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Towards the 21st century—the emerging importance of oligosaccharide synthesis

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Abstract: Recent progress in glycobiology has made the synthesis of oligosaccharides of prime importance for biomedical studies. Two such syntheses are described in order to illustrate our current research in this area. The first concerns the synthesis of oligosaccharides corresponding to the capsular polysaccharide of the yeast *Cryptococcos neoformans*, the second is the synthesis of a highly branched octasaccharide corresponding to the cell surface lipopolysaccharide of the Gram-negative bacterium *Moraxella catarrhalis*. In both syntheses extensive use was made of thioglycosides as glycosyl donors or as glycosyl donor precursors.

INTRODUCTION

Oligosaccharide structures, usually linked to proteins in glycoproteins and proteoglycans or to lipids in glycolipids participate in a large number of biological processes. These involve, *inter alia*, bacterial and viral recognition of specific cell structures as well as immune system recognition of invasive organisms. They are also involved in autoimmune processes. The enormous structural variability possible in oligosaccharide structures is the probable reason for Nature using them for the purpose of molecular recognition.

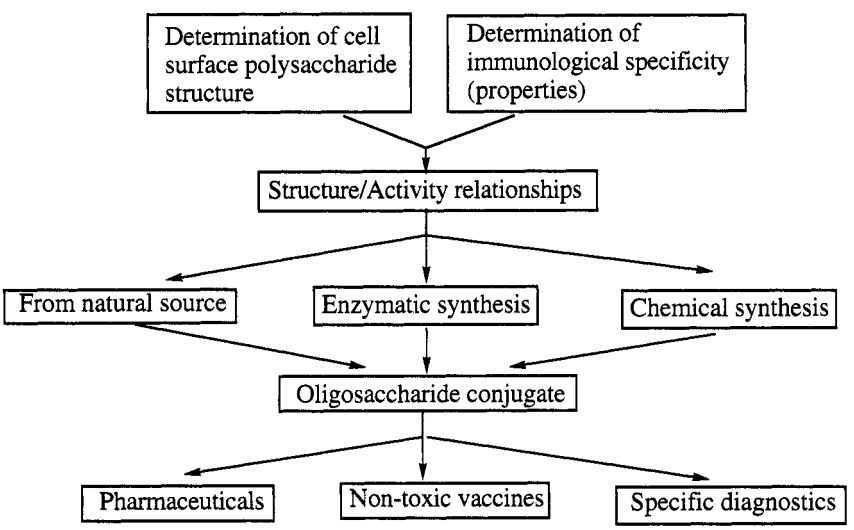

Fig. 1. Opportunities in glycobiological research.

Advances in biology, structural carbohydrate chemistry, and emerging knowledge of structure-activity relationships have made access necessary to these structures and to analogues of them. Thus, correlation of chemical structure with immunological specificity will clarify structure-activity relationships. Based on this knowledge, the relevant oligosaccharide may then be obtained either by suitable degradation and isolation from a natural source, or by synthesis. Transformation of the oligosaccharide into a glycoconjugate may then yield a specific diagnostic material, a non-toxic and highly specific vaccine, or a therapeutic product (Fig.1)

Considerable progress has been made in the last few years in the chemical synthesis of oligosaccharides and glycoconjugates. In the present review, two examples will be given of current research in our laboratory on syntheses of oligosaccharides of biomedical relevance.

SYNTHESES OF OLIGOSACCHARIDES CORRESPONDING TO CAPSULAR STRUCTURES OF *CRYPTOCOCCUS NEOFORMANS*

In humans with a fully functional immune system, the yeast *Cryptococcus neoformans* does not present much danger of infection. However, in patients with compromised immune systems such as in those with AIDS, resulting from HIV disease, infections by this opportunistic organism is a most serious and often terminal event. In biomedical studies of this disease, it became necessary to have access to oligosaccharides of defined structure obtainable by chemical synthesis.

