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# Synthesis and biodistribution studies of carbohydrate derivatives radiolabeled with technetium-99m

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ABSTRACT

Three carbohydrate derivatives, $MAG_3$-Gl, $MAG_3$-Ga, $MAG_3$-NG, were synthesized and radiolabeled in high yields. These substances were injected in health Swiss mice and their biodistribution were evaluated. Among them, $^{99m}$Tc-$MAG_3$-Ga displayed higher accumulation in hepatic tissue, due to the presence of specific receptors in the liver for this carbohydrate. Thus, the use of $^{99m}$Tc-$MAG_3$-Ga to assess hepatic function can be considered.



The surface of all mammalian cells is covered with a diverse range of complex carbohydrates attached to proteins and lipids that are embedded in the cell membrane. These oligo- and polysaccharides form a first line of interaction with the extracellular world, through their recognition by carbohydrate-binding proteins called lectins present on other mammalian cells, viruses, bacteria and bacterial toxins.[1,2] Carbohydrate–protein interactions are responsible for a wide range of biological phenomena including cancer cell metastasis, inflammation, and infections by bacteria and viruses.[3–6]

Lectins bind mono- and oligosaccharides reversibly and with high specificity. Each lectin molecule contains typically two or more carbohydrate-combining sites, that is, they are di- or polyvalent. They are found in most organisms, ranging from viruses and bacteria to plants and animals, and represent a heterogeneous group of oligomeric proteins that vary widely in size, structure, molecular organization, as well as in the constitution of their combining sites. In humans it is possible to found them in hepatocytes, reticuloendothelial cells, myocardial cells, and thrombin-activated platelets.[7–9]

Radioisotopes are useful to evaluate biodistribution of molecules in the body. This study is very important to obtain information about molecule–receptor interactions, and then, to produce radiopharmaceutical drugs able to be used as radiotracer and the molecules can be used as constituents of delivery systems.

Technetium-99m ($^{99m}$Tc) has been mostly used for labeling radiopharmaceuticals owing to its suitable physical and chemical characteristics and inexpensive isotope cost.[10–12]

Carbohydrates are generally weak ligands for chelating with $^{99m}$Tc. Therefore, functionalization with an external chelating group or the insertion of some functional groups is essential to obtain strong metal-binding compounds.[13] The mercaptoacetyl triglycine ($MAG_3$) is an efficient complexing agent for $^{99m}$Tc which has been used as tubular renal radiotracer.[14,15] Its structure has an carboxylic acid function that allows the coupling with some derivatives bearing an amino group.

The purpose of this study was to synthesize three $MAG_3$ derivatives bearing a carbohydrate moiety (D-glucose, *N*-acetylglucosamine, and D-galactose), radiolabel them with technetium-99m and evaluated their biodistribution in Swiss mice.

The *S*-benzoylmercaptoacetyl triglycine ($SBzMAG_3$) was synthesized according to Fritzberg, 1986,[14] and the carbohydrate derivatives were synthesized according to the procedure outlined in Scheme 1. Briefly, the 4-nitrophenyl β-D-glucopyranoside **1**, 4-nitrophenyl β-D-galactopyranoside **2**, and 4-nitrophenyl *N*-acetyl-β-D-glucosaminide **3** were reduced to the corresponding amines **4**, **5**, and **6**, respectively, using catalytic hydrogenation. The compounds **4**, **5**, and **6** were then reacted with $SBzMAG_3$, previously synthesized, using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDAC) as coupling agent to obtain $SBzMAG_3$-Gl **7**, $SBzMAG_3$-Ga **8**, and $SBzMAG_3$-NG **9**. All compounds were characterized by IR, $^1$H NMR, and $^{13}$C NMR spectroscopy.[16] The technetium-99m labeled $MAG_3$ carbohydrate-based complexes ($^{99m}$Tc-$MAG_3$-Gl, $^{99m}$Tc-$MAG_3$-Ga, and $^{99m}$Tc-$MAG_3$-NG) were prepared by ligand-exchange reaction with $^{99m}$Tc-tartarate at pH 6–8. In these conditions the benzoyl protecting group of **7**, **8**, and **9** is removed.[14]

