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Synthesis of 2-methoxypropyl benzene for epitope imprinting

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PURPOSE: To synthesize a novel, yet simple, compound for use in the development of a molecularly imprinted sensor for field-portable detection of harmful algal bloom toxins.

BACKGROUND: Harmful algal blooms (HABs) are occurring with increasing frequency and severity across the globe in part due to climate change and anthropogenic pollution (Bullerjahn et al. 2016). HABs produce several classes of toxins; however, microcystins (MCs) are the most commonly studied (Lone et al. 2015) and can be potent toxins with LD50s in the range of 50 µg/kg (Puddick et al. 2014). Sample analysis in laboratories, typically by high-pressure liquid chromatography tandem mass spectrometry (HPLC-MS/MS) or by Enzyme Linked Immunosorbent Assays (ELISAs) (USEPA 2015). These analytical techniques are highly sensitive and selective for the given toxins; however, the time it takes to collect, transfer, prepare, and analyze a sample before the data can be reported is significant; often, multiple days is the most expeditious.

Detecting whether a given algal bloom is toxic in near real-time is paramount to maintaining drinking water supplies and human health. A field-portable sensor could achieve this goal. A functional sensor requires a recognition element, a material that selectively interacts with the target analyte, which in electrochemical sensor applications is referred to as a transducer. Molecularly Imprinted Polymers (MIPs) are a promising technology in this regard as their specificity is approaching that of biological enzymes (Fernando et al. 2021). In fact, MIPs have been utilized with increasing frequency for the detection of algal toxins, especially MCs (Fernando et al. 2021).

Formation of the MIP can be accomplished directly on the surface of an electrode by utilizing an electropolymerizable monomer, such as *o*-phenylenediamine, in the presence of the analyte/template to fabricate the transducer element. Of the over 100 known MC based toxins each bears a common conserved moiety, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (ADDA). In fact, this moiety is also common amongst *nodularins* (ND) (Carmichael and An 1999). As such, if an electrode could be imprinted with this moiety conserved across all MC and ND toxins, a common sensor for these classes of chemicals could be fabricated. This is graphically represented in Figure 1.

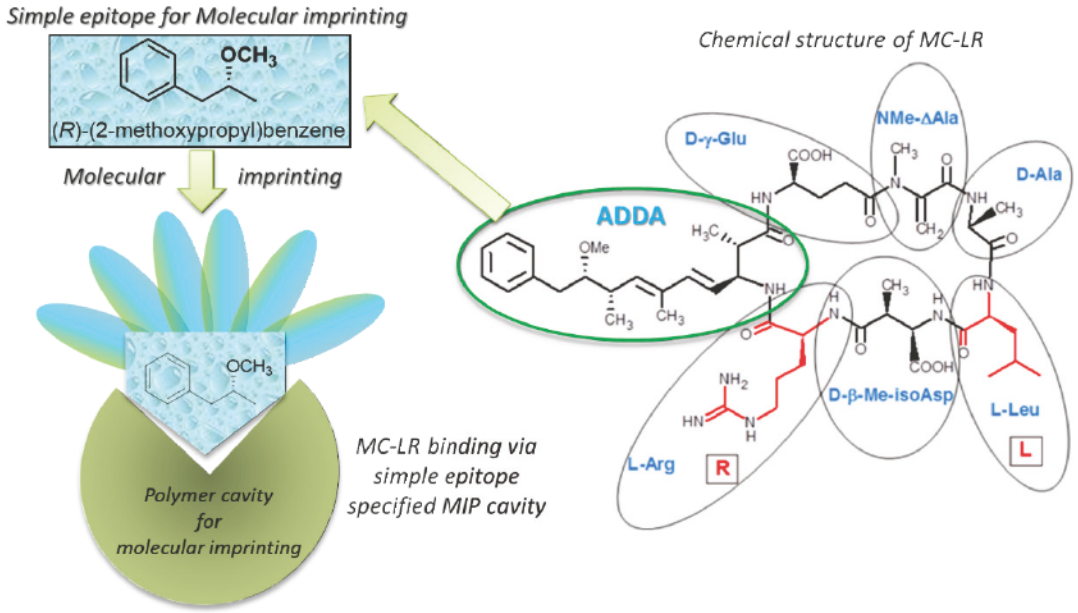
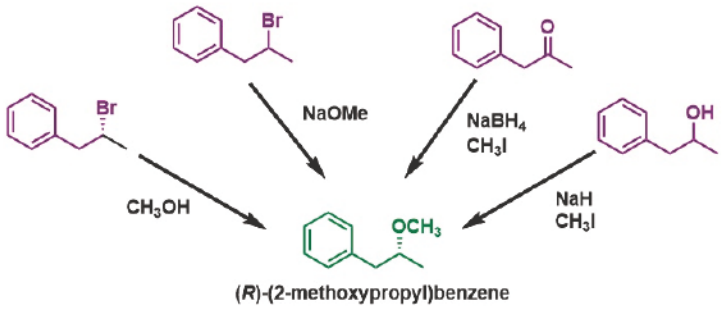


