Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary Information

A novel ^{18}F-labeled clickable substrate for targeted imaging of SNAP-tag expressing cells by PET *in vivo*

Authors

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Content

1. General Information

All *chemicals*, *reagents*, and *solvents* were of analytical grade and used without further purification unless otherwise specified. Solvents were purified and dried by literature procedures if necessary and handled under inert atmosphere.

Thin layer chromatography was conducted with TLC silica gel 60 F_{254} on aluminum sheets (Merck) as stationary phase in a saturated chamber at ambient temperature. Spots were visualized with UV light (254 nm or 365 nm) and with a cerium molybdate dipping bath [$Ce(SO_4)_2$ (1.8 g), $(NH_4)_6Mo_7O_{24}$ x 4 H_2O (45 g), conc. H_2SO_4 (45 g), H_2O (900 mL)] with additional heating using a heat gun at approximately 300 °C.

Compositions of the mobile phase and retention factor (R_f) of the compounds are given in the description of the synthetic procedures. As the retention factor (R_f) values strongly depend on the exact ratio of components of the eluent and some of these are highly volatile, the given retention factor values just represent approximate values.

Automated Flash column chromatography was performed on purfiFlash® XS520Plus (INTERCHIM, Montluçon France) with PF-50SIHP-F0330 or PF-50SIHP-F0120 columns (INTERCHIM, Montluçon France).

Preparative flash column chromatography was performed with silica gel 60 (40 - 63 µm, MACHEREY-NAGEL) as stationary phase, unless otherwise noted.

Lyophilisation of compounds after purification by reversed-phase HPLC or RP chromatography was performed using an Alpha 1-2 LD plus-freeze-dryer from CHRIST.

All *ESI-MS* determination were performed on a MicroTof (BRUKER DALTRONICS, Bremen) with loop inlet. Mass calibration was performed immediately before sample measurement on sodium formate clusters by quasi-internal calibration.

GC/MS measurements were performed with GC system GC-2010 coupled with mass spectrometer GCMS-QP2010 (SHIMADZU). Column Rxi-1ms (30.0 m length, 0.25 mm internal diameter, 0.25 µm thickness, RESTEK) was used and helium was used as carrier gas. Data acquisition was done with GCMS solution version 2.71 software (SHIMADZU).

^{1}H and ^{13}C NMR spectra were recorded with BRUKER Avance II 300, Bruker Avance II 400 or AGILENT DD2 600 spectrometers. Chemical shifts (δ) for ^{1}H and ^{13}C NMR spectra are given in ppm relative to TMS and are referenced to the solvent signal. Data for ^{1}H were reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m =multiplet), integration and coupling constant in Hz.

UV-spectra were measured on an U3010 spectrometer from HITACHI.

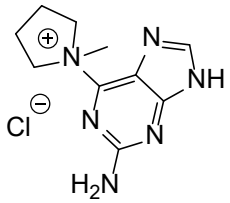
Fluorescence emission spectra were recorded on a F4500 fluorometer from HITACHI.

For the purification of the BG-conjugate with Dyomics® Dye DY549P1, a HPLC-system Azura from KNAUER was used: It consists of two Azura P2.1L pumps (100 mL pump head) and an Azura DAD 6.1L UV-detector.

ICP-OES measurements were performed to determine the residual copper concentrations in formulations for biological evaluation. An Acros ICP-OES (SPECTRO ANALYTICAL INSTRUMENTS GMBH, Kleve, Germany) with a radio frequency power of 1550 W with axial plasma view was used. For copper the lines at 327 nm and 396 nm were observed. The cool gas flow was fixed at 13 L/min and auxiliary as well as nebulizer gas flow was fixed at 0.8 L/min. To analyze the data the Smart Analyzer Vision software was used.

2. Synthesis

2.1. 1-(2-Amino-9H-purin-6-yl)-1-methylpyrrolidin-1-ium chloride (**1**)



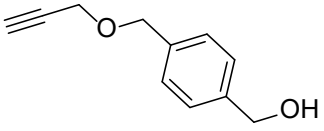
According to the literature-known procedure,[1,2] 6-chloroguanine (5 g, 33.1 mmol, 1 eq.) was dissolved in DMF (250 mL) for 35 min at 50 °C. After the reaction mixture cooled to room temperature, 1-mehtylpyrrolidine (11 mL, 99.3 mmol, 3 eq.) was added. The reaction mixture was stirred for 67 h at room temperature, before 125 mL acetone was added for complete precipitation. The white solid was filtered, washed with acetone and dried in vacuum to achieve 5.442 g (21.36 mmol, 65% [Ref.[1]: 66%]) of **1** as white solid.

MS (ESI+) = Calculated for $[C_{10}H_{15}N_6]^+$: 219.1353; found: 219.1358.

^{1}H-NMR (400 MHz, MeOD-d_4): δ = 2.18 (m, 2H), 2.35 (m, 2H), 3.72 (s, 3H), 3.98 (m, 2H), 4.81 (m, 2H), 8.17 (s, 1H).

^{13}C-NMR (101 MHz, MeOD-d_4): δ = 22.83, 52.75, 65.82, 117.65, 143.96, 153.03, 160.31, 161.10.

2.2. (4-((Prop-2-yn-1-yloxy)methyl)phenyl)methanol (**2**)



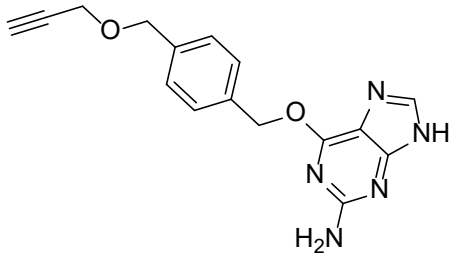
The reaction was adapted and modified from literature known procedures.[2,3] Briefly, in a 100 mL Schlenk round-bottom flask 1,4-benzenedimethanol (3 g, 21.71 mmol, 1.1 eq.) was dissolved in dry DMF (30 mL). NaH (1 g (60% in oil), 25 mmol, 1.28 eq.) was added over 15 min at 0 °C in small portions. The resulting suspension was stirred for 15 min before propargylbromide (2.9 g 19.54 mmol, 1 eq.) was added over 2 h at 0 °C. The crude mixture was stirred for 1 h at 25 °C. The reaction was quenched at 0 °C with ddH_2O, extracted with CH_2Cl_2, and purified by flash column chromatography (gradient: cyclohexane/ethyl acetate/methanol 6/4 to 6/4/1) to achieve 4-[prop-2-ynyloxymethyl]-benzyl (1.227 g, 6.96 mmol, 36% [Ref.[2]: 70%]) as brown oil.

MS (ESI+) = Calculated for $[C_{11}H_{12}O_2 + Na^+]^+$: 199.0730; found: 199.0726.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.80 (s, 1H), 2.47 (t, 1H, ^4J = 2.4 Hz), 4.17 (d, 2H, ^4J = 2.4 Hz), 4.61 (s, 2H), 4.68 (s, 2H), 7.35 (s, 4H).

^{13}C-NMR (101 MHz, $CDCl_3$): δ = 57.17, 65.20, 73.37, 74.81, 79.71, 127.22, 128.50, 136.80, 140.74.

2.3. O^6-(4-((Prop-2-yn-1-yloxy)methyl)benzyl)-9*H*-purine-2-amine (PYBG) (**3**)



The literature-known procedure to synthesize PYBG (**3**) was modified.[2] Briefly, a solution of **2** (872.29 mg 4.95 mmol, 3.6 eq.) in dry DMF (8 mL) was cooled to 0 °C before NaH (131.98 mg (60% in oil), 3.3 mmol, 2.4 eq.) was added in small portions over 10 min. Afterwards the mixture was stirred for 15 min at 0 °C. 1-(2-amino-9*H*-purin-6-yl)-1-methyl-pyrrolidinium chloride (**1**) (350 mg, 1.37 mmol, 1 eq.) and 4-dimethylaminopyridine (35.43 mg, 0.29 mmol, 0.21 eq.) were added and the reaction mixture was stirred for 1 h at 25 °C. Residual NaH was quenched by addition of 10 mL ddH_2O, the solvent was removed and the residue was purified by flash column chromatography (gradient: cyclohexane/ethyl acetate/methanol 6/4/1 to 6/4/2) to achieve PYBG (299.8 mg, 0.96 mmol, 71% [Ref.[2]: 72%]) as yellowish solid.

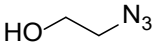
MS (ESI+) = Calculated for $[C_{16}H_{15}N_5O_2 + Na^+]^+$: 332.1118; found: 332.1119.

MS (ESI+) = Calculated for $[(C_{16}H_{15}N_5O_2)_2 + Na^+]^+$: 641.2343; found: 641.2352.

