Supporting Information

Discovery of clinical candidate BMS-906024, a potent pan-Notch inhibitor for the treatment of leukemia and solid tumors

Ashvinikumar V. Gavai,* Claude Quesnelle, Derek Norris, Wen-Ching Han, Patrice Gill, Weifang Shan, Aaron Balog, Ke Chen, Andrew Tebben, Richard Rampulla, Dauh-Rurng Wu, Yingru Zhang, Arvind Mathur, Ronald White, Anne Rose, Haiqing Wang, Zheng Yang, Asoka Ranasinghe, Celia D'Arienzo, Victor Guarino, Lan Xiao, Ching Su, Gerry Everlof, Vinod Arora, Ding Ren Shen, Mary Ellen Cvijic, Krista Menard, Mei-Li Wen, Jere Meredith, George Trainor, Louis J. Lombardo, Richard Olson, Phil S, Baran, John T. Hunt, Gregory D. Vite, Bruce S. Fischer, Richard A. Westhouse, and Francis Y. Lee

Contents of Supporting Information:

I. General Experimental Details

All non-aqueous reactions were carried out under an argon or nitrogen atmosphere at room temperature, unless otherwise noted. All commercial reagents and anhydrous solvents were purchased from Aldrich and were used without further purification or distillation, unless otherwise stated. Analytical thin layer chromatography (tlc) was performed on EM Science silica gel 60 F254 (0.25 mm). Compounds were visualized by UV light and/or stained with either p-anisaldehyde, potassium permanganate, or cerium molybdate solutions followed by heating. Flash column chromatography was performed on TeledyneISCO CombiFlash Rf instruments, using TeledyneISCO SiO2 columns of the appropriate

sizes, with gradients of solvents as indicated. Analytical high pressure liquid chromatography (HPLC) and LC-MS analyses were conducted using Shimadzu LC-10AS pumps and a SPD-10AV UV-vis detector set at 220 nm or 254 nm with MS detection performed with a Micromass Platform LC spectrometer. Analytical HPLC analyses were performed using the following conditions:

HPLC Method A: Sunfire C18 3.5 μ 4.6 x 150 mm column, solvent A: 5% acetonitrile – 95% water – 0.05% TFA, solvent B: 5% water – 95% acetonitrile – 0.05% TFA, flow rate 2 mL/min; linear gradient time = 12 min; start %B = 10, end %B = 100%, stop time 3 min.

HPLC Method B: Xbridge Phenyl 3.5 μ 4.6 x 150 mm column, solvent A: 5% acetonitrile – 95% water – 0.05% TFA, solvent B: 5% water – 95% acetonitrile – 0.05% TFA, flow rate 2 mL/min; linear gradient time = 12 min; start %B = 10, end %B = 100%, stop time 3 min.

HPLC Method C: YMC S5 ODS 4.6x50 mm column, solvent A: 10% methanol – 90% water – 0.2% H3PO4, solvent B: 10% water – 90% methanol – 0.2% H3PO4, flow rate 4 mL/min; linear gradient time = 3 min; start %B = 10, end %B = 100%, stop time 4 min.

HPLC Method D: Sunfire C18 3.5um, 3.0x150mm column, solvent A: 5% acetonitrile – 95% water – 0.05% TFA, solvent B: 95% acetonitrile – 5% water – 0.05% TFA, flow=0.5 mL/min, gradient from 10%B to 100%B over 15min, 254 nm detector.

HPLC Method E: Xbridge Phenyl 3.5um, 3.0x150mm column, solvent A: 5% acetonitrile – 95% water – 0.05% TFA, solvent B: 95% acetonitrile – 5% water – 0.05% TFA, flow=0.5 mL/min, gradient from 10%B to 100%B over 15min, 254 nm detector.

Chiral LC/Analytical SFC conditions: Column: Lux-Cellulose-2 (0.46 x 25cm), Mobile phase: 10% methanol in CO_2, Flow rate: 3 mL/min, wavelength: 220 nm; Temp.: 35°C.

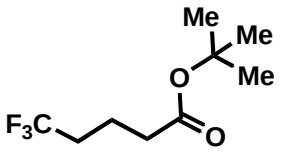
Preparative SFC chromatography (Lux-Cellulose-2 (3 x 25cm), 8% methanol in CO_2, 140 mL/min at 220 nm and 35°C; Sample in methanol, conc. = 70 mg/mL, Stack injection: 0.5 mL/9.2min. Fractions

containing product were concentrated, and dried overnight under vacuum to provide desired compounds.

NMR (^{1}H and ^{13}C) spectra were recorded on one of the following instruments: JEOL GSX-500 MHz or Bruker ARX-400 MHz spectrometers and calibrated using an internal reference. Elemental analyses were performed by Robertson Microlit laboratories and the results obtained are within 0.4% of the theoretical values. All compounds exhibited purity of >95% using analytical HPLC conditions given above. High resolution mass spectra (HRMS) were recorded on a ThermoFinnigan LTQ Orbitrap XL mass spectrometer using positive ion electrospray with an ESI Voltage of 5 kV.

II. Detailed Experimental Procedures for the Preparation of Compound 12 (BMS-906024)

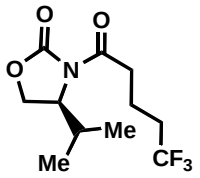
a. Preparation of tert-butyl 5,5,5-trifluoropentanoate



To a stirred solution of 5,5,5-trifluoropentanoic acid (5.00 g, 32.0 mmol) in THF (30 mL) and n-hexane (30 mL) at 0°C, was added tert-butyl 2,2,2-trichloroacetimidate (11.5 mL, 64.1 mmol). After 15 min of stirring, boron trifluoride etherate (0.4 mL, 3.2 mmol) was added, and the reaction mixture was allowed to warm to room temperature over 16 h. Solid sodium bicarbonate (5 g) was added and stirring continued for 30 min. The mixture was filtered over a pad of anhydrous magnesium sulfate, the solid was washed with hexanes (2 x 200 mL), and concentrated to 30 mL under reduced pressure at ambient temperature. The reaction mixture was again filtered, washed with hexane (5 mL), and concentrated under reduced pressure. The resulting oil was filtered through a 0.45 μm nylon membrane filter disk to

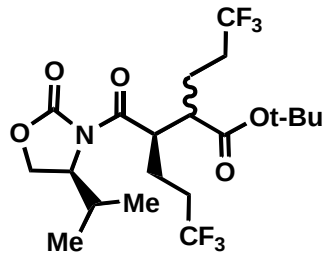
provide tert-butyl 5,5,5-trifluoropentanoate (6.6 g, 98%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (s, 9H), 1.74-1.83 (m, 2H), 2.00-2.13 (m, 2H), 2.24 (t, J = 7.28 Hz, 2H).

b. Preparation of (4S)-4-isopropyl-3-(5,5,5-trifluoropentanoyl)-1,3-oxazolidin-2-one (**13**)



To a stirred solution of 5,5,5-trifluoropentanoic acid (5.04 g, 32.3 mmol) in dichloromethane (50 mL) were added successively DMF (3 drops) and oxalyl chloride (3.4 mL, 38.8 mmol) over 5 min. The reaction mixture was concentrated under reduced pressure to give 5,5,5-trifluoropentanoyl chloride as pale yellow oil. To a solution of (S)-4-isopropyloxazolidin-2-one (4.18 g, 32.4 mmol) in THF (100 mL) at -78°C was added a solution of 2.5 M n-BuLi in hexanes (13.0 mL, 32.5 mmol) over 5 min. The reaction mixture was stirred at -78°C for 10 min and a solution of 5,5,5-trifluoropentanoyl chloride (5.64 g, 32.3 mmol) in THF (20 mL) was added via cannula over 15 min. The reaction mixture was stirred at 0°C for 15 min, and allowed to warm to ambient temperature over 16 h. Saturated aqueous ammonium chloride (50 mL) was added and the reaction mixture was extracted with ethyl acetate (2 x 100 mL). The combined organic layer was washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography (Teledyne ISCO CombiFlash Rf, 5 - 60% ethyl acetate in hexanes, RediSep silica gel 120 g). Concentration of appropriate fractions provided product **13** (7.39 g, 86%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.44 (1H, dt, J = 8.31, 3.53 Hz), 4.30 (1H, t, J = 8.69 Hz), 4.23 (1H, dd, J = 9.06, 3.02 Hz), 2.98 - 3.08 (2H, m), 2.32 - 2.44 (1H, m), 2.13 - 2.25 (2H, m), 1.88 - 2.00 (2H, m), 0.93 (3H, d, J = 7.05 Hz), 0.88 (3H, d, J = 6.80 Hz).

