

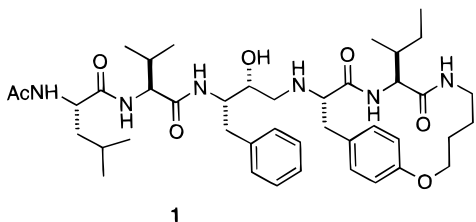
Synthesis and X-ray Crystallographic Structure of Leucine–Phenylalanyl Succinimide-Based Pseudopeptides

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Received September 17, 1996

The incorporation of a structural group, represented by X in Figure 1, which is known to inhibit a particular class of enzyme, into a peptide recognition sequence, has been the subject of much research. The aims of this work are to enhance the selectivity of an inhibitor toward a particular subclass of enzyme and also to produce families of peptide-based inhibitors to act as biological probes.¹ To date, a lot of this work has involved the incorporation of a transition-state analog, at position X in Figure 1, into a peptide sequence to produce potent inhibitors of proteases. For example, specific reversible inhibitors of serine proteases^{1,2} have been developed using peptidyl fluoromethyl ketones,³ peptidyl aldehydes,⁴ peptidyl carbamates,⁵ peptidyl keto esters,⁶ peptidyl boronic acids,⁷ and others.¹ Potent and selective inhibitors of the aspartyl proteases, renin⁸ and the HIV-protease,⁹ have also been developed by incorporating a peptide transition state isostere into a specific protease recognition sequence. For example the pseudopeptide **1**, which contains a centrally located hydroxyethylamine isostere, is a potent inhibitor of the HIV-1 protease (K_i 0.6 nM).¹⁰ Conformationally restricted structural elements, such as the macrocycle of **1**, have been introduced into peptide-based inhibitors in an attempt to enhance bioavailability and biostability.^{10,11}



Relatively few reports have appeared regarding the incorporation of an irreversible protease inhibitor into a peptide sequence. Some representative examples of

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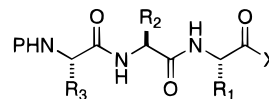
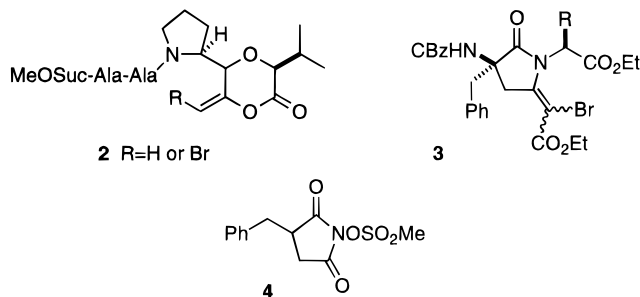


Figure 1. General structure of a peptidyl-based inhibitor of a protease.

irreversible inhibitors of serine proteases, with peptidic character, include the proline–valine pseudopeptide **2**,¹² phenylalanine-acylated enamino ester pseudodipeptides **3**,¹³ and peptidyl halo ketones.^{1,14} We now report on the synthesis and X-ray structure of peptidyl succinimide derivatives. The design of these compounds was based on simple succinimide compounds of the type **4**, initially developed by Groutas as general mechanism-based inhibitors of serine proteases.¹⁵



Results and Discussion

The method employed in the synthesis of the succinimide-based pseudodipeptides of the type **12** is outlined in Scheme 1. The oxazolidinone **5** was prepared from L-phenylalanine as previously described.¹³ Deprotonation of **5**, at C4, followed by alkylation with $\text{BrCH}_2\text{CO}_2\text{CHPh}_2$ and finally hydrolysis gave the acid **6** in good yield.¹³ Reaction of the acid **6** with *O*-benzylhydroxylamine in the presence of *N,N*-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) gave **7** in 81% yield. The ¹H NMR spectrum of the *N*-Cbz-protected amine **7** was run at an elevated temperature due to the existence of two conformational isomers at 23 °C. Cyclization of **7** with triethylamine at rt then gave the succinimide **9** in 99% yield. The free amine **8** was obtained in 73% yield by reaction of the protected amine **9** with *p*-toluenesulfonic acid under reflux. *N*-Acetyl-L-leucine was coupled onto **8** to give **10** in 65% yield, using DCC and HOBT. Deprotection of the benzyloxy group of **10** was achieved by catalytic hydrogenation to give the hydroxysuccinimide **11** which was then treated with methanesulfonyl chloride in the presence of diisopropylethylamine to give the desired pseudodipeptide **12**.

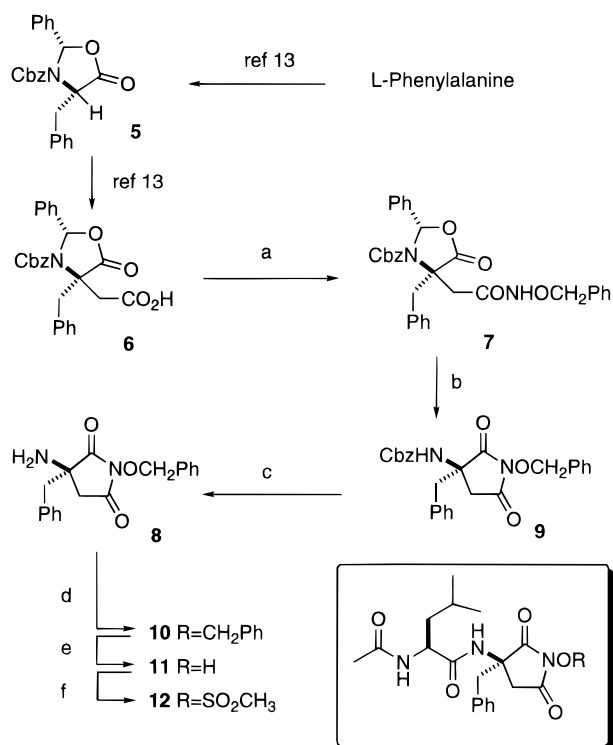
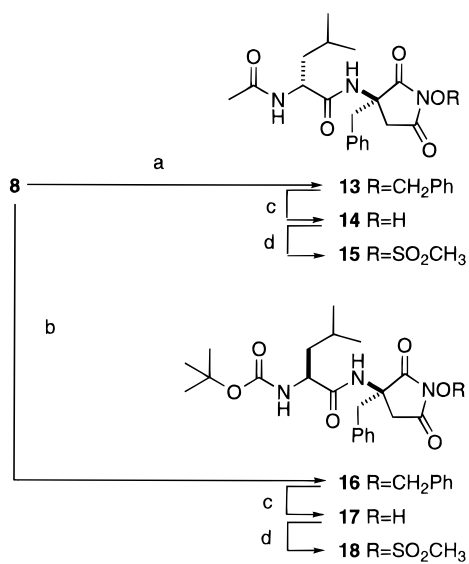
The epimeric pseudodipeptide, **15**, was prepared by coupling **8** with *N*-acetyl-D-leucine (step a, Scheme 2) followed by deprotection and *O*-mesylation as described

