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INCORPORATION OF AN AMINO FUNCTION IN A (1S,2S,3R)-3-HYDROXY-2-METHOXY-1-CYCLOHEXANE CARBOXYLIC ACID

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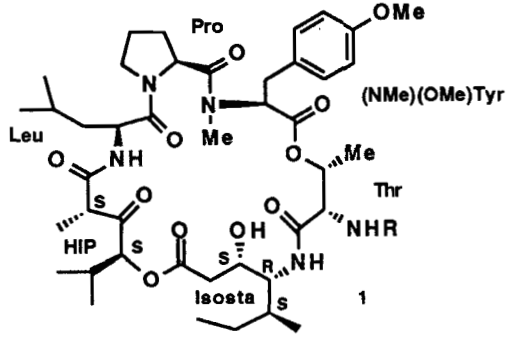
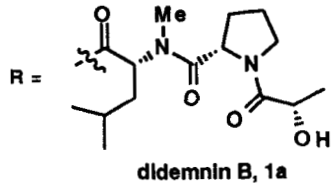
Abstract: *(1S,2S,3R)-3-Hydroxy-2-methoxy-1-cyclohexanecarboxylic acid was synthesized for the construction of an amino acid to be used in a constrained ring didemnin B analog (2). The amine functionality was introduced into the cyclohexane ring by reductive amination using sodium triacetoxyborohydride or by oxime formation followed by reduction.*





R = H·HCl

macrocycle, 1b

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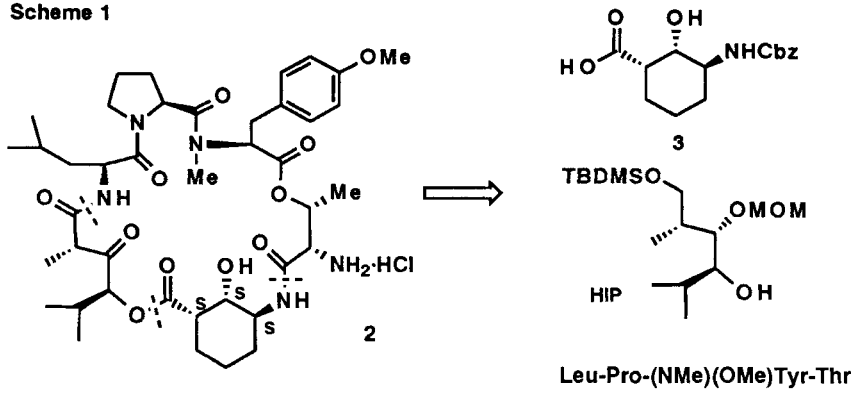
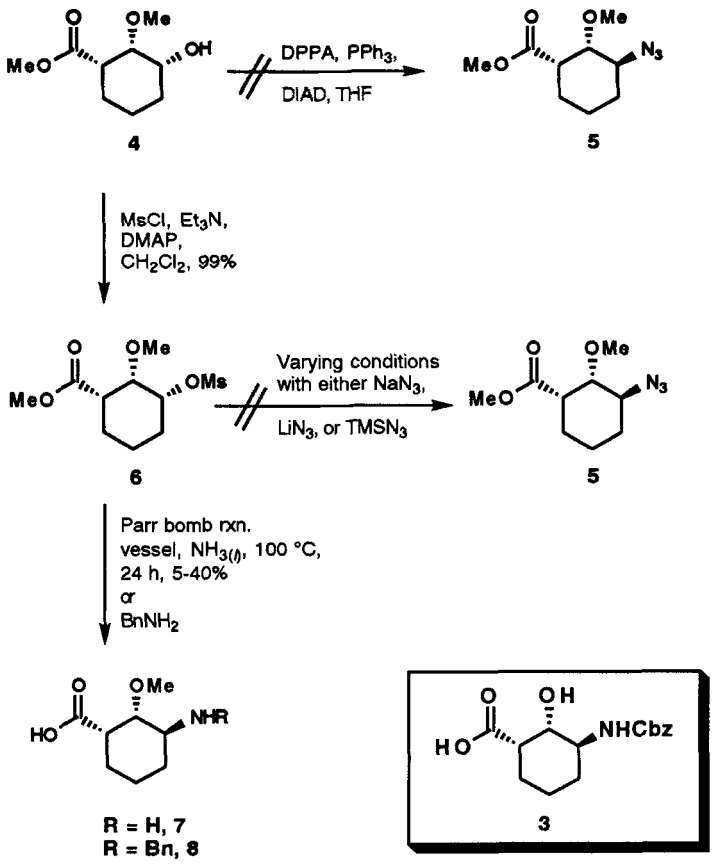
The didemnin class (**1**) of biologically active cyclodepsipeptides isolated from a marine tunicate has shown considerable antitumor, antiviral, and immunosuppressive activity.[1] Of all the members tested so far, didemnin B (**1a**), is the most active.[2-7] As part of our investigations designed to synthesize didemnin B analogs, we aimed to construct a constrained ring analog (**2**) of this natural product in order to determine the effect of modifying the isostatine hydroxyl group, a proposed bioactive structural feature of the macrocycle.[8] The isostatine side chain was tethered to the macrocycle by way of a cyclohexane ring to design a more rigid and structurally stable conformation: the fused ring system **2**. It has been reported that some peptide-like compounds in which (3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid (statine) was replaced with (3S,4S)-4-amino-3-hydroxy-5-cyclohexylpentanoic acid exhibited more promising profiles as antihypertensive agents.[9-11] Such changes can alter the biological activity of the molecules and help to determine binding site/conformation of active compounds. Therefore, we synthesized the hydroxy ester **4** as a key intermediate[8] for the amino acid **3**, needed for the construction of the macrocycle **2** (**Scheme 1**), using previous methodology.[12]

