Rates of Racemization and Coupling of Cysteine Active Ester Derivatives

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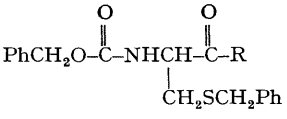
Summary Racemization and coupling rate constants of *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine active esters are reported and evaluated for use in peptide synthesis.

IN a previous communication,[1] we reported that *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine active esters do not racemize *via* a "β-elimination–re-addition" mechanism. Thus, the ready racemization of cysteine derivatives proceeds through α-hydrogen abstraction. In conjunction with this work, the effect of the most frequently used active ester groups on the rate of racemization as well as the rate of peptide bond formation of cysteine derivatives was studied.

TABLE 1. *The second-order racemization rate constants for the reaction of* N-*benzyloxycarbonyl*-S-*benzyl*-L-*cysteine active esters with triethylamine* [a,b]



Compound	where R is:	$k_{rac} \times 10^4$ (M^{-1} sec.$^{-1}$)
(I)[c]	O-*N*-succinimide	48·8 ± 2
(II)[c]	OC_6F_5	33·0 ± 6
(III)[c]	OC_6H_3-$(NO_2)_2$(2,4)	29·6 ± 2
(IV)[c]	OC_6H_3-$(NO_2)_2$(2,6)	29·0 ± 2
(V)[d]	$OC_6H_2Cl_3$(2,4,5)	4·88 ± 0·6
(VI)[c]	OC_6Cl_5	4·14 ± 0·2
(VII)[c]	OC_6H_4-*p*-NO_2	3·94 ± 0·3
(VIII)[c]	$OC_6H_2Cl_3$(2,4,6)	0·80 ± 0·05
(IX)[c]	OC_6Br_5	0·414 ± 0·02
(X)[c]	$OC_6H_2Br_3$(2,4,6)	0·1718 ± 0·001
(XI)[c]	OPh	0·0972 ± 0·002
(XII)[c,e]	-OEt	No racemization[f]
(XIII)	$-NHCH_2CO_2Et$	,, ,,

[a] 23 ± 1°, in tetrahydrofuran; [b] the concentration range of triethylamine was 0·22—0·36M; [c] the average of two experiments; [d] the average of four experiments; [e] 3·6 equiv. of triethylamine; [f] up to 7 days.

The second-order racemization rate constants for *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine active esters in tetrahydrofuran in the presence of triethylamine are presented in Table 1.[2] These values were shown to be true second-order rate constants by carrying out experiments at 1, 7, and 35 equiv. of triethylamine per mole of ester.

The second-order rate constants for the coupling of *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine active esters with valine methyl ester are given in Table 2. Column 3 in Table 2 indicates the time required for 90% completion of the coupling reactions. The cysteine phenyl ester did not give any observable reaction and the pentabromophenyl ester was insoluble in this solvent system.

TABLE 2. *The second-order coupling rate constants for the reaction of* N-*benzyloxycarbonyl*-S-*benzyl*-L-*cysteine active esters with valine methyl ester*[a]

Active ester	$k_2 \times 10^2$ (M^{-1} sec.$^{-1}$)	90% Reaction time (min.)
(II)[b,d]	40·4 ± 9	2·9
(III)[b,d]	18·4 ± 3	6·3
(I)[b,d]	5·44 ± 0·7	21
(IV)[b,e]	1·73 ± 0·2	67
(VI)[c,f]	1·72 ± 0·2	62[g]
(V)[b,f]	0·298 ± 0·03	385
(VII)[b,f]	0·105 ± 0·01	1088
(VIII)[b,f]	0·0626 ± 0·002	1856
(X)[b,f]	0·0215 ± 0·006	5310

[a] 23 ± 1°, in tetrahydrofuran; [b] the concentration of the active ester and valine methyl ester was 0·13M; [c] the concentration of this ester and valine methyl ester was 0·0845M; [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 0·0845M.

It is evident from these data that, even for the sterically hindered valine methyl ester, the required coupling time is often considerably less than that usually used in preparative work. The use of the required minimum coupling time would lessen the danger of racemization during peptide synthesis where α-hydrogen abstraction is the primary

mechanism for racemization. The data in Table 2 suggest that a fast-reacting *N*-protected active ester may be coupled with a slowly-reacting amino-acid active ester with a negligible amount of self-condensation of the latter. This variation of the "backing-off" procedure[3] would be very important for preparing intermediates for optically pure sequential polypeptides.[4] The activity of the esters in Table 2 is practically parallel with the rates of racemization reported in Table 1 with the exception of the hydroxysuccinimide ester.

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[2] Part of this work was presented at The First American Peptide Symposium, Yale University, New Haven, Connecticut, August 1968.
[3] M. Goodman and K. C. Steuben, *J. Amer. Chem. Soc.*, 1959, **81**, 3980.
[4] J. Kovacs, R. Giannotti, and A. Kapoor, *J. Amer. Chem. Soc.*, 1966, **88**, 2282.