ANALYSIS OF OLIGOSACCHARIDES. ^{29}Si AND ^{13}C NMR SPECTRA OF PERTRIMETHYLSILYLATED OLIGOSACCHARIDES DERIVED FROM XYLOPYRANOSE*

Eva PETRÁKOVÁ[a], Jan SCHRAML[b], Ján HIRSCH[a], Magdalena KVÍČALOVÁ[b], Jan ZELENÝ[b] and Václav CHVALOVSKÝ[b]

[a] *Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava and*
[b] *Institute of Chemical Process Fundamentals, Czechoslovak Academy of Sciences, 165 02 Prague-Suchdol*

Received August 13th, 1986

^{29}Si and ^{13}C chemical shifts are reported for a series of 30 pertrimethylsilylated oligosaccharides containing xylopyranosyl unit. The number of lines in ^{29}Si NMR spectra is in all cases in agreement with the number of hydroxyl groups present in the parent compound prior to trimethylsilylation. The results demonstrate the usefulness of ^{29}Si NMR spectroscopy for the analysis of oligosaccharides and other polyfunctional compounds. The validity of direct additivity of ^{13}C chemical shifts is tested on some model trisaccharides.

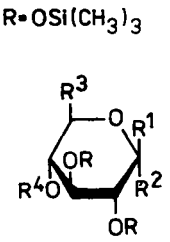
Analysis of polar functional groups by ^{29}Si NMR spectroscopy of their trimethylsilyl derivatives is a powerful method for the analysis of polyfunctional compounds and their mixtures[1]. Applications of this method to saccharides have included various monosaccharide derivatives[1-9] and a few di- and trisaccharides[10-13]. In the present paper the method is tested on an extensive series of oligosaccharides with known structure. All the studied oligosaccharides contain at least one xylopyranosyl unit. Various pertrimethylsilylated derivatives of methyl β-D-xylopyranoside were shown to have very similar conformer population ratio[10-12] and so one can rightly expect that direct additivity of substituent effects should hold for ^{13}C chemical shifts in such series of compounds. The extent to which the additivity is satisfied is tested here on a series of experimentally assigned chemical shifts in pertrimethylsilylated trisaccharides.

RESULTS AND DISCUSSION

The structures of all studied pertrimethylsilylated saccharides are shown in Scheme 1. For an easy reference the compound number indicates (the first digit) the number

* Taken in part from: Petráková E.: *Thesis*. Institute of Chemistry, Slovak Academy of Sciences, Bratislava 1983.

of monosaccharide units in the molecule (1, 2, and 3 for mono-, di-, and trisaccharides, resp.). The second digit identifies the position of the second or third monosaccharide unit on the main skeleton. The subsequent specifying letters have the following



1MA R^1=H, R^2=OCH_3, R^3=H, R^4=R

1MB R^1=OCH_3, R^2=H, R^3=H, R^4=R

1FA R^1=H, R^2=OR, R^3=H, R^4=R

1FB R^1=OR, R^2=H, R^3=H, R^4=R

1G R^1=H, R^2=OCH_3, R^3=$COOCH_3$, R^4=CH_3

meaning: M stands for methyl glycoside, F denotes 1-O-trimethylsilyl derivative, A and B indicate α and β anomers, resp., and G signifies the derivative of glucuronic acid. The relevant ^{29}Si and ^{13}C chemical shifts of all the studied compounds are gathered in Table I.

^{29}Si NMR *Chemical shifts.* Silicon-29 chemical shifts can be assigned to individual silicon atoms present in the molecule by heteronuclear chemical shift two-dimensional spectroscopy. The method is time consuming and requires resolved (at least partially) and assigned ^1H NMR spectra. In the course of the present work the 2D NMR method has been applied to several mono-, di-, and trisaccharides, the technical details of the work were published separately[10,11,13]. The assigned silicon chemical shifts provide important information about the compound with unknown structure because they identify the site of glycosidation in the parent oligosaccharide as the site without any trimethylsiloxy group directly attached to it. On the other hand, ^{29}Si chemical shifts are assigned in the compounds of known structure with the aim to establish empirical assignment rules that would be easy to use for assignment purposes also in the case of compounds with unknown structure. All our attempts to find either a relation between the chemical shift and structure or between the chemical shifts in closely related mono-, di-, and trisaccharides have failed apparently due to a small range of chemical shift values found in the oligosacharides. Therefore, the chemical shifts in the remaining compounds were left unassigned, they are denoted by a footnote *g* in the Table I.

