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Synthesis of glycans functioning as antigens of the ABO blood group system

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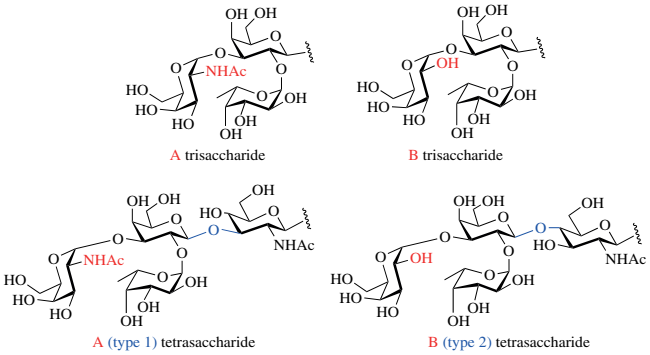
Since the discovery of carbohydrate nature of the antigens related to ABO blood group system in the middle of 20th century, an interest to synthetic approaches to these oligosaccharides is very high. ABH glycans are required for clinical applications, namely, immunoadsorption and blood typing, as well as for scientific investigations in the areas of hematology, immunology and glycobiology. In this article, the chemical syntheses of various ABH glycans and related structures are reviewed, and examples of their use for clinical purposes are collected.



Introduction

Since the discovery by W. M. Watkins and W. T. Morgan of the chemical structure of blood group ABH antigens in the early 60s,[1] the A and B oligosaccharides have perhaps become the most popular complex glycans in the synthetic chemistry of carbohydrates. On the one hand, this interest originates from a complicated manner of the synthesis of branched structures with α-fucosyl, α-galactosyl and α-*N*-acetylgalactosaminyl bonds. On the other hand, it is caused by the practical importance of A and B antigens for hematologists and, besides, by a complete lack of knowledge of their functions in a human organism. At today's level of understanding, the only function that all researchers agree on represents an increase in diversity. The diversity allows the entire human biological population to resist successfully the diseases caused by viruses and bacteria. The corresponding fighting mechanisms are different. For many bacteria, their specific adhesion to human cells occurs through bacterial lectins, which recognize one or another ABH glycan, and only a part of individuals is infected as a result, that is, the antigen is the carrier of the function. For glycosylated viruses, another mechanism is realized, where the function carriers are the antibodies, *viz.* so called natural antibodies, against the blood group antigens A or B, and they contribute to the elimination of viruses transmitted from the 'donor' to the 'recipient' of mismatch blood group.

The relatively recent discovery of the fact, that human galectins -4, -8 and -9 bind significantly better to glycans A and B compared with their canonical ligands of the oligolactosamine structure, further confused the understanding of the function of A/B glycans.

For a long time, it was believed that the antigenic determinants, *i.e.* the minimal structures recognized by the natural anti-A and anti-B antibodies, represent trisaccharides.[2] An investigation of the fine specificity of these antibodies using synthetic tetrasaccharides of types 1, 2, 3 and 4 has revealed that these trisaccharide moieties actually contact directly with the antigen-binding site of antibodies; however, tetrasaccharides represent the true antigenic determinants, and besides these specific antibodies, the blood group A donors have antibodies against trisaccharide A, but these antibodies are unable to bind to trisaccharide A as a partial structure of tetrasaccharide A.[2]

Note that ABH glycans have recently been found not only on erythrocytes, but also on other cells, primarily endothelial and

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epithelial, and therefore, they have been called 'histo-blood group antigens.' This fact was discovered with the help of monoclonal antibodies, which were well characterized using synthetic blood group glycans.

The presence of ABH glycans on endothelium greatly complicates the AB-incompatible organ transplantations, according to the same Landsteiner rules as are used in blood transfusion. However, if the corresponding recipient antibodies are removed, for example using a sorbent obtained on the basis of synthetic glycan, or a heart transplant is performed to an infant from two to six months old, who does not yet have natural antibodies, then the state of selective tolerance occurs, namely the recipient does not have antibodies to type 2 glycan specific for heart tissues, but does have antibodies to histo antigens of type 3 and 4 typical of other tissues.[3]

Actually, the composition of natural antibodies to ABH glycans is much more complex than is described in hematology textbooks. In addition to the features mentioned above, we also have noted the presence of antibodies in individuals of the O blood group, which bind both A and B glycans equally effective, since the epitope they recognize is located on the side of the glycan opposite to the 2-NHAc^{Gal} group of antigen A, *i.e.* to the only difference existing between A and B antigens. These antibodies were isolated using an affinity sorbent with glycan attached to the matrix by this particular group, making the AB epitope exposed and available for blood antibodies.[4]

The examples presented above demonstrate how synthetic glycans A and B have become an effective tool in this long and mysterious ABH story, although in fact there are immeasurably more analogous instances, and we only point a recent article clarifying the distinct involvement of A, B and O red blood cell carbohydrate chains in the promotion of infection by malarial plasmodium.[5] Apart from the purely academic interest in ABH glycans persisting over time, it is necessary to pay attention to their application in medicine, where absolutely different amounts are required, and to the appropriately scalable synthetic methods. We note the two main areas of their application. The first one is the removal of recipient antibodies during ABO-mismatched transplantation using Sepharose with immobilized glycans A and B,[6] and the second one is ABO blood typing, where synthetic glycolipids A and B are embedded in blood group O erythrocytes and as a result the erythrocytes with a standard content of A or B antigen are obtained, which is unattainable using natural A and B red blood cells.[7] The fact mentioned in the introduction, that the interest in the development of new syntheses of ABH structures is not something that is weakening but rather one that is growing, is illustrated by the above examples of biological and medical applications of synthetic oligosaccharides. Moreover, our long experience in this field allows us to suggest that the specificity of anti-A and anti-B natural human antibodies as well as other glycan-binding proteins is even more sophisticated, and therefore to solve the new challenges, further efforts will be required in the synthesis of oligosaccharides with complicated structure closer to the natural carbohydrate chains.

Structure of ABH glycans

Blood group ABH antigens are terminal fragments of carbohydrate components of glycolipids and glycoproteins presented on erythrocytes and some other tissues of a human organism. Numerous researches aimed at identifying their structure were conducted in the second half of 20th century, and their results have been reviewed.[8–10] It was established that minimal structural components (see Introduction) of all A and B

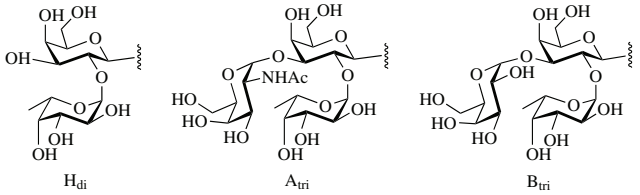


Figure 1 H disaccharide, A and B trisaccharides.

antigens represent the terminal branched trisaccharides Fucα(1→2)[GalNAcα(1→3)]Galβ, *i.e.* A trisaccharide or A_{tri}, and Fucα(1→2)[Galα(1→3)]Galβ, *i.e.* B trisaccharide or B_{tri} (Figure 1). Individuals with blood group O possess structures containing the terminal disaccharide Fucα(1→2)Galβ, *i.e.* H disaccharide or H_{di} (see Figure 1). In A, B and AB individuals, A_{tri} and/or B_{tri} moieties are synthesized from H_{di} by *N*-acetylgalactosaminyl transferase (GTA) and/or galactosyl transferase (GTB).

The way how the H_{di}, A_{tri} and B_{tri} moieties are linked to the carbohydrate backbone, *i.e.* core chain of the whole glycan, namely the position of glycosidic linkage with β-Gal and the next monosaccharide unit, can vary. To date, six types of ABH glycans have been identified[11] and their structures are listed in Table 1.

Table 1 Types of ABH glycans.

Type	Linkage of Galβ
1	Galβ1–3GlcNAcβ
2	Galβ1–4GlcNAcβ
3	Galβ1–3GalNAcα
4	Galβ1–3GalNAcβ
5	Galβ1–3Galβ
6	Galβ1–4Glcβ

The natural ABH antigens represent much more complex and structurally diverse glycans. They include one or more A_{tri}, B_{tri} or H_{di} fragments linked to the core chain. The composition of the core chain varies from short disaccharides with only one antigenic determinant to multiply branched oligosaccharides containing up to 50 monosaccharide units and bearing up to four antigenic determinants.[12] Note that some ABH glycans possess repetitive motifs, for instance A (type 3) antigen found on RBCs of individuals with blood subgroup A_1 includes two A_{tri} fragments linked to each other *via* Galβ1-3GalNAcα bond.[13] ABH oligosaccharides serve as glycan components of glycolipids or glycoproteins anchored to cell surface, and they can be found as well in biological liquids and excretions as soluble glycoproteins or as free reducing oligosaccharides.

The distribution of different types of ABH glycans in a human organism is tissue-specific, resulting from the genetic basis of their biosynthesis. The precursors of H disaccharide of various types can be fucosylated at the O(2) atom of galactose moiety by two fucosyltransferases, FUT I and FUT II, which are selective to different precursors. Activity of the corresponding two genes varies in different tissues, for instance, type 2 antigens are predominantly synthesized on RBCs, heart tissues and vascular endothelium, whereas type 1 structures are found in secretions. The details of the distribution of ABH glycans with different cores were observed by Oriol *et al.*,[14] Ravn *et al.*[15] and Jeyakanthan *et al.*[16]

Synthesis of A and B trisaccharides

A and B trisaccharides (see Figure 1) represent the terminal residues of all A and B blood group antigens. These compounds

have a branched structure, β-Gal unit being glycosylated not only with α-fucose at the O(2) atom, but also with α-galactosamine (or galactose) at the O(3) atom. Thus, there are two possible synthetic ways to trisaccharides A and B, namely the ones differing in the order of introduction of α-Fuc and α-GalNAc (or α-Gal) units.

The early synthetic efforts were aimed at B trisaccharide,[17,18] because the reliable methods for α-*N*-acetylgalactosaminylation required for synthesis of A_{tri} had still been unavailable. Both the galactosylation and fucosylatyion steps were carried out using benzylated glycosyl bromides under Lemieux conditions, namely S_N2 glycosylation with highly reactive β-bromide formed in equilibrium from the more stable α-bromide in the presence of Br^- ion.[19] Unfortunately, the Lemieux glycosylation provided the high yields of 65 and 95% only for fucosylation, while galactosylation proceeded much worse in 28 and 56% yields, the higher second yield value is due to the reversed order of the glycosylation steps, when the Gal unit was installed first.

Augé *et al.* proposed another galactosylation procedure employing benzylated galactosyl α-chloride as a donor and 3,4-*O*-dibutylstannylene derivative of galactose as a glycosyl acceptor.[20] 3,4-*O*-Dibutylstannylene group increased nucleophilicity of the O(3) atom as compared with free OH group, resulting in the higher yield of α-galactoside (74%). Nevertheless, the following reprotection of the obtained disaccharide led to 2,4-diol instead of 2-OH derivative, and further glycosylation of the diol by benzylated fucosyl bromide under the Lemieux conditions gave only 32% of the required protected B trisaccharide.

