Argonaut

Integrated Organic Synthesis and Purification

Workshop

American Chemical Society

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Argonaut Booth No. 322 - 325

Outline

Introduction	Julia Horak
Introduction to Purification Techniques for Expedited Organic Synthesis	Francis Allard

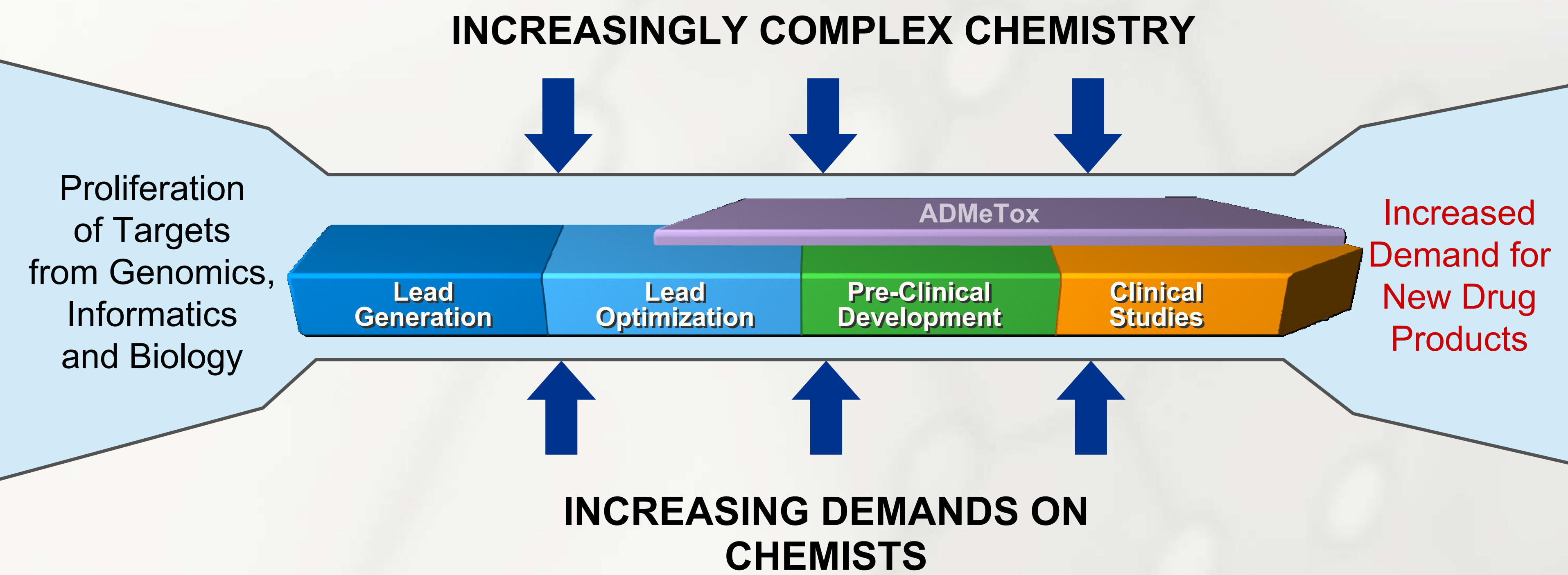
Integrated Strategies for Amine Synthesis and Purification

Flow through purification methods	Francis Allard
Integrated synthesis and purification – a case study	Sukanta Bhattacharyya
Reductive amination	Sukanta Bhattacharyya

Who is Argonaut?

- Founded in 1994
- Productivity Tools for Chemists
  - 10+ instrument & software products
  - 45+ chemical reagents
  - 2200+ installed systems
- Merged with Jones Chromatography January 2002
  - The synergy provides a unique combination of products to address integrated synthesis and purification challenges

The Chemistry Bottleneck



Argonaut's Solutions

ISOLUTE®

SPE Resins & Accessories

Analytical Method Development

Lead Generation | **Lead Optimization** | **Chemical Development**

Scavengers and Reagents

Advantage Series™

ISOLUTE®

SPE Sorbents & Accessories

Endeavor®

Flash Master™

Introduction to Purification Techniques for Expedited Organic Synthesis

- Catch and Release
- Scavenging
- Solid-Supported Liquid Extraction
- Flash Chromatography

Purification Challenges

- Purification often a bottleneck, even with small numbers of compounds
  - Traditional techniques are inefficient and time-consuming
    - Liquid-liquid extraction
    - Crystallization
  - Purifications can sometimes be difficult
    - *e.g.*, removing cyclohexylurea after DCC coupling reaction

Purification Techniques for Expedited Organic Synthesis

- Most reaction mixtures can be purified using one or more of three techniques
  - Resins
    - Polymer-supported reagents
    - Scavengers
  - Catch and Release
    - Solid phase extraction
    - Supported liquid extraction
  - Flash chromatography
  - Or, a combination of techniques

Polymer-Bound Scavengers and Reagents

- Bound scavengers are functional polymers designed to react with and bind excess reagents and/or byproducts
- Bound reagents are functional polymers designed to perform synthetic transformations in same way as solution counterparts

Catch and Release and Scavenging

- Catch and release methods and scavengers extract compounds onto functionalized or adsorptive media
  - Catch and release – reversible
    - Subsequent release into solution
  - Scavengers – irreversible
- Separation is based on major differences between compounds
- Purification is by filtration or simple passage through a column

Catch and Release vs. Scavengers

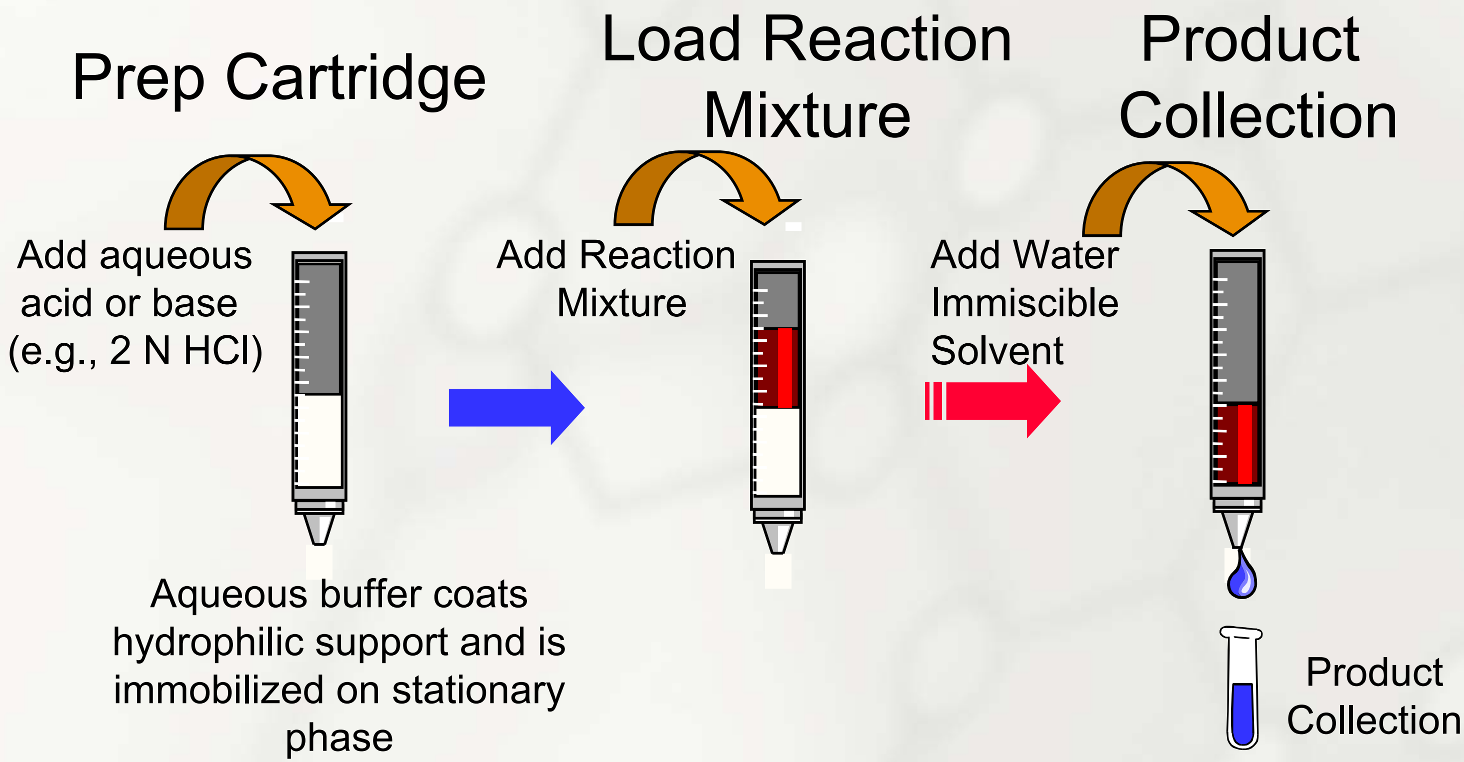
	Catch and Release	Scavengers
Function	Byproducts or impurities extracted from solution onto functionalized or adsorptive media (silica, polymer)	Byproducts or impurities with specific functionality removed from solution by reaction with a functionalized polymer
Mechanism	Ionic (acid-base), polarity	Covalent reaction
Mode of use	Reversible: ▪ Extract product onto media, elute impurities then release Irreversible: ▪ Extract impurity onto media, elute product	Irreversible: ▪ Extract impurities onto functional resin, elute product
Advantage	Fast interactions Flow-through cartridge format	Selective for specific functionality

