Asymmetric Synthesis of a C-3 Substituted Pipecolic Acid

Andrew J. Souers and Jonathan A. Ellman*

University of California at Berkeley, Department of Chemistry, Berkeley, California 94720

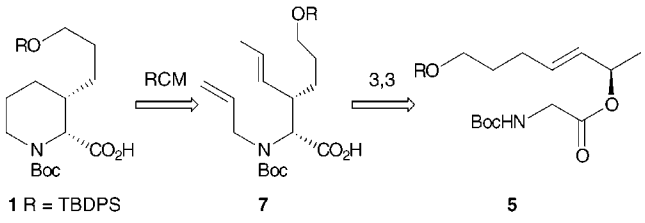
Received August 13, 1999

The design and synthesis of conformationally constrained amino acids has attracted considerable attention from the synthetic and medicinal chemistry communities.[1] The replacement of constrained amino acids for natural amino acids in both peptides and peptidomimetics is a proven strategy for probing the structural requirements of receptor-bound ligand conformations,[2] increasing potency of receptor−ligand interactions,[3] and imparting proteolytic stability.[4] In the course of our ongoing peptidomimetic efforts, we required access to enantiomerically and diastereomerically enriched pipecolic acid derivatives with substitution[5] at the C-3 position.[6] For our purposes, it was important not only that the compound be suitable for incorporation into solid-phase synthesis but also that a "functional handle" be present for postsynthetic modification. Based upon a recent report by Piscopio,[7] we designed and synthesized the novel amino acid **1** through a modular and efficient route that is highly amenable to analogue synthesis.

We envisioned the cyclic amino acid **1** coming from the *N*-allylated amino acid **7** via sequential ring closing metathesis and hydrogenation (Scheme 1). This densely functionalized amino acid would be accessed by the 3,3 Claisen rearrangement of chiral ester **5**, which is derived from chiral allylic alcohol **4** (Scheme 2) and *N*-Boc glycine.

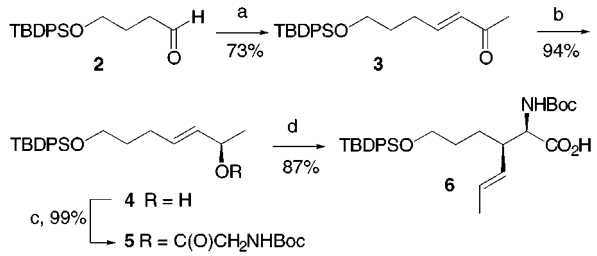
As illustrated in Scheme 2, the synthesis was initiated by Wittig condensation of known aldehyde **2**[8] and the ylide of acetonyltriphenylphosphonium chloride. Refluxing the reaction solution for 24 h in 3:1 dioxane/H_2O afforded the α,β-unsaturated ketone **3** in 73% yield. As expected, only the *E*-alkene isomer was detected by proton NMR spectroscopy. Asymmetric reduction of this ketone with (*S*)-2-methyl-CBS-oxazaborolidene[9] and catecholborane afforded the (*R*)-allylic alcohol **4** in 94% yield and 88% ee as determined by HPLC analysis of the corresponding Mosher esters.[10] The absolute configuration was inferred from ample literature precedent.[11]

Scheme 1



1 R = TBDPS **7** **5**

Scheme 2[a]



2 **3** **6**

[a] Key: (a) acetonyltriphenylphosphonium chloride (1.2 equiv), Na_2CO_3 (1.2 equiv), 3:1 dioxane/H_2O, reflux, 24 h; (b) catecholborane (1.98 equiv), CBS−(*S*)-oxazaborolidene (0.14 equiv), toluene, −78 °C; (c) *N*-Boc-Gly (1.2 equiv), DMAP (0.4 equiv), DIC (1.2 equiv), CH_2Cl_2; (d) LDA (3 equiv), THF, −20 °C → −78 °C then $ZnCl_2$/THF (1.2 equiv) −78 °C → rt, 10 h.