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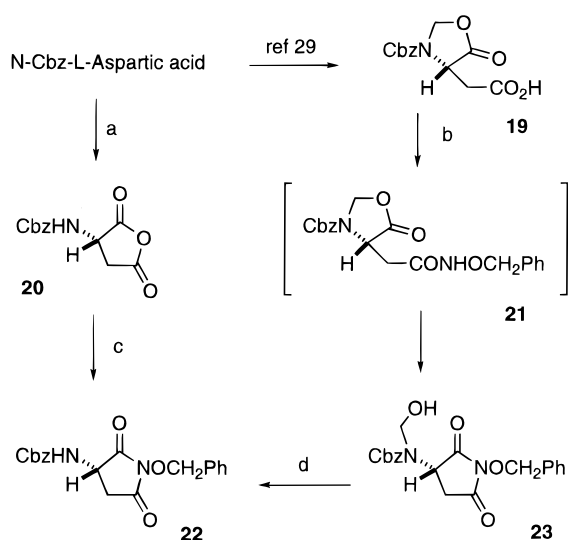
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Scheme 1^aScheme 2^a

above for **12**. The *N*-*t*-BOC-analog **18** was also prepared (Scheme 2) for biological testing and to allow entry into a series suitable for further elaboration toward the amino terminus of the peptide sequence. The synthetic methods presented in Schemes 1 and 2 provide a general method for the synthesis of pseudopeptides containing an *N*-[(alkylsulfonyl)oxy]succinimide moiety. The leucine-phenylalanine sequence of **12** was chosen to target the known specificity of α -chymotrypsin.¹

By comparison, the simple succinimide derivatives of the type **4** are prepared by reaction of the corresponding

Scheme 3^a

^a Key: (a) Ac₂O, reflux; (b) HCl.NH₂OCH₂Ph, Et₃N, HOBT, DCC, CH₂Cl₂, rt; (c) Et₃N, CH₂Cl₂, rt; (d) NH₂OCH₂Ph, toluene, reflux; (e) Et₃N, CH₂Cl₂, rt.

anhydride with *O*-benzylhydroxylamine.¹⁵ However, the anhydrides required to prepare compounds of the type **12** are not so readily available although there are reported examples of α -alkylated aspartic acid derivatives.¹⁶ The exception being *N*-Cbz-L-aspartic acid anhydride **20** which was reacted with with *O*-benzylhydroxylamine to give **22** in 72% yield (Scheme 3). Compound **22** was also prepared by a route analogous to that used in the preparation of **12**. In this case, the reaction of **19** (compare **6** in Scheme 1) with *O*-benzylhydroxylamine, gave **23** directly without the intermediacy of **21** (Scheme 3). Deformylation of **23** was accomplished, in a modest yield of 49%, by reaction with triethylamine at rt. Attempted deformylation of compounds of the type **23** under harsher reaction conditions¹⁷ gave reduced yields of the desired product. Compound **23** existed as a pair of conformational isomers by ¹H and ¹³C NMR spectroscopy.

An X-ray structural determination of **11** was carried out to determine its conformation. Structural studies of this type enable the compilation of accurate information regarding amino acid geometry and conformational flexibility among families of amino acids in different environments. Compound **11** is of particular significance in that the phenylalanine residue is alkylated at the α -position by incorporation into a succinimide ring. X-ray crystal structures of a few other conformationally constrained phenylalanine containing pseudopeptides have been reported^{18,19} as have the crystal structures of nonpeptidic *N*-hydroxysuccinimide derivatives, e.g. **24**,^{15c} and aspartic acid-derived peptidic imides.^{20,21}

A perspective drawing of **11**, with atomic labeling, is presented in Figure 2. The peptide backbone torsional

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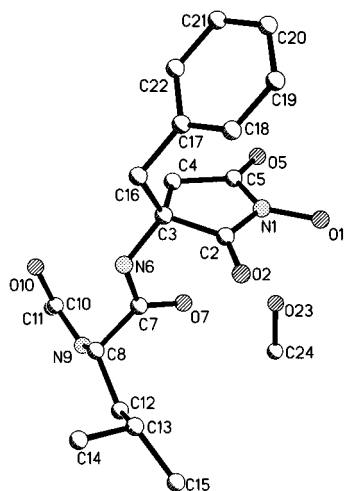
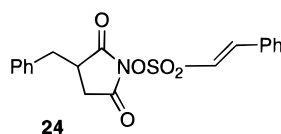


Figure 2. ORTEP diagram of **11**, with a cocrystallized molecule of methanol, showing the crystallographic numbering scheme.



angles (ω , φ , and ψ) and the amino acid side chain torsional angles (χ) are given in Table 1, with standard deviations given in parentheses. The absolute configuration of **11** was assigned as shown on the basis of crystallographic data and the known configuration of the starting amino acids, *i.e.* L-phenylalanine and L-leucine.

