Electronic Supplementary Material (ESI) for Chemical Communications

Development of an ^{11}C-labeled tetrazine derivative for rapid tetrazine-trans-cyclooctene ligation

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Comparison of bioorthogonal reaction kinetics

Table 1: Reported Bioorthogonal Rate Constants and Pharmacokinetic Parameters[1]

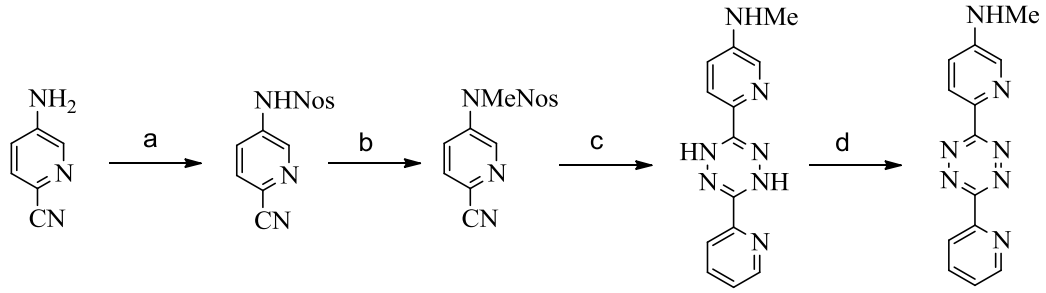
Reaction Type	Rate constant ($M^{-1}s^{-1}$)
Staudinger ligation	~ 0.002
Azide-Alkyne	1 - 0.0013
Nitrone-dipole-alkyne cycloaddition	12 - 32
Phen-TTA-norbornene	1.6(H_20, 20 °C)
Phen-TTA-trans-cycloocten (TCO)	6000 (H_20, 37 °C)
Pyridine-TTA-TCO	13090 (H_20, 37 °C)
Pyridine-TTA-strained TCO	22000 (MeOH, 25 °C)

Experimental Section

General. Chemicals were purchased from Acros, Fluka, Sigma, Tocris, or Merck. Recently, ABX started to purchase trans-cyclooctenol **11**. Unless otherwise stated, all chemicals were used without further purification. Flash chromatography was performed on silica gel 60 (35-70 µm). Microwave-assisted syntheses were carried out in a Biotage Initiator apparatus operating in single mode; the microwave cavity producing controlled irradiation at 2.45 GHz (BiotageAB, Uppsala, Sweden). The reactions were run in sealed vials (0.5–2.0 mL). These experiments were performed by employing magnetic stirring and a fixed hold time using variable power to reach it (over a period of 1-2 min) and then maintaining the desired temperature in the vessel for the programmed time period. The temperature was monitored by an IR sensor focused on a point on the reactor vial glass. The IR sensor was calibrated to internal solution reaction temperature by the manufacturer. Thin layer chromatography (TLC) was performed using plates from Merck (silica gel 60 F_{254} and aluminium oxide 60 F_{254}). ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Bruker AC 300 spectrometer. Chemical shifts are quoted as δ values (ppm) downfield from tetramethylsilane (TMS) internal standard. Infrared spectroscopy was performed on a Perkin Elmer FT-IR Spektrometer (Spectrum One). Melting points were determined on a Stanford Research Systems Optimelt system. LC-MS tests were performed on 6410 Triple Quad LC/MS instrument. Field desorption mass spectra (FD-MS) were recorded using a Finnigan MAT90 spectrometer and electrospray ionization mass spectrometry (ESI-MS) were performed on a ThermoQuest Navigator Instrument. HRMS were performed on a Brucker MicrOTOF instrument. Analytical high performance liquid chromatography (HPLC) measurements were performed on a Dionex system consisting of a P680A pump, a UVD 170U detector and a Scansys radiodetector. Chemical purity was checked either by HPLC or by GC. Lipophilicites were determined using a Dionex Ultimate 3000 HPLC equipped with a degasser, an autosampler, a column-oven and a UV-detector. High

resolution mass spectra (HRMS) were recorded on a Q-TOF Premier (Waters, USA) and Maxis Impact (BrukerDaltonics, Germany) spectrometer. [^{11}C]Methane was produced via the ^{14}N(p,α)^{11}C reaction by bombardment of an [^{14}N]N2 containing 10% H_2 target with a 17 MeV proton beam in a Scanditronix MC32NI cyclotron. Full spectral data of previously published compounds can be found in the indicated references.

