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Synthetic utility and reactivity of *N*-alkynylazoles

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Synthetic utility and reactivity of *N*-alkynylazoles

by

Bradford Lynd Gilbreath, B.S.

Thesis

Presented to the Faculty of the Graduate School

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Synthetic utility and reactivity of *N*-alkynylazoles

by

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The University of Texas at Austin, 2014

SUPERVISOR: Sean Kerwin

The chemistry of *N*-alkynylazoles is a newly emerging area of chemistry with particular interest in heterocycle synthesis. Synthetic preparations of this class of molecule have shown an increasing presence in literature, however, the synthetic utility of this class of molecule remains underexplored. Herein, it is shown that *N*-alkynlazoles are a versatile synthetic intermediate and may have utility as covalent modifiers of cysteine residues in polypeptides.

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Chapter 1: Introduction

The chemistry of ynamines and ynamides has received attention for numerous uses in synthetic transformations. There are considerable differences observed in the relativities of these two classes of molecules. A less studied class of *N*-alkynes is *N*-alkynylazoles. It would be expected that this class of molecule would exhibit a unique reactivity as well. Therefore, the interest herein is to begin exploration of the utility of *N*-alkynalzoles as synthetic intermediates.

Ynamines

Ynamines have a disseminated history stretching nearly seven decades before their existence was proven and widely accepted[1]. In 1958, Zaugg, et al.[2] were attempting to alkylate phenothiazine **1** with propargyl bromide to afford the propargyl product **2**. The attempted alkylation using sodium amide in xylenes failed which prompted the authors to use sodium hydride in DMF. From this reaction, Zaugg et al isolated the first ynamine **3** in 70% yield. These workers postulated that **3** was formed from the initial alkylation product **2** via isomerization under strongly basic conditions.

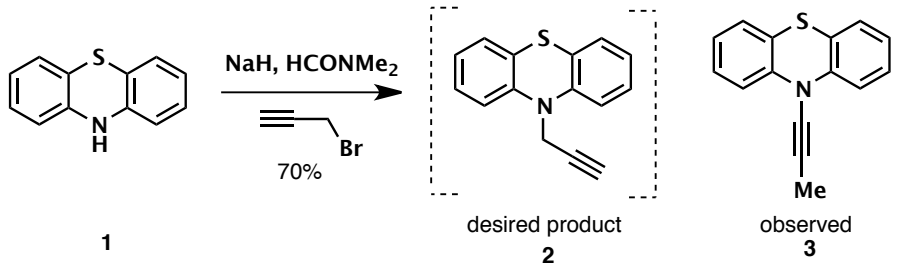


Fig. 1.1. First synthesis of an ynamine

The first synthesis of an ynamine (Fig 1.1) ushered in a new era of synthesis

employing these versatile intermediates. Before continuing with an historical perspective of synthetic routes to ynamines and related compounds, an overview of the synthetic utility of these compounds will be presented. Hsung, et al.[1] have eloquently categorized the reactivity of ynamines into two classes of addition reactions: regioselective additions and step-wise cycloadditions.

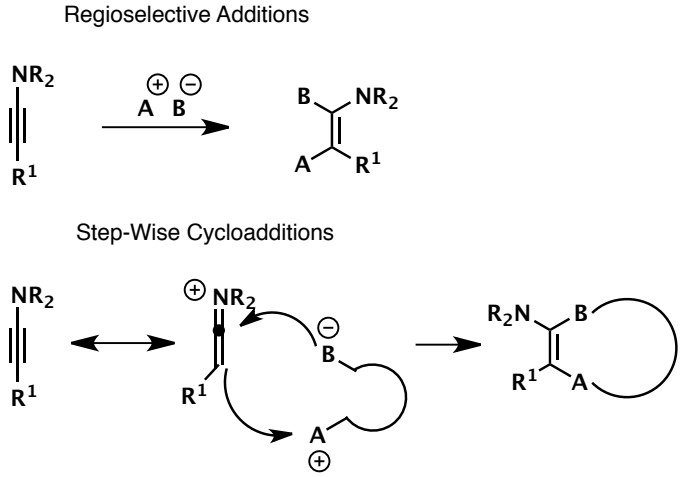


Fig. 1.2. Reactivity of ynamines[1]

The electronic nature of ynamines promotes highly regioselective addition reactions across the alkynyl triple bond due to the donating effect of the nitrogen lone pair.

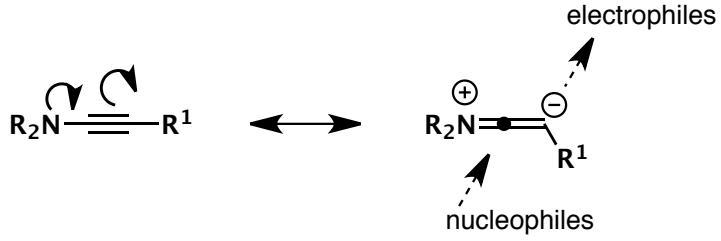


Fig. 1.3. Nitrogen lone pair donating character in ynamines[1]

Resonance easily explains the regioselectivity observed in such addition reactions. The

highly reactive nature of ynamines, with the tendency to easily undergo hydrolysis, is also readily evident when considering the effects of the electron donating nitrogen into the electron deficient alkyne.

Synthesis of ynamines

The first directed synthesis of an ynamine was achieved by Viehe[3] in 1963 by reacting **4** with lithium diethylamide resulting in **5** in 86% yield.

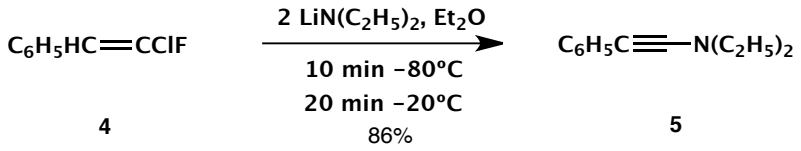


Fig. 1.4. First intended synthesis of ynamines

Viehe noted the method as providing access to a rather inaccessible class of molecules. A number of other diverse methods to synthesize ynamines have since appeared. In 1966, Viehe, et al.[4] reported the synthesis of alkylamines from α-halogeno iminium salts as well as ketene S,N-acetals by elimination reactions.

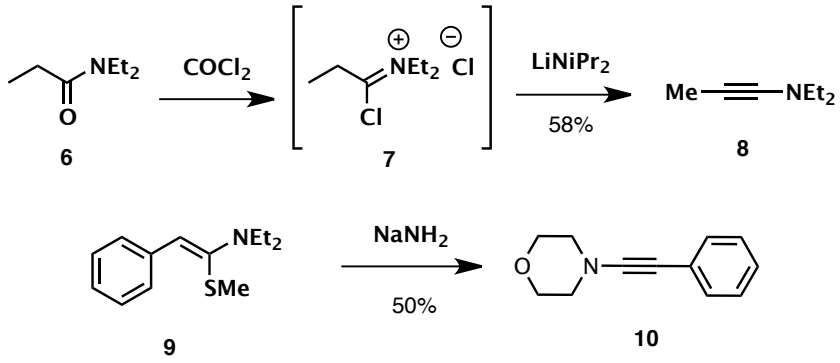


Fig. 1.5. Synthesis of ynamines from a-halogeno iminium salts and S,N-actals

Using the method which Weidinger, et al.[5] reported for the synthesis of α-halogenoiminium salts from carboxamides and phosgene, Viehe reacted the crude N,N-diethynyl-1-imidoyl chloride **7** with LDA to afford the ynamine **8** in good yield. Similar conditions transformed the S,N-acetal **9** to ynamine **10**, as reported by Elser, et al.[6], in moderate yield as well.

