Figure 1b

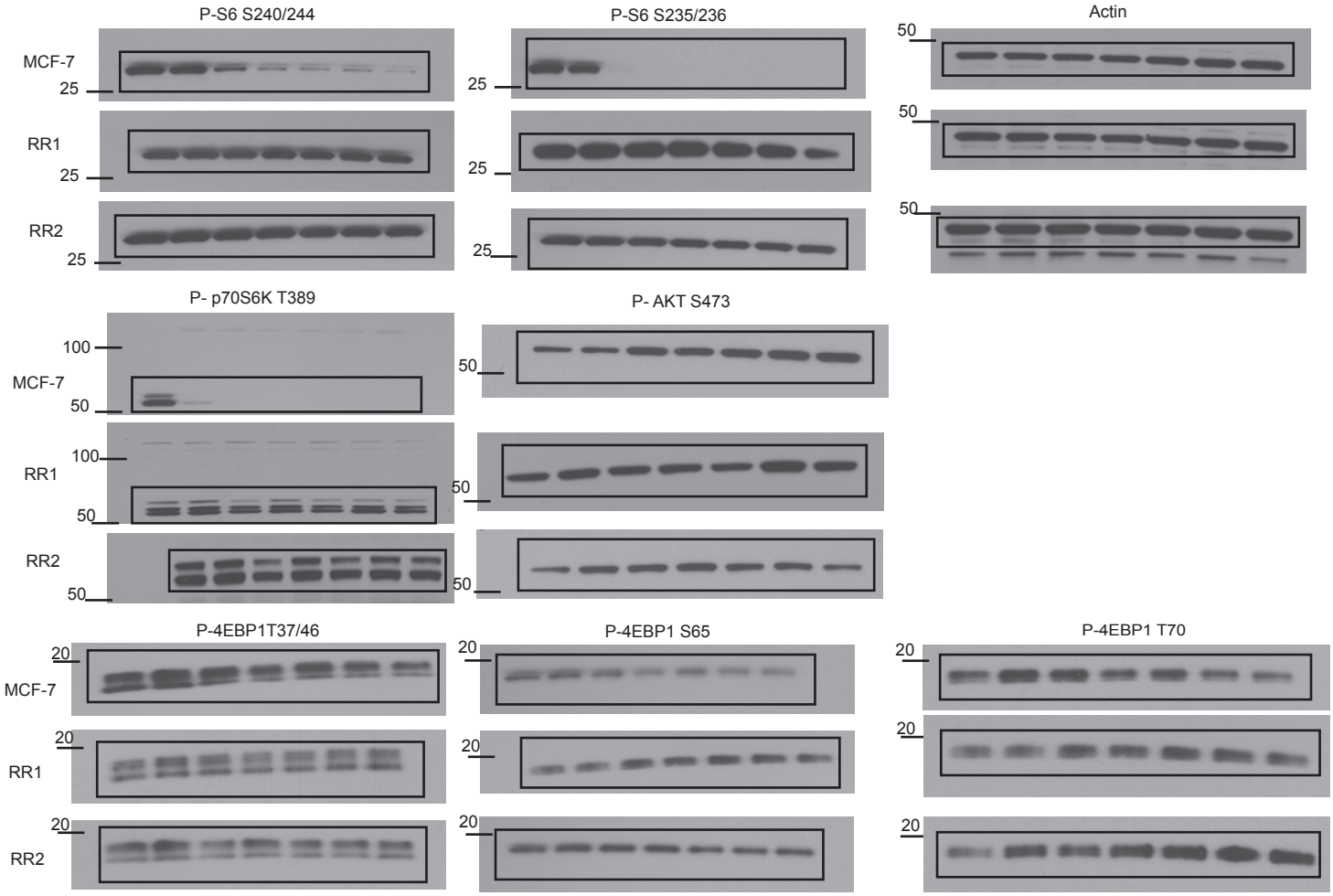
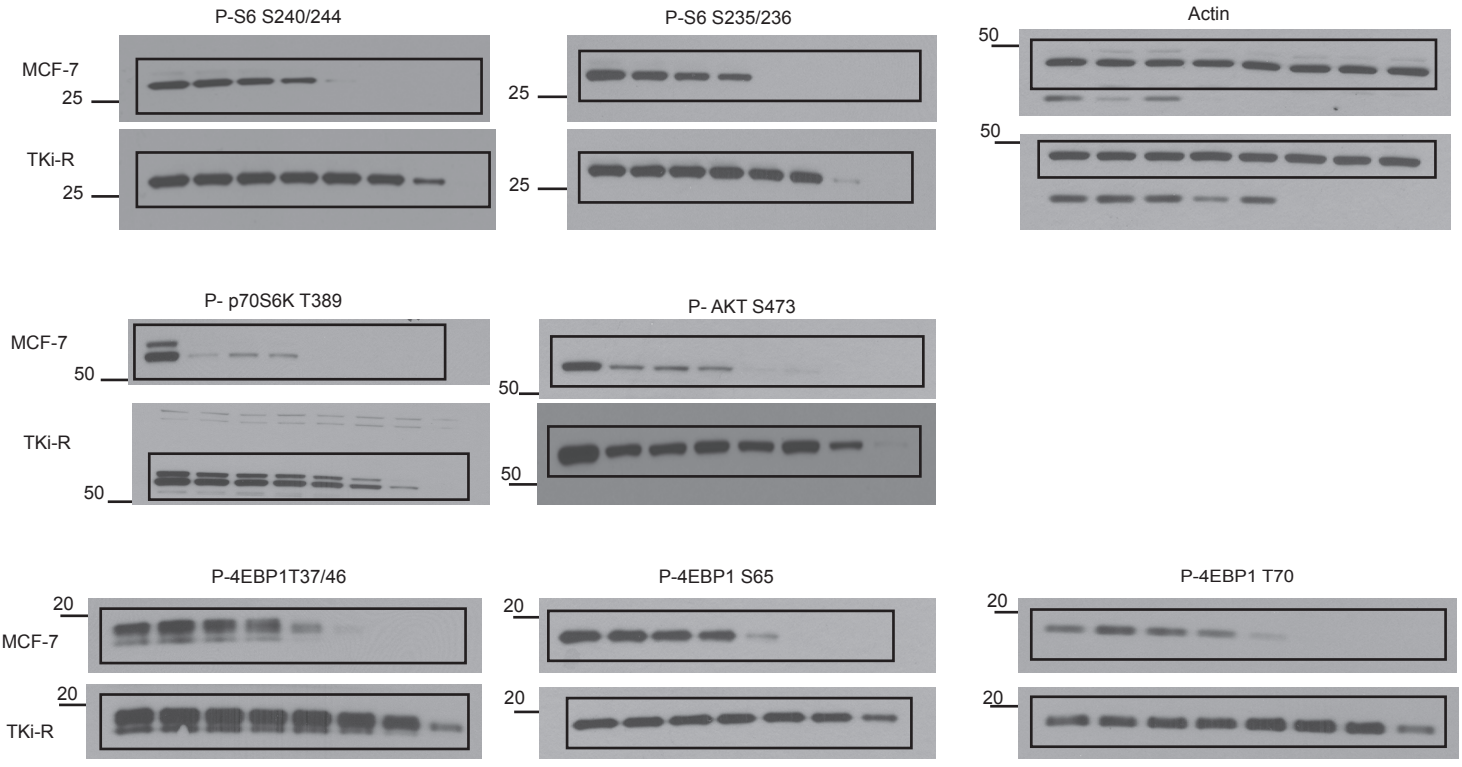


Figure 1c



Supplementary Figure 1: Uncropped blots from Figures 1b (top panels) and 1c (bottom panels)

Figure 2a

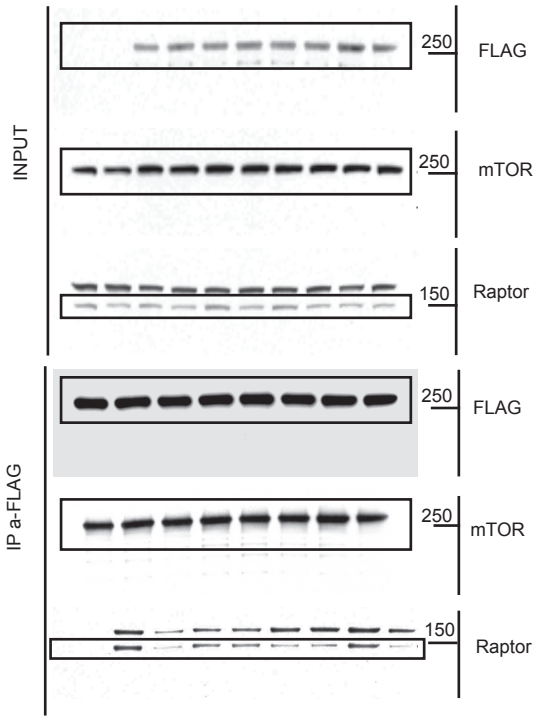
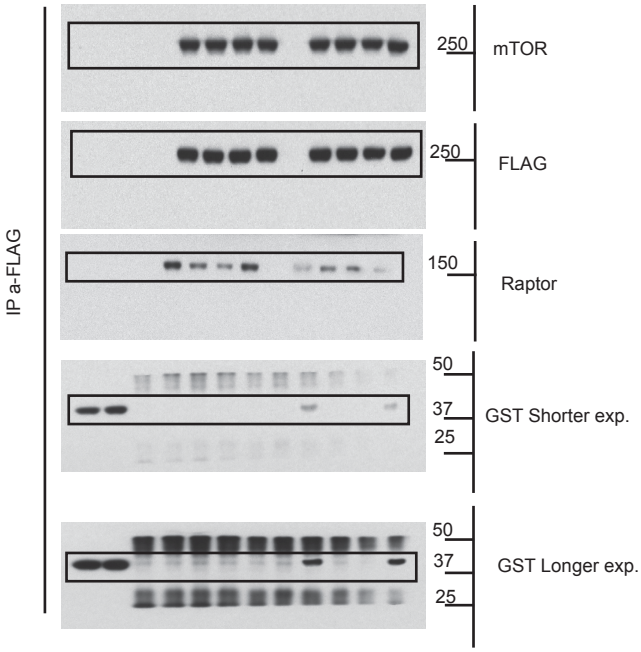


