Carbohydrate Research, 152 (1986) 81–87
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

2-DEOXY-1,3,4,5,6-PENTA-*O*-METHYL-2-(*N*-METHYLACETAMIDO)-D-GLUCITOL AND DERIVATIVES UNDERGO *C*-METHYLATION AT THE *N*-METHYLACETAMIDO GROUP ON REPEATED HAKOMORI METHYLATION

ULRICH ZÄHRINGER AND ERNST THEODOR RIETSCHEL
Forschungsinstitut Borstel, Institut für Experimentelle Biologie und Medizin, D-2061 Borstel (F.R.G.)
(Received May 13th, 1985; accepted for publication, November 27th, 1985)

ABSTRACT

After Hakomori methylation of 2-acetamido-2-deoxy-D-glucitol, the expected 2-deoxy-1,3,4,5,6-penta-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol (**3**) was identified by g.l.c.–m.s. as the major product, and two minor products, 2-acetamido-2-deoxy-1,3,4,5,6-penta-*O*-methyl-D-glucitol (**2**) and 2-deoxy-1,3,4,5,6-penta-*O*-methyl-2-(*N*-methylpropionamido)-D-glucitol (**4**), were present. The proportions and yields of these products were dependent on the reagent (sodium or potassium hydride) used for the preparation of the methylsulfinylmethanide. On Hakomori methylation of **2** and **3**, the *N*-methylpropionamido (**4**), *N*-methylisobutyramido, and traces of the *N*-methylpivalamido derivatives of 2-deoxy-1,3,4,5,6-penta-*O*-methyl-D-glucitol were formed. Using trideuteriomethyl iodide for methylation (*e.g.*, of **3**), it was found by g.l.c.–m.s. that the newly introduced methyl group(s) were located at the β-carbon of the *N*-methylacetamido group. Analogous results were obtained with 2-deoxy-4-*O*-[2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-D-glucopyranosyl]-1,3,5,6-tetra-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol.

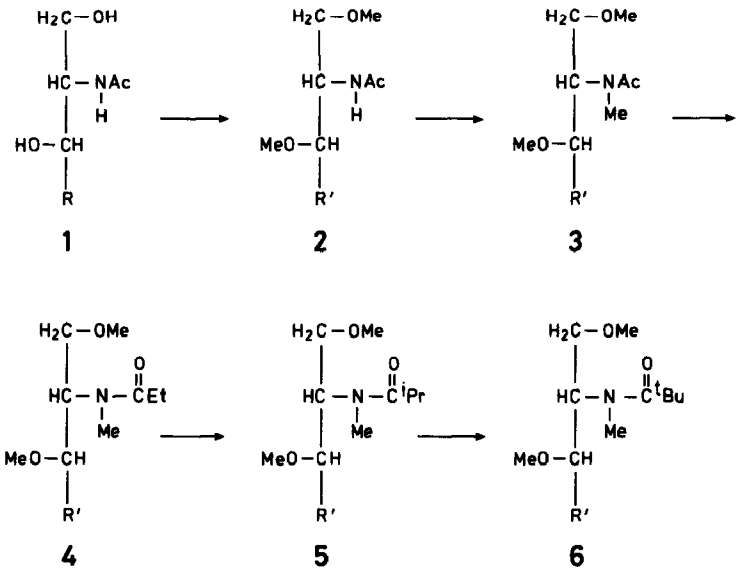
INTRODUCTION

Hakomori methylation[1] and modifications using potassium instead of sodium hydride for preparing the methylsulfinylmethanide[2] have been widely applied in structural studies of carbohydrates. In order to obtain unequivocal results, complete methylation is a prerequisite. However, a single treatment of complex oligosaccharides of low solubility in Me_2SO often results in incomplete methylation and repeated treatments are necessary[3].

In studies involving the repeated methylation of 2-acetamido-2-deoxy-D-glucitol (**1**), the expected product was formed together with other compounds having higher molecular weights and retention times in g.l.c. longer than that of 2-deoxy-1,3,4,5,6-penta-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol (**3**). We now report on the behaviour of 2-acetamido-2-deoxy-D-glucitol and 2-acetamido-4-*O*-

0008-6215/86/$ 03.50

(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucitol (di-*N*-acetylchitobi-itol) on repeated Hakomori methylation.



R = CHOHCHOHCH$_2$OH ; R' = CHOMeCHOMeCH$_2$OMe

EXPERIMENTAL

Methyl sulfoxide and methyl iodide were distilled before use and stored over molecular sieve (0.4 nm) and silver plates, respectively. Trideuteriomethyl iodide (99% D, Merck) and acetic anhydride (99% D, Ega-Chemie, Steinheim) were used without further purification.

