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Lanthanide(III) complexes of pyridine–tetraacetic acid-glycoconjugates: Synthesis and luminescence studies of mono and divalent derivatives

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ABSTRACT

A potent lanthanide chelate, fulfilling the requirements for the development of MRI contrast agents or luminescent probes, was armed with alkyne groups. We then implemented a click methodology to graft the bifunctional ligand to azide-containing glucoside and maltoside scaffolds. The resulting hydrophilic glycoconjugates retained the ligand binding capacity for Eu^{3+} or Tb^{3+} ion as evidenced by the number of bound water molecules to the lanthanide ion. Divalent Eu^{3+} and Tb^{3+} complexes were shown to double the brightness of the emitted fluorescent signal compared to its monovalent derivatives. Designing multivalent lanthanide luminescent probes would enable the fluorescent signal of labeled biomolecules to be enhanced.

The development of metal–carbohydrate conjugates for cellular imaging and clinical diagnosis is an extensive field of research. A wide range of glycoconjugates bearing contrast agents and radioelements, where the sugar moieties serve as biocompatible scaffolds or ligands to target specific organs, has been described. In nuclear medicine, several research groups have explored the development of glucose conjugates as new radiotracers in tumor oncology. Approaches generally consist of grafting stable radioactive technetium (Tc^{99m}) chelates to glycoside scaffolds with the aim of selectively targeting and irradiating cancer cells overexpressing glucose transporters (GLUTs).[1,2] Gadolinium complexes are commonly used in magnetic resonance imaging (MRI) to enhance local contrast. Starch and inulin polysaccharides bearing multiple Gd(III) were shown to exhibit both excellent biocompatibility and high molecular relaxivity leading to enhanced signals.[3,4] Interestingly, much shorter saccharide scaffolds, such as β-cyclodextrin and triaminoglycoside chitosan, functionalized with seven and three Gd-DTPA, respectively, were shown to enhance the relative signal intensity per Gd.[5,6]

Luminescent carbohydrate-based lanthanide probes for sensing biological substrates have been much less explored.[7] The photophysical properties of lanthanide probes are particularly suited for the time-gated luminescence detection of biological events.[8] They combine narrow bands in the visible region (Eu^{3+}, Tb^{3+}, Sm^{3+}, Dy^{3+}) and NIR region (Nd^{3+}, Yb^{3+}), long excited-state lifetimes (µs and ms range) and large Stokes's shifts.[9] The emission of lanthanide ions is usually carried out by an indirect process called the 'antenna effect' in which energy from the excited state of a sensitizer (a chromophoric group incorporated into a chelating unit) transfers to the excited states of the lanthanide ion, which emits in its emissive region.[10] The chelating unit is expected to form water-soluble lanthanide complexes of high kinetic stability and to provide an efficient shielding of the Ln^{3+} ion to prevent unwanted non-radiative deactivations from surrounding water molecules.[11] In the course of research on bimodal lanthanide probes suitable for optical and magnetic resonance imaging, we[12] and others[13] have shown that Ln^{3+} complexes derived from pyridine–tetraacetic acid possess the physical requirements for MRI (Ln = Gd) and optical imaging (Ln = Eu, Nd).

In this context, we describe herein a synthetic method to tether a new functionalized pyridine–tetraacetic ligand to glucoside and maltoside scaffolds (Fig. 1). We wanted to assess if the complexing abilities and the photophysical properties of such chelates were retained after their tethering to the sugar moieties. The alkynyl-armed pyridine chelate with pendant iminodiacetic groups was

