Novel synthesis of various orthogonally protected $\text{C}^\alpha$-methyl lysine analogues and biological evaluation of a Vapreotide analogue containing (S)-$\alpha$-methyl lysine


Supporting Information

Scheme 8: Stereochemical Configuration of 3b
Scheme 9: Absolute configuration of 3c and 3d
Scheme 10: Absolute configuration of 3e and 3f
Synthesis of (S) – 1-benzyl -3-ethyl-2-methyl-2-(2-(1,3—dioxoindolin-2-yl)malonate (26): A 250 mL round bottom flask was charged with 10g of 2b (31 mmol), 4.3 g of K₂CO₃ (31 mmol), 100 mL of anhydrous DMF, and a stirbar. A solution of 4.8g benzyl bromide (28 mmol) in 20 mL anhydrous DMF was slowly added over 15 minutes. The reaction was allowed to stir approximately 12 hr. under a nitrogen atmosphere. The reaction mixture was then diluted with 100 mL of water and the resulting mixture was washed with Et₂O (3 x 100 mL). The combined ether layer was washed with water (5 x 100 mL), washed with brine (2 x 100 mL), dried over MgSO₄, and the solvent was removed under reduced pressure. The product was purified by flash chromatography (40% Et₂O/Hexanes) providing 11g of 26 (27 mmol, 96%) as a colorless liquid. Rₐ = 0.2 (40% Et₂O/Hexanes). [α]₂⁴ = -3.08 (c = 1, CHCl₃).

IR (cm⁻¹): 2980, 1773, 1708. ¹H-NMR (CDCl₃, 400 MHz): δ 7.83 (m, 2H), 7.70 (m, 2H), 7.33 (m, 5H), 5.15 (m, 2H), 4.10 (m, 2H), 3.74 (m, 2H), 2.28 (m, 2H), 1.56 (s, 3H), 1.16 (t, 3H, J = 7 Hz). ¹³C-NMR (CDCl₃, 100 MHz): δ 171.4, 171.3, 168.0, 135.5, 134.0, 132.0, 128.5, 128.3, 128.1, 123.0, 67.0, 61.0, 52.0, 33.8, 33.8, 20.0, 14.0. HRMS [C₂₃H₂₃NO₆Na⁺]: calculated = 432.1417, found = 432.1406.

Synthesis of (R)-ethyl-3-methyl-2-oxopyrrolidine-3-carboxylate (27): A volume of 930 µL (10.2 mmol) 35% hydrazine in water was added to a solution of 3.8g (9.3 mmol) of 26 in 50 mL MeOH. The mixture was heated to reflux solvent overnight. A white precipitate was observed within an hour of reflux. The reaction mixture was allowed to cool to RT, and the resulting mixture was filtered. The filtrate was evaporated under reduced pressure. The resulting residue was taken up in CH₂Cl₂ and washed with water. The organic layer was dried over MgSO₄, evaporated under reduced pressure, and purified by column chromatography using 30%
Hexanes/EtOAc giving 1.2 g of a 10:1 mixture of 27a:27b as a white solid. The mixture was recrystallized in cold Et<sub>2</sub>O giving 1 g (6 mmol, 64.5%) of pure 27a as white crystals. R<sub>f</sub> (27a) = 0.31 (30% Hexanes/EtOAc). MP = 63 °C. [α]<sub>D</sub> = +19.0 (c = 2, MeOH). IR (cm<sup>-1</sup>): 3245, 2985, 1726, 1698, 1660. ¹H-NMR (CDCl<sub>3</sub>, 400 MHz): δ 7.06 (bs, 1H), 4.20 (m, 2H), 3.47 (m, 1H), 3.36 (m, 1H), 2.64 (m, 1H), 2.02 (m, 1H), 1.45 (s, 3H), 1.28 (t, 3H, J = 7 Hz). ¹³C-NMR (CDCl<sub>3</sub>, 100 MHz): δ 177.0, 172.0, 61.0, 51.0, 40.0, 34.0, 20.0, 14.0. HRMS [C<sub>8</sub>H<sub>13</sub>NO<sub>3</sub>Na<sup>+</sup>]: calculated = 194.0788, found = 194.0795.

