Unified Total Synthesis of Five Bufadienolides

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Supporting Information
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1. Experimental procedures

General method: All reactions sensitive to air or moisture were carried out under argon atmosphere in dry solvents unless otherwise noted. CH_2Cl_2, DMF, THF, and toluene were purified by Glass Contour solvent dispensing system (Nikko Hansen & Co., Ltd.). All other reagents were used as supplied. All reactions under heating conditions were performed in an oil bath. Analytical thin-layer chromatography (TLC) was performed using E. Merck Silica gel 60 F_{254} pre-coated plates (0.25 mm). Flash column chromatography was performed using 40-50 μm Silica Gel 60N (Kanto Chemical Co., Inc.), 40-63 μm Silica Gel 60 (Merck), or 32-53 μm Silica Gel BW-300 (Fuji Silysia Chemical Ltd.). Preparative thin-layer chromatography (PTLC) was performed using Merck silica gel 60 F_{254} pre-coated plates (0.5 mm). Preparative gel permeation chromatography (GPC) was performed on LaboACE LC-5060 (Japan Analytical Industry Co., Ltd.) equipped with JAIGEL-2HR column using UV (254 nm) and Refractive Index (RI) detectors in $CHCl_3$ with flow rate of 10.0 mL/min. High performance liquid chromatography (HPLC) was performed on a JASCO HPLC system (pump: JASCO PU-2086 Plus x2, detector: JASCO MD-4010, degasser: ERC Inc. ERC-3325, data analysis by JASCO ChromNAV 1.5.2.). Melting points were measured on Yanaco MP-J3 micro melting point apparatus and were uncorrected. Optical rotations were measured on JASCO P-2200 polarimeter at ambient temperature using the sodium D line. Infrared (IR) spectra were recorded on JASCO FT/IR-4100 spectrometer as a thin film on KBr or CaF_2. ^1H and $^{13}C\{^1H\}$ NMR spectra were recorded on JEOL JNM-ECX-500, JNM-ECZ-500, or JNM-ECS-400 spectrometer. Chemical shifts were denoted in ppm on the δ scale relative to residual solvent peaks as internal standard: $CHCl_3$ (δ 7.26 for ^1H NMR), $CDCl_3$ (δ 77.0 for $^{13}C\{^1H\}$ NMR), C_6D_5H (δ 7.16 for ^1H NMR), C_6D_6 (δ 128.0 for $^{13}C\{^1H\}$ NMR), CD_2HOD (δ 3.31 for ^1H NMR), CD_3OD (δ 49.0 for $^{13}C\{^1H\}$ NMR), D_3CSOCD_2H (δ 2.50 for ^1H NMR), and D_3CSOCD_3 (δ 39.52 for $^{13}C\{^1H\}$ NMR). Signal patterns are indicated as s, singlet; d, doublet; t, triplet; m, multiplet; br, broaden peak. The carbon numbering of compounds corresponds to that of vulgarobufotoxin (**4**) and 3-(*N*-succinyl argininyl) bufotalin (**5**), unless otherwise noted. High resolution mass spectra were measured on JEOL JMS-T100LP (ESI-TOF) or BRUKER DALTONICS microTOF II (ESI-TOF). X-ray crystallographic analysis was conducted using Inorganic Fine Crystal Structural Diffractometer VariMax Dual (Rigaku Co., Ltd.) with Mo radiation (λ = 0.71073 Å).

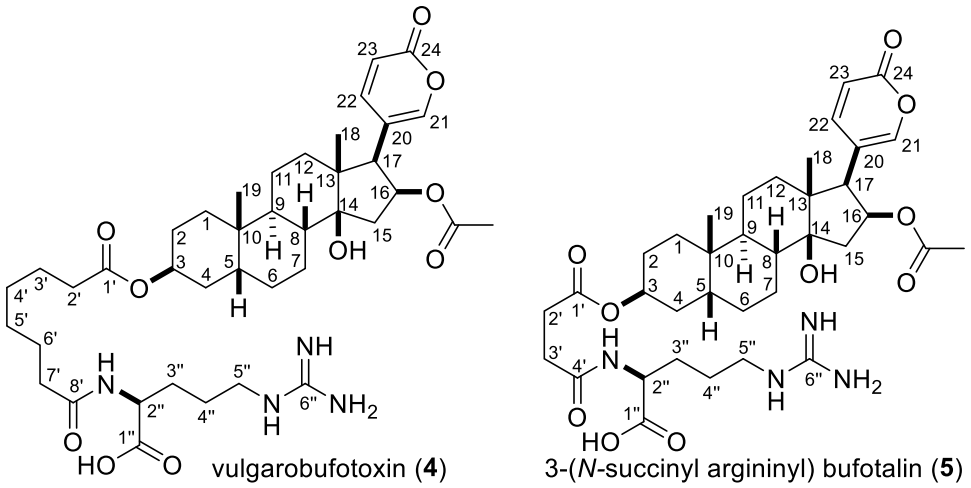
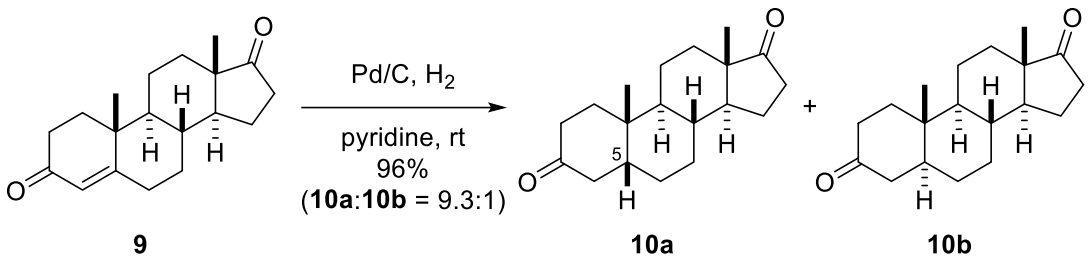
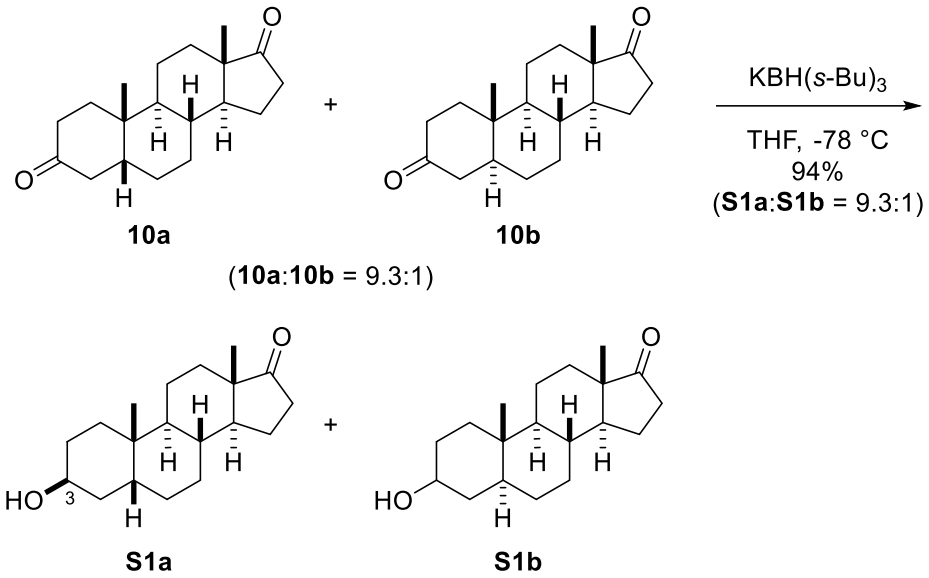


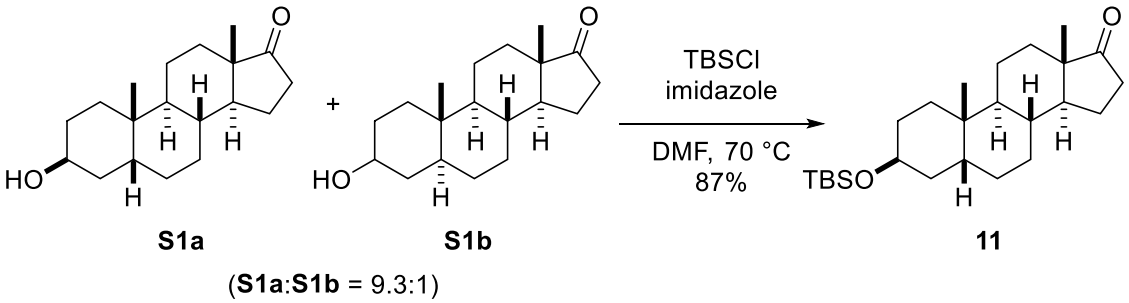
Figure S1. Numbering system of vulgarobufotoxin (**4**) and 3-(*N*-succinyl argininyl) bufotalin (**5**)



Diketones 10a [CAS: 1229-12-5][S1] **and 10b [CAS: 846-46-8].**[S2] A suspension of Pd/C (10 wt% Pd on carbon, 499 mg) in pyridine (15 mL) was stirred at room temperature for 30 min. Compound **9** (5.53 g, 19.3 mmol) was added to the suspension, and the resultant suspension was stirred under H_2 atmosphere (1 atm) at room temperature for 12 h. The reaction mixture was then filtered through a pad of Celite with CH_2Cl_2 (15 mL) and concentrated. After 1 M aqueous HCl (15 mL, 15 mmol) was added to the residue, the resultant mixture was extracted with CH_2Cl_2 (30 mL x6). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (100 g, hexane/CH_2Cl_2 = 3/1 to 0/1) to afford a 9.3:1 diastereomeric mixture of diketones **10a** and **10b** (5.34 g, 18.5 mmol) in 96% yield. The C5-stereochemistry of **10a** was determined by the X-ray crystallographic analysis of epoxide **6**: white solid. m.p. 121-123 °C. $[\alpha]_D^{27}$ +98 (*c* 0.96, $CHCl_3$). IR (film) 2934, 2861, 1738, 1712, 1452, 1377, 1266, 1248, 1051 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) *peaks of the major product **10a***: δ 0.89 (3H, s, H18), 1.07 (3H, s, H19), 1.30-1.80 (13H, m), 1.95-2.10 (6H, m), 2.41-2.48 (2H, m), 2.84 (1H, dd, *J* = 14.6, 14.6 Hz). $^{13}C\{^1H\}$ NMR (125 MHz, CD_3OD) *peaks of the major product **10a***: δ 14.2, 21.5, 22.7, 22.9, 25.7, 27.5, 32.8, 36.2, 36.4, 36.7, 37.9, 38.1, 41.8, 43.1, 45.8, 49.0 (deduced from HMBC), 52.4, 215.6, 223.5. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{19}H_{28}O_2Na$ 311.1987; Found 311.1976.

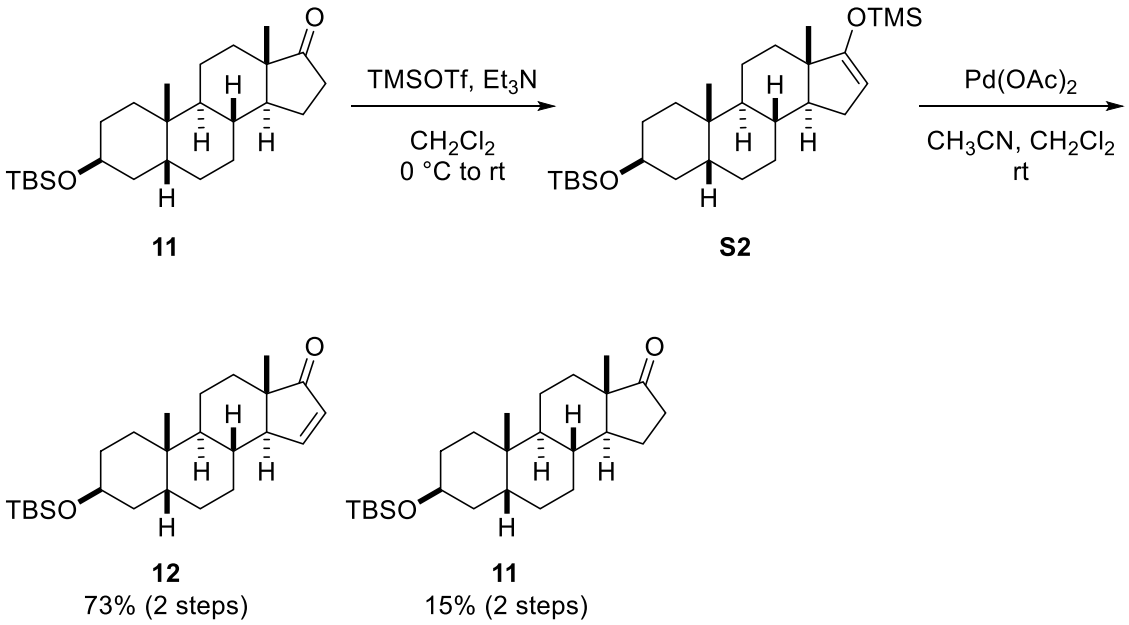


Ketones S1a [CAS: 571-31-3][S3] **and S1b.** KBH(*s*-Bu)$_3$ (1.0 M THF solution, 18.5 mL, 18.5 mmol) was added to a solution of a 9.3:1 diastereomeric mixture of diketones **10a** and **10b** (5.34 g, 18.5 mmol) in THF (185 mL) at -78 °C. After the reaction mixture was stirred at -78 °C for 5.5 h, 2.6 M aqueous NaOH (7.0 mL, 18 mmol) and aqueous H_2O_2 (30 wt%, 9.8 M, 7.0 mL, 69 mmol) were added to the mixture. The resultant mixture was concentrated and extracted with EtOAc (50 mL x5). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (150 g, hexane/EtOAc = 1/0 to 20/1 to 10/1) to afford a 9.3:1 diastereomeric mixture of ketones **S1a** and **S1b** (5.06 g, 17.4 mmol) in 94% yield. The C3-stereochemistry of **S1a** was determined by the X-ray crystallographic analysis of epoxide **6**: white solid. m.p. 163-166 °C. $[\alpha]_D^{27}$ +62 (*c* 1.10, $CHCl_3$). IR (film) 3218, 2929, 2859, 1738, 1451, 1405, 1324, 1197, 1031 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) *peaks of the major product **S1a***: δ 0.85 (3H, s, H18), 0.98 (3H, s, H19), 1.07-1.17 (1H, m), 1.19-1.42 (7H, m), 1.44-1.66 (7H, m), 1.73-1.82 (2H, m), 1.88-1.99 (3H, m), 2.06 (1H, ddd, *J* = 19.2, 9.4, 9.4 Hz), 2.43 (1H, dd, *J* = 19.2, 8.9 Hz), 4.11 (1H, dd, *J* = 2.8, 2.8 Hz, H3). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) *peaks of the major product **S1a***: δ 13.8, 20.3, 21.8, 23.8, 25.2, 26.3, 27.8, 29.9, 31.7, 33.4, 35.2, 35.3, 35.9, 36.5, 40.0, 47.9, 51.6, 66.9, 221.5. HRMS (ESI-TOF) [M+Na]$^+$ m/z: Calcd for $C_{19}H_{30}O_2Na$ 313.2143; Found 313.2140.



Ketone 11 [CAS: 65598-74-5].[S4] *t*-Butyldimethylsilyl chloride (TBSCl, 15.7 g, 104 mmol) was added to a solution of a 9.3:1 diastereomeric mixture of ketones **S1a** and **S1b** (15.1 g, 52.0

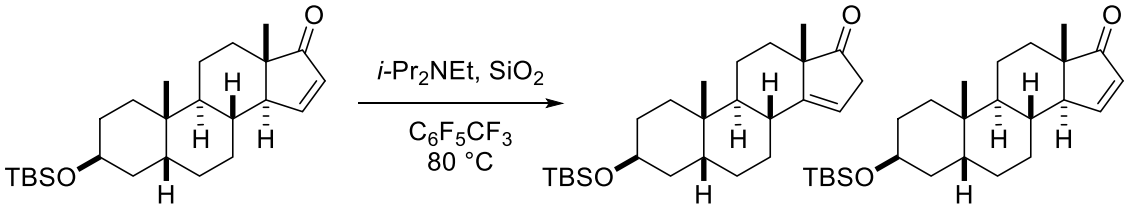
mmol), and imidazole (14.1 g, 207 mmol) in DMF (73 mL) at room temperature. After the reaction mixture was stirred at 70 °C for 15 h, saturated aqueous $NaHCO_3$ (100 mL) and H_2O (50 mL) were added to the mixture at 0 °C. The resultant mixture was extracted with Et_2O (100 mL x4). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (250 g, hexane/Et_2O = 100/1 then hexane/EtOAc = 3/1) to afford ketone **11** (18.2 g, 45.0 mmol) in 87% yield: white solid. m.p. 121-123 °C. $[\alpha]_D^{27}$ +70 (*c* 0.47, $CHCl_3$). IR (film) 2927, 2857, 1742, 1449, 1406, 1373, 1253, 1163, 1057, 1005 cm^{-1}. ^1H NMR (400 MHz, C_6D_6) δ 0.10 (6H, s, C*H*$_3$ of TBS), 0.62 (3H, s, H18), 0.88 (3H, s, H19), 0.94-1.41 (13H, m), 1.02 (9H, s, *t*-Bu of TBS), 1.47-1.56 (2H, m), 1.61-1.96 (6H, m), 2.16 (1H, dd, *J* = 19.2, 9.2 Hz, H16a), 4.02 (1H, brs, H3). $^{13}C\{^1H\}$ NMR (125 MHz, C_6D_6) δ -4.66, 13.7, 18.4, 20.6, 21.8, 24.0, 25.5, 26.1, 26.9, 28.8, 30.4, 32.4, 34.7, 35.3, 35.4, 35.7, 37.0, 40.4, 47.6, 51.4, 67.9, 218.2. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{25}H_{44}O_2SiNa$ 427.3008; Found 427.3014.



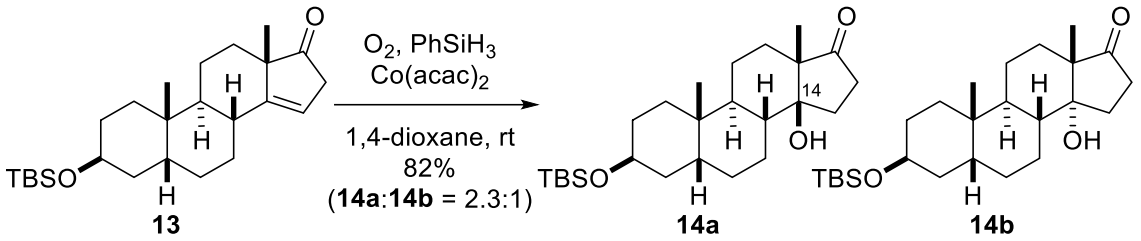
Enone 12 [CAS: 64647-72-9].[S4] Trimethylsilyl trifluoromethanesulfonate (TMSOTf, 24 mL, 0.13 mol) was added to a solution of ketone **11** (18.2 g, 45.0 mmol) and Et_3N (25 mL, 0.18 mol) in CH_2Cl_2 (900 mL) at 0 °C. After the reaction mixture was stirred at 0 °C for 20 min and at room temperature for 40 min, saturated aqueous $NaHCO_3$ (300 mL) was added to the mixture. The resultant mixture was extracted with hexane (600 mL x3). The combined organic layers were washed with brine (100 mL x2), dried over Na_2SO_4, filtered, and concentrated to afford the crude silyl enol ether **S2** [CAS: 914644-97-6][S4] (19.9 g), which was used in the next reaction without further purification.

$Pd(OAc)_2$ (12.1 g, 53.9 mmol) was added to a solution of the above crude silyl enol ether **S2** (19.9 g) in CH_3CN (720 mL) and CH_2Cl_2 (180 mL) at room temperature. After the reaction mixture was stirred at room temperature for 25 h, saturated aqueous $NaHCO_3$ (300 mL) was added to the mixture. The resultant mixture was filtered through a pad of Celite with EtOAc

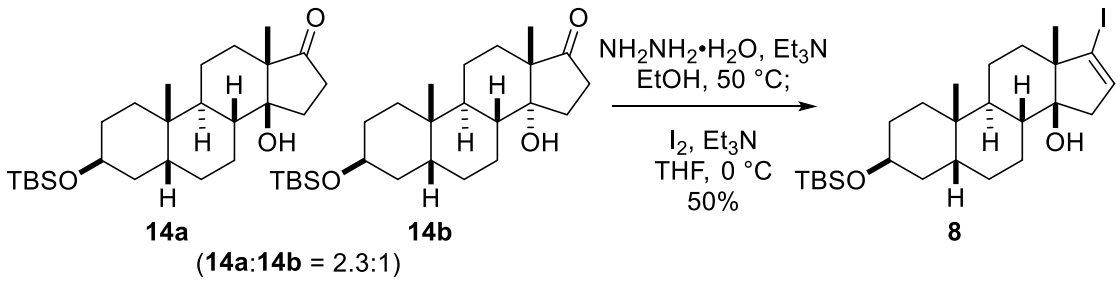
(300 mL) and extracted with EtOAc (300 mL x3). The combined organic layers were washed with brine (200 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (500 g, hexane/EtOAc = 100/1 to 20/1) to afford enone **12** (13.2 g, 32.8 mmol) and ketone **11** (2.66 g, 6.57 mmol) in 73% and 15% yields over 2 steps, respectively: white solid. m.p. 122-124 °C. $[\alpha]_D^{25}$ -17 (*c* 1.74, $CHCl_3$). IR (film) 2931, 2858, 1711, 1455, 1369, 1252, 1058, 1003 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.01 (6H, s, C*H*$_3$ of TBS), 0.88 (9H, s, *t*-Bu of TBS), 1.00 (3H, s), 1.04 (3H, s), 1.16-1.27 (3H, m), 1.38-1.58 (8H, m), 1.70-1.74 (1H, m), 1.81-1.99 (5H, m), 2.33 (1H, d, *J* = 11.2 Hz), 4.03 (1H, s, H3), 6.01 (1H, dd, *J* = 5.5, 3.0 Hz, H16), 7.52 (1H, d, *J* = 5.5 Hz, H15). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ -4.88, -4.86, 18.1, 20.0, 20.7, 23.9, 25.4, 25.8, 26.5, 28.5, 29.4, 29.9, 32.7, 34.4, 35.4, 36.5, 41.6, 51.2, 57.2, 67.1, 131.6, 158.8, 213.6. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{25}H_{42}O_2SiNa$ 425.2852; Found 425.2849.



Ketone 13 [CAS: 914644-98-7].[S4] *i*-Pr_2NEt (160 mL, 918 mmol) was added to a suspension of enone **12** (13.5 g, 33.5 mmol) and SiO_2 (50.3 g) in $C_6F_5CF_3$ (85 mL) at room temperature. After being stirred at 80 °C for 4 h, the reaction mixture was filtered through a pad of Celite with EtOAc (500 mL) and concentrated. The residue was purified by flash column chromatography on silica gel (500 g, hexane/EtOAc = 1/0 to 100/1 to 20/1) to afford ketone **13** (6.81 g, 16.9 mmol) and enone **12** (6.13 g, 15.2 mmol) in 50% and 45% yields, respectively: colorless oil. $[\alpha]_D^{26}$ +111 (*c* 0.38, $CHCl_3$). IR (film) 2930, 2860, 1747, 1639, 1449, 1376, 1254, 1158, 1057, 1009 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.00 (6H, s, C*H*$_3$ of TBS), 0.87 (9H, s, *t*-Bu of TBS), 0.98 (3H, s), 1.09 (3H, s), 1.20-1.26 (3H, m), 1.32-1.43 (5H, m), 1.48-1.55 (2H, m), 1.56-1.61 (2H, m), 1.74-1.82 (3H, m), 1.91-1.99 (1H, m), 2.17 (1H, brs), 2.79-2.85 (1H, m, H16a), 2.95-3.02 (1H, m, H16b), 4.00 (1H, s, H3), 5.47 (1H, d, *J* = 1.6 Hz, H15). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ -4.90, -4.87, 18.1, 19.9, 20.8, 23.1, 23.7, 25.8, 26.3, 28.6, 29.7, 33.5, 34.2, 35.5, 35.6, 36.4, 41.1, 41.4, 51.0, 67.2, 112.6, 154.1, 222.9. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{25}H_{42}O_2SiNa$ 425.2852; Found 425.2835.

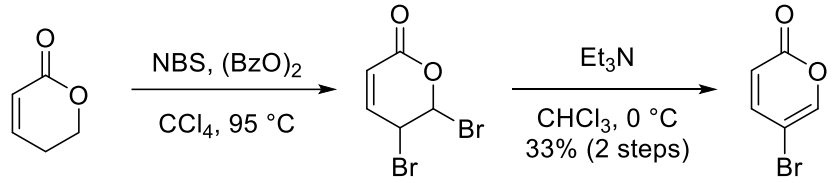


Ketones 14a and 14b. A solution of ketone **13** (10.1 g, 25.1 mmol) in 1,4-dioxane (450 mL) was bubbled with O_2 for 25 min at room temperature. Then cobalt(II) acetylacetonate ($Co(acac)_2$, 1.30 g, 5.06 mmol) was added to the mixture at room temperature. A solution of $PhSiH_3$ (9.3 mL, 75 mmol) in 1,4-dioxane (50 mL) was added to the mixture at room temperature over 1 h via a syringe pump under O_2 atmosphere (1 atm). After the reaction mixture was stirred under O_2 atmosphere (1 atm) at room temperature for 4 h, saturated aqueous $Na_2S_2O_3$ (200 mL) was added to the mixture. The resultant mixture was extracted with EtOAc (200 mL x4). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (500 g, hexane/EtOAc = 10/1 to 3/1) to afford a 2.3:1 mixture of ketones **14a** and **14b** (8.71 g, 20.7 mmol) in 82% yield. The C14-stereochemistry of **14a** was determined by the X-ray crystallographic analysis of epoxide **6**: white solid. m.p. 148-149 °C. $[\alpha]_D^{25}$ +21 (*c* 1.65, $CHCl_3$). IR (film) 3426, 2931, 2888, 2858, 1732, 1469, 1447, 1376, 1361, 1253, 1064 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.01 (6H, s, C*H*$_3$ of TBS), 0.87 (9H, s, *t*-Bu of TBS), 0.93 (3H x7/10, s, H19), 0.96 (1H x3/10, s, H19), 0.97 (3H x3/10, s, H18), 1.04 (3H x7/10, s, H18), 1.10-1.91 (18H and 1H x3/10, m), 2.13-2.21 (1H x7/10, m, H15a), 2.33-2.47 (2H, m, H16), 3.94-4.09 (1H, m, H3). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ -4.9, 12.8 (1C x7/10), 18.0 (1C x3/10), 18.1 (1C x7/10), 19.1 (1C x3/10), 19.6 (1C x7/10), 19.8 (1C x7/10), 19.9 (1C x3/10), 23.7 (1C x3/10), 23.8 (1C x7/10), 25.0 (1C x3/10), 25.8, 26.3 (1C x3/10), 26.4 (1C x7/10), 27.3 (1C x7/10), 28.5 (1C x3/10), 28.6 (1C x7/10), 29.7 (1C x7/10), 30.0 (1C x3/10), 30.2 (1C x3/10), 32.1 (1C x7/10), 33.0 (1C x7/10), 33.1 (1C x3/10), 33.2 (1C x3/10), 34.2, 35.2 (1C x7/10), 35.3 (1C x3/10), 35.9, 36.4 (1C x3/10), 38.0 (1C x3/10), 41.5 (1C x7/10), 52.7 (1C x3/10), 53.6 (1C x7/10), 67.0 (1C x7/10), 67.1 (1C x3/10), 81.5 (1C x3/10), 82.7 (1C x7/10), 219.2 (1C x3/10), 221.6 (1C x7/10), one $^{13}C\{^1H\}$ peak of **14a** was not observed. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{25}H_{44}O_3SiNa$ 443.2957; Found 443.2943.



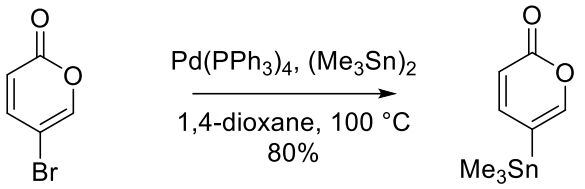
Vinyl iodide 8. $NH_2NH_2•H_2O$ (7.2 mL, 0.15 mol) and Et_3N (21 mL, 0.15 mol) were added to a solution of a 2.3:1 diastereomeric mixture of ketones **14a** and **14b** (3.14 g, 7.46 mmol) in EtOH (750 mL) at room temperature. After being stirred at 50 °C for 13 h, the reaction

mixture was concentrated. A solution of I_2 (5.73 g, 22.6 mmol) in THF (50 mL) was added dropwise to a solution of the residue and Et_3N (21 mL, 0.15 mol) in THF (300 mL) at 0 °C. After the reaction mixture was stirred at 0 °C for 40 min, saturated aqueous $Na_2S_2O_3$ (150 mL) and saturated aqueous $NaHCO_3$ (100 mL) were added to the mixture. The resultant mixture was extracted with EtOAc (150 mL x3). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (250 g, hexane/EtOAc = 1/0 to 70/1 to 10/1) to afford vinyl iodide **8** (1.98 g, 3.73 mmol) in 50% yield: white foam. $[\alpha]_D^{24}$ +12 (*c* 0.54, $CHCl_3$). IR (film) 3458, 2931, 2885, 2857, 1462, 1378, 1359, 1254, 1062 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.01 (6H, s, C*H*$_3$ of TBS), 0.80-1.05 (2H, m), 0.87 (9H, s, *t*-Bu of TBS), 0.93 (3H, s, H19), 1.03 (3H, s, H18), 1.15-1.26 (3H, m), 1.35-1.55 (6H, m), 1.61-1.97 (6H, m), 2.21 (1H, d, *J* = 16.6 Hz, H15a), 2.55 (1H, d, *J* = 16.6 Hz, H15b), 4.03 (1H, s, H3), 6.09 (1H, s, H16). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ -4.89, -4.87, 17.9, 18.0, 19.7, 21.1, 24.0, 25.8, 26.5, 28.7, 29.7, 34.2, 35.1, 36.0, 36.5, 37.5, 41.4, 42.6, 54.7, 67.1, 82.5, 111.5, 133.5. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{25}H_{43}IO_2SiNa$ 553.1975; Found 553.1955.



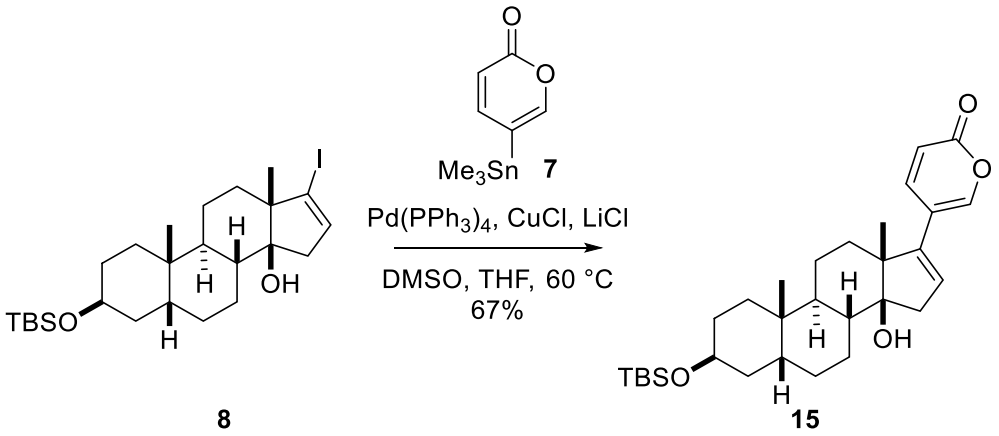
S3 **S4** **S5**

Bromide S5 [CAS: 19978-33-7].[S5] *N*-bromosuccinimide (NBS, 39.5 g, 222 mmol) and benzoyl peroxide ($(BzO)_2$, 75% purity, 1.36 g, 4.21 mmol) were successively added to a solution of lactone **S3** (9.0 mL, 0.11 mol) in CCl_4 (500 mL) at room temperature. After being stirred at 95 °C for 14.5 h, the reaction mixture was concentrated. The residue was purified by flash column chromatography on silica gel (250 g, hexane/EtOAc = 1/0 to 3/1) to afford the crude dibromide **S4** (24.6 g), which was used in the next reaction without further purification. Et_3N (74 mL, 0.53 mol) was added to a solution of the above crude dibromide **S4** (24.6 g) in $CHCl_3$ (500 mL) at 0 °C. After being stirred at 0 °C for 4 h, the reaction mixture was concentrated. The resulting mixture was filtered through a pad of silica gel (150 g, hexane/EtOAc = 1/1) and concentrated. The residue was purified by flash column chromatography on silica gel (500 g, hexane/EtOAc = 1/0 to 30/1 to 20/1) to afford bromide **S5** (6.31 g, 36.1 mmol) in 33% yield over 2 steps as white solid. The analytical data of **S5** were identical to those reported previously.[S6]



S5 **7**

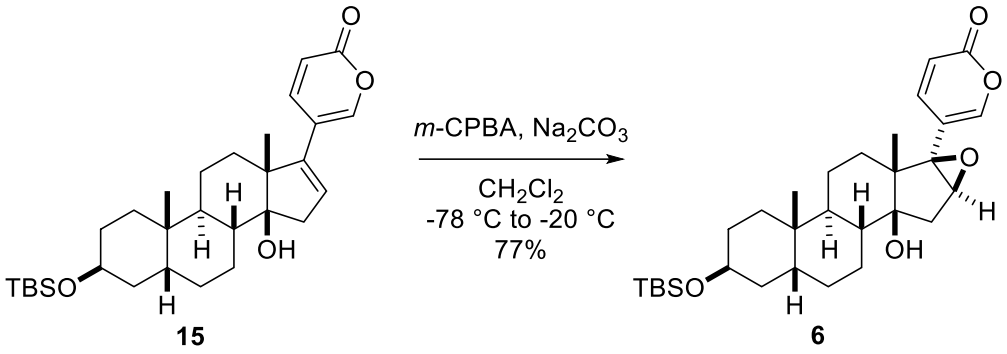
2-Pyrone 7 [CAS: 181866-08-0].[S6] $Pd(PPh_3)_4$ (1.91 g, 1.65 mmol) and $(Me_3Sn)_2$ (4.4 mL, 21 mmol) were added to a solution of bromide **S5** (2.84 g, 16.2 mmol) in 1,4-dioxane (41 mL), and then the mixture was degassed by the freeze-thaw procedure (x3). After being stirred at 100 °C for 12 h, the reaction mixture was concentrated. The residue was purified by flash column chromatography on silica gel (150 g, hexane/EtOAc = 1/0 to 20/1 to 10/1) to afford 2-pyrone **7** (3.34 g, 12.9 mmol) in 80% yield: colorless oil. IR (film) 2958, 2919, 2850, 1739, 1716, 1522, 1411, 1341, 1216, 1131 cm^{-1}. ^1H NMR (400 MHz, C_6D_6) δ -0.05 (9H, s, C*H*$_3$ of $SnMe_3$), 6.04 (1H, d, *J* = 9.2 Hz, H23), 6.45 (1H, dd, *J* = 9.2, 1.6 Hz, H22), 6.66 (1H, s, H21). $^{13}C\{^1H\}$ NMR (100 MHz, C_6D_6) δ -10.0, 112.0, 117.4, 147.1, 155.0, 160.8. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_8H_{12}O_2SnNa$ 282.9751; Found 282.9756.



