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 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.

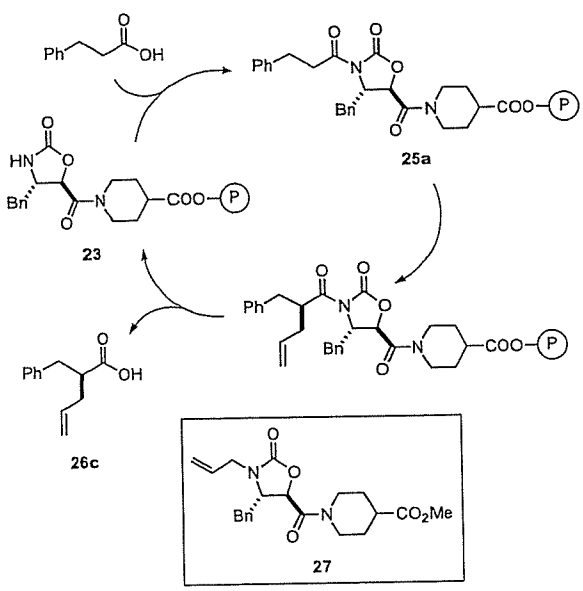


Figure 4. Recycling of the chiral auxiliary resin 23.

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.

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 (^{1}H and ^{13}C) were recorded on a JEOL JNM-AL300 (^{1}H: 300 MHz; ^{13}C:75.5 MHz) or a Varian UNITY INOVA 400NB (^{1}H: 400 MHz; ^{13}C: 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: $[\alpha]_D^T$ (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 (Na_2SO_4), 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 CaH_2 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 F_{254} 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 F_{254} 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·H_2O (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, Et_3N (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% $NaHCO_3$ aq, water (×2) and brine. After the organic layer was dried over Na_2SO_4, 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; ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (75.5 MHz, $CDCl_3$) 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; $[\alpha]_D^{26}=+16.3$ (c 0.64, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 3690, 3441, 3038, 1728, 1699, 1639, 1497, 1367, 1238, 1169, 698 cm^{-1}; HRMS (EI): found M^+ 496.2576, $C_{28}H_{36}N_2O_6$ requires M^+ 496.2573. Anal. Calcd for $C_{28}H_{36}N_2O_6$: 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 Et_3N (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% $NaHCO_3$ aq, water and brine. After the organic layer was dried over Na_2SO_4, 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; ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (75.5 MHz, $CDCl_3$) 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; $[\alpha]_D^{25}=-58.4$ (c 1.01, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 3030, 3020, 1774, 1730, 1666, 1231, 1207, 800, 791, 768, 714, 675 cm^{-1}; HRMS (EI): found M^+ 422.1843, $C_{24}H_{26}N_2O_5$ requires M^+ 422.1841. Anal. Calcd for $C_{24}H_{26}N_2O_5$: 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 Et_3N

(3.1 mL, 22.5 mmol) and trimethylacetylchloride (1.3 mL, 10.8 mmol) dropwise at −18 °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 $NaHCO_3$ 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 **10** as a white solid (4.7 g, 95%). R_f= 0.48 (*n*-hexane/AcOEt=1:1); mp 153–155 °C; ^{1}H NMR (400 MHz, $CDCl_3$). 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); ^{13}C NMR (75.5 MHz, $CDCl_3$) 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, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 1790, 1730, 1701, 1670, 1454, 1375, 1173, 718, 696 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, 71.51; 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 °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 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=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 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.18 (m, 15H), 5.14, 5.10 (2d, 0.5×2H, J=12.3 Hz), 5.09 (s, 0.5×2H), 4.88 (d, 0.16H, 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 1796, 1732, 1703, 1661, 1454, 1379, 1198, 1173, 772, 756, 727, 700, 679, 667 cm^{-1}; HRMS (EI): found M^+ 555.2478, $C_{33}H_{33}DN_2O_6$ requires M^+ 555.2479. Anal. Calcd for $C_{33}H_{33}DN_2O_6$: 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·H_2O (7.7 g, 50.4 mmol) in DMF (210 mL) was added EDC·HCl (9.7 g, 50.4 mmol) in parts at 0 °C. After stirring for 0.5 h at the same temperature, Et_3N (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% $NaHCO_3$ aq, water (×2) and brine. After the organic layer was dried over Na_2SO_4, 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 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.21 (m, 10H), 5.17, 5.12 (2d, 0.5×2H, J=12.5 Hz), 5.10 (s, 0.5×2H), 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, 0.5×9H), 1.38 (s, 0.5×9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) 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, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 3439, 3005, 1717, 1701, 1639, 1499, 1454, 1393, 1367, 1240, 1169, 700 cm^{-1}; HRMS (EI): found M^+ 496.2568, $C_{28}H_{36}N_2O_6$ requires M^+ 496.2573. Anal. Calcd for $C_{28}H_{36}N_2O_6$: 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 °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 Et_3N (8.4 mL, 60.5 mmol) dropwise at 0 °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% $NaHCO_3$ aq, water and brine. After the organic layer was dried over Na_2SO_4, 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 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.20 (m, 10H), 5.28 (br s, 0.5H,), 5.25 (br s, 0.5H), 5.14 (s, 0.5×2H), 5.12 (s, 0.5×2H), 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, 0.5×2H, 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, 128.3, 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 atomosphere. 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 μL, 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). Recrystalization 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; ^{1}H 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, 0.5×2H, 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, 0.5×3H, J= 7.0 Hz), 1.48 (d, 0.5×3H, 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 Kα radiation (λ=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) Å^3, Z=4, D_{calc}=1.280 g/cm^3, F_{000}=928.00, μ(Cu Kα)=7.10 cm^{-1}. Total of 1554 unique reflections (complete for $2\theta<110°$) 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; ^{1}H 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= 4.4 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; ^{1}H 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); ^{2}H 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, $C_{33}H_{33}DN_2O_6$ requires M^+ 555.2479. Anal. Calcd for $C_{33}H_{33}DN_2O_6$: 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. DIPCDI (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), HOBt·H_2O (1.8 g, 12.0 mmol), DMF (30 mL) and DIPCDI (1.9 mL, 12.0 mmol) were added, and the heterogenious 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-Test[47] 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 heterogenious 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-fluorenylmethoxy)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; ^{1}H 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, $C_{18}H_{22}N_2O_5$ requires M^+ 346.1528. Anal. Calcd for $C_{18}H_{22}N_2O_5 \cdot 0.25H_2O$: 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 (×5). The reaction was repeated once again, and the obtained resin was washed with DMF, $CHCl_3$ and MeOH (×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 (×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 $LiOH \cdot H_2O$ (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 (×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 $HOBt \cdot H_2O$ (1.2 equiv) and $EDC \cdot HCl$ (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); ^{1}H 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, $C_{10}H_{12}O_2$ requires M^+ 164.0837. Anal. Calcd for $C_{10}H_{12}O_2$: 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 ($CHCl_3$/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 ($CHCl_3$/MeOH=10:1); ^{1}H 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, $C_{11}H_{14}O_2$ requires M^+ 178.0994. Anal. Calcd for $C_{11}H_{14}O_2$: 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 ($CHCl_3$/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 ($CHCl_3$/MeOH=10:1); ^{1}H 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, $C_{12}H_{14}O_2$ requires M^+ 190.0994. Anal. Calcd for $C_{12}H_{14}O_2$: 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 ($CHCl_3$/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 ($CHCl_3$/MeOH=10:1); ^{1}H

