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SYNTHETIC ROUTES TO *N*-(1-DEOXY-D-FRUCTOS-1-YL)AMINO ACIDS BY WAY OF REDUCTIVE AMINATION OF HEXOS-2-ULOSES*

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ABSTRACT

There are described two routes to the synthesis of *N*-(1-deoxy-D-fructos-1-yl) derivatives of L-valine, L-leucine, L-methionine, L-phenylalanine, and 6-aminohexanoic acid. One route involved reductive amination of D-*arabino*-hexos-2-ulose in the presence of sodium cyanoborohydride; yields were limited by the formation of significant quantities of *N*-(1-deoxyhexitol-1-yl)amino acids, which were difficult to remove. This drawback was overcome in the second route, which involved reductive amination of 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-β-D-*arabino*-hexos-2-ulo-2,6-pyranose; following a deblocking step, *N*-(1-deoxy-D-fructos-1-yl) derivatives of the aforementioned amino acids were obtained in yields which were at least double those reported for the current procedure involving the reaction of an amino acid with D-glucose, and an Amadori type of rearrangement of the resulting D-glucosylamine.

INTRODUCTION

The amino groups of α-amino acids[2,3], peptides[4], and proteins[5] react with D-glucose to form D-glucosylamines, which undergo conversion into *N*-(1-deoxy-D-fructos-1-yl) derivatives by a self-catalyzed, Amadori rearrangement. Early studies of these processes were concerned with the intermediacy of *N*-(1-deoxy-D-fructos-1-yl)-L-amino acids in the browning, or Maillard, reaction in foodstuffs (see ref. 6). More recently, it has been shown[5,7] that *N*-(1-deoxy-D-fructos-1-yl) derivatives of mammalian proteins are formed *in vivo*. The process, which is called nonenzymic glycation (formerly termed, erroneously, "glycosylation"), may be important in the pathology of diabetes[7].

We now describe two routes to the synthesis of *N*-(1-deoxy-D-fructos-1-yl)

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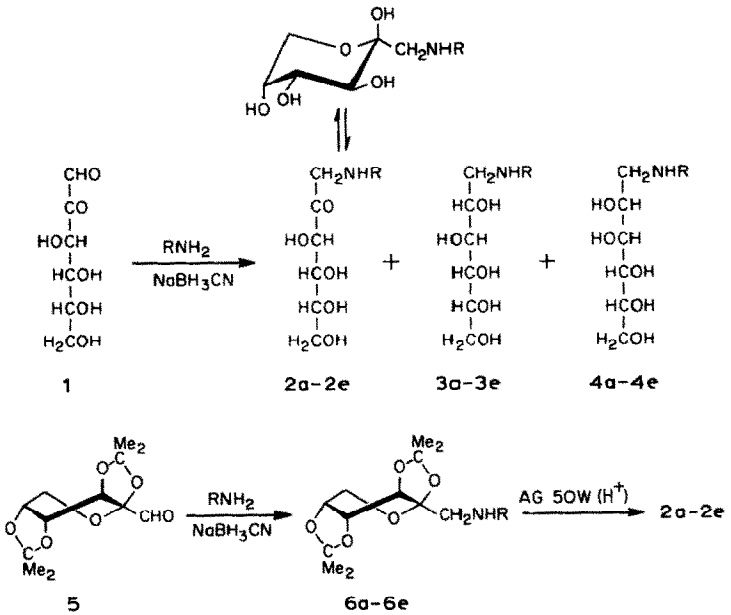
derivatives of amino acids; in our laboratory, such compounds, and their isotopically-labeled analogs, are needed for metabolic and related studies.

Yields of *N*-(1-deoxy-D-fructos-1-yl)amino acids obtained by reactions of D-glucose with amino acids were reported to be 27% or lower for pure, crystalline materials[2,3,*]. In a previous publication[8], we showed that *N*-(1-deoxyhexitol-1-yl)amino acids can be prepared by reductive amination of hexoses with amino acids in the presence of sodium cyanoborohydride. We now report that *N*-(1-deoxy-D-fructos-1-yl) derivatives of α-amino acids and 6-aminohexanoic acid can be synthesized in a similar manner from suitable hexos-2-ulose precursors, and that the method is a viable alternative to that involving Amadori rearrangement of a glucosylamine[2]. In particular, compounds **2a–2e** (see Scheme 1) were synthesized from D-*arabino*-hexos-2-ulose (**1**) or 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-β-D-*arabino*-hexos-2-ulo-2,6-pyranose (**5**).



