Dynamic Kinetic Resolution of *N*-Benzoyl-DL-Amino Acids *via* Peptide Bond Forming Reactions

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Abstract: Dynamic kinetic resolution (DKR) was demonstrated in the carbodiimide-mediated couplings of *N*-benzoyl-DL-amino acids with L-amino acid esters: the yields of the D-L-peptides significantly exceeded 50% in some cases. *N*-Benzoyl-DL-*t*-leucine afforded the D-L-peptide almost exclusively (up to 96% yield) in the reaction with methyl L-prolinate, which is the most efficient DKR obtained in the field of amino acids and derivatives.

Keywords: Dynamic kinetic resolution (DKR), carbodiimide-mediated couplings, *N*-benzoyl-DL-amino acids, 5(4*H*)-oxazolones.

INTRODUCTION

Kinetic resolution of racemic mixtures is still one of the most common ways of obtaining enantiomerically enriched compounds in industry in spite of the successful development of asymmetric syntheses. With this process, however, the maximum yield of one enantiomer is intrinsically only 50%. This limitation can be overcome by dynamic kinetic resolution (DKR) [1], where the enantiomers undergo rapid interconversion under the reaction conditions and the desired enantiomer can be theoretically obtained in a quantitative yield with enantiomeric excess approaching 100%. Therefore, increasing attention has been drawn to the discovery of DKR processes. *N*-Acyl-amino acids such as *N*-Benzoyl (Bz)-amino acids are known to be extremely prone to racemization during the coupling process. Their chiral lability is the basis for the well-known Young racemization test [2], which addresses the degree of racemization during the coupling of Bz-L-Leu with Gly-OEt by different coupling methods and conditions. This was followed by the Davies test utilizing the coupling of Bz-L-Val [3]. Of all racemization tests, those involving *N*-Bz-amino acids are known to be the most sensitive [4]. We have already reported [5] that in the EDC-mediated coupling of Bz-L-Val with L-Val-OMe in DMF the percentage of the D-L-isomer in the diastereomeric dipeptide products greatly exceeded 50% (D-L-isomer: 64%), and that the use of racemization-suppressing *N*-hydroxy additives such as HOBt [6] had only a limited effect (D-L-isomer: 48%). In view of these facts, we have come to an expectation that there will be a fair chance of discovering DKR during the peptide bond formation of *N*-Bz-DL-amino acids by the carbodiimide method to preferentially afford one of the diastereomers of the resulting peptide. In practice, we obtained the most efficient example of DKR of amino acid derivatives.

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MATERIALS AND METHODS

N-Bz-DL-amino acids were prepared using benzoyl chloride (Bz-Cl) under the Schotten-Baumann conditions [7]. They were converted to 5(4*H*)-oxazolones using DCC according to the literature [8]. The methyl esters of L-amino acids were mainly prepared as their hydrochlorides by the use of MeOH and thionyl chloride [9]. L-Pro-OMe·TosOH was purchased from Peptide Institute, Inc. Other chemicals were obtained from Wako Pure Chemical Industries, Tokyo Chemical Industry or Aldrich Chemical Co. All solvents were distilled and dried over molecular sieves prior to use.

Couplings of *N*-Benzoyl-DL-Amino Acids (Bz-DL-Xaa) with L-Amino Acid Methyl Esters (L-Xbb-OMe) by the EDC Method

Bz-DL-Xaa (0.15 mmol), L-Xbb-OMe·HCl (or L-Pro-OMe·TosOH) (0.15 mmol) and TEA (20.9 µl, 0.15 mmol) with or without an additive (see Table **1**) were suspended in DCM (1 ml) at 0 °C. After 15 min of stirring, EDC·HCl (28.8 mg, 0.15 mmol) and DCM (1 ml) were added at this temperature. The resulting mixture was stirred at 0 °C for 2 h and then at 25 °C for 48 h or 72 h. After evaporation of the solvent, followed by the addition of Bz-L-Val-L-Val-OEt (17.4 mg, 0.05 mmol) as an internal standard, the residue was distributed between ethyl acetate and 2M HCl, and the organic layer was further washed with 2M HCl, saturated brine, saturated $NaHCO_3$ and saturated brine again, and dried over Na_2SO_4. The residue after evaporation of the solvent was dissolved in acetonitrile and injected onto an ODS column for the HPLC separation of the diastereomers of the resulting peptide. At the same time the yield of the peptide formed was determined by the internal standard method.

