# The Dolastatins. 19. Synthesis of Dolaisoleuine[1]

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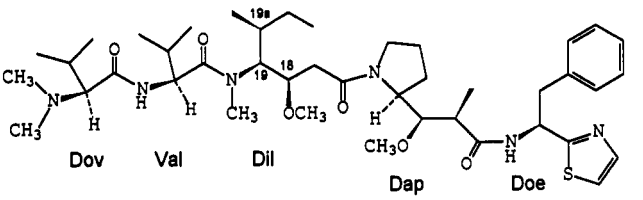
The synthesis of dolaisoleuine as its *tert*-butyl ester (Dil-OBu$^t$), a $\beta$-methoxy-$\gamma$-amino acid component of dolastatin 10, has been achieved employing as key step an aldol condensation between *N*-(benzyloxycarbonyl)-*N*-methyl-(*S*,*S*)-isoleucinal and *tert*-butyl acetate followed by *O*-methylation. The overall six-step reaction sequence to Dil proved to be convenient for routine preparation of this new amino acid and its stereochemical assignment as (3*R*,4*S*,5*S*)-*N*,*O*-dimethylisostatine.

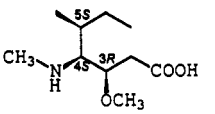
## Introduction

Dolastatin 10 (1)[2a] is one of a series of cytotoxic and antineoplastic peptides isolated from the Indian Ocean sea hare *Dolabella auricularia*.[2] The absolute configuration of this pentapeptide, a potent tubulin inhibitor[3] and powerful antineoplastic substance, has been firmly established by total synthesis.[4,5] Dolastatin 10 (1) is structurally quite unique and contains four unusual amino acids, namely dolavaline (Dov), dolaisoleuine (Dil), dolaproine (Dap), and dolaphenine (Doe), together with valine. In this paper we summarize our first approach to the synthesis of dolaisoleuine (Dil).[2a] Preparation of the other constituent amino acids and their assemblage to yield dolastatin 10 (1) is dealt with in previous reports.[1,4]

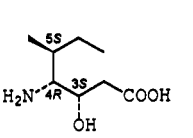
Dil (2), a $\beta$-methoxy-$\gamma$-amino acid, is structurally related to isostatine (3), a component of the didemnins[6] and to the leucine-type amino acid statine (4) found in pepstatin,[7] a lower plant constituent which inhibits proteases such as pepsin, renin, and cathepsin D. Because of its presence as a unit in several biologically active molecules and its use in the development of new inhibitors of aspartic proteinases,[8] statine has been the subject of much attention. A number of methods for stereoselective syntheses of statine and its analogues have been developed.[9–11] The



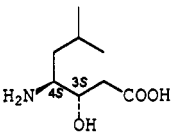

1, Dolastatin 10



2, Dil
[(3*R*,4*S*,5*S*)-*N*,*O*-Dimethylisostatine]



3, (3*S*,4*R*,5*S*)-Isostatine



4, Statine

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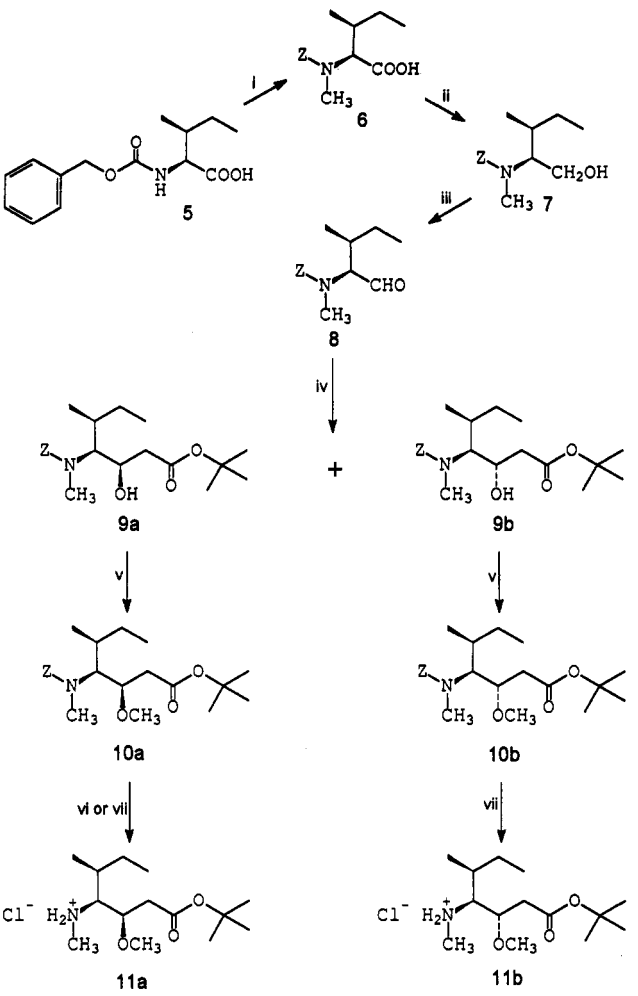
usual synthetic strategies have involved either an asymmetric aldol condensation[9] or formation of a β-keto ester followed by stereoselective reduction.[5b,c,10] The versatile utilization of chiral cyclic intermediates to give enantiomerically pure derivatives has been reported.[11] A stereocontrolled synthesis of *N,O*-dimethyl-γ-amino-β-hydroxy acids using an allyl Grignard reagent has also been described.[10e]