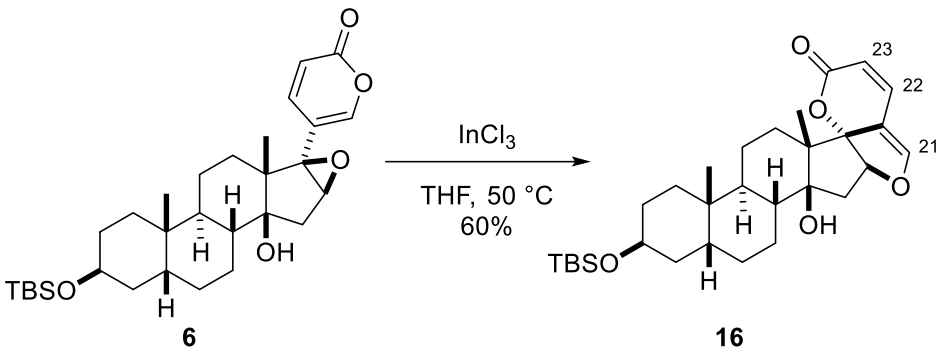
8 **15**

Pentacycle 15. $Pd(PPh_3)_4$ (3.24 g, 2.80 mmol), CuCl (2.75 g, 27.8 mmol), and LiCl (1.41 g, 33.3 mmol) were added to a mixture of vinyl iodide **8** (2.95 g, 5.56 mmol) and 2-pyrone **7** (4.80 g, 18.5 mmol). DMSO (49 mL) and THF (6.2 mL) were added to the mixture, and then the mixture was degassed by the freeze-thaw procedure (x3). After being stirred at 60 °C for 13 h, the reaction mixture was filtered through a pad of silica gel (200 g, hexane/EtOAc = 1/1) and concentrated. Then pH 7 phosphate buffer (200 mL) was added to the residue. The resultant mixture was extracted with Et_2O (200 mL x5). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography (a column consecutively packed with silica gel 150 g and 10 wt% KF contained silica gel 50 g, hexane/EtOAc = 1/0 to 10/1 to 2/1 to 1/1) to afford pentacycle **15** (1.87 g, 3.75 mmol) in 67% yield: white solid. m.p. 260 °C. $[\alpha]_D^{27}$ -58 (*c* 0.60, $CHCl_3$). IR (film) 3454, 2935, 2880, 2856, 1727, 1694, 1528, 1444, 1374, 1252, 1070, 833 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.01 (6H, s, C*H*$_3$ of TBS), 0.87 (9H, s, *t*-Bu of TBS), 0.94 (3H, s, H19), 1.03-1.09 (1H, m), 1.12 (3H, s, H18), 1.17-1.28 (4H, m), 1.35-1.49 (4H, m), 1.50-1.58 (2H, m),

1.62-1.73 (1H, m), 1.77-1.83 (3H, m), 1.86-1.93 (2H, m), 2.24 (1H, dd, *J* = 17.4, 3.2 Hz, H15a), 2.68 (1H, dd, *J* = 17.4, 1.8 Hz, H15b), 4.04 (1H, s, H3), 5.77 (1H, dd, *J* = 3.2, 1.8 Hz, H16), 6.31 (1H, dd, *J* = 9.8, 1.2 Hz, H23), 7.38 (1H, dd, *J* = 9.8, 2.8 Hz, H22), 7.438-7.444 (1H, m, H21). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$) δ -4.92, -4.88, 16.2, 18.0, 19.8, 21.2, 24.0, 25.8, 26.5, 28.7, 29.7, 34.2, 35.1, 35.9, 36.1, 38.6, 39.8, 40.9, 52.3, 67.1, 85.8, 115.9, 116.3, 125.0, 144.3, 145.1, 147.5, 161.2. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{30}H_{46}O_4SiNa$ 521.3058; Found 521.3060.

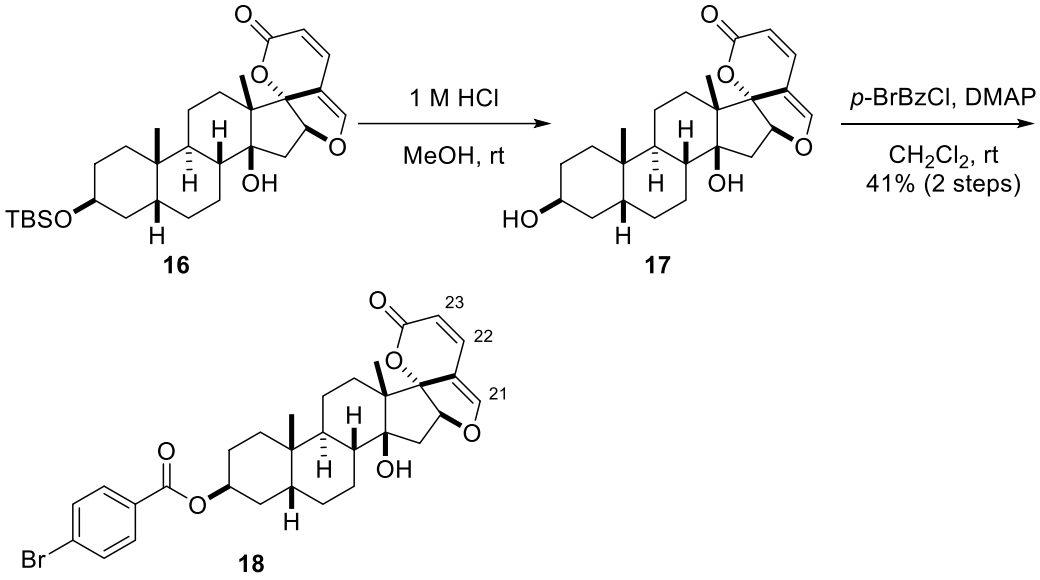


Epoxide 6. *m*-Chloroperoxybenzoic acid (*m*-CPBA, 77% purity, 965 mg, 4.31 mmol) was added to a solution of pentacycle **15** (1.65 g, 3.31 mmol) and Na_2CO_3 (465 mg, 4.39 mmol) in CH_2Cl_2 (83 mL) at -78 °C. After the reaction mixture was stirred at -20 °C for 5 h, saturated aqueous $Na_2S_2O_3$ (50 mL) was added to the mixture. The resultant mixture was extracted with EtOAc (50 mL x3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (20 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (100 g, hexane/EtOAc = 1/0 to 10/1 to 5/1 to 2/1) to afford epoxide **6** (1.32 g, 2.56 mmol) in 77% yield. The structure of **6** was determined by the X-ray crystallographic analysis (Page S39): colorless prism crystal (recrystallization from hexane/EtOAc by vapor diffusion method). m.p. 240-242 °C. $[\alpha]_D^{25}$ +10 (*c* 0.85, $CHCl_3$). IR (film) 3535, 3491, 3080, 2925, 2886, 2859, 1730, 1713, 1539, 1453, 1376, 1254, 1161, 1121 cm^{-1}. ^{1}H NMR (400 MHz, C_6D_6) δ 0.105 (3H, s, C*H*$_3$ of TBS), 0.112 (3H, s, C*H*$_3$ of TBS), 0.41-0.48 (1H, m), 0.82-0.94 (2H, m), 0.88 (3H, s, H19), 0.89 (3H, s, H18), 1.02 (9H, s, *t*-Bu of TBS), 1.05-1.13 (1H, m), 1.19-1.24 (3H, m), 1.29-1.33 (1H, m), 1.45-1.65 (5H, m), 1.69-1.86 (3H, m), 1.87-1.96 (2H, m), 2.10-2.14 (1H, m), 2.99 (1H, s, H16), 3.28 (1H, brs, O*H*), 4.11 (1H, brs, H3), 5.89 (1H, dd, *J* = 9.6, 1.2 Hz, H23), 6.61 (1H, dd, *J* = 9.6, 2.7 Hz, H22), 6.77 (1H, dd, *J* = 2.7, 1.2 Hz, H21). $^{13}C\{^{1}H\}$ NMR (100 MHz, C_6D_6) δ -4.7, -4.6, 13.9, 18.3, 20.0, 21.4, 24.2, 26.1, 26.9, 29.2, 30.1, 34.6, 34.8, 35.0, 35.4, 35.9, 36.4, 40.8, 46.4, 61.2, 67.7, 71.2, 80.6, 113.0, 115.9, 143.0, 151.1, 159.9. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{30}H_{46}O_5SiNa$ 537.3007; Found 537.3005.



6 **16**

Hexacycle 16. A solution of $InCl_3$ (40.1 mg, 181 μmol) in THF (0.60 mL) was added to a solution of epoxide **6** (24.6 mg, 47.8 μmol) in THF (1.0 mL) at room temperature. After the reaction mixture was stirred at 50 °C for 5 h, saturated aqueous $NaHCO_3$ (5 mL) was added to the mixture at room temperature. The resultant mixture was extracted with EtOAc (5 mL x3). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (5 g, hexane/EtOAc = 1/0 to 2/1 to 1/1) to afford alcohol **16** (14.9 mg, 28.9 μmol) in 60% yield: white solid. m.p. 235-237 °C. $[\alpha]_D^{23}$ +24 (*c* 0.37, $CHCl_3$). IR (film) 3398, 2932, 2858, 1685, 1626, 1452, 1253, 1128, 1066 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.01 (3H, s, C*H*$_3$ of TBS), 0.02 (3H, s, C*H*$_3$ of TBS), 0.88 (9H, s, *t*-Bu of TBS), 0.91 (3H, s, H19), 1.04 (3H, s, H18), 1.08-1.87 (17H, m), 2.08 (1H, d, *J* = 15.2 Hz, H15a), 2.50 (1H, dd, *J* = 15.2, 6.6 Hz, H15b), 4.05 (1H, brs, H3), 5.18 (1H, d, *J* = 6.6 Hz, H16), 5.72 (1H, d, *J* = 9.4 Hz, H23), 6.75 (1H, s, H21), 7.29 (1H, d, *J* = 9.4 Hz, H22). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ -4.9, 16.3, 18.1, 20.6, 20.8, 23.7, 25.8, 26.6, 28.6, 29.5, 33.2, 34.2, 35.2, 35.5, 35.9, 37.0, 42.0, 54.3, 67.1, 86.8, 93.3, 101.6, 111.8, 115.4, 136.7, 149.5, 163.6. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{30}H_{46}O_5SiNa$ 537.3007; Found 537.2983.

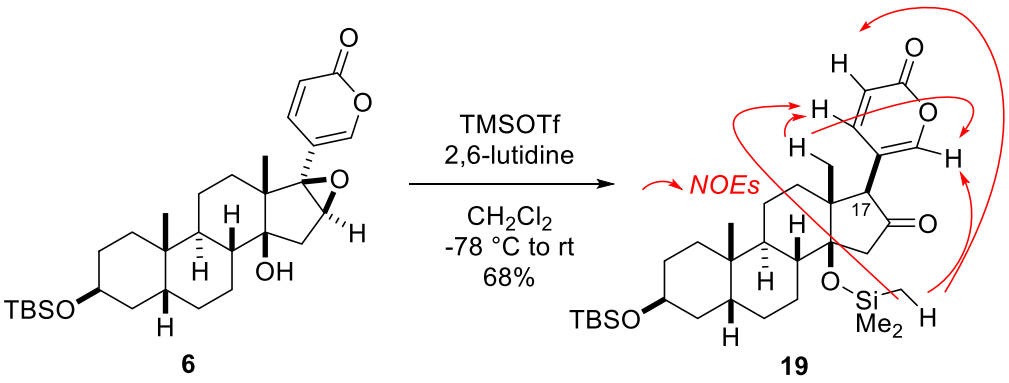


16 **17** **18**

***p*-Bromobenzoate 18.** 1 M aqueous HCl (270 μL, 270 μmol) was added to a solution of alcohol **16** (7.4 mg, 14 μmol) in MeOH (3.2 mL) at room temperature. After the reaction

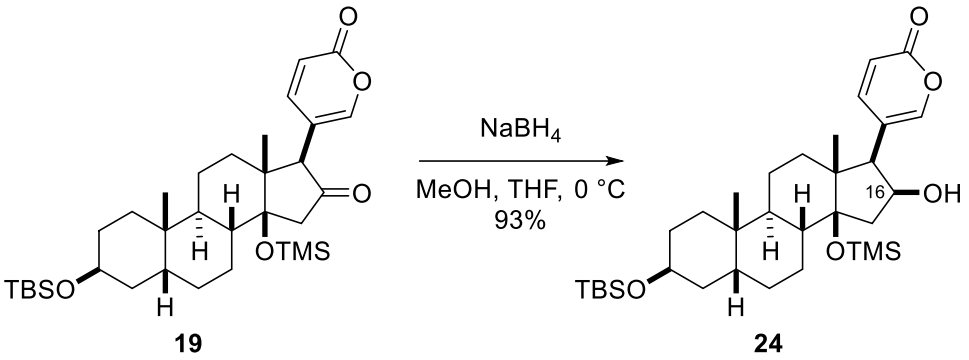
mixture was stirred at room temperature for 1.5 h, saturated aqueous $NaHCO_3$ (3 mL) was added to the mixture at 0 °C. The resultant mixture was extracted with EtOAc (3 mL x4). The combined organic layers were washed with brine (3 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (1 g, hexane/EtOAc = 1/0 to 1/1 to 1/2) to afford the crude diol **17** (4.8 mg), which was used in the next reaction without further purification: colorless amorphous. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{24}H_{32}O_5Na$ 423.2142; Found 423.2157.

p-BrBzCl (13.2 mg, 60.1 μmol) was added to a solution of the above crude diol **17** (4.8 mg) and *N*,*N*-dimethyl-4-aminopyridine (DMAP, 7.8 mg, 64 μmol) in CH_2Cl_2 (0.40 mL) at room temperature. After the reaction mixture was stirred at room temperature for 14 h, DMAP (7.5 mg, 61 μmol) and *p*-BrBzCl (12.5 mg, 57.0 μmol) were successively added to the mixture. After the reaction mixture was stirred at room temperature for 3 h, saturated aqueous $NaHCO_3$ (3 mL) was added to the mixture at 0 °C. The resultant mixture was extracted with EtOAc (3 mL x4). The combined organic layers were washed with brine (3 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (1 g, hexane/EtOAc = 1/0 to 2/1 to 1/2) to afford *p*-bromobenzoate **18** (3.3 mg, 5.7 μmol) in 41% yield over 2 steps. The structure of **18** was determined by the X-ray crystallographic analysis (Page S40): colorless plate crystal (recrystallization from hexane/EtOAc by vapor diffusion method). m.p. 201-203 °C. $[\alpha]_D^{24}$ +6.4 (*c* 0.17, $CHCl_3$). IR (film) 3496, 2938, 2872, 1711, 1633, 1453, 1389, 1277, 1115, 1012 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 0.86-2.09 (18H, m), 0.917 (3H, s, H18), 0.924 (3H, s, H19), 2.58 (1H, dd, *J* = 15.5, 6.3 Hz, H15a), 3.98 (1H, s, O*H*), 5.07 (1H, d, *J* = 6.3 Hz, H16), 5.23 (1H, brs, H3), 5.57 (1H, d, *J* = 9.2 Hz, H23), 7.07 (1H, s, H21), 7.50 (1H, d, *J* = 9.2 Hz, H22), 7.75 (2H, d, *J* = 7.8 Hz, aromatic), 7.89 (2H, d, *J* = 7.8 Hz, aromatic). $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d_6) δ 16.8, 20.1, 20.4, 23.6, 24.5, 26.1, 30.0, 30.5, 32.6, 34.4, 34.9, 36.4, 36.9, 41.3, 53.8, 71.5, 84.7, 92.6, 101.9, 111.2, 113.5, 127.2, 129.6, 131.1, 132.0, 138.4, 151.2, 163.2, 164.4. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{31}H_{35}BrO_6Na$ 605.1509 and 607.1489; Found 605.1493 and 607.1489.

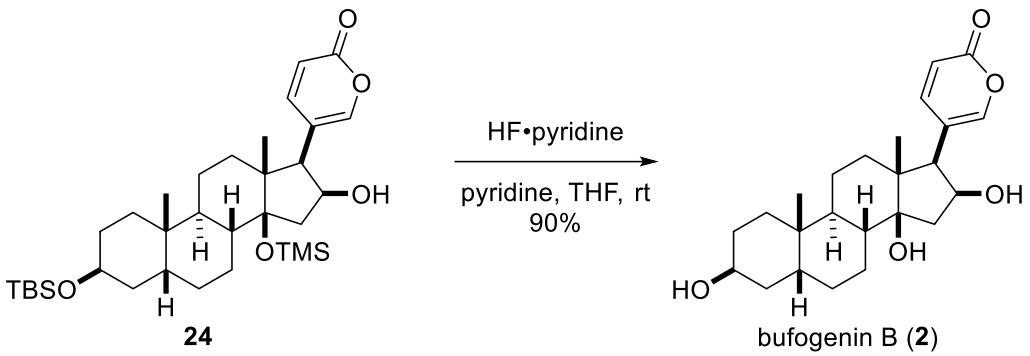


Ketone 19. TMSOTf (1.1 mL, 6.1 mmol) was added to a solution of epoxide **6** (1.32 g, 2.56 mmol) and 2,6-lutidine (1.5 mL, 13 mmol) in CH_2Cl_2 (26 mL) at -78 °C. After the reaction mixture was stirred at room temperature for 1 h, saturated aqueous $NaHCO_3$ (50 mL) was added to the mixture at -78 °C. The resultant mixture was extracted with EtOAc (50 mL x3). The

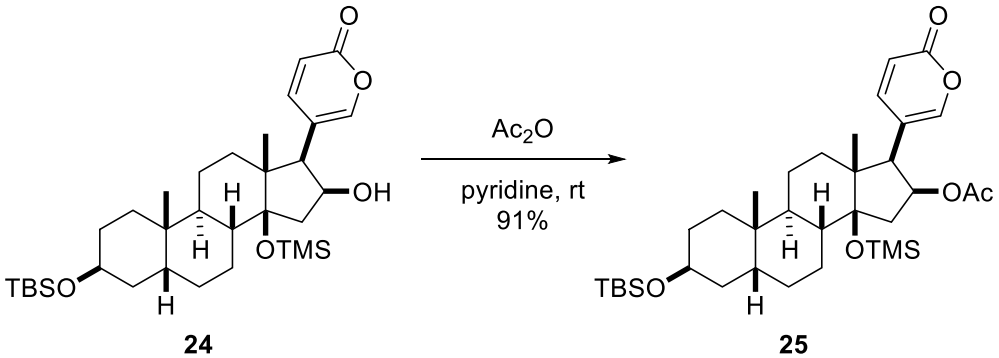
combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (50 g, hexane/EtOAc = 1/0 to 10/1 to 5/1) to afford ketone **19** (1.03 g, 1.75 mmol) in 68% yield. The C17-stereochemistry of **19** was determined by the NOE experiment: white solid. m.p. 138-140 °C. $[\alpha]_D^{25}$ +44 (*c* 0.54, $CHCl_3$). IR (film) 2930, 2858, 1747, 1540, 1448, 1380, 1253, 1062, 984, 838 cm^{-1}. ^1H NMR (400 MHz, C_6D_6) δ -0.01 (9H, s, C*H*$_3$ of TMS), 0.13 (3H, s, C*H*$_3$ of TBS), 0.14 (3H, s, C*H*$_3$ of TBS), 0.62 (3H, s, H18), 0.85-1.12 (4H, m), 0.87 (3H, s, H19), 1.04 (9H, s, *t*-Bu of TBS), 1.16-1.51 (7H, m), 1.55-1.82 (5H, m), 1.91-1.94 (1H, m), 2.14 (1H, d, *J* = 17.4 Hz, H15a), 2.36 (1H, s, H17), 2.42 (1H, d, *J* = 17.4 Hz, H15b), 4.08 (1H, brs, H3), 6.00 (1H, dd, *J* = 9.6, 1.4 Hz, H23), 6.72 (1H, brs, H21), 6.74 (1H, dd, *J* = 9.6, 2.8 Hz, H22). $^{13}C\{^1H\}$ NMR (100 MHz, C_6D_6) δ -4.7, -4.6, 2.8, 18.3, 18.4, 20.6, 23.6, 24.1, 26.1, 26.5, 28.8, 30.1, 34.6, 35.9, 36.2, 37.2, 39.9, 41.5, 47.97, 48.04, 61.4, 67.6, 88.3, 115.3, 116.1, 146.1, 151.8, 160.4, 214.1. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{33}H_{54}O_5Si_2Na$ 609.3402; Found 609.3374.



Alcohol 24. $NaBH_4$ (80.6 mg, 2.13 mmol) was added to a solution of ketone **19** (1.03 g, 1.75 mmol) in MeOH (17 mL) and THF (17 mL) at 0 °C. After the reaction mixture was stirred at 0 °C for 1.5 h, saturated aqueous NH_4Cl (50 mL) was added to the mixture. The resultant mixture was extracted with EtOAc (50 mL x3). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (50 g, hexane/EtOAc = 1/0 to 5/1 to 3/1) to afford alcohol **24** (954 mg, 1.62 mmol) in 93% yield. The C16-stereochemistry of **24** was determined by converting **24** to bufogenin B (**2**): colorless oil. $[\alpha]_D^{24}$ +13 (*c* 0.70, $CHCl_3$). IR (film) 3460, 2931, 2859, 1714, 1634, 1538, 1447, 1379, 1251, 1116, 1061, 994 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.02 (6H, s, C*H*$_3$ of TBS), 0.26 (9H, s, C*H*$_3$ of TMS), 0.81 (3H, s), 0.88 (9H, s, *t*-Bu of TBS), 0.91 (3H, s), 1.03-1.87 (17H, m), 1.93 (1H, d, *J* = 14.6 Hz, H15a), 2.24 (1H, dd, *J* = 14.6, 6.0 Hz, H15b), 2.54 (1H, d, *J* = 6.4 Hz, H17), 4.05 (1H, brs, H3), 4.29 (1H, dd, *J* = 6.4, 6.0 Hz, H16), 6.21 (1H, d, *J* = 9.8 Hz, H23), 7.30 (1H, brs, H21), 7.56 (1H, dd, *J* = 9.8, 1.7 Hz, H22). $^{13}C\{^1H\}$ NMR (100 MHz, C_6D_6) δ -4.7, -4.6, 3.1, 18.4, 18.7, 20.9, 23.9, 24.1, 26.1, 26.8, 28.9, 30.2, 34.7, 36.0, 36.3, 37.3, 41.4, 42.8, 43.1, 49.7, 59.1, 67.7, 73.7, 92.7, 113.2, 117.4, 149.1, 150.4, 161.5. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{33}H_{56}O_5Si_2Na$ 611.3558; Found 611.3537.

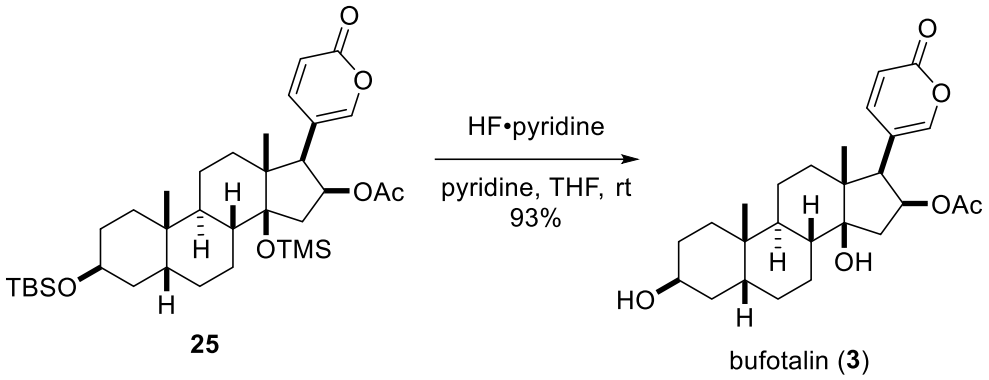


Bufogenin B (2) [CAS: 465-19-0]. A mixture of HF (70 wt% pyridine solution, 1.6 mL, 61 mmol) and pyridine (1.6 mL) was added to a solution of alcohol **24** (183 mg, 311 μmol) in THF (3.1 mL) at room temperature. After the reaction mixture was stirred at room temperature for 77 h, saturated aqueous $NaHCO_3$ (30 mL) was added to the mixture. The resultant mixture was extracted with EtOAc (30 mL x4). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (5 g, hexane/EtOAc = 1/1 to 1/5 to 1/2 then $CHCl_3$/MeOH = 5/1) to afford bufogenin B (**2**) (113 mg, 281 μmol) in 90% yield: colorless oil. $[\alpha]_D^{17}$ +44 (*c* 0.54, DMSO). IR (film) 3397, 2930, 2861, 1712, 1631, 1537, 1448, 1379, 1258, 1029 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 0.64 (3H, s, H18), 0.85 (3H, s, H19), 1.02-1.84 (18H, m), 2.44 (1H, dd, *J* = 14.4, 8.3 Hz, H15a), 2.63 (1H, d, *J* = 8.6 Hz, H17), 3.89 (1H, brs, H3), 4.16 (1H, s, O*H*), 4.19 (1H, brs, O*H*), 4.41-4.44 (1H, m, H16), 4.52 (1H, d, *J* = 4.1 Hz, O*H*), 6.12 (1H, d, *J* = 9.8 Hz, H23), 7.47 (1H, d, *J* = 2.3 Hz, H21), 8.08 (1H, dd, *J* = 9.8, 2.3 Hz, H22). $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d_6) δ 16.9, 20.9, 21.0, 23.9, 26.5, 27.6, 29.6, 33.2, 34.8, 35.0, 35.7, 39.6 (deduced from HMQC), 41.5, 42.8, 48.8, 57.6, 64.7, 70.6, 83.3, 111.0, 118.8, 150.3, 151.5, 161.7. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{24}H_{34}O_5Na$ 425.2298; Found 425.2293.



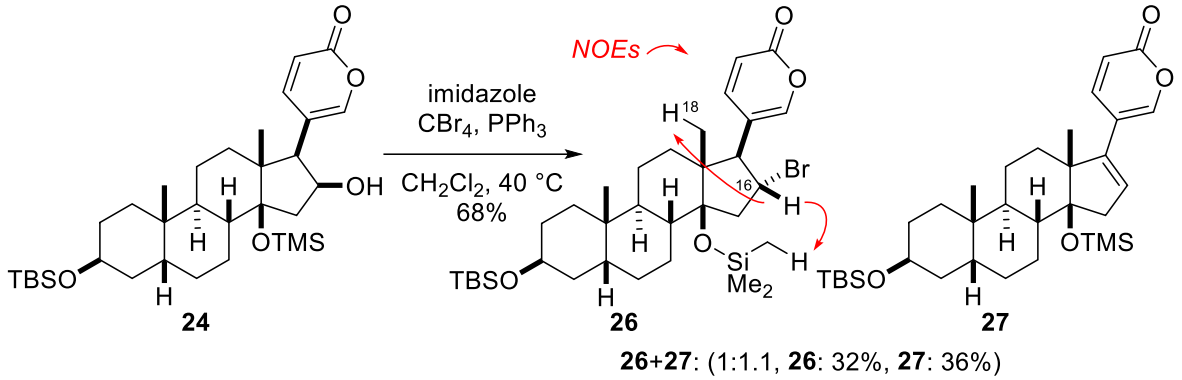
Acetate 25. Ac_2O (0.20 mL, 2.1 mmol) was added to a solution of alcohol **24** (157 mg, 267 μmol) in pyridine (2.7 mL) at room temperature. After the reaction mixture was stirred at room temperature for 18 h, saturated aqueous NH_4Cl (30 mL) was added to the mixture. The resultant mixture was extracted with EtOAc (30 mL x3). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (10 g, hexane/EtOAc = 1/0 to 10/1) to afford acetate **25** (153 mg, 242 μmol) in 91% yield: white solid. m.p. 160-163 °C. $[\alpha]_D^{24}$ +9.5

(*c* 0.76, $CHCl_3$). IR (film) 3523, 3439, 2926, 2858, 1746, 1730, 1714, 1638, 1539, 1448, 1378, 1361, 1249, 1059 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.01 (6H, s, C*H*$_3$ of TBS), 0.18 (9H, s, C*H*$_3$ of TMS), 0.76 (3H, s, H18), 0.87 (9H, s, *t*-Bu of TBS), 0.90 (3H, s, H19), 1.07-1.87 (18H, m), 1.89 (3H, s, C*H*$_3$CO), 2.45 (1H, dd, *J* = 15.6, 8.7 Hz, H15a), 2.79 (1H, d, *J* = 8.7 Hz, H17), 4.04 (1H, brs, H3), 5.46 (1H, dd, *J* = 8.7, 8.7 Hz, H16), 6.16 (1H, d, *J* = 9.8 Hz, H23), 7.22 (1H, d, *J* = 2.5 Hz, H21), 7.75 (1H, dd, *J* = 9.8, 2.5 Hz, H22). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ -4.89, -4.86, 3.1, 18.06, 18.12, 20.5, 21.0, 23.4, 23.9, 25.8, 26.6, 28.5, 29.7, 34.4, 35.7, 35.8, 37.0, 41.0, 41.56, 41.65, 50.5, 57.2, 67.1, 74.0, 90.3, 112.7, 116.8, 149.5, 150.7, 162.0, 170.0. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{35}H_{58}O_6Si_2Na$ 653.3670; Found 653.3643.



25 bufotalin (**3**)

Bufotalin (3) [CAS: 471-95-4]. A mixture of HF (70 wt% pyridine solution, 3.1 mL, 0.12 mol) and pyridine (3.1 mL) was added to a solution of acetate **25** (153 mg, 242 μmol) in THF (2.4 mL) at room temperature. After the reaction mixture was stirred at room temperature for 124 h, saturated aqueous $NaHCO_3$ (50 mL) was added to the mixture. The resultant mixture was extracted with EtOAc (50 mL x4). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (10 g, hexane/EtOAc = 1/1 to 1/3) to afford bufotalin (**3**) (100 mg, 225 μmol) in 93% yield: colorless oil. $[\alpha]_D^{24}$ +5.3 (*c* 1.28, $CHCl_3$). IR (film) 3468, 3057, 2935, 1713, 1633, 1537, 1448, 1378, 1250, 1136, 1092, 1037, 736 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.78 (3H, s, H18), 0.94 (3H, s, H19), 1.15-1.32 (5H, m, H1a, H6a, H7a, H11a, H12a), 1.37 (1H, d, *J* = 13.5 Hz, H4a), 1.42-1.61 (7H, m, H1b, H2, H8, H9, H11b, H12b), 1.75-1.78 (2H, m, H5, H7b), 1.81-1.92 (2H, m, H4b, H6b), 1.83 (1H, d, *J* = 15.8 Hz, H15a), 1.86 (3H, s, C*H*$_3$CO), 2.64 (1H, dd, *J* = 15.8, 8.9 Hz, H15b), 2.86 (1H, d, *J* = 8.9 Hz, H17), 4.14 (1H, brs, H3), 5.53 (1H, dd, *J* = 8.9, 8.9 Hz, H16), 6.19 (1H, d, *J* = 9.8 Hz, H23), 7.24 (1H, s, H21), 8.03 (1H, d, *J* = 9.8 Hz, H22). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 16.4, 20.9, 21.0, 21.1, 23.7, 26.3, 27.9, 29.5, 33.2, 35.3, 35.5, 35.9, 40.4, 40.8, 42.3, 49.4, 57.1, 66.7, 73.5, 84.4, 113.1, 116.8, 149.1, 150.9, 162.0, 170.0. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{26}H_{36}O_6Na$ 467.2404; Found 467.2396.

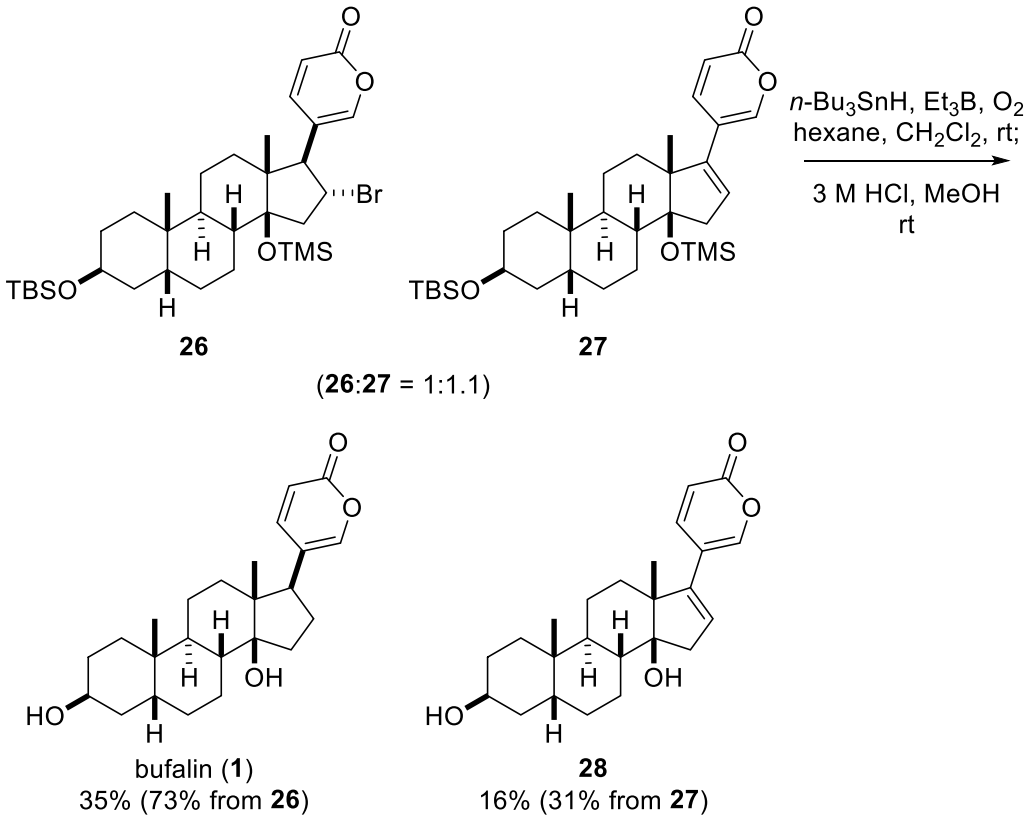


Bromide 26 and olefin 27. Imidazole (548 mg, 8.05 mmol), CBr_4 (1.31 g, 3.95 mmol), and PPh_3 (1.05 g, 4.00 mmol) were successively added to a solution of alcohol **24** (584 mg, 992 μmol) in CH_2Cl_2 (5.0 mL) at room temperature. After the reaction mixture was stirred at 40 °C for 21 h, imidazole (533 mg, 7.83 mmol), CBr_4 (1.35 g, 4.07 mmol), and PPh_3 (1.06 g, 4.04 mmol) were successively added to the mixture at room temperature. After the reaction mixture was stirred at 40 °C for 20 h, imidazole (542 mg, 7.96 mmol), CBr_4 (1.31 g, 3.95 mmol), and PPh_3 (1.04 g, 3.97 mmol) were successively added to the mixture at room temperature. After the reaction mixture was stirred at 40 °C for 9 h, saturated aqueous $NaHCO_3$ (50 mL) was added to the mixture at room temperature. The resultant mixture was extracted with EtOAc (50 mL x3). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (50 g, hexane/EtOAc = 1/0 to 50/1 to 10/1) to afford a 1:1.1 mixture of bromide **26** and olefin **27** (411 mg, 317 μmol for **26**, 357 μmol for **27**) in 32% and 36% yields, respectively. For characterizations of **26** and **27**, a small amount of the mixture was purified by GPC ($CHCl_3$). The C16-stereochemistry of **26** was determined by the NOE experiment.

26: colorless oil. $[\alpha]_D^{32}$ -31 (*c* 0.18, $CHCl_3$). IR (film) 2931, 2858, 1747, 1641, 1543, 1466, 1380, 1253, 1065, 990 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.02 (6H, s, C*H*$_3$ of TBS), 0.20 (9H, s, C*H*$_3$ of TMS), 0.70 (3H, s, H18), 0.88 (9H, s, *t*-Bu of TBS), 0.90 (3H, s, H19), 1.04-1.16 (1H, m), 1.23-1.34 (3H, m), 1.39-1.42 (3H, m), 1.46-1.58 (4H, m), 1.67-1.73 (3H, m), 1.81-1.89 (3H, m), 2.27-2.41 (2H, m, H15), 2.73 (1H, d, *J* = 8.0 Hz, H17), 4.07 (1H, brs, H3), 4.24 (1H, ddd, *J* = 11.2, 8.0, 8.0 Hz, H16), 6.29 (1H, d, *J* = 9.6 Hz, H23), 7.27-7.28 (1H, m, H21), 7.41 (1H, dd, *J* = 9.6, 2.5 Hz, H22). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ -4.8, 3.1, 18.1, 18.6, 20.9, 23.6, 23.8, 25.8, 26.6, 28.5, 29.7, 34.3, 35.7, 35.8, 37.1, 40.6, 43.4, 45.1, 50.6, 52.1, 63.2, 67.1, 90.8, 115.6, 118.1, 145.0, 149.4, 161.7. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{33}H_{55}BrO_4Si_2Na$ 673.2714 and 675.2694; Found 673.2744 and 675.2686.

