“Total Synthesis and Structural Confirmation of Chlorodysinosin A”

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Supporting Information (31 pages)

Experimental Procedures (S2-S8)

^1H and ^{13}C NMR Spectra (S9-S30)

Biological Activity (S31)

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General. Solvents were distilled under positive pressure of dry argon before use and dried by standard methods; THF and ether, from Na/benzophenone; and CH₂Cl₂, from CaCl₂. All commercially available reagents were used without further purification. All reactions were performed under nitrogen atmosphere. NMR (¹H, ¹³C) spectra were recorded on AMX-300, ARX-400, AV-400 and DMX-500 spectrometers. Low- and high-resolution mass spectra were recorded using fast atom bombardement (FAB) or electrospray techniques. Optical rotations were recorded in a 1 dm cell at ambient temperature. Analytical thin-layer chromatography was performed on pre-coated silica gel plates. Visualization was performed by ultraviolet light and/or by staining with ceric ammonium molybdate, ninhydrin, or potassium permanganate. Flash column chromatography was performed using (40-60 μm) silica gel at increased pressure.

(2*S*,3*S*)-3-Azido-1-(*tert*-butyldimethylsilyloxy)-4-methyl-pentan-2-ol (5a) and (2*S*,3*R*)-2-Azido-1-(*tert*-butyldimethylsilyloxy)-4-methyl-pentan-3-ol (5b)

To a solution of (2*R*,3*R*)-(3-isopropoxyiranyl)-methanol (8.00 g, 68.8 mmol) in 95 mL MeOEtOH was added NaN₃ (44.7 g, 0.69 mol), NH₄Cl (7.37g, 0.14 mol), and 5 ml of water. The mixture was heated to reflux for 24 h, cooled to RT, and the solvent removed under vacuum. The residue was then taken up in water, extracted with EtOAc, dried over Na₂SO₄, and concentrated. After drying under reduced pressure for 18 h, the resulting amber oil was taken up in 100 mL of CH₂Cl₂, cooled to 0 °C, and treated sequentially with Et₃N (14.4 mL, 0.10 mol), DMAP (0.20 g, catalytic), and TBSCl (11.42 g, 75.8 mmol). The solution was stirred at 0 °C for 2 h, diluted with CH₂Cl₂, washed with 1M HCl, dried over Na₂SO₄, and concentrated under vacuum. The crude residue was purified by flash chromatography over silica gel (8% EtOAc/hexane) to give **5a** and **5b** as a 1:1 mixture of regioisomers (14.1 g, 75% over 2 steps). ¹H NMR, mixture of regioisomers (300 MHz, CDCl₃) δ 3.98-3.84 (m, 2H), 3.78-3.63 (m, 2H), 3.57 (m, 1H), 3.39 (m, 1H), (dd, 1H, *J* = 4.4 Hz, *J* = 8.0 Hz), 2.54 (d, 1H, *J* = 5.5 Hz), 2.41 (d, 1H, *J* = 3.4 Hz), 2.08 (d of sept, 1H, *J* = 2.4 Hz, *J* = 6.8 Hz), 1.82 (d of sept, 1H, *J* = 1.4 Hz, *J* = 6.8 Hz), 1.03 (d, 3H, *J* = 6.9 Hz), 0.95-0.86 (m, 27H), 0.08 (m, 12H); ¹³C NMR, (100 MHz, CDCl₃) δ 71.1, 69.5, 63.9, 63.8, 30.1, 29.0, 25.7, 25.6, 20.3, 19.2, 18.1, 18.0, 16.6, 16.5, -5.6, -5.8; ESI/MS for C₁₂H₂₇N₃O₂Si calculated (M + H⁺) 274, found 274.

(2R,3S)- 2-(*tert*-Butyldimethylsilyloxymethyl)-3-isopropyl-aziridine (6).

