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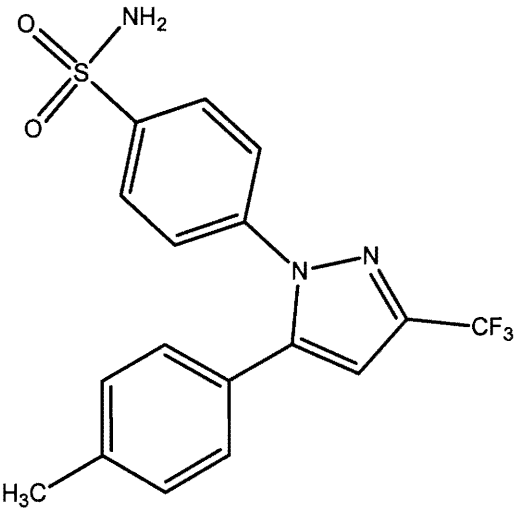
Background: Bayer originally produced aspirin, the first nonsteroidal anti-inflammatory drug (NSAID), in 1899. Today, more than 80 billion aspirin tablets are taken each year in the United States. Along with the popularity of over-the-counter NSAIDs, prescription NSAIDs have become the most widely prescribed drugs in the world. While the drugs relieve minor pain, decrease fever, and reduce inflammation, NSAIDs can have serious side effects when used for a long period of time. The more dangerous side effects include GI ulcers, bleeding, and renal failure. Since 25% of people who regularly use NSAIDs experience side effects, a better option for long-term treatment is needed. [1]

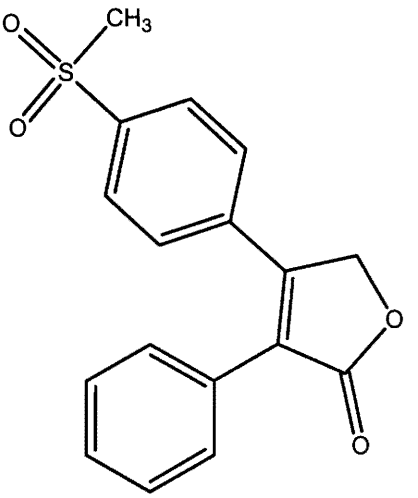
The negative side effects of NSAIDs are due to the inhibition of the constitutive enzyme prostaglandin H_2 synthase-1 (COX-1). This enzyme is responsible for the production of prostacyclin, which protects the gastric mucosa, and prostaglandin E_2, which is needed by the kidneys. The desired effects of NSAIDs—pain and fever relief—are brought about by the inhibition of COX-2, an isoenzyme of COX-1. COX-2 is induced after an injury or other inflammatory stimulus. It is responsible for the production of the prostaglandins that lead to inflammation. The problem with traditional NSAIDs is that these drugs inhibit the "good" COX-1 enzyme more efficiently than they inhibit the "bad" COX-2. In fact, aspirin is 10 to 100 times more effective against COX-1. [2]

This problem has now been solved by the introduction of selective COX-2 inhibitors. One of the first COX-2 inhibitors to be developed is o-(acetoxyphenyl)hept-2-ynyl sulfide (APHS). This drug is similar to aspirin in that it inactivates cyclooxygenase by acetylation, but it is 60 times as reactive against COX-2 and 100 times as selective as aspirin.[3] The first COX-2 inhibitor to be made available by prescription is celecoxib. The FDA approved celecoxib for the treatment of adult rheumatoid arthritis and osteoarthritis in December of 1998, and it is now sold under the trade name Celebrex. Another COX-2 inhibitor

approved for human use is rofecoxib—referred to commercially as Vioxx. It produces similar results as the celecoxib, and it is particularly beneficial for people who are sensitive to sulfonamide drugs. All of these drugs demonstrate the same analgesic and anti-inflammatory benefits of NSAIDs without the side effects of extensive COX-1 inhibition, and research indicates that COX-2 inhibitors may have even more uses. One probable use for COX-2 inhibitors is in the treatment of colorectal cancer—people with long-term NSAID use show a 40-50% reduction in colon cancer risk.[4]