Solid Supported Liquid Extraction

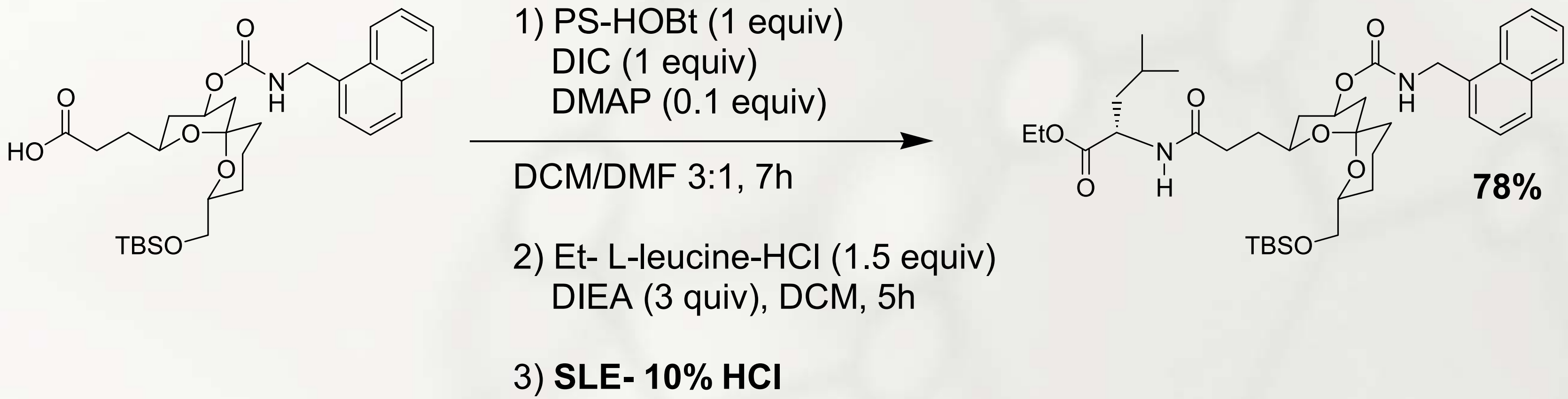
- Supported liquid extraction* (SLE)
  - Liquid-liquid partitioning in a column format
    - Same as liquid-liquid extractions in a separating funnel
  - Immobilize aqueous phase on stationary phase
    - Stationary phase = diatomaceous earth
  - Elute with water-immiscible solvent
  - Use to remove inorganic salts, amines and acids

*Johnson, C.R., *et al.*, *Tetrahedron*, **1998**, *54*, 4097

Solid Supported Liquid Extraction (SLE)



Complex Amide Synthesis



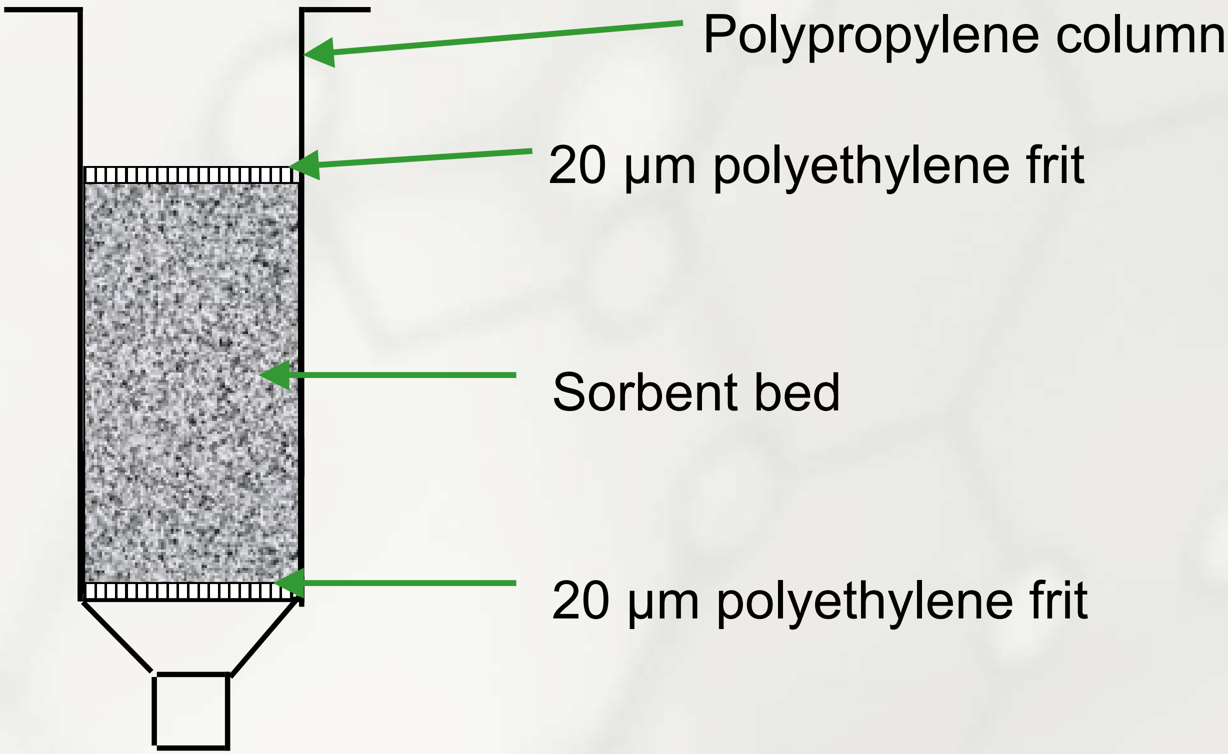
- Amide synthesis via resin-bound active ester intermediate using PS-HOBt(HL)
- Excess Et-Leucine-HCl, DIEA and DIEA-HCl removed by SLE with 10% aq. HCl

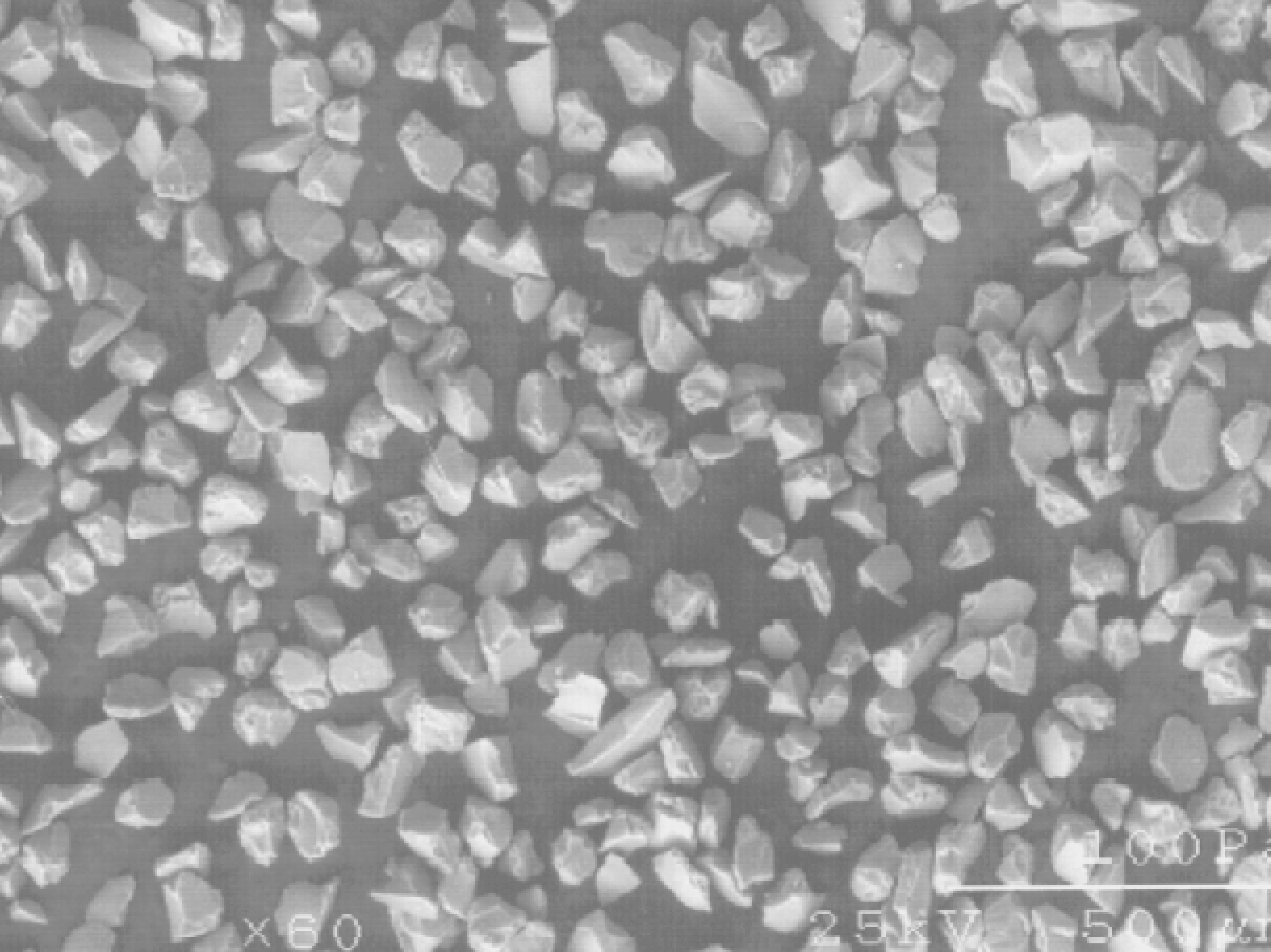
Kulkarni, B.A.; Roth, G.P.; Lobkovsky, E.; Porco,Jr. J.A. *J. Comb. Chem.* **2002**, *4*, 56.