Scheme 1



At this point in our synthesis, investigations were undertaken to find an appropriate transformation to incorporate the amino functionality into the cyclohexane ring of ester **4**. Amine formation *via* azide displacement proved to be an inefficient synthetic pathway, as shown in **Scheme 2**. Initially, alcohol **4** was

Scheme 2



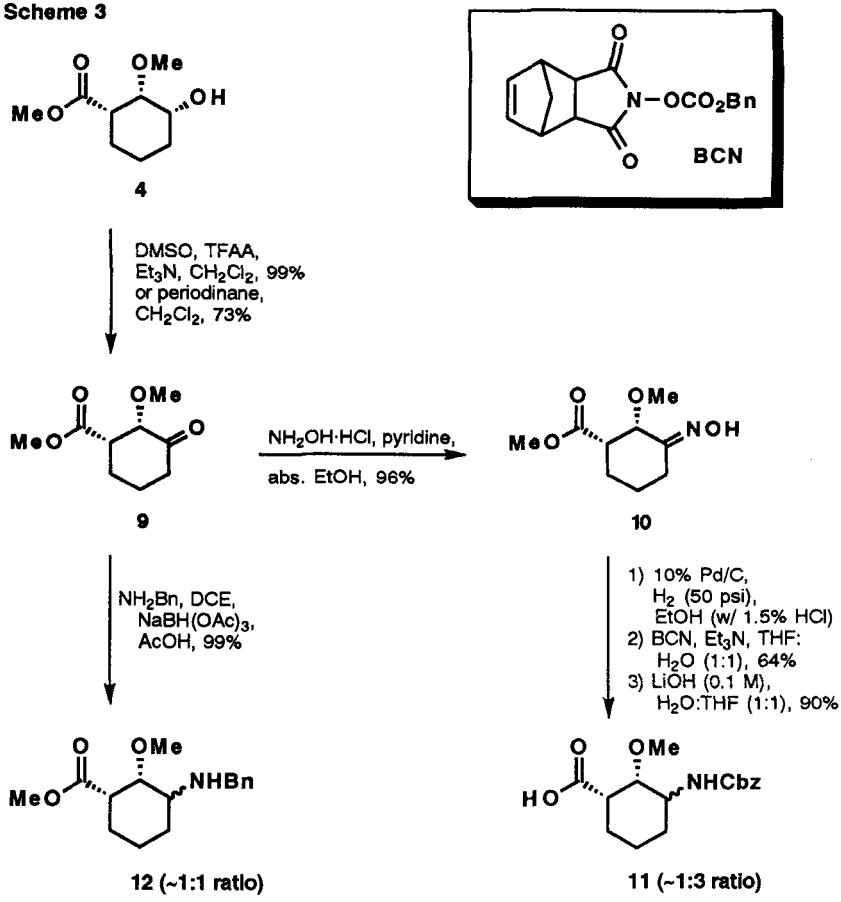
subjected to Mitsunobu conditions incorporating diphenylphosphorylazide (DPPA) and diisopropyl azodicarboxylate (isopropyl analog of DEAD);[13,14] however, no nitrogen functionality was incorporated into the ring, and only impure starting material was obtained. The alcohol was then mesylated (**6**) with the intent of

