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Note

Trialkylsilyl derivatives of cyclomaltoheptaose, cellulose, and amylose: rearrangement during methylation analysis

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Trialkylsilyl ethers are valuable protecting groups widely used in carbohydrate chemistry. The *tert*-butyldimethylsilyl (TBDMS) and the even larger thexyldimethylsilyl (thexyl = 1,1,2-trimethylpropyl, THxDMS) residues can be selectively introduced in the sterically less hindered primary position of sugar residues [1,2]. However, under more drastic conditions the secondary 2-OH can also be trialkylsilylated [3]. Properties of polysaccharide derivatives depend on the ds (degree of substitution) and on the distribution of the substituents. With regioselectively trialkylsilylated cellulose and amylose, highly uniform products having special properties are available [4]. The ds of such derivatives can be determined by elemental analysis (Si); however, the inclusion of $SiMe_2ROH$ from excess of reagent may cause inaccurate results. NMR spectroscopy does not allow determination of the molar ratio of all possible substitution patterns in the 4-linked glucosyl units [5]. We have already successfully applied standard methylation analysis and HPLC to per-6-*O*-THxDMS-celluloses after permethylation and hydrolysis [6]. In our investigations on the distribution pattern of other polysaccharide derivatives [7–10] we prefer to apply the reductive-cleavage method [11]. This procedure enables

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cleavage of the glycosidic bond, reduction, and acetylation to be performed in a one-pot reaction at room temperature. We now report on the analysis of 2,6-di-*O*-$SiMe_2R$ derivatives of cellulose, amylose, and cyclomaltoheptaose (β-cyclodextrin) by a modified procedure of standard methylation analysis and by reductive cleavage as an alternative.

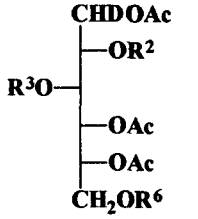
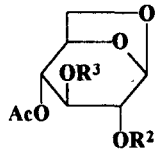
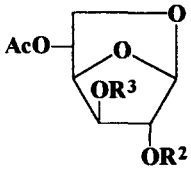
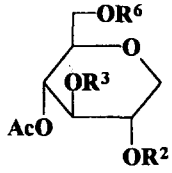
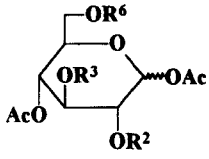
Cellulose was *O*-silylated with THxDMSCl or TBDMSCl starting under homogeneous conditions in dimethylacetamide (DMAc)–lithium chloride. β-Cyclodextrin derivatives were prepared as described [12]. The overall ds was determined by elemental analysis and by NMR spectroscopy, showing complete substitution at the primary position and 60–> 95% at O-2. Amylose was treated with TBDMSCl and imidazole in DMF at 80°C according to Fügedi [3].

Due to the lability of the trialkylsilyl residues under acidic conditions the derivatives were permethylated with sodium hydride and methyl iodide in THF prior to chemical degradation and subsequent GLC and GLC–MS analysis. The methyl substitution pattern determined in this way should indicate the complementary distribution of the original trialkylsilyl substituents.

Standard methylation analysis [13].—The permethylated trialkylsilyl derivatives were hydrolysed (2 M CF_3CO_2H, 120°C), reduced, and acetylated as described [6]. While derivatives of β-cyclodextrin and amylose could be analysed in this way, hydrolysis was incomplete for the cellulose silyl ethers with a complementary methyl-ds of < 1.3. Due to the drastic change in solubility after cleavage of the THxDMS-residues, the material partially precipitated. Pretreatment with 1.5 M MeOH/HCl at 90°C for 4 h was carried out to achieve better solubility of the trialkylsilyl ether. This procedure has also successfully been applied to hydrophobic starch benzoates [14] as well as other polysaccharides resistant to hydrolysis [15]. A three-step hydrolysis starting with 100% CF_3CO_2H at room temperature, followed by dilution to 9 M (100°C) and 2.7 M (120°C) [16], gave comparable results, while the two-step Saeman procedure (12 M H_2SO_4/1 M H_2SO_4) [17] was tedious and not so efficient.

