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THE SYNTHESIS OF BIPHENYLISOPROPYLOXYCARBONYL-AMINO ACID SALTS

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Abstract - A number of biphenylisopropyloxycarbonyl-blocked amino acids have been synthesized as their cyclohexylamine or dicyclohexylamine salts, and their properties described. A facile method for regenerating the Bpoc-amino acids from their salts is detailed, along with the conditions used to determine that the syntheses are free of racemization.

THE biphenylisopropyloxycarbonyl (Bpoc)[1–5] group is an extremely attractive α-amino blocking group for solid-phase peptide synthesis because its high acid lability ensures rapid and complete deprotection, even at very low acid concentrations. The opportunity to use weak trifluoroacetic acid in methylene chloride for deprotection also minimizes other solid-phase problems, such as premature loss of side-chain protection and cleavage of the peptide-resin bond. Nevertheless, literature references to the use of Bpoc amino acids have remained scarce, and a number of desirable Bpoc derivatives have not yet been synthesized. These facts reflect a drawback in the use of Bpoc protection: stored as free acids, Bpoc-amino acids undergo slow auto-catalyzed decomposition,[5] making it difficult to accumulate quantities for stock or for longer peptide syntheses.

To avoid this problem, we have found it advantageous to isolate Bpoc-amino acid derivatives as their mono- or dicyclohexylamine salts, as recommended by Sieber and Iselin.[1] Most of the salts crystallize cleanly from ether, more rapidly and in higher yield than the free acids, and show almost no decomposition after prolonged storage periods. Before coupling, the salts may be rapidly converted to the free acids by a simple, quantitative extraction procedure. And finally, as determined by the dipeptide method of Manning and Moore,[1,2] the synthesis, isolation and deprotection of Bpoc-amino acid salts may all be accomplished without racemization.

This paper describes the mono- or dicyclohexylamine salts of twenty naturally-occurring amino acids as N-α-Bpoc derivatives, with their physical properties and synthetic procedures. A method for conversion of the salts to the corresponding Bpoc-amino acid is presented, and the conditions used to evaluate their optical purity are listed.

DISCUSSION

Although some Bpoc-amino acids can be successfully stored as free acids for months at freezer temperatures,[2] samples of Bpoc-glycine, -alanine, -valine, and -phenylalanine that had been warmed repeatedly to room temperature for removal of aliquots showed extensive decomposition on thin-layer chromatography, while samples of Bpoc-O-benzyl-L-threonine and Bpoc-N$^{\epsilon}$-benzyloxycarbonyl-L-lysine

