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 Hg²⁺ 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).

\[
\begin{align*}
\text{NeuAc} & \rightarrow 6\text{Gal} \rightarrow 4\text{GlcNAc} \rightarrow 2\text{Man} \rightarrow 6\text{Man} \rightarrow 4\text{GlcNAc} \rightarrow 4\text{GlcNAc} \\
\text{NeuAc} & \rightarrow 6\text{Gal} \rightarrow 4\text{GlcNAc} \rightarrow 2\text{Man} \rightarrow 3\text{Man} \rightarrow 4\text{GlcNAc} \rightarrow 4\text{GlcNAc} \\
\text{Man} & \rightarrow 2\text{Man} \rightarrow 6\text{Man} \\
\text{Man} & \rightarrow 2\text{Man} \rightarrow 3\text{Man} \\
\text{Glc} & \rightarrow 2\text{Glc} \rightarrow 3\text{Glc} \rightarrow 2\text{Man} \rightarrow 2\text{Man} \rightarrow 6\text{Man} \rightarrow 4\text{GlcNAc} \rightarrow 4\text{GlcNAc}
\end{align*}
\]

1

2

779
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 AgOSO₂CF₃, HgBr₂-Hg(CN)₂, or TMSOSO₂CF₃ 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.
Synthetic approach to glycan chains of a glycoprotein and a proteoglycan

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 α-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.
Tributylstannylation of 13 to 14, and alkylation of 14 with benzyl bromide for 2 days at 90°C in the presence of Bu4NBr (Ref. 11) gave a 76% yield of the dibenzyl ether 15. Acetylation of 15 to give 16 and deacetylation of 16 with PdCl2 inaq AcOH-AcONa for 2h at 70°C (Ref. 12) afforded 17 in 83% yield from 15. Treatment of 17 with SOCl2 in the presence of catalytic amounts of DMF in CH2Cl2 for 2h at 20°C gave a quantitative yield of 11. Glycosidation of 12 (Ref. 13) with 11 in the presence of AgOSO2CF3 and powdered molecular sieves 4A for 16h at 20°C afforded a 62% yield of the chitobiosyl derivative 18. Deacetylation of 18 in boiling HCl-H2O-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). Benzilation 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 acetylated 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 CH2Cl2 at 0°C 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 CH2Cl2 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. PdCl2 catalysed deallylation of 26 gave a 58% yield of 27, which was treated with (i) 1:1 BuNH2-MeOH for 8 days at 90°C (ii) Ac2O-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

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.
Glycosidation of 31 (Ref. 17) with the lactosaminyl donor 32 (Ref. 18) in the presence of AgOSO₂CF₃—molecular sieves 4A in Cl(CH₂)₂Cl afforded a 94% yield of 33. Successive treatment of 33 with i) NaOMe—MeOH, ii) reflux in BuNH₂—MeOH (Ref. 19), iii) Ac₂O—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—CH₂Cl₂ gave a quantitative yield of 30.

Glycosidation of 9 with 6 molar equivalents of 30 in the presence of AgOSO₂CF₃—powdered molecular sieves 4A in 3:1 Cl(CH₂)₂Cl—toluene, and gel—chromatography of the product on Toyopearl HW 40 in 1:1 CH₂Cl₂—MeOH afforded a 55% 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—0 by 400 MHz ¹H 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 ¹H—nmr spectrum of synthetic 29 (Ref. 22) were also in good agreement with the target structure 29.

![Synthetic approach to glycan chains of a glycoprotein and a proteoglycan](image_url)

Fig. 1 400 MHz ¹H—nmr spectra of 29 taken at (a) 20°, (b) 60°.

a) Nmr spectra were recorded, for solutions in D₂O, with a JNM-GX400, or JNM-FX100 FT spectrometer. The values of δ, are expressed in p.p.m. downward from the internal standard, sodium 2,2,3,3-tetradeterio-4,4-dimethyl-4-silapentaneoate. The values of δ, are expressed in p.p.m. downward from tetramethylsilane, referenced indirectly with an internal standard of 1,4-dioxane(δ,67.40) 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) Me₂C(OMe)₂-TsOH in DMF, 15hr at 20°, (iii) Ac₂-pyridine. Deisopropylidenation of

![Diagram of carbohydrate structure](image)

Fig.2 400 MHz ¹H-nmr (at 20°) and 100.7 MHz ¹³C-nmr (at 20°) spectra of 6. The values of ¹J_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 HgBr2:Hg(CN)2-powdered molecular sieves 4A in Cl(CH2)2C1 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) H2, 10% Pd—C in 9:1 EtOH—H2O 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, respectively, by comparing the 1H nmr data of synthetic samples (Fig 2 and 3) with the reported data (Ref. 26).

