Carbohydrate Research, 187 (1989) 25–34
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

G.L.C.–M.S. OF *N*-(1-DEOXYALDITOL-1-YL)OCTADECYLAMINE DERIVATIVES IN THE ANALYSIS OF METHANOLYSATES OF NEOGLYCOLIPIDS OBTAINED BY REDUCTIVE AMINATION

JAN E. G. VAN DAM, AUGUSTINUS A. M. MAAS, JOHANNIS P. KAMERLING, AND JOHANNES F. G. VLIEGENTHART

Department of Bio-Organic Chemistry, Transitorium III, Utrecht University, P.O. Box 80075, NL-3508 TB Utrecht (The Netherlands)

(Received July 12th, 1988; accepted for publication, October 8th, 1988)

ABSTRACT

Hydrophobic conjugates of a series of aldoses have been prepared by reductive amination with octadecylamine and sodium cyanoborohydride, as model compounds for the analysis of reductively aminated oligosaccharides derived from capsular polysaccharides of *Streptococcus pneumoniae*. In the context of the methanolysis procedure for sugar analysis, g.l.c. and g.l.c.–m.s. (e.i.-mode) studies were carried out on the *N*-(1-deoxyalditol-1-yl)octadecylamine derivatives obtained after treatment with methanolic HCl, and subsequent *N*-acetylation and trimethylsilylation.

INTRODUCTION

Oligosaccharides derived from bacterial capsular polysaccharides may elicit specific protective antibodies against bacterial infections, when coupled to appropriate carriers[1–3]. For incorporation of carbohydrate antigens into liposomal membranes, coupling of the oligosaccharide moiety to a hydrophobic anchor is necessary[3]. Several methods for preparing covalently linked carbohydrate-conjugates have been described[4,5], including reductive amination of aldoses with cyanoborohydride for coupling to proteins[1,2,6–8], lipids[3,9,10], and synthetic polymers[6].

For the development of semi-synthetic vaccines to *Streptococcus pneumoniae*, based on reductive amination of oligosaccharide fragments derived from capsular polysaccharides[3], a screening method to examine the neoglycolipids prepared has been explored. Sugar analysis was incorporated using the methanolysis procedure, and we now present g.l.c. and e.i.-m.s. data for trimethylsilylated and *N*-acetylated *N*-(1-deoxyalditol-1-yl)octadecylamine derivatives obtained in the sugar analysis of model, reductively aminated saccharides, representing the native reducing sugar units (**4–10**) or alditols degraded by mild periodate oxidation (**1–3**).

0008-6215/89/$ 03.50

RESULTS AND DISCUSSION

A series of reductively aminated C-2 to C-6 aldoses has been analysed by the sugar analysis procedure based on methanolysis, *N*-acetylation, and trimethylsilylation[11]. A survey of the g.l.c. retention times of the resulting *N*-(1-deoxyalditol-1-yl)octadecylamine derivatives **1–10** is presented in Table I. In the case of the glucuronic acid conjugate **10**, four compounds **10a–10d** are formed, corresponding to the 1,6-lactam, the methyl ester, the 1,4-lactone, and the trimethylsilyl ester of the hexonic acid, respectively. For all conjugates, two accompanying peaks are present in the gas chromatograms, with shorter retention times (× 0.84 and × 0.93, respectively), due to contamination of the commercial octadecylamine with hexadecylamine (10%) and heptadecylamine (2%). In addition, when the alditol chain contains a primary hydroxyl group, the methanolysis procedure gives rise to *O*-acetylated products (~5%) with higher retention times (× 1.04)[11].

The e.i. mass spectra of the *N*-(1-deoxyalditol-1-yl)octadecylamines **1–10** have been recorded. Ions which contain the intact alkyl chain are listed in Table II, while ions which originate from the carbohydrate moiety are presented in Table III. Molecular ions in the spectra of **1–10** are absent or of very weak intensity (Table II). The most intense peaks in the high-molecular-mass region correspond to $[M - Me]^+$, explained as the loss of a Me group from one of the $SiMe_3$ substituents. Spectra of the linear compounds **1–9, 10b**, and **10d** show characteristic fragmentation patterns due to cleavages of the C–C bonds in the alditol moiety, as have been reported for the trimethylsilylated alditols[12,13], hexonic acids[14], and *N*-(1-deoxyhexitol-1-yl)amino acids[15]. As a typical example, the spectrum of **6** is depicted

