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Preparation and preliminary bioevaluation of a $^{99m}Tc(CO)_3$-glucose derivative prepared by a click chemistry route

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Development of a ^{99m}Tc-labelled glucose derivative as a single-photon emission computed tomography analogue to [^{18}F]-2-fluoro-2-deoxy-D-glucose (FDG) is considered of great interest. Herein, we present the synthesis and preliminary bioevaluation of a $^{99m}Tc(CO)_3$-glucose derivative. Derivatization of glucose at C2 was achieved using the so-called 'click chemistry', forming a histidine-like, 1,4-disubstituted triazole adequate as donor atom system for Tc(I)-tricarbonyl complexes. Synthesis of the intermediate azide derivative was achieved by conventional organic chemistry. Because of the efficiency and selectivity of this cycloaddition, labelling was performed in 'one pot', adding the tricarbonyl precursor *fac*-[$^{99m}Tc(CO)_3(H_2O)_3]^+$ to the same vial where the click reaction has taken place without any further purification. A single product with radiochemical purity higher than 90% was obtained. It was stable for at least 4 h in reaction milieu and exhibited high hidrophilicity (log *P* of −1.2) and low binding to plasma proteins (5 ± 1%). Biodistribution in C57BL/6 mice bearing induced Lewis murine lung carcinoma is characterized by low blood and liver uptake and rapid urinary excretion. ^{99m}Tc complex showed moderate tumour uptake but significant retention until 2 h post-injection. Soft tissue clearance was fast leading to significantly higher uptake in tumour in comparison to muscle ($p = 0.05$) at all time points. Overall, biodistribution of our compound was very similar to that of [^{18}F]-FDG. However, tumour uptake was significantly higher for [^{18}F]-FDG, probably because of high hidrophilicity of our derivative that may hinder cell penetration. Similarity to biodistribution of FDG is a promising outcome, and modifications of the chelator and linker might improve biological results.

Keywords: Tc-tricarbonyl complexes; glucose derivative; 'click chemistry'; tumour imaging agent

Introduction

Glucose is considered as a key molecule in metabolic evaluation of tumours, because a nearly universal property of primary and metastatic cancer is the upregulation of glycolysis, which can result in increased glucose consumption.[1] Therefore, availability of a ^{99m}Tc-labelled glucose derivative as a single-photon emission computed tomography analogue to the well-established positron emission tomography tracer [^{18}F]-2-fluoro-2-deoxy-D-glucose (FDG) is considered of great interest. Apart from the low costs and wide availability of ^{99m}Tc, the analogy in chemistry between technetium and rhenium, together with the fact that radioisotopes 186 and 188 of rhenium have appropriate nuclear properties for therapy (^{186}Re, $t_{1/2} = 90.6$ h, $E_{\beta máx.} = 1.1$ MeV, $E_\gamma = 137$ keV; ^{188}Re, $t_{1/2} = 17$ h, $E_{\beta máx.} = 2.1$ MeV, $E_\gamma = 155$ keV), contribute to this preference, because the studies of ^{99m}Tc complexes may be further expanded to the preparation of analogous rhenium compounds for therapy.

Although several groups have reported the preparation of ^{99m}Tc-labelled glucose and glucosamine analogues, to our knowledge, none of them has yet the expected properties, and further research in this area is still necessary.[2–7]

As part of our ongoing research in the application of new ^{99m}Tc moieties, namely Tc(V)-nitride, 4+1 mixed ligand Tc(III) and Tc(I)-tricarbonyl complexes, to the labelling of small biomolecules,[8–10] we present herein the preparation and preliminary bioevaluation as potential radiopharmaceutical for nuclear oncology of a $^{99m}Tc(CO)_3$-glucose derivative. Labelling was performed using the pendent approach in order to preserve the biological activity. Derivatization of glucose was achieved using the so-called 'click chemistry', a Huisgen's reaction, which occurs through a Cu(I)-catalyzed [3 + 2] cycloaddition of alkynes and azides forming a histidine-like, 1,4-disubstituted triazole adequate as donor atom system for Tc(I)-tricarbonyl complexes (Figure 1).[11–15]

Biodistribution studies in C57 mice bearing tumours induced by inoculation of 3LL cells of Lewis murine carcinoma are also presented.

