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Biological evaluation of glucose and deoxyglucose derivatives radiolabeled with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ core as potential melanoma imaging agents

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ABSTRACT

Glucose **9** and 2-deoxyglucose **10** were successfully synthesized and radiolabeled with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ intermediate in high yield. The complexes were characterized by HPLC and its stability with histidine over time was challenged. Cell uptake and biodistribution studies in melanoma-bearing C57BL/6 mice were performed. Both compounds showed accumulation in tumor tissue with high tumor-to-muscle ratios. Thus, D-glucose- and D-2-deoxyglucose-^{99m}Tc complex could be considered as agents for melanoma diagnosis.

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Glucose is the major energy source of cells, especially in heart and brain. Cancer cells are well known to display an enhanced carbohydrate uptake and consumption.[1,2] Due to the ideal decay properties ($t_{1/2}$ = 6 h, γ energy = 150 keV), low cost of production and on-site availability, ^{99m}Tc is the most frequently used radioisotope in nuclear medicine today.[9,10] Several groups have reported the preparation of glucose analogues labelled with ^{99m}Tc which demonstrate uptake into tumor tissue indicating that they are potential agents for use in metabolic imaging.[11–13] In addition, we recently described the use of ^{99m}Tc-glucarate as a potential agent for breast cancer imaging.[14]

Our group has recently started to develop potential radiopharmaceuticals for imaging melanoma. Melanoma is known as one of the most aggressive tumors in humans.[15,16] Its incidence has been steadily increasing over the past years, making it a serious problem for healthcare in several countries worldwide. Therefore, an early diagnosis of this disease together with an accurate assessment of its metastases could be the key for disease survival.[17,18] Several scintigraphic studies using monoclonal antibodies,[19] peptides,[20] iodobenzamides[21] and carbohydrates[22] have been developed over the past years to detect melanoma and localize metastatic lesions. While some of these biomolecules showed their potential in preliminary evaluations, only few of these agents achieved clinical significance. Therefore, our goal is to develop diagnostic imaging agents with high specificity and sensitivity towards melanoma, using B16F1 model for in vivo and in vitro experimentation. In this context, we propose the evaluation of ^{99m}Tc-glucose derivatives. Here, we report the potential use of two ^{99m}Tc labeled-glucose derivatives for melanoma diagnosis by evaluation in B16F1 melanoma-bearing C57BL/6 mice and cell uptake in B16F1 murine melanoma cells.

It is of great importance to achieve the effective complexation of the metal core with the biological active molecule without affecting its physiological properties. In the last years, the organometallic aqua complex $[^{99m}Tc(H_2O)_3(CO)_3]^+$ has been proposed as a versatile molecule to form the tricarbonyl core $[^{99m}Tc(CO)_3]^+$ moiety.[10,23] Tricarbonyl technetium-99m is an attractive core for the introduction of ^{99m}Tc into biomolecules because of its high chemical stability and small size. This precursor can easily form complexes with different di- or triligands by substitution of two or three water molecules, respectively. For the purposes of labeling glucose and 2-deoxyglucose with ^{99m}Tc, a hydrophilic iminodiacetic acid (IDA) chelate was attached to position C-1 separated by an ethylene linker (Scheme 1). Coordination of the tricarbonyl core is likely to be tridentate, via the two carboxylate oxygens and the tertiary amine of the IDA moiety.

