Study of the effect of the nature of the side chain in esters of α-amino acids on the diastereoselectivity of condensation with 5(4*H*)-oxazolone in the synthesis of dipeptides with N-terminal *N*-acetylphenylalanine *

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Conditions for fast racemization of 5(4*H*)-oxazolones prepared from *N*-acylphenylalanine were found. Reactions of 4-benzyl-2-methyl-5(4*H*)-oxazolone with amino acid esters proceed diastereoselectively to give predominantly dipeptides comprising *R*-phenylalanine. The diastereoselectivity increases with complication of the structure of the side chain in the amino acid esters.

Key words: dynamic kinetic resolution, dipeptides, amino acids, racemization, oxazolone, NMR.

Derivatives of *N*-acyl amino acids are of considerable interest as optically labile intermediates suitable for the syntheses involving dynamic kinetic resolution.[1—4] Particular attention is attracted by 5(4*H*)-oxazolones, which are readily formed upon dehydration of acylamino acids.[5]

The synthesis of peptides by the "mixed anhydride" approach using the classical two-step procedure, which includes activation of the acid component with alkyl chloroformate in the presence of a tertiary base (preparation of a mixed anhydride) and its subsequent condensation with the amino component, is known to be accompanied by substantial racemization.[6] Activated *N*-acylamino acids easily cyclize under the action of bases to give 5(4*H*)-oxazolones, which react with amino acid esters to give mixtures of diastereomeric peptides. If racemization of 5(4*H*)-oxazolone is fast, the stereochemical outcome of the reaction is determined by the relative rates of the reaction of 5(4*H*)-oxazolone stereoisomers with the amino component. Thus, the dipeptide formation represents a dynamic kinetic resolution.[3,4] The search for conditions that favor racemization and enhance the difference between the rates of interaction of oxazolone isomers with the amino component can open up the way to stereoselective synthesis of the diastereomers of dipeptides containing (*R*)-amino acids that are difficult to obtain.

The process diastereoselectivity depends on many factors including the structure of the amino component, the nature of the base, the solvent, *etc*. In this work, 5(4*H*)-oxazolones prepared from *N*-acylphenylalanine were used as models for studying the factors that determine the stereochemical outcome of their reactions with ethyl esters of amino acids differing in the structure of the side chain.

Results and Discussion

It is known that the use of an excess of a base used in the synthesis of peptides by the "mixed anhydride" method favors racemization. We used Et_3N (1.2 equiv) as the base in the preparation of dipeptides by this method starting from *N*-acetylphenylalanine **1** and ethyl esters of amino acids **4—7** (Scheme 1).

The fact that the process carried out under these conditions is accompanied by fast and complete racemization was confirmed by the formation of a diastereomer mixture of the same composition from both (*S*)- and (*RS*)-*N*-acetylphenylalanine. With a higher excess of Et_3N, the yield of dipeptides decreased. The resulting diastereomer mixtures were analyzed by HPLC (Table 1). For assignment of diastereomer mixtures in HPLC analysis, pure (*S*,*S*)-diastereomers **8a—11a** prepared by a known procedure were used.[6] In this case, *N*-methylmorpholine was taken as the base, the activation time was 2 min, and all components introduced in the reaction were taken in equimolar ratios. It should be noted that, despite all precautions under-

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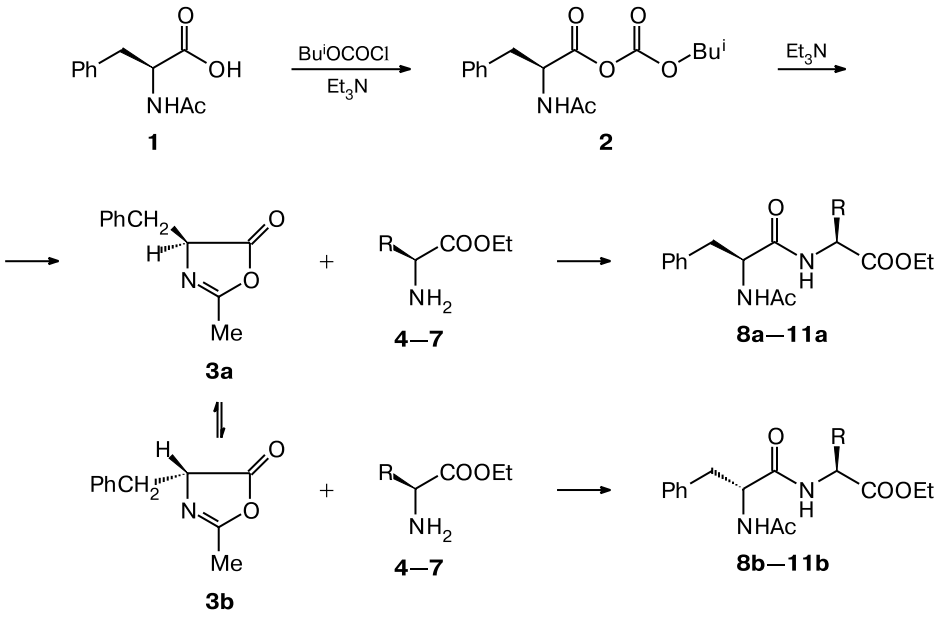
Scheme 1



