N-Phthaloylation of Chloro- and Hydroxy-2-amino-acids

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Because of displacement and amide–hydroxy neighbouring group reactions, 2-amino-acids bearing 4-substituents, (halogen, hydroxy) are difficult to acylate. New synthetic techniques designed to minimise these side reactions and to allow *N*-phthaloylation of chloro- and hydroxy-amino-acids are discussed. *N*-Phthaloylation of 4-chloro- and 4-hydroxylysines in aprotic solvents has been found to occur in high yield, and preferential attack at the 6-amino-function of these 4-substituted lysines has been observed.

THE successful photochlorination of amino-acids [1] has provided an efficient route to a new range of side-chain chlorinated derivatives. Such derivatives are potentially important intermediates in bringing about the further modification of the side chain through nucleophilic displacement [2] and elimination reactions [3] at the chlorinated carbon atom.

Before many of these modifications can be achieved, the nucleophilic character of both the amino- and carboxy-groups must be masked in order to prevent intramolecular displacement of the chlorine atom. *N*-Acylation and esterification are the most obvious means for such protection.

During acylation studies on *erythro*-4-chloro-L-lysine (I), we found that *N*-benzoylation, *N*-phthaloylation, and *N*-benzyloxycarbonylation, under the usual aqueous conditions was always accompanied by complete displacement of the halogen atom. The corresponding *NN*-diacyl-*threo*-4-hydroxy-L-lysine lactones (VII)—(IX) were the only products isolated, and then only in yields of less than 15%. Similar low yields were obtained when *threo*-4-hydroxy-L-lysine lactone (VI) was acylated under these conditions. In marked contrast, the corresponding acyl derivatives of lysine itself can be obtained in yields which exceed 80%.[4] These difficul-

[1] J. Kollonitsch, A. Rosegay, and G. A. Doldouras, *J. Amer. Chem. Soc.*, 1964, **86**, 1857; J. Kollonitsch, A. N. Scott, and G. A. Doldouras, *ibid.*, 1966, **88**, 3624.

[2] Y. Fujita, J. Kollonitsch, and B. Witkop, *J. Amer. Chem. Soc.*, 1965, **87**, 2030.

[3] S. Clark, R. C. Hider, and D. I. John, unpublished results.

[4] J. von Braun, *Ber.*, 1909, **42**, 839.

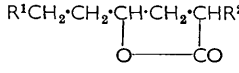
ties in acylating 4-substituted lysines can be attributed to neighbouring group reactions involving (*a*) halogen displacement in the chloro-amino-acid and/or in any *N*-acylated derivative, through intramolecular attack by carboxylate anion or amide, and (*b*) amide–hydroxy interaction (probably involving transacylation and subsequent hydrolysis) within the *N*-acyl hydroxy-amino-acids generated under the acylation conditions *via* lactone intermediates. Analogous examples have been noted of halogen displacement by carboxylate [5] and amide functions,[6] as well as labilisation of amides by hydroxy-groups.[7,8]

$R^1CH_2{\cdot}CH_2{\cdot}CHCl{\cdot}CH_2{\cdot}CHR^2{\cdot}CO_2R^3$

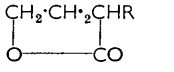
(I) $R^1 = R^2 = NH_2$, $R^3 = H$
(II) $R^1 = R^2 = NH_2$, $R^3 = Me$
(III) $R^1 = R^2 =$ phthalimido, $R^3 = Me$
(IV) $R^1 =$ phthalimido, $R^2 = NH_2$, $R^3 = Me$
(V) $R^1 =$ phthalimido, $R^2 =$ BzNH, $R^3 = Me$

$ClCH_2{\cdot}CH_2{\cdot}CHR{\cdot}CO_2Me$

(XII) $R = NH_2$
(XIII) R = phthalimido



(VI) $R^1 = R^2 = NH_2$
(VII) $R^1 = R^2 =$ BzNH
(VIII) $R^1 = R^2 =$ phthalimido
(IX) $R^1 = R^2 =$ ZNH
(X) $R^1 =$ phthalimido, $R^2 = NH_2$
(XI) $R^1 =$ phthalimido, $R^2 =$ BzNH



