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Short communication

Determination of coupling rate constants of *N*-t-Butyloxycarbonyl–amino acid-active esters

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Abstract

To determine the relative coupling rates of five commonly used active esters in peptide synthesis, *i.e.*, pentafluorophenyl (OPfp), pentachlorophenyl (OPcp), *p*-nitrophenyl (ONp), 2, 4, 5 trichlorophenyl (OTcp) and succinimidyl (OSu), of three Boc-amino acid (Ala, Phe and Cys (Bzl)), second-order coupling rate constants (K_c) were determined with L-valine methyl ester (Val-OMe) in THF 23°C. As expected, the active ester group had a significant effect on the K_c value, while the side chain of the amino acid had a less significant effect. The active esters Boc-amino acids of couple slower than the corresponding Z-amino-acid-active esters in most cases.

Key words: Coupling rates, amino acids, activated esters.

1. Introduction

Relative coupling rates of commonly used active esters in peptide synthesis would provide useful information and despite the need for such information little data exist in the literature[1–4].

Our group has reported the relative K_c values of five commonly used active esters (OPfp, OPcp, ONp, OTcp and OSu) of several Z- and Z-Gly-amino acids[5–7]. Besides the Z group, another very commonly used N-protecting group in peptide synthesis is the Boc group and in this paper we report our results on the K_c values of the above five active esters of three Boc amino acids (Ala, Phe and Cys (Bzl) with Val-OMe in THF. In addition we have compared the relative rate constants of the above three Boc- and Z-amino acids.

2. Experimental

Boc- and Z-amino-acid-active esters were prepared by the standard DCC method and the physical constants for most compounds agreed well with the literature values. The physical constants of the compounds whose values did not agree with the literature values are listed in Table I. Melting points were obtained on a Thomas-Hoover melting point apparatus

Table I
Physical constants of compounds whose values did not agree well with literature values

Compound	Value	MP(°C)	Sp. rotation	Elemental analysis (%)			
					C	H	N
Boc-Ala-OPfp	Revised	83–85	$[\alpha]_D^{23}$-42.8 (C 0.95, dioxane)	Calcd	47.33	3.97	3.94
				Found	47.35	3.97	3.8
	Lit (9)	83–85	$[\alpha]$-31.2 (c 1, dioxane)				
Boc-Ala-OSu	Revised	158–162	$[\alpha]$-53.5 (c 2, dioxane)	Calcd	50.34	6.34	9.79
				Found	50.15	6.30	9.54
	Lit (10)	148–144	$[\alpha]_D^{23}$-49 (c 2, dioxane)				
Boc-Phe-OTcp	Revised	117–119	$[\alpha]_D^{22}$-38.8 (c 2, DMF)	Calcd	54.01	4.53	3.15
				Found	54.01	4.59	3.12
	Lit (1)	122	$[\alpha]_D^{23}$-27.00 (c 2, DMF)				
Boc-Phe-OPcp	Revised	161–162.5	$[\alpha]_D^{23}$-39.9 (c 5.25, THF)	Calcd	46.76	3.50	2.72
				Found	46.79	3.46	2.60
	Lit (11)	148–150	$[\alpha]_D^{22}$-48 (c 2, EtoAc)				
Boc-Cys-(Bzl)OPcp	Revised	154–156	$[\alpha]_D^{23}$-46.5 (c 1.7, DMF)	Calcd	45.06	3.60	2.50
				Found	44.92	3.67	2.56
	Lit (11)	154	$[\alpha]_D^{22}$-38.2 (c 1.7, DMF)				
Boc-Cys(Bzl)-OTcp	Revised	76–78	$[\alpha]_D^{20}$-43.2 (c 1, DMF)				
	Lit (12)	77–78	$[\alpha]$ (Not reported)				

in open capillaries and are uncorrected. Optical rotations were determined on a Rudolph spectropolarimeter, Model 200S-34-800G. Kinetic data were obtained on a Perkin-Elmer Infracord using 0.1 mm NaCl-matched cells. The concentration of active ester at any determined time was calculated from absorbance data of the active ester around 5.6 u. In general, 7–10 data points were obtained during the course of the reaction. The reaction was determined to be of second order, since a plot of reciprocal of active ester concentration (M^{-1}) remaining at a given time (t) was linear, when initial equimolar conc. (0.13 M) of active ester and Val-OMe methyl was used. The second-order coupling rate constant was determined from the slope of the above plot. After the reactions were about 99% complete the products were isolated and characterized. The physical constants of the resulting dipeptides agreed well with the literature values.

