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Hydroxylysine containing glycoconjugates: an efficient synthesis of natural galactosylhydroxylysine (Gal-Hyl) and glucosylgalactosylhydroxylysine (Glu-Gal-Hyl) and of their (5*S*)-epimers

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Abstract—The paper reports the first chemical synthesis of α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-5-*O*-hydroxylysine, a biological marker of bone resorption and of its unnatural (5*S*)-epimer, starting from commercial sugars and amino acids. Moreover, the synthetic protocol set-up has resulted in a new procedure for the synthesis of the β-D-galactopyranosyl-5-*O*-hydroxylysine and its unnatural (5*S*)-epimer.

1. Introduction

Collagen is a family of structurally related proteins formed of a helix of three alpha chains, each of which has its own amino acid composition, genetic mechanism and tissue distribution. Some lysine (Lys) residues, specifically located in the collagen fibrils of bone and skin, are susceptible of post-translational hydroxylation and subsequent glycosylation by either β-D-galactopyranosyl or α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl moieties.[1,2] In the human body, during collagen breakdown, the glycosylated hydroxylysine residues are released into the serum as β-D-galactopyranosyl-5-*O*-hydroxylysine [(5*R*)-GaHL] **1** and α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-*O*-hydroxylysine [(5*R*)-GgaHL] **2** (Fig. 1). Then they are not recycled or significantly metabolized[3] but are quantitatively excreted into urine.[4]

Thus, glycosides **1** and **2** have attracted considerable attention as biochemical markers of total collagen turnover,[5] correlated to diseases of bone resorption,[6] useful for a better understanding of various pathologies and for clinical diagnosis. As a consequence, pure (5*R*)-GaHL **1** and (5*R*)-GGaHL **2** are essential for studies

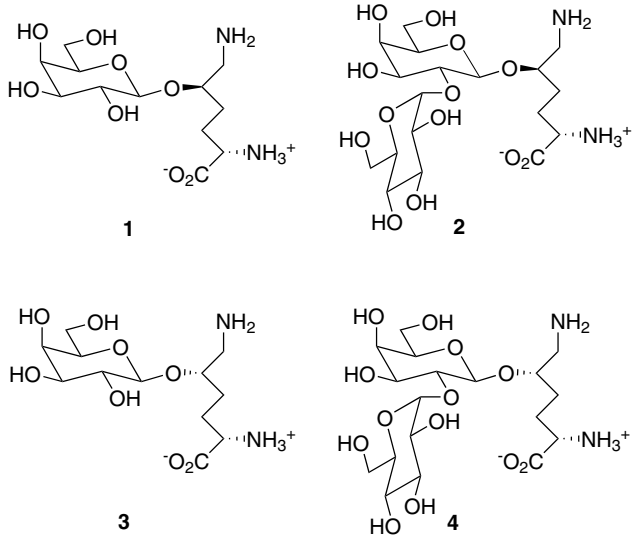

Figure 1.

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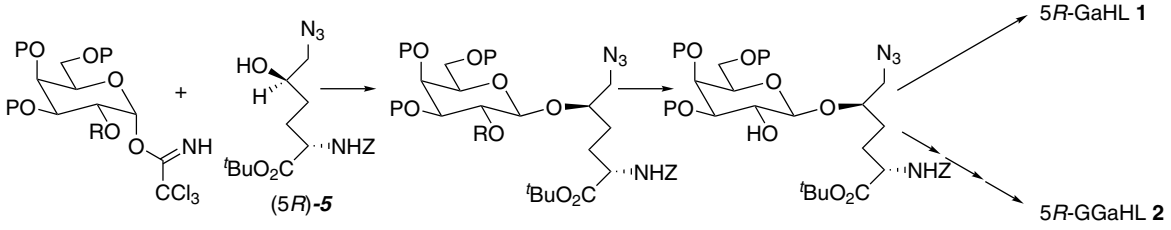
of collagen biochemistry and for the development of analytical methods to monitor the metabolic status of bone collagen. In fact, even if a large number of analytical methods for the quantitative detection of the glycosides **1** and **2** in biological samples has been reported,[7] only recently a racemic[8] and a homochiral[9] chemical syntheses of the (5*R*)-GaHL **1** have been described, while no synthesis of the (5*R*)-GGaHL **2** is to date available.[10,11] Thus the reference standards for physicochemical analyses are generally obtained from different natural sources,[12] in forms sometimes not completely free of contaminants. Moreover, the synthesis of the glycosides (5*R*)-GaHL **1** and of the (5*R*)-GGaHL **2** also represents an attractive synthetic target due to the interesting structural features of these glycoconjugated hydroxylysines. For this we enclosed the synthesis of glycosides (5*R*)-GaHL **1** and (5*R*)-GGaHL **2** in our programmes on the synthesis and the utilization of collagen metabolites.[13] In addition, we planned the synthesis of the glycosides (5*S*)-GaHL **3** and (5*S*)-GGaHL **4**, two glycosides of unnatural (5*S*)-hydroxylysine, epimers of the glycosides **1** and **2**, respectively. In fact, we considered that these unnatural epimers could be suitable internal standards for the detection of the natural metabolites **1** and **2**, in almost all the analytical methods reported, and in particular the more sophisticated liquid chromatography–tandem mass spectrometry (LC/MS/MS).[7a]

2. Results and discussion

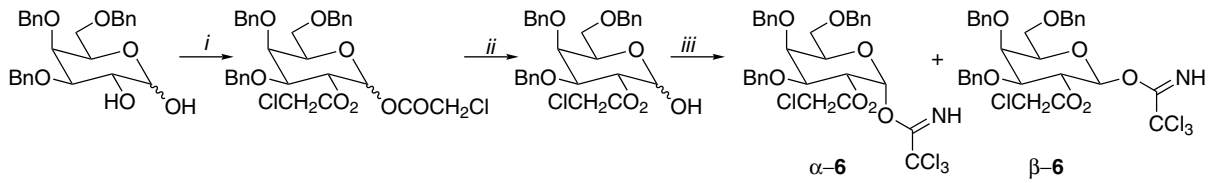
In planning our synthetic work for the preparation of the (5*R*)-GaHL **1** and of the (5*R*)-GGaHL **2** (Fig. 1), we decided to establish a common protocol, which could permit to approach the synthesis of both the (5*R*)-GaHL **1** and of the (5*R*)-GGaHL **2** and possibly of their (5*S*)-epimers **3** and **4**. With this in mind, we programmed to start the synthesis with the formation of a β-galactoside bond between a suitable *O*-glycosyl acceptor as the masked (5*R*)-5-hydroxy-L-lysine (5*R*)-**5** (Scheme 1), prepared by esterification, according to Kunz and co-workers[14] of the corresponding acid,[13d] and a galactosyl donor, conveniently activated, at the anomeric hydroxyl, to be used in a Schimdt[15] stereocontrolled protocol of β-glycosylation.

Our synthetic scheme prompted us to use as galactosyl acceptor the masked hydroxylysine (5*R*)-**5**, in place of other hydroxylysine esters, in order to avoid the protection, and the obligatory deprotection, of the ε-amino group of the amino acid and various troubles due to possible lactamizations. On the other hand, we decided to construct, as glycosyl donor, the galactosyl derivative α-**6** (Scheme 2), orthogonally protected as the chloroacetate at the 2-hydroxyl group. In fact, we considered that the trichloroacetimidate α-**6**, having a participating protecting group at the 2 position, could favour the formation of the desired β-anomeric bond. In addition, the chloroacetate group, after the glycosylation, could be selectively removed in the presence of the glycosidic bond, thus allowing to extend the saccharidic chain in the 2-direction.

With this in mind, we prepared the required glycosyl donor α-**6** from the easily available[16] 3,4,6-tri-*O*-benzyl-D-galactopyranose in three steps (Scheme 2). In fact, the preparation of α-**6** requires first the chloroacetylation of both the hydroxyl groups of the benzylated galactose, by reaction with chloroacetyl chloride, afford the 1,2-*O*-dichloroacetyl derivative as an 1:1 anomeric mixture (^{1}H NMR), then, a controlled treatment of the *O*-dichloroacetates mixture of with ammonia in acetonitrile[17] to allow the selective regeneration of the anomeric hydroxyl, thus affording the 3,4,6-tri-*O*-benzyl-2-*O*-chloroacetylgalactopyranose (as a chromatographically inseparable mixture of α and β epimers; 8:2; ^{1}H NMR) and finally, the activation of the glycosidic carbon by reaction with CCl_3CN, in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The first



Scheme 1. P = protecting group; R = acyl group removable with mild condition; Z = benzyloxycarbonyl group.



Scheme 2. Reagents and conditions: (i) $ClCH_2COCl$, Et_2O/Py, −10 to 25 °C, 2 h, 98%; (ii) NH_3, CH_3CN, 25 °C, 85%; (iii) Cl_3CCN, DBU, CH_2Cl_2, −30 °C, 4 h, 79%.

two reactions occur in nearly quantitative yields, while the formation of the desired trichloroacetimidate α-**6** was obtained, in the best case, in 79% yields. In all cases, the α-6 anomer was accompanied by its β-anomer β-**6**, which was isolated and completely characterized.