After coupling alcohol **4** with *N*-Boc-Gly to afford ester **5**, we employed a modified version of the Kazmaier 3,3 Claisen rearrangement procedure to set the two contiguous stereocenters of amino acid **6**.[12] Under the optimized conditions (see the Experimental Section), the rearrangement proceeded from the zinc-chelated *Z*-ester enolate to afford acid **6** in 85−90% yield. A single diastereomer was observed by HPLC and NMR analysis. On the basis of the literature precedent of Kazmaier[12] and Bartlett,[13] acid **6** was assigned as the syn diastereomer with *E*-alkene stereochemistry as would be expected from a chair transition state.

We then turned our attention to the selective electrophilic allylation of the Boc-nitrogen in the presence of the carboxylic acid moiety (Scheme 3). Although several different conditions have appeared in the literature,[14] the best reported yield for the allylation of a Boc-protected amino acid was 50%. It was found that allylation using allyl iodide and NaH in THF over several days proceeded in 62−68% yield on a multigram scale, with starting

(1) For recent reviews, see: (a) Gibson, S. E.; Thomas, N.; Guillo, N.; Tozer, M. J. *Tetrahedron*. **1999**, *55*, 585−615. (b) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699−1720. (c) Duthaler, R. O. *Tetrahedron* **1994**, *50*, 1539−1650.

(2) (a) Tellier, F.; Archer, F.; Brabet, I.; Pin, J.-P.; Azerad, R. *Bioorg. Med. Chem.* **1998**, *6*, 195−208. (b) Charette, A. B.; Cote, B. *J. Am. Chem. Soc.* **1995**, *117*, 12721−12732.

(3) Holladay, M. W.; Lin, C. W.; May, C. S.; Garvey, D. S.; Witte, D.; Miller, T. R.; Wolfram, C. A. W.; Nadzan, A. M. *J. Med. Chem.* **1991**, *34*, 455−457.

(4) Ogawa, T.; Yoshitomi, H.; Kodama, H.; Waki, M.; Stammer, C.; Shimohgashi, Y. *FEBS. Lett.* **1989**, *250*, 227.

(5) Swarbrick, M. E.; Gosselin, F.; Lubell, W. D. *J. Org. Chem.* **1999**, *64*, 1993−2002 and ref 18 therein.

(6) (a) Murray, P. J.; Starkey, I. D. *Tetrahedron Lett.* **1996**, *37*, 1875−1878. (b) Christie, B. D.; Rappoport, H. *J. Org. Chem.* **1985**, *50*, 1239−1246. (c) Angle, S. R.; Arnaiz, D. O. *Tetrahedron Lett.* **1989**, *30*, 515−518 and references therein.

(7) (a) Miller, J. F.; Termin, A.; Koch, K.; Piscopio, A. D. *J. Org. Chem.* **1998**, *63*, 3158−3159. (b) For related work, see: Burke, S. D.; Ng, R. A.; Morrison, J. A.; Alberti, M. J. *J. Org. Chem.* **1998**, *63*, 3160−3161.

(8) Ikeda, Y.; Uka, J.; Ikeda, N.; Yamamoto, H. *Tetrahedron.* **1987**, *43*, 731−741.

(9) (a) Corey, E. J.; Helal, C. J. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1986−2012.

(10) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519.

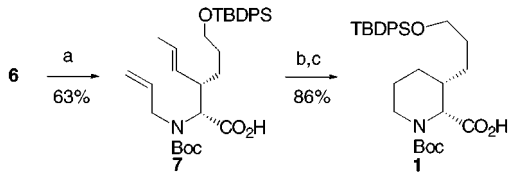
(11) For reductions of related α,β-unsaturated ketones, see: Lögers, M.; Overman, L. E.; Welmaker, G. S. *J. Am. Chem. Soc.* **1995**, *117*, 9139−9150. (b) Corey, E. J.; Bakshi, R. K.; Shibata, S.; Chen, C. P.; Singh, V. K. *J. Am. Chem. Soc.* **1987**, *109*, 7925−7926. (c) Corey, E. J.; Helal, C. J. *Tetrahedron Lett.* **1995**, *36*, 9153−9156. (d) Wipf, P.; Lim, S. *Chimia* **1996**, *50*, 157−167.

