

Figure 1. Reported polymer-supported Evans' chiral auxiliaries anchored at the 4-position of the oxazolidinone ring (A) and design of a new auxiliary anchored at the 5-position (B).

polymer-supported chiral Evans' oxazolidinone with a novel anchoring system onto the solid-support as a rapid communication.¹⁷ In this article, we describe the detailed design and synthesis of the polymer-supported chiral Evans' oxazolidinone and its reusability in Evans' asymmetric alkylation.

2. Results and discussion

2.1. Design of a new polymer-supported chiral oxazolidinone

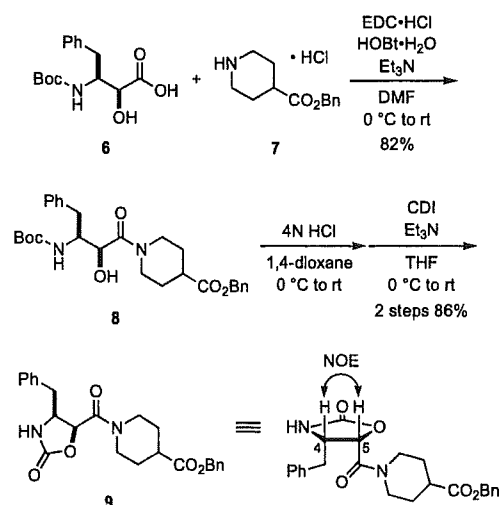
One of the common features of polymer-supported Evans' chiral auxiliary in all previous reports^{11–14} is that a chiral 4-substituted oxazolidin-2-one was connected to the solid-support through the chiral discriminating moiety at the 4-position of the oxazolidinone ring (Fig. 1A). This made us suspect that chiral control ability of Evans' oxazolidinone is influenced by the polystyrene backbone of the solid-support, leading to the low stereoselectivity in the asymmetric alkylation.^{11b} Hence, we proposed an alternative anchoring strategy, which leaves the crucial chiral discriminating moiety unmodified, and utilizes the external 5-position for connecting to the solid-support (Fig. 1B).

To prepare such a new oxazolidinone derivative, we focused on α -hydroxy- β -amino acids, which are routinely used in our laboratory as a core structure for the development of effective aspartic protease inhibitors.¹⁸ The unique structure of α -hydroxy- β -amino acids, in which three different functional groups, i.e. amino, hydroxyl and carboxyl groups, are located on two adjacent asymmetric carbon atoms gave us the idea. Namely, the known oxazolidinone formation¹⁹ at the 1,2-amino alcohol moiety of α -hydroxy- β -amino acid, (*2S,3S*)-3-amino-2-hydroxy-4-

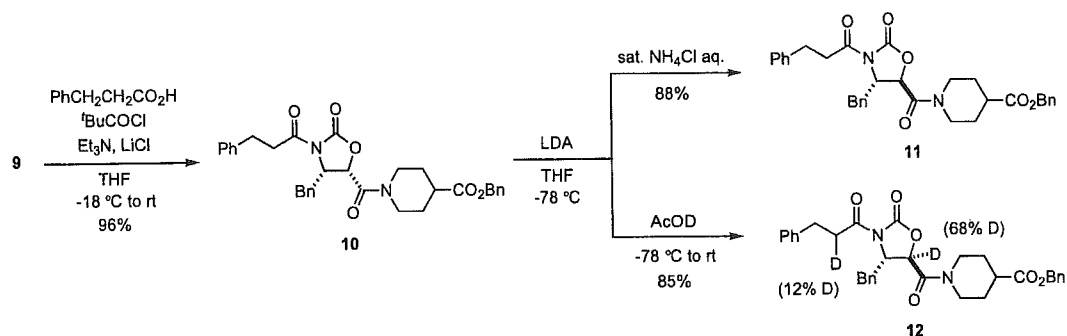
phenylbutanoic acid **3** (allophenylnorstatine, Apns),¹⁸ can afford a desired oxazolidinone derivative **4** with a benzyl substituent at the 4-position as a chiral discriminating group and a free carboxyl group at the 5-position which can connect with the solid-support (Fig. 1B). In addition, Burgess et al. pointed out that Wang resin had a better enantiomeric excess than Merrifield resin in asymmetric benzylation.^{11b} Since Wang resin has an additional benzyl moiety which has a space from the polystyrene backbone in comparison to Merrifield resin, we planned to employ both Wang resin and, as a further spacer, a piperidine-4-carboxylic acid. Thus, in the designed solid-supported auxiliary **5**, this spacer is connected to the carboxyl group at the 5-position of the oxazolidinone moiety by a tertiary amide bond and to Wang resin by an ester bond. This tertiary amide bond with no amide proton is stable under both acidic and basic conditions. The ester bond between the spacer and Wang resin can be formed by the standard methods.

2.2. Evaluation of new oxazolidinone derivatives in solution-phase model experiment

To understand the efficacy of designed solid-support chiral oxazolidinone **5** as a new chiral auxiliary, we first studied a solution-phase experiment, using a model oxazolidinone derivative **9** whose C-terminal is protected by a benzyl ester to mimic Wang resin. As Scheme 1 shows, **9** was synthesized by a three-step reaction. Namely, Boc-Apns-OH **6** was coupled to benzyl piperidine-4-carboxylate **7**²⁰ by the EDC-HOBt (EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, HOBt: 1-hydroxybenzotriazole) method²¹ to give dipeptide **8**,²² followed by the removal of the Boc group and CDI (1,1'-carbonyldiimidazole) treatment¹⁹ to afford the *cis*-configured oxazolidinone derivative **9** as a single isomer. During the cyclization reaction, neither aziridine nor 1,2-imidazolamine byproduct formation was observed.²³ The *cis*-configuration of **9** was confirmed by the coupling constant²⁴ between H-4 and H-5, and NOE experiments (Scheme 1 and Ref. 25). Synthesized **9** had



Scheme 1. Synthesis of a *cis*-configured oxazolidinone derivative **9** from Boc-Apns-OH **6**.

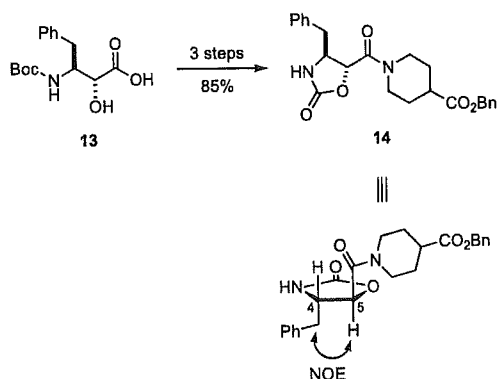


Scheme 2. Epimerization and deuterium incorporation to the *cis*-configured carboximide **9**.

coupling constants of $J_{4,5} = 7.9, 8.1$ Hz, which corresponded to the representative value of the *cis*-configured oxazolidinone.

Next, oxazolidinone **9** was *N*-acylated with 3-phenylpropionic acid by the mixed anhydride method to obtain carboximide **10** (Scheme 2).²⁶ Although there are three α -protons in **10**, the newly introduced carboximide α -proton was expected to be most acidic. Since it was reported that the imide-selective enolization of substrates with the both carboximide and ester structures was accomplished by careful base addition,²⁷ and that after asymmetric reaction, *N*-acyl fragments were selectively cleaved from the auxiliary by the imide-specific lithium hydroperoxide-mediated hydrolysis,²⁸ we proposed **9** as a chiral auxiliary that could be recovered and reused. However, its enolate formation gave a new compound even with a careful addition of LDA (1.2 equiv) to the cooled THF solution of **10** and a subsequent stirring for 0.5 h. This compound was an epimerized *trans*-configured carboximide **11**.²⁹ This result suggests that the α -proton of the carboxamide moiety was predominantly deprotonated by LDA to diminish the steric repulsion caused by *cis*-configuration.³⁰ Indeed, quenching lithium enolate generated in situ from **10** with acetic acid-*d* (99at.% D) afforded the deuterized **12** in 85% yield. The deuterium was incorporated mainly at the α -position of the carboxamide moiety (68% D) along with the α -position of the *N*-acylimide moiety (12% D).

This unexpected result prompted us to suggest that stable *trans*-configured oxazolidinone **14** was suitable for the auxiliary (Scheme 3). We synthesized **14** according to the procedure shown in Scheme 1, starting from Boc-Pns-OH **13** (Pns: phenylnorstatine), a *2R* isomer of **6**, in 85% yield (3 steps). The relative stereochemistry of **14** was analyzed by NMR. Coupling constants of $J_{4,5}$ were 5.1 and 5.3 Hz, which are well consistent with the known value in *trans*-configuration²⁴ and a strong NOE signal was observed between H-5 and two protons at the benzylic position.²⁵ In addition, the most stable conformation of **14** obtained from conformational analysis showed a dihedral angle of 136.4° between two methine hydrogens (H-4 and H-5). This value and Karplus curve reasonably supported the observed coupling constant. From these observations, the configuration between H-4 and H-5 in **14** was confirmed as *trans*. Furthermore, the absolute stereochemistry of **14** was confirmed as *4S,5R* by the X-ray crystal structural analysis of (*R*)-phenylethylamide **15**³¹ derived from **14** (Fig. 2). In addition, it was found that the piperidine-4-carboxylic acid spacer extended outside from the oxazolidinone core, suggesting that this spacer does not interfere with the asymmetric reaction.



Scheme 3. Synthesis of a *trans*-configured oxazolidinone derivative **14** from Boc-Pns-OH **13**.

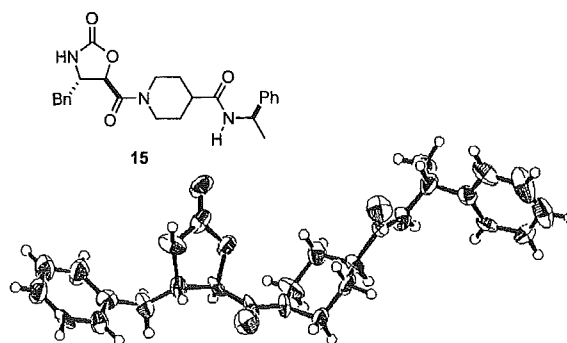
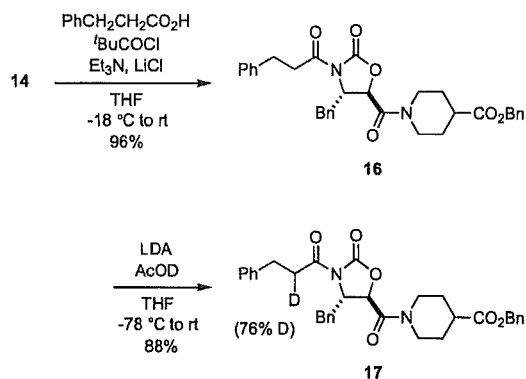


Figure 2. X-ray crystal structure of (*R*)-phenylethylamide **15**.

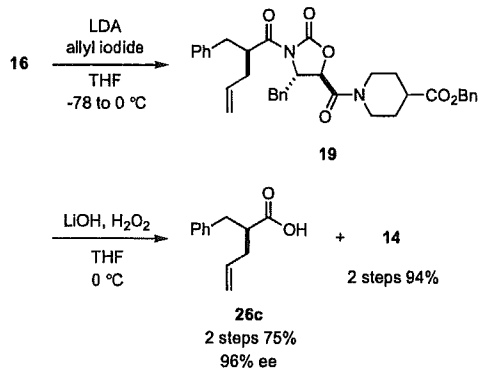
Next, we synthesized *N*-3-phenylpropionylated oxazolidinone **16** and subjected it to the deuterium labeling to confirm the enolization position (Scheme 4) by the same procedure described in Scheme 2. No particular change on TLC was observed during the enolization and the recovered product (88% yield) contained 76% of deuterized **17**, exclusively at the α -position of the desired carboximide moiety with unmodified **16**. This result suggests that the



Scheme 4. Deuterium incorporation to *trans*-configured carboximide **16**.

α -position of the imide *N*-acyl moiety in **16** has the most acidic α -proton, which is predominantly deprotonated by LDA. Self-condensation of **16** was not observed under this reaction condition.

With these positive observations, we examined the Evans' asymmetric allylation of the carboximide **16** as a model for alkylation (Scheme 5).¹⁵ Briefly, to a solution of **16** in THF was added LDA (1.2 equiv) dropwise at -78 °C. After stirring for 0.5 h, the generated *Z*-*O*-enolate **18**³² was treated with allyl iodide (3.0 equiv), and the temperature of the reaction mixture was gradually increased up to 0 °C over a period of 3 h. Resultant **19** was hydrolyzed by LiOH without any purification.²⁸ The desired α -allylated carboxylic acid **26c** was obtained in good yield (2 steps 75%, an average of 87% for each of the two steps in the reaction sequence) and high stereoselectivity (96% ee),³³ which were comparable to the standard Evans' asymmetric allylation in solution-phase.¹⁵ Oxazolidinone **14** was sufficiently recovered (94%) without epimerization, and no byproduct produced by the endocyclic cleavage of the oxazolidinone ring²⁸ was observed. These results proved that the *trans*-configured oxazolidinone **14** was effective as a chiral auxiliary and that the spacer moiety did not interfere with the asymmetric reaction.



Scheme 5. Asymmetric allylation of the *N*-3-phenylpropionylated carboximide **16**.

An energy minimization study of enolate intermediate **18** suggested that its conformation corresponds to that of the original chelation-controlled model proposed for standard

Evans' chiral auxiliary system (Fig. 3).^{15,34} Interestingly, this modeling also suggested that nucleophilic attack of the hydroperoxide anion to the oxazolidinone carbonyl for the endocyclic cleavage is effectively obstructed by the steric effect of the benzyl and carboxamide moieties.³⁵ From these data, we selected the structure of **14** originating from Pns as the candidate for solid-supported Evans' auxiliary.

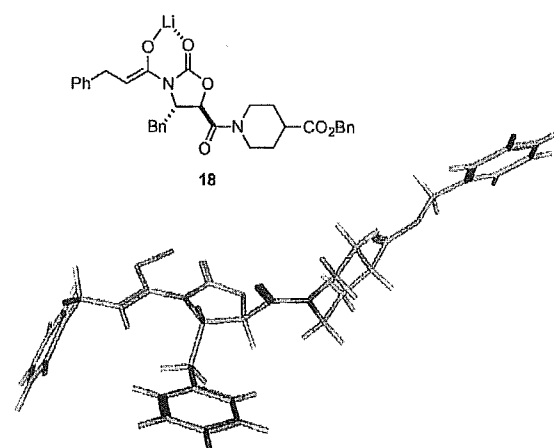


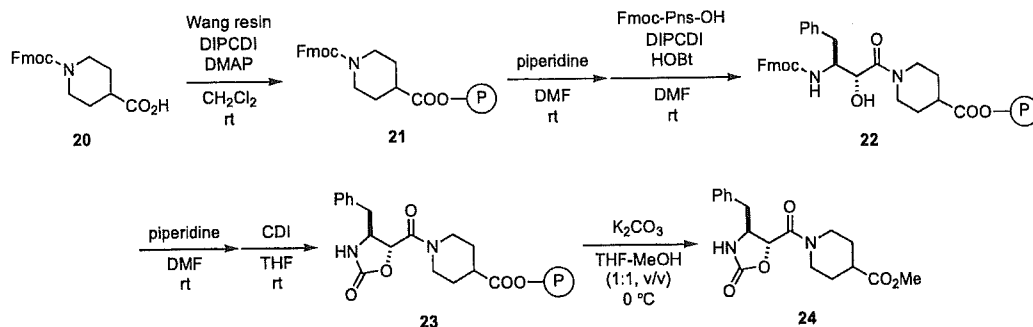
Figure 3. Energy minimization study of enolate intermediate **18**.