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 3.49 (t, 1H, ^{4}J = 2.4 Hz), 4.18 (d, 2H, ^{4}J = 2.4 Hz), 4.53 (s, 2H), 5.48 (s, 2H), 6.27 (s, 2H), 7.34 (d, 2H, ^{3}J = 8.1 Hz), 7.49 (d, 2H, ^{4}J = 2.4 Hz), 7.82 (s, 1H), 12.64 (s, 1H).

^{13}C-NMR (101 MHz, DMSO-d_6): δ = 57.33, 66.90, 70.90, 77.94, 80.63, 128.22, 128.89, 136.67, 137.86, 160.08.

2.4. 2-Azidoethan-1-ol (**6**)

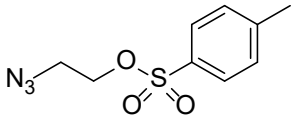


Since 2-azidoethanol (**6**) is a highly toxic, volatile and potentially explosive compound, it should not be isolated if not absolutely necessary.[4] It was not isolated herein. Even in solution, it must not be heated, especially in concentrated solutions.

Following a literature procedure,[5,6] 2-bromoethanol (14.18 mL, 200 mmol, 1 eq.) was solved in DMF (25 mL) and sodium azide (26.01 g, 400 mmol, 2 eq.) was added. The suspension was heated to 100 °C for 4 h in an explosion protected environment or an explosion protected cabinet. Afterwards the suspension was stirred at room temperature for 15 h and dichloromethane (200 mL) was added. The mixture was dried by addition over magnesium sulfate, filtered and used without further purification.

GC/MS (EI): m/z = 87.1; calculated for $[C_2H_5ON_3]^{+\bullet}$ ($[M]^{+\bullet}$): 87.1.

2.5. 2-Azidoethyl-(4-methylbenzene)-sulfonate (**7**)



A solution of 2-azidoethan-1-ol (**6**) in dichloromethane/DMF (~225 mL) obtained from the synthesis procedure described above was diluted with dichloromethane to give a total volume of 400 mL. It was assumed that the 2-bromoethanol had been completely converted (as confirmed by GC/MS). Triethylamine (41.6 mL, 300 mmol, 1.5 eq.) was added and the solution was stirred at room temperature for 15 min. Then, 4-methylbenzenesulfonic acid chloride (tosylchloride, 45.75 g, 240 mmol, 1.2 eq.) was added and the mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure. Diethyl ether was added (100 mL) and the mixture was filtered. The solid was washed with diethyl ether (3 x 100 mL), the combined organic phases were dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue was purified by column chromatography using cyclohexane:ethyl acetate 4:1 as the mobile phase. The pure product was isolated as a colorless liquid (29.32 g, 122 mmol, 61% [Ref.[6]: 66%])

R_f (TLC, cyclohexane:ethyl acetate 2:1) = 0.55

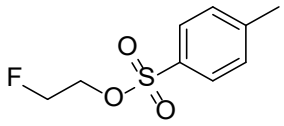
MS (ESI+) = Calculated for $[C_9H_{11}N_3O_3S + Na^+]^+$: 264.0413; found: 264.0421.

MS (ESI+) = Calculated for $[(C_9H_{11}N_3O_3S)_2 + Na^+]^+$: 505.0934; found: 505.0948.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.46 (s, 3H), 3.49 (t, 2H, ^3J = 5.1 Hz), 4.16 (t, 2H, ^3J = 5.1 Hz), 7.36 (d, 2H, ^3J = 8.3 Hz), 7.82 (d, 2H, ^3J = 8.3 Hz).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 21.7, 49.6, 68.1, 128.0, 130.0, 132.5, 145.3.

2.6. 2-Fluoroethyl-(4-methylbenzene)-sulfonate (**8**)



A solution of 2-fluoroethanol (1.06 mL, 18 mmol, 1 eq.) in pyridine (10 mL) was cooled to 0 °C and a solution of 4-methylbenzenesulfonic acid chloride (tosylchloride 3.77 g, 19.8 mmol, 1.1 eq.) in DCM (10 mL) was added to the cooled solution. The reaction mixture was stirred at room temperature overnight. Than hydrochloric acid (37%, 10 mL) in ice water (60 mL) was added carefully and the reaction mixture was extracted with DCM (3 x 10 mL). The organic layer was dried over magnesium sulfate and the solvents were removed under reduced pressure. The crude product was purified by column chromatography using cyclohexane:ethyl acetate 4:1. The product was isolated as a colorless oil (3.19 g, 14.6 mmol, (81%) [Ref.[7]: 99%]).

R_f (TLC, cyclohexane:ethyl acetate 4:1) = 0.22

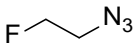
MS (ESI+) = Calculated for $[C_9H_{11}FO_3S + Na^+]^+$: 241.0305; found: 241.0299.

^{1}H NMR (300 MHz, $CDCl_3$) δ = 2.45 (s, 3H), 4.16 (dt, 2H, $^3J_{H,F}$ = 27.2 Hz), 4.57 (dt, 2H, $^3J_{H,F}$ = 47.1 Hz), 7.36 (d, 2H, $^3J_{H,H}$ = 8.3 Hz), 7.81 (d, 2H, $^3J_{H,H}$ = 8.3 Hz).

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 21.9, 68.7 (d, $^2J_{C,F}$ = 20.9 Hz), 80.7 (d, $^1J_{C,F}$ = 173.7 Hz), 128.2, 130.1, 132.8, 145.4.

^{19}F NMR (282 MHz, $CDCl_3$) δ = -224.6 (tt, 1F, $^2J_{H,F}$ = 47.1 Hz, $^3J_{H,F}$ = 27.2 Hz).

2.7. 1-Azido-2-fluorethane (**4**)



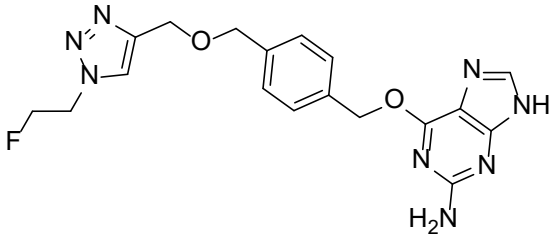
Since 1-azido-2-fluorethane (**4**) is a highly toxic, volatile and explosive compound, it should not be isolated and was not isolated herein. Even in solution, it must not be heated, especially in

concentrated solutions. The solutions are not stable but can be stored at -25 °C for at least one month.

To prepare a ~0.3 mmol/mL solution the following procedure was used:

Sodium azide (219 mg, 3.37 mmol, 3 eq.) was added to a solution of 2-fluoroethyl-(4-methylbenzene)-sulfonate (**8**) (245 mg, 1.12 mmol, 1 eq.) in DMF (3.5 mL) and the reaction mixture was stirred at room temperature overnight. After *full conversion (GC/MS),* the mixture was filtered and used for further steps without purification.

2.8. 6-((4-(((1-(2-Fluoroethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)oxy)-*9H*-purin-2-amine (**5**)



The alkyne derivative (**3**) (50 mg, 0.16 mmol, 1 eq.) and a solution of 1-azido-2-fluorethane (**4**) (~0.3 mmol/mL in DMF; 14 mg, 0.16 mmol, 0.54 mL of solution in DMF, 1 eq.) were stirred in DMF (2 mL). Copper(II)sulfate pentahydrate (8 mg, 32 µmol, 20 mol%) and sodium ascorbate (8 mg, 40 µmol, 25 mol%) were freshly dissolved in water (0.1 mL each). The two aqueous solutions were mixed and shaken until an orange color appeared. Then the catalyst was added to the alkyne/azide solution. The mixture was stirred at room temperature for 24 h. The residue was taken up with ethyl acetate (8 mL) and was washed with water (8 mL). The aqueous phase was extracted again with ethyl acetate (3 x 5 mL). The combined organic layers were washed with EDTA-solution (0.1 N, 5 mL) and brine (5 mL). The organic layer was dried over magnesium sulfate and the solvents were removed under reduced pressure. The residue was purified by column chromatography using dichloromethane:methanol 9:1 as mobile phase. The product was isolated as a slightly yellow oil (63 mg, 0.158 mmol, 98%).

R_f (TLC, chloroform:methanol 9:1) = 0.38

MS (ESI+) = Calculated for $[C_{18}H_{20}FN_8O_2 + H^+]^+$: 399.16878; found: 399.16839.

MS (ESI+) = Calculated for $[C_{18}H_{20}FN_8O_2 + Na^+]^+$: 421.15072; found: 421.14982.