Though the range of ^{29}Si chemical shifts (δ = 17·0–20·5) in pertrimethylsilylated oligosaccharides is small for establishing a reliable empirical assignment rule, the range is sufficient for resolution of a large number of lines which can be encountered in the spectra of silylated polysaccharides. With the spectral linewidths usually well below 0·5 Hz (depending on the magnetic field homogeneity and effectivness of broad-band proton decoupling) and with good proportionality between the signal intensity and silicon concentration (for the necessary precautions see ref.[14]), the number of silylated functional groups determined from ^{29}Si NMR spectra is very reliable. The structural sensitivity of the silicon chemical shift permits to use the ^{29}Si NMR spectra for differentiation between compounds in a way analogous to the use of finger-print region in IR spectroscopy (compare the two spectra shown in Fig. 1).

^{13}C NMR *Chemical shifts.* The skeletal carbon chemical shifts given in Table I were assigned either by one of the exact experimental methods (heteronuclear chemical shift 2D NMR correlation or selective proton decoupling) or according to the direct additivity rule.

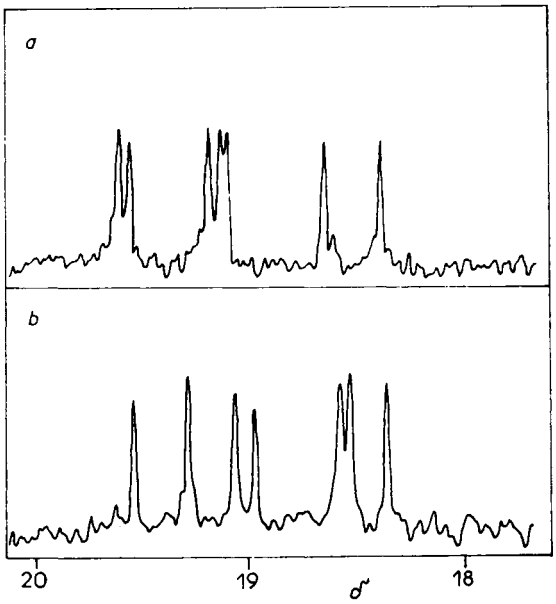


FIG. 1

^{29}Si NMR spectra of two pertrimethylsilylated trisaccharides *a* 34′MB and *b* 33′MB. (Note the difference in the appearance of the two spectra of structurally similar compounds.)

TABLE I

^{29}Si and ^{13}C NMR chemical shifts in pertrimethylsilylated xylopyranose derivatives[a]

Compd.[b]	Ring[b]	^{29}Si Chemical shifts				^{13}C Chemical shifts					Note
		Si-1	Si-2	Si-3	Si-4	C-1	C-2	C-3	C-4	C-5	
1 MA	A	—	18·66	18·39	18·32	100·32	74·85	73·61	71·87	61·86	[c]
1 MB	A	—	19·14	18·57	18·85	105·11	75·73	78·42	71·31	66·07	[d]
1 FA[e]	A	18·25	17·61	18·01	18·39	94·25	74·12	74·12	71·90	62·33	[f]
1 FB[e]	A	20·26	19·05	18·29	18·56	98·69	77·01	78·23	71·48	66·26	[f]
1 G	A	—	19·74[g]	19·56[g]	—	100·54	73·09	74·05	82·47	69·83	[h]
22 MA	A	—	—	19·58[g]	18·58[g]	105·06	74·50	76·19	71·96	66·07	[i]
	B	—	17·86[g]	17·57[g]	17·37[g]	96·84	74·10	73·38	71·96	61·97	
22 MB	A	—	—	18·88	18·49[j]	103·17	75·83[k]	79·81[k]	72·07[k]	66·42[k]	[d]
	B	—	18·62	18·31	18·28[j]	101·56	75·19	78·19	71·51[k]	65·90[k]	
22 FA[e]	A	19·41	—	17·94	17·72[j]	93·68	76·10	75·70	73·04[j]	61·50	
	B	—	19·01	18·43	18·40[j]	102·86	75·37	78·17	71·72[j]	66·23	[f]
22 FB[e]	A	19·94	—	18·45[j]	18·24[j]	96·60	78·17	79·41	72·03[j]	66·13[j]	
	B	—	18·26	18·43[j]	18·17[j]	101·64	75·51	78·33	71·87[j]	65·86[j]	
22 FG[l]	A	18·54[g]	—	19·21[g]	19·08[g]	91·59	78·08	73·50	72·23	62·32	[i]
	B	—	19·21[g]	19·08[g]	—	98·12	73·19	73·63	82·59	70·67	
22 G	A	—	—	19·57[g]	19·32[g]	104·60	75·72	73·22	71·82	65·86[m]	[i]
	B	—	18·78[g]	18·68[g]	—	97·07	72·89	74·94	82·78	69·62	
23 MA	A	—	19·65[g]	—	17·75[g]	104·90	74·23	78·54	71·96	65·42	[i]
	B	—	17·53[g]	17·42[g]	17·15[g]	96·93	73·85	73·85	72·16	62·03	