27: colorless oil. $[\alpha]_D^{23}$ +34 (*c* 0.39, $CHCl_3$). IR (film) 2933, 2887, 2858, 1747, 1447, 1249, 1225, 1153, 1088, 1062 cm^{-1}. ^1H NMR (400 MHz, C_6D_6) δ 0.08 (9H, s, C*H*$_3$ of TMS), 0.12 (6H, s, C*H*$_3$ of TBS), 0.82-0.99 (2H, m), 0.90 (3H, s, H19), 0.92 (3H, s, H18), 1.03 (9H, s, *t*-Bu of TBS), 1.14-1.52 (9H, m), 1.65-2.00 (6H, m), 2.18 (1H, dd, *J* = 17.4, 3.2 Hz, H15a), 2.35 (1H, dd, *J* = 17.4, 1.4 Hz, H15b), 4.04 (1H, brs, H3), 5.21 (1H, brs, H16), 5.97 (1H, dd, *J* = 9.8, 0.9 Hz, H23), 6.75 (1H, dd, *J* = 9.8, 2.5 Hz, H22), 7.07 (1H, d, *J* = 2.5 Hz, H21). $^{13}C\{^1H\}$ NMR

(100 MHz, C_6D_6) δ -4.7, -4.6, 2.9, 16.7, 18.4, 20.1, 21.9, 24.2, 26.1, 27.0, 29.0, 30.2, 34.6, 35.3, 36.5, 36.6, 38.6, 39.0, 42.7, 53.0, 67.8, 90.7, 115.6, 116.4, 123.9, 143.1, 146.0, 147.9, 160.0. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{33}H_{54}O_4Si_2Na$ 593.3453; Found 593.3429.

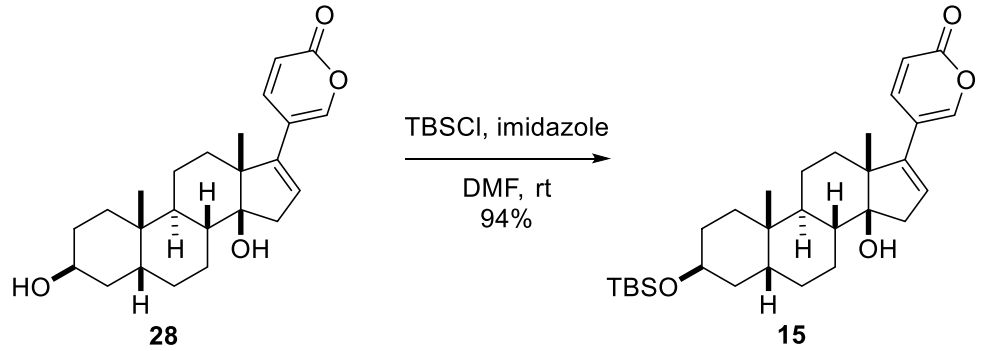


Bufalin (1) [CAS: 465-21-4] and diol 28. Et_3B (1.00 M hexane solution, 1.15 mL, 1.15 mmol) was added to a solution of a 1:1.1 mixture of bromide **26** and olefin **27** (297 mg, 230 μmol for **26**, 258 μmol for **27**), and *n*-Bu_3SnH (185 μL, 0.687 mmol) in hexane (8.1 mL) and CH_2Cl_2 (8.1 mL) at room temperature. The mixture was bubbled with O_2 for 3 min at room temperature. After the reaction mixture was stirred under O_2 atmosphere (1 atm) at room temperature for 2 h, Et_3B (1.00 M hexane solution, 1.15 mL, 1.15 mmol) and *n*-Bu_3SnH (185 μmol, 0.687 mmol) were successively added to the mixture. After the reaction mixture was stirred under O_2 atmosphere (1 atm) at room temperature for 2 h, a mixture of 3 M aqueous HCl (2.5 mL, 7.5 mmol) and MeOH (30 mL) was added to the mixture at room temperature. After the reaction mixture was stirred at room temperature for 20 h, saturated aqueous $NaHCO_3$ (50 mL) was added to the mixture. The resultant mixture was extracted with EtOAc (50 mL x4). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography (a column consecutively packed with silica gel 50 g and 10 wt% KF contained silica gel 25 g, hexane/EtOAc = 1/0 to 3/2 to 1/1 to 1/3) to afford bufalin (**1**) (65.2 mg, 169 μmol) and diol **28** (30.6 mg, 79.6 μmol) in 35% and 16% yields, respectively.

1: colorless oil. $[\alpha]_D^{20}$ +6.3 (*c* 0.97, $CHCl_3$). IR (film) 3456, 3059, 2933, 1711, 1538, 1450, 1255, 1134 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.70 (3H, s, H18), 0.95 (3H, s, H19), 1.10-1.65

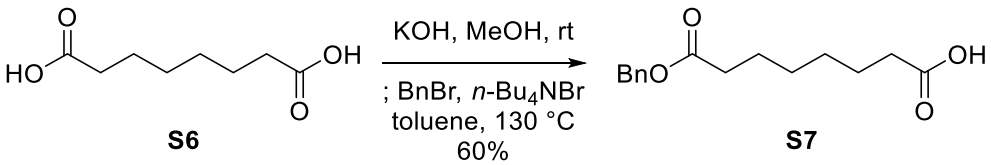
(13H, m, H1, H2, H4a, H6a, H7a, H8, H9, H11, H12), 1.66-1.80 (4H, m, H5, H7b, H15a, H16a), 1.86-1.96 (2H, m, H4b, H6b), 2.02-2.11 (1H, m, H15b), 2.15-2.23 (1H, m, H16b), 2.46 (1H, dd, *J* = 9.6, 6.4 Hz, H17), 4.14 (1H, brs, H3), 6.26 (1H, d, *J* = 9.8 Hz, H23), 7.23 (1H, d, *J* = 2.8 Hz, H21), 7.84 (1H, dd, *J* = 9.8, 2.8 Hz, H22). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$) δ 16.5, 21.4 (2C), 23.7, 26.5, 27.9, 28.7, 29.6, 32.7, 33.2, 35.3, 35.6, 35.9, 40.9, 42.3, 48.3, 51.2, 66.8, 85.4, 115.3, 122.7, 146.8, 148.5, 162.4. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{24}H_{34}O_4Na$ 409.2349; Found 409.2343.

28: white solid. m.p. 128-131 °C. $[\alpha]_D^{18}$ +73 (*c* 0.12, $CHCl_3$). IR (film) 3449, 3052, 2934, 2879, 1719, 1638, 1532, 1449, 1379, 1265, 1226, 1153, 1125, 1038 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (3H, s, H18), 1.06-1.99 (16H, m), 1.14 (3H, s, H19), 2.26 (1H, dd, *J* = 17.6, 3.2 Hz, H15a), 2.70 (1H, d, *J* = 17.6 Hz, H15b), 4.14 (1H, brs, H3), 5.77-5.79 (1H, m, H16), 6.33 (1H, dd, *J* = 9.6, 1.2 Hz, H23), 7.37-7.40 (1H, m, H22), 7.447-7.452 (1H, m, H21). $^{13}C\{^{1}H\}$ NMR (125 MHz, $CDCl_3$) δ 16.2, 19.8, 21.1, 23.9, 26.2, 27.9, 29.6, 33.3, 35.2, 35.93, 35.95, 38.6, 39.8, 40.7, 52.3, 66.8, 85.7, 116.0, 116.2, 125.0, 144.3, 145.1, 147.5, 161.2. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{24}H_{32}O_4Na$ 407.2193; Found 407.2197.



28 **15**

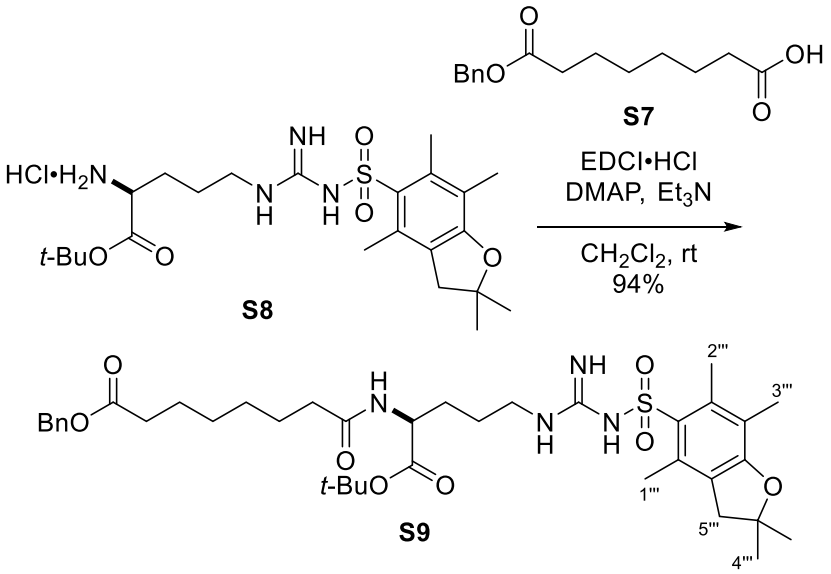
Alcohol 15 from diol 28. TBSCl (123 mg, 816 μmol) was added to a solution of diol **28** (15.1 mg, 39.3 μmol) and imidazole (104 mg, 1.53 mmol) in DMF (0.78 mL) at room temperature. After the reaction mixture was stirred at room temperature for 16 h, saturated aqueous $NaHCO_3$ (3 mL) was added to the mixture. The resultant mixture was extracted with Et_2O (3 mL x3). The combined organic layers were washed with brine (3 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (5 g, hexane/EtOAc = 1/0 to 1/1) to afford alcohol **15** (18.4 mg, 36.9 μmol) in 94% yield.



S6 **S7**

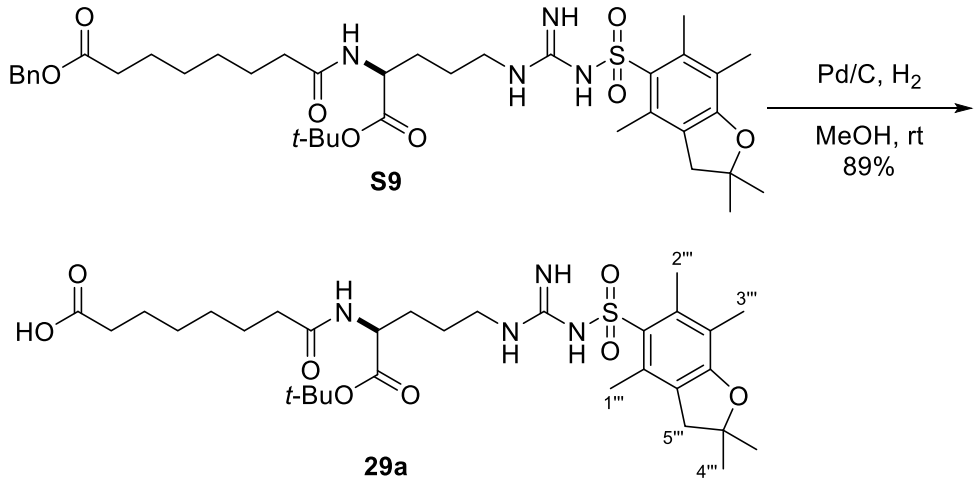
Carboxylic acid S7 [CAS: 15570-39-5].[S7] KOH (79.2 mg, 1.41 mmol) was added to a solution of suberic acid (**S6**, 258 mg, 1.48 mmol) in MeOH (11.4 mL) at room temperature. After being stirred at room temperature for 20 min, the reaction mixture was concentrated and dissolved in toluene (2.5 mL). *n*-Bu_4NBr (48.1 mg, 0.149 mmol) and benzyl bromide (BnBr,

0.20 mL, 1.7 mmol) were successively added to the solution at room temperature. After the reaction mixture was stirred at 130 °C for 13 h, 1 M aqueous HCl (1.7 mL, 1.7 mmol) was added to the mixture at room temperature. The resultant mixture was extracted with Et_2O (10 mL x3). The combined organic layers were washed with H_2O (10 mL) and brine (10 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (10 g, hexane/EtOAc = 1/0 to 3/1) to afford carboxylic acid **S7** (233 mg, 882 μmol) in 60% yield: colorless oil. IR (film) 3033, 2937, 2862, 1735, 1706, 1458, 1414, 1240, 1171 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 1.33-1.36 (4H, m, H4', H5'), 1.61-1.68 (4H, m, H3', H6'), 2.33 (2H, t, *J* = 7.5 Hz, H2'), 2.36 (2H, t, *J* = 7.5 Hz, H7'), 5.11 (2H, s, PhC*H*$_2$), 7.30-7.38 (5H, m, aromatic). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 24.4, 24.6, 28.56, 28.61, 33.9, 34.1, 66.1, 128.1, 128.5, 136.0, 173.6, 179.9. HRMS (ESI-TOF) $[M-H+2Na]^+$ m/z: Calcd for $C_{15}H_{19}O_4Na_2$ 309.1073; Found 309.1067.

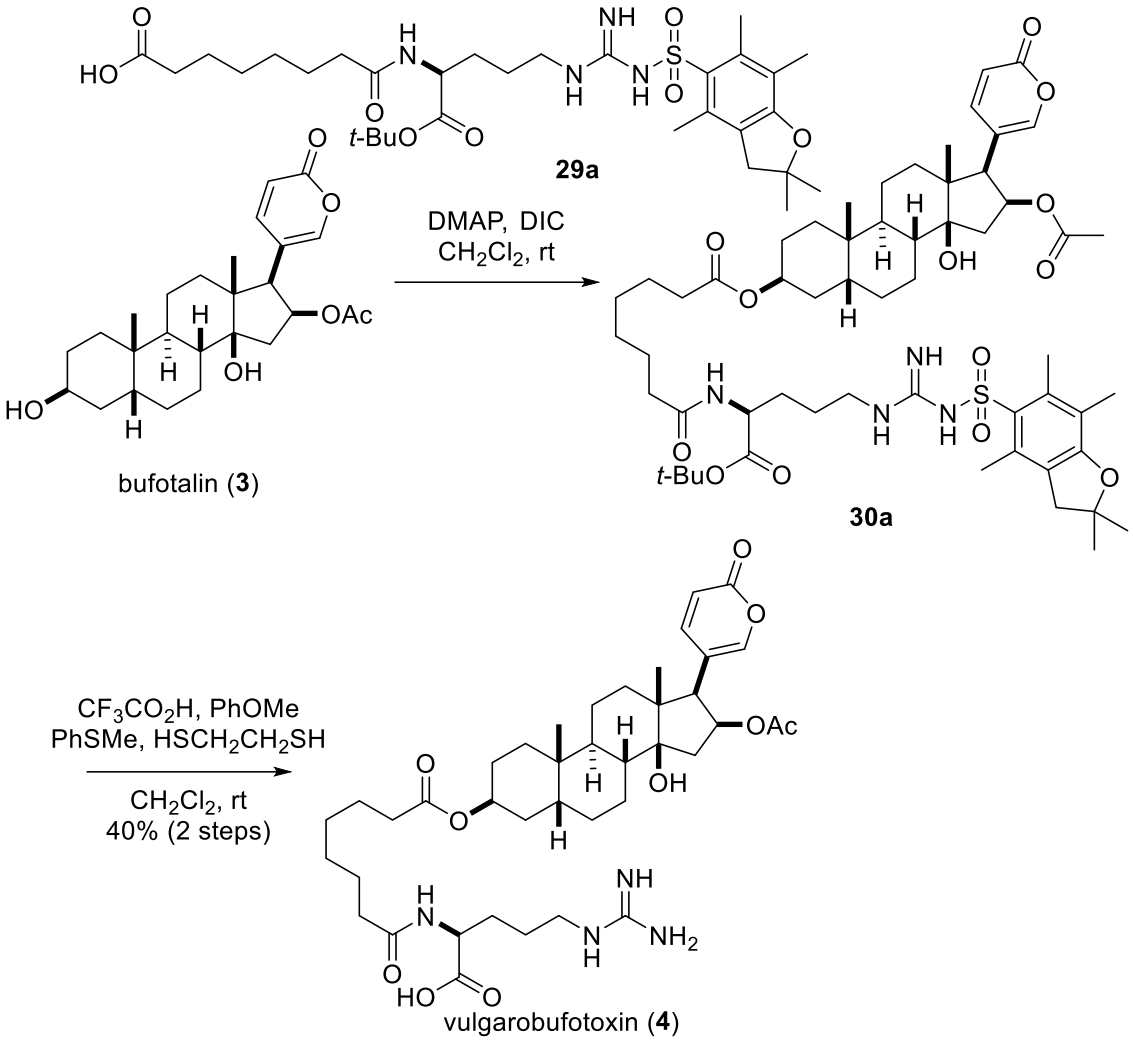


Benzyl ester S9. DMAP (14.9 mg, 122 μmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI•HCl, 87.6 mg, 457 μmol), and Et_3N (0.10 mL, 0.72 mmol) were successively added to a solution of amine hydrochloride **S8** (159 mg, 306 μmol) and carboxylic acid **S7** (121 mg, 458 μmol) in CH_2Cl_2 (1.5 mL) at room temperature. After the reaction mixture was stirred at room temperature for 19 h, saturated aqueous $NaHCO_3$ (5 mL) was added to the mixture. The resultant mixture was extracted with EtOAc (5 mL x4). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (10 g, hexane/EtOAc = 1/0 to 2/1 then $CHCl_3$/MeOH = 20/1) to afford benzyl ester **S9** (211 mg, 289 μmol) in 94% yield: colorless oil. $[\alpha]_D^{30}$ +5.4 (*c* 0.31, $CHCl_3$). IR (film) 3441, 3337, 2933, 2863, 1734, 1622, 1551, 1455, 1370, 1253, 1157, 1108 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 1.25-1.31 (4H, m), 1.44 (9H, s, *t*-Bu), 1.45 (6H, s, H4'''), 1.57-1.65 (7H, m), 1.78-1.84 (1H, m), 2.08 (3H, s, H3'''), 2.17-2.20 (2H, m), 2.34 (2H, t, *J* = 7.5 Hz), 2.51 (3H, s, H1'''), 2.57 (3H, s, H2'''), 2.94 (2H, s, H5'''), 3.21 (1H, brs, H5''a), 3.30 (1H, brs, H5''b), 4.37-4.41 (1H, m, H2''), 5.10 (2H, s, PhC*H*$_2$), 6.29 (1H, brs, N*H*), 6.33 (1H,

brs, N*H*), 6.39 (1H, d, *J* = 7.8 Hz, N*H*), 7.31-7.36 (5H, m, aromatic). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$) δ 12.4, 17.9, 19.2, 24.7, 25.1, 25.3, 27.9, 28.55, 28.65, 28.7, 34.1, 36.2, 40.6, 43.2, 66.1, 82.4, 86.3, 117.4, 124.5, 128.11, 128.15, 128.5, 132.2, 132.9, 135.9, 138.3, 156.1, 158.6, 171.4, 173.6 (2C), two $^{13}C\{^{1}H\}$ peaks were not observed. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{38}H_{56}N_4O_8SNa$ 751.3711; Found 751.3694.



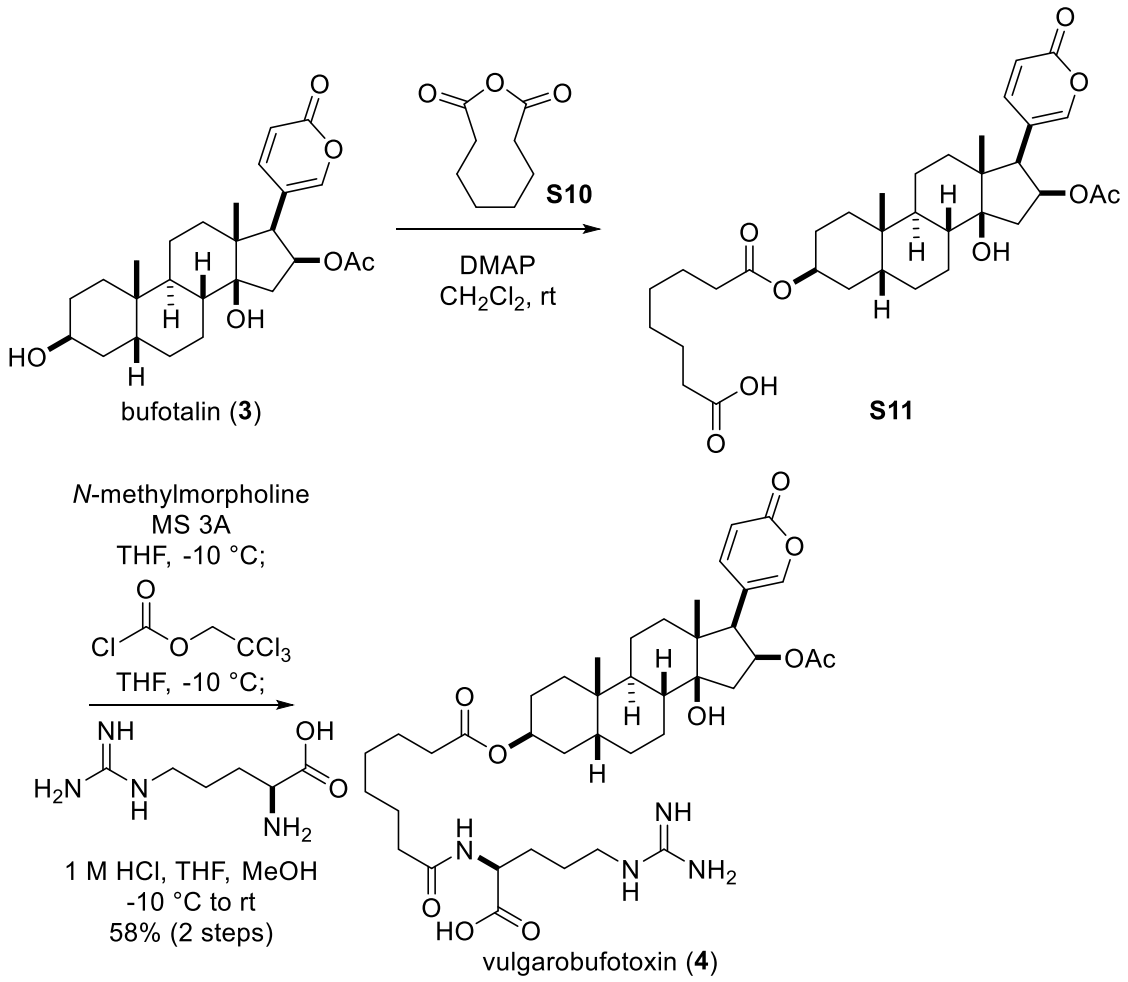
Carboxylic acid 29a. A suspension of benzyl ester **S9** (53.4 mg, 73.3 μmol) and Pd/C (5 wt% Pd on carbon, 10.7 mg) in MeOH (1.5 mL) was stirred under H_2 atmosphere (1 atm) at room temperature for 1 h. The reaction mixture was then filtered through a pad of Celite with EtOAc (10 mL) and concentrated. The residue was purified by flash column chromatography on silica gel (5 g, $CHCl_3$/MeOH = 1/0 to 30/1 to 10/1) to afford carboxylic acid **29a** (41.5 mg, 65.0 μmol) in 89% yield: colorless oil. $[\alpha]_D^{28}$ +7.6 (*c* 0.91, $CHCl_3$). IR (film) 3442, 3337, 2932, 2863, 1728, 1629, 1552, 1456, 1370, 1252, 1155, 1105 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 1.34-1.37 (4H, m), 1.44 (9H, s, *t*-Bu), 1.46 (6H, s, H4’’’), 1.53-1.67 (7H, m), 1.74-1.83 (1H, m, H3’’a), 2.08 (3H, s, H3’’’), 2.23 (2H, t, *J* = 7.3 Hz), 2.26-2.30 (2H, m), 2.51 (3H, s, H1’’’), 2.58 (3H, s, H2’’’), 3.00 (2H, s, H5’’’), 3.12-3.20 (2H, m, H5’’), 4.21 (1H, brs, H2’’), 8.16 (1H, d, *J* = 7.8 Hz, N*H*). $^{13}C\{^{1}H\}$ NMR (125 MHz, CD_3OD) δ 12.5, 18.4, 19.6, 25.91, 25.94, 26.8, 27.1, 28.3, 28.7, 29.8, 29.9, 34.9, 36.6, 41.5, 44.0, 54.2, 82.7, 87.7, 118.4, 126.0, 133.5, 134.4, 139.4, 158.1, 159.9, 172.8, 176.3, 177.6. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{31}H_{50}N_4O_8SNa$ 661.3242; Found 661.3260.



Vulgarobufotoxin (4) [CAS: 464-81-3]. Carboxylic acid **29a** (21.7 mg, 34.0 μmol), DMAP (1.0 mg, 8.2 μmol), and *N*,*N*'-diisopropylcarbodiimide (DIC, 5.0 μL, 32 μmol) were added to a solution of bufotalin (**3**) (12.6 mg, 28.3 μmol) in CH_2Cl_2 (0.28 mL) at room temperature. After the reaction mixture was stirred at room temperature for 15 h, saturated aqueous $NaHCO_3$ (3 mL) was added to the mixture. The resultant mixture was extracted with EtOAc (3 mL x3). The combined organic layers were washed with brine (3 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (1 g, hexane/EtOAc = 1/0 to 1/1 to 1/2 then $CHCl_3$/MeOH = 20/1) to afford the crude steroid **30a** (29.8 mg), which was used in the next reaction without further purification: colorless oil. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{57}H_{84}N_4O_{13}SNa$ 1087.5648; Found 1087.5644.

A mixture of CF_3CO_2H (720 μL), PhOMe (16 μL), PhSMe (40 μL), and $HSCH_2CH_2SH$ (24 μL) was added to a solution of a part of the above crude steroid **30a** (16.6 mg) in CH_2Cl_2 (1.5 mL) at room temperature. After the reaction mixture was stirred at room temperature for 4.5 h, toluene (20 mL) was added to the mixture and concentrated. The residue was purified by reversed-phase HPLC (column: Inertsil ODS-4 10 x 250 mm, eluent A: MeOH+0.05% CF_3CO_2H, eluent B: H_2O+0.05% CF_3CO_2H, A/B = 60/40, flow rate: 2.0 mL/min, detection: UV 254 nm, t_R = 21.3 min) to afford CF_3CO_2H salt of vulgarobufotoxin (**4**) (5.47 mg, 6.28

μmol) in 40% yield over 2 steps. Vulgarobufotoxin (**4**) was further purified by flash column chromatography on silica gel (0.5 g, CH_2Cl_2/MeOH/28 wt% aqueous NH_3 = 28/8/1) to compare the NMR data with those prepared by the Pettit's route:[S8] colorless oil. $[\alpha]_D^{28}$ +0.27 (c 0.32, MeOH). IR (film) 3332, 3179, 2931, 2862, 2360, 1711, 1538, 1450, 1379, 1246, 1201, 1135 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ 0.78 (3H, s), 0.98 (3H, s), 1.17-1.46 (13H, m), 1.55-1.74 (13H, m), 1.83-1.98 (3H, m), 1.85 (3H, C*H*$_3$CO), 2.01-2.06 (1H, m), 2.25 (2H, t, *J* = 7.7 Hz), 2.33 (2H, t, *J* = 7.5 Hz), 2.71 (1H, dd, *J* = 15.5, 8.9 Hz, H15a), 2.97 (1H, d, *J* = 8.9 Hz, H17), 3.16-3.26 (2H, m, H5''), 4.32 (1H, t, *J* = 5.8 Hz, H2''), 5.08 (1H, s, H3), 5.51 (1H, dd, *J* = 8.9, 8.9 Hz, H16), 6.21 (1H, d, *J* = 10.0 Hz, H23), 7.44 (1H, s, H21), 8.25 (1H, d, *J* = 10.0 Hz, H22). $^{13}C\{^1H\}$ NMR (125 MHz, CD_3OD) δ 17.1, 20.9, 22.2, 22.3, 24.3, 26.0, 26.1, 26.2, 26.8, 27.6, 29.9, 30.0, 30.8, 31.5, 31.7, 35.5, 36.2, 36.7, 37.1, 38.5, 41.1, 41.2, 42.0, 43.0, 50.8, 55.1 (deduced from HMQC), 58.2, 72.1, 75.7, 85.0, 113.2, 119.4, 152.1, 152.8, 158.6, 164.4, 171.9, 175.1, 175.6, 178.5 (deduced from HMBC). HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{40}H_{60}N_4O_{10}Na$ 779.4202; Found 779.4214.

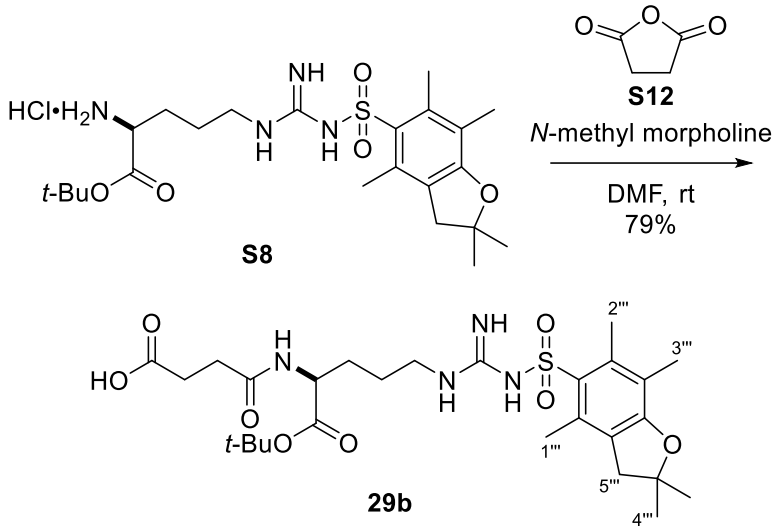


Vulgarobufotoxin (4) prepared by the Pettit's route.[S8] A solution of suberic acid (**S6**, 1.04 g, 5.97 mmol) in Ac_2O (2.1 mL) was stirred at 150 °C for 2 h. The reaction mixture was concentrated and azeotroped with toluene (2 mL x3) to afford the crude acid anhydride **S10**

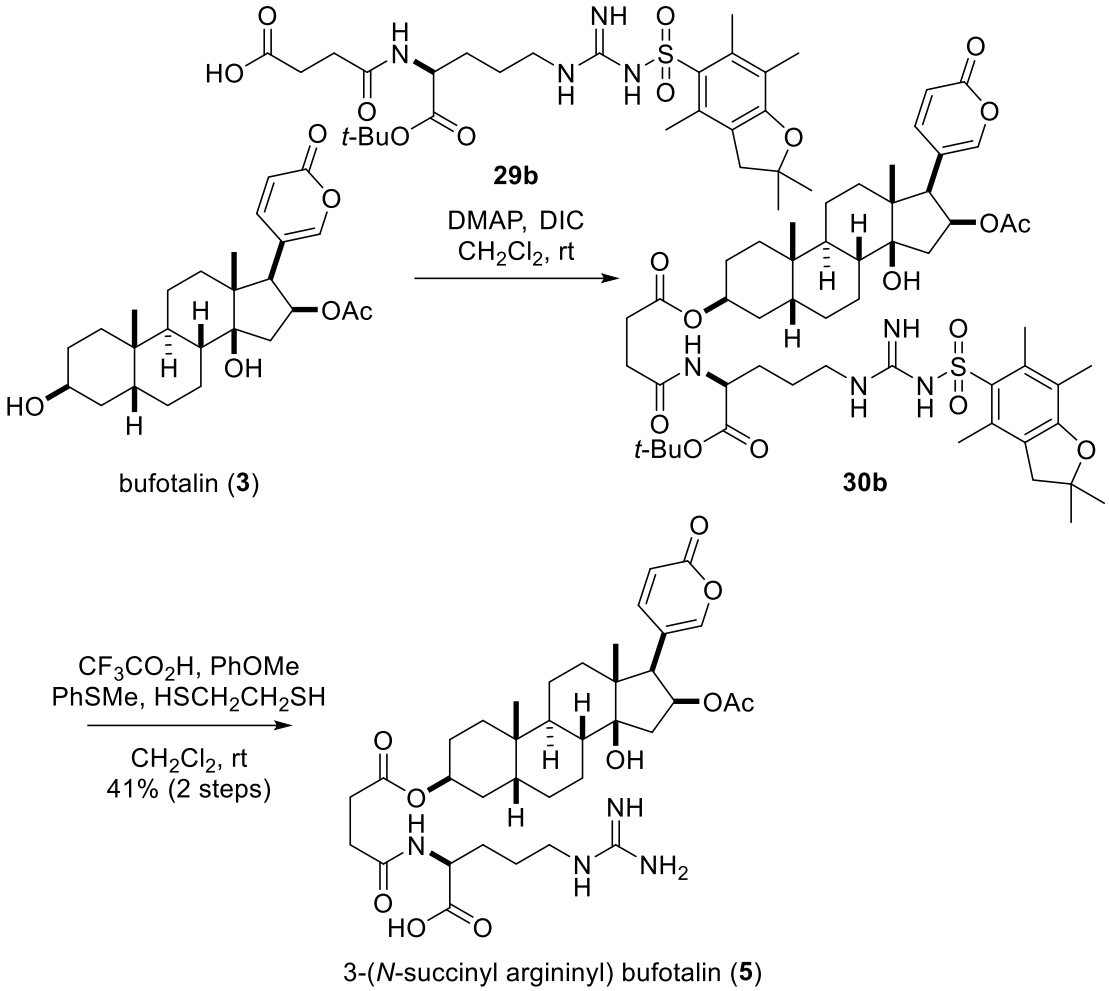
[CAS: 10521-06-9][S9] (995 mg) as white solid, which was used in the next reaction without further purification.

The above crude acid anhydride **S10** (3.6 mg) and DMAP (0.7 mg, 6 μmol) were added to a solution of bufotalin (**3**) (5.2 mg, 12 μmol) in CH_2Cl_2 (0.10 mL) at room temperature. After the reaction mixture was stirred at room temperature for 11 h, saturated aqueous NH_4Cl (3 mL) was added to the mixture. The resultant mixture was extracted with EtOAc (3 mL x4). The combined organic layers were washed with brine (3 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by PTLC ($CHCl_3$/MeOH = 20/1) to afford the crude steroid **S11** (7.1 mg), which was used in the next reaction without further purification: colorless oil. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{34}H_{48}O_9Na$ 623.3191; Found 623.3193.