Flash Chromatography

- Flash chromatography separates compounds based on differences in adsorption to or affinity for the column medium
  - Silica, NH_2, or C18 media
- Excellent way to separate structurally similar compounds
- Elution time is dependent on solvent employed
- Need to collect and analyze fractions post separation
  - Simplified by automated fraction collection and peak detection

An ISOLUTE Flash Column




100P
X60
25kV
500

Synthesis and Purification Workflow

Synthesis	Initial Purification	Final Purification
▪ Bound Reagents	▪ Scavengers ▪ Catch & Release	▪ Flash Chromatography

- Resins and catch and release replace liquid-liquid extraction and simplify mixture composition prior to chromatography
- Initial purification usually sufficient to proceed directly to next synthetic step

Synthesis and Purification Workflow

Integrated Strategies for Amine Synthesis

Why Amines?

- Amines and carboxamide derivatives are the most common structures in medicinal chemistry databases[1]
- 25% of drugs currently on the market are aliphatic tertiary amines[2]
- Secondary amines are useful intermediates
  - Additional diversity is easily added[3]
  - Used as pharmacophores,[4]
  - Versatile building blocks[5]

1. Ghose, Viswanadhan, Wendoloski, J. J. *J. Comb. Chem*. **1999**, *1*, 55.
2. Morphy et al., *J. Am. Chem. Soc.* **1997**, *119,* 3288.
3. See, for example: Bhattacharyya, Fan, Vo, Labadie, J. *Comb. Chem. & High Throughput Screening*, **2000**, *3*, 117.
4. Insaf, Witiak, *Synthesis*, **1999**, 435 and references therein.
5. Swamura, Ito, *Chem. Rev*. **1992**, *92*, 857; Togni, Venanzi, *Angew Chem. Int. Ed. Engl.* **1994**, *33*, 497.

Synthesis and Purification Workflow

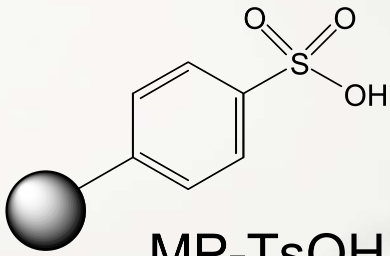
Flow Through Purification Methods

Synthesis	Initial Purification	Final Purification
▪ Bound Reagents	▪ Scavengers ▪ Catch & Release	▪ Flash Chromatography

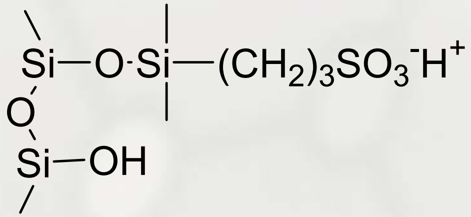
Amine Purification Using Flow Through Methods

- ISOLUTE® SCX-2 Cartridges
  - Silica based
  - Sorbent does not swell in solvent
  - 0.4 mmol/g capacity
  - Additional formats available, particularly large scale
- MP-TsOH Cartridges
  - Highly crosslinked macroporous polystyrene
  - Very stable under broad range of pH conditions
  - 2.5 mmol/g capacity
  - Limited swelling
    - Controlled by optimized manufacturing techniques

Bound Acid: MP-TsOH and ISOLUTE SCX-2



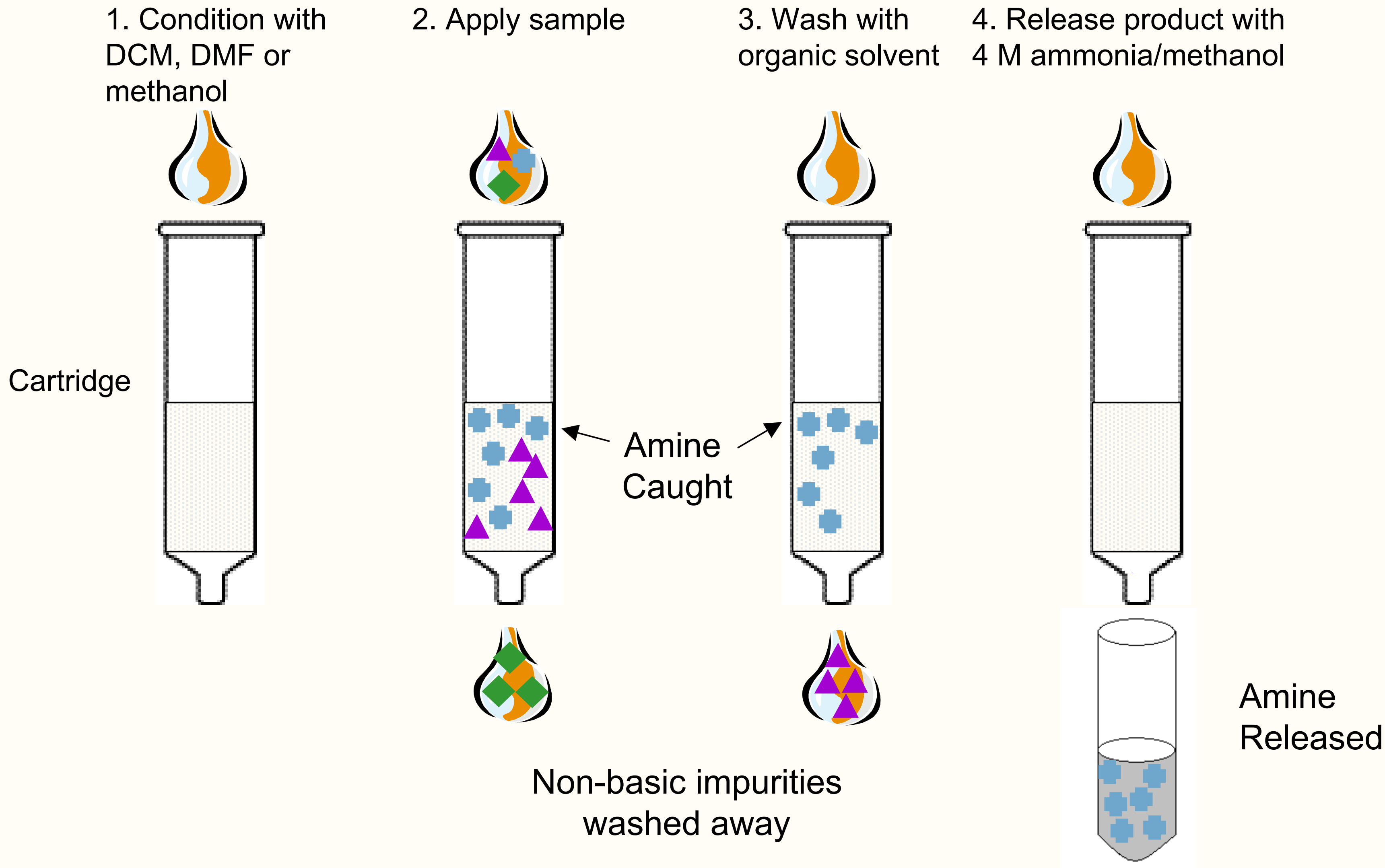
MP-TsOH



ISOLUTE SCX-2

- Bound sulfonic acid equivalents
  - Silica or highly crosslinked polystyrene based
- Scavenge amines, basic compounds
- Catch and Release purifications
  - Catch amines, basic heterocycles
  - Wash impurities
  - Release amine with ammonia/methanol

