Useful Intermediates for Synthesis of Dicarba Analogues of Cystine Peptides: Selectively Protected α-Aminosuberic Acid and α,α'-Diaminosuberic Acid of Defined Stereochemistry

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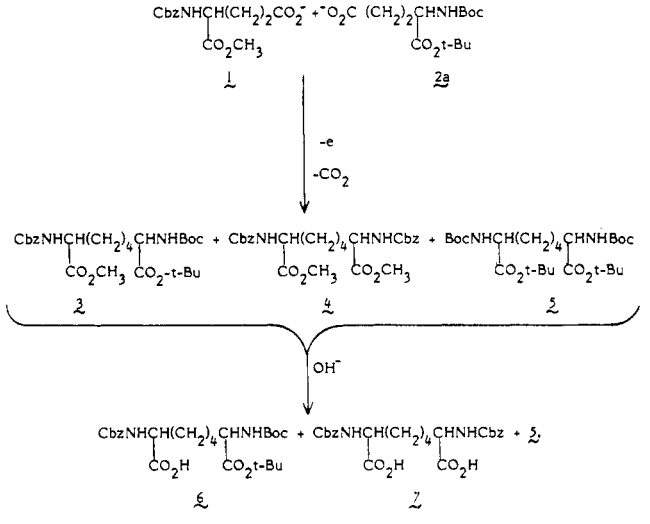
Practical procedures are described for synthesis of selectively protected α-aminosuberic acid and α,α'-diaminosuberic acid by the mixed Kolbe electrolytic decarboxylative dimerization of two different carboxylic acids. Stereochemistry is established by the choice of L- or D-glutamic acid precursors. The method is illustrated by the synthesis of α-*tert*-butyl N^{α}-Boc-$N^{\alpha'}$-Cbz-LL-α,α'-diaminosuberate (**6**) and α-*tert*-butyl ω-methyl N^{α}-Boc-D-α-aminosuberate (**10**) which can be used directly in the preparation of dicarba and desaminodicarba analogues of cystine peptides. Although statistically controlled mixtures are produced, facile procedures for isolation of the products have been worked out. No racemization of chiral centers was detected.

Dicarba and desaminodicarba analogues of biologically active cystine peptides such as oxytocin,[1-3] calcitonin,[4] and somatostatin[5-7] have shown high biological activity and enhanced metabolic and chemical stability owing to the absence of reducible disulfide linkages. The synthesis of this class of analogues has been difficult because of the lengthy multistep syntheses of the required, selectively blocked, amino acid intermediates. We wish to report a generally useful and practical method of synthesis of selectively blocked diamino- and aminosuberic acid derivatives as exemplified by the syntheses of **6** and **10**. Previously described[8] syntheses of selectively blocked diaminosuberic acid involved the preparation of a symmetrically blocked intermediate by the Kolbe electrolysis, followed by multiple deblocking and reblocking steps. Prior syntheses[9-11] of α-aminosuberic acid derivatives resulted in racemic products.

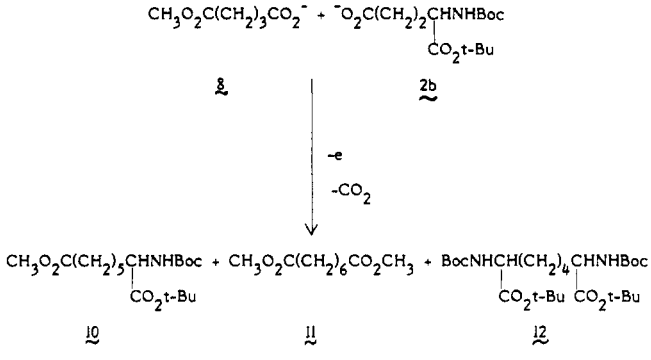
Our syntheses of **6** and **10**, on the contrary, made use of readily available selectively blocked glutamic acid derivatives to incorporate the desired protecting groups and stereochemistry prior to the Kolbe dimerization. Electrolysis is carried out on two differently blocked carboxylic acid derivatives, and the desired unsymmetrical dimer is separated from the two symmetrical dimers.

For synthesis of **6**, a stoichiometric mixture of α-*tert*-butyl *N*-Boc-L-glutamate (**2a**) and α-methyl *N*-Cbz-L-glutamate (**1**) was electrolyzed (Scheme I). Major products from the reaction, the desired unsymmetrical dimer **3** and the two symmetrical dimers **4** and **5**, were isolated as a mixture in 50% yield. These three products were only difficultly separable because of close solubility and polarity.

