HAWORTH MEMORIAL LECTURE. Experiments Directed Towards Glycoconjugate Synthesis*

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1 Introduction

Remarkable advances[1] in synthetic methods and strategies in the field of complex oligosaccharides have been made in the past decades. In this article, highlights of our synthetic studies on glycoconjugates will be described. Particular emphasis will be placed on strategies for the total syntheses of complex carbohydrates and glycoconjugates such as oligosaccharins, glycolipids, cycloglycans, glycoproteins, and proteoglycans.

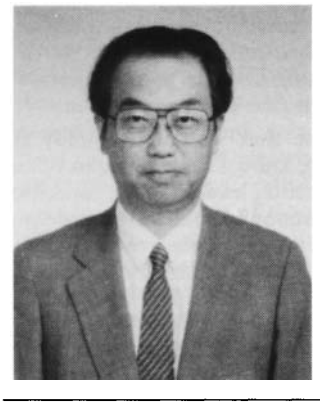
2 Exploitation of New Methods and Strategies

In 1976 a new method for highly regioselective and efficient acylation as well as alkylation was discovered by employing tin(IV)-mediated activation[2] of hydroxyl groups. This technology allowed the possibility of establishing synthetic strategies for the regioselective extension of glycan chains at the branching points of complex glycans.

A conceptually new approach to glycosylation was developed in 1987 by Y. Ito[3] employing alkyl phenylsulfenate as a new type of glycosyl acceptor which was readily activated in the presence of hard Lewis acid such as TMSOTf to generate a soft electrophile PhSOTf and a hard nucleophile ROTMS. Upon reaction with either glycals or thioglycosides, which may be regarded as soft nucleophiles, a high yield of O-glycosides was obtained under extremely mild conditions. Use of soft electrophile PhSeOTf[4] in order to activate S-glycosides was also developed and applied successfully in the synthesis of complex carbohydrate sequences. Activation of thioglycosides was also executed efficiently by S. Sato[5] in the presence of Bu^n_4NBr–$CuBr_2$–AgOTf (or $HgBr_2$) to give O-glycosides in a highly stereocontrolled manner.

In order to attain high stereocontrol for glycosylation using sialic acid derivatives as glycosyl donor, β-equatorially oriented phenylthio or phenylseleno groups[6] were introduced as a stereocontrolling auxiliary at C-3. This new strategy for the introduction of an α-D-NeuAc residue into carbohydrate sequences was recently implemented in the first total synthesis of disialosyl gangliosides, GD3.

Tomoya Ogawa received his Ph.D. (1967) from the University of Tokyo under Professor M. Matsui and began his career as an assistant at the same university in 1967. He moved to RIKEN in 1968, and was appointed head of the laboratory for synthetic cellular chemistry in 1979. For two years from 1972 he was a post-doctoral research fellow with Professor S. Hanessian at the University of Montreal. He returned to RIKEN in 1974. Since 1990 he has also been a professor of cellular biochemistry at the graduate school of the University of Tokyo.



In the case of alcohols with low reactivity such as ceramide derivatives, a major side-reaction led to the formation of orthoesters instead of 1,2-*trans* glycosides. To avoid this annoying outcome, both pivaloyl and trimethybenzoyl groups were introduced as O-2 auxiliaries in the glycosyl donor which eventually improved the coupling efficiency dramatically.[7]

3 Total Synthesis of Oligosaccharins

Some plant cell wall fragments have been chemically and physiologically characterized as oligosaccharins.[8] Oligogalacturonic acid (1), a pectin fragment, has been claimed to act as an endogenous regulator molecule which enhances the resistance of plant cells against invasive pathogens, and a fragment of hemicellulose xyloglucan (5) has been shown to be a factor controlling plant cell growth and differentiation.

In 1984, Y. Nakahara began synthetic studies on pectin fragment (1) with the aim of confirming its physiological functions in plants. The designed synthetic blocks (2), (3), and (4) proved to be highly efficient in executing α-stereoselective glycosylations under Mukaiyama conditions, first between (3) and (4) (63%) and then between the product of the latter coupling and (2) (64%). Eventually α-(1→4) linked dodecagalactoside was obtained[9] as a properly protected key intermediate, which was then oxidized, deprotected, and purified by FPLC to give (1) in 1989. Biotesting showed (1) to be physiologically equivalent to the natural sample in eliciting phytoalexin biosynthesis and accumulation in soybean.

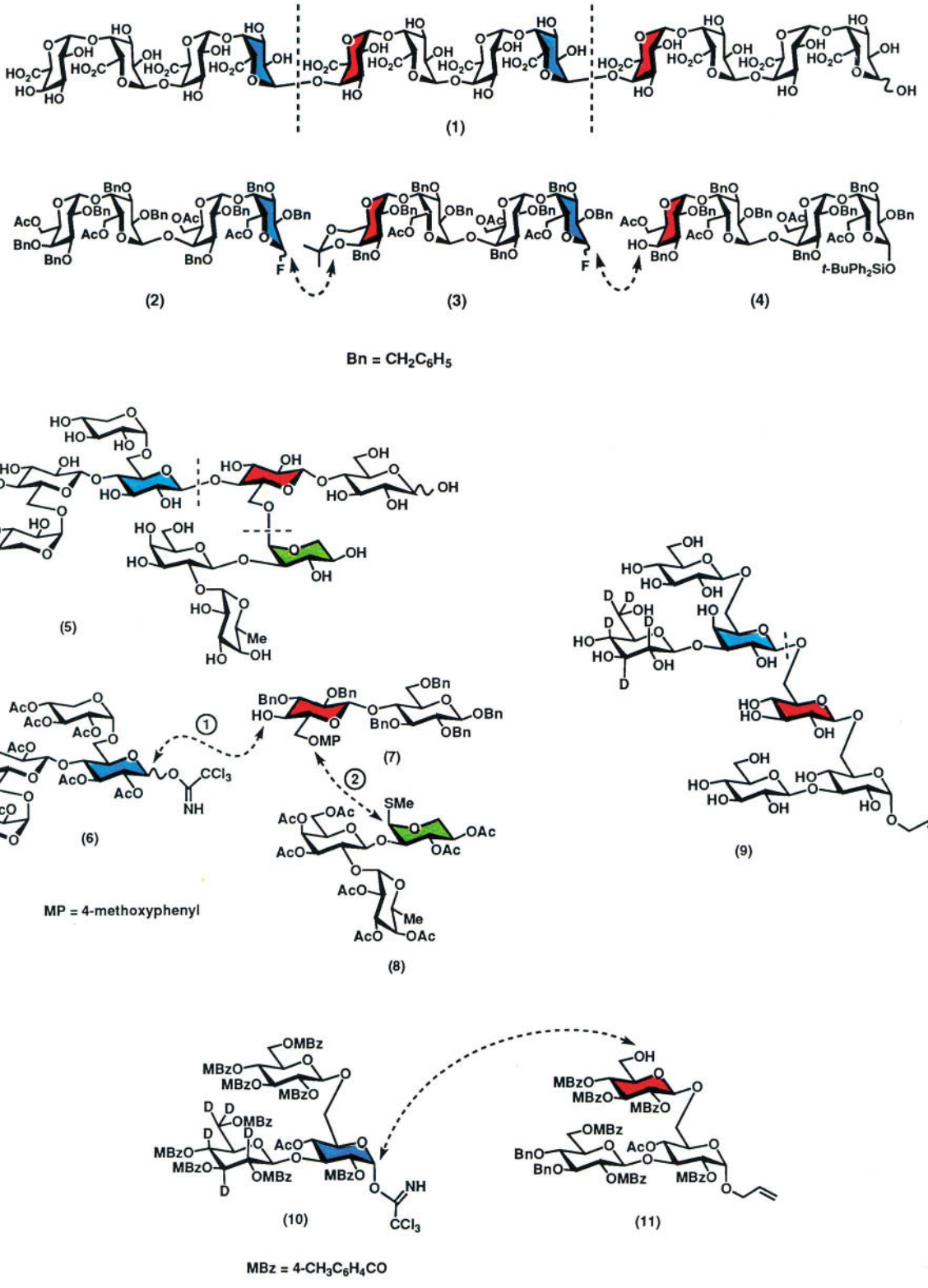
Another oligosaccharide (5), a fragment of xyloglucan, was reconstructed by K. Sakai[10] by coupling tetrasaccharide block (6) with disaccharide block (7) in high stereoselectivity and then coupling the hexasaccharide block so obtained with trisaccharide block (8), though in low stereoselectivity (α:β = 3:1). The product was deprotected to yield (5), which inhibited the auxin-stimulated growth of etiolated pea stem segments in the same manner as the natural product. In the course of these experiments directed toward a total synthesis of (5), an efficient way to achieve α-D-xylosylation at primary hydroxyl groups had to be developed. This was only possible using the novel copper-mediated activation procedure for thioglycosides mentioned above.

