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Stereoselective Synthesis of Dolastatin 10 and Its Congeners†

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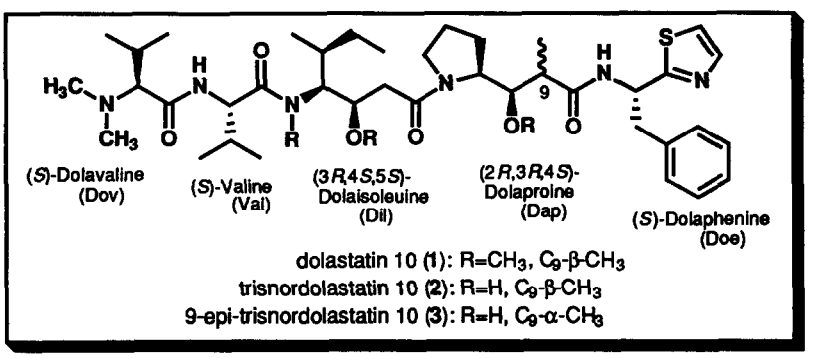
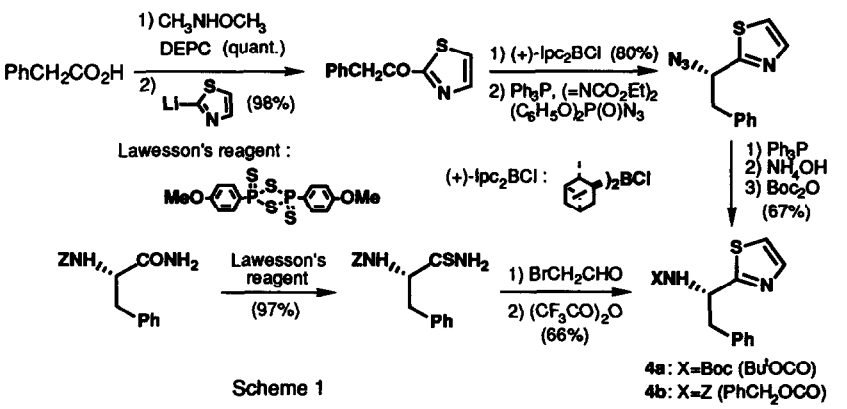
Abstract: Efficient synthesis of dolastatin 10 (**1**), a potent antitumor peptide from a sea hare *Dolabella auricularia*, has been achieved in a stereoselective manner. Trisnordolastatin 10 (**2**) and its C9-epimer (**3**) have also been synthesized.

Dolastatin 10 (**1**), isolated from an Indian Ocean sea hare *Dolabella auricularia*, shows powerful antineoplastic activity and is now expected to become a new anticancer agent.[1] In addition to its potent biological activity, the structural uniqueness as well as scarce availability in nature has led several groups[1b,2] including ours[3,4] to synthesize this peptide. We now wish to report the detail of our synthesis of dolastatin 10 (**1**) and its congeners : trisnordolastatin 10 (**2**) and 9-epi-trisnordolastatin 10 (**3**). The results of their biological investigation have also been described briefly.



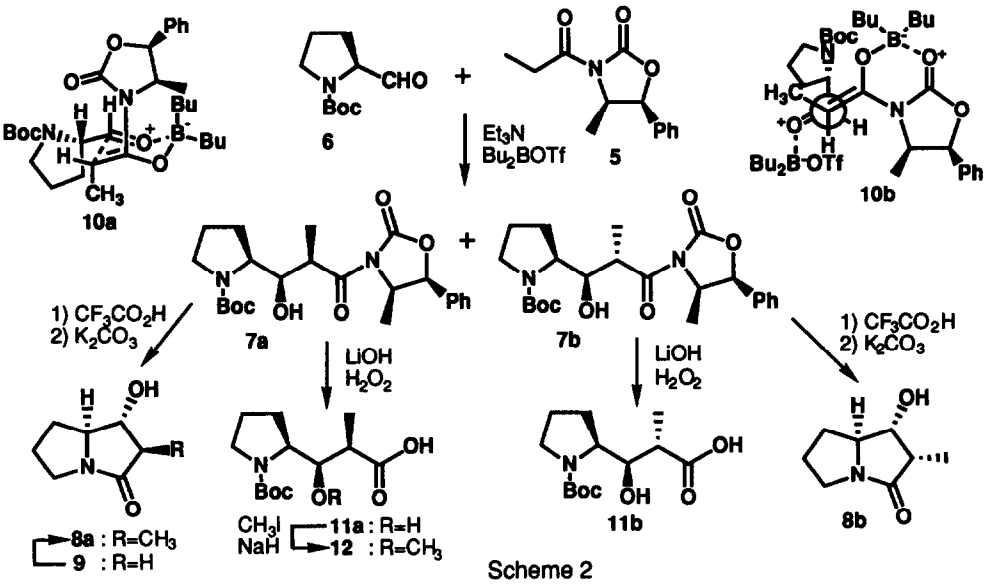


Scheme 1

†Dedicated with respect and deep appreciation to Professor Shun-ichi Yamada, one of the pioneers in the synthesis of optically active compounds, on the occasion of his 77th birthday (Ki-ju).

The significant problem is how to construct stereoselectively three unique units derived from α-amino acids : (*S*)-dolaphenine (Doe), (2*R*,3*R*,4*S*)-dolaproine (Dap), and (3*R*,4*S*,5*S*)-dolaisoleuine (Dil) units. We have already revealed[5] an efficient synthesis of the C-terminal Doe unit **4**, which is summarized in Scheme 1.

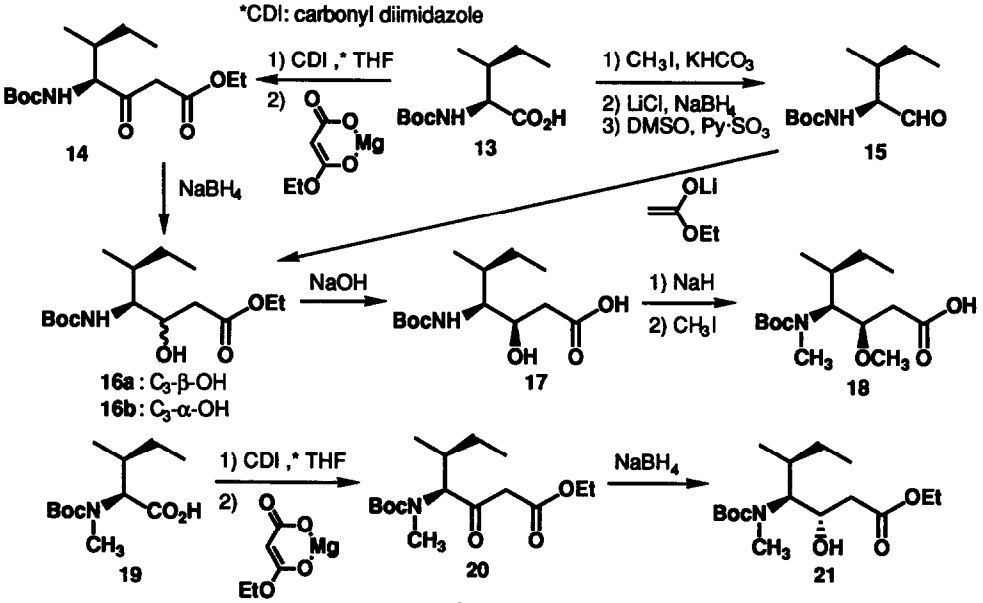
The Evans aldol methodology was adopted to construct the Dap unit **12**. When the N-propionyloxazolidinone **5** derived from (1*S*,2*R*)-norephedrine was allowed to react with Boc-(*S*)-prolinal (**6**)[6] by use of triethylamine and dibutylboron triflate, an interesting reversal of stereochemistry of the aldol products **7** was found to be dependent on the quantity of the reagents.[4,7] Only the expected syn-adduct **7a** was produced in excellent yield with complete diastereoselection when triethylamine was used in slight excess over dibutylboron triflate. However, when dibutylboron triflate existed in excess, the major product was the anti-adduct **7b**. The configurational assignment was made by transformation of the both adducts **7a** and **7b** to the corresponding pyrrolizidinone derivatives **8a** and **8b**, respectively, as shown in Scheme 2. The pyrrolizidinone **8a** was identical with the sample prepared by the methylation of the known lactam **9**[8] while the physical data of **8b** was identical with those reported.[1b] The stereochemical result of the aldol products may be explained by the closed transition state **10a** when an excess of triethylamine is used, while the open transition state **10b** will be dominant in the presence of an excess of dibutylboron triflate.[9] As we have already reported it,[7] the same phenomenon of the reversed stereochemistry was observed in the case of Z-(*S*)-prolinal, Boc- and Z-(*R*)-alaninal.[10] However, Boc-(*S*)-valinal, Boc-(*S*)-N-methylvalinal, Boc-(*S*)-isoleucinal, and benzaldehyde always afforded the syn isomers as the major products even when an excess of dibutylboron triflate was used. The remaining task to construct the Dap unit was removal of the chiral auxiliary from **7a** followed by O-methylation. The former was carried out by use of standard conditions[11] to give the carboxylic acid **11a**. Similarly the anti-isomer **7b** gave **11b**. Methylation of the alcoholic function was accomplished by treatment of **11a** with a large excess of methyl iodide and sodium hydride,[12] giving the required Dap unit **12** in good yield.



