

# Click Labeling Of $M(\text{CO})_3^+$ ( $M=\text{Re}, ^{99m}\text{Tc}$ )

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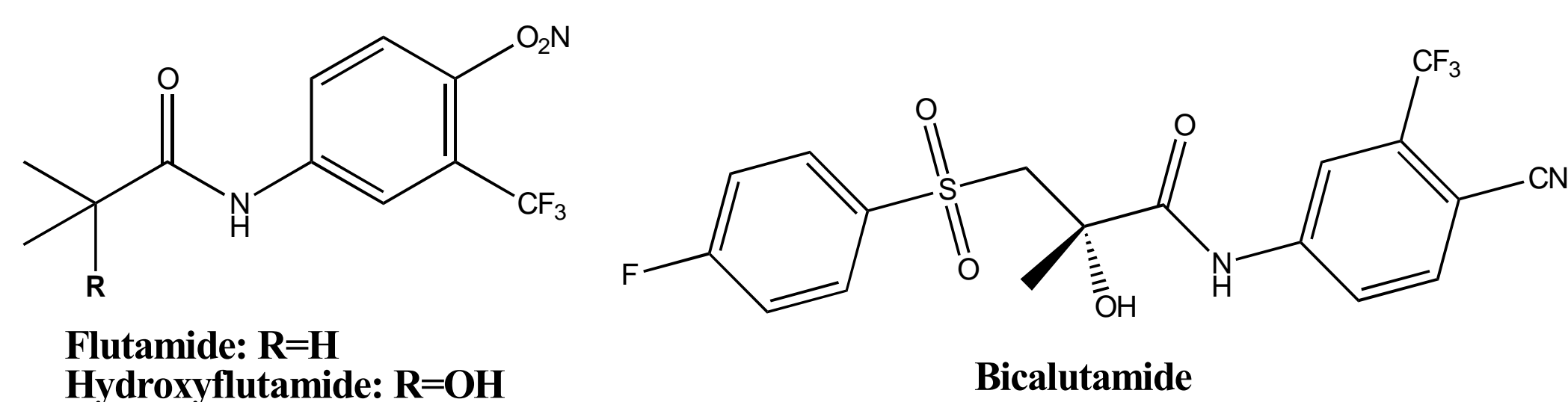
## Introduction

The motivation behind my research was to synthesize a Single Photon Emission Computed Tomography (SPECT) imaging molecule to target prostate cancer cells. The overarching goal was to investigate the androgen receptor (AR), over-expressed in early stage prostate cancer, with a composite AR-targeting molecule that would combine structural features of the potent AR-inhibitors, Bicalutamide and Flutamide, and would be capable of delivering the diagnostic radionuclide  $^{99m}\text{Tc}$  ( $E\gamma = 140 \text{ keV}$ ,  $I\gamma = 89\%$ ,  $t_{1/2} = 6 \text{ hours}$ ). (Figure 1)

It is hoped that functionalizing the AR-targeting molecule with the tridentate chelator to complex the organometallic  $^{99m}\text{Tc}(\text{OH}_2)_3(\text{CO})_3$  precursor will generate a targeting agent that converts from Flutamide to a higher affinity Bicalutamide derivative. In addition to improving the structural bioaffinity of the agents for the AR, the click reaction also provides a method to improve the specific activity of the radiolabeled product compared to the unlabeled product by utilizing chemical reactivity over complexation formation.