(XIV) $R = NH_2$
(XV) R = phthalimido

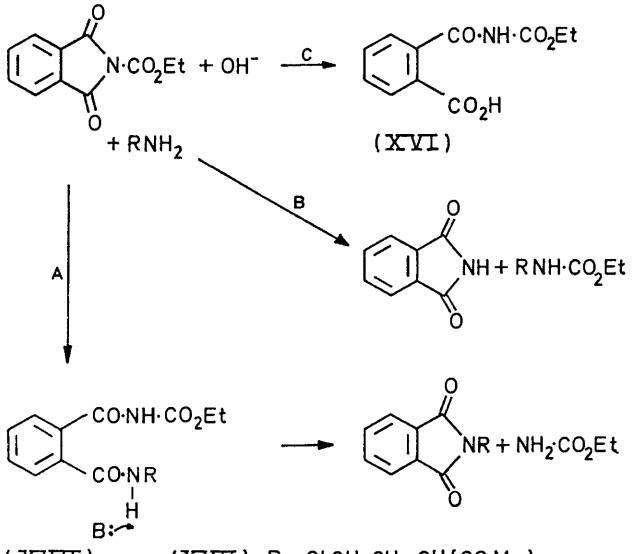
In order to overcome these interactions, it therefore became necessary to devise a new procedure for the *N*-acylation of chloro-amino-acids. In doing so, it was evident from our observations on 4-hydroxylysine lactone that we should also bear in mind its application to hydroxy-amino-acids, where a better technique was obviously desirable. The initial priority of excluding carboxylate anion participation could be easily achieved by ester formation, and probably the *N*-phthaloyl function was the most suitable *N*-acyl group for minimising amide interactions within the final *N*-acylated amino-acid ester products. Consequently, conditions for *N*-phthaloylation were sought under which ester groups were stable. A report [9] that dimethyl sulphoxide (DMSO) as solvent, in contrast to benzene, had successfully suppressed nucleophilic attack by morpholine at a lactone carbonyl group, suggested that this particular medium might also reduce any ester attack by amines during the phthaloylation. In a trial experiment, glycine ethyl ester hydrochloride, when subjected to phthaloylation with *N*-ethoxycarbonylphthalimide (NECP) (1·1 mol. equiv.) in DMSO containing triethylamine (1·1 mol. equiv.) yielded the *N*-phthaloyl derivative in 85% yield. The methyl esters of alanine, β-alanine, 2- and 4-aminobutyric acid, glutamic acid, methionine, phenylalanine, serine, threonine, and tyrosine, under the same conditions gave the corresponding *N*-phthaloyl derivatives in yields of 61—84%.

On applying this aprotic phthaloylation procedure to carboxy-protected chloro-amino-acids, we found that no halogen displacement occurred. Methyl 2-amino-4-chlorobutanoate (XII) gave the *N*-phthaloyl derivative (XIII) in 52% yield, and in the case of 4-chlorolysine methyl ester (II), use of 2·2 mol. equiv. of reagent gave both the *N*(6)-phthaloyl (IV) and *NN'*-diphthaloyl (III) derivatives in 26 and 32% yields, respectively. Confirmation that halogen displacement occurred through carboxylate anion participation was obtained when unesterified 4-chloro-L-lysine, under these conditions, gave an 80% yield of the *NN'*-diphthaloyl-4-hydroxy-L-lysine lactone (VIII). The high yield of *N*-acylated lactone obtained in this latter experiment also indicated that under aprotic conditions liberation of the hydroxy-function through lactone ring opening could be avoided, and thus any subsequent interference with the *N*-acylation reduced. This was further confirmed when an 88% yield of *N*-phthaloyl derivative was obtained from 4-hydroxyleucine lactone on treatment with NECP in DMSO, whereas extremely low yields were obtained under aqueous conditions. By application of the aprotic method we have also successfully achieved *N*-phthaloylation of the methyl esters of 3-chloroalanine, 4-chloronorvaline, 4-chloroglutamic acid, 2-amino-4-chlorobutanoic acid, and 4,4-dichloronorvaline as well as of the lactones of 4-hydroxylysine and 6-amino-4-hydroxyhexanoic acid. Yields were generally high and the use of NECP in DMSO avoids the disadvantages associated with this reagent in aqueous media, namely its relative insolubility in water and its susceptibility to attack by hydroxide ions yielding the phthalic acid derivative (XVI) (Scheme, route C). Efforts to improve the acylation through the use of pyridine, benzylamine, or *NN*-dimethylaniline as bases led, generally, to lower yields than with triethylamine; the reaction work-up was also complicated by the greater solubility of the corresponding hydrochlorides in DMSO. Phthalimide was isolated from these reactions, usually in about 10% yield, indicating a side reaction involving the ester carbonyl group of NECP (Scheme, route B).

