# Bifunctional Chelating Agents. Part 1. 1-(*p*-Aminophenethyl)-ethylenediaminetetra-acetic Acid

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The synthesis of a new bifunctional analogue of ethylenediaminetetra-acetic acid (EDTA), 1-(*p*-aminophenethyl)ethylenediaminetetra-acetic acid (13), from the amide of 2-amino-4-phenylbutyric acid (8) by reduction with diborane and acetylation to give compound (9), followed by nitration to afford the isomeric *NN'*-diacetyl-1-(*p*-nitrophenethyl)ethylenediamines [*para*- (10a) to *ortho*- (10b) ratio 9:1] of (10a) hydrolysis to compound (11), and tetra-alkylation to afford the acid (12), is described. This synthesis by-passes some of the inherent difficulties in the synthesis of the analogous 1-(*p*-nitrophenyl)-ethylenediamine system.

The synthesis of bifunctional analogues of EDTA has been undertaken by Meares and his co-workers [1] as an approach to covalent attachment of metal chelates to macromolecules.[2,3] Such chelates bind a wide range of ions, including those which act as radioactive tracers, and thus they may have applications in the radiolabelling of antibodies.[4] Meares *et al.* have prepared 1-(*p*-aminophenyl)ethylenediaminetetra-acetic acid (6a) and they bound it by diazotization to human serum albumin and bovine fibrinogen.[1] Hwang and Wase, working on erythrocytes and platelets, used the acid (6a) as a surface-labelling reagent.[5] Also, Meares and his co-workers used the bromoacetyl derivative bound to bleomycin [6,7] as a reagent with tumour-localizing properties. Later, Yeh *et al.*[8] prepared a series of EDTA analogues through amino-acid conversion *via* borane reduction of their amides and alkylation with bromoacetic acid. This series of compounds lacks the amino-function needed for diazotization and requires additional protection of the EDTA function during the coupling process to the biological macromolecules.

Repeating Meares' synthesis of 1-(*p*-aminophenyl)ethylene-diaminetetra-acetic acid (6a) according to Scheme 1, we found that the nitration reaction of *NN'*-diacetyl-1-phenylethylene-diamine (2) gave rise to a mixture of *para*- and *ortho*-isomers to which previous reference has not been given, in a ratio *ca.* 3 : 2 as shown by the $^1$H n.m.r. spectrum (aromatic region) of the crude product. The isomers were separated by crystallization to give the pure *para*-isomer (3a) (30% yield) and the pure *ortho*-isomer (3b) (20% yield), both characterized by their m.p.s and $^1$H n.m.r. spectra. Alternatively, the crude mixture of diamides (3a) and (3b) could also be subjected to acidic hydrolysis. The resulting *p*-nitrophenylethylenediamine dihydrochloride (4a) crystallizes upon cooling, whereas the more soluble *ortho*-isomer (4b) remains in solution and can be obtained after evaporation.

The question arose as to whether lowering the temperature of the nitration reaction would minimize the formation of the *ortho*-isomer † because of the expected steric hindrance of the α-acetamido-group when the reaction was repeated at −60 °C, and also at −20 °C, no appreciable change in the ratio of isomers was detected. The insensitivity to temperature variation in this case suggests that the α-acetamido-moiety of the molecule is involved in the formation of the *ortho*-isomer through an unstable *N*-nitro-amide intermediate (X) (see Scheme 2). Consequently the separation of the acetamido-group, by a chain of two or more carbon atoms, from the aromatic part of the molecule will diminish the formation of the *ortho*-isomer.