NMR (300 MHz, $CDCl_3$) 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); ^{13}C NMR (75.5 MHz, $CDCl_3$) 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, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 3308, 1719, 1217, 1200, 770, 700, 671 cm^{-1}; HRMS (EI): found M^+ 188.0835, $C_{12}H_{12}O_2$ requires M^+ 188.0837. Anal. Calcd for $C_{12}H_{12}O_2 \cdot 0.25H_2O$: 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 ($CHCl_3$/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 ($CHCl_3$/MeOH=10:1); ^1H NMR (300 MHz, $CDCl_3$) 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); ^{13}C NMR (75.5 MHz, $CDCl_3$) 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, $CHCl_3$): lit.,[50] $[\alpha]_D^{28}=+10.0$ (c 2.9, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 1732, 1717, 910, 777, 754, 739, 721, 700, 679, 652 cm^{-1}; HRMS (EI): found M^+ 236.1051, $C_{13}H_{16}O_4$ requires M^+ 236.1048. Anal. Calcd for $C_{13}H_{16}O_4$: 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, $CDCl_3$) 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); ^{13}C NMR (75.5 MHz, $CDCl_3$) 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, $CHCl_3$): lit.,[51] $[\alpha]_D^{22}=-30.1$ (c 1.00, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 1707, 1464, 1381, 1231, 893, 800, 694, 648 cm^{-1}; HRMS (EI): found M^+ 164.0830, $C_{10}H_{12}O_2$ requires M^+ 164.0837. Anal. Calcd for $C_{10}H_{12}O_2$: 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 ($CHCl_3$/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 ($CHCl_3$/MeOH=10:1); mp 60–62 °C; ^1H NMR (300 MHz, $CDCl_3$) 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); ^{13}C NMR (75.5 MHz, $CDCl_3$) 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, $CHCl_3$); FT-IR ($CHCl_3$) 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, $C_{10}H_{11}BrO_2$ requires M^+ 241.9942. Anal. Calcd for $C_{10}H_{11}BrO_2$: 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 ($CHCl_3$/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 ($CHCl_3$/MeOH=10:1); mp 101–103 °C; ^1H NMR (300 MHz, $CDCl_3$) 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); ^{13}C NMR (75.5 MHz, $CDCl_3$) 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, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 1713, 1607, 1522, 1464, 1381, 1348, 1231, 1097, 895, 733, 694, 648 cm^{-1}; HRMS (EI): found M^+ 209.0683, $C_{10}H_{11}NO_4$ requires M^+ for 209.0688. Anal. Calcd for $C_{10}H_{11}NO_4$: 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 ($CHCl_3$/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 ($CHCl_3$/MeOH=10:1); ^1H NMR (300 MHz, $CDCl_3$) 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); ^{13}C NMR (75.5 MHz, $CDCl_3$) 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, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 1709, 1474, 1383, 1103, 901, 870, 802, 725, 712, 677, 652 cm^{-1}; HRMS (EI): found M^+ 232.0055, $C_{10}H_{10}Cl_2O_2$ requires M^+ 232.0058. Anal. Calcd for $C_{10}H_{10}Cl_2O_2$: 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 ($CHCl_3$/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 ($CHCl_3$/MeOH=10:1); mp 30–31 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (75.5 MHz, $CDCl_3$)? 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, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 1732, 1599, 1495, 1238, 771, 750, 735, 691 cm^{-1}; HRMS (EI): found M^+ 192.0782, $C_{11}H_{12}O_3$ requires M^+ 192.0786. Anal. Calcd for $C_{11}H_{12}O_3$: 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 ($CHCl_3$/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 ($CHCl_3$/MeOH=10:1); ^{1}H NMR (300 MHz, $CDCl_3$) 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); ^{13}C NMR (75.5 MHz, $CDCl_3$) 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, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 1711, 1474, 901, 733, 698, 675, 667, 652 cm^{-1}; HRMS (EI): found M^+ 232.0054, $C_{10}H_{10}Cl_2O_2$ requires M^+ 232.0058. Anal. Calcd for $C_{10}H_{10}Cl_2O_2$: 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); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (75.5 MHz, $CDCl_3$) 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, $CHCl_3$); FT-IR ($CHCl_3$) ν_{max} 1753, 1746, 1655, 1456, 1437, 1175, 895, 648 cm^{-1}; HRMS (EI): found M^+ 386.1846, $C_{21}H_{26}N_2O_5$ requires M^+ 386.1841. Anal. Calcd for $C_{21}H_{26}N_2O_5$: C, 65.27; H, 6.78; N, 7.25; found: C, 64.99; H, 6.49; N, 7.47.

Acknowledgements

The authors would like to express our sincere gratitude to Dr. S. Ogawa (Kyoto Pharmaceutical University) for X-ray crystallographic analysis and in the NMR spectroscopy measurement. We also thank Dr. V. K. Sharma for the manuscript preparation. This research was supported in part by the 21st Century COE Program 'Development of Drug Discovery Frontier Integrated from Tradition to Proteome', the Frontier Research Program and grants from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan. On a final note, T. Kotake is grateful for Research Fellowships of JSPS for Young Scientists.

References and notes

1. (a) Procter, G. Asymmetric Synthesis; Oxford University Press: Oxford, 1996. (b) Gawley, R. E.; Aubé, J. *Principles of Asymmetric Synthesis*; Tetrahedron Organic Chemistry Series; Elsevier: Oxford, 1996; Vol. 14.
2. Ager, D. J.; Prakash, I.; Schaad, D. R. *Chem. Rev.* **1996**, *96*, 835–875.
3. Arvanitis, E.; Ernst, H.; Ludwig, A. A.; Robinson, A. J.; Wyatt, P. B. *J. Chem. Soc., Perkin Trans. 1* **1998**, 521–528.
4. Miyachi, H.; Nomura, M.; Tanase, T.; Suzuki, M.; Murakami, K.; Awano, K. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 333–335.
5. Crimmins, M. T.; She, J. *J. Am. Chem. Soc.* **2004**, *126*, 12790–12791.
6. (a) Gaulon, C.; Dhal, R.; Chapin, T.; Maisonneuve, V.; Dujardin, G. *J. Org. Chem.* **2004**, *69*, 4192–4202. (b) Shirokawa, S.; Kamiyama, M.; Nakamura, T.; Okada, M.; Nakazaki, A.; Hosokawa, S.; Kobayashi, S. *J. Am. Chem. Soc.* **2004**, *126*, 13604–13605.
7. (a) Obrecht, D.; Villalgordo, J. M. *Solid-supported Combinatorial and Parallel Synthesis of Small Molecular-weight Compound Libraries*; Tetrahedron Organic Chemistry Series; Elsevier: Oxford, 1998; Vol. 17. (b) Seneci, P. Solid Phase and Combinatorial Technologies; Wiley: New York, 2000.
8. McNamara, C. A.; Dixon, M. J.; Bradley, M. *Chem. Rev.* **2002**, *102*, 3275–3300.

