Derivatization of Glucose and 2-Deoxyglucose for Transition Metal Complexation: Substitution Reactions with Organometallic ^{99m}Tc and Re Precursors and Fundamental NMR Investigations

Jeannine Petrig,[a] Roger Schibli,*[a] Cécile Dumas,[a] Roger Alberto,[b] and P. A. Schubiger*[a]

Abstract: Synthetic strategies for the bifunctionalization of glucose and 2-deoxyglucose at position C-1 for transition metal coordination are reported. In particular organometallic technetium and rhenium complexes for potential use in diagnostic nuclear medicine were synthesized and investigated. Specifically, a common iminodiacetic acid (IDA) moiety was O-glycosidically connected through an ethylene spacer group to produce the pure α- (in case of 2-deoxyglucose) and β-anomer (in case of glucose). Reaction of the sugar derivatives with the organometallic precursor $[M(H_2O)_3(CO)_3]^+$ (M = ^{99m}Tc, Re) produced single products in high yield, which are water-soluble and water-stable. The displacement of the three water molecules of the metal precursor and thus the tridentate coordination of the metal-tricarbonyl core exclusively via the amine and the two carboxylic acid functionalities of the IDA chelate was verified by means of 1D and 2D ^{1}H NMR spectroscopy, mass spectrometry, and IR spectroscopy. The radioactive-labeled products (^{99m}Tc) proved their excellent stability in vitro in physiological phosphate buffer (pH = 7.4) and human plasma over a period of 24 h at 37 °C.

Keywords: bioinorganic chemistry · carbohydrates · rhenium · technetium · transition metals

Introduction

The coordination chemistry of transition metals with carbohydrate-based ligand systems in general has become a very active field in recent years. Even the reaction of native glucose with various transition metals produces complexes, which exhibit interesting biological features.[1–3] However, nonfunctionalized carbohydrates are generally weak ligands; therefore, functionalization with chelating groups leads to stronger metal-binding compounds. Such carbohydrate derivatives often contain polyamine-chelating systems such as tris(2-aminoethyl)amine,[4, 5] diaminopropane (dap), and diethylenediamine.[6] In most of these compounds at least one sugar hydroxy group is directly involved in the coordination sphere. Only recently a Pt^{II} complex with a glucose derivative was reported in which the metal is exclusively coordinated through a dap chelate and not through hydroxy functionalities.[7] The complex showed similar biological activity as cisplatin. All these examples are classical Werner-type complexes that exhibit no direct metal–carbon bond. The class of organometallic carbohydrate complexes has only recently attracted greater attention.[8] This, despite the fact that sugar moieties could enhance water-solubility useful, for example, for the development of water-soluble catalysts and the biocompatibility of traditionally hydrophobic organometallic compounds.[9, 10] On the other hand, organometallic metal cores often exhibit advantages in terms of kinetic inertness, stability, and size and thus could lead to the development of more efficient and stable compounds compared to "classical" inorganic complexes.

In terms of developing novel drugs for use in diagnostic and therapeutic nuclear medicine, it would be of great interest to label glucose with the transition metal isotope ^{99m}Tc to substitute the expensive but readily used ^{18}F-labeled 2-deoxyglucose (^{18}F-FDG) for localization of tumor and metastatic tissue. Due to the almost ideal decay properties ($t_{1/2}$ = 6 h, γ energy = 150 keV), low cost of production, and on-site availability, ^{99m}Tc is the most frequently used radioisotope in nuclear medicine today. However, complexes of Tc and

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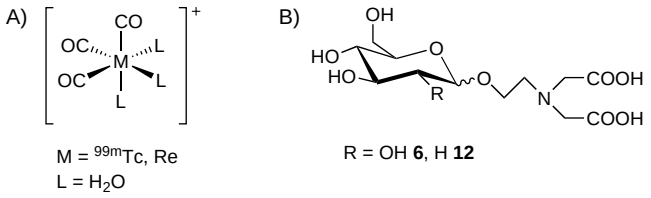
glucose and its derivatives are mentioned only sporadically in the literature.[11–14] The actual structures of these products were only poorly investigated and none of the complexes showed biological activity similar to ^{18}F-FDG, presumably due to the direct interactions/coordination of the hydroxy groups of the glucose with the Tc^{V} center. Our group has recently developed an elegant synthetic strategy to produce low-valent, organometallic ^{99m}Tc and ^{188}Re complexes of the general formula $[M(H_2O)_3(CO)_3]^+$ ($M = ^{99m}$Tc, ^{188}Re) for the radioactive labeling of small biomolecules such as central nervous system (CNS) receptor ligands or peptides (Scheme 1 A).[15, 16] The low-spin d^6-electron configuration of the