After radiolabeling the products were purified by column chromatography on Florisil mesh 60–100, using, first, acetone to

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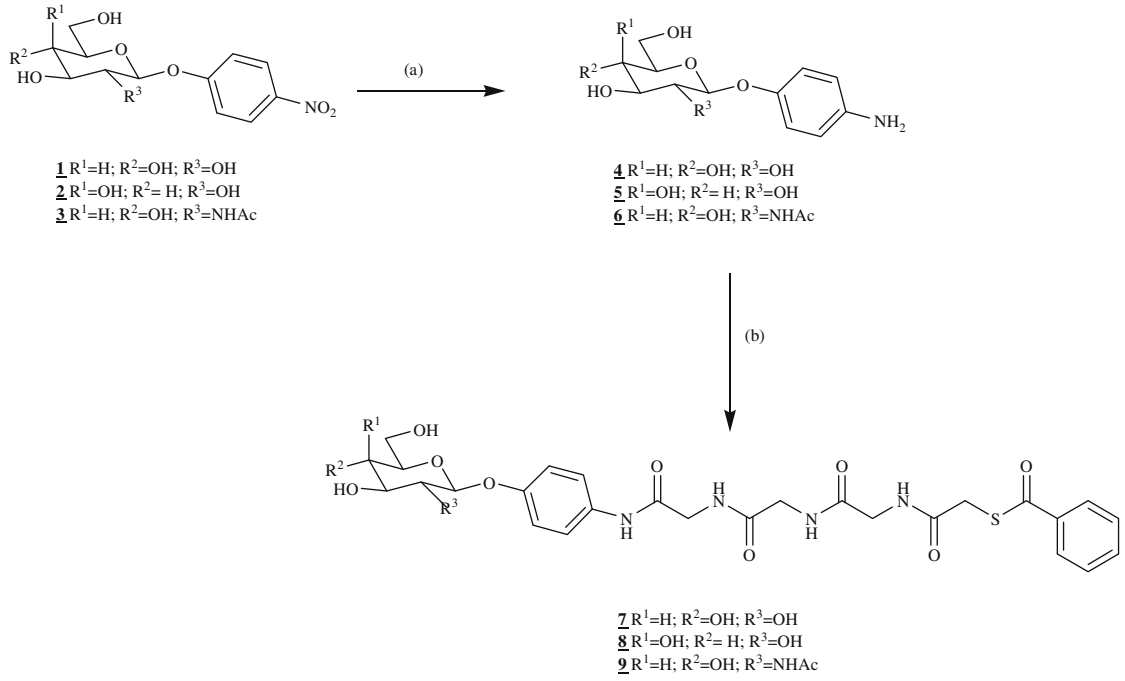


**Scheme 1.** Synthesis of carbohydrate-based $MAG_3$ derivatives **7–9**. Reagents: (a) $H_2$, Pd/C, solvent: methanol; (b) EDAC, $SBzMAG_3$, solvent: DMF.

remove $TcO_4^-$ and next, 0.9% saline to elute the $^{99m}$Tc-$MAG_3$-G. The radiolabeling yields of $^{99m}$Tc-$MAG_3$-Gl, $^{99m}$Tc-$MAG_3$-G $^{99m}$Tc-$MAG_3$-NG were determined by Instant Thin Layer Chromatograph (ITLC) on two systems: 0.9% saline to determine $TcO_2$ and acetone to determine $TcO_4^-$, as published elsewhere.[17,18] The radiochemical purities were higher than 90% for all compounds evaluated.

Biodistribution of these complexes were performed in Swiss mice (25–30 g) at 5, 15, 30, and 60 min post injection. The results are summarized in Tables 1–3.