Figure 2b



Supplementary Figure 2: Uncropped blots from Figures 2a and 2b

Figure 3c

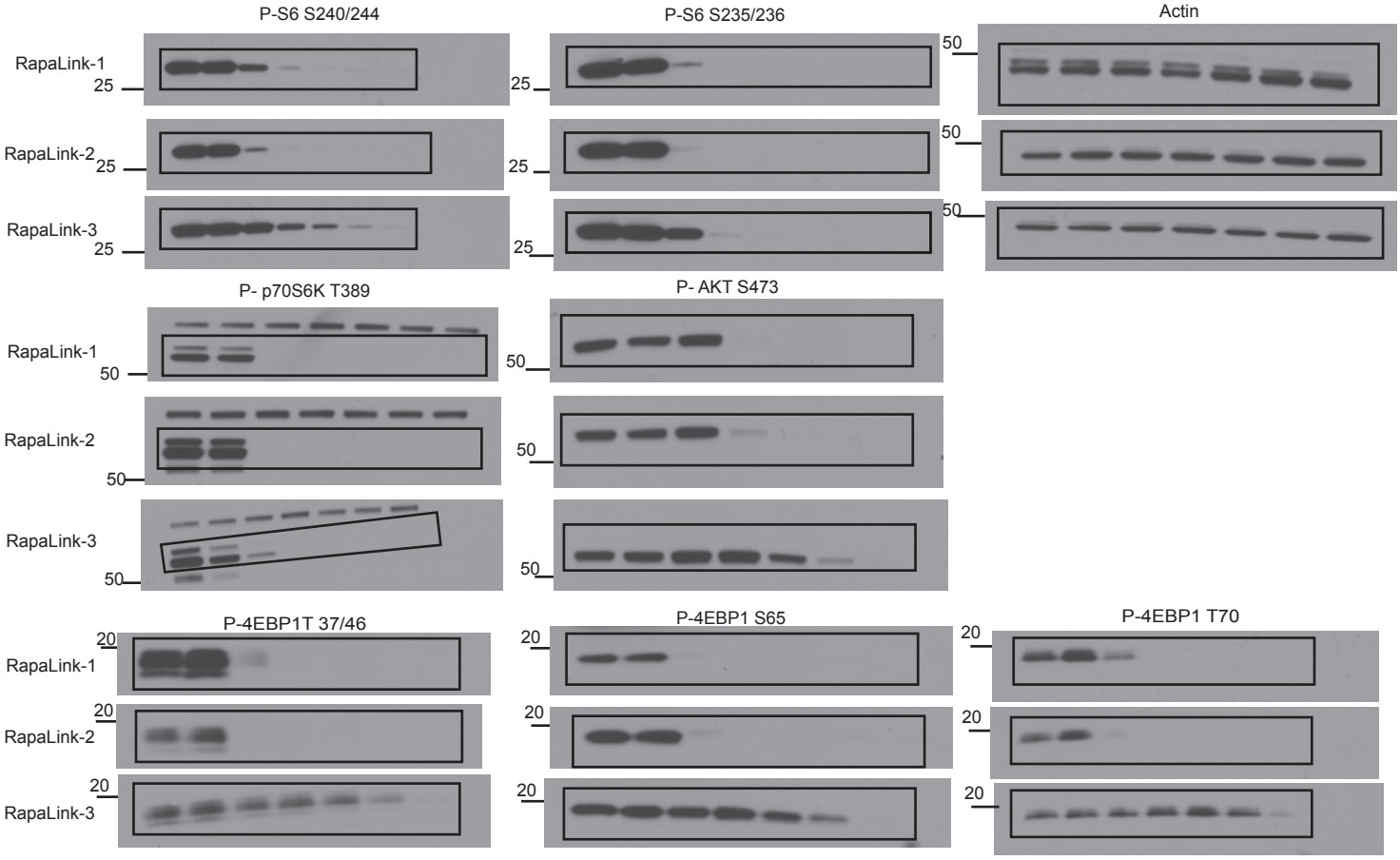
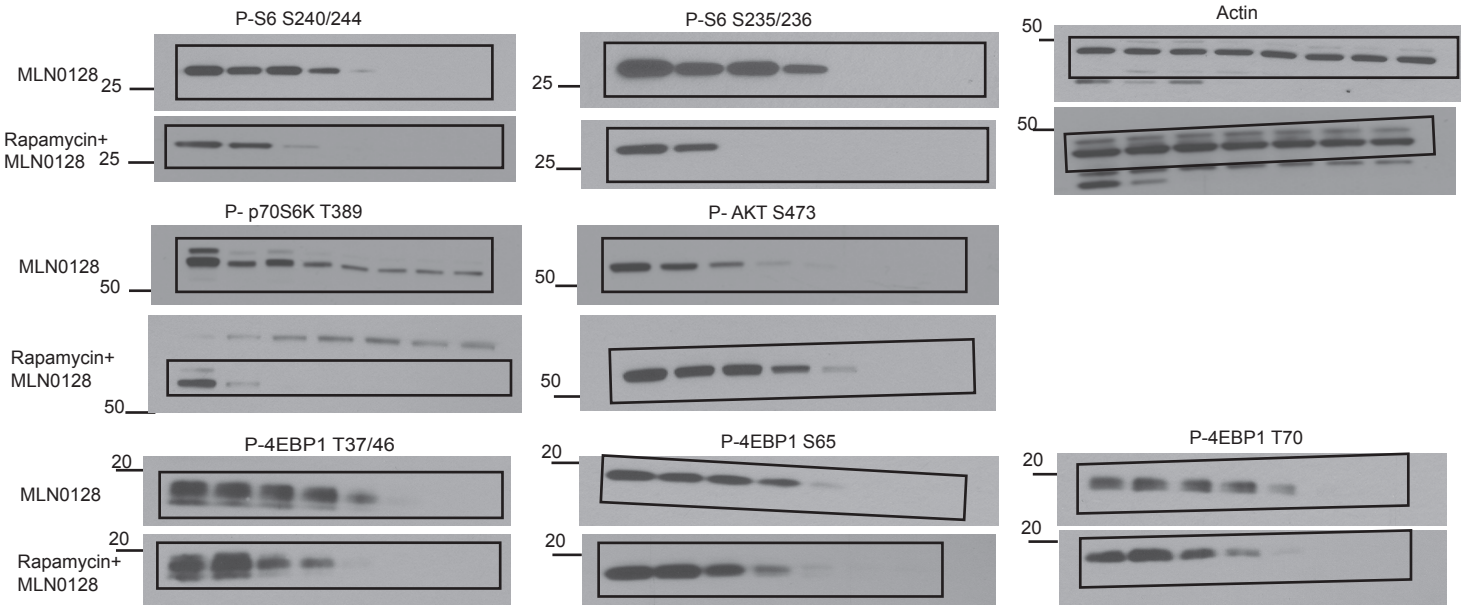
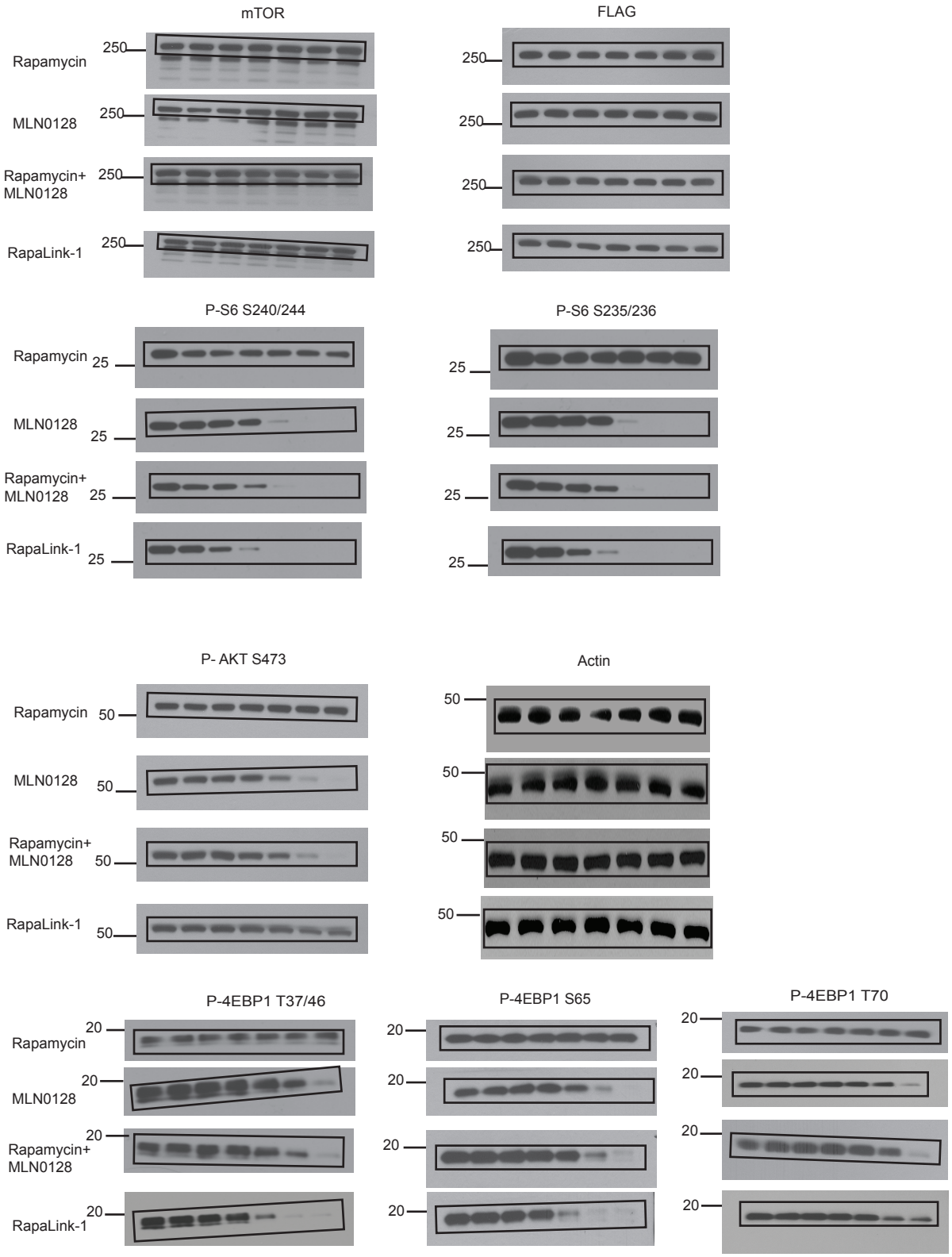


Figure 3d



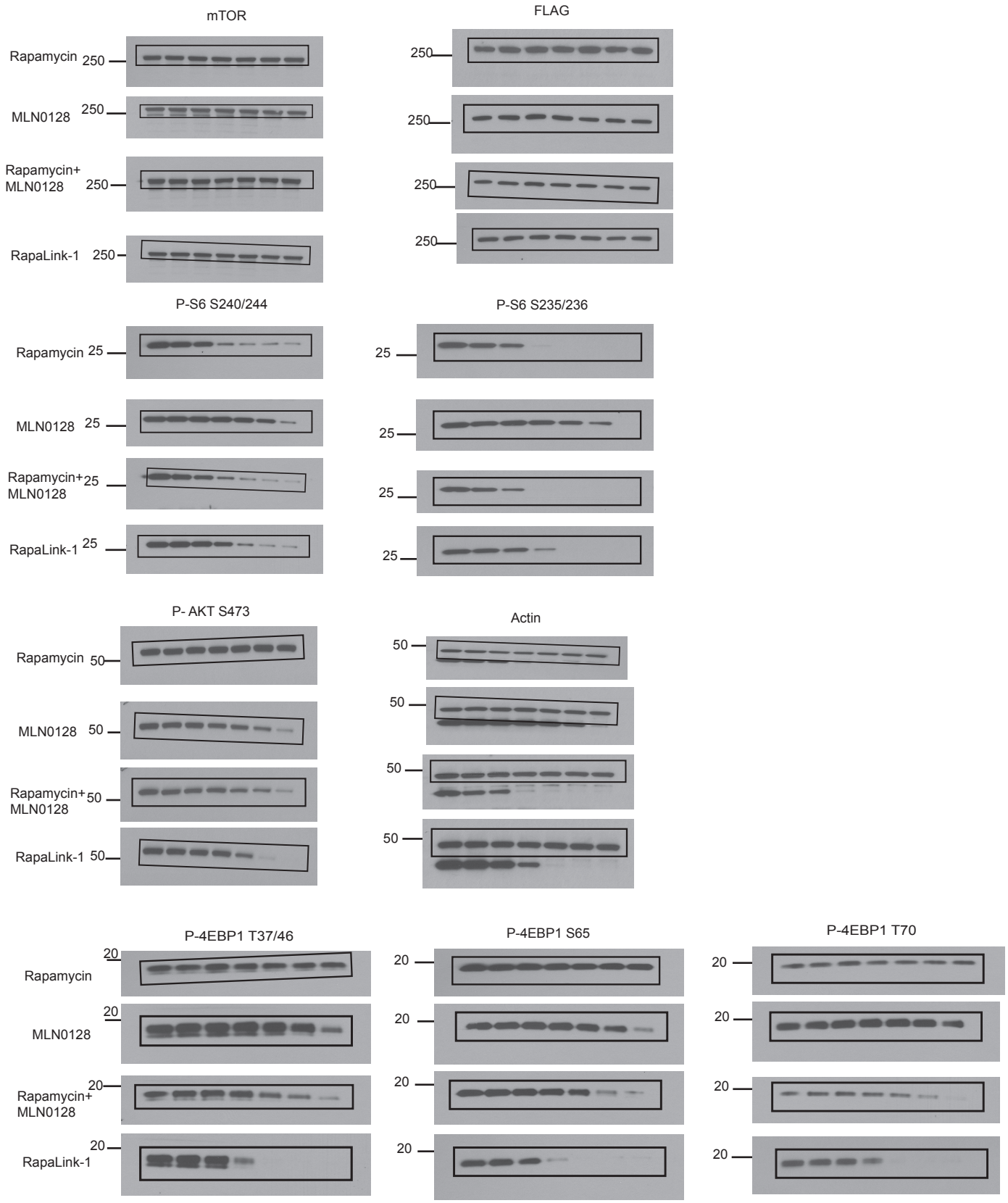
Supplementary Figure 3: Uncropped blots from Figures 3c (top panels) and 3d (bottom panel).