displacing the mesylate group with either an azide or benzylamine. Displacements with sodium, lithium, and trimethylsilyl azide were attempted in various solvents.[15-17] The conversion could not be effected by either increasing the polarity of the solvent (DMF or DMSO to EtOH:H_2O or MeOH:H_2O) or modifying the ionic character of the reaction (80% AcOH or $LiClO_4$)[18,19] to force the displacement by complexation.Varying the temperature and time of reaction did not affect the outcome, but higher temperatures resulted in decomposition. Since changes in solvents, alterations in temperature, and variations in the reaction time did not produce positive results, more vigorous conditions such as increased pressure were applied. Using a Parr bomb apparatus flushed with ammonia gas at an elevated temperature,[20] a modest yield of amino acid **7** was formed. Benzylamine was also employed as a nucleophile under some of the same conditions (increased temperature and pressure as well as varying solvents), but no displacement reaction afforded the desired compound **8** (**Scheme 2**).

Examination of a Dreiding model of mesylate **6** shows that if the displacement is to occur by an S_N2 mechanism or by a neighboring group assisted S_N1, attack of the nucleophile will be blocked by either steric hindrance or diaxial interactions with the ring hydrogens.

At this point, an alternative route *via* reductive amination was employed for amine formation. The alcohol (**4**) was oxidized under Swern conditions (**Scheme 3**). After obtaining ketone **9**, two approaches were tried. In one case an oxime (**10**) was formed and reduced under acidic hydrogenation conditions before being protected by a Cbz-group using N-[(benzyloxy)carbonyl]-5-norbornene-2,3-dicarboximide (BCN) to afford compound **11** (~1:3 ratio of diastereomers). This reagent is superior to benzyl chloroformate because it is less toxic. The second approach, reductive amination of the ketone with benzyl amine and sodium triacetoxyborohydride was also successful (**12**: ~1:1 ratio).[21,22] Although both

Scheme 3



strategies destroy the third stereogenic center, if a diastereomeric mixture of amines can be obtained, it can be separated at a later stage such as after esterification with the α–(α–hydroxyisovaleryl)propionyl unit (HIP) and both diastereomers can be used to make two new analogs. In addition, the major diastereomer (**3**) may also be obtainable through equilibrating conditions. At this point, unnatural cyclohexyl amino acid **3** is being coupled to the HIP alcohol to produce the lower portion of constrained ring macrocycle as pictured in **Scheme 1.** Investigations of the esterification and incorporation of this amino acid into the macrocycle are currently underway, and results will be published shortly.

Conclusion:

The deceptively simple transformation of an alcohol to an amino group was finally achieved using the reductive amination of ketone **9** with sodium triacetoxyborohydride or the hydrogenation of oxime **10** under acidic conditions. Nucleophilic displacement proved to be an impossible synthetic route due to the steric hindrance present in the cyclohexane ring. The unnatural amino acid **3** will be coupled (**Scheme 1**) to provide the macrocycle (**2**). This new didemnin analog will help define structural parameters which will be useful in the design of other bioactive analogs.