On aprotic phthaloylation of 4-hydroxylysine lactone, just as with 4-chlorolysine methyl ester, both the *N*(6)-phthaloyl (X) and *NN'*-diphthaloyl (VIII) derivatives were obtained. In each case, the structure of the monophthaloyl compounds was established through benzoylation of their free 2-amino-functions to yield the *N*(2)-benzoyl-*N*(6)-phthaloyl derivatives (XI) and (V). No compound resulting from monophthaloylation at the 2-amino-function was isolated. In previous attempts to obtain selective *N*(6)-acylation of 4-hydroxylysine lactone, *via* copper complex formation,[10] we were unable to detect any monoacylation product; only the diacyl derivative was isolated in very low yield (<0·1%). This probably arises through hydroxy-co-ordination with the copper(II) ions, which has also been observed to occur

[5] C. A. Kingsbury, *J. Amer. Chem. Soc.*, 1965, **87**, 5409.
[6] H. L. Goering, *J. Amer. Chem. Soc.*, 1951, **73**, 4737.
[7] T. C. Bruice and T. H. Fife, *J. Amer. Chem. Soc.*, 1962, **84**, 1973.
[8] Y. Shalitin and S. A. Bernhard, *J. Amer. Chem. Soc.*, 1964, **86**, 2291.
[9] H. E. Zuagg, F. E. Chadde, and R. J. Michaels, *J. Amer. Chem. Soc.*, 1962, **84**, 4567.
[10] A. Neuberger and F. Sanger, *Biochem. J.*, 1943, **37**, 515.

with serine[11] and 2-amino-4-hydroxybutanoic acid.[12] Furthermore, on account of neighbouring group interactions within the 4-substituted lysines, it was evidently not possible to utilise the alternative high pH technique of selective acylation of these compounds.[13] The preferential *N*(6)-phthaloylation of these compounds under aprotic conditions therefore represents an additional advantage of this technique. This selectivity probably results from the enhanced nucleophilic character of the 6-amino-function compared with that of the 2-amino-function of lysine in DMSO.[14] In an attempt to obtain exclusive monophthaloylation at the 6-amino-function, 4-hydroxy-L-lysine lactone was treated with 1 mol. equiv. each of NECP and triethylamine. However, both mono- and di-phthaloylation occurred, although the yield of the *N*(6)-phthaloyl derivative isolated was significantly increased under these conditions from 20 to 30%. These relatively easily isolated *N*(6)-phthaloyl derivatives might well be of value in allowing the incorporation of 4-chloro- and 4-hydroxy-lysine into peptides.



SCHEME

During the phthaloylation of methyl 2-amino-4-chlorobutanoate (XII) a crystalline intermediate was isolated. This compound on treatment with triethylamine gave 2-phthalimido-4-chlorobutanoate and had i.r. and n.m.r. absorptions characteristic of the diamide structure (XVII). Two analogous intermediate compounds (XVIIa and b) were also obtained from reactions of benzylamine and n-butylamine, respectively, with NECP. These compounds decomposed slowly at room temperature in methylene chloride solution but the decomposition rate was substantially increased on addition of triethylamine. The reaction followed pseudo-first-order kinetics and showed a strong dependance on the alkyl substituent (Table). The isolation of this type of intermediate, which has not previously been reported, together with kinetics of their decomposition, confirms the mechanism for aminolysis of NECP proposed by Nefkens *et al.*[15] (Scheme, route A).

TABLE

Rates of cyclisation in methylene chloride at 20°

Diamide	Base	k/l mol^{-1} s^{-1}
(XVIIa)	None	$(5{\cdot}0 \pm 0{\cdot}3) \times 10^{-6}$
(XVIIa)	Et_3N	$(2{\cdot}3 \pm 0{\cdot}1) \times 10^{-2}$
(XVIIb)	Et_3N	$(4{\cdot}5 \pm 0{\cdot}5) \times 10^{-3}$

EXPERIMENTAL

N.m.r. spectra were determined at 60 MHz with tetramethylsilane as internal standard. I.r. spectra of solid samples are for Nujol mulls. M.p.s were determined with a Kofler hot-stage apparatus. Chromatography was effected on Woelm basic alumina. DMSO was redistilled (72·5 °C, 12 mmHg) from and stored over 4 Å molecular sieves.