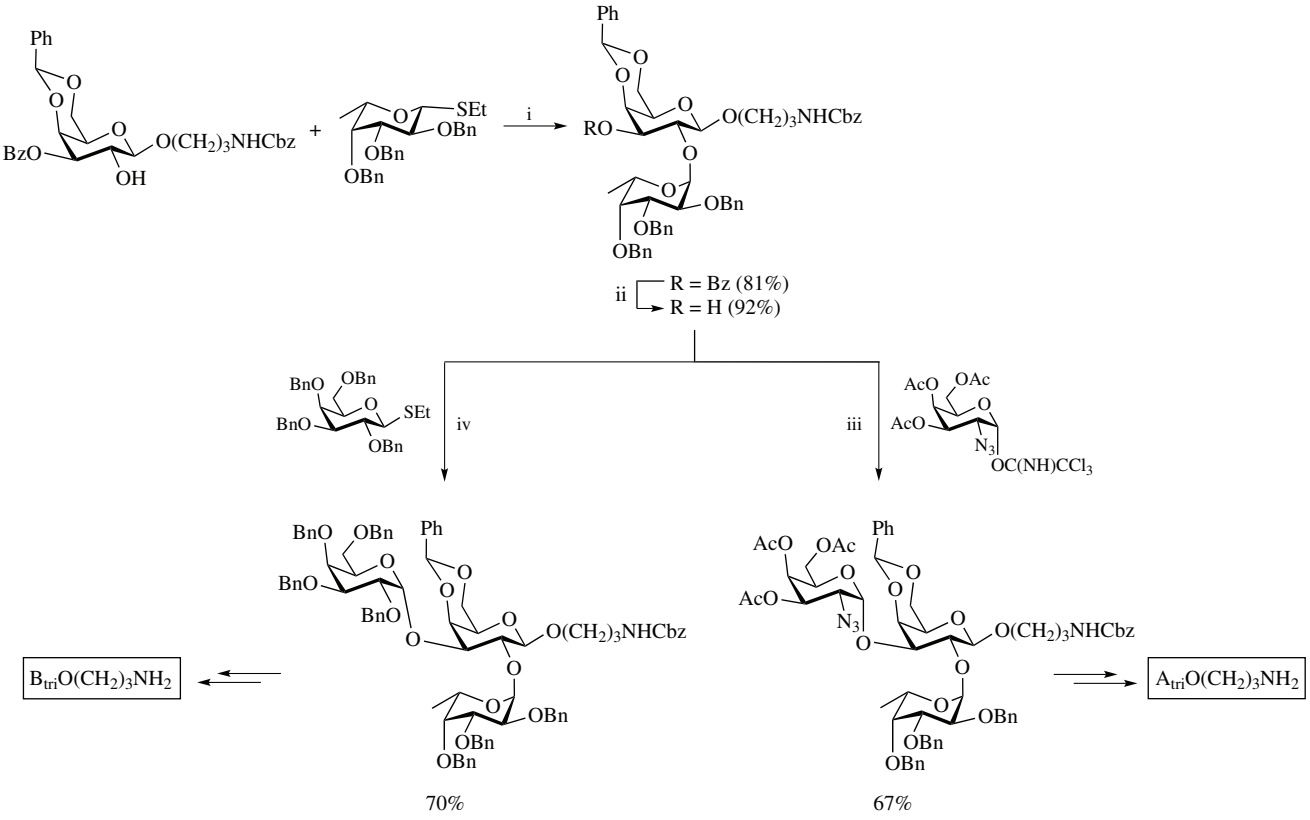
A remarkable progress in the synthesis of B trisaccharide was achieved with introduction of the imidate glycosylation procedure by Jacquinet and Sinaÿ, where benzylated *N*-methylacetimidates of fucose and galactose were used.[21] Both sequences of introduction of monosaccharide units were examined, affording the glycosylation products in yields of 84–91%.

In the late 1980s, the group of Norberg introduced thioglycoside donors into the synthesis of ABH oligosaccharides.[22,23] The glycosylation reactions were promoted by DMTSB or SO_2Cl_2/TfOH. In the synthesis of B trisaccharide only, the galactosylation and fucosylation steps were carried out using *p*-chlorobenzylated thioglycoside donors, resulting in 73% yield for each coupling step.[22] The *p*-chlorobenzyl protecting group was extensively used, since the *p*-chlorobenzylated derivatives of saccharides were easily crystallized, which facilitated their further isolation from reaction mixtures.

For the synthesis of both A and B trisaccharides, the group of Norberg proposed another approach. First, common H disaccharide precursor was obtained using the same *p*-chlorobenzylated thiofucoside in 61% yield. After debenzoylation of the 3-OH group, H disaccharide acceptor was either galactosylated with *p*-chlorobenzylated thiogalactoside, affording B trisaccharide (57%), or galactosaminylated using *p*-chlorobenzylated 2-azido-2-deoxygalactosyl bromide, resulting in protected A trisaccharide in 51% yield.[23]

Later, the same scheme employing common H disaccharide precursor was used by Korchagina *et al.*[24] The expected trisaccharides were obtained as 3-aminopropyl glycosides. The H disaccharide acceptor was synthesized *via* the Lemieux fucosylation of protected spacer armed galactose (79%) and then deacetylation of the O(3) atom. Trisaccharide A was obtained by glycosylation of the H disaccharide acceptor with acetylated β-chloride of 2-azidogalactose in 90% yield. The synthesis of B trisaccharide was accomplished using benzylated galactosyl bromide under the Helferich conditions, namely $Hg(CN)_2$ in C_6H_6, in 78% yield.

Recently, Karki *et al.* obtained A and B trisaccharides using a similar scheme with common H_{di} precursor.[25] This synthesis and the whole approach are illustrated in Scheme 1. Fucosylation of the 2-OH group of the spacer armed galactoside was carried out using benzylated SEt fucoside and promotion with NIS/TfOH (81%). After debenzoylation of the 3-OH group, the obtained



Scheme 1 *Reagents and conditions*: i, NIS, TfOH, PhMe, –15 ºC; ii, MeONa, MeOH; iii, TMSOTf, CH_2Cl_2, –20 ºC; iv, NIS, TfOH, CH_2Cl_2, –40 ºC.[25]

acceptor was either galactosylated by benzylated SEt galactoside (NIS/TfOH, 67% yield) or galactosaminylated by acetylated trichloroacetimidate of 2-azidogalactose using TMSOTf (70% yield) to afford B or A trisaccharides, respectively.

Synthesis of H trisaccharides

Blood group H trisaccharides possess a linear structure with common formula Fucα1-2Galβ1-Hex, where Hex denotes hexose, and are divided into six types, depending on the nature of the hexose and the position of bond between the Hex and Gal units (see Table 1). The disaccharide Galβ1-Hex is often referred to as core disaccharide.

The majority of chemical approaches to H trisaccharides are based on a two-step linear strategy including (i) galactosylation of the corresponding hexose monosaccharide (*i.e.* synthesis of core disaccharides) and (ii) reprotection followed by the fucosylation of the 2′-OH group of terminal galactose. Further the galactosylation and fucosylation steps are considered in detail.

Galactosylation

In early investigations[26–28] of the synthesis of H (type 1) and H (type 2) trisaccharides, the galactosylation was carried out *via* an orthoester method. Condensation of benzylated galactosyl ethyl orthoacetate or *tert*-butyl orthoacetate with the suitably protected glucosamine derivative led to type 1 or type 2 core disaccharides in 38–52% yields. The examples of orthoesters used in the synthesis of blood group antigens are limited to the above three early reports due to the low efficiency of orthoester donors in the glycosylation of secondary hydroxyl groups.

An application of galactosyl bromides as glycosyl donors led to much better results. Two types of reaction conditions were used for glycosylation by bromides, namely the Helferich method, when the reactions were carried out in $MeNO_2$ or in a mixture of $MeNO_2$ and C_6H_6 or PhMe in the presence of $HgCN_2$ and/or $HgBr_2$, and the condensation in CH_2Cl_2 in the presence of AgOTf.

The use of the Helferich conditions for the synthesis of type 1, 2 and 3 core disaccharides of H antigens has been described in several works.[29–35] Acetylated galactosyl bromide was employed in all instances. Type 1 core disaccharide[29–32] was obtained in 53% yield, while type 2[33,34] and type 3[35] core disaccharides were synthesized in 73–80% yields.

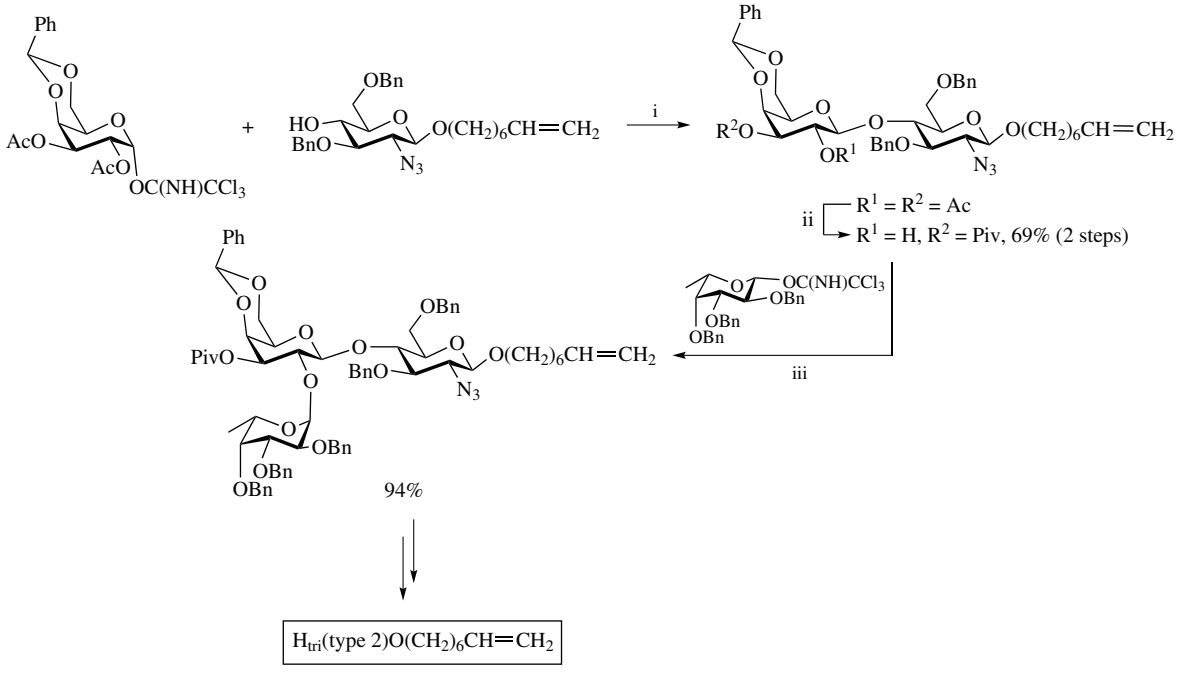
Hindsgaul and co-workers proposed galactosyl bromides bearing aroyl (benzoyl or *p*-nitrobenzoyl) substituent at the C(2) atom only, with all other hydroxyls benzylated, for the synthesis of lactosamine precursors of H (type 2) trisaccharides.[36] Condensation under the Helferich conditions provided the required stereoselectivity for β-isomer (76%). Glycosylation in the presence of AgOTf resulted in an anomeric mixture (β/α 3 : 2) in a lower yield of 60%. Nevertheless, the high potential of the AgOTf-promoted galactosylation was later demonstrated by Pazynina *et al.* in the synthesis of type 2 and type 4 core disaccharides,[37,38] where the required products were obtained in 68 and 64% yields for type 2 and type 4, respectively.

Thioglycosides were also successfully employed as glycosyl donors for the synthesis of H (type 1 and 2) glycans. Nifant'ev *et al.* synthesized the disaccharide precursor of H (type 1) antigen[39] *via* glycosylation of the 3-OH group in the glucosamine acceptor by acetylated ethylthiogalactoside in the presence of $NOBF_4$[40] in 76% yield. Galactosylation of the 4-OH group of glucosamine with thioglycosides was demonstrated in the work of Khatuntseva *et al.*, where lactosamine precursor of H (type 2) trisaccharide was obtained in 83% yield.[41]

Nilsson *et al.* also used thiogalactoside in the synthesis of H (type 2) trisachharide.[42] As described earlier for the synthesis of A_{tri} and B_{tri} by this group, *p*-chlorobenzyl protecting group was successfully used to facilitate the product isolation. Stereoselectivity of the reaction depends on conditions: single β-isomer (91%) was obtained using DMTST/CH_2Cl_2 at –10 ºC, whereas a mixture with β/α ratio of 8.8 : 1 (88%) was formed when MeOTf/Et_2O was used.

Karki *et al.* synthesized protected lactosamine by glycosylation of NPhth-glucosamine 3,4-diol with 2,3,4-tri-*O*-acetyl-6-*O*-benzyl SEt-galactoside. The reaction proceeded stereo- and regioselectively providing the required β1-4 glycoside in 92% yield.[25]

A cycle of three works published by Meloncelli *et al.* is one of the modern investigations of the chemical synthesis of blood group antigens.[43–45] A, B, and H antigens of all six types were obtained *via* the linear strategy using trichloroacetimidate glycosyl donors. The synthesis of H (type 2) trisaccharide is illustrated in Scheme 2. At the first step, core disaccharides of H (types 1–6) glycans were synthesized. The properly protected



Scheme 2 *Reagents and conditions*: i, TMSOTf, CH_2Cl_2; ii, MeONa, MeOH, then PivCl, pyridine; iii, TMSOTf, Et_2O.[43]

7-octenyl glycosides of 2-azido-2-deoxyglucose (for type 1 and 2 glycans), 2-azido-2-deoxygalactose (for type 3 and 4), galactose (for type 5) and glucose (for type 6) were used as glycosyl acceptors. The glycosylation reactions were carried out using 2,3-*O*-acetyl-4,6-*O*-benzylidenegalactosyl trichloroacetimidate in the presence of TMSOTf or $BF_3 \cdot Et_2O$ and provided 59–91% yields.

Fucosylation

After reprotection to deblock the 2′-OH group, the core disaccharides were subjected to fucosylation.

