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Short Communication

Coupling and racemization rate constants of alanine- and glycylalanine-active esters

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Abstract

Second-order racemization rate constants (k_r) were determined for the racemization of pentachlorophenyl (OPcp) and p-nitrophenyl (ONp) esters of N-carbobenzoxy-L-alanine (Z-L-Ala) and N-carbobenzoxyglycyl-L-alanine (Z-Gly-Ala) in the presence of TEA (triethylamine). The dipeptide-active esters showed the characteristic 5(4H)-oxazolone IR peak at 5.4μ during racemization. Second-order coupling rate constants (k_c) were determined for the above compounds with valine methyl ester. Significantly higher k_c/k_r ratios were obtained for dimers as compared to monomers and for OPcp than for ONp esters.

Key words: Racemization rates, coupling rates, dipeptides, active esters.

1. Introduction

Despite the general assumption that C-activated dipeptides racemize *via* oxazolone mechanism, conclusive evidence has been presented only in a few cases of Z-Gly-Phe dipeptides[1–4]. Our group has shown that Z-Gly-Cys(Bzl)-ONp and Z-Gly-Ser(Bzl)-OPcp racemize *via* α-H abstraction mechanism[5,6]. It appears that the presence of heteroatoms in the dipeptide side chain somehow prevents oxazolone formation. In order to obtain a better insight of dipeptide racemization mechanism, we have measured the k_r of ONp and OPcp esters of Z-Gly-Ala and checked for the presence of the oxazolone peak during racemization. In addition, we have reported the k_r values of the corresponding monomers and k_r and k_c values of the above monomers and dimers.

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2. Experimental

Boc- and Z-amino-acid-active esters were prepared by standard DCC method. Experimental details are given for the synthesis of new compounds and for literature preparations of known compounds which have been modified. Melting points were taken on a Thomas-Hoover melting point apparatus in open capillaries and are uncorrected. Optical rotations were determined on a Rudolph spectropolarimeter, Model 200S–340–8006. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y. and by Chemaltics, Inc., Tempe, Arizona.

The details of kinetic studies have been previously described[5,6]. Active esters were isolated by coupling with GlyOEt, after the racemization (about 90%) and coupling products were isolated by coupling with Val-OMe after the reactions were about 99% complete (Tables I and II).

2.1. *Synthesis of L-alanine nitrophenyl ester trifluoroacetate*

This was obtained by the removal of the Boc group from Boc-Ala-ONp with anhydrous trifluoroacetic acid(18 ml/0·01 mole of the active ester) followed by precipitation with anhydrous ether.

Table I
Physical constants of compounds isolated after coupling of Z-L-Ala and Z-Gly-L-Ala-active esters with ValOMe after the reaction had proceeded to 99° completion

Compound coupled	Compound isolated	Crude yield (°)	Crude MP, °C	MP after one recrystallization	$[\alpha]_D^{23,a}$
Z-Ala-ONp	Z-Ala-ValOMe	89	82–84.5	84–84.5	−37.83
Z-Ala-OPcp	···	96	79–81	84–84.5	−37.83
Z-Gly-Ala-ONp	Z-Gly-Ala-ValOMe	88	141–144	143–144	−48.93
Z-Gly-Ala-OPcp	··	99	140–144	143–144	−49.0

a = (c 1.4, MeOH).

Table II
Results and physical constants of compounds isolated after racemization (90°) in the presence of TEA followed by coupling with GlyOEt hydrochloride

Compound racemized	Product isolated	Crude yield (°)	Crude MP (°C)	MP after one recrystallization[a]
Z-Ala-ONp[b]	Z-DL-Ala-GlyOEt	64	75–79	80–82.5
Z-Ala-OPcp[b]	Z-DL-Ala-GlyOEt	75	77–81	81–82.5
Z-Gly-Ala-ONp[c]	Z-Gly-DL-Ala-GlyOEt	78	136–138	135–138
Z-Gly-Ala-OPcp[c]	Z-Gly-DL-Ala-GlyOEt	63	130–134	135–137

[a]Recrystallization solvent was CH_2Cl_2, Hexane.
[b]Ester racemized in the presence of 35 equivalents of TEA;
[c]Ester racemized in the presence of 7 equivalents of TEA.

The crude product (yield 85.9%) was recrystallized tetrahydrofuran-ether, mp 144–146°C, $[\alpha]_D^{23}$-12.86 (c 1.6, DMF), IR(KBr) 3.4 (ammonium salt), 5.65 (active ester) and 5.95 μ (acetate carbonyl).

Anal. Calc. for $C_{11}H_{11}F_3N_2O_6$: C, 40.75; H, 3.42; N, 8.64. Found: C, 40.95; H, 3.61; N. 8.90.

2.2. N-Carbobenzoxyglycyl-L-alanine p-nitrophenyl ester

The dipeptide was obtained by the coupling Z-Gly-OH and L-alanine *p*-nitrophenyl ester trifluoroacetate by mixed anhydride procedure. The crude product (85%) was recrystallized two times from ethylacetate-pentane, mp 107–108°C, $[\alpha]_D^{23}$-48.4 (c 2, EtoAc), IR (KBr) 5.63 (active ester) 5.93 and 6.52 μ (amide I and II, respectively).

The same compound reported in the literature[7] was obtained by DCC method, mp 105–106°C, $[\alpha]_D^{22}$-49.4 (c 2, EtOAc).

