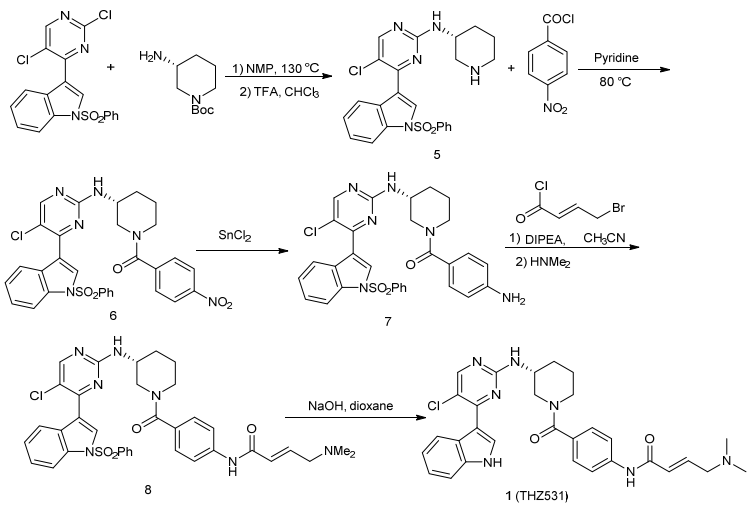
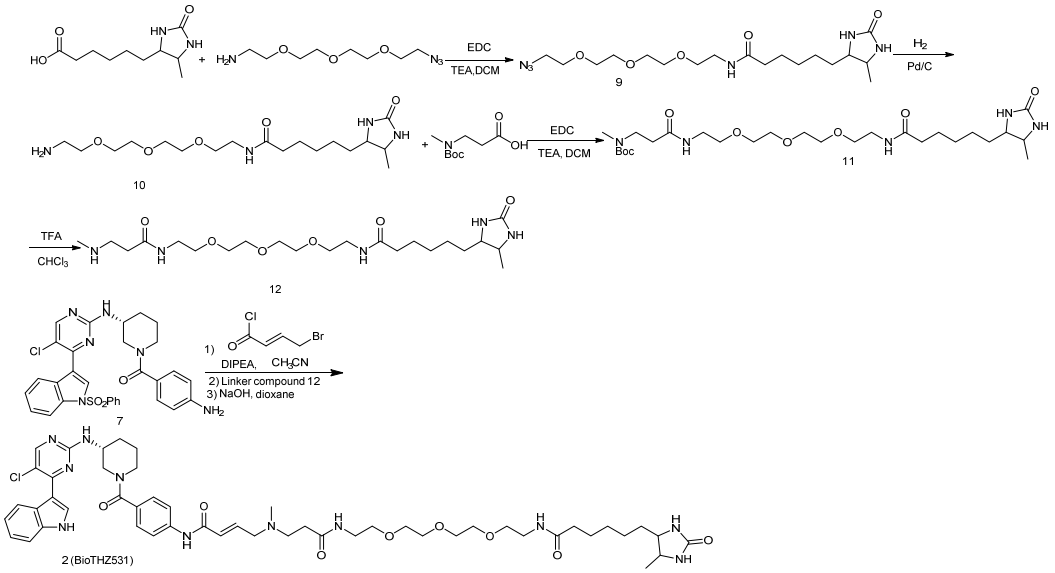
SUPPLEMENTARY NOTE

Chemistry

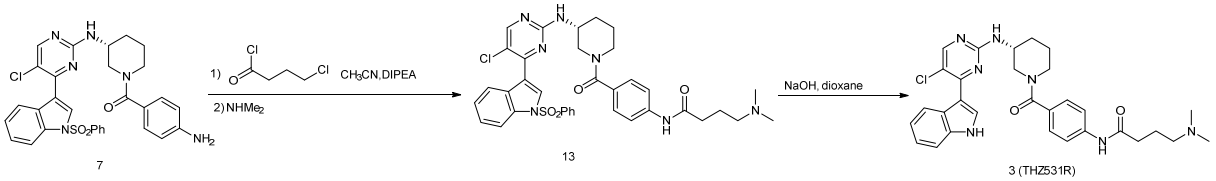
All solvents and reagents were used as obtained. 1H NMR spectra were recorded with a Varian Inova 600 NMR spectrometer and referenced to dimethylsulfoxide. Chemical shifts are expressed in ppm. Mass spectra were measured with Waters Micromass ZQ using an ESI source coupled to a Waters 2525 HPLC system operating in reverse mode with a Waters Sunfire C18 5 μm, 4.6 mm x 50 mm column. Purification of compounds was performed with either a Teledyne ISCO CombiFlash Rf system or a Waters Micromass ZQ preparative system. The purity was analyzed on an above-mentioned Waters LC-MS Symmetry (C18 column,4.6 mm x 50 mm, 5 μM) using a gradient of 5-95% methanol in water containing 0.05% trifluoroacetic acid (TFA). Detailed synthetic schemes and characterization data are presented below and elsewhere.[1]