During experimentation aimed at the elucidation of solution conformation of phytoalexin elicitor-active β-D-glucohexaoside, N. Hong developed a method for the stereocontrolled regiospecific introduction of deuterium atoms[11] to obtain deuterium labelled analogues such as (9). One of the reactions for the key stereoselective coupling was between deuterated trisaccharide donor (10) and trisaccharide accepter (11) which was carried out in 63% yield.

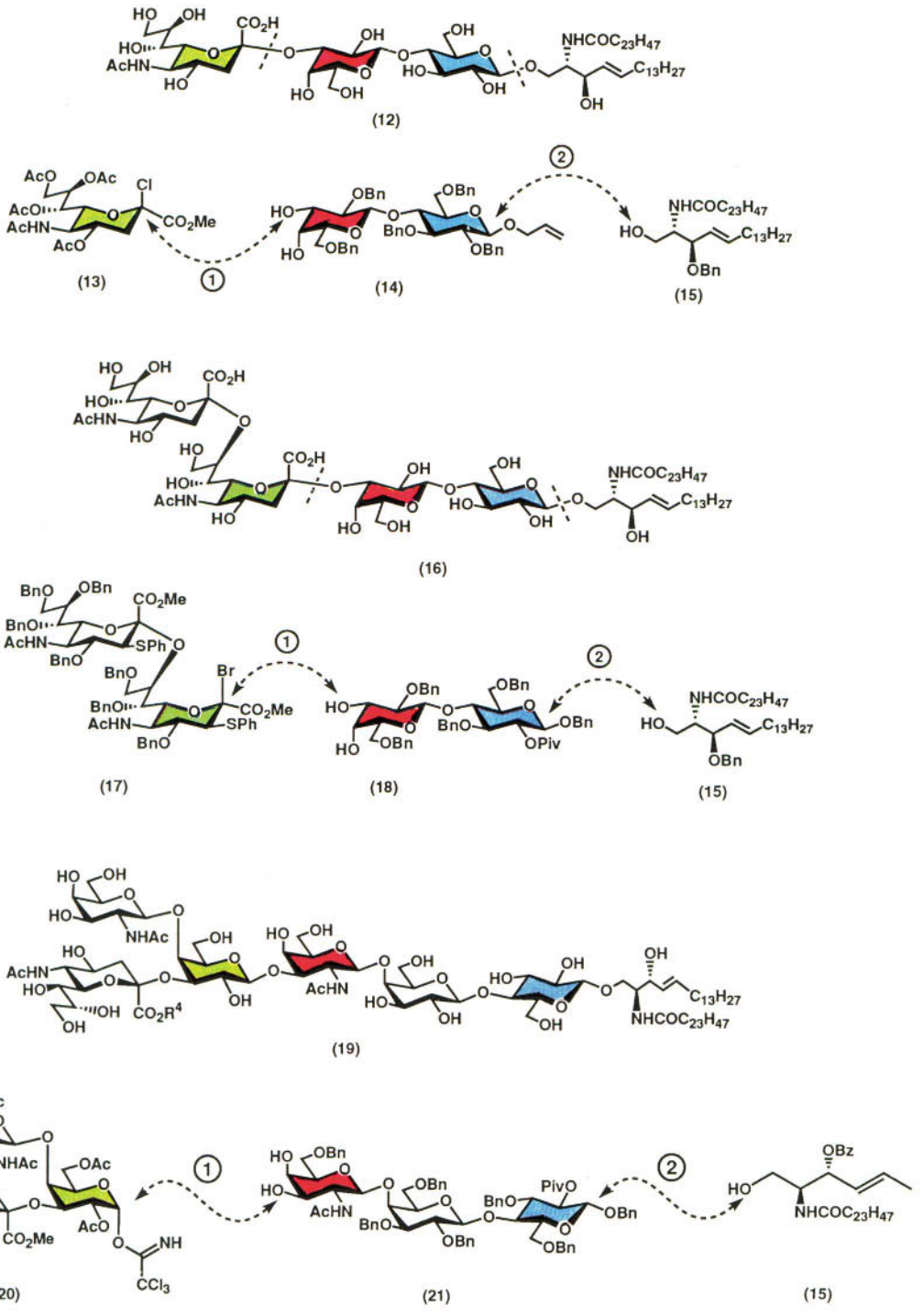
4 Total Syntheses of Glycolipids

Among many biologically significant glycosphingolipids we have studied so far, I will first focus here on the ganglio-

* Based on a Haworth Memorial Lecture delivered at the Spring Meeting of the Royal Society of Chemistry Carbohydrate Group on March 29th 1993 at the University of Dundee and at the Annual Chemical Congress of the Royal Society of Chemistry on April 7th 1993 at the University of Southampton.