Organic syntheses



Supplementary Scheme 1: Synthetic route for N-methyl-6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-amine (**4**): a) 2-nitrobenzenesulfonyl chloride, pyridine, 70 °C, 12h b) MeI, NaH, DMF, RT, 12h c) 2-cyanopyridine, N_2H_4, 90 °C, 12h d) DDQ, toluene

N-(6-cyanopyridin-3-yl)-2-nitrobenzenesulfonamide (1)

2-nitrobenzenesulfonyl chloride (1.989 g, 9 mmol) is added portionwise to a solution of 5-aminopicolinonitrile (1 g, 8.9 mmol) in pyridine (2 mL) and heated at 70 °C for 12 h. After this time, the residue is taken-up with water, extracted with ethyl acetate, dried, filtered, evaporated under reduced pressure to yield N-(6-cyanopyridin-3-yl)-2-nitrobenzenesulfonamide (2.64 g, 8.7 mmol, 98 %). The crude mixture was used without further purification. LC-MS (ESI): RT 5.931 min, m/z cald. for $C_{12}H_8N_4O_4S$ 304.03, found 305.0 [M] at 210 and 254 nm. R_f : 0.53 (EtOAc/Hepatne 1:1)

N-(6-cyanopyridin-3-yl)-N-methyl-2-nitrobenzenesulfonamide (2)

To a solution of N-(6-cyanopyridin-3-yl)-N-methyl-2-nitrobenzenesulfonamide (1.2 g, 3.947 mmol) in 50 mL dry DMF is added NaH (60% dispersion in mineral oil, 317.76 mg, 8 mmol). The mixture is stirred for 1 h at room temperature before iodomethane (0.9 mL, 12 mmol) is added. Afterwards, the solution is stirred for 12 h at room temperature and then diluted with EtOAc/H_2O and extracted with ethyl acetate. The combined organic layers are washed with water, then dried over Na_2SO_4 and

concentrated. Then, the residue was filtered through a silica pad using EtOAc and afterwards again concentrated. Finally, the crude mixture was dissolved in 2 mL EtOAc and 1 mL EtOH und precipitated with hexane to afford the pure product (529 mg, 1.66 mmol, 43%).^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.58 (1H, dd, J = 2.6 Hz), 7.84 – 7.63 (m, 6), 3.45 (3H, s). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 147.87, 140.41, 135.11, 134.07, 132.08, 131.75, 131.58, 130.22, 128.84, 126.06, 124.77, 116.93, 38.63. LC-MS (ESI): RT: 6.282 min, m/z cald. for $C_{13}H_{10}N_4O_4S$ 318.04, found 319.1 $[M+H]^+$ at 210 and 254 nm. R_f : 0.27 (EtOAc/PE 1:1)

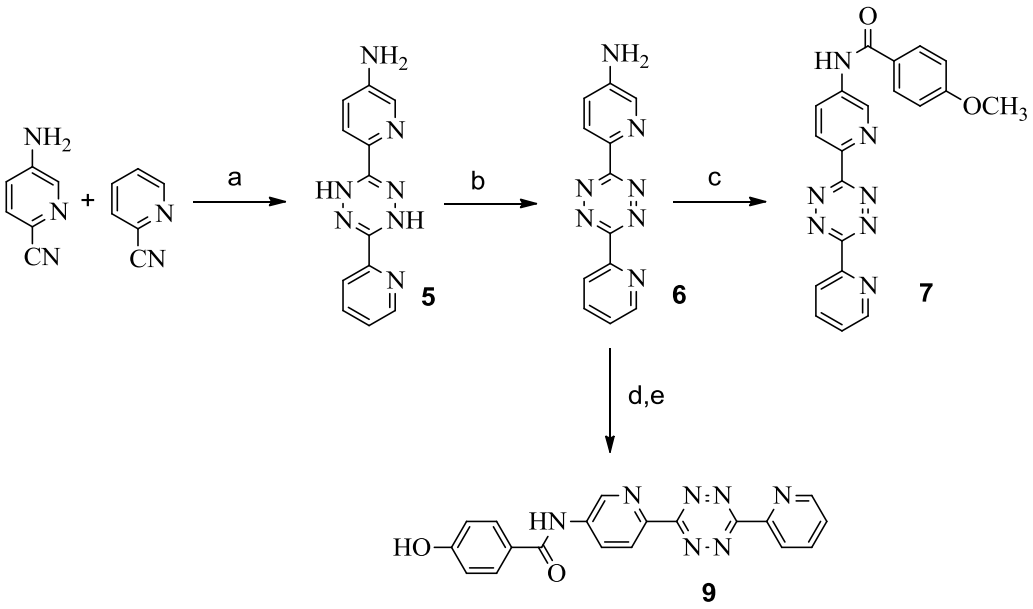
N-methyl-6-(6-(pyridin-2-yl)-1,4-dihydro-1,2,4,5-tetrazin-3-yl)pyridin-3-amine (3)