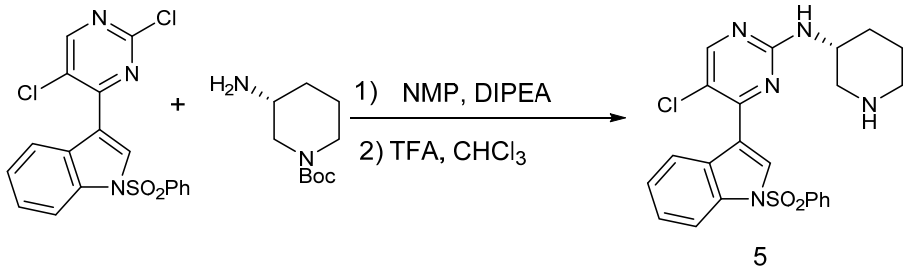
Scheme 1. The synthesis of THZ531



Scheme 2. The synthesis of BioTHZ531



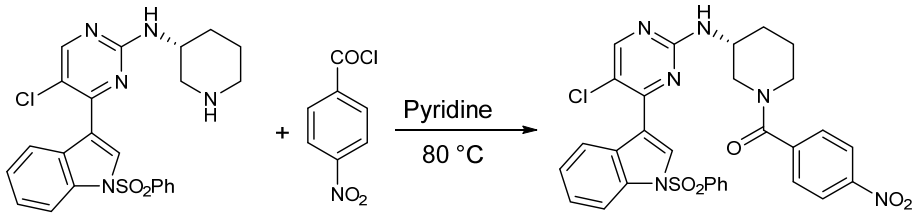
Scheme 3. The synthesis of THZ531R



(R)-5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)-N-(piperidin-3-yl)pyrimidin-2-amine (5)

To a solution of 3-(2, 5-dichloropyrimidin-4-yl)-1-(phenylsulfonyl)-1H-indole (402mg) in NMP (5.0mL) was added tert-butyl (R)-3-aminopiperidine-1-carboxylate (200 mg, 1.0 equiv) and diisopropylethylamine (129 mg, 1.0 equiv). The solution was heated for 2 h at 130 ^{O}C.The

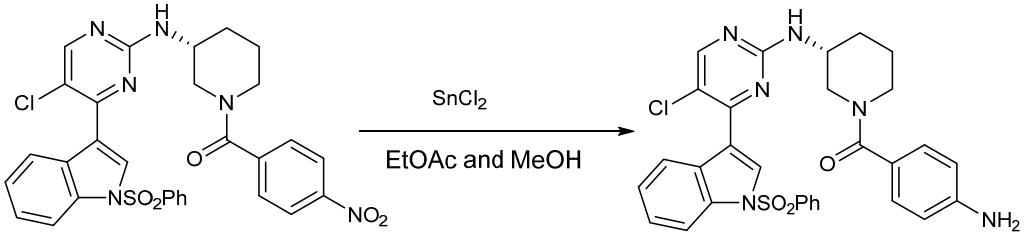
cooled solution was diluted with 100 mL of $CHCl_3$ and *i*-PrOH (4:1, 100 mL)) and then washed with water. After removing solvent, the crude was dissolved in $CHCl_3$ (10 mL) and then was treated with TFA (5 mL). After 30 min stirring at room temperature, the solvent was then removed. The separation by silica gel chromatography column with CH_2Cl_2/methanol (10/1) to give the product **5** (350 mg, 76%, two steps). LC-MS: *m/z* (M+H) 468.^{1}H NMR (500 MHz, DMSO) δ 8.70 (s, 2H), 8.54 (s, 1H), 8.48 (s, 1H), 8.29 (d, *J* = 8.2 Hz, 1H), 8.07 – 8.03 (m, 2H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.73 (t, *J* = 7.5 Hz, 1H), 7.63 (t, *J* = 7.9 Hz, 2H), 7.45 (dd, *J* = 11.3, 4.1 Hz, 2H), 7.37 (t, *J* = 7.6 Hz, 1H), 4.18 (td, *J* = 13.5, 6.9 Hz, 1H), 3.41 (s, 1H), 3.22 – 3.17 (m, 1H), 2.91 (dd, *J* = 21.6, 11.2 Hz, 2H), 2.04 (dd, *J* = 12.6, 3.8 Hz, 1H), 1.92 (d, *J* = 4.4 Hz, 1H), 1.80 – 1.56 (m, 2H).^{13}C NMR (125 MHz, DMSO) ^{13}C NMR (126 MHz, DMSO) δ 160.19, 158.72, 156.61, 137.33, 135.47, 134.50, 130.47 (2C), 129.85, 128.99, 127.35 (2C), 126.03, 124.61, 123.56, 117.82, 116.04, 113.51, 47.06, 45.65, 43.76, 28.57, 20.97.



