Accelerating Drug Discovery: Enabling Tools and Techniques

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Director R&D Chemistry
Biotage
Synthesis and Purification Workflow

- Synthesis
  - Bound Reagents

- Work up and initial Purification
  - Scavengers
  - Catch & Release

- Final Purification
  - Flash Chromatography
  - Preparative RP HPLC
What slows down drug discovery?

1. Target and Synthesis Design
2. Reaction
3. Work-up - usually extraction & evaporation
4. Purification - usually chromatography
5. Spectral Analysis Registration

Bottlenecks (3) and (4) become greater with microwave chemistry
The Tool Box
1. Polymer supported reagents
2. Polymer supported scavengers
3. Silica supported Reagents
4. Silica supported Scavengers
5. Microwave heating
6. Automated purification systems

How can we use these effectively
# Biotage Resin Reagents

<table>
<thead>
<tr>
<th>Bound Reagent</th>
<th>Solution Analog</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS-TsCl</td>
<td>p-toluenesulfonyl chloride</td>
<td>Catch &amp; Release</td>
</tr>
<tr>
<td>MP-TsOH</td>
<td>p-toluenesulfonic acid</td>
<td>Catch &amp; Release</td>
</tr>
<tr>
<td>PS-DIEA</td>
<td>Hindered tertiary amine</td>
<td>Amine base</td>
</tr>
<tr>
<td>PS-NMM</td>
<td>N-methyl morpholine</td>
<td>Non-benzylic base</td>
</tr>
<tr>
<td>PS-TBD</td>
<td>TBD</td>
<td>Strong Base</td>
</tr>
<tr>
<td>PS-DMAP</td>
<td>DMAP</td>
<td>Catalyst, Catch &amp; Release</td>
</tr>
<tr>
<td>MP-Carbonate</td>
<td>Ammonium carbonate</td>
<td>Base, Catch &amp; Release</td>
</tr>
<tr>
<td>PS-Triphenylphosphine</td>
<td>Triphenylphosphine</td>
<td>Mitsunobu/Wittig/Halogenation</td>
</tr>
<tr>
<td>PS-PPh₃-Pd</td>
<td>Triphenylphosphine Pd(0)</td>
<td>Palladium Catalyst</td>
</tr>
<tr>
<td>PS-Carbodiimide</td>
<td>DCC</td>
<td>Coupling Agent</td>
</tr>
<tr>
<td>PS-HOBt (HL)</td>
<td>HOBt</td>
<td>Coupling agent</td>
</tr>
<tr>
<td>MP-Borohydride</td>
<td>Sodium borohydride</td>
<td>Reducing Agent</td>
</tr>
<tr>
<td>MP-Cyanoborohydride</td>
<td>Sodium cyanoborohydride</td>
<td>Reducing agent</td>
</tr>
<tr>
<td>MP-Triacetoxyborohydride</td>
<td>Sodium triacetoxy borohydride</td>
<td>Reducing agent</td>
</tr>
<tr>
<td>MP-TsO-TEMPO</td>
<td>TEMPO</td>
<td>Oxidizing Agent</td>
</tr>
<tr>
<td>Electrophile</td>
<td>PS-Trisamine</td>
<td>MP-Trisamine</td>
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<tr>
<td>--------------------</td>
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<tr>
<td></td>
<td>MP-Carbonate</td>
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<tr>
<td></td>
<td>PS-Tosylhydrazide</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PS-Thiophenol</td>
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<td></td>
<td>PS-Triphenylphosphine</td>
<td></td>
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<tr>
<td>Nucleophile</td>
<td>PS-Isocyanate</td>
<td>MP-Isocyanate</td>
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<td></td>
<td>PS-Benzaldehyde</td>
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<td></td>
<td>PS-Tosyl chloride</td>
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<tr>
<td></td>
<td>MP-Tosic acid</td>
<td></td>
</tr>
<tr>
<td>Metal</td>
<td>MP-TMT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MP-DEAM</td>
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</table>
ISOLUTE® Si-Triamine