Chloroenamines have also been proven as precursors to ynamines by several groups. Several methods of introducing dichloroethenyl groups onto secondary amines allow access to ynamines by elimination. In 1970, Kundu, et al.[7] reported the synthesis of *N*-ethynylcarbazole **13**.

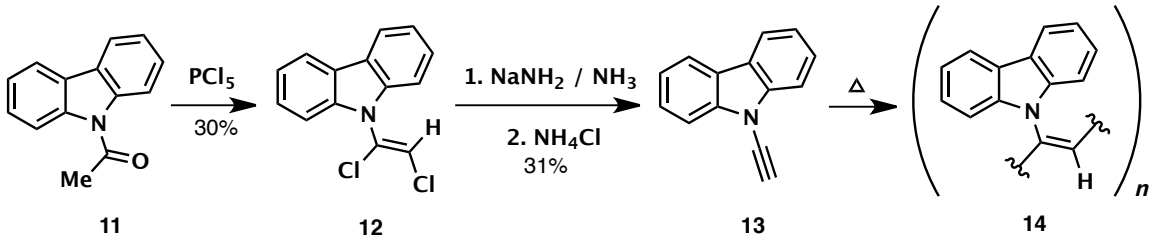


Fig. 1.6. Synthesis of ynamines from dichloroethenyl groups

N-acetylcarbazole **11** was reacted with phosphorus pentachloride giving N-(1,2-dichloroethenyl)carbazole **12** which was then treated with sodium amide in liquid ammonia followed by quenching with ammonium chloride to give the ynamine **13**. The purpose of this synthesis by Kundu, et al.[7] was to study the electronic properties of poly-*N*-ehtynylcarbazole **14**, which therein were inconsequential but nonetheless part of a larger interest in organic compounds with photoconductive properties.

Viehe has also reported nucleophilic substitution of halogens at acetylene bonds by amines as a direct route to ynamines[8] (Fig. 1.7).

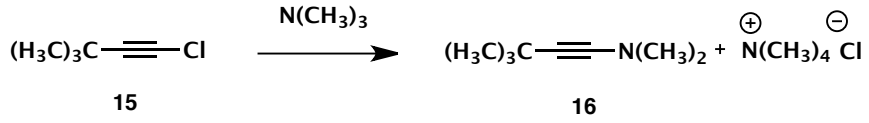


Fig. 1.7. Synthesis of ynamines by nucleophilic substitution

This reaction is analogous to earlier reports of haloalkyne nucleophilic displacement reactions toward the synthesis of acetylenic thioethers[9, 10, 11].

Additions to ynamines

Viehe was one of the earliest proponents for the use of ynamines in synthesis and published a concise report of additions to ynamines in 1964[12] which included cycloadditions, amidine formation, amidoxime formation and addition of alcohols among others (Fig. 1.8).

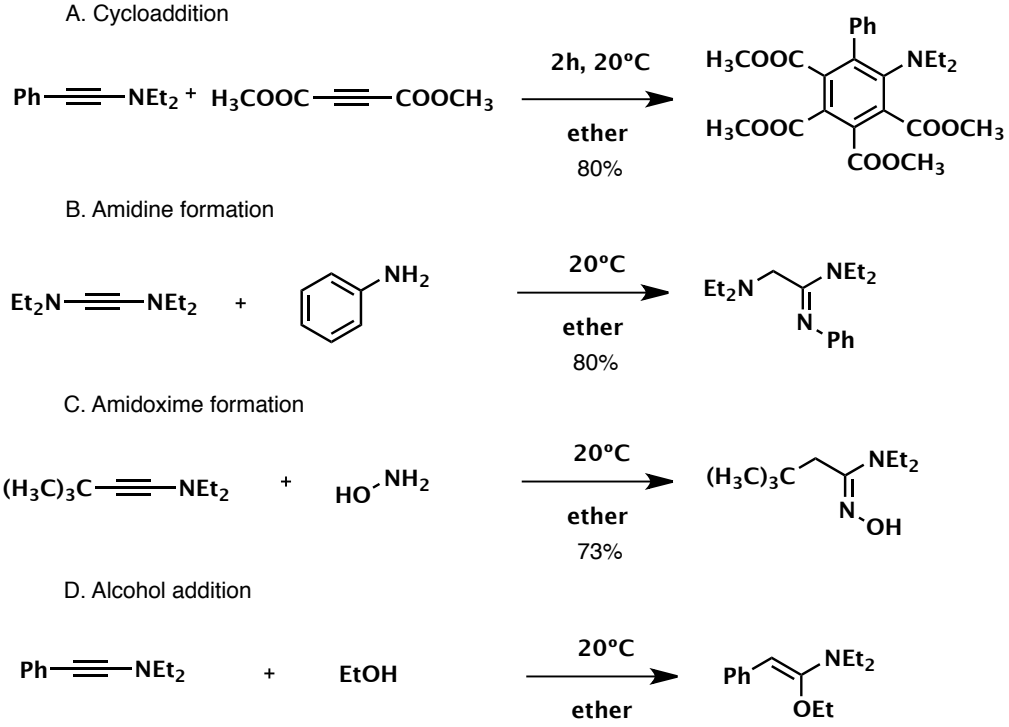


Fig. 1.8. Various additions to ynamines

Of special interest to Viehe was the observation of ynamines acting as dehydrating agents. Viehe, et al.[12] reported that the formation of anhydrides from carboxylic acids occurs more rapidly with alkynylamines than with dicyclohexylcarbodiimide or ethoxyacetylene.

Since the 1964 publication by Viehe, et al.[12], extensive work has gone into studying various cycloadditions to ynamines, as extensively reviewed by Hsung, et al.[1]. Various other cycloadditions of prominent interest include a [2+2] reaction reported by Himbert, et al.[13] between the *N*-alkynyl hydrazine **17** and the electron-deficient alkene **18** to afford the highly substituted butadiene **19** in good yields.

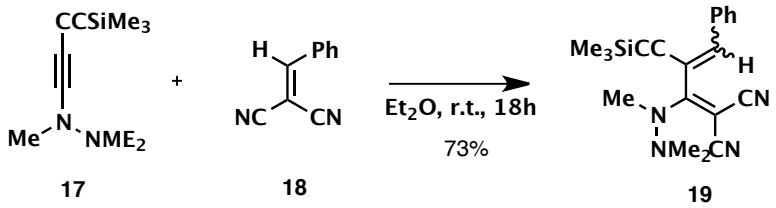


Fig. 1.9. Synthesis of substituted butadienes from ynamines

Cycloaddition reactions of ynamines are numerous and have shown much value as synthetic transformations. Aside from [2+2] cycloadditions, there have been numerous reports of [2+4], [2+2+1], and other cycloadditions as reviewed[1]. Interestingly, intramolecular reactions involving ynamines are much more rare. Genet and Kahn reported an intramolecular addition of a hydroxyl group to the ynamine **20** in 1980[14] which proceeded with high regioselectivity in 93% yield to afford the macrocyclic lactone **22** after hydrolysis.