Celecoxib



Rofecoxib

Introduction: Cancer of the colon and rectum is the third most diagnosed form of cancer in United States. Approximately 133,500 new cases of colorectal cancer were diagnosed in 1996, and in the same year about 54,900 people died as a direct result of this disease. Thanks to advances in treatment and a greater understanding of risk factors, the mortality rate for colorectal cancer is decreasing. Some important risk factors for the disease are age, a family history of colorectal cancer, sex, and inflammatory bowel disease, but approximately 75% of colon cancer patients have no known predisposing factors for colorectal cancer. Because these patients do not exhibit the established risk factors for colorectal cancer, they are

often not screened for the disease. In fact, the 1992 National Health Interview Survey found that only 17.3% of the American population above the age of 50 had been screened that year by fecal occult blood testing, a common screening method.[5]

Increased screening of the general population is needed because the chances of survival of colorectal cancer are greatly increased by early detection. When the disease is detected in its early stages, the available treatments are more effective. If the cancer involves only the bowel wall, the five-year survival rate is about 90%, but this rate falls to less than 10% if the cancer has metastasized. According to the Agency for Health Care Policy and Research (AHCPR), "the primary strategy for preventing colorectal cancer deaths is to detect and remove precursors of colorectal cancer or to detect and treat cancer in its earliest stages".[5]

The precursors of colorectal cancer mentioned above are the premalignant mucosal masses in the colon and rectum—otherwise known as adenomatous polyps. These colorectal cancer precursors are found in about 25% of the population by age 50. While the traditional screening methods are effective in detecting adenomatous polyps, they are often not employed. If the current screening methods are not being used enough, one solution is to develop new, easier screening methods. A suggestion of the AHCPR is to use the recent discoveries in the molecular biology of colorectal cancer to develop new screening interventions.[5]

One recent discovery in the molecular biology of colorectal cancer is the increased expression of the cyclooxygenase-2 (COX-2) enzyme. In 85% of human colorectal adenocarcinomas, COX-2 levels are 2 to 50 times greater in the cancer tissue than in surrounding tissue.[6] And, it is now thought that COX-2 is not simply present in colorectal cancer; the enzyme is actually needed for the

development of this cancer.[7] As mentioned earlier, the inhibition of COX-2 by NSAIDs, like aspirin, reduces human colorectal cancer risk by 40-50%.[6] With a specific COX-2 inhibitor, the chemopreventive effects of NSAIDs can be obtained without the GI side effects. In one study, celecoxib supplementation inhibited the incidence and multiplicity of colon tumors in rats by 93 and 97% respectively.[8] The chemopreventive benefits of celecoxib have led to its recent approval by the Food and Drug Administration as a supplement to standard treatment for familial adenomatous polyposis.

In addition to celecoxib's chemopreventive benefits, it may serve as a means to devise a new screening method. Not only is COX-2 found in much higher concentrations in colorectal cancer adenocarcinomas, the degree of expression may be related to the severity and stage of the disease. Greater COX-2 expression is found in larger tumors and in the later stages of the disease, and lymph node metastasis appears to increase COX-2 expression even further. Higher concentration of the enzyme also corresponds to decreased survival estimates.[9]

Because the COX-2 enzyme is inextricably linked to colorectal cancer, it can be used as a marker of the disease. This is why the focus of this research, the synthesis of a radiolabeled celecoxib derivative, might be useful. Labeling a known COX-2 inhibitor with ^{123}I should allow single-photon emission computer tomography (SPECT) of colorectal cancer. The ^{123}I-labeled celecoxib derivative would localize in the area of increased COX-2 expression caused by colorectal adenocarcinomas, and this localization would allow scintigraphy.