A mixture of regioisomeric azides **5a** and **5b** (12.2 g, 44.6 mmol) was dissolved in 90 mL of MeCN and treated with PPh₃ (12.9 g, 49.1 mmol). The solution was stirred at RT for 2 h, then heated to 50 °C for 18 h. After removal of MeCN under vacuum, the mixture was taken up in Et₂O, filtered through a pad of celite, and the filtrate concentrated under vacuum. The crude residue was purified by flash chromatography over silica gel (25% EtOAc/hexane) to give **6** as a colorless liquid (9.21 g, 90%). ¹H NMR, (400 MHz, CDCl₃) δ 3.70, (m, 2H), 1.95 (dd, 1H, *J* = 3.7 Hz, *J* = 7.2 Hz), 1.61 (dd, 1H, *J* = 3.1 Hz, *J* = 7.9 Hz), 1.22 (sept, 1H, *J* = 6.8 Hz), 1.12 (m, 1H), 1.05 (d, 3H, *J* = 6.6 Hz), 0.96 (d, 3H, *J* = 6.7 Hz), 0.89 (s, 9H), 0.08 (s, 6H); ¹³C NMR, (100 MHz, CDCl₃) δ 63.2, 41.1, 37.6, 32.4, 26.2, 20.8, 20.4, 18.6, -5.0; [α]_D -25.3 (*c* 1.0, CHCl₃); ESI/MS for C₁₂H₂₇NOSi calculated (M + H⁺) 230, found 230.

(2R,3S)- 2-(*tert*-Butyldimethylsilyloxymethyl)-3-isopropyl-1-(*tert*-butylsulfonyl)-aziridine (7d)

A solution of aziridine **6** (6.00 g, 26.2 mmol) in 100 mL of CH₂Cl₂ was cooled to 0 °C and treated with NEt₃ (36.5 mL, 0.261 mol), followed by dropwise addition of *tert*-butylsulfinyl chloride (6.81 mL, 54.9 mmol). The reaction was stirred at 0 °C until TLC showed consumption of the starting material (2 h). Upon completion, 100 mL of 10% aq. Na₂CO₃ was added, and the layers separated (note: acidic washes should be avoided as *t*-butyl sulfinamides are known to be unstable at low pH). The organic layer was then dried over Na₂SO₄, and concentrated under vacuum. Flash chromatography over silica gel (12% EtOAc/Hexane) and concentration in vacuo gave the pure sulfinyl aziridine which was directly taken up in 300 mL of CH₂Cl₂, cooled to 0 °C, and treated with *m*CPBA (5.42 g, 31.4 mmol). After the oxidation was complete by TLC (1 h), the mixture was filtered through a celite pad and the filtrate concentrated under vacuum. The crude residue was purified by flash chromatography over silica gel (10% EtOAc/hexane) to give **7d** as a colorless oil (8.03 g, 88% over 2 steps). ¹H NMR, (400 MHz, CDCl₃) δ 4.04 (m, 1H), 3.72 (dd, 1H, *J* = 7.1 Hz, *J* = 10.9 Hz), 2.75 (m, 1H), 2.53 (m, 1H), 1.90 (sept, 1H, *J* = 6.8 Hz), 1.44 (s, 9H), 1.16 (d, 3H, *J* = 6.6 Hz), 0.96 (d, 3H, *J* = 6.8 Hz), 0.88 (s, 9H), 0.08 (s, 6H); ¹³C NMR, (100 MHz, CDCl₃) δ 61.8, 59.8, 52.0, 46.6, 31.3, 28.3, 25.6, 24.0, 23.5, 20.4, 18.0; [α]_D +31.5 (*c* 1.0, CHCl₃); ESI/MS for C₁₆H₃₅NO₃SSi calculated (M + H⁺) 350, found 350.

(1*S*,2*R*)-tert-Butylsulfonic acid (2-chloro-1-hydroxymethyl-3-methyl-butyl)-amide (8).

A solution of **7d** (7.20 g, 20.6 mmol) in 50 mL MeCN was heated to reflux and treated with $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ (23.0 g, 61.8 mmol) in portions over 36 h (best results were obtained when 1 equiv. was added every 12 h). The mixture was then refluxed for an additional 36 h, cooled to RT and filtered through a celite pad with EtOAc rinsing. After concentration of the filtrate under vacuum, the crude residue could be readily crystallized from CH_2Cl_2 /hexane to give pure **7**. Alternatively, the crude material was purified by flash chromatography over silica gel (35% EtOAc/hexane) to give **8** as a white solid (4.48 g 80%). ^1H NMR, (300 MHz, CDCl_3) δ 4.48 (d, 1H, $J = 10.0$ Hz), 4.00-3.76 (m, 4H), 2.14 (sept, 1H, $J = 6.8$ Hz), 2.01 (bs, 1H), 1.42 (s, 9H), 1.07 (2d, 6H, $J = 6.4$ Hz, $J = 6.4$ Hz); ^{13}C NMR, (100 MHz, CDCl_3) δ 71.7, 61.8, 60.7, 60.6, 60.5, 60.0, 57.4, 31.3, 29.7, 24.3, 24.1, 20.2, 19.0 ; $[\alpha]_D -4.6$ (c 1.0, CHCl_3); ESI/MS for $\text{C}_{10}\text{H}_{22}\text{ClNO}_3\text{S}$ calculated ($M + \text{H}^+$) 272, found 272.

