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Enantioselective Synthesis of Peptides Using (1*R*)-3-Hydroxy-1,8,8-trimethyl-3-azabicyclo[3.2.1]octane-2,4-dione ((+)-*N*-Hydroxycamphorimide) an Active Ester Group[1)]

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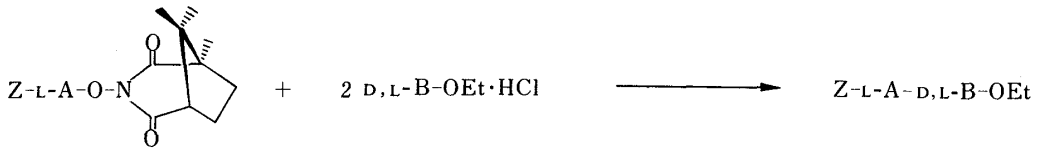
The enantioselective coupling reaction of *N*-protected amino acid (+)-*N*-hydroxycamphorimide esters (-OCamp) with racemic amino acid esters is discussed. The coupling reaction of several amino acids showed high enantioselectivity.

Keywords——enantioselective synthesis; (+)-*N*-hydroxycamphorimide; peptide; active ester; coupling reaction

Preparation of amides by the use of coupling reactions is very important in organic synthesis and many reagents for carrying our coupling reactions by one-pot procedures have been reported.[2)] In most cases, these coupling reactions are used to convert carboxylic acids into amides. Most of the coupling reagents first react with the carboxylic acids to give activated intermediates which undergo subsequent nucleophilic attack by amino groups. Therefore, if the ester group has chirality, aminolysis of the active ester should result in an enantioselective coupling reaction.

There have been several reports of enantioselective peptide synthesis. Gunster *et al.*[3)] reported the enantioselective acylation of racemic 1-phenylethylamine by the use of a trifluoroacetylated chiral polyamine. Recently, Teramoto *et al.*[4)] reported that *N*-hydroxytartarimide derivatives can be used as reagents for enantioselective peptide synthesis. Peptide synthesis by using these compounds showed high enantioselectivity for Z–Ala–Ala-OEt synthesis. We have also reported several active esterification reagents, carbonate,[5)] phosphate,[6)] and oxalate,[1,7)] which do not require a coupling reagent such as dicyclohexylcarbodiimide (DCC). As a part of our continuing research on the preparation of peptides under mild conditions, we now wish to report the enantioselective aminolysis of (+)-*N*-hydroxycamphorimide active esters of *N*-protected amino acid with several amino acids.

Natural dicarboxylic acid derivatives are good starting materials for optically active *N*-hydroxyimide derivatives. We synthesized (+)-*N*-hydroxycamphorimide (HOCamp) from D-camphoric acid for use as an optically active ester group by Larson's[8)] method.



A; Ala, Pro, Val. B; Ala, Val, Leu, Phe

Chart 1

TABLE I. Enantioselective Peptide Synthesis by Using (+)-*N*-Hydroxycamphorimide

Dipeptide[a]	Yield (%)	mp (°C)	$[\alpha]_D$ (EtOH, °C)	L-Content (%)	Optical yield[b] (%)	Base and catalyst mol eq	Reaction conditions
Z–L-Ala–Ala–OEt	89.4	114—115	−39.4° (c=1, 19)	93.7	87.3	$2NEt_3$	Room Temp. 4 weeks
	88.2	112—114	−40.5° (c=1, 20)	96.0	92.2	$2NEt_3$ CH_3COOH	Room temp. 2d
Z–L-Ala–Val–OEt	80.0	Oil	−15.5° (c=1, 24)	47.9	−4.3	$2NEt_3$	Room temp. 4 weeks
	99.0	Oil	−20.5° (c=1, 24)	66.0	31.9	$2NEt_3$ CH_3COOH	Room temp. 2d
Z–L-Ala–Leu–OEt	98.9	Oil	−19.1° (c=1, 26)	76.1	52.1	$2NEt_3$	Room temp. 3 weeks
	91.2	57—58	−38.3° (c=1, 25)	100	100	$2NEt_3$ $2CH_3COOH$	Room temp. 3d
Z–L-Ala–Phe–OEt	99.0	72—74	−15.1° (c=1, 24)	82.8	65.5	$2NEt_3$	Room temp. 4 weeks
	90.5	72—74	−15.3° (c=1, 22)	75.9	51.7	$2CH_3COONa$	Room temp. 2d
Z–L-Pro–Ala–OEt	92.0	Oil	−42.2° (c=1, 22)	42.7	−14.7	$2NEt_3$	Room temp. 3 months
	99.0	Oil	−46.0° (c=1, 22)	50.1	0.2	$2CH_3COONa$	Room temp. 5d
Z–L-Pro–Val–OEt	95.7	Oil	−31.4° (c=1, 25)	38.9	−22.2	$2NEt_3$	Refrigerator 3 months
	95.7	Oil	−38.1° (c=1, 24)	56.6	13.2	$2CH_3COONa$	Room temp. 5d
Z–L-Pro–Leu–OEt	82.1	Oil	−38.5° (c=1, 25)	67.5	34.9	$2NEt_3$	Refrigerator 3 months
	97.4	Oil	−35.5° (c=1, 24)	64.7	29.4	$2CH_3COONa$	Room temp. 1 week
Z–L-Pro–Phe–OEt	80.2	Oil	−31.9° (c=1, 25)	71.2	42.4	$2NEt_3$	Refrigerator 3 months
	94.3	Oil	−30.8° (c=1, 24)	54.6	9.1	$2CH_3COONa$	Room temp. 3 weeks
	99.0	Oil	−31.6° (c=1, 24)	66.7	33.3	$2CH_3COONa$ TosOH (Trace) $2CH_3COOH$	Room temp. 3 weeks
Z–L-Val–Ala–OEt	62.3	152—155	−28.0° (c=0.5, 27)	91.7	83.4	$2NEt_3$ $2CH_3COOH$	Room temp. 4 weeks
Z–L-Val–Phe–OEt	65.7	119—120	−22.9° (c=0.5, 27)	53.3	6.5	$2NEt_3$ $2CH_3COOH$	Room temp. 4 weeks

