mg Sorbent per Cartridge, 55-105 µm Particle Size, 50/pk (Waters, USA); preconditioning: 4 mL ethanol, 12 mL H_2O. $^{68}GaCl_3$ was obtained from a SnO_2-based $^{68}Ge/^{68}Ga$ generator (itG) (RadioMedix, USA).

HPLC-MS:

HPLC-MS purity analysis was carried out on an Agilent 1260 system (Phenomenex Luna, 5 µm C18(2) 100 Å, 100 × 2 mm, flow rate: 0.8 mL min^{-1}; UV detection at 220, 254 and 280 nm) coupled to an Agilent Technologies 6130 Quadrupole MS system using the following method:

Mobile Phases: A: H_2O (+ 0.1% formic acid, v/v) B: CH_3CN (+ 0.1% formic acid, v/v)

Method 1:

min	% A	% B
0	95	5
0.5	95	5
5.5	5	95
6.5	5	95
7	95	5
8	95	5

Preparative HPLC:

Preparative reversed-phase HPLC was carried out on an Agilent 1260 Infinity II system (Phenomenex Luna, 10 µm C18(2) 100 Å, 250 × 21.2 mm, flow rate: 15.0 mL min^{-1}; UV detection at 220, 254 and 280 nm) using the following methods:

Mobile Phases: A: H_2O (+ 0.1% TFA, v/v) B: CH_3CN (+ 0.1% TFA, v/v)

Method 2:

min	% A	% B
0	95	5
5	95	5
35	5	95
40	5	95
41	95	5
46	95	5

Method 3:

min	% A	% B
0	95	5
10	95	5
40	5	95
45	5	95
46	95	5
51	95	5

HPLC-ICP-MS

HPLC-ICP-MS was carried out on an Agilent 1260 HPLC system (Phenomenex Gemini 5µm C18 110 Å, 10 × 3.0 mm, flow rate: 1.0 mL min^{-1}) coupled to an Agilent 8800-QQQ ICP-MS system, using the following HPLC methods:

Mobile Phases: A: H_2O (+ 0.1% TFA, v/v) B: CH_3CN (+ 0.1% TFA, v/v)

Method 4:

min	% A	% B
0	95	5
1	95	5
12	5	95
13	5	95
14	95	5
15	95	5

Method 5:

min	% A	% B
0	95	5
1	95	5
9	5	95
10	5	95
11	95	5
12	95	5

Radio-HPLC

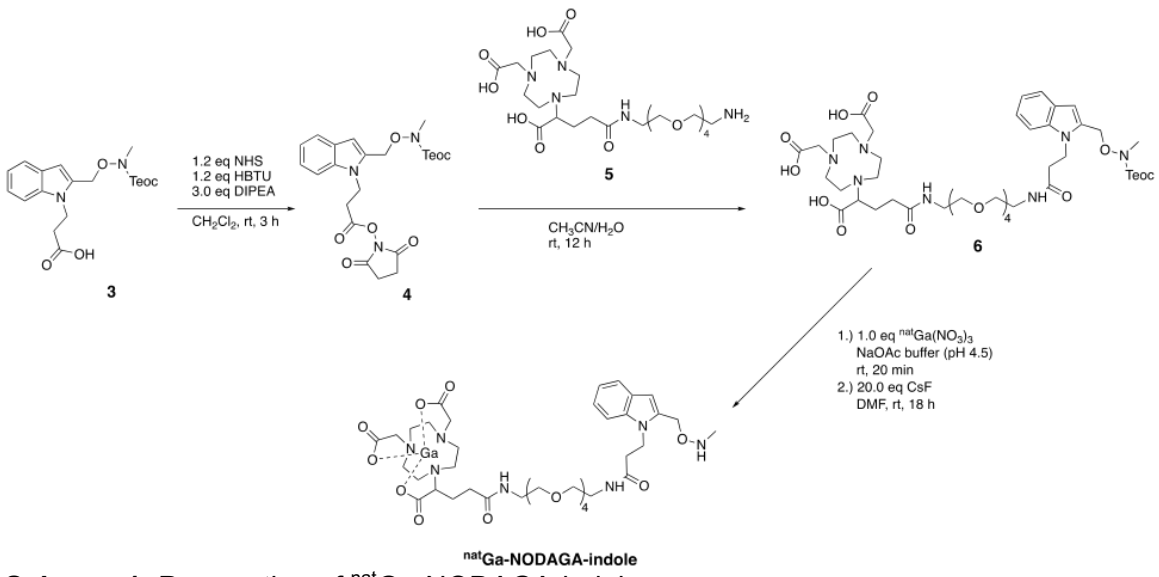
Radio-HPLC analysis was carried out on an Agilent 1100 Series HPLC unit (Kromasil, 5µm C4 100 Å, 150 × 4.6 mm, flow rate: 1.0 mL min^{-1}) with a Carroll/Ramsey radiation detector with a silicon PIN photodiode.

Mobile Phases: A: H_2O (+ 0.1% TFA, v/v) B: CH_3CN (+ 0.1% TFA, v/v)

Method 6:

min	% A	% B
0	95	5
4	95	5
23	5	95
26	5	95
27	95	5
30	95	5

Preparation of ^{nat}Ga-NODAGA-indole



Scheme 1. Preparation of ^{nat}Ga-NODAGA-indole.

Preparation of NODAGA-indole-Teoc (6)

3-(2-(3,8,8-trimethyl-4-oxo-2,5-dioxa-3-aza-8-silanonyl)-1*H*-indol-1-yl)propanoic acid (**3**) was synthesized according to Ref.[1] The starting material **3** (3.2 mg, 13 µmol, 1.25 eq relative to NODAGA-PEG$_4$-NH$_2$ (**5**)) was dissolved in CH_2Cl_2 (3 mL, dry) and *N*-Hydroxysuccinimide (NHS) (1.8 mg, 16 µmol, 1.5 eq), (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (6.1 mg, 13 µmol, 1.5 eq) , and *N*,*N*-diisopropylethylamine (DIPEA) (40 µmol, 7.0 µL, 3.75 eq, dry) were added. The reaction mixture was stirred at ambient temperature for 3 h. After extraction with H_2O (2 x 1.5 mL 0.1 M HCl solution, 1 x 1.5 mL H_2O), the organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The crude product 2,5-dioxopyrrolidin-1-yl 3-(2-(3,8,8-trimethyl-4-oxo-2,5-dioxa-3-aza-8-silanonyl)-1*H*-indol-1-yl)propanoate (**4**) was obtained as yellow oil and was used without further purification.

NODAGA-PEG$_4$-NH$_2$ (**5**) (11 µmol, 6.0 mg, 1.0 eq) was dissolved in H_2O (1 mL) and the pH was adjusted to 9.4 with DIPEA. Compound **4** prepared above was dissolved in CH_3CN (2 mL) and added quickly to the aqueous solution. The white suspension was stirred at ambient temperature for 12 h, filtered and purified by preparative HPLC (method 2) to isolate the title compound **6** as a colorless solid.

Yield over 2 steps: 9.5 mg (89 %)

LC/MS (ESI+): $C_{44}H_{73}N_7O_{15}Si$: m/z calcd 968.5 [M+H$^+$]; found 968.4 (M+H$^+$)

R_t (method 1): 4.21 min (purity > 99 %)

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.54 (d, *J* = 7.9 Hz, 1H), 7.41 (d, *J* = 7.5 Hz, 1H), 7.24 – 7.14 (m, 1H), 7.13 – 7.02 (m, 1H), 6.54 (s, 1H), 5.03 (s, 2H), 4.58 (br, 2H), 4.26 – 4.11 (m, 2H), 3.85 – 3.25 (m, 21H), 3.23 – 2.59 (m, 14H), 2.41 (br, 1H), 2.17 – 1.84 (m, 2H), 1.68 – 1.40 (m, 1H), 1.25 (s, 3H), 1.02 – 0.92 (m, 2H), 0.84 (br, 3H), 0.03 (s, 9H).