(12) Kazmaier, U. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 998−999. (b) Kazmaier, U.; Krebs, A. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2012−2014. (c) Kazmaier, U.; Maier, S. *Tetrahedron*. **1996**, *52*, 941−954.

(13) Bartlett, P. A.; Barstow, J. F. *J. Org. Chem.* **1982**, *47*, 3933−3941. (b) Bartlett, P. A.; Tanzella, D. J.; Barstow, J. F. *Tetrahedron Lett.* **1982**, *23*, 619−622.

10.1021/jo991293l CCC: $19.00
Published on Web 02/03/2000

Scheme 3[a]



[a] Key: (a) allyl iodide (1.5 equiv), NaH (3 equiv), THF, 0 °C → rt, 72 h; (b) Grubbs cat. (0.05 equiv), CH_2Cl_2; (c) 10% Pt/C, H_2, EtOAc, 50 psi, 16 h.

material accounting for the remaining mass. Attempts to accelerate the reaction rate by elevating the reaction temperature or by use of a more polar solvent, such as DMF, resulted in appreciable amounts of O-alkylation. No epimerization was detected in either the recovered starting material or product as determined by HPLC analysis. The densely functionalized amino acid **7** was then subjected to ring closing metathesis conditions using Grubb's phosphorylidine catalyst [$RuCl_2$(=CHPh)-(PCy_3)$_2$].[15] Although the reaction proceeded cleanly, column chromatography was insufficient to completely remove catalyst-derived impurities. The unpurified material was therefore directly submitted to the hydrogenation reaction at 50 psi H_2 with 10% Pt/C. After filtration over Celite, pure amino acid **1** was obtained in 86% overall yield for the two steps.

Two experiments were performed to rigorously establish the diastereomeric and enantiomeric purity of the final pipecolic acid **1.** First, acid **1** was converted to the methyl ester by diazomethane treatment. Upon subjection to base, an approximately 1:1 ratio of the methyl ester of **1** and the corresponding epimer were obtained as determined by HPLC−MS. HPLC analysis of the methyl ester of **1** clearly established that, to the limits of detection (<2%), this compound was not contaminated with the C-2 epimer**.** Second, acid **1** was coupled with both (*S*)-(−)-α- and (*R*)-(+)-α-methylbenzylamine. HPLC analysis of the (*S*)- and (*R*)-amide products established that the final product **1** was obtained in 94% enantiomeric purity.

In summary, we have demonstrated a versatile and efficient asymmetric synthesis route to constrained pipecolic acid derivatives. The overall synthesis yield of **1**, which starts from literature aldehyde **2**, is 32% over seven steps. The stereochemistry of the contiguous C-2 and C-3 stereocenters is relayed from scalemic allylic alcohol **4.** Additionally, other C-3 substituted pipecolic acids could easily be prepared by employing different aldehyde starting materials. The solid-phase incorporation of **1** into small molecule peptidomimetics and subsequent modification of the TBDPS ether via activation/displacement with nucleophiles will be reported in due course.

Materials and Methods

Reagents and General Methods. Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. When used as a reaction solvent, CH_2Cl_2 was distilled from CaH_2, and THF and dioxane were distilled under N_2 from sodium benzophenone ketyl, all immediately prior to use. Deoxygenation of nondistilled solvents and of reaction mixtures was achieved by bubbling N_2 or Ar through them for 15−20 min.

Chromatography was carried out using 230−240 mesh silica gel. Components were visualized either by ultraviolet light or *p*-anisaldehyde staining. Unless otherwise noted, all organic layers were dried over anhydrous Na_2SO_4, and all solvents were removed with a rotary evaporator under aspirator pressure. NMR spectra were recorded on a 500 MHz machine. NMR chemical shifts are expressed in ppm downfield, relative to internal solvent peaks. Coupling constants, *J*, are listed in hertz.[13]C NMR analyses of compounds **6**,**7**, and **1** were complicated by rotameric mixtures of amide rotamers and are not reported. Reversed-phase HPLC was performed on a Dynamax → HPLC system from Rainin using a Microsorb C_{18} column from Rainin. Normal-phase HPLC was performed on a Microsorb Si column from Rainin. Infrared spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrophotometer and taken as thin films on NaCl plates. Elemental analyses were performed by M−H−W Laboratories (Phoenix, AZ) or by the Microanalytical Laboratory at the University of California at Berkeley.