Furthermore, the major problem in Meares' synthesis of the nitrophenyl derivatives (5) is the very low yield of the alkylation reaction of the diamine (4a) with iodo- or bromo-acetic acid, which in our hands very rarely exceeded 30% and was more often than not even lower. This result stands out in comparison with a similar system with an unsubstituted aromatic ring which undergoes alkylation in 75% yield in 15 h, as reported by Okaku *et al.*[9]

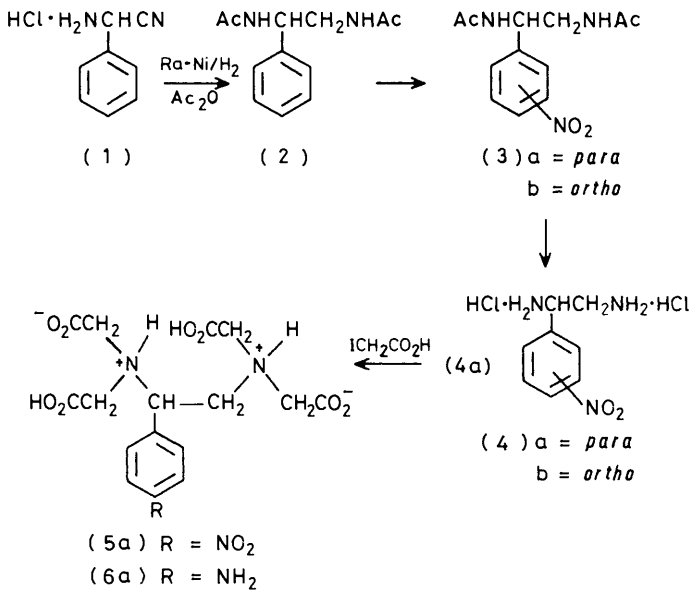
In the 1920's, Hanhorf and Ingold [10] studied the alkylation of *p*-nitrophenethylamines and they pointed to the easy formation of nitrostyrene as being a result of elimination of the quaternary β-amino-group. If quaternization of the amine in the β-position of compound (4a) competes with the alkylation of the sterically hindered amine group in the α-position, low yields would not be surprising.

To overcome *all* dificulties in the synthesis of EDTA analogues substituted by *p*-aminophenyl functions we proceeded to the synthesis of 1-(*p*-aminophenethyl)ethylene-diaminetetra-acetic acid, (13), another bifunctional agent with many prospective applications, but we expected that the separation between the chelating EDTA ligand and the functional aromatic ring, by a chain of three carbon atoms, would avoid risks of β-elimination and would result in improved yields in the nitration and alkylation steps; for this synthetic scheme, 2-amino-4-phenylbutyric acid was chosen as the starting material. The acid was converted through its methyl ester (7) into the amide (8) which was reduced with borane and acetylated to afford the diacetylamide (9) in 75% yield (Scheme 3).

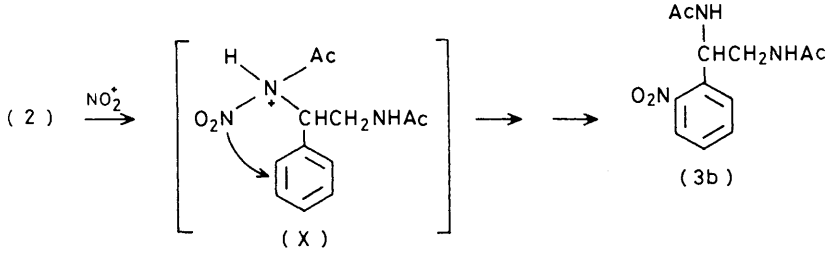
Nitration of the diacetylamide (9) occurred mainly at the *para*-position. The presence of the *ortho*-nitro-isomer (10b) could not be observed in the aromatic region ($^1$H n.m.r.) of the crude reaction product but it was detected in the spectrum of the concentrated mother liquor from which the main portion of the *para*-isomer (10a) had been removed by crystallization. The ratio of the isomers in the reaction mixture was estimated as 9 : 1. Again, as expected, the alkylation of the diamine (11) with bromoacetic acid was carried out smoothly and the corresponding ethylenediaminetetra-acetic acid (12) was obtained in 71% yield. The reduction of the nitro-compound (12) to the amine (13) produced the ' bifunctional chelating ' agent, available for coupling with macromolecules.