Reduction, N-acetylation, and methylation. — Reduction of 2-acetamido-2-deoxy-D-hexoses and chitobiose was carried out with either sodium borohydride or sodium borodeuteride as described[4]. *N*-Acyl derivatives of 2-amino-2-deoxy-D-glucose (GlcNPr, GlcN^{i}Bu, GlcNPiv*) were prepared with the corresponding anhydrides[5]. Methylation was performed according to Hakomori, using sodium[1] or potassium methylsulfinylmethanide[2]. If not stated otherwise, the latter reagent was used since it gave better yields of methylated products. Excess of the methylsulfinyl-methanide was tested for with triphenylmethane[6]. The 2-deoxy-1,3,4,5,6-penta-*O*-methyl-2-(*N*-methylacetamido)-D-hexitols and methylated chitobi-itol were purified by chromatography[7] on a column (15 × 1.5 cm) of LH-20 (Pharmacia) and then on a column (20 × 5 mm) of silica gel (elution with chloroform–methanol,

*Connotes the 2-propionamido, 2-isobutyramido, and 2-pivalamido derivatives, respectively.

75:25), and finally by semi-preparative reversed-phase h.p.l.c. to a purity of 99.5% as determined by analytical h.p.l.c. and g.l.c. (see below).

Chromatographic procedures and mass spectrometry. — Reversed-phase h.p.l.c. was performed using a DuPont pump (Model 870) equipped with a u.v. detector and a gradient controller (Model 8800). Samples were applied automatically by a WISP injector (Waters) and the effluent was monitored at 210 nm. Peak areas were integrated (SP 4100 integrator, Spectra Physics) and fractionation was effected with a BASIC programme-controlled fraction collector (Foxy, Colora).

2-Deoxy-1,3,4,5,6-penta-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol (**3**) could be separated from its acetamido analogue by using a semi-preparative Zorbax™ ODS (10 μm) column (25 cm × 9.4 mm i.d.). The solvents used for h.p.l.c. were methanol–water mixtures: *A*, 1:9, *B*, 3:1. The following gradient was used at 3.5 mL/min: 10% of solvent *B* isocratic for 5 min, then a hyperbolic gradient (exponent −4) to 75% of solvent *B* in 30 min, and remaining thereat for 10 min. Elution times: 2-acetamido-2-deoxy-1,3,4,5,6-penta-*O*-methyl-D-glucitol, 18.5 min; and its *N*-methyl derivative, 19.5 min. Methylated 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucitol could be isolated under similar conditions, using 75% of solvent *B* under isocratic conditions, with a retention time of 13.2 min.

G.l.c. was carried out with a Varian Aerograph (Model 3700) equipped with a capillary column (25 m × 0.32 mm i.d.) and chemically bonded OV-1 as liquid phase. The carrier gas was hydrogen at 1.3 mL/min and the split ratio was 1:50. The injector port and flame-ionisation detector temperature was 280°. Peak areas were recorded with a Hewlett–Packard reporting integrator (Model 3390A). Quantification of 2-deoxy-1,3,4,5,6-penta-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol was done by calibration of the flame-ionisation detector response-factor with highly purified, standard material.

G.l.c.–m.s. was performed with a Hewlett–Packard system (Model 5985) equipped with a fused-silica capillary column (10 m × 0.33 mm i.d.) to which SE-54 was chemically bonded. E.i.-m.s. was performed at 70 eV, an acceleration voltage of 1800 V, and a source temperature of 200°. For c.i.-m.s., methane was used as plasma at 0.66 mPa. The ion source (200°) was timed to optimal resolution by means of perfluorotributylamine. 2-Deoxy-1,3,4,5,6-penta-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol and its homologues were separated isothermally at 170° with helium as the carrier gas at 2.2 mL/min. Methylated di-*N*-acetylchitobi-itol and its *N*-methylacylamido analogues were separated by the following programme: 10 min at 260° and then to 280° at 3°/min.

RESULTS AND DISCUSSION

According to Phillips and Fraser[2], the use of potassium instead of sodium methylsulfinylmethanide for Hakomori methylation gave higher yields of 2-deoxy-1,3,4,5,6-tetra-*O*-methyl-2-(*N*-methylacetamido)-D-galactose. The yields of

