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Synthesis of RN277 and RN341

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We use this protocol and it's working

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Abstract

This protocol outlines the synthesis of the LRRK2-selective type-2 kinase inhibitors RN277 and RN341, including structural characterization using nuclear magnetic resonance (NMR) and mass spectrometry (MS).

Troubleshooting

Synthesis of 2,4-dichloro-5-iodo-7-trityl-7H-pyrrolo[2,3-d]pyrimidine

- 1 Dissolve 10.0 g of 2,4-dichloro-7H-pyrrolo[2,3-d]pyrimidine in 50 mL of anhydrous dichloromethane.
- 2 Add 15.6 g of N-iodosuccinimide in portions to the solution.
- 3 Stir the reaction at room temperature overnight.
- 4 Filter the precipitate and wash it with 20 mL of dichloromethane.
- 5 Resolve the white intermediate product in 50 mL of chloroform.
- 6 Add 10.7 mL of triethylamine to the solution.
- 7 Add 11.7 g of (chloromethanetriyl)tribenzene in portions.
- 8 Stir the reaction at room temperature overnight.
- 9 Remove the solvent and suspend the residue in methanol.
- 10 Filter the solid and wash it with 20 mL of methanol.
- 11 Collect 2,4-dichloro-5-iodo-7-trityl-7H-pyrrolo[2,3-d]pyrimidine as a white solid.
- 12 **Characterization:**
MS (ESI⁺): $m/z = 243.05$ [$C_{25}H_{16}Cl_2IN_3-C_6HCl_2IN_3$]⁺
¹H NMR (250 MHz, CDCl₃): $\delta = 7.43$ (s, 1H), 7.34 – 7.27 (m, 9H), 7.19 – 7.10 (m, 6H) ppm.

Synthesis of 4-(2-chloro-5-iodo-7-trityl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)morpholine

- 13 Combine 5.00 g of 2,4-dichloro-5-iodo-7-trityl-7H-pyrrolo[2,3-d]pyrimidine, 1.55 mL of morpholine, and 3.73 g of potassium carbonate in anhydrous dimethylformamide.
- 14 Stir the reaction mixture at room temperature overnight.
- 15 After the reaction is complete, add water to the mixture to precipitate the solid.
- 16 Filter the precipitated solid and wash it with water, followed by small amounts of cold ethanol.
- 17 Collect the product 4-(2-chloro-5-iodo-7-trityl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)morpholine as a white solid.
- 18 **Characterization:**
MS (ESI⁺): $m/z = 243.05$ [$\text{CPh}_3\text{-M}^+$]⁺
¹H NMR (250 MHz, CDCl₃): $\delta = 7.25 - 7.16$ (m, 9H), 7.14 (d, $J = 0.5$ Hz, 1H), 7.13 – 7.04 (m, 6H), 3.90 – 3.77 (m, 4H), 3.58 – 3.45 (m, 4H) ppm.

Synthesis of 2-chloro-5-iodo-N-methyl-7-trityl-7H-pyrrolo[2,3-d]pyrimidin-4-amine

- 19 Combine 5.00 g of compound 2,4-dichloro-5-iodo-7-trityl-7H-pyrrolo[2,3-d]pyrimidine, 8.99 mL of methylamine (2 M in THF), and 3.73 g of potassium carbonate in anhydrous dimethylformamide.
- 20 Stir the reaction mixture at room temperature overnight.
- 21 After the reaction is complete, add water to the mixture to precipitate the solid.
- 22 Filter the precipitated solid and wash it with water, followed by small amounts of cold ethanol.

23 Collect the product 2-chloro-5-iodo-*N*-methyl-7-trityl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine as a white solid.

24 **Characterization:**

MS (ESI⁺): $m/z = 308.9$ $[M-CPh_3^+ + 2H^+]^+$

¹H NMR (250 MHz, CDCl₃): $\delta = 7.22 - 7.13$ (m, 9H), $7.10 - 7.02$ (m, 6H), 6.88 (s, 1H), 6.05 (q, $J = 4.3$ Hz, 1H), 3.03 (d, $J = 4.9$ Hz, 3H) ppm.

