Asymmetric Induction during the Aminolysis of 5(4*H*)-Oxazolones from *N*-Benzoyl Amino Acids; Almost Specific Formation of One Epimer in the Reaction of the Oxazolone from *N*-Benzoyl-DL-*t*-leucine with Methyl L-Prolinate

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Synopsis. Asymmetric induction during the aminolysis of 5(4*H*)-oxazolones from *N*-benzoyl amino acids was investigated using a series of amino acid esters as amine nucleophiles. The reaction of the oxazolone from *N*-benzoyl-DL-*t*-leucine with methyl L-prolinate was found to produce the diastereomeric D-L isomer, almost specifically, under appropriate conditions.

The formation of a chirally labile 5(4*H*)-oxazolone has been regarded as one of the main causes of racemization during segment coupling in peptide synthesis.[1] When a racemic oxazolone is allowed to react with an optically active amino acid ester, the peptide product is usually not a 1:1 mixture of diastereomers because of the occurrence of asymmetric induction. However, such asymmetric induction, reported first by Weygand et al.,[2] has not hitherto been explicitly taken into account in the problem of racemization during the segment coupling of two chiral residues.[3] Recently, Benoiton et al.[4] have studied the solvent and temperature effects on asymmetric induction during the aminolysis of the oxazolones from Bz-DL-Leu[5] and Z-Gly-DL-Leu with N^{ε}-Z-L-lysine esters, and have showed that the diastereomeric D-L isomer is formed in excess in a non-polar solvent (dichloromethane or THF) and the L-L isomer is formed in excess in a polar solvent (DMF), the proportion of the L-L isomer increasing with decreasing temperature. On the contrary, in the aminolysis of the pseudooxazolones from *N*-trifluoroacetyl amino acids in THF, it has been reported that the L-L isomer is formed in excess when the esters of amino acids (except proline, as mentioned below) are used as amine nucleophiles.[2b,6] These results indicate that the direction of asymmetric induction depends not only on the solvents but also on the nature of the reactants. It is difficult to draw general conclusions concerning the direction and the extent of asymmetric induction, because relevant experimental data have so far been very limited. This is partly due to the restriction of the methods for the analysis of peptide diastereomers.

We have recently found that diastereomeric pairs of a variety of *N*-acyl dipeptide esters can be separated well by reversed phase high performance liquid chromatography (RP-HPLC).[7] Taking advantage of this procedure we set about to conduct a further study on asymmetric induction in peptide synthesis from the viewpoint of the substituent, solvent, and temperature effects. During the course of this investigation we found a reaction system which generates the largest degree of induction reported to date.

We first investigated asymmetric induction during the aminolysis of the oxazolone from Bz-DL-Val with a series of L-amino acid esters both in a representative non-polar solvent, dichloromethane, and in a representative polar solvent, DMF. As shown in Table 1, even in DMF it is only when L-alanine methyl ester was used as an amine nucleophile that the L-L isomer

Table 1. Asymmetric Induction during the Aminolysis of the 5(4*H*)-Oxazolones from Bz-DL-Val and Bz-DL-Leu with a Series of L-AA-OMe[a,b]

AA	5(4*H*)-Oxazolone from Bz-DL-Val		5(4*H*)-Oxazolone from Bz-DL-Leu
	in CH_2Cl_2	in DMF	in DMF
Ala	62	46	47
Abu	65	60	56
Val	67	65	64
Leu	67	63	59
Nle	78	60	55
Ile	64	66	64
Hep	63	60	57
Pro[c]	84	72	72
Phe	73	58	56
Tyr	66	64	
Phg	57	53	51
Ser	—[d]	—[d]	51
Met	74	51	

a) Data given as % D-L peptide formed. Duration and temperature of the reaction: 24 h at 5 °C. b) The amino acid esters were used in the form of hydrochloride in the presence of an equimolar amount of TEA. c) *p*-Toluenesulfonate. d) The diastereomers of the resulting peptide were not separated.

was formed in excess, while the D-L isomer was the predominant one in other cases. This was also the case with the aminolysis of the oxazolone from Bz-DL-Leu. When the solvent was changed from DMF to dichloromethane, the proportion of the D-L isomer increased in most cases, though there was observed one exception where L-isoleucine methyl ester was used as an amine nucleophile.

