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SYNTHESIS OF AN OCTASACCHARIDE FRAGMENT OF THE POLYLACTOSAMINE SERIES BY A BLOCKWISE APPROACH

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Summary : Block synthesis of an octasaccharide, β-(1-3) linked tetramer of N-acetyllactosamine, is reported. Intermediate di- and tetrasaccharides were converted either into trichloroacetimidates acting as glycosyl donors or into vicinal diols acting as glycosyl acceptors.

Human autoantibodies and hybridoma antibodies with anti-i blood-group activity recognize linear fragments of the poly-N-acetyllactosamine series, $(Gal\beta 1\text{-}4GlcNAc\beta 1\text{-}3)_n^1$. Recent studies[2] with chemically synthesized oligosaccharides have shown that antigenic determinants fitting the combining sites of anti-i antibodies are at least hexasaccharides. I and i antigens are known to be involved in cell differentiation and malignancy, but their exact size has still to be elucidated.

We have already reported[3] the synthesis of tetra- and hexasaccharide methyl β-glycosides of the polylactosamine series. We describe now an efficient preparation of an octasaccharide by a blockwise approach which virtually allows to prepare any of the compounds belonging to the above sequence.

Grundler and Schmidt[4] have recently shown that 1,2-trans-trichloroacetimidates of N-phthaloylaminosugars react with alcohols in the presence of a Lewis acid to give exclusively 1,2-trans-glycosides in high yields. Alkaline conditions (NaH or K_2CO_3 in dichloromethane) required for the preparation of trichloroacetimidates allow the presence of selectively removable protecting groups such as benzylidene or isopropylidene acetals. Imidates with acid-labile groups are good candidates for a blockwise approach of oligosaccharide synthesis, provided that activation by a Lewis acid does not affect the acetal functions or the glycosidic bonds. Previous experiments[4,5] have shown that trimethylsilyl trifluoromethanesulfonate (TMSOTf) gives excellent results to that respect.

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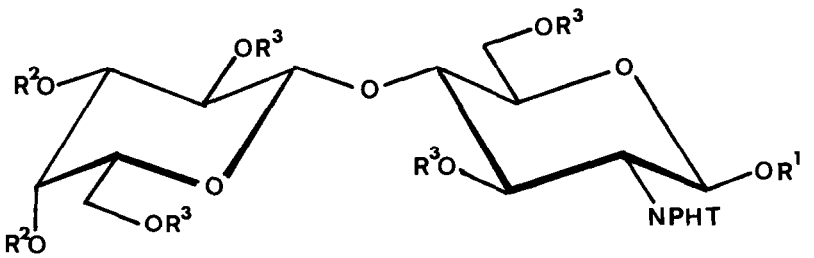
The disaccharide β-acetate 1 was readily obtained by N-phthaloylation and O-acetylation of crude lactosamine as previously described.[3] Treatment of 1 with benzyl alcohol and TMSOTf at room temperature afforded the β-benzyl glycoside [6] 2 in 91% yield, m.p. 185-186°C, $[\alpha]_D^{20}$ -9° (c, 2.1, $CHCl_3$). De-O-acetylation could be smoothly accomplished without opening of the phthalimido ring by treatment with 0.02 M sodium methoxide in methanol-dioxane (3:2, v/v) at 20°C for 3 h to give 3 : m.p. 246-248°C, $[\alpha]_D^{20}$ -66° (c, 1.04, pyridine).[7] Refluxing 3 with catalytic amounts of p-toluenesulfonic acid in acetone for 5 h gave a 3',4'-O-isopropylidene derivative 4 in 60% yield.[8] Acetylation with acetic anhydride-pyridine led to the crystalline key intermediate 5, m.p. 141-143°C, $[\alpha]_D^{20}$ + 9.5° (c 1.05, $CHCl_3$).[9]

Compound 5 was converted into a glycosyl donor 7 in two steps :

1. Hydrogenolysis of the O-benzyl group in 5 was quantitatively effected by transfer of hydrogen from cyclohexene in boiling ethanol in the presence of Pd/C[10] to give a crystalline β-hemiacetal 6, m.p. 138-140°C, $[\alpha]_d^{20}$ + 57° (c, 0.78, $CHCl_3$).
2. Treatment of 6 with trichloroacetonitrile and potassium carbonate in dichloromethane gave a β-trichloroacetimidate 7 in 66% yield, m.p. 143-145°C, $[\alpha]_D^{20}$ +58° (c, 0.55, $CHCl_3$).