The three chiral centers of Dil, with unknown configuration, presented a difficult synthetic problem. Initial clues to solving part of the stereochemical problem posed by the dolaisoleuine unit arose by comparing the relative chemical shifts of the alkyl side chain methyl signals in several *N*-methylisoleucine derivatives.[12] Analysis of the $^1$H-NMR spectra was revealing. With *N*-(benzyloxycarbonyl)-*N*-methyl-(*S,S*)-isoleucine and its enantiomer, *N*-(benzyloxycarbonyl)-*N*-methyl-(*R,R*)-isoleucine, the methyl triplet at δ 0.85 was found upfield relative to the methyl doublet at δ 0.95. In the spectra of the *allo* series [*N*-(benzyloxycarbonyl)-*N*-methyl-L-*allo*(*S,R*)- and *N*-(benzyloxycarbonyl)-*N*-methyl-D-*allo*(*R,S*)-isoleucine] the methyl triplet appeared at δ 0.97 and the doublet at δ 0.90. The $^1$H NMR spectrum of dolastatin 10 (1) displayed a methyl signal pattern similar to those of the former pair.[2a] Also, the D-*allo*-isoleucine-derived isostatine unit of the didemnins exhibited the latter pattern.[6a] Hence, dolaisoleuine was thought to be derived from either (*S,S*)- or (*R,R*)-isoleucine. On the bases of biosynthetic considerations and our prior structural determination of dolastatin 3,[2b] we assumed that dolaisoleuine contained the (*S,S*) configuration at the 4- and 5-positions. Thus, it was decided to synthesize both the (3*R*,4*S*,5*S*)- and (3*S*,4*S*,5*S*)-isomers. An aldol condensation between an *N*-protected *N*-methyl-(*S,S*)-isoleucinal and a suitable acetate equivalent that would give rise to easily separable isomers suitable for stereochemical analysis was undertaken.

Preparation of *N*-methyl-*N*-(benzyloxycarbonyl)-(*S,S*)-isoleucinal (8) from *N*-(benzyloxycarbonyl)-L-isoleucine (5) was accomplished according to Scheme 1. The *N*-methylation of Z-Ile (5) was performed without any detectable C-2 epimerization[13a,b] and in almost quantitative yield following the Benoiton[13c] procedure. The resulting *N*-methyl derivative (6) was reduced to alcohol 7 (90% yield) using borane–tetrahydrofuran.[14] Oxidation of alcohol 7 to aldehyde 8 was realized in good yield (and without any epimerization) by treatment with dimethyl sulfoxide and sulfur trioxide–pyridine complex.[15] Although aldehyde 8 was quite stable at freezing temperatures, it was unstable at room temperature. Hence, it was used immediately, without purification, in the next synthetic step.

The aldol condensation of aldehyde 8 with *tert*-butyl acetate (other less hindered esters proved troublesome in the subsequent synthesis of Val-Dil) was carried out using lithium diisopropylamide in tetrahydrofuran at −78 °C to

**Scheme 1**




[a] Key: (i) $CH_3I$, NaH, THF; (ii) $BH_3$–THF, THF; (iii) DMSO, $SO_3$–pyr, $Et_3N$; (iv) LDA, t-BuOAc, THF; (v) trimethyloxonium tetrafluoroborate, proton sponge, $ClCH_2CH_2Cl$, 4-Å molecular sieves; (vi) (a) 5% Pd/C, cyclohexene, MeOH; (b) 1 M HCl, 0 °C; (vii) (a) $H_2$, 5% Pd/C, EtOAc, MeOH; (b) 1 M HCl, −60 °C.

give a mixture of two diastereomers (**9a** and **9b**) in an approximate ratio of 4:3. The two compounds were separated easily by silica gel column chromatography. Hydroxy ester **9a** was initially methylated by treatment with boron trifluoride etherate in dichloromethane[16] followed by a large excess of diazomethane. For reasons of safety, especially in large-scale reactions, an alternative method was sought, and we found that use of trimethyloxonium tetrafluoroborate[17] led to methyl ether **10a** in comparable yields. Alcohol **9b** was methylated by the latter procedure to provide methyl ether **10b** in 71% yield.