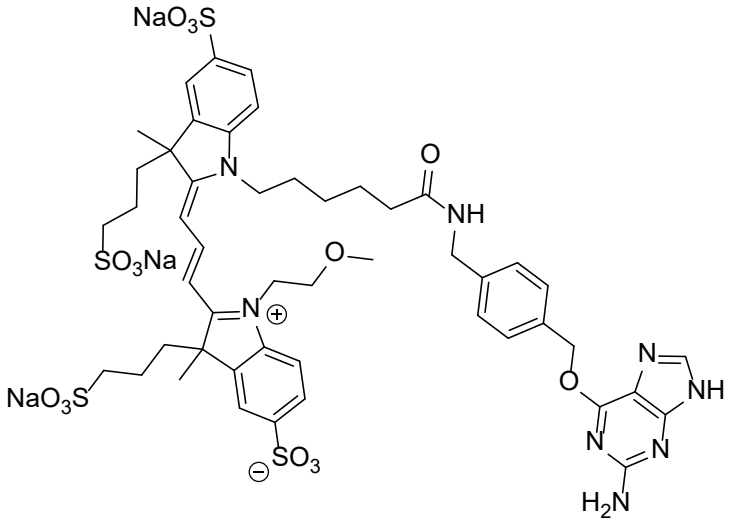
MS (ESI+) = Calculated for $[(C_{18}H_{20}FN_8O_2)_2 + Na^+]^+$: 819.31222; found: 819.31184.

^{1}H NMR (600 MHz, DMSO-d_6) δ = 4.55 (s, 2H), 4.58 (s, 2H), 4.70 (dt, 2H, $^3J_{H,F}$ = 60.1 Hz), 4.82 (dt, 2H, $^3J_{H,F}$ = 79.2 Hz), 5.48 (s, 2H), 6.27 (bs, 2H), 7.36 (d, 2H, $^3J_{H,H}$ = 7.9 Hz), 7.49 (d, 2H, $^3J_{H,H}$ = 7.9 Hz), 7.80-7.90 (m, 1H), 8.17 (s, 1H), 12.42 (s, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ = 50.0 (d, $^{2}J_{C,F}$ = 19.8 Hz), 62.8, 66.5, 71.0, 81.9 (d, $^{1}J_{C,F}$ = 168.2 Hz), 124.4, 127.6, 128.4, 136.0, 138.0, 144.1, 159.6, 162.3.

^{19}F NMR (564 MHz, $CDCl_3$) δ = -222.2 (tt, 1F, $^{2}J_{H,F}$ = 47.1 Hz, $^{3}J_{H,F}$ = 27.7 Hz).

2.9. Trisodium 2-((*E*)-3-((*Z*)-1-(6-((4-(((2-amino-9H-purin-6-yl)oxy)methyl)benzyl)amino)-6-oxohexyl)-3-methyl-5-sulfonato-3-(3-sulfonatopropyl)indolin-2-ylidene)prop-1-en-1-yl)-1-(2-methoxyethyl)-3-methyl-3-(3-sulfopropyl)-3H-indol-1-ium-5-sulfonate (*O*^{6}-Benzylguanin-NH2-DY549P1) (**9**), Benzylguanin coupled to Dye DY549P1 from FA. DYOMICS



The NHS-ester of DYOMICS® DY549P1 (1.0 mg, 0.96 µmol, 1 eq.) was dissolved in dry DMF (250 µL) and added to a solution of 6-((4-(aminomethyl)benzyl)oxy)-*9H*-purin-2-amine (1.3 mg, 4.81 µmol, 5 eq.) in a mixture of dry DMF (250 µL) and DIPEA (0.81 µL, 0.62 mg, 4.81 µmol, 5 eq.) carefully excluding air, water and light. The reaction was shaken for 24 h at room temperature. The solvent was removed under reduced pressure and the residue was re-dissolved in water (100 µL). The mixture was purified by HPLC (KNAUER Azura). The fraction containing the product was neutralized with sodium hydrogencarbonate solution (0.1N) to contain residual trifluoracetic acid. Solvents were removed (max. 30 °C) under reduced pressure and the aqueous residue was lyophilized and re-dissolved in water (2 mL). The solution was eluted to a C-18 Plus (SepPak®, FA. WATERS) cartridge (preconditioned with methanol (5 mL) and water (10 mL)) and washed with water (10 mL). The cartridge was eluted with methanol (10 mL), the solvent was removed (max. 30 °C) under reduced pressure and the residue was taken up with water and lyophilized. The product was isolated as a strongly pink powder (0.6 mg, 0.50 µmol, 52%).

*Table 1: HPLC purification parameters of compound **9***

Injection type / flow	Liquid / 5 mL/min
Equilibration / column type	5 CV / Eurospher 100-5 C18 HPLC column, 250 x 8 mm
Total runtime without equilibration time	16.0 min
Solvents, gradient	Acetonitril and Water (both containing 0.1 % TFA), From 0 min to 12.0 min 90% H_2O to 60% H_2O, than isocratic from 12.0 min to 14.0 min, gradient to 90% H_2O, from 14.0 min to 16.0 min.
Detection	Diode array detector

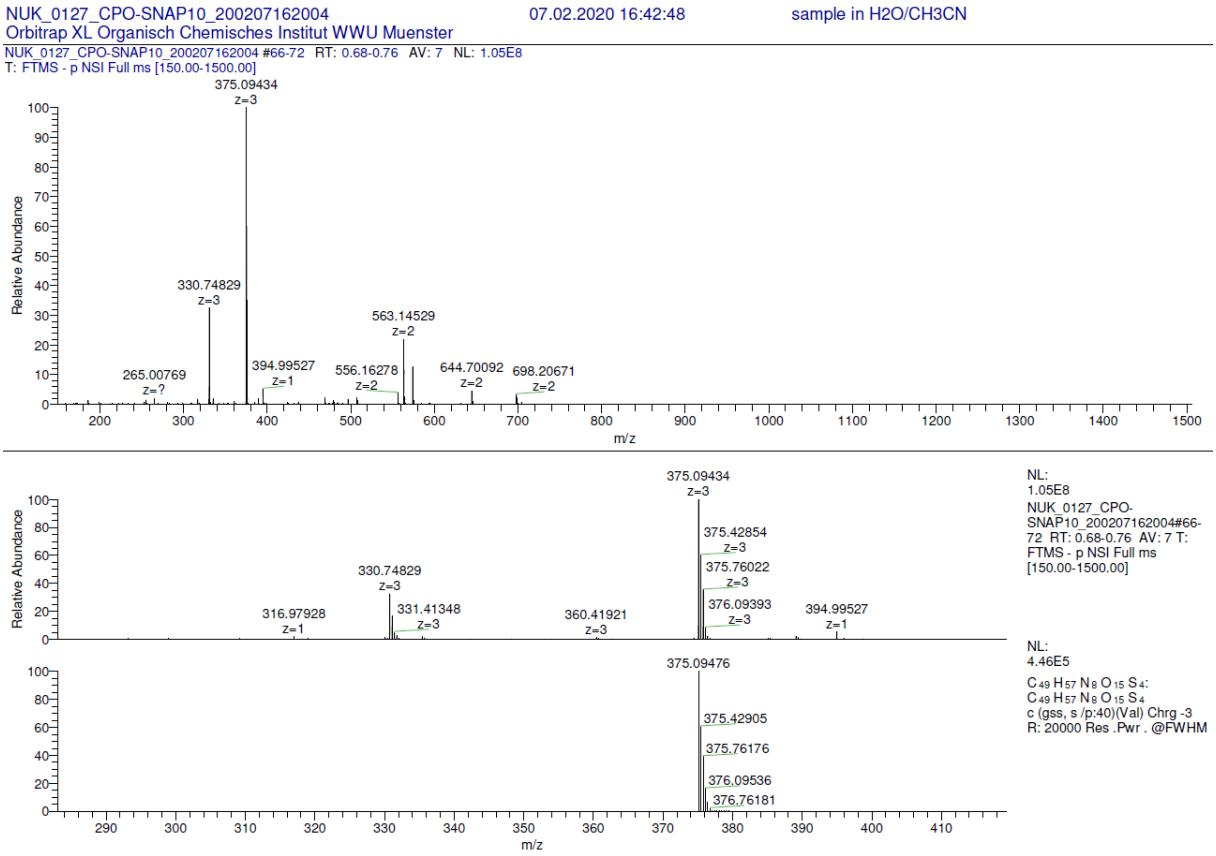
R_t (HPLC KNAUER Azura) = 6.65 min.

MS (ESI-) = Calculated for $[C_{49}H_{57}N_8O_{15}S_4]^{3-}$: 375.09476; found: 375.09434.

UV: λ_{max}: 548 nm.

FT: $\lambda_{max,emission}$: 573 nm.

Due to the low amount of compound and the pricing of the dye, NMR-spectroscopy was not possible.



