Donated Chemical Probe

AMPK/RSK1 Inhibitor Probe BAY-3827

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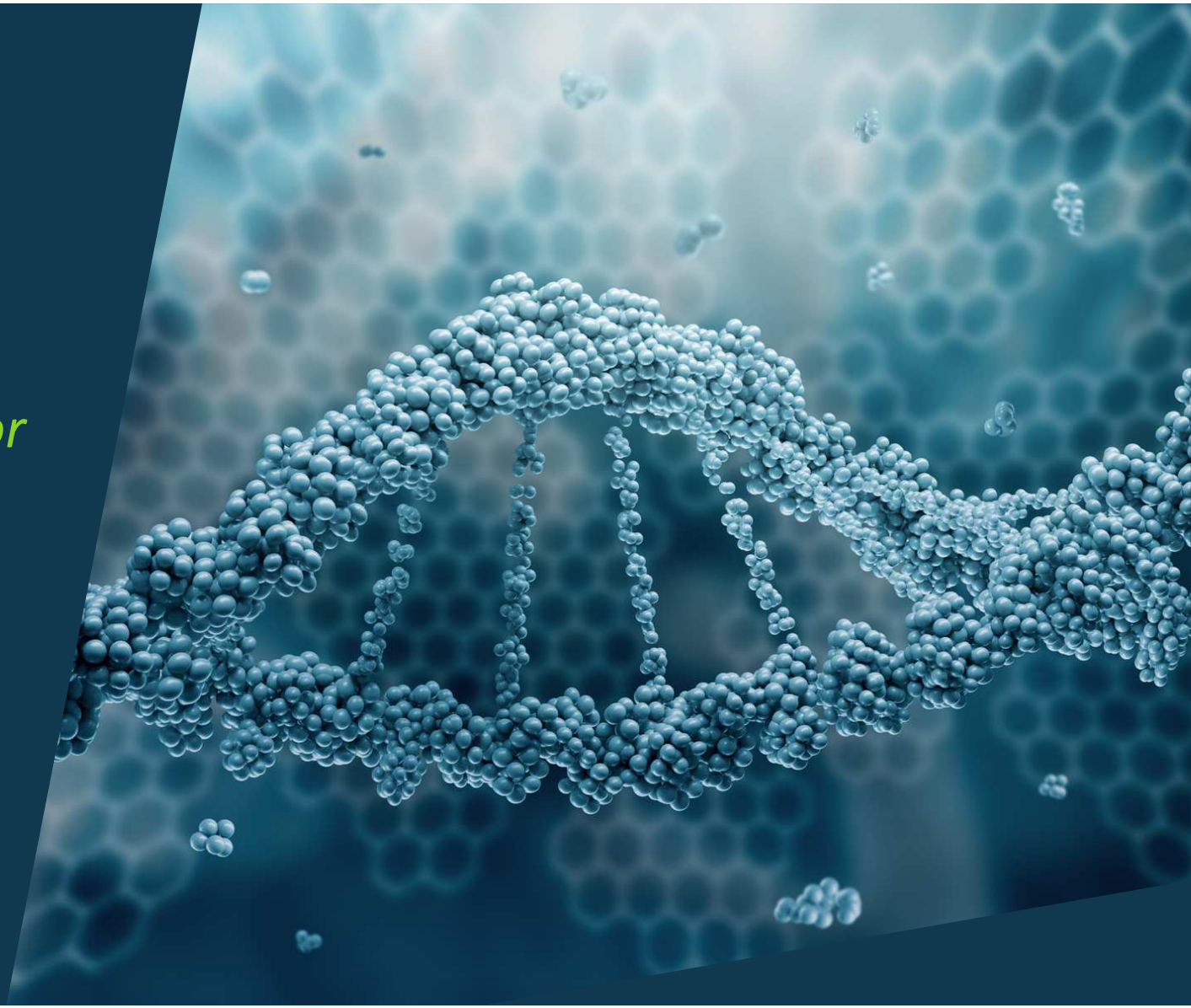
Clara Lemos & Volker Schulze



AMPK/RSK1 Probe BAY-3827

Disclaimer

// While populating the data for our Chemical Probe, we noticed that our AMPK Inhibitor is comparable active on RSK1, which was considered not relevant for the former oncology project