NN'-Dibenzoyl-4-hydroxy-L-lysine Lactone (VII) (*Preparation under Aqueous Conditions*).—4-Hydroxy-L-lysine lactone dihydochloride was dissolved in 5N-sodium hydroxide (3 ml). 5N-Sodium hydroxide (2 ml), and benzoyl chloride (1·55 g, 0·011 mol) were added alternately in 20 equal portions, during 30 min to the vigorously stirred solution. The resulting mixture was then acidified (pH 2·0) with dilute hydrochloric acid, and extracted with chloroform, to yield on evaporation an oil which solidified on cooling. Recrystallisation from benzene and light petroleum gave the *lactone* (VII) (0·15 g, 10%), m.p. 196—197° (Found: C, 68·0; H, 5·65; N, 7·85. $C_{20}H_{20}N_2O_4$ requires C, 68·2; H, 5·7; N, 7·9%); $\nu_{max.}$ 3300 (NH, amide), 1765 (γ-lactone CO) and 1660 and 1535 cm^{-1} (amide I and II); τ [$(CD_3)_2SO$] 2·0—2·5 (10H, benzamido), 1·1 and 1·5 (2H, amide NH), 5·15 (1H, CO·NH·C*H*R·CO$_2$R), 5·4 (1H, C*H*·O·CO$_2$R), 6·5 (2H, CO·NH·CH_2·C), and 7·6—8·1 (4H, C·CH_2·C).

Similarly, a low yield of the acylated derivative was obtained when 4-hydroxy-L-lysine lactone was treated with benzyloxycarbonyl chloride under identical conditions.

Benzoylation of 4-Chloro-L-lysine (*under Aqueous Conditions*).—4-Chloro-L-lysine dihydrochloride (1·26 g, 0·005 mol) was treated with sodium hydroxide and benzoyl chloride as for 4-hydroxylysine. A solid product was obtained after concentration. Recrystallisation from benzene and light petroleum gave *NN'*-dibenzoyl-4-hydroxylysine lactone (VII), (0·1 g, 6%), m.p. 196—197°. *NN'*-Dibenzoyloxycarbonyl-4-hydroxylysine (IX) was isolated when 4-chlorolysine was treated with benzoyloxycarbonyl chloride under similar conditions.

NN'-Diphthaloyl-4-hydroxy-L-lysine Lactone (VIII) (under *Aqueous Conditions*).—An aqueous solution (10 ml) of 4-hydroxy-L-lysine lactone (1·08 g, 0·005 mol), and sodium hydrogen carbonate (1·59 g, 0·015 mol) was added to finely ground NECP (2·07 g, 0·01 mol), and the mixture was vigorously stirred for 10 min. After rapid filtration, the solution was acidified with dilute hydrochloric acid to pH 4·0, and extracted with methylene chloride (100 ml);

[11] J. E. Letler and J. E. Bauman, *J. Amer. Chem. Soc.*, 1970, **92**, 437.
[12] K. M. Wellman, T. G. Mecca, W. Kungall, and C. R. Hare, *J. Amer. Chem. Soc.*, 1968, **90**, 805.
[13] J. Leclerc and L. Benoiton, *Canad. J. Chem.*, 1968, **46**, 1047.
[14] M. Frieden, *J. Amer. Chem. Soc.*, 1967, **89**, 4709.
[15] G. H. Nefkens, G. I. Tesser, and R. J. Nivard, *Rec. Trav. chim.*, 1960, **79**, 688.

evaporation gave an oil (0·41 g). A solution of this product in benzene was refluxed under a Dean–Stark apparatus for 12 h, and the residue obtained after evaporation was adsorbed on alumina (25 × 2 cm), and eluted with benzene–chloroform (1 : 1). The *lactone* (VIII) (0·31 g, 15%) had m.p. 226—227° (from benzene–light petroleum, 1 : 1) α_D (1% in $CHCl_3$) −10·4° (Found: C, 65·2; H, 4·05; N, 6·95. $C_{22}H_{16}N_2O_6$ requires C, 65·4; H, 4·0; N, 6·95%); $\nu_{max.}$ 1790, 1715 (phthalimido CO), and 1770 cm^{-1} (γ-lactone CO), τ 2·2 (8H, phthalimido), 6·06 (2H, CO·N·CH_2·C), 7·8 (2H, CH_2·CH·O·COR), 5·25 (1H, CH·O·CO_2R), 7·3—7·6 (2H, C·CH_2·C), and 4·8 (CO·N·CHR·CO_2R).

