Short Communication

Preparation of (*S*)-5-Amino-5-carboxy-*N,N,N*-trimethyl-1-pentaneaminium Chloride (L-Lysinebetaine Hydrochloride)

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L-Lysinebetaine provides a starting point for the biosynthesis of γ-butyrobetaine and carnitine.[1] It has also been claimed to increase cell proliferation and promote tumour growth.[2]

Published preparations of L-lysinebetaine are suited mainly for small-scale work or yield the compound in racemic form.[3–8] Small quantities of lysinebetaine as the dioxalate are also available commercially (Calbiochem, La Jolla, Calif., USA). Larger quantities are sometimes needed for the induction of trimethyllysine 3-hydroxylase (EC 1.14.11.) in *Pseudomonas* sp. AK1.[9]

We have earlier reported the large scale prepration of γ-butyrobetaine by methylation with *O*-methyl-*N,N'*-dicyclohexylisourea.[10] We have now developed this approach to the preparation of L-lysinebetaine.

Results and Discussion. N^{α}-Benzyloxycarbonyl-L-lysine is available via N^{ε}-benzylidene-L-lysine,[11,12] although the yield is vulnerable to increased reaction time and temperature and thus to scaling up.

A modified Eschweiler-Clarke methylation with sodium borohydride gave N^{ε},N^{ε}-dimethyl-N^{α}-benzyloxycarbonyl-L-lysine in 97 % yield. According to TLC and NMR the most homogeneous product was obtained when the formaldehyde was added in two lots, the sodium borohydride added as fast as possible, and the temperature kept between 0 and 10 °C. Hydrogenolysis of the dimethylated product gave pure N^{ε},N^{ε}-dimethyl-L-lysine hydrochloride after ion exchange chromatography in 97 % yield.

The quaternisation was carried out in good yield (>98 %) with the aforementioned *O*-methylisourea. Hydrogenolysis of the N^{α}-protected-L-lysinebetaine furnished quantitatively crude L-lysinebetaine which readily crystallized as the dihydrochloride upon treatment with hydrochloric acid.

According to ^{1}H NMR only one diastereomer was formed when a chiral shift reagent was added to a dimethyl sulfoxide-d_6 solution of the prepared compound. Treatment of neutralized L-lysinebetaine with oxalic acid to give a hemihydrate of L-lysinebetaine dioxalate allowed comparison with reported specific rotations.[13,14]

Elemental analysis of the crystalline product showed it to be the dihydrochloride. The overall yield from L-lysine including the crystallization was 57 %. Thus by the present procedure L-lysinebetaine is available in reasonable yield and adequate purity.

Experimental. ^{1}H NMR spectra were recorded with deuterium oxide as a solvent and sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 as the internal standard on a Bruker WH 270 instrument.

Ion exchange chromatography was performed on an AG 50W-X8, 200–400 mesh cation exchange resin in the H^+-form from Bio-Rad Laboratories, Richmond, Calif., USA. Compounds were eluted, either isocratically with 3 M hydrochloric acid or with an exponential gradient, at a

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linear flow rate of 0.34 cm min^{-1}. The gradient was obtained as follows: A certain volume, *vide infra,* of 6 M hydrochloric acid was allowed to slowly mix with the same volume of 1 M hydrochloride acid via a small piece of tubing. The resulting mixture was continuously stirred and pumped away to the column. Approximately 13 ml wet ion exchange resin and 120 ml eluent per g crude reaction mixture to be purified was used. Batches of at least 10 g could easily be purified.

Reactions and chromatography fractions were checked by TLC.[15] When specific rotations are reported *c* is given in g ml^{-1}.

N^{ε},N^{ε}-*Dimethyl*-N^{α}-*benzyloxycarbonyl*-L-*lysine.* About 15 ml of concentrated hydrochloric acid, 28.0 g (0.1 mol) N^{α}-benzyloxycarbonyl-L-lysine[11] and 250 ml of water are mixed. The resulting solution is cooled to 0 °C and 48 ml (0.5 mol) of 40 % aqueous formaldehyde are added. After a few min 32.8 g (0.87 mol) sodium borohydride is added at such a rate that excessive foaming is avoided. A second lot of 48 ml of 40 % aqueous formaldehyde is added when half of the sodium borohydride is consumed. The reaction temperature is kept at 5±5 °C, and the pH is continuously adjusted with concentrated hydrochloric acid and kept between 3 and 6. After completion of the reaction the pH is adjusted to 6.9, the reaction mixture taken to dryness, and the residue dissolved in absolute ethanol. The precipitated sodium chloride is then removed by filtration. This procedure is repeated once.

Addition of methanol followed by evaporation allows the removal of boric acid as methyl borate. Repetition twice of this last step furnishes a caramel-like product in 97 % yield, $[\alpha]_D^{26}$ −0.6° (*c* 0.25, glacial acetic acid).