N-methyl morpholine (6.0 μL, 55 μmol) was added to a suspension of the above crude steroid **S11** (7.1 mg) and activated molecular sieves 3A (MS 3A, 60.5 mg) in THF (0.50 mL) at -10 °C. After the reaction mixture was stirred at -10 °C for 10 min, trichloroethyl chloroformate (7.0 μL, 52 μmol) was added to the mixture. After the reaction mixture was stirred at -10 °C for 30 min, a solution of L-arginine (8.7 mg, 50 μmol) in 1 M aqueous HCl (15 μL, 15 μmol) and MeOH (150 μL) was added to the mixture. After being stirred at room temperature for 2 h, the reaction mixture was concentrated. The residue was purified by flash column chromatography on silica gel (0.5 g, CH_2Cl_2/MeOH/28 wt% aqueous NH_3 = 28/8/1) to afford vulgarobufotoxin (**4**) (5.2 mg, 6.9 μmol) in 58% yield over 2 steps: colorless oil. $[\alpha]_D^{28}$ -1.2 (*c* 0.26, MeOH). IR (film) 3386, 2932, 2861, 1712, 1537, 1378, 1246, 1201, 1134 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ 0.78 (3H, s, H18), 0.98 (3H, s, H19), 1.22-1.46 (13H, m), 1.55-1.74 (13H, m), 1.84-1.94 (3H, m), 1.85 (3H, s, C*H*$_3$CO), 2.01-2.06 (1H, m), 2.25 (2H, t, *J* = 7.7 Hz), 2.33 (2H, t, *J* = 7.5 Hz), 2.71 (1H, dd, *J* = 15.5, 8.9 Hz, H15a), 2.97 (1H, d, *J* = 8.9 Hz, H17), 3.17-3.23 (2H, m, H5''), 4.28-4.32 (1H, m, H2''), 4.61 (1H, brs, O*H*), 5.08 (1H, brs, H3), 5.51 (1H, dd, *J* = 8.9, 8.9 Hz, H16), 6.21 (1H, d, *J* = 10.1 Hz, H23), 7.44 (1H, s, H21), 8.11 (1H, s, N*H*), 8.25 (1H, d, *J* = 10.1 Hz, H22). $^{13}C\{^1H\}$ NMR (100 MHz, CD_3OD) δ 17.1, 20.9, 22.2, 22.3, 24.3, 25.9, 26.1, 26.3, 26.8, 27.6, 29.9, 30.0, 30.5, 31.5, 31.7, 35.5, 36.2, 36.7, 36.9, 38.5, 41.1, 41.2, 41.9, 43.0, 50.8, 53.9, 58.2, 72.1, 75.7, 85.0, 113.2, 119.4, 152.1, 152.8, 158.6, 164.4, 171.9, 175.1, 175.9, 176.6. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{40}H_{60}N_4O_{10}Na$ 779.4204; Found 779.4224.



Carboxylic acid 29b. Acid anhydride **S12** (10.4 mg, 104 μmol) and *N*-methyl morpholine (23 μL, 0.21 mmol) were successively added to a solution of amine hydrochloride **S8** (54.2 mg, 104 μmol) in DMF (0.20 mL) at room temperature. After the reaction mixture was stirred at room temperature for 4 h, saturated aqueous NH_4Cl (3 mL) was added to the mixture. The resultant mixture was extracted with EtOAc (3 mL x4). The combined organic layers were washed with brine (3 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (5 g, $CHCl_3$/MeOH = 1/0 to 30/1 to 10/1) to afford carboxylic acid **29b** (47.8 mg, 82.0 μmol) in 79% yield: colorless oil. $[\alpha]_D^{18}$ +16 (*c* 1.2, $CHCl_3$). IR (film) 3442, 3336, 2973, 2929, 2871, 1731, 1656, 1551, 1454, 1371, 1253, 1157, 1105 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 1.43 (9H, s, *t*-Bu), 1.45 (6H, s, H4’’’), 1.54-1.58 (2H, m, H4’’), 1.64-1.71 (1H, m, H3’’a), 1.82-1.88 (1H, m, H3’’b), 2.08 (3H, s, H3’’’), 2.43-2.64 (3H, m), 2.48 (3H, s, H1’’’), 2.54 (3H, s, H2’’’), 2.76-2.81 (1H, m), 2.94 (2H, s, H5’’’), 3.18 (2H, brs, H5’’), 4.44 (1H, brs, H2’’), 6.37 (2H, brs, N*H*), 6.88 (1H, brs, N*H*CO). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 12.4, 17.8, 19.2, 24.8, 27.9, 28.5, 29.4, 29.6, 30.5, 40.6, 43.1, 52.6, 82.4, 86.4, 117.5, 124.6, 132.2 (2C), 138.3, 156.3, 158.7, 171.2, 172.7, 176.0. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{27}H_{42}N_4O_8SNa$ 605.2616; Found 605.2594.

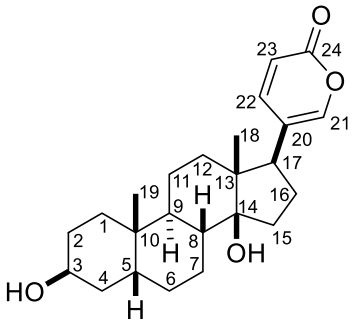


3-(*N*-succinyl argininyl) bufotalin (5) [CAS: 146996-65-8]. Carboxylic acid **29b** (8.92 mg, 15.3 μmol), DIC (3.0 μL, 19 μmol), and DMAP (0.54 mg, 4.4 μmol) were added to a solution of bufotalin (**3**) (5.61 mg, 12.6 μmol) in CH_2Cl_2 (0.25 mL) at room temperature. After the reaction mixture was stirred at room temperature for 12 h, saturated aqueous $NaHCO_3$ (3 mL) was added to the mixture. The resultant mixture was extracted with EtOAc (3 mL x3). The combined organic layers were washed with brine (3 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (0.5 g, $CHCl_3$/MeOH = 1/0 to 50/1) to afford the crude steroid **30b** (13.5 mg), which was used in the next reaction without further purification: colorless oil. HRMS (ESI-TOF) $[M+Na]^+$ m/z: Calcd for $C_{53}H_{76}N_3O_{13}SNa$ 1031.5022; Found 1031.5021.

A mixture of CF_3CO_2H (450 μL), PhOMe (10 μL), PhSMe (25 μL), and $HSCH_2CH_2SH$ (15 μL) was added to a solution of the above crude steroid **30b** (13.5 mg) in CH_2Cl_2 (1.5 mL) at room temperature. After the reaction mixture was stirred at room temperature for 4 h, toluene (20 mL) was added to the mixture and concentrated. The residue was purified by reversed-phase HPLC (column: Inertsil ODS-3 10 x 250 mm, eluent A: MeOH+0.05% CF_3CO_2H, eluent B: H_2O+0.05% CF_3CO_2H , A/B = 40/60, flow rate: 2.0 mL/min, detection: UV 254 nm, t_R = 32.4 min) to afford CF_3CO_2H salt of 3-(*N*-succinyl argininyl) bufotalin (**5**) (4.21 mg, 5.17 μmol) in 41% yield over 2 steps. 3-(*N*-succinyl argininyl) bufotalin (**5**) was further purified by flash

column chromatography on silica gel (first time: 0.5 g, EtOAc/EtOH/H_2O = 3/7/1; second time: 2.5 g, EtOAc/EtOH/H_2O = 3/7/1) to compare the NMR data with those in the literature:[S10] colorless oil. $[\alpha]_D^{25}$ -9.2 (*c* 0.12, MeOH). IR (film) 3355, 2933, 2869, 1714, 1651, 1594, 1380, 1248, 1025, 854 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 0.65 (3H, s, H18), 0.88 (3H, s, H19), 1.07-1.19 (2H, m), 1.23-1.78 (19H, m), 1.78 (3H, s, CH_3CO), 1.90-1.96 (1H, m), 2.38-2.46 (4H, m, H2', H3'), 2.67 (1H, dd, *J* = 16.9, 8.6 Hz, H15a), 2.88 (1H, d, *J* = 8.6 Hz, H17), 3.03 (2H, brs, H5''), 3.93 (1H, brs, H2''), 4.41 (1H, s, O*H*), 4.94 (1H, s, H3), 5.38 (1H, dd, *J* = 8.6, 8.6 Hz, H16), 6.19 (1H, d, *J* = 10.0 Hz, H23), 7.44-7.71 (2H, m, N*H*), 7.51 (1H, s, H21), 7.62 (1H, brs, N*H*), 8.18 (1H, d, *J* = 10.0 Hz, H22). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-d_6) δ 16.6, 20.66, 20.71, 20.8, 23.5, 24.3, 25.3, 26.1, 29.7, 29.8, 29.9, 30.2, 30.3, 34.6, 34.7, 36.6, 39.2 (deduced from HMQC), 40.0 (deduced from HMQC), 40.4, 41.3, 49.0, 53.6, 55.9, 70.0, 73.8, 82.6, 111.7, 117.3, 150.1, 151.4, 157.3, 161.2, 169.5, 169.7, 171.8, 175.9. HRMS (ESI-TOF) $[M+H]^+$ m/z: Calcd for $C_{36}H_{53}N_4O_{10}$ 701.3756; Found 701.3729.

2. Comparison of ^{1}H and $^{13}C\{^{1}H\}$ NMR data of reported and synthetic 1−5



bufalin (**1**)

Table S1. ^{1}H NMR data of reported[S11] and synthetic bufalin (**1**) in $CDCl_3$

No.	reported **1** (500 MHz)	synthetic **1** (400 MHz)[a]	Δ ($\delta_{reported}$ - $\delta_{synthetic}$)
	^{1}H [δ, multi, *J* (Hz)]	^{1}H [δ, multi, *J* (Hz)]	
1a	1.42 (m)[b]	1.24 (m)	0.18
1b	1.49 (m)	1.49 (m)	0.00
2a	1.52 (m)	1.52 (m)	0.00
2b	1.58 (m)	1.59 (m)	-0.01
3	4.14 (brs)	4.14 (brs)	0.00
4a	1.35 (m)	1.36 (m)	-0.01
4b	1.88 (m)	1.92 (m)	-0.04
5	1.75 (m)	1.76 (m)	-0.01
6a	1.27 (m)	1.27 (m)	0.00
6b	1.88 (m)	1.91 (m)	-0.03
7a	1.18-1.72 (m)	1.28 (m)	-
7b	1.18-1.72 (m)	1.72 (m)	-
8	1.51 (m)	1.53 (m)	-0.02
9	1.61 (m)	1.61 (m)	0.00
11a	1.18-1.72 (m)	1.17 (m)	-
11b	1.18-1.72 (m)	1.42 (m)	-
12a	1.39 (m)	1.36 (m)	0.03
12b	1.49 (m)	1.50 (m)	-0.01
15a	1.69 (m)	1.69 (m)	0.00
15b	2.06 (m)	2.04 (m)	0.02
16a	1.72 (m)	1.74 (m)	-0.02
16b	2.18 (m)	2.19 (m)	-0.01
17	2.46 (m)	2.46 (dd, 9.6, 6.4)	0.00
18	0.70 (s)	0.70 (s)	0.00
19	0.95 (s)	0.95 (s)	0.00

21	7.24 (d, 2.6)	7.23 (d, 2.8)	0.01
22	7.85 (dd, 9.7, 2.6)	7.84 (dd, 9.8, 2.8)	0.01
23	6.26 (d, 9.7)	6.26 (d, 9.8)	0.00

[a]Chemical shifts were determined by using ^{1}H-^{13}C HMQC spectra. [b]The δ value of this peak could be misreported.

Table S2. $^{13}C\{^{1}H\}$ NMR data of reported[S11] and synthetic bufalin (**1**) in $CDCl_3$

No.	reported **1** (125 MHz)	synthetic **1** (100 MHz)	Δ ($\delta_{reported}$ - $\delta_{synthetic}$)
	$^{13}C\{^{1}H\}$ (δ)	$^{13}C\{^{1}H\}$ (δ)	
1	29.62	29.59	0.03
2	27.86	27.87	-0.01
3	66.85	66.81	0.04
4	33.26	33.24	0.02
5	35.96	35.94	0.02
6	26.51	26.48	0.03
7	21.40	21.37	0.03
8	42.33	42.31	0.02
9	35.37	35.35	0.02
10	35.67	35.63	0.04
11	21.40	21.37	0.03
12	40.91	40.88	0.03
13	48.36	48.32	0.04
14	85.39	85.36	0.03
15	32.71	32.70	0.01
16	28.73	28.71	0.02
17	51.21	51.18	0.03
18	16.53	16.52	0.01
19	23.73	23.72	0.01
20	122.78	122.68	0.10
21	148.52	148.46	0.06
22	146.90	146.79	0.11
23	115.27	115.28	-0.01
24	162.49	162.40	0.09

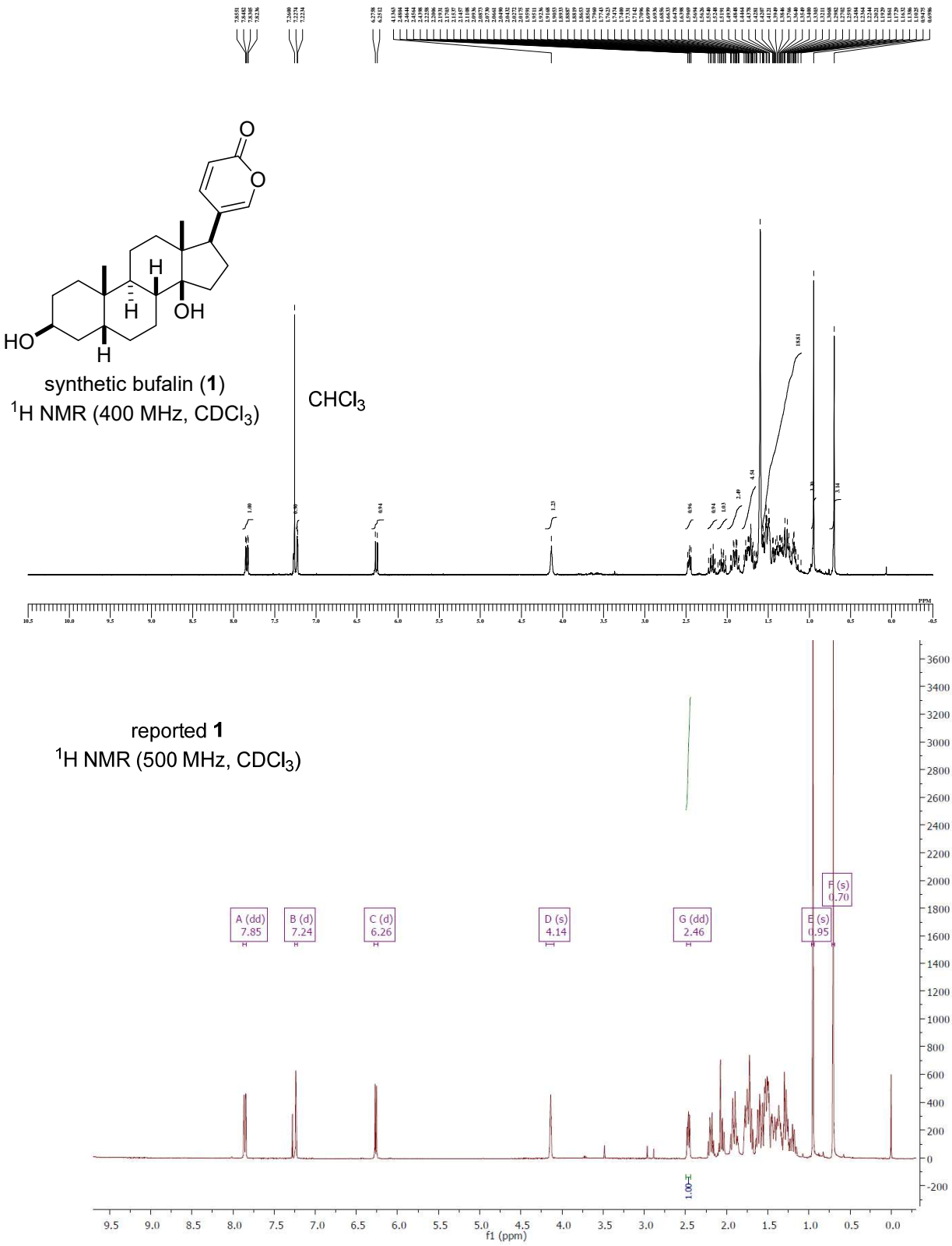


Figure S2. ^{1}H NMR spectra of synthetic **1** (400 MHz) and reported **1** (500 MHz)

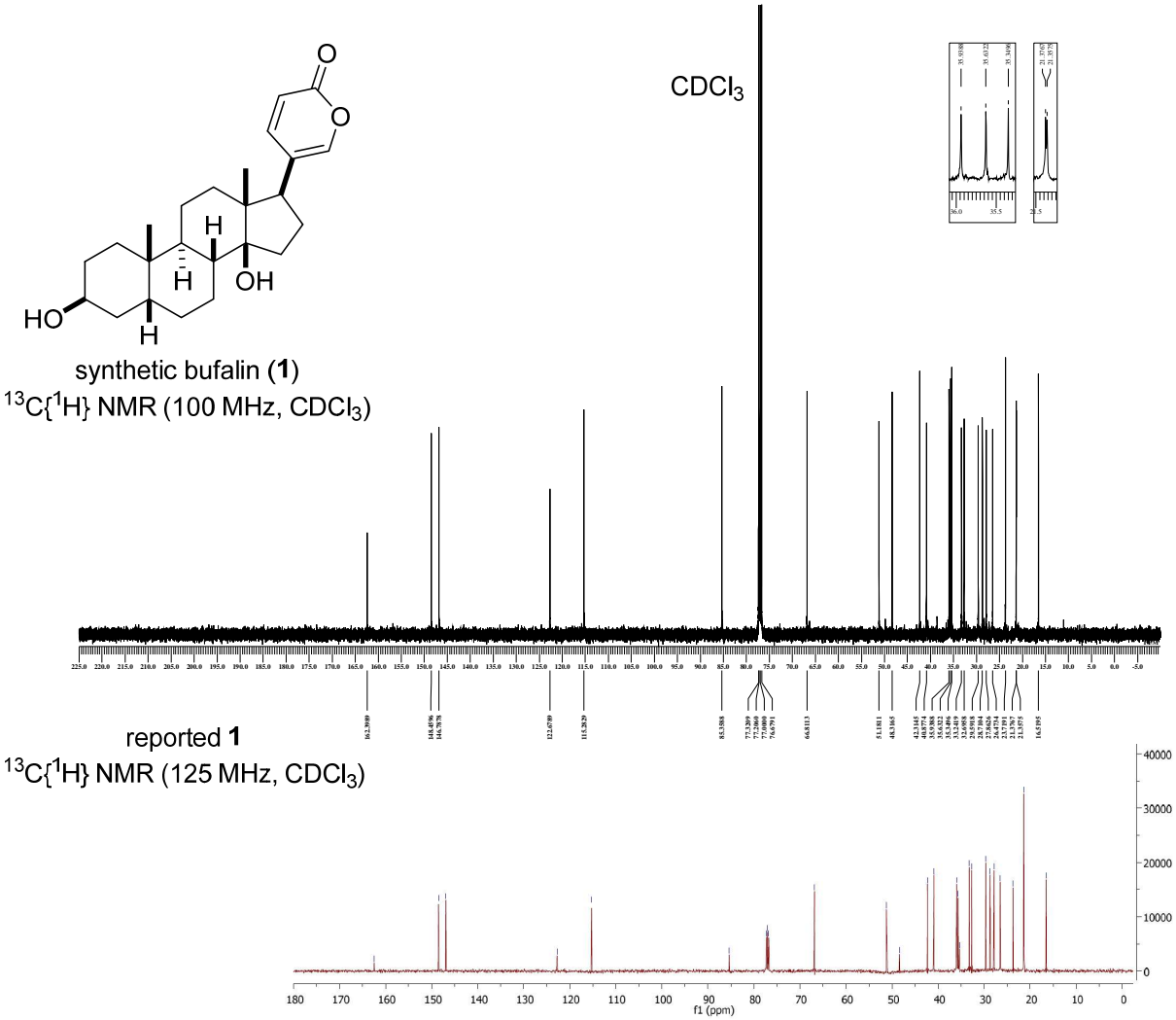
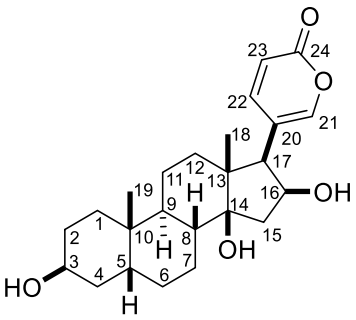


Figure S3. $^{13}C\{^{1}H\}$ NMR spectra of synthetic **1** (100 MHz) and reported **1** (125 MHz)

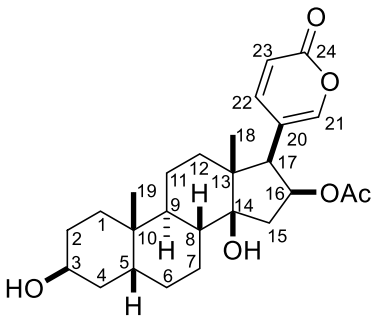


bufogenin B (**2**)

Table S3. ^{1}H NMR data of reported[S12] and synthetic bufogenin B (**2**) in DMSO-d_6

No.	reported **2**[a]	synthetic **2** (500 MHz)[b]	Δ ($\delta_{reported}$ - $\delta_{synthetic}$)
	^{1}H [δ, multi, *J* (Hz)]	^{1}H [δ, multi, *J* (Hz)]	
3	3.89 (brs)	3.89 (brs)	0.00
16	4.43 (m)	4.43 (m)	0.00
17	2.63 (d, 8.1)	2.63 (d, 8.6)	0.00
18	0.64 (s)	0.64 (s)	0.00
19	0.85 (s)	0.85 (s)	0.00
21	7.47 (d, 1.5)	7.47 (d, 2.3)	0.00
22	8.08 (dd, 9.7, 1.5)	8.08 (dd, 9.8, 2.3)	0.00
23	6.12 (d, 9.7)	6.12 (d, 9.8)	0.00

[a]Internal standard was not indicated. [b]Chemical shifts were determined by using ^{1}H-^{13}C HMQC spectra.



bufotalin (**3**)

Table S4. ^{1}H NMR data of reported[S13] and synthetic bufotalin (**3**) in $CDCl_3$

No.	reported **3** (500 MHz)[a]	synthetic **3** (500 MHz)[b]	Δ ($\delta_{reported}$ - $\delta_{synthetic}$)
	^{1}H [δ]	^{1}H [δ, multi, *J* (Hz)]	
1a	-	1.25 (m)	-
1b	1.49	1.49 (m)	0.00
2a	1.51	1.52 (m)	-0.01
2b	1.56	1.58 (m)	-0.02
3	4.14	4.14 (brs)	0.00
4a	1.37	1.37 (m)	0.00
4b	1.90	1.89 (m)	0.01
5	1.77	1.77 (brd, 12.6)	0.00
6a	1.29	1.29 (m)	0.00
6b	1.90	1.88 (m)	0.02
7a	1.21	1.20 (m)	0.01
7b	1.77	1.76 (m)	0.01
8	1.54	1.54 (m)	0.00
9	1.55	1.55 (m)	0.00
11a	1.21	1.20 (m)	0.01
11b	1.44	1.44 (m)	0.00
12a	1.28	1.29 (m)	-0.01
12b	1.56	1.56 (m)	0.00
15a	1.84	1.83 (d, 15.8)	0.01
15b	2.64	2.64 (dd, 15.8, 8.9)	0.00
16	5.54	5.53 (dd, 8.9, 8.9)	0.01
17	2.89	2.86 (d, 8.9)	0.03
18	0.78	0.78 (s)	0.00
19	0.98	0.94 (s)	0.04
21	7.25	7.24 (s)	0.01
22	8.03	8.03 (d, 9.8)	0.00

23	6.19	6.19 (d, 9.8)	0.00
Ac	1.87	1.86 (s)	0.01

[a]Internal standard was not indicated. [b]Chemical shifts were determined by using ^{1}H-^{13}C HMQC spectra.

Table S5. $^{13}C\{^{1}H\}$ NMR data of reported[S13] and synthetic bufotalin (**3**) in $CDCl_3$

No.	reported **3** (125 MHz)[a]	synthetic **3** (125 MHz)	Δ ($\delta_{reported}$ - $\delta_{synthetic}$)
	$^{13}C\{^{1}H\}$ (δ)	$^{13}C\{^{1}H\}$ (δ)	
1	29.56	29.54	0.02
2	27.91	27.88	0.03
3	66.73	66.71	0.02
4	33.27	33.23	0.04
5	35.88	35.85	0.03
6	26.37	26.34	0.03
7	21.14	21.11	0.03
8	42.32	42.27	0.05
9	35.55	35.52	0.03
10	35.29	35.26	0.03
11	21.06	21.04	0.02
12	40.42	40.38	0.04
13	49.39	49.36	0.03
14	84.41	84.39	0.02
15	40.83	40.79	0.04
16	73.57	73.54	0.03
17	57.18	57.13	0.05
18	16.44	16.44	0.00
19	23.71	23.70	0.01
20	116.83	116.84	-0.01
21	150.97	150.94	0.03
22	149.13	149.13	0.00
23	113.12	113.09	0.03
24	161.93	161.95	-0.02
CO of Ac	170.02	170.02	0.00
CH_3 of Ac	20.94	20.94	0.00

[a]Internal standard was not indicated.

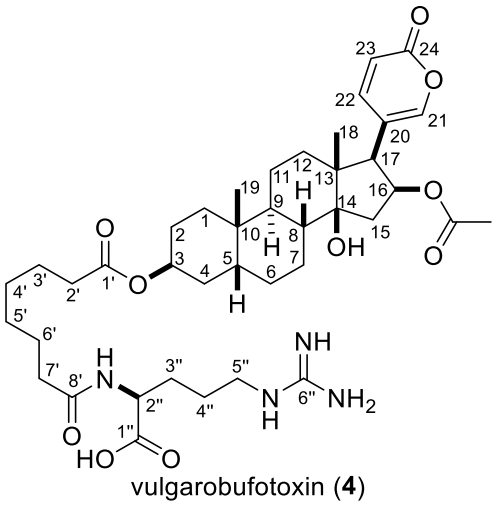


Table S6. ^{1}H NMR data of vulgarobufotoxin (**4**) prepared by our route and Pettit's route[S8] in CD_3OD

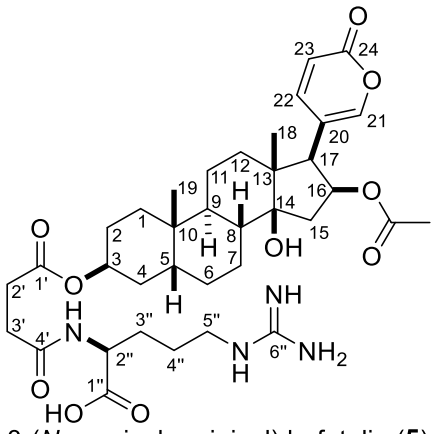
No.	synthetic **4** (a)[a] (our route, 500 MHz)	synthetic **4** (b)[a] (Pettit's route, 500 MHz)	Δ ($\delta_{synthetic\ (a)}$ - $\delta_{synthetic\ (b)}$)
	^{1}H [δ, multi, *J* (Hz)]	^{1}H [δ, multi, *J* (Hz)]	
3	5.08 (s)	5.08 (brs)	0.00
16	5.51 (dd, 8.9, 8.9)	5.51 (dd, 8.9, 8.9)	0.00
17	2.97 (d, 8.9)	2.97 (d, 8.9)	0.00
18	0.78 (s)	0.78 (s)	0.00
19	0.98 (s)	0.98 (s)	0.00
21	7.44 (s)	7.44 (s)	0.00
22	8.25 (d, 10.0)	8.25 (d, 10.1)	0.00
23	6.21 (d, 10.0)	6.21 (d, 10.1)	0.00
2''	4.32 (t, 5.8)	4.30 (m)	0.02
Ac	1.85 (s)	1.85 (s)	0.00

[a]Chemical shifts were determined by using ^{1}H-^{13}C HMQC spectra.

Table S7. $^{13}C\{^{1}H\}$ NMR data of vulgarobufotoxin (**4**) prepared by our route and Pettit's route [S8] in CD_3OD

synthetic **4** (a) (our route, 100 MHz)	synthetic **4** (b) (Pettit's route, 125 MHz)	Δ ($\delta_{synthetic\ (a)}$ - $\delta_{synthetic\ (b)}$)
$^{13}C\{^{1}H\}$ (δ)	$^{13}C\{^{1}H\}$ (δ)	
17.12	17.13	-0.01
20.92	20.93	-0.01
22.21	22.21	0.00
22.28	22.29	-0.01
24.31	24.31	0.00
25.95	25.94	0.01
26.12	26.12	0.00
26.22	26.30	-0.08
26.84	26.82	0.02
27.60	27.60	0.00
29.90	29.90	0.00
30.01	29.97	0.04
30.76	30.47	0.29
31.53	31.53	0.00
31.69	31.69	0.00
35.51	35.50	0.01
36.25	36.25	0.00
36.69	36.67	0.02
37.12	36.92	0.20
38.53	38.52	0.01
41.12	41.11	0.01
41.19	41.18	0.01
42.02	41.93	0.09
43.02	43.01	0.01
50.76	50.75	0.01
55.12	53.92	1.20
58.20	58.18	0.02
72.10	72.09	0.01
75.71	75.70	0.01
84.99	85.00	-0.01
113.15	113.16	-0.01
119.41	119.41	0.00

152.12	152.11	0.01
152.81	152.80	0.01
158.60	158.60	0.00
164.44	164.42	0.02
171.93	171.92	0.01
175.06	175.06	0.00
175.60	175.94	-0.34
178.50	176.61	1.89



3-(*N*-succinyl argininyl) bufotalin (**5**)

Table S8. ^{1}H NMR data of reported[S10] and synthetic 3-(*N*-succinyl argininyl) bufotalin (**5**) in DMSO-d_6

No.	reported **5** (600 MHz)[a,b]	synthetic **5** (500 MHz)[c]	Δ ($\delta_{reported}$ - $\delta_{synthetic}$)
	^{1}H [δ, multi, *J* (Hz)]	^{1}H [δ, multi, *J* (Hz)]	
3	4.95 (brs)	4.94 (s)	0.01
15a	2.67 (m)	2.67 (dd, 16.9, 8.6)	0.00
16	5.38 (dd, 8.4, 8.4)	5.38 (dd, 8.6, 8.6)	0.00
17	2.88 (d, 9.0)	2.88 (d, 8.6)	0.00
18	0.66 (s)	0.65 (s)	0.01
19	0.89 (s)	0.88 (s)	0.01
21	7.51 (s)	7.51 (s)	0.00
22	8.18 (d, 9.6)	8.18 (d, 10.0)	0.00
23	6.19 (d, 9.6)	6.19 (d, 10.0)	0.00
2"	3.92 (d, 7.2)	3.93 (brs)	-0.01
5"	3.03 (brs)	3.03 (brs)	0.00
OH	4.38 (s)	4.41 (s)	-0.03

[a]Internal standard was not indicated. [b]The ^{1}H NMR spectrum was provided by Professors Hui-Min Gao and Zhi-Min Wang. [c]Chemical shifts were assigned by using ^{1}H-^{13}C HMQC spectra.

Table S9. $^{13}C\{^{1}H\}$ NMR data of reported[S10] and synthetic 3-(*N*-succinyl argininyl) bufotalin (**5**) in DMSO-d_6

reported **5** (150 MHz)[a,b]	synthetic **5** (125 MHz)	Δ ($\delta_{reported}$ - $\delta_{synthetic}$)
$^{13}C\{^{1}H\}$ (δ)	$^{13}C\{^{1}H\}$ (δ)	
30.20	29.71	0.49
-	29.80	-
30.32	29.85	0.47
30.67	30.17	0.50
-	30.28	-
35.02	34.55	0.47
35.13	34.69	0.44
37.02	36.58	0.44
-	39.30	-
-	40.00	-
40.85	40.39	0.46
41.76	41.30	0.46
49.43	48.98	0.45
54.13	53.61	0.52
56.40	55.92	0.48
70.41	69.96	0.45
74.24	73.81	0.43
83.04	82.59	0.45
112.12	111.68	0.44
117.72	117.29	0.43
150.57	150.14	0.43
151.89	151.44	0.45
157.77	157.31	0.46
161.59	161.18	0.41
169.88	169.47	0.41
170.03	169.70	0.33
172.17	171.75	0.42
176.24	175.91	0.33

[a]Internal standard was not indicated. [b]The $^{13}C\{^{1}H\}$ NMR spectrum was provided by Professors Hui-Min Gao and Zhi-Min Wang.

