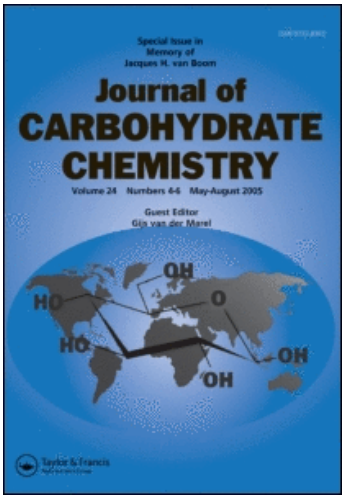

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COMMUNICATION

SYNTHESIS OF POLYLACTOSAMINE OLIGOMERS BY DISACCHARIDE POLYMERIZATION

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Polylactosamine chains, which consist of repeats of the disaccharide βGal(1→4)βGlcNAc(1 →3), are characteristic developmental and tumor associated carbohydrate markers found attached to both glycoproteins and glycolipids.[1,2] In addition to the accumulation of such sequences in a number of diseases, these structures are the immediate biosynthetic precursors to poly-Le^x determinants, {--βGal(1 →4)[αFuc(1 →3)βGlcNAc(1 →3)--}$_n$, which are recognized also as tumor associated antigens,[3-4] particularly in adenocarcinomas.[5]

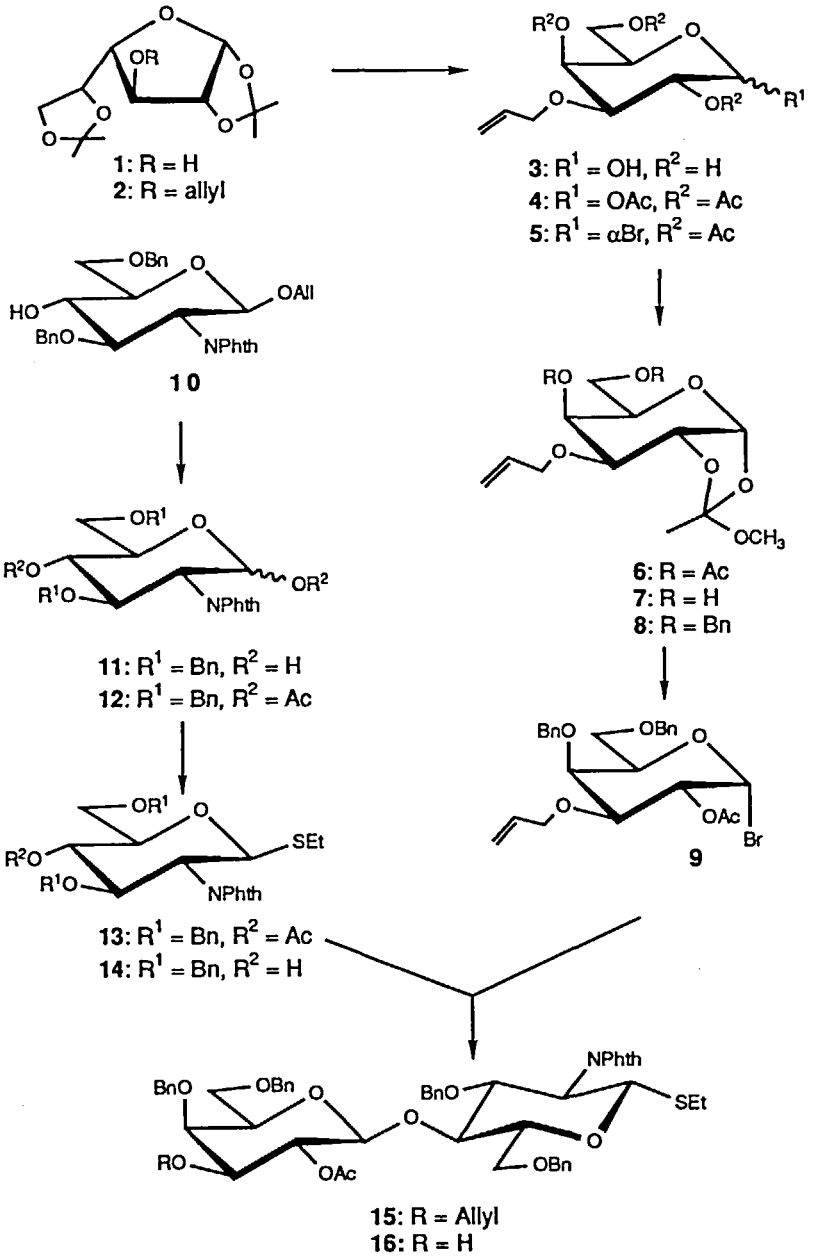
Several synthetic approaches to the preparation of polylactosamine and poly-Le^x oligomers have been reported. The assembly of polylactosamine oligomers by stepwise glycosylation using di- or tetrasaccharide glycosyl-donors has been described up to the octasaccharide.[6-8] Stepwise addition of suitably protected disaccharide derivatives has produced up to the trimer (a hexasaccharide) from which the 3-positions of the βGlcNAc residues could be selectively deprotected to allow chemical fucosylation.[8,9] Stepwise addition of preformed trisaccharide blocks has also been used to prepare a trimeric Le^x - containing glycolipid.[10] Holmes and Levery[11] have recently described a fucosyltransferase preparation from the adenocarcinoma cell line Colo 205 that could convert the glycolipid nLc_6 (which contains a lactosamine dimer sequence) to both mono- and difucosylated Le^x structures on scales of 1.0 - 2.5 mgs. Our own experience[12] with the readily purified human milk Lewis α(1 → 3/4)-fucosyltransferase in the preparative fucosylation of both βGal(1→3)βGlcNAc and βGal(1 →4)βGlcNAc structures suggests that this latter enzyme

