Microwave Chemistry and Solid Phase Synthesis

(a). Solid phase peptide synthesis

(b). Immobilized reagents

(c). Pd chemistry

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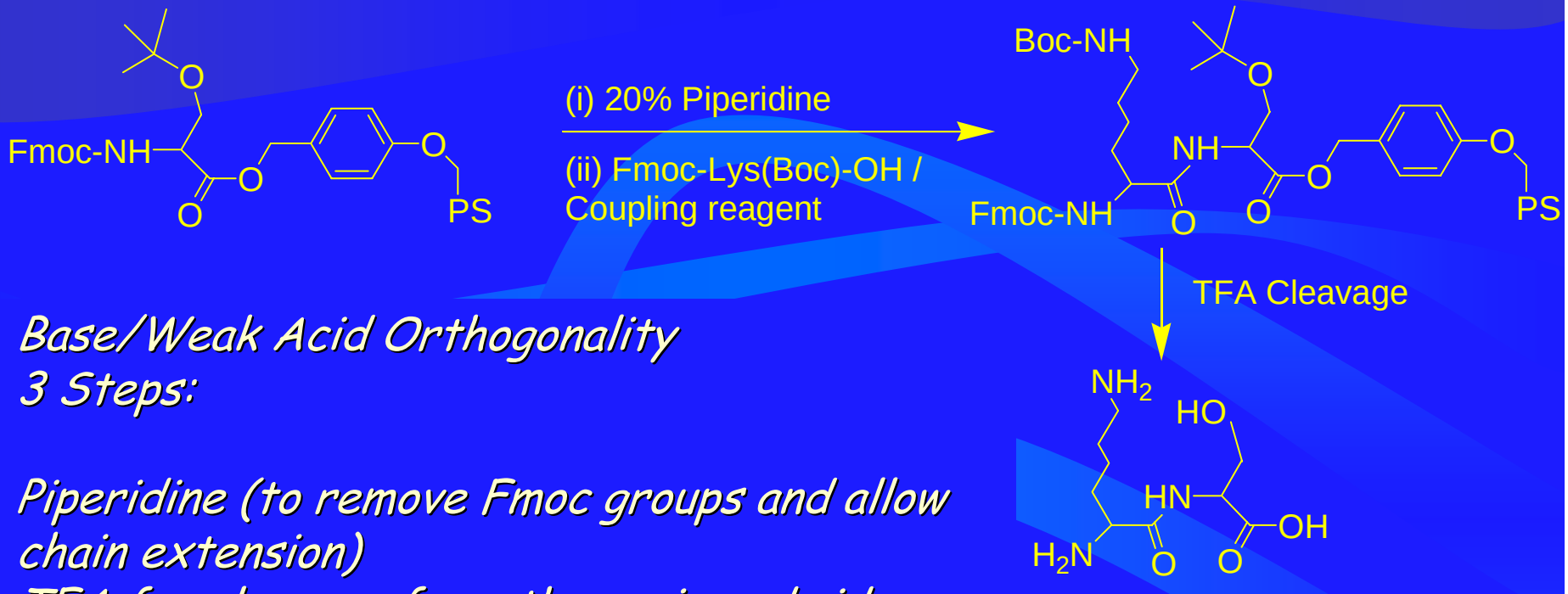
Solid Phase Peptide Synthesis - The Fmoc/Bu^t Route



Base/Weak Acid Orthogonality
3 Steps:

Piperidine (to remove Fmoc groups and allow chain extension)
TFA for cleavage from the resin and side chain deprotection!
Coupling of new amino acid to growing peptide chain

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Solid Phase Peptide Synthesis – Which Steps Might Benefit from Microwave Heating?

Piperidine (to remove Fmoc groups)

TFA for cleavage from the resin and side chain deprotection!

Coupling of new amino acid to growing peptide chain

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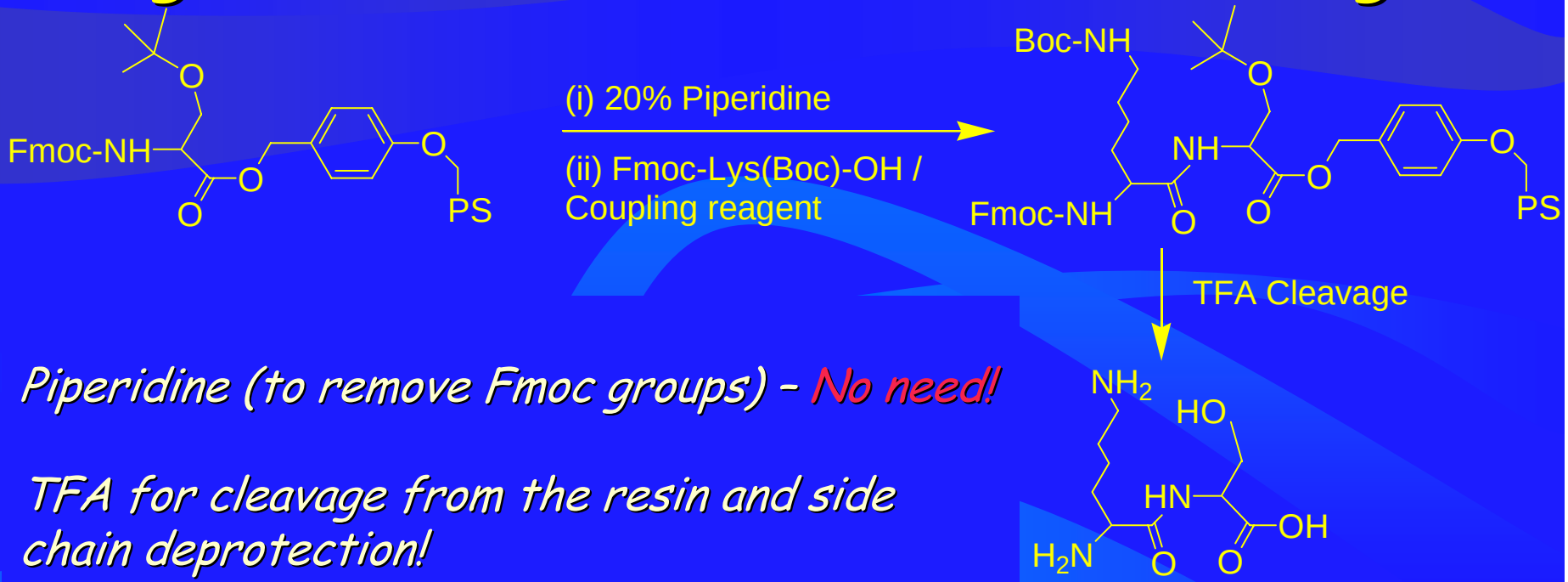
Solid Phase Peptide Synthesis – Which Steps Might Benefit from Microwave Heating?



Piperidine (to remove Fmoc groups) – No need!

TFA for cleavage from the resin and side chain deprotection!

