Potent Anticancer and Anti-inflammatory YD-3 Drug Synthesis: A Novel and Expedient Two Step Approach

I. Introduction

Life on this planet is built upon chains of carbon atoms which serve as the backbones of organic molecules that keep us alive and functioning. Nature has masterfully arranged these carbon-based molecules in ways that give living things their basic functions and structures. Organic chemistry has given scientists the limited option to mimic the creation of these carbon-based molecules. As advantageous and useful this may sound, there are still many challenges to overcome when it comes to synthesizing organic substances.

The use of noble metal catalysis has transformed the chemical industry. Of relevance to this study, since the discovery of Pd catalyzed cross-coupling reactions in the 70s [1-3], organic synthesis has experienced an unprecedented improvement in percent yield, milder reaction conditions, and reagent cost. The Mizoroki-Heck reaction[4-5], specifically, revolutionized the olefination process and opened up organic chemistry to a myriad of possibilities that would not have been possible before. Tsutomu Mizoroki and Richard F. Heck both independently used palladium catalysts to stitch together the cores of some complex organic molecules. Olefination in this context is the breaking of the carbon hydrogen bond to forge a new carbon-X molecule in which X can be oxygen, sulfur, nitrogen, carbon or a halogen. The importance of this breakthrough should not be understated. Before Mizoroki-Heck olefination, it was virtually impossible to meticulously break C-H bonds and attach new functional groups to that carbon. Because of this momentous discovery, the possibilities of new drugs that could be synthesized completely revolutionized and propelled modern medicine.

Due to the ubiquitous presence of C-H bonds in nature, this new olefination process is extremely useful especially in the pharmaceutical industry. However, there were some major issues with this technique: the selectivity of the olefination was awful, meaning that chemists were unable to target a specific C-H bond in a large molecule for modification; this made accurate synthesis rather difficult. However, the recently discovered ligand-mediated metal catalyst olefination fixed much of the selectivity dilemma [6-15]. By attaching organic ligands to the catalyst complex, it is considerably easier for chemists to manipulate the organic molecule to selectively execute C-H olefination. This distinct, novel method has paved the way for more advanced drug making and has made my personal drug synthesis experiment possible.

In recent years, the indazole molecule (Fig 1a) has proved to be effective in treating the most abominable diseases [16-27]. The practicality of indazole can be seen mainly from its derivatives, which demonstrate broad varieties of bio-active functions [28]. Indazole derivatives have been proven to have anti-inflammatory [29], anti-tumor [30], anti-HIV[31], anti-cancer [32], and anti-platelet activities [33], plus serotonin5-HT3 receptor antagonist activity[34], making it a medicinally valuable compound. This practicality inspired this study that was designed to look deeply into the indazole subject. Currently, the main challenge with C-H olefination in indazoles is the

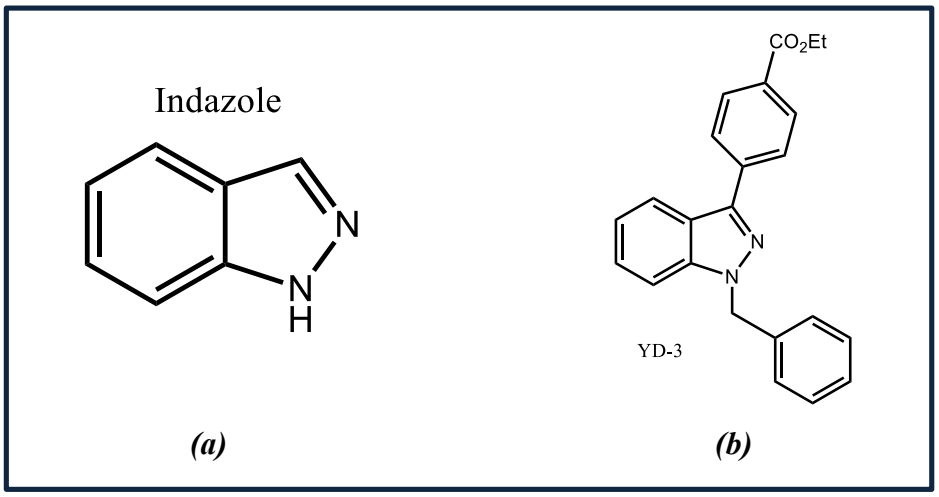


Figure 1 The molecular structure of Indazole (a) and YD-3(b)

unstable reaction mechanism caused by the adjacent N-N atoms which is why indole (an indazole derivative where there is only 1 nitrogen) is so much simpler to manipulate. However, with knowledge on the recent ligand mediated C-H olefination on pyridines (having a 5 carbon with 1 nitrogen ring structure), even the unstable derivatives of indazole could possibly be synthesized.

1-benzyl-3(ethoxycarbonylphenyl)-indazole (Figure 1b) is a new synthetic indazole derivative. It has been demonstrated to inhibit platelet aggregation in the blood stream and to hinder cell proliferation caused by the protein thrombin [35-36]. YD-3 is extremely useful given its thrombin inhibiting effects. Thrombin is a serine protease protein that is responsible for important parts in the coagulation cascade (blood clotting system) [37] and in angiogenesis (growth of new blood vessels from pre-existing ones). This specialized function offers a notably effective treatment in controlling cancer and cardiovascular disease symptoms[38]. Due to the fact that thrombin causes contents in the blood to coagulate, inhibiting it is beneficial for people with heart disease in that it drastically lowers the chance of further blood vessel blockage and coagulation in key parts of the cardiovascular system. Furthermore, since YD-3 inhibits thrombin, it also inhibits local angiogenesis [39], making it a direct anticancer product. Growing tumors rely on new blood vessels to supply nutrients for the cancerous cells to live and to proliferate. Since YD-3 inhibits thrombin which in turn inhibits angiogenesis, the growth of the cancer cells could be stunted or even be forced to decline. For YD-3 to be able to effectively treat the top two killer diseases in America, the aim of this study is to create an economically viable synthesis route targeted toward facilitating YD-3's widespread availability.

The traditional way of synthesizing YD-3 is not only ineffective but extremely expensive [16, 40-41]. Their methods depend on old and banal reaction procedures such as refluxing (distillation

technique) which prove to be time consuming and very limited. Previously, the synthesis of YD-3 required a vapid multi-step procedure while still having low yields. Most importantly, to modify any intermediate molecule, it would require beginning at step one and re-initiating the synthesis process, making the old method inefficient and tedious. The reaction pathway in Figure 2 illustrates the intimidating 8 step procedure.

