Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Supplementary Information

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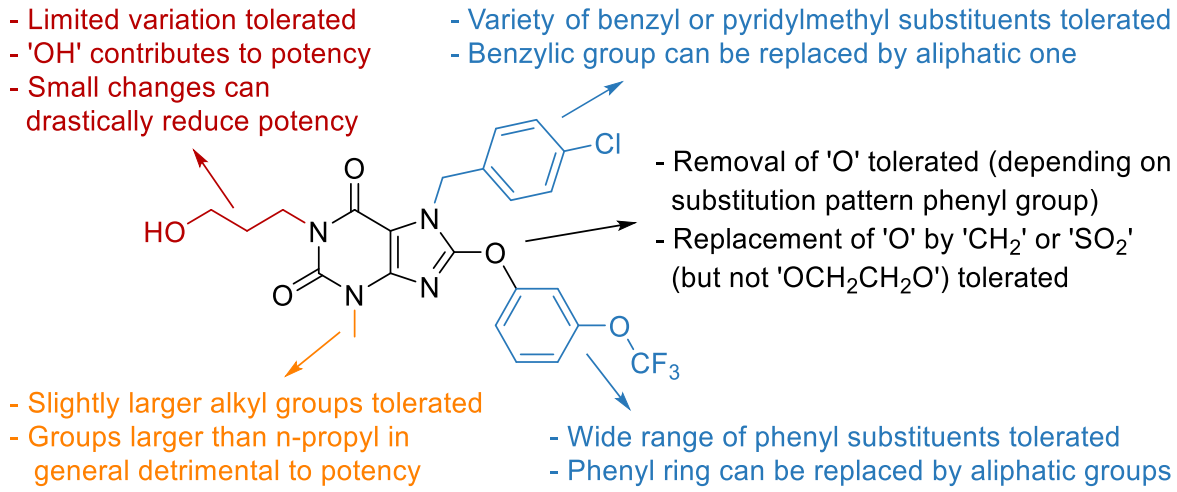
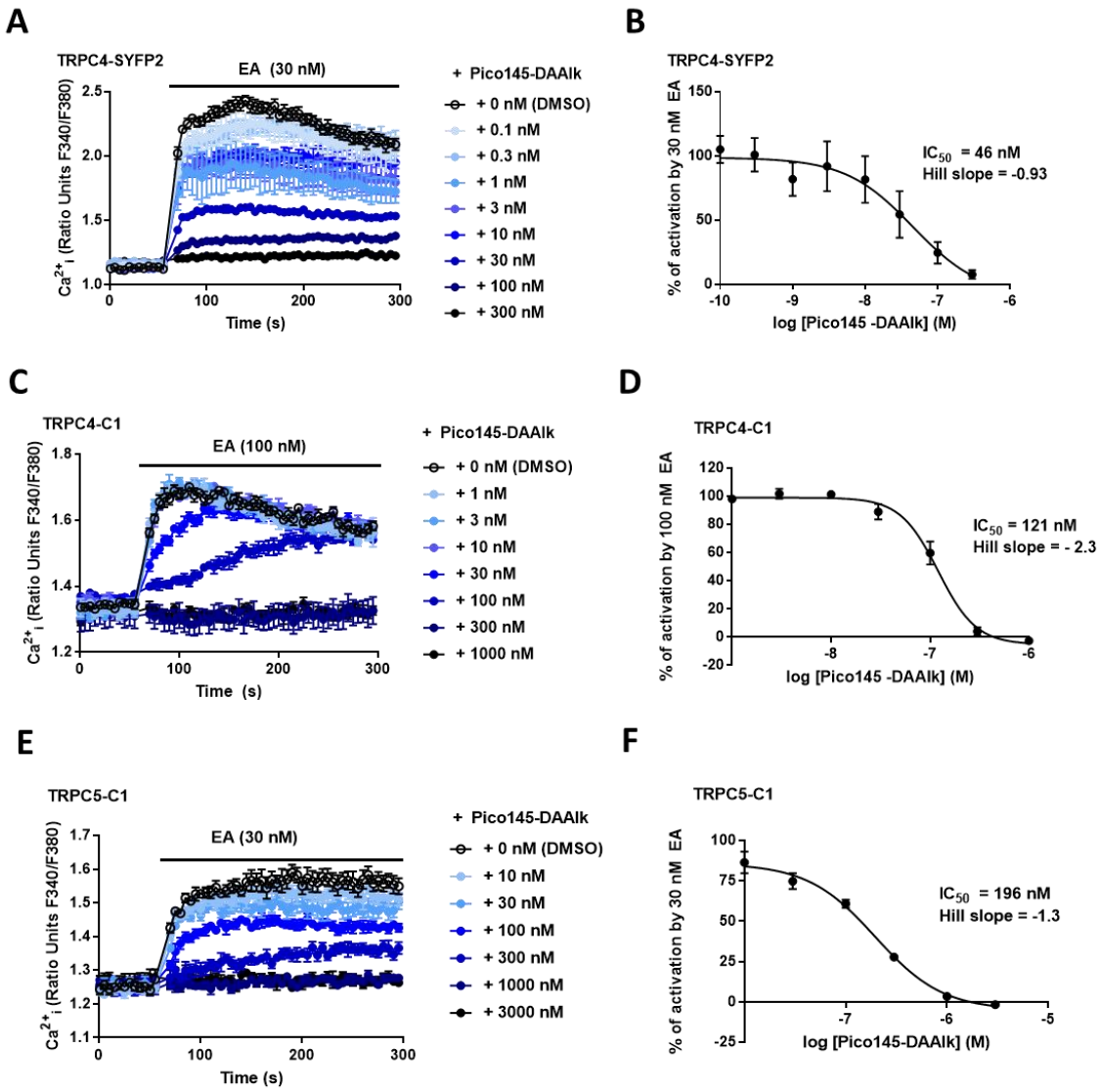
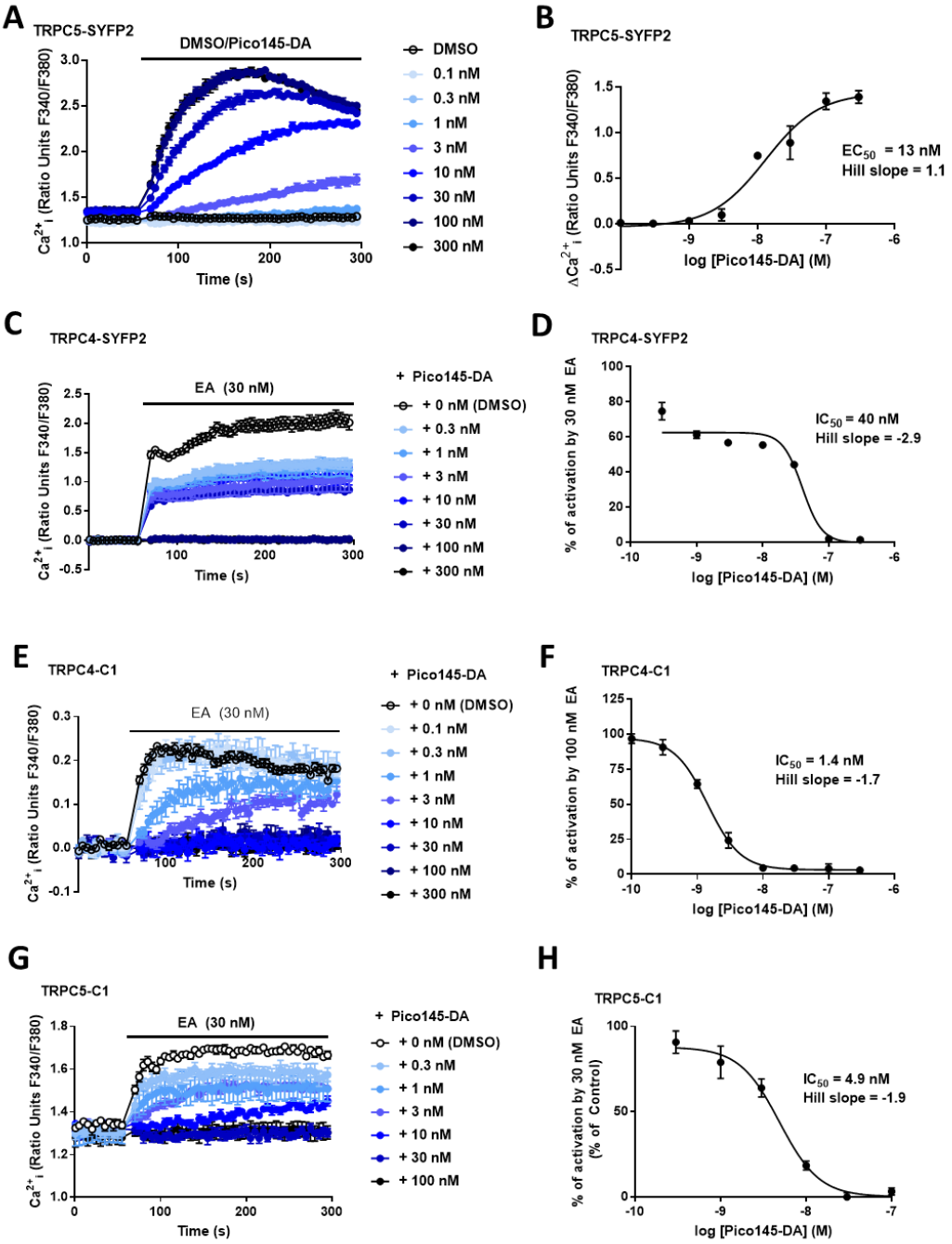
Supplementary Figures



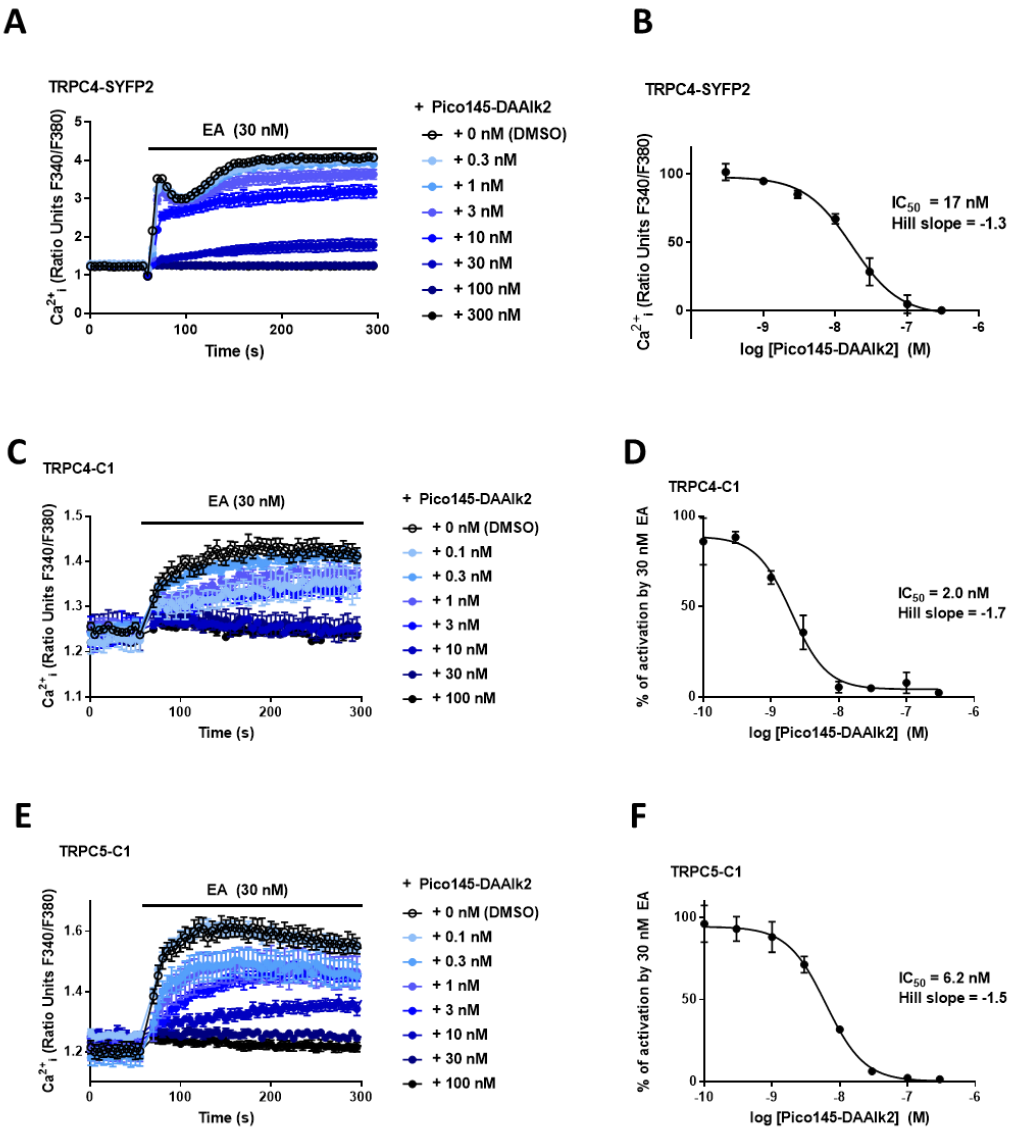
Supplementary Figure 1. Structure activity relationship of xanthine-based TRPC1/4/5 inhibitors based on semi-quantitative data reported in the patent literature.[1]



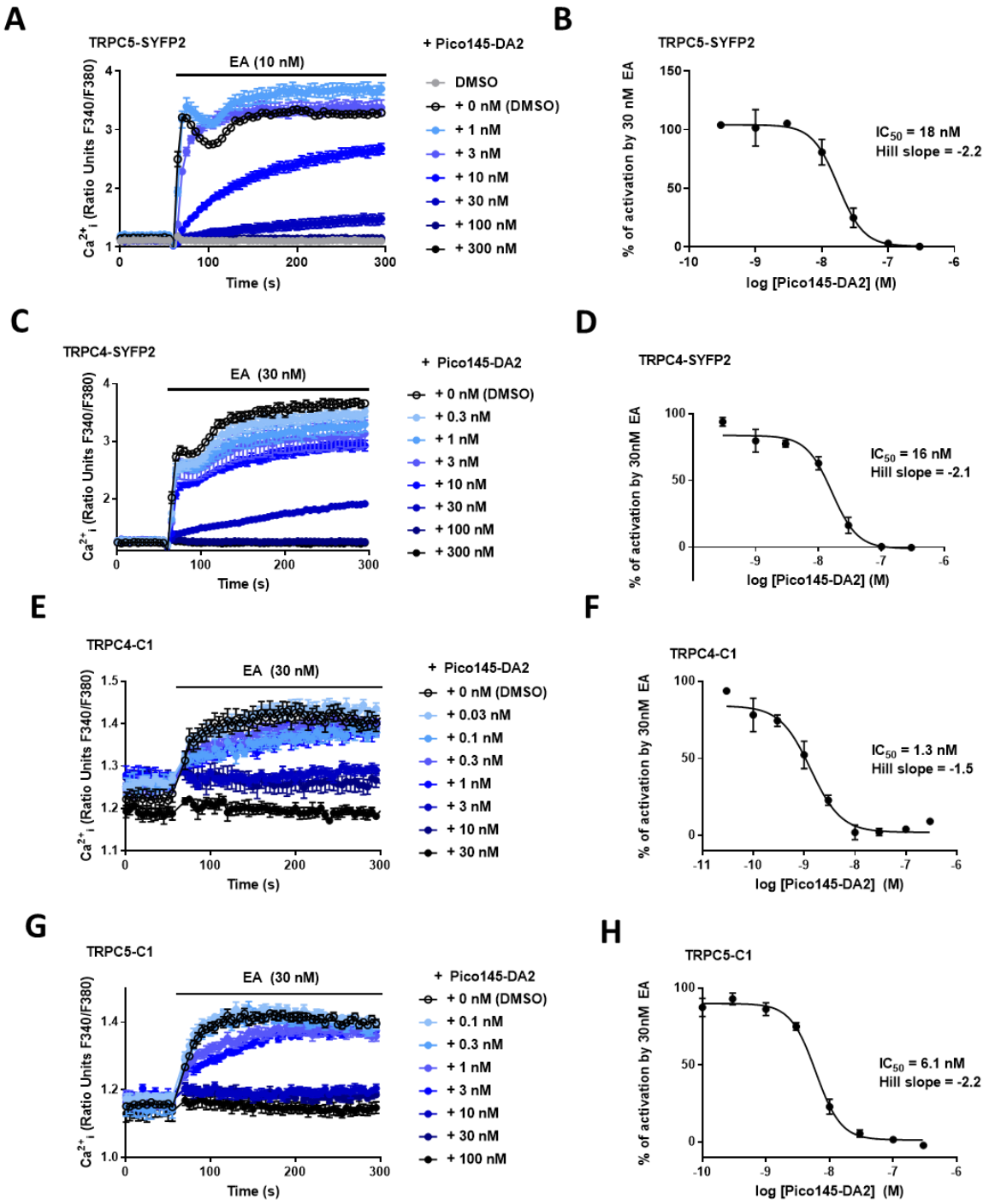
Supplementary Figure 2. Pico145-DAAlk **5** inhibits TRPC4-SYFP2, TRPC4-C1 and TRPC5-C1 channels. A,C,E) Representative traces from a single 96-well plate (N = 6) showing an inhibition of EA-mediated Ca^{2+} influx in response to 0.1-3000 nM Pico145-DAAlk **5** in HEK T-REx cells overexpressing TRPC4-SYFP2 (A), TRPC4-C1 (C) or TRPC5-C1 (E); B, D, F) Concentration-response data for experiments in (A), (C) and (E), showing mean responses ± SEM (n/N = 3/18). Responses were calculated at 150-200 s, compared to baseline at 0-55 s.



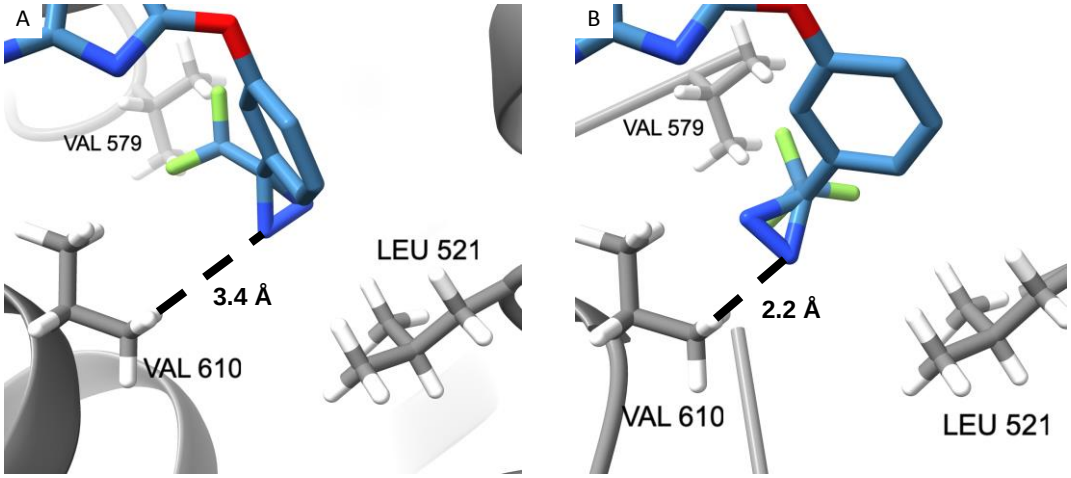
Supplementary Figure 3. Pico145-DA **4** activates TRPC5-SYFP2, and inhibits TRPC4-SYFP2, TRPC4-C1 and TRPC5-C1 channels. A) Representative traces from a single 96-well plate (N = 6) showing activation of Ca^{2+} influx by 0.1-300 nM Pico145-DA **4** in HEK T-REx cells overexpressing TRPC5-SYFP2. B) Concentration-response data for experiments in (A), showing mean responses ± SEM (n/N = 3/18). Responses were calculated at 150-200 s, compared to baseline at 0-55 s. C,E,G) Representative traces from a single 96-well plate (N = 6) showing an inhibition in EA-mediated Ca^{2+} influx in response to 0.1 to 3000 nM Pico145-DA **4**, in HEK T-REx cells overexpressing TRPC4-SYFP2 (C), TRPC4-C1 (E) or TRPC5-C1 (G); D, F, H) Concentration-response data for experiments in (A), (C) and (E), showing mean responses ± SEM (n/N = 3-4/18-24). Responses were calculated at 150-200 s, compared to baseline at 0-55 s.