(3*E*)-7-[[(1,1-Dimethylethyl)diphenylsilyl]oxy]-3-hepten-2-one (3). To a 250 mL three-neck flask were added Na_2CO_3 (1.99 g, 18.0 mmol), acetonyltriphenylphosphonium chloride (6.36 g, 17.9 mmol), and 60 mL of a 3:1 dioxane/H_2O solution. The mixture was heated to reflux for 45 min. A dropping funnel containing 4.90 g of aldehyde **2** (14.9 mmol) in 15 mL of dioxane was then added to the flask, and the aldehyde was added dropwise at a slow rate. The reaction mixture was allowed to proceed at reflux for 24 h. Upon cooling to room temperature, the solvents were evaporated, and the residue was taken up in 150 mL of EtOAc. After the addition of 50 mL of 1 M $NaHSO_4$, the mixture was partitioned in a separatory funnel. The aqueous layer was then washed with 1 M $NaHSO_4$ and brine, dried, and filtered. The organic extract was then concentrated in vacuo, and the yellow flakey residue was taken up in 80 mL of hexanes with a very small amount of EtOAc (ca 4−5 mL). After sitting for approximately 1 h, the resultant precipitate (triphenylphosphine oxide) was filtered and rinsed with hexanes. The filtrate was then concentrated and the residue loaded on a column and eluted with 12−25% EtOAc/hexanes to afford 4.01 g (73%) of the title compound as a yellow oil: IR (CH_2Cl_2) 3070, 2930, 2860, 1674 cm^{-1}; ^{1}H NMR (500 MHz, $CDC1_3$): δ; 1.06 (s, 9H), 1.68 (m, 2H), 2.22 (s, 3H), 2.35 (m, 2H), 3.69 (t, J = 6.1, 2H), 6.07 (d, J = 16.0, 1H), 6.79 (m, 1H), 7.37−7.45 (m, 6H), 7.66 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.2, 23.3, 26.8, 28.9, 30.9, 62.9, 127.6, 129.6, 131.5, 133.7, 135.5, 148.0, 198.6. Anal. Calcd for $C_{23}H_{30}O_2Si$: C, 75.36; H, 8.25. Found: C, 75.13; H, 8.58.

(2*R*,3*E*)-7-[[(1,1-Dimethylethyl)diphenylsilyl]oxy]-3-hepten-2-ol (4). To 4.01 g of enone **3** (11.0 mmol, dried by azeotroping 2× with toluene) in dry toluene (83 mL) at −78 °C was added a 1 M solution of (*S*)-2-methyl-CBS-oxazaborolidene (1.60 mL, 1.61 mmol) in toluene. After 5 min, a 1 M solution of catecholborane (2.30 mL, 21.7 mmol) in toluene was added over 20 min, and the solution was maintained at −78 °C for 6 h. The solution was then quenched with MeOH, slowly warmed to room temperature, and washed successively with 0.5 M NaOH (3×), 1 M $NaHSO_4$, and brine. After drying and filtering, the residue was loaded on a column and eluted with 15−30% EtOAc/hexanes to afford 3.78 g of a clear oil in 94% yield and in 88% ee as determined by Mosher ester formation according to the standard procedure.[8] Normal-phase HPLC analysis was performed with a 4.6 mm × 25 cm Si Microsorb column (Rainin, Walnut Creek, CA) and a flow rate of 1 mL/min with 99:1 hexanes−*i*-PrOH as the mobile phase ((*R*,*S*)-ester, R_f = 3.43 min; (*R*,*R*)-ester, R_f = 3.25 min); IR (CH_2Cl_2) 3349, 2930, 2860 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.05 (s, 9H), 1.24 (d, J = 6.33, 3H), 1.66 (m, 2H), 2.13 (m, 2H), 3.68 (t, J = 6.31, 2H), 3.71 (m, 1H), 4.22 (m, 1H), 5.50 (dd, J = 6.51, 15.4, 1H), 5.57 (m, 1H), 7.37−7.46 (m, 6H), 7.66−7.68 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.18, 23.3, 26.8, 28.3, 31.9, 63.1, 68.9, 127.6, 129.5, 130.5, 134.0, 134.4, 135.5. Anal. Calcd for $C_{23}H_{32}O_2Si$: C, 74.95; H, 8.75. Found: C, 75.06; H, 8.78.