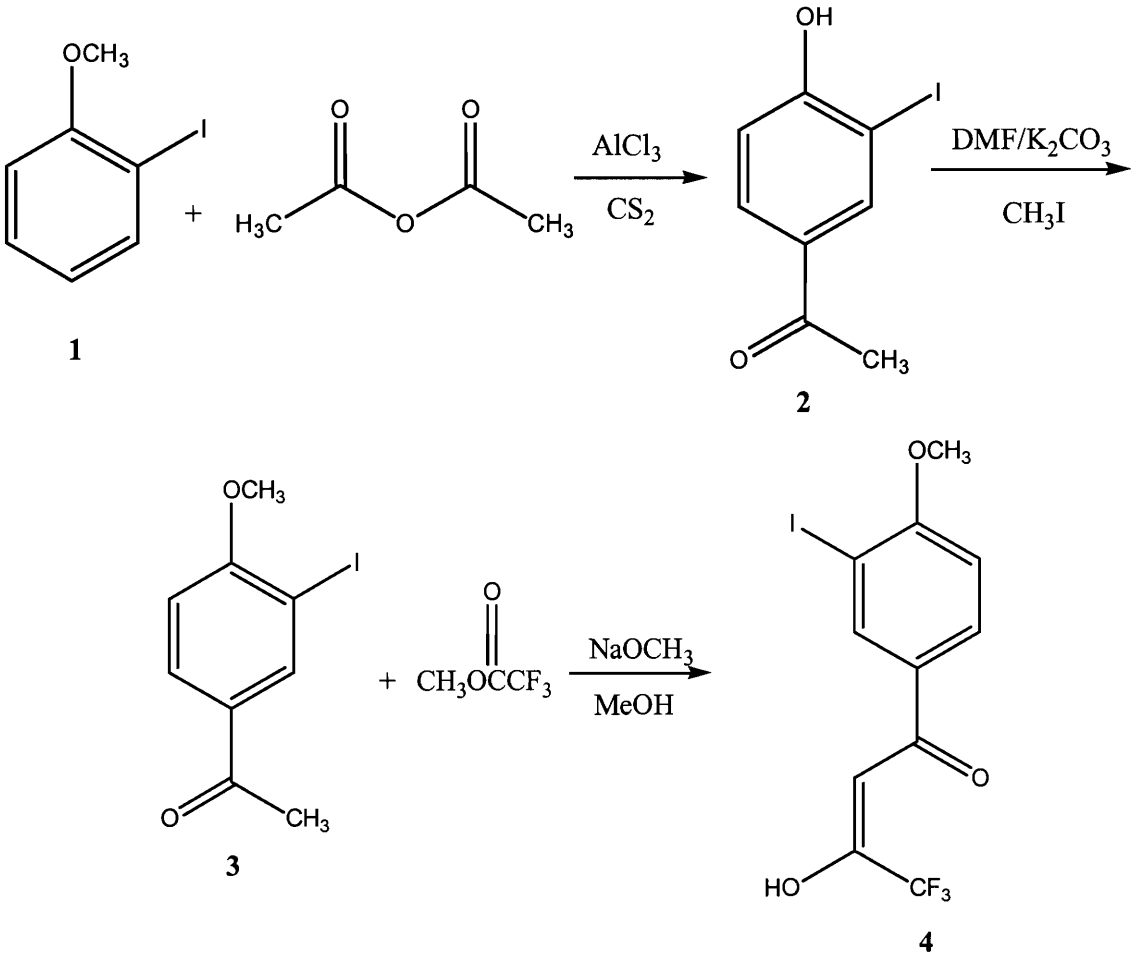
Chemistry: The development of a synthetic route to radiolabeled methyl and methoxy derivatives of celecoxib was originally planned; however, only the iodinated methoxy derivative was synthesized due to time constraints. The first synthetic plan required the iodination of 4-methoxyacetophenone, but this reaction

failed to produce the desired product. Finally, 2-iodoanisole, **1,** was chosen as the starting material for the synthesis.

4-[5-(3-Iodo-4-methoxyphenyl)-3-trifluoromethylpyrazol-1-yl] benzenesulfonamide, **6,** was synthesized from 2-iodoanisole, **1,** in four steps as shown in the **Scheme**. The reaction sequence could have been limited to three steps if the remethylation of 4-hydroxy-3-iodoacetophenone, **2,** had not been necessary. After the Friedel-Crafts acylation, the reaction mixture was allowed to stand at room temperature, which led to demethylation by the Lewis acid, aluminum chloride. The first step was the Friedel-Crafts acylation of the iodoanisole **1** to form 3-Iodo-4-methoxyacetophenone, **3,** and this was followed by a Claisen condensation with methyl trifluoroacetate. The 4,4,4-trifluoro-1-(3-iodo-4-methoxyphenyl)butane-1,3-dione, **4,** formed was then reacted with the sulfonamide **5** in ethanol to give the iodinated methoxy celecoxib derivative **6.** The final two steps in the **Scheme** were developed for use in the radioiodination sequence (which was not carried out due to time constraints).