a) The purities were checked by TLC on silica gel, and mass spectroscopy. *b*) Optical yield was calculated based on $[\alpha]_D$ of reference 9. Optical yield (%) = 100(LL − LD)/(LL + LD).[4]

N-Benzyloxycarbonyl (Z)-amino acid camphorimide ester was synthesized from Z-amino acid and (+)-*N*-hydroxycamphorimide with 1-ethyl-3-(3-*N*,*N*-dimethylaminopropyl)-carbodiimide hydrochloride (EDAC) in tetrahydrofuran (THF) at 0 °C. A-Amino acid *N*-hydroxycamphorimide ester reacted with 2 eq of racemic amino acid ethyl ester hydrochloride and NEt_3 in the presence of a catalytic amount of an acid or without the acid in acetonitrile. The reaction proceeded faster with an acid catalyst. For example, the reaction of Z–L-Ala-OCamp with racemic Ala-OEt hydrochloride for 4 weeks afforded Z–L-Ala–L-Ala-OEt in 87.3% optical yield in the presence of NEt_3 as a base, while the same reaction afforded

92.2% optical yield of Z–L-Ala–L-Ala-OEt in 2 d in the presence of NEt_3 and acetic acid as a catalyst (Table I). However, no clear relationship among acid catalyst, yield and reaction time was apparent. Steric hindrance of the (+)-camphorimide ring is thought to facilitate the selective reaction of Z–Ala-OCamp with the L-isomer of racemic alaninate. However, there seemed to be no clear correlation between enantioselectivity and the use of catalyst.

The optical yields of dipeptides were calculated by comparison of the specific rotations with those of authentic samples which were obtained by the Eintoph method by use of EDAC and *N*-hydroxy-5-norbornene-2,3-dicarboxyimide (HONb).[9]

Experimental

N-Benzyloxycarbonylamino acids were obtained commercially. Solvents were purified in the usual manner. All melting points are uncorrected. Infrared (IR) spectra were recorded on a JASCO A-2 spectrometer. Nuclear magnetic resonance (NMR) spectra were measured with a Varian T-60 spectrometer, using Me_4Si as an internal reference. Mass spectra (MS) were determined with a JMS-D-100 spectrometer at 75 eV by the direct inlet system. Specific rotations were measured with a JASCO model DIP-181 polarimeter.

General Procedures for the Preparation of Camphorimide Active Esters——*N*-Benzyloxycarbonyl-L-alanine *N*-hydroxycamphorimide Ester (Z–Ala-OCamp): EDAC (96 mg, 0.5 mmol) was added to an ice-cold solution of *N*-benzyloxycarbonyl-L-alanine (111.5 mg, 0.5 mmol) and *N*-hydroxycamphorimide (100 mg, 0.5 mmol) in 15 ml of dry THF. The mixture was stirred for 5 h and allowed to stand overnight. After evaporation of the THF *in vacuo*, the residue was extracted with EtOAc. The extract was washed with water, 1 N hydrochloric acid, water, 4% aqueous sodium hydrogen carbonate, and brine, then dried over sodium sulfate, and concentrated to dryness *in vacuo*. The residue was purified by column chromatography on silica gel to give Z–Ala-OCamp (322 mg, 80.1%), mp 102—103 °C. MS *m/z*: 402 (M^+). $[\alpha]_D^{21}$ −39.5° (*c* = 1, EtOH). IR ν_{max}^{KBr} cm^{-1}; 1800 (imide), 1750 (ester), 1720 (urethane). *Anal.* Calcd for $C_{21}H_{20}N_2O_6$: C, 62.67; H, 6.51; N, 6.96. Found: C, 62.79; H, 6.44; N, 6.82.