// We would like to make you aware that all cellular data focusses on our AMPK inhibitor

AMPK/RSK1 Probe BAY-3827

Scientific rationale – AMPK and myc-dependent tumors

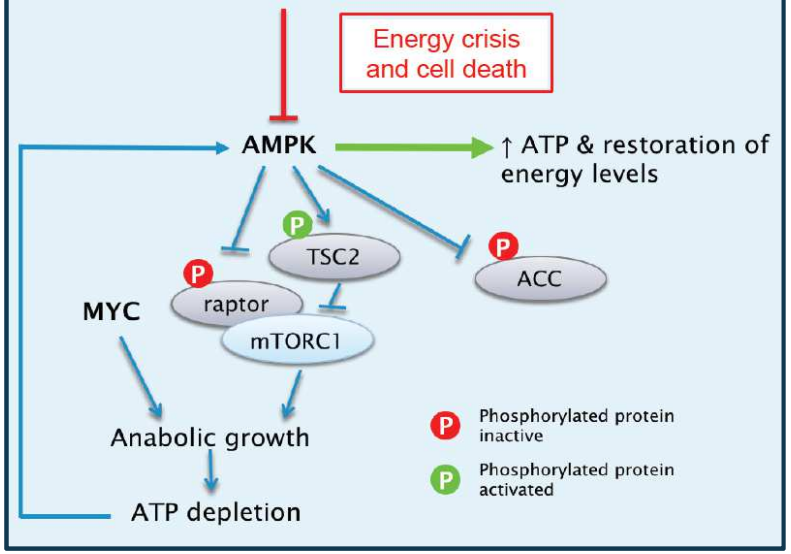
Rationale

- MYC dysregulation triggers increased anabolic activity, resulting in ATP depletion.
- AMPK is required to reduce these anabolic activities and to restore cellular energy levels.
- Without AMPK, the (tumor) cells run into an energy crisis and undergo apoptosis.

Validation (literature & in house data)

- AMPK knockdown induces apoptosis in a MYC-dependent fashion in U2OS-mycER cells *(Liu et al, 2012. Nature)*
- AMPK knockdown induces apoptosis in MYC-dependent BUT NOT in MYC-independent cells *(in house data)*
- AMPK is relevant for cell survival of MYC-dependent but not in MYC-independent tumor cells *(in house data)*

Signal Cascade



Disease Hypothesis: Inhibition of AMPK in MYC-dependent cells causes an energy crisis, resulting in apoptosis and cell death

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Compound C (Dorsomorphin), a literature known, unselective AMPK inhibitor

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The AMPK inhibitor Compound C is a potent AMPK-independent anti-glioma agent

Xiaona Liu,[1,2] Rishi Raj Chhipa,[1,2] Ichiro Nakano,[3] and Biplab Dasgupta[1,*]

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Abstract Go to:

AMPK is an evolutionarily conserved energy sensor important for cell growth, proliferation, survival and metabolic regulation. Active AMPK inhibits biosynthetic enzymes like mTOR and acetyl CoA carboxylase (required for protein and lipid synthesis, respectively) to ensure that cells maintain essential nutrients and energy during metabolic crisis. Despite our knowledge about this incredibly important kinase, no specific chemical inhibitors are available to examine its function. However, one small molecule known as Compound C (also called dorsomorphin) has been widely used in cell-based, biochemical and *in vivo* assays as a selective AMPK inhibitor. In nearly all these reports including a recent study in glioma, the biochemical and cellular effects of Compound C has been attributed to its inhibitory action towards AMPK. While examining the status of AMPK activation in human gliomas, we observed that glioblastomas (GBMs) express copious amount of active AMPK. Compound C effectively reduced glioma viability *in vitro* both by inhibiting proliferation and inducing cell death. As expected, Compound C inhibited AMPK; however, all the antiproliferative effects of this compound were AMPK-independent. Instead, Compound C killed glioma cells by multiple mechanisms including activation of the Calpain/Cathepsin pathway, inhibition of AKT, mTORC1/C2, cell cycle block at G2M and induction of necroptosis and autophagy. Importantly, normal astrocytes were significantly less susceptible to Compound C. In summary, Compound C is an extremely potent anti-glioma agent but we suggest that caution should be taken in interpreting results when this compound is used as an AMPK inhibitor.

