Enantioselective Total Synthesis of (-)-salinosporamide A (NPI-0052)

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Supporting Information

General Experimental Procedures. NMR spectra were collected using a 500 MHz Bruker Avance spectrometer using an inverse probe equipped with x,y,z-gradients, except for the ^{13}C NMR spectra, which were acquired with a broad-band observe probe. Data were acquired at 298 °K in CDCl_3 (referencing 7.24 ppm and 77.00 ppm), $\text{DMSO}-d_6$ (2.50 and 39.50 ppm), CD_3OD (3.30 and 49.00 ppm) or $\text{acetone}-d_6$ (2.04 and 29.80 ppm) for ^1H and ^{13}C -NMR, respectively. The LC-MS data were obtained from an Agilent HP1100 HPLC equipped with an Agilent PDA detector (the mobile phase was a mixture of CH_3CN and H_2O with TFA as an additive) and MSD system. The optical rotations were obtained from Rudolph Autopol-III automatic polarimeter and the melting point was from Mel-Temp apparatus.

Procedure to Synthesize Compounds 1-17:

Synthesis of Compound 7: To a suspension of D-serine methylester hydrochloride (375 g, 2.41 mol) in pentane (8 L) at rt were added *t*-butyl aldehyde (311.4 g, 3.616 mol) and Et_3N (268.3 g, 370 mL, 2.65 mol). The reaction mixture was refluxed O/N at 35 °C using Dean-Stark apparatus. The resulting reaction mixture was cooled to room temperature, filtered and the white solid was washed with pentane (1 L). The combined filtrate was concentrated under reduced pressure and dried under high vacuum to afford compound **7** (447 g, 99% yield) as a clear oil, which can be used without further purification. The structure was confirmed by ^1H -NMR (Fig-1).

Synthesis of *t*-butyl- α -allylacetate (Method A): To a solution of *t*-butylacetate (30 g, 0.19 mol) in dry THF (800 mL) at 0 °C was added *t*-BuOK (23.41 g, 95% w/w, 0.21 mol) and the solution was stirred for about 15 minutes. Allylbromide (18.39 g, 0.152 mol) was added and the solution was stirred at 0 °C for an additional 15 min. The reaction mixture was then allowed to warm to room temperature and stirred for about 5 hours under an atmosphere of N_2 . The above reaction mixture was then cooled to 0 °C, quenched with H_2O (300 mL), and extracted with EtOAc (3 x 200 mL). The combined organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (5 cm ID x 45 cm) using a solvent gradient of 100% hexanes (1.5 L) to 1.5% EtOAc /hexanes (3 L) to 2.5% EtOAc /hexanes (1 L) to 4% EtOAc /hexanes (700 mL) to afford pure compound *t*-butyl- α -allylacetate (14.5g, 38.5% yield). (Note: fractional distillation is the best method for purification of *t*-butyl- α -allylacetate; see Method B below for procedure.)

Synthesis of *t*-butyl- α -allylacetate (Method B): To a solution of *t*-BuOK (50g, 95% w/w, 0.42 mol) in dry THF (1.5 L) at 0 °C was added *t*-butylacetate (65 g, 0.41 mol) and the solution was stirred for about 15 minutes under an atmosphere of N_2 . Allylbromide (47g, 0.39 mol) was added slowly and the solution was stirred at 0 °C for about 20 hours. The reaction

mixture was allowed to warm to room temperature and stirred for additional 15 hours. The reaction mixture was then quenched with H₂O (1L) at 0 °C and extracted with EtOAc (3 x 0.5 L). The organic phase was dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by fractional distillation (130 °C oil bath, 90-95 °C bp) under high vacuum (12 mm Hg) to afford *t*-butyl- α -allylacetoacetate (54 g, 66% yield). ¹H-NMR (Fig-2): δ 5.68 (m, 1H), 5.03 (br dd, *J*=1, 17 Hz, 1H), 4.97 (br dd, *J*= 1, 10 Hz, 1H), 3.35 (t, *J*= 7.5 1H), 2.48 (br t, *J*=7.0, 2H), 2.16 (s, 3H), 1.39 (s, 9H).

Synthesis of Compound 8: To a solution of *t*-butyl- α -allylacetoacetate (606 g, 3.06 mol) in heptane (7 L) were added ethylene glycol (94 g, 15.28 mol) and PPTS (23 g, 0.09 mol). The reaction mixture was refluxed using Dean-Stark apparatus for 2 days, then cooled to room temperature. The reaction mixture was then neutralized with 13 mL of Et₃N and diluted with H₂O (5 L). The organic layer was separated, dried over MgSO₄ and concentrated under reduced pressure to afford 1,3-dioxolane derivative of *t*-butyl- α -allylacetoacetate (626 g, 0.18 mmol, 89% yield), which can be used for the next step without purification. ¹H-NMR (Fig-3): δ 5.72 (m, 1H), 5.06 (dd, *J*=1, 17 Hz, 1H), 4.97 (d, *J*= 10 Hz, 1H), 3.94 (m, 4H), 2.60 (dd, *J*=3.6, 11.5 Hz, 1H), 2.43 (m, 1H), 2.29 (m, 1H), 1.42 (s, 9H), 1.38 (s, 3H).

