Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry


# Supporting information for: Exploring isonitrile-based click chemistry

Henning Stöckmann,[†,‡] André A. Neves,[‡] Shaun R. Stairs,[†] Kevin M. Brindle,[‡] and Finian J. Leeper[*,†]

*University of Cambridge, Department of Chemistry, Lensfield Road, Cambridge CB2 1EW, UK, and Cancer Research UK Cambridge Research Institute, Li Ka Shing Centre, Robinson Way, Cambridge CB2 0RE, UK*

E-mail: fjl1@cam.ac.uk

Phone: ++44 (0) 1223 336 403. Fax: ++44 (0) 1223 336 362

[*]To whom correspondence should be addressed
[†]Department of Chemistry
[‡]Cancer Research UK Cambridge Research Institute

## Protein labelling

**(1) Synthesis of C2Am-*tert*-isonitrile** (Reaction of C2Am with **13**)

C2Am (62 $\mu$M) was reduced in HBS buffer (20 mM HEPES, 150 mM NaCl, pH 7.4) containing 10 mM DTT for 30 min at RT. Subsequently, reduced C2Am was buffer exchanged into HBN buffer (20 mM HEPES, 150 mM NaCl, 10 mM NaEDTA, pH 7.4) using 5-kDa spin filters (Vivaspin-20, Millipore), with a 10,000 dilution ratio. Reduced C2Am (62 $\mu$M) was then resuspended in HBS buffer in a 7 mL flask and the ligand (**13**, 283 mM in DMSO) added drop-wise to a final concentration of 616 $\mu$M. The reaction was performed at 4 °C for 18h, at 300 r.p.m. The final reaction mixture was filtered through 0.22$\mu$m cut-off filters (Millex-GV, PVDF membrane, Millipore) and analysed by MS.

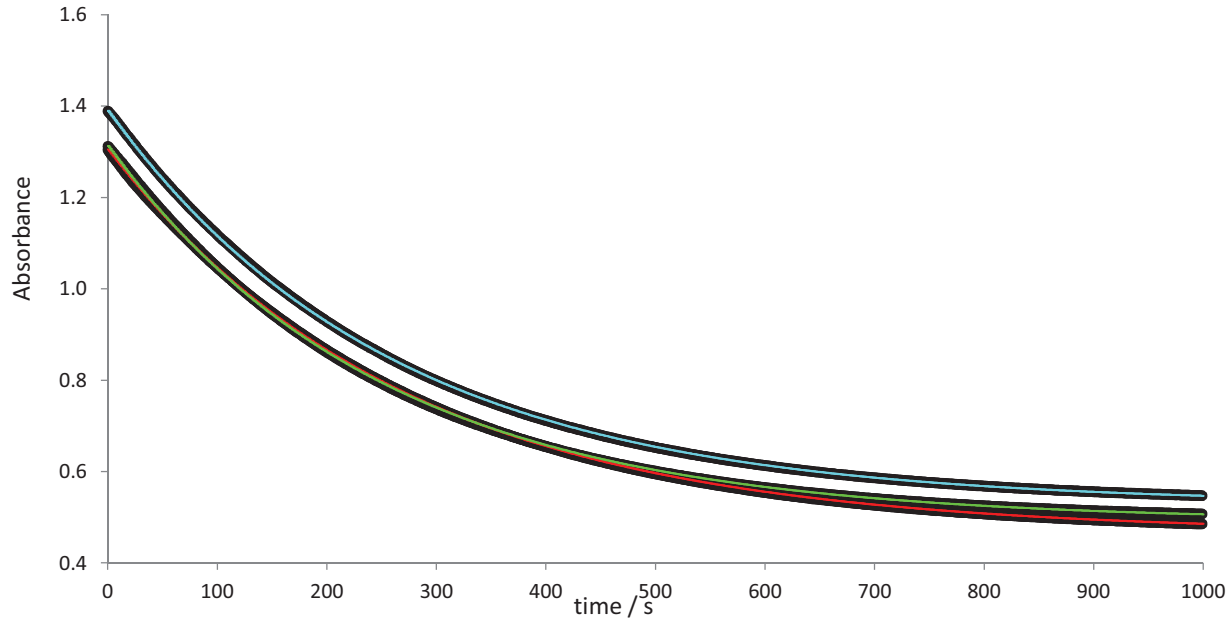
**(2) Reaction of C2Am-*tert*-isonitrile 14 with tetrazine-rhodamine 16**

A method similar to that used in (1) was followed. Tetrazine-rhodamine **16** was prepared as described below and dissolved in DMSO at 542 mM. **16** (540 $\mu$M, 9-fold excess) and C2Am-*tert*-isonitrile **14** (60 $\mu$M) were incubated for 18 h at RT and progress was assessed by MS.

## Representative kinetic evaluation of [4+1] cycloaddition reactions of 3,6-di-(2-pyridyl)-*s*-tetrazine 1 and isonitriles

Kinetic experiments for [4+1] cycloaddition reactions were performed under pseudo first order conditions by UV-Vis spectroscopy. The pseudo first order rate constants were determined by fitting the experimental data to the corresponding rate equation.

The reactions between isonitriles **2d** and **2e** and 3,6-di-(2-pyridyl)-*s*-tetrazine (**1**) were monitored by UV-Vis spectroscopy at 295 nm using the reagents in a 1:1000 molar ratio (tetrazine:isonitrile) in MeOH at 25 °C. The concentrations of the excess reagent (isonitrile) are given in the tables.




| Entry | k' | $A_{oo}$ | $A_0$ | $c_0$ / M | k / $s^{-1}$ x $M^{-1}$ |
|---|---|---|---|---|---|
| 1 | 0.00372 | 0.4652 | 1.3047 | 5.00E-02 | 0.0743 |
| 2 | 0.00386 | 0.5288 | 1.3897 | 5.00E-02 | 0.0772 |
| 3 | 0.00399 | 0.4912 | 1.3126 | 5.00E-02 | 0.0798 |
| | | | | **average** | **0.077** |
| | | | | **error** | **0.003** |

Figure S1: Kinetic evaluation of [4+1] cycloaddition reactions of isonitrile **2e** with 3,6-di-(2-pyridyl)-*s*-tetrazine (**1**) in MeOH. Top: Plots of absorbance *vs.* time from three different experiments (black) and best-fit exponential curves (blue, red and green). Bottom: Summary of data (pseudo first order rate, final absorbance, initial absorbance, concentration of excess substrate and second order rate constants) from three different experiments.

