

# Cyclization Kinetics and Biological Evaluation of an Anticancer 1,2-Dialkynylimidazole

Christophe Laroche,<sup>a</sup> Jing Li,<sup>a</sup> Cristina Gonzales,<sup>b</sup> Wendi M. David,<sup>b</sup> Sean M. Kerwin\*<sup>a</sup>

<sup>a</sup>*Division of Medicinal Chemistry, College of Pharmacy, The University of Texas at Austin, Austin, Texas 78712, USA. [skerwin@mail.utexas.edu](mailto:skerwin@mail.utexas.edu)*

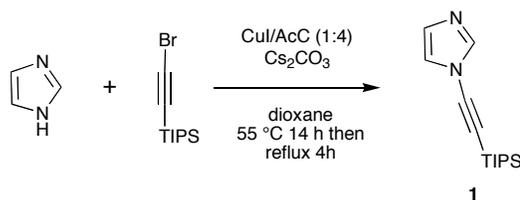
<sup>b</sup>*Department of Chemistry and Biochemistry, Texas State University, San Marcos, TX, 78666, USA*

## Table of Contents:

General Experimental	p. S2
Optimization of the Copper-catalyzed coupling	p. S2-S3
Characterization data for <b>1-3</b>	p. S4–S5
<sup>1</sup> H NMR Spectra for <b>1-3</b>	p. S6–S8
<sup>13</sup> C NMR Spectra for <b>1-3</b>	p. S9–S10
X-ray crystallography of <b>3</b>	p S11-S16
Cytotoxicity Studies of <b>3</b>	p S17-19
Apoptosis assay	p S20
DNA cleavage assay	p S21
Peptide adduct assay	p S22
Computational studies	p S23-32
Kinetic studies	p S33-S40
References	p S41-S42

**General:** All reactions were carried out under argon in oven-dried glassware with magnetic stirring. Unless otherwise noted, all materials were obtained from commercial suppliers and were used without further purification. CuI was purified by recrystallization<sup>1</sup> and Cs<sub>2</sub>CO<sub>3</sub> was heated several times with a heat gun in the reaction flask under vacuum prior to use. Tetrakis(triphenylphosphine) palladium was purified by recrystallization.<sup>1</sup> THF and 1,4-dioxane were distilled from sodium/benzophenone prior to use. Et<sub>3</sub>N was distilled from KOH prior to use. Unless otherwise noted, organic extracts were dried with Na<sub>2</sub>SO<sub>4</sub>, filtered through a fritted glass funnel, and concentrated with a rotary evaporator (20–30 mmHg). R<sub>f</sub> values are reported for analytical thin-layer chromatography (TLC) performed on EM Reagent 0.25 mm silica gel 60-F plates with UV light or KMnO<sub>4</sub> visualization. Flash chromatography was performed with EM Reagent silica gel (230–400 mesh) using the mobile phase indicated. Melting points (open capillary) are uncorrected. Unless otherwise noted, <sup>1</sup>H and <sup>13</sup>C NMR spectra were determined in CDCl<sub>3</sub> on a spectrometer operating at 400 and 100 MHz, respectively, and are reported in ppm using solvent as internal standard (7.26 ppm for <sup>1</sup>H and 77.0 ppm for <sup>13</sup>C). All mass spectra were obtained in the positive mode either by chemical ionization using methane as the ionizing gas or by electrospray ionization.

**Optimization of the Copper-catalyzed bromoalkyne coupling.** The following table summarizes the isolated yields of alkynylimidazole **1** obtained when imidazole was coupled to bromo-(triisopropylsilyl)-acetylene<sup>2</sup> under various reaction conditions. All reactions were carried out with a 1:4 ratio of CuI to 2-acetylcyclohexanone (AcC). Except where noted, all reactions were carried out on a 1 mmole scale.

**Table S1.** Condition optimization for the synthesis of **1**.

Entry	AcC	Cs <sub>2</sub> CO <sub>3</sub>	Bromoalkyne	CuI	Yield
1	20 mole %	2 equiv.	2 equiv.	5 mole %	82%
2	20 mole %	1.5 equiv.	2 equiv.	5 mole %	81%
3	20 mole %	1.1 equiv.	2 equiv.	5 mole %	74%
4	20 mole %	1 equiv.	2 equiv.	5 mole %	73%
5	20 mole %	2 equiv.	1.5 equiv.	5 mole %	74%
6	20 mole %	2 equiv.	1.1 equiv.	5 mole %	58%
7	20 mole %	2 equiv.	1 equiv.	5 mole %	55%
8	20 mole %	1.1 equiv.	1.1 equiv.	5 mole %	55%
9 <sup>a</sup>	4 mole %	1.1 equiv.	1.1 equiv.	1 mole %	70%
10 <sup>b</sup>	2 mole %	1.1 equiv.	1.1 equiv.	0.5 mole %	79%

a. Reaction time has been increased to 3 days at 55°C followed by 4H under reflux.  
 b. Reaction time has been increased to 6 days at 55°C followed by 2 days under reflux.

**Synthesis of 1-(triisopropylsilylethynyl)-1H-imidazole (1)** : A reaction flask under argon was charged with Cs<sub>2</sub>CO<sub>3</sub> (3.586 g, 11 mmol), CuI (10 mg, 0.05 mmol), the unsubstituted imidazole (680 mg, 10 mmol) and backfilled with argon. Dry 1,4-dioxane (20 mL) was added followed by bromo-(triisopropylsilyl)acetylene<sup>2</sup> (2.871 g, 11 mmol) and 2-acetylcyclohexanone (0.026 mL, 0.2 mmol). The solution was degassed by bubbling argon for 15 minutes. The mixture was heated to 55°C with an oil bath for 6 days and, then, refluxed for 2 days. The reaction was cooled to room temperature, quenched with 50 mL of a saturated NH<sub>4</sub>Cl solution and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent

was evaporated under reduce pressure and the residue was subjected to flash chromatography (0-10% EtOAc/hexane) to afford **1** (1.962 g, 79% yield) as a yellow oil. The spectral data obtained for **1** are in accord with the data previously reported.<sup>3</sup>

**Synthesis of 2-iodo-1-((triisopropylsilyl)ethynyl)-1*H*-imidazole (2)** : To a solution of 1-((triisopropylsilyl)ethynyl)-1*H*-imidazole (**1**) (1.240 g, 5 mmol) in THF (50 mL) under argon at -78°C was added *n*-BuLi (2.0 mL of 2.5 M solution in hexane, 5 mmol). The reaction mixture was stirred for 15 min at -78°C prior to the addition of powdered iodine (1.270 g, 5 mmol). After stirring for 30 min at -78°C, the mixture was quenched with a saturated aqueous solution of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (10 mL) and the temperature was allowed to rise to room temperature. The reaction mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×100 mL), the combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (0-5% EtOAc/hexane) to afford 1.552 g (83%) of **2** as a yellow liquid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.25 (1H, d, *J* = 1.6 Hz), 6.99 (1H, d, *J* = 1.6 Hz), 1.16-1.12 (21H, m); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 132.1, 125.6, 94.0, 91.6, 74.5, 18.6 (6C), 11.2 (3C); IR (neat) 2943, 2865, 2191, 1462, 1430, 1367, 1282, 1241, 1084, 1052, 883 cm<sup>-1</sup>; MS CI (*M* + 1) (375, 100 %); HRMS (CI) calc for C<sub>14</sub> H<sub>24</sub> N<sub>2</sub> Si I (*M* + H<sup>+</sup>) 375.0754, found 375.0754.