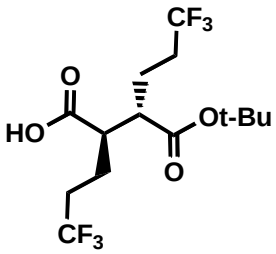
c. Preparation of tert-butyl (3R)-6,6,6-trifluoro-3-(((4S)-4-isopropyl-2-oxo-1,3-oxazolidin-3-yl)carbonyl)-2-(3,3,3-trifluoropropyl)hexanoate (**14**)

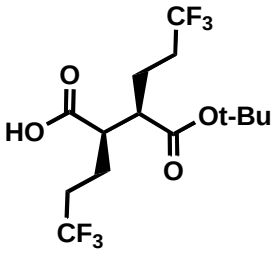


A 0.5 M solution of LDA was prepared by the addition of a solution of 2.5 M n-butyl lithium in hexanes (14.7 mL, 36.8 mmol) to a cold (-78°C) solution of diisopropylamine (5.3 mL, 37.2 mmol) in THF (59 mL) under nitrogen. The solution was stirred at 0°C for 15 min. A solution of 13 (2.45 g, 9.2 mmol) in toluene (15.3 mL) was added with stirring to dry lithium chloride (1.96 g, 46.2 mmol). The mixture was cooled to -78°C and the freshly prepared 0.5 M solution of LDA (21.0 mL, 10.5 mmol) was added. The reaction mixture was stirred at -78°C for 10 min, at 0°C for 10 min, and cooled to -78°C. The freshly prepared 0.5 M solution of LDA (37.0 mL, 18.5 mmol) was added to a cold (-78°C) solution of tert-butyl 5,5,5-trifluoropentanoate (3.41 g, 16.1 mmol) in toluene (15.3 mL). After 25 min of stirring at -78°C, this reaction mixture was transferred via cannula into the cold (-78°C) solution of the enolate of **13**. After an additional 5 min of stirring at -78°C, solid powdered bis(2-ethylhexanoyloxy)copper (9.02 g, 25.8 mmol) was rapidly added to the reaction vessel and the flask was rapidly recapped with a septum. The vessel was immediately removed from the cold (-78°C) bath and immersed into a warm (40°C) water bath with rapid swirling. The reaction mixture changed from the initial turquoise to brown color. After 20 min of stirring, the reaction mixture was poured into 5% aq. ammonium hydroxide (360 mL) and extracted with ethyl acetate (2 x 150 mL). The combined organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (Teledyne ISCO CombiFlash Rf, 0 to 60% ethyl acetate in hexanes, RediSep silica gel, 120 g). Concentration of appropriate fractions provided product **14** (2.87 g, 66%) as a pale yellow oil. ^{1}H NMR indicated that the product was a 1.6:1 mixture of diastereomers, as determined by integration

of the multiplets at 2.74 & 2.84 ppm. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.43 - 4.54 (2H, m), 4.23 - 4.35 (5H, m), 4.01 (1H, ddd, J = 9.54, 6.27, 3.51 Hz), 2.84 (1H, ddd, J = 9.41, 7.28, 3.64 Hz), 2.74 (1H, ddd, J = 10.29, 6.27, 4.02 Hz), 2.37 - 2.48 (2H, m), 2.20 - 2.37 (3H, m), 1.92 - 2.20 (8H, m), 1.64 - 1.91 (5H, m), 1.47 (18H, s), 0.88 - 0.98 (12H, m).

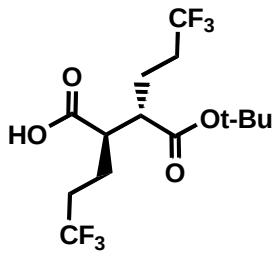
d. Preparation of (2R,3S)-3-(tert-butoxycarbonyl)-6,6,6-trifluoro-2-(3,3,3-trifluoropropyl)hexanoic acid (**15**) and (2R,3R)-3-(tert-butoxycarbonyl)-6,6,6-trifluoro-2-(3,3,3-trifluoropropyl)hexanoic acid (**18**)





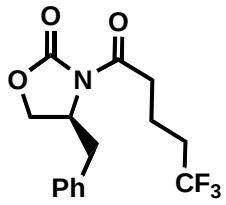
To a cold (0°C), solution-suspension of **14** (4.54 g, 9.5 mmol) in THF (140 mL) and water (42 mL) were added sequentially 30% aq. hydrogen peroxide (10.3 g, 91.0 mmol) and lithium hydroxide (685 mg, 28.6 mmol). The reaction mixture was stirred at 0°C for 1 h and allowed to warm up to ambient temperature over 1.5 h. Saturated aq. sodium bicarbonate (45 mL) and saturated aq. sodium sulfite (15 mL) were added, and the reaction mixture was concentrated under reduced pressure. The mixture was extracted with dichloromethane (3 x 150 mL) and the aqueous phase was acidified to pH 1 with 1M aq. HCl. Extraction with dichloromethane (3 x 150 mL) and ethyl acetate (150 mL) was followed by washing the combined organic layer with brine, drying (Na_2SO_4), filtration, and concentrated under reduced pressure to provide a mixture of the desired compounds (3.00 g, 86%) as colorless oil. Relative integration of the t-butyl peaks in ^{1}H NMR established a 1.7:1 ratio for **15** and **18**, respectively. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.76 - 2.84 (1H, m), 2.64 - 2.76 (3H, m), 2.04 - 2.35 (8H, m), 1.88 - 2.00 (4H, m), 1.71 - 1.83 (4H, m), 1.48 (9H, s), 1.46 (9H, s).

e. Preparation of (2R,3S)-3-(tert-butoxycarbonyl)-6,6,6-trifluoro-2-(3,3,3-trifluoropropyl)hexanoic acid (**15**)