3. X-ray structures and crystallographic data of 6 and 18

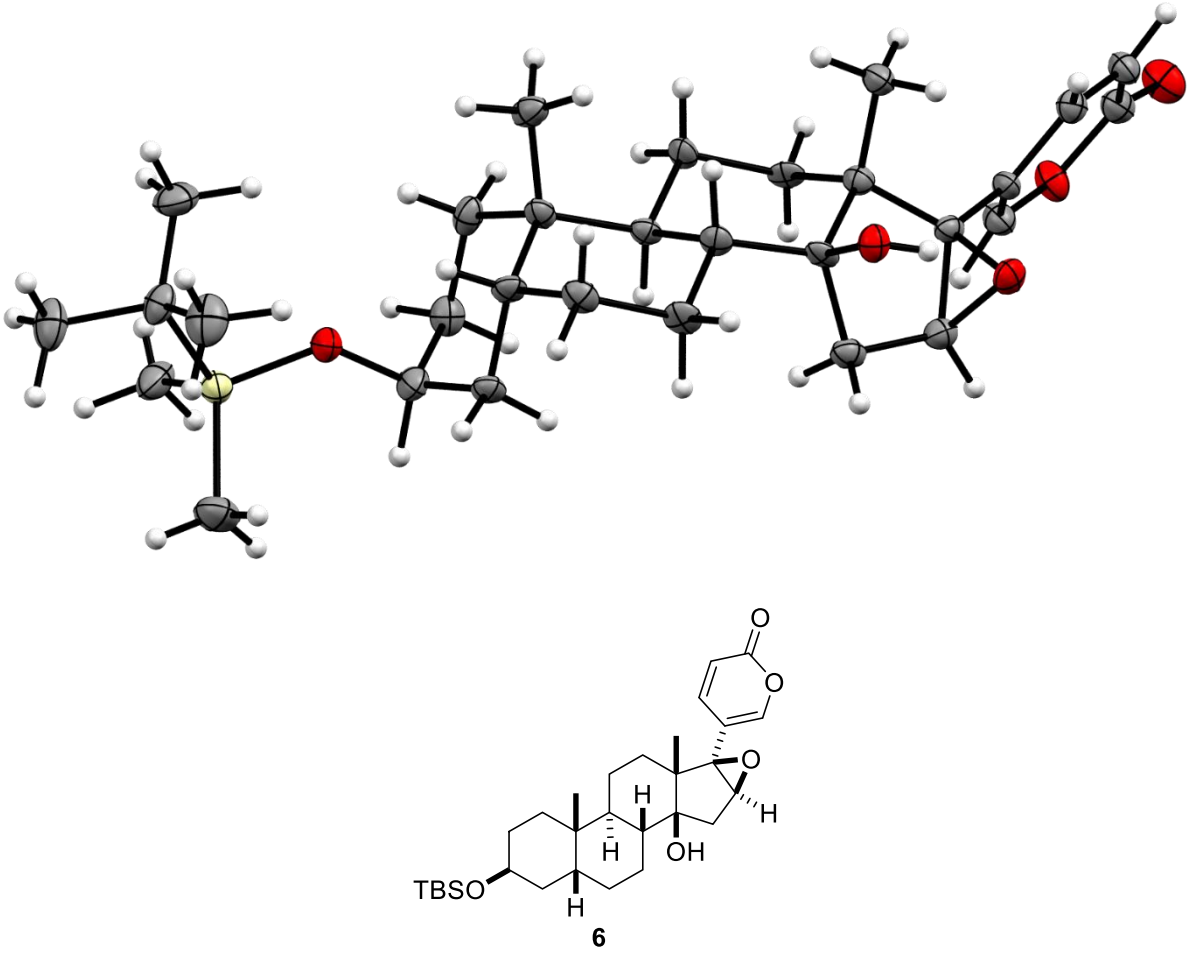


Figure S4. X-ray crystallographic structure of **6** (CCDC 2031223) with thermal ellipsoids at the 50% probability level (ORTEP)

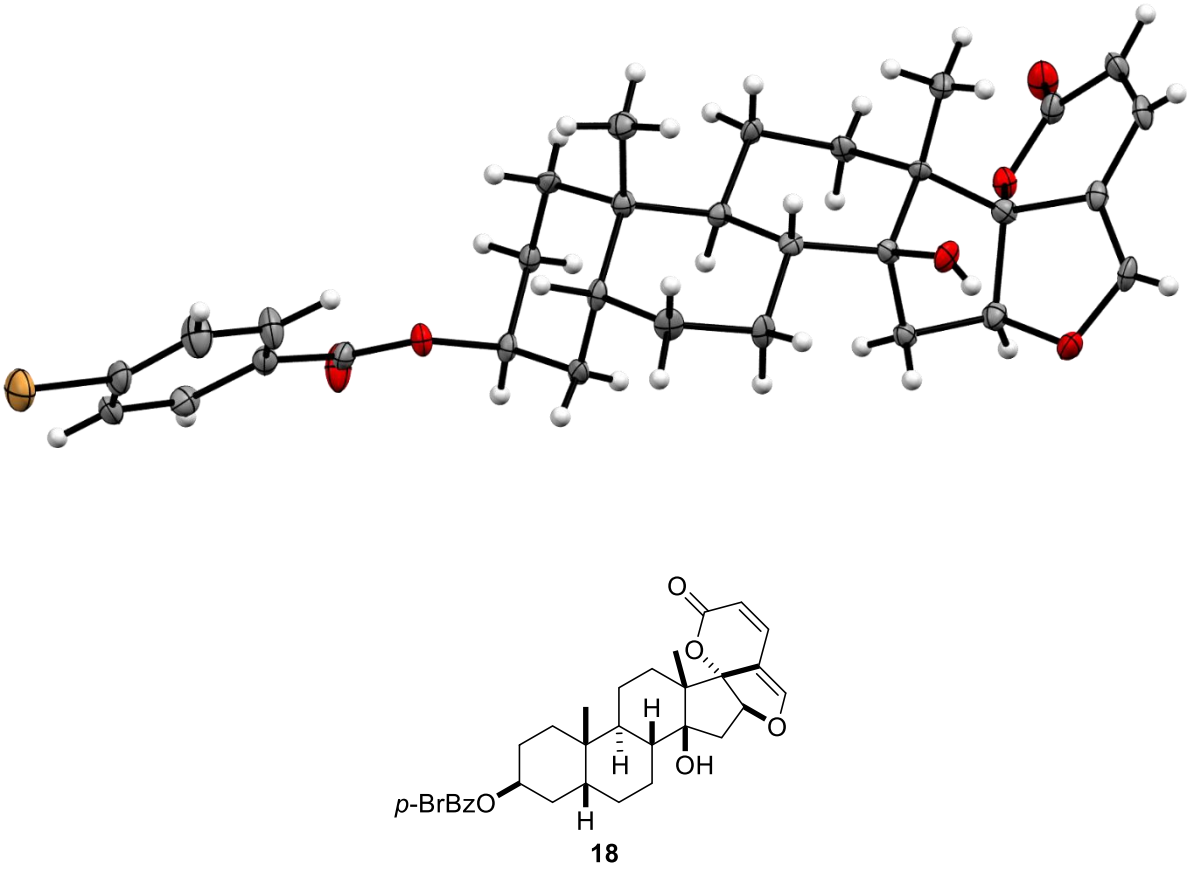


Figure S5. X-ray crystallographic structure of **18** (CCDC 2031224) with thermal ellipsoids at the 50% probability level (ORTEP)

Table S10. Crystal data and structure refinement for **6** and **18**

Compound	**6**	**18**
CCDC number	2031223	2031224
Molecular formula	$C_{30}H_{46}O_5Si$	$C_{31}H_{35}O_6Br$
Formula weight	514.78	583.52
Temperature (K)	93(2)	296(1)
Wavelength (Å)	0.71075	0.71073
Crystal color, habit	colorless, prism	colorless, plate
Crystal size (mm^3)	0.230 x 0.100 x 0.050	0.200 x 0.200 x 0.050
Crystal system	orthorhombic	orthorhombic
Space group	$P2_12_12_1$ (#19)	$P2_12_12_1$ (#19)
Unit cell dimensions		
a (Å)	7.152(3)	7.7359(3)
b (Å)	12.733(5)	12.9937(4)
c (Å)	31.445(12)	26.7557(14)
α (°)	90.0000	90.0000
β (°)	90.0000	90.0000
γ (°)	90.0000	90.0000
Volume ($Å^3$)	2864(2)	2689.43(19)
Z	4	4
Density (calculated) (g/cm^3)	1.194	1.441
μ(MoKα) (cm^{-1})	1.180	15.769
F(000)	1120.00	1216.00
Index ranges	–9<=h<9	–8<=h<10
	–16<=k<13	–17<=k<16
	–29<=l<40	–31<=l<35
Reflections collected	22448	18355
Independent reflections	6545	6093
R(int)	0.1066	0.0354
Completeness to theta	27.483°, 100%	29.094°, 100%
Max. and min. transmission	0.994, 0.773	1.000, 0.841
Refinement method	Full-matrix least-squares on F^2	
No. Observations (All reflections)	6545	6093
No. Variables	463	446
Reflection/Parameter Ratio	14.14	13.66
Goodness-of-fit on F^2	1.063	1.025
Residuals: R1 (I>2.00σ(I))	0.0758	0.0416
Residuals: R (All reflections)	0.1104	0.0544
Residuals: wR2 (All reflections)	0.1470	0.1031
Max. and min. peak in Final Diff. Map ($e^-/Å^3$)	0.25, –0.30	0.89, –0.49

4. Biological experiments

Cell culture. MCF-7 human breast cancer cells were obtained from the American Type Culture Collection (ATCC). The cells were maintained with growth medium [Dulbecco's Modified Eagle's Medium (DMEM) low glucose (D6046, Sigma) supplemented with 10 v/v% heat-inactivated fetal bovine serum, penicillin G (100 units/mL), and streptomycin (100 μg/mL)] under atmosphere of 5% CO_2 at 37 °C. The growth medium was refreshed every 2 or 3 days to reach 70-90% cell confluence.

Sulforhodamine B assay. Growth inhibitory activities of the tested compounds were evaluated according to the literature.[S14] Cell growth (%) was defined as follows:

$$\text{cell growth (\%)} = \frac{FL(sample) - FL(day0)}{FL(control) - FL(day0)} \times 100 \qquad \{FL(sample) \geq FL(day0)\}$$

$$\text{cell growth (\%)} = \frac{FL(sample) - FL(day0)}{FL(day0)} \times 100 \qquad \{FL(day0) > FL(sample)\}$$

FL = mean of the fluorescence intensity (Ex. 485 nm/Em. 585 nm)
*day*0 = time of addition of the tested compound as serial dilutions
control = control wells treated by vehicle (DMSO)

Various concentrations of compounds in the growth medium containing 2% DMSO were prepared by serial dilutions. MCF-7 cells were cultured in 6 cm cell culture dishes filled with the growth medium and harvested by trypsinization at 37 °C for 5-10 min. The collected cells were resuspended into the growth medium at 1.25 x 10^5 cells/mL. The cell suspension (100 μL/well) was seeded into the black polystyrene flat-bottom 96-well plates (sample-plates, 655090, Greiner bio-one). For calculating *FL*(*day*0), the same cell suspension (100 μL/well) was seeded into an independent 96-well plate (*day*0-plate). The sample-plate and *day*0-plate were incubated under atmosphere of 5% CO_2 at 37 °C for 24 h. Aliquots of the former medium (100 μL) containing compounds were added to each well of the sample-plate. The growth medium containing 2% DMSO (100 μL) was added to the wells for calculating *FL*(*control*) of the sample-plate and the wells for calculating *FL*(*day*0) of the *day*0-plate. To the cells in *day*0-plate was added an ice-cold solution of 30 w/v% trichloroacetic acid (TCA) in H_2O (100 μL/well). The *day*0-plate was incubated at 4 °C for 60 min, washed with H_2O (x4), dried, and stored at room temperature. The sample-plate was incubated at 37 °C under atmosphere of 5% CO_2 for 48 h. To the cells in the sample-plate was added an ice-cold solution of 30 w/v% TCA in H_2O (100 μL/well). The sample-plate was incubated at 4 °C for 60 min, washed with H_2O (x4), and dried. To each well of the *day*0-plate and sample-plate was added a solution of sulforhodamine B in H_2O (570 μg/mL, 100 μL/well). The fixed cells were stained at room temperature for 30 min in the dark. The cells were washed with AcOH/H_2O (1/99, x4) and dried. To the stained cells was added a solution of 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) in H_2O (10 mM, 200 μL/well). The plates were vortexed at room temperature for 10 min. The fluorescence (Ex. 485 nm/Em. 585 nm) of each well was measured on Spectra Max Gemini EM microplate reader (Molecular Devices). Growth inhibitory activities of the tested compounds were evaluated as 50% growth inhibitory

concentration (GI_{50}) values (nM) by means of three replicates. Sigmoidal curve fittings were performed on GraphPad Prism (GraphPad Software, Figure S6). The mean GI_{50} values and standard deviations of ouabain, **1**–**5**, and **28** were determined from three independent experiments (Table S11).

Table S11. Growth inhibitory activity of ouabain and bufadienolides

compound	GI_{50} [nM][a]
ouabain (control)	20.5 ± 1.1
ouabain[S15]	19.9 ± 3.6
1	13.3 ± 1.8
2	55.0 ± 5.7
3	28.2 ± 3.9
4	301 ± 36
5	1190 ± 340
28	2390 ± 670

[a]Three independent experiments were performed (mean ± SD).

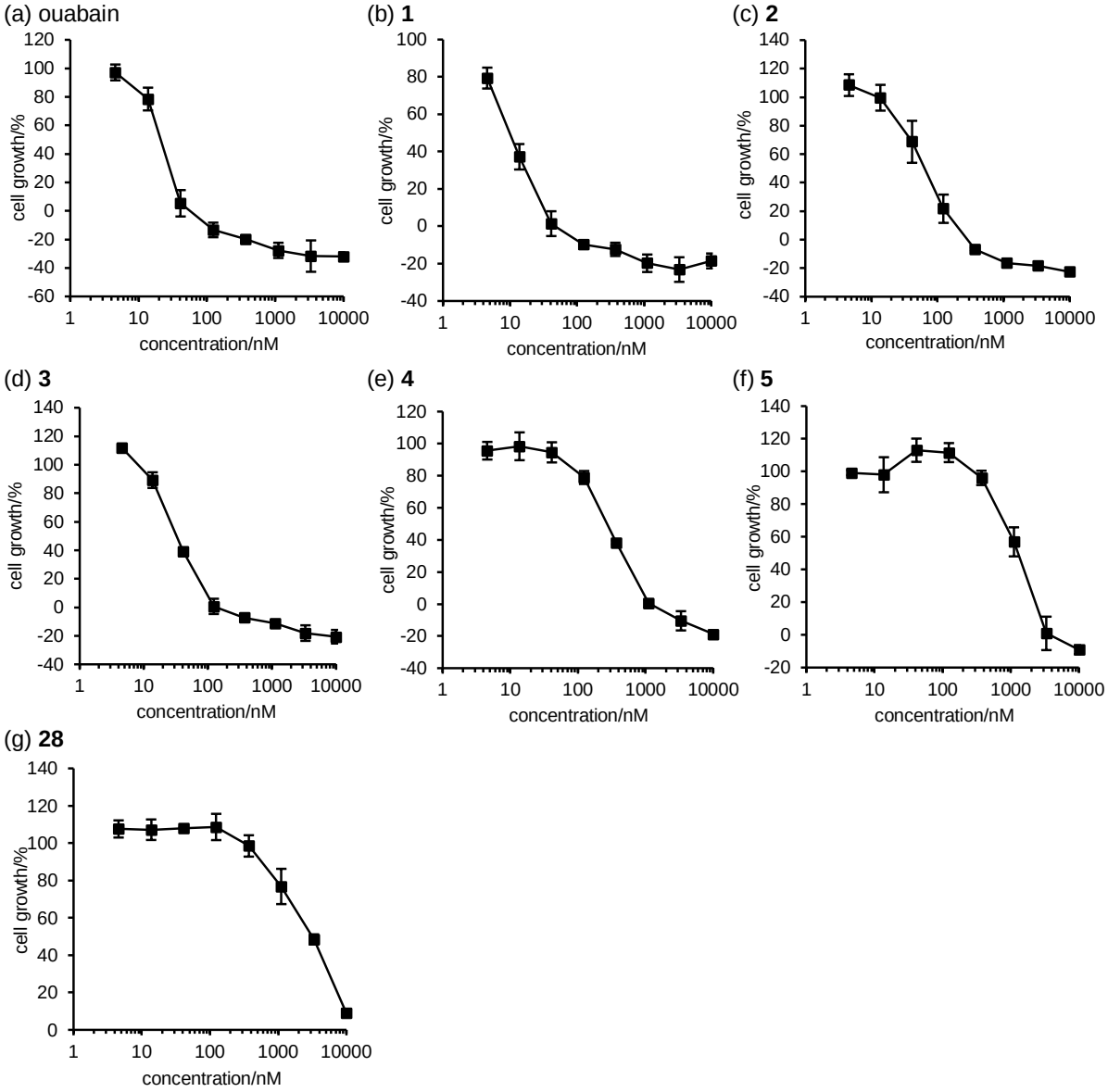


Figure S6. Representative concentration-response curves of ouabain, **1**–**5**, and **28**. Ouabain was used as a positive control. The cells were incubated for 48 h in the presence of compounds, and the cell growth (%) was evaluated. Each plot is displayed as mean ± SD of three replicates.

5. References

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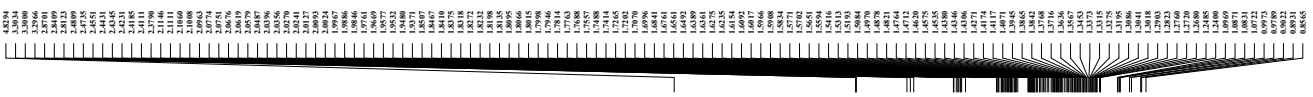
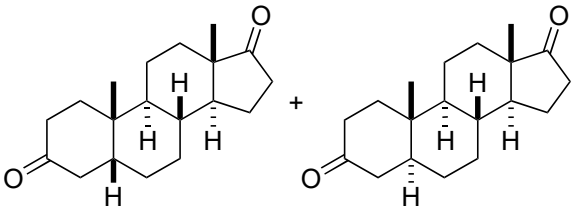
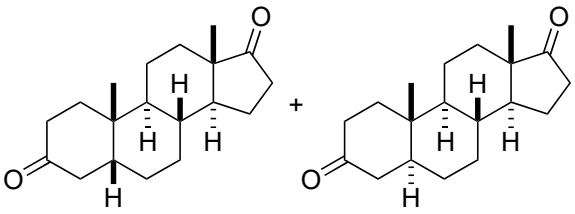




10a **10b**

10a:**10b** = 9.3:1

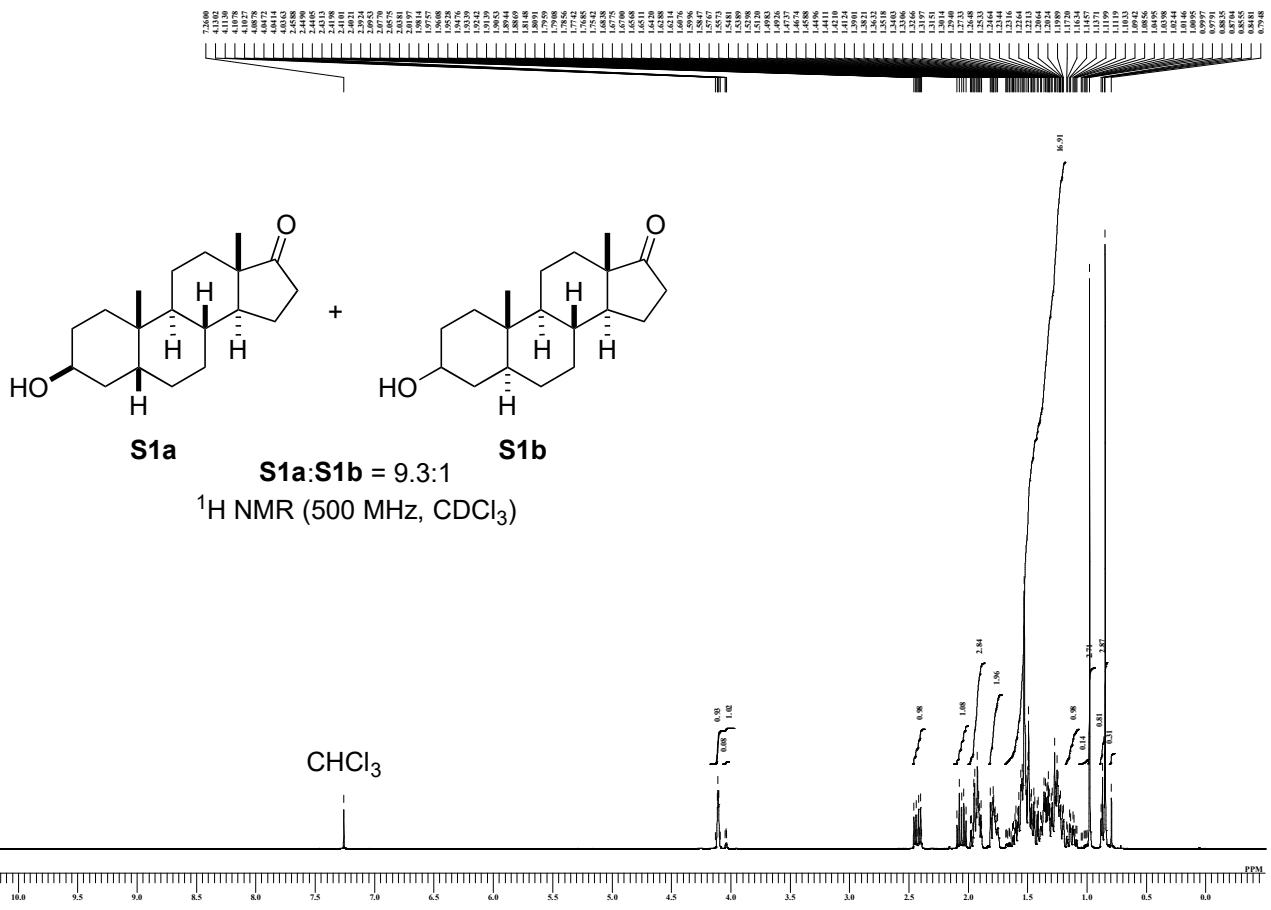
^{1}H NMR (500 MHz, CD_3OD)



10a **10b**

10a:**10b** = 9.3:1

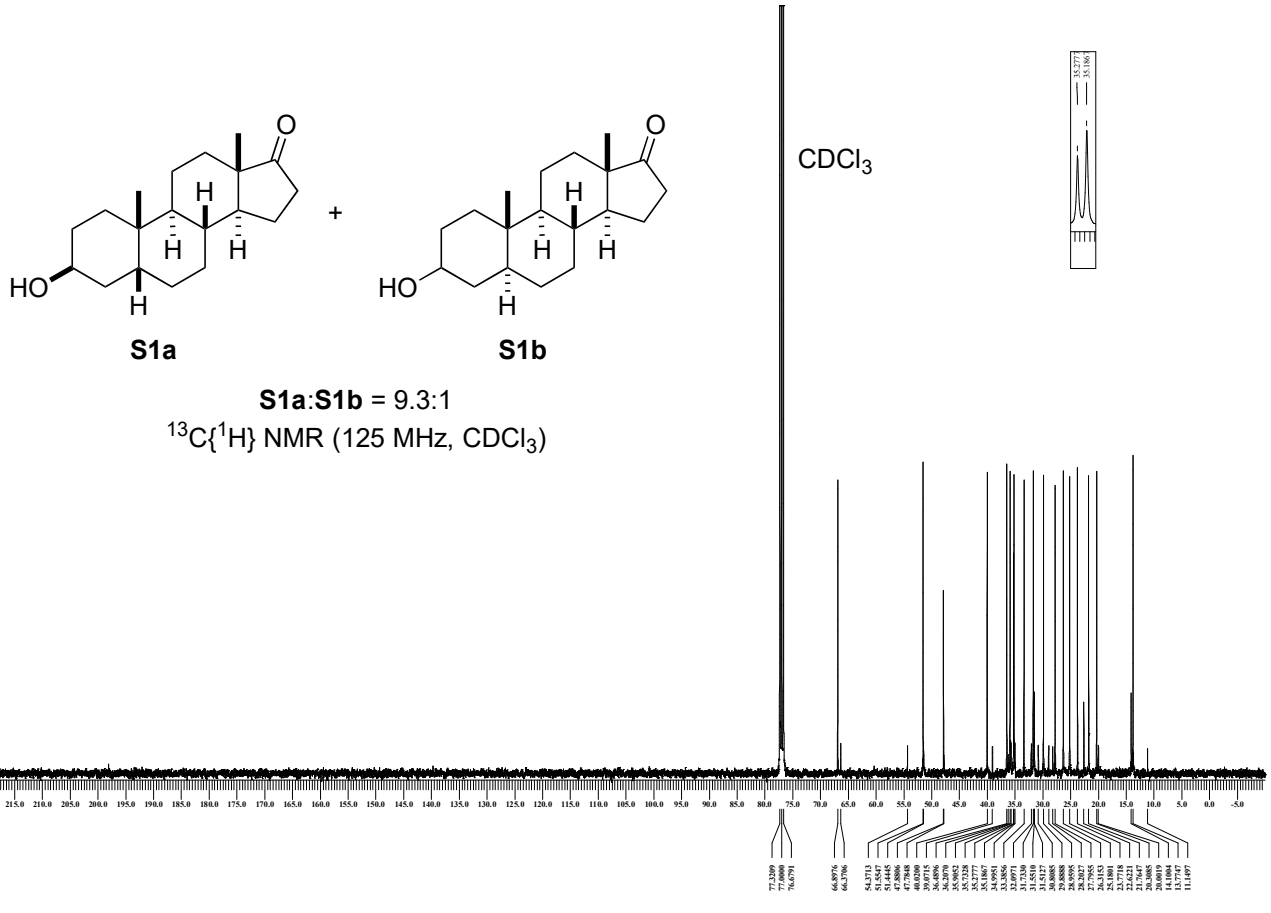
^{13}C{^{1}H} NMR (125 MHz, CD_3OD)



S1a **S1b**

S1a:**S1b** = 9.3:1

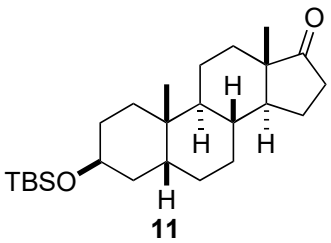
^{1}H NMR (500 MHz, $CDCl_3$)



S1a **S1b**

S1a:**S1b** = 9.3:1

^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$)



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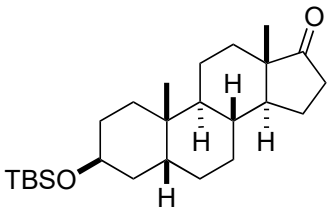
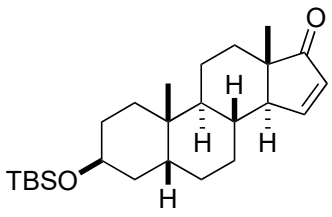
^{1}H NMR (400 MHz, C_6D_6)



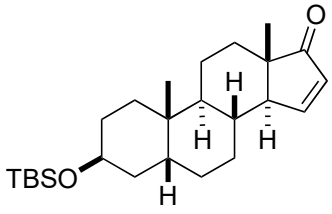
11

^{13}C{^{1}H} NMR (125 MHz, C_6D_6)



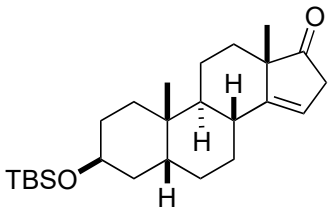
12

^{1}H NMR (400 MHz, $CDCl_3$)



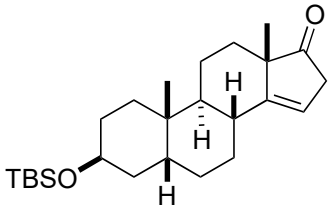
12

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$)



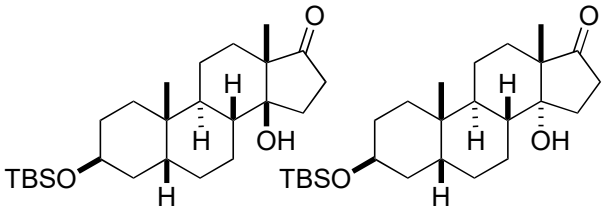
13

^{1}H NMR (400 MHz, $CDCl_3$)



13

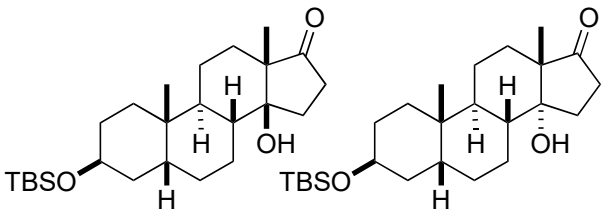
^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$)



14a **14b**

14a:**14b** = 2.3:1

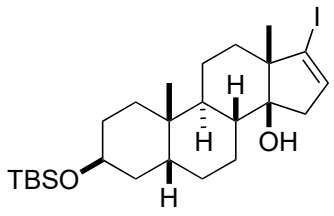
^{1}H NMR (400 MHz, $CDCl_3$)



14a **14b**

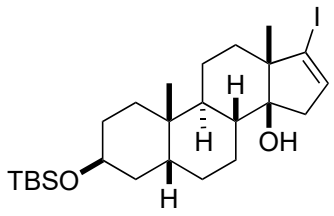
14a:**14b** = 2.3:1

$^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$)



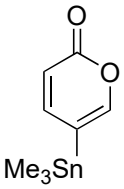
8

^{1}H NMR (500 MHz, $CDCl_3$)



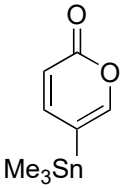
8

^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$)



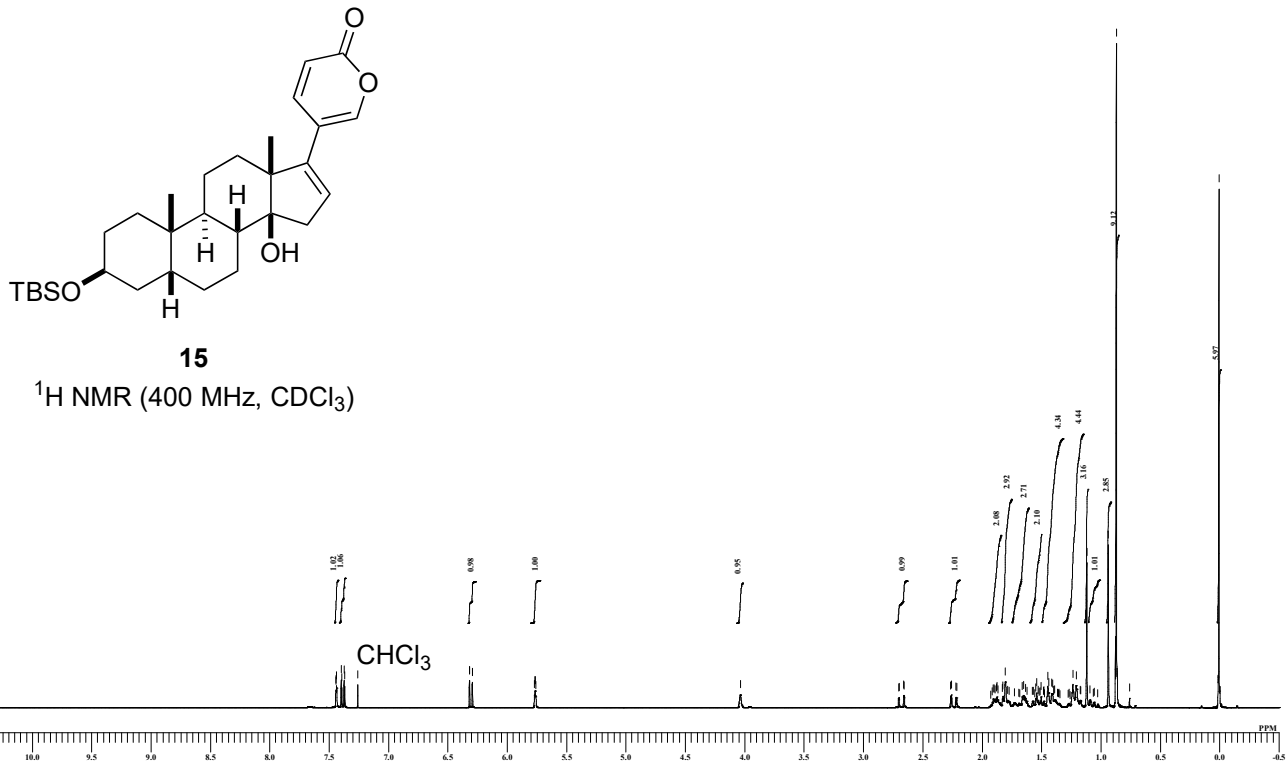
7

^{1}H NMR (400 MHz, C_6D_6)



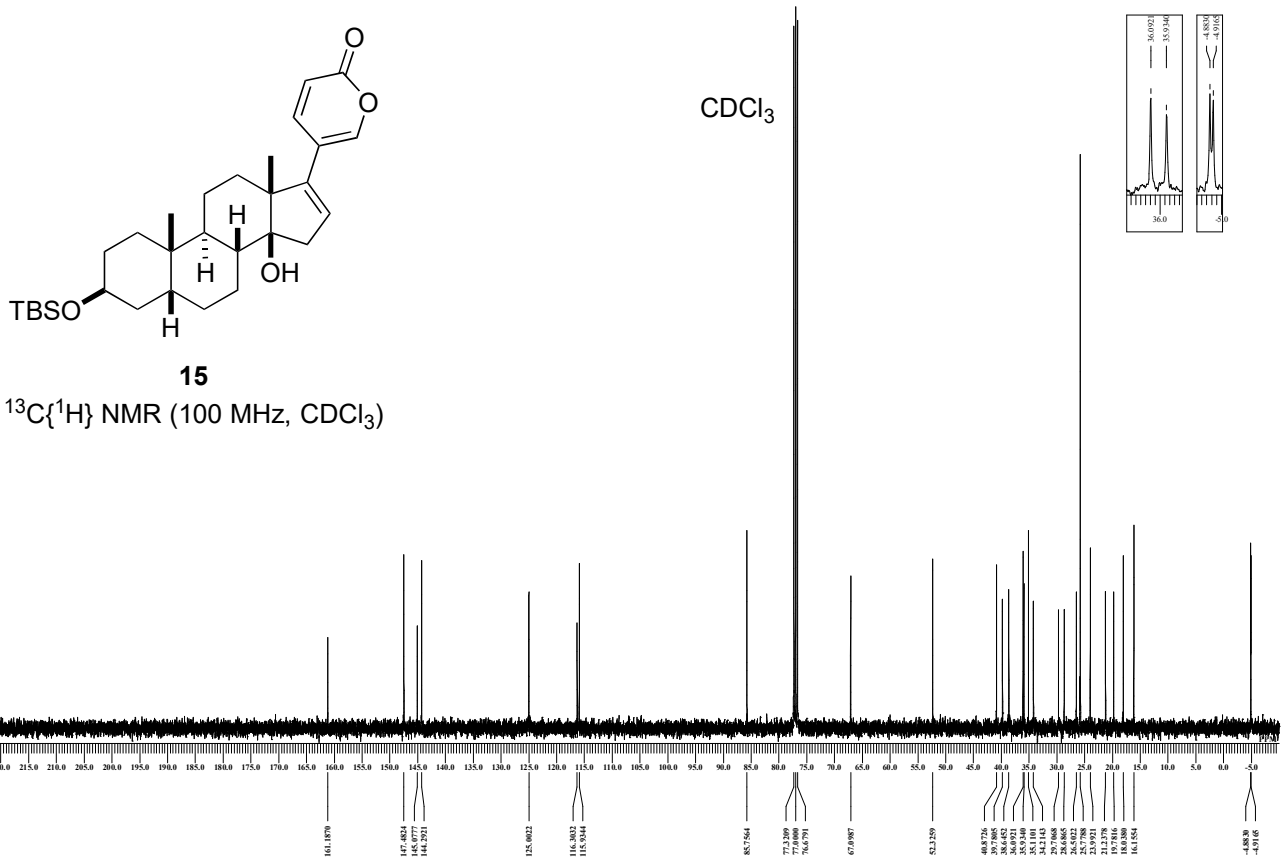
7

$^{13}C\{^{1}H\}$ NMR (100 MHz, C_6D_6)



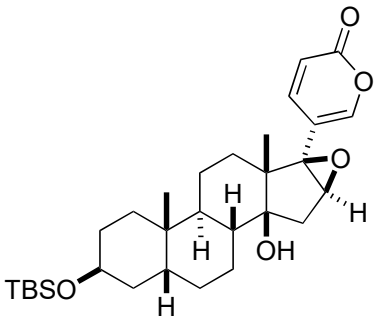
15

^{1}H NMR (400 MHz, $CDCl_3$)



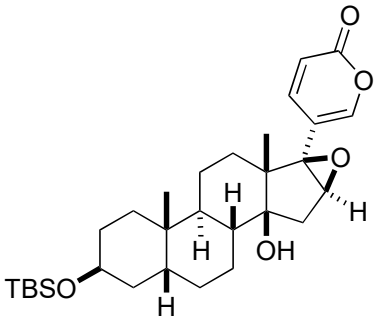
15

$^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$)



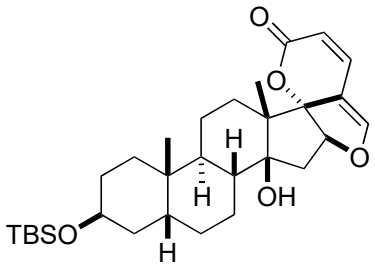
6

^{1}H NMR (400 MHz, C_6D_6)



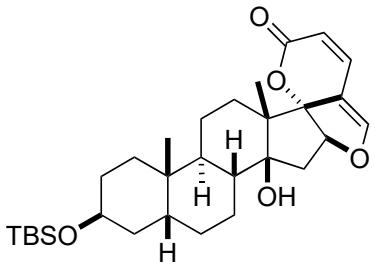
6

^{13}C{^{1}H} NMR (100 MHz, C_6D_6)



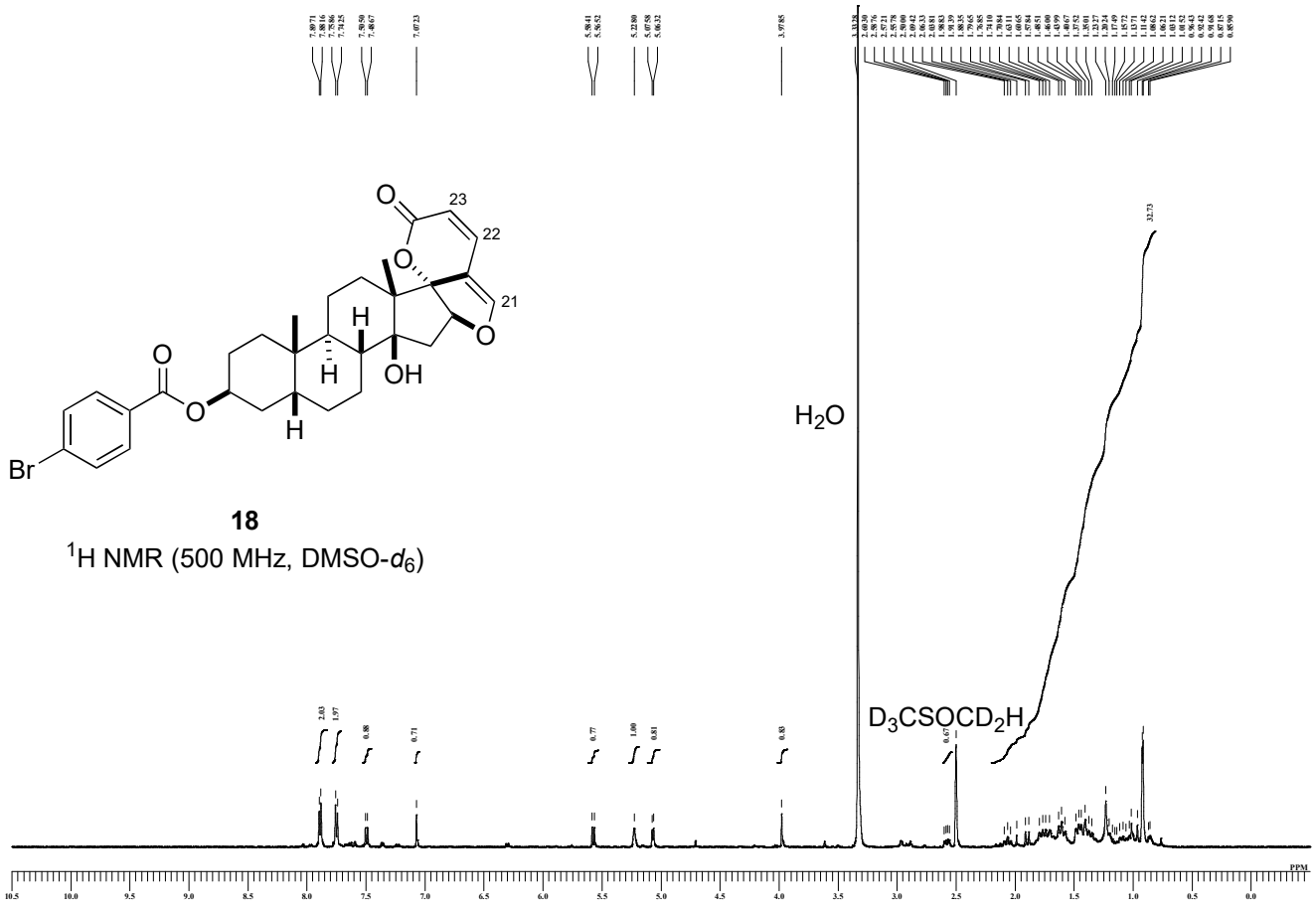
16

^{1}H NMR (500 MHz, $CDCl_3$)