Figure 4a MDA-MB-468 stable F2108L mTOR cells



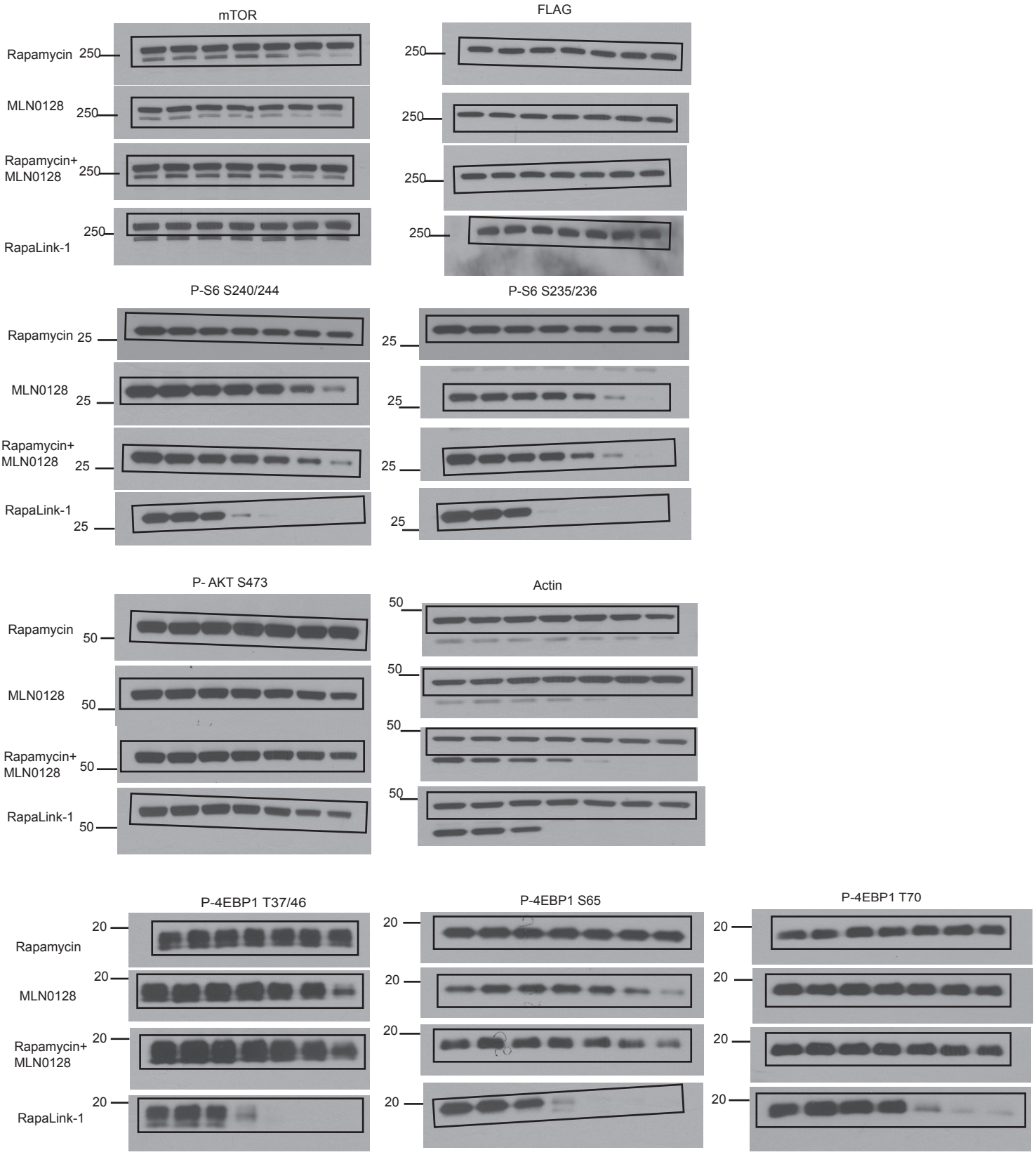
Supplementary Figure 4: Uncropped blots from Figure 4a.

Figure 4b MDA-MB-468 stable M2327I mTOR cells



Supplementary Figure 5: Uncropped blots from Figure 4b.

Figure 4e MDA-MB-468 stable F2108L/M2327I mTOR cells



Supplementary Figure 6: Uncropped blots from Figure 4e

MCF-7	Parental	Rapamycin Resistant		AZD8055 Resistant
mTOR	WT	RR1	RR2	TKi-R
Rapamycin IC50 (nM)	3.0	>500	>500	4.5
AZD8055 IC50 (nM)	33.1	24.5	26.9	478.6

Supplementary Table: Cell growth IC50 of the MCF-7 parental, RR1, RR2 and TKi-R cells
Cells were treated with either rapamycin or AZD8055 treatment and cell growth IC50 was determined as described in Figure 1d.

Supplementary Methods

RapaLink Synthesis

Abbreviations used in syntheses descriptions. AcOH: acetic acid, DME: 1,2-dimethoxyethane, DMF: *N*,*N*-dimethylformamide, DMSO: dimethylsulfoxide, dPEG: discrete poly-(ethylene glycol), EDCI: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, ESI: electrospray ionization, EtOAc: ethyl acetate, HOBt: 1-hydroxybenzotriazole, HPLC: high performance liquid chromatography, HR-MS: high resolution mass spectroscopy, LC–MS: liquid chromatography–mass spectrometry, LTQ-FT: linear trap quadrupole–Fourier transform, MeOH: methanol, NHS: N-hydroxysuccinimide, NMR: nuclear magnetic resonance, RP-HPLC: reverse phase–high performance liquid chromatography, THF: tetrahydrofuran, TLC: thin layer chromatography, TMS: tetramethylsilane.

Starting materials, reagents, and solvents for reactions were of reagent grade and were used as purchased. TLC was carried out using Merck Kieselgel 60, 63−200 mesh, F254 plates, or Fuji Silysia Chemical Ltd., 100−200 mesh, NH plates. Chromatographic purification was carried out using silica gel (Merck, 70−230 mesh) or basic silica gel (Fuji Silysia Chemical Ltd., DM1020, 100−200 mesh). RP-HPLC was carried out on a Waters Binary Gradient Module 2545 system equipped with an Agilent Zorbax 300-SB C18 column (5 μm, 4.6×250 mm) for analytical mode or a Waters XBridge Prep C18 column (5 μm, 30×250 mm) for preparative mode. The column was eluted with CH_3CN/water/0.1%formic acid (gradient mode), which was monitored by Waters Photodiode Array Detector 2998 (UV at $\lambda = 254$ nm). Yields were not optimized.

^1H NMR spectra for intermediates were recorded on a Varian Innova (400 MHz) spectrometer. ^1H NMR spectra, ^1H-^1H COSY, HSQC, and HMBC spectra for final compounds were recorded

on a Bruker Avance (800 MHz) spectrometer. ^{13}C NMR spectra were recorded on a Bruker Avance (500 MHz) spectrometer (500 MHz for ^{1}H, 126 MHz for ^{13}C). ^{1}H chemical shifts are reported in δ (ppm) as s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), m (multiplet) or br s (broad singlet) and are referenced to TMS as an internal standard. LC-MS (ESI-MS) spectra were recorded with a Waters 2695 separations module using a Waters ACQUITY UPLC BEH C18 1.7 μm column and were used to confirm ≥95% purity of each compound. Mobile phase A was 0.1% formic acid in ultrapure water. Mobile phase B was 0.1% formic acid in acetonitrile, which was increased linearly from 5% to 95% over 1.8 min and 95% over the next 0.3 min (flow rate: 0.6 mL/min). HR-MS analysis was conducted by QB3/Chemistry Mass Spectrometry Facility at UC Berkeley. Samples were analyzed by electrospray ionization with a mass measuring accuracy of 5 ppm using the LTQ-FT instrument.

Preparation of 2-(prop-2-yn-1-yloxy)ethanol. To a cooled ethane-1,2-diol (**2**) (150 mL) was slowly added NaH oil dispersion (43.2 g) over 1 h at −30 °C. The mixture was stirred at ambient temperature for additional 1 h. To the reaction mixture was slowly added 9.2 M propargyl bromide solution in toluene (45.47 g) over 30 min under cooling bath (−10 °C). The mixture was stirred at 50 °C for 60 h. It was then partitioned between EtOAc (400 mL) and water (200 mL). The aqueous layer was separated and extracted with EtOAc (200 mL). The organic layers were combined, washed with brine (100 mL) and dried over anhydrous $MgSO_4$. The insoluble was filtered and the filtrate was evaporated in vacuo. The crude material was purified by silica gel column chromatography (silica gel: 800 g, solvent: hexanes (2 L) followed by 50% EtOAc in hexanes (4L)). The desired fractions were combined and evaporated in vacuo to give the titled

compound (9.63 g, 31%) as a yellow oil. This material was used for the next reaction without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19–4.22 (2H, m), 3.73–3.79 (2H, m), 3.63–3.67 (2H, m), 2.45 (1H, t, J = 2.4 Hz), 1.96 (1H, t, J = 6.0 Hz).

Preparation of 2-(prop-2-yn-1-yloxy)ethyl trifluoromethanesulfonate 3. To a solution of 2-(prop-2-yn-1-yloxy)ethanol (1.50 g, 15.0 mmol) in CH_2Cl_2 (20 mL) was added 2,6-lutidine (2.44 mL, 21.0 mmol) followed by trifluoromethanesulfonic anhydride (3.15 mL, 18.7 mmol) at −50 °C under argon atmosphere. The mixture was stirred at −10 °C for 2 h. It was then partitioned between 50% EtOAc in hexanes (150 mL) and brine (15 mL). The organic layer was separated, washed with brine (15 mL) and dried over anhydrous $MgSO_4$. The insoluble was filtered and the filtrate was evaporated in vacuo. The crude material was purified by silica gel column chromatography (silica gel: 50 g, solvent 10% EtOAc in hexanes (500 mL)). The desired fractions were combined and evaporated in vacuo to give the titled compound (2.49 g, 72%) as a dark brown oil. This material was used for the next reaction without further purification.

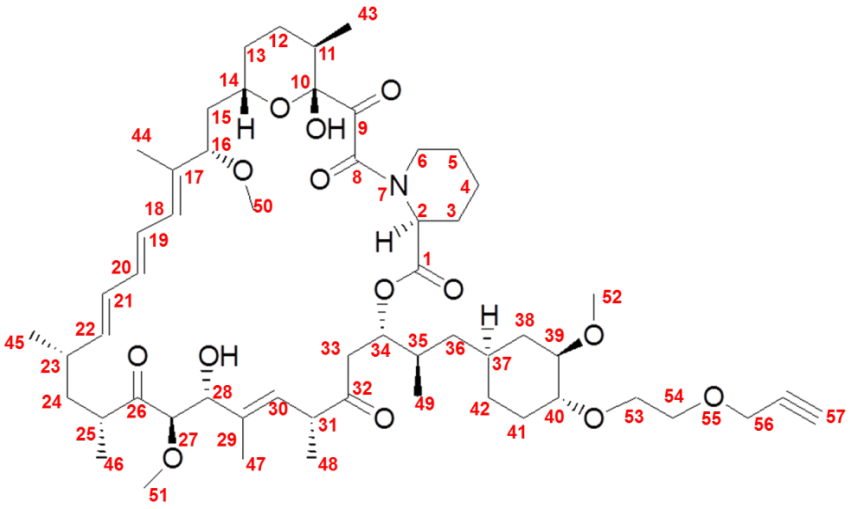
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.64–4.66 (2H, m), 4.22–4.25 (2H, m), 3.85–3.88 (2H, m), 2.48–2.49 (1H, m).

Preparation of 40-*O*-(2-(prop-2-yn-1-yloxy)ethyl)-rapamycin 4 as a precursor for the azide-alkyne cycloaddition reaction. To a solution of rapamycin (**1**) (652 mg, 0.714 mmol) in $CHCl_3$ (1.5 mL) were added a solution of 2-(prop-2-yn-1-yloxy)ethyl trifluoromethanesulfonate (**3**) (1.25 g, 5.35 mmol) in $CHCl_3$ (1.5 mL) and *N*,*N*-diisopropyl-*N*-ethylamine (6.2 mL, 35.7 mmol) at −10 °C under argon atmosphere. The mixture was stirred at 60 °C for 30 min. An additional amount of 2-(prop-2-yn-1-yloxy)ethyl trifluoromethanesulfonate (**3**) (1.25 g, 5.35 mmol) in

$CHCl_3$ (1.5 mL) was added. The mixture was stirred at 60 °C for additional 1 h. It was then cooled and partitioned between EtOAc (100 mL) and water (50 mL). The organic layer was washed with water (50 mL) and brine (2×25 mL), successively, and dried over anhydrous $MgSO_4$. The insoluble was filtered off and the filtrate was evaporated in vacuo. The crude material was purified by silica gel column chromatography (silica gel: 25 g, solvent: 20–80% EtOAc in hexanes). Desired fractions were combined and evaporated in vacuo. The obtained material was dissolved into 50% CH_3CN in water and lyophilized to give the titled compound (277 mg, 28%) as a colorless amorphous powder.