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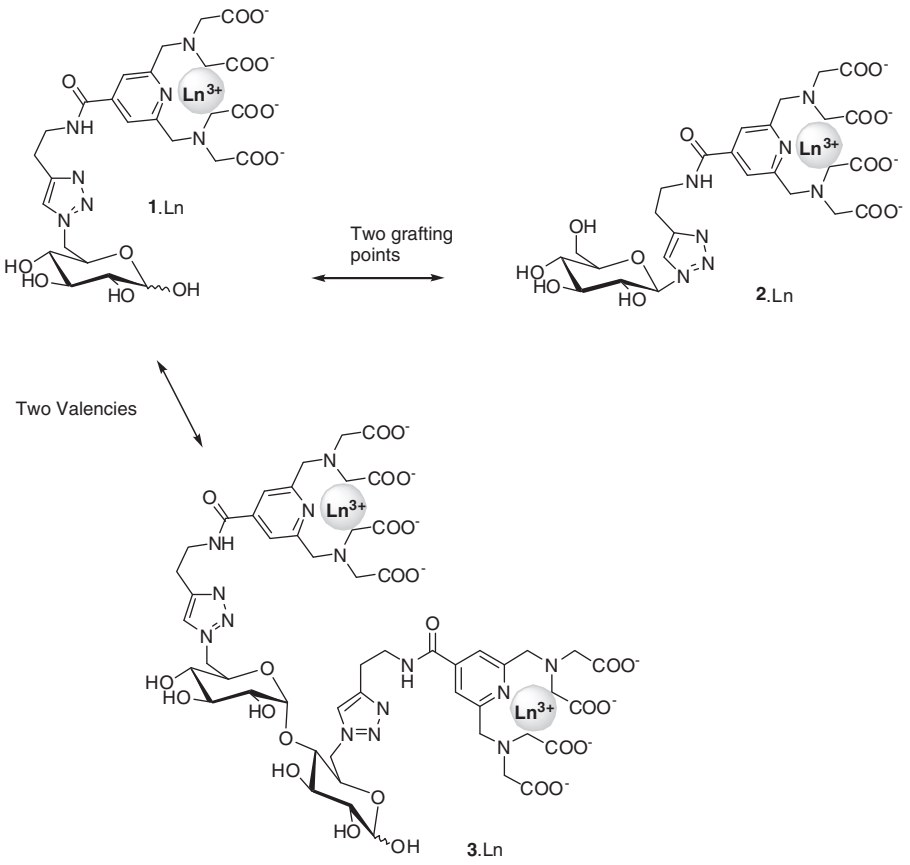


Figure 1. Structure of the lanthanide(III) complexes of the functionalized pyridine–tetraacetic acid glycoconjugates **1**, **2** and **3**.

grafted by click chemistry to hydrophilic and biocompatible glucoside and maltoside scaffolds. The bifunctional pyridine-based ligand was grafted onto either the C-6 or the anomeric position of the sugars (compounds **1** and **2**). In fact, the specific topologies adopted by the scaffold may prevent the access of water molecules to the inner-sphere coordination site, as previously described for Gd complexes.[14] The grafting position may therefore be detrimental to the efficiency of the probes. The divalent compound **3** was designed to accommodate two lanthanide ions. The modulation of the valency in **1** and **3** does not introduce new functional groups and should not significantly affect the hydrophilicity and spatial presentation of the pyridine-based chelate, because a critical glucoside fragment is repeated. The presence of several chelating agents tethered to a common scaffold is useful for delivering a larger payload of lanthanides to a specific target or for enhancing signal intensity with Gd ions.[3–6,15] We have recently reported the synthesis of a set of bifunctional carbohydrate cores with up to seven azides and bearing a terminal amino group.[16] These scaffolds may serve as valuable platforms for the delivery of lanthanide chelates to specific biological receptors. The chemical strategy developed here and the analysis of the photophysical properties of the new lanthanide–carbohydrate conjugates give first insights into the possibility of designing multivalent luminescent lanthanide chelates.

We first designed azido-functionalized carbohydrate scaffolds **4**[17]–**6** (Fig. 2). Compounds **5** and **6** were directly obtained from unprotected glucose and maltose, respectively, by a protocol that we previously implemented for the selective azidation of carbohydrates.[18] The 'one-pot' method consists of adding a PPh_3:CBr_4:NaN_3[19] mixture in a 2:2:10 ratio relative to the sugar unit, followed by acetylation of the crude mixture.