The first point to note regarding the conformation of **11** is that it lacks the typical H shape, commonly observed in the crystal structures of related dipeptides, where the two amino acid side chains are extended from the backbone and run approximately parallel.^{17,22–24} The phenylalanine CH_2Ph group of **11** is distorted from the standard H shape by adopting an unusual conformation as evidenced by a $\chi^{(2)}$ torsional angle of 173.9° (Table 1). An analysis of the literature on leucine–tyrosine crystal structures reveals that the $\chi^{(2)}$ torsion angle is typically in the range -50° to -80° .²² It should also be noted that **11** and the succinimide-based elastase inhibitor, **24**,^{15c} have vastly different conformation for the CH_2Ph groups, as determined by X-ray crystallography. The leucine side chain of **11** adopts a conformation ($\chi^{(1)}$ -176.3° , $\chi^{(2(1))}$ and $\chi^{(2(1))}$ 74.5° and -163.9° , Table 1) which is similar to that observed for one of three independent conformations (conformation A)¹⁹ adopted in the crystal structure of *N*-acetyl-L-Leu-L-Tyr-OMe. In this case the leucine side chain adopts the less common t(tg-) conformation.^{22,23}

As expected, the ω^0 and ω^1 torsion angles (Table 1) are consistent with planar peptide bonds. A φ and ψ map²³ for the observed conformation of **11** revealed it to be close to that of an antiparallel β sheet. Similar backbone conformations have been observed for related linear peptides containing a Leu-Tyr/Phe sequence.^{18,22–24} The observed structure of the imide of **11** is consistent with the published structures of peptidic imides.^{20,21} In particular, the succinimide ring of **11** is slightly puckered

Table 1. Torsion Angles for **11**

torsion		angle (deg)
C8–N9–C10–C1	ω^0	175.0(7)
C10–N9–C8–C7	φ^1	$-69.9(9)$
N6–C7–C8–N9	ψ^1	117.7(7)
C8–C7–N6–C3	ω^1	$-174.9(6)$
C2–C3–N6–C7	φ^2	$-53.0(8)$
N1–C2–C3–N6	ψ^2	129.6(6)
C13–C12–C8–N9	$\chi^{(1)}$	$-176.3(7)$
C8–C12–C13–C14	$\chi^{2(1)}$	74.5(9)
C8–C12–C13–C15	$\chi^{2(1)}$	$-163.9(7)$
N6–C3–C16–C17	$\chi^{(2)}$	173.9(6)
C22–C17–C16–C3	$\chi^{2(2)}$	91.9(9)
C18–C17–C16–C3	$\chi^{2(2)}$	$-85.1(8)$

with a N1–C2–C3–N6 (ψ^2) torsion angle of 129.6° which deviates slightly from a value of 120° that would correspond to a fully flat ring.²¹ The N1–C2 [1.370(9) Å] and N1–C5 [1.387(9) Å] bond lengths are significantly longer than N–C peptide bonds and φ^2 (C2–C3–N6–C7, Table 1) torsion angle is similar in magnitude to other succinimide peptides.²¹ Finally, the plane of the O7–C7–N6 peptide bond is approximately perpendicular (87°) to the succinimide ring.

Intermolecular hydrogen bonds are found in the crystal structure of **11**, between O5/N6, O5/O10, and O7/methanol (cocrystallized). Intramolecular hydrogen bonds were not evident as is the case in the crystal structures of related dipeptides.^{18,22–24} The above crystallographic determination provides data on the preferred conformations of succinimide-based pseudopeptides that are designed to probe the specificity of proteases.

Experimental Section

General Methods. Melting points are uncorrected. Optical rotations are given in units of 10^{-1} deg $\text{cm}^2 \text{g}^{-1}$. Preparative chromatography was carried out using a Chromatotron (Harrison Research Inc.) using glass plates coated with Merk type 60 PF₂₅₄ silica gel. Petroleum ether refers to the fraction of bp 60–70 °C.

X-ray crystallographic determination for compound 11: $\text{C}_{20}\text{H}_{29}\text{N}_3\text{O}_6$; MR 407.46; crystal dimensions $0.80 \times 0.40 \times 0.25$ mm; orthorhombic, a 12.364(7), b 12.565(7), c 13.756(12) Å; V 2137(3) Å³; space group $P2_12_12_1$; $Z = 4$, $F(000)$ 872; D_{calc} 1.266 mg/m^3 ; absorption coefficient 0.094 mm^{-1} ; θ range for data collection 2.21 – 22.50 ; index ranges $-13 \leq h \leq 13$, $0 \leq k \leq 13$, $0 \leq l \leq 14$; data/restraints/parameters 1605/0/272; goodness of fit on F^2 was 0.906; final R indices [for 1032 reflections with $I > 2\sigma(I)$] $R_1 = 0.0567$, $wR_2 = 0.1311$; R indices (all data) $R_1 = 0.0899$, $wR_2 = 0.1419$; largest difference peak and hole 0.265 and $-0.266 \text{ e} \text{ \AA}^{-3}$.

The unit cell parameters were obtained by least-squares refinement of the setting angles of 18 reflections with $10^\circ \leq 2\theta \leq 22^\circ$ from a Siemens P4 diffractometer using graphite-mono-chromatized Mo- $K\alpha$ radiation ($\lambda = 0.71073$ Å). A unique data set was measured at 158(2) K within $2\theta_{\text{max}} = 57^\circ$ limit (ω scans). Of the 1608 reflections obtained, 1605 were unique ($R_{\text{int}} = 0.2213$) and were used in the full-matrix least-squares refinement [SHELXL-93].²⁶ The intensities of three standard reflections, measured every 97 reflections throughout the 26 h data collection, showed 17.11% decay due to the instability of the crystal. The structure was solved by direct methods [SHELXS-86].²⁷ Hydrogen atoms were fixed in idealized positions. All non-hydrogen atoms were refined with anisotropic atomic displacement parameters. Neutral scattering factors and anomalous dispersion corrections for non-hydrogen atoms were taken from Ibers and Hamilton.²⁸ Full details of the X-ray structural

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determination of **11** have been deposited with the Cambridge Crystallographic Data Centre (CCDC).