A dry round-bottom flask was charged with 2-cyanopyridine (0.51 g, 4.88 mmol), N-(6-cyanopyridin-3-yl)-N-methyl-2-nitrobenzenesulfonamide (0.650 g, 4.88 mmol), and 64% hydrazine hydrate (0.947 mL, 26 mmol). The flask was fitted with a reflux condenser, and the mixture was heated to 90 °C for 12 h behind a blast shield. The reaction mixture was cooled to rt and the solvent evaporated. The solid was chromatographed twice using first EtOAc/Heptane (1:1) and then EtOAc to give the pure product (58 mg, 0.216 mmol, 4.44%) as an orange solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.57-8.53 (2H, m), 8.44 (1H, d, s), 8.02 (1H, dd, J_1 = 7.0 Hz, J_2 = 0.9 Hz,), 7.89 (1H, d, J = 2.6 Hz), 7.82 (1H, d, J = 8.5 Hz), 7.72 (1H, td, $J_{1,2}$ = 7.7 Hz, J_3 = 1.6 Hz), 7.53-7.25 (1H, m), 6.87 (1H, dd, J_1 = 8.6 Hz, J_2 = 2.8Hz), 4.17 (1H, bs), 3.88 (3H, s). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 148.51, 147.87, 147.41, 147.31, 146.40, 136.83, 136.01, 133.41, 124.93, 122.28, 121.30, 118.63, 30.45. LC-MS (ESI): RT: 5.718 min, m/z cald. for $C_{13}H_{13}N_7$ 267.12, found 268.1 $[M+H]^+$ at 210 and 254 nm. R_f : 0.7 (EtOAc/MeOH 10:1); 0.3 (EtOAc/Heptane 1:1)

N-methyl-6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-amine (4)

To a solution of N-methyl-6-(6-(pyridin-2-yl)-1,4-dihydro-1,2,4,5-tetrazin-3-yl)pyridin-3-amine (0.68 mg, 0.25 mmol) in anhydrous toluene (5 mL) under N_2 was added DDQ (0.115 g, 0.5 mmol). The reaction mixture was allowed to reflux and stir for 12 hr. The reaction mixture was concentrated under reduced pressure, and the crude residue was concentrated in vacuo onto deactivated silica gel[2] and chromatographed using a gradient (0-100%) of acetone in hexanes to afford the title compound (16 mg, 0.06 mmol, 24%) as a red solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.94-8.92 (1H, m), 8.67 (1H, dd, J_1 = 7.9 Hz, J_2 = 0.9 Hz), 8.61 (1H, d, J = 8.8 Hz), 8.37 (1H, d, J = 2.6 Hz), 7.96 (1H, td, $J_{1,2}$ = 7.8 Hz, J_3 = 1.8 Hz), 7.55-7.50 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.6 Hz), 3.01

(3H, s). LC-MS (ESI): RT: 4.828 min, m/z cald. for $C_{13}H_{11}N_7$ 265.11, found 266.1 $[M+H]^+$ at 210 and 254 nm. R_f : 0.36 (CH_2Cl_2/MeOH 15:1)



Supplementary Scheme 2: Synthetic route for **6**, **7** and **9**

6-(6-Pyridin-2-yl-1,4-dihydro-[1,2,4,5]tetrazin-3-yl)-pyridin-3-ylamine (5)

6-(6-Pyridin-2-yl-1,4-dihydro-[1,2,4,5]tetrazin-3-yl)-pyridin-3-ylamine was synthesized as reported by Blackman et al.[3]. R_f : 0.2 (EtOAc/MeOH 1 :1); 0.4 (THF/heptane 3:2)

3-(5-Aminopyridin-2-yl)-6-(pyridin-2-yl)-*s*-tetrazine (6)

6 was synthesized similar to the reported procedure by Blackman et al.[3]. In brief: To a solution of 3-(5-aminopyridin-2-yl)-6-(pyridin-2-yl)-1,4-dihydro-*s*-tetrazine (0.950 g, 3.8 mmol) in anhydrous toluene (25 mL) under N_2 was added DDQ (1.7 g, 7.5 mmol). The reaction mixture was allowed to reflux and stir for 12 hr. The reaction mixture was concentrated under reduced pressure, and the crude residue was concentrated in vacuo onto deactivated silica gel and chromatographed using a gradient (0-100%) of acetone in hexanes to give a red solid, that was further purified by column chromatography (EtOAC:MeOH 10:1) to afford the title compound (210 mg, 0.83 mmol, 22%) as a red solid. R_f : 0.2 (EtOAc/MeOH 10:1). For further analytical data see Blackman et al.[3].