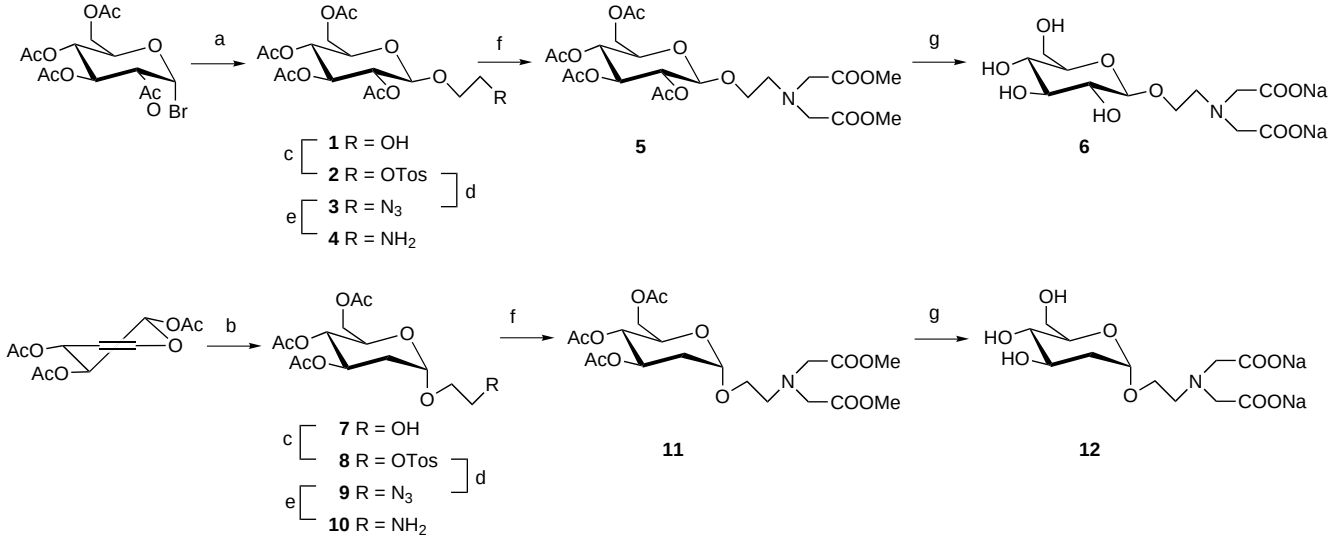


Scheme 1. A) Structure of organometallic precursor $[M(H_2O)_3(CO)_3]^+$ ($M = ^{99m}$Tc, Re). B) Structure of glucose/deoxyglucose derivatives for the labeling with organometallic precursor.

metal center gives rise to complexes of high kinetic inertness. The three substitution-labile water molecules are readily exchanged for suitable chelating systems containing amines and carboxylic acids. On the other hand the three CO ligands are stable to substitution and protect the metal center from ligand attack and/or back oxidation. Both properties give the complexes a high stability in biological environments. For the purposes of labeling glucose and 2-deoxyglucose with ^{99m}Tc and Re tricarbonyl a hydrophilic and simple iminodiacetic acid (IDA) moiety was attached to position C-1 separated by an ethylene linker (Scheme 1 B). The corresponding complexes are the first examples of organometallic carbohydrate compounds of Group 7 metals reported. The structure and the substitution behavior of the corresponding rhenium complexes were investigated by means of 1D and 2D ^{1}H NMR spectroscopy and mass spectrometry. Preliminary results of the in vitro stability of the corresponding radioactive ^{99m}Tc complexes are briefly mentioned.

Results and Discussion

Ligand synthesis: The bifunctionalization of glucose and 2-deoxyglucose at position C-1 with an iminodiacetic acid (IDA) moiety was accomplished in five steps (Scheme 2). All products were unambiguously characterized by means of ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry. For the synthesis of compound **6** acetobromoglucose was used as the starting material. A standard Königs–Knorr glycosylation using mercury(II) bromide and ethylene glycol gave the desired alcohol **1**. The glycosylation of tri-*O*-acetyl-D-glucal with ethylene glycol yielded the corresponding alcohol of the deoxyglucose (**7**). Both compounds **1** and **7** were tosylated to give **2** and **8**, respectively, and afterwards converted into the corresponding azides **3** and **9** using NaN_3. The reduction of the azides with hydrogen in the presence of Lindlar catalyst gave the corresponding amines **4** and **10** in almost quantitative yields. Finally the transition metal chelate was built up in one step by a double alkylation of the amine with methyl bromoacetate (**5** and **11**, respectively). Deprotection of the acetates with K_2CO_3 in methanol, which is suitable for hydrolyzing the methyl esters, unfortunately led to the hydrolysis of **5**/**11** at position C-1. Therefore, the deprotection was performed in two steps. First the acetates were removed with sodium methylate and then the esters were hydrolyzed with NaOH to form the disodium salt of the products **6** and **12**. In case of compound **6** the pure β-anomer and in case of **12** the pure α-anomer were produced. The compounds proved to be stable as solids for several weeks at room temperature in a desiccator.



Scheme 2. Bifunctionalization at position C-1 with an IDA moiety. a) Diethylene glycol, $HgBr_2$, molecular sieves, CH_2Cl_2, 71%; b) Diethylene glycol, acid resin, molecular sieves, acetonitrile, 40%; c) TosCl, pyridine, CH_2Cl_2, 72%; d) NaN_3, DMF, 92%; e) H_2, Lindlar catalyst, EtOH, 90%; f) methyl bromoacetate, NEt_3, THF, 50%; g) 1.) NaOMe, MeOH 2.) NaOH, H_2O, quantitatively.

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NMR investigations: The ^{1}H NMR spectra of compounds **6** and **12** revealed beside the signals of the sugar the singlet of the four equivalent protons of the IDA moiety at approximately $\delta = 3.2$ and additional signals of the protons of the ethylene spacer as expected (Figure 1 A). Interestingly, two of the four protons of the spacer in compounds **6** and **12** are nonequivalent and show multiplets centered around $\delta = 3.9$ and $\delta = 3.3$. Coupling with each other and the two other ethylene protons at $\delta = 2.86$ was verified in the 2D spectra. Based on the chemical shifts, the resonance at $\delta = 2.86$ can be assigned to NCH_2 protons and those at $\delta = 3.9$ and 3.3 to the OCH_2 protons. The asymmetry of the environments of the protons close to the sugar moiety originates from the restrained rotation around the glycosidic bonds.