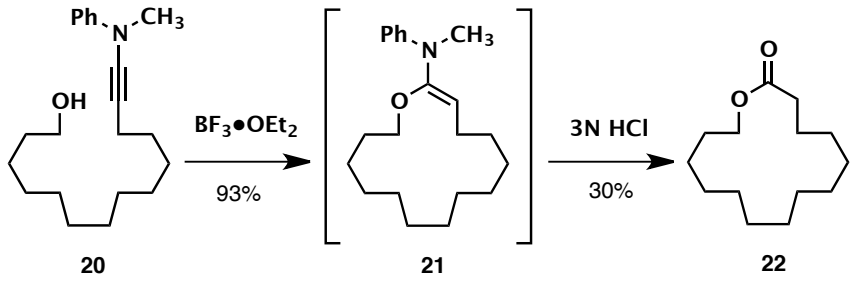


Fig. 1.10. Intramolecular hydroxyl addition to an ynamine

Ynamides

The first report of ynamines in 1958 by Zaugg, et al.[2] were formed from an isomerization reaction. Interestingly, similar conditions using the acridinone **23** afforded the *N*-propargyl substitution Zaugg expected but did not observe, rather than the corresponding ynamide. This lends to the difference in reactivity that is observed between ynamines and ynamides.

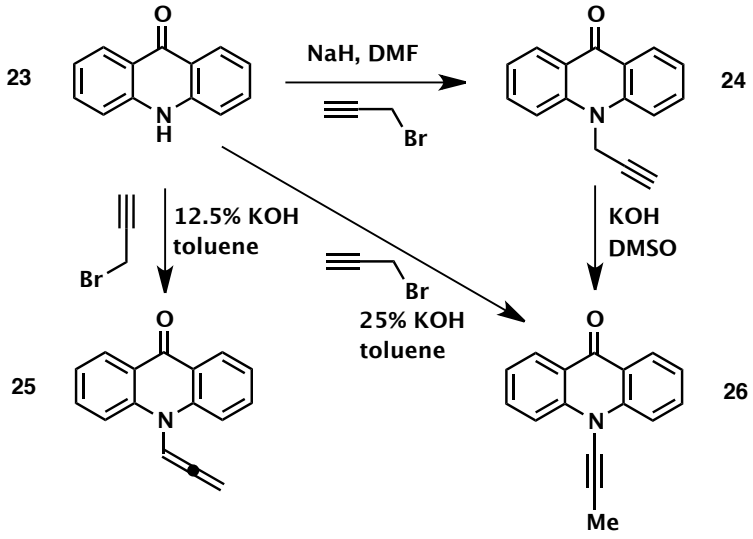


Fig. 1.11. Synthesis and isomerization reactivity of ynamides[2]

Similar reactions leading to the formation of ynamides have been more thoroughly

studied and it is evident that isomerization is dependent on the acidity of protons. Therefore, it may be assumed that the electron-withdrawing nature of ynamides, due to delocalization of the nitrogen lone-pair as observed in amide resonance structures, has a definite effect on the stability of ynamides over ynamines.

Ynamide synthesis

Similar to the synthesis of ynamines from *N*-acetyl functional groups[7], Bruckern[15] has reported the preparation of ynamides from *N*-formylsulfonamides **27**.

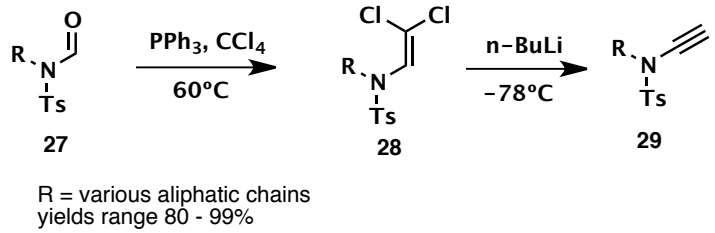


Fig. 1.12. Ynamide synthesis from N-formylation

Dichlorovinylamides **28** are reported to be efficiently prepared from the corresponding formamides **27** by reacting such with triphenylphosphine and tetrachloromethane. Elimination reactions of the dichlorovinylamides afford the ynamides **29** in good yields.

Iodonium salts have also been proven to provide access to ynamides. Feldman, et al.[16] reacted iodonium triflate salts with α-substituted ethyl-tosylamides **30**, **31** to produce ynamides **32**, **33** in moderate yields.

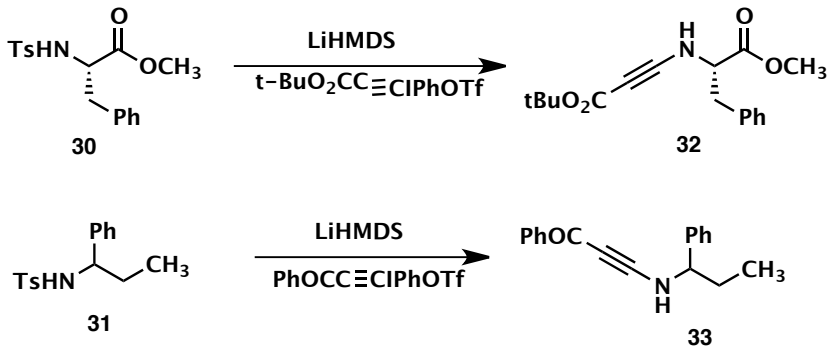


Fig. 1.13. Synthesis of ynamides from iodonium salts

Additions to ynamides

Intramolecular additions of ynamides have been studied more extensively than intramolecular additions to ynamines. Witulski, et al.[17] have generated some rare heterocycles based on Pauson-Khand intramolecular reactions. Witulski, et al. also reported the [2+2+1] cycloaddition of ynamides **34** to give products **35**/**36**.

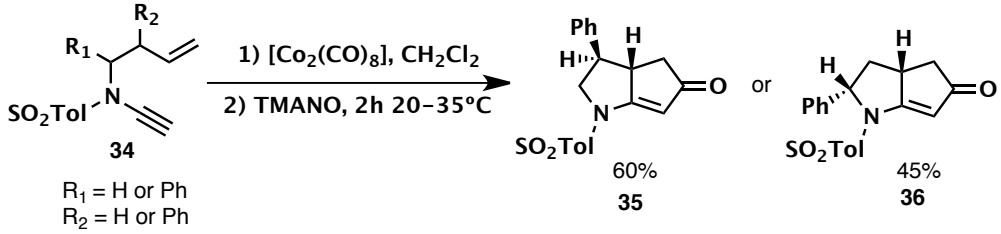


Fig. 1.14. Intramolecular cobalt catalyzed cycloaddition of ynamides

Witulski, et al.[18] have also reported the [2+2+2] Rhodium(I) catalyzed cycloadditions of ynamides, such as **37**, with acetylene for the synthesis of a variety of indolines e.g. **38**.

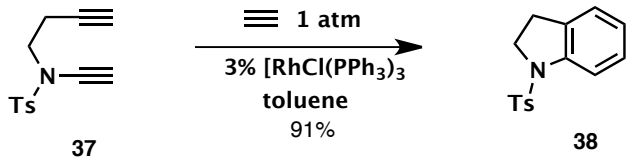


Fig. 1.15. Indoline synthesis from ynamides

Several ynamides with substituted alkynes were also shown to afford a number of highly regioselective indolines with yields ranging from 43 – 95%.

Ynamides have also recently appeared as intermediates in several total syntheses. Hsung, et al.[19] have illustrated a Bronsted acid-catalyzed stereoselective ynamide cyclization, which is the keteniminium variant of Pictet-Spengler cyclizations.