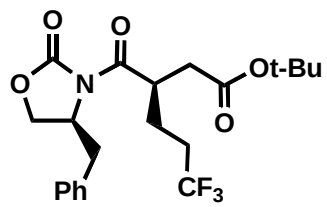
To a cold (-78°C), stirred solution of diisopropylamine (1.7 mL, 11.9 mmol) in THF (19 mL) under nitrogen was added a 2.5 M solution of n-BuLi in hexanes (4.8 mL, 12.0 mmol). The reaction mixture was stirred at -78°C for 5 min, then at 0°C for 15 min, and transferred via cannula drop wise (over 25 min) to a cold (-78°C) solution of the 1.7:1 ratio for **15** and **18** (1.99 g, 5.4 mmol) in THF (18 mL). The mixture was stirred at -78°C for 15 min, then at 24°C for 15 min, and again at -78°C for 15 min. A 1M solution of diethylaluminum chloride in hexanes (11.4 mL, 11.4 mmol) was added drop wise via syringe, and stirring continued at -78°C for 10 min, at 24°C for 15 min and again at -78°C for 15 min. Methanol (25 mL) was rapidly added, and the flask was swirled vigorously while warming to room temperature. The reaction mixture was concentrated to ~25% of the original volume. Ethyl acetate (100 mL), aq. 1M HCl (50 mL), and ice (75 g) were added. The aqueous layer was extracted with ethyl acetate (2 x 100 mL). The combined organics were washed with a solution of potassium fluoride (2.85 g) in water (75 mL) and aq. 1 M HCl (13 mL), and brine. Drying (Na_2SO_4), filtration, and concentration under reduced pressure provided the product (2.13 g, >99%) as a pale yellow oil. Relative integration of the t-butyl peaks in ^{1}H NMR established a 9:1 ratio for **15** and **18**, respectively. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.64 - 2.76 (2H, m), 2.04 - 2.35 (4H, m), 1.88 - 2.00 (2H, m), 1.71 - 1.83 (2H, m), 1.48 (9H, s).

f. Preparation of (4S)-4-benzyl-3-(5,5,5-trifluoropentanoyl)-1,3-oxazolidin-2-one (**16**)



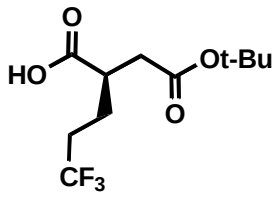
To a stirred solution of 5,5,5-trifluoropentanoic acid (4.5 g, 28.8 mmol) in dichloromethane (25 mL) were added successively DMF (3 drops) and oxalyl chloride (2.52 mL, 28.8 mmol) over 5 min. The reaction mixture was concentrated under reduced pressure to give 5,5,5-trifluoropentanoyl chloride as pale yellow oil. To a solution of (S)-4-benzyloxazolidin-2-one (3.80 g, 21.5 mmol) in THF (20 mL) at -78°C was added a solution of 2.5 M n-BuLi in hexanes (11.5 mL, 28.8 mmol) over 5 min. The reaction mixture was stirred at -78°C for 10 min and a solution of 5,5,5-trifluoropentanoyl chloride (5.03 g, 28.8 mmol) in THF (20 mL) was added via cannula over 15 min. The reaction mixture was stirred at -78°C for 45 min, and allowed to warm to ambient temperature. Saturated aqueous ammonium chloride (50 mL) was added and the reaction mixture was extracted with ethyl ether (2 x 100 mL). The combined organic layer was washed with 1M aq. sodium hydroxide, brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography over silica gel (220 g) eluting with a gradient from 20% to 100% dichloromethane in hexanes to give **16** (5.67 g, 84%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 - 7.39 (2H, m), 7.30 (1 H, d, J = 7.05 Hz), 7.18 - 7.25 (2H, m), 4.64 - 4.74 (1H, m), 4.17 - 4.27 (2H, m), 3.31 (1H, dd, J = 13.35, 3.27 Hz), 3.00 - 3.11 (2H, m), 2.79 (1H, dd, J = 13.35, 9.57 Hz), 2.16 - 2.28 (2H, m), 1.93 - 2.04 (2H, m).

g. Preparation of tert-butyl (3R)-3-(((4S)-4-benzyl-2-oxo-1,3-oxazolidin-3-yl)carbonyl)-6,6,6-trifluorohexanoate (**17**)



To a cold (-78°C), stirred solution of 16 (3.03 g, 9.6 mmol) in THF (20 mL) was added a 1 M solution of sodium hexamethyldisilazide in THF (10.6 mL, 10.6 mmol) under nitrogen. After 2 h of stirring at -78°C, tert-butyl 2-bromoacetate (5.62 g, 28.8 mmol) was added neat via syringe and stirring continued at -78°C for 6 h. The reaction mixture was allowed to warm up to ambient temperature and partitioned between saturated aq. ammonium chloride (50 mL) and ethyl acetate (100 mL). The organic phase was separated, and the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The combined organics were washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by flash chromatography (Teledyne ISCO CombiFlash Rf, 5% to 100% ethyl acetate in hexanes, RediSep SiO_2 120 g). Concentration of appropriate fractions provided **17** (2.79 g, 68%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (2H, d, J = 7.30 Hz), 7.24 - 7.32 (3H, m), 4.62 - 4.75 (1H, m), 4.15 - 4.25 (3H, m), 3.35 (1H, dd, J = 13.60, 3.27 Hz), 2.84 (1H, dd, J = 16.62, 9.57 Hz), 2.75 (1H, dd, J = 13.35, 10.07 Hz), 2.47 (1H, dd, J = 16.62, 4.78 Hz), 2.11 - 2.23 (2H, m), 1.90 - 2.02 (1H, m), 1.72 - 1.84 (1H, m), 1.44 (9 H, s).

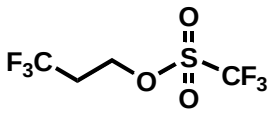
h. Preparation of (R)-2-(2-tert-butoxy-2-oxoethyl)-5,5,5-trifluoropentanoic acid



To a cold (0°C), stirred solution of 17 (2.0 g, 4.7 mmol) in THF (50 mL) and water (15 mL) were added successively 30% aq. solution of hydrogen peroxide (1.9 mL, 18.6 mmol) and a solution of lithium hydroxide (223 mg, 9.3 mmol) in water (2 mL). After 10 min, the reaction mixture was allowed to warm up to room temperature over 1h. The reaction was cooled to 0°C and saturated aq. sodium bicarbonate (25 mL) and saturated aq. sodium sulfite (25 mL) were added. After stirring for 15 min, the reaction tested negative for peroxide on a peroxide test strip. The mixture was concentrated to remove the majority of THF, washed with dichloromethane (3 x 50 mL), and the aqueous phase was acidified to pH

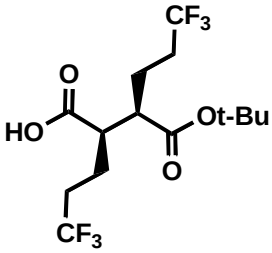
3 with 1M aq. HCl. Extraction with ethyl acetate (3 x 50 mL) was followed by washing with brine (2 x 25 mL), drying over anhydrous magnesium sulfate, and concentration under reduced pressure to provide the title compound (1.05 g, 83% yield) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.83 - 2.95 (1H, m), 2.62 - 2.74 (1H, m), 2.45 (1H, dd, J = 16.62, 5.79 Hz), 2.15 - 2.27 (2H, m), 1.88 - 2.00 (1H, m), 1.75 - 1.88 (1H, m), 1.45 (9H, s).

i. Preparation of 3,3,3-trifluoropropyl trifluoromethanesulfonate



To a cold (-25°C), stirred solution of 2,6-lutidine (18.4 mL, 158.0 mmol) in dichloromethane (120 mL) was added trifluoromethanesulfonic anhydride (24.9 mL, 147.0 mmol) over 3 min. After 5 min of additional stirring at -25°C, 3,3,3-trifluoropropan-1-ol (12.0 g, 105.0 mmol) was added over a period of 3 min. The reaction mixture was stirred at -25°C for 2 h and allowed to warm to ambient temperature over 1 h. The reaction mixture was concentrated to half of the original volume, and purified by silica gel column chromatography (330 g). Elution with dichloromethane afforded the title compound (13.74 g, 53%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.71 (2H, t, J = 6.15 Hz), 2.49 - 2.86 (2H, m).