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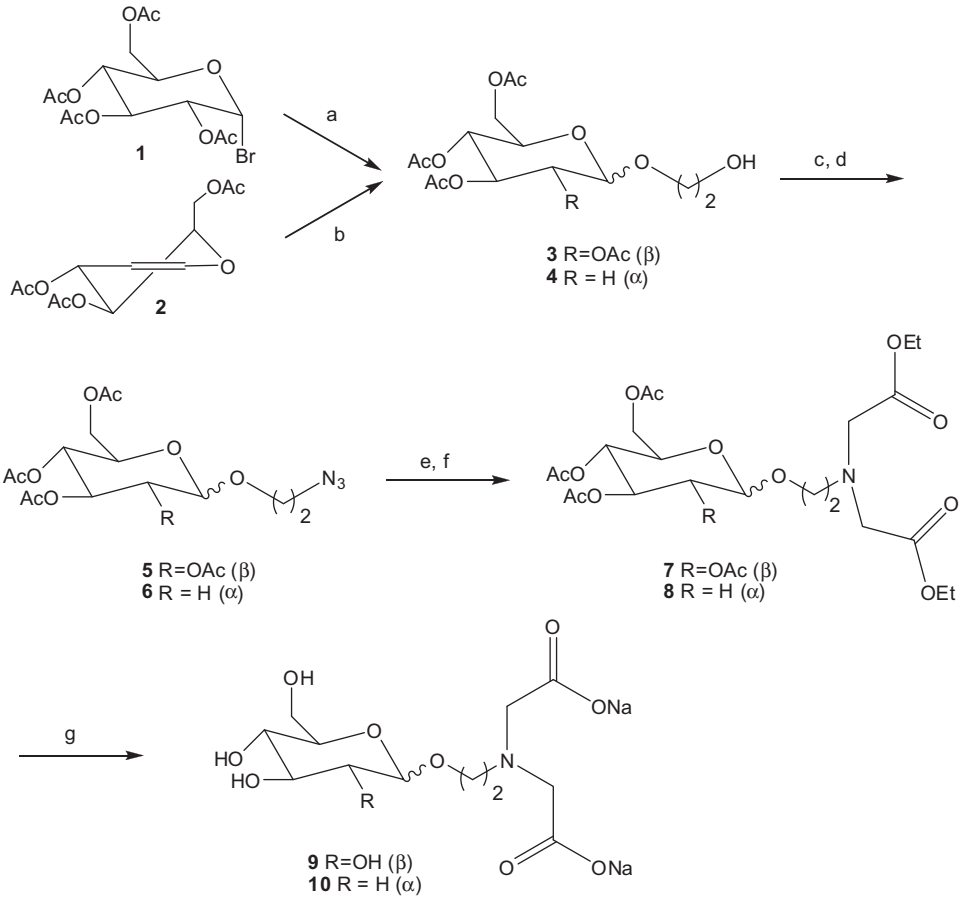
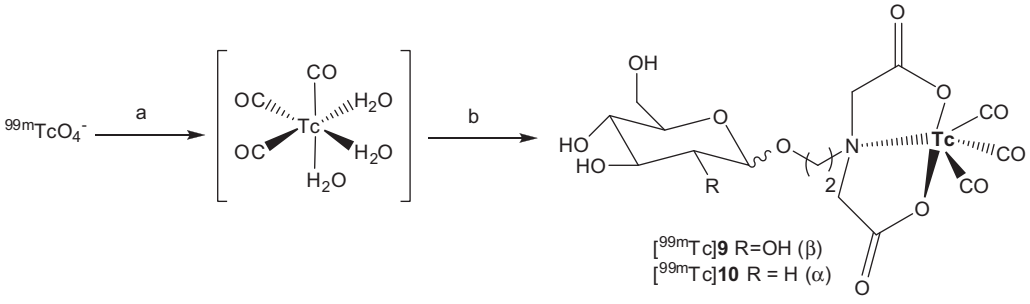


Scheme 1. Synthesis of glucose and 2-deoxyglucose derivatives. Reagents and conditions: (a) Diethylene glycol, $HgBr_2$, molecular sieves, CH_2Cl_2, 71%; (b) Diethylene glycol, acid resin (WPX-800), molecular sieves, acetonitrile, 40%; (c) TsCl, pyridine, CH_2Cl_2, **3** 72% **4** 60%; (d) NaN_3, DMF, 60 °C, 3 h, **5** 92% **6** 80%; (e) (i) Triphenylphosphine polymer-bound, CH_2Cl_2, (ii) H_2O; (f) Ethyl bromoacetate, Et_3N, THF, **7** 21% **8** 24%; over 2 steps; (g) (i) NaOMe, MeOH, (ii) NaOH, H_2O, quantitative yields.

Synthesis of ^{99m}Tc-glucose derivatives was performed using the method previously described with some modifications (Schemes 1 and 2).[24] Glycosylation of commercially available acetobromoglucose **1** and tri-*O*-acetyl-D-glucal **2** with ethylene glycol resulted in alcohols **3** and **4**, in good and moderate yield, respectively. The esters **7** and **8** were obtained through reduction-N-alkylation of the corresponding azides. To obtain the amines intermediates a Staudinger reaction has been adapted using a triphenylphosphine polymer-bound (TPP-PB).[25] The azide **5** and **6** were dissolved in dry THF and TPP-PB was added. After 24 h at room temperature, water was added and the reaction was stirred at room temperature for 6 h. Following standard work-up protocols and analysis, the desired amines were obtained and used in the next reaction without further purification.[26] Subsequently, the IDA moiety was built up in one step by a double alkylation of the corresponding amine with ethyl bromoacetate to yield **7** and **8**.[26] Finally, the desired disodium salts **9** and **10**, were obtained in quantitative yields by deprotection of the acetates in two steps.[24] First the acetates were removed with sodium methylate and then the esters were hydrolyzed with 1 M NaOH.