With the stating material in the hands, we performed the galactosylation of the masked hydroxylysine (5*R*)-**5** (Scheme 3) using as galactosyl donor the α-anomer α-**6** and *tert*-butyldimethylsilyl triflate as catalyst.[15] In these conditions, we obtained the β-*O*-galactoside **7**, which, in the crude product of the reaction, was not accompanied by any detectable quantity of the corresponding α-anomer or *ortho*-ester. On the contrary, the α-isomer was present, as a more polar companion (TLC and ESI-MS evidences) when the reaction was catalyzed by boron trifluoride etherate.

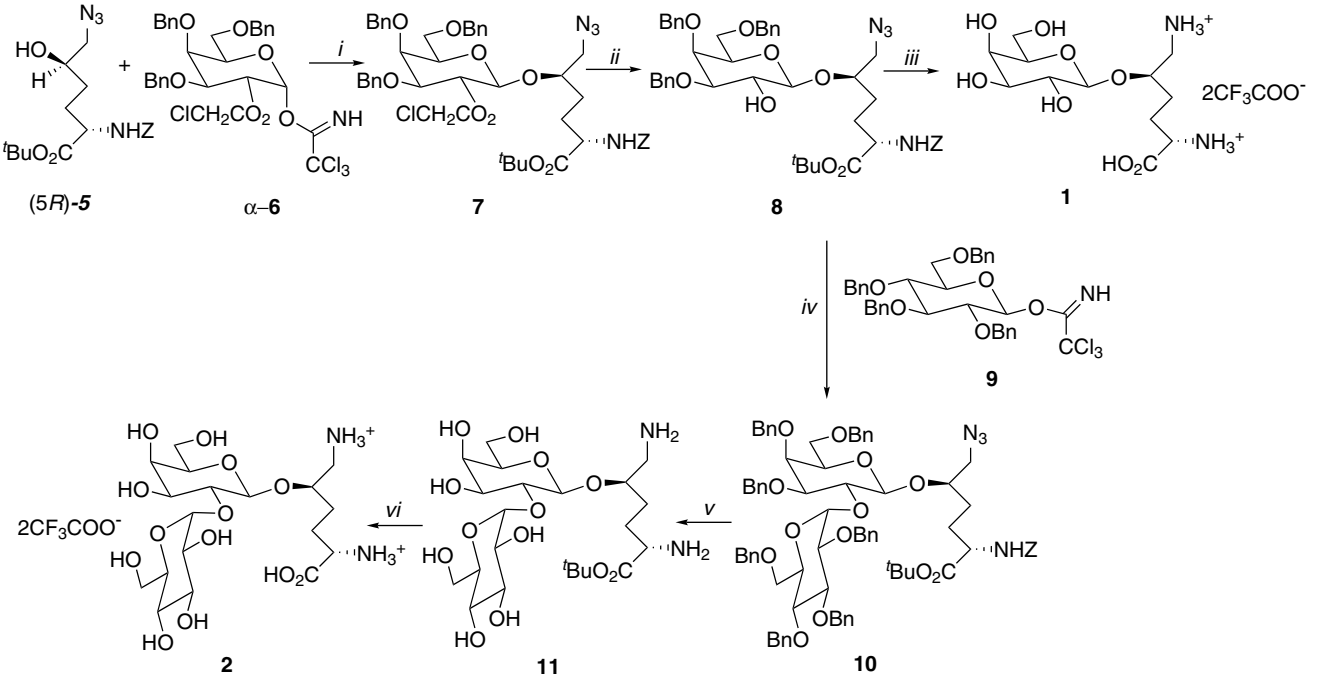
In a final preparation we used, as galactosyl donor, also the purified β-trichloroacetimidate β-**6** and obtained a super imposable result, thus supporting the conclusion that in the Schmidt glycosylation the geometry of the trichloroacetimidate is irrelevant.[15]

The successive selective regeneration of the 2-hydroxy group of **7** was performed by refluxing in methanol the chloroacetate **7** in the presence of $Zn(OAc)_2 2H_2O$. These very mild reaction conditions, were already found by us to be useful for the hydrolysis of a dichloroacetate in the synthesis of the glycoside etoposide.[18] The selective removal of the chloroacetate group generates the galactoside **8** having a free hydroxy group at position 2 of the galactose portion. This key intermediate **8** resulted useful not only for pursuing the synthesis of the (5*R*)-GaHL **1** but also for reaching the synthesis of the more complex disaccharidic glycoside (5*R*)-GGaHL **2** (Scheme 3).

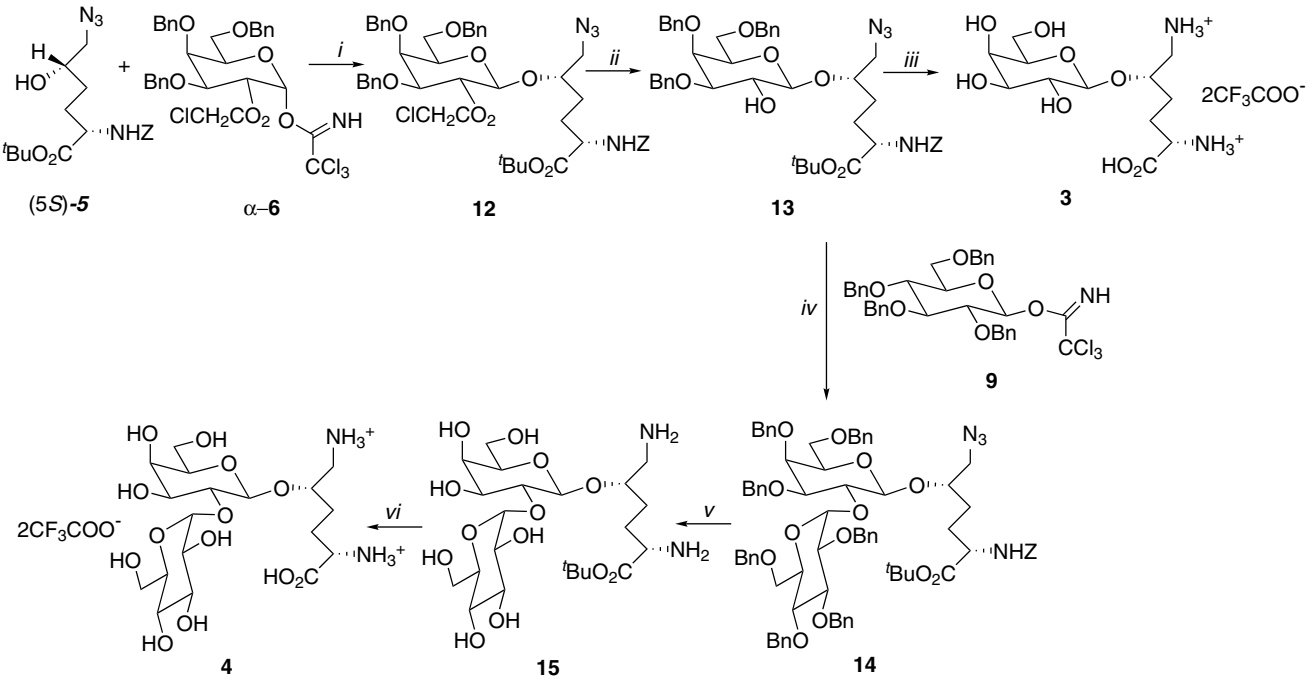
In fact, catalytic hydrogenation of the glycoside **8** causes the simultaneous generation of the amino group from the azide and the removal of the benzyl and benzyloxycarbonyl groups with formation of an amino *tert*-butyl ester, which, by simple treatment with aqueous trifluoracetic acid, affords the desired 5*R*-GaHL 1 as its trifluoracetate.

Obtainment of the disaccharide **2** required the formation of a α-glucosidic bond at the 2 position of the key intermediate **8**. This glucosylation has been affected, in the best conditions, coupling the acceptor **8** with *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)trichloroacetimidate **9**[19] in diethyl ether at 25 °C and in the presence of *tert*-butyldimethylsilyl triflate. In absence of a participant group at the 2 position of glycosyl donor, these conditions, as expected on the base of Wegmann and Schmidt work[20], allow to obtain with the higher-diastereoselectivity and the more convenient yield (45% after chromatographic purification), the protected diglycosidic compound **10**. Moreover also under these conditions, set-up by Wegmann and Schimdt[20] for the use of the glucosyl donor **9**, the compound **10** was accompanied by the undesired β-anomer (18% yield).

The correct α-stereochemistry at glucosidic anomeric carbon was confirmed by the observed chemical shift of the anomeric proton (δ = 5.62 ppm) and from its coupling constant (J = 3.5 Hz) with the adjacent H-2 proton in the glucopyranosidic ring.



Scheme 3. Reagents and conditions: (i) ^{t}BuMe$_2$SiSO$_3$CF$_3$, molecular sieves 3 A, Et$_2$O, 25 °C, 1 h, 52%; (ii) Zn(OAc)$_2$ 2H$_2$O, MeOH, reflux, 9 h, 93%; (iii) a. H$_2$, Pd/C, THF–EtOH, 1 atm, 25 °C, 90%; b. CF$_3$CO$_2$H–H$_2$O (95:5), 25 °C, 1 h, 92%; (iv) ^{t}BuMe$_2$SiSO$_3$CF$_3$, molecular sieves 3 A, Et$_2$O, 25 °C, 15 min, 45%; (v) H$_2$, Pd/C, THF–EtOH, 1 atm, 25 °C, 9 h, 90%; (vi) CF$_3$CO$_2$H–H$_2$O (95:5), 25 °C, 1 h, 92%.



