The Rates of Racemization and Peptide Bond Formation of Glutamic and Aspartic Acid Active Esters[1]

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Racemization and coupling rate constants of several *N*-carbobenzoxy-γ-methyl-L-glutamic acid and *N*-carbobenzoxy-β-methyl-L-aspartic acid active esters have been determined. The order of magnitude of coupling rate constants for both amino acid esters is comparable to that reported for the corresponding cysteine active esters. However, significant differences are observed for the racemization rates. The importance of the ratio of coupling to racemization rate constants is discussed.

It has been previously reported that racemization of *N*-carbobenzoxy-*S*-benzyl-L-cysteine active esters in organic solvents and in the presence of triethylamine occurs through α-hydrogen abstraction and proceeds with unusual facility.[2-4] It was also reported that the racemization for *N*-carbobenzoxy-*S*-benzyl-L-cysteine pentachlorophenyl ester and *N*-carbobenzoxy-L-phenylalanine pentachlorophenyl ester in a nonpolar solvent proceeds *via* isoracemization.[5,6] In addition the rate of coupling of the cysteine active ester derivatives with L-valine methyl ester was investigated.[3,4] From these data an important conclusion was drawn, that a fast coupling active ester which racemizes relatively slowly is probably the best choice for the synthesis of peptides and sequential polypeptides. Numerically this can be best expressed by the ratio of the rate constants of coupling (k_c) to racemization (k_{rac}); the larger this number, the smaller the racemization to be expected during coupling.

The fast racemization of the commonly used active esters of *N*-carbobenzoxy-*S*-benzylcysteine, as well as the reports of Anderson,[7] Liberek,[8] and others that several amino acid active ester derivatives racemize in the presence of a tertiary base, led us to study the effect of the side chain of amino acids on the rates of racemization and coupling. The results with glutamic and aspartic acids are reported in this paper.

Rates of Racemization of *N*-Carbobenzoxy-γ-methyl-L-glutamic and *N*-Carbobenzoxy-β-methyl-L-aspartic Acid Active Esters.—The racemization of several frequently used active esters of glutamic and aspartic acids was studied in tetrahydrofuran solution in the presence of triethylamine under the conditions described for cysteine active ester derivatives.[4] The results are given in Table I,[9] which contains the pseudo-first-order as well as the second-order racemization rate constants. These values were shown to be true second-order rate constants by carrying out experiments at 1, 7, and 35 equiv of triethylamine/mol of ester.

TABLE I

RACEMIZATION RATE CONSTANTS FOR THE REACTION OF *N*-CARBOBENZOXY-γ-METHYL-L-GLUTAMIC AND *N*-CARBOBENZOXY-β-METHYL-L-ASPARTIC ACID ACTIVE ESTERS WITH TRIETHYLAMINE[a-e]

R of Z-Glu-R (OMe)	$k_{rac} \times 10^6$, sec^{-1}	$k_{rac} \times 10^6$, M^{-1} sec^{-1}
OSu[d]	16.1 ± 2.5	45.0 ± 7.1
OPFP[d]	11.5 ± 1.1	32.2 ± 3.2
OTCP[c](2,4,5)	2.12 ± 0.01	5.93 ± 0.03
ONP[e]	1.08 ± 0.09	3.03 ± 0.27
OPCP[c]	0.701 ± 0.01	1.96 ± 0.04
R of Z-Asp-R (OMe)	$k_{rac} \times 10^6$, sec^{-1}	$k_{rac} \times 10^6$, M^{-1} sec^{-1}
OPFP[c]	87.0 ± 3.2	244 ± 8.9
OTCP[c](2,4,5)	12.6 ± 0.1	35.3 ± 0.27
ONP[c]	9.65 ± 0.8	27.0 ± 2.20
OPCP[c]	6.29 ± 0.33	17.6 ± 0.92

[a] 23 ± 1°, in tetrahydrofuran. [b] The concentration of triethylamine was 0.35 *M*; the concentration of active ester was 0.05 *M*. [c] The average of two experiments. [d] The average of four experiments. [e] The average of five experiments. [f] The OSu ester was not isolated in pure form.

