Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Model System for Irreversible Inhibition of Nek2: Thiol Addition to Ethynylpurines and Related Substituted Heterocycles

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Supplementary Information

General information

Quantitative ^{1}H NMR (^{1}H qNMR) measurements were obtained using a Bruker Avance III 500 MHz (11.74 τ) spectrometer operating at 500.303 MHz, equipped with a 5 mm broadband observe (BBO) probe. Experimental set-up and acquisitions were controlled using Topspin 2.1 (Bruker, Rheinstetten, Germany). Precision 5 mm diameter NMR tubes obtained from Bruker were used for all acquisitions. Experiments were carried out in DMSO-d_6 using solutions of a known concentration. ^{1}H NMR spectra were acquired as the summation of 4 transients and 1 equilibrating transient (signal unrecorded). Spectral data were collected at regular intervals for a defined number of experiments. Longitudinal relaxation (T1) measurements were carried out employing a standard 180-90 inversion recovery pulse sequence. The delay between transients was held constant at 10 s whilst the inversion delay was increased from 1 $\times 10^{-6}$ s (effectively zero) until full 90° relaxation of the slowest relaxing ^{1}H nucleus was observed resulting in zero net signal for the aforementioned proton. ^{1}H qNMR experiments were carried out using a composite 90°-180°-180° pulse, at a constant temperature of 24 °C. Samples were retained within the spectrometer for the duration of the experiment and maintained at a steady 20 Hz rotation throughout. The experiment was controlled using a multi-acquisition automation program, which included Fourier-transform and basic phase-correcting commands. Data were processed using standard Bruker phase-correcting algorithm. Line broadening was maintained at 0.30 Hz throughout and all baseline correction was applied from 10.0 to 0.0 ppm. Signals were integrated between defined chemical shift values at appropriate intervals throughout the course of the experiment. *Microsoft Excel* 2007 was used to manipulate raw data and to show data graphically.

Kinetic Analysis

$$\text{Thiol} + \text{DABCO} \underset{k'}{\overset{k}{\rightleftharpoons}} \text{Thiolate} + \text{DABCO}^+$$

$$\text{Thiolate} + \text{sm} \xrightarrow{k''} \text{Intermediate}$$

$$\text{Intermediate} + \text{DABCO}^+ \xrightarrow{k''} \text{Product} + \text{DABCO}$$

The reaction between thiol and DABCO is a rapid established equilibrium compared to the attack of thiolate on sm. The equilibrium equation for the reaction between DABCO and thiolate can be written as followed: $K = \frac{[\text{Thiolate}]_e[\text{DABCO}^+]_e}{[\text{Thiol}]_e[\text{DABCO}]_e} = \frac{k}{k'}$ (1)

After a short time the initial concentration of Thiol can be written as:

$$[\text{Thiol}]_0 = [\text{Thiol}]_e + [\text{Thiolate}]_e \quad (2)$$

The rate of the disappearance of the sm can be expressed as:

$$-\frac{d[sm]}{dt} = k''[sm][\text{Thiolate}]_e = k''[sm]K[\text{Thiol}]_e \frac{[\text{DABCO}]_e}{[\text{DABCO}^+]_e} \quad (3)$$

By replacing $[\text{Thiolate}]_e$ from equation 1 in equation 2, we obtain:

$$[\text{Thiol}]_0 = [\text{Thiol}]_e + K[\text{Thiol}]_e \frac{[\text{DABCO}]_e}{[\text{DABCO}^+]_e} \quad (4)$$

Therefore $[\text{Thiol}]_e$ can be expressed as:

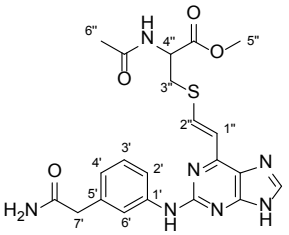
$$[\text{Thiol}]_e = \frac{[\text{Thiol}]_0}{1+K\frac{[\text{DABCO}]_e}{[\text{DABCO}^+]_e}} \quad (5)$$

By replacing $[\text{Thiol}]_e$ in equation 3 by its expression in equation 5, we have:

$$\frac{d[\text{product}]}{dt} = k''[sm]K[\text{Thiol}]_0 \frac{[\text{DABCO}]_e}{[\text{DABCO}^+]_e + K[\text{DABCO}]_e} = k_{app}[sm] \quad (6)$$

Isolation and Characterisation of 15

(*E*)-2-(3-((6-(2-((2,4,6-Trimethylbenzyl)thio)vinyl)-9*H*-purin-2-yl)amino)phenyl)acetamide (15)



2-(3-((6-Ethynyl-9*H*-purin-2-yl)amino)phenyl)acetamide **6** (50 mg, 0.17 mmol), *N*-acetylcysteine methyl ester (151 mg, 0.85 mmol) and DABCO (10 mg, 0.09 mmol) were solubilised in 8 mL of anhydrous DMF. The resulting solution was stirred at r.t. overnight. The solvent was then removed *in vacuo*. The crude product was purified by medium pressure chromatography on silica (EtOAc: MeOH 9:1) to give the product **15** as a yellow solid (41 mg, 0.09 mmol, 51 %).

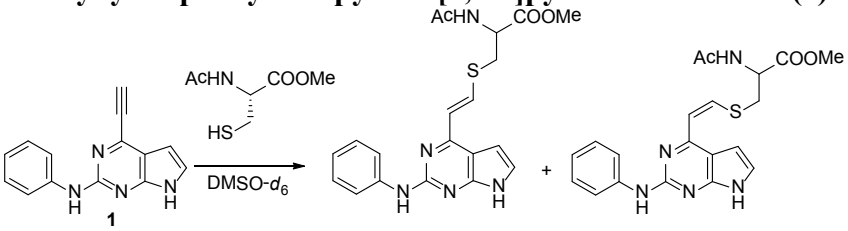
R_f: 0.55 (EtOAc: MeOH 9:1); Mp = 269-272 °C; UV λ_{max} (EtOH) 273 nm; IR (cm^{-1}) 3290, 3065, 2839, 2802, 1744, 1654, 1590; ^{1}H NMR (500 MHz, DMSO-d_6) ppm 1.88 (3H, s, H-6''), 3.27 (1H, dd, *J1* = 13.5 and 7.8 Hz, H-3''), 3.35 (2H, s, H-7'), 3.42 (1H, dd, *J1* = 13.5 and 7.8 Hz, H-3''), 3.68 (3H, s, H-5''), 4.60-4.64 (1H, m, H-4''), 6.79 (1H, d, *J* = 15.5 Hz, H-1''), 6.83 (1H, d, *J* = 7.8 Hz, H-4'), 6.86 (1H, br s, NH_2), 7.20 (1H, dd, *J* = 7.8 and 7.8 Hz, H-3'), 7.43 (1H, br s, NH_2), 7.69 (1H, d, *J* = 7.8 Hz, H-2'), 7.74 (1H, s, H-6'), 8.17 (1H, s, H-

8), 8.29 (1H, d, J = 15.5 Hz, H-2''), 8.57 (1H, d, J = 7.8 Hz, NH), 9.32 (1H, s, NH), 12.89 (1H, br s, N^9H); ^{13}C NMR (125 MHz, DMSO-d_6) ppm 22.3 (C-6''), 33.0 (C-3''), 42.6 (C-7'), 51.6 (C-4''), 52.3 (C-5''), 116.5 (C-2'), 119.1 (C-6'), 120.2 (C-1''), 121.4 (C-4'), 123.8 (C_q), 128.1 (C-3'), 136.5 (C_q), 138.8 (C-2''), 141.1 (C_q), 141.4 (C-8), 151.4 (C_q), 153.8 (C_q), 156.1 (C_q), 169.6 (CO), 170.7 (CO), 172.3 (CO); HRMS calcd for $C_{21}H_{24}N_7O_4S$ $[M+H]^+$ 470.1605, found 470.1602.

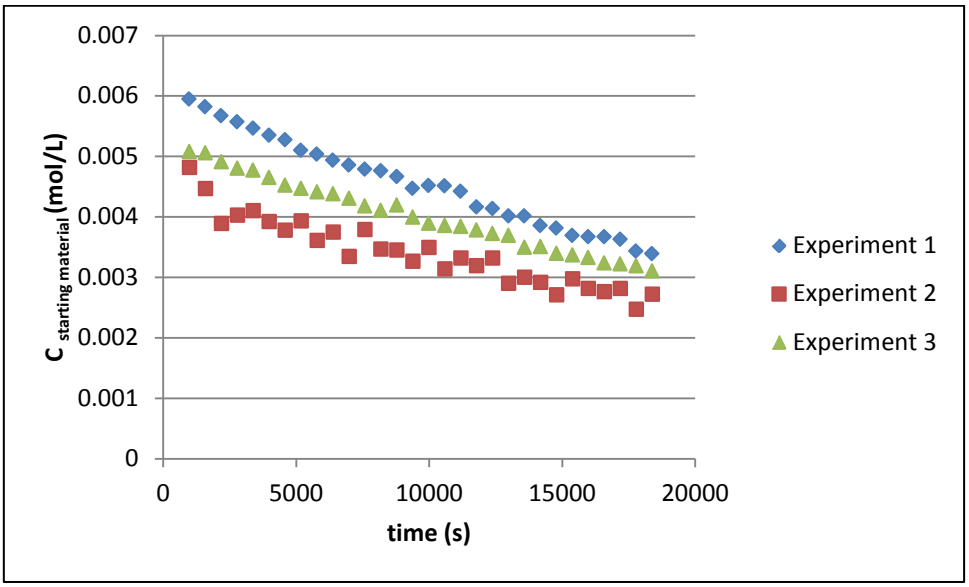
General procedure

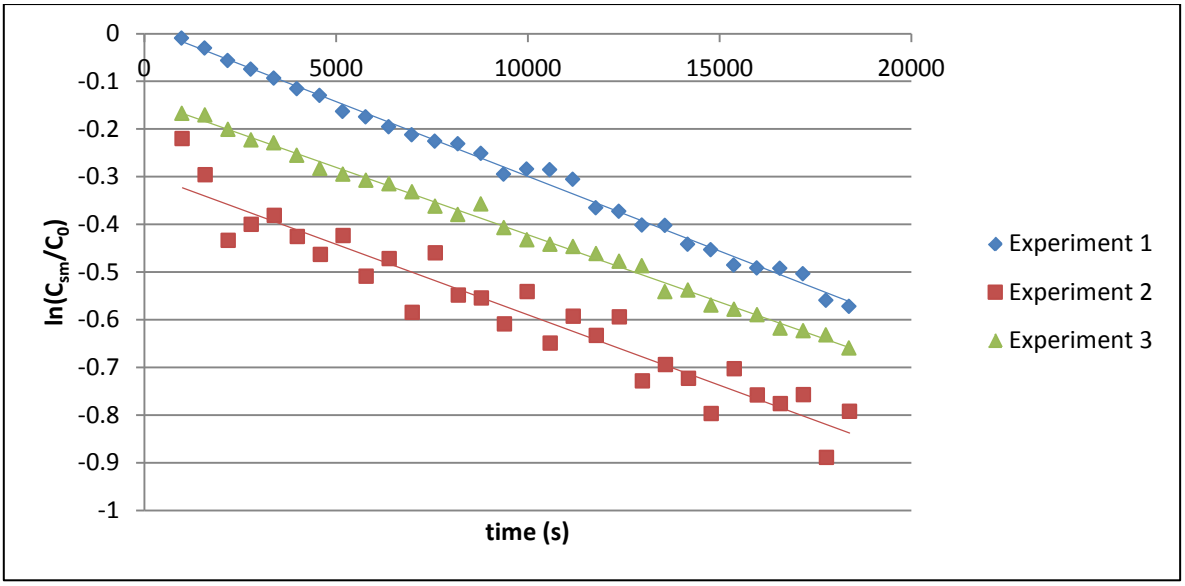
The 6-substituted heterocycle (690 µL from a stock solution in DMSO-d_6 containing 4.2 µmol of purine) was added to an excess of *N*-acetylcysteine methyl ester (7.48 mg, 42 µmol). The solution temperature was maintained at 24 °C (water-bath) before addition of a DMSO-d_6 solution (10 µL) containing DABCO (0.14 mg, 1.26 µmol) and DMF (0.33 µL, 4.2 µmol) to afford a final 6-substituted heterocycle concentration of 6 mM in a total volume of 700 µL. The NMR tube containing the reagents was quickly inverted several times to aid mixing and dissolution. The thoroughly mixed solution was inserted into the NMR magnet and the acquisition of quantitative ^{1}H NMR data was immediately initiated. The time between the addition of the DABCO/DMF-solution and the completion of the first ^{1}H qNMR experiment was monitored and subsequent time intervals between experiments were calculated based on the defined parameters

4-Ethynyl-*N*-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (1)



4-Ethynyl-*N*-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (**1**) (690 µL from stock solution in DMSO-d_6; 0.98 mg, 4.2 µmol) was treated according to general procedure. Disappearance of the broad singlet at 4.66-4.76 ppm was monitored as a function of time using the ^{1}H qNMR method outlined. Each spectrum was recorded every 10 min using 8 scans per experiment.