To a solution of 1,3-dioxolane derivative of *t*-butyl- α -allylacetoacetate (28g, 0.115 mol) in CH₂Cl₂ (28 mL) at 0 °C was added trifluoroacetic acid (TFA neat, 56 mL, 0.727 mol) and the solution was stirred for about 5 min. The reaction mixture was then allowed to warm to room temperature and stirred for one hour. The reaction mixture was diluted with CH₂Cl₂ (400 mL) and extracted with ice cold water (3 x 300 mL). The organic layer was dried over Na₂SO₄, concentrated under reduced pressure and dried under high-vacuum for about one hour (to remove the residual TFA) to afford compound **8** (15.5g, 72% yield) as light yellow oil, which can be used for the next step without purification. ¹H-NMR (Fig-4): δ 5.77 (m, 1H), 5.10 (br dd, *J*=1, 17 Hz, 1H), 5.02 (br d, *J*= 10 Hz, 1H), 4.00 (m, 4H), 2.76 (dd, *J*=3.8, 11.0 Hz, 1H), 2.43 (m, 2H), 1.41 (s, 3H).

Synthesis of Compound 9: To a solution of compound **8** (4.8g, 25.81 mmol) in dry CH₂Cl₂ (200 mL) at 0 °C were added Et₃N (7.82g, 77.42 mmol) and methanesulfonyl chloride (5.89g, 51.62 mmol) and the solution was stirred for about 10 min. Then compound **7** (5.31g, 28.4 mmol) was added, the reaction mixture was allowed to warm to room temperature slowly and stirred for about 15 hours. Then the reaction mixture was quenched with H₂O (200 mL) and extracted with CH₂Cl₂ (3 x 100 mL). The combined organic layer was dried over Na₂SO₄ and concentrated under reduced pressure to yield a mixture of two diastereomers (3:2 by LC-MS; Fig-5). The crude product was purified by silica flash chromatography (3 cm ID x 30 cm) using a solvent gradient of 19:1 (500 mL) to 9:1 (500 mL) to 17:3 (500 mL) to 4:1 (1.5 L) to 3:1 (1 L) hexanes/EtOAc to afford compound **9** (6g, 66% yield). ¹H-NMR (Fig-6); MS (ESI) *m/z* 356 [M+H]⁺.

Note: The product ratio was determined by proton NMR and LC-MS. The extracted ion chromatogram (*m/z* 356 [M+H]⁺) clearly indicated the presence of only two products of the expected mass, confirming that only 2 of the possible 8 diastereomers were present. The relative stereochemistry of **9** was assigned based on analysis of the deprotected product **6**.

Synthesis of Compound 6: To a solution of compound **9** (6 g, 16.9 mmol) in CH₃CN (350 mL) were added sodium iodide (3.3 g, 21.97 mmol) and cerium (III) chloride heptahydrate (9.45 g, 25.35 mmol) and the reaction mixture was stirred at 60-65 °C for 4 hours (the reaction progress can be monitored by LC-MS). The above reaction mixture was then quenched with water (200 mL) and extracted with EtOAc (3 x 150 mL). The combined organic layer (cloudy) was concentrated under reduced pressure to remove all of the CH₃CN/EtOAc, leaving about 20 mL of H₂O, which was further extracted with EtOAc (100 mL). The organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to afford compound **6** (4.4 g, 84% yield) as a mixture of two diastereomers (3:2; see Fig 7-9 for LC-MS, ¹H-NMR and NOESY), which can be used for the next step without purification. A portion of sample **6** (obtained from different lot) was further purified by reversed phase HPLC using a C-18 column (150 mm x 21 mm), and an isocratic solvent system of 40% acetonitrile in H₂O at a flow rate of 14.5 mL/min to afford compound **6a** and **6b** as pure samples.

Compound **6a**: ¹H-NMR (Fig-10) δ 5.73 (m, 1H), 5.34 (s, 1H), 5.12 (m, 1H), 5.05 (d, *J* = 10.1 Hz, 1H), 4.64 (d, *J* = 6.3 Hz, 1H), 4.53 (d, *J* = 8.2 Hz, 1H), 3.90 (t, *J* = 7.6 Hz, 1H), 3.80, (s, 3H), 3.67 (t, *J* = 7.6 Hz, 1H), 2.60 (m, 2H), 2.27 (s, 3H), 0.91 (s, 9H); MS (ESI) *m/z* 312 [M+H]⁺.

Compound **6b**: ¹H-NMR (Fig-11) δ 5.76 (m, 1H), 5.28 (s, 1H), 5.18 (br d, *J* = 17.3 Hz 1H), 5.08 (d, *J* = 10.1 Hz, 1H), 4.88 (m, 1H), 4.52 (d, *J* = 8.2 Hz, 1H), 3.88 (m, 1H), 3.81, (m, 1H), 3.76 (s, 3H), 2.88 (m, 1H), 2.63 (m, 1H), 2.21 (s, 3H), 0.86 (s, 9H); MS (ESI) *m/z* 312 [M+H]⁺.