In summary, detection and analysis of the inherent mechanistic difficulties in the synthesis of the 1-(*p*-nitrophenyl)-ethylenediamine system—particularly due to competing *ortho* substitution and sensitivity to basic conditions—has led to the design and synthesis of a system free from the above limitations.

† We wished to do this since the end product of the *ortho* series, compound (6b), was undesirable because it is not expected to be a good diazotization agent, owing to steric hindrance.




**Scheme 1**




**Scheme 2**

## Experimental

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

*NN'-Diacetyl-1-(p-nitrophenyl)ethylenediamine* (3a) *and NN'-Diacetyl-1-(o-nitrophenyl)ethylenediamine* (3b).—*NN'*-Diacetyl-1-phenylethylenediamine (2) [prepared from 1-phenylglycinonitrile (1) [11] by reduction according to the method of Meares [1] and acetylation] (5 g, 0.022 mol) was added, in small portions, to 90% $HNO_3$ (16 ml) at −40 °C. The reaction mixture was stirred for 4 h at −40 °C and was then poured onto a mixture of ice (100 g) and $Na_2CO_3$ (20 g) and extracted with ethyl acetate. The extract was dried and evaporated to yield a crude compound (5.3 g, 88%), m.p. 135—150 °C. Two recrystallizations from acetone–hexane gave the pure *para*-isomer (3a) (1.8 g, 30%), m.p. 172—174 °C (lit.,[1] 178—180 °C); δ ($CD_3OD$) 8.27 and 8.16, and 7.62 and 7.51 (total 4 H, $A_2B_2$ q, 4 × ArH), 5.40 (1 H, t, CH), 3.8 (2 H, m, $CH_2$), 2.00 (3 H, s, $CH_3$), and 1.90 (3 H, s, $CH_3$) (Found: C, 54.55; H, 5.7; N, 16.0. Calc. for $C_{12}H_{15}N_3O_4$: C, 54.33; H, 5.70; N, 15.84%).