For radiolabelling, precursor $[^{99m}Tc(H_2O)_3(CO)_3]^+$ was prepared[27] by boranocarbonate reduction from a saline solution of $Na^{99m}TcO_4^-$, using an IsoLink™ kit generously donated by Mallinckdrot Inc. (Scheme 2). Radioactive labeling of **9** and **10** was accomplished when $[^{99m}Tc(H_2O)_3(CO)_3]^+$ was added and the mixtures heated at 70 °C for 30 min, as described by Petrig et al.[24]



Scheme 2. Radiolabeled of glucose **9** and 2-deoxyglucose **10** analogues with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ intermediate. Reagents and conditions: (a) IsoLink kit, 100 °C, 30 min; (b) **9** or **10**, 70 °C, 30 min, PBS buffer pH 7.4.

The products were analyzed for their radiochemical purity by HPLC.[28] Radiochemical yield of [^{99m}Tc]**9** and [^{99m}Tc]**10** was 99.40% and 99.90%, respectively, immediately after the preparation. The specific radioactivity of the products was calculated to be 19.04–204.0 MBq/μmol and 18.5–77.62 MBq/μmol, respectively. HPLC analysis of the reaction mixtures showed the formation of main radiochemical species, at 8.3 and 8.9 min, corresponding to complexes [^{99m}Tc]**9** and [^{99m}Tc]**10**, respectively (Fig. 1), while that of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was found to be 3.8 min (result not shown).

To assess the stability of the complexes, challenge studies were done with histidine. The radiochemical purity was measured at different time points during the incubation with the amino acid, a potential competitor of metal binding in vivo (Table 1). [^{99m}Tc]**9** and [^{99m}Tc]**10** were added to a solution of histidine and incubated at 37 °C for 24 h, as described by Ferreira et al.[29] HPLC samples were analyzed at 1, 4 and 24 h of incubation of each complex. The tridentate ligand was expected to form a stable complex with the metal core, resistant to ligand exchange process. Only insignificant decomposition of the complexes occured over 24 h of incubation with 100-fold excess of histidine, suggesting a high in vivo stability. Percentage of the remaining [^{99m}Tc]**9** and [^{99m}Tc]**10** were 94.1% and 95.7%, respectively, shown by HPLC analysis after 24 h incubation (Table 1).

Partition coefficients were determined between 1-octanol and PBS at pH 7.4.[30] −1.60 ± 0.11 and −1.09 ± 0.02 values were found for [^{99m}Tc]**9** and [^{99m}Tc]**10**, respectively.

Biodistribution studies were carried out on C57BL/6 normal mice and C57BL/6 mice with B16F1 murine melanoma tumor, at 1.0 h post-injection, following procedure our group recently described.[31] Animal studies were carried out in compliance with the national laws related to the ethics during animal experimentation. B16F1 melanoma cells were inoculated subcutaneously in C57BL/6 mice and were used 10 days after. [^{99m}Tc]**9** and [^{99m}Tc]**10** were injected intravenously and the injected radioactivity was measured with a NaI(Tl) detector. Mice were sacrificed at 1 h post-injection. Tumor, other organs of interest and blood were collected, weighed and measured for radioactivity. The results are expressed as the percentage uptake of injected dose per gram of tissue (%ID/g). Tumor to muscle and tumor to blood ratios were calculated from the corresponding tissue concentrations. All data is presented as mean average ± standard deviation.

Biodistribution pattern for [^{99m}Tc]**9** and [^{99m}Tc]**10** in C57BL/6 normal mice is shown in Table 2. The complexes were rapidly eliminated through kidneys within an hour since more than 50% of the radioactivity was measured in urine and bladder: 56 ± 20% DI for [^{99m}Tc]**9** and 42 ± 8.2% DI for [^{99m}Tc]**10**. These results are in agreement with the hydrophilic log*P* values found, being [^{99m}Tc]**9** the highest hydrophilic one. Complexes also showed liver (7.2 ± 0.8% DI/g for [^{99m}Tc]**9** and 7.7 ± 1.3% DI/g for [^{99m}Tc]**10**) and intestine (16 ± 4.3% DI for [^{99m}Tc]**9** and 35 ± 6.6% DI for [^{99m}Tc]**10**) excretion, since activity accumulated in excretory organs within an hour and were rapidly cleared from blood and muscle. Uptake in other organs was insignificant. Biodistribution studies in melanoma-bearing C57BL/6 mice showed similar pattern to normal mice (Table 3). The complexes demonstrated tumor accumulation, reaching tumor to muscle ratios of 2.5 ± 0.7 and 2.0 ± 0.3 at 1 h post-injection for [^{99m}Tc]**9** and [^{99m}Tc]**10**, respectively (Table 4). Similar biodistribution patterns between these two complexes were observed.