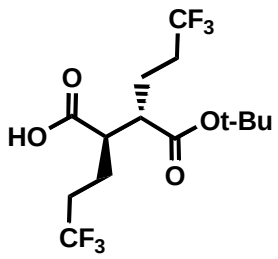
j. Preparation of (2R,3R)-3-(tert-butoxycarbonyl)-6,6,6-trifluoro-2-(3,3,3-trifluoropropyl)hexanoic acid (**18**)



To a cold (-78°C), stirred solution of (R)-2-(2-tert-butoxy-2-oxoethyl)-5,5,5-trifluoropentanoic acid (5.44 g, 20.1 mmol) in THF (60 mL) was added drop wise a 1.8 M solution of LDA in heptane/THF/ethylbenzene (24.6 mL, 44.3 mmol). After stirring for 2 h at -78°C, 3,3,3-trifluoropropyl

trifluoromethanesulfonate (6.44 g, 26.2 mmol) was added and stirring continued for 45 min. The reaction mixture was stirred at -25°C for 1h, and at 0°C for 45 min. 3,3,3-Trifluoropropyl trifluoromethanesulfonate (1.0 g, 4.1 mmol) was added and the reaction mixture was stirred for 20 additional min. The reaction was quenched with water and 1N aq. sodium hydroxide (2 x 50 mL) and washed with dichloromethane (2 x 50 mL). The organic layer was again extracted with 1N aq. NaOH (2 x 25 mL), and the aqueous layers were combined. The aqueous layer was cooled to 0°C, acidified with concentrated HCl to pH 2, and extracted with ethyl acetate (3 x 100 mL). The combined organics were washed with brine, dried over anhydrous sodium sulphate, and concentrated under reduced pressure to provide 18 (5.93 g, 80%) as a pale yellow solid. ^{1}H NMR showed a 1:5 mixture of **15** and **18** by integration of peaks for the t-Bu groups. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.81 (1H, ddd, J = 10.17, 6.32, 3.85 Hz), 2.63 - 2.76 (1H, m), 2.02 - 2.33 (4H, m), 1.86 - 1.99 (2H, m), 1.68 - 1.85 (2H, m), 1.47 (9H, s).

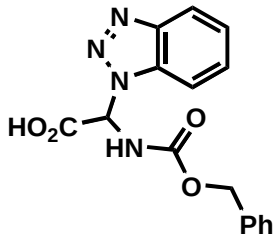
k. Preparation of (2R,3S)-3-(tert-butoxycarbonyl)-6,6,6-trifluoro-2-(3,3,3-trifluoropropyl)hexanoic acid (**15**)



A 1.8 M solution of LDA in heptane/THF/ethylbenzene (2.2 mL, 3.9 mmol) was added drop wise to a cold (-78°C) solution of **18** (645 mg, 1.8 mmol) in THF (6 mL). After stirring for 15 min, the reaction mixture was placed in a room temperature water bath. After 15 min of stirring, the reaction mixture was placed back in a -78°C bath and a 1 M solution of diethylaluminum chloride in hexane (3.9 mL, 3.9 mmol) was added slowly over 5 min and stirring continued for 15 min. The reaction mixture was stirred in a room temperature water bath for 10 min, at -78°C for 15 min, and methanol (8.0 mL, 198.0 mmol) was rapidly added. The reaction mixture was removed from -78°C bath and concentrated under reduced

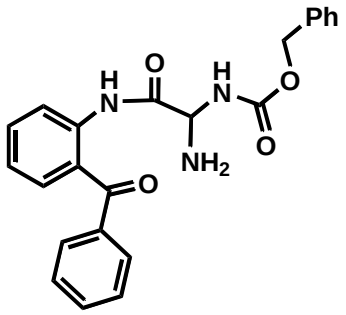
pressure. Ice (10 g) and aq. 1N HCl (16 mL, 16.0 mmol) were added and the reaction mixture was extracted with ethyl acetate (2 x 50 mL). The organic layer was washed with a solution of potassium fluoride (920 mg, 15.84 mmol) in water (25 mL) and aq. 1N HCl (4.5 mL). The organic layer was dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to provide 15 (540 mg, 90%) as a light yellow solid. ^{1}H NMR showed a 9:1 ratio of **15** and **18**, respectively. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.64 - 2.76 (2H, m), 2.04 - 2.35 (4H, m), 1.88 - 2.00 (2H, m), 1.71 - 1.83 (2H, m), 1.48 (9H, s).

l. Preparation of 2-(1H-benzo[d][1,2,3]triazol-1-yl)-2-(benzyloxycarbonylamino)acetic acid (**A**)



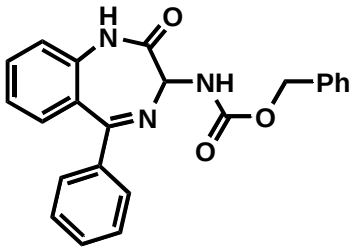
Benzotriazole (64.7 g, 540 mmol), glyoxylic acid (50.0 g, 540 mmol), and benzyl carbamate (82.1 g, 540 mmol) were refluxed in benzene (1500 mL) using a Dean-Stark apparatus. Approximately 18 mL of water was removed during 2 h of refluxing. The reaction mixture was allowed to cool to ambient temperature, filtered and the resulting solid was washed with MTBE. Drying under reduced pressure provided the desired compound (160 g, 90%) as a solid: HPLC: RT = 3.115 min (YMC S5 ODS 4.6x50 mm column, 4 min run w/ 4mL/min (0%-100% B) solvent B=90% MeOH-10% H_2O-0.2% H_3PO_4); MS (ES) = 327.10 $[M+1]^+$; ^{1}H NMR (400 MHz, DMSO-D_6) δ ppm 14.10 (s, 1 H) 9.30 - 9.43 (m, 1 H) 8.08 (d, J=8.31 Hz, 1 H) 7.91 - 8.03 (m, 1 H) 7.50 - 7.65 (m, 1 H) 7.39 - 7.49 (m, 1 H) 7.26 - 7.39 (m, 5 H) 7.17 - 7.25 (m, 1 H) 4.96 - 5.19 (m, 2 H) 3.12 - 3.54 (m, J=6.80 Hz, 1 H).

m. Preparation of benzyl 1-amino-2-(2-benzoylphenylamino)-2-oxoethylcarbamate (**B**)



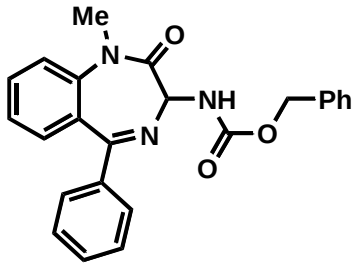
Oxalyl chloride (10.0 g, 80 mmol) was added drop wise to a cold (0°C) solution of 2-(1H-benzo[d][1,2,3]triazol-1-yl)-2-(benzyloxycarbonylamino)acetic acid (**A**) (25.0 g, 70 mmol) in THF (250 mL). A catalytic amount (1 drop) of DMF was added. After 15 min of stirring, a solution of 2-aminobenzophenone (13.6 g, 70 mmol) and N-methylmorpholine (15.5 g, 150 mmol) in THF (75 mL) were added drop wise and stirring continued at 0°C for 0.5 h. The reaction mixture was filtered and solid washed with THF. The filtrate was cooled to 0°C and ammonia (g) was bubbled through it for 0.5 h, resulting in formation of a precipitate. Methanol (400 mL) was added and bubbling continued for an additional 0.5 h. The reaction mixture was allowed to warm up to ambient temperature over 16 h. Concentration under reduced pressure was followed by addition of ethyl acetate (2000 mL). The organic layer was washed with aq. 1N sodium hydroxide and brine. Drying (sodium sulfate) and evaporation under reduced pressure afforded the desired product (28 g, 100%) as a solid. HPLC: RT = 1.95 min (Ascentis Express C18 50X2.1mm 2.7µm column, 4 min run w/ 1mL/min (0%-100% B) solvent B=98% acetonitrile-2% H_2O-10 mM ammonium formate); MS (ES) = 402 $[M-1]^-$.