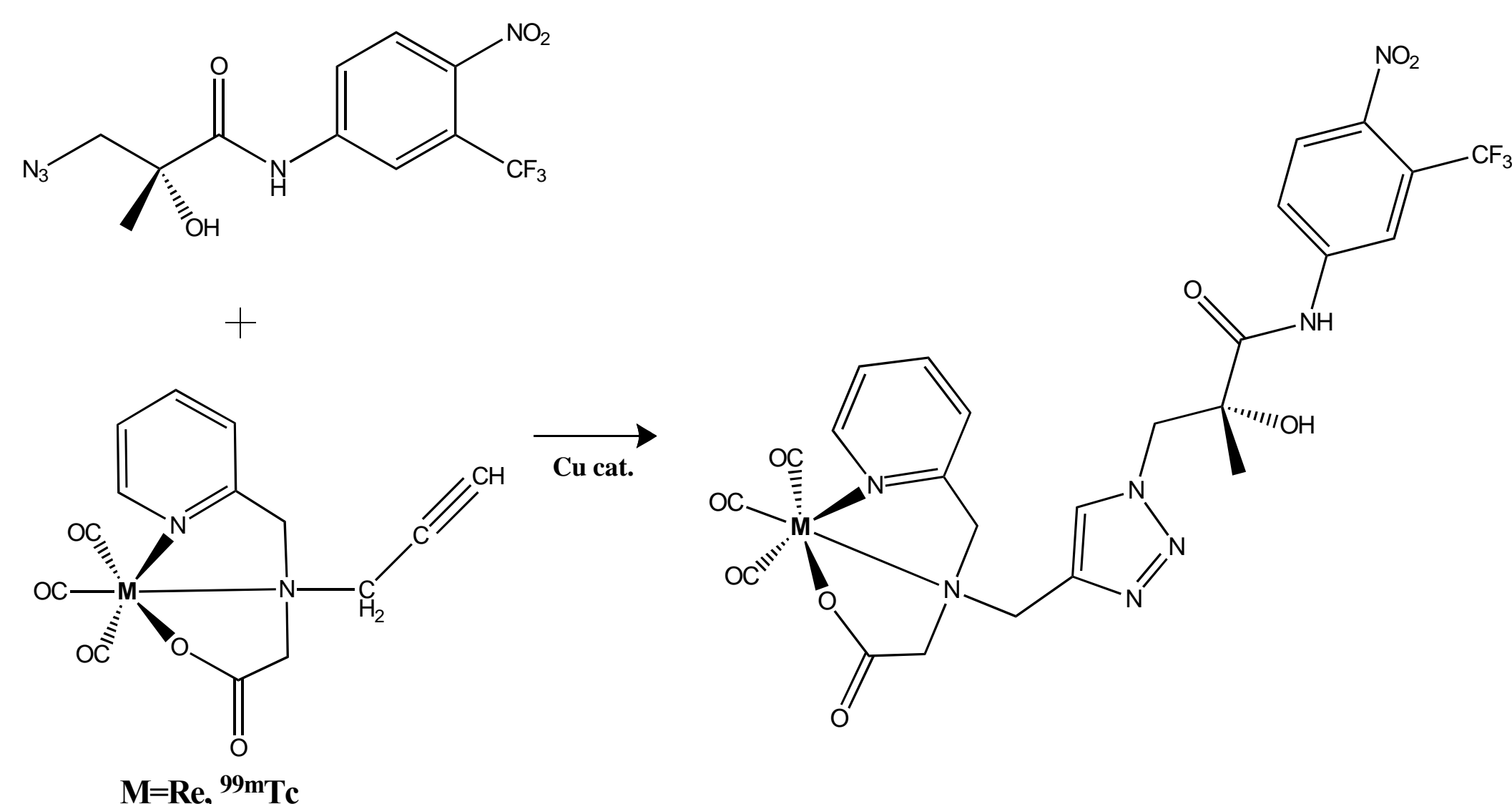
Hormonal therapies interfere with the growth of prostate cancer cells by reducing the uptake of testosterone and hindering growth in the cancer cells. Flutamide effectively binds to the androgen receptor inhibiting testosterone from binding to the active site, in which the cancerous cells either grow more slowly or stop growing altogether leading to apoptosis. Effectiveness of the flutamide drug against prostate cancer has its limitations as the cells develop a resistance to flutamide compound via hormone refractory syndrome. The lipophilic nature of the organometallic species would allow clearance through the liver providing optimal imaging of the prostate without occlusion from a high bladder or kidney clearance. These compounds would minimize any pharmacological effects by utilizing extremely low dose concentrations and limited exposure to cancer cells to guard against developing a drug resistance to hormone therapies.

Figure 1.