Synthesis of Compound 5: To a solution of compound **6** (26 g, 83.6 mmol) in dry THF (2.7 L) at rt was added *t*-BuOK (4.68 g, 41.8 mmol). The reaction mixture was stirred at rt for 15 min under an atmosphere of N₂ and then quenched with H₂O (900 mL) and extracted with EtOAc (3 x 400 mL). The combined organic phase was washed with saturated brine solution, dried over Na₂SO₄ and concentrated under reduced pressure. The reaction mixture was dissolved in 1:1 ether : hexanes (75 mL each) and transferred to a crystallization dish, where it was allowed to stand and crystallize. After an hour, the crystals (1st crop) were separated by decanting the mother liquor. The mother liquor was concentrated and the crystallization process was repeated as described above to yield a 2nd crop of crystals. The two crops of crystals were combined to obtain Compound **5** (13.5 g, 52% yield by crystallization). The mother liquor was chromatographed on a silica gel flash column (30 x 4 cm) using solvent gradient of 19:1 (500 mL) to 9:1 (1 L) to 17:3 (500 mL) hexanes/EtOAc to yield the dehydration product **25** (2.47 g) and the diastereomers **5** (3.05 g), **23** (250 mg as a mixture) and **24** (1.81 g). The combined yield of **5** is 64%. The ratio of products in the reaction mixture was determined by integration of the proton NMR and analytical HPLC data. The structures of **5** and **23** were confirmed by X-ray crystallography, and **24** was characterized by analysis of ¹H, ¹³C-NMR and NOESY spectra.

Compound **5**: Compound **5** was obtained as a colorless crystalline material, m.p. 113-114 °C (crystals obtained from 1:1; diethyl ether:hexanes); [α]_D²² 8.4 (c 0.96, CH₃CN); ¹H-NMR (Fig-12) δ 5.96 (m, 1H), 5.15 (br dd, *J* = 1.5, 17.2 Hz, 1H), 5.05 (d, *J* = 10.1 Hz, 1H), 4.93 (s, 1H), 4.50 (d, *J* = 8.9 Hz, 1H), 4.26 (d, *J* = 8.9 Hz, 1H), 3.77 (s, 3H), 3.10 (t, *J* = 6.7 Hz, 1H), 2.56 (m, 1H), 2.31 (m, 1H), 1.96 (s, 1H), 1.30 (s, 3H), 0.87 (s, 9H); ¹³C-NMR (Fig-13) δ 177.9, 171.8, 136.7, 116.6, 96.7, 80.4, 79.2, 68.0, 53.3, 52.6, 36.5, 27.9, 25.0 (3xCH₃), 23.0; MS (ESI) *m/z* 312 [M+H]⁺; HRMS (ESI) calcd for C₁₆H₂₆NO₅ [M+H]⁺: 312.1811; found: 312.1808 (Δ -1.0 ppm). COSY spectrum: see Fig 14. The structure of compound **5** was confirmed by x-ray crystallography (Fig-1 in publication). The details of the crystal structure of **5** may be obtained from Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (e-mail: deposit@ccdc.cam.ac.uk), quoting the depository number CCDC 622788.

Compound **23**: Compound **23** was further purified by reversed phase HPLC using a solvent gradient of 30% to 70% CH₃CN/H₂O over 30 min, at a flow rate of 14.5 mL/min to yield pure compound. ¹H-NMR (Fig-15) δ 5.88 (m, 1H), 5.09 (br dd, *J* = 1.5, 17 Hz, 2H), 4.9 (s, 1H), 4.52 (d, *J* = 9 Hz, 1H), 4.2 (d, *J* = 9 Hz, 1H), 3.77 (s, 3H), 2.68 (m, 1H), 2.51 (t, *J* = 7 Hz, 1H), 2.45 (m, 1H), 1.29 (s, 3H), 0.89 (s, 9H); MS (ESI) *m/z* 312 [M+H]⁺. The structure was confirmed by x-ray crystallography (Fig-16). The details of the crystal structure of **23** may be obtained from Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (e-mail: deposit@ccdc.cam.ac.uk), quoting the depository number CCDC 622789.

Compound **24**: $^1\text{H-NMR}$ (Fig-17): δ 5.93 (m, 1H), 5.16 (br dd, $J = 1, 17$ Hz, 1H), 5.06 (br d, $J = 10$ Hz, 1H), 4.88 (s, 1H), 4.58 (d, $J = 9.5$ Hz, 1H), 3.96 (d, $J = 9.5$ Hz, 1H), 3.79 (s, 3H), 3.43 (dd, $J = 6.3, 8.5$ Hz, 1H), 2.53 (m, 1H), 2.17 (m, 1H), 1.27 (s, 3H), 0.86 (s, 9H) and $^{13}\text{C-NMR}$ (Fig-18): δ 175.8, 171.5, 135.8, 116.9, 96.2, 80.9, 78.3, 68.8, 53.3, 52.6, 36.5, 28.8, 25.0, 20.2. MS (ESI) m/z 312 $[\text{M}+\text{H}]^+$. The relative stereochemistry was determined by NOESY (Fig-19).