Scheme 2

Preparation of the Dil unit was achieved from Boc-(*S*)-isoleucine (**13**) by a procedure analogous to our method for the preparation of the Boc-isostatine derivative.[13] Conversion of **13** to the corresponding imidazolide followed by treatment with the magnesium enolate of malonic acid half ester nearly quantitatively afforded the β-keto ester **14**, which was reduced with sodium borohydride to yield the hydroxy ester **16a** as the major product together with the diastereomer **16b** as a minor product in a ratio of 91 : 9. Obviously, this

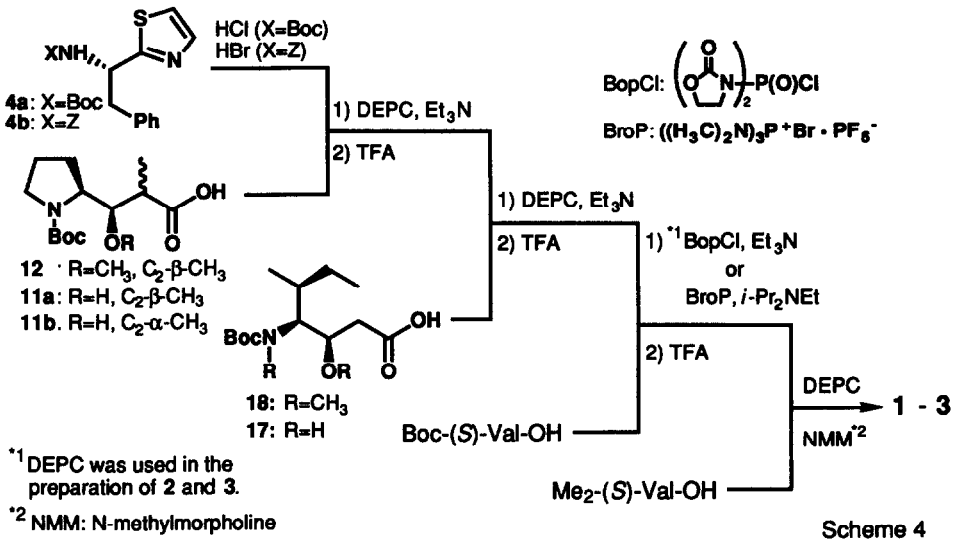
result of the borohydride reduction will be explained by the Cram's cyclic transition state. As an alternative approach, Boc-(*S*)-isoleucinal (**15**)[6] was allowed to react with the lithium enolate derived from ethyl acetate to give a mixture of the hydroxy esters **16a** and **16b** in a ratio of 38 : 62. The hydroxy ester **16a** separated on a column underwent alkaline hydrolysis to give the carboxylic acid **17**, which was treated with a large excess of sodium hydride and methyl iodide to give the required Dil unit **18**. Analogously, Boc-(*S*)-N-methylisoleucine (**19**)[12] was efficiently transformed to the β-keto ester **20**, of which the borohydride reduction resulted in the formation of the undesired hydroxy ester **21** as a sole isolable product.[14] The unit which remained to be synthesized was (*S*)-dolavaline (Dov), which was easily prepared from (*S*)-valine according to the literature.[15]



Scheme 3

Construction of dolastatin 10 (**1**) started from the C-terminal Doe unit in a stepwise manner, as shown in Scheme 4. Diethyl phosphorocyanidate (DEPC, $(C_2H_5O)_2P(O)CN$) and trifluoroacetic acid (TFA) were mainly used for the coupling and deprotection, respectively. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BopCl)[16] was used in place of DEPC for the coupling of Boc-(*S*)-valine with the Dil-Dap-Doe unit. Further investigation revealed that bromo tris(dimethylamino)phosphonium hexafluorophosphate (BroP)[17] gave more satisfactory result in this step. Dolastatin 10 (**1**) thus obtained was identical with the natural one by spectral (IR, ^{1}H-and ^{13}C-NMR) comparisons. Similarly, trisnordolastatin 10 (**2**) and its C9-epimer (**3**) were also synthesized by use of the norDap units **11a** and **11b**, respectively, in addition to the bisnorDil unit **17**. DEPC was used throughout the construction.

Cytotoxicity of dolastatin 10 (**1**), its congeners **2** and **3**, and their synthetic intermediates was evaluated against L1210 murine leukemia cells.[18,19] Dolastatin 10 (**1**) synthesized has remarkable cytotoxicity (IC_{50} = 2.95 x 10^{-4} μg/ml)[19] as reported.[1] Although trisnordolastatin 10 (**2**) shows cytotoxicity to some extent (10^{-1} μg/ml), its C9-epimer (**3**) has much weaker cytotoxicity (50 μg/ml). Interestingly, Boc-Val-Dil-Dap-Doe, the tetrapeptide lacking the N-terminal Dov unit of **1**, also shows strong cytotoxicity (4 x 10^{-3} μg/ml). These results might suggest the importance of the N,O,O-trimethyl functions and/or configuration at the C9 position to show cytotoxicity. The least unit which has cytotoxicity seems to be the Boc-tripeptide, Boc-Dil-Dap-Doe (11 μg/ml), lacking the N-terminal Dov-Val unit, since Boc-Dap-Doe shows little or no cytotoxicity (>100 μg/ml).



Scheme 4

The work described here will promise an efficient and large scale preparation of dolastatin 10 having significant antitumor activity. Further studies on structure-cytotoxicity relationships based on dolastatin 10 might promise the exploitation of a new antitumor drug.[20]

Experimental

Melting points were determined on a YAMATO MP-21 apparatus or a YANAGIMOTO micro melting point apparatus. Infrared spectra were measured with a JASCO IRA-2 or SHIMADZU FT IR-8100 spectrometer. ^{1}H-NMR spectra were recorded on a JEOL PMX-60, FX-100, EX-270, or GSX-400 spectrometer in $CDCl_3$ solution using tetramethylsilane as an internal standard, unless otherwise stated. Optical rotations were measured with a JASCO DIP-140 automatic polarimeter. Silica gel (BW-820MH or BW-200) was used for column chromatography and silica gel (Merck Art. 11695, Kieselgel 60 H or Wakogel FC-40) was used for flash column chromatography.

(*S*)-2-(1'-Amino-2'-phenylethyl)thiazole, (*S*)-dolaphenine ((*S*)-Doe). (1) Z-(*S*)-Dolaphenine (**4b**, Z-(*S*)-Doe)[5] (678 mg, 2 mmol) was treated with 25% HBr/AcOH (6 ml) at room temperature for 4 h. Volatiles were removed in vacuo. The residue was triturated with dry ether by decantation, dried under reduced pressure to give a slightly brown solid, which was dissolved in water (20 ml) and adjusted to pH 9 by the addition of saturated aqueous sodium hydrogen carbonate (40 ml). The resulting mixture was extracted with methylene chloride (50 ml x 3). The organic extracts were washed with 5% aqueous sodium thiosulfate (40 ml), water (40 ml), and saturated aqueous sodium chloride (40 ml), dried over sodium sulfate, and concentrated in vacuo to give (*S*)-dolaphenine ((*S*)-Doe) (408 mg, 99% yield) as a yellowish brown oil, which was used for the next step without further purification. (*S*)-Doe: IR (neat) ν_{max} cm^{-1}: 3370, 3300 (shoulder), 3070, 1600, 1500, 1450, 1120, 1060; ^{1}H-NMR δ 1.77 (2H, s), 2.84 (1H, dd, J=13Hz, 9Hz), 3.35 (1H, dd, J=13Hz, 5Hz), 4.48 (1H, dd, J=9Hz, 5Hz), 7.16 (6H, s), 7.65 (1H, d, J=3Hz).

(2) A mixture of Boc-(*S*)-dolaphenine (**4a**, Boc-(*S*)-Doe)[5] (30 mg, 0.1 mmol) and 10% HCl/MeOH (1 ml) was stirred at room temperature for 2 h. After concentration, the residual white solid was dissolved in water (20 ml) and the mixture was washed with ether (30 ml). The aqueous layer was adjusted to pH 10 with 28% aqueous ammonia and extracted with ether (20 ml x 2). The extracts were washed with saturated aqueous sodium chloride (20 ml), dried over sodium sulfate, and concentrated to give (*S*)-dolaphenine (19 mg, 95%).