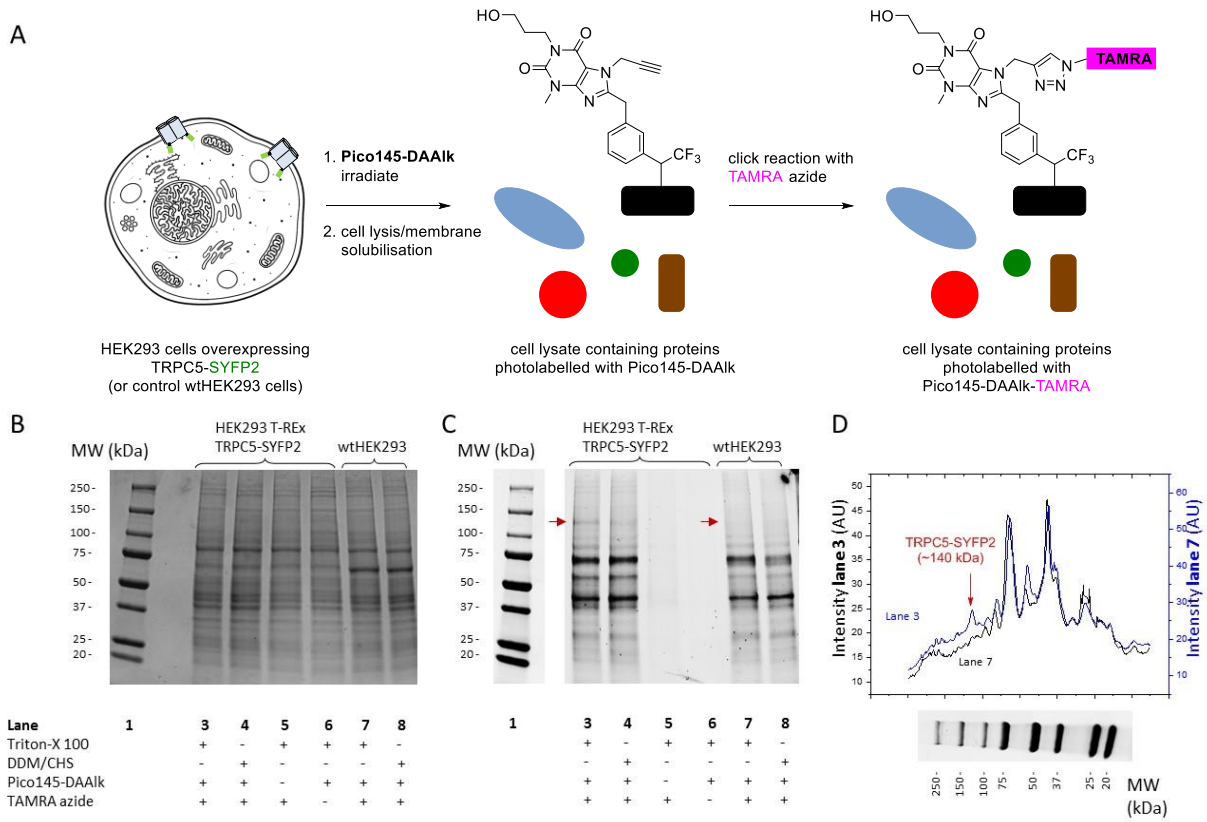
Supplementary Figure 4. Pico145-DAAlk2 **7** inhibits TRPC4-SYFP2, TRPC4-C1 and TRPC5-C1 channels. A,C,E) Representative traces from a single 96-well plate (N = 6) showing an inhibition in EA-mediated Ca^{2+} influx in response to 0.1-300 nM Pico145-DAAlk2 **7** in HEK T-REx cells overexpressing TRPC4-SYFP2 (A), TRPC4-C1 (C) or TRPC5-C1 (E); B, D, F) Concentration-response data for experiments in (A), (C) and (E) showing mean responses ± SEM (n=1-3/N=6-18). Responses were calculated at 250-295 s, compared to baseline at 0-55 s.



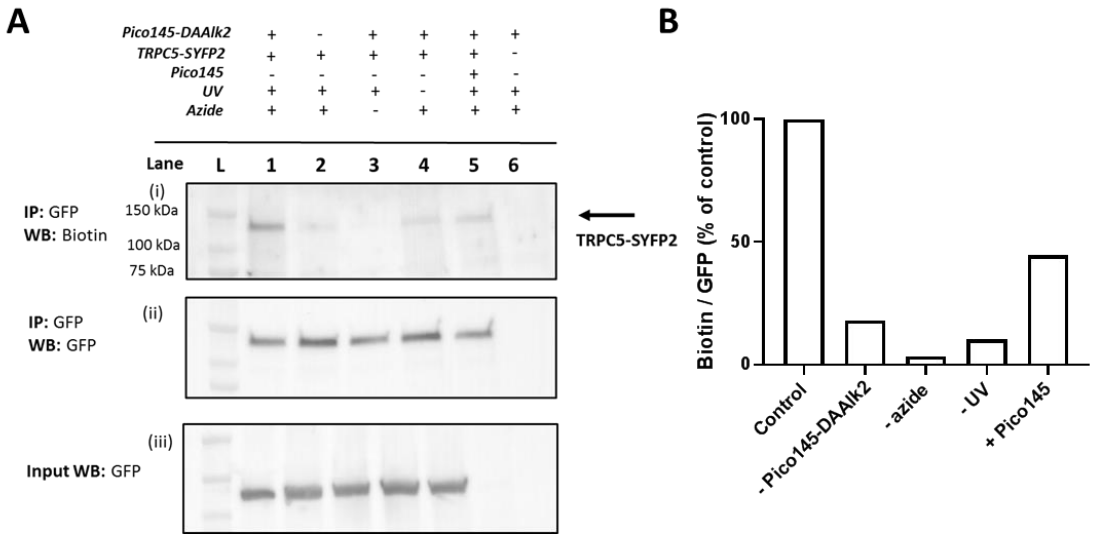
Supplementary Figure 5. Pico145-DA2 **6** inhibits TRPC5-SYFP2, TRPC4-SYFP2, TRPC4-C1 and TRPC5-C1 channels. A,C,E,G) Representative traces from a single 96-well plate (N = 6) showing an inhibition of EA-mediated Ca^{2+} influx in response to 0.1-300 nM Pico145-DA2 **6** in HEK T-REx cells overexpressing TRPC5-SYFP2 (A), TRPC4-SYFP2 (C), TRPC4-C1 (E) or TRPC5-C1 (G); B, D, F, H) Concentration-response data for experiments in (A), (C), (E) and (H) showing mean responses ± SEM (n=1-3/N=6-18). Response was calculated at 250-295 s, compared to baseline at 0-55 s.



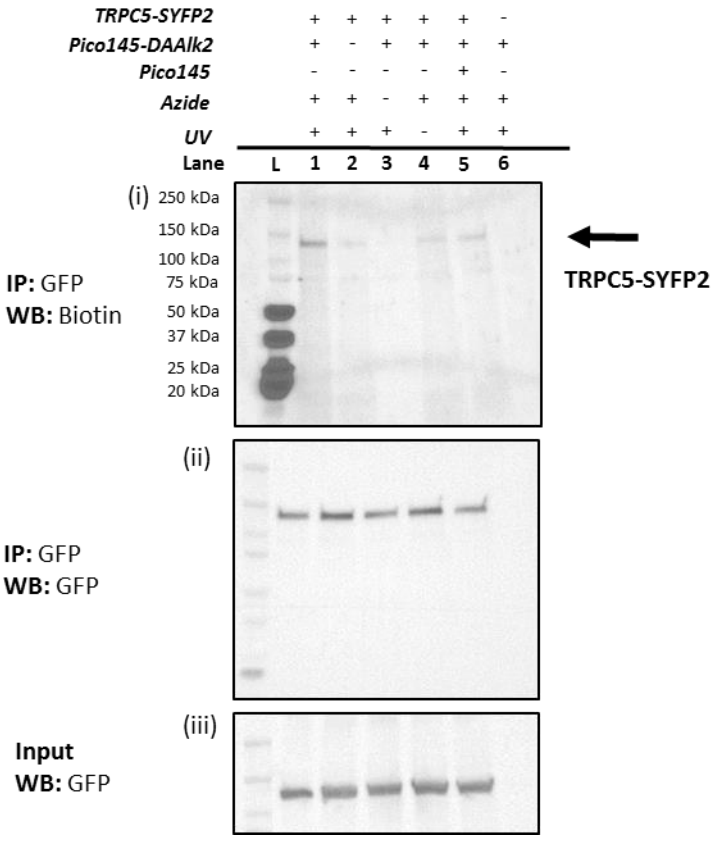
Supplementary Figure 6. Docking of Pico145-DA **4** suggest a requirement for its *C*-8 aryl substituent to twist in order to avoid clashes with nearby residues. A) Top-scoring docked pose of Pico145-DA **4** in TRPC5:C5 (PDB 6YSN; Pico145 ligand removed). The closest distance between the diazirine of **4** and V610 was measured. B) Pico145 in TRPC5:C5 (PDB 6YSN) was manually modified in the maestro GUI to give Pico145-DA. The diazirine group was minimised and the closest distance to V610 measured.



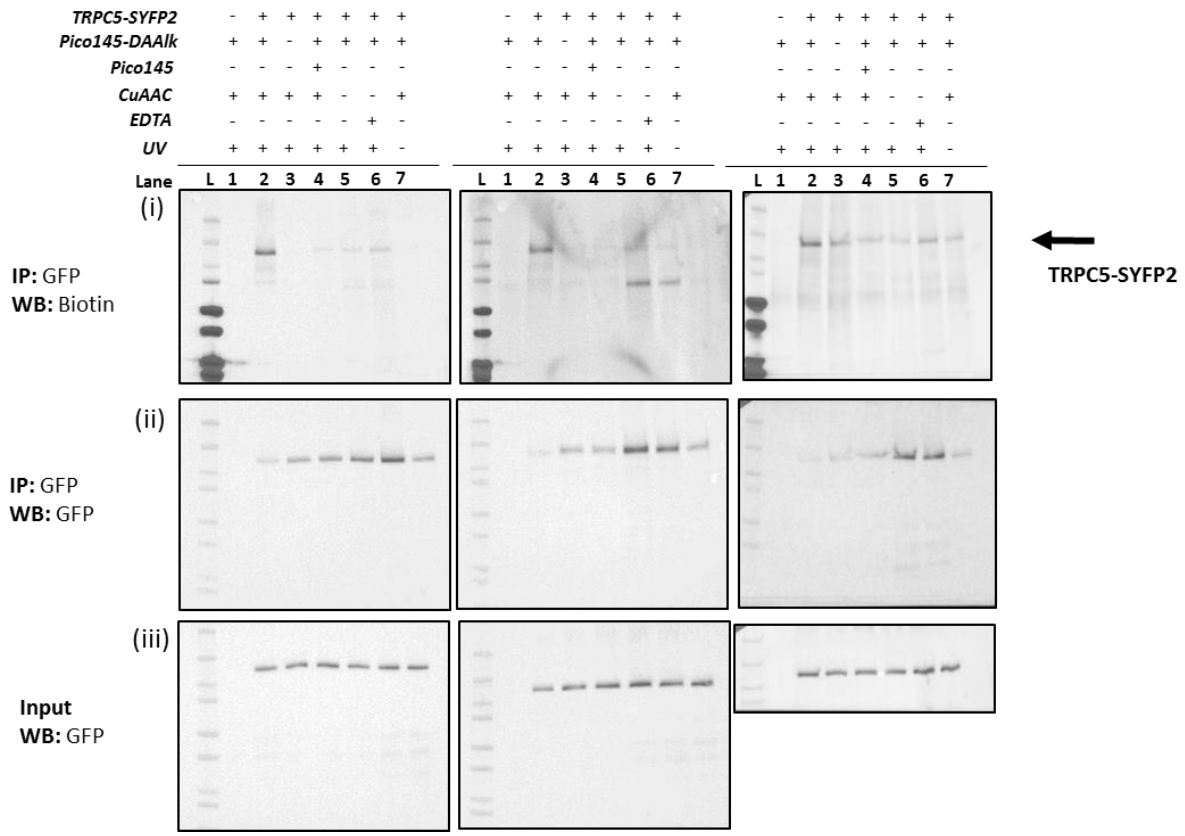
Supplementary Figure 7. Pico145-DAAlk **5** labels multiple proteins, including TRPC5-SYFP2, in HEK cells. A) HEK T-REx cells over-expressing TRPC5-SYFP2 (or wtHEK293 control cells) were incubated with Pico145-DAAlk **5** (300 nM, a large excess compared to the available TRPC5 in the HEK T-REx cells) and irradiated at 365 nm for 25 min using a UV crosslinker (Analytik Jena AG, Jena, Germany) at 0 °C for 25 minutes. Cells were washed, lysed (in lysis buffer with either Triton-X100 or DDM/CHS as detergents), and subjected to copper-catalysed alkyne-azide cycloaddition (click) reaction with TAMRA azide, resulting in TAMRA modification of proteins that had been photolabelled by Pico145-DAAlk. B) Coomassie Blue-stained SDS-PAGE gel analysis of cell lysates from PAL/click procedures. C) Fluorescent gel scan of SDS-PAGE gel shows TAMRA-labelled proteins only in samples that have been subjected to both Pico145-DAAlk/UV and click reaction with TAMRA azide (lanes 3, 4, 7 and 8, as compared to control lanes 5 and 6). The faint band at ~140 kDa is only present in TRPC5-SYFP-expressing cells (lanes 3 and 4 vs lanes 7 and 8), suggesting a direct interaction between Pico145-DAAlk and TRPC5-SYFP2. The band is slightly more intense in lane 3 than in lane 4, suggesting that Triton-X100 is better at solubilising TRPC5-SYFP2 than the milder detergents DDM/CHS. D) Quantification of fluorescent bands in lanes 3 and 7 in the SDS-PAGE gel. Fiji was used for densitometry analysis of lanes, and intensity profiles were plotted (normalised to most intense peaks). See Supplementary Methods for additional details.



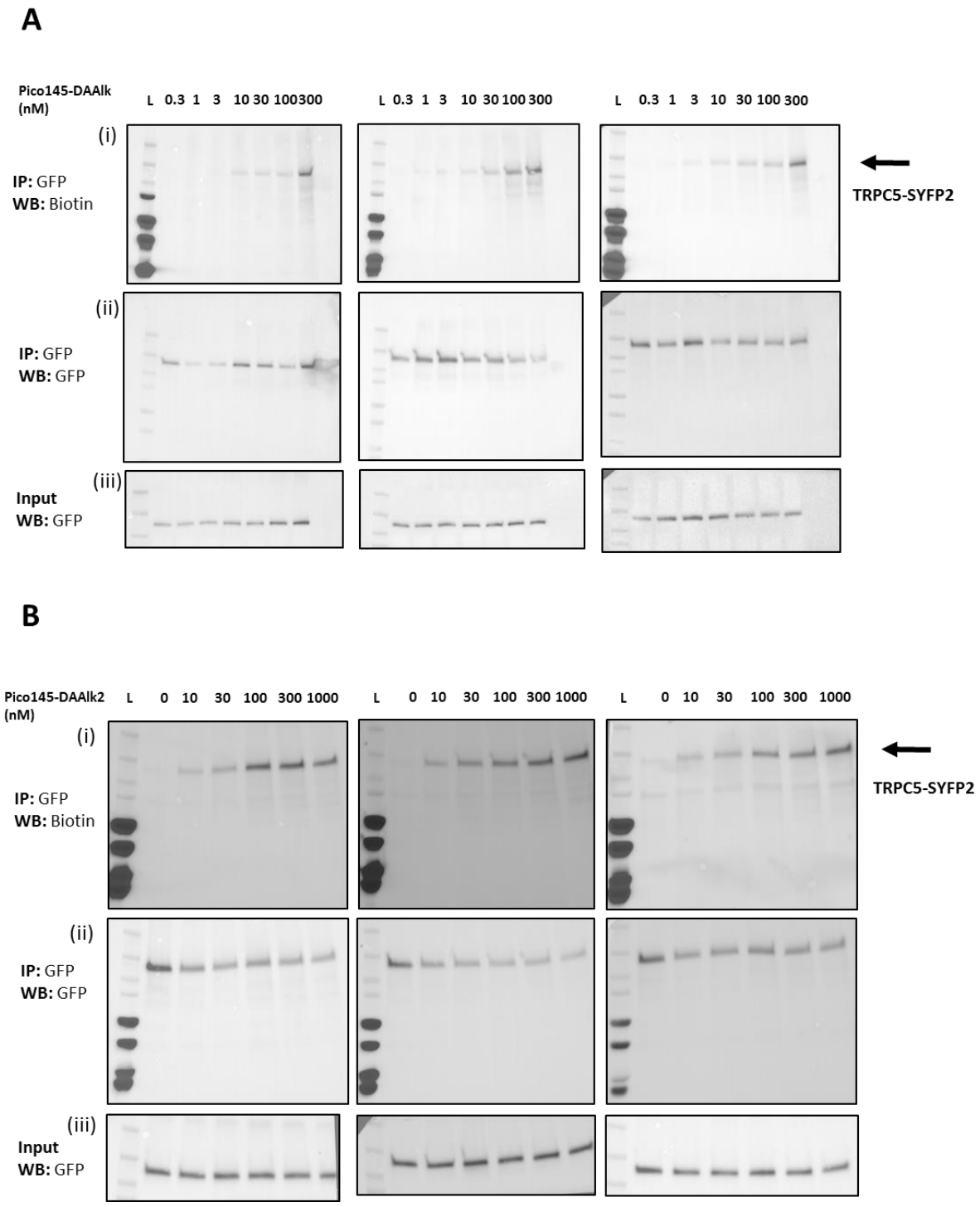
Supplementary Figure 8. Photoaffinity labelling of TRPC5 with Pico145-DAAlk2 **7**. A) Representative Western blot after experiment depicted in Figure 3, using Pico145-DAAlk **7** as the photoaffinity probe. Samples were blotted with Streptavidin-HRP (i) and anti-GFP (ii; loading control). Western blot with anti-GFP of input (pre-GFP trap) samples was performed to confirm successful TRPC5-SYFP2 expression in HEK T-REx cells (iii). The data show that photolabelling of TRPC5-SYFP2 in HEK T-REx cells (lane 1) is dependent on expression of TRPC5-SYFP2 (lane 6), treatment with Pico145-DAAlk2 **7** (300 nM, 30 min, 37 °C; lane 2), UV irradiation (365 nm, home-built LED photocrosslinker, 1 min; lane 4), and TAMRA-biotin azide (lane 3). In addition, Pico145 (100 nM) partially inhibits photoaffinity labelling with Pico145-DAAlk2, **7** (lane 5). Lane L indicates protein molecular weight ladder. B) Densitometry analysis of data presented in (A).



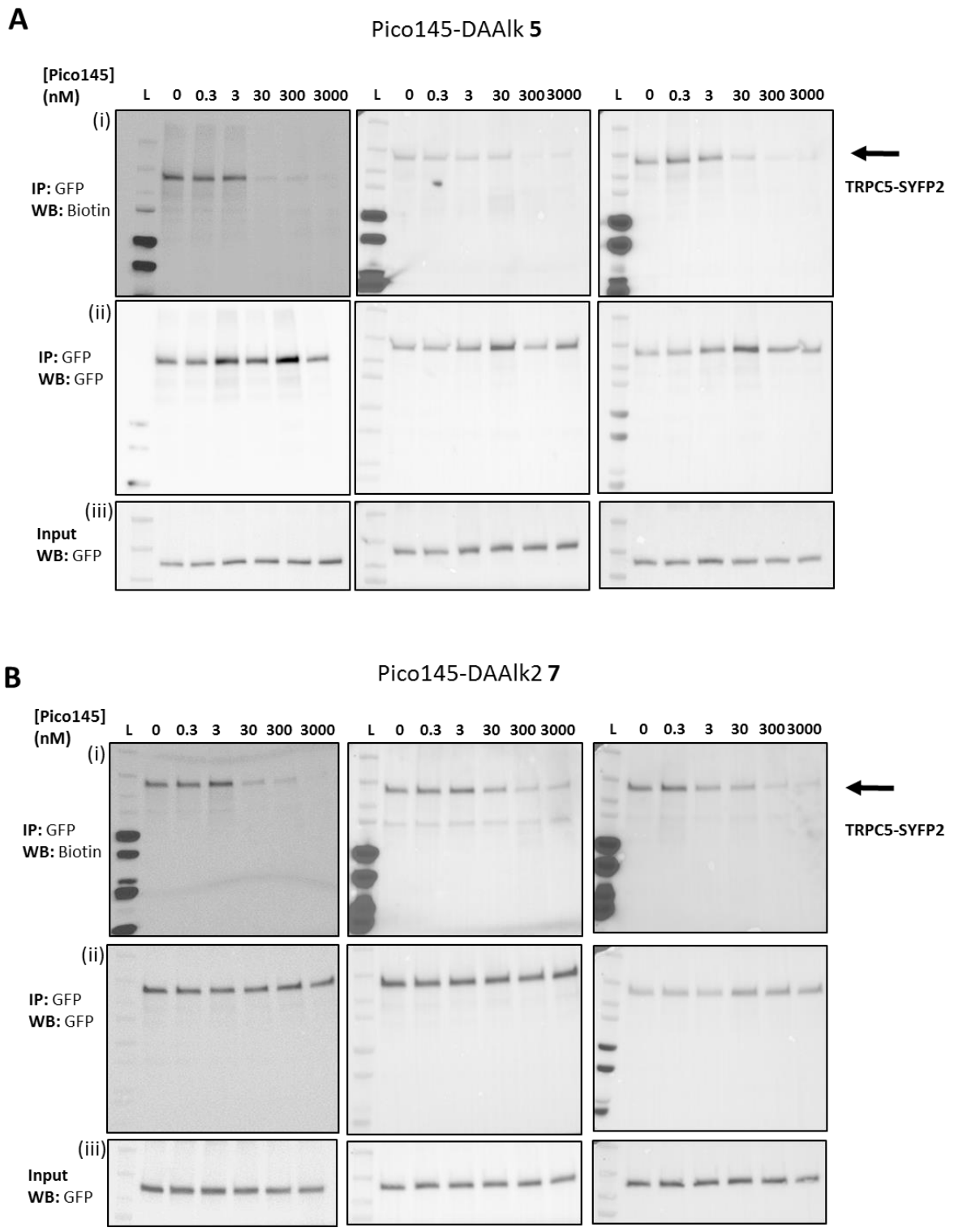
Supplementary Figure 9. Full Western blots for experiments shown in Supplementary Figure 6.