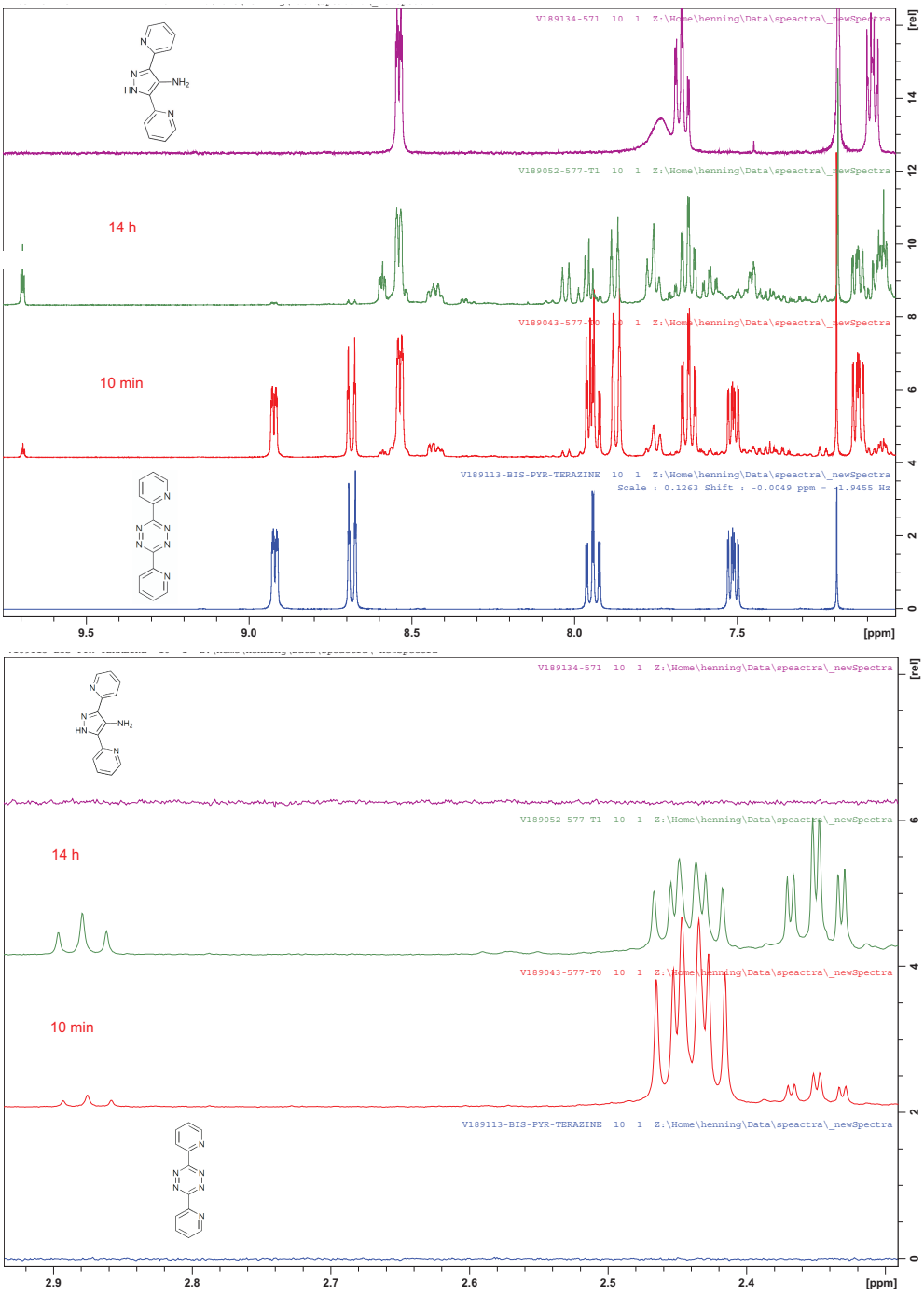



Figure S2: Reaction of 3,6-di-2-pyridyl-1,2,4,5-tetrazine (60 mM) with 1-pentyl isonitrile (120 mM) in $CDCl_3$ at 25 °C. $^1$H NMR spectra (400 MHz) were recorded after 10 min and 14 h and only the most relevant regions are shown. Also included are spectra of the pure starting tetrazine and aminopyrazole product. Peaks of particular interest are: for the imine **5b**, 2.45 (2H, td, *J* 11.3 & 4.8, C$\underline{H}_2$-CH=N) and 7.96 (1H, t, *J* 4.8, $CH_2$-C$\underline{H}$=N); for pentanal, 2.35 (2H, td, *J* 7.4 & 1.9, C$\underline{H}_2$-CH=O) and 9.69 (1H, t, *J* 1.9, $CH_2$-C$\underline{H}$=O).

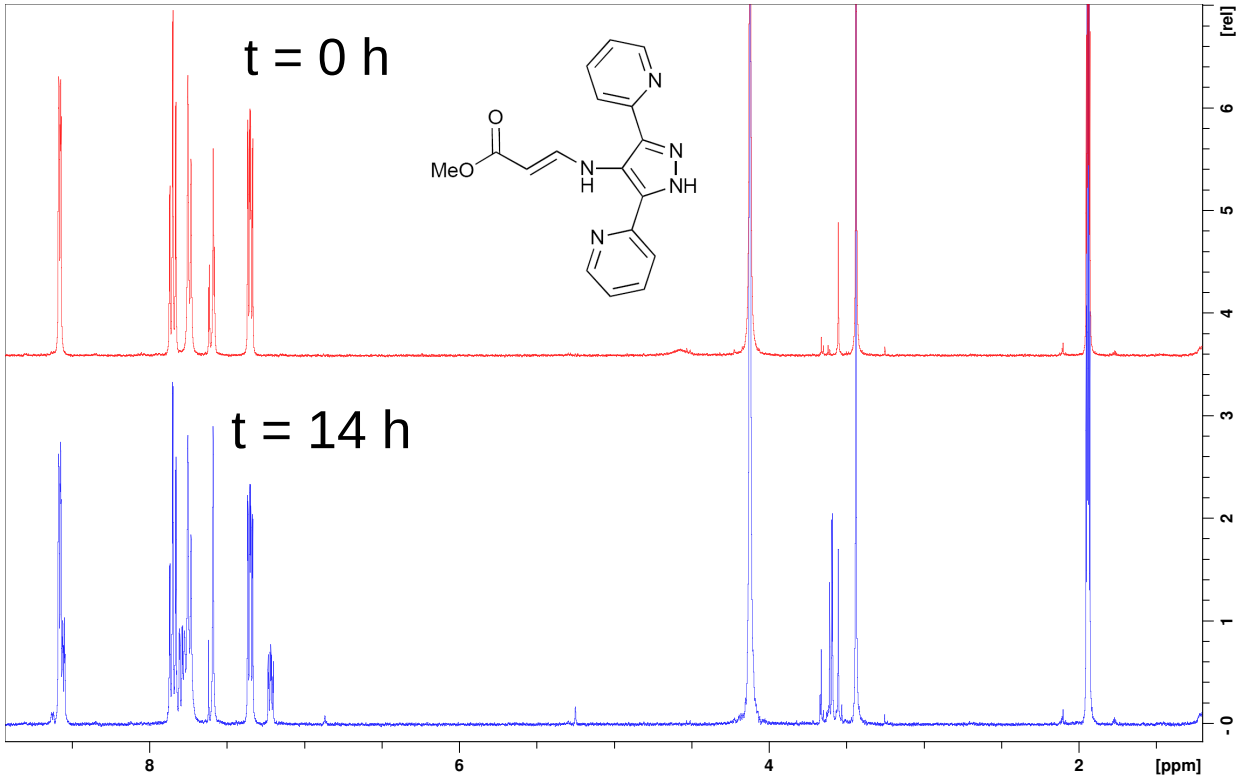



Figure S3: Degradation of tetrazine methyl isocyanopropionate adduct **8** in phosphate buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4$, 1.76 mM $KH_2PO_4$, $D_2O$, pH = 7.25-7.3 at 25 °C) / $CD_3CN$ (50:50). NMR spectra were taken at t = 0 h and t = 14 h.