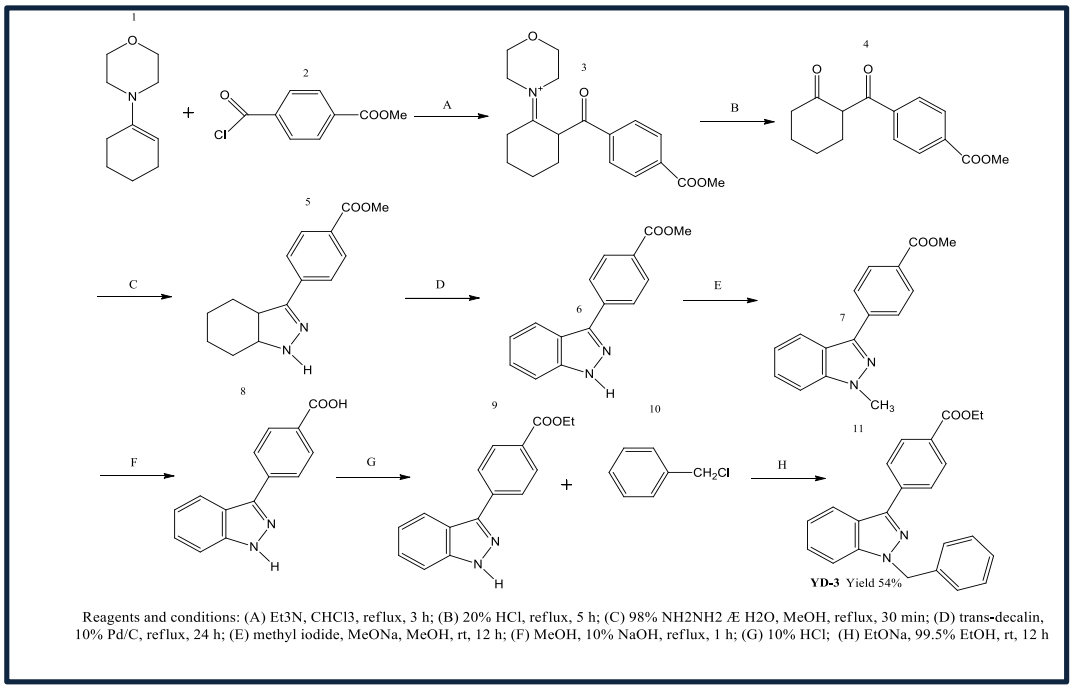


Figure 2. Old step by step synthesis of YD-3

To make YD-3 cheaper and more accessible commercially, this experimental study proposed a simple 2 step plan to synthesize the drug. First, the procedure starts with commercially available 97% indazole. Next, the constituents of YD-3 are stitched using a hybrid of the old SN2 substitution method and the new pyridine C-H olefination method, recently discovered. Each YD-3 constituent requires one step in the synthesis procedure. This allows formulation of the drug in 2 easy steps. The financial and material resources saved during the synthesis process will benefit not only the manufacturing of synthetic YD-3 but also ameliorate

the entire indazole synthesis procedure. Once this expedient 2 step approach is adopted, the novel ligand mediated, Pd-catalyzed C-H olefination process will completely change indazole drug synthesis making it more efficient than ever.

II. Materials and Methods

A. Basic Procedures

All materials used in the experiment were commercially available and handled in standard atmospheric conditions under fume hoods. The chemicals were used without further purification or any modification. A full list of equipment includes: 50mL/100mL/250mL round bottom flasks, 250 mL Erlenmeyer flasks, chromatography columns, hot plate stirrers, magnetic spin bars, electronic balance beams, glass funnels, medal clamps, oil pumps, rotary evaporators (rotovaps), vacuums, vacuum filter funnels with celite, vials, UV lamps, thin layer chromatography (TLC) plates, sep funnels, graduated cylinders, glass pipets, 20μL/50μL glass syringes, 1mL/5mL disposable syringes, and test tubes.

For reactions in this experiment, the general procedure for reaction setup was, for the most part, identical. All reactions were run under 1 atm air in sealed, high-pressure-rated test tubes. The tubes were heated in hot plate warmed oil baths calibrated by an external thermometer and agitated with a magnetic stirrer. Prior to starting the reactions, the plate was turned on and the oil bath was allowed to equilibrate to the desired temperature for half an hour. While the oil bath was being heated, the solid reagents (usually indazole/indazole derivative, base, metal catalyst, and ligand) were weighed and loaded into the tube along with a magnetic spin bar. After the temperature of the bath stabilized to the appropriate degree, the liquid ligand and solvent were loaded into the test tube via syringes. The loaded tube was tightly capped and immediately

placed above a magnetic stirrer to mix the contents for five minutes. Finally, the sealed tubes were allowed to react in the warmed oil bath for the set time period.

The workup of the products was relatively simple and straightforward. The contents of the tube were diluted and washed with ethyl acetate and poured through a celite vacuum filter into a 50 mL round bottle. The residue on the side of the tubes was collected via several ethyl acetate rinses and subsequent scraping with a metal rod. After pouring the entirety of the contents in the post-reaction tube into the celite filter, the resulting solution was concentrated via a rotovap (solvent removal via evaporation in vacuum). Then, depending on the procedure of the particular reaction, the concentrated solution was subjected to further purification and concentration.

To analyze products, 1H nuclear magnetic resonance (NMR) spectra were recorded on Bruker DRX-600, Bruker DRX-500, Varian Inova-400, Bruker AMX-400, and Varian Mercury-300 instruments. All were equipped for multi-nuclear 1D and 2D NMR capabilities along with offline data processing (MestReNova: NMR graph reading software). $CDCl_3$ was used as the solvent for all NMR samples. For yield determination, CH_2Br_2 was used as an internal standard (mostly to find yields for the condition optimization, catalyst, and ligand loading tests). The yields of products in the actual drug synthesis process were determined via stoichiometric calculation based on the weight of the products.

B. YD-3 Synthesis

The actual synthesis of the YD-3 drug molecule had 2 basic steps: (1) the SN2 reaction of indazole and benzyl bromide, and (2) the Pd-catalyzed arylation of the resulting indazole derivative from step 1.