TABLE 1. N^{α}-BIPHENYLISOPROPYLOXYCARBONYL-AMINO ACID SALTS

N^{α}-Bpoc-Amino acid	Protec group	Salt	Syn.[5] Proc.	Yield	M.p.	Lit. M.p.	TLC R_f† A	TLC R_f† B	Empirical Formula	Mol. Wt.	Elemental analyses Found C	Found H	Found N	Calc C	Calc H	Calc N
Ala	—	DCHA	1	85%	163°–65°	178°–80°[9]	0·71	0·52	$C_{31}H_{44}N_2O_4$	508·7	73·23	8·75	5·38	73·19	8·72	5·51
Arg	N^{ω}-Tos	CHA	2	63%	amorph.	—	0·72	0·43	$C_{35}H_{47}N_5O_6S$	665·8	63·40	7·35	10·31	63·14	7·12	10·52
Asn	N^{ω}-Mbh	CHA	2	82%	113°–18°	—	0·68	0·55	$C_{41}H_{49}N_3O_7$	695·8	70·85	7·00	5·96	70·77	7·10	6·04
Asp	β-O-Bzl	CHA	3	69%	148°–50°		0·74	0·53	$C_{33}H_{40}N_2O_6$	560·7	70·66	7·12	4·92	70·69	7·19	5·00
Cys	s-*p*-Mob	CHA	1	47%	154°–56°	—	0·74	0·46	$C_{33}H_{42}N_2O_5S$	578·7	68·43	7·46	4·76	68·49	7·32	4·84
Glu	γ-O-Bzl	CHA	3	56%	143°–45°	147–49°[8]	0·76	0·50	$C_{34}H_{42}N_2O_6$	574·7	71·32	7·44	4·79	71·05	7·37	4·87
Gln	N^{ω}-Mbh	CHA	1	70%	amorph.	—	0·71	0·42	$C_{42}H_{51}N_3O_7$	709·9	71·02	7·43	5·64	71·06	7·24	5·92
Gly	—	DCHA	2	60%	192°–93°	192°–93°	0·70	0·16	$C_{30}H_{42}N_2O_4$	494·7	72·62	8·67	5·50	72·84	8·56	5·66
His	—	—	*	41%	189° 90°	—	—	—	$C_{22}H_{23}N_3O_4$	393·4	66·78	5·97	10·63	67·16	5·89	10·68
His	N^{im}-Tos	CHA	*	75%	138°–40°	—	0·72	0·45	$C_{35}H_{42}N_4O_6S$	646·7	65·17	6·60	8·67	65·00	6·55	8·66
Ile	-	CHA	1	62%	230°–32°	—	0·76	0·56	$C_{28}H_{40}N_2O_4$	468·6	72·02	8·66	5·91	71·76	8·60	5·98
Leu	—	CHA	1	65%	148°–50°		0·80	0·45	$C_{28}H_{40}N_2O_4$	468·6	71·51	8·60	5·84	71·76	8·60	5·98
Lys	N^{ϵ}-Z	DCHA	*	75%	amorph.	—	0·77	0·50	$C_{42}H_{57}N_3O_6$	699·9	71·88	8·40	6·01	72·07	8·21	6·00
Met	—	DCHA	1	77%	141°–43°	143°–45°[10]	0·73	0·46	$C_{33}H_{48}N_2O_4S$	568·7	69·66	8·63	4·80	69·69	8·51	4·93
Met	S-O	DCHA	1	30%	156°–58°	160°–61°[8]	0·65	0·22	$C_{33}H_{48}N_2O_5S$	584·7	67·49	8·22	4·64	67·78	8·27	4·79
Phe	—	DCHA	1	62%	115°–17°	116°–19°[9]	0·74	0·63	$C_{37}H_{48}N_2O_4$	584·8	76·06	8·25	4·70	75·99	8·27	4·79
Pro	—	CHA	2	87%	154°–58°	—	0·72	0·46	$C_{27}H_{36}N_2O_4$	452·6	71·53	8·09	6·12	71·65	8·02	6·19
Ser	O-Bzl	CHA	1	66%	156°–59°	155°–56°[8]	0·72	0·59	$C_{32}H_{40}N_2O_5$	532·7	72·33	7·58	5·20	72·15	7·57	5·26
Thr	O-Bzl	CHA	1	60%	162°–64°	—	0·77	0·63	$C_{33}H_{42}N_2O_5$	546·7	72·26	7·94	4·93	72·50	7·74	5·12
Trp	—	DCHA	2	54%	amorph.	-	0·78	0·35	$C_{39}H_{49}N_3O_4$	623·8	75·07	8·23	6·33	75·09	7·92	6·74
Tyr	O-Bzl	CHA	*	32%	158°–60°	—	0·74	0·55	$C_{38}H_{44}N_2O_5$	608·8	74·74	7·24	4·61	74·97	7·29	4·60
Tyr	O-Cl_2Bzl	CHA	*	53%	160°–64°	—	0·76	0·48	$C_{38}H_{42}N_2O_5Cl_2 \cdot H_2O$	695·7	65·52	6·64	4·04	65·61	6·38	4·03
Val	-	CHA	2	59%	165°–69°	178°–80°[9]	0·72	0·28	$C_{27}H_{38}N_2O_4$	454·6	71·34	8·54	6·22	71·33	8·43	6·16

* Synthetic procedure described in full in this paper

† TLC on 250 μ silica gel G plates. Solvent system A: BuOH/pyridine/AcOH/H_2O (30:20:6:24). Solvent system B: $CHCl_3$/MeOH (4:1)

Abbreviations: Tos = tosyl; Mbh = 4,4′-methoxybenzhydryl; Bzl = benzyl; *p*-Mob = *para*-methoxybenzyl; Z = benzyloxycarbonyl; CHA = cyclohexylamine; DCHA = dicyclohexylamine

contained more than 50% deprotected amino acid after a year's uninterrupted storage at $-15°$. By contrast, the cyclohexyl- or dicyclohexylammonium salts of all the amino acid derivatives in Table 1 show only faint traces of decomposition a year after synthesis, having undergone repeated warming and recooling, while a sample of Bpoc-DL-tryptophan DCHA salt stored at room temperature was also intact. Neutralization of the α-carboxyl group and isolation of Bpoc-amino acids as amine salts is therefore a requisite when stock quantities of the derivatives are to be prepared and stored for extended periods of time.