(R) – 3- methyl-2-oxopyrrolidine-3-carboxylic acid (28): An amount of 1.6 g (9.4 mmol) of 27a was dissolved in 15 mL ethanol. A volume of 7 mL 1N NaOH was added to the reaction mixture. The solution was brought to reflux solvent for an hour. The solution was cooled and acidified with HCl to pH 4. The water layer was concentrated at 35 °C under high vacuum. A volume of 10 mL MeOH was added to the residue and stirred for 5 min. The MeOH layer was decanted from the remaining solid and concentrated in vacuo giving 1 g of 28 (6.9 mmol, 73%) as a white solid. MP = 155 °C. IR (cm<sup>-1</sup>) = 3363, 3368, 2975, 2906, 1749, 1722, 1704, 1636, 1485. R<sub>f</sub> = 0.17 (5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>). ¹H-NMR (CD<sub>3</sub>OD, 400 MHz): δ 3.35 (m, 1H), 3.25 (m, 1H), 2.49 (m, 1H), 1.95 (m, 1H), 1.27 (s, 3H). ¹³C-NMR (CD<sub>3</sub>OD, 100 MHz): δ 179.6, 175.7, 52.1, 40.7, 35.0, 20.3. ESI-MS [C<sub>6</sub>H<sub>10</sub>NO<sub>3</sub>]<sup>+</sup> = 143.1, observed = 143.2.

Benzyl (R)-3- methyl-2-oxopyrrolidin-3-ylcarbamate (29): An amount of 1.77 g (12.4 mmol) of 28 was dissolved in 50 mL of dry dichloroethane. A volume of 3.6 mL (26 mmol) Et<sub>3</sub>N was added followed by 3.1 mL (13.6 mmol) diphenylphosphorylazide (DPPA). The solution was allowed to stir for 2 hrs at RT and then heated to reflux solvent for 2 hr. A volume of 1.8 mL (17.4 mmol) benzyl alcohol was then added and the solution was allowed to reflux solvent over night. The dichloroethane layer was concentrated in
vacuo and the residue was purified by flash chromatography (40% EtOAc/Hexanes) giving 1.97g of 29 as a white wax (7.9 mmol, 64%). Rf = 0.10 (40% EtOAc/Hexanes). IR (cm⁻¹) = 3225, 1725, 1693, 1657, 1536. ¹H-NMR (CDCl₃, 400 MHz): δ 7.33 (m, 5H), 6.75 (bs, 1H), 5.55 (bs, 1H), 5.06 (m, 2H), 3.34 (m, 2H), 2.52 (m, 1H), 2.31 (m, 1H), 1.40 (s, 3H). ¹³C-NMR (CDCl₃, 100 MHz): δ 178.2, 155.2, 136.2, 128.7, 128.3, 128.2, 66.7, 57.2, 39.0, 34.8, 22.3. HRMS [C₁₃H₁₆N₂O₃Na⁺] = 271.1053, observed = 271.1047.

(R)- tert-butyl- 3- methyl- 2- oxopyrrolidin-3- ylcarbamate (30): An amount of 1.6 g (6.4 mmol) of 29 was dissolved in 25 mL MeOH in a pressure bottle. An amount of 0.16g Pd-C (10%) was added to the reaction mixture. The reaction mixture was allowed to shake under 20 psi H₂ pressure for 12 hr. The MeOH layer was filtered off through a Celite bed. The filtrate was concentrated in vacuo giving 0.66g of the free amine (5.8 mmol), which was then dissolved in 20 mL THF. A volume of 1.7mL (11.6 mmol) Et₃N was added to the reaction mixture. A solution of 1.5g (BOC)₂O (6.9 mmol) in 10 mL THF was added to the reaction mixture drop wise. The reaction mixture was allowed to stir over night at RT. The THF was concentrated and the resulting residue was extracted with Et₂O and water. The ether layer was concentrated and the residue was rinsed with hexane giving 0.83g of the 30 (3.9 mmol, 61% over two steps) as a white solid. The characterization of 30 complied with the literature.¹ [α]D²⁰ = -16 (c = 0.35, CHCl₃).

