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Laboratory Experiment

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Facilitating Students' Review of the Chemistry of Nitrogen-Containing Heterocyclic Compounds and Their Characterization through Multistep Synthesis of Thieno[2,3-*b*]Pyridine Derivatives

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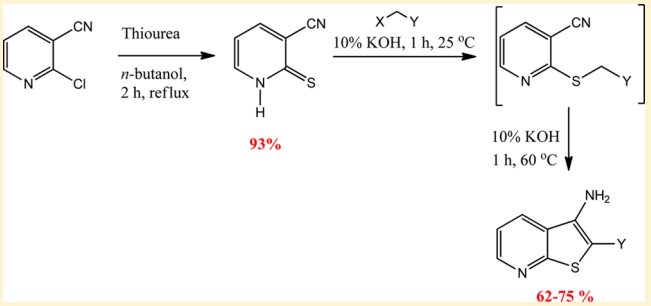
Supporting Information

ABSTRACT: A multistep synthesis of thieno[2,3-*b*]pyridine derivatives is described that is suitable for the upper-level undergraduate organic laboratory. This experiment exposes students to various hands-on experimental techniques as well as methods of product characterization such as IR and ^{1}H NMR spectroscopy, and mass-spectrometry. It also provides an opportunity for students to review important theoretical topics such as nucleophilic substitution, tautomerization, acidity of methylene-active compounds, base-promoted alkylation, and cyclization.



KEYWORDS: *Upper-Division Undergraduate, Organic Chemistry, Hands-On Learning/Manipulatives, Heterocycles, IR Spectroscopy, NMR Spectroscopy, Nucleophilic Substitution, Synthesis, Thin Layer Chromatography, Laboratory Instruction*

INTRODUCTION

Thienopyridines have attracted attention of many synthetic chemists over the years for their straightforward, mild synthetic pathways offering high yields and relatively pure products as well as their usefulness and practical applications in the biological and industrial fields.[1−6] Thienopyridine can appear as six isomeric structures, each differing according to their annealed positions.[1] Thieno[2,3-*b*]pyridine is one of the possible isomers and attracts scientific attention due to its wide range of biological utilities, including negative allosteric modulators of metabotropic GluR5 receptors,[2]anticancer agents,[3] anti-Alzheimers and anti-COX-2 activities,[4] and inhibitors of the human copper-trafficking proteins Atox1 and CCS.[7] Thieno[2,3-*b*]pyridines are also good synthons for the synthesis of different fused heterocyclic ring systems with a wide spectrum of biological activities.[1,6]

The chemistry of heterocyclic compounds is a large branch of modern organic chemistry. At the same time this topic is normally covered by one or two lectures during an organic chemistry undergraduate university course. Introducing a laboratory experiment related to the chemistry of heterocyclic compounds helps students focus on this topic and explore this branch of organic chemistry.

A laboratory experiment for the multistep synthesis of thieno[2,3-*b*]pyridine derivatives was adapted and modified to acquaint upper-division undergraduate organic chemistry students with the synthesis of heterocyclic compounds with fused rings.[8,9]Performing multistep syntheses in their undergraduate laboratory allows students to experience authentic organic chemistry research from technical procedures to detailed product analysis as well as performing retrosynthetic calculations. Several successful multistep syntheses have been reported in this journal but our proposed synthesis appears to be the first student laboratory experiment involving the synthesis of thieno[2,3-*b*]pyridine systems.[10−15] This experiment has been successfully used three times by 336 students in our third-year advanced organic chemistry courses. Students were required to submit a final individual report in a scientific journal style after completing their experiment.

EXPERIMENTAL SECTION

Students worked on this project individually, and each student was assigned to synthesize one of the final products **5**, **6**, or **8**, respectively (Scheme 1). Students were asked to perform retrosynthetic calculations for the desired amount of one of the assigned final products **5**, **6**, or **8** prior to coming to the laboratory (Supporting Information).

