An approach to O-linked sialoglycoprotein synthesis

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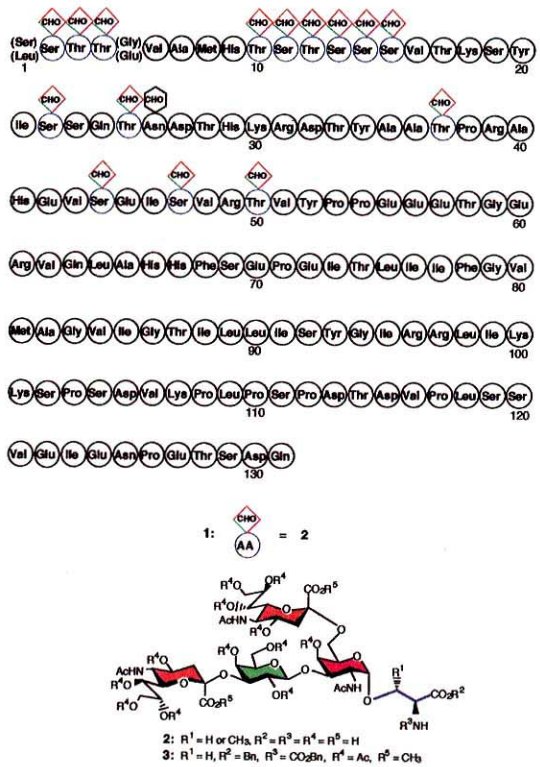
The synthesis of an O-linked sialoglycoprotein fragment was studied. In view of the advantages of a benzyl-based protection strategy, synthons **4**, **5**, **6**, **7**, and **8** were designed, and coupled via highly stereoselective glycosylations which led to **17** and **18** after manipulation of substituents. Using the key building blocks **17** and **18**, a dodecasaccharide-heptapeptide fragment of glycophorin A which involves the human M blood group epitope was synthesized for the first time.

In eukaryotic cells most membrane-bound and secretory proteins are modified by the attachment of oligosaccarides to the asparagine, serine, or threonine residues to form glycoconjugates which are called glycoproteins. It has been pointed out that some of those oligosaccharides contribute to the physicochemical properties of glycoproteins, protect glycoproteins against proteolytic attacks, and function in signal recognition in cell-cell, cell-membrane, and hormone-receptor interactions.[1,2] However, in most glycoproteins the biological functions of oligosaccharides remain uncertain.

Chemically synthesized oligosaccharides or oligosaccharide-bound peptide fragments are indispensable probes for the studies of glycobiology, because such homogeneous samples are hardly obtainable in enough amount from natural sources. A number of methodologies and technologies have been developed for oligosaccharide synthesis since methyl α-gentiobioside, β-D-Glc-(1 $\rightarrow$ 6)-α-D-Glc-OMe, was first synthesized by Helferich et al. in 1924.[3] The major technological developments in carbohydrate chemistry have been independent of those in peptide chemistry. At the present time, however, synthesis of glycopeptides has become accessible by employing the techniques suitably advanced for both oligosaccharide and peptide chemistry. In the techniques, are involved the employment of orthogonal protective groups and the choice of conditions effective to promote glycosylation and peptide coupling. It is noteworthy that the pioneering studies by Paulsen et al. in early 1980's have made significant contributions to the advancement of the researches in this field.[4] We describe herein our recent studies on the synthesis of an O-linked sialoglycopeptide.[5,6]

Glycophorin A (**1**) is a major transmembrane sialoglycoprotein of human erythrocyte and the N-terminal region is highly modified by O-glycosylation with sialic acid-containing tetrasaccharide chains (**2**) as shown in Scheme 1. The polymorphic N-terminal pentapeptide sequences correspond to the antigenic determinants of human MN blood type. The importance of the tetrasaccharide attachments in the MN antigenicity has been demonstrated by the evidences that enzymatic desialylation of glycophorin A or its proteolytically degraded fragments caused the loss of their MN antigenic activity and that resialylation with sialyltransferase restored the activity. The same tetrasaccharide is widely distributed as the carbohydrate portions of various human glycoproteins.

