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Synthesis of (±)-2-Formyl-5-(hydroxymethyl)pyrrole-1-norleucine. A Biologically Active Maillard Reaction Product Derived from Glucose and Lysine

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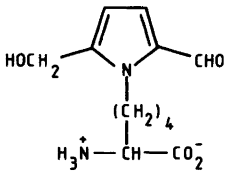
The title compound has been synthesized from pyrrole-2-carboxaldehyde, 1,4-dibromobutane and dimethyl acetamidomalonate by several routes. The preferred route proceeded *via* the known pyrrole-2,5-dicarboxaldehyde, the overall yield being 14 % from the mono- and 31 % from the dialdehyde.

The title compound (*1*) has been identified independently by Kato *et al.*[1] and by us[2] among the products formed in the reaction between D-glucose and L-lysine in boiling aqueous solution. It was reported recently that *1* is mutagenic[3] and inhibits certain enzymes, notably aminopeptidase N and carboxypeptidase A.[4] To facilitate further investigation of the biological activity of *1*, we have now developed a method for synthesizing this compound.

Kato *et al.* reported *1* as the L-form,[1] but our product was optically inactive and certainly racemic.[2] The synthetic *1* and its chiral precursors described here are, of course, also racemic.

The similar pyrrole derivative *2*, formed in the reaction between glucose and glycine, was synthesized in this laboratory by treating compound *3* or *4* (prepared from pyrrole-2-carboxaldehyde) with a large excess of ethyl bromoacetate and potassium carbonate, followed by reduction and deprotection.[5] However, it has been difficult to extend this



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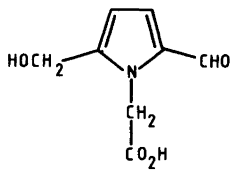

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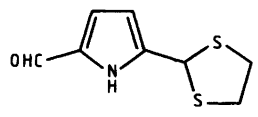

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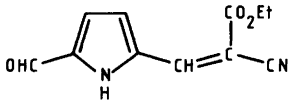

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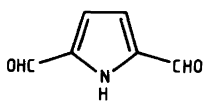

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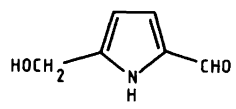

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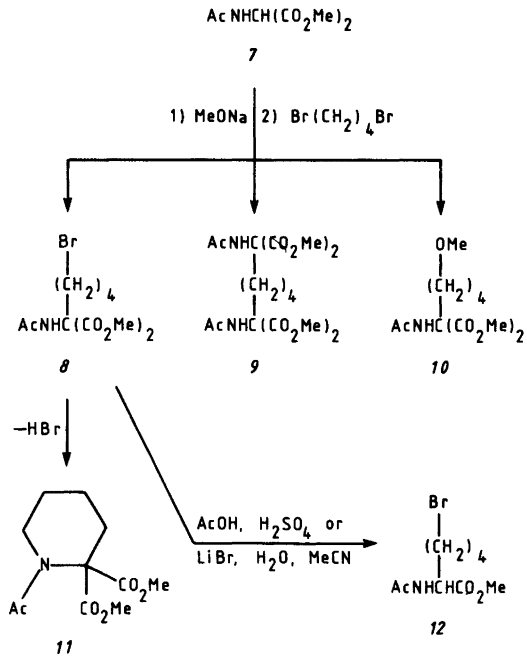


