New schemes for the synthesis of glycolipid oligosaccharide chains

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Abstract: The driving force for the constant improvement and development of synthetic methodologies in carbohydrate chemistry is the importance of natural oligosaccharide chains in numerous biological phenomena such as cell growth, differentiation, adhesion, etc. Here, we report our syntheses of the spacer-armed oligosaccharides of sialylated lacto- and neo-lacto-, globo-, ganglio-, and sulfoglucuronylparagloboside-series, which include new rationally designed synthetic blocks, efficient solutions for the stereoselective construction of glycosidic bonds, and novel protection group strategies.

INTRODUCTION

Natural oligosaccharides and glycoconjugates play a crucial role, acting as lectin receptors, in the process of cell adhesion. This makes synthetic oligosaccharides and neoglycoconjugates thereof (i.e., molecular probes in which an oligosaccharide is attached, via a spacer, to a label or carrier) indispensable tools for the research into the carbohydrate lectin interactions to determine the structural features responsible for specific recognition of carbohydrate ligands, define the binding topology, and understand the biology functions and mechanisms of action of the corresponding natural glycoconjugates [1].

Recent studies of the mechanisms of cell recognition have revealed the key role of various natural glycoconjugates. Glycolipids of sialylated lacto- and neo-lacto-, ganglio-, globo-, and sulfoglucuronylparagloboside-series are of great interest in this area as they act as differentiation, growth, cell adhesion, and signal transduction regulators [2].

In this communication, we overview the schemes which we recently developed for the syntheses of the linear and branched sialylated lacto- and neo-lacto-oligosaccharides 1–9, glycoprotein O-chains related tetrasaccharide 10, the gangliosides GM1 11 and Fuc-GM1 12, the globoside Gb 5 13, and the pentasaccharide sulfoglucuronyl paraglobosides 14 and 15 (glycolipid antigens HNK-1). The target compounds were obtained as the spacer-armed β-2-aminoethyl and β-3-aminopropyl glycosides suitable for further preparation of various labeled derivatives and neoglycoconjugates. The resulting molecular probes are being used to study the carbohydrate-binding proteins of selectin and galectin families, and the neurobiology processes mediated by HNK-1 carbohydrates.


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RESULTS AND DISCUSSION

The syntheses of the glycosyl ceramides related to the oligosaccharides 6 [3], 7 [4], 9 [5], 11 [6], 12 [7] 13 [8], and 14 [9,10] have been described earlier. Our syntheses of the spacer-armed oligosaccharides 1–15 involved new strategies in the design of the sialyl-galactosyl, glucuronyl-galactosyl, and galactosyl-galactosaminyl disaccharide blocks; efficient solutions for the stereoselective construction of the key (β-glucosaminyl, β-galactosaminyl, and β-glucuronyl) glycosidic bonds; novel protection group pathways, e.g., regioselective liberation of the 3-OH group in the glucuronic acid residue, and the choice of the masked form of spacer. As the latter, the 2-azidoethyl or 3-trifluoroacetamidopropyl agly-

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cons were selected in the present work. The availability of 2-azidoethyl glycosides from the corresponding allyl glycosides is an advantage, and we have documented the efficiency of such an approach to the syntheses of spacer-armed oligosaccharides [11].

Syntheses of sialylated lacto- and neolacto oligosaccharides 1–9 and glycoprotein O-chains related tetrasaccharide 10

The titled oligosaccharide chains, both linear and branched, have been of constant interest due to their unique biological activity, which is mainly associated with the presence of Neu5Ac residue. It has been well recognized that in the synthesis of sialylated oligosaccharides of these groups, the efficient and stereoselective construction of the Neu5Ac-α-(2→3)-Gal linkage is the main problem, and many approaches have been elaborated in order to improve the yield of the glycosylation with Neu5Ac donors [12–14].

