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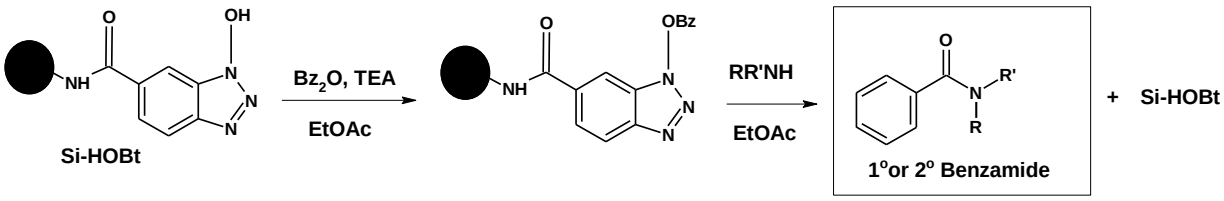
Amide Formation – A Microscale Experiment

Presented here is a detailed Experimental protocol utilizing Si-HOBt (solid phase reagent) for the synthesis of benzamide compounds with subsequent analysis via TLC for the Undergraduate Organic Laboratory Class. The experiment involves the conversion non-chromophoric amines to benzamides that can then be analyzed by TLC with UV detection. Multiple benzamide compounds can be quickly prepared with minimal amounts of solvents, no aqueous work-up and standard laboratory glassware. The experiment is geared towards providing students with their first hands on experience with **S**olid **P**hase **O**rganic **C**hemistry (SPOC).

In the first step of the experiment, Si-HOBt (pre-packaged in an SPE tube) is activated by the addition of benzoic anhydride to form the resin-bound activated benzoate 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 benzamide 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 Si-HOBt may be used in the undergraduate laboratory. The experimental protocol is readily modified to allow for the preparation of benzamide libraries, to identify mixtures of amines as well as for the collection and isolation of the benzamide products. If isolated, products can be characterized by melting point, GC, TLC, IR or NMR analysis.

Overall reaction scheme for the preparation of benzamides with Si-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
SPE-Si-HOBt	Aurora Analytics LLC	H1002-SPE-250	0.25g Si-HOBt, 4 mL capacity
Benzoic anhydride (note 2)	Sigma-Aldrich	385980	≥95%, MW 226.23 g/mole, CAS# 93-97-0
Triethylamine	Sigma-Aldrich	90335	TEA, ≥99.5%, MW 101.19 g/mole, d 0.726 g/mL, CAS# 121-44-8
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
Cyclohexylamine	Aldrich	240648	≥99.9%, MW 99.17 g/mole, d 0.867 g/mL, CAS# 108-91-8
Piperidine	Aldrich	411027	≥99.5%, MW 85.15 g/mole, d 0.862 g/mL, CAS# 110-89-4
Morhpoline	Aldrich	252360	≥99.0%, MW 87.12 g/mole, d 0.996 g/mL, CAS# 110-91-8)
Benzamide standards (note 3)	Aurora Analytics LLC		including N-1-propyl, N-1-butyl, N-1-dodecyl, N-cyclohexyl, N-piperidyl and N-morpholinyl
iso-Propylamine (note 4)	Aldrich	471291	≥99.5%, MW 59.11 g/mole, d 0.688 g/mL, CAS# 75-31-0, used to regenerate cartridge

Equipment and Supplies:

1) Ring stand with small three-fingered clamp
2) 50 mL beaker
3) 1.0 mL graduated pipettes
4) Small glass test tubes (12 x 75 mm or 13 x 100 mm, 2 required per experiment)
5) Graduated cylinder (10 mL)
6) Pasteur pipettes with bulb
7) 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: 50/50 EtOAc/hexanes (v/v)
f. UV lamp (254 nm)

Stock Solutions (note 5):

1) 0.5 M Benzoic anhydride stock solution
    a. 100 mL solution: Add 11.31 g benzoic anhydride to a 100 mL volumetric flask. Add 50 mL of EtOAc and swirl to dissolve the solid anhydride. Bring the volume of the solution to the 100 mL mark with EtOAc. Replace stopper and mix thoroughly. Transfer contents to an amber glass bottle for use and storage. Useful for up to 200 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 EtOAc and swirl to form a homogeneous solution. Bring the volume of the solution to the 100 mL mark with EtOAc. Replace stopper and mix thoroughly. Transfer contents to an amber glass bottle for use and storage. Useful for up to 200 experiments.
3) 0.5% w/v Benzamide 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 EtOAc and swirl to form a homogeneous solution. Bring the volume of the solution to the 25 mL mark with EtOAc. Replace stopper and mix thoroughly. Transfer contents to an amber glass vial/bottle for use and storage.
4) 0.5% w/v Amine solutions (note 6)
    a. 100 mL solution: Add 0.500 g of amine to a 100 mL volumetric flask. Add 50 mL of EtOAc and swirl to form a homogeneous solution. Bring the volume of the solution to the 100 mL mark with EtOAc. Replace stopper and mix thoroughly. Transfer contents to an amber glass bottle for use and storage. Useful for up to 100 experiments.
    b. Amount of amine required (mL)
        i. *n*-Propylamine: 0.695 mL
        ii. *n*-Butylamine: 0.680 mL
        iii. Cyclohexylamine: 0.577 mL
        iv. *n*-Dodecylamine : 0.620 mL
        v. Piperidine: 0.580 mL
        vi. Morpholine: 0.498 mL