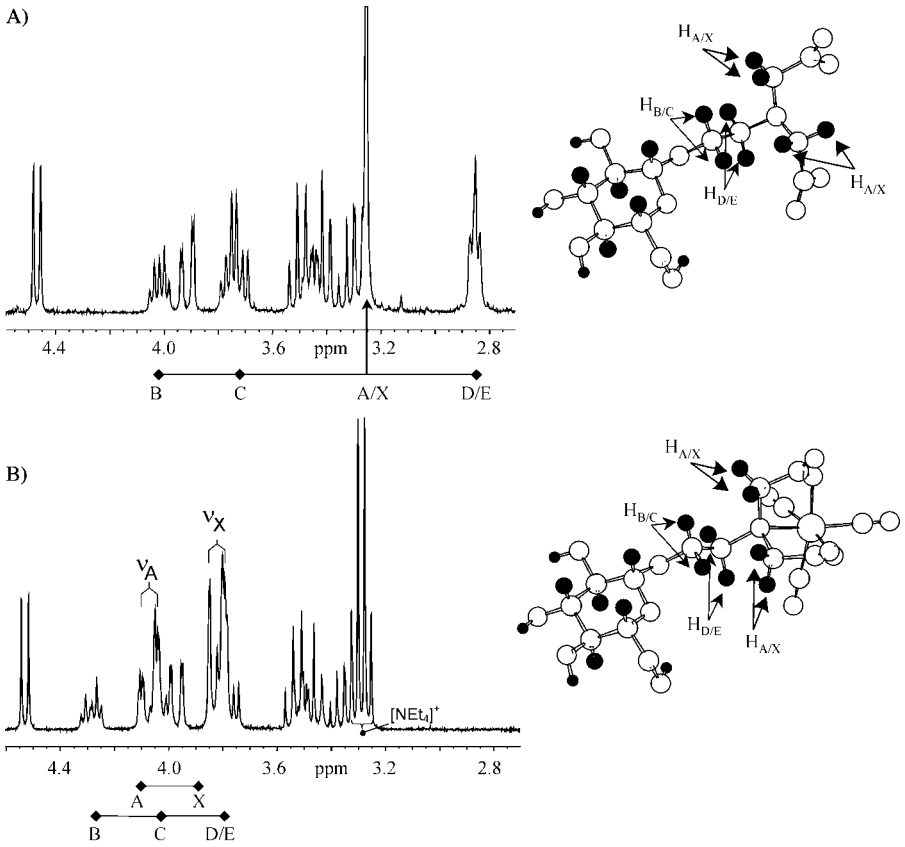

Figure 1. A) ^{1}H NMR spectrum (left) and three-dimensional (3D) model (right) of compound **6**. The proton signal of the metal chelate IDA and the ethylene linker with assignments (based on COSY experiments) are labeled. B) ^{1}H NMR spectrum (left) and 3D model (right) of complex **13a**. The AX spin system formed by the IDA protons is partially overlaid by signals of the sugar and/or the linker protons. Signals of $[NEt_4]^+$ originate from the organometallic precursor.

The bifunctionalized glucose derivatives **6** and **12** were treated with the organometallic precursor $[NEt_4]_2[Re(CO)_3Br_3]$ in water at 50 °C (Scheme 3). The coordination of the $Re(CO)_3$ core and, thus, the formation of the carbohydrate complexes **13a** and **14a**, takes place almost instantaneously as verified by means of HPLC (monitored at 254 nm) and from proton NMR spectra. The formation of side products was not observed. As a blank experiment, underivatized glucose and 2-deoxyglucose were also treated with $[NEt_4]_2[Re(CO)_3Br_3]$. In this case, no formation of stable products was observed under the same reaction conditions.

The tridentate coordination of the metal core through the IDA chelate was first verified in the NMR experiment. The coordination of the Re^I center causes the resonance of the four equivalent protons of the IDA moiety to disappear. At the same time the formation of an AX spin system with coupling constants around 16 Hz and an intensity equivalent to four protons was observed, shifted downfield by approximately 0.7 ppm (Figure 1 B). The NCH_2COO protons of the IDA moiety become nonequivalent upon rigid coordination to the metal center by virtue of their, now different chemical environments (see structure in Figure 1 B (right)). Similar observations were reported in case of the complexes $[Re(dppm)(NCMe)(CO)_3]ClO_4$ (dppm = bis(diphenylphosphanyl)methane) or $[Re(PADA)(CO)_3]$ (PADA = picolylamine *N,N*-diacetic acid).[15, 17] In the ^{1}H NMR spectrum of **13a** the AX pattern reveals additional splitting (ν_A: 1.3 Hz and 3 Hz; ν_X: 1.3 Hz). These couplings are presumably because of long-range couplings with protons of the ethylene linker and/or the glucose unit. The ^{1}H NMR spectrum of the complex **14a** showed an AX spin system with a coupling constant of 16.5 Hz. Since the signals are relatively broad, it can be assumed, that long-range couplings exist also in this complex but are not resolved.

Due to the electronic influence of the Re^I center also the resonance of the linker NCH_2 protons at $\delta = 2.8$ is shifted significantly downfield (approx. 1 ppm) compared to those in compounds **6** and **12**, respectively (Figure 1). Therefore, it is reasonable to assume that these protons are closer to the metal center. The signal of the linker protons closer to the sugar moiety shifted only slightly (0.1 – 0.2 ppm).

Since the hydroxy functionalities of a sugar are essential for biorecognition, the Tc/Re tricarbonyl center should not interfere with these functional groups. Coordination of the metal center to the hydroxy functionalities would be definitely observable in the ^{1}H NMR spectra due to significant chemical shifts of the anomeric protons. As evident from the ^{1}H NMR spectra of compounds **13a** and **14a** the $Re(CO)_3$ center has only a small effect on the ring protons. Thus, the metal center must be coordinated through the IDA moiety and not through hydroxy groups. Extension of the linker between sugar and chelating moiety can presumably minimize these influences even more.

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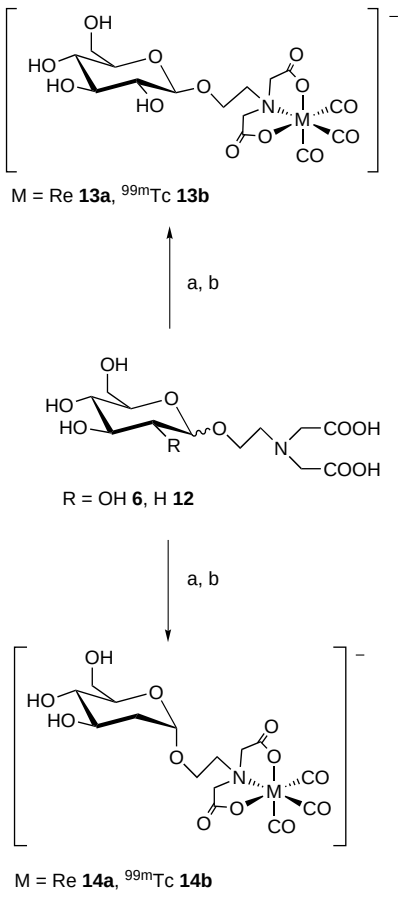

Scheme 3. a) $[NEt_4]_2[Re(CO)_3Br_3]$, H_2O, 50 °C, 2 h; b) $[^{99m}Tc(OH_2)_3(CO)_3]^+$, 10^{-4} M, 70 °C, 30 min, PBS buffer pH = 7.4.