As well as the partially methylated glucitol acetates (**1–7**) expected after reduction and acetylation, the formation of up to 18% of 1,6-anhydro-glucofuranose and -glucopyranose derivatives (**8–13**) was observed from the 6-*O*-THxDMS-glucosyl units [18,19]. They were included with the corresponding 6-*O*-acetylglucitols in the quantitative calculation.

Reductive cleavage.—When reductive cleavage was performed with 5 equiv each of Et_3SiH and Me_3SiOSO_2Me and 1 equiv of $BF_3 \cdot OEt_2$ [20], the yield of the 1,5-anhydroglucitols from the di-*O*-THxDMS (TBDMS) derivatives was much less than expected. From earlier investigations it was known that treatment of the reaction mixture with mixed-bed ion-exchange resin after quenching with MeOH can cause losses of material when the number of free hydroxy groups increases. Therefore, acetylation was directly performed with Ac_2O–CF_3CO_2H (10:1) as described [21]. When a large excess of reagents [40 equiv of Et_3SiH and Me_3SiOSO_2Me–$BF_3 \cdot OEt_2$ (1:1) or $Me_3SiOSO_2CF_3$–$BF_3 \cdot OEt_2$ (1:1)] was used, the molar ratio of the 2,4,6- or 3,4,6-tri-*O*-acetyl derivatives increased and reached up to 100% as compared to the hydrolytic procedure. However, besides the expected 1,5-anhydro-D-glucitols (**14–19**) the corresponding α,β-glucose acetates (**20–23**) were formed: up to 45% with Me_3SiOSO_2Me–

	R^2	R^3	R^6	Compound Number
	Me	Me	Me	1
	Ac	Me	Me	2
	Me	Ac	Me	3
	Me	Me	Ac	4
	Ac	Me	Ac	5
	Me	Ac	Ac	6
	Ac	Ac	Ac	7
	Me	Me	-	8
	Ac	Me	-	9
	Me	Ac	-	10
	Me	Me	-	11
	Ac	Me	-	12
	Me	Ac	-	13
	Me	Me	Me	14
	Ac	Me	Me	15
	Me	Me	Ac	16
	Ac	Me	Ac	17
	Me	Ac	Ac	18
	Ac	Ac	Ac	19
	Me	Me	Ac	20
	Ac	Me	Ac	21
	Me	Ac	Ac	22
	Ac	Ac	Ac	23

$BF_3 \cdot OEt_2$ and up to 98% with $Me_3SiOSO_2CF_3$–$BF_3 \cdot OEt_2$. The ratio of 1-acetate increased with the number of originally silylated OH groups. Obviously, the formation of 1,6-anhydroglucose derivatives, which are subsequently opened by the acetylating agent to give the 1-acetates, competes with the hydride transfer from the Et_3SiH. We had observed such intramolecular stabilisation of the oxocarbenium ion for hexakis(6-

Table 1
Results of standard methylation analysis (SMA) and reductive cleavage method (RCM) in the investigation of *O*-THxDMS-substituted β-cyclodextrins CD-1 and CD-2 and *O*-TBDMS-amylose (AS-1)