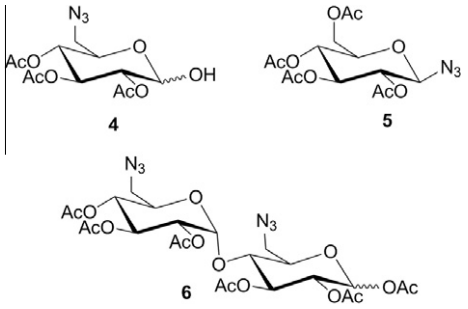
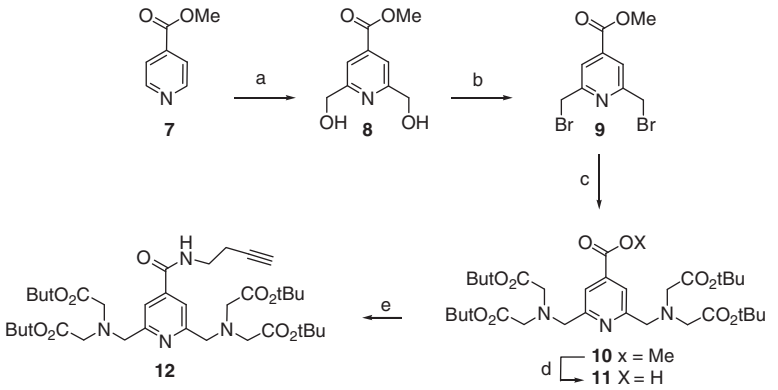


Figure 2. Structure of the azido-functionalized carbohydrates **4**–**6**.

We then focused our effort on the synthesis of the pyridine–tetraacetic acid ligand bearing an alkynyl group (Scheme 1). Scharbert and co-workers[20] previously described the radical alkylation of methyl isonicotinate with ammonium persulfate and silver salts with 30% yields. In our laboratory, these conditions led to compound **7** with less than 10% yield. We were delighted to see that a significantly higher yield of 60% was reached when the silver salt was substituted with iron dichloride. Phosphorus tribromide allowed the conversion of the diol **8** to **9** with 71% yield. The two bromide ions were successfully substituted by di-*tert*-butyliminodiacetate in acetonitrile to provide **10**. Selective hydrolysis of the methyl ester group led to the corresponding carboxylic acid **11**[21] quantitatively (overall yield: 40%). In a similar manner, Lebeau[22] obtained **11** in 4 steps starting from methyl isonicotinate; however, no characterization was indicated. After in situ activation of this group by PyBOP, and coupling with 3-butynyl amine,[23] the



Scheme 1. Synthesis of the alkynyl-armed bifunctional chelate **12**. Reagents and conditions: (a) $(NH)_4S_2O_8$ (10 equiv), $FeCl_2{\cdot}4H_2O$ (0.25 equiv), H_2SO_4 cat., MeOH/H_2O (1/1), 50 °C, 17 h, 60%; (b) PBr_3 (3 equiv), CH_3CN, 60 °C, 8 h, 71%; (c) di-*tert*-butyl iminodiacetate (2 equiv), Na_2CO_3, CH_3CN, 100 °C, 16 h, 95%; (d) K_2CO_3 (1 equiv), MeOH/H_2O (2/1), reflux, 2 h then HCl, quant.; (e) 3-butyn-1amine hydrochloride (1.3 equiv), DIPEA (5 equiv), PyBOP (1.6 equiv) CH_2Cl_2, rt, 24 h, 62%.

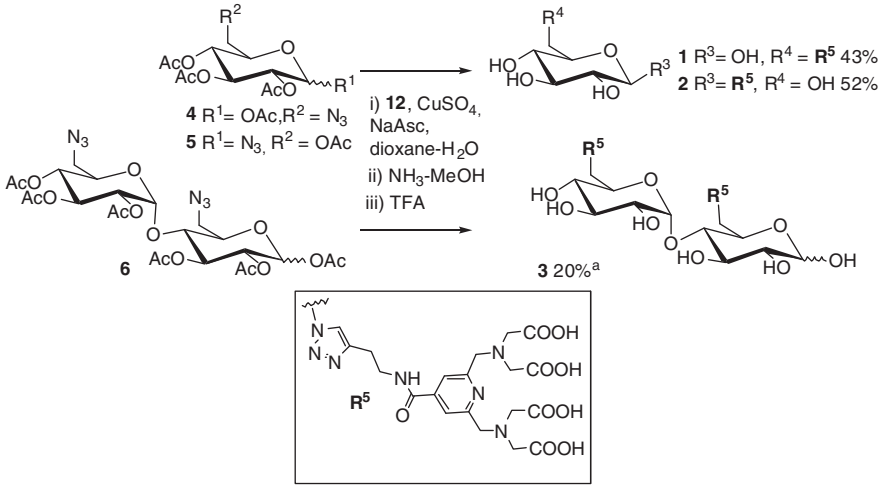
bifunctional chelate **12**[21] was obtained with 62% yield, after chromatographic purification on alumina.