Comparing the second-order racemization rate constant of glutamic and aspartic acid active esters with that of the cysteine active esters, it becomes evident that glutamic acid active esters racemize about 50–100 times and the aspartic acid active esters about 13–23 times slower than the corresponding cysteine active esters. It can be seen that the rate of racemization for both glutamic and aspartic acid active esters decreases in the order OPFP > OTCP > ONP > OPCP. Furthermore, this order is the same as that for the corresponding cysteine active esters,[4] with the exception of *p*-nitrophenyl and pentachlorophenyl esters, which racemize at the same rate in the case of cysteine.

Rates of Coupling of *N*-Carbobenzoxy-γ-methyl-L-glutamic Acid and *N*-Carbobenzoxy-β-methyl-L-aspartic Acid Active Esters with L-Valine Methyl Ester.—The rate of coupling was studied for these amino acid active esters with equimolar amounts of L-valine methyl ester in tetrahydrofuran. The dipeptide coupling products from each active ester were isolated and characterized. The second-order coupling rate constants are given in Table II together with the 99% reaction time. These rate constants were determined by following the disappearance of the active ester

(1) Part 6 of a series on racemization studies of amino acid derivatives. For parts 1–5 see ref 2–6.

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(8) (a) B. Liberek, *Tetrahedron Lett.*, 1103 (1963); (b) B. Liberek and Z. Grzunka, *ibid.*, 159 (1964).

(9) The following abbreviations have been used: Z = carbobenzoxy; Me = methyl; OSu = *N*-hydroxysuccinimidyl; OPFP = pentafluorophenyl; OTCP(2,4,5) = 2,4,5-trichlorophenyl; OPCP = pentachlorophenyl; ONP = *p*-nitrophenyl.

TABLE II

SECOND-ORDER COUPLING RATE CONSTANTS FOR THE REACTION OF *N*-CARBOBENZOXY-γ-METHYL-L-GLUTAMIC AND *N*-CARBOBENZOXY-β-METHYL-L-ASPARTIC ACID ACTIVE ESTERS WITH VALINE METHYL ESTER[a,b]

R of Z-Glu-R / OMe	$k_c \times 10^2$, M^{-1} sec^{-1}	99% reaction time, hr; active ester concentration 0.13 *M*	0.01 *M*
OPFP[d]	11.6 ± 2.2	1.82	24
OSu[e]	2.70 ± 0.67	7.85	102
OPCP[d]	0.164 ± 0.02	129	1680
OTCP[c](2,4,5)	0.100 ± 0.06	212	2750
ONP[c]	0.045 ± 0.008	470	6110
R of Z-Asp-R / OMe	$k_c \times 10^2$, M^{-1} sec^{-1}	99% reaction time, hr; active ester concentration 0.13 *M*	0.01 *M*
OPFP[c]	14.7 ± 0.9	1.44	19
OPCP[c]	0.737 ± 0.07	28.6	372
OTCP[c](2,4,5)	0.262 ± 0.01	80.8	1050
ONP[c]	0.072 ± 0.008	295	3830

[a] 23° ± 1, in tetrahydrofuran. [b] The concentration of the active ester and valine methyl ester was 0.13 *M*. [c] The average of two experiments. [d] The average of three experiments. [e] The average of five experiments.

carbonyl absorption peak in the infrared region between 5 and 6 μ.

The data presented in this table show that the order of magnitude of the rate constants for both amino acid active esters is comparable with that reported for the corresponding cysteine active esters.[4] In contrast to racemization, the side chain of the amino acid has no significant effect on the rate of coupling. The rate of coupling for all three amino acids investigated decreases with decreasing electron-withdrawing ability of the active ester groups in the following order: pentafluorophenyl > *N*-hydroxysuccinimide > pentachlorophenyl > 2,4,5-trichlorophenyl > *p*-nitrophenyl.