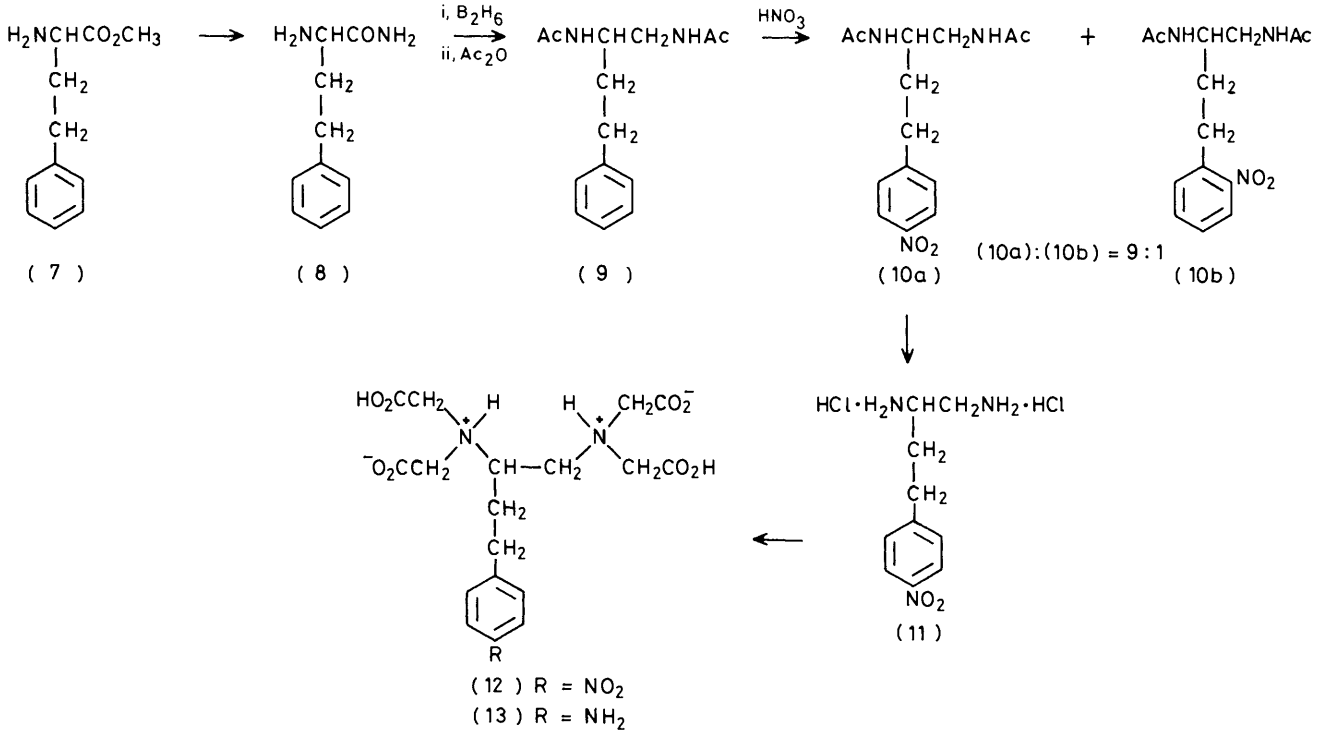
The mother liquor from the crystallization was concentrated and triturated with a small volume of ethyl acetate to produce a second crop of crystals. This fraction (1.25 g, 20%), m.p. 150—153 °C, exhibited the characteristic n.m.r. spectrum of the *ortho*-isomer (3b). Two recrystallizations to constant m.p. gave *compound* (3b) (530 mg), m.p. 168—169 °C; δ ($CD_3OD$) 8.23—8.10 and 7.71—7.58 (total 4 H, m, 4 × ArH), 2.00 (3 H, s, $CH_3$), and 1.90 (3 H, s, $CH_3$) (Found: C, 54.5; H, 5.8; N, 15.75. $C_{12}H_{15}N_3O_4$ requires C, 54.33; H, 5.70; N, 15.84%).

1-(p-*Nitrophenyl)ethylenediamine Dihydrochloride* (4a).—The diamide (3a) (1.1 g) was heated under reflux in a mixture of glacial acetic acid (5 ml) and concentrated HCl (7 ml) for 24 h. The mixture was cooled and crystals of the *title compound* were obtained (854 mg, 85%),* m.p. 235—245 °C (decomp.); δ ($D_2O$) 8.44 and 8.33, and 7.83 and 7.72 (total 4 H, $A_2B_2$ q, 4 × ArH) (Found: C, 37.7; H, 5.15; N, 16.9. $C_8H_{13}Cl_2N_3O_2$ requires C, 37.81; H, 5.16; N, 16.53%).

1-(o-*Nitrophenyl)ethylenediamine Dihydrochloride* (4b).—The diamide (3b) (300 mg) was heated under reflux in a mixture of glacial acetic acid (1.5 ml) and concentrated HCl (2.2 ml) for 24 h. The solvents were removed under reduced pressure and the residue was crystallized from ethanol to give the *title compound* (223 mg, 80%), m.p. 210—225 °C (decomp.); δ ($D_2O$) 8.58—7.82 (4 H, m, 4 × ArH) (Found: C, 37.6; H, 5.1; N, 16.7. $C_8H_{13}Cl_2N_3O_2$ requires C, 37.81; H, 5.16; N, 16.53%).

1-(p-*Nitrophenyl)ethylenediaminetetra-acetic Acid* (5a).—The dihydrochloride (4a) (0.61 g, 2.4 mmol) was dissolved in

* The dihydrochloride (4a) was obtained by Meares and his co-workers (ref. 1) in only 66% yield probably because they subjected a mixture of both the isomers (3a) and (3b) to hydrolysis.



