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SYNTHETIC APPROACH TO GLYCAN CHAINS OF A GLYCOPROTEIN AND A PROTEOGLYCAN

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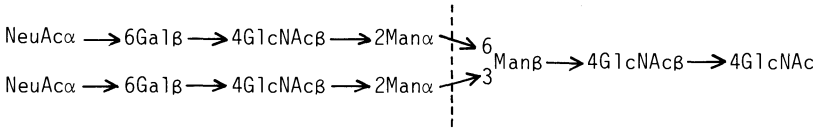
Abstract - Complex glycan chains of glycoproteins and proteoglycans present on cell surfaces are chosen for the target molecules of synthetic experiments. Practical and stereoselective approaches for their synthesis are developed by taking advantage of the presently available synthetic methodologies, such as (i) an efficient method for regioselective protection of hydroxyl groups via stannylation. (ii) an efficient method for glycosidation by use of Ag^{+} and Hg^{2+} salt in the presence of activated molecular sieves. (iii) a rational design of "oligosaccharide glycosyl acceptors and donors" based on retrosynthetic considerations for the purpose of stereoselective convergent type synthesis.

INTRODUCTION

Cell surface glycans are now generally accepted to be the molecular species which carry a variety of biological informations (Ref. 1). Synthetic studies on these complex molecules could not only provide the chemical evidences for or against the proposed stereostructures, but also be directed to supply enough amount of such synthetic glycan chains for the biological studies that may uncover the precise molecular mechanisms of recognition phenomena, such as cell-molecule, cell-cell, and cell-microbe interactions. In this paper we describe our approaches for the synthesis of following three glycans with branched chain structures. The glycans **1** and **2** are covalently linked to Asn residue of a protein through N-glycosidic bond, and classified as a complex and a high mannose type glycan, respectively (Ref. 2). On the other hand , **3** was proposed for the repeating unit of the cell surface proteomannan of Pyricularia oryzae (Ref. 3). A similar, highly branched mannan structure **4** was also proposed for the cell surface glycan of Saccharomyces cerevisiae (Ref. 4).



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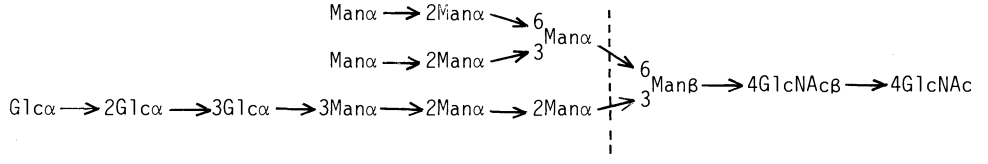

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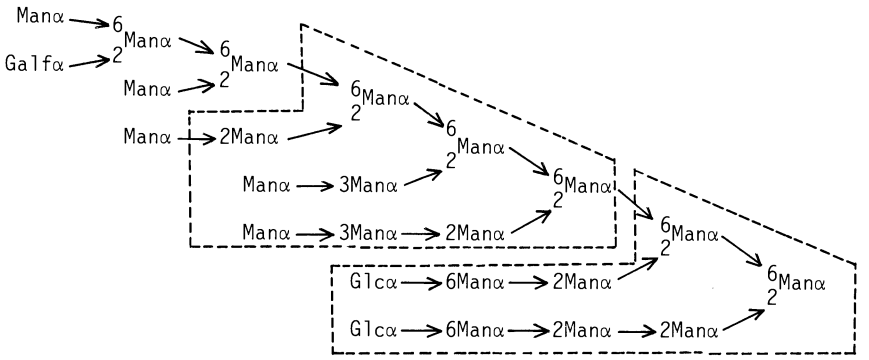

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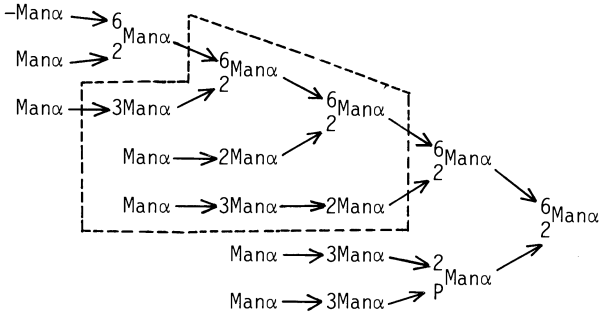

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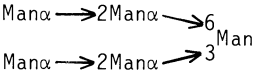
In designing the synthetic plan, following points are to be considered; i) control of the regiochemistry corresponding to the branching pattern of the target glycans, ii) control of the anomeric stereochemistry of the glycosyl residues involved in the glycan chain, iii) design of the synthetic intermediates efficient for a convergent type synthesis.
As we had developed a method for the regioselective enhancement of nucleophilicity of hydroxyl groups through stannylation (Ref. 5), this method was applied for the preparation of the regioselectively protected monosaccharide synthons. Allyl group was mainly used as a non-participating, temporary protective group (Ref. 6) throughout this work.
Control of the interglycosidic stereochemistry is based on the available stereoselective glycosidation methods using mainly $AgOSO_2CF_3$, $HgBr_2$-$Hg(CN)_2$, or $TMSOSO_2CF_3$ as the activating Lewis acid (Ref. 7). A rational design of the key intermediates is of great importance for a convergent type synthesis of glycans to be successful. In this project the target molecules are retrosynthesized at the α-glycosidic linkages, since the oligoglycosyl donors carrying no group capable of neighboring-participation at C-2 of the nonreducing-end-glycosyl residue are expected to meet serious difficulty in providing β-glycosidic stereochemistry.

SYNTHETIC APPROACH TO GLYCAN CHAINS OF A GLYCOPROTEIN

Retrosynthetic considerations of **1** and **2** led us to extract a trihexosyl unit **5**, common unit for both **1** and **2**, by disconnecting these molecules at the dotted lines. In order to perform elongation of the glycan chain, the trihexosyl unit 5 may be regarded as a key glycosyl acceptor. In this context, other remaining oligosaccharides **6**, **7**, and **8** should be designed as key glycosyl donors. In the following, our synthetic experiments directed toward a total synthesis of **1** and **2** based on this hypothesis will be discussed.

Manβ → 4GlcNAcβ → 4GlcNAc **5**

NeuAcα → 6Galβ → 4GlcNAcβ → 2Man **6**



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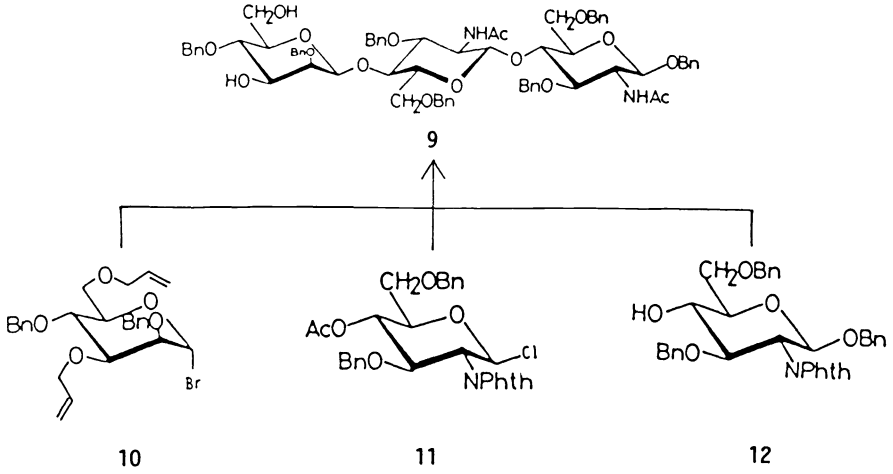
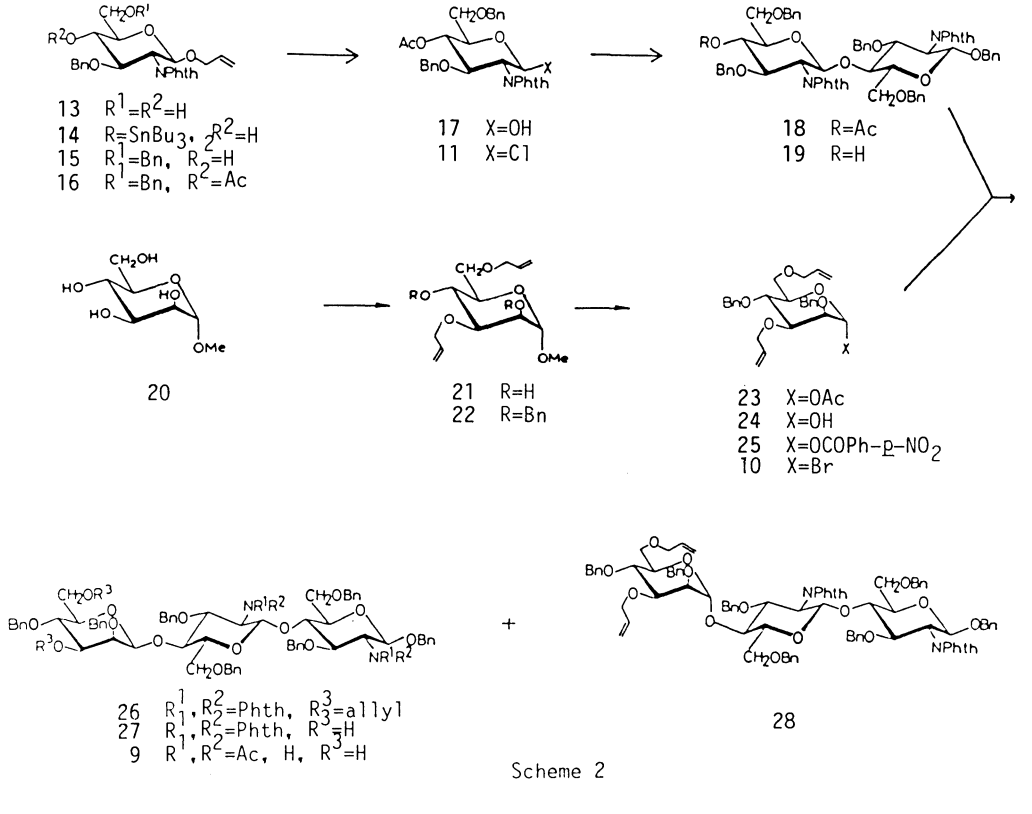
Glcα → 2Glcα → 3Glcα → 3Manα → 2Manα → 2Man **8**