(-)-(2*S*,4*R*)-4-Benzyl-4-[(benzyloxyamino)carbonyl]methyl-3-[(benzyloxy)carbonyl]-2-phenyl-1,3-oxazolidin-5-one (**7**). *O*-Benzyloxyamine hydrochloride (180 mg, 1.13 mmol), triethylamine (160 μ L, 1.15 mmol), and 1-hydroxybenzotriazole-1H₂O (173 mg, 1.13 mmol) were added to the carboxylic acid **6**¹³ (503 mg, 1.13 mmol) dissolved in dichloromethane (3 mL). The solution was stirred for 10 min at 0 °C under N₂ after which time *N,N*-dicyclohexylcarbodiimide (DCC) (235 mg, 1.14 mmol) was added. The mixture was stirred for a further 10 min at 0 °C and then for 6 h at rt under N₂. The reaction mixture was filtered and washed with 5% aqueous hydrochloric acid (25 mL) followed by water (2 \times 25 mL). The organic phase was dried (MgSO₄), and the residue was filtered and chromatographed using a 2 mm chromatotron plate, eluting with 35% ethyl acetate/65% petroleum ether to give **7** (507 mg, 81%). Compound **7** was recrystallized from ethyl acetate by diffusion with petroleum ether: mp 92–94 °C; FTIR (KBr) 3212, 3034, 1797, 1713, 1660 cm⁻¹; ¹H NMR (dioxane-*d*₆, 90 °C) δ 2.93 (d, *J* = 17.6 Hz, 1H), 3.50 (AB q, *J*_{AB} = 13.4 Hz, $\Delta\nu$ = 73.8 Hz, 2H, CH₂Ph), 3.50–3.65 (br, 1H), 4.98 (AB q, *J*_{AB} = 11.2 Hz, $\Delta\nu$ = 4.6 Hz, 2H, NOCH₂Ph), 5.13 (AB q, *J*_{AB} = 12.2 Hz, $\Delta\nu$ = 33.1 Hz, 2H, CO₂CH₂Ph), 6.48 (s, 1H), 6.52 (d, *J* = 7.8 Hz, 2H), 7.07 (br s, 1H), 7.12 (t, *J* = 7.3 Hz, 2H), 7.28–7.57 (m, 16H), 9.59 (br s, NH); ¹³C NMR (CDCl₃) δ 38.23, 41.85, 65.64, 67.25, 78.44, 79.52, 90.42, 127.46, 127.52, 127.86, 127.948, 128.18, 128.66, 128.90, 129.04, 129.26, 130.79, 134.78, 135.62, 152.56, 166.47, 172.83; $[\alpha]^{20}_D = -3^\circ$ (*c* = 1, CH₂Cl₂). Anal. Calcd for C₃₃H₃₀N₂O₆·1/4CH₃CO₂CH₂CH₃: C, 71.31; H, 5.63; N, 4.89. Found: C, 71.23; H, 5.60; N, 4.87.

(+)-(3*R*)-3-Benzyl-1-(benzyloxy)-3-[(benzyloxy)carbonyl]amino)succinimide (**9**). Triethylamine (0.5 mL, 3.59 mmol) was added to oxazolidinone **7** (190 mg, 0.345 mmol) dissolved in dichloromethane (4.5 mL), and the mixture was stirred for 2 h at rt under N₂. The reaction mixture was then washed with 5% aqueous hydrochloric acid (20 mL) followed by water (2 \times 20 mL). The organic phase was dried (MgSO₄), and the residue was chromatographed on a 1 mm chromatotron plate eluting with 25% ethyl acetate/75% petroleum ether to give **9** as a clear colorless oil (152 mg, 99%): FTIR (KBr) 3333, 3033, 1794, 1732, 1525 cm⁻¹; ¹H NMR (CDCl₃) δ 3.00 (AB q, *J*_{AB} = 20.1 Hz, $\Delta\nu$ = 14.0 Hz, 2H, CH₂CO), 3.03 (AB q, *J*_{AB} = 13.1 Hz, $\Delta\nu$ = 38.3 Hz, 2H, CH₂Ph), 4.94 (AB q, *J*_{AB} = 9.3 Hz, $\Delta\nu$ = 15.9 Hz, 2H, NOCH₂Ph), 5.09 (AB q, *J*_{AB} = 12.2 Hz, $\Delta\nu$ = 11.9 Hz, 2H, COCH₂Ph), 5.31 (s, 1H, NH), 7.14 (m, 3H), 7.30–7.38 (m, 10H), 7.45 (s, 2H); ¹³C NMR (CDCl₃) δ 37.53, 42.33, 57.48, 67.47, 78.82, 128.29, 128.44, 128.48, 128.61, 129.09, 129.20, 129.77, 130.07, 132.04, 133.42, 135.45, 154.94, 168.43, 172.34; $[\alpha]^{20}_D = +34^\circ$ (*c* = 0.5, CH₂Cl₂); HRMS (FAB, MK⁺) calcd for C₂₆H₂₄N₂O₅K 483.13222, found 483.13330.

(+)-(3*R*)-3-Amino-3-benzyl-1-(benzyloxy)succinimide (**8**). *p*-Toluenesulfonic acid (250 mg, 1.31 mmol) was added to **9** (291 mg, 0.66 mmol) dissolved in toluene (12 mL). The reaction mixture was heated to reflux for 2 h. The solvent was evaporated under reduced pressure, and the residue was taken up in dichloromethane (35 mL) and water (50 mL). The organic phase was separated, washed with water (2 \times 50 mL), and dried (MgSO₄), and the residue was purified by radial chromatography using a 1 mm plate and eluting with 75% ethyl acetate/25% petroleum ether to yield the amine **8** as a white solid (149 mg, 73%) which was recrystallized from ethyl acetate and petroleum ether: mp 109–111 °C; FTIR (KBr) 3385, 3030, 1786, 1718, 1600 cm⁻¹; ¹H NMR (CDCl₃) δ 2.61 (AB q, *J*_{AB} = 18.1 Hz, $\Delta\nu$ = 148.3 Hz, 2H, CH₂CO), 2.92 (AB q, *J*_{AB} = 13.2 Hz, $\Delta\nu$ = 51.5 Hz, 2H, CH₂Ph), 4.87 (AB q, *J*_{AB} = 10.3 Hz, $\Delta\nu$ = 25.4 Hz, 2H, NOCH₂Ph), 7.14 (m, 2H), 7.27–7.43 (m, 8H); ¹³C NMR (CDCl₃) δ 38.60, 44.72, 56.92, 78.59, 127.76, 128.52, 128.84, 129.45, 129.96, 130.09, 133.18, 134.11, 168.94, 175.71; $[\alpha]^{20}_D = +68^\circ$ (*c* = 1, CH₂Cl₂). Anal. Calcd for C₁₈H₁₈N₂O₃: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.62; H, 5.84; N, 9.12.