Experiment 1 ($R^2 = 0.99$; $k_{app} = 0.31 \times 10^{-4}$ s^{-1}; $t_{1/2} = 373$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
972	0.99	5.95	-0.01
1572	0.97	5.82	-0.03
2172	0.95	5.67	-0.06
2772	0.93	5.57	-0.07
3372	0.91	5.46	-0.09
3972	0.89	5.35	-0.12
4572	0.88	5.27	-0.13
5172	0.85	5.10	-0.16
5772	0.84	5.04	-0.17
6372	0.82	4.93	-0.20
6972	0.81	4.85	-0.21
7572	0.80	4.79	-0.23
8172	0.79	4.76	-0.23
8772	0.78	4.67	-0.25
9372	0.75	4.47	-0.29
9972	0.75	4.52	-0.28
10572	0.75	4.51	-0.29
11172	0.74	4.42	-0.31
11772	0.69	4.17	-0.37
12372	0.69	4.13	-0.37
12972	0.67	4.02	-0.40
13572	0.67	4.01	-0.40
14172	0.64	3.86	-0.44
14772	0.64	3.81	-0.45
15372	0.62	3.69	-0.49
15972	0.61	3.67	-0.49
16572	0.61	3.67	-0.49
17172	0.60	3.63	-0.50

17772	0.57	3.43	-0.56
18372	0.56	3.39	-0.57

Experiment 2 ($R^2 = 0.92$; $k_{app} = 0.30 \times 10^{-4}$ s^{-1}; $t_{1/2} = 385$ min)

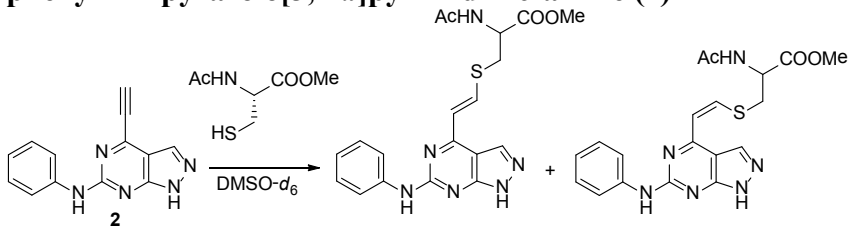
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
990	0.80	4.81	-0.22
1590	0.74	4.46	-0.30
2190	0.65	3.89	-0.43
2790	0.67	4.02	-0.40
3390	0.68	4.10	-0.38
3990	0.65	3.92	-0.43
4590	0.63	3.78	-0.46
5190	0.66	3.93	-0.42
5790	0.60	3.61	-0.51
6390	0.62	3.74	-0.47
6990	0.56	3.34	-0.58
7590	0.63	3.79	-0.46
8190	0.58	3.47	-0.55
8790	0.57	3.45	-0.55
9390	0.54	3.26	-0.61
9990	0.58	3.49	-0.54
10590	0.52	3.14	-0.65
11190	0.55	3.32	-0.59
11790	0.53	3.19	-0.63
12390	0.55	3.31	-0.59
12990	0.48	2.90	-0.73
13590	0.50	3.00	-0.69
14190	0.49	2.91	-0.72
14790	0.45	2.70	-0.80
15390	0.50	2.97	-0.70
15990	0.46	2.81	-0.76
16590	0.46	2.76	-0.78
17190	0.47	2.81	-0.77
17790	0.41	2.47	-0.89
18390	0.45	2.72	-0.79

Experiment 3 ($R^2 = 1.00$; $k_{app} = 0.28 \times 10^{-4}$ s^{-1}; $t_{1/2} = 413$ min)

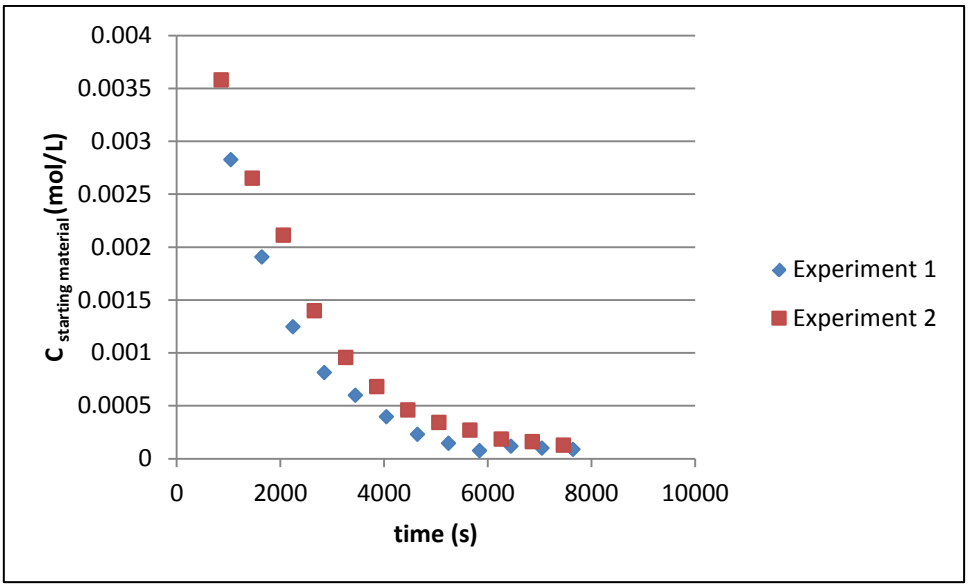
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
978	0.85	5.08	-0.17
1578	0.84	5.06	-0.17
2178	0.82	4.91	-0.20
2778	0.80	4.80	-0.22
3378	0.80	4.77	-0.23
3978	0.78	4.65	-0.26

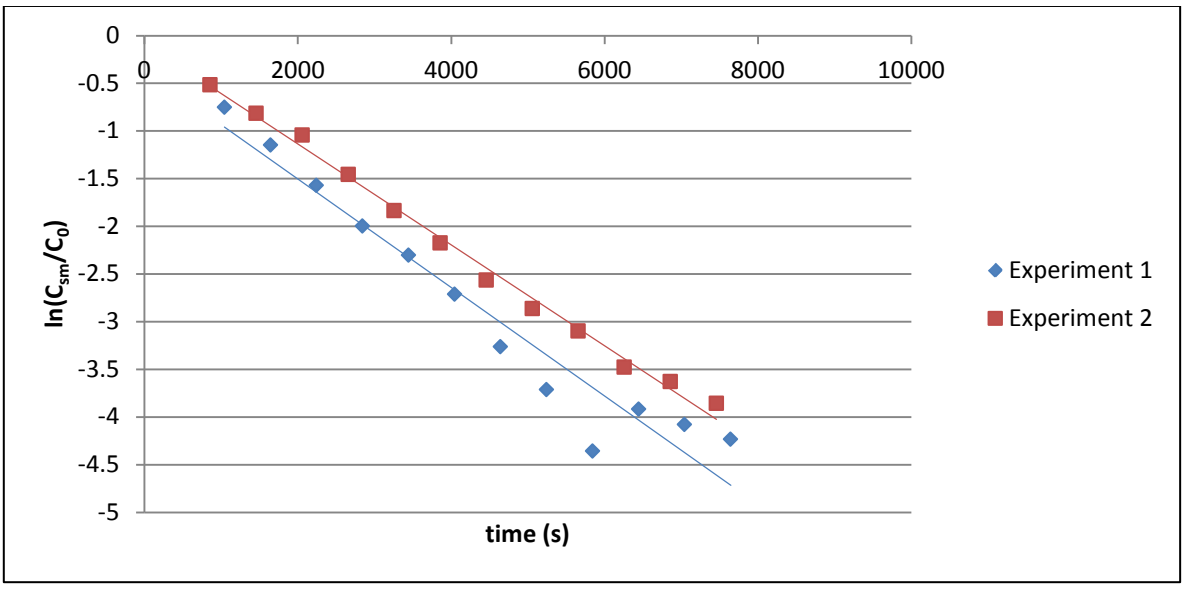
4578	0.75	4.52	-0.28
5178	0.75	4.47	-0.29
5778	0.74	4.41	-0.31
6378	0.73	4.38	-0.31
6978	0.72	4.31	-0.33
7578	0.70	4.18	-0.36
8178	0.68	4.11	-0.38
8778	0.70	4.20	-0.36
9378	0.67	3.99	-0.41
9978	0.65	3.89	-0.43
10578	0.64	3.86	-0.44
11178	0.64	3.84	-0.45
11778	0.63	3.78	-0.46
12378	0.62	3.72	-0.48
12978	0.62	3.69	-0.49
13578	0.58	3.49	-0.54
14178	0.58	3.51	-0.54
14778	0.57	3.40	-0.57
15378	0.56	3.37	-0.58
15978	0.56	3.33	-0.59
16578	0.54	3.24	-0.62
17178	0.54	3.22	-0.62
17778	0.53	3.19	-0.63
18378	0.52	3.10	-0.66

4-Ethynyl-*N*-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (2)



4-Ethynyl-*N*-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (**2**) (690 µL from stock solution in DMSO-d_6; 0.99 mg, 4.2 µmol) was treated according to general procedure. Disappearance of the broad singlet at 4.93-5.03 ppm was monitored as a function of time using the ^{1}H qNMR method outlined. Each spectrum was recorded every 10 min using 8 scans per experiment.





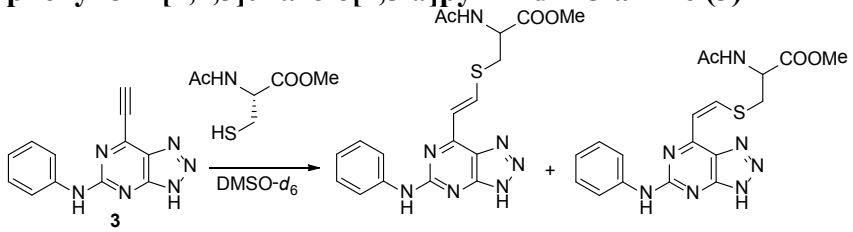
Experiment 1 ($R^2 = 0.94$; $k_{app} = 5.69 \times 10^{-4}$ s^{-1}; $t_{1/2} = 20$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
1044	0.47	2.83	-0.75
1644	0.32	1.92	-1.15
2244	0.21	1.25	-1.57
2844	0.14	0.81	-2.00
3444	0.10	0.60	-2.30
4044	0.07	0.40	-2.71
4644	0.04	0.23	-3.26
5244	0.02	0.15	-3.71
5844	0.01	0.08	-4.36
6444	0.02	0.12	-3.92
7044	0.02	0.10	-4.08
7644	0.02	0.09	-4.23