23 MB	A	—	19·42[k]	—	19·18[k]	105·05	76·13	80·21	69·06	66·45	*i*
	B	—	18·33	18·42	18·20[k]	102·38	75·27	78·59	71·86	66·19	
23 FA[e]	A	19·02	17·06	—	18·77	94·06	75·01	76·70	69·40	62·18	*f*
	B	—	18·10	18·28	18·00	102·34	75·37	78·67	71·89	66·16	
23 FB[e]	A	20·20	19·02	—	19·08	98·85	77·90	80·08	69·15	66·48	*f*
	B	—	18·28	18·43	18·18	102·21	75·37	78·52	71·89	66·16	
24 MA	A	—	19·20[g]	19·04[g]	—	104·86	76·25	81·03	75·94	64·78	*i*
	B	—	18·42[g]	18·14[g]	18·00[g]	101·28	74·42	73·51	71·97	62·60	
24 MB	A	—	19·59	19·15	—	105·29	75·06	76·49	75·00	62·91	*d*
	B	—	19·08	18·60	18·44	101·56	75·10	78·69	71·49	66·10	
24 FA[e]	A	18·28[g]	17·63[g]	18·35[g]	—	93·85	74·12	72·52	75·42	58·92	*f*,*i*
	B	—	18·60[g]	19·08[g]	18·39[g]	101·58	77·58	78·75	71·49	66·10	
24 FB[e]	A	19·98	19·25[j]	18·77	—	98·85	74·99[j]	76·35	75·09	62·92	*f*
	B	—	19·03[j]	18·52	18·39	101·58	77·58[j]	78·75	71·49	66·10	
32 MB	A	—	—	19·90	—	103·21	75·91	77·33	75·08	62·50	*n*
	B	—	19·81	18·69[j]	18·60	101·17	75·24	78·85[o]	71·36[o]	65·96[j]	
	C	—	19·22	18·36[j]	18·60	101·46	75·08	78·07[o]	71·43[o]	66·31[g]	
32 FA[e]	A	19·20[g]	—	19·17[g]	—	93·65	75·46	75·46	73·45	58·32	*p*
	B	—	19·14[g]	19·08[g]	18·56[g]	100·96	76·03	78·88	71·46	65·96	
	C	—	18·52[g]	18·48[g]	18·41[g]	102·67	75·25	78·10	71·66	66·20	
32 FB[e]	A	19·99[g]	—	18·63[g]	—	96·87	77·14	78·88	74·91	62·60	*p*
	B	—	19·08[g]	18·52[g]	18·45[g]	101·28	77·19	78·28	71·83	65·96	
	C	—	19·39[g]	18·35[g]	18·38[g]	101·58	75·25	78·28	71·46	66·07	
32 G	A	—	—	19·95[g]	—	104·57	75·41	76·36	73·52	62·59	*i*
	B	—	19·28[g]	19·28[g]	18·80[g]	101·83	74·86	78·77	71·41	66·12	
	C	—	18·58[g]	18·50[g]	—	97·14	72·90	73·24	83·01	69·82	
32′ M	A	—	19·35	19·64	—	105·30	75·78	76·68	75·10	63·18	*n*
	B	—	—	19·01	18·47	99·97	75·78	79·69	72·11	65·78	
	C	—	18·36	18·47	18·26	101·44	75·53	78·13	71·80	65·98	

TABLE I
(*Continued*)