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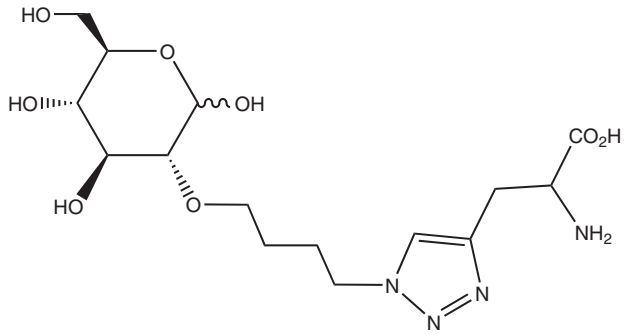

Figure 1. Glucose derivative bearing a 1,4 disubstituted triazole as donor system for ^{99m}Tc labelling.

Results and discussion

As a first step to achieve our goal, synthesis of a glucose derivative containing adequate donor groups for ^{99m}Tc labelling using the Tc-tricarbonyl core was pursued (Figure 1). The synthesis route used for the intermediate azide preparation is shown in Figure 2.

β-D-Pentaacetyl glucose was used as starting material; the anomeric carbon was protected using a piperidine group. According to the extensive study performed by R. Schibli *et al.*,[17] we decided to introduce a bromobutyl chain linker in C2 position by a two-step procedure. The first step involved the anomeric C protection with piperidine and selective hydrolysis of C2 to afford compound **1** by precipitation.[18] The following step consisted in a nucleophilic substitution with 1,4-dibromo butyl to obtain compound **2** in moderate yield.[19] Then, substitution of the bromine atom employing trimethylsylil azide allows us to obtain azide group necessary for the [3 + 2] cycloaddition in good yield. All intermediate products were obtained in reasonable yield and high purity as demonstrated by ^{1}H-NMR analysis. Elimination of the protective acetate groups of the hexose should be performed in order to obtain the final product. However, purification of the deprotected glucose derivative to eliminate salts that co-precipitated in the process is very difficult because of its high polarity. Consequently, we decided to perform deprotection *in situ* before each labelling without further purification.

The desired chelating system was introduced by means of the Huisgen's [3 + 2] cycloaddition, one of the most representative examples of 'click chemistry'. It consists of an innovative functionalization strategy for biomolecules because it is efficient, selective and devoid of side reactions. The mild reaction conditions are well suited for the modification of biomolecules, into which the required azide or alkyne functionalities were previously incorporated by standard synthetic transformations.[11] In addition, 1,4-disubstituted 1,2,3-triazoles share structural and electronic features with 1,4-disubstituted imidazoles of N^{ε}-derivatized histidines, which have been shown to be extraordinarily good chelators for organometallic cores of technetium.[12] Figure 3 depicts procedures used for deprotection and derivatization by 'click chemistry' of the glucose derivative containing an azide group (compound **3**).

This methodology has been applied recently by E. Benoist *et al.*[20] to the synthesis of C1 functionalized glucose derivatives containing various tridentate chelating systems. They have prepared an interesting series of ^{99m}Tc and Re complexes with promising biodistribution in normal animals. Another interesting aspect is that they demonstrated for the first time the possibility to graft a pre-chelated $M(CO)_3$ core directly onto a biomolecule by click chemistry. The reported data open new possibilities in the development of ^{99m}Tc-labelled glucose derivatives.

Our approach, however, was to take advantage of the high efficiency and selectivity of this cycloaddition, and consequently labelling was performed in 'one pot', directly adding the tricarbonyl precursor *fac*-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ obtained with radiochemical purity >90% (either by reduction of pertechnetate in the presence of CO(g) or by use of a lyophilized kit[16,21]) to the same vial where the click reaction has taken place without any separation or purification. A single product with a retention time of 14 min and radiochemical purity higher than 90% was obtained, as demonstrated by HPLC analysis. The proposed structure of the ^{99m}Tc complex is shown in Figure 4.

Stability for more than 4 h in reaction milieu was also determined by HPLC analysis.

The partition coefficient between 1-octanol and phosphate buffer pH 7.0 of the technetium complex was measured in order to assess their lipophilicity. A log *P* of −1.2 was obtained, indicating high hydrophilicity.

