*Inorg. Chem.* **2006**, *45*, 6979–6987


# Glucosamine Conjugates of Tricarbonylcyclopentadienyl Rhenium(I) and Technetium(I) Cores

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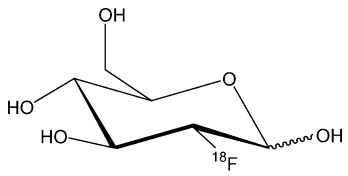
Received April 4, 2006

To obtain a $^{99m}$Tc glucose conjugate for imaging, double-ligand transfer (DLT) and related reactions were examined for the preparation of $CpM(CO)_3$ (Cp = cyclopentadienyl; M = Re, Tc) complexes with pendant carbohydrates at Cp. Tricarbonyl{*N*-(1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-$\beta$-D-glucopyranose)cyclopentadienyl carboxamide}rhenium(I) (**1a**) and tricarbonyl{*N*-(2-amino-2-deoxy-$\beta$-D-glucopyranose)cyclopentadienyl carboxamide}rhenium(I) (**2a**) were prepared. The compounds were fully characterized by mass spectrometry, elemental analysis, IR, and NMR spectroscopy. Full assignment of the NMR spectra verified the pendant nature of the glucosamine moieties in the solution state and that **2a** exists as both anomers. The solid-state structure of **2a** was determined by X-ray crystallography, again confirming the pendant nature of the glucosamine, but differing from the solution state in that the $\beta$ anomer crystallized preferentially (93%). Compound **2a** was determined to be a high-affinity competitive inhibitor ($K_i = 330 \pm 70$ $\mu$M) of the glucose metabolism enzyme hexokinase, demonstrating that it retains certain biological activity. The $^{99m}$Tc analogues **1b** and **2b** were prepared in moderate radiochemical yields by means of the single-ligand transfer (SLT) route, which is more pertinent to radiopharmaceutical synthesis.

## Introduction

$^{18}$F-2-Deoxy-2-fluoro-D-glucopyranose (FDG) is the preeminent radiotracer used in positron emission tomography (PET) to image glucose metabolism,[1] but its use is limited by cost and availability. Because glucose is a major energy source for the body, especially for the heart and brain, FDG is useful in several areas of medicine, such as cardiology[2] and neurology.[1] In addition, FDG is used in oncology as a tumor marker because of the elevated level of glucose metabolism in many tumor types.[3] Unfortunately, the radioisotope used in FDG, $^{18}$F, is cyclotron produced and has a short half-life, thereby limiting its availability. Hence, there is much interest in developing a radiopharmaceutical which resembles FDG but is more accessible and less expensive.




FDG

For the development of a more accessible FDG-like agent, $^{99m}$Tc is an optimal radioisotope. $^{99m}$Tc is currently used in >90% of nuclear medicine scans and is generator produced: both are significant factors in the wide availability and low cost of the isotope.[4] The physical properties of $^{99m}$Tc ($t_{1/2} = 6$ h, $\gamma = 141$ keV) are ideal for imaging with single-photon emission computed tomography (SPECT), the infrastructure for which is already available in most major hospitals. Clearly, the development of an FDG analogue

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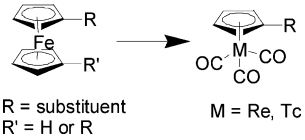
10.1021/ic0605672 CCC: $33.50 
Published on Web 07/21/2006

which incorporates this radiotracer would have a significant impact on health care cost and availability.

Research to combine the ideal properties of $^{99m}$Tc with the biological properties and applications of FDG has led to several groups developing $^{99m}$Tc glucose conjugates.[5−14] The initial investigations of these conjugates suffered from poor characterization[6,10,15] or interactions between the carbohydrate and metal,[5,16,17] which would likely negatively affect biological recognition of the carbohydrate. More recently, well-characterized complexes have been be prepared, radiolabeled in high yields, and have shown high stability.[7,9,12−14] Also, in vivo studies of a few of these compounds have shown promising biodistribution results and tumor uptake,[6,10,15] although the mechanism of uptake does not appear to mimic that of FDG or glucose. Further research is needed to develop a well-characterized glucose conjugate with high stability and optimal biological properties.

The "piano stool" organometallic core ($CpM(CO)_3$; Cp = cyclopentadienyl; M = Re(I), Tc(I)) combines stability, small size, and accessibility for preparing $^{99m}$Tc complexes.[18−23] This core is a neutral 18 electron species, with high stability resulting from the low-spin $d^6$ electron configuration, further stabilized by the Cp and CO ligands. Although many highly stable chelate systems have been developed for $^{99m}$Tc,[24,25] the small size of the piano stool core is advantageous for retaining biological activity, particularly when labeling small molecules such as glucose. Several routes to $CpM(CO)_3$ derivatives have been reported,[18−23] including one step radiolabeling procedures.[26] Clearly, the $CpM(CO)_3$ core is a very attractive candidate for the preparation of $^{99m}$Tc complexes containing glucose.

**Scheme 1.** General Double-Ligand Transfer (DLT)[21−23] and Single-Ligand Transfer (SLT)[26,27] Reactions[a]




[a] For DLT, $[MO_4]^-$, $CrCl_3$, $Cr(CO)_6$, MeOH, 1 h, 160 °C; for SLT, $[M(CO)_3(H_2O)_3]^+$, DMSO/$H_2O$, 4 h, 95 °C.

Two related synthetic routes, the double-ligand transfer (DLT)[21−23] and the single-ligand transfer (SLT),[26,27] have been reported for synthesizing $CpM(CO)_3$ containing complexes on both the macroscopic and tracer scales (Scheme 1). The DLT route was first reported by Wenzel[22,23] and has since been improved by Katzenellenbogen.[21] Advantages of this route include the short reaction time, important when using radiotracers with short half-lives, and the use of $[MO_4]^-$ (M = Re, Tc) as starting material, which is the form in which $^{99m}$Tc is acquired from hospital generators. More recently,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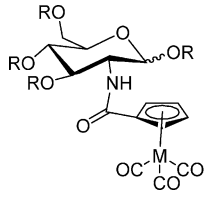
the SLT route has been reported,[26,27] which uses the $[M(CO)_3(H_2O)_3]^+$ core developed by Alberto and co-workers.[28] The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ complex can be prepared in high yield via a commercially available kit, and this removes the need for the carbonyl ligand donor and reducing agent used in the DLT. Both routes require a ferrocene derivative, from which the substituted Cp ring originates. By functionalization of the Cp ring with a biomolecule, the biomolecule can be incorporated into the final piano stool-type product. Because numerous ferrocenoyl glucose conjugates are known in the literature,[29−31] both DLT and SLT are potential routes to $^{99m}$Tc glucose-containing complexes.