Conclusion

Significance of the Ratio of Coupling and Racemization Rate Constants.—Comparison of the data presented in Tables I and II shows that the decreasing order of the racemization rate constants for the previously discussed active esters is not the same as the decreasing order of the coupling rate constants; this indicates that *the "activity" of an ester is not strictly parallel with its ability to racemize.* This is clearly indicated by the ratio of coupling to racemization rate constants, which is presented in Table III. For the conditions studied these ratios are considered to be important figures, since these numbers indicate the relative extent of racemization which can be expected during coupling by the α-hydrogen abstraction mechanism. The larger this number the smaller the amount of racemization to be expected during coupling. In Table II the times for 99% completion of coupling reactions are also given at two different concentrations of the active esters with 1 equiv of valine methyl ester. The 99% reaction times were calculated using the equation given in the Experimental Section. As the equation indicates, the 99% reaction time is inversely proportional to the initial concentration of the active ester; hence the amount of racemization in concentrated solution should be less extensive.

It is apparent from these data that the extent of racemization by α-hydrogen abstraction expected during coupling increases in the following order: pentafluorophenyl < pentachlorophenyl < *N*-hydroxysuccinimide < 2,4,5-trichlorophenyl < *p*-nitrophenyl esters. This order corresponds to that previously reported for the cysteine active esters.[4]

TABLE III

RATIO OF COUPLING TO RACEMIZATION RATE CONSTANTS[a]

R of Z-Glu-R / OMe	k_c/k_{rac}
OPFP	3590
OPCP	835
OSu	600
OTCP(2,4,5)	170
ONP	150
R of Z-Asp-R / OMe	k_c/k_{rac}
OPFP	1210
OPCP	840
OTCP(2,4,5)	150
ONP	55

[a] In this paper the simple ratio of coupling to second-order racemization rate constants, given in Table I and II, is used. In our previous paper[4] for the calculations of the ratios $^1/_2 k_{rac}$ (k_2) was used.

The influence of the side chain of the amino acid on the extent of racemization during coupling of the above active esters is reflected by the magnitude of k_c/k_{rac}. The decreasing order of k_c/k_{rac} for the three amino acids investigated so far is glutamic acid > aspartic acid > cysteine. For glutamic acid active ester derivatives the ratios are very large and therefore the choice of active ester is not so critical as for cysteine active ester derivatives.

Experimental Section

All melting points are uncorrected and were determined in a Thomas-Hoover melting point apparatus. The kinetics of racemization were studied on a Rudolph photoelectric polarimeter, Model 200S-340-80Q3. Coupling kinetics were studied using a Beckman Model IR-8 spectrophotometer. All kinetic studies were done in a constant-temperature room (23 ± 1°).

Solvents and Reagents.—Gc spectrograde tetrahydrofuran was stored over molecular sieves. Gc Spectrograde triethylamine was stored over sodium. The valine methyl ester was freshly distilled under vacuum.

Preparation of *N*-Carbobenzoxy-γ-methyl-L-glutamic Acid Pentafluorophenyl Ester.—1-Cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (12.71 g, 30 mmol) and pentafluorophenol (5.52 g, 30 mmol) were dissolved in 200 ml of methylene chloride at room temperature. The solution was cooled at 0° and 8.85 g (30 mmol) of *N*-carbobenzoxy-γ-methyl-L-glutamic acid was added. The reaction mixture was stirred at 0° for 4 hr, washed with 5% sodium bicarbonate, 1 *N* hydrochloric acid, and water, and dried over sodium sulfate. The solvent was evaporated under reduced pressure. The yellow oil solidified on trituration with hexane at −20°. It was recrystallized from ether–pentane: yield 8.75 g (63%); mp 59–60°; $[\alpha]^{22}D$ −17.4° (c 2.02, ethyl acetate). The infrared spectrum showed the characteristic active ester peak at 5.6 μ (KBr). *Anal.* Calcd for $C_{20}H_{16}NO_6F_5$: C, 52.27; H, 3.48; N, 3.02; F, 20.50. Found: C, 52.06; H, 3.57; N, 3.24; F, 20.31.

The above procedure was used for the preparation of the other active esters described below.

***N*-Carbobenzoxy-γ-methyl-L-glutamic Acid Pentachlorophenyl Esters.**—The crude yellow oily ester was triturated with absolute ethanol, giving a white solid, mp 110–113°, yield 10.2 g (81%). It was recrystallized from hot absolute ethanol: mp 120–121°; $[\alpha]^{22}D$ −15.4° (c 2.01, ethyl acetate); ir (KBr) 5.6 μ (active ester). *Anal.* Calcd for $C_{20}H_{16}NO_6Cl_5$: C, 44.39; H, 3.00;

N, 2.57; Cl, 32.49. Found: C, 44.15; H, 2.89; N, 2.76; Cl, 32.74.