R = Me (**4**, **8a,b**), CH_2Ph (**5**, **9a,b**), Pr^i (**6**, **10a,b**), $(CH_2)_2COOEt$ (**7**, **11a,b**)

Table 1. Some physicochemical properties of the synthesized dipeptides

Compound	M.p./°C	τ_R*/min	Mobile phase for HPLC
8a	153—155	5.67	Hexane—Pr^iOH—MeOH
8b		6.17	15 : 1 : 0.2
9a	154—155	4.20	Hexane—Pr^iOH—MeOH
9b		5.71	10 : 0.5 : 0.2
10a	119—121	3.97	Hexane—Pr^iOH—MeOH
10b		4.83	10 : 0.5 : 0.2
11a	135—137	5.46	Hexane—$CHCl_3$—
11b		6.06	Pr^iOH—MeCN
			40 : 3 : 3 : 3

* HPLC retention times of the diastereomers.

taken to suppress racemization, the dipeptides contained from 2 to 9% of the (*R*,*S*)-diastereomer. Recrystallization from acetone gave individual (*S*,*S*)-diastereomers. The structures of the compounds were proved by the data from elemental analysis and ^1H NMR spectroscopy (Table 2). The elemental analysis data are consistent with the structures of the products.

In all cases, the reaction gave diastereomer mixtures in which the (*R*,*S*)-diastereomer **8b—11b** of the corresponding dipeptide predominated (Table 3). The process diastereoselectivity depends on the nature of the side chain in the amino component. The amount of the (*R*,*S*)-diastereomer tends to increase following complication of the structure of the side chain in the amino component in the series Ala < Phe < Val < Glu. Thus, as in the case of 4-isopropyl-2-phenyl-5-oxazolone formed from *N*-benzoylvaline,[5] the (*R*)-isomer of 5(4*H*)-oxazolone (**3b**) reacts faster with (*S*)-amino esters, resulting in the predominant formation of the (*R*,*S*)-diastereomer of the dipeptide. (*S*)-Oxazolone (**3a**) reacts more slowly, and during the reaction it is converted into the (*R*)-isomer (**3b**). As a result of this dynamic kinetic resolution, the dipeptide diastereomer containing the *R*-phenylalanine predominates in the mixture (see Scheme 1).

^1H NMR and ^{13}C NMR spectroscopic studies gave direct evidence for the intermediate formation of 5(4*H*)-oxazolone. Following the addition of an equimolar amount of ethyl chloroformate to a solution of *N*-acetyl-(*S*)-phenylalanine and Et_3N in $CDCl_3$ at room temperature, a signal at δ 4.45 for the C(4)H proton of oxazolone as a doublet of doublets of quartets ($^3J_{C(4)H,CH_B} = 6.7$, $^3J_{C(4)H,CH_A} = 4.7$, $^5J_{C(4)H,CH_3} = 2.0$ Hz) was observed in the ^1H NMR spectrum, together with the signal with δ 4.73 corresponding to α-CH in the starting compound **1**. The doublet for the oxazolone methyl group is recorded at δ 2.09 and has a spin coupling constant of 2.0 Hz, whereas in the spectrum of the starting compound **1**, this signal occurs as a singlet at δ 1.96. The presence of long-range spin—spin interaction through five bonds between the C(4)H proton and the protons of the methyl group at C(2) was proved using a COSY-LR 2D experiment optimized for detecting long-range proton—proton coupling constants. This homoallylic interaction is a typical feature of the ^1H NMR spectra of

Table 2. Characteristic signals in the ^{1}H NMR spectra of dipeptides (δ, *J*/Hz)

