

SUPPORTING INFORMATION

## Photo-crosslinking of clinically relevant kinases using H89-derived photo-affinity probes

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Kinomescan activities of probe **3** and **4**

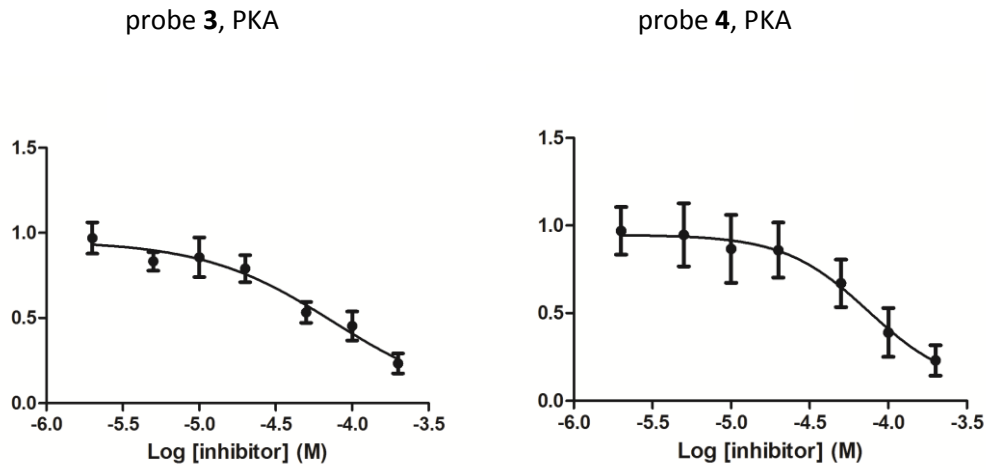
Dose dependency curves for probe **3** and **4**

<sup>1</sup>H and <sup>13</sup>C NMR spectra of all novel compounds

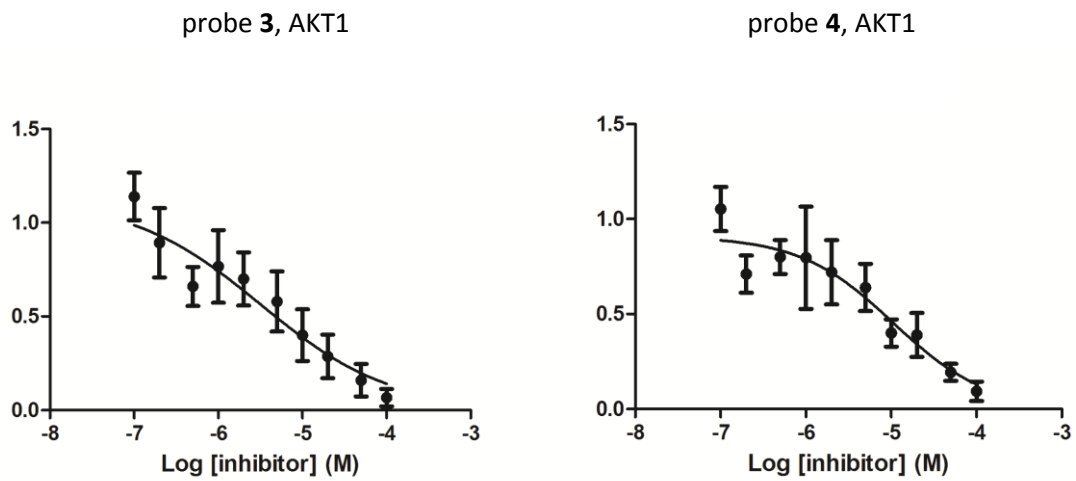
**Table S1:** Activities of probe **3** and **4** against a panel of kinases.<sup>1</sup>Activities are given as % residual kinase activity at 10  $\mu$ M probe.

Kinase	3	4	Kinase	3	4
PKAc- $\alpha$	1.5	3.4	TAOK1	72	52
AKT1	2.6	18	MEK2	73	72
ROCK2	3	1.7	FAK	74	100
AKT3	3.4	25	VEGFR2	75	85
PRKCH	5	2.8	CLK2	76	72
PRKCE	20	10	JNK1	78	82
PRKCD	23	14	TYK2	78	97
SNARK	23	24	TRKA	79	82
SGK3	31	32	AURKB	80	79
CDK11	35	78	GSK3B	80	74
PRKCI	35	35	PCTK1	80	84
CSNK1D	39	38	JNK3	81	89
MARK3	40	53	FGFR3	82	84
CDK7	43	48	PIK3CG	82	78
DYRK1B	45	74	PLK1	82	83
ALK	50	62	YANK3	82	88
FLT3	50	60	MAPKAPK2	83	95
MLCK	50	76	PIM1	83	81
CSF1R	51	96	ULK2	83	65
TSSK1B	53	56	ZAP70	83	94
AXL	54	74	DYRK1A	84	85
CHEK1	56	72	ERBB4	84	90
SRPK3	57	86	ERN1	85	57
CDK9	58	70	LKB1	85	94
PDGFRB	58	57	PAK4	86	98
PIP5K1A	58	37	ACVR1B	87	100
PDPK1	59	69	p38-beta	87	100
PLK4	59	57	CSNK1G3	89	91
RET	59	76	ERBB2	89	64
FGFR2	60	67	INSR	89	81
KIT	61	70	PAK2	89	96
AMPK- $\alpha$ 2	63	83	TGFBR1	89	88
DCAMKL1	63	86	PIM3	90	94
PRKCC	63	46	AURKA	91	69
EPHA2	65	66	JAK3	91	84
MAP3K4	65	68	PDGFRA	91	98
AKT2	66	91	RAF1	91	78
CDK2	66	92	BMPR2	92	89
CDK3	66	78	PIK3CA	93	94
ABL1-p	67	81	CSNK1G2	94	80
JAK2	67	90	MEK1	95	83
MET	67	55	PAK1	95	95
RSK2	67	72	BTK	96	92
TIE2	68	86	p38- $\alpha$	96	94
ERK1	69	70	HIPK2	97	95
JNK2	69	86	EGFR	98	100
PLK3	69	73	PIK3C2B	98	94
PIM2	70	62	IKK-beta	99	80
SRC	70	70	MKNK1	99	92
ADCK3	71	96	PIK4CB	99	97
MKNK2	71	71	BRAF	100	93
MST2	71	73	IKK-alpha	100	86
FLT1	72	69	MTOR	100	100
IGF1R	72	63	MYO3A	100	100
MLK1	72	69	RIOK2	100	88
MUSK	72	77			

<sup>1</sup> M. W. Karaman, S. Herrgard, D. K. Treiber, P. Gallant, C. E. Atteridge, B. T. Campbell, K. W. Chan, P. Ciceri, M. J. Davis, P. T. Edeen, R. Faraoni, M. Floyd, J. P. Hunt, D. J. Lockhart, Z. V. Milanov, M. J. Morrison, G. Pallares, H. K. Patel, S. Pritchard, L. M. Wodicka and P. P. Zarrinkar, *Nat. Biotech.*, 2008, **26**, 127-132.



