The Dolastatins. 17. Synthesis of Dolaproine and Related Diastereoisomers[1]

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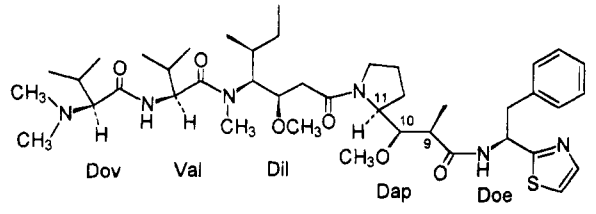
A special challenge in accomplishing the total synthesis of dolastatin 10 (**1**) entailed deducing the absolute configuration of the new β-methoxy-γ-amino acid component dolaproine (**2**) as 2*S*,2′*R*,3′*R* and devising a stereoselective synthesis. Synthesis of this unusual (*S*)-proline-derived unit as its *N*-*tert*-butoxycarbonyl derivative (**9b**) and three stereoisomers (**9a**, **9c**, **9d**) has been summarized. The diastereoisomers were assembled by an aldol condensation between aldehyde **5**, derived from (*S*)-proline, with chiral propionate **6**, followed by methylation and cleavage of the chiral directing ester group by hydrogenolysis to afford methyl ethers **9a**–**d**. The absolute stereochemistry of the diastereoisomers was determined by a combination of X-ray crystallographic analyses (of **9a** and lactam **11b** formed from isomer **7a**) and high-field (400 MHz) NMR studies. By using each of these isomers in a series of dolastatin 10 syntheses the stereochemistry of the dolaproine (**2**) unit of natural (−)-dolastatin 10 (**1**) was shown to be 2*S*,2′*R*,3′*R*.

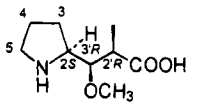
Introduction

After 12 years of intense investigation we succeeded (in 1984) in isolating[2] dolastatin 10 (**1**), a quite unique linear peptide from the western Indian Ocean sea hare *Dolabella auricularia*. We were subsequently able to complete the first total synthesis[3] of this powerful antineoplastic substance. Meanwhile, other synthetic approaches have been pursued,[4,5] and dolastatin 10 has been selected for clinical development. Because it contains nine asymmetric centers (one of 512 possible isomers) and the natural biosynthetic product was available in only trace quantities the absolute configuration was not determined when peptide **1** was originally isolated.[2] Development of a practicable total synthesis became necessary both in order to define the chiral centers and to provide a quantity sufficient for detailed biological evaluation and subsequent clinical trials.



1, Dolastatin 10

The new and unusual β-methoxy-γ-amino acid dolaproine (Dap, **2**) comprises the most complex unit of peptide **1** and appears to be produced by a biosynthetic aldol-type condensation of a proline-derived aldehyde and a propionate equivalent. *D. auricularia* has been found[1,2,6]



2, Dolaproine (Dap)

to preferentially utilize such aldol-derived amino acids for synthesizing powerful antineoplastic peptides. Besides γ-amino acid **2**, dolastatin 10 (**1**) contains dolaisoleuine (Dil),[3] another new β-methoxy-γ-amino acid which seems also to have arisen *via* an aldol-type condensation. Our synthesis of natural Dil has been summarized in a related contribution.[7] The β-methoxy-γ-amino acids Dil and Dap (**2**) are structurally related to both (3*S*,4*R*,5*S*)-isostatine,[8] a component of the antineoplastic didemnins,[9] and to the leucine type amino acid statine found in pepstatin,[10] a lower plant isolate which inhibits proteases such as pepsin, renin, and cathepsin D. Of the more than 500 naturally occurring amino acids currently known[11] only (to our knowledge) detoxinine,[12] a component of the detoxin class of *Streptomyces* depsipeptides, is similar to Dap (**2**) in being an aldol-type derivative of proline. A

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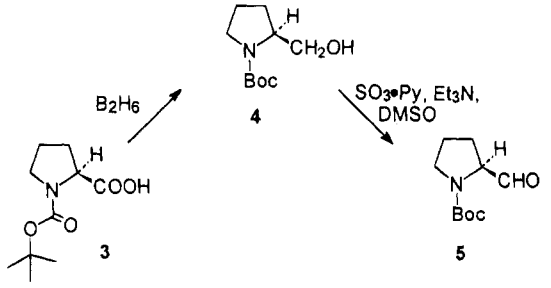
systematic approach to the synthesis of Dap (**2**) and three of its stereoisomers now follows.

One of the most common approaches to the synthesis of β-hydroxy-γ-amino acids involves an aldol condensation between an aldehyde derivative of the appropriate amino acid and a suitable enolate. Current advances in improving the aldol reaction offer a wide range of techniques.[13] A disadvantage is that mixtures of diastereomers which are difficult to separate may be formed. However, diastereoselective syntheses of statine, isostatine, and their analogs which make use of chiral enolates have been described.[10d,14] Wuts and Putt[15] have reported that the magnesium enolates of (*S*)- and (*R*)-2-acetoxy-1,1,2-triphenylethanol[16] provided excellent diastereoselectivity in the syntheses of statine and its C-3 epimer respectively. Similarly, in a synthesis of detoxinine Ewing and colleagues[12] found that the condensation of an (*S*)-prolinal derivative with (*S*)-2-acetoxy-1,1,2-triphenylethanol in the presence of magnesium bromide gave rise to a 3-fold excess of the desired aldol product, and the diastereomers were chromatographically separable.

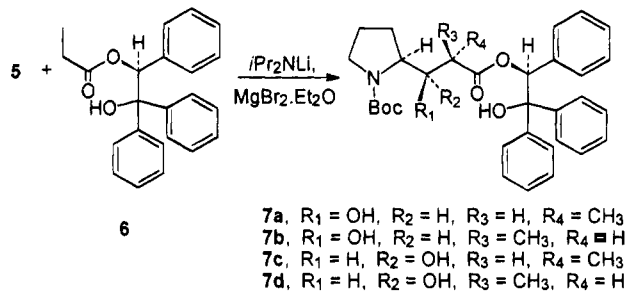
Dolaproine (**2**) is more complex than the amino acids mentioned above in that it contains three asymmetric centers where one resides in the proline ring. Since the proline moiety was thought likely to have the *S*-configuration we needed to select a propionate equivalent which, on condensation with *N*-protected (*S*)-prolinal (**3** → **5**), would produce all four diastereomers. In order to introduce the C-2 asymmetric methyl group, and to promote ready separation of the products, propionyl ester **6** was chosen.

Reduction of Boc-(*S*)-proline (**3**) to alcohol **4** was accomplished in good yield using diborane.[17] Oxidation of alcohol **4** to *N*-(*tert*-butoxycarbonyl)-(*S*)-prolinal (**5**)[18] was achieved without detectable racemization (in 92% yield) using dimethyl sulfoxide and sulfur trioxide–pyridine complex according to the Parikh–Doehring procedure.[19]

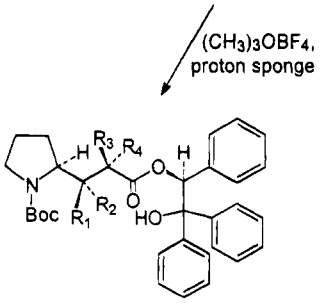


As noted earlier,[3] this oxidation could also be effected without obvious racemization according to the modified Swern[20] method, employing trifluoroacetic acid and dimethyl sulfoxide. Yields were higher under Parikh–Doehring conditions, and this method was generally used, particularly for multigram scale oxidations. The ^{1}H-NMR spectrum of aldehyde **5** in deuteriochloroform indicated the presence of two rotamers. Signals corresponding to the aldehyde proton appeared at δ 9.54 and at δ 9.45, the methine proton at δ 4.18 and δ 4.03, and the *tert*-butoxycarbonyl methyl groups at δ 1.46 and at δ 1.41. When the temperature was increased to 55 °C the signals coalesced. Changing the solvent to deuterioacetonitrile caused the room temperature spectrum to show one signal each for the aldehyde and methine protons but the *tert*-butoxycarbonyl methyl groups still appeared as two peaks.

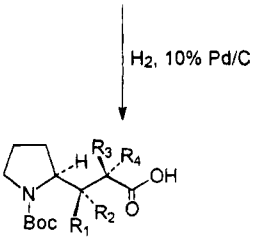
The required (*S*)-propionate chiral auxiliary **6** was prepared by reaction between (*S*)-methyl mandelate and phenylmagnesium bromide[12,16] followed by esterification to furnish (*S*)-2-(propionyloxy)-1,1,2-triphenylethanol (**6**) in good yield. Aldol condensation of aldehyde **5** with propionate **6** in tetrahydrofuran (at −95 °C) containing magnesium bromide[12] afforded a mixture of isomers in a ratio of 64:6:15:15 (**7a**:**7b**:**7c**:**7d**). Product composition



7a, R_1 = OH, R_2 = H, R_3 = H, R_4 = CH_3
7b, R_1 = OH, R_2 = H, R_3 = CH_3, R_4 = H
7c, R_1 = H, R_2 = OH, R_3 = H, R_4 = CH_3
7d, R_1 = H, R_2 = OH, R_3 = CH_3, R_4 = H



8a, R_1 = OCH_3, R_2 = H, R_3 = H, R_4 = CH_3
8b, R_1 = OCH_3, R_2 = H, R_3 = CH_3, R_4 = H
8c, R_1 = H, R_2 = OCH_3, R_3 = H, R_4 = CH_3
8d, R_1 = H, R_2 = OCH_3, R_3 = CH_3, R_4 = H



9a, R_1 = OCH_3, R_2 = H, R_3 = H, R_4 = CH_3
9b, R_1 = OCH_3, R_2 = H, R_3 = CH_3, R_4 = H
9c, R_1 = H, R_2 = OCH_3, R_3 = H, R_4 = CH_3
9d, R_1 = H, R_2 = OCH_3, R_3 = CH_3, R_4 = H

was dependent on temperature: when the reaction was carried out at −78 °C the ratio changed to 45:15:20:20.

