Supporting Information
for DOI: 10.1055/s-2008-1078270
© Georg Thieme Verlag KG Stuttgart · New York 2008
**Total Synthesis of Largazole**

Qi Ren, a Lu Dai, a Hui Zhang, a Wenfei Tan, a Zhengshuang Xu a,b and Tao Ye a,b

a Laboratory of Chemical Genomics, Peking University Shenzhen Graduate School, University Town of Shenzhen, Xili, Nanshan District, Shenzhen, China, 518055;
b Department of Applied Biology & Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong

Fax: (852) 22641912
E-mail: bctaoye@inet.polyu.edu.hk

**General Experimental**

All non-aqueous reactions were run under an inert atmosphere (nitrogen or argon) with rigid exclusion of moisture from reagents and all reaction vessels were oven-dried. Solvents were distilled prior to use: THF from Na/benzophenone, dichloromethane, DMF, triethylamine and diisopropylethylamine from CaH₂. NMR spectra were recorded on Bruker Advance DPX 300MHz, AV500MHz spectrometers. Chemical shifts are reported in parts per million (ppm), relative to either a tetramethylsilane internal standard or the signals due to the solvent. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad), integration and coupling constants. Low- and high- resolution EI and ESI mass spectra were obtained using a Finnigan MAT 95 mass spectrometer. Optical rotations were recorded on a Perkin Elmer 343 Polarimeter. TLC was carried out using pre-coated sheets (Qingdao silica gel 60-F₂₅₄, 0.2mm) which, after development, were visualized at 254nm, and/or staining in p-anisole, ninhydrin or phosphomolybdic acid solution followed by heating. Flash column chromatography was performed using the indicated solvents (with Rf = 1.5-2.0 for the desired component) on E. Qingdao silica gel 60 (230-400 mesh ASTM).

**Experimental procedures**

![Intermediate 13](image)

Intermedicate 13 (333.8mg, 0.8mmol) was dissolved in dichloromethane (5mL), 2-trimethylsilylethanol (354mg, 3.0mmol) was added via a syringe followed by a catalytic quantity of DMAP. The reaction mixture was stirred at room temperature for 14h before it was diluted with dichloromethane (50mL) and
successively washed with water (30mL) and brine (30mL). The organic phase was dried over Na₂SO₄ (anhydrous) and concentrated to leave an oil, which was purified with chromatograph on silica gel, using ethyl acetate – hexane (1:6) as eluant, to afford the desired product 5 (281mg, 94%) as a colorless oil. [α]_D = 1.5° (c, 1.8, MeOH); ¹H NMR (500 MHz, CDCl₃) δ: 5.67(td, 1H, J = 7.2Hz, 15.3Hz), 5.49(dd, 1H, J = 6.4Hz, 15.5Hz), 4.39-4.45 (m, 1H), 4.10-4.17(m, 2H), 3.56(t, 2H, J = 6.78Hz), 2.82(d, 1H, J = 4.1Hz), 2.38-2.49(m, 2H), 2.18(q, 2H, J = 7.4Hz), 0.90-0.96(m, 2H), 0.82(s, 9H), -0.02(br, 15H) ppm; ¹³C NMR (125 MHz, CDCl₃) δ: 172.4, 132.5, 132.8, 68.8, 62.9, 62.6, 41.6, 35.7, 25.9, 18.3, 17.3, -1.5, -5.2 ppm. HR-ESIMS m/z Calcd for C₁₈H₃₈NaO₄Si₂ [M+Na]⁺ 397.2206. Found: [M+Na]⁺ 397.2200.