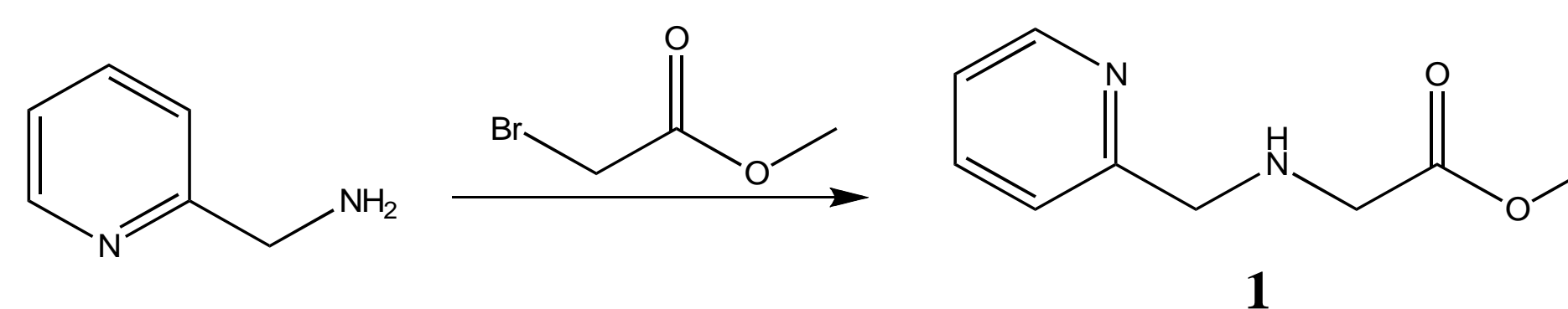
## My Research

My research investigated the feasibility of using an asymmetric tridentate chelator with an alkyne chain to complex a metal core. The alkyne ligand was prepared and characterized under normal conditions. The corresponding alkyne-metal complex was prepared with rhenium for standard chemical analysis and as a model for  $^{99m}\text{Tc}$  analogs. The alkyne provides a ready handle to "click" with an azide under mild conditions with a catalyzing agent. The rhenium-alkyne complex was clicked with benzyl azide as a model for the click reaction with the biologically active molecule hydroxyflutamide.



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## Synthesis of methyl 2-(pyridin-2-ylmethylamino)acetate

