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PARTIAL SYNTHESES OF THE CARBOHYDRATE CHAIN OF BRAIN GANGLIOSIDES

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Thirty five years have elapsed since the gangliosides were first isolated from pathological brain tissue[1]). Because of their apparent role in cerebral physiology and their remarkable immunochemical properties, this group of natural substances has become in recent years one of the most attractive topics in lipid research and much progress has been made in the early 1960's. A rapid and dramatic development in the chemistry of the major brain gangliosides which culminated in the complete elucidation of their structure led to the recognition that they have a common basic structure. They are built up from the same tetrasaccharide IV and differ only by the number of sialic acid residues. The establishment of the structure of these complex molecules has opened the way for synthetic studies.

I shall discuss the syntheses of several oligosaccharides which we have recently completed. I have also included a preliminary account of the work on this subject which is presently in progress in our laboratory.

The primary objective of our study in the ganglioside series has been the synthesis of fragments constructed in such a manner as to permit their combination to the tetrasaccharide IV or to the globoside I.

If we split the asialoganglioside I schematically as indicated by the dotted line, we obtain two fragments: the disaccharide galactosyl-(1→3)-N-acetyl-galactosamine (II) and lactosyl ceramide, the well known cytolipin H (fig. 1). Both fragments were synthesized earlier in our laboratory[2–4]), and at a first glance it would appear that they could be combined by a glycosidic bond to recover the asialoganglioside. This could be done in principle by the Koenigs-Knorr reaction, in which the bromide of II would react with the 4′-OH of III, assuming it were possible to protect all other hydroxyl groups. However, although such a combination may be feasible in the living cell, nature has made it difficult for the organic chemist to perform this reaction. The reason is that the hydroxyl group in position 4 of galactose has the axial orientation and is as such completely unreactive.

Let us now consider the fragments which have been obtained by hydrolysis

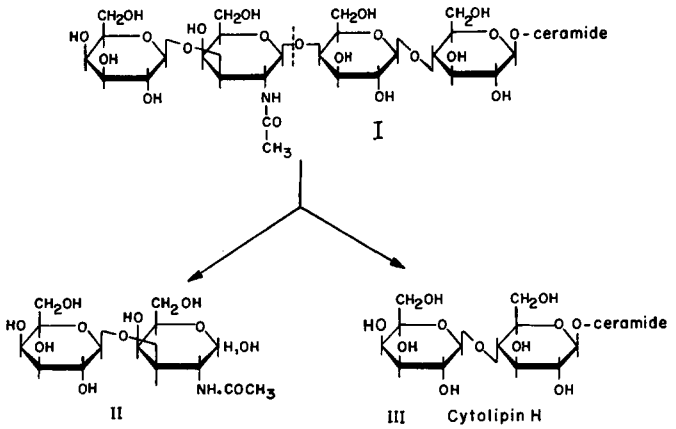


Fig. 1

of the tetrasaccharide chain[5]). In addition to the terminal disaccharide II, the following oligosaccharides have been identified among other fragments (fig. 2).

We decided to focus our attention first of all at the central disaccharide, namely N-acetylgalactosaminyl-(1→4)-galactose (V), for the following reason. The synthesis of amino sugar disaccharides of this type had not been possible. In fact, it posed for many years a special problem. For the preparation of a glycoside the Koenigs-Knorr reaction is generally employed, in

Gal(β1→3)GalNAc(β1→4)Gal(β1→4)Glu IV

GalNAc(β1→4)Gal V

GalNAc(β1→4)Gal(β1→4)Glu VI

Gal(β1→3)GalNAc(β1→4)Gal VII

Gal(β1→4)Glu

Fig. 2

which a fully acylated bromo sugar is condensed with an alcohol in the presence of an acid acceptor. However, with amino sugars the situation is complicated by a neighboring group participation. During its preparation such a bromide was found to undergo a rearrangement proceeding *via* an intermediate oxazoline which is cleaved by moisture to give eventually the hydrobromide VIII[6]) (fig. 3).