(4*R*,5*S*,2'*R*,3'*R*,2''*S*)-3-(3'-(N-*tert*-Butoxycarbonyl-2''-pyrrolidinyl)-3'-hydroxy-2'-methylpropanoyl)-4-methyl-5-phenyl-2-oxazolidinone (7a). To a precooled (2°C) solution of (4*R*,5*S*)-4-methyl-5-

phenyl-3-propionyl-2-oxazolidinone (**5**)[11a] (1.232 g, 5.3 mmol) in methylene chloride (20 ml) was added triethylamine (0.85 ml, 6.1 mmol), followed by the addition of freshly prepared dibutylboron triflate (1.4 ml, 5.5 mmol) dropwise at such a rate as to keep the internal temperature below 4°C. The mixture was stirred at 2°C for 45 min, then the ice bath was replaced with a dry ice-acetone bath. When the internal temperature dropped below -70°C, Boc-(*S*)-Pro-al (**6**)[6] (599 mg, 3.0 mmol) in methylene chloride (3 ml) and its washing (1 ml x 2) were added via a double-ended needle over a 5-min period below -60°C. The solution was stirred at -67°C for 2 h, at 2°C for 1 h, then at room temperature for 15 min. The reaction mixture was quenched with phosphate buffer (pH 7, 5 ml), followed by the addition of methanol (15 ml). To this solution was added 30% aqueous hydrogen peroxide-methanol (1:2, 18 ml) dropwise at such a rate as to keep the internal temperature below 10°C. After being stirred at 2°C for 1 h, the mixture was quenched with water (10 ml), then concentrated in vacuo. Water (10 ml) was added to the residue. The mixture was extracted with ether (80 ml x 3). The organic extracts were washed with 1 M aqueous potassium hydrogen sulfate (30 ml), water (30 ml), saturated aqueous sodium hydrogen carbonate (30 ml), and saturated aqueous sodium chloride (30 ml), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by flash column chromatography using ethyl acetate-hexane (1:4 - 1:3) as an eluent, giving the syn-aldol adduct **7a** (1.293 g, 99% yield) as a colorless foam: $[\alpha]_D^{23.5}$ -10.2° (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3444, 1784, 1694, 1393, 1196, 1121; ^{1}H-NMR δ 0.88 (3H, d, J=6.6Hz), 1.33 (3H, d, J=7.0Hz), 1.47 (s), 1.50 (s), 1.47-1.50 (9H, s), 1.81-1.95 (3H, m), 2.04 (1H, brs), 2.89 (0.5H, br, disappeared with D_2O), 3.24-3.34 (1H, m), 3.46-3.61 (1H, m), 3.90-3.97 (3H, m), 4.76 (1H, brt, J=6.2Hz), 5.68 (1H, d, J=7.0Hz), 7.28-7.44 (5H, m); FABMS (glycerin) m/z: 433 (M+1); Anal. Calcd for $C_{23}H_{32}N_2O_6$: C, 63.87; H, 7.46; N, 6.48. Found: C, 63.36; H, 7.34; N, 6.12.

(4*R*,5*S*,2'*S*,3'*R*,2"*S*)-3-(3'-(N-*tert*-Butoxycarbonyl-2"-pyrrolidinyl)-3'-hydroxy-2'-methylpropanoyl)-4-methyl-5-phenyl-2-oxazolidinone (7b). The boron enolate was prepared from (4*R*,5*S*)-4-methyl-5-phenyl-3-propionyl-2-oxazolidinone (**5**)[11a] (233 mg, 1.0 mmol), triethylamine (0.19 ml, 1.4 mmol), and freshly prepared dibutylboron triflate (0.38 ml, 1.5 mmol) according to the same procedure as that for **7a**. The chiral boron enolate was allowed to react with Boc-(*S*)-Pro-al (**6**)[6] (260 mg, 1.3 mmol). After the work-up analogous to the above and purification by flash column chromatography, the diastereomerically pure anti-aldol adduct **7b** (229 mg, 53% yield, 85% conversion yield) was obtained as a colorless foam accompanying the recovery of the propionyloxazolidinone (88 mg, 38% recovery). **7b**: $[\alpha]_D^{24}$ -9.34° (*c* 0.99, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3450, 1790, 1690, 1390, 1200, 1125, 1075; ^{1}H-NMR δ 1.00 (3H, d, J=5.9Hz), 1.37 (3H, d, J=6.4Hz), 1.45 (9H, s), 1.84 (3H, m), 2.15-2.20 (1H, m), 3.25-3.50 (3H, m), 3.71 (0.7H, d, J=10.1Hz, disappeared with D_2O), 3.95 (2H, brd, J=7.0Hz), 4.76 (1H, brt, J=6.6Hz), 5.56 (1H, d, J=7.2Hz), 7.26-7.44 (5H, m); FABMS (glycerin) m/z: 433 (M+1); Anal. Calcd for $C_{23}H_{32}N_2O_6$: C, 63.87; H, 7.46; N, 6.48. Found: C, 63.75; H, 7.26; N, 6.17.

(2*R*,3*R*,2'*S*)-3-(N-*tert*-Butoxycarbonyl-2'-pyrrolidinyl)-3-hydroxy-2-methylpropanoic acid (11a). To an ice-cooled solution of the syn-aldol adduct **7a** (1.258 g, 2.9 mmol) in tetrahydrofuran (44 ml) was added 30% aqueous hydrogen peroxide (1.5 ml, 14.7 mmol), followed by the addition of 0.4 N aqueous lithium hydroxide (14.5 ml, 5.8 mmol) at such a rate to keep the internal temperature below 4°C. After being stirred for 2.5 h, the mixture was quenched by the addition of 1 M aqueous sodium sulfite (16 ml, 16 mmol) below 5°C, and stirred at room temperature for 13.5 h. The resulting mixture was poured into cooled saturated aqueous sodium hydrogen carbonate (120 ml). The mixture was extracted with methylene chloride (50 ml x 3) to remove the oxazolidinone auxiliary. The combined methylene chloride extracts were dried over magnesium sulfate and concentrated in vacuo to give the oxazolidinone auxiliary (472 mg, 92% yield) as colorless crystals. The aqueous layer was cooled in an ice bath and acidified to pH 2 by the addition of 1 M aqueous potassium hydrogen sulfate (ca. 150 ml). After being salted out, the resulting mixture was extracted with ether (100 ml x 3). The combined ether extracts were washed with water (40 ml) and saturated aqueous sodium chloride (40 ml), dried over sodium sulfate, and concentrated in vacuo to give the carboxylic acid **11a** (757 mg, 95% yield) as a colorless solid, which was triturated with hexane and filtered. **11a**: mp 86°C (hexane); $[\alpha]_D^{22}$ -54.5° (*c* 1.0, MeOH); IR (KBr) ν_{max} cm^{-1}: 3425, 3250, 1730, 1680, 1170, 1130, 1040; ^{1}H-NMR (DMSO-d_6) δ 1.08 (3H, d, J=7.1Hz), 1.39 (9H, s), 1.69 (2H, m), 1.80-1.86 (1H, m), 1.91 (1H, m),

2.25 (1H, m), 3.11 (1H, m), 3.34 (1H, m), 3.67 (1H, m), 3.83 (1H, dd, J=7.6Hz, 3.7Hz), 4.5-5.5 (0.8H, br, disappeared with D_2O), 11.5-12.5 (0.5H, br, disappeared with D_2O); Anal. Calcd for $C_{13}H_{23}NO_5$: C, 57.13; H, 8.48; N, 5.12. Found: C, 57.11; H, 8.50; N, 4.50.

(2*S*,3*R*,2'*S*)-3-(N-*tert*-Butoxycarbonyl-2'-pyrrolidinyl)-3-hydroxy-2-methylpropanoic acid (11b). The anti-aldol adduct **7b** (432 mg, 1.0 mmol) was hydrolyzed with 30% aqueous hydrogen peroxide (0.5 ml, 4.9 mmol) and 0.4 N aqueous lithium hydroxide (5 ml, 2.0 mmol) according to the same procedure as that for **11a**. After treatment with 1 M aqueous sodium sulfite (6 ml, 6 mmol) and the usual work-up, the requisite carboxylic acid **11b** (269 mg, 99% yield) was obtained as a colorless solid, which was recrystallized from ether-hexane to give colorless crystals: mp 153-154°C ; $[\alpha]_D^{22}$ -94.7° (*c* 1.0, MeOH); IR (KBr) ν_{max} cm^{-1}: 3500, 1700, 1620, 1420, 1160; ^{1}H-NMR (DMSO-d_6) δ 1.02 (3H, d, J=7.0Hz), 1.41 (9H, s), 1.48-1.77 (2H, m), 1.81-1.89 (2H, m), 2.23 (1H, m), 3.14-3.21 (1H, m), 3.33 (1H, br), 3.76 (1H, br), 3.98 (1H, dd, J=17.9Hz, 9.4Hz), 4.73-5.02 (0.3H, br, disappeared with D_2O), 11.85-11.94 (0.3H, br, disappeared with D_2O); Anal. Calcd for $C_{13}H_{23}NO_5$: C, 57.13; H, 8.48; N, 5.12. Found: C, 56.76; H, 8.20; N, 4.99.

