Polypeptides. Part V.[1] The Use of t-Butyl 2,4,5-Trichlorophenyl Carbonate in the Synthesis of *N*-t-Butoxycarbonyl Amino-acids and their 2,4,5-Trichlorophenyl Esters

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t-Butyl 2,4,5-trichlorophenyl carbonate, conveniently prepared from phosgene in two stages, reacts cleanly with amino-acids in the presence of bases to give *N*-t-butoxycarbonyl amino-acids (and 2,4,5-trichlorophenol) in excellent yield. By co-extraction of the *N*-t-butoxycarbonyl amino-acid and 2,4,5-trichlorophenol from the acidified reaction mixture, followed by treatment of the extracts with *NN'*-dicyclohexylcarbodi-imide, high yields of the corresponding *N*-t-butoxycarbonyl amino-acid 2,4,5-trichlorophenyl esters are obtained. These active esters are mostly stable, crystalline solids which may be conveniently used in peptide synthesis.

THE reaction of various active esters of benzyl and t-butyl carbonate with the sodium salts of amino-acids to yield *N*-benzyloxycarbonyl- and *N*-t-butoxycarbonyl-amino-acids was reported by Frankel, Ladkany, Gillon, and Wolman,[2] and in a recent extension of this work it was found that, by adding *NN'*-dicyclohexylcarbodi-imide to the reaction mixture, the leaving group displaced in the reaction could be reincorporated, leading to active esters of both types of *N*-acylated amino-acids.[3] We have also investigated these reactions in the past and the results have been applied routinely in the large-scale preparation of amino-acid derivatives. The results of this experience may therefore be of interest.

Our general conclusions are as follows. First, the use of active esters of benzyl carbonate in the preparation of *N*-benzyloxycarbonyl amino-acids or their active esters is usually less convenient than methods already commonly used employing benzyloxycarbonyl chloride. Secondly, certain active esters of t-butyl carbonate are often superior to other commonly used reagents (*e.g.*, t-butoxycarbonyl azide) in the preparation of *N*-t-butoxycarbonyl amino-acids in that they are more conveniently prepared and stored, and often yield products in higher yield. The 2,4,5-trichlorophenyl ester of t-butyl carbonate is much more satisfactory than the *p*-nitrophenyl ester [4] in the latter respect and is our preferred reagent. However it should be noted that the yields quoted in the literature using t-butoxycarbonyl azide are often not the best obtainable. By strict control of reaction pH, Schnabel [5] was able to obtain the *N*-butoxycarbonyl derivatives of a wide range of amino-acids in uniformly high yield using the azide. In this case the advantage in the use of t-butyl 2,4,5-trichlorophenyl carbonate lies only in the ease of preparation of the reagent as compared with the azide (it is prepared from phosgene in two stages whereas the azide requires four stages). Finally, for the large-scale preparation of active esters of t-butoxycarbonyl amino-acids, the one-stage process whereby an active ester of t-butyl carbonate is treated with an amino-acid and the leaving group is re-utilised is superior to any other known process. We again prefer to use the 2,4,5-trichlorophenyl ester of t-butyl carbonate; after this has reacted with an amino-acid, the resulting *N*-t-butoxycarbonyl amino-acid and trichlorophenol are extracted together from the reaction mixture and the mixture is treated with *NN'*-dicyclohexylcarbodi-imide to give the 2,4,5-trichlorophenyl ester of the *N*-t-butoxycarbonyl amino-acid in 70—90% overall yield.

As a guide to model conditions for the reaction between amino-acids and t-butyl 2,4,5-trichlorophenyl carbonate, the reaction between L-leucine and the active ester was first explored. With aqueous t-butanol as the solvent the effect of six bases was examined and it was found that all except pyridine promoted the formation of *N*-t-butoxycarbonyl-L-leucine; the yield of product was greatest (93, 86%) using triethylamine or sodium carbonate, rather less (74, 68%) using magnesium oxide or sodium hydroxide, and least (40%) using sodium hydrogen carbonate. The reaction involving sodium hydroxide as base was little affected by temperature, but with other bases the reaction at room temperature was rather slow and overnight reaction at 45—50° or a two-hour reaction at 60—65° gave highest yields of the product. The use of less than two equivalents of triethylamine caused a decrease in yield. Aqueous t-butanol was rather more superior to aqueous dioxan in two cases where the effect of solvent was compared.

