The Highly Selective Sulfonylation of Cycloheptaamylose and Syntheses of Its Pure Amino Derivatives

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Mesitylenesulfonyl chloride reacted selectively with primary hydroxyl groups of cycloheptaamylose to give hexakis(6-*O*-mesitylsulfonyl)cycloheptaamylose (II) and heptakis(mesitylsulfonyl)cycloheptaamylose (I). The selectivity of mesitylenesulfonyl chloride in the preferential sulfonylation is 24 times larger than that of tosyl chloride. Pure hexakis(6-azido-6-deoxy)cycloheptaamylose (III) and hexakis(6-amino-6-deoxy)cycloheptaamylose (IV) were synthesized from II. Pure heptakis(6-amino-6-deoxy)cycloheptaamylose (VII) and mixture of positional isomers of hexakis(6-amino-6-deoxy)mesitylsulfonylcycloheptaamylose (VIII)* were obtained by the catalytic hydrogenation of the corresponding azido compounds V and VI,* which were themselves given by the reaction of I with sodium azide. These amino derivatives, IV, VII, and VIII,* showed significant antimicrobial activities against such gram-negative bacteria as Escherichia, Shigella, and Pseudomonas. These compounds also exhibited hypocholesterolemic effects in the chick when added in the diet for two weeks, probably through sequestration of intestinal bile acids.

Cycloamyloses have attracted increased interest in recent years[1] because of their ability to include a compound into their cavity utilizing hydrophobic interaction in an aqueous solution. In this respect, a variety of cycloamylose derivatives have been prepared as models of enzymes.[2] Therefore, the pure sulfonylesters of cycloamylose have been required as important key intermediates.

Lautsch and his co-workers[3] were the first to attempt the selective modification of one position in each D-glucose residue. They attempted to prepare heptakis(6-*O*-tosyl) and heptakis(6-*O*-mesyl)cycloheptaamyloses by using one molar equivalent of the corresponding sulfonyl chlorides per D-glucose residue.

Recently, Cramer and his co-workers[4] studied in detail the specific modification of each D-glucose residue of cycloamyloses at a primary carbon atom and claimed that hexakis- and heptakis(6-*O*-tosyl)cyclohexa and cycloheptaamyloses were obtained by the reaction of a 50% excess of tosyl chloride and the corresponding cycloamyloses.

Using this method with a 50% excess of tosyl cloride, Umezawa and Tatsuta[5] reported that the product was a mixture of tosylated cyclohexaamyloses and that pure hexakis(6-*O*-tosyl)cyclohexaamylose was obtained after purification on a silica gel column.

However, it is still uncertain whether tosylation occurred exclusively at the primary hydroxyl groups in all the residues as claimed by these workers, because the tosylation of monosaccharides, such as α-D-glucose[6] and methyl α-D-glucopyranoside,[7] and polysaccharides, such as amylose[8] and cellulose,[9] under similar conditions has been reported to cause further tosylation at some secondary hydroxyl groups. The tosyl esters in which all the tosyloxyl groups are attached to primary carbon atoms are obtained only by purification in the case of monosaccharides, while such tosyl esters can not be obtained in the case of polysaccharides. Thus, a functional modification of cycloamyloses has been prevented by difficulties in purifying the products and it has been practically impossible to obtain the pure products by the usual methods.

Pure sulfonylesters of cycloamyloses must have definitive numbers and established positions of sulfonyloxyl groups such as all at the C-2, C-3, or C-6 positions.

Both the number and the position of sulfonyloxyl groups are equivocal in Cramer's work, in which the term "hexakis" or "heptakis" represents only the average number of the tosyl groups of a complex mixture of the products. In Umezawa's work the number is evident upon the separation of the products, but the position has not been established. It seems most likely that they obtained a mixture of the positional isomers of hexakis(tosyl)cyclohexaamyloses, as we have clarified in the present work that the heptasulfonyl ester of cycloheptaamylose with a single spot in its thin-layer chromatography was found to be still a mixture of the positional isomers.