(1*R*,2*R*,8*S*)-Hexahydro-1-hydroxy-2-methyl-3H-pyrrolizin-3-one (8a). (1) The aldol adduct **7a** (131 mg, 0.30 mmol) was dissolved in trifluoroacetic acid (6 ml). The mixture was stirred at room temperature for 1 h and concentrated in vacuo. The residue was coevaporated with benzene. The resulting pinkish oil was dissolved in ethanol-water (1:2, 6 ml) and cooled to 0°C. Potassium carbonate (50 mg, 0.36 mmol) was added to this solution. The mixture was stirred at 0°C for 15 min then at room temperature for 1.5 h. Additional potassium carbonate (7 mg, 0.05 mmol) was added. Then the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo and extracted with methylene chloride (20 ml x 3). The combined organic extracts were dried over sodium sulfate and concentrated in vacuo. The resulting yellowish oil was purified by silica gel column chromatography using ethanol-ethyl acetate-methylene chloride (1:5:10) as an eluent to give the chiral oxazolidinone (52 mg, 97% yield) as colorless crystals and then the bicyclic compound **8a** (45 mg, 96% yield) as slightly reddish crystals, which were recrystallized from ethyl acetate-hexane to give colorless crystals: mp 101-102°C; $[\alpha]_D^{23.5}$ +4.53° (*c* 0.48, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3300, 1650, 1100, 1080; ^{1}H-NMR δ 1.21 (3H, d, J=7.1Hz), 1.45-1.55 (1H, m), 2.00-2.06 (2H, m), 2.15-2.22 (1H, m), 2.70-2.78 (1H, m), 3.02-3.09 (1H, m), 3.15-3.24 (1H, br, disappeared with D_2O), 3.55 (1H, dt, J=11.5Hz, 7.6Hz), 3.64-3.72 (2H, m).

(2) Ethyl (3*R*,2'*S*)-3-(N-*tert*-butoxycarbonyl-2'-pyrrolidinyl)-3-hydroxypropanoate (2.516 g, 8.8 mmol), prepared according to the reference 8, was treated with trifluoroacetic acid (10 ml) then cyclized under basic conditions (potassium carbonate (2.43 g, 18 mmol) in ethanol-water (2:1, 45 ml)) according to the same procedure as that for **8a**. After the usual work-up and purification, the bicyclic compound **9** (456 mg, 37% yield) was obtained as yellowish crystals, which were recrystallized from ethyl acetate-hexane.

The bicyclic compound **9** (13 mg, 0.09 mmol) thus obtained was dissolved in tetrahydrofuran (2 ml), and the solution was added to a precooled (-78°C) solution of LDA (prepared from diisopropylamine (56 µl, 0.4 mmol), 1.62 M solution of *n*-butyllithium in hexane (0.25 ml, 0.4 mmol), and tetrahydrofuran (1.5 ml)) via a double-ended needle under argon atmosphere. The mixture was stirred at -78°C for 1.25 h. The resulting mixture was added to a precooled (-78°C) solution of methyl iodide (0.18 ml, 2.8 mmol) in tetrahydrofuran (5 ml) via a double-ended needle. The mixture was stirred at -78°C for 1.75 h then at -20°C for 0.5 h. Acetic acid-tetrahydrofuran (1:20, 1 ml) was added to the reaction mixture, followed by the addition of water (10 ml). After being salted out, the mixture was extracted with methylene chloride (80 ml), dried over sodium sulfate, concentrated in vacuo, and purified by p-TLC (Merck Art. 5744, Kieselgel F_{254}). The requisite methylated compound **8a** (3 mg, 21% yield, 27% conversion yield) was obtained as colorless crystals accompanying the recovery of the starting material **9** (3 mg, 23% yield). The compound **8a** thus obtained was identical with the one derived from the syn-aldol adduct **7a** by spectral comparisons.

(1*R*,2*S*,8*S*)-Hexahydro-1-hydroxy-2-methyl-3H-pyrrolizin-3-one (8b). The aldol adduct **7b** (215 mg, 0.5 mmol) was deprotected with trifluoroacetic acid (10 ml). The resulting salt was cyclized under basic conditions (potassium carbonate (99 mg, 0.72 mmol) in ethanol-water (2:1, 9 ml)) according to the same procedure as that for **8a**. Accompanying the chiral oxazolidinone (91 mg, quantitative yield), the bicyclic

compound **8b** (62 mg, 80% yield) was obtained as slightly reddish crystals, which were recrystallized from ethyl acetate-hexane to give colorless crystals: mp 122°C lit.[1b] 121-122°C (acetone-cyclohexane); $[\alpha]_D^{23.5}$ -97.9° (*c* 0.51, $CHCl_3$) lit.[1b] $[\alpha]_D^{30}$ -115° (*c* 1.85, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3250, 1670, 1650, 1080; ^{1}H-NMR δ 1.27 (3H, d, J=7.5Hz), 1.50 (1H, dq, J=12.3Hz, 8.8Hz), 1.96-2.12 (2H, m), 2.14-2.21 (1H, m), 2.71 (quintet, J=7.5Hz), 2.71-2.8 (br, disappeared with D_2O), 2.71-2.8 (2H), 3.05 (1H, ddd, J=11.5Hz, 8.2Hz, 4.6Hz), 3.56 (1H, dt, J=11.5Hz, 7.7Hz), 3.76 (1H, dt, J=8.8Hz, 6.4Hz), 4.19 (1H, brq, changed to t with D_2O).

(2*R*,3*R*,2'*S*)-3-(N-*tert*-Butoxycarbonyl-2'-pyrrolidinyl)-3-methoxy-2-methylpropanoic acid (12). To a solution of the β-hydroxycarboxylic acid **11a** (137 mg, 0.50 mmol) and methyl iodide (0.48 ml, 7.6 mmol) in tetrahydrofuran (2.5 ml) was added sodium hydride (60% oil suspension, 81 mg, 2.0 mmol). After the mixture was stirred at 0°C for 18 h, saturated aqueous sodium hydrogen carbonate (20 ml) was added. The mixture was extracted with ether (30 ml) to remove the impurity. The aqueous layer was acidified to pH 3 by the addition of 1 M aqueous potassium hydrogen sulfate. After being salted out, the mixture was extracted with ethyl acetate (20 ml x 3). The combined organic extracts were washed with 5% aqueous sodium thiosulfate (saturated with sodium chloride, 10 ml) and saturated aqueous sodium chloride (10 ml), dried over sodium sulfate, and concentrated in vacuo to give the methyl ether **12** (128 mg, 89% yield) as a colorless oil: $[\alpha]_D^{24}$ -57.6° (*c* 2.1, MeOH) lit.[1b] $[\alpha]_D^{30}$ -40° (*c* 3.0, MeOH); IR (neat) ν_{max} cm^{-1}: 1694, 1651, 1100, 1006; ^{1}H-NMR δ 1.28 (3H, d, J=6.9Hz), 1.47 (9H, s), 1.74-1.94 (4H, m), 2.52 (1H, m), 3.19-3.25 (1H, m), 3.45 (3H, s), 3.81-3.96 (2H, m), 6.0-7.0 (0.5H, br, disappeared with D_2O); MS m/z: 287 (M^+).

Ethyl (4*S*,5*S*)-4-(N-*tert*-butoxycarbonylamino)-5-methyl-3-oxoheptanoate (14). To an ice-cooled solution of Boc-(*S*)-Ile-OH (**13**) (4.63 g, 20 mmol) in tetrahydrofuran (25 ml) was added N,N'-carbonyldiimidazole (3.56 g, 22 mmol). After evolution of gas, the resulting mixture was stirred at room temperature for 3.5 h. A 2.2 M solution of isopropylmagnesium bromide in ether (36.5 ml, 80 mmol) was added dropwise to a precooled (-10°C) solution of ethyl hydrogen malonate (5.29 g, 40 mmol) at such a rate to keep the internal temperature below 5°C. The mixture was stirred at room temperature for 1.5 h. This solution of the magnesium enolate was cooled in an ice bath, followed by the gradual addition of the imidazolide solution over a 1-h period via a double-ended needle at 5°C. The resulting mixture was stirred at 3°C for 30 min then at room temperature for 64 h. The reaction mixture was quenched by the addition of 10% aqueous citric acid (5 ml), and acidified to pH 3 with an additional 10% aqueous citric acid (110 ml). The solution was extracted with ethyl acetate-benzene (4:1) (150 ml x 3). The organic extracts were washed with water (50 ml), saturated aqueous sodium hydrogen carbonate (50 ml x 2), and saturated aqueous sodium chloride (50 ml), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography on silica gel using ethyl acetate-hexane (1:4) as an eluent to give the β-ketoester **14** (5.91 g, 98% yield) as a colorless oil: IR (neat) ν_{max} cm^{-1}: 3350, 1740, 1700, 1640 (shoulder), 1170, 1030; ^{1}H-NMR δ 0.8-1.0 (m), 1.03 (t, J=7.0Hz), 1.27 (t, J=7.0Hz), 0.8-1.27 (9H), 1.43 (9H, s), 1.5-2.2 (3H, m), 3.50 (2H, s), 4.17 (q, J=7.0Hz), 4.0-4.3 (m), 4.17-4.3 (3H), 4.7-5.2 (1H, m).