Table 1
Percentage of [^{99m}Tc]**9** and [^{99m}Tc]**10** remaining after incubation at 37 °C in 1 mM histidine for 1, 4 and 24 h

	1 h	4 h	24 h
[^{99m}Tc]**9**	99.5 ± 0.2	99.2 ± 0.2	94.1 ± 1.4
[^{99m}Tc]**10**	99.2 ± 0.7	97.8 ± 0.9	95.7 ± 1.6

Table 2
Biodistribution pattern of ^{99m}Tc-glucose complexes [^{99m}Tc]**9** and [^{99m}Tc]**10** in normal C57BL/6 mice, $n = 3$, at 60 min post-injection

	% ID/g 60 min Post-injection	
Organ	[^{99m}Tc]**9**	[^{99m}Tc]**10**
Blood	1.65 ± 0.07	1.2 ± 0.35
Liver	7.2 ± 0.8	7.7 ± 1.3
Heart	0.85 ± 0.21	0.28 ± 0.08
Lung	1.3 ± 0.4	1.1 ± 0.21
Spleen	0.42 ± 0.24	0.31 ± 0.18
Kidney	4.3 ± 1.7	1.8 ± 0.3
Thyroid	0.48 ± 0.1	0.31 ± 0.13
Muscle	0.14 ± 0.02	0.14 ± 0.05
Bone	0.16 ± 0.04	0.16 ± 0.07
Brain	0.19 ± 0.05	0.04 ± 0.01
	% of injected dose (% ID)	
Intestine	16 ± 4.3	35 ± 6.6
Urine and bladder	56 ± 20	42 ± 8.2

Tumor-cell uptake of [^{99m}Tc]**9** and [^{99m}Tc]**10** was preformed as described by Castelli et al.[31] B16F1 murine melanoma cells cultured in D-glucose free media were used in order to determinate whether the complexes are transported intracellularly via GLUT's. Figure 2 shows the result of this assay. Internalization of glucose **9** and deoxyglucose **10** labeled with ^{99m}Tc in melanoma cells was demonstrated by measuring the activity remaining within the cells at different time points. The results suggested that [^{99m}Tc]**9** and [^{99m}Tc]**10** are transported into cells. The highest internalizations reached 18% and 52% of the total activity after 90 min of incubation with [^{99m}Tc]**9** and 30 min with [^{99m}Tc]**10**, respectively. On the other hand, a blocking experiment using D-glucose was carried out in order to determinate whether the cellular uptake is GLUT's specific. Under these conditions, only [^{99m}Tc]**9** complex uptake was blocked (50% blockage) in presence of D-glucose, suggesting that internalization could be via GLUT's transporters.

In summary, labeling of glucose **9** and 2-deoxyglucose **10** via the ^{99m}Tc-tricarbonyl precursor was achieved in high yields (>99%). In our proposed design, the addition of a chelating moiety as IDA to the carbohydrate could minimize the perturbation of

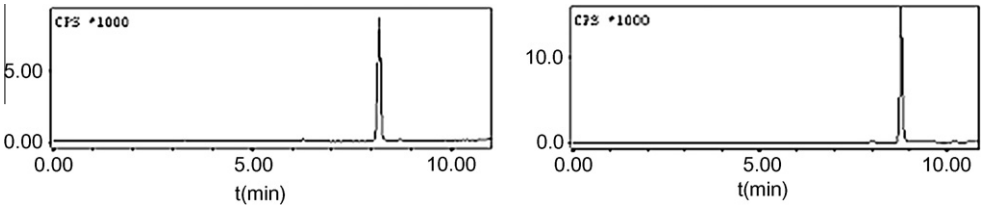


Figure 1. HPLC chromatographys obtain for [^{99m}Tc]**9** (left) and [^{99m}Tc]**10** (right) after radiolabeling procedure with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ of **9** and **10**. (Solvents: A = Water/TFA 0.1%, B = Acetonitrile/TFA 0.1%, Gradient: 100% A, 0% B time = 0 min, 0% A, 100% B *t* = 20 min, 100% A, 0% B *t* = 22 min, flow rate = 1 mL/min). CPS: counts per second.