The $^1$H NMR spectra of the Ile derivatives were of special interest. The spectrum of alcohol 7 ($CDCl_3$ solution) exhibited signals at δ 2.80 and δ 2.81 assigned to the *N*-methyl group. In depth variable temperature $^1$H NMR studies[12] showed this doubling effect was due to the presence of the bulky *N*-(benzyloxycarbonyl) group which gives rise to conformational isomers detectable at room temperature. An analogous phenomenon has been observed with the *tert*-butoxycarbonyl protecting group.[11f] In the $^1$H NMR spectrum of aldehyde 8 the aldehydic and H-α signals were doubled. The spectra of compounds **9a**,

(12) For purposes of comparison, a number of *N*-methylisoleucine derivatives were prepared by us; *cf.* Pettit, G. R.; Williams, M. D.; Srirangam, J. K.; Kantoci, D.; Hogan-Pierson, F.; Benoiton, N. L. *J. Chem. Soc., Perkin Trans. 1*, submitted for publication.

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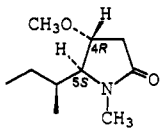
**9b**, **10a**, and **10b** were more complicated with doubling of the side-chain signals. A similar effect has been noted in the spectra of cyclo-L-isoleucyl-D-alloisoleucine.[18]

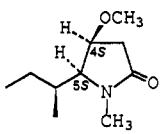
An $^1H$ NMR analysis of methyl ethers **10a** and **10b** revealed very valuable stereochemical information about dolaisoleuine (**2**). The signal for H-4 appears as a broad hump at $\delta$ 4.10 in the $^1H$ NMR spectrum of ester **10a**, very similar to the signal assigned to H-19 ($\delta$ 4.70) of dolastatin 10 (**1**). In contrast, H-4 of the two stable conformers of **10b** gives rise to a doublet of doublets at $\delta$ 3.69 and $\delta$ 3.85 ($J$ = 10.8 and 3.0 Hz). Such a difference in the spectra of the methyl ethers indicated isomer **10a** most likely to be the Dil derivative and that proved correct.

Selective deprotection of isomer **10a** was performed by careful (to avoid extensive lactam formation) hydrogenolysis using 5% palladium on carbon in ethyl acetate–methanol (3:1) followed by treatment with hydrogen chloride in ether to furnish hydrochloride **11a** (63% yield). During the hydrogenolysis lactam **12a** was obtained in 29% yield. Analogous reactions led to isomer **11b** from *N*-Z derivative **10b** (93% yield) with only a minor yield of lactam **12b**. Not surprisingly, when the reaction time was increased or when the *tert*-butoxy group was replaced by an ethoxy group, the $\gamma$-lactam was a major product. Lactam formation was reduced using a minimum amount of catalyst under anhydrous conditions. Hydrochloride salt **11a** was also derived from methyl ether **10a** by hydrogenolysis using internal hydrogen transfer with 5% palladium on carbon in methanol and cyclohexene (2:1) followed by treatment with ethereal hydrogen chloride; yields were higher, and pyrrolidinone **12a** formation was avoided.

Interestingly, the $^1H$ NMR spectra of the deprotected compounds gave evidence of only one conformer and gave further evidence for the *cis–trans* isomerism in the *N*-Z group at ambient temperature.[12] Lactams **12a** and **12b**




12a




12b

were very useful in establishing the stereochemistry of the aldol products by NMR spectroscopy. The $^1H$ NMR spectrum of isomer **12a** exhibited a doublet of doublets at $\delta$ 3.68 ($J$ = 7.0 and 1.7 Hz) for H-4 and at $\delta$ 3.46 ($J$ = 3.4 and 1.7 Hz) for H-5 whereas that of **12b** displayed a quartet at $\delta$ 4.04 ($J$ = 7.0 Hz) for H-4 and a doublet of doublets at $\delta$ 3.50 ($J$ = 7.0 and 3.6 Hz) for H-5. The spectrum of lactam **12b** also showed NOE enhancements of 6% (H-4 → H-5) and 7% (H-5 → H-4), but no NOE between those ring protons was found in the spectrum of isomer **12a**. The coupling constants and NOE enhancements revealed the *cis* arrangement of H-4 and H-5 in lactam **12b**. Thus, these protons were assigned as *trans* in isomer **12a**, and the absolute configuration of lactam **12a** was assigned 4*R*,5*S*,1′*S*. The configuration of **9a**, **10a**, and **11a** is therefore 3*R*,4*S*,5*S* and that of the **9b** series is 3*S*,4*S*,5*S*.

