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SYNTHESIS OF O-(2-O-α-D-GLUCOPYRANOSYL)-β-D-GALACTOPYRANOSIDE OF OPTICALLY PURE δ-HYDROXY-L-LYSYL-GLYCINE: A GLYCOPEPTIDE OF THE GLOMERULAR BASEMENT MEMBRANE

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Summary: The synthesis of the glycopeptide Glu-Gal-Hyl-Gly, which forms an essential part of the Glomerular Basement Membrane, is presented. The use of the levulinyl group as a protective group and the availability of the optically pure and fully-protected lactone of δ-hydroxy-L-Lysine (Hyl) will be put forward.

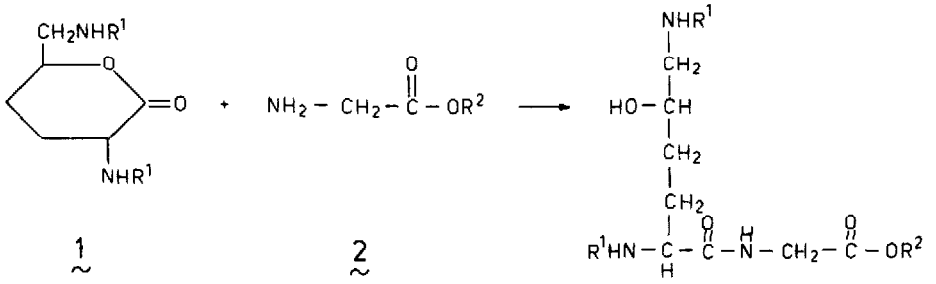
The disaccharide 2-O-α-D-glucopyranosyl-D-galactopyranose which is linked via a β-glycosidic bond with the alcoholic function of δ-hydroxy-L-Lysyl residue (L-Hyl) is part of the Glomerular Basement Membrane (GBM)[1]. A small glycopeptide composed of the same disaccharide and the peptide Hyl-Gly-Glu-Asp-Gly was also isolated[2] from GBM and reported to constitute the antigenic site of anti-GBM autoantibody, which is present in some patients with glomerulonephritis and Goodpasture's syndrome[3].

In this paper we wish to report, for the first time, the synthesis of O-(2-O-α-D-glucopyranosyl)-β-D-galactopyranoside of the optically pure δ-hydroxy-L-Lysyl-Glycine which presents the first part i.e. Glu-Gal-Hyl-Gly of the glycoproteine Glu-Gal-Hyl-Gly-Glu-Asp-Gly isolated from GBM.

The compound to be synthesized (i.e. compound 7c in the Scheme) contains one galactose unit the anomeric center of which is β-linked to the δ-hydroxy-L-Lysyl residue, while the neighbouring hydroxyl group is α-linked to glucose. For the introduction of the β-linkage we started[4] from the properly protected galactose bromide derivative 4 (R^3=Ac; R^4 = Lev). Compound 4 is protected with three acetyl and one levulinyl group, the former functions as a persistent and the latter as a temporary blocking group. The use of the levulinyl group not only favors, under the conditions of Helferich[5], the formation of a β-linkage with the partially protected dipeptide 3, but also opens the way to extend, by the specific removal of this group in the presence of the acetyl groups, the galactose derivative 5 in the 2-direction with the glucose derivative 6. The alcoholic functions of the glucose derivative 6 (see Scheme) are protected with the persistent benzyl groups, while the anomeric center is functionalized with the N-methyl-acetimidyl group. The nature of the latter group must ensure[6] the formation of the required α-interglycosidic linkage between the galactose and glucose moieties.

Another prerequisite in the total synthesis of the glycoprotein (i.e. 7c in the Scheme) is the availability of optically pure δ-hydroxy-L-Lysine (normal form). In our strategy we started from the properly protected (R^1=carbobenzoxy) lactone 1 of δ-hydroxy-L-Lysine (normal form). The latter was prepared by a slight modification[7] of the existing methods[8,9,10]. Furthermore, the absolute configuration of 1 (m.p. 145-147°C; $[\alpha]_D$ +28.9°, c 1 $CHCl_3$) was unambiguously ascertained by X-ray analysis[11].

