Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: *Asymmetry* 16 (2005) 3963–3969
Tetrahedron: *Asymmetry*

A stereoselective synthesis of (2*S*,3*R*)-β-methoxyphenylalanine: a component of cyclomarin A

Darren B. Hansen and Madeleine M. Joullié*

Department of Chemistry, University of Pennsylvania, 231 South 34th Street, Philadelphia, PA 19104, USA

Received 20 June 2005; revised 23 September 2005; accepted 4 October 2005

Abstract—A stereoselective synthesis of (2*S*,3*R*)-β-methoxyphenylalanine, an amino acid contained within the cyclic peptide cyclomarin A, was successfully synthesized from Lajoie's serine aldehyde.

1. Introduction

The presence of β-methoxy amino acids in biologically significant natural products[1–5] requires the stereoselective syntheses of these amino acids. Cyclomarin A is a novel cyclic peptide isolated from aestuarine actinomycete, cultured from a sediment sample collected in Mission Bay, California[5] (Fig. 1). Cyclomarin A is cytotoxic toward cancer cells with a mean IC_{50} of 2.6 μM against a panel of human cancer cell lines. Of greater interest is cyclomarin A's potent anti-inflammatory activity both in vitro and in vivo. The structure of cyclomarin A was deduced using a variety of spectroscopic methods. The number of unusual amino acids contained with cyclomarin A has attracted significant interest from the synthetic community,[6–9] culminating in the synthesis of cyclomarin C[10,11] a close congener of cyclomarin A and two separate syntheses of (2*S*,3*R*)-β-methoxyphenylalanine. The first synthesis of the desired β-methoxyphenylalanine was based on the Schöllkopf's chiral bis-lactam,[6] while the second is based on a stereoselective radical bromination.[10] En route to a total synthesis of cyclomarin A, we herein report the synthesis of (2*S*,3*R*)-β-methoxyphenylalanine based on Lajoie's serine aldehyde.

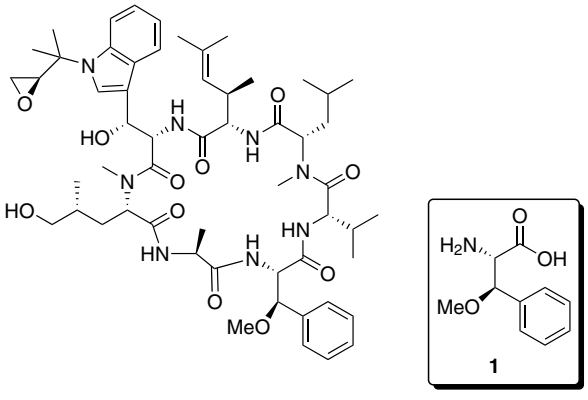


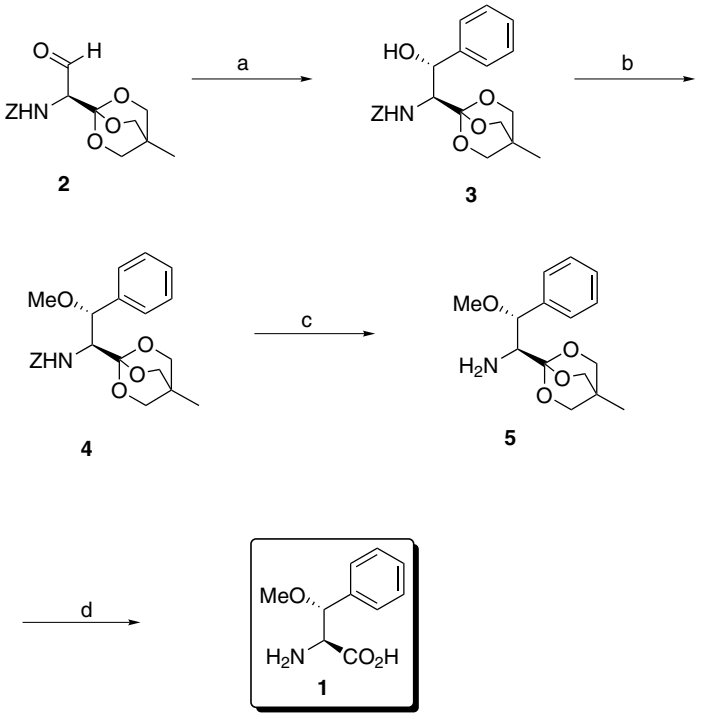
Figure 1. Cyclomarin A and (2*S*,3*R*) β-methoxyphenylalanine.

2. Results and discussion

Lajoie's serine aldehyde has proven to be a powerful intermediate in the synthesis of β-hydroxy and β-methoxy amino acids.[12–14] The method is general and allows for the generation of both *threo* and *erythro* diastereomers in a straightforward fashion. The serine aldehyde was prepared according to the literature procedures.[14,15] The addition of phenyl magnesium bromide to serine aldehyde afforded β-hydroxy phenylalanine intermediate **3** (Scheme 1). Methylation of the hydroxyl group utilized a combination of $Me_3O^+BF_4^-$ and proton sponge to give intermediate **4**.[16] Hydrogenolysis of the Cbz group gave amine **5**, which was amenable to chromatographic separation of the small amount of undesired diastereomer. Exposure to Lajoie's deprotection[12] conditions followed by ion-exchange chromatography allowed for the isolation of (2*S*,3*R*) β-methoxyphenylalanine **1**.

* Corresponding author. Tel.: +1 215 898 3158; fax: +1 215 573 9711; e-mail: mjoullie@sas.upenn.edu

0957-4166/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2005.10.029



Scheme 1. Reagents and conditions: (a) PhMgBr 61% yield, 90% de; (b) $Me_3O^+BF_4^-$, proton sponge®, 96%; (c) 10% Pd/C, H_2; (d) (1) 0.1% aqueous TFA, (2) 10% aqueous Cs_2CO_3, 88%.

