Carbohydrate Research, 128 (1984) 37–49
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHESIS OF *N*-(1-DEOXYHEXITOL-1-YL)AMINO ACIDS, REFERENCE COMPOUNDS FOR THE NONENZYMIC GLYCOSYLATION OF PROTEINS

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(Received July 1st, 1983; accepted for publication, August 30th, 1983)

ABSTRACT

The *N*-(1-deoxy-D-mannitol-1-yl) and *N*-(1-deoxy-D-glucitol-1-yl) derivatives of L-valine, L-alanine, L-threonine, and L-leucine were prepared by reductive amination of D-mannose and D-glucose with the appropriate amino acids, in the presence of sodium cyanoborohydride. N^{ε}-(1-Deoxy-D-mannitol-1-yl)- and N^{ε}-(1-deoxy-D-glucitol-1-yl)-L-lysine were prepared by similar reactions of hexoses with N^{α}-*tert*-butoxycarbonyl and N^{α}-benzyloxycarbonyl-L-lysine, followed by removal of the protecting groups. The structures were confirmed by ^{1}H-n.m.r. spectroscopy, which showed that each compound was completely free of its C-2 epimer. The synthetic compounds may be used as reference compounds for the identification of *N*-(1-deoxyhexitol-1-yl)amino acids formed when *N*-(1-deoxy-D-fructose-1-yl) groups of nonenzymically glycosylated proteins, of the hemoglobin A_{1c} type, are reduced with sodium borohydride, and the protein is subjected to acid-catalyzed hydrolysis.

INTRODUCTION

The free amino groups of several proteins, such as crystallin isolated from lens crystalline[1], serum albumin[2], hemoglobin[3], plasma lipoproteins[4], erythrocyte-membrane proteins[5], and collagen[6], react with D-glucose *in vivo* to form Schiff bases. The latter then undergo the Amadori rearrangement to give *N*-(1-deoxy-D-fructose-1-yl) derivatives of proteins[7]. The overall process, which occurs at an *N*-terminus or at the ε position of lysyl residues, is called "nonenzymic glycosylation". The resulting changes in the physical properties of proteins[8,9] may account for some of the complications of diabetes[10,11].

In order to determine the site of its attachment to a protein, the *N*-(1-deoxy-D-fructose-1-yl) residue is reduced with sodium borohydride to stabilize the carbohydrate–protein linkage. The product is hydrolyzed to free the resulting *N*-(1-

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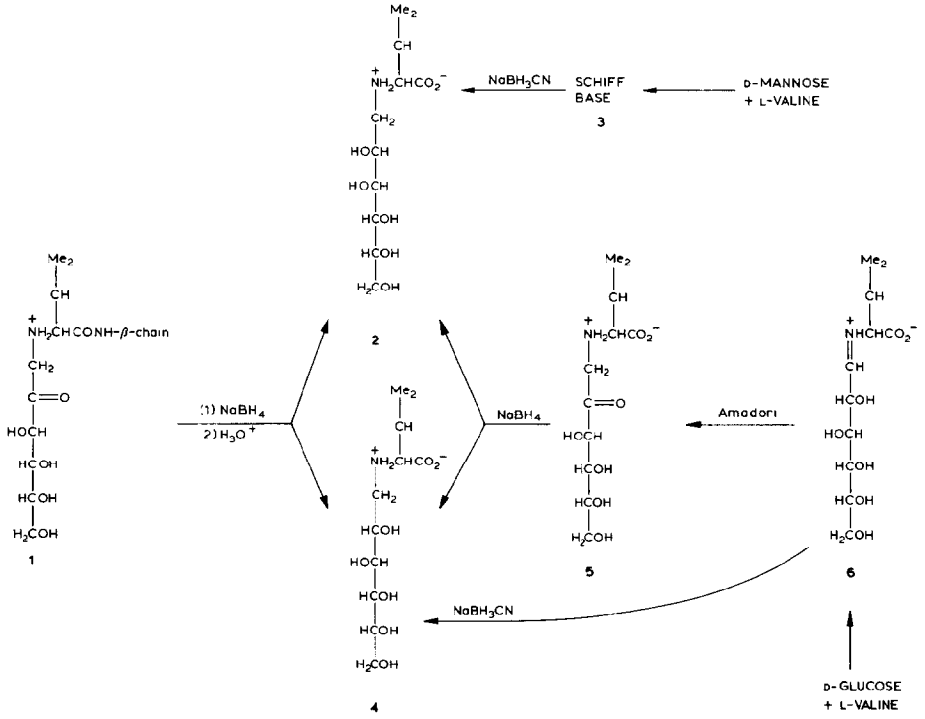


deoxyhexitol-1-yl)amino acid, which is then identified[12]. For example, reduction and hydrolysis of the β-chain of hemoglobin A_{1c} (**1**) gave a mixture of *N*-(1-deoxy-D-mannitol-1-yl)- and *N*-(1-deoxy-D-glucitol-1-yl)-L-valine (**2** and **4**, respectively), thus establishing that the carbohydrate residue was linked to the amino group of the *N*-terminal valine in the original polypeptide[13].