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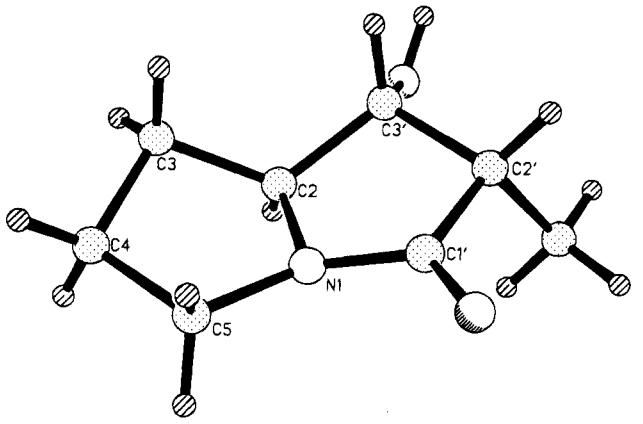


Figure 1. Computer-generated perspective drawing and X-ray numbering system of lactam 11b.

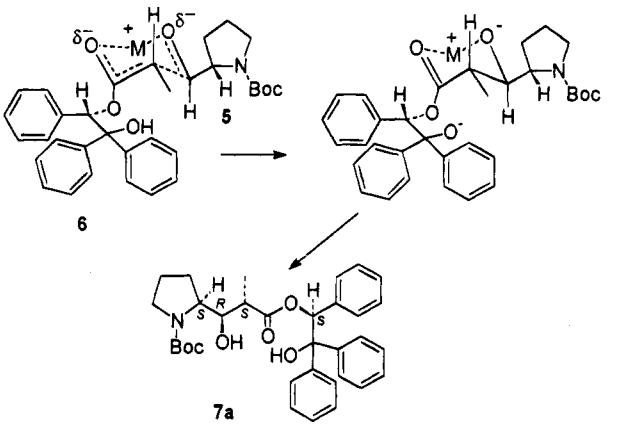
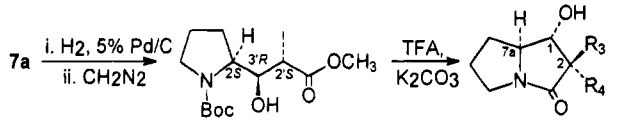


Figure 2. Chairlike transition state proposal for formation of isomer **7a**.

When the reaction was accomplished on a small scale the most polar and abundant of these isomers (**7a**) was reasonably easy to separate. However, with large quantities of reaction products (up to 40 g) it became necessary to develop an optimization chromatographic method[21] which led to excellent resolution. In order to define the stereochemistry of isomer **7a**, its methyl ester (**10**) was subjected to deprotection and cyclization with a trifluoroacetic acid → potassium carbonate sequence to yield lactam **11b** and the minor (presumably racemized) product **11a**. The ^{1}H NMR spectra of the products were



assigned on the basis of ^{1}H,^{1}H-COSY. A large coupling constant between H-1 and H-7a (J = 12 Hz) and a 6 Hz coupling constant between H-1 and H-2 in the spectrum of **11b** imply that H-1 and H-7a are *anti* while H-1 and H-2 are *syn* to each other. The assignment was supported by an NOE between H-1 and H-2 (H-1 → H-2, 6%; H-2 → H-1, 4%); there was no NOE between H-1 and H-7a. The absolute configuration of lactam **11b** was confirmed by X-ray crystal structure analysis (Figure 1), and thus the absolute configuration at the relevant chiral centers in aldol product **7a** was determined to be 2*S*,2′*S*,3′*R*.

With the procedure used, *anti* isomer **7a** was obtained with high selectivity, and the likely transition state[22] is shown in Figure 2. The experimental results suggested that in the presence of magnesium bromide the *E* enolate is preferentially formed[23] and the large substituents on the propionate and aldehyde induce reaction on the opposite face. A very helpful reviewer has suggested that the use of lithium diisopropylamide usually affords the (*E*)-enolate with or without magnesium bromide and the presence of magnesium may promote equilibration to the most thermodynamically stable products, prevent *E*/*Z* enolate equilibration, and/or through chelation hold the chiral auxiliary in place. At this stage the absolute configuration of Dap was still unknown and the major aldol product **7a** was employed for subsequent reactions leading to, as it turned out, an isodolastatin 10.[24]

The next synthetic step, methylation of isomer **7a**, was initially conducted with boron trifluoride etherate–diazomethane to afford methyl ether **8a**.[25] For large scale methylation this relatively unreliable and potentially dangerous procedure needed to be replaced and other methods were examined. Finally, trimethyloxonium tetrafluoroborate[26] in combination with proton sponge was found to be very suitable for both small and multigram scale methylation of alcohol **7a**. Deprotection of the C-terminal carboxyl group was achieved by hydrogenolysis to yield Dap isomer **9a**. An X-ray crystallographic analysis confirmed the 2*S*,2′*S*,3′*R* configuration (Figure 3) and, therefore, that the stereochemistry of 2′(*S*),3′(*R*)-iso-Dap methyl ester (**10**) had been retained in the major product of cyclization (**11b**). Since the other products of the aldol condensation (**7b**, **7c**, **7d**) were produced in small quantities relative to isomer **7a**, the aldol reaction was repeated using different propionate derivatives. Treatment of aldehyde **5** with *tert*-butyl, benzyl, or steroidal propionates or even an (*R*)-mandelate derivative yielded difficultly separable mixtures. Therefore, separation and utilization of the other (*S*)-mandelate-derived aldol products (**7b**, **7c**, **7d**) were investigated.

The aldol mixture was subjected to detailed chromatographic separation, and two of the diastereomers were resolved. The least polar compound (**7c**) and that next purified (**7d**) were methylated as above to afford Dap isomers **8c** and **8d**, respectively. Following de-esterification of esters **8c** and **8d**, the resultant *N*-(*tert*-butoxycarbonyl)-protected acids (**9c** and **9d**, respectively) were coupled with the other dolastatin 10 constituents.[24] Disappointingly, both gave rise to peptides isomeric with dolastatin 10 (**1**). Lactam **11c** was formed as a side product during the hydrogenolysis of **8d** when ethyl

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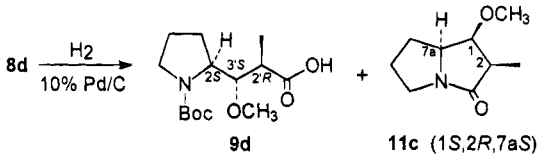
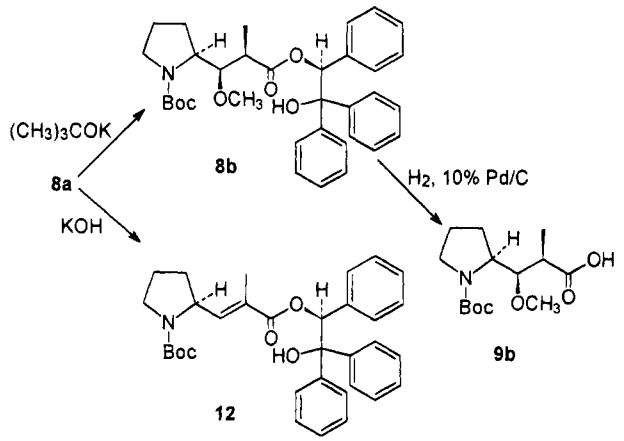
acetate was included in the solvent mixture. The ^1H NMR



spectrum of **11c** was unequivocally assigned on the basis of its two-dimensional COSY spectrum. Coupling constants of 8.3 Hz between H-1 and H-7a (*syn*) and 3.6 Hz between H-1 and H-2 (*syn*), corroborated by a NOE between H-1 and H-2 (H-1 → H-2, 2%; H-2 → H-1, 3%) and one between H-1 and H-7a (2%), implied an absolute stereochemistry of 1*S*,2*R*,7a*S* in **11c**, and therefore, **7d** was assigned to the 2*S*,2′*R*,3′*S* isomer.