(+)-(3*R*)-3-(*N*-Acetyl-L-leucylamino)-3-benzyl-1-(benzyloxy)succinimide (**10**). *N*-Acetyl-L-leucine (52 mg, 0.30 mmol) and 1-hydroxybenzotriazole-1H₂O (HOBT) (45 mg, 0.29 mmol) were added to the amine **8** (92 mg, 0.296 mmol) dissolved in dichloromethane (0.9 mL) at 0 °C under N₂. The mixture was stirred at 0 °C for 7 min after which DCC (62 mg, 0.30 mmol) was added. The mixture was stirred for 30 min at 0 °C and

then for a further 5.5 h at rt under N₂. The reaction mixture was diluted with dichloromethane, filtered, and washed successively with 5% aqueous hydrochloric acid (20 mL) and water (2 \times 20 mL). The organic phase was dried (Na₂SO₄), and the residue was chromatographed using a 1 mm chromatotron plate, eluting with 75% ethyl acetate/25% petroleum ether to give **10** as an oil (89 mg, 65%) which was crystallized from ethyl acetate by diffusion with petroleum ether: mp 177–178 °C; FTIR (KBr) 3299, 3064, 2958, 1795, 1731, 1645, 1548 cm⁻¹; ¹H NMR (CDCl₃) δ 0.93 (d, *J* = 6.4 Hz, 3H), 0.95 (d, *J* = 6.4 Hz, 3H), 1.51 (m, 1H), 1.67 (m, 2H), 2.01 (s, 3H), 2.90 (AB q, *J*_{AB} = 17.6 Hz, $\Delta\nu$ = 9.4 Hz, 2H, CH₂CO), 3.06 (AB q, *J*_{AB} = 13.2 Hz, $\Delta\nu$ = 48.2 Hz, 2H, C3CH₂Ph), 4.39 (q, *J* = 7.8 Hz, 1H), 4.92 (AB q, *J*_{AB} = 9.8 Hz, $\Delta\nu$ = 14.0 Hz, 2H, OCH₂Ph), 5.73 (d, *J* = 7.3 Hz, 1H, NH), 6.83 (s, 1H, NH), 7.16 (m, 2H), 7.33 (m, 6H), 7.47 (m, 2H); ¹³C NMR (CDCl₃) δ 22.06, 22.73, 23.05, 24.55, 37.35, 40.53, 41.92, 51.32, 57.36, 78.67, 128.28, 128.42, 129.05, 129.13, 129.68, 130.19, 131.92, 133.54, 168.48, 170.73, 171.69, 172.74; $[\alpha]^{20}_D = +5^\circ$ (*c* = 1, CH₂Cl₂). Anal. Calcd for C₂₆H₃₁N₃O₅: C, 67.08; H, 6.71; N, 9.03. Found: C, 66.90; H, 6.70; N, 9.08.

(+)-(3*R*)-3-(*N*-Acetyl-L-leucylamino)-3-benzyl-1-hydroxysuccinimide (**11**). A mixture of the 1-(benzyloxy)succinimide **10** (64 mg, 0.14 mmol) and 10% Pd on C (7 mg), in dry THF (4 mL), was stirred for 3.5 h under a hydrogen atmosphere. The reaction mixture was filtered and evaporated to dryness. The resulting solid was washed with ethyl acetate and redissolved in ethanol, and the solution was filtered and evaporated to give **11** as a white solid (24 mg, 47%). The residue from the ethyl acetate washings was crystallized from methanol by diffusion with diethyl ether to yield more **11** (5 mg, 10%): mp > 220 °C dec; FTIR (KBr) 3314, 2957, 1793, 1719, 1656, 1536 cm⁻¹; ¹H NMR (CD₃OD) δ 0.95 (d, *J* = 6.4 Hz, 3H), 0.99 (d, *J* = 6.4 Hz, 3H), 1.54 (m, 2H), 1.73 (hept, *J* = 6.8 Hz, 1H), 1.95 (s, 3H), 2.85 (s, 2H), 3.14 (AB q, *J*_{AB} = 13.2 Hz, $\Delta\nu$ = 59.7 Hz, 2H, CH₂Ph), 4.35 (dd, *J* = 6.8, 8.8 Hz, 1H), 7.19 (m, 2H), 7.28 (m, 3H); ¹³C NMR (CD₃OD) δ 22.45, 22.55, 23.60, 26.04, 37.83, 41.96, 42.64, 53.06, 59.13, 129.24, 130.11, 131.66, 134.16, 171.90, 173.73, 175.20, 175.38; $[\alpha]^{20}_D = +13^\circ$ (*c* = 0.2, CH₃OH); HRMS (FAB, MNa⁺) calcd for C₁₉H₂₅N₃O₅Na 398.16921, found 398.16909.