We then developed a method to tether **12** to the azido-functionalized carbohydrate cores **4**, **5** and **6** with a single purification step (Scheme 2). A copper-catalyzed azide alkyne cyclization (CuAAC) protocol was first performed in a mixture of dioxane and water.[24] We recently successfully applied this click protocol to the design of radiolabeled glucose conjugates.[2f] Stoichiometric (and not catalytic) amounts of copper sulfate were used for the cyclization, due to the high potency of the compounds to chelate and inactivate copper. The formation of such complexes was shown by ESI-MS analysis of the crude mixture. Extraction with an EDTA solution provided the protected glycoconjugates free of copper by a transchelation mechanism. The exclusive formation of the 1,4-regioisomers was confirmed by the large $\Delta(\delta_{C\text{-}4}-\delta_{C\text{-}5})$ values (>20 ppm) observed by ^{13}C NMR.[25] Protecting groups were sequentially removed using an aqueous solution of ammonia for the acetates and, after evaporation, by dissolution in pure TFA for the *tert*-butyl groups. Crude glycoconjugates were finally purified by preparative HPLC on a C-18 column to lead to pure **1**[26], **2** and **3**.

The Ln^{3+} complexes (Ln = Eu, Tb) of the glycoconjugates **1** and **2** were readily prepared by mixing equimolar quantities of the lanthanide salt ($LnCl_3$, $6H_2O$) in water. For glycoconjugate **3**, two equivalents of lanthanide salt were used. The solutions were then adjusted to a final concentration of 1×10^{-5} M in Tris buffer (pH 7.4, 50 mM). The UV–vis absorption spectra of the Eu and Tb complexes display broad bands in the UV domain with maxima at 276 nm; this absorption band is typical of the π–π^* transitions attributed to the pyridine moiety. The values of the absorption coefficients of the complexed glycoconjugates **1**, **2** and **3** ($\varepsilon \sim 2500$ vs 5600 M^{-1} cm^{-1}) are in agreement with the presence of one or two chelating agents grafted onto the carbohydrate scaffolds. Upon excitation in this absorption band, both europium and terbium complexes display sizeable and structured lanthanide emission luminescence in the visible spectra region (Fig. 3) characteristic of the lanthanide ion, indicating that the energy is absorbed by the chromophoric group and efficiently transferred to the chelated Ln^{3+} ion. The most intense peaks are the transitions $^5D_0 \rightarrow {}^7F_2$ (Eu^{3+}) and $^5D_4 \rightarrow {}^7F_5$ (Tb^{3+}) at 616 and 545 nm, respectively.

The luminescence lifetimes (τ) and quantum yields (Φ) were measured in aerated Tris buffer solutions at pH 7.4. As presented in Table 1, the luminescence properties (τ, Φ) of the pyridine-glycoconjugates are quite similar within the europium complexes **1**.Eu, **2**.Eu and **3**.Eu (τ = 0.39 ms, Φ 0.4–0.6%) and the terbium complexes **1**.Tb, **2**.Tb and **3**.Tb (τ = 1.18 ms, Φ 2.3–3%). The comparison of the dependence of the lifetimes of the complexes in H_2O (τ_H) and D_2O (τ_D) at 298 K allows an assessment of the hydration state of the Ln^{3+} using Parker's equation.[27]

The value of the hydration number q of 2 is in agreement with the seven complexation sites of the grafted chelate, and matches the literature value for the corresponding unfunctionalized pyridine chelate.[12] Additionally, the grafting position of the chelate to the sugar does not significantly modulate the luminescence properties and the sugar scaffolds do not seem to interfere with



Scheme 2. Synthetic procedure to obtain glycoconjugates **1**, **2** and **3**[a]. Yields given are for three steps after HPLC purification.