4-methoxy-N-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)benzamide (7)

127 mg p-anisoylchloride chloride (0.75 mmol) is added to a solution of **6** (56 mg, 0.223 mmol) in pyridine (2 mL) and heated at 130 °C for 5 min in a MW. The red solid was filtered off, washed

with H_2O and EtOAc. The red solid was isolated and basified with Et_3N and then filtered again to yield the title compound (84 mg, 0.218 mmol, 98%) as a pink solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 10.68 (1H, s), 9.25 (1H, d, *J* = 2.1 Hz), 8.92 (1H, t, *J* = 4.4 Hz), 8.66-8.64 (1H, m), 8.59-8.55 (2H, m), 8.14 (1H, td, *J1,2* = 7.8 Hz, 1.8 Hz), 8.02 (2H, d, *J* = 8.8 Hz), 7.71 (1H, d, J_1 = 6.7 Hz, J_2 = 4.7 Hz), 7.11 (2H, d, *J* = 9.1 Hz), 3.86 (3H, s) ^{13}C-NMR (100 MHz, $CDCl_3$, 80 °C): δ 166.15, 163.64, 163.42, 162.99, 151.03, 150.77, 144.67, 143.00, 139.25, 138.14, 130.38, 127.81,126.92,126.66, 125.12, 124.67, 114.34, 56.03. LC-MS (ESI): RT: 5.722 min, m/z 386.2 $[M+H]^+$ at 210 and 254 nm; HRMS (ESI) $[M+H^+]$ cald. for $C_{20}H_{16}N_7O_2$ 386.1365, found 386.1353.

4-(benzyloxy)-N-(6-(6-(pyridin-2-yl)-1,4-dihydro-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)benzamide (8)

4-(benzyloxy)benzoyl chloride (230 mg, 0.93 mmol) is added to 6-(6-Pyridin-2-yl-1,4-dihydro-[1,2,4,5]tetrazin-3-yl)-pyridin-3-ylamine (196 mg, 0.775 mmol) in pyridine (2 mL) and heated at 130 °C for 5 min in a MW. The mixture is diluted with 5 mL water, 5 mL ET_3N and 2 mL acetone, filtered, washed with 5 mL heptane and 2 mL acetone to yield the title compound (326 mg, 0.70 mmol, 92%) as a beige solid. The crude mixture was used without further purification. LC-MS (ESI): RT: 7.153 min, m/z cald. for $C_{26}H_{21}N_7O_2$ 463.18, found 464.4.1 $[M+H]^+$ at 210 and 254 nm.

4-hydroxy-N-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)benzamide (9)

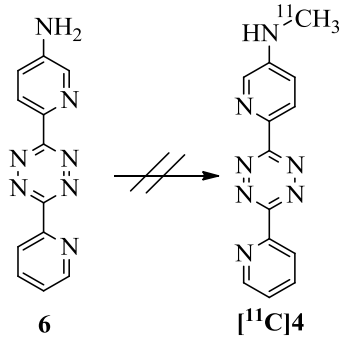
4-(benzyloxy)-N-(6-(6-(pyridin-2-yl)-1,4-dihydro-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)benzamide (118 mg, 0.25 mmol) were dissolved in 5 mL isopropanol. 150 mg Pd/C and 63 mg ammonium formate (1 mmol) were added and then heated to 70 °C for 20 min in a microwave oven. The crude mixture was concentrated and afterwards purified by flash chromatography (EtOAc: heptane: MeOH 6.6: 3.3: 2) to yield 4-hydroxy-N-(6-(6-(pyridin-2-yl)-1,4-dihydro-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)benzamide. This compound was used without further purification and dissolved in dry THF before 2,3-dichloro-5,6-dicyano- 1,4-benzoquinone (DDQ) (0.227 g, 1 mmol) in tetrahydrofuran was added dropwise. Although product formed during addition of the oxidant, the mixture was allowed to stir at room temperature for 6 h under an atmosphere of nitrogen. The precipitate was then filtered off, washed with cold tetrahydrofuran and MeOH, and finally dried to give **9** (77 mg, 0.21 mmol, 83 %) as a crystalline pink solid. MP: 249 – 251 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 10.61 (1H, s), 10.28 (1H, s), 9.26 (1H, s), 8.91 (1H, d, *J* = 4.4 Hz), 8.65-8.56 (3H, m), 8.14 (1H, t, $J_{1,2}$ = 7.2 Hz), 7.93 (2H, d, *J* = 8.5 Hz), 7.71 (1H, dd, J_1 = 6.7 Hz, J_2 = 5 Hz), 6.9 (2H, d, *J* = 8.5 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 166.39, 163.61, 163.40, 161.78, 151.20,

