Bifunctional Chelating Agents. Part 2.† Synthesis of 1-(2-Carboxyethyl)ethylenediaminetetra-acetic Acid by Ring Cleavage of a Substituted Imidazole

Janina Altman,* Nurit Shoef, Meir Wilchek, and Abraham Warshawsky
Departments of Biophysics and Organic Chemistry, Weizmann Institute of Science, Rehovot, Israel

The synthesis of 1-(2-carboxyethyl)ethylenediaminetetra-acetic acid, a new bifunctional analogue of ethylenediaminetetra-acetic acid (EDTA) is described. This is carried out *via* ring-opening benzoylation of ethyl imidazol-4-ylpropanoate, hydrogenation and hydrolysis to 4,5-diaminovaleric acid (DL-γ-ornithine) dihydrochloride, and subsequent alkylation.

Bifunctional chelating agents analogous to ethylenediaminetetra-acetic acid (EDTA) were first introduced by Meares and co-workers [1] and are of interest in radiolabelling,[2-5] and as tumour imaging reagents.[6,7]

The usual approach to the synthesis of EDTA analogues proceeds through the reduction of the appropriate nitrile [1] or amide [8-10] and subsequent alkylation. So far, 1-(*p*-aminophenyl),[1] 1-(*p*-aminobenzyl)-,[9] 1-(*p*-aminophenethyl)-,[10] and 1-(*p*-carboxymethoxybenzyl)-ethylenediaminetetra-acetic acid [8] have been prepared by this route.

In this work we wish to present an alternative approach to EDTA analogues based on the Bamberger ring cleavage dibenzoylation of a suitable substituted imidazole, under Schotten–Baumann reaction conditions (Scheme).

Although the Bamberger ring cleavage of imidazoles has been known for ninety years,[11] its kinetic and mechanistic aspects are still under investigation,[12,13] and the synthetic potential is still being explored.[14]

Special attention has been given to the Bamberger ring cleavage of imidazole during the modification of histidine-containing proteins.[15-18] For our purpose, the Bamberger ring cleavage of substituted imidazoles provides an intermediate containing both the 1,2-diamine system needed for building the chelating EDTA-part of the molecule, and the functions needed for attachment to biological macromolecules.

Ethyl imidazol-4(5)-yl propanoate (1) was chosen as a starting material. The ester was prepared by catalytic reduction of commercially available urocanic acid with palladium-on-charcoal ‡ and esterified by the thionyl chloride method. This sequence of reactions is shorter and simpler than the one described by Iizuka,[20] which involved monobenzyloxycarbonylation of urocanic acid, esterification with diazomethane, and then reduction.

When the ester (1) was treated with benzoyl chloride or benzyloxycarbonyl chloride in EtOAc–aqueous $NaHCO_3$, the unsaturated chain products (2a) and (2b) were obtained in 82 and 88% yield, respectively. The reaction required a large excess of acyl chloride to overcome the competing hydrolysis reaction, and prolonged reaction times. Interrupting the benzyloxycarbonylation after 2 h allowed the monoacyl intermediate, ethyl 3-[*N*-benzyloxycarbonylimidazol-4(5)-yl-propanoate], to be isolated in poor yield. Catalytic reduction of (2a) with palladium-on-charcoal in ethanol at 50 °C yielded ethyl 4,5-dibenzamidovalerate (3). Care must be taken to avoid an acidic solvent as the ene-amide adds water under acidic conditions, and hydrolyses to the oxo-amide (4) and benzamide. Hydrolysis of the ester (3) in refluxing hydrochloric acid–acetic acid gave the previously unknown 4,5-diaminovaleric acid (5) as the dihydrochloride (DL-γ-ornithine) in 89% yield, whereas hydrolysis of (3) under basic conditions at room temperature affected only the ester group, giving the diamide (6). Compound (2b) was hydrogenated in an attempt to remove the benzyloxycarbonyl groups and the double bond in one step, and give (5) directly. It seems, however, that the benzyloxycarbonyl groups were hydrogenated prior to the double bond, and the free ene-amine system then tautomerized to an imine which probably undergoes hydrolysis, as judged from the n.m.r. spectrum of the crude reaction mixture; this shows the presence, in 30% relative concentration, of a compound having a skeleton resembling the oxo-amide (4). The approach to the diamine (5) through the benzyloxycarbonyl derivative (2b) was, therefore, abandoned.