Mass spectrometric and IR spectroscopic investigations: Analysis of the mass spectra of compounds **13a** and **14a** showed intensive signals of the intact complex at *m*/*z* 606/608 and *m*/*z* 590/592, respectively. The difference of two mass units originates from the fact that rhenium exists as mixture of the two isotopes ^{185}Re/^{187}Re with almost similar natural abundance. In addition the mass spectrum of complex **14a** shows distinct fragmentations providing further evidence that the metal center is coordinated in the proposed fashion. Fragmentation of the compound **14a** occurs at position C-1, which produces the signals of *m*/*z* 428/430 ([*M* – 160]) lacking the sugar moiety. Further signals at *m*/*z* 400/402 correspond to the ′Re(IDA)(CO)$_3$′ fragment without the ethylene linker.

The pattern of the C–O stretching frequencies in the IR spectra confirm the facial arrangement of the three CO ligands in **13a** (2024 and 1906 cm^{-1}) and **14a** (2022 and 1892 cm^{-1}). In addition a single, strong absorption of the carboxylate functionalities with a significant blue-shift, due to the coordination of the metal, could be observed at 1636 cm^{-1} in both complexes.

In vitro characterization and stability of radioactive-labeled compounds: For fundamental radiopharmaceutical evaluation, the compounds **6** and **12** were also treated with the radioactive precursor $[^{99m}Tc(H_2O)_3(CO)_3]^+$ under physiological conditions (Scheme 3). Analysis of the products **13b** and **14b** by radioactive HPLC (the standard tool to characterize and analyze radioactive-labeled compounds) showed similar retention times as for the corresponding rhenium complexes **13a** and **14a**. Thus, it can be concluded that compound **6** and **12** produce with $[^{99m}Tc(H_2O)_3(CO)_3]^+$ complexes that are of identical structure to those of the rhenium analogues even under high diluted and nonstoichiometric conditions. The radioactive complexes **13b** and **14b** were tested in vitro in 1N physiological phosphate buffer (PBS, pH = 7.4) and human serum at 37 °C for 24 h. Only minor decomposition of the complex or back oxidation of the metal center to $^{99m}TcO_4^-$ was observed (< 5%) during this time. For an eventual application of these of similar compounds in diagnostic nuclear medicine, this stability would be sufficient. Details of the in vitro experiments will be published elsewhere.

Conclusion

Herein we present a synthetic strategy for the functionalization of glucose and 2-deoxyglucose at position C-1 with a nitrogen-containing side chain. The strategy can presumably be applied to functionalize other positions of monosaccharides and polysaccharides. Particularly the synthetic intermediates **4** and **10** may be useful for the formation of amine-containing ligand systems tailor-made for different metal centers. The usefulness of the bifunctionalization of the sugars for metal coordination with simple ligand systems was exemplified by reacting the derivatives with the organometallic precursors $[M(H_2O)_3(CO)_3]^+$ (M = ^{99m}Tc, Re). Whereas in the case of underivatized glucose and 2-deoxyglucose no formation of stable compounds was observed, the functionalized derivatives produced with $[M(H_2O)_3(CO)_3]^+$ single products in good yields. The novel organometallic ^{99m}Tc/Re carbohydrate complexes exhibit excellent stability under physiological in vitro conditions. These fundamental results provide the stimulus for future in vitro and in vivo experiments to determine biological affinity and activity of corresponding compounds. Furthermore, we believe that these results on labeled carbohydrate derivatives will provide additional insight for the progress and concept of bioinorganic (bioorganometallic) chemistry.

Experimental Section

General: All chemicals were purchased from Fluka or Aldrich and used without further purification. Solvents were purified and dried by standard procedures. $[NEt_4]_2[Re(CO)_3Br_3]$ was prepared as described in the literature.[18] The rhenium precursor dissolved readily in H_2O by substitution of all three bromides by water molecules to form $[Re(H_2O)_3(CO)_3]^+$ as described previously by our group.[18] All reactions were monitored by TLC on Silica Gel 60 F_{254} (Merck). Column chromatography was carried out on silica gel 60 0.062–200 mesh (Fluka). HPLC (gradient elution TEAP buffer/methanol) was done with a Merck/HITACHI L-600 Intelligent Pump with a UV detector Merck/HITACHI L-4000A and a radioactivity detector Berthold LB 506 C-1. Nucleosil 100–5 C-18 was used for the column. The NMR spectra were measured on a 300 MHz Varian Gemini 2000 spectrometer, and the peaks of the solvents were used as standard. The IR spectra were recorded on a Perkin-Elmer FT-IR 16PC. Mass spectra were measured on a Micromass VG Trio 2000 Operator or were

done by the MS service of the Organic Chemistry Department of the Federal Institute of Technology Zurich.

Synthesis of $[^{99m}Tc(H_2O)_3(CO)_3]^+$. The synthesis is described in detail in reference [15]; briefly: a sealed vial containing $NaBH_4$, Na,K-tartrate, and Na_2CO_3 was flushed for half an hour with CO gas. Afterwards TcO_4^- was added in 0.9% saline. The mixture was heated at 70 °C for 30 min and then neutralized with 1 M HCl. Yield > 98%.

Radioactive-labeling procedure: To 900 μL of a 10^{-4} M solution (physiological phosphate buffer pH = 7.4) of compounds **6** and **12**, respectively, 100 μL of a solution containing $[^{99m}Tc(H_2O)_3(CO)_3]^+$ was added. The mixture was heated at 70 °C for 30 min. The products were analyzed for their radiochemical purity (> 95%) by HPLC.