To recover the Bpoc-amino acids from their salts, the buffered washing method described in the Experimental section is used as presented, without altering solvent or buffer volumes for quantities up to 2 millimoles. When preparing amino acids for coupling, where completely quantitative recovery is not required, the back-washing may be limited to a single extraction of the combined aqueous phases. All of the Bpoc-amino acid salts described are readily soluble in CH_2Cl_2 (quantities of 2 millimoles in 25 ml), with the exception of Bpoc-glycine DCHA salt which does dissolve completely during the extraction procedure. Bpoc-O-Bzl-Tyr and Bpoc-O-Cl_2Bzl-Tyr, however, are less soluble in CH_2Cl_2 than their salts, and the wash volume for 2 millimoles of these two amino acids should be increased to 75 ml CH_2Cl_2 to prevent crystallization of the free acids. As the results of the titrations of acidified amino acid salts show, the Bpoc group is sufficiently stable at pH = 3·5 to allow essentially complete recovery of the free acids. The conversion of Bpoc-amino acid salts to the free acids, a rapid, quantitative and extremely simple extraction procedure, thus poses only minimal complications in the process of peptide synthesis.

While both the O-benzyl and O-2,6-dichlorobenzyl derivatives of tyrosine are presented in Table 1, the latter is probably to be preferred. First proposed by Yamashiro and Li,[14] O-2,6-dichlorobenzyltyrosine was found to be 50-fold more stable than O-benzyltyrosine, yet could be completely deprotected by HF. Using O-benzyltyrosine, this cleavage with liquid hydrogen fluoride has been found[15] to yield a mixture of tyrosine (63%) and 3-benzyltyrosine (37%). The ring-benzylated product can be cut to 13% by cleaving in 50% anisole/HF, while O-2,6-Cl_2Bzl-Tyr gives only 4% of 3-(2,6-Cl_2Bzl)-Tyr under the same conditions.[15]

Unlike any of the other amino acid derivatives, N^{α}-Bpoc-N^{ε}-Z-L-lysine DCHA salt was synthesized in DMSO using tetramethylguanidine as the base. The susceptibility of the benzyloxycarbonyl group to nucleophilic displacement is well known,[16] and N^{ε}-Z-lysine is, in fact, rapidly altered by alcoholic solutions of Triton B (N-benzyltrimethylammonium hydroxide, the base normally employed to solublize amino acids in these syntheses), with formation of a by-product which subsequently forms an unwanted Bpoc derivative. Since N^{α}-Bpoc-N^{ε}-Z-L-lysine DCHA salt could not be crystallized and was isolated as a glass without purification, the formation of all by-products must be rigorously avoided. Many other conditions for synthesizing this derivative were investigated: N^{ε}-Z-L-lysine was reacted with 2-*p*-biphenyl-2-propyl phenyl carbonate in CH_3CN with diisopropylethylamine, or in dioxane/water mixtures with NaOH; with Bpoc-azide in DMF containing Et_3N, or in neat pyridine, or in dioxane/water solution under pH stat conditions at varying pH. In almost all cases, starting material was quantitatively returned when the reaction solution was neutralized. Where there was any reaction at all, the yield was extremely small. With tetramethylguanidine and DMSO, however, the phenyl carbonate reacts smoothly

with N^{ϵ}-Z-L-lysine without producing any of the offending by-product, and affords the N^{α}-Bpoc derivative in high yield.