gangliosides GM_3 (12) and GD_3 (16). When M. Sugimoto started synthetic studies on these molecules around 1980, no clear experimental evidence had been reported for the introduction of sialic acid at secondary hydroxyl groups. We therefore first studied carefully the coupling reactions between sialosyl donor (13) and disaccharide glycosyl acceptor (14). In 1984 to our delight, after intensive purification of the reaction mixture, the (2→3)-linked trisaccharides were isolated for the first time (in 18% yield) and we were able to characterize them well spectroscopically.[12] Though the ratio of the products with α- and unnatural β-configuration was 1:2, this observation was clear evidence for the first time that we could employ the conventional sialosyl donor (13), known since 1966, to obtain not only a major elimination reaction leading to the formation of an undesired 2,3-dehydro-compound, but also the desired substitution at the C-2 position of (13) upon reaction with a rather sterically demanding alcohol such as (14). From the results of these experiments we deduced that the product obtained[13] by Shapiro in 1973 was in fact not the α- but β-sialylated compound. Encouraged by this observation, a total synthesis of the ganglioside GM_3 (12) was subsequently achieved for the first time in 1985. The ceramide part-structure

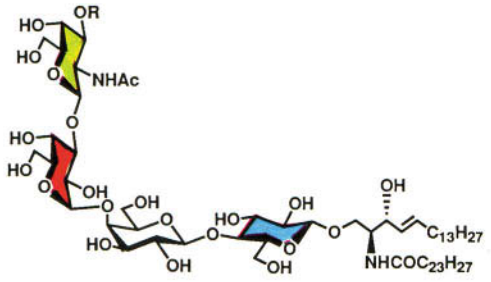


(15) was synthesized by K Koike from D-glucose in an efficient manner in 1984

In order to control the course of glycosylation sterically with a sialosyl donor, the novel glycosyl donor (17) was designed by Y Ito This proved to be a highly successful synthetic manoeuvre, and based on this advance the first total synthesis[14] of ganglioside GD_3 (16) as achieved in 1989 Coupling of disialosyl donor (17) with a disaccharide block (18) proceeded with high stereocontrol (α β = 60 1) in 48% yield Since the tetrasaccharide thus obtained from (17) and (18) carries a pivaloyl group as an auxiliary (*vide ante*) at the O-2 of the reducing end glucose residue, the coupling between ceramide derivative (15) and tetrasaccharide glycosyl donor was executed smoothly and the product was successfully transformed into the target compound (16)

A first total synthesis of a further chain-extended ganglioganglioside (19) was also achieved[15] in 1990 The coupling of (20) with (21) was carried out in the presence of BF_3 OEt_2 in $(CH_2Cl)_2$ to afford the desired $\beta(1\rightarrow3)$ linked hexasaccharide and the undesired $\beta(1\rightarrow4)$ linked isomer in 43 and 7% yield, respectively Functional group manipulations of the $\beta(1\rightarrow3)$ linked hexasaccharide, coupling with ceramide derivative (15), and final deprotection gave (19)

In addition to the studies on ganglio-gangliosides, other series of glycosphingolipids also attracted our synthetic curiosity Thus globo-series glycolipids such as SSEA-3 (22) (Stage Specific Embrionic Antigen-3) and Forssman antigen (23) were synthesized by S Nunomura for the first time in 1988[16] and 1989,[17] respectively Carbohydrate sequences (22) and (23) were synthesized by use of a common glycotriosyl acceptor (26) and glycobiosyl donors (24) and (25), respectively The coupling between the imidate (24) and a glycosyl acceptor (26) gave only



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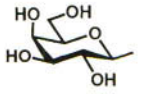
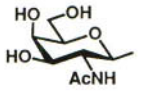
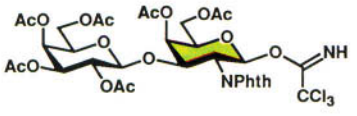


(23) R =





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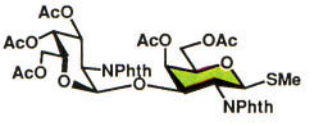

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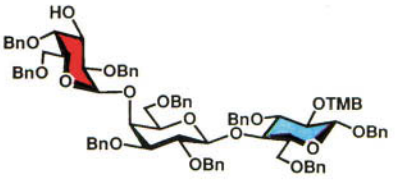
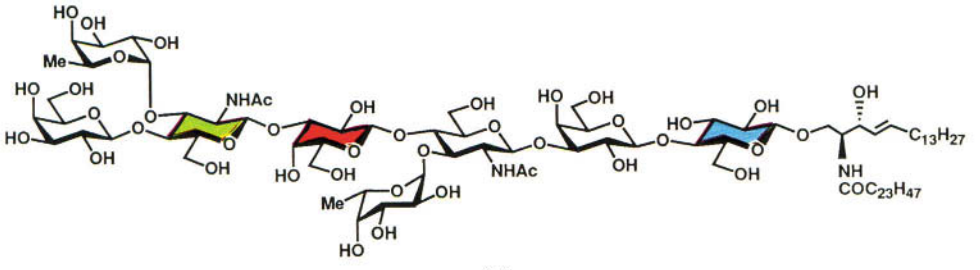


(26) (TMB = 2,4,6-trimethylbenzoyl)



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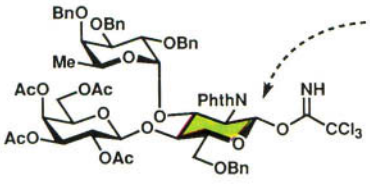

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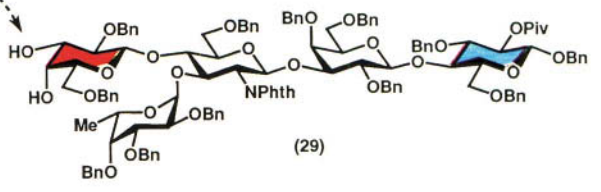

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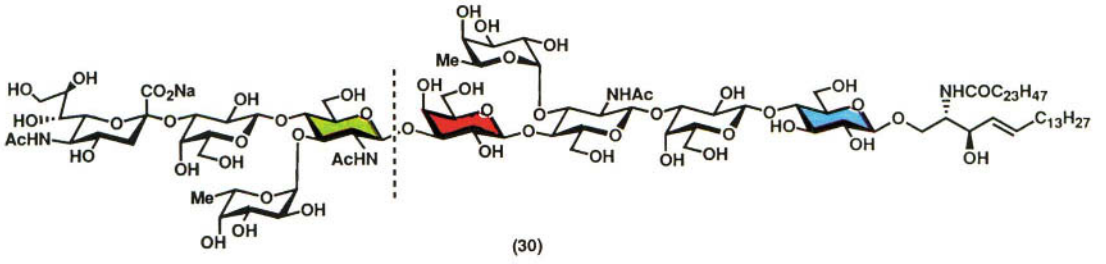
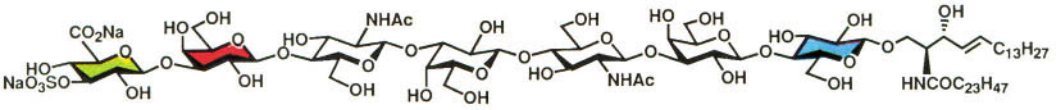


(30)

22% of the pentasaccharide based on (24). In fact, 44% of (24) was converted into an undesired rearranged anomeric trichloroacetamide. However, we were pleased to observe that Cu^{2+}-mediated glycosylation[5] of (26) with the thioglycoside (25) afforded a 78% yield of a mixture of α- and β-linked pentasaccharide in a ratio of 1:10. Couplings of the carbohydrate sequences with a ceramide derivative (15) were achieved in the usual way and the products were then converted into (22) and (23), respectively.