On the basis of this experience the standard conditions adopted for the reaction between amino-acids and t-butyl 2,4,5-trichlorophenyl carbonate involved the use of triethylamine and aqueous t-butanol at 60—65° for two hours (method A) or at 45—50° for eighteen hours (method B). The former conditions were used when the reactivity of the amino-acid was expected to be equal to or greater than that of L-leucine, and the latter when the reactivity was expected to be less. About 40 amino-acids were examined (see Table), comprising mainly α-amino-acids and partially protected α-amino-acids and also *N*-methyl amino-acids and β-amino-acids. The yields of t-butoxycarbonyl amino-acids were consistently good (often 90—95%) except in the case of the hydroxy-amino-acids; serine and threonine were largely unchanged and tyrosine gave a poor yield (38%) of

[1] Part IV, J. S. Morley, *J. Chem. Soc.* (*C*), 1967, 2410.
[2] M. Frankel, D. Ladkany, C. Gillon, and Y. Wolman, *Tetrahedron Letters*, 1966, 4765.
[3] Y. Wolman, D. Ladkany, and M. Frankel, *J. Chem. Soc.* (*C*), 1967, 689.
[4] G. W. Anderson and A. C. McGregor, *J. Amer. Chem. Soc.*, 1957, **79**, 6180.
[5] E. Schnabel, *Annalen*, 1967, **702**, 188.

N-t-butoxycarbonyl-L-tyrosine (isolated as the dicyclohexylammonium salt). Arginine and its derivatives were expected to require special conditions for smooth reaction and were not examined. In the case of amino-acid derivatives containing a further acidic group (*e.g.*, N^{α}-tosyl-α,β-diaminopropionic acid) additional triethylamine was used in the reaction with success.

We expected that the leaving group in these reactions (the 2,4,5-trichlorophenoxy-ion) could be re-utilised in the formation of 2,4,5-trichlorophenyl esters of the butoxycarbonyl amino-acids. This was in fact readily realised. After the reaction of an amino-acid with t-butyl trichlorophenyl carbonate under the standard conditions described above, the reaction mixture was acidified and the t-butoxycarbonyl amino-acid and 2,4,5-trichlorophenol were co-extracted usually into ethyl acetate; addition of *NN'*-dicyclohexylcarbodi-imide to the dried extracts lead to the formation of the 2,4,5-trichlorophenyl ester of the *N*-t-butoxycarbonyl amino-acid in yields averaging 80% (Table). These active esters, the use of which in peptide synthesis was first described by Boissonnas and his co-workers,[6,16] are generally crystalline solids which are stable in alcoholic solvents and on storage. Their reactivity towards amino-components is at least equal to that of the corresponding *p*-nitrophenyl esters. *N*-t-Butoxycarbonyl- and *N*-benzyloxycarbonyl-amino-acid 2,4,5-trichlorophenyl esters are indeed our preferred building-units in peptide synthesis; they have the general advantages of all active esters [17] and after reaction with an amino-component, the removal of 2,4,5-trichlorophenol from the product can usually be effected easily and quantitatively.

EXPERIMENTAL

The general explanations given in Part III [11] of this Series apply. Organic extracts were dried with anhydrous magnesium sulphate, and evaporations were carried out under reduced pressure, usually in a rotary evaporator.

2,4,5-Trichlorophenyl Chloroformate.—A stirred solution of phosgene (100 g., ~1 mole) in toluene (375 ml.) at −10° was treated with 2,4,5-trichlorophenol (170 g., 0·86 mole) followed by dimethylaniline (105 g., 0·87 mole) at such a rate that the reaction temperature was maintained at 5—10° during the addition (20 min.). The mixture was stirred at 22—24° overnight, ice (100 g.) was then added, and the suspension was filtered from *bis-(2,4,5-trichlorophenyl) carbonate* (9·5 g., 5%), m. p. 166—167° (from ethyl acetate) (Found: C, 37·3; H, 1·1; Cl, 50·4. $C_{13}H_4Cl_6O_3$ requires C, 37·1; H, 0·95; Cl, 50·5%). The organic layer in the filtrate was separated, washed with 10% brine (75 ml.), 2N-hydrochloric acid (75 ml.), and 10% brine (3 × 75 ml.), dried, and evaporated, yielding *2,4,5-trichlorophenyl chloroformate*. The pure compound (134·5 g., 60%), b. p. 93—95°/0·4—0·6 mm., m. p. 62—63°, was obtained by distillation of the crude product *in vacuo* (partial decomp. during distillation) (Found: C, 32·5; H, 0·5; Cl, 54·3. $C_7H_2Cl_4O_2$ requires C, 32·3; H, 0·8; Cl, 54·7%).