R = acetyl, M = Re (**1a**), M = $^{99m}$Tc (**1b**)
R = H, M = Re (**2a**), M = $^{99m}$Tc (**2b**)

In this work, we describe the modification of the DLT and SLT to obtain glucose derivative-containing compounds of the $CpM(CO)_3$ core. Another potential route for the preparation of $CpM(CO)_3$-containing glucose derivatives is via the synthesis of cyclopentadienyl ligands, which then can be reacted directly with the $[M(CO)_3(H_2O)_3]^+$ core.[19] This route was not considered because of the expected obstacles of preparing sodium cyclopentadienyl glucose conjugates. The synthesis of glucosamine−$CpM(CO)_3$ complexes on the macroscopic (M = Re) scale and radiotracer (M = $^{99m}$Tc) scale is described; the solid-state structure of **2a** was determined, and the biological activity related to the glucose-metabolism enzyme hexokinase was evaluated and compared to FDG to determine radiopharmaceutical potential.

## Experimental Section

**Materials and Methods.** Tricarbonyl(cyclopentadienyl carboxylic acid)rhenium,[32,33] $[NEt_4]_2[Re(CO)_3Br_3]$,[34] 1,1′-bis(methoxycarbonyl)ferrocene,[21] and 2-*N*-(1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-D-glucopyranose)-1-ferrocene carboxamide[29] were prepared as reported in the literature. Potassium perrhenate and $Re(CO)_5Br$ were purchased from Strem Chemicals (Newburyport, MA). The Isolink boranocarbonate kits were a gift from Mallinckrodt Inc. Saline solutions of $Na[^{99m}TcO_4]$ were obtained from the Vancouver Coastal Health-UBC Hospital. All other chemicals were purchased from Sigma Aldrich and used without further purification. Water was deionized, purified (Barnstead D9802 and D9804 cartridges), and distilled with a Corning MP-1 Mega-Pure Still. All solvents were HPLC grade and purchased from Fisher. $N_2$ gas was acquired from Praxair. Glassware used for all reactions was oven dried overnight. For high-temperature and high-pressure reactions, a cylindrical aluminum reaction block was used, with holes fitted for high-pressure thick-wall glass tubes (Ace Glass) and an IKA probe, with an aluminum cover that could be secured with screws. Thin-layer chromatography (TLC) was performed using silica TLC plates with aluminum backing (Merck), and silica column chromatography was performed using silica from Silicycle (Quebec City, PQ). All NMR solvents were purchased from Cambridge Isotope Laboratories.

$^1$H NMR spectra were recorded on Bruker AC200-E, AV-300, or AV-400 instruments at 200, 300.13, or 400.21 MHz, respectively. The NMR spectra were calibrated with the deuterated solvent used in each case, and $^1$H−$^1$H COSY 2D and TOCSY 1D NMR spectra were used to aid in the characterization of the compounds. Infrared spectra were recorded on an ATI Mattson Galaxy Series FTIR 5000 spectrophotometer as thin films on NaCl plates or as KBr disks. Mass spectra were obtained on a Macromass LCT (electrospray, ES-MS). Elemental analysis was performed at the University of British Columbia, Chemistry Department, by Mr. M. Lakha (Carlo Erba analytical instrument). UV−vis spectra and hexokinase inhibition assays were obtained on a Hewlett-Packard (HP) model 8453 diode array spectrophotometer, equipped with a kinetics software package. HPLC analysis was done on a Phenomenex Hydro-Synergi 4 $\mu$m C18 RP column using a Waters WE 600 HPLC system equipped with a 2478 dual wavelength absorbance UV detector and the Empower software package for the hexokinase substrate assays or a Knauer Wellchrom K1001 system equipped with a K-2501 UV absorption detector, a Kapintek radiometric well counter, and Peak simple software for the radiolabeling studies.

**Modification of DLT.** DLT was modified from the published methods[21] by variation of the solvent and/or temperature (Scheme 1). In brief, $KReO_4$ (29 mg, 0.10 mmol), $CrCl_3$ (80 mg, 0.50 mmol), $Cr(CO)_6$ (140 mg, 0.63 mmol), 1,1′-bis(methoxycarbonyl)ferrocene (90 mg, 0.30 mmol), and a small stir bar were added to a 4 mL pressure tube, which was then purged with $N_2$ gas. Solvent (1 mL) was added, and the pressure tube was sealed with a Teflon cap. The pressure tube was placed in an aluminum reaction block filled partially with high-temperature heating oil, and an aluminum cover was screwed on top of the block. The block was placed on a heating/stirring plate, stirring was started, and an IKA probe was inserted into an appropriate hole in the block, partially filled with high-temperature heating oil. The reaction temperature was set with the IKA probe. After the mixture was heated for 1 h at the desired reaction temperature, the heating was stopped, and the block was allowed to cool. Once the block was well below 100 °C, the aluminum cover was carefully removed, and the pressure tubes were gently transferred to a dry ice/methanol bath for 10 min and then to an ice bath for 10 min. The pressure tubes were opened, and the contents were transferred to a round-bottom flask using methanol. The solvent volume was reduced by rotary evaporation to a dark viscous residue. The residue was dissolved in dichloromethane, and the solution was clarified by filtration. The filtrate solvent volume was reduced by rotary evaporation, and the reaction yield was

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estimated by comparing the integration of the starting material and product resonances in the $^1$H NMR spectrum of the crude reaction mixture.

**SLT using Alternative Rhenium Starting Materials.** The SLT reaction was modified from that in the literature (Scheme 1).[27] A 4 mL pressure tube containing acetyl ferrocene (33 mg, 0.15 mmol) and rhenium starting material, either $Re(CO)_5Br$ (19 mg, 0.047 mmol) or $[NEt_4]_2[Re(CO)_3Br_3]$ (37 mg, 0.048 mmol), was purged with $N_2$ gas. Anhydrous methanol was added (1 mL), and the tube sealed with a Teflon cap before it was placed into the aluminum heating block. The reaction was stirred and heated at 160 °C for 1 h. After the aluminum block had cooled to less than 100 °C, the pressure tube was removed and cooled in an ice bath for 10 min. The yields were estimated from the $^1$H NMR spectra of the crude reaction mixtures by comparison of the integration of resonances from acetyl ferrocene and the expected product. Estimated yields were 40 and 16% for the reactions using $Re(CO)_5Br$ and $[NEt_4]_2[Re(CO)_3Br_3]$, respectively.