Compound 5 (262.3mg, 0.7mmol) and L-Fmoc-Val-OH (712.7mg, 2.1mmol) were dissolved in THF (10mL), DCC (433.3mg, 2.1mmol) and DMAP (3mg) was added at 0 °C. The reaction mixture was stirred at room temperature for 14h before it was poured into diethyl ether (100 mL). The ether solution was filtered through a pad of Celite and the filtrate was successively washed with saturated sodium bicarbonate (30mL), water (30mL) and brine (30mL). The organic phase was dried over Na₂SO₄ (anhydrous) and concentrated in vacuo to give an oil, which was purified by chromatograph on silica gel, using ethyl acetate – hexane (1:5) as eluant, to produce the desired product 3 (443.4mg, 91%) as a viscous oil. [α]_D = -23.7° (c, 0.8, MeOH); ¹H NMR (500 MHz, CDCl₃) δ: 7.76(d, 2H, J = 7.5Hz), 7.57-7.64(m, 2H), 7.38(t, 2H, J = 7.2Hz), 7.22(t, 2H, J = 7.4Hz), 5.85(dt, 1H, J = 6.8Hz, 14.7Hz), 5.69(q, 1H, J = 7.1Hz), 5.52(dd, 1H, J = 7.3Hz, 15.4Hz), 5.39(d, 1H, J = 8.9Hz), 4.33-4.43(m, 2H), 4.27-4.33(m, 1H), 4.23(t, 1H, J = 7.0Hz), 4.17(t, 2H, J = 8.5Hz), 3.60(t, 2H, J = 6.5Hz), 2.72(dd, 1H, J = 7.9Hz, 15.6Hz), 2.59(dd, 1H, J = 5.5Hz, 15.6Hz), 2.32(t, 2H, J = 6.5Hz), 2.15-2.19(m, 1H), 0.91-0.99(m, 8H), 0.88(s, 9H), 0.09(s, 3H), 0.04(s, 3H), 0.03(s, 9H) ppm. ¹³C NMR (125 MHz, CDCl₃) δ: 170.9, 169.6, 156.1, 143.9, 143.8, 141.3, 132.6, 128.1, 127.6, 127.0, 125.1, 119.9, 72.0, 67.0, 63.0, 62.3, 58.8, 47.2, 39.8, 35.7, 31.8, 25.9, 18.2 17.9, 17.3, -1.5, -5.3 ppm. HR-ESIMS m/z Calcd for C₁₈H₃₈NaO₄Si₂ [M+Na]⁺ 718.3571. Found: [M+Na]⁺ 718.3587.
Compound 7 (92.8mg, 0.5mmol) and compound 8 (119.7mg, 0.5mmol) was dissolved in degassed methanol (10mL), after triethylamine (50.5mg, 0.5mmol) was added, the solution was heated at 50 °C for 48h. All volatiles were removed in vacuo. The residue was purified by chromatograph on silica gel, using ethyl acetate – hexane (1:4) as eluant, to provide 4 (94.7mg, 51%) as a viscous oil. \[\alpha\]_D^20 = -5.8° (c, 1.0, MeOH); \(^1\)H NMR (300 MHz, CDCl₃) \(\delta\): 7.95 (s, 1H), 5.26 (br, 1H), 4.64 (d, 2H, \(J = 6.2\) Hz), 3.89 (d, 1H, \(J = 11.4\) Hz), 3.81 (s, 3H), 3.28 (d, 1H, \(J = 11.4\) Hz), 1.66 (s, 3H), 1.47 (s, 9H) ppm. \(^13\)C NMR (75 MHz, CDCl₃) \(\delta\): 173.6, 162.8, 155.5, 148.5, 133.0, 121.8, 84.5, 77.1, 52.9, 42.2, 41.4, 28.28, 23.93 ppm. HR-ESIMS \(m/z\) Calcd for C₁₅H₂₁N₃NaO₄Si₂ [M+Na]^+ 394.0871. Found: [M+Na]^+ 394.0889.

Compound 3 (30.3mg, 0.04mmol) in THF (3mL) was treated with diisopropylamine (20.2mg, 0.2mmol) at room temperature for 4h to remove the Fmoc protective group. The reaction mixture was concentrated in vacuo and then kept under high vacuum for 3 hours. At the same time, to a stirred solution of compound 4 (14.9mg, 0.04mmol) in THF−H₂O (1:1) mixture (1.5 mL) was added LiOH·H₂O (16.8mg, 0.4mmol) at 0 °C. After being stirred for 3 h at the same temperature, the reaction mixture was dissolved in EtOAc (5 ml) and the treated with HCl (0.5 ml, 1 M solution) at 0 °C. The organic layer was washed with water and brine and dried over anhydrous Na₂SO₄. Organic solvent was removed in vacuo and the residue was concentrated to dryness under vacuum to afford the corresponding acid.