For the first SN2 step of the synthesis, the phenyl group in the benzyl bromide molecule was substituted with the N-H bond in indazole (as seen by the red and green bonds in Fig. 3). To carry out this SN2 reaction, indazole was first added to the DMF (dimethylformamide) solvent in a 50mL round bottom flask along with potassium carbonate to be stirred into a near homogenous solution for 30 minutes at room temperature. Next, the reactant benzyl bromide was injected into the solution via a 3mL syringe. The round bottle was then placed in a 60 degree Celsius oil bath and left to stir for 12 hours (see figure 3).

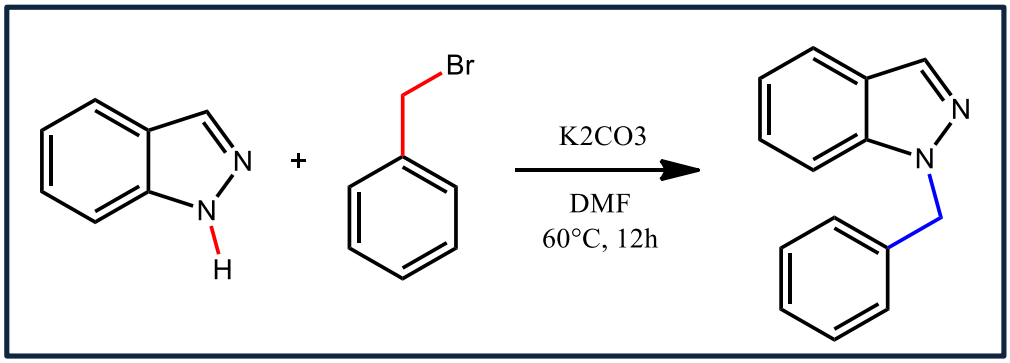


Figure 3. YD-3 Synthesis Step 1:Making the indazole derivative.

After the solution was left to react for half a day, the contents of the flask were poured through a standard vacuum filter to remove the potassium carbonate. The resulting solution contained the desired 1-benzyl-1H-1,2-benzodiazole ($C_{14}H_{12}N_2$) and its isomers. To isolate the desired product, the solution was concentrated via vacuum (rotovap) and ran through a 6:1 (ethyl acetate: hexane) solvent column chromatography. After product solution was separated into 20 test tubes, a thin layer chromatography (TLC) analysis was run on each tube to determine which ones had the desired product and how well the chemicals of the solution separated. After the tubes with the pure, desired product were identified, the contents of those tubes were poured into another 50 mL flask and concentrated via rotovap until all solvent was removed. Finally a 1H

NMR was run on the concentrated product to confirm the purity and structure of the product molecule. After confirmation on purity, the sample from the NMR tube was poured into a flask so that the $CDCl_3$ solvent could be eliminated and the final product could be weighed for yield calculation.

Reaction 2 was then started with the pure product from reaction 1 added to the corresponding reagents in this Pd-catalyzed reaction. Reaction setup was same as the general procedure mentioned above: all solid reagents (Cs_2CO_3 base, phenanthroline ligand, $Pd(OAc)_2$ catalyst) were loaded first into a sealable reaction test tube then all liquid reagents (reaction 1 product, Ethyl 4-iodobenzoate reactant, BTB solvent) were added. The contents were stirred for five minutes before being placing the test tube in a 160 degree Celsius oil bath for 48 hours. (See figure 4)

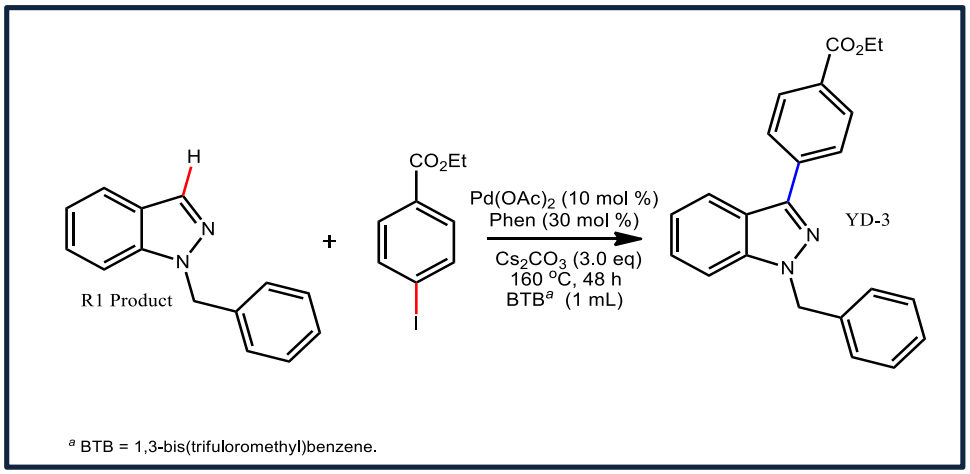


Figure 4. Second and final step synthesis of YD-3.

The workup for reaction 2 agreed with the basic workup procedures. The mixture of products after reaction 2 completed was first diluted/washed with ethyl acetate and then filtered using a celite/vacuum filter system. The resulting solution was then concentrated via rotovap. Next, the concentrated solution was analyzed using TLC plates using a 4:1 ethyl acetate: hexane

solvent. After inspection of the separation on the TLC plates, the decision to use the thin layer chromatography separation method was employed. After the TLC was run (ran 3 times using the same 4:1 ratio solvent), the silica of the product layer was scraped into a vial and then soaked with ethyl acetate. After stirring for 10 minutes (to dissolve all product still attached to the silica), the contents were filtered. The resulting solution was then concentrated and analyzed with 1H NMR. After purity was confirmed, the final product, YD-3, was poured into a vial and vacuumed to remove all solvents, leaving the final pure drug product.

C. Acknowledgements

All reaction procedures (setups, workups, etc) were done by me. During NMR analysis, specialists helped me obtain the mass spectroscopy and print the graphs. Also, the SciFinder research tool was introduced to me by lab staff through which I discovered the mechanics and possibilities of SN2 and Pd catalyzed reactions through literature referenced on the last page. As for lab procedures, lab staff instructed me on basic organic chemistry lab procedures such as TLC plating, reaction loading, rotovap use, and NMR tube preparation before I independently executed the methods.

