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BLOCK SYNTHESIS OF A HEXASACCHARIDE HAPTEN OF i BLOOD GROUP ANTIGEN

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Summary : Block synthesis of a hexasaccharide, β-(1→3) linked trimer of N-acetyllactosamine, is reported. This proved to be a potent inhibitor of anti-i antibodies.

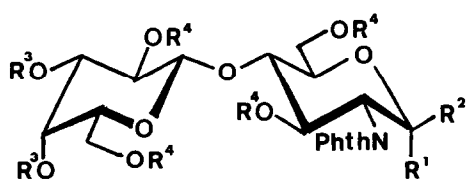
The chemical characterization of the human Ii blood-group system has recently gained renewed interest, when it was discovered that the I and i antigens are not only targets of cold agglutinins in some human diseases, but can be also considered as carbohydrate differentiation antigens showing marked changes during successive developmental stages of mammalian cells.[1] Anti-i antibodies are now known to be directed to linear sequences of the poly-N-acetyllactosamine series occurring on glycoproteins and glycolipids of cell membranes. The need of well-defined oligosaccharides for immunochemical investigations has prompted us to develop efficient chemical syntheses of oligosaccharides which are fragments of that series.[2]

The key intermediate in block syntheses of β-(1→3) dimer and trimer of N-acetyl-lactosamine was a phthalimido chloride 3 for which we devised a new preparation.[3] Crude lactosamine (2-amino-2-deoxy-4-O-β-D-galactopyranosyl-D-glucopyranose), easily obtained[4] from the commercially available disaccharide, 3-O-β-D-galactopyranosyl-D-arabinose, was treated with phthalic anhydride (2.1 equiv.) in aqueous acetone in the presence of sodium hydrogen carbonate (4.4 equiv.), then with acetic anhydride-pyridine to afford a 2:1 mixture of β- and α-acetates (1 and 2) in 60% yield. Catalytic amounts of perchloric acid in acetic anhydride brought about anomerization of the α-acetate 2 into the

more stable β-isomer 1, which was isolated in a 50% overall yield, m.p. 272°C, $|\alpha|_D$ + 33° (c 1.05, chloroform).[3] Compound 1 was converted into the desired chloride 3 by treatment with hydrogen chloride in acetic acid - acetic anhydride, m.p. 173.5°C, $|\alpha|_D$ + 30° (c 1.005, chloroform).

We also found that the selective removal of the anomeric O-acetyl group in the mixture of β- and α-acetates could be done by reaction of piperidine in tetrahydrofuran,[5] or more conveniently of hydrazine acetate in dimethylformamide,[6] to give the β-hydroxy derivative 4, m.p. 138-140°C, $|\alpha|_D$ + 34° (c 0.995, chloroform). Re-O-acetylation led then exclusively to the β-acetate 1. Alternatively, compound 4 could be directly transformed into chloride 3 by treatment with the Vilsmeyer reagent.[7]

A portion of chloride 3 was converted into the diol component 7 required for the coupling reaction through the following steps : a methyl β-D-glycoside 5 was prepared from 3 in 86% yield, m.p. 221-222°C, $|\alpha|_D$ + 18° (c 1.085, chloroform).[8] De-O-acetylation followed by treatment with acetone and catalytic amounts of p-toluene sulfonic acid led to a 3',4'-O-isopropylidene derivative (65% yield) identified by examination of the ^{1}H NMR spectrum of the O-acetylated compound 6, m.p. 148-150°C, $|\alpha|_D$ + 47° (c 1.06, chloroform) : signals for H-3' and H-4' were absent in the δ 4.8 - 5.4 region; H-2' appeared as a triplet at δ 4.87, J 7.5 Hz. Mild acidic hydrolysis converted 6 into diol 7 in 77% yield, m.p. 233-235°C, $|\alpha|_D$ - 44° (c 0.45, pyridine).



1 R^1= H, R^2= OAc, R^3= R^4= Ac

2 R^1= OAc, R^2= H, R^3= R^4= Ac

3 R^1= H, R^2= Cl, R^3= R^4= Ac

4 R^1= H, R^2= OH, R^3= R^4= Ac

5 R^1= H, R^2= OCH_3, R^3= R^4= Ac

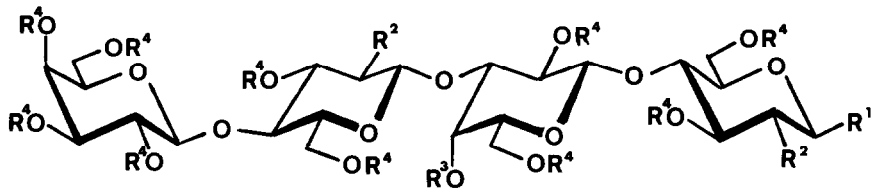
6 R^1= H, R^2= OCH_3, R^3,R^3= $(CH_3)_2C$, R^4= Ac