The amine (derived from 3) and the carboxylic acid (derived from compound 4) were dissolved in dichloromethane (10mL). Mukaiyama coupling reagent (20.9mg, 0.07mmol) and DIPEA(27.1mg, 0.2mmol) were added to the mixture at 0°C and the solution was stirred at room temperature for 20h. Saturated ammonium chloride (20mL) was used to quench the reaction and ethyl acetate (30mL X 3) was
used for extraction. The combined organic phase was further washed with saturated sodium bicarbonate (20mL) and brine (20mL), dried over sodium sulfate (anhydrous) and concentrated in vacuo to afford the crude product as a viscous oil. Further purification was performed by chromatograph on silica gel, using ethyl acetate – hexane (1:2) as eluant, to give the desired compound 2 (26.3mg, 90%) as colorless oil. \([\alpha]_D^{20} = -35.3^\circ \) (c, 1.5, MeOH); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.90 (s, 1H), 7.12(d, 1H, \(J = 9.0Hz\)), 5.75(td,1H, \(J = 6.9Hz\), 14.8Hz), 5.59(q, 1H, \(J = 7.2Hz\)), 5.45(dd, 1H, \(J = 7.4Hz\), 15.5Hz), 5.20(br, 1H), 4.56(d, 2H, \(J = 5.6Hz\), 4.42(dd, 1H, \(J = 4.7Hz\), 9.1Hz), 4.07-4.11(m, 2H), 3.71(d, 1H, \(J = 11.5Hz\)), 3.52(t, 2H, \(J = 6.8Hz\), 3.25(d, 1H, \(J = 11.5Hz\)), 2.64(dd, 1H, \(J = 7.8Hz\), 15.7Hz), 2.51(dd, 1H, \(J = 5.8Hz\), 15.6Hz), 2.16(q, 2H, \(J = 5.7Hz\), 2.06-2.10(m, 1H), 1.52(s, 3H), 1.39(s, 9H), 0.86-0.94(m, 2H), 0.76-0.82(m, 12H), 0.72(d, 3H, \(J = 6.9Hz\), -0.03(s, 9H), -0.04(s, 6H) ppm; \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 174.3, 170.3, 169.6, 163.1, 148.8, 132.4, 128.2, 121.2, 85.2, 71.9, 63.0, 62.3, 60.3, 56.9, 41.5, 39.8, 35.7, 31.1, 28.3, 25.9, 25.8, 24.7, 20.9, 19.0, 18.2, 17.4, 17.3, 14.1, -1.5, -5.3 ppm. HR-ESIMS m/z Calcd for C\(_{37}\)H\(_{64}\)N\(_4\)NaO\(_8\)S\(_2\)Si\(_2\) [M+Na]\(^+\) 835.3602. Found: [M+Na]\(^+\) 835.3598.

![Image](18.png)