A generalized structure for *Cryptococcus neoformans* serotypes A, B, C, D, and A-D is shown in Fig.2:

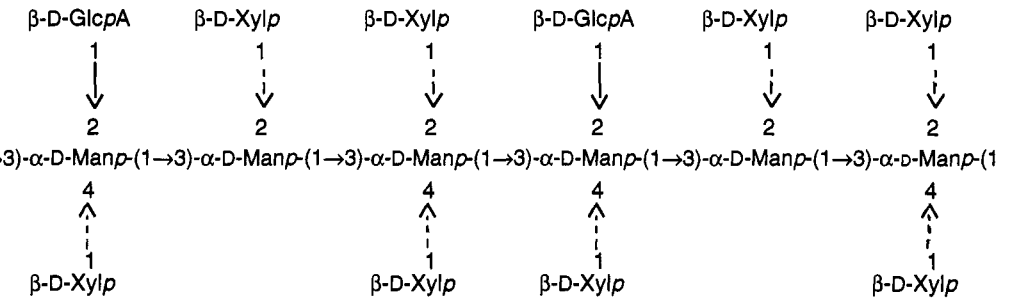

Fig.2. Generalized structure for *Cryptococcus neoformans* serotypes A, B, C, D, and A-D

All structures contain a (1→3)-linked backbone of α-D-mannopyranosyl residues. The serotypes differ in the glycosyl groups attached to this backbone. The glycosyl groups are β-D-xylopyranosyl or β-D-glucopyranosyluronic acid residues. Also, depending on the serogroups, *O-acetyl* groups, 2-10% by weight are present in the 6-positions of the mannosyl residues. The polysaccha-rides are not strictly regular. Several oligosaccharides corresponding to these structures have now been made. Syntheses of structures **I**, **II**, and **III** (Fig. 3), all containing a mannotriosyl backbone but differing in the glycosyl group on the central mannose (compounds **I** and **II**) and in having acetyl groups at the primary positions of the three mannoses (compound **III**) are discussed here. They were required for immunological studies and as NMR reference compounds in structural studies of *Cryprococcus neoformans* capsular polysaccharides.

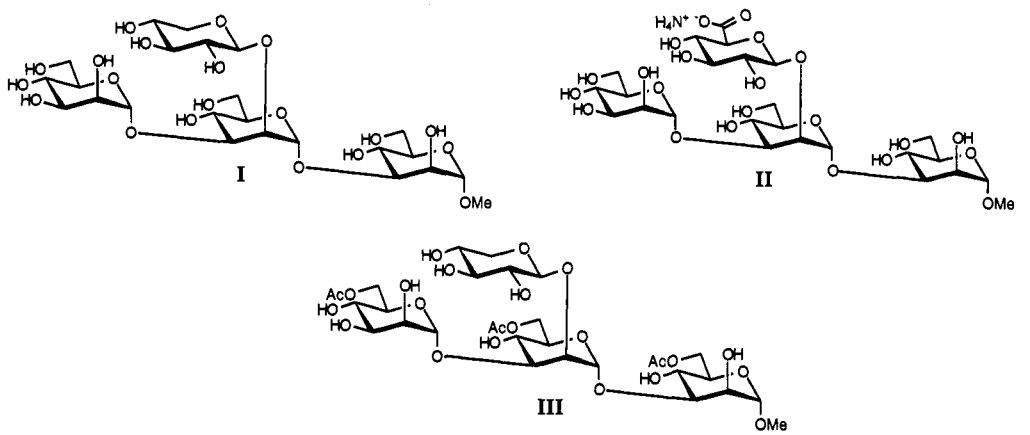

Fig.3. *Cryptococcus neoformans* oligosaccharides

The syntheses of these three oligosaccharides are summarized in Fig. 4. Monomeric starting materials were the thioglycoside and glycosyl bromide donors **1**, **7**, and **6** respectively, and the acceptor **2**. Starting materials in all three syntheses were the thioglycoside mannosyl donor **1** and the 3-OH mannoside acceptor **2**. Condensation using dimethyl(methylthio)sulfonium triflate (DMTST) as promotor yielded the disaccharide **3** which was debenzoylated to give the 2′,3′ diol **4**. To avoid excessive protecting group manipulation, diol **4** was subjected to stannylidene activation with dibutyltin oxide. Glycosidation of this intermediate with thioglycoside **1**, again under promotion with DMTST then gave the pivotal mannotriose **5** with a free 2'-OH. The corresponding 1→2 linked isomer was not present in the reaction mixture. If stannylidene activation was omitted and equimolar amount of **1** and **4** were used, the trisaccharide **5** was obtained in a similar yield, but due to the added presence of the 1→2 linked isomer from mannosylation at O'-2 of compound **4**, chromatographic purification was more cumbersome.