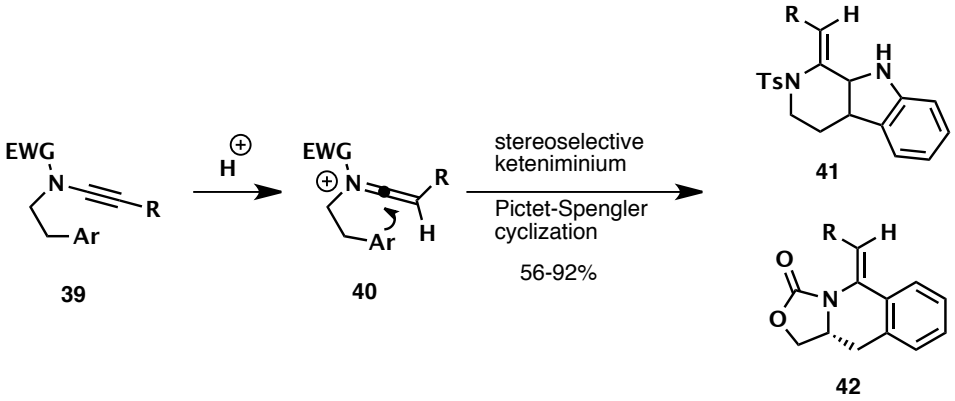


Fig. 1.16. Ynamide cyclization variant of the Pictet-Spengler reaction

Several arene-ynamides **39** tethered with aryl groups have afforded such complex heterocycles as **41** and **42**.

Cossy, et al.[20] have utilized an ynamide intermediate in the synthesis of the natural product lennoxamine.

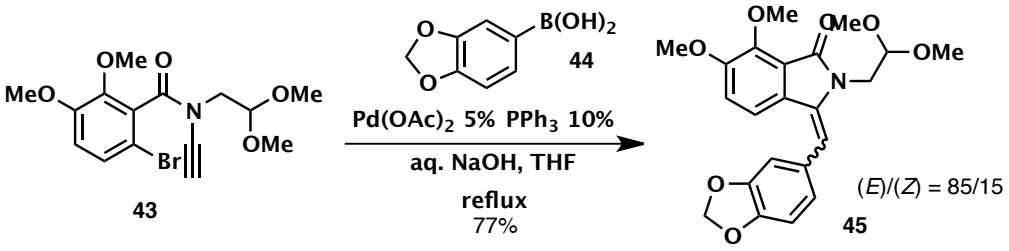


Fig. 1.17. Ynamide cyclization in a Heck-Suzuki-Miyaura reaction

A palladium-catalyzed Heck-Suzuki-Miyaura domino reaction of ynamide **43** in the presence of phenylboronic acid **44** afforded **45** in high yields leading to the natural product in two additional steps.

N-Alkynylazoles

Ynamines and ynamides have a relatively long chemical history in comparison to *N*-alkynylazoles. Ynamines have unstable character due to the donation of the nitrogen lone pair directly into the unsaturated alkyne p-orbitals. In comparison, ynamides have resonance delocalization and have proven to be more easily handled. *N*-alkynylazole resonance gives a hybrid effect in that the nitrogen lone pair can delocalize into the aromatic ring, and the extent of such delocalization dependent upon the electronic nature of the respective heterocycle variant.

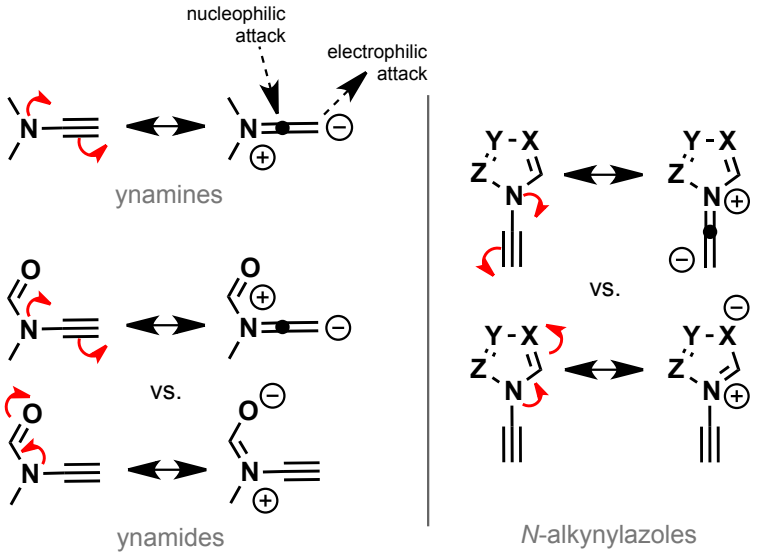


Fig. 1.18. Comparison of ynamine, ynamide, and *N*-alkynylazole resonance

The extent of nitrogen lone pair delocalization would be dependent on the heterocycle

and therefore reactivity would be expected to vary between *N*-alkynylazoles. Interestingly, additions of thiols to *N*-alkynes in pyrroles and imidazoles has been examined. Although the reaction conditions in these two instances are not identical

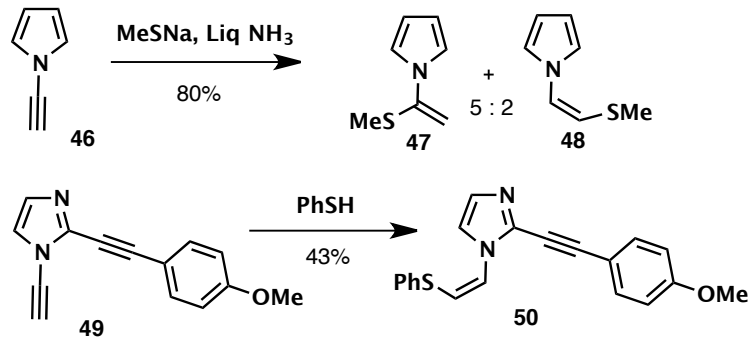


Fig. 1.19. Thiol additions to N-alkynylazoles

it is interesting that sodium methylthiolate adds to the *N*-alkyne of pyrrole **46** in both Markovnikov and anti-Markovnikov fashion whereas thiophenol adds to the *N*-alkyne of imidazole X in only anti-Markovnikov orientation.

N-alkynylazole synthesis

The first report of an *N*-alkynylazole was in 1985 by Brown, et al.[21]

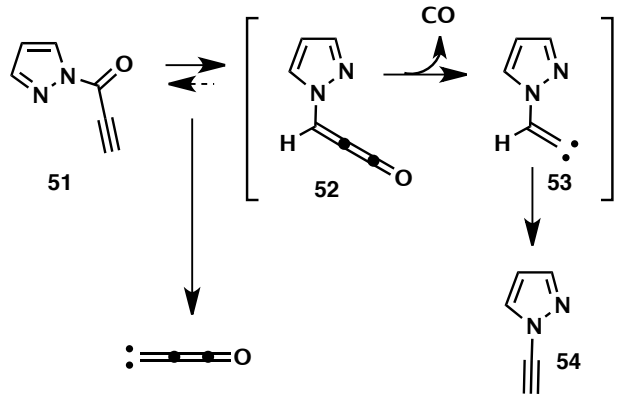


Fig. 1.20. Pyrolysis of propynoyl pyrazole leads to *N*-alkyne

It was proposed that 1-ethynylpyrazole **54** was formed via [3,2] sigmatropic rearrangement of the propynoyl moiety to **52** followed by decarbonylation to the carbene **53** and finally hydrogen migration to afford the *N*-alkyne **54**.

