the layers were separated. The aqueous layer was neutralized with sodium bicarbonate and extracted with three 50-ml portions of ether. The combined ether extracts were concentrated at reduced pressure on the steam bath.[18] If the residue contained a mixture of solid and an oil, it was washed with 10 ml of cold (0°) methanol and filtered immediately. If a solid was not formed, 5–10 ml of methanol was added and crystallization occurred upon refrigeration of the mixture. Recrystallization was effected with ethanol or benzene (see Table I).

(18) Conveniently, crystallization was found to be hastened if the ether extracts were not dried before removal of excess solvents, especially when *p*-methoxyaryl isoxazoles were synthesized.

Registry No.—**3b**, 1148-87-4; **3c**, 3672-51-3; **3d**, 24097-17-4; **3e**, 3672-52-4; **3f**, 24097-19-6; **3g**, 24097-20-9; **5a**, 22020-72-0; **5b**, 24097-22-1; **5c**, 24097-23-2; **6a**, 24097-24-3; **6b**, 24097-25-4; **7a**, 24097-26-5; **7b**, 24097-27-6; **8a**, 24162-37-0; **8b**, 18753-56-5.

Racemization of Amino Acid Derivatives. Rate of Racemization and Peptide Bond Formation of Cysteine Active Esters[1]

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Received December 1, 1969

It is demonstrated that N-carbobenzoxy-S-benzyl-L-cysteine active esters do not racemize *via* a "β-elimination–readdition" mechanism in the presence of triethylamine. Racemization and coupling rate constants of several N-carbobenzoxy-S-benzyl-L-cysteine esters are reported. The rate data indicate that (a) the required coupling time in some cases is considerably less than usually used in preparative work and (b) an N-protected amino acid containing a fast-coupling active ester can be joined with a slowly reacting active ester to yield optically pure carboxyl-activated intermediates which are useful for the preparation of high molecular weight sequential polypeptides. Comparison and evaluation of the coupling and racemization rate data allows selection of the "best suited" active ester for peptide bond formation under the conditions employed.

The most important problem in peptide synthesis is to avoid racemization. Racemization through an oxazolone[2] intermediate has been studied in detail and is well understood. However, some amino acid derivatives, where oxazolone formation is believed to be absent, have been found to racemize in the presence of base.[3] It has been suggested that racemization of these derivatives proceeds through α-hydrogen abstraction.[3] The unusual facility with which cysteine[4] and serine[5] derivatives racemize has been attributed to a "β-elimination–readdition" mechanism.[6] Even N-carbobenzoxy- and N-*t*-butoxycarbonyl-S-benzyl-L-cysteine active esters racemize in the presence of triethylamine.[3a,c]

In this paper we report studies on the mechanism of racemization and the comparison of the rates of racemization and peptide bond formation of N-carbobenzoxy-S-benzyl-L-cysteine active esters as a model for peptide synthesis. These studies led to important conclusions concerning the choice of an active ester for the synthesis of oligopeptides as well as high molecular weight sequential polypeptides.

Evaluation of the β-Elimination–Readdition Mechanism.[6]—The racemization of N-carbobenzoxy-S-benzyl-L-cysteine pentachlorophenyl ester with excess triethylamine was studied in the presence of benzyl mercaptan-^{35}S. The partially racemized active ester was isolated without any incorporation of radioactive sulfur.[7] On the other hand, racemization of N-carbobenzoxy-S-benzyl-L-cysteine *p*-nitrophenyl ester under identical conditions resulted in partially racemized N-carbobenzoxy-S-benzylcysteine thiobenzyl ester[8] (77% yield) which contained one equivalent of radioactive sulfur. The position of the sulfur-35 was established by hydrazinolysis of the thiobenzyl ester. The corresponding hydrazide showed complete absence of the incorporated sulfur-35.

These experiments clearly confirm that β-elimination–readdition is not the mechanism for the racemization of N-carbobenzoxy-S-benzyl-L-cysteine active esters under these basic conditions. This result leads one to conclude that racemization of cysteine derivatives proceeds through abstraction of the α hydrogen.

Racemization of N-Carbobenzoxy-S-benzyl-L-cysteine Active Esters.—The racemization of the active esters listed in Table I was carried out in tetrahydrofuran solution in the presence of triethylamine under strictly anhydrous conditions. When anhydrous solvents were used but manipulations were not carried out in a drybox, the racemization of some of the active esters was accompanied by hydrolysis which usually

(1) Part III of a series on racemization studies of amino acid derivatives. For parts I and II see ref 6.

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(6) Preliminary communications: J. Kovacs, G. L. Mayers, R. H. Johnson, and U. R. Ghatak, *Chem. Commun.*, 1066 (1968); J. Kovacs, G. L. Mayers, R. H. Johnson, R. E. Cover, and U. R. Ghatak, *ibid.*, 53 (1970).

(7) The readdition of benzyl mercaptan to N-carbobenzoxydehydroalanine pentachlorophenyl ester yielded racemic N-carbobenzoxy-S-benzylcysteine pentachlorophenyl ester. On the other hand, N-carbobenzoxydehydroalanine *p*-nitrophenyl ester on reaction with 1 equiv of benzyl mercaptan under similar conditions yielded a complex mixture, two components of which are N-carbobenzoxy-S-benzyl-DL-cysteine *p*-nitrophenyl ester and N-carbobenzoxy-S-benzyl-DL-cysteine thiobenzyl ester. When the above reaction was run with 2 equiv of benzyl mercaptan, N-carbobenzoxy-S-benzyl-DL-cysteine thiobenzyl ester was isolated in high yield.