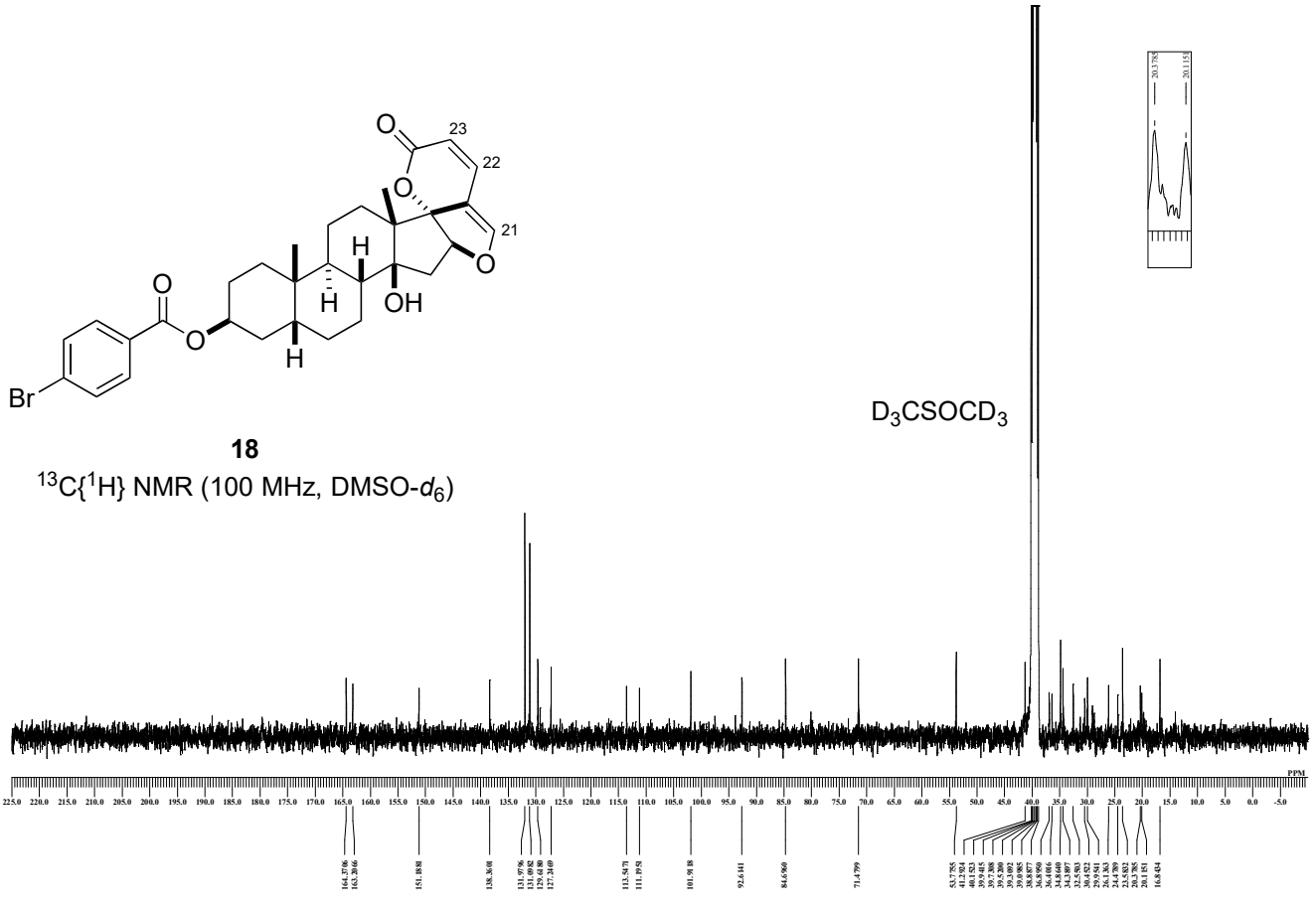
16

^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$)



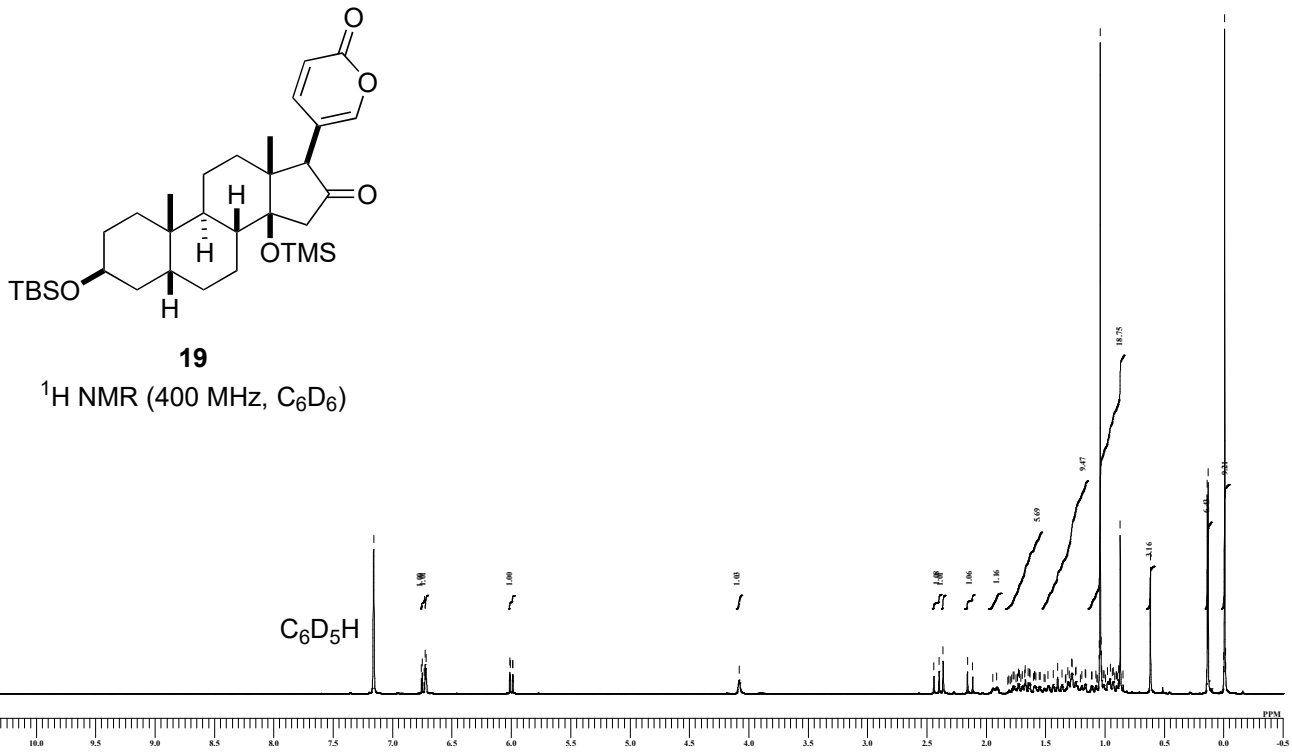
18

^{1}H NMR (500 MHz, DMSO-d_6)



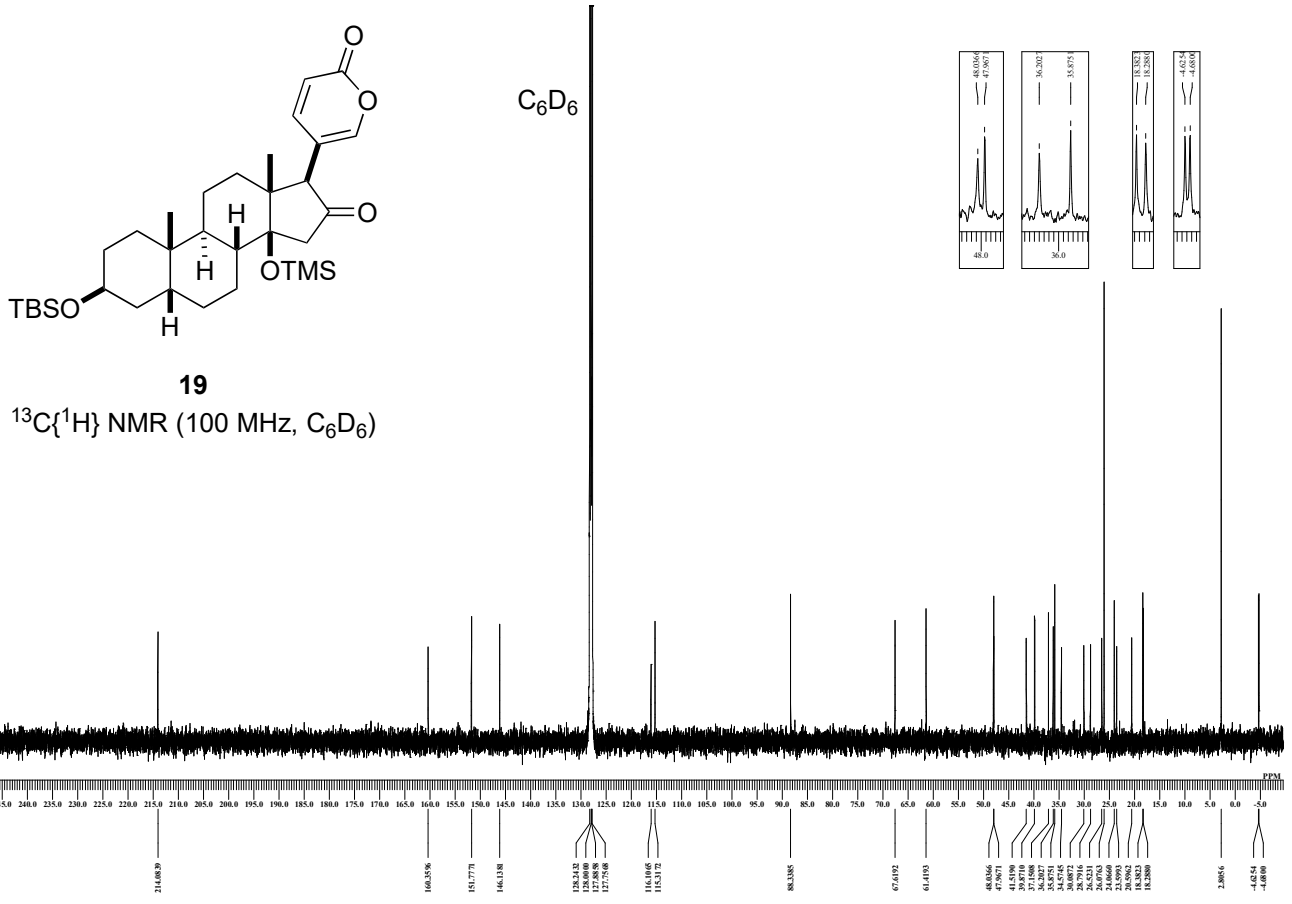
18

^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6)



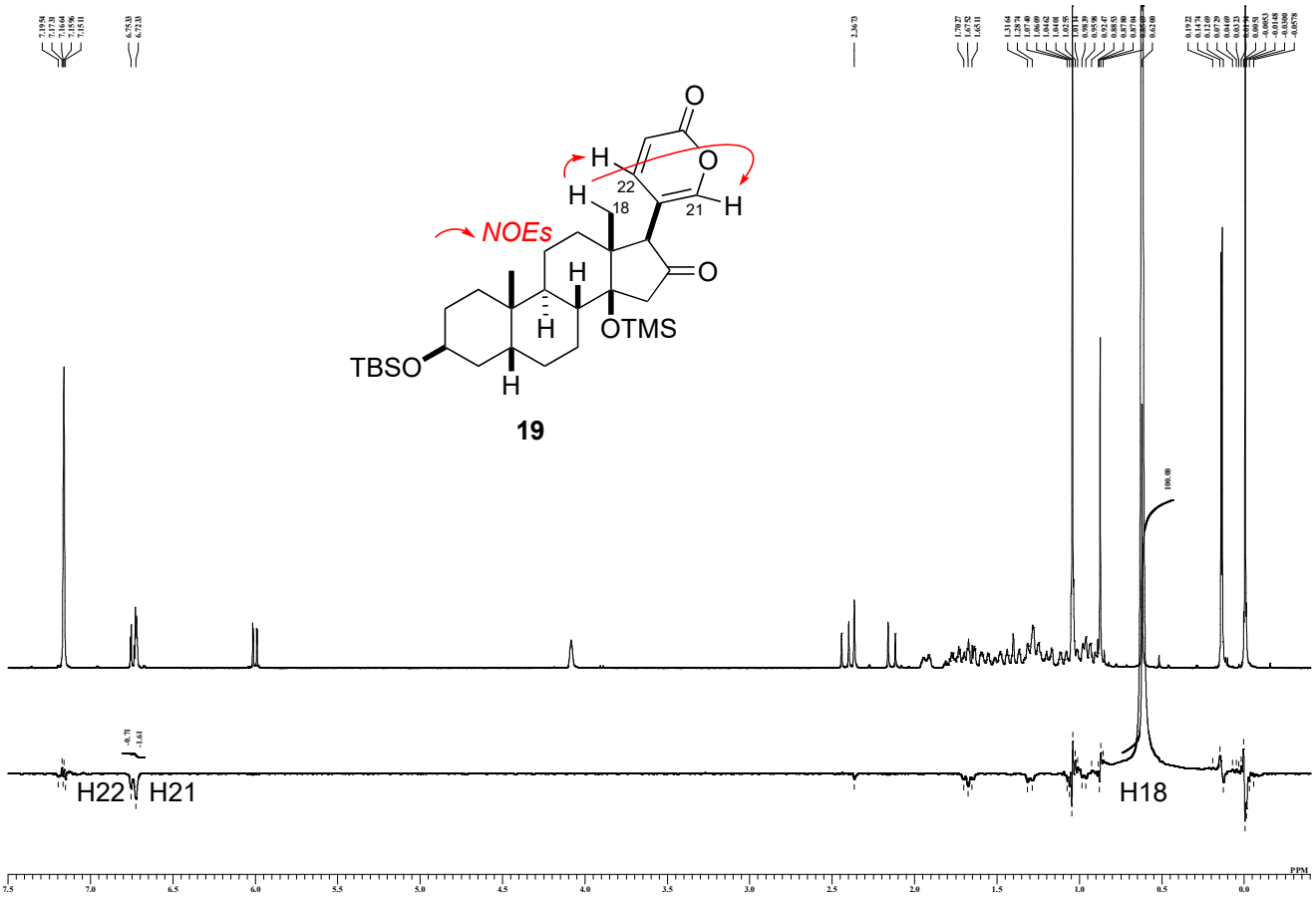
19

^{1}H NMR (400 MHz, C_6D_6)

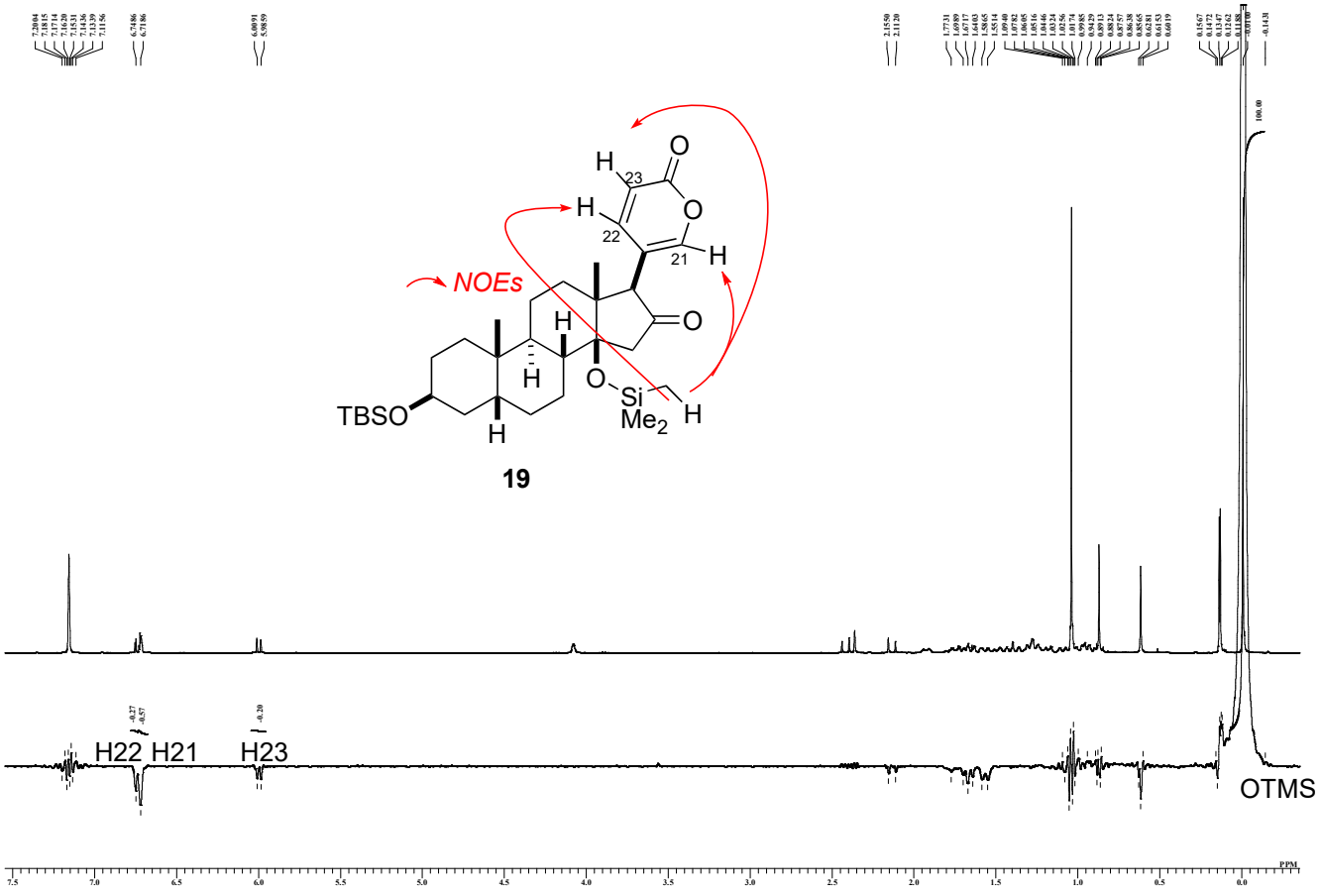


19

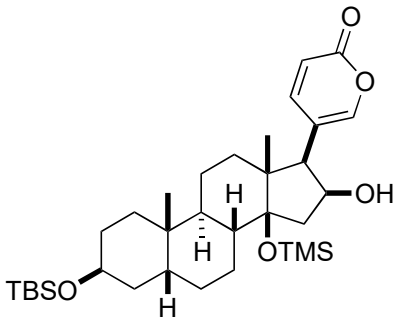
$^{13}C\{^{1}H\}$ NMR (100 MHz, C_6D_6)


NOEs
TBSO
OTMS
H22
H21
H18
PPM

19

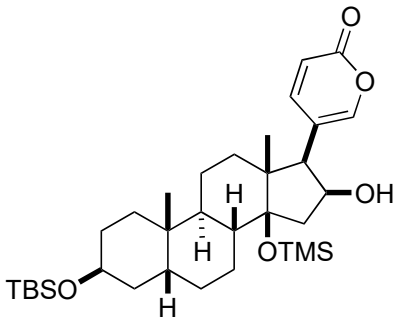

NOEs
TBSO
Si
Me2
H22
H21
H23
OTMS
PPM

19



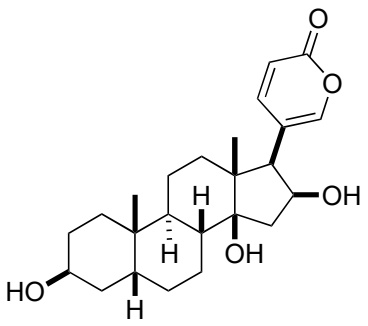
24

^{1}H NMR (400 MHz, $CDCl_3$)



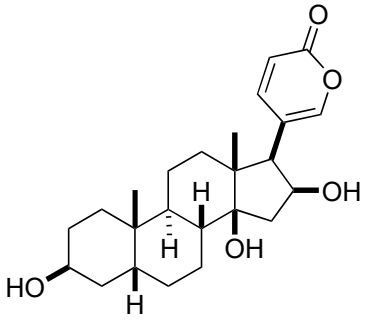
24

^{13}C{^{1}H} NMR (100 MHz, C_6D_6)



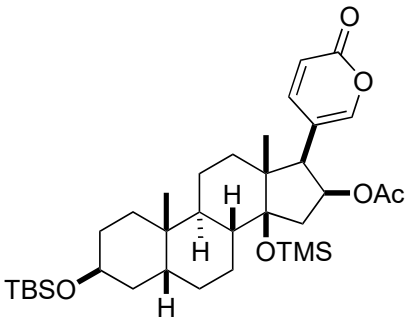
bufogenin B (**2**)

^{1}H NMR (500 MHz, DMSO-d_6)



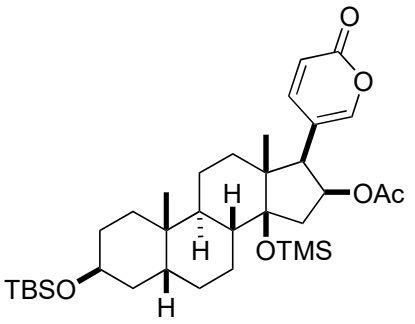
bufogenin B (**2**)

^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6)



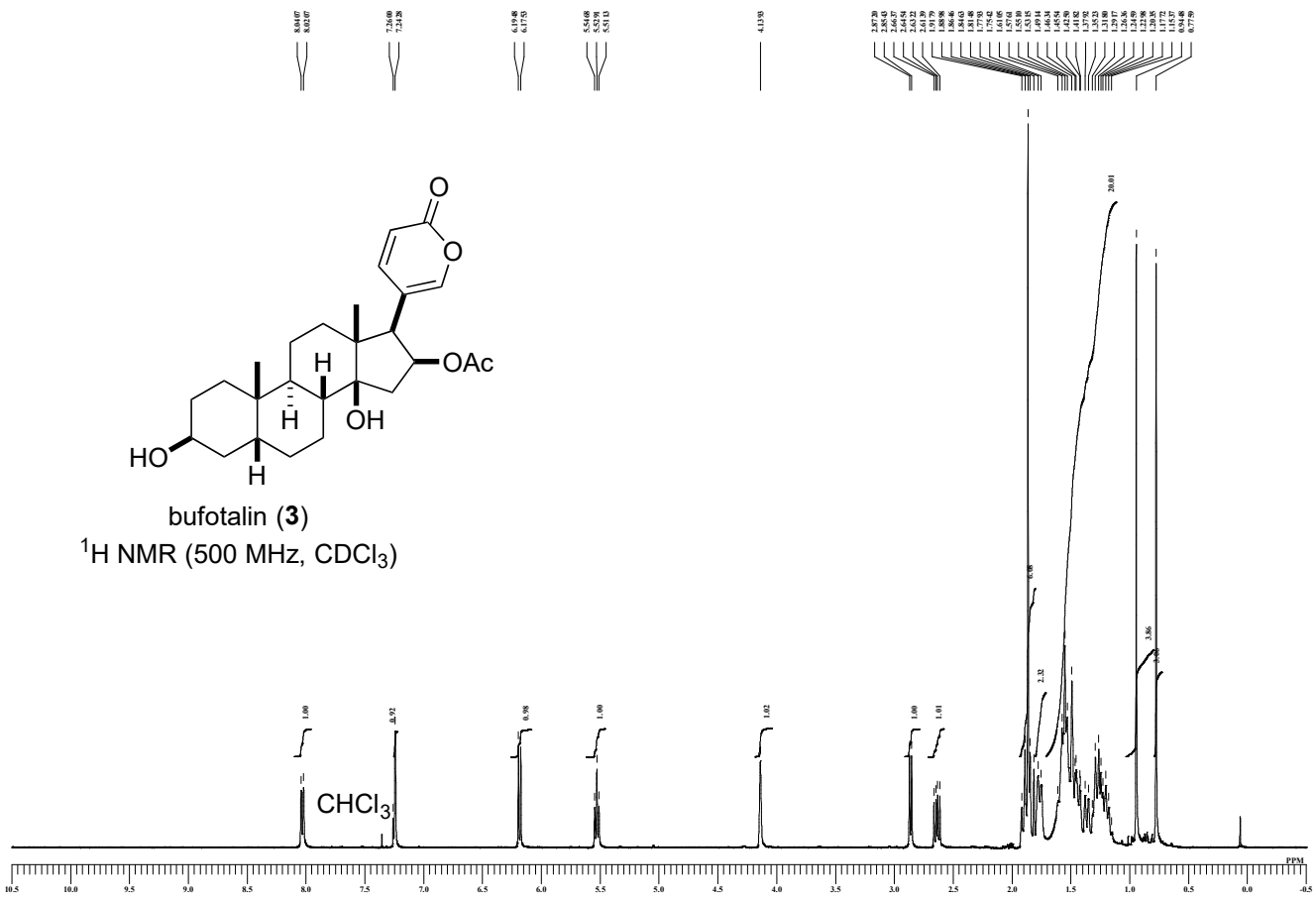
25

^{1}H NMR (400 MHz, $CDCl_3$)



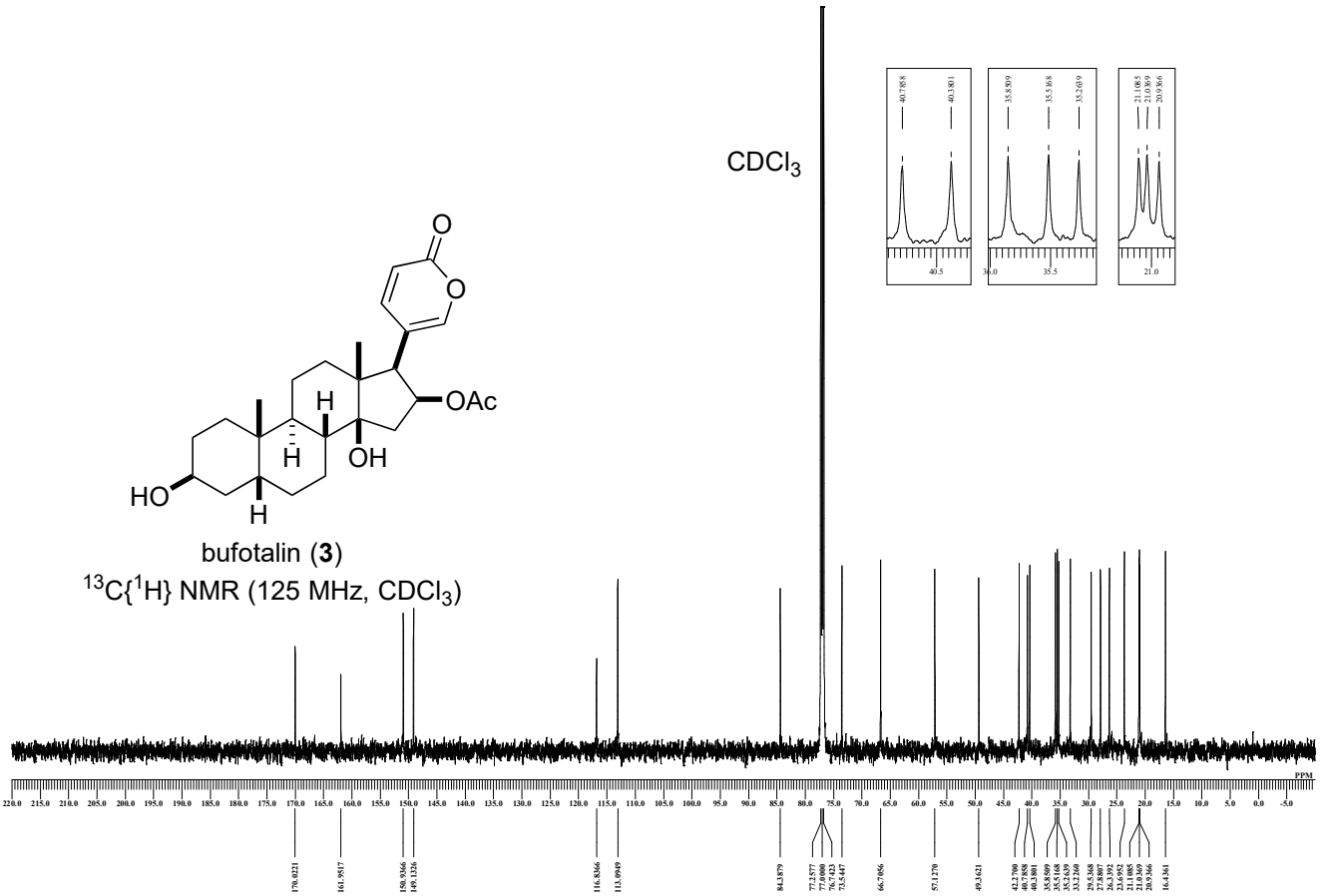
25

^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$)



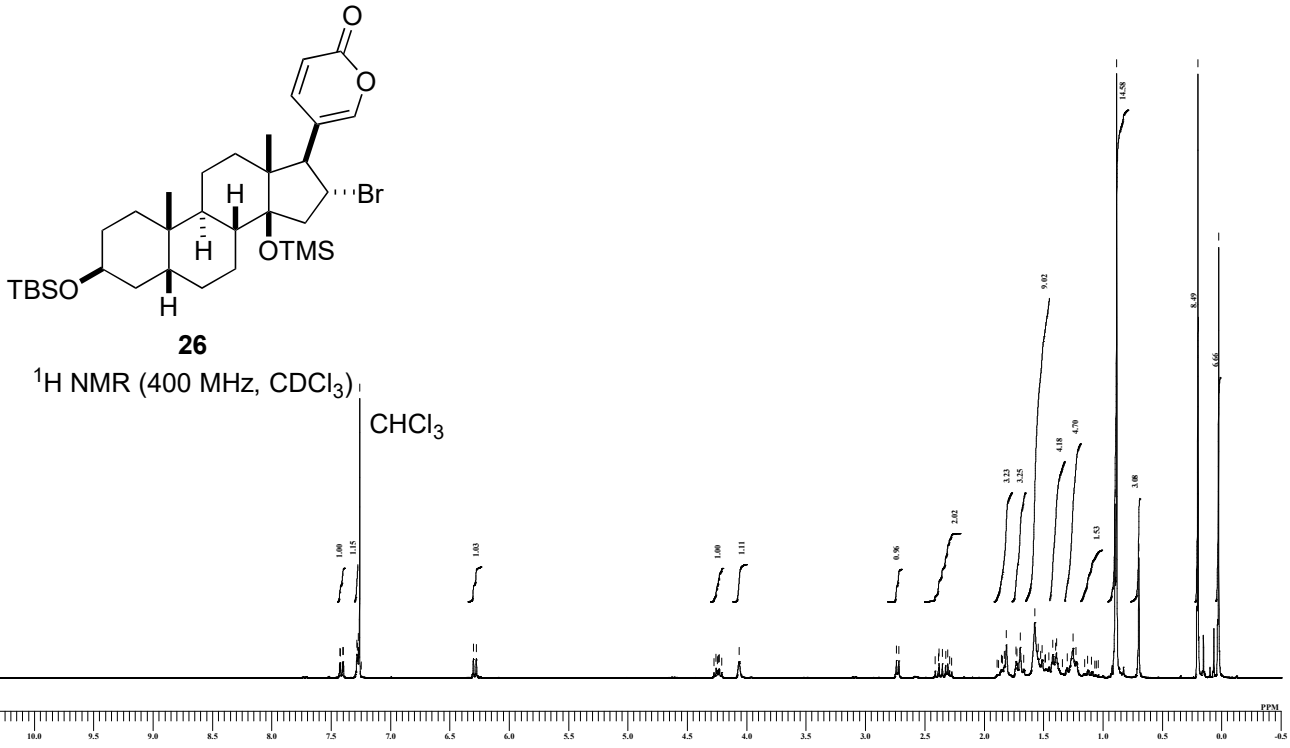
bufotalin (**3**)

^{1}H NMR (500 MHz, $CDCl_3$)



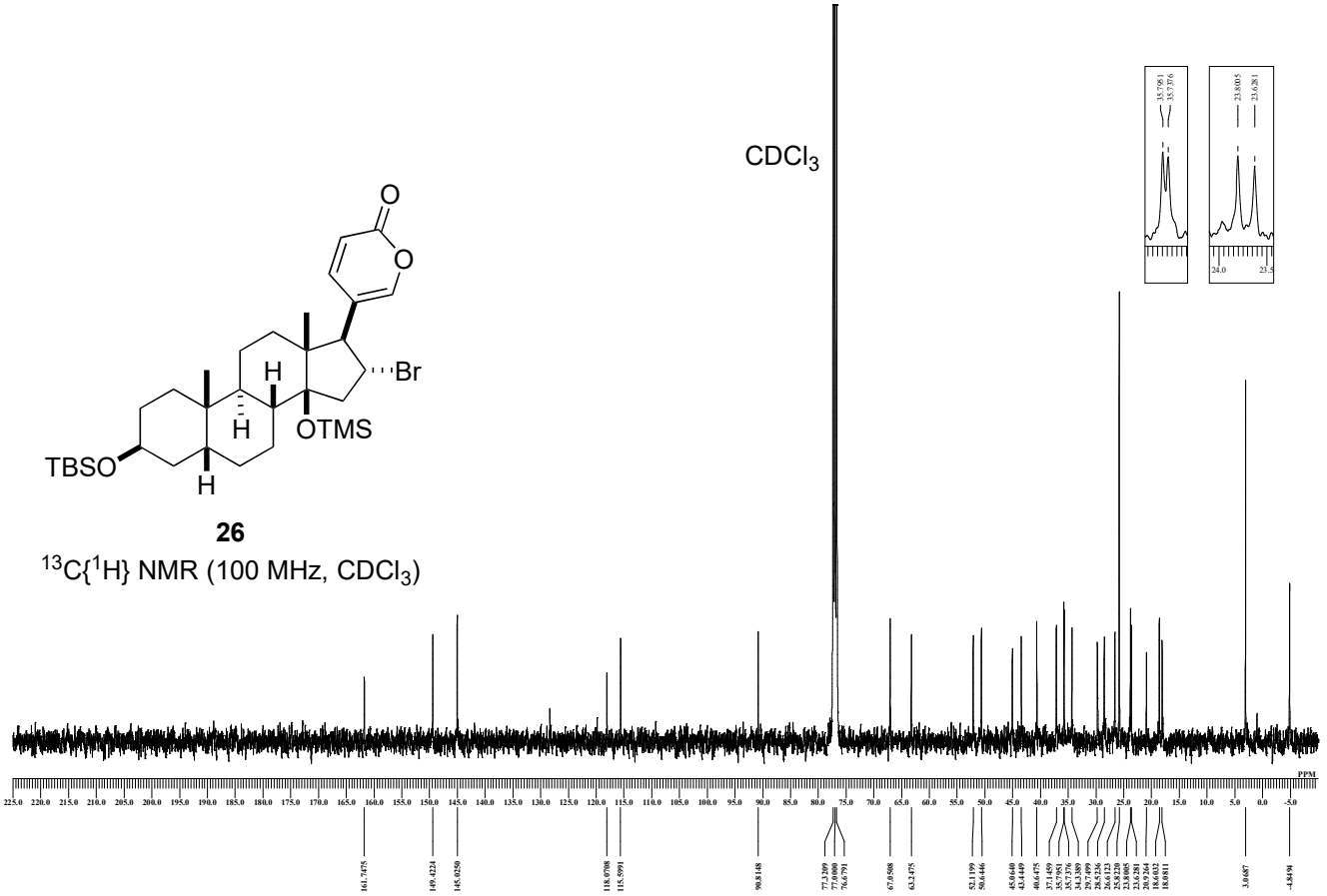
bufotalin (**3**)