Keywords: Glioma, Compound C, AMPK

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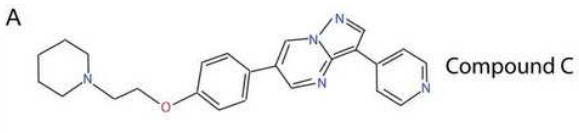

Compound C

	Compound C
AMPK in house assays; low / high ATP IC_{50}	320 nM / 8020 nM

- In the in house kinase panel out of 20 off-kinases 13 kinases were inhibited with an IC50 below 1 µM in low ATP kinases assays, 8 kinases were inhibited with IC50s below 300 nM.

Compound C is a known AMPK inhibitor, of rather low potency and critical kinase-selectivity, as well as literature known cellular off-target effects.

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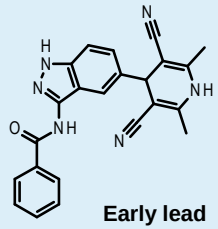
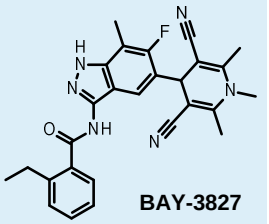
X-ray structure of early lead compound 6



Early lead compound 6

AMPK low / high ATP IC_{50}	18 nM / 280 nM
Mechan. pACC (IMR-32) IC_{50}	1560 nM



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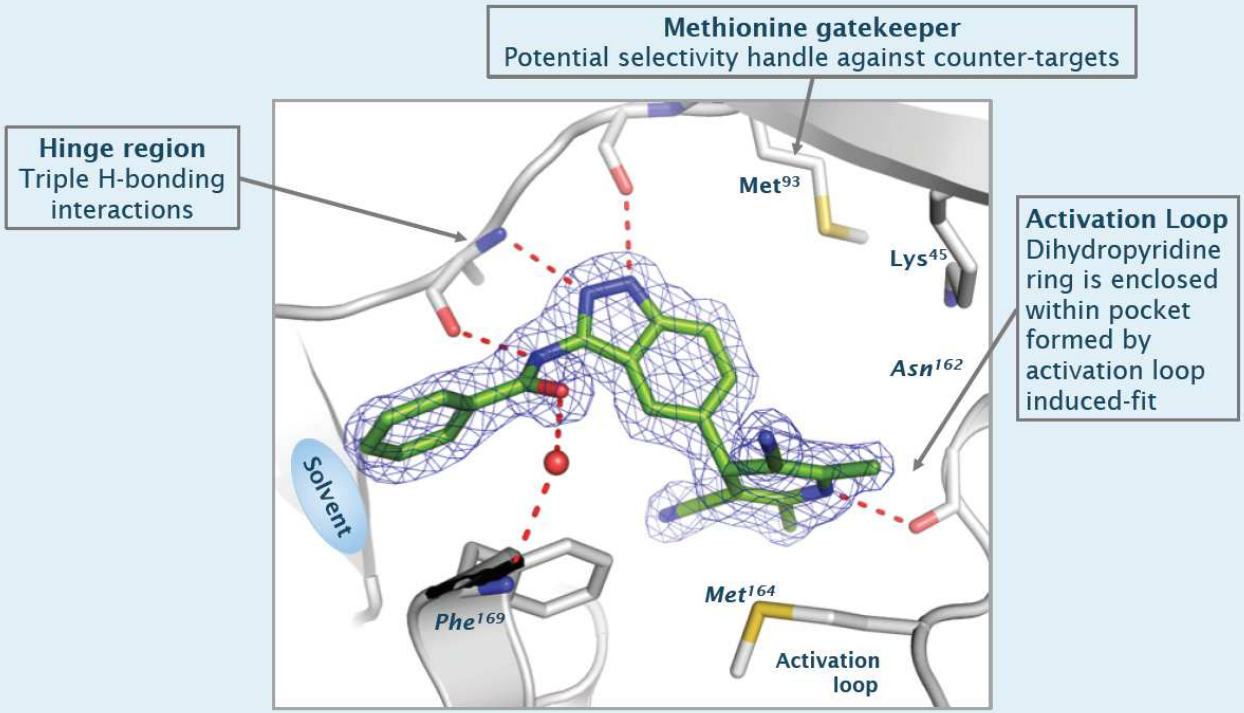
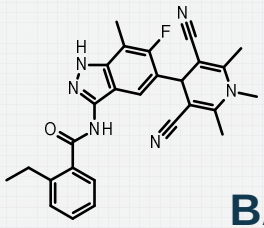
AMPK low / high ATP IC_{50}	1.4 nM / 15 nM
Mechan. pACC (IMR-32) IC_{50}	150 nM



