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A simple strategy for the synthesis of the glycosylated collagen biomarkers α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-*O*-pyridinoline (Glc-Gal-PYD), α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-5-*O*-hydroxylysine (Glc-Gal-Hyl) and of their unnatural epimers Glc-Gal-*epi*PYD and Glc-Gal-*epi*Hyl

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Abstract—A short synthetic strategy for the preparation of the α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-*O*-pyridinoline (Glc-Gal-PYD), of the α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-5-*O*-hydroxylysine (Glc-Gal-Hyl) and of their respective epimers, Glc-Gal-*epi*PYD and Glc-Gal-*epi*Hyl, useful for the analytical evaluation of collagen biomarkers in human sinovium and bone, is reported.
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1. Introduction

In some recent interesting work, Gineyts et al.[1] have demonstrated that α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-*O*-pyridinoline **1** (Glc-Gal-PYD **1**; Fig. 1), a glycoconjugate cross-link of collagen, is a biomarker strongly associated with the presence of osteoarthritis at the tibiofemoral and patellofemoral joints in men. Moreover urinary levels of Glc-Gal-PYD **1** are considered to be more specific markers of joint destruction progression in various diseases, including rheumatoid arthritis.[2–4]

Considering the biological relevance of Glc-Gal-PYD **1**, in previous work we realized the first synthesis of this biomarker, which is now available[5] for studies. Afterwards we started the synthesis of its epimer, the α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-*O*-*epi*pyridinoline **2** (Glc-Gal-*epi*PYD **2**), having an unnatural (5*S*)-hydroxylysine side chain, considering that this unnatural glycosylated pyridinoline was of wide interest as an internal standard, equally fluorescent, to set up suitable analytical methods for the quantitative evaluation of Glc-Gal-PYD **1** and of other glycosylated natural pyridinolines in biological media.

Herein we report the first synthesis of Glc-Gal-*epi*PYD **2** based on a relatively short procedure, which allows a parallel preparation of the natural epimer **1**, in a way which is shorter than that previously available. This synthetic strategy is also applied to the preparation of Glc-Gal-Hyl **3** and its unnatural epimer Glc-Gal-*epi*Hyl **4**.

2. Results and discussion

We have obtained the synthesis of Glc-Gal-*epi*PYD **2** according to a relatively short protocol (Scheme 1) which noticeably modifies the procedure already set up for the synthesis of the natural Glc-Gal-PYD **1** since it avoids the laborious initial separation of the diastereoisomeric masked hydroxylysines **5a** and **5b** required here.[5]

This was possible since we recently observed[6] that the epimeric galactosylated (5*S*)-hydroxylysine **6a** (Scheme 1) was easily separable by simple column chromatography from its known natural (5*R*)-epimer **6b**, thus offering the