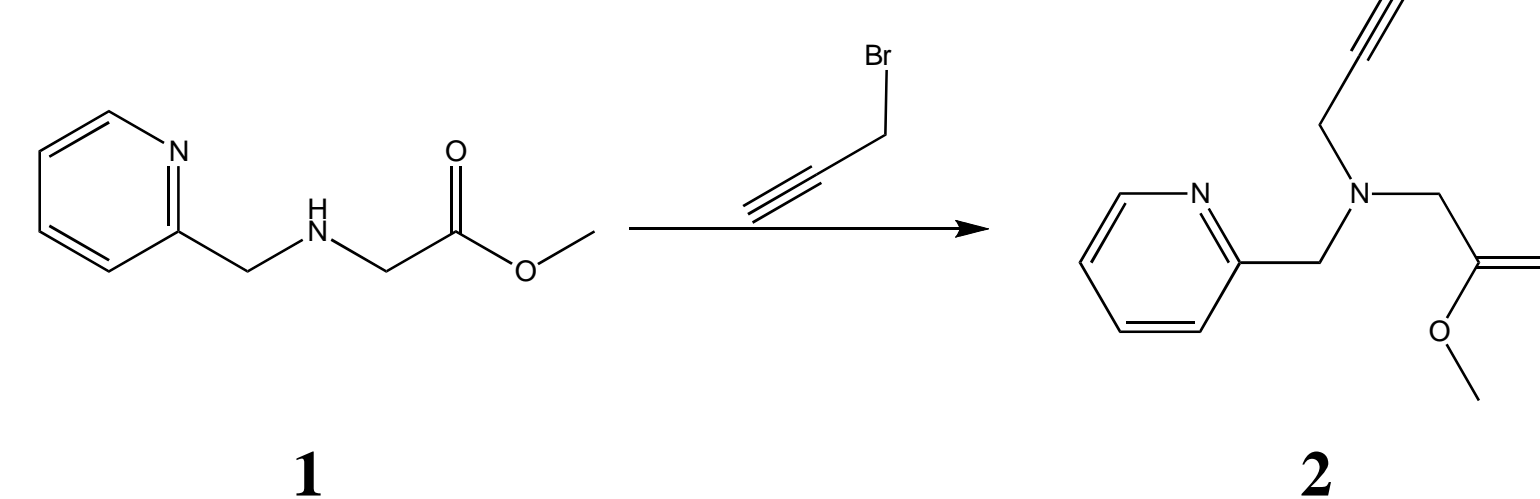


2-aminomethylpyridine (1.477 mL, 14.09 mmol) was dissolved in acetonitrile (60 mL) and stirred in an ice bath for 30 minutes. Methyl 2-bromoacetate (0.620 mL, 6.541 mmol) was dissolved in acetonitrile (10 mL) and added dropwise over the course of one hour. The reaction warmed to room temperature stirring overnight. The next morning a white precipitate was observed and filtered off. The solution was rotovapped to dryness and redissolved in dichloromethane. More white precipitate formed and was filtered off. The dichloromethane filtrate was loaded onto silica gel for column purification of **1** (gradient method: acetone and hexanes: start 100% hexanes going to 50% acetone-50% hexanes). (788 mg, 66.8%).  $^1\text{H NMR}$  [ $\delta$  (ppm),  $\text{CDCl}_3$ ] 8.55 (m, 1H), 7.64 (m, 1H), 7.29 (m, 1H), 7.16 (m, 1H), 3.93 (s, 2H), 3.72 (s, 3H), 3.48 (s, 2H), 2.22 (s, 1H).

•New purification method for large-scale preparations

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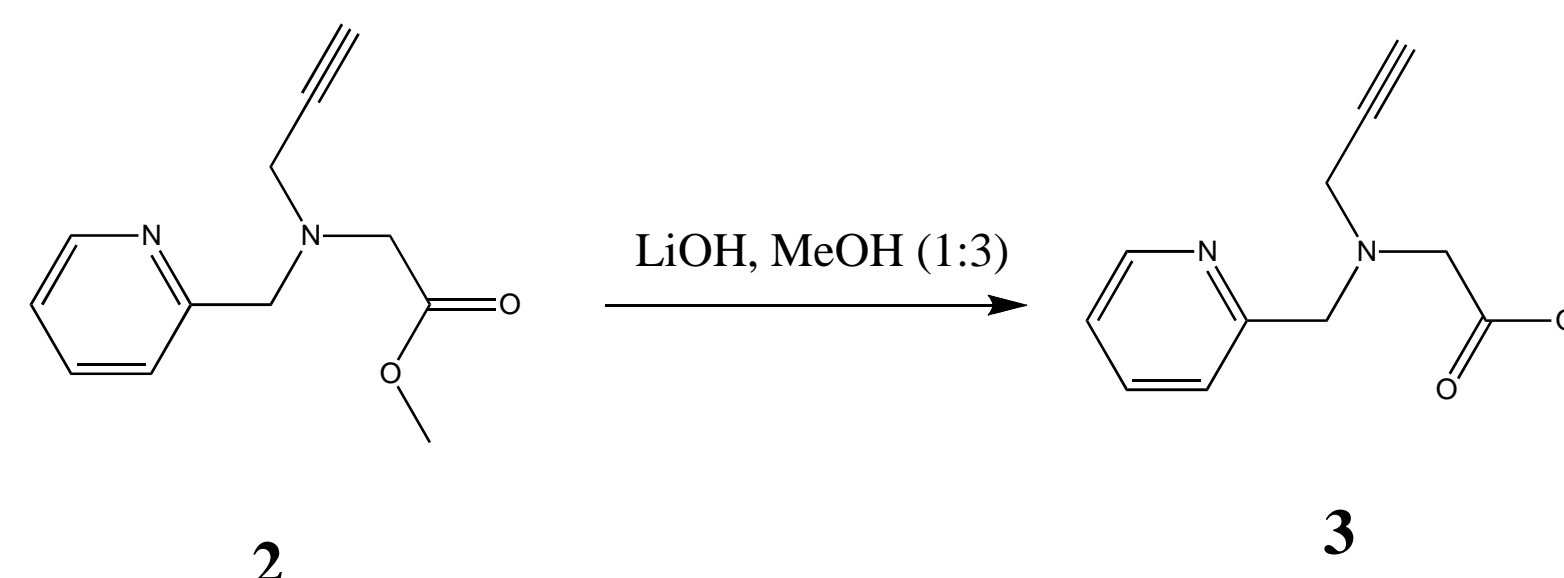
## Synthesis and Characterization of methyl 2-(prop-2-ynyl(pyridin-2-ylmethyl)amino)acetate