n. Preparation of (Z)-benzyl 2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-ylcarbamate (**C**)



A solution-suspension of benzyl 1-amino-2-(2-benzoylphenylamino)-2-oxoethylcarbamate (**B**) (28 g, 70 mmol) and ammonium acetate (24 g, 300 mmol) in glacial acetic acid (500 mL) was stirred at ambient temperature for 12 h and concentrated under reduced pressure. Ethyl acetate (125 mL) and MTBE (375 mL) were added and the mixture was cooled to 5°C. The resulting solid was filtered, washed with aq. 1N sodium hydroxide and water (to pH 7), and dried under reduced pressure to provide the desired product (14 g, 36%) as a solid. HPLC: RT = 1.93 min (Ascentis Express C8 50X2.1mm 2.7μm column, 4 min run w/ 1mL/min (0%-100% B) solvent B=90% acetonitrile-10% H_2O - 10 mM ammonium formate); MS (ES) = 386 $[M+1]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 9.14 (s, 1H), 7.51-7.58 (m, 3H), 7.31-7.49 (m, 10H), 7.16-7.24 (m, 2H), 6.65 (d, *J*=8.35 Hz, 1H), 5.36 (d, *J*=7.91 Hz, 1H), 5.18 (d, *J*=2.20 Hz, 2H).

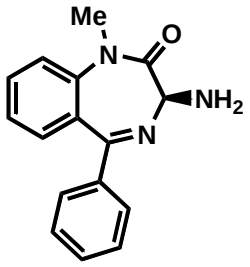
o. Preparation of (Z)-benzyl 1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-ylcarbamate (**D**)



Anhydrous potassium carbonate (7.5 g, 50 mmol) and iodomethane (5.7 g, 40 mmol) were added to a solution of (Z)-benzyl 2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-ylcarbamate (**C**) (14 g, 30 mmol) in DMF (140 mL). The mixture was stirred at ambient temperature for 5 h. Cold (0°C) water (100 mL) and ethyl acetate (200 mL) were added. The organic layer was washed with brine and concentrated under reduced pressure to afford the title compound (13 g, 89%) as a solid: HPLC: RT = 2.00 min (PUROSPHER@star RP-18 (4X55)mm,3μm column, 3 min run w/ 2.5 mL/min (0%-100% B) solvent B=90% acetonitrile-10% H_2O - 20 mM ammonium acetate); MS (ES) = 400 $[M+1]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.48-7.44 (m, 3H), 7.39 (m, 1H), 7.36-7.26 (m, 10H), 7.22 (m, 1H), 6.69 (d, *J*=8.4

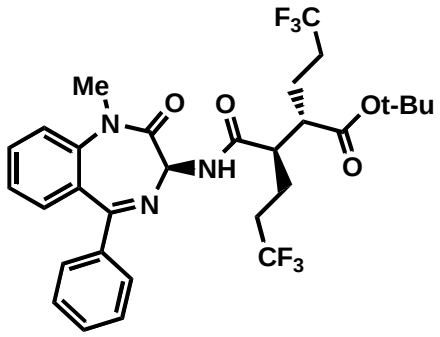
Hz, 1H), 5.33 (d, *J*=8.4Hz, 1H), 5.17 (d, *J*=12.4 Hz, 1H, A of AB pair of doublets), 5.13 (d, *J*=12.4 Hz, 1H, B of AB pair of doublets), 3.47 (s, 3H).

p. Preparation of (3S)-3-amino-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one (**E**)



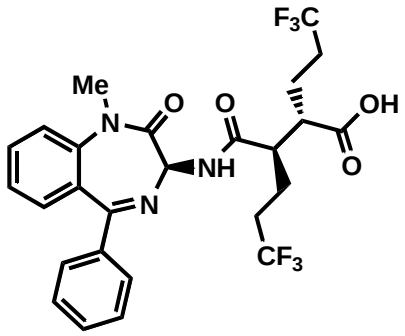
(Z)-Benzyl 1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-ylcarbamate (**D**) (100 g, 250 mmol) was stirred with 33% HBr in acetic acid (1000 mL) for 1 h at ambient temperature. MTBE (3000 mL) was added with vigorous stirring and the resulting solid was filtered. The solid was stirred in cold (0°C) water, filtered, and washed with aq. saturated sodium bicarbonate. The solid was dissolved in ethyl acetate (5000 mL), washed with water and brine, dried (sodium sulfate), and concentrated under reduced pressure to afford the title compound as a racemic mixture (50 g, 75%). The enantiomers were separated under chiral-SFC conditions using the following method: Chiralpak AS-H 5 x 25; Mobil phase: 30% MeOH + 0.1% diethylamine (DEA) in CO_2; Flow rate: 280 mL/min; Pressure: 100 bar; Temperature: 35°C. (3S)-3-amino-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one (S-enantiomer): HPLC: RT = 1.75 min (30% MeOH + 0.1% DEA in CO_2 on Chiralpak AS-H 4.6 x 250 mm, 3 mL/min, 35°C, 100 bar, 230 nm, 10μL injection); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 - 7.63 (2H, m), 7.55 (1H, ddd, J = 8.50, 7.11, 1.76 Hz), 7.40 - 7.47 (1H, m), 7.34 - 7.40 (3H, m), 7.31 (1H, dd, J = 7.81, 1.51 Hz), 7.14 - 7.22 (1H, m), 4.46 (1H, s), 3.44 (3H, s), 3.42 (2H, s); $[\alpha]_D$ = -155° (c = 1.9, MeOH) (Lit. $[\alpha]_D$ = -236°). The R-enantiomer was also obtained. HPLC: RT = 1.71 min; $[\alpha]_D$ = +165° (c = 2.1, MeOH) (Lit. $[\alpha]_D$ = +227°).

q. Preparation of tert-butyl (2S,3R)-6,6,6-trifluoro-3-(((3S)-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl)carbamoyl)-2-(3,3,3-trifluoropropyl)hexanoate (**20**)



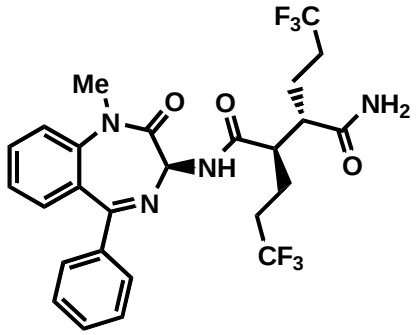
To a stirred solution of (3S)-3-amino-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one (**E**) (1.45 g, 5.5 mmol) and 15 (1.99 g, 5.4 mmol) in DMF (19 mL) were added O-benzotriazol-1-yl-N,N,N',N'-tetra-methyluronium tetrafluoroborate (1.79 g, 5.6 mmol) and triethylamine (3.0 mL, 21.5 mmol), and stirring continued for 16 h. The reaction mixture was poured into water (125 mL), the precipitated solid was collected by filtration, washed with water and dried to provide **20** (2.95 g, 89%) as a white solid. HPLC: RT = 2.00 min (Chromolith RP-18e 2.0x 50 mm column, 5 min run w/ 0.8 mL/min (0%-100% B) solvent B=90% methanol-10% H_2O – 0.1% TFA); MS (ES): m/z = 614 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.55 - 7.65 (3H, m), 7.44 - 7.52 (2H, m), 7.35 - 7.45 (4H, m), 5.52 (1H, d, J = 8.03 Hz), 3.48 (3H, s), 2.63 (2H, ddd, J = 9.35, 3.95, 3.76 Hz), 2.14 - 2.25 (4H, m), 1.90 - 2.03 (3H, m), 1.69 - 1.82 (1H, m), 1.51 (9H, s).