(-)-(3*R*)-3-(*N*-Acetyl-L-leucylamino)-3-benzyl-1-[(methanesulfonyl)oxy]succinimide (**12**). To a suspension of **11** (18 mg, 0.05 mmol) in dichloromethane (0.3 mL) was added diisopropylethylamine (11 μ L, 0.063 mmol). The suspension was cooled to 0 °C under N₂, methanesulfonyl chloride (6 μ L, 0.08 mmol) was added, and the mixture was stirred for 35 min at 0 °C. The mixture was diluted with dichloromethane (15 mL) and filtered to give starting material (4.5 mg, 25%). The filtrate was washed with cold water (10 mL), cold 5% aqueous hydrochloric acid (10 mL), and saturated aqueous sodium bicarbonate (10 mL); dried (Na₂SO₄); and evaporated to dryness under reduced pressure. The residue was further purified by a series of crystallizations from diethyl ether and also ethyl acetate/petroleum ether to give **12** (5.5 mg, 25%): mp 197–201 °C; FTIR (KBr) 3273, 3038, 2930, 1813, 1749, 1536 cm⁻¹; ¹H NMR (CDCl₃) δ 0.91 (d, *J* = 6.4 Hz, 3H), 0.94 (d, *J* = 6.4 Hz, 3H), 1.48 (m, 1H), 1.66 (m, 2H), 2.01 (s, 3H), 3.00 (s, 2H), 3.16 (AB q, *J*_{AB} = 13.2 Hz, $\Delta\nu$ = 59.8 Hz, 2H, CH₂Ph), 3.31 (s, 3H), 4.38 (q, *J* = 7.8 Hz, 1H), 5.70 (d, *J* = 7.4 Hz, 1H, NH), 6.97 (s, 1H, NH), 7.19 (m, 2H), 7.39 (m, 3H); ¹³C NMR (CDCl₃) δ 22.08, 22.75, 23.11, 24.61, 37.53, 40.02, 42.15, 51.28, 57.65, 128.57, 129.26, 130.17, 131.35, 165.99, 169.78, 170.79, 172.45; $[\alpha]^{20}_D = -36^\circ$ (*c* = 0.2, CH₂Cl₂); HRMS (FAB, MH⁺) calcd for C₂₀H₂₈N₃O₇S 454.16478, found 454.16471.

(+)-(3*R*)-3-(*N*-Acetyl-D-leucylamino)-3-benzyl-1-(benzyloxy)succinimide (**13**). A solution of the amine **8** (74 mg, 0.24 mmol) in dichloromethane (0.7 mL) was treated with *N*-acetyl-D-leucine (42 mg, 0.24 mmol), 1-hydroxybenzotriazole-1H₂O (38 mg, 0.25 mmol), and DCC (50 mg, 0.24 mmol) as described for **10** above. Purification by radial chromatography on a 1 mm plate eluting with 75% ethyl acetate/25% petroleum ether gave **13** (68 mg, 61%): mp 195–198 °C (ethyl acetate/petroleum ether); FTIR (KBr) 3263, 3036, 2934, 1796, 1732, 1634 cm⁻¹; ¹H NMR (CDCl₃) δ 0.89 (d, *J* = 6.3 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H), 1.45–1.71 (m, 3H), 2.08 (s, 3H), 2.91 (s, 2H), 3.00 (AB q, *J*_{AB} = 13.2 Hz, $\Delta\nu$ = 9.4 Hz, 2H, CH₂Ph), 4.41 (q, *J* = 8.3 Hz, 1H), 4.94 (AB q, *J*_{AB} = 9.8 Hz, $\Delta\nu$ = 14.6 Hz, 2H, OCH₂Ph), 5.69 (d, *J* = 7.8 Hz, 1H, NH), 7.19 (m, 3H), 7.34 (m, 6H), 7.47 (m, 2H); ¹³C NMR (CDCl₃) δ 21.01, 21.16, 22.88, 24.56, 37.40,

40.51 ($\times 2$), 51.02, 57.34, 78.60, 127.49, 128.30, 128.42, 129.13, 129.38, 130.27, 130.38, 133.52, 168.33, 171.56, 171.65, 174.51; $[\alpha]_D^{20} = +115^\circ$ ($c = 1.2$, CH_2Cl_2); HRMS (EI, M^+) calcd for $\text{C}_{26}\text{H}_{32}\text{N}_3\text{O}_5$ 465.22637, found 465.22698.

(+)-(3*R*)-3-(*N*-Acetyl-*D*-leucylamino)-3-benzyl-1-hydroxysuccinimide (14). A mixture of the 1-(benzyloxy)succinimide **13** (59 mg, 0.127 mmol) and 10% Pd on C (10 mg) in dry THF (4 mL) was stirred for 3.25 h under a hydrogen atmosphere. The reaction mixture was filtered, and filtrate was evaporated to dryness. The residue solid was washed with dichloromethane (3 mL), dissolved in methanol, and refiltered. Evaporation under reduced pressure gave **14** as an oil which crystallized from ethyl acetate and pentane (43 mg, 90%): mp 148–151 °C; FTIR (KBr) 3408, 1792, 1717, 1661 cm^{-1} ; ^1H NMR (CD_3OD) δ 0.92 (d, $J = 6.4$ Hz, 3H), 0.95 (d, $J = 6.4$ Hz, 3H), 1.55 (m, 2H), 1.63 (m, $J = 6.3$ Hz, 1H), 1.97 (s, 3H), 2.82 (AB q, $J_{\text{AB}} = 17.6$ Hz, $\Delta\nu = 24.8$ Hz, 2H, CH_2CO), 3.14 (AB q, $J_{\text{AB}} = 13.2$ Hz, $\Delta\nu = 50.1$ Hz, 2H, CH_2Ph), 4.43 (dd, $J = 6.8$, 8.7 Hz, 1H), 7.20 (m, 2H), 7.28 (m, 3H); ^{13}C NMR (CD_3OD) δ 22.32, 22.75, 23.63, 26.23, 37.77, 42.19, 42.55, 53.05, 58.94, 129.20, 130.13, 131.65, 134.43, 172.34, 173.64, 175.14, 175.56; $[\alpha]_D^{20} = +98^\circ$ ($c = 1.0$, CH_3OH); HRMS (FAB, MNa^+) calcd for $\text{C}_{19}\text{H}_{25}\text{N}_3\text{O}_5\text{Na}$ 398.16922, found 398.16930.

