Glucosamine conjugates bearing *N*,*N*,*O*-donors: potential imaging agents utilizing the $[M(CO)_3]^+$ core (M = Re, Tc)†

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The design rationale, synthesis and radiolabeling evaluation of four glucosamine conjugated ligands for the $[^{99m}Tc(CO)_3]^+$ core is described. The capability to bind the tricarbonyl core is initially demonstrated using the cold surrogate $[Re(CO)_3]^+$. The four compounds are competent chelates in binding $[^{99m}Tc(CO)_3]^+$ as labeling studies show, with yields ranging from 79 to 96% and the resulting complexes showing stability in the presence of competing chelates for 24 h at 37 °C. The rhenium complexes were tested for hexokinase-catalysed phosphorylation, and the technetium complexes were tested for GLUT-1 mediated cell uptake - they showed a small amount of uptake but it was not glucose dependent, suggesting that it was not *via* the GLUT-1 transporters.

Introduction

Carbohydrates are the principal energy source of the body's cells, tissues and organs. Since carbohydrates are broken down extracellularly into monosaccharides which are too polar to passively diffuse through cell membranes, monosaccharide transporters (collectively called glucose transporters or GLUTs) facilitate monosaccharide membrane crossing.[1,2] This family of transporters is also important in the success of FDG (2-deoxy-2-[^{18}F]-D-glucose) as a positron emission tomography (PET) agent in oncology. FDG is taken up into cells by way of the GLUTs, and is then phosphorylated by the enzyme hexokinase.[3,4] Once phosphorylated, FDG becomes trapped in cells because it is not a good substrate for any other cellular enzyme, and is negatively charged, meaning it cannot diffuse out of the cell. Phosphorylated FDG levels build up in cells with high concentrations of GLUTs and hexokinase, such as cancer cells. This, combined with the elevated glucose metabolism of many tumor cells,[5] leads to a larger amount of FDG being trapped in tumor cells compared to normal cells, giving a good tumor : background ratio and therefore good contrast in the PET images obtained.

FDG, however, has logistic and economic drawbacks associated with its radioisotope, ^{18}F. ^{18}F is cyclotron produced and has a relatively short half-life ($t_{1/2}$ = 110 min), significantly limiting its availability. Because of this there is interest in developing "FDG-like" radiopharmaceuticals that are more economical and readily accessible. Single Photon Emission Computed Tomography (SPECT) imaging, utilizing ^{99m}Tc in various chemical forms, is used in over 90% of nuclear medicine scans.[6] This is because ^{99m}Tc is generator produced and has near ideal physical properties ($t_{1/2}$ = 6 h, γ = 143 keV), making it both readily available and suitable for imaging. Additionally, the similarities between technetium's chemistry and bulk properties, and those of its third row congener rhenium provide both an analogue for macroscopic chemistry ($^{185/187}$Re) and the possibility of a therapeutic radiopharmaceutical based on the same molecular scaffold (^{186}Re $t_{1/2}$ = 3.68 d, β = 1.07 MeV, γ = 137 keV; ^{188}Re $t_{1/2}$ = 16.98 h, β = 2.12 MeV, γ = 155 keV). Unlike ^{123}I or ^{18}F which can be directly substituted for a hydrogen, carbon, nitrogen or oxygen atom in a biomolecule, technetium and rhenium are metals, and as such normally need to be chelated and "masked" before being utilized. The discovery of an easy synthesis of tricarbonyl technetium(I) by Alberto and coworkers[7] introduced a particularly useful radionuclide core for this type of work. This core is small, stable and inert and the three aqua ligands present following the kit preparation can easily be substituted for the desired chelate. Once formed these technetium tricarbonyl complexes are inert and stable, and the metal binding sphere relatively small, which is advantageous when appending biomolecules, for them to have the best chance of being recognized as the parent molecule *in vivo*, as is often the goal in this kind of work.

The general design for technetium-tagged tracers normally comprises a biomolecule responsible for the compound's efficacy and targeting, a spacer group between the biomolecule and the metal binding moiety, a chemical tether which will allow simple conjugation to the biomolecule and a strong chelator as the metal binding pocket. Many biomolecules have been labeled in this general way,[8] including proteins[9] and small molecules[10,11] as well as carbohydrates.[12-16] In this work, we have investigated the use of glucosamine as the biomolecule, as there is evidence describing its *in vivo* role in the transport and accumulation of compounds in tumors, as glucosamine conjugates have been reported to be both transported by GLUT transporters[17,18] and phosphorylated by hexokinase.[17,19] The primary amine of glucosamine lends itself to functionalization, and we have chosen to do this by conjugation to tridentate monoprotic proligands. We are particularly interested in assessing the system compatibility of a donor system we reported

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earlier,[20] which bears a pyridyl-*tert*-nitrogen-phenol moiety, and is capable of forming stable neutral complexes with $[^{99m}Tc(CO)_3]^+$.[20] The versatility of our approach renders itself useful for further evaluation of these types of systems. Our synthetic results are presented here, along with hexokinase catalysed phosphorylation and GLUT-mediated cell uptake studies.

Results and discussion

Tridentate proligands have been reported to form more stable complexes with the $[^{99m}Tc(CO)_3]^+$ core than bidentate proligands with equivalent donor groups, and they also possess higher stability *in vitro* and better *in vivo* clearance as compared to their bidentate counterparts.[21,22] Combining these findings with our interest in utilizing glucosamine as a biomolecule, we have designed and synthesized a series of monoanionic tridentate proligands conjugated to glucosamine for binding to the tricarbonyl core.

Synthesis

Amide coupling of **1** with *N*,*N*-dibenzylglycine using the water soluble carbodiimide, EDC, afforded the fully protected compound **2** (Scheme 1). Hydrogenolysis of the benzyl groups using hydrogen and palladium hydroxide on carbon as a catalyst with acetic acid as a solvent gave the acetate salt **3**. The ratio of acetic acid in the product differed from batch to batch such that before utilizing a particular batch for the succeeding step, the amount of acetic acid was quantified by ^{1}H NMR spectroscopy. Sufficient base (sodium carbonate) to neutralize this acid was added prior to addition of the appropriate aldehyde. 2-Pyridine carboxaldehyde or 1-methylimidazolecarboxaldehyde was added, and the corresponding imine formed and subsequently reduced to give the secondary amines **4** and **9**. Compound **4** was then used to prepare protected proligands **5** and **7** by reductive amination with salicylaldehyde and alkylation with methylbromoacetate, respectively. Compound **9** was used to prepare protected proligand **10** by reductive amination with salicylaldehyde. Proligands **6** and **11** were prepared in one step each by deacetylation of the acetyl protecting groups of **5** and **10** respectively with sodium methoxide. Proligand **8** was prepared in one step by addition of sodium hydroxide to **7** which deprotected both the methyl and the acetyl groups. These compounds displayed the expected spectroscopic properties, as analysed by infrared, ^{1}H and ^{13}C NMR spectroscopies and mass spectrometry, confirming their assigned structure. The building block approach to making these conjugates is manifested by easy modification of the binding motif donor sets as well as capability to change the length of the spacer between the amide functionality and the binding motif, and lends itself to extension to making more derivatives for SAR studies.

In the course of our study, we were also interested in investigating the effect of different connectivity between the glucosamine and the binding motif on their possible "glucose-like" uptake mechanism. There are amide linkages in proligands **6**, **8**, and **11**, so a less bulky secondary amine was investigated in proligand **19**.

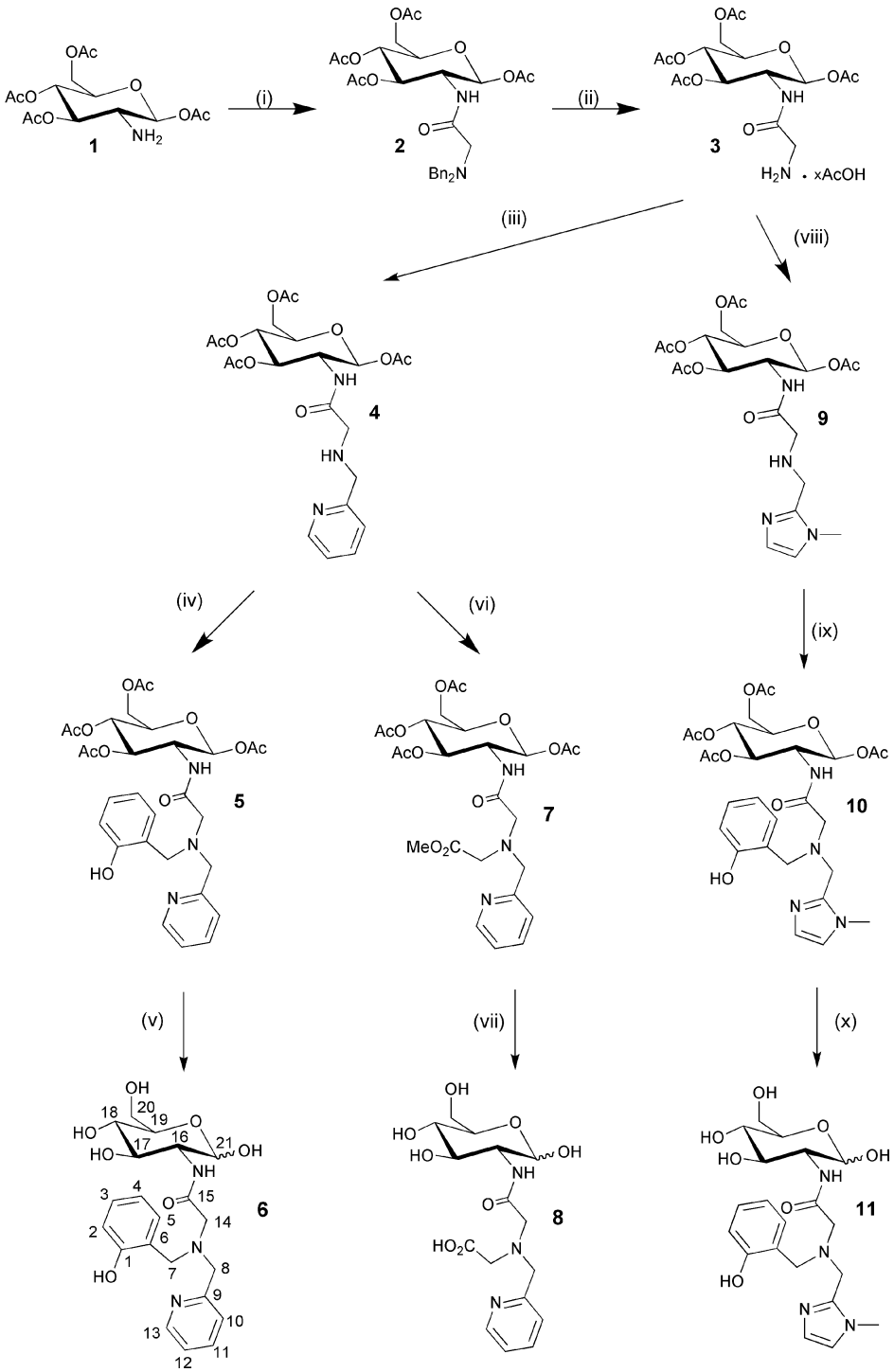
Reductive amination of ethanolamine with salicylaldehyde yielded **13** (Scheme 2). 2-(Dibenzylamino)ethanol **13** was then oxidized to the corresponding aldehyde **14** according to a previously reported procedure.[23] The aldehyde was unstable, so was used immediately following quantification by ^{1}H NMR spectroscopy. Reductive amination of **1** with **14** resulted in the fully protected compound **15**. Selective acetate deprotection with hydrazine acetate yielded the free C1-OH compound **16**. Silyl protection of **16** with TBDMS-Cl resulted in protected compound **17**. The changing of the protecting group at C1 of glucosamine was necessary as initial investigations using the C1-acetyl protected compound resulted in side reactions in the succeeding step and failure to furnish the desired product. Deprotection of the remaining acetyl protected alcohols was achieved by addition of NaOMe, yielding **18** which was further deprotected by H_2 using $Pd(OH)_2/C$ as a catalyst to yield amine derivative **19**. Sequential reductive aminations with 2-pyridinecarboxaldehyde and salicylaldehyde yielded C1 protected proligand **21**. Final silyl deprotection of **21** with benzyltrimethylammonium fluoride afforded proligand **22**.