Compound	Phe			Amino component		
	α-CH	β-CH_A	β-CH_B	α-CH	β-CH_n	γ-CH_n
8a	4.72 (dt, *J* = 8.0, *J* = 6.9)	3.06 (d, *J* = 6.9)		4.44 (dq, *J* = *J* = 7.1)	1.34 (d, *J* = 7.1)	—
8b	4.70 (td, *J* = 8.2, *J* = 6.2)	3.10 (dd, *J* = 13.6, *J* = 6.2)	2.99 (dd, *J* = 13.6, *J* = 8.2)	4.43 (dq, *J* = *J* = 7.2)	1.21 (d, *J* = 7.2)	—
9a	4.65 (dt, *J* = 7.5, *J* = 7.0)	3.02 (d, *J* = 7.0)		4.72 (ddd, *J* = 7.4, *J* = 6.3, *J* = 6.0)	2.98 (dd, *J* = 13.8, *J* = 6.3); 3.07 (dd, *J* = 13.8, *J* = 6.0)	
9b	4.69 (ddd, *J* = 7.9, *J* = 7.6, *J* = 7.0)	*	*	4.76 (dt, *J* = 8.1, *J* = 6.0)	2.90, 2.99 (both dd, *J* = 13.9, *J* = 6.0)	
10a	4.75 (dt, *J* = 7.6, *J* = 7.1)	3.06 (d, *J* = 7.1)		4.40 (dd, *J* = 8.5, *J* = 5.1)	2.10 (sept.d, *J* = 6.9, *J* = 5.1)	0.84, 0.88 (both d, *J* = 6.9)
10b	4.78 (ddd, *J* = 8.1, *J* = 8.0, *J* = 6.4)	3.11 (dd, *J* = 13.8, *J* = 6.4)	3.04 (dd, *J* = 13.8, *J* = 8.1)	4.38 (dd, *J* = 8.5, *J* = 4.7)	2.01 (sept.d, *J* = 6.9, *J* = 4.7)	0.75, 0.76 (both d, *J* = 6.9)
11a	4.71 (td, *J* = 7.0, *J* = 6.5)	3.08 (dd, *J* = 13.9, *J* = 6.5)	3.05 (dd, *J* = 13.9, *J* = 7.0)	4.49 (td, *J* = 7.6, *J* = 5.3)	2.15, 1.95 (both m)	2.30 (m)
11b	4.75 (td, *J* = 8.0, *J* = 6.5)	3.09 (dd, *J* = 13.7, *J* = 6.5)	3.03 (dd, *J* = 13.7, *J* = 8.0)	4.46 (td, *J* = 7.8, *J* = 4.9)	*	*

* The signals overlap with the corresponding signals of the *S*,*S*-isomer.

Table 3. Effect of the structure of the side chain of the amino component on the reaction diastereoselectivity

Compound		Content[a]		α[b]	Yield (%)
		S,*S*	*R*,*S*		
8a,b	Ac-Phe-(*S*)-AlaOEt	39.1	60.9	1.09	89.0
9a,b	Ac-Phe-(*S*)-PheOEt	31.3	68.7	1.36	71.7
10a,b	Ac-Phe-(*S*)-ValOEt	25.1	74.9	1.22	73.3
11a,b	Ac-Phe-(*S*)-Glu$(OEt)_2$	10.3	89.7	1.10	70.4

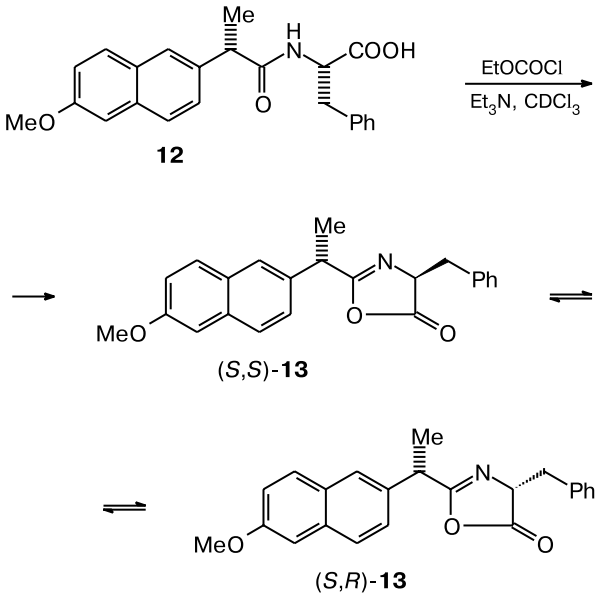
[a] The content of diastereomers in the final product according to HPLC data (%).
[b] Resolution coefficient.

5(4*H*)-oxazolones and has been described previously[7] for 2,4-dimethyl-5-oxazolone.

In the ^{13}C NMR spectrum of oxazolone **3**, the signal for C(4) is shifted downfield (δ 65.98) with respect to that for C(2) in the starting compound **1** (δ 54.31), whereas the signal for the methyl group is shifted upfield (δ 13.96 *vs.* δ 23.21 for the starting compound). The assignment of signals of protonated carbon atoms was confirmed by a HETCOR 2D experiment.