*Supplementary Figure 1: MS-ESI- Spectra of compound **9***

3. Radiosynthesis: General considerations

The *radiosynthesis* of the compound [^{18}F]**5** was carried out in a semiautomated fashion with a modified PET tracer radiosynthesiser TRACERLab FXFDG and the TRACERLab Fx software (both GE HEALTHCARE). The aqueous solutions of [^{18}F]fluoride were produced by irradiation of enriched [^{18}O]H_2O (enrichment min. 97 %) on a HP Eclipse cyclotron (CTI-SIEMENS) using a (max.) 10 MeV proton beam.

All *chemicals*, *reagents*, and *solvents* for the radiosynthesis of tracers were of analytical grade, purchased from commercial sources and used without further purification unless otherwise specified. Only solvents of pharmaceutical purity from ABX and Milli-Q®-water or water for injection from B. BRAUN were used for radiosynthesis.

Sep-Pak® C-18 Plus cartridges from WATERS were used for the *purification* of radiolabeled compounds. The cartridges were conditioned with ethanol (5 mL) followed by water (10 mL) prior to use.

In some cases, vessels were coated on the surface using Sigmacote® from SIGMA-ALDRICH.

Identification of labeled compounds was performed by co-injection of a non-radioactive [^{19}F]-containing reference compound on HPLC system B.

Radiochemical yields are based on the initially used activity and are generally decay corrected.

Radiochemical purity is the ratio of the fraction of product radioactivity and total radioactivity and was determined by analytical HPLC system B.

The *time of radiosynthesis* is given as the time between measurement of the starting activity and reconstitution of the tracer for application.

Measurements of total radioactivity for the determination of experimental log*D*-values were performed using a gamma-counter Wizard^2 2480 from PERKINELMER.

General measurements of radioactivity were done on an Isomed 2010-activimeter from MED Nuklearmedizintechnik.

For determination of the *molar (radio)activity* three linear calibration curves were created with different concentrations of the non-radioactive fluorinated reference compound **5** (2 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL (CH_3CN:H_2O 1:1)). Of each sample 20 µL were loaded on the analytical Radio-HPLC and the peaks in the UV-channel were integrated. Three calibration curves were determined by linear regression analysis of these measured values. This linear calibration curve was used to determine the amount of the compound (sum of non-radioactive and radiolabeled compound) in the product fraction after radiosynthesis and purification. The specific activity A_S and the molar activity A_M were calculated using the following equations:

$$A_S = \frac{A_C\left[\frac{MBq}{mL}\right]}{C\left[\frac{\mu g}{mL}\right]}; A_M = A_S\left[\frac{MBq}{\mu g}\right] \cdot M\left[\frac{\mu g}{\mu mol}\right]$$

Equation 1 and 2: Calculation of the specific and molar (radio)activities AS and AM. Ac is activity per mL of the activity of the formulated solution of the radiolabeled product and c is the concentration of the compound determined with the calibration curve.

Centrifugation was done in Eppendorf vessels (1.5 mL) in a MCF-2360 zentrifuge from LMS CONSULT GMBH & CO. KG.

Incubation of samples for the determination of serum stability was performed using a PST-60 HL plus thermo shaker from KISKER Biotech GmbH & Co. KG.

Separation, *purification* and determination of *radiochemical purity* of the compounds were performed by using semipreparative and analytical reversed-phase HPLC systems A and B (see below).

Semipreparative HPLC A: A WELLCHROME K-500 pump and a WELLCHROME K-501 pump, a K-2000 UV detector (HERBERT KNAUER GMBH), a NaI(Tl) Scintibloc 51 SP51 γ-detector (CRISMATEC), and a ACE 5 AQ column (10 mm × 250 mm). The recorded data was processed by the GINA Star software (RAYTEST ISOTOPENMESSGERÄTE GMBH).

Method:

Solvent A:	purified water (MilliQ®) + 0.1 % (V/V) trifluoroacetic acid
Solvent B:	acetonitrile + 0.1 % (V/V) trifluoroacetic acid
Gradient elution (% A):	0 - 2 min: 95 %; 2 - 16 min: gradient from 95 % to 5 %; 16 - 24 min: 5 %; 24 - 32 min: gradient from 5 % to 95 %.
Flow rate:	5,5 mL/min
Injection volume:	1000 µL
Detection:	UV: 254 nm
	γ-Counter: cpm

Analytical HPLC B: Two Smartline 1000 pumps and a Smartline UV detector 2500 (HERBERT KNAUER GMBH), a GabiStar γ-detector (RAYTEST ISOTOPENMESSGERÄTE GMBH) and a Nucleosil 100-5 C–18 column (4 mm × 250 mm). The recorded data was processed by the GINA Star software (RAYTEST ISOTOPENMESSGERÄTE GMBH).

Method:

Solvent A:	purified water (MilliQ®) + 0.1 % (V/V) trifluoroacetic acid
Solvent B:	acetonitrile + 0.1 % (V/V) trifluoroacetic acid
Gradient elution (% A):	0 - 9 min: gradient from 90 % to 10 %; 9 - 15 min: gradient from 10 % to 90 %.
Flow rate:	1,0 mL/min
Injection volume:	20 µL
Detection:	UV: 254 nm
	γ-Counter: cpm

Determination of the distribution coefficient ($logD_{exp}$). Following a method described by Prante *et al.* [8], the *distribution coefficient ($logD_{exp}$)* of the radioactive compound was determined in a two-phase system consisting of 1-octanol and PBS-buffer (pH = 7.4) to determine the lipophilicity. For this purpose, the corresponding ligand (~ 20 kBq) was dissolved in PBS-buffer (500 µL). 1-Octanol (500 µL) was added and the mixture was shaken at room temperature for 1 min. To achieve phase separation, the system was centrifuged for 2 min at 3000 rpm. Subsequently a part of the octanol phase (400 µL) was removed and buffer (400 µL) was added. The combined phases were shaken and layers were separated according to the description above. Aliquots of the buffer and octanol layers (3 × 100 µL from every layer) were taken to measure radioactivity in a γ-counter. The measurement provided the activity in counts per minute (cpm), the values were decay corrected. By calculating the quotient of $\frac{\text{cpm (1−Octanol)}}{\text{cpm (PBS)}}$ the *$logD_{exp}$* can be determined as $\log\frac{\text{cpm (1−Octanol)}}{\text{cpm (PBS)}}$.

The *serum stability* of the radioactive compound was evaluated by incubation in human and murine serum at 37 °C for up to 120 min. An aliquot of radioactive product (20 µL, ~5 MBq) in PBS-buffer was added to a sample of serum (200 µL), and the mixture was incubated at 37 °C. Samples of 20 µL each were taken after periods of 10, 30, 60, 90 and 120 min and quenched in MeOH/CH_2Cl_2 (1:1 (v/v), 100 µL) followed by centrifugation at 3000 rpm for 2 min. The clear solution was analyzed by analytical radio-HPLC system B.

3.1. Radiosynthesis of 6-((4-(((1-(2-[^{18}F]fluoroethyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)oxy)-9H-purin-2-amine [^{18}F]5 using the prostetic group 1-azido-2-[^{18}F]fluorethane

In a computer-controlled synthesizer, aqueous [^{18}F]fluoride anions (2646 – 5075 MBq) were passed through an anion exchange pre-conditioned cartridge (Sep-Pak® Light QMA, CO_3^{2-} as

counter ion). [^{18}F]Fluoride ions were eluted from the cartridge with a solution of Kryptofix®2.2.2 (20±1 mg, 53 µmol) and K_2CO_3 (40 µL, 1 M, 40 µmol) in water (200 µL) and CH_3CN (800 µL) into the reactor. The aqueous solution was carefully evaporated to dryness, first by heating in a stream of helium, then reducing the pressure and heating to higher temperature. First a temperature of 56 °C was applied for 5 min, then the temperature was increased to 84 °C and kept for 10 min.