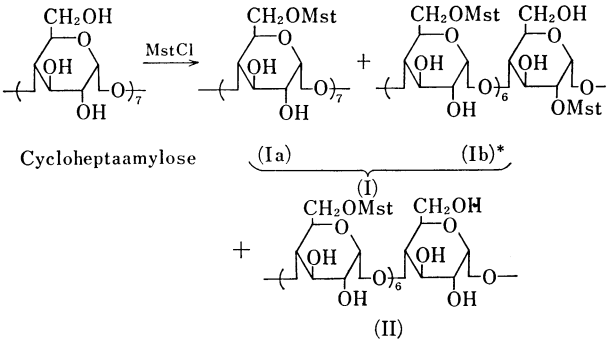
It seems almost impossible to obtain the pure sulfonyl esters without utilizing more highly selective sulfonylating agents than tosyl chloride or mesyl chloride.

Meanwhile, the use of mesitylenesulfonyl chloride (MstCl) as a selective sulfonylating agent has been reported. Palmer and his co-workers[10] reported that MstCl was a preferable sulfonylating agent in the case of secondary and tertiary hydroxyl groups of a steroid system. Furthermore, Creasey and Guthrie[11] found that MstCl reacted more selectively with one hydroxyl group of a vicinal secondary diol of α-D-glucopyranoside than did tosyl chloride, though Johnson *et al.*[12] showed in their aldosterone synthesis that the use of MstCl offered no significant advantage over the use of tosyl chloride for the selective esterification of a primary hydroxyl group in the presence of a secondary one.

In this paper we wish to report a highly selective sulfonylation of the primary hydroxyl groups of cycloheptaamylose using MstCl and describe the synthesis of some pure derivatives of cycloheptaamylose.

Results and Discussion

The selective esterification was carried out by adding a 20% excess of MstCl to a solution of cycloheptaamylose in dry pyridine at 0—5 °C and by then allowing the mixture to stand at room temperature for 3 days. The product was obtained as a white powder, and its

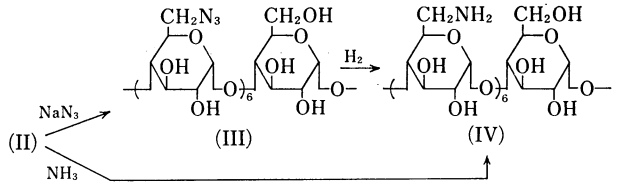


I: A mixture of Ia and Ib*.
Mst: 2,4,6-trimethylbenzenesulfonyl.
* This represents one of many kinds of possible positional isomers with regard to one secondary sulfonyl group.

thin-layer chromatography (TLC) gave only three spots (R_f; 0.79, 0.41, 0.02).

Compound Ib*, with an R_f value of 0.79, was separated on silica gel column and was established to be heptakis(mesitylsulfonyl)cycloheptaamylose by analyzing the sulfur content, while the one(II) with an R_f value of 0.41 was determined to be hexakis(mesitylsulfonyl)cycloheptaamylose in the same manner. A small amount of the compound with an R_f value of 0.02 may be pentakis(mesitylsulfonyl)cycloheptaamylose, though it was not further examined. The reaction of compound II with sodium azide in DMF at 80—85 °C, instead of at the 135 °C previously reported,[5] for 7 h led to the complete replacement of all the mesitylsulfonyloxyl groups by the azido groups.

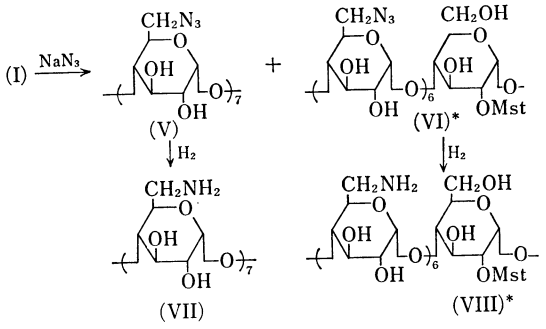
The IR absorption bands at 1600, 1350, 1190, and 1170 cm^{-1} due to the mesitylsulfonyloxyl group disappeared completely, while the one at 2100 cm^{-1} due to the azido group appeared. The azido compound obtained in this way was determined to be pure hexakis(6-azido-6-deoxy)cycloheptaamylose (III) with satisfactory physical properties. This indicates that II contains sulfonyloxyl groups only at the primary carbon atoms and none at the secondary ones, since a sulfonyloxyl group attached to a secondary carbon atom can hardly be replaced by an azido group under these reaction conditions.[13] Therefore, II must be pure hexakis(6-*O*-mesitylsulfonyl)-cycloheptaamylose.