Cartridges for Amine Purification by Catch and Release

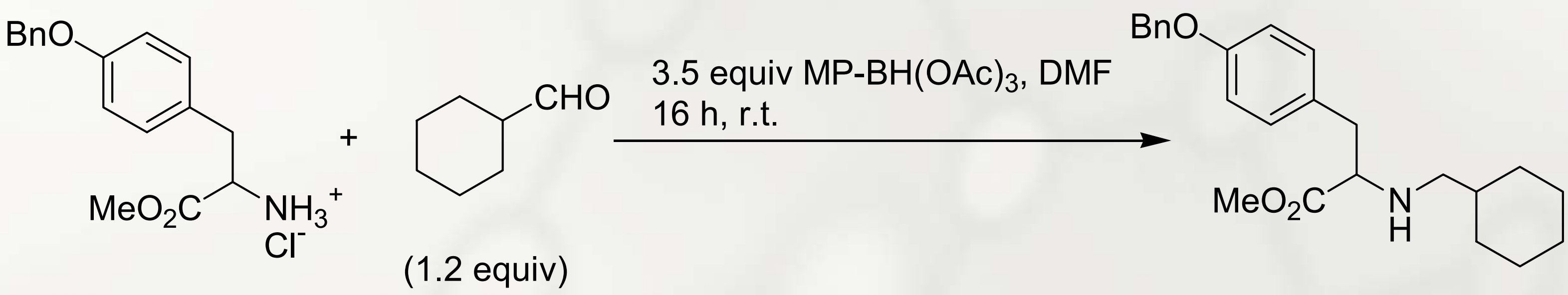


Amine Purification on MP-TsOH Cartridges

Amine	Resin (equiv)	Unretained Amine (%)		Released Amine (% Recovery)*
		DCM	DMF	
3-Phenylpropylamine	1.5	0	0	98
N-Methylbenzylamine	1.5	0	0	97
N,N-Dimethylbenzylamine	1.5	0	0	92
N-Methyl,N,N-di(2-phenethyl)amine	1.5	0	0	95
4-(2´-Dimethylaminoethyl)morpholine	3.0	0	0	96
2-Aminothiazole	1.5	0	0	96
Aniline	1.5	0	0	94
4-Nitroaniline	3.0	0	30	97

*From experiments using DCM as solvent

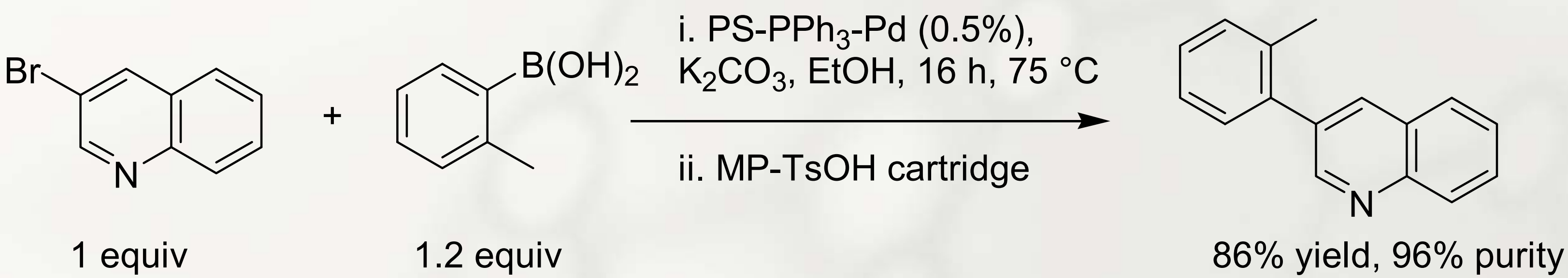
Solvent Exchange



Reductive alkylation of amino acid ester salt in 100% DMF

- MP-TsOH cartridges facilitate solvent exchange
- Excellent way to extract amines from DMF or DMSO
  - 100% retention
  - Subsequently concentrate amine from volatile ammonia/methanol solution
- Use as sample prep prior to direct application to flash column

Purification of Basic Heterocycles by Catch and Release Using MP-TsOH Cartridges



- MP-TsOH catch & release purifies end product after Suzuki coupling
  - Useful with both bound (PS-PPh_3-Pd) and conventional ($Pd(Ph_3P)_4$) catalysts

MP-TsOH and SCX-2 Cartridges for Purification by Catch and Release

- Retain aliphatic, aromatic and heterocyclic amines
- Retain even weakly basic amines
- Amines eluted in 4 M ammonia/methanol
  - Volatile, easy to remove
- Facilitate solvent exchange
- Very low leachables

Which Cartridge to Use?

- Both SCX-2 and MP-TsOH perform well for amine purification by catch and release
- Because the capacity of MP-TsOH is 6 – 9 times higher than SCX-2 it offers several advantages
  - Lower cartridge cost
  - Lower total solvent usage
  - Lower final sample volume
- MP-TsOH cartridges are a novel product from Argonaut that provide superior performance for amine purification

Synthesis and Purification Workflow

Selective Purification of Amines

Synthesis	Initial Purification	Final Purification
▪ Bound Reagents	▪ Scavengers ▪ Catch & Release	▪ Flash Chromatography

Selective Purification of Amines

- Mixtures of secondary and tertiary amines occur frequently, *e.g.,*
  - Alkylation reaction
  - Deprotection
  - Reductive amination
- Cartridges are useful for purifying amines
  - Cannot discriminate between amine classes
- Strategies to purify components in mixtures include:
  - Derivatization and purification
  - Selective scavenging
  - Flash chromatography

Synthesis and Purification Workflow

Derivatization and Purification

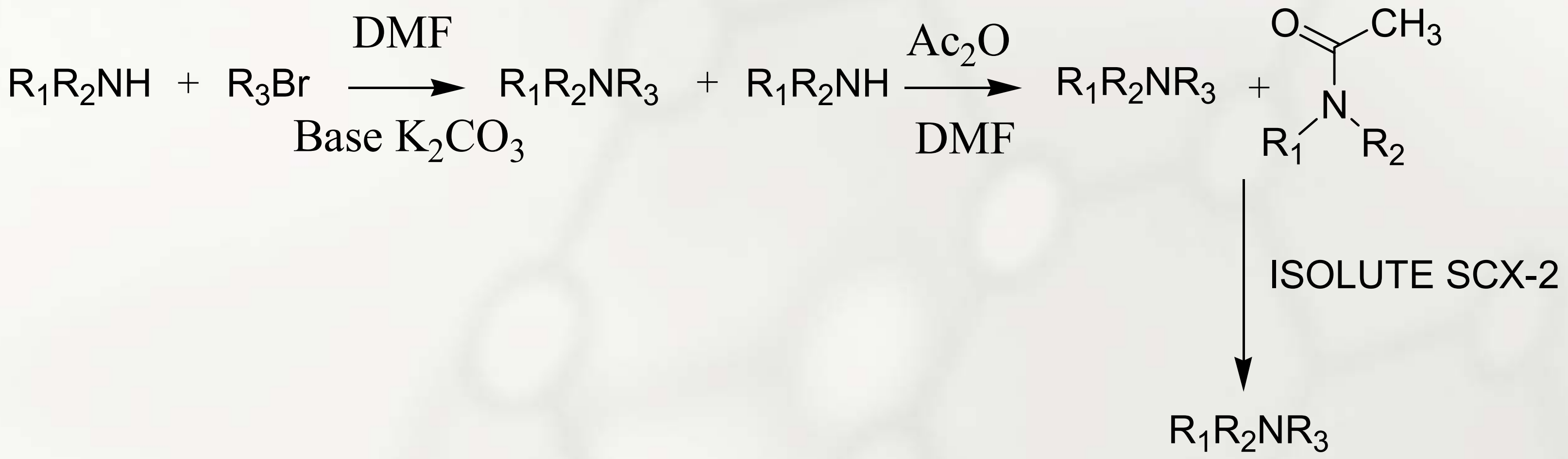
Synthesis	Initial Purification	Final Purification
▪ Bound Reagents	▪ Scavengers ▪ Catch & Release	▪ Flash Chromatography