**Tricarbonyl{*N*-(1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-$\beta$-D-glucopyranose)cyclopentadienyl carboxamide}rhenium(I) (1a).** 1-Hydroxybenzyltriazole (20 mg, 0.15 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (29 mg, 0.15 mmol), and tricarbonyl(cyclopentadienyl carboxylic acid)rhenium(I) (40 mg, 0.10 mmol) were weighed into a flask. The flask was purged with nitrogen gas; freshly distilled dichloromethane (15 mL) was added, and the reaction mixture was stirred at room temperature. After 3 h, 1,3,4,6-tetra-*O*-acetyl-$\beta$-D-glucosamine hydrochloride salt (44 mg, 0.12 mmol) and *N*,*N*-diisopropylethylamine (0.2 mL, 1.2 mmol) were added, and the reaction was refluxed overnight. The dark mixture was reduced on a rotary evaporator to a red oil, which was redissolved in dichloromethane (20 mL) and washed with water (3 × 30 mL). The organic layer was separated and dried with $MgSO_4$, and the solvent was removed with a rotary evaporator. The product was obtained as an off-white solid after purification by silica chromatography, eluted with 7:3 ethyl acetate/hexanes (rf ≈ 0.5), and dried in vacuo for 24 h (31 mg, 44%).$^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 2.04, 2.05, 2.07, 2.10 (4 s, 12H, C*H*$_3$COO), 3.85 (dd, 1H, *H*5, $^3J_{4,5}$ = 9.7 Hz, $^3J_{5,6a}$ = 2.6 Hz),4.12 (dd, 1H, *H*6a, $^2J_{6a,6b}$ = 12.6 Hz), 4.24 (dd, 1H, *H*6b, $^3J_{5,6b}$ = 4.9 Hz, $^3J_{6a,6b}$ = 12.54 Hz), 4.40 (dd, 1H, *H*2, $^3J_{1,2}$ = 9.1 Hz, $^3J_{2,3}$ = 10.4 Hz), 5.13 (t, 1H, *H*4, $^3J_{3,4}$ = 9.6 Hz, $^3J_{4,5}$ = 9.8 Hz), 5.23 (t, 1H, *H*3, $^3J_{2,3}$ = 10.5 Hz, $^3J_{3,4}$ = 9.6 Hz), 5.30, 5.36 (2d, 2H, *H*10, *H*10′, $^3J_{9,10}$ = 1.6 Hz), 5.65 (d, 1H, *H*1, $^3J_{1,2}$ = 8.8 Hz), 5.86, 5.94 (2d, 2H, *H*9, *H*9′, $^3J_{9,10}$ = 1.4 Hz), 6.32 (d, 1H, N*H*, $^3J_{NH,2}$ = 9.3 Hz). $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$ 20.54, 20.70, 20.78, 20.83 (*C*H$_3$COO) 52.94 (*C*2), 61.70 (*C*6), 67.88 (*C*3), 72.58 (*C*4), 72.90 (*C*5), 84.11, 85.61, 85.88, 87.40 (*C*9, *C*9′, *C*10, *C*10′), 92.65 (*C*1), 92.86 (*C*8), 162.32 (*C*7), 169.28, 169.58, 170.66, 171.97 (CH$_3$*C*OO), 192.13 (3 *C*O). IR (cm$^{-1}$, NaCl plate): 2027 (s), 1931 (s, br), ($\nu$(*fac*-Re(CO)$_3$), 1752 (s), ($\nu$(CH$_3$CO)), 1625 (s) ($\nu$(CONH)). MS (ESI+): *m*/*z* (relative intensity) 732, 730 ([M + Na]$^+$, 100). MS (ESI−): *m*/*z* (relative intensity) 708, 706 ([M − 1]$^-$, 28). Anal. Calcd for $C_{23}H_{24}NO_{13}Re$: C, 38.98; H, 3.41; N, 1.97. Found: C, 39.11; H, 3.30; N, 2.29.

**Tricarbonyl{*N*-(2-amino-2-deoxy-D-glucopyranose)cyclopentadienyl carboxamide}rhenium(I) Trihydrate (2a).** Hydroxybenzyltriazole (20 mg, 0.15 mmol), *N*,*N*′-dicyclohexylcarbodiimide (23 mg, 0.11 mmol), and tricarbonyl(cyclopentadienyl carboxylic acid)rhenium(I) (40 mg, 0.10 mmol) were weighed into a flask. After the flask was purged with nitrogen, freshly distilled dichloromethane (15 mL) was added, and the reaction mixture was stirred. After 3 h, glucosamine hydrochloride (23 mg, 0.11 mmol), *N*,*N*-diisopropylethylamine (0.2 mL, 1.2 mmol), and dimethylformamide (15 mL) were added to the reaction mixture, and the stirring was continued overnight. The volume was reduced on a rotary evaporator, and the mixture was vacuum dried to obtain a dark thick oil, which was dissolved in water (20 mL), filtered, and washed with water (10 mL). The aqueous filtrate was reduced on a rotary evaporator and vaccum dried, before purification by silica column chromatography, eluting with 8:2 ethyl acetate/methanol (rf ≈ 0.6). The off-white solid obtained was dried in vacuo for 24 h (32 mg, 66%). $^1$H NMR ($CD_3OD/D_2O$ 1:1, 400 MHz): $\delta$ 3.4−3.46 (m, 1.3H, *H*5$\alpha$, *H*6$\beta$), 3.60 (m, 0.3H, *H*5$\beta$), 3.7−3.8 (m, 2.6H, *H*2$\beta$, *H*4$\beta$, *H*6$\alpha$), 3.8−3.9 (m, 1.7H, *H*3, *H*4$\alpha$), 3.95 (dd, 0.7H, *H*2$\alpha$, $^3J_{1,2}$ = 3.2 Hz, $^3J_{2,3}$ = 10.7 Hz), 4.70 (d, 0.3H, *H*1$\beta$, $^3J_{1,2}$ = 8.4 Hz), 5.19 (d, 1H, *H*1$\alpha$, $^3J_{1,2}$ = 3.2 Hz), 5.59 (s, 2H, *H*10, *H*10′), 6.20, 6.25 (2s, 2H, *H*9, *H*9′). $^{13}$C NMR ($CD_3OD/D_2O$ 1:1, 75 MHz): $\delta$ 55.97 (*C*2$\alpha$), 58.54 (*C*2$\beta$), 62.46 (*C*6$\alpha$), 62.53 (*C*6$\beta$), 71.85 (*C*3$\alpha$), 72.01 (*C*3$\beta$), 72.17 (C5$\alpha$), 72.98 (*C*4$\alpha$), 75.33 (*C*5$\beta$), 77.68 (*C*4$\beta$), 86.29, 86.53, 87.84, 88.46 (*C*9$\beta$, *C*10$\beta$, *C*9′$\beta$, *C*10′$\beta$), 86.42, 86.73, 87.68, 88.58 (*C*9$\alpha$, *C*10$\alpha$, *C*9′$\alpha$, *C*10′$\alpha$), 92.29 (*C*1$\alpha$), 95.26 (*C*8$\alpha$), 95.57 (*C*8$\beta$), 96.61 (*C*1$\beta$), 165.46 (*C*7), 194.10 (3*C*O). IR (cm$^{-1}$, NaCl plate): 2028 (s), 1931 (s, br), ($\nu$(*fac*-Re(CO)$_3$), 1625 (s) ($\nu$(CONH)). MS (ESI+): *m*/*z* (relative intensity) 564, 562 ([M + Na]$^+$, 100), 542, 540 ([M + 1]$^+$, 45). MS (ESI−): *m*/*z* (relative intensity) 541 ([M]$^-$, 100). Anal. Calcd for $C_{15}H_{16}NO_9Re\cdot 3H_2O$: C, 30.30; H, 3.73; N, 2.36. Found: C, 30.61; H, 3.30; N, 2.49.