Reactions of the 5(4*H*)-Oxazolones from *N*-Benzoyl-DL-Amino Acids (Bz-DL-Xaa) with L-Pro-OMe

The 5(4*H*)-oxazolone (0.2 mmol) from Bz-DL-Xaa, L-Pro-OMe·TosOH (60.1 mg, 0.2 mmol) and Bz-L-Val-L-Val-OEt (17.4 mg, 0.05 mmol) as an internal standard were sus-

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pended in DCM (2 ml). After 15 min, a solution of TEA (27.9 µl, 0.2 mmol) in DCM (0.7 ml) was added and the reaction mixture was stirred at 25 °C for 72 h. An aliquot of the reaction mixture was withdrawn, diluted with acetic acid and then injected on to the HPLC column.

HPLC Analysis

HPLC analyses were performed on a GL Sciences PU610 instrument, equipped with a Rheodyne 8125 sample injector and a GL Sciences UV620 variable wavelength UV monitor. A Shimadzu C-R6A data processor was used for data acquisition and processing. Typical HPLC conditions were as follows: column, Inertsil ODS-3 (1.5 mm i.d. × 150 mm) (GL Sciences); mobile phase, 40–60% (by volume) MeOH aq.; flow rate 0.1 ml min^{-1}; column temperature, 30–40 °C; detection, UV at 254 nm.

RESULTS AND DISCUSSION

To investigate the possibility of DKR in the coupling process we took advantage of the good separation of the diastereomeric pairs of *N*-Bz-dipeptide esters by reversed-phase HPLC [10], which also permitted the precise determination of the coupling yield by the use of an internal standard compound. Table **1** shows the diastereomeric ratio (D-L : L-L) and the yields of the D-L- and L-L-peptides during the coupling of Bz-DL-Tle with L-Pro-OMe by the EDC method with or without an additive in DCM. The coupling reaction proceeded to completion within 48 h and the yield of the D-L-peptide surprisingly reached 96%, clearly demonstrating the occurrence of DKR (entry 1). The addition of some basic compounds was next examined in expectation of facilitating racemization. However, the use of DABCO and DBU [11] resulted in a slight retardation of the coupling reaction (entries 2 and 3). It is only when the chiral base, brucine, coexisted in the system that the yield of the D-L-peptide became only a little higher (97%) (entry 4). On the contrary, when the racemization-suppressing additive, HOBt, was employed, the yield of the D-L-peptide dropped to 89% (entry 5). The addition of DMAP [12] only resulted in a slight retardation of the coupling reaction (entry 6). The solvent effect on the same EDC-mediated coupling was next examined (Table **2**). In all the solvents used the D-L-peptide was produced in good to excellent yields far larger than 50%, consistent with the desired DKR process. However, the yield of the D-L-peptide was relatively low in the most polar solvent DMF and in the most apolar solvent *o*-xylene. A better solvent than DCM was not found.

Table 1. Coupling of Bz-DL-Tle with L-Pro-OMe by the EDC Method with or without an Additive in DCM[a]

			Yield (%)	
Entry	**Additive (equiv.)**	**D-L : L-L**	**D-L**	**L-L**
1	None	96 : 4	**96**	4
2	DABCO (0.67)	97 : 3	**94**	3
3	DBU (0.67)	97 : 3	**88**	3
4	Brucine (0.67)	97 : 3	**97**	3
5	HOBt (1)	90 : 10	**89**	10
6	DMAP (1)	96 : 4	**94**	4

[a]Reaction conditions: Bz-DL-Tle/L-Pro-OMe·TosOH/TEA/EDC·HCl, 1:1:1:1 equiv.; reactant concentration, 0.075 M.; duration and temperature, 2 h at 0 °C, then 48 h at 25 °C.