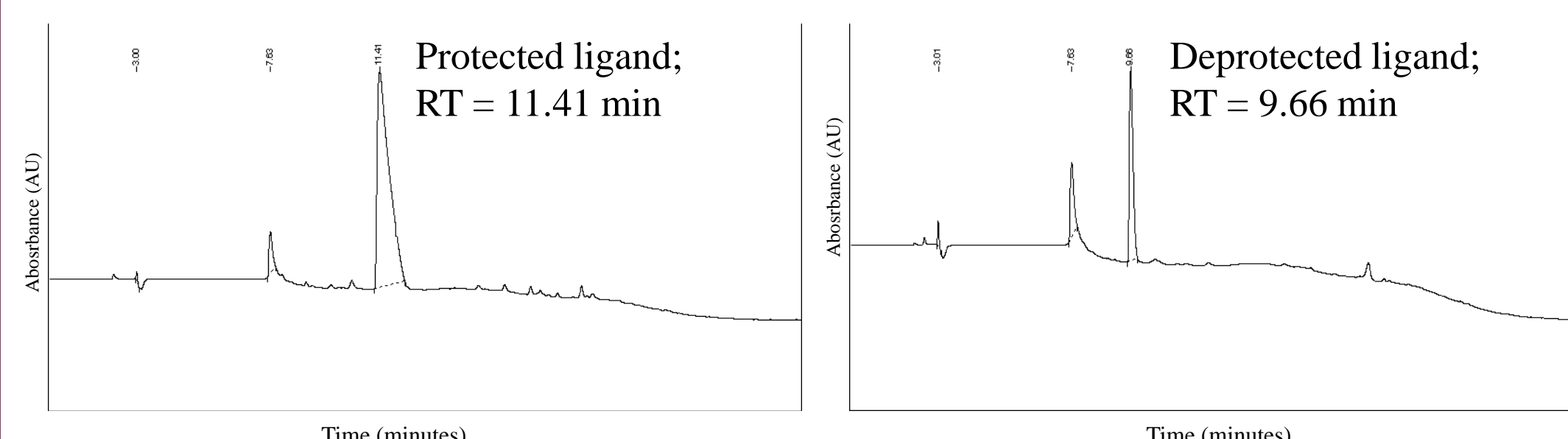
Compound **1** (200 mg, 1.110 mmol) was dissolved in acetonitrile (20 mL) and stirred. Potassium carbonate (307 mg, 2.221 mmol) was added. Propargyl bromide (0.119 mL, 1.337 mmol) was dissolved in acetonitrile (10 mL) and added dropwise. The reaction was refluxed overnight. The solution was rotovapped to dryness the following morning, redissolved in deionized water (50 mL) and extracted with dichloromethane (4 x 50 mL), then dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The dichloromethane filtrate was loaded onto silica gel for column purification of **2** (gradient method: acetone and hexanes: start 100% hexanes going to 35% acetone-65% hexanes). (132 mg, 54.5%).  $^{13}\text{C NMR}$  [ $\delta$  (ppm),  $\text{CDCl}_3$ ] 171.28, 158.37, 149.34, 136.79, 123.42, 122.45, 78.43, 73.97, 59.65, 54.18, 51.78, 43.04.