Supplementary Figure 10. Full Western blots for experiments shown in Figure 4.



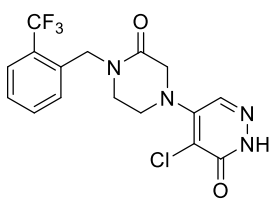
Supplementary Figure 11. Full Western blots for experiments shown in Figure 5. A) data for Pico145-DAAlk **5**; B) data for Pico145-DAAlk **7**.



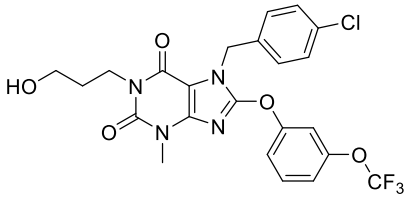
Supplementary Figure 12. Full western blots for experiments in Figure 6. A) data for Pico145-DAAlk **5**; B) data for Pico145-DAAlk **7**.

Supplementary Tables

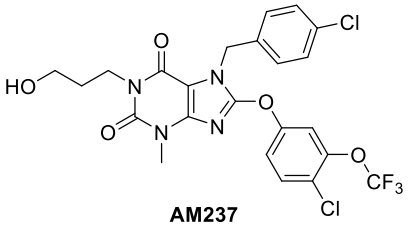
Supplementary Table 1. Calculated solubilities of TRPC5 modulators and photoaffinity probes.



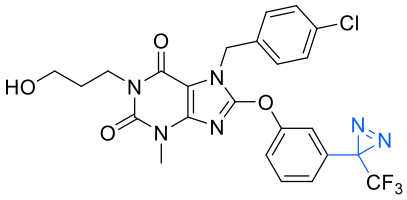
GFB-8438



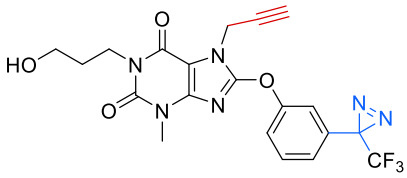
Pico145



AM237



Pico145-DA



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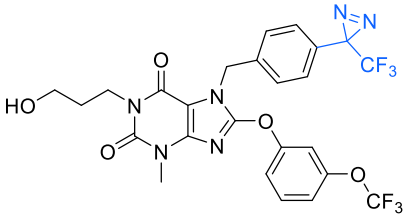
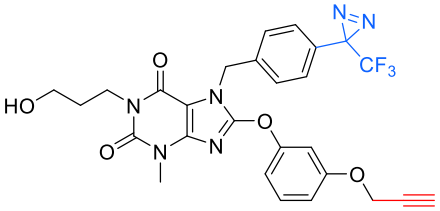


Pico145-DA2



Pico145-DAAlk2

compound ID	GFB-8438	Pico145	AM237	Pico145-DA	Pico145-DAAlk	Pico145-DA-2	Pico145-DAAlk-2
cLogS[a]	-3.737	-7.216	-7.743	-7.051	-5.237	-7.839	-7.265
cLogP[b]	2.072	5.454	5.834	4.630	3.054	5.147	4.719

[a] cLog S = log10 of calculated aqueous solubility in mol/L; [b] cLogP = log10 of calculated partition coefficient between n-octanol and water. Calculations were performed using QikProp v6.2 (rel 12) (Schrödinger) as described in the Methods.

Supplementary Methods

Initial photoaffinity labelling experiment (Supplementary Figure 7)

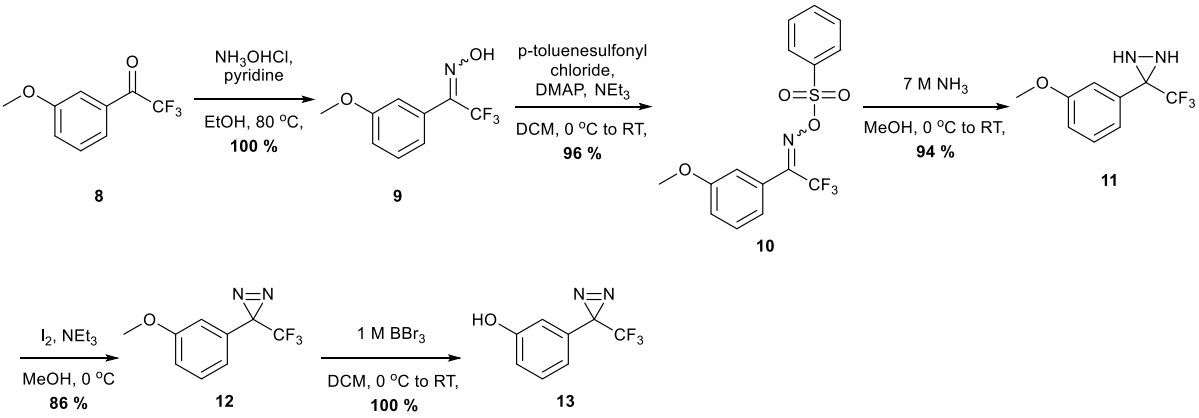
HEK-293 T-REx cells expressing inducible TRPC5-SYFP2 were plated onto poly-D-lysine coated 6-well plates (CorningTM CostarTM) and 1 μg·ml^{-1} tetracycline (tet+) was applied 18 h prior to experimentation to induce TRPC5-SYFP2 expression. WT HEK293 cells were used as a control for no expression of TRPC5. To treat cells for photoaffinity labelling, medium was removed and cells were washed once in DPBS. SBS containing 300 nM Pico145-DAAlk **5** or DMSO was then added to the cells, and cells were incubated at 37 °C for 5 min. Plates were placed on ice and irradiated at 365 nm for 25 minutes (UVP-Crosslinker cl-1000). Cells were washed with DPBS and lysed with Triton-X100 (1 x PBS, 0.1% SDS, 1% TritonX-100, 1 EDTA-free protease inhibitor cocktail (Thermo Scientific)) or DDM-CHS (1% DDM, 0.1% CHS, 100 nM NaCl, 20 mM Tris pH 7.5, EDTA free protease inhibitor cocktail) lysis buffer. The cells were transferred with gentle scraping to an Eppendorf and incubated in the lysis buffer at 4 °C for 1 h under constant mixing. Cell lysates were centrifuged at 10,000 ×g for 10 min at 4 °C in order to pellet large debris. The supernatant was transferred to a fresh tube and protein concentration measured using DC protein quantification. Lysate concentration was adjusted to 1 mg·ml^{-1}. CuAAC reagents were premixed in the following order and added to the lysate (50 μg) at the indicated final concentrations: 100 μM Fluor-Azide-545 (Sigma-Aldrich), 1 mM $CuSO_4$, 1 mM TCEP and 100 μM TBTA. Samples were incubated for 1 h with gentle agitation, at room temperature. Following CuAAC, EDTA was added to a final concentration of 10 mM and proteins were precipitated with ice-cold acetone (4 volumes) at -20 °C overnight, centrifuged at 16,000 ×g, 10 min, 4 °C and the pellet washed twice with ice-cold MeOH. Proteins were resuspended in equal volumes of 2% SDS in PBS and 2× SDS sample loading buffer (SLB: 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue and 0.125 M Tris HCl, pH 6.8) by vortexing. The lysates were separated by SDS-PAGE gel using 4-20% pre-cast gels (Mini-PROTEAN TGX, Bio-Rad) and Precision Plus Protein™ All Blue Prestained Protein Standards (Bio-Rad) were used as a molecular weight marker. Fluorescently modified proteins were analysed using a Molecular Imager ChemiDoc XRS System (Bio-Rad). After obtaining the fluorescence image, the same gel was stained with Coomassie Brilliant Blue (CBB, Bio-Rad) and visualised on a Molecular Imager ChemiDoc XRS System. Densitometric quantification of the fluorescencent bands was performed using Fiji.[2] Intensity profiles were normalised to most intense peaks before plotting).

General synthetic methods

All chemical reagents were purchased from commercial suppliers and used without further purification. Anhydrous solvents and liquid reagents were purchased in Sure/SealTM bottles. Flash column chromatography was carried out using silica (Merck Geduran silica gel, 35-70 μm particles). Thin layer chromatography was carried out on commercially available pre-coated aluminium plates (Merck silica 2 8 8 0 Kieselgel 60 F254). Analytical HPLC was performed on an Agilent 1290 Infinity Series equipped with a UV detector (set at 254 nm) and a Hyperclone C18 reverse phase column using MeCN/water (5→95% or 50→95%) containing 0.1% formic acid, at 0.5 mL min^{-1} over a period of five minutes. High resolution electrospray

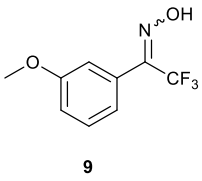
(ESI+) mass spectrometry was performed on a Bruker MaXis Impact QTOF mass spectrometer, and *m/z* values are reported in Daltons to four decimal places. ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded in deuterated solvents on a Bruker Avance 500 or Bruker Avance 400. Chemical shifts for ^{1}H and ^{13}C NMR are quoted in parts per million downfield of tetramethylsilane and referenced to residual solvent peaks ($CDCl_3$: ^{1}H = 7.26 ppm, ^{13}C = 77.16 ppm) and coupling constants (*J*) are reported to the nearest 0.1 Hz. The following abbreviations are used: s, singlet; d, doublet; t, triplet, q, quartet; m, multiplet. Chemical shifts for ^{19}F NMR are referenced to an external standard of trichloroflouromethane at 0 ppm.

Synthesis of diazirine building block 13



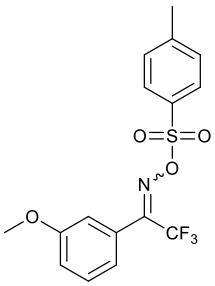
Supplementary Figure 13. Synthetic route to diazirine building block **13**.

N-[2,2,2-trifluoro-1-(3-methoxyphenyl)ethylidene]hydroxylamine 9[3]



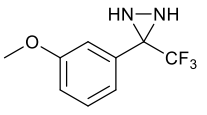
Hydroxylamine hydrochloride (340 mg, 4.90 mmol) was added to a stirred solution of 3'-methoxy-2,2,2-trifluoroacetophenone **8** (500 mg, 2.45 mmol) in anhydrous EtOH (8 mL) and anhydrous pyridine (2 mL). The reaction mixture was stirred while heating under reflux for 18 h, after which the mixture was allowed to cool to room temperature and the solvents were evaporated *in vacuo*. The residue was dissolved in Et_2O (30 mL), washed with water (2 × 20 mL), dried ($MgSO_4$) and concentrated *in vacuo,* to yield a pale orange oil (537 mg, quantitative). δH (500 MHz, $CDCl_3$) 7.39 (app. t, *J* 8.0 Hz, 1H), 7.10 (ddd, *J* 8.0, 1.9, 0.8, 1H), 7.07 (app. t, *J* 1.9, 1H), 7.00 (ddd, *J* 8.0, 1.9, 0.8, 1H), 3.83 (s, 3H); δC (125 MHz, $CDCl_3$) 159.6, 129.6, 128.6, 121.2 (q, *J* 274.3, CF_3), 120.9, 115.9, 114.3, 55.3, *C*=N-OH not observed; δF (235 MHz, $CDCl_3$) -66.5. Data consistent with literature.[3]

[2,2,2-trifluoro-1-(3-methoxyphenyl)ethylidene]amino 4-methylbenzene- 1-sulfonate 10[3] *p*-Toluenesulfonyl chloride (3.83 g, 20.1 mmol) was added to a solution of N-[2,2,2- trifluoro-1-(3-methoxyphenyl)ethylidene]hydroxylamine **9** (3.00 g, 14.7 mmol), DMAP (118 mg, 0.97 mmol) and anhydrous triethylamine (2.25 mL, 22.1 mmol) in DCM (70 mL) at 0 °C. The mixture was stirred for 3 h, while it was allowed to warm to room temperature, and then stirred for a further 16 h. The reaction mixture was washed with water (3 × 100 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude material was purified by column chromatography (SiO_2; hexane-DCM 7:3) to yield the product as a pale yellow solid (5.02 g, 96%). R_f 0.2 (3:7 DCM:hexane); δH (400 MHz, $CDCl_3$) 7.81 (d, *J* 8.3 Hz, 2H), 7.35-7.26 (m, 3H), 6.98 (dd, *J* 8.3, 2.0, 1H), 6.83 (app. d, *J* 8.3, 1H), 6.81 (t, *J* 2.0, 1H), 3.75 (s, 3H), 2.41 (s, 3H); δC (100 MHz, $CDCl_3$) 159.7, 154.1 (app. d, *J* 33.7), 146.3, 131.4, 130.2, 130.0, 129.4, 125.8, 120.7, 119.0 (q, *J* 277.7, CF_3), 117.4, 114.1, 55.6, 22.0; δF (235 MHz, $CDCl_3$) -66.9; ESI-HRMS: calcd. for $C_{16}H_{14}F_3NO_4SNa$ $[M+Na]^+$ 396.0488, found 396.0482. Data consistent with literature.[3]



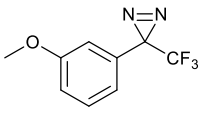
10

3-(3-methoxyphenyl)-3-(trifluoromethyl)diaziridine 11[4] [2,2,2-trifluoro-1-(3-methoxyphenyl)ethylidene]amino-4-methylbenzene-1-sulfonate **10** (1.60 g, 4.55 mmol) was dissolved in a 7 M solution of ammonia in methanol (15 mL) at 0 °C under a gaseous ammonia atmosphere. The reaction mixture was stirred vigorously to dissolve **10**, whilst bubbling ammonia through the solution. The reaction mixture was allowed to warm to room temperature after 6 h and stirred for a further 16 h at room temperature. The reaction was left open to allow the ammonia to evaporate, aqueous sodium bicarbonate (sat., 2 mL) was added to the mixture and a white precipitate formed. The white precipitate was filtered off and the crude product was extracted with DCM (30 mL) and concentrated *in vacuo*. The crude material was purified by column chromatography (SiO_2; hexane-EtOAc 85:15) to give the title compound as a colourless oil (860 mg, 94%). R_f 0.4 (DCM-hexane 9:1); δH (400 MHz, $CDCl_3$) 7.34 (app. t, *J* 8.0 Hz, 1H), 7.20 (ddd, *J* 8.0, 2.1, 0.9, 1H), 7.15 (app. t, *J* 2.1, 1H), 6.98 (ddd, *J* 8.0, 2.1, 0.9, 1H), 3.83 (s, 3H), 2.79 (d, *J* 8.8, 1H), 2.26 (d, *J* 8.8, 1H); δC (100 MHz, $CDCl_3$) 159.7, 133.0, 129.9, 123.5 (q, *J* 278.4, CF_3), 120.3, 115.8, 113.6, 58.0 (q, *J* 36.1, C-diaziridine), 55.4; δF (235 MHz, $CDCl_3$) -75.5; ESI-HRMS: calcd. for $C_9H_{10}F_3N_2O$ $[M+H]^+$ 219.0740, found 219.0675. Data consistent with literature.[4]



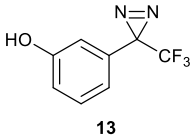
11

3-(3-methoxyphenyl)-3-(trifluoromethyl)-3H-diazirine 12[5] 3-(3-methoxyphenyl)-3-(trifluoromethyl)diaziridine **11** (1.60 g, 7.30 mmol) was dissolved in MeOH (10 mL) and stirred at 0 °C for 5 min. Triethylamine (1.50 mL, 11.0 mmol) was added to the reaction mixture and stirring continued for 5 min. Small portions of iodine (2.22 g in total, 8.76 mmol) were added until a deep red colour persisted. EtOAc (5 mL) was added to the solution and the mixture was washed with 1 M HCl (15 mL), aqueous sodium thiosulfate (10% w/w, 3 × 10 mL or until solution turned colourless) and brine (15 mL), dried (Na_2SO_4) and concentrated *in vacuo* to yield the title compound as a pale yellow oil (1.35 g, 86 %). R_f 0.1 (hexane-EtOAc 9:1); δH (500 MHz, $CDCl_3$)



12

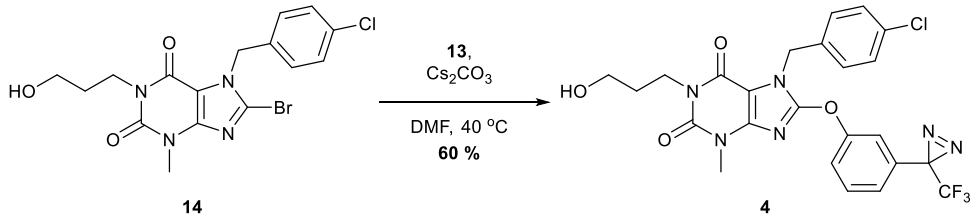
7.31 (app. t, *J* 8.1 Hz, 1H) 6.95 (dd, *J* 8.1, 2.1, 1H), 6.78 (app d, *J* 8.1, 1H), 6.70 (d, *J* 2.1, 1H), 3.81 (s, 3H); δC (125 MHz, $CDCl_3$) 159.9, 130.7, 129.9, 122.5 (q, *J* 274.6, CF_3), 118.9, 115.4, 112.4, 55.5, 28.6 (q, *J* 36.1, C-diazirine); δF (235 MHz, $CDCl_3$) -65.2; ESI-HRMS: calcd. for $C_9H_8F_3N_2O$ $[M+H]^+$ 217.0583, found 217.0599. Data consistent with literature.[5]



13

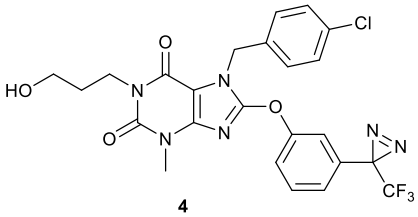
3-[3-(trifluoromethyl)-3H-diazirin-3-yl]phenol 13[6] BBr_3 (1.0 M in DCM, 7.40 mL, 7.40 mmol) was added dropwise to a solution of 3-(3-methoxyphenyl)-3-(trifluoromethyl)-3H-diazirine **12** (0.82 g, 3.70 mmol) in DCM (10 mL) over 5 min at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, then allowed to warm to room temperature and stirred for a further 12 h. Water (15 mL) was added to the mixture and the organic compounds were extracted with diethyl ether (3 × 20 mL). The combined organic layers were washed with water (3 × 20 mL) and brine (20 mL), and the ethereal layer was dried (Na_2SO_4) and concentrated *in vacuo* to yield the title compound as a brown oil (754 mg, quantitative). R_f 0.2 (hexane-EtOAc 9:1); δH (500 MHz, $CDCl_3$) 7.26 (t, *J* 8.0 Hz, 1H), 6.88 (dd, *J* 8.0, 2.1, 1H), 6.73 (dd, *J* 8.0, 2.1, 1H), 6.67 (t, *J* 2.1, 1H); δC (125 MHz, $CDCl_3$) δ 155.9, 131.0, 130.4, 122.2 (d, *J* 274.9, CF_3), 120.8, 118.1, 113.7, 28.4 (q, *J* 40.4, C-diazirine); δF (235 MHz, $CDCl_3$) -71.6; ESI-HRMS could not be obtained. Data consistent with literature.[6]

Synthesis of Pico145-DA 4



14 4

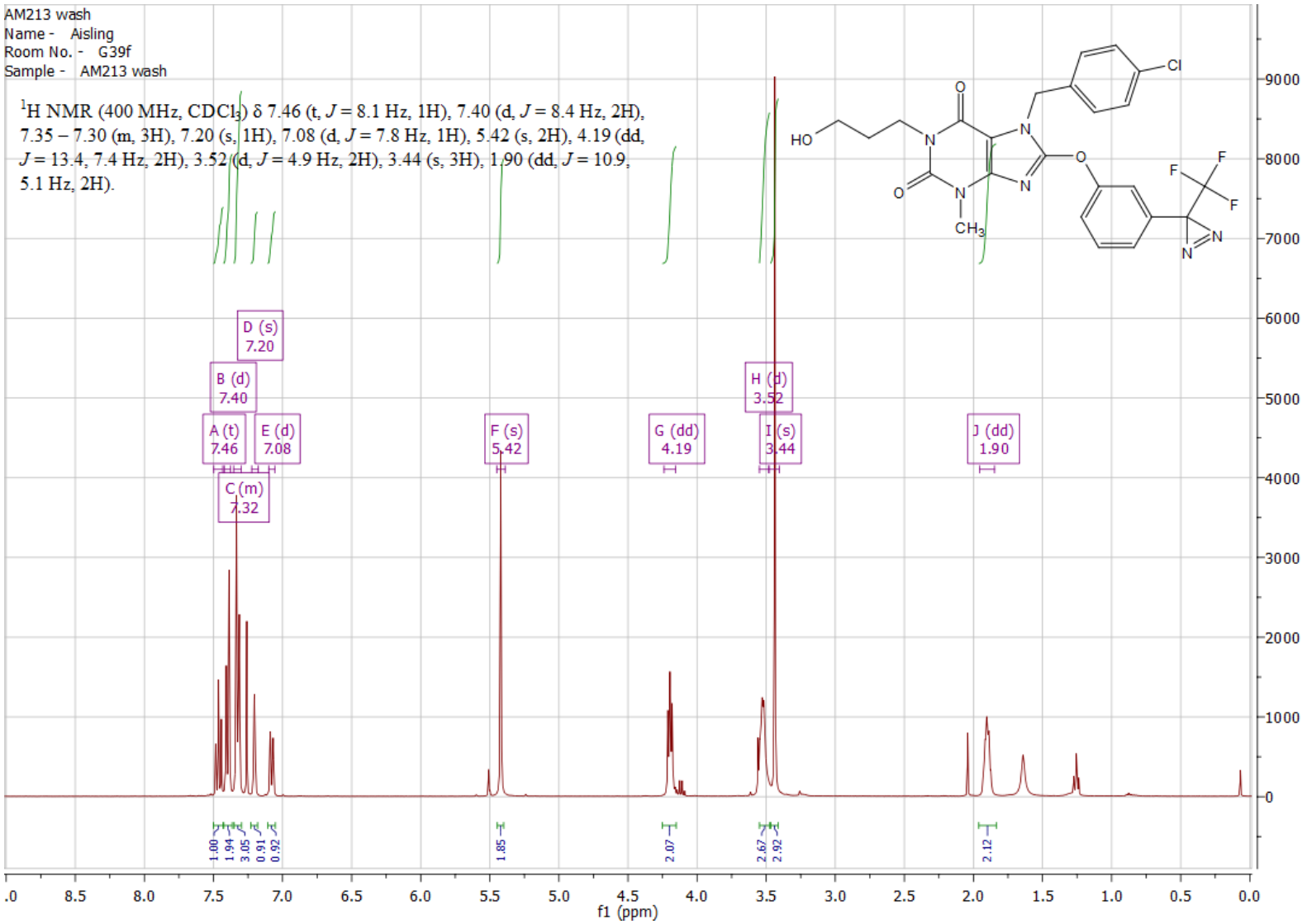
Supplementary Figure 14. Synthetic route to photoaffinity probe, Pico145-DA, **4**.