The key step in the synthesis is the electrophilic iododestannylation of 4-[5-(4-methoxy-3-trimethylstannanylphenyl)-3-trifluoromethylpyrazol-1-yl] benzenesulfonamide, **7**. Palladium catalyzed deiodostannylation with hexamethylditin in dioxane gave the tin precursor **7**. The tin compound **7** was then converted back to the iodinated celecoxib derivative **6** by heating with NaI and 0.3% peracetic acid. These are the conditions utilized for radioiodine incorporations involving $Na^{123}I$.

Scheme: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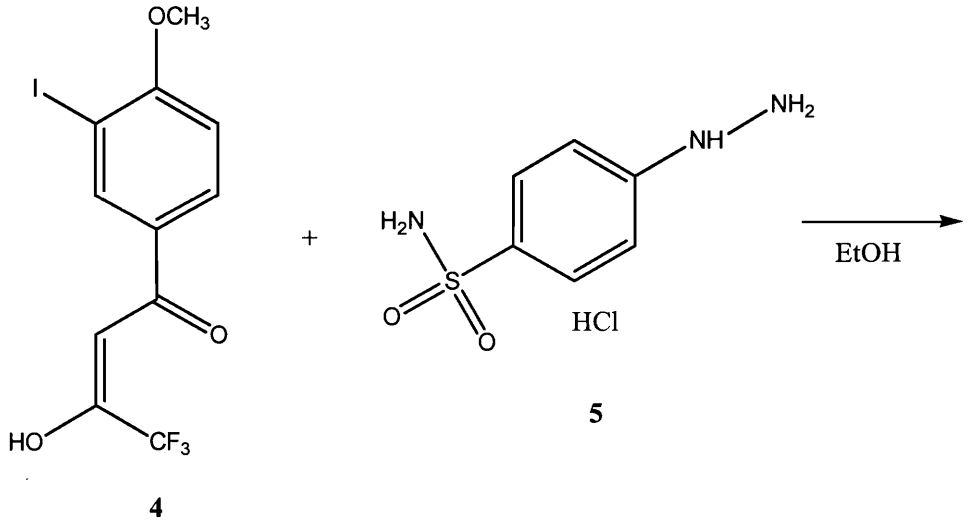
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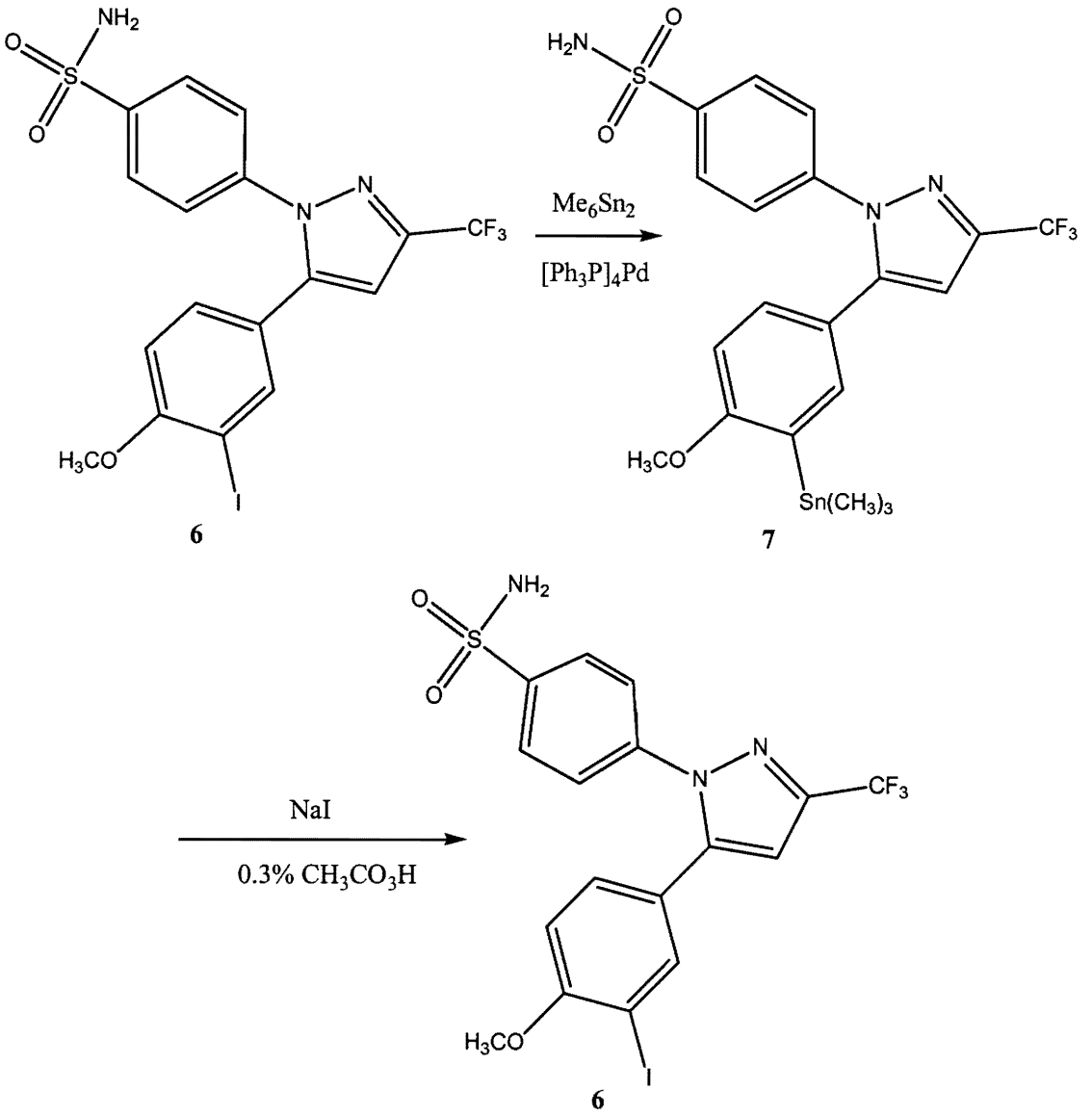