Hsung, et al.[22] have published the copper catalyzed coupling of alkynyl bromides with various heterocycles including pyrroles, indoles and benzimidazolidinones.

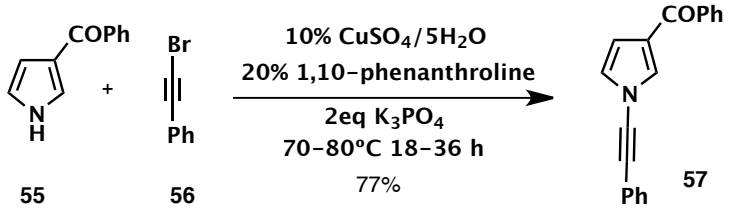


Fig. 1.21. Copper catalyzed coupling of pyrroles and bromoalkynes

Kerwin, et al.[23] have since published a copper catalyzed coupling reaction of bromoalkynes with various substituted imidazoles.

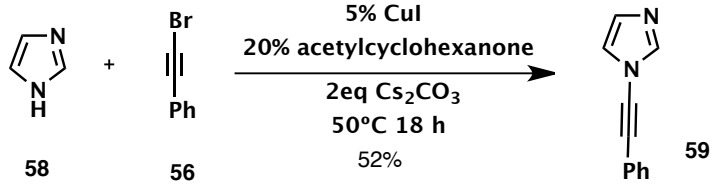


Fig. 1.22. Copper catalyzed coupling of imidazole and a bromoalkyne

Since then, numerous publications have emerged reporting similar conditions for various azoles.

Although there has been a recent surge in the number of publications reporting the preparation of *N*-alkynylazoles, the utility of this class of molecule remains unexplored. Therefore, the work presented herein forges a new area of reactions that lead to complex heterocycles by previously unknown methods.

Chapter 2: *N*-alkynylazole cyclizations

We first examined if the reagent-controlled regioselective hydroalkoxylation cyclization of 2-substituted 1-alkynylimidazoles also holds when the carbinol moiety is located at 5-position of the 1-alkynylimidazole.

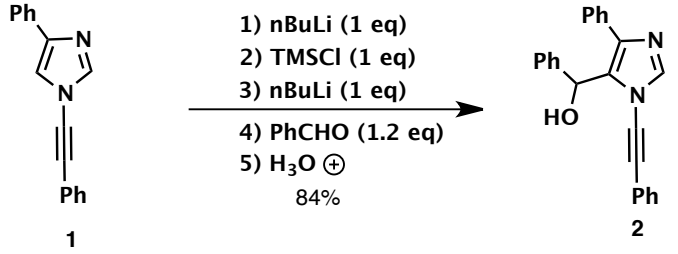


Fig.2.1 Preparation of 5-carbinol-functionalized 1-alkynylimidazole.

In order to access the required carbinol, we employed the sequential metalation-functionalization of the 4-phenyl-substituted 1-alkynylimidazole **1** (Fig. 2.1). In one pot, 1-alkynylimidazole **1** was deprotonated with *n*-BuLi at -78 °C to afford the 2-lithio species,[24] which was trapped with trimethylsilyl chloride. A second addition of base followed by trapping of the resulting 5-lithio species with benzaldehyde afforded the desired carbinol **2** in good yield after acidic work-up. Treating 1-alkynylimidazole **2** under the same conditions as employed for the regioselective 5-*exo-dig* cyclization of the 2-substituted isomer (5 mol % K_3PO_4 in CH_3CN under reflux) afforded the 5-*exo-dig* cyclization product **3** in 80% yield after chromatography (Fig.2.2). In contrast, treatment of **2** with 2 mol % $AuCl_3$ afforded exclusively the 6-*endo-dig* cyclization product **4** which was isolated in 92% yield after chromatography (Fig.2.2). The structures of **3** and **4** were assigned based on their ^1H and ^{13}C NMR. Thus, the reagent-controlled regioselective cyclization of 5-substituted 1-alkynylimidazole **2** mirrors that of the 2-substituted isomer.

This indicates that the subtle changes in geometry of the attacking hydroxyl group and the 1-alkynyl between isomers do not affect the origin or magnitude of the reagent-controlled regioselectivity.

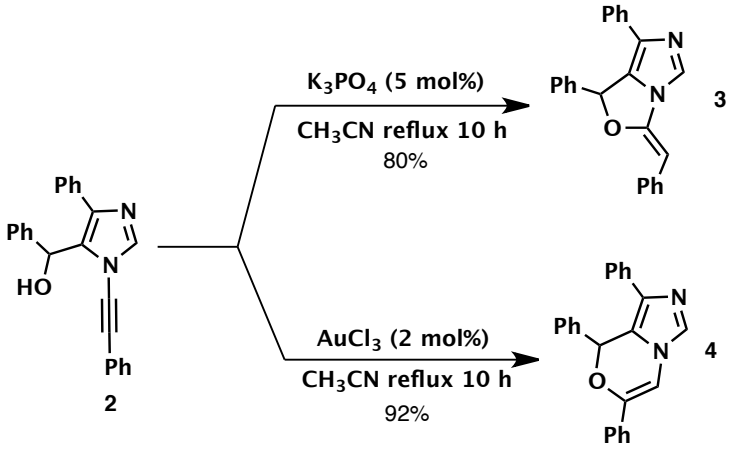


Fig.2.2 Regiocontrolled cyclization of 5-carbinol-functionalized 1-alkynylimidazole **5.**

Finally, we explored the use of 2-carbaldehyde 1-alkynylimidazoles as precursors to a variety of imidazo-fused heterocycles.

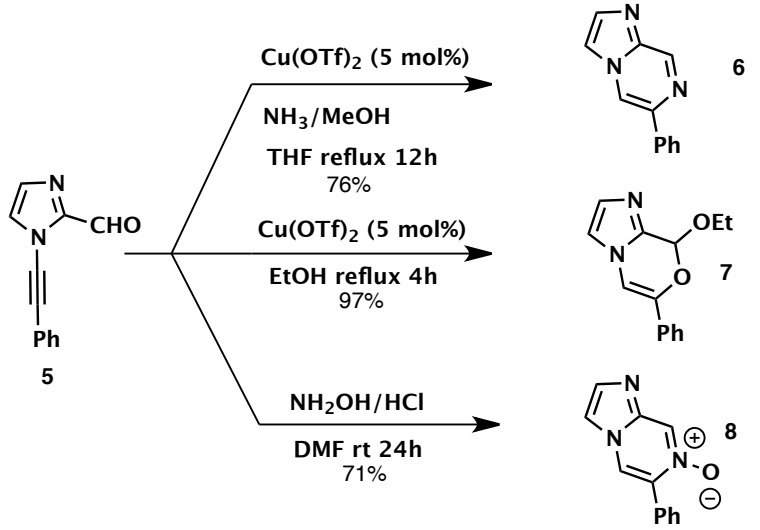


Fig.2.3 Exclusive *6-endo-dig* cyclizations of 2-carbaldehyde-functionalized 1-alkynylimidazole.