In the initial works on fucosylation, *i.e.* synthesis of terminal Fucα(1→2)Galβ fragment,[46,47] the glycosylation was carried out by acetylated fucosyl bromide under the classical Helferich conditions. Unsurprisingly, the total yields and stereoselectivity were not high, because the glycosyl donor possessed a participating acyl group at the C(2) atom, which facilitated the β-stereoselectivity instead of the required α one, and the yields of α-glycosides did not exceed 40%. Twenty years later Nifant'ev and co-workers demonstrated, that the replacement of acetyl groups with benzoyl ones in the glycosyl donor significantly increased both yield and stereoselectivity.[39] For instance, the condensation of benzoylated fucosyl bromide with type 1 disaccharide acceptor under the Helferich conditions provided a mixture of α- and β-glycosides with α/β ratio of 9 : 1, then the product of α-fucosylation was isolated in 71% yield.

Fucosyl bromide with nonparticipating benzyl group at the C(2) atom and *p*-nitrobenzoyl groups at the C(3) and C(4) ones was also proposed as glycosyl donor.[26,30,31,48] The acyl substituents at the C(3) or C(4) atoms of the flexible 6-deoxy cycle were supposed to form a bicyclic acyloxonium ion, in which β-side was blocked for a nucleophilic attack,[47] thus increasing the α-stereoselectivity of glycosylation. The results of application of this bromide were somewhat contradictory, this led to only 25% yield when reacted with type 1 core disaccharide in the presence of $Hg(CN)_2$, whereas in the condensation with very similar acceptor in the presence of $Hg(CN)_2/HgBr_2$ promoting system this bromide provided 65% yield of α-fucoside.

Introduced in the practice of glycosidic synthesis in the middle of 70s, the benzylated fucosyl bromide allowed the synthetic chemists to increase yields of α-fucosides significantly. The Lemieux fucosylation procedure using this bromide with halide ion catalysis remains one of the widely applied nowadays. These conditions were used in numerous works[27–29,34–37,42] and provided 63–94% yields of α-fucosides. A low outcome was obtained only once,[34] when the fucosylation of lactosamine acceptor proceeded in 50% yield under the Lemieux conditions and in 80% yield under the Helferich conditions. Possible reason for this poor result was described later.[36] *N*-Fucosyl imidates were supposed to be formed as by-products in the presence of Pr^i_2NEt, and the use of molecular sieves instead of Pr^iNEt_2 increased the yield of H trisaccharide from 44 to 94%. Nilsson *et al.* demonstrated[42] that the stereoselectivity of the Lemieux fucosylation was higher (90% of α-fucoside) than that of glycosylation with *p*-chlorobenzylated fucoside promoted with DMTSB (65% of α-anomer and 19% of β-anomer).

Glycosylation with the same benzylated fucosyl bromide in the presence of AgOTf was used by Pazynina *et al.* to obtain H (type 4) trisaccharide (72% yield).[38]

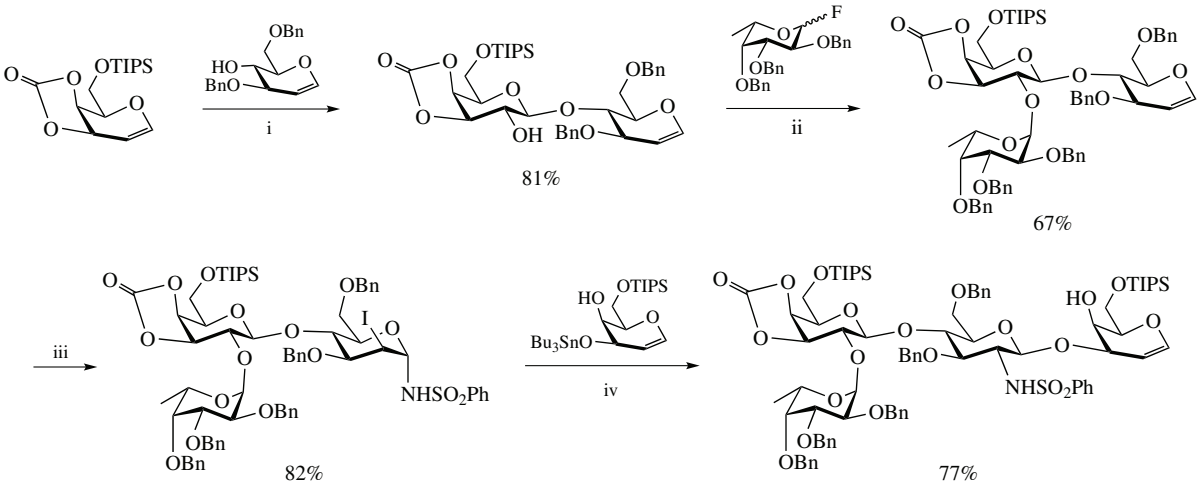
An example of successful use of thioglycoside as a fucosyl donor was described by Khatuntseva *et al.*,[41] where H (type 2) trisaccharide was obtained using benzylated ethylthiofucoside in 76% yield. The same fucosyl donor was used by Karki *et al.*,[25] where the glycosylation of 3,2′-OH lactosamine diol regioselectively provided H (type 2) trisaccharide in 83% yield.

Employment of an imidate method for the introduction of α-fucosyl unit in the synthesis of H glycans is also described.[33,43–45] Milat and Sinaÿ used benzylated *N*-methylacetimidate of fucose in the presence of TsOH (89% yield).[33] Meloncelli *et al.* applied benzylated β-trichloroacetimidate of fucose in the synthesis of H, A and B glycans of all six types, an example for H_{tri} (type 2) is shown in Scheme 2.[43–45] The reactions were carried out in Et_2O in the presence of TMSOTf, affording α-fucosides in 72–94% yields.

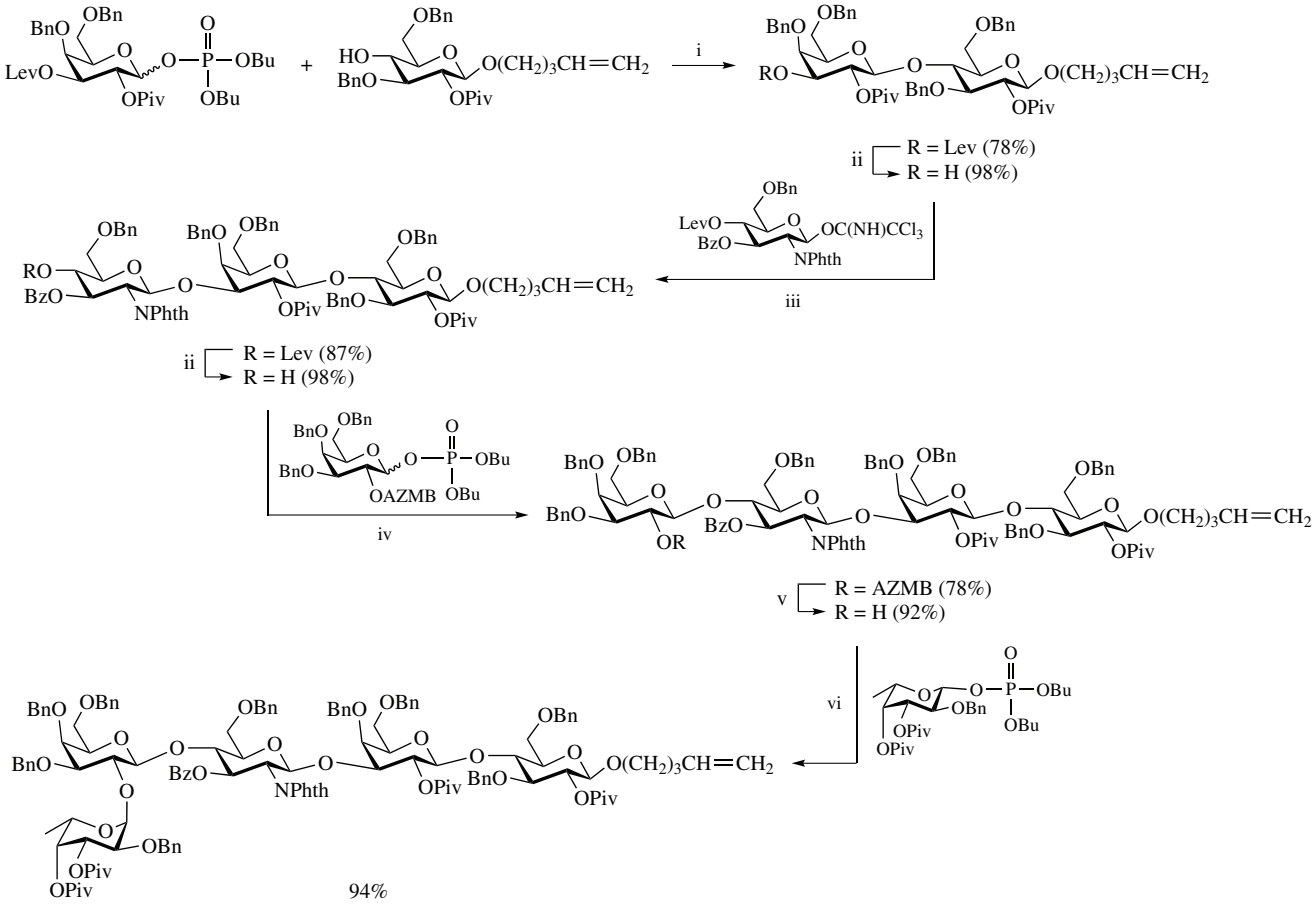
Synthesis of complex H oligosaccharides

Synthesis of more complex H glycans with core chain longer than the disaccharide one was accomplished by several groups.[49–52]

Danishefsky and co-workers developed a strategy based on the coupling of glycals, which was called glycal assembly approach. The method comprises the transformation of glycal, *i.e.* 1,2-unsaturated saccharide, to hexosyl or hexosaminyl donor and its further *in situ* coupling with another glycal as a glycosyl acceptor. The product is in turn activated and involved in the next coupling step as a glycosyl donor. Thus, the saccharide chain is elongated from the non-reducing end, contrary to classical linear oligosaccharide synthesis. This approach was successfully used for the synthesis of H (type 1) trisaccharide.[49] Condensation of protected galactal as glycosyl donor with 3,4-OH glucal diol as glycosyl acceptor resulted in disaccharide glycal (68%), which

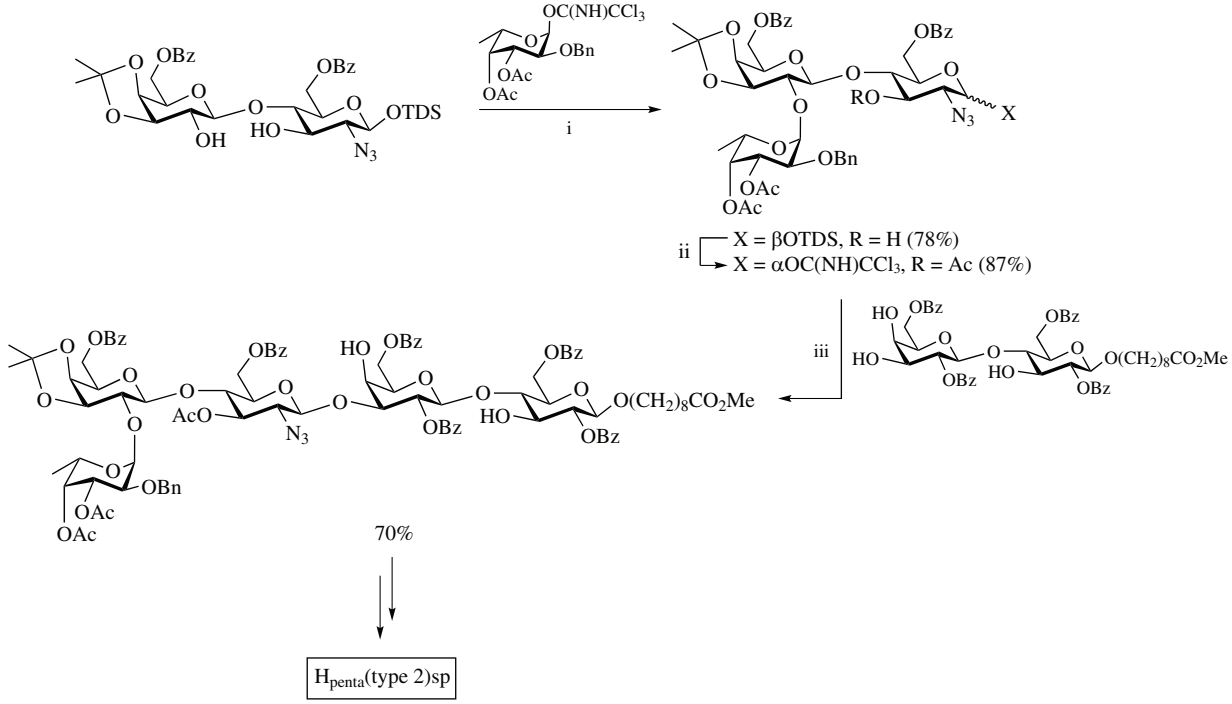


Scheme 3 *Reagents and conditions*: i, DMDO, CH_2Cl_2, then glycosyl aceptor, $ZnCl_2$, THF; ii, $Sn(OTf)_2$, DTBP, THF; iii, $I(coll)_2ClO_4$, $PhSO_2NH_2$, CH_2Cl_2; iv, $AgBF_4$, THF.[49,53]