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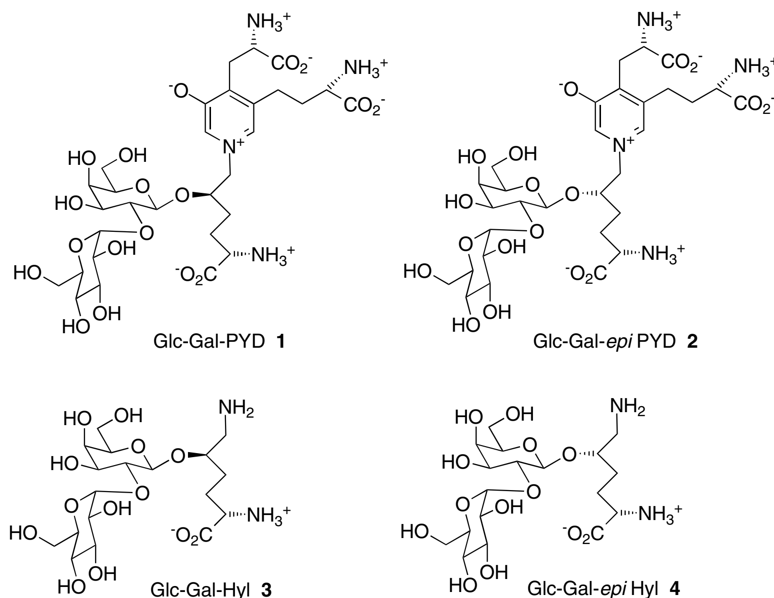
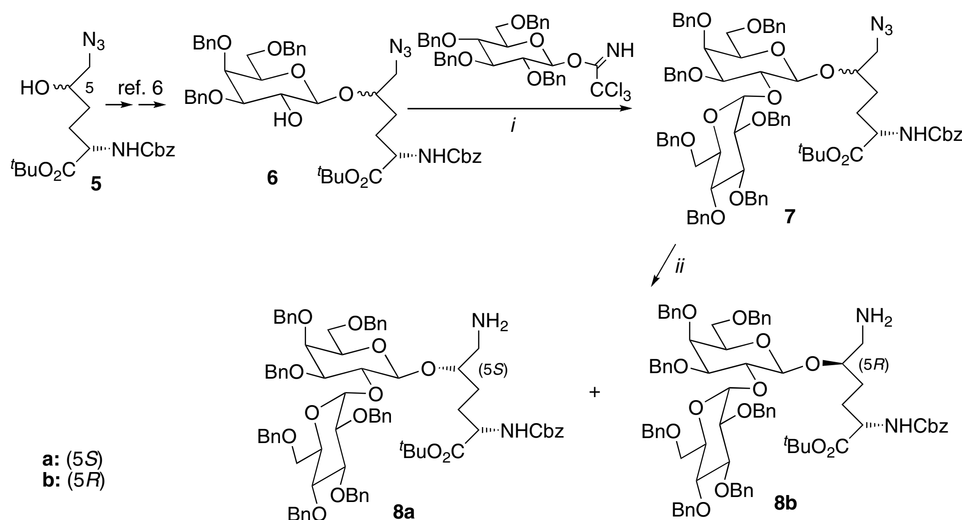


Figure 1. Glycosylated pyridinolines and hydroxylysines.

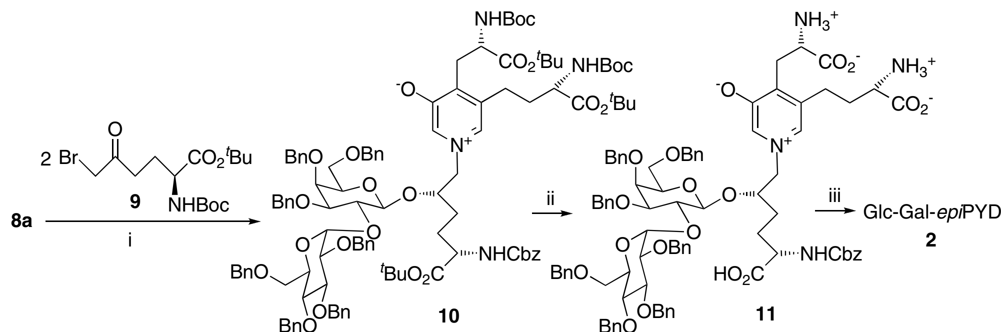


Scheme 1. Shortened parallel syntheses of glycosylated protected hydroxylysines. Reagents and conditions: (i) $^tBuMe_2SiSO_3CF_3$, molecular sieves 3 Å, Et_2O, rt, 1 h, 77%; (ii) (a) $SnCl_2$, PhSH, Et_3N, THF, rt, 1 h, (b) column chromatography: **8a** (41%), **8b** (39%).

possibility to start with these separated azides and perform an abbreviated synthesis of the glycoconjugate pyridinoline **1** and its epimer **2**. Moreover, we decided to postpone the separation of the epimeric intermediates to the level of the isomeric diglycosylated amines **8a** and **8b**, confident that the introduction of an additional saccharidic molecule could preserve or increase the differences of chromatographic behaviour between the (*R*)- and (*S*)-isomers. In effect, on reacting the mixture of epimeric (5*S*)- and (5*R*)-hydroazides **6a** and **6b** with a glucosyl donor (Scheme 2), we obtained first a chromatographically inseparable mixture of azides **7a** and **7b** and then after chemical reduction with $SnCl_2$–PhSH,[7] the mixture of amines **8a** and **8b** were easily separated by simple chromatography. At this point we reacted amine **8a** with the known bromoketone **9** in acetonitrile containing Na_2CO_3, according to our protocol.[6]