TABLE I

G.L.C. RETENTION TIMES (R_{ODA}) RELATIVE TO *N*-ACETYLOCTADECYLAMINE OF *N*-(1-DEOXYALDITOL-1-YL)OCTADECYLAMINE DERIVATIVES **1–10** AFTER METHANOLYSIS, *N*-ACETYLATION, AND TRIMETHYLSILYLATION

Compound[a]		R_{ODA}[b]
GlycolaldehydeH-ODA	**1**	1.29
D-GlyceraldehydeH-ODA	**2**	1.47
D-ErythroseH-ODA	**3**	1.66
D-RibH-ODA	**4**	1.96
L-RhaH-ODA	**5**	2.00
D-GlcH-ODA	**6**	2.46
D-GalH-ODA	**7**	2.48
D-ManH-ODA	**8**	2.32
D-GlcNAcH-ODA	**9**	2.68
D-GlcAH-ODA	**10a**	1.77
	10b	2.37
	10c	2.40
	10d	2.62

[a]D-GlcH = 1-deoxy-D-glucitol-1-yl, *etc.* [b]Temperature program: 160→280° at 6°/min.

TABLE II

FRAGMENT IONS CONTAINING THE INTACT ALKYL CHAIN AND THEIR RELATIVE PEAK INTENSITIES OBSERVED IN THE E.I. MASS SPECTRA OF THE TRIMETHYLSILYLATED (*N*-ACETYLATED) DERIVATIVES OF *N*-(1-DEOXYALDITOL-1-YL)OCTADECYLAMINES

Fragment ion	**1** m/z (%)	**2** m/z (%)	**3** m/z (%)	**4** m/z (%)	**5** m/z (%)	**6** m/z (%)	**9** m/z (%)	**10a** m/z (%)	**10b** m/z (%)	**10c** m/z (%)	**10d** m/z (%)
M^+				733(+)	747(+)		804(+)	717(18)	791(+)	687(1)	849(1)
$M^+ - HOSiMe_3$	337(13)	439(23)	541(1)	643(1)	657(+)			627(15)		597(3)	759(+)
$M^+ - 2 \times HOSiMe_3$			451(6)	553(2)	567(1)	655(+)		537(17)		507(4)	
$M^+ - Me$	412(18)	514(17)	616(8)	718(6)	732(3)	820(4)	789(10)	702(42)	776(8)	672(18)	834(12)
$M^+ - Me - HOSiMe_3$	322(18)			628(+)	642(+)		699(+)	612(7)		582(+)	744(+)
$M^+ - Me - 2 \times HOSiMe_3$								522(7)			
$M^+ - COCH_3$	384(12)		588(1)				761(2)		748(+)	644(2)	806(+)
$M^+ - OSiMe_3 - CH_2CO$	296(5)	398(64)		602(+)							
$M^+ - R^a$	324(21)	426(51)	528(3)	630(+)		732(+)					
$M^+ - R - HOSiMe_3$		336(12)	438(17)	540(17)		642(1)			642(+)		642(+)
$M^+ - R–OSiMe_3 - CH_2CO$			397(9)	499(2)	601(+)	601(+)					
$M^+ - R - 2 \times HOSiMe_3$						552(1)			552(2)		552(7)
$M^+ - RCHOSiMe_3$		324(5)	426(100)	528(7)	630(1)	630(1)			630(2)		630(3)
$M^+ - RCHOSiMe_3 - HOSiMe_3$				438(27)	540(59)	540(28)	509(5)		540(26)	540(31)[b]	540(33)
$M^+ - RCHOSiMe_3–OSiMe_3 - CH_2CO$				397(8)	499(5)	499(7)	468(18)		499(7)		499(8)
$M^+ - R(CHOSiMe_3)_2$			324(4)	426(100)	528(8)	528(10)	497(14)		528(12)		528(13)
$M^+ - R(CHOSiMe_3)_2 - HOSiMe_3$					438(12)	438(8)			438(10)	438(15)[b]	438(11)
$M^+ - R(CHOSiMe_3)_2–OSiMe_3 - CH_2CO$					397(21)	397(10)			397(13)	397(8)[b]	397(8)
$M^+ - R(CHOSiMe_3)_3$				324(3)	426(100)	426(100)	395(20)		426(100)	426(40)[b]	426(100)
$M^+ - R(CHOSiMe_3)_4$					324(3)	324(3)	324(4)[c]		324(11)	324(28)[b]	324(7)
$CH_2{=}NHC_{18}H_{37}$	282(100)	282(38)	282(17)	282(15)	282(16)	282(18)	282(17)		282(34)	282(57)	282(25)