Figure 1. Structure of MC-LR, Proposed epitope imprinting using 2-methoxypropylbenzene, and detection of MC-LR using the imprinted cavities.

This larger group is not commercially available and represents a significant challenge to be synthesized. Therefore, a smaller portion of the ADDA unit, (*R*)-(2-methoxypropyl)benzene, was identified as a target molecule for synthesis with many synthetic approaches available (Scheme 1). Although the synthesis of (*R*)-(2-methoxypropyl)benzene is relatively facile, separating the correct enantiomer, the rectus (*R*), may present some challenges to develop a sensor selective for the algal toxins of interest. Herein, we report the successful synthesis of the target molecule as a racemic mixture (a 50:50 mixture of the desired (*R*)- and byproduct (*S*)- enantiomers) are confirmed by utilizing two synthetic procedures (sodium borohydride method and sodium hydride method).



Scheme 1. Proposed synthetic routes for the synthesis of the desired epitope.

MATERIALS AND METHODS: All reagents were used as received. 1-phenyl-2-propanol (99%) was purchased from Acros Organics. Potassium *t*-butoxide (98%), anhydrous tetrahydrofuran (THF, >99.9%), and iodomethane (99.5%) were purchased from Sigma Aldrich (St. Louis, MO). Ethyl acetate (EtOAc, HPLC Grade) and ammonium chloride (Certified ACS) were purchased from Fisher

Scientific. Sodium sulfate (Na_2SO_4, 99%) was purchased from Mallinckrodt. Sodium hydride (NaH, 60% dispersion in mineral oil) was purchased from Spectrum Chemical Manufacturing Group.

Method A. 1-phenyl-2-propoanol (681 mg, 5 mmol) was dissolved in anhydrous THF (10 mL) in a dried 3-neck round bottom flask under nitrogen. Potassium *t*-butoxide (1.34 g, 12 mmol) was added to the reaction mixture and ultrasonicated. The reaction mixture was then placed in an ice bath for 15 min ensuring complete deprotonation of the alcohol. While maintaining the flask in the ice bath, iodomethane (650 µL, 10 mmol) was added dropwise under nitrogen. The reaction was allowed to stir overnight under nitrogen at room temperature. The solvent was removed via rotary evaporation under reduced pressure followed by the addition of EtOAc (60 mL), and the crude reaction mixture transferred to a separatory funnel. The crude reaction mixture was washed sequentially with DI water (2 × 50 mL) and twice with brine (2 × 50 mL). The aqueous layers were combined and extracted with EtOAc. The organic layers were combined and dried over Na_2SO_4 and the solids were removed *via* decantation. Percent yield for this reaction ranged from 50 – 60%.

Method B. NaH (182 mg, 4.55 mmol) was added to a dried 3-neck round bottom flask under nitrogen. The flask was placed in an ice bath and chilled prior to the addition of THF (10 mL). 1-phenyl-2-propanol (511 µL, 3.65 mmol) was dissolved in THF (26 mL) in a separate vial and added dropwise to the chilled 3-neck flask under nitrogen. The flask warmed to room temperature after the addition was complete and stirred for 30 minutes. Iodomethane (312 µL, 4.34 mmol) was added and the reaction stirred for 14 hours under nitrogen. The excess hydride was quenched with the addition of saturated NH_4Cl (35 mL) before transferring to a separatory funnel. The crude product mixture was extracted with diethyl ether (3 x 50 mL). The combined organic layers were washed with brine (2 x 50 mL) then dried over Na_2SO_4, and the solids were removed *via* decantation. Percent yield for this reaction ranged from 45 – 55%.

Purification. In both methods the combined organic solvent recovered from extraction steps were removed *via* rotary evaporation under reduced pressure and purified using an automated flash column chromatograph (Combiflash, Teledyne Isco, Lincoln, NE). A 40 g gold grade silica column was used. Flow rate was set to be 60 mL/min.

Characterization. Proton (^1H) and carbon (^{13}C) nuclear magnetic resonance spectra were collected on a Bruker Avance 300 MHz spectrometer. $CDCl_3$ was used as the deuterated solvent of choice.