r. Preparation of (2S,3R)-6,6,6-trifluoro-3-(((3S)-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl)carbamoyl)-2-(3,3,3-trifluoropropyl)hexanoic acid



To a cold (0°C), stirred solution of 20 (2.95 g, 4.8 mmol) in dichloromethane (20 mL) was added trifluoroacetic acid (20 mL, 260.0 mmol). The reaction was stirred for 1h at 0°C, and at room temperature for 2.5 h. Toluene (50 mL) was added, and the reaction mixture was concentrated under reduced pressure. The crude product was purified by flash chromatography (Teledyne ISCO CombiFlash Rf, 0% to 45% ethyl acetate in dichloromethane, RediSep SiO_2, 80g). Concentration of the appropriate fractions provided desired product (2.00 g, 75%) as a white solid: HPLC: RT = 2.77 min (Chromolith SpeedROD 4.6 x 50 mm (4 min grad) eluting with 10-90% aqueous MeOH over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 254 nm); MS (ES): m/z = 558 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.32 (1H, d, J = 8.03 Hz), 7.65 - 7.71 (1H, m), 7.50 - 7.60 (3H, m), 7.41 - 7.49 (2H, m), 7.39 (1H, dd, J = 7.91, 1.63 Hz), 7.23 - 7.35 (2H, m), 5.59 (1H, d, J = 8.03 Hz), 3.51 (3H, s), 2.81 (1H, ddd, J = 10.54, 6.90, 3.64 Hz), 2.67 - 2.76 (1H, m), 2.22 - 2.33 (3H, m), 1.99 - 2.12 (3H, m), 1.85 - 1.94 (1H, m), 1.79 (1H, ddd, J = 13.87, 7.84, 3.64 Hz).

s. Preparation of (2R,3S)-N-((3S)-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl)-2,3-bis(3,3,3-trifluoropropyl)succinamide (**12**)

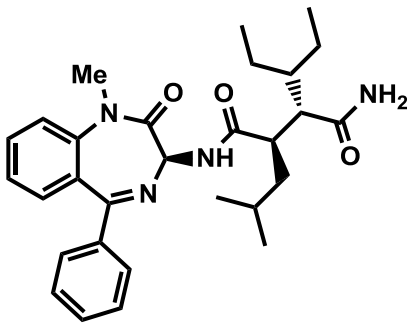


To a stirred solution of (2S,3R)-6,6,6-trifluoro-3-(((3S)-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl)carbamoyl)-2-(3,3,3-trifluoropropyl)hexanoic acid (3.46 g, 6.2 mmol) in DMF (25 mL) under nitrogen were added successively ammonium chloride (3.32 g, 62.1 mmol), EDC (3.55 g, 18.5 mmol), HOBT (2.85 g, 18.6 mmol), and triethylamine (16 mL, 115.0 mmol). The reaction was stirred for 16 h and poured into water (200 mL) with vigorous swirling. The solid was collected by

filtration, washed with water, and dried. The solid was purified by preparative SFC chromatography (Lux-Cellulose-2 (3 x 25cm), 8% methanol in CO_2, 140 mL/min at 220 nm and 35°C; Sample: 3.6 g in 50 mL methanol, conc. = 70 mg/mL, Stack injection: 0.5 mL/9.2min). Fractions containing product were concentrated, and dried overnight under vacuum to provide **12** (2.74 g, 79%) as a colorless solid: HPLC: RT = 9.60 min (HPLC Method D). Chiral LC/Analytical SFC conditions: Column: Lux-Cellulose-2 (0.46 x 25cm), Mobile phase: 10% methanol in CO_2, Flow rate: 3 mL/min, wavelength: 220 nm; Temp.: 35°C. RT = 9.21 min, Purity = 99.95%. MS (ES): m/z = 557 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (1H, d, J = 7.28 Hz), 7.71 - 7.80 (1H, m), 7.68 (2H, d, J = 8.78 Hz), 7.50 - 7.62 (3H, m), 7.45 (2H, t, J = 7.28 Hz), 7.29 - 7.40 (2H, m), 7.15 (1H, s), 5.30 (1H, d, J = 7.28 Hz), 3.39 (3H, s), 2.74 - 2.86 (1H, m), 2.02 -2.32 (3H, m), 1.45 - 1.79 (4H, m); $[\alpha]_D$ = -107.0° (5.73 mg/mL, DMSO). Elemental analysis: Theoretical: C: 54.11%; H: 4.70%; N: 10.06%; Actual: C: 54.06%; H: 4.90%; N: 10.08%. Karl Fisher Moisture: 0.48.

III. Experimental procedures and associated chemical data for compounds 3 - 11

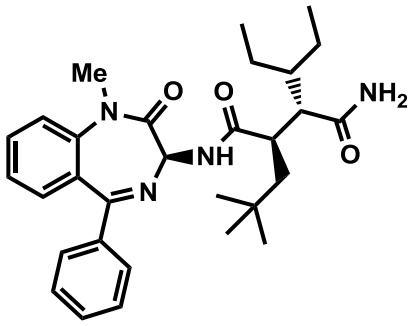
(2R,3S)-N-[(3S)-1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]-2-(2-methylpropyl)-3-(pentan-3-yl)butanediamide (**3**)



Compound **3** was prepared through an Ireland-Claisen route, and following methods as shown in US patent 7,053,084 B1 (Succinoylamino benzodiazepines as inhibitors of Aβ protein production, published May 30, 2006), affording 5.74 g of product: HPLC Ret. Time: 3.94 min (Purity >98%, Method C), ^{1}H

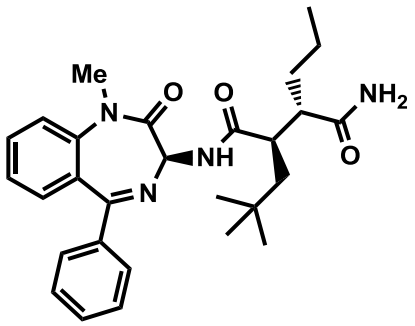
NMR (500 MHz, $CDCl_3$) δ 7.52 - 7.61 (m, 3 H), 7.41 - 7.48 (m, 1 H), 7.33 - 7.41 (m, 4 H), 7.20 - 7.26 (m, 1 H), 5.84 (s, 1 H), 5.52 (d, *J*=7.97 Hz, 1 H), 5.46 (s, 1 H), 3.41 - 3.48 (m, 3 H), 2.84 - 2.93 (m, 1 H), 2.61 (dd, *J*=8.66, 4.81 Hz, 1 H), 1.75 - 1.85 (m, 1 H), 1.53 - 1.73 (m, 4 H), 1.32 - 1.43 (m, 1 H), 1.19 - 1.30 (m, 2 H), 0.94 - 1.03 (m, 9 H), 0.87 (d, *J*=6.60 Hz, 3 H). $(M+H)^+$: 491. HRMS for $C_{29}H_{39}N_4O_3$ $(M+H)^+$ calculated: 491.3022; found: 491.3032.