A solution of azidoethyl tosylate (**7**) (20±1 mg, 83 µmol) in CH_3CN (500 µL) was added and the mixture was heated to 110 °C for 3 min. The formed 1-azido-2-[^{18}F]fluorethane ([^{18}F]**4**) was directly distilled from the reactor in a stream of helium into a flask containing the reaction mixture for the [3+2] dipolar cycloaddition within the 3 min reaction time. The flask was cooled to approximately -10 °C. Total received activity was determined.

rcy: 71 ± 6 % (d. c., n = 14)

t: 110 ± 49 min (n = 14)

The flask contained the alkyne **3** (5±1 mg) dissolved in DMF (500 µL) and a pre-reacted mixture of $CuSO_4 \cdot 5H_2O$ (10±1 mg dissolved in 100 µL H_2O) and sodium ascorbate (16±1 mg dissolved in 100 µL H_2O). The reaction mixture was stirred for 30 min. at 60 °C. Then, the mixture was added to ammonium buffer (1N NH_4Cl and 1N NH_4OH in water, 8 mL) and passed through a conditioned Sep-Pak® C18+ short cartridge. The cartridge was washed with water (10 mL). This step is crucial to break down most of the very stable copper-complex formed with the precursor as well as the ^{18}F-labeled product. The cartridge was processed further in different ways:

1. Elution with a mixture of dimethylformamide and triethylamine (9:1; 1 mL) and washing with water (1 mL). Doing so approximately one quarter of the trapped activity can be eluted.
2. Elution with ammonia in methanol (7 N, 5 mL) and washing with water (1 mL). Reduction of the solvent using reduced pressure. Doing so, most of the trapped activity can be eluted, but the reduction of the solvent is a time consuming extra step and the radioactive dose for the operator is considerably higher. This procedure was only performed when high resulting activities were necessary.

In both cases purification was performed by semi preparative radio-HPLC. The receiving flask contained sodium bicarbonate solution (8.4%, 100 µL) and sodium ascorbate (1 mg) to prevent acid-catalyzed decomposition and oxidative degradation, respectively. The [^{18}F]**5**-containing fraction was evaporated to dryness under reduced pressure and re-dissolved in sterile PBS buffer.

rcy: 13.6 ± 4.9 % (d. c., n = 14, total yield over two steps)

rcp: constantly >99 %, determined by analytical radio-HPLC and radio-TLC

t: 122 ± 10 min (n = 14)

A_m: 0.18 – 15.6 GBq/µmol

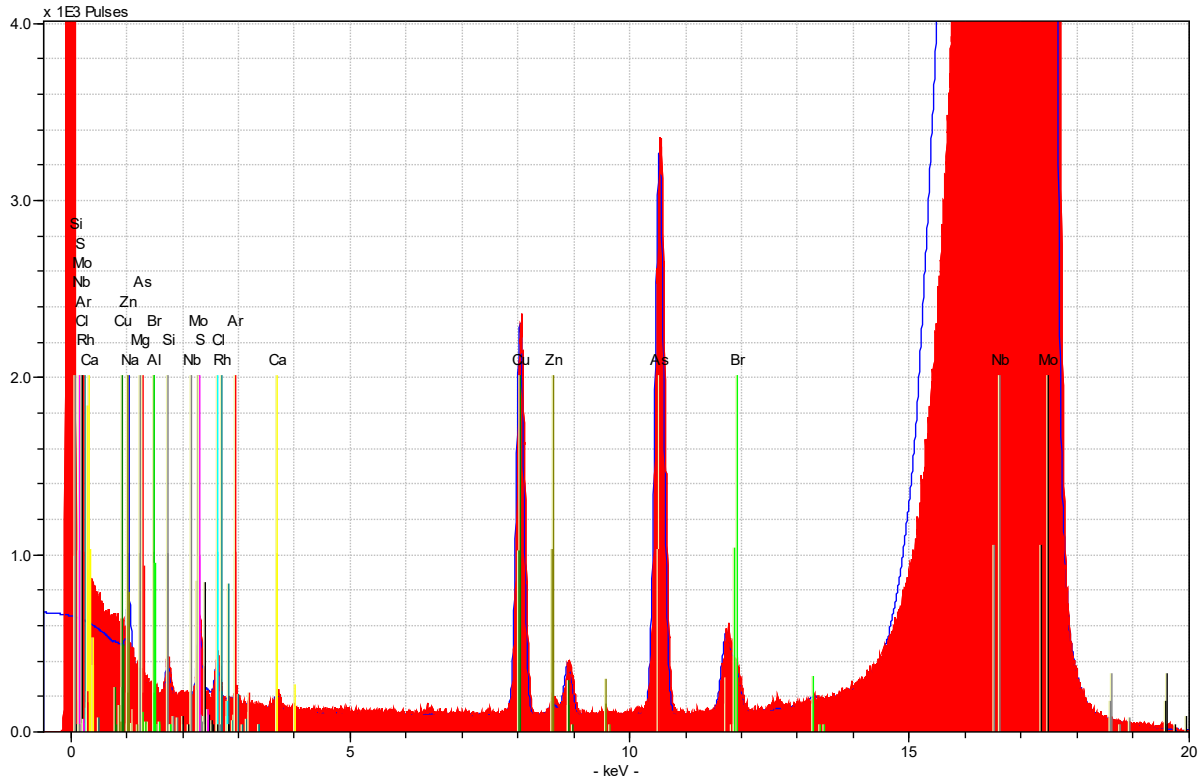
$logD_{7.4}$: 0.98 ± 0.12

t_r (semipreparative radio-HPLC): 8.7 min

t_r (analytical radio-HPLC): 7.2 min

R_f (Silica 60PF, DCM:MeOH 9:1, RAYTEST miniGita Gamma TLC Scanner): 0.33

Since both the precursor and the product formed very stable copper complexes, the concentration of copper in the final solutions were determined by ICP-OES in cooperation with the working group of Prof. Dr. Uwe Karst.

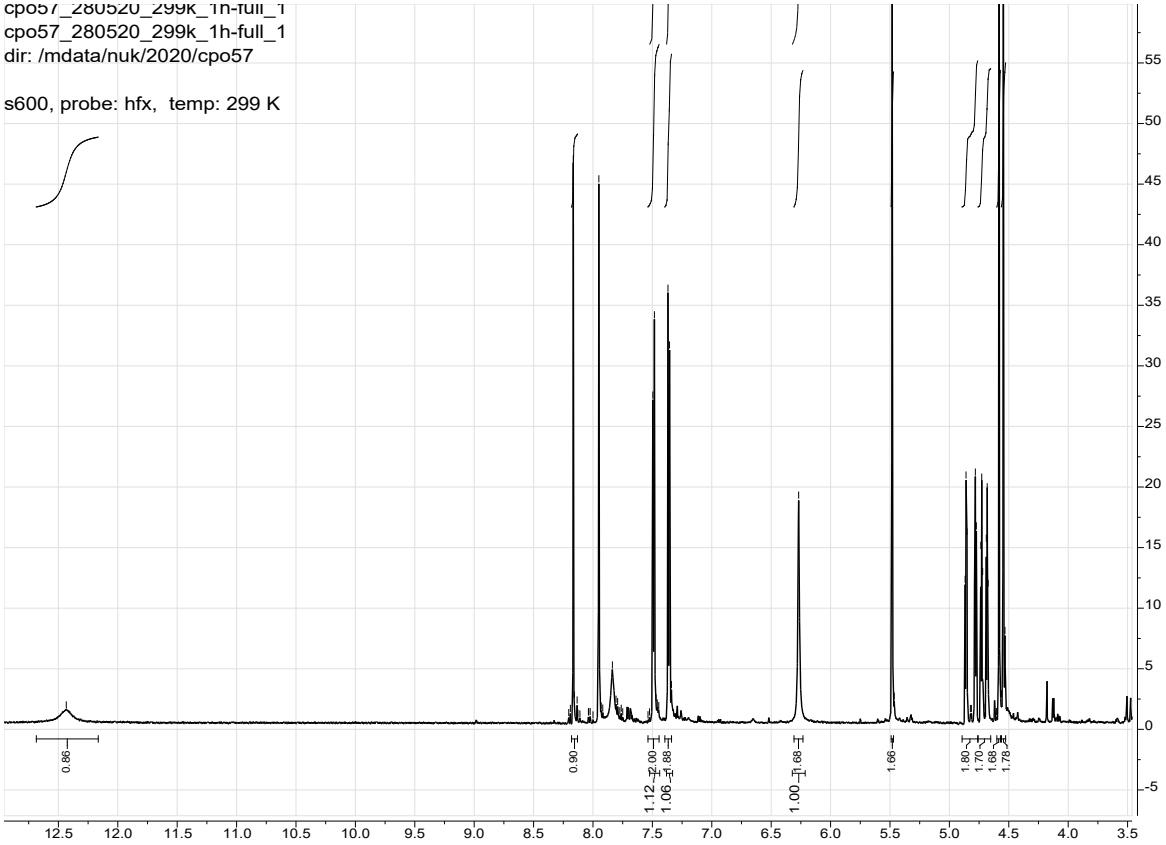


Supplementary Figure 2 ICP-OES spectrum of C18+ eluate after decay of [^{18}F]

