Aurora Analytics

Innovative Chemistry. Neoteric Solutions.

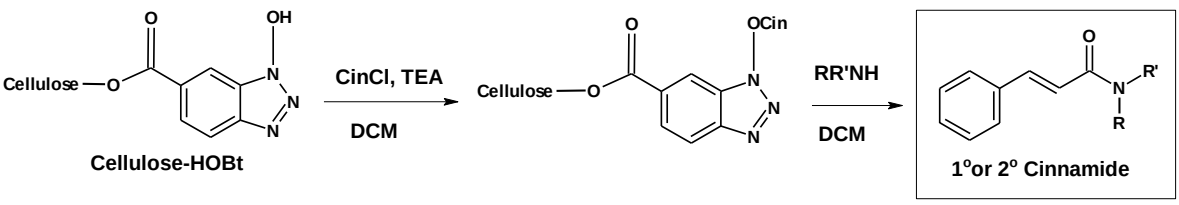
Thin Layer Chromatography – A Microscale Derivatization Experiment

Presented here is a detailed Experimental protocol for the Undergraduate Organic Laboratory Class utilizing Cellulose-HOBt (solid phase reagent) for the conversion of non-chromophoric amines to cinnamides that can then be analyzed by TLC with UV detection. Multiple cinnamide compounds can be quickly prepared with minimal amounts of solvents, no aqueous work-up and standard laboratory glassware. Derivatization of amines is a common technique that provides not only enhanced detection capability but improved chromatographic separations. The experiment is geared towards providing students not only with an understanding of TLC but also with their first hands on experience with **S**olid **P**hase **O**rganic **C**hemistry (SPOC).

In the first step of the experiment, a Cellulose-HOBt disk (in an SPE tube) is activated by the addition of cinnamoyl chloride to form the resin-bound activated cinnamate ester in a flow-through manner. The activated ester is then treated with a primary or secondary amine containing solution. The amine reacts to form the cinnamide compound, which is eluted from the solid-support. The eluent containing the product is readily analyzed for product formation by TLC analysis with UV detection.

The experiment provides an example of how Cellulose-HOBt may be used in the undergraduate laboratory to enhance the TLC experiment by incorporating a standard derivatization procedure. The experimental protocol is readily modified to allow for the preparation of cinnamide libraries and to identify mixtures of amines.

Overall reaction scheme for the preparation of cinnamides with Cellulose-HOBt:



Aurora Analytics LLC
1450 South Rolling Road, Baltimore, MD 21227
443-543-5799 | info@auroraanalytics.com | www.auroraanalytics.com

Experimental Procedure

Materials

Reagents (note 1):

Item	Vendor	Cat #	Description
Cellulose-HOBt disks	Aurora Analytics LLC	H1002-DSK-05	12.7 mm cellulose disks pre-loaded with HOBt
Cinnamoyl chloride	Sigma-Aldrich	C81101	98%, MW 166.60 g/mole, CAS# 102-92-1)
Triethylamine	Sigma-Aldrich	90335	TEA, ≥99.5%, MW 101.19 g/mole, d 0.726 g/mL, CAS# 121-44-8
Dichloromethane	Honeywell	LP300-4	Laboratory Plus, CAS# 75-09-2)
Ethyl acetate	EMD	EX0240-5	EtOAc, ACS reagent, CAS# 141-78-6
Hexanes	EMD	HX0299-5	ACS reagent, CAS# 110-54-3
n-Propylamine	Aldrich	240958	≥99%, MW 59.11 g/mole, d 0.719 g/mL, CAS# 107-10-8
n-Butylamine	Aldrich	471305	≥99.5%, MW 73.14 g/mole, d 0.740 g/mL, CAS# 109-73-9
n-Dodecylamine	Aldrich	D222208	98%, MW 185.35 g/mole, d 0.806 g/mL, CAS# 124-22-1
Pyrrolidine	Aldrich	83240	≥99.0%, MW 71.12 g/mole, d 0.852 g/mL, CAS# 123-75-1)
Morhpoline	Aldrich	252360	≥99.0%, MW 87.12 g/mole, d 0.996 g/mL, CAS# 110-91-08)
Ethanolamine	Aldrich	398136	≥99%, MW 61.08 g/mole, d 1.012 g/mL, CAS# 141-43-5)
Cinnamide standards (note 2)	Aurora Analytics LLC		including N-1-propyl, N-1-butyl, N-1-dodecyl, N-pyrrolidyl , N-morpholinyl and N-(2-hydroxyethyl)