Another important group of glycosphingolipids as targets for our synthetic studies was the so-called neolacto-series. S. Sato first approached the synthesis[18] of dimeric Le^x antigen (27). The crucial coupling between (28) and (29) was carried out in 62% yield in a highly regioselective manner. The octasaccharide intermediate was then converted into (27) in 9 steps in 15% overall yield. This bond disconnection strategy has also been applied[19] recently in the first synthesis of sialyl dimeric Le^x antigen (30).

Another neolacto-series glycosphingolipid, L2/HNK-1 antigen (31), isolated from the nervous system was synthesized[20] *via*



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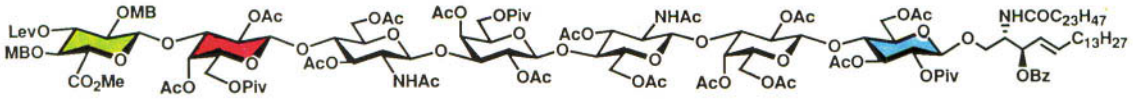

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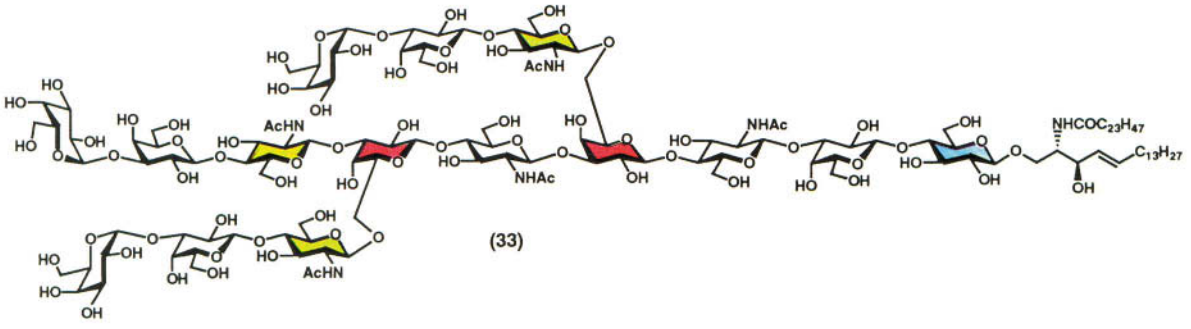

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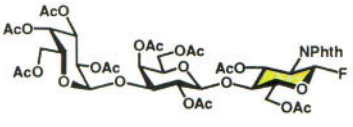

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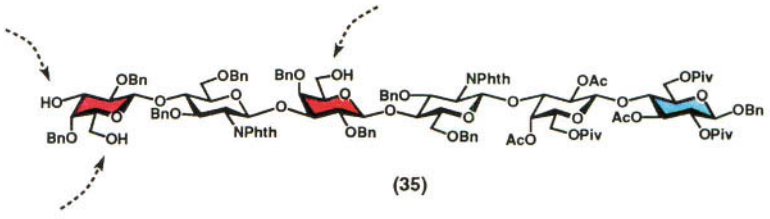

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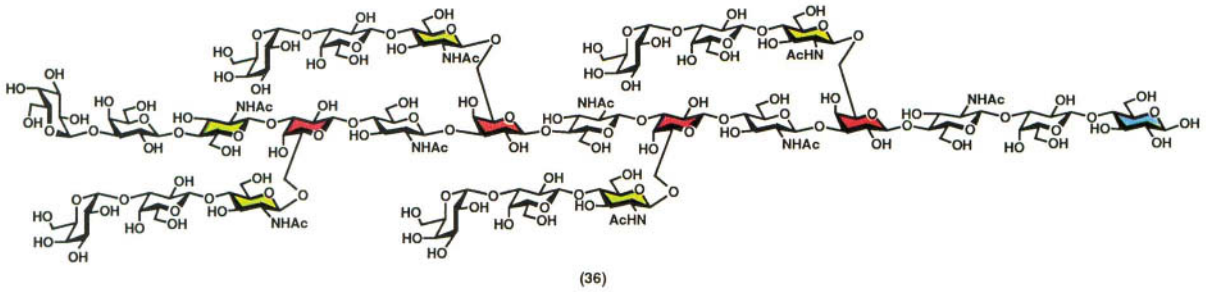

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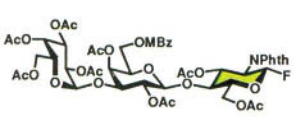