Coupling of new amino acid to growing peptide chain

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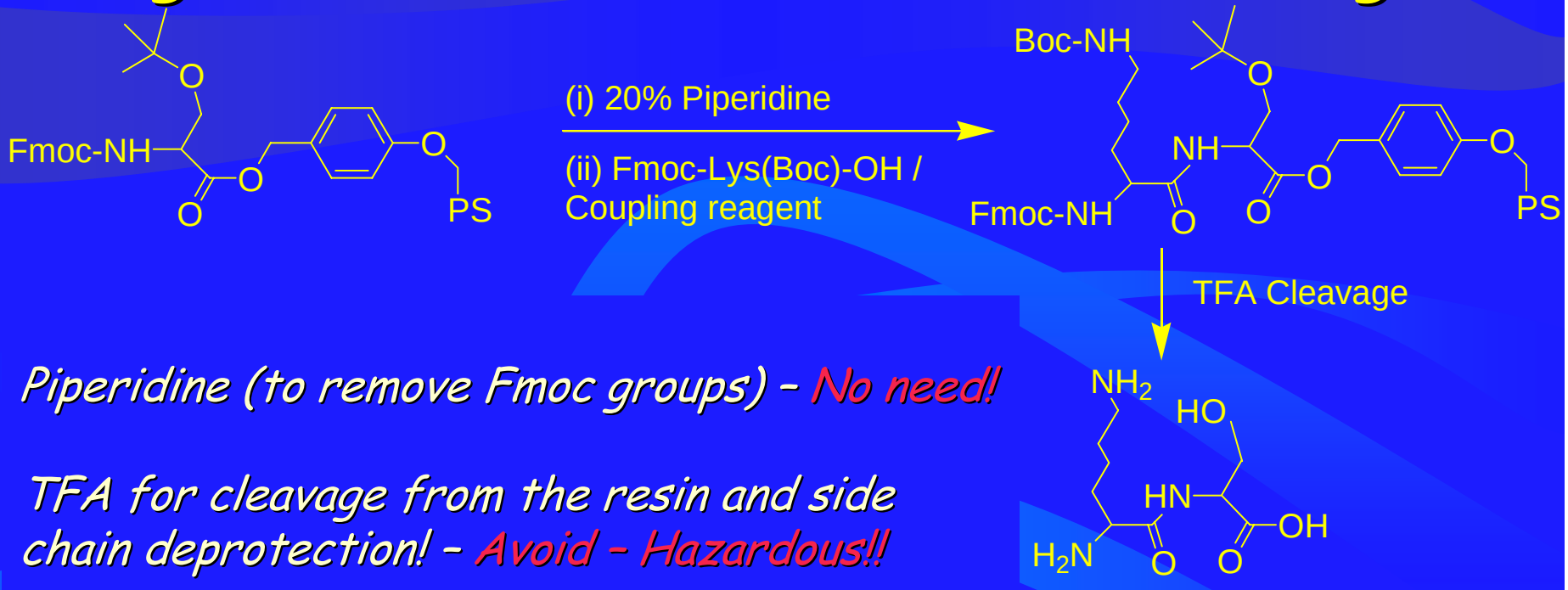
Solid Phase Peptide Synthesis – Which Steps Might Benefit from Microwave Heating?



Piperidine (to remove Fmoc groups) – No need!

TFA for cleavage from the resin and side chain deprotection! – Avoid – Hazardous!!

Coupling of new amino acid to growing peptide chain

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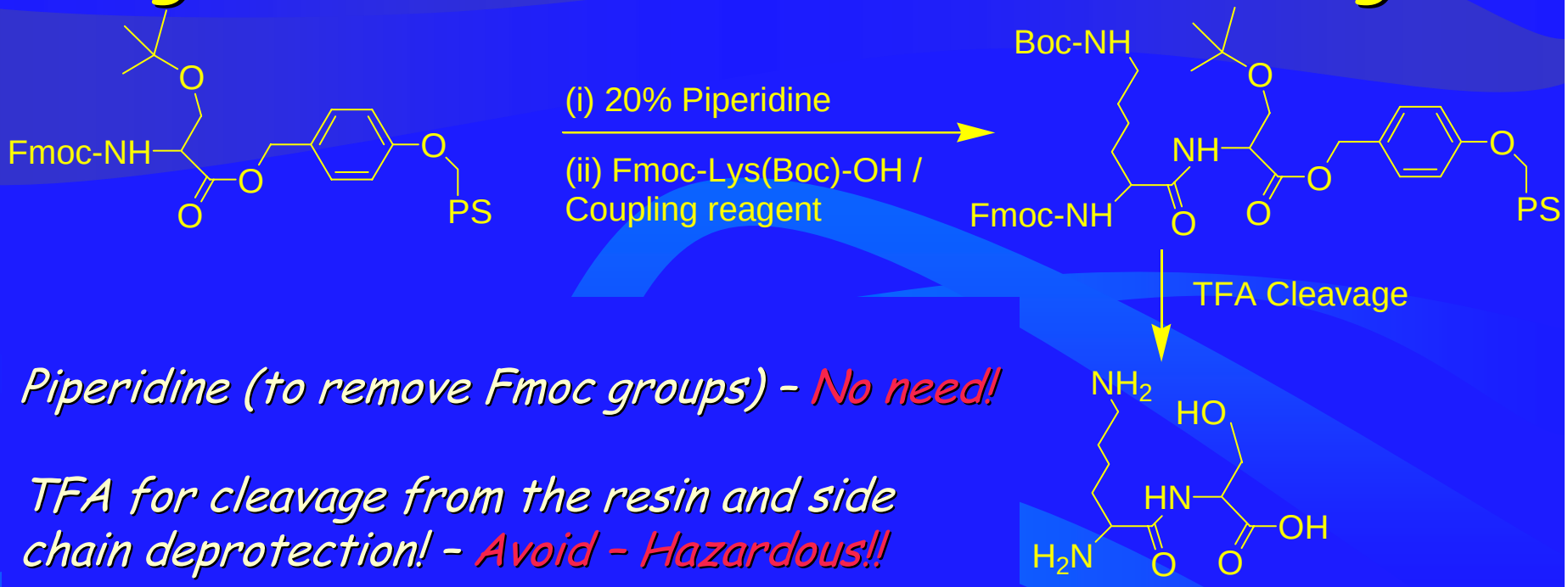
Solid Phase Peptide Synthesis – Which Steps Might Benefit from Microwave Heating?



Piperidine (to remove Fmoc groups) – No need!

TFA for cleavage from the resin and side chain deprotection! – Avoid – Hazardous!!

Coupling of new amino acid to growing peptide chain – Yes – Main cause of peptide synthesis failure

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(a). Peptide Chemistry and Microwave Heating

- Fmoc group stability and DMF decomposition
- Many coupling agents have short half-lives once added to a reaction mixture at 25°C (see Albericio, JOC, 1998 e.g. HATU and PyBOP – $t_{1/2}$ = 40 min at 25°C – so at 65°C, $t_{1/2}$ = 2.5 min).
- Avoid the use of exotic (and expensive) coupling regents – side reactions, short lived

- Do not heat Fmoc groups above 65°C
- DMF, DMA or NMP as solvent .
- Use DIC/HOBt for coupling.

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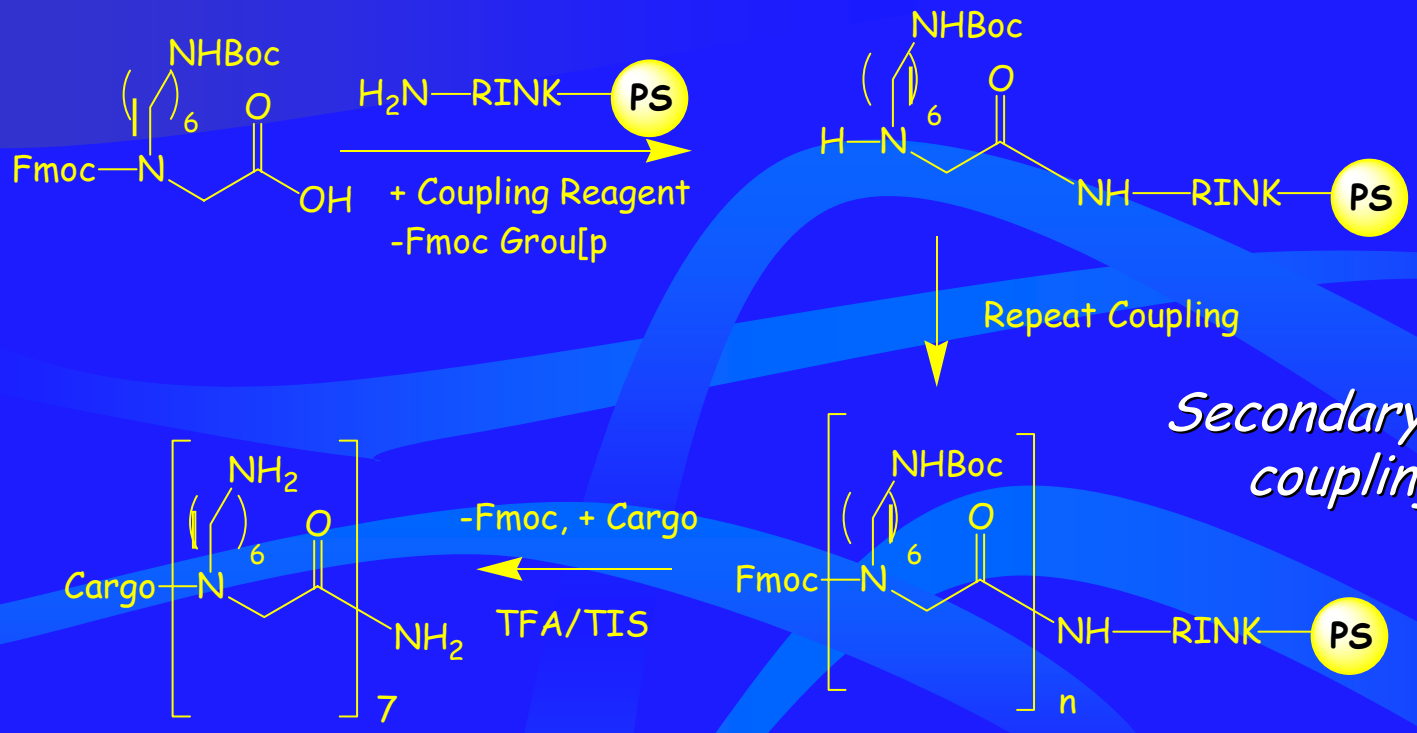
(a). Peptide Chemistry and Microwave Heating