Z–Pro-OCamp: 298 mg (69.6%), oil. MS *m/z*: 428 (M^+). $[\alpha]_D^{26}$ −29.8° (*c* = 1, EtOH). IR ν_{max}^{film} cm^{-1}; 1810 (imide), 1750 (ester), 1715 (urethane). *Anal.* Calcd for $C_{23}H_{28}N_2O_3$: C, 64.47; H, 6.59; N, 6.54. Found: C, 64.23; H, 6.56; N, 6.37.

Z–Val-OCamp: 362 mg (84.2%), foam. MS *m/z*: 430 (M^+). $[\alpha]_D^{26}$ −44.7° (*c* = 1, EtOH). IR ν_{max}^{film} cm^{-1}; 1800 (imide), 1750 (ester), 1720 (urethane). *Anal.* Calcd for $C_{23}H_{30}N_2O_6 \cdot 1/4H_2O$ (in a desiccator using silica gel); C, 63.51; H, 7.07; N, 6.44. Found: C, 63.38; H, 6.99; N, 6.23.

General Procedure for the Coupling of Benzyloxycarbonylamino Acid Camphorimide Esters and Racemic Amino Acid Esters——Z–L-Ala–Ala-OEt: A solution of D,L-alanine ethyl ester hydrochloride (76.8 mg, 0.5 mmol) and NEt_3 (50 mg, 0.5 mmol) in 5 ml of CH_3CN was added a solution of *N*-benzyloxycarbonyl-L-alanine *N*-hydroxycamphorimide ester (100 mg, 0.25 mmol) in 10 ml of CH_3CN, and the mixture was held at room temperature for 4 weeks. After evaporation of the CH_3CN *in vacuo*, the residue was extracted with EtOAc. The extract was washed with water, 1 N hydrochloric acid, water, 4% aqueous sodium hydrogen carbonate, and brine, then dried over sodium sulfate, and concentrated to dryness *in vacuo*. Purification was carried out by preparative thin-layer chromatography (TLC) on silica gel (acetone : benzene = 1 : 5) to give Z–Ala–Ala-OEt (281 mg, 87.3%), mp 114—115 °C. MS *m/z*: 322 (M^+). $[\alpha]_D^{19}$ −39.4° (*c* = 1, EtOH). IR ν_{max}^{film} cm^{-1}; 1750 (ester), 1690 (urethane). *Anal.* Calcd for $C_{16}H_{22}N_2O_5$: C, 59.61; H, 6.88; N, 8.69. Found: C, 59.60; H, 6.77; N, 8.76.

General Procedure for the Coupling of Benzyloxycarbonylamino Acid in the Presence of Catalytic Amount of Acid——Z–L-Ala–Ala-OEt: A mixture of D,L-alanine ethyl ester hydrochloride (79.8 mg, 0.5 mmol), NEt_3 (50 mg, 0.5 mmol), and CH_3COOH (30 mg, 0.5 mmol) in 5 ml of CH_3CN was added to a solution of *N*-benzyloxycarbonyl-L-alanine camphorimide ester (100.5 mg, 0.25 ml) in 10 ml of CH_3CN, and the mixture was held at room temperature for 2 d. Then it was treated as described above to give Z–L-Ala–Ala-OEt (297 mg, 92.2%), mp 112—114 °C. MS *m/z*: 322 (M^+). $[\alpha]_D^{20}$ −40.5° (*c* = 1, EtOH). IR ν_{max}^{film} cm^{-1}; 1750 (ester), 1690 (urethane). *Anal.* Calcd for $C_{16}H_{33}N_2O_5$: C, 59.61; H, 6.88; N, 8.69. Found: C, 59.69; H, 6.79; N, 8.75.