5 6

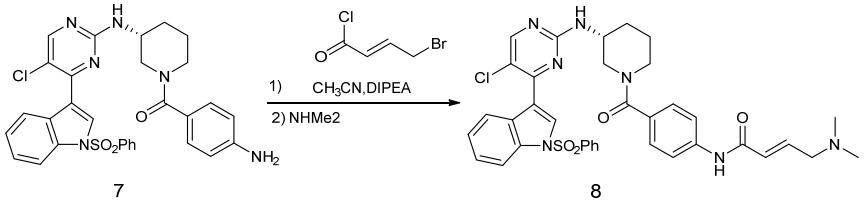
(R)-(3-((5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-yl)amino)piperidin-1-yl)(4-nitrophenyl)methanone (6) To a stirred solution of the compound 5 (467 mg) in pyridine (10 mL) was added 4-nitrobenzoyl chloride (180 mg, 1.0 equiv) and then was heated to 80 ^{O}C. The reaction mixture was stirred for 2 hours and then was concentrated under reduced pressure. The resulting crude was purified by silica gel column with CH_2Cl_2/methanol (10/1) to give the product **6** (450 mg, 73%). LC-MS: *m/z* (M+H) 617. ^{1}H NMR (500 MHz, DMSO) δ 8.49 (s, 1H), 8.31 (d, *J* = 41.7 Hz, 2H), 8.05 (d, *J* = 7.7 Hz, 2H), 8.00 (d, *J* = 8.3 Hz, 1H), 7.73 (t, *J* = 7.4 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 2H), 7.55 (s, 1H), 7.44 (t, *J* = 7.7 Hz, 1H), 7.37 (dd, *J* = 16.3, 8.5 Hz, 2H), 3.90 (s, 1H), 3.52 (s, 2H), 3.28 (s, 2H), 2.10 – 2.00 (m, 1H), 1.91 (s, 1H), 1.75 (s, 1H), 1.58

(s, 1H).[13]C NMR (125 MHz, DMSO) δ 168.01, 160.29, 158.51, 156.27, 148.12, 143.03, 137.36, 135.43, 134.48, 130.45 (2C), 129.71, 128.95, 128.49 (2C), 127.31 (2C), 126.00, 124.57, 123.62 (2C), 117.85, 115.58, 113.45, 50.52, 48.04, 42.61, 29.88, 22.67.