To further extend our work toward the synthesis of β-methoxyphenylalanine, we sought to synthesize the opposite diastereomer of **1** to showcase the power of this method. Dess–Martin oxidation of **3** provided β-keto intermediate **4** (Scheme 2). Reduction of the carbonyl with $LiBH_4$ under Felkin–Anh control gave clean inversion of the hydroxyl group to give diastereomer **7**. Methylation was accomplished as described earlier to afford **8**. Hydrogenolysis of the Cbz group then provided amine **9**. Exposure of amine **9** to Lajoie's deprotection conditions followed by ion-exchange chromatography allowed for the isolation of (2*S*,3*S*)-β-methoxyphenylalanine **10**.

Having synthesized both diastereomers of β-methoxyphenylalanine (β-MeO-Phe), we investigated whether it would participate in the initial coupling reactions necessary for the synthesis of cyclomarin A. (2*S*,3*R*)-β-Methoxyphenylalanine **1** was suitably protected in a two-step procedure. First the nitrogen was protected as a Cbz carbamate under Schotten–Baumen conditions. The crude *Z*-protected β-MeO-Phe was esterified with 2-(trimethylsilyl)ethanol (TMSE) to give the desired 2-(trimethylsilyl)ethyl ester **11** (Scheme 3) in good yield. Removal of the Cbz group proceeded without incident, and the crude amine was then coupled Cbz-Ala to provide dipeptide **12** in good yield.

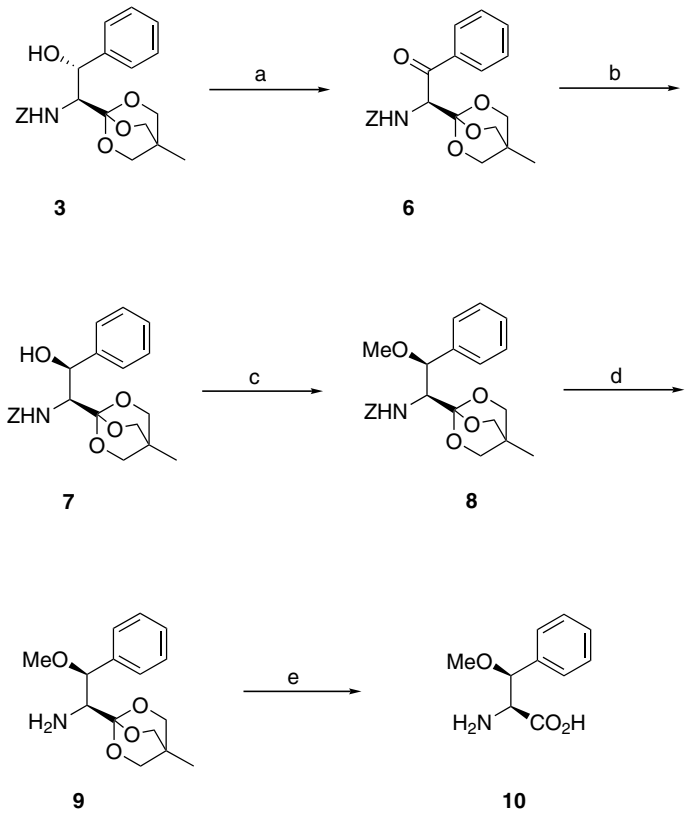
A different approach was investigated utilizing the coupling of the carboxyl group of the β-MeO-Phe **1**. In our previous synthesis of β-methoxytyrosine,[14] we had shown that the *ortho*-ester could be opened in the presence of the Cbz protected nitrogen without any accompanying racemization of the α-stereocenter. If the diol-ester could be cleaved, the resulting acid could then be coupled with other amino acids, extending Lajoie's methodology and shortening the total synthesis of cyclomarin A. Dipeptide **13** was prepared by coupling *Z*-Val with N(Me)-Leu-OTMSE. The *ortho*-ester intermediate **4** was deprotected with aqueous acetic acid to give crude a diol-ester, which was then saponified with aqueous lithium hydroxide to give the crude free acid. Hydrogenolysis of the Cbz group from **13** gave an amine, which was directly coupled with **4** to give a moderate yield of tripeptide **14** (Scheme 4).

3. Conclusion

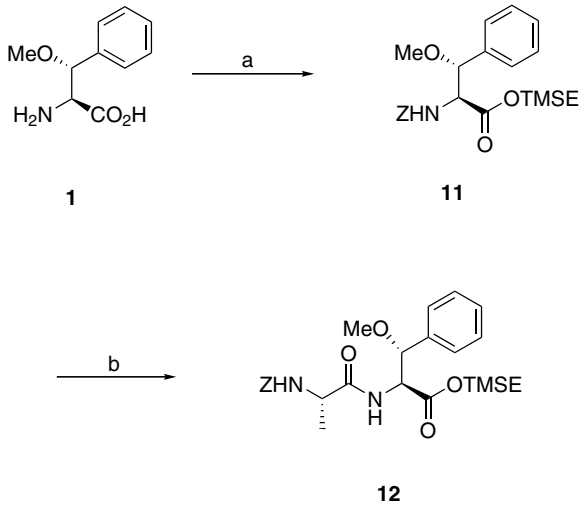
An efficient synthesis of (2*S*,3*R*)-β-methoxyphenylalanine **1** was achieved. The power of Lajoie's serine aldehyde was particularly evident in that it ultimately could be exploited to the synthesis of (2*S*,3*S*) diastereomer of β-methoxyphenylalanine **10** as well. The *ortho*-ester protecting group can be selectively removed to allow for the formation of tripeptide **14** found within cyclomarin A.

4. Experimental

Reactions requiring air-sensitive manipulations were conducted under an argon atmosphere. Methylene chloride was distilled from calcium hydride, tetrahydrofuran and diethyl ether were distilled from sodium/benzophenone. Analytical TLC was performed on 0.25 mm E. silica gel 60 F_{254} plates. Silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromato-



Scheme 2. Reagents and conditions: (a) Dess–Martin periodinane, 89%; (b) $LiBH_4$, 74% > 95% de; (c) $Me_3O^+BF_4^-$, proton sponge®, 73%; (d) 10% Pd/C, H_2; (e) (1) 0.1% aqueous TFA, (2) 10% aqueous Cs_2CO_3, 62% three steps.