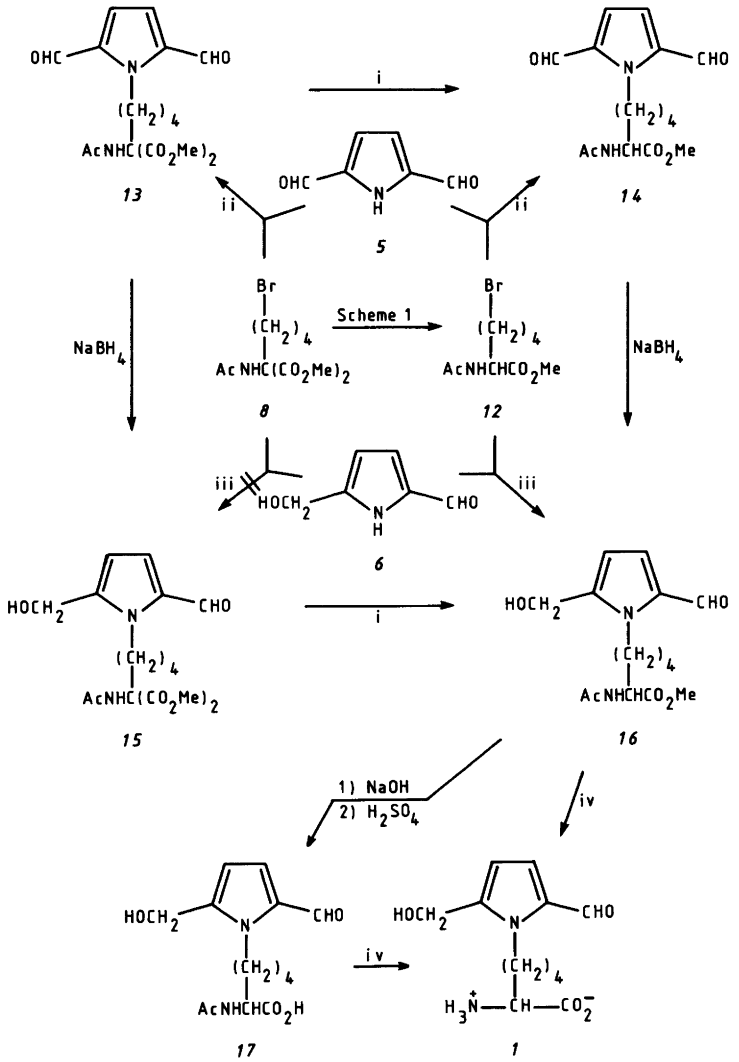
Scheme 1. Synthesis of 6-bromonorleucine derivatives (*8* and *12*).

method to homologues of *2*, but good results have been obtained by *N*-alkylation of the parent dialdehyde *5*.[6] Both *5*[7] and the related hydroxyaldehyde *6*[5] are readily available from pyrrole-2-carboxaldehyde *via 4*. To obtain *1*, we therefore planned to *N*-alkylate *5* or *6* with a suitable derivative of 6-bromonorleucine. A route from dimethyl acetamidomalonate (*7*) through the amido ester *8* was chosen (Scheme 1), since several compounds closely related to *8* have already been prepared,[8–10] and since the general procedure is well established.

RESULTS AND DISCUSSION

Compound *8* was prepared from *7*, 1,4-dibromobutane and sodium methoxide essentially as described for the corresponding diethyl ester.[8,9] The by-products *9* and *10* were obviously formed by further reaction of *8* with the sodium dimethyl acetamidomalonate or sodium methoxide, respectively (Scheme 1). Their formation was suppressed by using an excess of 1,4-dibromobutane. This procedure also served to minimize the expected[9] cyclization of *8* to the piperidine derivative *11* through base-induced elimination of hydrogen bromide. Although *11* was not isolated, its formation was indicated by ^{1}H NMR spectrometry.

The extra ester group in *8* may be removed before or after the *N*-alkylation. In the former case, a solution of *8* in glacial acetic acid was refluxed in the presence of sulfuric acid. The desired ester (*12*) was obtained in 35 % yield, *cf.* Ref. 11. Hydrolysis of *8* in alkali invariably led to a piperidine derivative through cyclization. A mixture of *12* and its iodo analogue in the approximate molar ratio 2:3 was obtained in about 50 % yield, when a solution of *8* in acetonitrile was refluxed in the presence of lithium iodide dihydrate, *cf.* Ref.



Scheme 2. Synthetic routes to the title compound (*1*). i=LiI · 2 H_2O in Me_2SO at 120 °C; ii=K_2CO_3 in MeCN at 80 °C; iii=$KOCMe_3$ in $HCONMe_2$ at 20 °C; iv=1) $Ba(OH)_2$, 2) CO_2, 3) H_2SO_4.

12. Of course, the halide mixture could be used for the *N*-alkylation without separation. A slightly poorer yield of pure *12* was obtained when lithium bromide was substituted for the lithium iodide. Refluxing in a higher-boiling solvent, such as *N,N*-dimethylformamide, again resulted in cyclization.