The catalytic hydrogenation of III gave pure hexakis-(6-amino-6-deoxy)cycloheptaamylose (IV) as a hexahydrochloride with satisfactory physical properties. IV was also obtained directly by the reaction of II with ammonia in methanol.

On the other hand, Ib* gave an azido compound (VI)* upon heating with sodium azide in DMF at 85—90 °C for 7 h. The product VI*, whose TLC gave almost a single spot (R_f=0.37), had the IR absorption bands due to the mesitylsulfonyloxyl group together with the band due to the azido group and gave a satisfactory analysis for a mixture of the positional isomers of hexakis(6-azido-6-deoxy)mesitylsulfonylcycloheptaamylose, in which the mesitylsulfonyl group is considered to bind with one among the fourteen secondary hydroxyl groups. Thus, Ib* was determined to be the positional isomers of hexakis(6-*O*-mesitylsulfonyl)-mesitylsulfonylcycloheptaamylose, in which six sulfonyl groups are attached to six primary hydroxyl groups, while one is attached to one among the fourteen secondary hydroxyl groups.

Meanwhile, Compound I, with the same R_f value as that of Ib*, was also obtained by washing the crude sulfonylation product with an appropriate solvent (see Experimental section). The azido compound obtained from I in the manner described above gave two spots in its TLC (R_f=0.01, 0.37); each component was separated on treatment with an appropriate solvent. The compound with an R_f value of 0.37 proved to be identical with the VI* previously obtained from Ib*. On the contrary, Compound V, with an R_f vaiue of 0.01, had the IR absorption bands at 2100 cm^{-1} due to the azido group, with no sulfonate band, and had seven azido groups, as evidenced by its elemental analysis. Therefore, V was determined to be pure heptakis(6-azido-6-deoxy)cycloheptaamylose. Thus, from these results, I, with the same R_f value as that of Ib*, was found to be a mixture of heptakis(6-*O*-mesitylsulfonyl)cycloheptaamylose (Ia) and Ib*. The latter had previously been obtained by the separation of the crude sulfonylation product by column chromatography. These results suggest that Ia, with its highly crowded and strained structure, may be easily hydrolyzed to the more stable II during separation on a silica gel column. Actually, the sulfonyl esters of cycloheptaamylose were not stable, and their TLC figures were changed almost completely when methanol solutions of the esters were allowed to stand at room temperature for two weeks.



The catalytic hydrogenation of V and VI* gave hydrochlorides of heptakis(6-amino-6-deoxy)cycloheptaamylose (VII) and positional isomers of hexakis (6-amino-6-deoxy) mesitylsulfonylcycloheptaamylose (VIII)* respectively.

The more highly selective sulfonylation of cycloheptaamylose with MstCl than with TsCl was clearly revealed by plotting the average number of the sulfonyloxyl groups against the reaction time. In dry pyridine,

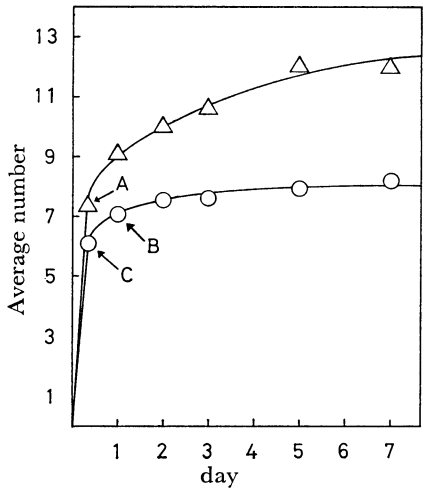


Fig. 1. Relation between average numbers of entered sulfonyloxyl groups and reaction time.
○: MstCl, △: TsCl.
[Numbers of primary, and secondary RSO_3; A: 6.2, 1.1; B: 6.6, 0.5; C: 6.1, 0].

cycloheptaamylose was esterified by the use of 4 eq-moles of the corresponding sulfonyl chlorides for the D-glucose residue at 15 °C.