Ethyl (3*R*,4*S*,5*S*)-4-(N-*tert*-butoxycarbonylamino)-3-hydroxy-5-methylheptanoate (16a) and its (3*S*)-epimer (16b). (1) To a solution of the β-ketoester **14** (119 mg, 0.4 mmol) in ethanol (6 ml) at -64°C was added sodium borohydride (76 mg, 2.0 mmol) in one portion. The reaction mixture was stirred for 5.5 h below -53°C, then quenched with 10% aqueous citric acid (2 ml). The resulting solution was acidified to pH 2 with an additional 10% aqueous citric acid (8 ml), followed by extraction with ethyl acetate (20 ml x 3). The organic extracts were washed with saturated aqueous sodium chloride (10 ml x 2), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography on silica gel using ethyl acetate-hexane (1:6 - 1:1) as an eluent to give the alcohols, **16a** and **16b** (105 mg, 88% yield). The product ratio (**16a**:**16b**=91:9) was determined by ^{1}H-NMR. **16a**: colorless crystals; Rf 0.31 (Merck Art. 5715, Kieselgel 60 F_{254}, ethyl acetate-hexane, 1:3); $[\alpha]_D^{24}$ +10.3° (*c* 0.98, MeOH); IR (KBr) ν_{max} cm^{-1}: 3550, 3280, 3050, 1720, 1695, 1670, 1540, 1175, 1065; ^{1}H-NMR δ 0.92 (t, J=7.1Hz), 0.94 (d, J=7.0Hz), 0.94-1.05 (m), 0.92-1.05 (7H), 1.28 (3H, t, J=7.1Hz), 1.44 (9H, s), 1.53-1.61 (1H, m), 1.80 (1H, m), 2.45 (1H, dd, J=16.5Hz, 9.0Hz), 2.57 (1H, dd, J=16.3Hz, 2.6Hz), 3.30 (1H, br, disappeared with D_2O), 3.54-3.59 (1H,

m), 4.00 (1H, brt, J=6.9Hz), 4.18 (2H, dq, J=7.1Hz, 1.5Hz), 4.41 (1H, d, J=9.7Hz); Anal. Calcd for $C_{15}H_{29}NO_5$: C, 59.38; H, 9.63; N, 4.62. Found: C, 59.12; H, 9.80; N, 4.46. **16b**: colorless viscous oil; Rf 0.33 (Merck Art. 5715, Kieselgel 60 F_{254}, ethyl acetate-hexane, 1:3); $[\alpha]_D^{24}$ -38.7° (*c* 1.0, MeOH); IR (neat) ν_{max} cm^{-1}: 3350, 1730, 1690, 1500, 1165, 1020; ^{1}H-NMR δ 0.89 (3H, t, J=7.4Hz), 0.96 (3H, d, J=6.8Hz), 1.11-1.20 (1H, m), 1.28 (3H, t, J=7.1Hz), 1.44 (9H, s), 1.52-1.67 (2H, m), 2.44 (1H, dd, J=16.9Hz, 2.8Hz), 2.55 (1H, dd, J=16.9Hz, 10.1Hz), 3.22 (t), 3.26 (br, disappeared with D_2O), 3.22-3.26 (2H), 4.17 (2H, q, J=7.1Hz), 4.26 (1H, d, J=9.9Hz), 4.84 (1H, d, J=10.1Hz); Anal. Calcd for $C_{15}H_{29}NO_5$: C, 59.38; H, 9.63; N, 4.62. Found: C, 59.19; H, 9.90; N, 4.43.

(2) To a precooled (-78°C) solution of LDA (prepared from diisopropylamine (0.21 ml, 1.5 mmol), 1.6 M solution of *n*-butyllithium in hexane (0.94 ml, 1.5 mmol), and tetrahydrofuran (1.5 ml)) was added ethyl acetate (0.15 ml, 1.5 mmol). The mixture was stirred at -78°C for 30 min. The aldehyde **15** (217 mg, 1.0 mmol) in tetrahydrofuran (1 ml) was added via a double-ended needle. The resulting mixture was stirred at -73°C for 10 min then quenched with 1 N hydrochloric acid (1.5 ml). After being adjusted to pH 2 with an additional 1 N hydrochloric acid, the reaction mixture was extracted with ethyl acetate (20 ml x 3). The organic extracts were washed with saturated aqueous sodium chloride (20 ml), dried over sodium sulfate, and concentrated in vacuo to give the alcohols, **16a** and **16b** (306 mg, quantitative yield). The product ratio (**16a**:**16b**=38:62) was determined by ^{1}H-NMR.

(3*R*,4*S*,5*S*)-4-(N-*tert*-Butoxycarbonylamino)-3-hydroxy-5-methylheptanoic acid (17). To an ice-cooled solution of the β-hydroxyester **16a** (1.22 g, 4.0 mmol) in ethanol (12 ml) was added 1 N aqueous sodium hydroxide (4.2 ml, 4.2 mmol). The mixture was stirred at 0°C for 30 min then at room temperature for 2 h. The resulting solution was acidified to pH 4 by the addition of 1 N aqueous hydrochloric acid (4.2 ml and a few drops), which was extracted with ethyl acetate-benzene (1:1) (40 ml x 3). The organic extracts were washed with 1 M aqueous potassium hydrogen sulfate (30 ml), and saturated aqueous sodium chloride (30 ml), dried over sodium sulfate, and concentrated in vacuo to give the β-hydroxy acid **17** (1.14 g, quantitative yield) as a colorless solid, which was recrystallized from ether-hexane: mp 101-105°C; $[\alpha]_D^{24}$ +10.3° (*c* 0.99, MeOH); IR (KBr) ν_{max} cm^{-1}: 3400, 3350, 1710, 1685, 1650, 1520, 1170, 1060; ^{1}H-NMR δ 0.88-2.0 (9H, m), 1.43 (9H, s), 2.43-2.67 (2H, m), 3.3-3.83 (1H, m), 3.83-4.3 (1H, m), 4.4-4.8 (1H, brd), 6.1-6.8 (2H, m, disappeared with D_2O); Anal. Calcd for $C_{13}H_{25}NO_5$: C, 56.71; H, 9.15; N, 5.09. Found: C, 56.61; H, 9.46; N, 4.98.

(3*R*,4*S*,5*S*)-4-(N-*tert*-Butoxycarbonyl-N-methylamino)-3-methoxy-5-methylheptanoic acid (18). To a solution of the β-hydroxycarboxylic acid **17** (55 mg, 0.2 mmol) in tetrahydrofuran (2 ml) was added sodium hydride (60% oil suspension, 56 mg, 1.4 mmol). The mixture was stirred at 0°C for 1h. Methyl iodide (0.19 ml, 3.0 mmol) was added to this mixture, which was stirred at 0°C for 40 h. After the same work-up as that for **12**, the N,O-dimethylated compound **18** (36 mg, 59% yield) was obtained as a yellowish oil: $[\alpha]_D^{23}$ -10.5° (*c* 0.97, MeOH); IR (neat) ν_{max} cm^{-1}: 3500, 3200-2900(br), 2971, 2933, 1736, 1693, 1153, 1101; ^{1}H-NMR δ 0.89 (t, J=7.3Hz), 0.90 (t, J=7.4Hz), 0.89-0.90 (3H), 0.97 (3H, d, J=6.8Hz), 1.06-1.13 (1H, m), 1.45 (s), 1.46 (s), 1.46-1.50 (m), 1.45-1.50 (10H), 1.78 (1H, br), 2.51 (dd, J=15.9Hz, 7.3Hz), 2.62 (dd, J=15.9Hz), 2.51-2.62 (2H), 2.70 (3H, s), 3.409 (s), 3.412 (s), 3.409-3.412 (3H), 3.89 (br), 3.89-4.1 (br), 3.89-4.1 (2H), 4.1-6.0 (1H, br, disappeared with D_2O); Anal. Calcd for $C_{15}H_{29}NO_5$: C, 59.38; H, 9.63; N, 4.62. Found: C, 58.86; H, 9.41; N, 4.47.

Ethyl (4*S*,5*S*)-4-(N-*tert*-butoxycarbonyl-N-methylamino)-5-methyl-3-oxoheptanoate (20). The β-ketoester **20** was prepared by the same procedure as that for **14** using Boc-(*S*)-MeIle-OH (**19**)[12] (243 mg, 1.0 mmol) as the starting material. Thus the ester **20** (273 mg, 87% yield) was obtained as a colorless oil: $[\alpha]_D^{24.5}$ -250° (*c* 1.0, EtOH); IR (neat) ν_{max} cm^{-1}: 1730, 1690, 1650 (shoulder), 1150, 1030; ^{1}H-NMR δ 0.90 (6H, d, J=6.4Hz), 1.0-1.1 (1H, m), 1.27 (t, J=7.1Hz), 1.2-1.5 (m), 1.2-1.5 (5H), 1.48 (9H, s), 2.63 (s), 2.70 (s), 2.63-2.70 (3H), 3.46 (ABq, J=15.6Hz), 3.48 (s), 3.46-3.48 (2H), 4.18 (2H, q, J=7.1Hz), 4.28 (d, J=10.6Hz), 4.59 (d, J=10.8Hz), 4.28-4.59 (1H); MS m/z: 315 (M^+).