HR-MS (ESI−) Calcd for $C_{56}H_{84}O_{14}N$ $(M-H)^-$ 994.5897. Found 994.5885 (Δ −1.24 ppm).

Table S1. NMR Analysis of Compound **4 as a precursor of RapaLink-1, -2, and -3**.



Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
1	*C*=O		169.2	2	N/A
2	*CH*	5.28 (br d, 4.8 Hz)	51.2	4a, 6a	3a,b

Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
3	*C*H_2	a: 2.35 (m) b: 1.74 (m)	27.1	2	3b, 2, 4b 3a, 2, 4b
4	*C*H_2	a: 1.77 (m) b: 1.47 (m)	20.7	2	4b, 3a, 5b 4a, 3a
5	*C*H_2	a: 1.74 (m) b: 1.49 (m)	25.3	3a, 6a	5b, 6b 5a, 4a, 6a,b
6	*C*H_2	a: 3.57 (m) b: 3.44 (m)	44.2	2, 4a,b	6b, 5b 6a, 5a,b
8	*C*=O	N/A	166.8	2, 6a	N/A
9	*C*=O	N/A	192.5	n.d.	N/A
10	O-*C*-OH	N/A	98.5	12, 43	N/A
11	*CH*	1.97 (m)	33.7	12, 43	12, 13a, 43
12	*C*H_2	1.59 (2H, m)	27.3	43	11, 13b
13	*C*H_2	a: 1.61 (m) b: 1.31 (m)	31.3	12, 15a	11, 13b 13a, 12, 14
14	*CH*-OC	3.87 (m)	67.2	12, 15a	13b, 15a, b
15	*C*H_2	a: 1.85 (m) b: 1.52 (m)	38.8	16	15b, 14, 16 15a, 14, 16
16	*CH*-OCH_3	3.66 (m)	84.4	15a, 18, 50	15a, b
17	-*C*=	N/A	135.5	15a, 19, 44	N/A
18	*CH*=C	5.96 (d, 9.6 Hz)	129.7	16, 20, 44	19
19	*CH*=C	6.38 (dd, 14.4, 10.8 Hz)	126.4	20, 21, 44	18, 20
20	*CH*=C	6.35 (dd, 16.6, 10.2 Hz)	133.7	18, 19, 21, 22	19, 21
21	*CH*=C	6.14 (dd, 15.2, 10.2 Hz)	130.2	19	20, 22

Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
22	*CH*=C	5.55 (dd, 14.8, 9.2 Hz)	140.2	20, 24b, 45	21, 23
23	*CH*	2.33 (m)	35.2	21, 22, 24a, 45	22, 24a, 45
24	*CH*$_2$	a: 1.49 (m) b: 1.21 (m)	40.2	22, 25, 45, 46	24b, 23 24a, 25
25	*CH*	2.74 (dd, 17.0, 5.8)	41.4	24a,b, 46	24b, 46
26	*C*=O	N/A	215.7	n.d.	N/A
27	*CH*-OCH$_3$	3.71 (m)	84.8	28, 51	28
28	*CH*-OH	4.18 (m)	77.3	27, 30, 47	27
29	*C*=C	N/A	136.1	28, 31, 47	N/A
30	*CH*=C	5.41 (d, 10.0 Hz)	126.8	28, 31, 47, 48	31
31	*CH*	3.34 (m)	46.6	30, 48	30, 48
32	*C*=O	N/A	208.2	31, 33a,b, 48	N/A
33	*CH*$_2$	a: 2.73 (m) b: 2.59 (m)	40.8	n.d.	33b, 34 33a, 34
34	*CH*-OCO	5.16 (dd, 10.0, 5.8 Hz)	75.7	33a,b, 49	33a,b, 35
35	*CH*	1.95 (m)	33.2	33a,b, 36a,b, 49	34, 36a,b, 49
36	*CH*$_2$	a: 1.19 (m) b: 1.11 (m)	38.3	34, 38b, 49	36b, 35, 37 36a, 35
37	*CH*	1.33 (m)	33.1	36a,b, 38a,b, 42b	36a, 38b, 42b
38	*CH*$_2$	a: 2.03 (m) b: 0.71 (m)	36.3	36a,b	38b, 39 38a, 37, 39
39	*CH*-OCH$_3$	3.06 (m)	83.2	38a,b,40, 52	38a,b, 40
40	*CH*-O-	3.13 (m)	83.3	38a,b, 39, 52, 53	39, 41b

Atom	Atom Type	δ ^{1}H Major (*3*:1)	δ ^{13}C Major (*3*:1)	HMBC C to H	^{1}H-^{1}H COSY
41	CH_2	a: 2.05 (m) b: 1.26 (m)	30.1	42b	41b 41a, 40, 42b
42	CH_2	a: 1.69 (m) b: 0.92 (m)	31.8	36a,b, 38a,b	42b 42a, 37, 41b
43	11-CH_3	0.94 (3H, d, 6.4 Hz)	16.3	11, 12	11
44	17-CH_3	1.65 (3H, s)	10.2	16, 18	n.d.
45	23-CH_3	1.05 (3H, d, 6.4 Hz)	21.6	22, 24a	23
46	25-CH_3	0.99 (3H, d, 6.4 Hz)	13.8	24a,b, 25	25
47	29-CH_3	1.74 (3H, s)	13.1	28, 30	n.d.
48	31-CH_3	1.10 (3H, d, 6.8 Hz)	16.0	30, 31	31
49	35-CH_3	0.91 (3H, d, 6.8 Hz)	16.9	36b	35
50	16-OCH_3	3.14 (3H, s)	55.9	16	n.d.
51	27-OCH_3	3.34 (3H, s)	59.5	27	n.d.
52	39-OCH_3	3.46 (3H, s)	58.0	39	n.d.
53	40-OCH_2-	3.72-3.79 (2H, m)	69.3	54, 40	54
54	-CH_2-O-	3.64-3.71 (2H, m)	69.6	53, 55	53, 55
55	-O-CH_2	4.20-4.22 (2H, m)	58.6	54, 57	54, 57
56	-*C*≡	N/A	79.8	55, 57	N/A
57	≡*CH*	2.41 (t, 2.2 Hz)	74.4	55	55

OH protons were not identified.

Preparation of *tert*-butyl (4-(4-amino-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)butyl) carbamate. To a suspension of 3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**5**) (2.11 g, 8.08 mmol) in DMF (25 mL) was added NaH oil dispersion (485 mg, 12.1 mmol) at 4 °C. The mixture was stirred at 4 °C for 30 min. To the reaction mixture was added *tert*-butyl (4-bromobutyl)carbamate (2.50 g, 8.92 mmol) in DMF (5 mL) at 4 °C. The mixture was stirred at

room temperature for 14 h. To the mixture was added water (100 mL) at room temperature. The mixture was cooled to 4 °C and stirred for 30 min. The resulting precipitate was collected by filtration. Drying the solid gave the titled compound (3.01 g, 86%) as a colorless powder.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (1H, s), 5.86 (2H, br s), 4.61 (1H, br s), 4.39 (2H, t, J = 7.2 Hz), 3.05–3.25 (2H, br s), 1.90–1.98 (2H, m), 1.44–1.55 (2H, m), 1.43 (9H, s).

LC-MS (ESI) m/z = 433.09 $(M+H)^+$.

Preparation of *tert*-butyl (4-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)butyl)carbamate 6. To a bi-phasic suspension of *tert*-butyl (4-(4-amino-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)butyl)carbamate (435 mg, 1.00 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[d]oxazol-2-amine (390 mg, 1.50 mmol), and Na_2CO_3 (530 mg, 5.00 mmol) in DME (10 mL) and water (5 mL) was added tetrakis(triphenylphosphine)palladium (0) (116 mg, 100 μmol) at room temperature under argon atmosphere. The mixture was stirred at 110 °C for 3 h. It was then cooled and partitioned between EtOAc (90 mL) and water (30 mL). The aqueous layer was separated and extracted with EtOAc (2×30 mL). The organic layers were combined, washed with brine (2×30 mL) and dried over anhydrous $MgSO_4$. The insoluble was filtered off and the filtrate was concentrated in vacuo. The crude material was purified by silica gel column chromatography (silica gel: 75 g, solvent: 50% EtOAc in hexanes (400 mL) followed by 20% MeOH in EtOAc (800 mL)). The desired fractions were combined and evaporated in vacuo. The obtained solid was recrystallized from MeOH/water to give the titled compound (332 mg, 76%) as a colorless solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (1H, s), 7.52 (2H, s), 7.46 (1H, d, J = 8.0 Hz), 7.41 (1H, s), 7.23 (1H, dd, J = 8.0, 1.2 Hz), 6.79 (1H, t, J = 5.6 Hz), 4.32 (2H, t, J = 5.6 Hz), 3.26–3.33 (2H, m), 2.88–2.96 (2H, m), 1.77–1.87 (2H, m), 1.35 (9H, s), NH_2 protons were not identified. LC-MS (ESI) m/z = 439.28 $(M+H)^+$.

Preparation of *N*-(4-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)butyl)-1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-amide 7. To a cooled liquid of TFA (3 mL) was added *tert*-butyl (4-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)butyl)carbamate (**6**) (300 mg, 0.68 mmol) at 4 °C. The mixture was stirred at ambient temperature for 1 h. It was then evaporated in vacuo. The oily residue was triturated with Et_2O for 10 min. The supernatant was removed and then the precipitate was triturated with 2 M hydrochloride in Et_2O solution (3 mL) for 30 min. The precipitate was collected by filtration under argon atmosphere. Drying the solid gave the salt of Boc-cleaved compound (362 mg). The obtained material (136 mg) was dissolved into DMF (4 mL). To the mixture was added triethylamine (146 μL, 1.05 mmol) followed by a solution of azide-dPEG8-NHS ester (Catalog number 10503, Quanta BioDesign, Ltd., Powell, OH USA) (200 mg, 0.35 mmol) in DMF (4 mL) under argon atmosphere. The mixture was stirred at room temperature for 13 h. It was then evaporated in vacuo. The residue was partitioned between 10% THF in EtOAc (100 mL) and brine (20 mL). The aqueous layer was separated and extracted with EtOAc (50 mL). The organic layers were combined and dried over anhydrous $MgSO_4$. The insoluble was filtered off and the filtrate was evaporated in vacuo. The resulting crude material was purified by silica gel column chromatography (silica gel: 25 g, 2–25% MeOH in CH_2Cl_2).

Desired fractions were combined and evaporated in vacuo to give the titled compound (145 mg, 72% in 2 steps) as a colorless wax.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (1H, s), 7.63 (1H, s), 7.38–7.40 (2H, m), 6.74 (1H, br s), 5.73 (1H, s), 4.46–4.48 (2H, m), 3.58–3.67 (31H, m), 3.39–3.41 (2H, m), 3.28–3.30 (2H, m), 2.45 (2H, br s), 2.01 (2H, br s), 1.59 (2H, br s), 4H protons were not identified.

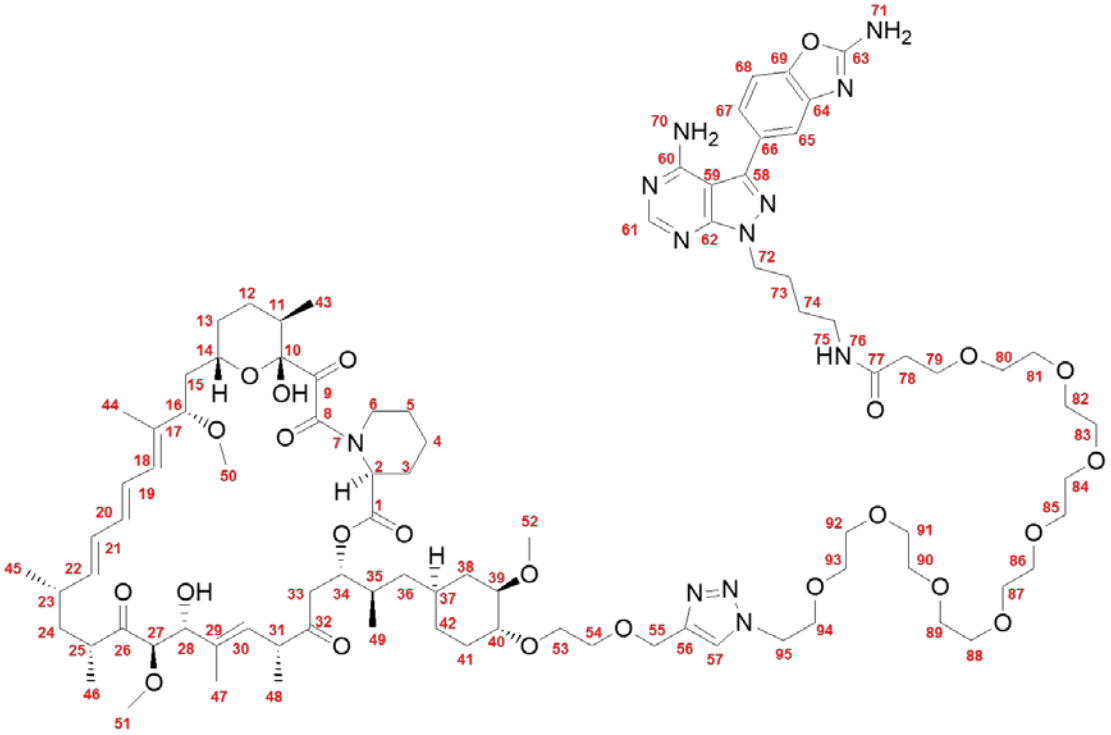
LC-MS (ESI) *m/z* = 786.34 $(M-H)^-$.