**Fig.S1:** Dose-dependent activity of PKA determined by FRET-based assay for probes 3 and 4.



**Fig.S2:** Dose-dependent activity of AKT1 determined by FRET-based assay for probes 3 and 4.

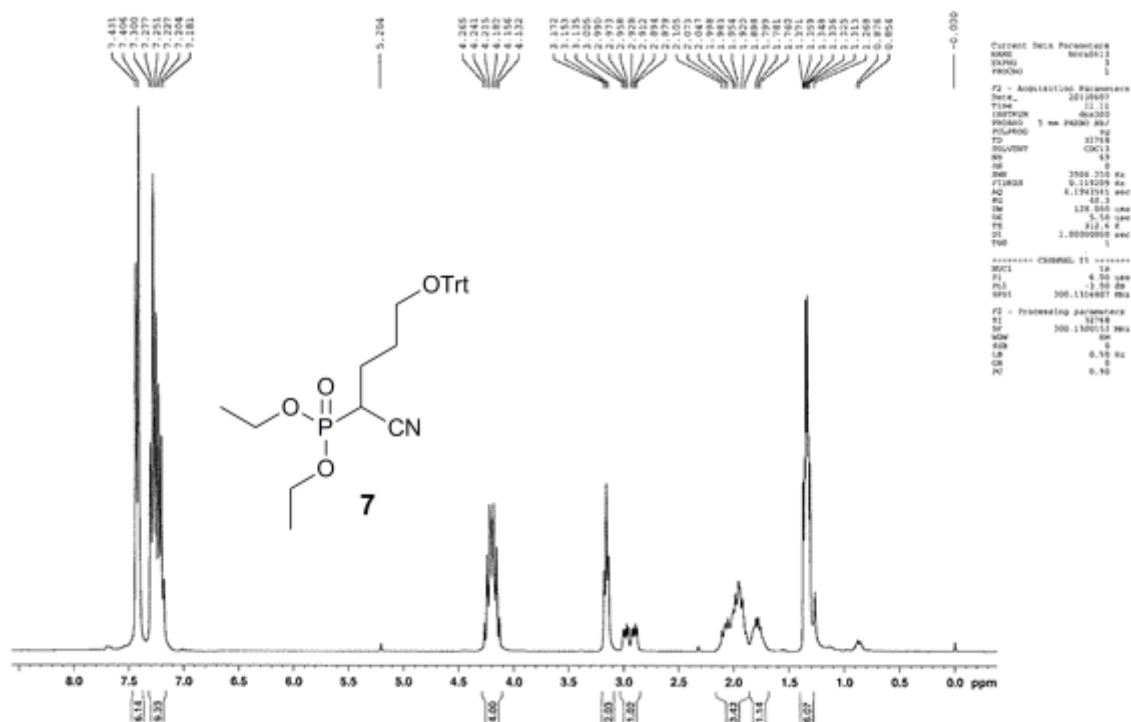


Fig.S3: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si)for (Diethyl (1-cyano-4-(trityloxy)butyl)phosphonate (7)).

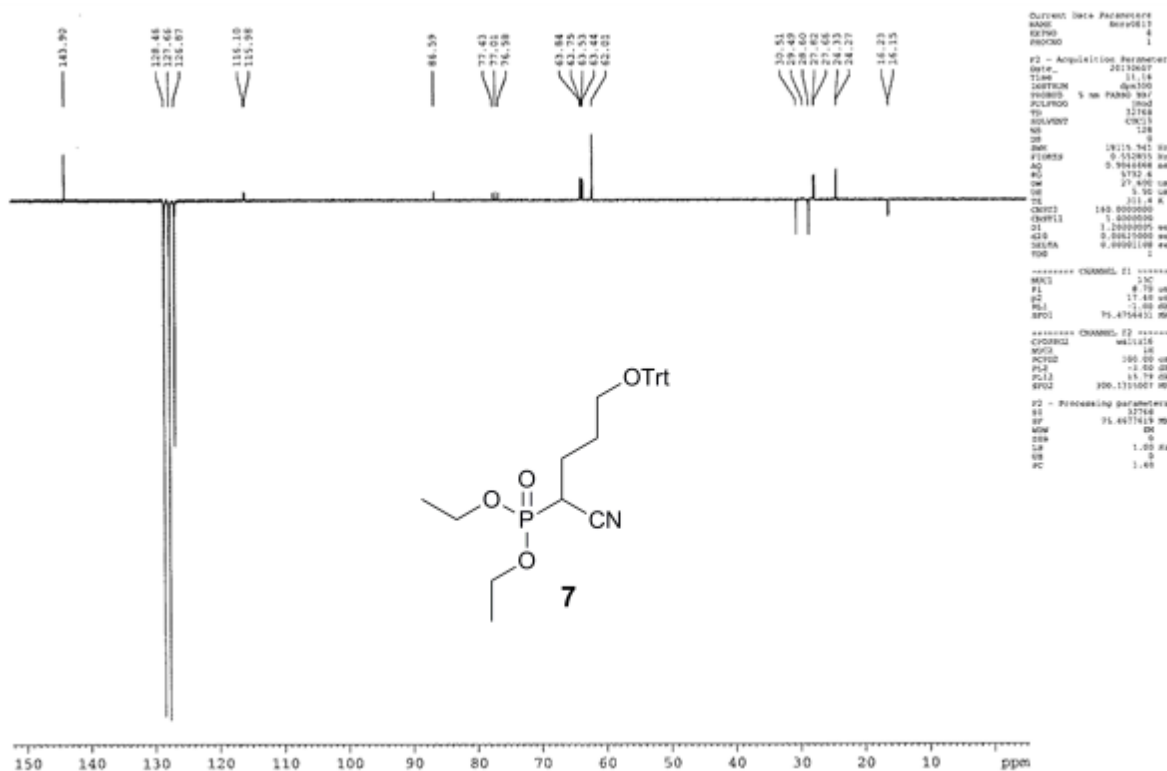
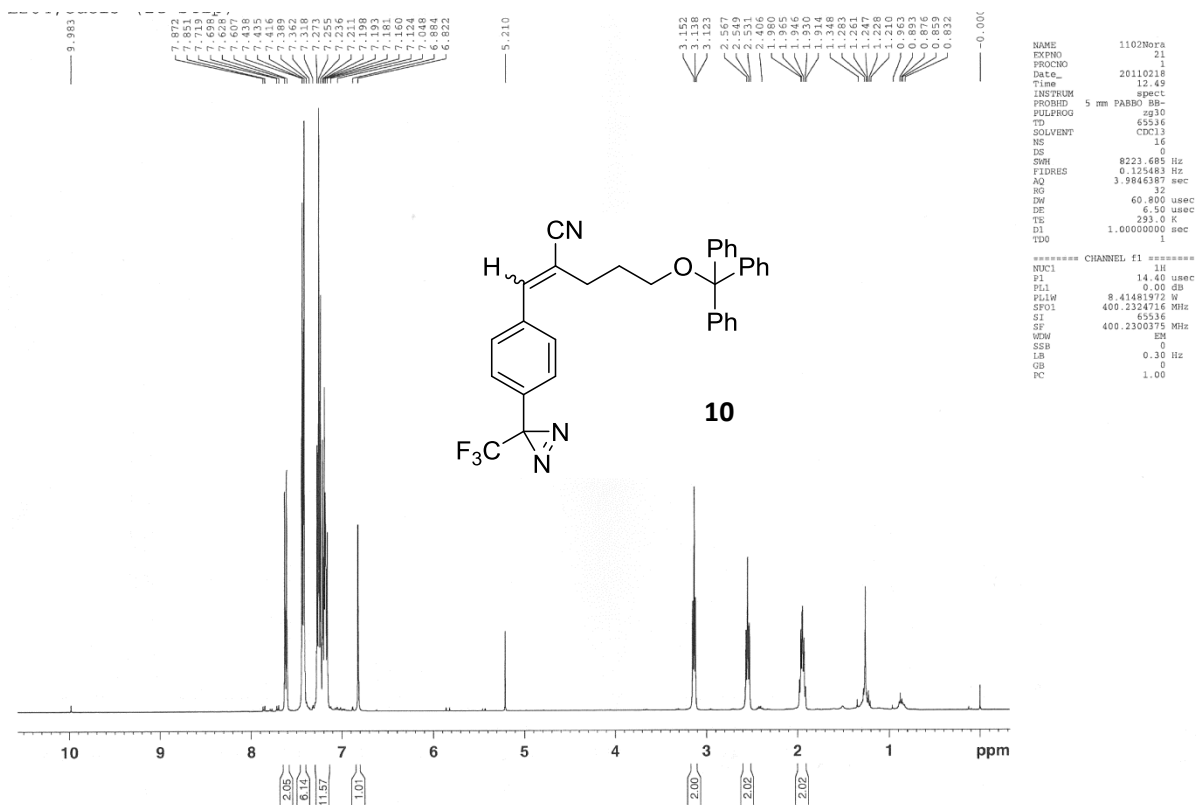
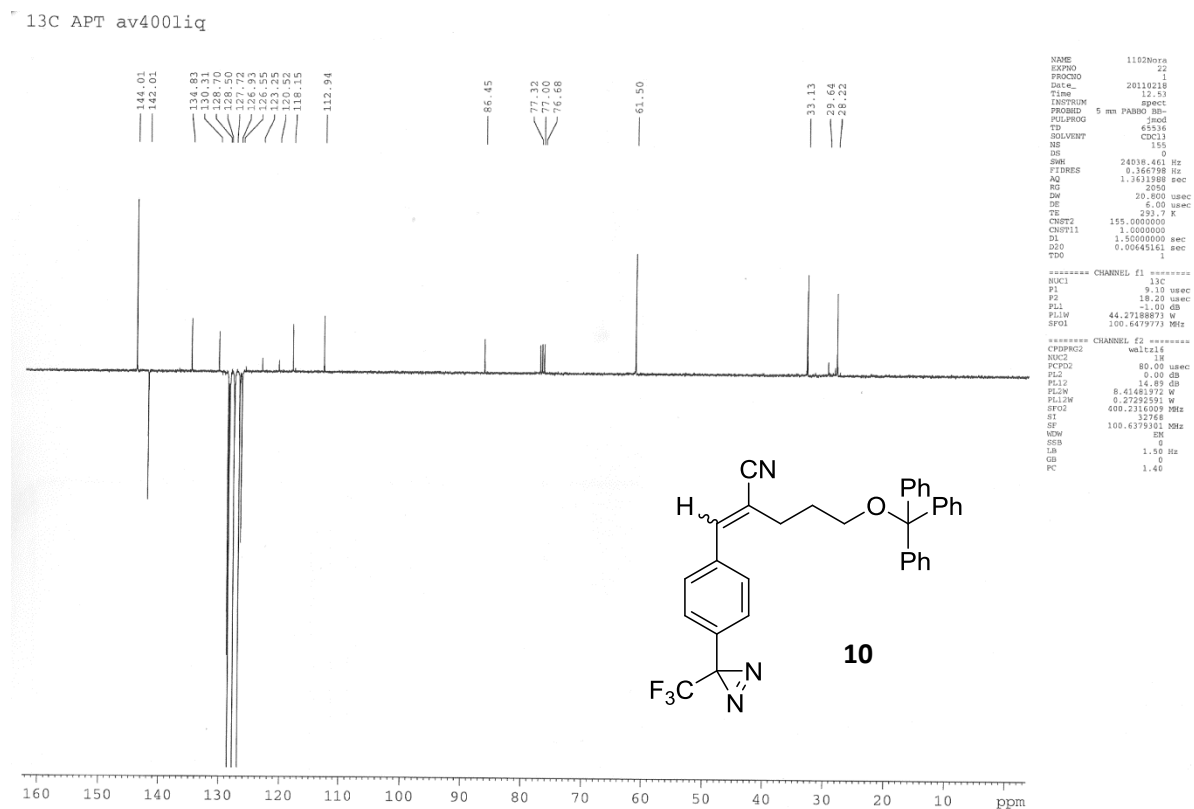