The synthesis, isolation and deprotection of the Bpoc-amino acid salts mentioned in Table 1 are all racemization-free, as determined by coupling the deprotected amino acids with the N-carboxyanhydrides of leucine or glutamic acid and chromatographing the diasteriomeric dipeptides produced.[12] By applying 2 micromoles of dipeptide to the amino acid analyzer, 0·05% of D-amino acid (or less, depending on the specific sample) was detectable in the presence of the L-form, and none of the Bpoc-amino acids examined gave any indication of D-amino acid content arising during the syntheses. Conditions for these determinations (see Table 2) were chosen so that, where necessary, samples containing 20 micromoles of dipeptide could be chromatographed without engulfing the L-D peak beneath the enlarged L–L, giving determinations sensitive to 0·005% or less. The procedure was also designed to use readily available buffers and to minimize the over-all time of the analyses.

TABLE 2. ELUTION CONDITIONS FOR DIASTERIOMERIC DIPEPTIDES[12]

D,L-amino-acid	L-NCA	pH	Column	Elution time (min.)			
				AA from NCA	Unreact. D,L-AA	L-D Dipep.	L-L Dipep.
ALA	Leu	4·25	58 cm.	56	36	91	110
ARG	Glu	5·26	58 cm.	21	>130	44	67
ASN	Leu	3·49	58 cm.	174	51	125	191
ASP	Leu	3·49	58 cm.	171	37	109	143
CYS (S-p-Mob)	Glu	4·25	58 cm.	26	253	154	205
GLU	Glu	3·49	58 cm.	52	52	76	104
GLN	Leu	3·49	58 cm	172	50	140	210
HIS	Glu	4·25	13 cm.	15	158	53	104
HIS	Glu	4·25	58 cm.	27	>430	205	430
ILE	Leu	4·25	58 cm.	61	57	191 allo 199	240
LEU	Leu	4·25	58 cm.	61	61	204	252
LYS	Glu	4·25	13 cm.	15	52	39	63
LYS	Glu	4·25	58 cm.	30	195	135	239
MET	Leu	4·25	58 cm.	61	51	157	180
PHE	Glu	4·25	58 cm.	26	98	72	101
PRO	Leu	4·25	58 cm.	61	38	106	139
SER	Leu	3·49	58 cm.	171	50	149	201
THR	Leu	3·49	58 cm.	173	48	150 allo* 166	189
TRP	Glu	4·25	58 cm.	26	323	215	373
TRP	Glu	4·25	13 cm.	14	83	56	92
TYR	Glu	4·25	58 cm.	27	90	68	96
VAL	Leu	4·25	58 cm.	61	42	139	157

Beckman Model 120B Amino Acid Analyzer with flow rate of 70 ml/h.[13]
Beckman AA-15 resin used in 58 cm column: Beckman PA-35 resin in 13 cm column
Buffers, prepared from Beckman concentrates, were 0·35N and contained 1 ml liquified phenol (88%)/liter

EXPERIMENTAL

M.ps were determined in a Thomas Hoover apparatus and are uncorrected. Microanalyses were performed at the Microanalysis Laboratory, Rockefeller University. Thin-layer chromatography utilized Analtech, Inc. 250-micron silica-gel G on glass plates in two solvent systems: BuOH/pyridine/AcOH/H_2O

(30:20:6:24), and $CHCl_3$/MeOH (4:1). The latter system is especially useful for separating Bpoc-amino acids from unprotected starting materials, which remain at the origin. R_f values in $CHCl_3$/MeOH are highly dependent on spot concentration and may vary from the quoted values. Spots were developed by preheating plates briefly to 80°, then spraying with ninhydrin (0·15%) in acetone containing 0·3% pyridine and 0·2% AcOH, and reheating.

L-Aspartic acid β-benzyl ester, N^{ε}-benzyloxycarbonyl-L-lysine, and L-methionine sulfoxide were purchased from Fox Chemical Co., Los Angeles; L-glutamic acid γ-benzyl ester from Schwarz/Mann, Orangeburg, New York; O-benzyl-L-serine from Cyclo Chemical, Los Angeles.

O-Benzyl-L-threonine was synthesized according to Mizoguchi *et al.*[6]; O-benzyl- and O-2,6-dichlorobenzyl-L-tyrosine according to Morley;[7] N^{G}-tosyl-L-arginine according to Ramachandran and Li;[8] s-*p*-methoxybenzyl-L-cysteine according to Frankel *et al.*[9] with physical data from Akabori *et al.*[10] N^{α}-(4,4'-methoxybenzhydryl)-L-glutamine and -L-asparagine, synthesized according to Konig and Geiger,[11] were the kind gifts of Dr. Su Sun Wang of the Hoffmann-LaRoche Co.