Compound **25**: $^1\text{H-NMR}$ (Fig-20) δ 5.81 (m, 1H), 5.04 (br dd, $J = 1.5, 7.5$ Hz, 1H), 5.02 (s, 1H), 4.78 (d, $J = 8.5$ Hz, 1H), 4.66 (s, 1H), 3.74 (s, 3H), 3.18 (d, $J = 8.5$ Hz, 1H), 2.97 (t, $J = 6.5$ Hz, 1H), 1.83 (s, 3H), 0.91 (s, 9H); $^{13}\text{C-NMR}$ (Fig-21) δ 178.4, 170.0, 151.9, 133.4, 132.8, 116.1, 96.9, 78.0, 70.5, 52.9, 35.2, 27.6, 24.7, 12.1; MS (ESI) m/z 294 $[\text{M}+\text{H}]^+$.

Synthesis of Compound 10: To a solution of compound **5** (10 g, 32.15 mmol) in THF/ H_2O (1:1, 600 mL) were added NMO (50% w/w aqueous solution, 15 mL, 64.3 mmol) and OsO_4 (2.5% wt.% in 2-methyl-2-propanol, 22 mL, 1.61 mmol). The resulting mixture was stirred at rt for 3 hours. Then NaIO_4 (20.6 g, 96.45 mmol) was added and the reaction mixture was stirred for an additional 2 hours at rt before diluting with ethyl acetate (250 mL) and washing with water (2x200 mL), saturated aqueous Na_2SO_3 (200 mL), and brine (200 mL). The organic layer was dried over MgSO_4 , concentrated under reduced pressure and dried under high vacuum to yield **10** (10 g, 99% yield) as a mixture of diastereomers which was used in the next step without any column chromatography. $^1\text{H-NMR}$ see Fig-22. MS (ESI) m/z 314 $[\text{M}+\text{H}]^+$.

Synthesis of Compounds 11: To a solution of compound **10** (10 g, 31.95 mmol) in dry THF (500 mL) was added $t\text{-BuOK}$ (4.29 g, 38.34 mmol) and the solution was stirred at room temperature for 20 min. Then benzyl bromide (6.56 g, 4.55 mL, 38.34 mmol) was added to the reaction mixture and stirred at rt for 6 hours. The reaction was quenched with H_2O (350 mL) and extracted with EtOAc (3 x 200 mL). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica flash column (3 cm ID x 45 cm) chromatography using a solvent gradient of 19:1 (300 mL) to 9:1 (300 mL) to 17:3 (300 mL) to 4:1 (250 mL) to 7:3 (350 mL) hexanes/EtOAc to afford compound **11** (8.8 g, 76% yield) as the major product and diastereomer of **11** (2.9 g, not a pure sample) as a minor product, with a combined yield of 91%. $^1\text{H-NMR}$, see Fig-23 for **11** and Fig-24 for diastereomer of **11**. The structure of **11** (crystallized from hexanes:ethylether; 1:1) was confirmed by X-ray crystallography (Fig-25). MS (ESI) m/z 404 $[\text{M}+\text{H}]^+$.

Compound 11: $^1\text{H-NMR}$ δ 7.27 (m, 5H), 5.19 (d, $J = 5.0$ Hz, 1H), 4.65 (d, $J = 11.4$ Hz, 1H), 4.65 (d, $J = 8.5$ Hz, 1H), 4.60 (s, 1H), 4.45 (d, $J = 12.0$ Hz, 1H), 4.21 (d, $J = 8.5$ Hz, 1H), 3.76 (s, 3H), 3.17 (d, $J = 8.5$ Hz, 1H), 2.60 (d, $J = 13.2$ Hz, 1H), 2.13 (m, 1H), 1.23 (s, 3H), 0.82 (s, 9H); MS (ESI) m/z 404 $[\text{M}+\text{H}]^+$.

Diastereomer of 11: $^1\text{H-NMR}$ δ 7.30 (m, 5H), 5.24 (dd, $J = 4.4, 6.3$ Hz, 1H), 4.77 (s, 1H), 4.72 (d, $J = 12.0$ Hz, 1H), 4.64 (d, $J = 8.5$ Hz, 1H), 4.45 (d, $J = 11.7$ Hz, 1H), 4.17 (d, $J = 8.5$ Hz, 1H), 3.78 (s, 3H), 3.36 (d, $J = 8.5$ Hz, 1H), 2.81 (ddd, $J = 1.0, 6.3, 14.2$ Hz, 1H), 2.13 (m, 1H), 1.37 (s, 3H), 0.86 (s, 9H); MS (ESI) m/z 404 $[\text{M}+\text{H}]^+$.