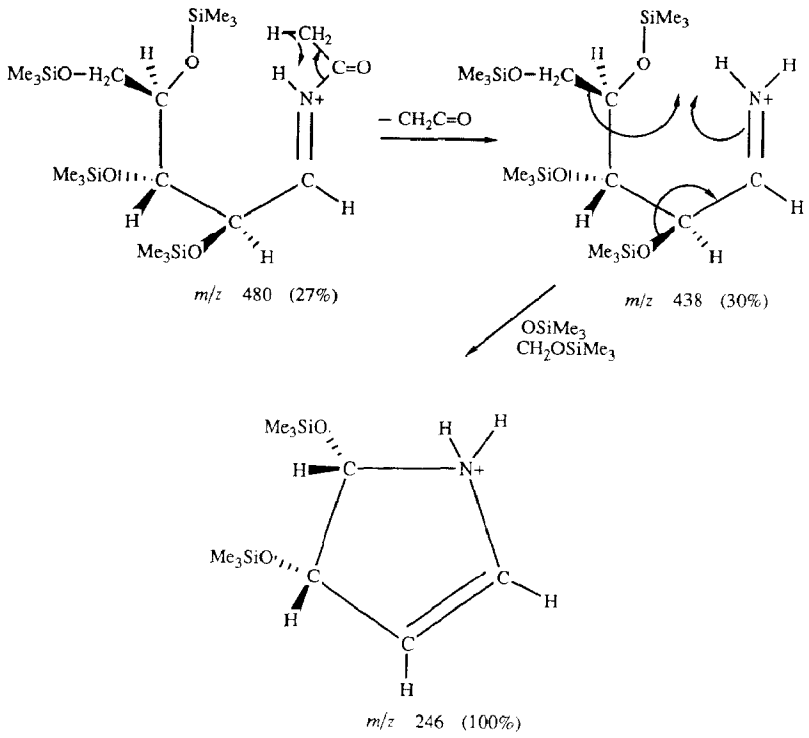
[a] **1–4, 6**, and **9**, R = CH_2OSiMe_3; **5**, R = CH_3; **10b**, R = COOMe; **10d**, R = $COOSiMe_3$. [b] For explanation, see Fig. 3. [c] $M^+ - R(CHOSiMe_3)_3 CHNHAc$.

TABLE III

FRAGMENT IONS ORIGINATING FROM THE ALDITOL MOIETY, AND THEIR RELATIVE PEAK INTENSITIES OBSERVED IN THE E.I. MASS SPECTRA OF THE TRIMETHYLSILYLATED (*N*-ACETYLATED) DERIVATIVES OF *N*-(1-DEOXYALDITOL-1-YL)OCTADECYLAMINES

Fragment ion[a]	m/z	**1**	**2**	**3**	**4**	**5**	**6**	**9**	**10a**	**10b**	**10c**	**10d**
$SiMe_3$	73	30	100	43	58	47	38	15	100	16	67	42
CH_2OSiMe_3	103	5	27	35	14	+	15	18	7	8	5	1
$Me_2SiOSiMe_3$	147		38	17	23	18	19	17	42	13	19	15
$CH(OSiMe_3)_2$	191			2	1	2	8		9	3	5	2
$(CHOSiMe_3)_2$	204	2	18	5	7	8	9	8	27	11	10	7
$H(CHOSiMe_3)_2$	205	1	50	6	30	2	18	17	5	4	5	3
$CH(CHOSiMe_3)_2$	217		27	33	14	42	27	26	43	41	100	23
$H(CHOSiMe_3)_3$	307			10	2	1	13	19	2	2	3	2
$CH(CHOSiMe_3)_3$	319				27		12	16	2			
$H(CHOSiMe_3)_4$	409				2		1	1	3	+	1	+
$CH(CHOSiMe_3)_4$	421						5		2	+	2	+

[a]Each positively charged.



