New Agents for *t*-Butyloxycarbonylation and *p*-Methoxybenzyloxycarbonylation of Amino Acids

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A new method for the preparation of *t*-butyloxycarbonyl amino acids and *p*-methoxybenzyloxycarbonyl amino acids has been devised. The former was obtained in satisfactory yields from amino acids and *t*-butyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate prepared from *t*-butyl alcohol and (4,6-dimethylpyrimid-2-ylthio)corbonyl chloride in dry pyridine, and the latter was also obtained from amino acids and *p*-methoxybenzyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate from *p*-methoxybenzyl alcohol and (*S*-4,6-dimethylpyrimid-2-ylthio)carbonyl chloride. These *t*-butyloxycarbonylation and *p*-methoxybenzyloxycarbonylation of amino acids are more convenient than the usual methods.

In recent years the *t*-butyloxycarbonyl (Boc) group[1] has found wide use for protecting the amino moiety of amino acids in peptide synthesis. Its merits are high selectivity and ease of removal by acidolysis (HCl in an inert solvent, or CF_3COOH), and at the same time resistance to hydrogenolysis, alkaline spilitting, hydrazinolysis, as well as absence of appreciable racemization.

The most widely employed reagents for the preparation of Boc-amino acids are Boc-azide,[2] Boc-Cl,[3] and *t*-butyl 2,4,5-trichlorophenyl carbonate,[4] which react with both the free amino acids and their esters in the presence of bases. But these methods suffer from various disadvantages, the strict control of reaction pH required in the case of Boc-azide, unstable property of Boc-Cl, and elaborate purification from 2,4,5-trichlorophenol of Boc-amino acids. A new water soluble agent (4-dimethylamino-1-*t*-butyloxycarbonylpyridinium chloride) for *t*-butyloxycarbonylation of amines was also reported.[5]

We wish to report a new, rapid and tidy method for the preparation of Boc-amino acids. Aminolysis of *t*-butyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate by the amino moiety of amino acids yields the corresponding Boc-amino acids in good yield; the 2-mercapto-4,6-dimethylpyrimidine liberated being acid soluble is easily separable from the Boc-amino acid, and the aminolysis is carried out at room temperature for 2—24 hr.

The *p*-methoxybenzyloxycarbonyl (Z(OMe))[6] group also has been widely used as a blocking substituent of the amino moiety of amino acids in peptide synthesis.[7] This group allows an easier removal by trifluoroacetic acid. The Z(OMe) group can be introduced with the use of *p*-methoxybenzyl 2,4,5-trichlorophenyl carbonate[8] or *p*-methoxybenzyloxycarbonyl azide.[9] These methods have been used in many cases, but the yields are not satisfactory. In the present investigation, *p*-methoxybenzyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate was very useful for the preparation of Z(OMe)-amino acids, too.

Results and Discussion

t-Butyl 4,6-Dimethylpyrimidyl-2-thiol Carbonate (IV) and p-Methoxybenzyl 4,6-Dimethylpyrimidyl-2-thiol Carbonate (V). 2-Mercapto-4,6-dimethylpyrimidine hydrochloride (I.HCl) was prepared from acetylacetone, thiourea, and concentrated hydrochloric acid in ethanol in the usual method.[10]

As was clear from the reaction schema shown below, the preparation of sodium 4,6-dimethylpyridine-2-thiolate (II) was described in experimental section in detail.

(4,6-Dimethylpyrimid-2-ylthio)carbonyl chloride(III) was prepared by adding a phosgene to the suspension of II in an inert solvent such as petroleum ether and toluene under cooling (−5——10°C). The resulting product was stable in a water free state at room temperature and solid below about 5°C, but decomposed at a temperature of about 100°C and hence could not be purified by distillation. In the sequent reaction, therefore, III was ordinarily used in a crude form. IV could be prepared readily from *t*-butyl alcohol and III in pyridine at room temperature and V also prepared from *p*-methoxybenzyl alcohol and III in the presence of pyridine in dry ether at room temperature. These reagents (IV and V) were considerably stable, pale yellow crystals and could be stored in a dark and cool place for six months without decomposition.

Boc-Amino Acids and Z(OMe)-Amino Acids. As a guide to model condition for the reaction between

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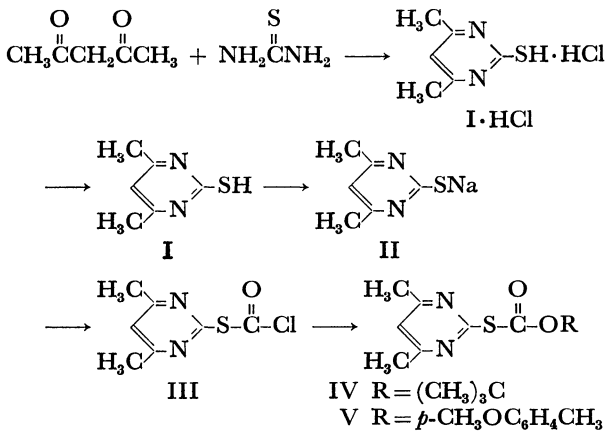

Fig. 1

amino acids and IV, the reaction between leucine and the active ester was first explored.