Reaction of proligands **6**, **8**, **11** and **22** with $[Re(H_2O)_3(CO)_3][Br]$ in refluxing ethanol afforded macroscopic quantities the non-radioactive rhenium surrogate compounds **6-Re(CO)$_3$**, **8-Re(CO)$_3$**, **11-Re(CO)$_3$** and **22-Re(CO)$_3$** (Scheme 3). The "cold" complexes have spectroscopic properties that confirm their expected structures.

As a representative example, **6-Re(CO)$_3$** showed significant shifts in both its ^{1}H and ^{13}C NMR spectra upon metal binding, compared to the spectra of proligand **6** (see Experimental section and ESI†). The ^{1}H NMR shifts due to the carbohydrate resonances were unchanged or showed very small shifts, confirming the pendant nature of the glucosamine moiety in solution. The ^{1}H NMR shifts of the aromatic resonances are indicative of metal binding, with the proton resonances all shifting significantly. In the spectra of **6-Re(CO)$_3$** the shifts of the aromatic protons upon metal binding were: H13 (8.49 to 8.84 ppm), H11 (7.77 to 8.07 ppm), H2 (7.50 to 7.70 ppm), H10/12 (7.13 to 7.53 ppm), H4 (7.28 to 7.35 ppm), and H3/5 (6.79 to 6.99 ppm). The same qualitative trend was also observed for **19-Re(CO)$_3$**, whereas for **8-Re(CO)$_3$** two of the aromatic protons shift upfield and two shift downfield, and for **11-Re(CO)$_3$** all the aromatic protons bar one shift downfield, while the proton on the imidazole ring adjacent to the coordinating nitrogen shifted upfield.

The ^{13}C NMR spectra (ESI†) are indicative of the expected coordination mode as well. The carbon resonances of the sugar regions shift very little, confirming that the glucosamine moiety remains pendant. The ^{13}C NMR spectrum of the ligand and the rhenium complex resulted in two sets of similar, yet distinct, resonances for the carbohydrate and spacer moieties, due to the presence of two anomers. As there is splitting in both the proligands and the complexes, it is likely that the observed splitting is due to the presence of anomers in both cases, rather than from the presence of Δ and Λ metal complex isomers. As the donor set is composed of three distinct donors, the complexes formed can be in either an A-B-C or a C-B-A *fac* arrangement, and although it is probable that both of these isomers form, it seems that they produce the same signals as each other in both the ^{1}H and ^{13}C NMR spectra.

The appearance of multiple carbon signals between 197.5 and 196.8 ppm in the ^{13}C NMR spectrum of **6-Re(CO)$_3$** indicates an arrangement of three carbonyl groups in a *fac* orientation around the metal centre. For the aromatic resonances, C9 and C6 shift upfield (158.0 from 159.8 ppm and 119.8 from 124.6 ppm, respectively) upon binding, while the other nine carbon resonances



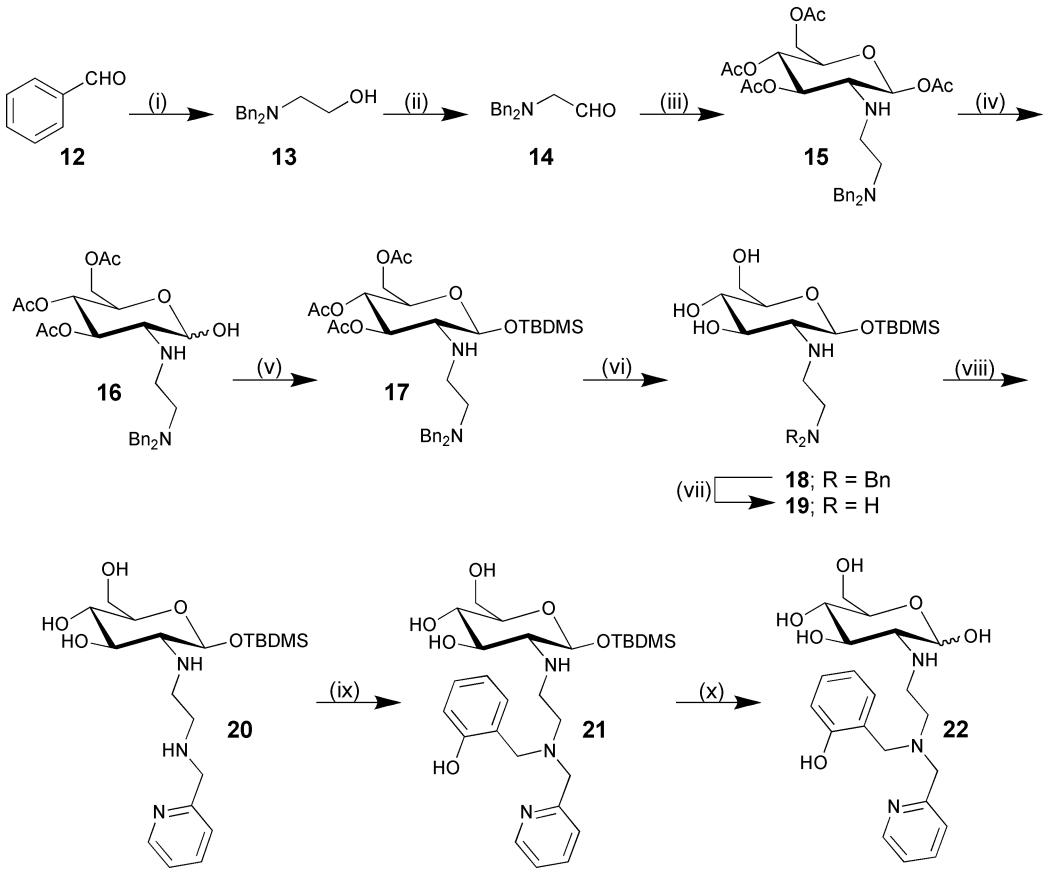
Scheme 1 *Reaction conditions:* (i) 1 equiv dibenzylglycine,[24] 1.1 equiv EDC·HCl, 1.1 equiv HOBT·H_2O, 0.1 equiv DMAP, DMF, 25 °C, 18 h; (ii) $Pd(OH)_2/C$, H_2, AcOH, 25 °C, 18 h; (iii) ~6 equiv Na_2CO_3, 1 equiv 2-pyridine carboxaldehyde, $ClCH_2CH_2Cl$, 25 °C, 18 h, 2.5 equiv $NaBH(OAc)_3$, 25 °C, 4 h; (iv) 3.1 equiv salicylaldehyde, 5.1 equiv $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, 25 °C, 20 h; (v) 5 equiv NaOMe, MeOH, 25 °C, 2 h; (vi) ~6 equiv Na_2CO_3, 2 equiv methylbromoacetate, CH_2Cl_2, 25 °C, 48 h; (vii) 1 M NaOH, 25 °C, 1 h; (viii) ~6 equiv Na_2CO_3, 1-methylimidazole, EtOH, 25 °C, 0.5 h, 2.5 equiv $NaBH_4$, 25 °C, 10 min; (ix) 2 equiv salicylaldehyde, 5.1 equiv $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, 25 °C, 20 h; (x) 5 equiv NaOMe, MeOH, 25 °C, 2 h.

shift downfield upon binding: C15 (174.5 to 182.8 ppm), C1 (158.0 to 160.7 ppm), C13 (149.7 to 154.0 ppm), C11 (139.0 to 142.5 ppm), C5 (132.6 to 134.9 ppm), C3 (130.3 to 132.7 ppm), C10 (124.9 to 127.4 ppm), C12 (124.1 to 125.7 ppm), C4 (120.6 to 121.2 ppm), and C2 (117.1 to 117.3 ppm). Diagnostic shifts occur for the methylene carbon resonances as well: C8 (60.5 to 69.2 ppm), C7 (56.7 to 68.2 ppm), C14 (58.1 to 62.6 ppm), whereas for C20, the resonance remains constant at 63.0 ppm, indicating the pendant nature of the glucosamine moiety, while providing evidence of binding of the tridentate unit. Further, the other

Table 1 Summary of labeling and stability results for ^{99m}Tc complex formation

Ligand	RT (Re compound)[a]	RT (^{99m}Tc compound)[a]	Radiochemical yield	Stability in cysteine–24 h[b]	Stability in histidine–24 h[b]
6	18.7 min	18.8 min	94%	99 ± 2%	98 ± 1%
8	12.9 min	13.2 min	94%	94 ± 2%	85 ± 3%
11	19.6 min	19.4 min	96%	100 ± 4%	94 ± 3%
22	16.2 min	16.3 min	79%	97 ± 3%	92 ± 3%

[a] HPLC conditions: 100% H_2O (with 0.1% TFA) linear gradient to 100% ACN at 30 min. [b] 37 °C.



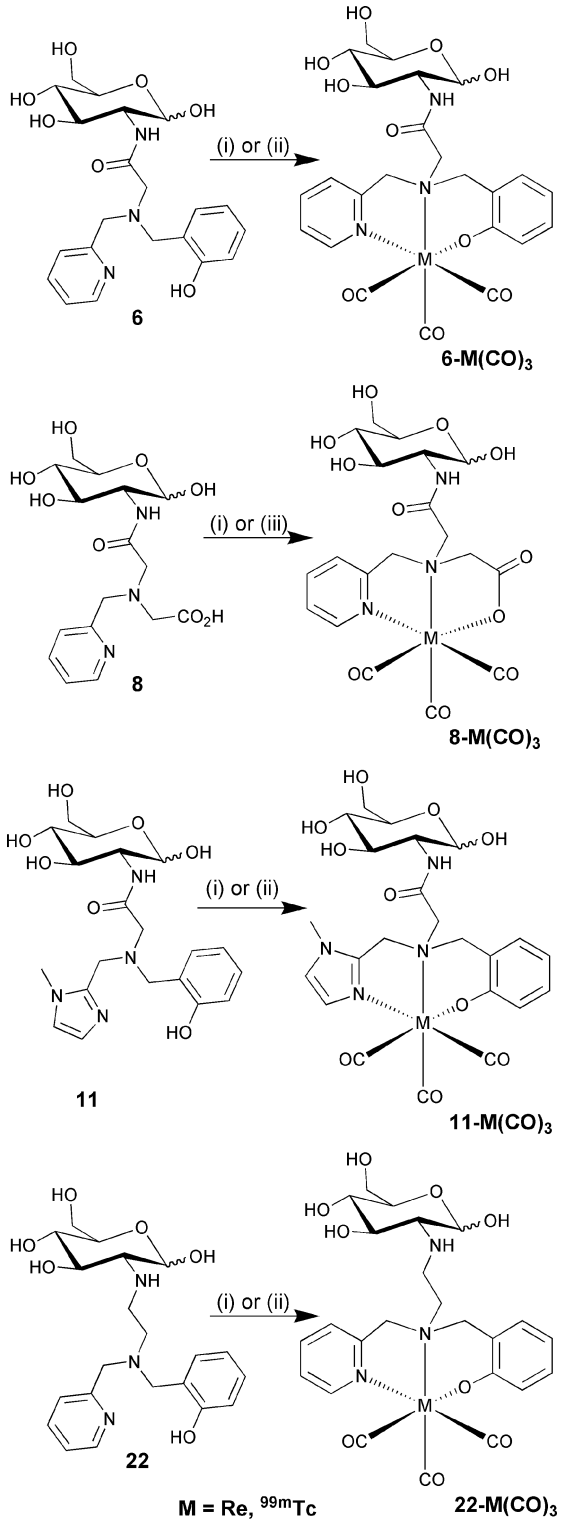
Scheme 2 *Reaction conditions:* (i) 2 equiv salicylaldehyde, $ClCH_2CH_2Cl$, 25 °C, 10 min, 4.3 equiv $NaBH(OAc)_3$, 25 °C, 16 h; (ii) 5.7 equiv Et_3N, 4.9 equiv SO_3-py complex, DMSO, 25 °C, 1 h;[23] (iii) 1.2 equiv **1**, $ClCH_2CH_2Cl$, 25 °C, 0.5 h, 2 equiv $NaBH(OAc)_3$, 25 °C, 16 h; (iv) 1.1 equiv NH_2NH_2-AcOH, DMF, 25 °C, 2.5 h; (v) 6.9 equiv imidazole, 4 equiv TBDMSCl, CH_2Cl_2, 25 °C, 2 h; (vi) 1.3 equiv NaOMe, MeOH, 25 °C, 2.5 h; (vii) $Pd(OH)_2/C$, H_2, MeOH, 25 °C, 24 h; (viii) 10 equiv Na_2CO_3, 1.05 equiv 2-pyridinecarboxaldehyde, MeOH, N_2, 25 °C, 24 h, 2.4 equiv $NaBH_4$, 25 °C, 24 h; (ix) 1 equiv salicylaldehyde, $ClCH_2CH_2Cl$, 25 °C, 1 h, 2 equiv $NaBH(OAc)_3$, 25 °C, 24 h; (x) 1.8 equiv benzyltrimethylammonium fluoride, THF, MeOH, 25 °C, 24 h.