N-*Phthaloylglycine Ethyl Ester* (*Preparation under Aprotic Conditions*).—Glycine ethyl ester hydrochloride (1·39 g, 0·01 mol), NECP (2·3 g, 0·011 mol), and triethylamine (1·42 ml, 0·011 mol) were dissolved in DMSO (20 ml). No effervescence occurred, but a precipitate of triethylamine hydrochloride gradually developed during 12 h at room temperature. The protected amine was isolated in two different ways:

(i) *Dilution with water.* After filtration, water (100 ml) was added to the solution, and the mixture was extracted with methylene chloride (2 × 50 ml). The extract was washed with water (2 × 100 ml) and dried ($MgSO_4$). Evaporation gave an oil, which was adsorbed on alumina (25 × 2 cm) and eluted with benzene (200 ml), followed by benzene–chloroform (1 : 1; 500 ml). The latter yielded a solid (2·4 g). Recrystallisation from ethanol gave N-*phthaloylglycine ethyl ester* (1·98 g, 85%) m.p. 102—102·5° (Found: C, 61·7; H, 4·75; N, 6·05. $C_{12}H_{11}NO_4$ requires C, 61·9; H, 4·15; N, 6·0%); $\nu_{max.}$ 1770 and 1720 (phthalimido CO), and 1735 cm^{-1} (ester CO), τ 2·1 (4H, phthalimido), 5·7 (2H, CO·N·CH_2·CO), and 5·9 and 8·9 (2H, and 3H, OEt).

(ii) *Removal of DMSO by distillation.* After filtration, the DMSO was removed by distillation (100 °C, 0·1 mmHg). The resulting paste was dissolved in chloroform, adsorbed on alumina (25 × 2 cm), and eluted with benzene (200 ml) to remove the final traces of DMSO. Elution was continued with benzene–chloroform (1 : 1; 500 ml), to yield a solid (2·2 g). Recrystallisation from ethanol gave N-phthaloylglycine ethyl ester (1·8 g, 76%), m.p. 102—102·5°. Method (i) was adopted generally. Method (ii) was used for the isolation of monoacylated lysine derivatives.

Analytical data for *N*-phthaloyl derivatives: N-*phthaloyl*-DL-*alanine methyl ester*, m.p. 70° (61%) (Found: C, 61·8; H, 4·6; N, 5·8. $C_{12}H_{11}NO_4$ requires C, 61·7; H, 4·7; N, 6·0%); N-*phthaloyl*-β-*alanine methyl ester*, m.p. 72° (68%) (Found: C, 61·9; H, 4·6; N, 5·9%); N-*phthaloyl*-DL-2-*aminobutyric acid methyl ester*, m.p. 65° (71%) (Found: C, 63·1; H, 5·5; N, 5·6. $C_{13}H_{13}NO_4$ requires C, 63·0; H, 5·3; N, 5·7%); N-*phthaloyl*-4-*aminobutyric acid methyl ester*, m.p. 87° (83%) (Found: C, 63·2; H, 5·4; N, 5·7%); N-*phthaloylglytamic acid dimethyl ester*, oil (67%) (Found: C, 59·3; N, 5·3; N, 4·5. $C_{15}H_{15}NO_6$ requires C, 59·1; H, 5·0; N, 4·6%); N-*phthaloyl*-L-*methionine methyl ester*, oil (75%), no satisfactory analysis obtained; N-*phthaloyl*-DL-*phenylalanine methyl ester*, m.p. 74—75° (79%) (Found: C, 69·4; H, 4·9; N, 4·3. $C_{18}H_{15}NO_4$ requires C, 69·8; H, 4·9; N, 4·5%); N-*phthaloyl*-DL-*serine methyl ester*, m.p. 110° (73%) (Found: C, 57·8; H, 4·4; N, 5·6. $C_{12}H_{11}NO_5$ requires C, 57·9; H, 4·4; N, 5·6%); N-*phthaloyl*-DL-*threonine methyl ester*, m.p. 95° (84%) (Found: C, 59·1; H, 4·9; N, 5·2. $C_{13}H_{13}NO_5$ requires C, 59·3; H, 4·9; N, 5·3%); N-*phthaloyl*-L-*tyrosine methyl ester*, m.p. 98—99° (78%) (Found: C, 65·9; H, 4·6; N, 3·9. $C_{18}H_{15}NO_5$ requires C, 66·4; H, 4·7; N, 4·3%); 3-*chloro*-N-*phthaloyl*-DL-*alanine methyl ester*, m.p. 69° (64%) (Found: C, 54·1; H, 3·8; Cl, 13·9; N, 5·5;. $C_{12}H_{10}ClNO_4$ requires C, 53·8; H, 3·8; Cl, 13·4; N, 5·2%); 4-*chloro*-N-*phthaloyl*-DL-2-*aminobutyric acid methyl ester*, m.p. 50° (52%) (Found: C, 55·1; H, 4·1; Cl, 12·8; N, 5·0. $C_{13}H_{12}ClNO_4$ requires C, 55·5; H, 4·3; Cl, 12·8; N, 5·0%); 4-*chloro*-N-*phthaloyl*-DL-*norvaline methyl ester*, oil (40%) (Found: C, 57·2; H, 5·1; Cl, 11·7; N, 4·5. $C_{14}H_{14}ClNO_4$ requires C, 56·9; H, 4·8; Cl, 12·0; N, 4·7%); 4-*chloro*-N-*phthaloyl*-L-*glutamic acid dimethyl ester*, oil (63%) (Found: C, 53·5; H, 4·2; Cl, 9·8; N, 3·8. $C_{15}H_{14}ClNO_6$ requires C, 53·2; H, 4·2; Cl, 10·5; N, 4·1%); 4,4-*dichloro*-N-*phthaloyl*-DL-*norvaline methyl ester*, m.p. 115° (71%) (Found: C, 44·7; H, 3·4; Cl, 20·1; N, 3·8. $C_{14}H_{13}Cl_2NO_4$ requires C, 51·0; H, 3·9; Cl, 21·5; N, 4·2%); N-*phthaloyl*-4-*hydroxy*-L-*leucine lactone*, m.p. 131° (88%) (Found: C, 63·5; H, 4·9; N, 5·4. $C_{14}H_{13}NO_4$ requires C, 64·0; H, 5·0; N, 5·4%); 6-*phthalimidohexan*-4-*olide*, m.p. 115—116° (65%) (Found: C, 64·75; H, 5·0; N, 5·45. $C_{14}H_{13}NO_4$ requires C, 64·85; H, 5·05; N, 5·40%).