The aldehyde **5**, prepared by deprotonation and trapping with DMF,[25] undergoes cyclization to the imidazolopyrazine **6** when treated with ammonia in MeOH/THF in the presence of catalytic $Cu(OTf)_2$ (Fig.2.3). Similarly, in the presence of catalytic $Cu(OTf)_2$ in ethanol, **5** is cleanly converted to the cyclic acetal **7**. Aldehyde **5** undergoes cyclization in the absence of metal catalyst when treated with hydroxylamine hydrochloride in DMF to afford the imidazolopyrazine *N*-oxide **8**. No products of 5-*exo-dig* cyclization were observed in any of these cyclizations. Thus, under the influence of catalytic Cu^{2+} or when the nucleophilic group involved in the cyclization is a sp^2-nitrogen, only products of *6-endo-dig* cyclization are observed.

Phenyl(4-phenyl-1-(2-phenylethynyl)-1*H*-imidazol-5-yl)methanol (2) : A stirred solution of 4-phenyl-1-(2-phenylethynyl)-1*H*-imidazole (**1**)[26] (324 mg, 1.32 mmol) in 20 mL of THF was cooled to -78 ºC. The following successive additions to the reaction solution were carried out while maintaining the reaction temperature at -78 ºC. *n*-BuLi (2 M in hexanes, 0.66 mL, 1.32 mmol) was added by syringe and the reaction stirred for 20 min. TMSCl (0.17 mL, 1.327 mmol) was added by syringe and the reaction stirred for 45 min. A second equivalent of *n*-BuLi (2 M in hexanes, 0.66 mL, 1.32 mmol) was added and the reaction stirred for 30 min. Benzaldehyde (0.162 mL, 1.592 mmol) was added by syringe and the reaction allowed to stir for 30 min. Aqueous 1 M HCl (10 mL) was added by syringe and the reaction was allowed to reach room temperature. The reaction mixture was extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried over Na_2SO_4, and the solvents were removed by reduced pressure giving a light

yellow oil which was purified by flash chromatography (0-5% EtOAc in hexanes). Purification afforded 390 mg (84%) of phenyl(4-phenyl-1-(2-phenylethynyl)-1*H*-imidazol-5-yl)methanol (**5**) as a white crystalline solid. R_f 0.6 (25% EtOAc in hexane); Mp 155-157 ºC; ^{1}H NMR δ 7.80 (1H, s), 7.67-7.61 (2H, m), 7.46-7.25 (11H, m), 7.19-7.14 (2H, m), 6.36 (1H, s), 3.15 (1H, s, O*H*); ^{13}C NMR δ 140.7, 140.4, 133.1, 131.3 (2C), 130.2, 128.8, 128.5 (2C), 128.4 (2C), 128.3 (2C), 128.1 (2C), 127.8, 127.5, 126.0 (2C), 120.9, 77.2, 73.7, 66.5; IR (KBr) 3172, 1741, 1597, 1410, 1236, 1204, 1038, 962, 770; MS 351 (M+1, 100%), 333 (M-18, 76%); HRMS calc for $C_{24}H_{19}N_2O$ ($M+H^+$) 351.1497, found 351.1495.

(*Z*)-3-benzylidene-1,3-dihydro-1,7-diphenylimidazo[1,5-*c*]oxazole (3) : A round bottom flask was charged with K_3PO_4 (3 mg, 0.014 mmol). To this was added, by syringe, a solution of phenyl(4-phenyl-1-(2-phenylethynyl)-1*H*-imidazol-5-yl)methanol (**2**) (100 mg, 0.285 mmol) in 10 mL of CH_3CN. The reaction was held at reflux for 10 h, cooled to room temperature, diluted with 10 mL H_2O, and extracted with CH_2Cl_2 (3×15 mL). The organic extracts were combined, dried over Na_2SO_4, and evaporated by reduced pressure yielding a light yellow oil which was purified by flash chromatography (0 – 6% EtOAc in hexanes). Purification afforded (*Z*)-3-benzylidene-1,3-dihydro-1,7-diphenylimidazo[1,5-*c*]oxazole (**3**) (86 mg, 87%) as a white crystalline solid. R_f 0.44 (10% EtOAc in hexane); Mp 190-191 ºC; ^{1}H NMR δ 7.94 (1H, s), 7.54-7.39 (9H, m), 7.32-7.13 (6H, m), 6.71 (1H, s), 5.63 (1H, s); ^{13}C NMR δ 142.8, 135.3, 133.7, 133.2, 132.4, 130.1, 129.2 (2C), 128.6 (2C), 128.5 (2C), 128.3 (2C), 128.2, 127.3 (2C), 127.0,

126.0, 125.8, 125.5 (2C), 85.37, 82.37; IR (KBr) 1702, 1598, 1462, 1418, 1268, 1152, 1054, 950, 696 cm^{-1}; MS 351 (M+1, 100%), 119 (M-232, 39%), 233 (M-118, 38%), 352 (M+2, 34%); HRMS calc for $C_{24}H_{19}N_2O$ ($M+H^+$) 351.1497, found 351.1496.

1,6,8-triphenyl-8*H*-imidazo[5,1-*c*][1,4]oxazine (4) : To a round bottom flask was added by syringe a solution of $AuCl_3$ (0.6 ml of 0.01 M in CH3CN, 0.006 mmol) followed by the addition of a solution of phenyl(4-phenyl-1-(2-phenylethynyl)-1*H*-imidazol-5-yl)methanol (**2**) (101 mg, 0.29 mmol) in 10 mL of CH_3CN and the resulting solution was degassed by sparging with argon. The reaction was heated under reflux for 10 h, cooled to room temperature, diluted with H_2O (10 mL), and extracted with CH_2Cl_2 (3×15 mL). The organic extracts were combined, dried over Na_2SO_4, and evaporated by reduced pressure yielding a light yellow oil which was purified by flash chromatography (0–2% EtOAc in hexanes). Purification afforded 1,6,8-triphenyl-8*H*-imidazo[5,1-*c*][1,4]oxazine (**4**) (92 mg, 91%) as a white crystalline solid. R_f 0.53 (10% EtOAc in hexane); Mp 142-143 ºC; ^{1}H NMR δ 7.78 (1H, s), 7.6-7.51 (4H, m), 7.37-7.22 (11H, m), 7.10 (1H, s), 6.83 (1H, s); ^{13}C NMR δ 141.7, 137.5, 136.3, 133.8, 132.9, 132.5, 129.1, 129.0, 128.7 (2C), 128.6 (2C), 128.5 (2C), 127.7 (2C), 126.9, 126.2 (2C), 124.4 (2C), 118.2, 101.5, 74.2; IR (KBr) 1683, 1615, 1492, 1457, 1402, 1285, 1219, 743, 691 cm^{-1}; MS 351 (M+1, 100%), 352 (M+2, 19%), 350 (M, 5%) 245 (M-105, 4%); HRMS calc for $C_{24}H_{19}N_2O$ ($M+H^+$) 351.1497, found 351.1497.