Experimental

General: All manipulations were conducted under an inert atmosphere (argon or nitrogen). All solvents were reagent grade. Anhydrous tetrahydrofuran (THF) was distilled from sodium/benzophenone. Dichloromethane (CH_2Cl_2) was distilled from calcium hydride (CaH_2). Organic acids and bases were reagent grade. All the other reagents were commercial compounds of the highest purity available. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel (60 F-254), plates (0.25 mm), precoated with a fluorescent indicator (0.50 mm plates were used for preparatory thin-layer chromatography). Visualization was effected with ultraviolet light, ninhydrin (0.3% w/v) in absolute ethanol containing 1% acetic acid, phosphomolybic acid reagent (7% w/v) in absolute ethanol, and anisaldehyde reagent (5% v/v) in absolute ethanol containing 5% sulfuric acid and 1% acetic acid. Flash column chromatography was carried out on Merck silica gel 60 particle size (0.040-0.063 mm). Proton and carbon magnetic resonance spectra (^{1}H-, ^{13}C-NMR) were recorded on either a Bruker AM-500 (500 MHz) or a Bruker AM-250 (250 MHz) Fourier transform spectrometer using $CDCl_3$ as the solvent. Chemical shifts were measured in parts per million (δ) relative to tetramethylsilane (TMS-0 ppm) or $CHCl_3$ as an internal reference (7.26 ppm for ^{1}H

and 77.0 ppm for ^{13}C). Coupling constants (J values) are in Hertz (Hz). Multiplicities are designated as singlet (s), broad singlet (bs), doublet (d), doublet of doublets (dd), doublet of doublet of doublets (ddd), doublet of triplets (dt), doublet of quartets (dq),triplet (t), triplet of doublets (td), quartet (q), and multiplet (m). Infrared spectra (IR) were obtained on a Perkin-Elmer Model 281-B or Perkin-Elmer Model 781 spectrometers. Liquids or oils were analyzed as neat films between sodium chloride plates or as $CHCl_3$ solutions in sodium chloride cells. Absorptions are reported in wave numbers (cm^{-1}), and their intensities are designated as strong (s), medium (m), or weak (w). The spectra are calibrated against the 1601 cm^{-1} band of a polystyrene film, and only the most prominent or characteristic absorptions are noted. Optical rotations (in degrees, °) were recorded on a Perkin-Elmer Model 241 polarimeter at the sodium D line. High resolution mass spectra (HRMS) were obtained on either a VG 70-70HS [a high resolution double focusing mass spectrometer using ammonia Chemical Ionization (CI) or Electron Impact (EI)] or a ZAB-E [using Fast Atom Bombardment (FAB), CI or EI]. The mass spectrometer was interfaced to VG/DEC 11-73 data systems. Gas chromatograms were obtained on a Hewlett Packard 5890 GC incorporating a HP-1 Crosslinked Methyl Silicone Gum capillary column. The elemental analysis was performed at the University of Pennsylvania Chemistry Department facility.

Methyl (1S,2S,3R)-3-hydroxy-2-methoxy-1-cyclohexane carboxylate (4). To a solution (0.5 M) of the previously prepared corresponding methoxy lactone (0.183 g, 1.17 mmol)[8] and MeOH (2.35 mL) at 0 °C was added K_2CO_3 (0.162 g, 1.17 mmol), and the mixture stirred at this temperature for 24 h and then at room temperature for 4 h. The resulting slurry was diluted with EtOAc (25 mL) and washed with 0.1 N HCl (3 mL) and saturated NaCl solution (3 mL). The resulting organic solution was dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by flash column chromatography eluting with