Scheme 1

A TRIHEXOSYL GLYCOSYL ACCEPTOR

A synthetic equivalent of **5** may be designed to be **9** which carries benzyl groups as a permanent protective group. This trihexosyl acceptor **9** was, in turn, retrosynthesized into monohexosyl synthons **10**, **11**, and **12** (Scheme 1). The two monohexosyl donors **10** and **11** were designed to be able to function as a β-D-Man and β-D-GlcNAc donors, respectively, according to the results of our preliminary experiments and of the elegant works reported by Paulsen et al (Ref. 8) and Lemieux et al (Ref. 9). Both monosaccharide synthons were synthesized by taking advantage of a regioselective alkylation method via trialkylstannylation (Ref. 10) as shown in Scheme 2.



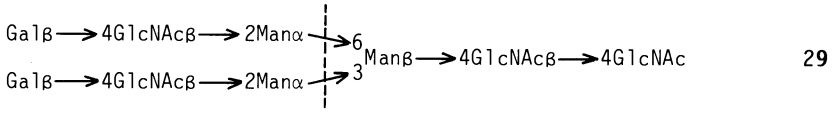
Scheme 2

Tributylstannylation of **13** to **14**, and alkylation of **14** with benzyl bromide for 2 days at 90° in the presence of $Bu_4N^+Br^-$ (Ref. 11) gave a 76% yield of the dibenzyl ether **15**. Acetylation of **15** to give **16** and deallylation of **16** with $PdCl_2$ in aq AcOH-AcONa for 2h at 70° (Ref. 12) afforded **17** in 83% yield from **15**. Treatment of **17** with $SOCl_2$ in the presence of catalytic amounts of DMF in CH_2Cl_2 for 2h at 20° gave a quantitative yield of **11**. Glycosidation of **12** (Ref. 13) with **11** in the presence of $AgOSO_2CF_3$ and powdered molecular sieves 4A for 16h at 20° afforded a 62% yield of the chitobiosyl derivative **18**. Deacetylation of **18** in boiling HCl-H_2O-acetone (Ref. 9) for 4 days gave an 82% yield of **19**.

Having the dihexosyl acceptor **19** prepared, we now turned to the synthesis of β-D-mannopyranosyl donor **10**. Commercially available methyl α-D-mannopyranoside **20** was first tributylstannylated and then alkylated with allyl bromide to give a 71% yield of **21** in a regioselective way (Ref. 14). Benzylation of **21** to give **22** and subsequent acetolysis of **22** afforded **23** in 75% yield from **21** (Ref. 15). Saponification of the acetate **23** gave hemiacetal **24** in 85% yield, which was acylated with p-nitrobenzoyl chloride to give an 82% yield of **25** together with a 17% yield of the β-anomer of **25**. Treatment of **25** with HBr in CH_2Cl_2 at 0° gave the desired unstable bromide **10**.

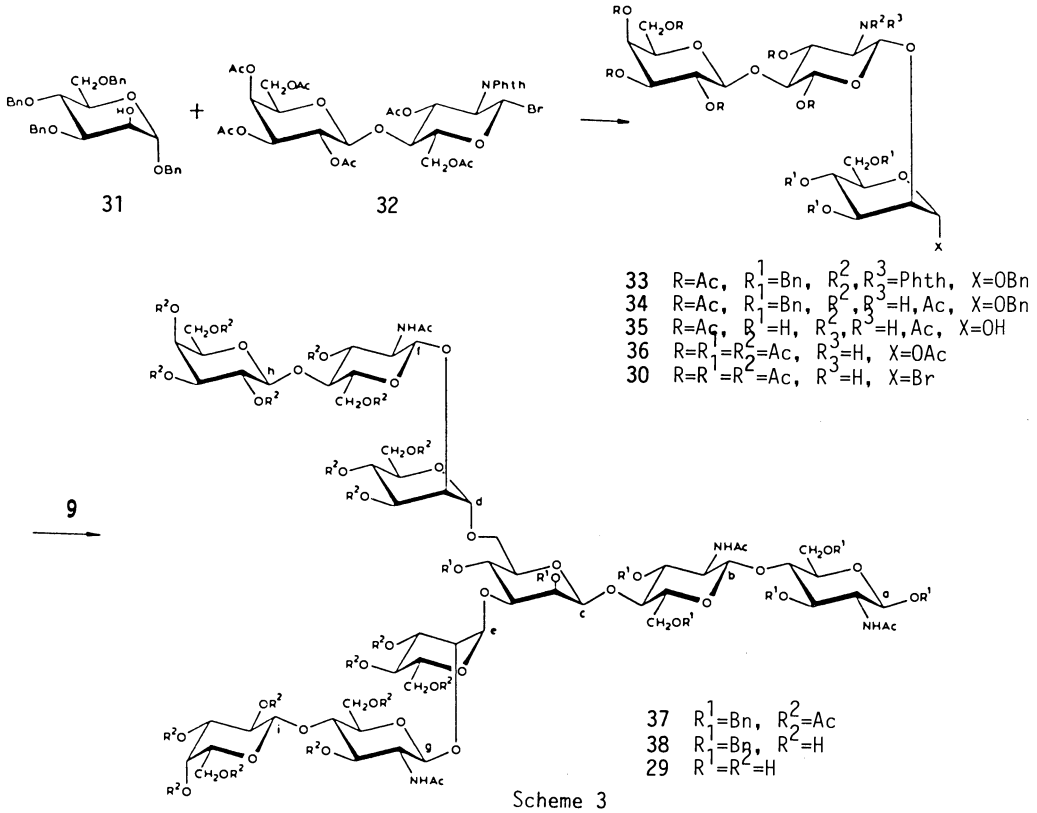
Glycosidation of the dihexosyl acceptor **19** with **10** in the presence of Ag silicate (Ref. 8) and powdered molecular sieves 4A in CH_2Cl_2 afforded a 40% yield of the β-anomer **26** along with a 36% yield of the α-anomer **28**. Judging from the difficulty in obtaining β-D-Man configuration from the glycosidation with the aglycon of low nucleophilicity such as **19**, the β:α ratio of 10:9 in this case seems to be satisfactory at the present moment. $PdCl_2$ catalysed deallylation of **26** gave a 58% yield of **27**, which was treated with (i) 1:1 $BuNH_2$-MeOH for 8 days at 90° (ii) Ac_2O-pyridine, (iii) NaOMe-MeOH, to give a 90% yield of the trihexosyl acceptor **9** (Ref. 16).

A NONAHEXOSYL UNIT OF A COMPLEX TYPE OF GLYCAN CHAINS



29

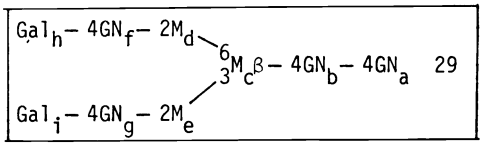
Having the key trihexosyl acceptor **9** synthesized, asialo oligosaccharide **29** of the glycan **1** was now chosen for the target in our synthetic experiments. The purpose is to examine the efficiency and the stereochemical outcome of a convergent type approach for the synthesis of **29** as a model of **1**. Therefore, we designed a trihexosyl donor **30** as the key intermediate, and the synthetic route for **29** via **30** was described in Scheme 3.



Scheme 3

Glycosidation of **31** (Ref. 17) with the lactosaminyl donor **32** (Ref. 18) in the presence of $AgOSO_2CF_3$-molecular sieves 4A in $Cl(CH_2)_2Cl$ afforded a 94% yield of **33**. Successive treatment of **33** with i) NaOMe-MeOH, ii) reflux in $BuNH_2$-MeOH (Ref. 19), iii) Ac_2O-pyridine, afforded a 91% yield of **34**. Catalytic hydrogenolysis of **34** in the presence of 10% Pd-C in AcOH at 80° to give **35**, and acetylation of **35**, afforded an 84% yield of **36**. Treatment of **36** with HBr in $AcOH-CH_2Cl_2$ gave a quantitative yield of **30**.

Glycosidation of **9** with 6 molar equivalents of **30** in the presence of $AgOSO_2CF_3$-powdered molecular sieves 4A in 3:1 $Cl(CH_2)_2Cl$-toluene, and gel-chromatography of the product on Toyopearl HW 40 in 1:1 CH_2Cl_2-MeOH afforded a 59% yield of the protected nonasaccharide **37**. Deacetylation of **37** with NaOMe-MeOH to give **38**, and hydrogenolysis of **38** with 10% Pd-C in AcOH at 80° afforded the target nonasaccharide **29**. The stereochemistry at the two anomeric carbon atoms, C-1d and C-1e, introduced by the last glycosidation step was each assigned to be α-D by 400 MHz ^{1}H nmr data of **29** (Fig 1). The spectrum contained two singlets, at δ 5.139 and 4.926 for H-1e and H-1d, respectively, in addition to a singlet for H-1c at δ 4.766, in good agreement with the data for both the natural (Ref. 20) and synthetic samples of similar structures (Ref. 21). Other aspects of the ^{1}H-nmr spectrum of synthetic **29** (Ref. 22) were also in good agreement wiith the target structure **29**.