^{13}C NMR (126 MHz, CD_3CN/CD_3OD (1:1)) δ = 176.2, 173.7, 171.2, 171.0, 159.6, 139.2, 135.5, 129.2, 124.2, 122.8, 121.6, 111.9, 72.1, 72.1, 71.9, 71.8, 71.0, 70.9, 69.1, 66.3, 41.9, 41.4, 41.0, 38.4, 37.8, 35.1, 25.7, 19.2, -0.6.

(one signal is located under the solvent peak at 118.3)

Preparation of ^{nat}Ga-NODAGA-indole

The starting material **6** (12.1 mg, 14 µmol, 1.0 eq) was dissolved in an aqueous sodium acetate solution (3 mL, pH 4.5) and $^{nat}Ga(NO_3)_3$ (3.6 mg, 14 µmol, 1.0 eq) in aqueous sodium acetate solution (1 mL, pH 4.5) was added. The reaction mixture was stirred at room temperature for 20 min and subsequently lyophilized.

The crude residue was dissolved in dimethylformamide (DMF) (1 mL, dry) and cesium fluoride (280 µmol, 20.0 eq, 43 mg) was added in portions. The reaction mixture was stirred at ambient temperature for 18 h, filtered and purified by preparative HPLC (method 3) to isolate ^{nat}Ga-NODAGA-indole as a colorless solid.

Yield: 6.1 mg (61 %) (^{nat}Ga-NODAGA-indole x TFA)

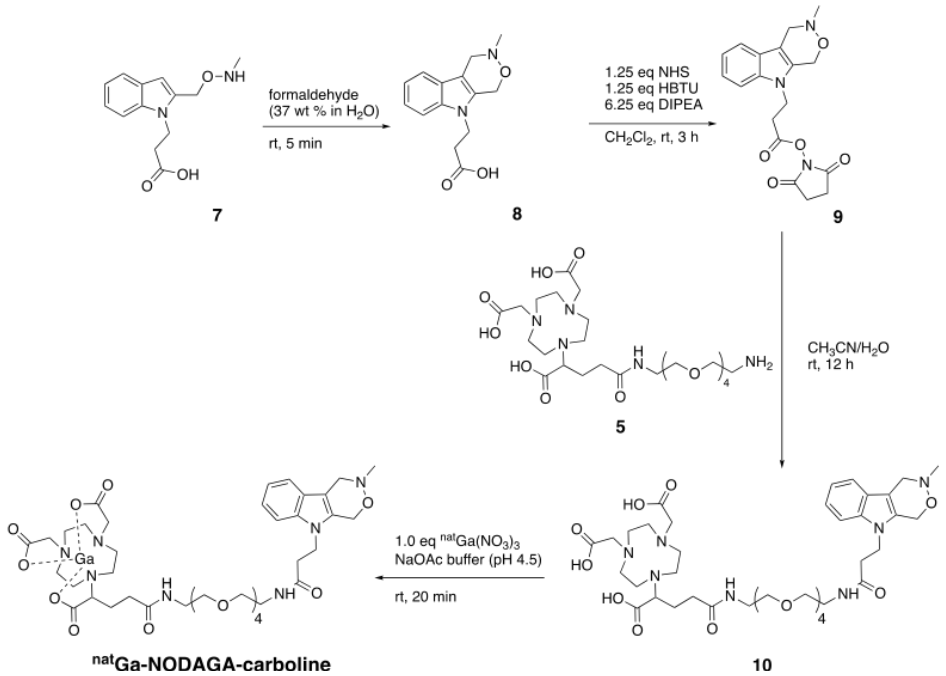
LC/MS (ESI+): $C_{38}H_{58}GaN_7O_{13}$: m/z calcd 890.3 [M+H$^+$]; found 890.1 (M+H$^+$)

R_t (method 1): 3.13 min (purity > 99 %)

^{1}H NMR (500 MHz, D_2O/CD_3OD (19:1)) δ = 7.74 (d, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 8.3 Hz, 1H), 7.42 – 7.36 (t, *J* = 7.5 Hz, 1H), 7.24 (t, *J* = 7.5 Hz, 1H), 6.83 (s, 1H), 5.31 (s, 2H), 4.63 (t, *J* = 6.4 Hz, 2H), 3.88 – 3.59 (m, 11H), 3.57 – 3.16 (m, 17H), 3.12 – 3.02 (m, 2H), 3.01 – 2.86 (m, 4H), 2.80 (t, *J* = 6.5 Hz, 2H), 2.62 (m, 2H), 2.46 (m, 2H), 2.38 – 2.24 (m, 2H), 1.82 (dt, *J* = 11.5, 6.0 Hz, 2H), 1.74 – 1.66 (m, 2H).

^{13}C NMR (126 MHz, D_2O/CD_3OD (19:1)) δ = 174.5, 174.0, 173.8, 172.6, 136.6, 126.0, 122.4, 120.6, 119.5, 109.6, 109.2, 105.4, 68.8, 68.7, 68.7, 68.6, 68.4, 67.9, 67.7, 66.3, 61.0, 60.9, 52.5, 52.4, 52.1, 51.7, 43.7, 39.0, 38.1, 38.1, 35.7, 35.1, 32.2.

Preparation of ^{nat}Ga-NODAGA-carboline



Scheme 2. Preparation of ^{nat}Ga-NODAGA-carboline.

Preparation of 3-(2-methyl-1,4-dihydro-[1,2]oxazino[5,4-*b*]indol-5(2*H*)-yl)propanoic acid (8)

3-(2-(((methylamino)oxy)methyl)-1*H*-indol-1-yl)propanoic acid (**7**) was synthesized according to Ref.[1] The starting material **7** (4.3 mg, 17 µmol, 1.0 eq) was dissolved in H_2O (1 mL) and formaldehyde (37 wt % in H_2O) (141 µL, 1.7 mmol, 100 eq) was added. The reaction mixture was stirred for 5 min at room temperature and purified by preparative HPLC (method 2) to afford the title compound **8** as a colorless solid.

Yield: 3.7 mg (83 %)

LC/MS (ESI+): $C_{14}H_{16}N_2O_3$: m/z calcd 261.3 [M+H$^+$]; found 261.1 (M+H$^+$)

R_t (method 1): 4.07 min (purity > 99 %)

^{1}H NMR (500 MHz, DMSO-d_6) δ = 7.45 (d, *J* = 8.2 Hz, 1H), 7.41 (d, *J* = 7.8 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 7.02 (t, *J* = 7.4 Hz, 1H), 5.08 (s, 2H), 4.24 (t, *J* = 6.7 Hz, 2H), 3.94 (br, 2H), 2.81 (s, 3H), 2.67 (t, *J* = 6.7 Hz, 2H).

^{13}C NMR (126 MHz, DMSO-d_6) δ = 172.4, 135.8, 132.9, 124.9, 120.8, 119.0, 117.6, 109.8, 105.4, 65.1, 54.1, 45.8, 34.2.

(one signal is located under the solvent peak at 39.5)

Preparation of NODAGA-carboline (10)

3-(2-methyl-1,4-dihydro-[1,2]oxazino[5,4-*b*]indol-5(2*H*)-yl)propanoic acid (**8**) (3.7 mg, 14 µmol, 1.25 eq relative to NODAGA-PEG$_4$-NH$_2$ (**5**)) was dissolved in CH_2Cl_2 (2 mL, dry) and *N*-Hydroxysuccinimide (NHS) (2.0 mg, 17 µmol, 1.5 eq), (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (6.6 mg, 17 µmol, 1.5 eq) , and *N*,*N*-diisopropylethylamine (DIPEA) (72 µmol, 12.5 µL, 6.25 eq, dry) were added. The reaction mixture was stirred at ambient temperature for 3 h. After extraction with H_2O (2 x 1.5 mL 0.1 M HCl solution, 1 x 1.5 mL H_2O), the organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The crude product **9** was obtained as yellow oil and was used without further purification.