6-phenylimidazo[1,2-*a*]pyrazine (6) : A round bottom flask was charged with $Cu(OTf)_2$

(14 mg, 0.038 mmol) to which a solution of 1-(2-phenylethynyl)-1*H*-imidazole-2-carbaldehyde (**5**)[21] (150 mg, 0.765 mmol) in 10 mL THF was added by syringe followed by the addition of a 2 M solution of ammonia in methanol (0.42 mL, 0.842 mmol). The mixture was heated under reflux for 12 h, allowed to cool to room temperature, diluted with H_2O (10 mL), and extracted with EtOAc (3×20 mL). The organic extracts were combined, dried over Na_2SO_4, and the solvents removed under reduced pressure. The residual yellow oil was purified by flash chromatography (0–25% EtOAc in hexanes) to afford 6-phenylimidazo[1,2-*a*]pyrazine (**6**) (114 mg, 76%) as a colorless crystalline solid. R_f 0.32 (75% EtOAc in hexane); Mp = 107-108 ºC; ^{1}H NMR δ 9.12 (1H, d, *J* = 1.6 Hz), 8.38 (1H, d, *J* = 1.6 Hz), 7.87-7.84 (2H, m), 7.75 (1H, s), 7.67 (1H, s), 7.44-7.34 (3H, m); ^{13}C NMR δ 143.2, 140.0, 139.8, 136.3, 136.0, 129.0 (2C), 128.8, 126.3 (2C), 115.0, 113.9; IR (KBr) 1521, 1485, 1459, 1439, 1325, 1317, 1144, 917, 778, 691; MS 196 (M+1, 100%), 195 (M, 12%), 194 (M-1, 1%); HRMS calc for $C_{12}H_{10}N_3$ ($M+H^+$) 196.0875, found 196.0878.

8-ethoxy-6-phenyl-8*H*-imidazo[2,1-*c*][1,4]oxazine (7) : A round bottom flask was charged with $Cu(OTf)_2$ (9 mg, 0.025 mmol) to which 1-(2-phenylethynyl)-1*H*-imidazole-2-carbaldehyde (**5**)[24] (100 mg, 0.51 mmol) in ethanol (10 mL) was added by syringe. The reaction was held at reflux for 4 h then allowed to cool to room temperature. H_2O (10 mL) was added, the mixture extracted with CH_2Cl_2 (3 × 15 mL), the organic extracts were combined and dried over Na_2SO_4, and the solvents removed under reduced pressure. The residual yellow oil was purified by flash chromatography (0–25% EtOAc in hexanes)

to afford 8-ethoxy-6-phenyl-8*H*-imidazo[2,1-*c*][1,4]oxazine (**7**) (120 mg, 97%) as a white

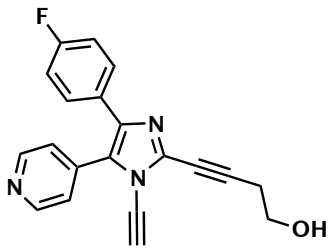
crystalline solid. R_f 0.51 (25% EtOAc in hexane); Mp = 86-87 ºC; ^{1}H NMR δ 7.63-7.61 (2H, m), 7.41-7.34 (3H, m), 7.14 (1H, d, *J* = 1.1 Hz), 7.09 (1H, s), 7.02 (1H, d, *J* = 1.3 Hz), 6.36 (1H, s), 4.05 (1H, quint, *J* = 11.9, 9.4 Hz), 3.87 (1H, quint, *J* = 11.9, 9.4 Hz), 1.25 (3H, t, *J* = 7.1 Hz); ^{13}C NMR δ 141.0, 137.4, 132.3, 129.6, 129.0, 128.7 (2C), 124.4 (2C), 115.4, 102.0, 94.6, 64.4, 15.2; IR (KBr) 3410, 3121, 2990, 2446, 1684, 1497, 1454, 1308, 1202, 1088, 1018, 970; MS 243 (M+1, 100%), 197 (M-46, 38%), 165 (M-78, 23%); HRMS calc for $C_{14}H_{15}N_2O_2$ ($M+H^+$) 243.1134, found 243.1137.

6-phenylimidazo[1,2-*a*]pyrazine 7-oxide (8) : A two-neck round bottom flask was charged with 1-(2-phenylethynyl)-1*H*-imidazole-2-carbaldehyde (**5**)[24] (50 mg, 0.25 mmol) to which was added by syringe DMF (10 mL) then hydroxylamine hydrochloride (53 mg, 0.76 mmol). The solution was stirred at room temperature for 14 h then diluted with H_2O (5 mL) and extracted with CH_2Cl_2 (3×15 mL). The organic extracts were combined, dried over Na_2SO_4, and evaporated by reduced pressure yielding a yellow oil that was purified by flash chromatography (50% EtOAc in hexanes). Purification afforded 6-phenylimidazo[1,2-*a*]pyrazine 7-oxide (**8**) (37 mg, 71%) as a yellow oil. ^{1}H NMR δ 8.75 (1H, s), 8.08 (1H, s), 7.73 (1H, d, *J* = 1.4 Hz), 7.65-7.59 (3H, m), 7.48-7.44 (3H, m); ^{13}C NMR δ 160.6, 156.8, 156.7, 149.4, 149.3, 148.4, 147.9, 147.2, 139.7, 133.1; IR (neat) 3380, 3081, 3059, 3025, 2924, 2850, 1694, 1600, 1492, 1450, 755, 698 cm^{-1};

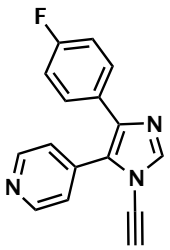
MS 212 (M+1, 90%), 196 (M-15, 100%); HRMS calc for $C_{12}H_{10}N_3O$ ($M+H^+$) 212.0824, found 212.0823.

Chapter 3: Covalent adduction of *N*-alkynylazoles to p38α

It has been previously shown that the *N*-alkynylazole **1** undergoes covalent adduction to p38α at the D-recruitment site.



1



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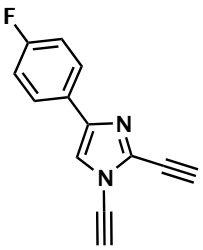

3

To determine the rate of this covalent adduction, **3** (25 μM) was incubated with p38α (5 μM) and aliquots were removed at various time points followed by "click" chemistry and in-gel fluorescence SDS-PAGE analysis. Quantification of the fluorescent bands' intensity as a function of time was fit to a first-order rate equation [$P = P_{max}(1-e^{-k't})$] to give a pseudo-first order rate for covalent adduction $k' = 0.11\ h^{-1}$. One issue that arose when studying the kinetics of the adduction of p38α by **3** was the possibility of adduction of p38α by **3** during the click reaction. In order to address this, we have carried out parallel experiments in which samples from adduction reactions were either directly treated under click reaction conditions, or first subjected to spin-column purification to remove unadducts **3** and then treated under click reaction conditions. There was no significant difference in the adducts formed under the two different conditions. Presumably, the click reaction of unadducted **3** is fast relative to adduct formation, and once unadducted **3** undergoes click reaction, it is no longer able to form adducts with p38α.