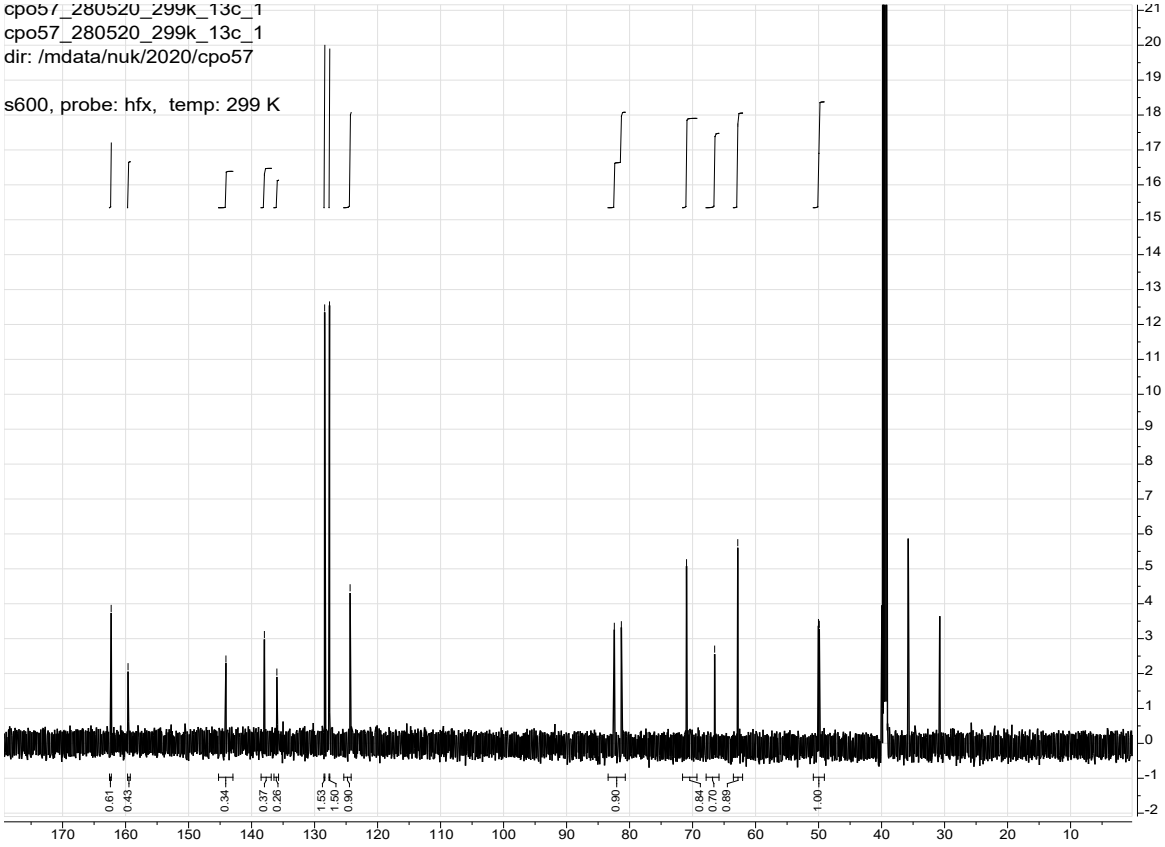
The total concentration of copper in the eluate of the C18+ cartridge was found to be 10.3 mg/L. When analyzing a sample after HPLC-purification a final copper-concentration below 23 µg/L was found.

4. NMR Spectra of new compounds

6-((4-(((1-(2-Fluoroethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)benzyl)oxy)-*9H*-purin-2-amine (5)



*Supplementary Figure 3: [1]H-NMR Spectra of compound **5***



*Supplementary Figure 4: ^{13}C-NMR spectra of compound **5***

*Supplementary Figure 5: ^{19}F-NMR spectra of compound **5***

5. *In vitro* and *in vivo* studies

Plasmids, nucleic acids, enzymes and bacteria

All DNA oligonucleotides and primers were synthesized by EUROFINS GENOMICS GERMANY GMBH. Sanger sequencing of plasmids was performed by EUROFINS GENOMICS GERMANY GMBH. pLV-EF1a-IRES-Blast was a gift from Tobias Meyer[9] (ADDGENE plasmid # 85133 ; http://n2t.net/addgene:85133 ; RRID:Addgene_85133). pSNAPf-ADRβ2 Control Plasmid was a gift from NEW ENGLAND BIOLABS & Ana Egana (ADDGENE plasmid # 101123 ; http://n2t.net/addgene:101123 ; RRID:Addgene_101123). psPAX2 and pMD2.G were a gift from Didier Trono (ADDGENE plasmid # 12260 ; http://n2t.net/addgene:12260 ; RRID:Addgene_12260; ADDGENE plasmid # 12259 ; http://n2t.net/addgene:12259 ; RRID:Addgene_12259). Sequences of primers and DNA oligonucleotides are listed in Supplementary Table S1. Q5® Hot Start High-Fidelity 2X Master Mix (NEW ENGLAND BIOLABS # M0494L) was used for PCR-amplification of all DNA fragments and oligonucleotides. NEBuilder® HiFi DNA Assembly Master Mix (NEW ENGLAND BIOLABS # E2621L) was used for fragment and vector ligation. Plasmids were expanded by heat-shock mediated transformation of recombinase-deficient E. coli K12 (NEB® Stable Competent E. coli, NEW ENGLAND BIOLABS # C3040H) grown on LB-agar plates containing 100 µg/mL ampicillin (SIGMA-ALDRICH # A9518). For mini-sized preparations, a single colony was inoculated in 4.5 mL of 100 µg/mL ampicillin containing LB medium and incubated for 24 h at 30°C and 350 rpm in an incubation shaker (INFORS Multitron). For medium-scale preparations, 250 mL of LB medium containing 100 µg/mL ampicillin was inoculated with a single bacterial colony for 30 h at 30°C and 250 rpm in baffled shaker flasks (THERMO FISHER # 4110-0250PK).

The SNAP-tag is a powerful tool for site-specific, covalent labeling of proteins. Proteins are usually tagged with the SNAP-tag as either C- or N-terminal fusion of the two proteins which can be separated by a short linker to aid proper folding of the fusion construct. In general, intracellular as well as membrane-proteins can be tagged with the SNAP-tag technology[11]. In this study, we chose the GPI-anchor motif of human CNTN1 in virtue of its small size and efficient GPI-anchoring[12], making it a favorable choice for lentiviral expression or applications where the cloning capacity of the viral vector is limited. To generate a GPI-anchored SNAP-tag fusion protein that is efficiently transported to the plasma-membrane, the signal peptide of the murine CD14 receptor and a HiBiT-tag (for luminescent detection of the protein, data not shown) was added N-terminally to SNAPf which was fused to the CNTN1 GPI-anchor motif at its C-terminus (Supplementary Figure 6).

The coding sequence of SNAPf was subcloned from pSNAPf-ADRβ2 Control Plasmid and inserted into pLV-EF1a-IRES-Blast in intermediate steps. Briefly, a synthetic fragment encoding the N-terminal signal peptide (amino acids 1-15, refseq NM_009841.4) of the murine CD14 receptor, a short linker (3 x GS) and the HiBiT-tag (PROMEGA CORPORATION) sequence followed by a second linker (oligo-1), was PCR-amplified with primer pair P1 and assembled into the PCR-amplified vector (primer pair P2) pLV-EF1a-Tk39-T2A-SNAPf-SMADT44-IRES-Blast (D.A.Depke, unpublished) through HiFi-assembly to generate pLV-EF1a-CD14sig-HiBiT-SNAPf-SMADT44-IRES-Blast. In a second step, this vector was PCR-amplified with primers P3 and assembled with the PCR-amplified synthetic fragment oligo-2, containing a fragment encoding the sequence motif of the GPI-anchor of the human contactin-1 gene (amino acids 994-1018, refseq NM_001843.4), yielding pLv-EF-1α-SNAPbiT-IRES-BSD.

Supplementary Figure 6: Schematic representation of the expression cassette of pLv-EF-1α-SNAPbiT-IRES-BSD. SNAPf is fused to a HiBiT-tag and the signal peptide of the murine CD14 receptor at its N-terminus to facilitate its transport to the plasma-membrane. At its C-terminus, the GPI-anchor motif of hCNTN1 was added, resulting in the attachement of a glycosylphosphatidylinositol to the protein and its insertion into the membrane. Proteins are separated by short linker sequences consisting of 3-4 GS residues to ensure proper folding of the fusionprotein (L). BSD: Blasticidin-S deaminase, IRES: internal ribosome entry site, WPRE: Woodchuck hepatitis virus post-transcriptional regulatory element.

Supplementary Table S1. Primers and oligonucleotides.