The complexes were rapidly excreted through kidneys, and they showed biodistribution pattern similar in almost all tissues. However, the D-galactose derivative $MAG_3$-Ga showed higher uptake in liver (Fig. 1).

In addition, 60 min post-injection, approximately, 58.0% of the $^{99m}$Tc-$MAG_3$-Ga was trapped in the liver, when compared with the initial time (5 min).This pattern do not occur with the others derivatives $^{99m}$Tc-$MAG_3$-Gl and $^{99m}$Tc-$MAG_3$-NG (Fig. 1). These complexes showed liver uptake 20.0% and 19.6%, respectively. Therefore, $^{99m}$Tc-$MAG_3$-Ga had higher uptake in liver than the other derivatives (about three times), probably due to the lectins present in hepatocytes that recognize this carbohydrate.

To confirm this hypothesis we performed a biodistribution study with receptor (lectin) blocking by coadministration of 10-fold cold D-galactose (Table 4). As shown, $^{99m}$Tc-$MAG_3$-Ga uptake in liver decreased considerably as compared to biodistribution data shown in Table 2, indicating competition for the receptor between cold D-galactose and $^{99m}$Tc-$MAG_3$-Ga. This result suggests that $^{99m}$Tc-$MAG_3$-Ga binds selectively to hepatocytes lectins (Fig. 2). Vera et al.[19] reported that a radiolabeled D-galactose derivative could be used to assessment of hepatocyte function and Kwon et al.,[20] verified this possibility in patients with hepatocellular carcinoma before and after hepatectomy. In this case, the D-galactose analog was used preoperatively to estimate the extent of hepatectomy and postoperatively to evaluate hepatic regeneration. Other studies were performed with similar purpose.[21–24]

**Table 1**
Biodistribution of $^{99m}$Tc-$MAG_3$-Gl in Swiss mice (%ID/g)[a]

| Tissue | 5 min | 15 min | 30 min | 60 min |
|---|---|---|---|---|
| Liver | 8.77 ± 1.22 | 5.29 ± 0.68 | 2.33 ± 0.17 | 1.82 ± 0.15 |
| Spleen | 0.99 ± 0.14 | 0.97 ± 0.05 | 0.42 ± 0.05 | 0.33 ± 0.04 |
| Kidney | 22.97 ± 3.91 | 8.06 ± 1.39 | 2.95 ± 0.41 | 1.24 ± 0.16 |
| Stomach | 1.74 ± 0.14 | 1.46 ± 0.20 | 0.77 ± 0.10 | 0.51 ± 0.06 |
| Heart | 1.66 ± 0.24 | 1.06 ± 0.13 | 0.53 ± 0.07 | 0.34 ± 0.04 |
| Lung | 2.11 ± 0.27 | 1.70 ± 0.20 | 0.75 ± 0.12 | 0.63 ± 0.08 |
| Blood | 3.28 ± 0.21 | 1.80 ± 0.21 | 0.76 ± 0.10 | 0.57 ± 0.06 |
| Bladder | 41.17 ± 5.40 | 71.30 ± 5.85 | 95.54 ± 3.05 | 94.24 ± 4.19 |

[a] All data are the mean percentage ($n$ = 5) of the injected dose of $^{99m}$Tc-$MAG_3$-Gl per gram of wet tissue, ± the standard deviation of the mean.