Synthesis of Compound 4: To a solution of compound **11** (5.3 g, 14.13 mmol) in dry THF (550 mL) was added lithium borohydride (2M solution in THF, 98.5 mL, 211.9 mmol) and the reaction mixture was stirred at rt. After 30 minutes of stirring, methanol (5 mL) was added to the reaction mixture at rt (room temperature was maintained by cooling the reaction mixture with a water bath). After 24 hours of additional stirring, the reaction mixture was quenched with H₂O (350 mL) and extracted with ethyl acetate (3 x 250 mL). The combined organic layer was washed with brine (250 mL), dried over Na₂SO₄ and concentrated under reduced pressure to afford a C-5 primary alcohol as a white solid (5 g, 94% yield), which can be used in the next step without any further purification. ¹H-NMR see Fig-26. MS (ESI) *m/z* 376 [M+H]⁺ and 398 [M+Na]⁺.

To a solution of the above prepared C-5 primary alcohol (5 g, 13.3 mmol) in dry CH₂Cl₂ (200 mL) were added NMO (4.67 g, 39.9 mmol) and TPAP (470.8 mg, 1.33 mmol). The resulting mixture was stirred at rt for 18 hours. The reaction mixture was then concentrated under reduced pressure and purified by silica flash chromatography (column 3 cm ID x 35 cm) using a solvent gradient of 19:1 (200 mL) to 9:1 (200 mL) to 17:3 (200 mL) to 4:1 (250 mL) to 7:3 (300 mL) hexanes/EtOAc to afford aldehyde **4** as a pale yellow oil (3.9 g, 79% yield). ¹H-NMR see Fig-27. MS (ESI) *m/z* 374 [M+H]⁺.

Synthesis of Cyclohex-2-enylzinc chloride: To a solution of 1,3-cyclohexadiene (0.96 g, 12 mmol, d = 0.84, 1143 uL) and Pd(PPh₃)₄ (462.2 mg, 0.4 mmol) in benzene (10 mL) under an atmosphere of nitrogen was added Bu₃SnH (1.16g, 4 mmol, d = 1.098, 1.06 mL) dropwise at rt and stirred for 15 minutes. The solvent was removed under reduced pressure and the resulting product was purified on silica flash chromatography (column 1.5 cm ID x 20 cm) using a solvent gradient of 10:0 (100 mL) to 19:1(100 mL) to 9:1 (100 mL) of hexanes/EtOAc to afford cyclohexenyltributyltin (3.5 g, 78.6% yield) as a clear liquid.

To a solution of the above prepared cyclohexenyltributyltin (0.92 g, 2.5 mmol) in THF (5 mL) at -78 °C under an atmosphere of nitrogen was added *n*BuLi (1 mL, 2.5 M solution in hexane, 2.5 mmol). After an additional 30 min stirring, ZnCl₂ (340 mg, 2.5 mmol, dissolved in 2 mL of THF) was added and stirring was continued at this temperature for 30 min at -78 °C to afford 2-cyclohexenylzinc chloride.

Synthesis of 5R,6R-cyclohexene derivative (C-6 epimer of 3): To a solution of aldehyde **4** (35 mg, 0.094 mmol) in 5 mL of THF at -78 °C, 1.2 mL of cyclohexenyl zinc chloride (freshly prepared) was added and stirred at -78 °C for about 3 hours. The reaction was quenched with H₂O (15 mL) and extracted with EtOAc (2 x 20 mL). The combined organic phase was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to yield a liquid residue. This residue was dissolved in 5 mL of hexanes and allowed to stand for an hour. A white solid was precipitated from the residue, which was separated by decanting the solvent. The solid material was further washed with hexanes (2 x 2 mL) and dried on high-vacuum to afford pure 5R,6R

cyclohexene derivative (C-6 epimer of **3**) (32 mg, 75% yield). $^1\text{H-NMR}$ see Fig-28 and $^{13}\text{C-NMR}$ see Fig-29. MS (ESI) m/z 456 $[\text{M}+\text{H}]^+$ and 478 $[\text{M}+\text{Na}]^+$. The stereochemistry was determined by X-ray crystallography (see Fig 30).

Synthesis of B-2-cyclohexen-1-yl-9-BBN: A solution of 9-borabicyclo[3.3.1]nonane (9-BBN) in THF (0.5 M, 40.0 mL, 20.0 mmol) was concentrated to 50% of the original volume using house-vacuum at 40 °C (on an oil bath). To this white cloudy suspension of 9-BBN in THF (1.0 M, 20.0 mL, 20.0 mmol) was added 1,3-cyclohexadiene (1.96 mL, 20.0 mmol) under an inert atmosphere at rt and stirred for 2 days to afford a solution of B-2-cyclohexen-1-yl-9-BBN in THF (1.0 M) which was directly used to couple with aldehyde **4**.