III. Results

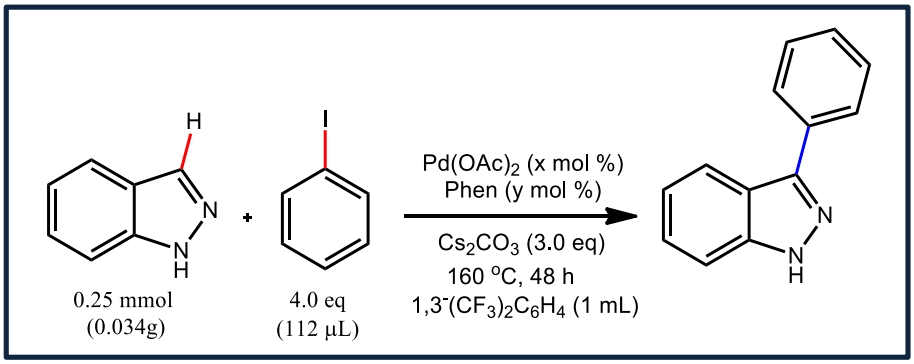
A. Testing the Catalytic Role

To test the extent of the effect that the catalyst palladium (II) acetate has on the second step reaction mechanism, reactions loaded with different amounts of catalyst were carried out to test for the catalytic role. Additionally, the test was used to identify the optimal amount of catalyst needed for the more efficient conversion from reagents to products for step 2 of YD-3

synthesis (step 1 requires no catalyst). The reaction procedure follows that of the general procedure described in the experimental section. The reactions were run in sealed tubes bathed in 160 degree Celsius oil with varying amounts of $Pd(OAc)_2$ and Phen with fixed amounts of base and solvent. Yields were determined by 1H NMR using CH_2Br_2 as an internal standard. The details and results of the reaction are listed in Table 1 below.

Evidently both $Pd(OAc)_2$ and Phen play vital roles in the reaction which explains the 0% yield obtained in entries 1, 2, 3 and 4. When both 10 mol% $Pd(OAc)_2$ and 10 mol% Phen (catalyst and ligand) were mixed, a 35% yield was achieved as calculated from NMR data (Figure 4). Further increasing $Pd(OAc)_2$ to 30 mol% has no large amplification effect on yield, indicating catalytic reaction to be the most efficient at 10 mol% $Pd(OAc)_2$.

Table 1. Testing the Catalytic Role: Conditions and Optimization.



Experiment	Entry	$Pd(OAc)_2$ (xmol %)	Base (3.0 eq)	Solvent (1mL)	Temperature (°C)	Phen (ymol %)	Yield (%)
Catalytic Test	1	0	Cs_2CO_3	1,3-$(CF_3)_2C_6H_4$	160	0	0
	2	0	Cs_2CO_3	1,3-$(CF_3)_2C_6H_4$	160	10	0
	3	0	Cs_2CO_3	1,3-$(CF_3)_2C_6H_4$	160	30	0
	4	10	Cs_2CO_3	1,3-$(CF_3)_2C_6H_4$	160	0	0
	5	10	Cs_2CO_3	1,3-$(CF_3)_2C_6H_4$	160	10	35
	6	30	Cs_2CO_3	1,3-$(CF_3)_2C_6H_4$	160	10	38

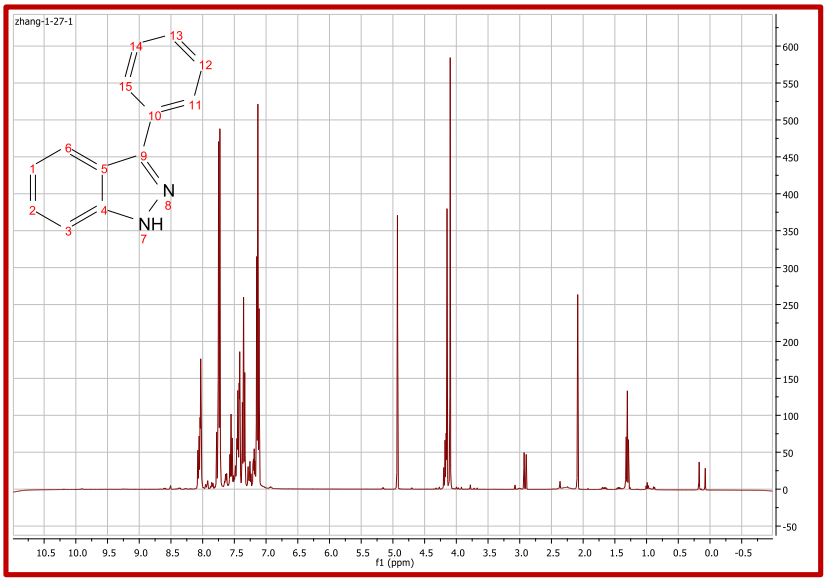
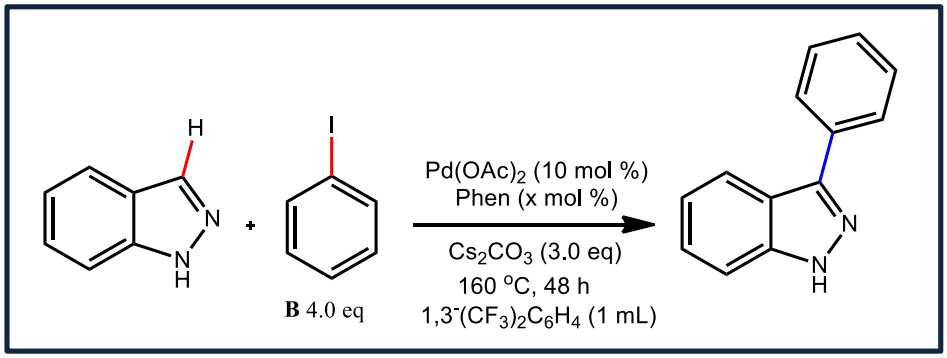


Figure 4. NMR graph of the product of the catalyst test with CH_2Br_2 as an internal standard to calculate yield.

B. Optimizing Ligand Loading

Similar to the catalytic tests, the ligand loading test was designed to explore the general role and effect of ligand amount in and on the reaction mechanism. Just like the catalyst test, these ligand loading reactions were used to observe which amount was optimal for reaction efficiency and high yield. Catalyst, base, solvent, and reactant amounts were kept constant so that the only independent variable was the amount of ligand loaded. Table 2 lists the full details of the reaction conditions. Yield was determined through 1H NMR mass spectroscopy and the same internal standard. As seen from the table, yield initially increases with increasing amount of ligand, but flattens out at 94% with 30 mol% ligand (Entry 9). The table also shows a subtle change between 30 mol% and 40 mol% ligand (Entry 9 and 10), indicating that 30 mol% is the most effective ligand amount.