(14) (a) Mouna, A. M.; Nguyen, C.; Rage, I.; Xie, J.; Nee, G.; Mazaleyrat, J. P.; Wakselman, M. *Synth. Commun.* **1994**, *24*, 2429−2435. (b) Hon, Y.; Chang, R. *Heterocycles* **1991**, *32*, 1089−1099. (c) Pitzele, B. S.; Hamilton, R. W.; Kudla, K. D.; Tsymbalov, S.; Stapelfeld, A.; Savage, M. A.; Clare, M. J.; Hammond, D. L.; Hansen Jr., D. W. *J. Med. Chem.* **1994**, *39*, 888−896.

(15) (a) Schwab, P.; Grubbs, R. M.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 100−110. (b) Dias, E. L.; Nguyen, S. T.; Grubbs, R. H. *J. Am. Chem. Soc.* **1997**, *119*, 3887−3897.

***N*-[(1,1-Dimethylethoxy)carbonyl]glycine (1*R*,2*E*)-6-1,1-Dimethylethyl)diphenylsilyl]oxy]-2-hexenyl Ester (5).** The alcohol **4** (3.76 g, 10.2 mmol) was dissolved in 51 mL of distilled CH_2Cl_2 and 2.15 g of *N*-Boc-Gly (12.3 mmol) was added, followed by 0.4 equiv of DMAP (0.50 g, 4.1 mmol). The solution was then cooled to 0 °C, and 1.2 equiv of DIC (1.92 mL, 12.3 mmol) was added via syringe. The solution was allowed to slowly warm to room temperature and was then stirred overnight. The solvents were evaporated in vacuo, and the residue was taken up in EtOAc. After being washed with 1 M $NaHSO_4$ (3×), saturated $NaHCO_3$ (3×), and brine, the organic layer was dried and filtered. After concentration in vacuo, the residue was loaded on a small plug of silica gel and eluted with 15% EtOAc/hexanes. The title compound (5.30 g) was obtained as a clear oil in 99% yield: IR (CH_2Cl_2) 3372, 2930, 2860, 1715 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.00 (s, 9H), 1.25 (d, J = 6.33, 3H), 1.42 (s, 9H), 1.65 (m, 2H), 2.14 (m, 2H), 3.66 (t, J = 6.20, 2H), 3.87 (m, 2H), 5.01 (br s, 1H), 5.36 (m, 1H), 5.45 (dd, J = 6.70, 16.0, 1H), 5.71 (m, 1H), 7.37−7.46 (m, 6H), 7.76−7.68 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.4, 18.5, 21.7, 22.8, 25.1, 26.5, 26.7, 30.0, 61.3, 70.7, 125.8, 127.3, 127.5, 127.8, 132.2, 133.8, 154.4, 168.4. Anal. Calcd for $C_{30}H_{43}NO_5Si$: C, 68.53; H, 8.24; N, 2.66. Found: C, 68.23; H, 8.03; N, 2.69.