With Dil derivative **11a** of known stereochemistry in hand we proceeded using the convergent synthesis previously outlined[4] to prepare natural dolastatin 10. In turn, this established the chirality of the Dil unit of dolastatin 10 as 18*R*,19*S*,19a*S*. Experiments directed at developing a very stereoselective synthesis of Dil are currently in progress.

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## Experimental Section

**General Procedures.** Refer to our earlier paper[1] for a summary of the general experimental procedures employed in this study.

***N*-(Benzyloxycarbonyl)-*N*-methyl-(2*S*,3*S*)-Ile (6).** The following procedure is a modification of one described by Benoiton.[13] To a cooled (0 °C) solution of *N*-(benzyloxycarbonyl)-L-isoleucine (**5**, 10g, 37.7 mmol) and methyl iodide (18.8 mL, 302 mmol) in freshly distilled tetrahydrofuran (150 mL) was added sodium hydride (60% dispersion, 5.42 g, 136 mmol). The mixture was stirred under argon at 0 °C for 15 min and at room temperature for 28 h. Ethyl acetate (100 mL) was added to the reaction mixture followed by water (300 mL). The aqueous layer was washed with ether (2 × 200 mL), acidified to pH 2 with concd hydrochloric acid, and extracted with ethyl acetate (1 × 400 mL, 3 × 200 mL). The organic phase was washed with water, and removal of solvent *in vacuo* yielded carboxylic acid **6** as a viscous oil[13c] (10.4 g, 99%). The oil did not require further purification and was used in the next reaction.

***N*-(Benzyloxycarbonyl)-*N*-methyl-(2*S*,3*S*)-isoleucinol (7).** Under an argon atmosphere borane–tetrahydrofuran complex (1.0 M, 70 mL) was slowly added with stirring to a cooled (0 °C) solution of carboxylic acid **6** (10.4 g, 37 mmol) in anhydrous tetrahydrofuran (200 mL). The mixture was stirred for 4 h, and reaction was discontinued by the careful addition of ice (50 g) followed by water (200 mL). The resultant mixture was extracted with ethyl acetate (3 × 300 mL). The organic phase was washed with brine, and solvent was removed *in vacuo* to yield a colorless liquid. Purification was accomplished by chromatography on a column of Sephadex LH-20 (eluant: methanol) to afford alcohol **7** (9 g, 90%): bp 196–198 °C (0.12 mmHg); $R_f$ 0.5 (5 mL of 3:2 acetone–hexane with 1 drop of acetic acid); $[\alpha]^{30}_D$ −11.3° (*c* 5.6, $CHCl_3$), after distillation $[\alpha]^{25}_D$ −8.3° (*c* 0.59, $CH_3OH$); IR (neat) 3425, 2964, 2877, 1695, 1454, 1342 $cm^{-1}$; EIMS *m/z* (relative intensity) 265 ($M^+$), HREIMS *m/z* (exact mass) 265.1675 ($M^+$, 2; calcd for $C_{15}H_{23}NO_3$ 265.1678). Anal. Calcd for $C_{15}H_{23}NO_3$: C, 67.90; H, 8.74. Found: C, 67.92; H, 8.74.

***N*-(Benzyloxycarbonyl)-*N*-methyl-(2*S*,3*S*)-isoleucinal (8).** To a solution of alcohol **7** (2.60 g, 9.88 mmol) in anhydrous dimethyl sulfoxide (10 mL) under nitrogen was added triethylamine (6.87 mL, 49.4 mmol). The mixture was stirred at room temperature for 15 min and cooled to 0 °C, and sulfur trioxide pyridine complex (7.85 g, 49.4 mmol) was added in one portion. The resultant reddish solution was stirred at 0–5 °C for 45 min, and oxidation was stopped by addition of water (50 mL). The aqueous mixture was extracted with diethyl ether (3 × 100 mL), and the organic phase was washed successively with 10% citric acid solution, water, saturated sodium bicarbonate solution, and brine. Removal of solvent *in vacuo* yielded chromatographically pure aldehyde **8** (2.4 g, 93%): bp 120–122 °C (0.05 mmHg); $R_f$ 0.39 (4:1 hexane–acetone); $[\alpha]^{25}_D$ −65.5° (*c* 0.41, $CHCl_3$); IR (neat) 2966, 2877, 2727, 1730, 1701, 1454, 1400, 1300, 1165 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) (two conformers) $\delta$ 9.69 and 9.63 (brs, 1 H, CHO), 7.34–7.24 (brm, 5 H, aromatic), 5.14 (brs, 2 H, $PhCH_2$), 4.27 and 4.04 (d, $J$ = 10 Hz, 1 H, $H^\alpha$), 2.87 and 2.85 (s, 3 H, $NCH_3$), 2.05–1.95 (m, 1 H, $H^\beta$), 1.45–1.35 (m, 1 H, $HCHCH_3$), 1.15–0.95 (m, 4 H, $HCHCH_3CHCH_3$), 0.90–0.80 (m, 3 H, $CH_2CH_3$). The $^1H$ NMR spectrum recorded in acetonitrile did not show evidence of conformational isomerism.