Scheme 4. Reagents and conditions: (i) $^{t}BuMe_2SiSO_3CF_3$, molecular sieves 3 A, Et_2O, 25 °C, 1 h, 50%; (ii) $Zn(OAc)_2$ $2H_2O$, MeOH, reflux, 9 h, 96%; (iii) a. H_2, Pd/C, THF–EtOH, 1 atm, 25 °C, 93%; b. CF_3CO_2H–H_2O (95:5), 25 °C, 1 h, 95%; (iv) $^{t}BuMe_2SiSO_3CF_3$, molecular sieves 3 A, Et_2O, 25 °C, 15 min, 42%; (v) H_2, Pd/C, THF–EtOH, 1 atm, 25 °C, 9 h, 92%; (vi) CF_3CO_2H–H_2O (95:5), 25 °C, 1 h, 95%.

Catalytic hydrogenation of the glycoside **10** cleaved the benzyl and benzyloxycarbonyl groups and generated the ε-amino group of the hydroxylysine aglycone, thus affording the *tert*-butyl esters **11**, which, by treatment with trifluoracetic acid, afforded the corresponding acid 5*R*-GGaHL **2**, isolated as ditrifluoracetate.

A parallel series of reactions starting with the galactose trichloroacetimidate α-**6** and the epimeric hydroxyazide (5*S*)-**5** allows to prepare the galactoside (5*S*)-GaHL **3** and the diglycoside (5*S*)-GGaHL **4** as trifluoroacetates (Scheme 4).

All reactions occur in a similar fashion and the conditions already set-up for the glycosylation of the (5*R*)-hydroxyazide (5*R*)-**5** and for its galactoside **8**, were found suitable for the preparation of the unnatural isomers **3** and **4**.

In particular the formation of the disaccharidic glycoside **14** appears to be independent from the stereochemistry of aglycone and, under the same conditions used for the (5*R*)-series, the compound **14** was obtained in 42% yield, accompanied by the β-anomer in 16% yield. Also in this case, the appropriate α-stereochemistry of the formed *O*-glucosidic bond was derived from the appropriate chemical shift ($\delta = 5.50$ ppm) and from its correct coupling constant ($J = 3.5$ Hz) with the adjacent H-2 proton.

3. Conclusions

In conclusion, our work makes available in a relatively easy way the two glycoconjugate (5*R*)-hydroxylysines **1** and **2** deriving from collagen breakdown and required in various biological studies. In addition, our work makes available the two glycoconjugate (5*S*)-hydroxylysines **3** and **4**, which appear very useful as internal standards in biological analyses directed to the quantification of glycosides (5*R*)-GaHL **1** and (5*R*)-GGaHL **2**.

More importantly, we feel that the present results are very attractive for assembling other more complex glycoside of cross-linked hydroxylysine (glycosylated pyridinolines) present in collagen,[21] adopting our previously reported protocols for the contraction of the 3-hydroxypyridine nucleus.[13] Work in this direction is ongoing in our laboratory and will be published in the due course.

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 for 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; bs, broad singlet; m, multiplet), coupling constant(s) in hertz, 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 correla-

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

Mass spectra were obtained using a Finnigan LCQdeca (ThermoQuest) ion trap mass spectrometer fitted 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 5 mL/min. The spray voltage was set at 5.0 kV in the positive and at 4.5 kV in the negative ion mode with a 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 or 0.2% ninhydrin in ethanol and heat as developing agent. E. Merck 230–400 mesh silica gel was used for flash column chromatography.[22] Usual work-up refers to washing the organic layer with water, drying over Na_2SO_4 and evaporating the solvent under reduced pressure.

4.2. *tert*-Butyl (2*S*,5*R*)-6-azido-2-benzyloxycarbonylamino-5-hydroxyhexanoate (5*R*)-5

A mixture of dicyclohexylcarbodiimide (3.7 g; 17.9 mmol), *tert*-butyl alcohol (2.1 mL; 22.0 mmol) and copper(I) chloride (19 mg) was stirred at room temperature for 5 days in order to obtain the *O*-*tert*-butyl-*N*,*N*′-dicyclohexylisourea. Then the mixture was diluted with CH_2Cl_2 (10 mL) and a solution of (2*S*,5*R*)-6-azido-2-benzyloxycarbonylamino-5-hydroxyhexanoic acid[13d] (700 mg; 2.17 mmol) in CH_2Cl_2 (20 mL) was added. The obtained mixture was then stirred at room temperature for 5 days, diluted with hexane (30 mL) and filtered on a short column of silica gel. The column was first eluted with hexane (400 mL) and then with diethyl ether (150 mL). Elution with diethyl ether afforded a partially purified product, which was then subjected to rapid chromatography (eluting with hexane/AcOEt; 70:30; v:v) to afford pure *tert*-butyl (2*S*,5*R*)-6-azido-2-benzyloxycarbonylamino-5-hydroxyhexanoate (5*R*)-**5** (731 mg; 89% yield): an oil; R_f 0.39 (silica gel, hexane/AcOEt; 60:40; v:v); $[\alpha]_D^{26} = +10.9$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.35–7.23 (5H, aromatics-H), 5.48 (1H, d, *J* = 7.7, N*H*), 5.08 (2H, s, OCH_2Ph), 4.29 (1H, m, 2-H), 3.77 (1H, m, 5-H), 3.29 (1H, dd, *J* = 12.4, 3.6, 6-Ha), 3.22 (1H, dd, *J* = 12.4, 6.8, 6-Hb), 2.72 (1H, d, *J* = 5.3, O*H*), 1.98 (1H, m, 3-Ha), 1.68 (1H, m, 3-Hb), 1.52 (2H, m, 4-H_2), 1.44 [9H, s, C(CH_3)$_3$]; ESI-MS (positive) *m*/*z*: 378.8 (39%; M+H$^+$), 401.0 (100%; M+Na$^+$), 402.0 (22%), 778.8 (48%; 2M+Na$^+$), 779.9 (21%). Anal. Calcd for $C_{18}H_{26}N_4O_5$: C, 57.13; H, 6.93; N, 14.81. Found: C, 57.1; H, 6.8; N, 14.7.

4.3. *tert*-Butyl (2*S*,5*S*)-6-azido-2-benzyloxycarbonylamino-5-hydroxyhexanoate (5*S*)-5

The ester was prepared starting with (2*S*,5*S*)-6-azido-2-benzyloxycarbonylamino-5-hydroxyhexanoic acid[13d] (800 mg; 2.48 mmol) in CH_2Cl_2 (23 mL) and using the procedure described above for the preparation of the epimer (5*R*)-**5**. After flash chromatography (eluting with hexane/AcOEt; 70:30; v:v,) the *tert*-butyl (2*S*,5*S*)-6-azido-2-benzyloxycarbonylamino-5-hydroxyhexanoate (5*S*)-**5** (817 mg; 87% yield): an oil; R_f 0.39 (silica gel, hexane/AcOEt; 60:40; v:v); $[\alpha]_D^{27} = +0.6$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.34–7.27 (5H, aromatics-H), 5.39 (1H, d, *J* = 7.5, N*H*), 5.08 (2H, s, OCH_2Ph), 4.27 (1H, m, 2-H), 3.76 (1H, m, 5-H), 3.32 (1H, dd, *J* = 12.4, 3.2, 6-Ha), 3.22 (1H, dd, *J* = 12.4, 7.1, 6-Hb), 2.30 (1H, d, *J* = 4.2, O*H*), 1.89 (1H, m, 3-Ha), 1.79 (1H, m, 3-Hb), 1.52 (2H, m, 4-H_2), 1.44 [9H, s, C(CH_3)$_3$]; ESI-MS (positive) *m*/*z*: 379.0 (37%; M+H$^+$), 401.0 (100%; M+Na$^+$), 402.0 (20%), 778.9 (46%; 2M+Na$^+$), 779.9 (19%). Anal. Calcd for $C_{18}H_{26}N_4O_5$: C, 57.13; H, 6.93; N, 14.81. Found: C, 57.2; H, 6.9; N, 14.7.