By this time it was clear that, assuming the original choice of (*S*)-proline was still correct, the required aldol product was the diastereomer most difficult to purify and present in lowest yield, namely, **7b**, and had either the 2′*S*,3′*S* or the 2′*R*,3′*R* configuration. The latter differed from the most abundant aldol product (**7a**) only at the, presumably, epimerizable α-carbon atom (position 2). Thus, it was decided to produce isomer **7a** in quantity and effect epimerization following methylation to ether **8a** in order to generate a 2*S*,2′*R*,3′*R*-Dap ester. Epimerization of the α-methyl group of isomer **8a** with potassium *tert*-butoxide under carefully controlled (−20 °C) conditions gave methyl ether **8b** in 57% yield. Treatment with potasium *tert*-butoxide at −78 °C yielded almost exclusively starting material (**8a**). In the presence of a cata-



lytic amount of water α,β-unsaturated ester **12** was formed as a side product, and when the reaction was carried out using potassium hydroxide in 95% ethanol at 0 °C only olefin **12** was produced. Since **8b** and **8c** are different compounds the absolute stereochemistry of esters **7c** and **8c** was assigned 2*S*,2′*S*,3′*S* and **8b** proved to be the desired intermediate.

When Dap ester **8b** was subjected to hydrogenolysis carboxylic acid **9b** was obtained in 91% yield. Coupling of this γ-amino acid with the other dolastatin 10 (**1**) units according to the synthesis previously outlined[3,24] gave a peptide identical to dolastatin 10 (**1**). In turn, this confirmed isomer **9b** as *N*-(*tert*-butoxycarbonyl)dolaproine and that the dolaproine unit of dolastatin 10 (**1**) possessed the 9*R*,10*R*,11*S* stereochemistry. With the chirality of Dap established we concentrated on a stereoselective synthesis, and that was nicely accomplished as described in a following contribution.[27]

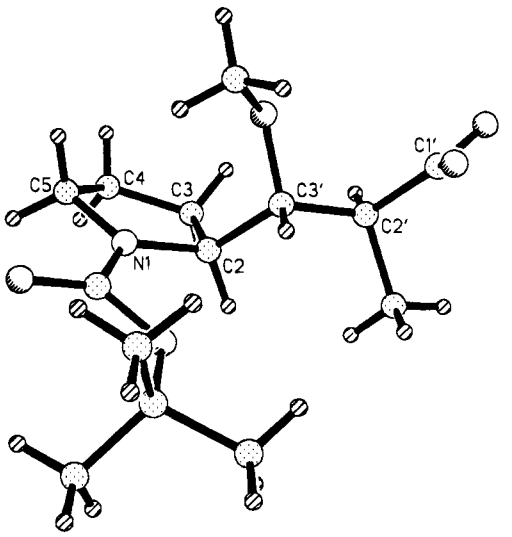


Figure 3. Computer-generated perspective drawing of carboxylic acid **9a**.

The three Dap isomers described here greatly assisted in establishing the Dap unit stereochemistry of dolastatin 10 (**1**) and were employed in initial structural probes directed at evaluating the effects of chiral changes in peptide **1** on cell growth inhibition.[24] An extension of these studies based on (*R*)-Pro is underway.

Experimental Section

All reagents were used as received from Sigma−Aldrich Chemical Co., solvents were redistilled. Solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate. Evaporation of solvents was performed under reduced pressure on a rotary evaporator at 40 °C. Thin layer chromatography was carried out with silica gel GHLF Uniplates (Analtech, Inc.). Both ambient column chromatography (Kieselgel 60, <0.063 and 0.063-0.200 mm) and "flash" chromatography (Kieselgel 60, 0.040−0.063 mm) were performed using silica gel supplied by E. Merck (Darmstadt).

Melting points are uncorrected and were determined on an Electrothermal 9100 apparatus. Optical rotations were recorded using a Perkin-Elmer 241 polarimeter. Ultraviolet spectra were obtained using a Hewlett-Packard 8540A UV/vis spectrophotometer, and infrared spectra were measured with a Nicolet FT-IR Model MX-1 unit. Nuclear magnetic resonance spectra were recorded on Varian Gemini 300 MHz (CHE-88-131109) and Bruker AM 400 instruments in $CDCl_3$ using tetramethylsilane as internal standard, unless otherwise stated. The EIMS mass spectra were recorded with a FINNIGAN-MAT 312 instrument (70 eV). The HREI and SP-SIMS (FAB) mass spectra were recorded with a Kratos MS 50 instrument in the NSF regional mass spectrometry facility at the University of Nebraska, Lincoln, NE. Elemental analyses were determined by Dr. A. W. Spang (Spang Microanalytical Laboratory, Eagle Harbor, MI). X-ray data collections were accomplished with an Enraf-Nonius CAD4 diffractometer.

***N*-(*tert*-Butoxycarbonyl)-(*S*)-proline (3).** To follow is a practical variation of earlier procedures for preparation of this useful Pro derivative.[28] Triethylamine (16.5 mL, 0.12 mmol) was added to an ice-cold suspension of (*S*)-proline (10.0 g, 0.09 mol) in dichloromethane (200 mL). Next, di-*tert*-butyl dicarbonate (27.2 g, 0.13 mol) in dichloromethane (10 mL) was added over 10 min, and the mixture was stirred at 0 °C for 2.5 h. The reaction was discontinued by addition of saturated aqueous citric acid (50 mL), and the organic phase was washed

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with brine (2 × 50 mL) and water (50 mL). Removal (*in vacuo*) of solvent yielded a crude product which was dissolved in hot acetyl acetate. Following addition of hexane (500 mL) Boc-(*S*)-Pro (**3**) crystallized from the cooled solution to yield 17.8 g, 95%: mp 138–140 °C (lit.[29] mp 134–136 °C), R_f 0.36, 1:1 methanol–ethyl acetate; ^{1}H NMR ($CDCl_3$) δ 7.5 (bs, 1 H), 4.4–4.1 (m, 1 H), 3.5–3.3 (m, 2 H), 2.0–1.8 (m, 4 H), 1.47 (s, 9 H).

***N*-(*tert*-Butoxycarbonyl)-(*S*)-prolinol (4).** To a solution of Boc-(*S*)-Pro (29.0 g, 0.14 mol) in tetrahydrofuran (200 mL, stirred under argon and cooled to 0 °C) was carefully added (under argon) borane–tetrahydrofuran complex (1 M, 270 mL). The mixture was stirred at 0 °C for 2 h and at room temperature for 1 h. Water (400 mL, at 0 °C) was added, and the aqueous mixture was extracted with ethyl acetate (300 mL). The organic phase was washed successively with brine (50 mL), saturated sodium bicarbonate solution (50 mL), and water (2 × 50 mL). Removal of solvent *in vacuo* yielded a viscous oil which was dissolved in dichloromethane (150 mL). The solution was filtered through a funnel of dry silica which was washed with dichloromethane. Evaporation (*in vacuo*) of the solvent yielded alcohol **4** as an oil (23.7 g, 87.5%) which spontaneously crystallized when freeze-dried: mp 58–60 °C (lit.[30] mp 57–58 °C); R_f 0.32, 3:1 hexane–ethyl acetate, 0.69, 6:1 chloroform–methanol; $[\alpha]^{25}_D$ −52.48° (*c* 1.61, CH_3OH) [lit.[30] $[\alpha]^{20}_D$ −47.5° (*c* 1.0, $CHCl_3$)]; IR (neat) 3387, 2974, 2879, 1693, 1674, 1408, 1170, 1126 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.92 (m, 1 H), 3.57 (m, 2 H), 3.42 (m, 1 H), 3.27 (m, 1 H), 1.96 (m, 1 H), 1.73 (m, 2 H), 1.56 (m, 1 H), 1.44 (s, 9 H); HREIMS *m/z* (rel intensity) 201 (M^+, 2), 170 (30), 128 (25), 114 (100), 70 (98). Anal. Calcd for $C_{10}H_{19}NO_3$: C, 59.68; H, 9.52; N, 6.96. Found: C, 59.42; H, 9.89; N, 6.94.

When the reduction was performed as above followed by purification by column chromatography (silica gel, 13–24 μm, 4 × 10 cm, dichloromethane) the alcohol crystallized and following recrystallization from hexane the yield was 32 g (76%), melting at 63.5–64 °C.

***N*-(*tert*-Butoxycarbonyl)-(*S*)-prolinal (5).** To a solution of alcohol **4** (37.3 g, 0.1856 mol) in dimethyl sulfoxide (250 mL stirred under argon at room temperature) was added triethylamine (90 mL) and stirring continued for 15 min. Before the addition of sulfur trioxide–pyridine complex (102.9 g, 0.65 mol, in batches over a 40 min period at 15–17 °C) the mixture was cooled (ice bath) and stirring was then continued under argon at 7–8 °C for 2.5 h. Following addition of ice (650 g) the aqueous mixture was extracted with dichloromethane (4 × 300 mL). The organic phase was washed successively with 50% citric acid solution (500 mL), water (500 mL), saturated sodium bicarbonate solution (500 mL), and water (500 mL). Removal of solvent *in vacuo* followed by freeze-drying yielded aldehyde **5** (34.0 g, 92%) as a dense oil which was used without further purification.