***tert*-Butyl (3*R*,4*S*,5*S*)- and (3*S*,4*S*,5*S*)-3-Hydroxy-4-[*N*-(benzyloxycarbonyl)-*N*-methylamino]-5-methyl heptanoate (9a and 9b).** Lithium diisopropylamide was prepared by slowly adding butyllithium (2.0 M, 4.0 mL, 8 mmol) in tetrahydrofuran (4 mL) to a well-stirred solution of diisopropylamine (1.2 mL, 8.5 mmol) in tetrahydrofuran (16 mL) at −78 °C under argon. The solution was stirred for 1.5 h and allowed to warm to −20 °C. Upon recooling to −78 °C *tert*-butyl acetate (1.1 mL, 8.17 mmol) was added (*via* syringe), and the resultant mixture was stirred under argon for 2 h with warming to −20 °C. To the recooled

(−78 °C) mixture was added (syringe) a solution of aldehyde 8 (1.66 g, 6.32 mmol) in tetrahydrofuran (5 mL), and stirring was continued at −78 °C for 1 h. The aldol reaction was discontinued by cautious treatment with ice-water (10 mL). Water (100 mL) was added, and the mixture was extracted with diethyl ether (1 × 100 mL, 2 × 50 mL). The ethereal solution was washed with water, solvent was evaporated *in vacuo*, and the viscous oily residue was fractionated on a column of silica gel (0.040–0.063 mm and elution with 7:1 hexane–acetone). First eluted was the 3*S*,4*S*,5*S*-isomer **9b** (0.833 g, 35%) as an oily liquid: bp 136–139 °C (0.05 mmHg); $R_f$ 0.38 (4:1 hexane–ethyl acetate); $[\alpha]^{25}_D$ −31.5° (*c* 2.41, $CHCl_3$); IR (neat) 3475, 2966, 2877, 1699, 1496, 1402, 1313, 1155, 1001 cm$^{-1}$; $^1$H NMR ($CDCl_3$) (two conformers in a ratio of ca. 1:2) δ 7.32–7.27 (m, 5 H, aromatic), 5.12–5.10 (s, 2 H, $PhCH_2$), 4.60–4.50 (m, 1 H, CH), 4.29–4.07 (m, 1 H, CH), 3.65–3.55 (m, 1 H, CH), 2.92 (s, 3 H, $NCH_3$), 2.80 (s, 1 H, OH), 2.24–2.22 (m, 2 H, $CH_2CO$), 2.06–2.04 (m, 1 H, CH), 1.44 and 1.42 (s, 9 H, *t*-Bu), 1.20 (m, 1 H, *H*CH), 1.04 (m, 1 H, *H*CH), 0.99 and 0.94 (d, *J* = 6.6 Hz, 3 H, $CH_3$), 0.84 and 0.79 (t, *J* = 7.5 Hz, 3 H, $CH_2CH_3$); EIMS *m/z* (relative intensity) 379 ($M^+$), 262, 234, 190, 144, 91 (100%); HRFABMS *m/z* (exact mass) 380.2425 [(M + H)$^+$; calcd for $C_{21}H_{34}NO_5$ 380.2437]. Anal. Calcd for $C_{21}H_{33}NO_5$: C, 66.47; H, 8.76; N, 3.69. Found: C, 66.33; H, 8.78; N, 3.78.

Following elution of a mixture of aldol products (**9b**, **9a**, 0.12 g, 5%) the 3*R*,4*S*,5*S* epimer **9a** (1.07 g, 45%) was obtained as an oily liquid: bp 146–149 °C (0.05 mmHg); $R_f$ 0.31 (4:1 hexane–ethyl acetate); $[\alpha]^{25}_D$ −2.9° (*c* 0.91, $CHCl_3$); IR (neat) 3462, 2966, 1699, 1456, 1367, 1317, 1153 cm$^{-1}$; $^1$H NMR ($CDCl_3$) (two conformers in a ratio of ca. 1:2) δ 7.36–7.24 (m, 5 H, aromatic), 5.10 (two overlapping AB quartets, 2 H, $PhCH_2$), 4.25 and 4.16 (m, 1 H, CH), 3.89 and 3.78 (m, 1 H, CH), 3.20–3.00 (brs, 1 H, OH), 2.87 and 2.76 (s, 3 H, $NCH_3$), 2.40–2.20 (m, 2 H, $CH_2CO$), 1.90 (m, 1 H, CH), 1.50 (m, 1 H, *H*CH), 1.42 and 1.41 (s, 9 H, *t*-Bu), 1.10–1.00 (m, 1 H, *H*CH), 0.98 and 0.93 (d, *J* = 6.8 Hz, 3 H, $CH_3$), 0.85 and 0.81 (t, *J* = 7.4 Hz, 3 H, $CH_2CH_3$); EIMS *m/z* (relative intensity) 379 ($M^+$), 306, 266, 234, 190, 144, 91 (100). Anal. Calcd for $C_{21}H_{33}NO_5$: C, 66.47; H, 8.76; N, 3.69. Found: C, 66.45; H, 8.75; N, 3.74.