Scheme I



Scheme II



Therefore, the methyl esters were hydrolyzed selectively, giving a mixture of the monobasic acid **6**, the dibasic acid **7**, and the neutral diester **5** which was separated readily by silica gel chromatography. The monobasic acid **6** was characterized by its NMR spectrum at 300 MHz and by elemental analysis of its dicyclohexylamine salt. Optical purity was established by removal of blocking groups to give LL-α,α'-diaminosuberic acid of the same rotation reported in an independent synthesis.[8]

Synthesis of **10** was accomplished by electrolysis of a mixture of α-*tert*-butyl *N*-Boc-D-glutamate (**2b**) and methyl glutarate (**8**) (Scheme II). Again the predominant products were three dimers, **10**, **11**, and **12**. The proportion of **10** was increased by the use of a twofold excess of methyl glutarate. In contrast to the diaminosuberic acid series, the desired unsymmetrical dimer **10** could be isolated and

(1) Rudinger, J.; Jost, K. *Experientia* **1964**, *20*, 570.
(2) Jost, K.; Rudinger, J. *Collect. Czech. Chem. Commun.* **1967**, *32*, 1229.
(3) Walter, R.; Yamanaka, T.; Sakakibara, S. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 1901.
(4) Sakakibara, S. In "Peptides, Proceedings of the Fifth American Peptide Symposium"; Goodman and Meienhofer, Eds.; Wiley: New York, 1977; p 436.
(5) Veber, D. F.; Strachan, R. G.; Bergstrand, S. J.; Holly, F. W.; Homnick, C. F.; Hirschmann, R.; Torchiana, M. L.; Saperstein, R. *J. Am. Chem. Soc.* **1976**, *98*, 2367.
(6) Emura, J.; Morikawa, T.; Takaya, T.; Sakakibara, S. In "Proceedings of the 14th Symposium on Peptide Chemistry"; Nakajima, T., Ed.; Protein Research Foundation: Osaka, Japan, 1977; p 36.
(7) Veber, D. F.; Holly, F. W.; Nutt, R. F.; Bergstrand, S. J.; Brady, S. F.; Hirschmann, R.; Glitzer, M. S.; Saperstein, R. *Nature (London)* **1979**, *280*, 512.
(8) Kellar, O. Dissertation, Institut für Molekularbiologie und Biophysik, E.T.H.; Zürich, 1974.
(9) Treibs, W.; Reinheckel, H. *Chem. Ber.* **1956**, *89*, 51.
(10) Farkasova, H.; Rudinger, J. *Collect. Czech. Chem. Commun.* **1965**, *30*, 3117.
(11) Hase, S.; Kiyoi, R.; Sakakibara, S. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 1266.

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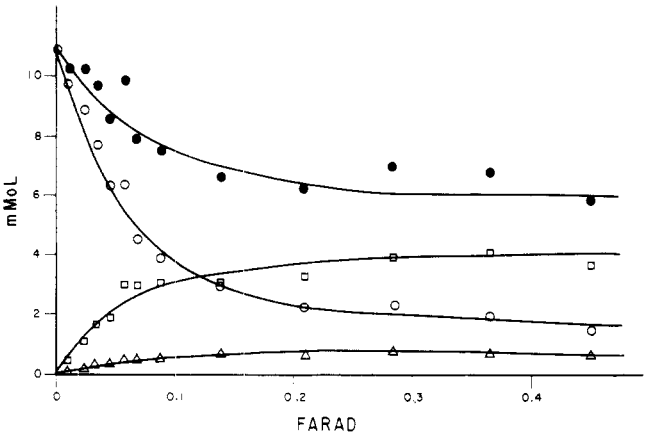

Figure 1. Formation of products and disappearance of starting material as a function of electrons introduced: ○, α-*tert*-butyl *N*-Boc-D-glutamate (**2b**); □, α-*tert*-butyl ω-methyl N^{α}-Boc-D-α-aminosuberate (**10**); △, di-*tert*-butyl di-Boc-DD-diaminosuberate (**12**); ● total of **2b**, **10**, and **12**.

purified by silica gel chromatography, without prior hydrolysis of the methyl esters. The isolated yield of **10** was 31% based on **2b**. The NMR spectrum of **10** was consistent with the structure and showed no unassignable resonances. Alkaline hydrolysis of **10** gave α-*tert*-butyl *N*-Boc-D-α-aminosuberate. The IR spectrum of this product was identical with that of the previously reported DL product.[5] For analysis by the Manning–Moore procedure,[12] **10** was hydrolyzed with 6 N HCl at 100 °C for 20 h. Reaction with the *N*-carboxy anhydride of glutamic acid gave only glutamyl-D-aminosuberic acid[5] as determined on an amino acid analyzer.