(8) This unexpected difference in the behavior between the *p*-nitrophenyl ester and the pentachlorophenyl ester in ester exchange reaction with benzyl mercaptan led to the investigation of the reaction of several other active esters with benzyl mercaptan. The data in Table IV suggest that steric effects may play a role in this ester exchange reaction.

manifested itself both as a residual rotation which remained practically constant for a considerable length of time and by diminution of the active ester carbonyl absorption in the infrared spectra.[9] Table I gives the second-order rate constants which were determined for the racemization of N-carbobenzoxy-S-benzyl-L-cysteine active esters.[10] 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

THE SECOND-ORDER RACEMIZATION RATE CONSTANTS FOR THE REACTION OF CARBOBENZOXY-S-BENZYL-L-CYSTEINE ACTIVE ESTERS[10] WITH TRIETHYLAMINE[a,b]

R of Z-Cys-R (BZL)	$k_{rac} \times 10^4$, M^{-1} sec^{-1}
-OSu[c]	48.8 ± 2
-OPFP[c]	33.0 ± 6
-ODNP (2,4)[c]	29.6 ± 2
-ODNP (2,6)[c]	29.0 ± 2
-OTCP (2,4,5)[d]	4.88 ± 0.6
-OPCP[c]	4.14 ± 0.2
-ONP[c]	3.94 ± 0.3
-OTCP (2,4,6)[c]	0.80 ± 0.05
-OPBP[c]	0.414 ± 0.02
-OTBP (2,4,6)[c]	0.1718 ± 0.001
-OPh[c]	0.0972 ± 0.002
-OEt[c,e]	No racemization[f]
-NHCH$_2$CO$_2$Et	No racemization[f]

[a] 23 ± 1°, in tetrahydrofuran. [b] The concentration range of triethylamine was 0.22–0.36 *M*. [c] The average of two experiments. [d] The average of four experiments. [e] 3.6 equiv of triethylamine. [f] Up to 7 days.

Aminolysis Rate Studies of N-Carbobenzoxy-S-benzyl-L-cysteine Active Esters.—The second-order rate constants for peptide bond formation between N-carbobenzoxy-S-benzyl-L-cysteine active esters and L-valine methyl ester are reported in Table II. L-Valine methyl ester was chosen for these reactions, since other methyl esters such as glycine and phenylalanine react too fast to be followed by the infrared techniques employed. Column 3 in Table II indicates the time required for 90% completion of the coupling reaction. The reaction between the phenyl ester and valine methyl ester was too slow to follow; the pentabromophenyl ester was insoluble in the solvent medium. The "activity" of esters in Table II is practically parallel with the rates of racemization recorded in Table I with the exception of the hydroxysuccinimide ester.

TABLE II

THE SECOND-ORDER COUPLING RATE CONSTANTS FOR THE REACTION OF N-CARBOBENZOXY-S-BENZYL-L-CYSTEINE ACTIVE ESTERS WITH VALINE METHYL ESTER[a]

R of Z-Cys-R (BZL)	$k_0 \times 10^2$, M^{-1} sec^{-1}	90% reaction time, min
-OPFP[b,d]	40.4 ± 9	2.9
-ODNP (2,4)[b,d]	18.4 ± 3	6.3
-OSu[b,d]	5.44 ± 0.7	21
-ODNP (2,6)[b,e]	1.73 ± 0.2	67
-OPCP[c,f]	1.72 ± 0.2	62[g]
OTCP (2,4,5)[b,f]	0.298 ± 0.03	385
-ONP[b,f]	0.105 ± 0.01	1088
-OTCP (2,4,6)[b,f]	0.0626 ± 0.002	1856
-OTBP (2,4,6)[b,f]	0.0215 ± 0.006	5310

[a] 23 ± 1°, in tetrahydrofuran. [b] The concentration of the active ester and valine methyl ester was 0.13 *M*. [c] The concentration of this ester and valine methyl ester was 0.0845 *M*. [d] The average of four experiments. [e] The average of three experiments. [f] The average of two experiments. [g] This value is based on an initial concentration of 0.0845 *M*.

As is evident from these data, even for the sterically hindered valine methyl ester, the required coupling time, in some cases, is considerably less than that usually used in preparative work. For example, from the reaction of N-carbobenzoxy-S-benzyl-L-cysteine pentafluorophenyl ester and valine methyl ester, the dipeptide was isolated in 90% yield after 5 min of reaction time. Peptide formation employing minimum required coupling time would lessen the danger of racemization. The rate data of Table II also indicate that a rapidly reacting N-protected active ester, such as N-carbobenzoxy-S-benzyl-L-cysteine pentafluorophenyl ester, may be coupled with slowly reacting amino acid active ester such as glycine *p*-nitrophenyl ester with a negligible amount of self-condensation of the latter. The N-protected dipeptide *p*-nitrophenyl ester was isolated in good yield in spite of the fact that the glycine active ester is expected to react the fastest in self-condensation. This variation of the "backing-off" procedure[11] would be very important for preparing intermediates for optically pure sequential polypeptides.[2g,12]

Rate Factors Influencing the Optical Purity of N-Carbobenzoxy Amino Acid Esters during Peptide Bond Formation.—Since a desired feature for peptide synthesis is racemization-free amide bond formation, a favorable ratio between coupling and racemization rates must exist if high molecular weight, optically pure polypeptides are to be obtained. The following general scheme was used to derive equations from which the optical purity of a peptide could be evaluated where

(9) In the study of the racemization of N-carbobenzoxy-S-benzyl-L-cysteine *p*-nitrophenyl ester, in which anhydrous tetrahydrofuran was used without manipulation being carried out in a drybox, atmospheric moisture was absorbed by the solvent. This led to hydrolysis and from the solution partially racemized N-carbobenzoxy-S-benzylcysteine was isolated. Under similar conditions a more striking example was observed during the racemization of N-carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester in chloroform solution (*c* 3.8) in the presence of 7 equiv of triethylamine; the apparent specific rotation changed from −7.4 to 12.1; N-carbobenzoxy-L-phenylalanine had a specific rotation of $[\alpha]^{23}D$ 87.6° (*c* 3.0, chloroform containing 7 equiv of triethylamine).

