Carbohydrate Research 346 (2011) 26–34

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

A Click procedure with heterogeneous copper to tether technetium-99m chelating agents and rhenium complexes. Evaluation of the chelating properties and biodistribution of the new radiolabelled glucose conjugates

Eric Benoist [a,b,*], Yvon Coulais [c], Mehdi Almant [d], José Kovensky [d], Vincent Moreau [d], David Lesur [d], Marine Artigau [a,b], Claude Picard [a,b], Chantal Galaup [a,b], Sébastien G. Gouin [d,†,*]

[a] Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique, SPCMIB, UMR 5068, 118, route de Narbonne, F-31062 Toulouse cedex 9, France
[b] Université de Toulouse;UPS;Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique, SPCMIB, UMR 5068, 118, route de Narbonne, F-31062 Toulouse cedex 9, France
[c] Laboratoire 'Traceurs et traitement de l'image', Faculté de Médecine de Purpan, EA 3033, 133, route de Narbonne, 31062 Toulouse cedex 9, France
[d] Laboratoire des Glucides UMR CNRS 6219, Institut de Chimie de Picardie, Faculté des Sciences, Université de Picardie Jules Verne, 33 rue Saint-Leu, 80039 Amiens Cedex 1, France

ARTICLE INFO

Article history:
Received 31 August 2010
Received in revised form 7 October 2010
Accepted 10 October 2010
Available online 20 October 2010

Keywords:
Glycoconjugates
Heterogeneous click chemistry
Bioconjugation
Metal complexes

ABSTRACT

An efficient protocol was developed to tether chelating agents and rhenium complexes onto a glucoside scaffold with a heterogeneous copper catalyst via click chemistry. The supported catalyst avoids the formation of unwanted copper complexes during the cyclisation step. The possibility to graft a pre-chelated $M(CO)_3$ core by click chemistry onto a biomolecule was highlighted for the first time. $^{99m}Tc(CO)_3$-gluco-conjugates displayed excellent in vitro stability, a fast in vivo blood clearance and a low specific organ uptake or long-term retention in spleen and stomach.

1. Introduction

Carbohydrates play a pivotal role in a host of biological events including molecular recognition, inflammation, tumour metastasis and viral or bacterial adhesion.[1] In particular, they provide a major energy source for life forms through the processing of glucose inside the cells. The energy requirement in many tumour types is significantly higher than in normal cells due to a rapid growing and altered metabolism. This feature has been extensively explored for the development of glucose conjugates as selective radiotracer for tumours in oncology. FDG (2-deoxy-2-[^{18}F]fluoro-D-glucose) has become an important radiopharmaceutical approved by the Food and Drug Administration to detect melanoma, lymphoma, breast and lung cancers by positron emission tomography (PET) scans.[2] FDG is internalized into the cells by the GLUT1 transporter and phosphorylated by hexokinase to a very polar product that cannot diffuse out of the cell. Applications are however limited by the short half life of the positron emitting fluorine-18 ($T_{1/2}$ = 109.8 min), which should be produced on site by expensive cyclotrons.

In this regard, the development of more readily available FDG analogues would be of high interest. The technetium-99 m radionuclide (^{99m}Tc) is extensively used in nuclear imaging with single photon emission computed tomography (SPECT), due to ideal imaging characteristics (140 keV γ emitter, relatively short half life of 6 h) combined with a convenient availability at low cost from the commercial ^{99}Mo/^{99m}Tc generator. A wide range of chelators is now available to efficiently coordinate diverse technetium species like TcO^{3+} and $Tc(CO)_3^+$ cores, and to form stable complexes under physiological conditions.[3] Numerous chemical strategies have been developed to graft such compounds onto biologically relevant carriers such as peptides,[4] antibodies[5] and carbohydrates.[6,7] In particular, several groups recently reported the synthesis of glucose derivatives containing ^{99m}Tc-chelating systems to substitute the expensive FDG in the localization of tumours.[8] These complexes were chemically grafted onto the different hydroxyl groups of the glucose scaffolds by glycosylation (C-1),[9] nucleophilic substitutions and reductive aminations (C-2, 3, 6),[10] or peptidic coupling when starting from glucose amine.[11] Despite the numerous attempts, a ^{99m}Tc based radiopharmaceutical that fulfils the criteria of a potent [^{18}F]-FDG surrogate has not been

* Corresponding authors.
E-mail addresses: benoist@chimie.ups-tlse.fr (E. Benoist), Sebastien.Gouin@univ-nantes.fr (S.G. Gouin).
† Present address: Université de Nantes, CEISAM, Chimie Et Interdisciplinarité, Synthèse, Analyse, Modélisation, UMR CNRS 6230, UFR des Sciences et des Techniques, 2, rue de la Houssinière, BP 92208, 44322 NANTES Cedex 3, France.

0008-6215/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2010.10.011

designed yet. It seems that the major drawback of this strategy is the presence of the technetium complex, which is too hindered to allow the glycoconjugate internalization into cells via the GLUT1 transporter.[12]

Nevertheless, carbohydrates attract much attention, as hydrophilic and biocompatible scaffolds for the development of molecular imaging agents,[13] or as vectors able to target carbohydrate binding proteins (lectins).[14] In this regard, the development of simple and efficient protocols to graft chelating agents or metal complexes onto carbohydrate scaffolds are of particular interest. In the present work we described a simple and efficient protocol to synthesize C-1 functionalized glucose derivatives-containing tridentate chelating systems via a copper catalysed azide–alkyne cyclization reaction (click reaction). We demonstrated for the first time the possibility to graft a pre-chelated $M(CO)_3$ core directly onto a biomolecule by click chemistry. The preparation and spectroscopic analysis of both cold $Re(CO)_3$ and radioactive $^{99m}Tc(CO)_3$ glycoconjugates are reported. In vitro histidine challenge experiments and first biodistributions of technetium glucoconjugates are also investigated.

2. Results and discussion

2.1. Synthesis of glucoconjugates and their corresponding metallic complexes (*M* = ^{99m}Tc, Re)

An ideal bifunctional chelating agent should (i) coordinate a metal ion with a high yield, (ii) form metal complexes with both high thermodynamic stability and in vivo kinetic inertness to minimize metal toxicity, (iii) be produced quickly, in a gram-scale and with an excellent overall yield. For the complexation of the $M(CO_3)^+$ core, recent in vitro and in vivo investigations showed that tridentate chelating systems are more appropriate.[15] A Tc- or Re-tricarbonyl complex with a tridentate ligand is less prone to cross-metallation due to its coordinative saturation and thermodynamic stability. Among these ligands, iminodiacetic acid (IDA) and di-(2-picolyl)amine (DPA) derivatives react avidly with the *fac*-$[M(CO)_3]^+$ core to form complexes with a stable octahedral coordination sphere. We first designed a set of alkynyl-armed chelating systems **1**–**4** based on DPA, IDA or analogous derivatives (Chart 1). Building blocks **1–3** could be considered as bifunctional chelating agents where the acetylenic arm allows conjugation to the glucopyranosyl azide by click chemistry. Recently, Schibli and co-workers reported that a 1,2,3-triazole ring formed during the cyclisation step is an effective binder for $M(CO_3)^+$ core.[16] Following their so-called 'click to chelate' approach, we synthesized pre-chelator **4**[17] that will potentially bind the metallic core through both nitrogen atoms and the formed triazole.

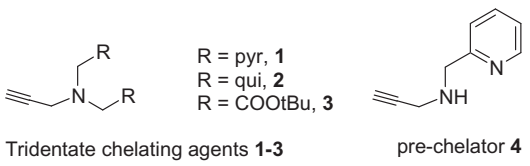


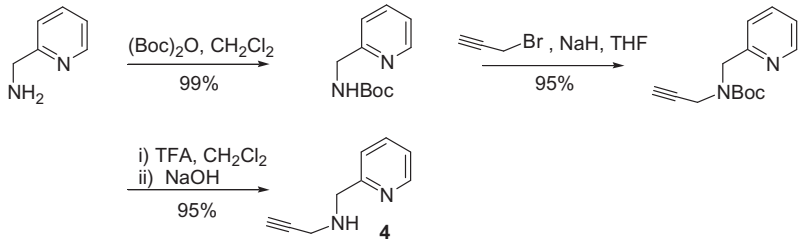
Chart 1. Design features of Tc-chelating agents.