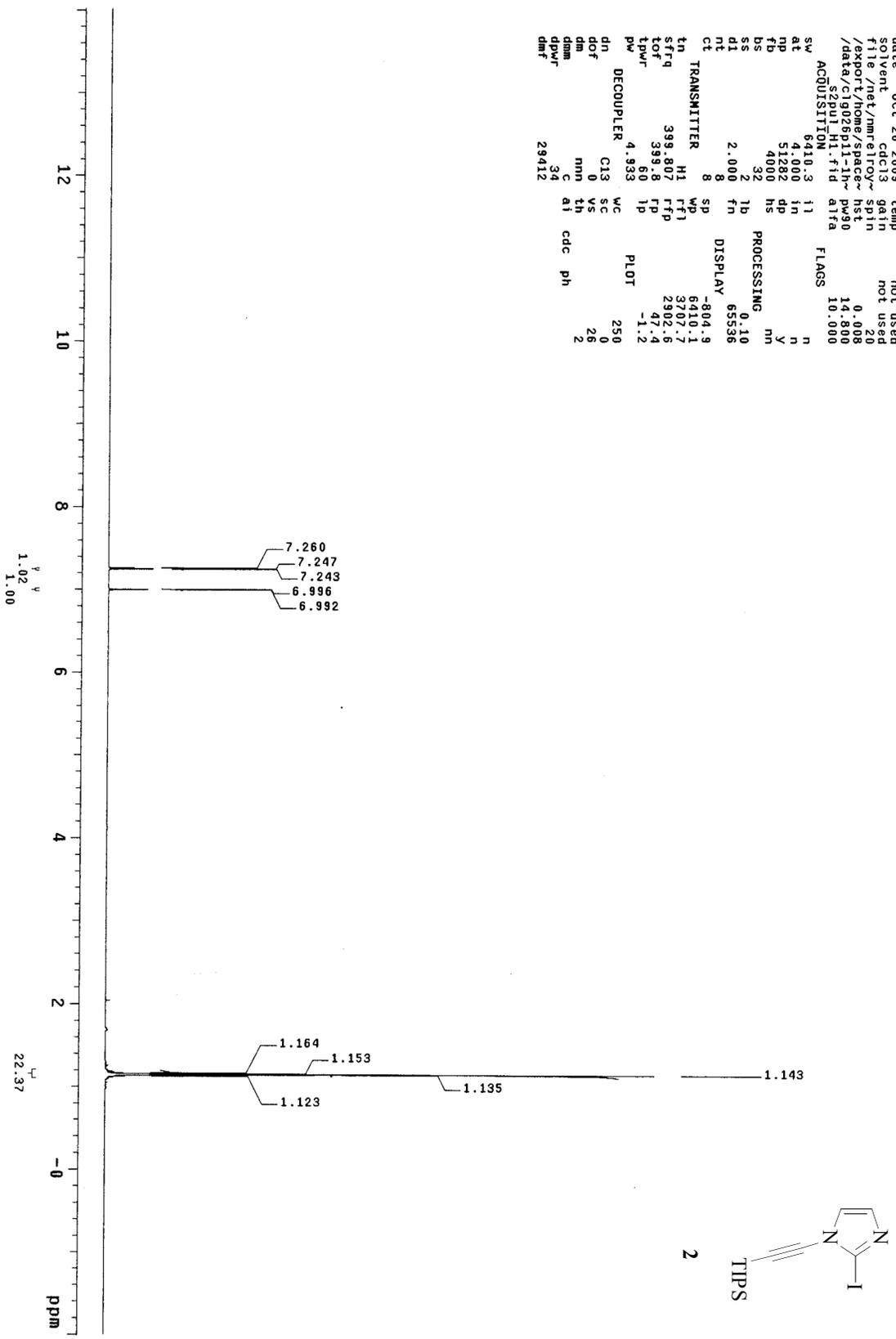
**Synthesis of 2-((4-methoxyphenyl)ethynyl)-1-((triisopropylsilyl)ethynyl)-1*H*-imidazole**: To a solution of 2-iodo-1-((triisopropylsilyl)ethynyl)-1*H*-imidazole **2** (1.552 mg, 4.15 mmol) in Et<sub>3</sub>N (40 mL) under argon was added 4-ethynylanisole (0.596 mL, 4.6 mmol), Pd(PPh<sub>3</sub>)<sub>4</sub> (48 mg, 0.0415 mmol) and CuI (16 mg, 0.083 mmol). The reaction mixture was degassed by bubbling argon for 15 minutes and stirred at 50°C for 2 hours. The solvent was removed under reduce

pressure and the residue was purified by flash chromatography (0-5% EtOAc/hexane) to afford 1.569 g (100%) of **3** as a yellow crystalline solid. m.p. 69-70°C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.49 (2H, d, *J* = 8.9 Hz), 7.13 (1H, d, *J* = 1.5 Hz), 7.03 (1H, d, *J* = 1.5 Hz), 6.87 (2H, d, *J* = 8.9 Hz), 3.84 (3H, s), 1.14-1.10 (21H, m); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 160.4, 135.8, 133.6 (2C), 129.4, 122.3, 114.0 (2C), 113.3, 93.7, 91.1, 76.6, 72.6, 55.3, 18.5 (6C), 11.1 (3C); IR (neat) 2943, 2865, 2360, 2341, 2204, 2183, 1606, 1528, 1462, 1291, 1252, 1173, 1111 cm<sup>-1</sup>; MS CI (M + 1) (379, 100 %); HRMS (CI) calc for C<sub>23</sub> H<sub>31</sub> N<sub>2</sub> O Si (M + H<sup>+</sup>) 379.2206, found 379.2199.

**Synthesis of 1-ethynyl-2-(2-(4-methoxyphenyl)-ethynyl)-1*H*-imidazole (3)** : To a solution of 2-((4-methoxyphenyl)ethynyl)-1-((triisopropylsilyl)ethynyl)-1*H*-imidazole (see above) (1.569 g, 4.15 mmol) in THF (40 mL) at -78°C was added TBAF (4.15 mL of 1 M solution in THF, 4.15 mmol) and the mixture was slowly warmed up to -40°C. The reaction mixture is quenched at -40°C with 20 mL of water and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×100 mL). The organic layers were combined, dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (0–20% EtOAc/hexane) to afford 831 mg (90%) of compound **4** as a yellow solid. The spectral data obtained for **4** are in accord with the data previously reported.<sup>4</sup> A second chromatography afforded analytically pure material. Anal. Calcd. For C<sub>14</sub> H<sub>10</sub> N<sub>2</sub> O: C, 75.66; H, 4.54; N, 12.60. Found: C, 75.81; H, 4.36; N, 12.54.

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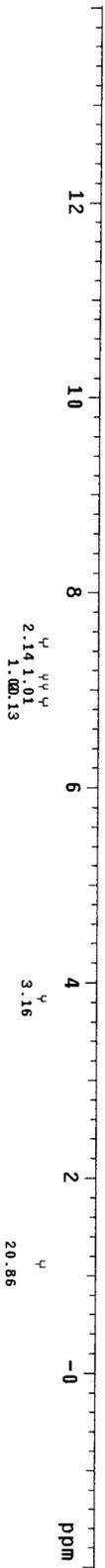
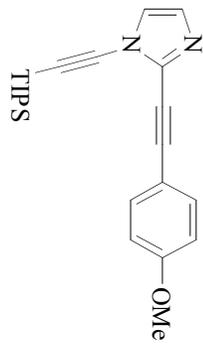
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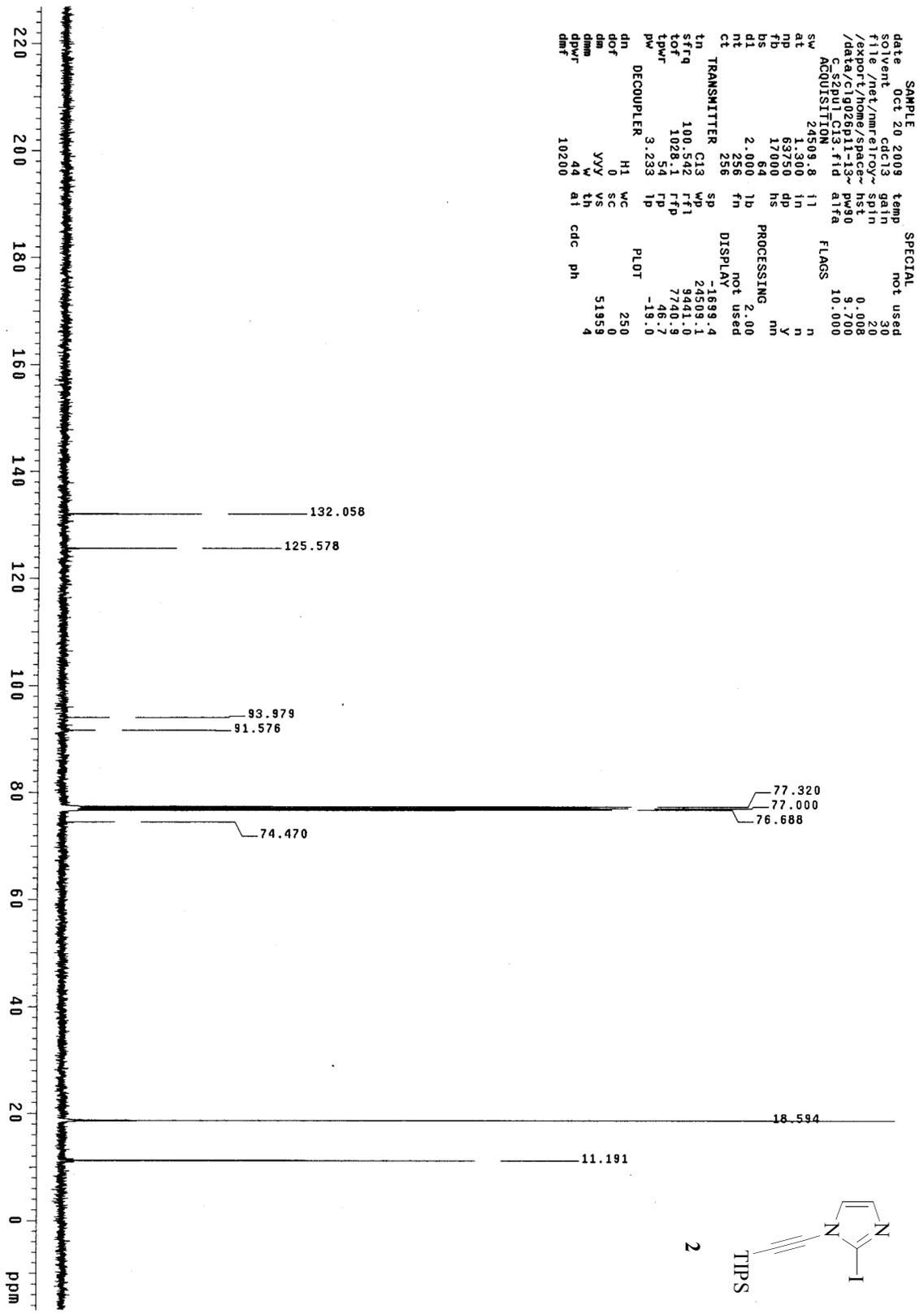
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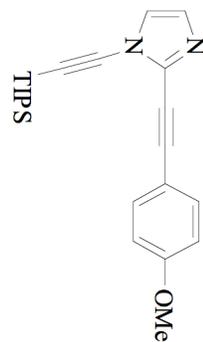
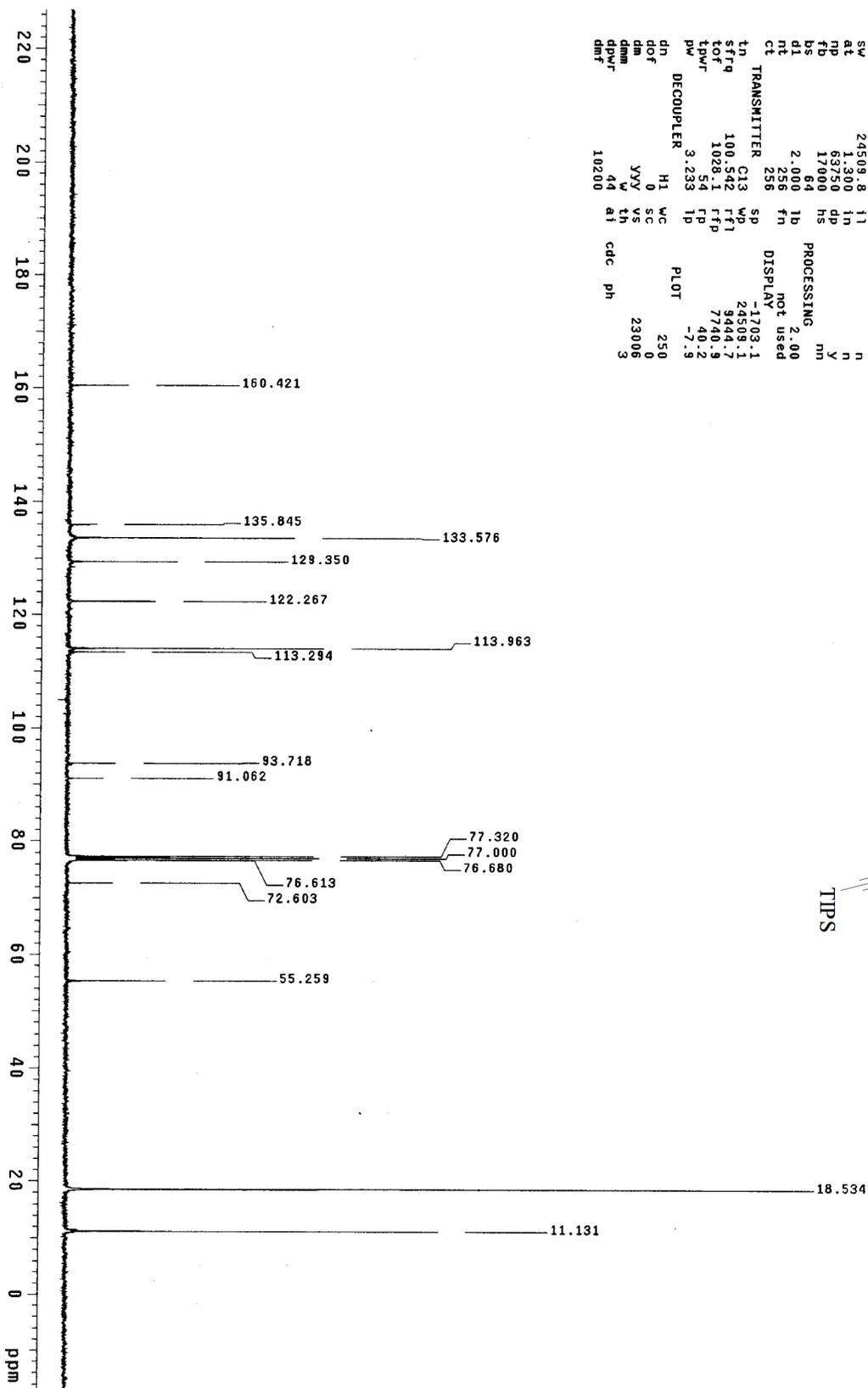