2.3. Solid-phase synthesis of Wang resin-supported chiral oxazolidinone **23**

In our previous communication,¹⁷ Wang resin-supported chiral oxazolidinone **23** was obtained by the carbodiimide-mediated coupling between Wang resin and the oxazolidinone-spacer unit prepared from **14** by hydrogenolysis. Since four-step solution-phase synthesis of this unit and its excess use (4 equiv) required for complete loading onto the resin were inefficient, in the present study we developed a more convenient synthetic route for **23** using Fmoc-based solid-phase method as shown in Scheme 6.³⁶ Namely, Fmoc-piperidine-4-carboxylic acid **20** was first loaded to Wang resin using the DIPCPI–DMAP (DIPCPI: 1,3-diisopropylcarbodiimide) method³⁷ in CH_2Cl_2 . After Fmoc-deprotection of **21** with 20% piperidine, Fmoc-Pns-OH was coupled by the DIPCPI–HOBt method to give the dipeptide resin **22** followed by removal of the Fmoc group. The resultant 1,2-amino alcohol moiety was converted to oxazolidinone with CDI. Methanolysis of **23** with potassium carbonate in anhydrous THF–MeOH yielded the corresponding methyl ester **24** as a single isomer (95% for 6 steps). During this synthesis of **21**, neither epimerization at the 5-position nor byproduct formation such as aziridine and 1,2-aminoimidazole was observed.²³ It is noteworthy that all reactions in Scheme 6 proceeded smoothly at room temperature within a few hours, and multi-gram quantity of the oxazolidinone resin **23** with high loading yield was efficiently synthesized in just a day.

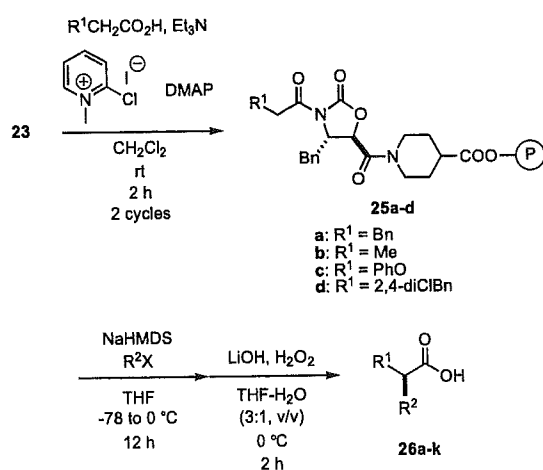
2.4. Solid-phase Evans' asymmetric alkylation with the oxazolidinone resin **23**

At first, we investigated the solid-phase Evans' asymmetric allylation of the *N*-3-phenylpropionylated carboximide

Scheme 6. Solid-phase synthesis of Wang resin-supported oxazolidinone resin **23**.

resin **25a**, which was prepared from **23** by Mukaiyama method (Scheme 7).³⁸ It was found that the use of NaHMDS (3 equiv) as a base and gradual increase of the temperature of reaction mixture up to 0 °C over a period of 12 h in the alkylation reaction were quite effective.³⁹ After quenching the reaction mixture with saturated NH₄Cl aq, the allylated carboximide resin was recovered, washed, then subjected to the LiOOH-mediated hydrolysis. The desired chiral α -allylated carboxylic acid **26c** was obtained with high stereoselectivity (96% ee), which was equal to the model experiment in solution-phase (Table 1, entry 3). The absolute configuration of acid **26c** was determined in comparison to the reported specific rotation,³³ suggesting

that the asymmetric alkylation on resin **25a** also proceeded in the same chelation-controlled model as the solution-phase method.¹⁵ During the hydrolytic cleavage, the ester linkage and oxazolidinone core were stable.⁴⁰ These encouraging results urged us to understand the generality of **23** in the Evans' asymmetric alkylation reaction. Several carboximide resins **25b–d** were prepared and subjected to the similar solid-phase alkylation reactions with a series of electrophiles (R²X).⁴¹ The results are summarized in Table 1. Favorably, not only highly reactive alkyl halides such as MeI and BnBr but also less reactive EtI reacted sufficiently under the same reaction conditions. Hydrolytic cleavage of the resultant resin afforded the corresponding chiral α -branched carboxylic acids **26a–k** with satisfying isolated yields (50–70%, for 3 steps) and enantiomeric excesses (84–97% ee).⁴² Especially, in the asymmetric benzylation of carboximide resin **25b**, stereoselectivity was found to be 97% ee (Table 1, entry 6), which was better than the value reported by Burgess et al.,^{11b} and was as high enough as in the corresponding solution-phase asymmetric alkylation utilizing the standard chiral 4-substituted oxazolidin-2-one.¹⁵ The relatively lower yield was due to the fact that the yield includes the three-step process from the oxazolidinone resin **23** to the final alkylated product **26**. We consider that yield for two steps (alkylation and hydrolysis) is similar to that of the solution-phase method, and average yield calculated for each step was reasonably acceptable (79–89%). We assume that these successful results are attributed to our new polymer-anchoring strategy based on the connection at the 5-position of the oxazolidinone ring. This liberates the chiral differentiating benzyl group from the polystyrene backbone of the resin, freeing the auxiliary



Scheme 7. Solid-phase asymmetric Evans' alkylation.

Table 1. Results of the solid-phase asymmetric Evans' alkylations

Entry	25	R ¹	R ² X	26	Yield ^a (%)	ee ^b (%)
1	25a	Bn-	MeI	26a	61(85)	85
2	25a	Bn-	EtI	26b	50(79)	88
3	25a	Bn-	Allyl-I	26c	68(88)	96
4	25a	Bn-	Propargyl-Br	26d	62(85)	96
5	25a	Bn-	BrCH ₂ CO ₂ Et	26e	62(85)	92
6	25b	Me-	BnBr	26f	70(89)	97
7	25b	Me-	4-BrBnBr	26g	68(88)	97
8	25b	Me-	4-NO ₂ BnBr	26h	55(82)	97
9	25b	Me-	2,4-diClBnI	26i	65(87)	97
10	25c	PhO-	Allyl-I	26j	50(79)	96
11	25d	2,4-diClBn-	MeI	26k	59(84)	84

^a Combined yield of 3 steps starting from oxazolidinone resin **23**. Value in the parenthesis is the average yield for each step.

^b Determined by chiral HPLC analysis after conversion to the corresponding (S)- α -methylbenzylamine-derived amides.

unit from the solid-support, which was not realized in the previous system based on the 4-position anchoring.

2.5. Recycling of the Wang resin-supported auxiliary 23

The recycling of the expensive auxiliary is one of the key points in the development of the polymer-supported chiral auxiliary. However, the recycling of the polymer-supported Evans' oxazolidinone has been reported in only one case of solid-phase 1,3-dipolar-cycloaddition,^{14b} with a considerable reduction of regio- and stereo-selectivity depending on the cycle number up to three, although the reason was unclear.

Hence, the ability of recycling of the Wang resin-supported chiral auxiliary 23 was studied in the solid-phase asymmetric allylation, mentioned above, to obtain α -allylated carboxylic acid 26c (Fig. 4). After the first cycle of allylation, the recovered chiral auxiliary resin 23 was washed and dried, then *N*-acylation with 3-phenylpropionic acid gave the corresponding carboximide resin 25a again. After the continuous second to fourth solid-phase asymmetric allylation, the desired product 26c was obtained in high enantioselectivity (96% ee each) (Table 2). Throughout these cycles, the product's stereoselectivity was maintained successfully, although the yield gradually decreased about

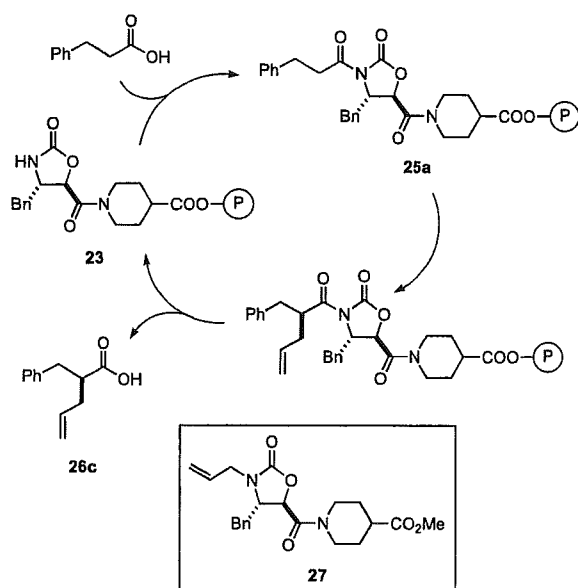


Figure 4. Recycling of the chiral auxiliary resin 23.

Table 2. Recycling of the Wang resin-supported chiral oxazolidinone 23 in Evans' asymmetric allylation

Cycle	Yield ^a (%)	ee ^b (%)
1	68	96
2	59	96
3	49	96
4	42	96

^a Combined yield of 3 steps starting from oxazolidinone resin 23.

^b Determined by chiral HPLC analysis after conversion to the corresponding (*S*)- α -phenylethylamides.

8% in each cycle. After the fourth cycle, the resin was cleaved by methanolysis to measure the amount of the residual auxiliary. Methyl ester 24, which corresponds to the chiral auxiliary on the resin, was obtained in 71% yield along with the 22% of undesired *N*-allylated oxazolidinone 27.⁴⁵ This indicated that the reduced yield obtained after recycling was due to the formation of byproduct 27, in which the substrate-loading site was completely blocked by the allyl group (Fig. 4). It is thought that this unfavorable side reaction was induced by the partial elimination of the *N*-acyl moiety during enolate-alkylation steps. In fact, from detailed analysis of our solution-phase model experiment, 6% of *N*-allylated byproduct formation was detected. Therefore, the reaction conditions should be carefully adjusted to minimize unfavorable *N*-alkylation of the oxazolidinone resin.

3. Conclusion

In the development of an efficient tool to prepare versatile chiral synthon, we designed and synthesized Wang resin-supported Evans' chiral oxazolidinone derivative based on the novel polymer-anchoring strategy, which utilizes the 5-position of the oxazolidinone ring. Solid-phase asymmetric Evans' enolate-alkylation reaction on this auxiliary resin proceeded successfully and a series of chiral α -branched carboxylic acids was obtained in high stereoselectivities (up to 97% ee), which are parallel to those obtained in the comparative classical solution-phase experiments. Therefore, this is the first successful example that Evans' asymmetric alkylation reaction proceeded efficiently on a solid-support. Furthermore, recycling of this polymer-bound chiral auxiliary was achieved by maintaining stereoselectivity of the product. This newly developed solid-support auxiliary provides a variety of chiral α -branched carboxylic acid derivatives, which would be valuable synthetic building blocks in Medicinal Chemistry.⁴⁴ These results also suggest the significance of the polymer-anchoring strategy of chiral auxiliary to perform the satisfactory asymmetric induction in solid-phase organic synthesis. Further application studies to other solid-phase Evans' asymmetric reactions are now in progress.

4. Experimental

4.1. General

NMR spectra (¹H and ¹³C) were recorded on a JEOL JNM-AL300 (¹H: 300 MHz; ¹³C: 75.5 MHz) or a Varian UNITY INOVA 400NB (¹H: 400 MHz; ¹³C: 100 MHz) spectrometer and the chemical shift values were expressed in parts per million downfield from tetramethylsilane (TMS) as an internal standard. All coupling constants (*J* values) were reported in Hertz (Hz). Infrared (IR) spectra were recorded using a Shimadzu FT-IR-8300 Fourier Transform Infrared Spectrophotometer. Melting points were taken on a micro hot-stage apparatus (Yanagimoto) and were uncorrected. Mass spectra (MS) were obtained by electron impact (EI) ionization methods on JEOL GCmate MS-BU20. Elemental analyses were done on a Perkin-Elmer Series

CHNS/O Analyzer 2400. Specific rotations were recorded on a Horiba High-speed Accurate Polarimeter SEPA-300 with a sodium lamp and are reported as follows: $[\alpha]_{\text{D}}^{\text{T}}$ (c g/100 mL, solvent). The enantiomeric excess was determined by chiral HPLC analysis with JASCO HPLC systems consisting of the following: pump, 880-PU; detector, 875-UV, measured at 230 nm; column, Chiralcel[®] OD normal phase column (4.6 × 250 mm; Daicel Chemical Ind., Ltd, Tokyo, Japan); mobile phase, *n*-hexane/EtOH; flow rate, 1.0 mL/min. Solvents used for HPLC analysis were of HPLC grade. Organic extracts were dried over sodium sulfate (Na₂SO₄), filtered, and concentrated using a rotary evaporator at <40 °C bath temperature. Solids and involatile oils were vacuum dried at <2 mmHg. Solution- and solid-phase asymmetric alkylation reactions were carried out under Ar atmosphere, using anhydrous THF in flame-dried glassware. In the case of solid-phase asymmetric alkylation reactions, immobilized substrates were agitated by a slow stirring under Ar atmosphere.

4.2. Materials

Commercially available chemicals were obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan), Nacalai Tesque, Inc. (Kyoto, Japan), Aldrich Chemical Co., Inc. (Milwaukee, WI) and Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan), and used without further purification. Exceptionally, triethylamine was distilled from CaH₂ under Ar atmosphere and stored over KOH (pellet). Dehydrated MeOH and THF were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan) and stored over pre-activated pellet-type molecular sieves 3A and 4A, respectively. Wang resin (0.80 mmol/g, styrene-1% DVB, 200–400 mesh) was purchased from Watanabe Chem. Ind., Ltd (Hiroshima, Japan). Boc-Apns-OH and H-Pns-OH were purchased from Nippon Kayaku (Tokyo, Japan). Boc- and Fmoc-Pns-OH were prepared from H-Pns-OH by the standard procedure. NaHMDS was used as supplied (Aldrich) as a solution in THF (1.0 M). Column chromatography was carried on Merck 107734 silica gel 60 (70–230 mesh). Analytical thin layer chromatography (TLC) was performed using Merck 105715 silica gel 60 F₂₅₄ precoated plates (0.25 mm thickness) and compounds were visualized by UV illumination (254 nm) and by heating after dipping in 10% ethanolic solution of phosphomolybdic acid or after spraying ca. 0.7% ethanolic solution of ninhydrin. Preparative TLC was done with Merck 105717 silica gel 60 F₂₅₄ plate (2.0 mm thickness).

4.3. Synthesis of *cis*-configured oxazolidinone **9** and *N*-3-phenylpropionylated carboximide **10**

4.3.1. Benzyl *N*-[(2*S*,3*S*)-3-[(*tert*-butoxycarbonyl)-amino]-2-hydroxy-4-phenylbutanoyl]piperidine-4-carboxylate **8.** To a solution of Boc-Apns-OH **6** (4.0 g, 13.5 mmol), benzyl piperidine-4-carboxylate HCl **7** (4.1 g, 16.2 mmol) and HOBt·H₂O (7.7 g, 16.2 mmol) in DMF (68 mL) was added EDC·HCl (3.1 g, 16.2 mmol) in parts at 0 °C. After stirring for 0.5 h at the same temperature, Et₃N (7.0 mL, 16.2 mmol) was added dropwise, then the reaction mixture was stirred overnight at room temperature. The solution was diluted with AcOEt and washed consecutively with 5% citric acid aq, 5% NaHCO₃ aq, water (×2) and

brine. After the organic layer was dried over Na₂SO₄, the solvent was removed under reduced pressure. The resulting white powder **8** (5.5 g, 82%) was used for the next reaction without any purification. *R*_f = 0.44 (*n*-hexane/AcOEt = 1:1); mp 37–39 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.41–7.14 (m, 10H), 5.16, 5.13 (2d, 0.5 × 2H, *J* = 12.3 Hz), 5.12 (s, 0.5 × 2H), 5.06 (br d, 0.5H, *J* = 8.4 Hz), 5.02 (br d, 0.5H, *J* = 9.0 Hz), 4.58 (d, 0.5H, *J* = 2.2 Hz), 4.55 (d, 0.5H, *J* = 2.2 Hz), 4.22–3.92 (m, 4H), 3.14, 3.06 (2ddd, 0.5 × 2H, *J* = 13.7, 11.2, 3.1 Hz), 2.88, 2.54 (2ddd, 0.5 × 2H, *J* = 13.4, 11.2, 3.1 Hz, partially overlapping with the next signal), 2.71–2.51 (m, 3H), 2.08–1.21 (m, 4H), 1.38 (s, 0.5 × 9H), 1.37 (s, 0.5 × 9H); ¹³C NMR (75.5 MHz, CDCl₃) δ 173.5, 173.5, 169.9, 169.6, 155.6, 137.8, 135.7, 129.2, 129.1, 128.6, 128.4, 128.3, 128.2, 128.1, 126.5, 126.4, 79.6, 77.2, 69.9, 69.8, 66.5, 54.1, 53.4, 44.1, 42.0, 42.0, 40.7, 34.4, 34.2, 28.3, 27.6; $[\alpha]_{\text{D}}^{26}$ = +16.3 (c 0.64, CHCl₃); FT-IR (CHCl₃) ν_{max} 3690, 3441, 3038, 1728, 1699, 1639, 1497, 1367, 1238, 1169, 698 cm⁻¹; HRMS (EI): found *M*⁺ 496.2576, C₂₈H₃₆N₂O₆ requires *M*⁺ 496.2573. Anal. Calcd for C₂₈H₃₆N₂O₆: C, 67.72; H, 7.31; N, 5.64; found: C, 67.69; H, 7.46; N, 5.58.