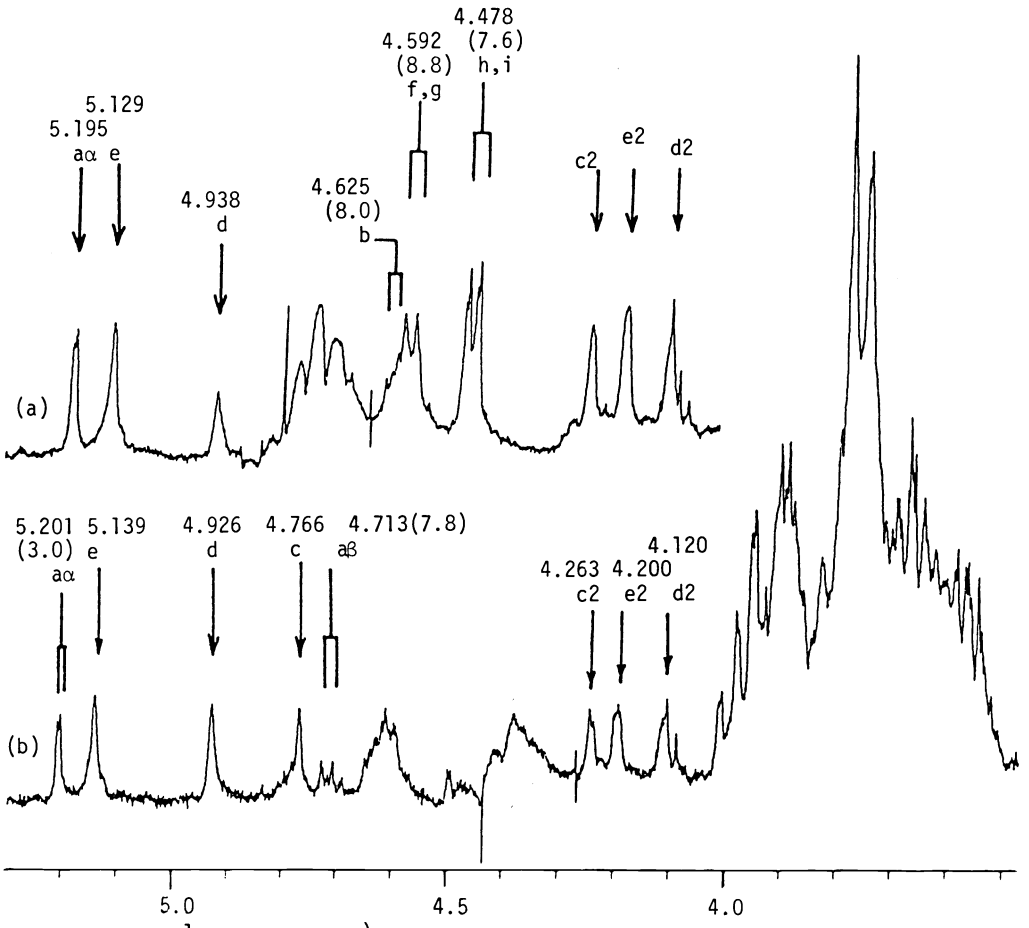


Fig.1 400 MHz ^{1}H-nmr spectra[a] of 29 taken at (a) 20°, (b) 60°.

a) Nmr spectra were recorded, for solutions in D_2O, with a JNM-GX400, or JNM-FX100 FT spectrometer. The values of δ_H are expressed in p.p.m. downward from the internal standard, sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate. The values of δ_C are expressed in p.p.m. downward from tetramethylsilane, referenced indirectly with an internal standard of 1,4-dioxane(δ_C67.40) through Fig.1-9.

A SIALYL TETRASACCHARIDE OF A COMPLEX TYPE OF GLYCAN CHAINS

As our experiments on the synthesis of asialononasaccharide **29** of a complex type of glycans could be performed successfully, we now turned our efforts to the synthesis of sialooligosaccharides of the glycoprotein glycan **1**. The target for this experiment was a sialyl tetrasaccharide **6**, which corresponds to the non-reducing end tetrasaccharide structure of **1**.

We designed the trihexosyl acceptor **43** as the key intermediate, expecting that the primary hydroxyl group of **43** should be selectively glycosylated with the readily available glycosyl donor **45** (Ref. 23).

The trihexosyl derivative **33** described in Scheme 3 was converted, via **40**, into the isopropylidene derivatives **41** and **42** in 69 and 11% yield, respectively, in 3 steps (i) NaOMe-MeOH, (ii) $Me_2C(OMe)_2$-TsOH in DMF, 15hr at 20°, (iii) Ac_2-pyridine. Deisopropylidenation of

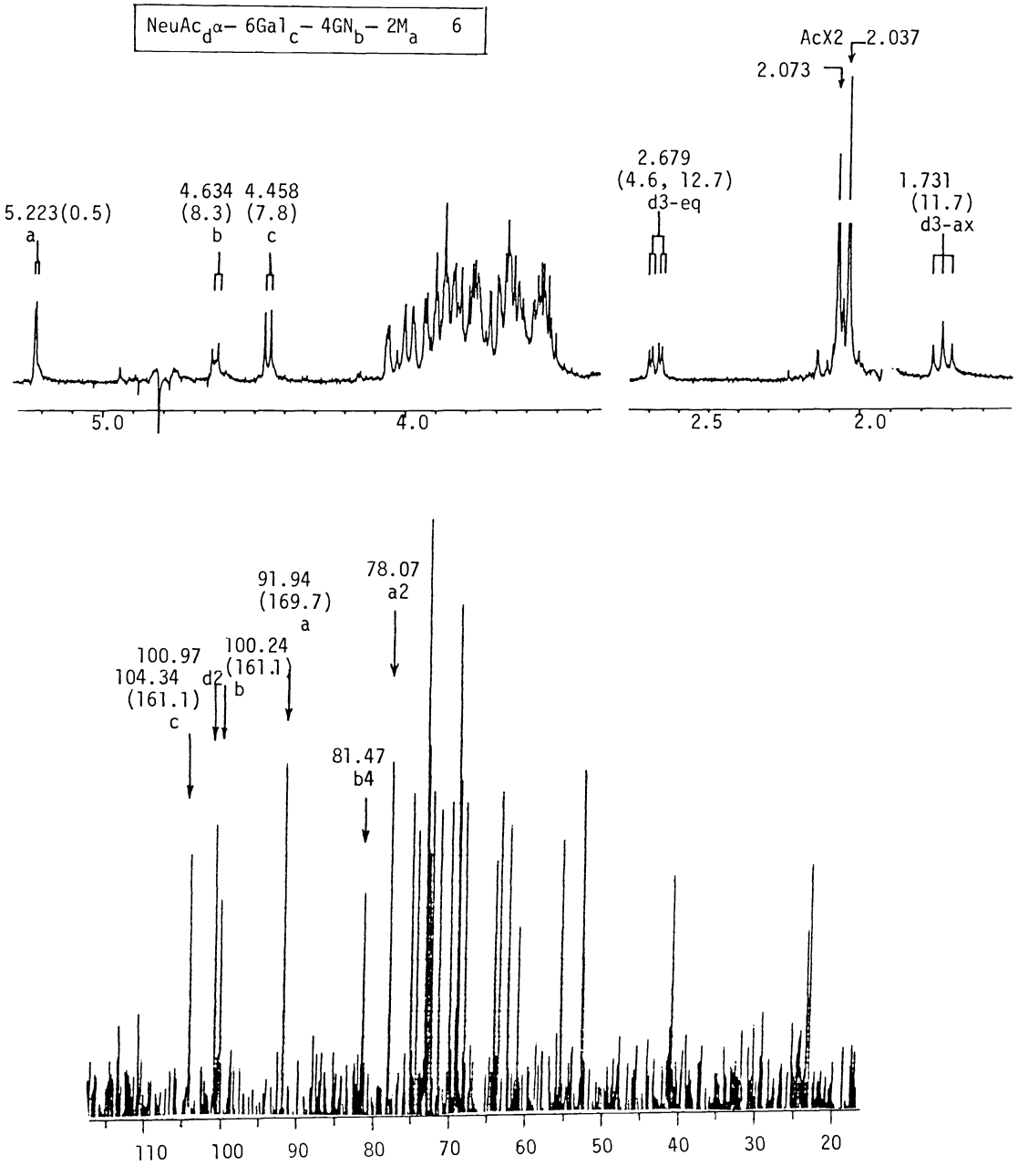


Fig.2 400 MHz ^{1}H-nmr$_3$(at 20°)$_1$and 100.7 MHz ^{13}C-nmr (at 20°) spectra of 6. The values of $^3J_{HH}$ and $^1J_{CH}$ are expressed in Hz in the parenthesis.

the major product **41** in AcOH-MeOH (1:1) afforded an 81% yield of the diol **43**. The isomeric diol **44** was obtained in a similar way from **42**. The structures of **43** and **44** were assigned according to the observation of the different reactivity of the hydroxyl groups. Thus, treatment of **43** with large excess of trityl chloride in pyridine for 21h at 20° afforded the 6-O-trityl derivative, while under the same condition **44** gave no tritylated product. Glycosidation of the trihexosyl acceptor **43** with the glycosyl donor **45** in the presence of 1:1:4 $HgBr_2$:$Hg(CN)_2$-powdered molecular sieves 4A in $Cl(CH_2)_2Cl$ for 4 days at 20° afforded a mixture of the anomers, in agreement with the low stereoselectivity reported previously (Ref. 24) for the glycosidation using the same donor. Separation over silicagel afforded **46** and **49** in 34 and 30% yields, respectively. Compounds **46** and **49** were separately submitted to the following deprotection steps [(i) NaOMe-MeOH, (ii) NaOH in 1:1 MeOH-THF, (iii) H_2, 10% Pd-C in 9:1 EtOH-H_2O at 60°, (iv) Sephadex G-25] to give the target tetrasaccharide **6** (80%) and the stereoisomer **39** (89%), respectively , via the compounds **47** and **48**, and via the compounds **50** and **51**. The anomeric configuration at C-2d of the tetrasaccharide **6** and **39** (Ref. 25) was assigned to be 2α and 2β, respectvely, by comparing the ^1H nmr data of synthetic samples (Fig 2 and 3) with the reported data (Ref. 26).