In our work we declined the construction of the sialyl-galactose disaccharide from monosaccharides as a laborious and not very efficient, in terms of overall yield, sequence. Within the alternative approach we studied the expeditious preparation of the sialyl-α-(2→3)-galactosyl disaccharide glycosyl donors from not monosaccharides but the trisaccharide sialyl-α-(2→3′)-lactose 16, which already contains the requisite sialyl-α-(2→3)-galactose fragment (Scheme 1). The trisaccharide 16 is an available compound which can be isolated from natural sources at laboratory [15] and industrial scale [16].

![Scheme 1 Transformation of 3′-sialyllactose trisaccharide into disaccharide glycosyl donors 19 and 20.](image)

In order to cleave selectively the acetal-type galactoside linkage in the trisaccharide 16 but remain intact the ketal-type sialoside one, acetylation of per-O-acetylated derivative 17 was studied, since the greater stability of sialoside linkage toward acetylation as compared to hexapyranoside ones had been mentioned, in contrast to the well-known ease of its acidic hydrolysis [17–19].

The sialyl-α-(2→3′)-lactose sodium salt 16 (the product of Neose Technologies, Inc.) was subjected to total acid-catalyzed O-acetylation (5 % H2SO4 in Ac2O at 40 °C) to give the expected acid in 96 % yield with no lactone formation, then the carboxy group was methylated with diazomethane into 17. Thorough experimentation with the nature and concentration of various protic and Lewis acidic catalysts (H2SO4, BF3·Et2O, Bu2BOTf, ZnCl2, AlCl3, FeCl3, SnCl4, TiBr4, TMSOTf) as well as the reaction temperature and time showed that boron-derived electrophiles were the reagents of choice. Thus, acetylation of the trisaccharide 17 in neat acetic anhydride in the presence of 10 % v/v of BF3·Et2O at 80 °C for 12 h gave the disaccharide 18 in 39 % yield, and acetylation in the presence of Bu2BOTf (10 equiv) at 70 °C for 3 h afforded 18 in 49 % yield.

The disaccharide 18 thus obtained was then easily transformed into the glycosyl-donors, trichloroacetimidate 19 and thioglycoside 20, by anomeric deacetylation with hydrazine acetate followed by treatment with Cl3CCN/DBU (→19, 76 %) or mercaptoysis with EtSH and BF3·Et2O (→20, 86 %), respectively. Compound 20 could also be obtained from the trichloroacetimidate 19 by the reaction with EtSH in the presence of TMSOTf and MS-4A (96%).

Thus, the disaccharide sialyl-galactosyl donors 19 and 20 were prepared from the readily available trisaccharide 16 in 5 and 4 steps in 30–40 % overall yields, respectively, which are much higher than those in known syntheses by coupling of N-acetylneuraminic acid and D-galactose blocks.

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The preparation of GlcNAc- and Fuc-GlcNAc-containing di-, tri-, and tetrasaccharide glycosyl acceptors 21–29 included the use of mono- and disaccharide donors with N-trichloroacetyl-D-glucosamine thioglycoside moiety. Systematic studies by others [20] and us [21] have revealed such a type of donors to be very efficient for stereospecific incorporating a β-D-GlcNAc residue into an oligosaccharide chain, even for glycosylations of low-reactive glycosyl acceptors with benzoyl-protected neighboring hydroxy groups. Furthermore, N-trichloroacetyl-protected sialyl-oligosaccharides can be deblocked directly in a single step by treatment with alkali, in contrast to N-phthaloyl-protected ones.

In order to achieve efficient introduction of protecting groups in the course of the preparation of compounds 21–29, efficient methods were developed for the benzylation of monosaccharide derivatives bearing base-labile N-trichloroacetyl group [21] and the opening of 4,6-O-benzylidene acetals of hexopyranosides into the corresponding 4-hydroxy,6-O-benzyl derivatives. Particularly, it was found [22] that the acidic reagent formed in situ from anhydrous AlCl$_3$ and H$_2$O in 3:1 ratio is much more efficient promoter for the reductive opening with Me$_3$N/B$_2$H$_3$ in tetrahydrofuran than the AlCl$_3$ alone as in the original procedure (see the refs. cited in [22]).