Reaction of 4-*Hydroxy*-L-*lysine Lactone Dihydrochloride with NECP.*—(a) 2 *Mol. equiv.* 4-Hydroxy-L-lysine lactone dihydrochloride (2·17 g, 0·01 mol) was treated with NECP (4·6 g, 0·022 mol) as for glycine ethyl ester. Isolation of the product by method (ii) yielded an oil, which dissolved on trituration with hot chloroform (30 ml). The cooled solution yielded N(6)-*phthaloyl*-4-*hydroxy*-L-*lysine lactone hydrochloride* (X) (0·62 g, 21%), m.p. 223—225° (decomp.), $\nu_{max.}$ 1805 (γ-lactone CO), 1785, and 1720 cm^{-1} (phthalimido CO), τ [$(CD_3)_2SO$] 2·0 (4H, phthalimido), 6·2 (2H, CO·N·CH_2·C), 7·8—8·0 (4H, CH_2·CH·O·COR), 5·35 [1H, CHR(NH_3^+)·CO_2R] 5·5 (1H, CH·O·COR), and 0·8—1·0 (3H, NH_3^+). After a number of unsuccessful attempts to recrystallise the monohydrochloride, an analytical sample was prepared by using Amberlite IR-120 resin. A methanolic solution of the monohydrochloride was adsorbed on the resin. The column was washed with methanol (500 ml) and eluted with methanolic 0·5N-hydrogen chloride (200 ml) to yield the monohydrochloride (Found: C, 54·4; H, 4·9; N, 9·2. $C_{14}H_{15}ClN_2O_4$ requires C, 54·1; H, 4·9; N, 9·1%).

The mother liquor from the initial crystallisation of the monohydrochloride (X) was adsorbed on alumina (25 × 2 cm) and eluted as before to yield *NN'*-diphthaloyl-4-hydroxylysine lactone (VIII) (2·4 g, 60%), m.p. 226—227°.

(b) 1 *Mol. equiv.* 4-Hydroxy-L-lysine lactone dihydrochloride (1·08 g, 0·005 mol) was treated with NECP (1·04 g, 0·005 mol) and triethylamine (0·68 ml, 0·005 mol) as in the previous preparation. Isolation by method (ii) yielded the lactone (X) (0·47 g, 32%), m.p. 223—225° (decomp)., and the lactone (VIII) (0·56 g, 28%).