glucosamine resonances C21, C17, C19, C18, and C16 shift little, if at all, upon binding: 92.7 to 91.9 ppm, 73.2 to 73.1 ppm, 73.0 to 72.0 ppm, 72.3 to 71.9 ppm, and 56.0 to 57.0 ppm, respectively. This qualitative trend of resonances near the predicted metal binding atoms shifting a significant amount and the carbohydrate resonances shifting much less so is also observed for **8-Re(CO)$_3$**, **11-Re(CO)$_3$** and **22-Re(CO)$_3$**.

Further evidence for complex formation is provided by IR spectroscopy and mass spectrometry of dilute MeOH solutions. For **6-Re(CO)$_3$**, **8-Re(CO)$_3$**, **11-Re(CO)$_3$** and **22-Re(CO)$_3$**, two carbonyl bands are present at 2032 and 1905 cm^{-1}, 2027 and 1910 cm^{-1}, 2028 and 1884 cm^{-1}, and 2029 and 1906 cm^{-1}, respectively, which are significantly higher than the rhenium precursor carbonyl stretches at 2000 and 1868 cm^{-1}. Rhenium exists as a mixture of isotopes, ^{185}Re and ^{187}Re (37:63) and thus gives rise to diagnostic isotope peak patterns in the mass spectra. ESI-MS+ of **6-Re(CO)$_3$**, showed *m/z* values for $[MH]^+$ of 702 ($M^{185}ReH^+$), 704 ($M^{187}ReH^+$), **8-Re(CO)$_3$** showed *m/z* values for $[MH]^+$ of 676 ($M^{185}ReH^+$), 678 ($M^{187}ReH^+$), **11-Re(CO)$_3$** showed *m/z* values for $[MH]^+$ of 705 ($M^{185}ReH^+$), 707 ($M^{187}ReH^+$) while **22-Re(CO)$_3$** showed *m/z* values for $[MH]^+$ 688 ($M^{185}ReH^+$), 690 ($M^{187}ReH^+$).

Labeling and stability

The ^{99m}Tc complexes formed in high radiochemical yields after reacting for 30 min (Table 1). The phenolate-based ligands required a base to give these short reaction times, whereas the acid-based ligand coordinated readily in PBS buffer at pH 7.4.



Scheme 3 *Reaction conditions:* (i) $[Re(H_2O)_3(CO)_3][Br]$, EtOH, Δ, 6–24 h; (ii) $[^{99m}Tc(H_2O)_3(CO)_3]^+$, 3 equiv NaOEt, EtOH, 0.5 h, 80 °C; (iii) $[^{99m}Tc(H_2O)_3(CO)_3]^+$, PBS, 0.5 h, 80 °C.

The phenolate ligands did coordinate to the metal without base, but not on a radiochemistry-appropriate timeframe.

The ^{99m}Tc complexes were analyzed using HPLC performed with coinjection of the thoroughly characterized analogous rhenium complex. Agreement of their retention times (RTs) supports the expected outcome of the technetium analogue having the same chemical structure as the rhenium complex. There is a slight difference between the retention times of the rhenium and technetium complexes because the UV (rhenium) and radiation (^{99m}Tc) detectors are connected in series. Percentage radiochemical yield was calculated by integration of all the peaks that appear in the radiation trace. The other major species seen in these HPLC traces was the unreacted starting material $[^{99m}Tc(H_2O)_3(CO)_3]^+$ (RT = 12 min).

All ^{99m}Tc complexes were tested for stability in excess concentrations of cysteine and histidine for 24 h at 37 °C (Table 1). Cysteine and histidine are strong, potentially tridentate ligands for the tricarbonyl core, and are found ubiquitously *in vivo*. There was no major degradation product seen, but rather several small peaks slowly grew in, suggesting the presence of several minor species. The compounds in this work all exhibit satisfactory stability to justify further investigation as molecular imaging agents, as they have been shown to be stable enough under relevant conditions. The phenolates showed greater stability under these challenge conditions than did the equivalent acid-based ligand, showing one potential benefit of using this ligating group.

A recent study found that a $[Tc(CO)_3]^+$ complex with two phenolate donors was not as stable to similar challenge conditions as were ligands made up of other binding group combinations.[25] It may be that the phenolate is best used in conjunction with two nitrogenous donors, as this provides a neutral binding sphere as well as a large nitrogen content, known to be for optimal $[Tc(CO)_3]^+$ binding.[26]

As the pK_a of the phenol is around 10, the reaction mixture needs to be made basic to enable sufficient ligand deprotonation for complex formation in a timely manner. In our systems this proceeded well and with no sign of degradation products, but these conditions may limit its general applicability. This potential drawback may be outweighed, however, by the increased lipophilicity of a phenol compared to an acid group, which in certain cases may be able to improve the biodistribution and solubilities of the resulting compound. As these biological properties can be very sensitive to small changes, this is a useful addition to the expanding knowledge on the coordination chemistry of technetium.

GLUT-1 cell uptake assay

Cell uptake of several ^{99m}Tc complexes was examined in LCC6-HER2 cells—a human breast cancer cell line with high surface concentrations of GLUT-1 glucose transporters.[27] Fig. 1 shows the results of this assay. The cell uptake of each of the ^{99m}Tc compounds examined was independent of glucose, meaning that the uptake was not due to transport *via* the GLUT-1 transporters. The percentage uptake for each of the compounds is quite low. For comparison, FDG, the positive control, showed uptake of around 3.4% of the total activity when in a glucose free environment, and 0.1% when in 5 mM glucose (results not shown). The trend of lipophilic compounds having higher uptake, suggests that passive diffusion or membrane association was responsible for the observed results. There was no significant difference between the uptake of the amine-linked and amide-linked compounds.

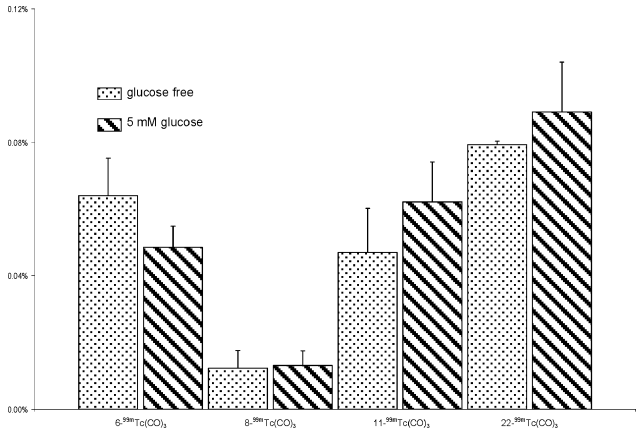


Fig. 1 Results of cell uptake studies – each compound's uptake was examined in a glucose free environment and in 5 mM glucose solution (average % of total activity in the cells after a 30 min incubation for four independent experiments are shown – error bars are standard deviations).

Hexokinase assay

Hexokinase catalyses the phosphorylation of glucose in the C-6 position, and is also known to phosphorylate certain glucose analogues.[3,17,19] We assessed whether our compounds were phosphorylated by hexokinase, an important factor when looking for a glucose-based imaging agent, as without phosphorylation to trap the compound, it can diffuse out of a cell just as quickly as it diffused in. Unfortunately none of the compounds tested were found to be phosphorylated by hexokinase, and therefore no differences could be identified between activities of the different compounds. This lack of activity may be because the metal-binding portions of the molecules are too bulky to allow the cleft of hexokinase to narrow as is required for phosphorylation to occur.

Conclusions

To our knowledge we were the first group to use the phenolate-based ligand system for the tricarbonyl core,[20,28] though others have shown interest in this donor system more recently.[25] The phenolate was investigated here as an anionic binding group to provide neutral complexes when bound to the monocationic tricarbonyl core. It has been demonstrated again here that the phenolate group is a suitable donor for the tricarbonyl core, as it binds to both the technetium and rhenium centers within a radiochemistry-appropriate timeframe and gives complexes that are stable to cysteine and histidine challenges. It is interesting to note that the phenolates are more stable in our hands under these physiologically relevant conditions than the equivalent acid compounds.

The assays performed on the compounds in this work, whether based on phenolates or other ligand types, showed the compounds not to be as biologically active as we had initially hoped. These compounds are likely too different from glucose for the enzymes to recognize and process them as they would their native substrates. In this case, that perturbation is too large to allow for a functional imaging agent to be formed. Work on compounds with longer linkers and different binding groups that is ongoing in our lab may circumvent these issues.

Experimental

Instruments and materials

All solvents and reagents were used as received from Sigma-Aldrich or Acros unless otherwise noted. $Re(CO)_5Br$ is commercially available (STREM). $[Re(H_2O)_3(CO)_3]Br$,[29] dibenzylglycine,[24] 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine **1**,[30] were prepared as described previously.

The analytical TLC plates were aluminium backed ultra pure silica gel 60, 250 μm, the glass backed preparative TLC plates 1000 μm, and the flash column silica gel (standard grade, 60 Å, 32-63 mm) used were all provided by Silicycle. ^1H and ^{13}C NMR, ^{13}C APT, ^1H-^1H COSY and ^1H-^{13}C HMQC spectra were recorded on a Bruker AV300, AV400 or DRX400 spectrometer. The NMR spectra are expressed on the δ scale and referenced to residual solvent peaks. Infrared spectra were recorded between 400 and 4000 cm^{-1} in transmission mode on a Nicolet 4700 FT-IR spectrophotometer as KBr discs at a resolution of ±4 cm^{-1} or on a Nicolet 6700 FT-IR spectrophotometer at a resolution of ± 0.09 cm^{-1}. Melting points were measured using a Mel-Temp by Laboratory Devices, Cambridge, Massachusetts. ESI mass spectra were recorded on a Micromass LCT instrument at the University of British Columbia, Department of Chemistry. High resolution mass spectra (Micromass LCT TOF-MS)) and elemental analysis (Carlo Erba analytical instrument) were provided by the Analytical Services Facility in the Department of Chemistry. HPLC analyses of cold compounds were done on a Phenomenex Synergi 4 μm C18 Hydro-RP column (4.6 × 250 mm) using a Waters WE 600 HPLC system equipped with a 2478 dual wavelength absorbance UV detector run using an Empower software package. HPLC analyses of radiolabeled complexes were performed on a Knauer Wellchrom K-1001 HPLC equipped with a K-2501 absorption detector and a Capintec radiometric well counter. A Phenomenex Synergi 4 μm C-18 Hydro-RP analytical column with dimensions of 4.6 × 250 mm was used. The identities of the novel compounds were confirmed by ^1H and ^{13}C NMR spectroscopy, high resolution mass spectrometry and infrared spectroscopy.