Although further studies of other glycosylated proteins depend upon the availability of *N*-(1-deoxyhexitol-1-yl)amino acids, *N*-(1-deoxy-D-mannitol-1-yl)- and *N*-(1-deoxy-D-glucitol-1-yl)-L-valine (**2** and **4**) are the only such compounds that have been synthesized and fully characterized. Their preparation involved a 3-day reaction of D-glucose with L-valine, when *N*-(1-deoxy-D-fructose-1-yl)-L-valine (**5**) was formed from the Schiff base **6**. Borohydride reduction of **5** gave an epimeric mixture of **2** and **4** which was partially resolved by ion-exchange chromatography[13]. The paucity of available reference compounds[13] is undoubtedly due to the length of time for the synthesis and the formation of pairs of epimers that are difficult to separate.

A more efficient method of synthesis of *N*-(1-deoxyhexitol-1-yl)amino acids using an approach which is similar to that used for the attachment of disaccharides to proteins[14] is described herein. A hexose is subjected to reductive amination by an amino acid in the presence of sodium cyanoborohydride. The Amadori rearrangement is avoided by reduction of the intermediate Schiff base *in situ*, thus giving a single epimer in each case, as exemplified by separate syntheses of **2** and **4** by way of **3** and **6**, respectively.

TABLE I

COMPOSITION AND PROPERTIES OF *N*-(1-DEOXYHEXITOL-1-YL)AMINO ACIDS

Compound	*Molecular formula*	*Anal.*						$[\alpha]_D^{22}$ *(degrees)*[a]	R_{Glc}	T[c]
		Calc.			*Found*					
		C	*H*	*N*	*C*	*H*	*N*			
N-(1-Deoxy-D-mannitol-1-yl)-										
L-valine (**2**)	$C_{11}H_{23}NO_7$	47.00	8.24	4.98	46.85	8.11	4.91	+9	1.41(*E*)	3.05
L-alanine	$C_9H_{19}NO_7$	42.68	7.56	5.53	42.67	7.49	5.48	+2	0.43(*E*)	2.63
L-threonine	$C_{10}H_{21}NO_8$	42.40	7.47	4.95	42.42	7.62	5.07	−1	0.77(*E*)	3.80
L-leucine	$C_{12}H_{25}NO_7$	48.80	8.53	4.74	48.72	8.42	4.81	+15	1.59(*E*)	3.20
L-lysine[d] (**14**)	$C_{12}H_{26}N_2O_7$	46.44	8.44	9.03	46.40	8.51	8.98	+6	0.34(*F*)	6.26
N-(1-Deoxy-D-glucitol-1-yl)-										
L-valine (**4**)	$C_{11}H_{23}NO_7$	47.00	8.24	4.98	46.94	8.27	5.04	+1	1.18(*E*)	3.13
L-alanine	$C_9H_{19}NO_7$	42.68	7.56	5.53	42.64	7.59	5.40	−13	0.39(*E*)	2.73
L-threonine	$C_{10}H_{21}NO_8$	42.40	7.47	4.95	42.31	7.60	4.84	−19	0.64(*E*)	3.93
L-leucine	$C_{12}H_{15}NO_7$	48.80	8.53	4.74	48.83	8.51	4.73	+1	1.43(*E*)	3.33
L-lysine[d] (**9**)	$C_{12}H_{26}N_2O_7$	46.44	8.44	9.03	46.53	8.24	9.05	+18	0.34(*F*)	6.34

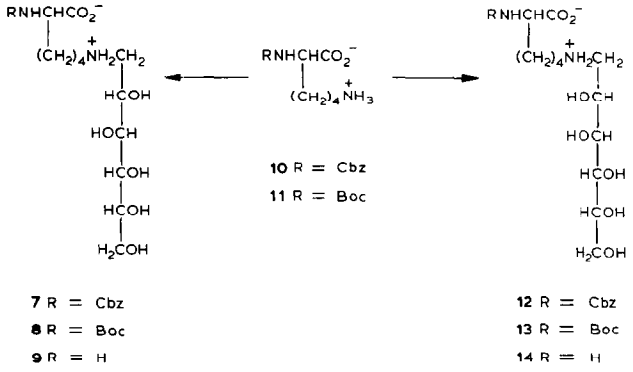
[a]For 1% solutions in 5M hydrochloric acid. [b]T.l.c. with developing solvent shown in parentheses. All compounds reacted with alkaline potassium permanganate. Lysine and alanine derivatives gave strong and weak ninhydrin reactions, respectively. [c]G.l.c. of *O*-$SiMe_3$ derivative formed with Me_3Si-imidazole; for definition of *T*, see Experimental. [d]1-Deoxyalditol group linked to ε nitrogen atom.

RESULTS AND DISCUSSION

Reactions of hexoses and disaccharides with proteins at 37°, *in vivo* and *in vitro*, occur very slowly because only a small proportion of the reacting hexose (or of the reducing residue of an oligosaccharide) is in the acyclic form[15]. In the present work, faster reactions of hexoses with neutral amino acids, in the presence of sodium cyanoborohydride, were achieved at 100°. Pure samples of the *N*-(1-deoxy-D-glucitol-1-yl) and *N*-(1-deoxy-D-mannitol-1-yl) derivatives of L-valine, L-alanine, L-threonine, and L-leucine were obtained in yields of 51–55%. Their properties are listed in Table I.