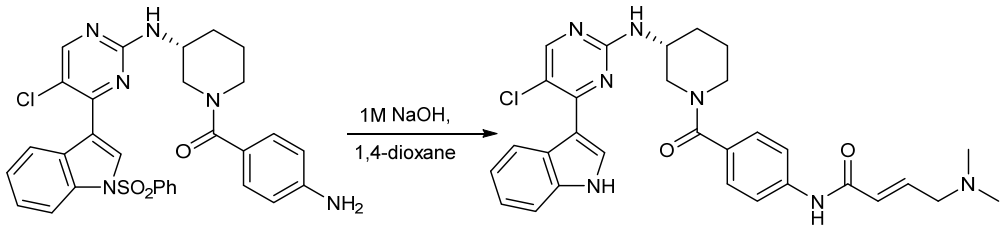
6 7

(R)-(4-aminophenyl)(3-((5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-yl)amino)piperidin-1-yl)methanone (7) The nitro compound 6 (617 mg) was suspended in ethyl acetate/methanol (5:1, 30 mL) and was treated with $SnCl_2$ (380 mg, 2.0 equiv). After stirring for 2 hours at 80 ^{O}C, the reaction mixture was cooled down to room temperature and poured onto saturated aqueous $NaHCO_3$. The mixture was stirred for 10 min followed by extraction with chloroform and 2-propanol (4:1, 100 mL). The organic layer was washed with water and brine, dried over sodium sulfate, filtered through a pad of celite and concentrated under reduced pressure. The resulting crude was purified by flash column with CH_2Cl_2/methanol (10/1) to provide the compound **7** (290 mg, 50%). LC-MS: *m/z* (M+H) 587. ^{1}H NMR (500 MHz, DMSO) δ 8.54 (s, 1H), 8.40 (s, 1H), 8.32 (d, *J* = 7.9 Hz, 1H), 8.09 – 8.03 (m, 2H), 8.00 (d, *J* = 8.3 Hz, 1H), 7.72 (t, *J* = 7.5 Hz, 1H), 7.62 (t, *J* = 7.9 Hz, 2H), 7.47 – 7.40 (m, 1H), 7.34 (t, *J* = 7.3 Hz, 1H), 7.20 (d, *J* = 8.2 Hz, 2H), 6.88 (s, 4H), 6.70 (d, *J* = 7.9 Hz, 2H), 4.07 (d, *J* = 11.7 Hz, 1H), 3.92 (s, 1H), 3.80 (d, *J* = 12.5 Hz, 1H), 3.10 (dt, *J* = 22.1, 7.5 Hz, 2H), 2.12 – 1.96 (m, 1H), 1.82 (dd, *J* = 9.0, 4.4 Hz, 1H), 1.67 (ddd, *J* = 16.5, 11.9, 3.8 Hz, 1H), 1.59 – 1.45 (m, 1H).[13]C NMR (125 MHz, DMSO) δ 170.41, 160.37, 158.53, 156.41, 146.65, 137.37, 135.40, 134.49, 130.44 (2C), 129.78, 129.20 (2C), 129.05, 127.33 (2C), 126.32, 125.95, 124.54, 123.72, 117.94, 115.57 (2C), 115.44, 113.41, 49.66, 48.26, 45.39, 30.52, 23.96.



7 8

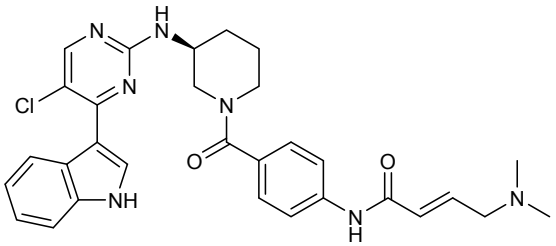
(R,E)-N-(4-(3-((5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-yl)amino)piperidine-1-carbonyl)phenyl)-4-(dimethylamino)but-2-enamide (8) To the solution of the compound 7 (60 mg) in acetonitrile (10 mL) was added diisopropylethylamine (13 mg, 1.0 equiv). The reaction mixture was cooled down to 0 ^{O}C and then treated with 4-chlorobut-2-enoyl chloride (54 mg, 3.0 equiv) in CH_2Cl_2. The reaction mixture was stirred for 10 min at 0 ^{O}C followed by adding dimethylamine in THF (1M, 2.0 mL). The reaction mixture was then warmed up to room temperature, stirred for 1 hour and then concentrated under reduced pressure. The resulting crude was purified by preparative HPLC to give the product **8** (38 mg, 55%) LC-MS: *m/z* (M+H) 698. ^{1}H NMR (500 MHz, DMSO) δ 10.38 (s, 1H), 8.53 (s, 1H), 8.37 (s, 1H), 8.30 (d, *J* = 6.9 Hz, 1H), 8.08 – 8.02 (m, 2H), 7.99 (d, *J* = 8.3 Hz, 1H), 7.72 (t, *J* = 7.5 Hz, 1H), 7.70 – 7.58 (m, 4H), 7.47 – 7.40 (m, 1H), 7.39 – 7.27 (m, 4H), 6.88 – 6.80 (m, 1H), 6.54 (d, *J* = 15.4 Hz, 1H), 5.95 (s, 1H), 3.95 (d, *J* = 6.6 Hz, 2H), 3.91 (s, 1H), 3.67 (d, *J* = 25.4 Hz, 1H), 3.29 – 3.09 (m, 2H), 2.81 (s, 6H), 2.56 (d, *J* = 8.7 Hz, 1H), 2.11 – 1.98 (m, 1H), 1.85 (s, 1H), 1.70 (dd, *J* = 9.4, 3.5 Hz, 1H), 1.54 (dd, *J* = 9.7, 3.8 Hz, 1H). ^{13}C NMR (125 MHz, DMSO) δ 169.74, 162.73, 160.33, 158.51, 156.38, 140.22, 137.35, 135.41, 134.47, 132.74, 131.95, 131.80, 130.44 (2C), 129.81, 129.04, 128.15 (2C), 127.32 (2C), 125.94, 124.55, 123.68, 119.42 (2C), 117.89, 115.50, 113.41, 57.35, 49.48, 48.17, 44.88, 42.54 (2C), 30.27, 23.65