Scheme 4 *Reagents and conditions*: i, TBSOTf, CH_2Cl_2, –78 °C; ii, N_2H_4·HOAc, CH_2Cl_2, MeOH; iii, TMSOTf, CH_2Cl_2, –20 °C; iv, TMSOTf, CH_2Cl_2, –78 °C; v, PBu_3, THF, H_2O; vi, TMSOTf, CH_2Cl_2, –20 °C.[51]

was then glycosylated with 2,3-*O*-benzyl-4-*O*-benzoylfucosyl fluoride in the presence of $AgClO_4/SnCl_2$ in 71% yield. The same method was applied to the synthesis of H (type 2) tetrasaccharide (Scheme 3). The assembly of core disaccharide and the fucosylation were carried out in the same way, but using monohydroxyl 4-OH acceptor. To turn the trisaccharide glycal into the glycoside of glucosamine, the authors employed so called 'sulfonamide glycosylation' protocol,[53] where glycal was transformed into 2-iodoglycosylsulfonamide, whose condensation with glycosyl acceptor was accompanied by migration of



Scheme 5 *Reagents and conditions*: i, $ZnCl_2$·OEt_2, CH_2Cl_2; ii, Ac_2O, pyridine, then TBAF, THF, then Cl_3CCN, DBU; iii, BF_3·OEt_2, CH_2Cl_2, *n*-C_6H_{14}, –10 °C.[52]

sulfonamide moiety to the C(2) atom of the donor. Unfortunately, the authors did not describe further derivatization or deprotection of the obtained H (type 1) trisaccharide and H (type 2) tetrasaccharide glycals.

Love *et al.* reported an application of dibutylphosphate glycosyl donors for the synthesis of H (type 2) glycans in the form of trisaccharide[50] and pentasaccharide[51] (Scheme 4). The usual linear synthetic scheme was chosen, dibutylphosphates were used as galactosyl and fucosyl donors with promotion by TBSOTf, and trichloroacetimidate was applied as glucosaminyl donor with promotion by TMSOTf. All the glycosylations proceeded almost quantitatively in 90–98% yields.

Contrary to the previous works, Windmüller and Schmidt obtained H (type 2) pentasaccharide *via* a block synthetic approach using trichloroacetimidate method of glycosylation (Scheme 5).[52] Regioselective fucosylation of 2′,3-diol lactose derivative provided H trisaccharide block (78%), which was converted into the corresponding glycosyl donor and then coupled with spacer armed lactose, affording H (type 2) pentasacchride in 70% yield. The high β-stereoselectivity of the [3+2] coupling, despite the presence of non-participating azido group at the C(2) atom of the glycosyl donor, should be emphasized.

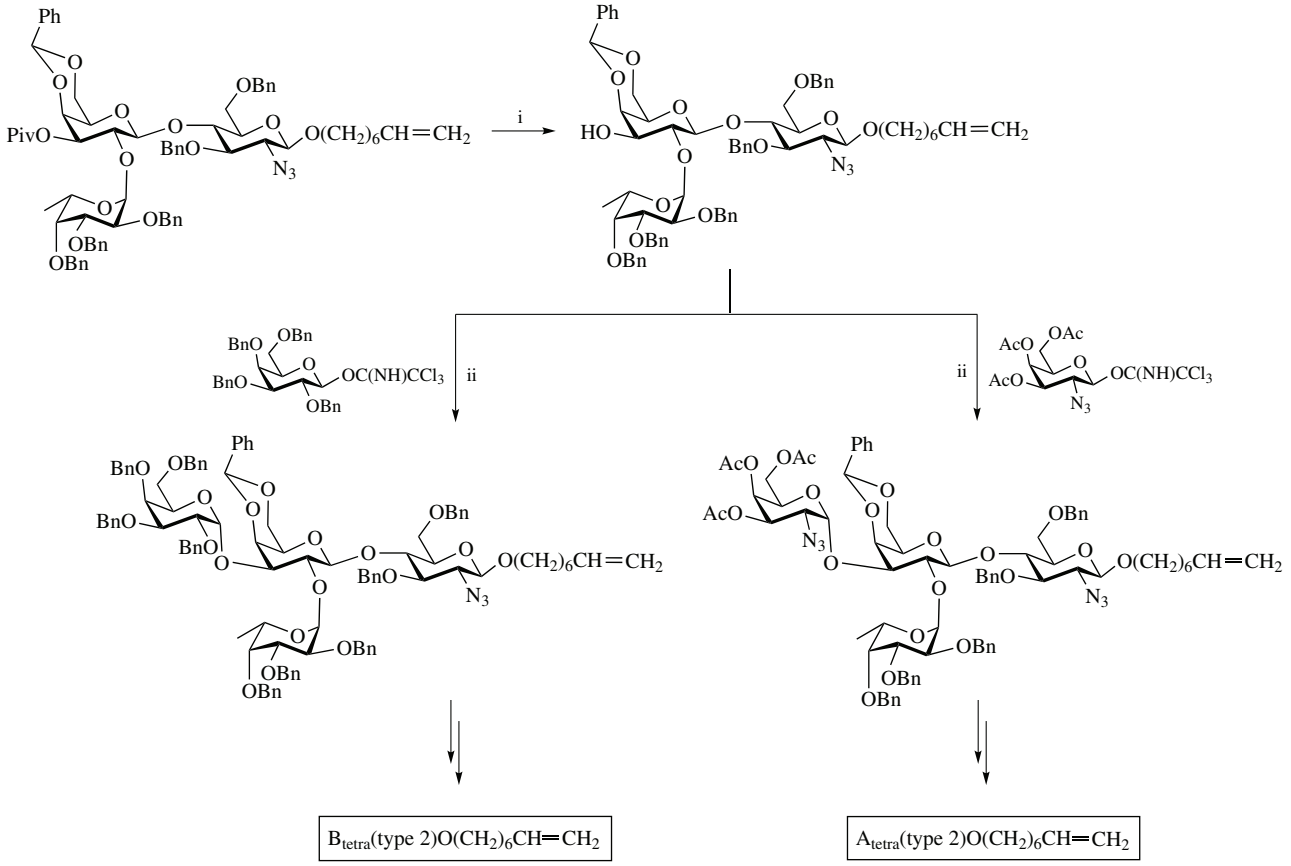
Synthesis of A and B tetrasaccharides

The classical scheme of the synthesis of A and B tetrasaccharides includes galactosaminylation or galactosylation of the 3′-OH group in the corresponding H trisaccharide glycosyl acceptors, the synthesis of the latter being described above. This route simulates the biosynthesis of these oligosaccharides, its main advantage being the ability to synthesize A, B and H glycans of one type by a single scheme, as illustrated in Scheme 6 for the synthesis of A_{tetra} (type 2) and B_{tetra} (type 2) saccharides by Melonchelli *et al.*[43]

In the most of the works considered, glycosyl halides were used as glycosylating agents and 2-azido-2-deoxygalactose served as a precursor for α-*N*-acetylgalactosamine, since the installation of α-glycosidic linkage required non-participating N_3 group at the C(2) atom of the glycosyl donor. Paulsen and Kolář proposed two methods for the synthesis of A tetrasaccharides using acylated 2-azido-2-deoxygalactosyl halides.[29,34] The first one was based on the glycosylation with acetylated $GalN_3$ β-chloride in the presence of $AgClO_4$ and Ag_2CO_3. This protocol resulted in A (type 1) tetrasaccharide in 46% yield. Application of the same procedure to the synthesis of A (type 2) glycan was later described by Khatuntseva *et al.*,[41] where the glycosylation of H (type 2) trisaccharide acceptor proceeded at both the 3′-OH group and the acetamido group at the C(2) atom of GlcNAc. To decompose an *N*-glycosyl imidate by-product, the mixture was treated by 90% TFA, and finally the yield of the required tetrasaccharide was 50%.

Another method for the synthesis of A tetrasaccharides, proposed by Paulsen and Kolář, consisted in glycosylation of 2-azido-2-deoxygalactose with acetylated α-bromide under the Helferich conditions. This approach allowed the authors to achieve better results as compared to the first one, namely A (type 1)[29] and A (type 2)[34] tetrasaccharides were obtained in 63 and 88% yields, respectively.

Bovin and co-workers carried out a detailed analysis of methods for α-galactosaminylation in the synthesis of blood group A oligosaccharides.[31] The best result was achieved using benzylated chloride of 2-azido-2-deoxygalactose in the presence of Ag_2CO_3 and AgOTf. The rejection of acetylated glycosyl donors originated from the possibility of the side reaction of aglycon acetylation. The application of the chosen method allowed the authors to obtain A (type 1) tetrasaccharide in 92% yield, while A (type 3) tetrasaccharide was synthesized



Scheme 6 *Reagents and conditions*: i, MeOLi, MeOH; ii, TMSOTf, Et_2O.[43]

in 42% yield in three steps, namely glycosylation, deblocking and the $N_3 \rightarrow NHAc$ transformation.[35] Synthesis of A (type 2) tetrasaccharide was performed in this laboratory much later and was carried out in a different way using benzylated bromide of azidogalactose in the presence of AgOTf (40% yield).[37]

The benzylated galactosyl bromide was the only glycosyl halide utilized for the synthesis of blood group B tetrasaccharides from H trisaccharides. The synthesis of B (type 1) tetrasaccharide with this donor was described by two groups, namely Paulsen and Kolář,[29] using the Helferich conditions (78% yield), and Bovin *et al.*,[30] with $AgClO_4$ and the additional diphenylcyclopropenyl activation of nucleophile[54] (60% yield).

The synthesis of B (type 2) tetrasaccharide from benzylated galactosyl bromide was performed by the same groups, *i.e.* Paulsen and Kolář,[34] using AgOTf and Ag_2CO_3 (82% yield), and Pazynina *et al.*,[37] where, unlike in other works, H trisaccharide 3′4′-diol was applied as the glycosyl acceptor, and galactosylation in the presence of AgOTf resulted in the required α1-3-glycoside in 50% yield and its α1-4-isomer in 28% yield.

B (type 3) tetrasaccharide was also synthesized by the Bovin's group,[35] where the glycosylation under the Helferich conditions proceeded in 84% yield.

The imidate strategy for the synthesis of B (type 2) tetrasaccharide was implemented by Milat and Sinaÿ,[33] the α-galactosylation with benzylated *N*-methylimidate afforded the required saccharide in 91% yield.