The chelate systems **1** and **3** were prepared in excellent yields (near 90%) by refluxing commercial di-(2-picolyl)amine or *tert*-butyl iminodiacetate with propargyl bromide in the presence of potassium carbonate in acetonitrile as previously described.[18] In a similar way, the reaction of propargyl amine with the chlorohydrate of 2-chloroquinoline gave the desired compound **2** in a fair yield of 62%. Particular interest arises in developing a fast access to such chelating systems, which can be either used to design radioimaging or fluorescent probes with technetium or rhenium, respectively. *N*-Propargyl-di-(2-picolyl)amine **4** was recently prepared by reductive amination with a moderate yield of 57%.[19] Consequently, we developed a more suitable synthetic pathway to prepare this compound in gram-scale starting from 2-(aminomethyl)pyridine, as outlined in Scheme 1. Following a three-step sequence already described for analogous derivatives,[20] the amine was first protected as a *tert*-Boc carbamate to avoid the formation of *N,N*-dipropargyl derivatives during the alkylation step. Subsequent treatment with an excess of sodium hydride in the presence of propargyl bromide, followed by amine deprotection with TFA, provided **4** with an overall yield of 89%.

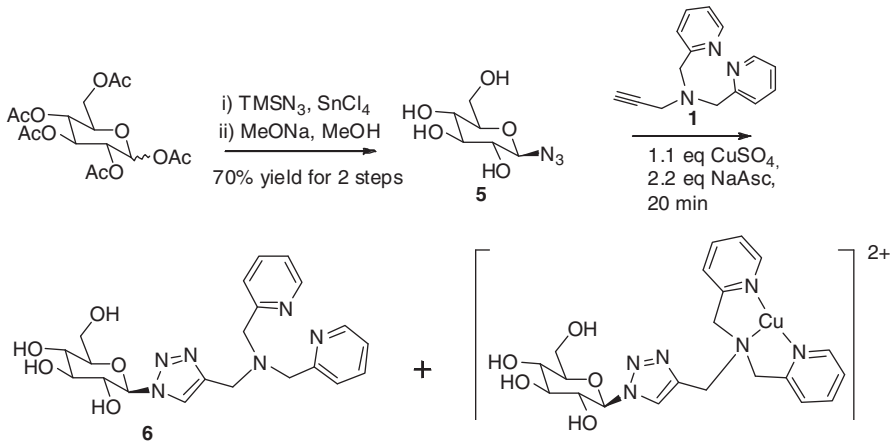
As proof of concept, two tricarbonylrhenium complexes were readily obtained by reacting equivalent amounts of ligands **1** or **2** and $Re(CO)_5Br$ in refluxing methanol. The co-ordination reactions were quantitative in 6 h and after purification led to mononuclear complexes isolated as bromine salts of general formula $[Re(CO)_3(L)][Br]$.

The glucopyranosyl azide[21] scaffold was easily obtained in two steps from glucose pentaacetate[22] (Scheme 2). Displacement of the anomeric acetate group with $TMSN_3$ in the presence of tin chloride followed by deprotection under Zemplén conditions (NaOMe in MeOH) led to **5** in 70% yield. The first copper(I)-catalysed cycloaddition with alkynyl-armed chelating agent **1** was performed in the presence of copper sulfate and sodium ascorbate in a mixture of *tert*-butanol and water to ensure the solubility of reagents. The reaction proceeded smoothly, leading to the expected cycloadduct **6** polluted by its copper complex, which was identified by ESI-MS during the experiment.

It was previously reported that treatment by a chelating resin (QuadraPure-IDA) allows the regeneration of the desired cycloadducts by transchelation of copper.[16i] However, this procedure will be ineffective for chelators possessing high kinetic inertness or stronger binding constants than the iminodiacetic groups attached to the resin. Performing the cyclization step with a supported or heterogenous source of copper(I) would be more appealing with this regard. Lipshutz and Taft have developed an efficient catalyst for the Huisgen [3+2] cyclization based on copper impregnated wood charcoal.[23] Interestingly, the authors claimed that the reaction takes place heterogeneously without any leaching of copper



Scheme 1. Synthetic pathway for the preparation of **4**.



Scheme 2. Synthetic pathway for the preparation of glycoconjugate **6**.

in solution as indicated by quantitative ICP-AES performed on crude products. Initial reaction trials with our substrates were disappointing as no traces of cycloadducts were observed after several days at room temperature. Earlier studies have shown that microwave irradiation could significantly improve the copper(I)-catalysed azide–alkyne cyclization (CuAAC) reaction kinetics compared to classical heating.[24] We were delighted to see that under microwave irradiation at 100 °C for 15 min, the expected glycoconjugate **6** could be isolated as a pure compound in 91% yield, after a simple filtration of the catalyst (Table 1). Additionally, ESI-MS analysis revealed that no detectable copper complex was present in the medium. The heterogeneous CuAAC was also highly regioselective yielding 1,4-disubstituted-1,2,3-triazoles identified by the large $\Delta(\delta_{C\text{-}4}-\delta_{C\text{-}5})$ values (14–23 ppm) observed in ^{13}C NMR for the different structures.[25] This procedure has been successfully applied to pre-chelator **4**, allowing conjugation and formation of the additional chelating group, simultaneously. When the reaction was conducted without catalyst, we obtained a mixture of 1,4- and 1,5-disubstituted-1,2,3-triazoles. Interestingly, the tridentate complex **1-Re** containing the $Re(CO)_3$ core can be directly introduced onto the carbohydrate scaffold, leading to **6-Re** with a moderate yield of 35% after HPLC purification. To our knowledge, this is the first example of a pre-chelated $M(CO)_3$ core directly grafted onto a biomolecule by click chemistry. This finding is of interest considering the existence of two radioactive isotopes of rhenium emitting both β-radiations that are able to kill tumoural cells (^{186}Re: $T_{1/2}$ = 90 h, $E(\beta^-)$ = 1.07 MeV, ^{188}Re $T_{1/2}$ = 17 h, $E(\beta^-)$ = 2.12 MeV). Additionally, rhenium exhibits very similar chemistry to technetium, and we expect that this reaction could also occur with this metal. A direct introduction of a pre-chelated Re or Tc core onto a biomolecule may be an alternative to the current labelling strategies. Therefore, the present procedure, can be considered as an extension of the 'click to chelate' concept described by Schibli for the synthesis of 1,2,3-triazole chelators and their in situ radiolabelling with technetium.[16]

Copper sulfate and sodium ascorbate[26] were a more efficient catalyst system for grafting **2** and **2-Re** onto the glucoside scaffold. It is noteworthy that glycoconjugate **8-Re** may be a potential fluorescent imaging agent for in vitro investigations of cellular uptake as suggested by a previous report of Zubieta and co-workers with a similar rhenium carbohydrate complex.[27]

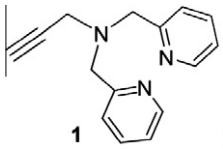
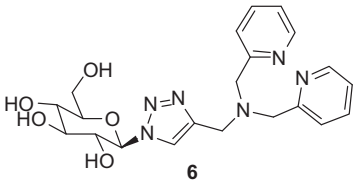
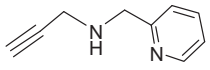
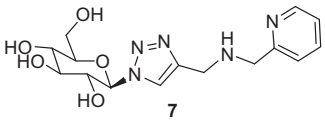
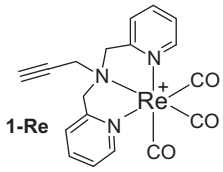
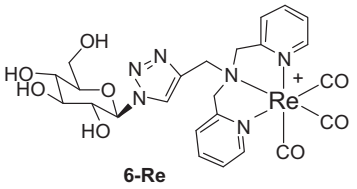
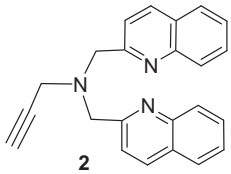
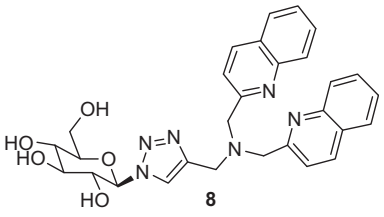
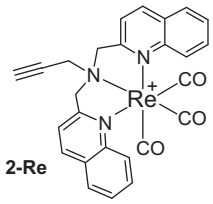
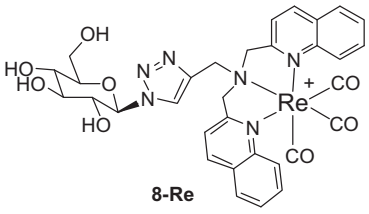
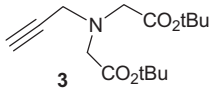
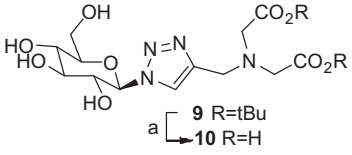
Surprisingly, the *tert*-butyl esters of product **9** were stable to acidic conditions and traces of these esters remain even after heating the mixture at 100 °C in pure trifluoroacetic acid. In contrast, saponification with NaOH followed by acidification with diluted HCl allowed a quantitative isolation of the expected product **10**.[28]