RESULTS AND DISCUSSION: The synthesis by both routes shows the formation of a new product when visualized by thin layer chromatography (TLC) shown in Figure 2. The products from each method generate a less polar group than the starting material. This is consistent with fundamental organic chemistry principles where ethers are less polar than alcohols. Therefore, on the TLC plate, the top spot corresponds to the desired ether compound and the smaller less prominent spot corresponds to any unreacted starting materials.

Figure 3 shows a typical combi-flash chromatograph. Both runs were conducted in EtOAc: Hexane mixtures. Starting materials and desired peaks are highlighted. The desired product was easy to purify due to the decreased polarity. The product came out of the silica column at 100% hexane between 1.0 and 3.0 minand appears as two peaks. This is potentially indicative of the different enantiomeric products; however, the flash chromatogram resolution is not sufficient to separate these two peaks. Unreacted starting materials eluted around 6.0 min with slight increase of ethyl

acetate to 10%. The remaining chromatogram shown in Figure 3 is residual solvent signal. Once the product was separated by combi-flash, TLC was performed to further ensure the correct product was separated via flash chromatography. Hence there is a possibility that these can be separated using liquid chromatography system.

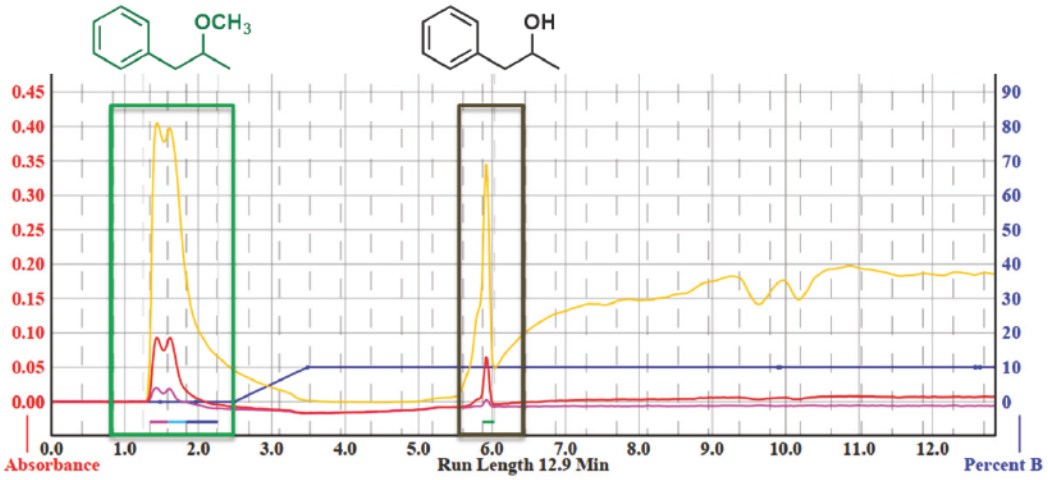


Figure 2. Flash chromatogram for desired product (green box) and starting material (black box). Three wavelengths were used to detect peaks, 254 nm (red trace), 280 nm (purple trace), and sum of all wavelengths from 200 to 400 nm (gold trace).

The concentrated oils were dissolved in deuterated chloroform, and the resultant nuclear magnetic resonance spectra for both proton (^{1}H) and carbon (^{13}C) are shown in Figure 4 and Figure 5, respectively.