4.3.2. Benzyl *N*-[(4*S*,5*S*)-4-benzyl-1,3-oxazolidin-2-one-5-carbonyl]piperidine-4-carboxylate **9.** Compound **8** (5.4 g, 10.9 mmol) was treated with 4 M HCl/dioxane (45.0 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 2.5 h. After the solvent was removed under reduced pressure, the obtained colorless oil was dissolved in anhydrous THF (110 mL). To this solution was added Et₃N (2.3 mL, 16.4 mmol) dropwise at 0 °C, followed by CDI (2.7 g, 16.4 mmol). The cloudy reaction mixture was stirred overnight at room temperature, diluted with AcOEt, and washed consecutively with 5% citric acid aq, 5% NaHCO₃ aq, water and brine. After the organic layer was dried over Na₂SO₄, the solvent was removed under reduced pressure and the residue was applied to silica-gel column chromatography (*n*-hexane/AcOEt = 1:10) to yield **9** as a white powder (4.0 g, 86% for 2 steps). *R*_f = 0.27 (*n*-hexane/AcOEt = 1:10); mp 136–137 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.40–7.14 (m, 10H), 5.41 (d, 0.5H, *J* = 7.9 Hz), 5.39 (d, 0.5H, *J* = 8.1 Hz), 5.15 (s, 0.5 × 2H), 5.14 (s, 0.5 × 2H), 4.98 (br s, 0.5H), 4.92 (br s, 0.5H), 4.46, 4.23 (2dtd, 0.5 × 2H, *J* = 13.6, 4.0, 1.5 Hz, partially overlapping with the next signal), 4.28–4.18 (m, 1H), 3.76 (m, 0.5 × 2H), 3.25, 3.11 (2ddd, 0.5 × 2H, *J* = 13.6, 10.3, 3.3 Hz), 2.92–2.53 (m, 3H), 2.87–2.71 (m, 0.5 × 2H, partially overlapping with the next signal), 2.05–1.93 (m, 2H), 1.80–1.62 (m, 2H); ¹³C NMR (75.5 MHz, CDCl₃) δ 173.4, 173.4, 163.8, 163.7, 157.4, 157.3, 135.8, 135.6, 129.2, 129.1, 129.1, 128.9, 128.6, 128.4, 128.1, 127.3, 127.2, 75.1, 74.9, 66.5, 55.5, 55.4, 44.1, 43.9, 41.5, 41.2, 40.8, 40.0, 37.4, 37.3, 28.1, 28.1, 27.6, 27.4; $[\alpha]_{\text{D}}^{25}$ = –58.4 (c 1.01, CHCl₃); FT-IR (CHCl₃) ν_{max} 3030, 3020, 1774, 1730, 1666, 1231, 1207, 800, 791, 768, 714, 675 cm⁻¹; HRMS (EI): found *M*⁺ 422.1843, C₂₄H₂₆N₂O₅ requires *M*⁺ 422.1841. Anal. Calcd for C₂₄H₂₆N₂O₅: C, 68.23; H, 6.20; N, 6.63; found: C, 68.14; H, 6.28; N, 6.49.

4.3.3. Benzyl *N*-[(4*S*,5*S*)-4-benzyl-(3-phenylpropionyl)-1,3-oxazolidin-2-one-5-carbonyl]piperidine-4-carboxylate **10.** To a solution of 3-phenylpropionic acid (1.8 g, 11.7 mmol) in anhydrous THF (30 mL) was added Et₃N

(3.1 mL, 22.5 mmol) and trimethylacetylchloride (1.3 mL, 10.8 mmol) dropwise at $-18\text{ }^{\circ}\text{C}$. The reaction mixture was stirred at the same temperature for 0.5 h, then anhydrous LiCl (420 mg, 9.9 mmol) was added, followed by the slow addition of a solution of oxazolidinone **9** (3.8 g, 9.0 mmol) in anhydrous THF (20 mL). After the addition was completed, the reaction mixture was stirred overnight at room temperature. The solution was poured into ice-cold satd NaHCO₃ aq and the organic phase was extracted with AcOEt, washed with water and brine, and dried over Na₂SO₄. The solvent was removed under reduced pressure, and the resulting oil was applied to silica-gel column chromatography (*n*-hexane/AcOEt=4:1) to yield the desired compound **10** as a white solid (4.7 g, 95%). R_f =0.48 (*n*-hexane/AcOEt=1:1); mp 153–155 $^{\circ}\text{C}$; ¹H NMR (400 MHz, CDCl₃). Major isomer δ 7.41–7.04 (m, 15H), 5.11–5.09 (m, 1H), 5.07 (s, 2H), 4.92–4.87 (m, 1H), 4.36–4.33 (m, 1H), 3.36–3.26 (m, 2H), 3.11–2.93 (m, 6H), 2.22 (tt, 1H, J =11.2, 3.7 Hz), 2.10 (td, 1H, J =12.6, 3.1 Hz), 1.89–1.85 (m, 1H), 1.63–1.38 (m, 3H); minor isomer δ 7.41–7.04 (m, 15H), 5.11–5.09 (m, 3H), 4.92–4.87 (m, 1H), 3.59 (ddd, 1H, J =13.6, 6.4, 4.0 Hz), 3.36–3.20 (m, 2H), 3.11–2.93 (m, 4H), 3.14, 2.87 (2ddd, 2H, J =13.4, 8.8, 3.7 Hz, partially overlapping with the next signal), 2.71–2.64 (m, 1H), 2.47–2.41 (m, 1H), 1.77–1.70 (m, 1H), 1.63–1.38 (m, 2H), 0.98–0.89 (m, 1H); ¹³C NMR (75.5 MHz, CDCl₃) δ 173.2, 172.9, 171.9, 171.9, 162.0, 161.9, 151.7, 151.6, 140.2, 135.7, 135.6, 135.5, 129.6, 129.5, 128.7, 128.6, 128.6, 128.5, 128.4, 128.4, 128.1, 127.2, 127.1, 126.3, 73.3, 66.5, 66.4, 57.6, 57.5, 43.3, 43.1, 41.1, 40.8, 40.4, 39.0, 36.9, 34.2, 34.2, 30.1, 27.5, 27.2, 26.8, 26.1; $[\alpha]_D^{25}$ = -25.2 (c 1.16, CHCl₃); FT-IR (CHCl₃) ν_{max} 1790, 1730, 1701, 1670, 1454, 1375, 1173, 718, 696 cm^{-1} ; HRMS (EI): found M^+ 554.2410, C₃₃H₃₄N₂O₆ requires M^+ 554.2416. Anal. Calcd for C₃₃H₃₄N₂O₆: C, 71.46; H, 6.18; N, 5.05; found: C, 71.51; H, 6.40; N, 4.84.

4.3.4. Deuterium labeling study of the carboximide **10**.

Under Ar atmosphere, the solution of the carboximide **10** (146.5 mg, 0.264 mmol) in anhydrous THF (2.6 mL) was cooled to $-78\text{ }^{\circ}\text{C}$ (MeOH-dry ice bath), and LDA (1.8 M solution in heptane/THF/ethylbenzene, 0.18 mL, 0.32 mmol) was added dropwise. After stirring for 0.5 h at the same temperature, acetic acid-*d* (99at.% D) (0.31 mL, 5.28 mmol) was added slowly and the reaction mixture was stirred for 1 h at room temperature. The solution was poured into ice-cold satd NH₄Cl aq and the organic phase was extracted with AcOEt, washed with 5% NaHCO₃ aq, water and brine, and dried over Na₂SO₄. The solvent was removed under reduced pressure, and the resulting oil was subjected to preparative TLC (*n*-hexane/AcOEt=3:2, 2 times development) to yield the products as a white powder (124.7 mg, 85%). The content of deuterium-incorporated **12** was detected by NMR. R_f =0.53 (*n*-hexane/AcOEt=1:1); mp 40–41 $^{\circ}\text{C}$; ¹H NMR (400 MHz, CDCl₃) δ 7.42–7.18 (m, 15H), 5.14, 5.10 (2d, 0.5 \times 2H, J =12.3 Hz), 5.09 (s, 0.5 \times 2H), 4.88 (d, 0.16H, J =4.6 Hz), 4.87 (d, 0.16H, J =4.4 Hz), 4.71–4.64 (m, 1H), 4.19 (dt, 0.5H, J =13.6, 4.2 Hz), 4.13 (dt, 0.5H, J =13.4, 4.2 Hz), 3.45–3.18 (m, 2.88H), 3.08–2.94 (m, 2H), 2.87–2.32 (m, 5H), 1.91–1.86 (m, 1H), 1.65–1.38 (m, 2H and 0.5H), 1.18–1.10 (m, 0.5H); ¹³C NMR (100 MHz, CDCl₃) δ 173.3, 173.3, 172.1, 172.1, 164.8, 164.7, 152.5, 152.4, 140.3, 140.3, 135.7, 135.6,

135.2, 129.6, 129.5, 129.3, 129.3, 128.6, 128.6, 128.5, 128.4, 128.4, 128.2, 128.1, 128.1, 127.7, 126.2, 71.8, 71.6, 71.5 (t, J =24.1 Hz), 66.5, 66.5, 59.2, 59.1, 58.9, 58.8, 43.5, 43.3, 41.7, 41.6, 40.4, 40.3, 37.8, 37.7, 37.1, 37.0, 30.2, 28.2, 28.0, 27.4, 27.3; $[\alpha]_D^{26}$ = -15.1 (c 1.55, CHCl₃); FT-IR (CHCl₃) ν_{max} 1796, 1732, 1703, 1661, 1454, 1379, 1198, 1173, 772, 756, 727, 700, 679, 667 cm^{-1} ; HRMS (EI): found M^+ 555.2478, C₃₃H₃₃DN₂O₆ requires M^+ 555.2479. Anal. Calcd for C₃₃H₃₃DN₂O₆: C, 71.33; H + D, 6.35; N, 5.04; found: C, 71.26; H + D, 6.06; N, 4.99.

4.4. Synthesis of *trans*-configured oxazolidinone **14** and *N*-3-phenylpropionylated carboximide **16**

4.4.1. Benzyl *N*-[(4*S*,5*R*)-4-benzyl-1,3-oxazolidin-2-one-5-carbonyl]piperidine-4-carboxylate **14**.

To a solution of Boc-Pns-OH **13** (12.4 g, 42.0 mmol), benzyl piperidine-4-carboxylate·HCl **7** (12.9 g, 50.4 mmol) and HOBt·H₂O (7.7 g, 50.4 mmol) in DMF (210 mL) was added EDC·HCl (9.7 g, 50.4 mmol) in parts at 0 $^{\circ}\text{C}$. After stirring for 0.5 h at the same temperature, Et₃N (7.0 mL, 50.4 mmol) was added dropwise, then the reaction mixture was stirred overnight at room temperature. The solution was diluted with AcOEt and washed consecutively with 5% citric acid aq, 5% NaHCO₃ aq, water (\times 2) and brine. After the organic layer was dried over Na₂SO₄, the solvent was removed under reduced pressure. The resulting white powder (20.0 g, 96%) was used for the next reaction without any purification. R_f =0.52 (*n*-hexane/AcOEt=1:1); mp 34–36 $^{\circ}\text{C}$; ¹H NMR (400 MHz, CDCl₃) δ 7.38–7.21 (m, 10H), 5.17, 5.12 (2d, 0.5 \times 2H, J =12.5 Hz), 5.10 (s, 0.5 \times 2H), 4.87 (br d, 0.5H, J =10.8 Hz), 4.71 (br d, 0.5H, J =10.3 Hz), 4.28–4.01 (m, 4H), 3.13–2.68 (m, 5H), 2.62–2.47 (m, 1H), 2.08–1.33 (m, 4H), 1.39 (s, 0.5 \times 9H), 1.38 (s, 0.5 \times 9H); ¹³C NMR (75.5 MHz, CDCl₃) δ 173.6, 173.3, 170.3, 155.3, 155.2, 137.9, 137.7, 135.8, 135.6, 129.3, 128.6, 128.5, 128.2, 128.1, 128.0, 126.7, 79.4, 66.9, 66.6, 66.3, 53.8, 53.1, 43.7, 43.3, 42.1, 41.7, 41.0, 40.2, 38.8, 38.6, 28.2, 27.5, 27.2, 27.1, 26.7; $[\alpha]_D^{25}$ = -20.0 (c 0.47, CHCl₃); FT-IR (CHCl₃) ν_{max} 3439, 3005, 1717, 1701, 1639, 1499, 1454, 1393, 1367, 1240, 1169, 700 cm^{-1} ; HRMS (EI): found M^+ 496.2568, C₂₈H₃₆N₂O₆ requires M^+ 496.2573. Anal. Calcd for C₂₈H₃₆N₂O₆: C, 67.72; H, 7.31; N, 5.64; found: C, 67.65; H, 7.31; N, 5.90.

Obtained dipeptide (20.0 g, 40.3 mmol) was treated with 4 M HCl/dioxane (140 mL) at 0 $^{\circ}\text{C}$, and the reaction mixture was stirred at room temperature for 2.5 h. After the solvent was removed under reduced pressure, the colorless oil obtained was dissolved in anhydrous THF (400 mL). To this solution was added Et₃N (8.4 mL, 60.5 mmol) dropwise at 0 $^{\circ}\text{C}$, followed by the addition of CDI (9.8 g, 60.5 mmol). The cloudy reaction mixture was stirred overnight at room temperature, diluted with AcOEt, and washed consecutively with 5% citric acid aq, 5% NaHCO₃ aq, water and brine. After the organic layer was dried over Na₂SO₄, the solvent was removed under reduced pressure and the residue was applied to silica-gel column chromatography (*n*-hexane/AcOEt=1:2) to yield **14** as a white powder (15.0 g, 88% for 2 steps). R_f =0.55 (*n*-hexane/AcOEt=1:5); mp 91–93 $^{\circ}\text{C}$; ¹H NMR (400 MHz, CDCl₃) δ 7.40–7.20 (m, 10H), 5.28 (br s, 0.5H), 5.25 (br s, 0.5H), 5.14 (s, 0.5 \times 2H), 5.12 (s, 0.5 \times 2H), 4.80 (d, 0.5H, J =5.3 Hz), 4.79 (d, 0.5H, J =5.1 Hz),

4.69–4.64 (m, 1H), 4.40–4.37 (m, 0.5H), 4.19 (dt, 0.5H, $J=13.6, 4.2$ Hz), 3.89–3.86 (m, 0.5H), 3.74–3.71 (m, 0.5H), 3.23, 3.0 (2br t, $0.5 \times 2H$, $J=11.2$ Hz, partially overlapping with the next signal), 3.06–2.77 (m, 3H), 2.67–2.55 (m, 1H), 1.99–1.59 (m, 4H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 173.7, 173.4, 164.4, 164.3, 156.9, 135.8, 135.8, 135.7, 129.1, 129.0, 128.6, 128.3, 128.1, 127.3, 76.8, 76.6, 66.5, 55.3, 44.8, 44.5, 42.0, 41.8, 41.0, 41.0, 40.9, 40.3, 28.4, 28.2, 27.5, 27.5; $[\alpha]_D^{27} = -91.2$ (c 1.28, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 3452, 3036, 3007, 1771, 1730, 1653, 1456, 1387, 1313, 1271, 1238, 1209, 1173, 1038, 1011, 756, 737, 698, 667 cm^{-1} ; HRMS (EI): found M^+ 422.1845, $C_{24}H_{26}N_2O_5$ requires M^+ 422.1842. Anal. Calcd for $C_{24}H_{26}N_2O_5$: C, 68.23; H, 6.20; N, 6.63; found: C, 67.99; H, 6.20; N, 6.55.