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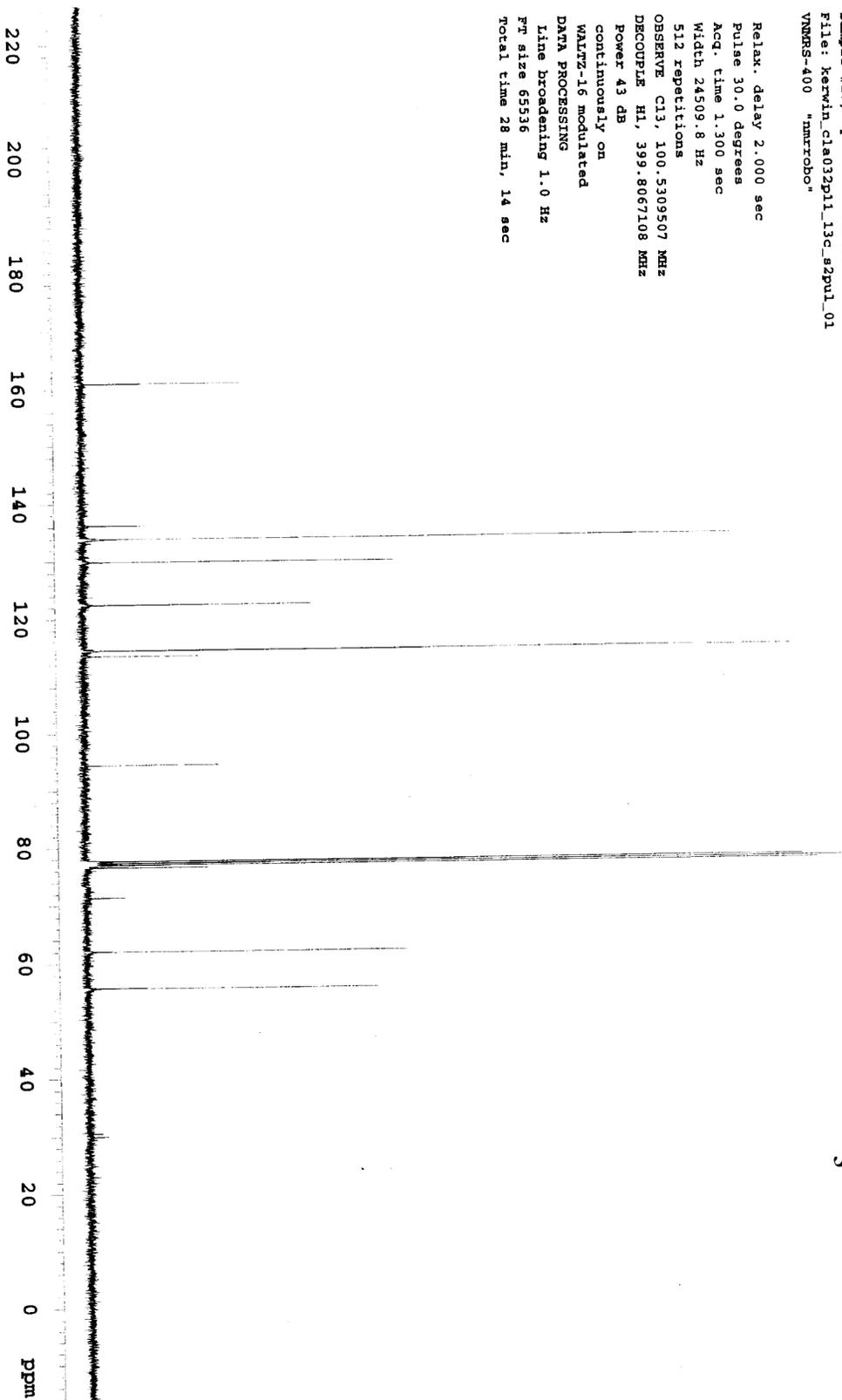
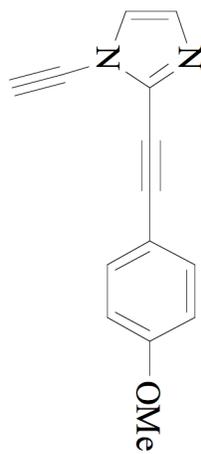
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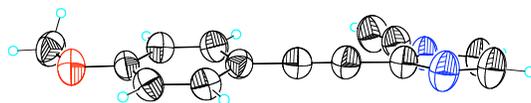
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### X-ray crystallography of 1-ethynyl-2-(2-(4-methoxyphenyl)-ethynyl)-1*H*-imidazole (**3**) :

Crystals grew as colorless laths by slow cooling of a water/acetonitrile (50/50) solution. The data crystal was cut from a larger crystal and had approximate dimensions; 0.43 x 0.06 x 0.05 mm. The data were collected at room temperature on a Nonius Kappa CCD diffractometer using a graphite monochromator with MoK $\alpha$  radiation ( $\lambda = 0.71073\text{\AA}$ ). A total of 217 frames of data were collected using  $\omega$ -scans with a scan range of  $1.2^\circ$  and a counting time of 75 seconds per frame. Data reduction were performed using DENZO-SMN.<sup>5</sup> The structure was solved by direct methods using SIR97<sup>6</sup> and refined by full-matrix least-squares on  $F^2$  with anisotropic displacement parameters for the non-H atoms using SHELXL-97.<sup>7</sup> The hydrogen atoms were observed in a  $\Delta F$  map and refined with isotropic displacement parameters. The function,  $\sum w(|F_o|^2 - |F_c|^2)^2$ , was minimized, where  $w = 1/[(\sigma(F_o))^2 + (0.053*P)^2]$  and  $P = (|F_o|^2 + 2|F_c|^2)/3$ .  $R_w(F^2)$  refined to 0.0922, with  $R(F)$  equal to 0.0460 and a goodness of fit,  $S$ , = 1.22. Definitions used for calculating  $R(F)$ ,  $R_w(F^2)$  and the goodness of fit,  $S$ , are given below.<sup>8</sup> The data were corrected for secondary extinction effects. The correction takes the form:  $F_{\text{CORR}} = kF_c/[1 + (9.2(13)\times 10^{-6}) * F_c^2 \lambda^3/(\sin 2\theta)]^{0.25}$  where  $k$  is the overall scale factor. Neutral atom scattering factors and values used to calculate the linear absorption coefficient are from the International Tables for X-ray Crystallography (1992).<sup>9</sup> All figures were generated using SHELXTL/PC.<sup>10</sup>



**Figure S1.** Side view of **3**. Displacement ellipsoids are scaled to the 50% probability level. The dihedral angle between the phenyl ring and the imidazole ring is  $11.8(2)^\circ$ .