***tert*-Butyl (3*R*,4*S*,5*S*)-3-Methoxy-4-[*N*-(benzyloxycarbonyl)-*N*-methylamino]-5-methylheptanoate (10a). Method A.** To a cooled (−78 °C) solution of heptanoic acid ester **9a** (1.9 g, 5.0 mmol) in dichloromethane (20 mL stirred under argon) was added boron trifluoride etherate (0.7 mL, 5.69 mmol). After 30 min an anhydrous solution of diazomethane (large excess, prepared from Diazald) in dichloromethane was added, and the mixture was stirred for 1 h. The polymethylene side products were removed by filtration, the filtrate was concentrated, and the residue was chromatographed (flash procedure on a column of silica gel, 0.040–0.063 mesh with 97:3 hexane–acetone) to afford methyl ether **10a** (1.15 g, 67% based on recovery of starting alcohol) as an oil: bp 192–193 °C (0.12 mmHg); $R_f$ 0.43 (4:1 hexane–ethyl acetate); $[\alpha]^{25}_D$ −11° (*c* 0.23, $CHCl_3$); IR (neat) 2968, 2827, 1734, 1701, 1454, 1367, 1313, 1153, 1101, 846, 769, 698 cm$^{-1}$; $^1$H NMR ($CDCl_3$, two conformers in a ratio of *ca.* 1:2) δ 7.32–7.25 (m, 5 H, aromatic), 5.10 and 5.09 (two overlapping AB quartets, $J_{AB}$ = 12.2 Hz, $\Delta\nu_{AB}$ = 15.6 Hz, 2 H, $PhCH_2$), 4.10 (m, 1 H, NCH), 4.00–3.90 and 3.90–3.79 (m, 1 H, OCH), 3.36 and 3.25 (s, 3 H, $OCH_3$), 2.75 and 2.74 (s, 3 H, $NCH_3$), 2.45–2.25 (m, 2 H, $CH_2CO$), 1.75–1.60 (m, 1 H, CH), 1.41 and 1.40 (s, 9 H, *t*-Bu), 1.50–1.35 (m, 1 H, *H*CH), 1.10–1.00 (m, 1 H, *H*CH), 0.94 and 0.89 (d, *J* = 6.7 Hz, 3 H, $CH_3$), 0.84 and 0.81 (t, *J* = 7.4 Hz, 3 H, $CH_2CH_3$); EIMS *m/z* (relative intensity) 393 ($M^+$), 281, 234 (100), 190, 91; HRFABMS *m/z* (exact mass) 394.2592 [(M + H)$^+$, 65; calcd for $C_{22}H_{36}NO_5$ 394.2594]. Anal. Calcd for $C_{22}H_{35}NO_5$: C, 67.15; H, 8.96. Found: C, 66.94; H, 9.05.

Method B. To a solution of ester 9a (1.3 g; 3.42 mmol) in dichloroethane (50 mL) under nitrogen at room temperature were added molecular sieves (4 Å; 1 g) followed by proton sponge (1.85 g, 8.63 mmol) and trimethyloxonium tetrafluoroborate (1.35 g; 9.13 mmol). The mixture was stirred at room temperature for 20 h. The solution was filtered, and the oily solid (2.45 g) obtained upon removal of solvent (*in vacuo*) was subjected to column chromatography on silica gel. Elution with hexane–ethyl acetate–acetone (8:1:1) led to the required product (10a, 1.17 g, 87%) and recovered alcohol 9a (0.05 g). The methyl ether specimens prepared by methods A and B were found to be identical by TLC and high-field $^1$H NMR.