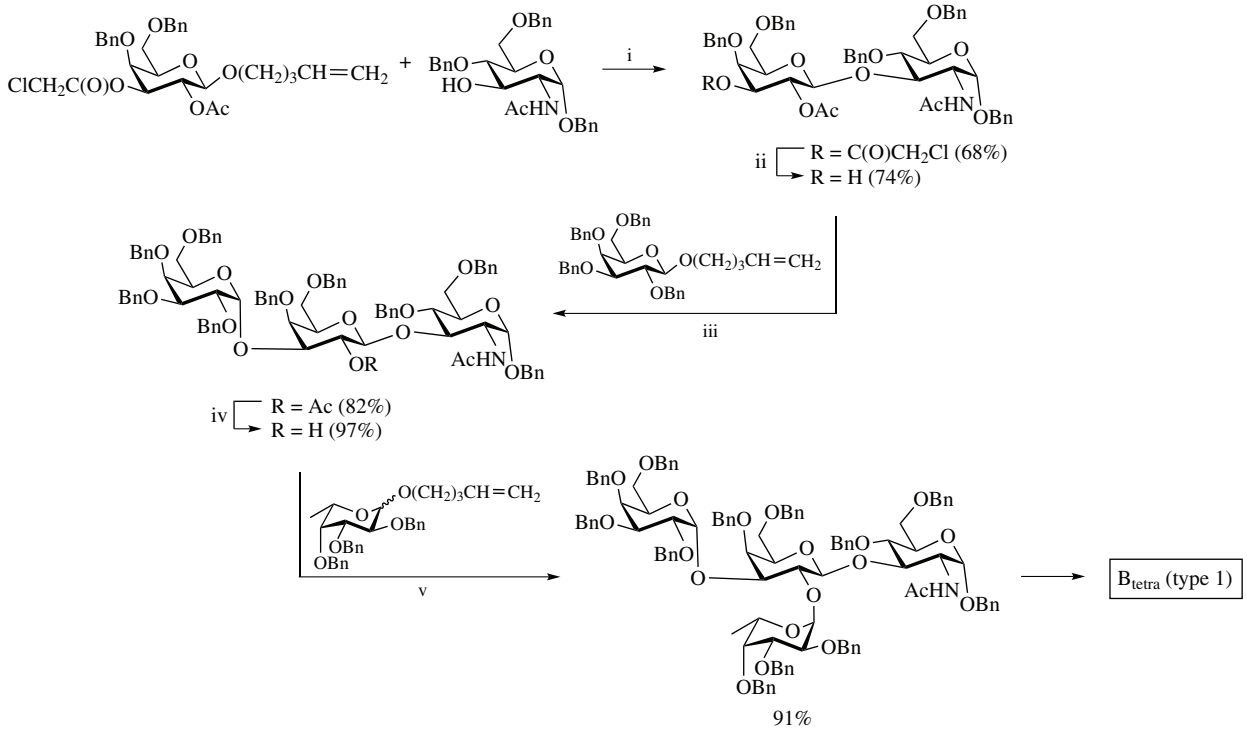
In the modern cycle of works authored by Meloncelli *et al.*,[43–45] A (type 1–6) and B (type 1–6) tetrasaccharides were obtained from the corresponding H trisaccharides using acetylated trichloroacetimidate of 2-azido-2-deoxygalactose and benzylated trichloroacetimidate of galactose for A and B antigens, respectively (see Scheme 6, example of type 2 glycans). The glycosylation yields for A (types 1–4) tetrasaccharides were 71–94%. For A (types 5 and 6) tetrasaccharides the combined yields of 51 and 47%, respectively, were demonstrated for the three steps, namely the glycosylation, the reduction of azido group and its following acetylation. B (types 1–6) tetrasaccharides were synthesized analogously in 60–86% yields.

Synthesis of A and B tetrasaccharides using nonclassical synthetic schemes

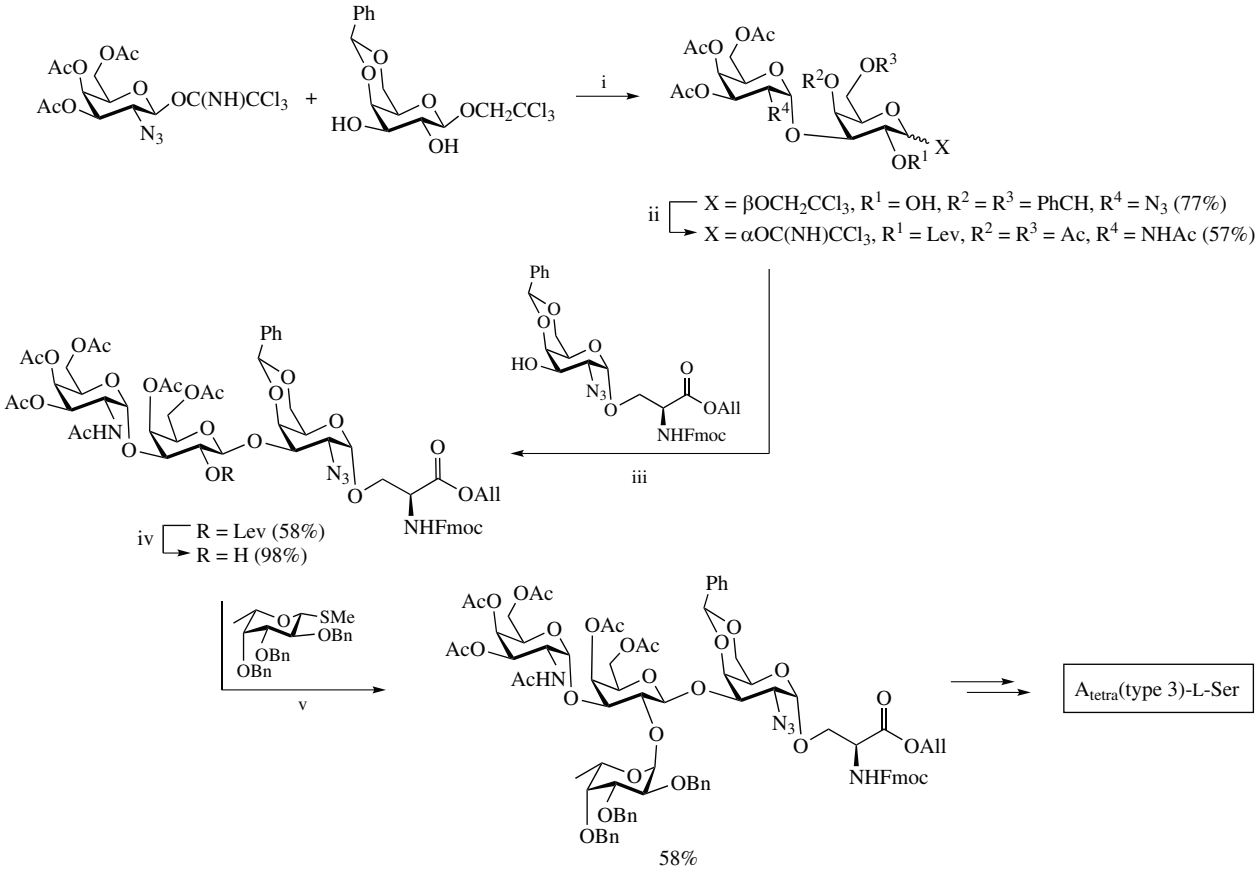
Some authors have chosen nontraditional way to the blood group A and B glycans. Udodong *et al.* reported the synthesis of B (type 1) tetrasaccharide based on the *n*-pentenyl glycoside strategy (Scheme 7).[55] The main difference of this approach from the classical scheme consisted in the sequence of the introduction of monosaccharide units, namely the Galα unit was installed to the 3′-OH group of the core disaccharide prior to the Fucα unit to the 2′-OH group. All the glycosylation reactions proceeded in high yields and stereoselectivity. Unfortunately, acid labile protecting groups, for instance benzylidene acetal, turned out to be unstable under the glycosylation conditions (NIS and Et_3SiOTf).

Macindoe *et al.* synthesized A (type 3) tetrasaccharide linked to L-serine as a structural motif found in O-chains of natural glycoproteins (Scheme 8).[56] At the first step, the terminal disaccharide block $GalN_3α1$-3Galβ was obtained (77%), then it was transformed into the corresponding trichloroacetimidate and coupled with the 3-OH group of the $GalN_3α1$-L-Ser acceptor (58% yield). Finally, the fucose unit was inserted using benzylated thioglycoside, in 58% yield for the required α-fucoside and 14% yield for the β-fucoside by-product.

Ryzhov *et al.* proposed a blockwise approach to A and B blood group glycans of types 1–4.[57–59] The target tetrasaccharides were synthesized *via* a [3+1] block scheme, where '3' denoted a glycosyl donor common for all A or B antigens, namely peracetylated trichloroacetimidate of A or B trisaccharide, and '1' was a properly protected glucosamine



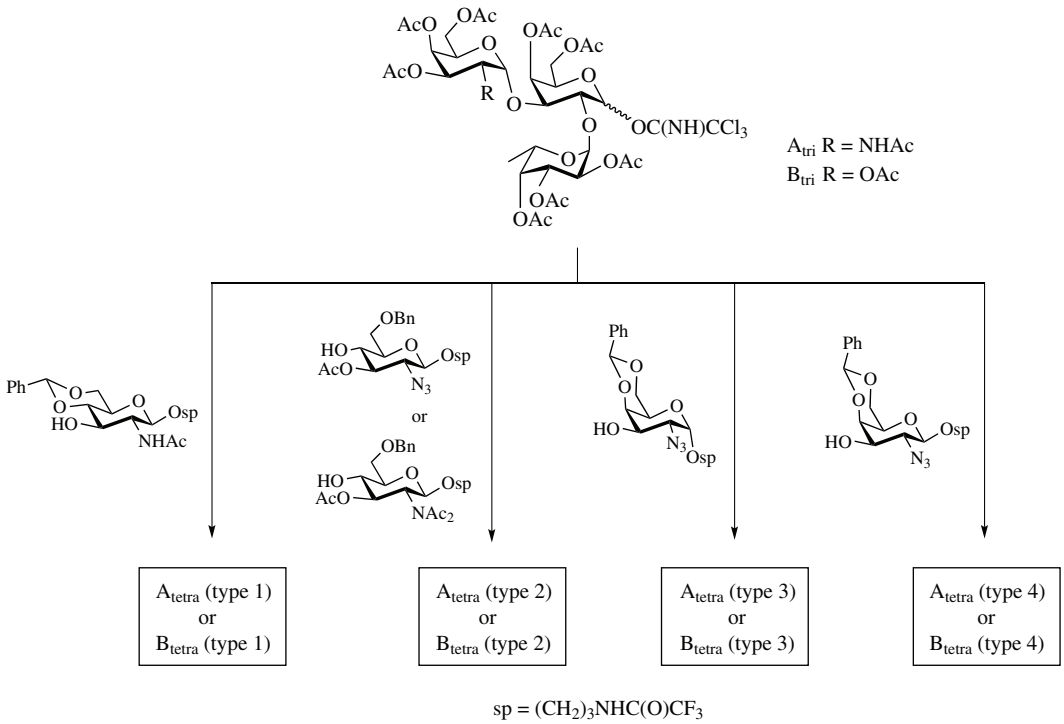
Scheme 7 *Reagents and conditions*: i, NIS, Et_3SiOTf, CH_2Cl_2; ii, N_2H_4·AcOH, CH_2Cl_2; iii, NIS, Et_3SiOTf, CH_2Cl_2; iv, NaOMe, MeOH, CH_2Cl_2; v, NIS, Et_3SiOTf, CH_2Cl_2.[55]



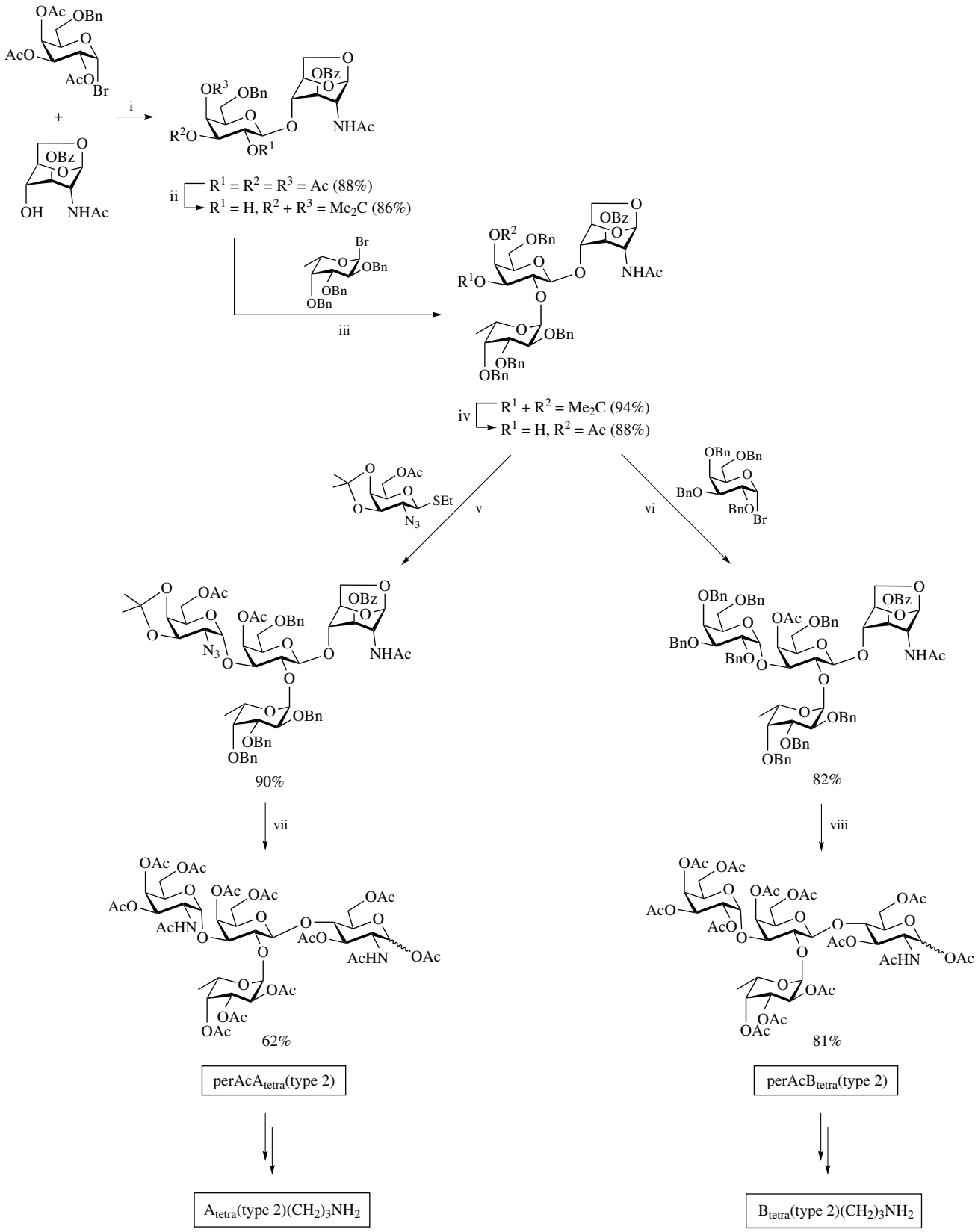
Scheme 8 *Reagents and conditions*: i, TMSOTf, CH_2Cl_2; ii, AcSH, CH_2Cl_2, then TFA, H_2O, CH_2Cl_2, then Ac_2O, DMAP, pyridine, then Zn, AcOH, THF, then Cl_3CCN, DBU, CH_2Cl_2; iii, TMSOTf, CH_2Cl_2, –20 °C; iv, N_2H_4·HOAc, PhMe; v, AgOTf, $CuBr_2$, Bu_4NBr, CH_2Cl_2, PhMe.[56]