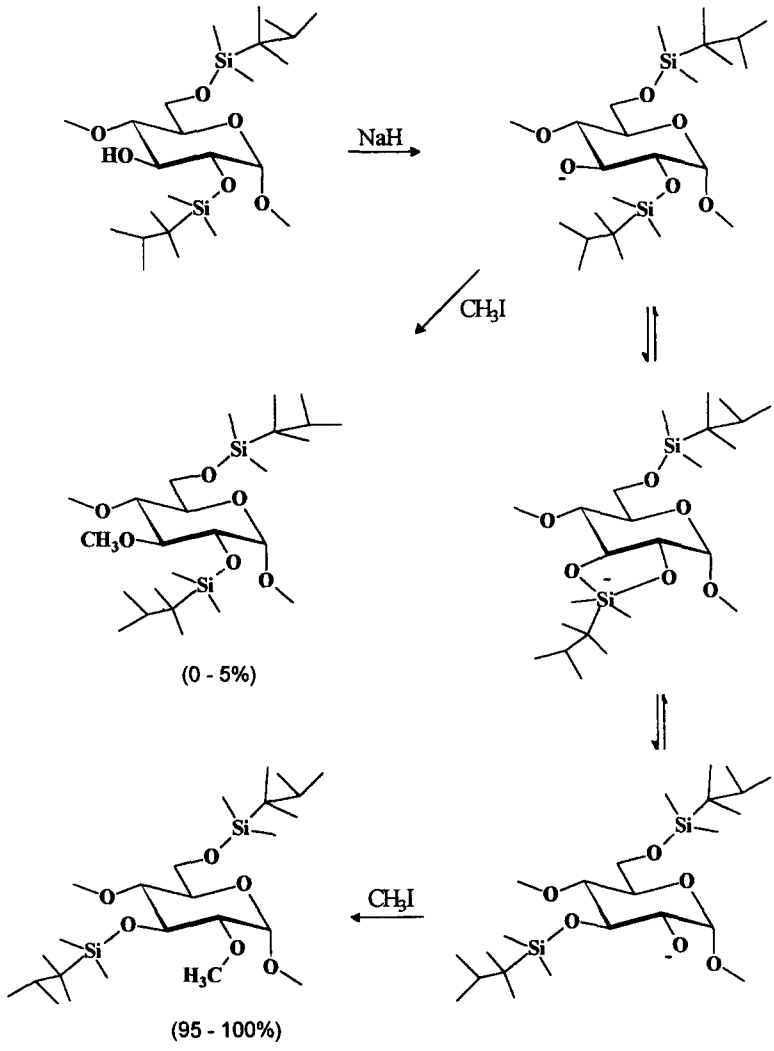
Substituted position [a] (mol%)	CD-1 SMA	CD-1 RCM	CD-2 SMA	CD-2 RCM	AS-1 SMA	AS-1 RCM
–	n.d. [b]	n.d.	n.d.	n.d.	5.00	5.05
3	0.52	n.d.	0.76	n.d.	4.80	2.00
6	20.68	24.46	2.83	2.11	24.57	25.96
2,6	1.17	n.d.	n.d.	n.d.	3.74	3.87
3,6	76.82	75.54	94.93	97.89	60.14	63.13
2,3,6	0.81	n.d.	1.48	n.d.	1.75	n.d.
ds (2) [c]	0.020	n.d.	0.015	n.d.	0.055	0.039
ds (3)	0.782	0.755	0.972	0.979	0.667	0.651
ds (6)	0.995	1.000	0.992	1.000	0.902	0.930
ds	1.80	1.76	1.98	1.98	1.62	1.62
Rearrangement [d]	98.5%	100%	100%	100%	94.6%	94.4%

[a] Acetylated positions in the degradation product, corresponding to silylated positions after the permethylation step.
[b] n.d. = Not detected.
[c] ds (n) = Degree of substitution of *O*-n by a silyl group after permethylation.
[d] Calculated from the ratio of "3,6" to ("2,6" + "3,6" + "3").

amino-6-deoxy-2,3-di-*O*-methyl)-α-cyclodextrin, while 6-*O*-linked galactofuranosides gave no 1,6-anhydrogalactose under reductive-cleavage conditions [18]. The best quantitative results were obtained with $Me_3SiOSO_2CF_3$ or its mixture with $BF_3 \cdot OEt_2$.