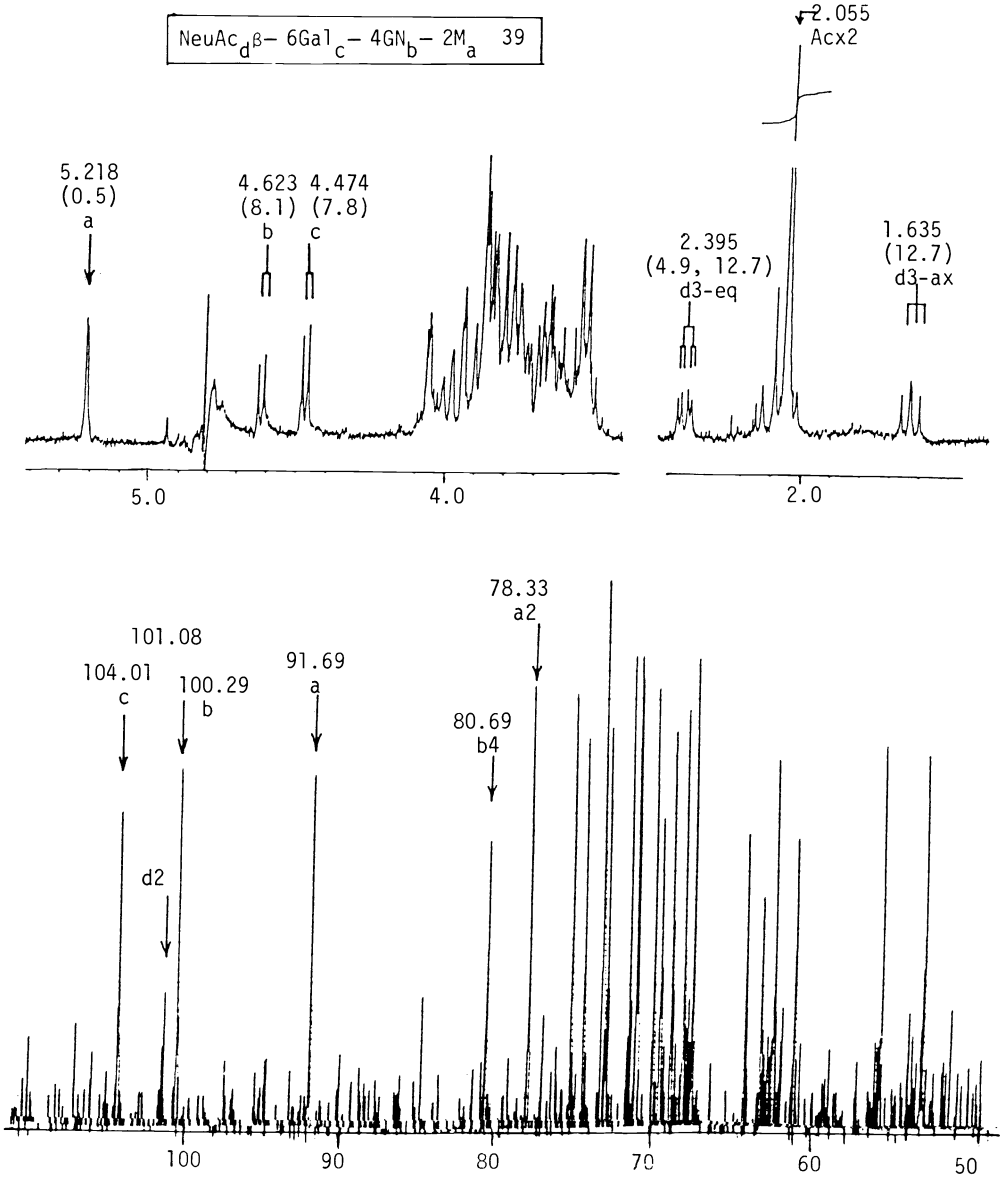
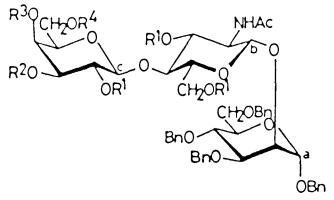
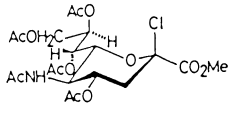


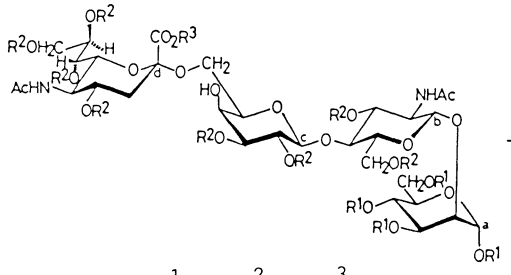
Fig. 3 400 MHz ^1H-nmr (at 20°) and 100.7 MHz ^{13}C-nmr (at 20°) spectra of 39

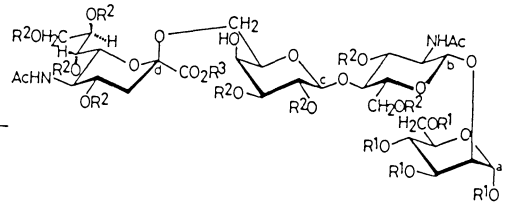




40 $R^1=R^2=R^3=R^4=H$
41 $R^1=R^2=Ac$, $R^3,R^4=CMe_2$
42 $R^1=R^4=Ac$, $R^2,R^3=CMe_2$
43 $R^1=R^2=Ac$, $R^3=R^4=H$
44 $R^1=R^4=Ac$, $R^2=R^3=H$

45





46 $R^1=Bn$, $R^2=Ac$, $R^3=Me$
47 $R^1=Bn$, $R^2=H$, $R^3=Me$
48 $R^1=Bn$, $R^2=R^3=H$
6 $R^1=R^2=R^3=H$

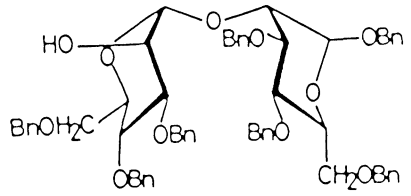
49 $R^1=Bn$, $R^2=Ac$, $R^3=Me$
50 $R^1=Bn$, $R^2=H$, $R^3=Me$
51 $R^1=Bn$, $R^2=R^3=H$
39 $R^1=R^2=R^3=H$

$Glc\alpha \longrightarrow 2Glc\alpha \longrightarrow 3Glc\alpha \longrightarrow 3Man\alpha \longrightarrow 2Man\alpha \longrightarrow 2Man$

8

$Glc\alpha \longrightarrow 2Glc\alpha \longrightarrow 3Glc\alpha \longrightarrow 3Man\alpha \longrightarrow X$

52



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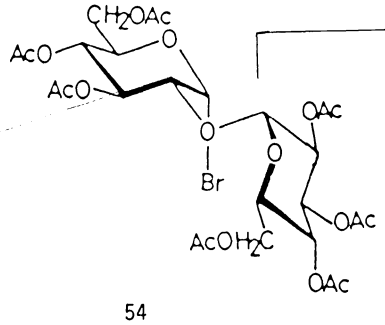


54

$H \longrightarrow 3Glc\alpha \longrightarrow 3Man\alpha \longrightarrow OBn$

55

Scheme 5

A LINEAR HEXAHEXOSYL UNIT OF A HIGH MANNOSE TYPE GLYCAN

In 1978, Li et al (Ref. 27) and in 1979, Liu et al. (Ref. 28) proposed the structure of the high-mannose type glycan **2**. In 1980, Ugalde et al. (Ref. 29) assigned the stereochemistry of the triglucopyranosyl part of **2** as Glc1α – 2Glc1α – 3Glc1α – 3 from the inhibition experiments with glucosidases. In order to provide synthetic support for the proposed structure **2**, we have studied an approach to a total synthesis of **2** with high regio- and stereo-control. According to retrosynthetic considerations, **2** was divided into three parts, namely **5**, **7**, and **8**. We now discuss a synthetic approach to a linear hexasaccharide **8**. Retrosynthetic considerations indicated that the target structure **8** might be reconstructed from the disaccharide synthons **53**, **54** and **55** (Scheme 5). As **53** (Ref. 17) and **54** (Ref. 30) are already available, we first describe the synthesis of **63**, which corresponds suitably protected disaccharide unit **55**.