Oligo/Primer	*Sequence (5' → 3')*
Oligo 1	ATGGAGAGAGTGCTTGGCCTGTTGCTGCTCCTGCTGGTTCACGC CGGATCTGGGTCTGGGAGTGTCTCCGGTTGGAGGCTCTTCAAGA AAATCTCCGGAGGTAGCGGCTCAGGAAGCGGCAGC
P1-*forward*	GGAAGCGGCAGCATGGACAAAGACTGCGAAATG
P1-reverse	CTCCATGGTGGCGTTCGCGATCTAGAGCCG
Oligo 2	GGGTCTGGCAGCAATGGGTCAGGGTCCAACACCACTGGATCAG GCAGTGGTGGATCTGGCGTTTCCCAGGTGAAGATCAGTGGTGCT CCCACACTCAGCCCTAGCCTGCTTGGACTGCTGTTGCCAGCCTT CGGCATTCTCGTCTACCTGGAGTTT
P3-forward	CCTGGAGTTTTAATGAATTCCTCGAGGGCGGC
P3-reverse	TGCCAGACCCACCTGCAGGACCCAGCCC

Coating of cell culture vessels. For gelantine coating of 100 mm petri dishes, 8 mL of a sterile 0.3% gelantine (SIGMA-ALDRICH # G9391) solution in dH_2O were added and plates were incubated overnight at 37°C in a tissue culture incubator. Then plates were washed 2 x with 10 mL of sterile dH_2O and air dried. Collagen-I was from THERMO FISHER (#A1048301) and diluted to 50 µg/mL in sterile 20 mM acetic acid in dH_2O. Volumes were scaled to a final amount of 5 µg/cm^2 of culture surface and cell culture vessels were incubated for 2 h at room temperature. The coating solution was removed, and plates were washed 2 x with sterile dH_2O.

Isolation and purification of nucleic acids. Mini-sized plasmid isolations were performed with the MACHEREY-NAGEL NucleoSpin™ Plasmid EasyPure kit (Macherey-Nagel™ # 740.727.250) according to the manufacturer's instructions. For midi-sized plasmid purifications and lentivirus production, the NucleoBond® Xtra Midi Plus EF kit (Macherey-Nagel™ # 740422.10) was used. Gel extractions of DNA fragments following electrophoresis in TAE-buffer (40 mM Tris-Base, 1 mM EDTA, 20 mM acetic acid, pH 8.0) were performed with the MACHEREY-NAGEl NucleoSpin™ Gel and PCR Clean-up Kit (Macherey-Nagel™ # 740609.50) following the manufacturer's instructions.

Cell culture. Gli36 glioblastoma cells and HEK293FT cells were grown in Dulbecco's Modified Eagle Medium (DMEM, high glucose, GlutaMAX™ Supplement, Gibco™ #10566016) supplemented with 1 % penicillin-streptomycin (P/S, SIGMA ALDRICH #P0781), 10 % fetal bovine serum (PAN-BIOTECH GMBH #1028162) in T-75 tissue-culture flasks (GREINER #2002380) in a 5% CO_2 atmosphere at 37°C. Cells were passaged every 2 – 3 days.

Production of lentivirus. HEK293FT producer cells were transfected by an optimized DNA-$CaPO_4$ method.[10] HEK293 FT cells were seeded at a density of 2.5 x 10^6 in 10 mL of fully supplemented medium in 0.3 % gelantine-coated 100 mm petri dishes (Corning® # 430167) 24 h before transfection. Prior to transfections, the medium was replaced with fully supplemented DMEM containing 10 µM chloroquine-diphosphate (SIGMA-ALDRICH #C6628). Plasmids pLv-EF-1α-SNAPbiT-IRES-BSD, psPax2 and pMD2.G were mixed at a molar ratio of 2:1:1 in a 1.5 mL reaction tube and sterile distilled water and 50 µl of an aqueous 2.5 M $CaCl_2$ solution were added to the tube to yield a final volume of 500 µL. A total of 18.7 µg DNA (340 ng/cm^2) was used per transfection reaction of cells grown in a 100 mm petri-dish. The plasmid solution was slowly and dropwise added to 500 µL of 2x HeBS (50 mM HEPES, 10 mM KCl, 12 mM dextrose, 280 mM NaCl, 1.5 mM Na_2PO_4, pH 7.05) in a 15 mL falcon tube under constant vortexing under aseptic conditions. The reaction was incubated for 10 min at room temperature and then added dropwise to the cells. On the next day, the medium was replaced with fresh DMEM, supplemented with 10% FBS, 1 % non-essential amino acids (MEM, THERMO FISHER # 11140035), 1 % sodium pyruvate (THERMO FISHER # 11360039) and 1% (P/S).

Lentivirus-containing supernatant was harvested 48 h after transfections. The supernatant was pooled in 50 mL Falcon tubes, centrifuged at 450 x g for 10 min to remove cellular debris and sterile filtered through 0.45 µm syringe filters. Polybrene (MERCK MILLIPORE # SLHP033RS) was added to a final concentration of 8 µg/mL and the solution was divided into 1 mL aliquots and stored at -80°C until use.

Generation of clonal cell lines. 3 x 10^5 Gli36 cells were seeded in 2 mL of fully supplemented medium. On the next day, polybrene (SIGMA-ALDRICH TR-1003-G) was added to the medium to a final concentration of 8 µg/mL and 200 µl of lentiviral supernatant was added to each well. Cells were cultured for 48 h, then the cells were washed 3 x with 5 mL of PBS (PAN-BIOTECH GMBH # P04-36500) and cultured for another 24 h in fresh medium. 72 h after transduction, cells were harvested, and 2 x 10^5 cells were seeded in 10 mL of fully supplemented DMEM in 100 mm petri dishes. On the next day, the medium was replaced with fully supplemented DMEM containing 25 µg/mL Blasticidin S (Invivogen # ant-bl-05). Cells were cultured for another 10 days in the presence of Blasticidin S and 5 colonies of cells stably expressing the transgene were isolated with cloning cylinders (MERCK MILLIPORE # TR-1004), detached with 50 µL of trypsin (Fisher Scientific # 11570626) and transferred to 96-well plates. Clonal lines

were expanded and transferred to larger culture vessels when they reached ~80% confluency. All experiments described in this study were performed with a single clonal cell line (Gli36ΔEα-SNAPbiT-BSD 208.C1).

Fluorescent labeling and pre-dosing of cells with [^{18}F]FTBG. 5 x 10^4 SNAP-tag$^+$ or SNAP-tag$^-$ cells Gli36 cells were seeded into each well of a collagen-I coated chambered glass slide (3 Well Chamber, removable, IBIDI #80381) and cultured for 24 h in fully supplemented medium. Cells were then incubated with medium containing 4 µM of cell-impermeable BG-Dy549P1 or fresh medium for 30 min at 37°C. Then, cells were washed 2 x with full medium and incubated for another 30 min in medium containing 4 µM of cell-permeable SNAP-Cell® 647-SiR (NEW ENGLAND BIOLABS # S9102S) or were left untreated. Following incubation with fluorescent benzylguanines, cells were washed 2 x with full medium, 2x with PBS and fixed with 4% paraformaldehyde (PFA) in saline (CARL ROTH #PO87.1) for 20 min at room temperature (RT). After fixation, cells were washed 2 x with PBS and permeabilized in PBS containing 10% FBS and 0.2% Tween-20 (SIGMA-ALDRICH # P2287) for 1 h at RT. Immunostainings with a monoclonal anti α-tubulin-AlexaFluor® 488 antibody (ABCAM # ab195887) at 1:200 dilution were performed overnight at 4°C in PBS containing 10% FBS and 0.2% Tween-20 on a rocker platform with gentle agitation. The next day, samples were washed 2 x with PBS, stained with DAPI (10 mg/mL in dH_2O, SIGMA ALDRICH # D9542) at a 1:3000 dilution in PBS for 5 minutes at RT, washed 2 x with PBS and mounted with Mowiol® (Mowiol solution: 6 g glycerol, 2.4 g Mowiol® 4-88, CARL ROTH # 0713.1) and 10 mm round glass coverslips. Images were acquired on a LEICA TCS SP8 confocal microscope.

For pre-dosing experiments, cells were incubated with 500 µL of full medium containing 200 kBq/mL [^{18}F]FTBG for 30 min at 37°C and washed 2 x with full medium before the cells were fluorescently labeled as described above. All experiments were performed in triplicates.