The key step of the preparation of the tetrasaccharide acceptor 29 was 3-O-fucosylation of ethyl 4,6-O-benzylidene-1-thio-2-trichloroacetamido-β-D-glycopyranoside [21] by 2-O-benzyl-3,4-di-O-benzoyl-α-L-fucopyranosyl trichloroacetimidate [23]. This coupling proceeded in good 69 % yield to give the desired α-linked 3-O-fucosyl-glycosaminide disaccharide stereoselectively without any formation of the side product of SET transfer, in contrast to some other related cases [21].

Study of glycosylation of acceptors 21–29 (Scheme 2) revealed that the optimal reaction conditions depended on the location of free OH-group to be glycosylated. Thus, the 3-O-glycosylation of the acceptors 21–23 by trichloroacetimidate 19 could be best performed when the 20 % excess of acceptor was used and the reaction was promoted by TMSOTf in CH$_2$Cl$_2$ in the presence of MS-4 Å at room temperature. In these conditions, the β-linked oligosaccharides 30, 31, and 34 were obtained in the yields of 71 % [21], 80 % [24], and 80 % [24], respectively.

On the contrary to above case, the 4-O-glycosylation with trichloroacetimidate 19 of the acceptors 24, 25, and 27 needed the use of excess of the donor 19 (2.1 equiv), performing the reaction in CH$_2$Cl$_2$ at low temperature of −20 °C, and promotion with BF$_3$Et$_2$O (0.1 equiv with respect to the imidate 19) in the presence of acid-washed molecular sieves MS AW-300. Under these conditions, the target β-linked oligosaccharides 33, 32, and 35 were obtained in the yields of 40 % [24], 56 % [24], and 81 % [21], respectively.
Study of another donor, thioglycoside 20, showed that it was more reliable than the imidate 19. Thus, the coupling of the tetrasaccharide acceptor 29 with the imidate 19 (3 equiv) gave in the best case the sialyl Lewis X hexasaccharide 39 in 50 % yield while the glycosylation of 29 by thioglycoside 20 (2.7 equiv) under promotion with NIS, TfOH, and MS-4 Å afforded to 39 in 65 % yield.

Glycosylation of the acceptor 28 with thioglycoside 20 deserves special comments. The reaction was promoted with NIS, TfOH, and MS-4 Å in CH2Cl2, and gave ca. 20 % yield of the expected pentasaccharide 36 together with another pentasaccharide product (ca. 30 %). The 1H and 13C NMR analysis of the latter with the use also of our previous observation [25] allowed determining its structure as the N-thioethylated derivative 37. Since this side N-thioethylation reaction could not be avoided, we sought for the conditions for the efficient 37 → 36 conversion and found that treatment with the excess of thiourea in 3:1 MeOH–AcOH for 30 min at room temperature was quite reliable protocol [26]. Thus, condensation of 20 with 28 followed by dethioethylation of the crude reaction products gave the pentasaccharide 36 in total yield of 48 %.

In the similar fashion, the glycosylation of the GlcNAc-(1→6)-GalNAc acceptor 26 promoted by NIS-TfOH-MSAW-300 in CH2Cl2, afforded, after treatment of the crude reaction products with thiourea, the tetrasaccharide 38 in 55 % yield.

In order to deblock the compounds 30, 35, 36, 38, and 39, they were first subjected to the simultaneous alkaline hydrolysis of methyl ester, O-acyl protections, and N-TCA group. Subsequent N-acetylation of the liberated amino group in the internal GlcN residue followed by the catalytic hydrogenolysis for de-O-benzylation and azido group reduction gave the target oligosaccharides 6, 7, 8, 10, and 9, respectively [21]. Compounds 31–34 were first de-O-benzylated by catalytic hydrogenolysis and then saponified with aqueous NaOH into the oligosaccharides 1, 5, 2, and 4, respectively. Compound 33 was also transformed into the 6-O-sulfated trisaccharide 3 by de-O-benzylation, selective 6-O-monosulfation of the corresponding 3,6-diol formed, and saponification.