N(2)-*Benzoyl*-N(6)-*phthaloyl*-4-*hydroxy*-L-*lysine Lactone* (XI).—Finely ground *N*(6)-phthaloyl-4-hydroxy-L-lysine lactone hydrochloride (X) (0·311 g, 0·001 mol) was added to a solution (10 ml) of benzoyl chloride (0·26 ml, 0·0022 mol) in pyridine. The hydrochloride had completely dissolved after 24 h. The solution was added to an alumina column (25 × 2 ml) and eluted with benzene (50 ml) and benzene–chloroform (1 : 1; 100 ml). Recrystallisation of the product from ethanol gave the *lactone* (XI) (0·35 g, 93%) as fine needles, m.p. 225—225·5°, α_D (1% in $CHCl_3$) −10·4° (Found: C, 66·85; H, 4·8; N, 7·45. $C_{21}H_{18}N_2O_5$ requires ,C, 66·7; H, 4·8; N, 7·4%); $\nu_{max.}$ 3270 (amide NH), 1780 (γ-lactone CO), 1710 and 1770 (phthalimido CO), and 1635 and 1535 cm^{-1} (amide I and II), τ [$(CD_3)_2SO$] 2·0 (4H, phthalimido), 2·0—2·4 (5H, benzamido), 6·15 (2H, CO·N·CH_2·C), 5·5 (1H,

CH·O·COR), 5·15 (1H, CO·NH·CH·CO_2·R), 0·9—1·0 (1H, amide NH), and 7·8—8·2 (4H, C·CH_2·C).

Attempted Preparation of 4-Chloro-NN′-diphthaloyl-L-lysine.—4-Chloro-L-lysine (2·53 g, 0·01 mol) was treated with NECP (2·3 g, 0·011 mol), and triethylamine (1·44 ml, 0·011 mol) as described for glycine ethyl ester. *NN′*-Diphthaloyl-4-hydroxy-L-lysine lactone (VIII) (3·1 g, 79%) was isolated by method (i).

N(6)-*Phthaloyl- and* NN′-*Diphthaloyl-4-chloro-L-lysine Methyl Esters* (IV) *and* (III).—4-Chloro-L-lysine methyl ester dihydrochloride (I) (2·67 g, 0·01 mol) was treated with NECP (4·6 g, 0·022 mol) and triethylamine (0·29 ml, 0·022 mol) as for glycine ethyl ester. Isolation by method (ii) yielded an oil, which on trituration with hot chloroform (30 ml) completely dissolved. The cooled solution yielded a white solid. Recrystallisation from ethanol gave N(6)-*phthaloyl-4-chloro-L-lysine methyl ester hydrochloride* (IV) (0·31 g, 26%), m.p. 190—191°, α_D (1% in MeOH) 15·2° (Found: C, 50·05; H, 4·95; N, 7·8. $C_{15}H_{18}Cl_2N_2O_4$ requires C, 49·9; H, 5·0; N, 77%), $\nu_{max.}$ 2800—2500 and 2040 (NH_3^+), 1775 and 1705 (phthalimido CO), and 1760 cm^{-1} (CO_2Me), τ 2·2 (4H, phthalimido), 6·3 (3H, CO_2·CH_3), 6·1 (2H, CO·N·CH_2·C), 5·7 (1H, CHCl), 5·8 [1H, CH·(NH_3^+)·CO_2Me], and 7·8 (4H, C·CH_2·C).

The mother liquor from the crystallisation of the monophthalimido-ester (IV) was adsorbed on alumina (25 × 2 cm) and eluted with benzene (100 ml) followed by benzene–chloroform (300 ml). The latter eluant yielded a thick oil (1·9 g), which solidified on trituration with warm ethanol. Recrystallisation from benzene and light petroleum gave NN′-*diphthaloyl-4-chloro-L-lysine methyl ester* (III) (1·51 g, 32%), m.p. 130—131°, α_D (1% in MeOH) −48·7° (Found: C, 60·2; H, 4·15; N, 6·15. $C_{22}H_{19}ClN_2O_6$ requires, C, 60·1; H, 4·2; N, 6·15%); $\nu_{max.}$ 1770 and 1700 (phthalimido CO), and 1740 cm^{-1} (esters CO); τ 2·1 (4H, phthalimido), 6·0 (2H, CO·N·CH_2·C), 5·7 (1H, CHCl), 4·7 (1H, CO·N·CH·CO_2·CH), 6·15 (3H, CO_2·CH_3), and 7·4—7·8 (4H, C·CH_2·C).