![Image](image-url)

**Fig. 3 400 MHz 1H-nmr (at 20°) and 100.7 MHz 13C-nmr (at 20°) spectra of 39**
Scheme 5

Glca → 2Glca → 3Glca → 3Manα → 2Manα → 2Man

40 $R_1^1 = R_2^2 = R_3^3 = R_4^4 = H$
41 $R_1^1 = R_2^2 = R_3^3 = R_4^4 = \text{Me}$
42 $R_1^1 = R_2^2 = R_3^3 = R_4^4 = \text{Me}$
43 $R_1^1 = R_2^2 = R_3^3 = R_4^4 = H$
44 $R_1^1 = R_2^2 = R_3^3 = R_4^4 = H$
45

46 $R_1^1 = \text{Bn}, R_2^2 = \text{Ac}, R_3^3 = \text{Me}$
47 $R_1^1 = \text{Bn}, R_2^2 = \text{H}, R_3^3 = \text{Me}$
48 $R_1^1 = \text{Bn}, R_2^2 = \text{H}, R_3^3 = \text{H}$
49 $R_1^1 = \text{Bn}, R_2^2 = \text{Ac}, R_3^3 = \text{Me}$
50 $R_1^1 = \text{Bn}, R_2^2 = \text{H}, R_3^3 = \text{Me}$
51 $R_1^1 = \text{Bn}, R_2^2 = \text{H}, R_3^3 = \text{H}$
52

Scheme 5

H → 3Glca → 3Manα → 0Bn

54

53

55
In 1978, Li et al. [Ref. 27] and in 1979, Liu et al. [Ref. 28] proposed the structure of the high-mannose type glycan \( \mathbf{2} \). In 1980, Ugalde et al. [Ref. 29] assigned the stereochemistry of the triglucopyranosyl part of \( \mathbf{2} \) as Glc\( \alpha \) - 2Glc\( \alpha \) - 3Glc\( \alpha \) from the inhibition experiments with glucosidases. In order to provide synthetic support for the proposed structure \( \mathbf{2} \), we have studied an approach to a total synthesis of \( \mathbf{2} \) with high regio- and stereo-control. According to retrosynthetic considerations, \( \mathbf{2} \) was divided into three parts, namely \( \mathbf{5}, \mathbf{7} \), and \( \mathbf{8} \). We now discuss a synthetic approach to a linear hexasaccharide \( \mathbf{8} \).

Retrosynthetic considerations indicated that the target structure \( \mathbf{8} \) might be reconstructed from the disaccharide synthons \( \mathbf{53}, \mathbf{54} \) and \( \mathbf{55} \) (Scheme 5). As \( \mathbf{53} \) [Ref. 17] and \( \mathbf{54} \) [Ref. 30] are already available, we first describe the synthesis of \( \mathbf{63} \), which corresponds suitably protected disaccharide unit \( \mathbf{55} \). A mixture of allyl 3-O-allyl-\( \beta \)- and 3-\( \alpha \)-D-glucopyranoside \( \mathbf{56} \), readily obtainable from 3-O-allyl-1,2,5,6-di-O-isopropylidene-\( \alpha \)-D-glucofuranose, was benzylated, to give \( \mathbf{57} \), and deallylation of \( \mathbf{57} \) with PdCl\(_2\) in aq AcOH-AcONa afforded a 61% yield of the tribenzyl ether \( \mathbf{58} \) as a mixture of the \( \alpha \)-and \( \beta \)-anomer in the ratio of 1:1. Acetylation of \( \mathbf{58} \), to give \( \mathbf{59} \), and treatment of \( \mathbf{59} \) with HCl in C\(_1\)(CH\(_2\))\(_2\)Cl gave a 70% yield of \( \mathbf{60} \). Glycosidation of \( \mathbf{61} \) [Ref. 31] with \( \mathbf{60} \) in the presence of Ag\( \text{OSO}_2\text{CF}_3\) [Ref. 32] - powdered molecular sieves 4A afforded a 72% yield of a mixture of \( \mathbf{62} \) and \( \mathbf{64} \) 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 \( \alpha \)-anomer \( \mathbf{62} \). Deacetylation of \( \mathbf{62} \) afforded \( \mathbf{63} \), which was glycosylated with the kajibiosyl donor \( \mathbf{54} \) in the presence of Ag\( \text{OSO}_2\text{CF}_3\)-powdered molecular sieves 4A to give a 59% yield of the protected tetrasaccharide \( \mathbf{65} \). Debenzylation of \( \mathbf{65} \) by catalytic hydrogen transfer [Ref. 34] with 10% Pd-C, HCOOH-MeOH gave \( \mathbf{66} \), and acetylation of \( \mathbf{66} \) afforded a 79% yield of the peracetylated tetrasaccharide \( \mathbf{67} \). Deacetylation of \( \mathbf{66} \) with NaOMe-MeOH gave free \( \alpha \)-saccharide \( \mathbf{68} \) (Fig. 4 for 400 MHz \( \text{'H NMR} \)). Treatment of \( \mathbf{67} \) with HBr-AcOH-CH\(_2\)Cl\(_2\) gave the...
tetrasaccharide donor 69 which corresponds to the synthet 52. The reaction of 69 with the
mangobiosyl acceptor 53 in the presence of Ag_2SO_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.

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.
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$_2$CF$_3$ 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$_2$CF$_3$ 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. $^1$H 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 $^1$H- and $^{13}$C-nmr data of 83 (Fig 7) with the reported one (Ref. 40). Now we need a mannosyl 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$_2$CF$_3$ in benzene afforded a 77% yield of 86 which was deprotected in a usual way to give the target mannononaoside 73 (Fig 8).

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.
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 reactivity 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 (● = OMe)
Synthetic approach to glycan chains of a glycoprotein and a proteoglycan

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.
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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