**Solid-State Structure Determination of 2a.** Crystals were grown by slow evaporation of a MeOH/$H_2O$ solution. X-ray data were collected and processed using a Bruker ×8 APEX diffractometer using graphite-monochromated Mo K$\alpha$ radiation ($\lambda$ = 0.71073 Å) to a maximum 2$\theta$ value of 55.8°. Data were collected and integrated using the Bruker SAINT software package[35] and corrected for Lorentz and polarization effects, as well as for absorption effects, using the multiscan technique (SADABS).[36] The X-ray structure was solved using direct methods (SIR92)[37] and expanded using Fourier techniques.[38] The material was a mixture of anomers, with 92% of the (R) configuration and 8% of the (S) configuration at C1. The atoms of the minor fragment were refined isotropically; all other non-hydrogen atoms were refined anisotropically, and the hydrogen atoms were included in calculated positions. All calculations were performed using the SHELXTL crystallographic software package.[39] The maximum and minimum peaks in the final differential Fourier map were 0.85 and −0.50 e$^-$/Å$^3$.

**Hexokinase Phosphorylation Assay.** Compound **2a** was qualitatively tested as a substrate for hexokinase; 10 $\mu$L of a $10^{-2}$ M solution of **2a** in 30 mM triethanolamine buffer (pH 6) was added to 1 mL of the assay mixture, containing 100 U/L hexokinase, 1 mM ATP, and 4 mM $MgCl_2$ dissolved in 30 mM triethanolamine buffer (pH 6). After it was incubated for 10 min or 24 h, the assay mixture was analyzed by analytical HPLC, using 30 mM $KH_2PO_4$ buffer as the solvent. The analyte was determined to be a substrate if the HPLC chromatogram showed the disappearance of the ATP peak (retention time = 7.0 min) and the appearance of the ADP peak (retention time = 9.8 min); **2a** was not a substrate for hexokinase.

(35) *SAINT*; Bruker AXS Inc.: Madison, WI, 1999.
(36) *SADABS*; Bruker AXS Inc.: Madison, WI, 1999.
(37) Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giocavazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. SIR92. *J. Appl. Crystallogr.* **1999**, *32*, 115−119.
(38) Beurskens, P. T.; Admiraal, G.; Beurskens, G.; Bosman, W. P.; Gleder, R. d.; Israel, R.; Smits, J. M. M. *The DIRDIF-94 Program System*; University of Nijmegen: Nijmegen, The Netherlands, 1994.
(39) *SHELXL*; Bruker AXS Inc.: Madison, WI, 1997.

**Hexokinase Inhibition Assay.** Compound **2a** and 2-deoxy-2-fluoro-D-glucopyranose (FDG) were tested for their capacity to inhibit glucose phosphorylation by hexokinase, and their inhibition constants were determined assuming competitive inhibition. In a cuvette, 500 $\mu$L of the glucose (HK) assay kit (Sigma) was pipetted into a solution containing either **2a** or FDG and glucose (total volume 750 $\mu$L). The kit contains HK and the ATP from which the phosphate is acquired, as well as 6-phosphate-glucose dehydrogenase and $NAD^+$. The reaction rate of glucose phosphorylation is measured via the second enzymatic reaction and the conversion of $NAD^+$ to NADH. The solution was monitored by UV spectroscopy at 340 nm every 30 s for 10 min to determine the reaction rate via the formation of NADH. The assay was repeated at several concentrations of **2a** or FDG (0−2850 mM) and glucose (20−140 mM). Linear regression $K_i$ values were determined, using Microsoft Excel, by first plotting the double reciprocal plot of 1/reaction rate versus 1/[substrate] (Lineweaver−Burke plot) for each concentration of **2** or FDG, then plotting the slope of each line versus the [inhibitor], and calculating the $K_i$ values as the −slope/intercept for the line. The error was determined using Microsoft Excel statistical methods for least squares approximations. A scheme illustrating the assay method and the graphs used to determine the $K_i$ values are available in Supporting Information.

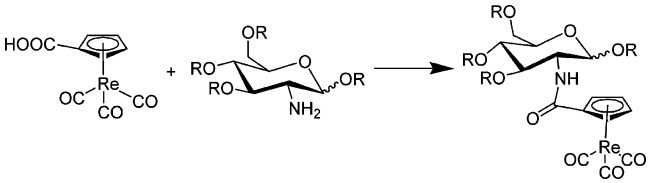
**$^{99m}$Tc Radiolabeling, Preparation of 1b and 2b.** Complexes were prepared using a modified SLT reaction.[27] $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared using the Isolink kits and 200 MBq of $[^{99m}TcO_4]^-$ in 1 mL of saline. A solution of 2-*N*-(1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-D-glucopyranose)-1-ferrocene carboxamide (0.5 mL, $10^{-2}$ M in DMSO) was sealed in a 3 mL vial and purged with $N_2$ gas; 100 $\mu$L of the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ solution was then added. The vial was heated to 60−80 °C for 1−3 h, cooled to room temperature, and analyzed by HPLC to verify the presence of **1b**. To prepare **2b**, 600 $\mu$L of 0.1 M sodium methoxide in methanol was added to the reaction vial, followed by 0.1 M HCl to neutralize it. The radiochemical yield was determined by HPLC (gradient 9:1 0.1% trifluoroacetic acid w/w in water/acetonitrile to 100% acetonitrile over 30 min) using **2a** as a standard.