**Table 2**
Biodistribution of $^{99m}$Tc-$MAG_3$-Ga in Swiss mice (%ID/g)[a]

| Tissue | 5 min | 15 min | 30 min | 60 min |
|---|---|---|---|---|
| Liver | 11.99 ± 1.00 | 11.49 ± 1.94 | 10.35 ± 0.57 | 6.95 ± 0.64 |
| Spleen | 1.59 ± 0.19 | 1.00 ± 0.22 | 0.58 ± 0.08 | 0.59 ± 0.12 |
| Kidney | 9.11 ± 2.17 | 5.48 ± 1.22 | 2.78 ± 0.50 | 2.47 ± 0.38 |
| Stomach | 1.14 ± 0.23 | 0.92 ± 0.16 | 0.62 ± 0.12 | 0.53 ± 0.05 |
| Heart | 1.82 ± 0.34 | 1.11 ± 0.18 | 0.63 ± 0.09 | 0.52 ± 0.07 |
| Lung | 2.61 ± 0.20 | 1.74 ± 0.43 | 0.97 ± 0.15 | 0.91 ± 0.08 |
| Blood | 4.12 ± 0.67 | 2.11 ± 0.49 | 1.07 ± 0.16 | 0.86 ± 0.07 |
| Bladder | 44.86 ± 6.98 | 81.05 ± 5.17 | 80.64 ± 9.71 | 86.37 ± 5.06 |

[a] All data are the mean percentage ($n$ = 5) of the injected dose of $^{99m}$Tc-$MAG_3$-Ga per gram of wet tissue, ± the standard deviation of the mean.

**Table 3**
Biodistribution of $^{99m}$Tc-$MAG_3$-NG in Swiss mice (%ID/g)[a]

| Tissue | 5 min | 15 min | 30 min | 60 min |
|---|---|---|---|---|
| Liver | 9.58 ± 0.81 | 6.92 ± 0.91 | 4.35 ± 0.25 | 1.88 ± 0.44 |
| Spleen | 2.01 ± 0.29 | 1.24 ± 0.17 | 1.10 ± 0.14 | 0.27 ± 0.02 |
| Kidney | 16.81 ± 2.24 | 6.25 ± 0.51 | 5.16 ± 1.20 | 1.93 ± 0.41 |
| Stomach | 1.06 ± 0.18 | 0.92 ± 0.16 | 0.76 ± 0.18 | 0.31 ± 0.05 |
| Heart | 2.56 ± 0.38 | 1.70 ± 0.24 | 1.66 ± 0.11 | 0.53 ± 0.10 |
| Lung | 4.00 ± 0.64 | 2.46 ± 0.23 | 1.98 ± 0.37 | 0.67 ± 0.12 |
| Blood | 7.33 ± 1.40 | 4.24 ± 0.47 | 3.86 ± 0.40 | 1.18 ± 0.24 |
| Bladder | 33.92 ± 7.56 | 76.27 ± 9.23 | 75.46 ± 13.62 | 89.07 ± 5.01 |

[a] All data are the mean percentage ($n$ = 5) of the injected dose of $^{99m}$Tc-$MAG_3$-NG per gram of wet tissue, ± the standard deviation of the mean.

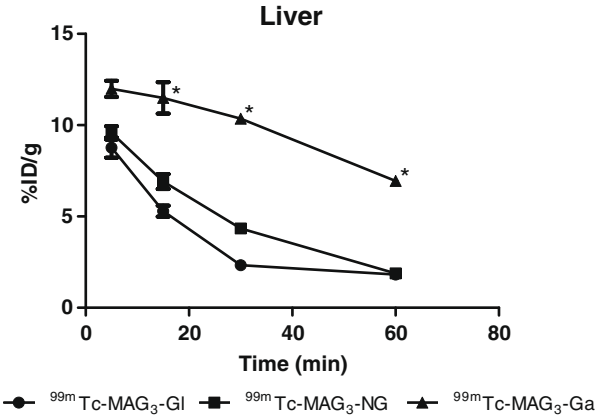


**Figure 1.** Amount of radioactivity in liver after intravenous administration of $^{99m}$Tc-MAG$_3$-GI, $^{99m}$Tc-MAG$_3$-NG, $^{99m}$Tc-MAG$_3$-Ga in Swiss mice. Results are expressed as means ± standard error. The asterisks indicate a statistically significant difference between $^{99m}$Tc-MAG$_3$-Ga and others derivatives ($p$ <0.05).