Compd.[b]	Ring[b]	^{29}Si Chemical shifts				^{13}C Chemical shifts					
		Si-1	Si-2	Si-3	Si-4	C-1	C-2	C-3	C-4	C-5	Note
32′ G	A	—	19·50[g]	19·29[g]	—	105·24	74·07	76·32	75·82	62·97	[i]
	B	—	—	19·14[g]	19·01[g]	100·66	76·14	76·58	72·23	65·80	
	C	—	18·50[g]	18·14[g]	—	97·21	73·32	73·32	82·63	70·01	
33 M	A	—	19·76	—	—	105·05	76·52	76·52	71·57	63·39	[n]
	B	—	19·47	18·37	18·25[j]	100·74	75·41	78·16	71·86	65·96	
	C	—	18·64	18·25	18·29[j]	101·32	75·51	78·31	71·86	65·96	
33′ M	A	—	18·54[q]	19·00	—	105·25	76·09	76·64	78·08	62·32	[n]
	B	—	19·29	—	19·01	101·02	76·09	70·42	69·20	66·27[j]	
	C	—	18·56	19·54[q]	18·38	101·87	75·39	78·42	71·83	66·17[j]	
34′ MB	A	—	19·63	19·12	—	105·27	75·98	76·36	74·61	62·75	[n]
	B	—	19·56	19·18	—	101·31	75·51	76·74	75·13	62·75	
	C	—	19·12	18·65	18·40	101·56	75·02	78·71	71·46	66·09	
35 M	A	—	—	18·98[g]	18·98[g]	103·12	75·81	79·89	72·05	66·02	[i]
	B	—	18·84[g]	18·84[g]	—	101·32	75·47	76·36	75·08	63·22	
	C	—	18·52[g]	18·37[g]	18·32[g]	101·51	74·81	78·78	71·53	65·84	
36 M	A	—	19·48[g]	—	19·34[g]	104·93	75·09	79·86	69·02	66·05	[i]
	B	—	19·01[g]	19·01[g]	—	102·13	76·33	76·81	75·42	62·05	
	C	—	18·52[g]	18·52[g]	18·32[g]	101·29	75·09	78·70	71·50	66·39	

[a] Chemical shifts in δ scale, approximate error $\pm 0{\cdot}04$ ppm. [b] For the structure of the compounds and ring labeling see Scheme 1. [c] Assigned by 2D NMR as described in ref.[10], ^3J(H-1, H-2) = 3·51 Hz. [d] Assigned by 2D NMR in ref.[10]. [e] Measured in mixture of anomers. [f] Assigned by 2D NMR in ref.[13]. [g] Not assigned. [h] Assigned by selective heteronuclear decoupling, ^3J(H-1, H-2) = 3·63 Hz. [i] Carbon-13 chemical shifts assigned by additivity rule only. [j] Assignments in the column may be interchanged. [k] Assignments may be interchanged. [l] Only α anomer present in the measured solution. [m] Assigned by APT experiment. [n] Assigned by 2D NMR in ref.[11]. [o] Assignments in the two columns may be interchanged in synchronism. [p] Assigned in ref.[11] by comparison only. [q] Assignment may be reversed.

The empirical direct additivity rule[15] can be expressed as[12]

$$\delta = \delta_0 + \sum_i \Delta_i ,$$

where δ and δ_0 are the chemical shifts of the corresponding nuclei in the given compound and in the reference molecule, respectively. When a parent hydrocarbon is chosen for the reference, the contributions of substituents Δ_i are called substituent chemical shifts (SCS), when some other more suitable compound is chosen for the reference, the increments are called derivatization chemical shifts (DCS). The DCS are assigned to substituents which differentiate the given compound from the chosen reference. Their values generally depend on the chosen reference compound, on the nucleus in question, on the nature and position of the substituent and on some other factors (usually undesirable as experimental conditions). According to the simple direct additivity rule it is assumed that the DCS value of a given substituent is independent of other substituents present in the studied compound.

In the field of NMR spectroscopy of saccharides the direct additivity rule is often used though stated in somewhat different terms like various methylation shift rules[16,17], O-substitution rules[16], glycosidation shifts[18], *etc.* All applications of these rules assume (though often only tacitly) that the studied molecule has the same conformation (or conformer ratio) as the reference compound and the compounds used for derivation of the DCS values. We have recently shown[12] that pertrimethylsilylation leads to conformational homogenization in an extensive series of derivatives of methyl β-D-xylopyranoside. The narrow range of proton–proton coupling constants ^3J(H-1, H-2) and almost constant ^{13}C chemical shifts of OCH_3 groups in the studied disaccharides[10] and trisaccharides[11] are also indicative of similar conformer populations within the series of compounds in which the chemical shifts were assigned by exact methods. Hence, these compounds provide good test for the validity of direct additivity, the deviations, if found, should not be caused by variations in conformer ratio but by some interactions.