9. Chung, C. W. Y.; Toy, P. H. *Tetrahedron: Asymmetry* **2004**, *15*, 387–399.
10. Hutchison, P. C.; Heightman, T. D.; Procter, D. J. *J. Org. Chem.* **2004**, *69*, 790–801.
11. (a) Allin, S. M.; Shuttleworth, S. J. *Tetrahedron Lett.* **1996**, *37*, 8023–8026. (b) Burgess, K.; Lim, D. *Chem. Commun.* **1997**, 785–786.
12. (a) Phoon, C. W.; Abell, C. *Tetrahedron Lett.* **1998**, *39*, 2655–2658. (b) Purandare, A. V.; Natarajan, S. *Tetrahedron Lett.* **1997**, *38*, 8777–8780.
13. Winkler, J. D.; McCoull, W. *Tetrahedron Lett.* **1998**, *39*, 4935–4936.
14. (a) Faita, G.; Paio, A.; Quadrelli, P.; Rancati, F.; Seneci, P. *Tetrahedron Lett.* **2000**, *41*, 1265–1269. (b) Faita, G.; Paio, A.; Quadrelli, P.; Rancati, F.; Seneci, P. *Tetrahedron* **2001**, *57*, 8313–8322. (c) Desimoni, G.; Faita, G.; Galbiati, A.; Pasini, D.; Quadrelli, P.; Rancati, F. *Tetrahedron: Asymmetry* **2002**, *13*, 333–337.
15. In the conventional solution-phase benzylation, >95% ee was normally obtained. Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737–1739.
16. Bew, S. P.; Bull, S. D.; Davies, S. G.; Savory, E. D.; Watkin, D. J. *Tetrahedron* **2002**, *58*, 9387–9401.
17. Kotake, T.; Rajesh, S.; Hayashi, Y.; Mukai, Y.; Ueda, M.; Kimura, T.; Kiso, Y. *Tetrahedron Lett.* **2004**, *45*, 3651–3654.
18. (a) Mimoto, T.; Kato, R.; Takaku, H.; Nojima, S.; Terashima, K.; Misawa, S.; Fukazawa, T.; Ueno, T.; Sato, H.; Shintani, M.; Kiso, Y.; Hayashi, H. *J. Med. Chem.* **1999**, *42*, 1789–1802. (b) Mimoto, T.; Hattori, N.; Takaku, H.; Kisanuki, S.; Fukazawa, T.; Terashima, K.; Kato, R.; Nojima, S.; Misawa, S.; Ueno, T.; Imai, J.; Enomoto, H.; Tanaka, S.; Sakikawa, H.; Shintani, M.; Hayashi, H.; Kiso, Y. *Chem. Pharm. Bull.* **2000**, *48*, 1310–1326.
19. Bunnage, M. E.; Davies, S. G.; Goodwin, C. J.; Ichihara, O. *Tetrahedron* **1994**, *50*, 3975–3986.
20. Benzyl piperidine-4-carboxylate was prepared from commercially available isonipecotic acid with $SOCl_2$ in BnOH. Ramachandran, J.; Li, C.-H. *J. Org. Chem.* **1963**, *28*, 173–177.
21. König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788–798.
22. In this condensation reaction, it was necessary to pay attention not to form the corresponding dimer of the acyl component, homobislactone, see: Hayashi, Y.; Kinoshita, Y.; Hidaka, K.; Kiso, A.; Uchibori, H.; Kimura, T.; Kiso, Y. *J. Org. Chem.* **2001**, *66*, 5537–5544.
23. (a) Mulvihill, M. J.; Cesario, C.; Smith, V.; Beck, P.; Nigro, A. *J. Org. Chem.* **2004**, *69*, 5124–5127. (b) Cutugno, S.; Martelli, G.; Negro, L.; Savoia, D. *Eur. J. Org. Chem.* **2001**, 517–522.
24. Hoffman, R. V.; Maslouh, N.; Cervantes-Lee, F. *J. Org. Chem.* **2002**, *67*, 1045–1056.
25. In addition, in the most stable conformation of **9** calculated by conformational analysis,[34] the dihedral angle between two methine hydrogens (H-4 and H-5) was 38.1°. This value and Karplus curve also supported the observed coupling constant. Furthermore, irradiation of H-5 in NMR gave a strong NOE for H-4. For a related example, Carter, P. H.; LaPorte, J. R.; Scherle, P. A.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1237–1239.
26. Ho, G.-J.; Mathre, D. J. *J. Org. Chem.* **1995**, *60*, 2271–2273.
27. Evans, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1989**, *111*, 1063–1072.
28. Evans, D. A.; Britton, T. C.; Ellman, J. A. *Tetrahedron Lett.* **1987**, *28*, 6141–6144.
29. Hoekstra, M. S.; Sobieray, D. M.; Schwindt, M. A.; Mulhern, T. A.; Grote, T. M.; Huckabee, B. K.; Hendrickson, V. S.; Franklin, L. C.; Granger, E. J.; Karrick, G. L. *Org. Process. Res. Dev.* **1997**, *1*, 26–38.
30. A steric repulsion between benzyl and carboxamide moieties in *cis*-configuration was thought to provide the ideal environment for chirality induction, because the conformation of benzyl moiety at the 4-position is restricted around *Re*-face of the enolate intermediate to avoid the steric repulsion by the carboxamide moiety at the 5-position.
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, ^{1}H NMR, $CDCl_3$), suggesting that the reaction proceeded via a *Z*-configured lithium enolate intermediate.
33. The absolute stereochemistry of **26c** was determined in comparison of its $[\alpha]_D$ value with the reported authentic data in Kurth, M. J.; Decker, O. H. W.; Hope, H.; Yanuck, M. D. *J. Am. Chem. Soc.* **1985**, *107*, 443–448. Enantiomeric excess of **26c** was determined by HPLC analysis after conversion to the corresponding (*S*)-phenethylamide. Vedejs, E.; Gingras, M. *J. Am. Chem. Soc.* **1994**, *116*, 579–588.
34. Energy minimization was performed by systematic conformation search on the MMFF94x force field using the Molecular Operating Environment modeling package (MOE 2004.03, Chemical Computing Group, Inc., Montreal, Canada), followed by MOPAC (PM3 program).
35. This effect may be similar to the shielding effect observed in 5,5-dimethyl-4-benzyloxazolidinone, so-called 'SuperQuats'. Davies, S. G.; Sanganee, H. J.; Szolcsanyi, P. *Tetrahedron* **1999**, *55*, 3337–3354.
36. *Fmoc Solid Phase Synthesis, A Practical Approach*; Chan, W. C., White, P. D., Eds.; Oxford University Press: Oxford, 2000.
37. Atherton, E.; Benoiton, N. L.; Brown, E.; Sheppard, R. C.; Williams, B. J. *J. Chem. Soc., Chem. Commun.* **1981**, 336–337.
38. Mukaiyama, T. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 707–721. Insufficient introduction (~60%) of the *N*-3-phenylpropionyl group was observed in single coupling. Hence, double coupling was employed.
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-Test[47] 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 H_2O_2 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) K_2CO_3, MeI, DMF, rt; (b) $NaBH_4$, CuCl, THF, 0 °C; (c) 1 N NaOH aq, MeOH, 50 °C. Unfortunately, simple hydrogenolysis of *trans*-2,4-dichlorocinnamic acid by H_2, 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.
45. Fan, H.-F. *Structure Analysis Programs with Intelligent Control*; Rigaku Corporation: Tokyo, Japan, 1991.
46. Beurskens, P. T.; Admiraal, G.; Beurskens, G.; Bosman, W. P.; de Gelder, R.; Israel, R.; Smits, J. M. M. *The DIRDIF-94 program system. Technical Report of the Crystallography Laboratory*; University of Nijmegen: The Netherlands, 1994.
47. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595–598.
48. Oppolzer, W.; Lienard, P. *Helv. Chim. Acta* **1992**, *75*, 2572–2582.
49. Meyers, A. I.; Knaus, G.; Kamata, K.; Ford, M. E. *J. Am. Chem. Soc.* **1976**, *98*, 567–576.
50. Cohen, S. G.; Milovanoviç, A. *J. Am. Chem. Soc.* **1968**, *90*, 3495–3502.
51. Yamada, S.; Terashima, S. *Chem. Pharm. Bull.* **1968**, *16*, 1816–1828.