## Results and Discussion

The initial attempts to use the DLT reaction with ferrocenyl carbohydrate conjugates encountered several difficulties. Methanol was determined to be the best solvent for the reaction by Katzenellenbogen and co-workers,[21] but in our system, when using ferrocenyl carbohydrate conjugates, decomposition of the ferrocenyl carbohydrate conjugates was observed. To overcome these difficulties, the DLT reaction conditions were modified to lower the reaction temperature and incorporate nonnucleophilic solvents. Trial reactions were done using 1,1′-bis(methoxy carbonyl)ferrocene, which has been successfully used in DLT previously.[21] Good yields of 65% were obtained using DMF at only 130 °C; DMF has been used in the DLT reaction with acetyl ferrocene but gave no yield of the desired product in this system.[21] Unfortunately, even at lower temperatures and using DMF, the ferrocenyl carbohydrate complexes yielded no desired product.

Because the SLT reaction, which also uses ferrocenyl compounds, uses a $[M(CO)_3^+]$-containing starting material, it requires neither a carbonyl donor nor a reducing agent, which may reduce the ferrocenyl carbohydrate conjugate decomposition observed in the DLT reaction. The reported

**Scheme 2.** Synthesis of **1a** and **2a**[a]




[a] Reaction conditions for R = Ac (**1a**), HOBt, EDC, diisopropylethylamine, $CH_2Cl_2$, reflux overnight; for R = H (**2a**), HOBt, DCC, diisopropylethylamine, $CH_2Cl_2$, DMF.

Re starting material used in the SLT, $[Re(CO)_6][BF_4]$, is not commercially available, so we investigated the potential of other $Re(I)(CO)_3$ starting materials.[27,40] The SLT reactions using acetyl ferrocene, $Re(CO)_5Br$, which is commercially available, and $[NEt_4]_2[Re(CO)_3Br_3]$, which is easily prepared,[34] gave comparable yields to the reported $[Re(CO)_6][BF_4]$ starting material.[26,27,40] Again, reactions with the ferrocenyl carbohydrate conjugates did not yield the desired product because of decomposition of the starting material.

Because the ferrocenyl carbohydrate conjugates were not stable under the DLT or SLT reaction conditions, an alternative synthesis route was considered (Scheme 2). An indirect DLT method has been reported for peptide labeling,[32,33] where the DLT reaction is used to prepare tricarbonyl(cyclopentadienyl methoxy carbonyl)rhenium(I): the methyl ester is converted to a carboxylic acid and then attached to the peptide by amide coupling. This method is attractive for the synthesis of aminoglucose conjugates, especially of glucosamine, which is known to have similar biochemistry to glucose and has been used in other promising Tc conjugates.[6,12] Tricarbonyl(cyclopentadienyl carboxylic acid)rhenium(I) was prepared, using the DLT reaction, by a literature method.[32,33] An activated ester was prepared in situ using HOBt with either DCC or EDC as activating agents. DCC (organic soluble) was used when preparing the nonprotected conjugate **2a** (water soluble) and EDC (water soluble) for the protected conjugate **1a** (organic soluble) to facilitate easy removal by extraction of the byproduct urea. Either glucosamine or 1,3,4,6-tetra-*O*-acetyl-$\beta$-D-glucopyranose was added with base to the reaction to form **2a** or **1a**, respectively. *N*,*N*′-Diisopropylethylamine was used as the base instead of pyridine or triethylamine because it can be easily removed from the product by evaporation under vaccum. The coupling reaction to form **1a** required heating to obtain acceptable yields, which may be attributed to the larger steric hindrance of the adjacent acetyl groups around the amine. After purification by column chromatography, **1a** and **2a** were isolated as off-white solids in 44 and 66% yields, respectively.

Both **1a** and **2a** were fully characterized by the usual methods. Positive electrospray mass spectra contained the mass peaks $[M + Na]^+$ at 100% relative intensity with the correct isotopic pattern for Re (62.6% $^{187}$Re and 37.4% $^{185}$Re). Elemental analysis confirmed the purity and bulk composition of the products. The elemental analysis of **2a**

(40) Top, S.; Masi, S.; Jaouen, G. The$\{Re(CO)_6]^+$ cation as a ligand-transfer reagent with ferrrocene derivatives. *Eur. J. Inorg. Chem.* **2002**, 1848−1853.

showed three water molecules associated with the compound: this is not surprising because free carbohydrate moieties often have associated water molecules, even after considerable drying. The IR spectra of the compounds confirmed both the presence of CO ligands and the formation of amide bonds. Two peaks diagnostic of C≡O stretches were found at 2027 and 1931 $cm^{-1}$: up to three peaks could be expected, but two peaks suggest three CO ligands with $C_{2v}$ local symmetry at the metal center (yielding two metal CO stretching frequencies, where one is doubly degenerate) or with two peaks overlapping to give the slightly broader peak at 1931 $cm^{-1}$. The IR spectrum of **1a** has peaks from both ester (1727 $cm^{-1}$) and amide (1625 $cm^{-1}$) functionalities because of the protecting acetyl groups and the amide link to the metal core, respectively. The IR spectrum of **2a** has only a peak for the amide functionality, confirming the formation of an amide bond at the C2 position of glucosamine and not an ester bond with the hydroxyl functional groups. The preference for amide bond formation was expected because of the higher nucleophilicity of primary amines compared to hydroxyls.

$^1H$ and $^{13}C$ NMR spectra were fully assigned for both compounds, confirm the pendant nature of the carbohydrate, and show the chirality induced by the carbohydrate. All expected resonances were observed, including one broad $^{13}C$ resonance near 200 ppm attributed to the CO ligands. For **2a**, the free hydroxyl group at the C1 position of the glucosamine is free to mutarotate between the α and $\beta$ anomers. As the $^1H$ resonances of the two anomers are overlapping and hard to differentiate, 1D TOCSY NMR was used, irradiating the C1 hydrogen atoms of each anomer in turn and acquiring the resonances associated within the same anomer ring system (Figure 1). Together with the 2D COSY spectrum, the complete assignment of hydrogen resonances for both anomers was accomplished. The coordination of the glucosamine moieties through the amine functionality is confirmed in the $^1H$ NMR spectra of each compound because the C2 hydrogen resonances are significantly shifted, up to 0.5 ppm downfield, from the resonances observed for free glucosamine. All other carbohydrate associated resonances are only slightly shifted, suggesting that the glucosamine is pendant and not interacting with the metal. The chirality of the glucosamine moiety induces splitting of the cyclopentadienyl (Cp) ring hydrogen and carbon resonances. In a substituted Cp ring, where the substituent is achiral, there are two sets of equivalent CH groups, and only two sets of hydrogen and carbon atom resonances are observed. In **1a** and **2a**, the substituent, glucosamine, contains many chiral centers. Compound **1a** is a single enantiomer, while two anomers are observed for **2a**. This chirality renders all the hydrogen and carbon atoms in the Cp rings inequivalent. For **1a**, four resonances are observed for the CH groups of the Cp ring in both the $^1H$ and $^{13}C$ NMR spectra (Figure 2). For **2a**, the spectra are further complicated by the two anomers, and 10 resonances are observed for the carbon atoms of the Cp ring (Figure 3). The $^1H$ NMR spectrum for **2a** does not resolve all eight hydrogen atoms of the Cp ring: three broad resonances are observed.