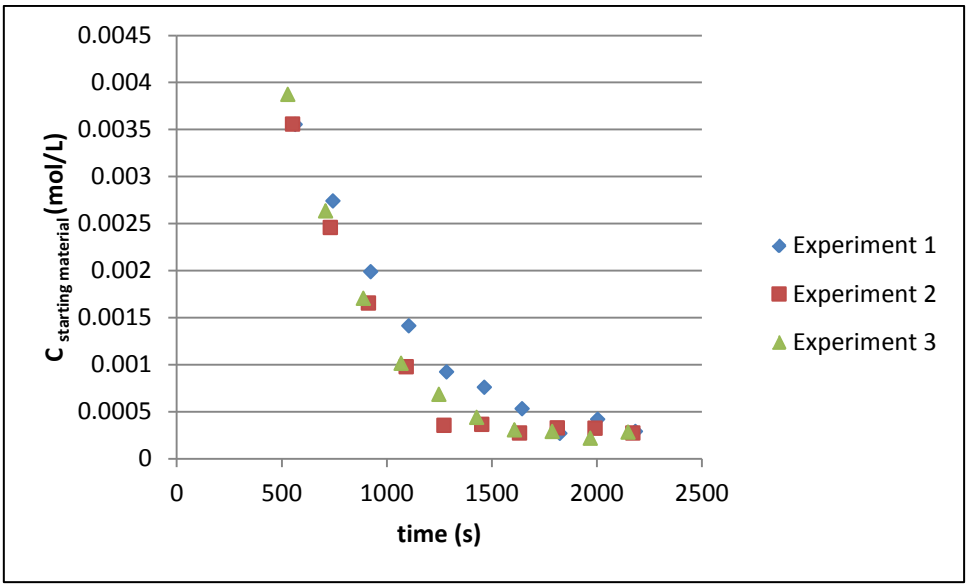
Experiment 2 ($R^2 = 0.99$; $k_{app} = 5.29 \times 10^{-4}$ s^{-1}; $t_{1/2} = 22$ min)

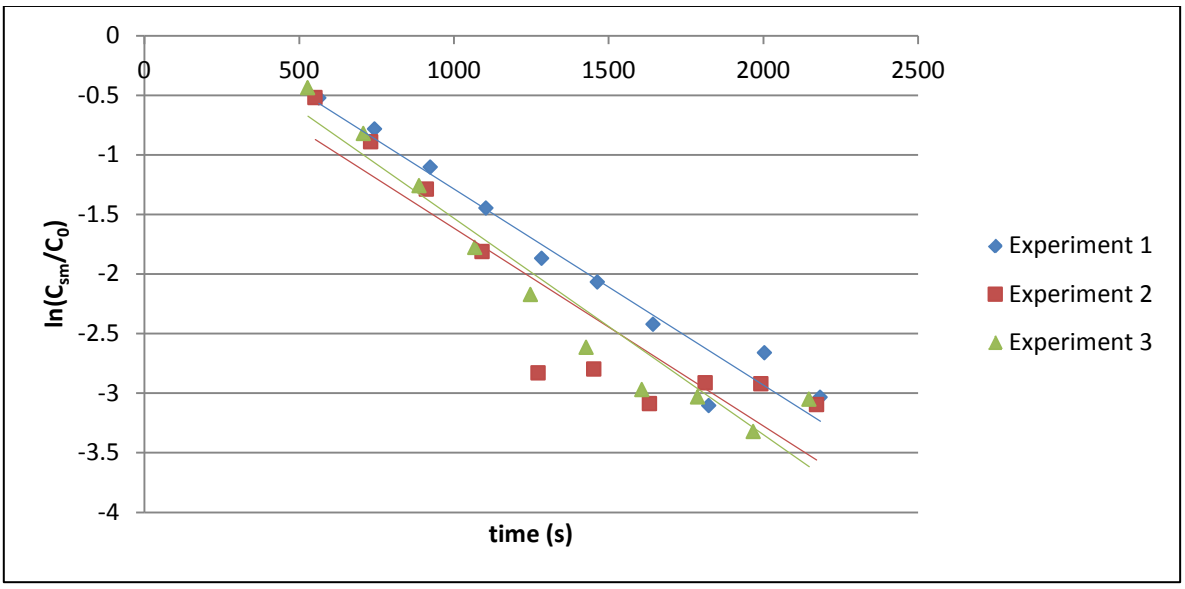
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
858	0.60	3.58	-0.52
1458	0.44	2.65	-0.82
2058	0.35	2.11	-1.04
2658	0.23	1.40	-1.46
3258	0.16	0.96	-1.84
3858	0.11	0.68	-2.18
4458	0.08	0.46	-2.57
5058	0.06	0.34	-2.86
5658	0.05	0.27	-3.10
6258	0.03	0.19	-3.48
6858	0.03	0.16	-3.63
7458	0.02	0.13	-3.86

7-Ethynyl-*N*-phenyl-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-5-amine (3)



7-Ethynyl-*N*-phenyl-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-5-amine (**3**) (690 µL from stock solution in DMSO-d_6; 0.99 mg, 4.2 µmol) was treated according to general procedure. Disappearance of the broad singlet at 5.14-5.24 ppm was monitored as a function of time using the ^{1}H qNMR method outlined. Each spectrum was recorded every 3 min using 4 scans per experiment.





Experiment 1 ($R^2 = 0.95$; $k_{app} = 16.46 \times 10^{-4}$ s^{-1}; $t_{1/2} = 7$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
564	0.59	3.55	-0.52
744	0.46	2.74	-0.78
924	0.33	1.99	-1.10
1104	0.24	1.41	-1.45
1284	0.15	0.92	-1.87
1464	0.13	0.76	-2.07
1644	0.09	0.53	-2.42
1824	0.05	0.27	-3.11
2004	0.07	0.42	-2.66
2184	0.05	0.29	-3.04

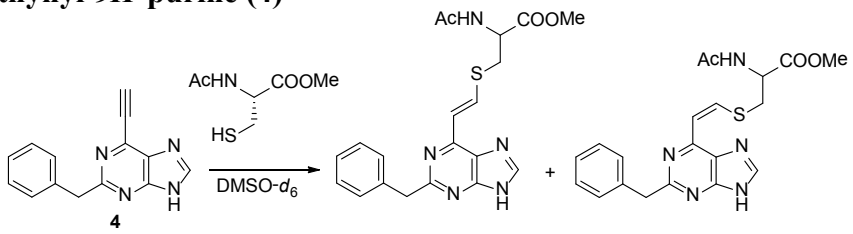
Experiment 2 ($R^2 = 0.83$; $k_{app} = 16.60 \times 10^{-4}$ s^{-1}; $t_{1/2} = 7$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
552	0.59	3.56	-0.52
732	0.41	2.46	-0.89
912	0.28	1.65	-1.29
1092	0.16	0.98	-1.81
1272	0.06	0.35	-2.83
1452	0.06	0.37	-2.80
1632	0.05	0.27	-3.09
1812	0.05	0.33	-2.91
1992	0.05	0.32	-2.92
2172	0.05	0.27	-3.10

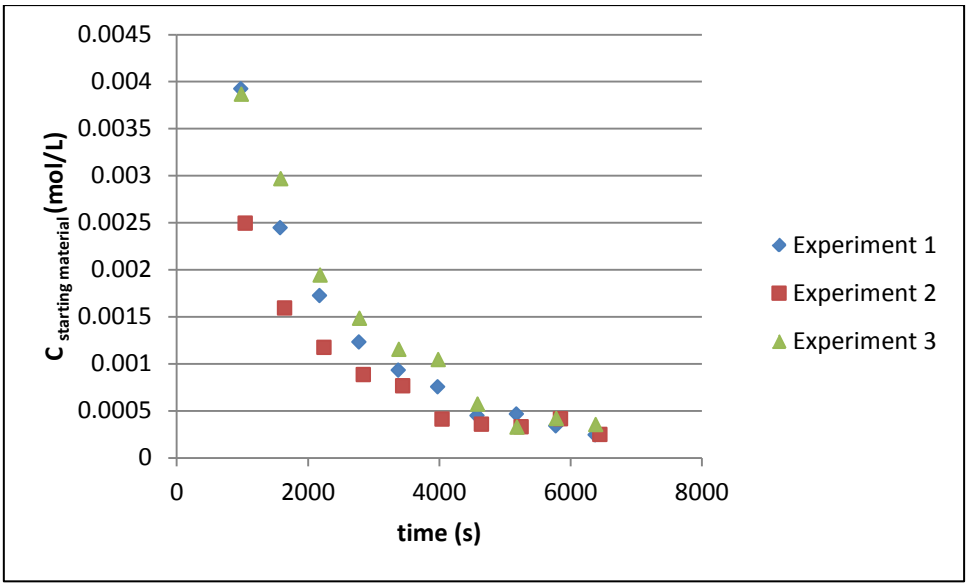
Experiment 3 ($R^2 = 0.93$; $k_{app} = 18.15 \times 10^{-4}$ s^{-1}; $t_{1/2} = 6$ min)

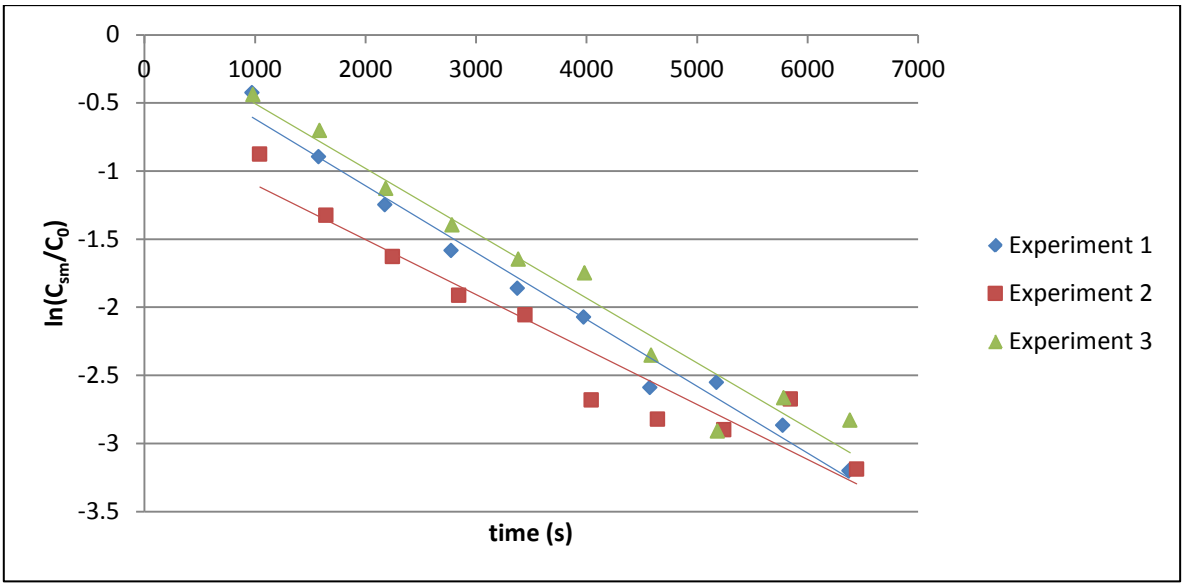
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
528	0.65	3.88	-0.44
708	0.44	2.64	-0.82
888	0.28	1.70	-1.26
1068	0.17	1.01	-1.78
1248	0.11	0.68	-2.17
1428	0.07	0.44	-2.62
1608	0.05	0.31	-2.97
1788	0.05	0.29	-3.03
1968	0.04	0.22	-3.32
2148	0.05	0.28	-3.05

2-Benzyl-6-ethynyl-9*H*-purine (4)



2-Benzyl-6-ethynyl-9*H*-purine (**4**) (690 µL from stock solution in DMSO-d_6; 0.98 mg, 4.2 µmol) was treated according to general procedure. Disappearance of the broad singlet at 4.89-4.99 ppm was monitored as a function of time using the ^{1}H qNMR method outlined. Each spectrum was recorded every 10 min using 8 scans per experiment.