NODAGA-PEG$_4$-NH$_2$ (**5**) (12 µmol, 7.6 mg, 1.0 eq) was dissolved in H_2O (1 mL) and the pH was adjusted to 9.4 with DIPEA. Compound **9** prepared above was dissolved in CH_3CN (2 mL) and added quickly to the aqueous solution. The white suspension was stirred at ambient temperature for 12 h, filtered and purified by preparative HPLC (method 2) to isolate the title compound **10** as a colorless solid.

Yield over 2 steps: 7.1 mg (71 %)

LC/MS (ESI+): $C_{39}H_{61}N_7O_{13}$: m/z calcd 836.4 [M+H$^+$]; found 836.4 (M+H$^+$)

R_t (method 1): 3.73 min (purity > 99 %)

^{1}H NMR (500 MHz,) δ = 7.63 (d, *J* = 7.7 Hz, 1H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 1H), 7.27 (t, *J* = 7.5 Hz, 1H), 5.41 (br, 2H), 4.43 (t, *J* = 6.4 Hz, 2H), 3.85 – 2.95 (m, 37H), 2.80 – 2.75 (m, 2H), 2.46 – 2.39 (m, 2H), 2.34 – 2.23 (m, 3H), 2.05 – 1.93 (m, 2H), 1.67 – 1.59 (m, 2H).

Preparation of ^{nat}Ga-NODAGA-carboline

The starting material **10** (6.7 µmol, 5.6 mg, 1.0 eq) was dissolved in an aqueous sodium acetate solution (3 mL, pH 4.5) and $^{nat}Ga(NO_3)_3$ (6.7 µmol, 1.7 mg 1.0 eq) in aqueous sodium acetate solution (1 mL, pH 4.5) was added. The reaction mixture was stirred at room temperature for 20 min and was subsequently purified by preparative HPLC (method 2).

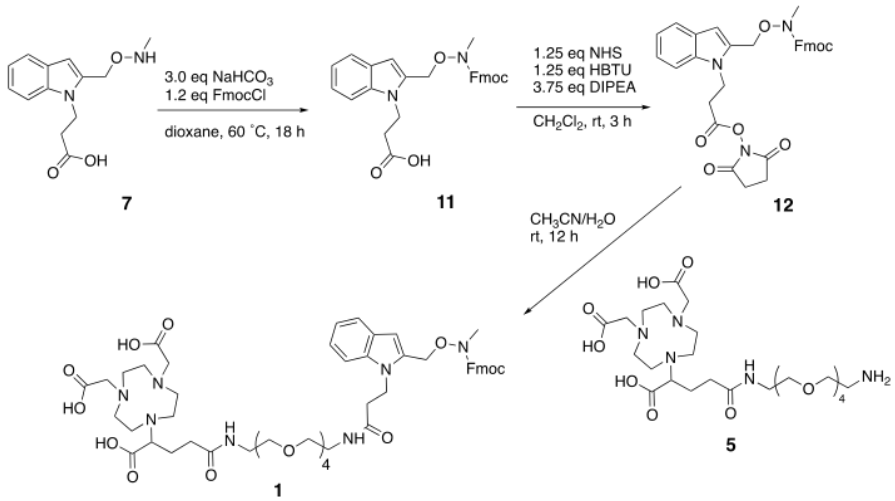
Yield: 5.2 mg (86 %)

LC/MS (ESI+): $C_{39}H_{58}GaN_7O_{13}$: m/z calcd 902.3 [M+H$^+$]; found 902.1 (M+H$^+$)

R_t (method 1): 3.50 min (purity > 99 %)

^{1}H NMR (500 MHz, D_2O/CD_3OD (19:1)) δ = 7.60 (d, *J* = 7.9 Hz, 1H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.32 (t, *J* = 7.5 Hz, 1H), 7.23 (t, *J* = 7.5 Hz, 1H), 5.24 (br, 2H), 4.41 (t, *J* = 6.4 Hz, 2H), 4.00 – 3.58 (m, 14H), 3.57 – 3.13 (m, 17H), 3.12 – 3.01 (m, 2H), 3.00 – 2.87 (m, 4H), 2.78 – 2.69 (m, 2H), 2.67 – 2.61 (m, 2H), 2.52 – 2.45 (m, 2H), 2.41 – 2.25 (m, 3H), 1.66 – 1.59 (m, 2H).
^{13}C NMR (126 MHz, D_2O/CD_3OD (19:1)) δ = 174.0, 174.0, 173.8, 172.5, 135.5, 131.2, 124.1, 120.9, 119.0, 117.2, 109.3, 104.9, 68.8, 68.7, 68.6, 68.6, 68.5, 68.0, 67.7, 61.0, 60.9, 60.9, 53.0, 52.5, 52.4, 52.2, 52.1, 51.7, 44.3, 43.2, 39.3, 38.2, 38.1, 35.5, 32.2.

Preparation of NODAGA-indole-Fmoc (1)



Scheme 3. Preparation of NODAGA-indole-Fmoc (**1**).

Preparation of 3-(2-((((((9*H*-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)oxy) methyl)-1*H*-indol-1-yl)propanoic acid (11)

3-(2-(((methylamino)oxy)methyl)-1*H*-indol-1-yl)propanoic acid (**7**) was synthesized according to Ref.[1] The starting material **7** (47 mg, 191 µmol, 1.0 eq) was dissolved in dioxane (10 mL) before $NaHCO_3$ (48 mg, 573 µmol, 3.0 eq) in H_2O (2 mL) and fluorenylmethyloxycarbonyl chloride (FmocCl) (59 mg, 229 µmol, 1.2 eq) were added. The reaction mixture was heated at 60 ˚C for 18 h. The reaction mixture was concentrated under reduced pressure and lyophilized. The crude residue was dissolved in EtOAc:hexanes (1:1), filtered and purified by silica gel chromatography (gradient: 20:1 → 2:1 hexanes:EtOAc with 2 % AcOH) yielding a colorless solid.
Yield: 68.3 mg (76 %)
LC/MS (ESI+): $C_{28}H_{26}N_2O_5$: m/z calcd 493.2 [M+Na^+]; found 493.1 (M+Na^+)
R_t (method 1): 5.84 min (purity > 99 %)
^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.77 (d, *J* = 7.6 Hz, 2H), 7.66 – 7.58 (m, 3H), 7.45 – 7.24 (m, 6H), 7.15 (t, *J* = 6.6 Hz, 1H), 6.55 (s, 1H), 4.96 (s, 2H), 4.61 – 4.47 (m, 4H), 4.27 (t, *J* = 6.5 Hz, 1H), 3.09 (s, 3H), 2.88 (t, *J* = 7.0 Hz, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ = 176.8, 157.7, 143.9, 141.7, 137.4, 133.1, 128.2, 127.7, 127.5, 125.3, 123.1, 121.7, 120.4, 109.8, 105.9, 68.1, 47.5, 39.2, 37.3, 34.9.

Preparation of NODAGA-indole-Fmoc (1)