7 R^1= R^3= H, R^2= OCH_3, R^4= Ac

Coupling of 3 with 7 using silver trifluoromethanesulfonate-s-collidine as promotor[9] occured at the more reactive 3'-position to give the crystalline

tetrasaccharide 8 in 64% yield, m.p. 203-205°C, $|\alpha|_D$ + 20.5° (c 0.83, chloroform). The newly formed β-(1→3) linkage was clearly demonstrated by the ^{1}H NMR signal of the anomeric proton in the internal D-glucosamine unit (δ 5.42, d, J 8.5 Hz), and by the two signals of equatorial H-4 of D-galactose units (δ 5.29 and 5.32, J 3.5 Hz) in the per-O-acetylated tetrasaccharide 9.

Sequential treatment of 8 with sodium methoxide in methanol, hydrazine hydrate in ethanol, acetic anhydride-pyridine, and final de-O-acetylation afforded the unprotected tetrasaccharide 10, crystallized as a trihydrate, m.p. 259-261°C, $|\alpha|_D^{20}$ - 10° (c 1.025, water); δ (D_2O, 400 MHz) : 4.71 (1H, d, J 7.5 Hz, H-1 of internal N-acetylglucosamine), 4.17 (1H, J 3 Hz, H-4 of internal D-galactose).



8 R^1= OCH_3, R^2= NPhth, R^3= H, R^4= Ac

9 R^1= OCH_3, R^2= NPhth, R^3= R^4= Ac

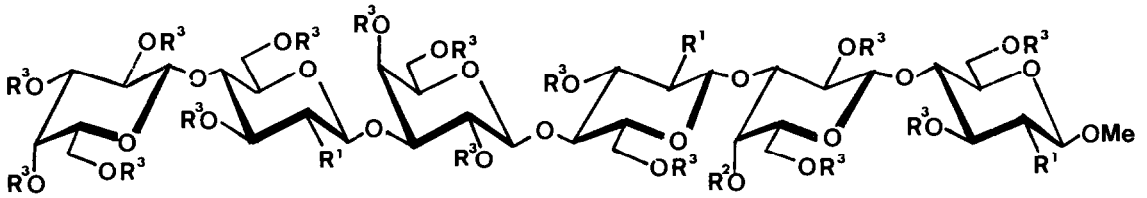
10 R^1= OCH_3, R^2= NHAc, R^3= R^4= H

11 R^1= OAc, R^2= NPhth, R^3= R^4= Ac

12 R^1= Cl, R^2= NPhth, R^3= R^4= Ac

The next block synthesis leading to the hexasaccharide trimer could be performed either by selective functionnalization of the terminal D-galactose unit in the above tetrasaccharide sequence, followed by coupling with chloride 3, or by activation of the tetrasaccharide into a chloride and coupling to diol 7; the latter route was selected, since it involved only two steps from 8 to 12. Besides the presence of N-phthaloyl groups in 8 allowed a clean acetolysis of the β-methyl glycosidic ending (0.6% conc. sulfuric acid in acetic acid - acetic anhydride overnight at 5°C) leading to the crystalline β-acetate 11 in nearly quantitative yield, m.p. 157-158°C, $|\alpha|_D$ + 21.5° (c 0.56, chloroform). Compound 11 was converted into the tetrasaccharide β-chloride 12, which was not purified, but immediately coupled to 7 in the presence of silver trifluoromethanesulfonate and s-collidine. A crystalline hexasaccharide 13 was obtained in 40% yield, m.p. 182-184°C, $|\alpha|_D$ + 16°

(c 0.44, chloroform); its ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) showed three distinct signals for the anomeric protons of the N-phthaloylglucosamine units (δ 5.18, 5.33, and 5.39, 3d, J 8.5 Hz). Sequential de-O-acetylation, hydrazinolysis, and N,O-acetylation gave 14, which showed in its ^{1}H NMR spectrum three signals for equatorial H-4 of the D-galactose units (δ 5.28, 5.29, and 5.32, J 3 Hz). De-O-acetylation of 13 afforded the desired hexasaccharide 15, purified by gel filtration chromatography and freeze-dried as an amorphous powder, $[\alpha]_D$ - 6° (c, 0.97, water).



13 R^1= NPhth, R^2= H, R^3= Ac 14 R^1= NHAc, R^2= R^3= Ac 15 R^1= NHAc, R^2= R^3= H

Details of immunochemical tests will be given elsewhere.[10] Briefly, hexasaccharide 15 appears as the most potent synthetic inhibitor known so far, being active in five i-anti-i systems. Tetrasaccharide 10 and the synthetic trisaccharide,[2] β-D-Gal-(1→4)-β-D-GlcNAc-(1→3)-D-Gal, are respectively 5 and 1000 times less active in the same assays.

References and Notes

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