Radiouptake-assays. For radiouptake assays, 5 x 10^4 SNAP-tag$^+$ or SNAP-tag$^-$ cells were seeded into the wells of collagen-I coated 24-well plates and incubated for 24 h at 37°C. The medium was replaced with fresh medium 4 hours before the assay was performed. Dilutions (0, 2, 10, 20, 50, 100, 150, 200 kBq/mL) of [^{18}F]FTBG were prepared in full medium supplemented with 25 mM HEPES. The culture supernatant was removed and 500 µL of each dilution was added to triplicate wells of the assay plates. Cells were incubated for 30 min at 37°C on a rocker platform with gentle shaking before the radiotracer containing solution was removed. The plates were washed 2 x with full medium and 1 x with PBS. 250 µL of trypsin were added to each well and the plates were incubated for 10 minutes at 37°C with gentle agitation. Then 500 µL of full medium were added to each well and the cell suspension was transferred to 2 mL reaction tubes. Wells were washed a second time with medium and the solution was pooled with the first harvest in 2 mL tubes. Radioactivity of the samples was

measured with a gamma-counter (Wizard2 gamma counter, PERKINELMER). All data was decay-corrected to the start of the incubation period with [^{18}F]FTBG.

For *in vitro* blocking studies, SNAP-tag$^+$ cells were incubated for 30 min with either 10 µM cell-permeable SNAP-Cell (NEW ENGLAND BIOLABS # S9106S) or 20 µM non-permeable SNAP-Surface (NEW ENGLAND BIOLABS # S9143S) substrates in full medium at 37°C or were left untreated in a tissue culture incubator. Cells were washed 2 x with full medium and incubated with 500 µL of 200 kBq/mL [^{18}F]FTBG in medium for another 30 min at 37°C with gentle agitation. Harvesting and scintillation counting was performed as described above.

***In vivo* studies.** Animals. All animal experiments were performed in accordance with the legal requirements of the European Community (Directive 2010/63/EU) and the corresponding German Animal Welfare Law (TierSchG, TierSchVersV) and were approved by the local authorizing agency (State Office for Nature, Environment and Consumer Protection North Rhine-Westphalia, Az. 84-02.04.2015.A410).

PET and CT scans. All PET scans were carried out on a high-resolution small animal PET scanner (32 module quadHIDAC, OXFORD POSITRON SYSTEMS LTD.) with uniform spatial resolution (<1 mm FWHM) and data was sampled in list mode and reconstructed as described elsewhere.[13] PET scans were performed for 60 minutes after i.v. injection of the radiotracer. CT scans were performed on a small animal CT scanner (Inveon, Siemens Medical Solutions). PET and CT images were coregistered manually by a landmark approach and data was analyzed in an in-house developed software (MEDgical) as described previously[14]. Quantification of the PET data was performed on the basis of manual segmentation of organs and tumor regions termed VOIs (Volumes-Of-Interest) throughout this manuscript.

Biodistribution studies were performed in female C57/BL6 mice (female, 12-15 weeks, 20–23 g). Mice were anesthetized with isoflurane/O_2 and a lateral tail vein catheter was placed using a 27G needle connected to 15 cm polyethylene tubing. 10 MBq of the radiotracer was injected as a bolus (100 µL compound flushed with 100 µL saline) via the tail vein and PET imaging was performed for 60 min.

Tumor studies were carried out in 7–10 week-old NMRI (Rj:NMRI-Foxn1nu/nu, JANVIER) mice. 2x10^6 SNAP-tag$^+$ and 2x10^6 SNAP-tag$^-$ were resuspended in 100 µL saline and injected subcutaneously into the left (SNAP-tag$^+$) and right (SNAP-tag$^-$) dorsal flanks/shoulder regions of each animal. Imaging experiments were performed 7-10 days post implantation and scans lasted for 60 min following i.v. injections of 12 MBq [^{18}F]FTBG.

Activity concentrations in PET data were normalized to the amount of the injected dose by the following calculation:

$$\%\frac{ID}{ml} = \frac{Voxel\ radioactivity\ (cps)}{Total\ radioactivity\ of\ the\ dataset\ (cps)} * 100\%$$

where ID is the injected dose and cps means counts/second.

Total radioactivity of a VOI was calculated as

$$\%ID = \%\frac{ID}{ml} * VOI\ total\ volume\ (ml)$$

Scintillation counting of [^{18}F]FTBG *ex vivo* organ distribution. Following PET and CT scans, mice were deeply anaesthetized with isoflurane/O_2 and animals were perfused with approximately 20 mL of saline via the left ventricle. Organs were harvested, weighed and radioactivity was measured with a gamma-counter. Data was decay-corrected and activity concentrations of organs were calculated as %ID/g (% Injected dose/g) as follows:

$$\%\frac{ID}{g} = \frac{\left(\frac{Radioactivity\ (MBq)}{Weight(g)}\right)}{Injected\ dose\ (MBq)} * 100\%$$

Tumors were weighed and cut into halves. One half of each tumor was used for scintillation counting, while the other half was used for autoradiography and histological stainings.

Autoradiography & histology. Tumors were frozen in Sakura Finetek™ Tissue-Tek™ O.C.T. Compound (Fisher Scientific # 12351753), cut into 40 µM sections on a cryostat and mounted on glass slides. Autoradiographic images were acquired on a Biospace µ-Imager for 8 h hours. Neighboring sections were stained with hematoxylin and eosin and imaged on a Nikon Eclipse NI-E upright light-microscope.

Statistical analysis. Statistical analysis was performed with GraphPad Prism 7 (La Jolla, CA, USA). Data was tested for normality by a Shapiro-Wilk test. To compare multiple groups at the same time, a One-way ANOVA or repeated measures ANOVA (RM ANOVA) with Tukey's post hoc test was performed. Single groups were tested for significant differences by using Student's t-test (normality test passed) or Mann-Whitney-U test (normality test failed). Statistical significance was defined as *$p<0.05$ and **$p<0.01$.

Supplementary Table S2. Ex vivo biodistribution analysis of [18F]FTBG @ 90 min p.i.

Tissue	Activity concentration (%ID/g)
Blood	0.29 ± 0.09
Serum	0.39 ± 0.12
Muscle	0.16 ± 0.05
Brain	0.10 ± 0.03
Heart	0.22 ± 0.07
Lung	0.30 ± 0.06
Spleen	0.47 ± 0.30
Kidney	2.79 ± 1.36
Urine	315.54 ± 112.46
Liver	0.66 ± 0.35
Intestine	66.56 ± 13.24

All data is shown as mean ± SD.

Consensus sequence of sequencing reads for pLV-EF1a-SNAPbiT-IRES-BSD:

GAGAGAGTGCTTGGCCTGTTGCTGCTCCTGCTGGTTCACGCCGGATCTGGGTCTGGGA
GTGTCTCCGGTTGGAGGCTCTTCAAGAAAATCTCCGGAGGTAGCGGCTCAGGAAGCGG
CAGCATGGACAAAGACTGCGAAATGAAGCGCACCACCCTGGATAGCCCTCTGGGCAAG
CTGGAACTGTCTGGGTGCGAACAGGGCCTGCACCGTATCATCTTCCTGGGCAAAGGAA
CATCTGCCGCCGACGCCGTGGAAGTGCCTGCCCCAGCCGCCGTGCTGGGCGGACCAG
AGCCACTGATGCAGGCCACCGCCTGGCTCAACGCCTACTTTCACCAGCCTGAGGCCAT
CGAGGAGTTCCCTGTGCCAGCCCTGCACCACCCAGTGTTCCAGCAGGAGAGCTTTACC
CGCCAGGTGCTGTGGAAACTGCTGAAAGTGGTGAAGTTCGGAGAGGTCATCAGCTACA
GCCACCTGGCCGCCCTGGCCGGCAATCCCGCCGCCACCGCCGCCGTGAAAACCGCCC
TGAGCGGAAATCCCGTGCCCATTCTGATCCCCTGCCACCGGGTGGTGCAGGGCGACCT
GGACGTGGGGGGCTACGAGGGCGGGCTCGCCGTGAAAGAGTGGCTGCTGGCCCACG
AGGGCCACAGACTGGGCAAGCCTGGGCTGGGTCCTGCAGGTGGGTCTGGCAGCAATG
GGTCAGGGTCCAACACCACTGGATCAGGCAGTGGTGGATCTGGCGTTTCCCAGGTGAA
GATCAGTGGTGCTCCCACACTCAGCCCTAGCCTGCTTGGACTGCTGTTGCCAGCCTTC
GGCATTCTCGTCTACCTGGAGTTTTAATGAATTCCTCGAGGGCGGCCGCTCTAGAGTCG
ACGGGCCGCGGTAACAATTGTTAACTAACTTAAGCTAGCAACGGTTTCCCTCTAGCGGG
ATCAATTCCGCCCCCCCCCCCTAACGTT

Full sequence of pLV-EF1a-SNAPbiT-IRES-BSD:

TATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCG
ATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATA
CGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCAC
CGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGG
TCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAG
TAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGT
CACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTT
ACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGT
CAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTC
TTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCAT
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