After exchange of the solvent (MeOH instead of MeCN), we obtained the protected Glc-Gal-*epi*PYD **10** in satisfactory yield (61%) and with the expected physico-chemical properties. In particular, the presence, in compound **10**, of the three amino acidic functionalities was supported by the ^{13}C resonances of the carboxylic groups, while that of the 3,4-hydroxypyridinium ring was supported by the ^{13}C resonances and the corrected UV absorption (λ_{max} 340.0 and 258.5 nm, in EtOH).[5]

The glycoconjugate compound **10** was then hydrolyzed by treatment with aqueous trifluoroacetic acid to afford tricarb-



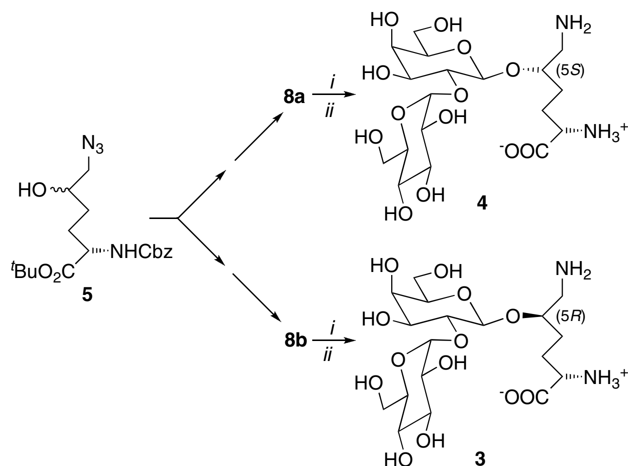
Scheme 2. Synthesis of glucogalacto*epi*pyridinoline. Reagents and conditions: (i) Na_2CO_3, MeCN, rt, 8 h, then Na_2CO_3, O_2, MeOH, rt, 72 h, 61%; (ii) TFA, rt, 1 h, 93%; (iii) H_2, $PdCl_2$, MeOH–H_2O–AcOH, rt, 16 h, 73%.

oxylic acid **11**, which was finally debenzylated by hydrogenolysis, in aqueous acidic medium under catalysis by $PdCl_2$, to afford the desired epimeric Glc-Gal-*epi*PYD **2**.

Glc-Gal-*epi*PYD **2** obtained showed physico-chemical properties (mass spectrum, ^{1}H NMR, etc.) in agreement with the assigned structure. These were very close to those of the natural Glc-Gal-PYD **1** obtained[5] from amine **8b**.

Due to these nearly superimposable physical properties of Glc-Gal-PYD **1** and Glc-Gal-*epi*PYD **2**, only after an extensive study of the HPLC behaviour of these epimers, under various conditions of analysis, were we able to find that they could be satisfactorily separated. This qualifies Glc-Gal-*epi*PYD **2** as a useful internal standard for HPLC analyses of Glc-Gal-PYD **1** in biological media.

Interestingly, our results also allow to obtain in a new simpler way the recently reported[8,9] Glc-Gal-hydroxylysines **3** and **4** (Scheme 3), two glycoconjugated amino acids, of wide interest in many biological studies. This additional possibility was demonstrated subjecting epimers **8a** and **8b** to two successive deblocking reactions (Scheme 3) and obtaining the corresponding glycosylated hydroxylysines **3** and **4**.



Scheme 3. Preparation of glycosylated hydroxylysines. Reagents and conditions: (i) H_2, Pd/C, THF–H_2O, rt, 9 h, 81%; (ii) TFA, rt, 1 h, 82%.

3. Conclusion

In conclusion, in this work we report the first synthesis of the unnatural Glc-Gal-*epi*PYD **2** together with a procedure which offers a simplified access to the preparation of the natural epimer Glc-GalPYD **1** and of the glycosylated hydroxylysines **3** and **4.** All of these glycoconjugated cross-links are now easily obtained for biochemical studies and can allow us to set up robust analytical HPLC methods.