The average number of the sulfonyloxyl groups introduced was determined by analyzing the sulfur content of the products which has been obtained from an aliquot of the reaction mixture. Furthermore, the positions of the sulfonyloxyl groups were determined by the transformation of the samples (A, B, and C) to azido compounds by the usual method. The number of the sulfonyloxyl groups remaining after this treatment should correspond to the number of the secondary sulfonyloxyl groups. The results are shown in Fig. 1. These data indicate that the preparation of pure heptakis(6-*O*-tosyl)cycloheptaamylose is almost impossible, while that of Ia or II is possible. As can be seen from the figure, both the sulfonylating reagents react with the six hydroxyl groups of cycloheptaamylose at a similar rate. After the six primary hydroxyl groups are esterified, however, subsequent sulfonylation becomes very slow in the case of MstCl, while TsCl reacts rapidly until eight hydroxyl groups are esterified. After 7 days, MstCl had reacted with eight hydroxyl groups, probably corresponding to seven primary and one secondary groups, while TsCl esterifies twelve hydroxyl groups, which may consist of seven primary and five secondary groups. This result indicates that bulky MstCl reacts less readily with a secondary hydroxyl group than does TsCl. It takes 5 h for TsCl and 120 h for MstCl to increase the sulfonylation number from six to eight; the latter process may involve one or two secondary hydroxyl groups. In conclusion, the selectivity of MstCl in the preferential sulfonylation of the primary hydroxyl groups is 24 times larger than that of TsCl. The surprisingly large selectivity may be due to the doughnut-shaped structure[14] of cycloheptaamylose, where the primary hydroxyl groups are located apart from the side of the torus, while the secondary hydroxyls are located directly on the other side of the torus.

Preliminary Biological Tests

It has been found that the amino compounds, IV, VII, and VIII*, showed significant antimicrobial activities, as tested by the two-fold dilution method in a heart-infusion agar medium, though it was earlier reported[5] that hexakis(6-amino-6-deoxy)cyclohexaamylose inhibited the growth of Bacillus subtilis PCI 219 at the concentration of 2000 μg/ml. These amino derivatives inhibited the growth of Staphylococcus aureus at concentrations of 25—100 μg/ml, of Escherichia coli at 12.5—50 μg/ml, of Shigella flexneri and sonnei at 1.56—100 μg/ml, and of Pseudomonas aeruginosa at 6.25—50 μg/ml.

It is also noteworthy that some of these compounds exhibited hypocholesterolemic activities, which were tested by feeding groups of 10 male one-day old chicks with a diet containing 0.2% cholesterol and a 1% test compound for two weeks, and by then determining the serum cholesterol using the method of Zak *et al.*,[15] with blood samples obtained through heart puncture. In this method, a mixture of IV and VII depressed the cholesterol level by 10—15% in comparison with the control groups. The details of the results will be reported elsewhere.

Experimental

All the melting points are uncorrected. The solutions were concentrated under reduced pressure at a bath temperature not exceeding 50 °C. The IR spectra were taken in Nujol mull on a Hitachi 215 spectrometer. The optical rotations were measured in a 0.5-dm tube with a Jasco DIP-180 polarimeter and were corrected as an anhydrous form if a compound had several molecules of adherent water. The TLC was performed on Merck TLC plate silica gel 60 F_{254}. Concentrated sulfuric acid was used as the spray reagent. The column chromatography was carried out by the use of Merck silica gel 60. The paper chromatography was conducted by using Merck Pre-Coated TLC Plates cellulose as a substitute for paper, and the substances were detected by the use of ninhydrin spray. The following solvent systems were used: for TLC, methanol–chloroform (3: 7) (Solvent A); for column chromatography, methanol–chloroform (1: 5) (Solvent B); for paper chromatography, 1-butanol–pyridine–acetic acid–water (1: 5: 2: 4) (Solvent C) and 1-propanol–pyridine–acetic acid–water (6: 4: 1: 3) (Solvent D).