4.4. Preparation of the *O*-(3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-α-D-galactopyranosyl)trichloroacetimidate α-6

4.4.1. Preparation of the 3,4,6-tri-*O*-benzyl-1,2-*O*-dichloroacetyl-D-galactopyranose. A solution of chloroacetyl chloride (3.7 mL; 46.4 mmol) in diethyl ether (20 mL) was slowly added to a solution of 3,4,6-tri-*O*-benzylgalactopyranose[16] (7.00 g; 15.6 mmol) and pyridine (6.27 mL; 77.6 mmol) in diethyl ether (80 mL), maintaining the solution temperature at −10 °C. After warming to 25 °C and a 2 h stirring, the reaction mixture was diluted with ethyl acetate (150 mL) and washed with a cold aqueous solution of citric acid (200 mL; 10%) and then with brine (3 × 100 mL). Usual work-up afforded the crude 3,4,6-tri-*O*-benzyl-1,2-*O*-dichloroacetyl-D-galactopyranose (9.20 g; 98% yield), as an oil sufficiently pure for direct use in the next reaction: R_f 0.29 and 0.23 (α and β epimers) (silica gel, hexane/AcOEt; 80:20; v:v); ^{1}H NMR showed what it was a mixture of α and β epimers in a 1:1 ratio. ^{1}H NMR ($CDCl_3$) for α-epimer: δ 6.39 (1H, d, *J* = 3.5, 1-H), 5.56 (1H, d, *J* = 10.5, 3.5, 2-H), 4.06 (1H, dd, *J* = 2.8, <1, 4-H), 3.93 (1H, dd, *J* = 10.5, 2.8, 3-H); for β-epimer: δ 5.63 (1H, d, *J* = 8.4, 1-H), 5.50 (1H, d, *J* = 9.8, 8.4, 2-H), 4.02 (1H, dd, *J* = 3.0, <1, 4-H), 3.60 (1H, dd, *J* = 9.8, 3.0, 3-H); ESI-MS (positive) *m*/*z*: 625.1 (100%), 626.2 (34%), 627.1 (64%), 628.1 (22%), 629.1 (11%), 641.2 (11%), 643.2 (7%). Anal. Calcd for $C_{31}H_{32}Cl_2O_8$: C, 61.70; H, 5.34. Found: C, 61.5; H, 5.4.

4.4.2. Preparation of the 3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-D-galactopyranose. The obtained 1,2-O-dichloroacetyl derivative (7.70 g; 12.8 mmol) dissolved in CH_3CN (200 mL), was added to a solution of ammonia in acetonitrile (70 mL; prepared by bubbling ammonia gas through the solvent at 25 °C for 10 min). The mixture was stirring at 25 °C until the starting material has disappeared (TLC, hexane/AcOEt; 80:20; v:v) and then concentrated at temperature <40 °C. After dilution with AcOEt (80 mL) and usual work-up, the residue was purified by flash chromatography (eluting with hexane/AcOEt; 70:30; v:v) to afford the 3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-D-galactopyranose as a mixture (5.72 g; 85% yield) of α and β epimers in a 80:20 ratio (^{1}H NMR): an oil; R_f 0.32 (silica gel, hexane/AcOEt; 70:30; v:v); $[\alpha]_D^{26} = +43.3$ (*c* 1, $CHCl_3$); ^{1}H NMR

($CDCl_3$) for α-epimer: δ 7.36–7.25 (15H, aromatics), 5.47 (1H, d, $J = 3.5$, 1-H); 5.35 (1H, dd, $J = 10.5$, 3.5, 2-H), 4.90 (1H, d, $J = 11.4$, benzylic), 4.69 (1H, d, $J = 12.2$, benzylic), 4.62 (1H, d, $J = 12.2$, benzylic), 4.53 (1H, d, $J = 11.4$, benzylic), 4.46 (1H, d, $J = 11.9$, benzylic), 4.40 (1H, d, $J = 11.9$, benzylic), 4.14 (1H, ddd, $J = 6.6$, 6.1, <1, 5-H), 4.04 (1H, d, $J = 15.4$, $ClCHHCO_2$), 3.98 (1H, dd, $J = 10.5$, 2.8, 3-H), 3.97 (1H, d, $J = 15.4$, $ClCHHCO_2$), 3.94 (1H, dd, $J = 2.8$, <1, 4-H), 3.57 (1H, dd, $J = 9.5$, 6.6, 6-Ha), 3.44 (1H, dd, $J = 9.5$, 6.1, 6-Hb); ESI-MS (positive) *m/z*: 549.2 (100%; $M+Na^+$), 550.2 (33%), 551.2 (39%), 565.3 (11%; $M+K^+$), 566.3 (3%), 567.3 (4%). Anal. Calcd for $C_{29}H_{31}ClO_7$: C, 66.09; H, 5.93. Found: C, 65.8; H, 5.8.

4.4.3. Preparation of the title compound α-6. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU; 248 μL; 0.0017 mmol) was added to a solution of the 3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-D-galactopyranose (4.38 g; 8.3 mmol) and trichloroacetonitrile (8.3 mL; 83.0 mmol) in CH_2Cl_2 (80 mL) at −30 °C. After a stirring of 4 h at −30 °C, the solvent was removed under reduced pressure and the residue was purified by flash column chromatography (eluting with hexane/AcOEt/Et_3N; 85:15:1; v:v:v) to afford the *O*-(3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-α-D-galactopyranosyl)trichloroacetimidate α-**6** (4.41 g; 79% yield) as an oil; R_f 0.56 (silica gel, hexane/AcOEt; 70:30; v:v); $[\alpha]_D^{26} = +66.7$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.51 (1H, s, =N*H*), 7.35–7.25 (15H, aromatics), 6.54 (1H, d, $J = 3.5$, 1-H); 5.57 (1H, dd, $J = 10.3$, 3.5, 2-H), 4.96 (1H, d, $J = 11.3$, benzylic), 4.72 (1H, d, $J = 12.2$, benzylic), 4.61 (1H, d, $J = 12.2$, benzylic), 4.60 (1H, d, $J = 11.3$, benzylic), 4.47 (1H, d, $J = 11.7$, benzylic), 4.41 (1H, d, $J = 11.7$, benzylic), 4.17 (1H, ddd, $J = 8.4$, 5.6, <1, 5-H), 4.11 (1H, dd, $J = 2.8$, <1, 4-H), 4.06 (1H, dd, $J = 10.3$, 2.8, 3-H), 3.90 (2H, s, $ClCH_2CO_2$), 3.65 (1H, dd, $J = 9.1$, 8.4, 6-Ha), 3.57 (1H, dd, $J = 9.1$, 5.6, 6-Hb); ESI-MS (positive) *m/z*: 692.4 (76%), 693.4 (21%), 694.2 (100%), 695.2 (31%), 696.1 (49%), 697.1 (15%), 698.0 (12%). Anal. Calcd for $C_{31}H_{31}Cl_4NO_7$: C, 55.46; H, 4.65; N, 2.09. Found: C, 55.5; H, 4.5; N, 2.1.

Further elution affords the *O*-(3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-β-D-galactopyranosyl)trichloroacetimidate β-**6** (0.610 g; 11% yield) as an oil; R_f 0.39 (silica gel, hexane/AcOEt; 70:30; v:v); $[\alpha]_D^{26} = +20.3$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.59 (1H, s, =N*H*), 7.36–7.25 (15H, aromatics), 5.71 (1H, d, $J = 8.1$, 1-H), 5.64 (1H, dd, $J = 9.8$, 8.1, 2-H), 4.92 (1H, d, $J = 11.6$, benzylic), 4.67 (1H, d, $J = 12.3$, benzylic), 4.60 (1H, d, $J = 11.6$, benzylic), 4.48 (1H, d, $J = 12.3$, benzylic), 4.46 (1H, d, $J = 11.6$, benzylic), 4.42 (1H, d, $J = 11.6$, benzylic), 4.03 (1H, dd, $J = 2.6$, <1, 4-H), 3.89 (1H, d, $J = 14.9$, $ClCHHCO_2$), 3.84 (1H, d, $J = 14.9$, $ClCHHCO_2$), 3.77 (1H, ddd, $J = 7.5$, 5.5, <1, 5-H), 3.68–3.61 (3H, overlapping, 3-H, 6-H2); ESI-MS (positive) *m/z*: 692.4 (76%), 693.4 (21%), 694.2 (100%), 695.2 (31%), 696.1 (49%), 697.1 (15%), 698.0 (12%). Anal. Calcd for $C_{31}H_{31}Cl_4NO_7$: C, 55.46; H, 4.65; N, 2.09. Found: C, 55.4; H, 4.6; N, 2.2.