***tert*-Butyl (3*S*,4*S*,5*S*)-3-Methoxy-4-[*N*-(benzyloxycarbonyl)-*N*-methylamino]-5-methylheptanoate (10b).** The 3*S*,4*S*,5*S* alcohol **9b** (1.62 g, 4.3 mmol) was methylated according to method B above (see **10a**) to give methyl ether **10b** (1.2 g, 71%) as a chromatographically homogeneous oil: bp 170 °C/0.1 mmHg (bulb to bulb); $[\alpha]^{25}_D$ −29° (*c* 3.3, $CHCl_3$); IR (NaCl) 2971, 2933, 1729, 1700, 1455, 1368, 1326, 1313, 1154, 1104 cm$^{-1}$; $^1$H NMR ($CDCl_3$) (two conformers in a ratio of *ca.* 1:2) δ 7.34–7.21 (m, 5 H, aromatic), 5.17–4.95 (m, 2 H, $PhCH_2$), 4.29 and 4.27 (m, 1 H, OCH), 3.85 and 3.69 (dd, *J* = 10.8 and 3.0 Hz, 1 H, NCH), 3.29 and 3.27 (s, 3 H, $OCH_3$), 2.81 and 2.80 (s, 3 H, $NCH_3$), 2.38–2.21 (m, 2 H, $CH_2CO$), 1.90–1.87 (m, 1 H, CH), 1.41 and 1.40 (s, 9 H, *t*-Bu), 1.31–1.23 (m, 2 H, $CH_2$), 0.94 and 0.89 (d, *J* = 6.6 Hz, 3 H, $CH_3$), 0.81 and 0.75 (t, *J* = 7.2 Hz, 3 H, $CH_2CH_3$); EIMS *m/z* 393 ($M^+$), 234, 185, 91 (100). Anal. Calcd for $C_{22}H_{35}NO_5$: C, 67.15; H, 8.96. Found: C, 66.49; H, 9.14.

***tert*-Butyl (3*R*,4*S*,5*S*)-3-Methoxy-4-(*N*-methylamino)-5-methylheptanoate Hydrochloride (11a). Method A.** To a solution of *N*-(benzyloxycarbonyl)dolaisoleuine *tert*-butyl ester (**10a**, 0.65 g, 1.65 mmol) in ethyl acetate–methanol (3:1, 15 mL) was added 5% palladium charcoal (0.20 g), and the mixture was hydrogenated for 16 h at ambient temperature and pressure. The catalyst was removed by filtration and the filtrate concentrated to dryness. The crude product was dissolved in diethyl ether (4 mL), and the solution was cooled to −60 °C and treated with an ethereal solution of hydrogen chloride (1 M, 2 mL) under anhydrous conditions. A precipitate formed immediately, and excess hydrogen chloride was removed by passing argon through the mixture. The amorphous hydrochloride salt **11a** (0.32 g, 65%) was collected and found to melt at 145–147 °C: $[\alpha]^{30}_D$ +7.3° (*c* 4.5, $CHCl_3$); IR (NaCl) 2963, 2930, 2877, 2823, 2765, 1726, 1591, 1482, 1457, 1427, 1393, 1366, 1155, 1091, 1082 cm$^{-1}$; $^{13}$C NMR ($CDCl_3$) δ 170.25 (CO), 81.47 (qC), 75.82 (CH), 66.27 (CH), 57.69 ($OCH_3$), 36.97 ($CH_2CO$), 34.63 ($NCH_3$), 33.92 (CH), 28.03 (3 × $CH_3$), 26.04 ($CH_2$), 15.78 ($CH_3$), 11.62 ($CH_3$); $^1$H NMR ($CDCl_3$) δ 9.55 and 8.95 (brs, 1 H, NH), 3.97 (m, 1 H, OCH), 3.36 (s, 3 H, $OCH_3$), 3.06 (brm, 1 H, NCH), 2.78 (brs, 3 H, $NCH_3$), 2.75–2.60 (m, 2 H, $CH_2CO$), 2.00 (m, 1 H, CH), 1.80–1.50 (m, 2 H, $CH_2$), 1.41 (s, 9 H, *t*-Bu), 1.09 (d, *J* = 6.8 Hz, 3 H, $CH_3$), 0.94 (t, *J* = 7.2 Hz, 3 H, $CH_3$). Anal. Calcd for $C_{14}H_{30}ClNO_3$: C, 56.83; H, 10.22. Found: C, 56.58; H, 10.31.