Amine nucleophiles had a profound effect on asymmetric induction. The most prominent was the fact that the proportion of the D-L isomer was exceedingly large both in dichloromethane and in DMF when L-proline methyl ester was used as an amine nucleophile. In the aminolysis of 2-trifluoromethyl-5(2*H*)-oxazolones, it has been reported that when an L-proline ester is used as an amine nucleophile, the direction of asymmetric induction is reversed, that is, the D-L isomer is formed predominantly in this case, in contrast to the cases using other L-amino acid esters which generate the L-L isomer in excess.[6] The direction of asymmetric induction during the aminolysis of 2-phenyl-5(4*H*)-oxazolones with proline methyl ester, however, was the same as that caused by the other amino acid esters as amine nucleophiles.

Next, using the proline ester as an amine nucleophile, we examined the aminolysis of the oxazolone derived from *t*-leucine (2-amino-3,3-dimethylbutanoic acid, Tle),[8] an unusual amino acid with a bulkier side chain than valine, in expectation of the generation of a higher degree of asymmetric induction. Thus, the oxazolone from Bz-DL-Tle was allowed to react with L-proline methyl ester in a variety of solvents at different temperatures (50 to −20 °C). The results are summarized in Table 2. Under all the conditions examined the D-L isomer was formed in excess to a large extent (≥70% D-L isomer), as expected. The effect of the solvent was extremely significant. Thus, the proportion of the D-L isomer was smallest in DMF and became largest in aromatic hydrocarbons. A similar effect of aromatic hydrocarbons was observed in the aminolysis (24 h at 0 °C) of other oxazolones with L-proline methyl ester as follows: the percentage of D-Val-L-Pro formed in the aminolysis of the oxazolone from Bz-DL-Val, 66 (DMF), 87 (toluene), 88 (*m*-xylene), 89 (*o*-xylene); percentage of D-aIle-L-Pro formed in the aminolysis of the oxazolone from Bz-DL-Ile, 79 (DMF), 92 (toluene), 92 (*m*-xylene), 92 (*o*-xylene). The effect of the temperature was also profound, especially in less apolar solvents. A lower temperature favored the formation of the D-L isomer in the present case. As a result of both the solvent and the temperature effects, the D-L isomer was formed almost specifically (97% D-L isomer) in the aminolysis of the oxazolone from Bz-DL-Tle in toluene or xylenes at −20 °C. This is the largest degree of asymmetric induction during the aminolysis of the oxazolones reported to date,[9] and makes a unique example of nearly exclusive formation of one epimer via the kinetic resolution of stereochemically labile enantiomers by a chiral reagent.

Table 2. Solvent and Temperature Effects on the Asymmetric Induction during the Aminolysis of the 5(4*H*)-Oxazolone from Bz-DL-Tle with L-Pro-OMe[a,b]

Solvent	Temp/°C			
	50	30	0	−20
DMF	70	76	82	86
Pyridine		79	86	88
CH_2Cl_2		82	92	95
Acetonitrile		84	91	95
Benzene	75	86		
Dioxane	74	89		
THF	87	89	92	93
Ethyl acetate		90	93	95
Toluene		90	95	97
Mesitylene		92	95	96
m-Xylene		92	96	97
o-Xylene	78	93	96	97

a) Data given as % D-L peptide formed. Duration of the reaction: 24 h. b) The amino acid ester was used in the form of *p*-toluenesulfonate in the presence of an equimolar amount of TEA.