Scheme 3. Reagents and conditions: (a) (1) Cbz-Cl, $NaHCO_3$; (2) EDAC, NEt_3, TMSE, DMAP, 79% two steps; (b) (1) 10% Pd/C, H_2; (2) *Z*-Ala, EDAC, NEt_3, 62% two steps.

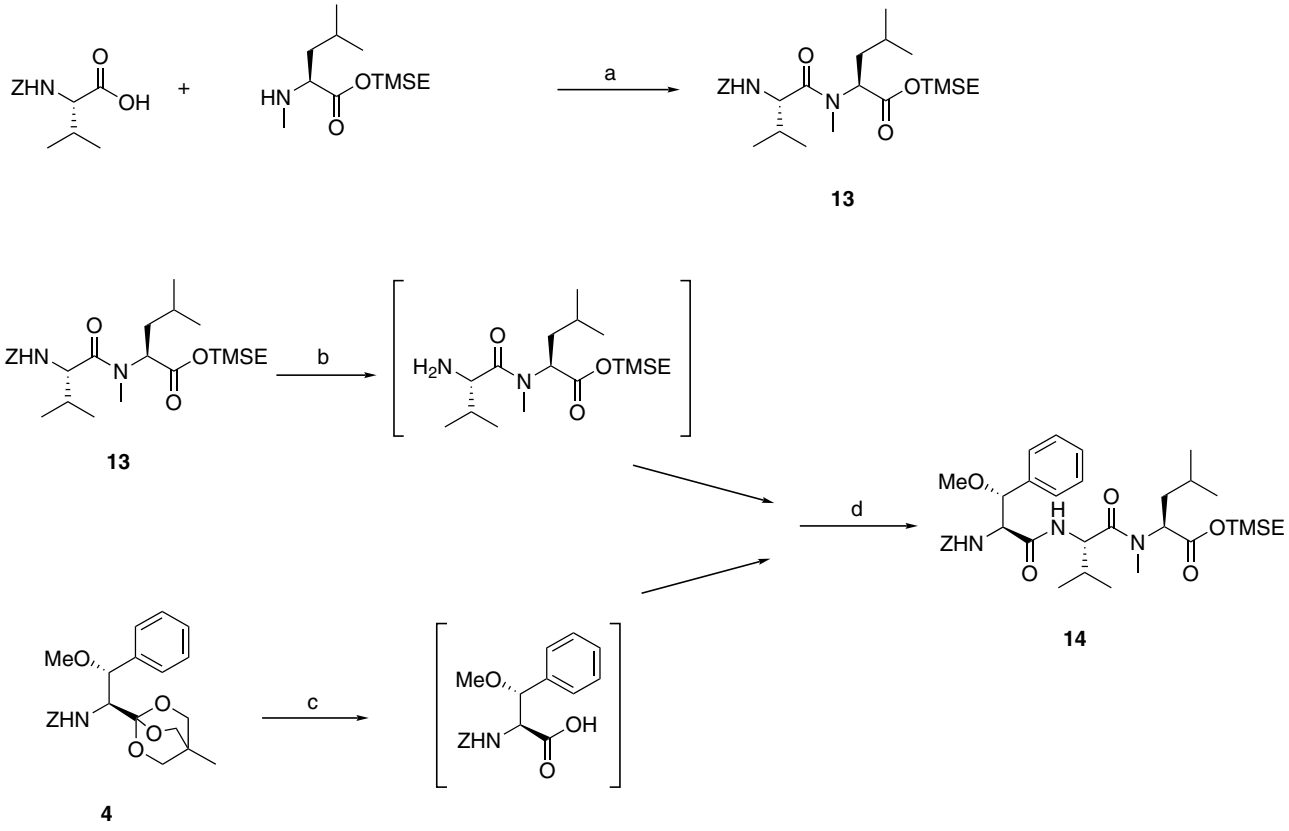
graphy. NMR spectra were recorded on a 500 MHz spectrometer and calibrated by using residual undeuterated solvent or TMS as an internal reference. Chemical shifts (δ) were measured in parts per million, and coupling constants (*J* values) in Hertz (Hz). Infrared spectra (IR) were recorded on an FT-IR spectrometer. High-resolution mass spectra (HRMS) were recorded using electrospray ionization (ESI). Optical rotations were recorded on a polarimeter at the sodium D line. Melting points were determined in an open capillary tube and were uncorrected.

4.1. 2-(4′-Methyl-2′,6′,7′-trioxabicyclo[2.2.2]octan-1′-yl)-2-benzyloxycarbonylaminoethanal (Lajoie's serine aldehyde) 2

Prepared according to the literature procedures.[14,15]

4.2. Benzyl-(1*S*,2*R*)-2-hydroxy-1-(4′-methyl-2′,6′,7′-trioxabicyclo[2.2.2]octan-1′-yl)-2-phenylethylcarbamate 3

Serine aldehyde **2** (30.9 mmol) was dissolved in anhydrous CH_2Cl_2 (300 mL), diluted with anhydrous Et_2O (300 mL) under argon and cooled to 0 °C. PhMgBr (3.0 M Et_2O, 35 mL, 105 mmol) was added and the reaction was allowed to stir for 5 min. The reaction was quenched at 0 °C with 5% aqueous NH_4Cl, and diluted with Et_2O (500 mL). The organic layer was separated and washed with water, then brine, dried over $MgSO_4$, and reduced in vacuo. The residue was purified by flash silica gel chromatography, eluting with 30–50% EtOAc–Hex to give a white powder (7.50 g, 61% yield, 90% de determined by integration of the α proton) : mp: 132–134 °C; $[\alpha]_D^{20} = -35.3$ (*c* 0.6, $CHCl_3$); TLC (50% EtOAc–Hex), $R_f = 0.36$; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.20 (m, 10H), 5.42 (d, $J = 10.3$, 1H), 5.34 (s, 1H), 5.00–4.91 (m, 1H), 4.86–4.79 (m, 1H), 4.09 (d, $J = 10.1$, 1H), 3.99 (s, 6H), 3.35 (s, 1H), 0.85 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.3, 140.0, 136.7, 128.4, 128.1, 127.8, 127.7, 127.3, 125.9, 108.8, 72.9,



Scheme 4. Reagents and conditions: (a) DCC, 48%; (b) Pd/C, H_2; (c) (1) aqueous HOAc; (2) LiOH; (d) EDAC, DIEA 45% over four steps.