Synthesis

2-((Bis(phenylmethyl)amino)acetamido)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranose, (2). *N*,*N*-Dibenzylglycine[24] (5.00 g, 0.0196 mol) was dissolved in hot DMF (125 mL). The resulting clear colorless solution was cooled in an ice bath. EDC·HCl (*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride) (4.14 g, 0.0216 mol), $HOBt·H_2O$ (1-hydroxybenzotriazole hydrate) (2.92 g, 0.0216 mol), and DMAP (4-dimethylaminopyridine) (0.239 g, 0.00196 mol) were added sequentially to the DMF solution. The reaction mixture was stirred at 0 °C for 30 minutes and to this solution was added 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine **1**[30] (6.81 g, 0.0177 mol). The ice-bath was removed and the clear colorless solution was stirred at room temperature. After 18 hours, the solvent was removed under reduced pressure, leaving behind a pale white residue. The residue was dissolved in CH_2Cl_2 (125 mL), washed with saturated aqueous Na_2CO_3 solution (3 × 40 mL), 1M HCl (3 × 40 mL) and then brine (2 × 30 mL). The organic layer was dried over anhydrous $MgSO_4$, and evaporated to dryness under reduced pressure. Column chromatography (silica-CH_2Cl_2/5% MeOH) was used to isolate **2**

as a white solid (9.25 g, 80% yield). $R_f = 0.56$ (silica-CH_2Cl_2/5% MeOH:); ^{1}H NMR (DMSO-d_6, 400 MHz, 25 °C, δ): 7.97 (d, J = 9.6 Hz, 1H), 7.37-7.21 (m, 10H), 5.99 (d, J = 8.4 Hz, 1H), 5.50 (t, J = 9.6 Hz, 1H), 4.91 (t, J = 10.0 Hz, 1H), 4.21 (dd, J = 12.4, 4.4 Hz, 1H), 4.10-3.97 (m, 3H), 3.53 (s, 4H), 2.93 (d, J = 16.0 Hz, 1H), 2.83 (d, J = 15.6 Hz, 1H), 2.01 (s, 3H), 1.98 (s, 3H), 1.82 (s, 3H), 1.78 (s, 3H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz, 25 °C, δ): 170.2, 170.0, 169.4, 169.3, 168.6, 137.7, 128.8, 128.2, 127.1, 91.7, 72.0, 71.5, 68.3, 61.5, 57.2, 55.8, 51.7, 20.5, 20.4, 20.3, 20.2 ppm; IR (KBr) ν_{max} (cm^{-1}): 1742 (s), 1663 (m), 1514 (m), 1371 (w), 1261 (m), 1225 (s), 1079 (m), 1039 (m), 740 (w); MS (ES+, 100% MeOH, 30V): m/z = 607 (MNa^+); HR-MS (ES+ of MNa^+) m/z calcd for $C_{30}H_{36}N_2O_{10}Na$: 607.2268, found 607.2267; Anal. Calcd. for $C_{30}H_{36}N_2O_{10}$: C, 61.63; H, 6.21; N, 4.79. Found: C, 61.90; H, 6.59; N, 5.11.

2-(2-Aminoacetamido)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl acetate, (3). Compound **2** (7.64 g, 0.0131 mol) was dissolved in glacial acetic acid (100 mL). $Pd(OH)_2$/C (0.800 g, 0.0057 mmol) was added in one portion, the flask was capped with a rubber septum and purged with H_2 from a balloon. The mixture was kept under a positive pressure of H_2 at room temperature for 18 h. The suspension was then filtered through a pad of celite and the solvent was removed under reduced pressure to yield yellow oil **3** (quantitative). $R_f = 0.05$ (silica-CH_2Cl_2/5% MeOH:); ^{1}H NMR (DMSO-d_6, 400 MHz, 25 °C, δ): 5.76 (d, J = 8.8 Hz, 1H), 5.22 (t, J = 10.0 Hz, 1H), 4.90 (t, J = 10.0 Hz, 1H), 4.19 (dd, J = 12.4 and 4.4 Hz, 1H), 4.06-3.97 (m, 3H), 3.39 (s, 2H), 2.04 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.92 (s, 3H), 1.87 (s, 30H, Acetic acid) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz, 25 °C, δ) 172.6, 170.2, 169.9, 169.8, 169.4, 169.0, 91.7, 72.1, 71.6, 68.2, 61.6, 52.2, 41.1, 21.5, 20.6, 20.6, 20.5, 20.4 ppm. MS (ES^+, 100% CH_3CN, 30 V): m/z = 427 (M+Na^+, 100%); HR-MS (ES^- of M^-) m/z calcd for $C_{16}H_{23}N_2O_{10}$ 403.1353, found 403.1354. *Note:* The IR spectrum was not obtained due to decomposition and EA was not obtained due to varying amounts of acetic acid present in the oily sample.

2-(((*N*-Pyridin-2-ylmethyl)amino)acetamido)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranose, (4). Compound **3** (6.0 g) was dissolved in $ClCH_2CH_2Cl$ (100 mL). Na_2CO_3 (34.2 g, 0.323 mol) and an equimolar amount of 2-pyridinecarboxaldehyde were added sequentially. The cloudy red solution was stirred for 18 h at room temperature. $NaBH(OAc)_3$ (6.85 g, 0.0323 mol) was then added in one portion and the resulting reaction mixture further stirred for an additional 4 h. The reaction was quenched by addition of saturated aqueous Na_2CO_3 solution (50 mL). The mixture was then partitioned and the aqueous layer further extracted with CH_2Cl_2 (2 × 25 mL). The combined organic extract was washed with brine (2 × 25 mL), then dried over anhydrous $MgSO_4$. After filtration of the drying agent, the filtrate was taken to dryness by rotary evaporation to yield crude **4**. Column chromatography (silica-CH_2Cl_2/5% MeOH) was used to isolate and purify **4** as a pale yellow solid (2.19 g, 43% yield). $R_f = 0.51$ (silica-CH_2Cl_2/10% MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz, 25 °C, δ): 8.49 (d, J = 7.8 Hz, 1H), 8.09 (d, J = 9.7 Hz, 1H), 7.75 (t, J = 7.5 Hz, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.25 (t, J = 5.8 Hz, 1H), 5.89 (d, J = 8.8 Hz, 1H), 5.37 (t, J = 9.6 Hz, 1H), 4.90 (t, J = 9.6 Hz, 1H), 4.19 (dd, J = 12.4 and 4.4 Hz, 1H), 4.08-3.94 (m, 3H), 3.65 (s, 2H), 3.09 (s, 2H), 2.03 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.92 (s, 3H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz, 25 °C, δ): 171.4, 170.1, 169.7, 169.3, 168.9, 159.4, 148.8, 136.5, 122.1, 121.9, 91.9, 72.2, 71.5, 68.2, 61.5, 53.9, 51.7, 51.5, 20.6, 20.5, 20.4, 20.4 ppm; IR (KBr) ν_{max} (cm^{-1}): 1747 (s), 1668 (m), 1522 (m), 1371 (w), 1229 (s), 1074 (m), 1041 (m), 598 (w); MS (ES+, 100% MeOH, 30 V): m/z = 496 (MH^+); HR-MS (ES+ of MH^+) m/z calcd for $C_{22}H_{30}N_3O_{10}$: 496.1931, found: 496.1930; Anal. Calcd for $C_{22}H_{29}N_3O_{10}$: C, 53.33; H, 5.90; N, 8.48. Found: C, 53.41; H, 6.20; N, 8.65.

2-((*N*-(2-Hydroxybenzyl)-*N*-(pyridin-2-ylmethyl)amino)acetamido)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranose, (5). Compound **4** (700 mg, 1.4 mmol) was dissolved in $ClCH_2CH_2Cl$ (15 mL). Salicylaldehyde (470 μL, 4.4 mmol) and $NaBH(OAc)_3$ (1.5 g, 7.2 mmol) were added sequentially and the reaction mixture was stirred at room temperature for 20 h. The reaction was quenched by addition of saturated aqueous Na_2CO_3 solution (10 mL). The mixture was then partitioned and the aqueous layer further extracted with CH_2Cl_2 (2 × 8 mL). The combined organic extract was washed with brine (2 × 10 mL), then dried over anhydrous $MgSO_4$. After filtration of the drying agent, the filtrate was taken to dryness by rotary evaporation to yield crude **5**. Column chromatography (silica-CH_2Cl_2/5% MeOH) was used to isolate and purify **5** as a yellow solid (0.670 g, 79% yield); $R_f = 0.74$ (silica-CH_2Cl_2/10% MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz, 25 °C, δ): 9.95 (s, 1H), 8.50 (d, J = 2.4 Hz, 2H), 7.72 (td, J = 7.8 and 1.8 Hz, 1H), 7.35 (d, J = 7.6 Hz, 1H), 7.27 (t, J = 6.6 Hz, 1H), 7.16-7.09 (m, 2H), 6.81-6.73 (m, 2H), 5.86 (d, J = 8.8 Hz, 1H), 5.35 (t, J = 9.6 Hz, 1H), 4.92 (t, J = 9.8 Hz, 1H), 4.21 (dd, J = 12.4 and 4.6 Hz, 1H), 4.09-4.00 (m, 3H), 3.66 (s, 2H), 3.59 (s, 2H), 3.09-2.98 (m, 2H), 2.01 (s, 3H), 1.98 (s, 3H), 1.92 (s, 3H), 1.83 (s, 3H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz, 25 °C, δ): 170.5, 170.0, 169.5, 169.3, 168.7, 158.2, 156.2, 148.8, 136.7, 130.8, 128.6, 123.1, 122.5, 122.3, 118.8, 115.4, 91.6, 72.0, 71.5, 68.1, 61.5, 58.2, 56.0, 53.7, 51.8, 20.5, 20.4, 20.4, 20.2 ppm; IR (KBr) ν_{max} (cm^{-1}): 3314 (m), 1754 (s), 1685 (m), 1522 (w), 1368 (m), 1221 (s), 1078 (m), 1040 (m), 760 (m); MS (ES+, 100% MeOH, 30 V): m/z = 602 (MH^+); HR-MS (ES+ of MH^+) m/z calcd for $C_{29}H_{36}N_3O_{11}$: 602.2350, found: 602.2348; Anal. Calcd. for $C_{29}H_{35}N_3O_{11}$: C, 57.90; H, 5.86; N, 6.98. Found: C, 58.04; H, 6.28; N, 7.29.