Experiment 1 ($R^2 = 0.98$; $k_{app} = 4.90 \times 10^{-4}$ s^{-1}; $t_{1/2} = 23.6$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
975	0.65	3.92	-0.42
1575	0.41	2.45	-0.90
2175	0.29	1.72	-1.25
2775	0.21	1.23	-1.58
3375	0.16	0.93	-1.86
3975	0.13	0.75	-2.07
4575	0.08	0.45	-2.59
5175	0.08	0.47	-2.55
5775	0.06	0.34	-2.87
6375	0.04	0.24	-3.20

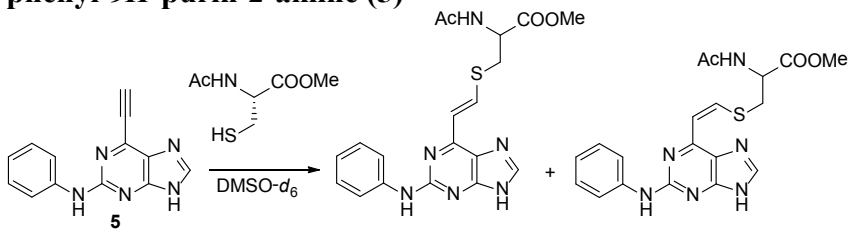
Experiment 2 ($R^2 = 0.92$; $k_{app} = 4.04 \times 10^{-4}$ s^{-1}; $t_{1/2} = 23.6$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
1044	0.42	2.49	-0.88
1644	0.27	1.60	-1.33
2244	0.20	1.18	-1.63
2844	0.15	0.89	-1.91
3444	0.13	0.77	-2.06
4044	0.07	0.41	-2.68
4644	0.06	0.36	-2.82
5244	0.06	0.33	-2.90
5844	0.07	0.41	-2.67
6444	0.04	0.25	-3.19

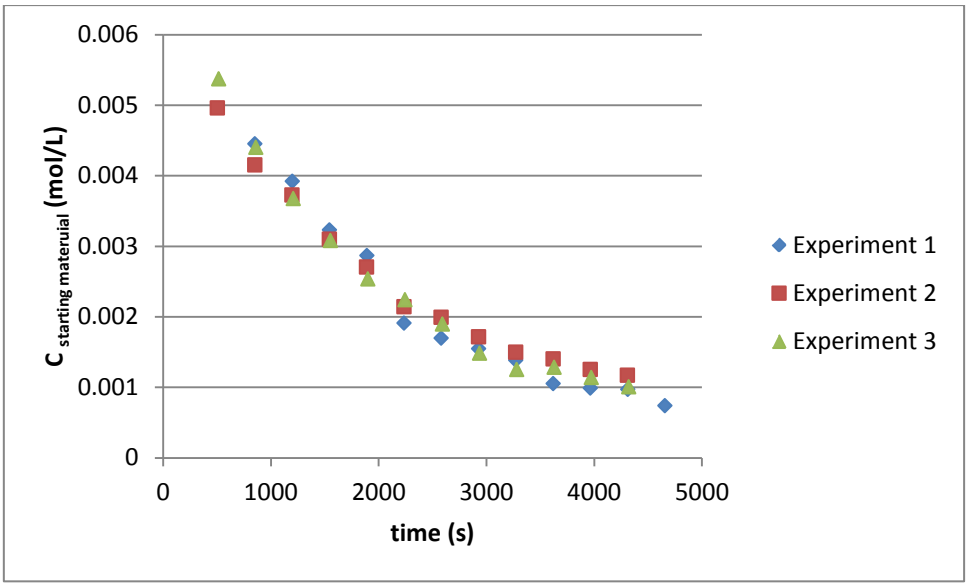
Experiment 3 ($R^2 = 0.96$; $k_{app} = 4.76 \times 10^{-4}$ s^{-1}; $t_{1/2} = 24.3$ min)

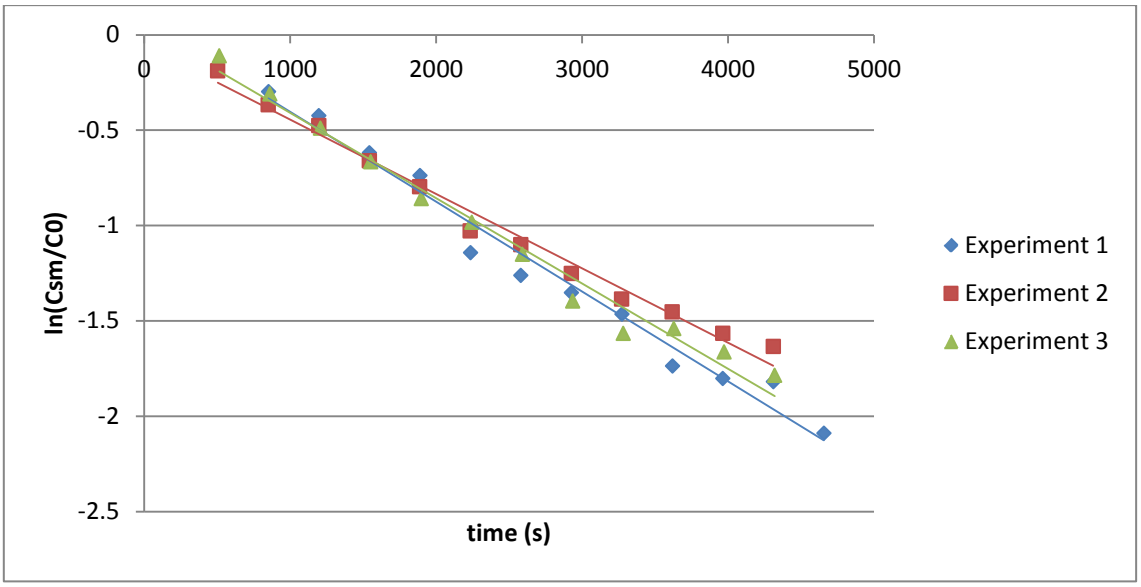
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
984	0.64	3.87	-0.44
1584	0.50	2.97	-0.70
2184	0.32	1.94	-1.13
2784	0.25	1.49	-1.40
3384	0.19	1.16	-1.65
3984	0.17	1.04	-1.75
4584	0.10	0.57	-2.35
5184	0.06	0.33	-2.91
5784	0.07	0.42	-2.66
6384	0.06	0.35	-2.83

6-Ethynyl-*N*-phenyl-9*H*-purin-2-amine (5)



6-Ethynyl-*N*-phenyl-9*H*-purin-2-amine (**5**) (690 µL from stock solution in DMSO-d_6; 0.99 mg, 4.2 µmol) was treated according to general procedure. Disappearance of the broad singlet at 4.81-4.91 ppm was monitored as a function of time using the ^{1}H qNMR method outlined. Each spectrum was recorded every 5.8 min using 4 scans per experiment.





No DABCO ($R^2 = 0.73$; $k_{app} = 1.92 \times 10^{-6}$ s^{-1}; $t_{½} = 6017$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
1200	1.00	6.02	0.00
4314	0.99	5.97	0.01
7774	0.96	5.76	0.04
11234	0.95	5.74	0.05
14694	0.97	5.85	0.03
18154	0.97	5.81	0.03
21614	0.95	5.71	0.05
25074	0.96	5.76	0.04
28534	0.93	5.60	0.07

Experiment 1 ($R^2 = 0.98$; $k_{app} = 4.71 \times 10^{-4}$ s^{-1}; $t_{1/2} = 24.5$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
851	0.74	4.45	-0.30
1197	0.65	3.92	-0.42
1543	0.54	3.23	-0.62
1889	0.48	2.87	-0.74
2235	0.32	1.91	-1.14
2581	0.28	1.70	-1.26
2927	0.26	1.55	-1.35
3273	0.23	1.38	-1.47
3619	0.18	1.05	-1.74
3965	0.17	0.99	-1.80
4311	0.16	0.97	-1.82
4657	0.12	0.74	-2.09

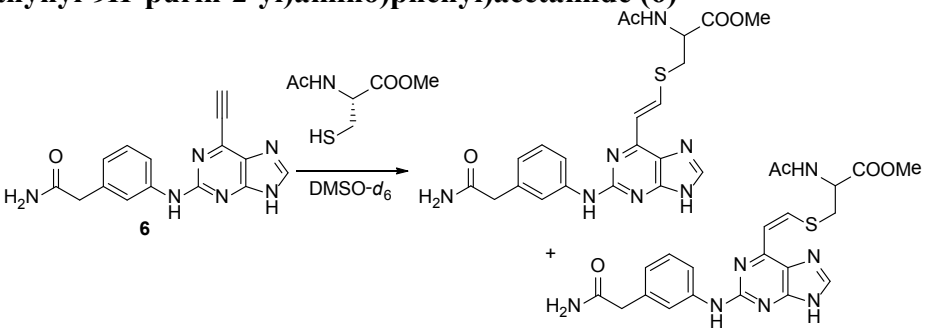
Experiment 2 ($R^2 = 0.99$; $k_{app} = 3.90 \times 10^{-4}$ s^{-1}; $t_{1/2} = 29.6$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
504	0.83	4.96	-0.19
850	0.69	4.15	-0.37
1196	0.62	3.73	-0.48
1542	0.52	3.10	-0.66
1888	0.45	2.70	-0.80
2234	0.36	2.14	-1.03
2580	0.33	2.00	-1.10
2926	0.29	1.71	-1.25
3272	0.25	1.50	-1.39
3618	0.23	1.40	-1.45
3964	0.21	1.25	-1.57
4310	0.20	1.17	-1.63

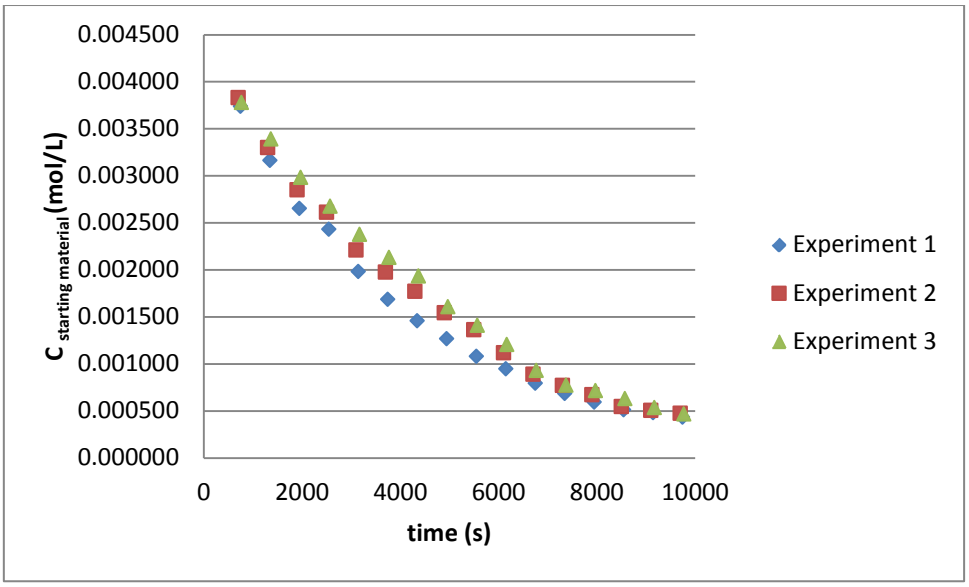
Experiment 3 ($R^2 = 0.98$; $k_{app} = 4.47 \times 10^{-4}$ s^{-1}; $t_{1/2} = 25.8$ min)

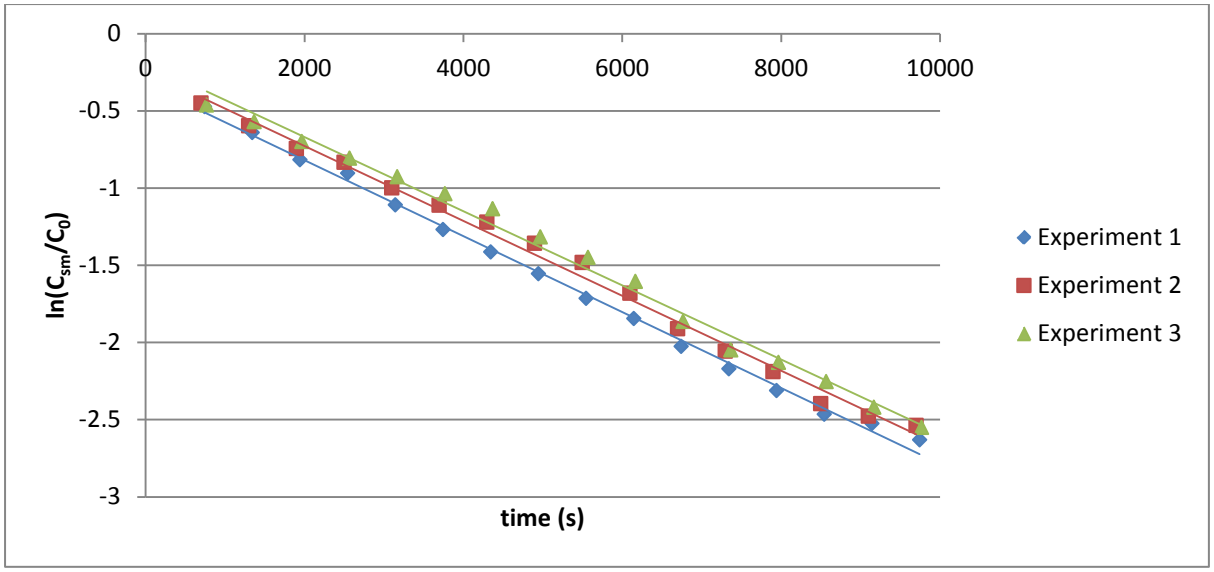
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
514	0.90	5.37	-0.11
860	0.73	4.40	-0.31
1206	0.61	3.68	-0.49
1552	0.51	3.08	-0.67
1898	0.42	2.54	-0.86
2244	0.37	2.24	-0.98
2590	0.32	1.90	-1.15
2936	0.25	1.48	-1.40
3282	0.21	1.25	-1.57
3628	0.21	1.29	-1.54
3974	0.19	1.14	-1.66
4320	0.17	1.01	-1.79

2-(3-((6-Ethynyl-9*H*-purin-2-yl)amino)phenyl)acetamide (6)



2-(3-((6-Ethynyl-9*H*-purin-2-yl)amino)phenyl)acetamide (**6**) (690 µL from stock solution DMSO-d_6, 1.23 mg, 4.2 µmol) was treated according to general procedure. Disappearance of the singlet at 4.71-4.91 ppm was monitored as a function of time using the ^{1}H qNMR method outlined. Each spectrum was recorded every 10 min using 8 scans per experiment.