**Scheme 3**

the minimum volume of doubly distilled water and the solution was brought to pH 9 with 7M KOH and heated to 50—55 °C. Bromoacetic acid (2.9 g, 20.89 mmol), neutralized to pH 7, was then added dropwise to the mixture during 5 h. The pH of the mixture was kept between 8.5—9 during the first 9 h, then between 9—9.5 for the next 13 h, and between 10—11 for a further 2 h. After being cooled the reaction mixture was brought to pH 7 with concentrated HCl and was chromatographed on a column (2 × 28 cm) of an anion-exchange resin (Dowex 01 × 8; 100—200 mesh; $HCO_2^-$ form [12]) and using linear gradient elution with 0—10M formic acid as eluant. Fractions (20 ml) were collected. The pure tetra-acid crystallized within the tubes containing fractions 20—32 (yield 350—400 mg, 35—40%), m.p. 172—174 °C (lit.,[1] 171—174 °C). It had $R_F$ 0.24 on silica t.l.c. [freshly prepared solution of EtOH–25% $NH_4OH$ (4 : 1) as developer].

1-(p-*Aminophenyl*)*ethylenediaminetetra-acetic Acid* (6a).—To a solution of the tetra-acid (5a) (94.4 mg, 0.22 mmol) in absolute ethanol (5 ml) were added triethylamine (200 µl) and 10% Pd–charcoal (30 mg). The mixture was hydrogenated under 1 atm of hydrogen overnight at room temperature and was then filtered and evaporated. The residue was dried *in vacuo*. Its n.m.r. spectrum indicated that it was a salt containing two equivalents of triethylamine: δ ($D_2O$) 7.29—7.02 (4 H, m, 4 × ArH), 3.72 (2 H, s, $COCH_2N$), 3.65 (6 H, s, 3 × $COCH_2N$), 3.19 (total 14 H: q, 6 × $CH_3CH_2N$ and s, $CHCH_2N$), and 1.28 (18 H, t, 6 × $CH_3$).

After a quantity of KOH had been added the sample was evaporated to dryness, the residue was redissolved in $D_2O$, and the n.m.r. spectrum was taken again. The aromatic region now exhibited a well formed quartet of the AA'BB' system: δ 7.98 and 7.88 (total 2 H, d, 2 × ArH), 6.85 and 6.75 (total 2 H, d, 2 × ArH), 3.34 (2 H, s, $COCH_2N$), 3.20 (6 H, s, 3 × $COCH_2N$), and 2.80br (2 H, s, $CH_2N$); $R_F$ 0.54 on silica t.l.c. [EtOH–25% $NH_4OH$ (4 : 1) as developer].

*Methyl* 2 *Amino-4-phenylbutyrate Hydrochloride* (7).—DL-2-Amino-4-phenylbutyric acid, prepared through amidoalkylation of styrene with a methyl α-methoxyhippurate (4.1 g, 0.018 mol) according to the method of Ben-Ishai *et al.*,[13] was dissolved in dry ice-cooled methanol. Freshly distilled thionyl chloride (14.7 g, 0.12 mol) was then added during 15 min, the ice-bath was removed, and the mixture was left at room temperature for 3 d. Dry diethyl ether (400 ml) was then added and the required hydrochloride salt precipitated out (3.7 g, 88%), m.p. 153—154 °C (lit.,[13] 150—152 °C).