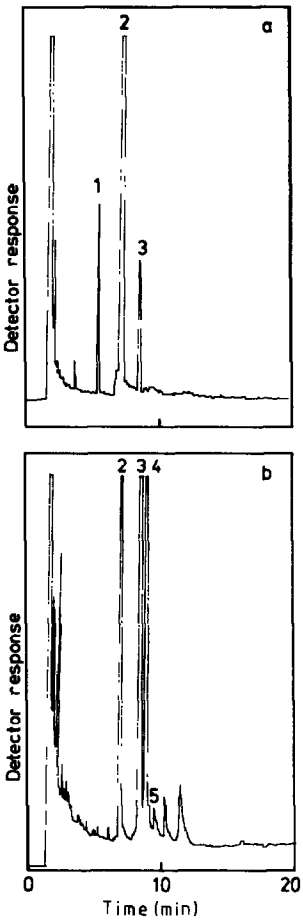
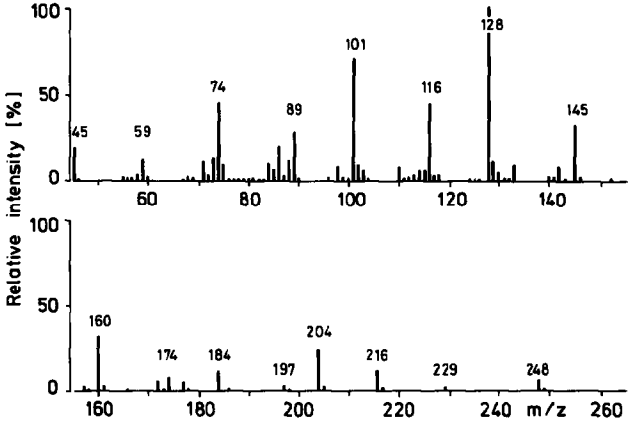


Fig. 1. Gas–liquid chromatograms obtained after (*a*) one-step and (*b*) two-step Hakomori methylation of 2-acetamido-2-deoxy-D-glucitol. G.l.c. was performed at 150° (see Experimental for details): methylated derivatives of 1, GlcNAc-ol; 2, GlcNMeAc-ol; 3, GlcNMePr-ol; 4, GlcNMe^{i}Bu-ol; and 5, GlcNMePiv-ol.

TABLE I

AMOUNT OF STARTING MATERIAL OBTAINED FROM GlcNAc-ol, GalNAc-ol, AND ManNAc-ol AFTER HAKOMORI METHYLATION

Counterion of methylsulfinylmethanide	*Amount of N-acyl derivatives formed (mol/mol)*								
	GlcN-ol			*GalN-ol*			*ManN-ol*		
	H | *-N-Ac*	*Me* | *-N-Ac*	*Me* | *-N-Pr*	*H* | *-N-Ac*	*Me* | *-N-Ac*	*Me* | *-N-Pr*	*H* | *-N-Ac*	*Me* | *-N-Ac*	*Me* | *-N-Pr*
Na^+	0.05	0.51	0.03	0.19	0.69	0.06	0.06	0.33	0.04
K^+	0.03	0.89	0.09	0.05	0.83	0.01	0.01	0.85	0.03



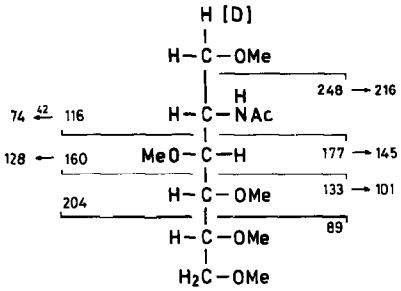


Fig. 2. E.i.-mass spectrum of 2-acetamido-2-deoxy-1,3,4,5,6-penta-*O*-methyl-D-glucitol. For the (1-^{2}H)-derivative, the fragments *m/z* 74, 116, 128, 160, and 204 are shifted to *m/z* 75, 117, 129, 161, and 205.

TABLE II

FRAGMENTATION (*m/z* VALUES WITH INTENSITIES IN BRACKETS) OF 2-DEOXY-1,3,4,5,6-PENTA-*O*-METHYL-2-(*N*-METHYLACYLAMIDO)-D-GLUCITOLS

		R			
		Acetyl	*Propionyl*	*Isobutyryl*	*Pivalyl*
	H [D] H-C-OMe	45	45	45	45
	Me H-C-N-R	42 130→ 88 (100) (77)	56 144→ 88 (100) (90)	70 158→ 88 (73) (100)	84 172→ 88 (100) (21)
145←177	MeO-C-H	32 174→142 (41) (62)	32 188→156 (28) (46)	202 (21)	216 (8)
101←133	H-C-OMe	218 (7)	232 (5)	246 (−)	260 (−)
89	H-C-OMe	262	276	290	304
45	H_2C-OMe	307	321	335	349

GlcNAc-ol, GalNAc-ol, and ManNAc-ol after Hakomori methylation with either sodium or potassium methylsulfinylmethanide, shown in Table I, accord with previous findings[2] in that better yields were obtained with the potassium salt. However, methylation of the 2-acetamido-2-deoxy-D-hexitols also yielded small amounts of methylated 2-deoxy-2-(*N*-methylpropionamido)-D-hexitols (Table I, peak 3 of Fig. 1a), indicating that *C*-methylation had also occurred.