acetone:chloroform (1:99) to provide **4** (0.155 g, 70%) as a clear waxy solid; m.p. 45-46 °C; R_f 0.24 (5:95-acetone:chloroform); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.16-1.26 (m, 1H), 1.54 (qd, J=4.0, 12.5 Hz, 1H), 1.66-1.73 (m, 3H), 1.74-1.80 (m, 1H), 2.28 (bs, 1H), 2.39 (t, J=9.2 Hz, 1H), 3.45 (s, 3H), 3.57 (dt, J=4.2, 11.0 Hz, 1H), 3.72 (s, 3H), 3.91 (t, J=2.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.5, 22.0, 29.5, 46.0, 51.6, 60.2, 71.8, 80.6, 173.9; IR (neat) 3100-3650 (s), 2960 (s), 2870 (m), 1730 (s), 1440 (m), 1370 (m), 1310 (m), 1265 (s), 1220 (s), 1185 (s), 1150 (s), 1110 (s), 1080 (s), 1010 (m), 970 (w), 940 (w), 900 (m), 880 (w), 855 (w), 840 (w), 810 (w), 765 (w) cm^{-1}; HRMS *m/z* calcd for $C_9H_{17}O_4$ (M+H): 189.1127, found 189.1138; $[\alpha]_D^{20}$ +6.52° (c=1.04, $CHCl_3$). The other epimeric ester was obtained in 25% yield.

Methyl (1S,2S,3R)-3-mesyloxy-2-methoxy-1-cyclohexane carboxylate (6). A solution of alcohol **4** (0.540 g, 2.87 mmol) in CH_2Cl_2 (25 mL) was cooled to 0 °C, and triethylamine (0.438 mL, 3.14 mmol) was added dropwise. Mesyl chloride (0.287 mL, 3.71 mmol) was then added dropwise followed by DMAP (0.103 g, 0.843 mmol). The reaction was stirred at 0 °C for 0.25 h and at rt for 2 h. The mixture was poured into ice/H_2O (~20 g) and extracted with EtOAc (2 x 100 mL). The resulting organic layers were washed with 10% HCl (20 mL) and saturated NaCl (20 mL) solutions, dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by flash column chromatography eluting with chloroform (100%) then acetone:chloroform (2:98) to obtain **6** (0.756 g, 99%) as an off-white glassy solid; m.p. 97-99 °C; R_f 0.69 (5:95-acetone:chloroform); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25-1.33 (m, 1H), 1.66-1.76 (m, 2H), 1.83-1.88 (m, 2H), 1.92-1.99 (m, 1H), 2.40 (dd, J=4.3, 12.4 Hz, 1H), 3.06 (s, 3H), 3.50 (s, 3H), 3.72 (s, 3H), 4.20 (s, 1H), 4.42 (dd, J=4.2, 12.1 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.6, 22.4, 26.1, 38.7, 46.3, 51.7, 60.8, 78.4, 82.2, 172.3; IR (neat) 3030 (w), 2960 (m), 2870 (w), 2850 (w), 1740 (s),

1440 (m), 1360 (s), 1270 (m), 1250 (w), 1225 (m), 1180 (s), 1140 (w), 1115 (m), 1080 (s), 1035 (m), 960 (s), 940 (s), 905 (m), 885 (m), 860 (m), 830 (m), 770 (w), 755 (w) cm^{-1}; HRMS *m/z* calcd for $C_{10}H_{19}O_6S$ (M+H): 267.0902, found 267.0899; $[\alpha]_D^{20}$ +40.3° (c=1.42, $CHCl_3$); Anal. Calcd for $C_{10}H_{18}O_6S$: C, 45.10; H, 6.81. Found: C, 44.59; H, 6.86.