Experiment 1 ($R^2 = 1.00$; $k_{app} = 2.50 \times 10^{-4}$ s^{-1}; $t_{1/2} = 46.2$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
744	0.62	3.74	-0.47
1344	0.53	3.16	-0.64
1944	0.44	2.65	-0.82
2544	0.41	2.43	-0.90
3144	0.33	1.98	-1.11
3744	0.28	1.69	-1.27
4344	0.24	1.46	-1.41
4944	0.21	1.27	-1.56
5544	0.18	1.08	-1.71
6144	0.16	0.95	-1.85
6744	0.13	0.79	-2.02
7344	0.11	0.68	-2.17
7944	0.10	0.59	-2.31
8544	0.09	0.51	-2.47
9144	0.08	0.48	-2.52
9744	0.07	0.43	-2.63

Experiment 2 ($R^2 = 0.99$; $k_{app} = 2.43 \times 10^{-4}$ s^{-1}; $t_{1/2} = 47.5$ min)

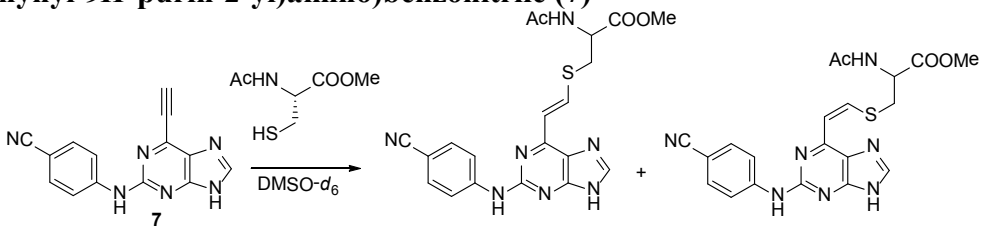
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
698	0.64	3.83	-0.45
1298	0.55	3.30	-0.60
1898	0.48	2.85	-0.74
2498	0.44	2.61	-0.83
3098	0.37	2.21	-1.00
3698	0.33	1.97	-1.11
4298	0.30	1.77	-1.22
4898	0.26	1.54	-1.36
5498	0.23	1.36	-1.48
6098	0.19	1.12	-1.68
6698	0.15	0.89	-1.91
7298	0.13	0.77	-2.06
7898	0.11	0.67	-2.19
8498	0.09	0.55	-2.40
9098	0.08	0.50	-2.48
9698	0.08	0.47	-2.54

Experiment 3 ($R^2 = 0.99$; $k_{app} = 2.40 \times 10^{-4}$ s^{-1}; $t_{1/2} = 48.1$ min)

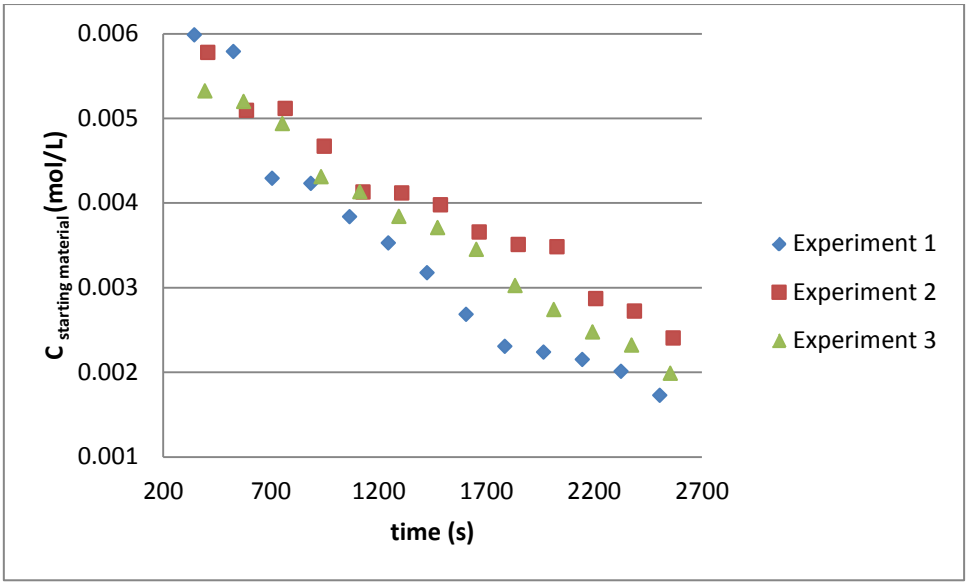
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
768	0.63	3.78	-0.46

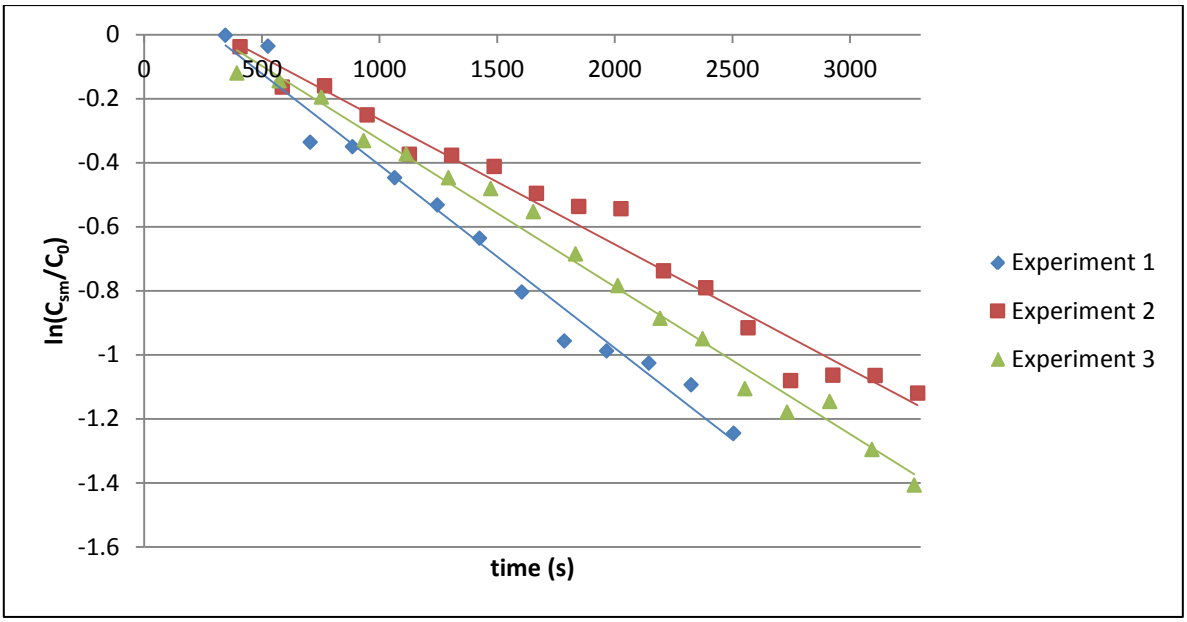
1368	0.57	3.39	-0.57
1968	0.50	2.98	-0.70
2568	0.45	2.68	-0.81
3168	0.40	2.38	-0.93
3768	0.36	2.13	-1.04
4368	0.32	1.93	-1.13
4968	0.27	1.61	-1.32
5568	0.24	1.41	-1.45
6168	0.20	1.21	-1.60
6768	0.16	0.93	-1.86
7368	0.13	0.77	-2.05
7968	0.12	0.71	-2.13
8568	0.11	0.63	-2.25
9168	0.09	0.53	-2.42
9768	0.08	0.47	-2.55

4-((6-Ethynyl-9*H*-purin-2-yl)amino)benzonitrile (7)



4-((6-Ethynyl-9*H*-purin-2-yl)amino)benzonitrile (**7**) (690 µL from stock solution DMSO-d_6; 1.09 mg, 4.2 µmol) was treated according to general procedure. Disappearance of the singlet at 4.87-4.97 ppm was monitored as a function of time using the ^{1}H qNMR method outlined. Each spectrum was recorded every 3 min using 4 scans per experiment.





Experiment 1 ($R^2 = 0.98$, $k_{app} = 5.71 \times 10^{-4}$ s^{-1}; $t_{1/2} = 20.2$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
345	1.00	5.99	-0.01
525	0.97	5.79	-0.04
705	0.72	4.29	-0.34
885	0.71	4.23	-0.35
1065	0.64	3.84	-0.45
1245	0.59	3.53	-0.53
1425	0.53	3.18	-0.64
1605	0.45	2.69	-0.80
1785	0.38	2.30	-0.96
1965	0.37	2.24	-0.99
2145	0.36	2.15	-1.03
2325	0.34	2.01	-1.09
2505	0.29	1.73	-1.25

Experiment 2 ($R^2 = 0.97$, $k_{app} = 3.90 \times 10^{-4}$ s^{-1}; $t_{1/2} = 29.6$ min)

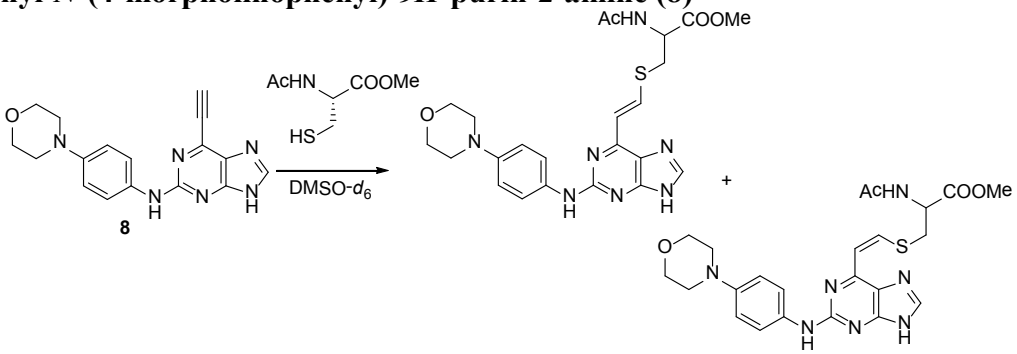
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
407	0.96	5.78	-0.04
587	0.85	5.09	-0.16
767	0.85	5.12	-0.16
947	0.78	4.67	-0.25
1127	0.69	4.13	-0.37
1307	0.69	4.12	-0.38
1487	0.66	3.98	-0.41
1667	0.61	3.66	-0.50
1847	0.58	3.51	-0.54
2027	0.58	3.48	-0.54
2207	0.48	2.87	-0.74
2387	0.45	2.72	-0.79
2567	0.40	2.40	-0.92
2747	0.34	2.04	-1.08
2927	0.35	2.07	-1.06
3107	0.35	2.07	-1.06
3287	0.33	1.96	-1.12

Experiment 3 ($R^2 = 0.97$, $k_{app} = 3.90 \times 10^{-4}$ s^{-1}; $t_{1/2} = 29.6$ min)