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In conclusion, the described above new synthetic methods enabled the efficient and stereo-selective preparation of the spacer-armed sialylated lacto- and neolacto-oligosaccharides.

**Syntheses of the spacer-armed oligosaccharide chains of gangliosides GM1 (11) and Fuc-GM1 (12) and globoside Gb₅ (13)**

The oligosaccharide chains of the gangliosides GM1 and Fuc-GM1 and the globoside Gb₅ (11–13) contain the common disaccharide fragment Gal-β-(1→3)-GalNAcβ. We have elaborated a new efficient glycosyl donor 44 for one-step introduction of this sequence into various oligosaccharide chains (Scheme 3).

In order to prepare the disaccharide glycosyl-donor, the galactosylation under different conditions of phenyl 2-azido-2-deoxy-1-thio-β-D-galactoside derivatives with free OH-groups at C3,4 was studied first [27]. However, these reactions resulted in preferential formation of (1→4)-linked disaccharides, but not the desired (1→3)-ones. In the alternative way, we also attempted to prepare the necessary disaccharide by glycosylation of phenyl thioglycoside 41 with acetobromogalactose 40 under various conditions. But no desirable product could be obtained due to the competitive reaction of phenylthio group transfer. Replacement of the phenylthio group by less nucleophilic p-nitrophenylthio one suppressed completely the aglycon transfer and allowed us to obtain the target disaccharide 43 in good 58 % yield. In order to convert the nonparticipating azido group into the participating trichloroacetamido one and enhance the reactivity of the thioglycoside, both nitrogen functions in 43 were subjected to simultaneous reduction with Zn in AcOH and subsequent bis-N-trichloroacetylation to give the donor 44 in 61 % yield [27].

The efficacy of the disaccharide donor 44 is illustrated by successful syntheses of the pentasaccharide Gb₅ [28] and GM1 [29]. The glycosylation of the trisaccharide acceptors 46 and 47 with 44 prompted by NIS-TIOH in CH₂Cl₂ in the presence of MS-4 Å at –30 to –40 °C afforded the pentasaccharides 48 and 50 in 73 and 85 % yield, respectively.
Further introduction of monosaccharide residues into the disaccharide 44 enabled the preparation of more complex oligosaccharide donors applicable to the synthesis of higher oligosaccharides. Thus, 44 was transformed into α-fucosylated analog 45 (61 % overall yield for 6 steps) by O-deacetylation (MeONa, MeOH), bis-4,6,4′,6′-O-benzylideneation [PhCH(OMe)2, CSA, DMF], selective mono-3′-O-benzoylation (BzCN, CH3CN, Et3N, 90 %), stereospecific α-fucosylation with 2-O-benzyl-3,4-di-O-benzoyl-α-L-fucopyranosyl trichloroacetimidate (TMSOTf, CH2Cl2, MS-4 Å at –30 °C, 93 % yield), deacetalation (80 % aq. AcOH, 80 °C), and O-acetylation (Ac2O, Py, DMAP). Subsequent coupling of the acceptor 46 with 45 (under the same conditions as described above) gave the hexasaccharide 49 (76 %) with the structure of Fuc-GM1 hexasaccharide.

Deprotection of the oligosaccharide 50 was performed by alkaline hydrolysis followed by N-acetylation, and subsequent catalytic hydrogenolysis as described above for the preparation of sialylated oligosaccharides.

Deprotection of the oligosaccharides 48 and 49 was also started from alkaline hydrolysis followed by N-acetylation. However, subsequent catalytic hydrogenolysis for O-debenzylation and simultaneous reduction of the azido group could be performed efficiently only in the presence of Boc2O [11]. The last step was the removal of Boc protection from the spacer amino group with aqueous CF3CO2H.