Although an excess of hexose was used in each preparation, ~7% of the original amino acid was always recovered after chromatography of the reaction mixture on Dowex 1 resin, even when the reaction time was extended beyond the usual 16 h. The incompleteness of the reaction may be due to a side reaction in which hydrogen cyanide adds to the intermediate Schiff base to give an *N*-(1-*C*-cyano-1-deoxyhexitol-1-yl)amino acid which, in the presence of acetic acid, is hydrolyzed back to hydrogen cyanide, the original hexose, and the amino acid by mechanisms suggested by Gidley and Sanders[16].

N^{ε}-(1-Deoxyhexitol-1-yl) derivatives of L-lysine were obtained by similar methods using the benzoxycarbonyl (Cbz) or *tert*-butoxycarbonyl (Boc) group to protect the α-amino group of lysine. For example, reductive amination of N^{α}-Cbz-L-lysine (**10**) with D-glucose or D-mannose, at 100°, was achieved within 4 h. In order to obtain acceptable yields of the N^{ε}-(1-deoxyhexitol-1-yl) derivatives **7** and **12**, it was necessary to avoid the use of an excess of hexose. This approach minimized further reactions of hexoses with **7** and **12**, in a manner similar to reductive aminations of aliphatic ketones[17] and disaccharides[14], where the proportion of each carbonyl compound was limited to avoid tertiary amine formation. The benzoxycarbonyl group of **7** and **12** was removed by hydrogen transfer from formic acid in the presence of palladium-coated, poly(ethylenimine) beads[18], to afford



10 R = Cbz
11 R = Boc

7 R = Cbz
8 R = Boc
9 R = H

12 R = Cbz
13 R = Boc
14 R = H

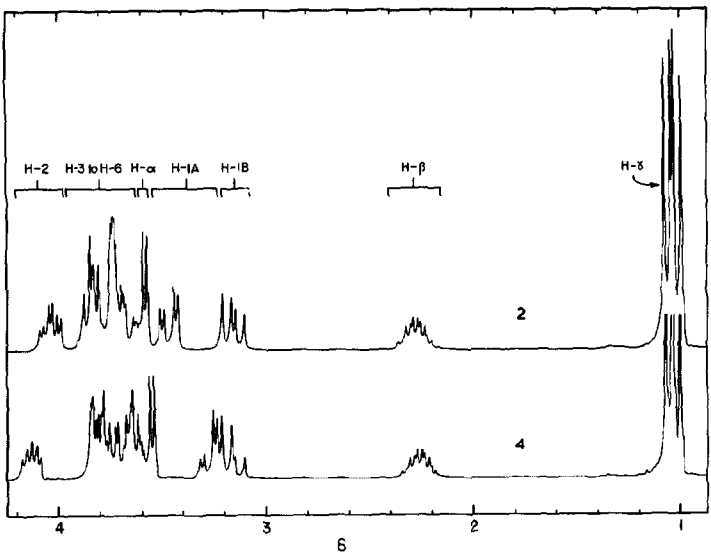


Fig. 1. ^{1}H-n.m.r. spectra, at 200 MHz, of compounds **2** and **4** in deuterium oxide.

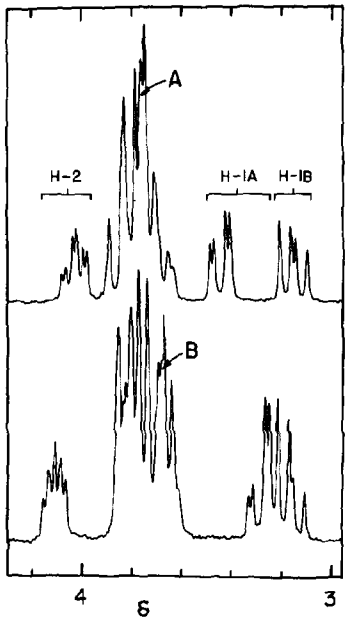


Fig. 2. Partial ^{1}H-n.m.r. spectra, at 200 MHz, of: (A) *N*-(1-deoxy-D-mannitol-1-yl)- and (B) *N*-(1-deoxy-D-glucitol-1-yl)-L-alanine in deuterium oxide.

TABLE II

^{1}H-N.M.R. CHEMICAL SHIFTS FOR *N*-(1-DEOXYHEXITOL-1-YL)AMINO ACIDS[a]

Compound	*H-1B*[b]	*H-1A*[b]	*H-2*[b]	*H-α*	*H-β*	*H-γ*	*H-δ*	*H-ε*
N-(1-Deoxy-D-mannitol-1-yl)-								
L-valine (**2**)	3.16	3.47	4.03	3.58(d)	2.28[c]	1.01, 1.06(d's)		
L-alanine	3.15	3.45	4.03	[d]	1.53(d)			
L-threonine	3.22	3.50	4.06	3.53(d)	4.10(dq)	1.34(d)		
L-leucine	3.15	3.43	4.02	[d]	[e]	[e]	0.96(d)	
L-lysine[f] (**14**)	3.00	3.30	3.93	[d]	1.75(m)	1.43(m)	1.75(m)	3.00(t)
Hydrochloride of **14**	3.14	3.44	4.00	4.00(t)	1.98(m)	1.54[g]	1.80[g]	3.15(t)
N-(1-Deoxy-D-glucitol-1-yl)-								
L-valine (**4**)	3.17	3.27	4.13	3.55(d)	2.26[c]	1.02, 1.06(d's)		
L-alanine	3.16	3.28	4.11	[d]	1.53(d)			
L-threonine	3.22	3.30	4.16	3.51(d)	4.11(dq)	1.33(d)		
L-leucine	3.15	3.27	4.11	[d]	[e]	[e]	0.96(d)	
L-lysine[f] (**9**)	3.01	3.11	4.03	[d]	1.74(m)	1.44(m)	1.74(m)	2.97(t)
Hydrochloride of **9**	3.18	3.27	4.10	4.00(t)	1.98(m)	1.54[g]	1.79[g]	3.17(t)