Scheme 1. Formation of fragment ion *m/z* 246 from *m/z* 480 in the e.i. mass spectrum of 2-acetamido-1,2-dideoxy-1-octadecylamino-3,4,5,6-tetra-*O*-trimethylsilyl-D-glucitol (**9**).

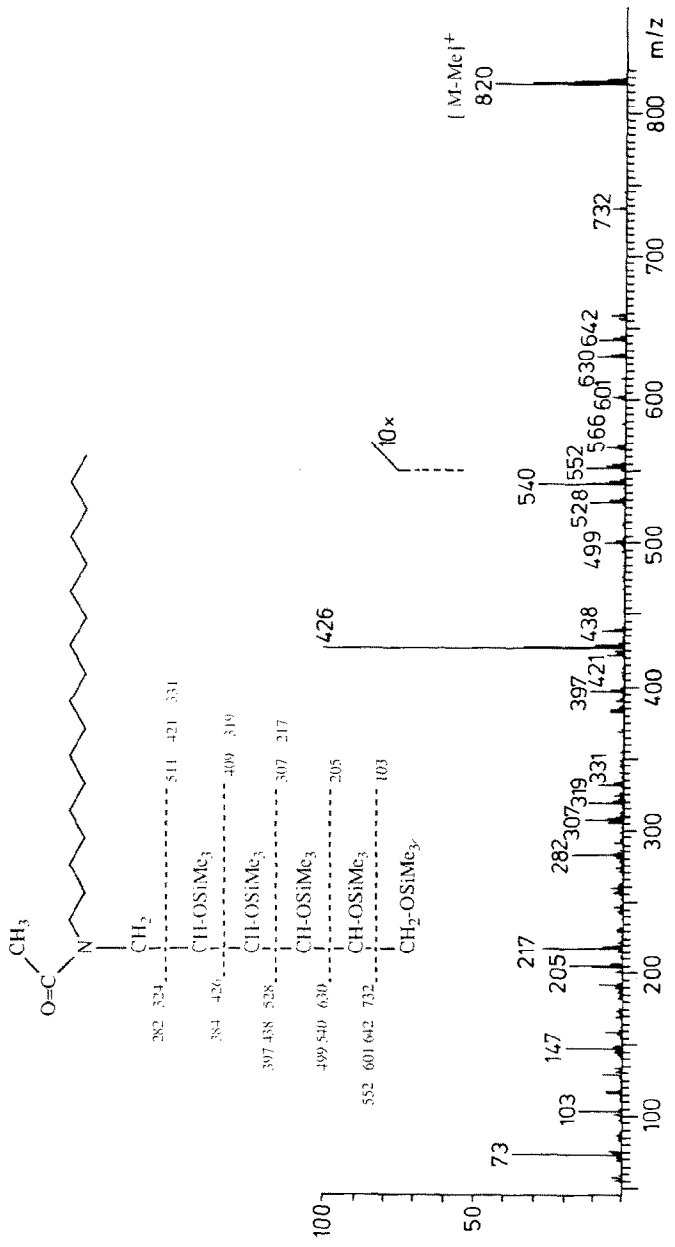


Fig. 1. E.i. (70 eV) mass spectrum of 1-deoxy-1-(*N*-octadecylacetamido)-2,3,4,5,6-penta-*O*-trimethylsilyl-D-glucitol (**6**).