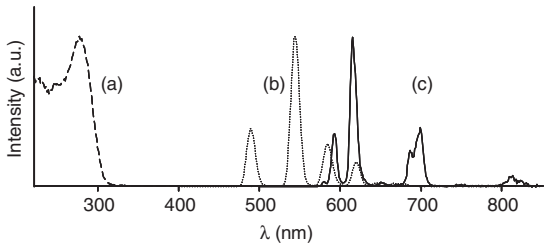


Figure 3. (a) Excitation (λ_{em} = 616 or 545 nm) and emission (λ_{exc} = 276 nm) spectra of **3**.Tb (b), **3**.Eu (c) in Tris buffer at 298 K.

Table 1
Absorption maxima wavelengths (λ_{max} in nm) and their corresponding molar absorption coefficients (ε in M^{-1} cm^{-1}), luminescence lifetimes (τ in ms), quantum yields (Φ in %), hydration state (q), brightness (B in M^{-1} cm^{-1}) for the Eu^{3+} and Tb^{3+} glycoconjugates in Tris buffer solution at 298 K (pH 7.4, 50 mM)

Compound	λ_{max} (ε)	τ_H[a]	τ_D[a]	Φ	q[b]	B[c]
1.Eu	276 (2420)	0.39	2.33	0.4	2.02	10
2.Eu	276 (2550)	0.39	2.30	0.6	2.02	15
3.Eu	276 (5600)	0.39	2.35	0.6	2.03	34
1.Tb	276 (2355)	1.18	2.45	2.3	1.90	54
2.Tb	276 (2415)	1.18	2.55	2.9	1.97	70
3.Tb	276 (5575)	1.18	2.46	3.0	1.90	167

[a] Data obtained in aerated Tris buffer, H_2O (H) or D_2O (D) solutions.
[b] For europium complexes: $q = 1.11(1/\tau_H - 1/\tau_D - 0.31)$; for terbium complexes: $q = 5(1/\tau_H - 1/\tau_D - 0.06)$.
[c] Determined at 276 nm.

Ln^{3+} complexation. Meade and co-workers[28] previously showed that such an effect could occur with specific sugar residues and the Gd ion. They reported that a galactopyranose sugar moiety tethered to a DO3A-Gd(III) complex could sterically prevent water access to the Gd ion.

The brightness value, corresponding to the product of the absorption coefficient at an excitation wavelength and the quantum yield, is indicative of the overall luminescence efficiency of the complexes. Grafting two pyridine-chelates to a sugar scaffold enhances the luminescence emission intensity by a factor of 2–3 compared to the corresponding mono-chelate. It seems, therefore, that the tethered lanthanide complexes do not self-quench in contrast to fluorescent organic probes.[29] Indeed, two fluorescent probes in close proximity may self-quench if their absorption and emission spectra overlap. In this case, the large Stokes shift of lanthanide complexes overcomes this undesirable self-quenching phenomenon.

A click methodology was implemented to graft an alkynyl pyridine-chelate to hydrophilic and biocompatible carbohydrate scaffolds. The resulting glycoconjugates were shown to retain their complexing abilities towards Eu^{3+} and Tb^{3+} with regard to the corresponding unfunctionalized chelate. The divalent conjugates **3**.Ln (Ln = Eu, Tb) were two to three times more fluorescent than their monovalent counterparts. Thus, labeling biomolecules with multivalent lanthanide probes may offer interesting opportunities to increase the detection threshold of specific targeted biomolecules. In further investigations, we will explore the relaxivity properties of the corresponding Gd-conjugates, and apply the synthetic methodology developed to the design of carbohydrate lanthanide probes with higher valency.