**Table S1.** Crystal data and structure refinement for **3**.

Empirical formula	C <sub>14</sub> H <sub>10</sub> N <sub>2</sub> O	
Formula weight	222.24	
Temperature	298(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P21/n	
Unit cell dimensions	a = 3.9680(4) Å	α = 90°.
	b = 10.7628(10) Å	β = 91.138(2)°.
	c = 27.402(2) Å	γ = 90°.
Volume	1170.02(18) Å <sup>3</sup>	
Z	4	
Density (calculated)	1.262 Mg/m <sup>3</sup>	
Absorption coefficient	0.082 mm <sup>-1</sup>	
F(000)	464	
Crystal size	0.43 x 0.06 x 0.05 mm	
Theta range for data collection	3.53 to 25.21°.	
Index ranges	-4 ≤ h ≤ 4, -12 ≤ k ≤ 11, -32 ≤ l ≤ 32	
Reflections collected	3467	
Independent reflections	2039 [R(int) = 0.0366]	
Completeness to theta = 25.21°	97.0 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F <sup>2</sup>	
Data / restraints / parameters	2039 / 0 / 195	
Goodness-of-fit on F <sup>2</sup>	1.197	
Final R indices [I > 2σ(I)]	R1 = 0.0460, wR2 = 0.0765	
R indices (all data)	R1 = 0.1410, wR2 = 0.0922	
Extinction coefficient	9.2(13) × 10 <sup>-6</sup>	
Largest diff. peak and hole	0.162 and -0.140 e.Å <sup>-3</sup>	

**Table S2.** Atomic coordinates ( $\times 10^4$ ) and equivalent isotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ) for **3**.  $U(\text{eq})$  is defined as one third of the trace of the orthogonalized  $U_{ij}$  tensor.

	x	y	z	$U(\text{eq})$
O1	2512(4)	2786(1)	1282(1)	65(1)
N1	7911(5)	3165(2)	4448(1)	59(1)
N2	9260(5)	1168(2)	4414(1)	72(1)
C1	8067(6)	2112(2)	4162(1)	57(1)
C2	9128(7)	2841(3)	4904(1)	70(1)
C3	9935(7)	1631(2)	4872(1)	76(1)
C4	7068(6)	2145(2)	3663(1)	61(1)
C5	6260(6)	2246(2)	3242(1)	59(1)
C6	5286(5)	2391(2)	2741(1)	51(1)
C7	3659(6)	3469(2)	2584(1)	57(1)
C8	2740(6)	3643(2)	2100(1)	54(1)
C9	3384(5)	2721(2)	1765(1)	50(1)
C10	5018(6)	1639(2)	1915(1)	55(1)
C11	5969(6)	1486(2)	2395(1)	55(1)
C12	790(9)	3895(3)	1112(1)	74(1)
C13	6673(6)	4297(2)	4303(1)	66(1)
C14	5634(7)	5256(2)	4170(1)	84(1)

**Table S3.** Bond lengths [Å] and angles [°] for **3**.

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O1-C9	1.365(2)	C6-C7	1.392(3)
O1-C12	1.448(3)	C7-C8	1.381(3)
N1-C13	1.369(3)	C7-H7	0.978(19)
N1-C2	1.377(3)	C8-C9	1.379(3)
N1-C1	1.380(3)	C8-H8	0.996(16)
N2-C1	1.311(2)	C9-C10	1.390(3)
N2-C3	1.372(3)	C10-C11	1.371(3)
C1-C4	1.418(3)	C10-H10	0.926(18)
C2-C3	1.344(3)	C11-H11	1.019(16)
C2-H2	0.97(2)	C12-H12A	0.95(3)
C3-H3	0.98(2)	C12-H12B	1.06(2)
C4-C5	1.197(3)	C12-H12C	0.98(3)
C5-C6	1.425(3)	C13-C14	1.167(3)
C6-C11	1.390(3)	C14-H14	0.99(2)
C9-O1-C12	117.6(2)	C5-C4-C1	176.2(2)
C13-N1-C2	127.3(2)	C4-C5-C6	179.0(2)
C13-N1-C1	125.9(2)	C11-C6-C7	117.9(2)
C2-N1-C1	106.73(18)	C11-C6-C5	121.9(2)
C1-N2-C3	105.15(18)	C7-C6-C5	120.3(2)
N2-C1-N1	111.0(2)	C8-C7-C6	121.5(2)
N2-C1-C4	128.2(2)	C8-C7-H7	120.9(11)
N1-C1-C4	120.8(2)	C6-C7-H7	117.6(11)
C3-C2-N1	105.4(2)	C7-C8-C9	119.5(2)
C3-C2-H2	130.6(14)	C7-C8-H8	118.9(11)
N1-C2-H2	124.0(14)	C9-C8-H8	121.6(11)
C2-C3-N2	111.7(2)	O1-C9-C8	124.26(19)
C2-C3-H3	127.9(13)	O1-C9-C10	115.9(2)
N2-C3-H3	120.2(12)	C8-C9-C10	119.8(2)

C11-C10-C9	120.1(2)
C11-C10-H10	122.3(12)
C9-C10-H10	117.5(12)
C10-C11-C6	121.1(2)
C10-C11-H11	119.3(10)
C6-C11-H11	119.5(10)
O1-C12-H12A	103.0(13)
O1-C12-H12B	110.1(11)
H12A-C12-H12B	114(2)
O1-C12-H12C	110.7(15)
H12A-C12-H12C	110(2)
H12B-C12-H12C	109(2)
C14-C13-N1	178.6(3)
C13-C14-H14	178.6(15)

**Table S4.** Torsion angles [°] for **3**.

---

C3-N2-C1-N1	-0.9(3)
C3-N2-C1-C4	178.0(3)
C13-N1-C1-N2	-177.4(2)
C2-N1-C1-N2	0.7(3)
C13-N1-C1-C4	3.6(4)
C2-N1-C1-C4	-178.3(2)
C13-N1-C2-C3	177.9(2)
C1-N1-C2-C3	-0.1(3)
N1-C2-C3-N2	-0.4(3)
C1-N2-C3-C2	0.8(3)
N2-C1-C4-C5	-156(4)
N1-C1-C4-C5	23(4)
C1-C4-C5-C6	-21(18)
C4-C5-C6-C11	165(15)
C4-C5-C6-C7	-14(15)
C11-C6-C7-C8	0.1(3)
C5-C6-C7-C8	178.9(2)
C6-C7-C8-C9	1.3(3)
C12-O1-C9-C8	-0.2(3)
C12-O1-C9-C10	179.7(2)
C7-C8-C9-O1	178.4(2)
C7-C8-C9-C10	-1.5(3)
O1-C9-C10-C11	-179.5(2)
C8-C9-C10-C11	0.4(3)
C9-C10-C11-C6	1.0(4)
C7-C6-C11-C10	-1.2(3)
C5-C6-C11-C10	-180.0(2)
C2-N1-C13-C14	157(11)
C1-N1-C13-C14	-26(11)

## Cytotoxicity:

The cytotoxicity assays were conducted at the NCI and were performed as described.<sup>11</sup> Briefly, cell suspensions that were diluted according to the particular cell type and the expected target cell density (5000-40,000 cells per well based on cell growth characteristics) were added by pipet (100  $\mu$  L) into 96-well microtiter plates. Inoculates were allowed a preincubation period of 24 h at 37° C for stabilization. Dilutions at twice the intended test concentration were added at time zero in 100- $\mu$  L aliquots to the microtiter plate wells. Test compounds were evaluated at five 10-fold dilutions with the highest well concentration being 100  $\mu$ M. Incubations lasted for 48 h in 5% CO<sub>2</sub> atmosphere and 100% humidity. The cells were assayed by using the sulforhodamine B assay.<sup>12,13</sup> A plate reader was used to read the optical densities, and a microcomputer program was used to process the optical densities into the GI<sub>50</sub> concentration, the concentration of test drug where  $100 \times (T - T_0)/(C - T_0) = 50$ , where the optical density of the test well after a 48-h period of exposure to test drug is T, the optical density at time zero is T<sub>0</sub>, and the control optical density is C. Additional information about the cell lines in the NCI60 panel including inoculation densities and doubling times can be found on the National Cancer Institute Department of Developmental therapeutics website at [http://dtp.nci.nih.gov/docs/misc/common\\_files/cell\\_list.html](http://dtp.nci.nih.gov/docs/misc/common_files/cell_list.html).