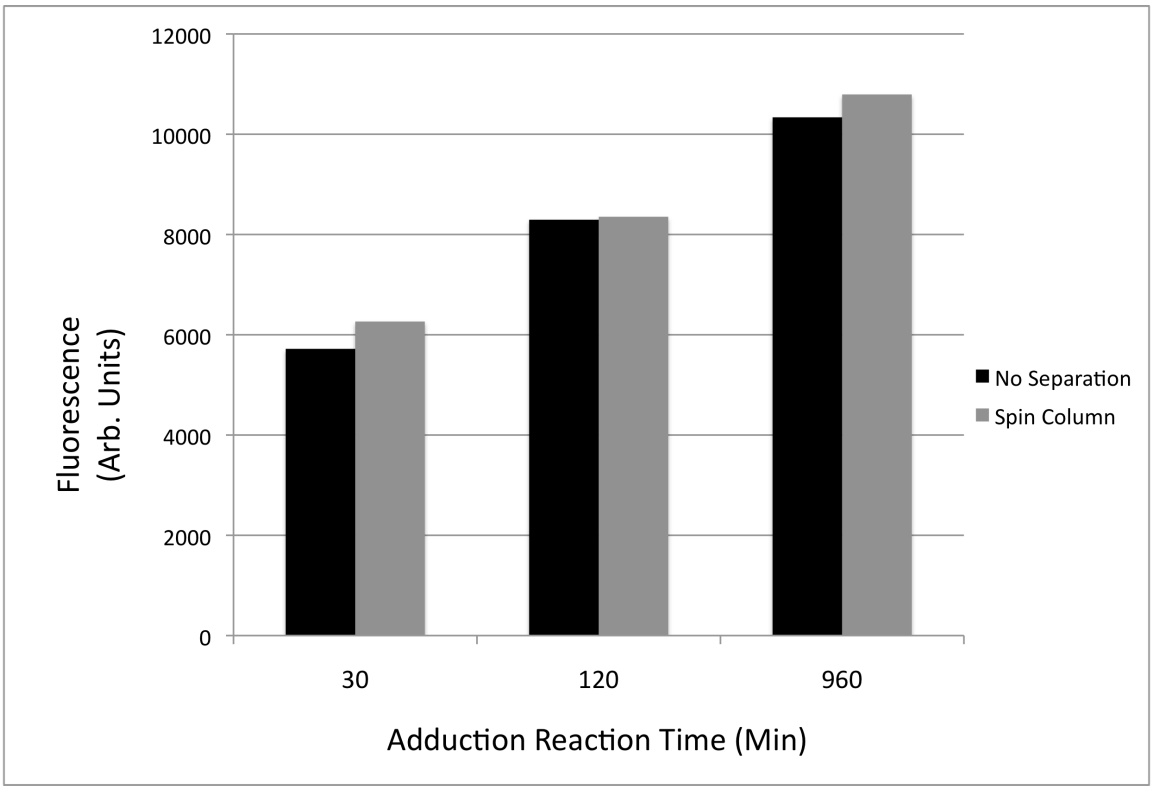

Fig.3.1 Comparison of p38α adduction by compound **3** with and without removal of non-adducted **3** prior to click chemistry. Black bars: Fluorescence intensity of bands from in-gel fluorescence SDS-PAGE analysis of click reactions run directly on adduction incubations containing 5 μM p38α and 100 μM **3** at 30, 120, and 960 min. Gray bars: Adduction reactions containing 5 μM p38α and 100 μM **3** at 30, 120, and 960 min were first subjected to spin column removal of unadducted **3** and then subjected to click reaction.

At higher concentrations of **3** (up to 200 μM), no rate saturation was observed. Thus, the non-covalent association of **3** with p38α is rather weak.

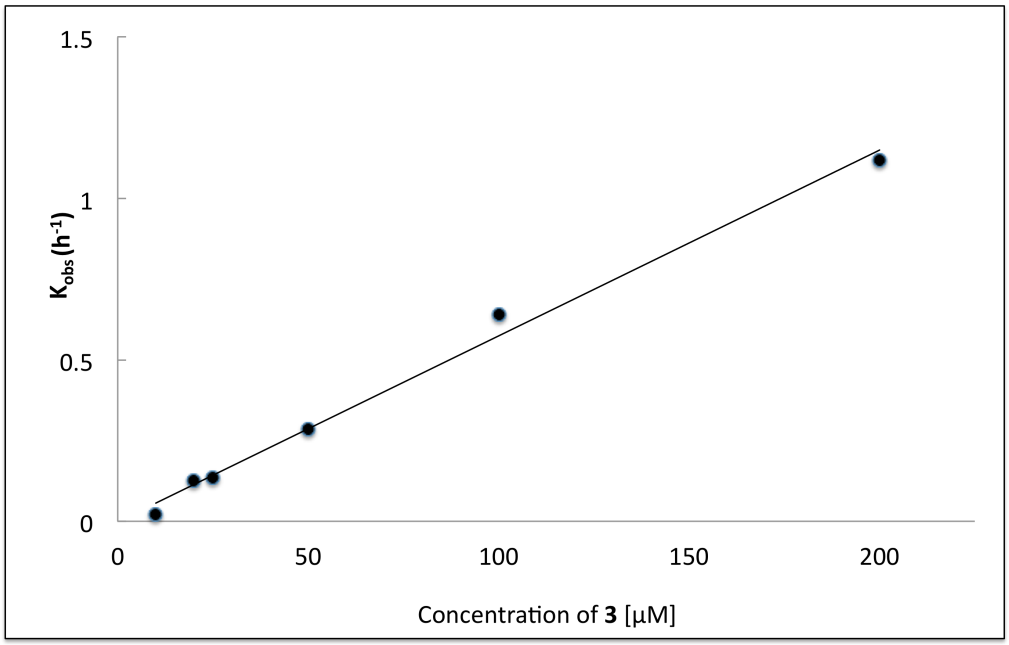

Fig.3.2 Concentration dependence of the observed pseudo-first order rate of p38α adduct formation by **3**.

Despite this, **3** is selective for adduction of Cys119, as demonstrated by competition experiments with **2**. A combination of weak binding at the docking site and kinetic preference for Cys119 adduction by the *N*-alkynylimidazole moiety may explain the selectivity of **3**. Also, the adduction of p38α by **3** is selectively blocked by ligands that bind to the recruitment site of the kinase.

We employed a fluorescent-based assay using probe **3** to screen for natural products that similarly recognize the DRS of p38α. Incubation of p38α (5μM) with **3**

(100 μM) was carried out in the presence of individual members of a small library of plant polyphenol natural products and synthetic analogs, each at 200 μM, for 16 h. The reaction mixtures were subjected to click reaction conditions and in-gel fluorescence analysis, and the inhibition of adduct formation by **3** was determined by quantification of the fluorescence bands.

Experimental

Adduct formation with DAIm 3, click reaction, and in-gel fluorescence PAGE analysis: Reaction mixtures (100 μL) containing 5 μM p38α, 100 μM **3** in 50 mM HEPES, pH 7.5, 1 mM EGTA, 2 mM DTT, 10 mM $MgCl_2$ were incubated at 25 °C for 16 h. Without further purification, aliquots of this reaction mixture were subjected to click reaction. To reaction mixtures containing 25 ng of p38α in 50 mM potassium phosphate buffer were added stock solutions of $CuSO_4$ (0.5 μL, 50 mM), tris(2-carboxyethyl)phosphine (0.5 μL, 50 mM), Alexa-594 azide (0.5 μL, 2.5 mM), and tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (1.65 μL, 1.5 mM). The click reaction was allowed to proceed at 25 °C for 16 h, quenched by the addition of 2X SDS loading buffer and heat inactivation at 95 °C for 10 min. The samples were analyzed by 10 % SDS PAGE. The gel was scanned by Typhoon Trio from GE healthcare and the data were analyzed by Image J software.

Competition Assay: Reaction mixtures (100 μL) containing 5 μM p38α , 100 μM **3,** and 100 μM test compound in 50 mM HEPES, pH 7.5, 1 mM EGTA, 2 mM DTT, 10 mM $MgCl_2$ were incubated at 25 °C for 16 h. Without further purification, aliquots of this

reaction mixture were subjected to click reaction and the samples were analyzed as shown above. The inhibition of adduct formation by **3** was determined by quantification of the fluorescence bands

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