The synthesis of Bpoc-amino acid salts. Most of the N^{α}-Bpoc-amino acids were synthesized essentially according to Sieber and Iselin, as modified by Wang and Merrifield,[5] utilizing either their 2-*p*-biphenyl-2-propyl phenyl carbonate Method 1 or Bpoc-azide Methods 2 and 3 as indicated in Table 1. However, after addition of the blocking group and precipitation with M citric acid by their methods, the Bpoc-amino acids were extracted into ether (*ca.* 200 ml for every 0·1 mole of product) which was then washed three times with $\frac{1}{2}$ volume of sodium citrate buffer, 0·5M in citric acid, pH = 3·5, and three times with the same volume of water to remove any unreacted amino acid. The ethereal solution of Bpoc-amino acid was then dried ($MgSO_4$) and filtered, and cyclohexylamine and dicyclohexylamine were added to small aliquots. In some cases, only one salt could be induced to crystallize, but where both could be obtained, the more highly crystalline salt or the one giving a higher yield was selected. The appropriate amine was then added to the bulk of the solution until it indicated pH = 8 when spotted on wet pH paper. Crystallization of the Bpoc-amino acid salt often occurred directly from the ether solution, but could be induced by decreasing the volume *in vacuo* at 20° or by addition of petroleum ether. To recrystallize, the salts were generally dissolved in a minimal quantity of MeOH and the solution diluted with ether to turbidity.

Exceptions to this general procedure are:

N^{α}-*Bpoc*-N^{G}-*tosyl*-L-*arginine* CHA *salt*: After synthesis and precipitation, the Bpoc-amino acid was dissolved in $CHCl_3$ since it is insoluble in ether. The solution was extracted, dried, neutralized with cyclohexylamine as above, and then all solvent was removed *in vacuo* at 20°. The residue was dissolved in CH_2Cl_2 and the product was precipitated by addition of ether and isolated by filtration.

N^{α}-*Bpoc*-N^{α}-(4,4'-*methoxybenzhydryl*)-L-*asparagine* CHA *salt*: The Bpoc-amino acid was dissolved in EtOAc for washing and neutralization, and the salt was crystallized by reducing the volume *in vacuo*, then adding three times that volume of ether and scratching to induce crystallization.

N^{α}-*Bpoc*-N^{α}-(4,4'-*methoxybenzhydryl*)-L-*glutamine* CHA *salt*: The Bpoc-amino acid was worked up in EtOAc, and after neutralization with cyclohexylamine, all solvent was removed *in vacuo* and the residue triturated with petroleum ether until completely solid.

Bpoc-glycine DCHA *salt*: The Bpoc-amino acid was worked up in CH_2Cl_2, and after adding dicyclohexylamine, the solution was reduced in volume *in vacuo* and ether added until crystallization was complete.

N^{α}-*Bpoc*-L-*histidine*: Histidine (15·5 g, 0·1 mole) was dissolved in 40% Triton B in MeOH (42 g) with mild heating, the MeOH removed *in vacuo*, and the residues dissolved in DMF (50 ml) which was then evaporated *in vacuo* at 50°. The oily residue was redissolved in DMF (50 ml), Et_3N (40·4, g, 0·4 mole) was added followed by Bpoc-azide (56·2 g, 0·2 mole), and the sample was stirred at 40° for $2\frac{1}{2}$ h. The bulk of the DMF was removed *in vacuo* at 50° and the oily residue triturated with water (*ca.* 150 ml), causing crystallization of Bpoc-L-histidine as plates. EtOAc (195 ml) was added to the suspension, which was stirred briefly and separated by filtration. The solid was redissolved in DMF, the solution evaporated *in vacuo* at 50°, the residue was triturated with water, and the resulting crystalline solid isolated by filtration, washing carefully with water, acetone, and 50% ether/acetone. After drying *in vacuo*, the sample weighed 16·1 g, m.p. = 189°–190°.