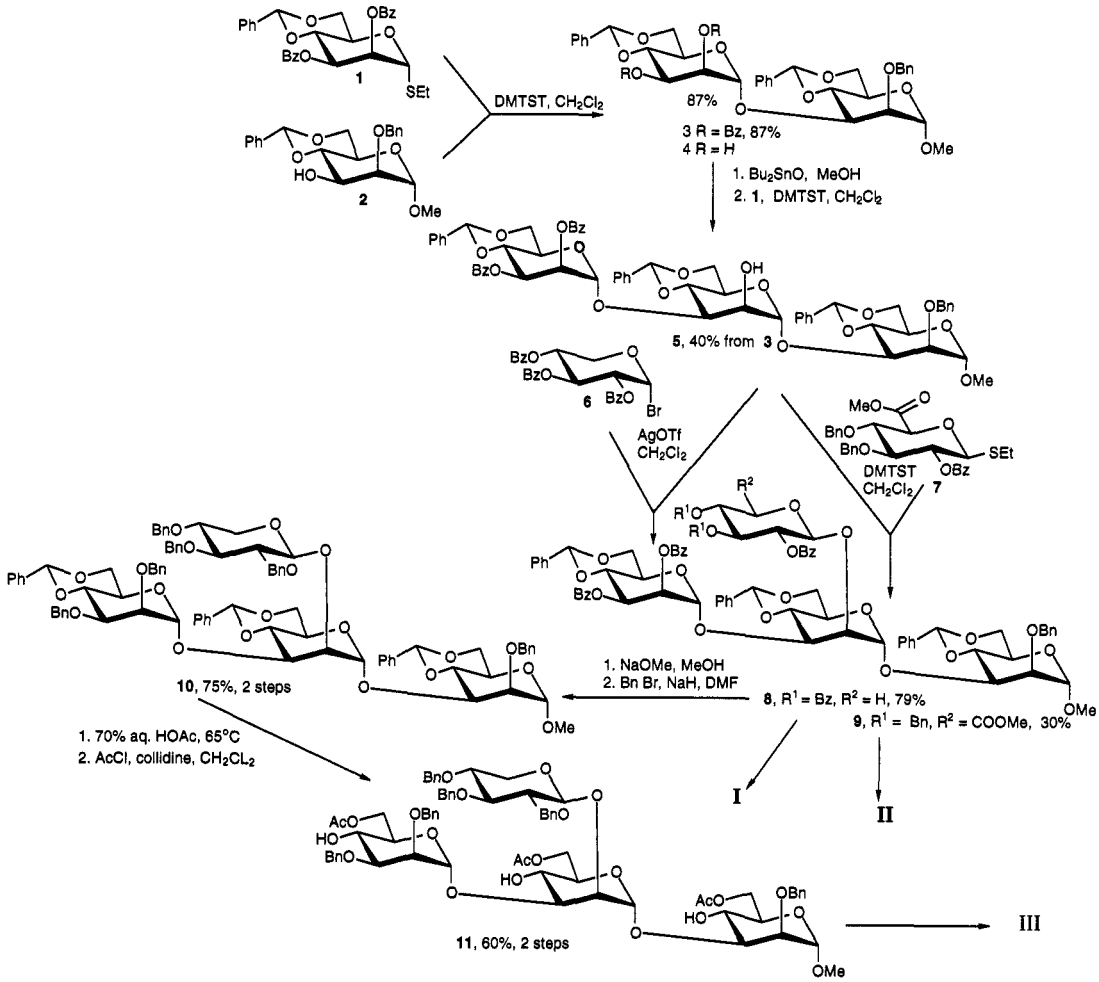

Fig. 4. Syntheses of *Cryptococcus neoformans* oligosaccharides

Condensation of the 2'-OH compound **5** with benzobromoxylose **6** under promotion with silver triflate gave the tetrasaccharide **8**. Deprotection by first debenzoylation and then catalytic hydrogenolysis then produced the target compound **I** in 77% yield from **8**. In order to obtain the second target compound **2**, the 2'-OH compound **5** was glycosylated with the thioglycoside donor **7** to produce the tetrasaccharide **9** which after deprotection by catalytic hydrogenolysis, debenzoylation and saponification was obtained as the ammonium salt **II** in 68% yield from **9.** In the route to target compound **III**, the three benzylidene groups were removed by mild acid treatment to leave free 4- and 6-OH groups on all three mannoses. Partial acetylation with acetyl chloride and collidine in dichloromethane then produced the 6,6',6''-triacetate **11**, which upon catalytic hydrogenolysis produced the target compound **III** in 77% yield from **11.**