Compound 17 (12.8mg, 0.02mmol), dissolved in dry methanol (2mL), was treated with potassium carbonate (2.8mg, 0.02mmol) at 0°C for 10 min. The reaction was quenched with saturated ammonium chloride (10mL) and extracted with ethyl acetate (20mL X 3). The combined organic phase was dried over anhydrous sodium sulfate and concentrated in vacuo. The resulted residue was dissolved in methanol – dichloromethane (v : v = 2 : 1, 5mL), and treated with DNTP (9.3mg, 0.03mmol) for 2h. Upon that time, 2-methylpropane-2-thiol (9mg, 0.1mmol) was added, followed by triethylamine (10.1mg, 0.1mmol). The reaction mixture was further stirred for 15min, then quenched with saturated ammonium chloride (15mL) and extracted with ethyl acetate (30mL X 3). The combined organic phases were dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified with chromatograph on silica gel, using ethyl acetate – hexane (1:8) as eluant, to produce the desired product 18 (6.9mg, 75%) as light yellow oil. \([\alpha]_D^{20} = -11.8^\circ \) (c, 0.4, MeOH); \(^1\)H NMR (300 MHz,CDCl\(_3\)) \(\delta\) 7.74-7.83(m, 2H), 7.58-7.67(m,
2H), 7.39-7.47(m 2H), 7.29-7.39(m, 2H), 5.85(dt, 1H, \( J = 6.8\text{Hz}, 14.9\text{Hz} \)), 5.69(q, 1H, \( J = 7.55\text{Hz} \)), 5.55(dd, 1H, \( J = 7.6\text{Hz}, 15.4\text{Hz} \)), 5.33(d, 1H, \( J = 9.1\text{Hz} \)), 4.36-4.48(m, 2H), 4.33(dd, 1H, \( J = 4.4\text{Hz}, 9.1\text{Hz} \)), 4.12-4.29(m, 3H), 2.54-2.81(m, 4H), 2.33-2.49(m, 2H), 2.12-2.28(m, 1H), 1.32(s, 9H), 0.97-1.02(m, 2H), 0.97(d, 3H, \( J = 7.0\text{Hz} \)), 0.88(d, 3H, \( J = 6.8\text{Hz} \)), 0.04(s, 9H) ppm. \( ^{13}\text{C} \) NMR (125 MHz, CDCl\(_3\)) \( \delta \): 170.9, 169.6, 156.1, 143.9, 143.8, 141.3, 133.5, 128.1, 127.7, 127.0, 125.1, 120.0, 71.9, 67.0, 63.1, 60.3, 58.8, 47.8, 47.2, 39.7, 31.8, 31.4, 29.9, 18.9, 17.3, -1.5 ppm. HR-ESIMS \( m/z \) Calcd for C\(_{36}\)H\(_{51}\)NNaO\(_6\)S\(_2\)Si [M+Na]\(^+\) 708.2825. Found: [M+Na]\(^+\) 708.2818.