[a] δ Values downfield from DSS. [b] The signals for H-1A, -1B, and -2 form an ABX system in which the H-2 signal (X) is split by coupling to H-3. [c] Doublet of septets. [d] Values not observed owing to overlap with signals from H-3, -4, -5, and -6 at δ 3.9–3.6. [e] H-β and H-γ gave a broad multiplet centered at δ 1.74. [f] 1-Deoxyalditol-1-yl group linked to ε nitrogen atom. [g] Broad quintet.

TABLE III

^{1}H-N.M.R COUPLING CONSTANTS (Hz) FOR *N*-(1-DEOXYHEXITOL-1-YL)AMINO ACIDS

Compound	$J_{1A,1B}$	$J_{1B,2}$	$J_{1A,2}$	$J_{2,3}$	$J_{\alpha,\beta}$	$J_{\beta,\gamma}$	$J_{\gamma,\delta}$	$J_{\delta,\varepsilon}$
N-(1-Deoxy-D-mannitol-1-yl)-								
L-valine (**2**)	12.6	8.6	3.7	8.6	4.3	7.2		
L-alanine	13.0	8.9	3.7	8.9	7.3			
L-threonine	13.0	8.3	3.6	8.3	7.3	6.7		
L-leucine	12.8	8.9	3.8	8.9	[a]	[a]	4.4	
L-lysine[b] (**14**)	12.9	9.3	3.3	9.3	[a]	[a]	[a]	~8
Hydrochloride of **14**	12.9	9.3	3.4	9.3	6.2	~8	~8	~8
N-(1-Deoxy-D-glucitol-1-yl)-								
L-valine (**4**)	12.9	9.8	3.3	5.0	4.3	7.2		
L-alanine	12.6	9.2	3.3	4.7	7.3			
L-threonine	13.0	9.1	3.6	4.8	7.3	6.6		
L-leucine	13.0	9.4	3.2	5.0	[a]	[a]	4.4	
L-lysine[b] (**9**)	13.0	9.3	3.9	4.6	[a]	[a]	[a]	~8
Hydrochloride of **9**	13.1	9.4	3.7	4.8	6.1	~8	~8	~8

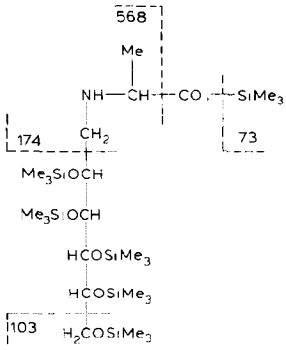
[a]Values not obtained owing to complexity of signals or overlap of signals from H-α and H-3–H-6.
[b]1-Deoxyalditolyl group attached to ε nitrogen atom.

compounds **9** and **14**, respectively*. The formation of **7** and **12** was accompanied by the production of hexitols that were not removed until after the deblocking step.

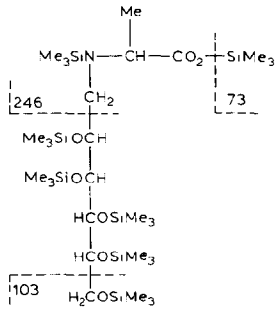
The conversion of N^{α}-Boc-L-lysine (**11**) into the N^{ε}-(1-deoxyhexitol-1-yl) derivatives **8** and **13** required a lower reaction-temperature, and therefore a longer time, than that used for the synthesis of **7** and **12**, in order to minimize the formation of unidentified by-products. Removal of the protecting group of **8** and **13** with hydrochloric acid gave samples of **9** and **14** having the same properties as those of samples originating from N^{α}-Cbz-L-lysine (**10**).

The structures of the *N*-(1-deoxyhexitol-1-yl)amino acids were confirmed by ^{1}H-n.m.r. spectroscopy (Figs. 1 and 2), and spectral parameters are listed in Tables II and III. Resonances attributable to protons of amino acid moieties were assigned by comparison with spectra of the corresponding free L-amino acids[19]. Coupling between H-2 of the carbohydrate residue and H-1A and H-1B was demonstrated by irradiation at the H-2 frequency (δ 4.0–4.2 region), when the eight lines attributable to H-1A and H-1B (δ 3.0–3.5 region) collapsed to form an AB quartet. The H-2 signal, which is part of an ABX system, is split further by coupling with H-3. The chemical-shift differences between H-1A and H-1B (~0.3 and 0.1 p.p.m. for *manno* and *gluco* compounds, respectively), and the values of $J_{2,3}$ (~8.5 and 5 Hz for *manno* and *gluco* compounds, respectively), are relatively independent of the structure of the amino acid portion of the molecule. In the δ 3.0–3.5 region of the

*Compound **9** has been synthesized by Schwartz and Gray[14], and Bunn *et al.*[3], but was not fully characterized in either case.