2-((*N*-(2-Hydroxybenzyl)-*N*-(pyridin-2-ylmethyl)amino)acetamido)-2-deoxy-*D*-glucopyranose, (6). Compound **5** (670 mg, 1.11 mmol) was dissolved in MeOH (5 mL) and NaOMe (310 mg, 5.7 mmol) was added in one portion. After stirring for 2 h at room temperature, the solvent was removed under reduced pressure to yield a pale white solid. Compound **6** (300 mg, 62% yield) was isolated and purified using column chromatography (silica-CH_2Cl_2/20% MeOH): $R_f = 0.34$ (silica-CH_2Cl_2/20% MeOH); ^{1}H NMR (MeOH-d_4, 400 MHz, 25 °C, δ): 8.55-8.45 (m, 1H), 7.81-7.75 (m, 1H), 7.51-7.49 (m, 1H), 7.30-7.27 (m, 1H), 7.15-7.11 (m, 2H), 6.83-6.76 (m, 2H), 5.06 (d, J = 3.5 Hz, 1H-β), 4.64 (d, 8.4 Hz, 1H-α), 3.90-3.62 (m, 8H), 3.35-3.27 (m, 2H), 3.23 (s, 2H) ppm. ^{13}C NMR (MeOH-d_4, 100 MHz, 25 °C, δ): 174.5, 159.7, 158.0, 149.6, 139.0, 132.3, 130.3, 124.9, 124.5, 124.0, 120.5, 117.1, 97.1 (α), 92.7 (β), 73.2, 73.0, 72.6, 63.0, 60.2, 58.0, 56.7, 55.8 ppm; IR (KBr) ν_{max} (cm^{-1}): 3386 (br, s), 1655 (s), 1597 (w), 1541 (w), 1489 (w), 1247 (m), 1104 (w), 1039 (m), 758 (s); MS (ES+, 100% MeOH, 30 V): m/z 434 (MH^+); HR-MS (ES+ of MH^+): m/z calcd for $C_{21}H_{28}N_3O_7$:

434.1927, found: 434.1925; Anal. Calcd. for $C_{21}H_{27}N_3O_7{\cdot}(H_2O)_{0.5}$: C, 56.77; H, 6.40; N, 9.46. Found: C, 56.52; H, 6.23; N, 9.21.

2-((*N*-(Pyridin-2-ylmethyl)-*N*-(methoxyethanoic acid)amino)-acetamido)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranose, (7). 2-((*N*-(Pyridin-2-ylmethyl)amino)acetamido)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranose (**4**) (0.950 g, 1.92 mmol) was dissolved in dry dichloromethane (15 mL). Na_2CO_3 (0.406 g, 3.84 mmol) and methyl bromoacetate (0.241 mL, 2.88 mmol) were added. The flask was evacuated and filled with argon, and the reaction mixture stirred at room temperature for 48 h. Water was added, the two layers were separated, and the organic layer was washed again with water, and once with brine, before being dried over $MgSO_4$. The drying agent was filtered off; the filtrate was evaporated on a rotary evaporator, and then purified by column chromatography on silica gel with 5% methanol in dichloromethane as eluent. The solvents were removed *in vacuo* to give an off white solid (0.790 g, 72% yield). R_f = 0.47 (silica-CH_2Cl_2/10% MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C, δ): 8.98 (d, J = 9.4 Hz, 1H), 8.60 (d, J = 4.5 Hz, 1H), 7.67 (ddd, J = 7.7 Hz, 7.6 Hz, 1.7 Hz, 1H), 7.23 (m, 2H), 5.84 (d, J = 8.8 Hz, 1H), 5.31 (dd, J = 10.2 Hz, 9.5 Hz, 1H), 5.13 (dd, J = 9.6 Hz, 9.8 Hz, 1H), 4.30 (m, 2H), 4.13 (dd, J = 2.0 Hz, 12.4 Hz, 1H), 3.88 (m, 3H), 3.71 (s, 3H), 3.40 (d, J = 3.1 Hz, 2H), 3.31 (s, 2H), 2.09, 2.02, 1.99, 1.94 (s, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C, δ): 171.90, 171.87, 170.89, 170.55, 169.59, 169.34, 157.77, 149.87, 137.08, 123.34, 123.00, 92.62, 73.12, 72.93, 68.43, 61.91, 59.60, 58.64, 54.76, 52.76, 51.99, 20.96, 20.93, 20.80, 20.78. IR ν_{max} (cm^{-1}): 3321 (w), 2958 (w), 1744 (s), 1663 (m), 1508 (m), 1390 (m), 1218 (s), 1038 (m). HR-MS (ES+ of MNa^+): *m/z* calcd for $C_{25}H_{33}N_3O_{12}Na$: 590.1962, found: 590.1948.

2-((*N*-(Pyridin-2-ylmethyl)-*N*-(ethanoic acid)amino)acetamido)-2-deoxy-*D*-glucopyranose, (8). 2-((*N*-(Pyridin-2-ylmethyl)-*N*-(methoxyethanoic acid) amino) acetamido)-1,3,4,6-tetraacetyl-2-deoxy-*D*-glucopyranose (**7**) (40 mg, 0.071 mmol) was suspended in 1 M NaOH (2 mL) and stirred vigorously. After about 5 min the solution became homogenous and slightly yellow. The reaction was complete according to ESI-MS after about 60 min. Amberlite CG-50 ion exchange resin was added and the resulting slurry stirred vigorously for 15 min, before the resin was filtered off. The aqueous filtrate was reduced to a yellow solid on the rotary evaporator before being taken up in a minimum amount of methanol and filtered to remove most of the NaOH. The methanolic solution was then purified by reverse phase HPLC (isocratic, H_2O with 0.1% TFA), and the solvents removed *in vacuo* to give a pale orange oil (15 mg, 55% yield). R_f = 0.33 (silica-MeOH). ^{1}H NMR (MeOH-d_4, 400 MHz, 25 °C, δ): 8.79 (s, 1H), 8.41 (dd, J = 7.4 Hz, 7.0 Hz, 1H), 7.93 (m, 1H), 7.86 (m, 1H), 5.10 (d, J = 3.1 Hz, 0.75H - α), 4.66 (d, J = 8.2 Hz, 0.25H - β), 4.41 (d, J = 4.7 Hz, 2H), 4.30, 4.02, 3.66–3.90, 3.50–3.64, 3.34–3.42 (m). ^{13}C NMR (MeOH-d_4, 100 MHz, 25 °C, δ): 173.79, 172.72, 162.63, 162.29, 156.53, 145.78, 145.66, 143.35, 126.71, 126.22, 96.40, 94.27, 92.10, 77.58, 75.38, 73.16, 72.76, 72.26, 71.95, 71.71, 68.21, 62.28, 61.88, 58.31, 58.23, 55.48, 57.31, 57.17. IR ν_{max} (cm^{-1}): 3272 (s, br), 2918 (m), 1667 (s, br), 1538 (m), 1417 (w), 1188 (s), 1031 (s). Purity assessed by HPLC: 96.5%. HR-MS (ES+ of MNa^+): *m/z* calcd for $C_{16}H_{23}N_3O_8Na$: 408.1383, found: 408.1388.

2-((*N*-(1-Methylimidazol-2-ylmethyl)amino)acetamido)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranose, (9). 2-(2-Amino-acetamido)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranose (**3**) (6.52 mmol) was dissolved in dry ethanol (20 mL), and an appropriate amount of sodium carbonate was added to neutralise the associated acetic acid, as determined by ^{1}H NMR spectroscopy of the starting material (4.60 g, 43.4 mmol). After ten minutes stirring to ensure all the acid was quenched, 1-methylimidazole-2-carboxaldehyde (0.770 g, 6.99 mmol) was added to the reaction. A nearly immediate colour change to green was observed, and within ten minutes the reaction was complete, as tested by TLC. Sodium borohydride (0.780 g, 21.1 mmol) was added and the reaction mixture stirred for ten minutes longer, as the green colour faded. The reaction mixture was quenched with water (30 mL), which also dissolved the excess sodium carbonate. The solution was extracted three times with dichloromethane (3 × 30 mL), the organic fractions were combined and washed once with water (30 mL), once with brine (30 mL), then dried over magnesium sulfate before being filtered and reduced to an oil on the rotary evaporator. This oil was purified using column chromatography (5% methanol in dichloromethane) and the solvents evaporated *in vacuo* to give an off white solid (0.81 g, 25% yield). R_f = 0.40 (silica-CH_2Cl_2/10% MeOH). ^{1}H NMR (MeOH-d_4, 400 MHz, 25 °C, δ): 7.67 (d, J = 9.0 Hz, 1H),6.95 (s, 2H), 6.84 (s, 2H), 5.80 (d, J = 8.7 Hz, 1H), 5.24 (dd, J = 9.4 Hz, 10.2 Hz, 1H), 5.13 (dd, J = 9.4 Hz, 9.7 Hz, 1H), 4.27 (m, 2H), 4.13 (dd, J = 2.2 Hz, 12.4 Hz, 1H), 3.83 (ddd, J = 9.8 Hz, 2.2 Hz, 4.7 Hz, 1H), 3.77 (d, J = 6.4 Hz, 2H, 3.63 (s, 3H), 3.29 (d, J = 4.6 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C, δ): 171.76, 170.66, 169.32, 144.21, 127.29, 121.31, 92.46, 72.81, 72.70, 67.89, 61.66, 52.73, 52.12, 44.98, 32.46, 20.90, 20.69, 20.56. IR ν_{max} (cm^{-1}): 3324 (w), 2953 (w), 2360 (m), 2341 (m), 1743 (s), 1673 (m), 1519 (m), 1367 (m), 1214 (s), 1034 (s), 728 (m). HR-MS (ES+ of MNa^+): *m/z* calcd for $C_{21}H_{30}N_4O_{10}Na$: 521.1860, found: 521.1863.

2-((*N*-(1-Methylimidazol-2-ylmethyl)-*N*-(2-hydroxybenzyl)-amino)acetamido)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-*D*-glucopy-ranose, (10). 2-((*N*-(1-Methylimidazol-2-ylmethyl)amino)acet-amido)-2-deoxy-*D*-glucopyranose (9) (0.800 g, 1.62 mmol) was dissolved in 1,2-dichloroethane (15 mL) and sodium carbonate (0.343 g, 3.24 mmol) was added. The reaction flask was purged for ten minutes with argon, salicylaldehyde (337 μL, 3.21 mmol) was added, and the reaction mixture stirred at room temperature for 30 min. Sodium triacetoxyborohydride (1.02 g, 4.83 mmol) was added and the reaction mixture stirred for 24 h. Upon completion of the reaction, as determined from loss of starting materials by TLC, the solvent was removed by rotary evaporator and the off white solid partitioned between dichloromethane and water (20 mL each). The layers were separated and the aqueous layer washed twice with dichloromethane (20 mL), the two fractions of which were then combined. These were dried with brine (30 mL) then $MgSO_4$, filtered and reduced in volume on the rotary evaporator. The yellow solution was purified by column chromatography on silica gel using 4% methanol in dichloromethane as an eluent, and the solvent was removed *in vacuo* to give an off white solid (0.626 g, 64% yield). R_f = 0.55 (silica-CH_2Cl_2/10% MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C, δ): 7.97 (d, J = 9.0 Hz, 1H), 7.14 (ddd, J = 7.2 Hz, 8.2 Hz,

1.5 Hz, 1H), 7.07 (d, $J = 1.1$ Hz, 1H), 6.96 (dd, $J = 7.4$ Hz, 1.3 Hz, 1H), 6.87 (s, 1H), 6.85 (s, 1H), 6.71 (dd, $J = 7.4$ Hz, 7.3 Hz, 1H), 5.72 (d, $J = 8.4$ Hz, 1H), 5.10 (dd, $J = 9.6$ Hz, 9.9 Hz, 1H), 5.00 (dd, $J = 9.8$ Hz, 9.4 Hz, 1H), 4.23 (dd, $J = 12.4$ Hz, 4.5 Hz, 1H), 4.02 (dd, $J = 2.1$ Hz, 12.6 Hz, 1H), 3.96 (dd, $J = 9.4$ Hz, 9.6 Hz, 1H), 3.84 (d, $J = 13.1$ Hz, 1H), 3.78 (ddd, $J = 4.5$ Hz, 2.0 Hz, 9.9 Hz, 1H), 3.67 (dd, $J = 15.6$ Hz, 14.1 Hz, 2H), 3.62 (d, $J = 13.1$ Hz, 1H), 3.52 (s, 3H), 3.18 (dd, $J = 16.7$ Hz, 10.6 Hz, 2H), 2.03, 1.94, 1.85, 1.76 (s, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C, δ): 171.71, 170.85, 170.26, 169.55, 169.04, 157.42, 145.28, 131.14, 129.88, 125.93, 122.35, 121.82, 119.06, 117.85, 92.04, 72.84, 72.56, 68.27, 61.85, 56.57, 56.49, 53.24, 50.27, 32.70, 20.95, 20.87, 20.76, 20.68. IR ν_{max} (cm^{-1}): 3274 (w), 1744 (s), 1674 (m), 1388 (m), 1211 (s), 1072 (m), 1035 (s), 760 (s). HR-MS (ES+ of MNa^+): *m/z* calcd for $C_{28}H_{36}N_4O_{11}Na$: 627.2278, found: 627.2288.