Experimental

Optical rotations were measured with a JASCO DIP-4 polarimeter. TLC and preparative TLC were performed on Merck Kieselgel $60F_{254}$ and Kieselgel GF_{254} (Type 60), respectively. The liquid chromatograph employed was a Shimadzu LC-3A instrument, equipped with a Shimadzu SPD-2A variable wavelength UV monitor and a Shimadzu Chromatopac C-R1A data processor. A prepacked ODS column (Cosmosil $5C_{18}$) consisted of a stainless-steel tube, 4.6 mm I.D. and 150 mm long. Bz-DL-Val-OH, Bz-DL-Leu-OH, Bz-DL-Ile-OH, and Bz-DL-Tle-OH[8b] were obtained as described by Greenstein and Winitz.[10] They were converted to 5(4*H*)-oxazolones using DCC or EDC·HCl according to methods described in the literature.[11,12]

HPLC Separation of the Diastereomers of *N*-Benzoyl Dipeptide Methyl Esters. Bz-DL-Val-OH (0.15 mmol) was coupled with H-L-AA-OMe·HCl (or TosOH) (where AA denotes the amino acid residues listed in Table 3) (0.15 mmol) in CH_2Cl_2 (2 ml) at 0 °C in the presence of TEA (0.14 mmol) by the DCC method. The neutral fraction was separated from the reaction mixture as usual. This fraction was submitted to a separation of the diastereomers by RP-HPLC under the conditions shown in Table 3. On the other hand, authentic samples to identify the L-L isomer on the chromatogram were prepared as follows: H-L-Val-L-AA-OMe·HCl (prepared via the coupling of Z-L-Val-OH (1 mmol) with H-L-AA-OMe·HCl (or TosOH) (1 mmol) in the presence of TEA (1 mmol) by means of EDC·HCl (1 mmol) and the subsequent debenzyloxycarbonylation by hydrogenation in the presence of 5% Pd-C and concd HCl) was dissolved in $CHCl_3$ (12 ml) and treated with benzoyl chloride (1.5 mmol) in the presence of TEA (2.2 mmol) for several hours. The neutral fraction was separated from the reaction mixture as usual. The capacity factors ($k'=(t_R-t_0)/t_0$, where t_R=retention time and t_0=void time) and the separation factors ($\alpha=k'_{D-L}/k'_{L-L}$) are listed in Table 3. The diastereomers of Bz-D/L-Val-L-AA-OMe for AA=Ser, Thr, Asp(OMe), Asn, Glu(OMe), Gln, Trp, and His could not be separated ($\alpha=1$) under any conditions examined.

Samples of a diastereomer mixture (L-L isomer + D-L isomer) and the corresponding L-L isomer for Bz-Leu-AA-OMe were prepared in the same manner as mentioned above.

Table 3. HPLC Separation[a] of the Diastereomers of Bz-Val-AA-OMe and Bz-Leu-AA-OMe

AA	Bz-Val-AA-OMe				Bz-Leu-AA-OMe			
	Conditions[b]	k'_{L-L}	k'_{D-L}	α	Conditions[b]	k'_{L-L}	k'_{D-L}	α
Ala	A	2.86	3.01	1.05	C	7.56	8.49	1.12
Abu	A	4.32	4.89	1.13	A	7.02	8.25	1.17
Val	B	3.42	4.05	1.18	B	5.41	6.45	1.19
Leu	B	5.76	6.70	1.16	B	8.46	10.23	1.21
Nle	B	6.37	7.36	1.16	B	9.41	11.37	1.21
Ile	B	5.61	6.68	1.19	B	8.52	10.42	1.22
Hep	B	11.63	13.69	1.18	B	16.94	20.85	1.23
Pro	A	4.45	3.86	1.15[c]	C	13.83	13.03	1.06[c]
Phe	B	5.86	6.77	1.16	B	8.75	9.98	1.14
Tyr	A	3.71	3.47	1.07[c]	A	6.06	5.43	1.12[c]
Phg	B	4.17	4.59	1.10	B	6.20	7.39	1.19
Ser	C	2.97	2.97	1.00	D	7.08	6.65	1.07
Met	A	5.76	6.24	1.08	A	9.60	10.19	1.10