The alkylation of compound (5) with bromoacetic acid at pH 9.5—10.5 gave the desired EDTA analogue (7) in 51% yield.

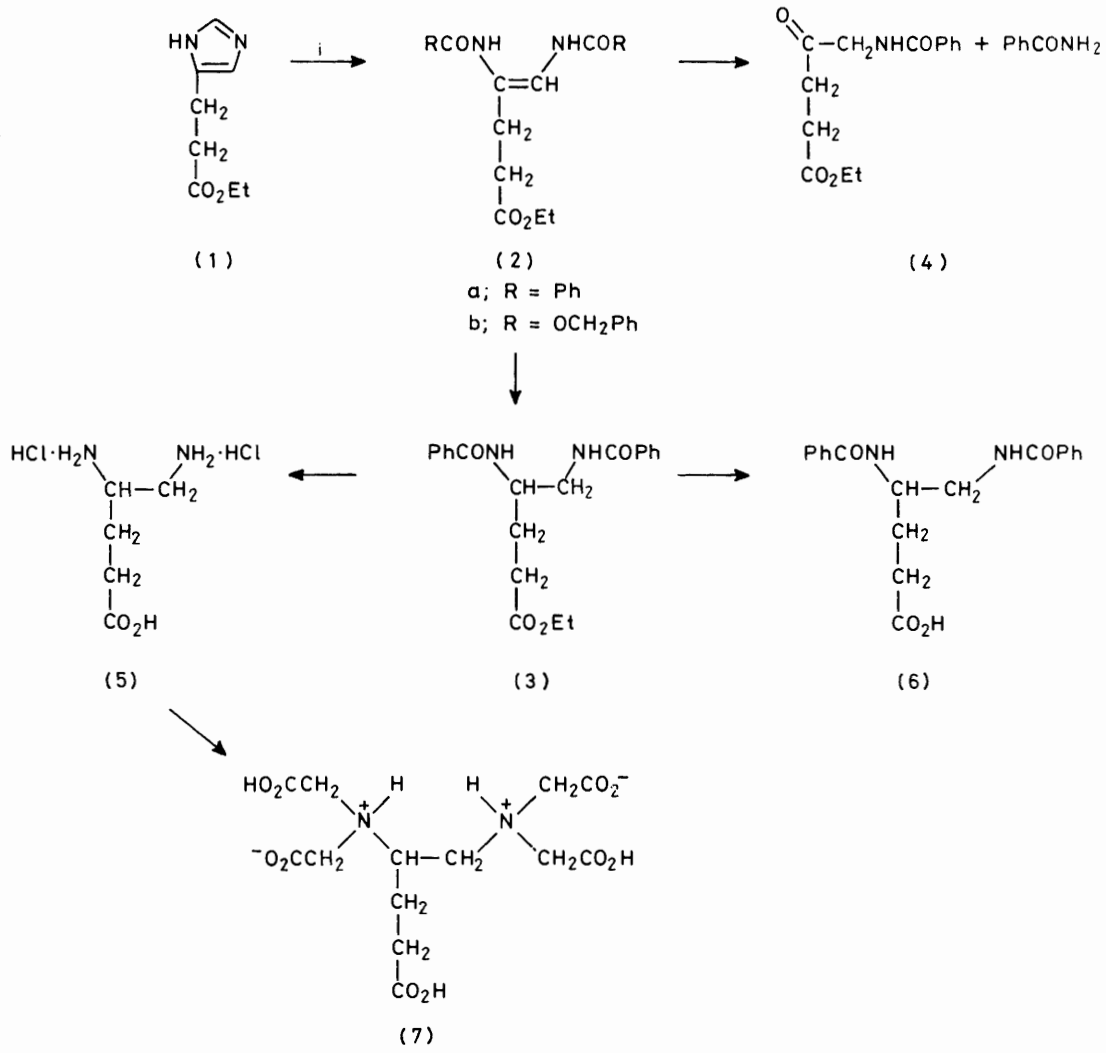
The Bamberger ring cleavage acylation reaction is a new approach to the synthesis of bifunctional chelating agents; the acid (7) produced in this case is well suited for conjugation to proteins and subsequent labelling with radioactive metals or with the lanthanide elements.

