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The first synthesis of glucosylgalactosyl hydroxylysine (Glu-Gal-Hyl) an important biological indicator of collagen turnover

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Abstract—This paper reports the first chemical synthesis of α-D-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl-*O*-hydroxylysine, a glycoside of hydroxylysine important as indicator of skin and bone collagen turnover, starting with commercial compounds.

The glucosylgalactosyl hydroxylysine **1** (Scheme 1) is a component of collagen, isolated from hydrolysates of various soluble collagens from marine sponges and from vertebrates.[1–5] The human glycoside, formed by a post-translational oxidation collagen lysine and successive glycosylation, is released during collagen breakdown and excreted in urine.[2,6]

Actually, glycoside **1** has attracted considerable attention both as component of collagen useful in research aimed at understanding how collagen induces rheumatoid arthritis[7] (an inflammatory disease characterized by cartilage degradation and bone erosion), and as indicator, together with the shorter glycoside (5*R*)-5-*O*-(β-D-galactopyranosyl)-5-hydroxy-L-lysine, of total collagen turnover.[8] Therefore, between some synthetic methods affording mono or diglycosylated hydroxylysine building blocks, useful in solid-phase synthesis of glycopeptides from collagen,[9] various analytical methods for the quantitative detection of the glycoside **1** or of galactosyl hydroxylysine in biological samples have been reported.[10–13] However, no synthesis of the free glucosylgalactosyl hydroxylysine **1** has, until now, been reported and the reference standards of this compound are obtained from different natural sources, in forms relatively free of contaminants.[2,5,14] Therefore, the accessibility of the glycoside **1** by synthesis represents an important target for the correct standardization of the analytical techniques normally used in clinical determinations of this biochemical marker.

On the other hand the availability of a simple protocol for the preparation of the glycoside **1** and of its congeners is of self-consistent interest due to the difficulty of assembling a glycosidic α-amino alcohol moiety in an intact amino acid, which in addition is prone to an easy lactamization. Herein we report the first chemical synthesis and complete characterization of the glycoside **1**[15] (Scheme 1). The synthesis starts from commercial amino acids and sugars and mimes the natural assembling of **1**, which is known to involve first the β-galactosylation of hydroxylysine residue and then the α-glucosylation, at position 2, of the formed galactoside. The first key step of the synthesis is the β-O-galactosylation of the hydroxyazide **2** (Scheme 1), a masked hydroxylysine. This reaction was efficiently performed, after various unsatisfactory attempts, using as galactosyl donor the differently protected galactose derivative **3** and the trichloroacetimidate protocol of Schmidt[16] (Scheme 1). The synthesis does not use an expensive protected natural L-hydroxylysine but starts from the protected amino acidic hydroxyazide **2**, prepared in pure form (Scheme 2), from L-lysine[17a] and from other more readily available amino acids (L-glutamic[17b] acid and glycine[17c]), according to our recent work on the synthesis of pyridinolines, other important biological

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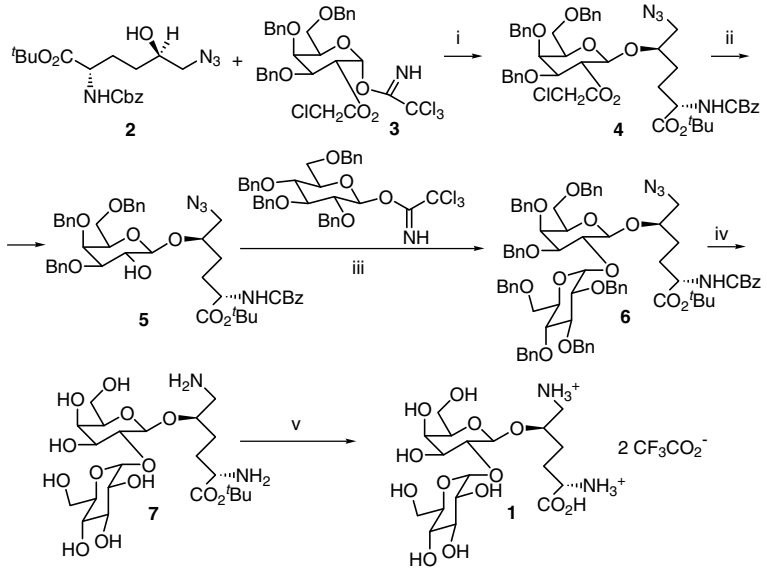
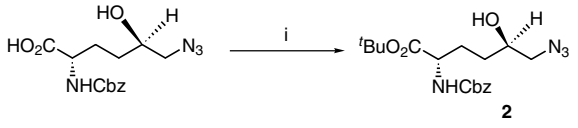