Equipment and Supplies:

1) SPE tubes, 8 mL with frit (Grace Alltech, Cat# 210208, 8 mL Extract-Clean tube, Cat# 211408, frit for 8 mL tube)
2) Ring stand with small three-fingered clamp
3) 50 mL beaker
4) Small glass test tubes (12 x 75 mm or 13 x 100 mm, 2 required per experiment)
5) 1.0 mL graduated pipettes
6) Graduated cylinder (10mL)
7) Pasteur pipettes with bulb
8) TLC Supplies
    a. TLC plates pre-cut to 4 cm x 6.7 cm (EMD Millipore Cat# 105554, Kieselgel 60_{F254}, 0.2 mm aluminum backed TLC plates)

b. Spotters (microcapillary tubes)
c. Elution chamber (150 mL beaker)
d. Aluminum foil
e. Solvent: 60/40 EtOAc/hexanes (v/v)
f. UV lamp (254 nm)

Stock Solutions (note 3):

1) 0.5 M Cinnamoyl chloride stock solution
    a. 100 mL solution: Add 8.33 g cinnamoyl chloride to a 100 mL volumetric flask. Add 50 mL of DCM and swirl to dissolve the solid chloride. Bring the volume of the solution to the 100 mL mark with DCM. Replace stopper and mix thoroughly. Transfer contents to an amber glass bottle for use and storage. Useful for up to 100 experiments.
2) 1.5 M Triethylamine stock solution
    a. 100 mL solution: Add 20.91 mL of TEA to a 100 mL volumetric flask. Add 50 mL of DCM and swirl to form a homogeneous solution. Bring the volume of the solution to the 100 mL mark with DCM. Replace stopper and mix thoroughly. Transfer contents to an amber glass bottle for use and storage. Useful for up to 100 experiments.
3) 0.5 % w/v Cinnamide standard solutions
    a. 25 mL solution: Add 0.125 g of the desired cinnamide compound to a 25 mL volumetric flask. Add 15 mL of DCM and swirl to form a homogeneous solution. Bring the volume of the solution to the 25 mL mark with DCM. Replace stopper and mix thoroughly. Transfer contents to an amber glass vial/bottle for use and storage.
4) 1% w/v Amine solutions (note 4)
    a. 100 mL solution: Add 1.000 g of amine to a 100 mL volumetric flask. Add 50 mL of DCM and swirl to form a homogeneous solution. Bring the volume of the solution to the 100 mL mark with DCM. Replace stopper and mix thoroughly. Transfer contents to an amber glass bottle for use and storage. Useful for up to 200 experiments.
    b. Amount of amine required (mL)
        i. *n*-Propylamine: 1.39 mL
        ii. *n*-Butylamine: 1.36 mL
        iii. *n*-Dodecylamine : 1.241 mL
        iv. Pyrrolidine: 1.174 mL
        v. Morpholine: 0.996 mL
        vi. Ethanolamine: 0.988 mL

Apparatus Set – up

To set up the apparatus, mount the 8 mL SPE tube, using a ring stand with a three-fingered clamp (figure 1). Add a Cellulose-HOBt disk (figure 2) to the SPE tube and push down carefully until it is flush with the frit in the SPE tube. Place a 50 ml beaker under the secured tube to collect the reaction products.

Figure 1

Figure 2

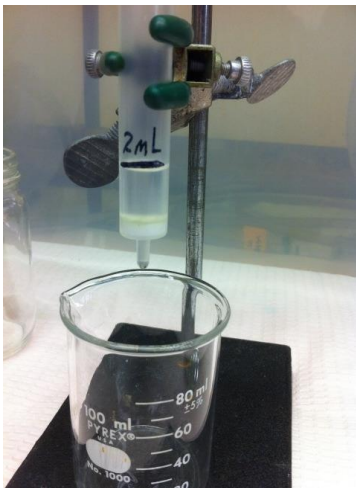


Figure 3

Procedure

Cinnamide Formation from Amine

1) Measure out 2 mL of DCM using a 10 mL graduated cylinder and transfer to the SPE tube. Allow the liquid to elute by gravity until no flow is observed (figure 3). Remove residual DCM in tube using a pipette bulb to generate gentle air pressure in tube to force the DCM to elute.