Fig.S4: <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)for (Diethyl (1-cyano-4-(trityloxy)butyl)phosphonate (7)).

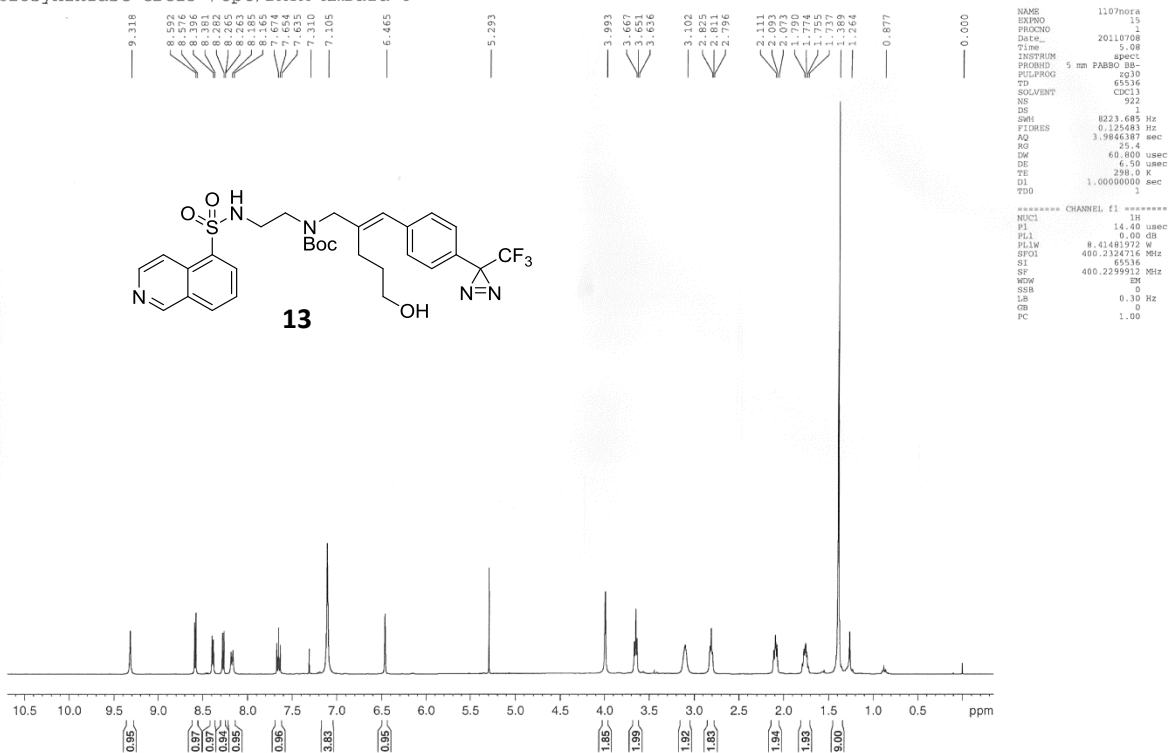


**Fig.S5:** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) for (E/Z)-2-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)-benzylidene)-5-(trityloxy)pentanenitrile (**10**).



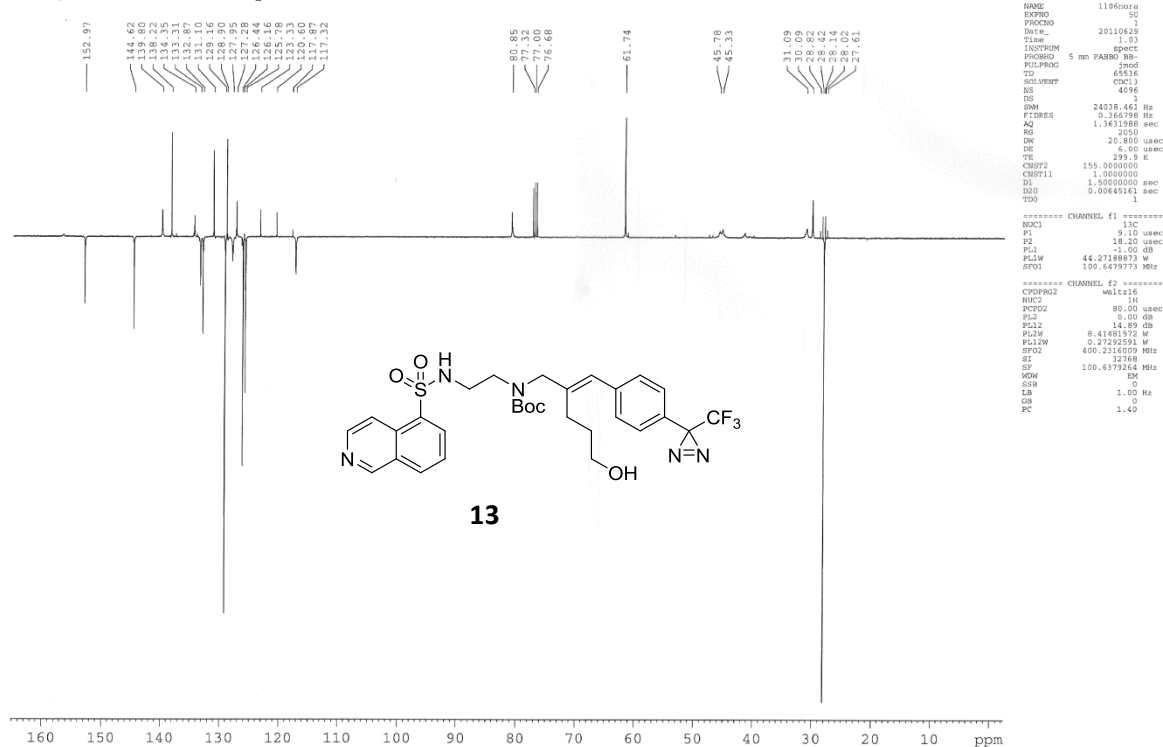
**Fig.S6:** <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) for (E/Z)-2-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)-benzylidene)-5-(trityloxy)pentanenitrile (**10**).