**Table S5.** Cytotoxicity of dialkynylimidazole **3** against a panel of human cancer cell lines.

<b>Cell Line</b>	<b>-Log(GI<sub>50</sub>)<sup>a</sup></b>
CCRF-CEM	5.77±0.07
HL-60	6.66±0.05
K-562	5.43±0.09
MOLT-4	5.2±3
RPMI-8226	5.8±0.4
SR	5.2±0.3
A549	5.1±0.4
EKVX	5.4±0.6
HOP-62	5.7±0.7
HOP-92	6.62±0.06
NCI-H226	4.83±0.09
NCI-N23	4.9±0.1
NCI-H322M	4.75±0.06
NCI-H460	4.99±0.05
NCI-H522	6.1±0.3
COLO 205	5.9±0.7
HCC-2998	5.2±0.6
HCT-116	5.33±0.03
HCT-15	5.0±0.1
HT29	5.8±0.1
KM12	5.415±0.01
SW-620	5.345±0.03
SF-268	5.75±0.03
SF-295	5.3±0.1
SF-539	6.5±0.3
SNB-19	4.81±0.08
SNB-75	6.2±0.5
U251	5.6±0.2
LOX IMVI	6.7±0.1
MALMe-3M	5.3±0.5
M14	4.91±0.06
SK-MEL-2	4.82±0.05
SK-MEL-28	5.1±0.3
SK-MEL-5	4.93±0.04
UACC-257	4.8±0.5
UACC-62	5.0±0.1
IGROV1	5.9±0.8
OVCAR-3	5.8±0.2
OVCAR-4	5.4±0.7
OVCAR-5	4.7±0.1
SK-OV-3	5.4±0.1
786-0	6.1±0.2
A498	4.82±0.04
ACHN	5.3±0.5
CAKI-1	6.1±0.3
SN12C	5.34±0.06

TK-10	4.96±0.04
UO-31	6.6±0.1
PC-3	6.1±0.3
DU-145	5.9±0.2
MCF7	5.76±0.02
NCI/ADR- RES	5.4±0.2
MDA-MB- 231	5.2±0.1
HS 578T	5.1±0.1
MDA-MB- 435	5.1±0.2
T-47D	5.6±0.1
Mean	5.48±0.05

<sup>a</sup> Negative Log of GI<sub>50</sub> (Molar), average of data for two independent assays, ± standard deviation.

**FACS analysis:** A549 Cells ( $2.0 \times 10^5$  cells) were plated in a 12-well plate in a final volume of 1 ml/well of F-12K with L-glutamine medium. After 24 h incubation at 37 °C in an atmosphere of 5 % CO<sub>2</sub>, cells were treated with 1 µl of compound **3** (5 µM, 2.5 µM, 1.25 µM and 0.625 µM). After 24 h, cells were rinsed with PBS and trypsinized. The cells were pelleted at 400 x g for 4 min, washed with annexin binding buffer (ABB), and pelleted again at 400 x g for 4 min. The media was aspirated and the cells were resuspended in ABB and stained with annexin V-FITC (5 µg/mL) for 8 min at room temperature. Propidium iodide (2 µg/mL) was added to the cell suspension. The cells were analyzed with Beckman Coulter flow cytometer.

**Supercoiled DNA Cleavage Assay:** The DNA cleavage efficiency was determined by incubation of **3** with solutions of supercoiled  $\Phi$ X174 plasmid DNA (50  $\mu$ M base pairs) in 50 mM *N,N,N*-tris(hydroxymethyl)aminomethane (Tris) buffer at pH 7. The reaction mixtures containing 100  $\mu$ M compound **3**, 100  $\mu$ M 1-propargyl-2-(2-(4-methoxyphenyl)ethynyl)pyridinium triflate<sup>14</sup> as positive control, or vehicle (13% (v/v) DMSO) were incubated for 12 h at 37 °C. DNA products were separated by agarose gel electrophoresis [1  $\times$  Tris-borate-*N,N,N',N'*-ethylenediaminetetraacetic acid (EDTA) (TBE) at 90 V for 1 h], stained with ethidium bromide (0.25  $\mu$ g/mL), and the images were analyzed using a fluorimager with ImageQuant software. The degree of cleavage of Form I DNA was determined using equation 2.

$$\text{Percent cleavage} = \frac{(2X[\text{Form III}]+[\text{Form II}])}{(2X[\text{Form III}]+[\text{Form II}]+[\text{Form I}])} \times 100 \quad (\text{eq 2})$$

The reported, normalized percent cleavage accounts for cleavage in control samples under the reaction conditions employed and this was calculated according to equation 3.

$$\text{Normalized percent cleavage} = \frac{\% \text{ cleavage (drug)} - \% \text{ cleavage (control)}}{100 - \% \text{ cleavage control}} \quad (\text{eq 3})$$

**Table S6.** DNA cleavage due to **3**

Compound	Normalized Percent DNA Cleavage
<b>3</b>	0.7 %
Positive control	100.0 %

### ***Peptide Incubation Procedure***

Bradykinin (1 mM, bradykinin triacetate salt, Sigma-Aldrich, St. Louis, MO) was incubated with **3** (1 mM) in Tris buffer (10 mM, pH 7.0, 2.5 % DMSO) at 70 °C for 24 hours. The control sample contained 250 μM bradykinin in Tris buffer (10 mM, pH 7.0, 2.5 % DMSO).

### ***Peptide MALDI Analysis***

Aliquots of the bradykinin incubated samples were analyzed by MALDI (at the University of Texas at Austin by Dr. Maria Person) following Zip-Tip (Millipore) reversed-phased cleanup. Aliquots were diluted 1:5 with  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA) matrix solution and a 1 μl aliquot of the matrix solution was spotted onto a MALDI target plate. A Voyager-DE Pro mass spectrophotometer (Applied Biosystems, Framingham, MA) in reflector mode, with external calibration of the instrument over a 700-4000  $m/z$  range was used to acquire spectra. An average of 3000 shots from a 337-nm nitrogen laser was applied.

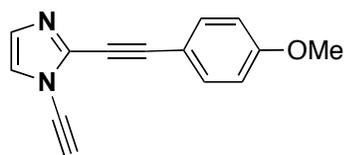
**Computational studies:** Calculations were carried out using Gaussian03<sup>15</sup> using the B3LYP hybrid functional and the 6-31G(d,p) basis set. All wavefunctions were checked for stability. Frequency calculations were carried out on all optimized structures to determine ZPE and enthalpies at 115 °C. The transition states all have one imaginary frequency along the mode corresponding to the bond breaking/formation, as confirmed by IRC following calculations. Calculations on the Bergman transition state **4**, the singlet diradical **5** and the transition state for the retro-Bergman cyclization **6** were performed using the broken-spin-symmetry, unrestricted formalism (Guess = Mix). It was found that using the quadratically convergent SCF optimization was important for maintaining the correct wavefunction during optimization. When spin contamination was noted in the resulting wavefunctions (**5** and **6** only), single-point calculations of the triplet state using the broken-spin-unrestricted geometries were carried out in order to correct the energy. The sum correction used the formula 1:

$$E_S = (1/x)E_{BS-S} - \{(1-x)/x\}E_T \quad (1)$$

Where E(S) is the sum-corrected energy of the singlet, E(BS-S) is the energy of the spin-contaminated singlet, E(T) is the energy of the triplet using the BS-S geometry, and x is:

$$x = (\langle S^2 \rangle_{BS-S} - \langle S^2 \rangle_T) / (\langle S^2 \rangle_S - \langle S^2 \rangle_T) \quad (2)$$

Where  $\langle S^2 \rangle_{BS-S}$  is the calculated spin contamination of the broken-spin Kohn-Sham orbitals,  $\langle S^2 \rangle_S$  and  $\langle S^2 \rangle_T$  are expectation values for the pure singlet and triplet states, respectively.