Synthesis of (S)- 2- methyl-ornithinedihydrochloride (31a): A volume of 30 mL 6N HCl solution was added to 1g of 4c (2.1 mmol) in a round bottom flask. The reaction mixture was heated to reflux solvent for 24 hr. The aqueous layer was evaporated to dryness under reduced pressure. The resulting gummy solid was triturated with EtOAc multiple times leading to 0.4g (1.8 mmol, 86%) of 31a as a white solid. All the characterization data of the product complied with the literature.² [α]D²⁴ = + 6.86 (c = 0.7, 4N HCl).
Synthesis of (S)-2- methyl-lysinedihydrochloride (31b): A volume of 30 mL 6N HCl solution was added in 1g of 4d (2.1 mmol) in a round bottom flask. The reaction mixture was heated to reflux solvent for 24 hr. The aqueous layer was evaporated to dryness under reduced pressure. The resulting gummy solid was triturated with EtOAc multiple times leading to 0.36g (1.5 mmol, 71%) of 31b as a white solid. All the characterization data of the product complied with the literature.\(^2\) \([\alpha]_D^{24} = +7.25\) (c = 1, 4N HCl).

General synthetic procedure for the formation 32a, and 32b: An amount of 3e/3f (1 equivalent) was dissolve in DMF under N\(_2\). A calculated amount of K\(_2\)CO\(_3\) (1.2 equivalent) was added to the solution. A measured volume of benzyl bromide (0.95 equivalents) was added to the reaction mixture. The reaction was allowed to stir over night under N\(_2\). Water was added to the reaction mixture and the aqueous layer was extracted with Et\(_2\)O (3 x 50 mL). The combined ether layer was given a water wash (10 x 50 mL). The Et\(_2\)O layer was dried over MgSO\(_4\), concentrates, and the residue was purified by flash chromatography (40% Et\(_2\)O/Hexanes) giving the product as a colorless oil.

(S)-1- benzyl 3- ethyl-2- methyl-2-(5-(1,3-dioxoisindolin-2-yl)pentyl)malonate (32a): 32a was synthesized following the general synthetic procedure for the formation of 32a/32b using 5g (14 mmol) of 3e. An amount of 5.2g of 32a (11.5 mmol, 82%) was obtained as a colorless viscous oil after purification (40% Et\(_2\)O/Hexanes). R\(_f\) = 0.16 (40% Et\(_2\)O/Hexanes). IR (cm\(^{-1}\)) = 2938, 1770, 1700. \(^1\)H-NMR (CDCl\(_3\), 400 MHz): \(\delta\) 7.83 (m, 2H), 7.70 (m, 2H), 7.32 (m, 5H), 5.15 (m, 2H), 4.11 (q, 2H, \(J = 7Hz\)), 3.64 (q, 2H, \(J = 7Hz\)), 1.85 (m, 2H), 1.64 (m, 2H), 1.41 (s, 3H), 1.28 (m, 4H), 1.15 (t, 3H, \(J = 7Hz\)). \(^13\)C-NMR (CDCl\(_3\), 100 MHz): \(\delta\) 172.2, 172.0,
(S)-1-benzyl-3-ethyl-2-methyl-2-(6-(1,3-dioxoisoindolin-2-yl)hexyl)malonate (32b): 32b was synthesized following the general synthetic procedure for the formation of 32a/32b using 5g (13.3 mmol) of 3f. An amount of 5.3g of 32b (11.4 mmol, 86%) was obtained as a colorless viscous oil after purification (40% Et2O/Hexanes). Rf = 0.30 (40% Et2O/Hexanes). IR (cm⁻¹) = 2936, 1771, 1706. ¹H-NMR (CDCl₃, 400 MHz): δ 7.83 (m, 2H), 7.70 (m, 2H), 7.32 (m, 5H), 5.15 (m, 2H), 4.11 (q, 2H, J = 7Hz), 3.64 (t, 2H, J = 7Hz), 1.85 (m, 2H), 1.64 (m, 2H), 1.41 (s, 3H), 1.30 (m, 4H), 1.17 (m, 5H). ¹³C-NMR (CDCl₃, 100 MHz): δ 172.3, 172.1, 168.4, 136.0, 134.0, 132.2, 128.5, 128.2, 128.0, 123.2, 66.7, 61.1, 54.0, 38.0, 35.5, 29.4, 28.5, 26.5, 24.2, 20.0, 14. HRMS [C₂₇H₃₁NO₆Na⁺] calculated = 488.2043, observed = 488.2030.