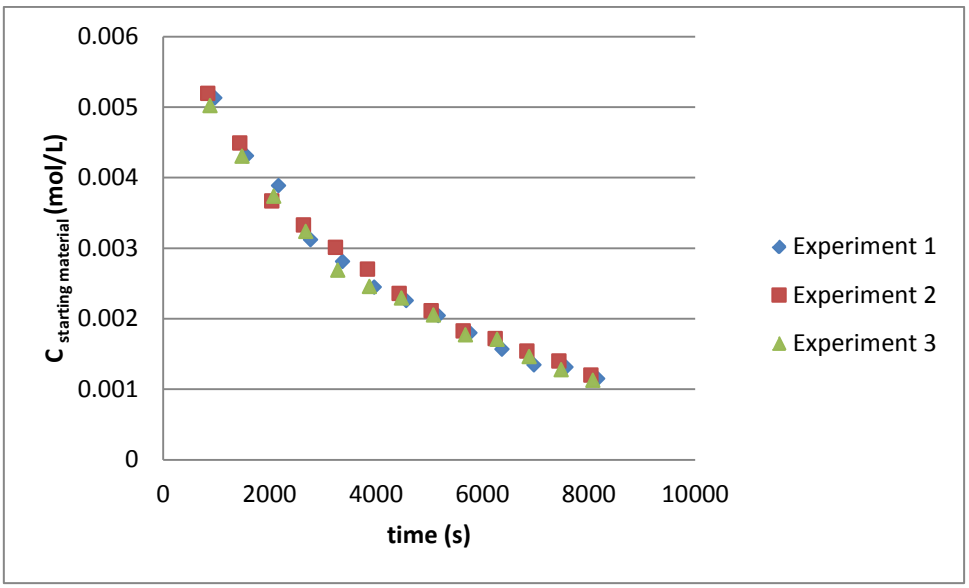
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
393	0.89	5.32	-0.12
573	0.87	5.20	-0.14
753	0.82	4.94	-0.19

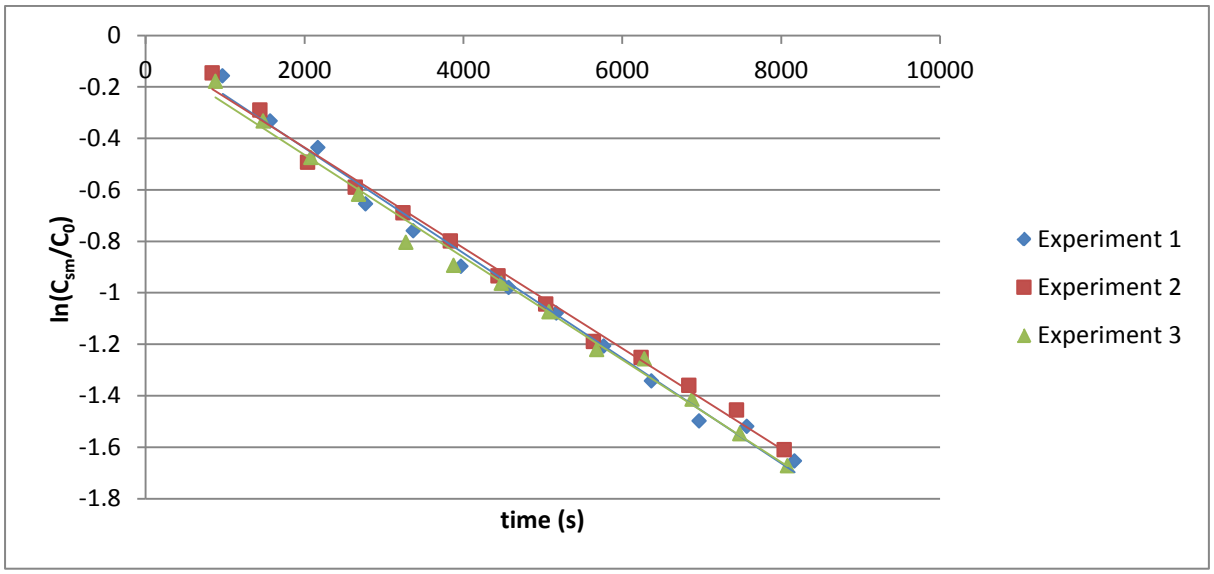
933	0.72	4.31	-0.33
1113	0.69	4.13	-0.37
1293	0.64	3.84	-0.45
1473	0.62	3.71	-0.48
1653	0.58	3.45	-0.55
1833	0.50	3.03	-0.68
2013	0.46	2.74	-0.78
2193	0.41	2.48	-0.89
2373	0.39	2.32	-0.95
2553	0.33	1.99	-1.11
2733	0.31	1.85	-1.18
2913	0.32	1.91	-1.15
3093	0.27	1.64	-1.30
3273	0.25	1.47	-1.41

6-Ethynyl-*N*-(4-morpholinophenyl)-9*H*-purin-2-amine (8)



6-Ethynyl-*N*-(4-morpholinophenyl)-9*H*-purin-2-amine (**8**) (690 µL from stock solution DMSO-d_6, 1.34mg, 4.2 µmol) was treated according to general procedure. Disappearance of the singlet at 4.74-4.84 ppm was monitored as a function of time using the ^{1}H qNMR method outlined. Each spectrum was recorded every 10 min using 8 scans per experiment.





Experiment 1 ($R^2 = 0.99$, $k_{app} = 2.04.10^{-4}$ s^{-1}; $t_{1/2} = 56.6$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
969	0.85	5.13	-0.16
1569	0.72	4.31	-0.33
2169	0.65	3.88	-0.44
2769	0.52	3.12	-0.65
3369	0.47	2.81	-0.76
3969	0.41	2.45	-0.90
4569	0.38	2.25	-0.98
5169	0.34	2.04	-1.08
5769	0.30	1.80	-1.21
6369	0.26	1.57	-1.34
6969	0.22	1.34	-1.50
7569	0.22	1.31	-1.52
8169	0.19	1.15	-1.65

Experiment 2 ($R^2 = 0.99$, $k_{app} = 1.95.10^{-4}$ s^{-1}; $t_{1/2} = 59.2$ min)

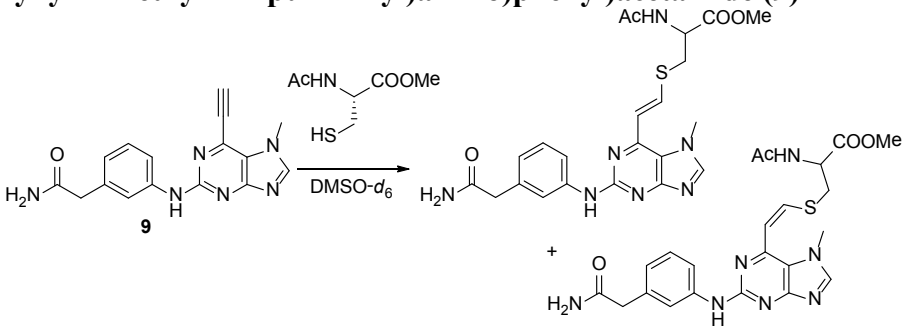
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
839	0.87	5.19	-0.15
1439	0.75	4.49	-0.29
2039	0.61	3.67	-0.49
2639	0.55	3.33	-0.58
3239	0.50	3.01	-0.69
3839	0.45	2.70	-0.80
4439	0.39	2.36	-0.93
5039	0.35	2.11	-1.04
5639	0.30	1.83	-1.19
6239	0.29	1.72	-1.25
6839	0.26	1.54	-1.36
7439	0.23	1.40	-1.46
8039	0.20	1.20	-1.61

Experiment 3 ($R^2 = 0.99$, $k_{app} = 1.99.10^{-4}$ s^{-1}; $t_{1/2} = 58.1$ min)

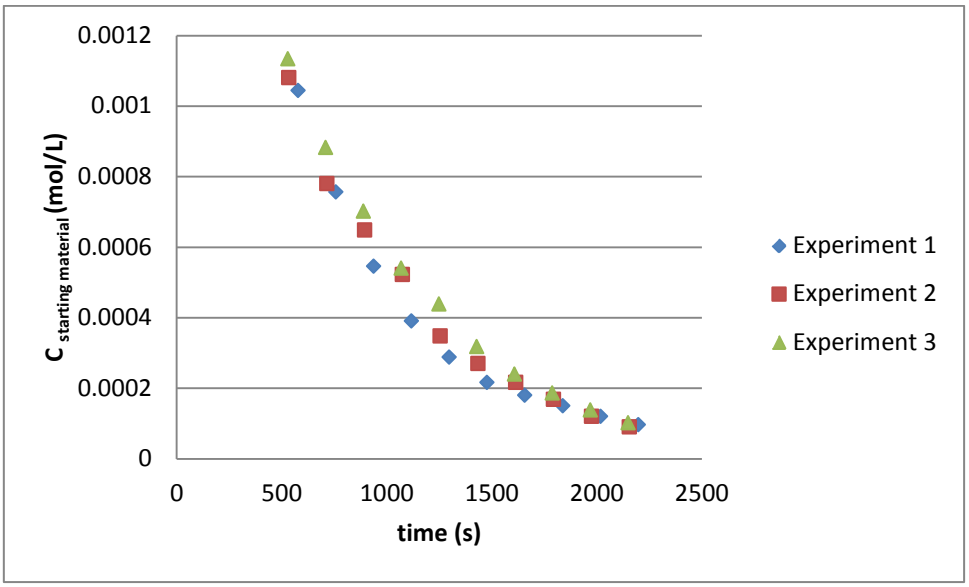
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
879	0.84	5.02	-0.18
1479	0.72	4.30	-0.33
2079	0.62	3.73	-0.47
2679	0.54	3.24	-0.62
3279	0.45	2.69	-0.80
3879	0.41	2.46	-0.89
4479	0.38	2.29	-0.96

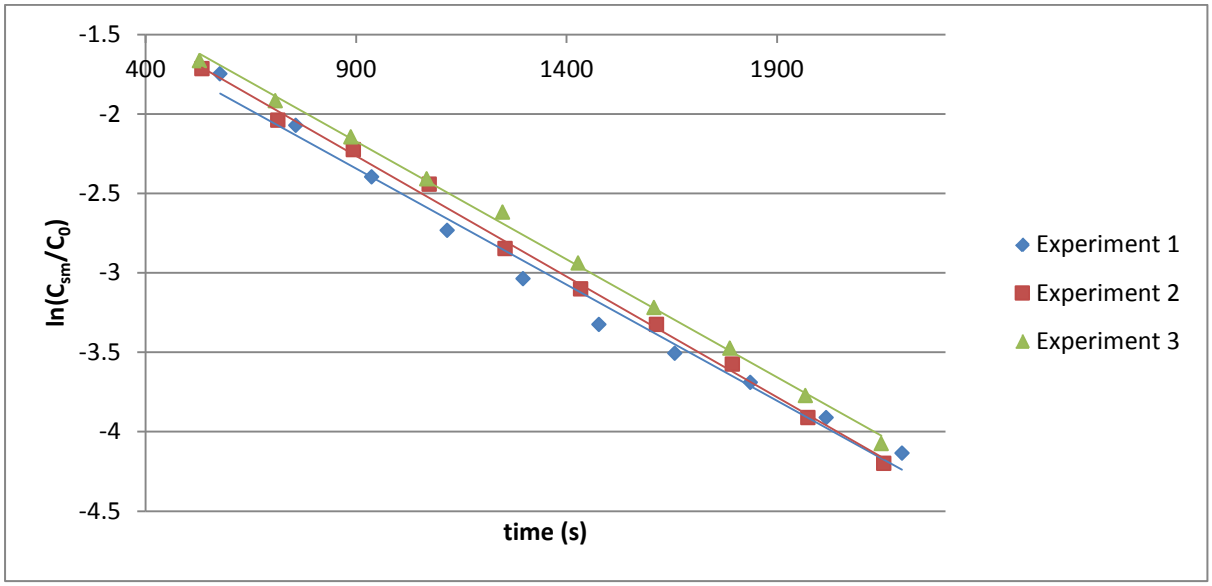
5079	0.34	2.05	-1.07
5679	0.30	1.77	-1.22
6279	0.28	1.71	-1.26
6879	0.24	1.46	-1.41
7479	0.21	1.28	-1.55
8079	0.19	1.13	-1.67

2-(3-((6-Ethynyl-7-methyl-7*H*-purin-2-yl)amino)phenyl)acetamide (9)



2-(3-((6-Ethynyl-7-methyl-7*H*-purin-2-yl)amino)phenyl)acetamide (**9**) (690 µL from stock solution DMSO-d_6, 1.29 mg, 4.2 µmol) was treated according to general procedure. Disappearance of the singlet at 4.90-5.10 ppm was monitored as a function of time using the ^{1}H qNMR method outlined. Each spectrum was recorded every 3 min using 4 scans per experiment.