a) Capacity factor, $k'=(t_R-t_0)/t_0$, where t_R=retention time and t_0=void time. Separation factor, $\alpha=k'_{D-L}/k'_{L-L}$. b) A: mobile phase, 50% MeOH aq; flow rate, 1.0 ml min^{-1}; column temp, 50 °C. B: mobile phase, 60% MeOH aq; flow rate, 1.0 ml min^{-1}; column temp, 30 °C. C: mobile phase, 45% MeOH aq; flow rate, 1.0 ml min^{-1}; column temp, 50 °C. D: mobile phase, 45% MeOH aq; flow rate, 0.8 ml min^{-1}; column temp, 50 °C. c) In this case, $\alpha=k'_{L-L}/k'_{D-L}$.

Their HPLC data are also listed in Table 3. The diastereomers of Bz-D/L-Leu-L-AA-OMe for AA=Thr, Asp(OMe), Asn, Glu(OMe), Gln, and Trp could not be separated under any of the conditions examined.

Samples of a diastereomer mixture, L-Ile-L-Pro + D-aIle-L-Pro, and the corresponding L-L isomer were prepared in the same manner as mentioned above: k' for D-aIle-L-Pro was 3.67 and k' for L-Ile-L-Pro 4.30, and α=1.17 under the conditions B in the footnote b of Table 3.

Bz-L-Tle-L-Pro-OMe and Bz-D-Tle-L-Pro-OMe. These were obtained through a treatment of H-L(or D)-Tle-L-Pro-OMe·HCl (prepared in the same manner as described above for the valyl peptide derivatives) with benzoyl chloride in the presence of *N,N*-diisopropylethylamine in $CHCl_3$. L-L Isomer: syrup, purified by preparative TLC on silica gel with $CHCl_3$-EtOAc (9:1); $[\alpha]_D^{25}$ −70.5° (*c* 1.5, MeOH); Anal. ($C_{19}H_{26}N_2O_4$) C, H, N. D-L Isomer: mp 123—124 °C (uncorrected) (EtOAc-petroleum ether); $[\alpha]_D^{22}$ −26.1° (*c* 1.0, MeOH); Anal. ($C_{19}H_{26}N_2O_4$) C, H, N. HPLC k'_{L-L}=5.20, k'_{D-L}=4.58, and α=1.14 (t_0=ca. 3.3 min) under the following conditions: mobile phase, 50% MeOH aq; flow rate, 1.0 ml min^{-1}; column temperature, 30 °C; detection, 254 nm; sensitivity range, 0.02 absorbance units full scale (a.u.f.s.).

Aminolysis of 5(4*H*)-Oxazolones with L-Amino Acid Methyl Esters. A 5(4*H*)-oxazolone (0.15 mmol) and H-L-AA-OMe·HCl (or TosOH) (where AA denotes the amino acid residues listed in Table 1) (0.15 mmol) were dissolved (or suspended) in 1.5 ml of solvent, and the mixture was placed into a thermostatted bath (−20 °C, 0 °C, 5 °C, 30 °C, or 50 °C). After 15 min, a solution of TEA (0.15 mmol) in 0.5 ml of the solvent was added and the reaction mixture was stirred for 24 h at the set temperature. After an addition of EtOAc (20 ml), the organic layer was separated, washed with 5% HCl, water, sat. $NaHCO_3$, and water, and dried over Na_2SO_4. One part of the residue obtained by the evaporation of the solvent was dissolved in MeOH and submitted to HPLC analysis. The results are summarized in Tables 1 and 2.

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5) Abbreviations given by the IUPAC-IUB Joint Commission (*Eur. J. Biochem.*, **138**, 9 (1984) are used throughout. Additional abbreviations: Bz, benzoyl; Z, benzyloxycarbonyl; THF, tetrahydrofuran; DMF, *N,N*-dimethylformamide; Abu, 2-aminobutanoic acid; Nle, norleucine; Hep, heptyline (2-aminoheptanoic acid); Phg, *C*-phenylglycine; aIle, alloisoleucine; DCC, dicyclohexylcarbodiimide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; TosOH, *p*-toluene-sulfonic acid; TEA, triethylamine.

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