The effects of bases, solvents, reaction time, and reaction temperature were examined and the following results were found.

With aqueous dioxane as the solvent, the effect of five bases was examined and it was found that all except pyridine promoted the formation of Boc-leucine: the yield of product was greatest using triethylamine (quantitatively), sodium hydroxide (88%) or sodium carbonate (85%) and least using sodium hydrogen

TABLE 1. EFFECT OF BASES IN THE PREPARATION OF BOC-LEUCINE (SHOWN BY % YIELD)[a]

AA/Base[b] (molar ratio)	Base				
	NaOH	$N(C_2H_5)_3$	Na_2CO_3	$NaHCO_3$	C_5H_5N
1.0/1.1	88	83	85	21	trace
1.0/1.5	96	100	—	—	—

a) Reaction condition: Solvent=50% aqueous dioxane; Reaction temperature=30—35°C; Reaction time=10hr
b) AA=amino acid

TABLE 2. EFFECT OF REACTION TIME AND SOLVENTS IN THE PREPARATION OF BOC-LEUCINE (SHOWN BY % YIELD)[a]

Base (AA/Base)	Solvent (50% aqueous)	Reaction time (hr)		
		2	5	10
NaOH	Dioxane	80	84	88
(1.0/1.1)	*t*-Butyl alcohol	74	86	86
	DMF[b]	—	86	91
$N(C_2H_5)_3$	Dioxane	—	90	100
(1.0/1.5)	*t*-Butyl alcohol	—	87	97
	DMF	—	99	100

a) Reaction temperature=30—35°C.
b) DMF=dimethylformamide.

TABLE 3. EFFECT OF REACTION TEMPERATURE IN THE PREPARATION OF BOC-LEUCINE (SHOWN BY %YIELD)[a]

Temperature (°C)	15—20	30—35	40—45
	79	99	92[b]

a) Reaction condition: Solvent=50% aqueous DMF; Base=triethylamine; AA/Base=1.0/1.5; Reaction time=5 hr.
b) Reaction time=2.5 hr.

TABLE 4. SYNTHESIS OF BOC-AMINO ACID DERIVATIVES

Amino acid derivative[a]	Yield(%)	Mp (°C) (Solvent)[e]	$[\alpha]_D^{20}(c, 1)$
Ala	99	81—82 (E-P)	−24.0 (AcOH)
Arg(NO_2)	100	123—125 (M-E-PA)	−6.8 (DMF)
Asp	72	116—117 (EA-PA)	−6.4 (MeOH)
Asn	82	175—177 (W)	−7.2 (DMF)
$(Cys)_2$	100	145—146 (EA)	−114.2 (AcOH)
Cys(S-Bzl)	95	66—68 (E-PA)	−43.7 (AcOH)
Glu	100	101—104 (EA-PA)	−15.6 (MeOH)
Gln	61	118—119 (EA-PA)	−2.8 (EtHH)
Gly	92	87—89 (EA-PA)	
His(N^{im}-Bzl)	98	180—182 (Py-EA-PA)	+24.8 (MeOH)
Hyp[b]	81	168—172 (M-E)	−27.9 (AcOH)
Ileu	100	68—71 (PA)	+2.5 (AcOH)
Leu[c]	97	69—74 (Et-W)	−25.0 (AcOH)
Lys[b]	94	132—138 (M-E)	−5.0 (AcOH)
Met[b]	100	134—138 (E-PA)	+15.2 (AcOH)
Phe	92	86—88 (E-PA)	−3.9 (AcOH)
Pro	96	136—137 (EA-PA)	−61.1 (AcOH)
Sar	87	89—90 (EA-PA)	
Ser[d]	85	90—93 (E-PA)	−4.9 (AcOH)
Ser (O-Bzl)	94	55—58 (E-PA)	+22.5 (EtOH)
Thr	90	74—77 (E-PA)	−7.3 (AcOH)
Trp	100	137—139 (EA-PA)	−22.9 (AcOH)
Tyr[b]	99	205—208 (M-E)	+3.8 (AcOH)
Tyr(O-Bzl)	95	109—111 (E-PA)	+16.6 (MeOH)
Val	98	77—79	−6.3 (AcOH)

a) All products were characterized by elementary analyses.
b) Dicyclohexylammonium salt.
c) Monohydrate.
d) Hemihydrate.
e) Abbreviations: E=ether; PA=petroleum ether; M, MeOH=methanol; EA=ethyl acetate; W=water; Py=pyridine; Et, EtOH=ethanol; AcOH=acetic acid; DMF=dimethylformamide.