The X-ray structure of early lead compound 6 in complex with the AMPKα2 domain proves binding to the ATP binding site of the kinase and allowed a better understanding of the binding mode

AMPK/RSK1 Probe BAY-3827

Technical In vitro profile



BAY-3827

POTENCY (IC_{50} [nM])	
AMPK low / high ATP IC_{50}	1.4 / 15
Mechan. pACC (IMR-32) IC_{50}	150
Mechan. pACC (Colo-320) IC_{50}	390
2D proli (IMR32) IC_{50}	24000
2D counter-proli (SK-N-F1) IC_{50}	22000

Properties & Physchem	
LogD @ pH 7.5	2.6
BEI / LLE (calc, AMPK low ATP)	19.5 / 6.1
Sw @ pH 6.5 [mg/L]	0.3
MW / MW corr / TPSA [g*mol / Å²]	469 / 454 / 109
Stability (r /h plasma, 4h) [%]	100 / 100
Protein binding [%] Mouse, female NMRI Williams E buffer	 3.8 31

***in vitro* DMPK Properties**

Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	24		231		9.7	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	liver mics (m / r / d / h)		5.0 / - / -		7 / - / -	
	rat hepatocytes		3.3		21	
	human hepatocytes					
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	> 10	3.3	7.7	> 10	1.6	0.42 yes
PXR	red					
CYP induction	--					

Selectivity	
In-house kinase panel	High selectivity see next slide
Eurofins @ 1 µM (kinase panel)	See next slide

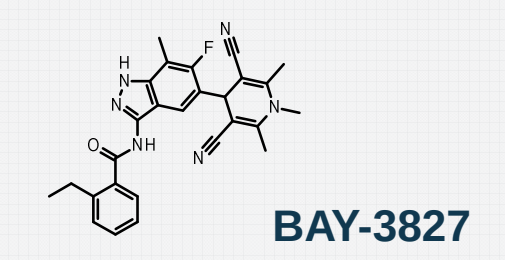
SAFETY	
Cytotox	
hERG IC_{50} [µM]	9.9

- BAY-3827 has high in vitro potency and selectivity
- BAY-3827 was found to exhibit drug-like properties.
- BAY-3827 has low metabolic stability and solubility. Moderate permeability & efflux

AMPK/RSK1 Probe BAY-3827

In house and Eurofins Kinase Profile

	BAY-3827 @ 1 µM	Eurofins IC50 [nM]	in house: IC50 [nM]
AMPKα2(h)	0	7	1,4
Rsk2(h)	0	52	
AMPKα1(h)	1	10	
Met(h)	1		790
Rsk4(h)	2		37
Rsk3(h)	5	24	
Flt3(h)	7		119
Rsk1(h)	7	9,1	
MSK1(h)	10	43	
MST3(h)	17	94	
Aurora-A(h)	34	480	1400



- BAY-3827 shows good selectivity in Eurofins kinase panel (329 off-kinases tested)
- BAY-3827 was tested against in-house kinase panel (~ 30 kinases tested)
- Selective against an in-house kinase panel and Eurofins Kinase panel: > 500 fold selectivity against 322 out of 329 off-kinases tested
- RSKs, Flt3, MSK1 and MST3 are most potently hit off-kinases