4. Experimental

4.1. General methods

Nuclear magnetic resonance spectra were recorded at 303 K on Bruker AM-500 spectrometer operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C. Chemical shifts are reported in parts per million (ppm, δ units) and are referenced to residual $CHCl_3$ ($\delta_H = 7.24$ ppm) and to $CDCl_3$ ($\delta_C = 77.0$ ppm) for solutions in $CDCl_3$ or to internal CH_3OD ($\delta_H = 3.30$ ppm and $\delta_C = 49.0$ ppm) for solutions in D_2O. ^{1}H NMR data are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; br s, broad singlet; m, multiplet), coupling constant(s) in Hz, assignment of proton(s). The ^{1}H and ^{13}C resonances were assigned by ^{1}H decoupling, ^{1}H–^{1}H COSY and ^{1}H–^{13}C correlation experiments. The carbon numeration used in the NMR description is that given in Figure 2.

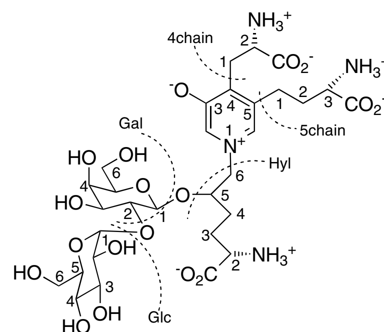


Figure 2. Carbon numeration used in this work.

Optical rotations were taken on a Perkin–Elmer 241 polarimeter and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}.

HPLC analyses were carried out on a ODS-2 column (Waters Spherosorb, 150 mm, 4.6 mm ID, 5 μm); elution was performed with 0.02 M heptafluorobutanoic acid (HFBA) in a water/MeCN solution containing variable ratio of these solvents and the detection was carried out by fluorescence ($\lambda_{ex} = 297$ nm; $\lambda_{emiss} = 380$ nm).

Mass spectra were carried out using a Finnigan LCQdeca (ThermoQuest) ion trap mass spectrometer equipped with an electrospray source (ESI). The spectra were collected in continuous flow mode by connecting the infusion pump directly to the ESI source. Solutions of compounds were infused at a flow rate of 10 μL/min. The spray voltage was set at 5.0 kV, operating in the positive ionization mode with capillary temperature of 220 °C. Full-scan mass spectra were recorded by scanning a *m*/*z* range of 100–2000.

All reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60 F_{254}) using UV light, 50% sulfuric acid, anisaldehyde–H_2SO_4–EtOH solution or 0.2% ninhydrin in ethanol and heat as developing agent. E. Merck 230–400 mesh silica gel was used for flash column chromatography.[10] Work-up refers to washing with water, drying with Na_2SO_4 and evaporation of the solvent.

4.2. Preparation of *tert*-butyl (2*S*,5*S*)- and (2*S*,5*R*)-6-amino-2-benzyloxycarbonylamino-5-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)]hexanoates 8a and 8b

4.2.1. Preparation of the epimeric mixture of (2*S*,5*S*)- and (2*S*,5*R*)-6-azido-2-benzyloxycarbonylamino-5-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyloxy)]hexanoate 7.

A mixture of epimeric galactosides **6**[6] (3.67 g; 4.52 mmol), *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl) trichloroacetimidate[8] (13.2 g; 19.3 mmol) and powdered molecular sieves (3 Å, 2 g), in anhydrous diethyl ether (150 mL), was stirred for 15 min at room temperature. Then *tert*-butyldimethylsilyl triflate (208 μL; 0.91 mmol) was added and stirring was continued for 1 h, at room temperature, under argon atmosphere. At this time, the powdered molecular sieves were filtered off and washed with AcOEt. The organic phase was worked up to afford a residue, which was purified by flash chromatography (eluting with hexane–AcOEt, 80:20 v/v) to give the mixture of epimers **7** (4.56 g; Y = 76%) as an oil. The product showed the correct elemental analysis and ^{1}H NMR in agreement with the presence of both known (5*S*) and (5*R*)-epimeric compounds.[8]

4.2.2. Preparation of *tert*-butyl (2*S*,5*S*)- and of (2*S*,5*R*)-6-amino-2-benzyloxycarbonylamino-5-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)]hexanoates 8a and 8b.