Experimental

M.p.s were determined on a Fisher-Johns melting point apparatus and are uncorrected. ^1H N.m.r. spectra were recorded on a Varian FT 80A spectrometer. I.r. spectra were obtained by using a Perkin-Elmer 467 spectrophotometer. Ether refers to diethyl ether.

Ethyl 3-Imidazol-4(5)-ylpropanoate (1).—Urocanic acid (imidazol-4-ylacrylic acid) (7 g, 0.05 mol) was dissolved in hot acetic acid (150 ml) and hydrogenated overnight, at room temperature, in a Parr apparatus in the presence of 10% Pd–C (350 mg), then filtered and evaporated. 3-Imidazol-4(5)-yl-propanoic acid was crystallized from ethanol–ether (6.9 g), m.p. 203—204 °C (lit.,[21] 203—208 °C). The acid (14 g, 0.1 mol) was suspended in absolute ethanol (140 ml), cooled in an ice-bath, and thionyl chloride (59 g, 0.49 mol) was added dropwise. The reaction mixture was then heated at 50 °C for 4 h and left at room temperature for 48 h. Dry ether (300 ml) and hexane (300 ml) were added and a yellow layer was formed. The upper layer was decanted, and the oily layer was treated again with ether–hexane to remove diethyl sulphite. Then ethyl acetate (500 ml) was added, followed by a small portion of solid $NaHCO_3$ until the mixture reached pH 8. Sodium sulphate was added and the mixture was left overnight, filtered, and concentrated, yielding the oily product (1) (15.7 g, 93%), $\nu_{max.}$ ($CHCl_3$) 3 440—3 000 (NH · · · N) and 1 710 cm^{-1} ($CO_2C_2H_5$); δ ($CDCl_3$) 10.90 (1 H, s, NH), 7.58 (1 H, s,

† Part 1, ref. 10.
‡ Schunack [19] performed the hydrogenation with Rh–C, and the esterification in boiling ethanol.



Scheme. *Reagents:* i, RCOCl, $NaHCO_3$

ImH), 6.81 (1 H, s, ImH), 4.12 (2 H, q, *J* 9 Hz, OCH_2), 2.98—2.57 (4 H, m, CH_2), and 1.23 (3 H, t, *J* 9 Hz, Me) (Found: C, 57.0; H, 7.2. Calc. for $C_8H_{12}N_2O_2$: C, 57.13, H, 7.19%).

Ethyl 3-[N-*Benzyloxycarbonylimidazol*-4(5)-*yl*]*propanoate*. —The ester (1) (1.68 g, 0.01 mol) was dissolved in ethyl acetate (30 ml). Benzyloxycarbonyl chloride (6 g, 0.035 mol) and 1M-$NaHCO_3$ (75 ml) were added simultaneously from two separate funnels during 30 min while being cooled in an ice-bath and stirred. The stirring was continued for 1 h with cooling and 1 h at room temperature, keeping the pH alkaline. The organic layer was separated, washed with water, and concentrated. The residue was adsorbed on a silica column (125 g, Merck). Benzyl alcohol was eluted with a mixture of 10—15% EtOAc–hexane, and the *product* was obtained upon elution with 25% EtOAc–hexane (530 mg, 17%), $\nu_{max.}$ ($CHCl_3$) 1 750 ($NCO_2C_7H_7$) and 1 725 cm^{-1} ($CO_2C_2H_5$); δ ($CDCl_3$ 8.03 (1 H, s, ImH), 7.37 (5 H, s, ArH), 7.15 (1 H, s, ImH), 5.32 (2 H, s, CH_2O), 4.10 (2 H, q, OCH_2), 2.90—2.53 (4 H, m, CH_2), and 1.23 (3 H, t, Me) (Found: C, 63.8; H, 6.2; N, 8.9. $C_{16}H_{18}N_2O_4$ requires C, 63.56; H, 6.00; N, 9.26%).