Experimental Section: All the reactions were performed using dry solvents in a nitrogen atmosphere. The starting materials were purchased from Aldrich Chemical Company. The products were purified by flash chromatography (SiO_2), and the ^1H spectra were taken with a Bruker AC 250 MHz NMR spectrometer. The chemical shifts are given in parts per million relative to tetramethylsilane. The melting points were recorded on an Electrothermal Digital Melting Point Apparatus and are uncorrected.

4-Hydroxy-3-iodoacetophenone (2)

2-Iodoanisole, **1,** (11.7 g, 0.050 mol) was dissolved in dry carbon disulfide (30 mL) and transferred to a 100 mL three-necked flask, equipped with a reflux condenser, a sealed stirrer unit, and a dropping funnel protected with a Drierite guard tube. Powdered anhydrous aluminum chloride (15 g, 0.11 mol) was added and the mixture was heated to gentle refluxing. Acetic anhydride (5.1 g, 0.50 mol) was added through the dropping funnel. Refluxing was continued for 2 hours, and the reaction mixture was left at room temperature overnight. The reaction mixture poured into a mixture of crushed ice (100 g) and concentrated hydrochloric acid (30 mL). After extraction with ether (2x100 mL), the combined organic extracts were washed with water and then 10% sodium hydroxide (50 mL). The sodium hydroxide extract was neutralized with 10% hydrochloric acid (50 mL). The aqueous portion was extracted with ether (2x100 mL) and the combined ethereal extracts were washed sequentially with water and brine. The solution was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure to give a pinkish solid. The crude yield was 9.80 g (74.8 %). ^{1}H NMR ($CDCl_3$): δ, 2.55 (s, 3H), 7.00 (d, 1H), 7.90 (d, 1H), and 8.30 (s, 1H).

3-Iodo-4-methoxyacetophenone (3)

4-Hydroxy-3-iodoacetophenone, **2,** (2.70 g, 10.3 mmol) was transferred to a double necked flask equipped with a reflux condenser and a septum protected with a Drierite guard tube. The solid was dissolved in dry DMF (80 mL). An excess of CH_3I (2.20 g, 15.5 mmol) and K_2CO_3 (2.17 g, 15.5 mmol) was added to the solution. The reaction mixture was stirred while heating at 60 °C for 24 hours. The mixture was then cooled to room temperature and the solvent removed under reduced pressure. The solid was purified by column chromatography with 3:1 petroleum ether/ethyl acetate as the mobile phase to obtain a light yellow product. The yield of pure product was 2.51 g (88%). ^{1}H NMR ($CDCl_3$): δ, 2.55 (s, 3H), 3.95 (s, 3H), 6.85 (d, 1H), 7.95 (d, 1H), and 8.35 (s, 1H).