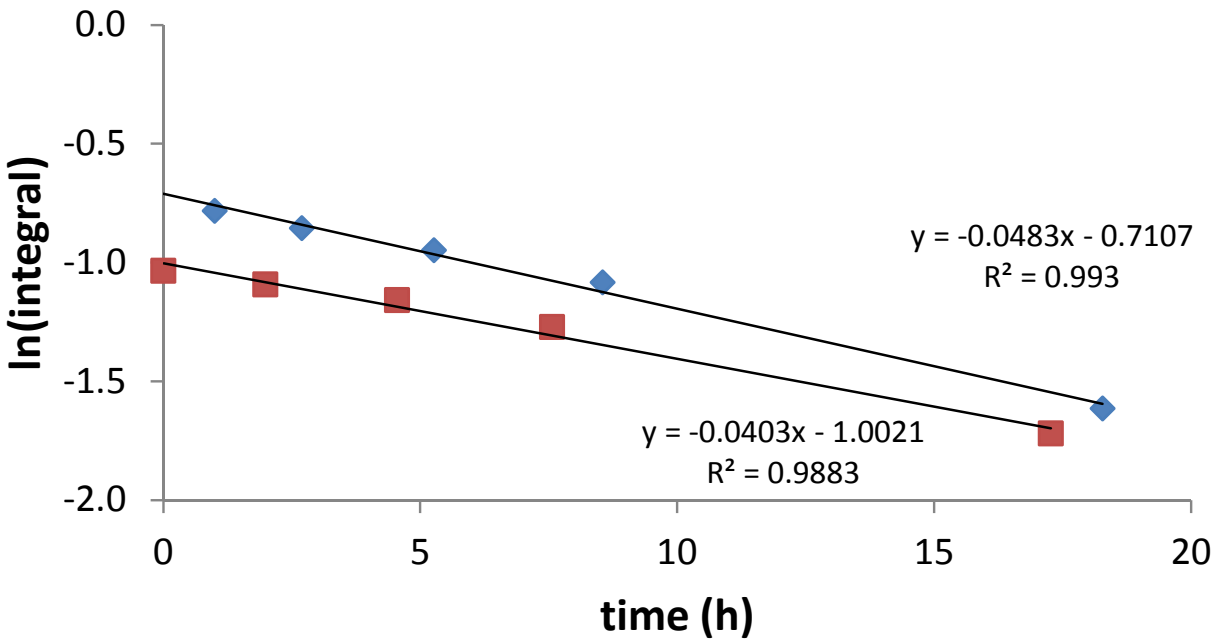



Figure S4: Degradation of tetrazine methyl isocyanopropionate adduct **8** in phosphate buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4$, 1.76 mM $KH_2PO_4$, $D_2O$, pH = 7.25-7.3 at 25 °C) / $CD_3CN$ (50:50). NMR spectra were taken at various time points. Half-life: 15.8 ± 2.0 h

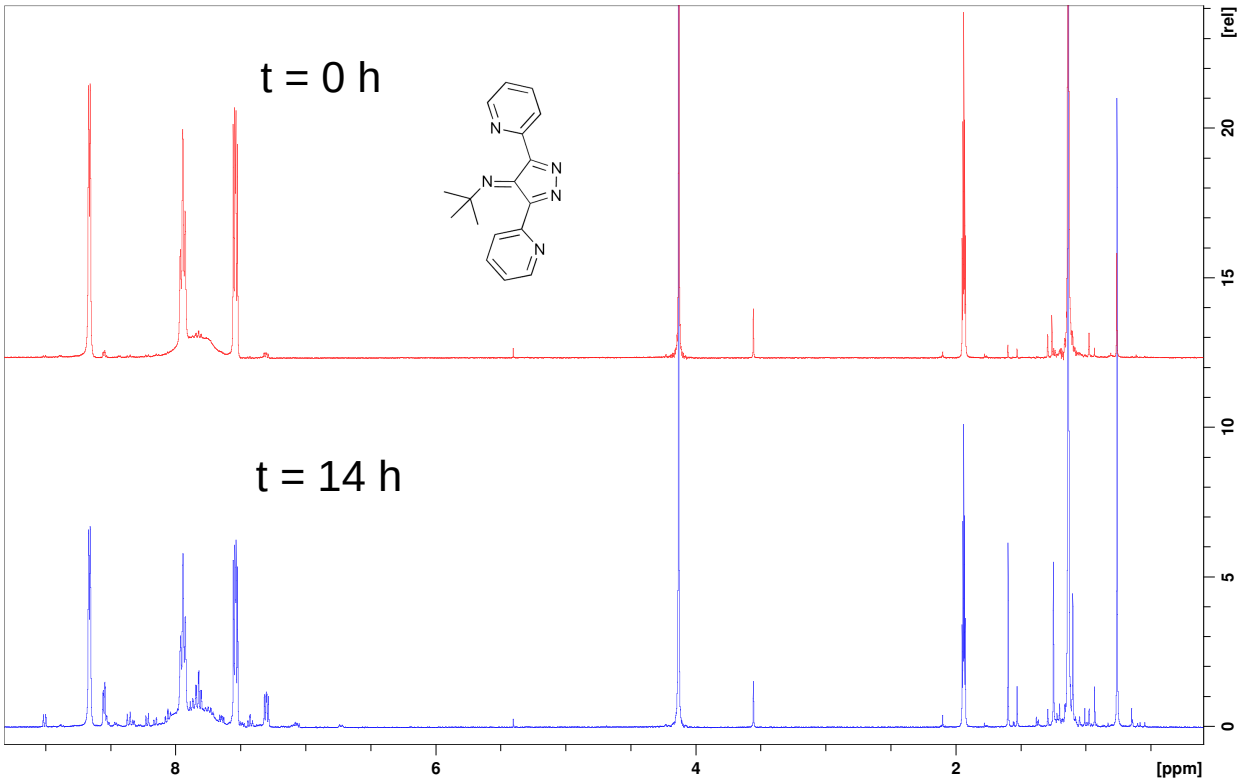



Figure S5: Degradation of tetrazine *tert*-butyl isonitrile adduct **5** in PBS / $CD_3CN$ (50:50). NMR spectra were taken at t = 0 h and t = 14 h.

