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ACTIVATION OF CARBOXYLIC ACIDS BY PYROCARBONATES. APPLICATION OF DI-tert-BUTYL PYROCARBONATE AS CONDENSING REAGENT IN THE SYNTHESIS OF AMIDES OF PROTECTED AMINO ACIDS AND PEPTIDES

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Abstract: Amides formation from protected amino acids and peptides was achieved in an easy and convenient one-pot procedure using di-tert-butyl pyrocarbonate as activating agent in the presence of pyridine and ammonium hydrogencarbonate. The method gave good yields and did not induce racemization during the amidation of urethane protected amino acids.

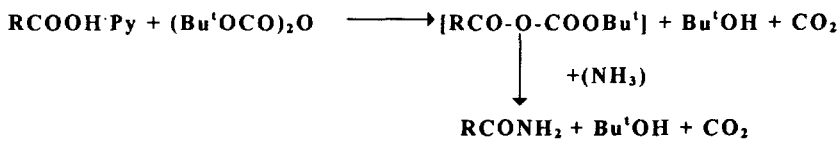
Carboxylic acid amides is a large class of natural and synthetic compounds, including, drugs and peptide hormones. Wide application of carboxamides stimulated an intensive search for methods of carboxylic acids amidation. Although much has been done in this field of synthetic organic chemistry, there is as yet no efficient method for carboxylic acid amidation.

The key problem of amide synthesis is the development of an adequate method for carboxyl group activation. Indeed, it is the choice of the carboxyl group activating reagent that determines a method's efficiency - in particular, its convenience, occurrence of side reactions and formation of by-products.

As an extension of the previous studies on carboxylic acids activation by dialkyl pyrocarbonates[1-3], we have attempted to employ these compounds as condensing reagents in the synthesis of amides of protected amino acids and peptides. The work was done with commercially available di-tert-butyl pyrocarbonate (Boc_2O), a reagent widely used for the introduction of the Boc-protecting group.

Several applications of Boc_2O are possible for the synthesis of carboxylic acid amides. One approach is to synthesize symmetrical anhydrides or active esters[2] with their subsequent ammonolysis. However, applying Boc_2O is more efficient if its interaction with the carboxylic acid proceeds in the presence of an ammonia derivative with which the pyrocarbonate reacts much slower than it does with carboxylate-ion. Then the mixed anhydride, formed as an intermediate, readily reacts with this ammonia derivative to give amide. Ammonium hydrogencarbonate[4] is most suitable for this purpose. If ammonium hydrogencarbonate is added to a mixture of carboxylic acid, Boc_2O and pyridine in a suitable solvent, a carboxamide is formed. Since the order of reagents' addition was of no importance for the yield of the target product, one-pot procedure appeared to be the most convenient. Possible pathways of initial reagents' conversion to final reaction products are presented in the scheme.

Scheme 1



Although the actual reaction process occurs in a much more complicated way and its precise mechanistic details are not yet clear, the method is simple[5], convenient and the yields of desired products are rather high. The results obtained are displayed in Table1.

Earlier it was shown that esterification of N-alkyloxycarbonyl derivatives of chiral amino acids with the Boc_2O - pyridine system was not accompanied by the racemization-induced loss of optical activity[6]. Detailed HPLC studies also confirmed the absence of racemization upon amidation of N-protected amino acids by the proposed procedure. It appears that Z-Pro-Phe-NH_2 is well separable from Z-Pro-D-PheNH_2 on RP HPLC C_{18} in 30% water acetonitrile[7]. In particular, HPLC of L-L dipeptide, obtained by acylation of L-PheNH_2 with Z-proline N-hydroxysuccinimide ester (or by the mixed anhydride method), missed the presence of any L-D diastereomer admixture. However, amidation of Z-Pro-Phe-OH led to formation of up to 10% of Z-Pro-D-PheNH_2. When Z-Pro-PheOH was amidated in the presence of copper(II) chloride[8], the racemization was as low as 0.6% and it was suppressed altogether by HOAt[9], however, amide yield was rather low (30%).