Preparation of 40-*O*-(2-((1-(32-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-27-oxo-3,6,9,12,15,18,21,24-octaoxa-28-azadotriacontyl)-1*H*-1,2,3-triazol-4-yl)methoxy)ethyl)-rapamycin (RapaLink-1). To a solution of *N*-(4-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)butyl)-1-azido-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-amide (**7**) (25.6 mg, 32.5 μmol) in MeOH (9 mL) was added 40-*O*-(2-(prop-2-yn-1-yloxy)ethyl)-rapamycin (**4**) (32.5 mg, 32.6 μmol). To the mixture were added 1 M aqueous $CuSO_4$ solution (120 μL, 120 μmol) and 1 M aqueous sodium ascorbate solution (60.0 μL, 60.0 μmol). The mixture was stirred at room temperature for 1 h. An additional amount of (**7**) (10.5 mg, 13.3 μmol) was added. The mixture was stirred for additional 2 h. It was then concentrated in vacuo. The crude material was partitioned between 20% THF in EtOAc (50 mL) and water (20 mL). The aqueous layer was separated and extracted with 20% THF in EtOAc (50 mL). The combined organic layer was dried over anhydrous $MgSO_4$. The mixture was passed through a pad of Celite (#545) using EtOAc. The filtrate was concentrated in vacuo and the crude material was purified by preparative RP-HPLC (20–95% CH_3CN in water containing 0.1% formic acid). The desired fractions were combined and lyophilized to give formic acid salt of the titled compound (13.3 mg, 22%) as a colorless amorphous powder.

LC-MS (ESI−) *m/z* = 1781.79 $(M-H)^-$.

HR-MS (ESI−) Calcd for $C_{91}H_{137}O_{24}N_{12}$ $(M-H)^-$ 1781.9874, Found 1781.9826 (Δ −2.70 ppm).

Table S2. NMR analysis of **RapaLink-1**.



Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
1	*C*=O	N/A	169.3	2	N/A
2	*CH*	5.28 (d, 5.6 Hz)	51.3	3a,b, 6a	3b
3	*CH_2*	a: 2.34 (m) b: 1.76 (m)	27.1	2, 5a	3b, 4b 3a, 2
4	*CH_2*	a: 1.78 (m) b: 1.47 (m)	20.7	2, 6a,b	4b, 5a,b 4a, 3a, 5a,b

Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
5	*C*H_2	a: 1.74 (m) b: 1.47 (m)	25.3	3a, 4b	5b, 4a,b 5a, 4a,b, 6b
6	*C*H_2	a: 3.56 (m) b: 3.43 (m)	44.2	2, 4a,b, 5b	6b 6a, 5b
8	*C*=O	N/A	166.7	2, 6a	N/A
9	*C*=O	N/A	196.3	n.d.	N/A
10	O-*C*-OH	N/A	98.5	11, 12, 43	N/A
11	*CH*	2.00 (m)	33.8	12, 43	12, 43
12	*C*H_2	1.60 (2H, m)	27.2	43	11, 13a,b
13	*C*H_2	a: 1.64 (m) b: 1.31 (m)	31.2	12, 15a	13b, 12 13a, 12, 14
14	*CH*-OC	3.88 (m)	67.2	12, 15a	13b, 15a,b
15	*C*H_2	a: 1.85 (m) b: 1.49 (m)	38.9	16	15b, 14, 16 15a, 14, 16
16	*CH*-OCH_3	3.65 (m)	84.3	15a, 18, 44, 50	15a,b
17	-*C*=	N/A	135.7	15a, 44	N/A
18	*CH*=C	5.98 (d, 11.2 Hz)	129.4	44	19
19	*CH*=C	6.39 (dd, 14.8, 10.8 Hz)	126.5	20, 21	18, 20
20	*CH*=C	6.31 (dd, 14.8, 10.4 Hz)	133.5	18, 19, 21, 22	19, 21
21	*CH*=C	6.14 (dd, 14.8, 10.4 Hz)	130.2	19	20, 22
22	*CH*=C	5.55 (dd, 15.4, 8.6 Hz)	140.0	20, 24a,b, 45	21, 23
23	*CH*	2.32 (m)	35.0	21, 22, 24a,b, 25, 45	22, 24a, 45

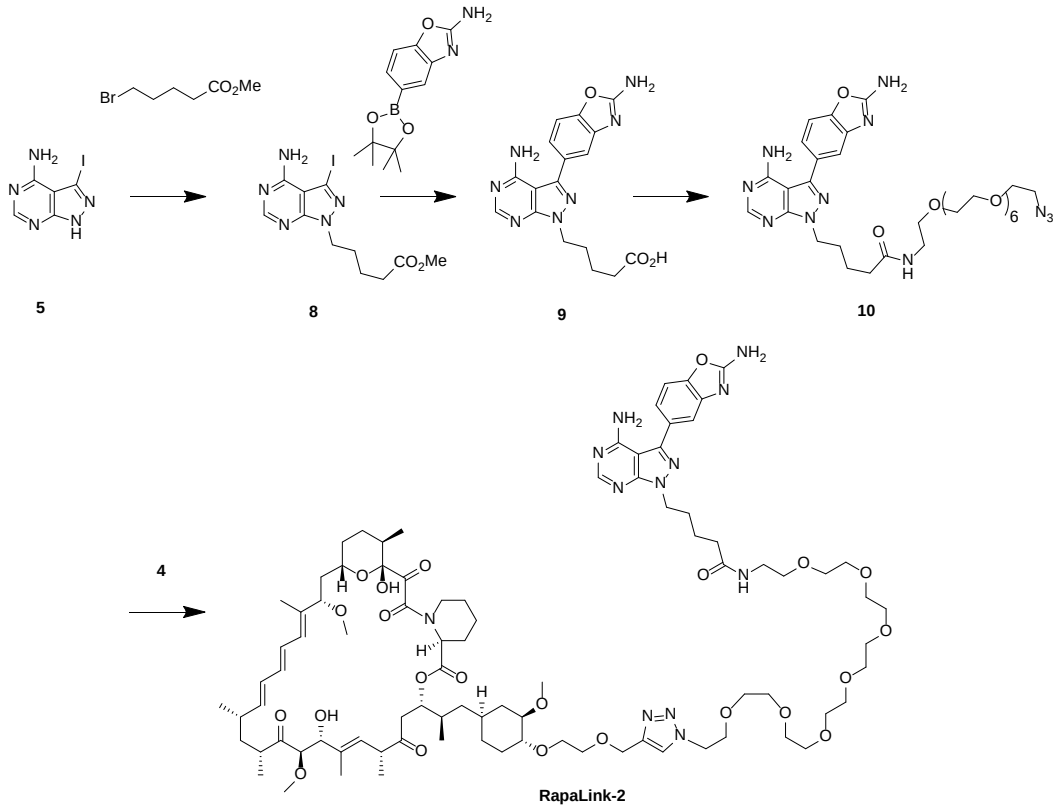
Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
24	*C*H$_2$	a: 1.47 (m) b: 1.21 (m)	40.3	22, 25, 45, 46	24b, 23 24a, 25
25	*CH*	2.70 (m)	41.5	24a,b, 46	24b, 46
26	*C*=O	N/A	n.d. (>210)	n.d.	N/A
27	*CH*-OCH$_3$	3.78 (m)	84.9	28, 51	28
28	*CH*-OH	4.20 (d, 4.8 Hz)	77.1	27, 30, 47	27
29	-*C*=	N/A	136.0	28, 31, 47	N/A
30	*CH*=C	5.42 (d,10.4 Hz)	126.5	28, 31, 47, 48	31
31	*CH*	3.29 (d,10.4 Hz)	46.6	30, 48	30, 48
32	*C*=O	N/A	208.3	30, 31, 33a,b, 48	N/A
33	*C*H$_2$	a: 2.70 (m) b: 2.58 (m)	40.7	n.d.	33b, 34 33a, 34
34	*CH*-OCO	5.16 (dd, 10.4,6.4 Hz)	75.6	33a,b, 49	33a,b, 35
35	*CH*	1.93 (m)	33.2	33a,b, 49	34, 36a,b, 49
36	*C*H$_2$	a: 1.17 (m) b: 1.09 (m)	38.4	34, 38b, 49	36b, 35, 37 36a, 35, 37
37	*CH*	1.33 (m)	33.0	36a,b, 38a,b, 42a,b	36a,b, 38b, 42a,b
38	*C*H$_2$	a: 2.02 (m) b: 0.69 (m)	36.3	36a,b, 42a	38b, 39 38a, 37, 39
39	*CH*-OCH$_3$	3.05 (m)	83.0	38a,b, 40, 52	38a,b, 40
40	*CH*-O-	3.11 (m)	83.1	38a,b, 39, 52, 53	39, 41a,b
41	*C*H$_2$	a: 2.01 (m) b: 1.24 (m)	30.0	42b.	41b, 40, 42b 41a, 40, 42b

Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
42	*C*H_2	a: 1.66 (m) b: 0.89 (m)	31.7	36b, 38a,b	42b, 37 42a, 37, 41a,b
43	11-*C*H_3	0.95 (3H, d, 6.7 Hz)	16.2	11, 12	11
44	17-*C*H_3	1.65 (3H, s)	10.2	16, 18	n.d.
45	23-*C*H_3	1.05 (3H, d, 6.4 Hz)	21.5	22, 24a,b	23
46	25-*C*H_3	0.99 (3H, d, 6.5 Hz)	13.6	24a,b, 25	25
47	29-*C*H_3	1.76 (3H, s)	13.4	28, 30	n.d.
48	31-*C*H_3	1.09 (3H, d, 6.7 Hz)	16.0	30, 31	31
49	35-*C*H_3	0.90 (3H, d, 6.8 Hz)	15.8	36a,b	35
50	16-O*C*H_3	3.13 (3H, s)	55.9	16	n.d.
51	27-O*C*H_3	3.33 (3H, s)	59.2	27	n.d.
52	39-O*C*H_3	3.43 (3H, s)	57.8	39	n.d.
53	40-O*C*H_2	3.72 (2H, m)	69.2	40, 54	54
54	-*C*H_2-O-	3.67 (2H, m)	70.2	53, 55	53
55	-O*C*H_2triazole	4.68 (2H, s)	64.6	54	n.d.
56	-*C*=	N/A	145.0	55, 57	N/A
57	=*CH*	7.76 (s)	123.8	55, 95	n.d.
58	PP-*C*	N/A	144.5	65, 67, 68	N/A
59	PP-*C*	N/A	98.5	61	N/A
60	PP-*C*-NH_2	N/A	157.5	n.d.	N/A
61	PP-*CH*	8.36 (s)	155.5	n.d.	n.d.
62	PP-*C*	N/A	154.1	61, 72	N/A
63	BO-*C*-NH_2	N/A	162.8	n.d.	N/A
64	BO-C	N/A	144.2	65, 68	N/A
65	BO-*CH*	7.62 (s)	116.3	67, 68	67

Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
66	BO-*C*	N/A	129.1	68	N/A
67	BO-*CH*	7.38 (dd, 8.4, 1.4 Hz)	121.6	65	65, 68
68	BO-*CH*	7.40 (d, 8.4 Hz)	109.6	65, 67	67
69	BO-*C*	N/A	149.3	65, 67, 68	N/A
72	N-*CH_2*	4.47 (2H, t, 6.8 Hz)	46.6	73, 74	73
73	*CH_2*	2.01 (2H, m)	27.2	72, 74, 75	72, 74
74	*CH_2*	1.58 (2H, m)	26.5	72, 73, 75	73, 75
75	*CH_2*-NHCO	3.30 (2H, m)	38.9	73, 74, 76	74
76	N*H*	6.77 (t, 4.8 Hz)	N/A	N/A	n.d.
77	*CO*	N/A	171.6	75, 76, 78, 79	N/A
78	*CH_2*	2.44 (2H, t, 5.8 Hz)	37.0	79	79
79	*CH_2*	3.69 (2H, t, 5.8 Hz)	67.4	78	78
80-93	O-(*CH_2CH_2*O)$_7$	3.54-3.64 (28H, m)	70.3-70.6	multi	multi-
94	O*CH_2*	3.87 (2H, t, 4.8 Hz)	69.5	95	95
95	*CH_2*-triazole	4.54 (2H, t, 4.8 Hz)	50.2	94	94

PP stands for pyrazolo[3,4-*d*]pyrimidine and BO stands for benzo[d]oxazole
OH protons and NHx protons were not identified.