Alternatively, 5 was transformed into the 3',4'-diol 8, a potential glycosyl acceptor, by mild acid hydrolysis (78% yield), m.p. 198-200°C, $[\alpha]_D^{20}$ -13° (c 0.91, $CHCl_3$).



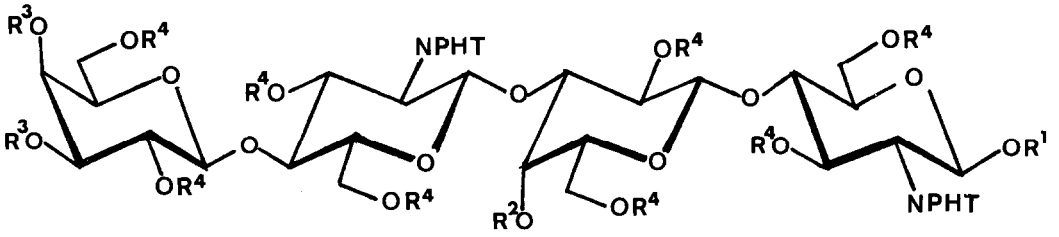
1 R^1= R^2= R^3= Ac
2 R^1= $CH_2C_6H_5$, R^2= R^3= Ac
3 R^1= $CH_2C_6H_5$, R^2= R^3= H
4 R^1= $CH_2C_6H_5$, R^2,R^2= $(CH_3)_2C$, R^3= H
5 R^1= $CH_2C_6H_5$, R^2,R^2= $(CH_3)_2C$, R^3= Ac
6 R^1= H, R^2,R^2= $(CH_3)_2C$, R^3= Ac
7 R^1= C:NH-CCl_3, R^2= $(CH_3)_2C$, R^3= Ac
8 R^1= $CH_2C_6H_5$, R^2= H, R^3= Ac

Glycosylation of 8 with 7 (20% excess) occurred in the presence of TMSOTfat -40° in <15 min. to give the crystalline tetrasaccharide 9 in 78% yield, m.p. 229-231°C, $[\alpha]_D^{20}$ +12° (c, 0.56, $CHCl_3$). 9 was conventionally O-

acetylated to give 10, the ^1H n.m.r. spectrum of which showed a characteristic signal for equatorial H-4' at δ 5.28 (J = 3 Hz), which was absent from the spectrum of 9, and a doublet at δ 5.34 (J = 8.5 Hz) corresponding to the anomeric proton of the internal D-glucosamine unit. A β-(1-3) linkage has thus been created, and no reactivity of the 4'-OH in 8 could be observed. Moreover, at -40°C the isopropylidene group is maintained and tetrasaccharide 10 becomes a convenient precursor for further block syntheses.

Hydrogenolysis of 10 was conducted as described for 5 and gave a tetrasaccharide β-hemiacetal 11, m.p. 153-156°C, $[\alpha]_D^{20}$ +35° (c, 0.45, $CHCl_3$), which was then converted into a β-trichloroacetimidate 12, m.p. 141-143°C, $[\alpha]_D^{20}$ +56° (c, 0.53, $CHCl_3$).

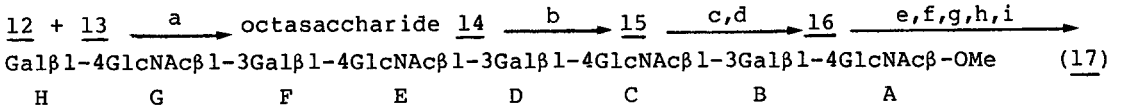
Alternatively, 10 was quantitatively transformed into the diol 13 by mild acid hydrolysis, m.p. 150-152°C, $[\alpha]_D^{20}$ +4° (c, 0.71, $CHCl_3$).