Ethyl (3*S*,4*S*,5*S*)-4-(N-*tert*-butoxycarbonyl-N-methylamino)-3-hydroxy-5-methylheptanoate (21). Prepared by the same procedure as that for **16a** and **16b** using the β-ketoester **20** (319 mg, 1.0 mmol) as the

starting material. The alcohol **21** (209 mg, 65% yield) was obtained as a colorless oil: IR (neat) ν_{max} cm^{-1}: 3430, 1730, 1720, 1690, 1670, 1160, 1030; ^{1}H-NMR δ 0.86-0.91 (3H, m), 1.00 (d, J=6.8Hz), 1.01 (d, J=6.6Hz), 1.00-1.01 (3H), 1.27 (t, J=7.1Hz), 1.29 (t, J=7.1Hz), 1.27-1.29 (3H), 1.0-1.12 (m), 1.34-1.45 (m), 1.0-1.45 (2H), 1.45 (s), 1.46 (s), 1.45-1.46 (9H), 2.0-2.2 (1H, m), 2.4-2.5 (2H, m), 2.85 (s), 2.87 (s), 2.85-2.87 (3H), 3.1-3.3 (1H, br, disappeared with D_2O), 3.48 (dd, J=10.5Hz, 7.1Hz), 3.57 (dd, J=6.8Hz, 2.7Hz), 3.48-3.57 (1H), 4.1-4.2 (2H, m), 4.3-4.35 (1H, m).

General procedure for the removal of the Boc function with trifluoroacetic acid. To a solution of the N-Boc amino acid or peptide (1.0 mmol) in methylene chloride (2.5 ml) was added trifluoroacetic acid (0.9 ml). After being stirred at room temperature for 4-17 h, the reaction mixture was concentrated in vacuo. Re-evaporation with benzene gave the deprotected product, which was immediately used for the next step without further purification.

General procedure for the peptide bond formation using the DEPC method. The carboxylic acid (1.0 mmol) and amine (1.0 mmol) components were dissolved in DMF (3 ml) and cooled to 0°C. DEPC (1.05 mmol) was added to this solution, followed by the addition of base (tertiary amine) (1.0 mmol). After being stirred at 0°C for 2 h and at room temperature for 4-24 h, the reaction mixture was diluted with ethyl acetate-benzene (2:1), washed with 1 M aqueous potassium hydrogen sulfate, water, saturated aqueous sodium hydrogen carbonate, and saturated aqueous sodium chloride, dried over sodium sulfate, and concentrated in vacuo to give the crude product.

Boc-(2*R*,3*R*,4*S*)-Dap-(*S*)-Doe. The dipeptide was prepared by the DEPC method as described above using Boc-(2*R*,3*R*,4*S*)-Dap (**12**) (127 mg, 0.44 mmol), (*S*)-Doe obtained above (129 mg, 0.63 mmol), DEPC (86 μl, 0.57 mmol), triethylamine (80 μl, 0.57 mmol), and DMF (1.5 ml). After the usual work-up, chromatographic purification on silica gel using ethyl acetate-hexane (1:1 - 2:1) as an eluent gave the dipeptide (204 mg, 98% yield) as a colorless solid, which was recrystallized from ethyl acetate-hexane: mp 131-132°C; $[\alpha]_D^{24}$ -76.5° (*c* 0.96, MeOH); IR (KBr) ν_{max} cm^{-1}: 3580, 3500, 3300, 1660, 1530, 1500, 1170, 1120; ^{1}H-NMR δ 1.16 (3H, s), 1.48 (9H, s), 1.59-1.63 (2H, m), 1.71-1.76 (2H, m), 2.28-2.40 (1H, m), 3.16-3.27 (2H, m), 3.36 (5H, s), 3.52 (1H, d, J=5.1Hz), 3.71-3.78 (1H, m), 5.62 (1H, m), 7.04 (1H, m), 7.14-7.28 (6H, m), 7.74 (1H, d, J=3.1Hz); FABMS (glycerin) m/z: 474 (M+1).

Boc-(3*R*,4*S*,5*S*)-Dil-(2*R*,3*R*,4*S*)-Dap-(*S*)-Doe. The tripeptide was prepared by the DEPC method as described above using **18** (120 mg, 0.40 mmol), the deprotected product from Boc-Dap-Doe (169 mg, 0.36 mmol), DEPC (66 μl, 0.43 mmol), triethylamine (60 μl, 0.43 mmol), and DMF (1.5 ml). The usual work-up gave the crude tripeptide (232 mg, 99% yield) as a colorless viscous oil, which solidified on standing: mp 58°C; $[\alpha]_D^{23}$ -71.0° (*c* 0.12, MeOH); IR (neat) ν_{max} cm^{-1}: 3480, 3290, 1690, 1680, 1660, 1610, 1540, 1150, 1100; ^{1}H-NMR δ 0.90 (3H, t, J=7.3Hz), 0.97 (3H, d, J=6.8Hz), 1.03-1.14 (1H, m), 1.14 (3H, d, J=7.2Hz), 1.45 (s), 1.46 (s), 1.45-1.46 (9H), 1.46-1.56 (1H, m), 1.62-1.83 (4H, m), 1.91-1.97 (1H, m), 2.32-2.49 (3H, m), 2.67 (s), 2.69 (s), 2.67-2.69 (3H), 3.24-3.38 (m), 3.335 (s), 3.339 (s), 3.37 (s), 3.38 (s), 3.24-3.38 (10H), 3.90 (1H, m), 4.10-4.15 (3H, m), 5.66 (1H, q), 7.19-7.27 (7H, m), 7.73 (1H, d, J=3.3Hz); FABMS (glycerin) m/z: 659 (M+1).

Boc-(*S*)-Val-(3*R*,4*S*,5*S*)-Dil-(2*R*,3*R*,4*S*)-Dap-(*S*)-Doe. (1) Boc-(*S*)-Val-OH (84 mg, 0.39 mmol) and the deprotected product from Boc-Dil-Dap-Doe (127 mg, 0.19 mmol) with trifluoroacetic acid were dissolved in methylene chloride (0.5 ml) and cooled to 0°C. BopCl (59 mg, 0.22 mmol) and triethylamine (85 μl, 0.61 mmol) were added to this solution. The mixture was stirred at 0°C for 39 h. The resulting mixture was diluted with benzene-ethyl acetate (1:3) (80 ml), washed with 1 M aqueous potassium hydrogen sulfate (20 ml), saturated aqueous sodium hydrogen carbonate (20 ml), and saturated aqueous sodium chloride (20 ml), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography on silica gel using ethyl acetate-hexane (2:1) as an eluent to give the title compound (86 mg, 59% yield, 97% conversion yield) as a colorless solid, which was recrystallized from ethyl acetate-hexane: $[\alpha]_D^{23.5}$ -64.6° (*c* 0.50, MeOH); IR (KBr) ν_{max} cm^{-1}: 3400, 3275, 1700, 1660 (shoulder), 1640, 1620, 1540 (shoulder), 1520, 1170, 1100; ^{1}H-NMR δ 0.88 (t, J=6.9Hz), 0.93 (d, J=6.6Hz), 0.98 (d, J=6.8Hz), 0.99 (d, J=6.6Hz), 0.95-1.06 (m), 0.88-1.06 (13H), 1.14 (3H, d, J=7.0Hz), 1.27-1.32 (1H, m), 1.43 (9H, s), 1.60-1.85 (4H, m), 1.86-2.05

(2H, m), 2.31-2.46 (3H, m), 2.75 (s), 3.00 (s), 3.14 (s), 2.75-3.14 (3H), 3.26-3.46 (m), 3.326 (s), 3.333 (s), 3.26-3.46 (11H), 3.89 (1H, dd, J=7.3Hz, 2.2Hz), 4.09-4.16 (2H, m), 4.39 (1H, dd, J=9.4Hz, 6.5Hz), 5.20 (1H, d, J=9.7Hz), 5.56 (1H, m), 7.10-7.27 (m), 7.20 (d, J=3.1Hz), 7.10-7.27 (7H), 7.73 (1H, d, J=3.1Hz); FABMS (glycerin) m/z: 758 (M+1).

(2) To a solution of the deprotected tripeptide (prepared from Boc-Dil-Dap-Doe (21 mg, 32 μmol), trifluoroacetic acid (50 μl), and methylene chloride (0.15 ml)), Boc-(*S*)-Val-OH (14 mg, 64 μmol), and BroP (19 mg, 50 μmol) in methylene chloride (0.2 ml) was added diisopropylethylamine (20 μl, 110 μmol). The mixture was shielded from light and stirred at 0°C for 10 min then at room temperature for 47 h. The resulting mixture was diluted with benzene-ethyl acetate (1:3) (80 ml), washed with 1 M aqueous potassium hydrogen sulfate (10 ml), water (10 ml), saturated aqueous sodium hydrogen carbonate (10 ml), and saturated aqueous sodium chloride (10 ml), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by p-TLC (Merck Art. 5744, Kieselgel 60 F_{254}) to give the title compound (22 mg, 91% yield).