70.8, 66.5, 58.6, 30.7, 14.3; IR (thin film) 3506 w, 3448 w, 2942 w, 2884 w, 1725 s br, 1513 m, 1448 w, 1396 w, 1331 w, 1290 m, 1225 m, 1196 m, 1090 m, 1049 s, 1020 s, 984 m, 726 m, 696 m; HRMS (EI): *m/z* calcd for $C_{22}H_{25}O_6NNa$ 422.1580, found 422.1564.

4.3. Benzyl-(1*S*,2*R*)-2-methoxy-1-(4′-methyl-2′,6′,7′-trioxabicyclo[2.2.2]octan-1′-yl)-2-phenylethylcarbamate 4

Compound **3** (7.80 g, 19.6 mmol) was dissolved in anhydrous CH_2Cl_2 (250 mL), under argon, and to the solution was added 4 Å mol. sieves (20.00 g), and proton sponge® (16.80 g, 78.00 mmol), followed by $MeO^+BF_4^-$ (10.00 g, 67.6 mmol). The reaction was allowed to stir under argon vigorously for 5 h. When the reaction was shown to be complete by TLC, it was diluted with EtOAc (750 mL) and filtered. The filtrate was washed sequentially with 10% aqueous $CuSO_4$, 5% aqueous NH_4Cl, satd $NaHCO_3$, and brine. It was then dried over $MgSO_4$ and the solution reduced in vacuo. The residue was purified by flash silica gel chromatography, eluting with 30% EtOAc–Hex to give a pale pink oil (7.71 g, 96% yield): $[\alpha]_D^{20} = -15.0$ (*c* 0.9, $CHCl_3$); TLC (50% EtOAc–Hex), $R_f = 0.44$; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.34–7.19 (m, 10H), 5.47 (d, $J = 10.3$, 1H), 5.06 (d, $J = 12.5$, 1H), 4.93 (d, $J = 12.5$, 1H), 4.66 (s, 1H), 3.97 (d, $J = 10.5$, 1H), 3.93 (s, 6H), 3.28 (s, 3H), 0.80 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.2, 140.0, 136.9, 128.3, 128.2, 127.7, 127.5, 126.5, 108.4, 79.6, 72.7, 66.3, 59.5, 57.4, 30.7, 14.4; IR (thin film) 3455 w, 3360 w, 2932 m, 2878 m, 1729 s, 1511 s, 1454 m, 1396 m, 1351 m, 1302 m, 1295 m, 1202 m, 1105 m, 1052 s, 1012 s, 913 w, 769 w, 726 m, 699 m, 627 m; HRMS (EI): *m/z* calcd for $C_{23}H_{27}O_6NNa$ 436.1736, found 436.1750.

4.4. (1*S*,2*R*)-2-Methoxy-1-(4′-methyl-2′,6′,7′-trioxabicyclo[2.2.2]octan-1′-yl)-2-phenylethanamine 5

Compound **4** (1.09 g, 2.64 mmol) was dissolved in EtOAc (40 mL), and diluted with EtOH (40 mL). After the addition of 10% $Pd(OH)_2/C$ (100 mg), the reaction was placed in a Parr bottle. The system was evacuated, filled with hydrogen gas (45 psi) and allowed to shake for 4 h. After the hydrogen gas was vented, the reaction was filtered through Celite, and the Celite pad was washed with EtOAc. The filtrate was reduced in vacuo and the residue purified by column flash silica gel chromatography, eluting with 50% acetone–Hex to give two products; a trace amount of the undesired diastereomer ($R_f = 0.27$, 50% acetone–Hex) and the major diastereomer (0.53 g, 72%) as a viscous oil: $[\alpha]_D^{20} = -16.8$ (*c* 1.5, $CHCl_3$); TLC (50% acetone–Hex), $R_f = 0.15$; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.33–7.28 (m, 5H), 4.48 (d, $J = 3.9$, 1H), 3.86 (s, 6H), 3.27 (s, 3H), 2.95 (d, $J = 3.9$, 1H), 0.78 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 141.1, 128.0, 127.2, 127.1, 108.7, 81.7, 72.6, 61.2, 57.2, 30.5, 14.4; IR (thin film) 2930 m, 2877 m, 1731 w, 1455 w, 1398 w, 1351 w, 1278 w, 1195 w, 1096 s, 1049 s, 1013 s, 987 s, 701 s; HRMS (EI): *m/z* calcd for $C_{15}H_{22}O_4N$ 280.1549, found 280.1557.

4.5. (2*S*,3*R*)-2-Amino-3-methoxy-3-phenylpropanoic acid (β-methoxyphenylalanine) 1

Amine **5** (180 mg, 0.64 mmol) was dissolved in water (10 mL) and TFA (0.1 mL) then added. The reaction was allowed to stir at room temperature for 45 min. The solution was reduced in vacuo, the remaining residue redissolved in water (10 mL), Cs_2CO_3 (1.00 g, 3.04 mmol) was added, and the reaction allowed to stir for 16 h. The reaction was acidified to pH ~ 2 with 6.0 M HCl. The total solution was loaded on approximately 15 cm^2 of IRC-50 (strong acid exchange resin, H^+ form, Rohm and Haas Corp.), washed with water until the pH of the column eluant was greater than 5.0. The column was eluted with 4% aqueous NH_4OH, all ninhydrin positive fractions were collected and reduced in vacuo to give a tan powder (110 mg, 88%): mp = 185–189 °C; $[\alpha]_D^{20} = -18.4$ (*c* 0.4, H_2O); ^1H NMR (D_2O, 500 MHz) δ 7.50–7.42 (m, 1H), 4.78 (d, J = 5.5, 1H), 3.85 (d, J = 5.5, 1H), 3.32 (s, 3H); ^{13}C NMR (D_2O, 125 MHz) δ 172.4, 136.8, 129.7, 129.6, 127.5, 81.7, 67.2, 61.1, 57.8; IR (KBr pellet) 3063 br s, 1617 s, 1392 s, 1362 m, 1089 m, 703 m; HRMS (EI): *m*/*z* calcd for $C_{10}H_{13}O_3NNa$ 218.0793, found 218.0802.