Table 1. Preparation of N-Protected Amino Acid and Dipeptide Amides RCO-NH$_2$

entry	RCO	solvent	yield %[b]	M.p. °C[c]	$[a]_D$,[d]	Lit. M.p.°C	data $[a]_D$	ref.
1	Boc-Ala	DO	83	126-127	-1,4	124-125	-2,7	4
2	Z-Ala	DO	78	136-137	-4,5[e]	130-131	-4,5[e]	10
3	Boc-Asp(OBzl)	DO	95	158-160	+7,8	157-160	-2,6	4
4	Glp	DMF	92	152-160	-19.0	-	-	-
5	Z-Glp	MeCN	84	156-157	+1.4	-	-	-
6	(Z-Cys)$_2$	DMF	87	193-194	-172[f]	199-201	-	11
7	Boc-Glu(OBzl)	DO	90	122-123	+4.1	120-122	+4.0	4
8	Z-Gly	DMF	80	134-135	-	138-139	-	-
9	Boc-Leu	DO	82	145-146	-11.1	144-146	-11.4	4
10	Fmoc-Leu	DO	70	138-139	-17.6	-	-	-
11	Boc-Met	DMF	88	120-121	-7.3	-	-	-
12	Boc-Phe	DO	80	142-144	+16.7	142-149	+16.7	4
13	Boc-D-Phe	MeCN	77	141-143	-14.5	-	-	-
14	Z-Phe	MeCN	92	163-164	-8.5	161-162	-2.6	4
15	Fmoc-Phe	DO	73	162-163	-18.2	-	-	-
16	Boc-Pro	DO	82	102-104	-49.4	104-106	-43.4	4
17	Boc-Trp	MeCN	93	137-138	+6.7	133-136	+7.7	4
18	Boc-Tyr(Boc)	MeCN	78	160-161	+9,8	-	-	-
19	Boc-Tyr(Bzl)	DO	76	170-172	+13.6	170-171	+16.0	4
20	Z-Val	DO	78	205-208	+22.9[f]	204-206	+25.5[f]	10
21	Z-Ala-Pro	DO	91	160-161	-40.2	-	-	-
22	Z-Ile-Pro	DO	83	114-115	-93.8	-	-	-
23	Z-Gly-Gly	DO	50	180-182	-	179-181	-	12
24	Z-Gly-Pro	DMF	90	145-146	-43.0	150-151	-	13
25	Z-Pro-Phe[14]	DO	75	170-172	-54.6	-	-	-
26	Z-Trp-Pro	DMF	92	97-98	-16.3	-	-	-
27	Z-Met-Pro	DO	76	136-137	-65.5	-	-	-

a) Each compound gave a single spot on TLC. Satisfactory data of microanalysis were obtained for each new compound within an error range of -+0.3%. b) Isolated yield; c) Uncorrected capillary melting points. d) c 1, EtOH at 18° C. e) c 1, MeOH, f) c 1, DMF.

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References and Notes

Abbreviations used are: DO, dioxane; MeCN, acetonitrile; MeOH, methanol; EtOH, ethanol, EA, ethyl acetate; DMF, dimethylformamide; Boc, tert-butyloxycarbonyl; Fmoc, 9-fluorenylmethyloxycarbonyl; Z, benzyloxycarbonyl; HOAt, 1-hydroxy-7-azabenzotriazole; RP HPLC, reversed phase high performance liquid chromatography; TLC, thin liquid chromatography. All amino acids are of the L-configuration (unless otherwise stated).

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5. Typical procedure: to a stirred solution of N-protected amino acid (10 mmol), pyridine (0.5 ml) and Boc_2O (3 g, 13 mmol) in an appropriate solvent (10-15 ml) ammonium hydrogencarbonate (1 g, 12.6 mmol) was added and the mixture was stirred for 4-16 h. Ethyl acetate or mixture chloroform with 10% n-propanol was added and after washings with water and 5% H_2SO_4 the solution was dried, the solvent was evaporated and the product was triturated with ether. In another variant the reaction mixture was diluted with water (30-40 ml), stirred until crystallization was completed, a residue was then collected by filtration, washed by water, dried and recrystallized as necessary.
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