Using the chemical shifts in pertrimethylsilylated methyl β-D-xylopyranoside (1 MB) as the reference, two types of DCS can be derived from the assigned chemical shifts in pertrimethylsilylated disaccharides. The DCS values of the first type (Table II) account for the subtitution of one trimethylsilyl group in 1MB by pertrimethylsilylated β-D-xylopyranosyl group; these DCS values can be used for calculation of the chemical shifts in the backbone of pertrimethylsilylated xylooligosaccharides. The DCS values of the second type (Table III) describe the change in the chemical shifts upon replacement of the glycosidic methyl group in 1 MB by pertrimethylsilylated methyl β-D-xylopyranosyl unit. Of course, the latter values depend on the position at which the unit is attached (the position of the glycosidic link); the values can be used for predicting the chemical shifts in the terminal xylopyranosyl rings

which are connected to the oligosaccharide backbone through the C-1 carbon atom.

Differences between experimental chemical shifts and chemical shifts calculated according to the direct additivity rule are shown in Table IV for pertrimethylsilylated trisaccharides which were measured as methyl glycosides. Obviously, the chemical shifts predicted according to the additivity are good enough to differentiate C-1 and C-5 carbon chemical shifts from the others and, in some cases, to assign the lines of these two types of carbon atoms to the rings but the accuracy of the predicted shifts is not sufficient for the assignment of C-2, C-3, and C-4 carbon lines. This is

TABLE II

^{13}C NMR derivatization chemical shifts (DCS) due to a replacement of a trimethylsilyl groups in pertrimethylsilylated methyl β-D-xylopyranoside (1MB) by pertrimethylsilylated β-D-xylopyranosyl residue[a]

Site of substitution	DCS on carbon				
	C-1	C-2	C-3	C-4	C-5
2	−1·94	0·10	1·39	0·76	0·35
3	−0·06	0·40	1·79	−2·25	0·38
4	0·18	−0·67	−1·93	3·69	−3·16

[a] DCS values in ppm units derived as a difference in C-n carbon chemical shifts in the ring A of the appropriate disaccharide derivative and in 1MB.

TABLE III

^{13}C derivatization chemical shifts (DCS) due to replacement of the 1-O-methyl group in pertrimethylsilylated methyl-β-D-xylopyranoside (1MB) pertrimethylsilylated n-O-xylopyranosyl residues[a]

n[b]	DCS on carbon				
	C-1	C-2	C-3	C-4	C-5
2	−3·55	−0·54	−0·23	0·20	−0·17
3	−2·73	−0·46	0·17	0·55	0·12
4	−3·55	−0·63	0·27	0·18	0·03

[a] DCS values in ppm derived as a difference in C-n carbon chemical shifts in the ring B of the appropriate disaccharide derivative and in 1MB. [b] Substituted position on the ring A of the disaccharide derivative.

serious limitation especially as it has been derived from the data obtained at low concentration and on compounds which are conformationally homogeneous. The disagreement between the calculated and experimental chemical shifts should be expected larger for other series of compounds. The deviations are notably large in the spectrum of 33M where apparently the proximity of two xylopyranosyl units (in 3-O- and 4-O- positions) leads to sterical interactions.

Analogous DCS values as discussed above for methyl glycosides can be derived for trimethylsilyl glycosides or for derivatives of glucuronic acid (series of compounds labelled F and G, resp.). In fact such values were used to predict the chemical shifts in the corresponding trisaccharides. In view of the above results the assignment based on additivity predictions should be considered as tentative only.

EXPERIMENTAL

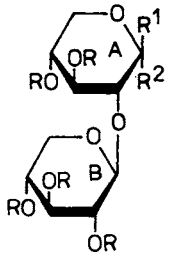
Compound preparation. The pertrimethylsilyl derivatives were prepared by pertrimethylsilylation of the parent saccharides which were either commercial products or were prepared by described procedures[19-32]. Various silylating procedures[33] have been used in our laboratory, the following three have been more or less standardized for silylation of methyl glycosides:

TABLE IV
Deviations of experimental ^{13}C chemical shifts from calculated values in trisaccharides[a]