15



16

spectrum of each 1-deoxy-D-mannitol-1-yl compound, lines attributable to H-1A or H-1B of the corresponding 1-deoxy-D-glucitol-1-yl compound were absent, and *vice versa,* showing that every compound was completely free of its epimer.

The possibility of identifying the *N*-(1-deoxyhexitol-1-yl)amino acids by combined gas-liquid chromatography–mass spectrometry (g.l.c.–m.s.) of their trimethylsilyl (Me_3Si) derivatives is of interest, as the technique should be suitable for characterization of small quantities of compounds in hydrolyzates of sodium borohydride-treated, glycosylated proteins. Studies with *N*-(1-deoxy-D-mannitol-1-yl)-L-alanine showed that Me_3Si-imidazole is a suitable per(trimethyl)silylation reagent, since it reacts with only the carboxyl and hydroxyl groups, to produce a single derivative **15**. A single derivative was also formed from each of the other compounds listed in Table I. Per(trimethyl)silylation with *N,O*-bis(trimethylsilyl)-trifluoroacetamide was less satisfactory, as it resulted in partial derivatization of the nitrogen atom, so that two compounds, **15** and **16**, were obtained, as shown by g.l.c.–m.s.

For confirmation of the structure of **15** and **16** by electron impact m.s. (e.i.m.s.), the most useful information was obtained from cleavages between the carboxyl and α carbon atoms, and between C-1 and C-2 of the carbohydrate residue. The masses of the resulting ions, coupled with the value for MH^+ obtained by chemical ionization m.s. (c.i.m.s.), gave clear proof that **15** and **16** are trimethylsilyl derivatives of an *N*-(1-deoxyhexitol-1-yl)alanine. Hence g.l.c.–m.s. appears to be a promising technique for identification of *N*-(1-deoxyhexitol-1-yl)amino acids.

We have therefore demonstrated that *N*-(1-deoxyhexitol-1-yl)amino acids can be synthesized directly from hexoses and amino acids. The procedure is straightforward, and protecting groups are required only when it is necessary to prevent the reaction of the hexose with a second nucleophilic group.

EXPERIMENTAL

General. — Radioactive compounds, obtained from New England Nuclear (Boston, MA 02118), had the following specific activities: L-[3,4-^{3}H]valine, 1.89 TBq/mmol; L-[3-^{3}H]alanine, 2.77 TBq/mmol; L-[G-^{3}H]threonine, 0.15 TBq/mmol; L-[4,5-^{3}H]leucine, 0.18 TBq/mmol; D-[U-^{14}]glucose, 0.15 TBq/mol; and D-[1-^{14}C]mannose, 1.85 TBq/mol. The ^{1}H-n.m.r. spectra of deuterium oxide solutions were obtained at 200 MHz with a Bruker CPX-200 spectrometer operating in the Fourier-transform mode. Chemical shifts are given in δ values downfield from the internal standard, sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS). AB and ABX systems were subjected to second-order analysis[20]. Trimethylsilyl derivatives for g.l.c. were prepared by treating each sample (1 mg) with trimethylsilylimidazole in pyridine (Tri-Sil Z; Pierce Chemical Co., Rockford, IL 61105; 1 mL) for 2 h at 50°, or with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.4 mL) and acetonitrile (0.4 mL) for 0.5 h at 130° (ref. 21). G.l.c. was performed with a Hewlett–Packard 5720 chromatograph equipped with a flame-ionization detector. The glass column (180 × 0.4 cm) contained 3% OV-17 on Chromosorb W (100–120 mesh), at 160–240°, increasing at 2°/min. The carrier gas, nitrogen, flowed at 45 mL/min. *T* is the retention time relative to that of 1,2,3,4,5,6-hexa-*O*-(trimethylsilyl)-D-glucitol. G.l.c.–m.s. was performed at the National Research Council of Canada, Ottawa, with a Hewlett–Packard 5985 combined gas chromatograph–mass spectrometer. The conditions for g.l.c. were similar to those described for the model 5720 chromatograph, except that the column temperature was raised from 160 to 230° at 5°/min. For e.i.m.s., the source temperature was 200° and the ionization potential 70 eV. The corresponding values for c.i.m.s., with isobutane, were 150° and 230 eV. Chromatographic solvent systems were as follows (v/v): (*A*) 1-propanol–5.9M ammonia, 22:3; (*B*) 21:4; (*C*) 17:3; (*D*) 4:1; (*E*) 41:9; and (*F*) 3:1. Column chromatography was performed with silica gel (60–200 mesh; Davison Chemical Co., Baltimore, MD). The radioactivity of column fractions was determined in the presence of Aquasol (New England Nuclear) with a Beckman LS 7500 counter. For t.l.c., silica gel G layers, 0.25-mm thick, were developed twice with solvent *E*, or once with solvent *F*, and sprayed with alkaline potassium permanganate[22] or ninhydrin. Concentrations of hexoses in reaction mixtures were determined by colorimetry, with the phenol–sulfuric acid reagent[23].