ISOLUTE® Si-Carbonate

ISOLUTE® Si-Ts-Hydrazine

ISOLUTE® Si-Thiol

ISOLUTE® Si-Propylsulfonic acid (SCX-2)

ISOLUTE® Si-EthylPhenyl sulfonic acid (SCX-3)
Surface Images of an MP-Resin
Bound Pd(0) Catalyst
- Stable to air, light and moisture
- Easy Handling
- Shelf-stable at room temperature
- Simplified product isolation
- Low Pd levels in product (< 100ppm)
PS-PPh$_3$-Pd: Suzuki Coupling (Traditional)

- Reactions performed under air, no inert conditions required
- Products isolated in high purity and yield
- Low palladium level in products (< 60 ppm)
PS-PPh₃-Pd & ISOLUTE® Si-Carbonate
Rapid Suzuki Reaction & Workup

Br

O₂N

OH

+ N

O₂N

OH

+ μW 130 °C, 10 min

EtOH / DME (1:1)

Cs₂CO₃

~ 5 mins

Total Synthesis + Purification = ~ 15 min

Ghassemi, S. ACS, Atlanta, GA, March 2006
PS-PPh$_3$-Pd and MP-BH$_4$: Reductive Deprotection of N-Alloc

- Alloc orthogonal to Boc, Fmoc and Cbz groups
- Current Pd(0) catalyst drawbacks:
  - Formation of N-allylated by-product
  - Aqueous work-up
  - High Pd level in products
- Reactions performed under air at rt, no inert condition
- Products isolated in high yield and purity
- Low palladium level in products (< 100 ppm)

1 mmol

\[
\begin{align*}
\text{NHR} & \quad \rightarrow \quad \text{RNH}_2 \\
\text{O} & \quad \text{O} \\
\text{O} &
\end{align*}
\]

- i. 2 mol% PS-PPh$_3$-Pd
- ii. 3 equiv MP-BH$_4$
- DCM:MeOH:H$_2$O (5:4:1); RT, 2 h
- iii. Filter through Na$_2$SO$_4$ plug
Key Microwave-Assisted Transformations
Solid-supported reagents/Scavengers

- Pd Catalyzed reactions
- **PS-Triphenylphosphine**
- Acid catalyzed Reactions
- Base Catalyzed reaction
- Amidation
- Reductive Amination
- Oxidation
PS-Triphenylphosphine

**Wittig Chemistry**
Alkene Synthesis

**Mitsunobu Chemistry**
Ether Synthesis

**Palladium Catalyzed Reactions**
C-C coupling

- **Chlorination**
  Formation of acid chlorides & alkyl halides

- **Cl<sub>3</sub>CCCN or CCl<sub>4</sub>
  Scavenging of Alkyl Halides

**DEAD, DIAD**

- **Formation of acid chlorides & alkyl halides**

**Pd(OAc)<sub>2</sub>**
### PS-Triphenylphosphine - Chlorination

R-OH or R-CO$_2$H $\xrightarrow{\text{PS-PPh}_3 \ (2 \ \text{equiv})}$ R-Cl or R-COCl

**CCl$_4$, reflux**

16 h

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Product</th>
<th>Yield %</th>
<th>% Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td>98</td>
<td>95</td>
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<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
<td>74</td>
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<td><img src="image6" alt="Image" /></td>
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<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
<td>100</td>
<td>100</td>
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<td><img src="image9" alt="Image" /></td>
<td><img src="image10" alt="Image" /></td>
<td>100</td>
<td>100</td>
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<tr>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
<td>73</td>
<td>90</td>
</tr>
</tbody>
</table>