(10) The following abbreviations have been used: Z = carbobenzoxy; BZL = benzyl; Su = N-hydroxysuccinimidyl; PFP = pentafluorophenyl; DNP (2,4) = 2,4-dinitrophenyl; DNP (2,6) = 2,6-dinitrophenyl; TCP (2,4,5) = 2,4,5-trichlorophenyl; PCP = pentachlorophenyl; NP = *p*-nitrophenyl; TCP (2,4,6) = 2,4,6-trichlorophenyl; PBP = pentabromophenyl; TBP (2,4,6) = 2,4,6-tribromophenyl. The known esters listed below were prepared by the method described for the pentachlorophenyl ester. N-hydroxysuccinimide ester, mp 90–91°, $[\alpha]^{22}D$ −58.5° (*c* 1.8, dioxane) {lit. mp 91–92°, $[\alpha]D$ −58° ± 2.9° (*c* 1.746, dioxane), private communication from Dr. G. W. Anderson}; pentafluorophenyl ester, mp 83–84°, $[\alpha]D$ −40.0° (*c* 2.05, tetrahydrofuran), prepared in this laboratory by J. Roberts; *p*-nitrophenyl ester, mp 94–95°, $[\alpha]^{22}D$ −43.6° (*c* 1, dimethylformamide) {lit. mp 93–94°, $[\alpha]^{22}D$ −42° (*c* 1, dimethylformamide), M. Bodanszky and V. du Vigneaud, *J. Amer. Chem. Soc.*, **81**, 2504 (1959)}; 2,4,6-trichlorophenyl ester, mp 110–111°, $[\alpha]^{23}D$ −61.0° (*c* 1, ethyl acetate {lit. mp 111–112°, $[\alpha]^{20}D$ −62° ± 2° (*c* 1, ethyl acetate), G. Kupryszewski, *Rocz. Chem.*, **37**, 593 (1963)}; phenyl ester, mp 100–101°, $[\alpha]^{23}D$ −10.9° (*c* 2, chloroform) {(lit. mp 100–102°, $[\alpha]D$ −11.0° (*c* 1, chloroform), G. Blotnz, J. Biernat, and E. Taschner, *Justus Liebigs Ann. Chem.*, **663**, 194 (1963)}.

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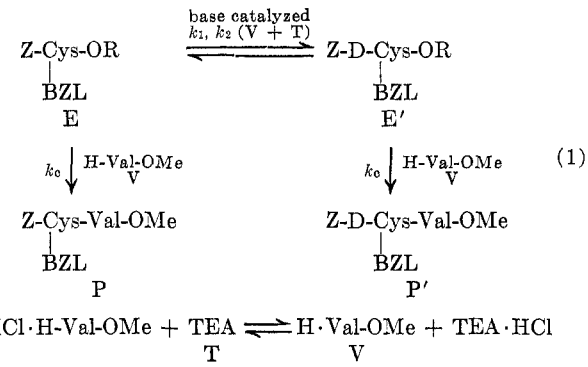
(12) (a) J. Kovacs, R. Giannotti and A. Kapoor, *ibid.*, **88**, 2282 (1966); (b) J. Kovacs, L. Kisfaludy, M. Q. Ceprini, *ibid.*, **89**, 183 (1967).



(1)

E is the starting ester, E′ is its enantiomer, P is the optically pure peptide, P′ is the undesired diastereomer, V is the coupling base, T is a base added to release V from its acid salt, k_c is the second-order coupling rate constant, and k_1 and k_2 are the interconversion rate constants of E and E′ for V and T, respectively.

The following assumption was made in deriving the rate expressions given below. The coupling rate constant, k_c, is identical for reactions of both E and E′.

$$\frac{dC_{E'}}{dt} = -(k_c + k_1)C_{E'}C_V - k_2C_{E'}C_T + k_2C_EC_T + k_1C_EC_V \quad (2)$$

$$\frac{dC_P}{dt} = k_cC_EC_V \quad (3)$$

$$\frac{dC_{P'}}{dt} = k_cC_{E'}C_V \quad (4)$$

from which one may write

$$\left(\frac{C_P}{C_{P'}}\right)_\infty = \frac{\int_0^\infty C_EC_V dt}{\int_0^\infty C_{E'}C_V dt} \quad (5)$$

Making the steady-state assumption for E′, solving for $(C_{E'}C_V)$, and substituting into eq 5, the product ratio becomes

$$\left(\frac{C_P}{C_{P'}}\right)_\infty = \frac{(k_c + k_1)\int_0^\infty C_EC_V dt}{\int_0^\infty (k_1C_EC_V + k_2(C_E - C_{E'})C_T)\,dt} \quad (6)$$

but, since in the steady state, $C_E \gg C_{E'}$, we may write

$$\left(\frac{C_P}{C_{P'}}\right)_\infty = \frac{(k_c + k_1)\int_0^\infty C_EC_V dt}{\int_0^\infty (k_1C_EC_V + k_2C_EC_T)dt} \quad (7)$$

If one further assumes that (a) equimolar amounts of T and the acid salt of V are present at the start of the reaction in a homogenous system and (b) T and V are in equilibrium throughout the reaction, then

$$C_T = KC_V \quad (8)$$

Substituting for C_T from eq 8 into eq 7 gives

$$\left(\frac{C_P}{C_{P'}}\right)_\infty = \frac{k_c + k_1}{k_1 + k_2K} \quad (9)$$

The values of $(C_P/C_{P'})_\infty$ expressed in eq 7 and 9 represent the maximum amount of the undesired diastereomer that could form under the experimental conditions assumed.