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## Synthesis of 2-(prop-2-ynyl(pyridin-2-ylmethyl)amino)acetic acid

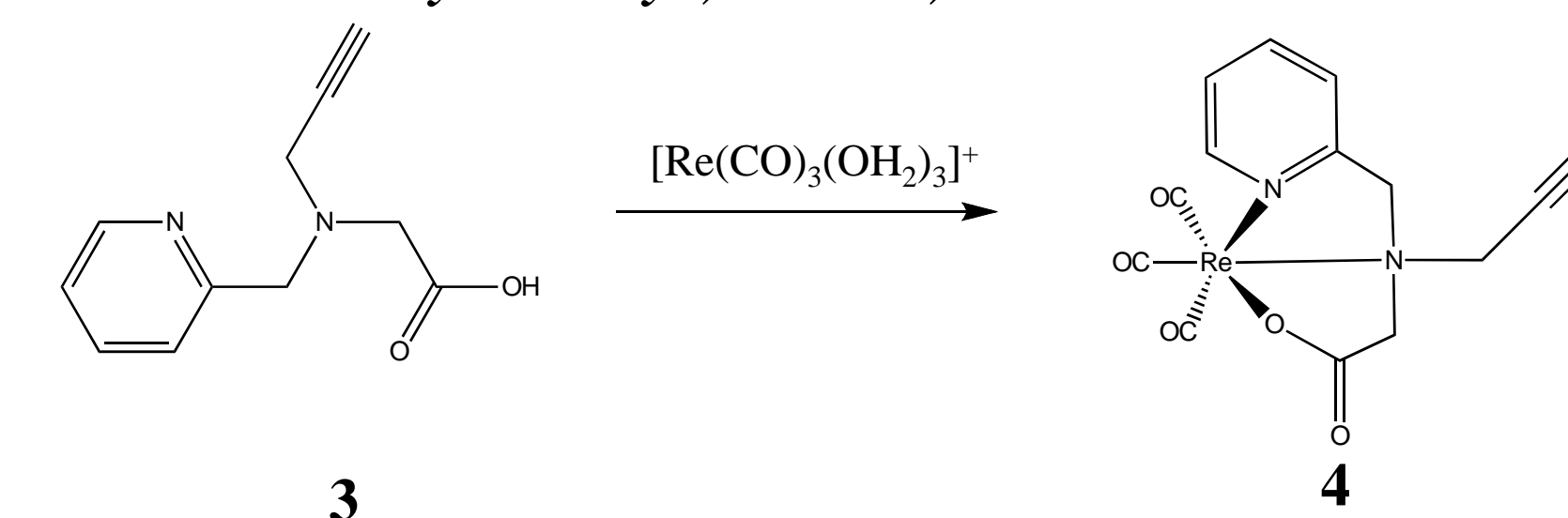


Compound **2** (132 mg, 0.605 mmol) was dissolved in methanol and 1.0 M lithium hydroxide (8 mL, 3:1) and stirred in an ice bath. The solution was stirred overnight as it warmed to room temperature. The following morning the solution was rotovapped to dryness, redissolved in deionized water (15 mL), and the pH adjusted from 11.84 to 6.49 using 1.0 M HCl and 1.0 M  $\text{NaHCO}_3$ . The solution was rotovapped to dryness and redissolved in dichloromethane and the insoluble salts were filtered off. Analytic HPLC was used to monitor the reaction, and quantitative yields of compound **3** are expected for this deprotection.



