

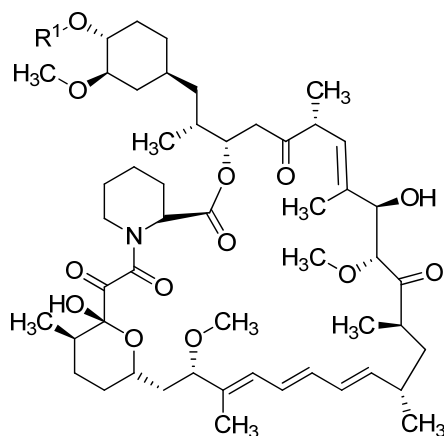
[0004] The chemical structure of BA9 compared to sirolimus and other sirolimus derivatives is provided in Figure 1. BA9 is structurally related to rapamycin (also known as sirolimus). The structure consists of the rapamycin 31-membered macrolide triene lactone ring with ethoxyethylation at position 40.

5 **[0005]** BA9, like sirolimus, binds to the intracellular immunophilin protein FKBP12. It is believed that the resulting macrolide/FKBP12 complex then binds, in a manner similar to sirolimus, to mTOR, a protein critical for cell cycle progression. Inactivation of mTOR results in suppression of several specific signal transduction pathways and arrest of the cell cycle at the G1 to S phase.

10 **[0006]** Given the therapeutic value of BA9 and other rapamycin derivatives, improved processes for preparation of this family of active agents is desired. The present invention addresses this and other needs.

BRIEF SUMMARY

15 **[0006a]** In one aspect of the present disclosure, there is provided a method for obtaining a purified, solid compound having a structure according to Formula I:



Formula I

wherein R^1 is selected from the group consisting of $R^a-(O)_d-R^b$, wherein R^a is C_{1-5} alkylene, R^b is C_{1-5} alkyl or C_{1-5} alkylene-OH, and the subscript d is an integer selected from 0-1; C_{1-5} alkyl; C_{6-10} aryl C_{1-5} alkylene; hydroxy C_{1-5} alkylene; C_{1-5} alkoxy C_{1-5} alkylene; acyl; acyl C_{1-5} alkylene; amino C_{1-5} alkylene; C_{1-5} alkylamino C_{1-5} alkylene; acylamino C_{1-5} alkylene; C_{1-5} alkoxycarbonylamino C_{1-5} alkylene; and C_{6-10} aryl;

the method comprising:

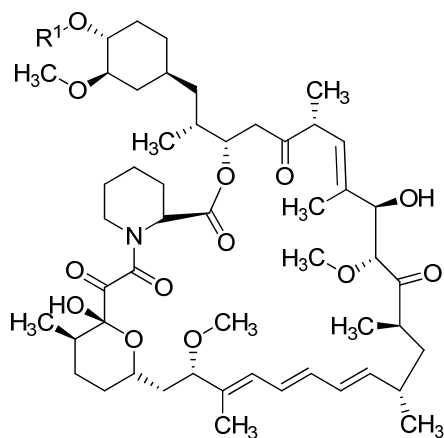
- 5 a) forming a mixture consisting essentially of a crude compound having a structure according to Formula I and a non-polar organic solvent under conditions sufficient to dissolve the compound, wherein the dielectric constant of the non-polar organic solvent is less than about 5, and wherein forming the mixture comprises heating the mixture;
- b) solidifying at least a portion of the compound having the structure according to Formula I, wherein the solidifying comprises cooling the mixture; and
- c) separating at least a portion of the solidified compound from the solvent in the mixture;
- 10 thereby obtaining the purified, solid compound.

[0006b] In another aspect of the present disclosure, there is provided a crystalline form of 40-O-(2-ethoxyethyl) rapamycin, wherein the X-ray powder diffraction pattern thereof shows one or more diffraction peaks at or around the angles (2θ) selected from the group consisting of 5.00° , 7.06° , 9.22° , 10.07° , 10.50° , 11.94° ,
15 12.71° , 13.15° , 14.73° , 16.33° , 16.80° , 17.07° , 18.01° , 18.57° , 19.42° , 19.81° , 20.16° , 20.44° , 20.93° , 21.55° , 22.29° , 22.58° , 23.92° , 24.26° , 24.83° , 25.17° , 26.32° , 27.48° , 28.60° , and 32.28° .