(*S*)-Dov-(*S*)-Val-(3*R*,4*S*,5*S*)-Dil-(2*R*,3*R*,4*S*)-Dap-(*S*)-Doe, dolastatin 10 (1). Dolastatin 10 (**1**) was constructed by the DEPC method as described above using Me_2-(*S*)-Val-OH, (*S*)-Dov (35 mg, 0.24 mmol), the deprotected product from Boc-Val-Dil-Dap-Doe (30 mg, 0.04 mmol) with trifluoroacetic acid, DEPC (8 μl, 0.05 mmol), N-methylmorpholine (26 μl, 0.24 mmol), and DMF (0.2 ml). After the usual work-up, purification by column chromatography on silica gel using ethanol-ethyl acetate-hexane (1:5:4) as an eluent gave dolastatin 10 (**1**) (28 mg, 90% yield) as a colorless solid, which was recrystallized from acetone-hexane: Rf 0.45 (Merck Art. 5715, Kieselgel 60 F_{254}, CH_2Cl_2-MeOH-H_2O-NH_4OH, 90:10:0.8:0.2) lit.[1a] Rf 0.43 (CH_2Cl_2-MeOH-H_2O-NH_4OH, 90:10:0.8:0.2); mp 104-107°C lit.[1b] mp 102-106°C (acetone-hexane); $[\alpha]_D^{23}$ -59.8° (*c* 0.035, MeOH), $[\alpha]_D^{24}$ -78.4° (*c* 0.88, MeOH) lit.[1b] $[\alpha]_D^{27}$ -57° (*c* 0.026, MeOH); IR (KBr) ν_{max} cm^{-1}: 3466, 1654, 1618, 1560, 1545, 1528, 1499, 1099; ^{1}H-NMR (CD_2Cl_2/$CHDCl_2$) δ 0.82 (3H, t, J=7.3Hz), 0.90 (d, J=6.6Hz), 0.94 (d, J=7.0Hz), 0.96 (d, J=7.0Hz), 0.98 (d, J=6.8Hz), 0.95-1.05 (m), 1.00 (d, J=6.8Hz), 0.90-1.05 (16H), 1.08 (3H, d, J=7.0Hz), 1.35-1.40 (1H, m), 1.54-1.64 (2H, m), 1.64-1.75 (m), 1.76-1.84 (m), 1.64-1.84 (3H), 1.91-2.01 (m), 2.03-2.13 (m), 1.91-2.13 (2H), 2.23 (s), 2.24 (s), 2.23-2.24 (6H), 2.29 (1H, m), 2.39 (d, J=6.8Hz), 2.40 (m), 2.39-2.40 (3H), 3.01 (3H, s), 3.24 (1H, dd, J=13.4Hz, 9.2Hz), 3.30 (s), 3.31 (s), 3.30-3.31 (6H), 3.41 (dd, J=13.4Hz, 6.1Hz), 3.37-3.45 (m), 3.41-3.45 (4H), 3.84 (1H, dd, J=8.2Hz, 1.7Hz), 3.98 (1H, m), 4.12 (1H, br), 4.76 (1H, dd, J=9.1Hz, 6.3Hz), 5.52 (1H, m), 6.80 (1H, d, J=9.0Hz), 7.19-7.26 (m), 7.25 (d, J=3.1Hz), 7.19-7.26 (7H), 7.72 (1H, d, J=3.3Hz); FABMS (glycerin) m/z: 785 (M+1); ^{13}C-NMR (CD_2Cl_2/$CHDCl_2$) δ 10.86, 14.45, 16.01, 18.01, 18.06, 19.77, 20.22, 24.95, 25.38, 26.17, 28.00, 30.62, 31.34, 33.49, 37.97, 41.40, 42.92 x 2, 44.72, 47.98, 52.95, 54.00, 54.27, 58.13, 59.78, 60.88, 76.90, 78.77, 81.95, 119.16, 127.03, 128.70 x 2, 129.76 x 2, 137.68, 142.74, 170.47, 171.56, 172.41, 173.79, 174.00.

Boc-(2*R*,3*R*,4*S*)-norDap-(*S*)-Doe. Obtained in quantitative yield as colorless crystals: mp 132°C (ether-hexane); $[\alpha]_D^{25}$ -78.9° (*c* 1.0, MeOH); IR (KBr) ν_{max} cm^{-1}: 3478, 3305, 3069, 1692, 1665, 1532, 1501, 1406, 1163, 1111; ^{1}H-NMR δ 1.14 (3H, brs), 1.47 (9H, s), 1.74-1.88 (4H, m), 2.41 (1H, brs), 3.20-3.26 (br), 3.23 (dd, J=13.8Hz, 7.8Hz), 3.36 (dd, J=13.7Hz, 6.4Hz), 3.45 (br), 3.20-3.45 (4H), 3.83 (1H, d, changed to t with D_2O), 4.11 (1H, quintet, J=7.1Hz), 4.74 (0.5H, br, disappeared with D_2O), 5.61 (1H, brd, J=6.2Hz), 7.12 (2H, d, J=6.4Hz), 7.13-7.27 (5H, m), 7.74 (1H, d, J=3.3Hz); Anal. Calcd for $C_{24}H_{33}N_3O_4S$: C, 62.72; H, 7.24; N, 9.14. Found: C, 62.37; H, 7.29; N, 8.72.

Boc-(3*R*,4*S*,5*S*)-bisnorDil-(2*R*,3*R*,4*S*)-norDap-(*S*)-Doe. Obtained in 98% yield as a colorless solid: IR (KBr) ν_{max} cm^{-1}: 3300 (br), 1690, 1650, 1610, 1530, 1250, 1170; ^{1}H-NMR δ 0.93 (d, J=6.8Hz), 0.95 (t, J=6.8Hz), 0.92-0.97 (m), 0.92-0.97 (7H), 1.20 (3H, d, J=7.0Hz), 1.42 (9H, s), 1.55-1.58 (1H, m), 1.69 (2H, m), 1.89-1.95 (2H, m), 2-3 (2H, br, disappeared with D_2O), 2.12 (1H, brm), 2.35-2.42 (1H, quintet), 2.51 (brdd), 2.54 (brdd), 2.51-2.54 (2H), 3.21-3.28 (m), 3.24 (dd, J=13.8Hz, 8.3Hz), 3.21-3.28 (2H), 3.34-3.41 (m), 3.41 (dd, J=13.8Hz, 6.0Hz), 3.34-3.41 (2H), 3.68 (2H, brs), 4.06-4.13 (1H, m), 4.25-4.29 (1H, m), 4.43 (1H, m, disappeared with D_2O), 5.63 (1H, dt, J=8.2Hz, 6.2Hz), 7.14 (d, J=8.2Hz, disappeared with D_2O), 7.17-7.25 (m), 7.14-7.25 (7H), 7.73 (1H, d, J=3.3Hz).

Boc-(*S*)-Val-(3*R*,4*S*,5*S*)-bisnorDil-(2*R*,3*R*,4*S*)-norDap-(*S*)-Doe. Obtained in 66% yield as a colorless

solid: $[\alpha]_D^{25}$ -58.5° (*c* 0.79, MeOH); IR (KBr) ν_{max} cm^{-1}: 3430, 3300, 1690, 1650, 1620, 1560, 1540, 1530, 1510, 1500, 1170, 1040; ^{1}H-NMR δ 0.85 (3H, d, J=7.0Hz), 0.95 (d, J=6.6Hz), 0.98 (d, J=6.8Hz), 0.94-0.99 (m), 0.94-0.99 (10H), 1.21 (3H, d, J=7.0Hz), 1.31-1.43 (1H, m), 1.47 (9H, s), 1.54-1.63 (1H, m), 1.66-1.80 (2H, m), 1.83-2.00 (2H, m), 2.16 (1H, br), 2.22-2.31 (1H, m), 2.53 (1H, brd), 2.59 (1H, m), 3.19 (1H, dd, J=13.7Hz, 8.8Hz), 3.25-3.36 (m), 3.41 (dd, J=13.7Hz, 6.4Hz), 3.25-3.41 (3H), 3.61 (1H, brdt), 3.76 (brt, J=5.4Hz), 3.79 (t, J=7.2Hz), 3.76-3.79 (2H), 4.07-4.22 (2H, m), 4.25-5.25 (1H, disappeared with D_2O), 5.45 (1H, d, J=7.5Hz), 5.66 (1H, dt, J=8.7Hz, 6.4Hz), 6.06 (1H, d, J=10.3Hz), 7.19-7.26 (6H, m), 7.81 (1H, d, J=3.1Hz), 7.96 (1H, d, J=8.4Hz); FABMS (glycerin) m/z: 716 (M+1).