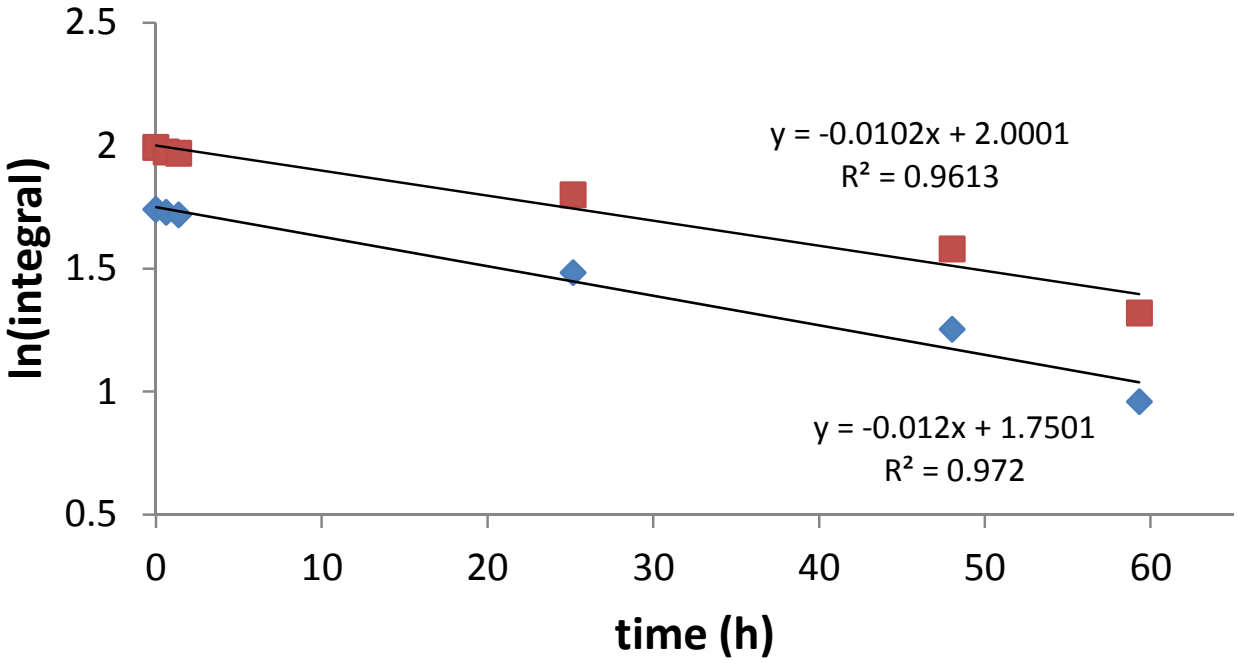



Figure S6: Degradation of tetrazine *tert*-butyl isonitrile adduct **5** in PBS / $CD_3CN$ (50:50). NMR spectra were taken at various time points. Half-life: 62.9 ± 7.2 h

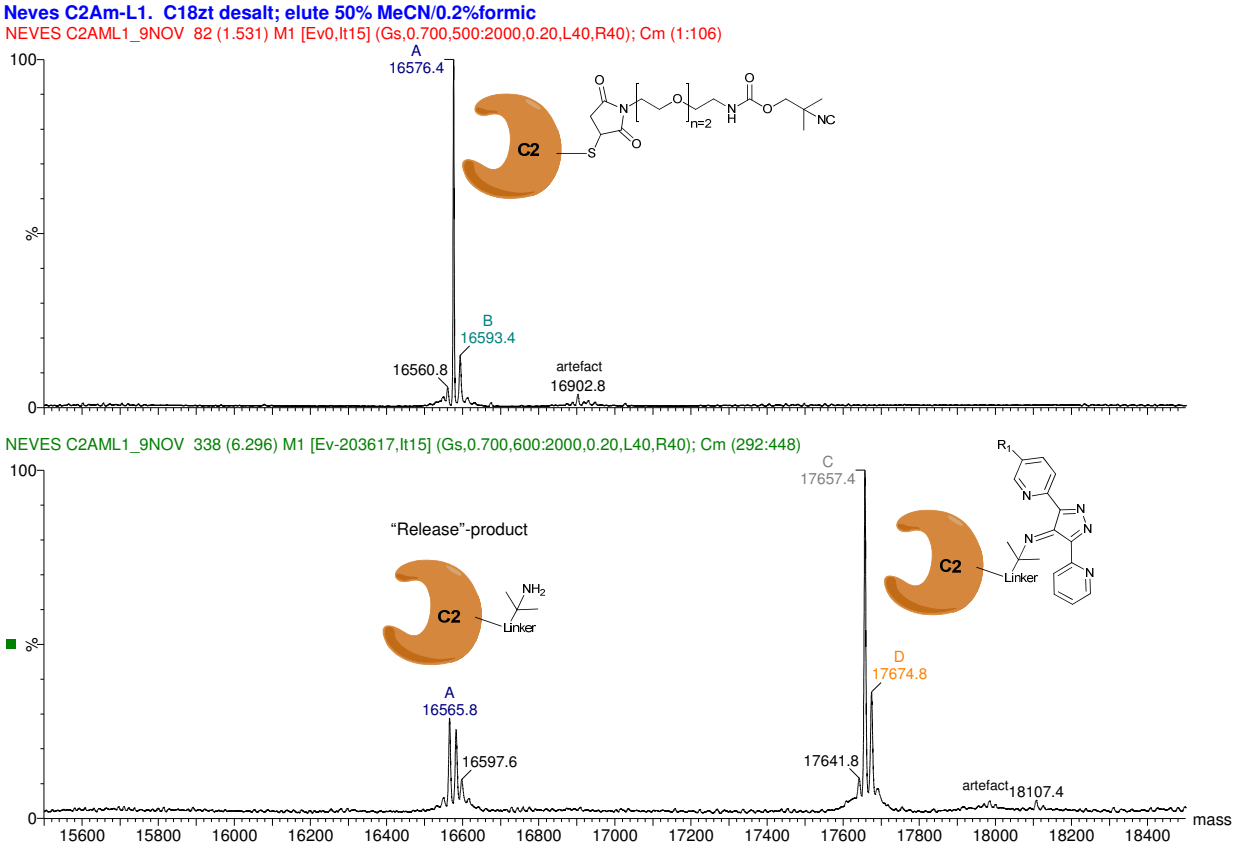



Figure S7: Reaction of C2Am with tetrazine-rhodamine: deconvoluted data (see Figure S8 and Figure S9 for the raw data).