Derivatization/Purification Techniques

- Goal
  - Selectively purify tertiary amine in presence of secondary amine
- Strategy
  - Derivatize the secondary amine selectively making it non-basic
  - Separate from the tertiary amine using ISOLUTE SCX-2 SPE
- Two routes for derivatization
  - Acetylate the secondary amine
    - Secondary amine is non-recoverable
  - Treat with Boc_2O, forming the carbamate of the secondary amine
    - Recover secondary amine by subsequent removal of Boc group

Thanks to Synthetic Technologies Group, Lilly Research Laboratories, Erl Wood, UK

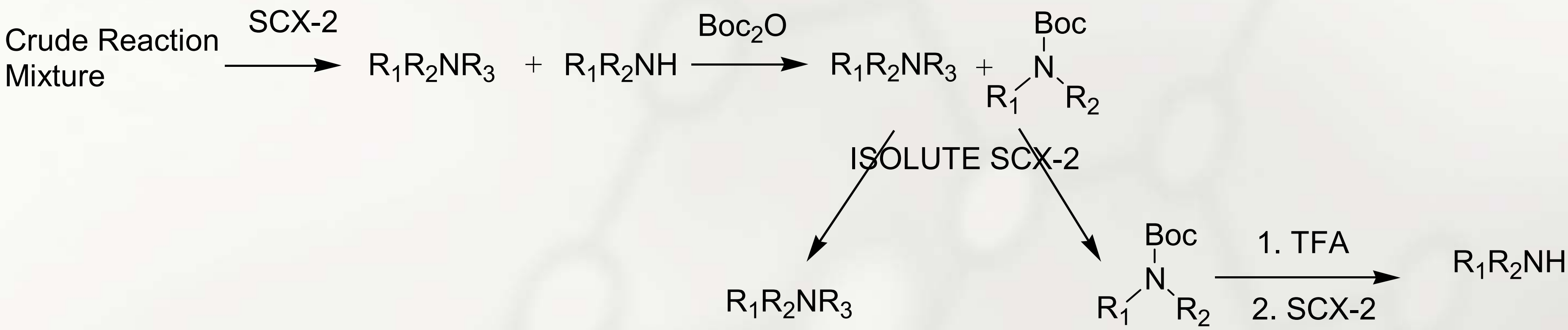
Secondary Amine Is Not Recovered



- Alkylation reaction yields secondary and tertiary amines
- Convert secondary amine to acetamide (non-basic) with 2 equiv acetic anhydride
- Selectively retain tertiary amine on ISOLUTE SCX-2
- Elute tertiary amine in 2 M ammonia/methanol solution
  - Concentrate by evaporation
  - Dissolve in solvent of choice

Thanks to Synthetic Technologies Group, Lilly Research Laboratories, Erl Wood, UK

Both Secondary and Tertiary Amines Recovered



- Useful with valuable secondary amine
- Separate secondary and tertiary amines from other reaction components with SCX-2
- Transform secondary amine to non-basic carbamate with Boc_2O
- Purify tertiary amine with SCX-2
- Deprotect secondary amine with TFA
- Release free base from SCX-2

Thanks to Synthetic Technologies Group, Lilly Research Laboratories, Erl Wood, UK

Derivatization/Purification Techniques

- Derivatization converts secondary amine to a non-basic derivative
- Tertiary amine recovered from ISOLUTE SCX-2 by catch and release
- Secondary amine may also be recovered using protection/deprotection followed by ISOLUTE SCX-2
- Compatible with flow-through processing using either columns or plates

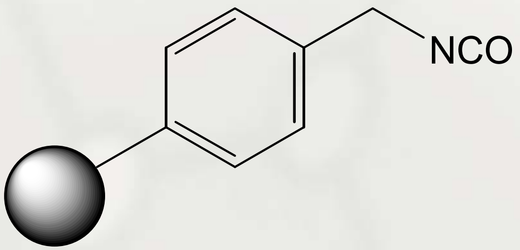
Synthesis and Purification Workflow

Amine Purification by Selective Scavenging

Synthesis	Initial Purification	Final Purification
▪ Bound Reagents	▪ Scavengers ▪ Catch & Release	▪ Flash Chromatography

Amine Selective Scavenging

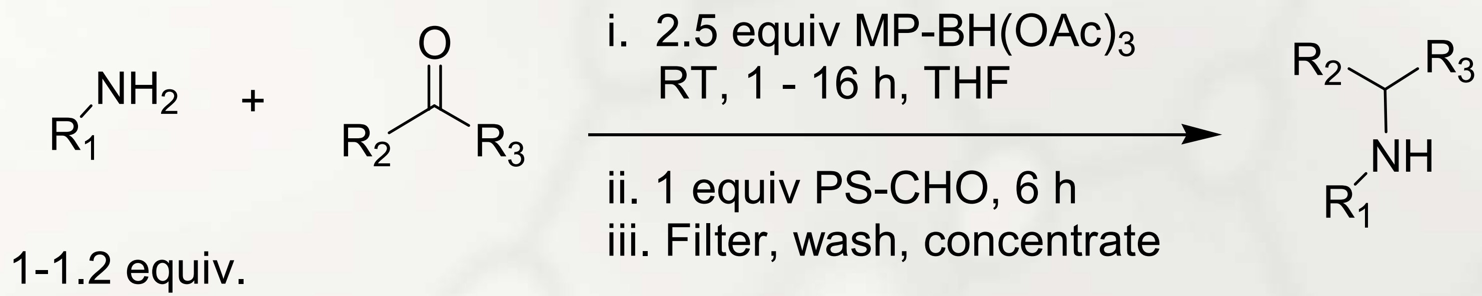
PS-Benzaldehyde



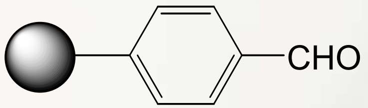
PS-Isocyanate

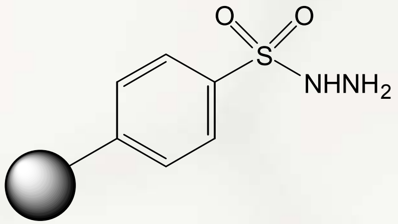
- PS-Benzaldehyde scavenges primary, but not secondary or tertiary amines
- PS-Isocyanate scavenge primary and secondary amines, but not tertiary amines
- Purified product obtained by filtration
- Scavenging is not reversible

Elimination of Excess Primary Amine



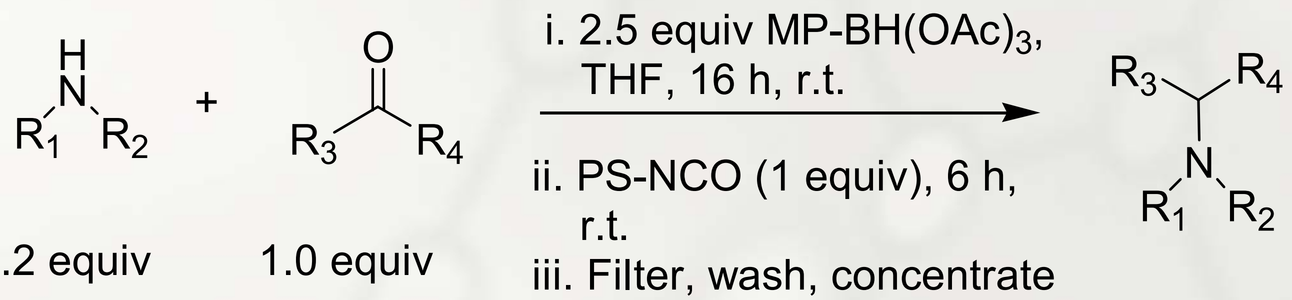
Secondary amine synthesis by reductive amination



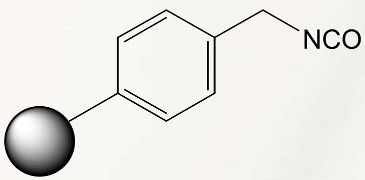


- PS-Benzaldehyde selectively scavenges excess primary amine
- Product secondary amine remains in solution
- PS-TsNHNH$_2$ scavenges carbonyls
- Use a PS-TsNHNH$_2$/PS-CHO cocktail if the reaction does not go to completion

Elimination of Excess Secondary Amine



Tertiary amine synthesis by reductive amination



- PS-Isocyanate scavenges primary and secondary amines, but not tertiary amines

Selective Amine Purification

- Two options
- Derivatization/Purification
  - Suited to flow-through processing
  - Amines recoverable
- Selective Scavenging
  - Product amine remains in solution
  - Purified by filtration away from scavenger resin
  - Amines not recoverable
  - Compatible with single pot syntheses

Synthesis and Purification Workflow

Case Study:
Synthesis and Purification of Secondary Amines Using Bound Reagents, Catch and Release, and Flash Chromatography

Synthesis	Initial Purification	Final Purification
▪ Bound Reagents	▪ Scavengers ▪ Catch & Release	▪ Flash Chromatography