In the light of the usefulness of the Kolbe synthesis in preparing the products described above, it seems surprising that this reaction is not used more frequently. Possibly a reason for this is an uncertainty in handling experimental problems.

We found that the following conditions are satisfactory and that the various important variables can be understood in terms of current theories of the mechanism and consideration of the operational requirements.

The mechanism of the coupling reaction is presumed to be formation of unstable radicals by loss of an electron from **1**, **2a**, **8**, and **2b** at the platinum anode. Loss of CO_2 gives radicals which, by dimerization, afford **3**, **4**, and **5** from **1** and **2a** and afford **10**, **11**, and **12** from **8** and **2b**. Competing side reactions are expected to be first order, the radical intermediate undergoing either decomposition or reaction with solvents. To optimize the desired bimolecular coupling reactions, high concentrations of reactants are thus required. In contrast to many electrolytic oxidations, the Kolbe reaction does not require precise control of voltage, but high current density does favor the coupling reactions. We used a current density of about 0.4 A/cm^2. A suitable reaction temperature was found to be 25 °C; a lower temperature led to a decrease in current density. A great deal of heat is generated by the 200–400 W in the small volume between the electrodes (2 mL). To dissipate this heat, efficient mixing and rapid heat transfer are required. The use of a glass vessel resulted in overheating due to inefficient heat transfer. The reaction is best run in a metal vessel which is in a cold bath at −70 °C. Reactions were carried out in a mixture of pyridine–methanol, which gave fewer side reactions than methanol alone, in contrast to literature reports.[13]

(12) Manning, J.; Moore, S. *J. Biol. Chem.* **1968**, *243*, 5591.

Figure 1 shows the time course of a typical reaction followed by amino acid analyses of the starting material and product. When conditions as described above were applied to the preparation of **10**, product was formed at the rate indicated in Figure 1. It is seen that the formation of amino- and diaminosuberic acid derivatives increases with current flow until about 30 equiv of electrons have been introduced, at which point **10** has been formed in 40% yield. No further formation of product was seen. These results are consistent with the view that, as the concentrations of α-*tert*-butyl *N*-Boc-D-glutamate diminished, current is consumed by solvent at an increasing rate and becomes the dominant reaction beyond 0.3 F. It also appears from this study that decomposition of the starting material forms the greatest part of the loss of yield of product. Electrolysis of the product would not appear to contribute much to the loss of yield.

These conditions provide a means for utilization of Kolbe electrolyses to make available optically pure, selectively protected diamino- and monoamino suberic acid derivatives for use in peptide synthesis.

Experimental Section[14]

α-Methyl Cbz-L-glutamate (1). A suspension of 31.3 g of Cbz-L-glutamic anhydride[15] in 1 L of methanol was stirred at 25 °C for 20 h. The solution was evaporated in vacuo to a thick oil (38.4 g) and the α- and γ-methyl esters (R_f 0.55 and 0.2; 80:20:2 $CHCl_3$–MeOH–H_2O) were separated by chromatography on 3.7 kg of silica 60 (E. Merck) packed in 80:20:1.3 $CHCl_3$–*i*-PrOH–H_2O. Elution with 80:20:1.3 followed by 60:30:5 $CHCl_3$–MeOH–H_2O yielded 23.5 g of the α-methyl ester, 3.58 g of mixed esters, and 10.3 g of the γ-methyl ester. Crystallization of the α-methyl ester from EtOAc–petroleum ether yielded product: mp 66–68 °C (lit.[16] 68–69 °C); $[\alpha]^{23}_D$ −25.2° (*c* 1, MeOH) [lit. $[\alpha]^{25}_D$ −25.9° (*c* 1, MeOH)].