^{1}H NMR: δ 1.39 (2H,m), 1.56–1.85 (4H,m), 2.83 (6H,s), 3.04 (2H,m), 3.97 (1H,m), 5.12 (2H,d), 7.43 (5H, broad s).

Hydrogenolysis of N^{ε},N^{ε}-dimethyl-N^{α}-benzyloxycarbonyl-L-lysine under similar conditions as described below followed by ion chromatography gives N^{ε},N^{ε}-dimethyl-L-lysine hydrochloride in 97 % yield (calculated as the dihydrochloride) as a colourless viscous oil, $[\alpha]_D^{26}$ +16.7 °C (*c* 0.05, 0.1 M hydrochloric acid). We were not successful in obtaining a crystalline sample as described in Ref. 7. M.p. 172 °C, D,L material.

^{1}H NMR: δ 1.49 (2H,m), 1.78 (2H,m), 1.95 (2H,m), 2.87 (6H,s), 3.16 (2H,m), 3.98 (1H,t).

N^{ε},N^{ε},N^{ε}-*Trimethyl*-N^{α}-*benzyloxycarbonyl*-L-*lysine hydrochloride.* N^{ε},N^{ε}-Dimethyl-N^{α}-benzyloxycarbonyl-L-lysine, (30.8 g 0.1 mol), is dissolved in a minimum amount of methanol and 26.4 g (0.11 mol) of crude *O*-methyl-*N,N'*-dicyclohexylisourea[10] are added. The reaction mixture is stirred for six days. About 125 ml of water are added and the stirring continued for additional 2 h. The urea is filtered off, 18 ml of concentrated hydrochloric acid added, and the filtrate taken to dryness yielding the hydrochloride quantitatively as a viscous oil, $[\alpha]_D^{26}$ −7.4° (*c* 0.02, water), pure enough to be used in the next step. A crystalline inner salt has been reported to give $[\alpha]_D^{26}$ −4° (*c* 2, water).[14]

^{1}H NMR: δ 1.39 (2H,m), 1.77 (4H,m), 3.06 (9H,s), 3.24 (2H,m), 3.99 (1H,m), 5.14 (2H,m), 7.44 (5H, broad s).

N^{ε},N^{ε},N^{ε}-*Trimethyl*-L-*lysine dihydrochloride.* N^{ε},N^{ε},N^{ε}-Trimethyl-N^{α}-benzyloxycarbonyl-L-lysine hydrochloride, (35.9 g 0.1 mol), is taken up in a minimum amount of water and about 1 g of palladised charcoal (10 %) and a small volume of concentrated hydrochloric acid is added. The reaction mixture is deareated and vigorously stirred under hydrogen at 70 °C. The pH is checked from time to time and kept below 6.5.

After the completed deprotection as monitored by TLC (less than 24 h) the catalyst is filtered off and the filtrate evaporated furnishing a crude product in more than 98 % yield. The crude product is taken up in 3 M hydrochloric acid and partly reevaporated leaving a viscous oil which spontaneously crystallizes upon standing in the refrigerator. Some absolute ethanol is added to increase the yield of crystals. They are then isolated by filtration and dried over P_2O_5/KOH *in vacuo.* Seeding crystals may be obtained if part of the crude product is ion exchange chromatographed giving an oil which is more prone to crystallize.

L-Lysinebetaine dihydrochloride may be reprecipitated from methanol or absolute ethanol followed by treatment with acetone. It starts sintering at 210 °C and melts with decomposition between 220 and 235–250 °C, depending on the batch (lit. D,L-material: 240, 246–248 and 260.2–261.2 °C)[4,6,7] $[\alpha]_D^{26}$ +15.7° (*c* 0.05, 0.1 M hydrochloric acid). Found: C 41.4; H 8.5; N 10.7; Cl

27.2. Calc. for $C_9H_{22}N_2O_2Cl_2$: C 40.8; H 8.8; N 10.6; Cl 26.3.

^1H NMR: δ 1.54 (2H,m), 1.88 (2H,m), 2.04 (2H,m), 3.12 (9H,s), 3.62 (2H,m), 4.14 (1H,t).

Splitting of signals was not observed when tris (3-trifluoroacetyl-*d*-camphorate)europium(III) was added to a dimethyl sulfoxide-d_6 solution of L-lysinebetaine dihydrochloride. The formation of a complex was, however, evident since an upfield shift by 0.15 for the methine proton could be observed. A dioxalate was prepared and recrystallized as described in Refs. 13 and 14, m.p. 143–147 °C, $[\alpha]_D^{24}$+10.4° (*c* 0.07, water). Literature values, m.p. 122–124 °C, $[\alpha]_D^{24}$+8.9° (*c* 0.7, water) and $[\alpha]_D^{18}$+10.8° (*c* 5, water).

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