(2*R*,3*R*,4*E*)-2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]propyl]-4-hexenoic Acid (6). A solution of LDA was prepared by the slow addition of *n*-BuLi (2.20 M, 14.0 mL, 30.6 mmol) to diisopropylamine (4.71 mL, 33.7 mmol) in 135 mL of THF at −20 °C. After being stirred for 20 min, the solution was cooled to −78 °C, and 35 mL of a 0.29 M solution of ester **5** (5.36 g, 10.2 mmol) in THF was slowly added. After 5 min, 24.5 mL of a $ZnCl_2$ solution in THF (0.500 M, 12.3 mmol) was added in one portion, and the reaction mixture was allowed to warm to room temperature over the course of 8−10 h. After this time, the reaction was quenched with 5 mL of 1 M $NaHSO_4$, and the solvents were removed in vacuo. The residue was taken up in 120 mL of EtOAc, washed with 1 M $NaHSO_4$ (3 × 50 mL) and brine, dried, and filtered. Column chromatography with 35−75% EtOAc/hexanes afforded 4.68 g (87%) of the title compound as a pale yellow oil: IR (CH_2Cl_2) 3058, 2918, 2860, 1715 cm^{-1}; ^1H NMR (500 MHz, MeOH-d_4) δ 1.02 (s, 9H), 1.39−1.46 (m, 2H), 1.43 (s, 9H), 1.62 (m, 2H), 1.66 (d, J = 6.03, 3H), 2.32 (m, 1H), 3.64 (m, 2H), 4.01 (m, 1H), 5.20 (m, 1H), 5.46 (m, 1H), 7.37−7.42 (m, 6H), 7.64−7.66 (m, 4H). Anal. Calcd for $C_{30}H_{43}NO_5Si$: C, 68.53; H, 8.24; N, 2.66. Found: C, 68.38; H, 8.40; N, 2.62.

(2*R*,3*R*,4*E*)-2-[[(1,1-Dimethylethoxy)carbonyl]-2-propenylamino]-3-[3-[[(1,1-dimethylethyl)diphenylsilyl]-oxy]propyl]-4-hexenoic Acid (7). The acid **6** (4.60 g, 8.76 mmol) was dissolved in 53 mL of THF and cooled to 0 °C. NaH (65%, 1.07 g, 26.7 mmol) was added in portions over a period of 45 min, followed by the addition of allyl iodide (1.22 mL, 13.4 mmol). The reaction mixture was allowed to warm to room temperature and then stirred vigorously for ca. 72 h. After this time, the reaction was quenched with 10 mL of 1 M $NaHSO_4$, and the solvents were evaporated. The residue was taken up in EtOAc, washed with 1 M $NaHSO_4$ (3×) and brine, dried, and filtered. Chromatography with 9:1 CH_2Cl_2/EtOAc with 1% AcOH afforded 3.10 g of the title compound in 63% yield: IR (CH_2Cl_2) 3070, 2918, 2860,1697 cm^{-1}; ^1H NMR (500 MHz, MeOH-d_4) δ 1.02 (s, 9H), 1.42 (s, 9H), 1.41−1.49 (m, 2H), 1.63 (m, 2H), 1.67 (d, J = 6.03, 3H), 2.54 (m, 1H), 3.67 (m, 2H), 3.96 (m, 2H), 4.54 (m, 1H), 5.05−5.19 (m, 3H), 5.52 (m, 1H), 5.85 (m, 1H), 7.39−7.46 (m, 6H), 7.65−7.67 (m, 4H). Anal. Calcd for $C_{33}H_{47}NO_5Si$: C, 70.05; H, 8.37; N, 2.48. Found: C, 70.08; H, 8.17; N, 2.50.