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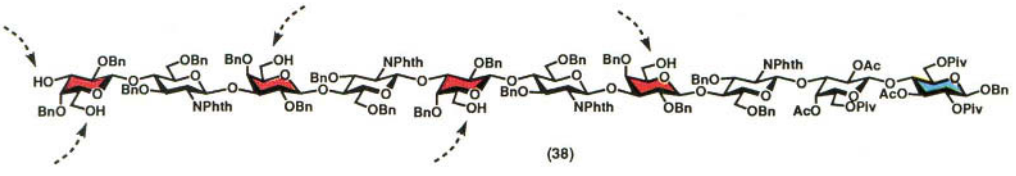
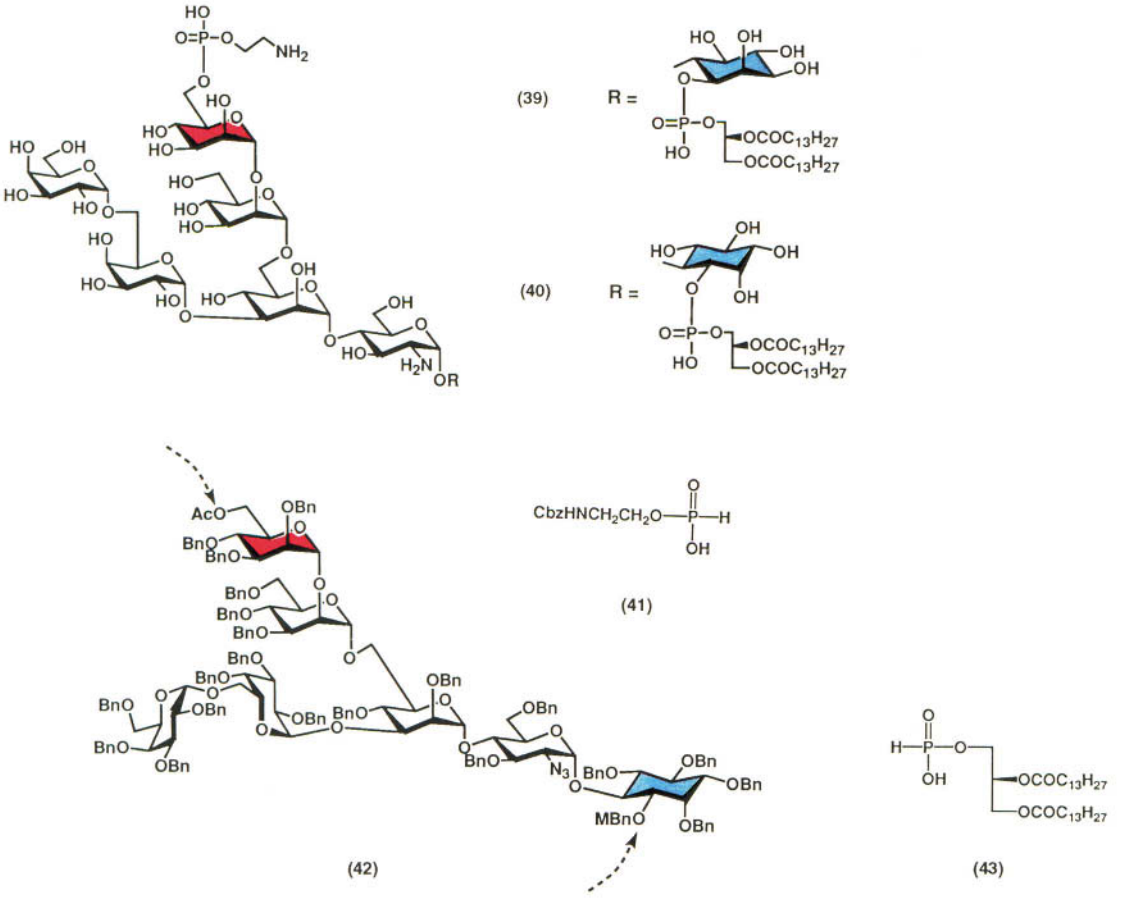


(38)

a key intermediate (32) by T. Nakano in 1991. Neolacto-series glycosphingolipid (33) with multiple branches was synthesized[21] by Y. Matsuzaki in 1992. Simultaneous couplings between a glycotriosyl donor (34) and three hydroxyl groups of the glycohexaosyl acceptor (35) were achieved by the Suzuki procedure[22] to give stereoselectively 71% of pentadecasaccharide, which was in turn converted into (33) in 8 steps in 5% overall yield.

In continuation of the neolacto-series syntheses, the complex pentaantennary structure of I-type glycan (36) was synthesized in 1993 by employing essentially the same strategy as used in the case of (33). Two key intermediates (37) and (38) were used as glycosyl donor and a glycosyl acceptor, respectively. After glycosylation, the desired protected pentacosasaccharide was isolated in 37% yield and was then completely deprotected in four steps in 37% overall yield to give (36).[23] The structure was confirmed by ^{1}H-NMR and FAB-MS.[24]

Apart from the glycosphingolipids so far discussed, glycosylphosphatidylinositol anchor (39) was a particularly challenging target for synthesis. In 1991 C. Murakata reported a first synthesis[25] of (39). In consequence of a personal communication,[26] however, we now have to revise our previous assignment for the synthetic structure of (39) to that of its diastereoisomer (40) which contains L-*myo*inositol instead of the D-enantiomer. Thus in fact a synthesis of *unnatural* GPI anchor (40) was carried out by us employing regioselectively protected heptasaccharide (42) and two hydrogen phosphonate synthons (41) and (43).



Another example I would like to note here is the recent synthetic study[27] on Nod factor glycolipid (44) carried out by S. Ikeshita. In this case, the introduction of the activated fatty acid (45) onto the carbohydrate backbone was planned to be the last step of the synthetic sequence. A deblocked key intermediate (46) was employed so that we could easily introduce a variety of fatty acids and thus study the biological specificity of interactions between host plants and microbes in terms of fatty acid structures.

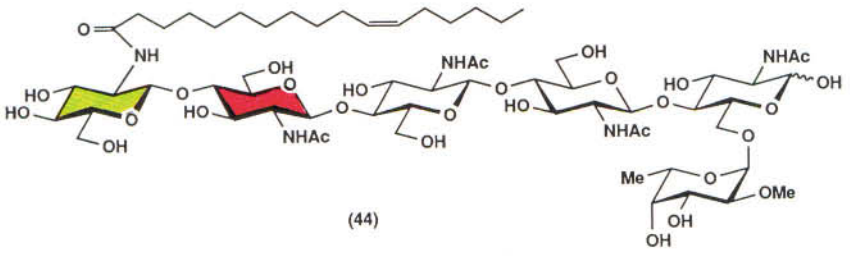
5 Synthesis of Cycloglycans

Cyclodextrins are a group of naturally occurring cycloglycans well known for their ability as host molecules to recognize hydrophobic guest molecules. In 1985, Y. Takahashi examined[28] for the first time *non-enzymatic* 'cycloglycosylation' of an acyclic glucohexaosyl fluoride (48) by utilizing Mukaiyama conditions and successfully isolated a cyclic product (in 21% yield) that was successfully converted into α-cyclodextrin (47). Thus the total synthesis of (47) was accomplished 70 years after Schardinger first described its properties in reliable detail in 1920. After completing our synthesis of γ-cyclodextrin in 1987, we then turned our efforts to the synthesis of other (1 → 4)-linked cyclooligosaccharides. By employing a linear thioglycoside such as (49) a high conversion into α-(1 → 4) linked cyclo*manno*hexaose (74%) was achieved by M. Mori in the presence of a soft electrophile such as PhSeOTf.[3] In addition, further transformation of the cycloglycan into functionalized cycloglycans such as (50) could now be executed.[29] By employing α-(1 → 4)-linked oligolactose (51) as the key intermediate, trigonally shaped cyclooligolactose (52) was prepared[30] by H. Kuyama in 1993. Further experiments have established synthetic routes to the homologous cyclooligolactoses (53) and (54). Energy minimized structures (55), (56), and (57) derived by T. Nukada using a modified MM2 program (Daikin Co.) indicated unique structures for synthetic cycloglycans (52), (53), and (54), respectively. Practical applications expected for these kinds of artificial cycloglycans remain to be shown.