In the case where racemization is rapid relative to coupling, and if the coupling rate constants differ for the L and D active esters,[13] eq 9 becomes

$$\left(\frac{C_P}{C_{P'}}\right)_\infty = \frac{k_c}{k_{c'}} \quad (10)$$

where k_c and k_c' are the coupling rate constants for esters E and E′. In our systems where racemization is slow relative to coupling, large differences between k_c and k_c' should not affect the validity of our conclusions.

In the model system studied, namely, the coupling of N-carbobenzoxy-S-benzyl-L-cysteine active esters with L-valine methyl ester hydrochloride in the presence of triethylamine in tetrahydrofuran solution at room temperature, the values of $(C_P/C_{P'})_\infty$ can be evaluated from the rate data presented in Tables I and II. The value that was used for k_2 in this evaluation was $^1/_2k_{rac}$ given in Table I. Inasmuch as experimental results show that even an excess of valine methyl ester causes no racemization during active ester coupling, k_1 in eq 9 may be neglected. Since K is a constant depending upon the reaction system, the ratios of k_c/k_2, which are presented in Table III indicate the relative extents to which these active esters are susceptible to racemization during coupling.

TABLE III
RATIO OF COUPLING AND RACEMIZATION RATES

R of Z-Cys-R BZL	k_c/k_2	$(C_P/C_{P'})_\infty$
-OPFP	245	62
-ODNP (2,4)	124	35
-OPCP	83	25
-OTBP (2,4,6)	25	11
-OSu	22	11
-OTCP (2,4,6)	16	9
-OTCP (2,4,5)	12	7.1
-ODNP (2,6)	12	6.7
-ONP	5.3	4.2

The values tabulated for $(C_P/C_{P'})_\infty$ in Table III were obtained from analog computer simulations for the coupling system containing 1 equiv of valine methyl ester hydrochloride, 1 equiv of N-carbobenzoxy-S-benzyl-L-cysteine active ester, and 2 equiv of triethylamine. Similar conditions are commonly present[12,14] in the synthesis of high molecular weight sequential polypeptides.

Standard analog programming techniques were used[15] employing the experimentally determined rate constants for each system. The initial concentrations for the active ester, the free valine methyl ester, and the triethylamine were all taken as 0.129 M. These initial conditions assume that triethylamine instantaneously releases the free valine methyl ester from its acid salt. The computer simulations permit the evaluation of the product ratios, $(C_P/C_{P'})_\infty$, without making the steady-state assumption.

As can be seen from Table III, the computer-derived product ratios parallel the k_c/k_2 ratios derived using the steady-state assumption; however, these ratios are not linearly related. This nonlinearity could be due to the fact that the steady-state assumption may be invalid for systems with small k_c/k_2 ratios and the fact that the two sets of computations are based on disparate assumptions about the nature of the equilibrium between the two bases.

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(15) A. S. Jackson, "Analog Computations," McGraw-Hill Book Co., New York, N. Y., 1960.

Since optimum synthesis conditions are those in which $(C_{P'})_\infty$ is minimized (racemization is small), the most desirable active ester would be one in which the product ratio is largest. Where racemization is critical, such as cysteine, these data indicate that the *p*-nitrophenyl ester, one of the active esters most frequently used in coupling, would produce more of the undesired diastereomer under these conditions than any of the other esters investigated.

Experimental Section

All melting points are uncorrected and were determined in a Thomas–Hoover melting point apparatus. The kinetics of racemization were studied either on the Rudolph photoelectric polarimeter, Model 200S-340-8006, or on the Cary Model 60 recording spectropolarimeter. Coupling kinetics were studied on a Beckman Model IR-8 spectrophotometer. All kinetic studies were done in a constant temperature room (23 ± 1°); no other thermostatting was used. All computer data were obtained from two slaved EAI TR-20 analog computers. Radioactive samples were counted on a Tri-Carb liquid scintillation spectrometer (Packard Model 2002).

Solvents and Reagents.—Tetrahydrofuran was purified by refluxing for 3 days over solid potassium hydroxide, then distilled. The distillate was refluxed over lithium aluminum hydride for one day, followed by distillation. The purified material was then stored over molecular sieves. Reagent grade triethylamine was converted to its hydrochloride salt, recrystallized from absolute ethanol, then liberated from its salt with aqueous sodium hydroxide, dried over solid potassium hydroxide, and distilled from sodium under nitrogen. The purified material was stored over sodium. The valine methyl ester was twice distilled under vacuum and stored in the freezer.

Preparation N-Carbobenzoxy-S-benzyl-L-cysteine Pentachlorophenyl Ester.—Dicyclohexylcarbodiimide (4.12 g, 20 mmol) and pentachlorophenol (5.33 g, 20 mmol) were dissolved in 100 ml of anhydrous ethyl acetate at room temperature. The solution was cooled to 0° in an ice bath and 6.9 g (20 mmol) of N-carbobenzoxy-S-benzyl-L-cysteine were added. The reaction mixture was stirred at 0° for 1 hr and then at room temperature for 1 hr. The dicyclohexylurea (DCU) was removed by filtration and washed with dioxane. The ethyl acetate and dioxane filtrates were combined and the solvent was removed *in vacuo*. The residue was redissolved in dioxane and filtered to remove residual DCU. The dioxane was removed *in vacuo;* the crude yield was 10.6 g (89%). It was recrystallized from dimethylformamide–methanol, yield 8.0 g (67%), mp 171–172°, (lit.[16] mp 171–172°), $[\alpha]^{23}D$ −38.9° (*c* 0.7, chloroform). The ir spectrum showed the characteristic active ester peak at 5.6 μ (KBr).

Anal. Calcd for $C_{24}H_{18}NO_4Cl_5$: C, 48.55; H, 3.06; N, 2.36; S, 5.40; Cl, 29.86. Found: C, 48.26; H, 2.87; N, 2.32; S, 5.56; Cl, 30.31.