2) Cellulose-HOBt Activation Step: In a small test tube (12x75 mm or 13x100 mm) combine 1 mL of cinnamoyl chloride stock (0.5 M) with 1 mL of 1.5 M triethylamine (TEA) stock solution (both are in DCM and are to be dispensed with dedicated 1.0mL graduated pipettes). The solution will turn yellow and a white vapor may be observed. Mix with a disposable glass Pasteur pipette and then transfer to the column using same pipette. Allow the liquid to elute by gravity until no flow is observed.

3) Measure out 2 mL of DCM using a 10 mL graduated cylinder. Use a Pasteur pipette to transfer the DCM to the SPE tube in portions to carefully rinse the top and sides of the tube in order to remove last traces of the cinnamoyl chloride solution. Allow the liquid to elute by gravity until no flow is observed. If elution rate is very slow, a pipette bulb may be used to generate air pressure in tube to force the DCM to elute.

4) Measure out 1 mL of DCM using a 10 mL graduated cylinder. Use a Pasteur pipette to rinse the outlet of the SPE tube to remove any solid residue from the activation step. Use care to ensure the rinse flows into the 50 mL beaker.

5) Measure out 2 mL of DCM using a 10 mL graduated cylinder and transfer to the SPE tube. Allow the liquid to elute by gravity until no flow is observed. If elution rate is very slow, a pipette bulb may be used to generate air pressure in tube to force the DCM to elute.

6) Repeat **Step 5** five times. After final rinse, remove residual DCM in tube using a pipette bulb to generate gentle air pressure in tube to force the DCM to elute.

7) Replace the beaker with a small test tube (12x75 mm) held in place in a test tube rack or standing in a 25 mL Erlenmeyer flask. The solution collected in the beaker is to be discarded in an appropriate organic liquid waste container.

8) Cinnamide Formation Step: Measure out 0.5 mL of the desired amine solution (known or unknown sample) using a 1.0 mL graduated pipette and transfer to the SPE tube. Allow the liquid to elute by gravity until no flow is observed. Collect final portion of amine solution by using a pipette bulb to generate gentle air pressure in tube to force the DCM to elute.

9) The solution collected in the test tube is used directly for TLC analysis. When completed, the sample solution is to be discarded in an appropriate organic liquid waste container.

TLC Analysis

1) TLC chamber: Add 10 mL of a 60/40 (v/v) solution of EtOAc and hexanes to a 150 mL beaker. Place a piece of aluminum foil over top of beaker as a cover as shown in Figure 4.

2) On a pre-cut TLC plate draw a horizontal line 1 cm from bottom of plate (origin) in pencil. Mark the TLC plate with pencil to indicate where sample(s) will be loaded. Draw a small vertical line across the origin where each solution is to be spotted. Label each spot.

3) Load sample by dipping the spotter into the sample solution and apply to TLC plate with gentle pressure on pre-marked spot (one to two spots may be applied). Allow the spot to air dry for one minute.

4) Place the plate in the TLC chamber and replace cover.

5) When solvent front moves to within 10 – 15 mm of top of plate, remove from chamber and mark solvent front with pencil.

6) Place plate under UV illumination and circle UV active spots with pencil. (note 5)

7) Calculate R_f value of each spot by dividing the distance the compound traveled from the origin by the distance the solvent front traveled from the origin. Use the center of the spot to mark the distance traveled.

8) Repeat procedure for different amines. Repeat procedure for the unknown. Compare the R_f value of the unknown to R_f values of known compounds (standards) to determine which amine was used for the procedure.

Figure 4

Figure 5. Order of Cinnamides: n-Pr, n-Bu, n-Dodecyl, 2-Hydroxyethyl, Pyrrolidinyl, Morpholinyl

Notes

1) Reagents sourced from different vendors are acceptable as long as the reagents are at least the same purity level or grade.
2) Cinnamide standards are available from Aurora Analytics, LLC.
3) Dichloromethane is readily replaced with 2-methyltetrahydrofuran (Me-THF) or tetrahydrofuran if preferred.
4) Prepare in fume hood with appropriate Personal Protective Equipment.
5) Other/additional amines with different TLC properties may be utilized as well
6) If the UV active spots are faint, the solution may be concentrated to a smaller volume using a stream of nitrogen gas in a fume hood. The TLC analysis can then be repeated with the more concentrated solution.