150.80, 144.51, 142.96, 139.56, 138.43, 130.77, 127.77, 127.23, 125.34, 125.03, 124.85, 115.78. LC-MS (ESI): RT 5.203 min, m/z 372.1 $[M+H]^+$ at 210 and 254 nm. R_f: 0.8 (MeOH); HRMS (ESI) $[MH^+]$ cald. for $C_{19}H_{14}N_7O_2$ 372.1209, found 372.1226.

Determination of Lipophilicities[4]

Lipophilicites were determined using a Dionex Ultimate 3000 UHPLC equipped with degasser, autosampler, column-oven and UV-detector. The eluent was 50:50 (v/v) 20 mM sodium phosphate-buffer (pH = 7.4) and MeOH. Injected volumes were 100 µL with a flow rate of 1 mL/min. The determination was carried out on a Luna C18(2) 5µm 100Å (150 mm × 4.6 mm, 5 µm) and UV detection was conducted at 254 nm. The logarithm of retention factor of reference compounds (phenol, acetophenone, *p*-cresol, benzene, toluene, chlorobenzene, benzophenone, naphthalene, diphenyl and phenanthrene) and tested compounds was calculated, and a plot of the reference values against their known logD values was used to intrapolate logD values for tested compounds.

Radiolabeling of [^{11}C]N-methyl-6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-amine: [^{11}C]4