α-*tert*-Butyl N^{α}-Boc-$N^{\alpha'}$-Cbz-α,α'-diaminosuberate (6). Sodium (0.25 g) was added to a solution of 8.0 g of α-methyl Cbz-L-glutamate and 8.6 g of α-*tert*-butyl Boc-L-glutamate (**2**) in 240 mL of methanol and 80 mL of pyridine. The mixture was treated electrolytically for 55 min at 20–25 °C with a current of 4 A at 100 V generated by a Kepco 0–100-V, 0–5-A power supply using 2.5 × 4 cm platinum electrodes which were spaced 2 mm apart. Mechanical stirring, a metal beaker as a reaction vessel, and a CO_2–isopropyl alcohol cooling bath at −40 °C were used to obtain adequate temperature control. Electrolysis was stopped when the pH of the reaction reached 7.2–7.6 as measured by moist pH paper (range 6–8). The solvent was evaporated in vacuo to an oil which partially redissolved in 100 mL of $CHCl_3$–EtOAc (95:5). The suspension was filtered, and the filtrate evaporated to a dark oil (16.75 g). The three dimeric coupling products (**3**, **4**, and **5**) were separated as a mixture from both the more mobile and the more polar byproducts by chromatography on 1.7 kg of silica gel 60 (E. Merck) packed in 95:5 $CHCl_3$–EtOAc, with elution with the same solvent at a flow rate of 100 mL/min. Column fractions (70 mL each) were evaluated by TLC, and fractions containing the mixture of dimers were combined and evaporated to dryness (7.82 g). TLC analysis showed dimethyl di-Cbz-diaminosuberate (**4**, R_f 0.29), N^{α}-Cbz-α-methyl-$N^{\alpha'}$-Boc-α'-*tert*-butyl diaminosuberate (**3**, R_f 0.36), and di-*tert*-butyl di-Boc-diaminosuberate (**5**, R_f 0.42) (Quantum Q_1 plates, 95:5 $CHCl_3$–EtOAc).

A solution of 7.8 g of the above mixture of blocked diaminosuberic acid derivatives (**3**, **4**, and **5**) in 157 mL of dioxane was treated at 25 °C with 78 mL of 1 N NaOH for 15 min with stirring. The turbid solution was neutralized with 4 mL of acetic acid, the volume reduced in vacuo to 50 mL, and the solution lyophilized. Separation was accomplished by chromatography on 1.5 kg of silica

(13) Baizer, M., Ed. "Organic Electrochemistry"; Marcell Decker: New York, 1973; p 476.

(14) TLC analyses were carried out on Analtech silica gel plates unless otherwise indicated.

(15) Greenstein, J. P.; Winitz, M. *Chem. Amino Acids* **1961**, *2*, 1094.

(16) Klieger, E.; Gibian, H. *Justus Liebigs Ann. Chem.* **1962**, *655*, 195.

gel 60 (E. Merck) packed in 80:20:2 $CHCl_3$–CH_3OH–NH_4OH, with elution with the same solvent at a flow rate of 140 mL/min. Fractions (70 mL) containing the desired product with R_f 0.39 (80:20:2 $CHCl_3$–CH_3OH–NH_4OH) were combined, and the solvent was evaporated in vacuo to give the ammonium salt of **6** which was converted to the acid by the addition of 100 mL of 5% citric acid. After extraction with three 100-mL portions of EtOAc, the combined extracts were dried over $MgSO_4$, filtered, and evaporated in vacuo to yield 2.7 g of **6**: $[\alpha]^{23}_D$ −18.2° (*c* 0.6, 50% HOAc); NMR (300 MHz, CD_3OD) δ 1.4–1.85 (m, 8, CH_2), 1.43 (s, 9, $C(CH_3)_3$), 1.45 (s, 9, $C(CH_3)_3$), 3.95 (dd, 1, *J* = 8.5, 5.0 Hz, $CHCO_2C(CH_3)_3$), 4.15 (dd, 1, *J* = 8.8, 4.8 Hz, $CHCO_2H$), 5.11 (s, 2, CH_2O), 7.32–7.4 (m, 5, Ar).

DCHA salt: mp 145–145.3 °C with a transition at 135 °C (ether–petroleum ether); $[\alpha]^{23}_D$ −13.9 (*c* 0.3, 50% HOAc).

Anal. Calcd for $C_{37}H_{61}N_3O_8$: C 65.75; H, 9.10; N, 6.22. Found: C, 65.90; H, 9.15; N, 6.16.