For analytical purposes a sample of aldehyde **5** (3.0 g) was purified by flash column chromatography (Kieselgel 60, 0.063–0.2 mm, 2.8 cm × 29 cm, eluant: 3:1 hexane–ethyl acetate, 650 mL): R_f 0.52, 3:1 hexane–ethyl acetate; $[\alpha]^{25}_D$ −99.5° (*c* 0.61, $CHCl_3$) [lit.[18] $[\alpha]_D$ −97° (*c* 1.0, $CHCl_3$)]; IR (neat) 3450, 2976, 2881, 2808, 2712, 1785, 1705, 1479, 1396, 1255, 1165, 1122 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.54 (bs, 0.4 H), 9.45 (d, 0.6 H), 4.18 (m, 0.4 H), 4.03 (m, 0.6 H), 3.48 (m, 2 H), 2.2–1.8 (m, 4 H), 1.46 (s, 3.6 H), 1.41 (s, 5.4 H). A similar procedure followed by column chromatography on Sephadex LH-20 (4 × 30 cm, eluant: methanol) gave aldehyde **5** in 87% yield.

(*S*)-1,1,2-Triphenyl-1,2-ethanediol. The following procedure represents a useful modification of earlier[12] reports. To a solution of phenylmagnesium bromide (3 M in diethyl ether, 170 mL, 0.51 mol) at 0 °C was added slowly (over 1 h) with stirring a solution of (*S*)-(+)-methyl mandelate (23.7 g, 0.14 mol) in diethyl ether (180 mL). The mixture was heated at reflux for 2 h, cooled to 0 °C, and slowly poured with stirring onto an ice-cold aqueous solution of sodium bisulfate (20%, 400 mL). The aqueous layer was extracted with ethyl acetate (3 × 100 mL), and the combined extract was washed sequentially with brine (50 mL), saturated sodium bicarbonate solution (50 mL), and water (50 mL). Removal of solvent (*in vacuo*) yielded an oily residue which was dissolved in ethanol (540 mL). Water (600 mL) was slowly added with stirring, and the mixture was retained at 0 °C for 16 h. The crystals which formed were collected and washed with aqueous ethanol (50%, 50 mL) to yield **(*S*)-1,1,2-triphenyl-1,2-ethanediol** (40.2 g, 97%): mp 114–117 °C (lit.[12] mp 111–117 °C); R_f 0.42, 3:1 hexane–ethyl acetate; ^{1}H NMR ($CDCl_3$) δ 7.7–7.1 (m, 15 H), 5.61 (d, J = 3.12 Hz, 1 H), 3.12 (s, 1 H), 2.44 (d, J = 3.48 Hz, 1 H).

In another experiment phenylmagnesium bromide (3 M, 59.7 mL) was added to a solution of (*S*)-methyl mandelate (8.2 g, 0.05 mmol) in tetrahydrofuran, and reaction was terminated by addition of ammonium chloride. Following extraction of the crude product as above purification was achieved by flash column chromatography [Whatman LPS-1, 13-24 μm, 0.7 bar, hexane–ethyl acetate (2:1)] to give (*S*)-1,1,2-triphenyl-1,2-ethanediol as a crystalline solid (11.6 g, 85%) melting at 116–117 °C.

(*S*)-2-(Propionyloxy)-1,1,2-triphenylethanol (6). Propionic anhydride (18 mL, 0.14 mol) was added (with stirring, over 30 min) to a solution of (*S*)-1,1,2-triphenyl-1,2-ethanediol (26.0 g, 0.09 mol) in pyridine (50 mL at 0 °C). The mixture was stirred for 30 min at 0 °C and at room temperature for an additional 23 h. The solid (**6**) which precipitated was collected by filtration and washed with acetone. More product (**6**) crystallized on concentration of the mother liquor and was also collected and washed with acetone. The filtrate was poured onto ice–water (2 L) and the water was decanted. When the oily residue was suspended in acetone (100 mL) a third crop of **(*S*)-2-(propionyloxy)-1,1,2-triphenylethanol** (**6**) crystallized. After being washed with acetone it was combined with the earlier collections (27.1 g, 73% in total): mp 217 °C; R_f 0.54, 3:1 hexane–ethyl acetate; $[\alpha]^{30}_D$ −203° (*c* 1.44, $CHCl_3$): ^{1}H NMR ($CDCl_3$) δ 7.7–7.1 (m, 15 H), 6.69 (s, 1 H), 2.79 (s, 1 H), 2.24 (q, J = 7.33 Hz, 2 H), 0.99 (t, J = 7.33 Hz, 3 H). Anal. Calcd for $C_{23}H_{22}O_3$: C, 79.75; H, 6.40. Found: C, 79.60, H, 6.30.

[2*S*-[2*R[(*R**),α(*R**),β(*S**)]]]-1-[(1,1-Dimethylethoxy)carbonyl]-β-hydroxy-α-methyl-2-pyrrolidinepropanoic Acid, 2-Hydroxy-1,2,2-triphenylethyl Ester (7a) and Diastereoisomers 7c,d.** A solution of diisopropylamine (1.4 mL, 10 mmol) in tetrahydrofuran (20 mL under argon) was cooled to −78 °C before addition of *n*-butyllithium (1.6 M in hexane, 5.6 mL, 9 mmol). Stirring was continued for 1.5 h. The mixture was allowed to warm to −20 °C and then recooled to −78 °C prior to addition of a suspension of ester **6** (1.4 g, 4 mmol) in tetrahydrofuran (15 mL). The mixture was stirred for 30 min at −78 °C and allowed to warm to room temperature over 2 h. The resultant yellow solution was recooled to −95 °C. At this point magnesium bromide etherate (freshly prepared from magnesium: 0.29 g, 12 mmol and dibromoethane: 0.52 mL, 6 mmol in 15 mL of tetrahydrofuran) was added. Stirring was continued for 1 h at −78 °C, the mixture was recooled to −95 °C, and a solution of aldehyde **5** (0.8 g, 4.0 mmol) in tetrahydrofuran (10 mL) was added. The reaction mixture was stirred for an additional 2 h at −95 °C followed by addition of saturated ammonium chloride solution (5 mL) at 0 °C. Diethyl ether (400 mL) was added, and the ethereal solution was washed with water (2 × 200 mL). Evaporation of solvent yielded a glassy solid. Analysis by HPLC [RP-8, 3 mm, 100 × 4.6 mm, eluant: acetonitrile–water (50–100%), 15 min gradient and elution at 1 mL/min] gave evidence of an isomer ratio of 64:6:15:15. The mixture was preadsorbed onto silica gel (10 g). Flash column chromatography (60 × 200 mm, eluant: 19:1 hexane–acetone) afforded unreacted ester (**6**, 0.22 g) followed by a mixture of diastereomers (**7b**, **7c**, **7d**, 0.76 g, 42%) and finally the major product (**7a**, 0.85 g, 47%) which separated from acetone–hexane as an amorphous powder: mp 128–130 °C; R_f 0.26, 8:4:1 hexane–chloroform–acetone; $[\alpha]^{30}_D$ −147 °C (*c* 2.45, $CHCl_3$); IR (NaCl) 3417, 2977, 1724, 1677, 1450, 1404, 1367, 1166, 754, 697 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 173.79, 164.00, 142.71, 128.59, 128.12, 127.63, 127.32, 126.90, 126.55, 80.30, 79.76, 79.44, 72.94, 59.48, 47.23, 42.36, 28.56, 25.69, 23.99, 14.27; ^{1}H NMR (DMSO-d_6, 100 °C) δ 7.494–7.063

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(m, 15 H), 6.533 (s, 1 H), 5.467 (s, 1 H), 4.054 (m, 1 H), 3.685 (dd, J = 4.0, 2.5 Hz, 1 H), 3.377 (m, 1 H), 3.165 (m, 1 H), 2.382 (pentet, J = 7.1 Hz, 1 H), 1.825 (m, 2 H), 1.624 (m, 2 H), 1.426 (s, 9 H), 0.836 (d, J = 7.1 Hz, 3 H); EIMS m/z (relative intensity) 545 (M^+, 0.1), 544 ($M^+ - 1$, 0.2), 527 ($M^+ - H_2O$, 4), 363 (100), 307 (98), 263 (95). Anal. Calcd for $C_{33}H_{39}NO_6$: C, 72.64; H, 7.20; N, 2.57. Found: C, 72.21; H, 7.16; N, 2.61.

Repeated flash chromatography of the diastereoisomeric mixture under the above conditions led to the isolation of **[2*S*-[2*R**[(*R**),α(*R**),β(*R**)]]]-1-[(1,1-dimethylethoxy)carbonyl]-β-hydroxy-α-methyl-2-methyl-2-pyrrolidinepropanoic acid, 2-hydroxy-1,2,2-triphenylethyl ester** (**7c**, 200 mg, 11%) as an amorphous powder from hexane–acetone: mp 68–70 °C; $[\alpha]^{26}_D$ −188° (c 3.0, $CHCl_3$); IR (NaCl) 3450, 1724, 1700, 1685, 1653, 1646, 1449, 1414, 1369, 1166, 697 cm^{-1}; ^1H NMR (DMSO-d_6, 84 °C) δ 7.602–7.035 (m, 15 H), 6.571 (s, 1 H), 5.610 (brs, 1 H), 5.520 (brd, 1 H), 3.824 (m, 1 H), 3.493 (dd, J = 12.0, 7.0 Hz, 1 H), 3.408 (m, 1 H), 3.139 (m, 1 H), 2.509 (pentet, 1 H), 1.779 (m, 2 H), 1.60 (m, 2 H), 1.420 (s, 9 H), 0.918 (d, J = 7.0 Hz, 3 H). Anal. Calcd for $C_{33}H_{39}NO_6$: C, 72.64; H, 7.20. Found: C, 72.34; H, 6.99.