総合論文

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*
木曽　良明

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

Yoshio Hayashi* and Yoshiaki Kiso

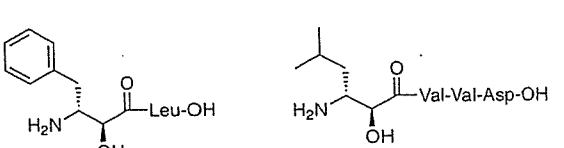
α-Hydroxy-β-amino acids are well known as inhibitory machinery for the development of protease inhibitors. In our ongoing efforts to develop effective aspartic protease inhibitors such as HIV-1 protease, malaria plasmepsin and human β-secretase inhibitors, the α-hydroxy-β-amino acids are also the critical 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 homobislactone 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, homobislactone, Evans' chiral auxiliary, asymmetric alkylation, solid-phase synthesis, difficult sequence, Aβ1-42, 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，タキンイド類にも存在し，抗腫瘍活性発現に必須な構造となっている[7]。一方，有機化学的見地からは，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



Bestatin　　Amastatin

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

* 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, (2*S*,3*S*)-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，しばしば溶媒に溶け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。

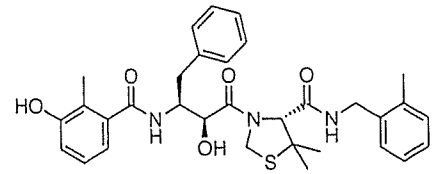
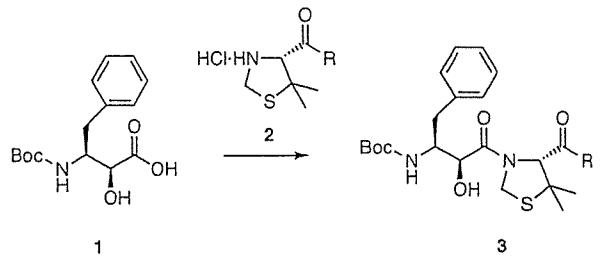
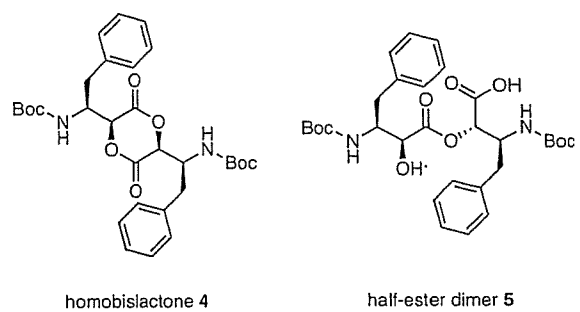


Fig. 2 Structure of HIV-1 protease inhibitor KNI-764.



Scheme 1



homobislactone 4　　half-ester dimer 5

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

Table 1 Homobislactone 4 formation under various coupling conditions.

Coupling Agents	Additives	Et_3N (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	-	33.3 ± 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), Et_3N or DIEA, rt. for 2 h.

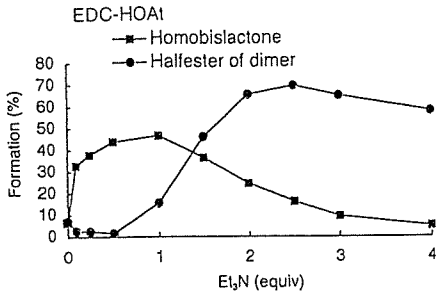


Fig. 4 Effect of base concentration on the byproduct formation.

その結果，この homobislactone 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-dimethylaminopropyl)carbodiimide hydrochloride, HOAt：1-hydroxy-7-azabenzotriazole)[13]での例を示すが，触媒量の Et_3N 添加が homobislactone 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 Et_3N の添加量を増やすと homobislactone 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 homobislactone 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 homobislactone 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

Homobislactone 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，homobislactone 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，homobislactone 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 homobislactone の生成は約 2% とわずかであった。これらの homobislactone 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。

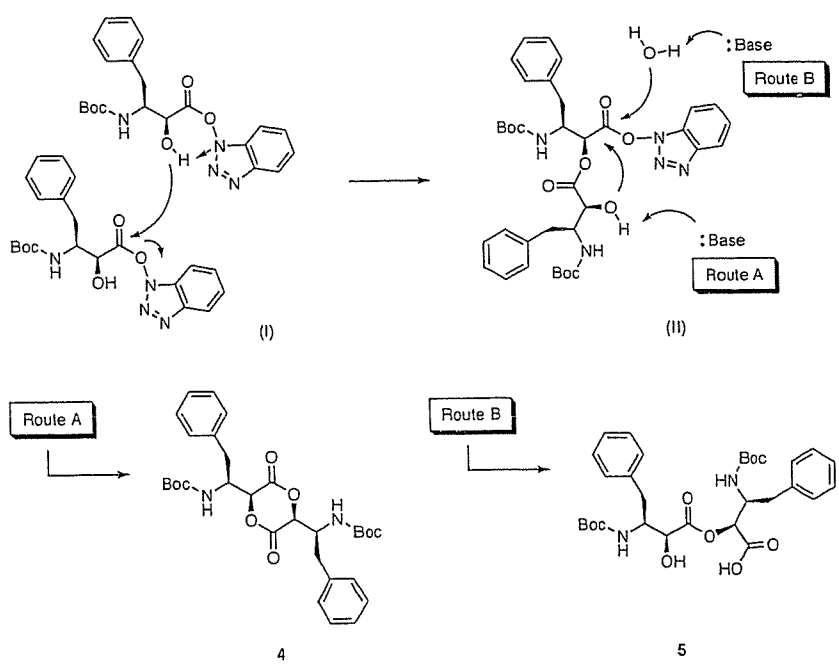


Fig. 5 Proposed mechanism of formation of homobislactone and half-ester of dimer.

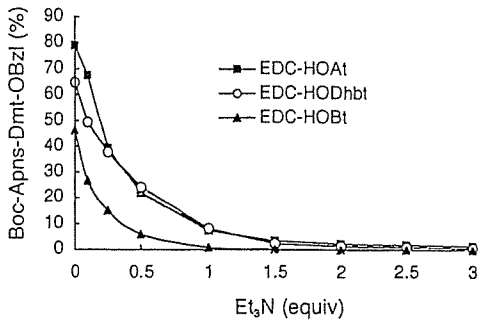


Fig. 6 Effect of base concentration on the yield of Boc-Apns-Dmt-OBzl 6.