Table 2. Testing the Ligand Loading: conditions and optimization.



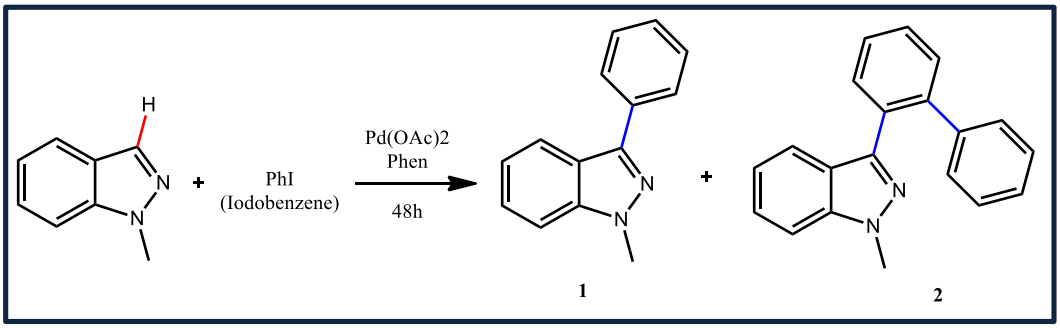
Experiment	Entry	$Pd(OAc)_2$ (mol %)	Base (3.0 eg)	Solvent (1mL)	Temperature (°C)	Phen (xmol %)	Yield (%)
Load test	7	10	Cs2CO3	1,3-$(CF_3)_2C_6H_4$	160	10	38
	8	10	Cs2CO3	1,3-$(CF_3)_2C_6H_4$	160	20	66
	9	10	Cs2CO3	1,3-$(CF_3)_2C_6H_4$	160	30	94
	10	10	Cs2CO3	1,3-$(CF_3)_2C_6H_4$	160	40	88

C. Optimization of Reaction Conditions

Further extensive screening on reaction conditions (amount of reagents that maximizes yield) proved that an appropriate base was critical to the second step of YD-3 synthesis (as showed at Table 3). Among a set of screened bases, K_3PO_4, CsF and Cs_2CO_3 worked well with relatively high yields. It was found that Cs_2CO_3 (entry 11) resulted in the best yields as the base with a total desired yield of 51%.

Furthermore, subsequent experiments showed that the solvent also affects the reaction yield in this arylation reaction. Further solvent screening showed the 1,3-bis(trifluoromethyl)benzene (BTB) was the best solvent, giving a 94% product yield. Through these screening tests, the conclusion was that a combination of Cs_2CO_3 base with BTB solvent gives the most efficient mechanism and optimal reaction conditions.

Table 3. Screen test for optimal reaction conditions.



Experiment	Entry	$Pd(OAc)_2$ (mol %)	Base (3.0 eg)	Solvent (1mL)	Temperature (ºC)	Phen (mol %)	Yield (%) 1(2)
Base Test	11	10	Cs2CO3	1,3-$(CF_3)_2C_6H_4$	160	30	51(14)
	12	10	CsF	1,3-$(CF_3)_2C_6H_4$	160	30	40(2)
	13	10	K3PO4	1,3-$(CF_3)_2C_6H_4$	160	30	37(6)
	14	10	K2HP4	1,3-$(CF_3)_2C_6H_4$	160	30	3(0)
Solvent Test	15	10	Cs2CO3	PhCl	160	30	89(3)
	16	10	Cs2CO3	PhH	160	30	85(3)
	17	10	Cs2CO3	PhCF3	160	30	87(0)
	18	10	Cs2CO3	1,3-$(CF_3)_2C_6H_4$	160	30	94(6)

D. Synthesis of the YD-3 Drug Molecule

The various test/optimization experiments have demonstrated that a 10 mol% $Pd(OAc)_2$ (0.006g), 30 mol% Phen (0.014g), 3 equivalents of Cs_2CO_3 base (0.25g), and 1 mL 1,3-bis(trifluoromethyl)benzene solvent gives the best yield for the Pd catalyzed reaction responsible for step 2 of YD-3 synthesis.

The synthesis of YD-3 requires 2 main steps as explained in the experimental section B: first the SN2 substitute reaction that adds a phenyl group to nitrogen 1 on indazole (Figure 5) and second the Pd-catalyzed reaction that is mediated by organic ligands which results in final arylation to form the YD-3 molecule.

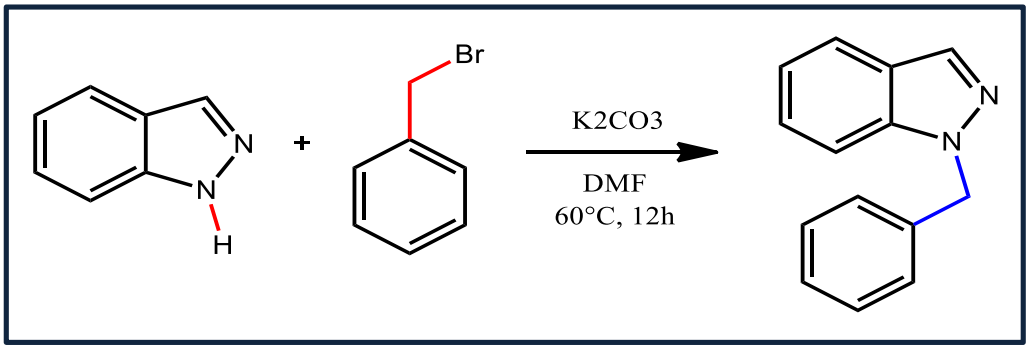


Figure 5. YD-3 synthesis: first step, reaction scheme.

In step 1, the bimolecular nucleophilic substitution (AKA SN2) reaction expels the electron-deficient group while replacing it with substituted electrophilic center (phenyl group in this case) in one step. In other words, benzyl bromide experiences nucleophilic substitution and form a new bond with indazole at the C1 site (see red line in Figure 5). Below is the NMR spectrum (Figure 6) for the SN2 reaction with a calculated yield of 60%.