(2*R*,1'*S*,2'*R*)-3-(tert-Butyldiphenylsilyloxy)-*N*-(2'-chloro-1'-hydroxymethyl-3'-methyl-butyl)-2-methoxy-propionamide (9).

A solution of **8** (0.40 g, 1.47 mmol) in 73 mL of CH_2Cl_2 was cooled to 0 °C and treated with anisole (3.20 mL, 29.4 mmol) and 5 equiv. of trifluoromethanesulfonic acid (0.65 mL, 7.36 mmol, 0.1 M final concentration). The solution was stirred at RT 18 h after which the volatiles were removed under vacuum. The residue was taken up in water, washed 3 times with Et_2O , and the aqueous solution frozen and lyophilized to give the triflate salt of the amine which was used directly. A solution of the crude amine salt in 10 mL CH_2Cl_2 was cooled to 0 °C and treated sequentially with 2,6-lutidine (0.71 mL, 7.36 mmol), 3*R*-(tert-butyldiphenylsilyloxy)-2-methoxy-propionic acid (0.58 g, 1.62 mmol), and PyBOP (0.84 g, 1.62 mmol), then allowed to warm to RT. After stirring at RT 18 h, the reaction was diluted with CH_2Cl_2 , washed with 1M HCl, 10% aq. Na_2CO_3 , and brine, dried over Na_2SO_4 and concentrated under vacuum. The crude residue was purified by flash chromatography over silica gel (35% EtOAc/hexane) to give **9** as a colorless oil (0.56 g, 77% over 2 steps). ^1H NMR, (400 MHz, CDCl_3) δ 7.69 (m, 4H), 7.46 (m, 6H), 7.18 (d, 1H, $J = 9.3$ Hz), 4.32 (m, 1H), 4.05 (m, 1H), 3.96 (m, 2H), 3.84 (t, 1H, $J = 6.6$ Hz), 3.79 (m, 1H), 3.71 (m, 1H), 3.39 (s, 3H), 2.63 (bs, 1H), 2.07 (sept, 1H, J

= 6.3 Hz), 1.10 (s, 9H), 1.00 (d, 3H, $J = 6.5$ Hz), 0.97 (d, 3H, $J = 6.7$ Hz); ^{13}C NMR, (100 MHz, CDCl_3) δ 170.5, 135.5, 135.4, 133.0, 129.6, 127.6, 82.8, 69.0, 63.2, 62.6, 58.2, 52.6, 30.7, 26.4, 20.6, 19.1, 17.4; $[\alpha]_{\text{D}}^{25} +23.1$ (c 1.0, CHCl_3); ESI/MS for $\text{C}_{26}\text{H}_{38}\text{ClNO}_4\text{Si}$ calculated ($\text{M} + \text{H}^+$) 492, found 492.

(2*S*,3*R*,2'*R*)-2-[3'-*tert*-Butyldiphenylsilyloxy-2'-methoxy-propionylamino]-3-chloro-4-methyl-pentanoic acid (10).