4.4.2. *N*-[*N*-[(4*S*,5*R*)-4-benzyl-1,3-oxazolidin-2-one-5-carbonyl]piperidine-4-carboxyl]-(*R*)-1-phenethyl amide

15. To a solution of oxazolidinone **14** (141.1 mg, 0.334 mmol) in MeOH (3.0 mL) and water (0.35 mL) was added 5% Pd-C (15.2 mg), and the reaction mixture was stirred for 3 h under H_2 atmosphere. The reaction mixture was purged with Ar, then filtered through a pad of Celite® with MeOH. After evaporation, the resulting oil was diluted with AcOEt, and washed consecutively with water and brine. After the organic layer was dried over Na_2SO_4 , the solvent was removed under reduced pressure. To a solution of this carboxylic acid in DMF (4.0 mL) was added HOBt· H_2O (61.3 mg, 0.401 mmol) and EDC·HCl (61.3 mg, 0.401 mmol) at 0 °C. After the mixture was stirred for 0.5 h at the same temperature, (*R*)- α -methylbenzylamine (51.6 μ L, 0.401 mmol) was added dropwise. The reaction mixture was stirred for overnight at room temperature, then diluted with AcOEt and washed with 5% citric acid aq, 5% $NaHCO_3$ aq, water and brine, and dried over Na_2SO_4 . The solvent was removed under reduced pressure and the resulting crude product was purified by preparative TLC ($CHCl_3/MeOH=10:1$, 2 times development) to yield amide **15** as a white powder (133.7 mg, 92% for 2 steps). Recrystallization of the obtained white powder from $CHCl_3$ afforded the white needles, which was analyzed by X-ray crystallography. $R_f=0.34$ ($CHCl_3/MeOH=10:1$); mp 190–191 °C; 1H NMR (400 MHz, $CDCl_3$) δ 7.37–7.20 (m, 10H), 5.75 (br d, 0.5H, $J=8.1$ Hz), 5.72 (br d, 0.5H, $J=8.4$ Hz), 5.13 (q, 0.5H, $J=6.8$ Hz), 5.11 (q, 0.5H, $J=7.0$ Hz), 5.06 (s, 0.5H), 5.05 (s, 0.5H), 4.81 (d, 0.5H, $J=5.5$ Hz), 4.79 (d, 0.5H, $J=5.7$ Hz), 4.69–4.64 (m, 1H), 4.56–4.52 (m, 0.5H), 4.45–4.39 (m, 0.5H), 3.95–3.99 (m, 0.5H), 3.87–3.82 (m, 0.5H), 3.16, 2.87 (2ddd, $0.5 \times 2H$, $J=14.3, 11.5, 2.9$ Hz, partially overlapping with the next signal), 3.01–2.67 (m, 3H), 2.39–2.29 (m, 1H), 1.94–1.54 (m, 4H), 1.50 (d, $0.5 \times 3H$, $J=7.0$ Hz), 1.48 (d, $0.5 \times 3H$, $J=6.8$ Hz); ^{13}C NMR (75.5 MHz, DMSO- d_6) δ 172.8, 165.6, 165.5, 157.4, 145.0, 144.8, 136.3, 136.2, 129.6, 129.5, 128.6, 128.3, 126.8, 126.6, 125.8, 74.3, 74.1, 55.8, 55.5, 47.6, 44.0, 41.4, 41.3, 28.9, 28.1, 27.7, 22.5; $[\alpha]_D^{25} = +9.4$ (c 1.05, MeOH); HRMS (EI): found M^+ 435.2157, $C_{25}H_{29}N_3O_4$ requires M^+ 435.2158.

4.4.3. Crystallography of amide 15. Diffraction data for **15** were collected on a Rigaku AFC7R diffractometer with graphite monochromated Cu K α radiation ($\lambda=1.54178$ Å)

and a rotating anode generator. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation. Formula $C_{25}H_{29}N_3O_4$, formula weight=435.52, orthorhombic, space group $P2_12_12_1$ (#19), $a=17.986(2)$, $b=23.841(2)$, $c=5.269(3)$ Å, $V=2259(1)$ Å³, $Z=4$, $D_{calc}=1.280$ g/cm³, $F_{000}=928.00$, $\mu(Cu K\alpha)=7.10$ cm⁻¹. Total of 1554 unique reflections (complete for $2\theta < 110^\circ$) was used in the solution and refinement of structure. The structure was solved by direct methods using SAPI91,⁴⁵ and expanded using Fourier techniques with DIRDIF94 program.⁴⁶ The final refinement was done by the full-matrix least-squares method with anisotropic thermal parameters for all non-hydrogen atoms, and hydrogen atoms were included but not refined. The final R value was 0.238 ($R_w=0.087$).

4.4.4. Benzyl *N*-[(4*S*,5*R*)-4-benzyl-(3-phenylpropionyl)-1,3-oxazolidin-2-one-5-carbonyl]piperidine-4-carboxylate

16. To a solution of 3-phenylpropionic acid (6.8 g, 45.2 mmol) in anhydrous THF (100 mL) was added Et_3N (12.2 mL, 87.0 mmol) and trimethylacetylchloride (5.2 mL, 41.8 mmol) dropwise at -18 °C. The reaction mixture was stirred at the same temperature for 0.5 h, then anhydrous LiCl (1.6 g, 38.3 mmol) was added, followed by the slow addition of a solution of oxazolidinone **14** (14.7 g, 34.8 mmol) in anhydrous THF (75 mL). After the addition was completed, the reaction mixture was stirred overnight at room temperature. The solution was poured into ice-cold satd $NaHCO_3$ aq and the organic phase was extracted with AcOEt, washed with water and brine, and dried over Na_2SO_4 . The solvent was removed under reduced pressure, and the resulting oil was applied to silica-gel column chromatography (n -hexane/AcOEt=4:1) to yield the desired compound **16** as a white solid (18.5 g, 96%). $R_f=0.52$ (n -hexane/AcOEt=1:1); mp 39–41 °C; 1H NMR (400 MHz, $CDCl_3$) δ 7.40–7.18 (m, 15H), 5.14, 5.10 (2d, $0.5 \times 2H$, $J=12.3$ Hz), 5.09 (s, $0.5 \times 2H$), 4.88 (d, 0.5H, $J=4.4$ Hz), 4.87 (d, 0.5H, $J=4.4$ Hz), 4.71–4.65 (m, 1H), 4.19 (dt, 0.5H, $J=13.7, 4.0$ Hz), 4.13 (dt, 0.5H, $J=13.4, 4.2$ Hz), 3.45–3.18 (m, 3H), 3.08–2.94 (m, 2H), 2.83–2.33 (m, 5H), 1.92–1.86 (m, 1H), 1.65–1.39 (m, 2H and 0.5H), 1.19–1.09 (m, 0.5H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 173.1, 171.9, 164.7, 164.5, 152.4, 152.3, 140.2, 135.6, 135.5, 135.0, 129.4, 129.3, 129.2, 129.1, 128.4, 128.3, 128.3, 128.2, 128.2, 127.9, 127.9, 127.5, 126.0, 71.7, 71.5, 66.3, 59.2, 58.8, 43.3, 43.1, 41.5, 41.4, 40.2, 40.1, 37.6, 37.5, 36.9, 36.9, 30.0, 28.0, 27.9, 27.2; $[\alpha]_D^{26} = -16.7$ (c 2.09, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 3040, 3007, 1794, 1728, 1701, 1659, 1497, 1454, 1379, 1310, 1292, 1263, 1244, 1171, 1103, 1078, 1030, 694 cm^{-1} ; HRMS (EI): found M^+ 554.2410, $C_{33}H_{34}N_2O_6$ requires M^+ 554.2416. Anal. Calcd for $C_{33}H_{34}N_2O_6$: C, 71.46; H, 6.18; N, 5.05; found: C, 71.28; H, 5.99; N, 5.34.

4.4.5. Deuterium labeling study of the carboximide 16.

Under Ar atmosphere, the solution of the carboximide **16** (142.4 mg, 0.257 mmol) in anhydrous THF (2.6 mL) was cooled to -78 °C (MeOH-dry ice bath), and LDA (1.8 M solution in heptane / THF / ethylbenzene, 0.17 mL, 0.31 mmol) was added dropwise. After stirring for 0.5 h at the same temperature, acetic acid- d (99at.% D) (0.30 mL, 5.14 mmol) was added slowly, then cooling bath was removed and the reaction mixture was stirred for 1 h at room

temperature. The solution was poured into ice-cold satd NH_4Cl aq and the organic phase was extracted with AcOEt, washed with 5% NaHCO_3 aq, water and brine, and dried over Na_2SO_4 . The solvent was removed under reduced pressure, and the resulting oil was subjected to preparative TLC (*n*-hexane/AcOEt=1:1) to yield the products as a white powder (125.7 mg, 88%). The content of deuterium-incorporated **17** was detected by NMR. $R_f=0.53$ (*n*-hexane/AcOEt=1:1); mp 39–40 °C; ^1H NMR (400 MHz, CDCl_3) δ 7.40–7.18 (m, 15H), 5.14, 5.10 (2d, 0.5×2H, $J=12.3$ Hz), 5.09 (s, 0.5×2H), 4.88 (d, 0.5H, $J=4.6$ Hz), 4.87 (d, 0.5H, $J=4.6$ Hz), 4.71–4.64 (m, 1H), 4.19 (dt, 0.5H, $J=13.6$, 4.0 Hz), 4.13 (dt, 0.5H, $J=13.2$, 4.0 Hz), 3.44–3.18 (m, 2.24H), 3.06–2.94 (m, 2H), 2.83–2.33 (m, 5H), 1.91–1.86 (m, 1H), 1.64–1.38 (m, 2H and 0.5H), 1.18–1.08 (m, 0.5H); ^2H NMR (400 MHz, CHCl_3) δ 3.32 (s, 0.76D); ^{13}C NMR (75.5 MHz, CDCl_3) δ 173.3, 172.1, 164.8, 164.6, 152.5, 152.4, 140.3, 135.7, 135.6, 135.2, 129.5, 129.5, 129.3, 129.3, 128.6, 128.5, 128.4, 128.4, 128.1, 127.7, 126.2, 71.8, 71.6, 66.5, 59.2, 58.9, 43.5, 43.3, 41.7, 41.6, 40.3, 37.8, 37.7, 37.1, 37.0, 36.7 (t, $J=19.9$ Hz), 30.1, 30.1, 28.2, 28.0, 27.3; $[\alpha]_D^{27} = -14.8$ (c 1.69, CHCl_3); FT-IR (CHCl_3) ν_{max} 1792, 1732, 1703, 1661, 1454, 1371, 1236, 1196, 1186, 1173, 797, 725, 700, 673 cm^{-1} ; HRMS (EI): found M^+ 555.2482, $\text{C}_{33}\text{H}_{33}\text{DN}_2\text{O}_6$ requires M^+ 555.2479. Anal. Calcd for $\text{C}_{33}\text{H}_{33}\text{DN}_2\text{O}_6$: C, 71.33; H+D, 6.35; N, 5.04; found: C, 71.17; H+D, 6.29; N, 5.01.

4.5. Preparation of the Wang resin-supported oxazolidinone **23** by Fmoc-based solid-phase synthesis

Wang resin (0.80 mmol/g resin) (5.0 g, 4.0 mmol) in a cap-fitted reaction vessel was washed with CH_2Cl_2 (20 mL, ×5), then Fmoc-piperidine-4-carboxylic acid **20** (4.2 g, 12.0 mmol) and CH_2Cl_2 (30 mL) were charged. DIPCDCI (1.9 mL, 12.0 mmol) was added, followed by the addition of DMAP (48.7 mg, 0.4 mmol). The heterogeneous reaction mixture was vigorously shaken for 2 h at room temperature, then filtered and washed with DMF (20 mL, ×5). The obtained white resin **21** was then washed with piperidine in DMF (20%, v/v) (20 mL, ×5) and treated with piperidine in DMF (20%, v/v) (30 mL) for 0.5 h at room temperature. The solvent and reagent were drained and the resin was washed with DMF (20 mL), CHCl_3 (20 mL), DMF (20 mL) (×5, sequentially). Next, Fmoc-Pns-OH (5.0 g, 12.0 mmol), $\text{HOBT} \cdot \text{H}_2\text{O}$ (1.8 g, 12.0 mmol), DMF (30 mL) and DIPCDCI (1.9 mL, 12.0 mmol) were added, and the heterogeneous reaction mixture was vigorously shaken for 2 h at room temperature, then filtered and washed with DMF (20 mL, ×5). The aliquot of the resultant resin **22** was applied to the Kaiser-Test⁴⁷ to check the reaction progress. Starting secondary amine resin was positive (pale orange), whereas the dipeptide-bound resin **22** was negative (colorless). The obtained resin **22** was washed with piperidine in DMF (20%, v/v) (20 mL, ×5) and treated with piperidine in DMF (20%, v/v) (30 mL) for 0.5 h at room temperature. The solvent and reagent were drained and the resin was washed with DMF (20 mL), CHCl_3 (20 mL), DMF (20 mL) (×5, sequentially). The obtained amino alcohol resin was washed with THF (20 mL, ×5), then CDI (1.9 g, 12.0 mmol) and anhydrous THF (30 mL) were added. The heterogeneous reaction mixture was vigorously shaken for 3 h at room temperature, then filtered and washed with THF (20 mL, ×5). Kaiser-Test of the starting primary amine

resin was positive (blue), whereas the oxazolidinone resin **23** was negative (colorless). The obtained resin was washed with CHCl_3 (20 mL) and MeOH (20 mL) (×5, sequentially), then overnight drying in vacuo afforded the desired pale yellowish oxazolidinone resin **23** (6.3 g) with loading rate of 0.61 mmol/g.

4.5.1. O-Wang resin-supported *N*-[(9*H*-9-fluorenyl-methoxy)carbonyl]piperidine-4-carboxylic acid **21.** FT-IR (KBr) ν_{max} 1736, 1719 cm^{-1} .

4.5.2. O-Wang resin-supported *N*-((2*R*,3*S*)-3-[(9*H*-9-fluorenylmethoxy)carbonyl]amino)-2-hydroxy-4-phenylbutanoyl)piperidine-4-carboxylic acid **22.** FT-IR (KBr) ν_{max} 3398, 1733, 1718, 1638 cm^{-1} .

4.5.3. O-Wang resin-supported *N*-[(4*S*,5*R*)-4-benzyl-1,3-oxazolidin-2-one-3-carbonyl]piperidine-4-carboxylic acid **23.** FT-IR (KBr) ν_{max} 1763, 1740, 1655 cm^{-1} .