Trisaccharide	Ring	C-1	C-2	C-3	C-4	C-5
32 MB	A	−0·14	0·75	−0·55	−0·68	−0·76
	B	−0·39	0·14	0·16	−0·13	−0·14
	C	−0·10	−0·11	−0·12	−0·08	0·41
33 M	A	−0·18	1·06	−1·76	−1·18	0·10
	B	−0·82	0·31	−0·53	0·37	−0·14
	C	−1·06	0·24	−0·28	0·00	−0·23
32′ M	A	0·01	0·72	0·19	0·10	0·27
	B	0·35	0·58	−0·39	−0·14	−0·67
	C	−0·12	0·34	−0·06	0·29	0·08
33′ M	A	−0·04	1·03	0·15	3·08	−0·59
	B	−0·48	0·59	−0·06	−0·04	−0·21
	C	−0·51	0·12	−0·17	−0·03	−0·02
34′ M	A	−0·02	0·92	−0·13	−0·39	−0·16
	B	−0·43	1·08	−0·02	−0·07	−0·19
	C	0·00	−0·08	0·02	−0·03	−0·01

[a] Deviation is defined here as the experimental value minus the calculated value, in ppm.

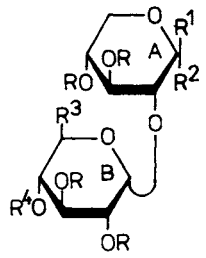
a) According to Klebe *et al.*[34] the saccharide was placed into a dry small reaction vessel equipped with a teflon septum seal. A 200% stoichiometric excess of N,O-bis(trimethylsilyl) acetamide (BSA) was added through a syringe. The reaction mixture was vibrationally stirred and heated at 60—70°C for 1 h. Excess reagents were distilled off *in vacuo* under a stream of dry nitrogen. (This method provided the desired products in the case of derivatives 1MA, 1MB, 22MB, 23MB, 24MB, 32MB, 32G, 33'M, 34'MB, and 36M.)



22 MB R^1=OCH_3, R^2=H

22 FA R^1=H, R^2=OR

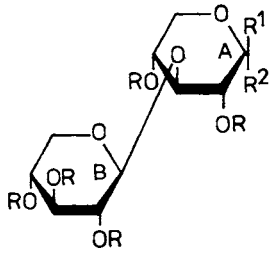
22 FB R^1=OR, R^2=H



22 MA R^1=OCH_3, R^2=H, R^3=H, R^4=R

22 G R^1=OCH_3, R^2=H, R^3=$COOCH_3$, R^4=CH_3

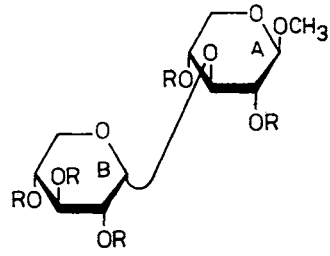
22 FG R^1=H, R^2=OR, R^3=$COOCH_3$, R^4=CH_3



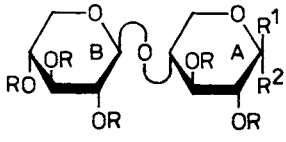
23 MB R^1=OCH_3, R^2=H

23 FA R^1=H, R^2=OR

23 FB R^1=OR, R^2=H



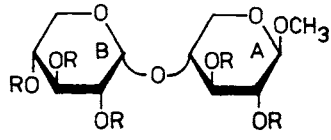
23 MA



24 MB R^1=OCH_3, R^2=H

24 FA R^1=H, R^2=OR

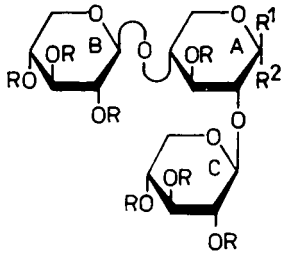
24 FB R^1=OR, R^2=H



24 MA

b) For silylation by trimethylchlorsilane (TMCS) in a heterogenous mixture with formamide and n-hexane we adopted the procedure described by Henylein and Koesters[35]. (The method was used for preparation of 1MA, 1MB, 1G, 22MA, 23MA, 23MB, 24MB, 32MB, 33M, 32'M, and 33'M.)

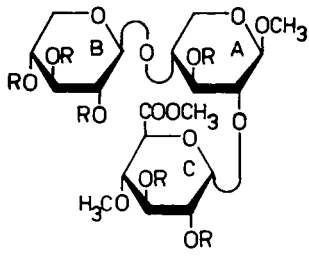
c) Combination of BSA and TMCS was used for silylation in the method described by Chambaz *et al.*[36,37]. (In this way compounds 24MA, 35M, and 22G were prepared.)