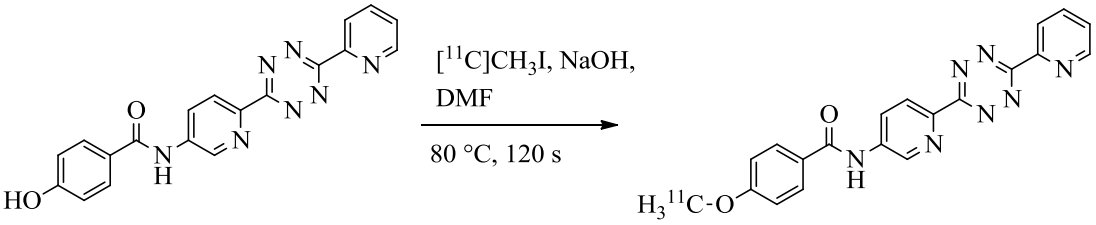


6 **[^{11}C]4**

Table 2: Tested radiolabeling conditions for the synthesis of **[^{11}C]4**

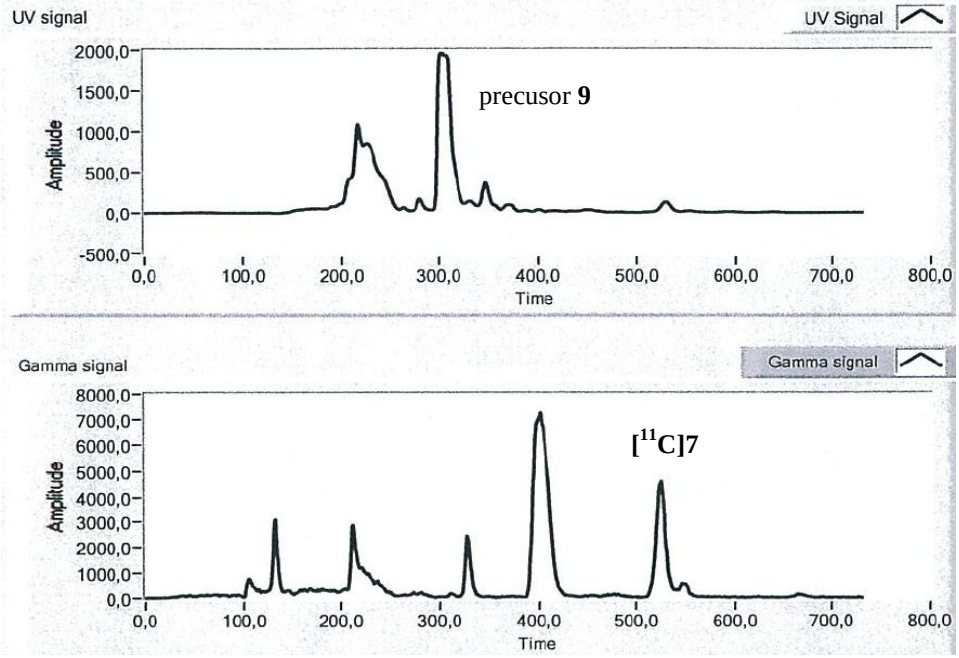
precursor	synthon	base	temperature	solvent	time	RCY
0.3 mg 6	[^{11}C]MeI	1 equiv. NaOH	60 °C	DMSO	5 min	-
0.3 mg 6	[^{11}C]MeI	1 equiv. NaOH	80 °C	DMSO	5 min	-
0.3 mg 6	[^{11}C]MeI	1 equiv. NaOH	80 °C	DMF	5 min	-
0.3 mg 6	[^{11}C]MeI	1 equiv. NaOH	80 °C	MeCN	5 min	-
0.3 mg 6	[^{11}C]MeI	1 equiv. NaOH	100 °C	DMSO	5 min	-
0.3 mg 6	[^{11}C]MeI	1 equiv. Cs_2CO_3	100 °C	DMSO	5 min	-
0.3 mg 6	[^{11}C]MeI	1 equiv. n-BuLi	100 °C	DMSO	5 min	-
0.3 mg 6	[^{11}C]MeI	1 equiv. Bu_4NOH	100 °C	DMSO	5 min	-
0.3 mg 6	[^{11}C]MeI	1 equiv. Bu_4NOH	100 °C	DMSO	10 min	-
0.3 mg 6	[^{11}C]MeI	1 equiv. Bu_4NOH	100 °C	DMSO	15 min	-
1.0 mg 6	[^{11}C]MeI	1 equiv. NaOH	100 °C	DMSO	5 min	-
3.0 mg 6	[^{11}C]MeI	1 equiv. NaOH	100 °C	DMSO	5 min	-
0.3 mg 6	[^{11}C]MeI	1 equiv. N	100 °C	DMSO	5 min	-
0.3 mg 6	[^{11}C]MeI	2 equiv. NaOH	100 °C	DMSO	5 min	-
0.3 mg 6	[^{11}C]MeI	1 equiv. NaOH	120 °C	DMSO	5 min	-
0.3 mg 6	[^{11}C]MeOTf	1 equiv. NaOH	80 °C	DMSO	5 min	-
0.3 mg 6	[^{11}C]MeOTf	1 equiv. NaOH	80°C	DMF	5 min	-
0.3 mg 6	[^{11}C]MeOTf	1 equiv. NaOH	80°C	MeCN	5 min	-

Radiolabeling of [^{11}C]4-methoxy-N-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)benzamide: [^{11}C]7