Table 2. Solvent Effect on the Coupling of Bz-DL-Tle with L-Pro-OMe by the EDC Method[a]

		Yield (%)	
Solvent	**D-L : L-L**	**D-L**	**L-L**
o-Xylene	92 : 8	**80**	7
Toluene	93 : 7	**92**	7
DCM	96 : 4	**96**	4
Chloroform	90 : 10	**89**	10
Ethyl acetate	92 : 8	**92**	8
Tetrahydrofuran	93 : 7	**93**	7
Acetonitrile	96 : 4	**95**	4
DMF	84 : 16	**66**	13

[a]Reaction conditions: Bz-DL-Tle/L-Pro-OMe·TosOH/TEA/EDC·HCl, 1:1:1:1 equiv.; reactant concentration, 0.075 M.; duration and temperature, 2 h at 0 °C, then 48 h at 25 °C.

Based on the above results, the *N*-Bz derivatives of several DL-amino acids (Bz-DL-Xaa) were allowed to react with L-Pro-OMe by the EDC method in DCM (Table **3**). With Val and Leu as the carboxyl components the results clearly demonstrate the DKR process, though the yields of the D-L-peptides were lower than that obtained with Tle. The influence of the amino components (L-Xbb-OMe) was next examined on the coupling of Bz-Tle, and the results are also included in Table **3**. It is only when Xbb was Leu besides Pro that the desired DKR process was unambiguously demonstrated. The coupling reactions were retarded to a great extent with other amino acids as the amino components.

It transpires that the formation of 5(4*H*)-oxazolone intermediates can account for much of the racemization occurring during the couplings of *N*-Acyl-amino acids or peptides [4]. The oxazolones racemize easily due to the low p*K*a value of the C-4 proton. They can undergo facile ring opening with a variety of nucleophiles to give amino acid derivatives. Thus, oxazolones make useful substrates for DKR. In fact, the DKR reactions of oxazolones have been reported mainly *via* enzymatic transformations such as lipase-catalyzed hydrolysis or alcoholysis [13, 14]. We previously reported on the asymmetric induction during the aminolysis of the oxazolones from *N*-Bz-DL-amino acids with a series of L-amino acid esters [10]. In some cases the diastereomeric ratios were found to go too far from 50:50, but it was obscure whether this was ascribed to DKR or not, because the yields of the peptide products were not quantified at that time. Therefore, the reactions of the oxazolones from *N*-Bz-DL-amino acids (Bz-DL-Xaa) with L-Pro-OMe in DCM were investigated in connection with the corresponding EDC-mediated couplings of Bz-DL-Xaa. The results are shown in Table **4**. The DKR processes were clearly observed in the reactions of the oxazolones from Val, Leu and Tle. Roughly speaking, the results are like those shown in Table **3** for the couplings by the EDC method. However, when Xaa was Tle or Phe, the yields of D-L-peptides were lower than those obtained by the EDC-mediated couplings.

In the EDC-mediated coupling of Bz-DL-Tle with L-Pro-OMe, the carboxyl component transforms into activated intermediate(s) (represented by Bz-Tle-X) by the action of EDC (Scheme **1**) [15]. The enantiomers of Bz-Tle-X un-

Table 3. Couplings of *N*-benzoyl-DL-Amino Acids (Bz-DL-Xaa) with L-Amino Acid Methyl Esters (L-Xbb-OMe) by the EDC Method in DCM[a]