t-Butyl 2,4,5-Trichlorophenyl Carbonate.—Crude 2,4,5-trichlorophenyl chloroformate (from 3·6 moles of trichlorophenol) in methylene chloride (400 ml.) was added over 1 hr. at 30—32° to a stirred solution of t-butanol (266 g., 3·6 mole) and quinoline (464 g., 3·6 mole) in methylene chloride (900 ml.). The mixture was stirred at 22—24° overnight, then ice–water (700 ml.) was added and the suspension was filtered from bis-(2,4,5-trichlorophenyl)carbonate (189 g., 25%). The organic layer in the filtrate was separated, washed with water (700 ml.) 2N-hydrochloric acid (2 × 700 ml.), and water (2 × 700 ml.), dried, and evaporated. Crystallisation of the residue from a mixture of methanol (900 ml.) and water (60 ml.) (charcoal) gave colourless plates of *t-butyl 2,4,5-trichlorophenyl carbonate* (610 g., 65%), m. p. 67—68·5° (Found: C, 44·2; H, 3·7; Cl, 36·3. $C_{11}H_{11}Cl_3O_3$ requires C, 44·4; H, 3·7; Cl, 35·8%). In other experiments on approximately the same scale the yields of bis(trichlorophenyl) carbonate were 20—37% and of t-butyl trichlorophenyl carbonate 55—65%.

Preparation of N-t-Butoxycarbonyl Amino-acids.—Method A. The amino-acid or amino-acid derivative (10 mmoles), t-butyl 2,4,5-trichlorophenyl carbonate (3·42 g., 11·5 mmoles), triethylamine (3·50 ml., 25 mmoles), water (8 ml.), and t-butanol (12 ml.) were stirred at 60—62° for 2 hr. After removal of most of the t-butanol *in vacuo*, water (15 ml.) was added and the mixture was acidified to pH 3 at 0° with concentrated aqueous citric acid and then extracted with ethyl acetate (1 × 15 ml., 2 × 8 ml.). The combined extracts were washed with N-sodium hydrogen carbonate (1 × 10 ml., 3 × 5 ml.) and the washings were acidified to pH 3 at 0° with aqueous citric acid. The product was collected by filtration or by extraction with ethyl acetate and recrystallised from the solvent or mixed solvents indicated in the Table. Where the product was water-soluble (BOC-Gln), the reaction mixture was acidified with acetic acid, evaporated to small volume, and extracted with n-butanol. After evaporation of the extracts the resulting residue was lyophilised and purified from the solvent indicated in the Table.

Method B. The procedure was as described in Method A but the reaction was carried out at 50—55° for 18 hr.

Method C. The procedure was as described in Method A but 4·90 ml. (35 mmoles) of triethylamine were used and the reaction was carried out at 50—55° for 18 hr.

Preparation of 2,4,5-Trichlorophenyl Esters of N-t-Butoxycarbonyl Amino-acids.—The amino-acid or its derivative (10 mmoles) was treated with t-butyl 2,4,5-trichlorophenyl carbonate (11·5 mmoles) by the method indicated in the Table. After removal of most of the t-butanol *in vacuo*, water (15 ml.) was added and the mixture was acidified to pH 3 at 0—5° with concentrated aqueous citric acid and then extracted with ethyl acetate (1 × 15 ml., 2 × 8 ml.). The combined extracts were washed with water (2 × 5 ml.), dried, and then treated at 0—5° with *NN'*-dicyclohexylcarbodi-imide (1·14 g., 10·5 mmoles). The mixture was kept overnight at 4°, filtered from dicyclohexylurea, and evaporated. The resulting residue was crystallised from the solvent indicated in the Table. Unless otherwise stated the yields given in the Table refer to that of the pure, recrystallised product. In the case of asparagine (Asn) and glutamine (Gln), the t-butoxycarbonyl amino-acids were isolated and then treated with 2,4,5-trichlorophenol (10% excess) and *NN'*-dicyclohexylcarbodi-imide (5% excess) in dry dimethylformamide at 0° for 4 hr. Many of these reactions have also been carried out on the molar scale with consistent results.