4.5. *tert*-Butyl (2*S*,5*R*)-6-azido-2-benzyloxycarbonylamino-5-(3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-β-D-galactopyranosyloxy)hexanoate 7

A mixture of *tert*-butyl (2*S*,5*R*)-6-azido-2-benzyloxycarbonylamino-5-hydroxyhexanoate (5*R*)-**5** (1.09 g; 2.88 mmol), *O*-(3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-α-D-galactopyranosyl)trichloroacetimidate α-**6** (3.3 g; 4.92 mmol) and powdered molecular sieves (3 Å, 500 mg) in anhydrous diethyl ether (120 mL) was stirred for 15 min at 25 °C. At this time *tert*-butyldimethylsilyl trifluoromethanesulfonate (330 μL; 1.44 mmol) was added and stirring was continued for 1 h under argon. At this time, the powdered molecular sieves were filtered off and washed with AcOEt. The organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (100 mL) and worked-up to afford a residue, which was purified by flash chromatography (eluting with hexane/AcOEt; 75:25; v:v) to give the title compound **7** (1.33 g; 52% yield): an oil; R_f 0.20 (silica gel, CH_2Cl_2/diisopropyl ether; 100:5; v:v); $[\alpha]_D^{26} = +7.9$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.33–7.23 (20H, aromatics), 5.34 (1H, dd, $J = 10.0$, 7.9, 2′-H), 5.33 (1H, d, $J = 7.7$, N*H*), 5.09 (1H, d, $J = 12.1$, part A of AB system, Cbz OC*H*HPh), 5.06 (1H, d, $J = 12.1$, part B of AB system, Cbz OCH*H*Ph), 4.90 (1H, d, $J = 11.6$, benzylic), 4.63 (1H, d, $J = 12.1$, benzylic), 4.56 (1H, d, $J = 11.6$, benzylic), 4.44 (1H, d, $J = 12.1$, benzylic), 4.43 (1H, d, $J = 11.7$, part A of AB system, benzylic), 4.43 (1H, d, $J = 7.9$, 1′-H), 4.41 (1H, d, $J = 11.7$, part B of AB system, benzylic), 4.18 (1H, m, 2-H), 4.01 (1H, d, $J = 14.7$, $ClCHHCO_2$), 3.92 (1H, dd, $J = 2.6$, <1, 4′-H), 3.92 (1H, d, $J = 14.7$, $ClCHHCO_2$), 3.63 (1H, m, 5-H), 3.59–3.54 (3H, overlapping, 5′-H, 6′-Ha, 6′-Hb), 3.50 (1H, dd, $J = 10.0$, 2.6, 3′-H), 3.37 (1H, dd, $J = 13.0$, 4.6, 6-Ha), 3.33 (1H, dd, $J = 13.0$, 5.9, 6-Hb), 1.82 (1H, m, 3-Ha), 1.61–1.51 (3H, overlapping, 3-Hb, 4-Ha, 4-Hb), 1.44 [9H, s, $C(CH_3)_3$]; ^{13}C NMR ($CDCl_3$): δ 171.0 (C-1), 165.9 ($ClCH_2CO_2$), 155.8 (NH*C*O), 101.5 (C-1′), 82.5 [$C(CH_3)_3$], 80.1 (C-3′), 78.3 (C-5), 74.5, 73.6, 72.1 ($3 \times OCH_2Ph$), 73.7 (C-5′), 73.1 (C-2′), 72.4 (C-4′), 68.6 (C-6′), 67.0 ($NHCO_2CH_2Ph$), 54.4 (C-6), 54.1 (C-2), 40.7 ($ClCH_2CO_2$), 28.4 (C-3), 27.9 [$C(CH_3)_3$], 27.7 (C-4); ESI-MS (positive) *m/z*: 909.4 (100%; $^{35}Cl-M+Na^+$), 910.4 (50%), 911.4 (43%; $^{37}Cl-M+Na^+$), 925.3 (23%; $^{35}Cl-M+K^+$), 926.4 (12%), 927.4 (11%; $^{37}Cl-M+K^+$). Anal. Calcd for $C_{47}H_{55}ClN_4O_{11}$: C, 63.61; H, 6.25; N, 6.31. Found: C, 63.5; H, 6.3; N, 6.3.

4.6. *tert*-Butyl (2*S*,5*R*)-6-azido-2-benzyloxycarbonylamino-5-(3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-β-D-galactopyranosyloxy)hexanoate 7 from the β-trichloroacetimidate β-6

Starting with the *tert*-butyl (2*S*,5*R*)-6-azido-2-benzyloxycarbonylamino-5-hydroxyhexanoate (5*R*)-**5** (165 mg; 0.44 mmol) and the *O*-(3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-β-D-galactopyranosyl)trichloroacetimidate β-**6** (500 mg; 0.75 mmol) and following the glycosylation procedure described above, the title compound **7** (190 mg; 49% yield) was obtained, identical in all respects with that described above.

4.7. *tert*-Butyl (2*S*,5*R*)-6-azido-2-benzyloxycarbonylamino-5-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyloxy)hexanoate 8

A mixture of chloroacetylated galactoside **7** (1.2 g; 1.35 mmol) and $Zn(CH_3COO)_2$ $2H_2O$ (648 mg; 2.95 mmol) in MeOH (30 mL) was refluxed for 9 h. Then the solvent was evaporated and the residue was dissolved in AcOEt (50 mL) and worked-up. Flash chromatography (eluting with hexane/AcOEt; 75:25; v:v) afforded the pure title compound **8** (1.02 g; 93% yield): an oil; R_f 0.35 (silica gel, hexane/AcOEt; 70:30; v:v); $[\alpha]_D^{26} = +0.7$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.37–7.26 (20H, aromatics), 5.76 (1H, d, *J* = 7.7, N*H*), 5.13–5.09 (2H, AB system, benzylic), 4.90 (1H, d, *J* = 11.6, benzylic), 4.69 (2H, s, benzylic), 4.60 (1H, d, *J* = 11.6, benzylic), 4.47 (1H, d, *J* = 11.7, benzylic), 4.43 (1H, d, *J* = 11.7, benzylic), 4.31 (1H, d, *J* = 7.7, 1′-H), 4.25 (1H, ddd, *J* = 7.7, 6.3, 6.3, 2-H), 3.93 (1H, ddd, *J* = 9.8, 7.7, 2.3, 2′-H), 3.85 (1H, dd, *J* = 2.5, <1, 4′-H), 3.74 (1H, m, 5-H), 3.59–3.54 (3H, overlapping, 5′-H, 6′-Ha, 6′-Hb), 3.41 (2H, d, *J* = 4.7, 6-H_2), 3.37 (1H, dd, *J* = 9.8, 2.5, 3′-H), 3.19 (1H, d, *J* = 2.3, 2′-OH), 1.95 (1H, m, 3-Ha), 1.85 (1H, m, 3-Hb), 1.68 (1H, m, 4-Ha), 1.63 (1H, m, 4-Hb), 1.45 [9H, s, C(C*H*$_3$)$_3$]; ^{13}C NMR ($CDCl_3$): δ 171.2 (C-1), 155.8 (NH*C*O), 103.8 (C-1′), 82.1 [*C*(CH$_3$)$_3$], 81.9 (C-3′), 78.1 (C-5), 74.3, 73.3, 72.6 (3 × O*C*H$_2$Ph), 73.6 (C-5′), 73.0 (C-4′), 71.3 (C-2′), 68.7 (C-6′), 66.8 (NHCO$_2$*C*H$_2$Ph), 54.6 (C-6), 54.0 (C-2), 27.8 [C(*C*H$_3$)$_3$], 27.7 (C-4), 27.5 (C-3); ESI-MS (positive) *m*/*z*: 833.4 (100%; M+Na$^+$), 834.4 (51%), 835.0 (15%), 849.5 (29%; M+K$^+$), 850.5 (15%). Anal. Calcd for $C_{45}H_{54}N_4O_{10}$: C, 66.65; H, 6.71; N, 6.91. Found: C, 66.5; H, 6.6; N, 6.8.

4.8. (2*S*,5*R*)-2,6-Diamino-5-(β-D-galactopyranosyloxy)hexanoic acid 1

The *tert*-butyl (2*S*,5*R*)-6-azido-2-benzyloxycarbonylamino-5-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyloxy)-hexanoic acid **8** (200 mg; 0.25 mmol) in tetrahydrofuran (8.6 mL) and ethanol (28.6 mL) was hydrogenated in the presence of palladium (generated from 30 mg of $PdCl_3$), at room temperature under 1 atm of hydrogen. After 6 h, the reaction was monitored on TLC (silica gel, MeOH/25% aqueous NH_3; 10:2; v:v), which indicated the presence of a single spot at R_f 0.21. Then the catalyst was filtered off on a pad of Celite and washed with methanol (15 mL). Evaporation of the solvent mixture under reduced pressure (below 40 °C) afforded a residue, which was dissolved into TFA–H_2O (1.5 mL; 95:5; v:v) and the resulting solution was stirred at room temperature for 1 h. The crude product obtained by evaporation of the solvent was dissolved in distilled water (3 mL) and washed with toluene (2 × 4 mL). The aqueous solution was co-evaporated with toluene under reduced pressure (below 40 °C) to afford the pure glucosylgalactosyl hydroxylysine **1** as ditrifluoroacetate (125 mg; 92% yield); a syrup; R_f 0.21 (silica gel, MeOH/25% aqueous NH_3; 10:2; v:v); $[\alpha]_D^{26} = -0.9$ (*c* 1, MeOH) [lit.[9] −1.4 (*c* 0.18, MeOH)]; ^{1}H NMR (D_2O): δ 4.42 (1H, d, *J* = 7.7, 1′-H), 4.10 (1H, dd, *J* = 6.3, 6.3, 2-H), 4.04 (1H, m, 5-H), 3.88 (1H, dd, *J* = 2.7, <1, 4′-H), 3.73–3.71 (2H, AB system, 6′-Ha, 6′-Hb), 3.66 (1H, ddd, *J* = 7.4, 4.7, <1, 5′-H), 3.61 (1H, dd, *J* = 10.0, 2.7, 3′-H), 3.51 (1H, dd, *J* = 10.0, 7.7, 2′-H), 3.17 (1H, dd, *J* = 13.1, 1.4, 6-Ha), 2.99 (1H, dd, *J* = 13.1, 9.5, 6-Hb), 2.20 (1H, m, 3-Ha), 2.00 (1H, m, 3-Hb), 1.78 (1H, m, 4-Ha), 1.72 (1H, m, 4-Hb); ^{13}C NMR (D_2O): δ 172.6 (C-1), 163.7 (q, J_{CF} = 36.2, $CF_3CO\overline{O}$), 117.3 (q, J_{CF} = 290.9, CF_3COO^-), 103.4 (C-1′), 76.7 (C-5), 76.4 (C-5′), 73.7 (C-3′), 72.1 (C-2′), 69.8 (C-4′), 62.3 (C-6′), 53.7 (C-2), 44.0 (C-6), 29.1 (C-4), 26.5 (C-3); ESI-MS (positive) *m*/*z*: 325.2 (100%; M+H$^+$), 326.2 (13%), 347.2 (27%; M+Na$^+$), 363.3 (5%; M+K$^+$). Anal. Calcd for $C_{12}H_{24}N_2O_8{\cdot}2CF_3COOH$: C, 34.79; H, 4.74; N, 5.07. Found: C, 34.7; H, 4.6; N, 5.1.