Ethyl 4,5-*Dibenzamidopent*-4-*enoate* (2a).—The ester (1) (9.2 g, 0.054 mol) was dissolved in ethyl acetate (140 ml) in a 2-l three-necked flask. Benzoyl chloride (15.7 g, 0.112 mol) dissolved in EtOAc (40 ml), and 1M-$NaHCO_3$ (380 ml) was added dropwise during 1 h with stirring and cooling in an ice-bath. The reaction mixture was stirred for 1 h, then further portions of benzoyl chloride (15.7 g, 0.112 mol) in EtOAc (40 ml), and 1M-$NaHCO_3$ (280 ml), were added slowly and simultaneously, followed by an additional portion of 1M-$NaHCO_3$ (200 ml). The reaction was stirred overnight. The organic layer was separated, concentrated, then redissolved in tetrahydrofuran (300 ml) and stirred with 10% $NaHCO_3$ (600 ml) for 24 h to decompose any *N*-formyl intermediate from the Bamberger cleavage and to remove the benzoic acid. Ethyl acetate (200 ml) was added, the organic layer separated, and the water layer extracted with more ethyl acetate. The joint organic extracts were dried (Na_2SO_4), concentrated, and the residue crystallized from ethyl acetate–hexane to give the *product* (2a) (16.24 g, 82.4%), m.p. 128—129 °C; $\nu_{max.}$ ($CHCl_3$) 1 700 ($CO_2C_2H_5$) and 1 640 cm^{-1} (CONH); δ ($CDCl_3$) 10.34 (1 H, d, *J* 11 Hz, NH), 9.01 (1 H, s, NH), 8.97—7.36 (10 H, m, ArH), 6.66 (10 H, d, *J* 11 Hz, CH), 4.16 (2 H, q, OCH_2), 2.76—2.51 (4 H, m, CH_2), and 1.27 (3 H, t, Me). The signals at δ 10.34 and 9.01 disappeared when D_2O and TFA (trifluoroacetic acid) were added, and the signal of the olefinic proton at δ 6.66 became a singlet (Found: C, 69.2; H, 6.1; N, 7.7. $C_{21}H_{22}N_2O_4$ requires C, 68.83; H, 6.05; N, 7.64%).

Ethyl 4,5-*Bis(dibenzyloxycarbonylamino)pent-4-enoate* (2b).—The ester (1) (1.68 g, 0.01 mol) was dissolved in ethyl acetate (30 ml) and cooled in an ice-bath. Benzyloxycarbonyl chloride (6 g, 0.035 mol) and 1M-$NaHCO_3$ (75 ml) were added from two funnels with stirring during 30 min, and the reaction mixture was left at room temperature overnight. The organic layer was separated, dried (Na_2SO_4), and concentrated, and the residue was chromatographed on a silica column (125 g). Benzyl alcohol was eluted with 10—15% ethyl acetate–hexane. Upon elution with 20% EtOAc–hexane, the *product* (2b) was obtained as an oil (3.74 g, 88%), $\nu_{max.}$ 3 420 (NH) and 1 730 cm^{-1} (CO); δ ($CDCl_3$) 7.31 (10 H, s, ArH), 7.06br (1 H, NH), 6.16 (1 H, d, *J* 12 Hz, CH), 5.09 (2 H, s, OCH_2), 5.08 (2 H, s, OCH_2), 4.15 (2 H, q, OCH_2), 2.39 (4 H, s, CH_2), and 1.28 (3 H, t, Me) (Found: C, 64.6; H, 6.3; N, 6.3. $C_{23}H_{26}N_2O_6$ requires C, 64.77; H, 6.15; N, 6.56%).