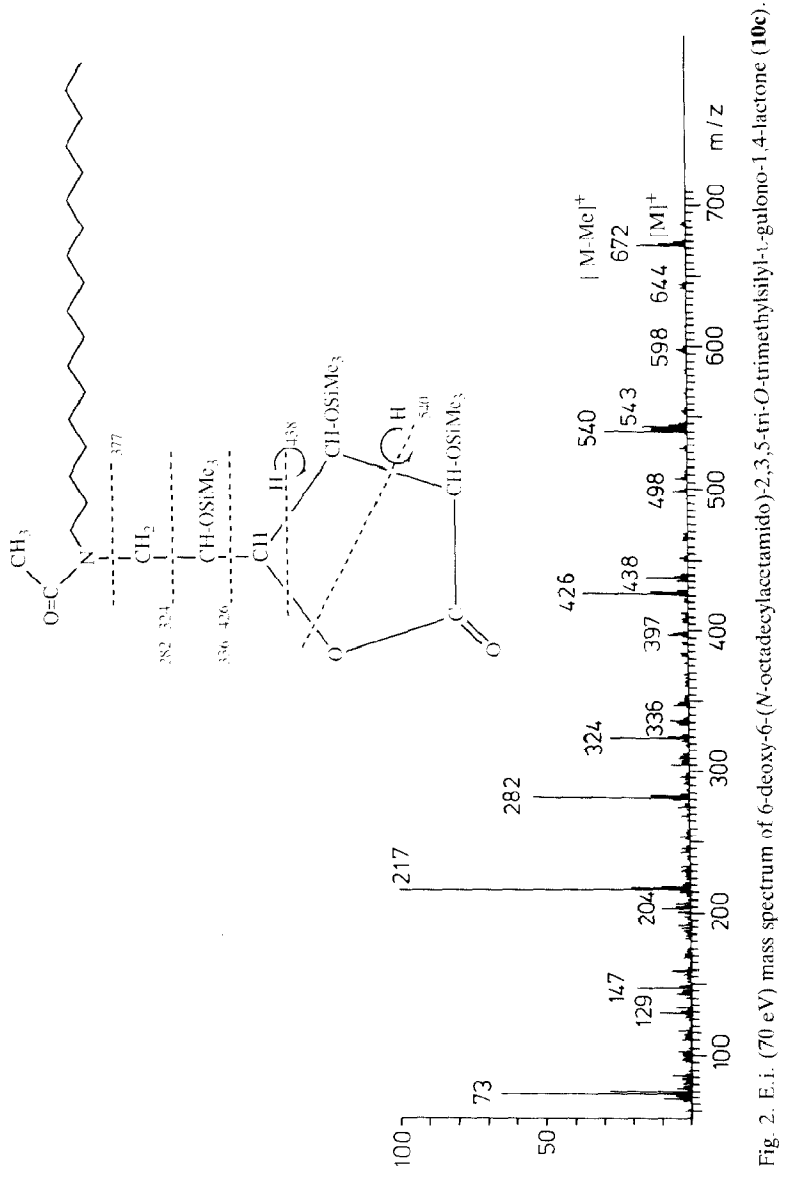


Fig. 2. E.i. (70 eV) mass spectrum of 6-deoxy-6-(*N*-octadecylacetamido)-2,3,5-tri-*O*-trimethylsilyl-L-gulono-1,4-lactone (**10c**).