N-Carbobenzoxy-γ-methyl-L-glutamic Acid N-Hydroxysuccinimide Ester.—The crude oil was crystallized from hot absolute ethanol and recrystallized twice from the same solvent: yield 8.5 g (70%); mp 107–108°; $[\alpha]^{22}D$ −24.0° (*c* 2, dioxane); ir (KBr) 5.5 μ (active ester). [This ester was prepared by a different method described by Anderson,[10] mp 107–108°, $[\alpha]^{22}D$ −23.3° (*c* 2, dioxane)].

N-Carbobenzoxy-γ-methyl-L-glutamic Acid 2,4,5-Trichlorophenyl Ester.—The crude oil was crystallized from hot absolute ethanol and recrystallized twice from the same solvent: yield 10.5 g (70%); mp 122–123°; $[\alpha]^{22.5}D$ −26.0° (*c* 2, dimethylformamide); ir (KBr) 5.6 μ (active ester). [This ester was prepared by a different method described by Pless and Boissonnas,[11] mp 123°, $[\alpha]^{22}D$ −26.0° (*c* 2, dimethylformamide)].

N-Carbobenzoxy-γ-methyl-L-glutamic Acid p-Nitrophenyl Ester.—The crude oil was crystallized from hot absolute ethanol and recrystallized twice from the same solvent: yield 9.6 g (80%); mp 107–108°; $[\alpha]^{22}D$ −32.3° (*c* 1, 95% acetic acid); ir (KBr) 5.6 μ (active ester). [This ester was prepared by a different method described by Klieger and Gibian,[12] mp 107–108°, $[\alpha]^{22}D$ −32.7° (*c* 1, 95% acetic acid)].

N-Carbobenzoxy-β-methyl-L-aspartic Acid Pentafluorophenyl Ester.—The crude oil was crystallized from hot absolute ethanol and recrystallized from the same solvent: yield 10.8 g (80%); mp 80–81°; $[\alpha]^{23}D$ −20.5° (*c* 2, tetrahydrofuran); ir (KBr) 5.6 μ (active ester). *Anal.* Calcd for $C_{19}H_{14}NO_6F_5$: C, 51.02; H, 3.16; N, 3.13; F, 21.24. Found: C, 51.30; H, 3.41; N, 3.27; F, 20.94.

N-Carbobenzoxy-β-methyl-L-aspartic Acid Pentachlorophenyl Ester.—The crude solid was recrystallized twice from hot absolute ethanol to give white crystals: yield 11.5 g (85%); mp 134–135°; $[\alpha]^{22}D$ −23.0° (*c* 2, tetrahydrofuran); ir (KBr) 5.6 μ (active ester). *Anal.* Calcd for $C_{19}H_{14}NO_6Cl_5$: C, 43.59; H, 2.66; N, 2.64; Cl, 33.45. Found: C, 43.46; H, 2.61; N, 2.89; Cl, 33.15.

N-Carbobenzoxy-β-methyl-L-aspartic Acid 2,4,5-Trichlorophenyl Ester.—The crude oil was crystallized from hot 2-propanol and recrystallized from the same solvent: yield 8.1 g (60%); mp 97–98°; $[\alpha]^{22}D$ −34.0° (*c* 2, tetrahydrofuran); ir (KBr) 5.6 μ (active ester). *Anal.* Calcd for $C_{19}H_{16}NO_6Cl_3$: C, 49.54; H, 3.50; N, 3.04; Cl, 23.09. Found: C, 49.66; H, 3.74; N, 3.15; Cl, 22.84.

N-Carbobenzoxy-β-methyl-L-aspartic Acid p-Nitrophenyl Ester.—The crude oil was crystallized from chloroform–hexane and recrystallized from the same solvent: yield 7.3 g (60%); mp 105–106°; $[\alpha]^{22}D$ −43.5° (*c* 2, dimethylformamide); ir (KBr) 5.6 μ (active ester). [This ester was prepared by a different method and described by Goodman and Boardman,[13] mp 105–106°, $[\alpha]^{25}D$ −43.7° (*c* 2, dimethylformamide)].