^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$)



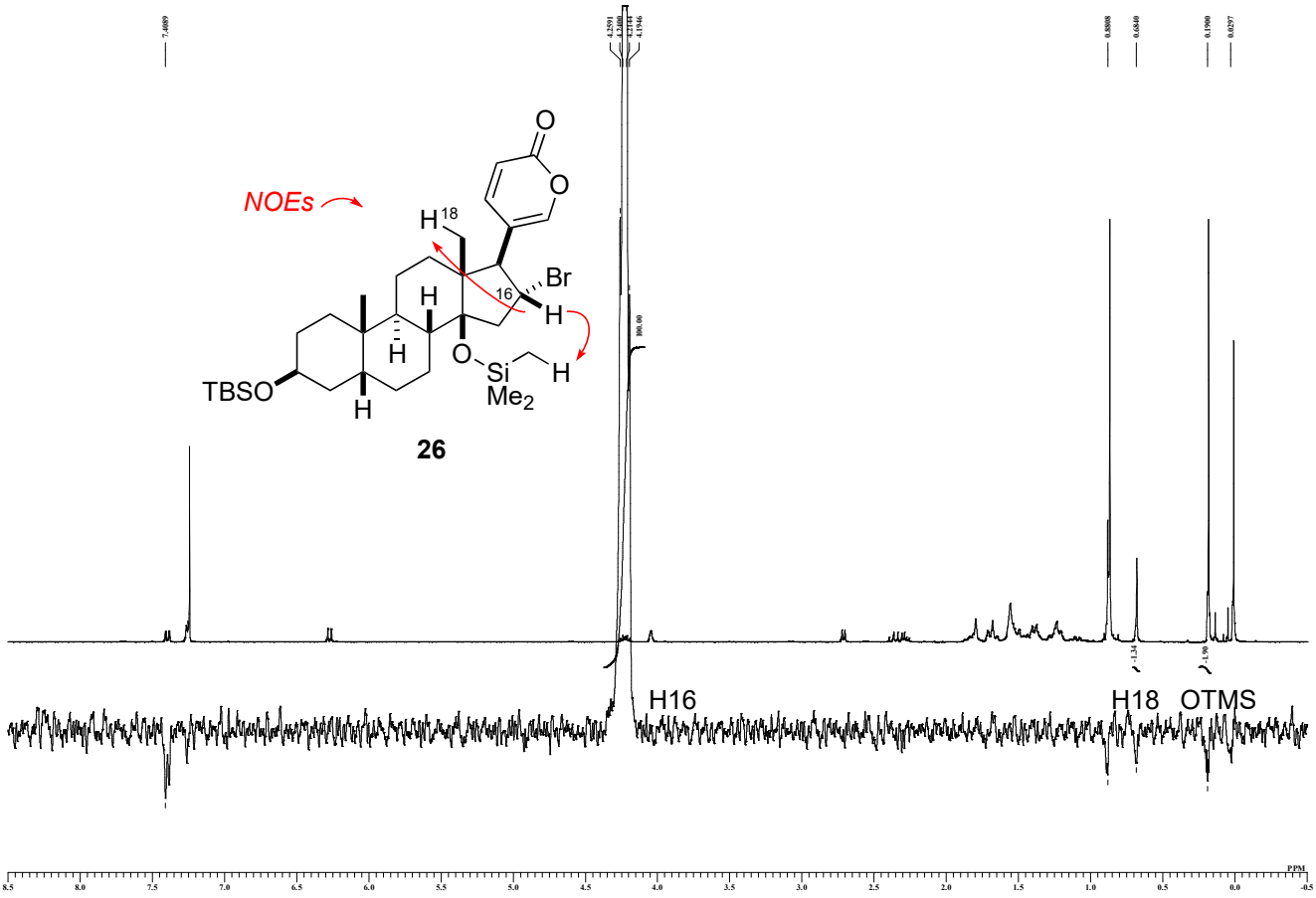
26

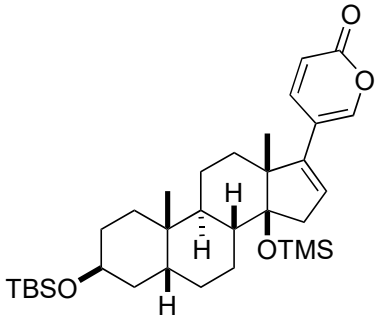
^{1}H NMR (400 MHz, $CDCl_3$)



26

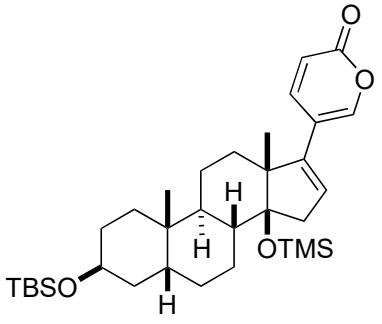
$^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$)


NOEs
H18
16
Br
H
H
H
O
Si
Me2
H
TBSO
H
O
O
26
H16
H18
OTMS



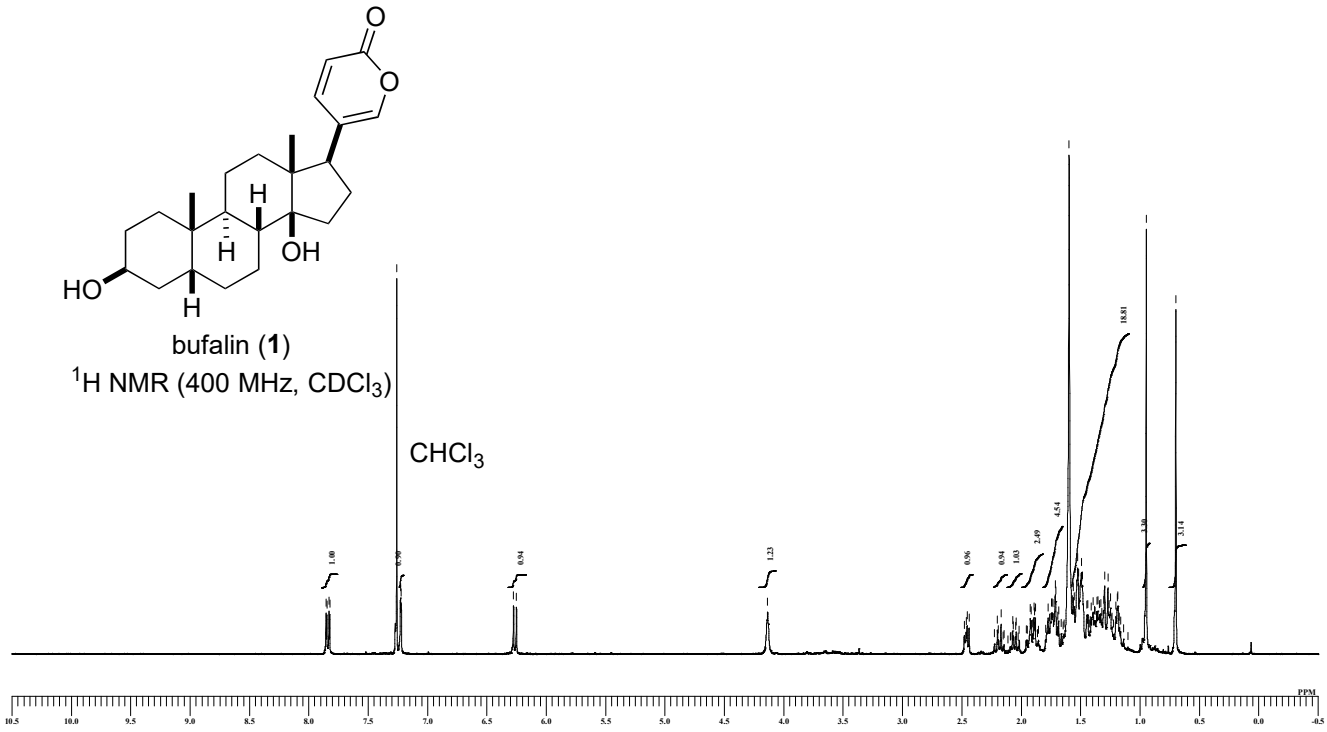
27

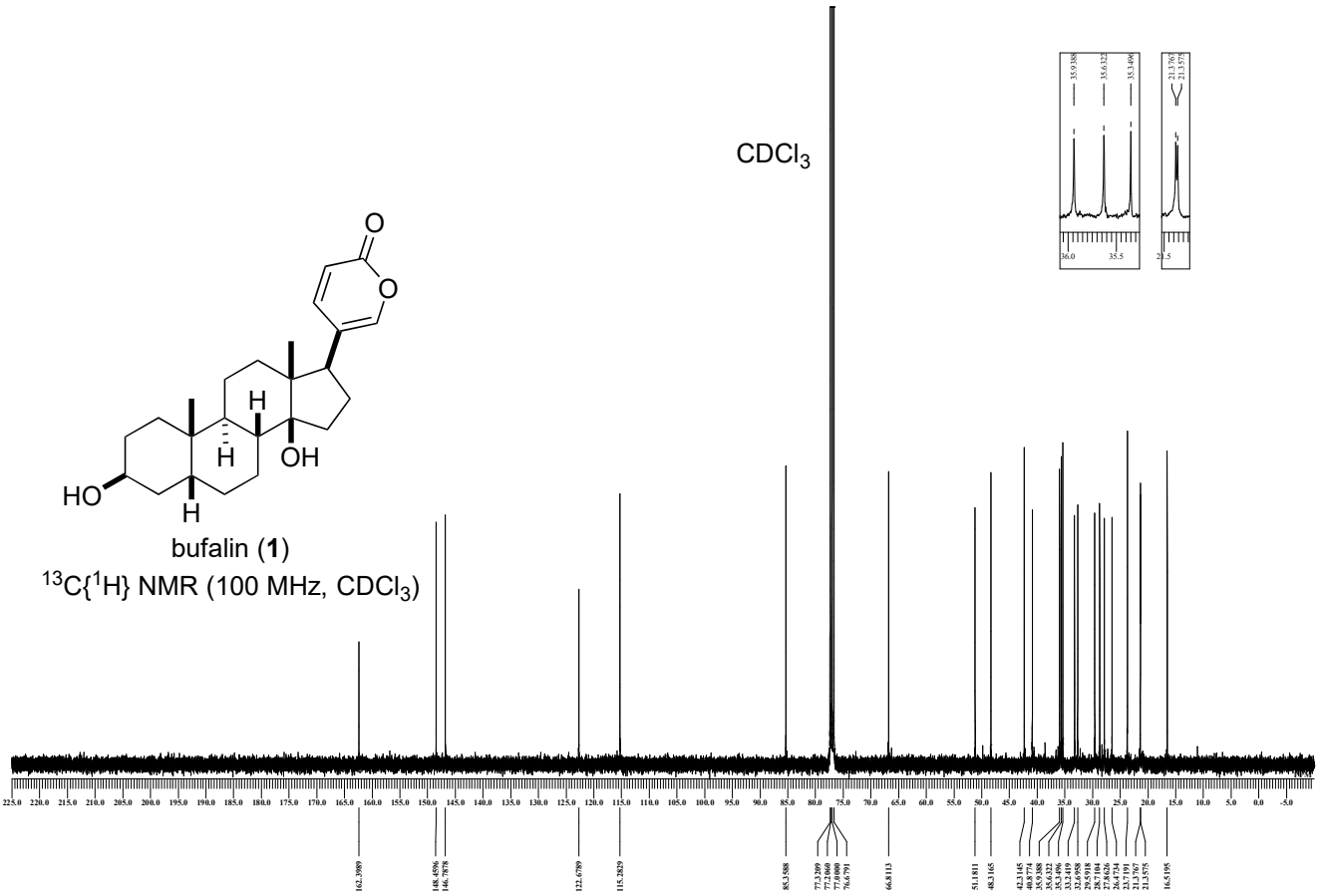
^{1}H NMR (400 MHz, C_6D_6)

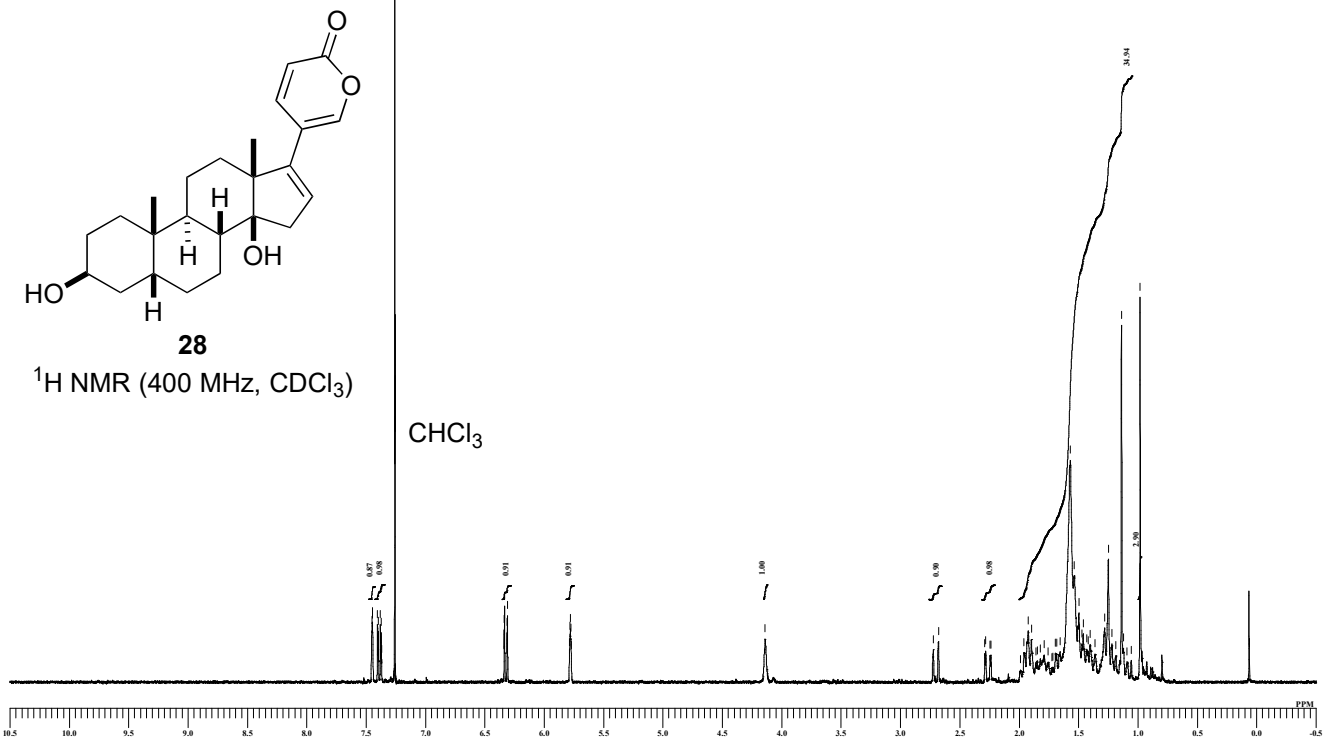


27

^{13}C{^{1}H} NMR (100 MHz, C_6D_6)

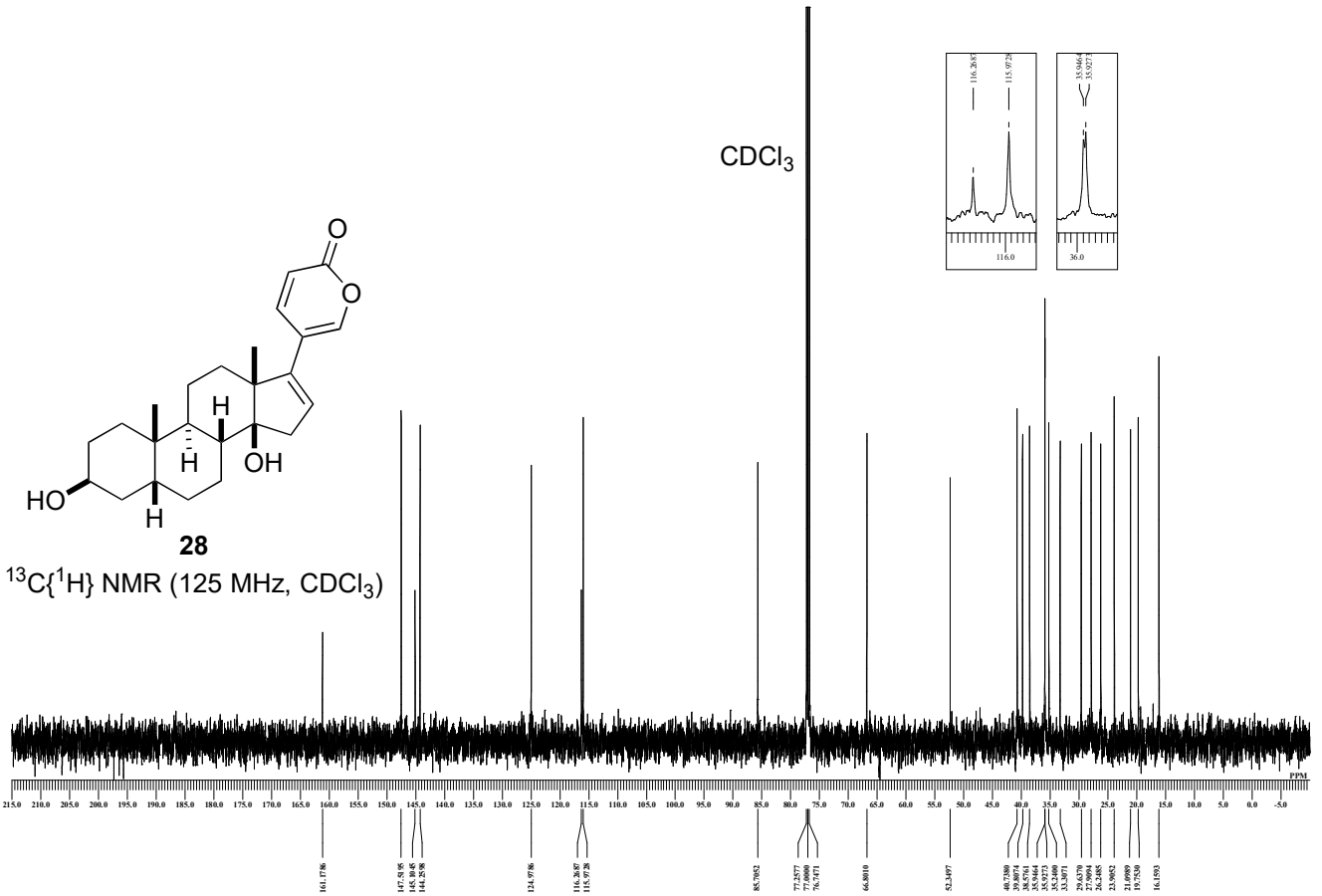

O
O
H
H
OH
HO
H
bufalin (1)
1H NMR (400 MHz, CDCl3)
CHCl3
PPM


O
O
H
H
OH
HO
H
bufalin (1)
13C{1H} NMR (100 MHz, CDCl3)
CDCl3



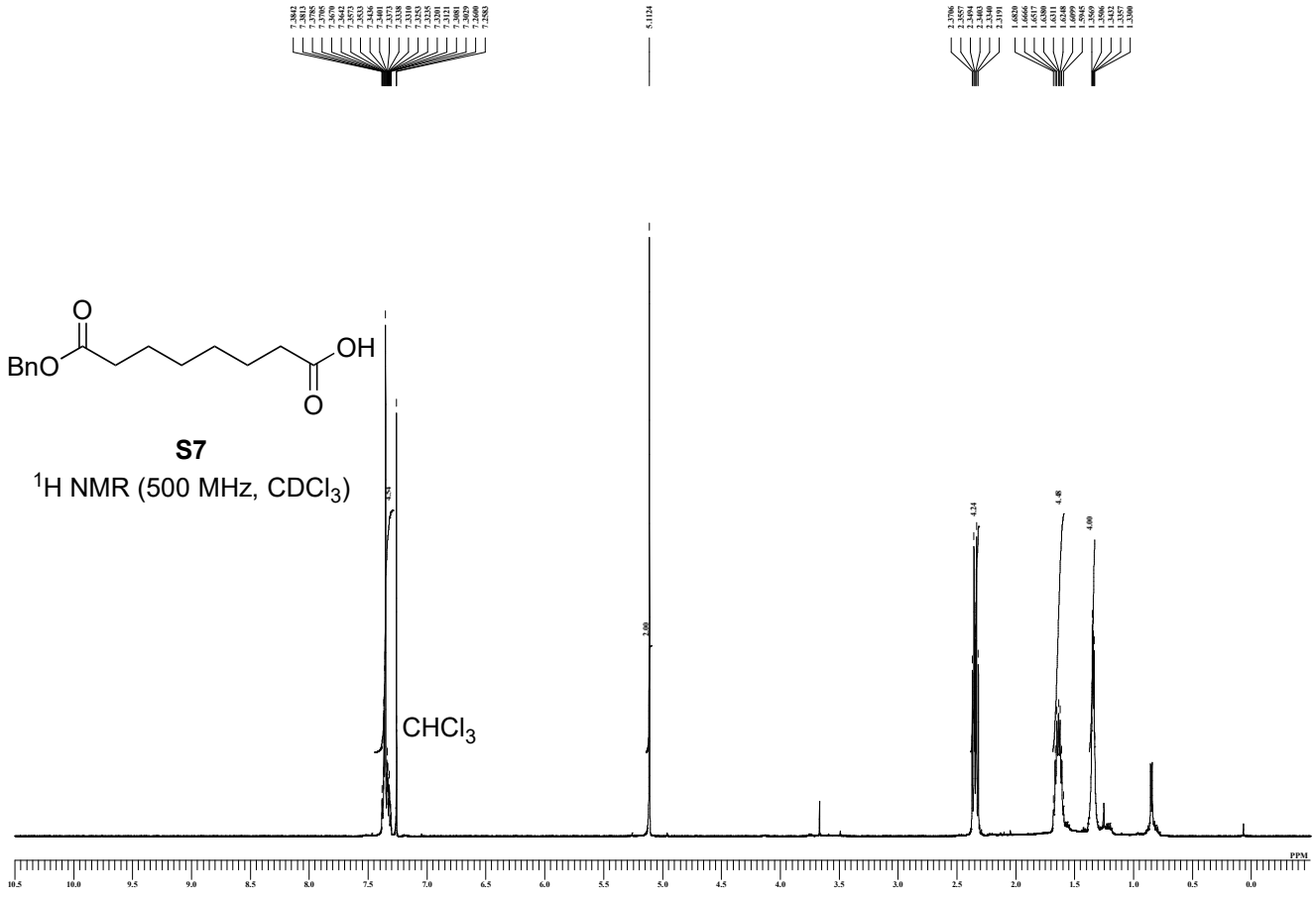
28

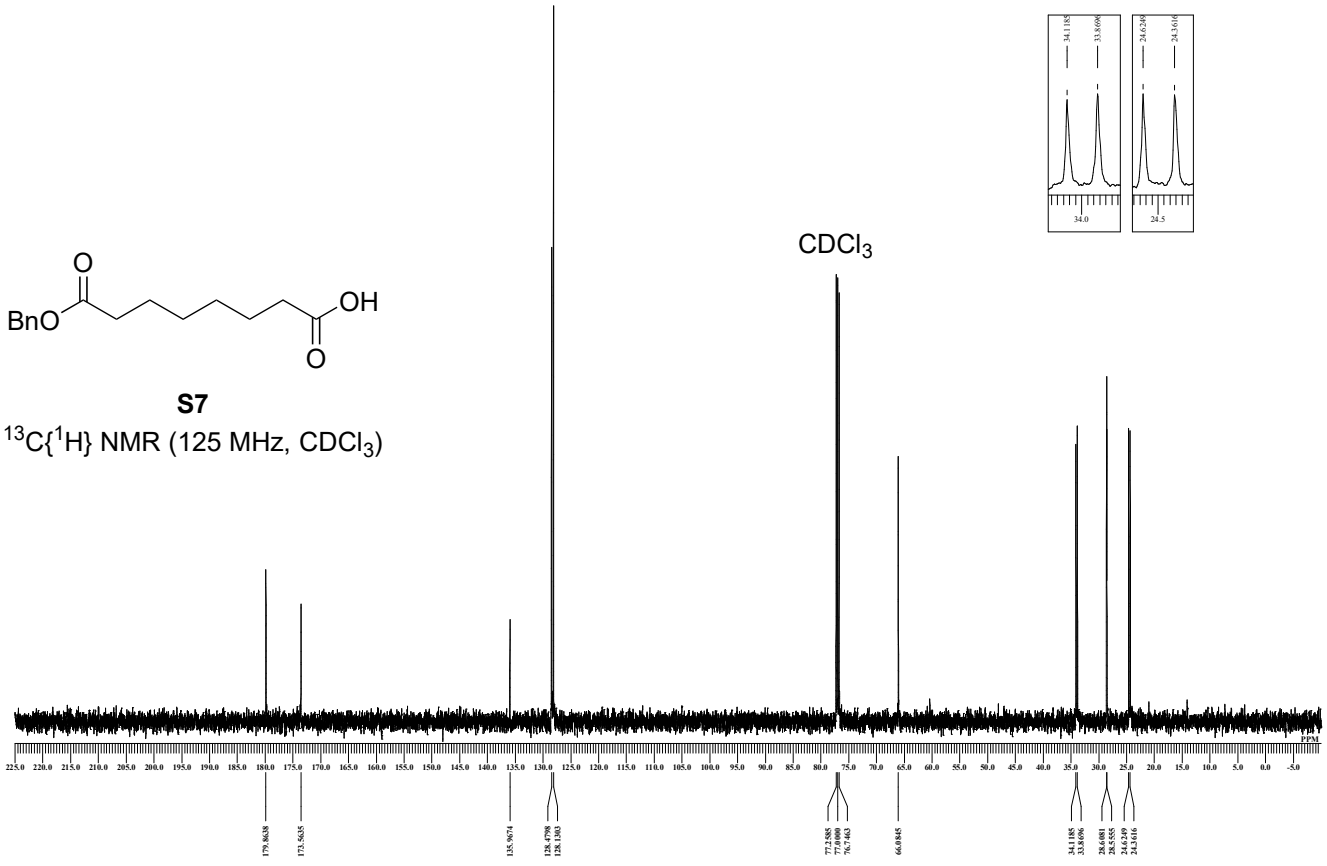
^{1}H NMR (400 MHz, $CDCl_3$)

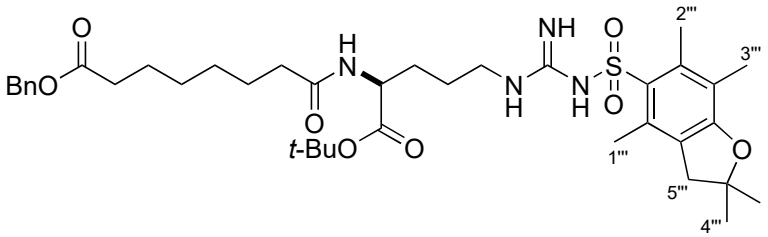


28

$^{13}C\{^{1}H\}$ NMR (125 MHz, $CDCl_3$)

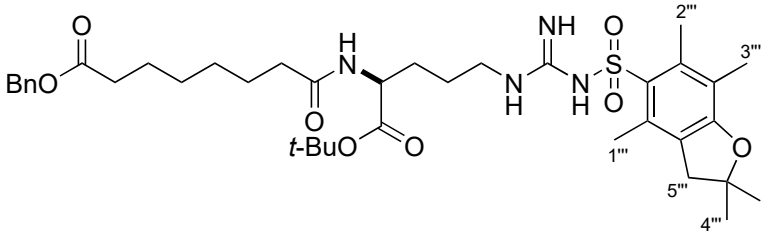

BnO
OH
O
O
S7
1H NMR (500 MHz, CDCl3)
CHCl3
PPM


BnO
OH
O
O
S7
13C{1H} NMR (125 MHz, CDCl3)
CDCl3
PPM



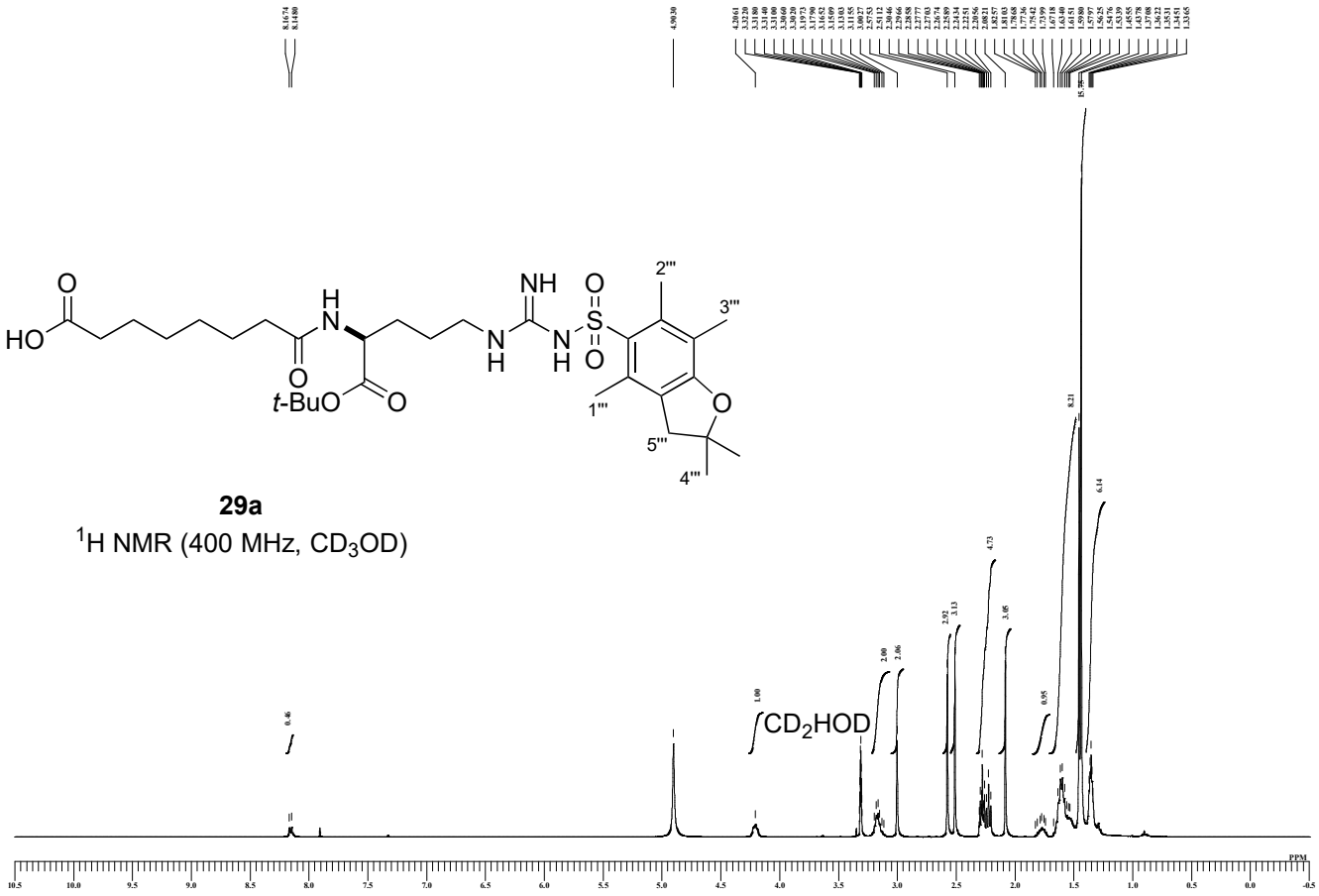
S9

^{1}H NMR (500 MHz, $CDCl_3$)



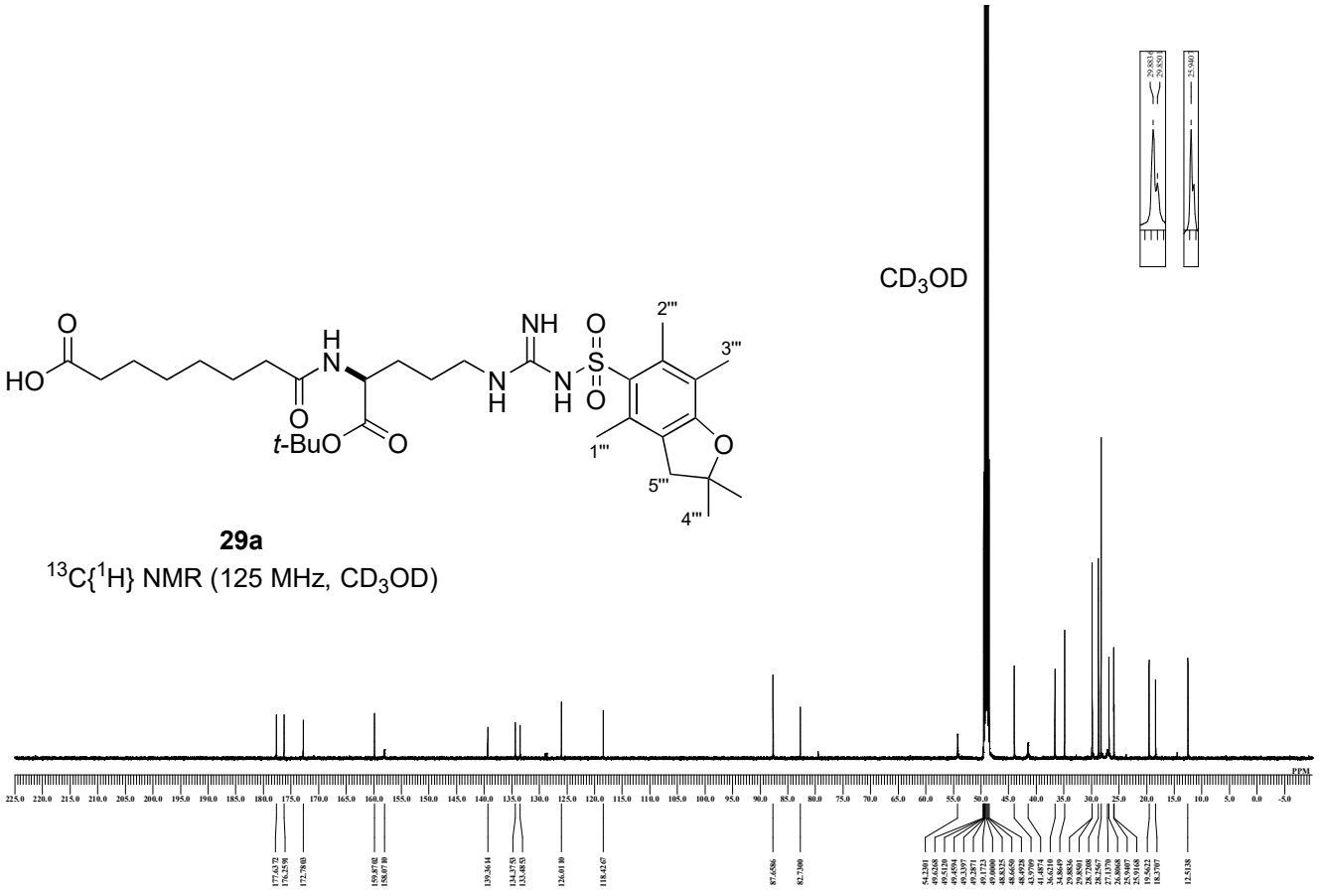
S9

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$)