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

N-Carbobenzoxydehydroalanine Pentachlorophenyl Ester.—The crude semisolid product was triturated with petroleum ether (bp 40–60°) and filtered giving a white solid, mp 129–131° (62% yield). It was recrystallized from ethyl acetate, mp 131–133°, λ_{KBr} 5.68 μ (active ester).

Anal. Calcd for $C_{17}H_{10}NO_4Cl_5$: C, 43.49; H, 2.15; N, 2.98; Cl, 37.75. Found: C, 43.45; H, 2.03; N, 2.86; Cl, 37.33.

N-Carbobenzoxydehydroalanine *p*-Nitrophenyl Ester.—This crude oily product was chromatographed on a column of silica gel (i.d. 2.5 cm, height 33 cm) using benzene–petroleum ether (bp 40–60°). The first two fractions afforded an oil which crystallized on standing. The solid was triturated with pentane and filtered, mp 66–69° (yield 32%). The material was recrystallized from ether–pentane, mp 70–71°, λ_{KBr} 5.72 μ (active ester).

Anal. Calcd for $C_{17}H_{14}N_2O_6$: C, 59.66; H, 4.12; N, 8.19. Found: C, 59.76; H, 4.47; N, 8.28.

N-Carbobenzoxy-S-benzyl-L-cysteine Pentabromophenyl Ester.—The crude ester melted at 189–191° (yield 93%). The ester was recrystallized from dimethylformamide–methanol, mp 196–197°, $[\alpha]^{22}D$ −40.9° (*c* 2.04, tetrahydrofuran), λ_{KBr} 5.65 μ (active ester).

Anal. Calcd for $C_{24}H_{18}NO_4SBr_5$: C, 35.43; H, 2.20; N, 1.72; S, 3.93; Br, 48.96. Found: C, 35.43; H, 2.20; N, 2.01; S, 3.69; Br, 48.21.

N-Carbobenzoxy-S-benzyl-L-cysteine 2,4,6-Tribromophenyl Ester.—The yield was 80%; recrystallization from ethyl acetate–pentane gave needles, mp 119–120°, $[\alpha]^{25}D$ −48.6° (*c* 3.24, tetrahydrofuran), λ_{KBr} −5.62 μ (active ester).

Anal. Calcd for $C_{24}H_{20}NO_4SBr_3$: C, 43.79; H, 3.06; N, 2.13; S, 4.87; Br, 36.42. Found: C, 43.96; H, 2.98; N, 2.05; S, 4.90; Br, 36.30.

N-Carbobenzoxy-S-benzyl-L-cysteine 2,4,5-Trichlorophenyl Ester.—The yield was 92%, mp 92–93°. It was recrystallized from ethyl acetate–hexane, mp 92–93°, $[\alpha]^{22}D$ −43.9° (*c* 2.5, tetrahydrofuran), λ_{KBr} 5.62 μ (active ester).

Anal. Calcd for $C_{24}H_{20}NO_4SCl_3$: C, 54.92; H, 3.84; N, 2.67; S, 6.11; Cl, 20.26. Found: C, 55.08; H, 4.15; N, 2.54; S, 5.77; Cl, 20.52.

N-Carbobenzoxy-S-benzyl-L-cysteine 2,4-Dinitrophenyl Ester.—The yield was 73%, mp 93–95°. It was recrystallized from absolute ethanol, mp 98–100°, $[\alpha]^{25}D$ −61.5° (*c* 2.54, tetrahydrofuran), λ_{KBr} 5.62 μ (active ester).

Anal. Calcd for $C_{24}H_{21}N_3O_8S$: C, 56.36; H, 4.14; N, 8.22; S, 6.27. Found: C, 56.65; H, 4.56; N, 8.31; S, 6.31.

N-Carbobenzoxy-S-benzyl-L-cysteine 2,6-Dinitrophenyl Ester.—The yield was 73%, mp 102–104°. It was recrystallized from absolute ethanol, mp 107–108°, $[\alpha]^{23}D$ −103.8° (*c* 1.8, tetrahydrofuran), λ_{KBr} 5.58 μ (active ester).

Anal. Calcd for $C_{24}H_{21}N_3O_8S$: C, 56.36; H, 4.14; N, 8.22; S, 6.27. Found: C, 56.58; H, 4.18; N, 8.30; S, 6.41.

N-Carbobenzoxy-S-benzyl-L-cysteine Thiobenzyl Ester.—The crude oily product was crystallized from methanol–water, mp 73–75° (recrystallization from ethanol did not change the melting point), yield 45%, $[\alpha]^{22}D$ −94.8° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{25}H_{25}NO_3S_2$: C, 66.49; H, 5.58; N, 3.10; S, 14.20. Found: C, 66.70; H, 5.59; N, 32.1; S, 13.85.

Racemization of N-Carbobenzoxy-S-benzyl-L-cysteine Pentachlorophenyl Ester in the Presence of Benzyl Mercaptan-^{35}S.—N-Carbobenzoxy-S-benzyl-L-cysteine pentachlorophenyl ester (891 mg, 1.5 mmol) was dissolved in 30 ml of anhydrous chloroform. Benzyl mercaptan-^{35}S (0.176 ml, 1.55 mmol) (the benzyl mercaptan-^{35}S was obtained from Nuclear Chicago and diluted with unlabeled benzyl mercaptan; the activity was 3300 cpm/μmol) and triethylamine (1.5 ml, 10.3 mmol) were added and the mixture was stirred at room temperature for 90 min. The triethylamine was neutralized with 1 ml of concentrated hydrochloric acid. The chloroform solution was washed with water until neutral and dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo*. The residue was triturated with pentane and filtered. The yield was 775 mg (87%), mp 164–166°. Recrystallization of a small portion from ethyl acetate raised the melting point to 168–169°, $[\alpha]^{25}D$ −0.8° (*c* 2, chloroform).