Aminolysis Rate Studies on Active Esters.—Calibration curves of the esters were obtained by measuring the net absorbancies of the active ester peak by the base-line method.[14] A tetrahydrofuran solution which was 0.13 *M* in active ester and 0.13 *M* in valine methyl ester[15] was used to study the aminolysis of all esters. The reactions were followed using a double-beam infrared spectrometer by monitoring the disappearance of the active ester carbonyl band in the 5.6-μ region. A sealed 0.1-mm BaF_2 cell was used for the solutions; a matched BaF_2 cell containing the solvent was in the reference beam. Conformance to Beer's law was checked for all esters studied throughout the pertinent concentration ranges.

For the slower reactions, the spectrum between 5 and 6 μ was scanned periodically throughout the reaction. At least ten data points were taken for each run.

For the faster reactions, the spectrometer was set on the absorbance maximum of the active ester carbonyl peak and the pen excursion at this wavelength was monitored as a function of time.[4] In all cases, the initial reading was taken within 20 sec of mixing. Using this technique, a minimum of ten data points were obtained for each run. The 99% reaction time was calculated using the equation $t_{99\%} = 99/k_c C_E^0$, where k_c = coupling rate constant, C_E^0 = initial ester concentration, and $t_{99\%}$ = time when the coupling reaction is 99% complete. This equation is valid only for the case where the initial concentration of the ester and the amine are identical.

For each active ester when the reaction time reached 90–95% completion the *N*-carbobenzoxy-β-methyl-L-aspartyl-L-valine methyl ester and *N*-carbobenzoxy-γ-methyl-L-glutamyl-L-valine methyl ester dipeptides were isolated and characterized. A typical procedure for the isolation of the dipeptides is illustrated by the preparation of *N*-carbobenzoxy-β-methyl-L-aspartyl-L-valine methyl ester.

N-Carbobenzoxy-β-methyl-L-aspartyl-L-valine Methyl Ester.—A 280-mg portion of *N*-carbobenzoxy-β-methylaspartic acid pentachlorophenyl ester (70 mg/ml, 0.13 *M*) and 68 mg of valine methyl ester (17 mg/ml, 0.13 *M*) were dissolved in 4 ml of tetrahydrofuran. After 14 hr the solvent was removed under vacuum and the residue was triturated with pentane–ether (9:1) to remove the phenol. After standing, the crystalline dipeptide was filtered, washed with pentane–ether (9:1), and dried at 35° over P_2O_5 under vacuum, yield 394 mg (94.6%), mp 72–74°. The crystalline material was dissolved in a minimum amount of hot ether and filtered and then pentane was added to slight turbidity. The recrystallized dipeptide was filtered, washed with pentane–ether (9:1), and dried under vacuum over P_2O_5 for 1 hr: yield 250 mg (64%); mp 73–75°; $[\alpha]^{24}D$ −15.2° (*c* 2, tetrahydrofuran). *Anal.* Calcd for $C_{19}H_{26}N_2O_7$: C, 57.86; H, 6.65; N, 7.10. Found: C, 57.62; H, 6.72; N, 6.93.

N-Carbobenzoxy-γ-methyl-L-glutamyl-L-valine Methyl Ester.—This dipeptide was prepared from *N*-carbobenzoxy-γ-methyl-L-glutamic acid 2,4,5-trichlorophenyl ester and used for analysis. *Anal.* Calcd for $C_{20}H_{28}N_2O_7$: C, 58.82; H, 6.91; N, 6.86. Found: C, 58.60; H, 6.94; N, 6.97.

The procedures used for the coupling of the remaining active esters with valine methyl ester are the same as that described above for *N*-carbobenzoxy-β-methyl-L-aspartyl-L-valine methyl ester. The coupling results for these esters are described in Table IV. All compounds which were not analyzed gave iden-

TABLE IV

RESULTS OF THE COUPLING OF GLUTAMIC AND ASPARTIC ACID ACTIVE ESTER DERIVATIVES WITH VALINE METHYL ESTER[a]