PhSH (2.25 mL, 21.9 mmol) and Et_3N (2.25 mL, 16.1 mmol) were added to a stirred solution of $SnCl_2$ (1.00 g, 5.29 mmol) in anhydrous THF (37 mL). After 10 min, the epimeric mixture of azides **7** (4.56 g, 3.42 mmol) was added. The analyte mixture was stirred at room temperature for 1 h. After this time, the solvent was evaporated and the crude residue was purified by flash chromatography. Elution with hexane–AcOEt (30:70, v/v) afforded first some by-products, then, with hexane–AcOEt–MeOH (30:70:2, v/v), 5*S*-isomer **8a** (1.83 g; Y = 41%), a glass: $[\alpha]_D^{20} = +40.5$ (*c* 1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.3–7.0 (aromatics), 5.51 (1H, d, *J* 3.5 Hz, H_{Glc}-1), 5.36 (1H, d, *J* 8.4 Hz, N–H), 4.60 (1H, d, *J* 7.7 Hz, H_{Gal}-1), 4.31 (1H, ddd, *J* 9.5, 3.0, 2.5 Hz, H_{Glc}-5), 4.19 (1H, m, H_{Hyl}-2), 4.10 (1H, dd, *J* 9.9, 7.7 Hz, H_{Gal}-2), 4.03 (1H, dd, *J* 9.5, 9.5 Hz, H_{Glc}-3), 3.96 (1H, dd, *J* 3.5, <1 Hz, H_{Gal}-4), 3.67 (1H, dd, *J* 9.5, 9.5 Hz, H_{Glc}-4), 3.63–3.55 (3H, overlapping, H_{Gal}-5, H_{Gal}-6a, H_{Gal}-6b), 3.60 (1H, dd, *J* 9.5, 3.5 Hz, H_{Glc}-2), 3.56 (1H, dd, *J* 9.9, 3.2 Hz, H_{Gal}-3), 3.38 (2H, br s, H_{Glc}-6a and H_{Glc}-6a), 2.58 (1H, dd, *J* 12.9, 6.6 Hz, H_{Hyl}-6a), 2.52 (1H, dd, *J* 12.9, 4.5 Hz, H_{Hyl}-6b), 1.81 (1H, m, H_{Hyl}-3a), 1.73 (1H, m, H_{Hyl}-3b), 1.51 (1H, m, H_{Hyl}-4a), 1.45 (1H, m, H_{Hyl}-4b), 1.37 [3H, s, $C(CH_3)_3$]; ^{13}C NMR ($CDCl_3$): δ 171.6 (C_{Hyl}-1), 155.9 ($NHCO_2Bn$), 138.8–127.0 (aromatics), 102.7 (C_{Gal}-1), 96.7 (C_{Glc}-1), 82.4 (C_{Glc}-3), 81.7 (CMe_3), 81.5 (C_{Gal}-3), 79.4 (C_{Glc}-2), 78.8 (C_{Hyl}-5), 78.3 (C_{Glc}-4), 73.4 (C_{Gal}-4), 73.3 (C_{Gal}-2), 73.1 (C_{Gal}-5), 69.9 (C_{Glc}-5), 68.5 (C_{Gal}-6), 68.1 (C_{Glc}-6), 54.2 (C_{Hyl}-2), 45.4 (C_{Hyl}-6), 28.4 (C_{Hyl}-4), 28.4 (C_{Hyl}-3), 27.9 [$C(CH_3)_3$]; ESI-MS (positive) *m*/*z*: 1307.5 (100%), 1308.5 (66%),1309.6 (33%), 1310.7 (9%; $M+H^+$). Anal. Calcd for $C_{79}H_{90}N_2O_{15}$: C, 72.57; H, 6.94; N, 2.14. Found: C, 72.73; H, 6.89; N, 2.19.

Elution with hexane–AcOEt–MeOH (30:70:5, v/v) afforded (5*R*)-isomer **8b** (1.74 g; Y = 39%). This compound showed the correct elemental analysis and the physico-chemical properties already described.[6]