2-Hydroxyethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (1): Powdered molecular sieves (UOP type 3A) and ethylene glycol (2.7 mL, 3.02 g, 48.6 mmol) were added to α-D-acetobromoglucose (2 g, 4.8 mmol) in dry CH_2Cl_2 (25 mL). After 15 min mercury(II) bromide (1.75 g, 4.8 mmol) was added and the mixture was stirred overnight. The mixture was diluted with CH_2Cl_2 (150 mL) and filtered over a pad of celite. The organic phase was washed with 5% KI (3 × 15 mL) and water (3 × 15 mL), dried over Na_2SO_4, and the solvent was evaporated. This yielded a **1** as a slightly yellowish oil (1.35 g, 71%) that was sufficiently pure for further synthesis. ^{1}H NMR ($CDCl_3$, 25 °C): δ = 5.22 – 4.976 (m, 3H), 4.55 (d, J = 8.0 Hz, 1H; H-1), 4.19 (d, J = 4.1 Hz, 1H), 3.78 (dd, J_{12} = 4.7 Hz J_{23} = 41.2 Hz, 1H; dd, J_{12} = 3.9 Hz J_{23} = 32.7 Hz, 1H), 3.76 – 3.69 (m, 1H), 2.09 2.05 2.03 2.00 (s, 12H; H_3CC=O);^{13}C NMR ($CDCl_3$, 25 °C): δ = 171.3 170.9 170.1 170.0 (H_3CC–O), 102.1 (C-1), 73.7, 73.3, 72.6, 72.0, 69.2, 67.0, 62.7, 62.6, 21.3, 21.2 (H_3CC=O). The spectroscopic data correspond to that in the literature.[19]

2-*p*-Tolylsulfonyloxyethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (2): Toluene-4-sulfonyl chloride (1.3 g, 6.8 mmol) was added in small portions to **1** (1.34 g, 3.4 mmol) in CH_2Cl_2 (15 mL) and pyridine (1.5 mL) at 0 °C, and the solution was stirred overnight at room temperature. The mixture was diluted to 60 mL and washed with 1N HCl, saturated $NaHCO_3$, and water. The organic phase was dried over Na_2SO_4 and the solvent evaporated. Column chromatography (SiO_2, ethyl acetate/isohexane 1:1) gave **2** as a white solid (1.23 g; 66%). ^{1}H NMR ($CDCl_3$, 25 °C): δ = 7.76 (d, J = 8.5 Hz, 2H; CH arom.), 7.35 (d, J = 8.0 Hz, 2H; CH arom.), 5.12 (dd, J_{23} = 9.6 Hz J_{34} = 41.9 Hz, 1H; H-3), 5.09 (dd, J_{45} = 9.3 Hz J_{34} = 42.0 Hz, 1H; H-4), 4.92 (dd, J_{12} = 8.0 Hz J_{23} = 9.6 Hz, 1H; H-2), 4.50 (d, J = 8.0 Hz, 1H; H-1), 4.25 – 4.06 (m, 4H), 4.00 – 3.94 (m, 1H), 3.83 – 3.75 (m, 1H), 3.69 – 3.64 (m, 1H),2.07 2.04 2.01 1.99 (s, 12H; H_3CC=O); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 171.3 170.8 170.1 170.0 (H_3CC=O), 145.7 133.6 (C arom.), 130.6 128.6 (CH arom.), 101.5 (C1-H),73.3, 72.5, 71.6, 69.2, 68.9, 67.9, 62.5, 22.3 ($H_3CC_{arom.}$), 21.4 21.2 (H_3CC=O) The spectroscopic data correspond to that in the literature.[20]

2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (3): Compound **2** (1 g, 1.83 mmol) and NaN_3 (0.48 g, 7.32 mmol) in DMF (20 mL) were heated overnight at 60 °C. After cooling, the mixture was diluted with water (50 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried over Na_2SO_4. Evaporation of the solvent yielded **3** as a yellowish solid (0.72 g; 94%). ^{1}H NMR ($CDCl_3$, 25 °C): δ = 5.20 (t, J = 9.3 Hz, 1H; H-4), 5.10 (t, J = 9.6 Hz, 1H; H-3), 5.01 (dd, J_{12} = 7.9 Hz J_{23} = 9.5 Hz; H-2), 4.59 (d, J = 8.0 Hz, 1H; H-1), 4.25 (dd, J_{6a5} = 4.7 Hz J_{6a6b} = 12.4 Hz, 1H; H-6a), 4.15 (dd, J_{6b5} = 2.5 Hz J_{6a6b} = 12.4 Hz, 1H; H-6b), 4.02 (ddd, J_{vic} = 3.5 Hz J_{vic} = 5.7 Hz J_{gem} = 10.4 Hz, 1H), 3.73 – 3.65 (m, 2H), 3.53 – 3.44 (m, 1H), 3.28 (ddd, J_{vic} = 3.5 Hz J_{vic} = 4.6 Hz J_{gem} = 13.5 Hz, 1H), 2.08 2.04 2.02 2.00 (s, 12H; H_3CC=O); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 171.3 170.9 170.0 (H_3CC=O), 101.4 (C1-H), 73.5, 72.6, 71.8, 69.2, 69.0, 51.2, 21.4 21.3 21.2 (H_3CC=O) The spectroscopic data correspond to that in the literature.[21]

2-Aminoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside toluene-4-sulfonic acid salt (4):[22] Toluene-4-sulfonic acid (0.73 g, 3.85 mmol) and Lindlar catalyst (0.88 g) were added to a solution of **3** (1.46 g, 3.5 mmol) in ethanol (40 mL). The mixture was hydrogenated at 1 atm H_2 for 30 h. The mixture was diluted with ethanol and the catalyst was filtered off over a pad of celite. The evaporation of the solvent gave **4** as a white solid (1.8 g; 90%). ^{1}H NMR ($CDCl_3$, 25 °C): δ = 7.72 (d, J = 8.0 Hz, 2H; CH arom.), 7.67 (br s, 2H; NH_2), 7.18 (d, J = 8.0 Hz, 2H; CH arom.), 5.10 (t, J = 9.3 Hz, 1H; H-4), 4.99 (t, J = 9.6 Hz, 1H; H-3), 4.90 – 4.84 (m, 1H; H-2), 4.46 (d, J = 8.0 Hz, 1H; H-1), 4.32 – 4.30 (m, 1H), 4.02 – 3.95 (m, 1H; 3H), 3.65 – 3.60 (m, 1H), 3.20 – 3.10 (m, 2H), 2.37 (s, 3H; $C_{arom.}$-CH_3), 2.02, 2.00, 1.97 (s, 12H; H_3CC=O); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 171.7 170.7 170.4 170.0 (H_3CC=O), 142.1 141.4 (C arom.), 129.7 126.6 (CH arom.) 101.7 (C1-H), 73.3, 72.6, 71.6, 69.2, 68.6, 62.0, 41.0, 22.0 ($H_3CC_{arom.}$) 21.4 21.3 21.2 (H_3CC=O); IR (KBr): $\tilde{\nu}$ = 3034 br, 1744 vs, 1624 w, 1526 w, 1440 w, 1374 s, 1268 vs, 1236 vs, 1126 s, 1090 s, 1040 vs, 11010 s, 908 w, 816 w, 682 m, 568 m cm^{-1}; MS (ES): m/z (%): 392 (100) [M+1].