Secondary amine makes coupling difficult

N-Alkylglycines for cell delivery

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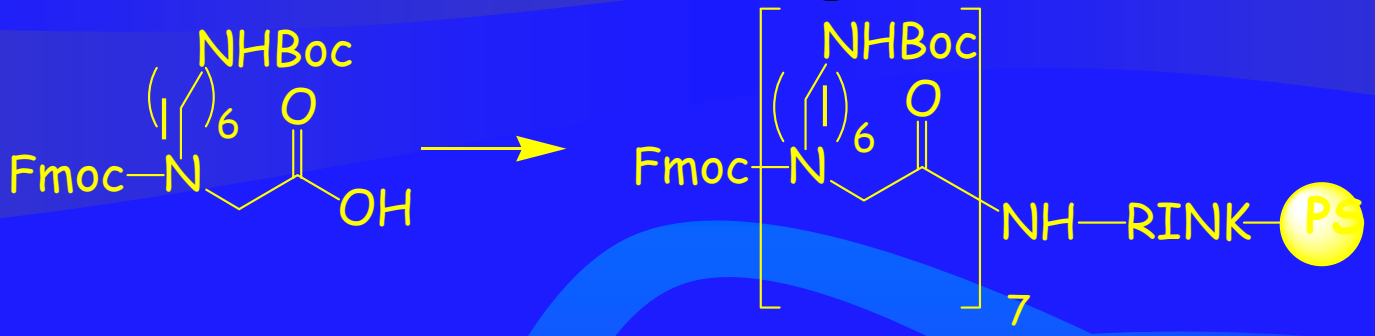
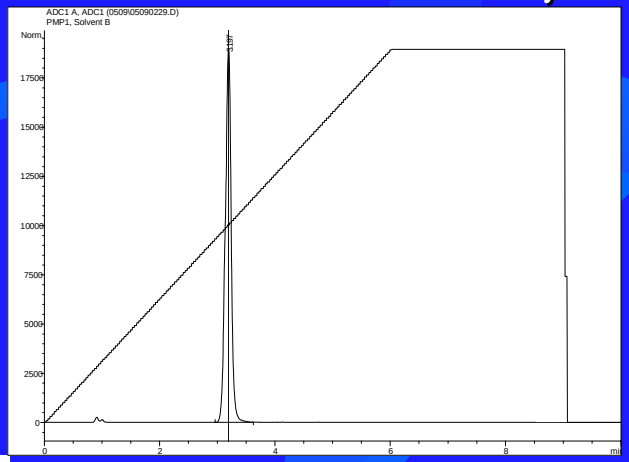
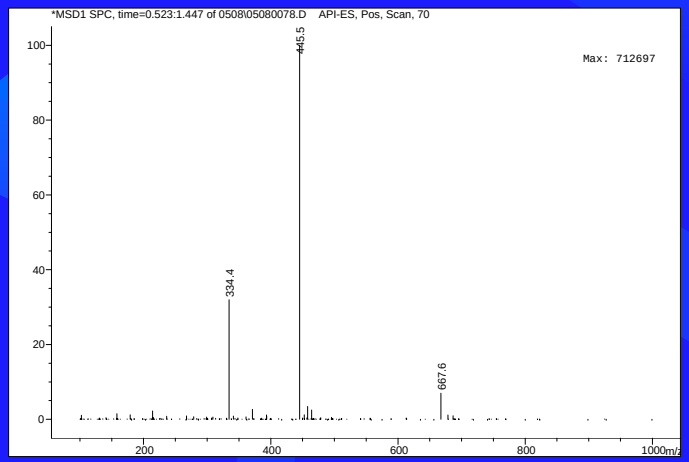
(a). Peptide Chemistry and Microwave Heating



Microwave heating: 60 °C, 10 minute couplings
DIC/HOBt (0.1M, 3equiv.)
20% Piperidine – 10 minutes





98% purity, ELS Detection
Single couplings

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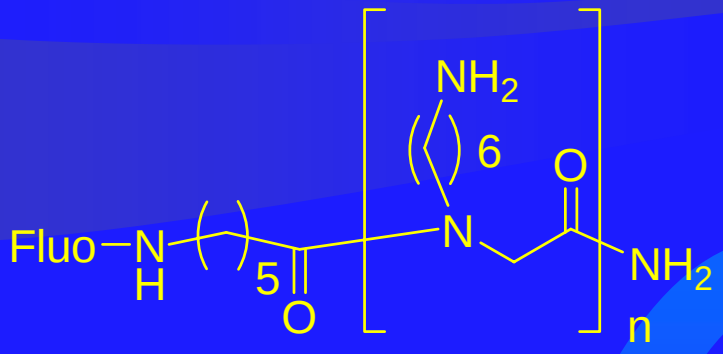
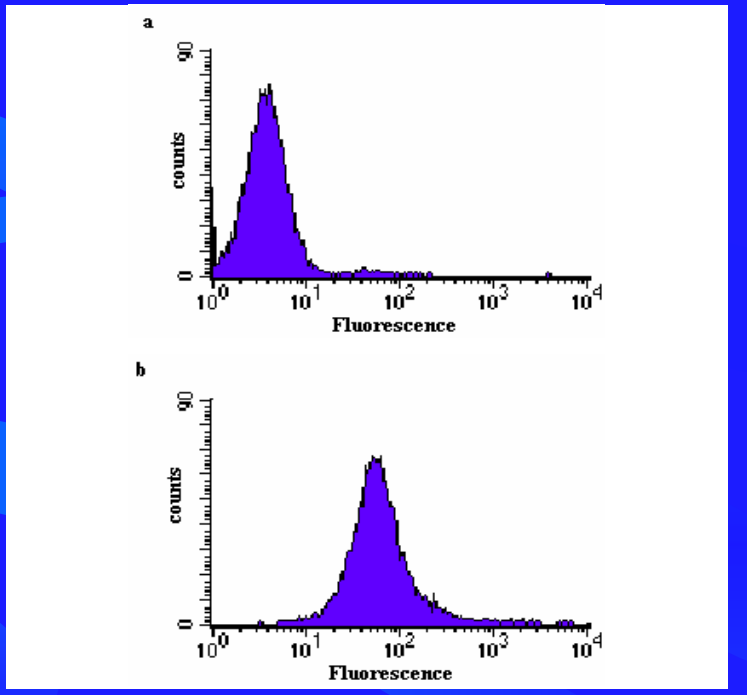
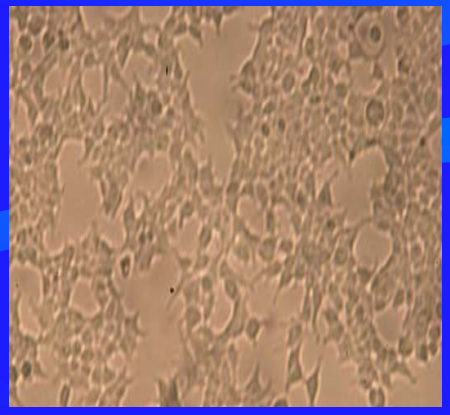
Cellular Delivery - Cell Permeable Peptoids