A mixture of allyl 3-O-allyl-α- and -β-D-glucopyranoside **56**, readily obtainable from 3-O-allyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose, was benzylated, to give **57**, and deallylation of **57** with $PdCl_2$ in aq AcOH-AcONa afforded a 61% yield of the tribenzyl ether **58** as a mixture of the α-and β-anomer in the ratio of 1:1. Acetylation of **58**, to give **59** and treatment of **59** with HCl in $Cl(CH_2)_2Cl$ gave a 70% yield of **60**. Glycosidation of **61** (Ref. 31) with **60** in the presence of $AgOSO_2CF_3$ (Ref. 32) -powdered molecular sieves 4A afforded a 72% yield of a mixture of **62** and **64** in the ratio of 3:1. In the presence of $Hg(CN)_2$-$HgBr_2$-powdered molecular sieves 4A (Ref. 33), however, the same glycosidation gave a 79% yield of the α-anomer **62**. Deacetylation of **62** afforded **63**, which was glycosylated with the kojibiosyl donor **54** in the presence of $AgOSO_2CF_3$-powdered molecular sieves 4A to give a 59% yield of the protected tetrasaccharide **65**. Debenzylation of **65** by catalytic hydrogen transfer (Ref. 34) with 10% Pd-C, HCOOH-MeOH gave **66**, and acetylation of **66** afforded a 79% yield of the peracetylated tetrasaccharide **67**. Deacetylation of **66** with NaOMe-MeOH gave free tetrasaccharide **68** (Fig 4 for 400 MHz ^{1}H NMR). Treatment of **67** with HBr-AcOH-CH_2Cl_2 gave the

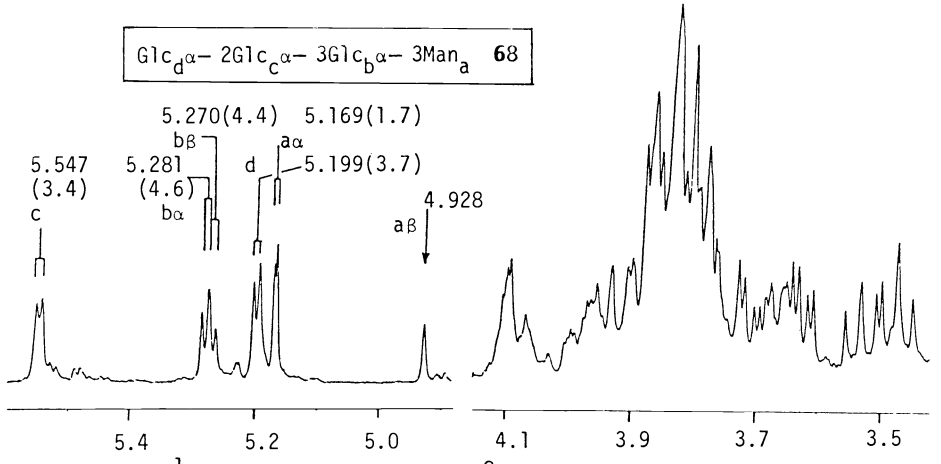


Fig.4 400MHz ^{1}H-nmr spectra of 68 at 20°.

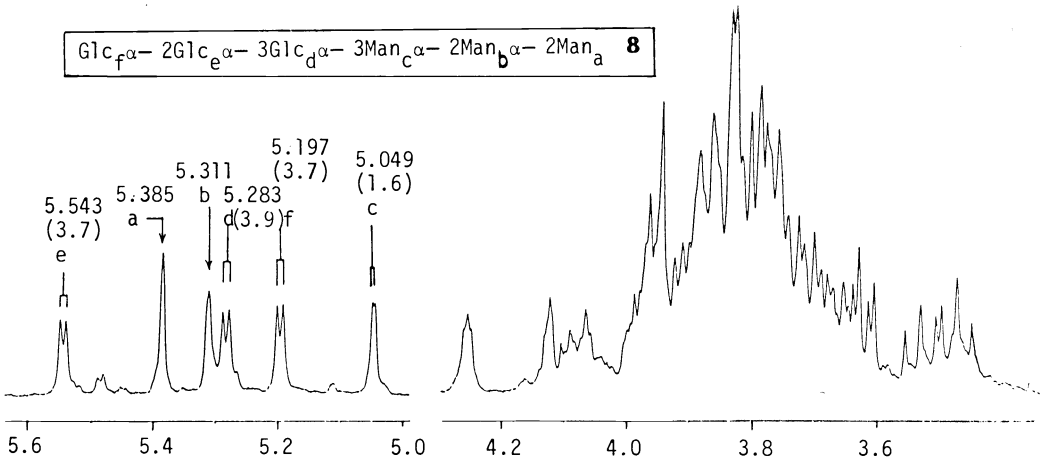
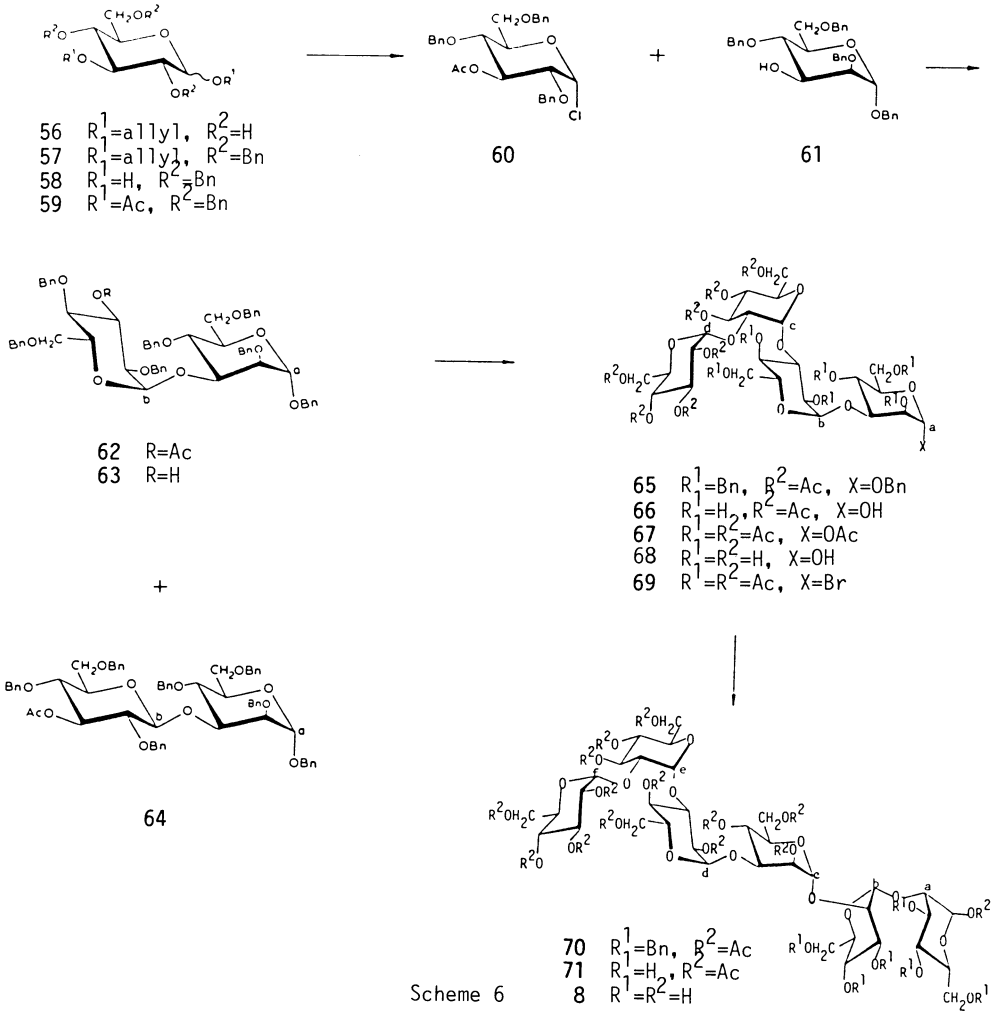


Fig. 5 400MHz ^{1}H-nmr spectra of 8 at 20°.

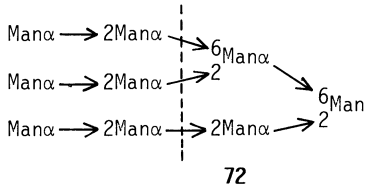
tetrasaccharide donor **69** which corresponds to the synthon **52**. The reaction of **69** with the mannobiosyl acceptor **53** in the presence of $AgOSO_2CF_3$-powdered molecular sieves 4A afforded a 56% yield of the protected hexasaccharide **70**, which was subjected to debenzylation and deacetylation to give the target, linear hexasaccharide **8** (Fig 5) (Ref. 35). Further experiments directed toward a reconstruction of the glycans **1** and **3** by use of the oligosaccharide intermediates derived from **5**, **6**, **7** and **8** are now under investigation.



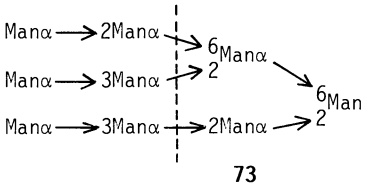
Scheme 6

SYNTHETIC APPROACH TO MICROBIAL CELL SURFACE MANNAN: BRANCHING NONAHEXOSYL UNIT MODELS

Microbial cell surface glycans play an important role, for example, in the interaction between higher plants and pathogenic microbes. In certain plants, such microbial glycans are known to induce defending responses within the plant tissues (Ref. 36). Knowledge of the molecular mechanisms for such biological interactions involving carbohydrates will certainly pave the way for new approaches to the plant protection. From this view point, we started our project concerning the synthetic studies of microbial cell wall glycans (Ref. 17). We describe here our experiments for the synthesis of nonahexosyl units **72**, **73** and **87**, which represent a part structure of microbial mannan structures **3** and **4**.