1-*O*-[2-(Dimethyl iminodiacetato)ethyl]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (5): Triethylamine (1.2 mL, 0.88 g, 8.7 mmol) and methyl bromoacetate (0.54 mL, 0.9 g, 5.88 mmol) were added to **4** (1.58 g, 2.8 mmol) in THF (30 mL) and the solution was refluxed overnight. After cooling, the mixture was filtered, diluted with CH_2Cl_2 (30 mL), and washed with water. The organic phase was dried over Na_2SO_4 and the solvent was evaporated. Column chromatography (SiO_2, ethyl acetate/isohexane 2:1) gave **5** as a yellowish solid (0.98 g; 65%). ^{1}H NMR ($CDCl_3$, 25 °C): δ = 5.17 (t, J = 9.3 Hz, 1H; H-4), 5.06 (t, J = 9.6 Hz, 1H; H-3), 4.95 (dd, J_{12} = 8.0 Hz J_{23} = 9.6 Hz, 1H; H-3), 4.57 (d, J = 8.0 Hz, 1H; H-1) 4.26 – 4.10 (m, 2H; H-6), 3.97 – 3.92 (m, 1H, H-5), 3.71 – 3.65 (m, 2H), 3.68 (s, 6H; $COOCH_3$), 3.59 (s, 4H; NCH_2COOCH_3), 3.01 – 2.94 (m, 2H), 2.07 2.03 2.00 1.98 (s, 12H; H_3CCOO); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 172.2 ($COOCH_3$), 171.3 170.9 170.1 170.0 (H_3CC=O), 101.2 (C1-H), 73.6, 72.4, 772.0, 69.9.7, 69.1, 62.6, 56.1, 54.4, 52.2, 21.4 21.3 (H_3CC=O); IR (KBr): $\tilde{\nu}$ = 2960 w, 2894 vw, 1758 vs, 1434 m, 1378 m, 1226 s, 1172 s, 1062 s, 1036 s, 946 w cm^{-1}; MS (FAB): m/z (%): 536 (100) [M+1].

[2-(Iminodiacetato)ethyl]-β-D-glucopyranosyl disodium salt (6): Sodium methanolate (0.067 g, 1.23 mmol) was added to a solution of **5** (0.66 g, 1.23 mmol) in dry methanol (33 mL). After the mixture had been stirred for 4 h at room temperature, glacial acetic acid (0.07 mL, 0.074 g, 0.37 mmol) was added and the solvent evaporated. The residue was dissolved in water (33 mL) and 1M NaOH (2.5 mL) was added. The mixture was stirred overnight at room temperature and the solvent was evaporated. After drying in a desiccator, **6** was obtained as an amorphous yellowish solid (0.56 g; 98%). The product was sufficiently pure for the labeling. ^{1}H NMR (D_2O, 25 °C): δ = 4.47 (d, J = 7.97 Hz, 1H; H-1), 4.05 – 3.98 (m, 1H; H-B), 3.91 (dd, J = 1.9 Hz J' = 12.4 Hz, 1H; H-6), 3.79 – 3.69 (m, 2H; H-6′ H-C) 3.51 (t, J = 9.1 Hz, 1H; H-3), 3.48 – 3.72 (m, 1H; H-5), 3.39 (t, J = 9.1 Hz, 1H; H-4), 3.33 – 3.27 (m, 1H; H-2), 3.26 (s, 4H; H-A/X), 2.87 -2.84 (m, 2H; H-D/E), 1.91 (s, 3H; NaOAc); IR (KBr): $\tilde{\nu}$ = 3424 br, 1584 s, 1410 s, 1334 w, 1222 w, 1162 vw, 1078 m, 1040 m, 626 w cm^{-1}; MS (ES): m/z (%): 338 (100) [M – 2Na+1].

(2-Hydroxyethyl)-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranoside (7):[23] Preparation of the acid resin: Ag 50W-X2 (50 g, H^+ form, 100 – 200 mesh, moisture content 72 – 84%) was washed with water (3 × 100 mL) until the filtrate was colorless and then with acetonitrile (10 × 70 mL). Afterwards it was dried in a desiccator over phosphorus pentoxide for 24 h to give the dry resin. Tri-*O*-acetyl-D-glucal (3 g, 11 mmol) and dry LiBr (3.35 g, 38.57 mmol) were dissolved in dry acetonitrile (36 mL). Powdered molecular sieves (2.4 g, UOP type 3A) and the acid resin (4.56 g) were added. After the mixture had been stirred for a few minutes, ethylene glycol (6.45 mL, 7.18 g, 275.47 mmol) was added. The mixture was stirred for a further 5 h and then filtered, the solution was neutralized with triethylamine, and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 and washed once with ice cold 1M HCl and once with saturated $NaHCO_3$. The organic phase was dried over Na_2SO_4 and the solvent was evaporated. Column chromatography (SiO_2, ethyl acetate/isohexane 2:1) gave **7** as a colorless oil (1.48 g; 40%). ^{1}H NMR ($CDCl_3$, 25 °C): δ = 5.31 (ddd, J = 5.5 Hz J' = 9.3 Hz, 1H; H-3), 4.99 (t, J = 9.6 Hz, 1H; H-4; br d, J = 3.3 Hz, 1H; H-1), 4.27 (dd, J_{6a} = 4.7 Hz J_{6a6b} = 12.1 Hz; H-6a), 4.06 (dd, J_{6b} = 2.2 Hz, J_{6a6b} = 12.1 Hz, 1H; H-6a), 4.03 – 3.98 (m, 1H; H-5), 3.76 – 3.70 (m, 2H), 3.61 – 3.55 (m, 1H), 2.28 (dd, $J_{12eq.}$ = 4.1 Hz $J_{2eq.2ax.}$ = 12.9 Hz; dd, J = 6.6 Hz J' = 13.2 Hz, 1H; H-2 equiv), 2.08 2.03 2.01 (s, 9H; 3 H_3CCOO), 1.83 (dd, $J_{12ax.}$ = 8.0 Hz $J_{2eq.2ax.}$ = 13.2 Hz; dd, J = 15.4 Hz J' = 12.9 Hz, 1H; H-2ax.); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 171.3 170.8 170.5 (H_3CC=O), 98.0 (C1-H), 70.3, 70.0, 69.6, 68.7, 63.1, 62.4, 35.6, 21.6 21.4 (H_3CC=O); IR ($CHCl_3$): $\tilde{\nu}$ = 2942 w, 1740 vs, 1370 m, 1239 vs, 1138 m, 1080 m, 1044 s, 1002 m, 984 m, 604 w cm^{-1}; MS (ES): m/z (%): 357 (100) [M+Na].

2-*p*-Tolylsulfonyloxyethyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranoside (8): Compound **8** was prepared according to the procedure described for **2**. Yield: 78%. ^{1}H NMR ($CDCl_3$, 25 °C): δ = 7.79 (d, J = 8.5 Hz, 2H; CH arom.), 7.36 (d, J = 8.2 Hz, 2H; CH arom.), 5.22 (dd J = 5.5 Hz J' = 11.5 Hz; dd, J = 5.2 Hz J' = 11.5 Hz, 1H; H-3), 4.96 (t, J =

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9.6 Hz, 1H; H-4), 4.90 (br d, J = 4.1 Hz, 1H; H-1), 4.25 (dd, J_{56a} = 4.7 Hz J_{6a6b} = 12.3 Hz; H-6a), 4.17 (t, J = 4.7 Hz, 2H; CH_2), 4.00 (dd, J_{56b} = 2.5 Hz, J_{6a6b} = 12.3 Hz, 1H; H-6a), 3.94–3.88 (m, 1H; H-5), 3.85–3.77 (m, 1H), 3.67–3.60 (m, 1H), 2.44 (s, 3H; $C_{arom.}$-CH_3) 2.17 (dd, $J_{12eq.}$ = 4.1 Hz $J_{2eq.2ax.}$ = 13.0 Hz; dd, J = 6.6 Hz J' = 12.9 Hz, 1H; H-2 eq.), 2.07 2.03 1.99 (s, 9H; 3 H_3CCOO), 1.83 (dd, $J_{12ax.}$ = 8.0 Hz $J_{2eq.2ax.}$ = 13 Hz; dd, J = 15.2 Hz J' = 12.9 Hz, 1H; H-2ax.); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 171.3 170.7 170.5 (H_3CC=O), 145.7 133.5 (C arom.), 130.6 128.6 (CH arom.), 97.8 (C1-H),69.8, 69.4, 96.3, 68.8, 65.8, 62.8, 35.3, 22.3 ($H_3CC_{arom.}$), 21.5 21.4 (H_3CC=O); IR ($CHCl_3$): $\tilde{\nu}$ = 2926 w, 1742 vs, 1368 m, 1194 m, 1176 m, 924 w, 602 w cm^{-1}; MS (ES): *m/z* (%): 511 (100) [M+Na].

2-Azidoethyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranoside (9): Compound **9** was prepared according to the procedure described for **3**. Yield: 80%. ^{1}H NMR ($CDCl_3$, 25 °C): δ = 5.33 (dd J = 5.5 Hz J' = 11.6 Hz; dd, J = 5.2 Hz J' = 11.6 Hz, 1H; H-3), 5.01 (t, J = 9.6 Hz, 1H; H-4; br d, J = 4.1 Hz, 1H; H-1), 4.31 (dd, J_{56a} = 4.7 Hz J_{6a6b} = 12.1 Hz; H-6a), 4.08 (dd, J_{56b} = 2.5 Hz, J_{6a6b} = 12.1 Hz, 1H; H-6a), 4.05–3.98 (m, 1H; H-5), 3.86–3.80 (m, 1H), 3.65–3.58 (m, 1H), 3.43 (t, J = 5.1 Hz, 2H; CH_2), 2.31 (dd, $J_{12eq.}$ = 4.1 Hz $J_{2eq.2ax.}$ = 13.1 Hz; dd, J = 6.6 Hz J' = 12.9 Hz, 1H; H-2 eq.), 2.09 2.05 2.02 (s, 9H; 3 H_3CCOO), 1.85 (dd, $J_{12ax.}$ = 7.97 Hz $J_{2eq.2ax.}$ = 13.1 Hz; dd, J = 15.4 Hz J' = 12.9 Hz, 1H; H-2ax.); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 171.1 170.5 (H_3CC=O), 97.9 (C1-H), 69.9, 69.5, 68.9, 67.2, 63.0, 51.1, 35.5, 21.6 21.4 (H_3CC=O); IR ($CHCl_3$): $\tilde{\nu}$ = 2108 s, 1742 vs, 1370 m, 1136 m, 1052 s, 984 w, 602 w cm^{-1}; MS (ES): *m/z* (%): 382 (100) [M+Na].

2-Aminoethyl-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranoside toluene-4-sulfonic acid salt (10): Compound **10** was prepared according to the procedure described for **4**. Yield: 95%. ^{1}H NMR ($CDCl_3$, 25 °C): δ = 7.87 (br s, 2H; NH_2), 7.70 (d, J = 8.0 Hz, 2H; CH arom.), 7.09 (d, J = 7.7 Hz, 2H; CH arom.), 5.24–5.16 (m, 1H; H-3), 4.90 (t, J = 9.6 Hz; H-4), 4.81 (br d, J = 2.5 Hz, 1H; H-1), 4.20 (dd, J_{56a} = 4.1 Hz J_{6a6b} = 12.4 Hz, 1H; H-6a), 3.93–3.86 (m, 2H; H-5 H-6b), 3.77–3.73 (m, 1H), 3.58–3.49 (m, 1H), 3.10 (br s, 2H), 2.34 (s, 3H; $C_{arom.}$-CH_3), 2.22–2.16 (m, 1H; H-2 eq.), 2.02 1.95 1.94 (s, 9H; H_3CC=O), 1.65–1.56 (m, 1H; H-2ax.); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 171.3, 170.8, 170.5 (H_3CC=O), 141.9, 141.2 (C arom.), 129.6, 126.6 (CH arom.) 97.9 (C1-H), 69.8, 69.6, 68.6, 64.1, 62.7, 40.0, 35.0, 22.0 (H_3C-$C_{arom.}$) 21.6, 21.4, 21.3 (H_3CC=O); IR (KBr): $\tilde{\nu}$ = 3450 br, 1744 vs, 1370 m, 1234 vs, 1126 s, 1038 s, 1012 s, 820 w, 686 m, 602 m cm^{-1}; MS (ES): *m/z* (%): 334 (100) [M+1].