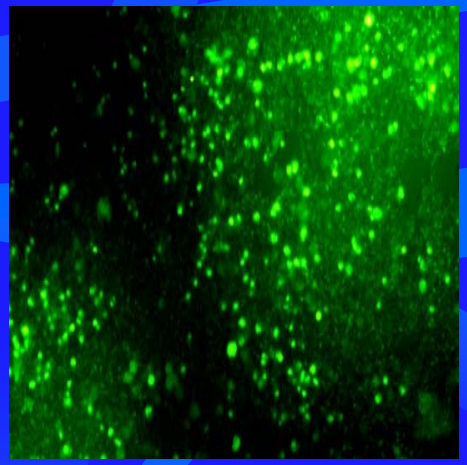


Flow cytometry analysis with HEK293T cells





HEK293T (human embryonic kidney) cells, general transmission image at low magnification

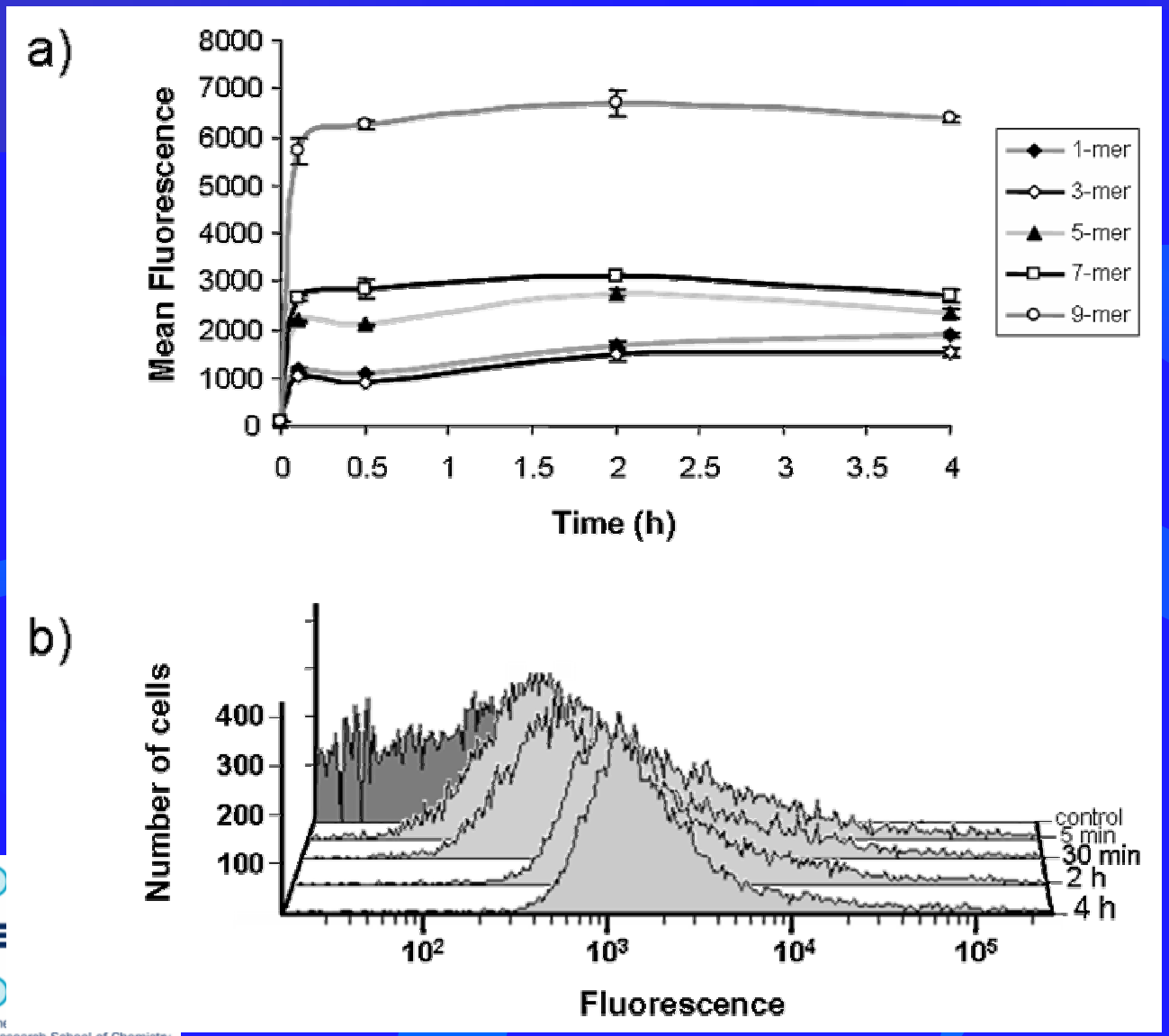


10μM, 37°C for 6h, fluorescent image at low magnification

Oligomer	% Incorporation
n = 3	99
n = 5	93 (25°C)
n = 5	97 (37°C)
n = 7	99

Peretto, I.; Sanchez-Martin, R. M.; Wang, X.-h.; Ellard, J.; Mittoo, S.; Bradley, M. *Chemical Communications* **2003**, 2312-2313

Cell Permeable Peptoids – Uptake



a) Comparative uptake of K562 cells with oligomers (n=1,3,5,7,9)

b) HeLa cells after incubation with 10 μM solution of compound (n=9).

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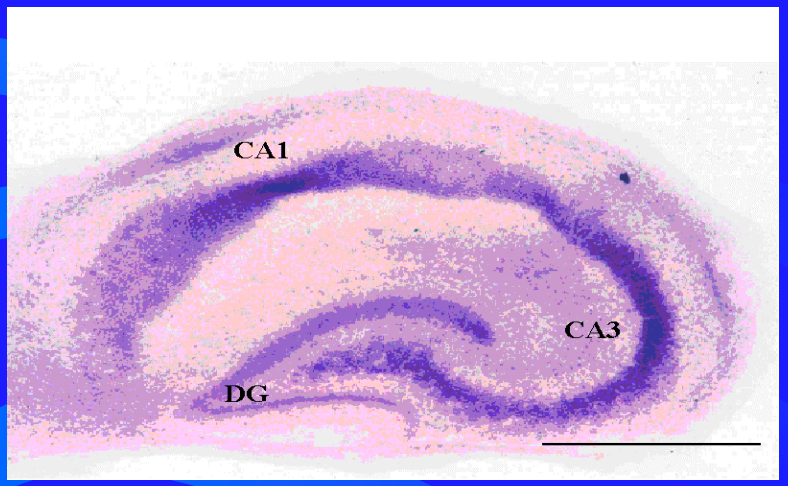
Cell Permeable Peptoids