In conclusion, the disaccharide block 44 was shown to be a reliable tool for the efficient construction of various oligosaccharides of globo- and ganglio series. Another versatility of the approach described is the possibility to employ the azido disaccharide 43, the precursor of 44, in the syntheses of the oligosaccharides with α-linked Gal-β-(1→3)-GalNAc disaccharide unit.

Syntheses of 3-O-sulfoglucuronylparaglobosides 14 and 15

Natural carbohydrates that bear HNK-1 epitope participate in neurite outgrowth and neural cells adhesion, play an important role in a number of other developmental processes of mammalian nervous system and are recognized by L- and P-selectins [30–32]. This epitope is present in glycolipid penta- or heptasaccharide chains 3-O-sulfo-GlcA{β1-[3Gal(β1-4)GlcNAc(β1-)]n3}Gal(β1-4)Glcβ (n = 1,2) [33] and in several glycoproteins and proteoglycans of neural tissues [32].

The bottleneck of the previous syntheses of the glycosyl ceramides related to the pentasaccharides 14 and 15 was multistep and laborious introduction of protecting groups into glucuronic acid in order to differentiate by temporary levulinoyl protection the OH-group at C3, which is sulfated in the target molecules [9,10].

Our synthesis of the pentasaccharides 14 and 15 [34–36] included selective liberation of the OH-group at C3 of glucuronic acid residue via 6,3-lactonization-methanolysis procedure (Scheme 4). This key feature allowed us to use the readily available disaccharide trichloroacetimidate 51 which does not contain any temporary protecting group at O3 of the GlcA residue, instead of much less available selectively protected derivatives which were used in known syntheses of HNK-1 related oligosaccharides [9,10]. Preparation of the disaccharide donor included glycosylation of allyl 2-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside with per-O-pivaloylated methyl glucuronyl bromide under Helferich conditions. This combination of protecting groups as well as the nature of glycosyl donor and promotor was found to be the most efficient [34,35].

Condensation of the disaccharide trichloroacetimidate 51 with the trisaccharide acceptors 52 and 53 gave the pentasaccharides 54 (82 %) and 55 (62 %) which were then saponified into the corresponding carboxyhexaols 56. Their lactonization by heating in Ac2O gave the per-O-acetylated derivatives of the type 57, which were then subjected to mild AcONa-catalyzed methanolysis to afford the 3-hydroxy derivatives 58 and 60 in 74 and 35 % overall yields, respectively. At last, O-sulfation into 59 and 61, followed by deprotection gave the spacer-armed pentasaccharide 15 and its propyl glycoside 14, respectively [35,36].
Further elaboration of the lactonization-methanolysis procedure gave ready access to other selectively benzoylated or pivaloilated derivatives of glucuronic acid including methyl (ethyl 2,4-di-O-benzoyl-1-thio-β-D-glucuronopyranoside)onate, methyl (allyl 2,4-di-O-benzoyl-β-D-glucuronopyranoside)onate, methyl (allyl 2,4-di-O-pivaloyl-β-D-glucuronopyranoside)onate, methyl (allyl 4-di-O-benzoyl-β-D-glucuronopyranoside)onate, and methyl (allyl 4-O-pivaloyl-1-thio-β-D-glucuronopyranoside)onate [37].

In conclusion, the lactonization-methanolysis procedure allowed straightforward and efficient synthesis of the 3-O-sulfoglucuronylparagloboside pentasaccharides 14 and 15, their fragments and nonsulfated analogs which are currently being used to study of the biosynthesis of HNK-1 positive carbohydrate chains and their role in neurobiology processes [38,39].

ACKNOWLEDGMENTS

This work was supported by Russian Foundation for Basic Research (Projects 01-03-33059a, 03-03-32556, 03-03-32567a, and 03-03-06290-MAC) and Russian Academy of Sciences (Grant 129-6-1999 for young researchers). 3′-Sialyllactose was kindly provided by Neose Technologies, Inc., Horsham, PA, USA.

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