Synthesis of RapaLink-2.



Scheme S1. Synthetic route to **RapaLink-2**.

Preparation of 5-(4-amino-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)pentanoic acid 8. To a suspension of 3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**5**) (2.11 g, 8.08 mmol) in DMF (25 mL) was added NaH oil dispersion (485 mg, 12.1 mmol) at 4 °C. The mixture was stirred at 4 °C for 30 min. To the reaction mixture was added methyl 5-bromopentanoate (1.79 g, 8.90 mmol) in DMF (5 mL) at 4 °C. The mixture was stirred at room temperature for 108 h. It was then partitioned between EtOAc (200 mL) and water (100 mL). The aqueous layer was separated and extracted with EtOAc (100 mL). The organic layers were combined, washed with brine (50 mL), and dried over anhydrous $MgSO_4$. The insoluble was filtered off and the filtrate was evaporated in vacuo. The obtained material was triturated with 20% EtOAc in hexanes (100 mL) for 15 min.

The resulting precipitate was collected by filtration. Drying the solid gave the titled compound (1.94 g, 64%) as a pale beige powder.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (1H, s), 4.27 (2H, t, J = 6.8 Hz), 3.56 (3H, s), 2.32 (2H, t, J = 7.6 Hz), 1.74–1.84 (2H, m), 1.40–1.50 (2H, m), 2H protons were not identified.

LC-MS (ESI) m/z = 376.14 $(M+H)^+$.

Preparation of 5-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)pentanoic acid 9. To a bi-phasic suspension of 5-(4-amino-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)pentanoic acid (**8**) (375 mg, 1.00 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[d]oxazol-2-amine (390 mg, 1.50 mmol), and saturated aqueous Na_2CO_3 solution (2.5 mL) in DME (10 mL) and water (2.5 mL) was added tetrakis(triphenylphosphine)palladium (0) (116 mg, 100 μmol) at room temperature under argon atmosphere. The mixture was stirred at 110 °C for 3 h. The reaction mixture was cooled to 60 °C and diluted with MeOH (10 mL) and THF (10 mL). To the reaction mixture was added 4 M aqueous LiOH solution (5 mL). The mixture was stirred at 60 °C for additional 2 h. It was then cooled and acidified using AcOH to adjust pH to be 3~4. The mixture was partitioned between EtOAc (200 mL) and water (10 mL). The organic layer was washed with brine (20 mL) and dried over anhydrous $MgSO_4$. The insoluble was filtered off and the filtrate was concentrated in vacuo. The crude material was purified by silica gel column chromatography (silica gel: 25 g, solvent: 2–30% MeOH in CH_2Cl_2). The desired fractions were combined and evaporated in vacuo. The obtained solid was triturated with 20% EtOAc in hexanes. The resulting precipitate was collected by filtration. Drying the solid gave the titled compound (207 mg, 56%) as a pale pink powder.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (1H, s), 8.24 (1H, s), 7.52 (2H, s), 7.46 (1H, d, J = 8.0Hz), 7.41 (1H, d, J = 1.6 Hz), 7.24 (1H, dd, J = 8.0, 1.6 Hz), 4.33 (2H, t, J = 6.8 Hz), 2.25 (2H, t, J = 7.2 Hz), 1.82–1.91 (2H, m), 1.44–1.53 (2H, m), 2H protons were not identified.

LC-MS (ESI) m/z = 368.22 $(M+H)^+$.

Preparation of 5-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-*N*-(23-azido-3,6,9,12,15,18,21-heptaoxatricosyl)pentanamide 10. To a solution of 5-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)pentanoic acid (**9**) (170 mg, 0.46 mmol) in DMF (4 mL) was added triethylamine (193 μL, 1.39 mmol) followed by azide-dPEG7-amine (Catalog number 10523, Quanta BioDesign, Ltd., Powell, OH USA) (219 mg, 0.56 mmol), EDCI (133 mg, 0.69 mmol), and HOBt (93.6 mg, 0.693 mmol). The mixture was stirred at room temperature for 6 h and then stirred at 40 °C for 13 h. It was then partitioned between EtOAc (100 mL) and water (50 mL). The aqueous layer was separated and extracted with EtOAc (100 mL). The organic layers were combined, washed with brine (20 mL), and dried over anhydrous $MgSO_4$. The insoluble was filtered off and the filtrate was evaporated in vacuo. The resulting crude material was purified by silica gel column chromatography (silica gel: 25 g, 2–25% MeOH in CH_2Cl_2). Desired fractions were combined and evaporated in vacuo to give the titled compound (286 mg, 83%) as a pale brown wax. This material was used for the next reaction without further purification.

LC-MS (ESI) m/z = 744.32 $(M+H)^+$.

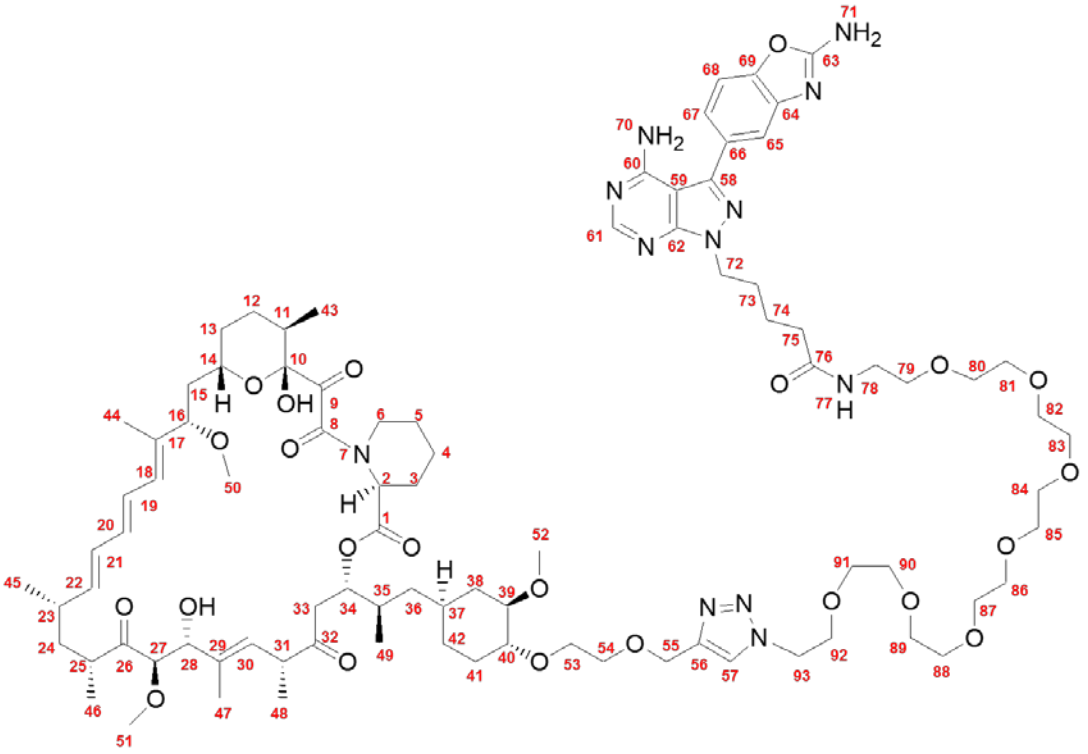
Preparation of 40-*O*-(2-((1-(29-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-25-oxo-3,6,9,12,15,18,21-heptaoxa-24-azanonacosyl)-1*H*-1,2,3-triazol-4-

yl)methoxy)ethyl)-rapamycin RapaLink-2. To a solution of 5-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-*N*-(23-azido-3,6,9,12,15,18,21-heptaoxatricosyl)pentanamide (**10**) (32.0 mg, 43.0 μmol) in MeOH (5 mL) and THF (1 mL) was added 40-*O*-(2-(prop-2-yn-1-yloxy)ethyl)-rapamycin (**4**) (32.5 mg, 32.6 μmol). To the mixture were added 1 M aqueous $CuSO_4$ solution (100 μL, 100 μmol) and 1 M aqueous sodium ascorbate solution (50.0 μL, 50.0 μmol). The mixture was stirred at room temperature for 2 h. It was then concentrated in vacuo. After removing the insoluble material by filtration through a pad of silica gel, the crude material was partitioned between 20% THF in EtOAc (50 mL) and water (5 mL). The organic layer was evaporated in vacuo. The resulting crude material was purified by preparative RP-HPLC (20–95% CH_3CN in water containing 0.1% formic acid). The desired fractions were combined and lyophilized to give formic acid salt of the titled compound (7.6 mg, 13%) as a colorless amorphous powder.

LC-MS (ESI−) *m/z* = 1737.69 $(M-H)^-$

HR-MS (ESI−) Calcd for $C_{89}H_{133}O_{23}N_{12}$ $(M-H)^-$ 1737.9612, Found 1737.9561 (Δ −2.94 ppm).

Table S3. NMR analysis of **RapaLink-2**



Atom	Atom Type	δ ^{1}H Major (3:1)	δ ^{13}C Major (3:1)	HMBC C to H	^{1}H-^{1}H COSY
1	*C*=O	N/A	169.3	2	N/A
2	*CH*	5.27 (d, 4.8 Hz)	51.3	4a, 6a	3b
3	CH_2	a: 2.33 (m) b: 1.74 (m)	27.1	2	3b, 4a,b 3a, 2, 4a,b
4	CH_2	a: 1.78 (m) b: 1.46 (m)	20.7	2, 6a	4b, 3a,b, 5a,b 4a, 3a,b, 5a,b
5	CH_2	a: 1.73 (m) b: 1.45 (m)	25.3	3a, 6a	5b, 4a,b, 6a,b 5a, 4a,b, 6a,b
6	CH_2	a: 3.54 (m) b: 3.41 (m)	44.2	2, 4a,b	6b, 5a,b 6a, 5a,b
8	*C*=O	N/A	166.8	2, 6a	N/A

Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
9	*C*=O	N/A	197.2	n.d.	N/A
10	O-*C*-OH	N/A	98.5	11, 12, 43	N/A
11	*CH*	1.99 (m)	33.9	12, 43	12, 13a, 43
12	*CH*$_2$	1.60 (m)	27.2	43	11, 13b
13	*CH*$_2$	a: 1.63 (m) b: 1.32 (m)	31.2	12, 15a.	13b, 11 13a, 12, 14
14	*CH*-OC	3.88 (m)	67.2	12, 15a, 16	13b, 15a,b
15	*CH*$_2$	a: 1.85 (m) b: 1.47 (m)	39.0	16	15b, 14, 16 15a, 14, 16
16	*CH*-OCH$_3$	3.66 (m)	84.3	15a, 18, 44, 50	15a,b
17	-*C*=	N/A	135.9	15a, 19, 44	N/A
18	*CH*=C	5.97 (d, 10.4 Hz)	129.4	20, 44	19
19	*CH*=C	6.38 (dd, 14.4, 11.2 Hz)	126.5	18, 20, 21, 22, 44	18, 20
20	*CH*=C	6.30 (dd, 14.4, 10.4 Hz)	133.5	18, 19, 21, 22	19, 21
21	*CH*=C	6.13 (dd, 15.2, 10.4 Hz)	130.2	19, 20	20, 22
22	*CH*=C	5.54 (dd, 15.2, 8.8 Hz)	140.0	20, 24a,b, 45	21, 23
23	*CH*	2.32 (m)	35.0	21, 22, 24a,b, 25, 45	22, 24a, 45
24	*CH*$_2$	a: 1.46 (m) b: 1.20 (m)	40.3	22, 25, 45, 46	24b, 23 24a, 25
25	*CH*	2.69 (m)	41.5	24a,b, 45, 46	24b, 46
26	*C*=O	N/A	213.4	n.d.	N/A
27	*CH*-OCH$_3$	3.78 (d, 5.6 Hz)	84.9	28, 51	28
28	*CH*-OH	4.20 (d, 4.8 Hz)	77.0	27, 30, 47	27