biosyn1Hfast CDCl3 /opt/DATA nmrafd 8



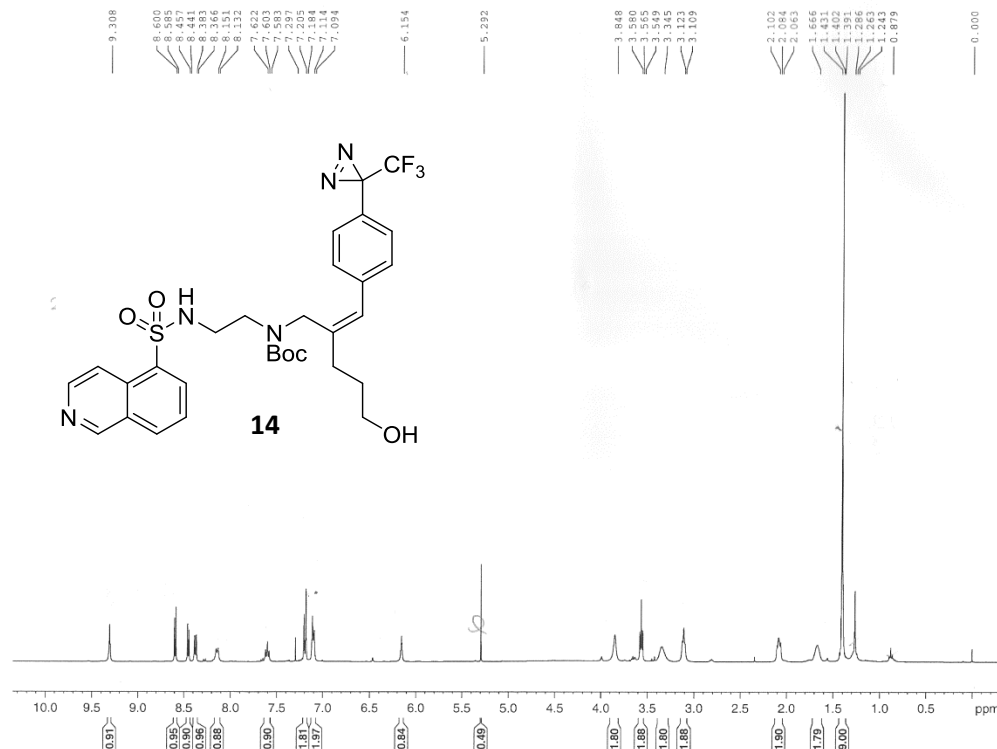
**Fig.S7:**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\text{Me}_4\text{Si}$ ) for tert-butyl(*E*)-(5-hydroxy-2-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzylidene)pentyl)(2-(isoquinoline-5-sulfonamido)ethyl)-carbamate (**13**).

biosynAPTfast CDCl3 /opt/DATA nmrafd 5



**Fig.S8:**  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ ) for tert-butyl(*E*)-(5-hydroxy-2-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzylidene)pentyl)(2-(isoquinoline-5-sulfonamido)ethyl)-carbamate (**13**).

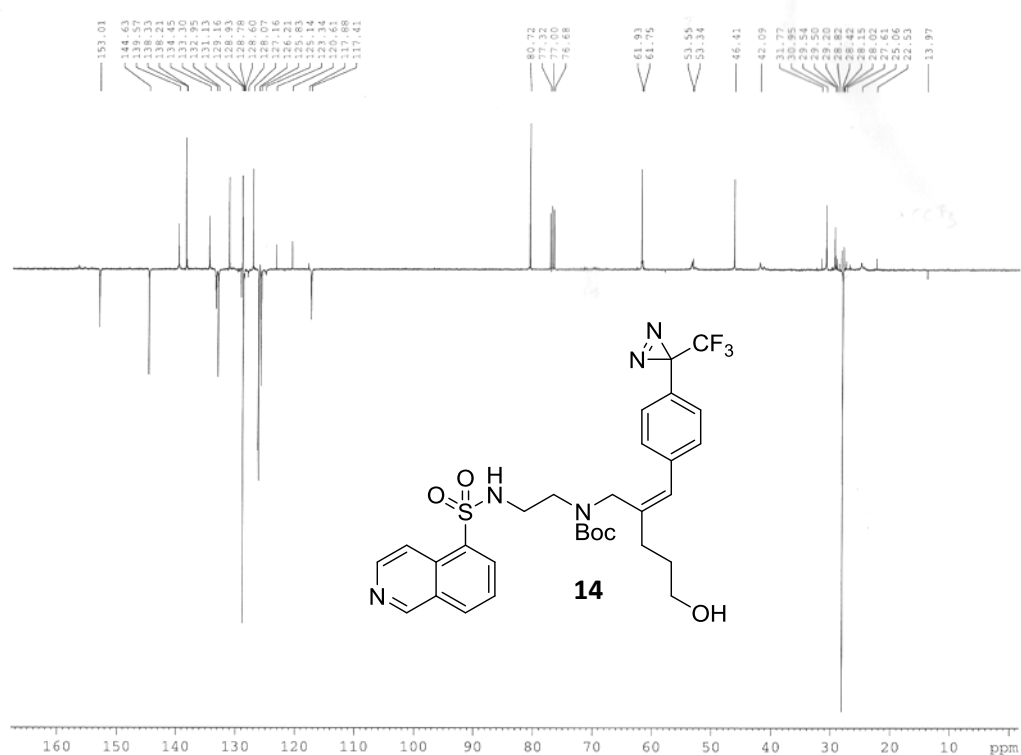
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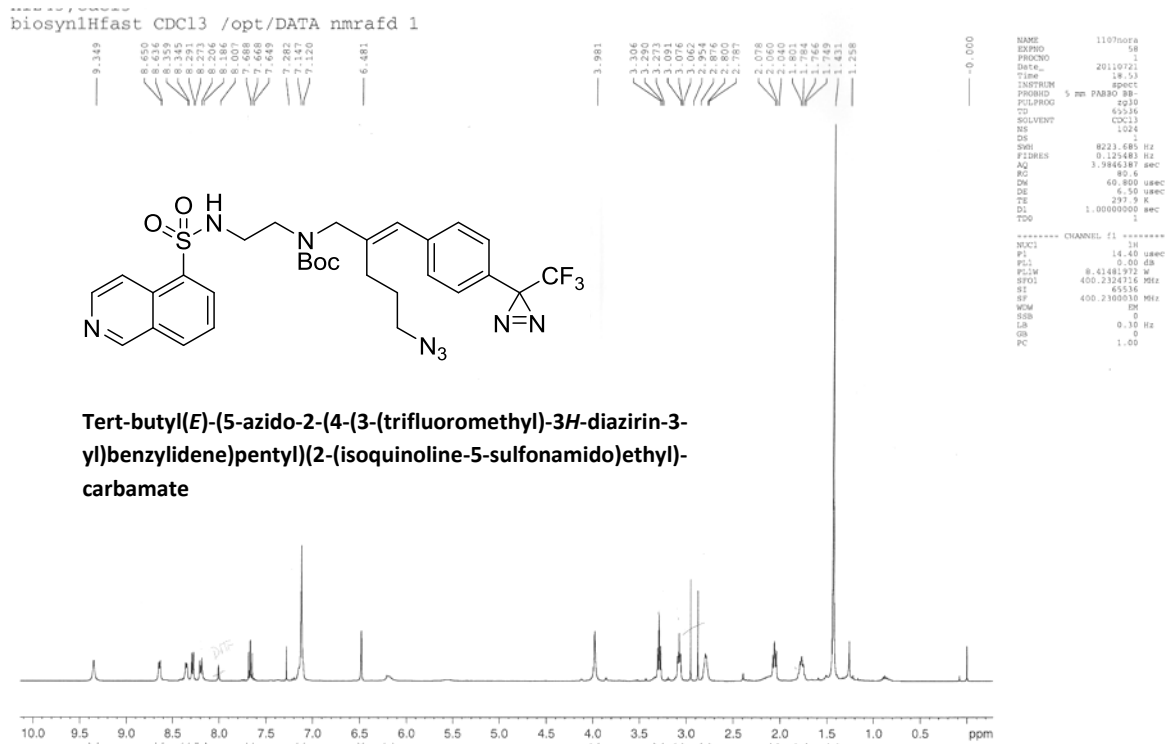
**Fig.S9:** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) for tert-butyl(Z)-(5-hydroxy-2-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzylidene)pentyl)(2-(isoquinoline-5-sulfonamido)ethyl)-carbamate (**14**)