4.6. (*S*)-Benzyl-1-(4′-methyl-2′,6′,7′-trioxabicyclo-[2.2.2]octan-1′-yl)-2-oxo-2-phenylethyl-carbamate 6

Compound **3** (1.00 g, 2.51 mmol) was dissolved in CH_2Cl_2 (50 mL), and Dess–Martin periodinane (1.60 g, 3.76 mmol) added. The reaction was allowed to stir at room temperature for 15 min. When the reaction was shown to be complete by TLC, it was quenched with satd $NaHCO_3$ (100 mL) and satd $Na_2S_2O_3$ (100 mL) and the mixture was stirred for 15 min until both layers were clear. The organic layer was separated, dried over $MgSO_4$, and reduced in vacuo. The residue was purified by flash silica gel chromatography, eluting with 40% EtOAc–Hex to give a pale oil (891 mg, 89% yield): $[\alpha]_D^{20} = -14.1$ (*c* 0.4, $CHCl_3$); TLC (50% EtOAc–Hex), $R_f = 0.48$; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.06 (d, J = 7.5, 2H), 7.56 (t, J = 7.4, 1H), 7.45 (t, J = 7.8, 2H), 7.35 (m, 5H), 5.92 (d, J = 9.1, 1H), 5.59 (d, J = 9.2, 1H), 5.10 (s, 2H), 3.86 (s, 6H), 0.75 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 195.1, 155.9, 136.7, 136.3, 133.2, 129.4, 128.9, 128.4, 128.2, 128.1, 107.3, 72.9, 67.0, 57.5, 30.7, 14.2; IR (thin film) 3436 w, 3366 w, 2954 w, 2884 m, 1725 s, 1690 s, 1590 w, 1507 s, 1467 w, 1449 m, 1355 m, 1320 m, 1296 m, 1214 s, 1049 s, 1002 s, 691 m; HRMS (EI): *m*/*z* calcd for $C_{22}H_{23}O_6NNa$, 420.1423 found 420.1413.

4.7. Benzyl-(1*S*,2*S*)-2-hydroxy-1-(4′-methyl-2′,6′,7′-trioxabicyclo[2.2.2]octan-1′-yl)-2-phenylethylcarbamate 7

Ketone **6** (891 mg, 2.25 mmol) was dissolved in CH_2Cl_2 (25 mL) and the solution diluted with MeOH (25 mL) under argon, and cooled to −78 °C. Then a solution of $LiBH_4$ (2.0 M in THF, 1.3 mL, 2.6 mmol) was added and the reaction allowed to stir at −78 °C for 6 h. When the reaction was complete by TLC, it was quenched with 5% aqueous NH_4Cl, diluted with CH_2Cl_2 (200 mL), and allowed to warm to room temperature. The organic layer was separated and dried over $MgSO_4$ and reduced in vacuo. The residue was purified by flash silica gel chromatography, eluting with 40% EtOAc–Hex to give a pale oil (660 mg, 74% yield): $[\alpha]_D^{20} = -15.5$ (*c* 0.7, $CHCl_3$); TLC (50% EtOAc–Hex), $R_f = 0.32$; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.36 (d, J = 7.1, 2H), 7.29–7.23 (m, 6H) 7.11 (d, J = 7.2, 2H), 4.97 (d, J = 12.4, 1H), 4.85–4.82 (m, 2H), 4.22–4.18 (m, 1H), 4.10 (s, 1H), 3.96 (s, 6H), 0.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) 155.7, 140.1, 136.5, 128.3, 127.8, 127.7, 108.7, 74.3, 72.8, 66.5, 58.7, 30.7, 14.2; IR (thin film) 3495 m, 3342 m, 2896 m, 1719 s, 1531 s, 1243 m, 991 s, 697 m; HRMS (EI): *m*/*z* calcd for $C_{22}H_{25}O_6NNa$ 422.1580, found 422.1583.

4.8. Benzyl-(1*S*,2*S*)-2-methoxy-1-(4′-methyl-2′,6′,7′-trioxabicyclo[2.2.2]octan-1′-yl)-2-phenylethylcarbamate 8

Compound **7** (2.68 g, 6.72 mmol) was dissolved in anhydrous CH_2Cl_2 (175 mL), proton sponge® (3.60 g, 16.8 mmol) was added, followed by $Me_3O^+BF_4^-$ (1.99 g, 13.4 mmol). The reaction was allowed to stir for 8 h at room temperature under argon. The reaction was diluted with EtOAc (500 mL) and filtered. The filtrate was washed with 10% aqueous $CuSO_4$, 5% aqueous NH_4Cl, satd $NaHCO_3$, brine, dried over $MgSO_4$, and reduced in vacuo. The residue was purified by flash silica gel chromatography, eluting with 40% EtOAc–Hex to give a pale pink oil (2.03 g, 73% yield): $[\alpha]_D^{20} = +7.2$ (*c* 0.6, $CHCl_3$); TLC (50% EtOAc–Hex), $R_f = 0.52$; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.38–7.23 (m, 5H), 5.15–5.06 (m, 2H), 4.73 (d, J = 10.7, 1H), 4.65 (d, J = 3.7, 1H), 4.47 (dd, J = 3.7, 10.7, 1H), 3.85–3.78 (m, 6H), 3.28 (s, 3H), 0.77 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.3, 138.2, 136.8, 128.3, 128.3, 127.9, 127.8, 127.5, 127.4, 107.8, 81.3, 72.5, 66.6, 57.6, 56.8, 30.5, 14.3; IR (thin film) 2933 m, 2878 m, 1732 s, 1514 m, 1397 m, 1315 m, 1218 m, 1100 m, 1012 s, 731 m, 700 m; HRMS (EI): *m*/*z* calcd for $C_{23}H_{28}O_6N$ 414.1917, found 414.1900.