Compounds were fully characterized by classical spectroscopic methods. Briefly, the NMR spectra revealed the characteristic signals of the sugar unit and the ligand or the chelating moiety. Due to the electronic influence of the tricarbonyl rhenium core, a significant downfield shift of the hydrogen resonances near the chelating N-atoms was observed. In such complexes, the protons of the methylene groups adjacent to the aromatic rings are non-equivalent. The ^{13}C NMR spectra of **6-Re** and **8-Re** show only two peaks near 195–197 ppm with the characteristic ratio of peak height of 2:1 for the *fac*-$[Re(CO)_3]^+$ core indicating that (i) two CO groups are magnetically equivalent, (ii) the coordination sphere exhibits mirror symmetry including one of the three facial carbonyl groups and the coordinated amino group, as previously reported for such chelating units.[27] Interestingly, the complex **7-Re** exhibited different analytical features. Firstly, the reaction of glycoconjugate **7** with $Re(CO)_5Cl$ in refluxing methanol, led to a mixture of two structural isomers in a 1:1 ratio. The observation of two diastereoisomers can only result from the formation of a complex where the ligand is bound in a bidentate fashion. This result is surprising as Schibli and co-workers having demonstrated recently that this kind of ligand acted as a tridentate chelate.[12] Unfortunately, it was not possible by HPLC to achieve isomeric resolution under a wide range of chromatographic conditions. 2D-NMR experiments suggested that the rhenium core was coordinated by the (2-aminomethyl)pyridine part of the glycoconjugate, giving a neutral complex of the general formula [$ReCl(CO)_3$(**7**)]. We hypothesized that the nitrogens on the triazole ring may be deactivated due to the electroattractive effect of the endocyclic oxygen atom.

2.2. In vitro and in vivo studies

The ^{99m}Tc-complexes were prepared by mixing the precursor *fac*-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ synthesized using the Isolink kit (Mallinckrodt Inc.), with compounds **6**, **7**, **8** or **10** in a methanol/buffer solution pH 3.4. After 30 min at 95 °C, $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was entirely consumed and a new product was observed on the radiochromatogram. The four radioconjugates were obtained in an excellent radiolabelling yield ranging from 95% for **6-Tc** and **7-Tc**, to 90% for **8-Tc** and **10-Tc**, respectively. As expected, the radiolabelling of **7** led to a mixture of two isomers in an approximate 1:1 ratio. To characterize the pure radiolabelled glycocomplexes, HPLC co-injections of ^{99m}Tc-radiocomplexes with the corresponding 'cold' rhenium complexes were performed. In all cases, the retention times of the complexes were analogous, as illustrated in Figure 1 for **6-Tc** and **6-Re**. These results evidenced that the structures of the formed complexes are similar for technetium at the tracer level or with rhenium at higher metal concentration.

Table 1
Copper catalysed formation of the glycoconjugates and glycocomplexes

Entry	Chelator/complex	Glycoconjugate/glycocomplex	Catalyst	Time	Yield (%)
1	 **1**	 **6**	C/Cu	15 min	91[b]
2	 **4**	 **7**	C/Cu	15 min	87[b]
3	 **1-Re**	 **6-Re**	C/Cu	8 h	35[c]
4	 **2**	 **8**	$CuSO_4$/NaAsc	15 min	70[b]
5	 **2-Re**	 **8-Re**	$CuSO_4$/NaAsc	45 min	26[c]
6	 **3**	 a **9** R=tBu **10** R=H	$CuSO_4$/NaAsc	30 min	Quant[b]

[a] NaOH/EtOH then HCl (99% yield).
[b] Based on the crude material.
[c] After isolation by preparative HPLC.

High stability of the metal complexes toward competitive ligands is of primary importance for the development of biologically relevant imaging probes. In vitro stability of the radiolabelled glycocomplexes was assessed over a 24 h period with a 250-fold molar excess of histidine, a high affinity competitive ligand for the $[^{99m}Tc(CO)_3]^+$ core.[29] No significant differences were observed for the tridentate chelates **6-Tc**, **8-Tc**, **10-Tc** and **7-Tc**. Less than 10% of ^{99m}Tc was dissociated from the complexes after 24 h incubation (data not shown). These results showed that the inertness of the tridentate Tc-complexes towards transchelation, and are consistent with previously reported histidine challenge assays showing the stability of DPA and IDA-frameworks.[30] They also confirm that the sixth coordination site of the bidentate complex **7-Tc** was occupied by a chlorine; a water molecule would have led to a higher exchange rate.[31]

Biodistribution studies in healthy male Wistar rats were performed to evaluate the organ uptakes and clearances of the $^{99m}Tc(CO)_3$-complexes **6-Tc**, **10-Tc** and **7-Tc** (mixture of two isomers). It is well known that the formation of metal complex isomers is detrimental for in vivo administration since the blood clearance and radiopharmaceutical biodistribution may be altered. Nevertheless, in this first assay, complex **7-Tc** was only used as a reference to compare the biological behaviours of this bidentate glycocomplex versus a tridentate complex (**6-Tc**, **10-Tc**). The results are expressed as percent of injected dose per organ (%ID/organ) at 5, 30 and 240 min post-injection, as shown in Table 2.

Complex **6-Tc** shows a high liver uptake (from 31% ID at 5 min to 18% ID at 240 min) and is preferentially eliminated by the hepatobiliary system, as revealed by the 45% ID at 240 min post-injection. Its elimination via the renal urinary excretion route is not significant (2% ID at 60 min) and after 240 min post-injection only 11.2% ID was detected in the urine. In contrast, complexes **7-Tc** and **10-Tc** exhibit modest hepatobiliary elimination and were mainly excreted via the renal pathway. Thus, complex **10-Tc** presented the highest concentration of radioactivity in urine at 240 min (36% ID), indicating its hydrophilic character, as expected for an anionic complex. More interestingly, the rapid clearance of these compounds from the blood-stream, reflected by the low blood

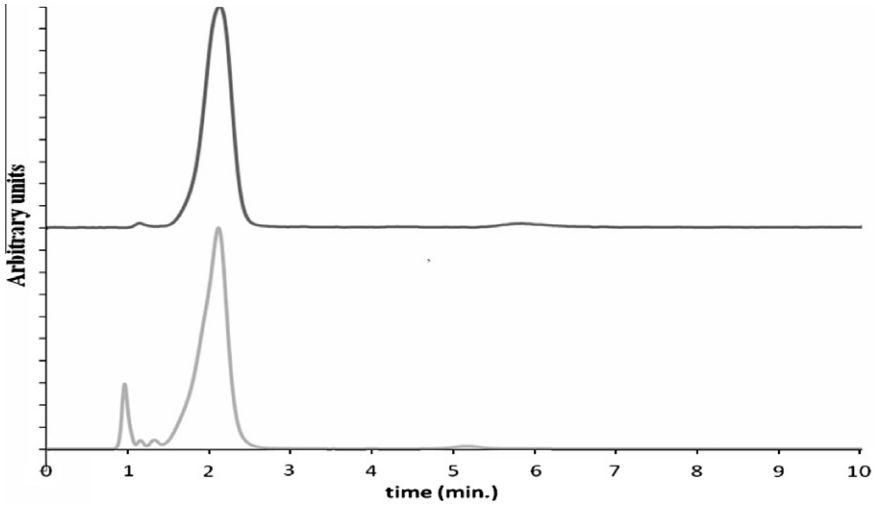


Figure 1. HPLC profiles of **6-Tc** (up, radiometric) and **6-Re** (bottom, UV/230 nm) (elution conditions: MN Nucleodur C18 ec column 125 × 4 mm, 5 μm; eluent: 0.1% TFA in H_2O/MeOH 60:40).

Table 2
Biodistributions of **6-Tc**, **7-Tc** and **10-Tc** in rats, at various time post-injection (the results are the average of at least three experiments)