General synthetic procedure for the formation of 33a, and 33b: A measured amount of 32a/32b (1 equivalent) was dissolved in methanol. A calculated amount of 35% N₂H₄.H₂O in water (1.2 equivalents) was added to the reaction mixture. The reaction mixture was heated to reflux solvent for 6 hrs. The reaction mixture was cooled to RT and the white precipitate was filtered off. The MeOH layer was concentrated in vacuo and the gummy solid was taken up in CH₂Cl₂ leading to more white precipitate. The white precipitate is again removed by filtration and the CH₂Cl₂ layer was again concentrated in vacuo giving pure product as colorless oil.

(S)-1-benzyl-3-ethyl-2-(5-aminopentyl)-2-methylmalonate (33a): 33a was prepared from 32a following the general synthetic procedure for the formation of 33a/33b using 5g of 32a (11 mmol). An amount of 3.3g (10.3 mmol, 94%) of 33a was obtained as a
colorless viscous oil. Rf = 0.12 (3% MeOH/CH2Cl2). IR (cm⁻¹) = 3100, 3000, 2938, 1724. ¹H-NMR (CDCl₃, 400 MHz): δ 7.32 (m, 5H), 5.15 (m, 2H), 4.11 (q, 2H, J = 7Hz), 2.65 (t, 2H, J = 7Hz), 1.87 (t, 2H, J = 8Hz), 1.61 (bs, 2H), 1.41 (m, 5H), 1.24 (m, 7H). ¹³C-NMR (CDCl₃, 100 MHz): δ 172.3, 172.2, 136.0, 128.5, 128.2, 128.0, 67, 61.2, 54.0, 42.0, 35.4, 33.5, 27.0, 24.0, 20.0, 14.0. HRMS [C₁₈H₂₇NO₄Na⁺] calculated = 344.1832, observed = 344.1823.

(S)-1-benzyl-3-ethyl-2-(6-aminohexyl)-2-methylmalonate (33b): 33b was prepared from 32b following the general synthetic procedure for the formation of 33a/33b using 5g of 32b (10.7 mmol). An amount of 3g (8.9 mmol, 83%) of 33b was obtained as a colorless viscous oil. Rf = 0.14 (3% MeOH/CH₂Cl₂). IR (cm⁻¹) = 3300, 2932, 1726. ¹H-NMR (CDCl₃, 400 MHz): δ 7.25 (m, 5H), 5.07 (m, 2H), 4.04 (q, 2H, J = 7Hz), 2.68 (bs, 2H), 2.61 (t, 2H, J = 7Hz), 1.77 (t, 2H, J = 7Hz), 1.33 (t, 2H, 57Hz), 1.14 (m, 9H). ¹³C-NMR (CDCl₃, 100 MHz): δ 171.3, 171.2, 135.0, 127.5, 127.2, 127.0, 66.0, 60.1, 53.0, 41.0, 34.5, 32.0, 28.5, 25.5, 23.1, 19.0, 13.0. HRMS [C₁₉H₂₉NO₄Na⁺] calculated = 358.1988, observed = 358.1983.