6 Glycoprotein Glycans

Glycans of glycoproteins are classified into two major groups: N-linked types in which glycan chains are linked to an Asn residue, and O-linked types in which glycan chains are linked to either a Ser or a Thr residue. Synthetic approaches towards typical structures (58) and (61) are highlighted here. The biantennary structure (58) carries two identical tetrasaccaride branches. Because of this characteristic, a tetrasaccharide block (59) and a trisaccharide block (60) were designed as glycosyl donor and glycosyl acceptor, respectively. The trichloroacetimidate (59) was coupled with diol (60) under Schmidt conditions to give the desired α-(1 → 6) and α-(1 → 3) linked undecasaccharide product in (56% yield) which was deprotected to afford (58). This crucial experiment completed the first total synthesis[31] of a very typical biantennary complex type N-glycan of glycoprotein in 1986.

Glycophorin A is one of the abundant glycoproteins embedded in the plasma membrane of human red blood cells and is rich in carbohydrate content. As part of our project on the synthesis of clustered O-linked glycans on a peptide core, a partial structure of glycophorine A which corresponds to the N-terminal heptapeptide (61) was chosen as a model target. Based on the novel strategy[14] for the α-stereoselective introduction of sialic acid as well as the conventional Fmoc strategy[32] for the peptide chain elongation, tetrasaccharide-serine/threonine blocks (62) and (63) were designed and subsequently synthesized in highly stereocontrolled manner by Y. Nakahara. A chain elongation from (64) by repeated use of (62) and (63) was carried out successfully. The product was finally deprotected[33] to give the N-terminal heptapeptide structure (61) that represents the human blood group M epitope. Based on these achievements in both N- and O-linked glycopeptide synthesis, we are continuing



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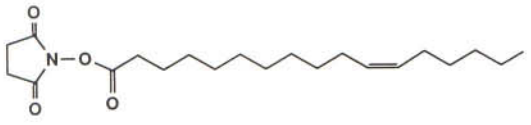

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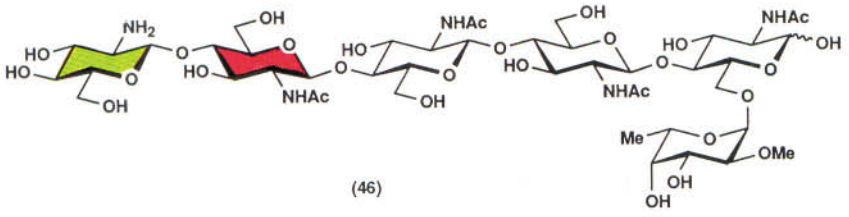

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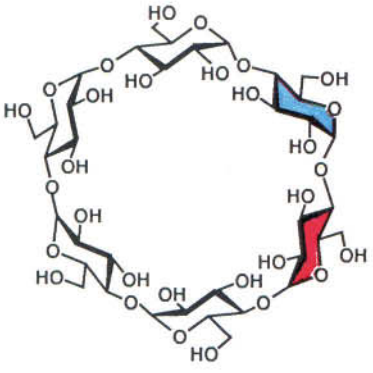

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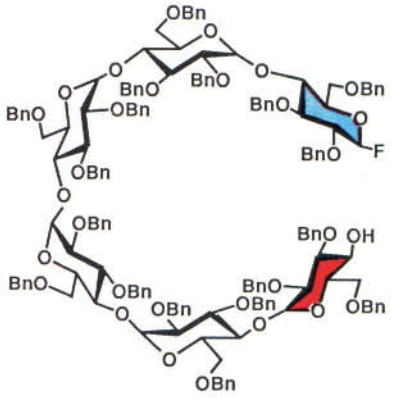

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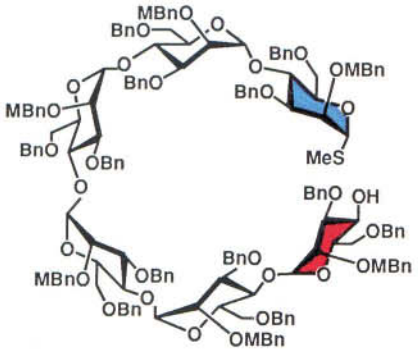
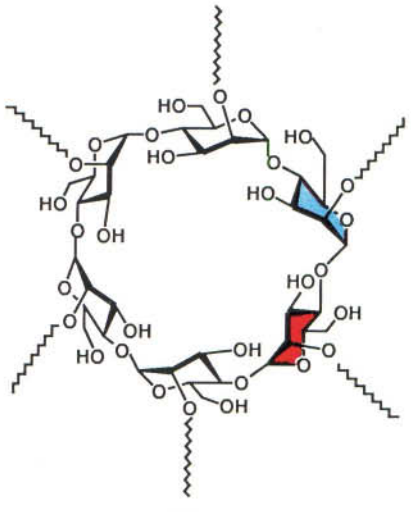


(49)

MBn = 4-MeO-$C_6H_4CH_2$



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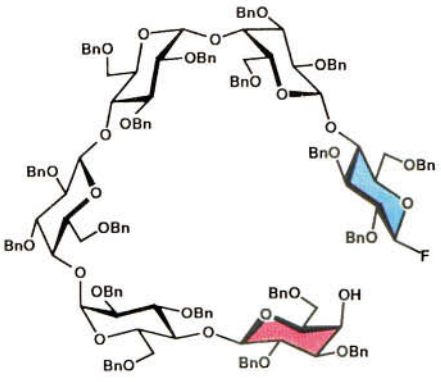

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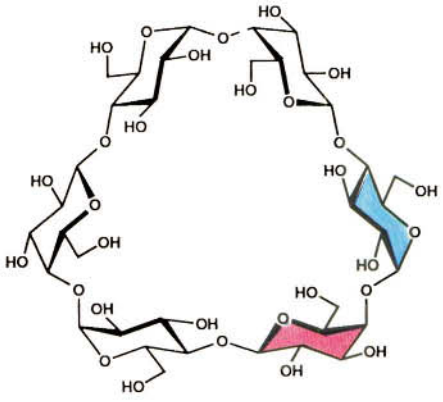

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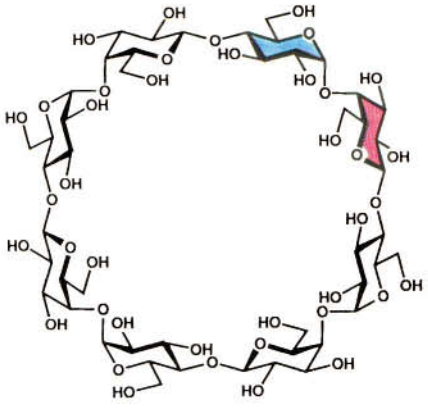

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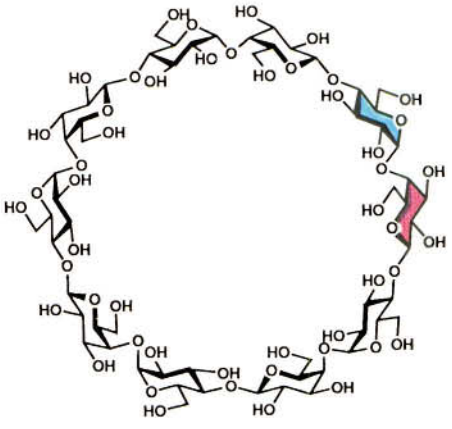