7 1 (THZ531)

(R,E)-N-(4-(3-((5-chloro-4-(1H-indol-3-yl)pyrimidin-2-yl)amino)piperidine-1-carbonyl)phenyl)-4-(dimethylamino)but-2-enamide (1) The compound 8 (70 mg) was dissolved in 1,4-dioxane (5 mL) and aqueous NaOH (1.0 M, 5 mL). The solution was allowed to stir at room temperature for 2 hours followed by neutralization with HCl (1M, 5 mL). The solution was then extracted with chloroform and 2-propanol (4:1, 30 mL) and was washed with water. The removal of solvent provided the crude which was purified by HPLC to give the final product **1** (25 mg, 43%). LC-MS 558 (M+1), ^{1}H NMR (500 MHz, DMSO-*d6*) 11.83 (s, 1H), 10.94 (s, 1H), 10.41 (s, 1H), 8.53 (s, 1H), 8.44 (s, 1H), 8.25 (s, 1H), 7.67 (d, *J* = 7.0 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 2H), 7.22 (t, *J* = 8.0 Hz, 1H), 7.16 (d, *J* = 8.0 Hz, 1H), 6.83 (m, 1H), 6.52 (d, *J* = 15.0 Hz, 1H), 4.02 (br, 2H), 3.93-3.80 (m, 3H), 3.20 (br, 2H), 2.80 (s, 6H), 2.10 (m, 1H), 1.86 (m, 1H), 1.71 (m, 1H), 1.61 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 169.89, 162.78, 160.36, 157.54, 153.27, 140.36, 136.78, 132.99, 132.70, 131.99, 131.65, 128.11 (2C), 126.74, 123.20, 122.96, 121.69, 119.42 (2C), 113.66, 112.59, 111.53, 57.19, 49.12, 48.30, 45.07, 42.31 (2C), 30.21, 23.51.

(*S*,*E*)-*N*-(4-(3-((5-chloro-4-(1*H*-indol-3-yl)pyrimidin-2-yl)amino)piperidine-1-carbonyl)phenyl)-4-(dimethylamino)but-2-enamide

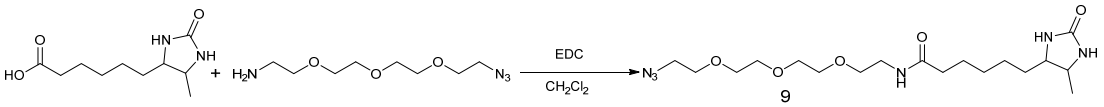


4 (THZ532)

Same synthetic method as compound 1 (THZ531)

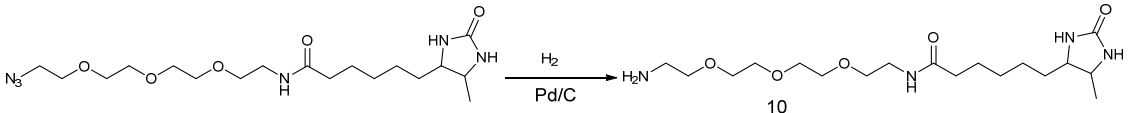
(S,E)-N-(4-(3-((5-chloro-4-(1H-indol-3-yl)pyrimidin-2-yl)amino)piperidine-1-carbonyl)phenyl)-4-(dimethylamino)but-2-enamide (4) LC-MS 558 (M+1), ^{1}H NMR (500 MHz, DMSO-*d6*) 11.83 (s, 1H), 10.94 (s, 1H), 10.41 (s, 1H), 8.53 (s, 1H), 8.44 (s, 1H), 8.25 (s, 1H), 7.67 (d, *J* = 7.0 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 2H), 7.22 (t, *J* = 8.0 Hz, 1H), 7.16 (d, *J* = 8.0 Hz, 1H), 6.83 (m, 1H), 6.52 (d, *J* = 15.0 Hz, 1H), 4.02 (br, 2H), 3.93-

3.80 (m, 3H), 3.20 (br, *2*H), 2.80 (s, 6H), 2.10 (m, 1H), 1.86 (m, 1H), 1.71 (m, 1H), 1.61 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 169.89, 162.78, 160.36, 157.54, 153.27, 140.36, 136.78, 132.99, 132.70, 131.99, 131.65, 128.11 (2C), 126.74, 123.20, 122.96, 121.69, 119.42 (2C), 113.66, 112.59, 111.53, 57.19, 49.12, 48.30, 45.07, 42.31 (2C), 30.21, 23.51.