The solid-state structure of **2a** shows the glucosamine moiety to be remote from the metal center, as well as the relatively small size of the core (Figure 4, Table 1). The metal center has an octahedral geometry, with the three carbonyl ligands facially coordinated at approximately 90° to one another. The bond lengths between the CO ligands and the metal complex were typical for Re(I)CO, as was the almost linear angle formed by each ReCO.[18,41,42] The planar Cp ligand occupied the other facial plane of the octahedron. The structure is consistent with the many piano stool-type complexes reported.[18,41,42] As expected from the spectroscopic data, the glucosamine moiety is covalently bonded to the Cp ring of the metal core via an amide bond. The carbohydrate is positioned above the piano stool core, well removed from the metal center, eliminating any possibility of interaction. The structure of the glucosamine moiety is relatively undistorted from its typical boat conformation. While two anomers in similar amounts were observed in the solution-state NMR spectra, the $\beta$ anomer crystallized preferentially, with only 7% of the α anomer present in the solid state. Since the anomer concentrations are different, other differences may also be present in the solution and solid-state structures. To verify important structural aspects, such as the pendant nature of the carbohydrate moiety in solution, the solid-state structure is not enough. Full characterization by nonambiguous NMR spectral assignments was also carried out (vide supra).

The interaction of **2a** with hexokinase (HK) was examined to evaluate the retention of glucosamine biological activity. What makes FDG an excellent imaging tracer is not how it mimics glucose, but how it differs from glucose.[3] FDG is transported throughout the body and into cells similarly to glucose. Once in the cells both FDG, glucose, and other hexoses, like glucosamine, are phosphorylated by hexokinase, the first enzyme in the Krebs cycle of glucose metabolism. After phosphorylation, glucose is further metabolized, but FDG is not a substrate for the subsequent enzymatic reaction and is instead trapped intracellularly.[3] This trapping mechanism allows FDG to accumulate, yielding high-target tissue-to-background ratios for quality imaging. Two assays were used to determine how **2a** interacts with HK. Compound **2a** was first tested as a substrate for HK by monitoring the coreaction of ATP to ADP, but no substrate activity was observed. In the second assay, the inhibitory ability of **2a** was determined by competition with glucose. The rate of glucose phosphorylation was measured alone or in the presence of **2a** at different concentrations; **2a** was determined to be a competitive inhibitor with $K_i = 330 \pm 70$ $\mu$M, suggesting that **2a** strongly binds the active site of the enzyme. The competitive inhibition constant for FDG was

(41) Bolm, C.; Kesselgruber, M.; Hermanns, N.; Hildebrand, J. P.; Raabe, G. Application of a planar chiral $\eta^5$-cyclopentadienylrhenium(I)-tricarbonyl complex in asymmetric catalysis: Highly enantioselective phenyl transfer to aldehydes. *Angew. Chem., Int. Ed.* **2001**, *40*, 1488−1490.

(42) Top, S.; Hafa, H. E.; Vessieres, A.; Quivy, J.; Vaissermann, J.; Hughes, D.; Mcglinchey, M. J.; Mornon, J.-P.; Thoreau, E.; Gerard, J. Rhenium carbonyl complexes of estradiol derivatives with high affinity for the estradiol receptor: An approach to selective organometallic radiopharmaceuticals. *J. Am. Chem. Soc.* **1995**, *117*, 8372−8380.