3-(2-((((((9*H*-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)oxy) methyl)-1*H*-indol-1-yl)propanoic acid (**11**) (10 mg, 21 µmol, 1.25 eq relative to NODAGA-PEG_4-NH_2 (**5**)) was

dissolved in CH_2Cl_2 (3 mL, dry) and *N*-Hydroxysuccinimide (NHS) (2.9 mg, 25 µmol, 1.5 eq), (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (9.7 mg, 25 µmol, 1.5 eq) , and *N*,*N*-diisopropylethylamine (DIPEA) (80 µmol, 14 µL, 3.75 eq, dry) were added. The reaction mixture was stirred at ambient temperature for 3 h. After extraction with H_2O (2 x 1.5 mL 0.1 M HCl solution, 1 x 1.5 mL H_2O), the organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The crude product (**12**) was obtained as yellow oil and was used without further purification.
NODAGA-PEG_4-NH_2 (**5**) (17 µmol, 11 mg, 1.0 eq) was dissolved in H_2O (1 mL) and the pH was adjusted to 9.4 with DIPEA. Compound **12** prepared above was dissolved in CH_3CN (2 mL) and added quickly to the aqueous solution. The white suspension was stirred at ambient temperature for 12 h, filtered and purified by preparative HPLC (method 2) to isolate the title compound **1** as a colorless solid.
Yield over 2 steps: 10.3 mg (58 %)
LC/MS (ESI+): $C_{53}H_{71}N_7O_{15}$: m/z calcd 1046.5 [$M+H^+$]; found 1046.4 ($M+H^+$)
R_t (method 1): 4.70 min (purity > 99 %)
^{1}H NMR (500 MHz, CD_3CN) δ = 7.88 (d, *J* = 7.4 Hz, 2H), 7.68 (d, *J* = 7.4 Hz, 2H), 7.60 (d, *J* = 7.6 Hz, 1H), 7.51 – 7.41 (m, 3H), 7.40 – 7.33 (m, 2H), 7.24 (t, *J* = 8.2 Hz, 1H), 7.10 (t, *J* = 7.7 Hz, 1H), 6.86 (br, 2H), 6.46 (s, 1H), 4.89 (s, 2H), 4.60 – 4.49 (m, 4H), 4.36 – 4.29 (m, 1H), 3.86 (s, 2H), 3.76 – 3.62 (m, 3H), 3.59 – 3.39 (m, 15H), 3.38 – 3.29 (m, 4H), 3.29 – 3.21 (m, 2H), 3.19 – 2.72 (m, 16H), 2.70 – 2.60 (m, 2H), 2.36 (s, 2H).
^{13}C NMR (126 MHz, CD_3CN) δ = 173.0, 160.5, 158.5, 145.5, 142.7, 138.6, 134.9, 129.3, 128.8, 128.7, 126.5, 123.8, 123.8, 122.4, 121.5, 121.5, 121.1, 121.1, 111.5, 106.4, 71.4, 71.3, 71.3, 71.3, 71.2, 71.2, 71.1, 68.5, 68.5, 48.6, 41.4, 40.6, 40.4, 37.9, 37.5, 33.7.

Radiosynthesis

Preparation of ^{68}Ga-NODAGA-carboline

To a solution of ligand: NODAGA-carboline (**10**) (0.5 mM, 70 µL) were added an aqueous sodium acetate solution (3 M, pH 4.5, 180 µL) and $^{68}GaCl_3$ in HCl solution (0.15 M, 1.0 mL, ~1.5 mCi), and the reaction mixture was heated to 60 ˚C for 5 min (radiochemical yield > 99 %, as determined by radio-HPLC analysis). The crude mixture was loaded onto a Sep-Pak C_{18} Plus Light Cartridge. After flushing the Sep-Pak with 4 mL H_2O, the ^{68}Ga-labeled species was eluted with ethanol (1 mL) and concentrated under reduced pressure. The ^{68}Ga-labeled product was dissolved in phosphate buffered saline (1 X) and filtered (sterile filter, pore size: 0.2 µm) (~800 µCi, radiochemical purity > 99 %, as determined by radio-HPLC analysis, overall radiochemical yield without decay correction: ~53 %).

Preparation of ^{68}Ga-NODAGA-indole

To a solution of ligand: NODAGA-indole-Fmoc (0.5 mM, 100 µL) were added an aqueous sodium acetate solution (3 M, pH 4.5, 270 µL) and $^{68}GaCl_3$ in HCl solution (0.15 M, 1.5 mL, ~2.3 mCi), and the reaction mixture was heated to 60 ˚C for 5 min (radiochemical yield > 99 %, as determined by radio-HPLC analysis). The crude mixture was loaded onto a Sep-Pak C_{18} Plus Light Cartridge. After flushing the Sep-Pak with 4 mL H_2O, the ^{68}Ga-labeled species was eluted with ethanol (1 mL) and concentrated under reduced pressure. The crude residue was treated with 20 % piperidine in dimethylformamide (DMF) (150 µL, dry) and polymer-bound piperazine (5 mg) for 5 min at 60 ˚C. Subsequently, the reaction mixture was loaded onto a Sep-Pak C_{18} Plus Long Cartridge. After flushing the Sep-Pak with 12 mL HCl solution (10 mM), the ^{68}Ga-labeled product was eluted with ethanol (6 mL) and concentrated under reduced pressure. The ^{68}Ga-labeled product was dissolved in

phosphate buffered saline (1 X) containing hydroxylamine wang resin (0.5 mg per mL). The mixture was filtered (sterile filter, pore size: 0.2 µm) into a sterile vial that was flushed with argon beforehand (~600 µCi, radiochemical purity > 95 %, as determined by radio-HPLC analysis, overall radiochemical yield without decay correction: ~26 %).

Aorta binding assay

Porcine aorta was purified using the mild method reported by Umeda et al.[2]

25 mg of lyophilized aorta were treated for 24 h at 37 ˚C with either ^{nat}Ga-NODAGA-indole or ^{nat}Ga-NODAGA-carboline in PBS buffer (200 µM) with a total volume of 500 µL. After 24 h, the aorta segments were washed 3 times in fresh PBS buffer to remove any non-specifically bound probe. The aorta segments were then digested in conc. HNO_3 for 12 h at 37 ˚C and the ^{69}Ga concentration in each sample was then determined using an Agilent 8800 ICP-MS system.
For the blocking study, 25 mg of lyophilized aorta were treated for 24 h at 37 ˚C with 3-(2-(((methylamino)oxy)methyl)-1*H*-indol-1-yl) propanoic acid (**7**) in PBS buffer (4.0 mM) with a total volume of 500 µL. After 24 h, the aorta segments were washed 3 times in fresh PBS buffer to remove any non-specifically bound **7**. Afterwards, the aorta tissue was treated with either ^{nat}Ga-NODAGA-indole or ^{nat}Ga-NODAGA-carboline again, using the same procedure as described above.

Plasma stability

A 50 µL aliquot of either ^{nat}Ga-NODAGA-indole or ^{nat}Ga-NODAGA-carboline in PBS buffer (2.0 mM) was added to 300 µL of human blood plasma. The mixture was then incubated for 24 h at 37 °C. An aliquot (50 µL) of this mixture was diluted with 250 µL PBS buffer, and filtered using a 0.2 µm filter. A 15 µL aliquot of this filtrate was analyzed using HPLC-ICP-MS (method 5). For both probes, there was no evidence of degradation.

Animal protocol

All experiments and procedures were performed in accordance with the National Institutes of Health's "Guide for the Care and Use of Laboratory Animals" and were approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee.

Animal lung fibrosis model

In the bleomycin model (BM), pulmonary fibrosis was induced in 6 to 8-week old male C57/BL6 mice (Charles River Laboratories, Wilmington MA) by administering a single intratracheal dose of bleomycin (Fresenius Kabi, Lake Zurich, Il) (1.0 unit/kg), prepared in sterile phosphate-buffered saline (PBS) (50 µL). Sham mice were intratracheally injected with PBS. After 13 to 15 days after bleomycin (or PBS) instillation, animals were used for biodistribution and PET imaging.

A total of 24 C57/BL6 mice were included to study pulmonary fibrosis:

A. Intratracheal bleomycin instillation, imaged after 13-15 days (n=14), 8 mice were injected with ^{68}Ga-NODAGA-indole (14 days after bleomycin) and 6 mice were

injected with ^{68}Ga-NODAGA-carboline (4 mice: 13 days after bleomycin; 2 mice: 15 days after bleomycin).

B. Intratracheal PBS instillation as Sham control (n=10), 6 mice were injected with ^{68}Ga-NODAGA-indole, 4 mice were injected with ^{68}Ga-NODAGA-carboline.

All animals were sacrificed 120 min after injection for ex-vivo analysis.