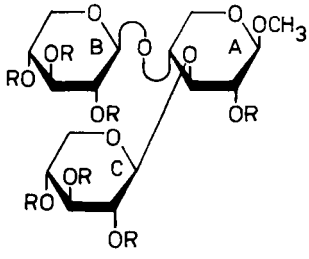
32 MB R^1=OCH_3, R^2=H

32 FA R^1=H, R^2=OR

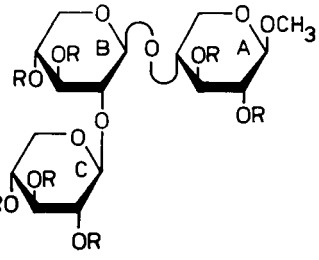
32 FB R^1=OR, R^2=H



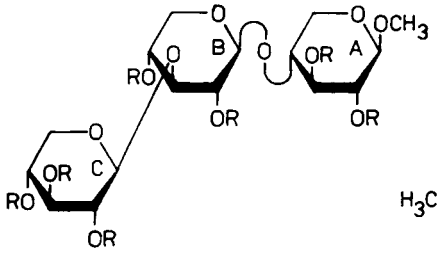
32 G



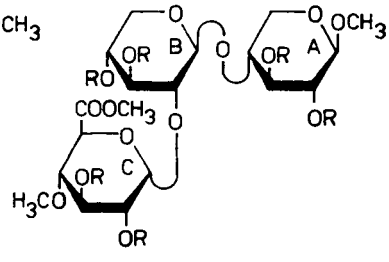
33 M



32' M



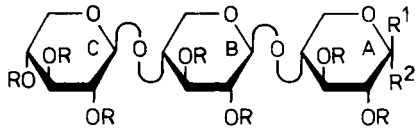
33' M



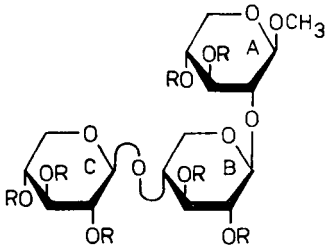
32' G

The intensity and the number of lines in the ^{29}Si NMR spectra of saccharides which could undergo anomerization through the silylation reaction varied with the method of silylation used and with the duration of the reaction. It was found[13] that reproducible results could be obtained only by the method *a*) with overall reaction time control. For that reason all the compounds designated by F (*e.g.* 1FA and 1FB) were prepared by this method and their spectra measured in anomeric mixtures.

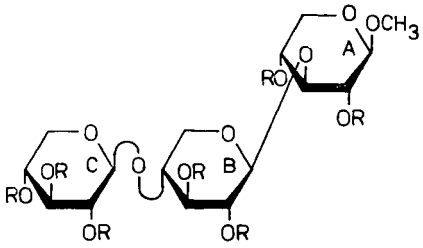
NMR *measurements.* The samples were measured in deuteriochloroform solutions containing 1—2% (v/v) of hexamethyldisilane (HMDSS) which served as an internal secondary reference ($\delta(^{29}Si) = -19{\cdot}79$). The solvent (Institute of Nuclear Research, Swierek, Poland) was dried over molecular sieve (Fischer 5A). The reported data were obtained from approximately 0·05M solutions, which were contained in carefully dried 5 mm tubes closed by septum caps.



34 MB $R^1 = OCH_3$, $R^2 = H$



35 M



36 M

In place of 34 MB read 34′ MB.

All the spectra were measured on a Varian XL-200 spectrometer operating at 50·3 and 39·7 MHz for ^{13}C and ^{29}Si NMR spectra, respectively. The length of 90° pulses was 7—9 μs in the observation chanel and 50 μs in the decoupler chanel. Standard software (H-2Z version) was used. ^{13}C NMR spectra were measured in 8—16 kHz spectral widths using 32 K words of memory for FID accumulation, the spectra were referenced to the central line of deuterichloroform ($\delta = 76{\cdot}99$). ^{29}Si NMR spectra were measured by gated decoupling method[38] or by routine decoupled refocused INEPT (ref.[39]). The two methods gave the same chemical shifts and intensity ratios, the letter method gave better signal-to-noice ratio. The spectra were measured in 4 kHz spectra width with acquisition time of 1 s and 16 k of memory used for FID accumulation. The FIDs were usually exponentially weighted with the line broadening of 1—3 Hz.