DL-2-*Amino-4-phenylbutyramide* (8).—A solution of the ester-hydrochloride (7) (3.7 g) in $CHCl_3$ (200 ml) was shaken with 10% aqueous $Na_2CO_3$ (200 ml). The organic layer was separated and dried ($Na_2SO_4$) and was then evaporated to dryness. The residue was transferred, in the minimum volume of methanol, into a pressure bottle, ammonia-saturated dry methanol (20% w/v; 60 ml) was added, and the mixture was left for 48 h at room temperature. The solvent was then removed and the crude residue (2.57 g) was crystallized from chloroform–hexane to afford the *amide* (8) (2.36 g, 82.8%), m.p. 89—90 °C; $\nu_{max.}$ ($CHCl_3$) 1 670 $cm^{-1}$ ($CONH_2$); δ ($CDCl_3$) 7.38—7.13 (5 H, m, ph), 5.7br (2 H, s, $CONH_2$), 3.37 (1 H, q, CH), 2.75 (2 H, t, $PhCH_2$), 2.18—1.66 (2 H, m, $CHCH_2$), and 1.56 (2 H, s, $NH_2$). The signals at δ 5.7 and 1.56 disappeared when $CD_3OD$ and TFA (trifluoroacetic acid) were added. (Found: C, 67.3; H, 7.9; N, 15.75. $C_{10}H_{14}N_2O$ requires C, 67.38; H, 7.92; N, 15.73%).

NN'-*Diacetyl*-1-*phenethylethylenediamine* (9).—The amide (8) (2.136 g, 0.012 mol) was dissolved in tetrahydrofuran (THF) (100 ml; distilled from $LiALH_4$) and the solution was placed in a tube (20 × 4 cm) to which a two-necked adaptor was connected. Through one neck was inserted a gas-dispersion tube attached to a borane generator built according to Zweifel and Brown.[14] The other neck was attached to a

condenser. A tube from its outlet led to a bubbler containing mercury and acetone.

After the system had been flushed with dry $N_2$, a slow stream of borane [generated from $NaBH_4$ (11.3 g) and boron trifluoride–diethyl ether (124 g)] was bubbled through the solution for 8 h. After the addition of borane was terminated, the mixture was heated at 60 °C overnight. The contents were then transferred to a 500 ml bottle, and methanol (25 ml) was added with caution. The solvents were removed and, to the well dried residue, acetonitrile (80 ml; distilled over $P_2O_5$), triethylamine (16.4 ml), and acetic anhydride (4.9 ml) were added. The mixture was kept overnight at room temperature and then heated under $N_2$ at 50 °C for 24 h. All solvents were evaporated off under reduced pressure, the residue was dissolved in ethyl acetate, and the solution was shaken with 10% aqueous $NaHCO_3$ (15 ml). The organic layer was dried and evaporated to dryness. The residual product was crystallized from ethyl acetate–hexane to afford the *title compound* (2.23 g, 75%), m.p. 154—155 °C; $\nu_{max.}$ ($CHCl_3$) 1 660 $cm^{-1}$ ($COCH_3$); δ ($CDCl_3$) 7.35—7.12 (5 H, m, Ph), 6.37br (1 H, s, NH), 6.26 (1 H, d, NH), 4.0 (1 H, m, CH), 3.43—3.17 (2 H, m, $CH_2N$), 2.67 (2 H, t, $PhCH_2$), 1.94 (6 H, s, 2 × $COCH_3$), and 1.76 (2 H, m, $CHCH_2$) (Found: C, 67.6; H, 8.1; N, 11.4. $C_{14}H_{20}N_2O_2$ requires C, 67.71; H, 8.12; N, 11.29%).

*NN'-Diacetyl-1-(p-nitrophenethyl)ethylenediamine* (10a).—Compound (9) (2.4 g, 9.4 mmol) was added in small portions to 95% $HNO_3$ and the mixture was stirred for 3 h at −40 °C. The cold nitration mixture was then poured onto a mixture of crushed ice and solid $Na_2CO_3$ and was extracted with ethyl acetate. The crude product (2.75 g, 99%), obtained after evaporation of the extract, had m.p. 138—145 °C; its $^1H$ n.m.r. spectrum showed a well formed $A_2B_2$ system indicating that the *ortho*-isomer (10b) did not form more than 10% of the product. Crystallization from acetone–hexane gave the pure *para-isomer* (10a) (1.13 g, 41%), m.p. 172—173 °C; $\nu_{max.}$ ($CHCl_3$) 1 660 ($NHCOCH_3$) and 1 350 $cm^{-1}$ ($NO_2$); δ ($CDCl_3$) 8.19 and 8.08, and 7.48 and 7.37 (total 4 H, $A_2B_2$ q, 4 × ArH), 6.3br (2 H, s, NH), 4.0 (1 H, m, CH), 3.3 (2 H, m, $CH_2N$), 2.8 (2 H, m, $ArCH_2$), 1.98 (6 H, s, 2 × $COCH_3$), and 1.86 (2 H, m, $CHCH_2$) (Found: C, 56.7; H, 6.4; N, 13.85. $C_{14}H_{19}N_3O_4$ requires C, 57.1; H, 6.48; N, 14.28%).