Organ	%ID/organ								
	6-Tc			**7-Tc**			**10-Tc**		
	5 min	30 min	240 min	5 min	30 min	240 min	5 min	30 min	240 min
Carcass	32.03 ± 1.82	15.23 ± 7.06	5.07 ± 1.51	54.04 ± 1.52	25.26 ± 2.44	8.25 ± 3.41	50.23 ± 1.85	34.72 ± 5.61	5.36 ± 1.29
Liver	30.81 ± 2.70	26.26 ± 2.13	17.67 ± 3.06	**11.54 ± 1.50**	**19.76 ± 1.47**	**20.56 ± 0.85**	8.15 ± 0.67	3.82 ± 0.44	1.68 ± 0.26
Intestine	**17.42 ± 6.62**	**35.68 ± 6.54**	**45.21 ± 12.52**	4.06 ± 0.34	3.91 ± 1.13	10.04 ± 1.50	4.61 ± 0.92	10.58 ± 2.83	12.88 ± 8.54
Stomach	3.04 ± 2.29	2.90 ± 1.36	3.07 ± 1.52	0.63 ± 0.04	1.33 ± 0.72	0.99 ± 0.19	0.67 ± 0.14	2.82 ± 2.46	3.93 ± 1.61
Pancreas	2.46 ± 0.03	1.89 ± 0.08	1.60 ± 0.32	0.95 ± 0.14	1.68 ± 0.13	2.03 ± 0.64	0.75 ± 0.01	0.40 ± 0.07	0.17 ± 0.04
Heart	0.11 ± 0.00	0.05 ± 0.02	0.02 ± 0.01	0.30 ± 0.09	0.10 ± 0.02	0.02 ± 0.01	0.30 ± 0.01	0.13 ± 0.04	0.03 ± 0.01
Lungs	0.39 ± 0.06	0.18 ± 0.07	0.09 ± 0.02	0.69 ± 0.12	0.29 ± 0.01	0.08 ± 0.04	0.71 ± 0.15	0.33 ± 0.08	0.07 ± 0.02
Kidneys	8.22 ± 3.64	2.05 ± 0.71	0.89 ± 0.40	14.71 ± 2.37	5.46 ± 0.56	1.64 ± 0.11	**13.24 ± 5.58**	**7.39 ± 2.55**	**1.18 ± 0.24**
Spleen	0.06 ± 0.01	0.08 ± 0.03	0.02 ± 0.01	0.17 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.13 ± 0.01	0.05 ± 0.02	0.03 ± 0.01
Brain	0.03 ± 0.01	0.04 ± 0.01	0.01 ± 0.01	0.05 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.04 ± 0.03	0.01 ± 0.01	0.02 ± 0.01
Blood	**4.99 ± 0.43**	**1.35 ± 0.69**	**0.26 ± 0.11**	**13.24 ± 0.25**	**5.04 ± 1.01**	**0.65 ± 0.37**	**16.25 ± 1.40**	**6.01 ± 0.28**	**0.86 ± 0.13**

activity, indicated their high stability against metal exchange reactions with blood proteins. Additionally, the spleen and stomach values were low, indicating minimal in vivo oxidation of technetium to pertechnetate or reduction to colloidal Tc. Altogether, these results highlight that the new glycoconjugates are able to shield and stabilize ^{99m}Tc in vivo. In addition, the only significant difference between the tridentate complexes **6-Tc** and **10-Tc** and the bidentate one concerns the liver uptake. No specific uptake or long-term retention was observed in other organs or tissues for complexes **6-Tc** and **10-Tc**. In contrast, **7-Tc** exhibited a high liver uptake after 240 min, as illustrated by the 21% ID at 240 min post-injection.

2.3. Conclusion

We have developed an efficient protocol to graft ^{99m}Tc-chelating agents and rhenium complexes onto a glucose scaffold. ^{99m}Tc$(CO)_3$-complexes **6-Tc** and **10-Tc** displayed in vitro stability against histidine exchange reactions, a fast blood clearance, and low specific uptake or long-term retention in spleen and stomach revealing a high in vivo stability. More interestingly, the feasibility to directly tether a pre-chelated Re$(CO)_3$ core by click chemistry was highlighted for the first time. The heterogeneous cyclization protocol developed may also be applicable to other metallic cores and more complex carbohydrate scaffolds. Work in this direction is currently being performed in our group.

3. Experimental

3.1. General methods

All purchased materials were used without further purification. Methylene chloride was distilled from calcium hydride and tetrahydrofuran over sodium and benzophenone. Analytical thin layer chromatography (TLC) was carried out on Merck D.C.-Alufolien Kieselgel 60 F_{254}. Flash chromatography (FC) was performed on GEDURAN SI 60, 0.040–0.060 mm pore size using distilled solvents. *N*-Propargyl-di-(2-picolyl)amine **1** and 3-[bis(*tert*-butoxycarbonylmethyl)amino]-prop-1-yne **3** were prepared as described previously.[18] Re$(CO)_5$Br was purchased from Aldrich Chemical Company; ^{1}H, and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 300 (75.5) MHz with a Bruker AC-300 spectrometer or at 500 (125) MHz with a Bruker Avance DRX 500, and chemical shifts are reported in parts per million relative to tetramethylsilane or a residual solvent peak ($CHCl_3$: ^{1}H: δ 7.26, ^{13}C: δ 77.2). Peak multiplicity is reported as: singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), sextet (s), multiplet (m) and broad (br). Mass spectra were obtained on a Perkin–Elmer Sciex API 365 (electrospray), a DSQ2 Thermofisher (chemical ionisation) or a Nermag 10-10 (FAB) mass spectrometer. High Resolution Mass Spectra (HRMS) were obtained by Electrospray Ionisation (ESI) on a Micromass-Waters Q-TOF Ultima Global. Optical rotations were measured on a 343 Perkin–Elmer automatic polarimeter at 20 °C

in a 1 cm cell in the stated solvent; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1} (concentration, *c*, given as g/100 mL). Microwave irradiation was performed in a CEM Discover apparatus (300 W).

Preparative reversed-phase HPLC was accomplished on a Waters PREP LC 4000 chromatography system with a (DEDL) PL-ELS 1000 photodiode array detector or a Waters 600E gradient chromatograph coupled to a UV–visible detector (ICS) and a γ-detector (Raytest) for the ^{99m}Tc-compounds. Non-radioactive HPLC samples were purified on a preparative Prevail C-18 column (2.2 × 25 cm) for **10**, an NH_2 Alltech Altima 5 μM C-18 column for **6**, **7**, **8**, **6-Re**, **8-Re** and a Phenomenex Luna C-18 column for **7-Re**. Gradient A: the mobile phase was H_2O (solvent A) and CH_3CN (solvent B). The gradient consisted of 100% A for 5 min then 100% A–100% B in 35 min (20 mL/min flow rate). Gradient B: the mobile phase was $HCOONH_4$ buffer, 10 mM, pH 4 (solvent A) and CH_3CN (solvent B). The gradient consisted of 90% A–50% A in 18 min then 50% A–90% A in 2 min (20 mL/min flow rate).

RP-HPLC analysis and purification of ^{99m}Tc-complexes were carried out on a Nucleodur C18 end capped RP column (Macherey-Nagel analytical column 125 × 4 mm, 5 μm). The RP-HPLC conditions were as follows: flow rate was 1 mL/min, *eluent 1* (for complexes **6-Tc**, **7-Tc** and **10-Tc**) was 0.1% TFA in H_2O/MeOH 80:20 (solvent A) and 0.1% TFA in H_2O/MeOH 40:60 (solvent B) and gradient system was 0–10 min, 100% A–100% B; 10–15 min, 100% B; 15–20 min, 100% B–100% A, *eluent 2* (for complexes **8-Tc**) was 0.1% TFA in H_2O/MeOH 52:48 and isocratic gradient. In all runs, the wavelength used for UV detection was 230 nm.

Caution: Sodium azide, when inhaled, is highly toxic. Precautions must be taken when weighing the material, such as using a powder mask and a teflon spatula (metallic spatula may cause explosion). The azidation reactions should be performed behind a plastic shield due to the potential explosion. In acidic pH hydrazoic acid (HN_3) may be formed, which may explode and/or, when inhaled, may cause intoxication.

3.2. 3-[Bis(2-quinolinylmethyl)amino]-prop-1-yne 2

2-Chloromethylquinoline monochloride (1.5 g, 7 mmol) and potassium carbonate (4.83 g, 35 mmol) were added to acetonitrile (20 mL) and stirred at room temperature for 15 min. Propargyl amine (0.24 mL, 3.5 mmol) was added and the mixture stirred at 75 °C overnight. The solvent was evaporated and the residue purified by column chromatography on silica gel (CH_2Cl_2–MeOH 98:2). The product was isolated as a brown powder (730 mg, 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (4H, m, CH_{Ar}), 7.50 (6H, m, CH_{Ar}), 7.24 (2H, m, CH_{Ar}), 4.08 (4H, s, CH_2), 3.46 (2H, m, NCH_2), 2.33 (1H, t, J_4 2.3 Hz, ≡CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.5, 147.1, 127.6 (C_{Ar}), 136.5, 129.5, 129.4, 127.5, 126.2, 122.0 (CH_{Ar}), 78.7 (C_{Ar}), 73.7 (≡CH), 60.3 (NCH_2), 38.0 (NCH_2); MS (DCI/NH_3): 338 $[M+H^+]^+$.

3.3. *N*-Boc-2-picolylamine

According to a modification of a literature procedure,[20] to a solution of di-*tert*-butyl dicarbonate (21.88 g, 0.1 mol) in CH_2Cl_2 (200 mL) at 0 °C, 2-(aminomethyl)pyridine (10.30 mL, 0.1 mol) was added dropwise. The resulting solution was stirred at room temperature overnight, and then diluted with 100 mL of CH_2Cl_2. The organic layer was washed twice with 0.1 M NaOH (100 mL), dried over Na_2SO_4 and evaporated to give a pale yellow oil (20.70 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (1H, m, CH_{pyr}), 7.70 (1H, m, CH_{Ar}), 7.31–7.17 (2H, m, CH_{Ar}), 4.37 (2H, d, *J* 5.5 Hz, CH_2), 1.40 (9H, s, CH_3); MS (DCI/NH_3): 209 $[M+H^+]^+$.