9

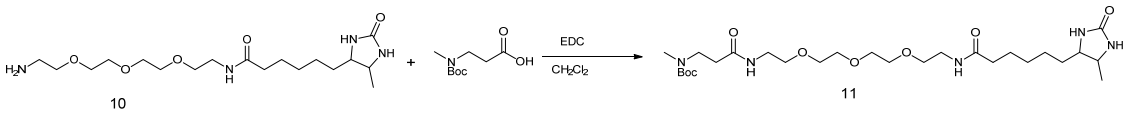
N-(2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethyl)-6-(5-methyl-2-oxoimidazolidin-4-yl)hexanamide (10) Desthiobiotin (430 mg, 2.0 mmol), 11-Azido-3,6,9-trioxaundecan-1-amine (435 mg, 2.0 mmol) and 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (576 mg, 3.0 mmol), were dissolved in methylene chloride (10 mL) and trimethylamine (380 uL, 3.0 mmol) was added at 0°C. The mixture was stirred at room temperature for 6 hours. The reaction solution was added with water and extracted with chloroform and isopropanol (4:1). The organic layer was dried with sodium sulfate and the solvent was removed under the reduced pressure to give a crude product which was used directly in the next step.



9 10

N-(2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethyl)-6-(5-methyl-2-oxoimidazolidin-4-yl)hexanamide the crude from above reaction was dissolved in methanol (10 mL) and then 10% Pd on carbon (50 mg) was added. The mixture solution was stirred for 3 hours with hydrogen balloon at room temperature. The solution was filtered to remove catalyst and solvent was removed under reduced pressure. The product was purified by silica gel column with CH_2Cl_2/methanol (10/1) to provide the product **10** (233 mg, 30%). LC-MS: *m/z* (M+H) 389. ^{1}H NMR (500 MHz, MeOD) δ 3.85 (dd, *J* = 7.8, 6.5 Hz, 1H), 3.76 – 3.70 (m, 1H), 3.71 – 3.64 (m, 8H), 3.62 – 3.50 (m, 4H), 3.40 (d, *J* = 5.5 Hz, 1H), 3.34 (dt, *J* = 3.3, 1.6 Hz, 1H), 2.81 (dd, *J* =

7.2, 3.4 Hz, 2H), 2.25 (dd, *J* = 15.2, 7.9 Hz, 2H), 1.72 – 1.60 (m, 2H), 1.59 – 1.50 (m, 2H), 1.50 – 1.27 (m, 4H), 1.14 (d, *J* = 6.5 Hz, 3H).



10 11

tert-butyl methyl(22-(5-methyl-2-oxoimidazolidin-4-yl)-3,17-dioxo-7,10,13-trioxa-4,16-diazadocosyl)carbamate (11) The compound **10** (388 mg, 1.0 mmol) 3-((tert-butoxycarbonyl)(methyl)amino)propanoic acid (260 mg, 1.3 mmol) and 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (370 mg, 2.0 mmol) in methylene chloride (15 mL) and then trimethylamine (270 uL, 2.0 mmol) was added at 0°C. The mixture solution was stirred for 6 hours. The solvent was removed under the reduced pressure and the residue was purified by purified by silica gel column with CH_2Cl_2/methanol (10/1) to provide the product **11** (120mg, 21%) LC-MS: *m/z* (M+H) 574.37

^{1}H NMR (500 MHz, MeOD) δ 3.85 (dd, *J* = 7.9, 6.5 Hz, 1H), 3.76 – 3.62 (m, 8H), 3.59 – 3.50 (m, 5H), 3.40 (d, *J* = 5.5 Hz, 3H), 3.34 (dt, *J* = 3.3, 1.6 Hz, 1H), 3.21 – 3.12 (m, 1H), 2.89 (s, 3H), 2.45 (t, *J* = 6.9 Hz, 2H), 2.36 (s, 2H), 2.24 (t, *J* = 7.5 Hz, 2H), 1.69 (dq, *J* = 29.6, 7.5 Hz, 2H), 1.53 (ddd, *J* = 7.5, 5.2, 2.7 Hz, 2H), 1.49 (s, 9H), 1.45 – 1.30 (m, 3H), 1.15 – 1.10 (m, 3H).