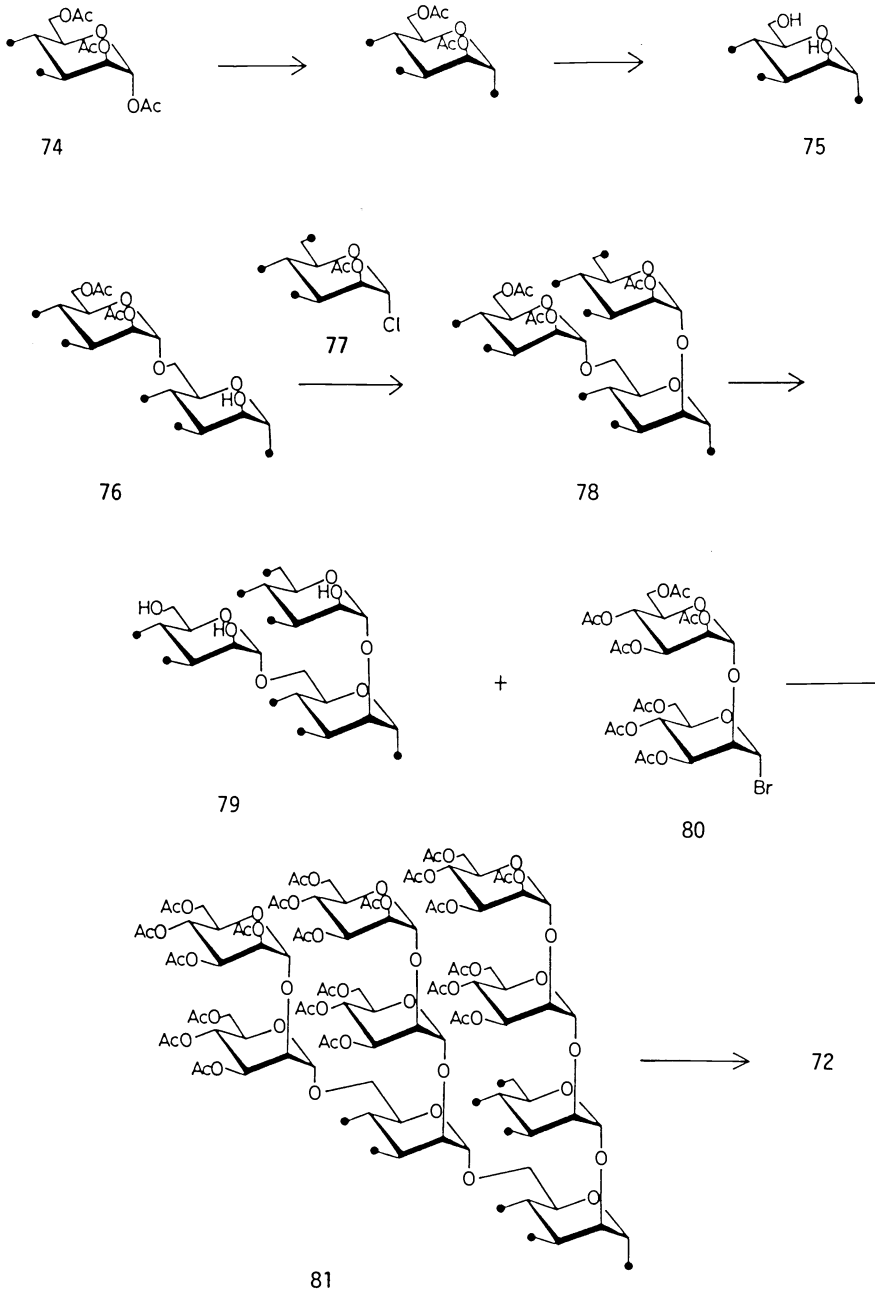


72



73

As for the synthetic plan of M9 models 72 and 73, we disconnected these glycans by dotted lines and designed two dihexosyl glycosyl donors **80** and **85** as well as a trihexosyl glycosyl acceptor **79**, based on the retrosynthetic considerations. The reconstruction of **72** and **73** was performed by using these key intermediates as follows. Treatment of the triacetate **74** (Ref. 37) with TMS triflate (Ref. 38) and benzyl alcohol, and deacetylation of the product afforded diol **75** (78%). Glycosidation of **75** at the primary hydroxyl group again with **74** in the presence of TMS triflate gave **76** and the undesired trisaccharide in 59 and 10% yields, respectively. Glycosidation of **76** with **77** (Ref. 37 and 39) in the presence of $AgOSO_2CF_3$ afforded a 66% yield of **78** which was deacylated to give the key glycosyl acceptor **79**. Even though the mannobiosyl donor **80** was reported to give α-stereochemistry in the reaction with C-2-OH of the mannopyranosyl residue (Ref. 17), it is uncertain that the same donor **80** can also give α-stereochemistry with a primary hydroxyl group at C-6 of **79**. The result of a partial glycosidation of **79** at the primary hydroxyl group which will be described later on clearly demonstrated that the stereochemical outcome in this case was also α-configuration.



Scheme 7 (●— = OBn)

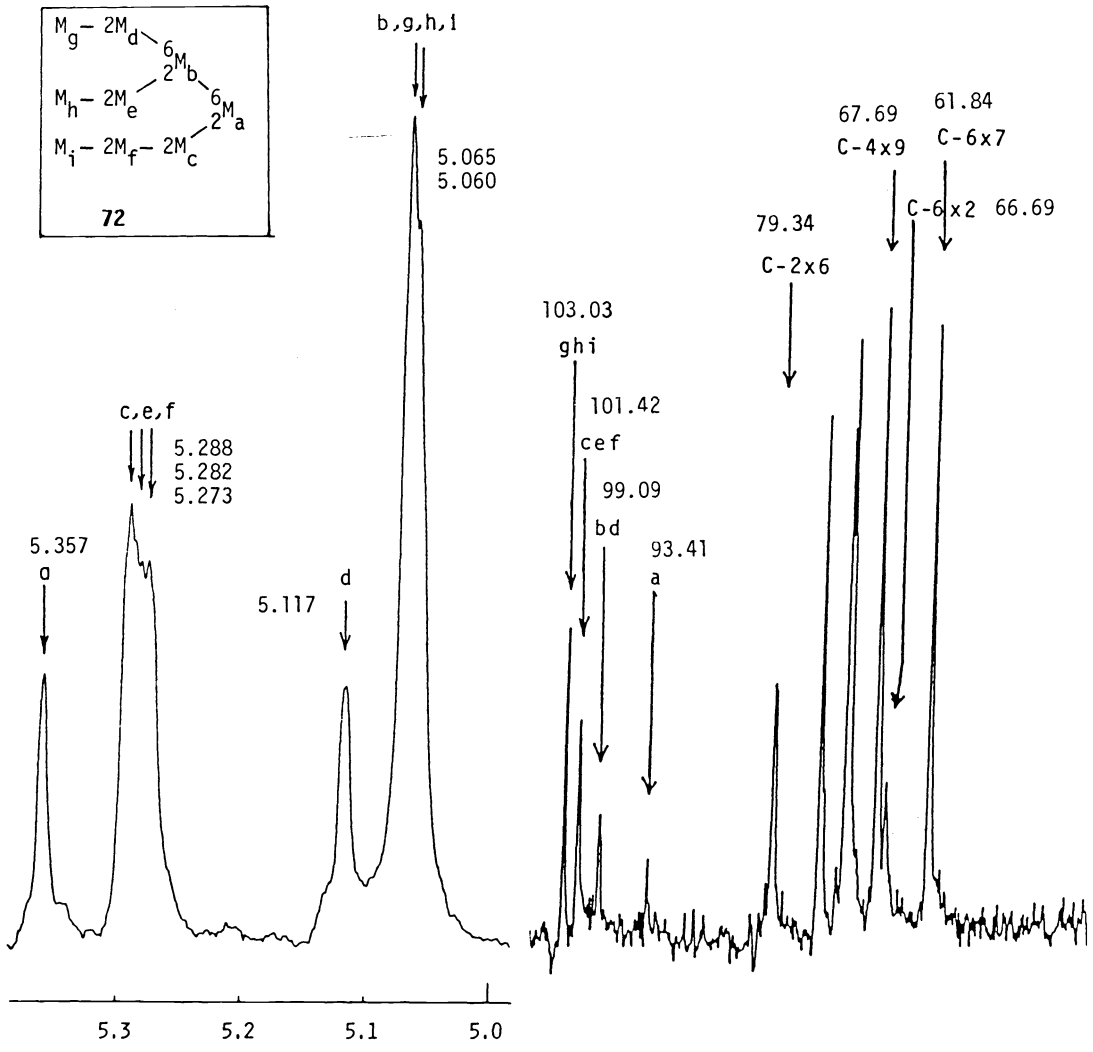


Fig.6 400MHz ^{1}H-nmr (52°, left) and 22.5MHz ^{13}C-nmr (20°,right) spectra of 72.