Small-animal PET-CT imaging and analysis

Animals were anesthetized with isoflurane (1 - 1.5 %) and placed on a heating pad in a small-animal PET/CT scanner (Triumph, TriFoil Imaging), equipped with inhalation anesthesia. After placement of an in-dwelling catheter in the femoral vein for probe administration, the probe was given as a bolus. A whole-body CT was obtained either immediately before or immediately after the PET acquisition. PET and CT images were reconstructed using the LabPET software (TriFoil Imaging). For the reconstruction of the PET images a volumetric maximum likelihood estimation method with 30 iterations was used. The PET data were decay corrected and analyzed using the AMIDE software package.[5] For the time-activity curves, volumes of interest (VOIs) were drawn over various organs on whole-body coronal images. Within the VOIs, the radioactivity concentration was obtained from mean pixel values and converted to counts per milliliter per minute and then divided by the injected dose (ID) to obtain an imaging VOI-derived percentage of the injected radioactive dose per cubic centimeter of tissue (% ID/cc).

Biodistribution protocol

The left lung, blood, urine, heart, liver, left rectus femoris muscle, spleen, small intestine, kidneys, tail, gall bladder and left femur bone were collected from all animals. The right lung was inflated and fixed with 10% formalin, embedded in paraffin, and cut into 5 µm sections. Tissue sections were stained with Picrosirius Red with a counterstain of Fast Green. Images were acquired using a Nikon TE2000 microscope. The other organs were weighed, and radioactivity in each tissue was measured on a gamma counter (Wizard2Auto Gamma, PerkinElmer). Tracer distribution is presented as % ID/gram for all organs. The radioactivity in the left lung is also reported as % ID/lung.

Hydroxyproline assay

The hydroxyproline assay was performed as previously reported.[3]

Allysine assay

The allysine assay was performed as previously reported.[4]

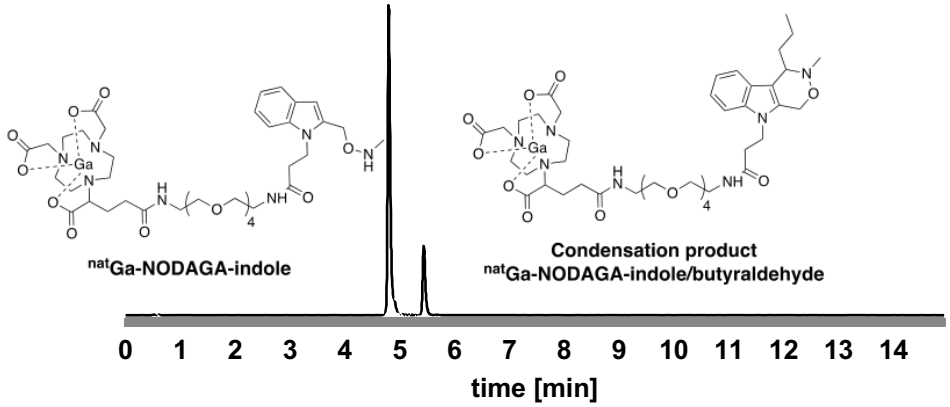


Figure S1. HPLC-ICP-MS trace (method 4) of crude material from reaction of ^{nat}Ga-NODAGA-indole with butyraldehyde at 300 K in PBS (pH 7.4). ^{nat}Ga-NODAGA-indole and the condensation product ^{nat}Ga-NODAGA-indole/butyraldehyde elute at 4.82 min and 5.46 min, respectively.

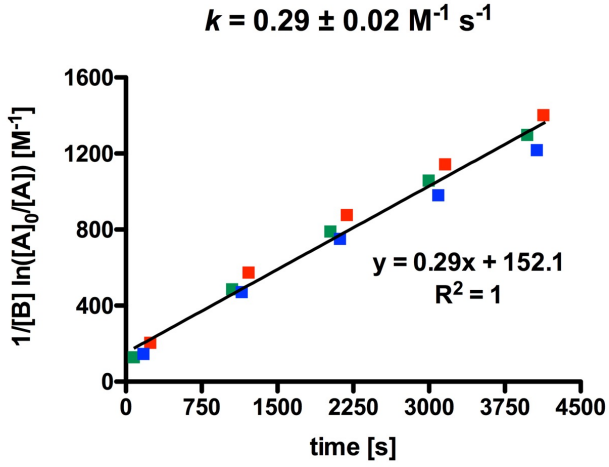


Figure S2. Results from HPLC-ICP-MS kinetic experiments on the reaction of ^{nat}Ga-NODAGA-indole (A) with butyraldehyde (B) at 300 K in PBS (pH 7.4) under pseudo first order conditions ($[A]_0$ = 100 μM, [B] = 1.0 mM). Each symbol color represents an independent experiment.

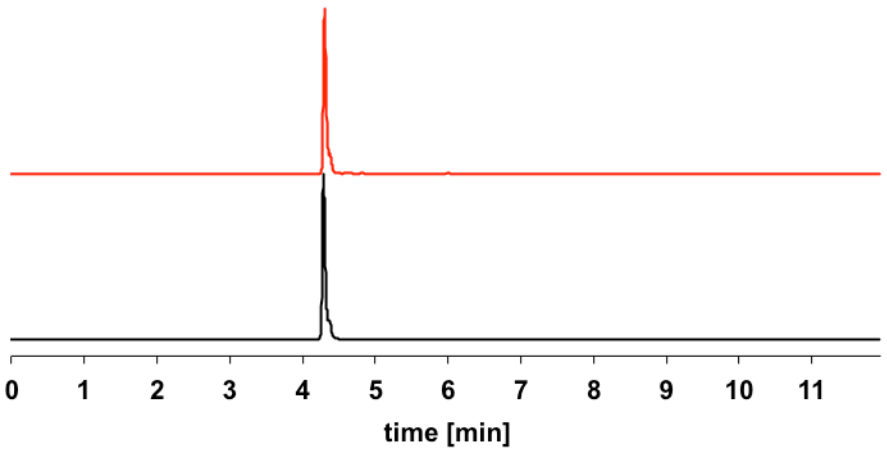


Figure S3. Normalized HPLC-ICP-MS traces (method 5) of ^{nat}Ga-NODAGA-indole in PBS (bottom trace, black) and ^{nat}Ga-NODAGA-indole after incubation in human blood plasma at 310 K for 24 h (top trace, red). ^{nat}Ga-NODAGA-indole elutes at 4.32 min. There is no evidence of dechelated ("free") gallium or any small molecule degradation species of ^{nat}Ga-NODAGA-indole which have different retention times than ^{nat}Ga-NODAGA-indole.

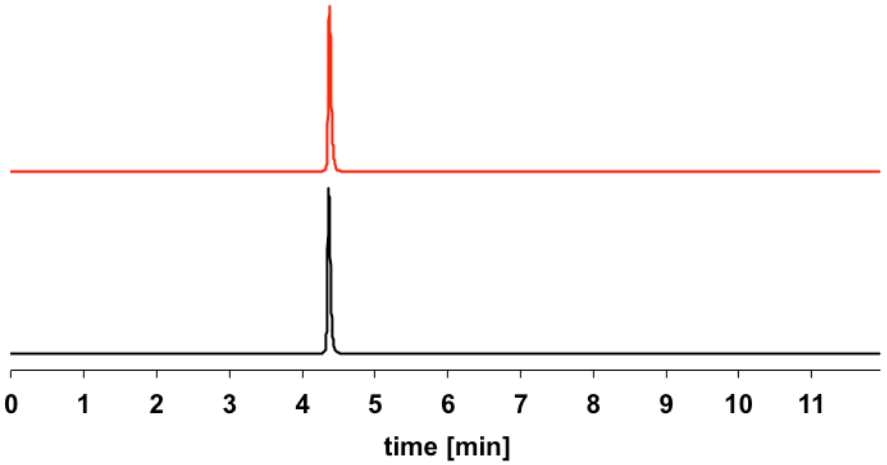


Figure S4. Normalized HPLC-ICP-MS traces (method 5) of ^{nat}Ga-NODAGA-carboline in PBS (bottom trace, black) and ^{nat}Ga-NODAGA-carboline after incubation in human blood plasma at 310 K for 24 h (top trace, red). ^{nat}Ga-NODAGA-carboline elutes at 4.40 min. There is no evidence of dechelated ("free") gallium or any small molecule degradation species of ^{nat}Ga-NODAGA-indole which have different retention times than ^{nat}Ga-NODAGA-indole.