Scheme 1

2a–4a, and **6a** R = $-CH(CHMe_2)CO_2H$ (L-valine);
2b–4b, and **6a** R = $-CH(CH_2CHMe_2)CO_2H$ (L-leucine);
2c–4c, and **6c** R = $-CH(CH_2CH_2SMe)CO_2H$ (L-methionine);
2d–4d, and **6d** R = $-CH(CH_2Ph)CO_2H$ (L-phenylalanine);
2e–4e, and **6e** R = $-CH(CH_2)_4CO_2H$ (6-aminohexanoic acid).

RESULTS AND DISCUSSION

Reductive amination of D-*arabino*-hexos-2-ulose (**1**) by L-valine in the presence of sodium cyanoborohydride resulted in the formation of a 7:3 mixture of

*The synthesis of esters of *O*-isopropylidene derivatives of *N*-(1-deoxy-D-fructos-1-yl)amino acids from 2,3:4,5-di-*O*-isopropylidene-1-*O*-trifluoromethanesulfonyl-D-fructopyranose was reported[18], however the products were not deprotected.

N-(1-deoxy-D-fructos-1-yl)-L-valine (**2a**) and the C-2 reduction products, **3a** and **4a**, respectively. The processing of the reaction mixture involved acidification and passage through a column of a cation-exchange resin. A partial ^{1}H-n.m.r. spectrum is shown in Fig. 1A. Significantly, the proportions of **2a**, **3a**, and **4a** were not changed if the solution was acidified and acetone was added to decompose the excess of cyanoborohydride rapidly, prior to the resin-adsorption step. Hence, the reduction of the carbonyl group at C-2 occurred during the initial reaction-period, and not during subsequent processing.

The absence of reductive amination of the carbonyl group at C-2 of **1** was established as follows. In the ^{1}H-n.m.r. spectrum of the reaction mixture, no signals were present in the anomeric-proton region at[9] 5.0–5.5 p.p.m., indicating the absence of 2-amino-2-deoxyhexose derivatives of the type formed by the Heyns rearrangement of ketosylamines. The absence of a slow-moving component on paper chromatograms indicated that reductive amination had probably not occurred at C-1 and C-2 of the same molecule. The removal of the byproducts, **3a** and **4a**, was difficult. Although **4a** and **2a** could not be separated by silica gel, a partial

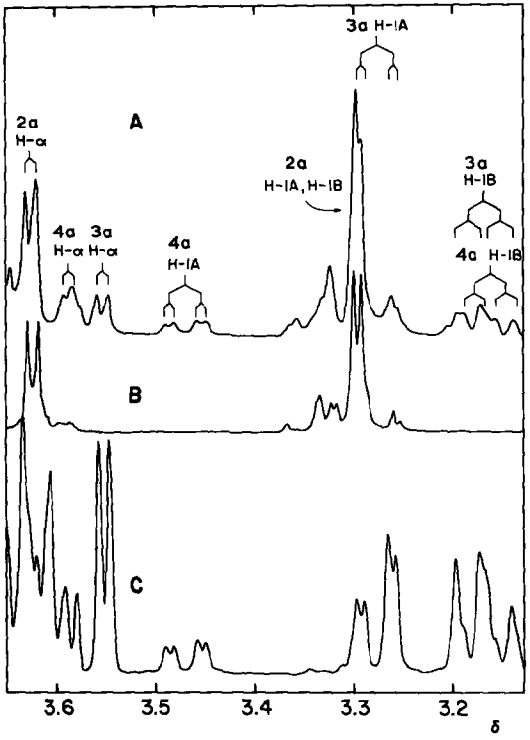


Fig. 1. Partial ^{1}H-n.m.r. spectra, at 400 MHz, of (A) a mixture of **2a**, **3a**, and **4a** formed by reductive amination of **1** with L-valine; (B) a sample of **2a** isolated from the mixture by cellulose chromatography; and (C) a mixture of **3a** and **4a** formed by reduction of **2a** using sodium borohydride. Line assignments are based on previous interpretations of the spectra of **2a** (ref. 3), **3a** (ref. 8), and **4a** (ref. 8).