This behavior has for a long time made the amino sugar disaccharides

synthetically inaccessible. It was recognized that this sort of rearrangement depends largely on the nature of the substituent of the amino group and that the acetyl group is not suitable for this purpose. Various substituents have been tried by several authors[7–11]) with only limited success. The resulting bromides gave satisfactory results only with reactive alcohols, such as methanol, benzyl alcohol or the primary hydroxyl group of sugars. Since the availability of the central disaccharide was a prerequisite for the continuation of our studies, considerable efforts were made to achieve its synthesis. We visualized that protection of the amino group by a suitable electrophilic substituent should weaken the nucleophilic attack of the carbonyl oxygen on the anomeric center (as indicated in fig. 3) and thus minimize the tendency

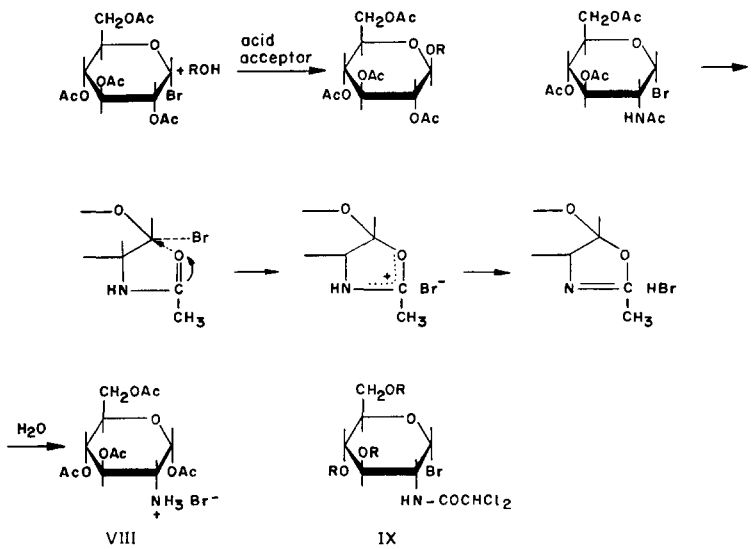


Fig. 3

of oxazoline formation. Following this trend of thought we eventually found that the introduction of the powerful electron-attracting dichloroacetyl group had the desired effect. It led to a bromide of type IX[12]), which met the three important requirements of stability, reactivity and deblocking of the amino group under mild conditions. The high reactivity was demonstrated by the fact that it reacted with benzyl alcohol in almost quantitative yield.

As to the second component of the disaccharide, namely, the galactose moiety, I have already mentioned that the hydroxyl group in position 4 is not reactive. It is generally accepted that equatorial hydroxyls are more reactive than axial ones. In the stable C1 conformation of galactopyranose

the 4-OH has an axial orientation. By conversion into the alternative 1C conformation the 4-OH acquires the equatorial position and becomes more reactive. Such a situation exists in 1,6-anhydrogalactose. Consequently, for the coupling with the amino sugar we used a suitably substituted galactosan. The synthesis of the disaccharide proceeded then as follows (fig. 4). Galactosamine hydrochloride was treated with dichloroacetic anhydride and the intermediate polyacyl derivative was selectively saponified to N-dichloroacetylgalactosamine X. The free hydroxyl groups were benzoylated. It is noteworthy that although complete benzoylation of galactosamine is more difficult to achieve, we preferred it to the facile acetylation, since the benzoyl