REFERENCES

1. Schraml J., Pola J., Jancke H., Engelhardt G., Černý M., Chvalovský V.: This Journal *41*, 360 (1976).
2. Gale D. J., Haines A. H., Harris R. K.: Org. Magn. Reson. *7*, 635 (1975).
3. Haines A. H., Harris R. K., Rao R. C.: Org. Magn. Reson. *9*, 432 (1977).

4. Schraml J., Včelák J., Černý M., Chvalovský V.: This Journal *48*, 2503 (1983).
5. Schraml J., Petráková E., Pihar O., Hirsch J., Chvalovský V.: Org. Magn. Reson. *21*, 666 (1983).
6. Gale D. J., Evans N. A.: Org. Magn. Reson. *21*, 567 (1983).
7. Liepinsh E., Sekacis I., Lukevics E.: Magn. Res. Chem. *23*, 10 (1985).
8. Nardin R., Vincendon M.: J. Magn. Reson. *61*, 338 (1985).
9. Schraml J., Kučar Š., Zelený J., Chvalovský V.: This Journal *50*, 1176 (1985).
10. Schraml J., Petráková E., Pelnař J., Kvíčalová M., Chvalovský V.: J. Carbohydr. Chem. *4*, 393 (1985).
11. Schraml J., Petráková E., Hirsch J.: Magn. Res. Chem. *25*, 75 (1987).
12. Schraml J., Petráková E., Pihar O., Hirsch J., Chvalovský V.: This Journal *48*, 1829 (1983).
13. Schraml J., Petráková E., Hirsch J., Čermák J., Chvalovský V., Teeäär R., Lippmaa E.: This Journal, in press.
14. Schraml J., Blechta V., Kvíčalová M., Nondek L., Chvalovský V.: Anal. Chem. *58*, 1892 (1986).
15. Wehrli F. W., Wirthlin T.: *Interpretation of Carbon-13 NMR Spectra*, p. 39. Heyden, London 1976.
16. Gorin P. A. J.: Adv. Carbohydr. Chem. Biochem. *38*, 13 (1981).
17. Shashkov A. S., Chizhov O. S.: Bioorg. Khim. *2*, 437 (1976).
18. Gorin P. A. J.: Carbohydr. Res. *39*, 3 (1975).
19. Kováč P.: Carbohydr. Res. *31*, 323 (1973).
20. Kováč P., Palovčík R.: Chem. Zvesti *32*, 501 (1978).
21. Kováč P.: Chem. Zvesti *33*, 365 (1979).
22. Hirsch J., Kováč P.: Carbohydr. Res. *77*, 241 (1979).
23. Kováč P.: This Journal *44*, 928 (1979).
24. Kováč P., Petráková E.: Chem. Zvesti *34*, 537 (1980).
25. Kováč P.: This Journal *45*, 892 (1980).
26. Kováč P., Hirsch J.: Carbohydr. Res. *79*, 303 (1980).
27. Kováč P.: Chem. Zvesti *34*, 234 (1980).
28. Petráková E., Kováč P.: Chem. Zvesti *35*, 551 (1981).
29. Petráková E., Kováč P.: Chem. Zvesti *35*, 699 (1981).
30. Hirsch K., Kováč P., Alföldi J., Mihálov V.: Carbohydr. Res. *88*, 146 (1981).
31. Kováč P., Petráková E., Kočiš P.: Carbohydr. Res. *93*, 144 (1981).
32. Hirsch J., Petráková E.: Chem. Zvesti *38*, 409 (1984).
33. *Pierce Handbook and General Catalog 1979—80*, p. 153. Pierce Chemical Company, Rockford, III, 1978.
34. Klebe J. F., Finkbeiner H., White D. M.: J. Am. Chem. Soc. *88*, 3390 (1966).
35. Henylein F. A., Koesters B.: Ber. *92*, 1638 (1959).
36. Chambaz E. M., Horning E. C.: Anal. Lett. *1*, 201 (1967).
37. Chambaz E. M., Maume G., Maume B., Horning E. C.: Anal. Lett. *1*, 749
38. Levy G. C., Cargioli J. D. in the book: *Nuclear Magnetic Resonance Spectroscopy of Nuclei other than Protons* (T. Axenrod and G. A. Webb, Eds), p. 251. Wiley, New York 1974.
39. Schraml J.: This Journal *48*, 3402 (1983).

Translated by the author (J. S.).