A solution of alcohol **9** (0.481 g, 0.978 mmol) in 5.7 mL MeCN was cooled to 0°C and treated, over 30 min, with a solution of 1 mg of CrO_3 dissolved in 5.8 mL of 0.1 M H_5IO_6 in wet MeCN (99.3/0.7 MeCN/ H_2O). TLC indicated consumption of the starting material within 30 min, and the reaction was quenched by adding 5.8 mL of sat. aq. NaHCO_3 . The MeCN was removed under vacuum, the aqueous mixture treated with 2 mL of 0.5 M aq. NaOH , and washed with Et_2O . The aqueous layer was then acidified to $\text{pH} < 3$ with 1M aq. HCl and extracted with EtOAc . The organic extracts were dried over Na_2SO_4 and concentrated under vacuum to give carboxylic acid **10** as an off-white foam (0.401 g, 78%) which was sufficiently pure for use in the following step. ^1H NMR, (300 MHz, MeOD) δ 7.65 (m, 4H), 7.58 (d, 1H, $J = 9.0$ Hz), 7.38 (m, 6H), 5.14 (dd, 1H, $J = 4.4$ Hz, $J = 9.1$ Hz), 3.93 (m, 2H), 3.39 (t, 1H, $J = 3.3$ Hz), 3.29 (dd, 1H, $J = 4.3$ Hz, $J = 8.4$ Hz), 3.41 (s, 3H), 2.24 (m, 1H), 1.08 (d, 3H, $J = 6.6$ Hz), 1.02 (m, 12H); ^{13}C NMR, (100 MHz, CDCl_3) δ 172.3, 136.8, 136.7, 134.5, 134.4, 131.0, 128.9, 84.0, 70.3, 64.5, 58.7, 56.4, 32.2, 27.5, 20.9, 20.2, 19.0; $[\alpha]_{\text{D}}^{25} +30.8$ (c 1.0, MeOH); ESI/MS for $\text{C}_{26}\text{H}_{36}\text{ClNO}_5\text{Si}$ calculated ($\text{M} + \text{H}^+$) 506, found 506.

Compound 16a.

A solution of carboxylic acid **10** (0.046 g, 0.091 mmol) and (2*S*,5*S*,6*S*,3*aR*,7*aS*)-5,6-bis(methoxy)methoxy-octahydroindole-2-carboxylic acid methyl ester (**15**, 0.025 g, 0.082 mmol) in 1 mL of CH_2Cl_2 was cooled to 0°C and treated with recrystallized DEPBT (0.037 g, 0.123 mmol), and 2,6-lutidine (0.024 mL, 0.246 mmol). The reaction was stirred at 0°C for 4h, then diluted with CH_2Cl_2 and washed with 1M HCl and 10% aq.

Na₂CO₃ (note: reaction times longer than 4 hr led to significant elimination of HCl from the coupled product). The organic layer was dried over Na₂SO₄, evaporated under vacuum, and the crude residue purified by flash chromatography over silica gel (30% EtOAc/hexane) to give **16a** as a white foam (0.041 g, 57%). ¹H NMR, (400 MHz, CDCl₃) δ 7.65 (m, 4H), 7.36 (m, 6H), 7.17 (d, 1H, *J* = 9.9 Hz), 5.15 (t, 1H, *J* = 10.4 Hz), 4.83 (d, 1H, *J* = 6.7 Hz), 4.75-4.51 (m, 3H), 4.39 (m, 2H), 3.95 (m, 4H), 3.70 (m, 2H), 3.70 (s, 3H), 3.41 (s, 3H), 3.37 (s, 3H), 3.35 (s, 3H), 2.42 (m, 2H), 2.26 (m, 2H), 2.12 (m, 2H), 1.96 (t, 1H, *J* = 14.0 Hz), 1.80 (m, 1H), 1.09 (s, 9H), 0.99 (d, 3H, *J* = 6.5 Hz), 0.78 (d, 3H, *J* = 6.8 Hz); ¹³C NMR, (100 MHz, CDCl₃) δ 172.9, 170.5, 169.1, 136.1, 136.0, 133.8, 133.5, 130.2, 130.1, 128.1, 96.1, 95.2, 83.5, 83.4, 72.8, 69.2, 63.9, 59.2, 58.8, 56.1, 56.0, 55.0, 52.6, 52.5, 35.9, 33.2, 28.7, 27.2, 26.7, 26.0, 21.2, 19.8, 15.4; [α]_D +47.8 (c 1.0, CHCl₃); ESI/MS for C₄₀H₅₉ClN₂O₁₀Si calculated (M + H⁺) 791, found 791.

Compound 16b.