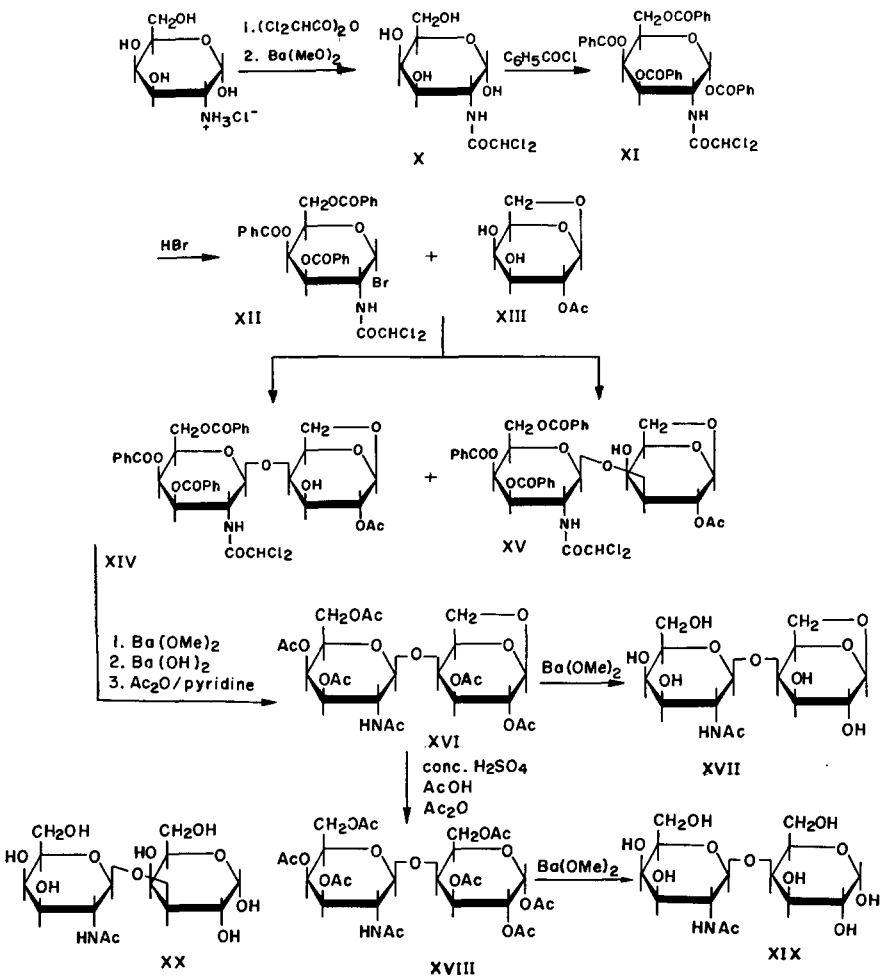


Fig. 4

groups are claimed to impart to the bromides a certain stability[13]). The bromide XII was now condensed with 2-acetylgalactosan XIII in order to bring about the glycosidic linkage with position 4. It was found, however, that the axial OH in position 3 had also reacted, though to a smaller extent. We thus obtained a mixture of the 1→4 and 1→3 isomers XIV and XV in a ratio of 3:2. They migrated closely on TLC, but we were able to separate them by two or three columns and crystallization. The total yield amounted to 50%. The continuation of the synthesis is demonstrated on the desired 1→4 isomer. Selective removal of the ester groups in XIV with anhydrous methanolic barium methoxide was followed by hydrolysis of the dichloroacetyl group with aqueous barium hydroxide, and the resulting product was acetylated to give compound XVI. The primary effect of these three operations was the exchange of the dichloroacetyl group by the acetyl group. At this stage we proved the 1→4 linkage by periodate oxidation of the deacetylated disaccharide XVII which consumed two moles of the reagent. In contrast, the 1→3 isomer consumed only one mole of periodate. Opening of the anhydro ring of XVI by means of sulfuric acid in the presence of acetic anhydride was accompanied by total acetylation. Hydrolysis of the polyacetate XVIII completed the synthesis of N-acetylgalactosaminyl-(1→4)-galactose (XIX). By the same sequence of reactions we obtained the 1→3 isomer XX. A disaccharide of this structure has been isolated by Yamakawa and his associates[14]) from hydrolyzates of kidney glycolipids. Both compounds had an identical m.p., but differed somewhat in optical rotation.

The synthesis of the disaccharide XIX opened the way for further studies in the ganglioside series. Our next objective was now to lengthen the carbohydrate chain to the trisaccharide VI inherent in the Tay-Sachs ganglioside. This means connecting the anomeric hydroxyl with carbon 4 of glucose. However, it is well known that the 4-OH of glucose exhibits rather low reactivity, even though it has the equatorial orientation. This is probably due to the steric hindrance of neighboring substituents, especially in position 5. It was felt that 1,6-anhydroglucose would perhaps behave more satisfactorily. On the basis of our experience with galactose, we tried in this case to prevent the formation of two isomers by using the disubstituted glucosan XXII in which only one hydroxyl group is available for reaction. The preparation of this compound involves a number of steps and will be described in a forthcoming paper.