Reason for the Study

- After organic synthesis chromatography is often necessary
- This may require:
  - Preliminary purification – usually liquid-liquid extraction
  - Solvent exchange
- The purpose of this study was to develop flow through methods to integrate pre-chromatography workup with the final purification

Synthesis of Secondary Amines: Background

- Selective mono-N-alkylation of primary amines remains the most direct approach
- Conventional base-promoted alkylation reactions of primary amines using alkyl halides or sulfonates are generally inefficient
- Suffer from poor chemo-selectivity due to the competing over-alkylation reactions

Chemo-Selective Synthesis of Secondary Amines

$$RNH_2 + R^1(CH_2)_nBr \xrightarrow[\text{DMF, 23 °C}]{\text{CsOH, 4 Å Mol Sieves}} RNH(CH_2)_nR^1$$

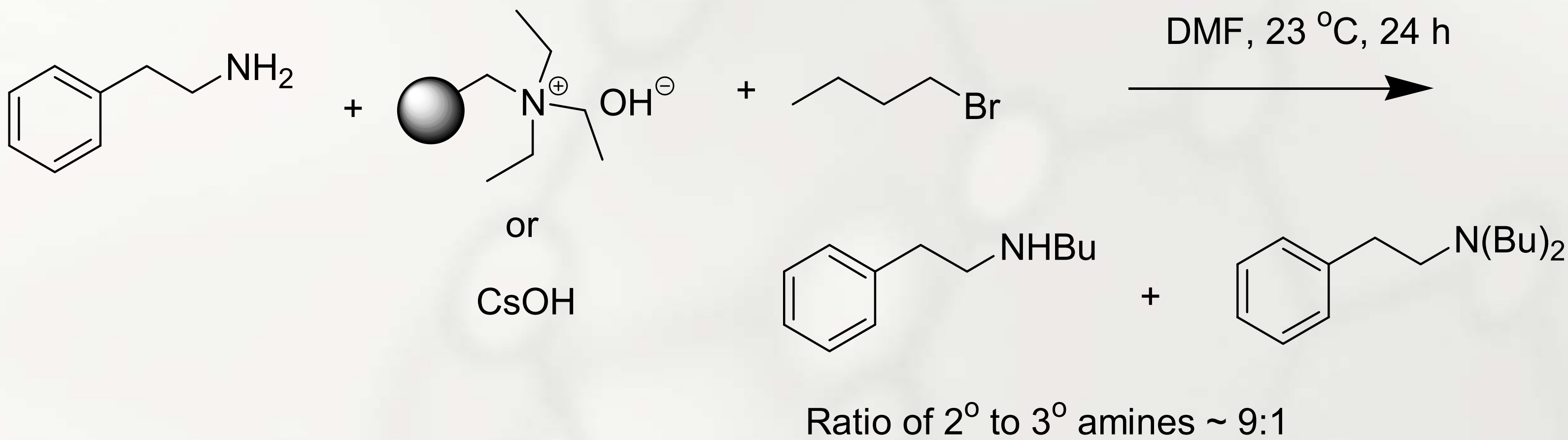
- Advantages
  - Direct N-alkylation to secondary amine
  - CsOH controls over-alkylation reaction
- Limitations
  - Requires aqueous quenching & liquid-liquid extraction
  - Chromatographic separation
  - CsOH is very moisture sensitive, inconsistent results

Salvatore, Nagle, Jung, *J. Org. Chem*, **2002**, *67*, 674.

Chemo-Selective Synthesis of Secondary Amines

- Plan
  - Isolate product mixture by simple filtration
  - Purify amine mixture by catch and release using MP-TsOH cartridge
    - Remove non-basic impurities and allow solvent (DMF) exchange
  - Release the amine mixture onto an ISOLUTE FL cartridge and remove solvent
  - Separate primary, secondary and tertiary amines by flash chromatography
- In addition
  - Identify a bound base for selective N-alkylation of 1^o amines

Chemo-Selective Synthesis of Secondary Amines



Chemoselective Mono-N-Alkylation of Phenethylamine

- Reaction performed with CsOH, also with a bound base[1]
- Bound base easier to handle than CsOH
  - Product mixture isolated by simple filtration of the resin

1. Under development by Argonaut Technologies

Post-Reaction Workup

- MP-TsOH cartridge
  - Purifies product secondary amine, tertiary amine and unreacted primary amine away from non-basic reaction byproducts
  - Replaces DMF with volatile ammonia/methanol
- ISOLUTE FL cartridge
  - Facilitates amine transfer to flash column
  - Solvent evaporation
- Flash chromatography
  - Separates primary, secondary and tertiary amines

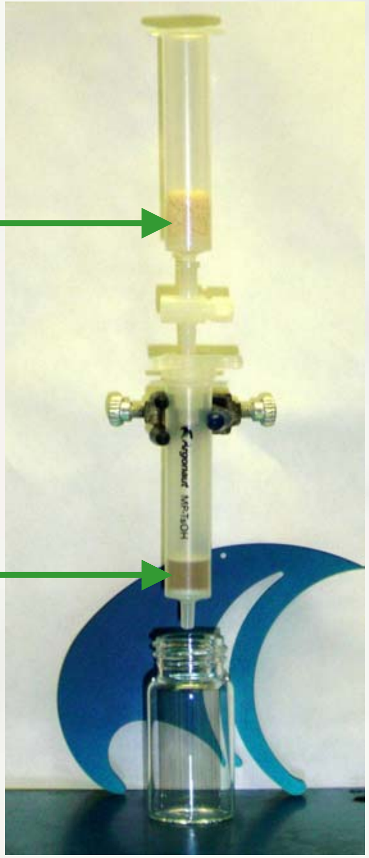
Post-Reaction Workup

- Filter reaction mixture
- Transfer from round bottom flask to empty ISOLUTE cartridge body
- Flitrate applied directly to MP-TsOH Cartridge

Amine Purification and Solvent Removal

Reaction mixture with mol sieves (or resin-bound base)