could similarly be used in the conversion of polylactosamines to poly-Le^x chains. We therefore turned our attention to the preparation of the required substrates for this enzyme, i.e. a series of polylactosamine backbones of defined chain-length.

Hashimoto et al.[13] have reported that chemical polymerization of thioethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside resulted in the production of a series of protected chitooligosaccharides. We now report a similar approach to the preparation of the target polylactosamine oligomers which makes use of a controlled polymerization of the partially protected disaccharide **16** which was synthesized by standard procedures. The galactosyl residue in **16** was prepared starting from 1,2;5,6-di-*O*-isopropylidene-α-D-galactofuranoside (**1**)[14] which was *O*-allylated (71%) to give **2**. Removal of the isopropylidene groups in **2** using 90% trifluoroacetic acid furnished **3** which was *O*-acetylated to give **4** (51%). Treatment of **4** with $TiBr_4$ gave bromide **5** (80%) from which orthoester **6** (72%) was prepared by reaction with Bu_4NBr and methanol in nitromethane containing 2,6-lutidine. Removal of the acetate groups in **6** by saponification furnished **7** (80%) which was benzylated (BnBr/NaH, DMF) to yield **8** (86%). Compound **8** could be converted to the bromide **9** (acetyl bromide/Et_4NBr,[15] 87%) for subsequent reaction.

Compound **16** was prepared by condensation of **9** with **14**. The preparation of **14** began with the known 3,6-di-*O*-benzyl derivative **10**[16] from which the allyl group was removed[17] by isomerization followed by hydrolysis. Diol **11** thus obtained (70%) was acetylated (Ac_2O/pyr, 95%) and the resulting diacetate **12** was treated with ethanethiol in the presence of $BF_3{\cdot}Et_2O$ to furnish thioglycoside **13** (80%). Zemplen deacetylation of **13** then gave alcohol **14** (80%). Condensation of **9** with **14** in the presence of silver triflate/collidine at -20 °C furnished disaccharide **15** (85%) from which the 3'-O-allyl group was removed[17] in 80% yield. Analytically pure **16** [^{1}H NMR ($CDCl_3$) δ 4.94 (dd, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 10.0 Hz, H-2'), 4.52 (d, H-1'), 3.43 (ddd, $J_{3',4'}$ = 3.5 Hz, $J_{3',OH}$ = 10.0 Hz, H-3'), 2.17 (d, OH), 2.08 (s, Ac),1.17 (t, Me)], the "monomer" for chemical polymerization, was thus prepared on a one gram scale.