3.4. *N*-Boc,*N*-propargyl-2-picolylamine

To a solution of *N*-Boc-2-picolylamine (11.3 g, 54.3 mmol) in freshly distilled THF (200 mL) at 0 °C, sodium hydride (5.2 g, 217 mmol) was added gently in portions. The solution was stirred for 20 min and treated dropwise with propargyl bromide (80 wt % in toluene, 7.9 mL, 70.6 mmol). After stirring for 3 h at room temperature, the solution was quenched cautiously with water (100 mL). The aqueous layer was extracted twice with CH_2Cl_2 (200 mL). The combined extracts were washed with brine (3×) and dried over Na_2SO_4. The crude product was purified by chromatography on silica gel (CH_2Cl_2–MeOH 98:2). The product was isolated as a pale yellow oil (12.7 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (1H, m, CH_{Ar}), 7.68 (1H, m, CH_{Ar}), 7.22–7.13 (2H, m, CH_{Ar}), 4.56 (2H, s, CH_2), 4.10 (2H, m, CH_2), 2.22 (1H, t, J_4 2.4 Hz, ≡CH), 1.49 (9H, m, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.3 (CO), 155.1 (C_{Ar}), 149.3, 136.6, 122.1, 120.9 (CH_{Ar}), 80.7 (CCH), 79.3 (C_{tBu}), 79.2 (≡CH), 57.7 (NCH_2), 36.5 (NCH_2), 28.3 (CH_3); MS (DCI/NH_3): 247 $[M+H^+]^+$.

3.5. *N*-Propargyl-2-picolylamine 4

N-Boc,*N*-propargyl-2-picolylamine (4.75 g, 19.3 mmol) was dissolved in CH_2Cl_2–TFA (1:1; 15 mL) and stirred at room temperature for 3H, then the solvent was removed. The free amine was obtained by dissolving the residue in 0.5 M NaOH (10 mL) and extracting into $CHCl_3$ (3 × 30 mL). The organic phases were collected, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (CH_2Cl_2–MeOH 95:5) to give **4** as a pale yellow oil (2.67 g, 95%). Analytical data were in agreement with the literature.[17]

3.6. General procedure for the synthesis of $Re(CO)_3$ complexes

Complexes **1-Re** and **2-Re** were prepared by a substitution route from commercial $Re(CO)_5Br$. Ligand **1** or **2** (1 mmol) and $Re(CO)_5Br$ (406 mg, 1 mmol) were dissolved in MeOH (10 mL) and stirred at 65 °C for 6 h. After cooling to room temperature, the brown solution was evaporated to dryness. The residue was purified by column chromatography on silica gel (CH_2Cl_2–MeOH 95:5) to give the desired complex of general formula $[Re(CO)_3L][Br]$.

3.7. Complex 1-Re

237 mg of **1** gave **1-Re** as a brown powder (430 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.64 (2H, m, CH_{Ar}), 7.92 (2H, m, CH_{Ar}), 7.82 (2H, m, CH_{Ar}), 7.20 (2H, m, CH_{pyr}), 5.97 (2H, d, *J* 18 Hz, CH_2), 4.66 (2H, d, *J* 18 Hz, CH_2), 4.48 (2H, m, NCH_2), 2.78 (1H, t, J_4 2.5 Hz, ≡CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.4 (C≡O), 161.6 (C_{Ar}), 151.0, 140.4, 125.9, 125.5 (CH_{Ar}), 80.2 (CCH), 76.1 (≡CH), 67.7 (NCH_2), 58.2 (NCH_2); MS (ES^+): 506/508 $[(\mathbf{1\text{-}Re})-Br^-]^+$.

3.8. Complex 2-Re

337 mg of **2** give **2-Re** as a brown powder (300 mg, 41%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (2H, m, CH_{Ar}), 8.38 (2H, m, CH_{Ar}), 7.93–7.83 (6H, m, CH_{Ar}), 7.68 (2H, m, CH_{Ar}), 6.10 (2H, d, *J* 17.9 Hz, CH_2), 5.33 (2H, d, *J* 17.9 Hz, CH_2), 4.76 (2H, m, NCH_2), 2.72 (1H, t, J_4 2.5 Hz, ≡CH); ^{13}C NMR (75 MHz, MeOD) δ 195.7, 193.6 (C≡O), 164.7, 146.8, 128.5 (C_{Ar}), 141.4, 132.7, 128.5, 128.1, 127.8, 119.5 (CH_{Ar}), 79.3 (CCH), 76.4 (≡CH), 68.8 (NCH_2), 56.0 (NCH_2); MS (FAB^+): 606/608 $[M-Br^-]^+$.

3.9. 2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranosyl azide 5

1,2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranoside (6 g, 15 mmol) was dissolved in dry DCM (200 mL) at rt. $TMSN_3$ (5.25 mL, 39 mmol) and stannic chloride (900 μL, 7.6 mmol) were added dropwise to the solution and the mixture was stirred for 15 min at room temperature under Ar. $NaHCO_3$ (7.68 g, 90 mmol) was added and the mixture diluted with CH_2Cl_2 (200 mL) prior to extraction with sat $NaHCO_3$ (300 mL) and water (300 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (6:4 cyclohexane–EtOAc) to give pure 2,3,4,6-penta-*O*-acetyl-β-D-glucopyranosyl azide (4 g, 70% yield).[32]

This compound (1 g, 2.68 mmol) was dissolved in technical grade MeOH (50 mL) and one molar sodium methanolate solution (1 mL) was added. The mixture was stirred for 8 h and $120H^+$ resin was added until the pH reached 5. The resin was filtered and the filtrate evaporated under reduced pressure to give pure glucopyranosyl azide **5** (542 mg, 99% yield) without purification. Analytical data were in agreement with the literature.[21]

3.10. General procedure for the heterogeneous Click chemistry

Compounds **6**, **7**, **6-Re** were obtained accordingly. The catalyst was synthesized by immobilisation of copper nanoparticles onto charcoal as previously described.[20] Alkynyl compound **1**, **4** or **1-Re** (146 μmol) and glucopyranosyl azide **5** (146 μmol) were dissolved in a mixture of *t*-BuOH and H_2O (5:1; 1.2 mL). Catalyst C/Cu (35 mg) was added and the mixture was stirred at 100 °C under microwave irradiation until TLC or ESI-MS indicated total disappearance of the starting materials. The mixture was filtered over a pad of Celite and rinsed with methanol. The filtrate was evaporated under reduced pressure to give the expected cycloadducts.

3.11. Glucoconjugate 6

$[\alpha]_D^{20}$ −7 (*c* 0.1, MeOH); R_t = 23.68 min (gradient A); ^{1}H NMR (500 MHz, DMSO) δ 8.48 (2H, d, *J* 4.0 Hz, CH_{Ar}), 8.27 (1H, s, CH_{Trz}), 7.76 (2H, dd, *J* 7.6 Hz, *J* 1.6 Hz, CH_{Ar}), 7.58 (2H, d, *J* 7.8 Hz, CH_{Ar}), 7.25 (2H, dd, CH_{Ar}), 5.52 (1H, d, $J_{1,2}$ 9.2 Hz, H-1), 5.36 (1H, d, *J* 5.8 Hz, OH), 5.26 (1H, br, OH), 5.13 (1H, d, OH), 4.63 (1H, t, *J* 4.8 Hz, OH), 3.70 (8H, m, 2 × CH_2, CH_{2Trz}, H-2, H-6), 3.44 (2H, m, H-3, H-6′), 3.50 (1H, H-4), 3.25 (1H, br, H-5); ^{13}C NMR (125 MHz, DMSO-d_6): δ 159.0 (C_{Ar}), 148.9 (C_{Ar}), 143.4 (Cq_{Trz}), 136.6 (C_{Ar}), 122.9 (CH_{Trz}), 122.6, 122.1 (C_{Ar}), 87.5 (C-1), 80.0, 77.0 (C-3, C-4), 72.1 (C-2), 69.6 (C-5), 60.7 (C-6), 58.7 (CH_2), 48.1 (CH_2); HRMS (ES+): found 465.1861 $C_{21}H_{26}N_6O_5Na$ requires 465.1862 $[M+Na^+]^+$.