From Rana, S., Gooding, O., Labadie, J. *2003* Unpublished optimized procedures
PS-Triphenylphosphine Microwave-assisted Chlorination

\[
\begin{align*}
\text{BnO} & \quad \text{BnO} \\
\text{OH} & \quad + \\
\text{NOH} & \quad \text{NH}_2 \\
\end{align*}
\]

\[
\begin{align*}
\text{77-98\%} & \\
\end{align*}
\]

(i) PS-PPh$_3$ (3 equiv); CCl$_3$CN (1.5 equiv)  
100 $^\circ$C / 5 min

(ii) DIEA (2 equiv); Aldoxime; THF  
150 $^\circ$C / 15 min

Key Microwave-Assisted Transformations
Solid-supported reagents/Scavengers

- Pd Catalyzed reactions
- PS-Triphenylphosphine
- **Acid catalyzed Reactions**
- Base Catalyzed reaction
- Amidation
- Reductive Amination
- Oxidation
Bound Acid: MP-TsOH
ISOLUTE® Si-TsOH

- Bound sulfonic acid equivalent
- Applications:
  - Acid catalyst
  - Cleavage of acid sensitive groups eg BOC-
  - Scavenger for amines & basic compounds
  - Catch and Release purifications
## Solid-supported TsOH Microwave-assisted Pyrazole Synthesis

![Chemical Structures](image)

**Table:**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acid</th>
<th>Method</th>
<th>Temp</th>
<th>time</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p-TsOH</td>
<td>Non-µW</td>
<td>100 °C</td>
<td>7 h</td>
<td>95 %</td>
</tr>
<tr>
<td>2</td>
<td>p-TsOH</td>
<td>µW</td>
<td>160 °C</td>
<td>5 min</td>
<td>61 %</td>
</tr>
<tr>
<td>3</td>
<td>Si-TsOH SO₃H</td>
<td>Non-µW</td>
<td>100 °C</td>
<td>6 h</td>
<td>84 %</td>
</tr>
<tr>
<td>4</td>
<td>Si-TsOH SO₃H</td>
<td>µW</td>
<td>160 °C</td>
<td>5 min</td>
<td>95 %</td>
</tr>
</tbody>
</table>

Simultaneous BOC-deprotection & Amine Purification

**Acidic Si-phenylsulfonic acid (Si-TsOH)**
- spontaneously remove BOC- protected amine group
- purify the free amines via “Catch & Release” mechanism

Unsymmetrical sulfamides

* Ghassemi, S. et al. Molecular Diversity, 2005, 9, 295-299
Key Microwave-Assisted Transformations
Solid-supported reagents/Scavengers

• Pd Catalyzed reactions
• PS-Triphenylphosphine
• Acid catalyzed Reactions
• **Base Catalyzed reaction**
• Amidation
• Reductive Amination
• Oxidation
PS-DIEA, Si-Carbonate, Si-TsOH
Rapid Amidation and workup

\[
\text{Br-} \text{C}_6\text{H}_4\text{COOH} + \text{C}_6\text{H}_5\text{NH} \xrightarrow{\text{PS-DIEA, HATU, } \mu\text{W, 110-150 °C}} \text{6-12 min} \xrightarrow{\text{Catch & Release}} \\
\text{Br-} \text{C}_6\text{H}_4\text{CONH}_2 + \text{C}_6\text{H}_5\text{NH}
\]

59% yield
97% Purity

Ghassemi, S. ACS, Atlanta, GA, March 2006
Key Microwave-Assisted Transformations
Solid-supported reagents/Scavengers

- Pd Catalyzed reactions
- PS-Triphenylphosphine
- Acid catalyzed Reactions
- Base Catalyzed reaction
- **Amidation**
- Reductive Amination
- Oxidation
Amide Synthesis: Reagent Comparison

- **PS-Carbodiimide - Coupling Agent**
  - One-step amide synthesis
  - Scavenging may be required
  - Rearrangement to acylisourea can be problematic --- Can be solved by addition of HOBt