4.5.4. Methanolysis of the oxazolidinone resin **23 to afford the methyl *N*-[(4*S*,5*R*)-4-benzyl-1,3-oxazolidin-2-one-5-carbonyl]piperidine-4-carboxylate **24**.** Oxazolidinone-loaded resin **23** (129.9 mg, 0.083 mmol) was swollen in anhydrous THF (0.85 mL) and anhydrous MeOH (0.85 mL), then potassium carbonate (22.9 mg, 0.166 mmol) was added in one portion at 0 °C. The heterogeneous reaction mixture was gently stirred for 2 h at room temperature. The reaction was quenched by the addition of satd NH_4Cl aq, and the resultant resin was removed by filtration. The filtrate was extracted with AcOEt, and washed with water and brine, then dried over Na_2SO_4 . After solvent removal, the remaining crude oil was purified by preparative TLC (*n*-hexane/AcOEt=1:10) to yield the oxazolidinone methyl ester **24** as a white solid (27.4 mg, 95% in 6 steps from Wang resin). $R_f=0.30$ (*n*-hexane/AcOEt=1:5); mp 39–40 °C; ^1H NMR (400 MHz, CDCl_3) δ 7.36–7.21 (m, 5H), 5.73 (br s, 1H), 4.82 (d, 0.5H, $J=5.5$ Hz), 4.80 (d, 0.5H, $J=5.5$ Hz), 4.67–4.62 (m, 1H), 4.39–4.34 (m, 0.5H), 4.19 (dt, 0.5H, $J=13.6$, 4.0 Hz), 3.83–3.79 (m, 0.5H), 3.70–3.65 (m, 0.5H, partially overlapping with the next signal), 3.70 (s, 0.5×3H), 3.68 (s, 0.5×3H), 3.20, 3.00 (2ddd, 0.5×2H, $J=14.1$, 10.6, 3.1 Hz, partially overlapping with the next signal), 2.99–2.78 (m, 3H), 2.61–2.50 (m, 1H), 1.96–1.54 (m, 4H); ^{13}C NMR (75.5 MHz, CDCl_3) δ 174.3, 174.1, 164.5, 164.4, 157.1, 135.8, 135.7, 129.1, 128.8, 127.1, 76.4, 76.3, 55.4, 55.3, 51.8, 44.7, 44.4, 41.9, 41.7, 40.8, 40.7, 40.6, 40.1, 28.3, 28.1, 27.4; $[\alpha]_D^{25} = -104.4$ (c 0.55, CHCl_3); FT-IR (CHCl_3) ν_{max} 3454, 3007, 2955, 1771, 1732, 1655, 1456, 1437, 1383, 1317, 1269, 1240, 1194, 1177, 1040, 1015, 760, 745 cm^{-1} ; HRMS (EI): found M^+ 346.1526, $\text{C}_{18}\text{H}_{22}\text{N}_2\text{O}_5$ requires M^+ 346.1528. Anal. Calcd for $\text{C}_{18}\text{H}_{22}\text{N}_2\text{O}_5 \cdot 0.25\text{H}_2\text{O}$: C, 61.61; H, 6.46; N, 7.98; found: C, 61.99; H, 6.26; N, 7.96.

4.6. General procedure for *N*-acylation of the Wang resin-supported oxazolidinone resin **23**, solid-phase asymmetric alkylation, lithium hydroperoxide-mediated hydrolysis, and the derivatization to the (*S*)-phenylethylamide for enantiomeric excess determination

Oxazolidinone-loaded resin **23** in a polystyrene reactor was washed with CH_2Cl_2 (×5), then the corresponding

carboxylic acid (3.0 equiv), 2-chloro-1-methylpyridinium iodide (3.0 equiv) and anhydrous CH_2Cl_2 (0.08 mmol resin/mL) were added. The mixture was shaken for 10 min, followed by the addition of Et_3N (5.0 equiv) and DMAP (0.3 equiv). The reaction mixture was shaken for 2 h at room temperature and filtered, then the resultant resin was washed with CH_2Cl_2 ($\times 5$). The reaction was repeated once again, and the obtained resin was washed with DMF, CHCl_3 and MeOH ($\times 5$, sequentially), then overnight drying in vacuo afforded the desired carboximide resin **25**. Under Ar atmosphere, carboximide resin **25** in a glass reaction vessel was swollen in THF (20 mL/mmol resin) for 10 min at room temperature, and the heterogeneous mixture was cooled to -78°C (MeOH-dry ice bath), followed by the dropwise addition of 1.0 M THF solution of NaHMDS (3.0 equiv). After continuously stirring for 1 h at the same temperature, the corresponding alkyl halide (10.0 equiv) was added. The temperature of the reaction mixture was gradually increased up to 0°C over 12 h with gentle stirring, then quenched by the addition of satd NH_4Cl aq, and tri-phase reaction mixture was stirred for additional 15 min. at 0°C . The resultant resin was separated from the reaction mixture by filtration, followed by washing with THF- H_2O (1:1), THF and MeOH ($\times 5$, sequentially). Then, the resin was dried well in the desiccator under reduced pressure for 3 h. THF- H_2O (3:1, v/v) (0.05 mmol resin/mL) was added to the α -alkylated carboximide resin, and the resin was swollen for 10 min. at 0°C . Next, 30% aqueous H_2O_2 (6.0 equiv) and $\text{LiOH}\cdot\text{H}_2\text{O}$ (3.0 equiv) were added. After gentle stirring for 2 h at the same temperature, the reaction was quenched by the addition of 1.5 N NaHSO_3 aq, and the deacylated resin was filtered off. The filtrate was acidified to pH 2 with 1 N HCl aq, and extracted with AcOEt. The extract was washed with brine, and dried over Na_2SO_4 . After removal of the solvent under reduced pressure, the residue was purified by preparative TLC to yield the desired α -alkylated carboxylic acids **26**. The recovered oxazolidinone resin **23** was washed with THF, CHCl_3 and MeOH ($\times 5$, sequentially), then dried in the desiccator under reduced pressure. Determination of the enantiomeric excess of the obtained carboxylic acids **26** was carried out by derivatization to the corresponding (*S*)-phenylethyl amides and chiral HPLC analysis. To a 0.05 M solution of the acids **26** in DMF was added $\text{HOBt}\cdot\text{H}_2\text{O}$ (1.2 equiv) and EDC·HCl (1.2 equiv) at 0°C . The mixture was stirred for 0.5 h at the same temperature, and (*S*)-phenylethylamine (1.2 equiv) was added dropwise. The reaction mixture was stirred overnight at room temperature, then diluted with AcOEt and washed with 5% citric acid aq, 5% NaHCO_3 aq, water and brine, and dried over Na_2SO_4 . The solvent was removed under reduced pressure, and the resulting amide was subjected to the HPLC analysis without any purification. Enantiomeric excess was calculated from the peak areas of the corresponding two diastereomers.

4.6.1. (*S*)-2-Benzylpropanoic acid 26a. The title compound **26a** was obtained according to the general procedure using the oxazolidinone resin **23** (277.5 mg, 0.169 mmol). Purification by preparative TLC (*n*-hexane/AcOEt=1:1) gave **26a** as a colorless oil (16.8 mg, 61% yield in 3 steps from oxazolidinone resin **23**). $R_f=0.63$ (*n*-hexane/AcOEt=1:1); ^1H NMR (300 MHz, CDCl_3) δ 7.32–7.17 (m, 5H), 3.08 (dd, 1H, $J=13.0$, 6.1 Hz), 2.83–2.71 (m, 1H), 2.67 (dd, 1H, $J=13.0$, 7.9 Hz), 1.18 (d, 3H, $J=6.8$ Hz); ^{13}C NMR

(75.5 MHz, CDCl_3) δ 181.7, 139.0, 129.0, 128.4, 126.4, 41.1, 39.3, 16.5; $[\alpha]_D^{28}=+20.6$ (c 0.87, CHCl_3); lit.,⁴⁸ $[\alpha]_D=+25.5$ (c 1.00, CHCl_3); FT-IR (CHCl_3) ν_{max} 3038, 2980, 1709, 1454, 1238, 719, 698, 675 cm^{-1} ; HRMS (EI): found M^+ 164.0838, $\text{C}_{10}\text{H}_{12}\text{O}_2$ requires M^+ 164.0837. Anal. Calcd for $\text{C}_{10}\text{H}_{12}\text{O}_2$: C, 73.15; H, 7.37; found: C, 73.25; H, 7.47. Enantiomeric excess was 85% ee determined by chiral HPLC analysis of the corresponding (*S*)- α -methylbenzylamine-derived amide with Chiralcel[®] OD normal phase column (*n*-hexane/EtOH=30/1, 1.0 mL/min, 230 nm), major isomer=13.1 min, minor isomer=16.8 min.

4.6.2. (*S*)-2-Benzylbutanoic acid 26b. The title compound **26b** was obtained according to the general procedure using the oxazolidinone resin **23** (193.8 mg, 0.118 mmol). Purification by preparative TLC ($\text{CHCl}_3/\text{MeOH}=10:1$) gave **26b** as a colorless oil (10.6 mg, 50% yield in 3 steps from oxazolidinone resin **23**). $R_f=0.53$ ($\text{CHCl}_3/\text{MeOH}=10:1$); ^1H NMR (300 MHz, CDCl_3) δ 7.30–7.16 (m, 5H), 2.98 (dd, 1H, $J=13.6$, 7.7 Hz), 2.75 (dd, 1H, $J=13.6$, 6.8 Hz), 2.66–2.57 (m, 1H), 1.72–1.54 (m, 2H), 0.96 (t, 3H, $J=7.3$ Hz); ^{13}C NMR (75.5 MHz, CDCl_3) δ 181.3, 139.1, 128.9, 128.4, 126.4, 48.8, 37.7, 24.7, 11.6; $[\alpha]_D^{26}=+30.7$ (c 0.86, benzene); lit.,⁴⁹ $[\alpha]_D^{24}=+34.7$ (c 8.45, benzene); FT-IR (CHCl_3) ν_{max} 1707, 1462, 1383, 1096, 899, 696, 652 cm^{-1} ; HRMS (EI): found M^+ 178.0999, $\text{C}_{11}\text{H}_{14}\text{O}_2$ requires M^+ 178.0994. Anal. Calcd for $\text{C}_{11}\text{H}_{14}\text{O}_2$: C, 74.13; H, 7.92; found: C, 73.99; H, 7.99. Enantiomeric excess was 88% ee determined by chiral HPLC analysis of the corresponding (*S*)- α -methylbenzylamine-derived amide with Chiralcel[®] OD normal phase column (*n*-hexane/EtOH=30/1, 1.0 mL/min, 230 nm), major isomer=11.3 min, minor isomer=16.1 min.

4.6.3. (*S*)-2-Benzyl-4-pentenoic acid 26c. The title compound **26c** was obtained according to the general procedure using the oxazolidinone resin **23** (302.1 mg, 0.184 mmol). Purification by preparative TLC ($\text{CHCl}_3/\text{MeOH}=10:1$) gave **26c** as a colorless oil (23.8 mg, 68% yield in 3 steps from oxazolidinone resin **23**). $R_f=0.50$ ($\text{CHCl}_3/\text{MeOH}=10:1$); ^1H NMR (300 MHz, CDCl_3) δ 7.31–7.16 (m, 5H), 5.78 (ddt, 1H, $J=17.1$, 10.3, 7.0 Hz), 5.12–5.05 (m, 2H), 3.03–2.94 (m, 1H), 2.82–2.72 (m, 2H), 2.44–2.25 (m, 2H); ^{13}C NMR (75.5 MHz, CDCl_3) δ 180.7, 138.8, 134.7, 128.9, 128.5, 126.5, 117.5, 46.9, 37.3, 35.6; $[\alpha]_D^{26}=+24.0$ (c 1.27, CHCl_3); lit.,³³ $[\alpha]_D^{25}=+19.2$ (c 12.2, CHCl_3); FT-IR (CHCl_3) ν_{max} 3084, 3067, 3038, 1709, 922, 802, 775, 764, 746, 739, 729, 721, 700, 675, 667 cm^{-1} ; HRMS (EI): found M^+ 190.0989, $\text{C}_{12}\text{H}_{14}\text{O}_2$ requires M^+ 190.0994. Anal. Calcd for $\text{C}_{12}\text{H}_{14}\text{O}_2$: C, 75.76; H, 7.42; found: C, 75.50; H, 7.50. Enantiomeric excess was 96% ee determined by chiral HPLC analysis of the corresponding (*S*)- α -methylbenzylamine-derived amide with Chiralcel[®] OD normal phase column (*n*-hexane/EtOH=30/1, 1.0 mL/min, 230 nm), major isomer=11.6 min, minor isomer=15.2 min.

4.6.4. (*S*)-2-Benzyl-4-pentynoic acid 26d. The title compound **26d** was obtained according to the general procedure using the oxazolidinone resin **23** (206.9 mg, 0.126 mmol). Purification by preparative TLC ($\text{CHCl}_3/\text{MeOH}=10:1$) gave **26d** as a colorless oil (14.7 mg, 62% yield in 3 steps from oxazolidinone resin **23**). $R_f=0.44$ ($\text{CHCl}_3/\text{MeOH}=10:1$); ^1H

NMR (300 MHz, CDCl₃) δ 7.31–7.20 (m, 5H), 3.09 (dd, 1H, $J=13.4, 6.6$ Hz), 2.99–2.85 (m, 2H), 2.44 (dd, 2H, $J=6.4, 2.6$ Hz), 2.06 (t, 1H, $J=2.6$ Hz); ¹³C NMR (75.5 MHz, CDCl₃) δ 179.5, 138.1, 129.0, 128.5, 126.7, 80.9, 70.6, 45.9, 36.3, 20.0; $[\alpha]_D^{26} = -10.9$ (c 1.24, CHCl₃); FT-IR (CHCl₃) ν_{\max} 3308, 1719, 1217, 1200, 770, 700, 671 cm⁻¹; HRMS (EI): found M⁺ 188.0835, C₁₂H₁₂O₂ requires M⁺ 188.0837. Anal. Calcd for C₁₂H₁₂O₂·0.25H₂O: C, 74.78; H, 6.54; found: C, 75.14; H, 6.57. Enantiomeric excess was 96% ee determined by chiral HPLC analysis of the corresponding (*S*)- α -methylbenzylamine-derived amide with Chiralcel[®] OD normal phase column (*n*-hexane/EtOH=30/1, 1.0 mL/min, 230 nm), major isomer=16.4 min, minor isomer=18.6 min.

4.6.5. (*R*)-2-Benzyl-4-ethoxy-4-oxobutanoic acid 26e. The title compound **26e** was obtained according to the general procedure using the oxazolidinone resin **23** (259.8 mg, 0.158 mmol). Purification by preparative TLC (CHCl₃/MeOH=10:1) gave **26e** as a colorless oil (23.1 mg, 62% yield in 3 steps from oxazolidinone resin **23**). $R_f=0.41$ (CHCl₃/MeOH=10:1); ¹H NMR (300 MHz, CDCl₃) δ 7.32–7.17 (m, 5H), 4.11 (q, 2H, $J=7.2$ Hz), 3.21–3.10 (m, 2H), 2.83–2.74 (m, 1H), 2.64 (dd, 1H, $J=17.0, 8.9$ Hz), 2.41 (dd, 1H, $J=17.0, 4.6$ Hz), 1.22 (t, 3H, $J=7.2$ Hz); ¹³C NMR (75.5 MHz, CDCl₃) δ 179.5, 171.7, 137.9, 129.1, 128.6, 126.8, 60.8, 42.8, 37.4, 34.8, 14.1; $[\alpha]_D^{26} = +10.6$ (c 1.15, CHCl₃); lit.,⁵⁰ $[\alpha]_D^{28} = +10.0$ (c 2.9, CHCl₃); FT-IR (CHCl₃) ν_{\max} 1732, 1717, 910, 777, 754, 739, 721, 700, 679, 652 cm⁻¹; HRMS (EI): found M⁺ 236.1051, C₁₃H₁₆O₄ requires M⁺ 236.1048. Anal. Calcd for C₁₃H₁₆O₄: C, 66.09; H, 6.83; found: C, 65.93; H, 6.81. Enantiomeric excess was 92% ee determined by chiral HPLC analysis of the corresponding (*S*)- α -methylbenzylamine-derived amide with Chiralcel[®] OD normal phase column (*n*-hexane/EtOH=30/1, 1.0 mL/min, 230 nm), major isomer=15.7 min, minor isomer=16.6 min.

4.6.6. (*R*)-2-Benzylpropanoic acid 26f. The title compound **26f** was obtained according to the general procedure using the oxazolidinone resin **23** (236.5 mg, 0.144 mmol). Purification by preparative TLC (*n*-hexane/AcOEt=1:1) gave **26f** as a colorless oil (16.6 mg, 70% yield in 3 steps from oxazolidinone resin **23**). $R_f=0.63$ (*n*-hexane/AcOEt=1:1); ¹H NMR (300 MHz, CDCl₃) δ 7.31–7.17 (m, 5H), 3.08 (dd, 1H, $J=13.0, 6.1$ Hz), 2.80–2.70 (m, 1H), 2.67 (dd, 1H, $J=13.0, 7.9$ Hz), 1.18 (d, 3H, $J=6.8$ Hz); ¹³C NMR (75.5 MHz, CDCl₃) δ 182.3, 139.0, 129.0, 128.4, 126.4, 41.2, 39.3, 16.5; $[\alpha]_D^{28} = -30.7$ (c 1.04, CHCl₃); lit.,⁵¹ $[\alpha]_D^{22} = -30.1$ (c 1.00, CHCl₃); FT-IR (CHCl₃) ν_{\max} 1707, 1464, 1381, 1231, 893, 800, 694, 648 cm⁻¹; HRMS (EI): found M⁺ 164.0830, C₁₀H₁₂O₂ requires M⁺ 164.0837. Anal. Calcd for C₁₀H₁₂O₂: C, 73.15; H, 7.37; found: C, 72.94; H, 7.31. Enantiomeric excess was 97% ee determined by chiral HPLC analysis of the corresponding (*S*)- α -methylbenzylamine-derived amide with Chiralcel[®] OD normal phase column (*n*-hexane/EtOH=30/1, 1.0 mL/min, 230 nm), major isomer=16.8 min, minor isomer=13.1 min.