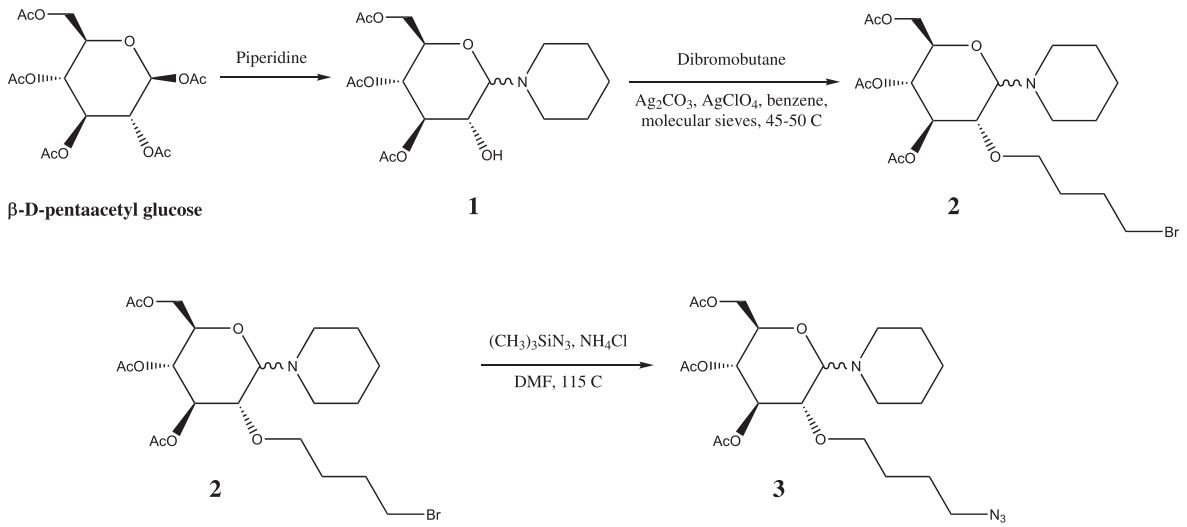

Figure 2. Synthesis route for the preparation of azide derivative of glucose (compound **3**).

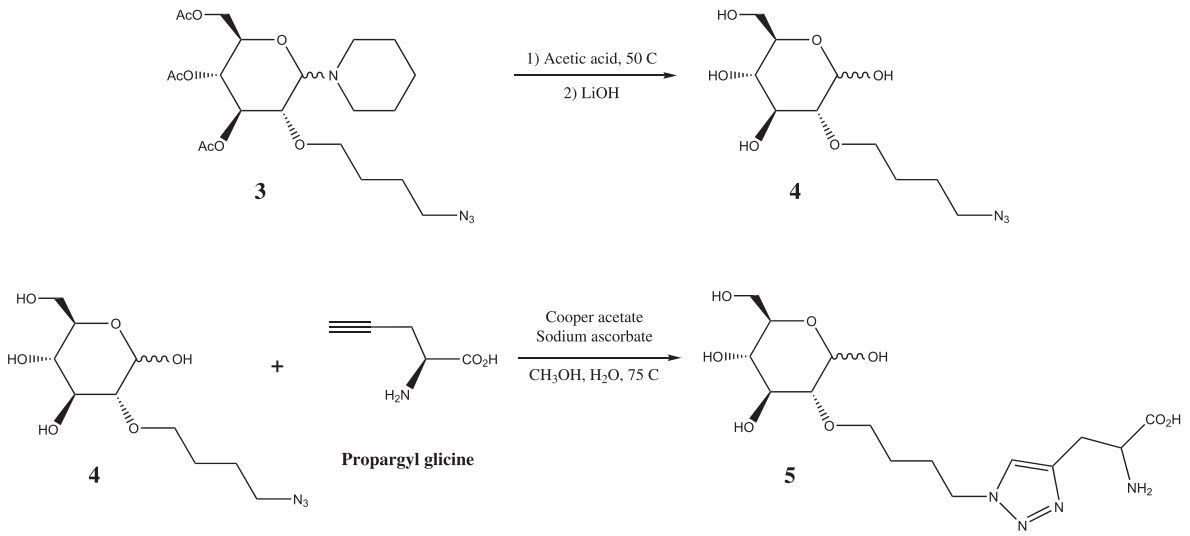

Figure 3. Elimination of protective groups of glucose derivative **3** and introduction of donor system by 'click chemistry'.

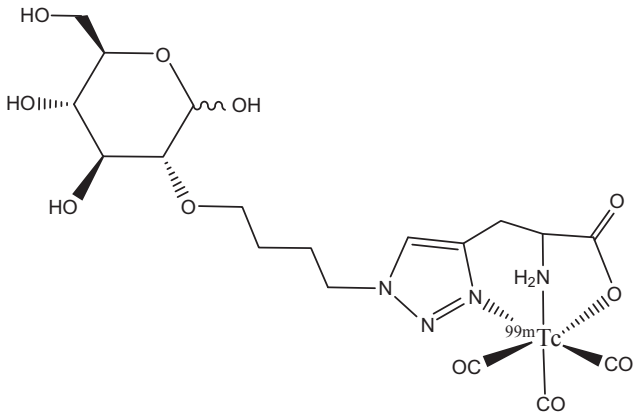

Figure 4. Proposed structure of ^{99m}Tc-labelled glucose derivative.