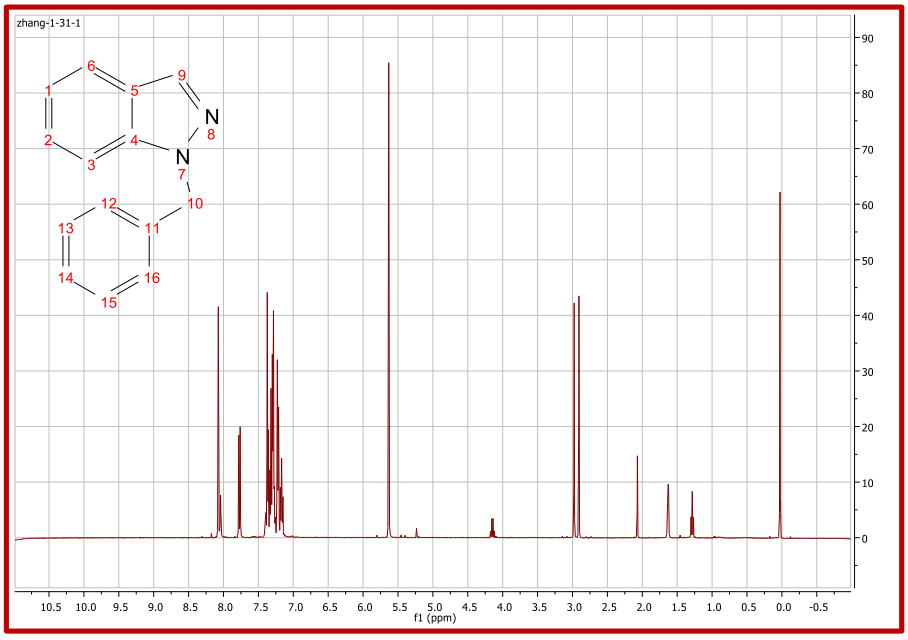


Figure 6. YD-3 synthesis: first step, NMR spectrum.

For the second catalytic reaction to form YD-3, the optimal conditions (as mentioned before) for the reaction were used. Figure 7 shows the NMR results from the complete setup and Figure 8 shows the complete reaction scheme along with yield.

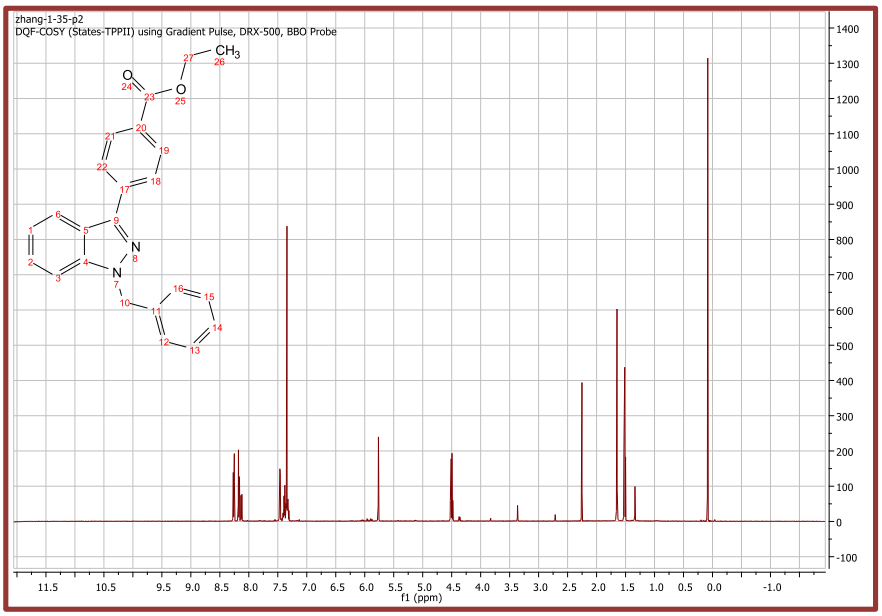


Figure7. YD-3 synthesis: second and last step, 64% yield determined by 1H NMR using CH_2Br_2 as an internal standard.

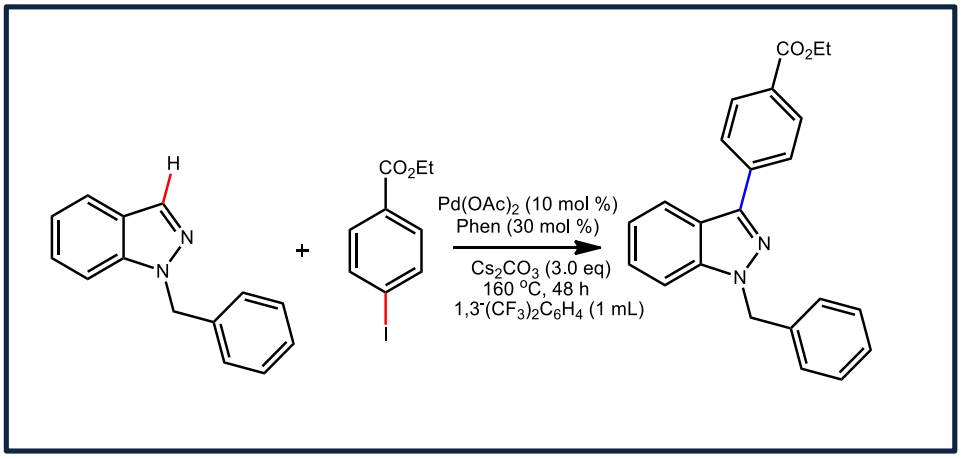


Figure 8. YD-3 Drug Synthesis second and last step, reaction scheme

After concentration and purification to isolate YD-3, a total of 0.05g of pure product was recovered. Taking into consideration to overall miniscule scope of the reactions in this experiment, this outcome is extremely successful. The drug yield of this last reaction was determined to be 64%.

IV. Discussion

First step in YD-3 synthesis can be explained by an interchange mechanism as showed in Figure 9. The reaction process is a type of nucleophilic substitution, where a lone pair of electrons from a nucleophile attacks an electron deficient electrophilic center and forms a chemical bond while expelling another group, the leaving group. Thus the incoming group replaces the leaving group in one step. Since two reacting species are involved in the slow, rate-determining step of the reaction, this leads to the name bimolecular nucleophilic substitution.