4.3. Preparation of completely protected α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-*O*-5-epipyridinoline 10

The protected bromoketone **9** (654 mg, 1.72 mmol) was added to a solution of amine **8a** (900 mg, 0.69 mmol) in CH_3CN (45 mL) containing Na_2CO_3 (1.35 g, 12.7 mmol), and the mixture was stirred at room temperature under nitrogen for 8 h. At this time was observed the disappearance of the starting glycosylated amine **8a** and of the initially formed monoalkylated product (TLC:CH_2Cl_2–MeOH, 100:5, v/v; $R_f = 0.43$ and 0.79, respectively). The solvent was then evaporated under reduced pressure and the crude residue was dissolved in MeOH (45 mL) and shaken under a slight pressure of oxygen (1.3 atm) and at room temperature for 72 h. After this time, the mixture was diluted with dichloromethane (50 mL) and filtered on a pad of Celite. After evaporation of the solvent we obtained a crude residue which was chromatographed on silica gel and eluted with CH_2Cl_2–MeOH (100:4, v/v) to afford the protected glycosylated pyridinoline **10** (790 mg; Y = 61%) as a glass: $[\alpha]_D^{20} = +34.9$ (*c* 1, CH_2Cl_2); UV λ_{max}-(EtOH)/nm (ε/dm^3 mol^{-1} cm^{-1}), 258.5 (5200), 340 (5950); ^{1}H NMR ($CDCl_3$): δ 7.32–7.04 (aromatics), 5.48 (d, *J* 7.7 Hz, N–H), 5.33 (1H, d, *J* 3.5 Hz, H_{Glc}-1), 4.39 (1H, d, *J* 7.7 Hz, H_{Gal}-1), 4.31 (1H, ddd, *J* 9.5, 3.0, 2.5 Hz,

H_{Glc}-5), 4.14 (1H, m, H_{Hyl}-2), 4.07 (1H, dd, *J* 9.6, 7.7 Hz, H_{Gal}-2), 4.06 (1H, dd, *J* 9.5, 9.5 Hz, H_{Glc}-3), 3.81 (1H, m, H_{Hyl}-5), 3.70 (1H, dd, *J* 9.5, 9.5 Hz, H_{Glc}-4), 3.63 (1H, dd, *J* 9.5, 3.5 Hz, H_{Glc}-3), 3.56 (1H, dd, *J* 9.6, 3.5 Hz, H_{Gal}-3), 3.46 (1H, dd, *J* 3.5, <1 Hz, H_{Gal}-4), 3.30 (2H, m, H_{Glc}-6a and H_{Glc}-6b), 1.46, 1.45, 1.38 [27H, 3 × s, 3 × $C(CH_3)_3$], 1.44 [18H, s, 2 × $C(CH_3)_3$]; ^{13}C NMR ($CDCl_3$): δ 171.2, 171.1, 171.0 (3 × *C*OO^{t}Bu), 168.7, 155.6, 143.2 (3 × pyridinium ring carbons), 156.0 (NH*C*OO), 102.3 (C_{Gal}-1), 97.1 (C_{Glc}-1), 56.6, 53.7, 53.6 (3 × aminoacid α-carbons), 28.4, 28.3, 27.93, 27.91, 27.8 [5 × $C(CH_3)_3$]; ESI-MS (positive) *m*/*z*: 1885.7 (80%), 1886.6 (100%), 1887.6 (60%), 1888.5 (10%; M+H$^+$). Anal. Calcd for $C_{109}H_{136}N_4O_{24}$: C, 69.41; H, 7.27; N, 2.97. Found: C, 69.52; H, 7.15; N, 3.06.

4.4. Preparation of the α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-*O*-epipyridinoline 2, by successive regeneration of protecting functionalities

4.4.1. Preparation of the partially protected Glc-Gal-*epi*PYD 11. The completely protected Glc-Gal-*epi*PYD **10** (600 mg, 0.318 mmol) was dissolved in CF_3COOH–H_2O (12 mL, 95:5, v/v) and stirred at room temperature for 1 h. Evaporation of the solvent under reduced pressure afforded a crude residue formed by the partially protected Glc-Gal-*epi*PYD **11** as tri-trifluoroacetate salt (550 mg; Y = 93%): a glass. The ^{1}H NMR spectrum was recorded to verify the complete cleavage of Boc groups and of the *tert*-butyl esters.