The several investigated routes to *1* are shown in Scheme 2. All routes had the last steps in common, *i.e.*, the deprotection of the amido ester *16*, either *via* the amido acid *17* or directly to *1*. In either case, the acetyl group was removed with barium hydroxide.[13] Since the deprotection of *16* presented no problems and proceeded in good yield, only the routes to *16* will be discussed:

Route 1: *6*+*8*→*15*→*16*
Route 2: *6*+*12*→*16*
Route 3: *5*+*12*→*14*→*16*
Route 4: *5*+*8*→*13*→*14*→*16*
Route 5: *5*+*8*→*13*→*15*→*16*

If the synthesis of *5*[7] or *6*[5] from *4* and the conversion *8*→*12* are included, each route involves four steps. However, *6* is obviously less acidic than *5* and was not *N*-alkylated by *8* even under vigorous conditions, thus ruling out route 1. The strong base required for *N*-alkylation in route 2 led to extensive cyclization of *12*. To compensate for this, a large excess of *12* had to be used, making route 2 less attractive. It is also important to note that *13* and *15* were stable and crystalline solids, whereas *14* and *16* were obtained as syrups, which decomposed within a few weeks. Before the reduction *14*→*16* in routes 3 and 4, at least partial purification of *14* proved to be necessary. Before hydrolysis to *1*, *16* had to be separated from large amounts of *12* and its cyclization products in route 2 or from *14* and/or overreduced products in routes 3 and 4. By contrast, the crude *16*, obtained from *15* in route 5, was hydrolysed without purification to *1*, which was obtained in *ca.* 58 % yield (calc. on *15*). We therefore prefer route 5. The synthetic *1* was identical (TLC, ^{1}H and ^{13}C NMR) with samples obtained from glucose and lysine.[1,2]

EXPERIMENTAL

General

Materials. Compounds *4* and *5* were prepared from pyrrole-2-carboxaldehyde.[7] Compound *6* was prepared from *4*[5] and compound *7* *via* dimethyl (hydroxyimino)malonate.[14] When the latter substance was prepared, the stirring was continued for 3 h at 20 °C after the nitrite addition. Other chemicals were commercial samples. Solvents were freshly distilled before use. All solvent mixtures were prepared on a volume basis (v/v). The light petrol boiled at 40–60 °C.

Chromatography. Column chromatography (CC) was performed on silica gel (Merck 60, 230–400 mesh), often using the "flash" technique (FC).[15] All separations were monitored by TLC on silica gel (Riedel-de Haën, SIF) with the same eluent as in CC. After the TLC plates had been inspected in UV light, phloroglucinol–hydrochloric acid,[16] ethanolic ninhydrin or silver nitrate in acetone–2-phenoxyethanol, 20:1, was used as spray reagent.

Spectrometry. The high-resolution mass spectrum of *16* was recorded at 90 eV with a V.G. ZAB instrument at the Institute of Medical Chemistry, University of Gothenburg, using electron impact (EI). Low-resolution mass spectra were recorded for $m/z > 42$ at 70 eV with a Finnigan 4021 instrument, using EI and direct insertion. The spectra of *9* and *10* were also recorded with chemical ionization (CI) and ammonia as reaction gas. NMR spectra were recorded at 89.60 (^{1}H) or 22.53 MHz (^{13}C) with a Jeol FX-90Q instrument. Where the data are listed below, Nos. 2–5 refer to the pyrrole nucleus, and α–ε to the norleucine skeleton.

Other methods. Evaporations were performed at reduced pressure below 40 °C. Melting points were determined under a Kolfer hot-stage microscope and are corrected.

Syntheses according to Scheme 1

Dimethyl acetamido-(4-bromobutyl)malonate (8). Sodium (2.3 g, 100 mmol) was dissolved in dry methanol (50 ml). To the refluxing solution was added *7* (19 g, 100 mmol) in small portions, followed by 1,4-dibromobutane (43 g, 200 mmol) in one portion. The mixture was refluxed for 3 h, concentrated, treated with water (50 ml) and extracted with chloroform

(3×50 ml). The extract was washed with water (50 ml), filtered from a little *9* and evaporated. The residue was dissolved in boiling carbon tetrachloride (*ca.* 20 ml). Light petrol (*ca.* 140 ml) was added to the stirred solution, until a faint turbidity appeared. Crystallization at 0 °C yielded crude *8* (15 g, 47 %), containing little or no *10*. Pure *8*, m.p. 90 °C, was obtained by recrystallization in the same way. Anal. $C_{11}H_{18}BrNO_5$: C, H, Br, N. MS, *m/z* (rel. int.): 43 (100), 222 (55), 224 (53), 264 (26), 266 (24), 82 (23), 55 (21), 162 (18), 164 (17), 54 (11),..., 323 (0.6, M), 325 (0.6, M). ^{1}H NMR ($CDCl_3$): δ 1.3 (γ-CH_2, m), 1.86 (δ-CH_2, quintet), 2.04 (Ac, s), 2.35 (β-CH_2, m), 3.39 (ε-CH_2, t), 3.78 (2 OMe, s), 6.8 (NH, broad s); $|J|$6.5 (δ,ε) Hz.