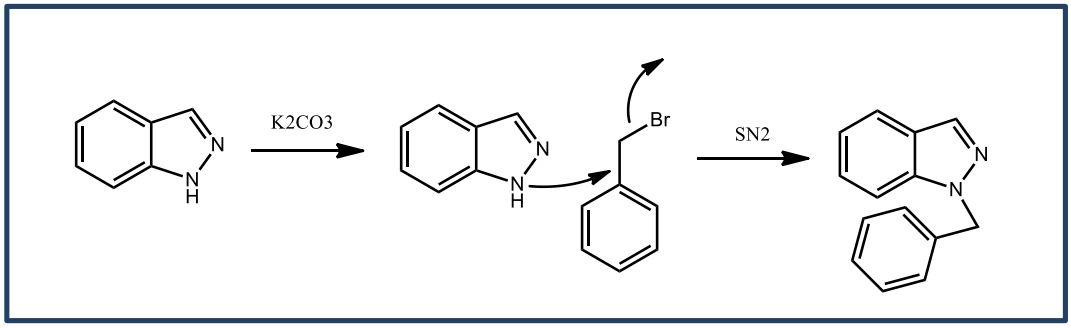


Figure 9. YD-3 synthesis, first reaction mechanism

The second step in YD-3 synthesis involves C-H activation. As shown in Figure 10, indazole first coordinates to the Pd metal center through its sp2 N atom. Then, C-H activation occurs to generate the active indazole-Pd intermediate. After oxidative addition of this intermediate with aryl iodide and reductive elimination, the reaction produced YD-3. During this process, the

ligand mediates the coordination and dissociation of indazole, further affecting the activity of the C-H activation step. Due to the unstability of indazole, the solvent selection is extremely

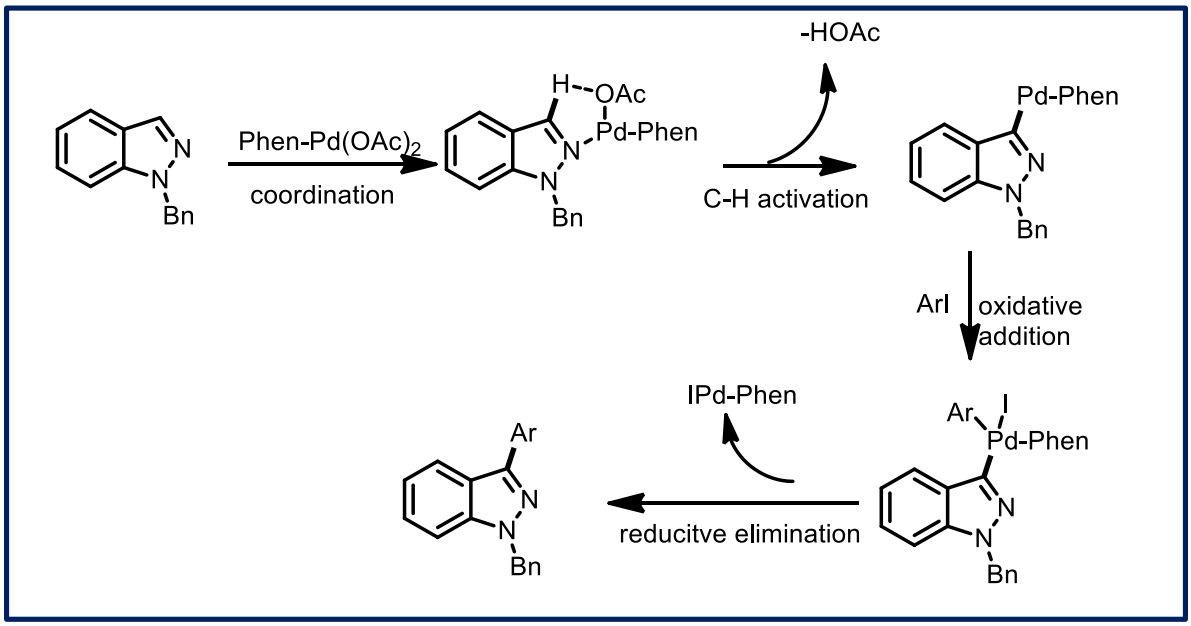


Figure 10. Reaction mechanism of direct C-H activation of indazole.

important. In polar solvents, such as DMF and DMSO, indazole decomposes easily and always gives low material balance. On the other hand, non-polar solvents including toluene, chlorobenzene and 1,3-bis(trifluoromethyl)benzene worked well with the arylation reaction complex, thus giving good yields.

V. Conclusion and Future Work

In summary, for the first time a Pd-catalyzed C-H arylation protocol was used for the arylation of indazoles by using a novel $Pd(OAc)_2$-Phen catalyst system. Our study has verified

high levels of reactivity with this catalyst as a result of the strong bidentate coordination of the Phen ligand, which would destabilize the N-bound coordination mode of the indazole substrates with Pd. The enhanced dissociation rate would increase the local concentration of the indazole substrate around the Pd catalyst with various orientations with respect to the Pd center. The N-bound indazole substrate dissociates from Pd, assisted by the ligand. Indazole subsequently reorients itself to bind to Pd through the π system, which triggers C-H activation to form the aryl-Pd(II) species. This finding provides a new approach for developing Pd-catalyzed C-H functionalization of indazoles, which is previously considered to be nonactive. As an application of the new approach, YD-3 has expeditiously been synthesized only in two steps, in comparison with conventional 5 steps synthesizes. The protocol established in this study paves a path for Indazole motif related drug synthesis consistent with both economic and environmental constraints. Based on this reaction protocol, further reaction investigation, such as olefination with olefins, carbonylation with carbon monoxide, and amination with amines or amides would be possible. All the potential reactions will greatly facilitate the indazole related drug development.