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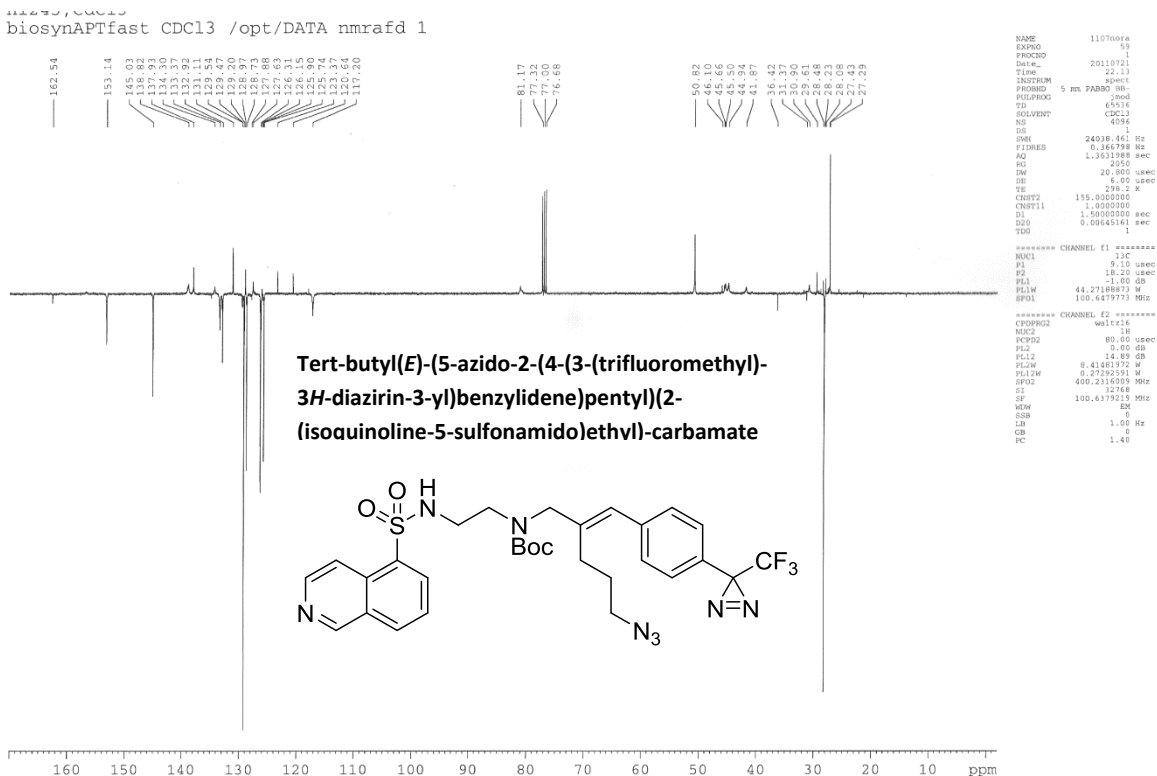


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NUC2      13C
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D1        1.0000000 sec
D2        1.0000000 sec
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TD0       1
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**Fig.S10:** <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) for tert-butyl(Z)-(5-hydroxy-2-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzylidene)pentyl)(2-(isoquinoline-5-sulfonamido)ethyl)-carbamate (**14**).

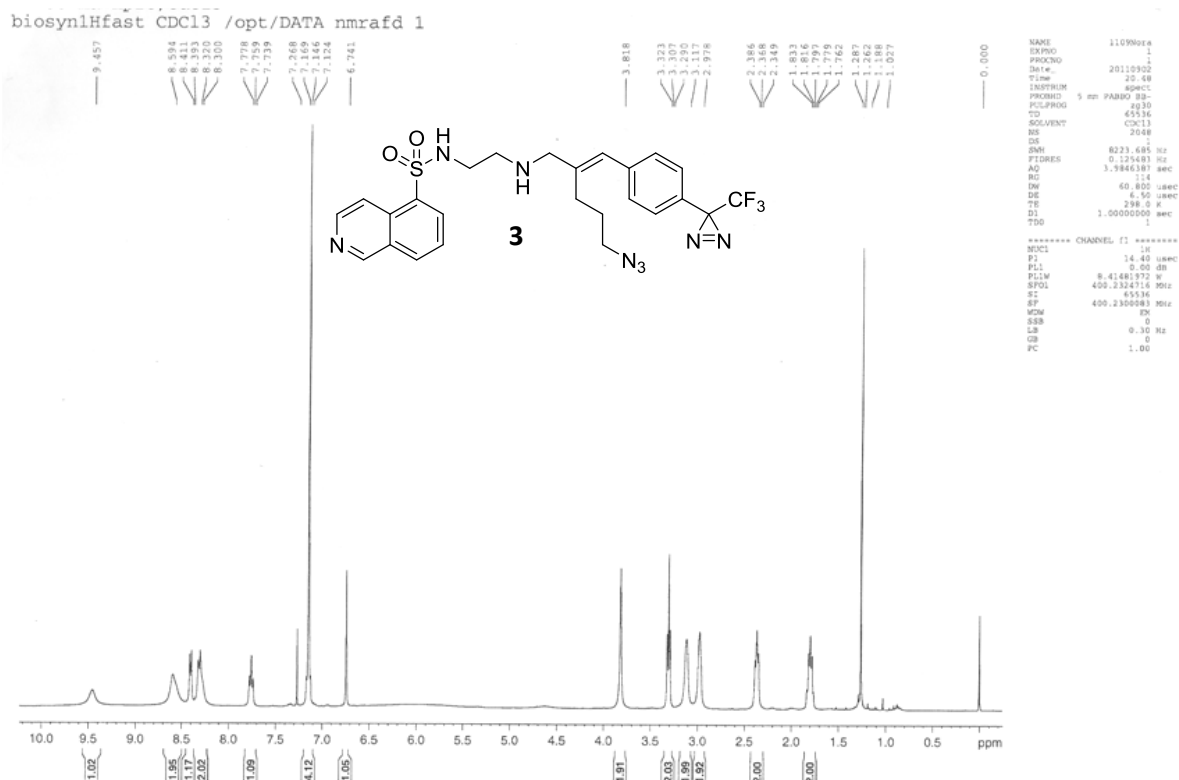


**Fig.S11:** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) for tert-butyl(*E*)-(5-azido-2-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzylidene)pentyl)(2-(isoquinoline-5-sulfonamido)ethyl)-carbamate.