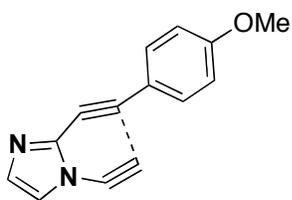
**3**

B3LYP/6-31G(d,p) = -724.092919 au

B3LYP/6-31G(d,p) Zero Point Corrected Energy = -723.889938

NIMAG = 0

C	-5.02197	-1.28432	0.00032
C	-5.09974	0.08172	0.00018
N	-3.71647	-1.71231	0.00029
N	-3.78156	0.52841	-0.00004
C	-2.97271	-0.62103	0.00003
C	-3.36828	1.81459	-0.00027
C	-1.56627	-0.54303	-0.00012
C	-0.35238	-0.46972	-0.00014
C	-3.00744	2.96611	-0.00046
C	1.06683	-0.39720	-0.00009
C	1.72652	0.84409	0.00036
C	1.84992	-1.57322	-0.00047
C	3.11715	0.92140	0.00042
C	3.23211	-1.50241	-0.00040
C	3.87842	-0.25514	0.00002
H	-5.84134	-1.98917	0.00050
H	-5.92271	0.77799	0.00020
H	-2.68584	3.98084	-0.00068
H	1.13781	1.75554	0.00068
H	1.35522	-2.53868	-0.00081
H	3.59339	1.89428	0.00080
H	3.84102	-2.40013	-0.00069
O	5.23918	-0.29731	0.00005
C	5.95415	0.93005	0.00014
H	7.01107	0.66125	0.00011
H	5.73303	1.52637	-0.89389
H	5.73321	1.52613	0.89443



4

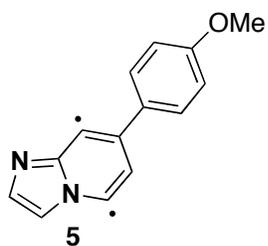
BS-UB3LYP/6-31G(d,p) = -724.0441887 au

BS-UB3LYP/6-31G(d,p) Zero Point Corrected Energy = -723.8422217 au

NIMAG = 1

S<sup>2</sup> = 0.0000

C	5.21418	-0.56920	0.28958
C	4.84072	0.67904	-0.13613
N	4.13351	-1.38365	0.50904
N	3.46214	0.67788	-0.21864
C	3.05900	-0.68818	0.21962
C	2.56608	1.60974	-0.53989
C	1.68596	-0.86905	0.27855
C	0.65060	-0.15661	0.07333
C	1.29348	1.55801	-0.53103
C	-0.79992	-0.17648	0.01870
C	-1.58285	0.88320	0.49731
C	-1.45890	-1.30896	-0.50776
C	-2.97576	0.83237	0.45538
C	-2.84089	-1.36999	-0.54878
C	-3.61333	-0.29791	-0.07087
H	6.22029	-0.92881	0.45220
H	5.40932	1.55619	-0.39904
H	0.42223	2.08046	-0.87565
H	-1.09755	1.75269	0.92868
H	-0.86643	-2.13772	-0.88000
H	-3.54730	1.66731	0.84178
H	-3.35245	-2.23818	-0.95038
O	-4.96121	-0.45653	-0.16115
C	-5.79919	0.58955	0.31114
H	-6.82253	0.25299	0.14179
H	-5.65120	0.77196	1.38271
H	-5.62935	1.52246	-0.24059



Singlet

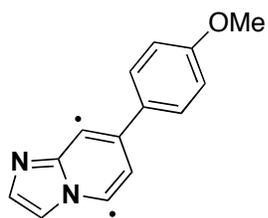
BS-UB3LYP/6-31G(d,p) = -724.068188357 au

BS-UB3LYP/6-31G(d,p) Zero Point Corrected Energy = -723.8635184 au

NIMAG = 0

$S^2 = 0.8852$

C	5.11752	-0.76573	0.23610
C	4.85031	0.56085	-0.01659
N	3.98595	-1.52146	0.33234
N	3.47430	0.66439	-0.09025
C	2.96918	-0.70118	0.13942
C	2.62526	1.67323	-0.30732
C	1.58539	-0.79169	0.11216
C	0.69924	0.21724	-0.08132
C	1.28765	1.54204	-0.33610
C	-0.76868	0.04149	-0.06492
C	-1.64244	1.08946	0.24929
C	-1.33234	-1.21720	-0.35803
C	-3.02635	0.90875	0.26547
C	-2.70295	-1.41166	-0.34335
C	-3.56520	-0.34728	-0.03454
H	6.09494	-1.21329	0.35512
H	5.48651	1.41964	-0.15674
H	0.63180	2.37111	-0.57041
H	-1.24673	2.06443	0.51596
H	-0.67462	-2.04336	-0.60994
H	-3.66614	1.74463	0.52120
H	-3.13603	-2.37896	-0.57504
O	-4.89607	-0.63889	-0.05206
C	-5.81705	0.39864	0.25032
H	-6.80908	-0.04761	0.17130
H	-5.67401	0.78228	1.26839
H	-5.73721	1.23003	-0.46146



Triplet

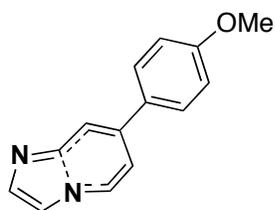
UB3LYP/6-31G(d,p) = -724.0611134 au

UB3LYP/6-31G(d,p) Zero Point Corrected Energy = -723.8557324 au

NIMAG = 0

S<sup>2</sup> = 2.0092

C	5.10750	-0.80878	0.23130
C	4.87068	0.53158	0.00199
N	3.95865	-1.54157	0.30166
N	3.49673	0.64574	-0.07376
C	2.97225	-0.67434	0.12014
C	2.64148	1.66851	-0.28416
C	1.57735	-0.75802	0.08792
C	0.69018	0.26782	-0.08846
C	1.29226	1.56964	-0.30705
C	-0.77346	0.07176	-0.07157
C	-1.66187	1.11433	0.22030
C	-1.32006	-1.19957	-0.34012
C	-3.04295	0.91473	0.24207
C	-2.68759	-1.41391	-0.31795
C	-3.56412	-0.35545	-0.02933
H	6.07490	-1.27873	0.34764
H	5.52200	1.38240	-0.11749
H	0.68097	2.44025	-0.51456
H	-1.27986	2.10124	0.46222
H	-0.65139	-2.02122	-0.57912
H	-3.69486	1.74699	0.47846
H	-3.10779	-2.39126	-0.53042
O	-4.89090	-0.66595	-0.03893
C	-5.82608	0.36509	0.24092
H	-6.81185	-0.09653	0.17266
H	-5.68794	0.77342	1.25004
H	-5.75811	1.18143	-0.48928



6

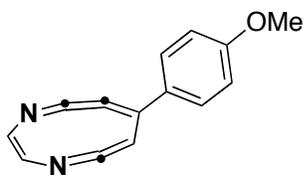
BS-UB3LYP/6-31G(d,p) = -724.0619073au

BS-UB3LYP/6-31G(d,p) Zero Point Corrected Energy = -723.8596013 au

NIMAG = 1

$S^2 = 0.4813$

C	5.10046	-0.74796	0.23824
C	4.83286	0.57319	-0.03309
N	3.99890	-1.54204	0.35818
N	3.48561	0.78071	-0.12162
C	2.91529	-0.86602	0.17797
C	2.61243	1.69591	-0.31286
C	1.58217	-0.80910	0.11576
C	0.71160	0.21377	-0.08063
C	1.27178	1.54650	-0.34124
C	-0.75952	0.04014	-0.06286
C	-1.63117	1.08193	0.27687
C	-1.32585	-1.21117	-0.38129
C	-3.01542	0.90380	0.28829
C	-2.69708	-1.40233	-0.37335
C	-3.55726	-0.34334	-0.04192
H	6.08795	-1.17267	0.35573
H	5.52035	1.39142	-0.18585
H	0.58926	2.35212	-0.57437
H	-1.23296	2.04814	0.56977
H	-0.66866	-2.03311	-0.64715
H	-3.65300	1.73436	0.56591
H	-3.13176	-2.36383	-0.62537
O	-4.88891	-0.63146	-0.06595
C	-5.80730	0.40084	0.26090
H	-6.80048	-0.04095	0.17118
H	-5.66359	0.75977	1.28787
H	-5.72525	1.24880	-0.43075



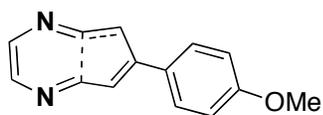
7

B3LYP/6-31G(d,p) = -724.105519au

B3LYP/6-31G(d,p) Zero Point Corrected Energy = -723.901462 au

NIMAG = 0

C	5.00362	-0.76631	0.09202
C	4.98351	0.61669	-0.02973
N	3.95567	-1.61530	0.16163
N	3.81786	1.28850	-0.08891
C	2.74412	-1.22283	0.11054
C	2.65388	1.50573	-0.12401
C	1.52494	-0.91404	0.07089
C	0.68769	0.18823	-0.02285
C	1.29546	1.48024	-0.14067
C	-0.78372	0.04764	-0.01857
C	-1.66071	1.12984	0.13858
C	-1.35290	-1.23356	-0.16956
C	-3.04519	0.96004	0.13525
C	-2.72413	-1.41911	-0.17666
C	-3.58623	-0.32098	-0.02671
H	5.98554	-1.22851	0.14177
H	5.89382	1.20012	-0.07422
H	0.73403	2.39579	-0.26831
H	-1.27357	2.13246	0.29048
H	-0.68789	-2.08371	-0.28442
H	-3.68521	1.82419	0.26649
H	-3.15819	-2.40599	-0.29797
O	-4.91864	-0.60491	-0.04771
C	-5.83858	0.46541	0.10582
H	-6.83192	0.01762	0.05618
H	-5.71692	0.96724	1.07406
H	-5.73581	1.20578	-0.69764



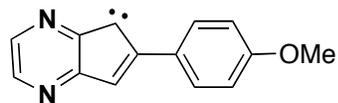
8

B3LYP/6-31G(d,p) = -724.0806223 au

B3LYP/6-31G(d,p) Zero Point Corrected Energy = -723.8779263 au

NIMAG = 1

C	5.10526	0.67782	0.14350
C	5.07466	-0.70744	-0.04179
N	3.99614	1.40954	0.26043
N	3.91028	-1.35236	-0.15020
C	2.81736	0.86817	0.13861
C	2.74002	-0.82833	-0.06488
C	1.51819	0.96868	-0.06663
C	0.60359	-0.09125	-0.07059
C	1.40783	-1.24875	-0.15103
C	-0.85015	0.02443	-0.05826
C	-1.67733	-1.09670	0.09961
C	-1.46714	1.28496	-0.19317
C	-3.06573	-0.97962	0.12175
C	-2.84472	1.41650	-0.17027
C	-3.65864	0.28268	-0.01350
H	6.05224	1.19827	0.24760
H	5.98200	-1.30124	-0.05503
H	1.09749	-2.28022	-0.26568
H	-1.23448	-2.08012	0.22226
H	-0.84521	2.16568	-0.32976
H	-3.67061	-1.86933	0.24806
H	-3.32083	2.38534	-0.27700
O	-5.00041	0.51444	-0.00622
C	-5.87628	-0.59309	0.14787
H	-6.88630	-0.18222	0.12488
H	-5.76156	-1.31585	-0.66966
H	-5.71596	-1.10443	1.10529