The hexaacetate XVIII was converted into the bromide XXI which was condensed with 2,3-diacetylglucosan (fig. 5). In view of our earlier observation[3]) that the reactivity of bromosugars decreases with the length of the carbohydrate chain, a yield of 20% of the trisaccharide XXIII seemed quite satisfactory. Opening of the anhydro ring was done as shown before to give

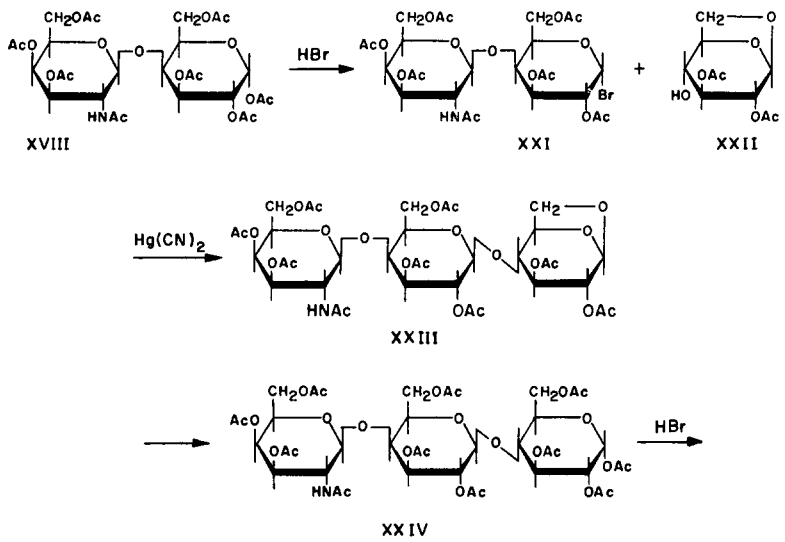


Fig. 5

the polyacetate XXIV whose molecular weight was corroborated by mass spectrometry. The final reaction, of the bromide XXV with ceramide to yield the Tay-Sachs globoside XXVIII as depicted in fig. 6, is under investigation.

Simultaneously we have studied an approach to the trisaccharide VII. It should be emphasized here that we made it a rule to explore the synthetic procedures in this series by means of models with the inexpensive glucosamine. A logical starting material for such a model synthesis appeared to be the polyacetyl derivative (XXIX) of galactosyl-(1→3)-N-acetylglucosamine[15]). However, the N-acetyl derivative as such will not lead to a stable bromide, since it would undergo rearrangement in a manner I have shown before. An attempt was, therefore, made to introduce the stabilizing dichloroacetyl group as follows. The hydroxyamide was treated with thionyl chloride, a reaction which was expected to give the oxazoline XXX. The ring was to be opened by acid to obtain the free amine XXXI which would then be dichloroacetylated. However, the compound obtained after treatment with thionyl chloride did not show the properties of an oxazoline, and we eventually identified it as the chloride XXXII. Thus, chlorination of the OH had taken place instead of oxazoline formation. Chlorides have been used in the Koenigs-Knorr reaction; they are more stable than the bromides, but have the disadvantage of being considerably less reactive. An attempt was nevertheless made to condense the chloride with 2,3-diacetylgalactosan and we obtained a low yield of the trisaccharide XXXIV.