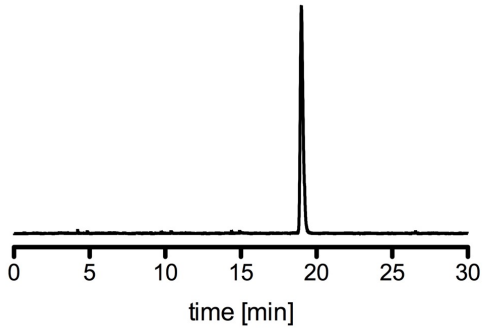


Figure S5. Radio-HPLC trace (method 6) of ^{68}Ga-NODAGA-indole-Fmoc (radiochemical yield > 99 %). ^{68}Ga-NODAGA-indole-Fmoc elutes at 19.00 min.

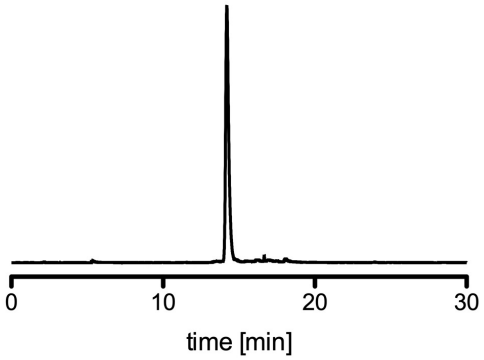


Figure S6. Radio-HPLC trace (method 6) of ^{68}Ga-NODAGA-indole. ^{68}Ga-NODAGA-indole elutes at 14.21 min.

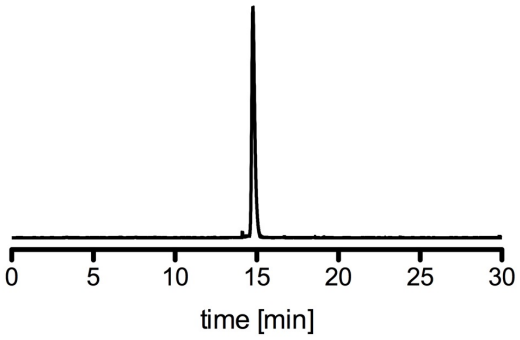


Figure S7. Radio-HPLC trace (method 6) of ^{68}Ga-NODAGA-carboline (radiochemical yield > 99 %). ^{68}Ga-NODAGA-carboline elutes at 14.76 min.

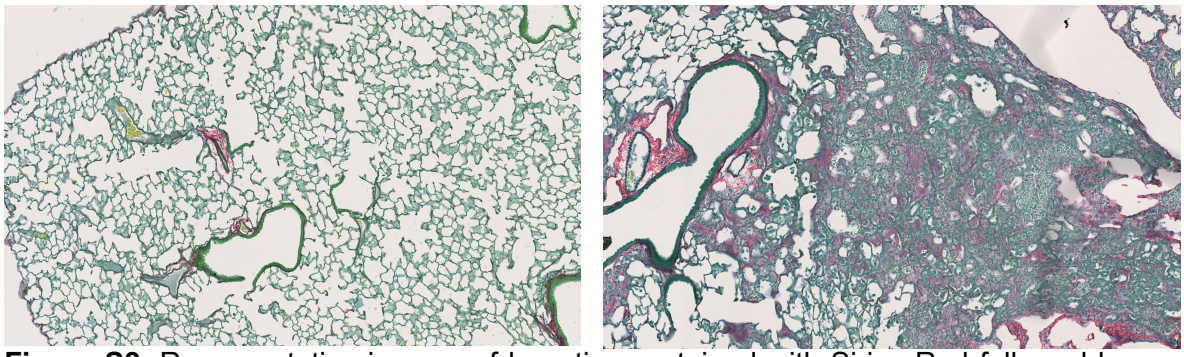


Figure S8. Representative images of lung tissue stained with Sirius Red followed by a fast green counter stain (magnification, x200) for sham- (left) and bleomycin-treated animals (right) (day 14 after bleomycin instillation). Note the increased density in the image on the right and extensive areas of red (fibrosis) staining.

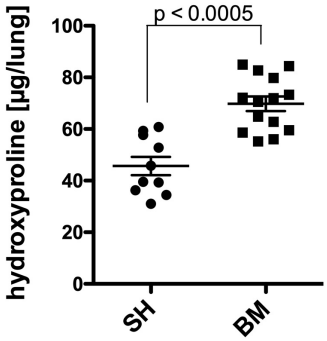


Figure S9. Lung hydroxyproline (collagen marker) levels measured in the left lung, expressed as µg/lung of sham (SH) (n=10) and bleomycin-injured (BM) (n=14) mice. Bleomycin-injured mice exhibit a 1.5-fold higher hydroxyproline content in their lung tissue compared to sham animals. Data were analyzed by a two sided unpaired t-test.

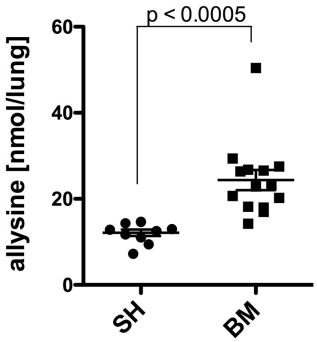


Figure S10. Lung allysine levels measured in the left lung, expressed as nmol/lung of sham (SH) (n=10) and bleomycin-injured (BM) (n=14) mice. Bleomycin-injured mice exhibit a 2.0-fold higher allysine content in their lung tissue compared to sham animals. Data were analyzed by a two sided unpaired t-test.

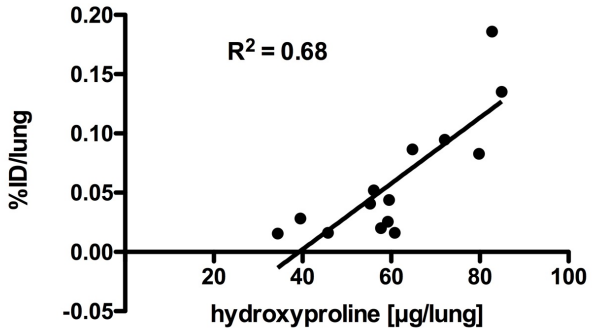


Figure S11. Correlation between uptake of ^{68}Ga-NODAGA-indole in lungs from sham- (n=6) and bleomycin-treated (n=8) mice, expressed in %ID/lung and the hydroxyproline content in the lung tissue (R^2 = 0.68, p = 0.0003)