Tetramethyl 1,6-bis(acetamido)-1,1,6,6-hexanetetracarboxylate (9). The procedure described in the preceding paragraph was repeated with less 1,4-dibromobutane (11 g, 50 mmol), which was added dropwise over 1 h. After refluxing for another 3 h and treatment with water, the sparingly soluble *9* was collected, washed with methanol and recrystallized from 1-butanol, m.p. 215–216 °C. Anal. $C_{18}H_{28}N_2O_{10}$: C, H, N. MS, *m/z* (rel. int.): 43 (100), 244 (24), 212 (19), 109 (17), 202 (11), 331 (10), 169 (9), 272 (9), 142 (8), 157 (8). With CI, the base peak appeared at *m/z* 433 ($M+H^+$). ^{1}H NMR ($CDCl_3$): δ 1.08 (γ- and γ'-CH_2, m), 2.04 (2 Ac, s), 2.27 (β- and β'-CH_2, m), 3.78 (4 OMe, s), 6.77 (2 NH, broad s).

Dimethyl acetamido-(4-methoxybutyl)malonate (10). The filtrate obtained after collecting *9* (see preceding paragraph) was extracted with chloroform. The extract was washed with water and evaporated. The residue was extracted with boiling carbon tetrachloride. Some *7* remained undissolved. Addition of light petrol to the new extract and cooling yielded crystalline *10*, m.p. 50–53 °C. Anal. $C_{12}H_{21}NO_6$: C, H, N. MS, *m/z* (rel. int.): 43 (100), 45 (49), 142 (41), 82 (39), 44 (22), 55 (17), 114 (14), 184 (13), 71 (12), 59 (11). With CI, the base peak appeared at *m/z* 276 ($M+H^+$) and the second strongest peak at *m/z* 293 ($M+H^++NH_3$). ^{1}H NMR ($CDCl_3$): δ 1.0–1.7 (γ- and δ-CH_2, m), 2.04 (Ac, s), 2.34 (β-CH_2, m), 3.31 (ε-OMe, s), 3.35 (ε-CH_2, t), 3.78 (2 CO_2Me, s), 6.78 (NH, broad s); $|J|$ 6.2 (δ,ε) Hz.

(±)-Methyl 2-acetamido-6-bromohexanoate (12). A. Crude *8* (6.5 g, 20 mmol) was dissolved in glacial acetic acid (10 ml), containing concentrated sulfuric acid (1.0 g), *cf.* Ref. 11. The solution was refluxed until TLC analysis showed no more *8* (*ca.* 3 h). After cooling, the solution was poured on to ice. The mixture was extracted with dichloromethane. The extract was washed with 1 M sodium hydrogen carbonate followed by water, dried over sodium sulfate and evaporated. FC (EtOAc–light petrol, 5:2) of the residue yielded *12* (1.85 g, 35 %). After crystallization from toluene, *12* melted at 75.5–76.5 °C. Anal. $C_9H_{16}BrNO_3$: C, H, Br, N. MS, *m/z* (rel. int.): 43 (100), 164 (79), 166 (75), 208 (51), 206 (50), 88 (38), 56 (19), 84 (15), 131 (12), 60 (11),..., 265 (0.9, M), 267 (0.9, M). ^{1}H NMR ($CDCl_3$): δ 1.3–2.0 (β-, γ- and δ-CH_2, m), 2.04 (Ac, s), 3.40 (ε-CH_2, t), 3.76 (OMe, s), 4.64 (α-H, broad q), 6.25 (NH, broad d); $|J|$6.4 (δ,ε),7.5 (*HCNH*) Hz.

B. A solution of *8* (3.2 g, 10 mmol) in acetonitrile (15 ml) was refluxed with lithium iodide dihydrate (2.7 g, 20 mmol) for 48 h and evaporated. The residue was treated with water and extracted with chloroform. The extract was washed with water and evaporated. FC (EtOAc–light petrol, 5:2) of the residue yielded a mixture (1.45 g, *ca.* 50 %) of *12* and its 6-iodo analogue. Their approximate molar ratio was 2:3 according to the ^{1}H NMR spectrum, which revealed the iodine compound at δ 3.18 (ε-CH_2, t). Pure *12* was obtained in a similar experiment with lithium bromide (1.7 g, 20 mmol) and water (0.72 g, 40 mmol) instead of the iodide, but the reaction was slower (*ca.* 30 % of *8* remained after refluxing for 72 h).