N^{α}-*Bpoc*-N^{im}-*tosyl*-L-*histidine* CHA *salt*: Bpoc-L-histidine (16·0 g) was added to a solution of Na_2CO_3 (14·3 g, a 3-fold molar excess) in 50% aqueous dioxane (300 ml), and the suspension stirred while *p*-TsCl (8·5 g) was added. Stirring was maintained for 4 h at room temp, during which time solid precipitated. The mixture was ether extracted (1 × 300 ml, 4 × 100 ml), and the aqueous layer acidified to pH = 4 with 1M citric acid. The oily precipitate was dissolved in CH_2Cl_2 (400 ml), the organic solution washed with sodium citrate buffer, 0·5M in citric acid, pH = 3·5 (2 × 100 ml), and with water (4 × 100 ml), dried ($MgSO_4$)

and filtered, and cyclohexylamine (4·0 g) added. After evaporating *in vacuo* to a volume of *ca.* 30 ml, ether (100 ml) was added to the solution and the Bpoc-N^{im}-tosyl-L-histidine cyclohexylamine salt was allowed to completely crystallize at 0°. The product, isolated by filtration and dried *in vacuo*, was recrystallized from concentrated MeOH solution by adding ether. Yield = 19·6 g, m.p. = 138°–140°.

N^{α}-*Bpoc*-N^{ϵ}-*benzyloxycarbonyl*-L-*lysine* DCHA *salt*: N^{ϵ}-benzyloxycarbonyl-L-lysine (2·8 g) was suspended in DMSO (25 ml), tetramethylguanidine (3·6 g, a 3-fold molar excess) was added, followed by 2-*p*-biphenyl-2-propyl phenyl carbonate (3·3 g), and the slurry was stirred at 50° for 3 h. The solution was increased in volume to 150 ml by water and CH_2Cl_2 extracted. The extracts were evaporated to dryness *in vacuo* at 35°; the remaining oily residue dissolved in a mixture of 1M NaOH (15 ml), water (20 ml) and dioxane (10 ml); and then ether washed (4 × 10 ml) to remove unreacted phenyl carbonate. The aqueous layer was neutralized with 1M citric acid to pH = 3. The precipitated oil was ether extracted (4 × 10 ml), the combined ethereal solutions washed with citrate buffer, 0·5M in citric acid, pH = 3·5 (4 × 10 ml), then dried ($MgSO_4$) and filtered. Dicyclohexylamine was added to the solution until it became basic. The volume of the solution was reduced *in vacuo* to *ca.* 20 ml, and petrol ether added, precipitating an oil. The supernatant solution was decanted, the oil dissolved in ether and again precipitated by the addition of petrol ether. The precipitation was repeated once again. The oil was triturated with repeated small quantities of petrol ether until a gummy solid. It was then dried to a glass by evacuation under high vacuum. Yield = 5·3 g.

Bpoc-L-*methionine* DCHA *salt* was recrystallized from CH_2Cl_2 by adding ether.

N^{α}-*Bpoc*-O-*benzyl*-L-*threonine* CHA *salt*: The precipitated free acid was worked up in CH_2Cl_2. The salt crystallized from conc MeOH solution on adding petroleum ether to turbidity.

Bpoc-L-*tryptophan* DCHA *salt*: After addition of the Bpoc group and precipitation, the Bpoc-L-tryptophan was dissolved in CH_2Cl_2, washed with buffer, dried, and neutralized with dicyclohexylamine. Solvent was evaporated *in vacuo*, the residue dissolved in EtOAc, and petrol ether added to precipitate product. Supernatant solvent was decanted and the oil was triturated with petrol ether until it solidified and could be filtered.

N^{α}-*Bpoc*-O-*benzyl*-L-*tyrosine* CHA *salt*: Due to its insolubility, O-benzyl-L-tyrosine (7·1 g) was only partially dissolved in Triton B (15·0 ml) and MeOH (20 ml). The alcohol was removed *in vacuo*, DMF (15 ml) was added to the residue and the suspension evaporated under high vacuum at 50°. This procedure was repeated with THF (15 ml) and again with DMF (15 ml). DMF (30 ml) and THF (30 ml) were then added to the residue, followed by 2-*p*-biphenyl-2-propyl phenyl carbonate (9·6 g, a 10% molar excess), and the suspension stirred at 50° for 5 h, during which time most solids dissolved. Remaining solids were removed by filtration and the filtrate distributed between water and ether (250 ml each). The aqueous layer was washed with ether (4 × 100 ml), cooled on ice and acidified with 1M citric acid to pH = 4. Ether (250 ml) was added, and after stirring, the suspension was filtered to remove undissolved solids. The organic layer was washed with citrate buffer, 0·5M in citric acid, pH = 3·5 (3 × 100 ml), then with water (3 × 100 ml), dried ($MgSO_4$) and filtered. Cyclohexylamine was added until the solution produced a basic reaction when spotted on wet pH paper, and then all solvent was removed *in vacuo*. The residue was dissolved in MeOH, the solution concentrated and ether added to the point of turbidity. The product crystallized on standing cold, and was isolated by filtration. Yield = 5·1 g. (recrystallization from MeOH and ether).