Table 5. Synthesis of Z(OMe)-amino acid derivatives

Amino acid derivative[a)]	Yield (%)	Mp (°C) (Solvent)[c)]	$[\alpha]_D^{20}$(*c*, 1)
Ala	95	80—82 (EA–PA)	−12.2 (AcOH)
Arg(NO_2)	97	149—150 (M–E–PA)	−5.3 (MeOH)
Asp	82	125—127 (EA–PA)	+9.7 (AcOH)
Asn	80	160—163 (W)	−5.5 (MeOH)
Cys(S–Bzl)	94	90—92 (EA–PA)	−52.6 (MeOH)
Glu	100	114—116 (EA–PA)	−7.1 (AcOH)
Gln	90	146—147 (W)	−10.0 (MeOH)
Gly	100	96—98 (EA–PA)	
His(N^{im}–Bzl)	97	203—206 (W)	+6.8 (AcOH)
Ileu	95	63—65 (E–PA)	+8.1 (EtOH)
Leu[b)]	99	156—160 (M–E)	−6.9 (MeOH)
Met	99	74—75 (EA–PA)	−23.8 (MeOH)
Phe	93	90—91 (E–PA)	+6.4 (AcOH)
Pro[b)]	98	145—148 (M–PA)	−23.7 (MeOH)
Ser	82	98—100 (EA–PA)	+6.7 (AcOH)
Ser(O–Bzl)	80	72—74 (E–PA)	+15.4 (EtOH)
Thr[b)]	96	175—177.5 (M–E)	+7.7 (MeOH)
Trp	98	114—116 (EA–PA)	+3.5 (AcOH)
Tyr[b)]	94	122—124 (M–E)	+26.7 (MeOH)
Val	99	62—64 (E–PA)	+9.8 (DMF)

a) All products were characterized by elementary analyses.
b) Dicyclohexylammonium salt.
c) Abbreviations: The same as in Table 4.

carbonate (21%) shown in Table 1. Aqueous dioxane and dimethylformamide were rather more superior to other aqueous solvents, and a 10 hr reaction at 30—35°C gave the highest yield of the product shown in Table 2. As shown in Table 3, a five hours reaction gave 79% yield at a temperature of 15—20°C, 99% yield at 30—35°C.

On the bases of these experiences, the standard condition adopted for the reaction between amino acids and IV involved the use of triethylamine and aqueous dioxane or dimethylformamide at 30—35°C or room temperature for overnight. About twenty amino acids were examined and the yields of Boc-amino acids were consistently good as shown in Table 4.

Z(OMe)-amino acids were prepared from V and free amino acids by the same method as described for Boc-amino acids. The products obtained were shown in Table 5.

Experimental

Melting points were all determined in a Yanagimoto electric micromelting point apparatus and are uncorrected, and optical rotations were measured with a JASCO automatic polarimeter DIP-SL. All amino acids were L-configuration in this report.

4,6-Dimethylpyrimidyl-2-thiol Chloroformate (III). A solution of 128 g (3.2 mol) of sodium hydroxide in 600 ml of water was mixed with 420 g (3.0 mol) of I, and the resulting mixture was heated to completely dissolve the I and then allowed to cool. The resulting solution was charged into 15 l of acetone, whereby a sodium salt of I was precipitated. The precipitated sodium salt was recovered by filtration and then dried at 120°C for 24 hr to obtain 462 g (95%). Subsequently, 324 g (2.0 mol) of the above-mentioned sodium salt (II) was added as it was, *i.e.* in the form of solid to 1185 g of petroleum ether, and to the resulting suspension was added to 297 g of phosgene (3.0 mol) at −15—−10°C, and the mixture was reacted with stirring at room temperature for 4 hr. After completion of the reaction, excess phosgene was removed by evaporation at 40—50°C while injecting nitrogene into the reaction liquid. Thereafter, the precipitate formed was filtered and then the petroleum ether was removed by evaporation *in vacuo*, to obtain about 365 g of III in crude yield of 90%.

t-*Butyl* S-*4,6-Dimethylpyrimidin-2-ylthiolcarbonate (IV).* Into a solution of 267 g (3.6 mol) of *t*-butyl alcohol in 752 g of pyridine was dropped under stirring and cooling to −5—0°C 364 g (1.9 mol) of the crude III, and the resulting mixture was reacted at 20—25°C for 3 hr. After completion of the reaction, deposited pyridine hydrochloride was separated by filtration, and the filtrate was charged with 2000 ml of water and then extracted 3 times each with 800 ml of petroleum ether. The petoleum ether layer was sufficiently washed with a cold 1N aqueous hydrochloric acid solution, washed twice with a saturated aqueous sodium chloride solution and dried over anhydrous sodium sulfate, and then the petroleum ether was evaporated under reduced pressure, wherby the crystal was obtained. The crystal was washed with a small amount of cold *n*-heptane and then dried to obtain 274—319 g (60—70%) of IV. Mp 50—51°C.