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21. Selected spectral data for **11** and **12**. Compound **11**: NMR ^1H ($CDCl_3$, 300 MHz) δ (ppm): 1.45 (s, 36H: tBu); 3.53 (s, 8H: CH_2COO); 4.13 (s, 4H, CH_2py); 8.11 (s, 2H, H_{py}). NMR ^{13}C ($CDCl_3$, 75 MHz) δ (ppm): 28.1 (CH_3); 52.5 (NCH_2COO); 55.8 (NCH_2py); 81.4 (Cq tBu); 121.6 ($C_{3,5\text{-py}}$); 142.75 ($C_{4\text{-py}}$); 158.9 ($C_{2,6\text{-py}}$); 167.9 (COOH); 170.5 (COOtBu). MS (ES^+) = 660.3 [M+Na^+]; 638.1 [M+H^+]. Compound **12**: NMR ^1H ($CDCl_3$, 300 MHz) δ (ppm): 1.44 (s, 36H: tBu); 2.03 (t, 1H, J = 2.4 Hz: $HC\equiv$); 2.52 (td, 2H, J = 6.6 Hz, J = 2.7 Hz: $CH_2C\equiv$); 3.47 (s, 8H: CH_2COO); 3.60 (q, 2H, J = 6.6 Hz: CH_2NH); 4.06 (s, 4H, CH_2py); 6.94 (m, 1H, N*H*); 7.91 (s, 2H, H_{py}). NMR ^{13}C ($CDCl_3$, 75 MHz) δ (ppm): 19.3 ($CH_2C\equiv$); 28.1 (CH_3); 38.7(CH_2NH); 56.0 (NCH_2COO); 59.8 (NCH_2py); 70.1 ($\equiv CH$); 81.2 (Cq tBu); 81.4 ($CH_2C\equiv$); 118.6 ($C_{3,5\text{-py}}$); 142.7 ($C_{4\text{-py}}$); 159.9 ($C_{2,6\text{-py}}$); 166.2 (CONH); 170.5 (COOtBu). MS (ES^+) = 711.4 [M+Na^+] (100); 689.4 [M+H^+] (55) HR-MS (TOF ES^+) = calculated for M+H^+ = 689.4126; found = 689.4111.
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26. Experimental procedure and analytical data for **1**. Compound **5** (10 mg, 26.8 μmol) and **13** (20.3 mg, 29.4 μmol) were dissolved in a dioxane/water mixture (2/0.5 mL). Copper sulfate (4.2 mg, 26.3 μmol) and sodium ascorbate (10 mg, 54 μmol) were added and the mixture was stirred at 70 °C for 45 min under μW irradiation. The mixture was evaporated under reduced pressure, dissolved in dichloromethane (10 mL), and the organic layer washed with an aqueous solution of ethylenediamine tetraacetic acid trisodium salt (500 mg in 10 mL) and water (10 mL). The organic layer was dried over $MgSO_4$, filtered

and evaporated under reduced pressure. The residue was dissolved in a methanolic solution of ammonia 7 M (5 mL) and the solution was stirred for 3 h. The solvent was evaporated under reduced pressure and the residue dissolved in pure TFA (10 mL). The mixture was stirred for 12 h at rt. The solvent was evaporated and the residue purified by HPLC on a C18 column. The elution gradient was 100% water—TFA (0.1%) for 10 min then a linear gradient to CH_3CN 100% for 90 min. The residue was lyophilized to lead to pure **1** (8 mg, 43%) as a white powder.

$[\alpha]_D$ = +9 (c = 0.2, H_2O); Tr = 18.6 min; ^{1}H NMR (300 MHz, DMSO) δ = 9.05 (1H, br, NH), 8.16 (1H, s, H_{Trz}), 7.90 (2H, s, H_{Pyr}), 5.48 (1H, d, J = 9 Hz, H-1), 4.24 (4H, s, CH_2N), 3.70–3.20 (18H, m, H-2,-3,-4,-5,-6, 6 × CH_2), 2.90 (2H, br, CH_2NHCO), ^{13}C NMR (75 MHz, DMSO): δ = 171.4 (CO), 164.5 (NHCO), 156.6 (C_{Pyr}), 144.5 (C_{Trz}), 144.0 (C_{Pyr}), 121.5 (CH_{Trz}), 120.5 (C_{Pyr}), 87.4 (C1), 79.9, 76.9, 72.1, 69.8 (C2,-3,-4,-5), 60.8, 60.2 (C6, CH_2), 58.1, 54.4, 25.3 (CH_2); HRMS (ES+): found 670.2331 $C_{26}H_{36}N_7O_{14}$ requires 670.2320.

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