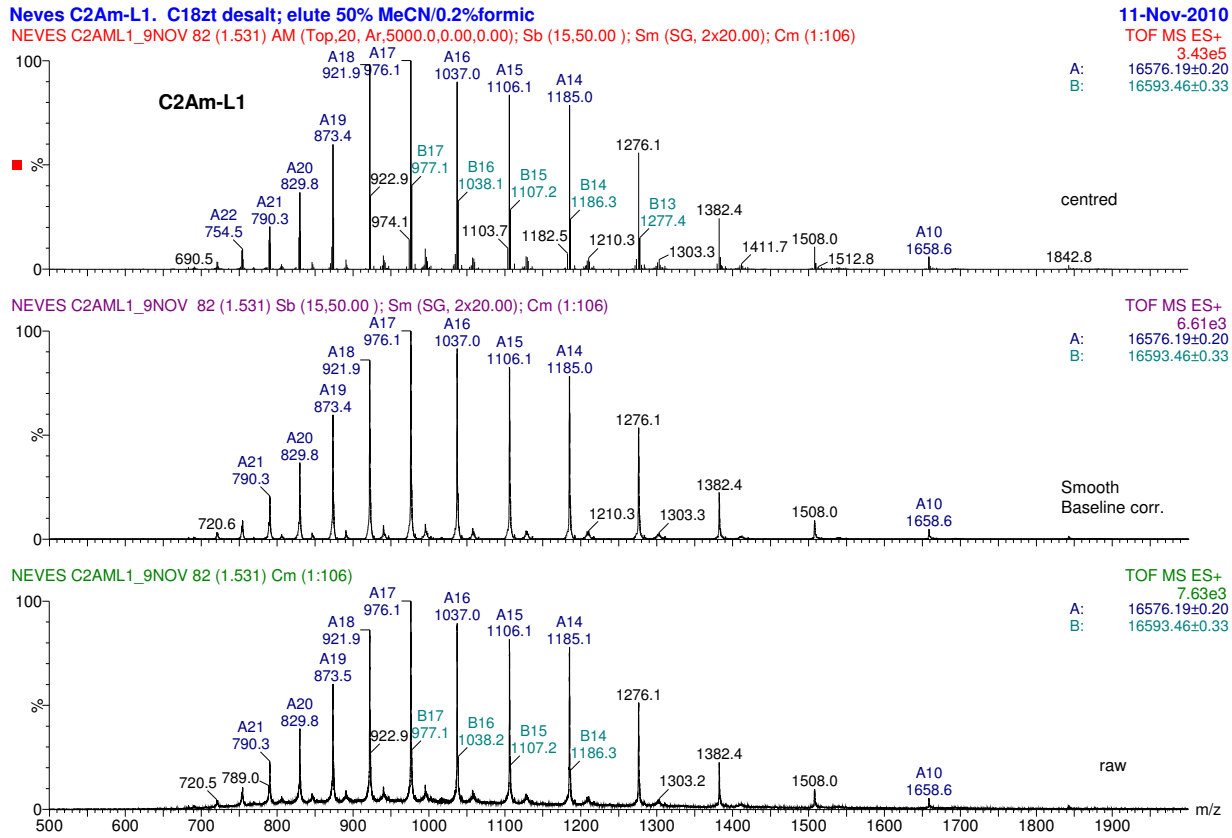



Figure S8: Reaction of C2Am with tetrazine-rhodamine: raw data for the upper spectrum in Figure S7.

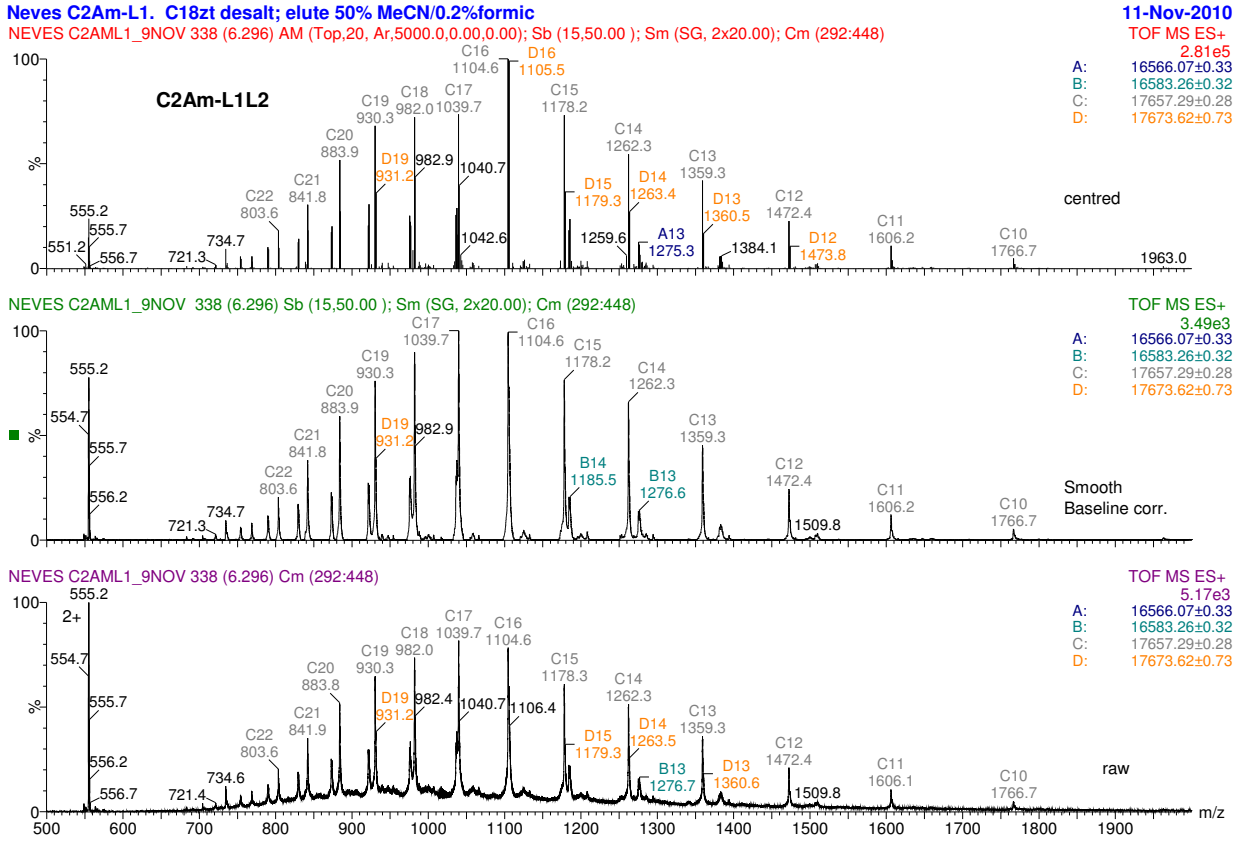


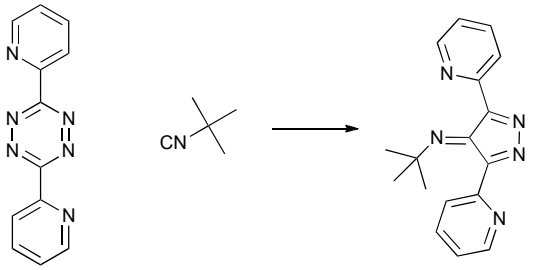

Figure S9: Reaction of C2Am with tetrazine-rhodamine: raw data for the lower spectrum in Figure S7.

## General methods and materials

NMR spectra were recorded on the following instruments: Bruker DRX500, Bruker Avance BB, Bruker Avance TCI, Bruker AM400 and Bruker DRX400. All chemical shifts are quoted in ppm, relative to tetramethylsilane, using the residual solvent peak as a reference standard. All coupling constants are quoted in Hz. Infrared spectra were recorded on a Perkin Elmer Spectrum One (FT-IR) spectrophotometer. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier TOF mass spectrometer with electrospray and modular Lockspray interface. Analytical thin layer chromatography (TLC) was carried out on Merck Kieselgel 60 F254 plates with visualisation by ultra violet light (254 nm), potassium permanganate or ninhydrin or phosphomolybdic acid /$Ce(SO_4)_2$ dip. Flash column chromatography was carried out using Merck Kieselgel 60 (230-400 mesh). HPLC was carried out on a Varian ProStar system, UV detection at 254 nm, Phenomenex Jupiter C18 column, particle size 5 $\mu$m. LC-MS analysis was performed on a Waters 2795 system, UV detection at 254 nm, Supelco ABZ+plus column, 3.3cm x 4.6mm, particle size 3 $\mu$m. All solvent mixtures are reported as % vol/vol unless otherwise stated. Reagents and solvents were purified using standard means. All other chemicals were used as received unless noted otherwise. Extractive procedures were performed using distilled solvents and evaporation of solvents was performed under reduced pressure. All aqueous solutions used were saturated. Unless otherwise stated, all non-aqueous reactions were carried out under an argon atmosphere using anhydrous conditions and oven-dried glassware. Standard techniques were employed for handling air-sensitive materials.