			Yield (%)	
Xaa	**Xbb[b]**	**D-L : L-L**	**D-L**	**L-L**
Ala	Pro	89 : 11	44	5
Val	Pro	91 : 9	**82**	8
Leu	Pro	89 : 11	**77**	9
Tle	Pro	96 : 4	**96**	4
	Ala	60 : 40	37	25
	Val	67 : 33	42	21
	Leu	60 : 40	**50**	33
	Phe	57 : 43	39	30
Phe	Pro	86 : 14	42	6

[a]Reaction conditions: Bz-DL-Xaa/L-Xbb-OMe·HCl (or TosOH)/TEA/EDC·HCl, 1:1:1:1 equiv.; reactant concentration, 0.075 M; duration and temperature, 2 h at 0 °C, then 72 h at 25 °C.

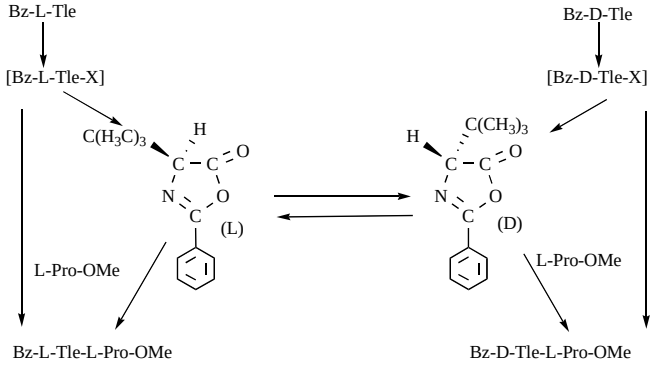
[b]Used in the form of hydrochlorides. L-Pro-OMe was used in the form of *p*-toluenesulfonate.

Table 4. Reactions of the 5(4*H*)-Oxazolones from *N*-Benzoyl-DL-Amino Acids (Bz-DL-Xaa) with L-Pro-OMe in DCM[a]

		Yield (%)	
Xaa	**D-L : L-L**	**D-L**	**L-L**
Ala	86 : 14	48	8
Val	87 : 13	**82**	12
Leu	85 : 15	**74**	13
Tle	90 : 10	**88**	10
Phe	85 : 15	33	6

[a]Reaction conditions: 5(4*H*)-oxazolone from Bz-DL-Xaa/L-Pro-OMe·TosOH/TEA, 1:1:1 equiv.; reactant concentration, 0.074 M; duration and temperature, 72 h at 25 °C.

dergo isomerization mainly *via* the enantiomeric oxazolones which interconvert each other quite rapidly. Then the peptide products are produced mainly *via* the oxazolones and the reaction of the D-oxazolone with L-Pro-OMe must be much faster than that of the L-oxazolone. The same is probably true with the reactions of the enantiomers of Bz-Tle-X. As a consequence, the D-L-peptide can be formed almost exclusively. It is worthwhile to note that this is the most efficient DKR observed in the field of amino acids and derivatives including oxazolones reported to date [14] and makes a unique example of nearly exclusive formation of one epimer *via* the non-enzymatic DKR of stereochemically labile enantiomers by a chiral reagent.



Scheme 1. Possible mechanism for the EDC-mediated coupling of Bz-DL-Tle with L-Pro-OMe affording the D-L-peptide almost exclusively. Bz-Tle-X denotes the activated species derived from Bz-Tle.

ABBREVIATIONS

EDC = 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

DMF = *N*,*N*-Dimethylformamide

HOBt = 1-Hydroxybenzotriazole

DCC = *N*,*N*'- dicyclohexylcarbodiimide

TosOH = *p*-Toluenesulfonic acid

TEA = Triethylamine

DCM = Dichloromethane

ODS = Octadecylsilanized

HPLC = High performance liquid chromatography

Tle = *t*-Leucine (2-amino-3,3-dimethylbutanoic acid)

DABCO = 1,4-Diazabicyclo[2.2.2]octane

DBU = 1,8-Diazabicyclo[5.4.0]undec-7-ene

DMAP = 4-(Dimethylamino)pyridine.

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