- **PS-HOBt - Active Esters**
  - Two-step process
  - Amine = limiting reagent in acylation, affords high purity amides
  - Storable reactive intermediate
PS-Carbodiimide & ISOLUTE® Si-Carbonate
Rapid Acylation & Purification of Amines

\[
\text{I-COOH} + \text{NH}_3 + \text{tSO-I} \xrightarrow{\text{PS-Carbodiimide}} \text{HOBt, DIEA} \xrightarrow{\mu W, 100 \degree C, 5 \text{ min}} \text{Product}
\]

\[
\text{HOBr} + \text{Acid}
\]

Reaction Mixture

Product

Total Synthesis + Purification \sim 10 \text{ mins}

99% yield

98 % Purity

Ghassemi, S. ACS, Atlanta, GA, March 2006
PS-Carbodiimide: Microwave Assisted Synthesis

\[ \text{O} \quad \text{OH} \quad + \quad R_1^1R_2^2\text{NH} \quad \xrightarrow{1) \text{PS-Carbodiimide, HOBt, DMA, uW, 100 }^\circ\text{C, 5min}} \quad \text{NR}_1^1R_2^2 \quad \xrightarrow{2) \text{Si-Carbonate}} \]

98% Yield
98% purity
< 15 min

\[ R_1^1R_2^2\text{NH} = \]

- BnNH₂
- O
- Ph
- Me
- Me

- High yield, high purity using filtration, Si-CO₃ cartridge based purification

- D. Sauer et.al. Organic Letters 2003, 24, 4721-4724
Amides From Active Ester Resins

Key Microwave-Assisted Transformations
Solid-supported reagents/Scavengers

- Pd Catalyzed reactions
- PS-Triphenylphosphine
- Acid catalyzed Reactions
- Base Catalyzed reaction
- Amidation
- **Reductive Amination**
- Oxidation
Bound Reagents
Reductive Amination

**MP-BH(OAc)$_3$**
- Tolerates acid-sensitive groups: ketals, acetals
- Secondary amines isolated as acetate
- Tertiary amines as free base

**MP-BH$_3$CN**
- Requires acetic acid
- Similar reactivity and scope
- Masked toxicity
- Very little pressure build up with MAOS

**MP-BH$_4$ /Ti(iOPr)$_4$**
- Suppresses over-alkylation with reactive carbonyls
- Enables use of:
  - sterically hindered carbonyls ie. adamantyl ketones
  - enolizable ketones eg acetophenone
- Titanium isopropoxopoxide scavenged by PS-DEAM
Synthesis of Secondary Amines

- **Typical solution-phase protocol**

  \[ R_1 \text{NH}_2 + R_2 \text{R}_3 \overset{\text{1.4 equiv. NaBH(OAc)}_3}{\text{RT, 1-16 h}} \rightarrow \begin{array}{c} R_2 \text{R}_3 \\ \text{1-1.2 equiv.} \end{array} + \begin{array}{c} \text{R}_1 \text{NH} \\ \text{R}_2 \text{R}_3 \end{array} \]

  
  
  i. 1.4 equiv. NaBH(OAc)$_3$
  
  (1 equiv. HOAc)
  
  RT, 1-16 h
  
  ii. 1M NaOH
  
  iii. Etheral extraction
  
  iv. Wash, dry
  
  v. Column purification

  Pure product

- **Expedited bound reagent protocol**

  \[ R_1 \text{NH}_2 + R_2 \text{R}_3 \overset{\text{2.5 equiv MP-BH(OAc)}_3}{\text{RT, 1 - 16 h, THF}} \rightarrow \begin{array}{c} R_2 \text{R}_3 \\ \text{1-1.2 equiv.} \end{array} + \begin{array}{c} \text{R}_1 \text{NH} \\ \text{R}_2 \text{R}_3 \end{array} \]