Cellular uptake of peptoid (n = 7) by a hippocampal slice culture



Thionin stained hippocampal slice showing all the cell layers

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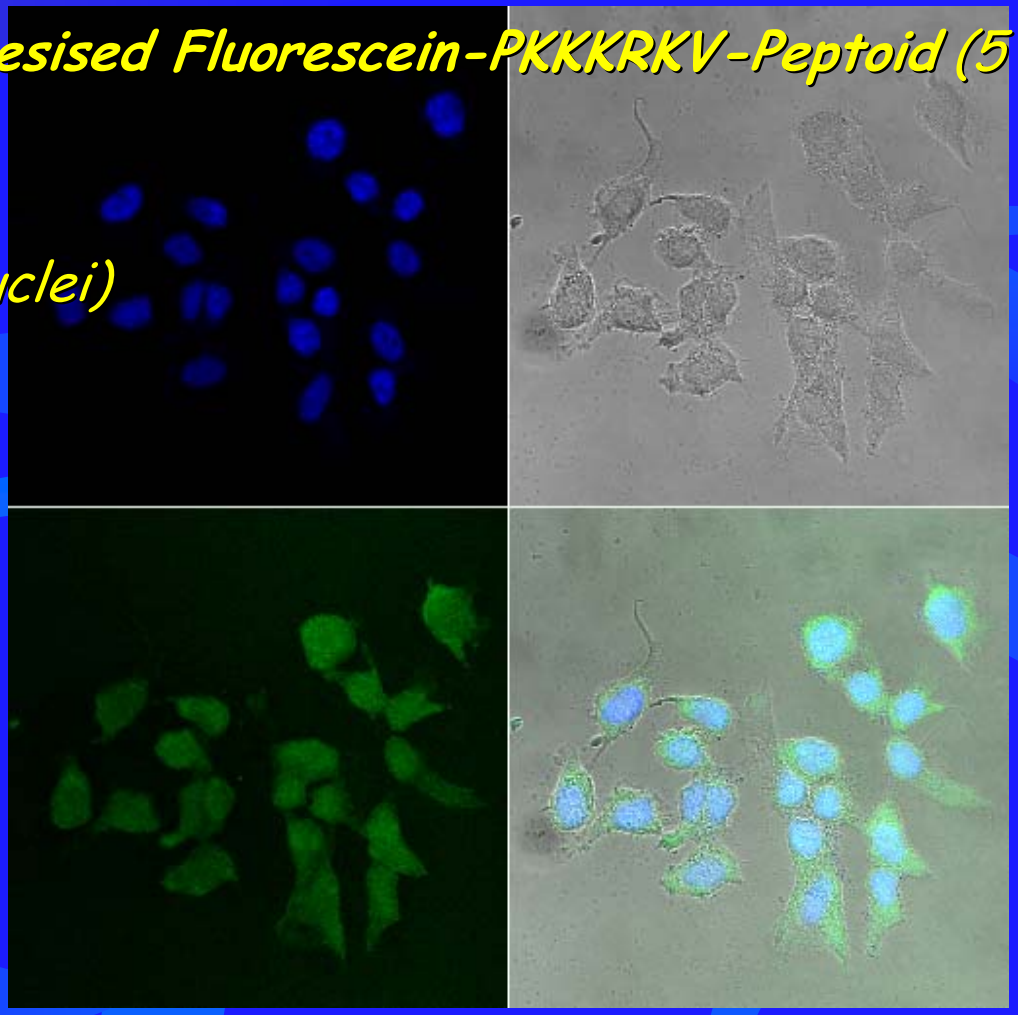
Directed Chemistry in Cells

Nuclei labelling in live cells

Synthesised Fluorescein-PKKKRKV-Peptoid (5 mer)

(DAPI stained nuclei)



Delivery within 30 mins

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Can we deliver α-MSH into Cells and Tissue?

a-MSH : a-Melanocyte Stimulating Hormone
(Ac-SYSMEHFRWGKPV-NH_2)

- Cell impermeable.
- Binds the Melanocortin 1 receptor (MC1R).
- Stimulates the pigmentation of melanocytes and melanoma cells.
- Immunomodulatory activity (suppression of lymphocyte proliferation in vitro at 10^{-13} M to 10^{-11} M).

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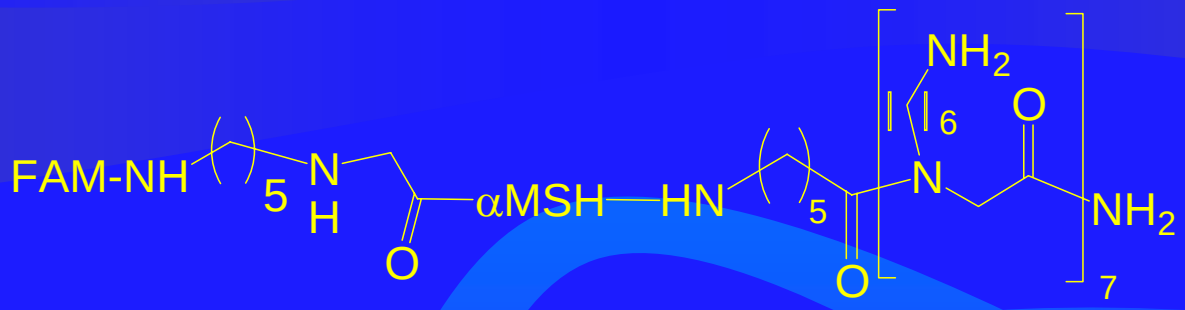
(a). Peptide Chemistry and Microwave Heating



Addition of 17-mer peptide (αMSH)

Microwave heating: 60 °C, 10 minute couplings DIC/HOBt (0.1M, 3equiv.)

Coupling with 5(6)-carboxyfluorescein (again 10 minutes and quantitative)

Isolated yield of peptide = 45% (>98% per step)

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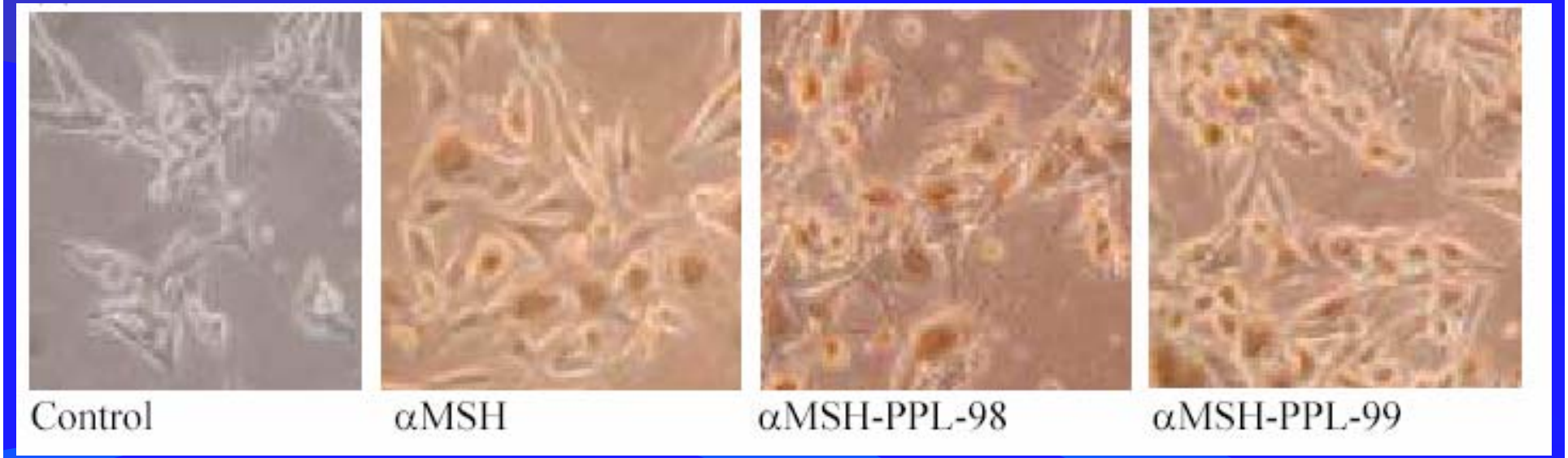
Pigmentation Assay