or galactosamine acceptor (Scheme 9). The main advantage of this approach was the possibility to obtain a complete library of A and B glycans of the four types with minimal number of steps and an economy in time, labor and starting materials, due to the use of the common trisaccharide moiety. The drawbacks comprised the impossibility to obtain H trisaccharides and the modest yields of the [3+1] coupling in the synthesis of A (type 2) and B (type 2) tetrasaccharides, namely 35–55%, while the coupling yields exceeded 80% for the types 1, 3 and 4.

To overcome the above problem on the way to A (type 2) and B (type 2) tetrasaccharides, this group elaborated another

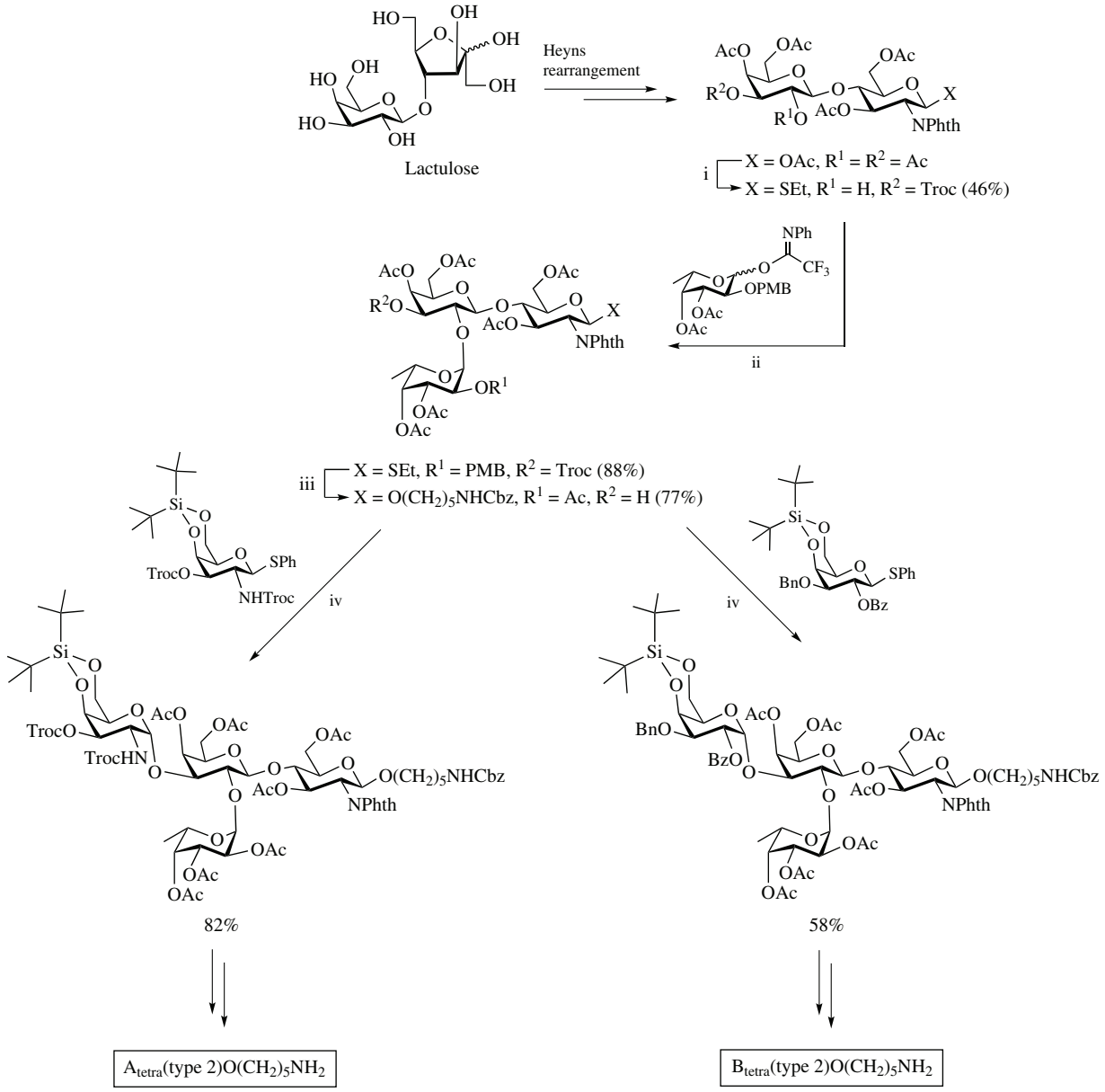


Scheme 9 *Reagents and conditions*: TMSOTf, MeCN for all the 1–4 types.[57–59]



Scheme 10 *Reagents and conditions*: i, $HgBr_2$, $Hg(CN)_2$, $C_2H_4Cl_2$; ii, HCl, MeOH, then Ac_2O, $NaHCO_3$ aq., then $Me_2C(OMe)_2$, CSA, CH_2Cl_2; iii, Et_4NBr, $C_2H_4Cl_2$; iv, TFA, CH_2Cl_2, then $MeC(OEt)_3$, TsOH, CH_2Cl_2, then AcOH; v, NIS, TfOH, Et_2O; vi, AgOTf, TMU, $C_2H_4Cl_2$; vii, TFA, CH_2Cl_2, then MeONa, MeOH, then H_2, Pd/C, Ac_2O, MeOH, then Ac_2O, DMAP, pyridine, then H_2SO_4, Ac_2O, AcOH, then NaOAc; viii, MeONa, MeOH, then H_2, Pd/C, then Ac_2O, DMAP, pyridine, then H_2SO_4, Ac_2O, AcOH, then NaOAc.[60]

protocol providing the synthesis of target compounds in multigram scale.[60] The scheme used the classical linear approach, but 1,6-anhydro-*N*-acelyglucosamine was chosen as the starting unit (Scheme 10). The 1,6-anhydro cycle served as a temporary protection for the anomeric center and simplified the isolation of intermediate compounds, since they crystallized easily. The Galβ and Fucα units were introduced *via* the corresponding glycosyl bromides under the Helferich and Lemieux conditions, respectively. Further attachment of the Galα moiety to obtain B (type 2)



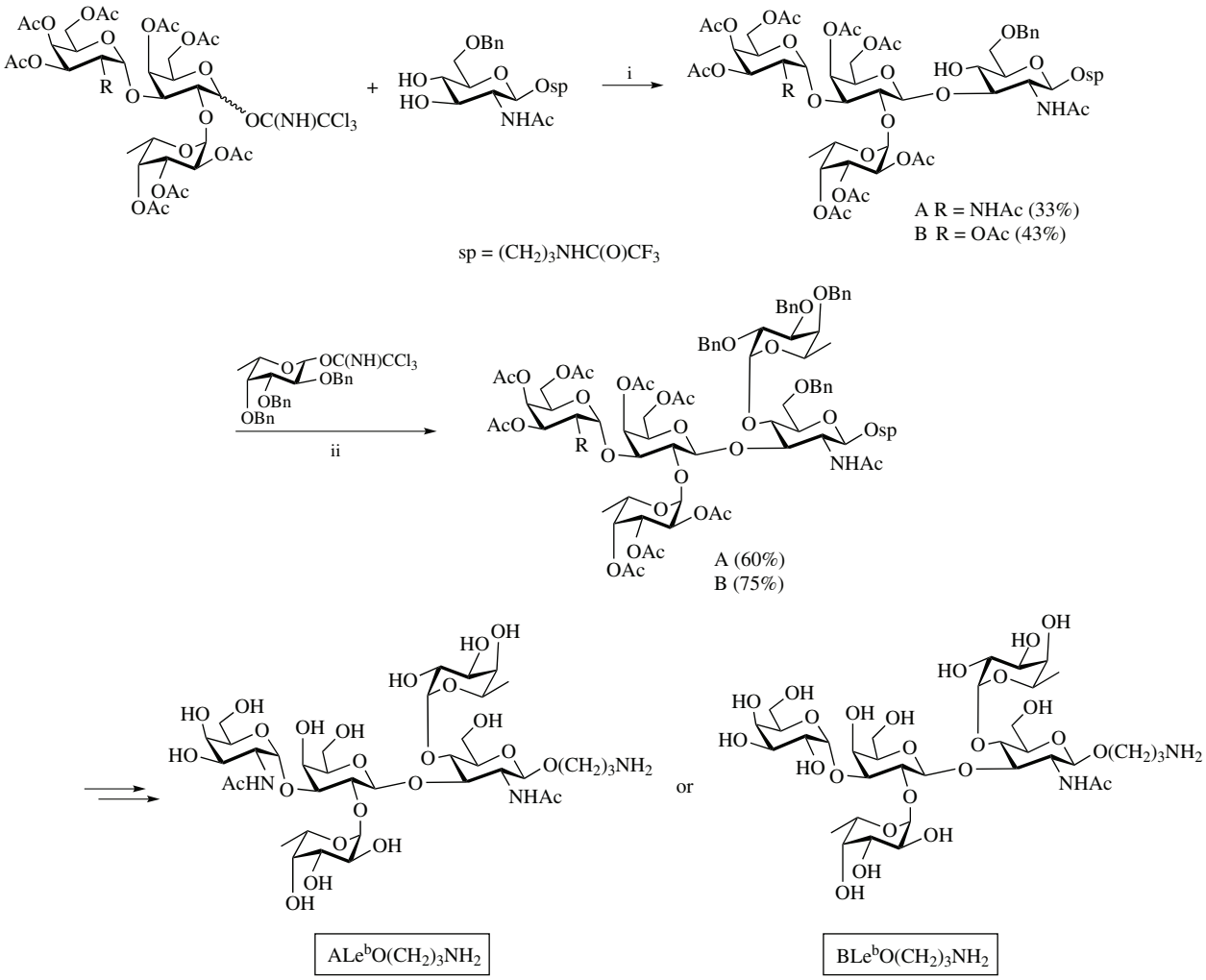
Scheme 11 *Reagents and conditions*: i, EtSH, $BF_3{\cdot}Et_2O$, $C_2H_4Cl_2$, then NaOMe, MeOH, CH_2Cl_2, then butane-2,3-dione, CSA, $MeC(OMe)_3$, MeOH, then Ac_2O, DMAP, pyridine, then TFA, TrocCl, Bu_2SnCl_2, PEMP, acetone; ii, TMSOTf, CPME, CH_2Cl_2; iii, TFA, CH_2Cl_2, then Ac_2O, pyridine, then $HO(CH_2)_5NHCbz$, NIS, TfOH, CH_2Cl_2, then Zn, AcOH, $C_2H_4Cl_2$; iv, NIS, TfOH, CH_2Cl_2.[63]

tetrasaccharide was carried out by benzylated galactosyl bromide in the presence of AgOTf. To obtain A (type 2) tetrasaccharide, different galactosaminyl donors were tested. The best results were demonstrated when 6-*O*-acetyl-3,4-*O*-isopropylidene thioglycoside of 2-azido-2-deoxygalactose was used (89% yield, α/β 24 : 1). The high stereoselectivity was achieved *via* the conformational assistance of isopropylidene acetal, which prevented the nucleophilic attack from the β-side in the structurally rigid bicyclic glycosyl cation.[61,62] The 1,6-anhydro cycle was opened after the complete assembly of tetrasaccharides, thus enabling the introduction of any aglycon, as well as the transformation of the obtained glycans to glycosyl donors and further synthesis of higher complex blood group oligosaccharides.