AMPK/RSK1 Probe BAY-3827

2D proli with AMPK-selective Cpds in Myc-dependent cells

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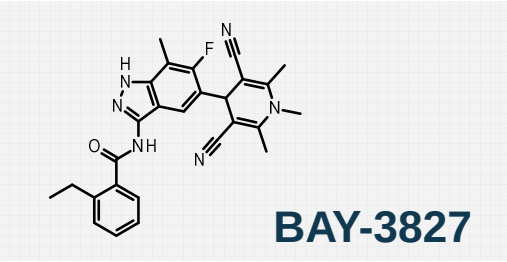
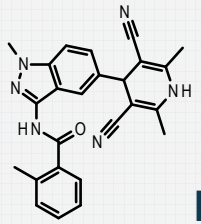
Method	Test system	Cpd 19 BAY-3827
AMPK (low/high ATP)	IMR-32	1.4 / 15 nM
Aurora A (low ATP)	IMR-32	480 nM
pACC HTRF	IMR-32	150 nM
Proliferation assay	COLO 320DM	≈ 30 µM
	LS-174T	> 30 µM
	Ramos	12 µM
	SNU-16	12 µM
	SU-DHL-10	13 µM
	Oci-Ly-7	12 µM
	JJN3	≈ 30 µM
	COLO 201, control	16 µM
	IMR-32	24 µM
	IMR-5/75	21 µM
	SK-N-F1, control	22 µM



The potent and selective AMPK inhibitor BAY-3827 did not inhibit cell proliferation in cancer cell lines with dysregulated MYC signaling

AMPK/RSK1 Probe BAY-3827

In vitro profile of Negative Control BAY-974



BAY-974

POTENCY; IC_{50} [nM]	
AMPK low / high ATP IC_{50}	>20000 / > 20000
Mechan. pACC (IMR-32) IC_{50}	> 30000
Mechan. pACC (Colo-320) IC_{50}	> 30000
2D proli (IMR32) IC_{50}	> 30000
2D counter-proli (SK-N-F1) IC_{50}	> 30000

Properties & Physchem	
LogD @ pH 7.5	2.2
BEI / LLE (calc, AMPK low ATP)	-- / --
Sw @ pH 6.5 [mg/L]	7.3
MW / MW corr / TPSA [g*mol / Å^2]	422 / 422 / 107
Stability (r /h plasma, 4h) [%]	-- / --

in vitro DMPK Properties						
Caco2 permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	9		315		35	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	liver mics (m / r / d / h)		- / - / -		- / - / -	
	rat hepatocytes		-		-	
	human hepatocytes					
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	-	-	-	-	-	-
PXR	-					
CYP induction	--					

Selectivity	
In-house kinase panel	No kinases significantly inhibited
Eurofins @ 1 µM (kinase panel)	tbd

SAFETY	
Cytotox	--
hERG IC_{50} [µM]	--

- BAY-974 is inactive against AMPK in vitro and was found to exhibit drug-like properties
- BAY-974 has moderate-low solubility, permeability and efflux
- Negative control BAY-974 shows no significant kinase inhibition in in-house kinase panel (~ 30 kinases tested)

AMPK/RSK1 Probe BAY-3827

Summary / Conclusion

Probe criteria	
Inhibitor/agonist potency: goal is < 50 nM (IC50, Kd)	**Surpasses criteria**; High potency in biochemical AMPK assay with IC50 = 1.4 nM @ 10 µM ATP;15 nM @ 2 mM ATP
Selectivity within target family: goal is > 30-fold	**Surpasses criteria**; Selectivity > 500 fold against 322 out of 329 off-kinases tested
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	-
On target cell activity for cell-based targets: goal is < 1 micromolar IC50/EC50	**Surpasses criteria**; Active in cellular mechanistic assay (150 nM) demonstrating cellular target engagement
On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture	Additional proof for on target activity: ATP-competitive binding mode demonstrated (X-ray available)
Neg ctrl: in vitro potency – > 100 times less; Cell activity – >100 times less potent than the probe	**Surpasses criteria**; BAY-974 inactive (IC_{50} > 30µM)

We ask for acceptance of AMPK/RSK1 inhibitor BAY-3827 as chemical probe, accompanied by BAY-974 as negative control

AMPK/RSK1 Probe BAY-3827

Project Team / Acknowledgement

Benjamin Bader
Clara Christ
Clara Lemos
Volker Schulze
Simon Holton
Pascale Lejeune
Christian Lechner
Christian Hildebrandt

Andrea Hägebarth
Marcus Bauser

Bernard Haendler
Patrick Steigemann
Oliver Politz
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Philip Lienau
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Lara Kuhnke
Hans Briem
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Peter Spreyer
Cora Scholten
Léa Bouché

