Convenient Synthesis of *N*-Protected Amino Acid Amides[1)]

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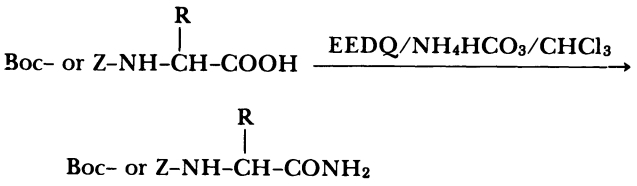
Synopsis. Boc- or Z-amino acids were conveniently amidated at room temperature by a one-pot procedure employing ammonium hydrogencarbonate and *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline in yields of 81—96%. The benzyl ester-type protector for the side chains of aspartic acid and glutamic acid was not affected by the procedure.

Amino acid amides, an important starting material in peptide synthesis, are usually prepared by ammonolysis of amino acid esters, or by coupling of *N*-protected amino acids with ammonia.[2)] The former procedure is somehow inconvenient, since ammonia, a reagent often troublesome for handling, is required and the reaction is rather slow. In the latter, care must be paid to prevent possible racemization during activation of the amino acids, especially when mixed anhydride method[3)] was employed for the purpose.

We report here that by addition of ammonium hydrogencarbonate and *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ),[4,5)] a mild mixed anhydride-type coupling reagent stable to amines,[5)] Boc- or Z-amino acids in chloroform are conveniently converted to the corresponding amides at room temperature.



The reaction proceeded smoothy with gentle evolution of carbon dioxide, although ammonium hydrogencarbonate is scarcely soluble in the solvent. The amides were readily isolated in satisfactory yields after removal of the excess ammonium hydrogencarbonate by washing the reaction mixture with water. By this procedure, the benzyl ester-type protecting group for the β- or γ-carboxylic acid part of aspartic acid or glutamic acid, respectively, was not affected. No racemization occurred during the amidation when an *N*-protected amino acid with a urethane-type protector was employed as the starting compound. However, this method cannot be applied to the preparation of a peptide amide consisting of an optically active amino acid at the C-terminal, since in a test preparation, Boc-L-leucyl-L-leucine gave a considerable amount of the diastereomeric product (14%), Boc-L-leucyl-D-leucinamide. The data are summarized in Table 1.

Experimental

Melting points were uncorrected. Merck precoated silicagel plates were used for TLC analysis with a solvent system of chloroform–MeOH (19:3). For HPLC analysis, a LiChrosorb RP-18 column (4×250 mm) was employed with a solvent system of MeOH–water (1:1) at a flow rate of 1.83 ml min^{-1}. The column effluent was monitored at 220 nm, and the peak area was recorded by a Shimadzu C-R3A integrator.

Preparation of Boc-Glu(OBzl)-NH$_2$ (General Procedure).

Table 1. Preparation of *N*-Protected Amino Acid Amides

Product[a)]	Yield/%	Mp θ_m/°C	Solvent for recrystallization	$[\alpha]_D^{26}$/° (*c* 1, EtOH)
Boc-Ala-NH$_2$	89	124—125	AcOEt–hexane	−2.7
Boc-Val-NH$_2$[b)]	88	155—157	AcOEt–hexane	<±0.05
Boc-Leu-NH$_2$	82	144—146	AcOEt–hexane	−11.4
Boc-Ile-NH$_2$	88	166—167	AcOEt–hexane	−3.0
Boc-Ser(Bzl)-NH$_2$	81	101—102	AcOEt–hexane	+27.8
Boc-Thr(Bzl)-NH$_2$	91	135—136	AcOEt–hexane	+38.6
Boc-Lys(Z)-NH$_2$[c)]	89	137—142	AcOEt–hexane	+1.7
Boc-Asp(OBzl)-NH$_2$	89	157—160	AcOEt	−2.6
Boc-Glu(OBzl)-NH$_2$	90	120—122	AcOEt–hexane	+4.0
Boc-Phe-NH$_2$	91	142—149	AcOEt–hexane	+16.7
Z-Phe-NH$_2$[d)]	94	161—162	AcOEt–hexane	−2.6
Boc-Tyr(Bzl)-NH$_2$	96	170—171	AcOEt	+16.0
Boc-Trp-NH$_2$	85	133—136	AcOEt–hexane	+7.7
Z-Trp-NH$_2$[e)]	96	188—189	AcOEt	−4.0
Boc-Pro-NH$_2$	81	104—106	Ether–hexane	−43.4