Disaccharide alcohol **16** (1.0 gm) was polymerized essentially as described by Hashimoto et al.[13] by reaction in dichloromethane (15 mL) in the presence of methyl triflate (5 eq) at -15°C for 24 h. Baseline separation of the product protected oligomers **16** - **20** was achieved by chromatography twice on Iatrobeads using sequentially ethyl acetate-hexane (2:3) and (1:1) as the solvents. This procedure yielded dimer **17** [198 mg, 20%, ^{1}H NMR[18] ($CDCl_3$): δ 2.22 (d, J = 10 Hz, OH), 2.09 and 1.69 (each s, Ac)], trimer **18** [102 mg, 10%: δ 2.16 (d, J = 10 Hz, OH), 2.09,1.73 and 1.60 (each s, Ac)], tetramer **19**



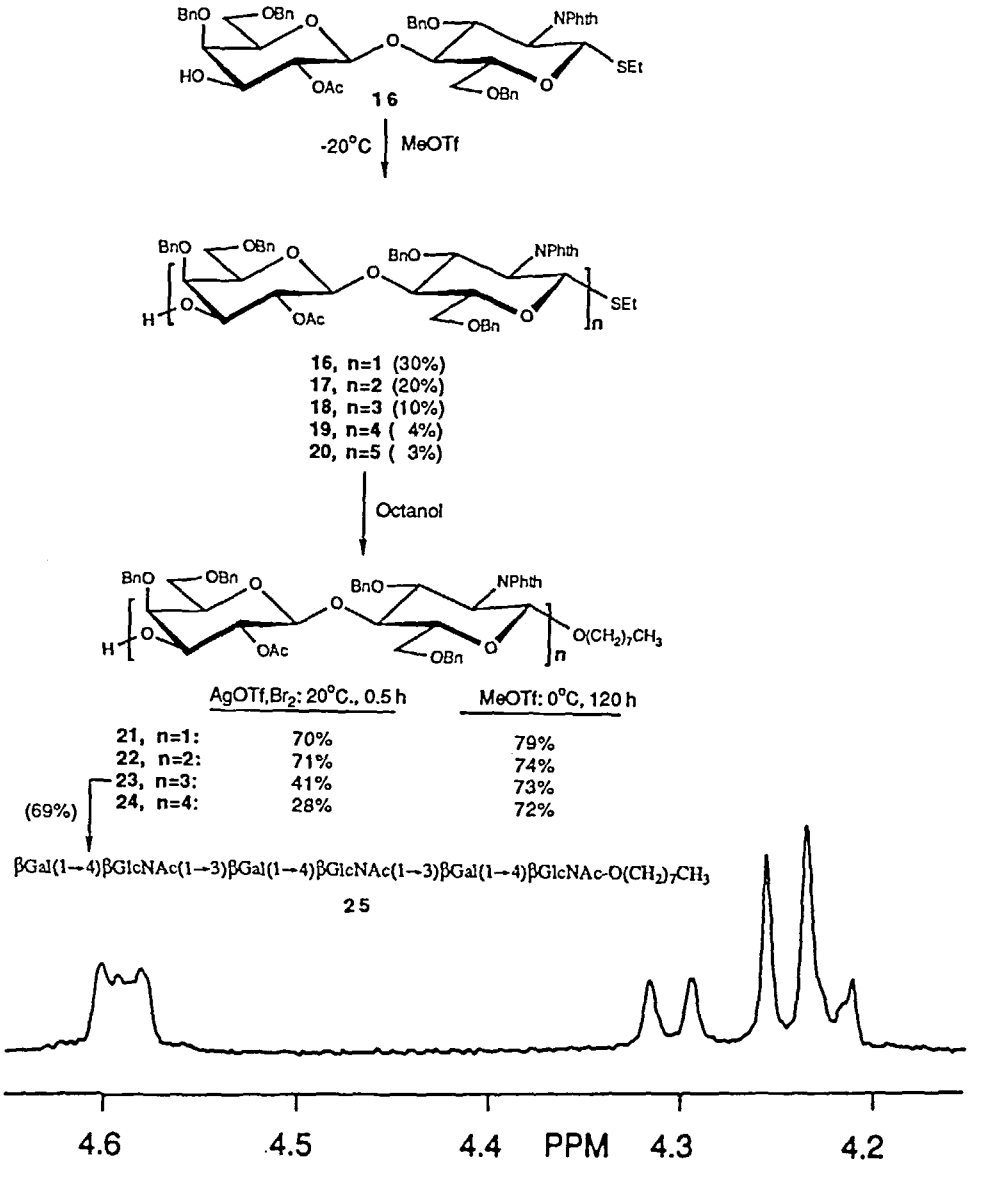


Figure 1. Partial 360 MHz ^{1}H NMR spectrum (60 °C DMSO-d6:D_2O 4:1, DMSO-d5 = δ 2.490) of hexasaccharide **25**. The chemical shifts for the anomeric protons are δ 4.591 (H-1" and H-1""), 4.304 (H-1"""), 4.244 (H-1' and H-1"') and 4.220 (H-1). Other signals (not shown) include δ 1.825 (2xAc), 1.786 (Ac) and 0.799 (Me).

[42 mg, 4%: δ 2.18 (d, J = 10 Hz, OH), 2.09,1.72, 1.70 and 1.69 (each s, Ac)] and pentamer **20** [29.8 mg, 3%: δ 2.15 (d, J = 10 Hz, OH), 2.09, 1.72, 1.70,1.69 and 1.65 (each s, Ac)]. Unreacted **16** (301 mg, 30%) was also recovered. Increased reaction times resulted in the production of much larger polymers which were not characterized.

Oligomers **16 - 19** proved to be efficient glycosyl donors suitable for the addition of polylactosamine blocks of defined length to an appropriate acceptor. In this study, we attempted only the glycosylation of l-octanol with the objective of using the product octyl glycosides in simple fucosyltransferase assays.[19] Two different procedures for the glycosylations were evaluated. Using the conditions of Kihlberg and Bundle[20] ($AgOSO_2CF_3/Br_2$) the glycosylations of octanol with **16 -19** (20-40 mg scales) were rapid (0.5 hr at 20 °C) and provided **21** (70%), **22** (71%), **23** (41%) and **24** (28%). The reasons for the yields decreasing with increasing chain length are not clear but the products seemed to be decomposing