(*S*)-Dov-(*S*)-Val-(3*R*,4*S*,5*S*)-bisnorDil-(2*R*,3*R*,4*S*)-norDap-(*S*)-Doe, trisnordolastatin 10 (2). Obtained in 43% yield as a colorless solid: mp 98-100°C; $[\alpha]_D^{24}$ -52.9° (*c* 0.29, MeOH); IR (KBr) ν_{max} cm^{-1}: 3430, 3320, 1654, 1647, 1560, 1542, 1508, 1499, 1035; ^{1}H-NMR δ 0.87 (d, J=7.0Hz), 0.90 (d, J=6.8Hz), 0.92 (t), 0.96 (d, J=6.8Hz), 0.99 (d, J=6.8Hz), 1.01 (d, J=7.0Hz), 0.87-1.01 (m), 0.87-1.01 (19H), 1.21 (3H, d, J=7.0Hz), 1.21-1.36 (1H, m), 1.52-1.77 (3H, m), 1.80-1.89 (1H, m), 1.91-1.98 (1H, m), 2.08-2.17 (2H, m), 2.19-2.31 (m), 2.26 (s), 2.19-2.31 (7H), 2.46-2.54 (3H, m), 3.20 (1H, dd, J=13.8Hz, 8.9Hz), 3.25-3.31 (1H, m), 3.34-3.40 (1H, m), 3.46 (1H, dd, J=13.9Hz, 6.2Hz), 3.65 (1H, br, changed to t with D_2O), 3.99 (1H, brt, changed to dd (J=6.2Hz, 4.2Hz) with D_2O), 4.07-4.14 (3H, m), 4.53 (1H, br, disappeared with D_2O), 5.30 (1H, br, disappeared with D_2O), 5.64 (1H, dt, J=8.5Hz, 6.2Hz), 6.11 (1H, d, J=9.7Hz), 6.79 (1H, d, J=7.5Hz, disappeared with D_2O), 7.18-7.27 (6H, m), 7.60 (1H, d, J=8.4Hz), 7.75 (1H, d, J=3.3Hz); FABMS (glycerin) m/z: 744 (M+1).

Boc-(2*S*,3*R*,4*S*)-norDap-(*S*)-Doe. Obtained in 96% yield as a colorless solid: mp 131-132°C; $[\alpha]_D^{22.5}$ -81.6° (*c* 0.87, MeOH); IR (KBr) ν_{max} cm^{-1}: 3410, 3300, 3100, 3030, 1690 (shoulder), 1650, 1550, 1500, 1170, 1110; ^{1}H-NMR δ 1.16 (3H, brd, J=6.0Hz), 1.46 (9H, s), 1.73-1.75 (2H, m), 1.88-1.90 (1H, m), 2.18 (1H, m), 2.55 (1H, br), 3.23 (dd, J=13.9Hz, 8.6Hz), 3.28 (dd, J=8.1Hz, 3.7Hz), 3.23-3.28 (2H), 3.37 (3H, br), 3.69 (1H, br, disappeared with D_2O), 3.93 (1H, m), 5.57 (1H, m), 7.14-7.28 (6H, m), 7.71 (1H, d, J=2.9Hz), 8.41 (1H, d); FABMS (glycerin) m/z: 460 (M+1).

Boc-(3*R*,4*S*,5*S*)-bisnorDil-(2*S*,3*R*,4*S*)-norDap-(*S*)-Doe. Obtained in 85% yield as colorless crystals: mp 131°C (ethyl acetate-hexane); $[\alpha]_D^{24.5}$ -59.6° (*c* 1.0, MeOH); IR (KBr) ν_{max} cm^{-1}: 3430, 3300, 1690, 1645, 1620, 1525; ^{1}H-NMR δ 0.87-0.95 (m), 0.93 (d, J=7.1Hz), 0.95 (t, J=6.8Hz), 0.87-0.95 (7H), 1.16 (3H, d, J=7.2Hz), 1.41 (9H, s), 1.53-1.58 (1H, m), 1.72-1.80 (2H, m), 1.95-2.11 (3H, m), 2.42 (1H, quintet, J=6.8Hz), 2.52 (2H, brs), 2.5-3.2 (1.5H, br, disappeared with D_2O), 3.24 (1H, dd, J=13.8Hz, 8.2Hz), 3.39 (1H, dd, J=13.7Hz, 6.0Hz), 3.30-3.40 (2H, m), 3.68 (2H, d, J=3.7Hz, changed to s with D_2O), 4.05 (1H, t), 4.17-4.21 (1H, m), 4.42-4.45 (1H, m), 5.63 (1H, dt, J=8.1Hz, 6.1Hz), 7.15-7.27 (6H, m), 7.64 (1H, d, J=8.2Hz), 7.71 (1H, d, J=3.1Hz); Anal. Calcd for $C_{32}H_{48}N_4O_6S \cdot H_2O$: C, 60.54; H, 7.78; N, 8.82. Found: C, 60.57; H, 7.62; N, 8.71.

Boc-(S)-Val-(3*R*,4*S*,5*S*)-bisnorDil-(2*S*,3*R*,4*S*)-norDap-(*S*)-Doe. Obtained in 66% yield as a colorless oil: $[\alpha]_D^{25}$ -56.9° (*c* 0.39, MeOH); IR (neat) ν_{max} cm^{-1}: 3300, 3075, 1690, 1680, 1640, 1620, 1550, 1520, 1500; ^{1}H-NMR δ 0.88 (d, J=6.8Hz), 0.92 (d, J=7.0Hz), 0.98 (d, J=6.8Hz), 0.98 (t), 0.88-0.98 (m), 0.88-0.98 (13H), 1.18 (3H, d, J=7.2Hz), 1.45 (9H, s), 1.56-1.64 (1H, m), 1.69-1.78 (2H, m), 1.88-2.06 (2H, m), 2.11-2.25 (2H, m), 2.38 (1H, dq, J=7.0Hz, 6.0Hz), 2.50 (2H, d, J=3.9Hz), 3.24 (dd, J=13.8Hz, 8.0Hz), 3.39 (dd, J=13.8Hz, 6.0Hz), 3.29-3.39 (m), 3.24-3.39 (4H), 3.69 (1H, br), 3.80 (1H, dd, J=7.3Hz, 6.1Hz), 4.04-4.18 (3H, m), 4.86 (2H, d, J=7.3Hz and br (disappeared with D_2O), 1H disappeared with D_2O), 5.09 (1H, br, disappeared with D_2O), 5.63 (1H, dt, J=8.1Hz, 6.0Hz), 6.07 (1H, d, J=10.3Hz), 7.16-7.27 (6H, m), 7.66 (1H, d, J=8.1Hz), 7.72 (1H, d, J=3.1Hz); FABMS (glycerin) m/z: 716 (M+1).

(*S*)-Dov-(*S*)-Val-(3*R*,4*S*,5*S*)-bisnorDil-(2*S*,3*R*,4*S*)-norDap-(*S*)-Doe, 9-epi-trisnordolastatin 10 (3). Obtained in 96% yield as a colorless solid: mp 105°C (decomp.); $[\alpha]_D^{23}$ -79.0° (*c* 0.096, MeOH); IR (neat) ν max cm^{-1}: 3300, 3060, 1640, 1620 (shoulder), 1540, 1150, 1060, 1040; ^{1}H-NMR δ 0.87 (d, J=7.0Hz), 0.91-0.94 (m), 0.92 (d, J=6.8Hz), 0.97 (d, J=6.8Hz), 0.99 (d, J=6.8Hz), 1.02 (d, J=6.8Hz), 0.87-1.02 (18H), 1.17 (3H, d, J=7.2Hz), 1.60 (2H, br), 1.69-1.80 (2H, m), 1.93-1.96 (1H, m), 2.03-2.14 (3H, m), 2.16-2.25 (1H, m), 2.25 (6H, s), 2.36-2.42 (m), 2.46-2.55 (m), 2.36-2.55 (4H), 3.24 (1H, dd, J=13.8Hz, 8.2H), 3.32-3.42 (m),

3.39 (dd, J=14.1Hz, 6.2Hz), 3.32-3.42 (3H), 3.71-3.73 (1H, m), 3.98 (1H, brd, changed to t (J=5.2Hz) with D_2O), 4.08 (1H, m, changed to d (J=7.1Hz) with D_2O), 4.15-4.18 (1H, m), 4.71 (1H, br, disappeared with D_2O), 4.91 (1H, d, J=8.2Hz), 5.62 (1H, dt, J=8.0Hz, 6.0Hz), 6.01 (1H, d, J=10.4Hz), 6.78 (1H, d, J=7.7Hz), 7.16-7.26 (6H, m), 7.64 (1H, d, J=8.6Hz), 7.72 (1H, d, J=3.3Hz); FABMS (glycerin) m/z: 744 (M+1).

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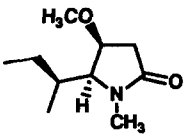

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