**General synthetic procedure for the formation of 34a, and 34b:** 34a/34b were synthesized from 33a/33b following a literature procedure.³ A measured amount of 33a/33b (1 equivalent) was dissolved in 24 mL 2:1 2.5M NaOH/EtOH mixture. The solution was cooled to 0°C and a measured amount of NH₂OSO₃H (2 equivalent) was added to the solution. The solution was stirred at 0°C for 35 minutes. At that point an additional amount of NH₂OSO₃H (1 equivalent) and 5 mL 2.5 M NaOH were added to the reaction mixture. The reaction was allowed to stir at 0°C for another 90 minutes and then allowed to warm to RT overnight. The reaction mixture was
acidified to pH 1. The aqueous layer was extracted with Et₂O (3 x 50 mL). The combined organic layer was washed with brine, dried over MgSO₄, concentrated in vacuo, and purified in 1:1 Et₂O/Hexanes giving the product as a colorless oil.

(R)- 2-(ethoxycarbonyl)-2-methylheptanoic acid (34a): 34a was prepared from 33a following the general synthetic procedure of making 34a/34b using 3g of 33a (9.3 mmol). An amount of 1.2g of 34a was obtained (5.5 mmol, 59%) after purification. R_f = 0.49 (50% Et₂O/Hexanes). IR (cm⁻¹) = 2956, 2930, 2871, 1705. [α]D₂⁵ = + 3.15 (c = 2, CH₂Cl₂). ¹H-NMR (CDCl₃, 400 MHz): δ 10.38 (bs, 1H), 4.21 (q, 2H, J = 7Hz), 1.87 (m, 2H), 1.44 (s, 3H), 1.27 (m, 9H), 0.88 (t, 3H, J = 7Hz). ¹³C-NMR (CDCl₃, 100 MHz): δ 178.0, 172.5, 61.5, 53.6, 35.7, 32.0, 24.0, 22.3, 20.0, 14.0, 13.9. HRMS [C₁₁H₂₀O₄Na⁺] calculated = 239.1255, observed = 239.1253.

(R)- 2-(ethoxycarbonyl)-2-methyloctanoic acid (34b): 34b was prepared from 33b following the general synthetic procedure of making 34a/34b using 3g of 33b (8.9 mmol). An amount of 1.3g pure 34b was obtained (5.6 mmol, 63%) after purification. R_f = 0.51 (50% Et₂O/Hexanes). IR (cm⁻¹) = 2955, 2927, 2858, 1705. [α]D₂⁵ = + 2.2 (c = 1, CH₂Cl₂). ¹H-NMR (CDCl₃, 400MHz): δ 4.21 (q, 2H, J = 7Hz), 1.87 (m, 2H), 1.44 (s, 3H), 1.28 (m, 11H), 0.88 (t, 3H, J = 7Hz). ¹³C-NMR (CDCl₃, 100MHz): δ 178.0, 172.6, 61.6, 53.6, 35.8, 31.4, 29.4, 24.2, 22.6, 20.0, 14.1, 14.0. HRMS [C₁₂H₂₂O₄Na⁺] calculated = 253.1410, observed = 253.1409.