Three 4 h laboratory periods were required to finish the experiment. During the first lab period 2-thioxo-1,2-dihydropyridine-3-carbonitrile **2** was synthesized upon reflux of 2-chloronicotinonitrile **1** with equimolar amounts of thiourea in *n*-butanol. Product **2** began to precipitate as yellow needles after 1 h of reflux and the reaction was completed in 2−2.5 h. This synthesis was monitored by thin layer chromatography (TLC) until the complete disappearance of the initial compound **1** was observed. Product **2** was separated by the

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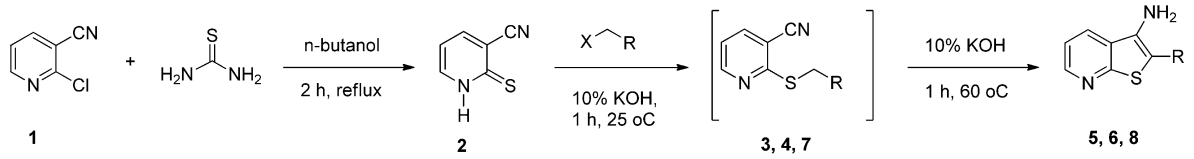
Scheme 1. Synthetic Route for the Synthesis of Thieno[2,3-*b*]pyridine Derivatives



Compounds 3, 5: X = Cl; R= (CO)NH2
Compounds 4, 6: X = Br; R = (CO)OC_2H_5.
Compounds 7, 8: X = Cl, R = CN

end of the first lab period, and normally no purification was required. Full characterization of this product (IR sample, ^{1}H NMR spectrum, melting point, and physical properties) was partially performed at the end of first lab period and at the beginning of the second week of the project. The resulting thione **2** has a good chromophore group and therefore the UV−vis analysis could also be done if time permitted. At the beginning of the second lab period students initially combined compound **2** with equimolar amounts of the assigned alkyl halide and potassium hydroxide solution in DMF at room temperature. The reaction was monitored by TLC until the complete formation of intermediate products **3**, **4**, and **7**. This reaction required 1−2 h to complete and its rate depended on the structure of the initial halide. The second aliquot of potassium hydroxide was added after the completion of the first step of the reaction was confirmed by TLC analysis. The reaction mixture was then heated with stirring at 60 °C for about 1 h. Final products **5**, **6**, or **8** were isolated at the end of second lab period, or at the beginning of the third lab period. Their recrystallization from ethanol, or methanol, was completed during the third lab period along with characterization by melting point, IR samples, ^{1}H NMR spectra, and mass-spectrometry. Detailed procedures are described in the Supporting Information.

Alternatively, thieno[2,3-*b*]pyridines can be synthesized by the reaction of chloropyridine **1** with commercially available thiols with methylene active groups.[1,5,6] This method is one step shorter but less versatile as the list of all available thiols with methylene active groups is not extensive. It is important to mention that the synthetic route for the formation of compound **8** described in this article produced better yields compared to the method described in the most recent research paper.[5]

HAZARDS

All chemicals and solvents must be handled with care according to the information available on their Material Safety Data Sheets (MSDS). Personal protection, such as safety glasses, gloves, and lab coats, must be worn during the experiment. All procedures must be carried out under a well-ventilated fume hood. Discharge of chemicals used in this project into the environment must be avoided. *n*-Butanol, acetone, hexanes, and ethanol are flammable liquids and must be kept away from flames. Hexanes is a known neurotoxin. DMF is hazardous in case of skin and eye contact and by inhalation and ingestion. Thiourea (CAS # 62-56-5) is a potential carcinogen and teratogen and must be handled with extreme care. Ethyl bromoacetate (CAS # 105-36-2) is combustible and acutely toxic; gloves must be worn at all times when dealing with this chemical. 2-Chloroacetamide (CAS # 79-07-2) and chloroacetonitrile (CAS # 107-14-2) are both toxic by ingestion and skin and eye irritants and must be handled with extreme care. 2-Chloro-3-pyridinecarbonitrile (CAS # 6602-54-6) and 1,2-dihydro-2-thioxo-3-pyridinecarbonitrile (CAS # 52505-45-0) are irritants and toxic if inhaled or ingested. Potassium hydroxide is corrosive. MSDS data are not available for the products. Therefore, students should handle them as hazardous following standard safety protocols.