Further elution afforded **[2*S*[2*R**[(*R**),α(*S**),β(*R**)]]]-1-[(1,1-dimethylethoxy)carbonyl]-β-hydroxy-α-methyl-2-pyrrolidinepropanoic acid, 2-hydroxy-1,2,2-triphenylethyl ester** (**7d**, 190 mg, 10%) as an amorphous powder from acetone–hexane: mp 88–90 °C; $[\alpha]^{30}_D$ −150° (c 5.3, $CHCl_3$); IR (NaCl) 3350, 2910, 1720, 1650, 1375, 1140, 1010, 730 cm^{-1}; ^1H NMR (DMSO-d_6, 84 °C) δ 7.483–7.071 (m, 15 H), 6.531 (s, 1 H), 5.616 (s, 1 H), 4.350 (brs, 1 H), 3.689 (m, 1 H), 3.571 (dd, J = 10.9, 5.2 Hz, 1 H), 3.380 (m, 1 H), 3.105 (m, 1 H), 2.517 (m, 1 H), 1.761 (m, 2 H), 1.646 (m, 1 H), 1.540 (m, 1 H), 1.402 (s, 9 H), 0.940 (d, J = 7.0 Hz, 3 H). Anal. Calcd for $C_{33}H_{39}NO_6$: C, 72.64; H, 7.20; N, 2.57. Found: C, 72.73; H, 7.50; N, 2.59.

Another useful variation of this experiment was developed for obtaining isomer **7a**. A solution of diisopropylamine (13 mL, 93 mmol) in tetrahydrofuran (80 mL) was stirred (under nitrogen and cooled to −78 °C) while n-butyllithium (1.6 M in hexane, 50 mL, 80 mmol) was added over 20 min. Stirring at −78 °C was continued for 15 min, and ester **6** (10.0 g, 29 mmol) was added in one portion. The mixture was stirred for 30 min at −78 °C and for 2 h while the temperature adjusted to room temperature. The resultant yellow solution was recooled to −78 °C prior to addition of magnesium bromide etherate (12.0 g, 46 mmol). Stirring was continued for 30 min, and then the mixture was cooled to −95 °C before the addition (over 20 min) of a solution of aldehyde **5** (8.0 g, 40 mmol) in tetrahydrofuran (15 mL). The temperature was maintained at −95 to −100 °C during the addition. The reaction mixture was stirred for an additional 3 h at −95 °C, potassium bisulfate solution (20%, 100 mL) was added after warming to room temperature, adjustment to pH 6.5 was effected with 10% potassium bisulfate, and water (100 mL) was added. The aqueous mixture was extracted with ethyl acetate (3 × 100 mL), and the combined extract was washed with water (100 mL). The procedure was repeated three times. Removal of solvent from the total extract yielded a residue (80 g) which was separated by column chromatography (Kieselgel 60, <0.063 mm, 9.5 × 57 cm, eluant: 32.3:53.4:14.3[21] hexane–chloroform–acetone). Removal of solvent from the fractions eluted at 2.2–3.1 L yielded the pure 2*S*,2′*S*,3′*R*,1″*S* isomer (**7a**, 30.5 g, 46%): mp 128–130 °C.

[2*S*-[2*R[(*R**),α(*R**),β(*S**)]]]-1-[(1,1-Dimethylethoxy)carbonyl]-β-methoxy-α-methyl-2-pyrrolidinepropanoic Acid, 2-Hydroxy-1,2,2-triphenylethyl Ester (8a). Method A.** To a solution of alcohol **7a** (0.60 g) in dichloromethane (10 mL, stirred under argon at −78 °C) was added boron trifluoride etherate (0.15 mL). After 30 min diazomethane (large excess, prepared from Diazald) in anhydrous dichloromethane was added, and 1 h later the polymethylene side products were removed by filtration. The filtrate was concentrated, and the residual solution was subjected to flash column chromatography (eluant: 9:1 hexane–acetone) to give methyl ether **8a** (0.38 g; 62%) as a powder which crystallized from acetone–hexane in rosettes: mp 130–132 °C; R_f 0.57 (3:4:1 hexane–chloroform–methanol); $[\alpha]^{30}_D$ −169° (c 4.15, $CHCl_3$); IR (NaCl) 3500, 2977, 1723, 1693, 1449, 1392, 1367, 1166, 1111, 753, 696 cm^{-1}; ^1H NMR (DMSO-d_6, 60 °C) δ 7.480–7.056 (m, 15 H), 6.582 (s, 1 H), 3.900 (m, 1 H), 3.699 (t, J = 7.0 Hz, 1 H), 3.400 (m, 1 H), 3.156 (s, 3 H), 3.111 (m, 1 H), 2.374 (pentet, J = 7.0 Hz, 1 H), 1.761 (m, 3 H), 1.640 (m, 1 H), 1.434 (s, 9 H), 0.794 (d, J = 7.0 H, 3 H); ^1H NMR ($CDCl_3$) δ 7.4–7.0 (m, 15 H), 6.78 and 6.66 (2s, 1 H), 4.27 and 3.86 (2d, J = 6.17, 10.55 Hz, 1 H), 3.73 (m, 1 H), 3.5 and 3.2 (2m, 1 H), 3.22 and 3.21 (2s, 3 H), 3.12 and 3.0 (2m, 1 H), 2.63 and 2.38 (2m, 1 H), 1.85 (m, 1 H), 1.70 (m, 3 H), 1.47 and 1.46 (2s, 9 H), 0.92 and 0.84 (2d, J = 7.2, 6.98 Hz, 3 H); HRFABMS m/z 566.3094, $[M + Li]^+$; calcd for $C_{34}H_{41}NO_6Li$ 566.3094. Anal. Calcd for $C_{34}H_{41}NO_6$: C, 72.96; H, 7.38. Found: C, 72.48; H, 7.41.

Method B. Trimethyloxonium tetrafluoroborate (8.9 g, 60 mmol) and proton sponge (12.8 g, 60 mmol) were added to a solution of alcohol **7a** (10.9 g, 20 mmol) in dichloroethane (200 mL under argon), and the mixture was stirred at room temperature for 23 h. A further 47.2 g of alcohol **7a** was divided into five batches, and repeat reactions were carried out as above. All the products were pooled, and removal of solvent *in vacuo* yielded a yellow solid which was fractionated by column chromatography (Kieselgel 60, <0.063 mm, 1600 g, 9.5 × 57 cm, eluant: 3:4:1 hexane–chloroform–acetone[21]) to yield methyl ether **8a** (37.6 g, 63%) as a powder. Crystallization from acetone–hexane afforded rosettes, mp 130–132 °C, identical (TLC, NMR) to the sample prepared according to method A. Further elution of the column yielded starting material (**7a**, 3.8 g).

[2*S*-[2*R[(*R**),α(*R**),β(*R**)]]]-1-[(1,1-dimethylethoxy)carbonyl]-β-methoxy-α-methyl-2-pyrrolidinepropanoic Acid, 2-Hydroxy-1,2,2-triphenylethyl Ester (8c).** To a solution of ester **7c** (0.18 g, 0.33 mmol) in anhydrous dichloromethane (2 mL, stirred under nitrogen at room temperature) was added proton sponge (0.40 g, 1.2 mmol of 1 mL of dichloromethane) followed by a suspension of trimethyloxonium tetrafluoroborate (0.25 g, 1.2 mmol) in dichloromethane (4 mL). The mixture was stirred at room temperature for 24 h. Additional trimethyloxonium tetrafluoroborate (0.10 g) and proton sponge (0.20 g) were added and stirring continued for 12 h. The solution was filtered through Celite, and the solid phase was washed with dichloromethane. The combined filtrate was washed sequentially with saturated citric acid solution (2 × 100 mL), saturated potassium bisulfate solution (2 × 100 mL), and water (100 mL). Removal (*in vacuo*) of solvent yielded a residue which was separated by flash column chromatography (eluant: 9:1 hexane–acetone) to afford methyl ether **8c** (75 mg, 41%) as an amorphous powder from acetone–hexane: mp 143–145 °C; $[\alpha]^{26}_D$ −141° (c 1.5, $CHCl_3$); IR (NaCl) 3360, 1734, 1691, 1450, 1400, 1394, 1367, 1166, 1115, 1093, 698 cm^{-1}; ^1H NMR (DMSO-d_6, 84 °C) δ 7.501–7.049 (m, 15 H), 6.577 (s, 1 H), 5.630 (brs, 1 H), 3.93 (m, 1 H), 3.446 (dd, J = 8.9, 4.4 Hz, 1 H), 3.374 (m, 1 H), 3.117 (m, 1 H), 2.884 (s, 3 H), 2.550 (pentet, J = 7.0 Hz, 1 H), 1.820 (m, 2 H), 1.680 (m, 2 H), 1.407 (s, 9 H), 0.831 (d, J = 7.0 Hz, 3 H). Anal. Calcd for $C_{34}H_{41}NO_6$: C, 72.96; H, 7.38; N, 2.57. Found: C, 72.69; H, 7.41; N, 2.41.