The facile access to the trisaccharide **5** using a minimum of protecting group manipulation thus made it possible to obtain the three target compounds **I**, **II**, and **III** from the same precursor.

SYNTHESIS OF AN OCTASACCHARIDE CORRESPONDING TO PART OF THE OUTER CELL WALL STRUCTURE OF *MORAXELLA CATARRHALIS* SEROTYPE A

The Gram negative bacterium *Moraxella catarrhalis* is now recognized as a common cause of respiratory tract and middle ear infections. In order to develop diagnostics and vaccines, oligosaccharides were needed corresponding to its outer cell wall lipopolysaccharide structure shown in Fig. 5. Several oligosaccharides corresponding to the right-hand part of the structure have been made, all glycosidically linked to a spacer which makes attachment to proteins possible, thereby obtaining artificial antigens. Here will be described the synthesis of an octasaccharide comprising the structure shown, but without the two 3-deoxy-D-*manno*-octulosonic acid units (Fig. 5).

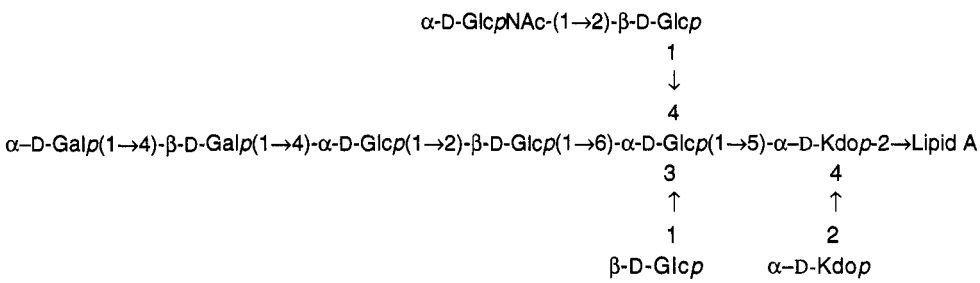

Fig. 5. The cell wall lipopolysaccharide structure of *Moraxella catarrhalis* seroptype A

The synthesis started from the disaccharide thioglycoside **12** available from previous work. Its benzoyl groups were replaced with benzyls and the product **13** was then attached by an α–D-glycosidic bond to the *p*-trifluoroacetamido spacer using DMTST in ether, taking advantage of a non-participating benzyl group at O-2. Mild acidic hydrolysis removed the benzylidene group in **14** to give the 2,3-diol **15** which was converted into the 6-*O*-*tert*.butyldimethylsilyl ether (TBDMS ether) **16.** Silver triflate promoted glycosylation with 3,4,6-tri-*O*-benzyl-2-*O*-chloroacetyl-α-D-glucopyranosyl bromide **18**, obtained in turn from the thioglycoside **17** produced the trisaccharide **19**, from which the monochloroacetyl group was removed, giving **20**, which now was glycosylated with 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl bromide (**22**), in turn obtained from the thioglycoside **21**, using silver triflate promotion to obtain the tetrasaccharide. The TBDMS group in the product **23** was removed and the 6-OH compound **24** was glycosylated with the glycosyl bromide **18** (above) under silver triflate promotion. The chloroacetyl group in the product **25** was removed by hydrazinedithiocarbonate without removing the three acetyl groups. The pentasaccharide **25** is the first major building block in the synthesis. There now remained the synthesis of the trisaccharide building block. Thiomethyl β-lactoside (**27**) was 4',6'-benzylidenated and the product **28** was then fully benzylated to give **29.** Regioselective reductive opening of the benzylidene group in **29** the gave the 4'-OH compound **30** which was glycosylated under silver triflate promotion with the galactosyl chloride **31**. The thioglycoside **32** was converted into the bromo sugar **33** (Fig. 6). Finally the two blocks **26** and **33** were condensed in diethyl ether in the presence of silver triflate to produce the final α-D-glycosidic linkage and 282 mg of the octasaccharide **34**. The final steps in the synthesis were: (1) Deacetylation with NaOMe-MeOH. (2) Catalytic hydrogenolysis to remove the benzyl groups and to reduce the azido group to an amino group. (3) *N*-acetylation by treatment with Ac_2O in MeOH. The total yield for these last 3 steps, including chromatographic purification was 36%.