Compound 18 (54.9mg, 0.08mmol) in acetonitrile (3mL) was treated with diethylamine (5.9mg, 0.08mmol) at room temperature for 3h. The reaction mixture was concentrated \textit{in vacuo} and kept under high vacuum for 3 hours. The above amine was mixed with the carboxylic acid derived from compound 4 (33.5mg, 0.09mmol) and then dissolved in dichloromethane (10mL). Mukaiyama coupling reagent (47.8mg, 0.16mmol) and DIPEA (43.4mg, 0.32mmol) were added to the mixture at 0\(^\circ\)C and the solution was stirred at room temperature for 20h. Saturated ammonium chloride (20mL) was used to quench the reaction and ethyl acetate (30mL X 3) was used for extraction. The combined organic phases were further washed with saturated sodium bicarbonate (20mL) and brine (20mL), dried over sodium sulfate (anhydrous) and concentrated \textit{in vacuo} to give a viscous oil. Further purification was performed by silica gel chromatograph, using ethyl acetate – hexane (1:2) as eluant, to afford the desired compound 2 (57.8mg, 91%) as a colorless oil. \( [\alpha]_{20}^{\text{D}} = -31.8 \degree \) (c, 0.5, MeOH); \(^1\text{H} \) NMR (500 MHz, CDCl\(_3\)) \( \delta \): 7.98(s, 1H), 7.19(d, 1H, \( J = 9.0\text{Hz} \)), 5.83(td, 1H, \( J = 6.7\text{Hz}, 14.8\text{Hz} \)), 5.67(q, 1H, \( J = 7.1\text{Hz} \)), 5.53(dd, 1H, \( J = 7.4\text{Hz}, 15.4\text{Hz} \)), 4.64(d, 2H, \( J = 5.41\text{Hz} \)), 4.50(dd, 1H, \( J = 4.7\text{Hz}, 9.0\text{Hz} \)), 4.17(dd, 2H, \( J = 7.9\text{Hz}, 9.2\text{Hz} \)), 3.78(d, 1H, \( J = 11.5\text{Hz} \)), 3.33(d, 1H, \( J = 11.5\text{Hz} \)), 2.73(dd, 1H, \( J = 7.6\text{Hz}, 15.7\text{Hz} \)), 2.70(t, 2H, \( J = 7.4\text{Hz} \)), 2.61(dd, 1H, \( J = 6.0\text{Hz}, 15.6\text{Hz} \)), 2.37-2.42(m, 1H), 2.33(t, 1H, \( J = 6.6\text{Hz} \)), 2.13-2.19(m, 1H), 1.84-1.88(m, 1H), 1.60(s, 3H), 1.47(s, 9H), 1.31(s, 9H), 0.98(t, 2H, \( J = 8.6\text{Hz} \)), 0.86(d, 3H, \( J = 6.8\text{Hz} \)), 0.81(d, 3H, \( J = 6.8\text{Hz} \)),
0.03(s, 9H) ppm. $^{13}$C NMR (125MHz, CDCl$_3$) $\delta$: 174.4, 170.3, 169.6, 163.2, 155.6, 148.8, 133.3, 128.3, 127.5, 121.3, 85.2, 71.8, 63.1, 57.0, 47.8, 41.9, 41.5, 39.8, 39.6, 31.9, 31.2, 30.0, 28.3, 27.0, 24.8, 19.0, 17.5, 17.4, -1.5 ppm. HR-ESIMS $m/z$ Calcd for C$_{35}$H$_{58}$N$_4$NaO$_7$S$_4$Si [M+Na]$^+$ 825.2855. Found: [M+Na]$^+$ 825.2858.