Experiment 1 ($R^2 = 0.99$, $k_{app} = 14.62.10^{-4}$ s^{-1}; $t_{1/2} = 7.9$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
577	0.17	1.04	-1.75
757	0.13	0.76	-2.07
937	0.09	0.55	-2.40
1117	0.07	0.39	-2.73
1297	0.05	0.29	-3.04
1477	0.04	0.22	-3.32
1657	0.03	0.18	-3.51
1837	0.03	0.15	-3.69
2017	0.02	0.12	-3.91
2197	0.02	0.10	-4.14

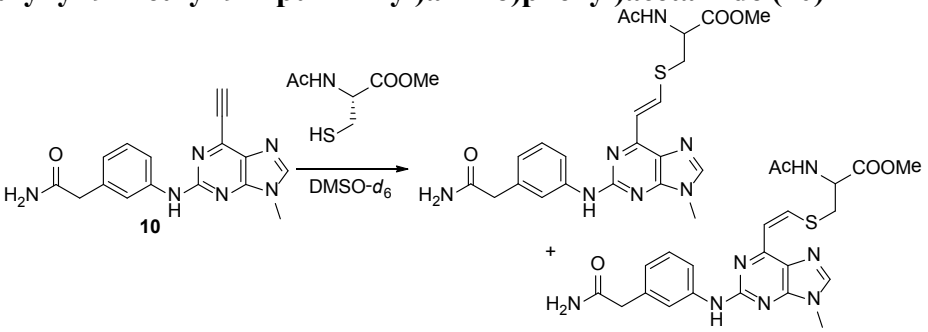
Experiment 2 ($R^2 = 1.00$, $k_{app} = 15.19.10^{-4}$ s^{-1}; $t_{1/2} = 7.8$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
534	0.18	1.08	-1.71
714	0.13	0.78	-2.04
894	0.11	0.65	-2.23
1074	0.09	0.52	-2.44
1254	0.06	0.35	-2.85
1434	0.05	0.27	-3.10
1614	0.04	0.22	-3.32
1794	0.03	0.17	-3.58
1974	0.02	0.12	-3.91
2154	0.02	0.09	-4.20

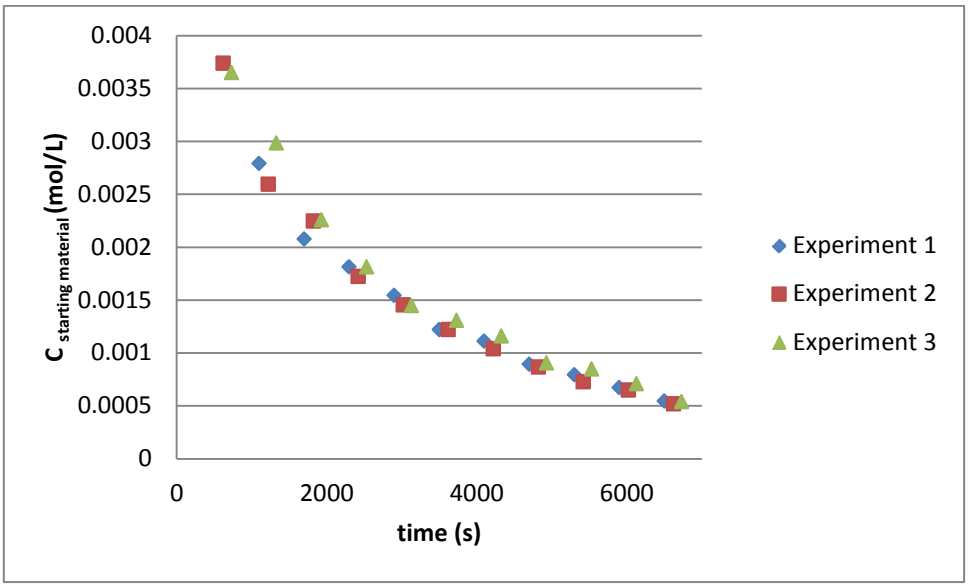
Experiment 3 ($R^2 = 1.00$, $k_{app} = 14.83.10^{-4}$ s^{-1}; $t_{1/2} = 7.8$ min)

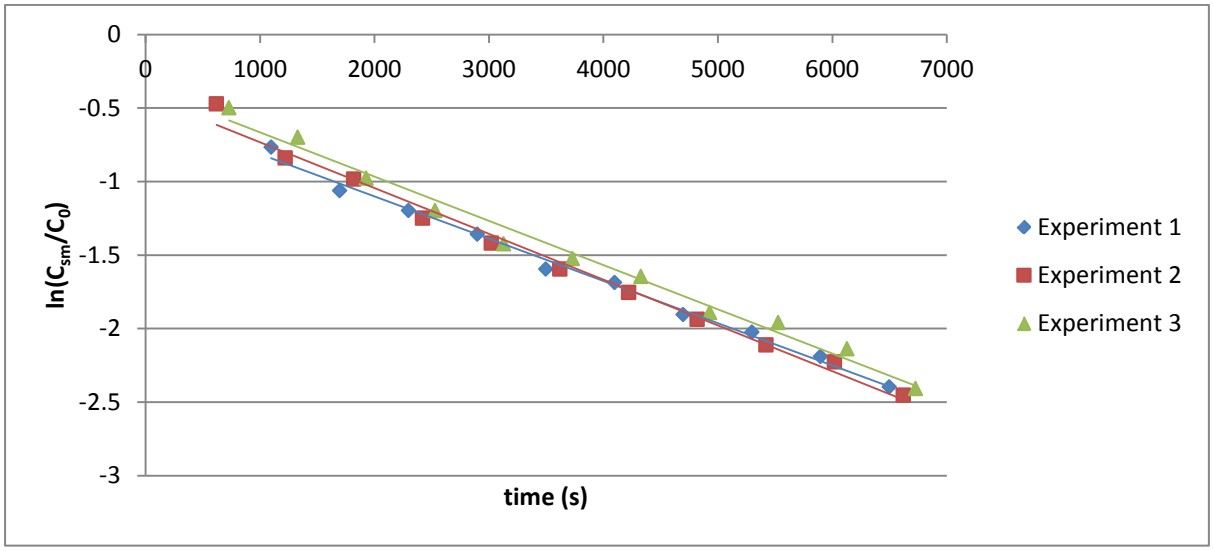
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
528	0.19	1.13	-1.67
708	0.15	0.88	-1.92
888	0.12	0.70	-2.15
1068	0.09	0.54	-2.41
1248	0.07	0.44	-2.62
1428	0.05	0.32	-2.94
1608	0.04	0.24	-3.22
1788	0.03	0.19	-3.47
1968	0.02	0.14	-3.78
2148	0.02	0.10	-4.07

2-(3-((6-Ethynyl-9-methyl-9*H*-purin-2-yl)amino)phenyl)acetamide (10)



2-(3-((6-Ethynyl-9-methyl-9*H*-purin-2-yl)amino)phenyl)acetamide (**10**) (690 µL from stock solution DMSO-d_6, 1.29 mg, 4.2 µmol) was treated according to general procedure. Disappearance of the singlet at 4.74-4.94 ppm was monitored as a function of time using the ^{1}H qNMR method outlined. Each spectrum was recorded every 10 min using 8 scans per experiment.





Experiment 1 ($R^2 = 0.99$, $k_{app} = 2.87.10^{-4}$ s^{-1}; $t_{1/2} = 40.3$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
1097	0.47	2.79	-0.77
1697	0.35	2.08	-1.06
2297	0.30	1.81	-1.20
2897	0.26	1.54	-1.36
3497	0.20	1.22	-1.59
4097	0.19	1.11	-1.69
4697	0.15	0.89	-1.90
5297	0.13	0.79	-2.02
5897	0.11	0.67	-2.19
6497	0.09	0.55	-2.40

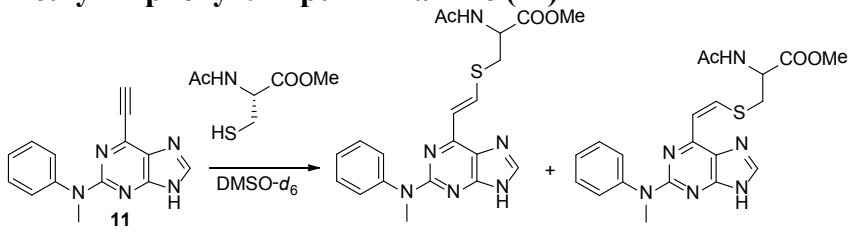
Experiment 2 ($R^2 = 0.99$, $k_{app} = 3.11.10^{-4}$ s^{-1}; $t_{1/2} = 37.2$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
620	0.62	3.74	-0.47
1220	0.43	2.59	-0.84
1820	0.37	2.24	-0.98
2420	0.29	1.72	-1.25
3020	0.24	1.45	-1.42
3620	0.20	1.22	-1.59
4220	0.17	1.04	-1.75
4820	0.14	0.86	-1.94
5420	0.12	0.73	-2.11
6020	0.11	0.65	-2.23
6620	0.09	0.52	-2.45

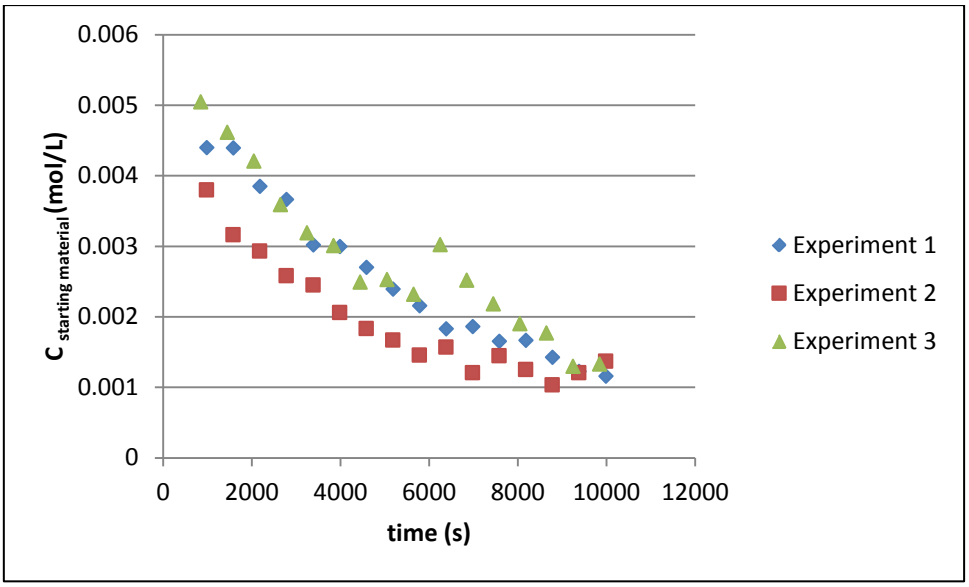
Experiment 3 ($R^2 = 0.99$, $k_{app} = 3.01.10^{-4}$ s^{-1}; $t_{1/2} = 38.4$ min)