N-*(1-Deoxy-*D-*mannitol-1-yl)-*L-*valine* (**2**). — A solution of L-valine (117 mg, 1.0 mmol) and sodium cyanoborohydride (94 mg, 1.5 mmol) in water (4 mL) was adjusted to pH 7.0 with 0.2M hydrochloric acid. An aqueous solution (5 mL) of D-mannose (540 mg, 3.0 mmol) and D-[1-^{14}C]mannose (5 μCi) was then added. The resulting solution was kept for 16 h at 100° in a sealed reaction vial, adjusted to pH 9.8 with 2M sodium hydroxide, and applied to a column (2 × 15 cm) of Dowex 1-X8 (OAc^-) anion-exchange resin, previously rinsed with 88mM ammonia until the effluent was at pH 9. The column was washed with 88mM ammonia (90 mL) to remove sodium ions and unreacted D-mannose, followed by water (112 mL) to re-

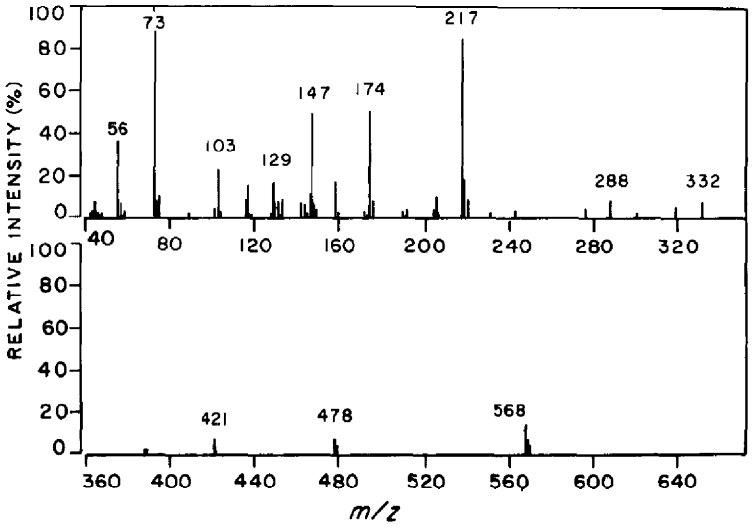


Fig. 3. Electron-impact mass spectrum of **15**.

move ammonia. Aqueous acetic acid (2%) was passed through the column. Radioactive effluent fractions were pooled and evaporated to dryness. The residue (300 mg) was composed of the title compound (R_{Glc} 1.41; solvent *E*; $KMnO_4$ spray) contaminated with valine (R_{Glc} 4.82; ninhydrin spray). G.l.c. of a sample treated with trimethylsilylimidazole showed a major component having *T* 3.05 (product) and a minor, unidentified component having *T* 1.17. A portion (200 mg) of the crude product was supplemented with L-[3,4-^{3}H]valine (37 kBq), and chromatographed on a column of silica gel. Valine (monitored by ^{3}H radioactivity) and compound **2** (monitored by ^{14}C radioactivity) were eluted with solvents *A* and *B*, respectively. Pure **2** (102 mg, 54%) was obtained by recrystallization from 4:1 ethanol–water. Physical constants, analytical data, and n.m.r. parameters are given in Tables I–III.

Other N-*(1-deoxy-*D*-hexitol-1-yl) derivatives of* L-*valine,* L-*alanine,* L-*leucine, and* L-*threonine.* — The compounds (see Table I) were prepared from L-valine, L-alanine, L-leucine, and L-threonine with D-[U-^{14}C]glucose or D-[1-^{14}C]mannose, under the conditions described for the preparation of **2**. The appropriate ^{3}H-amino acid was added before silica gel column chromatography, in which the following solvents were used: for (1-deoxyhexitol-1-yl)valines and (1-deoxyhexitol-1-yl)leucines, *A* and *B*; and for (1-deoxyhexitol-1-yl)alanines and (1-deoxyhexitol-1-yl)threonines, *C* and *D*. All compounds were crystallized from 4:1 ethanol–water, and were obtained in yields of 52–58%. Their properties are listed in Tables I–III.

G.l.c.–m.s. of per(trimethylsilyl)derivatives of N-*(1-deoxy-*D*-mannitol-1-yl)-*L*-alanine.* — Treatment of *N*-(1-deoxy-D-mannitol-1-yl)-L-alanine with trimethylsilylimidazole gave **15**, *T* 2.63; c.i.m.s.: *m/z* 686.5 (MH^+; calc. for $C_{27}H_{68}NO_7Si_6$, 686.4); e.i.m.s. (Fig. 3): *m/z* 568 (M − CO_2SiMe_3), 478 (568 − Me_3SiOH), 217

[$(Me_3SiO{=}CH{-}CH{=}CHOSiMe_3)^+$], 147 [$(Me_3SiO{=}SiMe_2)^+$], 174 [$(CH_2{=}NH{-}CHMe{-}CO_2SiMe_3)^+$], 103 [$(CH_2{=}SiMe_3)^+$], and 73 [$(Me_3Si)^+$].