4,4,4-Trifluoro-1-(3-iodo-4-methoxyphenyl)butane-1,3-dione (4)[10]

3-Iodo-4-methoxyacetophenone, **3,** (1.17 g, 4.24 mmol) was transferred to a double necked flask equipped with a reflux condenser and a septum protected with a Drierite guard tube. The solid was dissolved in dry methanol (2.7 mL) and sodium methoxide (1.3 mL, 25% solution in methanol) was added. The mixture was stirred for 5 minutes. Methyl trifluoroacetate (0.60 mL, 5.0 mmol) was then added. After refluxing for 24 hours, the reaction mixture was cooled to room temperature. The solvent was removed under reduced pressure and the reaction was quenched with HCl (3N, 11 mL). The product was extracted with ethyl acetate (4x7.5 mL), and the extracts dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under reduced pressure, and the brown oil was purified by column chromatography using a 3:1 petroleum ether/ethyl acetate mobile phase. This gave 0.74 g (66%) of a light pink solid. ^{1}H NMR ($CDCl_3$): δ, 4.00 (s, 3H), 6.50 (s, 1H), 6.90 (d, 1H), 7.95 (d, 1H), and 8.40 (s, 1H).

4-[5-(3-Iodo-4-methoxyphenyl)-3-trifluoromethylpyrazol-1-yl] benzenesulfonamide (6)[10]

Dione **4** (1.27 g, 3.41 mmol) was transferred to a double necked flask equipped with a reflux condenser and a septum protected with a Drierite guard tube. The compound was dissolved in dry ethanol (50 mL) and 4-sulfonamidophenylhydrazine hydrochloride, **5,** (0.90 g, 4.0 mmol) was added to the solution. The reaction mixture was then refluxed and stirred for 20 hours. The solution was cooled to room temperature and solvent was removed under reduced pressure. The product was purified by a flash chromatography column (SiO_2) using petroleum ether/ethyl acetate (2:1) as the mobile phase; a yellow solid was obtained. The iodinated methoxy celecoxib derivative **6** weighed 1.16 g (65%): melting point 183-185 °C. ^{1}H NMR ($CDCl_3$): δ, 3.69 (s, 3H), 4.89 (s, 2H), 6.73 (s, 1H), 6.74 (d, 1H, J = 8.5 Hz), 7.02 (s, 1H, J = 8.5 Hz), 7.47 (d, 2H, J = 8.5 Hz), 7.75 (s, 1H), 7.92 (d, 2H, J = 8.5 Hz).

4-[5-(4-Methoxy-3-trimethylstannanylphenyl)-3-trifluoromethylpyrazol-1-yl] benzenesulfonamide (7)

The iodinated methoxy celecoxib derivative **6** (0.55 g, 1.1 mmol) was placed in a double necked flask equipped with a reflux condenser and flushed with nitrogen. Anhydrous 1,4-dioxane (12 mL), hexamethylditin (0.540 g, 1.65 mmol), and tetrakis(triphenylphosphine)palladium (~0.05 g) were added sequentially. The reaction mixture was stirred at reflux for 2.5 hours. The solution was cooled to room temperature and filtered. The solvent was removed under reduced pressure, and the tin compound **7** was purified by column chromatography with 2:1 petroleum ether/ethyl acetate as the mobile phase. A yellowish gel was obtained in a yield of 0.50 g (81%). ^{1}H NMR ($CDCl_3$): δ, 0.27 (s with Sn satellites, J Sn-CH = 54 Hz, 9H), 3.61 (s, 3H), 4.91 (s, 2H), 6.73 (s, 1H), 6.75 (d, 1H, J = 8.5 Hz), 7.14 (d, 1H, J = 8.5 Hz), 7.24 (d, 1H), 7.50 (d, 2H, 8.5 Hz) and 7.94 (d, 2H, J = 8.5 Hz).