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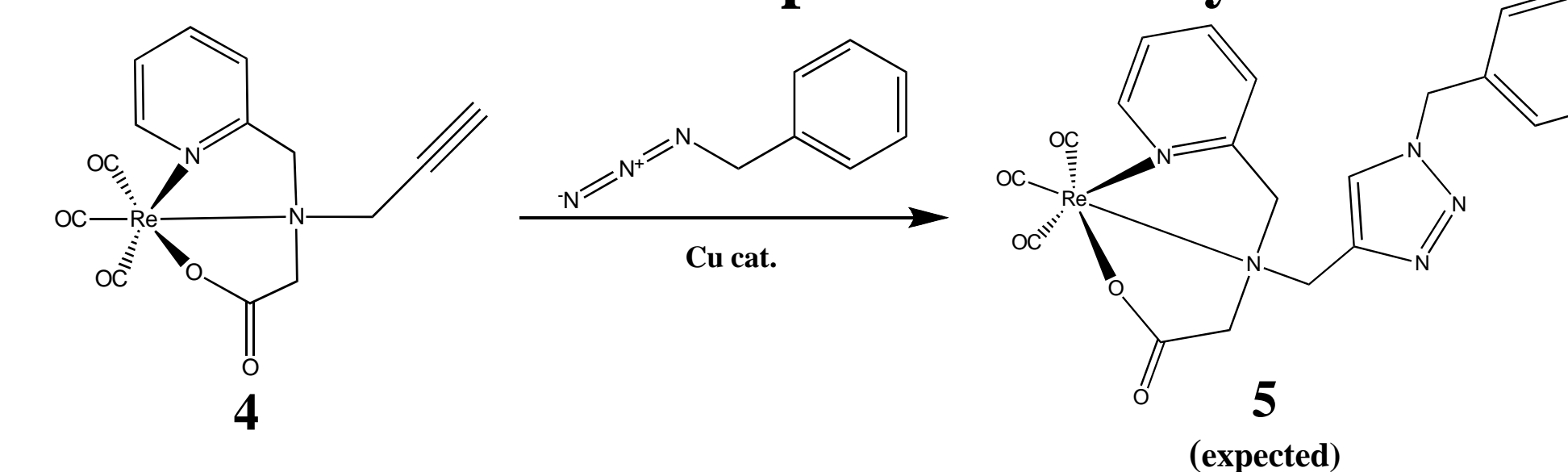
## Rhenium Complexation with 2-(prop-2-ynyl(pyridin-2-ylmethyl)amino)acetic acid



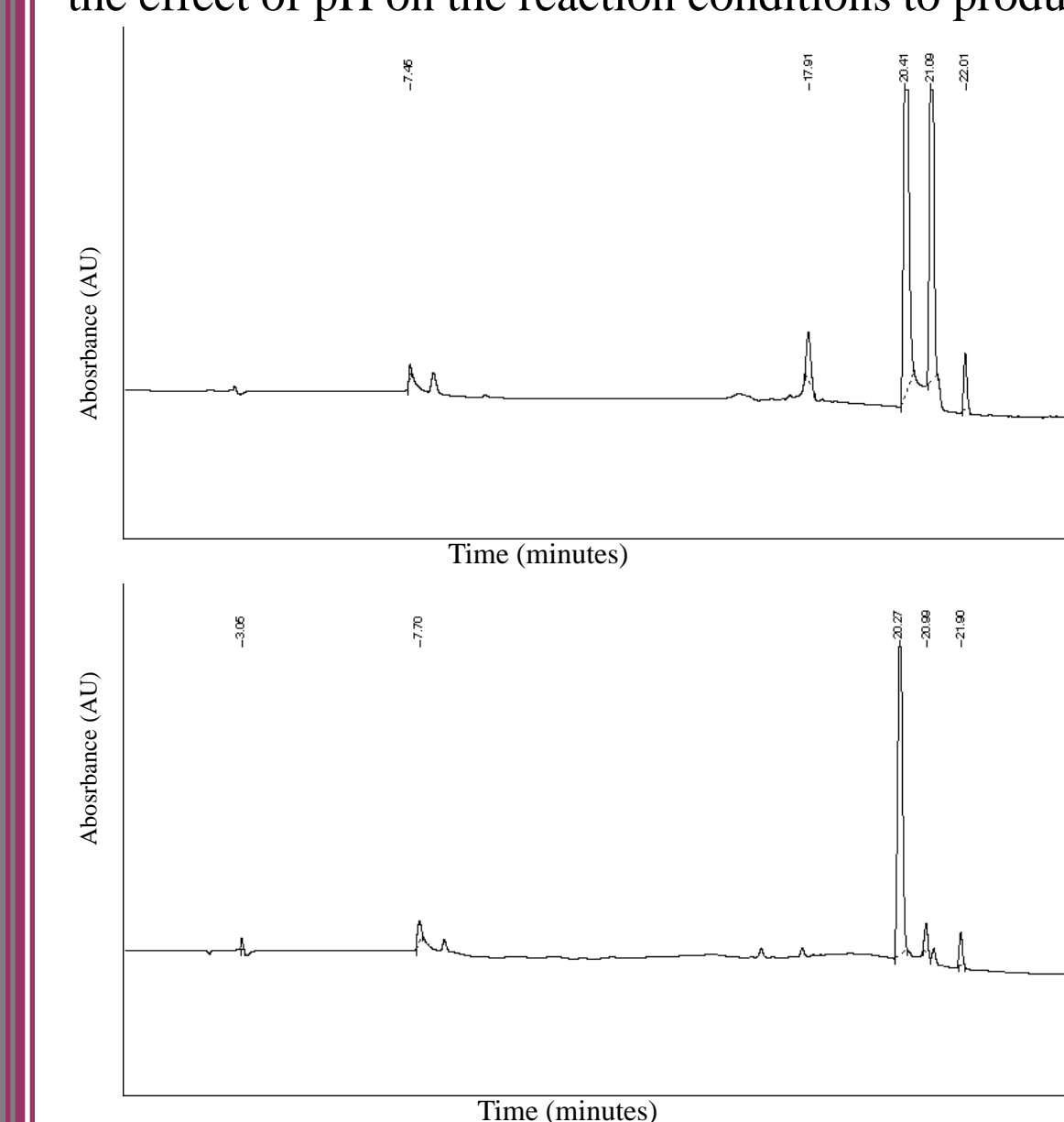
A crude sample of compound **3** (~124 mg) was dissolved in deionized water (10 mL) and stirred. 0.1 M  $[\text{Re}(\text{CO})_3(\text{OH}_2)_3]^+$  (3.000 mL, 0.300 mmol) was added and the pH of the solution was adjusted to 6. The solution was stirred under reflux over the weekend. The reaction cooled to room temperature and a brown precipitate was observed. The solution was rotovapped and redissolved in a minimal amount of deionized water and refrigerated. The precipitate, compound **4**, was filtered on a glass frit and washed with cold water.  $^1\text{H NMR}$  [ $\delta$  (ppm),  $\text{CDCl}_3$ ] 8.82 (d, 1H), 8.10 (m, 1H), 7.76 (d, 1H), 7.55 (t, 1H), 4.71 (dd, 2H,  $J=15.44$ ), 4.38 (ddd, 2H,  $J=16.48$ ), 3.75 (dd, 2H,  $J=17.17$ ), 3.25 (t, 1H).