Syntheses according to Scheme 2

Dimethyl acetamido-[4-(2,5-diformyl-1-pyrrolyl)butyl]malonate (13). A solution of *5* (4.9 g, 40 mmol) and *8* (13.0 g, 40 mmol) in dry acetonitrile (200 ml) was stirred and refluxed with potassium carbonate (8.3 g, 60 mmol) for 24 h, filtered and evaporated. FC ($CHCl_3$–EtOAc, 1:1) of the residue yielded *13* (10.9 g, 75 %). After crystallization from toluene, *13* melted at 110–112 °C. Anal. $C_{17}H_{22}N_2O_7$: C, H, N. MS, *m/z* (rel. int.): 142 (100), 123 (50), 82 (49), 43 (47), 55 (18), 165 (17), 184 (13), 54 (13), 121 (12), 197 (10),..., 366 (2, M). ^{1}H NMR ($CDCl_3$): δ 1.2 (γ-CH_2, m), 1.7 (δ-CH_2, m), 2.05 (Ac, s), 2.32 (β-CH_2, m), 3.78 (2 OMe, s), 4.72 (ε-CH_2, t), 6.89 (NH, broad s), 6.98 (3- and 4-H, s), 9.81 (2 CHO, s); $|J|$7.4 (δ,ε) Hz.

(±)-Methyl α-acetamido-2,5-diformylpyrrole-1-hexanoate (14). *A.* A solution of *5* (3.7 g, 30 mmol) and *12* (8.0 g, 30 mmol) in dry acetonitrile (150 ml) was stirred and refluxed with potassium carbonate (15 g) for 16 h, filtered and evaporated. A solution of potassium dihydrogen phosphate (15 g) in water (50 ml) was added to the residue. The mixture was extracted with chloroform. The extract was washed with water and evaporated. FC (EtOAc–$CHCl_3$, 5:1) of the residue yielded *14* (5.5 g, 60 %) as a brown syrup. MS, *m/z* (rel. int.): 43 (100), 189 (28), 134 (27), 162 (26), 249 (10), 190 (9), 136 (9), 122 (9), 88 (9), 150 (8),..., 308 (6, M). ^{1}H NMR ($CDCl_3$): δ 1.3–2.0 (β-, γ- and δ-CH_2, m), 2.03 (Ac, s), 3.74 (OMe, s), 4.65 (α-H and ε-CH_2, m), 6.2 (NH, broad d), 6.97 (3- and 4-H, s), 9.82 (2 CHO, s);|*J*|8.3 (*HCNH*) Hz.

B. A solution of *13* (370 mg, 1.0 mmol) in dimethyl sulfoxide (5 ml) was stirred with lithium iodide dihydrate (340 mg, 2.0 mmol) at 120 °C. After 2 h, another 340 mg of the lithium salt was added. Stirring was continued for another 2 h at 120 °C. TLC then indicated complete conversion of *13* to *14*. The latter was isolated by CC (EtOAc–$CHCl_3$, 8:1). The product was identical (TLC, MS, ^{1}H NMR) with that obtained by method *A*.

Dimethyl acetamido-(4-[2-formyl-5-(hydroxymethyl)-1-pyrrolyl]butyl)malonate (15). Sodium borohydride (95–190 mg, 2.5–5.0 mmol) * was added in one portion to a stirred solution of *13* (3.7 g, 10.0 mmol) in methanol (100 ml). After 15 min, glacial acetic acid (1.5 g) was added. The solution was evaporated. The residue was treated with water (30 ml) and extracted with chloroform (3×50 ml). The extract was washed with a little water and evaporated. FC (EtOAc–$CHCl_3$, 4:1) of the residue yielded *15* (2.6 g, 71 %) as an oil, which crystallized overnight, m.p. 103.5–105 °C. Anal. $C_{17}H_{24}N_2O_7$: C, H, N. MS, *m/z* (rel. int.): 43 (100), 108 (25), 297 (24), 82 (22), 80 (21), 55 (21), 249 (16), 219 (16), 44 (15), 162 (14),..., 368 (11, M). ^{1}H NMR (CD_3OD): δ 1.2 (γ-CH_2, m), 1.7 (δ-CH_2, m), 2.00 (Ac, s), 2.23 (β-CH_2, m), 3.73 (2 OMe, s), 4.32 (ε-CH_2, t), 4.60 (5-CH_2, s), 6.24 (4-H, d), 6.97 (3-H, d), 9.40 (CHO, s);|*J*|4.1 (3,4), 7.6 (δ,ε) Hz.