a) Each compound gave a single spot on TLC. Satisfactory data of microanalysis was obtained for each compound within an error range of ±0.3%. b) Mp 156—157 °C in lit;[6)] mp 157—158 °C in lit.[7)] c) Mp 142 °C, $[\alpha]_D^{25}$ +1.6° (*c* 1, MeOH) in lit.[8)] d) Mp 161—162 °C in lit;[9)] mp 164—165 °C, $[\alpha]_D^{20}$ −4.9° (*c* 1.5, 80% AcOH) in lit;[10)] mp 163—164 °C, $[\alpha]_D^{25}$ −6.3° (*c* 2, DMF) in lit.[11)] e) Mp 187—188 °C in lit.[12)]

Boc-Glu(OBzl)-OH (0.675 g, 2 mmol), EEDQ (0.544 g, 2.2 mmol), and ammonium hydrogencarbonate (0.474 g, 6 mmol) were suspended in chloroform (5 ml), and the mixture was stirred overnight at room temperature. The mixture was then washed with water (2 ml×1, 1 ml×1) and evaporated to give a solid, which was recrystallized from ethyl acetate-hexane; 0.603 g (90%); mp 120—122 °C. Found: C, 61.00; H, 7.39; N, 8.22%. Calcd for $C_{17}H_{24}O_5N_2$: C, 60.70; H, 7.19; N, 8.33%.

Preparation of Boc-Ala-NH_2 and Boc-Pro-NH_2. Prepared according to the general procedure with modification: the aqueous washing phase was extracted with chloroform (2 ml×6) to recover the water soluble products. The desired amides were obtained from the combined organic layer.

Preparation of Z-Trp-NH_2. Prepared according to the general procedure with modification: a larger volume of chloroform (25 ml) was used as the solvent because of the poor solubility of the product.

Racemization Test. a) In Amidation of Amino Acid: Boc-Leu-NH_2 (0.326 g, 1.42 mmol) prepared by the general procedure described above was dissolved in 4 M† HCl/dioxane (5 ml) at room temperature. After 15 min, the mixture was evaporated and the residue was triturated in ether to give a solid (0.232 g), which was then dissolved in chloroform (10 ml) and triethylamine (0.20 ml, 1.44 mmol). To this solution was added Boc-Leu-ONSu (0.473 g, 1.44 mmol) and the mixture was stirred overnight at room temperature. The mixture was then washed successively with 0.1 M HCl (2 ml×1, 1 ml×1), water (2 ml), 0.5 M $NaHCO_3$ (2 ml×1, 1 ml×1), and water (2 ml×2). An aliquot (0.002 ml) of the chloroform solution was analyzed by the HPLC to determine the ratio of Boc-Leu-Leu-NH_2 (the retention time of the authentic sample, 24 min) and Boc-Leu-D-Leu-NH_2 (30 min). Similarly Boc-Phe-NH_2 obtained was converted to Boc-Leu-Phe-NH_2 and the purity of the product was also examined by the HPLC (the retention time of an authentic Boc-Leu-Phe-NH_2, 34 min; Boc-Leu-D-Phe-NH_2, 44 min). In either case, no peak corresponding to the D-amino acid containing peptide was observed (less than 0.2%).

b) In Amidation of Peptide: Boc-Leu-Leu-OH (0.344 g, 1 mmol), EEDQ (0.272 g, 1.1 mmol), and ammonium hydrogencarbonate (0.237 g, 3 mmol) were allowed to react in chloroform (5 ml) overnight at room temperature. The reaction mixture was washed with water (2 ml×1, 1 ml×1) and evaporated to give an oil, which was then dissolved in AcOEt. By addition of hexane, a viscous oil was separated. The oil collected by decantation was dissolved in MeOH and analyzed by the HPLC.

† 1 M=1 mol dm^{-3}.

References

1) Amino acids with no prefix are of L-configuration. Following abbreviations were used; Boc=*t*-butoxycarbonyl, Z=benzyloxycarbonyl, Bzl=benzyl ether, OBzl=benzyl ester, ONSu=*N*-hydroxysuccinimide ester.

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