**Table 4**
Biodistribution of $^{99m}$Tc-MAG$_3$-Ga in Swiss mice with blocked receptor (%ID/g)[a]

| Tissue | 5 min | 15 min | 30 min | 60 min |
|---|---|---|---|---|
| Liver | 12.54 ± 0.42 | 7.61 ± 0.69 | 5.82 ± 1.12 | 2.43 ± 0.13 |
| Spleen | 1.40 ± 0.24 | 1.15 ± 0.21 | 0.52 ± 0.09 | 0.17 ± 0.04 |
| Kidney | 10.66 ± 2.03 | 9.19 ± 2.28 | 3.07 ± 0.10 | 1.88 ± 0.16 |
| Stomach | 0.42 ± 0.14 | 0.83 ± 0.07 | 1.32 ± 0.82 | 0.39 ± 0.15 |
| Heart | 1.77 ± 0.12 | 2.06 ± 0.34 | 0.67 ± 0.16 | 0.23 ± 0.12 |
| Lung | 2.53 ± 0.77 | 2.71 ± 0.14 | 1.00 ± 0.09 | 0.48 ± 0.02 |
| Blood | 3.05 ± 0.67 | 3.58 ± 0.54 | 1.10 ± 0.19 | 0.53 ± 0.03 |
| Bladder | 60.19 ± 20.33 | 81.37 ± 8.78 | 80.28 ± 22.10 | 52.64 ± 11.50 |

[a] All data are the mean percentage ($n$ = 5) of the injected dose of $^{99m}$Tc-MAG$_3$-Ga per gram of wet tissue, ± the standard deviation of the mean.

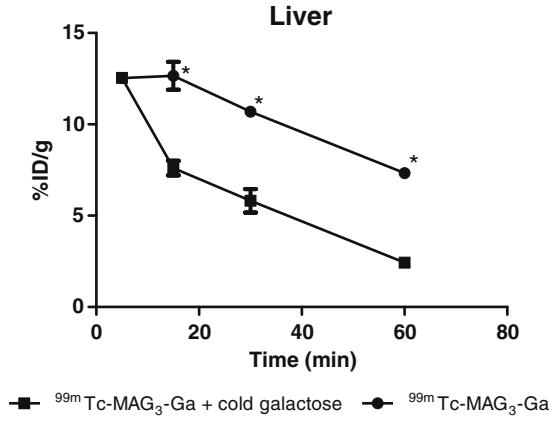


**Figure 2.** Amount of radioactivity in liver after intravenous administration of $^{99m}$Tc-MAG$_3$-Ga and $^{99m}$Tc-MAG$_3$-Ga + cold galactose in Swiss mice. Results are expressed as means ± standard error. The asterisks indicate a statistically significant difference between $^{99m}$Tc-MAG$_3$-Ga and $^{99m}$Tc-MAG$_3$-Ga + cold galactose ($p$ <0.05).

In summary, three novel carbohydrate-based MAG$_3$ derivatives were synthesized and labeled with technetium-99m successfully. The complex $^{99m}$Tc-MAG$_3$-Ga displayed selective uptake by the liver and can be considered as a potential imaging agent for evaluation of hepatic function.