as evidenced by streaking on tlc examination. Similar yields were obtained when the reaction was performed at 0 °C. Glycosylation of octanol with the decasaccharide **20** has not yet been attempted since too little material was available to allow a proper evaluation of optimum reaction conditions. When the glycosylations of octanol were performed on similar scales using methyl triflate as the promoter at 0 °C, the reaction took much longer (5 days) and the yields were substantially improved, especially for the longer oligomers: **21** [79%: ^{1}H NMR ($CDCl_3$):δ 5.08 (d, J = 8 Hz, H-1), 2.19 (d, J = 10 Hz, OH), 2.07 (s, Ac)], **22** [74%: δ 5.14 (d, J = 8 Hz, H-1), 2.17 (d, J = 10 Hz, OH), 2.09 and 1.68 (each s, Ac)], **23** (73%: δ 5.16 (d, J = 8 Hz, H-1), 2.19 (d, J = 10 Hz, OH), 2.09, 1.72 and 1.66 (each s, Ac)] and **24** (72%: δ 5.16 (d, J = 8Hz, H-1), 2.18 (d, J = 10 Hz, OH), 2.09, 1.72, 1.70 and 1.65 (each s, Ac)].

The octyl hexasaccharide **23** (15 mg), a potential precursor to the trimeric Le^x structure, was deprotected by sequential treatment with hydrazine-hydrate in MeOH (75 °C,15 hrs) followed by reacetylation (Ac_2O/pyr) and purification by chromatography. Zemplen deacetylation followed by hydrogenation over 5% Pd/C in MeOH then furnished hexasaccharide **25** (4.7 mg, 69% from **23**) whose molecular weight was confirmed by FAB-MS ($M+Na^+$ = 1248). Compound **25** was poorly soluble in water and its ^{1}H-NMR spectra was recorded in DMSO-d6:D_2O at 60 °C, conditions typical for glycolipids. The anomeric region of the ^{1}H NMR spectrum of **25** is presented in Figure 1 which shows the level of anomeric purity of products obtainable by the polymerization process. The signals for the βGlcNAc anomeric protons were all second order but the vicinal coupling constants were near 7.5 Hz and all of the βGal residues had $J_{1,2}$ = 8.0 Hz. The characteristic chemical shifts are given in the Figure legend. No other signals for anomeric protons were seen in the NMR spectrum of **25** confirming that all of the sugar residues were β-linked.