[2*S*-[2*R[(*R**),α(*S**),β(*R**)]]]-1[(1,1-Dimethylethoxy)carbonyl]-β-methoxy-α-methyl-2-pyrrolidinepropanoic Acid, 2-Hydroxy-1,2,2-triphenylethyl Ester (8d).** The preceding methylation procedure (see **8c**) was performed using ester **7d** (1.40 g, 2.56 mmol) to yield methyl ester **8d** (0.94 g, 72%) as an amorphous powder: mp 95–96 °C; $[\alpha]^{26}_D$ −142° (c 3.2, $CHCl_3$); IR (NaCl) 3390, 1733, 1684, 1392, 1367, 1169, 1111, 1093, 753, 698 cm^{-1}; ^1H NMR (DMSO-d_6, 84 °C) δ 7.534–7.087 (m, 15 H), 6.575 (s, 1 H), 5.574 (s, 1 H), 3.836 (m, 1 H), 3.373 (dd, J = 10.8, 3.2 Hz, 1 H), 3.346 (m, 1 H), 3.069 (m, 1 H), 3.030 (s, 3 H), 2.520 (m, 1 H), 1.735 (m, 2 H), 1.569 (m, 2 H), 1.390 (s, 9 H), 0.955 (d, J = 7.0 Hz, 3 H). Anal. Calcd for $C_{34}H_{41}NO_6$: C, 72.96; H, 7.38; N, 2.57. Found: C, 73.03; H, 7.71; N, 2.56.

[2*S*-[2*R[(*R**),α(*S**),β(*S**)]]]-1-[(1,1-Dimethylethoxy)carbonyl]-β-methoxy-α-methyl-2-pyrrolidinepropanoic Acid, 2-Hydroxy-1,2,2-triphenylethyl Ester (8b). Method A.** A solution of freshly prepared potassium *tert*-butoxide in tetrahydrofuran (1 M, 0.65 mL) was added to a solution of

propionate **8a** (0.165 g, 0.3 mmol) in tetrahydrofuran (2 mL, at −20 °C under nitrogen). The solution was stirred, becoming bright yellow, until warmed to −15 °C (2−5 min), and then the reaction was terminated with saturated citric acid solution (6 mL). The mixture was poured into saturated citric acid solution (10 mL) and extracted with dichloromethane (3 × 10 mL), and the combined extract was washed with water (3 × 10 mL). Removal of solvent *in vacuo* yielded an oil (0.16 g) which was subjected to flash column chromatography (eluant: 19:1 hexane−acetone) to afford Dap ester **8b** (90 mg, 57%) as fine crystals from acetone−hexane: mp 130−132 °C; R_f 0.36, 7:1:1 hexane−chloroform−ethyl acetate; $[\alpha]^{30}_D$ −155 °C (*c* 0.01, $CHCl_3$); IR (NaCl) 3420, 2977, 1733, 1674, 1408, 1367, 1244, 1166, 757, 698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.689−6.995 (m, 15 H), 6.845 (s, 1 H), 5.687 (s, 1 H), 4.172 (dd, J = 10.4, 1.1 Hz, 1 H), 3.689 (m, 1 H), 3.373 (s, 3 H), 3.180 (m, 1 H), 3.020 (m, 1 H), 2.641 (distorted dq, J = 7.0, 1.1 Hz, 1 H), 2.200 (m, 1 H), 1.913 (m, 1 H), 1.650 (m, 2 H), 1.521 (s, 9 H), 1.150 (d, J = 7.0 Hz, 3 H); HRFABMS *m/z* 566.3101, $[M + Li]^+$; calcd for $C_{34}H_{41}NO_6Li$ 566.3094. Anal. Calcd for $C_{34}H_{41}NO_6$: C, 72.96; H, 7.38; N, 2.50. Found: C, 72.73; H, 7.68; N, 2.47.

Method B. The following procedure was employed for preparing larger quantities of Dap ester **8b**. A solution of propionate **8a** (2.0 g, 3.5 mmol) in tetrahydrofuran (20 mL) was stirred under nitrogen and cooled to −20 °C. Finely crushed potassium *tert*-butoxide was added in two batches (2 × 0.5 g, 9 mmol) with a 5 min interval. The mixture was stirred for a total of 15 min at −20 °C and then diluted with citric acid solution (50%, 20 mL). Water (20 mL) was added, the mixture was extracted with dichloromethane (3 × 20 mL), and crude product was isolated. The reaction was repeated six times as shown above using a total of 36 g of propionate **8a** (3 × 2 g, 4 g, 8 g, 16 g), and yields were found to improve with increasing scale. The total combined product was separated by column chromatography (Kieselgel 60, <0.063 mm, 1600 g, 9.5 × 57 cm, eluant: 7:1:1 hexane−chloroform−ethyl acetate[21]) to afford Dap ester **8b** (14.9 g, 41%).

[2*S*-[2*R,α(*R**),β(*S**)]]-1-[(1,1-Dimethylethoxy)carbonyl]-β-hydroxy-α-methyl-2-pyrrolidinepropanoic Acid, Methyl Ester (10).** A mixture of hydroxy ester **7a** (0.50 g, 0.92 mmol) and 5% palladium-on-carbon (0.50 g) in ethyl acetate−methanol (1:1, 115 mL) was hydrogenated at ambient temperature and pressure for 36 h. The catalyst was collected on Celite and washed with ethyl acetate. Solvent was removed *in vacuo*, and the residue was dissolved in diethyl ether and treated with excess diazomethane. Removal of the ether yielded a viscous oil which was preadsorbed onto silica gel and chromatographed on a silica column. Elution with 97:3 hexane−ethyl acetate afforded ester **10** as an oil (0.18 g, 68%): $[\alpha]^{30}_D$ −64° (*c* 2.35, $CHCl_3$); IR (NaCl) 3450, 2975, 2953, 1741, 1694, 1394, 1367, 1194, 1167, 1119, 1107 cm^{-1}; ^{13}C NMR ($CDCl_3$) (all the signals were either very broad or doubled due to the presence of stable conformers at ambient temperature) δ 176.14, 156.0, 79.65, 73.95, 59.85 and 59.50, 51.75, 47.54 and 47.21, 42.84 and 42.32, 28.49, 25.37, 24.27, 14.68; ^1H NMR ($CDCl_3$) δ 4.100−3.950 (m, 2 H), 3.708 (s, 3 H), 3.269 (m, 2 H), 2.545 (pentet, J = 7.0 Hz, 1 H), 1.905 (m, 2 H), 1.829 (m, 1 H), 1.747 (m, 1 H), 1.463 (s, 9 H), 1.266−1.230 (m, 3 H, this signal appeared as a doublet in DMSO-d_6 at 65 °C, J = 7.0 Hz); HRFABMS *m/z* 294.1886, $[M + Li]^+$; calcd for $C_{14}H_{25}NO_5Li$ 294.1893. Anal. Calcd for $C_{14}H_{25}NO_5$: C, 58.50; H, 8.77; N, 4.87. Found: C, 58.31; H, 8.85; N, 4.77.

[1*R*-(1α,2α,7aα)]-Hexahydro-1-hydroxy-2-methyl-3-oxo-1*H*-pyrrolizine (11b). To a solution of ester **10** (0.14 g, 0.48 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (1.5 mL) at 0 °C. The solution was stirred under nitrogen for 1 h, solvent was evaporated under a stream of nitrogen, and water (3 mL) was added to the residue followed by potassium carbonate (0.20 g). The mixture was heated on a steam bath for 15 min, allowed to cool to room temperature, and extracted with ethyl acetate (3 × 20 mL), and the combined extract was washed with water (20 mL). Removal of solvent yielded an oily residue which was fractionated by flash column chromatography (elution with 3:1 hexane−ethyl acetate). First eluted was the racemized minor product, **[1*R*-(1α,2β,7aα)]-hexahydro-1-hydroxy-2-methyl-3-oxo-1*H*-pyrrolizine (11a**, 8.3 mg, 12%), fine needles from acetone−hexane: mp 100−101 °C; $[\alpha]^{25}_D$ +2.0 (*c* 3.7, CH_3OH); IR (NaCl) 3335, 2976, 2949, 2880, 1681, 1659, 1457, 1447, 1367, 1107 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 173.97, 82.15, 66.44, 49.00, 41.72, 29.95, 26.64, 12.66; ^1H NMR ($CDCl_3$) δ 3.667 (m, 2 H), 3.572 (m, 2 H), 2.725 (pentet, J = 7.0 Hz, 1 H), 2.170 (m, 1 H), 2.000 (m, 2 H), 1.488 (m, 1 H), 1.213 (d, J = 7.0 Hz, 3 H); HREIMS *m/z* 155.0943, M^+; calcd for $C_8H_{13}NO_2$ 155.0947.