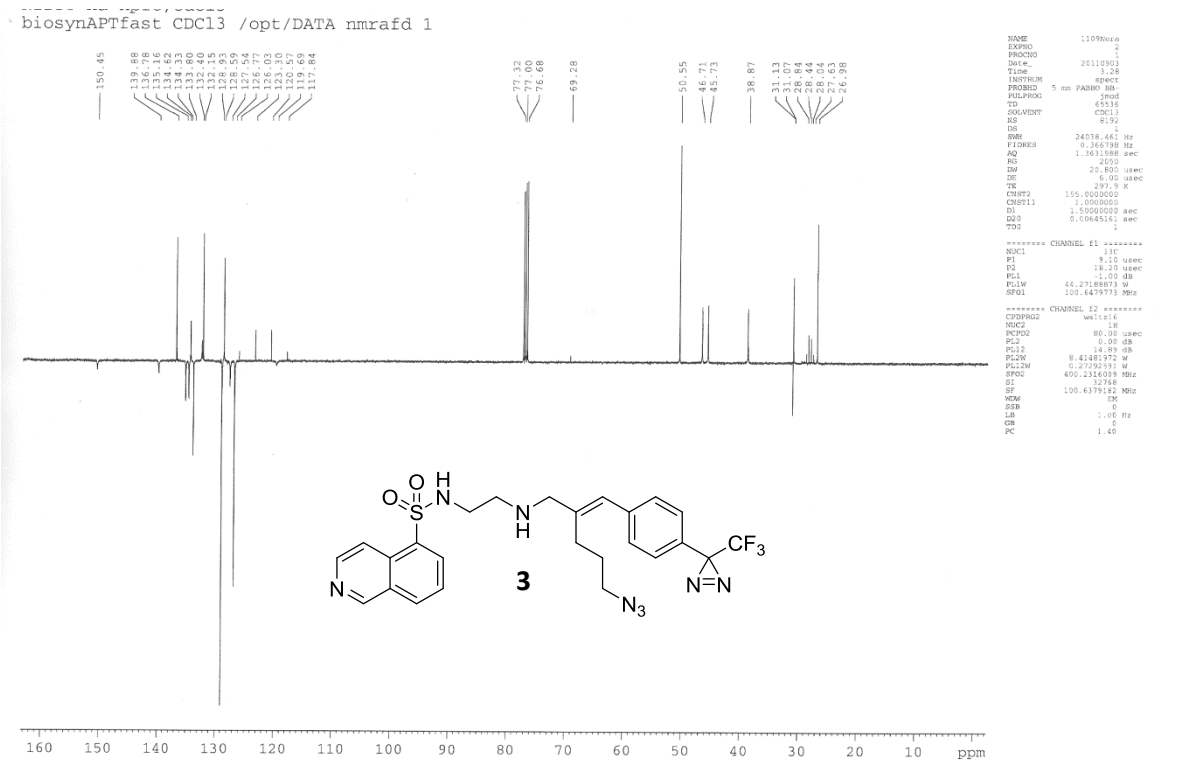


**Fig.S12:** <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) for tert-butyl(*E*)-(5-azido-2-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzylidene)pentyl)(2-(isoquinoline-5-sulfonamido)ethyl)-carbamate.



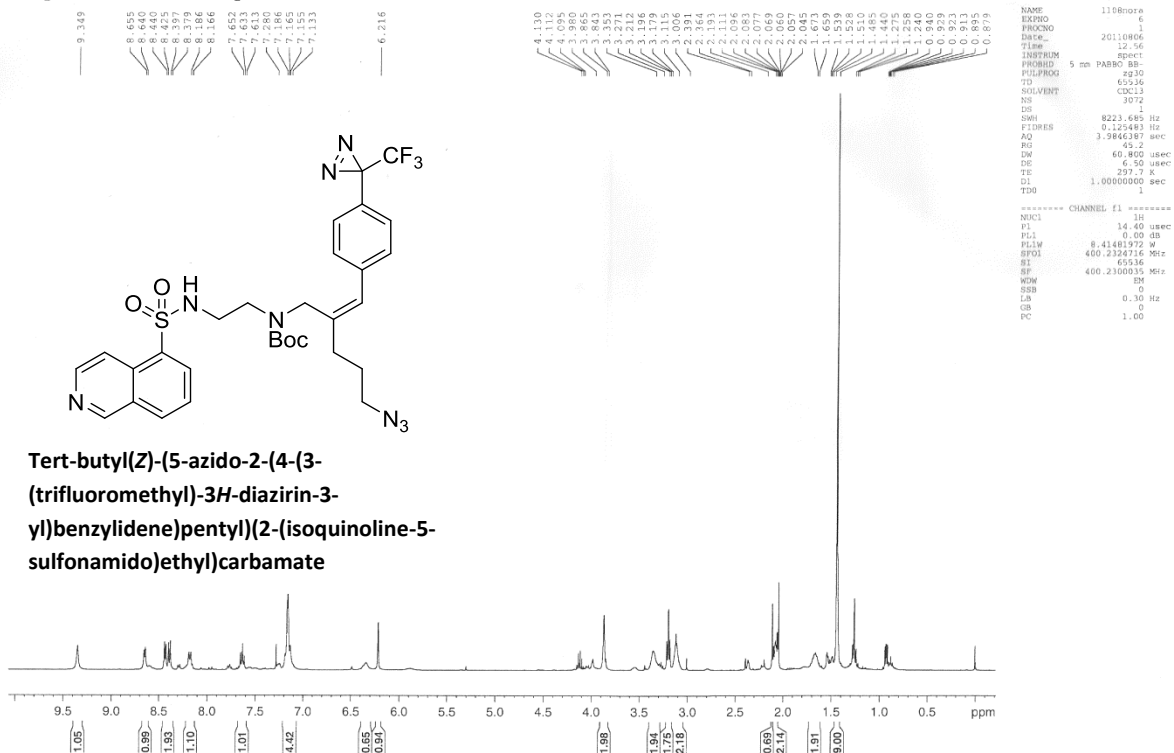


**Fig.S13:**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\text{Me}_4\text{Si}$ ) for (*E*)-*N*-(2-((5-azido-2-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzylidene)pentyl)amino)ethyl)isoquinoline-5-sulfonamide (**3**).



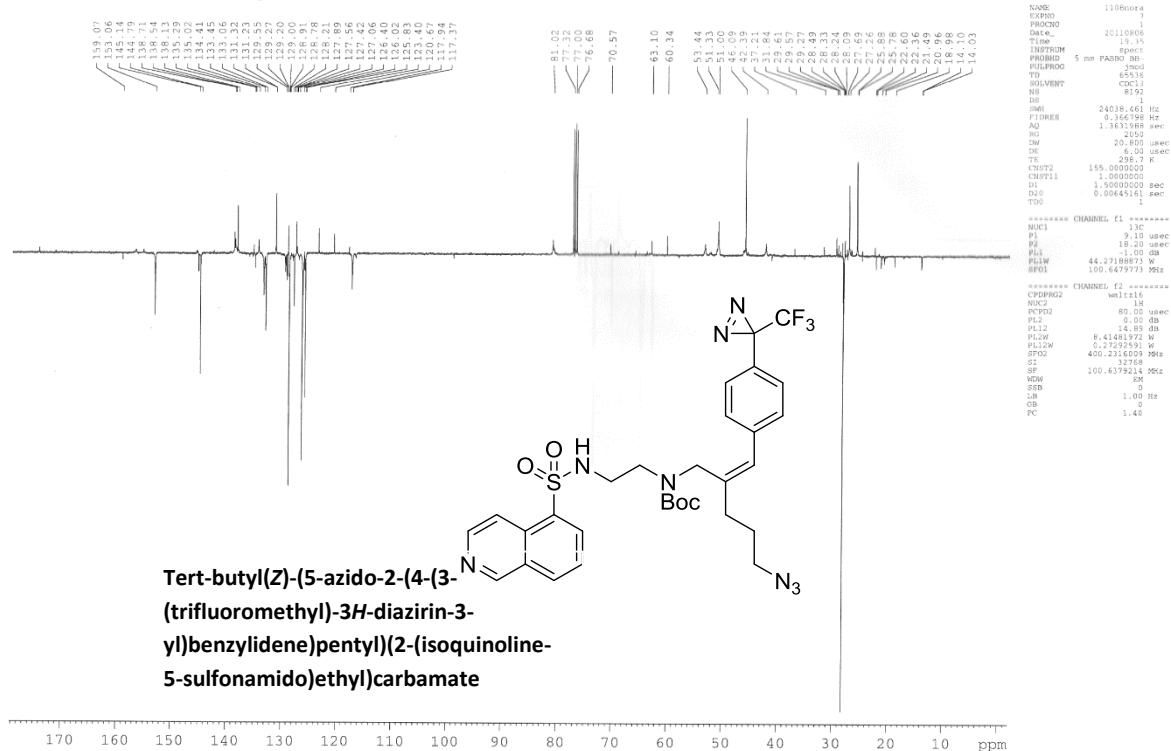
**Fig.S14:**  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ ) for (*E*)-*N*-(2-((5-azido-2-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzylidene)pentyl)amino)ethyl)isoquinoline-5-sulfonamide (**3**).