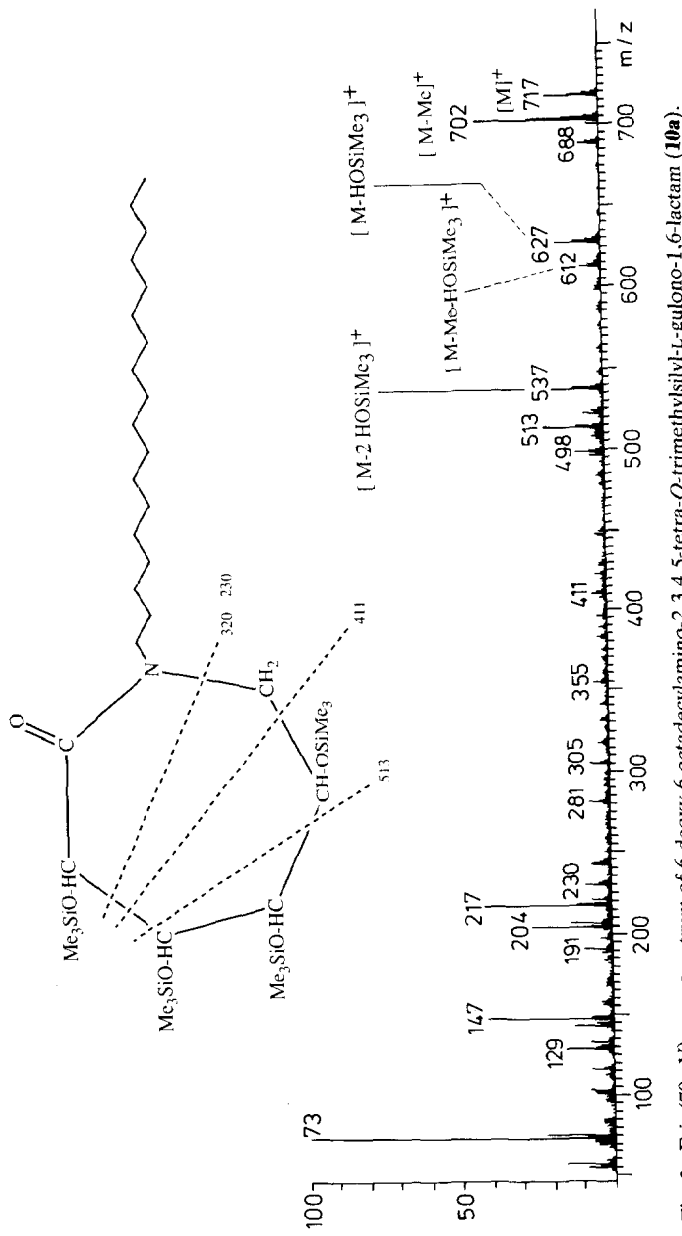


Fig. 3. E.i. (70 eV) mass spectrum of 6-deoxy-6-octadecylamino-2,3,4,5-tetra-*O*-trimethylsilyl-L-gulono-1,6-lactam (**10a**).

in Fig. 1. The spectrum of **10c** (Fig. 2) shows the fragmentation pattern of a trimethylsilylated hexono-1,4-lactone[16]. No mass-spectral data are available for trimethylsilylated hexono-1,6-lactam derivatives like **10a** (Fig. 3). Stereochemical differences are not expressed in the mass spectra of compounds **6–8**. Comparison of these spectra shows only minor differences in the relative intensities of some ions. Fragmentations of M^+ and of the primary fragment ions occur by the loss of one or more $HOSiMe_3$ molecules and/or an $OSiMe_3$ radical. Elimination of ketene and an $OSiMe_3$ radical, eventually together with part of the alditol chain, is typical for the *N*-acetylated compounds (*e.g.*, Table II, *m/z* 397, 499, or 601). Within the aliphatic chain, fragmentations are hardly observable. The presence of the intact alkyl chain is reflected in the base peak at *m/z* 426 in the mass spectra of **3–8, 10b**, and **10d**, representing the alkyl chain retaining C-1 and C-2 of the alditol moiety. In all spectra, except that for **10a**, the intact alkyl chain retaining C-1 of the alditol-moiety gives rise to a peak with moderate intensity at *m/z* 324 $[CH_2{=}\overset{+}{N}(COCH_3)\text{–}(CH_2)_1\text{-}CH_3]$. Subsequent elimination of ketene leads to *m/z* 282. Common for trimethylsilyl derivatives of carbohydrates[13] are the fragment ions *m/z* 305 $[CHOSiMe_3{=}COSiMe_3\text{–}CHOSiMe_3]^+$, 217 $[CH(CHOSiMe_3)_2]^+$, 204 $[(CHOSiMe_3)_2]^+$, 191 $[CH(OSiMe_3)_2]^+$, 147 $[SiMe_3OSiMe_2]^+$, 103 $[CH_2OSiMe_3]^+$, 89 $[OSiMe_3]^+$, and 73 $[SiMe_3]^+$, and will not be discussed further.

As compared to the alditol analogues **1–8, 10b**, and **10d**, the presence of the acetamido group at C-2 in **9** has a definite influence on the fragmentation pattern. Cleavage of the C-2–C-3 bond with charge localised on the alkylamine fragment in **9** leads to a peak of much lower intensity (20% for *m/z* 395) as compared to the base peak fragmentation in the other acyclic compounds. The peak at *m/z* 438 (30%), also present in the spectra of the other acyclic compounds, results from elimination of CH_3CONH_2 from *m/z* 497 $[M - H(CHOSiMe_3)_3]^+$ and of ketene from *m/z* 480 $[M - CH_2N(COCH_3)\text{–}(CH_2)_{17}CH_3]^+$. The base peak in the spectrum of **9** is *m/z* 246 $(C_{10}H_{24}NO_2Si_2)^+$, which corresponds to a stable pyrrole ring, formed from *m/z* 480 as illustrated in Scheme 1. The fragment at *m/z* 246, although of lower intensity, is also found in the spectrum of trimethylsilylated 2-acetamido-2-deoxyglucitol[13] with and without a D-atom at C-1, indicating that cleavage of the C-1–C-2 bond is involved in its formation. As usual for trimethylsilylated amino sugars, the analogue of *m/z* 217 is present at *m/z* 186 (22%) $[CHOSiMe_3\text{–}CH{=}CHNHCOCH_3]^+$.