4-[5-(3-Iodo-4-methoxy-phenyl)-3-trifluoromethyl-pyrazol-1-yl]-benzensulfonamide (6) from Stannane precursor

To a solution of tin precursor **7** (11. 4 mg, 0.02 mmol) in methanol (0. 3 mL) was added a solution of sodium iodide (6.0 mg, 0.04 mmol) in water (0. 1 mL). Peracetic acid (1.0 mL, 0.3 % methanolic solution, 0.04 mmol) was slowly added and the reaction mixture stirred for 10 min. Excess iodine was decomposed by a drop of 10% sodium thiosulfate solution. The progression of the reaction was followed on TLC by comparing with an authentic sample. A major portion of the starting tin precursor was transformed to the iodo compound **6**.

Conclusions: The iodinated methoxy celecoxib derivative **6** was synthesized and regenerated after stannylation via an oxidative iodination suitable for the preparation of radioiodinated celecoxib derivative **6**. The fact that the iodinated

compound was easily regenerated indicates that the radiolabeling will be successful. Radiolabeling experiments are currently in progress.

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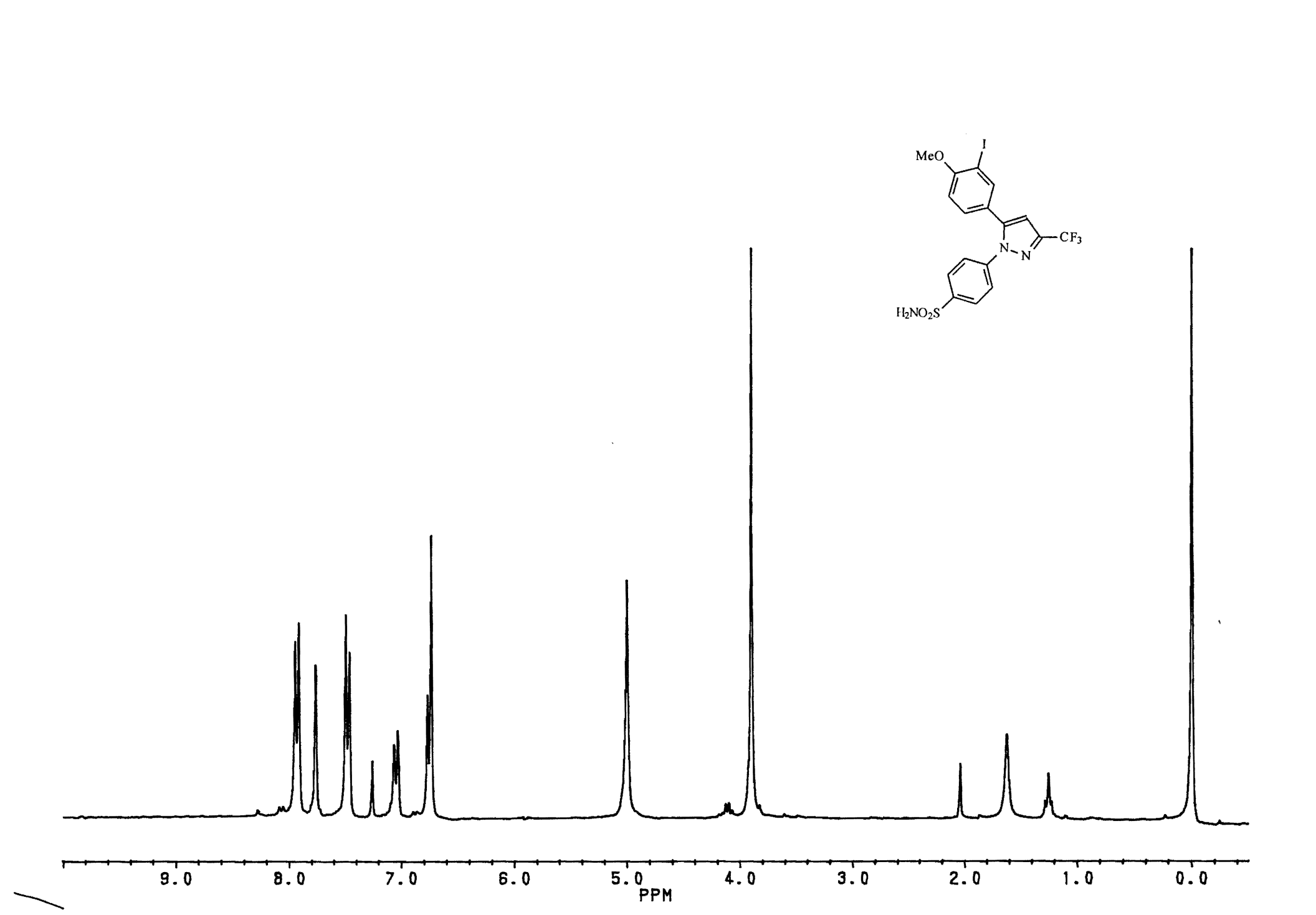
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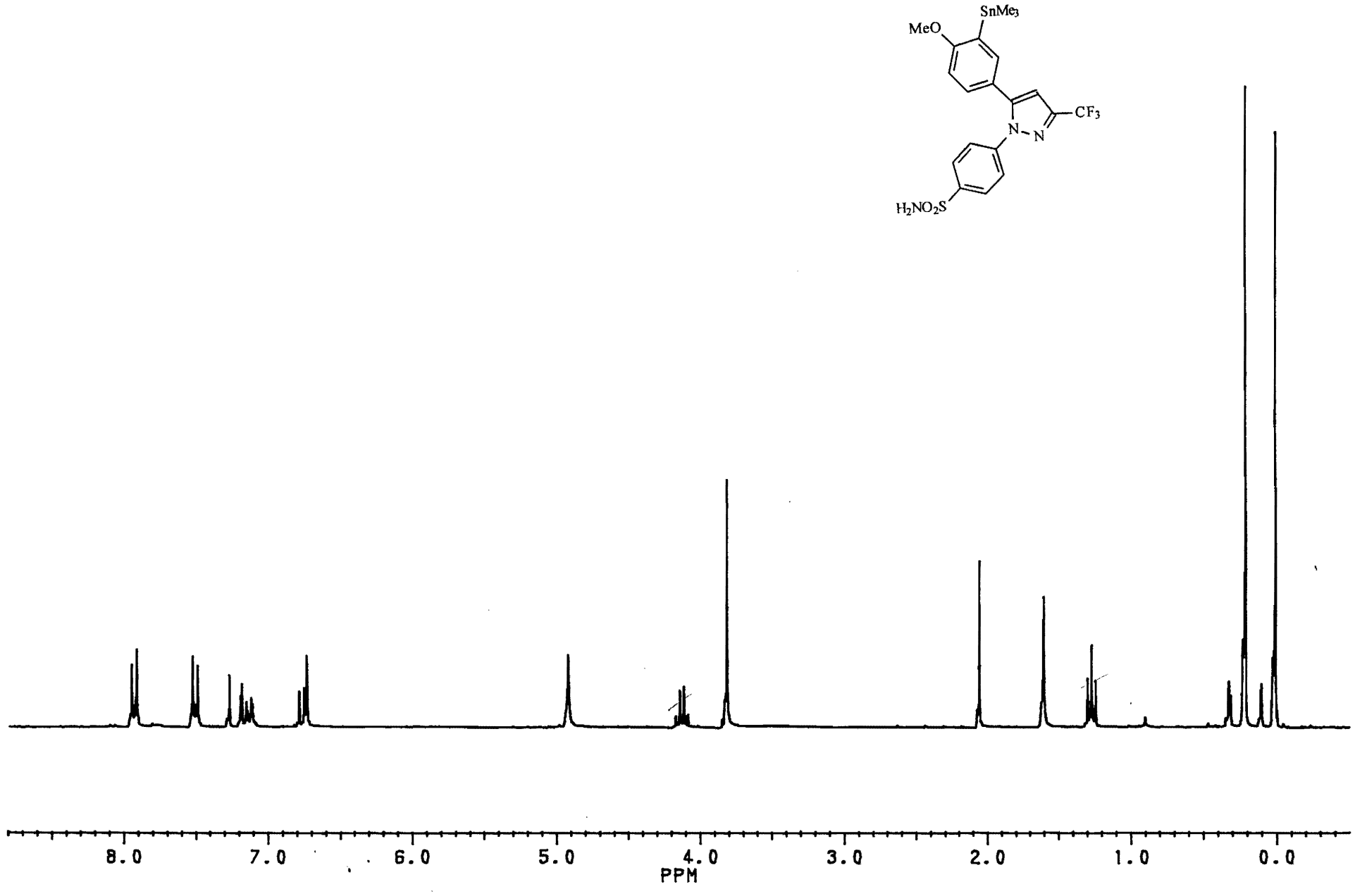
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