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References and Notes

1) This constitutes Part XII of a series entitled "Studies on Activating Methods of Functional Groups." Part XI:

K. Takeda, K. Tsuboyama, K. Yamaguchi, and H. Ogura, *J. Org. Chem.*, **50**, 273 (1985).
2) For general reviews see: *a*) M. A. Ogliaruso and J. F. Wolfe, "The Chemistry of Acid Derivatives," Part I, ed. by S. Patai, John Wiley and Sons, New York, 1979; *b*) M. Bodanszky, Y. S. Hausner, and M. A. Ondetti, "Peptide Synthesis," John Wiley and Sons, New York, 1976; *c*) M. Bodanszky, "The Peptides," Vol. I, ed. by E. Gross and J. Meienhofer, Academic Press, New York, 1979, p. 105.
3) E. J. Gunster and R. C. Schulz, *Makromol. Chem.*, **181**, 643 (1980).
4) T. Teramoto, M. Deguchi, T. Kurosaki, and M. Okawara, *Tetrahedron Lett.*, **22**, 1109 (1981).
5) H. Ogura, T. Kobayashi, K. Shimizu, K. Kawabe, and K. Takeda, *Tetrahedron Lett.*, **1979**, 4745.
6) H. Ogura, S. Nagai, and K. Takeda, *Tetrahedron Lett.*, **21**, 1967 (1980).
7) K. Takeda, I. Sawada, A. Suzuki, and H. Ogura, *Tetrahedron Lett.*, **24**, 4451 (1983).
8) H. O. Larson and E. K. W. Wat, *J. Am. Chem. Soc.*, **85**, 827 (1963).
9) Authentic samples of dipeptides for specific rotation were synthesized by the Eintoph method, using *N*-hydroxy-5-norvornen-2,3-dicarboxyimide and EDAC (N. Izumiya, K. Noda, C. B. Antinses, *Arch. Biochem. Biophys.*, **144**, 237 (1971)). These compounds showed satisfactory IR, MS, and elemental analysis data. Z–L-Ala–L-Val–OEt; mp 82—83 °C. $[\alpha]_D^{20}$ −29.9° (*c*=1, EtOH). Z–L-Ala–D-Val–OEt: mp 77.5—79.5 °C. $[\alpha]_D^{24}$ −2.3° (*c*=1, EtOH). Z–L-Ala–L-Leu–OEt: mp 60.5—62.0 °C. $[\alpha]_D^{24}$ −38.1° (*c*=1, EtOH). Z–L-Ala–D-Leu–OEt: mp 119—120.5 °C. $[\alpha]_D^{24}$ +41.3° (*c*=1, EtOH). Z–L-Ala–L-Phe–OEt: mp 91—92 °C. $[\alpha]_D^{27}$ −14.6° (*c*=1, EtOH). Z–L-Ala–D-Phe–OEt: mp 86.5 °C. $[\alpha]_D^{25}$ −17.5° (*c*=1, EtOH). Z–L-Pro–L-Ala–OEt: mp 79—82 °C. $[\alpha]_D^{25}$ −72.3° (*c*=1, EtOH). Z–L-Pro–D-Ala–OEt: mp 61.5—62 °C. $[\alpha]_D^{24}$ −19.8° (*c*=1, EtOH). Z–L-Pro–L-Val–OEt: oil. $[\alpha]_D^{24}$ −54.5° (*c*=1, EtOH). Z–L-Pro–D-Val–OEt: mp 56—57 °C. $[\alpha]_D^{24}$ −16.7° (*c*=1, EtOH). Z–L-Pro–L-Leu–OEt: 62.5—64 °C. $[\alpha]_D^{24}$ −73.7° (*c*=1, EtOH). Z–L-Pro–D-Leu–OEt: mp 119.5—121 °C. $[\alpha]_D^{25}$ +34.5° (*c*=0.5, EtOH). Z–L-Pro–D-Phe–OEt: mp 65—68 °C. $[\alpha]_D^{24}$ −27.2° (*c*=0.5, EtOH). Z–L-Val–L-Ala–OEt: mp 163—163.5 °C. $[\alpha]_D^{27}$ −32.7° (*c*=0.5, EtOH). Z–L-Val–D-Ala–OEt: mp 146—146.5 °C. $[\alpha]_D^{27}$ +23.8° (*c*=0.5, EtOH). Z–L-Val–L-Phe–OEt: mp 135—135.5 °C. $[\alpha]_D^{27}$ −31.5° (*c*=0.5, EtOH). Z–L-Val–D-Phe–OEt: mp 137.5—140 °C. $[\alpha]_D^{27}$ −13.1° (*c*=0.5, EtOH). Z–L-Ala–L-Ala–OEt: $[\alpha]_D^{25}$ −42.4° (*c*=1, EtOH).[6] Z–L-Ala–D-Ala–OEt: $[\alpha]_D^{25}$ +4.8° (*c*=1, EtOH).[6] Z–L-Ala–L-Val–OEt: mp 82—83 °C. $[\alpha]_D^{20}$ −19.9° (*c*=1, EtOH).[10] Z–L-Pro–L-Phe–OEt: mp 65—68 °C. $[\alpha]_D^{22}$ −33.8° (*c*=1, EtOH).[11]
10) D. S. Kemp, S.-L. H. Choong, and L. Pekaar, *J. Org. Chem.*, **39**, 3841 (1974).
11) H. Aoyagi, K. Arakawa, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **41**, 433 (1968).