N(2)-*Benzoyl*-N(6)-*phthaloyl-4-chloro-L-lysine Methyl Ester* (V).—The ester (IV) (0·356 g, 0·01 mol) was added to a solution (30 ml) of benzoyl chloride (0·26 ml, 0·02 mol) in pyridine. After 12 h at room temperature the solution was added to an alumina column (10 × 1 cm) and eluted with benzene (50 ml) followed by benzene–chloroform (1 : 1; 100 ml) to yield a solid. Recrystallisation from benzene and light petroleum gave N(2)-*benzoyl*-N(6)-*phthaloyl-4-chloro-L-lysine methyl ester* (V) (0·30 g, 61%), m.p. 159—160°, α_D (1% in MeOH) +12·0° (Found: C, 61·9; H, 5·05; N, 6·65. $C_{22}H_{21}ClN_2O_5$ requires, C, 62·0; H, 4·96; N, 6·55%), $\nu_{max.}$ 3250 (amide NH), 1647 and 1545 (amide I and II), 1725 and 1785 (phthalimido CO) and 1742 cm^{-1} (ester CO), τ 2·1 (4H, phthalimido), 2·1—2·5 (5H, benzamido), 6·2 (2H, CO·N·CH_2·C), 6·1 (1H, CHCl), 4·95 (1H, CO·NH·CH·CO_2·CH_2), 2·8 (1H, amide NH), and 7·5—8·0 (4H, C·CH_2·C).

Isolation of Diacyl Intermediate (XVII).—During the preparation of methyl 4-chloro-2-phthalimidobutanoate (XIII), the intermediate (XVII) was precipitated from the reaction mixture. Recrystallisation from chloroform gave a microcrystalline product, m.p. 114—118°, $\nu_{max.}$ 3140, 3200, and 3280 (amide NH), 1760 and 1770 (ester CO), and 1540 and 1650 cm^{-1} (amide I and II); τ 2·2 (4H, aromatic CH), 5·8 (2H, CO_2·CH_2), 6·2 (3H, CO_2·CH_3), 6·4 (2H, CH_2Cl), 7·6 (2H, CH_2·CH_2Cl), and 8·8 (3H, CO_2·CH_2·CH_3).

Triethylamine (0·01 g, 0·1 mmol) was added to a solution (6 ml) of compound (XVII) (0·371 g, 1 mmol) in DMSO and the solution was stirred overnight. Work-up by method (i) gave methyl 4-chloro-2-phthalimidobutanoate (XIII), m.p. 50° (0·26 g, 90%).

Reaction of Benzylamine with NECP.—Benzylamine (0·26 ml, 0·025 mol) was added to a solution in benzene (10 ml) of NECP (0·6 g, 0·0027 mol). A precipitate developed immediately. The solid was recrystallised from chloroform and light petroleum to give the *diamide* (XVIIa) (0·7 g, 65%), m.p. 147—148°; $\nu_{max.}$ 3000—3200 (NH, diacylamide), 1770 and 1705 (diacyl CO), 1650 and 1550 (amide I and II), and 3370 cm^{-1} (amide NH), τ 2·2 (4H, aromatic), 2·0 (1H, NH of diacylamide), 3·0 (1H, amide NH), 5·5 (2H, N·CH_2Ph), 2·5 (5H, Ph), and 8·5 and 5·8 (3H, and 2H, OEt).

Reaction of n-Butylamine and NECP.—n-Butylamine (0·27 ml, 0·0025 mol) was added to a benzene solution of NECP. The precipitate was rapidly filtered off and recrystallised from chloroform and light petroleum to give the diamide (XVIIb) (0·62 g, 53%), m.p. 132—134°; $\nu_{max.}$ 3000—3200 (NH of diacylamide), 1770 and 1725 (diacylamide CO), 1555 and 1645 (amide I and II), and 3325 cm^{-1} (amide NH), τ 2·2 (4H, aromatic), 2·0—3·0 (2H, amide NH), 6·25 (2H, CO·NH·CH_2·C), 7·5 (2H, CH_2·CH_2·NHR), 7·5 (2H, CO·NH·CH_2·C), 8·7 (4H, C·CH_2·C), and 9·1 (3H, CH_3·C).

Decomposition of the Intermediates (XVIIa and b) *in the Presence of Base.*—Methylene chloride, used as a solvent for these reactions, was stored over anhydrous potassium carbonate, in the absence of light. The reactions were followed by measurement of the u.v. absorbance at 325 nm of 0·01M-solutions of the diacylamides.

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