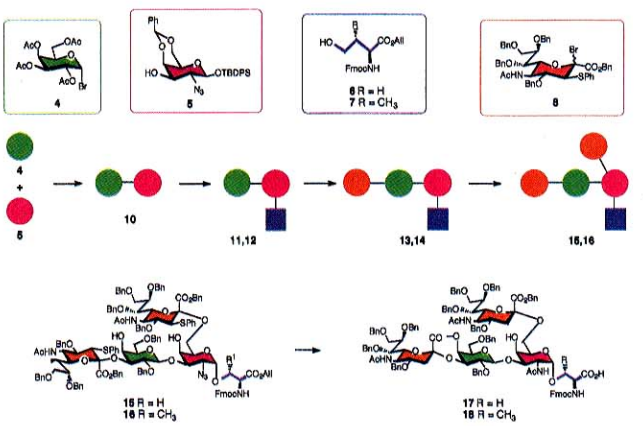


Scheme 1

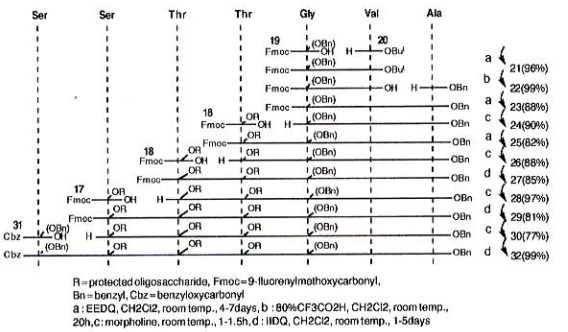
Several groups have synthesized glycophorin A fragment analogs, especially corresponding to the regions bearing the carbohydrate cluster, as the synthetic prototype of complex glycoproteins, since the complete structure of this glycoprotein had been reported early in the history of glycoprotein-structural studies.[7,8] However, when we initiated our studies, there had been no reported examples where sialic acid was involved in the synthesis of glycopeptides. The synthesis of sialyl glycosides, though important in carbohydrate chemistry, had not been studied so much, because sialic acid had been considerably expensive to use even in the laboratory scale preparation. In 1988, the first synthesis of a sialic acid-containing tetrasaccharide linked to L-serine (**3**) was achieved in this laboratory.[9] The synthesis, however, led us to the unsatisfactory results particularly with respect to

the low stereoselectivity in glycosylation between the carbohydrate donor and the serine derivative, and the synthesized compound was not suitably protected for further elongation of the peptide chain. The recent advances in glycosylation methodology prompted us to search for a more efficient route to the tetrasaccharide derivatives. A novel approach to the sialoglycopeptide was designed based on the Fmoc strategy of peptide synthesis in combination with benzyl protection for both hydroxyl and carboxyl functionalities. The benzyl protection allows non-basic, mild conditions for the ultimate deprotective stage, which should eliminate any undesirable cleavage of the carbohydrate-peptide linkage and minimize amino acid racemization.