Treatment of *N*-(1-deoxy-D-mannitol-1-yl)-L-alanine with *N,O*-bis(trimethylsilyl)trifluoroacetamide gave **15**, together with **16** (*T* 3.62) in a ratio of 1.5:1. C.i.m.s. of **16**: *m/z* 758.8 (MH^+; calc. for $C_{30}H_{76}NO_7Si_7$, 758.4); e.i.m.s. of **16**: *m/z* (relative intensities) 640–247, no ions, 246(100) [$(CH_2{=}N(SiMe_3){-}CHMe{-}CO_2SiMe_3)^+$], 147(18) [$(Me_3SiO{=}SiMe_2)^+$], and 73(38) [$(TMS)^+$].

Some of the assignments just given were based upon previous interpretations of mass spectra of trimethylsilyl derivatives of alditols[24] and amino acids[25].

N^{α}-*Cbz*-N^{ε}-*(1-Deoxy*-D-*mannitol-1-yl)*-L-*lysine* (**12**). — An aqueous solution (1.5 mL) of N^{α}-Cbz-L-lysine (**10**; 140 mg, 0.5 mmol) and sodium cyanoborohydride (38 mg, 0.8 mmol) was adjusted to pH 7.0 with 0.1M hydrochloric acid, and mixed with an aqueous solution (0.25 mL) of D-mannose (90 mg, 0.5 mmol) and D-[1-^{14}C]mannose (90 kBq). The solution was kept at 100° for 4 h, when no D-mannose was detectable by colorimetry. T.l.c. of the reaction mixture (solvent *F*): R_{Glc} 0.12–0.71 ($KMnO_4$ spray; by-products), R_{Glc} 0.98 ($KMnO_4$ spray; mannitol), R_{Glc} 2.0 ($KMnO_4$ spray; **12**), and R_{Glc} 2.9 (ninhydrin spray; **10**). The mixture was applied to a column of silica gel; **10** (ninhydrin-positive) and **12** (radioactive) were eluted with solvents *A* and *C*, respectively. The solution of **12** was evaporated to dryness. The residue was dissolved in water and lyophilized to give a powder (92 mg, 41%) containing **12** (R_{Glc} 2.0) and a small proportion of mannitol (R_{Glc} 0.98); ^{1}H-n.m.r.: δ 1.12–1.35 (m, 2 H, 2 H-γ), 1.38–1.70 (m's, 4 H, 2 H-β, 2 H-δ), ~2.91 (t, partial overlap with 2.98 signal, 2 H, $J_{\delta,\varepsilon}$ ~8 Hz, 2 H-ε), 2.98, 3.25 (AB of ABX, 8 lines, 2 H, $J_{1A,1B}$ 13.0, $J_{1A,2}$ 3.2, $J_{1B,2}$ 9.9 Hz, H-1B, H-1A), 3.44–3.83 (m's, 6.5 H, H-α, -3, -4, -5, and 2 H-6, remainder due to H's of mannitol), 3.83 (X of ABX, ddd, 1 H, $J_{2,3}$ 8.6 Hz, H-2), 4.94, 5.00 (ABq, 2 H, *J* 12.5 Hz, PhCH_2), and 7.27 (s, 5 H, Ph).

N^{α}-*Boc*-N^{ε}-*(1-Deoxy*-D-*mannitol-1-yl)*-L-*lysine* (**13**). — An aqueous solution (1.5 mL) of N^{α}-Boc-L-lysine (**11**; 123 mg, 0.5 mmol) and sodium cyanoborohydride (38 mg, 0.6 mmol) was adjusted to pH 7.0 with 0.1M hydrochloric acid, and mixed with an aqueous solution (0.25 mL) of D-mannose (90 mg, 0.5 mmol) and D-[1-^{14}C]mannose (20 kBq). The solution was maintained for 72 h at 37°, after which it contained 12 μmol of D-mannose. T.l.c. (solvent *F*) R_{Glc} 0.13–0.75 (by-products), R_{Glc} 0.98 (mannitol), R_{Glc} 2.0 (**13**), and R_{Glc} 2.8 (**11**). Compound **13**, contaminated with mannitol, was obtained as a powder (96 mg, 47%) by silica gel chromatography as described for the purification of **12**: ^{1}H-n.m.r.: δ 1.34–1.50 (m, overlaps 1.42 signal, 2 H, 2 H-γ), 1.42 (s, 9 H, Bu^t), 1.56–1.85 (m's, 4 H, 2 H-β, 2 H-δ), ~3.11 (t, overlaps 3.12 signal, 2 H, $J_{\delta,\varepsilon}$ ~8 Hz, 2 H-ε), 3.12, 3.43 (AB of ABX, 8 lines, 2 H, $J_{1A,1B}$ 13.2, $J_{1A,2}$ 3.2, $J_{1B,2}$ 9.3 Hz, H-1B, -1A), 3.60–3.92 (m's, 6.6 H, H-α, -3, -4, -5, and 2 H-6, remainder due to H's of mannitol), and 3.99 (X of ABX, ddd, 1 H, $J_{2,3}$ 8.2 Hz, H-2).