General synthetic procedure for the formation of 35a, and 35b: A measured amount of 34a/34b (1 equivalent) was dissolved in 3 mL of H₂O, and 1 mL of acetone was added to the solution. A solution of Et₃N (1.2 equivalent) in 1 mL acetone was added to the reaction mixture drop wise followed by a solution of methylchloroformate (1.55 equivalent) in 1 mL acetone. The reaction was allowed to stir for 30 minutes at RT. A solution of NaN₃ (1.56 equivalent) in 3 mL H₂O was added to the reaction mixture and the
mixtures was stirred for 2 hr. The reaction mixture was then poured into 25 mL of ice cold water. The water layer was extracted with ether (3 x 50 mL). The combined ether layer was dried over MgSO₄, concentrated in vacuo giving the acylazide as a colorless oil. The acylazide was dissolved in toluene and heated to reflux solvent for 2 hr. The toluene was concentrated in vacuo giving the isocyanate as yellowish oil. A volume of 10 mL 4M HCl was added to the isocyanate and the mixture was heated to reflux solvent for 4 hr. The water layer was concentrated under reduced pressure giving the (S)-α-alkyl-alaninehydrochloride as a pale yellowish solid. The 35a/35b HCl salt was then dissolved in MeOH and NaHCO₃ was added portion wise to neutralize it to (S)-α-alkyl-alanine (35a/35b). The MeOH layer was filtered and concentrated giving 35a/35b as a white solid.

**Synthesis of (S)-α-pentylalanine (35a):** 35a was prepared following the general synthetic procedure for the formation of 35a/35b using 1g of 34a (5 mmol). An amount of 0.5g (3 mmol, 60%) of (S)-α-pentylalanine (35a) was obtained as a white solid after neutralization. All the characterization data of 35a complied with the literature.⁴, ⁵ [α]D²⁵ = + 4.1 (c = 1, MeOH).

**Synthesis of (S)-α-hexylalanine (35b):** 35b was prepared following the general synthetic procedure for the formation of 35a/35b using 1g of 34b (4.6 mmol). An amount of 0.55g (3.4 mmol, 74%) of (S)-α-pentylalanine (35b) was obtained as a white solid after neutralization. All the characterization data of 35b complied with the literature.⁴, ⁵ [α]D²⁵ = + 6.7 (c = 0.15, MeOH).

**Specific Binding of Vapreotide analogue (25) against IMR 32 cells:** Table 2 illustrates the specific binding experiments of Vapreotide analogue (25) against IMR 32 human neuroblastoma cells that are known to over express SSTR2.⁶ In the binding assay ¹¹¹⁴In-Pentetreotide, which is known to effectively bind to SSTR2, was used as the radio ligand (Hot ligand). In addition, Octreotide
acetate, which is known to have high selectivity for SSTR2, was used as a positive control (Cold ligand 1) to compete with the $^{111}$In-Pentetreotide. The Vapreotide analogue (25, Cold ligand 2) was allowed to compete with the $^{111}$In-Pentetreotide as well (Cold ligand 2). Cells were harvested, washed and counted in gamma counter to determine the quantity of the $^{111}$In-Pentetreotide (CPM) bound to the cells. The specific binding of each cold ligand was determined from the equation below based on the amount of $^{111}$In-Pentetreotide bound to the IMR 32 cells as obtained from the gamma counter.

Specific binding of Octreotide Acetate in CPM (cold ligand 1) = competitive binding of $^{111}$In-Pentetreotide and Octreotide Acetate in CPM (Hot + Cold 1) – binding of $^{111}$In-Pentetreotide in CPM (Hot)

Specific Binding of Vapreotide analogue (25, cold ligand 2) = competitive binding of $^{111}$In-Pentetreotide and Vapreotide (35) in CPM (Hot + Cold 2) – binding of $^{111}$In-Pentetreotide in CPM (Hot)
**Competitor:** Octreotide Acetate (Cold 1)

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**Competitor:** Vapreotide (Cold 2) (25, Figure 2) with (S)-α-methyl-α-lysine

|          | 3272      | 4284             | -78.3                                  |
|          | 4062      | 3415             |                                        |
|          | 3574      | 3444             |                                        |
| Mean CPM | 3636      | 3714.3           |                                        |
| Standard Deviation | 398.6 | 493.6            |                                        |

**Total CPM added** 348,315

**Background CPM** 40

**Radio Ligand Used** $^{111}$In-Pentetreotide (Hot)

| Table 2: Specific binding assay of the Vapreotide (25) against IMR 32 cell line |
HPLC purification (Figure 2)
References

Chiral HPLC traces of PLE hydrolyzed half-esters
X = Impurities