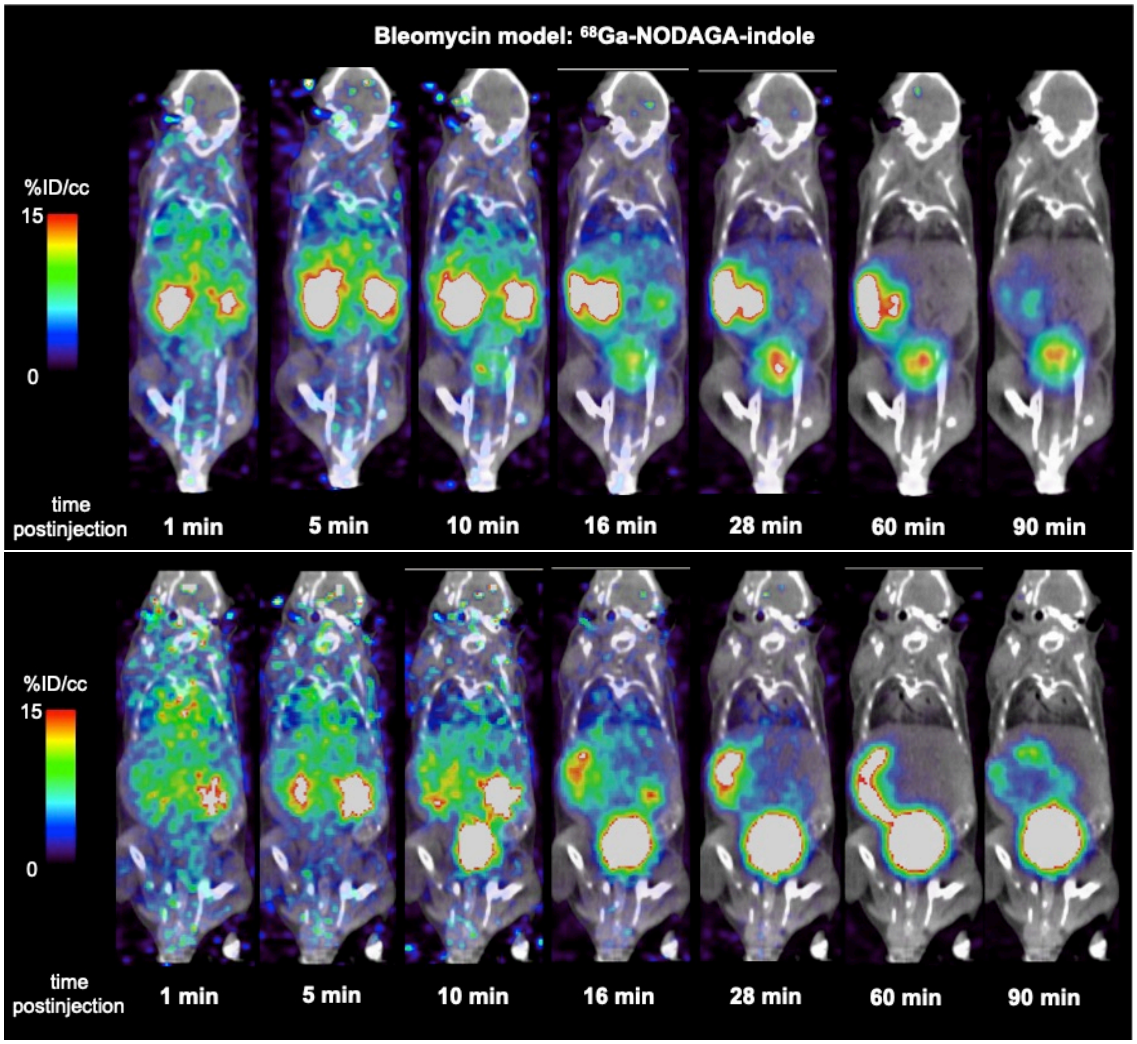


Figure S12. Representative fused PET/CT images of a bleomycin injured mouse showing two different coronal image slices after administration of ^{68}Ga-NODAGA-indole as a function of time. Grayscale image shows CT image, and color scale image shows PET image. The color scale gives the injected radioactive dose per cubic centimeter of tissue (% ID/cc). Following probe injection, ^{68}Ga-NODAGA-indole immediately distributes throughout the body and is rapidly eliminated via the kidneys (top panel) and has cleared out of the kidneys after 60 min. The bottom panel shows rapid uptake into the liver and then fast clearance (after 10 min) from the liver into the intestines. Note also the accumulation from the kidneys into the urinary bladder (bottom, bottom panel) starting at 10 minutes and increasing with time.

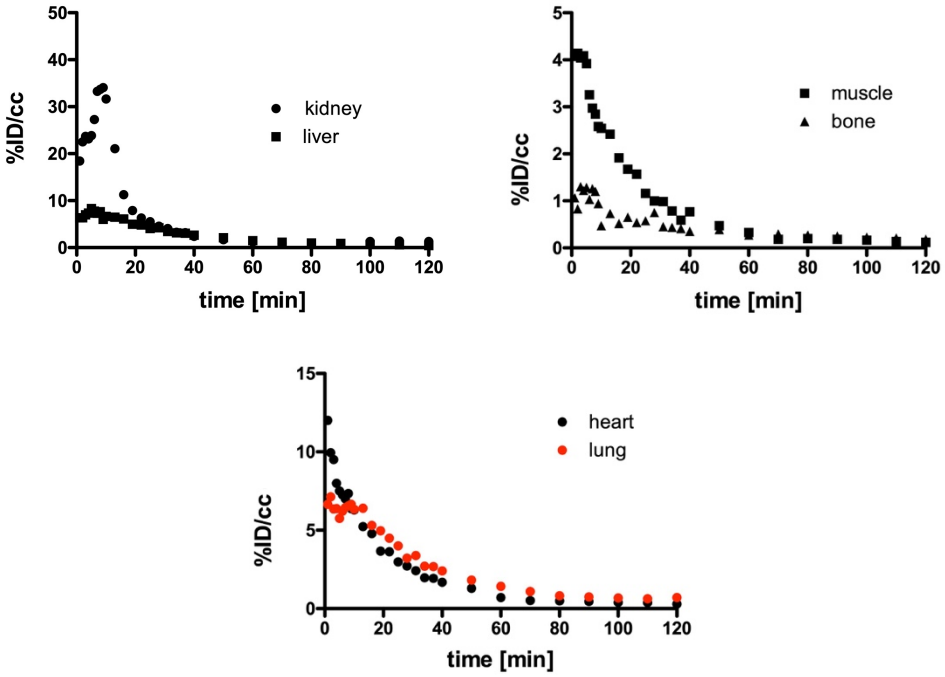


Figure S13. Time-activity curves of various organs from dynamic PET imaging in bleomycin-injured mice (n=2) after ^{68}Ga-NODAGA-indole injection.