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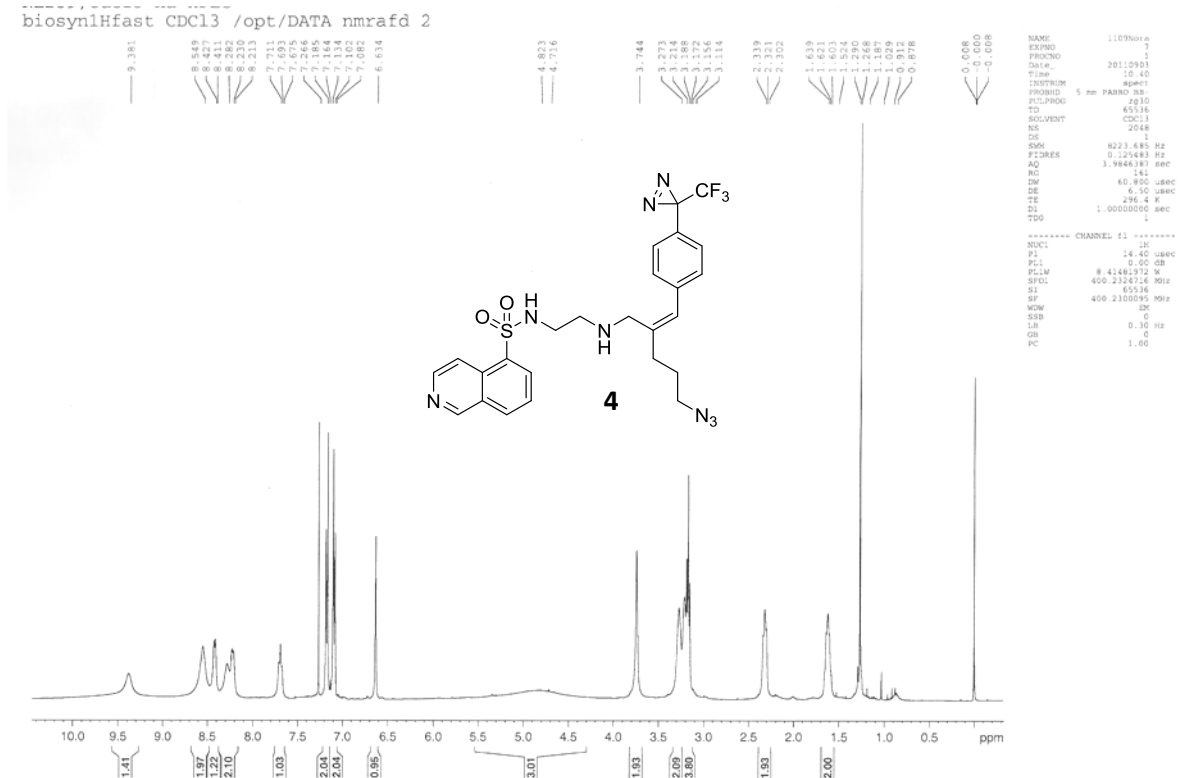


**Fig.S15:** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) for tert-butyl(Z)-(5-azido-2-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzylidene)pentyl)(2-(isoquinoline-5-sulfonamido)ethyl)-carbamate.

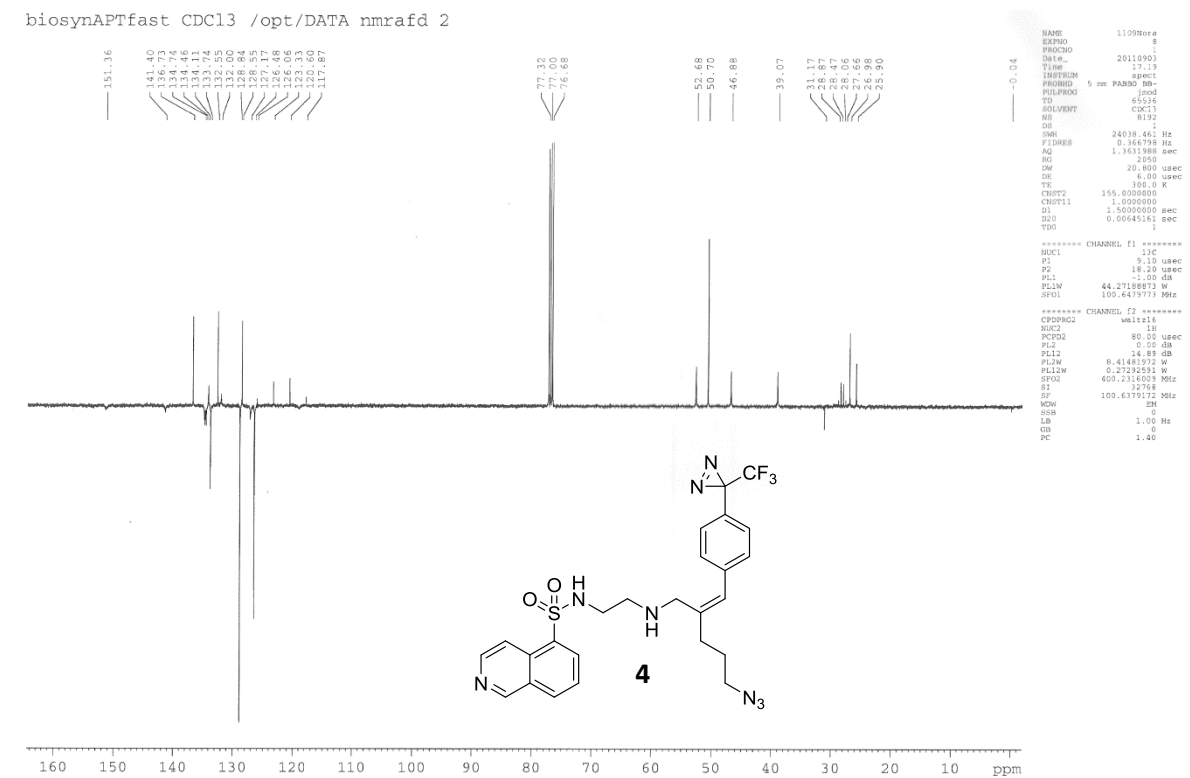
biosynAPTfast CDCl<sub>3</sub> /opt/DATA nmrafd 7



**Fig.S16:** <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) for tert-butyl(Z)-(5-azido-2-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzylidene)pentyl)(2-(isoquinoline-5-sulfonamido)ethyl)-carbamate.



**Fig.S17:** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) for (Z)-N-(2-((5-azido-2-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzylidene)pentyl)amino)ethyl)isoquinoline-5-sulfonamide (**4**).



**Fig.S18:** <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) for (Z)-N-(2-((5-azido-2-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzylidene)pentyl)amino)ethyl)isoquinoline-5-sulfonamide (**4**).