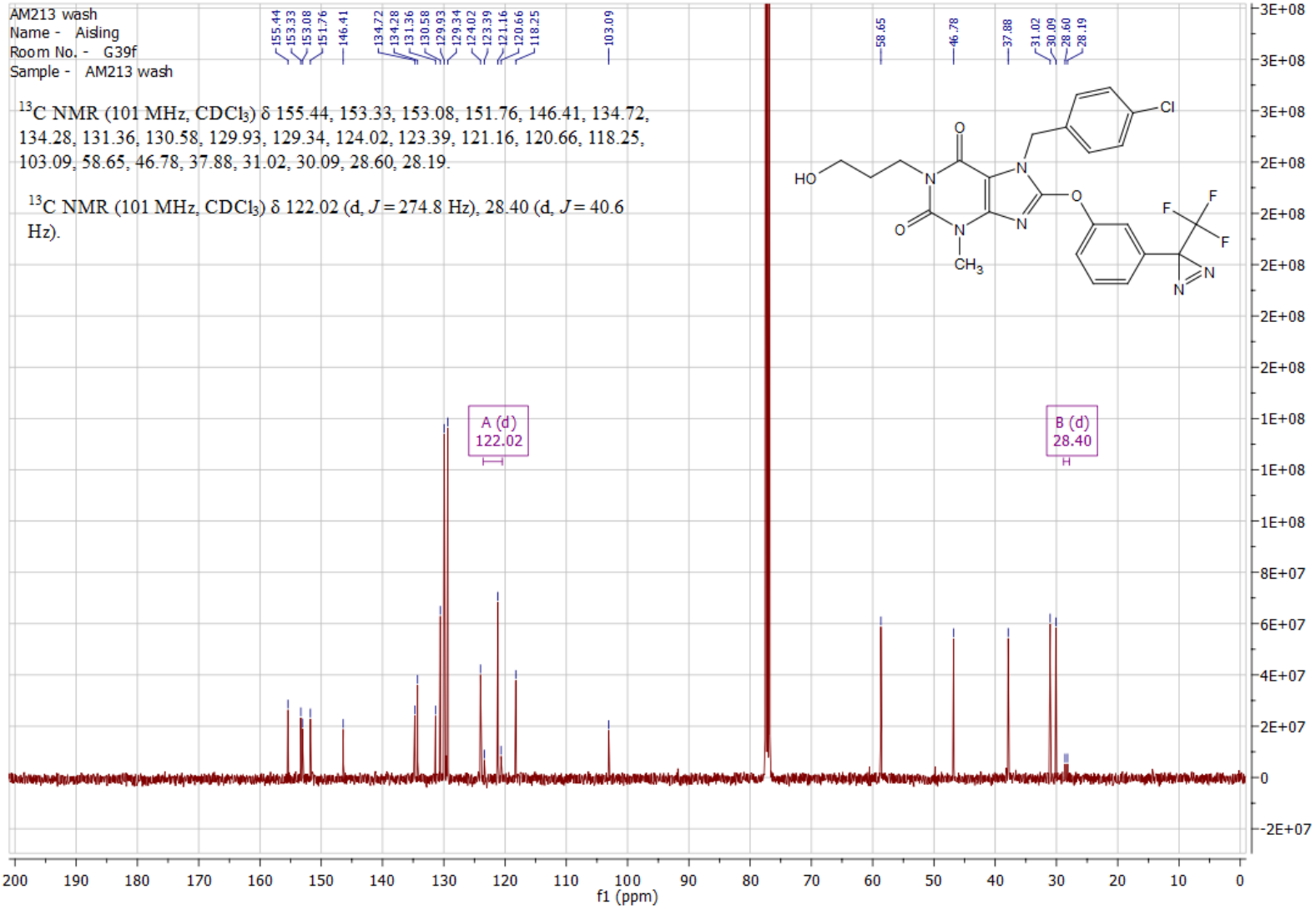
4

7-[(4-chlorophenyl)methyl]-1-(3-hydroxypropyl)-3-methyl-8-{3-[3-(trifluoromethyl)-3H-diazirin-3-yl]phenoxy}-2,3,6,7-tetrahydro-1H-purine-2,6-dione (Pico145-DA) 4 Cs_2CO_3 (286 mg, 0.81 mmol) was added to a solution of 8-bromo-7-[(4-chlorophenyl)methyl]-1-(3-hydroxypropyl)-3-methyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione, **14** (prepared as described in Rubaiy et al., 2017)[7] (289 mg, 0.67 mmol) in DMF (6 mL). After 5 min, 3-[3-(trifluoromethyl)-3H-diazirin-3-yl]phenol **13** (150 mg, 0.74 mmol) was added to the reaction mixture, and the reaction mixture was heated to 40 °C for 1 h. The mixture was cooled, partitioned between EtOAc (10 mL) and water (10 mL). The organics were washed with aqueous LiCl (1 N, 3 × 10 mL), dried (Na_2SO_4) and concentrated *in vacuo* to yield the title compound (220 mg, 60 %). R_f 0.2 (7:3 EtOAc:hexane); δH (400 MHz, $CDCl_3$) 7.46 (t, *J* 8.1 Hz, 1H), 7.40 (d, *J* 8.4, 2H), 7.35 – 7.30 (m, 3H), 7.20 (s, 1H), 7.08 (d, *J* 7.8, 1H), 5.42 (s, 2H), 4.19 (dd, *J* 13.4, 7.4, 2H), 3.52 (d, *J* 4.9, 2H), 3.44 (s, 3H), 1.90 (dd, *J* 10.9, 5.1, 2H); δC (101 MHz, $CDCl_3$) 155.4, 153.3, 153.1, 151.8, 146.4, 134.7, 134.3, 131.4, 130.6, 129.9, 129.3, 124.0, 122.0 (q, *J* 274.8, CF_3), 121.2, 118.3, 103.1, 58.7, 46.8, 37.9, 31.0, 30.1, 28.4 (q, *J* 40.6, C-

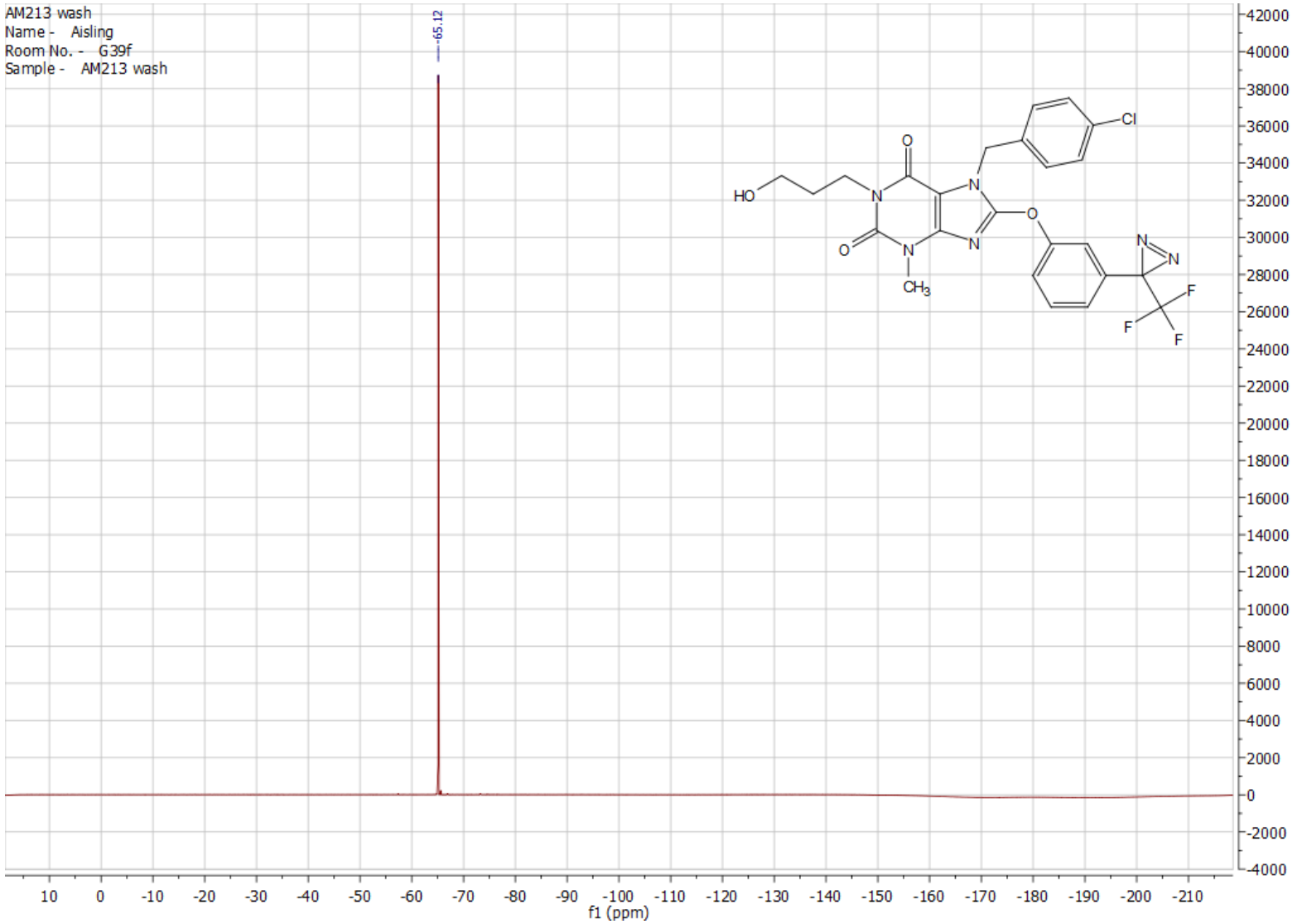
diazirine); δF (235 MHz, $CDCl_3$) -65.1; ESI-HRMS: calcd. for $C_{24}H_{21}ClF_3N_6O_4$ $[M+H]^+$ 549.1259, found 549.1266; HPLC: RT = 1.93 min.



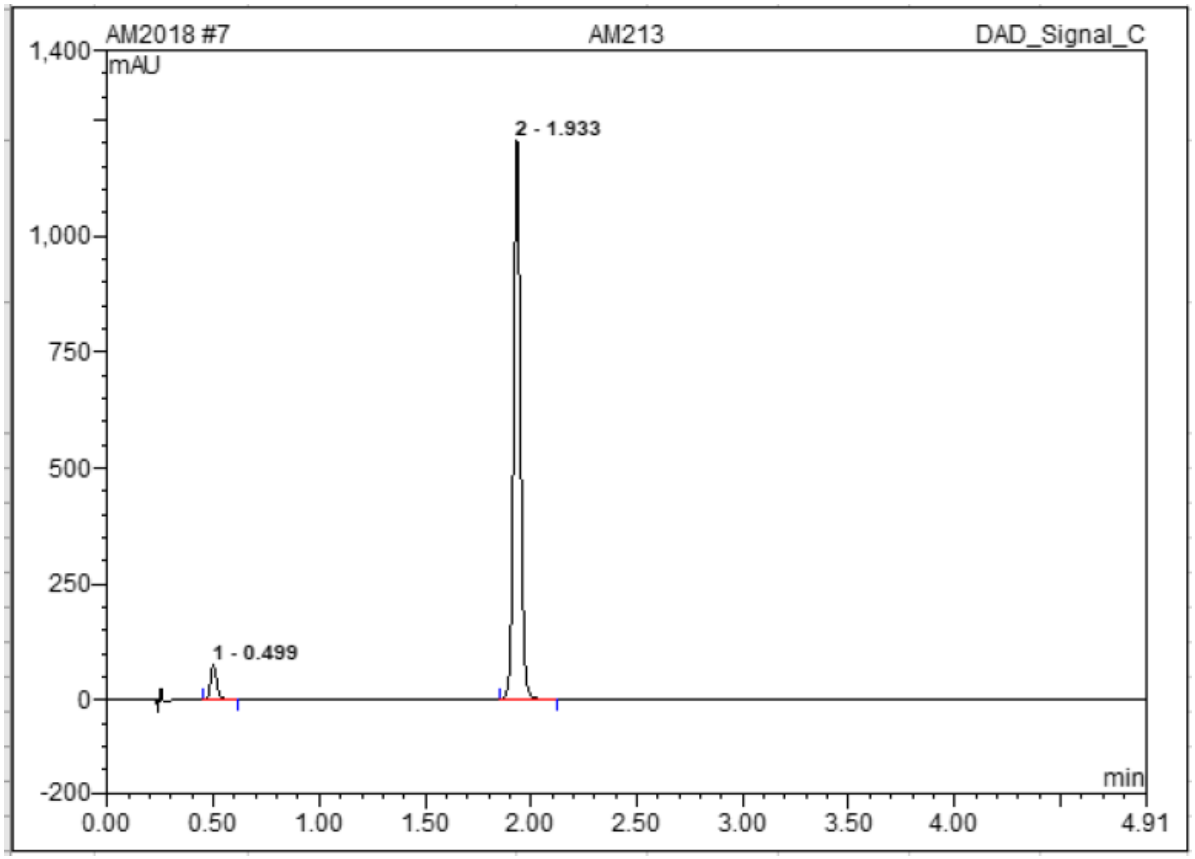
Supplementary Figure 15. ^{1}H NMR spectrum of Pico145-DA **4**.



Supplementary Figure 16. ^{13}C NMR spectrum of Pico145-DA **4**.

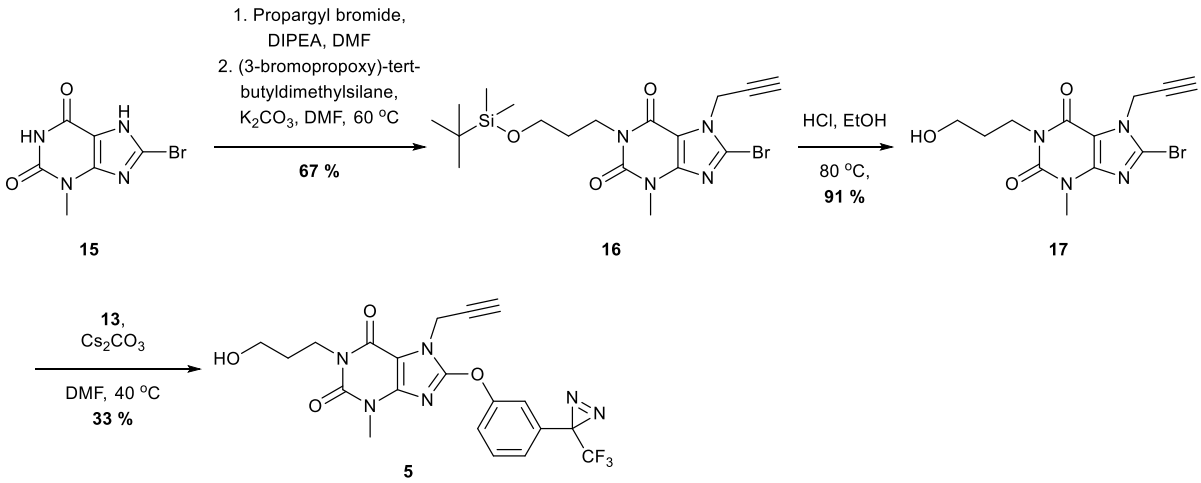


Supplementary Figure 17. ^{19}F NMR spectrum of Pico145-DA **4**.

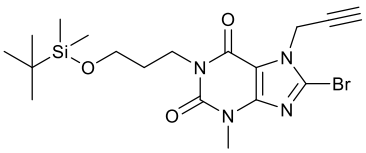


Supplementary Figure 18. HPLC chromatogram of Pico145-DA **4** (MeCN/water 50-95%).

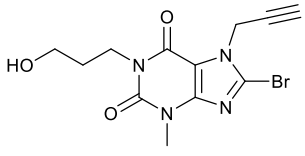
Synthesis of Pico145-DAAlk 5



Supplementary Figure 19. Synthetic route to Pico145-DAAlk **5**.


16

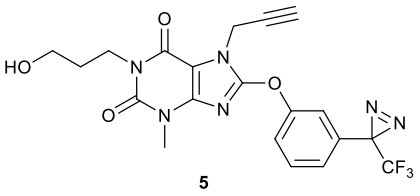
8-bromo-1-{3-[(tert-butyldimethylsilyl)oxy]propyl}-3-methyl-7-(prop-2-yn-1-yl)-2,3,6,7-tetrahydro-1H-purine-2,6-dione 16 Propargyl bromide (80% in toluene, 1.36 mL, 12.2 mmol) was added to a solution of 8-bromo-3-methyl-1H-purine-2,6(3H,7H)-dione, **15**, (3 g, 12.2 mmol) and N,N-diisopropylethylamine (2.14 mL, 12.2 mmol) in DMF (25 mL) and the reaction mixture was stirred for 16 h. Water (30 mL) was added to the reaction mixture and the crude product was isolated by filtration, washed with water (10 mL), ethanol (10 mL) and ether (10 mL). The crude product was carried forward without purification. All crude material was dissolved in DMF (30 mL), K_2CO_3 (1.6 g, 12.2 mmol) and (3-bromopropoxy)-tert-butyldimethylsilane (2.97 g, 12.2 mmol) were added, and the mixture was stirred at 60 °C for 16 h. The reaction mixture was poured into water (60 mL) and the organics were extracted with EtOAc (4 × 100 mL), washed with aqueous LiCl (1 N, 2 × 50 mL), brine (50 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The crude material was purified by column chromatography (SiO_2; hexane-EtOAc 95:5 →1:1) to yield the title compound as a white solid (3.8 g, 67 %). R_f 0.3 (7:3 hexane:EtOAc); δH (400 MHz, $CDCl_3$) 5.15 (d, *J* 2.5 Hz, 2H), 4.07 (t, *J* 7.2, 2H), 3.69 (t, *J* 6.2, 2H), 3.51 (s, 3H), 2.38 (t, *J* 2.5, 1H), 1.85 (tt, *J* 7.2, 6.2, 2H), 0.84 (s, 9H), 0.00 (s, 6H); δC (100 MHz, $CDCl_3$) 154.1, 151.0, 148.2, 127.4, 108.5, 75.7, 74.3, 61.3, 39.4, 36.5, 31.0, 29.8, 25.9, 18.3, 0.0; ESI-HRMS: calcd. for $C_{18}H_{27}BrN_4O_3SiNa$ $[M+Na]^+$ 477.0928, found 477.0920.