[0006c] In a further aspect of the present disclosure, there is provided a crystalline form of 40-O-(2-ethoxyethyl) rapamycin, wherein the differential scanning calorimetry
20 thermogram thereof shows one or more local minima at around 138°C and around 192°C .

[0006d] In another aspect of the present disclosure, there is provided a crystalline form of 40-O-(2-ethoxyethyl) rapamycin, wherein the infrared spectrum thereof shows one or more peaks at or around the wavenumbers selected from the group consisting of 2967.1 cm^{-1} , 2931.7 cm^{-1} , 2863.3 cm^{-1} , 1745.9 cm^{-1} , 1718.8 cm^{-1} , 1645.7 cm^{-1} , 1619.0 cm^{-1} , 1451.2 cm^{-1} , 1378.2 cm^{-1} , 1189.4 cm^{-1} , 1073.9 cm^{-1} , and 988.1 cm^{-1} .
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[0007] The present disclosure also provides method for obtaining a purified, solid compound having a structure according to Formula I:



Formula I

wherein R¹ is selected from the group consisting of H; R^a-(O)_d-R^b, wherein R^a is C₁-
5 salkylene, R^b is C₁₋₅salkyl or C₁₋₅salkylene-OH, and the subscript d is an integer
selected from 0-1; C₁₋₅salkyl; C₆₋₁₀arylC₁₋₅salkyl; hydroxyC₁₋₅salkyl;
C₆₋₁₀arylC₁₋₅salkoxy; C₁₋₅salkoxyC₁₋₅salkyl; acyl; acylC₁₋₅salkyl; aminoC₁₋₅salkyl; C₁-
salkylaminoC₁₋₅salkyl; acylaminoC₁₋₅salkyl; C₁₋₅salkoxycarbonylaminoC₁₋₅salkyl; and
C₆₋₁₀aryl.

[0008] The method includes: a) forming a mixture comprising a crude compound having a structure according to Formula I and a non-polar organic solvent under conditions sufficient to dissolve the compound; b) solidifying at least a portion of the compound having the structure according to Formula I; c) separating at least a portion
5 of the solidified compound from the solvent in the mixture; and thereby obtaining the purified, solid compound.

[0009] In another example, the present disclosure provides for a crystallized form of BA9.

10 BRIEF DESCRIPTION OF THE DRAWINGS

[0010] **Figure 1** shows the chemical structures of sirolimus, Biolimus A9, and related derivatives.

[0011] **Figure 2** shows a scheme for the preparation of rapamycin derivatives and purification of the derivatives according to the methods of the invention.

15 [0012] **Figure 3** shows an X-ray powder diffraction (XRPD) pattern observed for BA9 Lot 2A.

[0013] **Figure 4** shows a differential scanning calorimetry (DSC) thermogram observed for BA9 Lot 2A.

[0014] **Figure 5** shows an XRPD pattern observed for BA9 Lot 2B.

20 [0015] **Figure 6** shows a DSC thermogram observed for BA9 Lot 2B.

[0016] **Figure 7** shows an optical micrograph observed for BA9 Lot 2B.

[0017] **Figure 8** shows a dynamic vapor sorption (DVS) kinetic plot observed for BA9 Lot 2B.

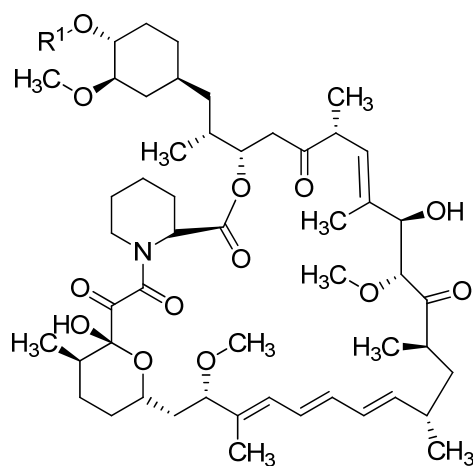
[0018] **Figure 9** shows a XRPD pattern overlay observed for BA9 Lot 2B after a
25 DVS experiment and a reference pattern of BA9 Form I.

[0019] **Figure 10** shows an overlay of the XRPD patterns obtained from solvent slurries and a reference pattern of BA9 Form I.

[0020] **Figure 11** shows an overlay of the XRPD patterns obtained in anti-solvent crystallization experiments and a reference pattern of BA9 Form I.