[6] E. Sandrin and R. A. Boissonnas, *Helv. Chim. Acta*, 1963, **46**, 1637.

N-t-Butoxycarbonyl amino-acids and their 2,4,5-trichlorophenyl esters

	BOC-Amino-acid [a]					BOC-Amino-acid-OCP [a]						
Amino-acid [a]	Method [b]	Yield	M. p. [c]	Solvent [d]	M. p. (lit.)	Yield	M. p.	Solvent [d]	M. p. (lit.) or formula	Analysis [e] C	H	N
Ala	A	81	81—82°	EtAc–pet	80—82° [5]	76	85—86°	Pet	81—82° [6]			
			144—145 (DCH) [f]	EtAc								
β-Ala	A	79	75—76	EtAc–pet	73—74 [7]	73	98—99	Cyclohex.	94—95 [7]			
Asp(OBu^t)	B	93	Syrup		63—64 [8]	72	76—77	Pet		48·6	5·1	2·9
			144—145 (DCH)		139·5—142 [9]				$C_{19}H_{24}Cl_3NO_6$	48·7	5·2	3·0
Asn	B	77	180—181	Water	181—182 [8] 188 [6]	84	179—180	EtOH	182 [4]	43·8	4·1	7·0
									$C_{15}H_{17}Cl_3N_2O_5$	43·8 [g]	4·15 [g]	6·8 [g]
Cys(Bzl)	B	95	65—66	Et_2O–pet	63—65 [4,5]	86	77—78	Pet		51·4	4·5	2·8
									$C_{21}H_{22}Cl_3NO_4S$	51·3	4·5	2·85
Cys(Me)	A	98	Syrup			82	92—93	Pet		43·4	4·5	3·4
									$C_{15}H_{18}Cl_3NO_4S$	43·5	4·4	3·4
Cys(Et)	A	99	Syrup			80	68—70	Pet		45·2	4·5	3·2
									$C_{16}H_{20}Cl_3NO_4S$	44·8	4·45	3·3
Z-Dap	See Exptl.	94	144—145 [h]	EtAc–pet		90	149—150	EtAc–pet		51·0	4·4	5·5
			194—195 (DCH) [h]	EtOH					$C_{22}H_{23}Cl_3N_2O_6$	51·0	4·5	5·4
Tos-Dap	C	94	126—127 [h]	EtAc–pet								
Z-Dab	B	98	Syrup			89	138—139	EtOH		52·4	4·6	5·2
									$C_{23}H_{25}Cl_3N_2O_6$	51·9	4·7	5·3
Dab(Z)	B		Syrup			64	124—125	EtOH		52·1	4·6	5·3
			108—109 (DCH) [i]	Cyclohex.					$C_{23}H_{25}Cl_3N_2O_6$	51·9	4·7	5·3
Eth	A	96	Syrup			82	83—84	Cyclohex.		45·8	4·9	3·2
									$C_{17}H_{22}Cl_3NO_4S$	46·1	5·0	3·1
Glu(OBu^t)	B	93	106—108	EtAc–pet	110—112 [5]	91	114—115	Pet	114·5—115 [10]			
Gln	B	85	Foam		116—118 [5,15]	45	161—162	Benzene		45·5	4·7	6·7
									$C_{16}H_{19}Cl_3N_2O_5$	45·1	4·5	6·6
Gly	A	88	93—94	EtAc–pet	94—95 [5]	86	107—108	Pet	106—107 [6]			
HomoCys(Bzl)	B	97	Syrup			84	111—112	Cyclohex.		52·7	4·7	2·9
									$C_{22}H_{24}Cl_3NO_4S$	52·3	4·8	2·8
Ile	A	93	Syrup		66—68 [5]	85	69—70	EtOH		49·8	5·5	3·3
			124—125 (DCH) [j]	Pet				(−20°)	$C_{17}H_{22}Cl_3NO_4$	49·7	5·4	3·4
Leu	A	93	83—84 [k]	Pet	78—81 [5]	95	Syrup			50·0	5·6	3·2
									$C_{17}H_{22}Cl_3NO_4$	49·7	5·4	3·4
Lys(Z)	A	97	Syrup			86	98—99	EtOH		53·7	5·2	5·0
			110—111 (DCH) [l]	EtAc	110—111 [5]				$C_{25}H_{29}Cl_3N_2O_6$	53·6	5·2	5·0
Z-Lys	A	80	Syrup		76—78 [5]	74	76—78	Cyclohex.		53·7	5·1	5·0
			153—154 (DCH)						$C_{25}H_{29}Cl_3N_2O_6$	53·6	5·2	5·0
Lys(Tos)	C	94	Syrup									
			144—145 (DCH)		143—144 [5]							
Tos-Lys	C	92	141—142 [m]	Benzene								
DL-3-Amino-isobut.	A	87	88—89 [n]	EtAc–pet		76	78—79	Cyclohex.		46·9	4·6	3·6
									$C_{15}H_{18}Cl_3NO_4$	47·1	4·7	3·7
Met	A	100	Syrup		47—49 [12]	81	91—91·5	Cyclohex.	89—90 [7]			
D-Met	A	100	Syrup			83	90—91	Cyclohex.	89—90 [11]			
N-Me-Ala	B	92	90—91 [p]	Cyclohex.	54—55 [5]	95	Syrup			47·4	4·9	3·5
									$C_{15}H_{18}Cl_3NO_4$	47·0	4·75	3·65
DL-4-Me-Trp	A					65	146—147	EtOH		55·3	4·6	5·5
									$C_{23}H_{23}Cl_3N_2O_4$	55·5	4·7	5·6
DL-5-Me-Trp	A					82	178—179	EtAc–pet		55·2	4·6	5·6
									$C_{23}H_{23}Cl_3N_2O_4$	55·5	4·7	5·6
DL-6-Me-Trp	A					55	154—155	EtAc–Et_2O		55·2	4·7	5·5
									$C_{23}H_{23}Cl_3N_2O_4$	55·5	4·7	5·6
Orn(Z)	A	98	Syrup		Syrup [5]	85	145—146	EtOH		53·1	5·0	5·1
									$C_{24}H_{27}Cl_3N_2O_6$	52·7	5·0	5·1
Z-Orn	A	84	99—100	Et_2O–pet	101 [13]	79	129—130	EtOH		52·9	4·9	5·2
			132—133 (DCH)	EtAc	133 [13]				$C_{24}H_{27}Cl_3N_2O_6$	52·7	5·0	5·1
Phe	A	90	83—85	Et_2O–pet	84—86 [5]	78	120—121	EtOH	122 [6]			