17

8-bromo-1-(3-hydroxypropyl)-3-methyl-7-(prop-2-yn-1-yl)-2,3,6,7- tetrahydro-1H-pur ine-2,6-dione 17 8-bromo-1-{3-[(tert-butyldimethylsilyl)oxy]propyl}-3-methyl-7-(prop-2-yn-1-yl)-2,3,6,7-tetrahydro-1H-purine-2,6-dione **16**, (1.0 g, 2.2 mmol) and aqueous HCl (conc., 4 mL) were suspended in EtOH (20 mL) and heated under reflux for 1.5 h. The reaction mixture was concentrated *in vacuo* and the crude material was triturated (Et_2O, 20 mL), filtered and washed with Et_2O (2 × 5 mL), and then concentrated *in*

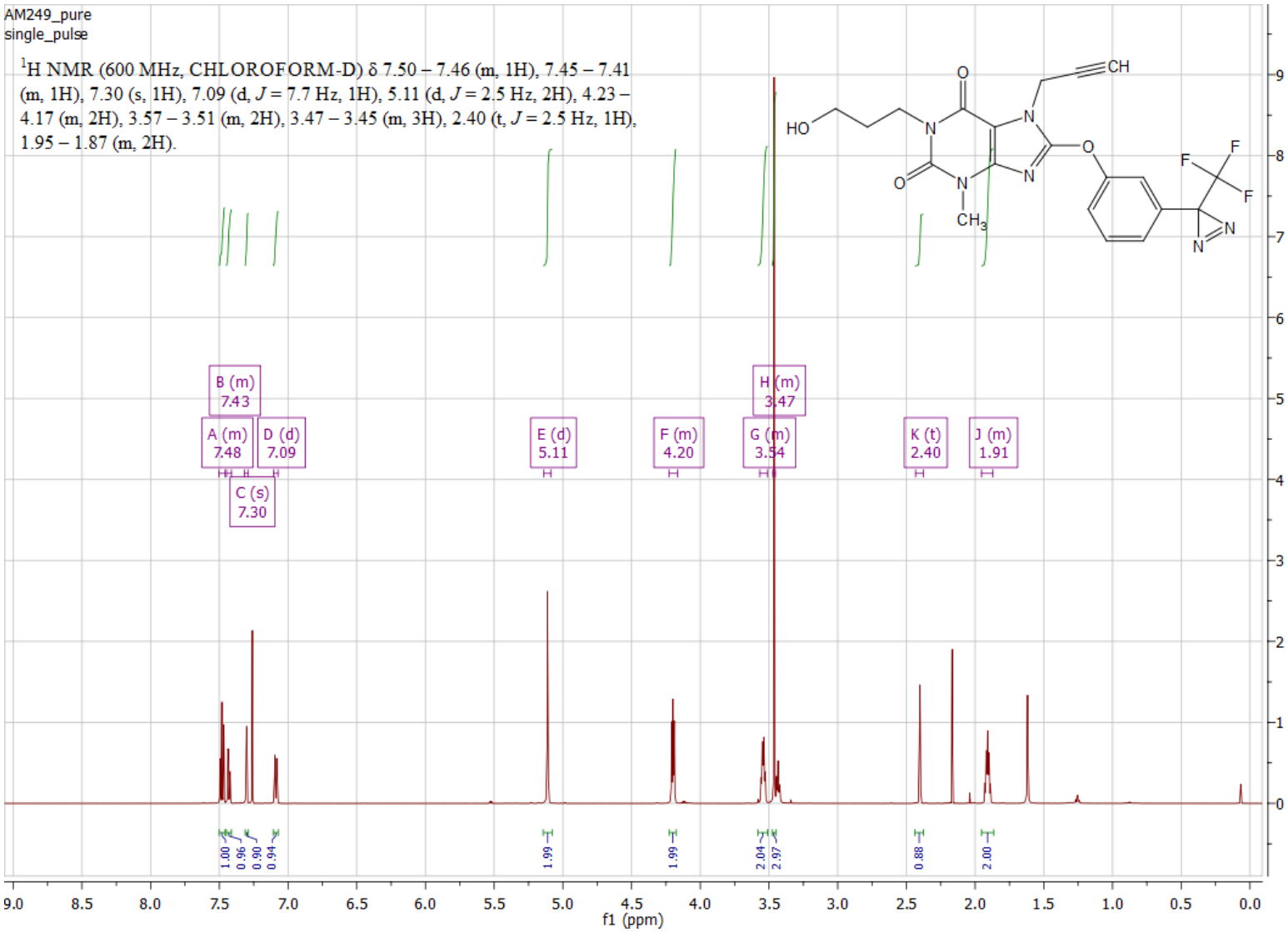
vacuo to yield the title compound as a white solid (682 mg, 91 %). δH (400 MHz, $CDCl_3$) 5.18 (d, *J* 2.5 Hz, 2H), 4.19 (t, *J* 5.8, 2H), 3.56 (s, 3H) 3.54 (t, *J* 5.7, 2H), 2.43 (t, *J* 2.5, 1H), 1.90 (tt, *J* 5.8, 5.7, 2H) δC (100 MHz, $CDCl_3$) 154.8, 151.5, 148.6, 128.4, 108.4, 75.5, 74.6, 58.8, 38.2, 36.6, 35.6, 30.2; ESI-HRMS: calcd. for $C_{12}H_{13}BrN_4O_3Na$ $[M+Na]^+$ 363.0063, found 363.0057.

1-(3-hydroxypropyl)-3-methyl-7-(prop-2-yn-1-yl)-8-{3-[3-(trifluoromethyl)-3H-diazirin-3-yl]phenoxy}-2,3,6,7-tetrahydro-1H-purine-2,6-dione (Pico145-DAAlk) 5

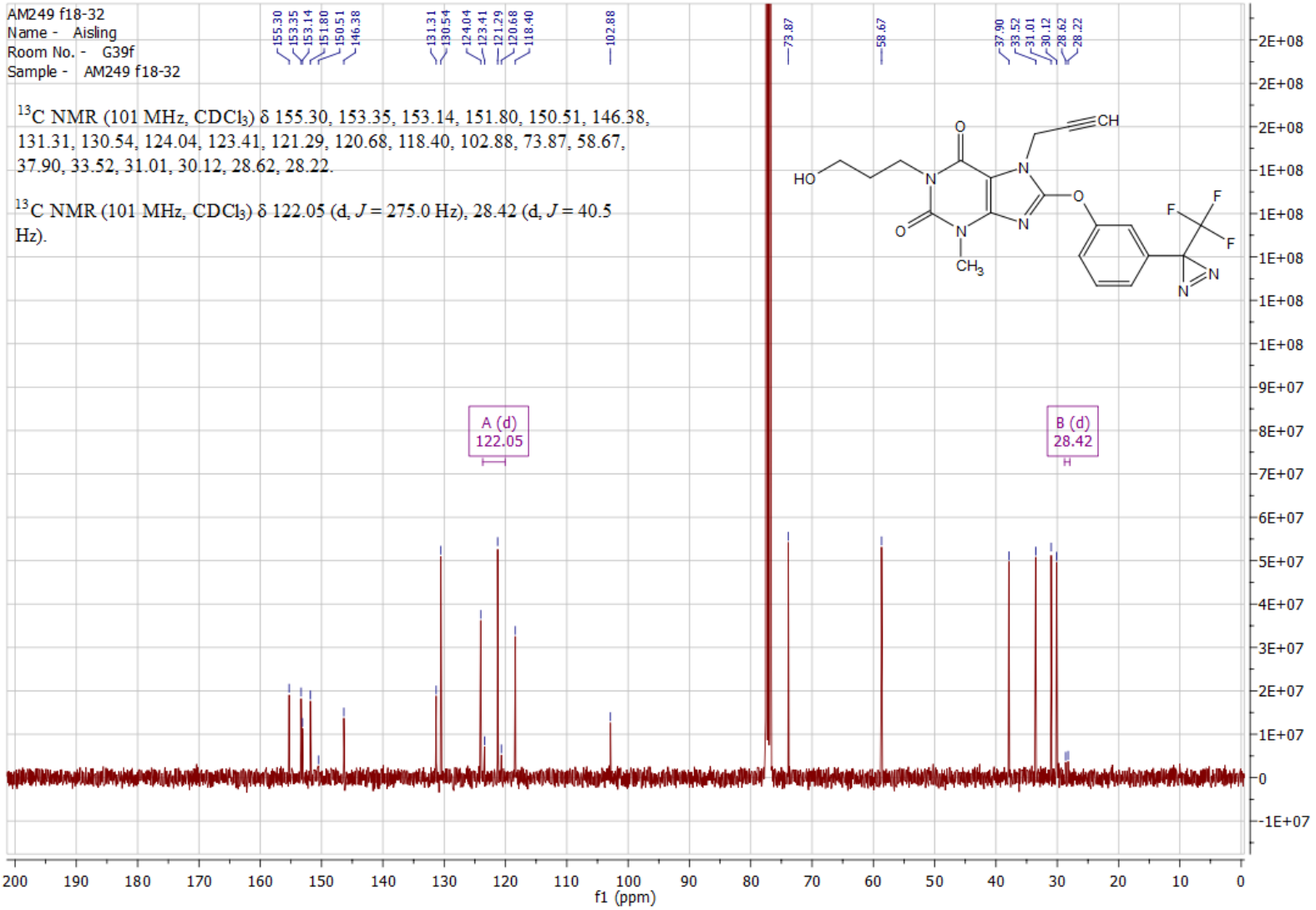


5

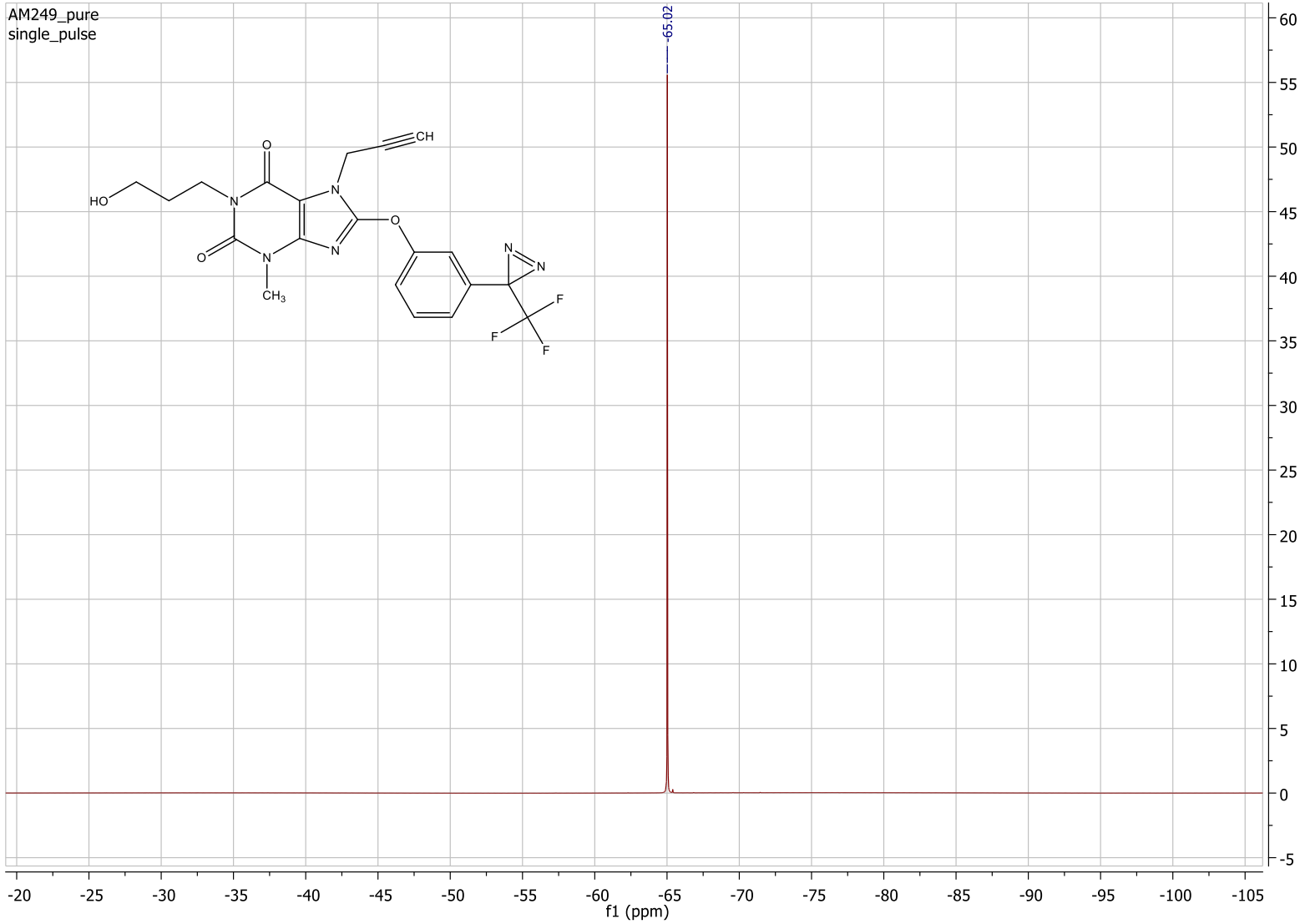
Cs_2CO_3 (381 mg, 1.08 mmol) was added to a solution of 8-bromo-1-(3- hydroxypropyl)-3-methyl-7-(prop-2-yn-1-yl)-2,3,6,7-tetrahydro-1H-purine-2,6-dione **17** (307 mg, 0.90 mml) in DMF (8 mL). After 5 min, 3-[3-(trifluoromethyl)-3H-diazirin- 3-yl]phenol **13** (200 mg, 0.99 mmol) was added and the reaction mixture was heated to 40 °C for 1 h. The mixture was cooled, partitioned between EtOAc (10 mL) and water (10 mL). The organics were washed with aqueous LiCl (1 M, 3 × 10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude material was purified by column chromatography (SiO_2; eluting with 3:7 EtOAc:hexane) to yield the *title compound* as an off-white solid (135 mg, 33 %). R_f 0.3 (3:7 EtOAc:hexane); δH (600 MHz, $CDCl_3$) 7.50 – 7.46 (m, 1H), 7.45 – 7.41 (m, 1H), 7.30 (s, 1H), 7.09 (d, *J* 7.7 Hz, 1H), 5.11 (d, *J* 2.5, 2H), 4.23 – 4.17 (m, 2H), 3.57 – 3.51 (m, 2H), 3.47 – 3.45 (m, 3H), 2.40 (t, *J* 2.5, 1H), 1.95 – 1.87 (m, 2H), 1.62 (s, 1H); δC (101 MHz, $CDCl_3$) 155.3, 153.4, 153.1, 151.8, 150.5, 146.4, 131.3, 130.5, 124.0, 122.1 (q, *J* 275.0, CF_3), 121.3, 118.4, 102.9, 73.9, 58.7, 37.9, 33.5, 31.0, 30.1, 28.4 (q, *J* 40.5, C-diazirine); δF (235 MHz, $CDCl_3$) -65.0; ESI-HRMS: calcd. for $C_{20}H_{18}F_3N_6O_4$ $[M+H]^+$ 485.1156, found 485.1154, HPLC: RT = 0.85 min.



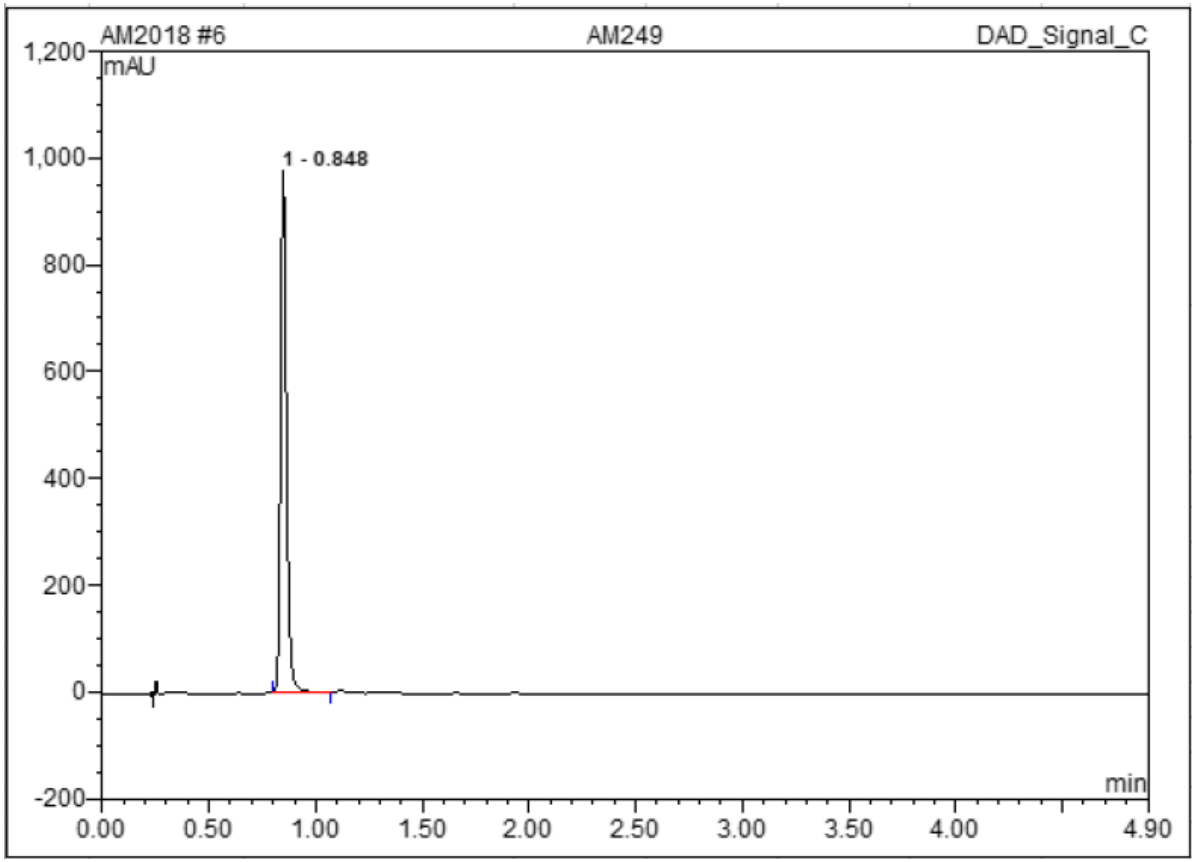
Supplementary Figure 20. ^{1}H NMR spectrum of Pico145-DAAlk **5**.



Supplementary Figure 21. ^{13}C NMR spectrum of Pico145-DAAlk **5**.

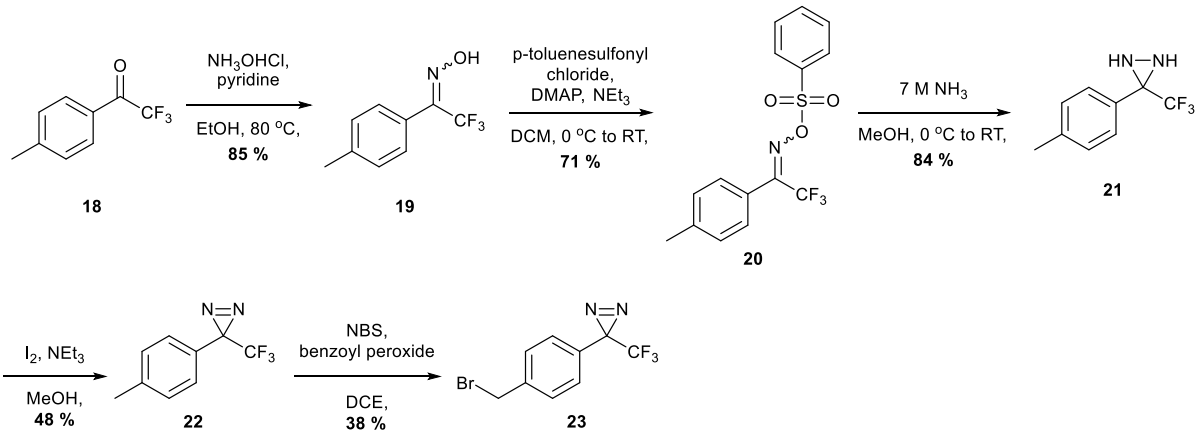


Supplementary Figure 22. ^{19}F NMR spectrum of Pico145-DAAlk **5**.



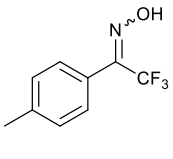
Supplementary Figure 23. HPLC chromatogram of Pico145-DAAlk **5** (MeCN/water 50-95%).

Synthesis of diazirine building block 23



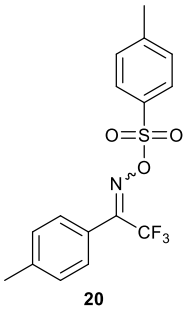
Supplementary Figure 24. Synthetic route to diazirine building block **23**.