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16. Compound **7**: mp 213.2–214.4 °C, $[\alpha]_D$ −21.7 ($c$ 2.5, DMSO). IR ($\nu_{máx}$, cm$^{-1}$): 3272, 3081, 2928, 1638, 1074, 1019. $^1$H NMR (DMSO-$d_6$, 400 MHz, $\delta$) 9.67 (s, 1H), 8.50 (t, 1H), 8.28 (t, 1H), 8.21 (t, 1H), 7.93 (d, 2H), 7.70 (t, 1H), 7.57 (t, 2H), 7.50 (d, 2H), 6.98 (d, 2H), 5.28 (d, 1H), 5.05 (d, 1H), 4.99 (d, 1H), 4.78 (d, 1H), 4.55 (t, 1H), 3.88 (s, 2H), 3.86 (d, 2H), 3.80 (d, 2H), 3.78 (d, 2H), 3.71–3.67 (m, 1H), 3.50–3.46 (m, 1H), 3.32–3.12 (m, 4H). $^{13}$C NMR (DMSO-$d_6$, 400 MHz, $\delta$), 190.4 (1C), 169.4–167.2 (4C), 153.4 (1C), 136.0 (1C), 134.1 (1C), 133.0 (1C), 129.2 (2C), 126.9 (2C), 120.5 (2C), 116.5 (2C), 100.8 (1C), 77.0 (1C), 76.7 (1C), 73.3 (1C), 69.8 (1C), 60.8 (1C), 42.6–42.2 (3C), 32.5 (1C).
Compound **8**: mp 213.6–215.1 °C, $[\alpha]_D$ −14.9 ($c$ 2.5, DMSO). IR ($\nu_{máx}$, cm$^{-1}$): 3280, 3082, 2922, 1639, 1071, 1026. $^1$H NMR (DMSO-$d_6$, 400 MHz, $\delta$) 9.66 (s, 1H), 8.50 (t, 1H), 8.28 (t, 1H), 8.20 (t, 1H), 7.93 (d, 2H), 7.70 (t, 1H), 7.56 (t, 2H), 7.51 (d, 2H), 6.98 (d, 2H), 5.26 (d, 1H), 4.99 (d, 1H), 4.97 (d, 1H), 4.78 (d, 1H), 4.54 (t, 1H), 3.87 (s, 2H), 3.85 (d, 2H), 3.79 (d, 2H), 3.78 (d, 2H), 3.71–3.68 (m, 1H), 3.50–3.44 (m, 1H), 3.24–3.13 (m, 4H). $^{13}$C NMR (DMSO-$d_6$, 400 MHz, $\delta$), 190.1 (1C), 169.3–167.3 (4C), 153.4 (1C), 136.0 (1C), 134.1 (1C), 133.0 (1C), 129.1 (2C), 127.0 (2C), 120.4 (2C), 116.4 (2C), 101.2 (1C), 77.4 (1C), 77.0 (1C), 73.6 (1C), 70.1 (1C), 61.1 (1C), 42.9–42.6 (3C), 32.8 (1C).
Compound **9**: mp 198.9–200.1 °C, $[\alpha]_D$ −17.8 ($c$ 2.5, DMSO). IR ($\nu_{máx}$, cm$^{-1}$): 3266, 3084, 1639, 1074, 1027. $^1$H NMR (DMSO-$d_6$, 400 MHz, $\delta$) 9.69 (s, 1H), 8.51 (t, 1H), 8.28 (t, 1H), 8.21 (t, 1H), 7.94 (d, 2H), 7.79 (d, 1H), 7.70 (t, 1H), 7.57 (t, 2H), 7.50 (d, 2H), 6.92 (d, 2H), 5.30 (d, 1H), 5.05 (d, 1H), 4.97 (d, 1H), 4.90 (d, 1H), 3.89 (s, 2H), 3.85 (d, 2H), 3.75 (d, 2H), 3.74 (d, 2H), 3.71–3.66 (m, 1H), 3.51–3.47 (m, 1H), 3.30–3.18 (m, 4H), 1.82 (s, 3H). $^{13}$C NMR (DMSO-$d_6$, 400 MHz, $\delta$), 190.3 (1C), 169.3–167.1 (5C), 153.5 (1C), 135.9 (1C), 134.1 (1C), 133.2 (1C), 129.2 (2C), 126.9 (2C), 120.5 (2C), 116.7 (2C), 99.6 (1C), 77.2 (1C), 74.1 (1C), 70.3 (1C), 60.8 (1C), 55.5 (1C), 42.5 (2C), 42.2 (1C), 32.4 (1C), 23.1 (1C).
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