Selective Sulfonylation of Cycloheptaamylose; *Heptakis(mesitylsulfonyl)cycloheptaamylose (I and Ib*)*; *Hexakis(6-O-mesitylsulfonyl)cycloheptaamylose (II).* Into a solution of cycloheptaamylose (32.7 g, containing 0.8% water) in dry pyridine (320 ml), we stirred MstCl (55.6 g, 1.2 eq-moles for the D-glucose residue) at 0—5 °C. After 3 h, the mixture was allowed to stand for 3 days at room temp. To the mixture 20 ml of cold water was stirred in, drop by drop, under cooling after which the new mixture was allowed to stand for 1 h at room temp. The mixture was then poured into a large volume of a dil-HCl solution under cooling to obtained a white precipitate which was collected and washed with water; yield, 66.0 g (S, 9.07%). TLC with Solvent A proved the product to be composed of three components with R_f values of 0.79, 0.41, and 0.02. These products (33.0 g) were chromatographed on a silica gel column (60×700 mm) with Solvent B. The substance with an R_f value of 0.79 was obtained as a white powder and

was determined to be Ib*; yield, 5.0 g; mp 187.0 °C (decomp), $[\alpha]_D^{20}$ +70.8 ° (*c* 1.0, chloroform), IR spectrum: 1600 (phenyl), 1350, 1185, 1170 (sulfonate) cm^{-1}. Found: C, 52.20; H, 5.89; S, 9.40%. Calcd for $C_{105}H_{140}O_{49}S_7$: C, 52.33; H, 5.81; S, 9.30%.

The substance with an R_f value of 0.41 was also obtained as a white powder and was determined to be II; yield, 12.5 g; mp 189.5 °C (decomp), $[\alpha]_D^{20}$ +76.2 ° (*c* 1.0, chloroform), IR spectrum; 1600 (phenyl), 1355, 1185, 1180 (sulfonate) cm^{-1}. Found: C, 51.64; H, 5.91; S, 8.76%. Calcd for $C_{96}H_{130}O_{47}S_6$: C, 51.75; H, 5.84; S, 8.63%.

The powdered mixture of the crude sulfonyl esters (33.0 g) described above was suspended in a mixture of ethyl acetate (800 ml) and ethanol (200 ml) at 50 °C for 1 h. To the mixture, ethyl acetate (500 ml) was then added, and the mixture was stirred for 10 h at room temperature and subsequently filtered. The remaining powder was suspended in a mixture of the same solvent; to the mixture we then added ethyl acetate, and then we stirred and filtered the solution. This treatment was repeated two more times. The filtrate (about 5 l) was then concentrated, and the residue was suspended in a hot ethyl acetate (2 l) for 30 min, stirred at room temperature overnight, and filtered. To the filtrate ether was added, drop by drop, until the clear solution became slightly cloudy after which it was allowed to stand overnight. After the precipitate had then been filtered off, the filtrate was concentrated, the residue was dissolved in hot ethyl acetate (1.5 l), and ether was added. These procedures were repeated two further times, after which the white precipitate I was obtained by adding ether to the last residue. This was practically pure heptakis(mesitylsulfonyl)cycloheptaamylose, as determined by TLC (R_f=0.79), though a trace of II (R_f=0.41) was still present; yield, 1.4 g; mp 177—181 °C (decomp), $[\alpha]_D^{20}$ +69.8 ° (*c* 1.0 chloroform). Found: C, 52.66; H, 6.06; S, 9.38%. The substance with an R_f value of 0.02 was not further examined.

Hexakis(6-azido-6-deoxy)cycloheptaamylose (III). To a solution of II (3.0 g) in dry *N,N*-dimethylformamide (30 ml), sodium azide (3.0 g) was added after which the mixture was heated with stirring at 80—85 °C for 7 h. The reaction mixture was then poured into a large volume of cold water the white precipitate thus obtained was collected and washed with water; yield, 1.60 g (92.5%); mp above 230 °C (decomposed slowly); IR spectrum: 2100 (azido) cm^{-1}, no sulfonate band. Found: C, 39.07; H, 5.26; N, 19.24%. Calcd for $C_{42}H_{64}N_{18}O_{29}$: C, 39.25; H, 4.98; N, 19.63%.