2.3. N-Carbobenzoxyglycyl-L-alanine pentachlorophenyl ester

The dipeptide was synthesized by coupling Z-Gly-OH and L-alanine pentacholorophenyl ester hydrobromide by mixed anhydride procedure.

The crude product (yield 81%) was recrystallized two times from tetrahydrofuran-pentane, mp 177–179°C, $[\alpha]_D^{23}$-35.30 (c 1.99, dioxane), IR (KBr) 5.60 (active ester), 6.01 (amide I), and 6.518 μ (amide II).

The above compound reported in literature[8] was synthesized by different methods as shown below:

Method of synthesis	MP (°C)	$[\alpha]_D^{25,a}$
DCC	172–174	−25.6
Complex	178–179	−34.9
CCL COOPcp	182–183	−6.00

a. In dioxane, C = 2.

3. Rates of racemization and coupling of Z-L-Ala- and Z-Gly-L-Ala-active esters

As can be observed from the experimental and predicted[9] k_r and k_c values of compounds (Tables II and IV), the relative k_r values of Z-L-Ala-ONp are 2·47 times higher than that of Z-Ala-L-OPcp, and the relative k_c values of Z-Ala-OPcp are 3·37 times higher than that of Z-Ala-ONp.

The relative k_c rate of the dimers to monomers (b/c in Table IV) is about 8 for both the esters and the k_r ratios are 58.1 and 141 for the ONp and OPcp esters, respectively (b/c in Table I). While the coupling ratio of dimer/monomer is a little difficult to explain, the racemization ratio is not, since it is known that activated dimers are easier to racemize

Table III
Experimental* and predicted second-order racemization rate constants (k_r) for the reaction of Z-L-Ala[13] and Z-Gly-L-Ala-active esters with TEA in THF at 23°C

Compound	$k_r \times 10^{-6}$ $M^{-1}sec^{-1}$ (b)	Compound	$k_r \times 10^{-6}$ $M^{-1}sec^{-1}$ (c)	b/c
Z-Ala-ONp	2.1 ± 0.05 (1.3)	Z-Gly-Ala-ONp	122 ± 7.1 (139)	58.1
Z-Ala-OPcp	0.825 ± 0.02 (0.825)	Z-Gly-Ala-OPcp	116 ± 2.73 (87)	141

*Each result is the average of two experiments using two different concentrations of TEA and one concentration of active ester (0.05 M). Values in parentheses are predicted values based on the additivity principle.

Table IV
Experimental*, predicted second-order coupling rate constants (k_c) and k_c/k_r for the reaction of Z-L-Ala[10] and Z-Gly-L-Ala-active esters with L-valine methyl ester in THF at 23°C

Compound	$k_r \times 10^{-2}$ $M^{-1}sec^{-1}$ (b)	k_c/k_r	Compound	$k_r \times 10^{-2}$ $M^{-1}sec^{-1}$ (c)	b/c	k_c/k_r
Z-Ala-ONp	0.15 ± 0.001 (0.11)	714	Z-Gly-Ala-ONp	1.3 ± 0.6 (0.95)	8.7	107
Z-Ala-OPcp	0.50 ± 0.03 (0.50)	6060	Z-Gly-Ala-OPcp	3.7 ± 1.1 (4.3)	7.3	319

*Each result is the average of two experiments using equimolar concentration of active ester and ValoMe (0.13), except in the case of Z-Gly-Ala-OPcp where concentration was 0.05 M. Values in parentheses are predicted values based on the additivity principle.

than the activated monomers due to the formation of the intermediate, 5(4H)-oxazolone, from the former. In the case of these dipeptides, characteristic 5(4H)-oxazolone peak at 5·4 μ in IR was noted in the presence of TEA. This evidence, together with the reasonably good agreement of the experimental with predicted k_r values[9], indicates that the racemization proceeds *via* 5(4H)-oxazolone mechanism for these dipeptides.

As can be observed (Table IV), significantly higher k_c/k_r values are obtained with the OPcp ester than with the widely used ONp ester for both monomers and dimers. Since optical purity of a peptide can be optimized by maximizing k_c/k_r ratio[9], it appears that it is safer (less racemization) to use, for the coupling of alanine monomers and dimers, the OPcp esters than the widely used ONp esters.

Our hypothesis on a smaller degree of racemization expected with the OPcp ester than with the ONp ester was tested by coupling monomers and dimers of these esters with valine methyl ester. The kinetics of these reactions was studied and after the reaction had proceeded to 99% completion the peptides were isolated and characterized. If a smaller

degree of racemization occurred with the OPcp esters than with the ONp esters, we would expect to isolate products with higher specific rotation. The results of these experiments (Table II) and the specific rotation data indicate no differences between these values. However, comparing the degree of racemization produced by different coupling methods, based on comparisons of optical rotations of final products, it is not considered to be a sensitive method and, hence, our conclusions on the lack of difference in racemization production by these esters should be considered approximate. In order to verify the existence of smaller differences in racemization produced by these esters, sensitive methods such as isotopic dilution procedure should be used[4,11].

Although in the case of alanine which has very low tendency to racemize, the k_c/k_r values did not prove to be a parameter sensitive enough to help choose a better coupling ester, it can be quite indicative of racemization tendency of amino acids. In the case of histidine-active esters our group has reported the lowest k_c/k_r than for any other amino acid studied. The lowest k_c/k_r value indicates an extremely high tendency to racemize and this is supported by literature studies on the unusually high racemization (50%) produced during the couplings of Z-His[12,13].

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