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## Click Rhenium complex with benzyl azide



Compound **4** (34 mg, 0.0708 mmol) was partially dissolved in methanol (1 mL) and combined in a small vial with benzyl azide (0.010 mL, 0.0775 mmol) dissolved in methanol (1 mL). Sodium ascorbate (3 mg, 0.0151 mmol) was dissolved in a separate vial in deionized water (1 mL), and copper (II) acetate (2 mg, 0.0110 mmol) was dissolved in another vial in deionized water (1 mL). The sodium ascorbate solution was added to the reaction vial, followed by addition of the copper (II) solution and stirred and heated at  $70^\circ\text{C}$ . After 45 minutes, two products were formed, which suggests that coordination of the triazole may have occurred in addition to expected compound **5**. After stirring and heating overnight, no change was evident. Taking 1 mL of the reaction solution and acidifying to  $\sim\text{pH } 2$  resulted in one product. This suggests that future work should investigate the effect of pH on the reaction conditions to produce the desired coordination.



After 45 minutes: no starting material (19.16 min); two products at 20.41 min, 21.09 min; excess benzyl azide at 22.01 min

With acidification: no starting material (19.16 min); converted to one product at 20.27 min

## Conclusions and Future Work:

The asymmetric alkyne ligand is useful for complexing metal cores and clicking with azides under mild conditions compatible with hydroxyflutamide. The next steps will be developing the click reaction of the rhenium complex under new pH conditions, clicking with the azide of hydroxyflutamide, radio-labeling with  $^{99m}\text{Tc}$ , and testing the compounds *in vivo* with prostate cancer cell lines DU-145 (AR-positive) and PC-3 (AR-negative).

## Acknowledgements :

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