The first step in the synthesis of 7c involved the aminolysis[12,13] of lactone 1 with



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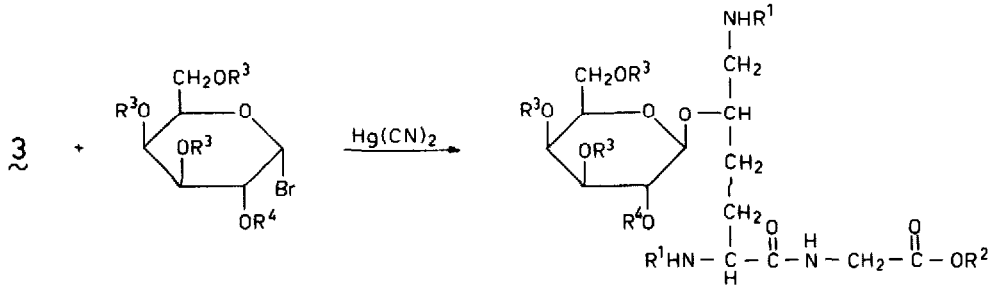
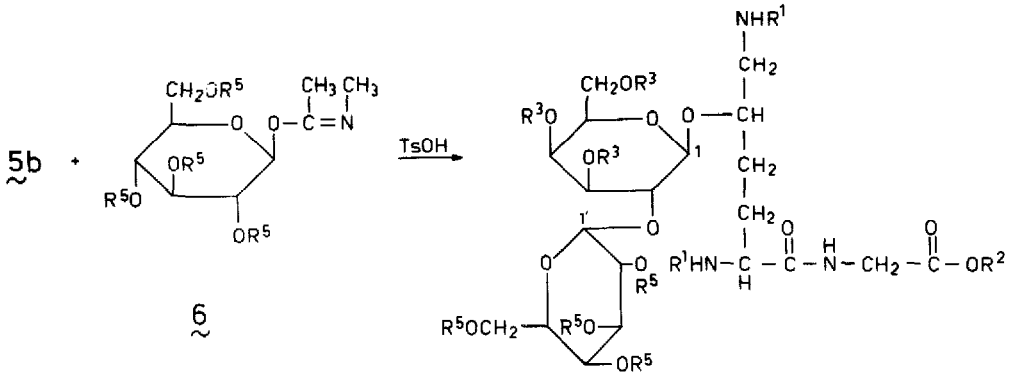


4 R^4 = Lev

5 a: R^4 = Lev
b: R^4 = H



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R^1 = $-C(=O)-O-CH_2-\emptyset$
R^2 = Bu^t
R^3 = Ac
R^4 = Lev = $CH_3-C(=O)-(CH_2)_2-C(=O)-$
R^5 = Benzyl

7 a: R^2 = H
b: R^2 = R^3 = H
c: R^2 = R^3 = R^5 = H ; R^1 = H(·HCl)

Scheme

the t-butyl-ester of glycine 2[14]. The advantage adherent to the use of the t-butyl protecting group will be explained later. Thus aminolysis of 1 (3.4 mmol) with 2 (4.0 mmol) in dry dioxane (7 ml) during 20 hr at 70°C gave, after work-up and purification by column chromatography, 3 (2.5 mmol) as a crystalline product[15], m.p. 98-99°C, $[\alpha]_D$ -9.8 (c 1 in $CHCl_3$).

For the introduction of the β-glycosidic bond we chose, as mentioned before, the conditions of Helferich. Thus, condensation of the bromo derivative 4 (4.8 mmol) with 3 (2.4 mmol) in dry toluene (30 ml) in the presence of $Hg(CN)_2$ (4.8 mmol) afforded, after work-up and purification by column chromatography, glycopeptide 5a (R^4=Lev; 0.72 mmol) as a homogeneous glass[16]. Removal of the levulinyl group was performed[4] by treating 5a (R^4=Lev; 0.72 mmol) in dry pyridine (7 ml) with a solution of hydrazine hydrate (1.0 M) in pyridine/acetic acid (3:2, v/v, 7 ml). Quenching of the reaction mixture with pentane -2,4-dione (1.4 ml) afforded, after work-up and purification by column chromatography, 5b (R^4=H; 0.46 mmol) as a homogeneous glass[16].