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っては，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' oxazolidinone[16]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。従って，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-carboxilic acid をリンカーとして挿入することにした。

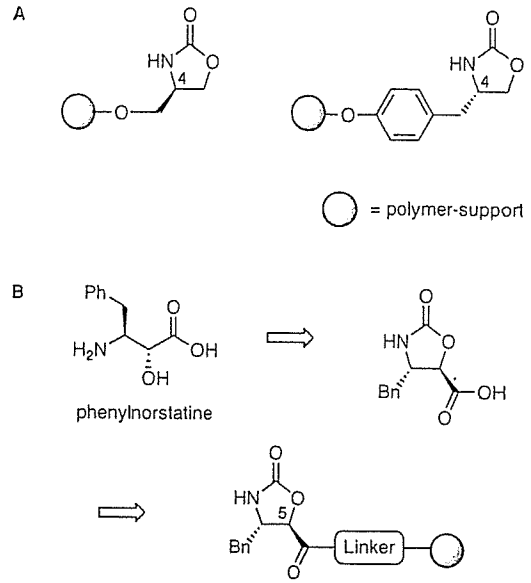
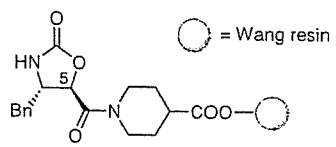


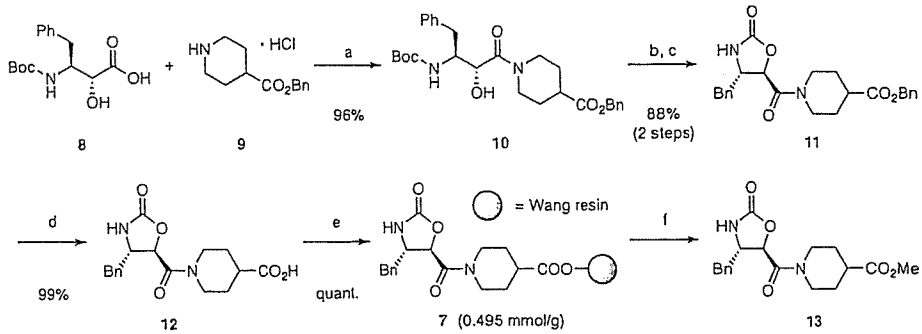
Fig. 7 Solid-supported Evans-type chiral auxiliaries.



7

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

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 LiOOH[20]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, Et_3N, DMF, 0 ℃ to rt; b) 4 M HCl/1,4-dioxane, 0 ℃ to rt; c) Et_3N, CDI, THF, 0 ℃ to rt; d) H_2, Pd/C, MeOH-H_2O, rt; e) Wang resin, DIPCDI, DMAP, DMF, rt; f) K_2CO_3, THF-MeOH, 0 ℃.

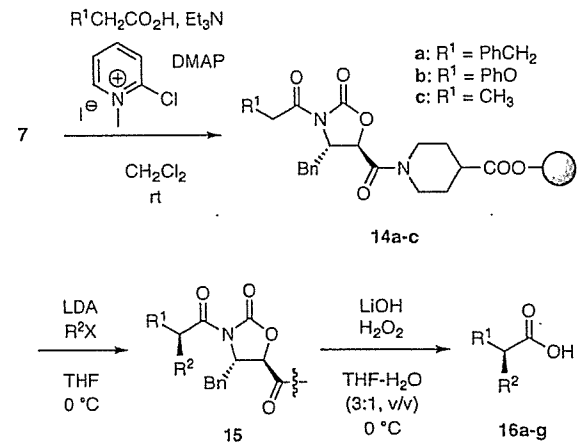
Scheme 2

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，DIPCDI-DMAP 法(DIPCDI: 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 ℃にて一定時間撹拌した。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^2X	16	Yield (%)[a,c]	Ee (%)[b,c]
1	14a	MeI	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		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

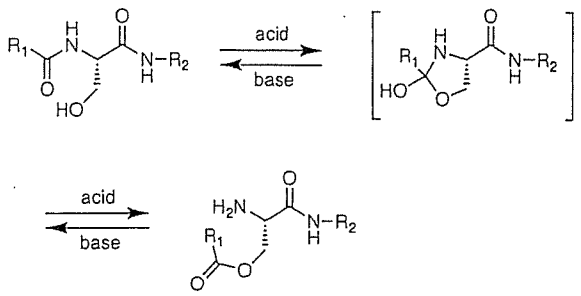


Fig. 9 N–O or O–N intramolecular acyl migration in peptides.

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が多く知られている。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。

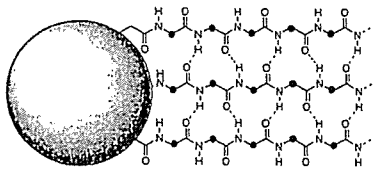


Fig. 10 β-Sheet formation of peptides in the solid phase peptide synthesis.

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-methoxybenzyl(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。

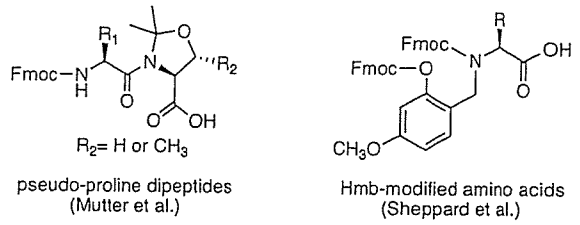


Fig. 11 β-Sheet breakers for the synthesis of difficult sequence-containing peptides.

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。

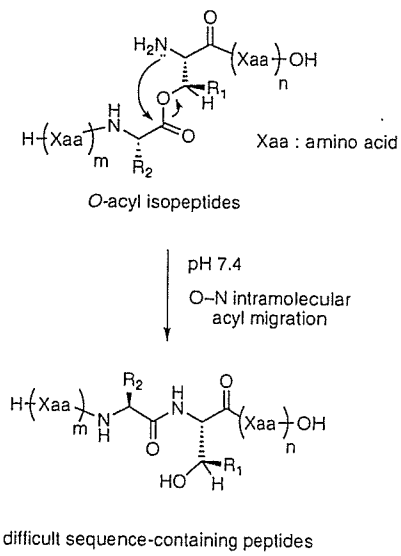
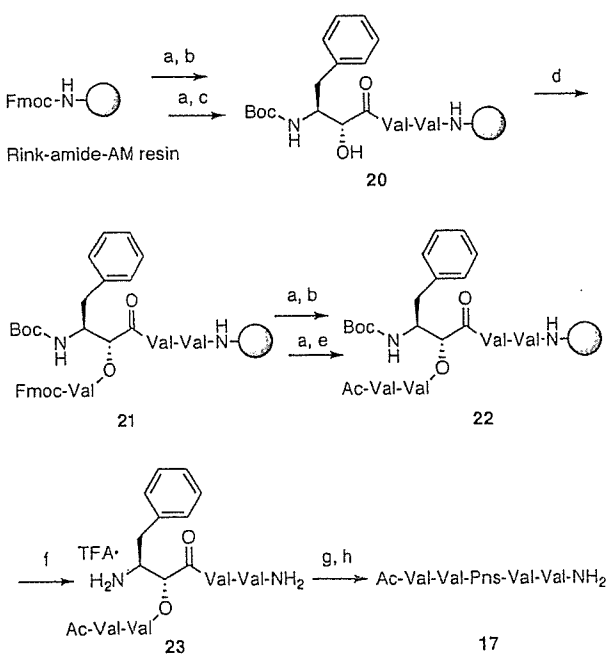


Fig. 12 "*O*-acyl isopeptide method" for the synthesis of difficult sequence-containing peptides.