1-(1,1-Dimethylethyl) Hydrogen (2*R*,3*R*)-3-[3-[[(1,1-Dimethylethyl)diphenylsilyl]oxy]propyl]-1,2-piperidinedicarboxylate (1). To a 250 mL flask were added 3 g (5.31 mmol) of amino acid **7** and 71 mL of CH_2Cl_2. The solution was degassed, and 218 mg (0.270 mmol, 5%) of Grubbs phosphorylidine catalyst was added. The solution turned bright purple upon addition, and was stirred at room temperature overnight. The reaction solution was then concentrated in vacuo, and the contents were loaded on a short plug of silica gel. Elution with CH_2Cl_2 afforded the product in 86% yield by mass. The resultant oil was slightly discolored by ruthenium complexes, although they were undetectable by NMR and TLC. The oil was then taken up in 50 mL of EtOAc and added to a 150 mL Parr shaker bottle. After the addition of 1.78 g of 10% platinum on carbon (50% water weight, 0.460 mmol), the mixture was purged with hydrogen and then shaken for 16 h at 50 psi. After the mixture was filtered over Celite, the pure product was isolated as a colorless oil in 86% yield over the two steps (2.40 g, 4.57 mmol): IR (CH_2Cl_2) 2932, 2858, 1698 cm^{-1}; ^1H NMR (500 MHz, MeOH-d_4) δ 1.14 (s, 9H), 1.36 (m, 1H), 1.51−1.55 (m, 2H), 1.52 (s, 9H), 1.67−1.74 (m, 6H), 3.26 (m, 1H), 3.4 (m, 1H), 3.75 (m, 1H), 3.94 (m, 1H), 4.69−4.80 (m, 1H), 7.42−7.51 (m, 6H), 7.69−7.78 (m, 4H). Anal. Calcd for $C_{30}H_{43}NO_5Si$: C, 68.53; H, 8.24; N, 2.66. Found: C, 68.39; H, 8.10; N, 2.73.

LC-MS Analysis of Amino Ester. To assess the diastereomeric purity of acid **1**, a small aliquot was converted to the corresponding methyl ester by diazomethane treatment. An aliquot was injected on a reversed-phase LC-MS with a Zorbax SB-C_{18} column (2.1 mm × 5 cm, Agilent Technologies, Wilmington, DE) (0.5 mL/min, 10%-100% CH_3CN/H_2O over 10 min, 254 nm) to afford a single peak (R_f = 11.9 min, mass + Na = 562.3). A 20 mg (0.030 mmol) aliquot of the methyl ester was then taken up in 0.5 mL of THF and the solution cooled to −78 °C. After addition of 0.06 mL of a 1.2 M KHMDS/THF solution (0.070 mmol), the reaction solution was stirred at −78 °C for 20 min. Following this, 0.5 mL of a saturated NH_4Cl solution was added, and the mixture was allowed to warm to room temperature. The mixture was then extracted twice with EtOAc, the organic layers were combined and dried, and the resultant solution was evaporated to afford a clear oil. The oil was taken up in CH_3CN, and a small aliquot was injected on an LC-MS using the previously described parameters. Analysis afforded two peaks in a roughly 1:1 ratio (R_f = 11.9 min, mass + Na = 562.3, and 12.0 min, mass + Na = 562.3), demonstrating that the C-2/C-3 diastereomer was not present in acid **1** to the levels of detection (<2%) by HPLC analysis.

HPLC Analysis of α-Methylbenzyl Amide Derivatives. To establish the enantiomeric purity of final amino acid **1**, a 30 mg (0.060 mmol) aliquot was coupled with (*S*)-(−)-α-methylbenzylamine (15 mg,0.12 mmol) using HATU (46 mg, 0.12 mmol) and *i*-Pr_2EtN (42 μL, 0.24 mmol) in CH_2Cl_2. Upon disappearance of the starting material by TLC, the solution was washed with 1 M $NaHSO_4$, dried, and filtered. Evaporation of the volatiles afforded a clear oil. The same procedure was performed with (*R*)-(+)-α−methylbenzylamine. Co-injection of the (*S*)- and (*R*)-amide products on a reversed-phase Microsorb C_{18} column (4.6 mm × 25 cm, Rainin, Walnut Creek) [1 mL/min, 10−50% CH_3CN/H_2O (0.1% TFA) over 30 min, 254 nM] provided two peaks with retention times of 34.8 and 35.3 min. Individual HPLC analysis of the (*R*)-amide showed the same two peaks (34.8 and 35.3 min) in a 32.5:1 ratio, respectively. As expected, similar analysis of the (*S*)-amide showed the same two peaks in a 1:32 ratio. The enantiomeric excess of amino acid **1** is therefore 94%.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (GM53696). A.J.S. would like to thank Pharmacia-Upjohn for financial support.

JO991293L