## Synthetic procedures

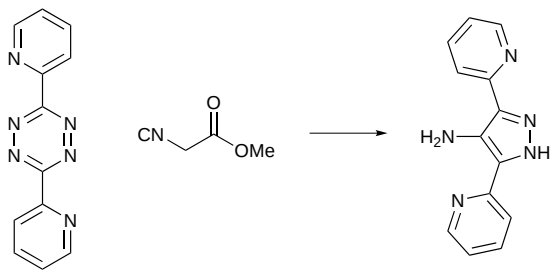
**4-*t*-Butylimino-3,5-di(pyridin-2-yl)-4*H*-pyrazole** (**5f**, tetrazine *tert*-butyl isonitrile adduct)




To a solution of 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine (55 mg, 233 $\mu$mol, 1.0 eq) in DCM (2 mL) was added *tert*-butyl isonitrile (19 mg, 233 $\mu$mmol, 1.0 eq) with stirring at room temperature. When the reaction had gone to completion (colour change from pink to red, < 1 h), the precipitate was collected and dried to give the desired product (60 mg, 88% yield) as a red solid, mp 166-168 °C. Note: The reaction could also be performed in THF/$H_2O$ (1:1), but in this case the solvent was removed and the product crystallised from DCM/$Et_2O$ (85% yield).

$\delta_H$(500 MHz, $CDCl_3$) 8.74 (2H, br s), 7.97 (2H, br s), 7.80 (2H, app t, $J$=6.7), 7.41-7.35 (2H, m), 1.26 (9H, s); $\delta_C$(125 MHz, $CDCl_3$) 159.34, 150.31 (br s), 149.02 (br s), 136.95 (br s), 136.05 (br s), 126.70 (br s), 124.74, 62.61, 30.61; $\nu_{max}$(solid)/$cm^{-1}$ 1584, 1564, 1543, 1465, 1434, 1426, 1362; $\lambda_{max}$(MeCN)/nm 221, 360; Calcd. for $C_{17}H_{17}N_5$: C, 70.08; H, 5.88; N, 24.04. Found: C, 69.92; H, 5.87; N, 23.99;

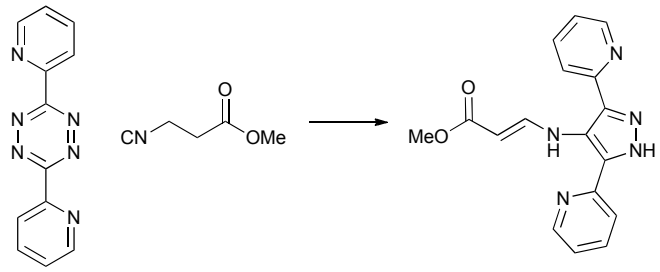
**3,5-Di(pyridin-2-yl)-1H-pyrazol-4-amine (6)**



To a solution of 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine (110 mg, 466 $\mu$mol, 1.0 eq) in MeOH/$H_2O$ (1:1, 2 mL) was added methyl isocyanoacetate (92 mg, 931 $\mu$mmol, 2.0 eq) with stirring at room temperature. When the reaction had gone to completion (colour change from pink to yellow, < 1 h), the precipitate was collected and dried to give the desired product (95 mg, 86% yield) as a yellow solid, mp 238-240 °C.

$\delta_H$(400 MHz, DMSO−$d_6$) 13.13 (1H, s), 8.63-8.50 (2H, m), 8.03-7.70 (4H, m), 7.30-7.10 (2H, m), 5.99 (2H, s); $\delta_C$(100 MHz, DMSO−$d_6$) 154.69, 150.32, 149.14, 148.41, 136.96, 136.71, 136.61, 131.59, 124.57, 121.08, 120.42, 118.81, 117.81; $\nu_{max}$(solid)/cm$^{-1}$ 3464, 3355, 2871, 1600, 1587, 1462, 1423; $\lambda_{max}$(MeCN)/nm 255, 343; *m/z* (EI) 238.1095 (M+H$^+$. $C_{13}H_{12}N_5$ requires 238.1087).

**(E)-Methyl 3-(3,5-di(pyridin-2-yl)-1H-pyrazol-4-ylamino)acrylate** (**8**, tetrazine methyl isocyanopropionate adduct)

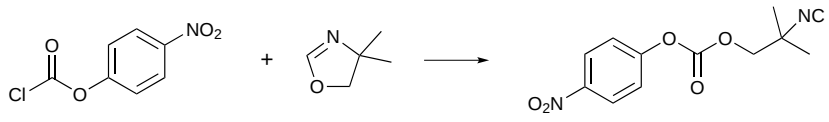



To a solution of 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine (110 mg, 466 $\mu$mol, 1.0 eq) in DCM (2 mL) was added methyl isocyanopropionate (105 mg, 931 $\mu$mmol, 2.0 eq) with stirring at room temperature. When the reaction had gone to completion (colour change from pink to colourless, < 1 h), the precipitate was collected and dried to give the desired product (130 mg, 87% yield) as a white solid, mp 190–192 °C. Notes: 1) The reaction could also be performed in THF/$H_2O$ (1:1), but in this case the solvent was removed and the product crystallised from DCM/hexanes. 2) The reaction yields the *trans*-olefin, which readily isomerises to the *cis*-olefin in $CDCl_3$. In addition, hydrogen-deuterium exchange at the $\alpha$-olefinic carbon was observed at pH 7.4.

$\delta_H$(400 MHz, $CDCl_3$) 12.20 (1H, s, pyrazole-N<u>H</u>), 8.98 (1H, br s, N<u>H</u>-CH=CH-$CO_2$Me), 8.63 (2H, app d, *J*=4.0, pyr-<u>H</u>), 8.10-7.50 (2H, br s, pyr-<u>H</u>), 7.77 (2H, td, *J*=7.7, 1.7, pyr-<u>H</u>), 7.64 (1H,

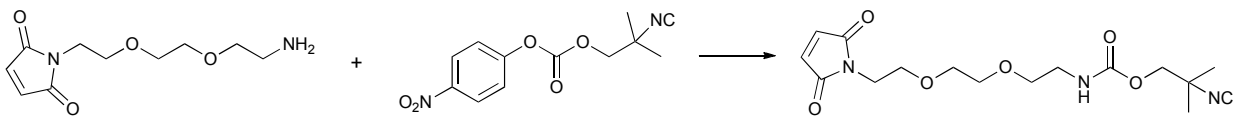
dd, *J*=9.5, 13.3, C<u>H</u>=CH-$CO_2$Me), 7.26-7.21 (2H, m, pyr-<u>H</u>), 4.86 (1H, br d, *J* = 13.3, CH=C<u>H</u>-$CO_2$Me), 3.59 (3H, s, $CO_2$<u>Me</u>); $\delta_C$(100 MHz, $CDCl_3$) 169.33, 148.80, 146.50, 137.05, 122.68, 121.12, 92.84, 50.72; $\nu_{max}$(solid)/$cm^{-1}$ 3244, 1693, 1621, 1593, 1570, 1541, 1482, 1434, 1322; $\lambda_{max}$(MeCN)/nm 256, 285; *m/z* (EI) 322.1308 ($M+H^+$. $C_{17}H_{16}N_5O_2$ requires 322.1299).