Synthesis of 3-(2-chloro-4-morpholino-7-trityl-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)aniline

25 Combine 5.50 g 4-(2-chloro-5-iodo-7-trityl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)morpholine (9.06 mmol), 1.37 g (3-aminophenyl)boronic acid (9.97 mmol), potassium phosphate (3 eq.), XPhos (0.05 eq.), and XPhos Pd G2 (0.05 eq.) in a mixture of dioxane and water (4:1).

26 Heat the reaction mixture to 80 °C and stir for 4 hours.

27 After the reaction is complete, add water to the mixture to precipitate the solid.

28 Extract the reaction mixture with DCM and evaporate the solvent.

29 Collect the crude orange product and recrystallize it from ethanol.

30 Obtain 3-(2-chloro-4-morpholino-7-trityl-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)aniline as a white solid.

31 **Characterization:**

MS (ESI⁺): $m/z = 572.20$ $[M+H]^+$

¹H NMR (250 MHz, CDCl₃): $\delta = 7.25 - 7.02$ (m, 16H), 6.96 (s, 1H), 6.69 (d, $J = 7.5$ Hz, 1H), 6.61 (s, 1H), 6.52 (d, $J = 7.8$ Hz, 1H), $3.50 - 3.40$ (m, 4H), $3.28 - 3.19$ (m, 4H) ppm.

Synthesis of 5-(3-aminophenyl)-2-chloro-*N*-methyl-7-trityl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine

- 32 Combine 3.80 g 2-chloro-5-iodo-N-methyl-7-trityl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (6.90 mmol), 1.13 g (3-aminophenyl)boronic acid (8.28 mmol), potassium phosphate (3 eq.), XPhos (0.05 eq.), and XPhos Pd G2 (0.05 eq.) in a mixture of dioxane and water (4:1).
- 33 Heat the reaction mixture to 80 °C and stir for 4 hours.
- 34 After the reaction is complete, add water to the mixture to precipitate the solid.
- 35 Filter the precipitate from the reaction mixture.
- 36 Recrystallize the solid from ethanol.
- 37 Obtain 5-(3-aminophenyl)-2-chloro-N-methyl-7-trityl-7H-pyrrolo[2,3-d]pyrimidin-4-amine as a white solid.
- 38 **Characterization:**
MS (ESI⁺): $m/z = 516.15 [M+H]^+$
¹H NMR (250 MHz, DMSO-*d*₆): $\delta = 7.39 - 7.03$ (m, 15H), 6.84 – 6.64 (m, 2H), 6.63 – 6.45 (m, 3H), 5.88 (q, $J = 4.3$ Hz, 1H), 5.24 (s, 2H), 2.86 (d, $J = 4.7$ Hz, 3H) ppm.

Synthesis of 3-(tert-butyl)-1-(p-tolyl)-1H-pyrazol-5-amine

- 39 Dissolve 5.00 g of p-tolylhydrazine and 5.12 g of 4,4-dimethyl-3-oxopentanenitrile in 50 mL of anhydrous methanol.
- 40 Heat the reaction mixture to 80 °C and stir overnight.
- 41 After confirming quantitative conversion, remove the solvent under reduced pressure.
- 42 Collect the crude product 3-(tert-butyl)-1-(p-tolyl)-1H-pyrazol-5-amine as a pale yellow solid.
- 43 Use the crude without further purification for further synthesis.

44 Characterization:

MS (ESI⁺): $m/z = 230.15$ [M+H]⁺

¹H NMR (250 MHz, DMSO-*d*₆): $\delta = 7.55 - 7.32$ (m, 1H), 5.71 (s, 1H), 2.40 (s, 1H), 1.32 (s, 2H), 1.09 (s, 1H) ppm.