References

(1) B. M. Trost, S. A. Godleski, J. P. Genet, J. Am. Chem. Soc. 1978, 100, 3930 .
(2) P. S. Baran, E. J. Corey, J. Am. Chem. Soc. 2002, 124, 7904.
(3) N. K. Garg, D. D. Caspi, B. M. Stoltz, J. Am. Chem. Soc. 2004, 126, 9552.
(4) Mizoroki, T.; Mori, K.; Ozaki, A. Bull. Chem. Soc. Jpn. 1971, 44, 581.
(5) Heck, R. F.; Nolley, J. P., Jr. J. Org. Chem. 1972, 37, 2320.
(6) J. K. Stille, Angew. Chem. 1986, 98, 504; Angew. Chem. Int. Ed. Engl. 1986, 25, 50.
(7) R. F. Heck in Comprehensive Organic Synthesis, Vol. 4 (Eds.: B. M. Trost, I. Fleming), Pergamon, Oxford, 1991, p. 833;
(8) N. Miyaura, A. Suzuki, Chem. Rev. 1995, 95, 2457;
(9) Metal-Catalyzed Cross- Coupling Reactions (Eds.: F. Diederich, P. J. Stang),Wiley-VCH, New York, 1998.
(10) T.-Y. Luh, M.-K. Leung, K.-T. Wong, Chem. Rev. 2000, 100, 3187; f) T. Hiyama, J. Organomet. Chem. 2002, 653, 58;
(11) E.-i. Negishi, Q. Hu, Z. Huang, M. Qian, G. Wang, Aldrichimica Acta 2005, 38, 71; h) B. M. Trost, M. L. Crawley, Chem. Rev. 2003, 103, 2921;
(12) K. C. Nicolaou, P. G. Bulger, D. Sarlah, Angew. Chem. 2005, 117, 4516; Angew. Chem. Int. Ed. 2005, 44, 4442;
(13) D. S. Surry, S. L. Buchwald, Angew. Chem. 2008, 120, 6438; Angew. Chem. Int. Ed. 2008, 47, 6338; k) J. F. Hartwig, Nature 2008, 455, 314;
(14) S. E. Denmark, C. S. Regens, Acc. Chem. Res. 2008, 41, 1486.
(15) D.W. Old, J. P. Wolfe, S. L. Buchwald, J. Am. Chem. Soc. 1998, 120, 9722;
(16) Hua-Sin Chen,Sheng-Chu Kuo,Che-Ming Teng, Fang-Yu Lee,etal. Bioorganic & Medicinal Chemistry, 2008,16:1262–1278.
(17) Elguero J, Katritzky AR, Rees CW. In Comprehensive Heterocyclic Chemistry, 1984, 5:167.
(18) Kovach EG, Barnes DE. J. Am. Chem. Soc, 1954, 76:1176.
(19) Song JJ, Yee NK. Org. Lett, 2000, 2: 519.
(20) Song JJ, Yee NK. Tetrahedron Lett, 2001, 41:2937.
(21) Frontana-Uribe BA, Moinet C. Tetrahedron, 1998, 54: 3197.
(22) Stadlbauer W. Sci. Synth, 2002, 12: 227.
(23) Akazome M, Kondo T, Watanabe YJ. Org. Chem, 1994, 59:3375.
(24) Caron S, Vazquez E. Synthesis, 1999, 588.
(25) Fernandez PA, Bellamy T, Kling M, Madge DJ, Selwood DL. Heterocycles, 2001, 55:1813.
(26) Yoshida T, Matsuura N, Yamamoto K, Doi M, Shimada K, Morie T, Kato S. Heterocycles, 1996, 43: 2701.
(27) Jukin K, Hsu MC, Fernando D, Leanna MR.. J. Org. Chem, 2006, 71:8166.
(28) Brase, S.; Gil, C.; Knepper, K. *Bioorg. Med. Chem.* 2002, *10*, 2415.
(29) Runti, C.; Baiocchi, L. *Int. J. Tissue React.* 1985, *7*, 175. (b) Bistochi, G. A.; De Meo, G.; Pedini, M.; Ricci, A.; Brouilhet, H.; Bucherie, S.; Rabaud, M.; Jacquignon, P. *Farmaco*, *Ed. Sci.* 1981, *36*, 315. (c) Picciola, G.; Ravenna, F.; Carenini, G.; Gentili, P.; Riva, M. *Farmaco*, *Ed. Sci.* 1981, *36*, 1037.
(30) Keppler, B. K.; Hartmann, M. *Met.-Based Drugs* 1994, *1*, 145.

(31) (a) Sun, J.-H.; Teleha, C. A.; Yan, J.-S.; Rodgers, J. D.; Nugiel, D. A. *J. Org. Chem*. 1997, *62*, 5627. (b) Rodgers, J. D.; Johnson, B. L.; Wang, H.; Greenberg, R. A.; Erickson-Viitanen, S.; Klabe, R. M.; Cordova, B. C.; Rayner, M. M.; Lam, G. N.; Chang, C.-H. *Biomed. Chem. Lett.* 1996, *6*, 2919.

(32) De Lena, M.; Lorusso, V.; Latorre, A.; Fanizza, G.; Gargano, G.; Caporusso, L.; Guida, M.; Catino, A.; Crucitta, E.; Sambiasi, D.; Mazzei, A. *Eur. J. Cancer* 2001, *37*, 364.

(33) Lee, F.-Y.; Lien, J.-C.; Huang, L.-J.; Huang, T.-M.; Tsai, S.-C.; Teng, C.-M.; Wu, C.-C.; Cheng, F. C.; Kuo, S.-C. *J. Med. Chem.* 2001, *44*, 3747.

(34) Harada, H.; Morie, T.; Hirokawa, Y.; Terauchi, H.; Fujiwara, I.; Yoshida, N.; Kato, S. *Chem. Pharm. Bull.* 1995, *43*, 1912.

(35) Eidt JF, Allison P, Noble S, etal.Thrombin is an important mediator of platelet aggregation in stenosed canine coronary arteries with endothelial injury. J Clin Invest, 1989, 84: 18-27.

(36) McNamara CA, Sarembock IJ, Gimple LW, etal. Thrombin stimulates proliferation of cultured rataortic smooth muscle cells by a proteolytically activated receptor. J Clin Invest, 1993, 91: 94-98.

(37) Folkman J. What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst,1990, 82:4-6.

(38) Fidler IJ, Ellis LM. The implications of angiogenesis for the biology and therapy of cancer metastasis. Cell, 1994, 79:185-188.

(39) Seiple IB, Su S, Rodriguez RA,Gianatassio R, Fujiwara Y,Sobel AL, Baran PS. For radical-based methods to effect arylation of pyridines. J. Am. Chem. Soc, 2010, 132: 13194.

(40) Peng CY, Pan SL, Guh JH, Liu YN, Chang YL, Kuo SC, Lee FY, Teng CM. The indazole derivative YD-3 inhibits thrombin-induced vascular smooth muscle cell proliferation and attenuates intimal thickening after balloon injury. Thromb Haemost, 2004, 92:1232-1239.

(41) For earlier examples, see: (a) Elguero, J. In *Comprehensi*V*e Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds.; Pergamon: New York, 1984; Vol. 5, p 167.