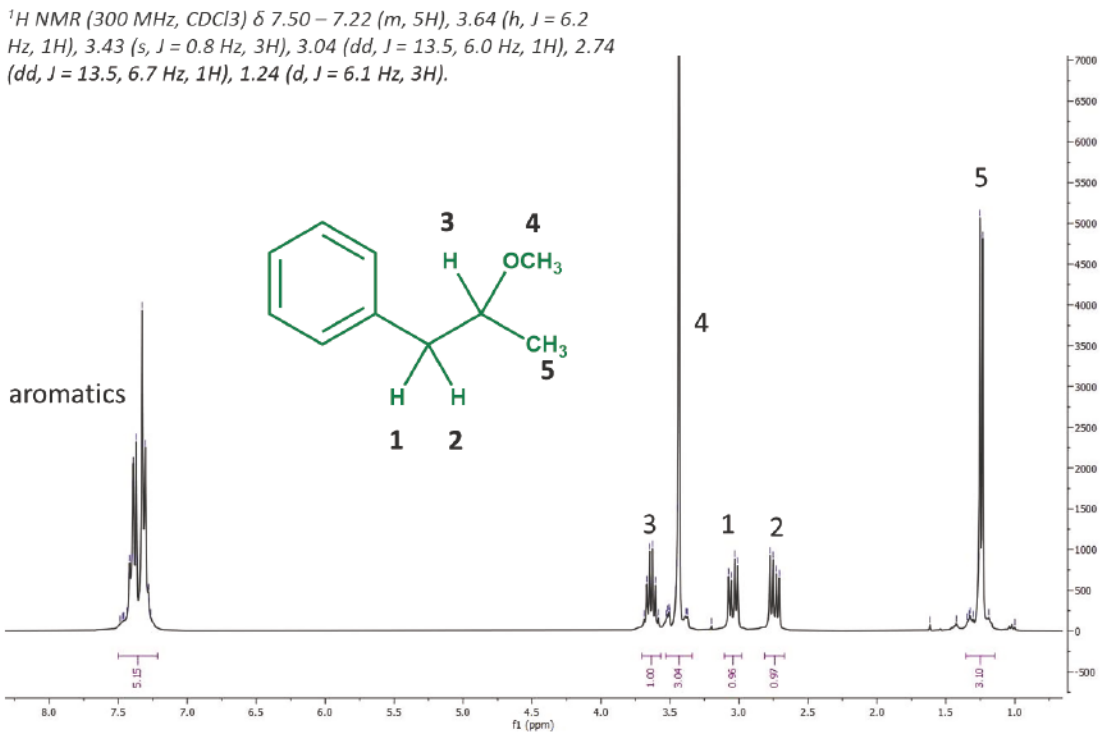


Figure 3. Proton (^{1}H) NMR spectrum of (2-methoxy)propyl benzene in $CDCl_3$.

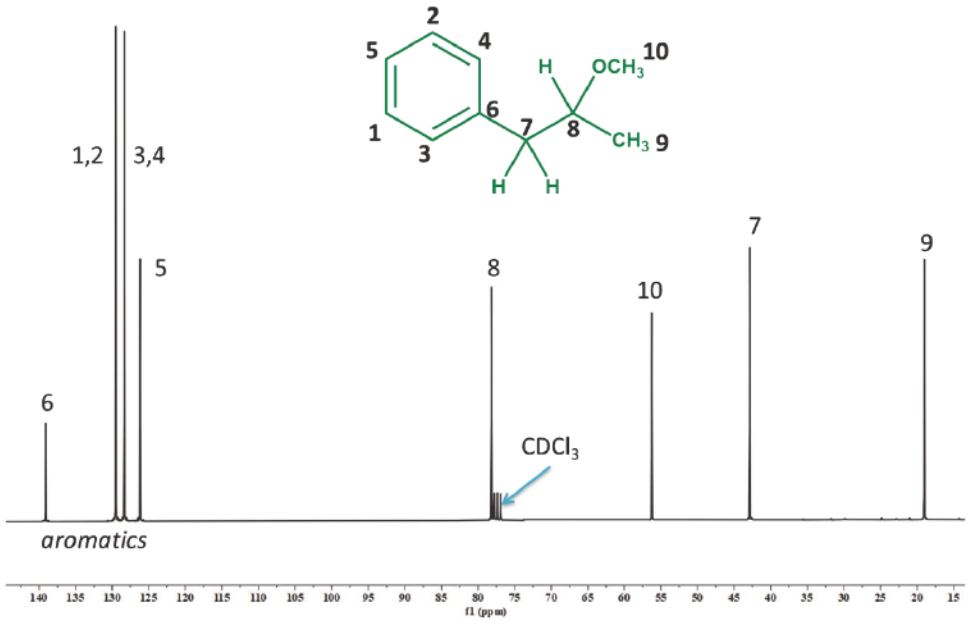


Figure 4. Carbon (^{13}C) NMR spectrum of (2-methoxy)propyl benzene in $CDCl_3$.

The spectra of the purified products from methods A and method B are shown in Figure 6 overlaid with the starting material common to both methods, (2-hydroxy)propyl benzene, in Figure 5. The most significant change observed in the spectra is the splitting of the peak at ~2.75 and 3.00 ppm,

the benzylic position. This is indicative of the prochirality of the product where the 2-bond coupling is being observed. Due to this, the peaks are true doublets of doublets (dd). The addition of a peak at ~3.4 ppm shows the successful methylation of the hydroxyl group.