Binding to plasma proteins was studied using size exclusion chromatography. Ideally, low protein binding is required in order to ensure adequate pharmacokinetics of the potential radiopharmaceuticals. Additionally, only the unbound fraction of the radiotracer will penetrate cells and other biological membranes.[22] A relatively low protein binding of $5 \pm 1\%$ was obtained, correlating with the high *in vitro* stability and low lipophilicity of this complex.

Glucose derivative bearing the azide group (**3**) and propargylglicine were also labelled with ^{99m}Tc and analysed by HPLC. Substitution was above 90% when propargylglicine was used as ligand, but HPLC revealed three major peaks with longer retention times than our labelled glucose derivative. On the other hand, when compound **3** was used as ligand, substitution of tricarbonyl precursor was negligible. Radiochromatograms of all labelled species are shown in Figure 5.

In order to assess the potentiality of our approach for the design of a potential radiopharmaceutical for nuclear oncology, the biodistribution of the ^{99m}Tc complex was evaluated at 30 min, 1 h and 2 h post-injection in C57BL/6 mice bearing tumours induced by inoculation of 3LL Lewis murine lung carcinoma cells. Cells were inoculated subcutaneously in the right limb, and biodistribution studies were performed 20 to 30 days after inoculation when tumours have adequate size.

Table 1 summarizes the results expressed as % dose/organ in the most significant organs as a function of time.

Results are in full agreement with physicochemical properties of the ^{99m}Tc complex. Biodistribution is characterized by low blood and liver uptake. Excretion occurs rapidly through the urinary tract as expected for a compound with low lipophilicity. Thyroid and stomach activities are very low indicating minimal *in vivo* reoxidation. Uptake in other organs was negligible. Uptake in tumour is moderate at all time points, probably because of high hydrophilicity that may hinder cell penetration.

Table 2 summarizes *in vivo* tumour uptake expressed as % dose/g as well as tumour/muscle ratio.

^{99m}Tc complex demonstrated moderate tumour uptake ($0.49 \pm 0.03\%$ dose/g at 0.5 h post-injection) and significant retention until 2 h ($0.35 \pm 0.01\%$ dose/g). Soft tissue clearance is fast, and this was reflected in tumour/muscle ratios, which were very favourable in the studied period (1.8 ± 0.1 at 0.5 h to 2.75 ± 0.06 at 2 h). Statistical analysis demonstrated that uptake in tumour was significantly higher in comparison to muscle ($p = 0.05$) at all time points.

Although the uptake of various ^{99m}Tc-labelled glucose derivatives in tumour have been reported earlier, their comparison is very difficult because of the heterogeneity of the type of tumours used. However, analysis of previously reported data[5–7,23] showed that tumour uptake in most of them is low, in the same order of ^{99m}Tc-labelled glucose derivative **5**, specially at longer retention times and even those that have higher tumour uptake[3,24] have low tumour/blood ratio.

In order to compare with the gold standard, biodistribution of [^{18}F]-FDG (provided by CUDIM, Uruguayan Centre for Molecular Imaging) in the same animal model and the same experimental conditions was also performed. Results are shown in Tables 3 and 4.

Although the overall biodistribution of our compound is very similar to that of [^{18}F]-FDG and tumour/muscle ratios at longer biodistribution times are also comparable, the total tumour uptake and the tumour dose/gramme are significantly higher for [^{18}F]-FDG, probably because of high hidrophilicity that may hinder cell penetration. Although ^{18}F-FDG is even more