t. (2R,3S)-2-(2,2-Dimethylpropyl)-N-[(3S)-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]-3-(pentan-3-yl)butanediamide (**4**)



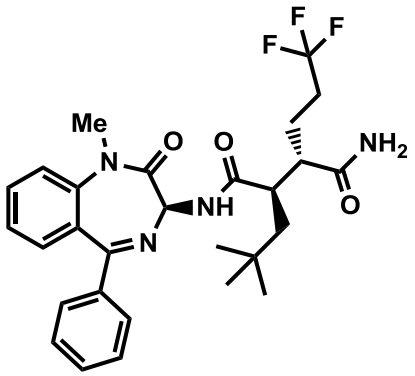
Compound **4** was prepared in a route analogous to Compound **3**, using 4,4-dimethylpentanoic acid, providing 12 mg of the product: HPLC Ret. Time: 10.09 min (Purity 98%, Method A), 9.43 min (Purity 98%, Method B). ^1H NMR (400MHz, DMSO-d_6) δ 9.21 (d, *J*=7.8 Hz, 1H), 7.77 - 7.66 (m, 2H), 7.59 - 7.53 (m, 2H), 7.51 (d, *J*=7.3 Hz, 1H), 7.49 - 7.42 (m, 2H), 7.35 (d, *J*=3.8 Hz, 2H), 7.14 (br s, 1H), 6.75 (s, 1H), 5.34 (d, *J*=8.1 Hz, 1H), 3.00 (br t, *J*=10.6 Hz, 1H), 2.07 (br d, *J*=8.1 Hz, 2H), 1.65 (dd, *J*=10.3, 13.6 Hz, 1H), 1.50 - 1.36 (m, 1H), 1.19 - 1.04 (m, 3H), 0.91 (br d, *J*=4.3 Hz, 4H), 0.87 (s, 9H), 0.82 (br t, *J*=7.1 Hz, 4H). $(M+H)^+$: 505.

u. (2R,3S)-2-(2,2-Dimethylpropyl)-N-[(3S)-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]-3-propylbutanediamide (**5**)



Compound **5** was prepared via an alkylation route similar to Compound **12**, beginning with the 4,4-dimethylpentanoyl oxazolidinone analogous to Compound **16**, and using allyl bromide in Preparation j. Obtained 28 mg of product: HPLC Ret. Time: 9.03 min (Purity 95.0%, Method A), 8.65 min (Purity 95%, Method B). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 - 7.67 (m, 2 H), 7.46 - 7.57 (m, 2 H), 7.36 - 7.45 (m, 4 H), 7.27 - 7.31 (m, 1 H), 6.14 (s, br, 1 H), 5.54 (d, *J*=8.0 Hz, 1 H), 5.40 (s, br, 1 H), 3.49 (s, 3 H), 2.60 - 2.68 (m, 1 H), 2.51 (t, *J*=10.0 Hz, 1 H), 1.88 (dd, *J*=14.0, 10.5 Hz 1 H), 1.76-1.84 (m, 1 H), 1.43-1.74 (m, 4 H), 1.37 (d, *J*=14.0 Hz 1 H), 1.00 (t, *J*=7.3 Hz 3 H), 0.91 (s, 9 H). $(M+H)^+$: 477.

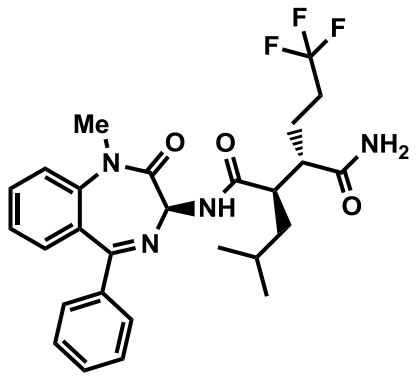
v. (2R)-2-(2,2-Dimethylpropyl)-N-[(3S)-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]-3-(3,3,3-trifluoropropyl)butanediamide (**6**)



Compound **6** was prepared via the oxidative enolate coupling route similar to compound **12**, beginning with the 4,4-dimethylpentanoyl oxazolidinone analogous to Compound **13**. Obtained 5.9 mg of product: HPLC Ret. Time: 9.66 min (Purity 99%, Method A), 9.17 min (Purity 99%, Method B). ^{1}H NMR (500MHz, DMSO-d_6) δ 9.46 (d, *J*=8.8 Hz, 1H), 7.75 - 7.71 (m, 1H), 7.70 - 7.66 (m, 1H), 7.55 - 7.50 (m, 3H), 7.48 (s, 1H), 7.45 - 7.39 (m, 2H), 7.39 - 7.31 (m, 2H), 7.02 (s, 1H), 5.37 (d, *J*=8.2 Hz, 1H), 3.38 (s,

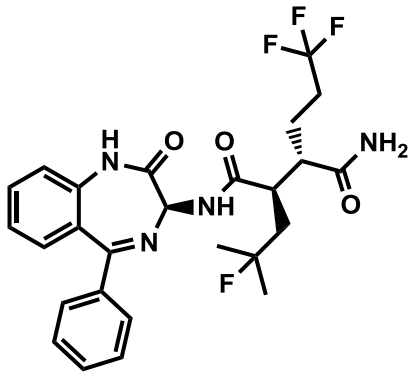
3H), 2.88 (t, *J*=10.2 Hz, 1H), 2.29 - 2.10 (m, 4H), 1.87 - 1.76 (m, 1H), 1.72 - 1.60 (m, 2H), 0.83 (s, 9H). $(M+H)^+$: 531. HRMS for $C_{28}H_{34}F_3N_4O_3$ $(M+H)^+$ calculated: 531.2583; found: 531.2591.

w. (2R,3S)-N-[(3S)-1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]-2-(2-methylpropyl)-3-(3,3,3-trifluoropropyl)butanediamide (**7**)



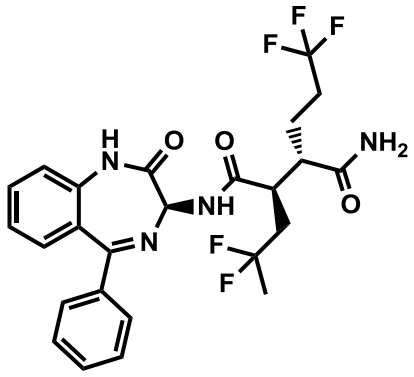
Compound **7** was prepared via the oxidative enolate coupling route similar to compound **12**, beginning with the 4-methylpentanoyl oxazolidinone analogous to Compound **13**. Obtained 21 mg of product: HPLC Ret. Time: 8.80 min (Purity 96%, Method D), 9.16 min (Purity 97%, Method E). ^{1}H NMR (400MHz, DMSO-d_6) δ 9.39 (d, *J*=8.4 Hz, 1H), 7.76 - 7.70 (m, 1H), 7.69 - 7.64 (m, 1H), 7.57 - 7.47 (m, 4H), 7.47 - 7.40 (m, 2H), 7.38 - 7.30 (m, 2H), 6.97 (s, 1H), 5.37 (d, *J*=8.1 Hz, 1H), 3.38 (s, 3H), 2.83 (td, *J*=10.8, 3.0 Hz, 1H), 2.34 (td, *J*=10.2, 3.6 Hz, 1H), 2.28 - 2.07 (m, 2H), 1.85 - 1.71 (m, 1H), 1.69 - 1.57 (m, 1H), 1.54 - 1.44 (m, 2H), 1.06 - 0.96 (m, 1H), 0.90 (d, *J*=6.4 Hz, 3H), 0.79 (d, *J*=6.4 Hz, 3H). $(M+H)^+$: 517. HRMS for $C_{27}H_{32}F_3N_4O_3$ $(M+H)^+$ calculated: 517.2427; found: 517.2437.