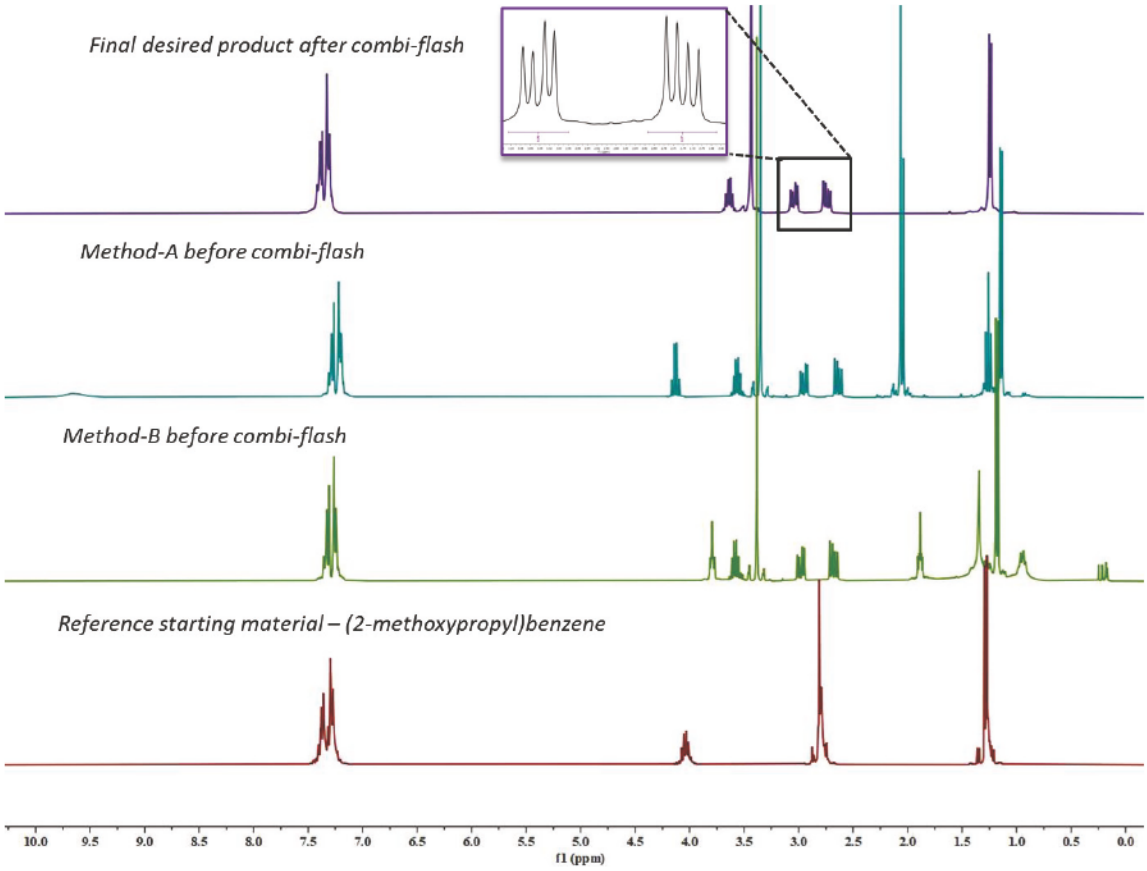


Figure 5. Stacked ^{1}H NMR spectra of all the steps as mentioned in the above figure.

SUMMARY: The synthesis of the target molecule utilizing two synthetic approaches has been successfully demonstrated. The simplicity of each method will enable scaling-up of the synthesis if the need arises for a larger quantity. Although commercially available, 2-methoxypropyl benzene, is only sold as a racemic mixture which was isolated here. Future efforts may require the isolation of the *R*- enantiomer to achieve the necessary selectivity. The synthetic product will be utilized in future studies to develop a molecularly imprinted polymer (MIP) *via* an electropolymerization procedure. This enables the fabrication of a transducer element, effectively recognizing the (2-methoxy)propyl benzene molecule. As this molecule is a smaller subset of the conserved ADDA unit in all MC and ND algal toxins, the sensor will be tested for efficacy in detecting these harmful chemicals.

ADDITIONAL INFORMATION: This technical note was prepared with equal contributions by Dr. Lee C. Moores, Research Chemist, Environmental Laboratory (EL), US Army Engineer Research and Development Center (ERDC) (Lee.C.Moores@usace.army.mil), Dr. PU Ashvin Fernando, Senior Scientist, Bennett Aerospace Inc. Mr. Garrett George, Research Physical Scientist, EL, ERDC assisted with the synthesis and purification of the novel compound and drafting of this TN.

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