2-((*N*-(1-Methylimidazol-2-ylmethyl)-*N*-(2-hydroxybenzyl)-amino)acetamido)-2-deoxy-*D*-glucopyranose, (11). 2-((*N*-(1-Methylimidazol-2-ylmethyl)-*N*-(2-hydroxybenzyl) amino) acetamido)-2-deoxy-1,3,4,6-tetraacetyl-*D*-glucopyranose (**10**) (0.225 g, 0.372 mmol) was dissolved in methanol (10 mL). An excess of NaOMe (0.110 g, 20.4 mmol) was added and the reaction mixture stirred at room temperature for two hours. Amberlite CG-50 ion exchange resin was added, and after ten minutes of rapid stirring, was removed by filtration. The filtrate was reduced in volume on a rotary evaporator, and the resulting yellow-orange oil purified by reverse phase HPLC (H_2O with 0.1% TFA to 100% ACN over 30 min). The solvents were removed *in vacuo* to give a pale yellow oil (0.040 g, 25% yield). $R_f = 0.24$ (silica-CH_2Cl_2/20% MeOH). ^{1}H NMR (MeOD, 400 MHz, 25 °C, δ): 7.28–7.32 (m, 2H), 7.18–7.20 (m, 1H), 7.09–7.14 (m, 1H), 6.76–6.82 (m, 2H), 5.09 (d, $J = 3.4$ Hz, 0.63H - α), 4.67 (d, $J = 8.3$ Hz, 0.37H - β), 4.00–4.10 (m, 2H), 3.79–3.95 (m, 4H) 3.74 (s, 3H), 3.72 (s, 2H), 3.44 (s, 2H)3.35–3.43 (m, 2H). ^{13}C NMR (MeOD, 100 MHz, 25 °C, δ): 173.64, 173.24, 157.74, 157.59, 146.42, 133.31, 133.15, 131.20, 131.12, 125.48, 123.97, 121.25, 121.09, 119.65, 116.92, 97.23, 92.98, 78.49, 76.31, 73.54, 73.01, 72.93, 72.65, 63.20, 63.12, 59.96, 59.89, 58.88, 56.00, 57.61, 57.55, 49.50, 35.26, 35.18. IR ν_{max} (cm^{-1}): 3293 (s, br), 2942 (m, br), 1671 (s), 1609 (w), 1531 (m), 1459 (m), 1210 (s), 1133 (s), 757 (m). Purity assessed by HPLC: 97.8%. HR-MS (ES+ of MNa^+) *m/z* calcd for $C_{20}H_{28}N_4O_7Na$: 459.1856, found 459.1848.

2-(Dibenzylamino)ethanol, (13). To a solution of ethanolamine (**12**) (1.007 g, 16.49 mmol) in $ClCH_2CH_2Cl$ (100 mL) was added salicylaldehyde (3.4 mL, 33.46 mmol) and the resulting mixture stirred at ambient temperature. After 10 minutes, $NaBH(OAc)_3$ (15 g, 70.77 mmol) was added. After 16 hours, the reaction was quenched by addition of saturated aqueous Na_2CO_3 solution (100 mL). The mixture was then partitioned and the aqueous layer further extracted with CH_2Cl_2 (2 × 100 mL). The combined organic extract was washed with brine (150 mL) and dried over $MgSO_4$. After filtration of the drying agent, the filtrate was taken to dryness by rotary evaporation. **13** was purified and isolated as a light yellow oil by column chromatography (silica-hexanes/10% EtOAc to hexanes/20% EtOAc) in 83% yield: $R_f = 0.15$ (silica-hexanes/10% EtOAc); ^{1}H NMR (DMSO-d_6, 400 MHz, 25 °C, δ): 7.36-7.20 (m, 10H), 4.39 (t, $J = 5.3$ Hz, 1H), 3.57 (s, 4H), 3.49 (m, 2H), 2.47 (t, $J = 6.6$ Hz, 2H) ppm; Alternatively, **13** is commercially available from TCI America as 95% pure by GC.

2-(Dibenzylamino)acetaldehyde, (14)[23]. To a solution of **13** (3.5 g, 14.5 mmol) in DMSO (70 mL) was added triethylamine (11.5 mL, 82.5 mmol). Sulfur trioxide-pyridine complex (11.2 g, 70.4 mmol) in DMSO (70 mL) was added dropwise and the reaction mixture was stirred at ambient temperature. After 1 hour, diethyl ether (200 mL), saturated ammonium chloride solution (100 mL) and water (100 mL) was added. The mixture was partitioned and the aqueous layer further extracted with diethylether (3 × 100 mL). The combined organic extract was washed with brine (100 mL) and dried over $MgSO_4$. After the drying agent was filtered, the filtrate was taken to dryness by rotary evaporation. The target compound was not purified further as it is highly unstable. A DMSO-d_6 NMR sample was however taken to verify the presence of the aldehyde moiety (9.5 (s) ppm).

2-(2-Bis(phenylmethyl)-1,2-diaminoethyl)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranose, (15). Compound **1** was free-based by dissolving **1·HCl** (5 g, 13.0 mmol) in CH_2Cl_2 (100 mL) and saturated aqueous Na_2CO_3 solution (150 mL), and stirring vigorously for 0.5 h. The resulting mixture was then partitioned and separated, then the aqueous phase was further extracted with CH_2Cl_2 (3 × 60 mL). The organic layers were combined and reduced to dryness by rotary evaporation under reduced pressure yielding 4.20 g, 12.1 mmol. This was dissolved in $ClCH_2CH_2Cl$ (80 mL), and added to a solution of **14** (~10.35 mmol, quantified by ^{1}H NMR) in $ClCH_2CH_2Cl$ (40 mL). The resulting mixture was stirred at ambient temperature for 0.5 h before addition of $NaBH(OAc)_3$ (4.4 g, 20.8 mmol). After 16 h, the reaction was quenched with saturated aqueous Na_2CO_3 solution (100 mL). The layers were then partitioned and the aqueous phase extracted with CH_2Cl_2 (2 × 100 mL). The combined organic extracts was washed with brine (100 mL) and dried over anhydrous $MgSO_4$. The solution was then taken to dryness by rotary evaporation yielding crude **15** as dark brown oil. Column chromatography (silica-CH_2Cl_2/10% CH_3CN) was used to isolate and purify **15** as light yellow oil (2.52 g, 44% yield): $R_f = 0.60$ (silica-CH_2Cl_2/10% CH_3CN); ^{1}H NMR (DMSO-d_6, 400 MHz, 25 °C, δ): 7.31-7.21 (m, 10H), 5.65 (d, $J = 8.4$ Hz, 1H), 5.15 (t, $J = 9.6$ Hz, 1H), 4.81 (t, $J = 9.7$ Hz, 1H), 4.15-4.11 (m, 1H), 4.01-3.91 (m, 2H), 3.50 (q, $J = 10.7$ Hz, 4H), 2.75-2.60 (m, 3H), 2.36 (t, $J = 6.2$ Hz, 2H), 2.00 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.89 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, D1 = 2 s, 25 °C, δ): 170.5, 170.3, 169.8, 169.3, 139.6, 129.0, 128.6, 127.3, 94.3, 73.5, 71.6, 69.0, 62.1, 61.1, 57.9, 53.7, 44.8, 21.2, 21.1, 21.0, 20.9 ppm; IR (NaCl) ν_{max} (cm^{-1}): 1752 (s), 1367 (w), 1222 (s), 1069 (m), 1040 (m), 751 (w); MS (ES+, 100% CH_3CN, 30V): $m/z = 571$ (MH^+); HR-MS, (ES+ of MH^+) *m/z* calcd for $C_{30}H_{39}N_2O_9$: 571.2656, found: 571.2657.

2-(2-Bis(phenylmethyl)-1,2-diaminoethyl)-2-deoxy-3,4,6-tri-*O*-acetyl-β-*D*-glucopyranose, (16). To a solution of **15** (1.65 g, 2.89 mmol) in DMF (5 mL) was added NH_2NH_2·AcOH (0.30 g, 3.3 mmol). The resulting solution was stirred at ambient temperature for 2.5 h followed by addition of EtOAc (17 mL). The mixture was then washed with ice-cold saturated aqueous Na_2CO_3 solution (4 × 40 ml), brine (20 mL) and dried over anhydrous $MgSO_4$. The solvent was removed by rotary evaporation to yield crude **16** as white hygroscopic solid (~80% yield, quantified by ^{1}H NMR):

R_f = 0.22 (silica-CH_2Cl_2/10% CH_3CN); ^{1}H NMR (DMSO-d_6, 300 MHz, 25 °C, δ): 7.32-7.21 (m, 10H), 5.18 (br s, 1H), 5.06 (t, J = 9.8 Hz, 1H), 4.79 (t, J = 9.5 Hz, 1H), 4.15-3.95 (m, 3H), 3.60 (d, J = 13.7 Hz, 2H), 3.41 (d, J = 13.7 Hz, 2H), 2.83-2.80 (m, 1H), 2.66-2.52 (m, 2H), 2.46-2.35 (m, 2H), 2.00 (s, 3H), 1.96 (s, 3H), 1.89 (s, 3H) ppm; MS (ES+, 100% CH_3CN, 30V): m/z = 529 (MH^+); HR-MS, (ES+ of MH^+) m/z calcd for $C_{28}H_{37}N_2O_8$: 529.2550, found: 529.2551.

2-(2-Bis(phenylmethyl)-1,2-diaminoethyl)-2-deoxy-1-*tert*-butyldimethylsilyl-3,4,6-tri-*O*-acetyl-β-*D*-glucopyranose, (17). To a solution of **16** (7.88 mmol) in CH_2Cl_2 (25 mL) was added imidazole (3.7 g, 54.3 mmol) and *tert*-butyldimethylsilyl chloride (4.81 g, 31.9 mmol). After stirring for 2 h at ambient temperature, the reaction was quenched by addition of water (50 mL) and the mixture partitioned. The aqueous layer was further extracted with CH_2Cl_2 (3 × 25 mL) and the combined organic extract was washed with brine (20 mL), and dried over anhydrous $MgSO_4$. After the drying agent was filtered, the filtrate was taken to dryness by rotary evaporation yielding crude **17** which was isolated and purified by column chromatography (silica-CH_2Cl_2 to CH_2Cl_2/5% CH_3CN) as a yellow oil in 55% yield; R_f = 0.25 (silica-CH_2Cl_2/5% CH_3CN); ^{1}H NMR (DMSO-d_6, 400 MHz, 25 °C, δ): 7.31-7.21 (m, 10H), 4.99 (t, J = 9.6 Hz, 1H), 4.74-4.68 (m, 2H), 4.11-4.06 (m, 1H), 3.98-3.95 (m, 1H), 3.84-3.78 (m, 1H), 3.50 (q, J = 13.6 Hz, 4H), 2.89-2.85 (m, 1H), 2.72-2.64 (m, 1H), 2.43-2.37 (m, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.89 (s, 3H), 0.84 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, D1 = 1.5 s, 25 °C, δ): 170.0, 169.9, 169.5, 139.1, 128.5, 128.1, 126.8, 98.0, 73.4, 70.5, 69.4, 63.4, 62.3, 57.6, 53.2, 45.0, 25.6, 20.6, 20.5, 20.4, 17.6, -4.2, -5.2 ppm; IR (NaCl) ν_{max} (cm^{-1}): 3477 (m), 3319 (m), 2900 (br), 2118 (m), 1951 (m), 1770 (s), 1674 (m), 1651 (m), 1603 (m), 1585 (m), 1513 (s), 1495 (s), 1471 (s), 1455 (s), 1092 (m); MS (ES+, 100% MeOH, 30V): m/z = 643 (MH^+), 665 (MNa^+); HR-MS, (ES+ of MH^+) m/z calcd for $C_{34}H_{51}N_2O_8Si$: 643.3415, found: 643.3411.