9 **[^{11}C]7**

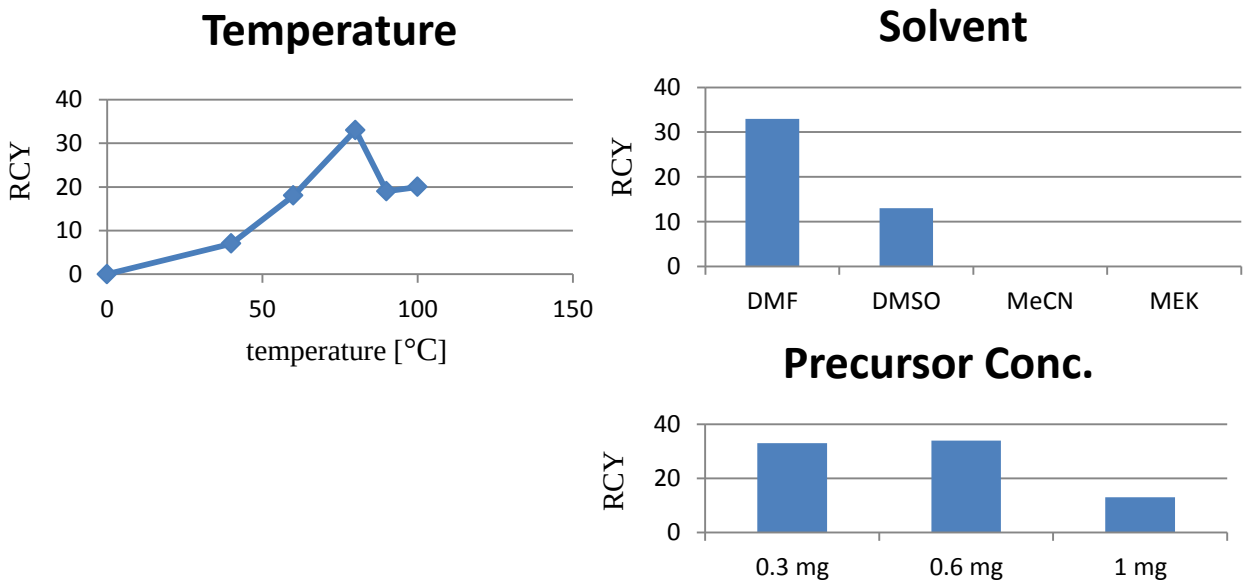
[^{11}C]MeI produced using a fully automated system was transferred in a stream of helium to a 1.1-mL vial containing the labeling precursor (0.3– 0.4 mg), 0.8 µL 2N NaOH (2 equiv.) and DMF (300 µL). The resulting mixture was heated at 80 °C for 120 s and then purified by HPLC on a Luna 5 µm C18(2) 100 Å column (Phenomenex Inc.) (250 × 10 mm; 40:60 acetonitrile: 0.1% phosphoric acid; and flow rate, 6 mL/min, retention times: **[^{11}C]7** = 510 sec; precursor **(9)** = 300 sec). The fraction corresponding to the labeled product was collected in sterile water (150 mL), and the resulting solution was passed through a solid-phase C18 Sep-Pak extraction column (Waters Corp.), which had been preconditioned with ethanol (10 mL), followed by isotonic sodium chloride solution (20 mL). The cartridge was flushed with sterile water (3 mL). Then, the trapped radioactivity was eluted with ethanol (3 mL), followed by isotonic sodium chloride solution (3 mL) into a 20-mL vial containing phosphate buffer (9 mL, 100 mM, pH 7), giving a 15 mL solution of **[^{11}C]7** with a pH of approximately 7. In a total synthesis time of 50–60 min, 0.4–0.8 GBq of **[^{11}C]7** was produced (radiochemical yield 33 ± 5 %). Analytical data are included in the manuscript.



Supplementary Scheme 3: Semi-preparative HPLC chromatograms of the precursor **9** and **[^{11}C]7**

Table 3: Tested radiolabeling conditions for the synthesis of **[^{11}C]7**

precursor	synthon	base	temperature	solvent	time	RCY
0.3 mg 9	[^{11}C]MeI	2 equiv. NaOH	40 °C	DMF	2 min	7
0.3 mg 9	[^{11}C]MeI	2 equiv. NaOH	60 °C	DMF	2 min	18
0.3 mg 9	[^{11}C]MeI	2 equiv. NaOH	80 °C	DMF	2 min	33
0.3 mg 9	[^{11}C]MeI	2 equiv. NaOH	90 °C	DMF	2 min	19
0.3 mg 9	[^{11}C]MeI	2 equiv. NaOH	100 °C	DMF	2 min	20
0.3 mg 9	[^{11}C]MeI	2 equiv. NaOH	80 °C	DMSO	2 min	13
0.3 mg 9	[^{11}C]MeI	2 equiv. NaOH	80 °C	MeCN	2 min	-
0.3 mg 9	[^{11}C]MeI	2 equiv. NaOH	80 °C	MEK	2 min	-
0.3 mg 9	[^{11}C]MeI	2 equiv. Et_3N	80 °C	DMF	2 min	-
0.3 mg 9	[^{11}C]MeI	2 equiv. K_2CO_3	80 °C	DMF	2 min	24
0.3 mg 9	[^{11}C]MeI	2 equiv. Cs_2CO_3	80 °C	DMF	2 min	5
0.3 mg 9	[^{11}C]MeI	2 equiv. Bu_4NaOH	80 °C	DMF	2 min	12
0.6 mg 9	[^{11}C]MeI	2 equiv. NaOH	80 °C	DMF	2 min	34
1.0 mg 9	[^{11}C]MeI	2 equiv. NaOH	80 °C	DMF	2 min	13
0.3 mg 9	[^{11}C]MeI	1 equiv. NaOH	80 °C	DMF	2 min	24
0.3 mg 9	[^{11}C]MeI	4 equiv. NaOH	80 °C	DMF	2 min	-
0.3 mg 9	[^{11}C]MeOTf	2 equiv. NaOH	80 °C	DMF	2 min	27
0.3 mg 9	[^{11}C]MeOTf	2 equiv. NaOH	80 °C	DMF	2 min	25