The final step in the synthesis of the fully-protected glycopeptide 7, which consisted of the introduction of the α-glycosidic bond between C-1' of the glucose derivative 6 and the hydroxyl function at C-2 of the galactose derivative 5b, was accomplished by applying the methodology originally devised by Sinaÿ et al.[6]. Thus, a solution of 5b (R^4=H; 0.45 mmol) in dry nitromethane (10 ml) was treated with 6 (R^5=Benzyl; 2.0 mmol) and anhydrous p-toluenesulphonic acid (TsOH; 1.1 mmol) in the presence of powdered 4Å molecular sieves (1 gr). The solution was stirred for 48 hr at 20°C and, after work-up and purification by column chromatography, the fully-protected glycopeptide 7 (0.25 mmol) was isolated as a homogeneous glass[16].

Complete deblocking of the fully-protected glycopeptide 7 was performed in three distinct stages. Firstly, short trifluoroacetic acid treatment of 7 removes the t-butyl group to give homogeneous[17] 7a (R^2=H) having a free carboxyl function. The selective removal of the t-Butyl group has the additional advantage that partially-protected 7a so obtained can be extended at the carboxyl end with a properly protected tripeptide (i.e., Glu-Asp-Gly), to afford the naturally occurring glycopeptide of the Glomerular Basement Membrane (GBM). Secondly, deblocking of the base-labile acetyl groups from 7a with triethylamine/methanol/water, afforded homogeneous 7b (R^3=R^3=H)[17]. Finally, catalytic hydrogenolysis (Pd/C)-removal of carbobenzoxy (R^1) and benzyl (R^5) groups - of 7b afforded, after purification by SP-Sephadex C-25 ion-exchange chromatography using aqueous ammonium formate (pH 4.5) as the eluting solvent followed by lyophilization of the pure fractions, the di-formate salt of 7c (R^2=R^3=R^5=H; R^1=H,HCOOH; normal, L-form) as a white powder in a yield of 80% (based on fully-protected 7). The identity and homogeneity of glycopeptide 7c was ascertained by ^{13}C-n.m.r. spectroscopy, paper chromatography, paper electrophoresis and HPLC. Further, the optical purity of 7 and 7c obtained above was also corroborated indirectly by comparing the ^{13}C-n.m.r. data of the anomeric carbon atoms C-1 and C-1' of these compounds with those obtained from the corresponding glycopeptides 7 and 7c, which were synthesized starting from a racemic mixture of 1 (allo-form). Thus 7 and 7c derived from 1 (normal, L-form) showed the following δ-values: C-1; 101.65 and 101.47: C-1'; 95.83 and 97.46, respectively. The δ-values observed for the same carbon atoms of 7 and 7c containing the allo, D- and allo, L-form of δ-hydroxy-Lysine are: C-1; 102.08 and 101.47: C-1'; 98.95, 96.22 and 98.95, 97.44, respecti-

vely. The appearance of two signals for C-1' in the latter two glycoprotein derivatives is indicative for the optical impurity of these compounds[18].

Attempts to link the glycopeptide 7b (R^2=R^3=H) with the properly protected tripeptide Glu-Asp-Gly, and, furthermore, the coupling of fully-deblocked 7c with bovine serum albumin (BSA) and the characterization of rabbit antibodies raised against the coupling product, are presently under investigation.

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15. Satisfactory C/H/N analytical data were obtained for this compound.
16. The identity of this compound was ascertained by ^{1}H- and ^{13}C-n.m.r. spectroscopy.
17. The homogeneity of this compound was established by paper chromatography and paper electrophoresis.
18. The δ-values observed for the corresponding carbon atoms i.e.: C-1; 102.35, 101.71 and 101.44, respectively; C-1'; 98.89, 95.86 and 98.98, 97.44, respectively, of 7 and 7c which contain the normal,D- and normal,L-form of δ-hydroxy-Lysine are also indicative for the optical impurity of these glycoproteine derivatives.

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