TABLE I

CHEMICAL SHIFTS[a] FOR COMPOUNDS **6a**, **6d**, **6e**, AND **2a–2e**[b]

Parameter	**6a**	**6d**	**6e**	**2a**	**2b**	**2c**	**2d**	**2e**
H-1A(d)	2.90	3.03[c]	2.89	3.32	3.33	3.30[c]	3.26	3.29[c]
H-1B(d)	3.82		2.84	3.27	3.25		3.23	
H-3(d)	4.36	4.31	4.29	3.75	3.75	3.75	3.70	3.72
H-4(dd)	4.72	4.68	4.66	3.87	3.88	3.88	3.85	3.87
H-5(m)	4.43	4.34	4.33	3.99	3.99	3.99	3.97	4.01
H-6A(dd)	3.89	3.88	3.90	4.01	4.01	4.02	3.99	4.00
H-6B(dd)	3.72	3.71	3.72	3.74	3.74	3.74	3.72	3.76
$O\diagdown CMe_2^d$	1.40, 1.43,	1.33, 1.36,	1.82, 1.89,	—	—	—	—	—
$O\diagup$	1.50, 1.56	1.43, 1.52	1.92, 1.98	—	—	—	—	—
H-α	2.95d	3.59t	2.17t	3.63d	3.71t	3.76t	3.99t	2.21t
H-β	2.88m	2.96d	1.90m	2.26m	1.80m	2.17m	3.25d	1.59[e]
H-γ	0.90d, 0.97d	—	1.77[e]	1.01d, 1.03d	1.80m	2.63m	—	1.37[e]
H-δ	—	—	2.03[e]	—	0.95d	—	—	1.73[e]
H-ε	—	—	2.63t	—	—	—	—	3.11t
S-Me	—	—	—	—	—	2.12s	—	—
Ph	—	7.30m	—	—	—	—	7.37m	—

[a]In p.p.m. downfield from DSS. [b]Signals of spectra of **2a–2e** are assigned to the major component [β-pyranose; $^1C_4$(D) conformation], based on ref. 10. Weak signals at δ 3.2–3.4 (m), 4.08 (t), and 4.20 (d), present in spectra of **2a–2e**, are attributable to furanose forms[10]. [c]Apparent singlet. [d]Four singlets. [e]Quintet.

TABLE II

COUPLING CONSTANTS (Hz) FOR COMPOUNDS **6a, 6d, 6e,** AND **2a–2e**

Coupling constant	**6a**	**6d**	**6e**	**2a**	**2b**	**2c**	**2d**	**2e**
$J_{1A,1B}$	12.1	[a]	11.8	12.8	12.8	[a]	13.0	[a]
$J_{3,4}$	2.0	~2	2.6	9.9	9.9	9.9	10.0	10.0
$J_{4,5}$	8.0	8.0	8.0	3.4	3.4	3.2	3.3	3.2
$J_{5,6A}$	<1.0	<1.0	<1.0	1.2	[a]	[a]	1.1	1.2
$J_{5,6B}$	[a]	[a]	[a]	2.0	2.1	2.1	2.0	2.0
$J_{6A,6B}$	13.5	13.0	13.2	12.6	12.6	12.6	12.5	12.6
$J_{\alpha,\beta}$	6.5	7.0	7.5	4.3	5.7	6.5	7.5	7.6
$J_{\beta,\gamma}$	7.0	—	7.5	7.2	[a]	[a]	—	7.6
$J_{\gamma,\delta}$	—	—	7.5	—	4.5	—	—	7.6
$J_{\delta,\varepsilon}$	—	—	7.5	—	—	—	—	7.6

[a]Value not obtained.

separation of **3a** and **4a** from **2a** was achieved by column chromatography on cellulose, to afford a sample of the latter, in 35% yield from L-valine. The ^{1}H-n.m.r. parameters for **2a** (see Tables I and II; partial spectrum in Fig. 1B) were in close agreement with reported values[3]. The identity of **2a** was confirmed by borohydride reduction to form the known[8] *N*-(1-deoxyhexitol-1-yl) derivatives, **3a** and **4a** (see the ^{1}H-n.m.r. spectrum in Fig. 1C).

Similar results were obtained when three other α-amino acids, and 6-aminohexanoic acid, were used for the reductive amination of **1**, to form compounds **2b–2e**. Pure products were obtained in each case, but yields (see Table III)

TABLE III

PURIFICATION METHODS, YIELDS, AND PROPERTIES OF COMPOUNDS **2a–2e**

Compound	*Solvents (cellulose column)*[a]	*% Yield*[b]			*Crystallization solvent*	R_{Glc}	$[\alpha]_D^{22}$ *(degrees)*[e]
		From **1**	*From* **5**				
		[c]	[c]	[d]			
2a	*B,A*	35	73	58(27)	MeOH–H_2O	1.76	−40(−43)
2b	*B* only	32	79	41(14)	1-BuOH[f]	2.58	−40(−37)
2c	*B,A*	25	81	53(21)	1-BuOH–EtOH[f]	1.91	−34(−34)
2d	*C,B*	32	63	49(24)	1-BuOH[f]	2.35	−31(−33)
2e	*B,A*	34	57	38	1-BuOH–MeOH[f]	2.09	−47