  
  
  i. 2.5 equiv MP-BH(OAc)$_3$
  
  RT, 1 - 16 h, THF
  
  ii. 1 equiv PS-CHO, 6 h
  
  iii. Filter, wash, concentrate

  Pure product
**MP-CNBH$_3$**

**Reductive Amination**

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Amine</th>
<th>Carbonyl</th>
<th>Conv. Yield</th>
<th>$\mu$W Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Amine structure" /></td>
<td><img src="image" alt="Carbonyl structure" /></td>
<td>79%</td>
<td>98%</td>
</tr>
<tr>
<td><img src="image" alt="Amine structure" /></td>
<td><img src="image" alt="Carbonyl structure" /></td>
<td>91%</td>
<td>85%</td>
</tr>
<tr>
<td><img src="image" alt="Amine structure" /></td>
<td><img src="image" alt="Carbonyl structure" /></td>
<td>39%</td>
<td>77%</td>
</tr>
<tr>
<td><img src="image" alt="Amine structure" /></td>
<td><img src="image" alt="Carbonyl structure" /></td>
<td>63%</td>
<td>79%</td>
</tr>
<tr>
<td><img src="image" alt="Amine structure" /></td>
<td><img src="image" alt="Carbonyl structure" /></td>
<td>69%</td>
<td>73%</td>
</tr>
</tbody>
</table>