A solution of ester **16a** (0.400 g, 0.505 mmol) in 8 mL of 1,2-DCE was treated with 20 equiv. of solid Me₃SnOH over 48 h while stirring at 75°C. Best results were obtained when 5 equiv. of reagent were added every 12 h. After TLC indicated a complete reaction, the mixture was partitioned between DCM and 1M HCl and the organic layer was washed with 5 portions of 1M HCl. Drying over Na₂SO₄ and evaporation under vacuum afforded the crude carboxylic acid which contained residual Me₃SnOH. The crude material could be purified by flash chromatography over silica gel (100% EtOAc to 10% MeOH/EtOAc) to give **16b** as a white foam (0.305 g, 78%). ¹H NMR, (400 MHz, DMSO-*d*₆) δ 7.68 (d, 1H, *J* = 9.6 Hz), 7.63 (m, 4H), 7.45 (m, 6H), 5.04 (t, 1H, *J* = 10.3 Hz), 4.77 (d, 1H, *J* = 6.6 Hz), 4.62 (m, 3H), 4.25 (m, 1H), 4.12 (m, 2H), 3.85 (m, 4H), 3.64 (m, 1H), 3.37 (m, 3H), 3.27 (m, 3H), 3.24 (m, 3H), 2.28 (m, 4H), 2.11 (qd, 1H, *J* = 6.1 Hz, *J* = 8.1 Hz), 1.98 (m, 1H), 1.83 (dd, 2H, *J* = 14.3 Hz, *J* = 28.6 Hz), 0.98 (s, 9H), 0.85 (d, 3H, *J* = 6.5 Hz), 0.75 (d, 3H, *J* = 6.7 Hz); ¹³C NMR, (100 MHz, DMSO-*d*₆) δ 173.9, 170.2, 168.5, 136.1, 136.0, 133.8, 130.9, 128.8, 96.4, 94.9, 83.3, 72.9, 72.7, 69.5, 64.3, 59.4, 58.3, 55.9, 55.1, 52.4, 35.9, 33.4, 28.6, 27.5, 26.7, 26.1, 21.4, 18.9, 15.7; [α]_D +35.5 (c 1.0, CHCl₃); ESI/MS for C₃₉H₅₇ClN₂O₁₀Si calculated (M + H⁺) 777, found 777.

Compound 18a.

A solution of carboxylic acid **16b** (0.050 g, 0.064 mmol) and amine **17** (0.046 g, 0.129 mmol) in 1 mL of DCM was cooled to 0°C and treated with PyBOP (0.050 g, 0.096 mmol) and 2,6-lutidine (0.021 mL, 0.193 mmol). After stirring 4 h the reaction was diluted with DCM and washed with 1M HCl and sat. aq. NaHCO₃. The organic layer was dried over Na₂SO₄ and concentrated under vacuum to give the crude product. Purification by flash chromatography over silica gel (65% to 75% EtOAc/Hex) gave **18a** as a white foam (0.066 g, 93%). ¹H NMR, (400 MHz, CDCl₃) δ 7.65 (m, 4H), 7.38 (m, 6H), 7.19 (d, 1H, *J*=9.4 Hz), 5.51 (s, 1H), 5.09 (t, 1H, *J*=9.0 Hz), 4.72 (d, 1H, *J*=6.6 Hz), 4.64 (m, 3H), 4.51-4.20 (m, 6H), 4.12-3.85 (m, 4H), 3.74 (m, 1H), 3.65 (m, 1H), 3.38 (m, 9H), 2.71 (m, 1H), 2.45-1.82 (m, 12H), 1.49 (m, 18H), 1.10 (s, 9H), 0.98 (d, 3H, *J*=6.3 Hz), 0.83 (d, 3H, *J*=6.6 Hz); ¹³C NMR, (100 MHz CDCl₃) δ 171.1, 169.9, 169.4, 153.8, 135.5, 135.4, 133.1, 129.7, 127.6, 119.3, 95.5, 94.8, 82.6, 72.6, 72.0, 68.1, 62.7, 60.2, 57.9, 55.5, 55.1, 52.3, 37.4, 34.8, 32.0, 31.1, 28.4, 28.3, 28.2, 28.0, 26.6, 26.1, 25.4, 20.6, 19.1, 14.7; [α]_D +43.4 (*c* 0.5, MeOH); ESI/MS for C₅₆H₈₅ClN₆O₁₃Si calculated (*M* + *H*⁺) 1114, found 1114.

Compound 18b.