Heating of I and Ib with Sodium Azide: Heptakis(6-azido-6-deoxy)cycloheptaamylose (V) and Hexakis(6-azido-6-deoxy)mesitylsulfonylcycloheptaamylose (VI)*.* To a solution of I (1.2 g) in dry *N,N*-dimethylformamide (20 ml) sodium azide (1.2 g) was added; there after the mixture was heated with stirring at 85—90 °C for 7 h and then poured into a large volume of cold water. A white precipitate (0.60 g) was collected, washed with water, and dried; it showed two spots in its TLC with Solvent A (R_f 0.37 and 0.01). The precipitate was dissolved in a mixture of methanol (20 ml) and *N,N*-dimethylformamide (20 ml), and then diisopropyl ether was added slowly. The white precipitate which separated was collected. This treatment was repeated twice more. A white powder (0.20 g) thus obtained was determined to be V; mp above 220 °C (decomposed slowly); IR spectrum: 2100 (azido) cm^{-1}, no sulfonate band. Found: C, 38.24; H, 5.04; N, 21.54%. Calcd for $C_{42}H_{63}N_{21}O_{28}$: C, 38.50; H, 4.81; N, 22.46%.

The filtrate was concentrated, the residue was dissolved in ethyl acetate, and the solution was treated with active charcoal and filtered. To the filtrate, diisopropyl ether was added, and the white precipitate VI*, whose TLC gave only one spot (R_f=0.37), was collected; yield, 0.3 g; mp 211—216 °C (decomp). IR spectrum; 2100 (azido), 1600 (phenyl), 1185, 1175 (shoulder) (sulfonate) cm^{-1}. Found: C, 41.41; H, 5.16; N, 17.74; S, 2.23%. Calcd for $C_{51}H_{74}O_{31}N_{18}S$: C, 41.75; H, 5.05; N, 17.19; S, 2.18%. Compound VI* was also obtained by heating Ib* with sodium azide in *N,N*-dimethylformamide at 85—90 °C for 7 h.

Hexakis(6-amino-6-deoxy)cycloheptaamylose (IV). A sample (1.20 g) of III was suspended in methanol (200 ml) containing 5%-HCl aq (20 ml), and the mixture was hydrogenated with platinum dioxide (300 mg) under 3 atm of hydrogen pressure at 10—20 °C for 2 days. After the removal of the catalyst, the filtrate was concentrated. To the residue ethanol was added, and the precipitate which was thus separated was collected and dissolved in a small amount of water. The solution was lyophilized, and the hexahydrochloride of IV was obtained as a white powder; yield, 1.15 g (91%); mp 190.5—191.5 °C (decomp). $[\alpha]_D^{20}$ +136.4 ° (*c* 1.0, H_2O), R_f=0.37 with Solvent C. IR spectrum; 3300 (ν OH, NH, broad), 1600 (δ as NH_3^+), 1550 (δ s NH_3^+) cm^{-1}. Found: C, 34.05; H, 6.13; N, 5.64; Cl, 14.23%. Calcd for $C_{42}H_{82}O_{29}N_6Cl_6 \cdot 7H_2O$: C, 34.22; H, 6.52; N, 5.70; Cl, 14.46%.

A small quantity of IV was hydrolyzed with 3M hydrochloric acid in a sealed tube for 4 h at 100 °C, and the mixture was concentrated. The hydrolyzate was then paper-chromatographed with Solvent D and detected by ninhydrin. The chromatogram was completely identical (R_f=0.27, violet; R_f=0.63, yellow) with that obtained from the hydrolysis of 6-amino-6-deoxy-α-methylglucoside (mp 200—201 °C, lit,[16] mp 195—200 °C, R_f=0.41 with Solvent D).