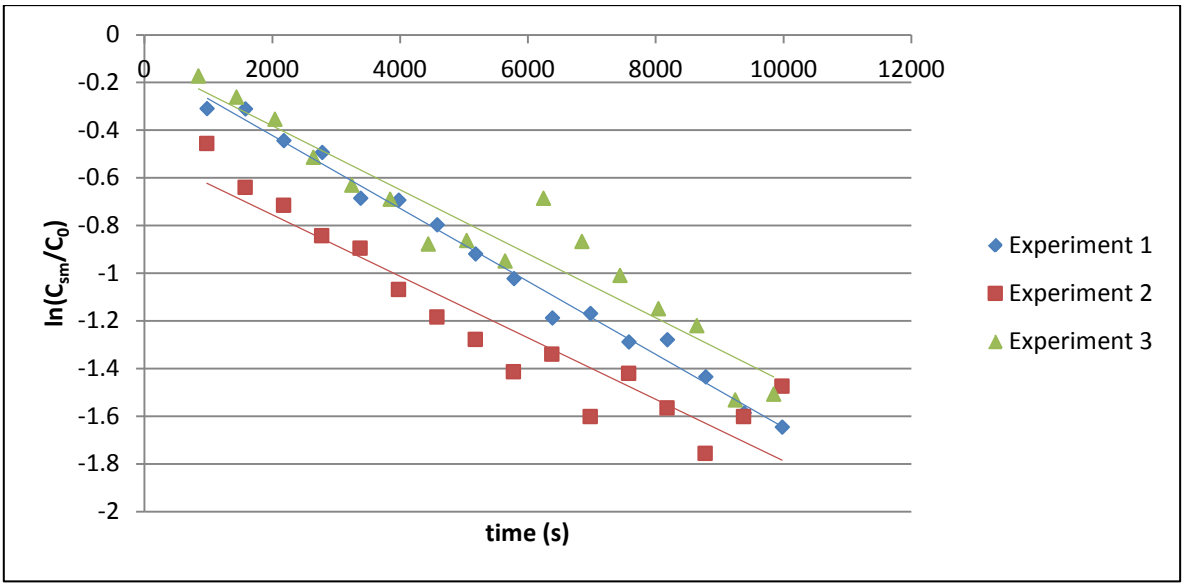
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
0.728	0.61	3.65	-0.50
1328	0.50	2.98	-0.70
1928	0.38	2.26	-0.98
2528	0.30	1.81	-1.20
3128	0.24	1.45	-1.42
3728	0.22	1.31	-1.52
4328	0.19	1.16	-1.65
4928	0.15	0.91	-1.89
5528	0.14	0.85	-1.96
6128	0.12	0.71	-2.14
6728	0.09	0.54	-2.41

6-Ethynyl-*N*-methyl-*N*-phenyl-9*H*-purin-2-amine (11)



6-Ethynyl-*N*-methyl-*N*-phenyl-9*H*-purin-2-amine (**11**) (690 µL from stock solution DMSO-d_6, 1.05 mg, 4.2 µmol) was treated according to general procedure. Disappearance of the singlet at 4.70-4.80 ppm was monitored as a function of time using the ^{1}H qNMR method outlined. Each spectrum was recorded every 10 min using 8 scans per experiment.





Experiment 1 ($R^2 = 0.99$, $k_{app} = 1.53.10^{-4}$ s^{-1}; $t_{1/2} = 75.5$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
985	0.73	4.40	-0.31
1585	0.73	4.39	-0.31
2185	0.64	3.85	-0.44
2785	0.61	3.66	-0.49
3385	0.50	3.02	-0.69
3985	0.50	3.00	-0.69
4585	0.45	2.70	-0.80
5185	0.40	2.39	-0.92
5785	0.36	2.16	-1.02
6385	0.31	1.83	-1.19
6985	0.31	1.86	-1.17
7585	0.28	1.65	-1.29
8185	0.28	1.67	-1.28
8785	0.24	1.43	-1.44
9385	0.20	1.23	-1.59
9985	0.19	1.16	-1.65

Experiment 2 ($R^2 = 0.89$, $k_{app} = 1.29.10^{-4}$ s^{-1}; $t_{1/2} = 89.6$ min)

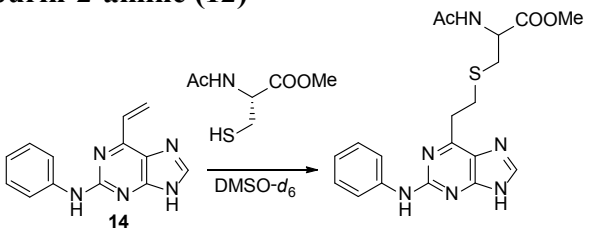
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
978	0.63	3.80	-0.46
1578	0.53	3.16	-0.64
2178	0.49	2.93	-0.72
2778	0.43	2.58	-0.84
3378	0.41	2.45	-0.90
3978	0.34	2.06	-1.07
4578	0.31	1.84	-1.18
5178	0.28	1.67	-1.28
5778	0.24	1.46	-1.41
6378	0.26	1.57	-1.34
6978	0.20	1.21	-1.60
7578	0.24	1.45	-1.42
8178	0.21	1.25	-1.57
8778	0.17	1.04	-1.76
9378	0.20	1.21	-1.60
9978	0.23	1.37	-1.48

Experiment 3 ($R^2 = 0.91$, $k_{app} = 1.34.10^{-4}$ s^{-1}; $t_{1/2} = 86.2$ min)

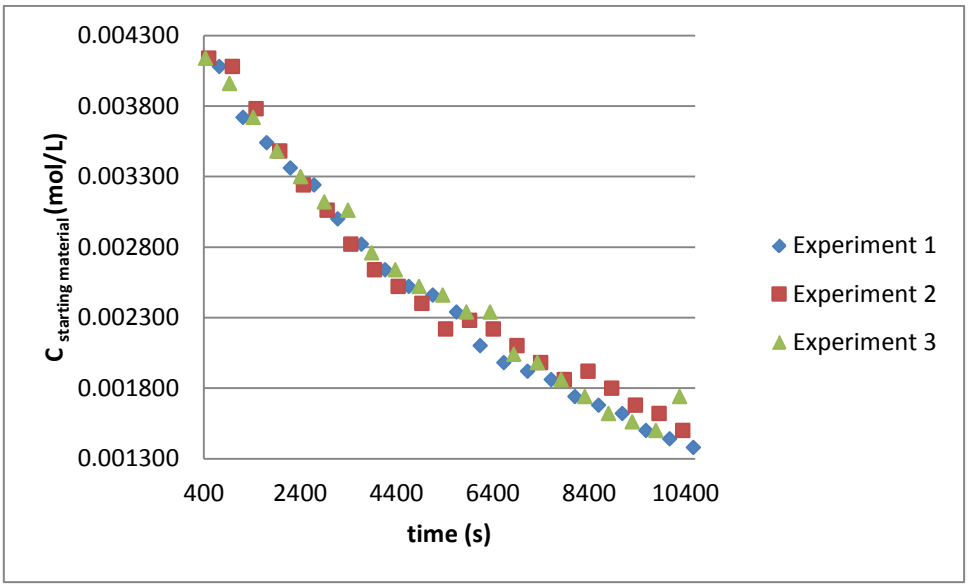
Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
846	0.84	5.05	-0.17

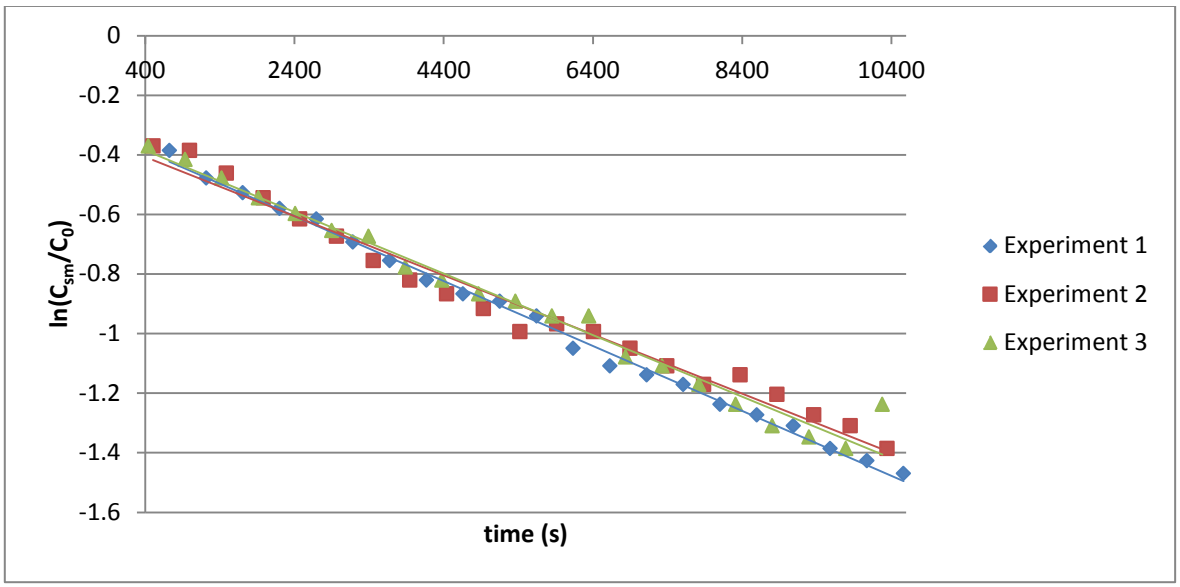
1446	0.77	4.62	-0.26
2046	0.70	4.21	-0.35
2646	0.60	3.59	-0.51
3246	0.53	3.19	-0.63
3846	0.50	3.01	-0.69
4446	0.42	2.49	-0.88
5046	0.42	2.53	-0.86
5646	0.39	2.32	-0.95
6246	0.50	3.02	-0.69
6846	0.42	2.52	-0.87
7446	0.36	2.18	-1.01
8046	0.32	1.90	-1.15
8646	0.30	1.77	-1.22
9246	0.22	1.30	-1.53
9846	0.22	1.33	-1.51

N-Phenyl-6-vinyl-9*H*-purin-2-amine (12)



N-Phenyl-6-vinyl-9*H*-purin-2-amine (**12**) (690 µL from stock solution in DMSO-d_6; 1.0 mg, 4.2 µmol) was treated according to general procedure. Disappearance of the characteristic vinyl proton (H^a) signal at 5.90-5.96 ppm was monitored as a function of time. Each spectrum was recorded every 4.1 min using 4 scans per experiment.
Note: Not all data are shown as more than 100 were collected.





Experiment 1 ($R^2 = 1.00$, $k_{app} = 1.09.10^{-4}$ s^{-1}; $t_{1/2} = 106$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
723	0.68	4.08	-0.39
1215	0.62	3.72	-0.48
1707	0.59	3.54	-0.53
2199	0.56	3.36	-0.58
2691	0.54	3.24	-0.62
3183	0.50	3.00	-0.69
3675	0.47	2.82	-0.76
4167	0.44	2.64	-0.82
4659	0.42	2.52	-0.87
5151	0.41	2.46	-0.89
5643	0.39	2.34	-0.94
6135	0.35	2.10	-1.05
6627	0.33	1.98	-1.11
7119	0.32	1.92	-1.14
7611	0.31	1.86	-1.17
8103	0.29	1.74	-1.24
8595	0.28	1.68	-1.27
9087	0.27	1.62	-1.31
9579	0.25	1.50	-1.39
10071	0.24	1.44	-1.43
10563	0.23	1.38	-1.47

Experiment 2 ($R^2 = 0.98$, $k_{app} = 0.99.10^{-4}$ s^{-1}; $t_{1/2} = 116.7$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
503	0.69	4.14	-0.37
995	0.68	4.08	-0.39
1487	0.63	3.78	-0.46
1979	0.58	3.48	-0.54
2471	0.54	3.24	-0.62
2963	0.51	3.06	-0.67
3455	0.47	2.82	-0.76
3947	0.44	2.64	-0.82
4439	0.42	2.52	-0.87
4931	0.40	2.40	-0.92
5423	0.37	2.22	-0.99
5915	0.38	2.28	-0.97
6407	0.37	2.22	-0.99
6899	0.35	2.10	-1.05
7391	0.33	1.98	-1.11
7883	0.31	1.86	-1.17
8375	0.32	1.92	-1.14
8867	0.30	1.80	-1.20

9359	0.28	1.68	-1.27
9851	0.27	1.62	-1.31
10343	0.25	1.50	-1.39

Experiment 3 ($R^2 = 0.98$, $k_{app} = 1.03.10^{-4}$ s^{-1}; $t_{1/2} = 112.2$ min)

Time (s)	Baseline corrected integral	Concentration (mM)	$Ln(C_{sm}/C_0)$
0	1.00	6.00	0.00
440	0.69	4.14	-0.37
932	0.66	3.96	-0.42
1424	0.62	3.72	-0.48
1916	0.58	3.48	-0.54
2408	0.55	3.30	-0.60
2900	0.52	3.12	-0.65
3392	0.51	3.06	-0.67
3884	0.46	2.76	-0.78
4376	0.44	2.64	-0.82
4868	0.42	2.52	-0.87
5360	0.41	2.46	-0.89
5852	0.39	2.34	-0.94
6344	0.39	2.34	-0.94
6836	0.34	2.04	-1.08
7328	0.33	1.98	-1.11
7820	0.31	1.86	-1.17
8312	0.29	1.74	-1.24
8804	0.27	1.62	-1.31
9296	0.26	1.56	-1.35
9788	0.25	1.50	-1.39
10280	0.29	1.74	-1.24