x. (2R,3S)-2-(2-Fluoro-2-methylpropyl)-N-[(3S)-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]-3-(3,3,3-trifluoropropyl)butanediamide (**8**)



Compound **8** was prepared via the oxidative enolate coupling route similar to compound **12**, beginning with the 4-fluoro-4-methylpentanoyl oxazolidinone analogous to Compound **13**. Obtained 150 mg of product: HPLC Ret. Time: 8.23 min (Purity 98%, Method A), 8.14 min (Purity 97%, Method B). ^{1}H NMR (500MHz, METHANOL-d_4) δ 7.62 (ddd, *J*=1.5, 7.1, 8.3 Hz, 1H), 7.55 - 7.45 (m, 3H), 7.41 - 7.33 (m, 3H), 7.33 - 7.28 (m, 1H), 7.28 - 7.21 (m, 1H), 5.43 (s, 1H), 3.35 (s, 2H), 3.01 - 2.93 (m, 1H), 2.55 - 2.45 (m, 1H), 2.31 - 2.11 (m, 3H), 1.96 (dt, *J*=5.4, 10.1 Hz, 2H), 1.75 - 1.61 (m, 1H), 1.43 - 1.29 (m, 6H). $(M+H)^+$: 521. HRMS for $C_{26}H_{29}F_4N_4O_3$ $(M+H)^+$ calculated: 521.2176; found: 521.2187.

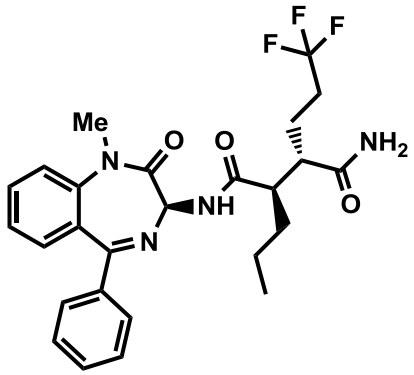
y. (2R,3S)-2-(2,2-Difluoropropyl)-N-[(3S)-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]-3-(3,3,3-trifluoropropyl)butanediamide (**9**)



Compound **9** was prepared via the oxidative enolate coupling route similar to compound **12**, beginning with the 4,4-difluoropentanoyl oxazolidinone analogous to Compound **13**. Obtained 49 mg of product: HPLC Ret. Time: 8.35 min (Purity 98%, Method A), 8.26 min (Purity 98%, Method B). ^{1}H NMR (400 MHz, METHANOL-d_4) δ 7.55 - 7.68 (1 H, m), 7.43 - 7.57 (3 H, m), 7.33 - 7.44 (3 H, m), 7.19 - 7.34 (2 H, m), 5.32 - 5.49 (1 H, m), 2.96 - 3.13 (1 H, m), 2.48 - 2.62 (1 H, m), 2.30 - 2.48 (1 H, m), 2.11 - 2.29

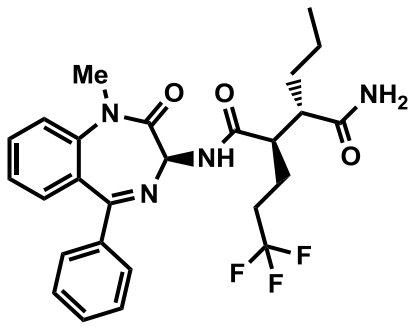
(2 H, m), 1.84 - 2.06 (3 H, m), 1.50 - 1.71 (3 H, m). $(M+H)^+$: 525. HRMS for $C_{25}H_{26}F_5N_4O_3$ $(M+H)^+$ calculated: 525.1925; found: 525.1937.

z. (2R,3S)-N-[(3S)-1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]-2-propyl-3-(3,3,3-trifluoropropyl)butanediamide (**10**)



Compound **10** was prepared via the oxidative enolate coupling route similar to compound **12**, beginning with the pentanoyl oxazolidinone analogous to Compound **13**. Obtained 65 mg of product: HPLC Ret. Time: 8.86 min (Purity 100%, Method A). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (1 H, d, *J*=8.1 Hz), 7.70 - 7.78 (1 H, m), 7.64 - 7.70 (1 H, m), 7.50 - 7.59 (4 H, m), 7.40 - 7.47 (2 H, m), 7.30 - 7.39 (2 H, m), 7.02 (1 H, s), 5.35 (1 H, d, *J*=8.1 Hz), 3.38 (3 H, s), 2.70 - 2.80 (1 H, m), 2.38 (1 H, td, *J*=10.3, 3.6 Hz), 2.05 - 2.26 (2 H, m), 1.67 - 1.80 (1 H, m), 1.53 - 1.64 (1 H, m), 1.14 - 1.49 (4 H, m), 0.83 (3 H, t, *J*=6.9 Hz). $(M+H)^+$: 503. HRMS for $C_{26}H_{30}F_3N_4O_3$ $(M+H)^+$ calculated: 503.2270; found: 503.2281.

aa. (2R,3S)-N-[(3S)-1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]-3-propyl-2-(3,3,3-trifluoropropyl)butanediamide (**11**)



Compound **11** was prepared via an alkylation route similar to Compound **12**, beginning with Compound **16**, and using allyl bromide in Preparation j. Obtained 36 mg of product: HPLC Ret. Time: 9.03 min (Purity 98%, Method A), 8.66 min (Purity 100%, Method B). ^{1}H NMR (400 MHz, DMSO-d6) δ 9.42 (1 H, d, *J*=7.3 Hz), 7.71 - 7.79 (1 H, m), 7.65 - 7.70 (1 H, m), 7.52 - 7.61 (4 H, m), 7.43 - 7.51 (2 H, m), 7.31 - 7.39 (2 H, m), 6.95 (1 H, br. s.), 5.27 (1 H, d, *J*=7.3 Hz), 3.39 (3 H, s), 2.66 - 2.77 (1 H, m), 2.53 - 2.64 (1 H, m), 2.31 - 2.42 (1 H, m), 2.14 - 2.30 (1 H, m), 1.52 - 1.64 (2 H, m), 1.39 - 1.50 (1 H, m), 1.04 - 1.29 (3 H, m), 0.81 (3 H, t, *J*=6.9 Hz). $(M+H)^+$: 503. HRMS for $C_{26}H_{30}F_3N_4O_3$ $(M+H)^+$ calculated: 503.2270; found: 503.2282.

IV. Human Notch constructs

Human constructs were generated by PCR using standard molecular biology techniques and verified by sequencing. All constructs were generated in the pCDNA3.1+ Hyg vector (Invitrogen, Carlsbad, CA) and contain an N-terminal signal sequence, Notch coding sequence and a C-terminal c-myc tag (7 copies of EQKLISEEDL). The Notch coding sequence included begins N-terminal to the putative S2 cleavage site and contains the transmembrane and cytoplasmic domains. Coding sequence in the human Notch1 construct includes amino acids 1714-2555 (Accession NP_060087.3); a M1737V mutation within the transmembrane domain was added to suppress internal translation initiation. Coding sequence in the human Notch-2 construct includes amino acids 1645-2471 (Accession NP_077719.2). Coding sequence in the human Notch-3 construct includes amino acids 1622-2321 (Accession NP_000426.2). Coding sequence in the human Notch-4 construct includes amino acids 1415-2003 (Accession NP_004548.3).