[a]Fractions (10 mL) were collected. Elution of **2b** started at fraction 95. Elution of other compounds began at about fraction 185, following a change of solvent at fraction 160. [b]Based upon original amino acid. [c]As aqueous solution; not crystallized. [d]After crystallization. Yields reported[2,3] for syntheses involving Amadori rearrangement of D-glucosylamines are shown in parentheses. [e](c 1.0, water); lit. values[2,3] in parentheses. [f]Anhydrous solvents.

were limited by the formation, and difficulty of subsequent removal, of *N*-(1-deoxyhexitol-1-yl)amino acids.

The problem of the partial reduction of the carbonyl group at C-2 was obviated in the second route to the target compounds, which involved reductive amination of **5** (see Scheme 1). The di-*O*-isopropylidene intermediates, **6a–6e**, were obtained relatively rapidly, and in high yield, presumably because, in this case, the aldehyde group is not involved in a hemiacetal ring. The 4,5-*O*-isopropylidene group was readily removed, but stronger conditions were required for removal of the 2,3-*O*-isopropylidene group, presumably because of the special polar environment at the anomeric center[11], and the presence of the protonated nitrogen atom. Because no *N*-(1-deoxyhexitol-1-yl)amino acids, and only small proportions of colored by-products, were formed, chromatographic purification was simple and efficient. Overall yields of **2a–2e** from amino acids (see Table III) were at least double those obtained previously[2,3].

EXPERIMENTAL

General. — D-*arabino*-Hexos-2-ulose was prepared from the bis(phenylhydrazone)[12]. 2,3:4,5-Di-*O*-isopropylidene-*aldehydo*-β-D-*arabino*-hexos-2-ulo-2,6-pyranose (**5**) was prepared, in 80% yield, by oxidation of 2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranose[13] using dimethyl sulfoxide–trifluoroacetic anhydride[14]. The ^{1}H-n.m.r. parameters for **5** were in close agreement with those reported for a sample of **5** which had been obtained by an oxidation using the chromium trioxide–dipyridine complex[15]. ^{1}H-N.m.r. spectra of deuterium oxide solutions were measured at 400 MHz with a Bruker AM400 instrument. Chemical shifts are given in p.p.m. downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS). Paper chromatograms (descending; Whatman No. 1) were developed with 5:2:1 (v/v) 1-butanol–acetic acid–water and visibilized with alkaline silver nitrate[16] or ninhydrin. R_{Glc} refers to the rate of migration relative to that of D-glucose. *N*-(1-Deoxy-D-fructosyl) compounds were purified by chromatography on a column (2 × 40 cm) of Whatman CF-11 cellulose. The following solvents (v/v) were used: (*A*) ethyl acetate–acetic acid–water, 9:2:2; (*B*) 18:3:2; and (*C*) 36:4:3. *N*-(1-Deoxy-D-fructos-1-yl) compounds were estimated by the phenol–sulfuric acid colorimetric procedure[17]. For **2a–2d**, molar color yields were 10% of that obtained from D-glucose. For **2e**, the molar color yield was 1% of that of D-glucose.

*N-(1-Deoxy-*D*-fructos-1-yl)-*L*-valine* (**2a**) *from* D-arabino-*hexos-2-ulose* (**1**). — A solution of L-valine (117 mg, 1.0 mmol), sodium cyanoborohydride (75 mg, 1.2 mmol), and D-*arabino*-hexos-2-ulose (712 mg, 4.0 mmol) in water (7 mL) was adjusted to pH 7 with 0.2M sodium hydroxide, and kept for 16 h at 38° in a sealed reaction-vial. The solution was adjusted to pH 2.5 with 0.5M hydrochloric acid, and applied to a column (2 × 12 cm) of AG 50W (H^+) cation-exchange resin, previously rinsed with 2.5M acetic acid, in a well-vented hood. The column was washed with 2.5M acetic acid (100 mL) to remove unreacted **1**. Decomposition of cyanoboro-

hydride occurred at this stage. Elution with a solution (pH 5.3) of pyridine (0.23M) and acetic acid (0.14M) gave a mixture of **2a** (151 mg by phenol–sulfuric acid assay; R_{Glc} 1.76), **3a** (R_{Glc} 1.51), and **4a** (R_{Glc} 1.51). The ^{1}H-n.m.r. spectrum (see Fig. 1A) indicated that the ratios of **2a, 3a,** and **4a** were 5.1:1.3:1, respectively. Partial separation of **2a** from **3a** and **4a** was achieved by cellulose-column chromatography (see Table III). The resulting sample of **2a** (98 mg, 35%) was stored as an aqueous solution. Its ^{1}H-n.m.r. spectrum (see Fig. 1B) showed an absence of signals attributable to **3a** and **4a**.