Compound 19 (24.1mg, 0.03mmol) was treated with trifluoroacetic acid – dichloromethane (v : v = 1 : 1, 2mL) at room temperature for 3h. After the reaction mixture was concentrated in vacuo and dried under high vaccum for three hours, the residue was dissolved in dimethyl formamide (40mL). To this solution, HATU (114mg, 0.3mmol), HOAT (40.8mg, 0.3mmol) and DIPEA (58mg, 0.45mmol) were added and the reaction mixture was stirred for 48h. Solvent and other volatiles were removed under high vacuum and the residue was dissolved in ethyl acetate (100mL) and washed successively with water (20mL) and brine (20mL). The organic phase was dried over anhydrous sodium sulfate and concentrated in vacuo to provide crude product, which was purified with chromatograph on silica gel, using ethyl acetate – hexane (2:1) to afford the desired cyclic peptide 20 (10.7mg, 61%) as oil. $[\alpha]_D^{20} = 17.5 ^\circ$ (c, 0.2, MeOH); $^1$H NMR (500MHz, CDCl$_3$) $\delta$: 7.75(s, 1H), 7.18(d, 1H, $J = 9.3$Hz), 6.50(dd, 1H, $J = 2.7$Hz, 9.0Hz), 5.88(dd, 1H, $J = 6.9$Hz, 14.6Hz), 5.65-5.71(m, 1H), 5.54(dd, 1H, $J = 6.7$Hz, 15.5Hz), 5.26(dd, 1H, $J = 9.4$Hz, 17.6Hz), 4.60(dd, 1H, $J = 3.6$Hz, 9.4Hz), 4.27(dd, 1H, $J = 3.1$Hz, 17.6Hz), 4.03(d, 1H, $J = 11.3$Hz), 3.28(t, 1H, $J = 10.5$Hz), 2.85(dd, 1H, $J = 9.9$Hz, 16.3Hz), 2.67-2.75(m, 3H), 2.37-2.46(m, 2H), 2.06-2.14(m, 1H), 1.86(s, 3H), 1.32(s, 9H), 0.68(d, 3H, $J = 6.9$Hz), 0.53(d, 3H, $J = 6.9$Hz) ppm. $^{13}$C NMR (125MHz, CDCl$_3$) $\delta$: 173.5, 169.3, 168.8, 167.9, 164.5, 147.4, 132.8, 128.1, 124.1, 84.3, 71.9, 57.7, 47.8, 43.2, 41.0, 40.4, 39.4, 34.0, 31.8, 29.9, 24.1, 18.8, 16.6 ppm. ESIMS $m/z$ at 585.17(100.0%), 607.15(88.8%). HR-ESIMS $m/z$ Calcd for C$_{25}$H$_{37}$N$_4$O$_4$S$_4$ [M+H]$^+$ 585.1698. Found: [M+H]$^+$ 585.1689.
Cyclodepsipeptide 20 (9.9mg, 0.02mmol) was dissolved in degassed THF-H₂O (v:v = 4:1, 2mL) and treated with tri-\(n\)-butylphosphine (6.1mg, 0.03mmol) at room temperature for 6h. The reaction solution was made up to 50mL with ethyl acetate and dried over anhydrous sodium sulfate. The free thiol intermediate was obtained after removal of solvent in vacuo. The thiol intermediate was then dissolved in dichloromethane (5mL), DIPEA (21.9mg, 0.17mmol) and octanoyl chloride (22mg, 0.136 mmol) was added at 0 °C followed by a catalytic quantity of DMAP. The reaction mixture was stirred at room temperature for 10min and then quenched by saturated sodium bicarbonate (5mL). Dichloromethane (30mL X 3) was used for extraction. The combined organic phases were dried over anhydrous sodium sulfate and concentrated in vacuo to give the crude product. Purification with chromatograph on silica gel, using ethyl acetate – hexane (2:1), provided the target molecule 1 (8.2mg, 0.0132 mmol 78%). \[\alpha\]D\textsubscript{20} = 18.5° (c, 0.2, MeOH); \(\text{\textsuperscript{1}H NMR}\) (500MHz, CDCl\textsubscript{3}) \(\delta\): 7.76 (s, 1H), 7.15(d, 1H, \(J = 9.3Hz\)), 6.46(dd, 1H, \(J = 2.6Hz, 9.5Hz\)), 5.80-5.84(m, 1H), 5.65-5.68(m, 1H), 5.51(dd, 1H, \(J = 7.1Hz, 15.5Hz\)), 5.29(dd, 1H, \(J = 9.4Hz, 17.6Hz\)), 4.61(dd, 1H, \(J = 3.3Hz, 9.2Hz\)), 4.27(dd, 1H, \(J = 2.8Hz, 17.6Hz\)), 4.05(d, 1H, \(J = 11.3Hz\)), 3.28(d, 1H, \(J = 11.3Hz\)), 2.90(t, 2H, \(J = 7.2Hz\)), 2.86(dd, 1H, \(J = 10.5Hz, 16.5Hz\)), 2.68(dd, 1H, \(J = 2.0Hz, 16.3Hz\)), 2.53(t, 2H, \(J = 7.4Hz\)), 2.29-2.33(m, 2H), 2.07-2.13(m, 1H), 1.87(s, 3H), 1.62-1.66(m, 2H), 1.25-1.30(m, 8H), 0.87(t, 3H, \(J = 6.8Hz\)), 0.69(d, 3H, \(J = 7.0Hz\)), 0.51(d, 3H, \(J = 7.1Hz\)); \(\text{\textsuperscript{13}C NMR}\) (75MHz, CDCl\textsubscript{3}) \(\delta\): 199.4, 173.5, 169.4, 168.9, 167.9, 164.6, 147.4, 132.7, 128.4, 124.2, 84.4, 72.1, 57.7, 44.1, 43.3, 41.1, 40.4, 34.2, 32.2, 31.6, 29.0, 28.9, 27.9, 25.6, 24.2, 22.6, 18.9, 16.6, 14.0 ppm. \(\text{ESIMS } m/z\) at 623.23(44.5%), 645.21(100.0%). \(\text{HR-ESIMS } m/z\) Calcd for C\textsubscript{29}H\textsubscript{43}N\textsubscript{4}O\textsubscript{5}S\textsubscript{3} [M+H]\(^+\) 623.2396. Found: [M+H]\(^+\) 623.2371.
R4-28 Chloroform-D Bruker DPX 300

ppm (t1)

R4-28 Chloroform-D Bruker DPX 300

ppm (t1)