**Conventional condition:**
1. THF
2. Room temp, 16 h

**Microwave condition:**
1. DCM
2. 110 $^\circ$C, 5-7 min

Pannagiotis, P. www.biotagepathfinder.com

---

Frqyhqwlrqdo#frqglwrq = (1) THF  
(2) room temp, 16 h

Plfurzdyh#frqglwrq = (1) DCM  
(2) 110 $^\circ$C, 5-7 min

---
MP-CNBH₃ – Reductive Amination
Purification Strategies - summary

**Scenario 1**

```
R-N-Ar
R2 R3

2° or 3° amine
Product
```

```
“Catch & Release”

```

```
Excess Carbonyl

```

```
MP-TsOH

```

```
Product
NH₃⁺ SO₃⁻

```

```
NH₃-MeOH

```

```
Product
NH₃⁺
2° or 3° amine

```

```
R2

```

```
Excess Carbonyl

```

**Scenario 2**

```
R-N-Ar
R2 R3

2° amine
Product
```

```
Ar-NH₂

```

```
Excess 1° amine

```

```
SO₃H

```

```
Product
NH₃⁺ SO₃⁻

```

```
NH₃-MeOH

```

```
Product
NH⁺
2° amine

```

```
R3

```

```
Excess Carbonyl

```

**Scenario 3**

```
R-N-Ar
R2 R3

3° amine
Product
```

```
Ar-NH

```

```
Excess 2° amine

```

```
OCN

```

```
PS-Isocyanate

```

```
MP-Isocyanate

```

```
Product
NH⁺
3° amine

```

```
R2

```

```
xs 2° amine

```

```
xs 1° amine

```

```
Ar-N

```

```
R

```

```
Product
OCN

```

```
PS-Benzaldehyde

```

```
OHC

```

```
PS-Benzaldehyde

```

```
N

```

```
H

```

```
N

```

```
Ar

```

```
R

```

```
xs 1° amine

```

```
R2

```

```
R3

```

```
R

```

```
Ar

```

```

```
```

Biotage
Key Microwave-Assisted Transformations
Solid-supported reagents/Scavengers

- Pd Catalyzed reactions
- PS-Triphenylphosphine
- Acid catalyzed Reactions
- Base Catalyzed reaction
- Amidation
- Reductive Amination
- Oxidation
MP-TsO-TEMPO
Bound Oxidizing Agent

MP-TsO-TEMPO is a bound oxoammonium sulfonate

- Oxidation of benzylic, allylic, acetylenic and cyclic secondary alcohols
- Highly controlled reaction. No over-oxidation to acid.
- Stable
- Resin is a mixture of active oxoammonium and reduced hydroxylammonium species.
### Oxidation of Alcohols

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Method</th>
<th>Solvent</th>
<th>Temp</th>
<th>Time</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Alcohol 1" /></td>
<td>Non-µW</td>
<td>CH(_3)CN</td>
<td>rt</td>
<td>16 h</td>
<td>95 %</td>
</tr>
<tr>
<td><img src="image2.png" alt="Alcohol 2" /></td>
<td>µW</td>
<td>DCM</td>
<td>100 °C</td>
<td>10 min</td>
<td>100 %</td>
</tr>
<tr>
<td><img src="image3.png" alt="Alcohol 3" /></td>
<td>Non-µW</td>
<td>CH(_3)CN</td>
<td>rt</td>
<td>16 h</td>
<td>99 %</td>
</tr>
<tr>
<td><img src="image4.png" alt="Alcohol 4" /></td>
<td>µW</td>
<td>DCM</td>
<td>60 °C</td>
<td>2 h</td>
<td>100 %</td>
</tr>
<tr>
<td><img src="image5.png" alt="Alcohol 5" /></td>
<td>Non-µW</td>
<td>CH(_3)CN</td>
<td>rt</td>
<td>16 h</td>
<td>70 %</td>
</tr>
<tr>
<td><img src="image6.png" alt="Alcohol 6" /></td>
<td>µW</td>
<td>DCM</td>
<td>60 °C</td>
<td>5 min</td>
<td>93 %</td>
</tr>
<tr>
<td><img src="image7.png" alt="Alcohol 7" /></td>
<td>Non-µW</td>
<td>DCM</td>
<td>rt</td>
<td>16 h</td>
<td>70 %</td>
</tr>
<tr>
<td><img src="image8.png" alt="Alcohol 8" /></td>
<td>µW</td>
<td>DCM</td>
<td>60 °C</td>
<td>1.5 min</td>
<td>100 %</td>
</tr>
<tr>
<td><img src="image9.png" alt="Alcohol 9" /></td>
<td>Non-µW</td>
<td>DCM</td>
<td>rt</td>
<td>16 h</td>
<td>100 %</td>
</tr>
<tr>
<td><img src="image10.png" alt="Alcohol 10" /></td>
<td>µW</td>
<td>DCM</td>
<td>60 °C</td>
<td>2.5 min</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Lundin, R. z z z lerwjhdsdkiqghuilfrp
MP-TMT – New Palladium Scavenger

Macroporous polystyrene-2,4,6-trimercaptotriazine

- Bound TMT ligand on macroporous resin
- Scavenges Pd(II) and Pd(0), ligated palladium
- Effective in aqueous and non-aqueous solutions
- Useful for compound polishing
- Reduces residual palladium to low ppm levels
Palladium Scavenging: Advances

- Scavenges ligated palladium
- Aqueous and non-aqueous
- Next generation backbone

\[
\text{HO-Br} + \text{B(OH)₂} \xrightarrow{\text{PdCl₂(PPh₃)₂}} \text{Product + PdLₙ}
\]

Aq. \(\text{Na}_2\text{CO}_3\), Toluene
80 °C, 24 h, inert atmosphere
work-up

\[
\text{HO-OMe} \rightarrow \text{HO-OMe}
\]

5-10 equiv, rt, stir 4-16 h

33400 PPM Pd (ICP) <190 PPM Pd (ICP)

\[
\%	ext{ scav 2 equiv resin with time}
\]

Biotage
SPE
Solid Phase Extraction

• Purification------Catch and release technology
Bound Acid: MP-TsOH

- Bound sulfonic acid equivalents
  - Highly cross-linked polystyrene based

- Scavenges 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

1. Condition with DCM, DMF or methanol
2. Apply sample
3. Wash with organic solvent
4. Release product with 4 M ammonia/methanol

Non-basic impurities washed away
Thank you for your attention