N^{α}-*Cbz*-N^{ε}-*(1-Deoxy*-D-*glucitol-1-yl)*-L-*lysine* (**7**). — This compound was prepared by the method described for the preparation of **12**, except that D-glucose

and D-[U-^{14}C]glucose replaced D-mannose and D-[1-^{14}C]mannose. The product (95 mg, 43%) contained **7** (R_{Glc} 2.0) and a small proportion of D-glucitol (R_{Glc} 0.88, solvent *F*); ^{1}H-n.m.r.: δ 1.12–1.30 (m, 2 H, 2 H-γ), 1.37–1.70 (m's, 4 H, 2 H-β, 2 H-δ), ~2.88 (t, partial overlap with 2.96 signal, 2 H, $J_{\delta,\varepsilon}$ ~8 Hz, 2 H-ε), 2.96, 3.05 (AB of ABX, 8 lines, 2 H, $J_{1A,1B}$ 13.1, $J_{1A,2}$ 3.7, $J_{1B,2}$ 9.0 Hz, H-1B, -1A), 3.41–3.85 (m's 6.8 H, H-α, -3, -4, -5, and 2 H-6, remainder due to H's of D-glucitol), 3.93 (X of ABX, ddd, 1 H, $J_{2,3}$ 5.2 Hz, H-2), 4.92, 4.98 (ABq, 2 H, J 12.5 Hz, PhCH_2), and 7.26 (s, 5 H, Ph).

N$^{\alpha}$-*Boc*-N$^{\varepsilon}$-*(1-Deoxy*-D-*glucitol-1-yl)*-L-*lysine* (**8**). — The conditions of the reaction were similar to those used for the synthesis of **13**, but D-mannose and D-[1-^{14}C]mannose were replaced by D-glucose and D-[U-^{14}C]glucose. After treatment for 72 h at 37°, 0.1 mmol of D-glucose had not reacted. The treatment was therefore continued for another 60 h, when no D-glucose remained. The final product was a powder (93 mg, 45%) that contained **8** (R_{Glc} 2.0) and a small proportion of D-glucitol (R_{Glc} 0.88, solvent *F*); ^{1}H-n.m.r.: δ 1.34–1.50 (m, overlaps 1.42 signal, 2 H, 2 H-γ), 1.42 (s, 9 H, Bu^t), 1.56–1.85 (m's, 4 H, 2 H-β, 2 H-δ), ~3.11 (t, partial overlap with 3.16 signal, 2 H, $J_{\delta,\varepsilon}$ ~8 Hz, 2 H-ε), 3.16, 3.25 (AB of ABX, 8 lines, 2 H, $J_{1A,1B}$ 13.0, $J_{1A,2}$ 3.5, $J_{1B,2}$ 9.5 Hz, H-1B, -1A), 3.58–3.93 (m's, 6.5 H, H-α, -3, -4, -5, and 2 H-6, remainder due to H's of D-glucitol, and 4.09 (X of ABX, ddd, 1 H, $J_{2,3}$ 5.1 Hz, H-2).

N$^{\varepsilon}$-*(1-Deoxy*-D-*mannitol-1-yl)*-L-*lysine* (**14**). — (*a*) *From* **12**. A solution of **12** (60 mg, 0.135 mmol) in 7:14:29 (v/v) formic acid–methanol–water (4 mL) was agitated with palladium-coated poly(ethylenimine) beads[18] (Pierce Chemical Co.) for 2 h in an atmosphere of nitrogen, the mixture was filtered, and the filtrate concentrated to dryness. T.l.c. and g.l.c. of the residue (41 mg) showed that it contained **14**, contaminated with a small proportion of mannitol. Crude **14** was dissolved in 5mM hydrochloric acid and applied to a column of Dowex 50W (H^+) cation-exchange resin. Mannitol and **14** were eluted from the resin with 5mM hydrochloric acid and 0.7M ammonia, respectively. Pure **14** (36 mg, 81%) was obtained by removal of ammonia, addition of water, and lyophilization. The properties are shown in Tables I–III. For conversion into the hydrochloride, a solution of **14** (20 mg) in 2M hydrochloric acid (1 mL) was lyophilized. The ^{1}H-n.m.r. spectral parameters are shown in Tables II and III.

(*b*) *From* **13**. Compound **13** (70 mg, 0.170 mmol) was dissolved in 2M hydrochloric acid (1 mL), and the solution kept for 15 min. Water (5 mL) was added, and the solution lyophilized to afford a glass (51 mg) that contained the hydrochloride of **14** and mannitol. Pure **14** (44 mg, 83%) was obtained by chromatography on a column of Dowex 50W (H^+) cation-exchange resin, as described under (a). The properties of the sample were identical to those of the sample prepared from **12**.

N$^{\varepsilon}$-*(1-Deoxy*-D-*glucitol-1-yl)*-L-*lysine* (**9**). — The protecting groups of **7** and **8** were removed by the methods described for the preparation of **14**, to give pure **9**, a powder, in yields of 80 and 83%, respectively. The properties of **9** are shown in Tables I–III.

ACKNOWLEDGMENTS

The authors are grateful to the Canadian Diabetes Association for financial support of this work. We thank Dr. M. B. Perry, National Research Council of Canada, for recording the g.l.c.–m.s. data, and Mrs. A. V. Johnson for technical assistance.

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