Hara *et al*. proposed another interesting approach (Scheme 11).[63] This group synthesized type 2 core disaccharide (lactosamine) from lactulose (4-*O*-β-D-galactopyranosyl-D-fructose) *via* the Heyns rearrangement,[64–66] as this way appeared to be synthetically simple, required only three steps and used lactulose as an inexpensive available starting material. The product obtained was converted to ethyl thioglycoside and, after the reprotection to liberate the 2′-OH group, was glycosylated by fucosyl *N*-phenyl trifluoroacetimidate (88%), and finally the spacer arm aglycon was installed. Another interesting synthetic example was the regioselective introduction of the Troc group at the C(3′) atom.[67] After the H (type 2) trisaccharide acceptor was obtained, the galactosaminyl or galactosyl units were installed using the conformationally rigid SEt glycosides. The rigidity of the glycosyl donors was achieved using the 4,6-*O*-di-*tert*-butylsilylene group.[68] This conformational assistance provided high α-stereoselectivity of both glycosylation reactions, despite the presence of participating NHTroc and OBz substituents at the C(2) atom of glycosyl donors. The A (type 2) and B (type 2) tetrasaccharides were obtained in 82 and 58% yields, respectively.

Synthesis of chimeric Lewis/ABH glycans

The Lewis antigens are a family of fucosylated oligosaccharides structurally related to ABH glycans.[69] This family includes trisaccharide Le^a and terasaccharide Le^b, both comprising type 1



Scheme 12 *Reagents and conditions*: i, TMSOTf, CH_2Cl_2, MeCN; ii, TMSOTf, CH_2Cl_2, Et_2O.[70]

core structure. Trisaccharide Le^x and tetrasaccharide Le^y, despite their names, do not represent conventional Lewis antigens and are composed of type 2 core structure. The most complex representatives of this family are so called chimeric Lewis/ABH oligosaccharides ALe^b, BLe^b, ALe^y and BLe^y, which possess structural features of both A and B glycans [GalNAcα or Galα at the C(3) atom of Galβ] and Lewis glycans [Fucα at the C(3) or C(4) atoms of GlcNAcβ].

The first effort to synthesize chimeric Lewis/ABH glycans was made by Ryzhov *et al.*[70] The [3+1] block scheme described earlier, which employs *N*-acetylglucosamine 3,4-diol as a glycosyl acceptor, could provide both β1-3 and β1-4 glycosides, and the fucosylation of the remaining OH group would in turn give A(B)Le^b or A(B)Le^y pentasaccharides, respectively. Unfortunately, only β1-3 glycosides were isolated in moderate yields of 33 and 43% for A and B ones, respectively, whereas the β1-4 products were obtained in minor amounts. The fucosylation of the O(4) atom in GlcNAc moiety by benzylated fucosyl trichloroacetimidate afforded A Lewis b and B Lewis b pentasaccharides (Scheme 12).

To obtain ALe^y and BLe^y pentasaccharides, Pazynina *et al.* chose the linear synthetic strategy[71] close to the synthesis of A (type 2) and B (type 2) tetrasaccharides. Lactosamine 3,2′-diol was fucosylated under the Lemieux conditions with the excess of glycosyl donor, affording Le^y tetrasaccharide,[37] which was further glycosylated at the preliminary deblocked 3-OH group of the Galβ unit by benzylated bromides of 2-azido-2-deoxygalactose (for ALe^y, 50% yield) or galactose (for BLe^y, 71% yield) in the presence of AgOTf (Scheme 13).

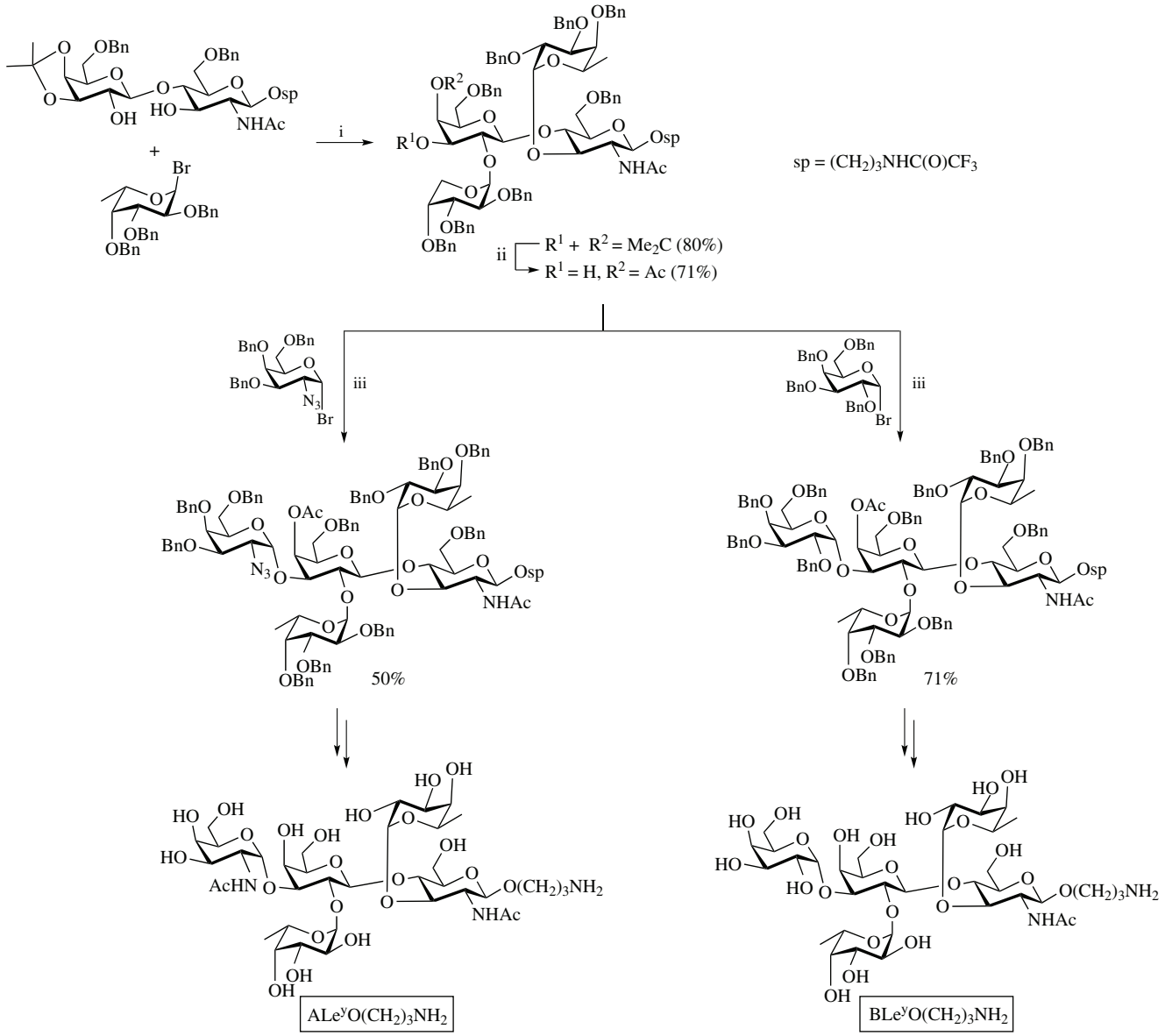
Synthesis of complex A and B glycans

The chemical synthesis of blood group B (type 4) hexasaccharide was reported by Zimmermann *et al.* (Scheme 14).[72] The synthesis was based entirely on the trichloroacetimidate glycosylation approach. At the first step, type 4 core disaccharide was obtained and converted to 2′,3′-diol. The Galα and Fucα units were then sequentially installed using the difference in reactivity for the 3'- and 2′-OH groups. The assembled tetrasaccharide was transferred to glycosyl trichloroacetimidate and reacted with lactose glycosyl acceptor (49% yield).

A project aimed at the synthesis of A (type 2) and B (type 2) biantennary tetradecasaccharides is now ongoing in the Ryzhov's group. The synthetic route involves the coupling of the A (type 2) and B (type 2) tetrasaccharide glycosyl donors obtained from the corresponding acetates[60] (see above) and the branched 3′,6′-tri(lactosamine) acceptor (Scheme 15).

Function–spacer–lipid constructs and kodecytes in blood typing

Recently, the function–spacer–lipid constructs (FSLs) have been developed, which represent artificial glycolipids consisting of three structural motifs, namely the functional part (glycan of interest), the spacer arm and the lipid part (usually dioleoylphosphatidyl ethanolamine, DE) capable of incorporating into cell membrane.[73,74] A key feature of FSLs is the presence of an 'active' spacer arm, which provides the molecule with required properties. The spacer allows one to vary such parameters of the molecule as the rigidity, the distance between the glycan and the lipid parts, and also improves the solubility in



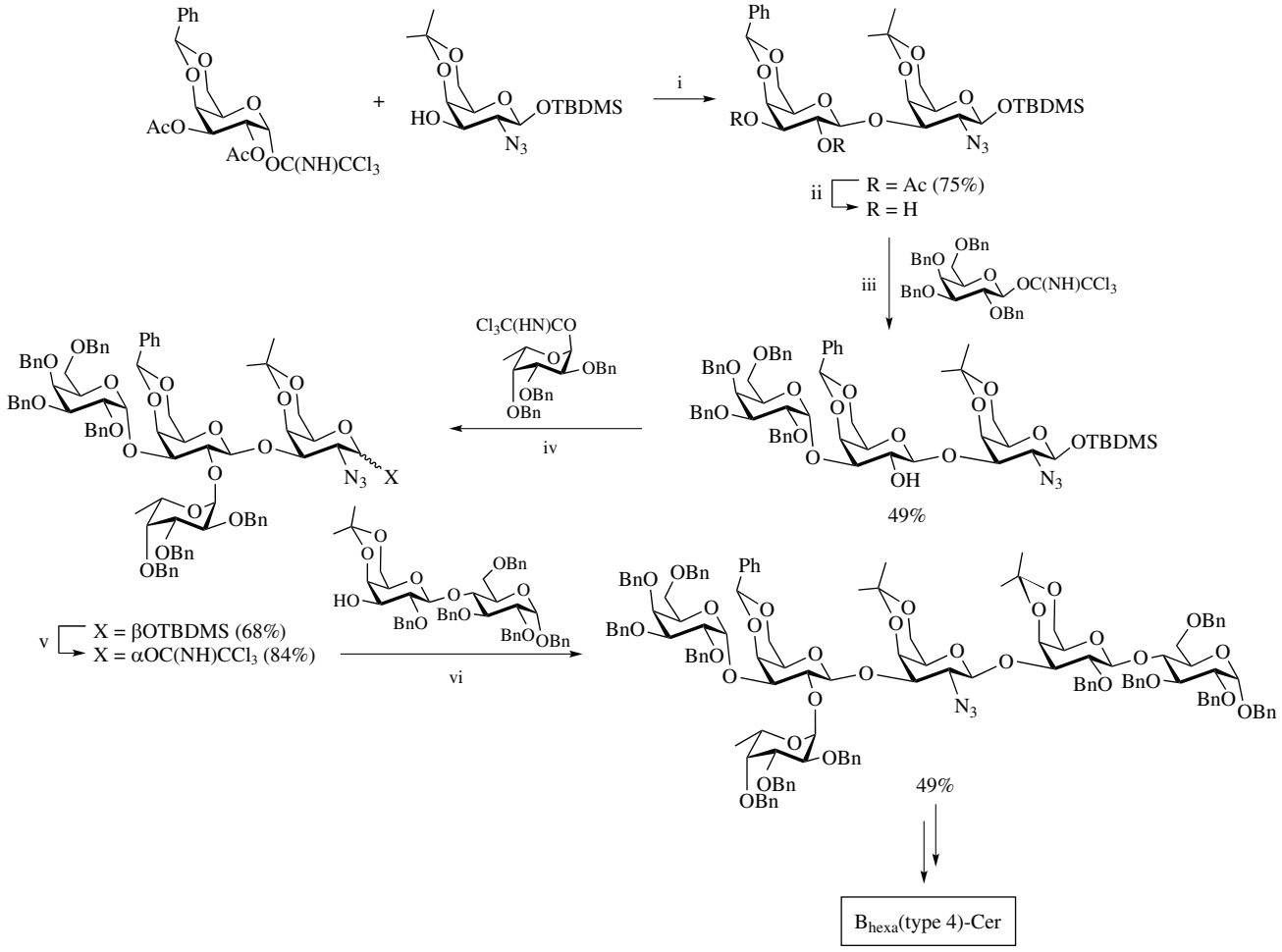
Scheme 13 *Reagents and conditions*: i, Et_4NBr, Pr^i_2NEt, CH_2Cl_2; ii, TFA, $CHCl_3$, then $MeC(OEt)_3$, TsOH, MeCN, then AcOH; iii, AgOTf, TMU, CH_2Cl_2.[37,71]

aqueous medium. FSLs can be incorporated into the cell membrane without damaging or killing the cells. This insertion proceeds in a bespoke manner, since the density of the inserted glycans can be varied and estimated. The technology employing the incorporation of FSLs into the living cells is known as KODE technology and the modified cells are known as kodecytes.