Cyclodextrin trialkylsilyl ethers.—Standard methylation analysis of mainly 2,6-di-*O*-THxDMS- or -*O*-TBDMS-substituted β-cyclodextrins surprisingly gave 1,3,4,5,6-penta-*O*-acetyl-2-*O*-methyl-D-glucitol (**6**) as the main product and different amounts of 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol (**4**) besides some minor components resulting from other possible substitution patterns or from slight undermethylation (see Table 1), and the corresponding 1,6-anhydrogluco-pyranose and -furanose **10** and **13** mentioned above. Reductive cleavage of the same samples yielded the 1,5-anhydro-D-glucitols **18** (2-*O*-Me) and **16** (2,3-di-*O*-Me) together with the corresponding glucose acetates **20** and **22**.

The formation of the 2-*O*-methyl- instead of the 3-*O*-methyl-D-glucose derivatives from the 2,6-di-*O*-$SiMe_2R$ glucosyl units indicates rearrangement of the $SiMe_2R$ group under the alkaline methylation conditions. The well-known 1,2-migration of TBDMS residues [22,23] was investigated for monosilyl ethers of polyols in methanolic K_2CO_3 by Mulzer and Schöllhorn [24] and was shown to be a thermodynamically controlled process. For methyl 4,6-*O*-benzylidene-2-*O*-TBDPS-α-D-glucopyranoside, a 1:2 equilibrium mixture with the 3-*O*-TBDPS derivative was obtained. Manna et al. observed a smooth rearrangement of the TBDMS group in 2,3-*O*-isopropylidene-5-*O*-TBDMS-6-*O*-tosyl-D-mannofuranose when treated with NaH in DMF, yielding the corresponding 1,6-anhydro-4-*O*-TBDMS-mannopyranose [19]. Recently, Icheln et al. obtained heptakis(2-*O*-allyl-3,6-di-*O*-TBDMS)-β-cyclodextrin from the 2,6-di-*O*-TBDMS derivative



Scheme 1.

with NaH and allyl bromide in DMF [25], which is in agreement with our results. The 2-alkoxide is removed from the equilibrium by the fast reaction with the alkyl halide, causing finally complete migration of the TBDMS group to position 3 (see Scheme 1).

Quantitative results from both methods are shown for two selected *O*-THxDMS-cyclodextrins (CD-1 and CD-2) in Table 1. The ds of CD-1 and CD-2 was 1.80 and 1.98, respectively. The yield of the 2-*O*-methylated anhydroglucitol **18** obtained by the reductive-cleavage method was 85–100% as calculated by comparison with the data from standard methylation analysis.

Table 2
Results of standard methylation analysis (SMA) and reductive cleavage method (RCM) in the investigation of *O*-THxDMS-substituted celluloses CS-1, CS-2, CS-3, and CS-4

Substituted position [a] (mol%)	CS-1 SMA	CS-1 RCM	CS-2 SMA	CS-2 RCM	CS-3 SMA	CS-3 RCM	CS-4 SMA	CS-4 RCM
2	1.15	traces	0.28	n.d. [b]	n.d.	n.d.	0.37	n.d.
6	22.13	20.44	11.73	12.32	15.75	14.61	29.06	25.11
2,6	71.31	75.77	83.42	86.46	77.58	81.75	65.68	72.77
3,6	4.54	3.80	3.10	1.22	5.37	3.64	3.54	2.12
2,3,6	0.87	n.d.	1.47	n.d.	1.30	n.d.	1.35	n.d.
ds (2) [c]	0.734	0.580	0.852	0.865	0.789	0.818	0.674	0.732
ds (3)	0.054	0.038	0.046	0.012	0.067	0.036	0.049	0.021
ds (6)	0.989	1.000	0.997	1.000	1.000	1.000	0.996	1.000
ds	1.78	1.80	1.89	1.88	1.86	1.85	1.72	1.75
Rearrangement [d]	6.0%	4.8%	3.6%	1.4%	6.5%	4.3%	5.1%	2.8%

[a] Acetylated positions in the degradation product, corresponding to silylated positions after the permethylation step.
[b] n.d. = Not detected.
[c] ds (n) = Degree of substitution of *O*-n by a silyl group after permethylation.
[d] Calculated from the ratio of "3,6" to ("2,6" + "3,6").