Methyl (1S,2S,3S)-3-amino-2-methoxy-1-carboxylate cyclohexane (7). Mesylate **6** (19.6 mg, 73.6 μmol) and liquid ammonia (1.56 mL, 70.5 mmol) were combined in a small steel Parr bomb cooled to -78°C. The sealed apparatus was heated to 100 °C for 24 h, and then cooled to -78 °C before opening and releasing excess ammonia gas. The chamber was diluted with Et_2O (10 mL) and 10% NaOH (3 mL). The aqueous layer was neutralized with 10% HCl to pH 7, and then extracted with EtOAc (2 x 15 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to provide **7** (5.1 mg, 40%); R_f 0.48 (10:90-methanol:chloroform); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.63-2.10 (m, 7H), 2.35 (q, J=11.2 Hz, 27.2 1H), 3.43 (s, 3H), 3.91 (d, J=7.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.31, 25.36, 26.2, 36.8, 37.6, 58.8, 71.0, 179.9; IR ($CHCl_3$) 3520 (w), 2400-3460 (m), 2950 (s), 1685 (s), 1455 (m), 1410-1440 (m), 1290 (m), 1250 (m), 1155 (w), 1085 (m), 1040 (w), 1000 (w), 980 (w), 890 (w) cm^{-1}; HRMS *m/z* calcd for $C_8H_{16}NO_3$ (M+H): 174.1130, found 174.1135.

Methyl (1S,2S)-2-methoxy-3-oxo-1-cyclohexane carboxylate (9). Trifluoroacetic anhydride (0.827 mL, 5.83 mmol) in CH_2Cl_2 (2.00 mL) was added dropwise to a solution of DMSO (0.550 mL, 7.76 mmol) in CH_2Cl_2 (4.00 mL), at -78 °C. The resulting mixture was stirred at -78 °C for 20 min, and alcohol **4** (0.292 g, 1.55 mmol) in CH_2Cl_2 (4 mL) was added dropwise. After 1.5 h, triethylamine (1.63 mL, 11.7 mmol) in CH_2Cl_2 (2 mL) was added dropwise. After 1 h at ambient temperature, the reaction was diluted with Et_2O (80 mL). The organic solution was washed with 5% HCl (10 mL), 5% $NaHCO_3$ (10 mL), and

saturated NaCl (10 mL) solutions. The organic layer was dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by flash column chromatography eluting with ethyl acetate:petroleum ether (20:80) to afford **9** (0.286 g, 99% yield) as a clear light yellow oil; R_f 0.43 (30:70-ethyl acetate:petroleum ether); ^1H NMR (500 MHz, $CDCl_3$) δ 1.65-1.72 (m, 1H), 1.94-1.99 (m, 1H), 2.03-2.16 (m, 2H), 2.27 (dt, J=6.1, 13.9 Hz, 1H), 2.66 (ddd, J=5.7, 9.6, 19.5 Hz, 1H), 2.94-2.97 (m, 1H), 3.36 (s, 3H), 3.72 (s, 3H), 3.82 (d, J=3.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.2, 23.8, 38.2, 48.5, 51.9, 58.0, 83.6, 171.8, 208.6; IR (neat) 2960 (m), 2830 (w), 1740 (s), 1450 (m), 1435 (m), 1380 (w), 1335 (w), 1315 (w), 1300 (w), 1255 (m), 1200 (m), 1170 (m), 1135 (w), 1105 (m), 1080 (w), 1060 (w), 1035 (w), 1020 (w), 990 (w), 950 (w), 915 (w), 880 (w), 850 (w), 780 (w), 750 (w), 705 (w) cm^{-1}; HRMS *m/z* calcd for $C_9H_{18}NO_4$: 204.1236, found 204.1230; $[\alpha]_D^{20}$ -15.4° (c=2.00, $CHCl_3$).