Scheme 1. Reagents and conditions: (i) *t*-$BuMe_2SiSO_3CF_3$, molecular sieves 3A, Et_2O, 25 °C, 1 h, 52%; (ii) $Zn(OAc)_2{\cdot}2H_2O$, MeOH, reflux, 9 h, 93%; (iii) *t*-$BuMe_2SiSO_3CF_3$, molecular sieves 3A, Et_2O, 25 °C, 1 h, 45%; (iv) H_2, Pd/C, THF–EtOH, 1 atm, 25 °C, 90%; (v) CF_3CO_2H–H_2O (95:5), 25 °C, 1 h, 92%.

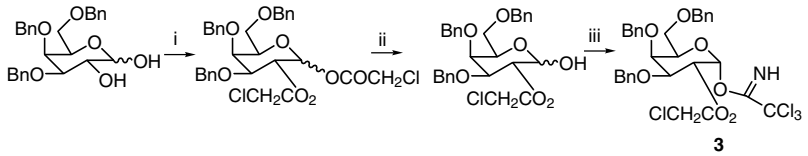


Scheme 2. Reagents and conditions: (i) *O*-*tert*-butyl-*N*,*N*′-dicyclohexylisourea, CH_2Cl_2, rt, 5 days, 89%.

markers of collagen turnover.[18] More importantly, the use of the hydroxyazide **2**, in place of hydroxylysine, avoided the protection, and the obligatory deprotection, of the ε-amino group of this amino acid, and various troubles due to its possible lactamization.[19] The preparation of the *tert*-butyl ester **2** from the parent acid was performed using the procedure reported by Liebe and Kunz,[20] which was very efficient.

As glycosyl donor was chosen the galactosyl trichloroacetimidate **3** (Scheme 3), with the 2-hydroxy group protected as chloroacetate. This protective group was selected both for its capacity to favour, as neighbouring group participation, the formation of the desired β-anomeric linkage and for its cleavability, under nearly neutral conditions, which could open the way to extend the obtained galactose derivative **4** in the 2-direction. Under the best conditions found, *tert*-butyldimethylsilyl triflate promoted the galactosylation of the hydroxyazide **2**, in diethyl ether at 25 °C, affording the β-O-galactoside **4** in 52% yield.[21] In the crude product of the reaction, the obtained β-O-galactoside **4** is not accompanied by any detectable quantity of the corresponding α-anomer or *ortho*-ester. On the contrary, the α-isomer is present, as a more polar companion (TLC and ESI-MS evidences) when the reaction is catalyzed by boron trifluoride. As expected, the regeneration of the 2-hydroxy group of **4**, affording compound **5**, was performed in quantitative yields, by refluxing the chloroacetate **4** with $Zn(OAc)_2{\cdot}2H_2O$ in methanol. These very mild reaction conditions, we found useful for dichloroacetate hydrolysis in the synthesis of the glycoside etoposide,[22] allows the preservation of all the remaining functions of the derivative **4**. The successive α-glucosylation of **5** was performed using the *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)trichloroacetimidate and *tert*-butyldimethylsilyl triflate, which in this case should favour[23] the formation of the α-anomer **6** which, in fact, was obtained in 45% yield after chromatographic purification.[24] At this point, the strategic choice of the protective groups, has permitted the simultaneous cleavage of the benzyl and benzyloxycarbonyl groups and the generation of the ε-amino group of the hydroxylysine aglycone by catalytic hydrogenation.

The formed glucosylgalactosyl hydroxylysine *tert*-butyl ester **7** was finally treated with trifluoroacetic acid to



Scheme 3. Reagents and conditions: (i) $ClCH_2COCl$, Et_2O–Py, −10 to 25 °C, 2 h, 98%; (ii) 25% aqueous NH_3, THF, 1 h, 85%; Cl_3CCN, DBU, CH_2Cl_2, −30 °C, 4 h, 79%.

afford the target glycoconjugate **1** as ditrifluoroacetate salt.

Our work makes available in a relatively easy way the commercially unavailable hydroxylysine glycoconjugate **1**, and facilitates its use in biological researches on collagen. More generally, we feel that present results appear to be very attractive for the assembling, via our previously reported protocols,[17,18] of other more complex glycoside of cross-linked hydroxylysine (glycosylated pyridinolines) present in collagen.[25]

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