Cellulose trialkylsilyl ethers.—In contrast, standard methylation analysis of celluloses mainly 2,6-di-*O*-substituted with THxDMS residues gave 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methyl-D-glucitol (**5**) as the main product accompanied by the 2,3-di-*O*-methylated glucitol **4** and only small amounts of the 2-*O*-methyl product **6**, resulting from rearrangement or original silylation in position 3 to such a small degree that it was not detected by NMR. The 1,6-anhydroglucose derivatives **8**, **9**, **11**, and **12** as well as traces of compounds **2** and **7** were also detected. About 1% of the hexaacetate **7** may at least partially arise from undermethylated 2,6-di-*O*-THxDMS-glucosyl residues.

After reductive cleavage the 1,5-anhydro-D-glucitols **16**, **17**, and **18** and the α,β-glucose acetates **20** and **21** were obtained. Quantitative results are given for four cellulose derivatives (CS-1, CS-2, CS-3, and CS-4) in Table 2. With $Me_3SiOSO_2CF_3$–$BF_3 \cdot OEt_2$ (1:1) the apparent amount of di-*O*-substitution was always higher, presumably a result of demethylation with this strong Lewis acid mixture, as observed earlier. With Me_3SiOSO_2Me–$BF_3 \cdot OEt_2$ (1:1) the yield of the 3-*O*-methylated degradation products was still only 58–99% as compared to data obtained from standard methylation analysis. In Table 2 the results from the degradation with $Me_3SiOSO_2CF_3$ and its mixture with $BF_3 \cdot OEt_2$ are shown.

In contrast to the α-linked cyclodextrin derivatives the 2-*O*-THxDMS ethers of the β-linked cellulose underwent only up to 6% rearrangement, provided that partial undermethylation of a 6-*O*-THxDMS-glucosyl unit is excluded and that the 2-*O*-methyl derivative does not arise from original 3-*O*-silylation. Therefore, it was of interest to look at the behaviour of the corresponding ethers of the α-linked glucan amylose.

Amylose trialkylsilyl ether.—Investigation of permethylated amylose mainly substituted with TBDMS in positions 2 and 6 proved that nearly complete rearrangement had

occurred during methylation with NaH and CH_3I in THF. Therefore, it can be concluded that the configuration of the glucosidic linkage is responsible for the event of the alkylation reaction of 2,6-di-*O*-SiR_3 derivatives. Results for one sample with a ds of 1.62 are given in Table 1 (AS-1).

An intermolecular migration of the trialkylsilyl group to O-3 of a neighbouring glucosyl residue seems to be unlikely, while the intramolecular rearrangement of a silyl group from O-2 to O-3 in methyl 4,6-*O*-benzylidene-α-glucopyranoside has already been proved [24].

1. Conclusion

The substitution pattern of trialkylsilyl ethers of cyclodextrins, celluloses, and amyloses with a ds of 1.5–2.0 was determined by standard methylation analysis including a modified hydrolysis step for cellulose samples, and by the reductive-cleavage method. The latter method suffered from losses of higher-substituted glucose units, but gave comparable quantitative results when a high excess of Me_3SiOSO_2Me–$BF_3 \cdot OEt_2$ (1:1) was applied. However, the formation of non-reduced glucose acetates was then favoured. The 2-*O*-THxDMS groups in the α-linked glucans rearranged to the 3-OH group during methylation, yielding nearly quantitatively the 2-*O*-methylated product, while the corresponding β-glucan derivative (cellulose) gave the 3-methyl ether as the main product. The combination of thermodynamic (rearrangement) and kinetic control (ether formation) results in the quantitative formation of the 2-*O*-methyl-3,6-di-*O*-$SiMe_2R$-substituted products in the case of α-glucans and therefore offers a valuable access to selectively 2-*O*-alkylated derivatives.