N-(2,2,2-trifluoro-1-(4-methylphenyl)ethylidene)hydroxylamine 19[8]


19

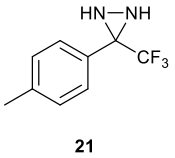
Hydroxylamine hydrochloride (1.80 g, 26.6 mmol) was added to a solution of 4'-methyl-2,2,2-trifluoroacetophenone **18** (2.00 mL, 13.3 mmol) in anhydrous ethanol (40 mL) and anhydrous, distilled pyridine (10 mL). The reaction mixture was stirred for 18 h under reflux, and then concentrated *in vacuo*. The residue was dissolved in Et_2O (50 mL), washed with H_2O (3 x 50 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give a clear oil (2.31 g, 85 %). R_f 0.3 (4:6 EtOAc:hexane); δH (500 MHz, $CDCl_3$) 7.46 (d, *J* 8.1 Hz, 2H), 7.30 (d, *J* 8.1, 2H), 2.42 (s, 3H); δC (101 MHz, $CDCl_3$) 146.8 (q, *J* 32.0, C=N-OH), 140.4, 129.3, 129.2, 128.8, 121.2 (q, *J* 274.4, CF_3), 21.6; δF (376 MHz, $CDCl_3$) -66.3; ESI: m/z 204.3 $[M+H]^+$; ESI-HRMS: calcd. for $C_9H_9F_3NO$ $[M+H]^+$ 204.0631, found 204.0629. Data consistent with literature.[8]

2,2,2-trifluoro-1-(4-methylphenyl)ethylidene)amino-4-methylbenzenesulfonate, 20[8]


20

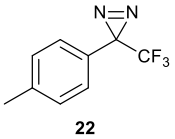
p-Toluenesulfonyl chloride (2.96 g, 15.5 mmol) was added to a solution of N-(2,2,2-trifluoro-1-(4-methylphenyl)ethylidene)hydroxylamine **19** (2.30 g, 11.3 mmol), DMAP (0.14 g, 1.13 mmol) and anhydrous, distilled trimethylamine (1.73 mL, 12.4 mmol) in anhydrous DCM (40 mL) at 0 °C. The mixture was stirred for 3 h, while it was allowed to warm to room temperature, and then stirred at room temperature overnight. The reaction mixture was washed with water (3 x 50 mL), dried over Na_2SO_4, concentrated *in vacuo* and purified by column chromatography (SiO_2; 6:4 DCM:hexane) to give a white solid; (2.85 g, 71 %). R_f 0.5 (6:4 EtOAc:hexane); δH (500 MHz, $CDCl_3$) 7.91 – 7.87 (m, 2H), 7.39 (d, *J* 8.2 Hz, 2H), 7.32 (d, *J* 8.2, 2H), 7.27 (d, *J* 9.6 Hz, 2H), 2.48 (s, 3H), 2.41 (s, 3H); δC (126 MHz, $CDCl_3$) 146.1, 142.2, 131.2, 129.8, 129.5, 129.3, 128.5, 121.7, 21.8, 21.5 (CF_3 and C=N not observed due to signal intensity and ^{13}C-^{19}F splitting); δF (376 MHz, $CDCl_3$) -66.6; ESI: m/z 358.3 $[M+H]^+$; ESI-HRMS: calcd. for $C_{16}H_{15}F_3NO_3S$ $[M+H]^+$ 358.0719, found 358.0717. Data consistent with literature.[8]

3-(4-methylphenyl)-3-(trifluoromethyl)diaziridine 21 2,2,2-trifluoro-1-(4-methylphenyl)ethylidene)amino-4-methylbenzenesulfonate **20** (2.80 g , 7.97 mmol) was dissolved in a 7 M solution of ammonia in methanol (30 mL) at 0 °C under a gaseous ammonia atmosphere. The reaction mixture was stirred vigorously to dissolve the starting material whilst bubbling ammonia through the solution. The solution was allowed to warm to room temperature after 6 h and stirred for a further 16 hours. Aqueous sodium bicarbonate (sat., 4 mL) was added to the reaction mixture, leading to precipitation of a white solid, which was removed by vacuum filtration. The filtrate was extracted with DCM (50 mL), washed with water (3 x 50 mL) and brine (1 x 25 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give an orange solid (0.86 g, 84 %). R_f 0.6 (1:9 EtOAc:hexane); δH (400 MHz, $CDCl_3$) 7.49 (d, *J* 8.1 Hz, 2H), 7.23 (d, *J* 8.1, 2H), 2.76 (d, *J* 8.8, 1H), 2.38 (s, 3H), 2.19 (d, *J* 8.8, 1H); δC (101 MHz, $CDCl_3$) 140.3, 130.4, 129.4, 128.0, 123.6 (q, *J* 278.2, CF_3), 57.9 (q, *J* 35.9, C-diaziridine), 21.3; δF (376 MHz, $CDCl_3$) -75.7; ESI: m/z 203.3 $[M+H]^+$; ESI-HRMS: calcd. for $C_9H_{10}F_3N_2$ $[M+H]^+$ 203.0791, found 203.0791.



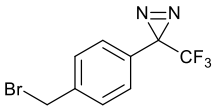
21

3-(4-methylphenyl)-3-(trifluoromethyl)diazirine 22 3-(4-methylphenyl)-3-(trifluoromethyl)diaziridine **21** (0.80 g, 3.96 mmol) was dissolved in methanol (20 mL), the flask wrapped in foil and the mixture stirred at 0 °C for 5 min. Distilled triethylamine (0.83 mL, 5.94 mmol) was added to the mixture and stirring continued for 5 min. Iodine crystals were added slowly until the solution was consistently red-brown in colour. EtOAc (5 mL) was added to the solution and the mixture was washed with 1 M HCl (5 mL). The organic fractions were washed with aqueous sodium thiosulfate (10 %, 5 mL) and brine (5 mL), dried over Na_2SO_4, concentrated *in vacuo*, and purified by column chromatography (SiO_2; 9:1 hexane:EtOAc) to give an orange oil (0.38 g, 48 %). R_f 0.6 (1:9 EtOAc:hexane); δH (500 MHz, $CDCl_3$) 7.22 (d, *J* 7.9 Hz, 2H), 7.12 (d, *J* 7.9, 2H), 2.39 (s, 3H); δC (126 MHz, $CDCl_3$) 140.0, 129.7, 126.6, 126.3, 122.4 (q, *J* 274.5, CF_3), 28.6 (q, *J* 40.3, C-diazirine), 21.3; δF (376 MHz, $CDCl_3$) -65.5.



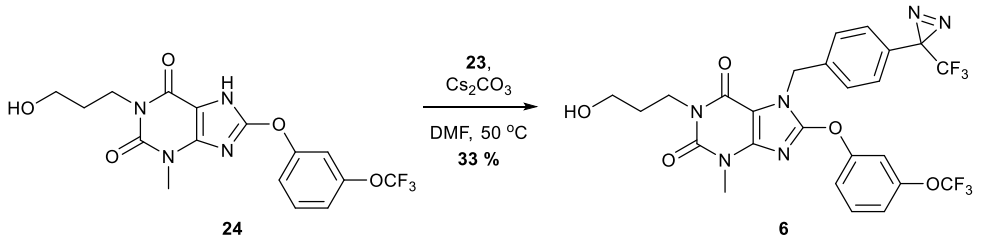
22

3-(4-(bromomethyl)phenyl)-3-(trifluoromethyl)diazirine, 23[8] Dibenzoyl peroxide (12 mg, 0.05 mmol) was added to a solution of 3-(4-methylphenyl)-3-(trifluoromethyl)diazirine **22** (0.20 g, 1.00 mmol) and N-bromosuccinimide (0.21 g, 1.20 mmol) in 1,2-dichloroethane (5 mL) and the resulting mixture was stirred at 70 ˚C for 2 h. The solution was cooled to room temperature, filtered, and the solid washed with DCM (5 mL). The filtrate was concentrated *in vacuo* and purified by column chromatography (SiO_2; hexane) to give a white solid (0.11 g, 38 %). R_f 0.3 (hexane); δH (400 MHz, $CDCl_3$) 7.34 (d, *J* 8.3 Hz, 2H), 7.09 (d, *J* 8.3, 2H), 4.38 (s, 2H); δC (101 MHz, $CDCl_3$)) δ 138.4, 128.4, 128.3, 125.9, 121.0 (q, *J* 274.7, CF_3), 31.0, 27.3 (q, *J* 40.6, C-diazirine); δF (376 MHz, $CDCl_3$) -65.2; ESI: m/z 279.34 $[M+H]^+$. Data consistent with literature.[8]

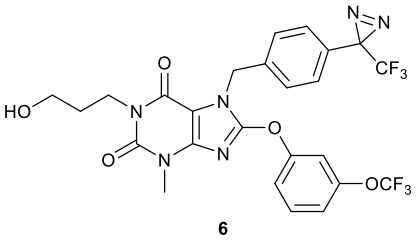


23

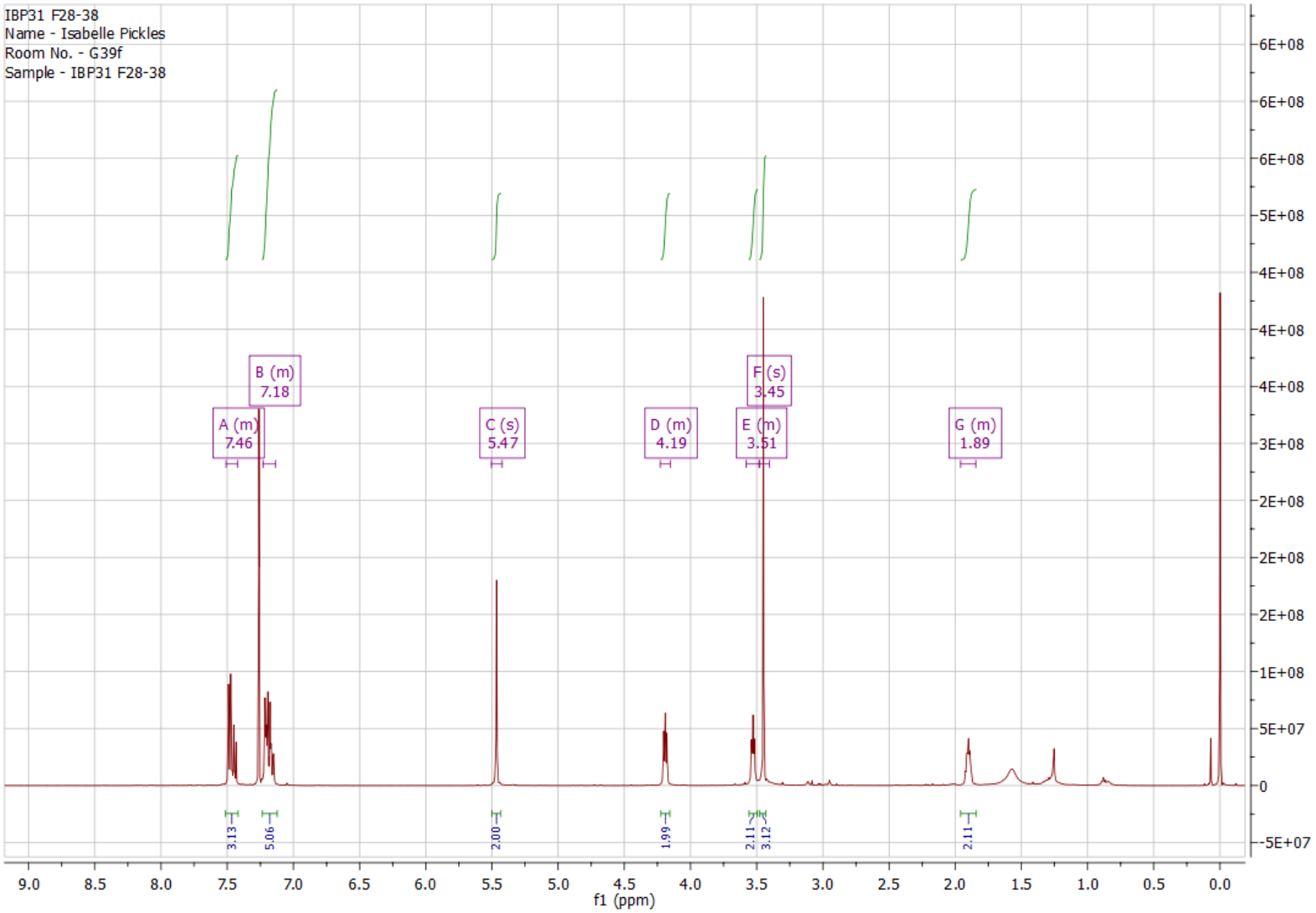
Synthesis of Pico145-DA2 6



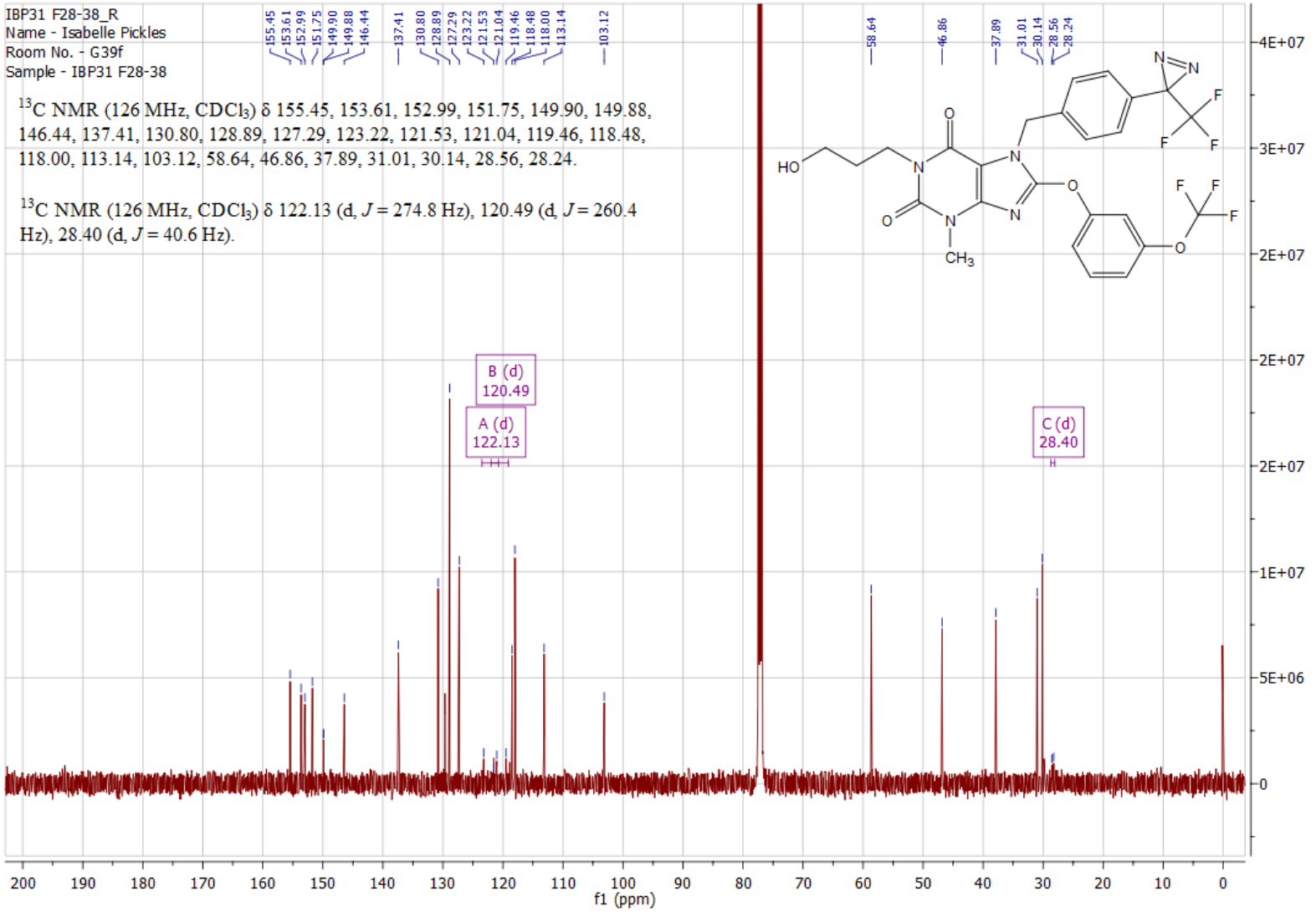
Supplementary Figure 25. Synthetic route to Pico145-DA **6**.



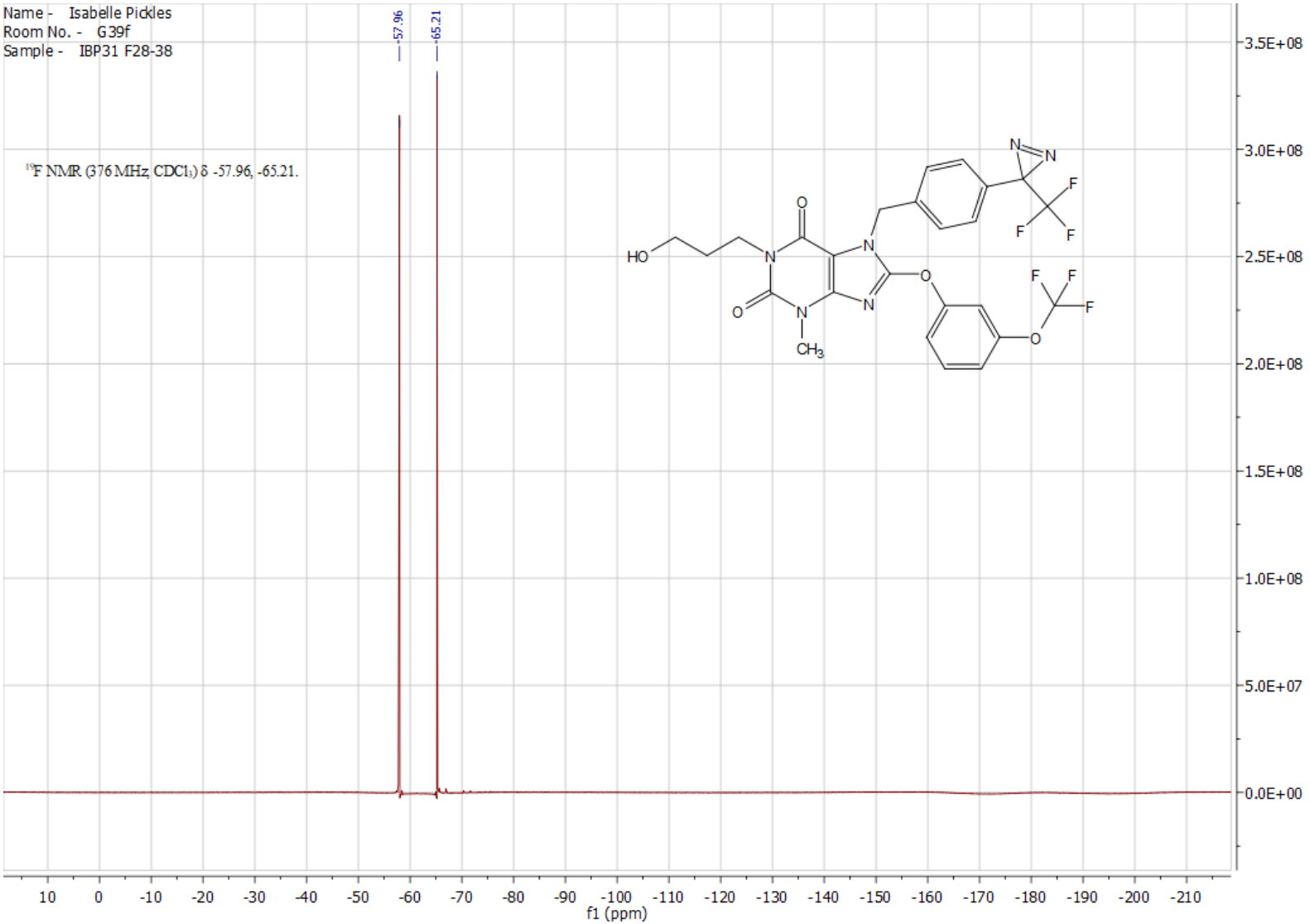
1-(3-hydroxypropyl)-3-methyl-7-((4-(3-methyldiazirin-3-yl)phenyl)methyl)-8-(3-(trifluoromethoxy)phenoxy)purine-2,6-dione (Pico145-DA2) 6 To a solution of 7-((4-(3-tert-butyldiazirin-3-yl)phenyl)methyl)-1-(3-hydroxypropyl)-3-methyl-8-(3-(trifluoromethoxy)phenoxy)purine-2,6-dione **24** (prepared according to Rubaiy et al., 2017)[7] (0.03 g, 0.07 mmol) and Cs_2SO_4 (0.05 g, 0.14 mmol) in DMF (5 mL), 3-(4-(bromomethyl)phenyl)-3-(trifluoromethyl)diazirine **23** (0.02 g, 0.08 mmol) was added and the solution was stirred at 50 °C overnight. The product was poured into water (20 mL) and extracted with EtOAc (4 x 20 mL), washed with water (2 x 20 mL), aqueous LiCl (1 N, 2 x 20 mL) and brine (20 mL), dried over Na_2SO_4, concentrated *in vacuo*, and purified by column chromatography (SiO_2; 6:4 hexane:EtOAc) to yield a white residue (0.014 g, 33 %). R_f 0.3 (6:4 EtOAc:hexane); δH (500 MHz, $CDCl_3$) 7.51 – 7.42 (m, 3H), 7.23 – 7.13 (m, 5H), 5.47 (s, 2H), 4.23 – 4.15 (m, 2H), 3.58 – 3.48 (m, 2H), 3.45 (s, 3H), 1.96 – 1.84 (m, 2H), hydroxypropyl -O*H* not detected; δC (126 MHz, $CDCl_3$) 155.5, 153.6, 153.0, 151.8, 149.9, 146.4, 137.4, 130.8, 129.64, 128.9, 127.3, 122.1 (q, *J* 274.8, CF_3), 120.5 (q, *J* 260.4, CF_3) 118.5, 118.0, 113.1, 103.1, 58.6, 46.9, 37.9, 31.0, 30.1, 28.4 (q, *J* 40.6, C-diazirine); δF (376 MHz, $CDCl_3$) -58.0, -65.2; ESI: m/z 599.10 $[M+H]^+$; ESI-HRMS: calcd. for $C_{25}H_{21}F_6N_6O_5$ $[M+H]^+$ 599.1472, found 599.1479; HRMS: RT = 4.17 min.