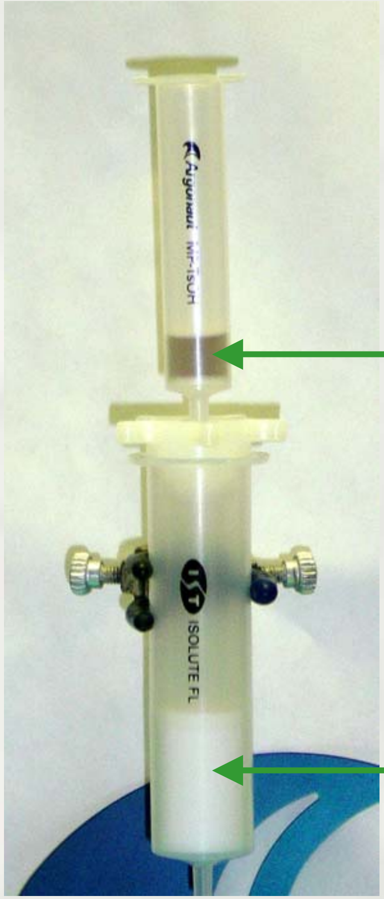
MP-TsOH Cartridge



Catching Amine Mixture on MP-TsOH Cartridge

MP-TsOH Cartridge

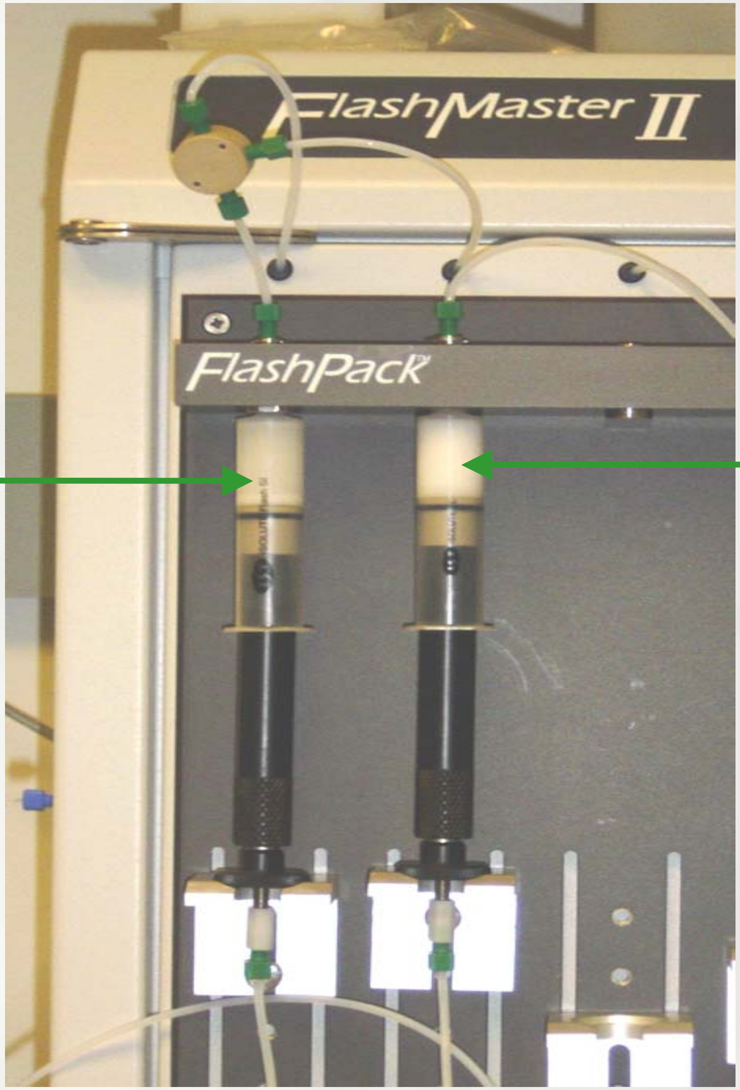
ISOLUTE FL Cartridge



Release of Amine Mixture onto ISOLUTE FL Cartridge

Flash Chromatography on FlashMaster II

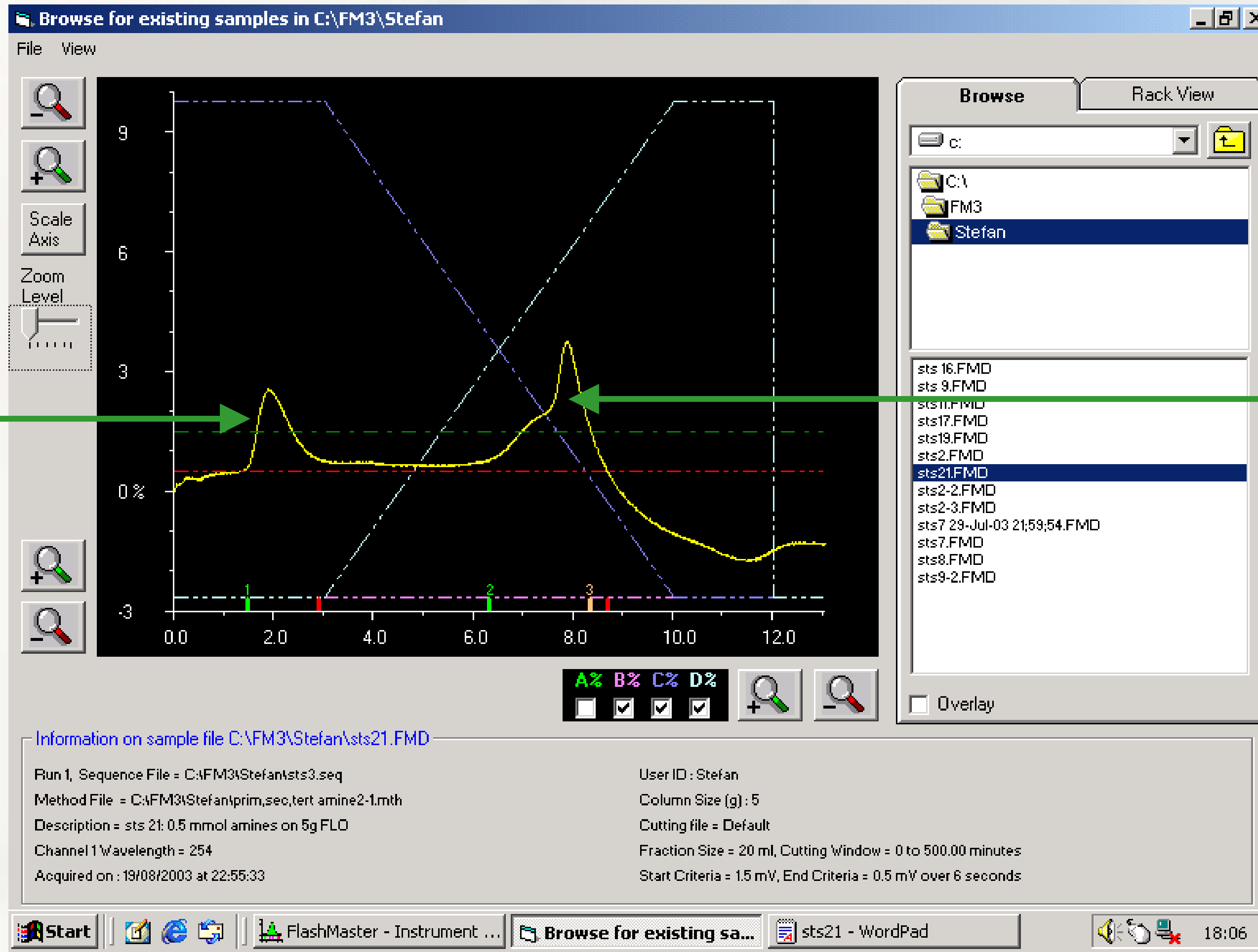
Flash chromatography column



Amine mixture retained on ISOLUTE FL Cartridge

Secondary and Tertiary Amine Separation by Flash Chromatography

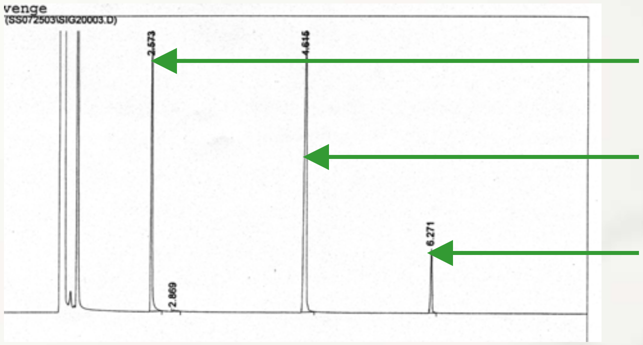
Tertiary amine



Secondary amine

Ethyl acetate/methanol gradient

Purification Results: GC Analysis

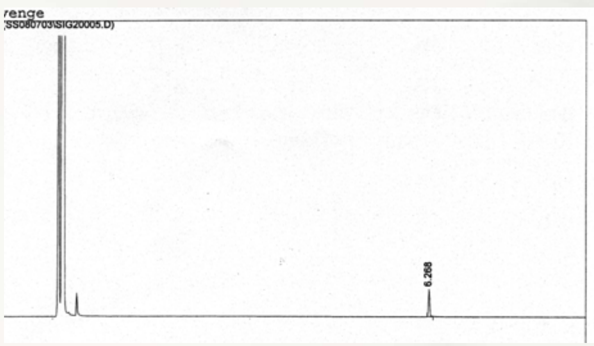


Primary Amine

Secondary Amine

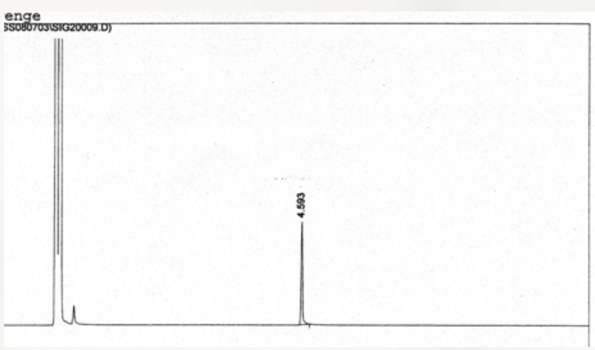
Tertiary Amine

Crude Reaction Mix



Tertiary Amine

First Band to Elute from Flash Chromatograph



Secondary Amine

Second Band to Elute from Flash Chromatograph

Contrast with Traditional Workup Procedures

- Prior to flash chromatography
  - Liquid-liquid extraction
  - Drying
  - Concentration

Liquid-Liquid Extractive Workup

Drying – Concentration

Case Study Conclusions

- Demonstrated integrated synthesis, workup and purification of secondary amines
  - Synthesis
    - Highly chemoselective mono-N-alkylation of primary amines with CsOH or tetraalkylamonium hydroxide resin
  - Purification
    - SPE of the amine mixture on MP-TsOH cartridge exchanges DMF solvent and removes any excess alkyl halide
    - ISOLUTE FL removes solvent and facilitates loading on to flash column
  - Flash Chromatography
    - Separation of amine mixture using FlashMaster II

Synthesis and Purification Workflow

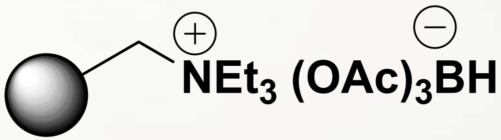
Amine Synthesis by Reductive Amination

Synthesis	Initial Purification	Final Purification
Bound Reagents	Scavengers Catch & Release	Flash Chromatography