RESULTS AND DISCUSSION

The first step in the synthetic pathway was the synthesis of thiolactam **2**. Students obtained **2** with a yield of 85−97% (Table 1) and decent level of purity. Product **2** could exist in two tautomeric forms; the thione isomer was more stable under normal conditions.[1] Formation of the thiolactam tautomer was confirmed by the IR spectrum which had a secondary amide peak around 3340 cm^{-1}. The ^{1}H NMR spectrum in DMSO-d_6 had a singlet at 14.24 ppm for the thioamide NH group which supported the proposed structure. Representative student spectra can be found in the Supporting Information. Students started their second lab period with alkylation of **2** using their assigned alkyl halide and one equivalent of potassium hydroxide. This reaction was relatively quick, typically taking about 30 min, and was monitored by TLC until disappearance of **2** in the reaction mixture. The reaction could be quenched by adding a sufficient amount of ice-cold water which caused the precipitation of the intermediate compounds **3**, **4**, and **7**. These compounds can be separated by vacuum filtration. In our case, students did not perform separation and characterization of **3**, **4**, and **7**. Instead, they immediately proceeded with the cyclization step by adding one more equivalent of potassium hydroxide solution to the reaction mixture and raising the temperature up to 60 °C. The cyclization was completed within 1 h. TLC analysis showed an absence of the intermediate spot in the reaction mixture. Products were isolated by the addition of ice cold water to the reaction mixture and purified by recrystallization from ethanol or methanol. Yields for the final products were not as high as for the thiolactam **2**, but they were in a good range of 65 to 72% depending on the initial alkyl halide used for this step (Table 1). Structures of compounds **5**, **6**, and **8** were confirmed with ^{1}H NMR in DMSO-d_6 by noting the appearance of primary amino group at 7.00−7.18 ppm and

Table 1. Typical Practical Yield for Each Compound Obtained by Students

Compound	Avg. Student Yields, %[a]	Student Yield Range, %[a]
2	93	85−97
5	65	33−88
6	57	24−86
8	72	45−92

[a]Percentage yields based on experimental trials for three classes of 112 students per class.

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absence of the methylene (CH_2) shift at 4 ppm typical for the intermediates **3**, **4**, and **7**.[8] Students' analysis of the IR spectra of **5**, **6**, and **8** showed the presence of NH_2 peaks and disappearance of the C≡N stretch of precursor **2** at 2211 cm^{-1} (Supporting Information). Most students were able to finish this experiment with adequate yield (Table 1) and purity. They reported their practical data in a formal laboratory report format using ChemDraw and bibliographic writing programs. Students correctly characterized intermediate and final products using physical property analysis and IR, MS (by molecular ion and base peak comparison with known compounds available through online databases), and ^1H NMR spectral data. The students' assignments and final reports demonstrated that they understood the concept of multistep organic synthesis, are capable of analyzing practical data, and can write their reports using modern software available in chemistry.

■ CONCLUSION

The described laboratory experiment introduced students to multistep synthesis of thieno[2,3-*b*]pyridine derivatives adopted from scientific literature. Preparing prelab notes and performing the laboratory experiment helped students to learn more about the chemistry of heterocyclic compounds, multistep synthesis of fused aromatic systems, as well as their biological activity and applications in modern technology. The experiment exposed students to different hands-on laboratory techniques, retrosynthetic calculations, and provided an opportunity to analyze practical results and discuss them in a formal laboratory report. We believe that skills and knowledge gained performing this experiment will be useful in the students' future in chemistry.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00909.

Students' handouts, notes to the instructor, experimental data for each compound, list of chemical and reagents (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Litvinov, V. P.; Dotsenko, V. V.; Krivokolysko, S. G. Thienopyridines: synthesis, properties, and biological activity. *Russ. Chem. Bull.* **2005**, *54* (4), 864−904.

(2) Nógrádi, K.; Wágner, G.; Domány, G.; Bobok, A.; Magdó, I.; Kolok, S.; Mikó-Bakk, M. L.; Vastag, M.; Sághy, K.; Gyertyán, I.; Kóti, J.; Gál, K.; Farkas, S.; Keserű, G. M.; Greiner, I.; Szombathelyi, Z. Thieno[2,3-b]pyridines as negative allosteric modulators of metabotropic GluR5 receptors: Lead optimization. *Bioorg. Med. Chem. Lett.* **2015**, *25* (8), 1724−1729.