Ethyl 4,5-*Dibenzamidovalerate* (3).—The hydrogenation of 4,5-dibenzamidopent-4-enoate (2a) (5 g) was carried out in ethanol (150 ml) with 10% Pd–C (900 mg) in a Parr Apparatus, with external heating to 50 °C with a lamp during 20 h. The solution was filtered and concentrated, the residue recrystallized from ethyl acetate to give the *product* (3) (4.8 g, 95%), m.p. 161—162 °C; $\nu_{max.}$ ($CHCl_3$) 3 300 (NH), 1 720 ($CO_2C_2H_5$), and 1 640 cm^{-1} (CONH); δ ($CDCl_3$) 7.86—7.32 (10 H, s, ArH), 4.32 (1 H, t, CHN), 4.12 (2 H, q, OCH_2), 2.63 (2 H, t, CH_2N), 2.51 (2 H, t, *J* 8 Hz, CH_2CO_2), 2.00 (2 H, q, *J* 8 Hz, CH_2), and 1.22 (3 H, t, Me) (Found: C, 68.4; H, 6.6; N, 7.5. $C_{21}H_{24}N_2O_4$ requires C, 68.57; H, 6.57; N, 7.60%).

Ethyl 5-*Benzamido*-4-*oxovalerate* (4).—The unsaturated ester (2a) (500 mg) was dissolved in a mixture of ethanol (40 ml) and 1M-HCl (1.5 ml) and brought to reflux. The reaction was monitored by t.l.c. on silica plates using EtOAc–hexane as eluant. After 48 h all the starting compound had disappeared. The reaction mixture was cooled, neutralized by addition of solid $NaHCO_3$, concentrated, redissolved in a minimum volume of chloroform, dried (Na_2SO_4) and adsorbed on a silica column (20 g). Upon elution with a mixture of 30% EtOAc–pentane, the pure ketone (4) (50 mg) was obtained, m.p. 93—94 °C, $\nu_{max.}$ ($CHCl_3$) 3 380 (NH), 1 720 (CO), and 1 640 cm^{-1} (CONH); δ ($CDCl_3$) 7.88—7.30 (5 H, m, ArH), 7.15br (1 H, NH), 4.38 (2 H, d, *J* 6 Hz, $HNCH_2CO$), 4.12 (2 H, q, *J* 9 Hz, OCH_2), 2.87—2.67 (4 H, m, CH_2), and 1.24 (3 H, t, *J* 9 Hz, Me). The signal at δ 7.15 disappeared when D_2O and TFA were added and the signal at δ 4.38 became a singlet (Found: C, 64.0; H, 6.5; N, 5.2. $C_{14}H_{17}NO_4$ requires C, 63.86; H, 6.51; N, 5.31%).

Elution with 40% EtOAc–pentane gave, according to n.m.r., more of the ketone (4), together with benzamide. Two recrystallizations from ethyl acetate–hexane gave the pure benzamide, m.p. 123—124 °C, no depression in the m.p. when mixed with the authentic sample.

4,5-*Diaminovaleric Acid Dihydrochloride* (γ-*Ornithine*) (5).—The saturated ester (3) (5 g) was refluxed for 24 h in a mixture of glacial acetic acid (50 ml) and 36% hydrochloric acid (120 ml) (bath temperature 145 °C). The solvents were removed under reduced pressure, the residue was triturated several times with dry ether to remove the benzoic acid and quickly recrystallized from absolute methanol (30 ml) and absolute ether (30 ml) upon cooling. [Prolonged standing in methanol must be avoided since the acid dihydrochloride (5) tends to undergo esterification.] The *products* (5) (2.5 g, 88%) had m.p. 174—175 °C; $\nu_{max.}$ 1 680 cm^{-1}; δ (D_2O) 3.12 (1 H, quintet, *J* 8 Hz, CHN), 3.36 (2 H, d, *J* 8 Hz, CH_2N), 2.68 (2 H, t, *J* 9 Hz, CH_2CO_2H), and 2.19—1.88 (2 H, m, CH_2) (Found: C, 29.3; H, 6.9; N, 13.6. $C_5H_{14}Cl_2N_2O_2$ requires C, 29.28; H, 6.88; N, 13.66%).