WHAT IS CLAIMED IS:

1. A method for obtaining a purified, solid compound having a structure according to Formula I:



Formula I

wherein R^1 is selected from the group consisting of $R^a-(O)_d-R^b$, wherein R^a is C_{1-5} alkylene, R^b is C_{1-5} alkyl or C_{1-5} alkylene-OH, and the subscript d is an integer selected from 0-1; C_{1-5} alkyl; C_{6-10} aryl C_{1-5} alkylene; hydroxy C_{1-5} alkylene; C_{1-5} alkoxy C_{1-5} alkylene; acyl; acyl C_{1-5} alkylene; amino C_{1-5} alkylene; C_{1-5} alkylamino C_{1-5} alkylene; acylamino C_{1-5} alkylene; C_{1-5} alkoxycarbonylamino C_{1-5} alkylene; and C_{6-10} aryl;

the method comprising:

- a) forming a mixture consisting essentially of a crude compound having a structure according to Formula I and a non-polar organic solvent under conditions sufficient to dissolve the compound, wherein the dielectric constant of the non-polar organic solvent is less than about 5, and wherein forming the mixture comprises heating the mixture;
 - b) solidifying at least a portion of the compound having the structure according to Formula I, wherein the solidifying comprises cooling the mixture; and
 - c) separating at least a portion of the solidified compound from the solvent in the mixture;
- thereby obtaining the purified, solid compound.

2. The method of claim 1, wherein the non-polar organic solvent is selected from the group consisting of hexane, heptane, methyl *t*-butyl ether, ligroin, octane, cyclohexane, and mixtures thereof.

3. The method of claim 2, wherein the non-polar organic solvent is hexane.
4. The method of claim 2, wherein the non-polar organic solvent is heptane.
5. The method of claim 1, wherein forming the mixture in step a) comprises heating the mixture to a temperature of from about 35 °C to about 100 °C.
6. The method of claim 1, wherein forming the mixture in step a) comprises heating the mixture to reflux.
7. The method of claim 1, wherein cooling the mixture in step b) comprises cooling the mixture to a temperature of from about -78 °C to about 25 °C.
8. The method of claim 7, wherein cooling the mixture in step b) comprises cooling the mixture to a temperature of about 15 °C.
9. The method of claim 1, wherein the purified, solid compound is obtained in a crystalline form.
10. The method of claim 1, further comprising:
 - d) solubilizing the solidified compound in a polar organic solvent to form a solution;
 - e) contacting the solution with water to precipitate at least a portion of the compound; and
 - f) drying the precipitated compound.

11. The method of claim 10, wherein the polar organic solvent is selected from the group consisting of methanol, ethanol, isopropanol, *t*-butanol, tetrahydrofuran, and acetone.
12. The method of claim 11, wherein the polar organic solvent is methanol.
13. The method of claim 10, wherein the drying is conducted under reduced pressure.
14. The method of claim 1, further comprising:
 - i) contacting the compound with an anti-solvent composition; and
 - ii) obtaining a crystalline solid form of the compound.
15. The method of claim 10, further comprising:
 - i) contacting the compound with an anti-solvent composition; and
 - ii) obtaining a crystalline solid form of the compound.
16. The method of claim 14, wherein the compound is purified and solid.
17. The method of claim 14, wherein the compound is purified.
18. The method of claim 14, wherein the compound is solid.
19. The method of claim 14, wherein the anti-solvent composition comprises hexane, heptane, methyl *t*-butyl ether, ligroin, octane, cyclohexane, and mixtures thereof.
20. The method of claim 1, wherein R^1 is $R^a-(O)_d-R^b$.
21. The method of claim 20, wherein R^1 is selected from the group consisting of CH_2-CH_2-OH and $CH_2-CH_2-O-CH_2-CH_3$.
22. The method of claim 21, wherein R^1 is $CH_2-CH_2-O-CH_2-CH_3$.

23. The method of claim 1, wherein the purity of the purified, solid compound is increased by from about 1% to about 20% with respect to the crude compound.

24. The method of claim 1, wherein the purity of the purified, solid compound is increased by from about 1% to about 10% with respect to the crude compound.

25. The method of claim 1, wherein the purity of the purified, solid compound is increased by about 3% with respect to the crude compound.