A solution of compound **18a** (0.060 g, 0.054 mmol) in 0.540 mL of THF was cooled to 0°C and treated with a 1M solution of TBAF in THF (0.269 mL, 0.269 mmol). The reaction was stirred at RT for 15 min, diluted with 1M HCl, and extracted with DCM. The organic layers were dried over Na₂SO₄ and concentrated under vacuum. Purification by flash chromatography over silica gel (100% EtOAc to 5% MeOH/EtOAc, then 10% MeOH/DCM) gave **18b** as a white foam (0.040 g, 85%). ¹H NMR (400 MHz, CDCl₃) δ 7.12 (d, 1H, *J*=9.0Hz), 6.39 (s, 1H), 5.55 (s, 1H), 5.10 (t, 1H, *J*=10.2Hz), 4.86 (d, 1H, *J*=6.6Hz), 4.65 (m, 3H), 4.35 (m, 5H), 4.12 (m, 1H), 3.83 (m, 4H), 3.44 (s, 6H), 3.39 (m, 2H), 3.37 (s, 3H), 2.99 (m, 1H), 2.69 (dd, 1H, *J*=12.6Hz, *J*=23.1Hz), 2.34 (m, 2H), 2.12

1H), 2.69 (dd, 1H, $J=12.6\text{Hz}$, $J=23.1\text{Hz}$), 2.34 (m, 2H), 2.12 (m, 5H), 1.83 (m, 2H), 1.66 (s, 9H), 1.51 (s, 9H), 1.40 (m, 1H), 1.06 (d, 3H, $J=6.7\text{Hz}$), 1.01 (m, 3H). ^{13}C NMR, (100 MHz, $\text{DMSO}-d_6$) δ 171.2, 170.3, 169.2, 135.6, 119.8, 119.7, 95.5, 94.8, 81.9, 72.6, 72.1, 68.0, 62.0, 60.4, 58.9, 58.1, 55.4, 55.1, 52.3, 37.4, 35.0, 32.3, 28.5, 28.4, 28.1, 26.0, 25.4, 24.0, 20.6, 20.0, 19.6, 14.8, 13.5; $[\alpha]_D^{25} +50.2$ (c 1.0, CHCl_3); ESI/MS for $\text{C}_{40}\text{H}_{67}\text{ClN}_6\text{O}_3$ calculated ($\text{M} + \text{H}^+$) 875, found 875.

Chlorodysynosin A (2).

A solution of alcohol **18b** (0.018 g, 0.021 mmol) in 1 mL of DCM was treated with Bu_2SnO (0.002 g, 0.008 mmol) and $\text{SO}_3\cdot\text{Pyr}$ complex (0.023 g, 0.144 mmol) and stirred at RT 18 h. The crude mixture was filtered through celite and the filtrate concentrated under vacuum. The residue was then re-subjected to the sulfation conditions (with the above amounts) and stirred an additional 6 h. The mixture was again filtered through celite and evaporated to give the crude sulfate as a glassy solid. To this solid was added 2 mL DCM and 0.222 mL of > 99.9% TFA. The reaction was stirred at RT 6 h, diluted with EtOAc , and concentrated under vacuum without heating. The crude deprotected peptide was then taken up in 1.5 mL of 4:1 $\text{MeCN}:\text{H}_2\text{O}$. Purification by preparative RP-HPLC chromatography on C_{18} column (20% to 55% $\text{MeCN}:\text{H}_2\text{O}$ with 0.1% TFA, linear gradient over 30 min) yielded a solution of pure **2** in $\text{MeCN}:\text{H}_2\text{O}$. The MeCN was removed under vacuum and the aqueous solution frozen and lyophilized to give chlorodysynosin A as a white amorphous solid (5.1 mg, 38% overall yield from alcohol **18b**). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 7.81 (d, 1H, $J=8.8$ Hz, amide NH), 7.61 (t, 1H, $J=5.0$ Hz, amide NH), 7.17 (s, 3H, guanidine NH), 5.63 (s, 1H), 4.90 (m, 1H, $J_a=9.0$ Hz, $J_b=8.0$ Hz, $J_c=1.4$ Hz), 4.31 (m, 1H), 4.12 (m, 6H), 3.95 (dd, 1H, $J_a=10.7$ Hz, $J_b=2.6$ Hz), 3.90 (dd, 1H, $J_a=6.2$ Hz, $J_b=2.7$ Hz), 3.80 (dd, 1H, $J_a=10.7$ Hz, $J_b=6.3$ Hz), 3.66 (m, 1H), 3.59 (bs, 2H, OH), 3.57 (m, 1H), 3.31 (m, 1H), 3.29 (s, 3H), 3.09 (m, 1H), 2.39 (m, 1H), 2.31-2.13 (2m, 3H), 2.06 (m, 3H), 1.95 (m, 3H), 1.57 (d, 1H, $J=14.4$ Hz), 0.98 (d, 3H, $J=6.6$ Hz), 0.92 (d, 3H, $J=6.5$ Hz); ^{13}C NMR, (100 MHz, $\text{DMSO}-d_6$) δ 170.9, 168.9, 167.9, 154.0, 136.0, 119.3, 80.6, 68.5, 68.4, 67.1, 65.9, 59.8, 57.1, 55.3, 54.6, 54.1, 52.0, 36.4, 35.2, 33.4, 28.4, 27.9, 27.6, 26.6, 20.6, 15.0; HRMS for $\text{C}_{26}\text{H}_{43}\text{ClN}_6\text{O}_{10}\text{S}$ calculated ($\text{M} + \text{H}^+$) 667.25227, found 667.25042.