**2-Isocyano-2-methylpropyl (4-nitrophenyl) carbonate (11)**



*n*-BuLi (3.36 mL, 1.6 M in hexanes, 5.04 mmol, 1.0 eq.) was added slowly to 4,4-dimethyl-2-oxazoline (0.5 g, 5.04 mmol, 1.0 eq.) in 10 ml dry THF at -78 °C. The solution was stirred for 1 h at -78 °C and quickly transferred *via* cannula to a stirred solution of *para*-nitrophenylchloroformate (1.02 g, 5.04 mmol, 1.0 eq.) in THF (10 mL) that had been cooled to -78 °C. After 30 minutes the solution was allowed to reach 25 °C. The solution was washed with water and saturated aqueous NaCl and dried over anhydrous $Na_2SO_4$. Removal of the solvent yielded an off-white solid, mp 94–96 °C, (1.1 g, 83% yield), which was used in the following reactions without further purification. $R_f$ (50% EtOAc : 50% hexanes) 0.65; $\delta_H$(100 MHz, $CDCl_3$) 8.29 (2H, d, *J*=9.2), 7.40 (2H, d, *J*=9.2), 4.23 (2H, s), 1.53 (6H, s); $\delta_C$(100 MHz, $CDCl_3$) 156.65 (t, *J*=4.0), 155.21, 152.14, 145.59, 125.38, 121.73, 73.58, 56.14 (t, *J*=6.0), 25.78; $\nu_{max}$(film)/$cm^{-1}$ 3083, 2987, 2144 (NC), 1764, 1618, 1593, 1521, 1494;*m/z* (EI) 265.0813 ($M+H^+$. $C_{12}H_{13}N_2O_5$ requires 265.0819).

**Maleimido *tert*-isonitrile linker 13**



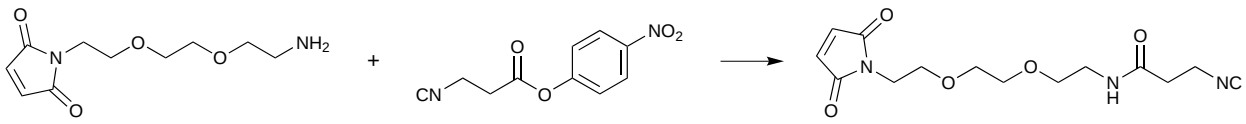
To a solution of O-(2-Aminoethyl)-O'-(2-maleimidoethyl)ethylene glycol trifluoroacetate salt (69 mg, 0.2 mmol, 1.0 eq) in DCM (2 mL) were added 2-isocyano-2-methylpropyl (4-nitrophenyl)

carbonate **11** (54 mg, 0.2 mmol, 1.0 eq) and Hünig's base (71 $\mu$L, 0.4 mmol, 2.0 eq) with stirring at room temperature. When the reaction had gone to completion (TLC, 12 h), the solvent was removed and the residue subjected to column chromatography (50% to 100% EtOAc in hexanes) to give the desired product (65 mg, 91% yield) as a clear oil.

$R_f$ (100% EtOAc) 0.20;

$\delta_H$(500 MHz, $CDCl_3$) 6.70 (2H, s), 4.02 (2H, s), 3.72 (2H, t, *J*=5.6), 3.63 (2H, t, *J*=5.6), 3.61-3.57 (2H, m), 3.57-3.53 (2H, m), 3.51 (2H, t, *J*=5.2), 3.36 (2H, q, *J*=5.2), 1.41 (6H, s); $\delta_C$(125 MHz, $CDCl_3$) 170.71, 155.81, 155.06 (t, *J*=4.4), 134.20, 70.30, 69.89, 69.86, 67.90, 56.68 (t, *J*=5.7), 40.97, 37.08, 25.82; $\nu_{max}$(film)/$cm^{-1}$ 3364, 2874, 2137 (NC), 1704, 1531, 1407; *m/z* (EI) 376.1496 ($M+Na^+$. $C_{16}H_{23}N_3O_6Na$ requires 376.1485).

**Maleimido isocyanopropionamide linker 15**




To a solution of O-(2-aminoethyl)-O'-(2-maleimidoethyl)ethylene glycol trifluoroacetate salt (110 mg, 321 $\mu$mol, 1.0 eq) in DCM (2 mL) were added 4-nitrophenyl 3-isocyanopropanoate (85 mg, 386 $\mu$mol, 1.2 eq, prepared as described below) and Hünig's base (112 $\mu$L, 112 $\mu$mol, 2.0 eq) with stirring at room temperature. When the reaction had gone to completion (LC-MS, 12 h), the solvent was removed and the residue subjected to column chromatography (50% to 100% EtOAc in hexanes) to give the desired product (52 mg, 52% yield) as a clear viscous oil. Note: The compound is unstable at room temperature and must be stored at -20 °C.

$R_f$ (100% EtOAc) 0.20; $\delta_H$(500 MHz, $CDCl_3$) 6.72 (2H, s), 3.77-3.70 (3H, m), 3.67-3.61 (2H, m), 3.60-3.56 (3H, m), 3.56-3.53 (2H, m), 3.53-3.49 (2H, m), 3.48-3.39 (2H, m), 2.68-2.44 (2H, m); $\delta_C$(125 MHz, $CDCl_3$) 171.62, 170.84, 156.85 (t, *J*=5.5), 134.24, 70.19, 69.86, 69.60, 67.94, 39.31, 37.31, 35.84, 35.06, 34.09; $\nu_{max}$(film)/$cm^{-1}$ 3311, 2921, 2872, 2154, 1706, 1664, 1544, 1436, 1408; *m/z* (EI) 310.1415 ($M+H^+$. $C_{14}H_{20}N_3O_5$ requires 310.1403).

**4-Nitrophenyl 3-formamidopropanoate**

A stirred solution of 3-formamidopropanoic acid (2.8 g, 23.9 mmol, 1 eq), 4-nitrophenol (3.3 g, 23.9 mmol, 1 eq) and *N*,*N*'-dicyclohexylcarbodiimide (4.93 g, 23.9 mmol, 1 eq) in DMF (25 mL) was stirred at r.t. When the reaction had finished (TLC, typically $<$ 12 h), *N*,*N*'-dicyclohexylurea was removed by filtration. The solvent was removed and the residue subjected to flash chromatography (100% EtOAc) to give the product as an off-white solid (5.0 g, 88% yield), mp 91-94 °C.