		Dipeptide			
Registry no.	Active ester	Yield, mg (%)	Mp, °C, crude	Mp, °C,[b] recrystd	$[\alpha]^{22}D$, deg (*c* 1, THF)
39993-89-0	Z-Asp(OMe)-OPCP	394 (94)	72–74[c]	73–75	−15.2
39993-91-4	Z-Asp(OMe)-OTCP(2,4,5)	215 (89)	72-73	73–75	−15.0
39993-92-5	Z-Asp(OMe)-OPFP	252 (98)	74–75		−15.1
3330-39-0	Z-Asp(OMe)-ONP	88 (82)	73–75	74–75	−14.8
25613-46-1	Z-Glu(OMe)-OPCP	200 (76)	87–88[d]	89–91	−7.0
39993-96-9	Z-Glu(OMe)-OTCP(2,4,5)	150 (71)	91–92	91–92	−7.5
39993-97-0	Z-Glu(OMe)-OPFP	125 (60)	88–89	89–90	−7.0
5672-80-0	Z-Glu(OMe)-ONP	76 (50)	89–90	90–91	−7.0
39538-31-3	Z-Glu(OMe)-OSu	148 (93)	87–88	89–90	−6.5

[a] The concentration of each active ester and the valine methyl ester was 0.13 *M* in tetrahydrofuran. [b] The recrystallization of all dipeptides was carried out as described for the preparation of Z-(OMe)-Asp-Val-OMe. [c] Registry no., 39993-90-3. [d] Registry no., 4823-98-7.

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tical ir spectra with those of the analyzed dipeptides in both series.

Racemization Rate Studies on Active Esters.—The racemization rate studies were carried out in tetrahydrofuran solution with active ester concentrations of 0.05 *M* and 1, 7, and 35 equiv of triethylamine at 23 ± 1°. The preparation and storage of all solutions for the rate studies using 7 equiv of triethylamine were carried out in a glove bag under a dry nitrogen atmosphere. The racemization experiments with 1 and 35 equiv of triethylamine were performed using anhydrous solutions in the open atmosphere. All kinetics were followed at 589 nm. The first observed rotations were taken within 5 min of mixing the reagents. The pseudo-first-order data for the 7 equiv of triethylamine were plotted and found to be linear up to 90% racemization for all the esters. The second-order rate constants listed in Table I were obtained by dividing the pseudo-first-order rate constants by the triethylamine concentration. An unweighted linear least-squares computer program was routinely used to evaluate all kinetic data.

The second-order racemization rate constants for 1 and 35 equiv of triethylamine were calculated from the initial rates and were identical within experimental error with those obtained from the racemization with 7 equiv of triethylamine.

For the experiments with 1 and 35 equiv of triethylamine, after 95% reaction time the tetrahydrofuran solutions were evaporated under vacuum and the residues were used for racemate identification. The racemized active esters were analyzed using infrared spectroscopy and thin layer chromatography. The residue from the 1-equiv experiments was compared in chloroform solution with the pure L isomer; in all cases the ir spectra of the DL isomers were essentially identical with that of the L isomer. A thin layer chromatogram ($CHCl_3$–MeOH, 9:1) showed the DL compound and a very small amount of phenol which may have resulted from the hydrolysis of the active esters during the course of the experiments. In the case of 35 equiv of triethylamine experiments the thin layer chromatograms indicated more extensive hydrolysis. The extent of hydrolysis seems to be parallel with the reactivity of the ester. This was supported by infrared spectra, which showed the free carboxyl group absorptions.

One racemized active ester from each series was isolated, recrystallized, and characterized by elemental analysis.

Racemized *N*-Carbobenzoxy-γ-methylglutamic Acid Pentachlorophenyl Ester.—The residue from the racemization experiment with 35 equiv of triethylamine was triturated with ether and filtered, mp 118–120°. After two recrystallizations from methanol the compound was dried over P_2O_5 under vacuum at 75° for 2 hr, mp 119–120°, $[\alpha]^{23}D$ 0.00 (*c* 2, ethyl acetate). *Anal.* Calcd for $C_{20}H_{16}NO_6Cl_5$: C, 44.19; H, 2.97. Found: C, 43.70; H, 2.97.