Dominik Mumberg
Carl Friedrich Nising
Franz von Nussbaum

Enrico Stasik
Carolin Pohle
Franziska Scholze
Sebastian Schulze
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External Collaboration
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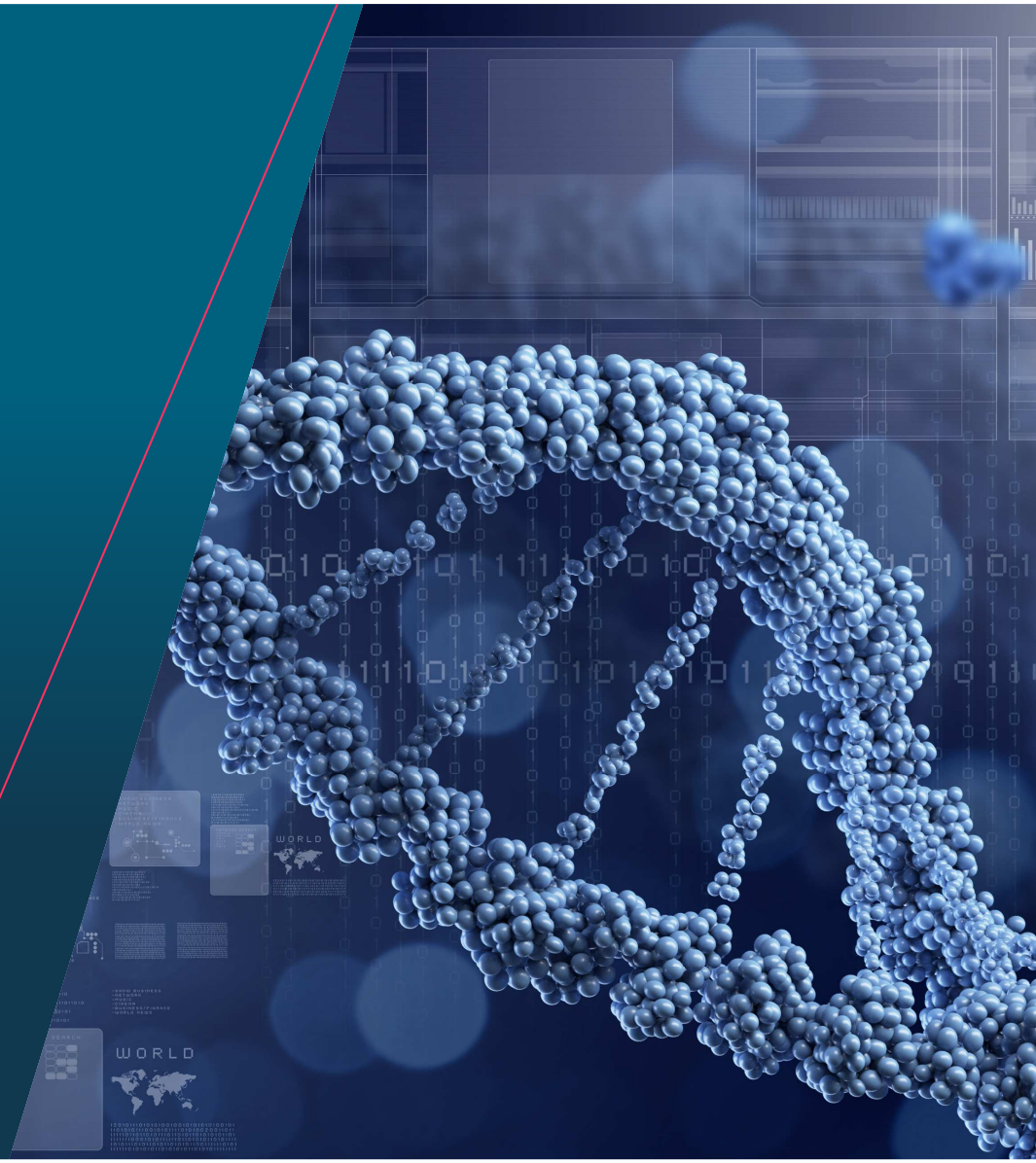
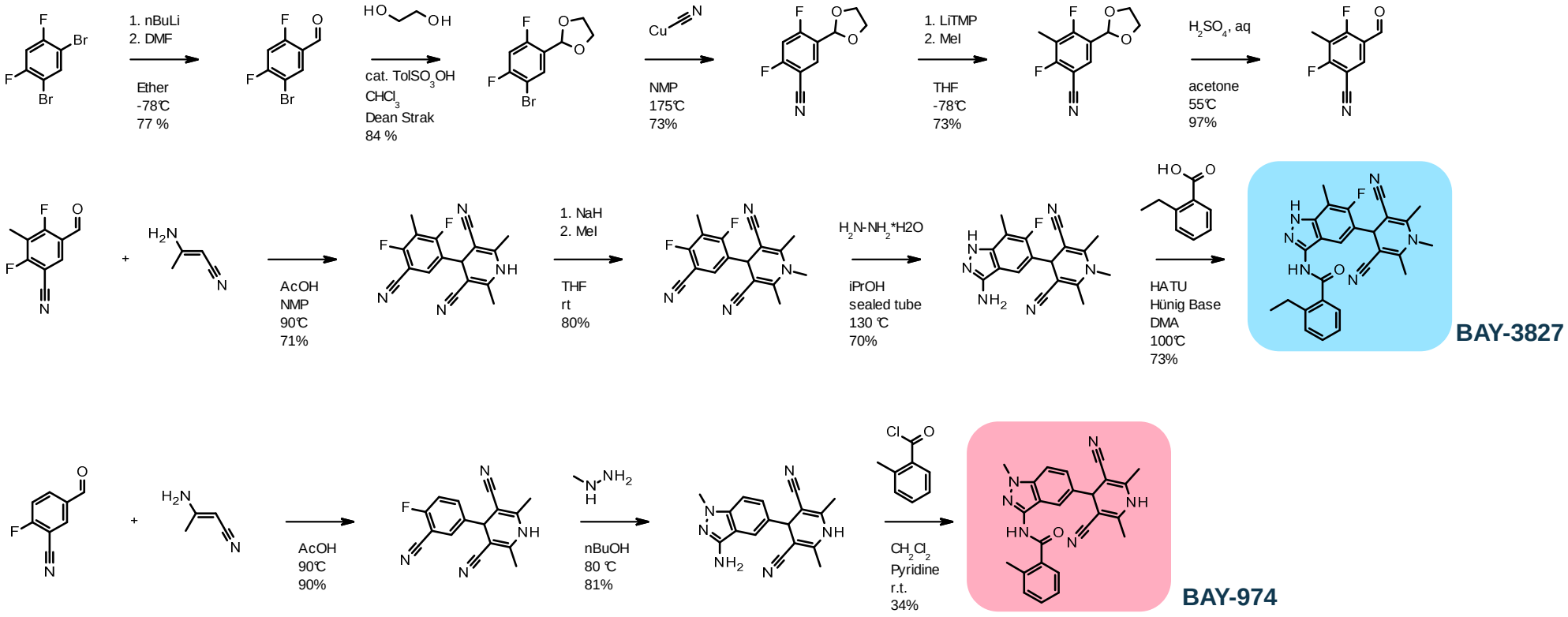
Thank You