26. A crystalline form of 40-*O*-(2-ethoxyethyl) rapamycin, wherein the X-ray powder diffraction pattern thereof shows one or more diffraction peaks at or around the angles (2θ) selected from the group consisting of 5.00°, 7.06°, 9.22°, 10.07°, 10.50°, 11.94°, 12.71°, 13.15°, 14.73°, 16.33°, 16.80°, 17.07°, 18.01°, 18.57°, 19.42°, 19.81°, 20.16°, 20.44°, 20.93°, 21.55°, 22.29°, 22.58°, 23.92°, 24.26°, 24.83°, 25.17°, 26.32°, 27.48°, 28.60°, and 32.28°.

27. The crystalline form of claim 26, wherein the X-ray powder diffraction pattern thereof shows 10 or more diffraction peaks at or around the angles (2θ) selected from the group consisting of 5.00°, 7.06°, 9.22°, 10.07°, 10.50°, 11.94°, 12.71°, 13.15°, 14.73°, 16.33°, 16.80°, 17.07°, 18.01°, 18.57°, 19.42°, 19.81°, 20.16°, 20.44°, 20.93°, 21.55°, 22.29°, 22.58°, 23.92°, 24.26°, 24.83°, 25.17°, 26.32°, 27.48°, 28.60°, and 32.28°.

28. The crystalline form of claim 26, wherein the X-ray powder diffraction pattern thereof shows 25 or more diffraction peaks at or around the angles (2θ) selected from the group consisting of 5.00°, 7.06°, 9.22°, 10.07°, 10.50°, 11.94°, 12.71°, 13.15°, 14.73°, 16.33°, 16.80°, 17.07°, 18.01°, 18.57°, 19.42°, 19.81°, 20.16°, 20.44°, 20.93°, 21.55°, 22.29°, 22.58°, 23.92°, 24.26°, 24.83°, 25.17°, 26.32°, 27.48°, 28.60°, and 32.28°.

29. The crystalline form of claim 26, having an X-ray powder diffraction pattern substantially in accordance with Fig. 5.
30. The method of claim 10, wherein the purified, solid compound is obtained in an amorphous form.
31. A crystalline form of 40-*O*-(2-ethoxyethyl) rapamycin, wherein the differential scanning calorimetry thermogram thereof shows one or more local minima at around 138 °C and around 192 °C.
32. The crystalline form of 40-*O*-(2-ethoxyethyl) rapamycin according to claim 31, wherein the thermogram shows local minima at 137.9 °C and 191.6°C.
33. The crystalline form of 40-*O*-(2-ethoxyethyl) rapamycin according to claim 31, wherein the thermogram is substantially in accordance with FIG. 6.
34. A crystalline form of 40-*O*-(2-ethoxyethyl) rapamycin, wherein the infrared spectrum thereof shows one or more peaks at or around the wavenumbers selected from the group consisting of 2967.1 cm⁻¹, 2931.7 cm⁻¹, 2863.3 cm⁻¹, 1745.9 cm⁻¹, 1718.8 cm⁻¹, 1645.7 cm⁻¹, 1619.0 cm⁻¹, 1451.2 cm⁻¹, 1378.2 cm⁻¹, 1189.4 cm⁻¹, 1073.9 cm⁻¹, and 988.1 cm⁻¹.
35. The crystalline form of 40-*O*-(2-ethoxyethyl) rapamycin according to claim 34, wherein the infrared spectrum shows five or more peaks at or around the wavenumbers selected from the group consisting of 2967.1 cm⁻¹, 2931.7 cm⁻¹, 2863.3 cm⁻¹, 1745.9 cm⁻¹, 1718.8 cm⁻¹, 1645.7 cm⁻¹, 1619.0 cm⁻¹, 1451.2 cm⁻¹, 1378.2 cm⁻¹, 1189.4 cm⁻¹, 1073.9 cm⁻¹, and 988.1 cm⁻¹.
36. The crystalline form of 40-*O*-(2-ethoxyethyl) rapamycin according to claim 34, wherein the infrared spectrum shows ten or more peaks at or around the wavenumbers selected from 2967.1 cm⁻¹, 2931.7 cm⁻¹, 2863.3 cm⁻¹, 1745.9 cm⁻¹, 1718.8 cm⁻¹, 1645.7 cm⁻¹, 1619.0 cm⁻¹, 1451.2 cm⁻¹, 1378.2 cm⁻¹, 1189.4 cm⁻¹, 1073.9 cm⁻¹, and 988.1 cm⁻¹.
37. The crystalline form of 40-*O*-(2-ethoxyethyl) rapamycin according to claim 34, wherein the infrared spectrum is substantially in accordance with FIG. 12.