Anal. Calcd for $C_{24}H_{18}NO_4SCl_5$: C, 48.55; H, 3.06; N, 2.36. Found: C, 48.83; H, 3.36; N, 2.50.

The recrystallized pentachlorophenyl ester (10 mg) was dissolved in 10 ml of toluene (scintillation grade); this solution was used to prepare samples for scintillation counting. The scintillator solution was *p*-bis[2-(5-phenyloxazolyl)]benzene (0.4%) and 2,5-diphenyloxazole (0.005%) made up in scintillation grade toluene. The counting showed the active ester had 3.6 ± 2 cpm/μmol.

Racemization of N-Carbobenzoxy-S-benzyl-L-cysteine *p*-Nitrophenyl Ester in the Presence of Benzyl Mercaptan-S^{35}.—N-Carbobenzoxy-S-benzyl-L-cysteine *p*-nitrophenyl ester (1.4 g, 3 mmol) was dissolved in 60 ml of chloroform. Benzyl mercaptan-^{35}S (activity 3300 cpm/μmol, 0.351 ml, 3 mmol) and triethylamine (3 ml, 21.5 mmol) were added and the mixture was stirred at room temperature for 90 min. The triethylamine was neutralized with 2 ml of concentrated hydrochloric acid. The chloroform solution was washed with water until neutral and dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo* at room temperature. The residue was an oil, which was dissolved in ethyl acetate and precipitated with pentane to yield five fractions. These fractions were monitored by tlc. Fraction 1 (365 mg, mp 89–97°) was primarily N-carbobenzoxy-S-benzyl-cysteine thiobenzyl ester contaminated with some starting material and *p*-nitrophenol. Fractions 2

(16) Previously reported mp 171–173° and $[\alpha]^{21}D$ −34.3 (*c* 0.7, chloroform): J. Kovacs, M. Q. Ceprini, C. A. Dupraz, and G. N. Schmit, *J. Org. Chem.*, **32**, 3696 (1967).

TABLE IV

REACTION OF CARBOBENZOXY-S-BENZYL-L-CYSTEINE ACTIVE ESTERS WITH BENZYL MERCAPTAN IN THE PRESENCE OF TRIETHYLAMINE

Starting Ester X of Z-Cys-X (BZL)	Product X of Z-Cys-X (BZL)	Yield, %	Mp, °C	Registry no.
OSu	SBZL[a]	80[b]	75–77	
ONP	SBZL[a]	83[b]	73–76	
OPFP	SBZL[a]	83[b]	74–76	
ODNP (2,4)	SBZL[a]	86[b]	75–76	
OTCP[c] (2,4,5)	SBZL[a]	82[b]	72–76	
OTCP[c] (2,4,6)	OTCP[e] (2,4,6)	90[d]	107–109	5276-82-4
OTBP[c] (2,4,6)	OTBP[e] (2,4,6)	92[d]	110–112 (softening above 98°)	24164-39-4
OPBP[c]	OPBP[e]	87[d]	192–193	24164-49-6
OPCP	OPCP	87	168–169	

[a] Products were identified by comparison of the ir spectrum in chloroform with those of the optically pure L isomers and also by tlc. [b] Once crystallized from ether–hexane. [c] Tetrahydrofuran as reaction solvent. [d] Crude solid washed with *n*-hexane. [e] Characterized by elemental analysis and ir spectrum.

and 3 (679 mg) showed only the thiobenzyl ester on tlc. Fractions 4 and 5 were mainly *p*-nitrophenol and were discarded. Fractions 2 and 3 were combined and recrystallized from absolute methanol. The yield was 205 mg, mp 77–78°, $[\alpha]^{22}D$ −25.4° (*c* 1, dimethylformamide). This sample of thiobenzyl ester exhibited 3139 ± 25 cpm/μmol. The sample gave an identical infrared spectrum in chloroform solution with that of an authentic sample of N-carbobenzoxy-S-benzyl-L-cysteine thiobenzyl ester.

Anal. Calcd for $C_{25}H_{25}NO_3S_2$: C, 66.49; H, 5.58; N, 3.10; S, 14.20. Found: C, 66.30; H, 5.96; N, 3.36; S, 13.94.

A portion of the recrystallized thiobenzyl ester (167 mg, 0.37 mmol) was dissolved in 1 ml of absolute ethanol. Hydrazine, 95% (0.06 ml), was added and the reaction mixture was allowed to stand overnight. The reaction mixture was diluted with 20 ml of ether and then pentane to precipitate the hydrazide. The hydrazide was filtered and washed with pentane. The yield was 132 mg (99%). The hydrazide was recrystallized from ether–pentane containing 1 ml of methanol. The white crystalline solid was filtered, mp 120–121°. The identity of this compound was established by comparison of its ir spectrum in chloroform solution with that of an authentic sample of N-carbobenzoxy-S-benzyl-L-cysteine hydrazide. The hydrazide (10 mg) was dissolved in 24 ml of scintillation grade toluene and 1 ml of absolute methanol. This sample of hydrazide showed 4 ± 0.6 cpm/μmol.

Reaction of N-Carbobenzoxydehydroalanine Pentachlorophenyl Ester with Benzyl Mercaptan.—N-Carbobenzoxydehydroalanine pentachlorophenyl ester (469 mg, 1 mmol) was dissolved in 20 ml of chloroform. Benzyl mercaptan (124 mg, 1 mmol) and triethylamine (1 ml, 7.1 mmol) were added to the solution and the mixture was stirred at room temperature for 105 min. The solvent and triethylamine were removed *in vacuo* at room temperature. The solid residue was triturated with pentane, filtered, and washed with pentane. N-Carbobenzoxy-S-benzyl-DL-cysteine pentachlorophenyl ester was isolated in 79% yield, mp 164–165°.