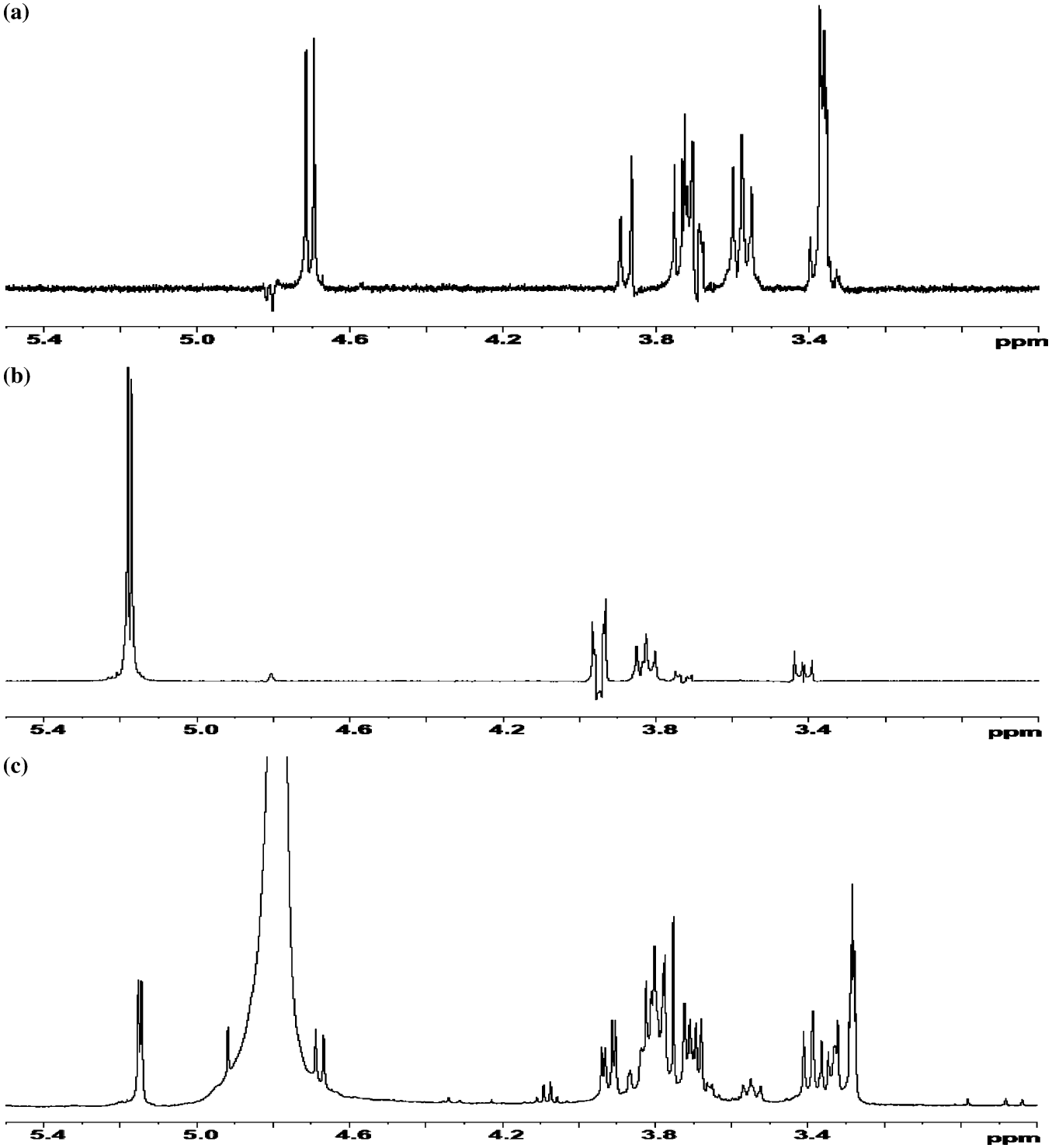



**Figure 1.** TOCSY $^1$H NMR spectra (400 MHz, $CD_3OD/D_2O$ 1:1) of **2a** for the $\beta$ (a) and $\alpha$ (b) anomers and the overall $^1$H NMR spectrum (c) for the region of glucose hydrogen atom resonances.

determined using the same method and found to be $K_i = 1060 \pm 80\ \mu$M, suggesting that **2a** is a better inhibitor than FDG and binds to the enzyme active site either more strongly or more easily. Although FDG is a substrate for HK, it competes with glucose for the enzyme active site and is therefore also an inhibitor. The inhibition of HK by FDG could be measured because the phosphorylation of glucose was monitored by a secondary reaction for which FDG and its phosphorylated product are not substrates. Although **2a** does not act like FDG and is not a substrate for HK, the high affinity competitive inhibition of **2a** suggests it retains some glucose-like biological activity. Compound **2a** may be a substrate for certain glucose transporters (which may be less sensitive to substrate modifications).

Because **2a** may have biological properties of interest, development of the radiochemistry was undertaken. The indirect DLT route, which was used to prepare **1a** and **2a** on the macroscopic scale, is not ideal for radiopharmaceutical

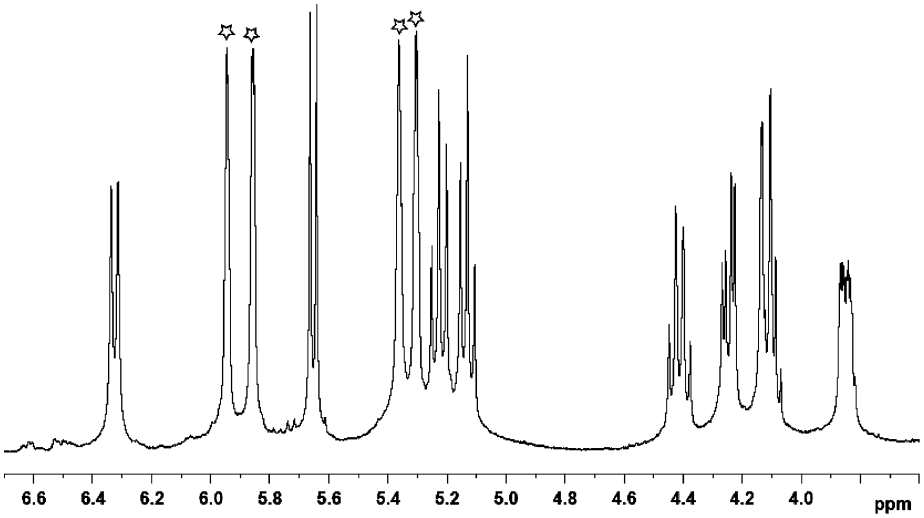



**Figure 2.** $^{1}$H NMR spectrum (300 MHz, $CDCl_3$) of **1a** showing 4 resonances (stars) associated with the inequivalent hydrogen atoms on the cyclopentadienyl ring.

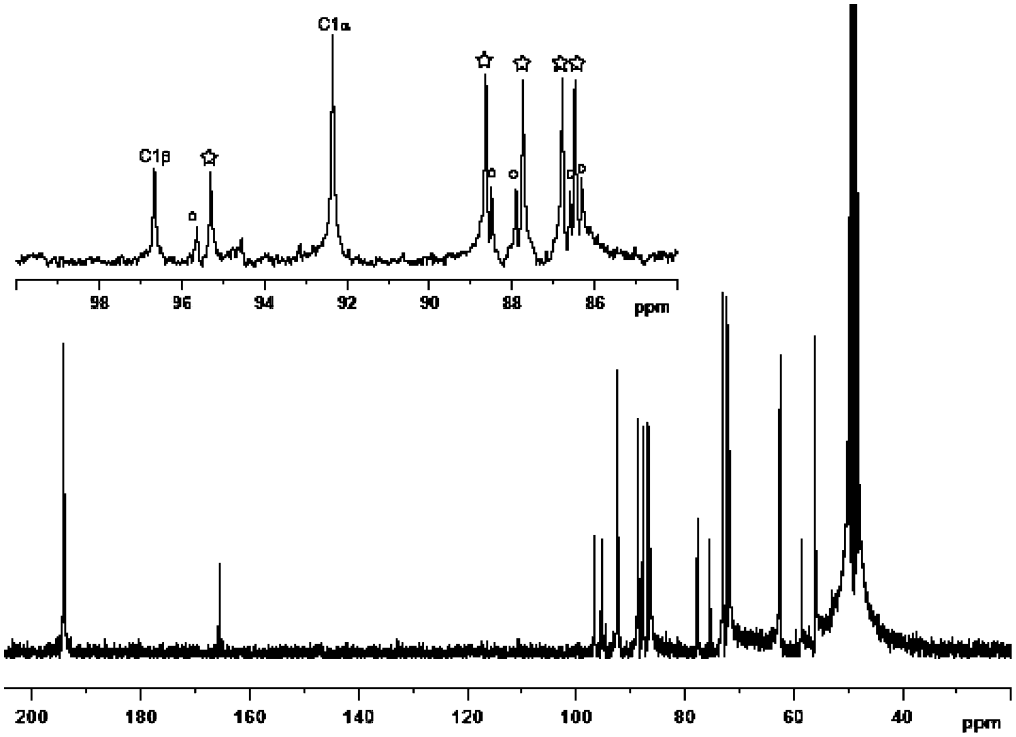



**Figure 3.** $^{13}$C NMR spectrum (75 MHz, $CD_3OD/D_2O$ 1:1) of **2a** with 5 major ($\alpha$ anomer, stars) and 5 minor ($\beta$ anomer, circles) resonances resulting from the inequivalent carbon atoms on the cyclopentadienyl ring.