2-(2-Bis(phenylmethyl)-1,2-diaminoethyl)-2-deoxy-1-*tert*-butyldimethylsilyl-β-*D*-glucopyranose, (18). Compound **17** (2.8 g, 4.36 mmol) was dissolved in MeOH (40 mL). NaOMe (0.3 g, 5.56 mmol) was then added and the reaction mixture stirred at ambient temperature. After 2.5 hours, the reaction was neutralized by the addition of Sigma-Aldrich Mixed Bed Resin TMD-8 hydrogen and hydroxide form until no color change was observed upon addition of further resin. The used up resin was then filtered out and the filtrate taken to dryness by rotary evaporation. Compound **18** was isolated as a white solid (1.75 g, 78% yield) by column chromatography (silica-CH_2Cl_2/1% MeOH to CH_2Cl_2/5% MeOH): R_f = 0.20 (silica-CH_2Cl_2/10% MeOH); ^{1}H NMR (DMSO-d_6, D_2O, 400 MHz, 25 °C, δ): 7.33-7.19 (m, 10H), 4.32 (d, J = 7.6 Hz, 1H), 3.65-3.53 (m, 3H), 3.43-3.37 (m, 3H), 3.09-3.02 (m, 3H), 2.95-2.89 (m, 1H), 2.75-2.69 (m, 1H), 2.48-2.36 (m, 2H), 2.07 (t, J = 8.0 Hz, 1H), 0.82 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, D1 = 1.5 s, 25 °C, δ): 139.3, 128.6, 128.1, 126.7, 99.6, 77.2, 75.1, 70.4, 65.8, 61.2, 57.5, 53.4, 46.8, 25.7, 17.6, -3.8, -5.2 ppm; IR (KBr) ν_{max} (cm^{-1}): 3422 (br), 2928 (m), 2856 (m), 1496 (m), 1453 (m), 1257 (m), 1174 (m), 1078 (s), 1029 (m), 839 (s); MS (ES+, 100% MeOH, 30V): m/z = 517 (MH^+), 539 (MNa^+); HR-MS, (ES+ of MNa^+) m/z calcd for $C_{28}H_{44}N_2NaO_5Si$: 539.2917, found: 539.2914.

2-(1,2-Diaminoethyl)-2-deoxy-1-*tert*-butyldimethylsilyl-β-*D*-glucopyranose, (19). To a solution of **18** (0.58 g, 1.12 mmol) in MeOH (10 mL) was added $Pd(OH)_2$/C (0.15 g). The reaction was then stirred at ambient temperature under a positive H_2 atmosphere using an H_2-filled balloon. After 24 hours, the mixture was filtered through a MeOH pre-washed pad of celite. The resulting filtrate was reduced to dryness by rotary evaporation yielding a light yellow solid (0.375 g, 99% yield). ^{1}H NMR (DMSO-d_6, D_2O 400 MHz, 25 °C, δ): 4.35 (d, J = 7.6 Hz, 1H), 3.43-3.39 (m, 2H), 3.15-3.01 (m, 3H), 2.86-2.78 (m, 1H), 2.70-2.61 (m, 2H), 2.56-2.52 (m, 1H), 2.06 (t, J = 8.4 Hz, 1H), 0.85 (s, 9H), 0.07 (s, 6H) ppm; IR (KBr) ν_{max} (cm^{-1}): 3406 (br), 2954 (m), 2929 (m), 2882 (m), 2957 (m), 1655 (w), 1637 (w), 1618 (w), 1473 (m), 1390 (w), 1255 (m), 1172 (m), 1079 (s), 840 (s), 782 (s); MS (ES+, 100% ACN, 30V): m/z = 337 (MH^+); HR-MS, (ES+ of MH^+) m/z calcd for $C_{14}H_{33}N_2O_5Si$: 337.2159, found: 337.2158.

2-((2-(*N*-Pyridin-2-ylmethyl))diaminoethyl)-2-deoxy-1-*tert*-butyldimethylsilyl-β-*D*-glucopyranose, (20). To a solution of **19** (0.37 g, 1.1 mmol) in MeOH (10 mL) was added 2-pyridinecarboxaldehyde (0.11 mL, 1.15 mmol) and Na_2CO_3 (1.17 g, 11.04 mmol). The mixture was then stirred at ambient temperature under a N_2 atmosphere. After 24 hours, $NaBH_4$ (0.1 g, 2.64 mmol) was added and the mixture further stirred at ambient temperature. After another 24 hours, the solid was filtered and the filtrate reduced to dryness by rotary evaporation. Compound **20** was isolated and purified using column chromatography (silica-CH_2Cl_2/20% MeOH to CH_2Cl_2/19% MeOH/1% Et_3N) as light yellow solid (0.21 g, 45% yield): R_f = 0.23 (silica-CH_2Cl_2/20% MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz, 25 °C, δ): 8.47 (d, J = 4.3 Hz, 1H), 7.73 (t, J = 7.6 Hz, 1H), 7.43 (d, J = 7.9 Hz, 1H), 7.24-7.21 (m, 1H), 4.89 (d, J = 4.1 Hz, 1H), 4.34-4.32 (m, 2H), 3.76 (s, 2H), 3.64-3.63 (m, 1H), 3.44-3.33 (m, 1H), 3.07-3.03 (m, 3H), 2.95-2.85 (m, 1H), 2.75-2.65 (m, 1H), 2.65-2.52 (m, 3H), 2.09 (t, J = 8.7 Hz, 1H), 0.86 (s, 9H), 0.76 (s, 6H) ppm; IR (KBr) ν_{max} (cm^{-1}): 3404 (br), 2930 (m), 2852 (m), 2604 (m), 2498 (m), 1474 (m), 1443 (m), 1453 (m), 1398 (m), 1074 (s), 1078 (s), 1038 (s), 839 (s), 783 (m); MS (ES+, 100% MeOH, 30V): m/z = 428 (MH^+), 450 (MNa^+); HR-MS, (ES+ of MH^+) m/z calcd for $C_{20}H_{38}N_3O_5Si$: 428.2581, found: 428.2579.

2-((2-(2-Hydroxybenzyl)-2-(pyridin-2-ylmethyl)diaminoethyl)-2-deoxy-1-*tert*-butyldimethylsilyl -β-*D*-glucopyranose, (21). To **20** (0.21 g, 0.49 mmol) in $ClCH_2CH_2Cl$ (5 mL) was added salicylaldehyde (0.05 mL, 0.49 mmol). The resulting solution was then stirred at ambient temperature. After 1 hour, $NaBH(OAc)_3$ (0.21 g, 0.99 mmol) was added and the mixture further stirred at ambient temperature. After 24 hours, the reaction was quenched by addition of saturated aqueous Na_2CO_3 solution (2 mL). The mixture was then partitioned and the aqueous layer further extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phase was taken to dryness by rotary evaporation affording crude **21**. Purification was achieved by column chromatography (silica-CH_2Cl_2/5% MeOH to CH_2Cl_2/10% MeOH), yielding **21** as light yellow solid (0.12 g, 44% yield): R_f = 0.53 (silica-CH_2Cl_2/10% MeOH); ^{1}H NMR (DMSO-d_6, D_2O, 400 MHz, 25 °C, δ): 8.46 (d, J = 4.8 Hz, 1H), 7.74 (d, J = 7.6 Hz, 1H), 7.38 (d, J = 7.6 Hz, 1H), 7.26 (t, J = 6.0 Hz, 1H), 7.15 (d, J = 7.2 Hz, 1H), 7.05 (t, J = 7.6 Hz, 1H), 6.72 (m, 2 H), 4.36 (d, J = 7.6 Hz, 1H), 3.67-3.56 (m, 5H), 3.40 (d, J = 8.8 Hz, 1H), 3.06-2.94 (m, 4H), 2.78-2.72 (m, 1H),

2.53-2.51 (m, 2H), 2.10 (m, 1H), 0.79 (s, 9H), 0.02 (s, 3H), -0.003 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, D1 = 2 s, 25 °C, δ): 158.8, 156.8, 149.1, 137.4, 130.3, 128.7, 123.9, 123.4, 122.8, 119.3, 115.9, 99.4, 77.3, 75.1, 70.6, 65.8, 61.4, 59.3, 54.5, 53.6, 46.5, 26.1, 18.0, -3.4, -4.8 ppm; IR (KBr) ν_{max} (cm^{-1}): 3422 (br), 2921 (m), 2856 (m), 1591 (m), 1489 (m), 1473 (m), 1460 (m), 1254 (m), 1078 (s), 1029 (m), 839 (s), 783 (m), 755 (s); MS (ES+, 100% MeOH, 30V): m/z = 534 (MH$^+$), 556 (MNa$^+$); HR-MS, (ES+ of MH$^+$) m/z calcd for $C_{27}H_{44}N_3O_6Si$: 534.2999, found: 534.2997.

2-((2-(2-Hydroxybenzyl)-2-(pyridin-2-ylmethyl)diaminoethyl)-2-deoxy-β-*D*-glucopyranose, (22). To **21** (0.21 g, 0.39 mmol) in THF (5 mL) and MeOH (1 mL) was added benzyltrimethylammonium fluoride (0.12 g, 0.71 mmol). The resulting mixture was then stirred at ambient temperature. After 24 hours, the solvent was removed by rotary evaporation and **22** was isolated by column chromatography (silica-CH_2Cl_2/10% MeOH to CH_2Cl_2/20% MeOH) as a white solid (0.10 g, 60% yield): R_f = 0.20 (silica-CH_2Cl_2/20% MeOH); ^{1}H NMR (DMSO-d_6, D_2O, 400 MHz, 25 °C, δ): 8.50 (m 1H), 7.76 (m, 1H), 7.40 (m, 1H), 7.27 (m, 1H), 7.15 (m, 1H), 7.08 (t, J = 7.6 Hz, 1H), 6.74 (m, 2 H), 5.02 (d, J = 3.3 Hz, 0.73 H), 4.31 (d, J = 7.8 Hz, 0.26H), 3.79-3.45 (m, 7H), 3.07-3.02 (m, 2H), 2.89-2.71 (m, 2H), 2.56-2.52 (m, 2H), 2.26-2.23 (m, 0.73H), 2.15-2.05 (m, 0.25H) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, D1 = 2 s, 25 °C, δ): 158.6, 158.4, 156.8, 156.7, 148.8, 148.7, 136.8, 136.7, 130.0, 129.8, 128.2, 128.1, 123.7, 123.5, 123.1, 123.0, 122.3, 122.2, 118.7, 118.6, 115.6, 97.8, 90.3, 76.9, 75.3, 72.2, 72.0, 70.8, 70.5, 65.0, 62.3, 61.2, 58.7, 54.4, 53.8, 53.1, 52.9, 45.8, 44.1 ppm; IR (KBr) ν_{max} (cm^{-1}): 3421 (br), 1596 (s), 1571 (s), 1458 (s), 1409 (s), 1252 (m), 1041 (s), 755 (s); MS (ES+, 100% MeOH, 30V): m/z = 420 (MH$^+$); HR-MS, (ES+ of MH$^+$) m/z calcd for $C_{21}H_{30}N_3O_6$: 420.2135, found: 420.2133; Anal. Calcd. for $C_{21}H_{29}N_3O_6{\cdot}(CH_3)_2CO{\cdot}(CH_3OH)_{0.3}$: C, 59.91; H, 7.49; N, 8.63. Found: C, 59.68; H, 7.46; N, 8.95.