3. Results and discussion

3.1. *Coupling of Boc-amino-acid-active esters*

The second-order K_c of Z- and Boc-amio-acid-active esters with Val-OMe in THF at 23°C is reported in Table II. The values listed in parentheses are predicted rate constants based on the additivity principle[6].

Table II
Experimental and predicted second-order coupling rate constants (K_c) for the reaction of Z- and Boc-amino-acid-active esters with Val-OMe

Z-L-amino-acid-active ester[6]	$K_c \times 10^{-2}\,M^{-1}\,sec^{-1}$	Boc-L-amino-acid-active ester	$K_c \times 10^{-2}\,M^{-1}\,sec^{-1}$	$K_c(Z)$ / K_c (Boc)
Z-Ala-OSu	7.7 (6.7)	Boc-Ala-OSu	4.3 ± 0.35 (5.7)	1.8
Z-Ala-OPfp	19.3 (18.7)	Boc-Ala-OPfp	16.3 ± 1.72 (17.4)	1.2
Z-Ala-OTcp	0.30 (0.27)	Boc-Ala-OTcp	0.29 ± 0.005 (0.29)	1.1
Z-Ala-ONp	0.15 (0.11)	Boc-Ala-ONp	0.11 ± 0.018 (0.1)	1.4
Z-Ala-OPcp	0.506 (0.506)	Boc-Ala-OPcp	0.27 ± 0.008 (0.47)	1.9
Z-Phe-OSu	4.0 (4.7)	Boc-Phe-OSu	2.2 ± 0.15 (4.0)	1.8
Z-Phe-OPfp	11.9 (13.1)	Boc-Phe-OPfp	11.6 ± 0.38 (12.3)	1.0
Z-Phe-OTcp	0.20 (0.19)	Boc-Phe-OTcp	0.16 ± 0.02 (0.17)	1.3
Z-Phe-ONp	0.03 (0.08)	Boc-Phe-ONp	0.05 ± 0.01 (0.07)	0.60
Z-Phe-OPcp	0.29 (0.35)	Boc-Phe-OPcp	0.30 ± 0.05 (0.33)	0.97
Z-Cys(Bzl)-OSu	5.4 (8.6)	Boc-Cys(Bzl)-OSu	3.4 ± 0.34 (8.0)	1.6
Z-Cys(Bzl)-OPfp	40.4 (22.9)	Boc-Cys(Bzl)-OPfp	44.3 ± 4.0 (22.7)	0.92
Z-Cys(Bzl)-OTcp	0.30 (0.34)	Boc-Cys(Bzl)-OTcp	0.22 ± 0.005 (0.32)	1.4
Z-Cys-(Bzl)-ONp	0.11 (0.15)	Boc-Cys(Bzl)-ONp	0.13 ± 0.032 (0.13)	0.85
Z-Cys(Bzl)-OPcp	1.7 (0.66)	Boc-Cys(Bzl)-OPcp	0.67 ± 0.02 (0.61)	3.2

Most experiments were conducted at equimolar ratios of active ester and amine at 0.13 M conc. and results are the average of two experiments.

3.2. *Effect of the active ester group*

Since the active ester is directly attached to the carbonyl site, a very significant effect can be expected. As can be noted from Table I, the K_c values of the active esters of the three Boc-amino acids decrease as follows: OPfp > OSu > OPcp > OTcp > ONp except in the case of Ala where OPcp ≃ OTcp.

The above coupling rate also represents the order of electron-withdrawing ability of groups except in the case of pentafluorophenyl which has roughly the same electron-withdrawing ability of the pentachlorophenyl group. However, the OPfp esters couple significantly faster than the OPcp esters as shown in this study and other studies reported by our laboratory[4–7]. This property of pentafluorophenyl esters is attributed to neighbouring group participation by fluorine as well as to the excellent solvation of pentafluorophenyl leaving group[8].

In addition, it is interesting to note that the *p*-nitrophenyl esters, one of the active esters most commonly used in peptide synthesis, couple significantly slower than lesser used OPfp and OPcp esters.

3.3. *Effect of the amino-acid side chain*

Since the side chain of the amino acid is not directly attached to the carbonyl carbon, the site of the new bond formation, a less significant effect on the K_c value is expected than

by variation of the active ester group. This can be confirmed by this study and other studies reported by our group[5–7]. The K_c values of Boc-amino acids showed no definite trends in the K_c values.

3.4. *Effect of the N-protecting group*

As can be observed from the coupling ratio of the Z- and Boc-amino acids (Table II), the Z-amino active esters couple faster than the corresponding Boc-amino-acid-active esters in most cases. The difference is probably due to steric factors.

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