Synthesis of 2,2,2-trichloroethyl (3-(tert-butyl)-1-(p-tolyl)-1H-pyrazol-5-yl)carbamate

45 Prepare a solution of 9.39 g of 3-(tert-butyl)-1-(p-tolyl)-1H-pyrazol-5-amine in 100 mL of ethyl acetate and water in a 2:1 ratio.

46 Cool the reaction mixture to 0 °C. And add 4.09 g of sodium hydroxide to the cooled solution.

47 Slowly add 6.76 mL of trichloroethylchloroformate to the mixture.

48 Allow the reaction mixture to warm to room temperature and stir for 1 hour.

49 After the reaction is complete, separate the layers and wash the organic layer with brine.

50 Collect 2,2,2-trichloroethyl (3-(tert-butyl)-1-(p-tolyl)-1H-pyrazol-5-yl)carbamate as a pale brown solid.

51 Characterization:

MS (ESI⁺): $m/z = 404.05$ [M+H]⁺

¹H NMR (500 MHz, DMSO-*d*₆): $\delta = 9.90$ (s, 1H), 7.37 (d, $J = 8.4$ Hz, 2H), 7.26 (d, $J = 8.3$ Hz, 2H), 6.27 (s, 1H), 4.85 (s, 2H), 2.34 (s, 3H), 1.28 (s, 9H) ppm.

Synthesis of 1-(3-(tert-butyl)-1-(p-tolyl)-1H-pyrazol-5-yl)-3-(3-(2-chloro-4-morpholino-7H-pyrrolo[2,3-d]pyrimidin-5-yl)phenyl)urea (RN277)

52 Dissolve 150 mg of 3-(2-chloro-4-morpholino-7-tryl-7H-pyrrolo[2,3-d]pyrimidin-5-yl)aniline (262 μ mol) and 159 mg of 2,2,2-trichloroethyl (3-(tert-butyl)-1-(p-tolyl)-1H-pyrazol-5-yl)carbamate (393 μ mol) in 4 mL of anhydrous dimethylformamide.

53 Add 137 μ L of diisopropylethylamine to the solution.

- 54 Heat the reaction mixture to 60 °C and stir overnight.
- 55 After the reaction is complete, remove the solvent.
- 56 Dissolve the crude intermediate in 3 mL trifluoroacetic acid.
- 57 Stir the reaction mixture overnight.
- 58 Pour the mixture into a 4 M potassium carbonate solution.
- 59 Extract the resulting mixture with ethyl acetate and remove the solvent.
- 60 Purify the crude product via reverse-phase flash chromatography.
- 61 Collect RN277 as a white solid.

62 **Characterization:**

MS (ESI⁺): $m/z = 585.25$ [M+H]⁺

¹H NMR (500 MHz, DMSO-*d*₆): $\delta = 12.27$ (d, $J = 2.3$ Hz, 1H), 9.11 (s, 1H), 8.35 (s, 1H), 7.58 (t, $J = 1.7$ Hz, 1H), 7.43 (d, $J = 2.5$ Hz, 1H), 7.42 – 7.38 (m, 2H), 7.36 – 7.28 (m, 4H), 7.09 (dt, $J = 7.2, 1.5$ Hz, 1H), 6.36 (s, 1H), 3.48 – 3.41 (m, 4H), 3.24 – 3.19 (m, 4H), 2.37 (s, 3H), 1.27 (s, 9H) ppm.

¹³C NMR (101 MHz, DMSO-*d*₆): $\delta = 160.52, 160.20, 153.87, 151.53, 151.15, 139.63, 137.08, 136.78, 136.05, 135.63, 129.67, 129.11, 124.32, 122.66, 121.63, 117.78, 116.41, 116.10, 100.72, 95.05, 65.28, 49.12, 32.00, 30.20, 20.58$ ppm.