Methyl (1S,2S)-3-(N-benzylamino)-2-methoxy-1-cyclohexane carboxylate (12). To a solution of ketone **9** (80.0 mg, 0.430 mmol) and 1,2-dichloroethane (DCE, 4.00 mL) was added benzylamine (51.7 μL, 0.473 mmol) followed by acetic acid (24.6 μL, 0.430 mmol). Sodium triacetoxyborohydride (137mg, 0.645 mmol) was then added to the mixture. The reaction was stirred at rt for 4 h. GC analysis as well as TLC showed that the reaction was complete. The solution was quenched with saturated $NaHCO_3$ solution (4 mL), and the product was extracted with EtOAc (3 x 15 mL), dried ($MgSO_4$), filtered, and concentrated to afford **12** (118 mg, 99%); [GC conditions: Initial temp. 50 °C, Final temp. 225 °C, Rate 10 °C/min., R_f: ketone **9**, 13.95; $BnNH_2$, 8.84; product (2 diast.), 23.83 and 24.90]; R_f 0.60 (10:90-methanol:chloroform); ^1H NMR (500 MHz, $CDCl_3$) δ 1.24-1.53 (m, 6H), 1.70-1.92 (m, 6H), 2.27 (td, J=1.9, 7.8 Hz, 1H), 2.52 (dt, J=2.8, 12.0 Hz, 1H), 2.65 (bs, 2H), 2.92 (td, J=4.1, 10.2 Hz, 1H), 3.13 (dd, J=3.4, 7.1 Hz, 1H), 3.21 (s, 3H), 3.44 (s, 3H), 3.50 (dd, J=3.5, 9.6 Hz, 1H),

3.63-3.67 (m, 1H), 3.67 (s, 3H), 3.71 (s, 3H), 3.85-3.90 (m, 2H), 4.08-4.12 (m, 2H), 7.21-7.37 (m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.6, 21.5, 23.6, 26.83, 26.85, 27.8, 43.9, 46.8, 50.2, 50.8, 51.3, 51.5, 51.6, 56.4, 59.1, 60.3, 78.3, 81.4, 126.8, 126.9, 127.5, 128.0, 128.1, 128.2, 128.3, 128.4, 128.45, 128.5, 140.1, 140.6, 174.2, 175.7; IR (neat) 3330 (w), 3090 (w), 3070 (w), 3030 (w), 2950 (s), 2870 (m), 1740 (s), 1600 (w), 1495 (w), 1435-1465 (m), 1370 (m), 1310 (m), 1260 (m), 1200 (m), 1165 (m), 1130 (m), 1090 (s), 1040 (w), 1030 (m), 990 (w), 960 (w), 935 (w), 900 (w), 880 (w), 840 (w), 735 (m), 700 (m) cm^{-1}; HRMS *m/z* calcd for $C_{16}H_{24}NO_3$ (M+H): 278.1756, found 278.1764; $[\alpha]_D^{20}$ +37.2° (c=1.15, $CHCl_3$).

Methyl (1S,2S)-3-(N-hydroxylamino)-2-methoxycyclohexane carboxylate (10). To a solution of ketone **9** (290 mg, 1.56 mmol) and absolute ethanol (14.5 mL) was added hydroxylamine hydrochloride (141 mg, 2.03 mmol) followed by dropwise addition of pyridine (0.328 mL, 4.06 mmol). The solution was refluxed for 2 h and then cooled to rt before removing the ethanol by distillation. The residue was diluted with cold H_2O (10 mL) and the viscous oil obtained was extracted with EtOAc (100 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated to give **10** (310 mg, 99% yield); R_f 0.39 (5:95-acetone:chloroform); ^1H NMR (250 MHz, $CDCl_3$) δ 1.30-1.45 (m, 1H), 1.78-2.10 (m, 5H), 2.56 (dt, J=5.3, 15.0 Hz, 1H), 3.21 (s, 3H), 3.75 (s, 3H), 4.13 (d, J=3.0 Hz, 1H), 9.12 (bs, 1H); IR ($CHCl_3$) 3580 (m), 3160-3500 (m), 3000 (m), 2950 (s), 2870 (m), 2830 (m), 2410 (w), 1735 (s), 1435 (s), 1370 (m), 1320 (m), 1305 (m), 1175-1260 (s), 1135 (m), 1105 (s), 1085 (s), 1060 (m), 1040 (m), 1015 (m), 965 (m), 945 (m), 900 (s), 880 (w), 840 (m), 650 (w) cm^{-1}; HRMS *m/z* calcd for $C_9H_{15}NO_4$ (M+): 201.1001, found 201.1012.