4.6.7. (*R*)-3-(4-Bromophenyl)-2-methylpropanoic acid 26g. The title compound **26g** was obtained according to the general procedure using the oxazolidinone resin **23** (185.5 mg, 0.113 mmol). Purification by preparative TLC

(CHCl₃/MeOH=10:1) gave **26g** as a white powder (18.6 mg, 68% yield in 3 steps from oxazolidinone resin **23**). $R_f=0.55$ (CHCl₃/MeOH=10:1); mp 60–62 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.41 (d, 2H, $J=8.4$ Hz), 7.06 (d, 2H, $J=8.4$ Hz), 3.01 (dd, 1H, $J=13.0, 6.4$ Hz), 2.77–2.68 (m, 1H), 2.64 (dd, 1H, $J=13.0, 7.5$ Hz), 1.18 (d, 3H, $J=6.8$ Hz); ¹³C NMR (75.5 MHz, CDCl₃) δ 181.1, 138.0, 131.5, 130.7, 120.3, 40.9, 38.7, 16.6; $[\alpha]_D^{26} = -26.4$ (c 1.02, CHCl₃); FT-IR (CHCl₃) ν_{\max} 3030, 1711, 1466, 1381, 1231, 1215, 1097, 893, 800, 787, 750, 733, 725, 696, 677, 654 cm⁻¹; HRMS (EI): found M⁺ 241.9949, C₁₀H₁₁BrO₂ requires M⁺ 241.9942. Anal. Calcd for C₁₀H₁₁BrO₂: C, 49.41; H, 4.56; found: C, 49.56; H, 4.66. Enantiomeric excess was 97% ee determined by chiral HPLC analysis of the corresponding (*S*)- α -methylbenzylamine-derived amide with Chiralcel[®] OD normal phase column (*n*-hexane/EtOH=50/1, 1.0 mL/min, 230 nm), major isomer=30.8 min, minor isomer=27.5 min.

4.6.8. (*R*)-3-(4-Nitrophenyl)-2-methylpropanoic acid 26h. The title compound **26h** was obtained according to the general procedure using the oxazolidinone resin **23** (256.2 mg, 0.156 mmol). Purification by preparative TLC (CHCl₃/MeOH=10:1) gave **26h** as a pale yellowish powder (21.2 mg, 65% yield in 3 steps from oxazolidinone resin **23**). $R_f=0.44$ (CHCl₃/MeOH=10:1); mp 101–103 °C; ¹H NMR (300 MHz, CDCl₃) δ 8.16 (d, 2H, $J=8.8$ Hz), 7.36 (d, 2H, $J=8.8$ Hz), 3.15 (dd, 1H, $J=16.5, 9.9$ Hz), 2.86–2.77 (m, 2H), 1.23 (d, 3H, $J=6.6$ Hz); ¹³C NMR (75.5 MHz, CDCl₃) δ 181.0, 146.9, 146.7, 129.8, 123.7, 40.8, 39.0, 16.8; $[\alpha]_D^{25} = -36.9$ (c 1.14, CHCl₃); FT-IR (CHCl₃) ν_{\max} 1713, 1607, 1522, 1464, 1381, 1348, 1231, 1097, 895, 733, 694, 648 cm⁻¹; HRMS (EI): found M⁺ 209.0683, C₁₀H₁₁NO₄ requires M⁺ for 209.0688. Anal. Calcd for C₁₀H₁₁NO₄: C, 57.41; H, 5.30; N, 6.70; found: C, 57.58; H, 5.39; N, 6.72. Enantiomeric excess was 97% ee determined by chiral HPLC analysis of the corresponding (*S*)- α -methylbenzylamine-derived amide with Chiralcel[®] OD normal phase column (*n*-hexane/EtOH=20/1, 1.0 mL/min, 230 nm), major isomer=33.2 min, minor isomer=37.5 min.

4.6.9. (*R*)-3-(2,4-Dichlorophenyl)-2-methylpropanoic acid 26i. The title compound **26i** was obtained according to the general procedure using the oxazolidinone resin **23** (251.2 mg, 0.153 mmol). Purification by preparative TLC (CHCl₃/MeOH=10:1) gave **26i** as a pale yellowish oil (25.3 mg, 71% yield in 3 steps from oxazolidinone resin **23**). $R_f=0.56$ (CHCl₃/MeOH=10:1); ¹H NMR (300 MHz, CDCl₃) δ 7.37 (m, 1H), 7.16–7.15 (m, 2H), 3.12 (dd, 1H, $J=12.8, 6.6$ Hz), 2.90–2.82 (m, 1H), 2.79 (dd, 1H, $J=12.8, 7.2$ Hz), 1.22 (d, 3H, $J=6.8$ Hz); ¹³C NMR (75.5 MHz, CDCl₃) δ 181.9, 135.4, 134.9, 133.0, 132.0, 129.4, 127.0, 39.3, 36.3, 16.8; $[\alpha]_D^{27} = -44.9$ (c 1.00, CHCl₃); FT-IR (CHCl₃) ν_{\max} 1709, 1474, 1383, 1103, 901, 870, 802, 725, 712, 677, 652 cm⁻¹; HRMS (EI): found M⁺ 232.0055, C₁₀H₁₀Cl₂O₂ requires M⁺ 232.0058. Anal. Calcd for C₁₀H₁₀Cl₂O₂: C, 51.53; H, 4.32; found: C, 51.68; H, 4.44. Enantiomeric excess was 97% ee determined by chiral HPLC analysis of the corresponding (*S*)- α -methylbenzylamine-derived amide with Chiralcel[®] OD normal phase column (*n*-hexane/EtOH=70/1, 1.0 mL/min, 230 nm), major isomer=24.2 min, minor isomer=21.7 min.

4.6.10. (R)-2-Phenoxy-4-pentenoic acid 26j. The title compound **26j** was obtained according to the general procedure using the oxazolidinone resin **23** (284.0 mg, 0.173 mmol). Purification by preparative TLC (CHCl₃/MeOH=10:1) gave **26j** as a white solid (16.7 mg, 50% yield in 3 steps from oxazolidinone resin **23**). $R_f=0.48$ (CHCl₃/MeOH=10:1); mp 30–31 °C; ¹H NMR (400 MHz, CDCl₃) δ 9.19 (br s, 1H), 7.31–7.25 (m, 2H), 7.02–6.98 (m, 1H), 6.90 (dd, 2H, $J=8.8, 1.1$ Hz), 5.91 (ddt, 1H, $J=17.0, 10.3, 7.0$ Hz), 5.21 (dd, 1H, $J=17.0, 1.6$ Hz), 5.16 (dd, 1H, $J=10.3, 1.6$ Hz), 4.72 (t, 1H, $J=6.2$ Hz), 2.72–2.76 (m, 2H); ¹³C NMR (75.5 MHz, CDCl₃) δ 176.5, 157.4, 131.9, 129.6, 122.1, 119.0, 115.3, 75.9, 36.8; $[\alpha]_D^{28} = +7.9$ (c 1.96, CHCl₃); FT-IR (CHCl₃) ν_{\max} 1732, 1599, 1495, 1238, 771, 750, 735, 691 cm⁻¹; HRMS (EI): found M⁺ 192.0782, C₁₁H₁₂O₃ requires M⁺ 192.0786. Anal. Calcd for C₁₁H₁₂O₃: C, 68.74; H, 6.29; found: C, 68.49; H, 6.34. Enantiomeric excess was 96% ee determined by chiral HPLC analysis of the corresponding (*S*)- α -methylbenzylamine-derived amide with Chiralcel[®] OD normal phase column (*n*-hexane/EtOH=50/1, 1.0 mL/min, 230 nm), major isomer=8.3 min, minor isomer=9.8 min.

4.6.11. (S)-3-(2,4-Dichlorophenyl)-2-methylpropanoic acid 26k. The title compound **26k** was obtained according to the general procedure using the oxazolidinone resin **23** (208.5 mg, 0.127 mmol). Purification by preparative TLC (CHCl₃/MeOH=10:1) gave **26k** as a colorless oil (17.4 mg, 59% yield in 3 steps from oxazolidinone resin **23**). $R_f=0.52$ (CHCl₃/MeOH=10:1); ¹H NMR (300 MHz, CDCl₃) δ 7.37 (m, 1H), 7.17–7.16 (m, 2H), 3.12 (dd, 1H, $J=12.8, 6.6$ Hz), 2.90–2.80 (m, 1H), 2.79 (dd, 1H, $J=12.8, 7.3$ Hz), 1.22 (d, 3H, $J=6.8$ Hz); ¹³C NMR (75.5 MHz, CDCl₃) δ 181.8, 135.4, 134.9, 133.0, 132.1, 129.4, 127.0, 39.2, 36.3, 16.8; $[\alpha]_D^{27} = +34.7$ (c 0.95, CHCl₃); FT-IR (CHCl₃) ν_{\max} 1711, 1474, 901, 733, 698, 675, 667, 652 cm⁻¹; HRMS (EI): found M⁺ 232.0054, C₁₀H₁₀Cl₂O₂ requires M⁺ 232.0058. Anal. Calcd for C₁₀H₁₀Cl₂O₂: C, 51.53; H, 4.32; found: C, 51.93; H, 4.62. Enantiomeric excess was 85% ee determined by chiral HPLC analysis of the corresponding (*S*)- α -methylbenzylamine-derived amide with Chiralcel[®] OD normal phase column (*n*-hexane/EtOH=70/1, 1.0 mL/min, 230 nm), major isomer=21.7 min, minor isomer=24.2 min.

4.6.12. Reuse of the oxazolidinone resin 23 in solid-phase Evans' asymmetric allylation, and methanolysis of the oxazolidinone resin recovered after three-times recycling. Starting from the oxazolidinone resin **23** (298.9 mg, 0.182 mmol), reaction sequence (*N*-acylation with 3-phenylpropionic acid, asymmetric allylation, and LiOOH-mediated hydrolysis) was repeated three times according to the procedure for synthesizing carboxylic acid **26c**. Then, oxazolidinone-loaded resin **23** recovered after three-times recycling was subjected to the methanolysis condition following the same procedure for synthesizing ester **24**. After the reaction, the resultant crude oil was purified by preparative TLC (*n*-hexane/AcOEt=1:5) to yield the methyl ester **24** (45.3 mg, 72% calculated from the loading rate of the starting oxazolidinone resin **23**) and *N*-allylated oxazolidinone methyl ester **27** as a pale yellowish viscous oil (16.1 mg, 23% calculated by the loading rate of the starting oxazolidinone resin **23**). $R_f=$

0.47 (*n*-hexane/AcOEt=1:5); ¹H NMR (400 MHz, CDCl₃) δ 7.35–7.17 (m, 5H), 5.78 (dddd, 1H, $J=17.2, 10.3, 7.3, 4.8$ Hz), 5.26–5.19 (m, 2H), 4.70 (d, 0.5H, $J=4.4$ Hz), 4.69 (d, 0.5H, $J=4.6$ Hz), 4.66–4.60 (m, 1H), 4.30 (dtd, 0.5H, $J=3.4, 4.0, 1.5$ Hz), 4.24–4.21 (m, 0.5H), 4.20–4.17 (m, 0.5H), 4.15–4.10 (m, 0.5H), 3.68–3.51 (m, 2H), 3.69 (s, 0.5×3H), 3.67 (s, 0.5×3H, partially overlapping with the next signal), 3.15–2.71 (m, 4H), 2.56–2.45 (m, 1H), 1.91–1.38 (m, 4H); ¹³C NMR (75.5 MHz, CDCl₃) δ 174.3, 174.0, 164.5, 164.4, 156.0, 155.9, 135.3, 135.2, 131.6, 129.2, 128.9, 128.9, 127.3, 127.2, 118.9, 118.8, 73.5, 73.3, 57.0, 56.9, 51.8, 45.2, 44.7, 44.4, 41.9, 41.7, 40.7, 40.2, 38.1, 37.9, 28.4, 28.1, 27.5, 27.4; $[\alpha]_D^{26} = -85.9$ (c 1.19, CHCl₃); FT-IR (CHCl₃) ν_{\max} 1753, 1746, 1655, 1456, 1437, 1175, 895, 648 cm⁻¹; HRMS (EI): found M⁺ 386.1846, C₂₁H₂₆N₂O₅ requires M⁺ 386.1841. Anal. Calcd for C₂₁H₂₆N₂O₅: C, 65.27; H, 6.78; N, 7.25; found: C, 64.99; H, 6.49; N, 7.47.

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31. Crystallographic data (excluding structural factors) for the structure **15** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 259416. Copies of the data can be obtained, free of charge, on application to CDCC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
32. The quenching study of lithium enolate generated from a similar derivative, *N*-propionylated carboximide, with TBSOTf, afforded the *O*-silyl enol ether as a single isomer in 82% yield. The irradiation to its olefinic methyl group gave a clear NOE enhancement for *tert*-butyldimethyl moiety (400 MHz, ¹H NMR, CDCl₃), suggesting that the reaction proceeded via a *Z*-configured lithium enolate intermediate.
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39. Other bases such as LiHMDS and KHMDS were less effective, and lower conversion was observed in the reaction below –20 °C.
40. (a) Preliminary stability test of **23** against LiOOH treatment revealed that ester linkage is sufficiently inert under the basic condition up to 8 h at 0 °C, and oxazolidinone ring was also stable enough. Kaiser-Test⁴⁷ of the recovered resin was completely negative, indicating that there is no free amino group caused by the oxazolidinone ring opening. Indeed, hydrolysis of the ester moiety was observed only in the H₂O₂ free condition. (b) Methanolysis of the recovered oxazolidinone resin **23** afforded the corresponding methyl ester **24** in 94% without any epimerization. Additionally there is no contamination of endo-cleavage byproduct as well as in the case of solution-phase model experiment.