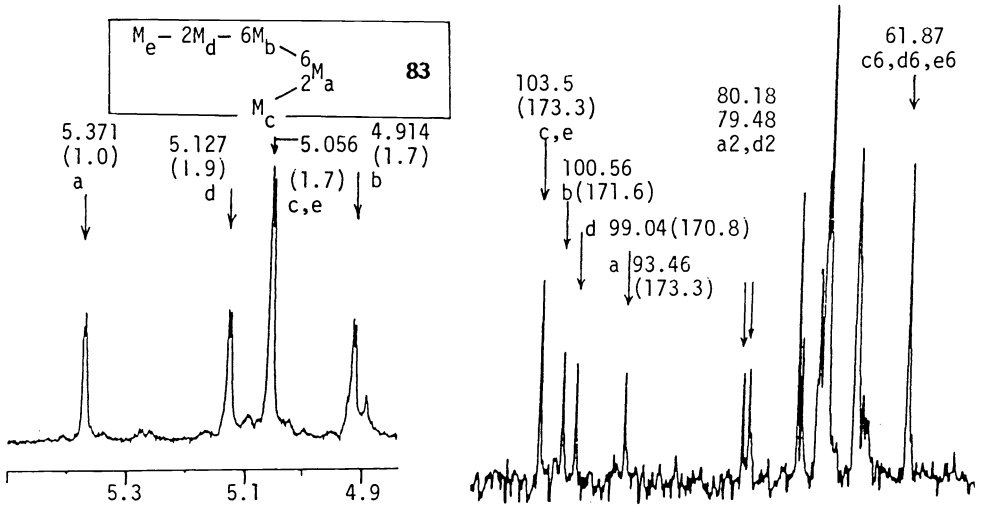
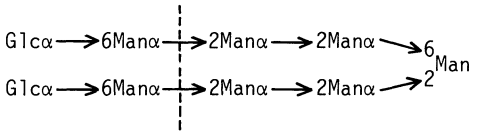


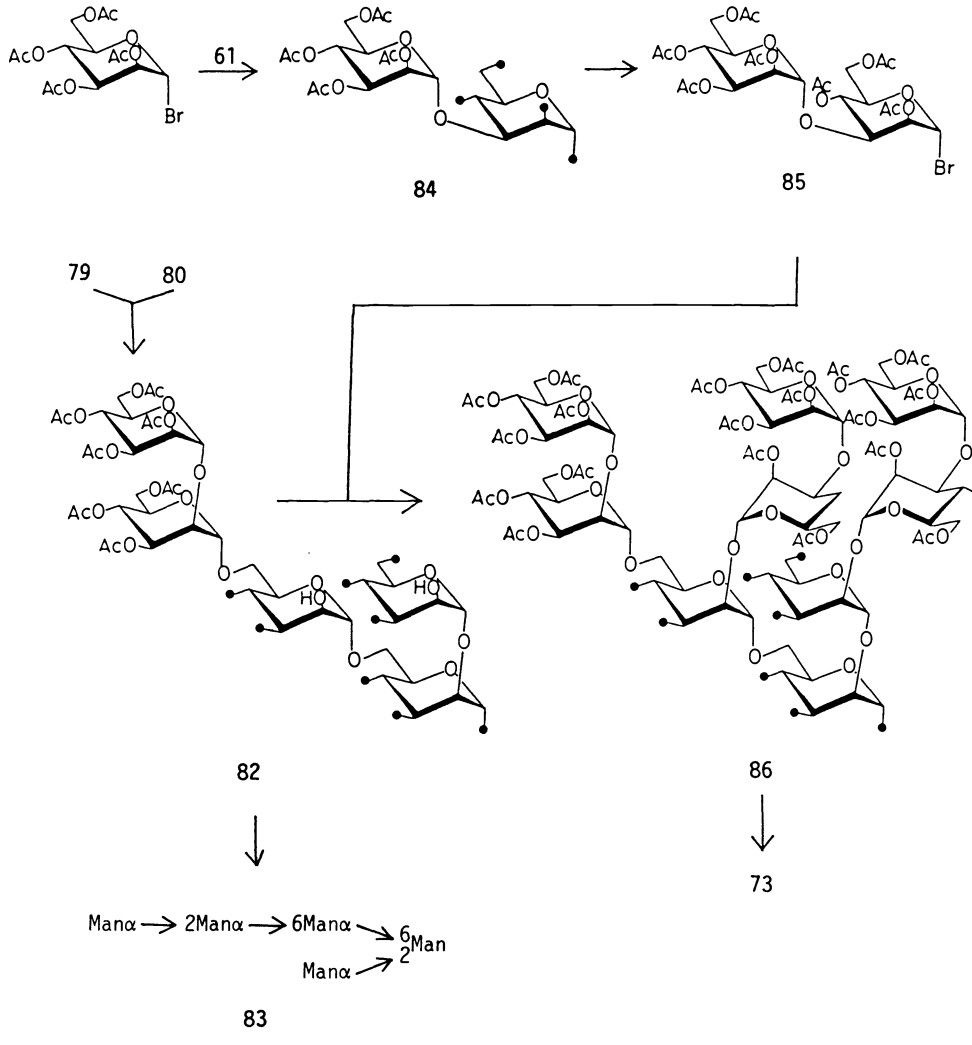
Fig.7 400MHz ^{1}H-nmr (60°,left) and 22.5MHz ^{13}C-nmr (20°,right) spectra of 83.

Glycosidation of **79** with an excess of **80** in benzene in the presence of $AgOSO_3CF_3$ and purification of the product by gel-chromatography gave a 56% yield of protected nonamannoside **81.** Deacetylation and catalytic hydrogenolysis afforded a target glycan **72.** ^1H And ^{13}C nmr spectrum was shown in Fig.6.
A partial glycosidation of **79** at the primary hydroxyl with **80** is required for the synthesis of **73** and a 57% yield of the protected pentamannoside **82** was obtained which was deprotected in a usual way to give free pentasaccharide **83.** The structure of **83** was assigned by comparing ^1H- and ^{13}C-nmr data of **83** (Fig 7) with the reported one (Ref. 40). Now we need a mannobiosyl donor **85** for further elongation of the glycan chain, which was readily prepared from **61** via **84.** The glycosidation of **82** with **85** in the presence of $AgOSO_2CF_3$ in benzene afforded a 77% yield of **86** which was deprotected in a usual way to give the target mannononaoside **73** (Fig 8).



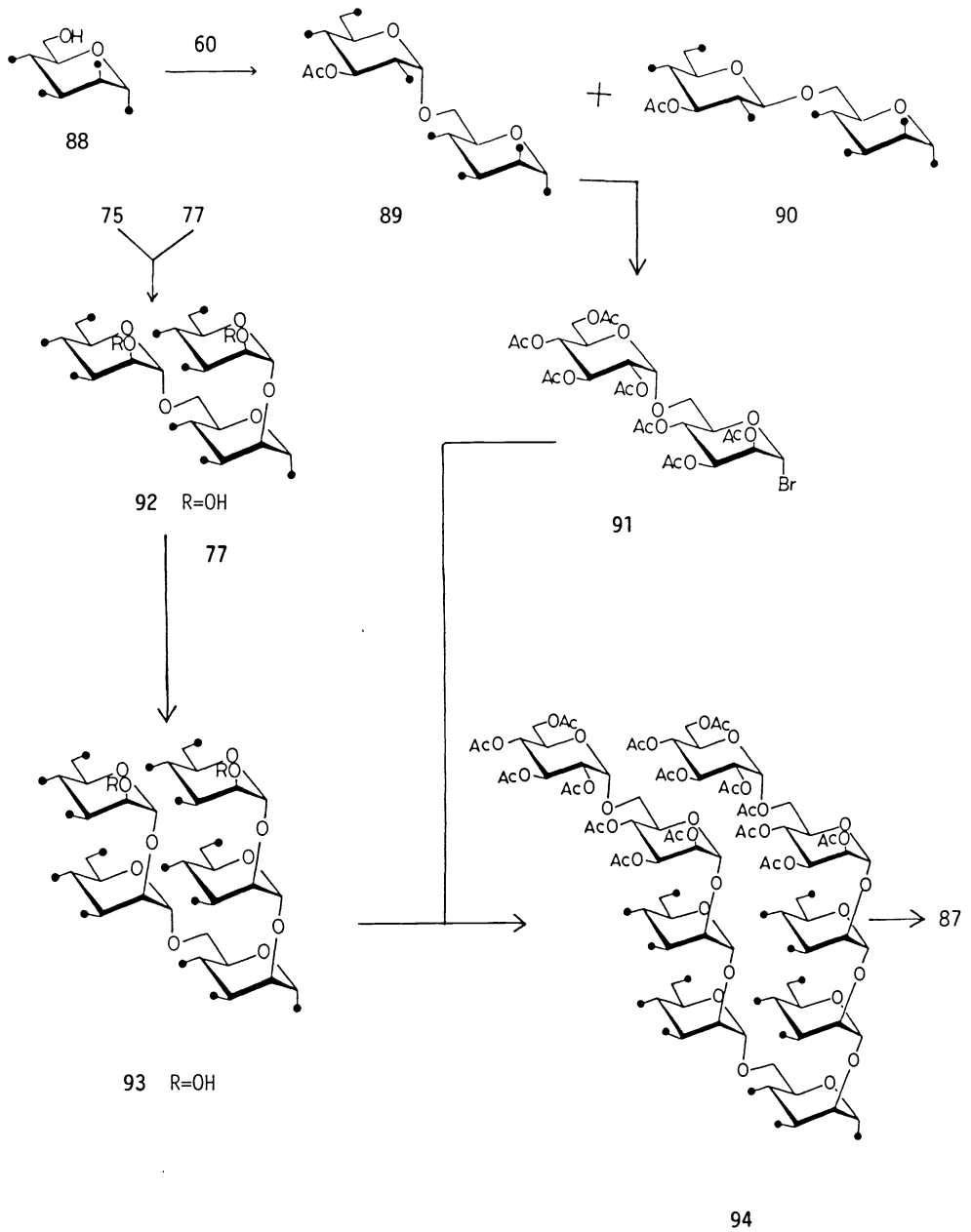
87

Now we turned to the G2M7 model **87.** The target structure **87** was disconnected at the dotted line to give a glycosyl donor **91** and a glycosyl acceptor **93.** The two key intermediates **91** and 93 for the reconstruction of **87** were prepared as follows.