TABLE (*Continued*)

Amino-acid [a]	BOC-Amino-acid [a] Method [b]	Yield	M. p. [c]	Solvent [d]	M. p. (lit.)	BOC-Amino-acid-OCP [a] Yield	M. p.	Solvent [d]	M. p. (lit.) or formula	Analysis [e] C	H	N
D-Phe	A					79	120—121	EtOH		54·0	4·5	3·1
									$C_{20}H_{20}Cl_3NO_4$	54·1	4·5	3·15
Pro	A	92	135—137	EtAc–pet	136—137 [4]	90	54—55	Pet	52—54 [2]			
Trp	A	97	143—144	EtAc–pet	143—144 [7]	83	134·5—135	EtAc	132—133 [7]			
D-Trp	A	95	143—144	EtAc–pet	143—144 [7]	86	133—134	EtAc	132—133 [7]			
Tyr	B	38	212 dec. (DCH) [g]	EtOH–EtAc	136—138 [4] 96—98 [5]							
Tyr(Bzl)	B	82	106—108	EtAc–pet	109—110 [14]	79	116—117	Cyclohex.		58·8	4·8	2·4
									$C_{27}H_{26}Cl_3NO_5$	58·8	4·7	2·55 [r]
Val	A	90	Syrup		77—79 [4]	91 [t]	58—60	Pet (−20°)	$C_{16}H_{20}Cl_3NO_4$	48·5	5·1	3·5
			165—166 (DCH) [s]	EtOH–Et_2O						48·4	5·1	3·5