Heptakis(6-amino-6-deoxy)cycloheptaamylose (VII). A sample (200 mg) of V was subjected to catalytic hydrogenation with platinum dioxide (100 mg) in a mixture of methanol (200 ml) and 3%-HCl aq (5 ml) and then treated in a manner similar to that described above. The heptahydrochloride of VII was obtained as a white powder; yield, 150 mg (70%); mp 182—185 °C (decomp). $[\alpha]_D^{20}$ +131 °C (*c* 1.0, H_2O). R_f=0.30 with Solvent C. IR spectrum: 3300 (ν OH, NH, broad), 1600 (δ as NH_3^+), 1500 (δ s NH_3^+) cm^{-1}. Found: C, 33.41; H, 6.32; N, 6.21; Cl, 16.03%. Calcd for $C_{42}H_{84}O_{28}N_7Cl_7 \cdot 8H_2O$: C, 33.02; H, 6.55; N, 6.42; Cl, 16.28%.

Positional Isomers of Hexakis(6-amino-6-deoxy)mesitylsulfonylcycloheptaamylose (VIII). A sample (600 mg) of VI* was hydrogenated with platinum dioxide (150 mg) in methanol (50 ml) containing 5%-HCl aq (10 ml) in a manner similar to that described above; the hexahydrochloride of VIII* was thus obtained as a white powder; yield, 550 mg (88%); mp 187—188 °C (decomp). $[\alpha]_D^{20}$ +112 ° (*c* 1.0, H_2O). R_f=0.69 with Solvent C. IR spectrum: 3300 (broad, ν OH, NH), 1605 (δ as NH_3^+), 1505 (δ s NH_3^+), 1195, 1175 (shoulder) (sulfonate) cm^{-1}. Found: C, 36.20; H, 5.91; N, 5.24; Cl, 13.51; S, 1.94%. Calcd for $C_{51}H_{92}O_{31}N_6Cl_6S \cdot 7H_2O$: C, 36.98; H, 6.40; N, 5.08; Cl, 12.87; S, 1.93%.

Reaction of II with Ammonia in a Methanol Solution. A sample (6.0 g) of II was dissolved in a 10%-NH_3–methanol solution (100 ml), and the mixture was heated at 70—75 °C in a sealed tube for 3 days. After the reaction mixture had then been cooled to 0—10 °C, the slightly yellow crystals which were thus separated were collected and washed with methanol. The crystals were dissolved in water (30 ml), treated with active charcoal, and lyophilized. The free base of IV was thus obtained as a white powder; yield, 2.7 g (89%); mp above 230 °C. IR spectrum; 3300, 3350 (ν OH, NH), 1600 (δ NH_2) cm^{-1}. Found: C, 42.75; H, 6.52; N, 6.79%. Calcd for $C_{42}H_{76}O_{29}N_6 \cdot 3H_2O$: C, 42.64; H, 6.94; N, 7.11%.

The aqueous solution of the base was neutralized with hydrochloric acid to pH 2 and then concentrated. The white precipitate of the hexahydrochloride was obtained by adding ethanol to the residue. The physical properties of the hexahydrochloride thus obtained were identical with those of the hexahydrochloride which was obtained by the hydrogenation of III.

More Highly Selective Sulfonylation of Cycloheptaamylose with MstCl than with TsCl. Into a solution of cycloheptaamylose (9.97 g, containing 2.5% water) in dry pyridine (200 ml) we quickly stirred a solution of the corresponding sulfonylating agent (MstCl, 55.47 g, TsCl 48.36 g; 4.0 eq-moles respectively) in dry pyridine (100 ml) at 15.0 °C; the mixture was then kept at 15.0±0.5 °C during experiments. An aliquot portion (10—15 ml) of the reaction mixture was taken up from time to time and was quenched with 1 ml of cold water. After 3 h the aliquot was poured into a large volume of water to give a white precipitate, which was collected. The average number of entering sulfonyloxyl groups was determined by analyzing the sulfur content of the precipitate. The results are shown in Fig. 1. The samples (A, B, and C) were all heated with sodium azide in DMF at 80 °C for 7 h, and the average numbers of the sulfonyloxy groups remaining and of the azido group replacing them were analyzed. The average number of the azido groups introduced corresponds to the number of the primary sulfonyloxyl groups, while that of the sulfonyloxy groups remaining represents the number of secondary sulfonyloxy groups in the samples (A, B, and C).

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