4.9. (1*S*,2*S*)-2-Methoxy-1-(4′-methyl-2′,6′,7′-trioxabicyclo[2.2.2]octan-1′-yl)-2-phenylethanamine 9

Compound **8** (2.04 g, 7.30 mmol) was dissolved in EtOAc (25 mL) and diluted with EtOH (25 mL). After addition of 10% $Pd(OH)_2/C$ (0.20 g), the mixture was placed in a Parr bottle. The system was evacuated and filled with hydrogen gas (45 psi), and the reaction mixture was shaken for 4 h. The Parr bottle was vented and the reaction mixture was filtered through Celite, the Celite pad was then washed with EtOAc. The filtrate was reduced in vacuo to give a clear viscous oil that was recrystallized from EtOAc. (1.75 g, 86% yield): mp = 96–98 °C; $[\alpha]_D^{20} = -6.5$ (*c* 1.1, $CHCl_3$); TLC (50% EtOAc–Hex), $R_f = 0.20$; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.46–7.27 (m, 5H), 4.80 (d, J = 4.1, 1H), 3.82 (s, 6H), 3.77 (d, J = 4.2, 1H), 3.30 (s, 3H), 0.76 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) 135.4, 128.3, 128.3, 128.1, 106.2, 79.1, 58.2, 57.1, 30.6, 14.1; IR (thin film) 3370 m, 2936 s, 2881 s, 1744 m, 1593 m, 1496 m, 1456 w, 1354 w, 1273 w, 1209 w, 1099 s, 1048 s, 1026 s, 728

w, 704 m; HRMS (EI): *m/z* calcd for $C_{15}H_{22}O_4N$ 280.1549, found 280.1537.

4.10. (2*S*,3*S*)-2-Amino-3-methoxy-3-phenylpropanoic acid (β-methoxyphenylalanine) 10

Amine **9** (1.75 g, 6.23 mmol) was dissolved in water (50 mL), TFA (0.5 mL) was added and the reaction allowed to stir at room temperature for 45 min. The solution was reduced in vacuo. The remaining residue was redissolved in water (10 mL), and Cs_2CO_3 (3.00 g, 9.20 mmol) added. The reaction was allowed to stir for 16 h and acidified to pH ~ 2 with 6.0 M HCl. The total solution was loaded on approximately 50 cm^2 of IRC-50 (strong acid exchange resin, H^+ form, Rohm and Haas Corp.), washed with water until the pH of the column eluant was greater than 5.0. The column was eluted with 4% aqueous NH_4OH, all ninhydrin positive fractions were collected and reduced in vacuo to give a tan powder (884 mg, 72%): $[\alpha]_D^{20} = +34.5$ (*c* 0.6, H_2O); ^1H NMR (D_2O, 500 MHz) δ 7.49–7.34 (m, 5H), 4.91 (d, $J = 3.9$, 1H), 4.16 (d, $J = 3.9$, 1H), 3.39 (s, 3H); ^{13}C NMR (D_2O, 125 MHz) δ 171.5, 135.1, 129.7, 129.5, 127.6, 67.2, 59.4, 57.4; IR (KBr pellet) 3142 br, 1734 m, 1617 s, 1529 s, 1455 m, 1309 s, 1273 m, 1098 s, 1075 m, 967 m, 749 s, 704 s, 570 m; HRMS (EI): *m/z* calcd for $C_{10}H_{13}O_3NNa$ 218.0793, found 218.0797.

4.11. (2*S*,3*R*)-*N*-Cbz-β-Methoxyphenylalanine(OTMSE) ester 11

β-Methoxyphenylalanine **1** (1.17 g, 6.02 mmol) was dissolved in satd $NaHCO_3$ (60 mL), $NaHCO_3$ (0.51 g, 6.1 mmol) added, followed by Cbz-Cl (1.3 mL, 9.2 mmol). The reaction was allowed to stir overnight at room temperature. The reaction was washed twice with Et_2O and the aqueous layer was acidified to pH ~ 2 with 6.0 M HCl. The reaction was extracted three times with EtOAc. The organic extracts were combined and washed with brine, dried over $MgSO_4$, and reduced in vacuo to give a pale oil that was dissolved in CH_2Cl_2 and cooled to 0 °C under argon. Then NEt_3 (1.7 mL, 12 mmol), DMAP (100 mg), EDAC (2.30 g, 12.0 mmol), and 2-trimethylsilylethanol (3.0 mL, 12 mmol) were added. The reaction was allowed to slowly warm to room temperature and stirred overnight. The reaction was diluted with Et_2O (250 mL), washed with 1.0 N HCl, water, satd $NaHCO_3$, and brine. The solution was dried over $MgSO_4$ and reduced in vacuo. The residue was purified by flash silica gel chromatography, eluting with 10% EtOAc–Hex to give a clear oil (2.04 g, 79% yield): $[\alpha]_D^{20} = -15.2$ (*c* 0.7, $CHCl_3$); TLC (10% EtOAc–Hex), $R_f = 0.22$; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.34–7.26 (m, 10H), 5.57 (d, $J = 9.3$, 1H), 5.00 (s, 2H), 4.82 (d, $J = 2.7$, 1H), 4.52 (dd, $J = 2.9$, 9.4, 1H), 4.30–4.27 (m, 2H), 3.27 (s, 3H), 1.03–0.95 (m, 2H), 0.12–0.02 (m, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.2, 156.0, 137.0, 136.3, 128.3, 127.9, 127.8, 127.7, 126.7, 82.5, 66.6, 63.9, 59.8, 57.4, 17.2; IR (thin film) 3355 w, 2953 m, 1730 s, 1508 m, 1250 m, 1103 m, 1059 m, 838 m, 698 m; HRMS (EI): *m/z* calcd for $C_{23}H_{31}O_5NNaSi$ 452.1869, found 452.1851.