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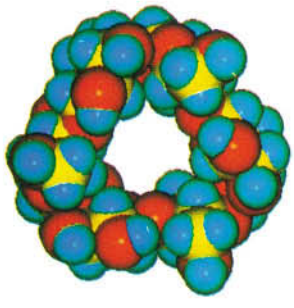

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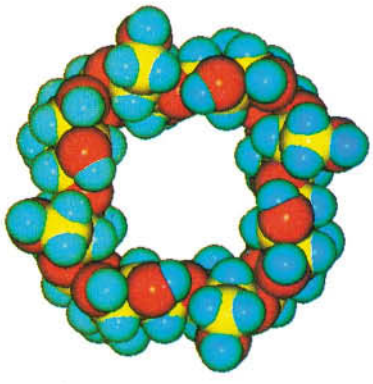

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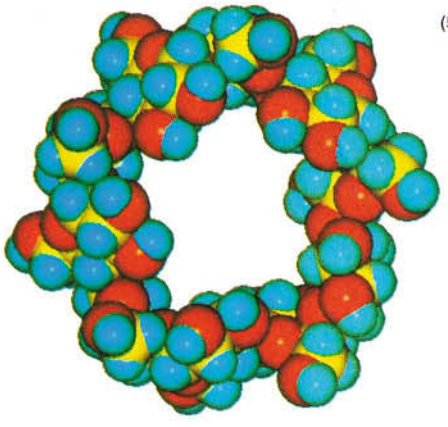

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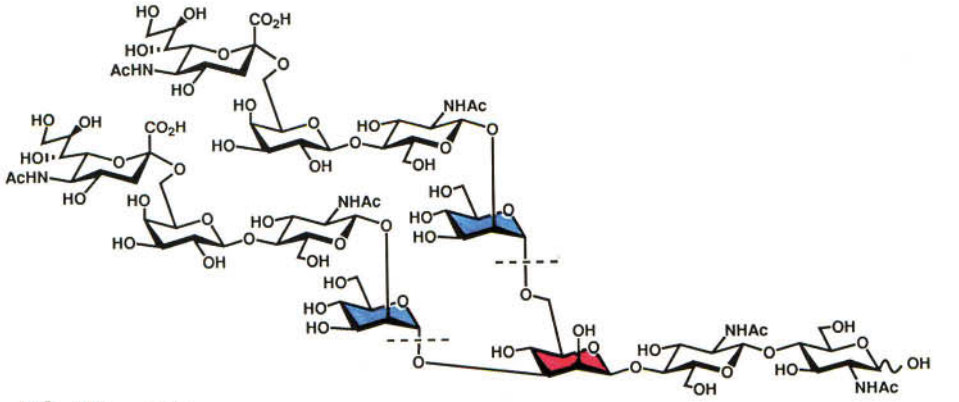

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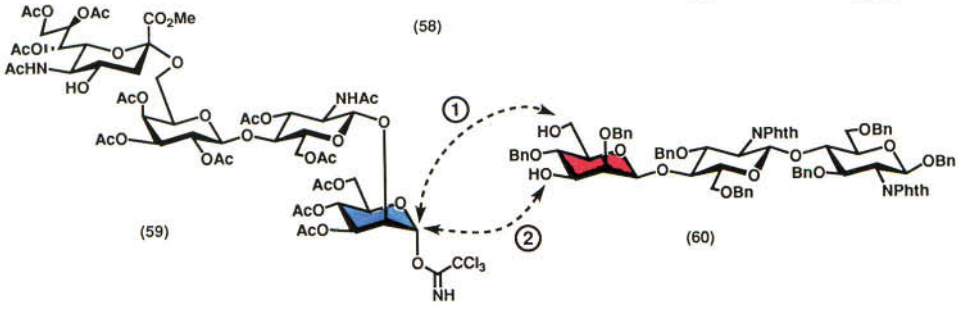
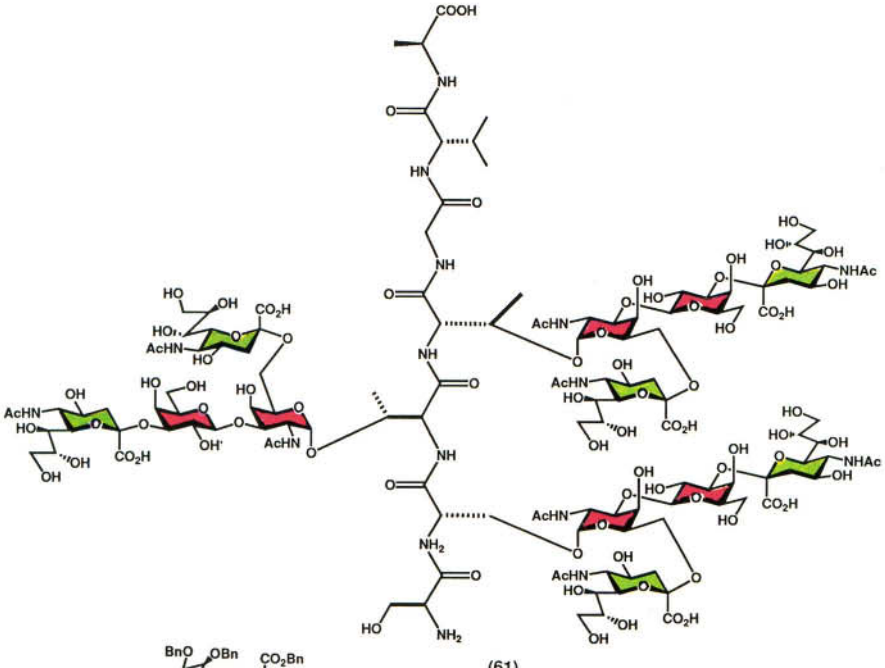


(59) (60)



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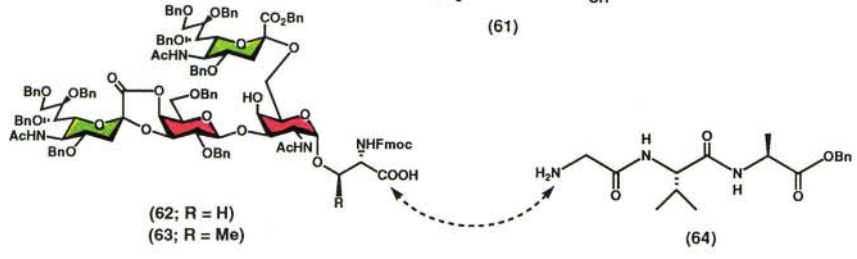


(62; R = H)
(63; R = Me)

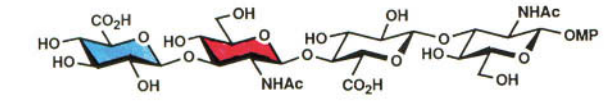
(64)

our synthetic challenge of dissecting the structure and function of biologically active glycoproteins.