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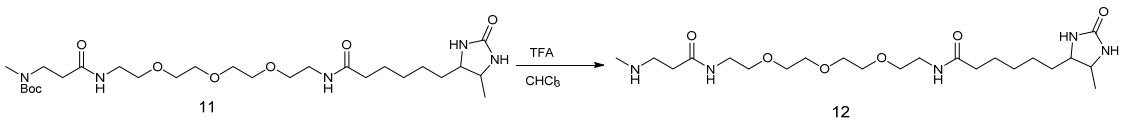
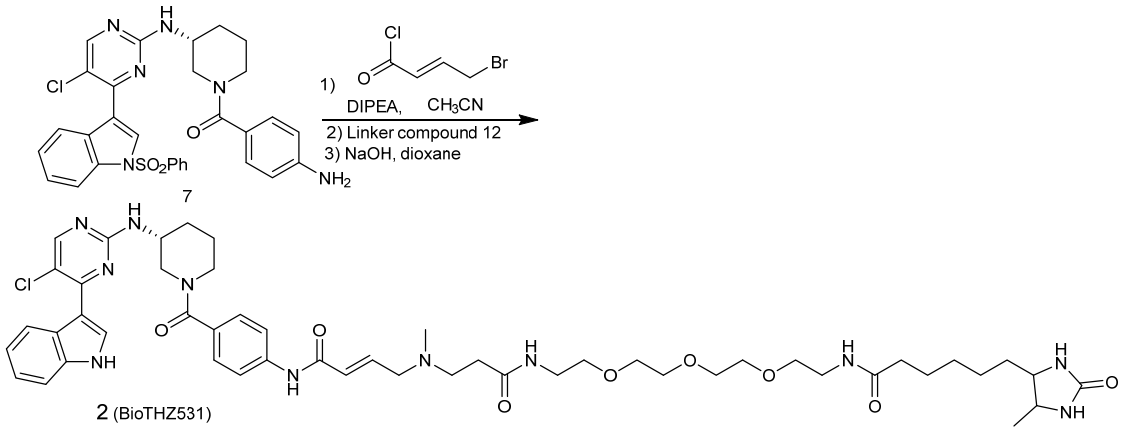


11 12

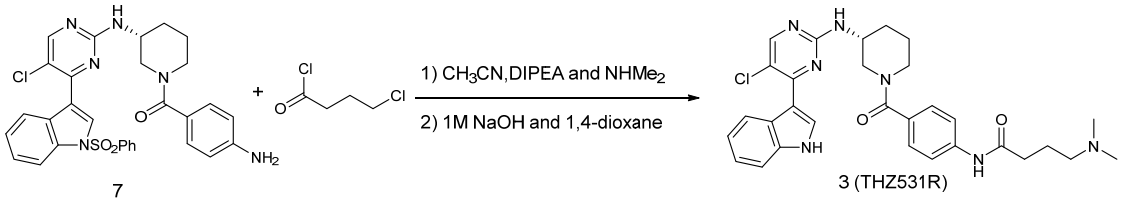
6-(5-methyl-2-oxoimidazolidin-4-yl)-N-(5-oxo-9,12,15-trioxa-2,6-diazaheptadecan-17-yl)hexanamide Crude compound 11 was dissolved in chloroform (10 mL) and then trifluoroacetic acid (5 mL) was added. The solution was stirred at room temperature for 1 hour and then solvent was removed under the reduced pressure. The residue was purified by

preparative LC-MS with methanol and water (1% TFA) as eluent to provide compound 11 (165 mg, 35% for two steps). LC-MS: *m/z* (M+H) 474.