Reductive Amination

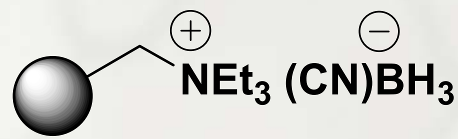
- Reductive amination of carbonyl compounds is a powerful tool in constructing diverse series of amines
- Resin-bound reagents and scavengers simplify purification of product amine
  - Excess substrate and reaction byproducts are removed by filtration

Resins for Reductive Amination



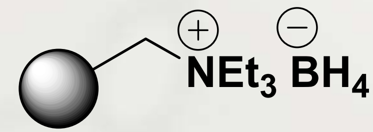
1.8 - 2.4 mmol/ g

MP-Triacetoxyborohydride



2-3 mmol/ g

MP-Cyanoborohydride

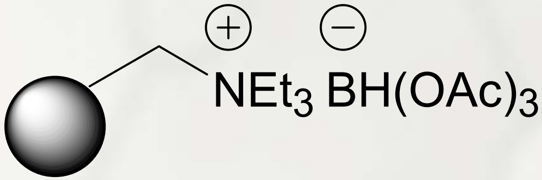


3-3.2 mmol/ g

MP-Borohydride

- Macroporous resin
  - Inert scaffold for the chemical reagent
  - Efficient reagent delivery
  - Removed by simple filtration
  - Limited swelling
  - Ease of use and handling
- Stable

MP-Triacetoxyborohydride[1]

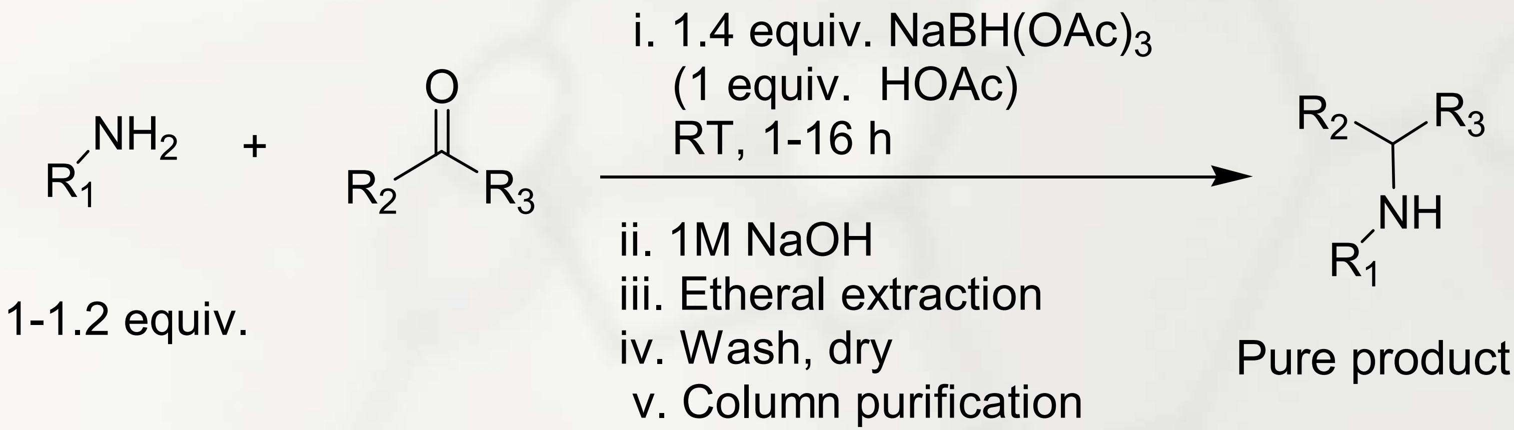


- Solid supported analog of $NaBH(OAc)_3$
  - Higher yields with fewer by-products[2]
- Effective under neutral conditions
- Compatible with acid labile groups (acetals/ketals)
- Easy handling and delivery
- Product isolated by filtration, THF rinse

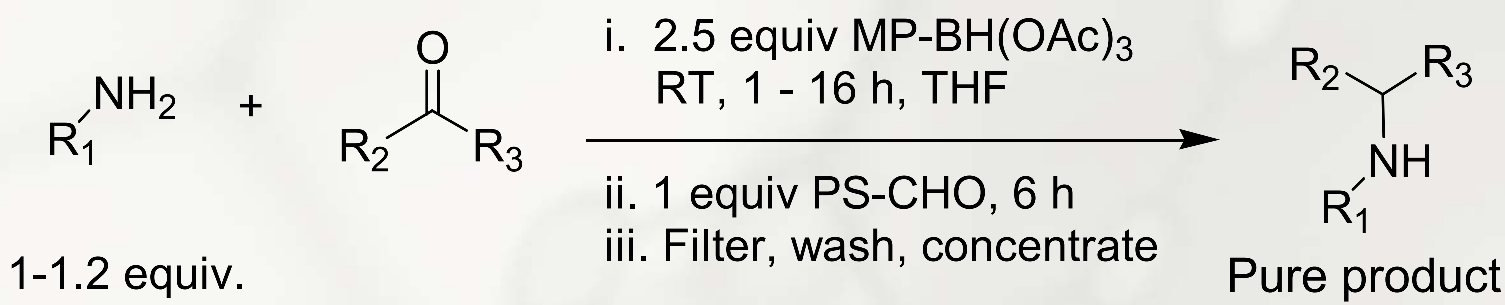
1. Bhattacharyya, S., Rana, S., Gooding, O.W., Labadie, J. *Tetrahedron Lett.* **2003,** *44,* 4957-4960.
2. Abdel-Majid A. F. et al. *J. Org. Chem*. 1996, *61*, 3849

MP-Triacetoxyborohydride: Synthesis of Secondary Amines

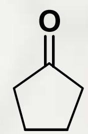
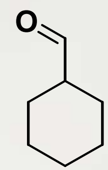
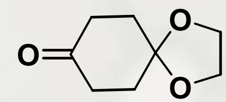
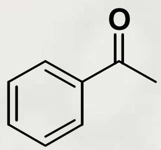
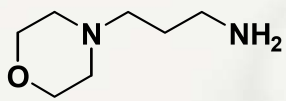
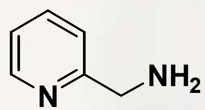
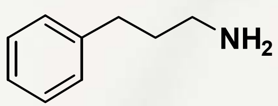
- Typical solution-phase protocol



- Expedited bound reagent protocol



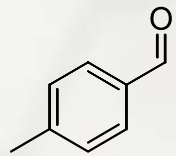
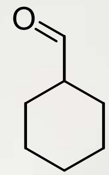
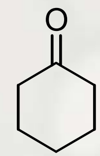
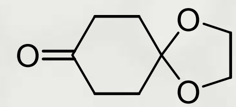
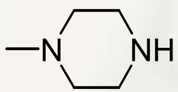
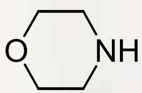
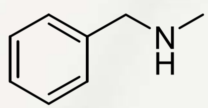
MP-Triacetoxyborohydride: Synthesis of Secondary Amines

				
	93 (100)	77(84)*16*	77 (100)	69(98)
	93 (98)	90(96)*3*	91(100)	76(27)
	100 (100)	94 (88)*12*	54(100)	39(91)

% yield (% purity) *% overalkylation*

- Primary amine used in excess
- Scavenged by PS-Benzaldehyde at the end of the reaction
- Acid-labile ketal works well
- Product isolated as acetate salt
- Free amine generated by catch and release on MP-TsOH cartridges

MP-Triacetoxyborohydride: Synthesis of Tertiary Amines

				
	97(100)	92(99)	88(100)	85(100)
	63(100)	90(99)	69(100)	64(100)
	67(100)	77(99)	82(100)	76(100)

%yield (%purity)

- Secondary amine used in excess
- Scavenged by PS-Isocyanate at end of reaction
- Product isolated as free amine
- Negligible boron leaching observed
- If amine is limiting reagent use MP-TsOH cartridges for purification

Reductive Amination: Summary

- MP-BH(OAc)$_3$ (neutral, mild conditions)
  - Compatible with acid-sensitive groups, e.g. ketals, acetals
- MP-BH$_3$CN
  - Requires acetic acid
  - More aggressive conditions
- MP-BH$_4$
  - Reduce pre-formed imine to minimize over-alkylation
  - Use with Ti(O^{i}Pr)$_4$ to suppress over-alkylation

Integrated Synthesis and Purification – Conclusions

- The new modular approach to synthesis and purification uses:
  - Resin-bound reagents
  - Scavengers
  - Catch and release cartridges
- Use singly or in combination to eliminate the need for time-consuming purification procedures
- Flash chromatography used when necessary for further purification

Modular Approach to Synthesis and Purification

Synthesis	Initial Purification	Final Purification
▪ Bound Reagents	▪ Scavengers ▪ Catch & Release	▪ Flash Chromatography

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