[a] The abbreviations for amino-acid residues and protecting groups and their mode of use are in accordance with the suggestions of the Committee on Nomenclature which reported at the Fifth European Peptide Symposium [*Peptides. Proc. Fifth European Peptides Symp.*, ed. G. T. Young, Pergamon, London, 1963, p. 261] with the modifications adopted by I.U.P.A.C. (*Inform. Bull.*, I.U.P.A.C., 1966, No. 25, p. 32). Unless otherwise stated all amino-acids are of L-configuration. OCP = 2,4,5-Trichlorophenyl ester, 3-amino-isobut. = 3-aminoisobutyric acid. [b] See Experimental section for description of the methods. [c] DCH = Dicyclohexylammonium salt. [d] Pet. = light petroleum, b. p. 60—80°, cyclohex. = cyclohexane, EtOH (−20°) = ethanol cooled to −20°, Pet (−20°) = light petroleum cooled to −20°. [e] Found figures are given first and the required figures underneath. [f] Found: C, 64·9; H, 10·3; N, 7·5. $C_8H_{15}NO_4,C_{12}H_{23}N$ requires C, 64·8; H, 10·3; N, 7·6%. [g] Calculated. [h] See Experimental section for analysis. [i] Found: C, 65·8; H, 9·1; N, 7·7. $C_{17}H_{24}N_2O_6,C_{12}H_{23}N$ requires C, 65·3; H, 8·9; N, 7·9%. [j] Found: C, 66·6; H, 10·7; N, 6·7. $C_{11}H_{21}O_4,C_{12}H_{23}N$ requires C, 67·0; H, 10·7; N, 6·8%. [k] Hemi-hydrate. [l] Found: C, 66·3; H, 9·3; N, 7·5. $C_{19}H_{28}N_2O_6,C_{12}H_{23}N$ requires C, 66·1; H, 9·2; N, 7·5%. [m] Found: C, 54·0; H, 7·1; N, 6·7. $C_{18}H_{28}N_2O_6S$ requires C, 54·0; H, 7·0; N, 7·0%). [n] Found: C, 53·0; H, 8·5; N, 6·9. $C_9H_{17}NO_4$ requires C, 53·2; H, 8·4; N, 6·9%. [p] Found: C, 53·5; H, 8·5; N, 6·8. $C_9H_{17}NO_4$ requires C, 53·2; H, 8·4; N, 6·9%. [q] Found: C, 67·8; H, 9·3; N, 5·6. $C_{14}H_{19}NO_5,C_{12}H_{23}N$ requires C, 67·5; H, 9·1; N, 6·05%. [r] Found: Cl, 19·5. Required: Cl, 19·35%. [s] Found: C, 66·3; H, 10·6; N, 6·9. $C_{22}H_{42}N_2O_4,C_{12}H_{23}N$ requires C, 66·3; H, 10·6; N, 7·0%. [t] Yield refers to the crude product.

N$^{\alpha}$-*Tosyl*-*N*$^{\beta}$-*t-butoxycarbonyl*-L-α,β-*diaminopropionic Acid*.—In addition to the preparation using t-butyl 2,4,5-trichlorophenyl carbonate described in the Table, the following method was also satisfactory. *N*$^{\alpha}$-Tosyl-L-α,β-diaminopropionic acid (25·8 g., 0·1 mole) in N-sodium hydroxide (350 ml.) was treated with t-butoxycarbonyl azide (34·9 ml., 0·25 mole) in dioxan (200 ml.), and the mixture was stirred at 45—50° for 48 hr. After removal of the dioxan *in vacuo*, the solution was extracted twice with ether (backwashing with water) and then acidified at 0—10° to pH 3—4 with concentrated aqueous citric acid. The solid, isolated by extraction with ethyl acetate, was crystallised from a mixture of ethyl acetate (100 ml.) and light petroleum (b. p. 60—80°) 200 ml., yielding prismatic needles of the required *acid* (33·1 g., 92%), m. p. (variable) 125—126 to 128—129° (efferv.) (unchanged after further recrystallisation), $[\alpha]_D^{22}$ −71·1° (*c* 4·0 in N-sodium hydroxide), R_{FD} 0·66, R_{FF} 0·69 (Found: C, 50·0; H, 6·1; N, 7·5%; neutralisation equiv., 349. $C_{15}H_{22}N_2O_6S$ requires C, 50·2; H, 6·2; N, 7·8%; neutralisation equiv., 358). N.m.r. ($CDCl_3$), τ −1·1 (1H, broad singlet, CO_2H), 2·1—2·9 (4H, AA′BB′ pattern, aromatic protons), 4·1 (1H, multiplet, N*H*·CH·CO_2H), 4·7 [1H, broad, N*H*·CO·OC$(CH_3)_3$], 6·1 (1H, multiplet, NH·C*H*·CO_2H), 7·63 (3H, singlet, CH_3 of aromatic ring), and 8·6 [9H, singlet, $(CH_3)_3$C·O]. The *dicyclohexylammonium salt*, prepared in ethanol, had m. p. 218—219° (decomp.) (Found: N, 7·8. $C_{15}H_{22}N_2O_6S,C_{12}H_{23}N$ requires N, 7·8%). In a previous experiment the acid obtained had m. p. (variable) 139—140 to 154—155°; it was otherwise identical with the products obtained as described above or in the Table, and gave a dicyclohexylammonium salt of m. p. 218—219° (decomp.).