4.12. (*S*)-*N*-Cbz-Ala-(2*S*,3*R*)-*N*-Cbz-β-methoxyphenylalanine(OTMSE)-ester 12

Cbz-β-MeO-Phe-(OTMSE) ester **11** (50.0 mg, 0.11 mmol) was dissolved in EtOAc (5 mL) and diluted with EtOH (5 mL) in a Parr hydrogenation flask. After the addition of 10% Pd/C (10.0 mg), flask was evacuated, then filled with hydrogen (1 atm) and stirred for 4 h. The reaction was evacuated and the mixture filtered through Celite, the Celite pad was then washed with EtOAc. The filtrate was reduced in vacuo and the residue was combined with CH_2Cl_2 (5 mL), (*S*)-*N*-Cbz-Ala (38.0 mg, 0.17 mmol), followed by NEt_3 (0.031 mL, 0.22 mmol), and DMAP (5.0 mg). The reaction was placed under argon and cooled to 0 °C. EDAC (33.0 mg, 0.17 mmol) was then added and the reaction allowed to slowly warm to room temperature and stirred overnight. The reaction was diluted with Et_2O (30 mL), washed with 1.0 M aqueous citric acid, water, satd $NaHCO_3$, brine, dried over $MgSO_4$, and reduced in vacuo. The residue was purified by flash silica gel chromatography, eluting with 20–30% EtOAc–Hex to give a clear oil (34 mg, 62% yield): $[\alpha]_D^{20} = -12.0$ (*c* 2.0, $CHCl_3$); TLC (20% EtOAc–Hex), $R_f = 0.11$; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.25 (m, 10H), 6.58 (d, $J = 8.9$, 1H), 5.29 (br s, 1H), 5.13 (s, 2H), 4.83 (s, 1H), 4.74 (d, $J = 9.1$, 1H), 4.27–4.24 (m, 3H), 3.28 (s, 3H), 1.34–1.30 (m, 3H), 1.03–0.99 (m, 2H), 0.11–0.00 (m, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.8, 169.8, 155.6, 136.9, 136.2, 128.5, 128.3, 128.1, 128.1, 128.0, 126.6, 82.3, 66.8, 64.0, 57.7, 57.5, 50.2, 18.6, 17.3, −1.7; IR (thin film) 3315 br m, 2953 m, 1729 s, 1669 s, 1507 s, 1454 m, 1250 s, 1097 m, 860 m, 838 m, 699 m; HRMS (EI): *m/z* calcd for $C_{26}H_{36}O_6N_2NaSi$ 523.2240, found 523.2238.

4.13. (*S*)-*N*-Cbz-Val-(*S*)-*N*-Me-Cbz-Leu(OTMSE) ester 13

(*S*)-Cbz-Val (2.60 g, 10.33 mmol) was dissolved in CH_2Cl_2 (10 mL) under argon and cooled to 0 °C. DCC (2.13 g, 10.33 mmol) was added and the reaction allowed to stir for 1 min before adding DMAP (50.0 mg), and (*S*)-*N*-Me-leucine(OTMSE) ester (1.05 g, 6.88 mmol). The reaction was allowed to stir at 0 °C for 1 h and warmed to room temperature and stirred overnight. The reaction was diluted with Et_2O (50 mL), and placed in the freezer (−20 °C) overnight. The mixture was filtered at low temperature, and the filtrate then washed with 1.0 M HCl, water, satd $NaHCO_3$, brine, dried over $MgSO_4$, and reduced in vacuo. The residue was purified by flash silica gel chromatography, eluting with 10–20% EtOAc–Hex to give a clear oil (1.60 g, 62% yield): $[\alpha]_D^{20} = -16.1$ (*c* 0.4, $CHCl_3$); TLC (10% EtOAc–Hex), $R_f = 0.16$; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.36–7.29 (m, 5H), 5.51 (d, $J = 9.1$, 1H), 5.30 (dd, $J = 5.0$, 10.7, 1H), 5.09 (s, 2H), 4.54 (dd, $J = 6.1$, 9.1, 1H), 4.20–4.15 (m, 2H), 3.00 (s, 3H), 2.08–2.04 (m, 1H), 1.78–1.64 (m, 2H), 1.48–1.45 (m, 1H), 1.03–0.86 (m, 14H), 0.04–0.00 (m, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.7, 171.7, 156.4, 136.4, 128.5, 128.0, 127.9, 66.8, 63.5, 55.9, 54.7, 36.9, 31.2, 31.2, 24.8, 23.2, 21.4, 19.5, 17.4, 17.3, −1.6; IR (thin film)

3304 br m, 2956 s, 1732 s, 1645 s, 1524 m, 1469 m, 1410 m, 1251 m, 1176 m, 1026 w, 860 m, 838 m, 697 w; HRMS (EI): *m*/*z* calcd for $C_{25}H_{42}O_5N_2NaSi$ 501.2761, found 501.2770.