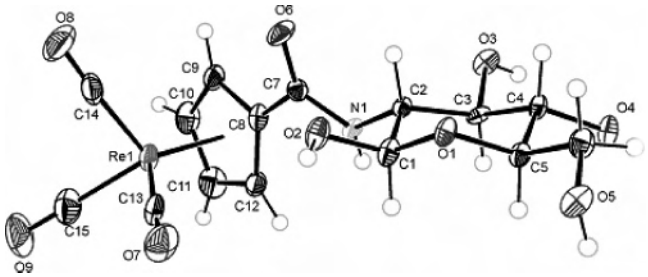


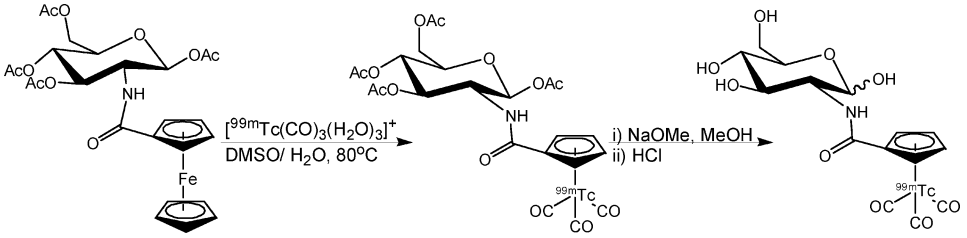

**Figure 4.** ORTEP view of **2a** showing the atom labeling scheme (50% thermal ellipsoids).

preparation because it requires several steps, several purifications, and gives only moderate yields. In comparison, the SLT route requires fewer manipulations, only two steps with no purification between them, and may give higher radiochemical yields and purity in a shorter time. Although the SLT route was not successful using ferrocenyl carbohydrate complexes on the macroscopic scale, the reaction may be feasible on the tracer scale as milder temperatures have been reported for the $^{99m}$Tc SLT.[27] Attempts to prepare $^{99m}$Tc analogues, **1b** and **2b**, from the corresponding ferrocenyl carbohydrates gave mixed results. For the preparation of **2b**

**Table 1.** Selected Bond Lengths (Å) and Angles (deg) in **2a**

| | | | |
|---|---|---|---|
| $Re-C(O)_{av}$ | 1.903 | $C(O)-Re-C(O)_{av}$ | 89.6 |
| $C-O_{av}$ | 1.161 | $Re-C-O_{av}$ | 177.5 |
| $C(Cp)-C(Cp)_{av}$ | 1.420 | C(8)−C(7)−N(1) | 116.39(4) |
| C(7)−O(6) | 1.236(6) | C(8)−C(7)−O(6) | 120.8(4) |
| C(7)−N(1) | 1.341(5) | O(6)−C(7)−N(1) | 122.9(4) |

**Scheme 3.** Preparation of $^{99m}$Tc Complexes **1b** and **2b**




from *N*-(2-deoxy-2-amino-D-glucopyranose)ferrocenyl carboxamide, no product formation was observed. For the preparation of **1b**, the product formed in yields ranging from 45 to 80% depending on time and temperature (Scheme 3). The highest yields were obtained at temperatures of 60−80 °C and reaction times between 2.5 and 3.5 h. The complex was identified and radiochemical yields determined by HPLC using the nonradioactive Re complexes for comparison. At the tracer-level concentrations of the radiolabeled complexes, other methods of characterization are precluded.

Since **2b** could not be prepared directly, it was acquired by removal of the acetyl protecting groups from **1b** (Scheme 3). In the HPLC trace of **1b**, numerous impurities were present with similar retention times to that of **1a**, possibly complexes wherein the four acetyl protecting groups were only partially removed. After complete deacetylation, these impurities disappeared, and the radiochemical yield of the **2b** increased proportionally to 78%. Although in the coinjection HPLC trace, the UV−vis peak attributed to **2a** agrees well with the radiation peak attributed **2b**, the UV−vis trace of **2a** has two peaks because of the two anomers present, while only one product peak is observed in the radiation trace of **2b** suggesting that only one anomer is present for the $^{99m}$Tc complex. Because the acetylated compound is anomerically pure, initially only one anomer is formed upon deacetylation. If the equilibration of anomers via mutarotation is slow, only the initially formed anomer may be observed for the $^{99m}$Tc complex.

Similar chemistry was attempted with $^{186}$Re, a potentially therapeutic radioisotope. $[^{186}Re(CO)_3(H_2O)_3]^+$ was prepared via literature methods[16,43] and then used in the SLT reaction as described above. Unfortunately, Re is more susceptible to reoxidation than Tc, and after the extended period of heating required for the SLT reaction, no product had formed, while a significant amount of $[^{186}ReO_4]^-$ was present. The more timely and difficult method used on the macroscopic scale may be necessary to prepare the $^{186}$Re complex.

(43) Park, S. H.; Seifert, S.; Pietzsch, H.-J. Novel and efficient preparation of precursor $[^{188}Re(OH_2)_3(CO)_3]^+$ for the labeling of biomolecules. *Bioconjugate Chem.* **2006**, *17*, 223−225.

## Conclusions

This work demonstrates the utility of the $CpM(CO)_3$ core in the labeling of highly functionalized and sensitive biomolecules, such as glucosamine. The glucosamine conjugates **1a** and **2a** were prepared and fully characterized. The solid-state structure of **2a** shows the small size of the $CpM(CO)_3$ core and the pendant nature of the glucosamine moiety, remote from the metal. Preliminary in vitro evaluation of **2a** shows that it retains some biological activity because it is a high-affinity competitive inhibitor of hexokinase with a $K_i$ value approximately three times smaller than that for FDG. It was necessary to use different methods for macroscopic (Re, indirect DLT) and tracer (Tc, SLT) syntheses; however, $^{99m}$Tc analogues **1b** and **2b** were prepared in significant radiochemical yields. $^{99m}$Tc-labeled **2b** is expected to be highly stable in vivo and may be useful as a tumor marker or glucose transporter imaging agent; further optimization of this system and in vivo testing is planned.

**Acknowledgment.** The authors would like to thank Mallinckrodt for the Isolink Kits, Vancouver Coastal Health-UBC Hospital for $[^{99m}TcO_4]^-$, and the Natural Sciences and Engineering Research Council of Canada (NSERC) for a Strategic Grant, an Undergraduate Student Research Award (J.S.) and, in collaboration with MDS Nordion Inc., an Industrial Post-Graduate Scholarship (C.L.F.).

**Supporting Information Available:** A scheme and graphical data for the hexokinase assays and X-ray crystallography data. This material is available free of charge via the Internet at http://pubs.acs.org.

IC0605672