In summary, protected thioethyl glycosides of polylactosamine oligomers, up to at least the decasaccharides, can be prepared by condensation polymerization of a suitably protected βGal(1→4)βGlcNAc disaccharide. The protected thioglycosides produced in this manner are efficient glycosyl donors for the preparation of polylactosamine glycosides of defined chain length. The feasibility of converting the synthetic polylactosamine oligomers to poly-Le^x structures using the human milk Lewis (αl → 4) fucosyltransferase has been evaluated so far only in a preliminary way. The trimeric N-acetyllactosamine hexasaccharide 25 was found to be a substrate for the human milk enzyme in radioactive Sep-Pak assays.[18] Prolonged incubation of this trimer appeared to result in the production of a trifucosylated nonasaccharide as evidenced by the the appearance of a major ion of mass 1687 ($M+Na^+$) in the fast-atom bombardment mass spectrum of a crude incubation product. Scale-up to allow the isolation of this product and its identification by NMR spectroscopy is currently in progress.

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REFERENCES

1. M. Fukuda, *Biochim. Biophys. Acta*, **780**, 119 (1985).
2. S.-I. Hakomori, *Chem. Phys. Lipids*, **42**, 209 (1986).
3. J. W. Dennis, *Cancer Surveys*, **7**, 573 (1988).
4. S.-I. Hakomori, *Advances Cancer Res.*, **52**, 257 (1989).
5. A. K. Singhal, T. F. Orntoft, E. Nudelman, S. Nance, L. Schibig, M. R. Stoud, H. Clausen and S.-I. Hakomori, *Cancer Res.*, **50**, 1375 (1990).
6. J. Alais and A. Veyrieres, *Tetrahedron Lett.*, **24**, 5223 (1983).
7. J. Alais and A. Veyrieres, *Tetrahedron Lett.*, **28**, 3345 (1987).
8. M. Nilsson and T. Norberg, *J. Carbohydr. Chem.*, **8**, 613 (1989).
9. M. Nilsson and T. Norberg, *Carbohydr. Res.*, **183**, 71 (1988).
10. K. C. Nicolaou, T. J. Caulfield, H. Kataoka and N. A.. Stylianides, *J. Amer. Chem. Soc.* **112**, 3693 (1990).
11. E. H. Holmes and S. B. Levery, *Arch. Biochem. Biophys.* **274**, 633 (1989).

12. M. M. Palcic, A. P. Venot, R. M. Ratcliffe and O. Hindsgaul, *Carbohydr. Res.* **190**, 1 (1989).

13. H. Hashimoto, Y. Abe, S. Horito and J. Yoshimura, *J. Carbohydr. Chem.* 8, 307 (1989).

14. M. M. A. Abdel Rahman, *Egypt. J. Chem.*, **19**, 887 (1976).

15. O. Hindsgaul, T. Norberg, J. LePendu and R. U. Lemieux, *Carbohydr. Res.*,**109**, 109 (1982).

16. P. J. Garegg and H. Hultberg, *Carbohydr. Res.* 93, C-10 (1981).

17. E. J. Corey and J. W. Suggs, *J. Org.Chem.*, **38**, 3224 (1973).

18. ^{1}H NMR spectra were recorded at 360 MHz. The spectra were second-order and very complex and only characteristic features are reported and assignments are tentative. The remaining signals and their intensities were in accord with the structures assigned.

19. M. M. Palcic, L. D. Heerze, M. Pierce and O. Hindsgaul, *Glycoconjugate J.* **5**, 49 (1988).

20. J. O. Kihlberg, D. A. Leigh and D. R. Bundle, *J. Org. Chem.*, **55**, 2860 (1990).