3.12. Glycoconjugate 7

$[\alpha]_D^{20}$ −11 (*c* 0.1, MeOH); R_t = 26.73 min (gradient A); ^{1}H NMR (300 MHz, CD_3OD) δ 8.49 (1H, br, CH_{Ar}), 8.13 (1H, s, CH_{Trz}), 7.80 (1H, t, *J* 7.5 Hz, CH_{Ar}), 7.47 (1H, d, *J* 6.9 Hz, CH_{Ar}), 7.30 (1H, br, CH_{Ar}), 5.62 (1H, d, $J_{1,2}$ 9.1 Hz, *H*-1), 3.91–3.85 (6H, m, H-2, H-6, $2CH_2$), 3.72 (1H, dd, H-6′), 3.60–3.50 (3H, m, H-3, H-4, H-5); ^{13}C NMR (75 MHz, CD_3OD): δ 159.9 (C_{Ar}), 149.9 (CH_{Ar}), 146.9 (NC=CH_{Trz}), 138.8, 124.2 (CH_{Ar}), 123.8 (NC=*C*H$_{Trz}$), 123.6 (*C*H$_{Ar}$), 89.6 (*C*-1), 81.1, 78.4, 74.0, 70.9 (C-2, 3, 4, 5), 62.4 (C-6), 54.4, 44.3 ($2xCH_2NH_2$); HRMS (ES+): found 374.1428 $C_{15}H_{21}N_5O_5Na$ requires 374.1440 $[M+Na^+]^+$.

3.13. Glycocomplex 6-Re

$[\alpha]_D^{20}$ −9 (*c* 0.1, MeOH); R_t = 22.28 min (gradient A); ^{1}H NMR (500 MHz, DMSO) δ 8.82 (2H, s, CH_{Ar}), 8.63 (1H, s, CH_{Trz}), 7.97 (2H, dd, *J* 7.8 Hz, *J* <1 Hz, CH_{Ar}), 7.55 (2H, d, *J* 7.8 Hz, CH_{Ar}), 7.40 (2H, s, CH_{Ar}), 5.67 (1H, d, $J_{1,2}$ 9.2 Hz, H-1), 5.40 (3H, br, OH), 5.10 (2H, t, C*H*H$_{Ar}$), 5.02 (2H, m, CH_{2Trz}), 4.64 (3H, dd, 2 × CH*H*$_{Ar}$ + OH), 4.58 (1H, d, *J* 17 Hz, CH*H*$_{Ar}$), 3.77 (2H, m, H-2, H-6), 3.49 (3H, m, H-3, H-4, H-6′), 3.49 (2H, m, H-5, H-6′); ^{13}C NMR (125 MHz, DMSO-d_6): δ 196.1, 195.2 (CO), 160.3 (C_{Ar}), 151.8 (C_{Ar}), 140.5 (C_{Ar}), 139.4 (C_{Ar}), 125.9, 125.7 (CH_{Trz}, C_{Ar}), 123.5 (C_{Ar}), 87.7 (C-1), 80.0, 76.7 (C-3, C-4), 72.4 (C-2), 69.6 (C-5), 67.5, 67.4 (CH_{2Ar}), 62.8, 60.7 (CH_2); HRMS (ES+): found 711.1356 $C_{24}H_{26}N_6O_8Re$ requires 711.1342 $[M-Br^-]^+$.

3.14. General procedure for the Click chemistry with $CuSO_4$–NaAsc

Compounds **8**, **8-Re** and **9** were synthesized using this procedure. Alkynyl compound **2**, **2-Re** or **3** (73 μmol) and glucopyranosyl azide **5** (73 μmol) were dissolved in a dioxane–H_2O mixture (5:1; 1.2 mL). $CuSO_4$ (14 μmol) and sodium ascorbate (30 μmol) were added and the mixture was stirred at 100 °C for 30 min under microwave irradiation. The mixture was filtered on a pad of Celite and rinsed with MeOH. The filtrate was evaporated under reduced pressure to give the expected cycloadduct.

3.15. Glycoconjugate 8

$[\alpha]_D^{20}$ −10 (*c* 0.1, H_2O); R_t = 36.66 min (gradient A); ^{1}H NMR (300 MHz, CD_3OD) δ 8.27 (1H, s, CH_{Trz}), 8.18 (2H, d, *J* 8.5 Hz, CH_{Ar}), 7.92 (2H, d, CH_{Ar}), 7.78 (2H, d, *J* 8.4 Hz, CH_{Ar}) 7.75 (2H, d, *J* 8.4 Hz, CH_{Ar}), 7.65 (2H, t, CH_{Ar}), 7.48 (2H, t, *J* 7.0 Hz, CH_{Ar}), 5.64 (1H, d, $J_{1,2}$ 9.2 Hz, H-1), 3.98–3.87 (8H, m, H-2, H-6, 2 × CH_{2Ar}, CH_{2Trz}), 3.72 (1H, dd, $J_{6,6'}$ 11 Hz, $J_{5,6'}$ 3 Hz, H-6′), 3.62–3.50 (3H, m, H-3,4,5); ^{13}C NMR (75 MHz, CD_3OD): δ 161.2 (C_{Ar}), 148.9 (C_{Ar}), 145.1 (N*C*=CH_{Ar}), 138.5, 130.9, 128.9, 128.8, 127.6 (4CH_{Ar}, C_{Ar}), 124.7 (NC=*C*H$_{Trz}$), 122.5 (CH_{Ar}), 89.6 (C-1), 81.1, 78.5, 74.1, 70.9 (C-2, 3, 4, 5), 62.3 (C-6), 61.1 ($2CH_{2Ar}$), 50.2 (CH_{2Trz}); HRMS (ES+): found 565.2151 $C_{29}H_{30}N_6O_5Na$ requires 565.2175 $[M+Na^+]^+$.

3.16. Glycocomplex 8-Re

$[\alpha]_D^{20}$ −13 (*c* 0.1, MeOH); R_t = 21.02 min (gradient A); ^{1}H NMR (300 MHz, CD_3OD) δ 8.68 (1H, s, CH_{Trz}), 8.52 (4H, br, CH_{Ar}), 8.00 (3H, m, CH_{Ar}), 7.87 (1H, t, *J* 8 Hz, CH_{Ar}), 7.66 (2H, t, *J* 8 Hz, CH_{Ar}), 7.60 (2H, dd, *J* 8 Hz, *J* 5 Hz, CH_{Ar}), 5.77 (1H, d, $J_{1,2}$ 9 Hz, H-1), 5.45 (1H, m, *CH*H), 5.24 (2H, s, CH_{2Trz}), 4.90 (3H, m, 3 × CH*H*$_{Ar}$), 4.03 (2H, m, H-2, H-6), 3.70 (1H, dd, $J_{6,6'}$ 11 Hz, $J_{5,6'}$ 3 Hz, H-6′), 3.65–3.55 (3H, m, H-3, H-4, H-5); ^{13}C NMR (75 MHz, CD_3OD): δ 197.2, 195.2 (CO), 166.0, 148.1 (C), 142.9 (CH_{Ar}), 141.2 (NC=CH_{Trz}), 133.6, 133.5, 130.9, 129.8, 129.5 (CH_{Ar}), 127.5 (NC=*C*H$_{Trz}$), 121.3 (*C*H$_{Ar}$), 89.7 (*C*-1), 81.2, 78.1 (C-3, C-4), 74.2 (C-2), 70.7 (C-5), 69.6, 62.3, 62.1 (CH_2); HRMS (ES+): found 811.1647 $C_{32}H_{30}N_6O_8Re$ requires 811.1655 $[M-Br^-]^+$.

3.17. Glycoconjugate 9

$[\alpha]_D^{20}$ −6 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (300 MHz, CD_3OD) δ 8.12 (1H, br, CH_{Trz}), 5.61 (1H, d, $J_{1,2}$ 9.2 Hz, H-1), 4.03–3.30 (12H, m, H-2_{glu} to H-6_{glu}, 3 × CH_2), 1.45 (18H, s, 6 × CH_3); ^{13}C NMR (75 MHz, CD_3OD): δ 171.8 (CO), 145.1 (NC=CH_{Trz}), 124.6 (NC=*C*H$_{Trz}$), 89.5 (C-1), 82.4, 78.3 (C-3, C-4), 74.0 (C-2), 70.8 (C-5), 62.3 (C-6), 55.7 (CH_{2Ar}+CH_{Trz}), 28.4 (CH_3); HRMS (ES+): found 511.2364 $C_{21}H_{36}N_4O_9Na$ requires 511.2380 $[M+Na^+]^+$.