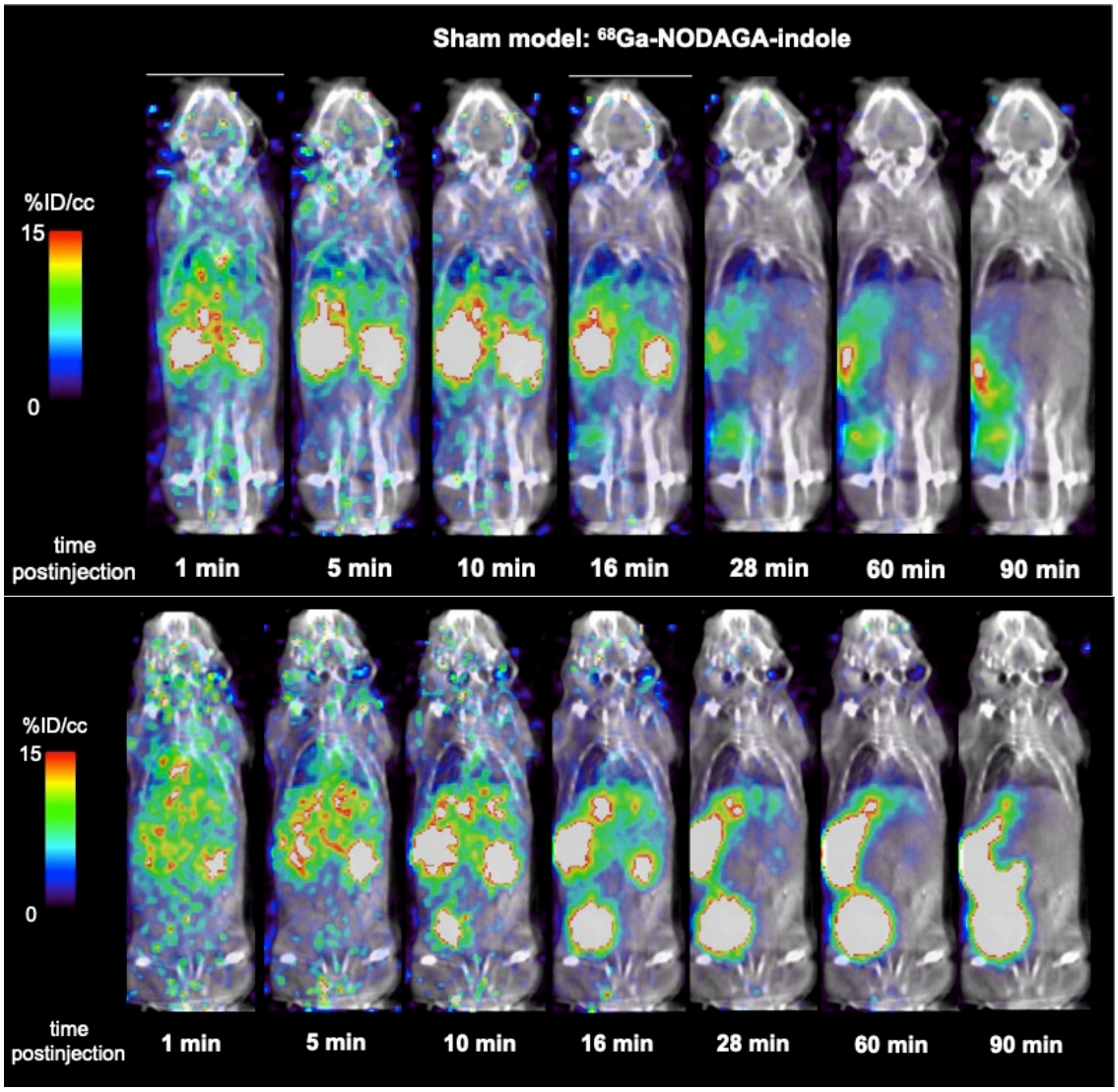


Figure S14. Representative fused PET/CT images of a sham injured mouse showing two different coronal image slices after administration of ^{68}Ga-NODAGA-indole as a function of time. Grayscale image shows CT image, and color scale image shows PET image. The color scale gives the injected radioactive dose per cubic centimeter of tissue (% ID/cc). Following probe injection, ^{68}Ga-NODAGA-indole immediately distributes throughout the body and is rapidly eliminated via the kidneys (top panel) and has cleared out of the kidneys after 60 min. The bottom panel shows rapid uptake into the liver and then fast clearance (after 10 min) from the liver into the intestines. Note also the accumulation from the kidneys into the urinary bladder (bottom, bottom panel) starting at 10 minutes and increasing with time. The whole body biodistribution and pharmacokinetics are very similar to the animal shown in Figure S12.

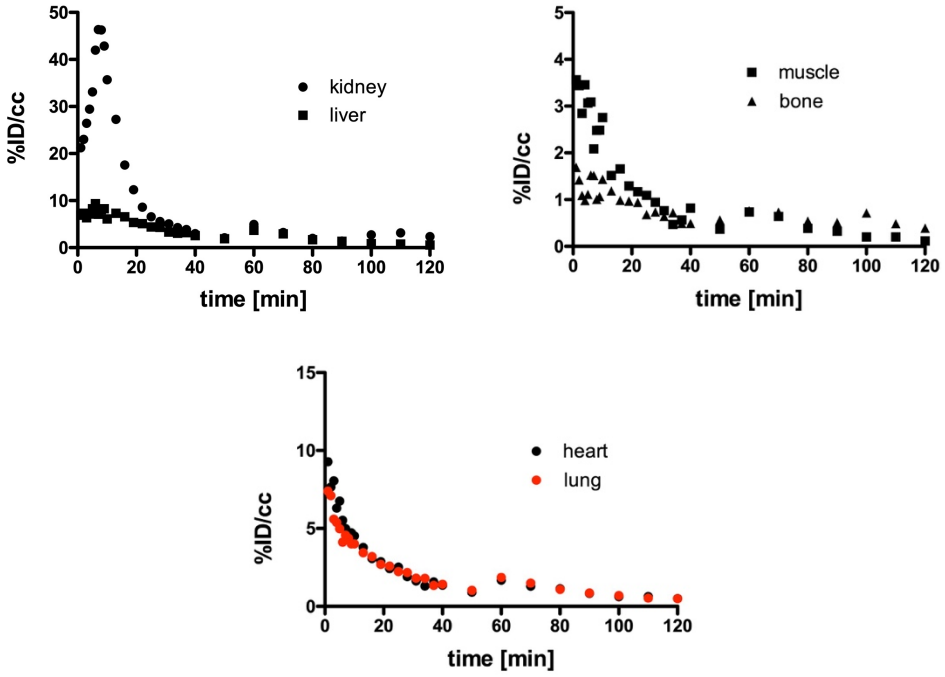


Figure S15. Time-activity curves of various organs from dynamic PET imaging in sham-treated mice (n=2) after ^{68}Ga-NODAGA-indole injection.

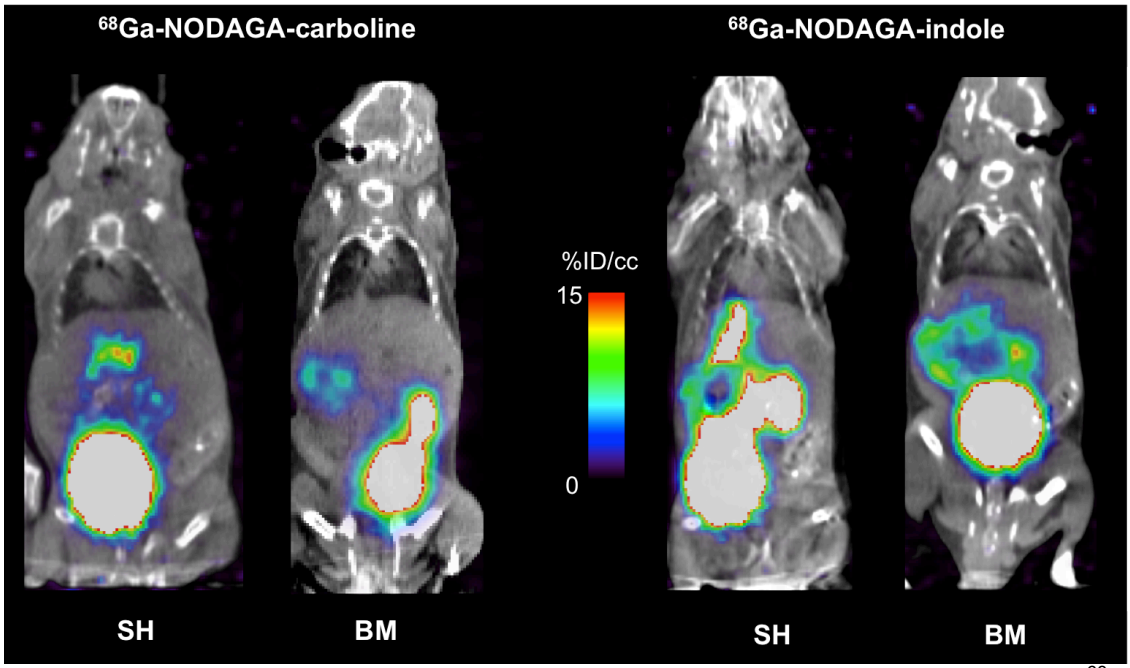


Figure S16. Representative fused PET/CT images 110 min post-injection of ^{68}Ga-NODAGA-carboline (left) or ^{68}Ga-NODAGA-indole (right) in sham- (SH) and bleomycin-treated (BM) animals indicating that most of the injected dose is in the urinary bladder and intestines at this time point. Grayscale image shows CT image, and color scale image shows PET image. The color scale gives the injected radioactive dose per cubic centimeter of tissue (% ID/cc). Note, that the PET scale used here (0 – 15 %ID/cc) was

chosen to show the high concentrations of activity in the intestines and urinary bladder and is different from that used in Figure 3 (0 – 4 %ID/cc) making it difficult to visualize the difference in lung uptake between the probes.

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(3) Waghorn, P. A.; Jones, C. M.; Rotile, N. J.; Koerner, S. K.; Ferreira, D. S.; Chen, H. H.; Probst, C. K.; Tager, A. M.; Caravan, P. *Angew. Chem. Int. Ed.* **2017**, *56* (33), 9825-9828.
(4) Waghorn, P. A.; Oliveira, B. L.; Jones, C. M.; Tager, A. M.; Caravan, P. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2017**, *1064*, 7-13.
(5) Loening, A. M.; Gambhir, S. S.; Mol. Imaging. **2003**, *2*, 131-137