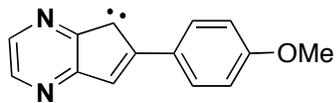
9

B3LYP/6-31G(d,p) = -724.0892107 au

B3LYP/6-31G(d,p) Zero Point Corrected Energy = -723.8846367 au

NIMAG = 0

C	5.06083	0.57667	0.43504
C	5.04438	-0.76944	0.07163
N	3.95589	1.35819	0.42582
N	3.91843	-1.42469	-0.29829
C	2.84708	0.70366	0.11927
C	2.81948	-0.69471	-0.19225
C	1.51183	1.12604	-0.26354
C	0.60349	0.04429	-0.12867
C	1.41946	-1.06348	-0.39331
C	-0.85491	0.10660	-0.09583
C	-1.62335	-1.03383	0.17336
C	-1.52612	1.32874	-0.30218
C	-3.01523	-0.97752	0.22119
C	-2.90768	1.39842	-0.25533
C	-3.66604	0.24435	0.00427
H	5.99441	1.05780	0.71312
H	5.96579	-1.34579	0.06354
H	1.09563	-1.98909	-0.85952
H	-1.12637	-1.97961	0.36949
H	-0.94068	2.21711	-0.51792
H	-3.57619	-1.87882	0.43612
H	-3.43091	2.33431	-0.42025
O	-5.01576	0.41874	0.02996
C	-5.83983	-0.71073	0.28114
H	-6.86680	-0.34476	0.25248
H	-5.71026	-1.48405	-0.48632
H	-5.63906	-1.14582	1.26816



Triplet

UB3LYP/6-31G(d,p) = -724.1071007 au

UB3LYP/6-31G(d,p) Zero Point Corrected Energy = -723.9019807 au

NIMAG = 0

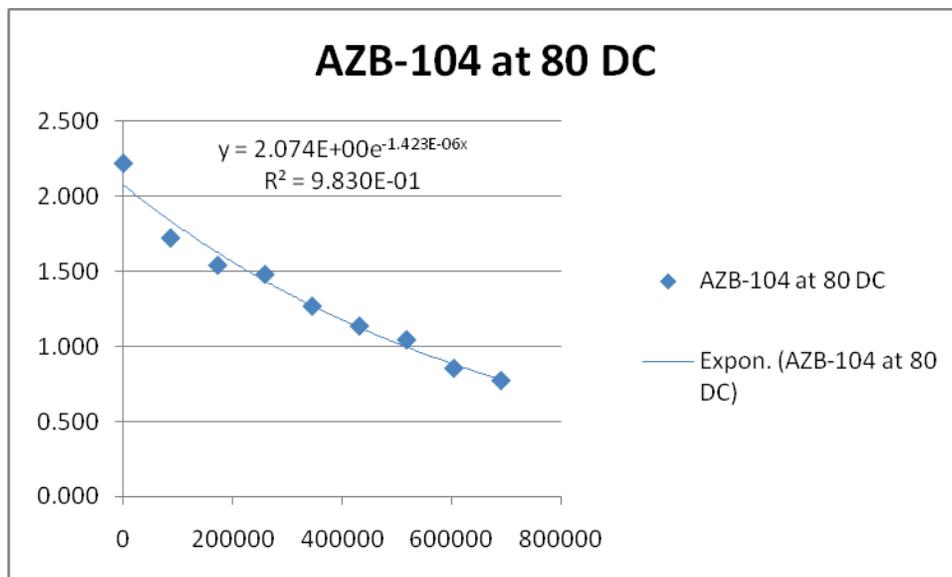
$S^2 = 2.0312$

C	-5.10899	0.63613	0.00008
C	-5.06890	-0.75759	-0.00007
N	-3.99809	1.40442	0.00016
N	-3.91527	-1.46253	-0.00015
C	-2.86461	0.71906	0.00008
C	-2.81707	-0.72143	-0.00008
C	-1.48261	1.11814	0.00011
C	-0.60479	0.00323	-0.00001
C	-1.42232	-1.13846	-0.00013
C	0.85841	0.07508	-0.00001
C	1.66267	-1.07305	0.00017
C	1.50571	1.32735	-0.00019
C	3.05403	-0.99139	0.00016
C	2.88697	1.42488	-0.00020
C	3.67609	0.26390	-0.00003
H	-6.06207	1.15813	0.00014
H	-5.99081	-1.33332	-0.00013
H	-1.09755	-2.17193	-0.00027
H	1.20305	-2.05655	0.00035
H	0.90572	2.23294	-0.00034
H	3.63818	-1.90364	0.00031
H	3.38375	2.38926	-0.00035
O	5.02352	0.46270	-0.00005
C	5.87353	-0.67517	0.00014
H	6.89319	-0.28812	0.00009
H	5.72154	-1.29270	0.89425
H	5.72156	-1.29298	-0.89378

**Kinetic study of the thermolysis of 1-ethynyl-2-(2-(4-methoxyphenyl)-ethynyl)-1*H*-imidazole (3) :** A solution of **3** (0.05 mmol) and 5,6-benzoquinoline (0.05 mmol) in 3 mL of 1,4-cyclohexadiene was prepared. 300  $\mu$ L aliquots of this solution were heated at different temperatures in sealed tubes. At regular time period, an aliquot was cooled down and a small quantity (around 30  $\mu$ L) was transferred. The 1,4-cyclohexadiene was evaporated under vacuum. The residue was solubilised in 1 mL of an acetonitrile/water (50/50) mixture. This solution was injected in a Thermo-Finnigan LTQ XL LC-MS apparatus using a C18 short pad column. The solutions were eluted at 0.5 mL/min. using a gradient of acetonitrile (5-95%, 4 min.) in water followed 95 % acetonitrile (1.5 min.). The mass spectra were recorded in positive mode using ESI as ionization technique and subsequently analyzed using the software Xcalibur.

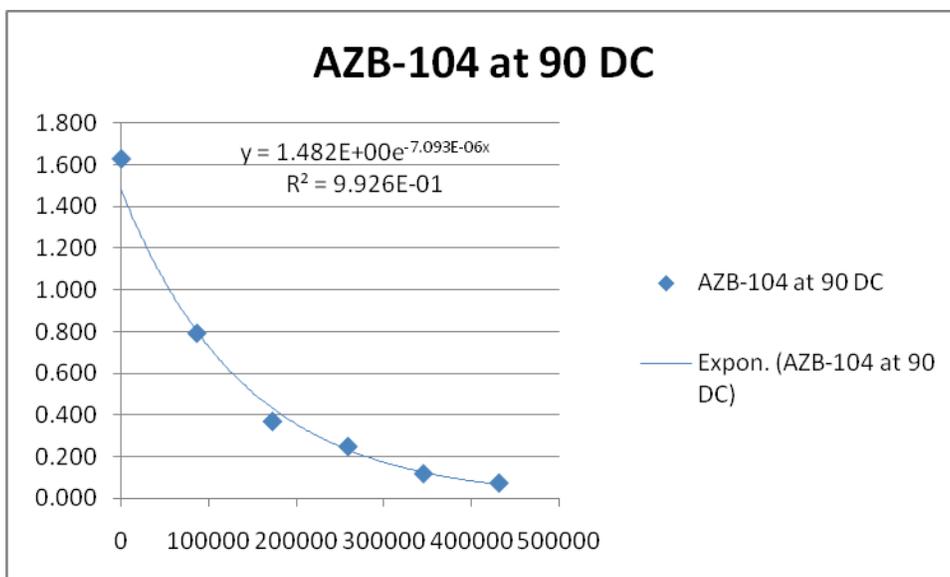
Thermolysis at 80°C.