In contrast to **1–9**, sugar analysis of the glucuronic acid conjugate **10** yields, in principle, four g.l.c. peaks with R_{ODA} values of 1.77, 2.37, 2.40, and 2.62, respectively. When the *N*-acetylation step is omitted, the major product corresponds to the peak R_{ODA} 1.77 (**10a**, Fig. 3) and is identified as a seven-membered ring structure, as is known for caprolactam (ε-lactam). Lactams are known to be formed under the influence of hexamethyldisilazane[17] as silylating reagent. The peak with R_{ODA} 2.40 (**10c**, Fig. 2) is identified as the γ-lactone. The peak with R_{ODA} 2.37 of the methyl ester derivative (**10b**) disappears when methanolysis is omitted, whereas the intensity of that with R_{ODA} 2.62 of the trimethylsilyl ester (**10d**) in-

creases. It has to be noted that, during methanolysis, the carboxyl group is not completely esterified. Starting from D-glucurono-6,3-lactone, a similar mixture of products is obtained.

The mass spectrum of the 1,6-lactam **10a** (Fig. 3) contains relatively intense ions at *m/z* 717 (M^+, 18%) and at 702 ($M^+ - Me$, 42%). Both ions give rise to subsequent elimination of one or two $HOSiMe_3$ molecules. The relatively intense peaks at *m/z* 513 and 411 can be explained as $[M - (CHOSiMe_3)_3]^+$ and $[M - (CHOSiMe_3)_2]^+$, respectively. It is evident that the ring structure does not permit the formation of fragment ions *m/z* 324 and 282, which occur in all spectra discussed so far. On the other hand, for **10a**, increased fragmentations in the alkyl chain are observed. In the high-molecular-mass region of the spectrum of **10c** (Fig. 2), peaks are found for M^+ at *m/z* 687, $[M - Me]^+$ at *m/z* 678, and $[M - COCH_3]^+$ at *m/z* 644. The occurrence of a 1,4-lactone form is supported by the ion at *m/z* 426 formed by rupture of the bond between the side chain and the ring (C-2–C-3)[16]. Formation of fragment ions *m/z* 540 and 438 requires ring opening and transfer of a proton (see Fig. 2), whereas *m/z* 543 is the result of a $OSiMe_3$ transfer $[COOSiMe_3–CHOSiMe_3–CH_2–N(COCH_3)–(CH_2)_{17}CH_3]^+$.

EXPERIMENTAL

Reductive amination. — For the preparation of the *N*-(1-deoxyalditol-1-yl)-octadecylamine derivatives, aqueous solutions (1 mL) of sugar (1 mmol) and $NaCNBH_3$ (5 mmol) were added dropwise to a magnetically stirred solution of octadecylamine (5 mmol) in tetrahydrofuran (5 mL). The pH was kept at pH 8–9 by the addition of triethylamine. Incubations were carried out at room temperature or at 40–45° for 5 to 14 days. The progress of the reaction was monitored by t.l.c. (Silica Gel $60F_{254}$, Merck) with 6:5:5 1-butanol–pyridine–water as solvent system, using orcinol–sulfuric acid and ninhydrin spray detection. When carbohydrate starting-material had disappeared, the mixture was worked-up by destroying the excess of cyanoborohydride with a few drops of 0.5M HCl (in an adequately ventilated hood) followed by co-evaporation of boric acid with methanol (2×). The crude residue was washed repeatedly with chloroform to remove the excess of octadecylamine, taken up in 3:1 chloroform–methanol and filtered. Further purification of 25-mg samples was performed on a high-performance silica column (17 × 1.5 cm, Polygosyl 60-4063, particle size 40–63 μm, Macherey–Nagel), eluted with 65:32:6 chloroform–methanol–water. Neoglycolipid-containing fractions were detected by a spot test on t.l.c. plates (orcinol–sulfuric acid and ninhydrin spray reagents). Purity was checked with h.p.t.l.c. (Silica Gel 60, Merck), using the same eluent and detection methods.