2. Experimental

General.—Acetic anhydride, $CHCl_3$, CH_2Cl_2, DMF, THF, $NaBD_4$, NaH, imidazole, TBDMSCl, triethylsilane, and CF_3CO_2H (all analytical grade) were purchased from Merck (Darmstadt, Germany); DMAc, $Me_3SiOSO_2CF_3$, and Me_3SiOSO_2Me were from Fluka (Neu-Ulm, Germany); BF_3 etherate and pyridine were from Aldrich (Steinheim, Germany).

Trialkylsilyl ethers.—Trialkylsilyl ethers of cellulose (Avicel^R) were prepared from a solution in DMAc–LiCl [5% (w/w) cellulose, 8% (w/w) LiCl] with THxDMSCl (3–10 equiv/"anhydroglucose" unit) and pyridine (1.1 equiv/"anhydroglucose" unit) at room temparature. β-Cyclodextrin derivatives were prepared with THxDMSCl from a solution in DMF, which was saturated with ammonia at −15°C [12]. The TBDMS-ether of amylose was prepared with TBDMSCl and imidazole in DMF at 80°C according to Fügedi [3]. Methylation was performed with 10 equiv of NaH and CH_3I in dry THF for 2 days [6].

Methanolysis–hydrolysis procedure.—To the permethylated trialkylsilyl ethers (2.3 mg) in a V-vial was added 1.5 M MeOH/HCl (0.6 mL), and the mixture was stirred in a heating block at 90°C for 4 h. After evaporation to dryness the residue was submitted

to acid hydrolysis with 2.0 M CF_3CO_2H at 120°C for 3 h. Reduction (with $NaBD_4$) and acetylation were carried out as described [26].

Hydrolysis with trifluoroacetic acid.—Three-step hydrolysis with CF_3CO_2H was performed according to Fengel et al. [16] modified by Nehls (personal communication). To the cellulose derivative (2 mg), CF_3CO_2H (120 μL) was added and left at room temperature until the mixture became clear. The acid was diluted to 9 M and heated for 15 min to 100°C. The cooled solution was further diluted to 2.7 M and stirred at 120°C for 2 h. The residue was reduced and acetylated.

Saeman hydrolysis.—Two-step hydrolysis with H_2SO_4 (first 12 M at room temperature for 30 min, followed by 1 M at 120°C for 2 h) was performed according to Selvendran et al. [17].

Reductive cleavage.—To the permethylated trialkylsilyl ethers (2.3 mg) in $CHCl_3$ (200 μL) in a screw-cap vial were added Et_3SiH (40 equiv/glucosidic bond) and Lewis acid [Me_3SiOSO_2Me–$BF_3 \cdot OEt_2$ (1:1), $Me_3SiOSO_2CF_3$–$BF_3 \cdot OEt_2$ (1:1), or $Me_3SiOSO_2CF_3$ alone, 40 equiv], and the mixture was kept at room temperature for 3–20 h. The degradation products were acetylated with CF_3CO_2H–Ac_2O (100 μL, 1:10) for 30 min at 50°C. After 5 min at room temperature the solutions were twice washed with aqueous $NaHCO_3$, and the organic phase was dried and analysed by GLC.

GLC.—GLC was carried out on a Carlo Erba Fractovap 4160 gas chromotograph, equipped with an on-column injection system, a CP-Sil 8 CB Chrompack capillary column (25 m × 0.25 mm) and a 2-m retention gap, a flame ionisation detector, and a Merck Hitachi D-2500 Chromatointegrator. Hydrogen was used as carrier gas. Response factors (for multiplication of the peak areas) were calculated according to the ECR-concept [27,28]. Temperature program: 70°C, 1 min isothermal; 20°C/min to 130°C; then 4°C/min to 290°C.

GLC–MS.—Mass spectra were obtained with a VG Analytical VG/70-250S instrument. For CIMS ammonia was used as reactant gas. All compounds were identified by comparison of their mass spectra and GLC retention times with reference compounds or known data [7,18,29].

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