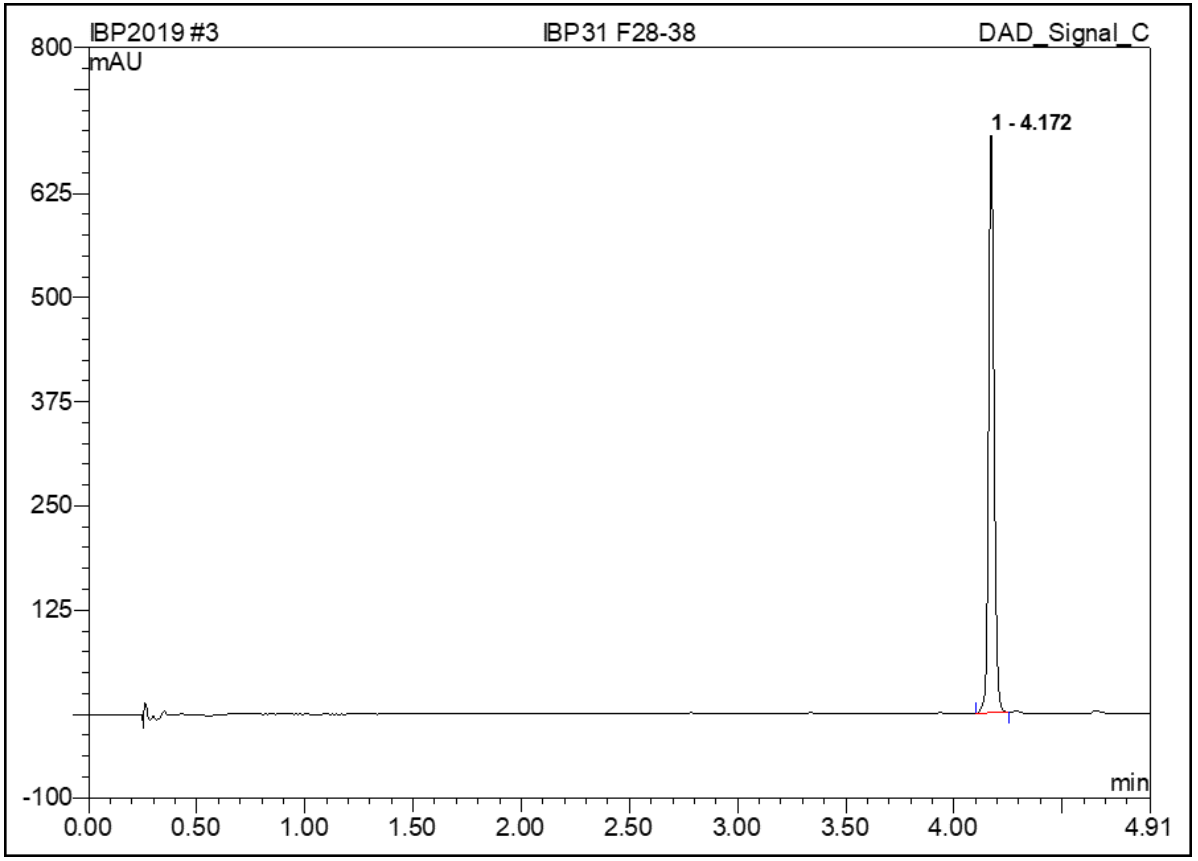
Supplementary Figure 26. ^{1}H NMR spectrum of Pico145-DA2 **6**.



Supplementary Figure 27. ^{13}C NMR spectrum of Pico145-DA2 **6**.

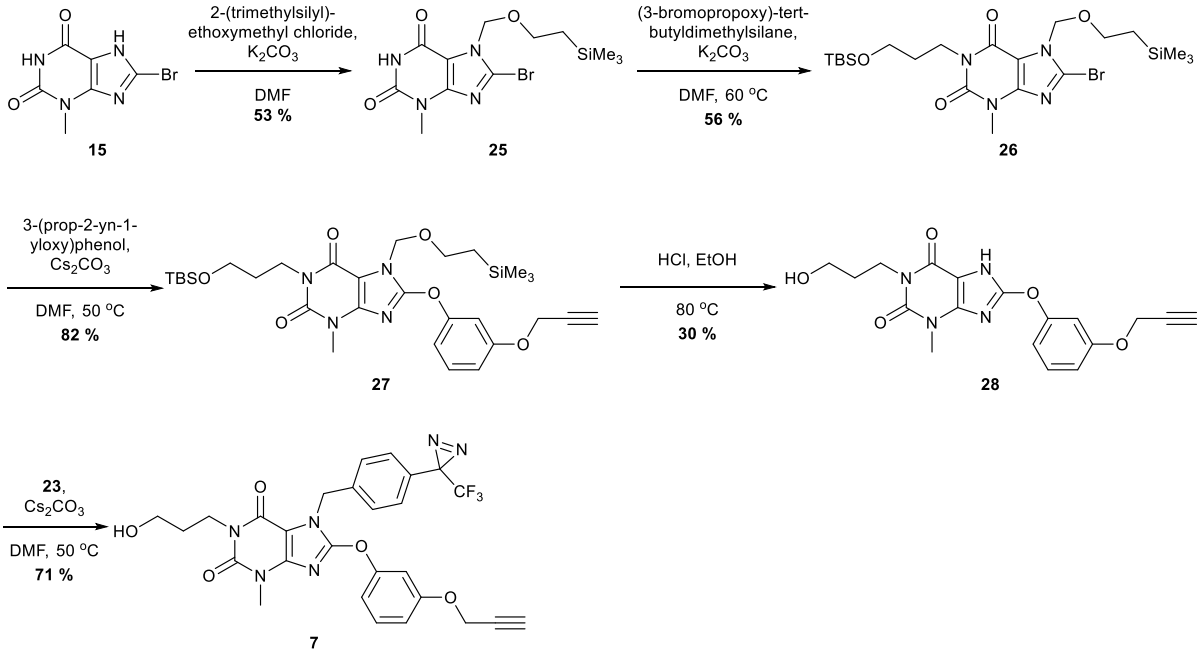


Supplementary Figure 28. ^{19}F NMR spectrum of Pico145-DA2 **6**.

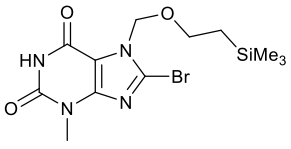


Supplementary Figure 29. HPLC chromatogram of Pico145-DA2 **6** (MeCN/water 5-95%).

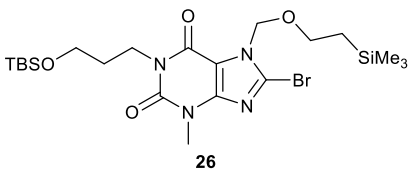
Synthesis of Pico145-DAAlk2 7



Supplementary Figure 30. Synthetic route to Pico145-DAAlk **7**.

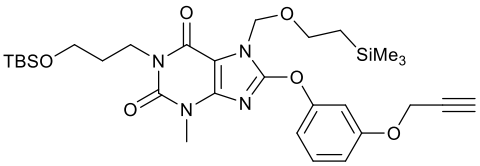


8-bromo-3-methyl-7-(2-(trimethylsilyl)ethoxymethyl)-1H-purine-2,6-dione 25 2-(trimethylsilyl)ethoxymethyl chloride (0.29 mL, 1.63 mmol) was added dropwise to a solution of 8-bromo-3-methyl-1,7-dihydropurine-2,6-dione **15** (0.40 g, 1.63 mmol) and K_2SO_4 (0.45 g, 3.26 mmol) in DMF (4 mL) at 0 °C, and the reaction was stirred at room temperature overnight. The solution was partitioned between EtOAc (25 mL) and water (25 mL) and the aqueous layer extracted with EtOAc (3 x 25 mL). The combined organic fractions were washed with aqueous LiCl (1 N, 3 x 25 mL) and brine (25 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give a pale pink solid (0.36 g, 53 %). R_f 0.5 (8:2 EtOAc:hexane); δH (500 MHz, $CDCl_3$) 5.68 (s, 2H), 3.69 (m, 2H), 3.54 (s, 3H), 0.94 (2H, m), -0.01 (s, 9H); δC (101 MHz, $CDCl_3$) 154.9, 152.0, 151.5, 130.5, 110.7, 76.5, 68.8, 30.7, 19.2, 0.0; ESI: m/z 373.17 $[M-H]^-$; ESI-HRMS: calcd. for $C_{12}H_{19}BrN_4NaO_3Si$ $[M+Na]^+$ 397.0302, found 397.0302.



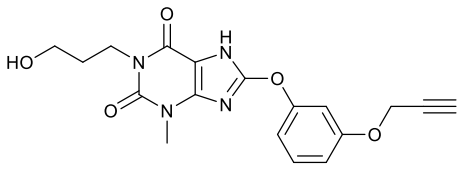
8-bromo-1-(3-((tert-butyldimethylsilyl)oxy)propyl)-3-methyl-7-((2-(trimethylsilyl)ethoxy)methyl)purine-2,6-dione 26 To a solution of 8-bromo-3-methyl-7-(2-(trimethylsilyl)ethoxymethyl)-1H-purine-2,6-dione **25** (0.35 g, 0.93 mmol) in DMF (5 mL), K_2SO_4 (0.13 g, 0.93 mmol) and (3-bromopropoxy)-tert-butyldimethylsilane (0.23 mL, 1.05 mmol) were added and the reaction mixture stirred at 60 °C overnight. The solution was poured into water (50 mL) and extracted with EtOAc (4 x 50 mL). The organic fractions were washed with aqueous LiCl (1 N, 2 x 50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give an orange solid, which was purified by column chromatography (SiO_2; 4:6 hexane:EtOAc) to give a colourless oil (0.45 g, 56 %). R_f 0.6 (6:4 EtOAc:hexane); δH (500 MHz, $CDCl_3$) 5.71 (s, 2H), 4.10

(m, 2H), 3.71 (m, 4H), 3.56 (s, 3H), 1.86 (m, 2H), 0.94 (m, 2H), 0.87 (s, 9H), 0.03 (s, 6H), -0.02 (s, 9H); δC (101 MHz, $CDCl_3$) 154.1, 151.1, 148.3, 128.5, 109.2, 74.7, 67.0, 61.2, 39.3, 31.0, 30.0, 26.0, 18.4, 17.9, -1.3, -5.2; ESI: m/z 547.51 $[M+H]^+$; ESI-HRMS: calcd. for $C_{21}H_{39}BrN_4NaO_4Si_2$ $[M+Na]^+$ 569.1585, found 569.1575.



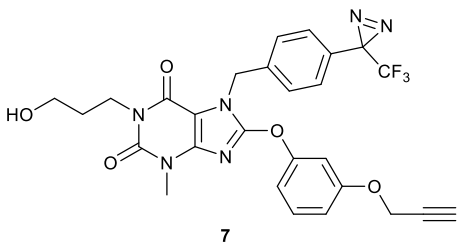
27

1-(3-((tert-butyldimethylsilyl)oxy)propyl)-3-methyl-8-(3-(prop-2-yn-1-yloxy)phenoxy)-7-((2-(trimethylsilyl)ethoxy)methyl)purine-2,6-dione 27 3-(prop-2-yn-1-yloxy)phenol[9] (0.29 g, 1.98 mmol) was added to a suspension of 8-bromo-1-(3-tert-butyldimethylsilyloxypropyl)-3-methyl-7-(2-(trimethylsilyl)ethoxymethyl)purine-2,6-dione **26** (0.98 g, 1.80 mmol) and Cs_2CO_3 (1.27 g, 3.6 mmol) in DMF (10 mL) and the reaction stirred at 80 °C for 7 h. The reaction mixture was allowed to cool to room temperature and then stirred overnight. The reaction mixture was diluted with water (50 mL) and extracted with EtOAc (4 x 50 mL). The combined organic fractions were washed with aqueous LiCl (1 N, 2 x 50 mL) and brine (25 mL), dried over Na_2SO_4, concentrated *in vacuo*, and purified by column chromatography (SiO_2; 6:4 hexane:EtOAc) to give an orange oil (0.91 mg, 82 %). R_f 0.7 (6:4 EtOAc:hexane); δH (500 MHz, $CDCl_3$) 7.32 (t, *J* 8.3 Hz, 1H), 6.99 – 6.91 (m, 2H), 6.91 – 6.85 (m, 1H), 5.69 (s, 2H), 4.70 (d, *J* 2.4, 2H) 4.14 – 4.08 (m, 2H), 3.78 – 3.72 (m, 4H), 3.55 (s, 3H), 2.53 (t, *J* 2.33, 1H), 1.92-1.82 (m, 2H), 1.00 – 0.94 (m, 2H), 0.88 (s, 9H), 0.03 (s, 6H), -0.03 (s, 9H); ESI: m/z 615.37 $[M+H]^+$.



28

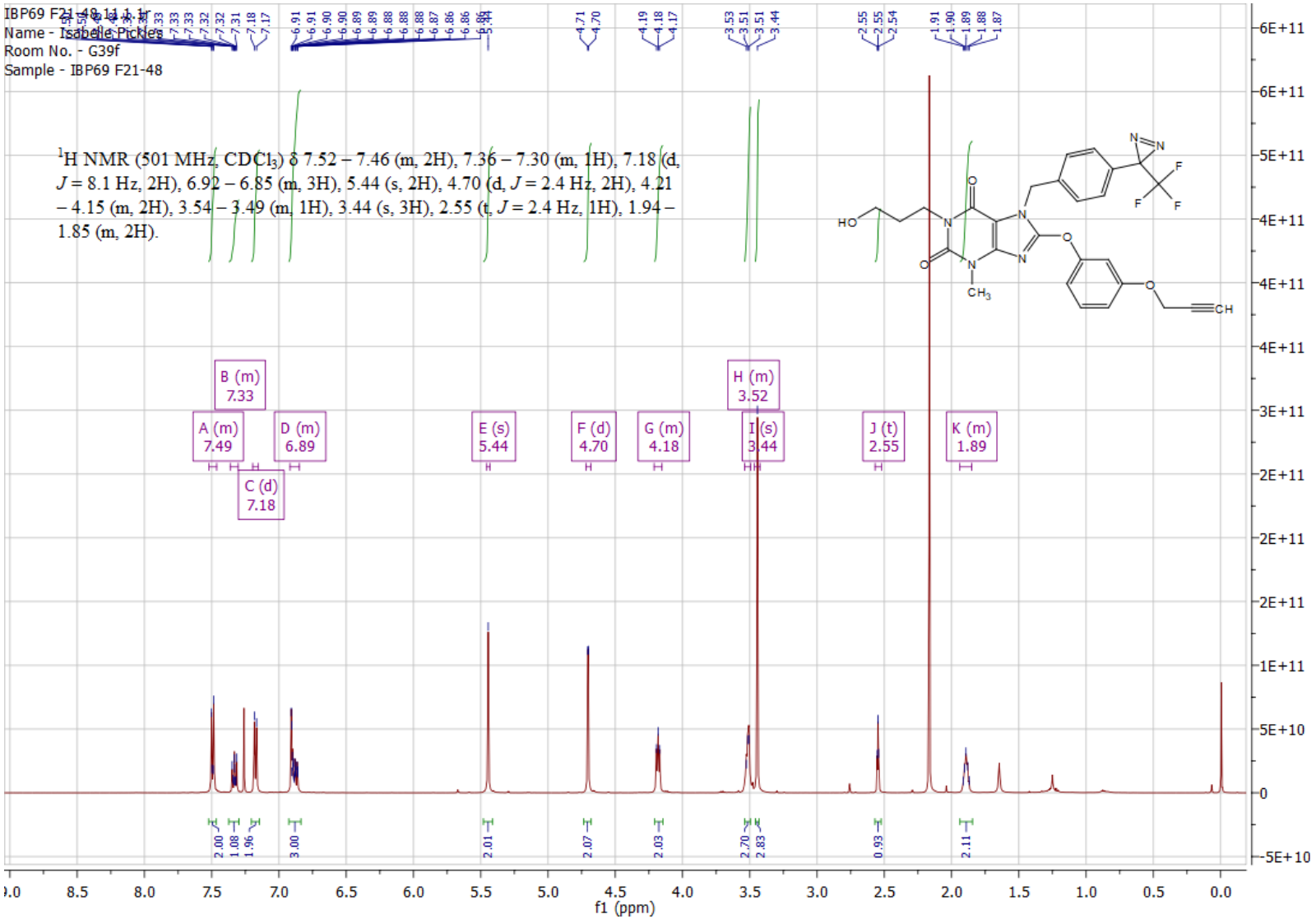
1-(3-hydroxypropyl)-3-methyl-8-(3-(prop-2-yn-1-yloxy)phenoxy)-7H-purine-2,6-dione 28 To a solution of 1-(3-((tert-butyldimethylsilyl)oxy]propyl)-3-methyl-8-(3-(prop-2-yn-1-yloxy)phenoxy)-7-((2-(trimethylsilyl)ethoxy)methyl)purine-2,6-dione **27** (0.90 g, 1.45 mmol) in EtOH (5 mL), concentrated aqueous HCl (1 mL) was added and the resulting solution was heated at reflux for 1 h. The solvents were removed *in vacuo*, the product was extracted using EtOAc (4 x 20 mL) and washed with water (3 x 20 mL) and brine (20 mL). The solution was dried over Na_2SO_4, concentrated *in vacuo*, and purified by column chromatography (SiO_2; 6:4 EtOAc:hexane) to give a white solid (0.16 g, 30 %). δH (500 MHz, $CDCl_3$) 7.31 (t, *J* 8.2 Hz, 1H), 7.00 – 6.92 (m, 2H), 6.86 (ddd, *J* 8.2, 2.4, 0.8, 1H), 4.69 (d, *J* 2.4, 2H), 4.18 (t, *J* 6.0, 2H), 3.55 – 3.51 (m, 2H), 3.51 (s, 3H), 2.55 (t, *J* 2.4, 1H), 1.94 – 1.85 (m, 2H); δC (126 MHz, $CDCl_3$) 158.6, 155.5, 155.1, 154.1, 151.6, 147.7, 130.3, 112.6, 112.2, 107.0, 103.1, 78.0, 76.1, 58.6, 56.1, 38.2, 30.8, 30.4; ESI: m/z 371. 09 $[M+H]^+$; ESI-HRMS: calcd. for $C_{18}H_{19}N_4O_5$ $[M+H]^+$ 371.1350, found 371.1353.



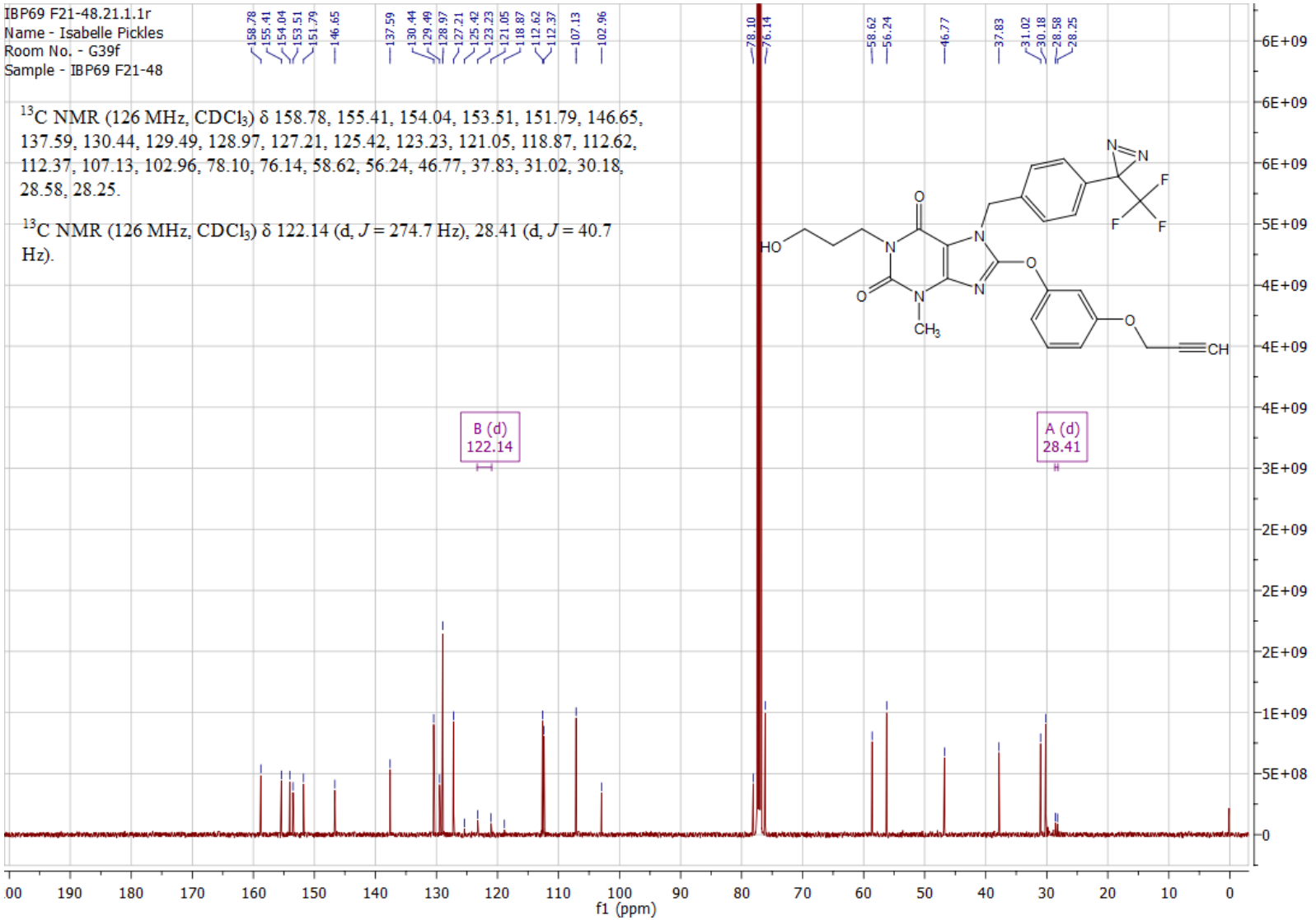
7

1-(3-hydroxypropyl)-3-methyl-7-((4-(3-methyldiazirin-3-yl)phenyl)methyl)-8-(3-(prop-2-yn-1-yloxy)phenoxy)purine-2,6-dione (Pico145-DAAlk2) 7 To a solution of 1-(3-hydroxypropyl)-3-methyl-8-(3-(prop-2-yn-1-yloxy)phenoxy)-7H-purine-2,6-dione **28**, (0.05 g, 0.14 mmol) and Cs_2SO_4 (0.10 g, 0.28 mmol) in