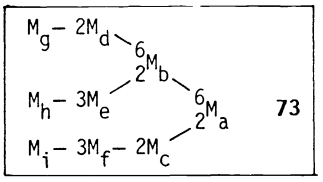


Scheme 8 (●— = OBn)

Glycosidation of **88** with **60** in the presence of $Hg(CN)_2$ afforded the α-anomer **89** as a major product in 53% yield along with a 20% yield of the β-anomer **90.** The lower stereoselectivity observed in this glycosidation using the glycosyl donor **60** compared with that of **60** with **61** (Scheme 6) may be due to the higher reactvity of C6-OH of **88** (Ref. 7). **89** was converted into the bromide **91** in a usual way in 60% yield.
Next the designed glycosyl acceptor **93** was prepared in a straight-forward manner using two monosaccharide synthons **75** and **77.** Thus, glycosidation of **75** with **77** in the presence of $AgOSO_2CF_3$ and subsequent deacylation afforded the diol **92** which was subjected again to the same reaction sequence to afford the desired **93** in 25% overall yield from **75.** Finally, the reaction between the donor **91** and the acceptor **93** in the presence of $AgOSO_2CF_3$ afforded a 60% yield of the protected nonasaccharide **94** which was deprotected to give the target nonasaccharide **87.** The structure of **87** was assigned by the synthetic sequence and confirmed by the nmr data (Fig 9, 10).



Scheme 9 (●— = OBn)



73

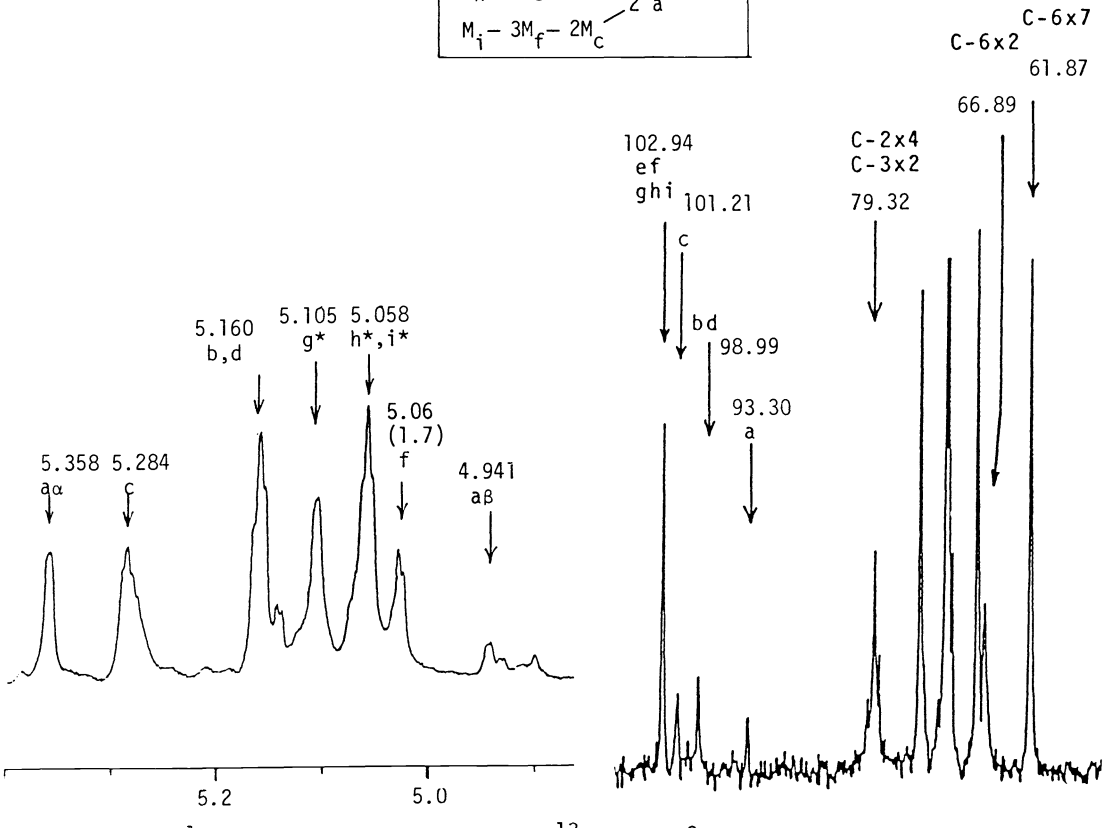
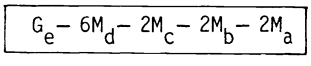


Fig.8 400MHz ^{1}H-nmr (60°, left) and 22.5MHz ^{13}C-nmr (20°, right) spectra of 73.



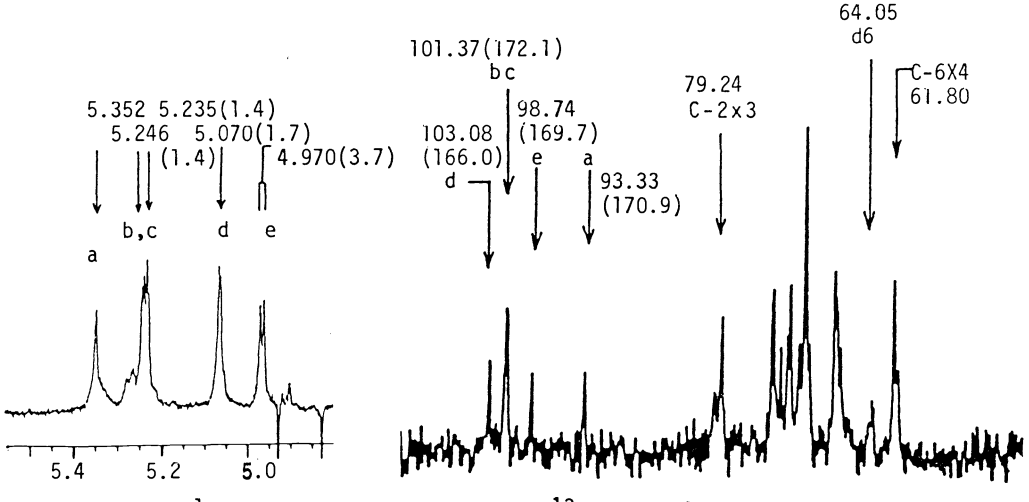


Fig.9 400MHz ^{1}H-nmr (60°,left) and 22.5MHz ^{13}C-nmr (20°,right) of Glcα–6Manα– 2Manα– 2Manα– 2Man which was prepared by using the donor **91**.

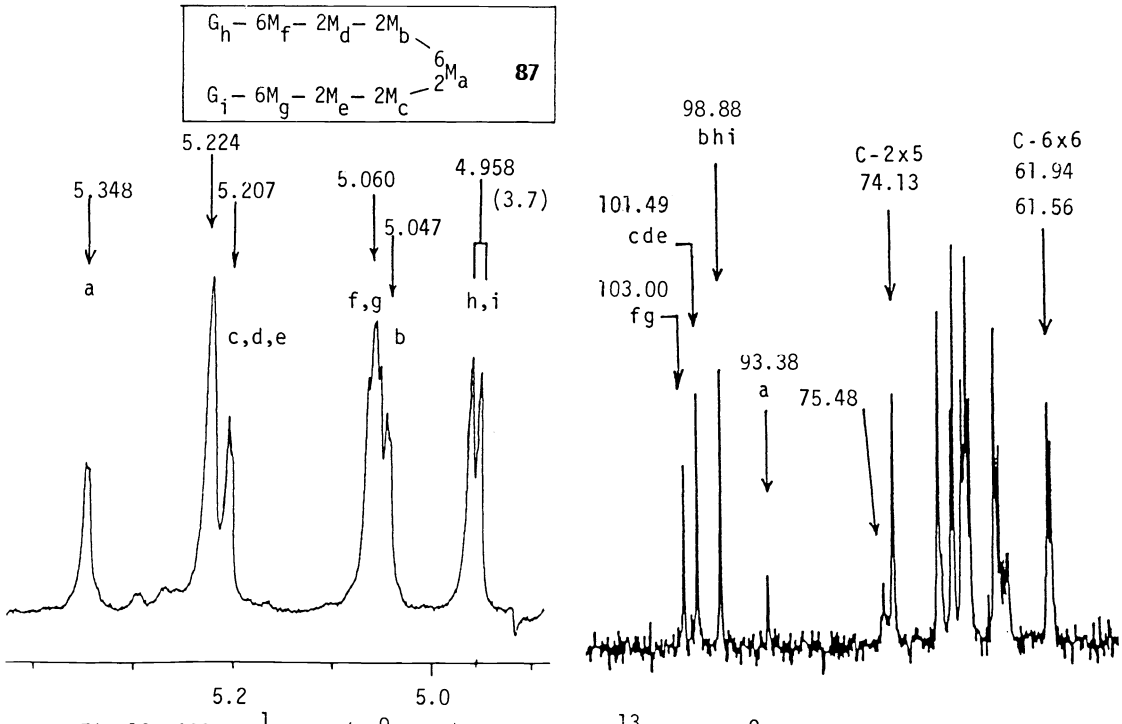


Fig.10 400MHz ^{1}H-nmr (60°,left) and 22.5MHz ^{13}C-nmr (20°,right) spectra of **87**.

CONCLUSION

Based on a rational design of the key intermediate oligosaccharide donors and acceptors by disconnecting the target glycan chains at interglycosidic linkages of α-D configurations, a convergent type approach for the synthesis of complex glycans could be efficiently executed. In preparing regioselectively protected monosaccharide synthons and oligosaccharide intermediates, partial stannylation methods were proved to be quite efficient and practical.

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