N$^{\alpha}$-*Benzyloxycarbonyl*-*N*$^{\beta}$-*t-butoxycarbonyl*-L-α,β-*diaminopropionic Acid*.—A vigorously stirred solution of *N*$^{\alpha}$-tosyl-*N*$^{\beta}$-t-butoxycarbonyl-L-α,β-diaminopropionic acid (Table) (35·8 g., 0·1 mole) in liquid ammonia (1 l.) (prepared without special drying precautions) was cooled in a bath at −50° and treated with small portions of freshly cut sodium until a persistent (for 10 min.) blue colour was obtained (~11 g. of sodium required). Sufficient ammonium hydrogen carbonate was then added to discharge the blue colour and the solution was allowed to evaporate. The residue was dissolved in ice–water and lyophilised, yielding a white powder, R_{FA} 0·40, R_{FC} 0·31 (single ninhydrin positive spots). A solution of this product in ice–water (150 ml.) and acetone (20 ml.) was treated dropwise at 0—10° with benzyloxycarbonyl chloride. When the pH fell to 9·5—10, simultaneous addition of 4N-sodium hydroxide was commenced to maintain a pH of 10—10·5. After 75 min. the pH was steady (a total of 23 ml. of benzyloxycarbonyl chloride was required). The mixture was extracted twice with ether

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(backwashing with water), then acidified at 0—5° with concentrated aqueous citric acid and extracted thrice with ethyl acetate. The combined ethyl acetate extracts were washed with 10% aqueous citric acid and water, then dried and evaporated, yielding the *acid* (28·23 g., 83%), m. p. 137—139°; one crystallisation from a mixture of ethyl acetate (200 ml.) and light petroleum (b. p. 60—80°) (400 ml.) gave the pure product (20·02 g., 60% based on N^{α}-tosyl-N^{β}-t-butoxycarbonyl-L-α,β-diaminopropionic acid), m. p. 145—146° (efferv.), $[\alpha]_D^{24}$ −8·6° (*c* 0·99 in methanol), $[\alpha]_D^{22}$ −20·2° (*c* 4·0 in N-sodium hydroxide) R_{FA} 0·84, R_{FC} 0·62, R_{FD} 0·61, R_{FF} 0·72 (Found: C, 56·9; H, 6·8; N, 8·2. $C_{16}H_{22}N_2O_6$ requires C, 56·8; H, 6·6; N, 8·3%). The *dicyclohexylammonium salt*, prepared in ethanol–ether, had m. p. 194—195°, $[\alpha]_D^{22}$ +0·5° (*c* 4·0 in methanol) (Found: N, 8·0; $C_{16}H_{22}N_2O_6,C_{12}H_{23}N$ requires N, 8·1%). N.m.r. ($CDCl_3$), τ 1·7 (2H, broad, NH_2^+ of dicyclohexylammonium), 2·6 (5H, singlet, aromatic protons), 4·1 (1H, doublet, J = 7 c./sec., $NH{\cdot}CH{\cdot}CO_2^-$), 4·65 [1H, broad, $NH{\cdot}CO{\cdot}O(CH_3)_3$], 4·9 (2H, singlet, $PhCH_2O$), 6·0 (1H, multiplet, $NH{\cdot}CH{\cdot}CO_2^-$), 6·5 (2H, multiplet, $CH_2{\cdot}NH$), 7·05 (2H, multiplet, two CH of cyclohexyl rings), 7·9—9·05 (20H, broad multiplet, CH_2's of cyclohexyl rings), and 8·6 [9H, singlet, $(CH_3)_3C{\cdot}O$].

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