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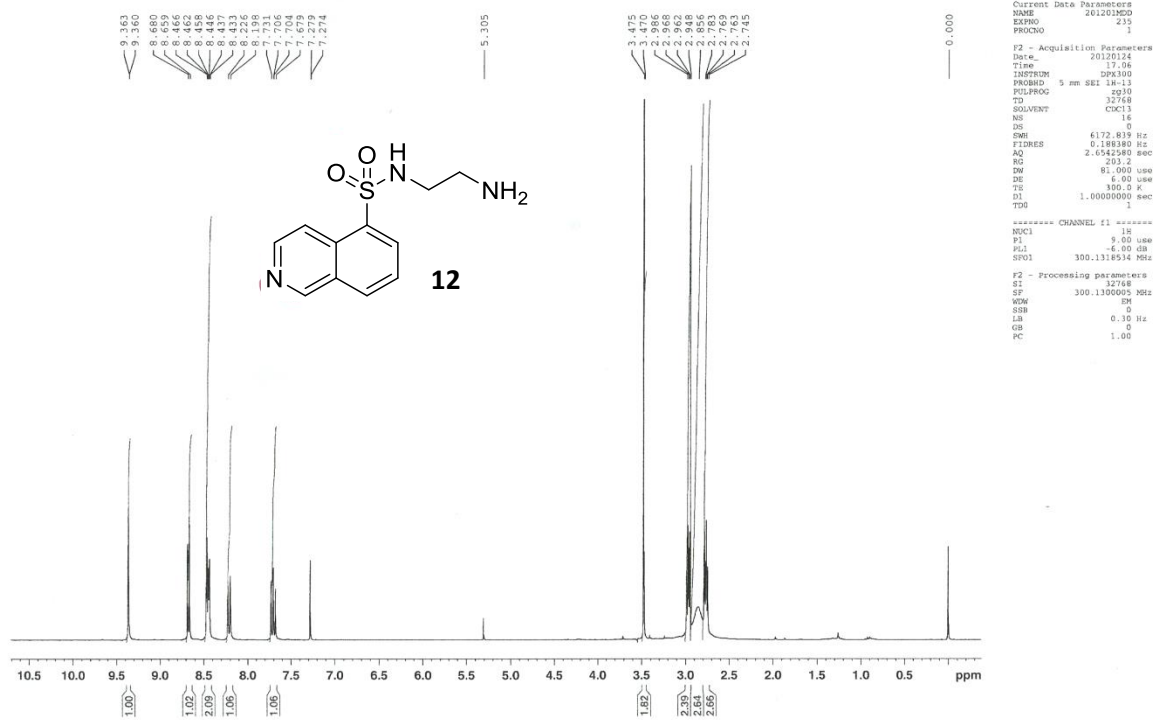


Fig.S19:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\text{Me}_4\text{Si}$ ) for (*N*-(2-aminoethyl)isoquinoline-5-sulfonamide (**12**).

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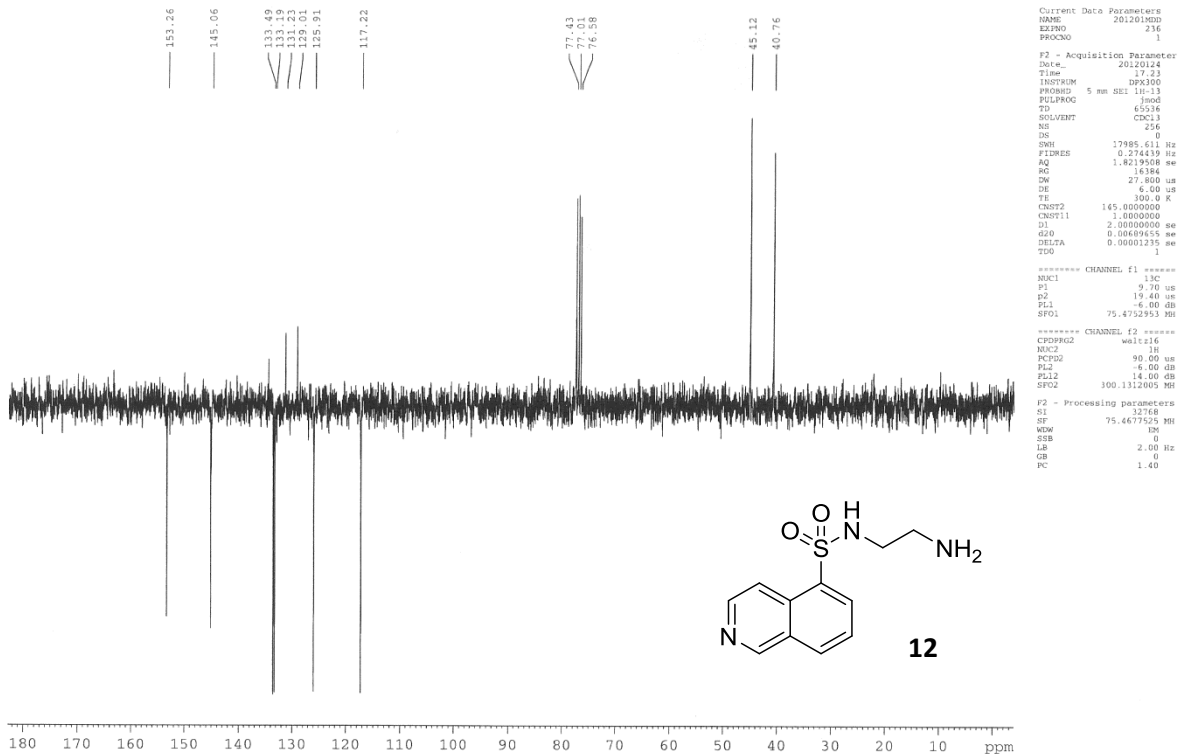
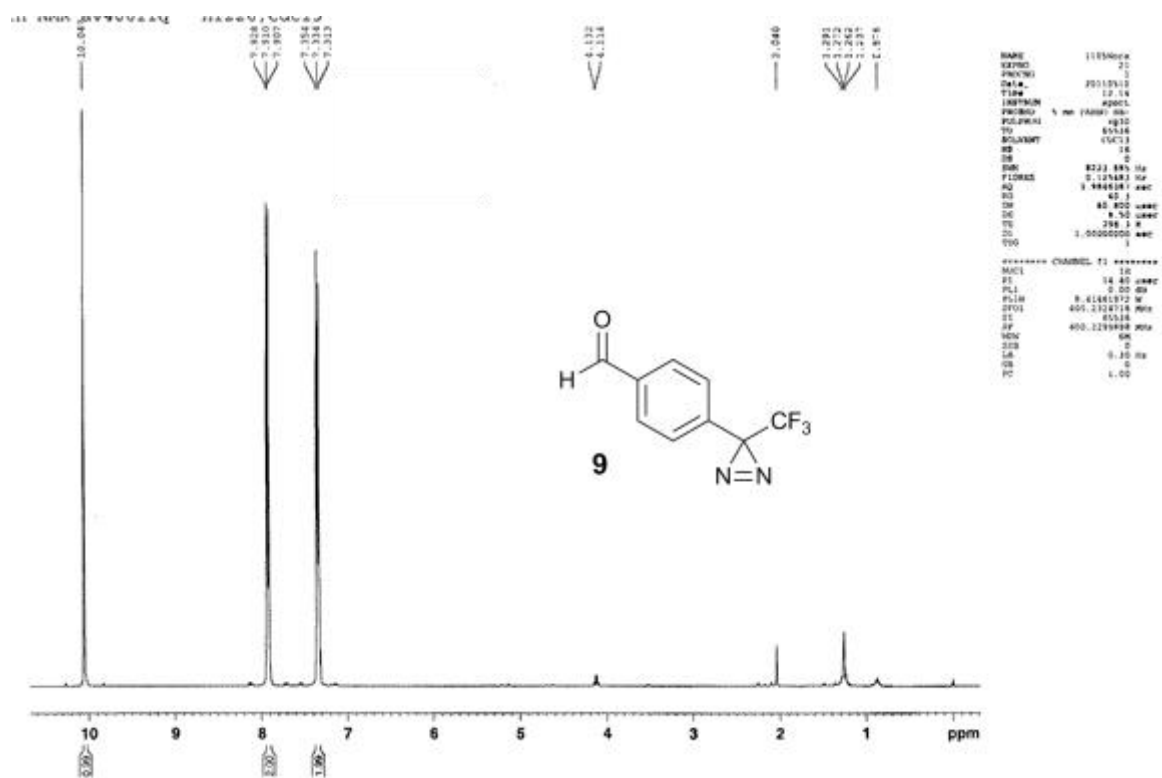


Fig.S20:  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ ) for (*N*-(2-aminoethyl)isoquinoline-5-sulfonamide (**12**).



**Fig.S21:**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\text{Me}_4\text{Si}$ ) for 4-(3-(Trifluoromethyl)-3H-diazirin-3-yl)benzaldehyde (9).



**Fig.S22:**  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ ) for 4-(3-(Trifluoromethyl)-3H-diazirin-3-yl)benzaldehyde (9).