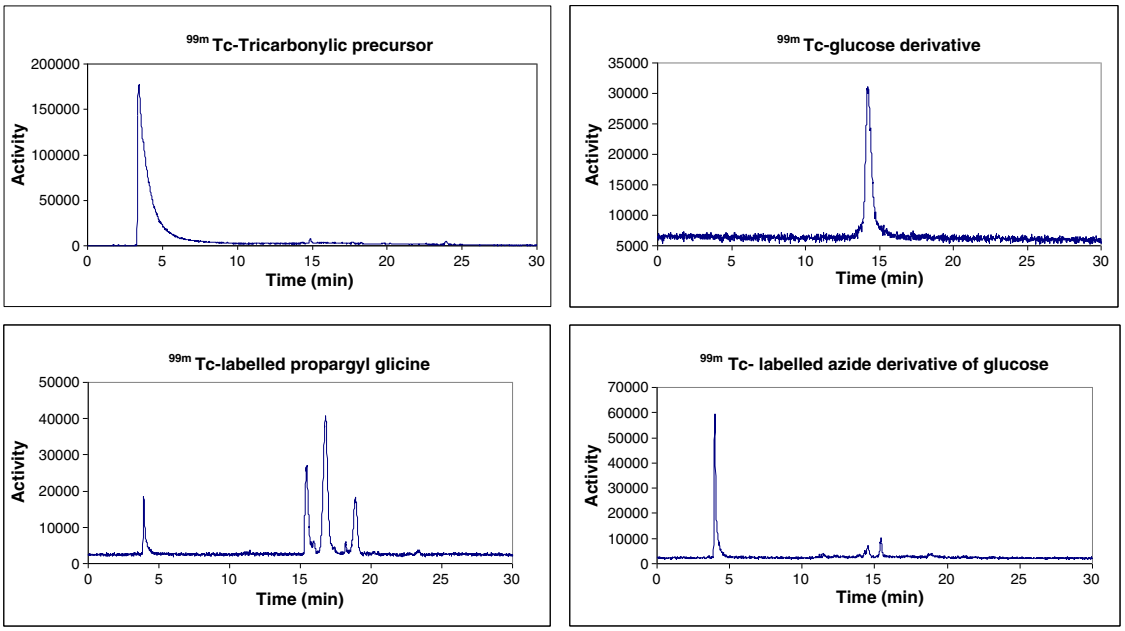

Figure 5. Radiochromatograms of various stages of ^{99m}Tc labelling of glucose derivatives.

Table 1. Biodistribution results of $^{99m}Tc(CO)_3$-glucose derivative in C57BL/6 bearing induced Lewis murine lung carcinoma

	% Injected dose		
Organ	0.5 h	1.0 h	2.0 h
Blood	2.15 ± 0.15	2.06 ± 0.43	0.82 ± 0.09
Liver	8.07 ± 0.38	5.61 ± 0.65	3.02 ± 0.44
Kidneys	2.08 ± 0.28	0.93 ± 0.23	0.47 ± 0.04
Thyroid	0.08 ± 0.05	0.12 ± 0.07	0.10 ± 0.02
Muscle	2.57 ± 0.81	2.19 ± 0.74	0.83 ± 0.09
Tumour	0.20 ± 0.06	0.25 ± 0.12	0.10 ± 0.07
Stomach	1.94 ± 0.70	1.29 ± 0.55	0.98 ± 0.08
Intestine	10.94 ± 2.19	16.30 ± 5.73	16.88 ± 4.25
Bladder + urine	67.65 ± 0.01	73.10 ± 5.10	70.20 ± 3.10

Each value represents the mean percentage ($n = 3$) of the injected dose/organ of tissue, ±standard deviation of the mean.

Table 2. Uptake in tumour of $^{99m}Tc(CO)_3$-glucose derivative in C57BL/6 bearing induced Lewis murine lung carcinoma

	% Injected dose/gramme		
Organ	0.5 h	1.0 h	2.0 h
Blood	1.38 ± 0.10	1.45 ± 0.35	0.70 ± 0.20
Muscle	0.27 ± 0.08	0.26 ±0.10	0.13 ± 0.05
Tumour	0.49 ± 0.03	0.55 ± 0.05	0.35 ± 0.01
Tumour/muscle	1.80 ± 0.11	2.13 ± 0.15	2.75 ± 0.06

Each value represents the mean percentage ($n = 3$) of the injected dose/g of wet tissue, ±standard deviation of the mean.

hydrophilic than ^{99m}Tc-labelled glucose derivative **5** (log $P = -2.21$ determined using the same experimental conditions), FDG is taken up by cells through the specific transporter GLUT-1 and not by passive diffusion. To the best of our knowledge, there is no experimental evidence that any of the ^{99m}Tc-labelled glucose derivatives developed so far can be recognized by the transporter, and consequently, cell penetration might be due to passive diffusion.

Experimental

General

All laboratory chemicals were reagent grade and were used without further purification. Solvents for chromatographic analysis were HPLC grade. [^{99m}Tc]$NaTcO_4$ was obtained from a commercial generator (Tecnonuclear S.A., Buenos Aires, Argentina). Carbonyl labelling agent Isolink was provided by Covidien (Dublin, Ireland). NMR spectra were obtained in the indicated deuterated solvent using a Bruker Avance DPX 400 Spectrometer (Massachusetts, USA). Chemical shifts are reported as δ values (parts per million) relative to residual protons of deuterated solvent. Coupling constants are reported in Hertz (Hz). The

Table 3. Biodistribution results of [^{18}F]-FDG in C57BL/6 bearing induced Lewis murine lung carcinoma