(±)-Methyl α-acetamido-2-formyl-5-(hydroxymethyl)pyrrole-1-hexanoate (16). *A.* Potassium *t*-butoxide (450 mg, 4.0 mmol), *6* (125 mg, 1.00 mmol) and *12* (1.33 g, 5.0 mmol) were dissolved in *N,N*-dimethylformamide (30 ml). The solution was evaporated after 5 days. Toluene was added to the residue and the mixture was evaporated. CC (EtOAc–$CHCl_3$, 8:1) of the residue yielded *16* (130 mg, 42 %) as an oil. MS, *m/z* (rel. int.): 43 (100), 55 (71), 134 (64), 84 (60), 57 (56), 83 (51), 82 (51), 162 (45), 97 (43), 98 (40),..., 310 (9, M); M, obs. 310.153, calc. for $C_{15}H_{22}N_2O_5$ 310.153. ^{1}H NMR (CD_3OD): δ 1.2–2.0 (β-, γ- and δ-CH_2, m), 1.97 (Ac, s), 3.70 (OMe, s), 4.34 (ε-CH_2, broad t), 4.6 (α-H, m, overlap), 4.60 (5-CH_2, s), 6.24 (4-H, d), 6.97 (3-H, d), 9.39 (CHO, s);|*J*|3.9 (3,4), 7.3 (δ,ε) Hz.

B. Compound *14* (1.23 g, 4.0 mmol) was reduced as described for *13*→*15*. CC (EtOAc–$CHCl_3$, 8:1) yielded *16* (*ca.* 0.9 g, 70 %), identical (TLC, MS, ^{1}H NMR) with a sample prepared by method *A*.

C. A solution of *15* (370 mg, 1.00 mmol) in dimethyl sulfoxide (5 ml) was treated with lithium iodide dihydrate for 4 h, as described for *13*→*14*. TLC (EtOAc–MeOH, 9:1) indicated complete conversion of *15* to *16*. The reaction mixture was used immediately for preparation of *1*.

(±)-N$^{\alpha}$-Acetyl-2-formyl-5-(hydroxymethyl)pyrrole-1-norleucine (17). A solution of *16* (3.1 g, 10 mmol) in methanol (100 ml) was mixed with aq. 1 M sodium hydroxide (30 ml). After 1 h, the solution was acidified to pH 4 with 1 M sulfuric acid and evaporated. The residue was further dried by evaporation with carbon tetrachloride and extracted twice with boiling methanol. Evaporation of the extract yielded *17* (2.3 g, 78 %) as a light-brown amorphous solid. ^{1}H NMR (CD_3OD): δ 1.1–2.0 (β-, γ- and δ-CH_2,m), 1.97 (Ac,s), 4.26 (α-H, t, overlap), 4.34 (ε-CH_2, broad t), 4.62 (5-CH_2, s), 6.24 (4-H, d), 6.96 (3-H, d), 9.40 (CHO, s); |*J*|4.0 (3,4), 7.4 (δ,ε) Hz.

(±)-2-Formyl-5-(hydroxymethyl)pyrrole-1-norleucine (1). Pure *16* or *17* (*ca.* 500 mg, 1.65 mmol) and barium hydroxide octahydrate (2.1 g, 6.6 mmol) were dissolved in hot water (15 ml), freshly boiled to expel dissolved carbon dioxide, *cf.* Ref. 13. The solution was refluxed for 24 h, cooled to 20 °C, saturated with carbon dioxide and filtered from barium carbonate.

* The optimum amount of borohydride depends on its quality. This amount was determined in a similar small-scale reduction, where the borohydride was added gradually and the disappearance of *13* monitored by TLC.

The filtrate was acidified to pH 4.5 with 1 M sulfuric acid, filtered from barium sulfate and evaporated. FC (MeCN–H_2O–95 % EtOH, 9:2:2) of the residue yielded *1* (270–275 mg, 65 %). The ^1H and ^{13}C NMR spectra of the product agreed with those of *1* obtained from glucose and lysine.[1,2] When a solution of crude *16*, freshly prepared by method *C* from *15* (370 mg, 1.00 mmol), was used as starting material, the alkaline hydrolysate was thoroughly washed with chloroform to remove most of the dimethyl sulfoxide. The yield of *1* was 145–150 mg (57–59 % calc. on *15*).

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