N-*(1-Deoxy-2,3:4,5-di-*O*-isopropylidene-β-*D*-fructopyranos-1-yl) derivatives of* L*-valine,* L*-phenylalanine, and 6-aminohexanoic acid* (**6a, 6d,** *and* **6e**, *respectively).* — The amino acid (1 mmol), water (5 mL), and sodium cyanoborohydride (126 mg, 2 mmol) were added to a solution of 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-β-D-*arabino*-hexos-2-ulo-2,6-pyranose (**5**; 516 mg, 2 mmol) in methanol (5 mL). The resulting solution, in an unstoppered flask, was kept for 6 h at 70°, during which time, approximately one-half of the solvent (mostly methanol) was lost by evaporation. The solution was cooled to room temperature, adjusted to pH 1.5 with hydrochloric acid, and stirred for 0.5 h to decompose the excess of cyanoborohydride. If necessary, the solution was readjusted to pH 1.5 with hydrochloric acid. It was then applied to a column of Dowex 50W (H^+) cation-exchange resin (10 mL) in water. Water (100 mL) and 0.5M ammonia (300 mL) were passed through the column successively. The ammoniacal effluent was concentrated, to give colorless crystals (70–80%) of **6a, 6d,** or **6e**. The structure of each compound was confirmed by ^{1}H-n.m.r. spectroscopy (see Tables I and II), and by subsequent conversion into the deprotected compound, **2a, 2d,** or **2e** (see later).

N-*(1-Deoxy-*D*-fructos-1-yl)-*L*-valine* (**2a**) *from* **5**. — L-Valine (117 mg, 1 mmol) was treated with **5** and sodium cyanoborohydride, as described in the previous paragraph. Water (8 mL) and AG 50W (H^+) cation-exchange resin (8-mL bed-volume) were added to the reaction solution, and the temperature was maintained for 4 h at 70° (occasional stirring) in an unstoppered flask in the hood. The excess of cyanoborohydride was completely decomposed at this stage. The reaction mixture, including the resin, was transferred to the surface of a column (2 × 12 cm) of AG 50W (H^+) resin which had been prerinsed with 2.5M acetic acid. The column was processed as described for the preparation of **2a** from **1**, and final purification was effected by cellulose-column chromatography (see Table III), to afford an aqueous solution of **2a** (204 mg, 73%). The ^{1}H-n.m.r. spectrum indicated that the sample was almost pure, although traces of silver nitrate-positive substances, R_{Glc} 1.10 and 1.35, were detected on heavily-loaded chromatograms. Pure **2a** (162 mg, 58%) was obtained by crystallization from methanol containing a small quantity of water. The ^{1}H-n.m.r. data (see Tables I and II) and the value for the specific rotation (see Table III) were in close agreement with data reported in the literature[2,3].

N-*(1-Deoxy-*D*-glucitol-1-yl)-*L*-valine* (**3a**) *and* N-*(1-deoxy-*D*-mannitol-1-yl)-*L*-valine* (**4a**) *from* **2a**. — A sample of **2a** (20 mg; prepared from **1**) was dissolved in

0.1M sodium hydrogencarbonate solution (1 mL) and reduced with sodium borohydride (15 mg). The excess of borohydride was decomposed with acetic acid, and the resulting boric acid was removed as the methyl ester. Purification by chromatography on Dowex 1 anion-exchange resin[8] gave a product (17 mg), R_{Glc} 1.51. Comparison of its ^{1}H-n.m.r. spectrum (see Fig. 1C) with spectra of the individual epimers[8] showed that it was a mixture of **3a** and **4a** in the ratio of 2.6:1.

*N-(1-Deoxy-D-fructos-1-yl) derivatives of L-leucine, L-methionine, L-phenylalanine, and 6-aminohexanoic acid (**2b**, **2c**, **2d**, and **2e**, respectively).* — These compounds were prepared from **1** and **5** by the methods described for the synthesis of **2a**. By-products, including the *N*-(1-deoxyhexitol-1-yl)-amino acids formed in syntheses commencing from **1**, were removed by cellulose chromatography. ^{1}H-N.m.r. parameters (see Tables I and II) and specific rotations (see Table III) of **2b**, **2c**, and **2d** were similar to published values[2,3].

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