S91 melanoma cells in culture

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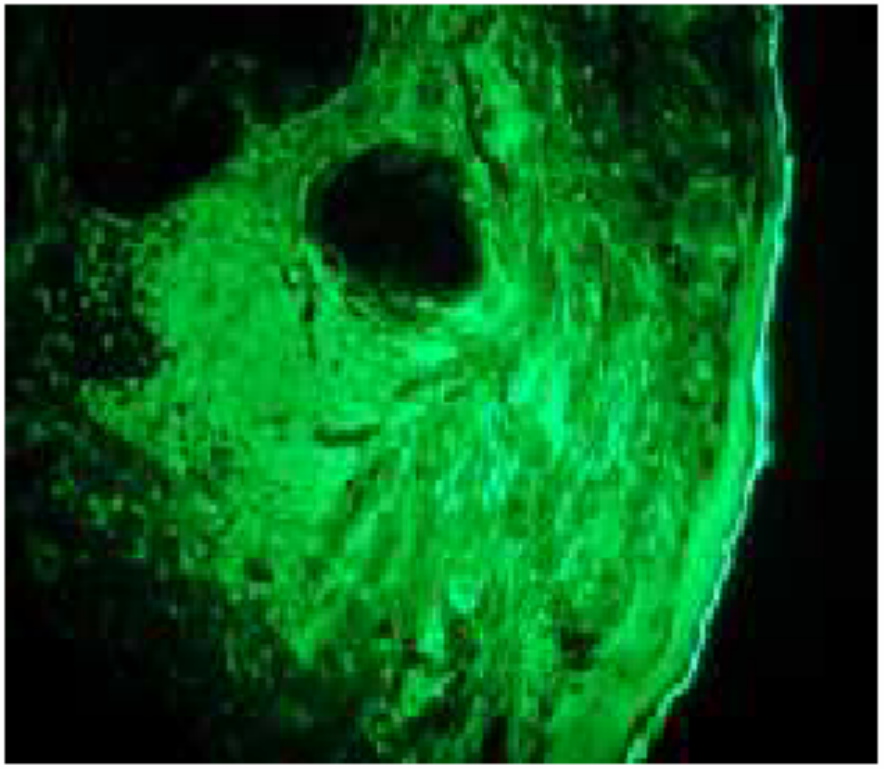
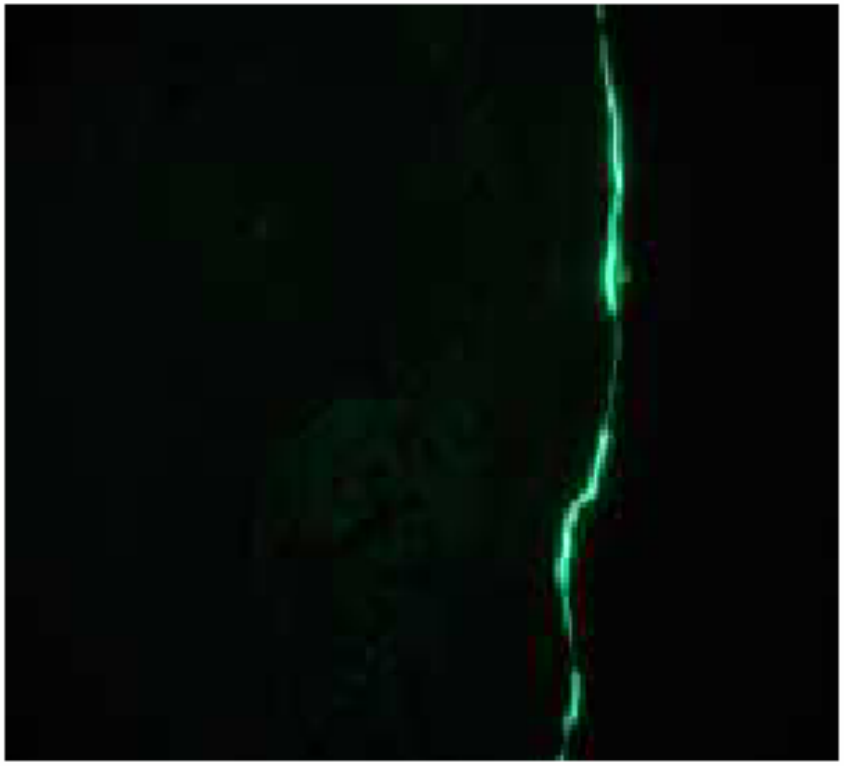
Human Skin - Delivery





Fluo-α-MSH-Peptoid (99) (3h, 10^{-3} M)

Fluo-α-MSH (3h, 10^{-3} M)

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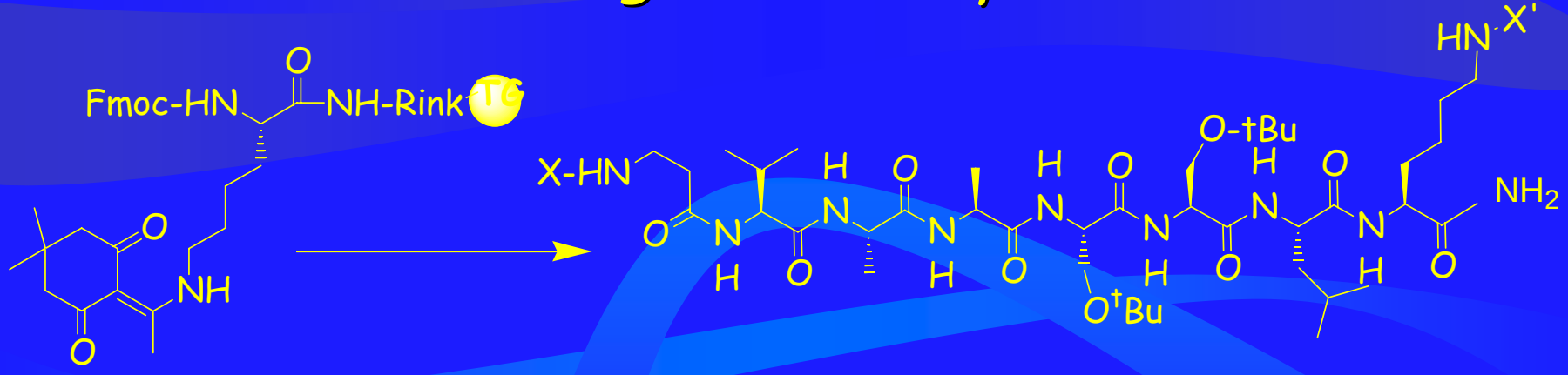
(a). Peptide Chemistry and Microwave Heating - FRET Peptides

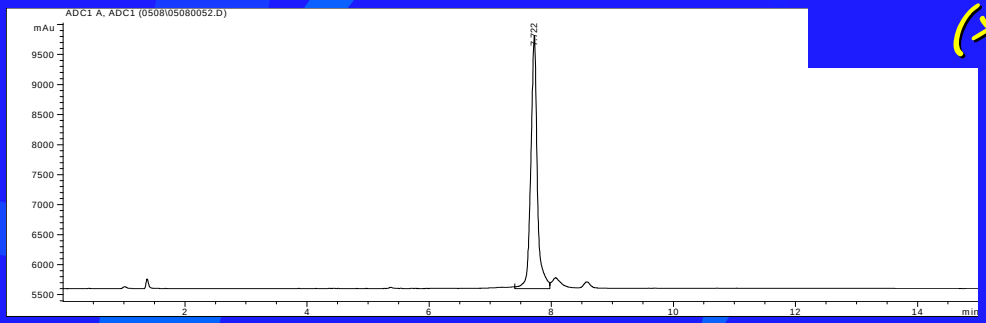


X = Dabcyl; X' = FAM

X = Fmoc-3-(NO_2)Tyr; X' = 2-Abz

10 minute couplings: 60°C, DMF, DIC/HOBt
All Fluorophores coupled easily (10 minutes).

Dabcyl/FAM Peptide (>90% purity)



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(a). Peptide Chemistry and Microwave Heating

60°C is optimal (rapid couplings and no by-products)

Single couplings needed in all cases tested to date

DIC/HOBt performed excellently under these conditions

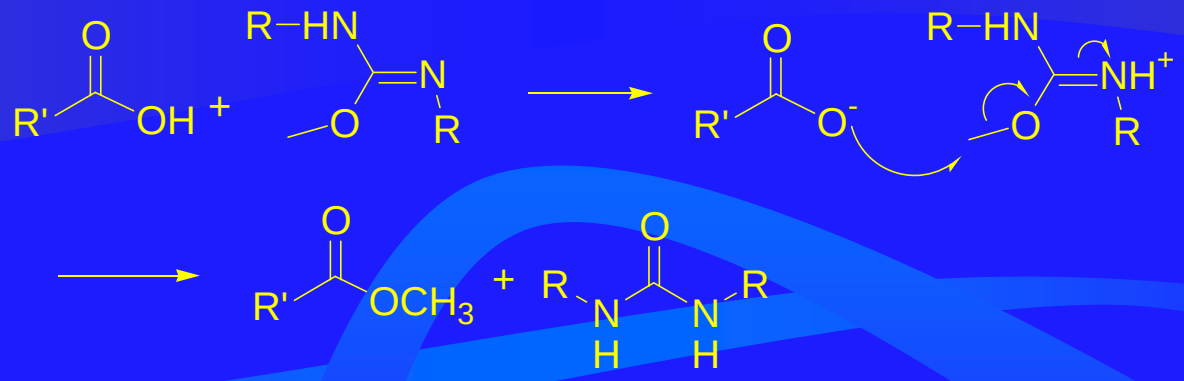
Avoids the use of exotic coupling reagents with their problems and side-reactions

Excellent method for coupling poorly reactive acids such as fluorophores

Time needed reduced form 1 week to 1 day with Peptoid-Peptide conjugate

Arrhenius based acceleration

Isoureas as Alkylating Agents (CCE)



Isoureas have been used to prepare esters from carboxylic acids. They are tolerant of other functional groups in the molecule but the urea by-product can be difficult to remove

Low toxicity, but also lower reactivity than other alkylating agents

Use of a solid-supported isourea?