2-(Methyliminodiacetato)ethyl-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranoside (11): Compound **11** was prepared according to the procedure described for **5** and **10**. Yield: 66%. ^{1}H NMR ($CDCl_3$, 25 °C): δ = 5.27 (dd, J = 5.5 Hz J' = 11.5 Hz; dd, J = 5.2 Hz J' = 11.3 Hz, 1H; H-3), 4.97 (t, J = 9.6 Hz; H-4), 4.94 (br d, J = 1.9 Hz, 1H; H-1), 4.31 (dd, J_{56a} = 4.4 Hz J_{6a6b} = 12.1 Hz, 1H; H-6a), 4.02 (dd, J_{56b} = 2.2 Hz J_{6a6b} = 12.1 Hz, 1H; H-6b), 3.99–3.94 (m, 1H; H-5), 3.79–3.72 (m, 1H), 3.70 (s, 6H; $COOCH_3$), 3.62 (s, 4H; N-CH_2-COO-), 3.52–3.50 (m, 1H) 2.98 (t, J = 5.5 Hz, 2H) 2.20 (dd, J_{12eq} = 4.4 Hz $J_{2eq.2ax.}$ = 12.9 Hz; dd, J = 6.6 Hz J' = 12.2 Hz, 1H; H-2 eq.), 2.07 2.02 1.99 (s, 9H; 3 H_3CCOO), 1.80 (dd, $J_{12ax.}$ = 8.1 Hz $J_{2eq.2ax.}$ = 12.7 Hz; dd, J = 15.4 Hz J' = 12.9 Hz, 1H; H-2ax.); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 172.4 ($COOCH_3$), 171.4 170.7 (H_3CC=O), 97.7 (C1-H), 73.6, 70.4, 69.7, 67.6, 63.0, 56.3, 54.3, 52.3, 35.6, 21.6, 21.4 (H_3CC=O): IR ($CHCl_3$): $\tilde{\nu}$ = 1742 vs, 1368 w, 1050 m cm^{-1}; MS (FAB): *m/z* (%): 478 (100) [M+1].

2-(Iminodiacetato)ethyl-2-deoxy-α-D-*arabino*-hexopyranoside disodium salt (12): Compound **12** was prepared according to the procedure described for **6**. Yield: 98%. ^{1}H NMR (D_2O, 25 °C): δ = 5.00 (d, J = 3.3 Hz, 1H; H-1), 3.91–3.90 (m, 1H; H-3) 3.87 (dd, J = 2.2 Hz J' = 12.4 Hz, 1H; H-6), 3.83–3.74 (m, 2H; H-6′ H-B), 3.68–3.56 (m, 2H; H-5 H-C), 3.34 (t, J = 9.5 Hz, 1H; H-4) 3.27 (s, 4H; H-A/X), 2.89–2.85 (m, 2H; H-D/E) 2.20–2.14 (m, 1H; H-2), 1.92 (s, 3H; NaOAc), 1.74–1.64 (m, 1H; H-2′); IR (KBr): $\tilde{\nu}$ = 3422 br, 1584 vs, 1410 s, 1334 m, 1266 w, 1196 m, 1126 m, 1064 m cm^{-1}; MS (ES): *m/z* (%): 322 (100) [M − 2Na+1].

[2-(Iminodiacetato)ethyl]-β-D-glucopyranosyltricarbonylrhenate sodium salt (13a): The disodium salt **6** (42 mg, 0.09mmol) was dissolved in water (5 mL), and $[NEt_4]_2[Re(CO)_3Br_3]$ (70 mg, 0.09 mmol) was added. After 2 h at 50 °C the solvent was evaporated and the residue was dried overnight in a desiccator. CH_2Cl_2 was added to the residue to dissolve most of the formed tetraethylammonium bromide. The precipitate was filtered and dried in vacuum. Yield 90%. ^{1}H NMR (D_2O, 25 °C): δ = 4.53 (d, J = 8.0 Hz, 1H; H-1), 4.34–4.23 (m, 1H; H-B), 4.10–4.01 (m, 1H; H-C) 4.00 (2 d, J = 16.8 Hz, 2H; H-A), 3.97 (dd, J = 1.9 Hz J' = 12.4 Hz; 1H, H-6), 3.82 (2d, J = 16.8 Hz, 2H; H-X), 3.82–3.74 (m, 3H; H-6′ H-D/E), 3.54 (t, J = 9.1 Hz, 1H; H-3) 3.53–3.48 (m, 1H; H-5), 3.44 (t, J = 9.1 Hz, 1H; H-4) 3.38–3.33 (m, 1H; H-2), 1.91 (s, 3H; NaOAc) IR (KBr): $\tilde{\nu}$ = 3444 br, 2024 vs, 1906 vs, 1636 vs, 1396 m, 1076 m, 1038 m cm^{-1}; MS (ES): *m/z* (%): 606, 608 (100) [M^-].

2-(Iminodiacetato)ethyl-2-deoxy-α-D-*arabino*-hexopyranosyltricarbonylrhenate sodium salt (14a): Compound **14a** was prepared according to the procedure described for **13a**. Yield: 81%. ^{1}H NMR (D_2O, 25 °C): δ = 5.06 (d, J = 2.5 Hz; 1H), 4.06 (d, J = 16.5 Hz, 2H; H-A),4.00–3.89 (m, 5H), 3.58–3.72 (m, 4H), 3.75 (d, J = 16.5 Hz, 2H; H-X), 3.69–3.63 (m, 1H), 3.34 (t, J = 9.5 Hz, 1H; H-4), 2.25–2.20 (m, 1H; H-2a), 1.78–1.72 (m, 1H; H-2b); IR (KBr): $\tilde{\nu}$ = 3422 br, 2022 s, 1892 vs, 1636 s, 1394 m, 1062 m, 1028 m cm^{-1}; MS (ES): *m/z* (%): 592, 590 (100) [M^-], 430, 428 (80) [$C_9H_8NO_5Re^-$].

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