Anal. Calcd for $C_{24}H_{18}NO_4SCl_5$: C, 48.55; H, 3.06; N, 2.36. Found: C, 48.75; H, 3.43; N, 2.42.

Ester Exchange Reaction of N-Carbobenzoxy-S-benzyl-L-cysteine Active Ester with Benzyl Mercaptan in the Presence of Triethylamine.—A typical example is given below. To a solution of 0.221 g (0.5 mmol) of N-carbobenzoxy-S-benzyl-L-cysteine N-hydroxysuccinimide ester in dry chloroform (10 ml) at room temperature, 0.06 ml (0.5 mmol) of benzyl mercaptan and 0.5 ml (3.6 mmol) of dry triethylamine were added. After 3 hr the reaction mixture was cooled and diluted with 10 ml of chloroform. The organic phase was washed with 1 *N* hydrochloric acid and water and dried over sodium sulfate. The solvent was evaporated under reduced pressure. The colorless thick oil solidified on trituration with ether–hexane. It was recrystallized from the same solvent affording 191 mg (80%) of the thiobenzyl ester, mp 75–77°. Identity of this compound was proved by comparison of its ir spectrum in chloroform solution with that of an authentic sample and also by tlc. See Table IV.

Racemization Rate Studies on Active Esters.—All operations needed for preparation of the solutions for these rate studies were carried out in a glove bag under a dry nitrogen or helium atmosphere. The concentration of the active esters in tetrahydrofuran was between 0.314 and 0.514 *M*. The racemization was initiated by adding 7 equiv of triethylamine to this solution. All kinetics were followed at 589 mμ. The first reading was taken within 2 min of mixing the reagents. The pseudo-first-order plots were linear up to 90% racemization for all the esters except for the 2,4-dinitrophenyl ester which was linear up to 60–70% racemization. The second-order rate constants listed in Table I were obtained by dividing the pseudo-first-order rate constants by the triethylamine concentration. The racemization rate studies with 1 and 35 equivalents of triethylamine were run in a similar manner, but the data are not reported.

Aminolysis Rate Studies on Active Esters.—A tetrahydrofuran solution which was 0.13 *M* in N-carbobenzoxy-S-benzyl-L-cysteine active ester and 0.13 *M* in valine methyl ester was used to study the aminolysis of all esters except the pentachlorophenyl ester where 0.0845 *M* solutions were used.

The courses of 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 NaCl cell was used for the sample solutions; a matched NaCl cell containing the solvents 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. Net absorbances were estimated using the base-line method. At least 10 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. In all such cases, the initial reading was taken within 20 sec of mixing. Using this technique, a minimum of ten data points was obtained for each run. With this technique rate constants up to 1.0 M^{-1} sec^{-1} can be easily estimated.

Coupling of N-Carbobenzoxy-S-benzyl-L-cysteinyl Pentafluorophenyl Ester with Valine Methyl Ester.—N-Carbobenzoxy-S-benzyl-L-cysteine pentafluorophenyl ester (1.5 g, 2.9 mmol) was added to 402 mg (3.1 mmol) of valine methyl ester dissolved in 22.8 ml of tetrahydrofuran and the solution was stirred for 5 min at room temperature and worked up in the usual manner, yield 90%. The residue was triturated with pentane and the solid was filtered and washed thoroughly with pentane, yield 1.06 g (80%), mp 78–79°. Recrystallization from ethyl acetate pentane raised the melting point to 79–80°, $[\alpha]^{22}D$ −30.1° (*c* 2, tetrahydrofuran).

Anal. Calcd for $C_{24}H_{30}N_2O_5S$: C, 62.86; H, 6.59. Found: C, 62.85; H, 6.47.

Racemization Study of N-Carbobenzoxy-S-benzyl-L-cysteine *p*-Nitrophenyl Ester during Coupling.—A solution of the *p*-nitrophenyl ester (2.98 g, 6.4 mmol) and 1.7 ml (12.8 mmol) of valine methyl ester in 50.0 ml of tetrahydrofuran was allowed to react for 200 hr. The rotation of the solution after 200 hr was α 1.274. The following solution was prepared as a control: 0.117 g (0.256 mmol) of the aforementioned dipeptide, 0.0356 g (0.256 mmol) of *p*-nitrophenol, and 0.0329 g (0.256 mmol) of

valine methyl ester dissolved in 2.0 ml of tetrahydrofuran. The value of this solution was −1.259 indicating there was not any significant racemization.

N-Carbobenzoxy-S-benzyl-L-Cysteinylglycine p-Nitrophenyl Ester.—Glycine *p*-nitrophenyl ester hydrobromide (0.277 g, 0.001 mmol) was dissolved in dimethylformamide (2 ml) and tetrahydrofuran (20 ml) was added, followed by the addition of N-carbobenzoxy-S-benzyl-L-cysteine pentafluorophenyl ester (0.511 g, 0.001 mmol). The solution was cooled to −10°, and triethylamine (0.14 ml, 0.001 mol) was added. After 10 min at −10° and 20 min at room temperature, the mixture was filtered. Ethyl acetate (25 ml) was added to the filtrate, and the solution washed with saturated sodium chloride. The dried solution was evaporated and the residue triturated with ether. Filtration gave 0.364 g (70%) of a crude product, mp 150–153°. This material was dissolved in 20 ml of hot methanol and tetrahydrofuran mixture (1:1) and diluted with an equal volume of ether. On cooling a small amount of white solid separated which was discarded. The filtrate on dilution with an excess of hexane afforded the desired dipeptide, 0.260 g, mp 158–159°, $[\alpha]^{22}D$ −26.85 (*c* 2, tetrahydrofuran).

Anal. Calcd for $C_{26}H_{25}N_3SO_7$: C, 59.65; H, 4.81; N, 8.02. Found: C, 59.30; H, 4.88; N, 8.29.