Synthesis of Compound 3: To a solution of the aldehyde **4** (3.9 g, 10 mmol) in THF (60 mL) at -78 °C was added the 9-cyclohex-2-enyl-9-BBN solution in THF (1 M, 15.6 mL, 15.6 mmol). The reaction mixture was allowed to warm to rt over 1.5 hr and stirred for an additional 10 hours at rt. Ethanolamine (3.15 mL, 52.0 mmol) was then added to the above reaction mixture and stirring continued for an additional 8 hours at rt. The reaction mixture was then filtered through a short silica plug, concentrated under reduced pressure and the resulting residue was purified by silica flash chromatography using a solvent gradient of EtOAc/hexanes (5-20%) to afford 5R,6S-cyclohexene derivative **3** as a white solid (3.66 g, 8.0 mmol, 80.4%). $^1\text{H-NMR}$ see Fig-31 and $^{13}\text{C-NMR}$ see Fig-32. MS (ESI) m/z 456 $[\text{M}+\text{H}]^+$ and 478 $[\text{M}+\text{Na}]^+$. HRMS (ESI) m/z 456.2750 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{27}\text{H}_{38}\text{NO}_5$, 456.2750, $\Delta = 0.0$ ppm), m/z 478.2569 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{27}\text{H}_{37}\text{NO}_5\text{Na}$, 478.2549, $\Delta = -4.3$ ppm). The structure of **3** (crystals obtained from 1:1 EtOAc/hexanes) was confirmed by X-ray crystallography (Fig-33).

Synthesis of Compound 12: To a solution of **3** (400 mg, 0.88 mmol) in THF (20 mL) was added aqueous HCl (0.5 N, 2 mL). The reaction mixture was warmed to 60 °C and stirred for 10 hours at this temperature. The above reaction mixture was diluted with H_2O (20 mL), then extracted with EtOAc (2 X 20 mL) and CH_2Cl_2 (3 X 20 mL). The combined organic phase was dried over MgSO_4 and concentrated under reduced pressure. The crude residue was re-dissolved in THF/ H_2O (2:1; 22.5 mL); then NaBH_4 (100 mg, 2.63 mmol) was added and stirred at rt for 30 min. The reaction mixture was diluted with H_2O (20 mL) and extracted with EtOAc (2 X 20 mL) and CH_2Cl_2 (3 X 20 mL), and the organic phase was dried over MgSO_4 and concentrated under reduced pressure to afford the C-13 primary alcohol of **12** as crude white solid (260 mg, 81%) which can be used in the next step without purification. $^1\text{H-NMR}$ see Fig-34 and $^{13}\text{C-NMR}$ see Fig-35. MS (ESI) m/z 368.3 $[\text{M}+\text{H}]^+$.

To a solution of the above prepared C-13 primary alcohol of **12** (120 mg, 0.33 mmol) in CH_2Cl_2 (5 mL) were added Et_3N (120 μl , 0.86 mmol) and benzoyl chloride (BzCl , 60 μl , 0.52 mmol). The reaction mixture was stirred at rt for 10 hours. Then the reaction mixture was directly concentrated under reduced pressure and the resulting product was purified by silica flash chromatography (EtOAc in hexanes, 10% to 30%) to afford benzoyl derivative **12** (136 mg, 0.29 mmol, 87%). $^1\text{H-NMR}$ see Fig-36. MS (ESI) m/z 472.3 $[\text{M}+\text{H}]^+$.

Synthesis of Compound 13: To a solution of **12** (136 mg, 0.29 mmol) in $\text{CF}_3\text{CH}_2\text{OH}$ (2 mL) were added 1,3-propanedithiol (200 μl , 2 mmol) and a catalytic amount of aqueous HCl (12N, 10 μL). The reaction mixture was stirred at 60 $^\circ\text{C}$ for 4 hr, concentrated under reduced pressure and the resulting crude product was then purified by silica flash chromatography (EtOAc in hexanes, 20% to 80%) to afford **13** (110 mg, 94%;). $^1\text{H-NMR}$ see Fig-37 and $^{13}\text{C-NMR}$ see Fig-38. MS (ESI) m/z 404.3 $[\text{M}+\text{H}]^+$.

Synthesis of Compound 14: To a solution of **13** (70 mg, 0.17 mmol) in CH_2Cl_2 (2 mL) were added Et_3N (480 μL , 3.47 mmol) and TMSCl (220 μL , 1.74 mmol) and the solution was stirred at rt for 12 hours. The reaction was quenched with saturated aqueous NaHCO_3 (5 mL) and extracted with CH_2Cl_2 (3 x 5 mL). The combined organic phase was dried over MgSO_4 , concentrated under reduced pressure and then purified by silica flash chromatography (EtOAc in hexanes, 20% to 80%) to afford **14** (44 mg, 53% yield). $^1\text{H-NMR}$ see Fig-39 and $^{13}\text{C-NMR}$ see Fig-40. MS (ESI) m/z 476.3 $[\text{M}+\text{H}]^+$.