Synthesis of L-Re(CO)$_3$ complexes. Equivalent amounts of ligand and [Re(H$_2$O)$_3$(CO)$_3$]Br in EtOH was stirred under reflux conditions for between 6 and 24 h. The solvent was then removed by rotary evaporation and the pure complex was isolated using either column chromatography, HPLC or preparative TLC.

Preparation of 6-Re(CO)$_3$. Refluxed for 24 h then solated as a pale white solid (72% yield) by column chromatography (silica-CH_2Cl_2/20% MeOH). R_f = 0.27 (silica-CH_2Cl_2/20% MeOH). ^{1}H NMR (MeOH-d_4, 400 MHz, 25 °C, δ): 8.85 (m, 1H), 8.07 (m, 1H), 7.70 (m, 1H), 7.53 (m, 2H), 7.35 (t, J = 8.3 Hz, 1H), 7.00 (m, 2H), 5.06-4.95 (m, 4H), 4.91-4.83 (m, 1H), 4.49-4.39 (m, 1H), 3.82-3.55 (m, 5H), 3.50-3.33 (m, 1H), 3.23-3.15 (m, 1H); ^{13}C NMR (MeOH-d_4, 100 MHz, 25 °C, δ): 197.5, 196.8, 182.8, 182.3, 182.1, 160.7, 160.3, 158.0, 154.0, 153.8, 142.5, 142.2, 134.9, 132.7, 127.4, 125.7, 125.4, 121.2, 119.8, 119.6, 117.3, 95.8, 91.9, 91.8, 78.2, 78.0, 75.3, 74.9, 73.1, 72.1, 72.0, 71.9, 71.8, 69.2, 69.1, 68.2, 68.1, 63.0, 62.7, 62.6, 62.5, 59.4, 57.1, 57.0. IR (KBr) ν_{max} (cm^{-1}): 3386 (br, m), 2032 (s), 1905 (s), 1618 (m), 1458 (w), 764 (w). MS (ES+, 100% MeOH, 30V): m/z = 702 (M^{185}ReH$^+$), 704 (M^{187}ReH$^+$); HR-MS (ES+ of MH$^+$): m/z calcd for $C_{24}H_{27}N_3O_{10}{}^{187}$Re: 704.1254, found: 704.1256; Anal. Calcd. for $C_{24}H_{26}N_3O_{10}Re{\cdot}(HBr)_{1.5}$: C, 34.98; H, 3.36; N, 5.10 Found: C, 34.75; H, 3.51; N, 4.88.

Preparation of 8-Re(CO)$_3$. Refluxed for 6 h, then purified as an off-white solid by semi-preparative HPLC (H$_2$O with 0.1% TFA to 100% ACN over 30 min) ((66% yield). R_f = 0.12 (silica-CH_2Cl_2/20% MeOH). ^{1}H NMR (MeOH-d_4, 400 MHz, 25 °C, δ): 8.81 (d, J = 5.4 Hz, 1H), 8.09 (dd, J = 7.8 Hz, 7.8 Hz, 1H), 7.73 (d, J = 7.6 Hz, 1H, 7.55 (dd, J = 7.40 Hz, 5.8 Hz, 1H), 5.16–5.33, 4.34–4.70, 4.06–4.12, 3.65–3.91, 3.25–3.53 (m). ^{13}C NMR (MeOH-d_4, 100 MHz, 25 °C, δ): 197.8, 197.0, 196.4, 183.6, 180.5, 168.4, 159.8, 159.6, 152.2, 140.4, 125.8, 123.9, 95.7, 93.7, 91.3, 76.9, 74.8, 72.1, 71.9, 71.3, 70.9, 69.4, 69.2, 69.1, 69.0, 68.8, 68.7, 61.5, 60.9, 57.1, 54.4. IR ν_{max} (cm^{-1}): 3292 (w, br), 2360 (m), 2341 (m), 2027 (m), 2015 (s), 1910 (s), 1863 (s), 1635 (m, br), 774 (m). Purity assessed by HPLC: 97.1%. HR-MS (ES+ of MNa$^+$): m/z calcd for $C_{19}H_{22}N_3O_{11}Na^{187}Re$: 678.0710, found: 678.0704.

Preparation of 11-Re(CO)$_3$. Refluxed for 8 h then purified to a pale yellow oil by semi-preparative HPLC (H$_2$O with 0.1% TFA to 100% ACN over 30 min) (43% yield). R_f = 0.30 (silica-CH_2Cl_2/20% MeOH). ^{1}H NMR (MeOD, 400 MHz, 25 °C, δ): 7.53 (d, J = 7.4 Hz, 1H), 7.35 (dd, J = 7.1 Hz, J = 7.1 Hz, 1H), 7.20 (d, J = 5.8 Hz, 1H), 7.14 (s, 1H), 6.98 (m, 2H), 5.10 (d, J = 3.3 Hz 0.45H - α), 4.40 (s, 0.55H - β), 4.66–4.95, 4.08–4.14, 3.64–3.81, 3.01–3.55, (m, 13H), 3.64 (s, 3H). ^{13}C NMR (MeOD, 100 MHz, 25 °C, δ): 197.94, 197.44, 197.35, 196.55, 196.35, 181.29, 181.23, 158.24, 158.20, 152.17, 152.04, 135.03, 135.00, 133.00, 129.61, 125.88, 125.80, 121.49, 120.15, 120.01, 117.58, 96.19, 92.53, 91.99, 78.46, 75.69, 75.18, 73.51, 73.44, 72.36, 72.27, 72.13, 72.05, 68.18, 67.92, 65.29, 64.81, 64.43, 62.92, 62.74, 59.14, 58.95, 57.47, 57.42, 35.40, 35.28, 35.21. IR ν_{max} (cm^{-1}): 3232 (m, br), 2360 (w), 2028 (s), 1884 (s), 1672 (m), 1620 (s), 1198 (s), 1134 (s), 757 (m), 527 (s). Purity assessed by HPLC: 95.9%. HR-MS (ES+ of MH$^+$) m/z calcd for $C_{23}H_{28}N_4O_{10}{}^{187}$Re: 707.1363, found 707.1365.

Preparation of 22-Re(CO)$_3$. Refluxed for 24 h then isolated by preparative TLC (silica-CH_2Cl_2/20% MeOH) as white solid (87% yield). R_f = 0.31 (silica -CH_2Cl_2/20% MeOH). ^{1}H NMR (DMSO-d_6, D_2O, 400 MHz, 25 °C, δ): 8.97-8.74 (m, 1H), 8.15 (t, J = 7.6 Hz, 1H), 7.78 (t, J = 8.0 Hz, 1H), 7.65-7.48 (m, 2H), 7.32 (t, J = 7.6 Hz, 1H), 7.00-6.98 (m, 1H), 6.93 (t, J = 7.6 Hz, 1H), 4.96-4.91 (m, 0.71H), 4.83-4.75 (m, 2H), 4.65-4.57 (m, 0.23H), 4.53-4.33 (m, 2H), 4.11-3.50 (m, 4H), 3.26-3.17 (m, 2H), 3.11-2.85 (m, 2H), 2.62-2.51 (m, 1H), 2.28-2.04 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz, 25 °C, δ): 196.2, 196.0, 195.9, 160.0, 159.8, 156.8, 154.2, 153.8, 141.1, 141.0, 140.9, 140.8, 134.2, 131.2, 126.4, 126.2, 119.5, 118.3, 115.9, 97.3, 94.2, 90.9, 77.6, 77.5, 76.4, 73.3, 72.4, 71.4, 71.2, 70.5, 68.3, 68.0, 65.8, 60.8, 60.7, 60.0, 59.9, 55.0, 54.7, 53.3, 53.0, 52.8,; IR (KBr) ν_{max} (cm^{-1}): 3386 (br, m), 2029 (s), 1906 (s), 1609 (m), 1458 (m), 1033 (m), 762 (m); MS (ES+, 100% MeOH, 30V): m/z = 688 (M^{185}ReH$^+$), 690 (M^{187}ReH$^+$); HR-MS (ES+ of MH$^+$): m/z calcd for $C_{24}H_{29}N_3O_9{}^{187}$Re: 690.1461, found: 690.1460; Anal. Calcd. for $C_{24}H_{28}N_3O_9{\cdot}(HBr)_{0.1}{\cdot}H_2O$: C, 36.60; H, 3.97; N, 5.34 Found: C, 36.70; H, 3.92; N, 5.17.

General procedure for radiolabeling with [^{99m}Tc(H$_2$O)$_3$(CO)$_3$]$^+$. The organometallic precursor [^{99m}Tc(H$_2$O)$_3$(CO)$_3$]$^+$ was prepared from a saline solution of Na[^{99m}TcO$_4$] (1 mL, 100 MBq) using an Isolink(tm) kit generously provided by Mallinckrodt (now Covidien). Briefly, a 1 mL saline solution of Na[^{99m}TcO$_4$] was added to an Isolink(tm) kit, and the vial was heated to reflux for 20 min. Upon cooling, ~0.12 mL of a 1 M HCl solution was added to adjust the pH between 9-10. To 0.50 mL of compound **6**, **8**, or **22** (10^{-3} M) in EtOH, was added 3 equiv of NaOEt (0.15 mL, 10^{-2} M

in EtOH) followed by 0.10 mL of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ solution. Alternatively, to 0.5 mL of **11** in PBS solution at pH 7.4 was added 0.10 mL of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ solution. The resulting solutions were heated to 80 °C for 0.5 h and cooled to ambient temperature prior to HPLC injection.

Cysteine and histidine challenge experiments. To either cysteine or histidine (1 mM, 0.9 mL) in PBS buffer solution at pH 7.4 was added a solution of ^{99m}Tc complex (final ligand concentration 10^{-5} M). The samples were incubated at 37 °C for 24 h and were then analyzed by HPLC.

GLUT-1 cell uptake studies

These experiments were performed in a manner similar to that of Schibli *et al*,[31] and were carried out in LCC6-HER2 cells, a human breast cancer cell line chosen for its overexpression of the glucose transporter GLUT-1.[27] Suspensions of cells were prepared in 1% PBS (pH 7.4) to a concentration of 1×10^6 cells/mL, and 0.5 mL was aliquoted into 1.5 mL Eppendorf vials (5×10^5 cells per vial). Either 15 μCi of a test ^{99m}Tc complex or 30 μCi of ^{18}F-FDG (positive control) was added in 500 μL PBS solution, and the vial gently inverted. Each compound was added to the cells under both glucose-free and 5 mM D-glucose conditions with each condition being tested in quadruplicate. The resulting suspensions were incubated at 37 °C for 30 min with shaking at 400 RPM. Following incubation, the vials were centrifuged at 1100 RPM for 5 min then 900 μL of the supernatant was removed. Cold PBS solution (900 μL) was added, and gently mixed with the cells. The centrifuging and washing procedure was repeated four times in total to give the original supernatant and three washing supernatants. Finally the cells were vortexed in cold PBS (1 mL) to remove them from the vial wall then transferred to a tube for gamma counting. The activity in each cell sample was divided by the sum of all the supernatants for that sample to give a percentage of the activity associated with the cells.

Hexokinase phosphorylation studies

These were performed in a manner similar to that previously reported.[32] Briefly, a 5 mM aqueous solution of test compound (100 μL) was added to a reaction vial to give a final concentration of 0.5 mM test compound, 10 U/mL hexokinase, 1 mM ATP, 4 mM $MgCl_2$ in a 30 mM TEOA (triethanolamine, pH = 6) buffer. The resulting solutions were mixed gently by inverting each vial three times, and then incubated for 16 h at 37 °C. Each solution (20 μL) was analysed by HPLC to determine whether any of the ATP had been converted to ADP, as would accompany the phosphorylation of a substrate. Using a Phenomenex Synergi 4 μm Hydro-RP 80 Å column (250 × 4.6 mm) and eluting with 30 mM KH_2PO_4, the retention time of ATP was 8.5 min, whereas that of ADP was 12.5 min.

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