Continued elution of the silica gel gave the major product (**11b**, 40 mg, 59%) as prisms from acetone−cyclohexane: mp 121−122 °C; $[\alpha]^{30}_D$ −115° (*c* 1.85, $CHCl_3$); IR (NaCl) 3251, 2985, 2979, 2934, 1677, 1444, 1312, 1089 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 176.72, 74.81, 66.83, 47.04, 41.51, 29.75, 26.75, 10.26; ^1H NMR ($CDCl_3$) δ 4.195 (dd, J = 12.0, 6.0 Hz, 1 H), 3.744 (ddd, J = 12.4, 8.7, 6.3 Hz, 1 H), 3.571 (dt, J = 11.6, 7.7 Hz, 1 H), 3.043 (ddd, J = 12.0, 8.4, 4.7 Hz, 1 H), 2.720 (pentet, J = 7.6 Hz, 1 H), 2.170 (dddd, J = 17.5, 12.6, 8.6 Hz, 1 H), 2.035 (m, 2 H), 1.482 (ddd, J = 17.5, 12.6, 8.6 Hz, 1 H), 1.267 (d, J = 7.6 Hz, 3 H); HREIMS *m/z* 155.0952, M^+; calcd for $C_8H_{13}NO_2$ 155.0947. Anal. Calcd for $C_8H_{13}NO_2$: C, 61.91; H, 8.44; N, 9.03. Found: C, 61.57; H, 8.63; N, 8.77.

X-ray Crystallographic Analysis of Lactam 11b. A colorless crystal (0.33 × 0.2 × 0.33 mm) of lactam **11b** that separated from acetone−cyclohexane solution was used in the data collection. Crystal data: $C_8H_{13}NO_2$, monoclinic space group $P2_1$, with a = 5.420(1) Å, b = 10.556(1) Å, c = 7.238(1) Å, β = 95.007(7)°, V = 412.57 Å, λ (Cu Kα) = 1.541 84 Å, ϱ_o = 1.248 g cm^{-3}, ϱ_o = 1.248 g cm^{-3} for Z = 2 and FW = 155.09. All reflections corresponding to a complete hemisphere, with $2\Theta \leq 130°$, were measured at 26 ± 1 °C using the $\omega/2\Theta$ scan technique. After Lorentz and polarization corrections, merging of equivalent reflections and rejection of systematic absences, 1643 unique reflections ($F_o > 3\sigma(F_o)$) were used in the structure determination and refinement. No absorption corrections were made in the data. Direct methods structure determination was readily accomplished with MULTAN-80.[31] All non-hydrogen atoms were revealed in the initial solution. Subsequent refinement calculations were performed with CRYSTALS.[32] The hydrogen atom coordinates were calculated at optimum positions. Full-matrix least-squares anisotropic refinement on all non-hydrogen atoms, with hydrogen atom coordinates and isotropic temperature factors fixed in the final cycle, yielded standard crystallographic residuals of R = 0.053 and R_w = 0.058 (anomalous dispersion corrections and extinction parameter included in the refinement). On the basis of the known absolute stereochemistry of the chiral carbon adjacent to the nitrogen atom, the complete absolute stereochemistry of all chiral centers in lactam **11b** could be assigned as follows (using the Dap numbering system): 2*S*,2′*S*,3′*R*. A computer-generated drawing is shown in Figure 1.[33,35]

[2*S*-[2*R(*R**)]]-1-[(1,1-Dimethylethoxy)carbonyl]-α-methyl-2-pyrrolidine-2-propenoic Acid, 2-Hydroxy-1,2,2-triphenylethyl Ester (12).** To a solution of propionate **8a** (0.80 g) in tetrahydrofuran (20 mL, cooled to 0 °C) was added potassium hydroxide (0.80 g) in 96% ethanol (10 mL). The mixture was stirred at 0 °C for 20 min, and at that point 50% citric acid solution (10 mL) was added followed by water (10 mL). The mixture was extracted with dichloromethane (3 × 10 mL), and solvent was removed from the combined extract to yield a residue. The product was isolated by flash column chromatography (silica gel 0.040−0.063 mm, 3.2 × 41 cm, eluant; 7:1:1 hexane−chloroform−ethyl acetate) to provide olefin **12** (0.65 g, 87%) as crystals from acetone−heptane: mp 191.4−192.2 °C; R_f 0.08, 7:1:1 hexane−chloroform−ethyl acetate; R_f 0.27, 4:1 hexane−acetone; $[\alpha]_D$ −184° (*c* 1.4, $CHCl_3$);

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(32) Watkin, D. J.; Carruthers, J. R.; Betteridge, P. W. *Crystals User Guide*; Chemical Crystallography Laboratory, University of Oxford: Oxford, England, 1985.

(33) Preparation of Figures 1 and 3 was done with "SHELXTL-PLUS", Sheldrick, G. Siemens Analytical X-Ray Instruments, Inc., Madison, WI 53719.

IR (KBr) 3533.8, 2978.3, 1699.4, 1396.6, 1255.7, 1163.1, 1091.8, 887.3, 748.4, 698.3 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 166.89, 154.47, 144.68, 144.61, 143.27, 136.22, 128.54, 128.25, 127.96, 127.52, 127.44, 127.20, 126.76, 126.52, 80.26, 79.35, 78.58, 55.08, 46.29, 32.16, 28.03, 23.66, 12.22; ^1H NMR ($CDCl_3$) δ 7.51–7.47 and 7.31–6.99 (m, 15 H), 6.72 (s, 1 H), 6.67 (d, J = 9 Hz, 1 H), 4.35 (m, 1 H), 3.45 (m, 1 H), 3.35 (m, 1 H), 2.73 (s, 1 H), 2.04 (hextet, J = 6.5, 12.3 Hz, 1 H), 1.79 (m, 1 H), 1.76 (s, 9 H), 1.55 (hextet, J = 6.7, 12.2 Hz, 1 H), 1.37 (m, 1 H), 1.17 (s, 3 H); MS m/z (relative intensity) 527 (M^+, 0.03), 345 (11.85), 289 (15.56), 184 (14.00), 183 (86.23), 167 (11.40), 165 (12.86), 155 (18.51), 154 (23.38), 140 (13.23), 139 (100), 138 (86.49), 114 (13.03), 110 (17.73), 109 (11.22), 106 (12.93), 105 (71.31), 91 (19.28) 77 (29.25), 71 (10.03), 70 (12.47), 67 (10.04), 57 (55.66), 55 (12.90), 51 (11.64). Anal. Calcd for $C_{33}H_{37}NO_5$: C, 75.12; H, 7.07; N, 2.66. Found: C, 75.16; H, 7.26; N, 2.65.

[2*S*-[2*R,α(*R**),β(*S**)]]-1-[(1,1-Dimethylethoxy)carbonyl]-β-methoxy-α-methyl-2-pyrrolidinepropanoic Acid (9a).** Hydrogen was passed (48 h) through a mixture of ester **8a** (0.41 g) in ethyl acetate–methanol (3:1, 50 mL) and 10% palladium-on-carbon (0.15 g) at ambient temperature and pressure. The catalyst was collected on Celite and washed thoroughly with ethyl acetate. Removal of solvent *in vacuo* from the combined filtrate yielded an oil. Silica gel column chromatography (eluant: 7:3 → 0:1 hexane–acetone) afforded carboxylic acid **9a** (0.17 g, 80.7%). Recrystallization from acetone–hexane gave rods: mp 105–108 °C; R_f 0.35 (5:1:1 chloroform–methanol–ethyl acetate); $[\alpha]^{30}_D$ −107.5 ° (*c* 0.4, $CHCl_3$); IR (NaCl) 2934, 1695, 1654, 1417, 1400 cm^{-1}; ^1H NMR (DMSO-d_6, 120 °C) δ 3.885 (dd, J = 9.2, 2.2 Hz, 1 H), 3.846 (m, 1 H), 3.449 (m, 1 H), 3.315 (s, 3 H), 3.180 (m, 1 H), 2.360 (dq, J = 9.2, 7.2 Hz, 1 H), 1.910 (m, 2 H), 1.810 (m, 1 H), 1.700 (m, 1 H), 1.400 (s, 9 H), 1.000 (d, J = 7.2 Hz, 3 H); HRFABMS m/z 294.1900, $[M + Li]^+$; calcd for $C_{14}H_{25}NO_5Li$ 294.1893. Anal. Calcd for $C_{14}H_{25}NO_5$: C, 58.52; H, 8.79; N, 4.87. Found: C, 58.53; H, 8.91; N, 4.81.