4.14. (2*S*,3*R*)-*N*-Cbz-β-Methoxyphenylalanine-(*S*)-Val-(*S*)-*N*-Me-Leu(OTMSE) ester 14

Compound **4** (1.00 g, 2.41 mmol) was dissolved in dioxane (15 mL), and AcOH (12 mL) was added followed by water (13 mL). The reaction was allowed to stir for 10 min and the solution reduced in vacuo. The residue was redissolved in toluene (30 mL) and reduced in vacuo twice. The remaining residue was dissolved in dioxane (10 mL), diluted with MeOH (10 mL), and water (10 mL), cooled to 0 °C followed by the addition of 1.0 M aqueous LiOH (3.6 mL, 3.61 mmol). The reaction was allowed to warm slowly to room temperature and stir overnight. The reaction was acidified with 6.0 M aqueous HCl until the pH was 2–3 and then extracted three times with EtOAc. The organic layers were combined and washed with brine, dried over $MgSO_4$, and reduced in vacuo to give the crude acid. In a Parr flask, dipeptide **13** (1.20 g, 2.50 mmol) was dissolved in MeOH (20 mL) and 10% Pd/C (220 mg) was added to the solution. The flask was evacuated and filled with hydrogen (1 atm) and allowed to stir for 3 h. The mixture was filtered through Celite, the Celite pad washed with EtOAc, the filtrate was combined and reduced in vacuo to give the crude dipeptide amine. The crude dipeptide amine and crude acid were combined in CH_2Cl_2 (10 mL), DMAP (10 mg), EDAC (647 mg, 3.37 mmol), DIEA (0.84 mL, 4.82 mmol) were added and the reaction allowed to stir overnight under argon. The reaction was diluted with EtOAc (100 mL) and washed with 1.0 M HCl, water, satd $NaHCO_3$, brine. The solution was dried over $MgSO_4$ and reduced in vacuo. The residue was purified by flash silica gel chromatography, eluting with 20% EtOAc–Hex to give a clear oil (710 mg, 45% yield four steps): $[\alpha]_D^{20} = -9.5$ (*c* 0.8, $CHCl_3$); TLC (20% EtOAc–Hex), $R_f = 0.20$; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.47–7.26 (m, 10H), 5.53 (d, $J = 8.2$, 1H), 5.36 (dd, $J = 4.9$, 10.6, 1H), 5.06 (d, $J = 12.1$, 1H), 4.97 (d, $J = 12.2$, 1H), 4.19–4.84 (m, 2H), 4.50 (dd, $J = 3.1$, 8.1, 1H), 4.24–4.14 (m, 2H), 3.31 (s, 3H), 3.01 (s, 3H), 2.17–2.09 (m, 1H), 1.80–1.62 (m, 2H), 1.53–1.36 (m, 1H), 1.03–0.86 (m, 14H), 0.04 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.0, 171.6, 168.7, 155.9, 126.8, 136.2, 128.4, 128.3, 128.0, 127.9, 126.7, 126.6, 81.8, 66.9, 63.5, 59.8, 57.4, 54.5, 54.0, 36.9, 31.5, 31.1, 24.8, 23.2, 21.4, 19.5, 17.4, 17.2, −1.7; IR (thin film) 3316 br w, 2955 m, 1732 s, 1641 s, 1506 m, 1409 w, 1250 m, 1221 m, 1100 w, 1059 w, 858 w, 839 w, 698 w; HRMS (EI): *m*/*z* calcd for $C_{35}H_{53}O_7N_3NaSi$ 678.3351, found 678.3562.

Acknowledgments

We thank NSF (CHEM-**0130958**) and the University of Pennsylvania for their support.

References

1. Ford, P. W.; Gustafson, K. R.; McKee, T. C.; Shigematsu, N.; Maurizi, L. K.; Pannell, L. K.; Williams, D. E.; Dilip de Silva, E.; Lassota, P.; Allen, T. M.; Van Soest, R.; Andersen, R. J.; Boyd, M. R. *J. Am. Chem. Soc.* **1999**, *121*, 5899–5909.
2. Zampella, A.; D'Auria, M. V.; Paloma, L. G.; Casapullo, A.; Minale, L.; Debitus, C.; Henin, Y. *J. Am. Chem. Soc.* **1996**, *118*, 6202–6209.
3. Zampella, A.; Randazzo, A.; Borbone, N.; Luciani, S.; Trevisi, L.; Debitus, C.; D'Auria, M. V. *Tetrahedron Lett.* **2002**, *43*, 6163–6166.
4. Tada, H.; Tozyo, T.; Terui, Y.; Hayashi, F. *Chem. Lett.* **1992**, 431–434.
5. Renner, M. K.; Shen, Y. C.; Cheng, X. C.; Jensen, P. R.; Frankmoelle, W.; Kauffman, C. A.; Fenical, W.; Lobkovsky, E.; Clardy, J. *J. Am. Chem. Soc.* **1999**, *121*, 11273–11276.
6. Sugiyama, H.; Shioiri, T.; Yokokawa, F. *Tetrahedron Lett.* **2002**, *43*, 3489–3492.
7. Sugiyama, H.; Yokokawa, F.; Aoyama, T.; Shioiri, T. *Tetrahedron Lett.* **2001**, *42*, 7277–7280.
8. Tarver, J. E.; Joullié, M. M. *J. Org. Chem.* **2004**, *69*, 815–820.
9. Tarver, J. E.; Terranova, K. M.; Joullié, M. M. *Tetrahedron* **2004**, *60*, 10277–10284.
10. Wen, S.-J.; Yao, Z. J. *Org. Lett.* **2004**, *6*, 2721–2724.
11. Wen, S.-J.; Hu, T.-S.; Yao, Z.-J. *Tetrahedron* **2005**, *61*, 4931.
12. Blaskovich, M. A.; Evindar, G.; Rose, N. G. W.; Wilkinson, S.; Luo, Y.; Lajoie, G. A. *J. Org. Chem.* **1998**, *63*, 3631–3646.
13. Blaskovich, M. A.; Lajoie, G. A. *J. Am. Chem. Soc.* **1993**, *115*, 5021–5030.
14. Hansen, D. B.; Wan, X. B.; Carroll, P. J.; Joullié, M. M. *J. Org. Chem.* **2005**, *70*, 3120–3126.
15. Rose, N. G. W.; Blaskovich, M. A.; Evindar, G.; Wilkinson, S.; Luo, Y.; Fishlock, D.; Reid, C.; Lajoie, G. A. *Org. Synth.* **2003**, *79*, 216–227.
16. Ireland, R. E.; Liu, L. B.; Roper, T. D.; Gleason, J. L. *Tetrahedron* **1997**, *53*, 13257–13284.