Racemized *N*-Carbobenzoxy-β-methylaspartic Acid Pentachlorophenyl Ester.—This ester was isolated similarly to the glutamic acid active ester which is described above. The crude compound, mp 125–126°, was recrystallized from methanol–water and a second time from methanol–ether, mp 122–124°, $[\alpha]^{23}D$ −0.9 (*c* 2, tetrahydrofuran). *Anal.* Calcd for $C_{19}H_{14}NO_6Cl_5$: C, 43.09; H, 2.66. Found: C, 42.71; H, 2.78.

Registry No.—*N*-Carbobenzoxy-γ-methyl-L-glutamic acid, 4652-65-7; *N*-carbobenzoxy-β-methyl-L-aspartic acid, 3160-47-2; triethylamine, 121-44-8; valine methyl ester, 4070-48-8; 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate, 4641-47-8; pentafluorophenol, 771-61-9; *N*-carbobenzoxy-γ-methylglutamic acid pentachlorophenyl ester racemate, 39994-03-1; *N*-carbobenzoxy-β-methylaspartic acid pentachlorophenyl ester racemate, 39994-04-2.

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Reactions of *tert*-Butyl Trimethylsilyl Carbonate and of Bistrialkylsilyl Carbonates with Amino Acids. Carbon-13 Chemical Shifts in Carbonates and Silyl Carbonate Derivatives

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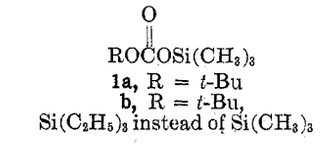
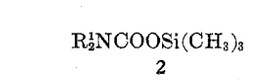
A number of bistrialkylsilyl carbonates, $R_3SiOC(=O)OSiR_3'$, have been prepared. The recently described *tert*-butyl trimethylsilyl carbonate (**1a**) has been shown to react with α-amino acid esters to form the corresponding silylurethanes, $RC(NHCOOR')HCOOR''$, where $R' = Si(CH_3)_3$. Under more drastic conditions, the completely silylated amino acid derivatives are formed. All of the silylated derivatives are hydrolyzed by moist ether to the parent amino acids or esters; with L-tyrosine, complete silylation followed by hydrolysis with moist ether regenerates the L-tyrosine, with no evidence of racemization. Bistriethylsilyl carbonate (**3c**) and *tert*-butyl triethylsilyl carbonate (**1b**) yield the same silylurethane from glycine ethyl ester. ^{13}C chemical shifts have been measured, and assignments of chemical shifts to specific types of carbon have been made for a series of carbonate esters, di- and tricarbonates, silyl carbonates, and *t*-BOC and other urethanes derived from glycine ethyl ester. As the number of carbonate groups in the molecule increases, the chemical shifts of the carbonyl carbons move to higher field. The presence of sulfur (in place of oxygen) next to the carbonyl carbon moves its chemical shift downfield ~16–18 ppm. Other regularities are noted.

Recently[1] we described the preparation of *tert*-butyl trimethylsilyl carbonate (**1a**) and related compounds;



1a, R = *t*-Bu
b, R = *t*-Bu, $Si(C_2H_5)_3$ instead of $Si(CH_3)_3$



2

it was shown that amines attacked **1a** to form the corresponding silylurethanes, $R_2'NCOOSi(CH_3)_3$, rather than the carbon urethanes (*t*-BOC derivatives), $R_2NCOOC(CH_3)_3$. Acid chlorides, however, attacked **1a** to form anhydrides, such as $ROC(=O)OC(=O)R^1$ (R = *t*-Bu, R^1 = CH_3 or OC_2H_5), presumably with the elimination of $ClSi(CH_3)_3$.

The present paper describes the reaction of **1a** with amino acids or their esters to form silylated derivatives analogous[2] to **2**. In a companion study in this labora-

(1) Y. Yamamoto and D. S. Tarbell, *J. Org. Chem.*, **36**, 2954 (1971).

(2) Similar silylated derivatives of amino acids, prepared in other ways, have been reported by H. R. Kricheldorf, *Synthesis*, 259 (1970); *Justus Liebigs Ann. Chem.*, **748**, 101 (1971); and earlier papers. Silylation of several amino acids by bis(trimethylsilyl)trifluoroacetamide for vpc analysis is reported by K. Bergstrom and J. Gurtler, *Acta Chem. Scand.*, **25**, 175 (1971), and references cited therein.