For quantitative estimation of the relative rate of formation and racemization of oxazolone during the reaction, *N*-[(*S*)-2-(6-methoxy-2-naphthyl)propionyl]-(*S*)-phenylalanine[8] (**12**) was taken as the model compound, which was converted into oxazolone (**13**) in the presence of Et_3N in $CDCl_3$ at room temperature (Scheme 2). The structure of compound **13** was confirmed by ^{1}H NMR spectroscopy.

Scheme 2



The reaction gave diastereomeric oxazolones (*S*,*S*)-**13** and (*S*,*R*)-**13**, which can be distinguished in the ^{1}H NMR spectra owing to the presence of two asymmetric centers. The triplets of doublets for C(4)H of the oxazolone fragment are characteristic signals: δ 4.49 ($^3J_{C(4)H,CH_2}$ = 5.2, $^5J_{C(4)H,CH\text{-}Nap}$ = 1.0 Hz) and δ 4.51 ($^3J_{C(4)H,CH_2}$ = 5.2, $^5J_{C(4)H,CH\text{-}Nap}$ = 2.1 Hz) for (*S*,*S*)-**13** and (*S*,*R*)-**13**, respectively.

When the reaction was carried out with a stoichiometric amount of Et_3N, the total content of oxazolones de-

termined 10 min after mixing the reactants was 40% and the (*S*,*S*)-**13** to (*S*,*R*)-**13** ratio was 83 : 17. After 8 h, the total content of oxazolones increased to 60% and the (*S*,*S*)-**13** to (*S*,*R*)-**13** ratio was 60 : 40. After 30 h, complete racemization was observed. In the reaction carried out with a 20% molar excess of Et_3N, both the formation and racemization rates of oxazolone substantially increased. As soon as 10 min after mixing the reactants, the total content of oxazolone **13** was 98%. This was accompanied by complete racemization.

The conditions found can be used for stereodirected synthesis of stereoisomers of the dipeptides containing difficult-to-prepare *R* amino acids.

Experimental

^1H and ^{13}C NMR spectra were recorded on a Bruker DRX 400 spectrometer (400 and 100 MHz, respectively). HPLC of the compounds synthesized was carried out on a Milikhrom 4-UF chromatograph using Silasorb-60 as the sorbent, a 64×2 mm column, detection at 230 nm, and the elution rate of 200 μL min^{-1}. The melting points were measured on a Boetius hot stage and were not corrected.

N-[(*S*)-2-(6-Methoxy-2-naphthyl)propionyl]-(*S*)-phenylalanine[8] and *N*-acetyl-(*S*)-phenylalanine[9] were synthesized by previously described procedures. Esterification of amino acids with ethanol was carried out by a known procedure.[10]

Synthesis of a mixture of compounds 8a,b—11a,b (general procedure). Triethylamine (0.61 mL, 4.34 mmol) and Bu^iOCOCl (0.48 mL, 3.62 mmol) were added dropwise with stirring to a solution of *N*-acetyl-(*S*)-phenylalanine (0.75 g, 3.62 mmol) in THF (17 mL) cooled to −12 °C. The reaction mixture was stirred for 30 min at −13 °C, and a mixture prepared from an (*S*)-amino acid ethyl ester hydrochloride (3.62 mmol) and Et_3N (0.51 mL, 3.62 mmol) in THF (9 mL) cooled to −10 °C was added. The reaction mixture was stirred for 1 h at −12 °C, for 1 h at 0 °C, and for 3 h at ~20 °C, and kept without stirring for 17 h. The precipitate was filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in chloroform, washed with a 5% solution of $NaHCO_3$, water, 5% HCl, and water, and dried with Na_2SO_4. The chloroform solution was concentrated *in vacuo* to dryness. The residue was analyzed by ^1H NMR spectroscopy and HPLC.

Detection of oxazolones 3a,b and 13 (general procedure). Triethylamine (0.05 or 0.06 mmol) and EtOCOCl (0.005 mL, 0.05 mmol) were added to a solution of *N*-acetyl-(*S*)-phenylalanine or compound **12** (0.05 mmol) in $CDCl_3$ (1.0 mL). The ^1H NMR spectra were recorded at specified intervals, and the yields of oxazolones **3a,b** (**13**) were determined based on the ratio of the integral intensities of the proton signals of the C(2)H fragment of phenylalanine and the C(4)H fragment of oxazolones.

Oxazolones **13**. The proton signal of the C(2)H fragment of the starting compound **12** was observed at δ 4.65 (ddd), the proton signal of the C(4)H fragment of oxazolones **13** occurred at δ 4.49 and 4.51 (both td). The diastereomer ratio in a mixture of (*S*,*S*)-**13** and (*R*,*S*)-**13** was determined from the integral intensities of the signals of the C(4)H proton.

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