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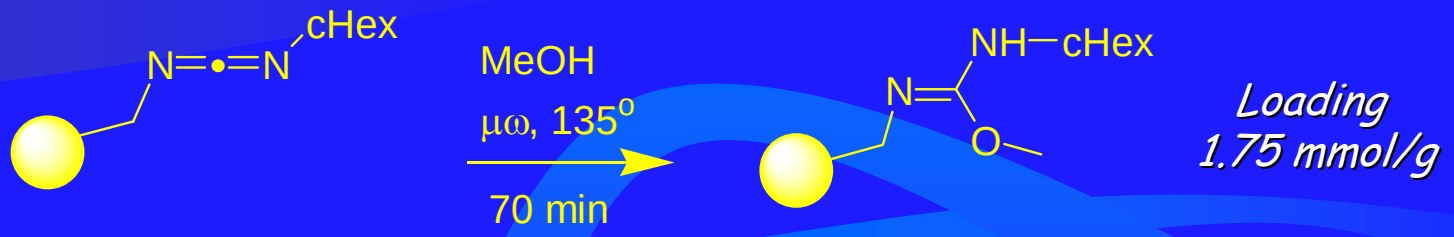
(b). Polymer-Supported O-alkyl-isoureas



Loading 1.75 mmol/g

- *Microwave heating with carbodiimide resins*
- *No other reagents apart from methanol are used (no purification problems)*
- *Easily followed by IR*

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Brono Linclau and Stefano Crosignani, Alessander Chighine

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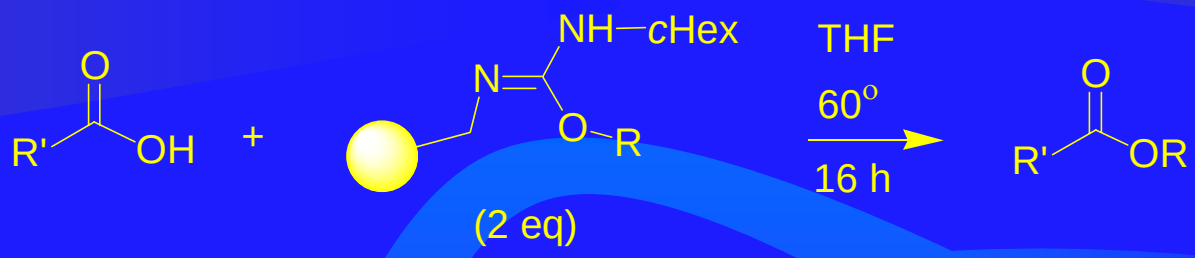
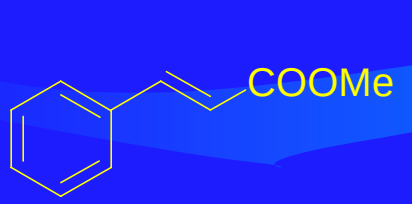
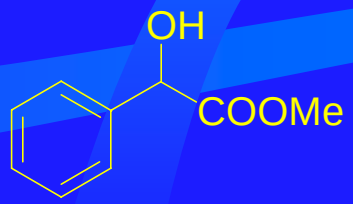
(b). Synthesis of Esters

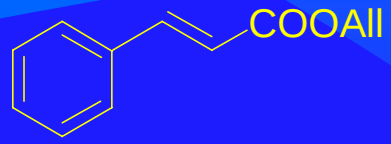




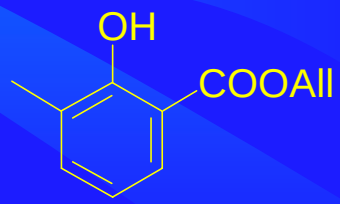
83% (purity >95%)



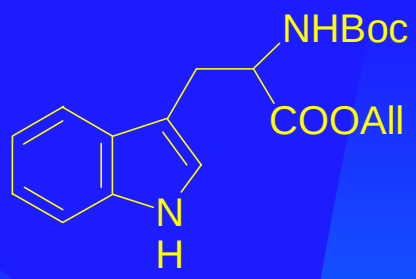
85% (>95%)



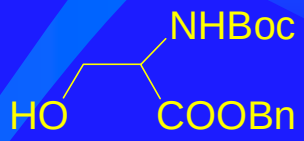
82% (>95%)



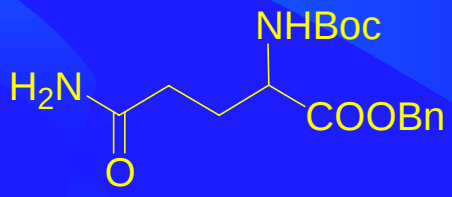
90% (>95%)



93% (>95%)



99% (>95%)



98% (>95%)

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Org. Lett. **2003**, *5*, 853-856

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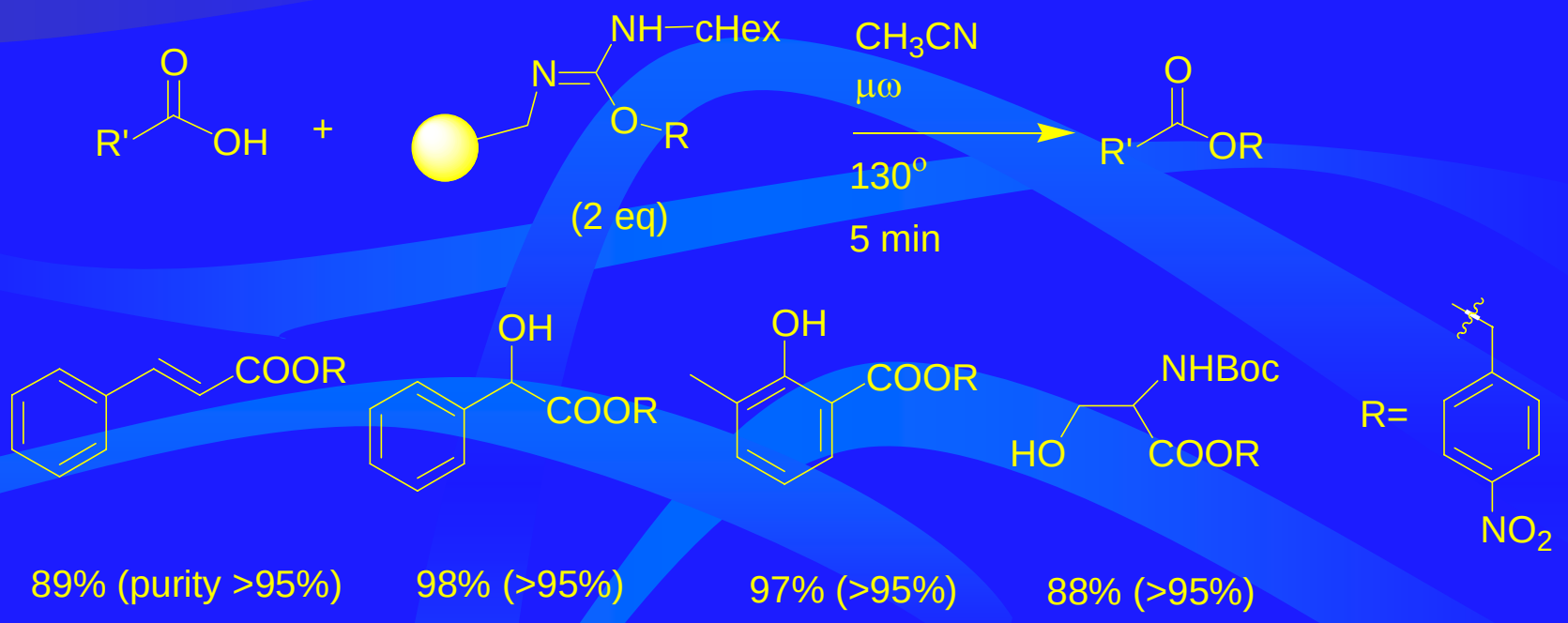
(b). Microwave-Assisted Synthesis of Esters