4.9. *tert*-Butyl (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 10

A mixture of galactoside **8** (1.61 g; 1.98 mmol), *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)trichloroacetimidate **9**[19,20] (2.66 g; 3.96 mmol) and powdered molecular sieves (3 A; 500 mg) in anhydrous diethyl ether (120 mL) was stirred for 15 min at room temperature. Then *tert*-butyldimethylsilyl triflate (227 μL; 0.99 mmol) was added and stirring was continued, at room temperature, for 1 h under argon. The powdered molecular sieves were filtered off and washed with AcOEt. The organic phase was washed with a saturated $NaHCO_3$ aqueous solution (100 mL) and worked-up to afford a residue, which was purified by flash chromatography (eluting with hexane/AcOEt; 80:20; v:v) to give the title compound **10** (1.19 g; 45% yield) as an oil; R_f 0.28 (silica gel, hexane/AcOEt; 80:20; v:v); $[\alpha]_D^{29} = +25.6$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.38–7.06 (20H, aromatics), 5.62 (1H, d, *J* = 3.5, 1″-H), 5.20 (1H, d, *J* = 7.9, N*H*), 5.08–4.45 (16H, benzylic) 4.63 (1H, d, *J* = 7.7, 1′-H), 4.33 (1H, ddd, *J* = 10.0, 2.2, 2.2, 5″-H), 4.21 (1H, dd, *J* = 10.0, 7.7, 2′-H), 4.14 (1H, m, 2-H), 4.01 (1H, dd, *J* = 9.5, 9.5, 3″-H), 3.99 (1H, dd, *J* = 3.0, <1, 4′-H), 3.88 (1H, m, 5-H), 3.69 (1H, dd, *J* = 10.0, 9.5, 4″-H), 3.63 (1H, dd, *J* = 9.5, 3.5, 2″-H), 3.64–3.61 (3H, overlapping, 5′-H, 6′-Ha, 6′-Hb), 3.62 (1H, dd, *J* = 10.0, 3.0, 3′-H), 3.54 (1H, dd, *J* = 12.8, 4.0, 6-Ha), 3.45–3.39 (2H, AB system, 6″-Ha, 6″-Hb), 3.35 (1H, dd, *J* = 12.8, 3.9, 6-Hb), 1.75–1.78 (2H, overlapping, 3-Ha, 4-Ha), 1.61–1.54 (2H, overlapping, 3-Hb, 4-Hb), 1.44 [9H, s, C(C*H*$_3$)$_3$]; ESI-MS (positive) *m*/*z*: 1355.6 (100%; M+Na$^+$), 1356.6 (82%), 1357.6 (40%), 1358.5 (11%), 1371.5 (31%; M+K$^+$), 1372.5 (24%), 1373.5 (13%), 1374.4 (4%). Anal. Calcd for $C_{79}H_{88}N_4O_{15}$: C, 71.15; H, 6.65; N, 4.20. Found: C, 71.0; H, 6.7; N, 4.2.

4.10. *tert*-Butyl (2*S*,5*R*)-2,6-diamino-5-[α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyloxy]hexanoate 11

The protected compound **10** (300 mg; 0.22 mmol) in tetrahydrofuran (39 mL) and ethanol (114 mL) was hydrogenated in the presence of palladium (10% on activated carbon, 300 mg) at room temperature under 1 atm of hydrogen. After 9 h, the reaction was monitored on

TLC (silica gel, MeOH/25% aqueous NH_3; 10:1.5; v:v), which indicated the presence of a single spot at R_f 0.27. Then the catalyst was filtered off on a pad of Celite and thoroughly rinsed with a methanolic solution of distilled water (20 mL; 5:1; v:v). Evaporation of the combined filtrates under reduced pressure (below 40 °C) 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 (below 40 °C) to give the title compound **11**, (110 mg; 90% yield) as a syrup; R_f 0.27 (silica gel, MeOH/25% aqueous NH_3; 10:1.5; v:v); $[\alpha]_D^{27} = +25.9$ (*c* 1, MeOH); ^{1}H NMR (D_2O): δ 5.35 (1H, d, *J* = 3.6, 1″-H), 4.63 (1H, d, *J* = 7.5, 1′-H), 4.12 (1H, m, 5-H), 4.03 (1H, ddd, *J* = 9.5, 1.6 and 1.6, 5″-H), 4.02 (1H, dd, *J* = 6.4, 6.4, 2-H), 3.90 (1H, dd, *J* = 3.0, <1, 4′-H), 3.80 (1H, dd, *J* = 12.2, 1.6, 6″-Ha), 3.75–3.62 (6H, overlapping, 3′-H, 5′-H, 6′-H_2, 3″-H, 6″-Hb), 3.68 (1H, dd, *J* = 9.5, 7.5, 2′-H), 3.50 (1H, dd, *J* = 9.9, 3.6, 2″-H), 3.39 (1H, dd, *J* = 9.5, 9.5, 4″-H), 3.19 (1H, dd, *J* = 13.3, 1.9, 6-Ha), 3.01 (1H, dd, *J* = 13.3 and 9.9, 6-Hb), 2.10 (1H, m, 3-Ha), 2.00 (1H, m, 3-Ha), 1.85 (1H, m, 4-Ha), 1.69 (1H, m, 4-Hb), 1.48 [9H, s, C(C*H*$_3$)$_3$]; ^{13}C NMR (D_2O): δ 170.1 (C-1), 102.4 (C-1′), 98.6 (C-1″), 86.9 [*C* (CH_3)$_3$], 76.1, 75.8 (C-2′, C-3″), 75.1 (C-5), 73.8 (C-3′), 72.6 (C-5″), 72.5 (C-5′), 72.3 (C-2″), 70.4 (C-4″), 69.9 (C-4′), 62.1 (C-6′), 61.4 (C-6″), 54.0 (C-2), 43.6 (C-6), 28.7 (C-4), 28.1 [C(CH_3)$_3$], 26.3 (C-3); ESI-MS (positive) *m/z*: 543.1 (100%; M+H^+), 544.1 (23%), 565.1 (23%; M+Na^+), 566.2 (6%). Anal. Calcd for $C_{22}H_{42}N_2O_{13}$: C, 48.70; H, 7.80; N, 5.16. Found: C, 48.8; H, 7.8; N, 5.1.

4.11. (2*S*,5*R*)-2,6-Diamino-5-[α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyloxy]hexanoic acid 2

The *tert*-butyl ester **11** (300 mg; 0.55 mmol) was dissolved into TFA–H_2O (3.0 mL; 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 **2** as ditrifluoroacetate (363 mg; 92% yield); a syrup; R_f 0.22 (silica gel, MeOH/25% aqueous NH_3; 10:3; v:v); $[\alpha]_D^{26} = +21.9$ (*c* 1, MeOH); ^{1}H NMR (D_2O): δ 5.33 (1H, d, *J* = 3.6, 1″-H), 4.61 (1H, d, *J* = 7.5, 1′-H), 4.10 (1H, m, 5-H), 4.07 (1H, dd, *J* = 6.5, 6.5, 2-H), 4.02 (1H, ddd, *J* = 9.7, 1.5 and 1.5, 5″-H), 3.89 (1H, dd, *J* = 2.3, <1, 4′-H), 3.80 (1H, dd, *J* = 11.3, <1, 6″-Ha), 3.74–3.66 (6H, overlapping, 3′-H, 5′-H, 6′-H_2, 3″-H, 6″-Hb), 3.65 (1H, dd, *J* = 9.5, 7.5, 2′-H), 3.49 (1H, dd, *J* = 9.9, 3.6, 2″-H), 3.38 (1H, dd, *J* = 9.7, 9.7, 4″-H), 3.18 (1H, dd, *J* = 12.9, <1, 6-Ha), 3.00 (1H, dd, *J* = 12.9 and 10.5, 6-Hb), 2.14 (1H, m, 3-Ha), 2.00 (1H, m, 3-Hb), 1.87 (1H, m, 4-Ha), 1.71 (1H, m, 4-Hb); ^{13}C NMR (D_2O): δ 172.4 (C-1), 163.7 (q, J_{CF} = 34.3, CF_3*C*OO$^-$), 117.3 (q, J_{CF} = 289.9, *C*F_3COO$^-$), 102.5 (C-1′), 98.5 (C-1″), 76.1, 75.8 (C-2′, C-3″), 75.2 (C-5), 73.8 (C-3′), 72.6 (C-5″), 72.4 (C-2″), 72.2 (C-5′), 70.4 (C-4″), 69.9 (C-4′), 62.0 (C-6′), 61.3 (C-6″), 53.4 (C-2), 43.6 (C-6), 28.8 (C-4), 26.2 (C-3); ESI-MS (positive) *m/z*: 487.0 (100%; M+H^+), 488.1 (19%), 489.1 (4%), 509.2 (38%; M+Na^+), 525.1 (5%; M+K^+), 973.0 (7%; 2M+H^+). Anal. Calcd for $C_{18}H_{34}N_2O_{13}$·2CF_3COOH: C, 36.98; H, 5.08; N, 3.92. Found: C, 37.0; H, 5.2; N, 3.9.