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，

Ac-Val-Val-Pns-Val-Val-NH_2(17)を選択し，通常のFmoc固相合成法および"*O*-アシルイソペプチド法"の比較検討を実施した。通常のFmoc固相合成法では，目的物と同等量のFmoc-Val-Val-Pns-Val-Val-NH_2 18およびH-Val-Val-Pns-Val-Val-NH_2 19が副生した(図13A)。これは，Fmoc基の除去およびカップリングが不完全であったために生成したものと考えられる。また表3に示すように，ペプチド17のHPLC精製は，低い溶解性のため困難であり，収率は7%であった。一方，"*O*-アシルイソペプチド法"(スキーム4)では，Pnsヒドロキシ基へのFmoc-Val-OHの導入時に3.2%のラセミ化が観察されたが，以降の固相合成過程において，本エステル結合の開裂は観察されなかった。そして*O*-アシルイソペプチド23が主生成物として効率良く得られた(図13B)。この結果は，ペプチド中に挿入された分岐エステル構造が，樹脂上でのdifficult sequenceに起因する悪影響を排除し，カップリング・脱保護効率改善をもたらしたことを意味している。さらに，23は水およびメタノールに対し良好な溶解性を示したことから，HPLCによる精製が容易であり，高い収率(58%)で得られた(表3)。そして最後に，精製した*O*-アシルイソペプチド23をpH 7.4リン酸緩衝液(PBS)に溶解させると，半減期<1 minという早いO—N分子内アシル基転位反応により，目的のペンタペプチドアミド17が沈殿物として効率良く(総収率54%)得られた(図14)[10c]。



a) 20% Piperidine/DMF, 20 min; b) Fmoc-Val-OH, DIPCDI, HOBt, DMF, 2 h; c) Boc-Pns-OH, DIPCDI, HOBt, DMF, 2 h; d) Fmoc-Val-OH, DIPCDI, DMAP, CH_2Cl_2, 16 h×2; e) Ac_2O, TEA, DMF, 2 h; f) TFA-*m*-cresol-thioanisole-H_2O (92.5 : 2.5 : 2.5 : 2.5), 90 min; g) preparative HPLC (a linear gradient of CH_3CN in 0.1% aqueous TFA); h) PBS, pH 7.4, 25 ℃.

Scheme 4

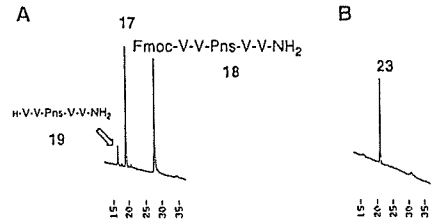


Fig. 13 HPLC profiles of crude deprotected peptides; (A) conventional solid phase peptide synthesis, (B) *O*-acyl isopeptide method.

Table 3 Solubility and yield of 17 and 23.

	H_2O-solubility		MeOH-solubility		Yield
Compd	mg / mL[a]	Ratio[b]	mg / mL[a]	Ratio[b]	(%)
17	0.008± 0.001	1	0.065 ± 0.019	1	7
23	59.4 ± 13.6	7500	277 ± 84	4300	58

[a] Values are means ± SD of three experiments. [b] Ratio=solubility of *O*-acyl isopeptide/solubility of parent peptide.

このように，"*O*-アシルイソペプチド法"は5残基のアミノ酸からなるdifficult sequence含有モデルペプチドの合成において，顕著な収率改善をもたらしたことから，difficult sequence含有ペプチドの新規効率的合成手法になりうることが示唆された。

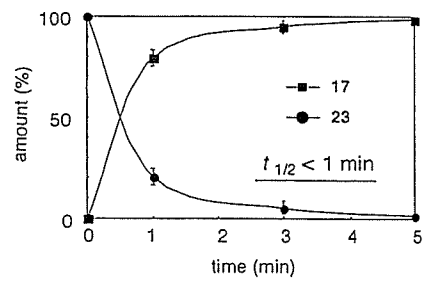


Fig. 14 Migration of *O*-acyl isopeptide 23 to 17 in PBS (pH 7.4, 25 ℃).

3.2 O—N分子内アシル基転位反応によるアミロイドベータペプチド(Aβ)1-42の合成

"*O*-アシルイソペプチド法"により低分子のα-ヒドロキシ-β-アミノ酸を含む，difficult sequence含有ペプチドの合成が成功したことから，次に高分子difficult sequence含有ペプチドの合成に挑戦した。標的difficult sequence含有ペプチドとして，その合成の難しさが指摘されているアミロイドβペプチド(Aβ)1-42[26]に注目した。本ペプチドはアミロイド形成において重要な役割を演じ，アルツハイマー病の病因と考えられており[27]，化学合成Aβ1-42の効率的供給は，Aβ1-42と発病の因果関係を詳細に解明する上で，重要なポイントである。しかしながら，本ペプチドは非常に疎水性の高いペプチドであり，種々の溶媒中で不溶性の凝集体を形成するため，一般的な固相合成法では収率・純度は極めて低く，さらにHPLCによる精製は溶媒への低溶解性と

ブロードな溶出のために困難を極める。

Aβ1-42 は 25, 26 位に Gly-Ser 残基を有するため，この部分で *O*-アシル体とすると，ペプチド 17 の合成で，観察されたラセミ化の問題を回避できる。そこでイソペプチド"26-*O*-アシルイソ Aβ1-42"(24)の合成と，それに続く O－N 分子内アシル基転位反応を利用した Aβ1-42 への変換を検討した(図 15)。

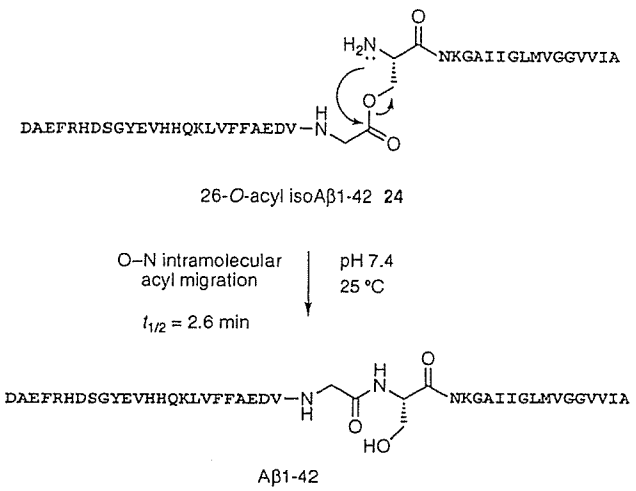


Fig. 15 Synthesis of Aβ1-42 via the O–N intramolecular acyl migration reaction of 26-*O*-acyl isoAβ1-42.

O-アシルイソ体 24 は，トリチル樹脂を用いる Fmoc 固相合成法にて，先に述べたモデル *O*-アシルイソペプチド 23 と同様の方法で合成した。得られた保護イソペプチド樹脂は，TFA-*m*-cresol-thioanisole-H_2O による脱保護，NH_4I-dimethylsulfide による還元を経て，HPLC により精製した。粗生成 24 の HPLC 分析から，合成過程において，Aβ26-42(SNKGAIIGLMVGGVVIA)の副生(1.6%)が若干認められたが，分取 HPLC 精製では，Aβ1-42 に特有のブロードな溶出でなく，シャープなピークとして溶出されたため，容易に精製することができ，結果 33.6%の良好な収率で *O*-アシルイソ体 24 が得られた。なお，通常の Fmoc 型固相合成法での Aβ1-42 の収率は 7.2%であった。精製した 24(TFA 塩)の水溶性は 15 mg mL^{-1} で，Aβ1-42(0.14 mg mL^{-1})に比べ 100 倍高い値を示した。興味深い点は，42 残基の Aβ1-42 ペプチド鎖中に，わずか 1 カ所のイソペプチド構造を導入することで，difficult sequence の悪影響を受けずに固相合成効率および水溶性を顕著に改善できたことである。これは凝集性の元凶となる特異な 2 次構造が，*O*-アシルイソペプチド構造の導入により形成され難くなったためと考えられる。本結果から，"*O*-アシルイソペプチド法"は，比較的高分子の difficult sequence 含有ペプチドの効率的合成法にも応用可能であることが示された。一方，*O*-アシルイソペプチド体 24 はリン酸緩衝液(pH 7.4) 中において，O－N 分子内アシル基転位反応により目的の Aβ1-42 へと定量的に化学変換された(図 16)。転位反応の半減期は 2.6 分と非常にスムーズであった。また，pH 4.9 では半減期 3 時間，pH 2.0 では変換が起こらず，明らかに変換は pH 依存的であった。さらに，24 の TFA 塩(固体)は 4 ℃の保存において安定であった。Aβ1-42 は，DMSO などの保存溶液中でさえも高頻度に凝集体を形成することから，このような不均一な Aβ1-42 を利用した生物学的研究には大変問題がある[28]。しかし，*O*-アシルイソ体 24 を利用すれば溶解性およびこの凝集性の問題を解決でき，生物評価系に 24 を添加することで，*in situ* でインタクトな Aβ1-42 を迅速に産生させることが可能になると考えられる。従って，アルツハイマー病の研究において，26-*O*-アシルイソ Aβ1-42(24)は有益な新規研究ツールになると期待される[10a, b]。