Synthesis of Compound 15: To a solution of **14** (120 mg, 0.25 mmol) in CH_2Cl_2 (5 mL) was added Dess-Martin periodinane (118 mg, 0.278 mmol) and the reaction mixture was stirred at rt for 2 hours. The reaction was quenched with saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (3 mL) and saturated aqueous NaHCO_3 (2 mL) and extracted with CH_2Cl_2 (2 x 5 mL). The combined organic phase was dried over MgSO_4 and concentrated under reduced pressure to afford the crude aldehyde as a white powder, which was used for the next step without purification.

To a solution of the above freshly prepared aldehyde in *t*-BuOH/H₂O (2:1; 4.5 mL) was added NaH₂PO₄ (400 mg, 3.33 mmol) and the reaction mixture was cooled to 0 °C. Then 2-methyl-2-butene (2M in THF, 3.70 mL, 7.57 mmol) and NaClO₂ (175 mg, 1.93 mmol) were added sequentially and stirred at 0 °C for 1.5 hours. The reaction mixture was then diluted with brine (5 mL) and extracted with EtOAc (2 x 5 mL). The aqueous phase was acidified with HCl (0.5 M) to pH 3.0 and extracted with CH₂Cl₂ (3 x 5 mL). The combined organic phase was dried over MgSO₄ and concentrated under reduced pressure to yield a white solid residue which was purified by reversed phase HPLC using an ACE 5μ C18 column (150 x 21 mm ID) and a solvent gradient of 10% to 100% CH₃CN/H₂O/0.05% TFA over 22 min, holding at 100% CH₃CN/0.05% TFA for 3 min at a flow rate of 14.5 mL/min to afford the carboxylic acid, **15** (66 mg, 63% yield over two steps). ¹H-NMR see Fig-41 and ¹³C-NMR see Fig-42. MS (ESI) *m/z* 418.2 [M+H]⁺.

Synthesis of Compound 16: To a solution of carboxylic acid **15** (100 mg, 0.24 mmol) in MeOH (2.5 ml) was added K₂CO₃ (99.5 mg, 0.72 mmol) and the reaction mixture was stirred at rt for 15 hours. Then aqueous HCl (400 μl, 1.0 N) was added to this reaction mixture which was directly concentrated and dried under high vacuum to afford the C-13 primary alcohol of **15** as a white residue, which was directly used for the next step without further purification.

To a solution of the above prepared C-13 primary alcohol of **15** in CH₃CN (2.0 ml) were added pyridine (1.5 ml) and BOPCl (183 mg, 0.72 mmol) and the reaction mixture was stirred at rt for 2 hours. Then the reaction mixture was concentrated under reduced pressure and purified by silica flash chromatography (EtOAc in hexanes, 10% to 100%) to afford β-lactone **16** (42.5 mg, 60% overall yield from **15**). ¹H-NMR see Fig-43 and ¹³C-NMR see Fig-44. MS (ESI) *m/z* 296 [M+H]⁺.

Synthesis of Compound 2: To a solution of β-lactone **16** (35 mg, 0.118 mmol) in CH₃CN (250 μL) were added pyridine (250 μL) and Ph₃PCl₂ (80 mg, 0.24 mmol) and the reaction mixture was stirred at rt for 18 hours. Then the reaction mixture was concentrated under reduced pressure and purified by silica gel chromatography using a EtOAc/hexanes gradient (5% to 20%) to afford **2** (21 mg, 57% yield). See Fig-45 and Fig-46 for ¹H and ¹³C-NMR spectra. MS (ESI) *m/z* 314 [M+H]⁺. HRMS (ESI) *m/z* 314.1151 [M+H]⁺ (calcd for C₁₅H₂₁ClNO₄, 314.1159, Δ = -2.4 ppm).

Synthesis of Compound 17: To a solution of compound **2** (10 mg, 32 μmol) in CH_2Cl_2 (4 mL) in a round bottom flask (25 mL) were added Dess-Martin periodinane (20.35 mg, 48 μmol). The reaction mixture was stirred at rt for about 2 hours then quenched with saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (5 mL) and saturated aqueous NaHCO_3 (5 mL), and then extracted with CH_2Cl_2 (2 x 5 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude product was then purified by silica flash column (0.4 cm ID x 3 cm) chromatography using a solvent gradient of 19:1 (5 mL) to 9:1 (5 mL) to 17:3 (5 mL) to 4:1 (10 mL) hexanes/EtOAc to afford **17** (6 mg, 60% yield). ^1H NMR see Fig-47; MS (ESI), m/z 312 $[\text{M}+\text{H}]^+$ and 334 $[\text{M}+\text{Na}]^+$.