X-ray Crystal Structure Determination of Carboxylic Acid 9a. A colorless crystal (0.1 × 0.2 × 0.45 mm) of carboxylic acid **9a**, grown from acetone–methanol solution, was used in the X-ray data collection. Crystal data: $C_{14}H_{25}NO_5$, orthorhombic space group $P2_12_12_1$, with a = 10.027(1) Å, b = 10.763(2) Å, c = 15.332(2) Å, V = 1655.1 Å^3, λ (Cu Kα) = 1.541 84 Å, ϱ_o = 1.157 g cm^{-3}, ϱ_c = 1.153 g cm^{-3} for Z = 4 and FW = 287.36. All reflections corresponding to a complete octant, with $2\Theta \leq 130°$, were measured at 26 ± 1 °C using the $\omega/2\Theta$ scan technique. After Lorentz and polarization corrections, merging of equivalent reflections, and rejection of systematic absences, 1439 unique reflections ($F_o > 3\sigma(F_o)$) were used in the structure determination and refinement. An empirical absorption correction was made using the ψ scan technique.[34,35] Direct methods were used in the structure determination using MULTAN-80.[31] All non-hydrogen atoms were revealed in the initial solution. Refinement was performed with CRYSTALS.[32] The hydrogen atom coordinates were calculated at optimum positions. Full-matrix least-squares anisotropic refinement on all non-hydrogen atoms, with hydrogen atom coordinates and isotropic temperature factors fixed in the final cycle, yielded standard crystallographic residuals of R = 0.062 and R_w = 0.061 (anomalous dispersion corrections and extinction parameter included in the refinement). On the basis of the known absolute stereochemistry of the chiral carbon adjacent to the nitrogen atom, the complete absolute stereochemistry of all chiral centers in isomer **9a** was assigned as follows (using Dap numbering system): 2*S*,2′*S*,3′*R*. A computer-generated drawing of the carboxylic acid is shown in Figure 3.[33]

[2*S*-[2*R,α(*R**),β(*R**)]]-1-[(1,1-dimethylethoxy)carbonyl]-β-methoxy-α-methyl-2-pyrrolidinepropanoic Acid (9c).** The benzyl oxygen bond of ester **8c** (0.20 g, 0.36 mmol) was cleaved by hydrogenolysis in ethyl acetate–methanol as described above (see **9a**). The crude product was purified by flash column chromatography (eluant: 4:1:1 acetone–methanol–hexane followed by 4:1 acetone–methanol) to afford acid **9c** (80 mg, 79%) as an amorphous solid: mp 69–70 °C; $[\alpha]^{26}_D$ −23° (*c* 0.75, $CHCl_3$); IR (NaCl) 2976, 2934, 1689, 1685, 1609, 1457, 1419, 1402, 1368, 1167 cm^{-1}; ^1H NMR (DMSO-d_6, 120 °C) δ 4.073 (dd, J = 7.0, 5.4 Hz, 1 H), 3.490 (dd, J = 7.5, 5.4 Hz, 1 H), 3.422 (m, 1 H), 3.329 (s, 3 H), 3.200 (m, 1 H), 2.420 (pentet, J = 7.0 Hz, 1 H), 1.823 (m, 4 H), 1.440 (s, 9 H), 1.052 (d, J = 7.0 Hz, 3 H); HRFABMS m/z 310.1634, $[M + Na]^+$; calcd for $C_{14}H_{25}NO_5Na$ 310.1630.

[2*S*-[2*R,α(*S**),β(*R**)]]-1-[(1,1-Dimethylethoxy)carbonyl]-β-methoxy-α-methyl-2-pyrrolidinepropanoic Acid (9d).** Hydrogenolysis of ester **8d** (0.70 mg, 1.25 mmol) employing 10% palladium-on-carbon (0.20 g) in methanol (80 mL) was conducted at ambient temperature and pressure for 15 h and product isolated as recorded above for ester **9a**. Silica gel column chromatography with 19:1 acetone–methanol as eluant led to carboxylic acid **9d** (0.31 g, 86%) as an amorphous powder from acetone–hexane: mp 84–85 °C; $[\alpha]^{30}_D$ −58° (*c* 2.1, $CHCl_3$); IR (NaCl) 2950, 1700, 1640, 1395, 1160, 1095, 900 cm^{-1}; ^1H NMR (DMSO-d_6, 84 °C) 4.010 (m, 1 H), 3.681 (t, J = 7.0 Hz, 1 H), 3.373 (m, 1 H), 3.320 (s, 3 H), 3.170 (m, 1 H), 2.435 (pentet, J = 7.0 Hz, 1 H), 1.867 (m, 2 H), 1.767 (m, 2 H), 1.421 (s, 9 H), 1.118 (d, J = 7.0 Hz, 3 H). Anal. Calcd for $C_{14}H_{25}NO_5$: C, 58.52; H, 8.79; N, 4.87. Found: C, 58.39; H, 9.13; N, 4.99.

In another experiment hydrogen was passed through a mixture of ester **8d** (0.23 g; 0.42 mmol) in ethyl acetate–methanol (1:1, 20 mL) and 10% palladium-on-carbon (0.36 g) at ambient temperature and pressure for 24 h. The catalyst was collected on Celite and washed with ethyl acetate. Removal of solvent *in vacuo* from the combined filtrate followed by flash column chromatography (9:1 dichloromethane–methanol as eluant) afforded acid **9d** (34 mg, 27%) identical (^1H NMR, TLC in 3:1 hexane–acetone + 1 drop of acetic acid) with the product (**9d**) obtained above. Continued elution of the column with 9:1 dichloromethane–methanol yielded **[1*S*-(1β,2β,7aα)]-hexahydro-1-methoxy-2-methyl-3-oxo-1*H*-pyrrolizine (11c**, 44.8 mg, 52%) as an amorphous powder from acetone–hexane: mp 166–9 °C; $[\alpha]^{26}_D$ +20° (*c* 1, $CHCl_3$); IR (NaCl) 2915, 1738, 1205, 1120, 1100, 1070, 930 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.921 (dd, J = 8.2, 3.6 Hz, 1 H), 3.661 (brdd, J = 16.5, 8.6 Hz, 1 H), 3.494 (s, 3 H), 3.438 (m, 2 H), 2.712 (dq, J = 7.3, 3.6 Hz, 1 H), 2.113 (m, 2 H), 2.037 (m, 1 H), 1.782 (m, 1 H), 1.218 (d, J = 7.3 Hz, 3 H); HREIMS m/z 169.1102, $[M]^+$; calcd for $C_9H_{15}NO_2$: 169.1103.

Boc-Dap (9b). To a suspension of 10% palladium-on-carbon (0.30 g) in anhydrous methanol (3 mL, under nitrogen at room temperature) was added with stirring a solution of ester **8b** (0.30 g) in methanol (2 mL). The system was evacuated, flushed three times with hydrogen, and stirred vigorously under hydrogen for 12 h. The solution was passed through a short silica gel column (and product eluted with acetone followed by methanol) to remove the catalyst and achieve partial purification. Removal of solvent yielded ***N*-(*tert*-butoxycarbonyl)dolaproine (9b**, 0.15 g, 94%)[27] as a solid. Recrystallization (twice) from acetone–hexane produced needles: mp 138–142 °C; R_f 0.62, 40:40:1 hexane–ethyl acetate–acetic acid; R_f 0.30, 5:1:1 chloroform–methanol–ethyl acetate; $[\alpha]_D$ −61.4° (*c* 0.5, CH_3OH); IR (NaCl) 2976, 1696, 1685, 1675, 1669, 1663, 1653, 1640, 1594, 1457, 1423 cm^{-1}; ^{13}C NMR (major conformer) ($CDCl_3$) δ 180.65, 155.16, 83.40, 80.28, 61.49, 59.80, 46.89, 43.18, 28.73, 26.28, 24.20, 13.71; ^1H NMR (DMSO-d_6, 100 °C) δ 3.915 (brm, 1 H), 3.833 (brm, 1 H), 3.426 (m, 1 H), 3.322 (s, 3 H), 3.154 (m, 1 H), 2.174 (brm, 1 H), 1.943 (m, 1 H), 1.850 (m, 2 H), 1.662 (m, 1 H), 1.447 (s, 9 H), 1.111 (d, J = 6.3 Hz, 3 H); ^1H NMR ($CDCl_3$) δ 3.99 and 3.87 (m, 1 H), 3.79 (m, 1 H), 3.52 (m, 1 H), 3.44 (s, 3 H), 3.21 (m, 1 H), 2.55 (m, 1 H), 1.87 (m, 3 H), 1.71 (m, 1 H), 1.44 (s, 9 H), 1.24 (d, 3 H); HRFABMS m/z 294.1901, $[M + Li]^+$; calcd for $C_{14}H_{25}NO_5Li$ 294.1893.

For larger scale reactions 10% palladium-on-carbon (10 g) was added to a solution of ester **8b** (13.2 g) in ethanol (300

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(35) The author has deposited atomic coordinates for lactam **11b** and acid **9a** with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

mL), and hydrogen was passed through the mixture with vigorous stirring for 20 h. The catalyst was collected and washed with ethanol, and the solvent was removed (*in vacuo*). The reaction was repeated as above on another aliquot of ester **8b** (1.7 g), and the combined reaction products were separated by column chromatography on silica gel LPS-1 (13–24 μm, 3.5 × 50 cm). Elution with 6:1 hexane–acetone followed by 4:1 dichloromethane–methanol yielded Boc-Dap (**9b**, 6.9 g, 91%).

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