In order to prepare the more reactive bromide, we studied another approach,

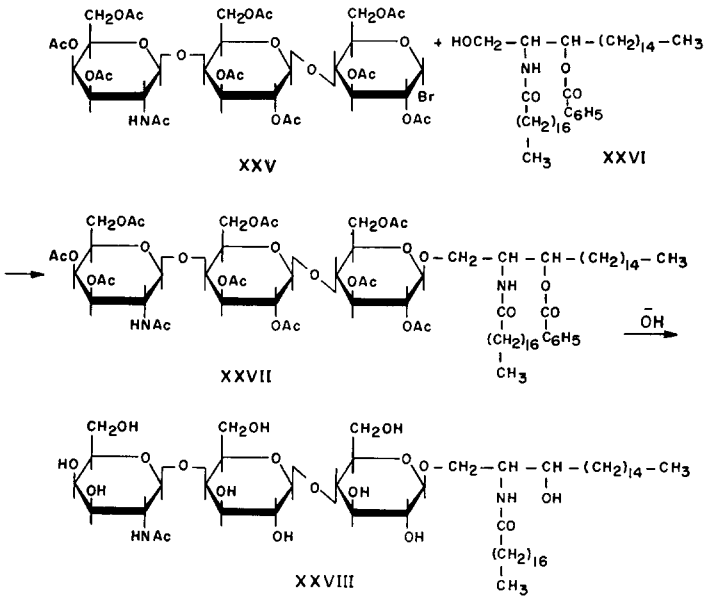


Fig. 6

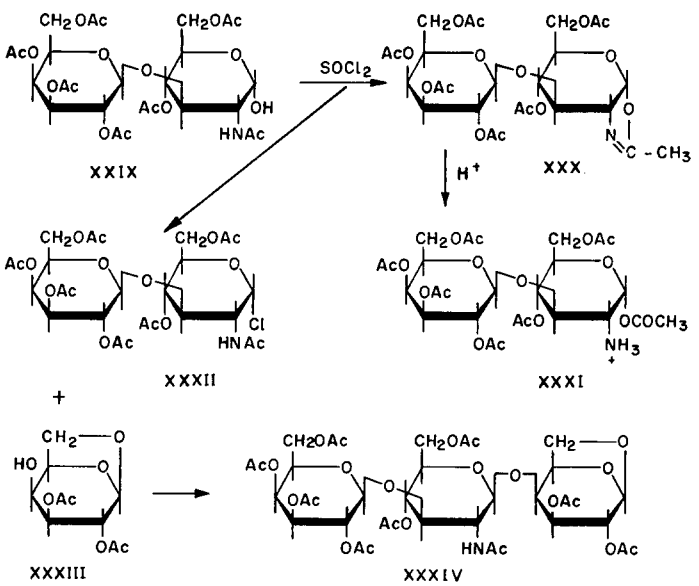


Fig. 7

likewise with glucosamine. To this end we employed the benzyloxycarbonyl derivative XXXVII which was prepared recently by Takanashi *et al.*[16]). After hydrolysis of the protective benzylidene group, the free hydroxyls were benzoylated, whereupon both the carbobenzoxy and the benzyl groups were removed by catalytic hydrogenation. The free amino alcohol XXXIX was now treated with dichloroacetic anhydride to give the bis-dichloroacetyl derivative which was converted into the desired bromide XLI. It can be seen that this bromide has essentially the same structure as that of the galactosamine I have described before, and was expected to be both stable and reactive. However, when we condensed it with diacetylgalactosan as shown in fig. 7 for the chloride, only a low yield of the trisaccharide was formed. There is evidence to suggest that this failure was due to a deficient conversion of XL to XLI under the conditions applied.