Synthesis of NPI-0052 (1): To a solution of **17** (8 mg, 25.7 μmol) in DMSO (0.5 mL) in a round bottom flask (25 mL), 800 μL of potassium phosphate buffer (150 mM, pH 6.9), 16 mg of ketoreductase EXP-B1Y, 1.6 mg of glucose dehydrogenase (GDH), 400 μL of glucose (50 mM) and 400 μL of NAD^+ (1 mM) were added. The above reaction mixture was stirred at 37-39 $^\circ\text{C}$ for about 40 min and then extracted with EtOAc (2 x 15 mL); the combined organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. This afforded about 6.2 mg of **NPI-0052** as a crude product which was purified by silica gel flash chromatography (2.5 cm ID x 4 cm) using a solvent gradient of 9:1 to 4:1 to 7:3 to 3:2 to 1:1 (20 mL each) EtOAc/hexanes to afford pure **NPI-0052** (4.5 mg, 56%). $[\alpha]_{\text{D}}^{-70}$ (c 0.05, CH_3CN). The ^1H NMR (see Fig-48) and ^{13}C NMR (see Fig-50) spectra were in complete agreement with those of an authentic sample of NPI-0052 (Fig-49 and Fig-51 respectively). MS (ESI) m/z 314 $[\text{M}+\text{H}]^+$. HRMS (ESI) m/z 314.1173 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{15}\text{H}_{21}\text{ClNO}_4$, 314.1159, $\Delta = 4.5$ ppm).

Prior to this, the conversion of **17** to **1** was demonstrated on a semisynthetic product with 85-90% yield after purification. Ref: R.R. Manam, V.R. Macherla, B.C.M. Potts, *Tetrahedron Lett.* **2007**, 48, 2537-2540.

Fig-1: ¹H-NMR of Compound 7

Fig-2: ¹H-NMR of *t*-butyl- α -allylacetoacetate

Fig-3: ^1H -NMR of 1,3-dioxolane derivative of *t*-butyl- α -allylacetoacetate

Fig-4: ^1H -NMR of Compound **8** in CDCl_3

Fig-5: LC-MS of Compound **9**

Fig-6: ^1H -NMR of Compound **9** in CDCl_3

Fig-7: LC-MS of Compound **6**

Fig-8: ^1H -NMR of Compound **6** in CDCl_3

Fig-9: NOESY of Compound **6** in CDCl_3

Fig-10: ^1H -NMR of Compound **6a** in CDCl_3

Fig-11: ^1H -NMR of Compound **6b** in CDCl_3

Fig-12: ¹H-NMR of Compound **5** in CDCl₃

Fig-13: ¹³C-NMR of Compound **5** in CDCl₃

Fig-14: ^1H - ^1H COSY of Compound **5** in CDCl_3

Fig-15: ^1H NMR of Compound **23** in CDCl_3

Fig-16. ORTEP plot of the X-ray crystal structure of **23**

Fig-17: ^1H NMR of Compound **24** in CDCl_3

Fig-18: ^{13}C NMR of Compound **24** in CDCl_3

Fig-19: NOESY of Compound **24** in CDCl_3

Fig-20: ^1H NMR of Compound **25** in CDCl_3

Fig-21: ^{13}C NMR of Compound **25** in CDCl_3

Fig-22: ^1H NMR of Compound **10** in CDCl_3

Fig-23: ^1H NMR of Compound **11** in CDCl_3

Fig-24: ^1H NMR of diastereomer of **11** in CDCl_3

Fig-25. X-ray crystal structure of **11**

Fig-26: ^1H NMR of Compound C-5 primary alcohol in CDCl_3

Fig-27: ^1H NMR of Compound 4 in CDCl_3

Fig-28: ^1H NMR of Compound C-6 epimer of **3** in CDCl_3

Fig-29: ^{13}C NMR of Compound C-6 epimer of **3** in CDCl_3

Fig-30: X-ray Crystal Structure of C-6 epimer of **3**

Fig-31: ^1H NMR of Compound **3** in CDCl_3

Fig-32: ^{13}C NMR of Compound 3 in CDCl_3

Fig-33: X-Ray Crystal Structure of Compound 3

Fig-34: ¹H NMR of C-13 primary alcohol of **12** in CDCl₃

Fig-35: ¹³C NMR of C-13 primary alcohol of **12** in CDCl₃

Fig-36: ^1H NMR of Compound **12** in CDCl_3

Fig-37: ^1H NMR of Compound **13** in CDCl_3

Fig-38: ¹³C NMR of Compound **13** in CDCl₃

Fig-39: ¹H NMR of Compound **14** in CDCl₃

Fig-40: ^{13}C NMR of Compound **14** in CDCl_3

Fig-41: ^1H NMR of Compound **15** in CD_3OD

Fig-42: ^{13}C NMR of Compound **15** in CD_3OD

Fig-43: ^1H NMR of Compound **16** in acetone-d_6

Fig-46: ^{13}C NMR of Compound **2** in CDCl_3

Fig-47: ^1H NMR of Compound **17** in CDCl_3

Fig-48: ^1H NMR of **NPI-0052 (1)** (synthetic) in DMSO- d_6

Fig-49: ^1H NMR of **NPI-0052 (1)** - comparison with authentic sample

Fig-50: ^{13}C NMR of NPI-0052 (1) (synthetic) in DMSO-d6

Fig-51: ^{13}C NMR of NPI-0052 (1) (synthetic) – comparison with authentic sample