AMPK/RSK1 Probe BAY-3827

Chemical Synthesis of BAY-3827 and of Negative Control BAY-974



BAY-3827 was synthesized in a linear sequence of 9 steps; BAY-974 was synthesized in a linear sequence of 3 steps.

AMPK/RSK1 Probe BAY-3827

Methods

- AMPK kinase assays were performed at both low (10 µM) and high (2 mM) ATP concentrations. Aurora A kinase assays were performed at low ATP concentrations (10 µM).

- The cellular mechanistic assay was performed in IMR-32 neuroblastoma cells using a commercially available phospho-ACC HTRF® kit (Cisbio). This assay was used to determine the level of ACC phosphorylated at Ser79 (pACC) in cell lysates.

- Caco-2 permeability was determined with an assay using Caco-2 cells purchased from DSMZ (Germany). Permeability (Papp) was measured in the apical to basolateral (A->B) and basolateral to apical (B->A) directions. The basolateral (B) to apical (A) efflux ratio was calculated using the formula Papp (B-A) / Papp (A-B).

- Proliferation assays were performed in a panel of cells with dysregulated C-MYC (COLO 320DM, LS-174T, Ramos, SNU-16, SU-DHL-10, OCI-LY7 and JJN-3) or N-MYC (IMR-32 and IMR-5/75). Colo201 and SK-N-F1 were used as control cell lines (without C-MYC/N-MYC dysregulation).