4,5-*Dibenzamidovaleric Acid* (6).—The ester (3) (100 mg, 0.3 mmol) was dissolved in methanol (10 ml). 1M-KOH (0.3 ml) was added and the mixture left at room temperature for 24 h. The solvent was evaporated, water added, acidified with 6M-HCl, and extracted with ethyl acetate. The organic layer was dried and evaporated and the residue was crystallized from ethyl acetate to give the *product* (6) (68 mg, 73.5%), m.p. 226—227 °C; $\nu_{max.}$ (Nujol) 1 700 (CO_2H) and 1 640 cm^{-1} (CONH); δ (CD_3OD) 7.9—7.2 (10 H, m, ArH), 3.7—3.5 (2 H, m, CH_2N), 2.45 (2 H, t, *J* 8 Hz, CH_2CO_2), and 2.00 (2 H, m, CH_2) (Found: C, 67.0; H, 6.1. $C_{19}H_{20}N_2O_4$ requires C, 67.04; H, 5.92%).

1-(2-*Carboxyethyl*)*ethylenediaminetetra-acetic Acid* (7).—All glassware used in this experiment was washed in twice-distilled water. A solution of the dihydrochloride (5) (1.045 g, 5 mmol) in water (5 ml) (neutralized to pH 7 with 5M-KOH) in a three-necked flask in which a glass electrode was immersed, was treated with bromoacetic acid (3.475 g, 25 mmol) which had previously been neutralized to pH 7 with 5M-KOH and 1M-KOH towards the end-point of the neutralization. The mixture was then brought to pH 10 with 5M-KOH and heated to 50 °C (bath). The pH of the mixture was kept between 9.5—10 during the first 9 h, then brought to pH 11 using 1M-KOH and left overnight with heating, with no further addition of alkali. After being cooled, the reaction mixture was acidified to pH 2.5 with 6M-HCl, water was removed under reduced pressure and the residue was triturated several times with hot ethanol to remove glycolic acid and then twice recrystallized from twice-distilled water. After the first recrystallization it was let to stand overnight in the refrigerator, and after the second recrystallization, at room temperature. The *product* (7) (800 mg) was obtained, m.p.182—184 °C; $\nu_{max.}$ (Nujol) 1 740 and 1 720—1 670 cm^{-1}; δ (D_2O) 3.86 (4 H, s, NCH_2CO_2H), 3.72 (4 H, s, $NCH_2CO_2^-$), 3.28—3.47 (2 H, m, CH_2N), 2.48 (2 H, t, CH_2CO_2H), and 2.2—1.5 (2 H, m, CH_2); δ (D_2O–TFA) 4.20 (4 H, s, $NCH_2CO_2^-$), 3.81 (4 H, s, NCH_2CO_2DH); δ (D_2O–K_2CO_3) 3.61br (4 H, s, $NCH_2CO_2^-$), 3.55 (2 H, s, $NCH_2CO_2^-$), and 3.50 (2 H, s, $NCH_2CO_2^-$) (Found: C, 40.8; H, 5.7; N, 7.4. $C_{13}H_{20}N_2O_{10}$·H_2O requires C, 40.84; H, 5.80, N, 7.34%). Ethanol solutions from triturations and the mother-liquor from crystallizations were combined, evaporated, redissolved, brought to pH 6.5 and chromatographed on a column (1.5 × 21 cm) of an anion-exchange resin (AG 1-X4; HCO_2^- form [22]) using linear gradient elution with 1—4M-formic acid as eluant. Fractions (20 ml) were collected. The penta acid (7) was found in fractions 27—36. After their lyophilisation and crystallization further product (170 mg) was obtained, m.p. 182—184 °C, bringing the total yield to 51%.

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