	% Injected dose		
Organ	0.5 h	1.0 h	2.0 h
Blood	5.52 ± 0.48	2.98 ± 0.18	2.03 ± 0.69
Liver	5.07 ± 0.54	3.21 ± 0.06	3.05 ± 0.08
Kidneys	1.67 ± 0.06	0.95 ± 0.15	0.84 ± 0.18
Thyroid	0.15 ± 0.04	0.24 ± 0.07	0.13 ± 0.03
Muscle	18.32 ± 0.11	27.74 ± 1.89	24.62 ± 4.75
Tumour	17.32 ± 0.18	19.72 ± 3.29	18.75 ± 4.05
Stomach	1.31 ± 0.09	0.78 ± 0.01	1.40 ± 0.05
Intestine	7.09 ± 0.91	8.09 ± 0.58	6.42 ± 0.70
Bladder + urine	22.70 ± 3.87	6.85 ± 0.49	5.62 ± 0.70

Each value represents the mean percentage ($n = 3$) of the injected dose/organ of tissue, ±standard deviation of the mean.

Table 4. Uptake in tumour of [^{18}F]-FDG in C57BL/6 bearing induced Lewis murine lung carcinoma

	% Injected dose/gramme		
Organ	0.5 h	1.0 h	2.0 h
Blood	3.85 ± 0.06	1.96 ± 0.08	1.64 ± 0.12
Muscle	1.99 ± 0.02	2.84 ±0.30	2.92 ± 0.42
Tumour	8.04 ± 0.06	7.95 ± 0.87	8.19 ± 1.39
Tumour/muscle	4.04 ± 0.02	2.80 ± 0.21	2.80 ± 0.31

Each value represents the mean percentage (n = 3) of the injected dose/g of wet tissue, ± standard deviation of the mean.

multiplicity is defined by s (singlet), t (triplet) or m (multiplet). HPLC analysis was developed on an LC-10 AS Shimadzu Liquid Chromatography System (Kyoto, Japan) using a reverse phase column Phenomenex Luna 5 μm, C18 column (4.6 × 150 mm). Elution was performed with a binary gradient system at 1.0 mL/min flow rate using triethylamine-phosphate buffer pH 2.5 as mobile phase A and methanol as mobile phase B; the elution profile was as follows: 0 to 3 min 100% A; 3 to 6 min linear gradient to 25% B; 6 to 9 min linear gradient to 34% B; 9 to 20 min linear gradient to 100% B; 20 to 27 min 100% B; and 27 to 30 min linear gradient 0% B. Detection was accomplished either with a photodiode array detector (SPD-M10A, Shimadzu) that recorded UV–vis spectra on flux or with a '3 × 3' NaI (Tl) crystal scintillation detector. Activity measurements were performed either in a Dose Calibrator, Capintec (Ramsey, NJ, USA) CRC-5R or in a scintillation counter, '3 × 3' NaI (Tl) crystal detector associated to an ORTEC monochannel analyzer.

Synthesis

*N-(3,4,6-Tri-O-acetyl-β-D-glucopyranosyl)piperidine (**1**)*

β-D-Pentaacetyl glucose (20 g, 0.05 mol) was cooled to 20 °C, and piperidine (23 mL, 0.23 mol) was added slowly. The reaction was kept at 20–25 °C for 30 min until the suspended solution became clear, and after 15 min, precipitation began. The reaction was cooled to 0 °C, and diethylether (80 mL) was added. After 4 h stirring, the reaction mixture was allowed to reach room temperature and filtered. The solid was rinsed with diethylether and cold ethanol and dried 'in vacuo'. Yield: 39%. ^{1}H-NMR (400 MHz, $CDCl_3$): δ: 5.11 (t, 1H, 4-CH), 4.94 (t, 1H, 3-CH), 4.21 (dd, 1H, 6-CH_2), 4.08 (dd, 1H, 6-CH_2), 3.87 (d, 1H, 1-CH), 3.69 (t, 1H, 2-CH), 3.62–3.56 (m, 1H, 5-CH), 2.93–2.86 (m, 2H, CH_2–N), 2.64–2.56 (m, 2H, CH_2–N), 2.06 (s, 6H, 2x CH_3), 2.01 (s, 3H, CH_3), 1.62–1.44 (m, 6H, 3xCH_2).