3.18. Glycoconjugate 10

Compound **9** (300 mg, 0.615 mmol) was dissolved in ethanol (12 mL). 2 M NaOH (12 mL) was added while stirring, and the mixture was heated at 60 °C for 12 h. After evaporation under reduced

pressure, the residue was dissolved in water (12 mL) at 0 °C. 6 M HCl was added until the solution became acidic. The mixture was evaporated under reduced pressure and the dry residue dissolved in absolute ethanol (12 mL). After salts were filtered on a frit, the filtrate was evaporated under reduced pressure leading to **10** quantitatively as a hygroscopic solid. $[\alpha]_D^{20}$ −5 (*c* 0.1, H_2O); R_t = 4.54 min (gradient A); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.23 (1H, s, CH_{Trz}), 5.52 (1H, d, $J_{1,2}$ 8.6 Hz, H-1), 4.08 (2H, br, CH_2), 3.72–3.33 (9H, m, H-2,3,5,6,6′, 2 × CH_2), 3.23 (1H, br, H-4); ^{13}C NMR (75 MHz, DMSO-d_6): δ 171.0 (CO), 141.9 (NC=CH_{Trz}), 123.8 (NC=CH_{Trz}), 87.5 (C-1), 80.0, 76.8 (C-3, C-4), 72.2 (C-2), 69.5 (C-5), 60.7 (C-6), 53.4 (CH_2CO_2H), 48.2 (CH_{2Trz}); HRMS (ES+): found 399.1121 $C_{13}H_{20}N_4O_9Na$ requires 399.1128 $[M+Na^+]^+$.

3.19. Glycocomplex 7-Re

Glycoconjugate **7** (100 mg, 0.284 mmol) and $Re(CO)_5Cl$ (113 mg, 0.312 mmol) were dissolved in MeOH (10 mL) and stirred at 65 °C for 6 h. After cooling to room temperature, the solution was evaporated to dryness and purified by preparative C-18 HPLC to give the desired complex **7-Re** as a white powder. (140 mg, 75% yield); R_t = 5.79/6.03 min (gradient B); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.75 (1H, m, CH_{Ar}), 8.51 (1H, m, CH_{Trz}), 8.02 (1H, t, *J* 7.5 Hz, CH_{Ar}), 7.66 (1H, d, *J* 7.0 Hz, CH_{Ar}), 7.42 (1H, br, CH_{Ar}), 5.61 (1H, d, $J_{1,2}$ 8.7 Hz, *H*-1), 4.76 (2H, s, CH_2), 4.54 (1H, m, C*H*H), 4.36 (1H, m, C*H*H), 3.65–3.22 (6H, m, H-3, H-4, H-5, H-6, H-6′); ^{13}C NMR (75 MHz, DMSO-d_6): δ 196.5–195.5 (CO), 161.4 (C_{Ar}), 153.4 (CH_{Ar}), 142.6 (NC=CH_{Ar}), 141.4, 127.3 125.7 (CH_{Ar}), 123.2 (NC=CH_{Trz}), 89.2 (*C*-1), 81.4, 80.1, 77.1, 73.2 (C-2, 3, 4, 5), 62.9 (CH_2N), 60.9 (C-6), 51.8 (CH_2N). All the peaks are doubled due to the presence of the two isomers. MS (ES+): 622.3 $[M-Cl^-]^+$. Anal. Calcd for $C_{18}H_{21}N_5O_8ReCl$: C, 32.90; H, 3.22. Found: C, 32.34; H, 3.27.

3.20. Radiolabelling of glycoconjugates

$Na[^{99m}TcO_4]$ was eluted from a ^{99}Mo/^{99m}Tc generator (Mallinckrodt Inc.) using 0.9% saline solution. The precursor $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared from pertechnetate (600 μL eluate) using an Isolink kit®.[33] The mixture was stirred for 30 min at 95 °C and then cooled down.

To a solution of the glycoconjugate **6**, **7**, **8** or **10** (1 mg/mL in methanol, 100 μL) placed in a borosilicate vial under nitrogen, were added successively 200 μL of an aqueous acetic acid buffer 0.2 M pH 3.4 and 65 μL of the freshly prepared $[^{99m}Tc(CO)_3(H_2O)_3]^+$ solution. The vial was sealed with a teflon-lined cap and the mixture was heated at 95 °C for 30 min. Upon cooling, the resulting complex was analysed and purified with the HPLC system described above. The radiolabelling yields ranged from 95% for **6-Tc** and **7-Tc** to 90% for **8-Tc** and 80% for **10-Tc**. The radiochemical purity assessed by ITLC was >95% after RP-HPLC purification for each complex. The retention time of **6-Tc**, **7-Tc**, **8-Tc** and **10-Tc** were 6.44 min, 5.08/5.60 min, 8.13 min and 4.69 min, respectively.

3.21. Histidine challenge assay

Each radiolabelled glycoconjugate was subject to transchelation in the presence of histidine as an assay of label stability. Corresponding HPLC purified ^{99m}Tc-complexes dissolved in a mixture of MeOH–H_2O (3:7 v/v) were diluted with an equivalent volume of a freshly prepared PBS solution of L-histidine at 1 mg/mL solution (≈250:1 histidine/complex molar ratio). The solutions were stirred and incubated at 37 °C for various time intervals (4 and 24 h). Periodically incubated aliquots were removed and analysed by RP-HPLC.

3.22. Biodistribution in healthy rats

All experiments were carried out in compliance with French laws relating to the conduct of animal experimentation. Before being used in the animal studies, purified ^{99m}Tc-complex solution was filtered through a 0.22 μm sterile filter (Millipore®) and diluted with sterile saline solutions. Healthy male Wistar rats (200–250 g) anaesthetised with Nesdonal® were sacrificed at 5, 30 and 240 min post-injection (p.i.) (n = 3) after an intra-jugular injection of 300 μL of the diluted tracer solution **6-Tc**, **7-Tc** or **10-Tc**. The organs of interest (liver, spleen, heart, lungs, kidneys and brain) were dissected, weighed and their radioactivity was measured in a Packard autogamma counter. Results, expressed as percentage of injected dose per organ (%ID/organ) were summarized in Table 2. For total blood radioactivity calculation, blood mass is assumed to be 7% of total body mass.

Acknowledgements

This work was carried out with financial support from the French Agence Nationale de la Recherce (ANR JC07_183019), the Centre National de la Recherche Scientifique, the Ministère Délégué à l'Enseignement Supérieur et à la Recherche and the Conseil Régional de Picardie.