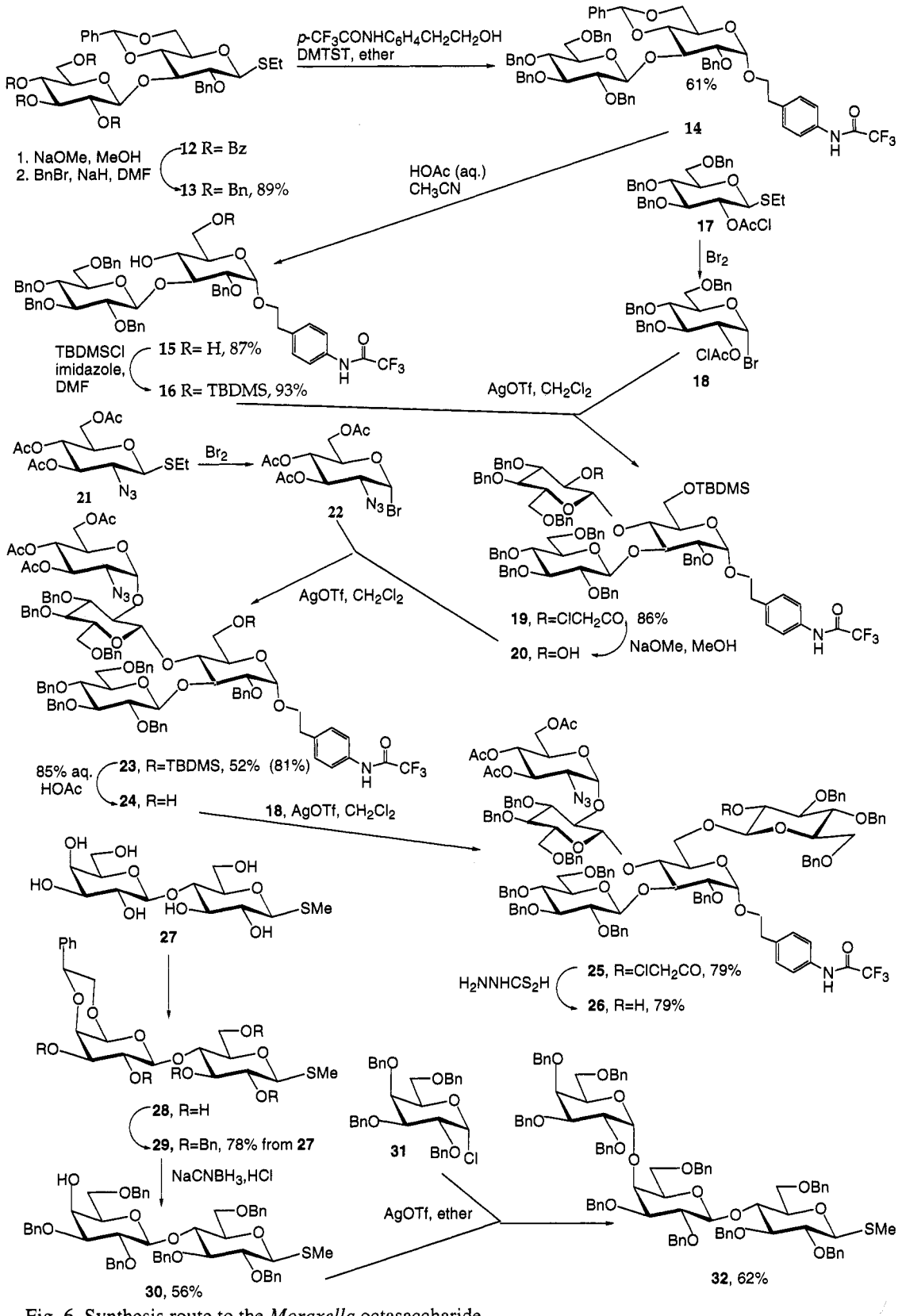

Fig. 6. Synthesis route to the *Moraxella* octasaccharide.