NMR Data for natural chlorodysynosin A (see ref 7b): ^1H NMR ($\text{DMSO}-d_6$) 7.77 (d, 1H, $J=8.7$ Hz, amide NH), 7.55 (dd or t, 1H, $J=5.8$, amide NH), 7.13 (bs, 3H, guanidine NH), 5.61 (s, 1H), 4.87 (dd, 1H, $J_a=10.2$ Hz, $J_b=9.1$ Hz), 4.76 (d, 1H, $J=2.6$ Hz, OH), 4.65 (d, 1H, $J=2.6$ Hz, OH), 4.29 (m, 1H), 4.11 (m, 2H), 4.11 (m, 1H), 4.10 (m, 2H), 4.06 (m, 1H), 3.93 (dd, 1H, $J_a=10.8$ Hz, $J_b=2.6$ Hz), 3.87 (dd, 1H, $J_a=6.2$ Hz, $J_b=2.6$ Hz), 3.78 (dd, 1H, $J_a=10.8$ Hz, $J_b=6.2$ Hz), 3.66 (bs, 1H), 3.55 (bs, 1H), 3.26 (s, 3H), 3.26 (m, 1H), 2.35 (m, 1H), 2.24 (m, 2H), 2.17 (m, 1H), 2.07 (m, 1H), 2.04 (m, 1H), 1.93 (m, 2H), 1.91 (m, 1H), 1.55 (d, 1H, $J=15.1$ Hz), 0.95 (d, 3H, $J=6.4$ Hz), 0.90 (d, 3H, $J=6.5$ Hz). ^{13}C NMR, ($\text{DMSO}-d_6$) δ 170.9, 168.9, 167.8, 154.1, 136.0, 119.3, 80.6, 68.5 (2), 67.1, 65.9, 59.8, 57.1, 55.3, 54.6, 54.1, 52.0, 36.3, 35.2, 33.4, 28.4, 27.9, 27.6, 26.6, 20.6, 15.0.

Compound 6

71.671

61.772

60.637

60.531

60.008

57.413

31.264

29.725

24.109

20.209

18.968

170.456

135.504
135.408
133.049
129.617
129.563
127.548

82.805

69.007

63.145

62.526

58.170

52.625

30.623

26.623

20.529

19.111

17.470

S18

S19

172.874
170.465
169.043

136.099
135.946
133.769
133.502
130.181
130.105
128.140

96.094
95.180

83.430

72.751
69.159
63.859
59.153
58.763
56.091
55.985
54.985
52.565
52.495
35.895
33.220
28.673
27.267
27.167
26.639
25.987
21.128
19.733
15.371

S22

S23

Biological Results (IC ₅₀)	Thrombin	Factor Xa	Factor VIIa	Trypsin
Dysinosin A (1)	46 nM	5 μ M	326 nM	--
Chlorodysinosin A (2)	5.7 nM	1.54 μ M	39 nM	37 nM

Molecular Dynamics. Molecular dynamics simulations were performed with MacroModel (Schroedinger Inc., New York, USA, www.schroedinger.com) using MMFF94 forcefield and GBSA implicit solvation. The X-ray starting conformation was carefully minimized and equilibrated in the forcefield before a 1 ns conformational sampling.

Figure 3A (left). Overlay of the bound conformation of **1** (yellow) with the minimized energy conformer obtained from modeling (green), showing a distinct Leu side-chain conformation; **B** (middle). Range of allowed χ^1 angles obtained from modeling for **1** (red data points) and **2** (black data points); **C** (right). Overlay of the bound conformation of **2** (yellow) with the minimized energy conformer obtained from modeling (purple), showing close conformational overlap.