Table 3
Biodistribution pattern of ^{99m}Tc-glucose complexes [^{99m}Tc]**9** and [^{99m}Tc]**10** in B16F1 melanoma-bearing C57BL/6 mice, $n = 3$, at 60 min post-injection

	% ID/g 60 min Post-injection	
Organ	[^{99m}Tc]**9**	[^{99m}Tc]**10**
Blood	1.6 ± 1.3	2.3 ± 0.28
Liver	3.9 ± 0.99	8.7 ± 1.3
Heart	0.31 ± 0.14	0.38 ± 0.06
Lung	0.67 ± 0.49	1.5 ± 0.57
Spleen	0.27 ± 0.11	0.38 ± 0.22
Kidney	2.3 ± 0.95	3.5 ± 0.71
Thyroid	0.39 ± 0.14	0.41 ± 0.16
Muscle	0.12 ± 0.02	0.29 ± 0.08
Bone	0.17 ± 0.04	0.46 ± 0.30
Brain	0.046 ± 0.006	0.15 ± 0.02
Tumor	0.31 ± 0.23	0.40 ± 0.28
	% of injected dose (% ID)	
Intestine	19 ± 4.1	46 ± 6.2
Urine and bladder	65 ± 7.6	16 ± 2.1

Table 4
Tumor/blood and tumor/muscle ratios for ^{99m}Tc-glucose complexes ([^{99m}Tc]**9** and [^{99m}Tc]**10**) at 60 min post-injection

	Tumor/blood	Tumor/muscle
[^{99m}Tc]**9**	0.21 ± 0.05	2.5 ± 0.3
[^{99m}Tc]**10**	0.24 ± 0.02	2.0 ± 0.7

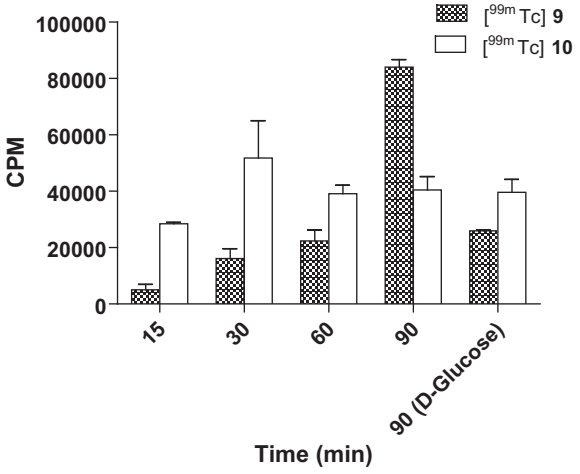


Figure 2. Tumor-cell uptake assay of ^{99m}Tc-glucose complexes ([^{99m}Tc]**9** and [^{99m}Tc]**10**) by B16F1 cells at 37 °C during 15, 30, 60 and 90 min. Blocking experiment was performed by incubation with D-glucose for 90 min. Total radioactivity is presented as count per minute (CPM).

structure and function. Other chelating systems as MAG_3 or MAMA, are also suitable options for radiolabeling glucose with technetium-99m as describe by Chen et al.[13] Those complexes presented similar biodistribution patterns, tumor uptake and partition coefficients than the complexes described in this Letter. [99mTc]**9** and [99mTc]**10** complexes were evaluated for its cell internalization and biodistribution in C57BL/6 mice bearing B16F1 murine melanoma model. The high in vitro uptake of [99mTc]**10** which was not dependent of glucose, supporting the idea that different affinity glucose transporters isoforms or passive transport may contribute to glucose transport across the cell membrane. Biodistribution studies showed that both complexes behave similar and have almost identical tumor to muscle and tumor to blood ratios meaning that deoxyglucose have the same distribution in vivo than glucose. The results presented here justify further investigations in animals and humans of these complexes as potential melanoma imaging agents.