(3) Arabshahi, H. J.; Leung, E.; Barker, D.; Reynisson, J. The development of thieno[2,3-b]pyridine analogues as anticancer agents applying in silico methods. *MedChemComm* **2014**, *5* (2), 186−191.

(4) Attaby, F. A.; Abdel-Fattah, A. M.; Shaif, L. M.; Elsayed, M. M. Anti-Alzheimer and anti-COX-2 activities of the newly synthesized 2,3-bipyridine derivatives (II). *Phosphorus, Sulfur Silicon Relat. Elem.* **2010**, *185* (3), 668−679.

(5) Okuda, K.; Ide, R.; Uramaru, N.; Hirota, T. Polycyclic N-Heterocyclic Compounds. Part 84: Reaction of N-(pyrido[3′,2′:4,5]-thieno[3,2-d]pyrimidin-4-yl)amidines or N-(pyrido[2′,3′:4,5]furo[3,2-d]pyrimidin-4-yl)amidines with Hydroxylamine Hydrochloride. *J. Heterocyclic Chem.* **2015**, *52* (3), 880−887.

(6) Hung, J. M.; Arabshahi, H. J.; Leung, E.; Reynisson, J.; Barker, D. Synthesis and cytotoxicity of thieno[2,3-b]pyridine and furo[2,3-b]pyridine derivatives. *Eur. J. Med. Chem.* **2014**, *86*, 420−437.

(7) Wang, J.; Luo, C.; Shan, C.; You, Q.; Lu, G.; Elf, S.; Zhou, Y.; Wen, Y.; Vinkenborg, J. L.; Fan, J.; Kang, H.; Lin, R.; Han, D.; Xie, Y.; Karpus, J.; Chen, S.; Ouyang, S.; Luan, C.; Zhang, N.; Ding, H.; Merkx, M.; Liu, H.; Chen, J.; Jiang, H.; He, C. Inhibition of Human Copper Trafficking by a Small Molecule Significantly Attenuates Cancer Cell Proliferation. *Nat. Chem.* **2015**, *7* (12), 968−979.

(8) Kaigorodova, E. A.; Konyushkin, L. D.; Mikhailichenko, S. N.; Vasilin, V. K.; Sazonov, A. V.; Kul'nevich, V. G. Synthesis and reactions of 3-cyano-6-methoxymethyl-4-methyl-2(1H)- Pyridinethione. *Chem. Heterocycl. Compd.* **1999**, *35* (3), 293−296.

(9) Kaigorodova, E. A.; Konyushkin, L. D.; Mikhailichenko, S. N.; Vasilin, V. K.; Kul'nevich, V. G. Synthesis of substituted 2-alkyl(aryl)thio-3-cyanopyridines and 3-aminothieno[2,3-b]pyridines. *Chem. Heterocycl. Compd.* **1996**, *32* (10), 1234−1238.

(10) Sichula, V. A. Synthesis of 10-Ethyl Flavin: A Multistep Synthesis Organic Chemistry Laboratory Experiment for Upper-Division Undergraduate Students. *J. Chem. Educ.* **2015**, *92* (9), 1539−1542.

(11) Marchetti, L.; DeBoef, B. Solution-Phase Synthesis of Dipeptides: A Capstone Project That Employs Key Techniques in an Organic Laboratory Course. *J. Chem. Educ.* **2015**, *92* (9), 1536−1538.

(12) Demare, P.; Regla, I. Synthesis of two Local Anesthetics from Toluene: An Organic Multistep Synthesis in a Project-Oriented Laboratory Course. *J. Chem. Educ.* **2012**, *89* (1), 147−149.

(13) Schatz, P. F. Synthesis of Chrysanthemic Acid. *J. Chem. Educ.* **1978**, *55* (7), 468−470.

(14) Peters, D. G.; Ji, C. A Multistep Synthesis for an Advanced Undergraduate Organic Chemistry Laboratory. *J. Chem. Educ.* **2006**, *83* (2), 290−291.

(15) Chen, X. Y.; Li-Sen Sun, L. S.; Gao, X.; Sun, X. W. Diastereoselective Allylation of N-tert-Butanesulfinyl Imines: An Asymmetric Synthesis Experiment for the Undergraduate Organic Laboratory. *J. Chem. Educ.* **2015**, *92* (4), 714−718.

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