Registry No.—N-Carbobenzoxydihydroalanine pentachlorophenyl ester, 24164-70-3; N-carbobenzoxydehydroalanine *p*-nitrophenyl ester, 24164-71-4; N-carbobenzoxy-S-benzyl-L-cysteine pentabromophenyl ester, 24164-49-6; N-carbobenzoxy-S-benzyl-2,4,6-tribromophenyl ester, 24164-39-4; N-carbobenzo-S-benzyl-2,4,5-trichlorophenyl ester, 24164-40-7; N-carbobenzoxy-S-benzyl-2,4-dinitrophenyl ester, 23180-03-2; N-carbobenzoxy-S-benzyl-2,6-dinitrophenyl ester, 24164-42-9; N-carbobenzoxy-S-benzylthiobenzyl ester, 24164-43-0; (±)-N-carbobenzoxy-S-benzylcysteinepentachlorophenyl ester, 24164-44-1; (±)-N-carbobenzoxy-S-benzylcysteine thiobenzyl ester, 24164-45-2; N-carbobenzoxy-S-benzylcysteine thiobenzyl ester hydrazide, 24164-46-3; N-carbobenzoxy-S-benzyl-L-cysteinyl pentafluorophenyl ester valine methyl ester dipeptide, 24215-87-0; N-carbobenzoxy-S-benzyl-L-cysteinylglycine *p*-nitrophenyl ester, 7669-99-0.

Acknowledgment.—This work was supported by grants from the National Institutes of Health (GM No. 06579 and 08795). We wish to thank Professor H. Horan for the infrared spectra.

The Rotational Barrier in 1,8-Diarylnaphthalenes[1a]

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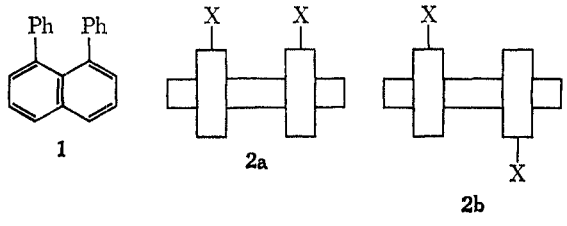
Received November 26, 1969

A series of 1-phenyl-8-(3-substituted phenyl)naphthalenes, **5–8**, have been synthesized to examine the question of the ease of rotation of the aryl rings in 1,8-diarylnaphthalenes. Although a number of 1,8-diphenylnaphthalenes with substituents in the phenyl rings exhibit temperature-dependent nmr spectra, the spectra are normally too complex for simple interpretation. However, the nonequivalence of the methyl signals in the low-temperature nmr spectrum of the derivative **8a** with a *meta* 2-hydroxy-2-propyl substituent provides unambiguous evidence for the rotation of the substituted phenyl ring in this substance. The free energy of activation ($\Delta G^{\ddagger}$) for this rotation is calculated to be 16 kcal/mol at 25°.

Various evidence[2,3] indicates the favored conformation of 1,8-diphenylnaphthalene (**1**) to be one in which the two phenyl rings are parallel to one another and perpendicular to the plane of the naphthalene ring as illustrated by a top view of the molecule in structure **2**. Consideration of the dimensions of such molecules as discerned from molecular models and the limited X-ray crystallographic data available[2] suggests the existence of a substantial energy barrier to rotation of the phenyl rings and led us to expect that *cis* (**2a**) and *trans* (**2b**)



1 **2a** **2b**

isomers of 1,8-di(*ortho*- or *meta*-substituted phenyl)-naphthalenes might be isolated. In fact, we were completely unsuccessful in this attempt and instead isolated a series of di-*meta*-substituted compounds as single crystalline substances.[3] Nmr and dipole moment data obtained from certain of these compounds suggested that equilibration of the two geometrical isomers **2a** ⇌ **2b** may be relatively rapid in solution with an energy barrier to rotation on the order of 10 kcal/mol. Since this energy barrier seemed unusually low and the interpretation of our data for these disubstituted compounds (**2**) was not unambiguous, we have sought more convincing evidence about this rotation barrier. This paper describes the preparation of a series of monosubstituted diphenylnaphthalenes (Scheme I) and appropriate nmr measurements which clearly demonstrate the rotation of the substituted phenyl ring in solution at 25°.

The synthetic route (Scheme I) followed our earlier pattern[3] in which the unsaturated ketone **3** was converted to a diene **4** which was dehydrogenated to the diarylnaphthalene **5**. This aryl chloride **5** was converted to the cyanide **6** with CuCN in HMP[4] and then hydrolyzed to the acid **7a**. This acid **7a** (pK^*_{MCS} = 7.04)[5] is slightly less acidic than the corresponding monoarylnaphthalene derivative **9a** (pK^*_{MCS} = 6.50–6.56)[3,5] possibly reflecting the increased steric hindrance to solvation of the carboxylate anion from acid **7a**.

(1) (a) This research has been supported by Public Health Service Grant 1-R01-CA10933 from the National Cancer Institute; (b) National Institutes of Health Predoctoral Fellow, 1968–1970.

(2) For a review, see V. Balasubramaniyan, *Chem. Rev.*, **66**, 567 (1966).

(3) (a) H. O. House and R. W. Bashe, II, *J. Org. Chem.*, **30**, 2942 (1965); **32**, 784 (1967). (b) H. O. House, R. W. Magin, and H. W. Thompson, *ibid.*, **28**, 2403 (1963), and references therein.

(4) H. O. House and W. F. Fischer, Jr., *ibid.*, **34**, 3626 (1969).

(5) The values pK^*_{MCS} are the apparent pK_a values in a mixture of 20% water and 80% Methyl Cellosolve: W. Simon, *Angew. Chem., Int. Ed. Engl.*, **3**, 661 (1964).