Time (sec)	Time (days)	5,6-benzoquinoline integration (X)	starting material integration (Y)	Y/X
0	0	8182955	18134471	2.216
86400	1	7698944	13239298	1.720
172800	2	7181236	11043442	1.538
259200	3	4239026	6260280	1.477
345600	4	4553130	5762703	1.266
432000	5	8169505	9254838	1.133
518400	6	13689264	14263671	1.042
604800	7	9431677	8033551	0.852
691200	8	5038709	3881692	0.770
777600	9	2438006	2214870	0.908
864000	10	4152084	2969028	0.715



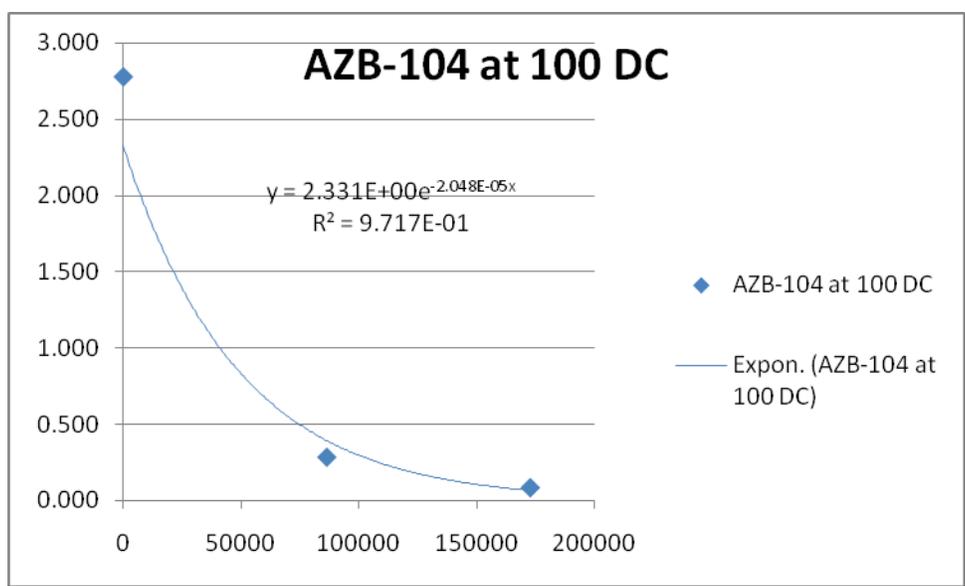
1. Thermolysis at 90°C.

Time (sec)	Time (days)	5,6-benzoquinoline integration (X)	starting material integration (Y)	Y/X
0	0	12224285	19920116	1.630
86400	1	14625957	11598617	0.793
172800	2	14002560	5189759	0.371
259200	3	12848088	3215462	0.250
345600	4	19243193	2308927	0.120
432000	5	17320280	1299154	0.075



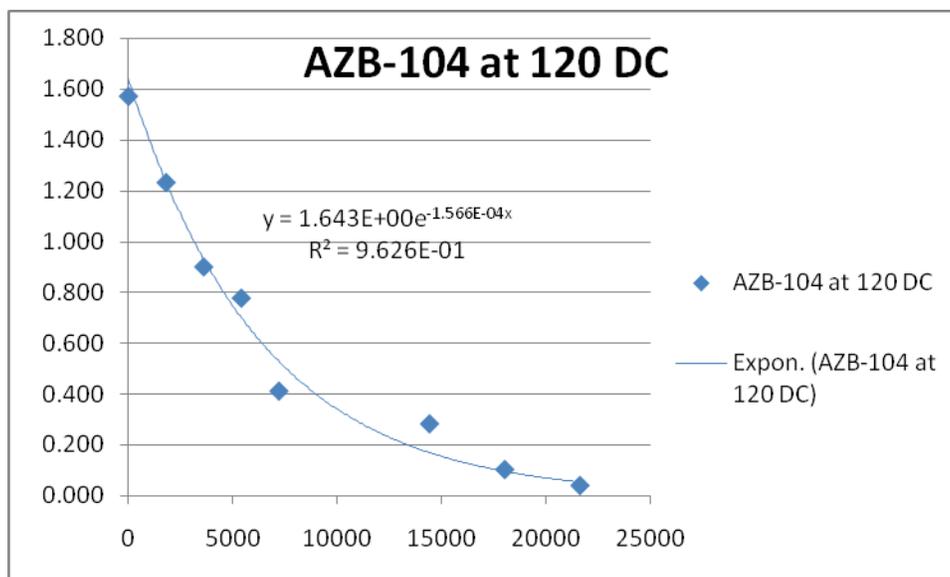
2. Thermolysis at 100°C.

Time (sec)	Time (days)	5,6-benzoquinoline integration (X)	starting material integration (Y)	Y/X
0	0	2805645	7784867	2.775
86400	1	17792365	4988766	0.280
172800	2	11403444	919322	0.081



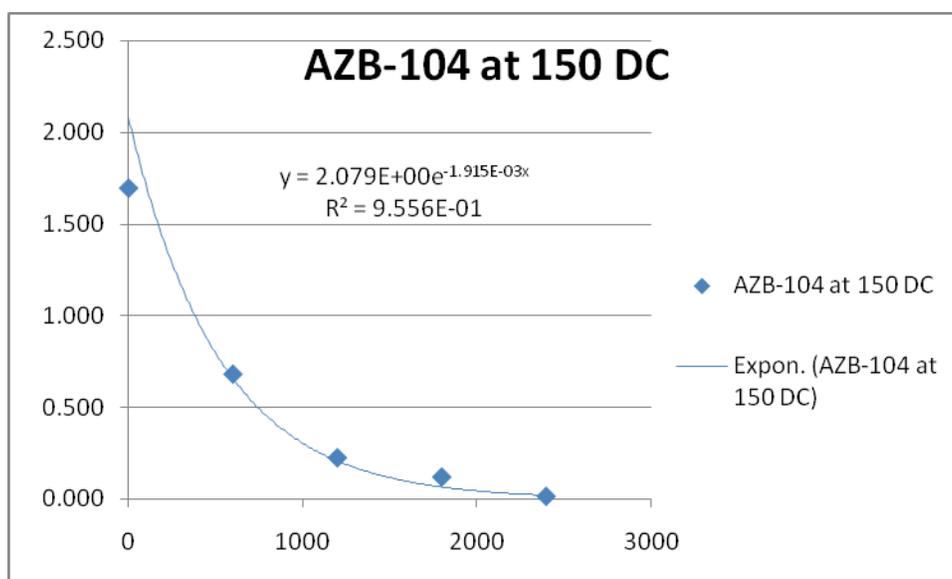
3. Thermolysis at 120°C.

Time (sec)	Time (days)	5,6-benzoquinoline integration (X)	starting material integration (Y)	Y/X
0	0	9754500	15334631	1.572
1800	30	16865350	20793040	1.233
3600	60	8937990	8051357	0.901
5400	90	9696795	7542287	0.778
7200	120	11001612	4536730	0.412
14400	240	10293585	2916984	0.283
18000	300	15300130	1584819	0.104
21600	360	15522996	633270	0.041



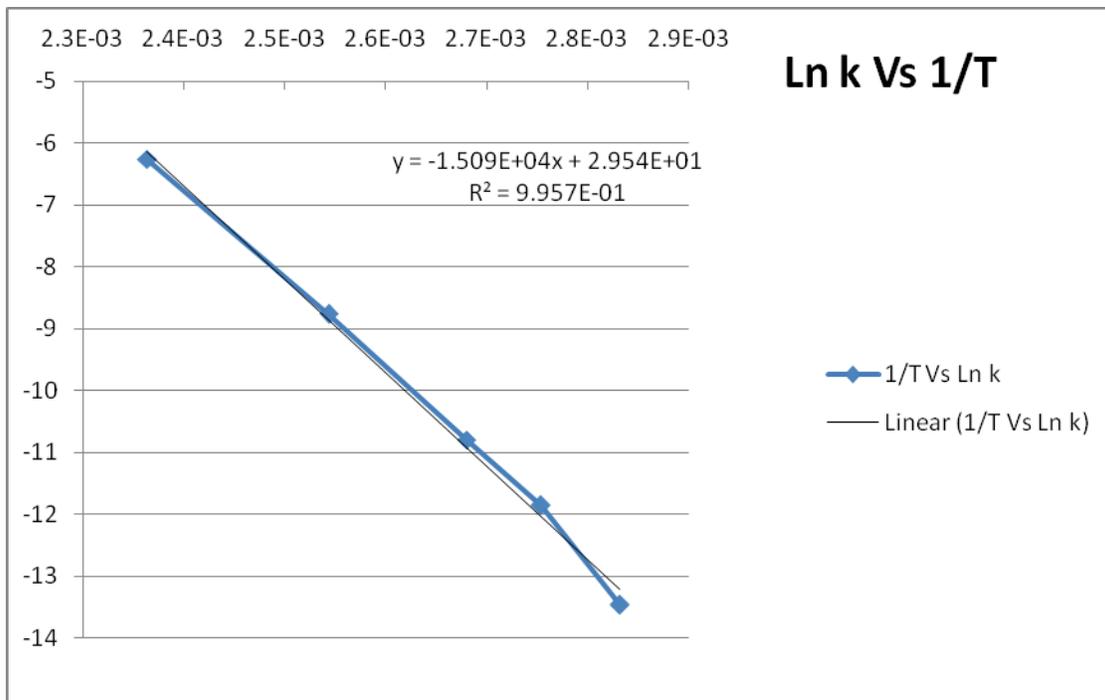
4. Thermolysis at 150°C.

Time (sec)	Time (minutes)	5,6-benzoquinoline integration (X)	starting material integration (Y)	Y/X
0	0	10860955	18416645	1.696
600	10	12905737	8776789	0.680
1200	20	14934566	3345537	0.224
1800	30	17979219	2126414	0.118
2400	40	16926621	220085	0.013



5. Arrhenius plot.

Ln k	k	Temperature (°C)	1/T (K)
13.4627	1.423E-06	80	0.00283
11.8564	7.093E-06	90	0.00275
10.7961	2.048E-05	100	0.00268
8.76182	1.566E-04	120	0.00254
6.25804	1.915E-03	150	0.00236



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 $R(F) = \sum (|F_o| - |F_c|) / \sum |F_o|$  for reflections with  $F_o > 4\sigma(F_o)$ .  
 $S = [ \sum w(|F_o|^2 - |F_c|^2)^2 / (n - p) ]^{1/2}$ , where n is the number of reflections and p is the number of refined parameters.
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