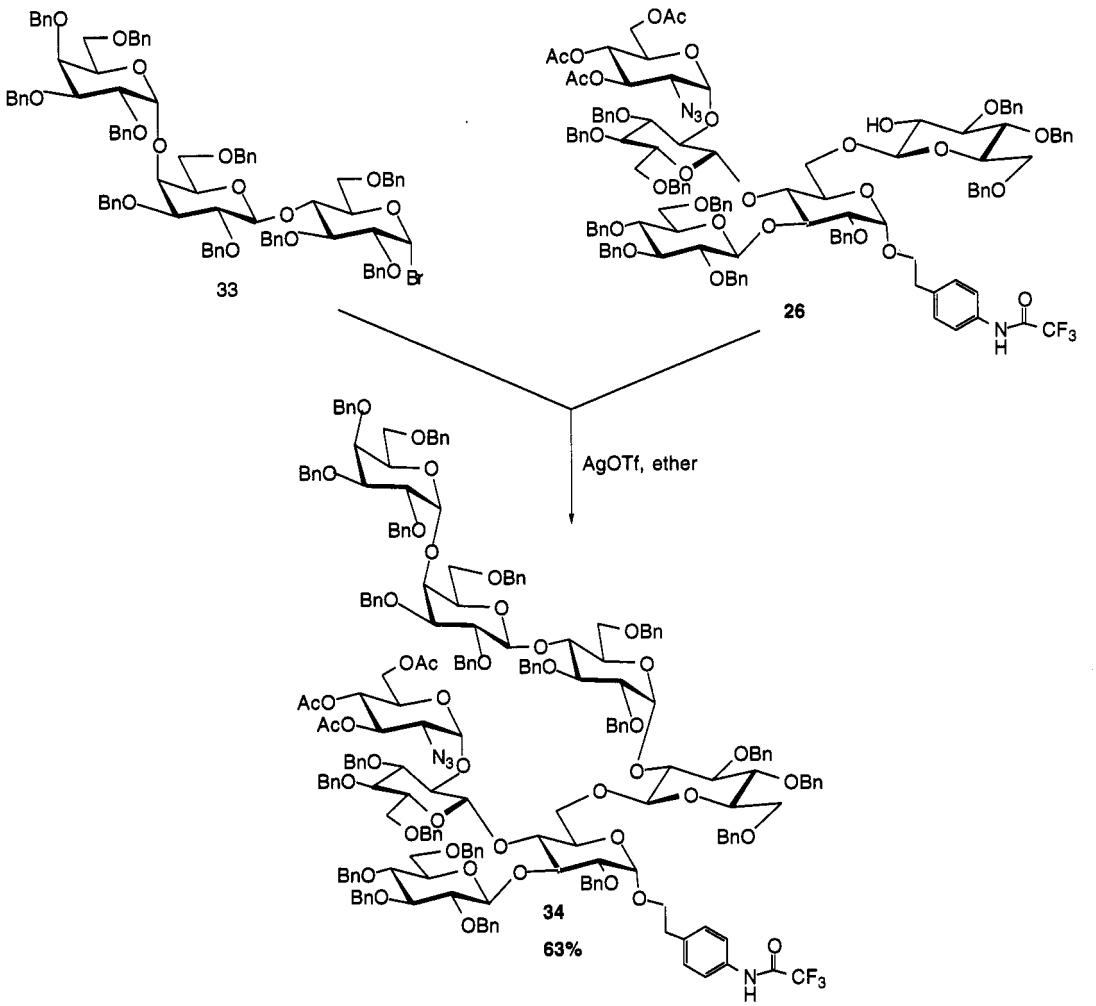

Fig. 6. Final glycosylation step in the octasaccharide synthesis

Acknowledgements

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References

The work on *Cryptococcus* and *Moraxella* oligosaccharides is fully described and all pertinent references are given in ref. 1-2 and in ref. 3-4, respectively.

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