(+)-(3*R*)-3-(*N*-Acetyl-*D*-leucylamino)-3-benzyl-1-(methanesulfonyloxy)succinimide (15). A solution of **14** (45 mg, 0.12 mmol, 1 equiv) in dichloromethane (0.6 mL) was treated with diisopropylethylamine (1.1 equiv) and methanesulfonylchloride (1.5 equiv) as described for **11** above to give **15** as an oil (51 mg, 93%): FTIR (KBr) 3263, 3065, 2959, 1811, 1755, 1645 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.88 (d, $J = 6.4$ Hz, 3H), 0.92 (d, $J = 6.4$ Hz, 3H), 1.49 (m, 1H), 1.59 (m, 1H), 1.69 (m, 1H₂), 2.08 (s, 3H), 3.02 (s, 2H), 3.11 (AB q, $J_{\text{AB}} = 13.1$ Hz, $\Delta\nu = 30.5$ Hz, 2H, CH_2Ph), 3.30 (s, 3H), 4.41 (q, $J = 8.3$ Hz, 1H), 5.69 (br d, $J = 7.3$ Hz, 1H, NH), 7.23 (m, 2H), 7.39 (m, 3H); ^{13}C NMR (CDCl_3) δ 21.07, 22.90, 23.01, 24.55, 37.34, 39.47, 40.23, 40.62, 51.13, 58.11, 127.85, 128.69, 130.28, 131.45, 166.78, 170.11, 171.92, 174.72; $[\alpha]_D^{20} = +85^\circ$ ($c = 0.9$, CH_2Cl_2); HRMS (FAB, MNa^+) calcd for $\text{C}_{20}\text{H}_{27}\text{N}_3\text{O}_7\text{SNa}$ 476.14677, found 476.14676.

(+)-(3*R*)-3-[*N*-(*tert*-Butoxycarbonyl)-*L*-leucylamino]-3-benzyl-1-(benzyloxy)succinimide (16). A solution of the amine **8** (127 mg, 0.41 mmol) in dichloromethane (1 mL) was treated with *N*-*t*-BOC-*L*-leucine (105 mg, 0.42 mmol), 1-hydroxybenzotriazole \cdot H_2O (67 mg, 0.44 mmol) and DCC (89 mg, 0.43 mmol) as described for **10** above. Purification by radial chromatography on a 1 mm silica gel chromatotron plate, eluting with 30% ethyl acetate/70% petroleum ether gave **16** (210 mg, 98%) as an oil: FTIR (KBr) 3302, 2959, 1732, 1659 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.93 (d, $J = 4.9$ Hz, 3H), 0.95 (d, $J = 4.9$ Hz, 3H), 1.44 (s, 9H), 1.6–1.7 (m, 3H), 2.91 (AB q, $J_{\text{AB}} = 17.5$ Hz, $\Delta\nu = 14.9$ Hz, 2H, CH_2CO), 3.06 (AB q, $J_{\text{AB}} = 13.2$ Hz, $\Delta\nu = 32.5$ Hz, 2H, $\text{C}_3\text{CH}_2\text{Ph}$), 4.05 (q, $J = 8.3$ Hz, 1H), 4.75 (d, $J = 6.8$ Hz, 1H, NH), 4.95 (AB q, $J_{\text{AB}} = 9.7$ Hz, $\Delta\nu = 12.1$ Hz, 2H, OCH_2Ph), 6.76 (s, 1H, NH), 7.16 (m, 2H), 7.34 (m, 6H), 7.48 (m, 2H); ^{13}C NMR (CDCl_3) δ 21.61, 22.77, 24.39, 28.13, 37.20, 40.50, 41.72, 52.32, 57.20, 78.56, 80.07, 128.04, 128.26, 128.85, 128.94, 129.55, 130.01, 131.84, 133.39, 155.95, 168.39, 171.64, 173.44; $[\alpha]_D^{20} = +11^\circ$ ($c = 2$, CH_2Cl_2); HRMS (EI, M^+) calcd for $\text{C}_{29}\text{H}_{38}\text{N}_3\text{O}_6$ 523.26824, found 523.26907.

(+)-(3*R*)-3-[*N*-(*tert*-Butoxycarbonyl)-*L*-leucylamino]-3-benzyl-1-hydroxysuccinimide (17). A mixture of the *N*-(benzyloxy)succinimide **16** (180 mg, 0.344 mmol) and 10% Pd on C (13 mg) in dry THF (5 mL) was stirred under a hydrogen atmosphere for 3 h. The mixture was filtered, and the solvent was evaporated under reduced pressure. The residue was washed with ethyl acetate (3 mL), and the filtrate was evaporated to give **17** as an oil (132 mg, 89%): mp 283–285 °C (ethyl acetate/petroleum ether); FTIR (KBr) 3306, 2961, 1792, 1717, 1651 cm^{-1} ; ^1H NMR (CD_3OD) δ 0.94 (d, $J = 6.3$ Hz, 3H), 0.97 (d, $J = 6.3$ Hz, 3H), 1.43 (s, 9H), 1.48 (m, 2H), 1.72 (hept, $J = 6.3$ Hz, 1H), 2.86 (s, 2H), 3.14 (AB q, $J_{\text{AB}} = 13.1$ Hz, $\Delta\nu = 54.9$ Hz, 2H), 4.10 (br t, $J = 7.3$ Hz, 1H), 6.73 (d, $J = 7.5$ Hz, 1H, NH), 7.19 (m, 2H), 7.29 (m, 3H), 8.70 (s, 1H, NH); ^{13}C NMR (CD_3OD) δ 22.41, 23.64, 25.98, 28.97, 37.83, 42.17, 42.63, 54.01, 59.05, 80.83, 129.23, 130.09, 131.60, 134.08, 158.17, 171.85, 175.12, 176.07; $[\alpha]_D^{20} = +21^\circ$ ($c = 2.1$, CH_3OH). Anal. Calcd for $\text{C}_{22}\text{H}_{31}\text{N}_3\text{O}_6$: C, 60.96; H, 7.21; N, 9.69. Found: C, 61.02; H, 7.36; N, 9.71.