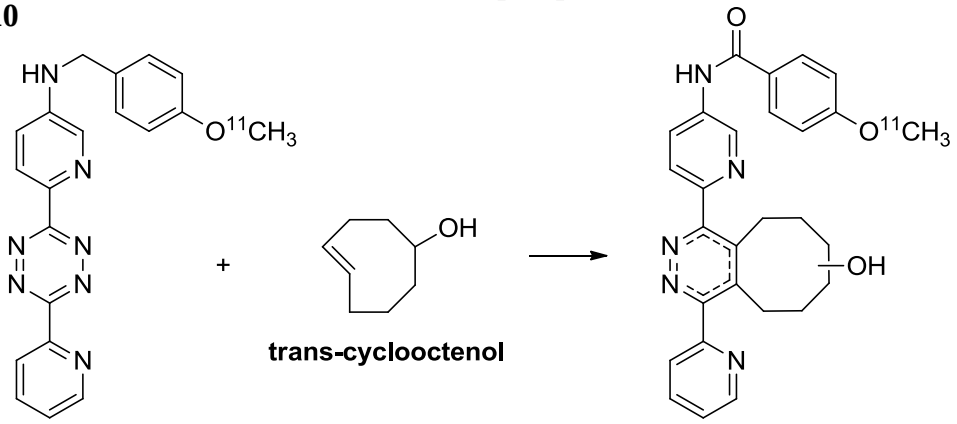
Supplementary Scheme 5: Temperature, solvent and precursor concentration influence on the labeling process of **[^{11}C]7**

Synthesis of reference cmpd 10

1 equiv. of **7** and 1 equivalent of trans-cyclooctenol **11** are dissolved in DMSO. Thereby, a color change from red to yellow can immediately be observed. The crude mixture was analyzed by LC-MS and HRMS. LC-MS (ESI): RT 5.3 min, m/z 482.2 $[M+H]^+$ at 210 and 254 nm; HRMS (ESI) $[MH^+]$ cald. for $C_{19}H_{14}N_7O_2$ 482.21867, found 482.21959. In addition, NMR showed that the double bond of trans-cyclooctenol had disappeared suggesting complete consumption of trans-cyclooctenol (see selected NMR and mass spectrometry data).

Radiolabeling of [^{11}C]10

[^{11}C]10: Mixture of various isomers



[^{11}C]7

[^{11}C]7 was directly eluted from the C18 Sep-Pak extraction column (Waters Corp.) with 1 mL DMSO into a 7 mL vial containing 1 mg of trans-cyclooctenol **11** dissolved in 1 mL DMSO. Afterwards, the mixture was immediately analyzed via HPLC (Luna 5 µm C18(2) 100Å column (Phenomenex Inc.) (150 x 4.6 mm (50:50 acetonitrile: IP-buffer (5mM Na-decanesulfonate, 25 mM phosphate buffer); pH=2.6 adjusted with H_3PO_4, flow rate: 2 mL/min).

References

1. C.S. McKay, J.A. Blake, J. Cheng, D.C. Danielson and J.P. Pezacki, *Chem. Commun.*, 2011,**47**, 10040-10042; N.K. Devaraj, G.M. Thurber, E.J. Keliher, B. Marinelli, and R. Weissleder, *PNAS*, **2012**, 109 4762–4767; S.S. van Berkel, M.B.van Eldijk, and J.C. van Hest. *Angew Chem*, 2011, **12**, 8806-8827; M.T. Taylor, M.L. Blackman, O. Dmitrenko, and J.M.Fox, *J. Am. Chem. Soc.*, 2011, **133**, 9646–9649; J.M. Baskin, J.A. Prescher, S.T. Laughlin, N.J. Agard, P.V. Chang, I.A. Miller, A. Lo, J.A. Codelli, and C. Bertozzi, *PNAS*, 2007, **104**, 16793–16797; W. Chen, D. Wang, C. Dai, D. Hamelberg and B. Wang, *Chem. Commun.*, 2012, **48**, 1736-1738.
2. P. Panne and J.M. Fox. *J. Am. Chem. Soc.*, 2007, **129**, 22 (see Supporting information)
3. M.L. Blackman, M. Royzen, and J.M. Fox, *J. Am. Chem. Soc.***,** 2010, ***46***, 13518– 13519.
4. OECD Guideline for Testing of Chemicals, 117, adopted 30.03.89