A Robust Synthesis of N\textsuperscript{\alpha}-(Carboxymethyl)-L-lysine (CML)

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Dedicated to Prof. Dr. Werner Schroth on the occasion of his 80th birthday. Ad multos annos!

Abstract: N\textsuperscript{\alpha}-(Carboxymethyl)-L-lysine is easily obtained by reductive amination of glyoxylic acid with N\textsuperscript{\alpha}-acetyl-L-lysine in the presence of a palladium catalyst and hydrogen.

Key words: amino acids, medicinal chemistry, hydrogenation, aminations, reductions

The reaction of reducing sugars with side-chain amino groups of proteins (the Maillard reaction) gives rise to intermediate compounds which further undergo intramolecular rearrangement reactions. Finally, stable so-called advanced glycation (i.e., nonenzymatic glycosylation) end products (AGEs)\textsuperscript{1,2} are obtained.

Several AGEs have been isolated and characterized, among them pentosidine,\textsuperscript{2} glucosepane\textsuperscript{3} and N\textsuperscript{\alpha}-(carboxymethyl)-L-lysine\textsuperscript{4} (CML; 1). Baynes and Thorpe\textsuperscript{5} have suggested that AGEs contribute in vivo to pathophysiological situations associated with long-term complications of ageing and diabetes. In addition, there is some evidence\textsuperscript{6} that dietary CML significantly contributes to the overall CML (protein bonded) in vivo, thus leading to the induction of inflammatory reactions in diabetic patients upon application of an AGE-rich diet.

Quite recently, CML has been used as a marker\textsuperscript{7} for profiling dairy products and, after coupling of CML to specific antibodies, an ELISA assay has been devised\textsuperscript{8} for the histochemical analysis of distal tubular epithelial cells of diabetic rats. In addition, CML has been identified\textsuperscript{9} as a marker for diabetic patients upon application of an AGE-rich diet.

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Besides isolation\textsuperscript{12} from biological material, e.g. Sagittaria pygmaea, several syntheses of 1 have been reported; the yields were quite often rather low (or not indicated) and purification procedures including HPLC had to be applied. Thus, alkylation\textsuperscript{12–14} of N\textsuperscript{\alpha}-acetyl-lysine with bromoacetaldehyde sodium salt or iodoacetate followed by suitable deprotection furnished approximately 20% of 1 after preparative HPLC purification. Small-scale, multistep sequences\textsuperscript{15} utilizing formylations yielded approximately 30–40% of 1. Approaches starting from N\textsuperscript{\alpha}-acetyllysine used either purification sequences via dibutyl esters (followed by preparative HPLC)\textsuperscript{16} or high-pressure hydrogenation\textsuperscript{17} of intermediates. In comparison, an enzymatic route\textsuperscript{18} gave a 10% yield of 1.

In order to access multigram amounts of 1 we set out to develop a robust, simple and inexpensive synthesis. Alkylation reactions of N\textsuperscript{\alpha}-acetyl- or N\textsuperscript{\alpha}-Boc-L-lysine invariably gave low yields and their workup in order to obtain pure material was rather tedious. Hence, a route utilizing a reductive amination sequence seemed more appropriate. Experiments using sodium borohydride, lithium borohydride or sodium cyanoborohydride failed to give good yields, but we finally succeeded using a heterogeneous palladium catalyst. Thus, reaction of glyoxylic acid with N\textsuperscript{\alpha}-acetyl-L-lysine in water, followed by a palladium-on-carbon catalysed hydrogenation at 5 atm, acidic hydrolysis and ion-exchange chromatography, furnished crystalline CML (1) in 46% yield (Scheme 1).

The reaction was monitored by TLC inspection on silica gel GF254 plates. NMR spectra were recorded on a Varian Gemini 2000 instrument (500 MHz). The chemical shifts (\(\delta\)) are reported in ppm and coupling constants (\(J\)) in Hz. Mass spectra were obtained on a Finnigan MAT LLQ instrument. Melting points were measured on a Leica Galen III instrument and are uncorrected. For the FT-IR data a Spectrum 1000 instrument, for the optical rotation data a 341 Polarimeter and for the UV/Vis data a Lambda 14 spectrometer (all from Perkin-Elmer) were used.

\(N\textsuperscript{\alpha}-(Carboxymethyl)-L-lysine (1)\)

A soln of glyoxylic acid monohydrate (1.27 g, 13.8 mmol) and \(N\textsuperscript{\alpha}-\)acetyl-L-lysine (1.98 g, 10.5 mmol) in H\(_2\)O (20 mL) was treated with 1 M aq NaOH (ca. 10 mL) to reach pH 8.7. The solution was

\[\text{HOOC}\text{-}N\text{-}\text{CH}_{2}\text{-COOH} \rightarrow \text{HOOC}\text{-}N\text{-}\text{CH}_{2}\text{-COOH} \]

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transferred together with 10% Pd/C (200 mg) into an autoclave and hydrogenated (5 atm) for 24 h. The mixture was filtered through a PES membrane (0.22 μm) and the filtrate was concentrated under reduced pressure. The residue was dissolved in H2O (100 mL) and the solution was concentrated to dryness. This procedure was repeated three times. The crude product was dissolved in H2O (100 mL) and filtered over silica gel (40 g). The silica gel was flushed with H2O (600 mL) and the combined filtrates were concentrated under reduced pressure to a total volume of 100 mL, then filtered through a PES membrane (0.22 μm) and concentrated under reduced pressure. The remaining residue was purified by ion-exchange chromatography [column: 3 × 40 cm, 60 g, Dowex 50WX8, 200–400 mesh, H+ form suspended in H2O (200 mL)]. The column was activated by rinsing with 6 M HCl (500 mL, ca. 240 mL/h) and H2O (500 mL, ca. 240 mL/h), and conditioned with 0.01 M HCl (350 mL, 100 mL/h). The residue, dissolved in H2O (ca. 20 mL), was applied and the column was again flushed with 0.01 M HCl (350 mL, 30 mL/h). Elution was accomplished by consecutively treating the column with increasing concentrations of HCl (1 M HCl: 500 mL, 1.5 M HCl: 500 mL, 2 M HCl: 700 mL; all ca. 30 mL/h). During the elution, fractions of 10–15 mL were collected. Detection of the product was accomplished by spraying the TLC plates with 0.1% ninhydrin in EtOH followed by incubation in a drying oven (10 min, 90 °C). The combined product fractions were concentrated to dryness to give the product (750 mg, 1.78 mmol) as colourless crystals. Another crop was obtained by repeating the column treatment 407 [2 M – H]+, 429 [2 M – 2 H + Na]+, 451 [2 M – 3 H + 2 Na]+.

UV/Vis (MeOH): λmax (log ε) = 210 nm (3.31).


References