An application of the KODE technology to O erythrocytes (*i.e.* lacking A or B antigens) provides the corresponding A and B kodecytes. The advantages of these kodecytes over natural RBCs are the following: (i) kodecytes possess only one type of A or B antigen with known synthetically determined structure, while the natural cells carry a wide diversity of A or B glycans; (ii) the antigen density on the surface of kodecytes can be varied and estimated, which allows us to obtain standard cells for practical application, while the properties of natural red blood cells always depend on the individual characteristics of a person; and (iii) the use of different spacer arms allows us to change the immunological presentation of glycan on the cell surface.

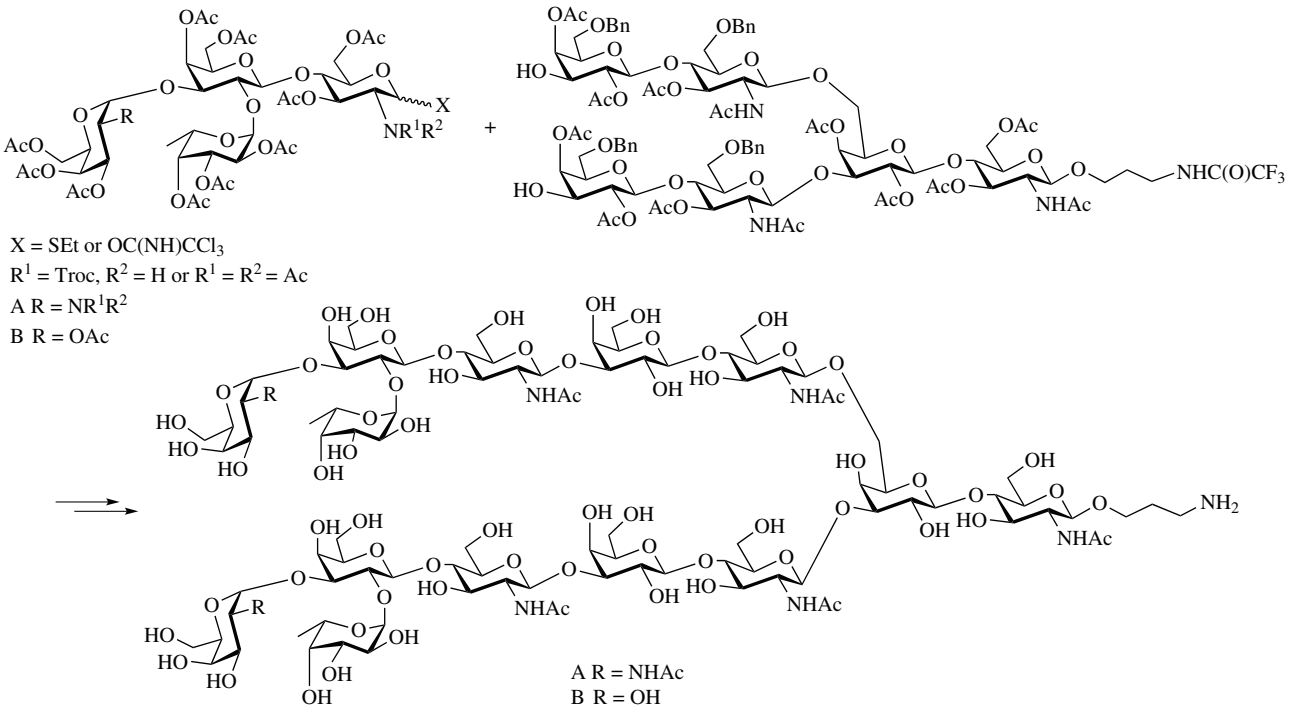
The three examples of FSLs carrying A_{tetra} (type 2) antigen are shown in Figure 2. The A_{tetra}(type 2)–Ad–DE construct possesses a short (2 nm) adipoyl spacer arm. In this case, the glycan moiety is situated close to the cell membrane. The A_{tetra}(type 2)–CMG–DE construct has longer (7 nm) oligopeptide spacer arm, which consists of glycine and carboxymethylglycine (CMG) residues. The presence of carboxyl groups along the chain provides the structural rigidity of the spacer arm, thus glycans are distanced away from the membrane into the thickness of the cell glycocalyx, and their availability for binding with antibodies is increased. The $[A_{tetra}(type\ 2)]_3$–T17–DE FSL has a trivalent spacer built of four CMG fragments, one of the vertices of the formed tetrahedron is linked to the lipid DE part, and the three other ones are connected to the functional glycans. Note that the distance between glycans in this construct corresponds to the one between two Fab-fragments of immunoglobulins. Such a construct mimics complex branched ABH glycans and enables the true multivalent interaction with antibodies.

The A and B FSLs were used for the mapping of fine specificity of the commercial monoclonal reagents employed for ABO typing in the clinical practice.[75] The two types of experiments were carried out, namely using the kodecytes and the FSLs printed on paper. Later, similar investigation was conducted for Lewis antigens, including chimeric Le/ABH structures.[76] The use of kodecytes allowed us not only to reveal specificities of anti-Lewis antibodies, but also to estimate the ratio of Le^b antigen levels on various RBCs, thus comparing the agglutination of these cells with the agglutination of kodecytes



Scheme 14 *Reagents and conditions*: i, $BF_3{\cdot}Et_2O$; ii, MeONa, MeOH; iii, TMSOTf; iv, TMSOTf; v, TBAF, THF, then Cl_3CCN, DBU; vi, $ZnCl_2{\cdot}Et_2O$.[72]

possessing different density of FSLs. The same approach was later proposed for the measurement of ABO antibodies level in blood before the ABO-incompatible transplantation.[77] The data on agglutination of kodecytes having different level of FSLs with undiluted plasma of patients potentially provides better accuracy than the traditional plasma dilution approach. Kodecytes were also used for an investigation of the exact mechanism of agglutination.[78] The agglutination of natural blood group A



Scheme 15

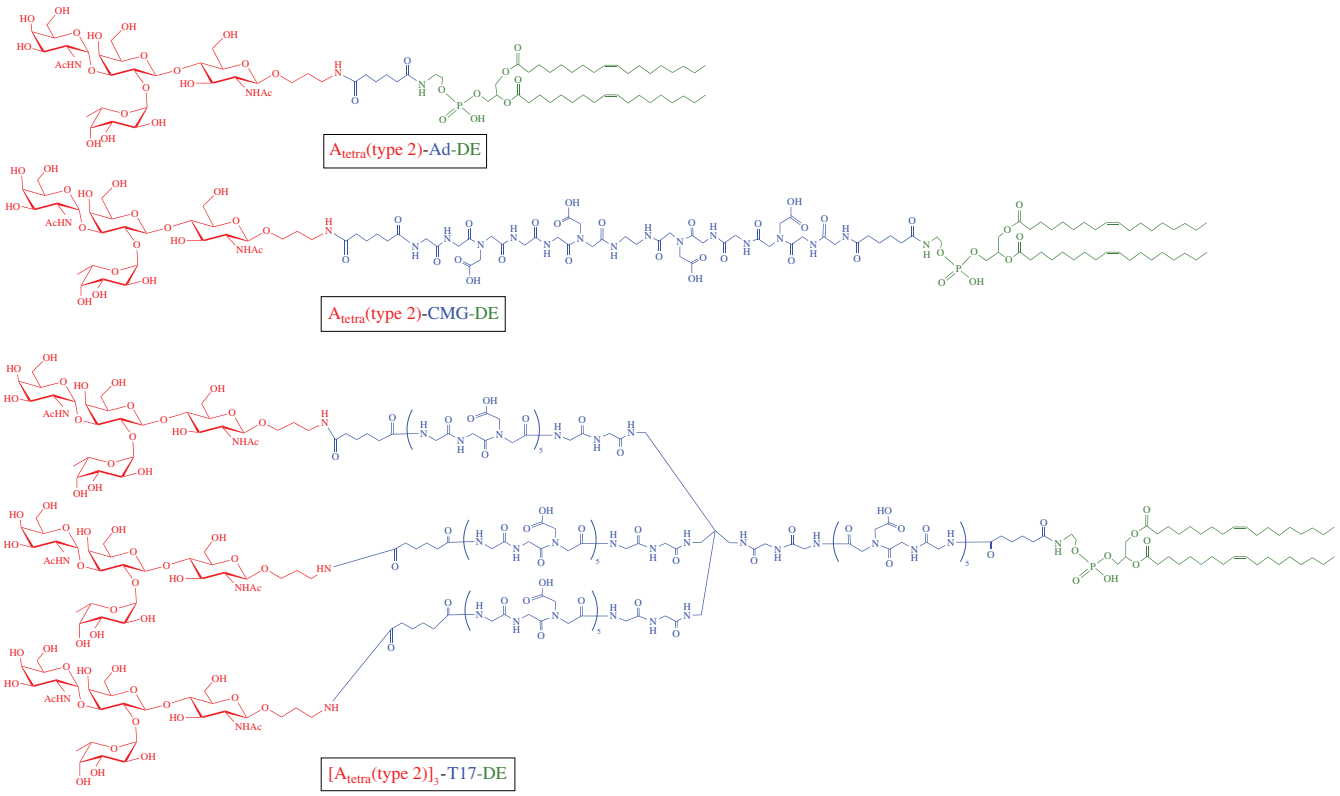


Figure 2 Function–spacer–lipid constructs carrying A$_{tetra}$(type 2) antigen.

erythrocytes and O erythrocytes with cells chemically modified with A (type 2) tetrasaccharide, as well as kodecytes bearing A$_{tetra}$(type 2)–Ad–DE with anti-A antibodies, was compared. The chemical modification occurred mainly on the periphery of the glycocalyx, whereas in kodecytes the antigens were situated closer to the membrane and thus their binding to antibodies was significantly influenced by steric factors. This allows us to make a confident conclusion about the predominant role of glycoproteins in the process of agglutination.

Clinical application of immunosorbents bearing A and B antigens

The presence of ABH glycans on endothelium is the main obstacle on the way to the ABO-incompatible organ transplantation. In this case the removal of the recipient anti-A or anti-B antibodies is required as the only currently practicable therapeutic approach. Nonselective methods for this removal, for instance plasmapheresis, have several drawbacks including the loss of essential plasma components and the possibility of virus infection or allergic reaction. In some cases, the plasmapheresis requires a splenectomy prior to the transplantation due to the key role of spleen in anti-A/B antibody production. The proposed use of immunosorbents bearing A or B antigens is much more selective, accurate and, therefore, gentle procedure, since only anti-A or anti-B antibodies are removed from the patient's plasma. Thus, it enables to avoid the splenectomy. Each immune adsorption session reduces the antibodies level by two to three titre steps.[79] Typically, four pre-operative and three post-operative immune adsorption sessions are performed.

Different types of A and B immunosorbents were available on the market in different periods of time. Almost all of them contain synthetic trisaccharides A or B as antigens/ligands, but differ in matrix. In Glycosorb, the trisaccharides are covalently linked to Sepharose,[80] in Synsorbs they are covalently bound to silica particles,[81] in BioSorbents A and B the trisaccharides are coupled to the macroporous glass beads *via* polyacrylamide.[6]

The use of A and B immunosorbents allows for ABO-incompatible transplantations of bone marrow and various organs, for example kidney,[82] liver,[83] lung[84] and heart,[85] as routine procedures with a high rate of survival.

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