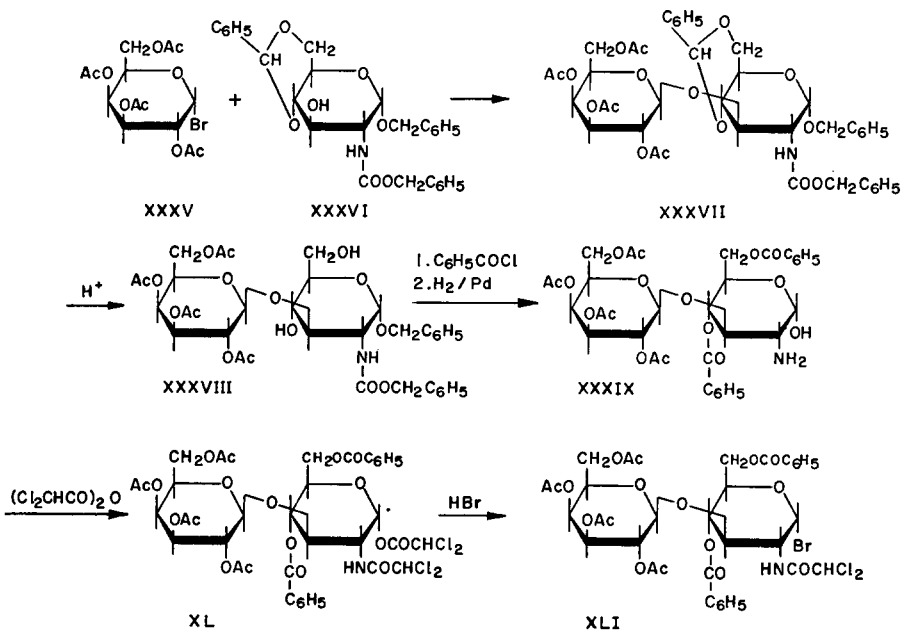


Fig. 8

Both routes which are based on a common principle, namely attaching galactose to the terminal disaccharide, had been designed as model procedures. At this point we decided to explore the synthesis of the true natural trisaccharide VII containing galactosamine. The structure of the intermediate compound XVII (fig. 9), in contrast to its glucosamine analogue, permits selective protection of the 3′- and 4′-positions, since acetone reacts exclusively with *cis*-vicinal hydroxyls. The isopropylidene derivative XLII was obtained

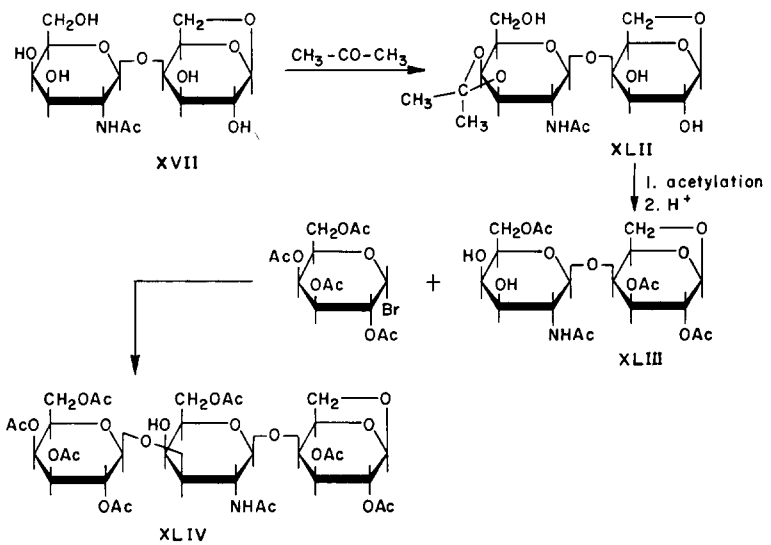


Fig. 9

in fairly good yield. Removal of the ketal group was accompanied by partial deacetylation which hampered somewhat the continuation of the synthesis of XLIV, which is in progress.

I shall now deal with the most simple fragment found in hydrolysates of gangliosides, namely lactose. The purpose of this study was to explore the possibilities of an unambiguous glycosidic substitution at position 3′, which would provide a model for the introduction of sialic acid. Very little work has been published on the selective substitution of lactose. The trityl derivatives are not known, nor were we successful in our attempts to prepare them. We succeeded, however, in preparing the isopropylidene derivative of the β-benzyl lactoside XLVI which, after acid treatment, gave the disaccharide XLVII (fig. 10). The 3′-OH reacted selectively with the bromide to give the trisaccharide XLVIII. Deacetylation followed by hydrogenation led to the final trisaccharide XLIX[17]). Admittedly, this bromide is not a very good model for the highly sensitive and less reactive bromide of N-acetylneuraminic acid, but the method provides some basis for the synthesis of sialyl derivatives.