*N-(3,4,6-Tri-O-acetyl-2-O-(6-bromobutyl)β-D-glucopyranosyl) piperidine (**2**)*

Compound **1** (2 g, 5.35 mmol) was dissolved in benzene (50 mL) at room temperature. Dibromobutane (1.77 g, 8.20 mmol), molecular sieves (5 g), Ag_2CO_3 (3.7 g) and $AgClO_4$ (1.66 g) were added. The reaction mixture was heated to 50–55 °C and allowed to react protected from light for 5 days. After reaching room temperature, the precipitate was filtrated through celite and rinsed with benzene. Benzene was evaporated *in vacuo*, and the residue was purified by flash column chromatography (petroleum ether : ethyl acetate 2:1). Yield: 40% ^{1}H-NMR (400 MHz, $CDCl_3$): δ: 5.03 (t, 1H, 3-CH), 4.95 (t, 1H, 4-CH), 4.20 (dd, 1H, 6-CH_2), 4.01 (dd, 1H, 6-CH_2), 3.94–3.85 (m, 2H, 1-CH, 2-CH), 3.85 (t, 2H, CH_2N_3), 3.54–3.47 (m, 1H, 5-CH), 3.41 (dt, 2H, CH_2O), 2.90–2.85 (m, 2H, CH_2N), 2.68–2.63 (m, 2H, CH_2N), 2.07 (d, 6H, 2xCH_3), 2.01 (s, 3H, CH_3), 1.92–1.81 (m, 2H, CH_2), 1.70–1.40 (m, 12H, 6xCH_2).

*(2R,3S,4R,5R)-2-(Acetoxymethyl)-5-(4-azidobuthyl)-6-(piperidin-1-yl)-tetrahydro-2H-pyran-3,4-diyl diacetate (**3**)*

To a stirred solution of **2** (2.0 g, 4.06 mmol) in dry dimethylformamide (30 mL), trimethyl-silylazide (805 mg, 7.00 mmol) and NH_4Cl (400 mg, 7.00 mmol) were added at room temperature, and the suspension was stirred at 115 °C under nitrogen for 4 h. The solution was poured onto ice water and extracted with Et_2O. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified with a silica column using petroleum ether : ethyl acetate (1:1) as eluent, to yield compound **3**. Yield: 65%. ^{1}H-NMR (400 MHz, $CDCl_3$): δ: 5.03 (t, 1H, 3-CH), 4.95(t, 1H, 4-CH), 4.20 (dd, 1H, 6-CH_2), 4.01 (dd, 1H, 6-CH_2), 3.94–3.85 (m, 2H, 1-CH, 2-CH), 3.85 (t, 2H, CH_2N_3), 3.54–3.47 (m, 1H, 5-CH), 3.41 (dt, 2H, CH_2O), 2.90–2.85 (m, 2H, CH_2N), 2.68–2.63 (m, 2H, CH_2N), 2.07 (d, 6H, 2xCH_3), 2.01 (s, 3H, CH_3), 1.92–1.81 (m, 2H, CH_2), 1.70–1.40 (m, 12H, 6xCH_2).

Radiolabelling

Deprotection

Compound **3** (22 mg, 4.68 mmol) was dissolved in acetone–water solution (2:1) (4.5 mL) and glacial acetic acid (1.0 μL, 1.00 mmol) was added. After the solution was stirred for 1 h at 50 °C, methanol was added (3.76 mL), and the reaction mixture was neutralized with a lithium hydroxide solution (0.5 M, 2 mL). The mixture was stirred overnight, and the solvent was evaporated under reduced pressure. The residue containing compound **4** (Figure 3) was dissolved in methanol and used for labelling without further purification.

*Preparation of triazole derivative of glucose derivative (**5**)*

A solution of propargylglycine in water (1.131 mg/mL, 50.0 μL) was mixed with a solution of the deprotection mixture in methanol (containing 1.286 mg/mL of compound **4**, 65.0 μL). Copper acetate solution in water (1.996 mg/mL, 7.5 μL) and sodium ascorbate in water (1.980 mg/mL, 15.0 μL) were added and the mixture heated for 1 h at 75 °C. After cooling, the reaction mixture was diluted with PBS buffer (300 μL).

Preparation of fac-[^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ complex

Using a kit formulation

^{99m}Tc-sodium pertechnetate (185–1850 MBq, 1 mL) was added to an Isolink kit (Covidien, USA) and the mixture incubated at 100 °C for 30 min. After cooling, pH was adjusted to 7.0 with 1 N HCl solution. Complex formation was checked by HPLC analysis as indicated in the general experimental section. Retention time: 4.0 min. Radiochemical purity > 90%.

Using CO (g)

The ^{99m}Tc-precursor complex was prepared according to a previously described method[16] as follows: Na/K tartrate (20.0 mg), Na_2CO_3 (4.0 mg) and $NaBH_4$ (7.0 mg) were placed in a vial. The vial was sealed and flushed with carbon monoxide for 30 min. ^{99m}Tc-sodium pertechnetate (185–1850 MBq, 1 mL) was added and the mixture incubated at 75 °C for 30 min. After cooling, pH was adjusted to 7.0 with 1 N HCl solution. The complex formation was checked by HPLC analysis as indicated in the general experimental section. Retention time: 4.0 min. Radiochemical purity > 90%.