Following collection of the hydrochloride salt the filtrate was concentrated and the residue chromatographed on a column of silica gel. Elution with hexane–acetone (4:1) afforded (4*R*,5*S*,1′*S*)-*N*-methyl-5-(1′-methylpropyl)-4-methoxypyrrolidin-2-one (**12a**, 90 mg, 29%) as a viscous oil: $[\alpha]^{30}_D$ −6° (*c* 0.9, $CHCl_3$); IR (NaCl) 1696, 1664, 1620, 1533, 1515, 1502, 1456, 1445, 1368, 1228 cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 3.68 (dd, *J* = 7.0, 1.7 Hz, 1 H, OCH), 3.46 (dd, *J* = 3.4, 1.7 Hz, 1 H, NCH), 3.29 (s, 3 H, $OCH_3$), 2.81 (s, 3 H, $NCH_3$), 2.55 (dd, *J* = 17.8, 7.0 Hz, 1 H, *H*CH), 2.39 (brd, *J* = 17.8 Hz, 1 H, *H*CH), 1.78 (m, 1 H, CH), 1.45 (m, 1 H, *H*CH), 1.32 (m, 1 H, *H*CH), 1.00 (t, *J* = 7.6 Hz, 3 H, $CH_3$), 0.71 (d, *J* = 7 Hz, 3 H, $CH_3$); EIMS *m/z* (relative intensity) 185 ($M^+$), 128 (100), 96, 71, 55; HREIMS *m/z* (exact mass) 185.1415 ($M^+$, 3; calcd for $C_{10}H_{19}NO_2$ 185.1416).

Method B. To a solution of ester 10a (7.35 g, 18.7 mmol) in anhydrous methanol (40 mL, under nitrogen) was added cyclohexene (20 mL) followed by 5% palladium on carbon (7.3 g). The temperature was immediately raised to reflux, and stirring was continued for 7 min. The solution was filtered quickly through Celite and concentrated under reduced pressure. Diethyl ether (10 mL) was added to the crude residue followed by an ethereal solution of hydrogen chloride (1 M, 15 mL). The resultant mixture was cooled to 0 °C and retained at that temperature for 16 h. The hydrochloride salt 11a was collected by filtration (7.0 g, 90%). The hydrochloride salts prepared by methods A and B were found to be identical by TLC and high-field $^1$H NMR.

***tert*-Butyl (3*S*,4*S*,5*S*)-3-Methoxy-4-(*N*-methylamino)-5-methylheptanoate Hydrochloride (11b).** The 3*S*,4*S*,5*S* methyl ether **10b** (0.68 g, 1.73 mmol) was hydrogenated in ethyl acetate (10 mL)–methanol (3 mL) employing method A (described above, see **11a**) to give hydrochloride **11b** (0.48 g, 93%) as an amorphous powder: mp 153–155 °C; $[\alpha]^{28}_D$ +35° (*c* 1.0, $CHCl_3$); IR (NaCl)

2971, 1728, 1481, 1472, 1465, 1366, 1219, 1155, 1111, 1097, 773 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 3.99 (m, 1 H, OCH), 3.47 (s, 3 H, $OCH_3$), 3.06 (dd, $J$ = 6.5, 3.0 Hz, 1 H, NCH), 2.81 (s, 3 H, $NCH_3$), 2.80 (dd, $J$ = 16.0, 6.3 Hz, 1 H, *H*CHCO), 2.53 (dd, $J$ = 16.0, 3.8 Hz, 1 H, *H*CHCO), 1.93 (m, 1 H, CH), 1.61 (m, 2 H, $CH_2$), 1.45 (s, 9 H, *t*-Bu), 1.21 (d, $J$ = 6.8 Hz, 3 H, $CH_3$), 0.95 (t, $J$ = 7.4 Hz, 3 H, $CH_3$). Anal. Calcd for $C_{14}H_{30}ClNO_3$; C, 56.83; H, 10.22. Found: 56.37; H, 10.58.

The filtrate obtained following recovery of the salt was concentrated and subjected to column chromatography on silica gel. Elution with hexane–acetone (17:3) afforded (4*S*,5*S*,1′*S*)-*N*-methyl-5-(1′-methylpropyl)-4-methoxypyrrolidin-2-one (**12b**, 15 mg) as a viscous oil: $^1H$ NMR ($CDCl_3$) $\delta$ 4.04 (q, $J$ = 7.0 Hz, 1 H, OCH), 3.50 (dd, $J$ = 7.1 and 3.6 Hz, 1 H, NCH), 3.25 (s, 3 H, $OCH_3$), 2.78 (s, 3 H, $NCH_3$), 2.47 (dd, $J$ = 16.8, 7.7 Hz, 1 H, *H*CHCO), 2.38 (ddd, $J$ = 16.8, 7.0, 0.6 Hz, *H*CHCO), 1.81 (m, 1 H, CH), 1.48–1.31 (m, 2 H, $CH_2$), 0.96 (d, $J$ = 7.0 Hz, 3 H, $CH_3$), 0.91 (t, $J$ = 7.4 Hz, 3 H, $CH_3$); HREIMS $m/z$ (exact mass) 185.1418 ($M^+$; calcd for $C_{10}H_{19}NO_2$ 185.1416).

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