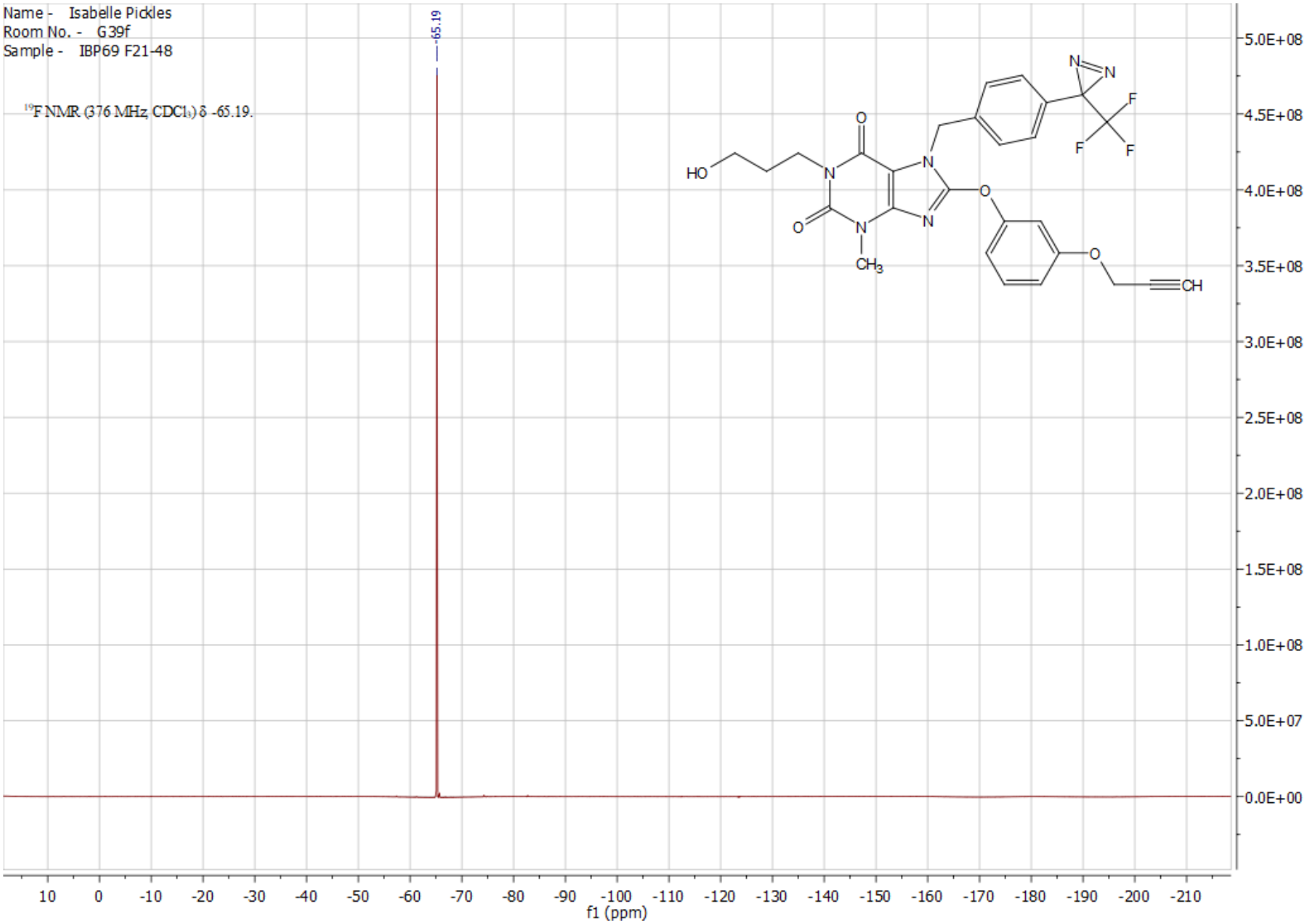
DMF (5 mL), 3-(4-(bromomethyl)phenyl)-3-(trifluoromethyl)diazirine **23** (0.05 g, 0.16 mmol) was added and the solution was stirred at 50 °C overnight to yield a brown solution. The product was poured into water (20 mL) and extracted with EtOAc (4 x 20 mL), washed with water (2 x 20 mL), aqueous LiCl (1 N, 2 x 20 mL) and brine (20 mL), dried over Na_2SO_4, concentrated *in vacuo*, and purified by column chromatography (SiO_2; 6:4 hexane:EtOAc) to yield a white residue (0.058 g, 71 %). R_f 0.2 (6:4 EtOAc:hexane); δH (501 MHz, $CDCl_3$) 7.52 – 7.46 (m, 2H), 7.36 – 7.30 (m, 1H), 7.18 (d, *J* 8.1 Hz, 2H), 6.92 – 6.85 (m, 3H), 5.44 (s, 2H), 4.70 (d, *J* 2.4, 2H), 4.21 – 4.15 (m, 2H), 3.54 – 3.48 (m, 2H), 3.44 (s, 3H), 2.55 (t, *J* 2.4, 1H), 1.94 – 1.85 (m, 2H), hydroxypropyl -*H* not detected; δC (126 MHz, $CDCl_3$) 158.8, 155.4, 154.0, 153.5, 151.8, 146.7, 137.6, 130.4, 129.5, 129.0, 127.2, 122.1 (q, J 274.7, CF_3), 112.6, 112.4, 107.1, 103.0, 78.1, 76.1, 58.6, 56.2, 46.8, 37.8, 31.0, 30.2, 28.4 (q, J 40.7, C-diazirine); δF (376 MHz, $CDCl_3$) -65.2; ESI: m/z 569.09 $[M+H]^+$; ESI-HRMS: calcd. for $C_{27}H_{24}F_3N_6O_5$ $[M+H]^+$ 569.1755, found 569.1763; HPLC: RT = 3.77 min.



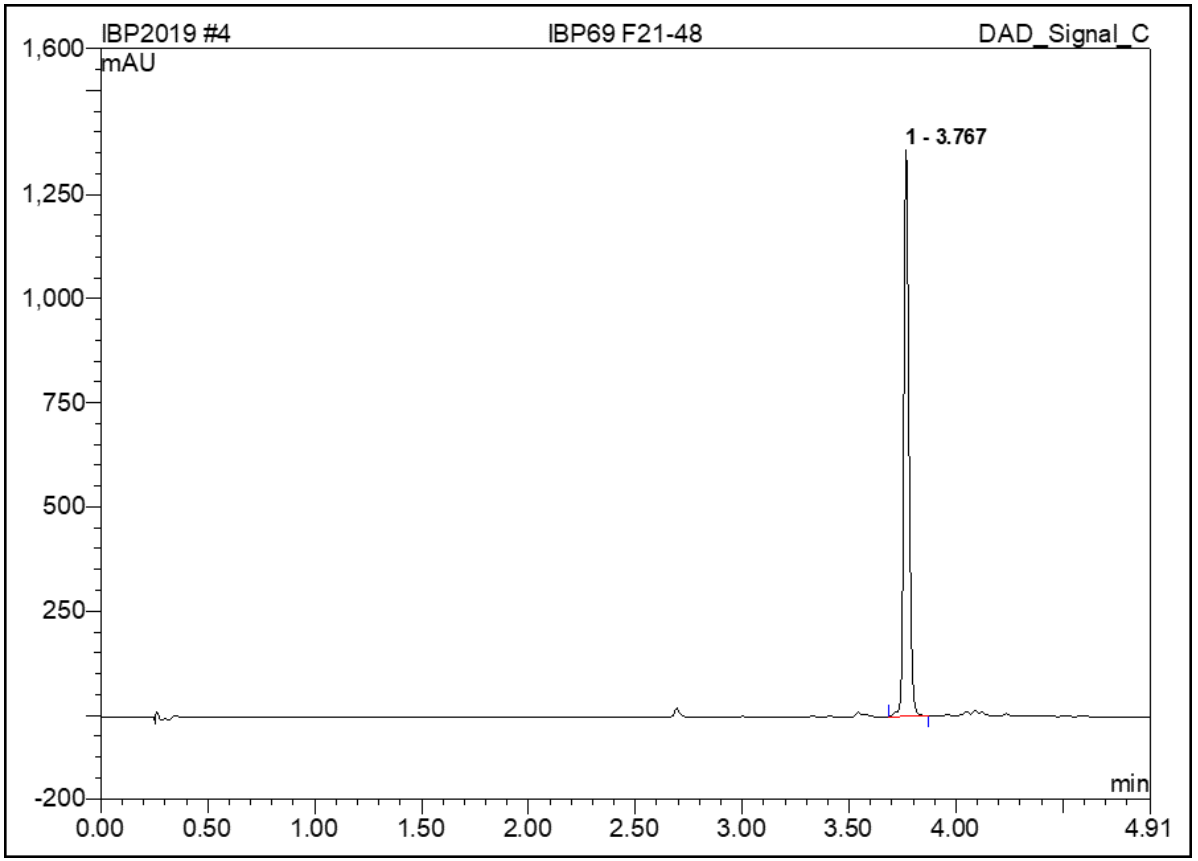
Supplementary Figure 31. ^{1}H NMR spectrum of Pico145-DAAlk2 **7**.



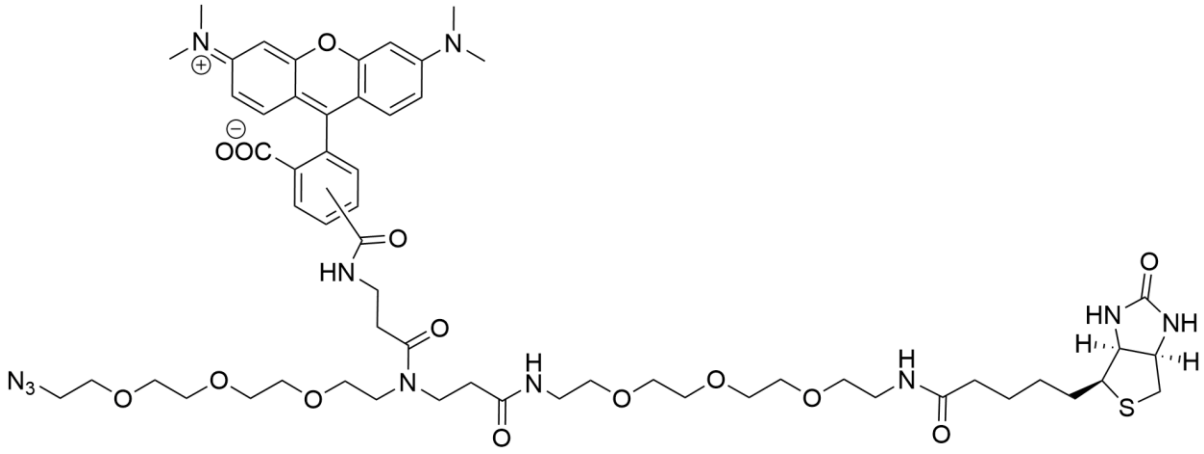
Supplementary Figure 32. ^{13}C NMR spectrum of Pico145-DAAlk2 **7**.



Supplementary Figure 33. ^{19}F NMR spectrum of Pico145-DAAlk2 **7**.

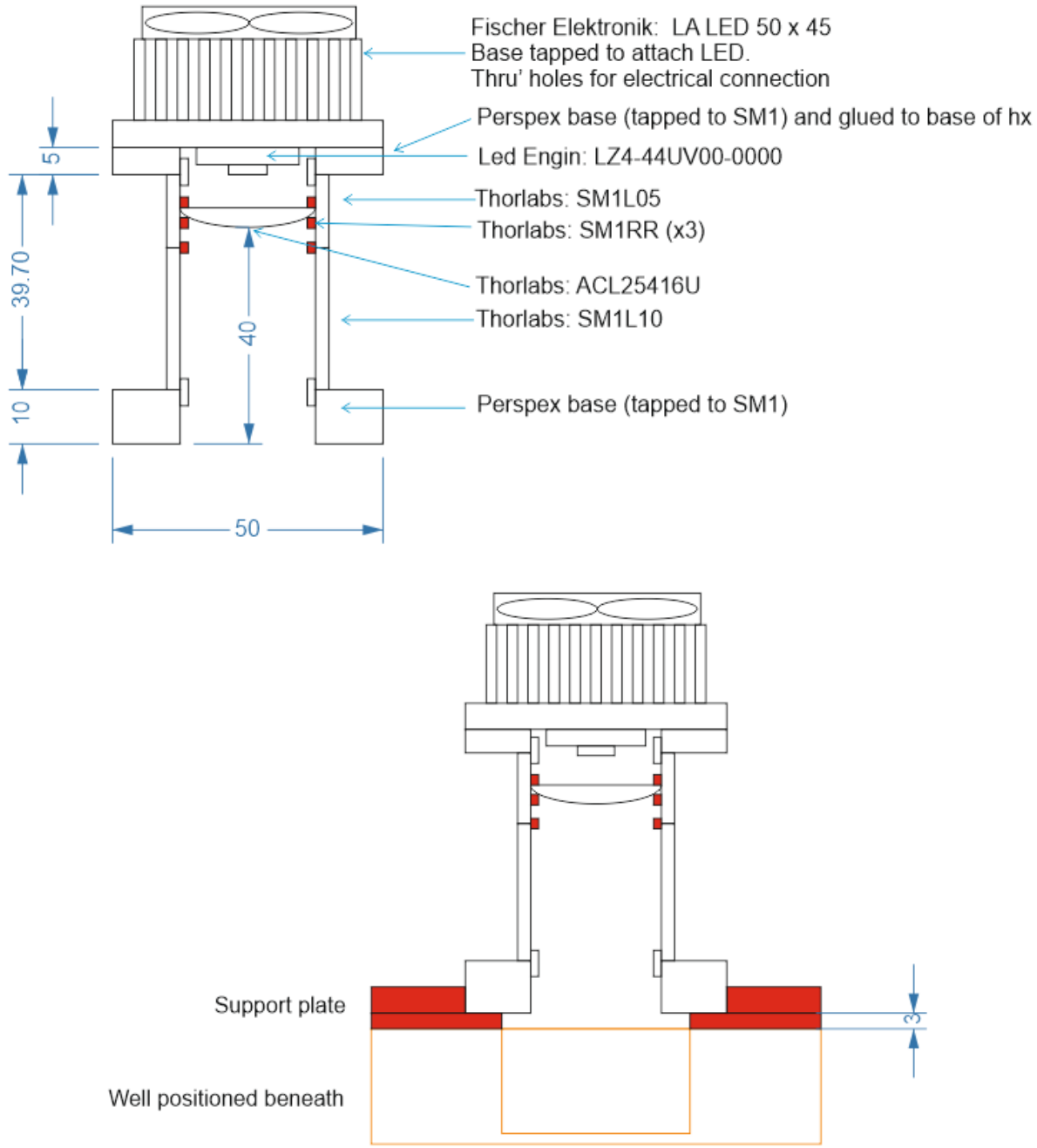


Supplementary Figure 34. HPLC chromatogram of Pico145-DAAlk2 **7** (MeCN/water 5-95%).



Supplementary Figure 35. Structure of commercial TAMRA biotin azide from Click Chemistry Tools (Scottsdale, AZ, USA). Taken from https://clickchemistrytools.com/product/tamra-biotin-azide/ (accessed: 16/02/2020).

LED photocrosslinker setup

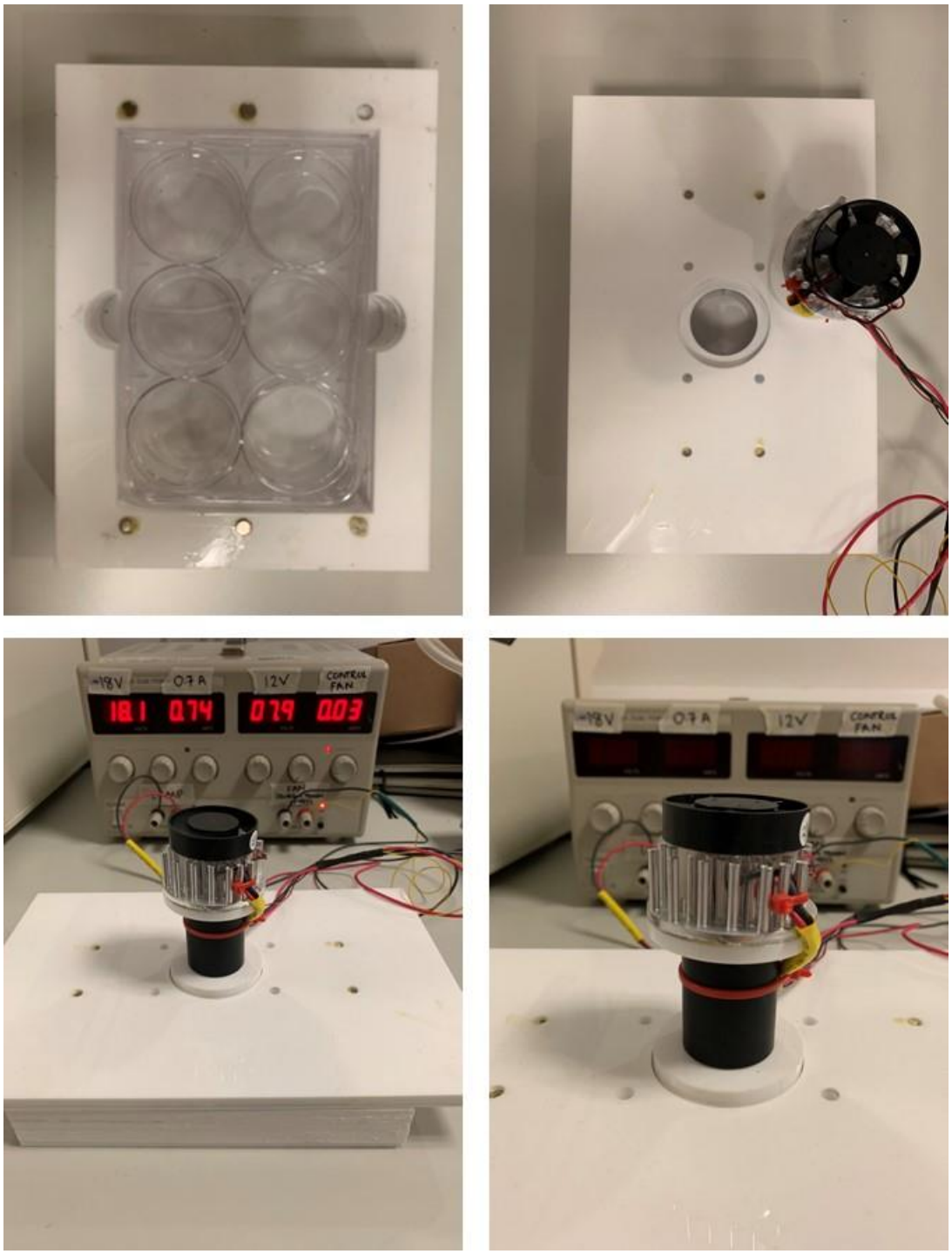


Supplementary Figure 36. Schematics of the LED photocrosslinker designed and built for this work. Measurements in mm.

The LED photocrosslinker used in photoaffinity labelling experiments with Pico145-DAAlk2 **7** was based on an earlier model built for irradiation of samples in Eppendorf vials.[10] The design was adapted to allow irradiation of wells in a 6-well plate.

The UV irradiator was built according to Supplementary Figure 34. A fan and pin fin heat exchanger were used to dissipate heat from the UV LED. A retaining Perspex ring, tapped to SM1, was glued to the base of the heat exchanger, allowing connection of an SM1L05 lens tube, in which was positioned a collimating lens held in place with two retaining rings. A third

retaining ring was used to join the upper lens tube to the lower lens tube. The lower lens tube was screwed to a Perspex base ring that was tapped to SM1.



Supplementary Figure 37. Pictures of the LED-based photocrosslinker used in this work.

This portable unit could then be positioned on a support plate, with a single opening (allowing verification that the unit was correctly positioned above the well; Supplementary Figure 34). This support plate sat above the wells, contained within a simple light-tight box, laser cut from sheets of Perspex that were then bonded together, such that the support plate sat just above the top of the 6-well plate (without a lid; Supplementary Figure 35). The support plate could

be slid so that the LED sat above any of the well openings, with enough material to prevent light egress. To power the assembly a dual output bench supply was used. The LED was run under voltage control of 18 V, and the fan was run at its nominal 12 V.

TRPC plasmid sequences used for generation of stable HEK T-REx cell lines

pcDNA4/TO/TRPC5-SYFP2

blue = hTRPC5
green = SYFP2

GACGGATCGGGAGATCTCCCGATCCCCTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGC
ATAGTTAAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAA
ATTTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCG
TTTTGCGCTGCTTCGCGATGTACGGGCCAGATATACGCGTTGACATTGATTATTGACTAGTTATTA
ATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTAC
GGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTAT
GTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAAC
TGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG
GTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTAC
ATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGG
ATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTT
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pcDNA4/TO/TRPC4-SYFP2

blue = hTRPC4β
green = SYFP2

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pcDNA4/TO/TRPC5-TRPC1

blue = hTRPC5
green = hTRPC1

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pcDNA4/TO/TRPC4-TRPC1

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