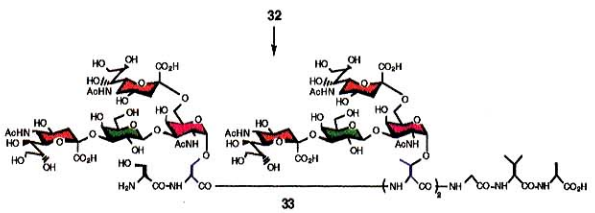
The synthetic pathway to the key building blocks, tetrasaccharide-amino acid conjugates, is outlined in Scheme 2, where the compounds **4**, **5**, **6**, **7**, and **8** were chosen as the synthons of each component. Glycosylation of **5** with **4** produced the β-linked disaccharide (**10**) under the stereocontrol by neighboring group participation of a 2-O-acetyl group in the glycosyl donor **4**. The disaccharide **10** was converted into the glycosyl fluoride through the manipulations of the protective groups in 8 steps. The fluoride was activated with the promoter of Cp_2ZrCl_2-$AgClO_4$[10] to react with the serine **6** and threonine derivatives **7**. The glycosylation displayed good α-selectivity ($\alpha : \beta = 7 \sim 10 : 1$) to give **11** and **12** in 80% yield. Stereocontrol in sialyl glycoside formation (sialylation) had been a long-standing question. However, in 1988, Ito and Ogawa obtained an efficient solution to the problem by introducing a stereo-directing phenylthio group into the C-3 position of a sialic acid-donor molecule.[11] According to this sialylation method, two sialic acid residues were stereoselectively introduced to the disaccharides **11** (and **12**) using a Hg salt promoter in the separate steps via **13** (and **14**) to give **15** (and **16**). Those reactions gave the α-glycosides exclusively. The tetrasaccharides **15** and **16** were converted, via 1) azide-acetamide transformation, 2) deallylation, and 3) desulfurization, into **17** and **18**, which are appropriately protected for the use as building blocks of a carboxylic acid component in oligopeptide synthesis. The synthesis of an N-terminal heptapeptide containing M blood group epitope was undertaken in solution according to the Fmoc strategy. The peptides were synthesized with EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline) or IIDQ (2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline) as the coupling agent, the procedure being illustrated in Scheme 3. The N-terminal Fmoc group was



Scheme 2



Scheme 3



Scheme 4

removed with morpholine in high yield after each elongation of the peptide bond. All the coupling reactions proceeded smoothly without any difficulty caused by increasing molecular weights of the reactants. To complete the synthesis, deprotection of the heptapeptide **32** was performed. (Scheme 4) All the benzyl-ether, -ester, and benzyloxycarbonyl groups were simultaneously cleaved by hydrogenolysis and the tri-lactone was readily hydrolyzed with a mild base ($NaHCO_3$, pH 7.5-8) present in the hydrogenation media to produce the target dodecasaccharide-heptapeptide molecule **33**, quantitatively. The homogeneity of the synthetic sample was confirmed by ion-exchange chromatography and the structure was assigned by ^{1}H-NMR and FAB mass spectroscopy.

We have now established the synthesis of a carbohydrate-clustered O-linked sialoglycoprotein fragment which involves the human M blood group epitope. Approaches to the more complex sialoglycoprotein molecules of biological interest and developements of the practical solid-phase synthesis of those sialic acid-containing glycopeptides will be the coming challenges.

References

1) J. Montreuil: Adv. Carbohydr. Chem. Biochem. **37**, 157 (1980).
2) D. L. Blithe: Trends in Glycosci. Glycotech. **5**, 81 (1993).
3) B. Helferich and J. Becker: Ann. **440**, 1 (1924).
4) H. Paulsen: Chem. Soc. Rev. **13**, 15 (1984).
5) Y. Nakahara, H. Iijima, and T. Ogawa: Tetrahedron Lett. **20**, 3321 (1994).
6) Y. Nakahara, H. Iijima, and T. Ogawa: Carbohydr. Lett. in press.
7) M. Tomita, H. Furthmayer, and V. T. Marchesi: Biochemistry **17**, 4756 (1978).
8) P. D. Siebert and M. Fukuda: Proc. Natl. Acad. Sci. USA **83**, 1665 (1986).
9) H. Iijima and T. Ogawa: Carbohydr. Res. **186**, 107 (1989).
10) K. Suzuki, H. Maeta, T. Matsumoto, and G. Tsuchihashi: Tetrahedron Lett. **29**, 3567 (1988).
11) Y. Ito and T. Ogawa: ibid. p. 3987.