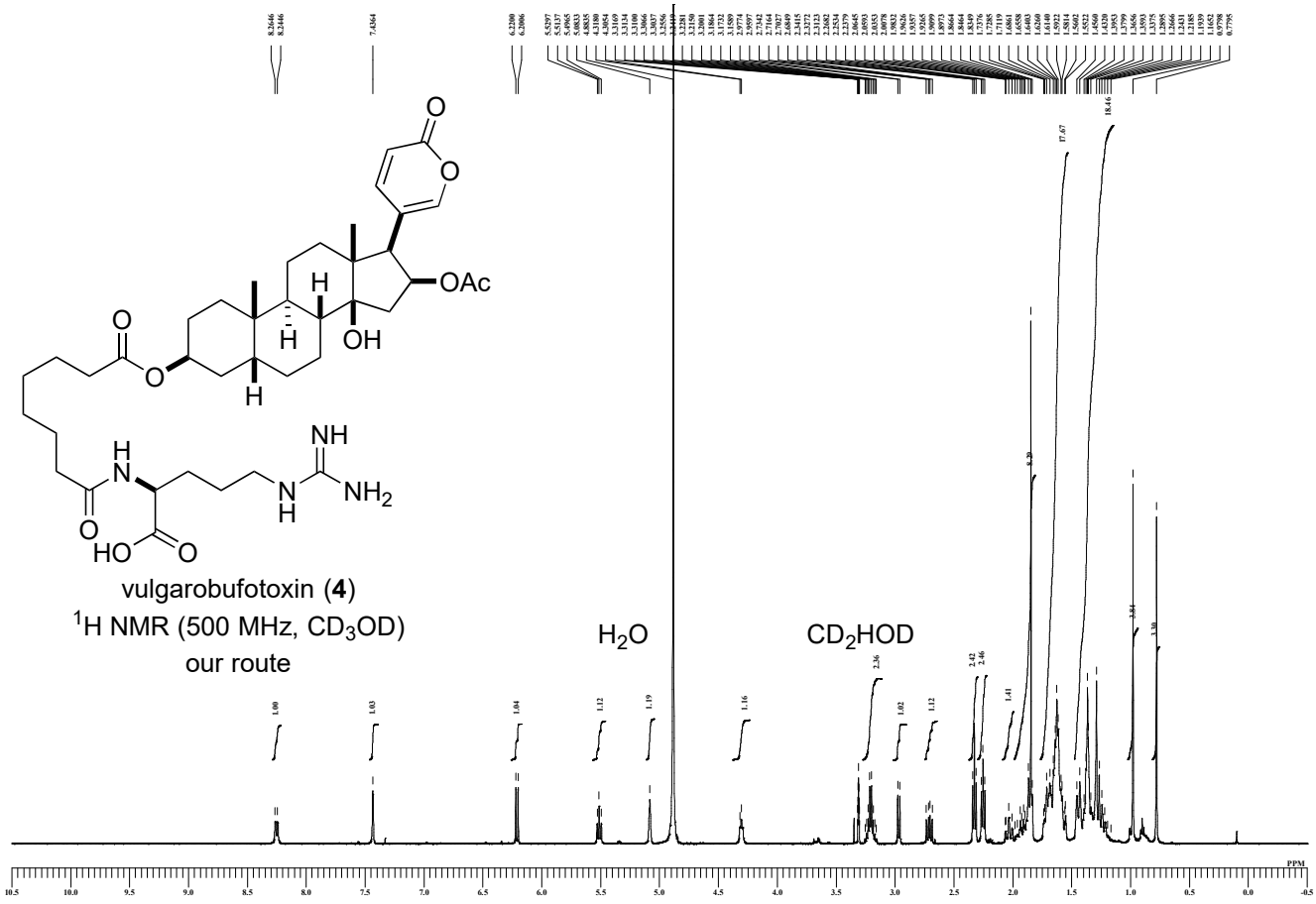
29a

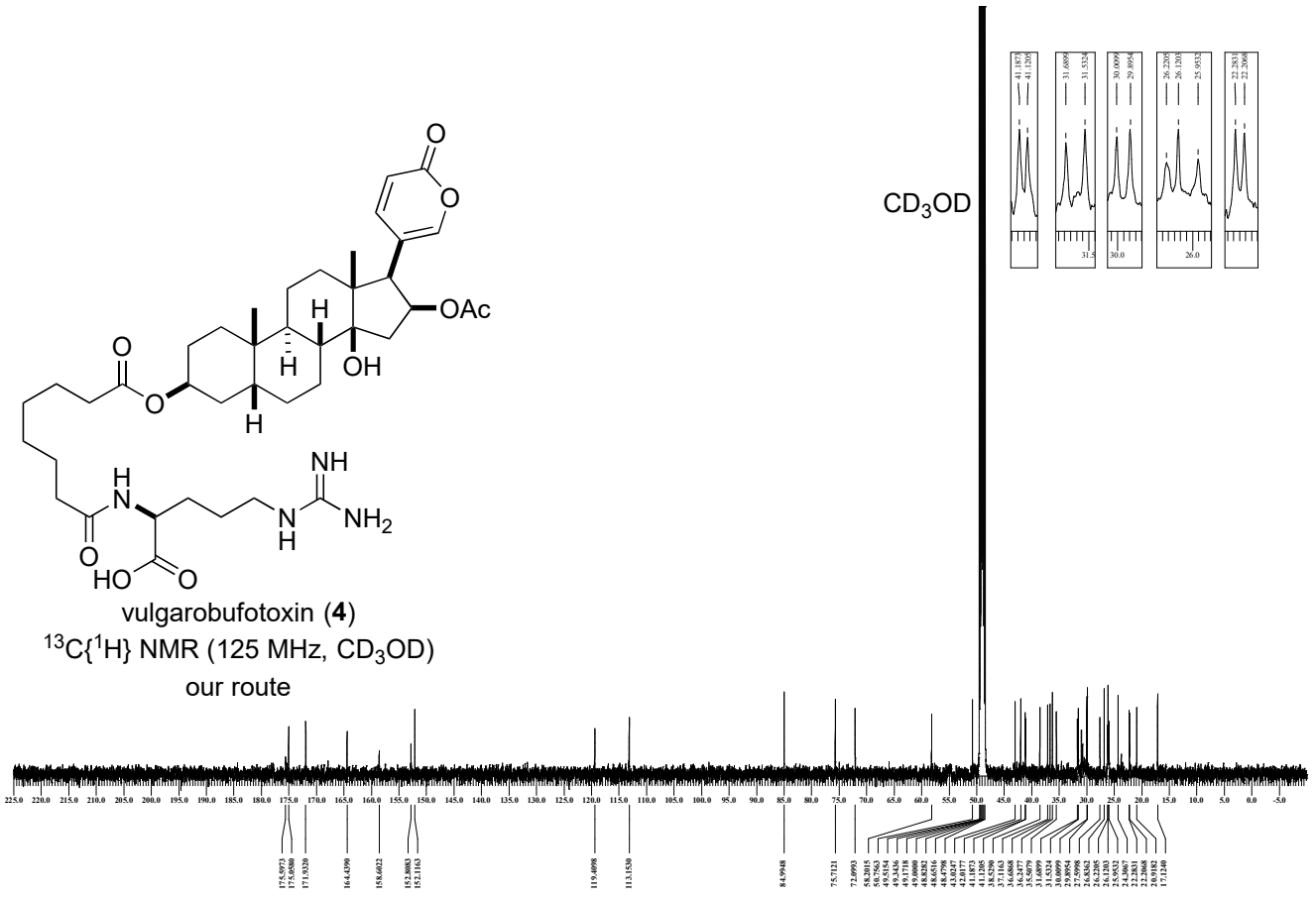
^{1}H NMR (400 MHz, CD_3OD)

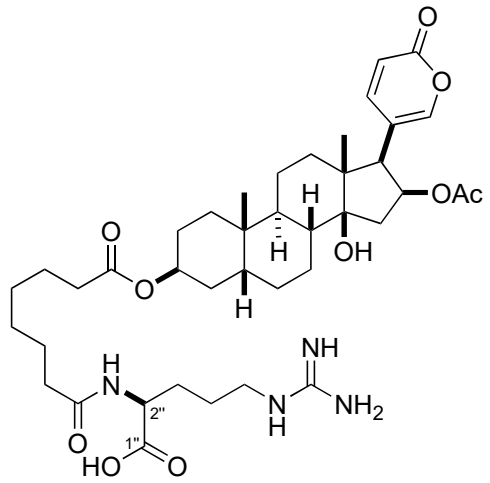


29a

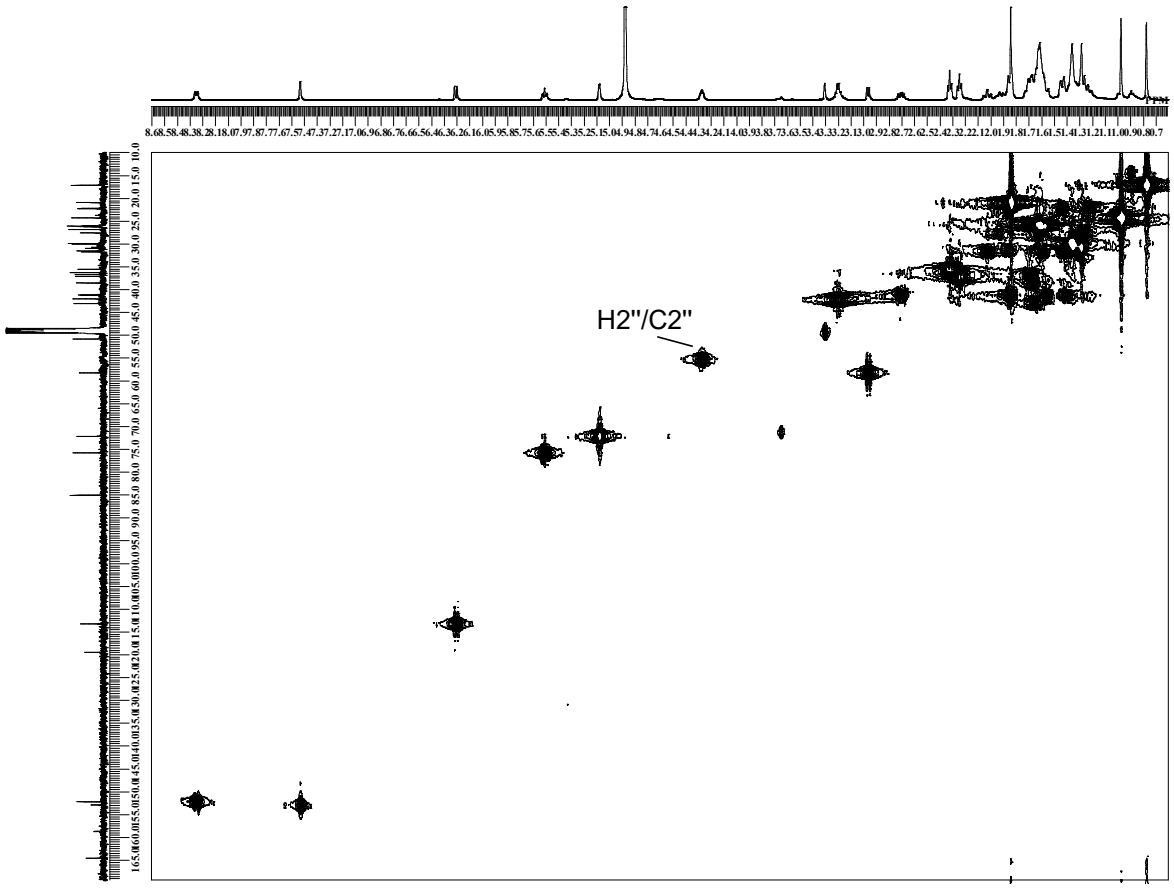
$^{13}C\{^{1}H\}$ NMR (125 MHz, CD_3OD)


vulgarobufotoxin (4)
1H NMR (500 MHz, CD3OD)
our route
H2O
CD2HOD
PPM

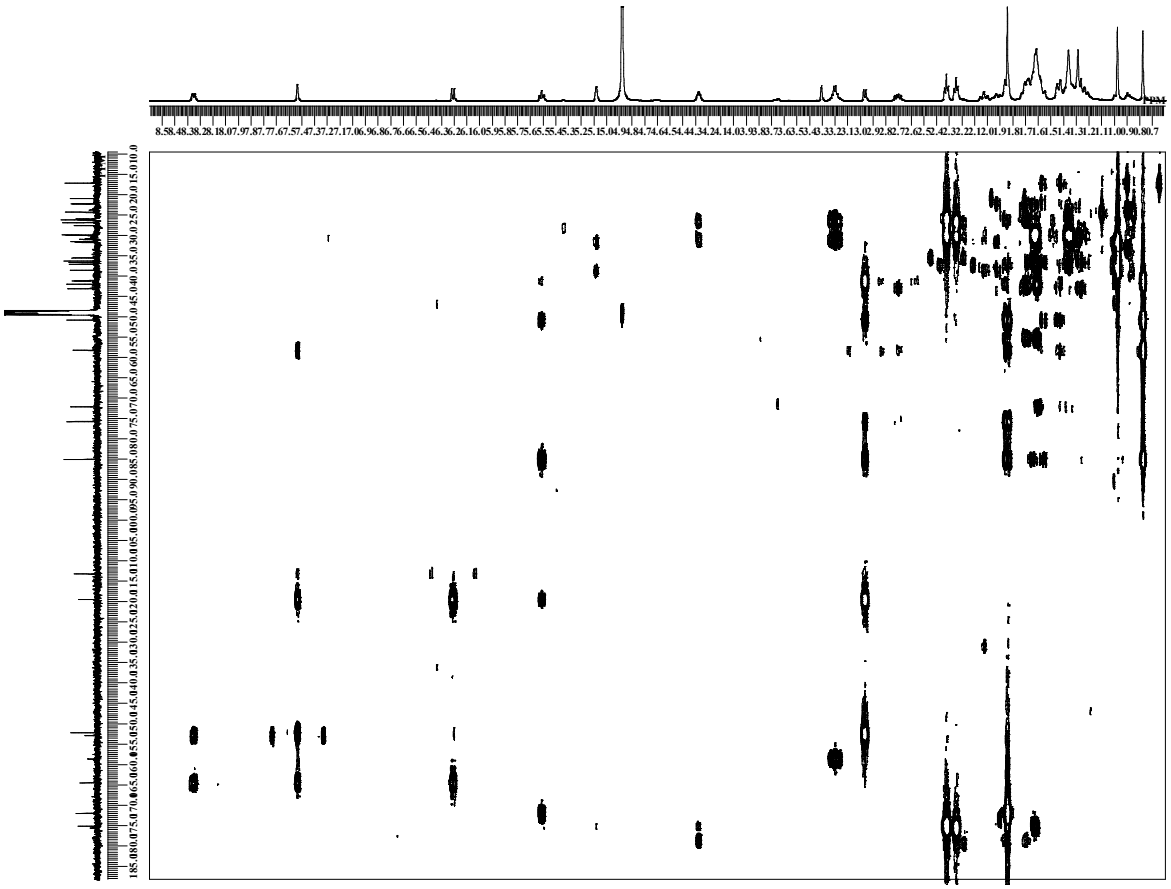

vulgarobufotoxin (4)
13C{1H} NMR (125 MHz, CD3OD)
our route
CD3OD

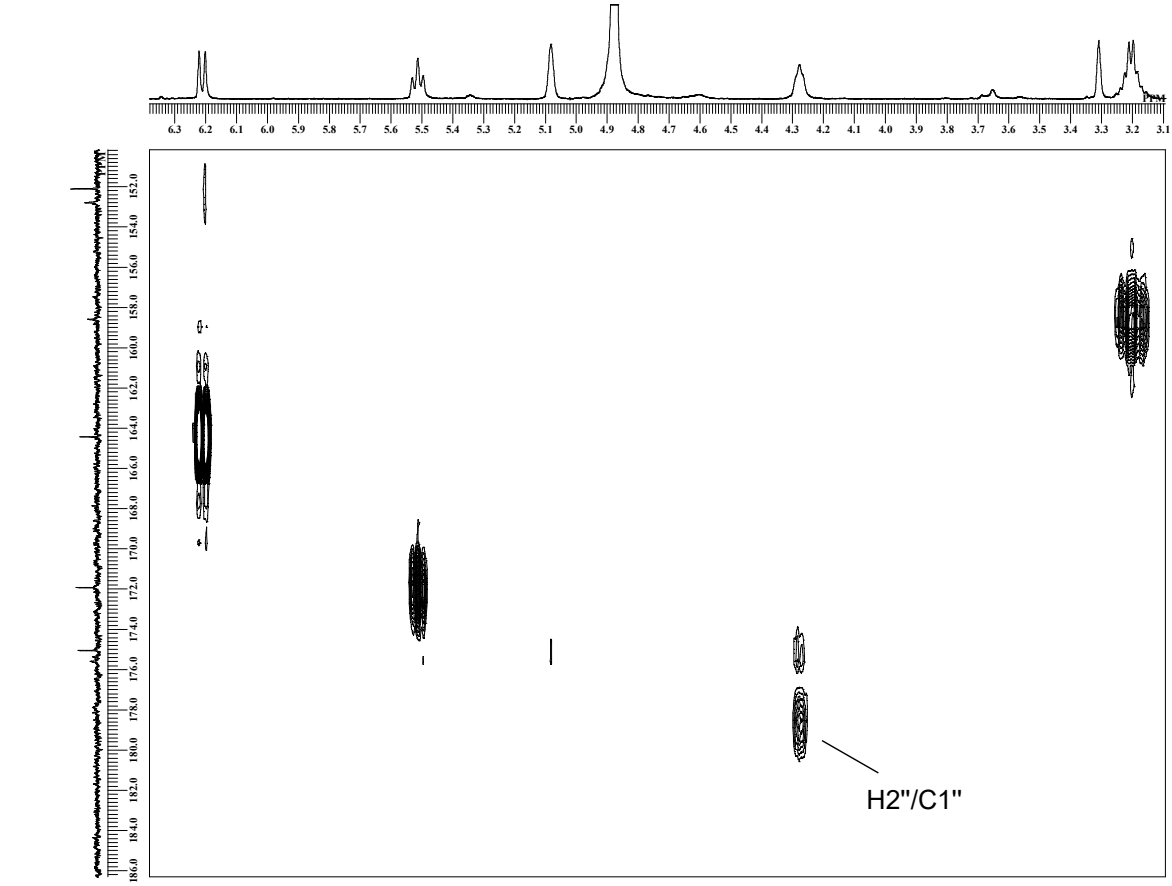


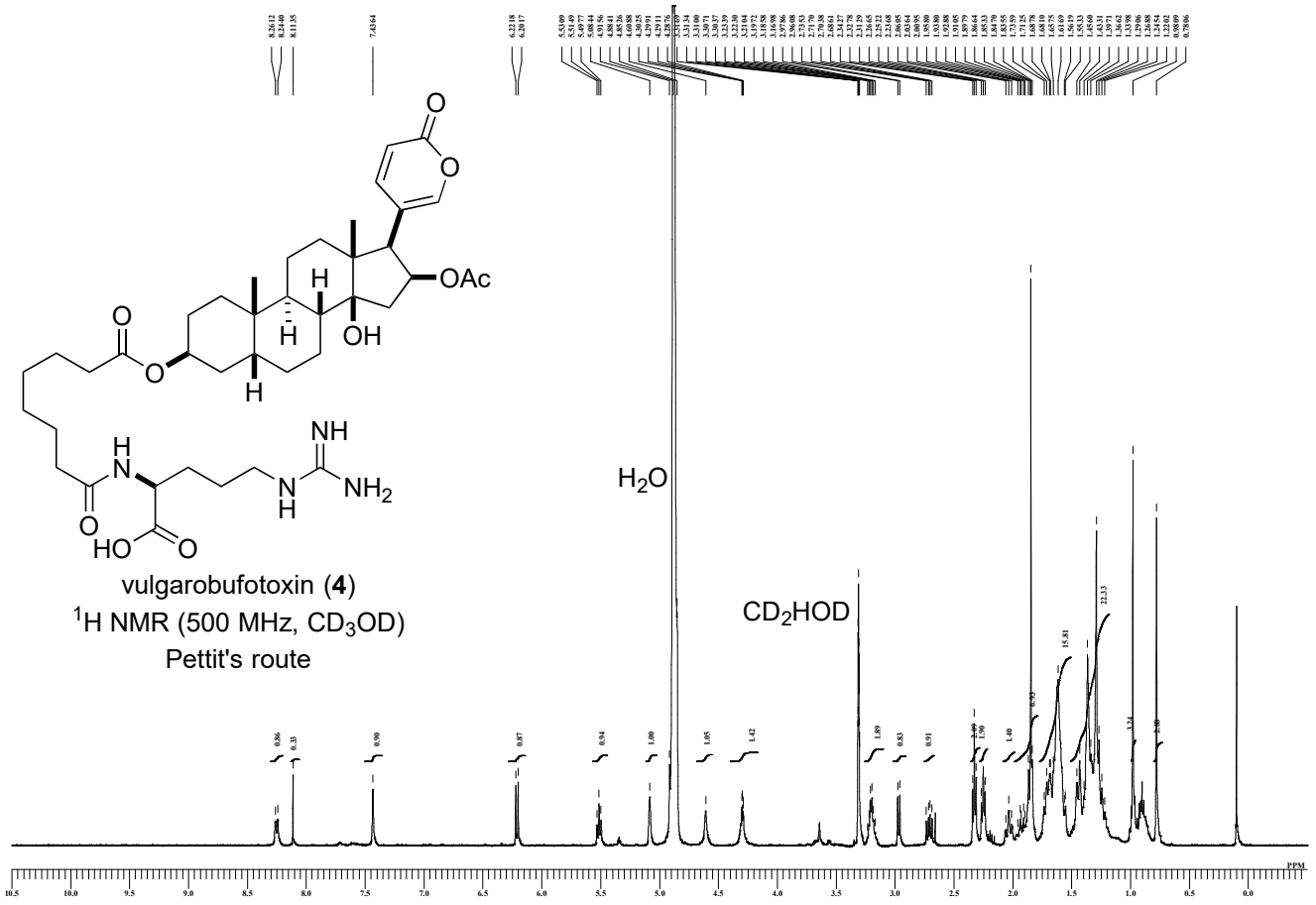
vulgarobufotoxin (**4**)
HMQC (500 MHz, CD_3OD)
our route



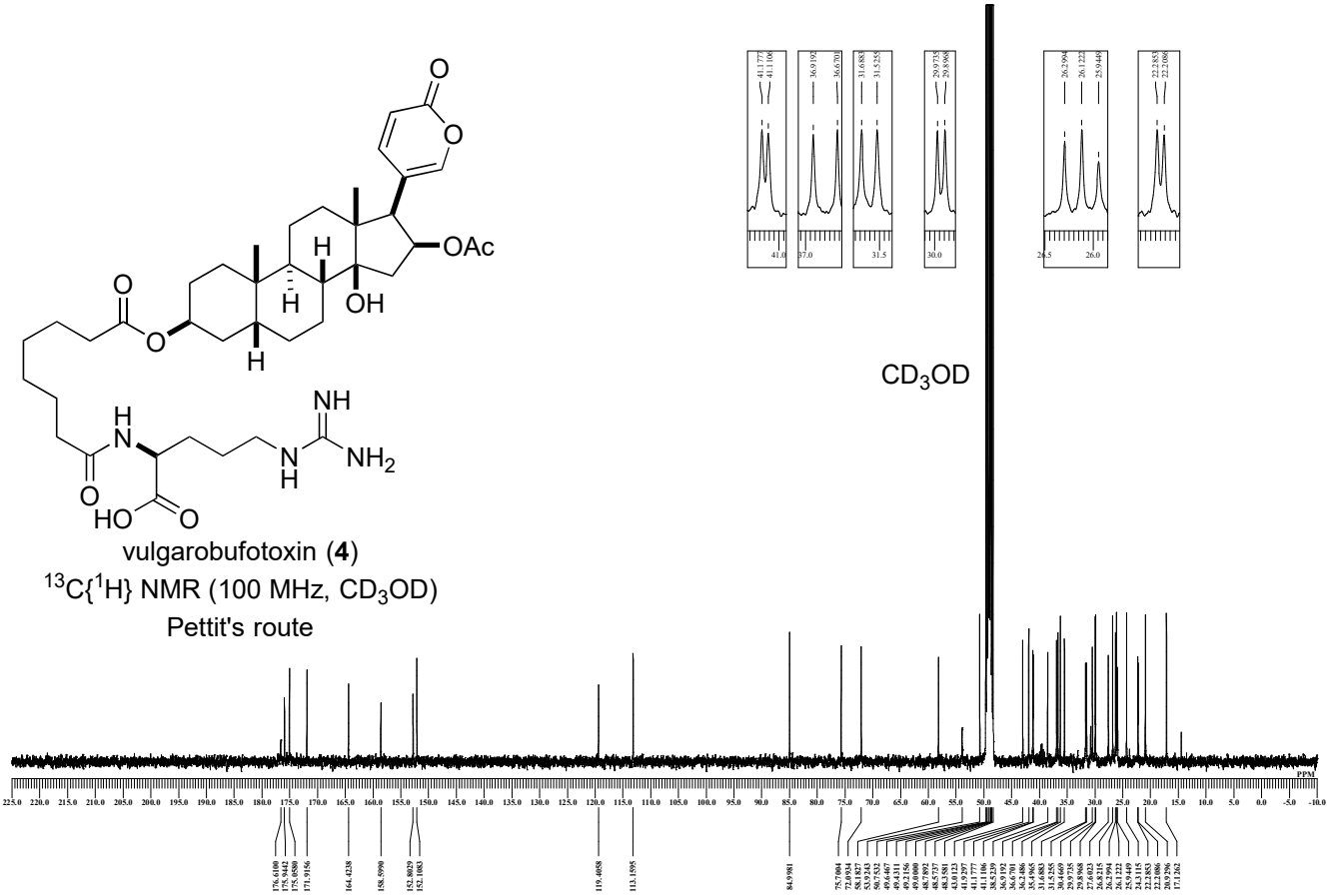
HMBC (500 MHz, CD_3OD)



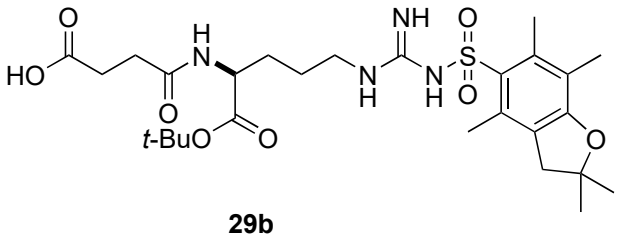




vulgarobufotoxin (**4**)
^{1}H NMR (500 MHz, CD_3OD)
Pettit's route

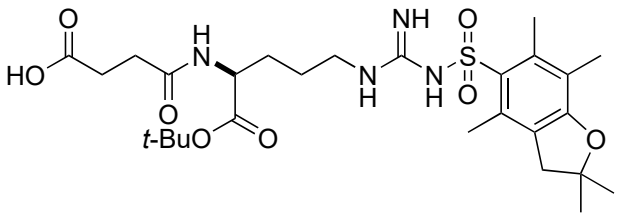


vulgarobufotoxin (**4**)
$^{13}C\{^{1}H\}$ NMR (100 MHz, CD_3OD)
Pettit's route



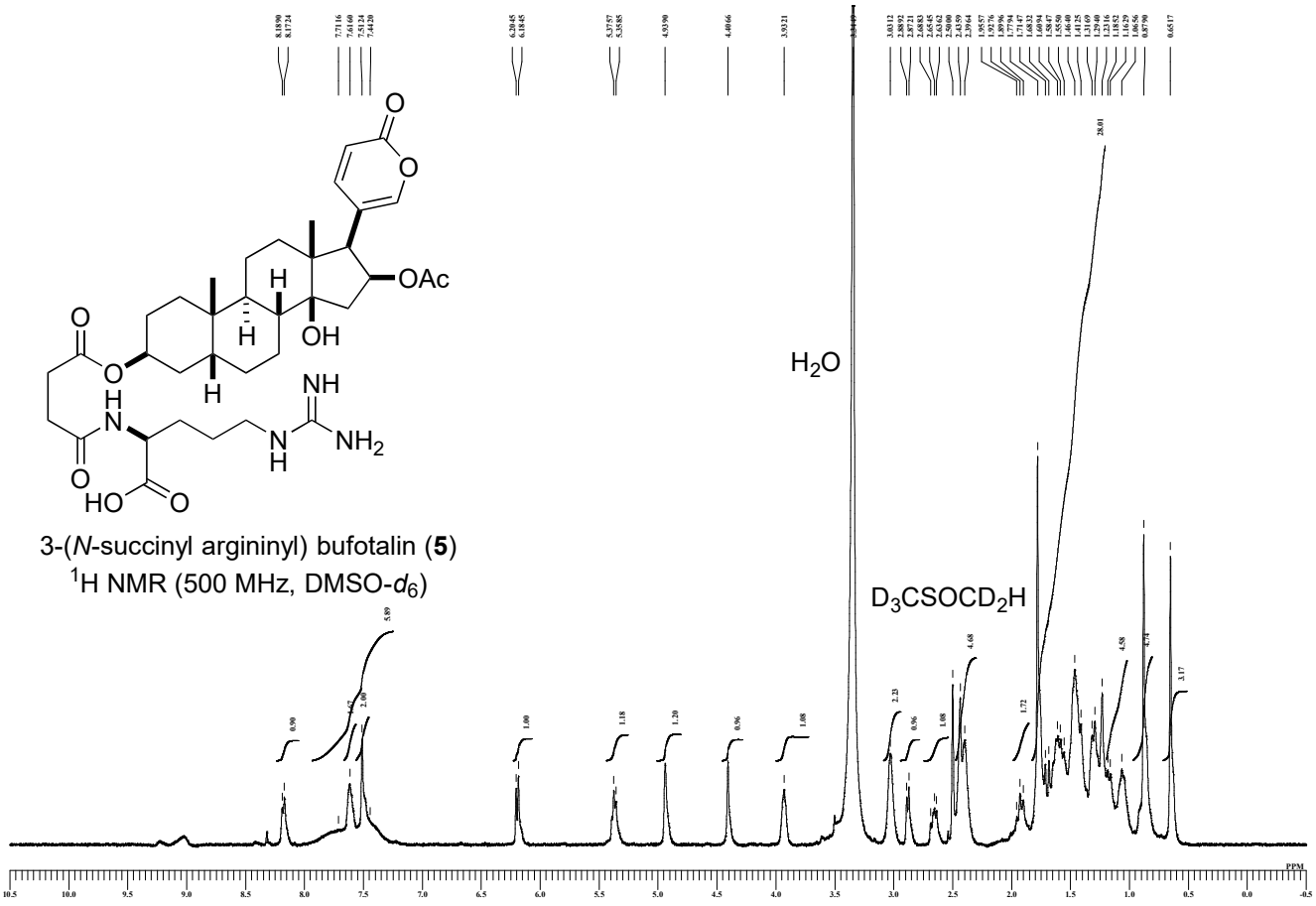
29b

^{1}H NMR (500 MHz, $CDCl_3$)

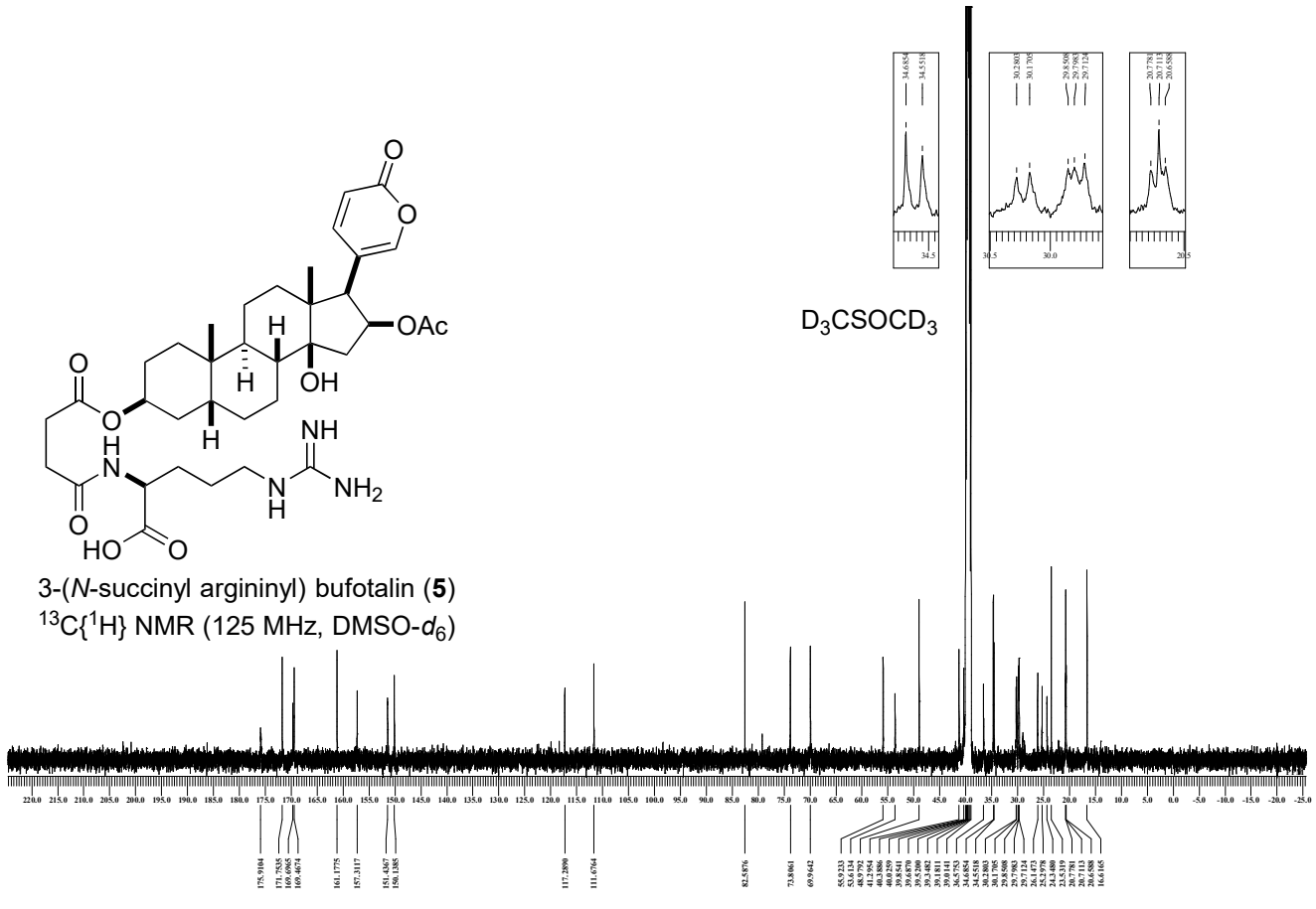


29b

$^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$)



3-(*N*-succinyl argininyl) bufotalin (**5**)
^{1}H NMR (500 MHz, DMSO-d_6)



3-(*N*-succinyl argininyl) bufotalin (**5**)
^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6)