Synthesis of 5-(3-aminophenyl)-2-(ethylthio)-N-methyl-7-trityl-7H-pyrrolo[2,3-d]pyrimidin-4-amine

- 63 Suspend 300 mg of 5-(3-aminophenyl)-2-chloro-N-methyl-7-trityl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (581 μ mol) and 489 mg of sodium ethyl thiolate (5.81 mmol) in 3 mL

of anhydrous dimethylformamide.

64 Heat the reaction mixture to 90 °C using a microwave reactor. Stir the mixture for 1 hour.

65 Add water to the reaction mixture to precipitate the product.

66 Filter the precipitate and wash the collected solid with 20 mL of ethanol.

67 Collect and dry the product to obtain 5-(3-aminophenyl)-2-(ethylthio)-*N*-methyl-7-trityl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine.

68 **Characterization:**

MS (ESI⁺): m/z = 542.20 [M+H]⁺

¹H NMR (250 MHz, CDCl₃): δ = 9.35 – 9.06 (m, 16H), 8.78 – 8.49 (m, 4H), 7.10 (q, J = 4.4 Hz, 1H), 5.73 (s, 2H), 4.86 (d, J = 4.8 Hz, 3H), 4.30 (q, J = 7.2 Hz, 2H), 2.78 (t, J = 7.2 Hz, 3H) ppm.

Synthesis of 1-(3-(tert-butyl)-1-(p-tolyl)-1H-pyrazol-5-yl)-3-(3-(2-(ethylthio)-4-(methylamino)-7H-pyrrolo[2,3-*d*]pyrimidin-5-yl)phenyl)urea (RN341)

69 Dissolve 122 mg of 5-(3-aminophenyl)-2-(ethylthio)-*N*-methyl-7-trityl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (225 μ mol) and 146 mg of 2,2,2-trichloroethyl 3-(tert-butyl)-1-(p-tolyl)-1H-pyrazol-5-yl)carbamate (360 μ mol) in 4 mL of anhydrous dimethylformamide.

70 Add 63 μ L of diisopropylethylamine to the solution.

71 Heat the reaction mixture to 60 °C and stir overnight.

72 After the reaction is complete, remove the solvent.

73 Dissolve the crude intermediate in 3 mL trifluoroacetic acid.

74 Stir the reaction mixture overnight.



- 75 Pour the mixture into a 4 M potassium carbonate solution.
- 76 Extract the resulting mixture with ethyl acetate and remove the solvent.
- 77 Purify the crude product via reverse-phase flash chromatography.
- 78 Collect RN341 as a white solid.

79 **Characterization:**

MS (ESI⁺): $m/z = 555.25$ [M+H]⁺

¹H NMR (500 MHz, DMSO-*d*₆): $\delta = 11.65$ (d, $J = 1.9$ Hz, 1H), 9.11 (s, 1H), 8.40 (s, 1H), 7.50 (t, $J = 1.6$ Hz, 1H), 7.40 (d, $J = 8.3$ Hz, 2H), 7.33 (dd, $J = 8.0, 2.3$ Hz, 3H), 7.25 (dd, $J = 8.1, 1.0$ Hz, 1H), 7.09 – 7.05 (m, 2H), 6.38 (s, 1H), 5.78 (q, $J = 4.5$ Hz, 1H), 3.10 (q, $J = 7.3$ Hz, 2H), 2.92 (d, $J = 4.7$ Hz, 3H), 2.36 (s, 3H), 1.34 (t, $J = 7.3$ Hz, 3H), 1.27 (s, 9H) ppm.

¹³C NMR (101 MHz, DMSO-*d*₆): $\delta = 162.19, 160.51, 156.25, 151.85, 151.65, 139.64, 137.14, 136.81, 136.07, 135.65, 129.67, 129.56, 124.41, 122.12, 118.72, 118.54, 116.66, 115.82, 97.25, 94.88, 31.99, 30.21, 27.59, 24.20, 20.57, 15.20$ ppm.