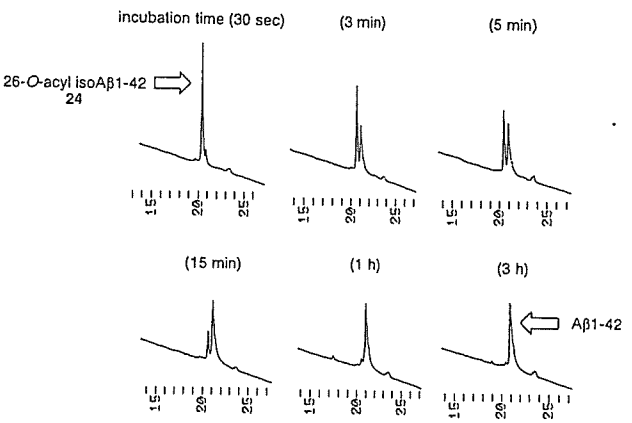


Fig. 16 HPLC profiles of the conversion of 26-*O*-acyl isoAβ1-42 (24) to Aβ1-42 via O–N intramolecular acyl migration in PBS (pH 7.4, 25 ℃).

4. 創薬化学における α-ヒドロキシ-β-アミノ酸を用いた研究展開

Taxoid 類は，微小管に作用し細胞周期を抑制することで抗癌作用を示すが，中でも paclitaxel 25(Taxol®)および docetaxel(Taxotere®)は，化学療法剤として癌治療に多大な貢献をしている[29]。しかし，最近これらの薬剤に対する薬剤耐性腫瘍の出現が問題となっており，新たな taxoid 系医薬品の開発が盛んに行われている。一方，共通化学構造であるタキサン骨格は疎水性が高いために，taxoid 類は一般に極端に水溶性が悪い。例えば，25 は注射剤であるが，極めて難水溶性 (0.00025 mg mL^{-1}) のため，溶解補助剤として界面活性剤 Cremophor EL が用いられる。しかし，この補助剤には過敏症反応を起こす副作用が知られている[30]。従って，taxoid 系化合物の溶解性改善は QOL の観点からも意義深い。そのため，すでに 20 種類以上もの paclitaxel 水溶性プロドラッグが報告されているが，実用化には至っ

ていない[3l]。これらはすべて，C-2′位またはC-7位水酸基に高極性補助基を付加することで水溶性を改善しているが，親薬物への変換に際して補助基部分が副生成物となることから，常に補助基に対する副作用を懸念する必要がある。

筆者らは，有機化学に基づいて薬剤学的付加価値の高い分子の創製を目指す"chemical pharmaceutics"の研究展開として，前節で述べた，O−N分子内アシル基転位反応に注目して，水溶性補助基を利用しない新しいタイプの水溶性プロドラッグを考案している。図17に示すように，すでに，α-ヒドロキシ-β-アミノ酸構造を有するHIV-1プロテアーゼ阻害剤の"O−N分子内アシル基転位型"水溶性プロドラッグを報告している[11c-g]。これらは，親化合物のO-アシルイソ体であり，塩形成可能なアミノ基が存在するために水溶性を改善できる。例えば，HIV-1プロテアーゼ阻害剤KNI-727(図17)の分子中央部Apns残基で，相当するO-アシル型プロドラッグ26にすると，水溶性はKNI-727より8,600倍上昇するとともに，生理的条件下では数分で副反応なく完全にKNI-727に変換された[11d]。一方，酸性条件下では安定で，塩酸塩(固体)として長期保存可能であった。

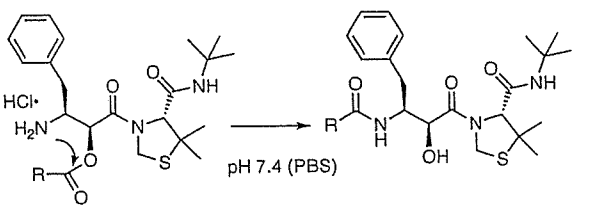


Fig. 17 Water soluble prodrug of HIV-1 protease inhibitors based on O−N intramolecular acyl migration.

筆者らは，このプロドラッグ戦略が，類似したα-ヒドロキシ-β-アミノ酸を例外なく分子内に有する一連のtaxoid類でも，難水溶性の問題を網羅的に解決できるのではないかと考えた。すなわちtaxoidの2′-O-acyl isoformを合成すれば，水溶性プロドラッグとして効果的に機能するのではないかと考え，paclitaxel 25の2′-O-benzoyl isoformであるisotaxel 27をデザインした(図18)。しかし，同様なベンゾイル構造を有するKNI-565(図17)のプロドラッグ28が比較的長い半減期(30分)を示したことから[11d]，isotaxel 27の合成に先立ち，タキサン環部分をシクロヘキサン環に変更した27のモデル化合物を合成し，生理的条件下での半減期を測定したところ，12分であった。これは，O−Nアシル基転位反応における5員環遷移状態で，置換基同士の立体配置が立体障害の大きな*cis*となる28に比べ，27のモデル化合物では，立体障害が小さい*trans*配置になるため，転位反応がより早く進行したものと考えられる。得られた半減期は，静脈点滴後，比較的速やかに親化合物を放出できる長さであったことから，実際にスキーム5に示すルートで，27の合成を行った。