Found: C, 54.88; H, 6.64; N, 11.57; S, 13.29%. Calcd for $C_{11}H_{16}O_2N_2S$: C, 54.98; H, 6.71; N, 11.66; S, 13.34%.

p-*Methoxybenzyl* S-*4,6-Dimethylpyrimidin-2-ylthiolcarbonate (V).* 244 g (2.2 mol) of *p*-methoxybenzyl alcohol and 167 g (2.1 mol) of pyridine were dissolved in 2600 ml of ether. Into the resuting solution was dropped under stirring and cooling to −5—0°C 324 g of the crude III obtained in the above-mentioned procedure, and the resulting mixture was reacted at room temperature for 3 hr. After completion of the reaction, the reaction liquid was once with cold water, twice with a 1N aqueous citric acid solution or a 1N aqueous hydrochloric acid solution cooled to 0°C and a saturated aqueous sodium chloride solution, and dried over anhydrous sodium sulfate and then the ether was evaporated *in vacuo* to obtain 340—388 g (70—80%) of the pale yellow crystal of V. Mp 58—60°C (recrystallized from ether–petroleum ether).

Found: C, 58.98; H, 5.31; N, 9.22; S, 10.48%. Calcd for $C_{15}H_{16}O_3N_2S$: C, 59.19; H, 5.30; N, 9.20; S, 10.54%.

t-*Butyloxycarbonyl Amino Acids and* p-*Methoxybenzyloxycarbonyl Amino Acids (General Method).* 0.10 mol of amino acid

and 0.15 mol (21 ml) of triethylamine were added to 55 ml of water. To the resulting mixture of 0.11 mol of IV or V in 55 ml of dioxane or dimethylformamide, and then the mixture was reacted under stirring at room temperature for several hours. (2—24 hr) After completion of the reaction 150 ml of water was added to the reaction mixture, and unreacted carbonate was extracted twice with 200 ml of ethyl acetate. Subsequently, the aqueous layer was cooled to 0°C and adjusted to pH 2 by addition of a 5N aqueous hydrochloric acid cooled to 0°C, and then extracted once with 150 ml of ethyl acetate and twice with 80 ml of ethyl acetate. Thereafter, the ethyl acetate layers were united together, washed thrice with 100 ml of a 5% aqueous hydrochloric acid cooled to 0°C, and twice with 100 ml of a saturated aqueous sodium chloride solution and dried over anhydrous sodium sulfate (if necessary, using active carbon for decolorization.) and then the ethyl acetate was evaporated *in vacuo*, the resulting solid was recrystallized from the solvent indicated in Tables 4 and 5.

The preparations of Boc-aspagine, Boc-N^{im}-benzylhistidine, Boc-serine, and Boc-threonine were different from the general method in the following parts.

Boc-asparagine. In this procedure, an aqueous layer adjusted to pH 2 was allowed to stand overnight in a cold place (0—5°C) instead of extraction from ethyl acetate, whereby the crystal was deposited. The crystal was recovered by filtration and then washed with cold water to obtain 19.0 g (81.9%) of Boc-asparagine. Mp 175—177°C $[\alpha]_D^{20}=-7.2°$ (*c* 1, DMF).

Boc-N^{im}*-benzylhistidine.* This compound was similarly prepared in Boc-asparagine except the following part. In this procedure, pH of an aqueous layer was adjusted to pH 5.4 by acetic acid instead of adjusting pH 2 by a cooled 5N aqueous hydrochloric acid. Yield 33.9 g (98.3%). Mp 180—182°C. $[\alpha]_D^{20}=+24.8°$ (*c* 1, methanol).

Boc-serine. After the aqueous layer was adjusted to pH 2 by addition of a cooled 5N aqueous hydrochloric acid, sodium chloride was added to this mixture. Thereafter, the ethyl acetate layer were united together, washed thrice with 100 ml of a cooled 5% aqueous hydrochloric acid saturated by sodium chloride and dried over anhydrous sodium sulfate and decolorized with active carbon and evaporated *in vacuo* to obtain 17.3 g (84.6%). Mp 90—93°C. $[\alpha]_D^{20}=-4.9°$ (*c* 1, acetic acid).

Boc-threonine was similarly prepared in above described procedure.