4.12. *tert*-Butyl (2*S*,5*S*)-6-azido-2-benzyloxycarbonylamino-5-(3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-β-D-galactopyranosyloxy)hexanoate 12

Starting with the *tert*-butyl (2*S*,5*S*)-6-azido-2-benzyloxycarbonylamino-5-hydroxyhexanoate (5*S*)-**5** (900 mg; 2.38 mmol) and the *O*-(3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-α-D-galactopyranosyl)trichloroacetimidate α-**6** (2.72 g; 4.05 mmol) and following the glycosylation procedure described above for the epimeric ester (5*R*)-**5**, the title compound **12** (1.06 g; 50% yield) was obtained, after flash chromatography (eluting with hexane/AcOEt; 75:25; v:v): an oil R_f 0.20 (silica gel, CH_2Cl_2/diisopropyl ether; 100:5; v:v); $[\alpha]_D^{27} = +1.7$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.35–7.23 (20H, aromatics), 5.34 (1H, dd, *J* = 9.8, 7.7, 2′-H), 5.31 (1H, d, *J* = 7.2, N*H*), 5.06 (2H, s, Cbz OCH_2Ph), 4.89 (1H, d, *J* = 11.6, benzylic), 4.65 (1H, d, *J* = 12.1, benzylic), 4.54 (1H, d, *J* = 11.6, benzylic), 4.47 (1H, d, *J* = 7.7, 1′-H), 4.46 (1H, d, *J* = 11.7, benzylic), 4.44 (1H, d, *J* = 12.1, benzylic), 4.38 (1H, d, *J* = 11.7, benzylic), 4.22 (1H, m, 2-H), 3.99 (1H, d, *J* = 14.7, ClC*H*HCO$_2$), 3.95 (1H, dd, *J* = 2.8, <1, 4′-H), 3.90 (1H, d, *J* = 14.7, ClCH*H*CO$_2$), 3.71 (1H, m, 5-H), 3.63 (1H, ddd, *J* = 7.7, 7.7, <1, 5′-H), 3.59–3.55 (2H, overlapping, 6′-Ha, 6′-Hb), 3.52 (1H, dd, *J* = 9.8, 2.8, 3′-H), 3.27 (1H, dd, *J* = 12.6, 3.5, 6-Ha), 3.13 (1H, dd, *J* = 12.6, 6.3, 6-Hb), 1.87 (1H, m, 3-Ha), 1.75 (1H, m, 3-Hb), 1.52–1.48 (2H, overlapping, 4-Ha, 4-Hb), 1.37 [9H, s, C(C*H*$_3$)$_3$]; ^{13}C NMR ($CDCl_3$): δ 171.4 (C-1), 166.1 (ClCH_2*C*O$_2$), 155.9 (NH*C*O), 100.2 (C-1′), 82.1 [*C*(CH_3)$_3$], 80.1 (C-3′), 76.8 (C-5), 74.5, 73.5, 72.0 (3 × OCH_2Ph), 73.5 (C-5′), 73.0 (C-2′), 72.6 (C-4′), 68.3 (C-6′), 66.7 (NHCO$_2$*C*H$_2$Ph), 54.6 (C-6), 53.7 (C-2), 40.8 (Cl*C*H$_2$CO$_2$), 28.3 (C-4), 28.2 (C-3), 28.0 [C(*C*H$_3$)$_3$]; ESI-MS (positive) *m/z*: 909.4 (100%; ^{35}Cl−M+Na^+), 910.4 (51%), 911.3 (43%; ^{37}Cl−M+Na^+), 925.4 (25%; ^{35}Cl−M+K^+), 926.4 (12%), 927.4 (10%; ^{37}Cl−M+K^+). Anal. Calcd for $C_{47}H_{55}ClN_4O_{11}$: C, 63.61; H, 6.25; N, 6.31. Found: C, 63.6; H, 6.2; N, 6.2.

4.13. *tert*-Butyl (2*S*,5*S*)-6-azido-2-benzyloxycarbonylamino-5-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyloxy)hexanoate 13

Starting with chloroacetylated galactoside **12** (900 mg; 1.01 mmol) and following the procedure described above for the selective hydrolysis of the epimer **7**, the title compound **13** (748 mg; 91% yield) was obtained after flash chromatography (eluting with hexane/AcOEt; 75:25; v:v): an oil; R_f 0.26 (silica gel, hexane/AcOEt; 70:30; v:v); $[\alpha]_D^{30} = -1.9$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.34–7.23 (20H, aromatics), 5.35 (1H, d, *J* = 8.1, N*H*), 5.06 (2H, s, benzylic), 4.87 (1H, d, *J* = 11.6, benzylic), 4.72–4.66 (2H, AB system, benzylic), 4.57 (1H, d, *J* = 11.6, benzylic), 4.44 (1H, d, *J* = 11.9, benzylic), 4.37 (1H, d, *J* = 11.9, benzylic), 4.30 (1H, d, *J* = 7.7, 1′-H), 4.23 (1H, m, 2-H), 3.91 (1H, ddd, *J* = 9.8, 7.7, 2.3, 2′-H), 3.89 (1H, dd, *J* = 2.6, <1, 4′-H), 3.78 (1H, m, 5-H), 3.62–3.54 (3H, overlapping, 5′-H, 6′-Ha, 6′-Hb), 3.41 (1H, dd, *J* = 9.8, 2.8, 3′-H), 3.37 (1H, dd,

J = 12.8, 3.7, 6-Ha), 3.26 (1H, dd, J = 12.8, 6.3, 6-Hb), 2.57 (1H, d, J = 2.3, 2′-OH), 1.89 (1H, m, 3-Ha), 1.80 (1H, m, 3-Hb), 1.59 (2H, m, 4-H_2), 1.38 [9H, s, C(CH_3)$_3$]; ^{13}C NMR ($CDCl_3$): δ 171.3 (C-1), 155.9 (NH*C*O), 102.8 (C-1′), 81.9 (C-3′), 81.8 [*C*(CH$_3$)$_3$], 77.4 (C-5), 74.4, 73.3, 72.5 (3 × O*C*H$_2$Ph), 73.5 (C-5′), 73.0 (C-4′), 71.3 (C-2′), 68.5 (C-6′), 66.7 (NHCO$_2$*C*H$_2$Ph), 54.3 (C-6), 53.8 (C-2), 28.3 (C-4), 28.1 (C-3), 27.8 [C(*C*H$_3$)$_3$]; ESI-MS (positive) *m/z*: 833.4 (100%; M+Na$^+$), 834.4 (51%), 835.0 (15%), 849.5 (25%; M+K$^+$), 850.5 (13%). Anal. Calcd for $C_{45}H_{54}N_4O_{10}$: C, 66.65; H, 6.71; N, 6.91. Found: C, 66.6; H, 6.7; N, 6.8.

4.14. (2*S*,5*S*)-2,6-Diamino-5-(β-D-galactopyranosyloxy)hexanoic acid 3

Starting with the *tert*-butyl (2*S*,5*S*)-6-azido-2-benzyloxycarbonylamino-5-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyloxy)hexanoate **13** (250 mg; 0.31 mmol) and following the same procedure above reported for the (2*S*,5*R*)-isomer **8**, the title compound **3** as ditrifluoroacetate was obtained (153 mg; 90% yield) as a syrup; R_f 0.22 (silica gel, MeOH/25% aqueous NH_3; 10:2; v:v); $[\alpha]_D^{27} = +21.3$ (*c* 1, MeOH); ^{1}H NMR (D_2O): δ 4.46 (1H, d, J = 7.7, 1′-H), 4.12 (1H, dd, J = 6.3, 6.3, 2-H), 4.10 (1H, m, 5-H), 3.88 (1H, dd, J = 2.8, <1, 4′-H), 3.73–3.70 (2H, AB system, 6′-Ha, 6′-Hb), 3.66 (1H, ddd, J = 6.5, 5.7, <1, 5′-H), 3.62 (1H, dd, J = 10.2, 2.8, 3′-H), 3.53 (1H, dd, J = 10.2, 7.7, 2′-H), 3.20 (1H, dd, J = 13.3, 2.5, 6-Ha), 3.12 (1H, dd, J = 13.3, 8.3, 6-Hb), 2.09 (2H, m, 3-H_2), 1.85 (1H, m, 4-Ha), 1.79 (1H, m, 4-Hb); ^{13}C NMR (D_2O): δ 172.7 (C-1), 163.7 (q, J_{CF} = 36.2, CF_3*C*OO$^-$), 117.3 (q, J_{CF} = 290.9, *C*F$_3$COO$^-$), 103.7 (C-1′), 77.5 (C-5), 76.3 (C-5′), 73.7 (C-3′), 72.0 (C-2′), 69.6 (C-4′), 62.0 (C-6′), 53.5 (C-2), 43.5 (C-6), 28.4 (C-4), 26.2 (C-3); ESI-MS (positive) *m/z*: 325.2 (100%; M+H$^+$), 326.2 (13%), 347.2, (30%; M+Na$^+$), 363.3 (4%; M+K$^+$). Anal. Calcd for $C_{12}H_{24}N_2O_8{\cdot}2CF_3COOH$: C, 34.79; H, 4.74; N, 5.07. Found: C, 34.8; H, 4.7; N, 5.1.