Periodate oxidation. — Compounds **1–3** were obtained by mild periodate oxidation of $NaBH_4$-reduced lactose (0.28mM) with 0.55mM $NaIO_4$ in 40mM aqueous imidazole (pH 6.5) at 0°. After 1 h, the reaction was worked-up by the addition of ethanol, centrifugation, and evaporation. The mixture of products was coupled to octadecylamine as described above, and subjected to sugar analysis.

Analytical methods. — Sugar analysis[11] of neoglycolipids was carried out, after methanolysis (methanolic M HCl; 24 h, 85°), *N*-acetylation, and trimethylsilylation, on a column (2 m × 3.5 mm, i.d.) packed with 3.8% of SE-30 on Chromosorb W/AW, at a N_2 flow-rate of 30 mL/min and a temperature program of 160→280° at 6°/min, using a Varian Aerograph 2700 gas chromatograph.

G.l.c.–m.s. was performed on a Carlo Erba GC/Kratos MS80/Kratos DS55 system (electron energy, 70 eV; accelerating voltage, 2.7 kV; ionising current, 100 μA; ion-source temperature, 280°; BP1 capillary column). H.r.p. determination of exact masses was performed with the same equipment, using a resolution power of ~4000.

ACKNOWLEDGMENTS

We thank Mrs. Anca van der Kerk-Van Hoof and Mr. Cees Versluis for recording the mass spectra, and Dr. W. Heerma for valuable discussions. The financial support of Centrascience B.V., Etten-Leur (The Netherlands) is gratefully acknowledged.

REFERENCES

1 S. B. SVENSON AND A. A. LINDBERG, *J. Immunol. Methods*, 25 (1979) 323–335.
2 H. SNIPPE, A. J. VAN HOUTE, J. E. G. VAN DAM, M. J. DE REUVER, M. JANSZE, AND J. M. N. WILLERS, *Infect. Immun.*, 40 (1983) 856–861.
3 H. SNIPPE, J. E. G. VAN DAM, A. J. VAN HOUTE, J. M. N. WILLERS, J. P. KAMERLING, AND J. F. G. VLIEGENTHART, *Infect. Immun.*, 43 (1983) 842–844.
4 J. D. APLIN AND J. C. WRISTON, *C.R.C. Crit. Rev. Biochem.*, 10 (1981) 259–306.
5 C. P. STOWELL AND Y. C. LEE, *Adv. Carbohydr. Chem. Biochem.*, 37 (1980) 225–281.
6 G. R. GRAY, *Arch. Biochem. Biophys.*, 163 (1974) 426–428.
7 R. ROY, E. KATZENELLENBOGEN, AND H. J. JENNINGS, *Can. J. Biochem. Cell Biol.*, 62 (1984) 270–275.
8 B. A. SCHWARTZ AND G. R. GRAY, *Arch. Biochem. Biophys.*, 181 (1977) 542–549; B. J. KAMICKER, B. A. SCHWARTZ, R. M. OLSON, D. C. DRINKWITZ, AND G. R. GRAY, *ibid.*, 183 (1977) 393–398.
9 H. WIEGANDT AND W. ZIEGLER, *Hoppe-Seyler's Z. Physiol. Chem.*, 355 (1974) 11–18.
10 C. WOOD AND E. A. KABAT, *J. Exp. Med.*, 154 (1981) 432–449.
11 J. P. KAMERLING AND J. F. G. VLIEGENTHART, *Cell Biol. Monogr.*, 10 (1982) 95–125.
12 G. PETERSON, *Tetrahedron*, 25 (1969) 4437–4443.
13 J. P. KAMERLING AND J. F. G. VLIEGENTHART, in A. M. LAWSON (Ed.), *Clinical Biochemistry; Mass Spectrometry: Application in Clinical Chemistry*, de Gruyter, Berlin, 1989, pp. 171–258.
14 G. PETERSON, *Tetrahedron*, 26 (1970) 3413–3428.
15 D. J. WALTON, W. A. SZAREK, D. B. MACLEAN, AND R. V. GERARD, *Carbohydr. Res.*, 137 (1985) 31–38.
16 G. PETERSON, O. SAMUELSON, K. ANJOU, AND E. VON SYDOW, *Acta Chem. Scand.*, 21 (1967) 1251–1256.
17 R. PELLGATA, M. PINZA, AND G. PIFFERI, *Synthesis*, (1978) 614–616.