(1S,2S)-3-(N-benzyloxycarbonylamino)-2-methoxy-1-cyclohexane carboxylic acid (11). Oxime **10** (102 mg, 0.507 mmol) was dissolved

in EtOH (3 mL w/ 1.5% conc. HCl) and to this solution was added $Pd(OH)_2$ (191 mg, 5.07 mmol - dried at 56 °C in drying pistol). The reaction mixture was shaken under an atmosphere of hydrogen (50 psi) for ~4 h and then filtered through Celite to remove the catalyst; the Celite was then washed with a large excess of EtOH. The filtrate was dried (Na_2SO_4), filtered, and concentrated. The residue was azeotroped with toluene (4 x 20 mL) to provide the hydrochloride salt of methyl (1S,2S)-3-amino-2-methoxy-1-cyclohexane carboxylate (112 mg, 99% yield); R_f 0.15 (10:90-methanol:chloroform); HRMS *m/z* calcd for $C_9H_{18}NO_3$ (M-Cl): 188.1287, found 188.1299.

To a solution (0.05M) of the above hydrochloride (113 mg, 0.505 mmol) in THF:H_2O (1:1, 10.0 mL) at 0 °C was added N-[(benzyloxy)carbonyl]-5-norbornene-2,3-dicarboximide (BCN, 174 mg, 0.556 mmol), followed by dropwise addition of triethylamine (0.211 mL, 1.52 mmol). The reaction was stirred at room temperature for 18 h and then concentrated to half volume. The residue was extracted with Et_2O (50 mL). The resulting organic layer was washed with 5% HCl (5 mL), 5% $NaHCO_3$ (5 mL), and saturated NaCl (5 mL) solutions, dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by flash column chromatography eluting with acetone:chloroform (1:99) to afford methyl (1S,2S)-3-(N-benzyloxycarbonylamino)-2-methoxy-1-cyclohexane carboxylate (115 mg, 64% yield) as a clear oil; R_f 0.54 (5:95-acetone:chloroform); ^1H NMR (500 MHz, $CDCl_3$) δ 1.25-1.29 (m, 1H), 1.47 (dq, J=3.7, 12.6 Hz, 1H), 1.62-1.79 (m, 4H), 2.39 (d, J=12.1 Hz, 1H), 3.35 (s, 3H), 3.59-3.65 (m, 1H), 3.70 (s, 3H), 3.98 (s, 1H), 5.06 (d, J=8.7 Hz, 1H), 5.11 (s, 2H), 7.31-7.36 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.0, 23.1, 26.8, 46.2, 51.5, 52.9, 60.4, 66.7, 79.1, 128.0, 128.1, 128.5, 136.5, 155.6, 173.6; HRMS *m/z* calcd for $C_{17}H_{24}NO_5$ (M+H): 322.1659, found 322.1650.

A cooled solution (0.1 M) of LiOH (5.00 mL) was added dropwise to a solution of the protected cyclohexylamino acid ester (93.0 mg, 0.289 mmol) in THF (5.00 mL) at 0 °C. The reaction was stirred at room temperature for 18 h, at which time the solution was concentrated to one half volume. The aqueous residue was washed with Et_2O (2 x 5 mL) and the combined organic layers were back extracted with saturated $NaHCO_3$ (10 mL) solution. All aqueous layers were combined and acidified to pH 2 with 1 M $KHSO_4$. The aqueous layer was then extracted with Et_2O (3 x 30 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and concentrated to afford **11** (80.5 mg, 90% yield); R_f 0.069 (5:95-acetone:chloroform); ^1H NMR (500 MHz, $CDCl_3$) δ 1.25-1.31 (m, 1H), 1.43-1.51 (m, 1H), 1.62-1.79 (m, 4H), 2.43 (d, J=12.3 Hz, 1H), 3.42 (s, 3H), 3.61-3.68 (m, 1H), 4.04 (s, 1H), 5.11 (s, 2H), 5.09-5.18 (m, 1H), 7.31-7.36 (m, 5H); HRMS *m/z* calcd for $C_{16}H_{21}NO_5$ (M+): 307.1420, found 307.1440.

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