Acknowledgments

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26. General procedure for preparation of esters **7** and **8**: Azide **5** or **6** (1 equiv) was dissolved in dry CH_2Cl_2 (24 mL/mmol) and triphenylphosphine polymer-bound (Sigma, 2 equiv, 3 mmol/g) was added. After the mixture had been shaked for 24 h at room temperature, water (0.38 mL/mmol) was added and then was shaked for a further 6 h. The mixture was filtered, washed with CH_2Cl_2 and the solvent was evaporated in vacuo to obtain the corresponding amines, that were sufficiently pure by TLC analysis for further synthesis. Immediately, triethylamine (2.2 equiv) and ethyl bromoacetate (2.2 equiv) were added to a solution of the corresponding amines in dry THF (17 mL/mmol). The solution was refluxed for 5 h and then was stirred overnight at room temperature under N_2 atmosphere. The mixture was filtered, diluted with CH_2Cl_2 and washed with water. The organic phases were dried over Na_2SO_4 and the solvent was evaporated in vacuo. The crude was subjected to column cromatography (SiO_2, mixtures of Hexane/Ethyl acetate 3:7) to afford **7** and **8**. **(7)**: 23%, yellowish oil, ^{1}H-RMN ($CDCl_3$): $\delta = 5.21$ (t, $J = 9.2$ Hz, 1H; H-3), 5.09 (t, $J = 9.6$ Hz, 1H; H-4), 5.00 (t, $J = 8.0$ Hz, 1H; H-2), 4.63 (d, $J = 8.0$ Hz, 1H; H-1), 4.29 (dd, $J = 4.8$ Hz, $J' = 4.0$ Hz, 1H; H-6a), 4.18 (c, $J = 7.2$ Hz, 4H; OCH_2CH_3), 4.12 (m, 1H; H-6b), 3.99 (m, 1H; OCH_2CH_2N), 3.71 (m, 2H; H-5 OCH_2CH_2N), 3.57 (s, 4H; $NCH_2C{=}O$), 3.04 (m, 2H; OCH_2CH_2N), 2.08, 2.04, 2.02, 1.99 (s, 12H; $CH_3C{=}O$), 1.28 (t, $J = 7.2$ Hz, 6H; OCH2CH_3). ^{13}C-RMN ($CDCl_3$): $\delta = 7.5$ (OCH_2CH_3), 53.5 (OCH_2CH_2N), 55.2 (C-5), 56.3 ($NCH_2C{=}O$), 61.6 (OCH_2CH_3), 62.5 (C-6), 68 (C-4), 69.2 (OCH_2CH_2N), 71.7 (C-2), 73.1 (C-3), 101.5 (C-1), 170.3 ((C=O)Me), 171.6 ((C=O)Et). **(8)**: 37%, yellowish oil, ^{1}H-RMN ($CDCl_3$): $\delta = 5.32$ (m, 1H; H-3), 5.04 (m, 2H; H-1 H-4), 4.36 (dd, $J = 4.4$ Hz $J' = 4.4$ Hz, 1H; H-6a), 4.22 (c, $J = 6.8$ Hz, 4H; OCH_2CH_3), 4.07 (d, $J = 2.0$ Hz, 1H; H-6b), 4.04 (m, 1H; H-5), 3.80 (m, 1H; OCH_2CH_2N), 3.63 (s, 4H; $NCH_2C{=}O$), 3.61 (m, 1H; OCH_2CH_2N), 3.05 (m, 2H; OCH_2CH_2N), 2.26 (dd, $J = 0.8$ Hz $J' = 1.2$ Hz, 1H; H-2 ec.), 2.11, 2.06, 2.02 (s, 9H; $CH_3C{=}O$), 1.87 (ddd, $J = 3.6$ Hz $J = 7.8$ Hz $J'' = 3.6$ Hz, 1H; H-2ax), 1.31 (t, $J = 6.8$ Hz, 6H; OCH2CH_3). ^{13}C-RMN ($CDCl_3$): $\delta = 14.1$ (OCH_2CH_3), 35.5 (C-2), 54.5 (OCH_2CH_2N), 56.7 ($NCH_2C{=}O$), 60.3 (OCH_2CH_3), 62.5 (C-6), 67.2 (C-5), 67.7 (OCH_2CH_2N), 69.7 (C-4), 98.0 (C-1), 170.1 ((C=O)Me), 171.5 ((C=O)Et).
27. Na[$^{99m}TcO_4^-$] (1 mL, 200 MBq) from ^{99m}Tc/^{99}Mo generator was added to Isolink™ kit. The solution was incubated in water bath at 100 °C for 20 min and then was neutralized with 1 mL of 0.1 N HCl solution. Label was controlled by HPLC as described in this Letter.

28. HPLC: Agilent 1200 system control equipped with gamma detector Raytest, Column C18 Thermo Scientific ODS HYPERSIL 300 × 4.6 mm, particle size 10 μ.
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