The optical purity was established by the total removal of blocking groups and comparison of the optical rotation of the product with the published data[8] for diaminosuberic acid. A solution of 85.5 mg of α-*tert*-butyl $N^{\alpha'}$-Cbz-N^{α}-Boc-α,α′-diaminosuberate in 2 mL of CH_2Cl_2 was treated at 25 °C with a stream of HBr for 1 h. The solution was flushed with a stream of nitrogen for 10 min, and ether was added to precipitate diaminosuberic acid dihydrobromide (58.8 mg): R_f 0.18 (7:2:1 *i*-PrOH–NH_4OH–H_2O), 0.39 (90:10 MeOH–NH_4OH); $[\alpha]^{23}_D$ +41.8° (*c* 0.2, 6 N HCl) (the diaminosuberic acid concentration of the rotation solution was determined by the amino acid analysis) [lit.[8] +37° (*c* 1.5, 6 N HCl), +41.1° (*c* 1, 6 N HCl)].

α-*tert*-Butyl ω-Methyl D-α-Aminosuberate (10). Sodium (0.25 g) was added to a solution of 7.9 g (54.0 mmol) of methyl glutarate (**8**) and 8.2 g (27.0 mmol) of α-*tert*-butyl *N*-Boc-D-glutamate (**2b**) in 240 mL of MeOH and 80 mL of pyridine, and the reaction mixture was electrolyzed as above. The reaction was followed by TLC using Analtech microplates and the solvent system 80:20:2 $CHCl_3$–CH_3OH–NH_4OH. The reaction was allowed to proceed at 24–25 °C for 1.75 h at which time >95% of the protected D-glutamic acid had been consumed as shown by TLC. The dark brown solution was concentrated in vacuo at 30 °C to a viscous oil (14.83 g). The oil, partially dissolved in 25 mL of $CHCl_3$, was centrifuged and the supernatant applied to a silica gel 60 (700 g) column. The column was eluted with $CHCl_3$–EtOAc (97:3), 125-mL fractions were collected, and those fractions shown to contain pure product **10**, as evaluated by TLC ($CHCl_3$–EtOAc, 95:5) using ninhydrin, were combined and concentrated in vacuo to yield a light yellow oil: 2.97 g; $[\alpha]^{20}_D$ +18.05° (*c* 1.7, MeOH); NMR (300 MHz, $CDCl_3$) 1.35–1.75 (m, 8, CH_2), 1.43 (s, 9, $C(CH_3)_3$), 1.46 (s, 9, $C(CH_3)_3$), 2.30 (t, 2, *J* = 7.5 Hz, CH_2CO), 3.66 (s, 3, OCH_3), 4.17 (dd, 1, *J* = 8.5, 6.5 Hz, CH–N), 5.03 (d, 1, *J* = 8.5 Hz, NH). Acid hydrolysis gave D-α-aminosuberic acid as determined on an amino acid analyzer.

For further characterization, **10** was treated with saturated HCl–EtOAc at 0 °C for 15 min to give the crystalline product, ω-methyl D-α-aminosuberate hydrochloride: mp 210–216 °C dec (lit.[2] mp 222–226 °C dec); $[\alpha]^{20}_D$ −15.77° (*c* 1.55, 5 N HCl). The NMR spectrum showed only the expected resonances and no *tert*-butyl signal. Hydrolysis of **10** in 1 N NaOH–THF gave α-*tert*-butyl N^{α}-Boc-D-α-aminosuberate: mp 75–76.5 °C; $[\alpha]^{20}_D$ +19.55° (*c* 1.33, MeOH); the IR was identical with that of the corresponding DL compound;[5] the NMR showed only expected resonances.

Rate Study. In the manner described above, 27 mmol of methyl glutarate and 13.5 mmol of α-*tert*-butyl Boc-D-glutamate were electrolyzed. Aliquots of 2 mL were withdrawn every 5 min for 1 h, every 10 min for the next 2 h, and every 15 min for the last 0.5 h. Fractions were analyzed after acid hydrolysis for glutamic acid, D-aminosuberic acid, and α,α′-DD-diaminosuberic acid on an amino acid analyzer. Amounts of these three compounds are plotted against moles of electrons (farads) which is calculated from the amperage and the time at which the aliquots were withdrawn.

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Registry No. 1, 5672-83-3; **2a**, 24277-39-2; **2b**, 73872-71-6; **3**, 63061-85-8; **4**, 73872-72-7; **5**, 73872-73-8; **6**, 63061-86-9; **6** DCHA salt, 73924-76-2; **8**, 1501-27-5; **10**, 70717-73-6; Cbz-L-glutamic anhydride, 4124-76-9; γ-methyl Cbz-L-glutamate, 4652-65-7; diaminosuberic acid dihydrobromide, 73924-77-3; ω-methyl D-α-aminosuberate hydrochloride, 70774-08-2; α-*tert*-butyl N^{α}-Boc-D-α-aminosuberate, 70717-72-5.