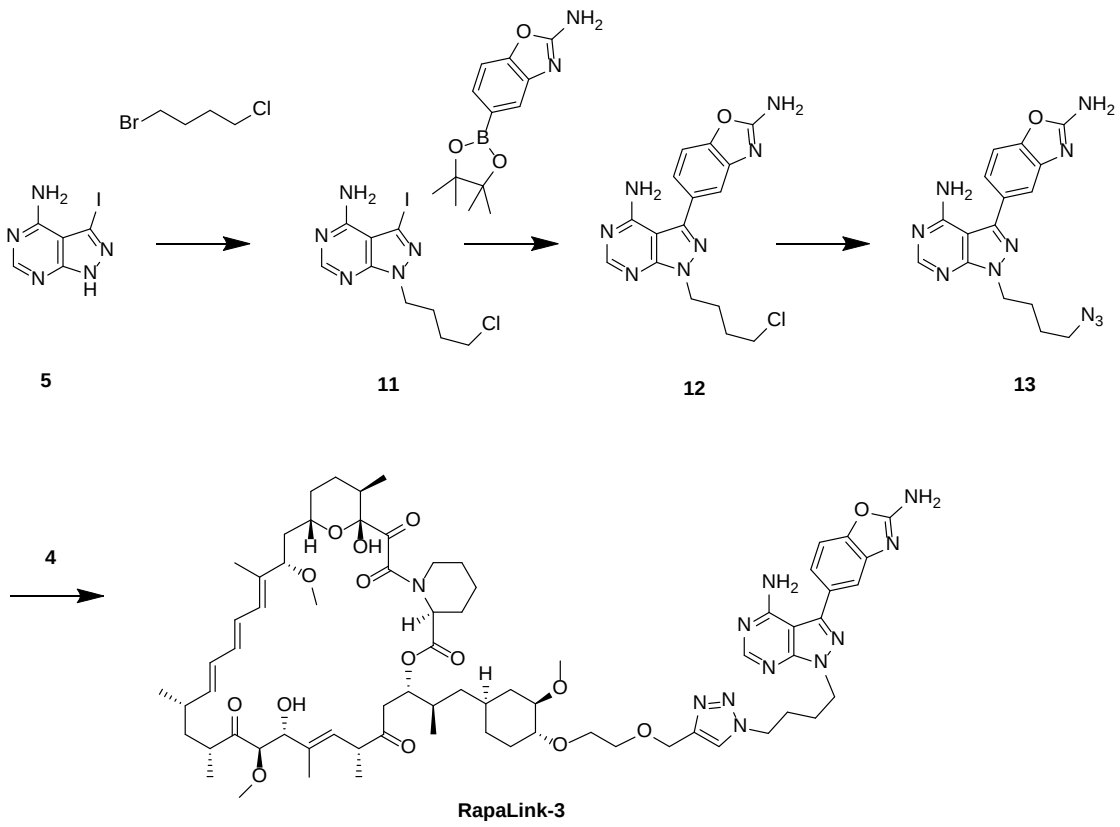
Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
29	-*C*=	N/A	136.0	28, 30, 31, 47	N/A
30	*CH*=C	5.43 (d,10.4 Hz)	126.5	28, 31, 47, 48	31
31	*CH*	3.32 (m)	46.6	30, 48	30, 48
32	*C*=O	N/A	208.3	30, 31, 33a,b, 48	N/A
33	*CH*$_2$	a: 2.69 (m) b: 2.57 (m)	40.6	n.d.	33b, 34 33a, 34
34	*CH*-OCO	5.15 (dd, 10.4, 5.6 Hz)	75.6	33a,b, 49	33a,b, 35
35	*CH*	1.92 (m)	33.2	33a,b, 36a,b, 49	34, 36a,b, 49
36	*CH*$_2$	a: 1.16 (m) b: 1.08 (m)	38.4	38b, 49	36b, 35, 37 36a, 35, 37
37	*CH*	1.32 (m)	33.0	36a,b, 38a,b, 42b	36a,b, 38b, 42a,b
38	*CH*$_2$	a: 2.01 (m) b: 0.70 (m)	36.3	36a,b	38b, 39 38a, 37
39	*CH*-OCH$_3$	3.05 (m)	83.0	38a,b, 40, 52	38a, 41a
40	*CH*-O-	3.11 (m)	83.1	38a,b, 39, 52	41b
41	*CH*$_2$	a: 2.01 (m) b: 1.23 (m)	30.0	42b	41b, 39, 42a 41a, 40, 42b
42	*CH*$_2$	a: 1.65 (m) b: 0.90 (m)	31.7	36a,b, 38a,b	42b, 37, 41a 42a, 37, 41b
43	11-*CH*$_3$	0.94 (3H, d, 6.4 Hz)	16.2	11, 12	11
44	17-*CH*$_3$	1.65 (3H, s)	10.2	16, 18	n.d.
45	23-*CH*$_3$	1.05 (3H, d, 7.2 Hz)	21.5	22, 23, 24a,b	23

Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
46	25-*C*H_3	0.98 (3H, d, 6.4 Hz)	13.6	24a,b, 25	25
47	29-*C*H_3	1.75 (3H, s)	13.4	28, 30	n.d.
48	31-*C*H_3	1.09 (3H, d, 6.4 Hz)	16.0	30, 31	31
49	35-*C*H_3	0.90 (3H, d, 6.4 Hz)	15.8	34, 36a,b	35
50	16-O*C*H_3	3.14 (s)	55.9	16	n.d.
51	27-O*C*H_3	3.33 (s)	59.2	27	n.d.
52	39-O*C*H_3	3.43 (s)	57.8	39	n.d.
53	40-O*C*H_2	3.74 (2H, m)	69.2	40, 54	54
54	-*C*H_2-O-	3.67 (2H, m)	70.2	53, 55	53
55	-O*C*H_2triazole	4.68 (2H, s)	64.6	54	57
56	-*C*=	N/A	145.0	55, 57	N/A
57	=*CH*	7.76 (s)	123.8	55, 93	55
58	PP-*C*	N/A	144.4	65, 67, 68	N/A
59	PP-*C*	N/A	98.4	61	N/A
60	PP-*C*-NH_2	N/A	157.9	61	N/A
61	PP-*CH*	8.36 (s)	155.8	n.d.	n.d.
62	PP-*C*	N/A	154.3	61, 72	N/A
63	BO-*C*-NH_2	N/A	162.8	n.d.	N/A
64	BO-C	N/A	144.0	65, 68	N/A
65	BO-*CH*	7.62 (s)	116.3	67, 68	67
66	BO-*C*	N/A	129.3	68	N/A
67	BO-*CH*	7.38 (d, 8.0 Hz)	121.7	65	65, 68
68	BO-*CH*	7.40 (d, 8.0 Hz)	109.6	65, 67	67

Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
69	BO-*C*	N/A	149.2	65, 67, 68	N/A
72	N-*CH*$_2$	4.45 (2H, t, 6.8 Hz)	46.5	73, 74	73
73	*CH*$_2$	2.01 (2H, m)	29.1	72, 74, 75	72, 74
74	*CH*$_2$	1.70 (2H, m)	22.8	72, 73, 75	73, 75
75	*CH*$_2$-CO	2.27 (2H, t, 7.6 Hz)	35.8	73, 74	74
76	*CO*	N/A	172.8	74, 75, 78	N/A
77	N*H*	6.55 (br)	N/A	N/A	N/A
78	*CH*$_2$	3.41 (2H, t, 5.0 Hz)	39.2	79	79
79	*CH*$_2$	3.51 (2H, t, 5.0 Hz)	70.0	78	78
80-91	O-(*CH*$_2$*CH*$_2$O)$_6$	3.54-3.64 (24H, m)	70.2-70.6	multi	multi
92	O*CH*$_2$	3.87 (2H, t, 5.2 Hz)	69.5	93	93
93	*CH*$_2$-triazole	4.53 (2H, t, 5.2 Hz)	50.2	92	92

PP stands for pyrazolo[3,4-*d*]pyrimidine and BO stands for benzo[d]oxazole
OH protons and NHx protons were not identified.

Synthesis of RapaLink-3.



Scheme S2. Synthetic route to **RapaLink-3**.

Preparation of 1-(4-chlorobutyl)-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine 11. To a suspension of 3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**5**) (2.11 g, 8.08 mmol) (prepared by a similar method to that described in *Nature Chemical Biology*, **2008**, 691–699) in DMF (25 mL) was added NaH oil dispersion (485 mg, 12.1 mmol) at 4 °C. The mixture was stirred at 4 °C for 30 min. To the reaction mixture was added 1-bromo-4-chlorobutane (1.45 g, 8.46 mmol) at 4 °C. The mixture was stirred at room temperature for 14 h. To the mixture was added water (25 mL) at room temperature. The mixture was cooled to 4 °C and stirred for 30 min. The resulting precipitate was collected by filtration. The obtained crude material was purified by silica gel column chromatography (silica gel: 40 g, solvent: 20–100% EtOAc in hexanes, 0–30% MeOH in EtOAc, and then DMF). Desired fractions were combined and evaporated in vacuo. The obtained

DMF solution (ca.100 mL) including desired material was diluted with water (150 mL). The resulting suspension was stirred at 4 °C for 30 min. The precipitate was collected by filtration. Drying the solid gave the titled compound (2.01 g, 71%) as a pale beige solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (1H, s), 4.30 (2H, t, J = 6.8 Hz), 3.65 (2H, t, J = 6.8 Hz), 1.85–1.95 (2H, m), 1.61–1.70 (2H, m), NH_2 protons were not identified.

LC-MS (ESI) m/z = 352.05 $(M+H)^+$.

Preparation of 5-(4-amino-1-(4-chlorobutyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[d]oxazol-2-amine 12. To a bi-phasic suspension of 1-(4-chlorobutyl)-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**11**) (703 mg, 2.00 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[d]oxazol-2-amine (780 mg, 3.00 mmol) (prepared by a similar method to that described in WO2010/051042A1), and saturated aqueous Na_2CO_3 solution (10 mL) in DME (30 mL) and water (10 mL) was added tetrakis(triphenylphosphine)palladium (0) (232 mg, 200 μmol) at room temperature under argon atmosphere. The mixture was stirred at 110 °C for 3 h. It was then cooled and partitioned between EtOAc (200 mL) and water (100 mL). The aqueous layer was separated and extracted with EtOAc (100 mL). The organic layers were combined, washed with brine (50 mL) and dried over anhydrous $MgSO_4$. The insoluble was filtered off and the filtrate was concentrated in vacuo. The crude material was purified by silica gel column chromatography (basic silica gel: 25 g, solvent: 20% MeOH in EtOAc (100 mL)). The desired fractions were combined and the obtained solid was triturated with EtOAc (50 mL) for 30 min. The precipitate was collected by filtration. Drying the solid gave the titled compound (445 mg, 62%) as a pale beige solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (1H, s), 7.53 (2H, s), 7.47 (1H, d, J = 8.0 Hz), 7.41 (1H, br s), 7.25 (1H, dd, J = 8.4, 1.2 Hz), 4.37 (2H, t, J = 6.8 Hz), 3.67 (2H, t, J = 6.8 Hz), 1.93–2.02 (2H, m), 1.67–1.76 (2H, m), NH_2 protons were not identified.

LC-MS (ESI) m/z = 358.20 $(M+H)^+$.

Preparation of 5-(4-amino-1-(4-azidobutyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[d]oxazol-2-amine 13. To a solution of 5-(4-amino-1-(4-chlorobutyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[d]oxazol-2-amine (**12**) (140 mg, 0.391 mmol) in DMF (3

mL) were added sodium azide (33.0 mg, 0.507 mmol) and potassium iodide (12.0 mg, 72.3 μmol) at room temperature. The mixture was stirred at 70 °C for 6 h. It was then cooled and partitioned between EtOAc (100 mL) and water (20 mL). The aqueous layer was separated and extracted with EtOAc (50 mL). The organic layers were combined, washed with brine (20 mL), and dried over anhydrous $MgSO_4$. The insoluble was filtered off and the filtrate was evaporated in vacuo. The obtained material was triturated with EtOAc (5 mL) for 15 min. The precipitate was collected by filtration. Drying the solid gave the titled compound (121 mg, 85%) as a pale beige powder.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (1H, s), 7.53 (2H, s), 7.47 (1H, d, J = 8.0 Hz), 7.41 (1H, d, J = 1.6 Hz), 7.24 (1H, dd, J = 8.0, 1.6 Hz), 4.37 (2H, t, J = 6.8 Hz), 3.36 (2H, t, J = 6.8 Hz), 1.86–1.95 (2H, m), 1.48–1.57 (2H, m), NH_2 protons were not identified.