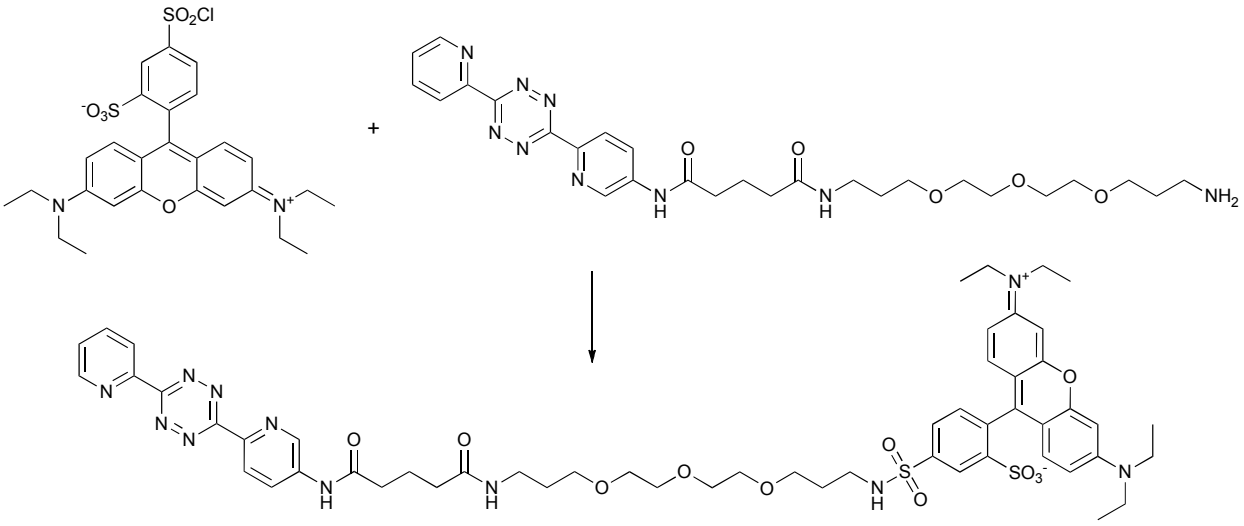
$R_f$ (100% EtOAc) 0.40; $\delta_H$(400 MHz, $CDCl_3$) 8.22 (2H, d, *J*=9.0), 7.26 (2H, d, *J*=9.0), 3.65 (2H, q, *J*=6.2), 3.04 (2H, t, *J*=6.2); $\delta_C$(100 MHz, $CDCl_3$) 169.99, 161.50, 155.05, 145.44, 125.25, 122.43, 34.31, 33.46; $\nu_{max}$(solid)/$cm^{-1}$ 3248, 3051, 2930, 2856, 1749, 1713, 1658, 1619, 1592, 1523, 1487, 1438; *m/z* (EI) 239.0658 ($M+H^+$. $C_{10}H_{11}O_5N_2$ requires 239.0662).

**4-Nitrophenyl 3-isocyanopropanoate**

A stirred solution of 4-nitrophenyl 3-formamidopropanoate (1.1 g, 4.6 mmol, 1.0 eq) and $NEt_3$ (3.2 mL, 23.1 mmol, 5.0 eq) in DCM (10 mL) was cooled to -50 °C. $POCl_3$ (646 $\mu$L, 6.9 mmol, 1.5 eq) was added drop-wise. When the reaction had finished (TLC, typically $<$ 1 h), the mixture was poured into ice-cold (dry ice) phosphate buffer (1 M, pH 7) and extracted with DCM (2 x). The combined organic phases were washed with brine, concentrated and the residue subjected to flash chromatography (gradient elution from 0% to 10% $Et_2O$ in DCM) to give the product as a colourless oil (0.8 g, 80% yield).

$R_f$ (50% EtOAc in hexanes) 0.44; $\delta_H$(400 MHz, $CDCl_3$) 8.28 (2H, d, *J*=9.2), 7.31 (2H, d, *J*=9.2), 3.82 (2H, t, *J*=6.6), 3.04 (2H, t, *J*=6.6); $\delta_C$(100 MHz, $CDCl_3$) 167.26, 158.51 (t, *J*=4.6), 154.74, 145.69, 125.37, 122.36, 36.99 (t, *J*=7.1), 34.27; $\nu_{max}$(film)/$cm^{-1}$ 3116, 3089, 2856, 2153, 1755, 1615, 1594, 1515, 1491, 1438, 1414, 1341; *m/z* (EI) 221.0555 ($M+H^+$. $C_{10}H_9O_4N_2$ requires 221.0557).

**Tetrazine-rhodamine conjugate 16**



To a solution of N1-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-N5-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)glutaramide (25 mg, 44 $\mu$mol, 1.0 eq, synthesised as described by Rossin et al.[1]) in DCM (2 mL) was added Sulforhodamine B acid chloride (25 mg, 44 $\mu$mol, 1.0 eq) and Hünig's base (39 $\mu$L, 220 $\mu$mol, 5.0 eq) with stirring at room temperature. When the reaction had gone to completion (LC-MS, typically $<$ 12 h), the solvent was removed and the residue subjected to HPLC (gradient elution from 5% MeCN to 95% MeCN in water) to give the desired product (38 mg, 78% yield) as a pink solid.

$\delta_H$(500 MHz, MeOD) 8.98 (1H, d, *J*=2.4), 8.85 (1H, d, *J*=4.7), 8.72 (1H, d, *J*=7.9), 8.64 (1H, d, *J*=1.9), 8.62 (1H, d, *J*=8.7), 8.34 (1H, dt, *J*=8.7, 2.5), 8.14 (1H, dd, *J*=7.8, 1.7), 8.11 (1H, dd, *J*=8.0, 1.9), 7.71 (1H, ddd, *J*=7.6, 4.8, 1.0), 7.57 (1H, d, *J*=8.0), 7.07 (2H, d, *J*=9.5), 6.94 (2H, dd, *J*=9.5, 2.4), 6.81 (2H, d, *J*=2.4), 3.68-3.56 (16H, m), 3.54 (2H, t, *J*=6.0), 3.52 (2H, t, *J*=6.0), 3.24 (2H, t, *J*=6.7), 3.11 (2H, t, *J*=6.7), 2.43 (2H, t, *J*=7.4), 2.25 (2H, t, *J*=7.4), 1.95 (2H, q, *J*=7.6), 1.82-1.70 (4H, m), 1.95 (2H, t, *J*=7.2); $\delta_C$(125 MHz, MeOD) 175.20, 174.27, 164.60, 164.44, 159.21, 157.75, 157.08, 151.53, 151.24, 147.23, 145.11, 143.98, 142.61, 140.34, 139.52, 135.32, 133.66, 132.56, 129.37, 128.24, 128.14, 127.65, 126.12, 125.58, 115.23, 115.04, 96.96, 71.58, 71.55, 71.25, 69.96, 69.38, 46.82, 41.67, 37.91, 37.00, 36.21, 30.88, 30.33, 22.69, 12.87;

$\nu_{max}$(solid)/cm$^{-1}$ 2869, 1648, 1586, 1530, 1464, 1415, 1393, 1334; *m/z* (EI) 1108.2910 (M+H$^{+}$. $C_{54}H_{65}N_{11}O_{11}S_2$ requires 1109.2984).

## References

(1) Rossin, R.; Verkerk, P. R.; van den Bosch, S. M.; Vulders, R. C. M.; Verel, I.; Lub, J.; Robillard, M. S. *Angew. Chem. Int. Ed.* **2010**, *49*, 3375–3378.