*Labelling of triazole derivative of glucose (**5**)*

Neutralized *fac*-[^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ precursor (50 μL) was added to the reaction mixture obtained as result of the aforementioned described deprotection procedure and heated for 1 h at 100 °C. Complex formation was checked by HPLC analysis. Retention time: 14.0 min. Radiochemical purity > 90%.

*Labelling of propargylglycine and azide derivative of glucose (**4**)*

Neutralized *fac*-[^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ precursor (50 μL) was mixed with propargylglicine in water (1.131 mg/mL, 50 μL) and heated for 1 h at 100 °C. Complex formation was checked by HPLC analysis as described in the general experimental section. Neutralized *fac*-[^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ precursor (50 μL) was mixed with deprotected azide derivative of glucose **4** and heated for 1 h at 100 °C. Complex formation was checked by HPLC analysis.

Physicochemical evaluation

Stability in labelling milieu

^{99m}Tc complex was incubated in the labelling milieu at room temperature, and the radiochemical purity was assessed by HPLC for up to 4 h after labelling.

Protein binding studies

^{99m}Tc complexes (25 μL) were incubated with human plasma (475 μL) at 37 °C for up to 120 min. At 30 and 120 min, aliquots (50 μL) were added to MicroSpin G-50 columns (GE Healthcare Failfield, Connecticut, USA), which have been pre-spun at 2000 *g* for 1 min. Columns were centrifuged again at 2000 *g* for 2 min, and the collected elute and the column were counted in a NaI(Tl)-scintillation counter. Protein bound tracer was calculated as the percentage of activity eluted from the column.

Lipophilicity

Lipophilicity was studied through the apparent partition coefficient between 1-octanol and phosphate buffer (0.125 M, pH 7.4). In a centrifuge tube, containing 2 mL of each phase, 0.1 mL of the ^{99m}Tc complex solution was added, and the mixture was shacked on a Vortex mixer and finally centrifuged at 5000 r.p.m. for 5 min. Three samples (0.2 mL each) from each layer were counted in a gamma counter. The partition coefficient was calculated as the mean value of each cpm/mL of 1-octanol layer divided by that of the buffer. Lipophilicity was expressed as log *P*.

Animal studies

All animal studies were approved by the Ethics Committee of the Faculty of Chemistry from Uruguay.

Biodistribution in animals bearing induced tumours

A culture of 3LL Lewis murine lung carcinoma cells was expanded and treated with trypsine previous to inoculation. A cell suspension in PBS containing 3×10^6 cells was prepared and injected subcutaneously in the right limb of C57BL/6 mice (8–10 weeks old). The animals developed palpable tumour nodules 20–30 days later ($1.5 \times 0.5 \times 0.5$ cm) and were used for biodistribution studies.

Three animals per group were injected via a lateral tail with either ^{99m}Tc compound or [^{18}F]-FDG (0.1 mL, 0.037–0.37 MBq). At different intervals after injection, the animals were sacrificed by neck dislocation. Whole tumour and samples of blood and muscle were collected, weighed and assayed for radioactivity. Results were expressed as % dose/organ and % dose/g tissue.

Conclusions

A glucose derivative suitable for labelling with ^{99m}Tc through the formation of a Tc(I)-tricarbonyl complex was successfully prepared. The introduction of the histidine-like, 1,4-disubstituted triazole used as chelator by 'click chemistry' and the labelling by substitution of the precursor were performed in one pot without further purification. The procedure is fast and simple, and a single product with adequate radiochemical purity was obtained. The ^{99m}Tc complex was stable in labelling milieu and exhibited high hidrophilicity and low protein binding. 'In vivo' biodistribution profile in mice bearing induced tumours was very favourable. Although tumour uptake was low, retention up to 2 h post-injection was considerable, and favourable tumour/muscle ratios were achieved. Although synthesis and evaluation of various ^{99m}Tc-glucose derivatives have been reported, most of them have been derivatized in C1 instead of C2, probably because of easier synthetic conditions.[3,4,22] Comparison of biological results is difficult because of the heterogeneity in animal models used. No comparison with [^{18}F]-FDG in the same experimental conditions has been reported to our knowledge. Our data, on the other hand, indicate that in spite of moderate tumour uptake, similarity to biodistribution of [^{18}F]-FDG is a promising outcome. Further modifications of the chelator and linker to increase lipophilicity might improve biological results.

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Conflict of Interest

The authors did not report any conflict of interest.

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