N-((E)-20-((4-((R)-3-((5-chloro-4-(1H-indol-3-yl)pyrimidin-2-yl)amino)piperidine-1-carbonyl)phenyl)amino)-16-methyl-13,20-dioxo-3,6,9-trioxa-12,16-diazaicos-18-en-1-yl)-6-(5-methyl-2-oxoimidazolidin-4-yl)hexanamide (2) To the solution of the compound **7** (60 mg) in acetonitrile (10 mL) was added diisopropylethylamine (13 mg, 1.0 equiv). The reaction mixture was cooled down to 0 ℃ and then treated with 4-chlorobut-2-enoyl chloride (54 mg, 3.0 equiv) in CH_2Cl_2. The reaction mixture was stirred for 10 min at 0 ^{O}C and propanol (4:1, 30 mL) and was washed with water. The removal of solvent provided the crude which was used then dissolved in THF (3.0 mL). To this solution, the free amino compound 12 (94.0 mg, 0.2 mmol) was added and the solution were then heated for 2 hours at 50℃. The reaction mixture was then cooled down to room temperature. The solvent was removed under reduced pressure and the residue was dissolved again in 1,4-dioxane (2.0 mL) followed by adding 1M NaOH solution (2.0 mL). The solution was stirred at room temperature for 2 hours and then was quenched with 1M HCl solution (2.0mL), extracted with chloroform/2-propanol (4/1, vol/vol, 20 mL). The organic layer was washed with water, brine and dried over sodium sulfate. The crude after removing the solvent was purified by HPLC to provide BioTHZ531 as TFA salt (15.0 mg, 15%) LC-MS: *m/z* (M+H) 987,^{1}H NMR (500 MHz, DMSO-*d6*) 11.67 (s, 1H), 10.23 (s, 1H), 8.54 (s, 1H), 8.39 (s,

1H), 8.21 (s, 1H), 8.05 (s, 1H), 7.65 (d, *J* = 7.0 Hz, 2H), 7.55 (br, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.22 (t, *J* = 8.0 Hz, 1H), 7.16 (t, *J* = 8.0 Hz, 1H), 7.06 (br, 1H), 6.83 (m, 1H), 6.52 (d, *J* = 15.0 Hz, 1H), 4.20-3.20 (m, 20H), 2.80 (s, 3H), 2.65 (t, *J* = 7.0 Hz, 2H), 2.08 (m, 3H), 1.86 (m, 1H), 1.71 (m, 1H), 1.58 (m, 1H), 1.51 (m, 2H), 1.40-1.22 (m, 4H), 1.00 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d6) δ 173.76, 170.70, 169.77, 163.10, 160.22, 158.68, 158.38, 157.41, 140.41, 136.63, 131.44, 131.08, 128.15, 126.81, 123.27, 122.76, 121.01, 119.18, 114.07, 112.24, 111.66, 55.75, 55.54, 55.20, 50.81, 48.27, 35.77, 34.38, 33.41, 30.63, 29.99, 29.21, 25.96, 25.30, 24.00, 19.75, 15.90.



(R)-N-(4-(3-((5-chloro-4-(1H-indol-3-yl)pyrimidin-2-yl)amino)piperidine-1-carbonyl)phenyl)-4-(dimethylamino)butanamide (3) The free amino compound **7** (60mg) was dissolved in acetonitrile (2.0 mL) was added diisopropylethylamine (20.0 uL) and 4-chlorobutanoyl chloride (30 mg, 2.0 equiv) in dichloromethane (1.0 mL). After stirring for 5 min, dimethylamine (1M, 2.0 mL)) in THF was added and the reaction mixture was then heated to 50 °C for 2 hours. After removal of solvent, the crude was then treated with 1,4-dioxane (2.0 mL) and 1 M NaOH solution (2.0 mL) and then the solution was stirred at room temperature for 2 hours. After quench with 1M HCl (2.0 mL), the solution was extracted with chloroform/2-propanol (4:1, 20 mL). The organic layer was washed with water, brine and dried with $MgSO_4$.The concentration under reduced pressure to give the crude produce which was purified by HPLC to provide THZ531R (**3)** as TFA salt (16.0 mg, 60%) LC-MS: *m/z* (M+H) 560 ^{1}H NMR (500 MHz, DMSO-*d6*) 11.67 (s, 1H), 9.95 (s, 1H), 9.34(br, 1H), 8.53 (s, 1H), 8.40 (s, 1H), 8.21 (s, 1H), 7.67 (d, *J* = 7.0 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 2H), 7.22 (t, *J* = 8.0 Hz, 1H), 7.16 (d, *J* = 8.0 Hz, 1H), 7.04 (br, 1H), 3.99 (br, 2H), 3.13 (m, 5H), 2.80 (s, 6H), 2.44 (t, J =7.0 Hz), 2.10

(m, 1H), 1.98 (m, 2H), 1.85 (m, 1H), 1.71 (m, 1H), 1.58 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 170.66, 169.81, 160.24, 158.66, 157.46, 140.40, 136.62, 131.43, 131.07, 128.14 (2C), 126.80, 123.25, 122.78, 121.03, 119.18 (2C), 114.09, 112.24, 111.65, 57.14, 49.40, 48.27, 45.06, 42.99 (2C), 33.36, 30.61, 23.97, 20.17.