4.15. *tert*-Butyl (2*S*,5*S*)-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 14

Starting with *tert*-butyl (2*S*,5*S*)-6-azido-2-benzyloxycarbonylamino-5-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyloxy)hexanoate **13** (1.81 g; 2.23 mmol), and following the procedure described above for the glycosylation of the epimer **8**, the title compound **14** was obtained (1.25 g; 42% yield) as a pure oil after flash chromatography (eluting with hexane/AcOEt; 80:20; v:v); R_f 0.28 (silica gel, hexane/AcOEt; 80:20; v:v); $[\alpha]_D^{32} = +31.7$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.37–7.05 (20H, aromatics), 5.50 (1H, d, J = 3.5, 1″-H), 5.28 (1H, d, J = 8.4, N*H*), 5.08–4.35 (16H, benzylic) 4.55 (1H, d, J = 7.7, 1′-H), 4.30 (1H, ddd, J = 10.2, 2.3, 2.3, 5″-H), 4.21 (1H, m, 2-H), 4.10 (1H, dd, J = 9.8, 7.7, 2′-H), 4.01 (1H, dd, J = 9.5, 9.5, 3″-H), 3.96 (1H, dd, J = 2.5, <1, 4′-H), 3.79 (1H, m, 5-H), 3.67 (1H, dd, J = 10.2, 9.5, 4″-H), 3.61 (1H, dd, J = 9.5, 3.5, 2″-H), 3.64–3.53 (3H, overlapping, 5′-H, 6′-Ha, 6′-Hb), 3.58 (1H, dd, J = 9.8, 2.5, 3′-H), 3.41–3.37 (2H, AB system, 6″-Ha, 6″-Hb), 3.20 (1H, dd, J = 12.6, 5.1, 6-Ha), 3.00 (1H, dd, J = 12.6, 6.1, 6-Hb), 1.83 (1H, m, 3-Ha), 1.68 (1H, m, 3-Hb), 1.58 (1H, m, 4-Ha), 1.43 (1H, m, 4-Hb), 1.37 [9H, s, C(CH_3)$_3$]; ESI-MS (positive) *m/z*: 1355.6 (100%; M+Na$^+$), 1356.6 (80%), 1357.6 (39%), 1358.5 (13%), 1371.5, (31%; M+K$^+$), 1372.5 (26%), 1373.5 (13%), 1374.4 (4%). Anal. Calcd for $C_{79}H_{88}N_4O_{15}$: C, 71.15; H, 6.65; N, 4.20. Found: C, 71.0; H, 6.6; N, 4.1.

4.16. *tert*-Butyl (2*S*,5*S*)-2,6-diamino-5-[α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyloxy]hexanoate 15

Starting with *tert*-butyl (2*S*,5*S*)-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 **14** (300 mg; 0.22 mmol) and following the procedure described above for the hydrogenolysis of the epimer **10**, the title compound **15** was obtained (112 mg; 92% yield) as a syrup; R_f 0.27 (silica gel, MeOH/25% aqueous NH_3; 10:1.5; v:v); $[\alpha]_D^{27} = +41.1$(*c* 1, MeOH); ^{1}H NMR (D_2O): δ 5.21 (1H, d, J = 3.2, 1″-H), 4.62 (1H, d, J = 7.9, 1′-H), 4.18 (1H, m, 5-H), 4.08 (1H, dd, J = 6.0, 6.0, 2-H), 4.00 (1H, ddd, J = 9.8, 1.6 and 1.6, 5″-H), 3.92 (1H, dd, J = 2.5, <1, 4′-H), 3.80 (1H, dd, J = 11.5, 1.5, 6″-Ha), 3.76–3.64 (6H, overlapping, 3′-H, 5′-H, 6′-H_2, 3″-H, 6″-Hb), 3.61 (1H, dd, J = 9.7, 7.5, 2′-H), 3.58 (1H, dd, J = 10.5, 3.2, 2″-H), 3.43 (1H, dd, J = 9.8, 9.8, 4″-H), 3.25 (1H, dd, J = 12.6, 1.9, 6-Ha), 3.16 (1H, dd, J = 12.6 and 8.0, 6-Hb), 2.09 (2H, ddd, J = 7.2, 7.2, 7.2, 3-H_2), 1.86 (1H, m, 4-Ha), 1.75 (1H, m, 4-Hb), 1.50 [9H, s, C(CH_3)$_3$]; ^{13}C NMR (D_2O): 169.8 (C-1), 103.0 (C-1′), 100.2 (C-1″), 86.8 [*C*(CH$_3$)$_3$], 79.2 (C-2′), 76.2 (C-3″), 76.1 (C-5), 73.9 (C-3′), 73.0 (C-5″), 72.5 (C-5′), 72.4 (C-2″), 70.3 (C-4″), 69.9 (C-4′), 62.0 (C-6′), 61.2 (C-6″), 54.0 (C-2), 43.2 (C-6), 28.5 (C-4), 28.1 [C(*C*H$_3$)$_3$], 26.3 (C-3); ESI-MS (positive) *m/z*: 543.1 (100%; M+H$^+$), 544.1 (25%), 565.2 (22%; M+Na$^+$), 566.2 (6%). Anal. Calcd for $C_{22}H_{42}N_2O_{13}$: C, 48.70; H, 7.80; N, 5.16. Found: C, 48.6; H, 7.7; N, 5.2.

4.17. (2*S*,5*S*)-2,6-Diamino-5-[α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyloxy]hexanoic acid 4

Starting with *tert*-butyl (2*S*,5*S*)-2,6-diamino-5-[α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyloxy]hexanoate **15** (300 mg; 0.55 mmol) and following the procedure described above for the hydrolysis of the analogue **11**, the title compound **4** was obtained as ditrifluoroacetate (355 mg; 90% yield); a syrup; R_f 0.22 (silica gel, MeOH/25% aqueous NH_3; 10:3; v:v); $[\alpha]_D^{27} = +33.4$ (*c* 1, MeOH); ^{1}H NMR (D_2O): δ 5.20 (1H, d, J = 3.6, 1″-H), 4.59 (1H, d, J = 7.2, 1′-H), 4.14 (1H, m, 5-H), 3.98 (1H, ddd, J = 9.8, 4.8, 2.9, 5″-H), 3.90–3.87 (2H, overlapping, 2-H, 4′-H), 3.77–3.65 (5H, overlapping, 5′-H, 6′-H_2, 6″-H_2), 3.69 (1H, dd, J = 10.0, 3.5, 3′-H), 3.66 (1H, dd, J = 10.5, 10.0, 3″-H), 3.59 (1H, dd, J = 10.0, 7.2, 2′-H), 3.55 (1H, dd, J = 10.5, 3.6, 2″-H), 3.41 (1H, dd, J = 10.0, 9.8, 4″-H), 3.22 (1H, dd, J = 13.6, 2.9, 6-Ha), 3.14 (1H, dd, J = 13.6, 7.2, 6-Hb), 2.02 (2H, ddd, J = 7.2, 7.2, 7.2, 3-H_2), 1.83 (1H, m, 4-Ha), 1.74 (1H,

m, 4-Hb); ^{13}C NMR (D_2O): δ 174.1 (C-1), 163.7 (q, $J_{CF} = 34.3$, CF_3COO^-), 117.0 (q, $J_{CF} = 289.9$, CF_3COO^-), 102.7 (C-1′), 99.9 (C-1″), 78.7 (C-2′), 76.0 (C-5 and C-3″), 73.6 (C-3′), 72.7 (C-5″), 72.3 (C-5′), 72.1 (C-2″), 70.0 (C-4″), 69.7 (C-4′), 61.7 (C-6′), 60.9 (C-6″), 54.4 (C-2), 42.8 (C-6), 28.2 (C-4), 26.1 (C-3); ESI-MS (positive) *m/z*: 487.0 (100%; $M+H^+$), 488.1 (20%), 489.1 (3%), 509.2 (36%; $M+Na^+$), 525.1 (4%; $M+K^+$), 973.0 (6%; $2M+H^+$). Anal. Calcd for $C_{18}H_{34}N_2O_{13}\cdot 2CF_3COOH$: C, 36.98; H, 5.08; N, 3.92. Found: C, 37.1; H, 4.9; N, 3.9.

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