4.4.2. Preparation of the α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-*O*-epipyridinoline 2. The partially protected Glc-Gal-*epi*PYD **11** (780 mg, 0.419 mmol) was dissolved in 390 mL of a mixture of MeOH–water–glacial acetic acid (8:2:1, v/v/v) and hydrogenated in the presence of $PdCl_2$ (150 mg) at room temperature and atmospheric pressure. When the hydrogen absorption was completed (16 h), the catalyst was separated by filtration over a pad of Celite. The solvent was eliminated under reduced pressure to afford a residue, which was recovered by water and then passed through a Dowex (50WX8-200) column (20 mL) and eluted with a 1 M solution of NH_3 in H_2O–MeOH (1:1, v/v). The solution was freeze-dried to obtain Glc-Gal-*epi*PYD **2** (225 mg; Y = 73%), an amorphous material, which showed $[\alpha]_D^{20} = +17.4$ (*c* 0.5, H_2O); ^{1}H NMR (D_2O) δ: 8.22, 8.17 (2H, 2 × br s, pyridinium ring protons), 5.19 (1H, d, *J* 3.5 Hz, H_{Glc}-1), 4.50 (1H, d, *J* 7.5 Hz, H_{Gal}-1), 4.20 (1H, m, H_{Hyl}-5), 4.16 (1H, dd, *J* 6.0 and 6.0 Hz, H_{5chain}-3), 4.02 (1H, dd, *J* 6.5 and 6.5 Hz, H_{Hyl}-2), 3.84 (1H, dd, *J* 3.5 and <1 Hz, H_{Gal}-4), 3.39 (1H, dd, *J* 9.5 and 7.5 Hz, H_{Gal}-2), 3.54 (1H, dd, *J* 9.5 and 3.5 Hz, H_{Glc}-2), 3.40 (1H, dd, *J* 9.5 and 9.5 Hz, H_{Glc}-4), 3.00 (1H, m, H_{5chain}-1a), 2.89 (1H, m, H_{5chain}-1b); ^{13}C NMR δ_C (CD_3OD): 172.5, 172.4, 171.7 (3 × CO_2H), 155.5 (C-3), 141.5, 141.0 (C-4 and C-5), 132.4, 130.8 (C-2 and C-6), 102.1 (C_{Gal}-1), 98.2 (C_{Glc}-1), 78.2 (C_{Hyl}-5), 77.4 (C_{Gal}-2). ESI-MS (positive) *m*/*z*: 754.6 (25%), 753.2 (100%; M+H$^+$). Anal. Calcd for $C_{30}H_{48}N_4O_{18}$: C, 47.87; H, 6.43; N, 7.44. Found: C, 47.96; H, 6.28; N, 7.51.

4.5. Comparison of HPLC behaviour of Glu-Gal-Pyd 1 and of Glu-Gal-*epi*Pyd 2

A sample of each compound was dissolved in water and analyzed using the best chromatographic conditions to separate compound **1** from its epimer **2**: the HPLC column has been reported previously in a general method, the mobile phase was a solution of water–CH_3CN (93:7, v/v) containing heptafluorobutyric acid (0.02 M), the flow rate was 1 mL/min and the detection was achieved by fluorescence. Glucosyl-galactosyl-*O*-epipyridinoline **2** was eluted after 45.5 min while glucosyl-galactosyl-*O*-pyridinoline **1** was eluted after 48.4 min showing a satisfactory peak resolution ($R_s > 1$).

4.6. Preparation of (2*S*,5*S*)-2,6-diamino-5-[α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyloxy]hexanoic acid 4

Amine **8a** (200 mg; 0.153 mmol) dissolved in tetrahydrofuran (25 mL) and ethanol (75 mL) was hydrogenated in the presence of palladium (200 mg, 10% on activated carbon) at room temperature under 1 atm of hydrogen. After 9 h, the catalyst was filtered on a pad of Celite and thoroughly rinsed with a solution of MeOH–distilled water (10 mL; 5:1, v/v). Evaporation of the combined filtrates at low temperature (<30 °C) and under reduced pressure afforded a residue, which was dissolved in distilled water (2 mL) and washed with toluene (2 × 4 mL). The aqueous solution was co-evaporated with toluene under reduced pressure. The residue was dissolved in wet trifluoroacetic acid (1 mL, TFA–H_2O, 95:5, v/v) and the resulting solution was stirred at room temperature for 1 h. Evaporation of the solvent afforded the pure title compound **4** as a ditrifluoroacetate (88 mg; Y = 81%), having the physico-chemical properties identical with those already observed by us.[8]

4.7. Preparation of (2*S*,5*R*)-2,6-diamino-5-[α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyloxy]hexanoic acid 3

Starting with amine **8b** (200 mg; 0.153 mmol) and following the procedure described above for the deprotection of the epimeric amine **8a**, the title compound **3** was obtained as a ditrifluoroacetate (89 mg; Y = 82%) identical in all respects with those already reported by us.[8]

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