Evaporation of the crystallization mother liquid afforded a residue (1.62 g) now enriched in the *ortho*-isomer (10b) (estimated to comprise *ca.* 20% of the product by the $^1H$ n.m.r. spectrum of the aromatic region). Further crystallization of this mixture yielded more *para*-isomer (10a).

*1-(p-Nitrophenethyl)ethylenediamine Dihydrochloride* (11).—The diamide (10a) (588 mg, 2 mmol) was refluxed for 20 h in a mixture of acetic acid (3 ml) and concentrated HCl (4.5 ml). The solvents were evaporated off under reduced pressure and the residue was dissolved in absolute alcohol and was precipitated with diethyl ether. The hygroscopic *dihydrochloride* was obtained quantitatively and showed ($D_2O$) 8.24 and 8.13, and 7.54 and 7.43 (total 4 H, $A_2B_2$ q, 4 × ArH), 3.78—3.55 (1 H, m, CH), 3.43—3.35 (2 H, $CH_2N$), 3.03—2.83 (2 H, m, $ArCH_2$), and 2.26—1.96 (2 H, m, $CHCH_2$) (Found: Cl, 24.0; N, 13.85. $C_{10}H_{17}Cl_2N_3O_2 \cdot H_2O$ requires Cl, 23.61; N, 13.99%).

*1-(p-Nitrophenethyl)ethylenediaminetetra-acetic Acid* (12).—All glassware used in this experiment was washed in doubly distilled water. A solution of the dihydrochloride (11) (430 mg, 1.43 mmol) in water (2 ml) (neutralized to pH 7 with 1M KOH) in a three-necked flask in which a glass electrode was immersed was treated with bromoacetic acid (1 g, 7 mmol), which had previously been neutralized to pH 7 with 6M KOH and 1M KOH towards the end-point of the neutralization. The mixture was then brought to pH 10.5 with 6M KOH and was then heated to 50 °C (bath). The pH of the mixture was kept between 9.5—10.5 during the heating. Absorption of alkali was fast during the first hour and was complete after 4.5 h. The mixture was left at 50 °C overnight and was then cooled in an ice-bath and acidified with 6M HCl. The desired product precipitated at between pH 4—2.5. The mother liquid was decanted and the reduced tetra-acid (12) was crystallized from doubly distilled water as its *monohydrate* (467 mg, 71%), m.p. 188—189 °C; $\nu_{max.}$ (KBr) 1 660—1 710 ($CO_2^-$ and $CO_2H$) and 1 330 $cm^{-1}$ ($NO_2$); δ ($D_2O$ + $K_2CO_3$) 8.22 and 8.11, and 7.49 and 7.38 (total 4 H, $A_2B_2$ q, 4 × ArH) (Found: C, 47.15; H, 5.4; N, 9.15. $C_{18}H_{23}N_3O_{10} \cdot H_2O$ requires C, 47.06; H, 5.49; N, 9.17%).

*1-(p-Aminophenethyl)ethylenediaminetetra-acetic acid* (13).—Catalytic reduction of the nitro-compound (12) was performed under analogous conditions to those of the reduction of compound (5a). The reduced product exhibited an AA'BB' pattern centred at δ 7 in its $^1H$ n.m.r. spectrum ($D_2O$ + $K_2CO_3$).

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