When 2-deoxy-1,3,4,5,6-penta-*O*-methyl-2-(*N*-methylacetamido)-D-glucitol (**3**) was treated under the conditions of Hakomori methylation, additional g.l.c. peaks were found (Fig. 1b; peaks 3 and 4, *T* 8.4 and 9.0 min; *cf.* 7.0 min for the starting material). C.i.-m.s. of these products gave $(M + 1)^+$ ions which were 14 and 28 mass units higher, respectively, than $(M + 1)^+$ of the starting material (*m/z* 308, peak 2 of Fig. 1b). This suggested that one and two methyl groups, respectively, had been introduced. When the methylation was conducted with trideuteriomethyl iodide, the $(M + 1)^+$ ions were shifted by 17 and 34 mass units, respectively. The products in peaks 3 and 4 (Fig. 1b) were identical (*T* values, g.l.c.–m.s.) with those obtained on Hakomori methylation of 2-deoxy-2-propionamido-D-glucitol and 2-deoxy-2-isobutyramido-D-glucitol, respectively. The identity of 2-deoxy-1,3,4,5,6-penta-*O*-methyl-2-(*N*-methylpivalamido)-D-glucitol (**6**) (Fig. 1b; peak 5, *T* 9.5 min) as a minor product of the methylation of the *N*-methylacetamido derivative was also established in this way. The e.i.-mass spectra fragmentation pattern shown in Fig. 2 for 2-acetamido-2-deoxy-1,3,4,5,6-penta-*O*-methyl-D-glucitol (**2**) is characteristic of all the compounds studied (Table II).

When the hepta-*O*-methyl derivative of di-*N*-acetylchitobi-itol, prepared from reduced chitobiose[8] and purified by reversed-phase l.c., was subjected to Hakomori methylation, then, of the 4^2 expected derivatives, 6 could be separated on capillary g.l.c. and identified by g.l.c.–m.s. (Table III). The interpretation of the mass spectra was based on published data[8].

The yields of products obtained on methylation of 2-acetamido-2-deoxy-1,3,4,5,6-penta-*O*-methyl-D-glucitol were not determined, but ~70% of the starting material was transformed into the *N*-methylacetamido derivative and 8% of the

TABLE III

CHARACTERISTIC MASS-SPECTRAL FRAGMENTS OF THE *N*-METHYLACYLAMINO HOMOLOGUES[a] OBTAINED AFTER METHYLATION OF METHYLATED DI-*N*-ACETYLCHITOBI-ITOL

Peak	*GlcN*		*GlcN-ol*	
		m/z		*m/z*
1	Acetyl	260 (228)	Acetyl	130 (88), 276
2	Acetyl	260 (228)	Propionyl	144 (88), 280
3	Propionyl	274 (242)	Acetyl	130 (88), 276
4	Propionyl	274 (242)	Propionyl	144 (88), 280
5	Propionyl	274 (242)	Isobutyryl	158 (88), 294
6	Isobutyryl	288 (256)	Propionyl	144 (88), 280

[a]Peak numbers correspond to the g.l.c. elution profile.

N-methylpropionamido derivative (**4**) was formed. Methylation of the *N*-methylacetamido derivative gave 60% of the *N*-methylpropionamido (**4**) and *N*-methylisobutyramido (**5**) derivatives. Thus, the *N*-methyl group plays an important role in the *C*-methylation observed during a second Hakomori methylation.

ACKNOWLEDGMENTS

We thank Dr. Michael Jensen for valuable suggestions, and Ingolf Keller and Hermann Moll for technical assistance. Part of this investigation was supported by a grant from the Fonds der Chemie.

REFERENCES

1 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
2 L. R. PHILLIPS AND B. A. FRASER, *Carbohydr. Res.*, 90 (1981) 149–152.
3 P. J. HARRIS, R. J. HENRY, A. B. BLAKENEY, AND B. A. STONE, *Carbohydr. Res.*, 127 (1984) 59–73.
4 S. HASE AND E. T. RIETSCHEL, *Eur. J. Biochem.*, 63 (1976) 93–99.
5 D. HORTON, H. MAYER, AND R. MONTGOMERY, *Biochem. Prep.*, 11 (1966) 1–3.
6 H. RAUVALA, *Carbohydr. Res.*, 72 (1979) 257–260.
7 K. STELLNER, H. SAITO, AND S. HAKOMORI, *Arch. Biochem. Biophys.*, 155 (1973) 464–472.
8 M. JENSEN, D. BOROWIAK, H. PAULSEN, AND E. T. RIETSCHEL, *Biomed. Mass. Spectrom.*, 6 (1979) 559–565.