41. 3-(2,4-Dichlorophenyl)propionic acid was prepared from *trans*-2,4-dichlorocinnamic acid in the following three-step reaction sequence (3 steps, 87%): (a) K₂CO₃, MeI, DMF, rt; (b) NaBH₄, CuCl, THF, 0 °C; (c) 1 N NaOH aq, MeOH, 50 °C. Unfortunately, simple hydrogenolysis of *trans*-2,4-dichlorocinnamic acid by H₂, 10% Pd–C in EtOH resulted in not only reduction of olefin moiety, but also de-chlorination at the 2-position on the aromatic ring. 2,4-Dichlorobenzyl iodide was prepared by iodination of the corresponding alcohol with NaI/Amberlyst. Tajbakhsh, M.; Hosseinzadeh, R.; Lasemi, Z. *Synlett* **2004**, 4, 635–638.
42. Absolute configuration of the products was determined in comparison to the specific rotations in literature; otherwise, corresponding authentic samples were prepared using (*S*)-4-benzyl-2-oxazolidinone. Yields were calculated from the original loading of Wang resin.
43. This type of side reaction is known in the standard Evans' chemistry and thought that the enolate intermediate partially decomposes via a ketene-pathway, see Ref. 15.
44. (a) Carboxylic acid **26c** is known as a key component of α -chymotripsin inhibitor, see: Kim, D. H.; Li, Z.-H.; Lee, S. S.; Park, J.; Chung, S. J. *Bioorg. Med. Chem.* **1998**, 6, 239–249. (b) Carboxylic acid **26e** is known as a key component of carboxypeptidase inhibitor, see: Byers, L. D.; Wolfenden, R. *Biochemistry* **1973**, 12, 2070–2078. (c) Carboxylic acid **26i** and **26k** are known as a key acyl component of potent γ -secretase inhibitor, see: Churcher, I.; Ashton, K.; Butcher, J. W.; Clarke, E. E.; Harrison, T.; Lewis, H. D.; Owens, A. P.; Teall, M. R.; Williams, S.; Wrigley, J. D. J. *Bioorg. Med. Chem. Lett.* **2003**, 13, 179–183.
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α -ヒドロキシ- β -アミノ酸を基盤 とした有機化学・創薬化学研究

林 良雄*
木曾 良明

Organic Chemistry and Medicinal Chemistry Based on α -Hydroxy- β -amino Acids

Yoshio Hayashi* and Yoshiaki Kiso

α -Hydroxy- β -amino acids are well known as inhibitory machinery for the development of protease inhibitors. In our ongoing efforts to develop effective aspartic protease inhibitors such as HIV-1 protease, malaria plasmepsin and human β -secretase inhibitors, the α -hydroxy- β -amino acids are also the critical core structures. In addition, the unique structure of these amino acids, in which three different functional groups, i.e. amino, hydroxyl and carboxyl groups, are located on the two adjacent asymmetric carbon atoms, also has interesting features to create new functional molecules useful in both organic chemistry and medicinal chemistry. In this article, organic and medicinal chemical applications based on the chemistry of α -hydroxy- β -amino acids will be presented, including 1) byproduction of homobis lactone during the carboxyl group activation of *N*-protected- α -hydroxy- β -amino acids, 2) development of α -hydroxy- β -amino acid derived new solid-supported Evans' chiral auxiliary for asymmetric synthesis, 3) development of a novel and efficient method for the synthesis of difficult sequence-containing peptides, and 4) O-N intramolecular acyl migration of α -hydroxy- β -amino acids for the development of water-soluble prodrugs of taxoids (isotaxoids).

Key words: α -hydroxy- β -amino acids, HIV-1 protease inhibitors, homobis lactone, Evans' chiral auxiliary, asymmetric alkylation, solid-phase synthesis, difficult sequence, A β 1-42, O-N intramolecular acyl migration, water-soluble prodrugs, paclitaxel

はじめに

α -ヒドロキシ- β -アミノ酸は、プロテアーゼ阻害剤の構成分子としてよく知られた異常アミノ酸である。天然に存在するその代表的な例としては、3-amino-2-hydroxy-4-phenylbutanoic acid (AHPBA) や 3-amino-2-hydroxy-5-methylhexanoic acid (AHMHA) が挙げられる。これらの異常アミノ酸は、梅澤濱夫先生・青柳高明先生らにより、天然から見出されたアミノペプチダーゼ阻害剤ベスタチン¹⁾やアマスタチン²⁾に含まれ、阻害作用発現における中心的役割を担っている。ベスタチンは、ウベニメクスという抗がん剤としても知られている。

また、 α -ヒドロキシ- β -アミノ酸類は、レニン阻害剤³⁾・HIV-1 プロテアーゼ阻害剤⁴⁾・マラリアプラスメプシン阻害剤⁵⁾・アルツハイマー病の原因と考えられる

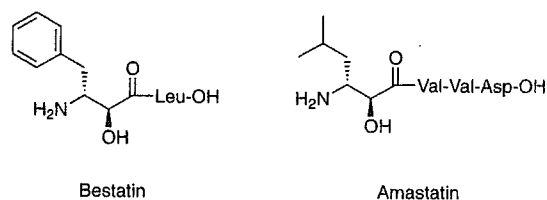


Fig. 1 α -Hydroxy- β -amino acid-containing natural protease inhibitors.

β -セクレターゼの阻害剤⁶⁾等のアスパラギン酸プロテアーゼ阻害剤のコア構造として利用され、創薬化学における重要な分子の1つとして認知されている。筆者らの研究室では、長年にわたりこの α -ヒドロキシ- β -アミノ酸に注目したアスパラギン酸プロテアーゼ阻害剤開発研究に挑戦している。さらにこの異常アミノ酸は、タキソイド類にも存在し、抗腫瘍活性発現に必須な構造となっている⁷⁾。一方、有機化学の見地からは、アミノ基・ヒドロキシ基・カルボキシル基の3種類の官能基が近接する構造のため、機能性有機分子創製の合成素子としても魅力的である。筆者らは、このような α -ヒドロキシ- β -アミノ酸のユニークな特徴に注目し、有機化学および創薬化学の分野において複数の研究を展開している。有機化学分野では、 α -ヒドロキシ- β -アミノ酸を酸成分とす

* 京都薬科大学 創薬科学フロンティア研究センター 21世紀COEプログラム 薬品化学教室 (607-8412 京都市山科区御陵四丁野町1)

* Department of Medicinal Chemistry, Center for Frontier Research in Medicinal Science, 21st Century COE program, Kyoto Pharmaceutical University (Yamashina-ku, Kyoto 607-8412, Japan)

るアミド結合形成反応の解析⁸⁾, 本分子を利用した新規ポリマー固定型 Evans 不斉補助基の開発⁹⁾, さらにこの異常アミノ酸をモデルとした O-N 分子内アシル基転位反応を精査することにより, ペプチド化学において合成の難しさが指摘されている difficult sequence 含有ペプチドの新規合成法“O-アシルイソペプチド法”の開発¹⁰⁾である。また創薬化学分野では, O-N 分子内アシル基転位反応を利用し, α -ヒドロキシ- β -アミノ酸残基を分子内に有する難水溶性薬剤である HIV-1 プロテアーゼ阻害剤の水溶性プロドラッグや, パクリタキセルの水溶性プロドラッグ“イソタキソイド”の開発である¹¹⁾。本総合論文では, これら最近筆者らが実施した研究について紹介したい。

1. α -ヒドロキシ- β -アミノ酸をアシル成分とするアミド結合形成反応の解析

α -ヒドロキシ- β -アミノ酸は, プロテアーゼ阻害作用を発現する中心的分子として, 酵素の触媒中心に作用する。従って, その立体化学は特に重要であり, すでに数多くの α -ヒドロキシ- β -アミノ酸の不斉合成研究が報告されている¹²⁾。一方, このような α -ヒドロキシ- β -アミノ酸を含むペプチドミメティック型プロテアーゼ阻害剤の合成では, 比較的反応性の低い α 位 2 級ヒドロキシ基を保護せずに, このアミノ酸を酸成分とするアミド結合形成反応に供される。ところが, かさ高いアミン成分との縮合では不規則な収率低下が見られ, その原因は精査されていなかった。

1.1 Boc-Apns-OH の活性化における homobislactone の形成

α -ヒドロキシ- β -アミノ酸を有する HIV-1 プロテアーゼ阻害剤は複数報告されているが⁴⁾, 当研究室で開発された強力な HIV-1 プロテアーゼ阻害剤 KNI-764 (JE-2147, AG-1776, SM-319777, 図 2)^{4a)}は, 活性発現に必須な hydroxymethylcarbonyl (HMC) 構造として α -ヒドロキシ- β -アミノ酸の一種である allophenylnorstatine [Apns, (2S,3S)-AHPBA], およびかさ高いイミノ酸の (*R*)-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid [Dmt] からなる Apns-Dmt 構造を P1 および P1' 部位に有する。この骨格を構築するための Boc-Apns-OH 1 と H-Dmt-R 2 の縮合も収率低下が起こる一例であった。筆者らは, 収率低下の要因として, 酸成分 1 の活性化段階に注目した。そして, Boc-Apns-Dmt-R 3 の合成をモデルとし, 収率低下の原因を検討した。

保護ペプチド 3 の合成時には, しばしば溶媒に溶解し難い白色沈殿の生成が認められた。その化学構造を解析したところ, 二分子の Boc-Apns-OH 1 からなる homobislactone 4 であることがわかった。この分子が生成す

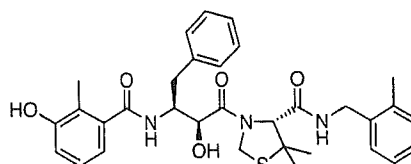
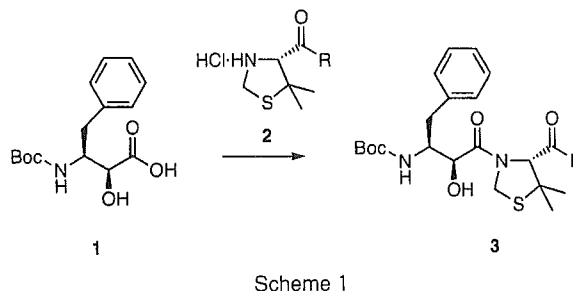


Fig. 2 Structure of HIV-1 protease inhibitor KNI-764.



るには, 縮合条件下で 1 の α 位水酸基が活性化される必要がある。筆者らはメカニズムを検討するために, 種々の縮合条件下での反応を HPLC を用いて解析した。

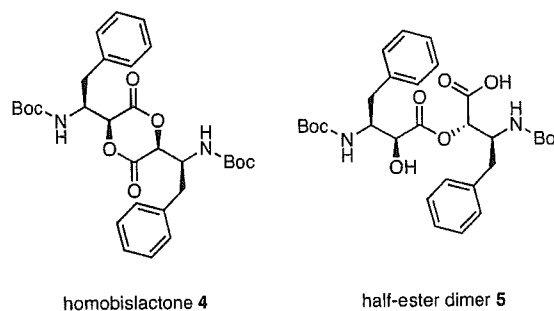


Fig. 3 Structure of homobislactone and its half-ester dimer.

Table 1 Homobislactone 4 formation under various coupling conditions.

Coupling Agents	Additives	Et ₃ N (eq)	DIEA (eq)	Formation of 4 ^a (%) ^a
EDC	-	-	-	0
EDC	-	1.0	-	0
EDC	HOAt	-	-	6.0 ± 0.4
EDC	HOAt	1.0	-	47.6 ± 1.2
EDC	HOAt	-	1.0	48.7 ± 1.1
EDC	HOBt	-	-	6.0 ± 0.2
EDC	HOBt	1.0	-	42.4 ± 1.2
EDC	HOBt	-	1.0	41.2 ± 0.2
EDC	HODhbt	-	-	<1.0
EDC	HODhbt	1.0	-	39.3 ± 2.1
EDC	HOSu	-	-	0
EDC	HOSu	1.0	-	3.3 ± 0.4
EDC	DMAP	-	-	3.1 ± 0.2
EDC	DMAP	1.0	-	<1.0
BOP	HOBt	2.0	-	54.5 ± 3.6
PyBOP	HOBt	2.0	-	58.9 ± 1.6
HBTU	-	2.0	-	24.1 ± 2.8

^a Yields were calculated by HPLC analysis. Values are the mean ± SEM of three independent experiments. Reaction conditions: 1 (1 eq), coupling agent (1.0 or 1.2 eq), additive (1 eq), Et₃N or DIEA, rt. for 2 h.

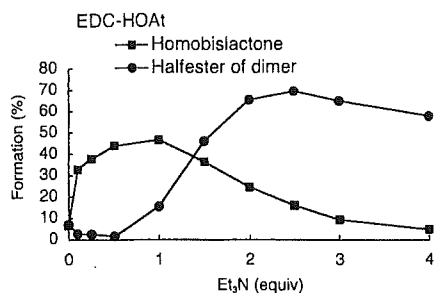


Fig. 4 Effect of base concentration on the byproduct formation.

その結果、この homobis lactone は特にベンゾトリアゾールあるいはベンゾトリアジン型活性エステルへと 1 を活性化する過程において副生することが明らかになった(表 1)。また、塩基の影響を検討したところ、図 4 に EDC-HOAt 法 (EDC: 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride, HOAt: 1-hydroxy-7-azabenzotriazole)¹³⁾での例を示すが、触媒量の Et₃N 添加が homobis lactone の副生を促進するとともに、1 当量の添加ではその生成量が最大となることが判明した。興味深いことに、さらに Et₃N の添加量を増やすと homobis lactone は減少し、代わって half-ester dimer 5 が優位に生成した。この結果から、これらの副産物生成のメカニズムとしては、図 5 に示すように、生成する活性エステル体の benzotriazole 1 位窒素原子が隣接基関与により α 位ヒドロキシ基の求核性を高め、別の活性エステルと反応し、次いで、もう一方のヒドロキシ基が反応することで 6 員環 homobis lactone を形成したと考えられる(図 5, Route A)。一方、過剰の塩基の存在下で

は、(II)の段階で活性エステルの分解が優先し 5 が形成されたと考えられる(図 5, Route B)。このような homobis lactone の副生は、他の α -ヒドロキシ- β -アミノ酸、例えば Boc-Pns-OH [Pns: phenylnorstatine, (2*R*, 3*S*)-AHPBA] や Boc-Chns-OH [Chns: cyclohexylnorstatine, (2*R*, 3*S*)-3-amino-2-hydroxyl-4-cyclohexylbutanoic acid] でも観察されたことから、本異常アミノ酸に共通した副反応であると考えられる。

1.2 アミド形成反応の最適化

Homobis lactone の副生とアミド結合形成反応での収率低下の関係を検討するために、Boc-Apns-Dmt-OBzl 6 の合成をモデルに HOAt, HODhbt (3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine)¹⁴⁾, HOBt (1-hydroxybenzotriazole)¹⁵⁾ を additive とする EDC 法を検討したところ、塩基の添加は、濃度依存的に収率の低下を招き、1.5 当量以上の塩基の存在では、全く目的物が得られなかった(図 6)。この結果は、塩基濃度の上昇に依存した副産物の増加と良く一致しており、homobis lactone などの副生が α -ヒドロキシ- β -アミノ酸を酸成分とするアミド結合形成反応での収率低下の主因であることが示唆された。効率的なアミド結合形成は、homobis lactone 4 の副生が最も少ない塩基を添加しない条件下で、EDC-HOAt 法のような強い活性化法を用いることで達成された(図 6)。また、この際の homobis lactone の生成は約 2% とわずかであった。これらの homobis lactone 副生に関する解析データは Apns-Dmt コア構造のみならず、 α -ヒドロキシ- β -アミノ酸を酸成分とするアミド結合の効率的な合成に有用な知見を与えると思われる。

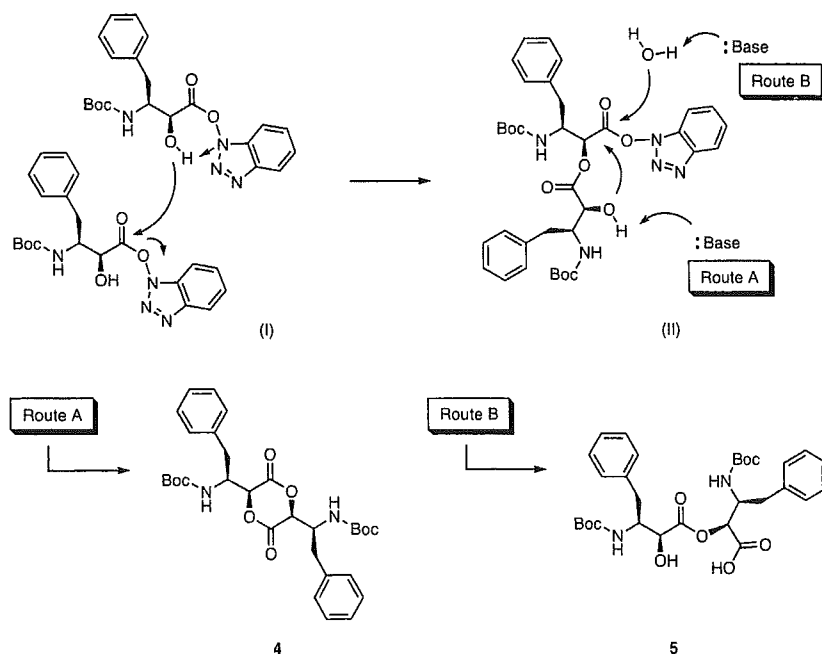


Fig. 5 Proposed mechanism of formation of homobis lactone and half-ester of dimer.