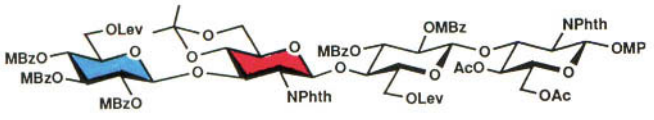
7 Glycosaminoglycans and Proteoglycans

Glycan structures that belong to proteoglycan or glycosaminoglycan (GAG) can be classified into four major groups, (i) hyaluronan, (ii) chondroitin and dermatan sulfate, (iii) heparin and heparan sulfate, and (iv) keratan sulfate. From our synthetic studies on these glycans, three examples are highlighted as follows. A synthetic approach taken by T. Slaghek towards a hyaluronic acid fragment tetrasaccharide (65) was designed by use of a key intermediate (66) that was prepared from each monosaccharide units. Oxidative conversion of (66) into (65) was executed in seven steps in 44% overall yield.[34]

Synthetic studies have also been directed toward the carbo-



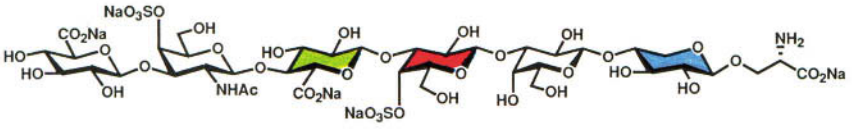
(65)



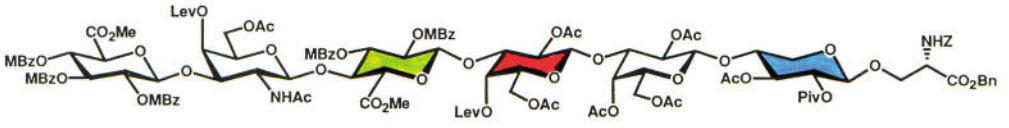
(66)

hydrate sequence (67) linked to a peptide backbone *via* a serine residue in chondroitin sulfate. Towards this goal, a key intermediate (68) was designed for the purpose of the regioselective introduction of the two sulfate groups of (67). Compound (68) was reconstructed from (69), (70), and (71). First two trisaccaride blocks (69) and (70) were coupled in the presence of $(Bu_4N)_2CuBr_4$ and AgOTf in 75% yield. The hexasaccharide that was isolated was further transformed into the key intermediate (68) in eight steps in 32% overall yield. Regioselective introduction of sulfate into (68) and complete deprotection to give (67) was executed[35] by F. Goto in five steps in 36% overall.

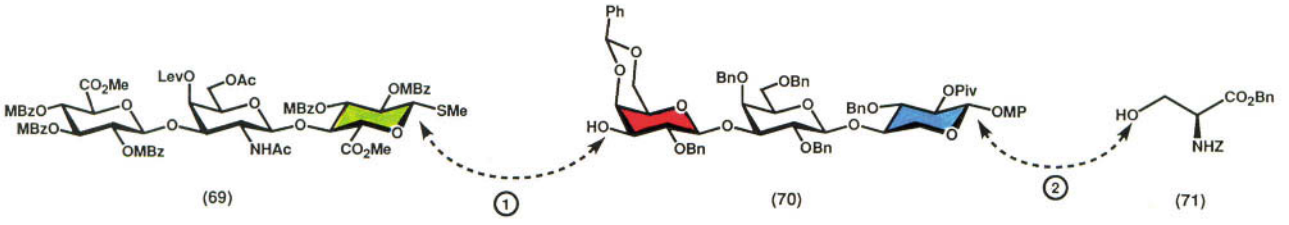
A dermatan sulfate hexasaccharide sequence (72) was shown to have high affinity towards heparin cofactor II.[36] Synthetic experiments directed towards (72) were carried out by designing



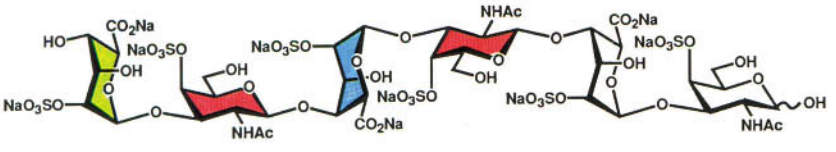
(67)



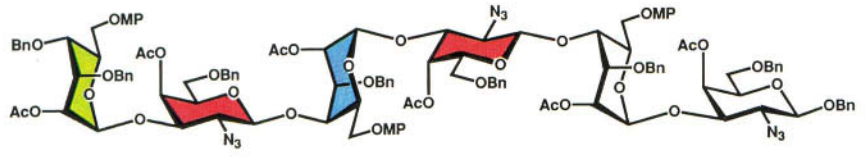
(68)



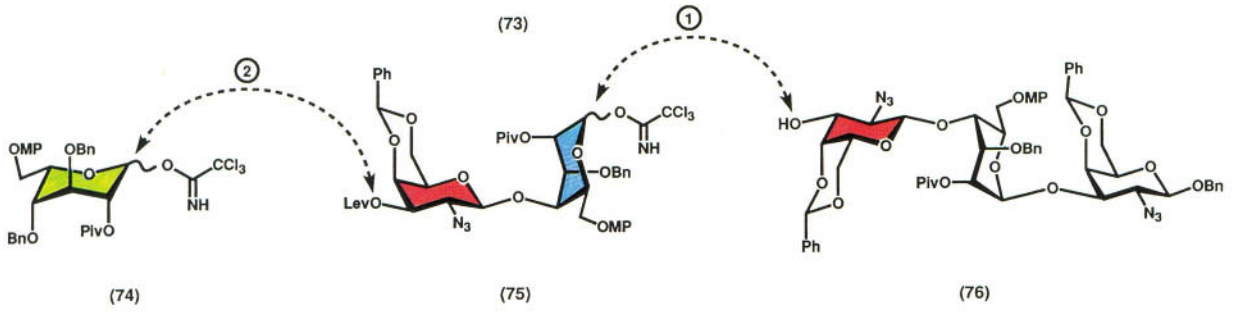
(69) (70) (71)



(72)



(73)



(74) (75) (76)

(73) as a key precursor molecule, which was in turn synthesized from two glycosyl donors (74) and (75), and a glycosyl acceptor (76) Transformation of (73) into (72) was performed in eight steps in 11% overall yield Unambiguous synthesis[37] of (72) afforded supporting evidence for the proposed structure of the biologically active domain of dermatan sulfate

In summary, my co-workers and myself have developed versatile and unambiguous synthetic approaches towards complex structures of carbohydrate sequences that occur in Nature

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