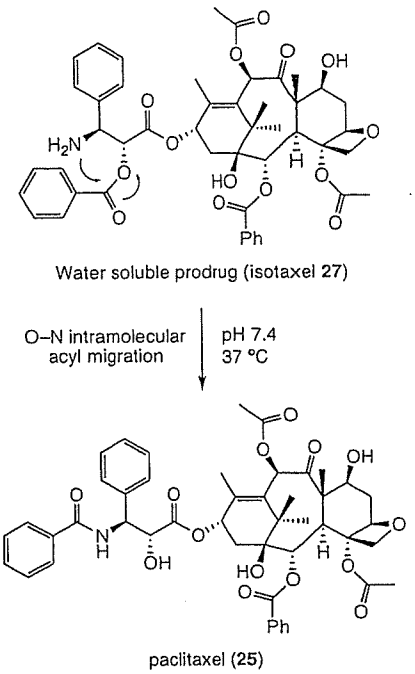
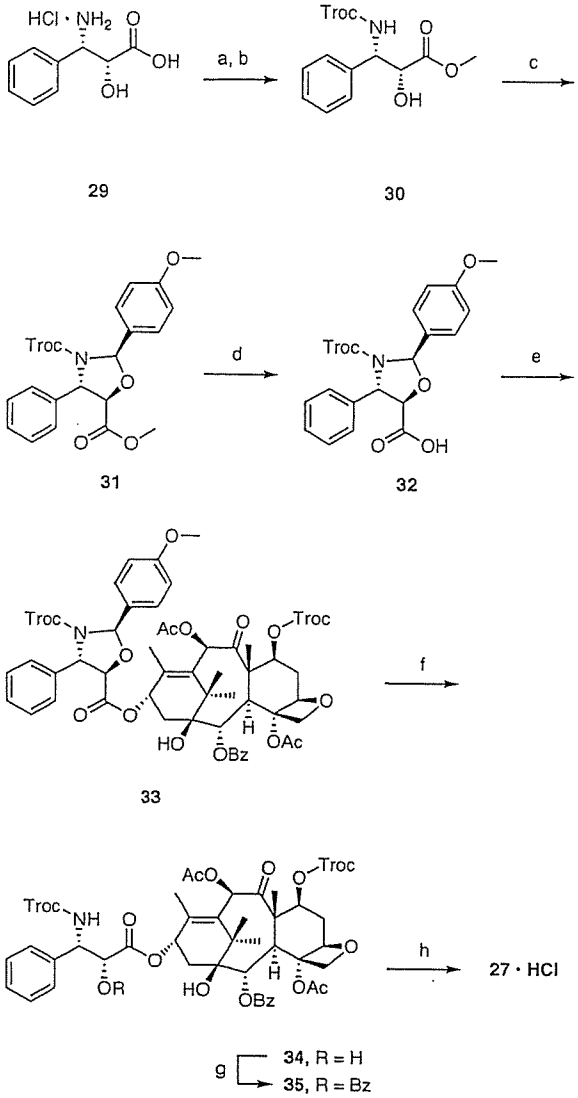


Fig. 18 New water soluble prodrug of paclitaxel.

すなわち，市販の(2*R*, 3*S*)-phenylisoserine·HCl 2から調製したN^{β}-Troc-phenylisoserine methyl ester 3をPPTS触媒下に4-methoxybenzaldehyde dimethyacetalと反応させ1,3-oxazolidine誘導体31とし，次いでメチルエステルを加水分解後，得られたカルボン酸誘導体32をDCC-DMAP法で7-Troc-baccatin IIIと縮合させた。このエステル化はほぼ定量的に進み，またC-2′位のラセミ化も観察されなかった。得られた化合物33のoxazolidine環をPTSで分解後に，2′-ヒドロキシ基に安息香酸をEDC-DMAP法で導入し，さらに，つのTroc基をZn-AcOHで除去し，12 mM HClを溶出液とするHPLC精製にて，目的のisotaxel塩酸塩27を総収率58%で得た。

27は親化合物25に比べ水溶性が1,800倍(0.45 mmL^{-1})上昇するとともに，生理的条件下(pH 7.4, 37 ℃において副反応を伴うことなく，完全に親薬物に変換された(図19)。また，この半減期はpH 7.4においては分であり，先に述べたモデル実験に近い値で，静脈投後の全身への薬物の送達には適度な値と思われる。方，半減期はpH依存的であり，pH 4.9では252分pH 2.0では転位は起こらなかった(図20)。また，塩塩として固体状態では，冷蔵での長期保存が可能である。さらに，静脈投与可能な0.035%クエン酸生理食



a) Succinimidyl-2, 2, 2-trichloroethylcarbonate, NaOH, $NaHCO_3$, dioxane, rt, 1 h; b) $SOCl_2$, MeOH, 0 ℃ to rt, 14 h, 97% over two steps; c) 4-methoxybenzaldehyde dimethyl acetal, PPTS, toluene, distillation 30 min., 92%; d) KOH, MeOH, rt, 30 min. 99%; e) 7-Troc-baccatin III, DCC, DMAP, toluene : CH_2Cl_2 2 : 1, rt, 3 h, 98%; f) PTS, MeOH, rt, 24 h, 94%; g) benzoic acid, EDC·HCl, DMAP, CH_2Cl_2, rt, 2 h, 92%; h) Zn (dust), MeOH : AcOH 1 : 1, rt, 4 h, then HCl, 77%.

Scheme 5

水(pH 4.0)[32]では，室温3時間のインキュベーションでも，親化合物25の生成は3%以下であったことから，isotaxel 27には，実用性があると思われる。一方，副作用の原因ともなる水溶性補助基を有しないことから，新しいタイプのタキソイドプロドラッグ(イソタキソイド)として開発される可能性がある。

筆者らは，さらに本水溶性プロドラッグ手法の一般性を検証するために，最近，他のタキソイド誘導体canadensolのO−N分子内アシル基転位型水溶性プロドラッグの合成も行い，良好な結果を得ている[11a]。また，taxoid類では，docetaxelのように，アミノ基の修飾基として，カルバメートを有するものがある。これらの誘導体の水溶性プロドラッグ体は，炭酸エステル構造を有し，O−N分子内アシロキシ基転位反応で親化合物に変換される必要がある。最近の検討ではBoc基を有するdocetaxelでは，一部アシロキシ基の加水分解が起こるが，それ以外のカルバメート型誘導体では，O−N分子内アシロキシ基転位反応により，適度な半減期で，副生物なしに親化合物に変換されることを見出している[11h]。これらの誘導体の中には，次世代の抗腫瘍剤として期待されるpaclitaxel耐性腫瘍に対して有効な化合物もあり，今後，本水溶性プロドラッグ(イソタキソイド)の実用的な応用例となる可能性がある。

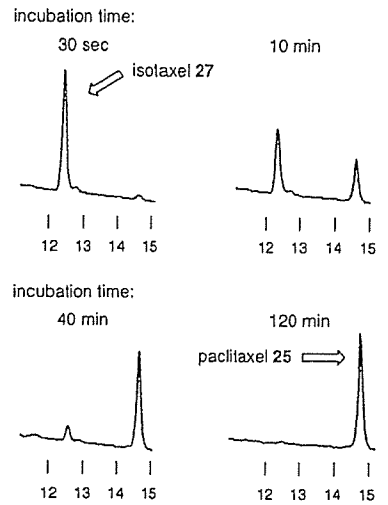


Fig. 19 HPLC profile of O−N benzoyl migration of isotaxel 27 in PBS (pH 7.4, 37 ℃).

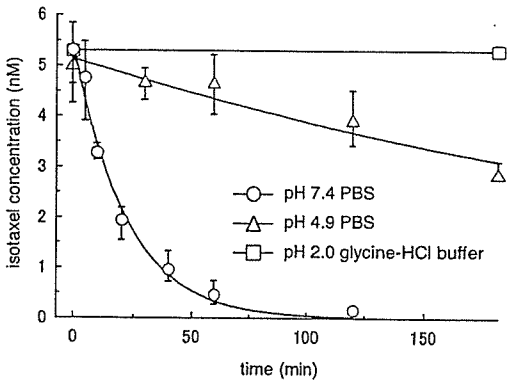


Fig. 20 Migration of isotaxel 27 in different pH conditions (PBS, pH 7.4, 37 ℃).

おわりに

筆者らは，α-ヒドロキシ-β-アミノ酸の分子機能に注目することで，有機化学・創薬化学での新規な応用研究を展開してきた。特に，α-ヒドロキシ-β-アミノ酸の特性の1つであるO−N分子内アシル基転位反応に注目することで，difficult sequence含有ペプチドの新しい合成手法までたどり着くことができた。また，水溶性プロ