Apparatus Set–up

To set up the apparatus, mount the 4 mL SPE tube containing Si-HOBt, using a ring stand with a three-fingered clamp (see figures 1 and 2). Place a 50 mL beaker under the secured tube to collect the reaction products.

Figure 1

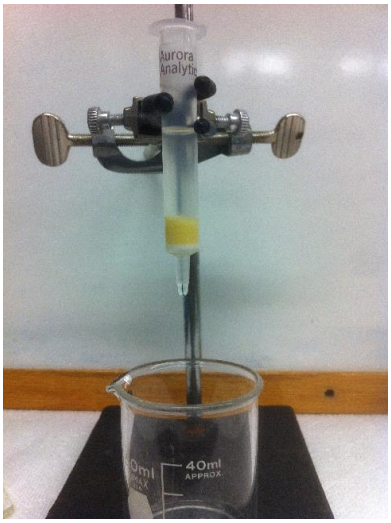


Figure 2

P**rocedure**

Benzamide Formation from Amine

1) Measure out 2 mL of EtOAc 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 EtOAc to elute. DO NOT LET THE TOP OF THE SILICA GET DRY!

2) Si-HOBt Activation Step: In a small test tube (12x75 mm or 13x100 mm) combine 0.5 mL of benzoic anhydride stock (0.5 M) with 0.5 mL of 1.5 M triethylamine (TEA) stock solution (both are in ethyl acetate (EtOAc) and are to be dispensed with dedicated 1.0mL graduated pipettes). Mix with a disposable glass Pasteur pipette and then transfer to the column using same pipet. Allow the liquid to elute by gravity until no flow is observed. DO NOT LET THE TOP OF THE SILICA GET DRY!

3) Measure out 2 mL of EtOAc 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 EtOAc to elute. DO NOT LET THE TOP OF THE SILICA GET DRY!

4) Repeat **Step 3** three times.

5) Replace the beaker with a small glass test tube. The solution collected in the beaker is to be discarded in an appropriate organic liquid waste container.

6) Benzamide Formation Step: Measure out 1.0 mL of the desired amine solution (known or unknown sample) 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 EtOAc to elute. DO NOT LET THE TOP OF THE SILICA GET DRY!

7) Measure out 0.5 mL of EtOAc 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 EtOAc to elute.

8) The solution collected in the test tube may be 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 50/50 (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 3.

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 7)

7) Calculate Rf 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 Rf value of the unknown to Rf values of known compounds (standards) to determine which amine was used for the procedure.

Figure 3

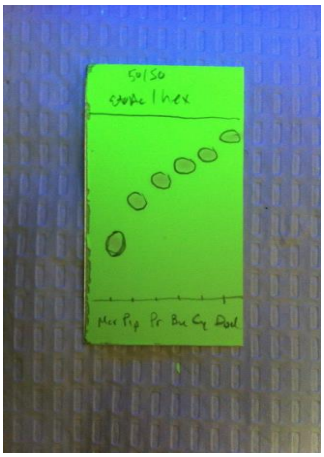


Figure 4. Order of Benzamides: Morpholino, Piperidinyl, n-Pr, n-Bu, Cyclohexyl, n-Dodecyl

Notes

1) Reagents sourced from different vendors are acceptable as long as the reagents are at least the same purity level or grade.
2) Benzoyl chloride may be substituted for benzoic anhydride in the experiment if desired. If benzoyl chloride is substituted the solvent should changed to dichloromethane (DCM).
3) Benzamide standards are available from Aurora Analytics LLC.
4) To regenerate the Si-HOBt cartridge, place a 50 ml beaker beneath the cartridge to collect the waste and add 2 mL of 10% (v/v) *iso*-propylamine in EtOAc. Allow this to drain through the silica, applying GENTLE pressure with a pipette bulb, but **do not** allow the liquid to go below the top frit. Repeat this two more times. Fill the tube with pure EtOAc, allowing this to drain through the silica, applying GENTLE pressure with a pipette bulb. Then push air through the column to dry the silica.
5) Prepare in fume hood with appropriate Personal Protective Equipment.
6) Other/additional amines with different TLC properties may be utilized as well
7) 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.