9 R^1= $CH_2C_6H_5$, R^2= H, R^3,R^3= $(CH_3)_2C$, R^4= Ac
10 R^1= $CH_2C_6H_5$, R^2= R^4= Ac, R^3,R^3= $(CH_3)_2C$
11 R^1= H, R^2= R^4= Ac, R^3, R^3= $(CH_3)_2C$
12 R^1= C:NH-CCl_3, R^2= R^4= Ac, R^3, R^3= $(CH_3)_2C$
13 R^1= $CH_2C_6H_5$, R^2= R^4= Ac, R^3= H

Glycosylation of 13 by 12 at -40°C in the presence of TMSOTf gave the crystalline octasaccharide 14 in 50% yield, m.p. 252-255°C, $[\alpha]_D^{20}$ -10° (c 0.7, $CHCl_3$). O-Acetylation of 14 gave 15, the ^1H n.m.r. spectrum of which showed in the δ 5.2-5.5 region four signals corresponding to the anomeric protons of the β-linked glucosamine units (J = 8.5 Hz) and three signals corresponding to the equatorial H-4 of the internal galactose units (J = 3 Hz); the spectrum of 14 has only six protons in that region.

Octasaccharide 15 was converted into a methyl β-glycoside 16 (65% yield) by hydrogenolysis followed by treatment with methanesulfonic anhydride, s-collidine and methanol.[12] Sequential treatment with dilute trifluoroacetic acid, sodium methoxide in methanol, hydrazine hydrate in boiling ethanol, acetic anhydride in pyridine, and final de-O-acetylation afforded the unprotected octasaccharide methyl β-glycoside 17 in 45% yield, $[a]_D^{20}$ -21.3° (c, 0.375, H_2O).[13]



Reagents : a) TMSOTf, -40°C; b) Ac_2O-pyridine; c) Pd-C-cyclohexene; d) Ms_2O-s-collidine-MeOH; e) CF_3CO_2H; f) 0.02 M NaOMe-MeOH; g) N_2H_4-H_2O-EtOH; h) Ac_2O-pyridine; i) 0.1 M NaOMe-MeOH.

Details of immunochemical tests will be given elsewhere.

References and notes

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6. Compound 2 was first prepared from lactal hexaacetate by the azidonitration route, R.U. Lemieux, S.Z. Abbas, M.H. Burzynska, and R.M. Ratcliffe, Can. J. Chem., 60, 63 (1982); m.p. 187-188°C, $[\alpha]_D^{25}$ -9.6° (c, 2.5, $CHCl_3$).

7. All new compounds showed satisfactory elemental analysis.

8. The reaction mixture contained small amounts of the 4',6'-O-isopropylidene isomer and of 3 which could be both easily separated from 4 by column chromatography on silicagel (ether-methanol, 9:1, v/v), then recycled to give more 4.

9. Signals for H-3' and H-4' were absent in the δ 4.8-5.4 region of the ^1H n.m.r. spectrum of 5, whereas 2 gives two signals in that region for H-3' and H-4', respectively at δ 4.94 (dd, 1 H, $J_{2',3'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz) and 5.31 (d, 1 H).

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11. R.R. Schmidt, J. Michel, and M. Roos, Liebigs Ann. Chem., 1343 (1984).

12. J. Leroux and A.S. Perlin, Carbohydr. Res., 67, 163 (1978); although an octasaccharide trichloroacetimidate could be easily obtained, its reaction with methanol and TMSOTf at -40°C was not clean and gave poor yields of methyl β-glycoside.

13. ^1H n.m.r. data (D_2O) : δ 4.732, 4.729, 4.726 (3d, 3H, J = 8 Hz, H-1 C,E,G), 4.511, 4.496, 4.489 (3d, 5 H, J = 8 Hz, H-1 A, B, D, F, H), 4.191 (d, 3H, J = 3 Hz, H-4 B, D, F), 3.964 (d, 1 H, J = 3 Hz, H-4 H), 3.534 (s, 3 H, O-CH_3), 2.064 (s, 12 H, NHAc).

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