LC-MS (ESI) m/z = 365.21 $(M+H)^+$.

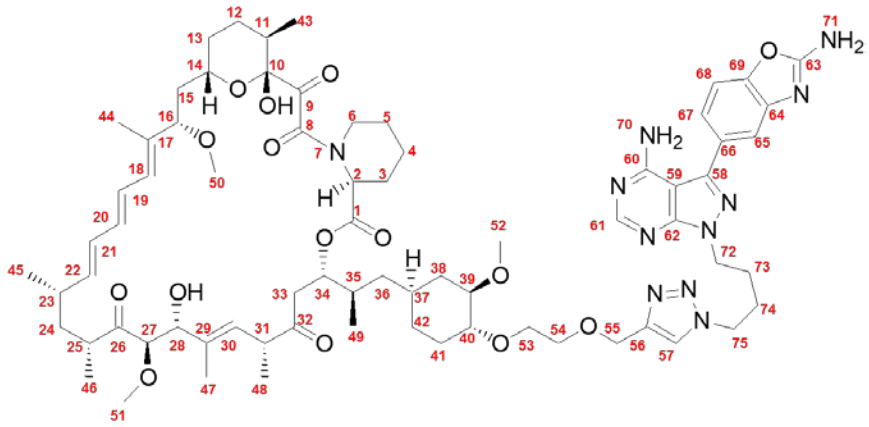
Preparation of 40-*O*-(2-((1-(4-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)butyl)-1*H*-1,2,3-triazol-4-yl)methoxy)ethyl)-rapamycin (RapaLink-3) To a solution of 5-(4-amino-1-(4-azidobutyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzo[d]oxazol-2-amine (**13**) (16 mg, 43.9 μmol) in a mixed solvent of MeOH (8 mL) and CH_2Cl_2 (4 mL) was added 40-*O*-(2-(prop-2-yn-1-yloxy)ethyl)-rapamycin (**4**) (32.5 mg, 32.6 μmol). To the mixture were added 1 M aqueous $CuSO_4$ solution (100 μL, 100 μmol) and 1 M aqueous sodium ascorbate solution (100 μL, 100 μmol). The mixture was stirred at room temperature for 4 h. It was then concentrated in vacuo. The crude material was partitioned between 20% THF in EtOAc (20 mL) and water (5 mL). The aqueous layer was separated and extracted with 20% THF in EtOAc (10 mL). The organic layers were combined, washed with brine (20 mL), and dried over anhydrous

$MgSO_4$. The mixture was dissolved into DMSO (4 mL) and 50% CH_3CN in water (4 mL) and the solution was passed through a pad of Celite (#545). The filtrate was purified by preparative RP-HPLC (20–95% CH_3CN in water containing 0.1% formic acid). The desired fractions were combined and lyophilized to give formic acid salt of the titled compound (9.1 mg, 19%) as a colorless amorphous powder.

LC-MS (ESI−) *m/z* = 1358.47 $(M-H)^-$.

HR-MS (ESI−) Calcd for $C_{72}H_{100}O_{15}N_{11}$ $(M-H)^-$ 1358.7406, Found 1358.7372 (Δ −2.49 ppm).

Table S3. NMR analysis of **RapaLink-3**.



Atom	**Atom Type**	**δ ^{1}H Major (3:1)**	**δ ^{13}C Major (3:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
1	*C*=O	N/A	169.3	2	N/A
2	*CH*	5.28 (d, 5.6 Hz)	51.3	4a, 6a	3b
3	CH_2	a: 2.33 (m) b: 1.73 (m)	27.0	2, 5a	3b, 4a,b 3a, 2, 4a,b
4	CH_2	a: 1.78 (m) b: 1.48 (m)	20.7	2, 6a,b	4b, 3a,b, 5a,b 4a, 3a,b, 5a,b

Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
5	*C*H_2	a: 1.72 (m) b: 1.46 (m)	25.3	3a, 4a, 6a,b	5b, 4a,b 5a, 4a,b, 6a,b
6	*C*H_2	a: 3.54 (m) b: 3.41 (m)	44.2	2, 4a, 5b	6b, 5b 6a, 5b
8	*C*=O	N/A	166.7	2, 6a,b	N/A
9	*C*=O	N/A	193.3	n.d.	N/A
10	O-*C*-OH	N/A	98.6	11, 12, 43	N/A
11	*CH*	2.01 (m)	34.0	43	12, 43
12	*C*H_2	1.60 (2H, m)	27.2	11, 43	11, 13a,b
13	*C*H_2	a: 1.66 (m) b: 1.30 (m)	31.3	12, 15a	13b, 12 13a, 12, 14
14	*CH*-OC	3.91 (m)	67.2	12, 15a, 16	13b, 15a,b
15	*C*H_2	a: 1.87 (m) b: 1.44 (m)	39.2	16	15b, 14, 16 15a, 14, 16
16	*CH*-OCH_3	3.65 (m)	84.1	15a,b, 18, 50	15a,b
17	-*C*=	N/A	136.2	15a, 19, 44	N/A
18	*CH*=C	5.98 (d, 10.8 Hz)	129.1	16, 20, 44	19
19	*CH*=C	6.38 (dd, 14.2, 10.8 Hz)	126.6	20, 21	18, 20
20	*CH*=C	6.30 (dd, 14.4, 10.2 Hz)	133.3	18, 19, 21, 22	19, 21
21	*CH*=C	6.13 (dd, 14.8, 10.4 Hz)	130.3	19, 20	20, 22
22	*CH*=C	5.53 (dd, 14.8, 8.8 Hz)	139.7	20, 24a,b, 45	21, 23
23	*CH*	2.32 (m)	35.0	21, 22, 24a,b, 45	22, 24a, 45

Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
24	*C*H_2	a: 1.43 (m) b: 1.20 (m)	40.3	22, 25, 45, 46	24b, 23 24a, 25
25	*CH*	2.65 (m)	41.6	24a,b, 46	24b, 46
26	*C*=O	N/A	214.6	n.d.	N/A
27	*CH*-OCH_3	3.84 (d, 4.2 Hz)	85.0	28, 51	28
28	*CH*-OH	4.24 (d, 4.0 Hz)	76.8	27, 30, 47	27
29	*C*=C	N/A	135.9	28, 30, 47	N/A
30	*CH*=C	5.45 (d, 9.6 Hz)	126.2	28, 31, 47, 48	31
31	*CH*	3.30 (m)	46.6	30, 48	30, 48
32	*C*=O	N/A	208.3	31, 33a,b, 48	N/A
33	*C*H_2	a: 2.68 (m) b: 2.55 (m)	40.5	n.d.	33b, 34 33a, 34
34	*CH*-OCO	5.16 (m)	75.6	33a,b, 36a,b, 49	33a,b, 35
35	*CH*	1.91 (m)	33.3	33a,b, 36b, 49	34, 36b, 49
36	*C*H_2	a: 1.16 (m) b: 1.07 (m)	38.5	38b, 49	36b, 37 36a, 35
37	*CH*	1.32 (m)	33.0	36b, 38a,b, 42b	36a, 38b, 42a,b
38	*C*H_2	a: 1.99 (m) b: 0.69 (m)	36.4	36a,b	38b, 39 38a, 37, 39
39	*CH*-OCH_3	3.02 (m)	83.0	38a,b, 40, 52	38a,b, 40
40	*CH*-O-	3.09 (m)	83.1	38a,b, 39, 52, 53	39, 41a,b
41	*C*H_2	a: 2.00 (m) b: 1.22 (m)	30.0	42b	41b, 40, 42a,b 41a, 40, 42b

Atom	**Atom Type**	**δ ^{1}H Major (*3*:1)**	**δ ^{13}C Major (*3*:1)**	**HMBC C to H**	**^{1}H-^{1}H COSY**
42	*C*H_2	a: 1.64 (m) b: 0.88 (m)	31.6	36a,b, 38a,b	42b, 37, 41a 42a, 37, 41a,b
43	11-*C*H_3	0.95 (3H, d, 6.6 Hz)	16.2	11, 12	11
44	17-*C*H_3	1.66 (3H, s)	10.2	16, 18	n.d.
45	23-*C*H_3	1.05 (3H, d, 6.6 Hz)	21.4	22, 24a,b	23
46	25-*C*H_3	0.98 (3H, d, 6.4 Hz)	13.4	24a,b, 25	25
47	29-*C*H_3	1.79 (3H, s)	13.7	28, 30	n.d.
48	31-*C*H_3	1.07 (3H, d, 6.6 Hz)	15.9	30, 31	31
49	35-*C*H_3	0.89 (3H, d, 6.6 Hz)	15.7	34, 36b	35
50	16-O*C*H_3	3.14 (3H, s)	55.9	16	n.d.
51	27-O*C*H_3	3.33 (3H, s)	58.9	27	n.d.
52	39-O*C*H_3	3.40 (3H, s)	57.8	39	n.d.
53	40-O*C*H_2	3.72 (2H, m)	69.2	40, 54	54
54	-*C*H_2-O-	3.64 (2H, m)	70.2	53, 55	53
55	-O*C*H_2triazole	4.67 (2H, s)	64.7	54	57
56	-*C*=	N/A	145.4	55, 57	N/A
57	=*CH*	7.55 (s)	122.4	55, 57	55
58	PP-*C*	N/A	143.7	68	N/A
59	PP-*C*	N/A	98.5	61	N/A
60	PP-*C*-NH_2	N/A	158.0	61	N/A
61	PP-*CH*	8.35 (s)	155.8	n.d.	n.d.
62	PP-*C*	N/A	154.4	61, 72	N/A
63	BO-*C*-NH_2	N/A	162.8	n.d.	N/A

Atom	Atom Type	δ ^{1}H Major (*3*:1)	δ ^{13}C Major (*3*:1)	HMBC C to H	^{1}H-^{1}H COSY
64	BO-C	N/A	144.8	65, 67	N/A
65	BO-*CH*	7.63 (s)	116.2	67	67
66	BO-*C*	N/A	129.2	68	N/A
67	BO-*CH*	7.40 (br d, 7.8 Hz)	121.8	65	65, 68
68	BO-*CH*	7.42 (d, 7.8 Hz)	109.8	65	67
69	BO-*C*	N/A	149.2	65, 67	N/A
72	N-CH_2	4.49 (2H, m)	46.1	73, 74	73
73	CH_2	2.01 (2H, m)	26.6	72, 74, 75	72, 74
74	CH_2	1.95 (2H, m)	27.4	72, 73, 75	73, 75
75	CH_2	4.41 (2H, t, 6.8 Hz)	49.6	57, 73, 74	74

PP stands for pyrazolo[3,4-*d*]pyrimidine and BO stands for benzo[d]oxazole
OH protons and NHx protons were not identified.

Primers used for site-directed mutagenesis:

Target	Sequence
FLAG-MTOR-NOTI-F:	5’-ATAAGAATGCGGCCGCATGGATTACAAGGATGACGACGAT AAGCTTGGAACCGGACCTGCC-3’
FLAG-MTOR-MLUI-R:	5’-CGACGCGTTTACCAGAAAGGGCACCAGCCAATATA-3’
A2034V-F:	5’-GGAAGAGGTATCTCGTTTGTACTTTGGGGA-3’
A2034V-R:	5’-AACGAGATACCTCTTCCAGGCCTTCATGC-3’
F2108L-F:	5’-TCATGTGTTACGACGAATCTCAAAGCAG-3’
F2108L-R:	5’-ATTCGTCGTAACACATGATAATAGAGGTCCCAGGCT-3’
S2215Y-F:	5’-GCCAATGACCCAACATATCTTCGGAA-3’
S2215Y-R:	5’- TGAGGTTTTTCCGAAGATATGTTGGGTCATT-3’
L2230V-F:	5'-TCATCCCTGTATCGACCAACTC-3'
L2230V-R:	5'- TTGGTCGATACAGGGATGACAGCGT-3'
M2327I-F:	5’-TAGCGGTCATTTCAATGGTTGGGTATAT-3’
M2327I-R:	5’-CCATTGAAATGACCGCTAAAGAACGGGTAT-3’
E2388Q-F:	5’-GACCAATGCTATGCAGGTTACAG-3’
E2388Q-R:	5’-TCCAGGCCTGTAACCTGCATAGCAT-3’

V2406A-F:	5'-CAGTGATGGAGGCGCTGCGAGAGCACAA-3'
V2406A-R:	5'- GCTCTCGCAGCGCCTCCATCACTGTGT-3'