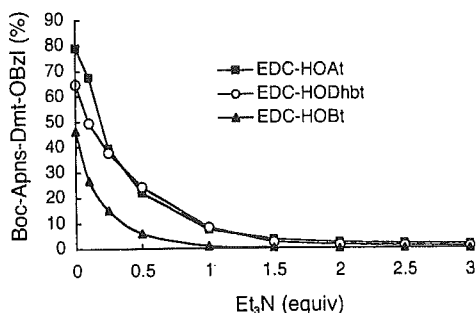


Fig. 6 Effect of base concentration on the yield of Boc-Apns-Dmt-OBzl 6.

2. α -ヒドロキシ- β -アミノ酸を母核とする新規ポリマー固定型 Evans 不斉補助基の開発

創薬では薬剤分子を構成する各官能基の精密な空間配置を実現する光学活性合成素子が必要不可欠であり、ことにコンビナトリアル化学の時代にあつては、構造多様性を兼ね備えて、それらを迅速に合成できる固相合成手法の開発が必要となる。Evans' oxazolidinone¹⁶⁾は、多様な光学活性合成素子の調製や天然物の不斉全合成に汎用されており、実用的な不斉導入試薬として広く認知されている。従つて、固相合成に適用できれば、各種不斉反応を利用した光学活性低分子化合物ライブラリーの構築などに、有効な手法を提供できると考えられる。

ところが、本不斉補助基を固相合成に適用した報告は意外と少ない¹⁷⁾。そして、代表的な反応の1つである Evans 不斉アルキル化では、立体選択性が最高でも 90% ee 程度(ベンジル化)と低いこと¹⁸⁾、また、樹脂への固定化において副反応が指摘されており¹⁹⁾、本不斉導入試薬の本来の威力は既報の固相合成では十分に発揮されていない。この原因の1つとして、ほとんどの報告例において、面選択性を制御する oxazolidinone 環 4 位の置換基部分(chiral discriminating group)が樹脂への固定化に利用されていることが挙げられる(図 7A)。この場合、chiral discriminating group は樹脂の影響を直接に受けることになる。そこで筆者らは、Evans 不斉補助基の新たな固定化法として、不斉誘導にあまり大きく影響しないと思われる oxazolidinone 環 5 位を利用することを考え、 α -ヒドロキシ- β -アミノ酸 phenylnorstatine (Pns)に注目した(図 7B)。

2.1 新規ポリマー固定型 Evans 不斉補助基の合成

Pns はそのアミノエタノール構造をカルボニル基で架橋することにより、5 位にカルボキシル基を有する oxazolidinone 誘導体へと容易に変換可能である。そして、このカルボン酸で樹脂に固定化することを考え、図 8 に示すような新規ポリマー固定型 oxazolidinone 7 をデザインした。用いる固相担体には、不斉反応に使用する THF などの各種有機溶媒に対して良好な膨潤性を示

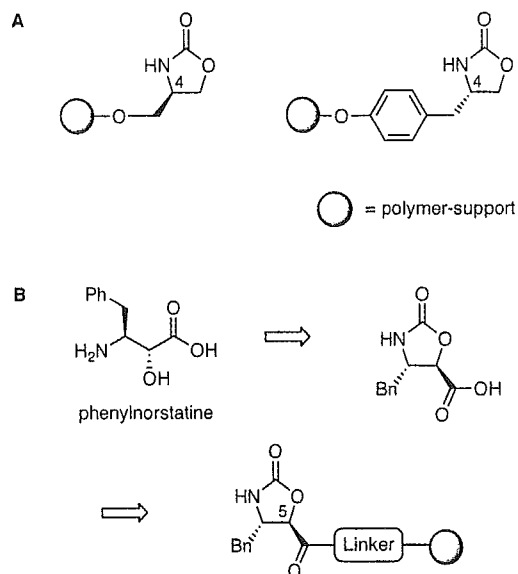


Fig. 7 Solid-supported Evans-type chiral auxiliaries.

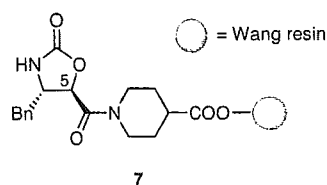
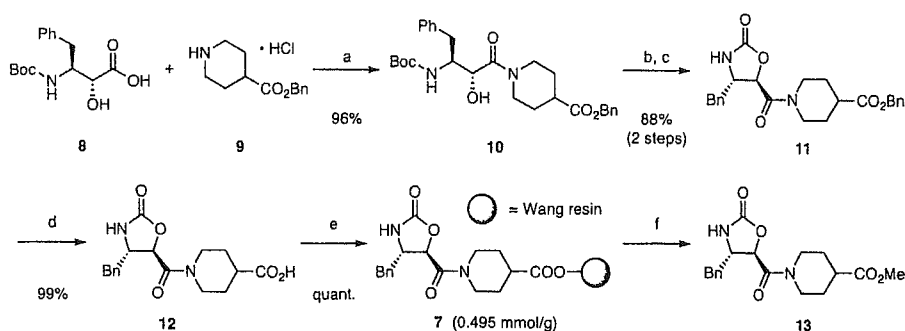


Fig. 8 New solid-supported Evans-type chiral auxiliary.

す Wang 樹脂を選択し、補助基構造が高分子担体から距離を保つように、piperidine-4-carboxylic acid をリンカーとして挿入することにした。

そして、まず新規 oxazolidinone 誘導体自体の反応性を通常の液相モデル実験で検証するために、スキーム 2 に示すように、Boc-Pns-OH 8 から調製した *trans* 配置型 oxazolidinone 誘導体 11 を用い、3-フェニルプロピオン酸あるいはプロピオン酸で *N*-アシル化し、 α 位不斉アルキル化反応を検討したところ、不斉誘起能を十分に有することがわかった(表 2)。このことから、oxazolidinone 環 5 位の修飾は、不斉誘起能に影響しないことが示唆された。また、不斉アルキル化後のアシル基は、Evans 不斉補助基においてよく用いられる LiOOH²⁰⁾ による加水分解反応で、oxazolidinone 環の開環およびリンカーと樹脂間のエステル結合の開裂を伴うことなく、効率的に切り出された。これは、本ポリマー固定型 Evans 不斉補助基が、再利用可能であることを示唆している。Pns の α 位エピマーである Apns でも、同様な oxazolidinone 体を合成し、不斉アルキル化反応を検討したが、この場合は、LDA 等の塩基処理で、オキサゾリジン環 5 位プロトンの引き抜きが起こり、5 位の異性化が進行することがわかった。Apns より誘導された oxazolidinone は、4 および 5 位の置換基が *cis* 配置となり、立体的に不安定になるためと考えられる。なお、



a) EDC, HOBT, Et₃N, DMF, 0 °C to rt; b) 4 M HCl/1,4-dioxane, 0 °C to rt; c) Et₃N, CDI, THF, 0 °C to rt; d) H₂, Pd/C, MeOH-H₂O, rt; e) Wang resin, DIPCIDI, DMAP, DMF, rt; f) K₂CO₃, THF-MeOH, 0 °C.

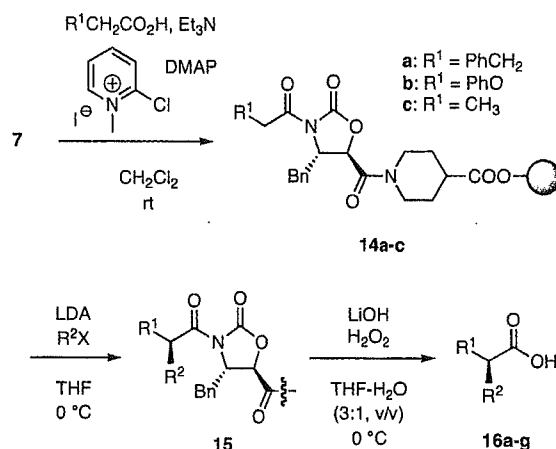
Scheme 2

Pns では、異性化は観察されなかった。

以上のことから、Pns から誘導されたカルボン酸誘導体 12 を用いて、DIPCIDI-DMAP 法 (DIPCIDI: 1,3-diisopropylcarbodiimide)²¹⁾ により Wang 樹脂への固定化を実施した (スキーム 2)。その結果、望むポリマー固定型 oxazolidinone 7 が定量的に得られた。なお、この導入率は、樹脂とリンカー間のエステル結合をメタノリシスすることにより、対応するエステル 13 を切り出し、その重量および Wang 樹脂の最初の置換量 (0.80 mmol/g) から算出した。

2.2 新規ポリマー固定型 Evans 不斉補助基を用いる不斉アルキル化反応

スキーム 3 に示すように、ポリマー固定型 Evans 不斉補助基 7 を *N*-アシル化して得たイミド樹脂 14 を用い、既報では不斉収率の点で良好な結果が得られていない固相 Evans 不斉アルキル化を試みた。Ar 雰囲気下、THF 中にて 14 を膨潤させた後、LDA によりリチウムエノラートを発生させ、次いでアルキル化剤を添加し、0 °C にて一定時間攪拌した。樹脂をろ取・洗浄・乾燥し、次いで LiOOH を用いるイミド選択的加水分解反応を行うことで²⁰⁾、表 2 に示すように、目的とするキラルなカルボン酸 16 が良好な通算収率で得られるとともに、期待どおり生成物の立体選択性は通常の液相法に匹敵する十分に高いレベルであった。この結果は、Evans 不斉アルキル化反応を固相上にて高立体選択的に実現した初めての例であり、既報の問題点¹⁸⁾を解決するものであると考えている。また、これら実験結果は、従来の固相 Evans 不斉反応の立体選択性を高めただけでなく、試薬の固定化に際し、樹脂へ不斉補助基をアンカリングする位置の重要性を強く示唆しており、固相担持型試薬の新しい開発に知見を与えるものと思われる。現在、固相上での本不斉補助基の簡便合成をはじめ、固相ヒドラジノ化、フッ素化などの各種固相不斉変換反応への応用を検討するとともに、補助基樹脂の回収・再利用についても良好な結果を得ている^{9b)}。



Scheme 3 Solid-phase Evans' asymmetric alkylation of the carboximide resin 14.

Table 2 Solid-phase Evans' asymmetric alkylations.

Entry	14	R ² X	16	Yield (%) ^{a,c}	Ee (%) ^{b,c}
1	14a	Mel	16a	48 (62)	85 (86)
2	14a	EtI	16b	50 (64)	88 (89)
3	14a		16c	54 (66)	96 (96)
4	14a		16d	51 (64)	94 (95)
5	14a	Br-CH ₂ -CO ₂ Et	16e	47 (60)	92 (90)
6	14b		16f	38 (48)	96 (96)
7	14c	BnBr	16g	40 (57)	97 (98)

^a Combined yield of 3 steps from 7, based on the initial loading rate of Wang resin. ^b Determined by HPLC analysis after conversion to the corresponding (*S*)- α -methylbenzylamine-derived amides. ^c Value in the parenthesis is the result of the solution-phase model experiment.

3. O-N 分子内アシル基転位反応を利用した difficult sequence 含有ペプチドの新規合成法開発

α -ヒドロキシ- β -アミノ酸では、隣接した炭素原子上にアミノ基とヒドロキシ基が存在しているため、一方にアシル基が存在する場合は、5員環遷移状態を経由して *N*-O/O-N 分子内アシル基転位反応を起こす可能性が

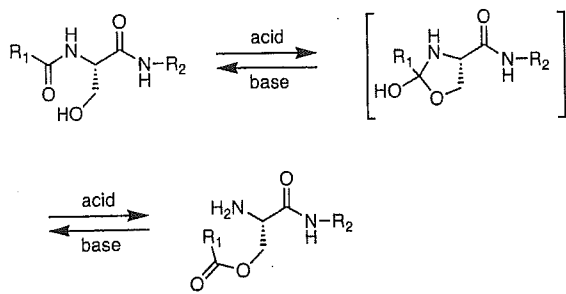


Fig. 9 N-O or O-N intramolecular acyl migration in peptides.

ある。この転位反応は、両官能基が同様な位置関係にあるセリン・スレオニンのようなアミノ酸を含むペプチドにおいて良く知られている。

すなわち、図9に示すように、ペプチドを強酸で処理すると、本来のN-アシル構造はセリン部分でN-O shiftと呼ばれる分子内転位反応によりO-アシルイソペプチド構造に異性化する。一方、生成したO-アシル体は、中性から弱塩基性の水溶液中で速やかにO-N分子内アシル基転位反応を起こし、逆に元のペプチドに戻ることができる。筆者らは、このO-N分子内アシル基転位反応に着目し、 α -ヒドロキシ- β -アミノ酸を含む difficult sequence 含有モデルペプチドの合成を行うことで、difficult sequence 含有ペプチドの新規合成法を開発した。

今日ペプチド合成は、自動固相合成機を用いることで、容易に達成できるイメージがあるが、アミノ酸配列によっては合成困難なものも多く知られている。これらは difficult sequence 含有ペプチドと呼ばれ、合成途上反応性が著しく低下し、純度・収率ともに満足な結果が得られない。原因は、特に固相法において、ペプチド鎖間の疎水相互作用および水素結合により形成される β -sheet 構造による微小な凝集体の生成によると考えられている(図10)²³⁾。また、このようなペプチドでは、溶媒への低溶解性とブロードな溶出のため、HPLCによる精製が困難を極める。

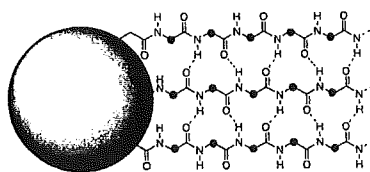


Fig. 10 β -Sheet formation of peptides in the solid phase peptide synthesis.

この問題を解決するための方法として、Mutterらは pseudo-proline と呼ばれる Ser/Thr から誘導された oxazolidine あるいは Cys から誘導される thiazolidine を含むジペプチド誘導体を固相合成のビルディングブロックとして開発している²⁴⁾。また、Sheppardらは、主鎖のアミド窒素保護基として 2-hydroxy-4-methoxy-

benzyl (Hmb) を報告している²⁵⁾。これらの特殊なビルディングブロックは、ペプチド鎖が固相上で β -sheet を形成することを妨げるもので、 β -sheet breaker と呼ばれている(図11)。しかしながら、これらを用いる固相合成では、予め相応するアミノ酸誘導体を準備する必要がある。

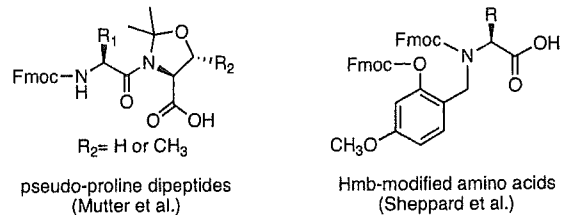


Fig. 11 β -Sheet breakers for the synthesis of difficult sequence-containing peptides.

筆者らが考案した新規合成法“O-アシルイソペプチド法”は、図12に示すように、まず塩形成可能なアミノ基を有するO-アシルイソペプチドを固相法で合成、HPLC精製した後、pH 7.4のリン酸緩衝液中でO-N分子内アシル基転位反応により目的のペプチドに変換するものである。

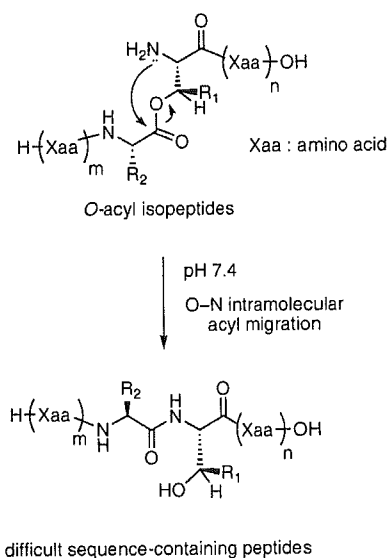


Fig. 12 “O-acyl isopeptide method” for the synthesis of difficult sequence-containing peptides.

3.1 α -ヒドロキシ- β -アミノ酸有する difficult sequence 含有ペプチドの合成

筆者らの研究室で研究されているペプチド性の α -ヒドロキシ- β -アミノ酸含有プロテアーゼ阻害剤には、difficult sequence を有するものがあり、通常の固相合成において目的物が得られないことがある。そこで、O-N分子内アシル基転位反応が可能な α -ヒドロキシ- β -アミノ酸を有する difficult sequence 含有モデルペプチドの合成により“O-アシルイソペプチド法”の有効性を検証した。モデルペプチドとして、