(-)-(3*R*)-3-[*N*-(*tert*-Butoxycarbonyl)-*L*-leucylamino]-3-benzyl-1-(methanesulfonyloxy)succinimide (18). A sus-

pension of **17** (50 mg, 0.12 mmol) in dichloromethane (0.5 mL) was treated with diisopropylethylamine (1.1 equiv) and methanesulfonyl chloride (1.6 equiv) as described for **12** above to give **18** as an oil (43 mg, 73%): FTIR (KBr) 3312, 2961, 1813, 1755, 1682 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.91 (d, $J = 5.8$ Hz, 3H), 0.94 (d, $J = 5.8$ Hz, 3H), 1.43 (s, 9H), 1.58–1.68 (m, 3H), 3.01 (s, 2H), 3.15 (AB q, $J_{\text{AB}} = 13.2$ Hz, $\Delta\nu = 46.7$ Hz, 2H, CH_2Ph), 3.33 (s, 3H), 4.06 (br q, $J = 8.4$ Hz, 1H), 4.78 (br d, $J = 6.7$ Hz, 1H, NH), 6.94 (br s, 1H, NH), 7.19 (m, 2H), 7.37 (m, 3H); ^{13}C NMR (CDCl_3) δ 21.68, 22.79, 24.43, 28.10, 37.38, 39.62, 40.31, 41.76, 52.26, 57.51, 80.26, 128.32, 129.08, 130.07, 131.33, 155.90, 166.12, 169.81, 173.45; $[\alpha]_D^{20} = -12^\circ$ ($c = 1.2$, CH_2Cl_2); HRMS (FAB, MNa^+) calcd for $\text{C}_{23}\text{H}_{33}\text{N}_3\text{O}_6\text{SNa}$ 534.18864, found 534.18747.

(-)-(S)-1-(Benzyloxy)-3-[[benzyloxy]carbonyl]amino]succinimide (22). **Method A.** A mixture of *N*-Cbz-*L*-aspartic acid (2 g, 7.4 mmol) was refluxed in acetic anhydride (3.5 mL, 0.037 mmol) for 1.5 h to give **20** as a white solid which was washed with diethyl ether (3×5 mL) (1.69 g, 90%). A solution of *O*-benzylhydroxylamine (444 mg, 3.61 mmol) in toluene (2 mL) was added to a refluxing solution of the anhydride **20** (0.90 g, 3.61 mmol) in toluene (5 mL). The mixture was refluxed for 1 h and then filtered through Na_2SO_4 , and the filtrate was evaporated. The residue was taken up into ethyl acetate (40 mL) and washed with 10% aqueous sodium hydrogen carbonate (40 mL), followed by water (2×40 mL). The organic phase was dried (Na_2SO_4), and solvent removed to yield **22** as a white solid (0.921 g, 72%): mp 142–143 °C; ^1H NMR (CDCl_3) δ 2.77 (dd, $J = 5.4$, 17.8 Hz, 1H), 3.05 (dd, $J = 9.1$, 17.8 Hz, 1H), 4.21 (m, 1H), 5.11 (AB q, $J_{\text{AB}} = 12.2$ Hz, 2H), $\Delta\nu = 6.7$ Hz, 5.14 (s, 2H), 5.47 (br d, $J = 6.3$ Hz, 1H), 7.30–7.41 (m, 8H), 7.50 (br s, 2H); ^{13}C NMR (CDCl_3) δ 33.88, 47.58, 67.56, 78.96, 133.22, 135.53, 155.77, 168.79, 170.23; $[\alpha]_D^{20} = -2^\circ$ ($c = 1$, CH_2Cl_2); HRMS (FAB, MH^+) calcd for $\text{C}_{19}\text{H}_{19}\text{N}_2\text{O}_5$ 355.12939, found 355.12970. **Method B.** To a solution of the oxazolidinone **19**²⁹ (0.207 g, 0.74 mmol) in dichloromethane was added triethylamine (104 μL , 0.75 mmol), *O*-benzylhydroxylamine hydrochloride (0.118 mg, 0.74 mmol), and HOBT (0.114 mg, 0.74 mmol). After the solution was stirred for 10 min, DCC (0.163 mg, 0.79 mmol) was added and the mixture stirred at rt for 18 h. The mixture was filtered, and the filtrate diluted with dichloromethane (15 mL) and washed successively with 5% aqueous hydrogen chloride (20 mL) and water (2×20 mL). The organic phase was dried (MgSO_4), and solvent was removed to give **23** which was not purified further: ^{13}C NMR (CDCl_3) δ 33.03, 33.71, 52.58, 53.40, 68.24, 68.83, 72.87, 73.34, 78.34, 78.88, 128.12, 128.49, 128.62, 128.66, 128.77, 128.84, 129.24, 129.29, 129.56, 129.85, 133.38, 135.27, 154.56, 154.66, 168.50, 169.11, 169.78, 170.37; HRMS (FAB, MK^+) calcd for $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_6\text{K}$ 423.09583, found 423.09580. Compound **23** (108 mg, 0.281 mmol) was treated with triethylamine (0.9 mL, 6.46 mmol) in dichloromethane (8.1 mL) at rt for 3 h. The mixture was washed with 5% aqueous hydrogen chloride (10 mL) and water (2×10 mL). The organic phase was dried (MgSO_4), and the residue was chromatographed on a 1 mm silica gel chromatotron plate eluting with 50% ethyl acetate/50% petroleum ether to give **22** (49 mg, 49%), data as recorded above. Further elution gave recovered starting material **23** (41 mg, 38%).

Acknowledgment. This work was partially supported by research grants from the Marsden Fund and the Foundation for Research Science and Technology (New Zealand). We thank Professor W. T. Robinson (Department of Chemistry, University of Canterbury, New Zealand) for help with the X-ray crystallography.

Supporting Information Available: ^1H NMR spectra of compounds **9**, **12–16**, **18**, and **22** (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO961781I