References

1. For a review: (i) Hanessian, S.; Mascitti, V.; Rogel, O. *J. Org. Chem.* **2002**, *67*, 3346–3354; (ii) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637–674; (iii) Gabius, H.-J. *Adv. Drug Delivery Rev.* **2004**, *56*, 421–424.
2. Fowler, J. S.; Wolf, A. P. *Acc. Chem. Res.* **1997**, *30*, 181–188.
3. (i) Bartholomä, M.; Valliant, J.; Maresca, K. P.; Babich, J.; Zubieta, J. *Chem. Commun.* **2009**, 493–512; (ii) Banerjee, S. R.; Maresca, K. P.; Franscesconi, L.; Valliant, J.; Babich, J. W.; Zubieta, J. *Nucl. Med. Biol.* **2005**, 1–20; (iii) Schibli, R.; Schubiger, P. A. *Eur. J. Nucl. Med.* **2002**, *29*, 1529–1542; (iv) Dilworth, J. R.; Parrot, S. J. *Chem. Soc. Rev.* **1998**, *27*, 43–55; (v) Hashimoto, K.; Yoshihara, K. *Top. Curr. Chem.* **1996**, *176*, 79.
4. (i) Stephenson, K. A.; Reid, L. C.; Zubieta, J.; Babich, J. W.; Kung, M.-P.; Kung, H. F.; Valliant, J. F. *Bioconjugate Chem.* **2008**, *19*, 1087–1094; (ii) Schaffer, P.; Gleave, J. A.; Lemon, J. A.; Reid, L. C.; Pacey, L. K.; Farncombe, T. H.; Boreham, D. R.; Zubieta, J.; Babich, J. W.; Doering, L. C.; Valliant, J. F. *Nucl. Med. Biol.* **2008**, *35*, 159–169; (iii) Le Gal, J.; Benoist, E. *Eur. J. Org. Chem.* **2006**, 1483–1488; (iv) Karra, S. R.; Schibli, R.; Gali, H.; Katti, K. V.; Hoffman, T. J.; Higginbotham, C.; Sieckman, G. L.; Volkert, W. A. *Bioconjugate Chem.* **1999**, *10*, 254–260.
5. Orlova, A.; Nilsson, F. Y.; Wikman, M.; Widström, C.; Ståhl, S.; Carlsson, J.; Tolmachev, V. *J. Nucl. Med.* **2006**, *47*, 512–519.
6. (i) Karube, Y.; Katsuno, K.; Takaka, J.; Matsunaga, K.; Haruno, M.; Kuroki, M.; Arakawa, F.; Kanda, H. *Nucl. Med. Biol.* **1996**, *23*, 753–759; (ii) Palestro, C. J.; Kipper, S. L.; Welland, F. L.; Love, C.; Tomas, M. B. *Radiology* **2002**, *223*, 758–764.
7. Yang, D. Y.; Kim, C.-G.; Schechter, N. R.; Azhdarinia, A.; Yu, D.-F.; Oh, C.-S.; Bryant, J. L.; Won, J.-J.; Kim, E. E.; Podoloff, D. A. *Radiology* **2003**, *226*, 465–473.
8. For a review: (i) Bowen, M. L.; Orvig, C. *Chem. Commun.* **2008**, 5077–5091; (ii) Ferreira, C. L.; Bayly, S. R.; Green, D. E.; Storr, T.; Barta, C. A.; Steele, J.; Adam, M. J.; Orvig, C. *Bioconjugate Chem.* **2006**, *17*, 1321–1329; (iii) Petrig, J.; Schibli, R.; Dumas, C.; Alberto, R.; Schubiger, P. A. *Chem. Eur. J.* **2001**, *7*, 1868–1873; (iv) Baly, S. R.; Fisher, C. L.; Storr, T.; Adam, M. J.; Orvig, C. *Bioconjugate Chem.* **2004**, *15*, 923–926; Branco de (v) Barros, A. L.; Cardoso, V. N.; Das Graças Mota, L.; Amaral Leite, E.; Cristina de Oliveira, M.; José Alves, R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2497–2499.
9. (i) Gottschaldt, M.; Bohlender, C.; Müler, D.; Klette, I.; Baum, R. P.; Yano, S.; Schubert, U. S. *Dalton Trans.* **2009**, 5148–5154; (ii) Mikata, Y.; Shinohara, Y.; Yoneda, K.; Nakamura, Y.; Esaki, K.; Tanahashi, M.; Brudzin, I.; Hirohara, S.; Yokoyama, M.; Mogami, K.; Tanase, T.; Kitayama, T.; Takashiba, K.; Nabeshima, K.; Takagi, R.; Takatani, M.; Okamoto, T.; Kinoshita, I.; Doe, M.; Hamazawa, A.; Morita, M.; Nishida, F.; Sakakibara, T.; Orvig, C.; Yano, S. *J. Org. Chem.* **2001**, *66*, 3783–3789.
10. (i) Dumas, C.; Petrig, J.; Frei, L.; Spingler, B.; Schibli, R. *Bioconjugate Chem.* **2005**, *16*, 421–428; (ii) Dumas, C.; Schibli, R.; Schubiger, P. A. *J. Org. Chem.* **2003**, *68*, 512–518.
11. Ferreira, C. L.; Ewart, C. B.; Bayly, S. R.; Patrick, B. O.; Steele, J.; Adam, M. J.; Orvig, C. *Inorg. Chem.* **2006**, *45*, 6979–6987.
12. Schibli, R.; Dumas, C.; Petrig, J.; Spadola, L.; Scapozza, L.; Garcia-Garayoa, E.; Schubiger, P. A. *Bioconjugate Chem.* **2005**, *16*, 105–112.
13. (i) Song, Y.; Kohlmeir, E. K.; Meade, T. J. *J. Am. Chem. Soc.* **2008**, *130*, 6662–6663; (ii) Bammer, R.; de Crespigny, A. J.; Howard, D.; Seri, S.; Hashiguchi, Y.; Nakatani, A.; Moseley, M. E. *Magn. Reson. Imaging* **2002**, *22*, 619–624; (iii) Bryson, J. M.; Chu, W.-J.; Lee, J.-H.; Reineke, T. M. *Bioconjugate Chem.* **2008**, *19*, 1505–1509.

14. (i) André, J. P.; Geraldes, C. F. G. C.; Martins, J. A.; Merbach, A. E.; Prata, M. I. M.; Santos, A. C.; de Lima, J. J. P.; Tóth, E. *Chem. Eur. J.* **2004**, *10*, 5804–5816; (ii) Ziegler, T.; Hermann, C. *Tetrahedron Lett.* **2008**, *49*, 2166–2169.
15. (i) Schlibi, R.; Schwarzbach, R.; Alberto, R.; Ortner, K.; Schmalle, H.; Dumas, C.; Egli, A.; Schubiger, P. A. *Bioconjugate Chem.* **2002**, *13*, 750–756; (ii) Schibli, R.; La Bella, R.; Alberto, R.; Garcia-Garayoa, E.; Ortner, K.; Abram, U.; Schubiger, P. A. *Bioconjugate Chem.* **2000**, *11*, 345–351.
16. (i) Mindt, T. L.; Struthers, H.; Brans, L.; Anguelov, T.; Schweinsberg, C.; Maes, V.; Tourwé, D.; Schibli, R. *J. Am. Chem. Soc.* **2006**, *128*, 15096–15097; (ii) Mindt, T. L.; Müller, C.; Melis, M.; de Jong, M.; Schibli, R. *Bioconjugate Chem.* **2008**, *19*, 1689–1692; For a review see: (iii) Struthers, H.; Mindt, T. L.; Schibli, R. *Dalton Trans.* **2010**, *39*, 675–696.
17. Struthers, H.; Viertl, D.; Kosinski, M.; Spingler, B.; Buchegger, F.; Schibli, R. *Bioconjugate Chem.* **2010**, *21*, 622–634.
18. Camp, C.; Dorbes, S.; Picard, C.; Benoist, E. *Tetrahedron Lett.* **2008**, *49*, 1979–1983.
19. Struthers, H.; Spingler, B.; Mindt, T. L.; Schibli, R. *Chem. Eur. J.* **2008**, *14*, 6173–6183.
20. Kempf, D. J.; Codacovi, L.; Wang, X. C.; Kohlbrenner, W. E.; Wideburg, N. E.; Saldivar, A.; Vasavanonda, S.; Marsh, K. C.; Bryant, P.; Sham, H. L.; Green, B. E.; Betebenner, D. A.; Erickson, J.; Norbeck, D. W. *J. Med. Chem.* **1993**, *36*, 320–330.
21. (i) Praly, J.-P.; Senni, D.; Faure, R.; Descotes, G. *Tetrahedron* **1995**, *51*, 1697–1708; (ii) Wilkinson, B. L.; Bornaghi, L. F.; Houston, T. A.; Innocenti, A.; Supuran, C. T.; Poulsen, S.-A. *J. Med. Chem.* **2006**, *49*, 6539–6548.
22. This compound was synthesized as described in: Cohen, R. B.; Tsou, K. C.; Rutenburg, S. H.; Seligman, A. M. *J. Biol. Chem.* **1952**, *195*, 239–249.
23. Lipshutz, B.; Taft, B. R. *Angew. Chem., Int. Ed.* **2006**, *45*, 8235–8238.
24. (i) Bouillon, J. C.; Meyer, A.; Vidal, S.; Jochum, A.; Chevolot, Y.; Cloarec, J.-P.; Praly, J.-P.; Vasseur, J.-J.; Morvan, F. *J. Org. Chem.* **2006**, *71*, 4700–4702; (ii) Appukkuttan, P.; Dehaen, W.; Fokin, V. V.; Van der Eycken, E. *Org. Lett.* **2004**, *6*, 4223–4225.
25. Rodios, N. A. *J. Heterocycl. Chem.* **1984**, *21*, 1169–1173.
26. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.
27. Banerjee, S. R.; Babich, J. W.; Zubieta, J. *Inorg. Chim. Acta* **2006**, *359*, 1603–1612.
28. Gouin, S. G.; Gestin, J.-F.; Morandeau, L.; Segat-Dioury, F.; Meslin, J.-C.; Deniaud, D. *Org. Biomol. Chem.* **2005**, *3*, 454–461.
29. Egli, A.; Alberto, R.; Tannahill, L.; Schibli, R.; Abram, U.; Schaffland, A.; Waibel, R.; Tourwe, D.; Jeannin, L.; Iterbeke, K.; Schubiger, P. A. *J. Nucl. Med.* **1999**, *40*, 1913–1917.
30. (i) Storr, T.; Fisher, C. L.; Mikata, Y.; Yano, S.; Adam, M. J.; Orvig, C. *Dalton Trans.* **2005**, 654–655; (ii) Schibli, R.; Netter, M.; Scapozza, L.; Birringer, M.; Schelling, P.; Dumas, C.; Schoch, J.; Schubiger, P. A. *J. Organomet. Chem.* **2003**, *668*, 67–74.
31. Bourkoula, A.; Paratvatou-Petsotas, M.; Papadopoulos, A.; Santos, I.; Pietzsch, H.-J.; Livaniou, E.; Pelecanou, M.; Papadopoulos, M.; Pirmettis, I. *Eur. J. Med. Chem.* **2009**, *44*, 4021–4027.
32. Szilàgyi, L.; Györgydeàk, Z. *Carbohydr. Res.* **1985**, *143*, 21–41.
33. (i) Alberto, R.; Schibli, R.; Schubiger, A. P.; Abram, U.; Pietzsch, H. J.; Johannsen, B. *J. Am. Chem. Soc.* **1999**, *121*, 6076–6077; (ii) Alberto, R.; Ortner, K.; Weatley, N.; Schibli, R.; Schubiger, A. P. *J. Am. Chem. Soc.* **2001**, *123*, 3135–3136.