Before concluding, I would like to point to an aspect which may have important repercussions in future biochemical research on gangliosides. We have been making extensive use of the reactive bromide which is stabilized by the dichloroacetyl group. In the course of our investigations we encountered amino sugar disaccharides which were so labile to alkali that any attempt to remove the dichloroacetyl group by cold barium hydroxide resulted in complete cleavage of the glycosidic bond. We eventually found

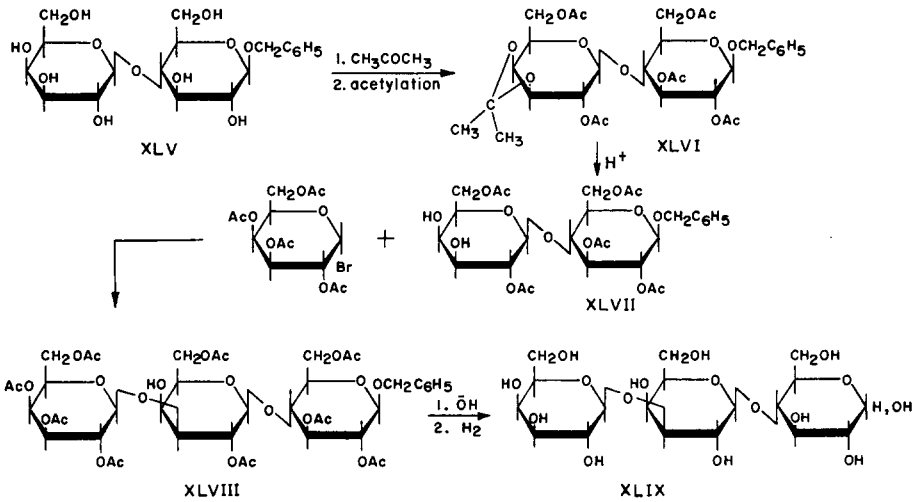
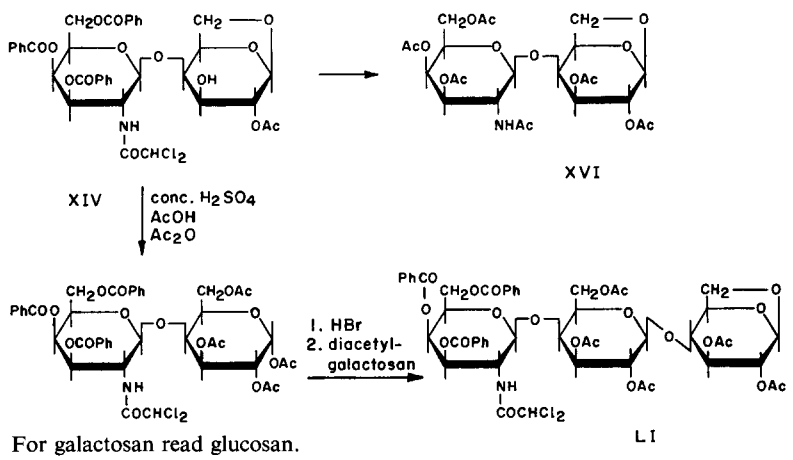


Fig. 10



For galactosan read glucosan.

Fig. 11.

that the dichloroacetyl group could be smoothly converted into acetyl group by hydrogenolysis of the chlorine atoms. In fig. 4, describing the synthesis of the central disaccharide, we have seen that it required three steps to convert the dichloroacetyl group into the acetyl group (XIV–XVI). Opening of the anhydro ring in XVI is effected under comparatively drastic conditions in the presence of concentrated sulfuric acid, and is invariably accompanied by a considerable scission of the glycosidic bond which may reduce the yield to a minimum. We have found very recently that if the reaction is carried

out directly with the dichloroacetyl derivative XIV, practically no scission occurs, and the yield of L is almost quantitative (fig. 11). This result, together with the catalytic reduction of the dichloroacetyl group, provide an important improvement in the synthesis of the disaccharide. However, these observations may have a more far-reaching consequence. If we continue the synthesis with L following the sequence of reactions shown in figs. 5 and 6 we may arrive at a Tay-Sachs globoside in which the dichloroacetyl group is retained. Hydrogenolysis of the chlorine atoms with tritium would provide a labeled substrate which might be essential in the study of the metabolism of gangliosides.

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