N^{α}-*Bpoc*-O-2,6-*dichlorobenzyl*-L-*tyrosine* CHA *salt*: O-2,6-dichlorobenzyl-L-tyrosine was solublized normally by Triton B, and the reaction with 2-*p*-biphenyl-2-propyl phenyl carbonate was run at 50° for 3 h. After extracting the solution with ether and neutralizing with citric acid, the resulting suspension of solids was shaken with EtOAc (250 ml) and undissolved solids removed by filtration. The organic layer was washed with citrate buffer, 1M in citric acid, pH = 3·5 (3 × 100 ml), then with water (3 × 100 ml), dried ($MgSO_4$) and filtered. Cyclohexylamine was added until the solution produced a basic reaction when spotted on wet pH paper. The solution was concentrated *in vacuo* to *ca.* 150 ml and the product allowed to crystallize at 4°, Yield = 11·0 g. Recrystallization from MeOH with a small amount of CH_2Cl_2 added to aid the initial solution, or from CH_2Cl_2/ether.

Recovery of Bpoc-amino acids from their CHA *or* DCHA *salts.* (i) Bpoc-L-alanine DCHA salt (0·02032 g, $4{\cdot}00 \times 10^{-5}$ moles) was dissolved in 20 ml of CH_2Cl_2 and the solution washed with sodium citrate buffer, 0·5M in citric acid, pH = 3·5 (3 × 10 ml), which was back-washed with CH_2Cl_2. The combined CH_2Cl_2 solutions were then washed with 3 × 10 ml of water, which was back-washed with CH_2Cl_2, and the combined organic solutions were dried ($MgSO_4$) and filtered. This solution of Bpoc-amino acid could then be used directly in peptide synthesis.

To estimate the recovery, the solution was evaporated to dryness at 20°. The residue was taken up in

DMF (15 ml), 2 drops of bromothymol blue (1% in EtOH) were added, and the solution was titrated with 0·0100N NaOH. The end point was observed at 3·96 ml added NaOH (99%).

(ii) Bpoc-L-leucine CHA salt (0·01872 g, $4·00 \times 10^{-5}$ moles) required 4·14 ml 0·0100N NaOH (103%).

(iii) N^{a}-Bpoc-O-Bzl-l-serine CHA salt (0·02128 g., $4·00 \times 10^{-5}$ moles) required 4·28 ml 0·0100N NaOH (107%).

(iv) N^{a}-Bpoc-N^{ϵ}-Z-L-lysine DCHA salt (0·02806 g, $4·00 \times 10^{-5}$ moles) required 4·00 ml 0·0100N NaOH (100%).

(v) A blank titration required 0·01 ml 0·0100N NaOH to produce an end point (2·5%).

The Determination of the Optical Purity of Bpoc-Amino acid Salts. A sample of each amino acid (20 micromoles) was deprotected by dissolving in 10% TFA in CH_2Cl_2 for 15 min, or if removal of side-chain protection was required, by dissolving in liquid HF for $\frac{1}{2}$ h at room temp. The residue was dissolved in 2 ml of sodium borate buffer, 0·4M in boric acid, pH = 10·2, and derivatized with 24 micromoles of the N-carboxyanhydride of L-leucine or L-glutamic acid according to the procedure of Manning and Moore.[12]

The resulting solution of diasteriomeric dipeptides was diluted to a volume of 10 ml with water, and a 1 ml aliquot was injected onto a Beckman Model # 120B amino acid analyzer under conditions of column length and pH of eluting buffer as detailed in Table 2

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