- The use of acetonitrile instead of THF allows faster heating
- Similar results obtained for methyl, benzyl and allyl esters

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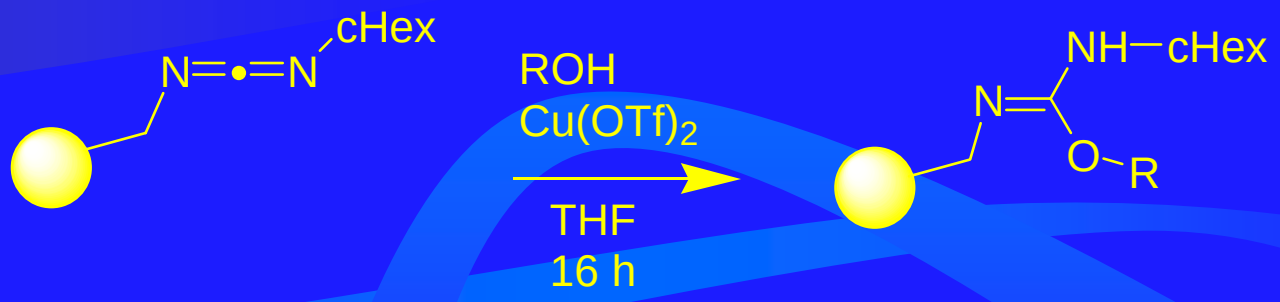
(b). Synthesis of PS-isoureas with Copper (II) catalysis



- The reaction of other alcohols with the carbodiimide can also be performed at RT using $Cu(OTf)_2$ as catalyst (3-5% w/w)
- The copper catalyst removed form the resin using TMEDA.
- Washing solution is blue coloured when copper is present, it is easy to detect when the salt is completely removed

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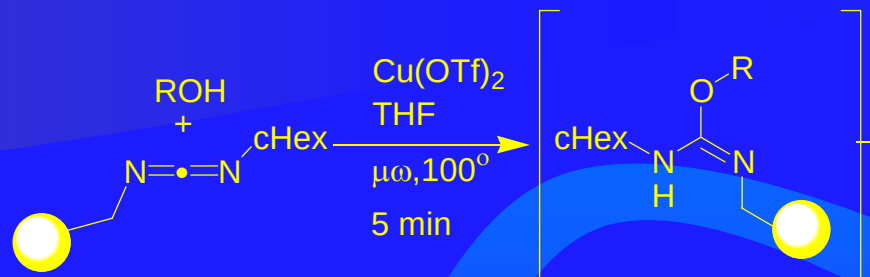
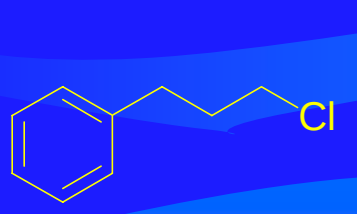
(b). Supported Reagents - PS-isoureas and the synthesis of haloalkanes





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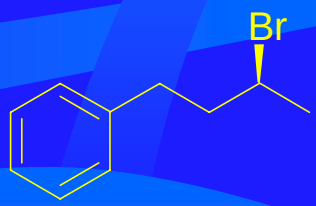
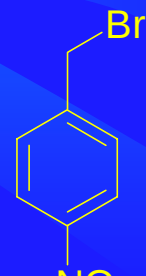


88%

92%



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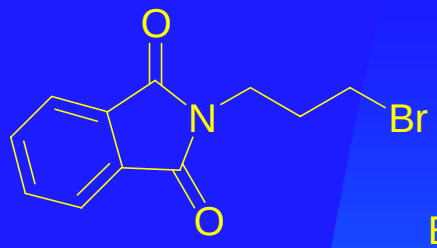
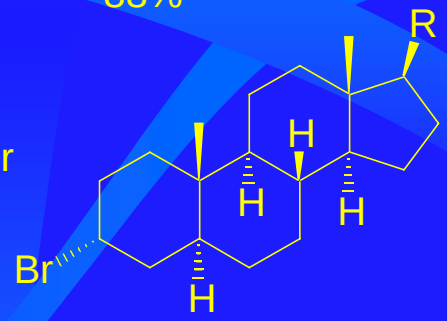
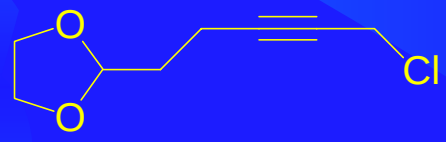


89%



80%



82%

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Stefano Crosignani, Chem. Commun. 2003, 260-261.

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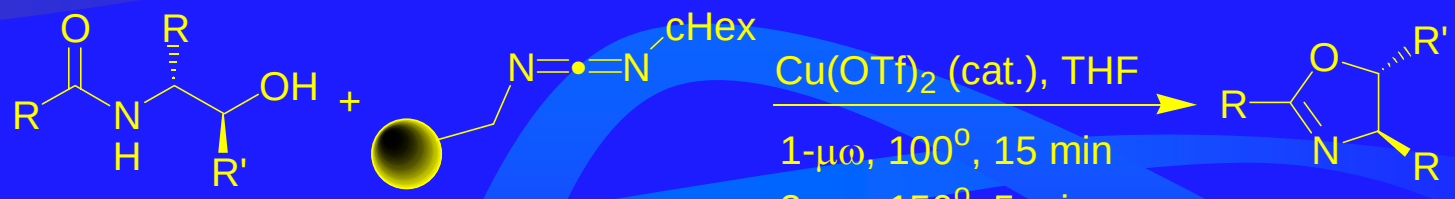
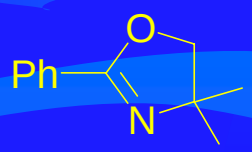
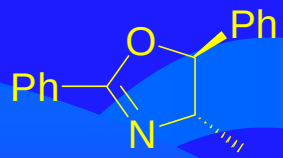
(b). Oxazoline Synthesis via PS-Carbodiimide

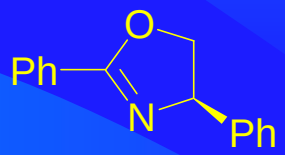




70%



76%



84%

- *Products isolated in good yields and excellent purities (>95%)*
- *Filtration on short alumina plug used to remove copper catalyst*

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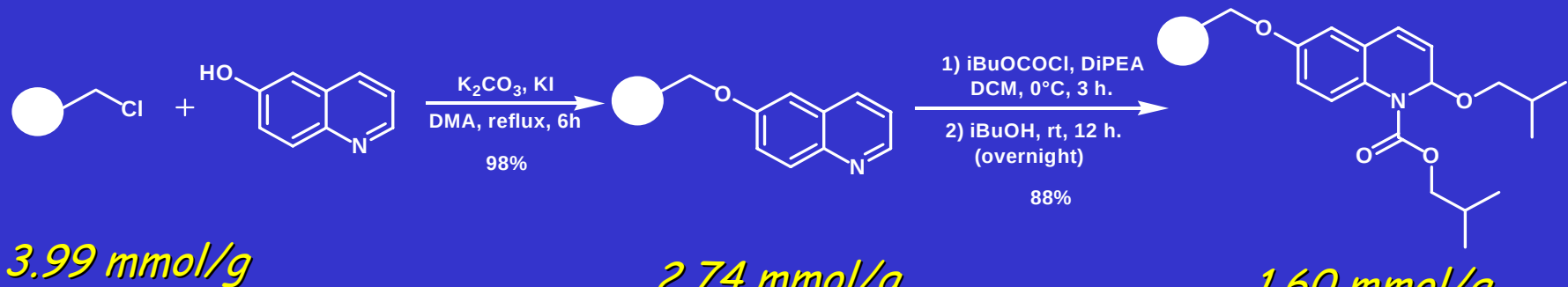
(b). Synthesis of Polymer-Supported IIDQ

Three steps from Merrifield resin :



3.99 mmol/g

2.74 mmol/g

1.60 mmol/g

86% overall conversion

No requirement of a pre-activation step

- *The order of addition of the amine, acid or coupling agent makes no difference to the efficiency of the coupling reaction*

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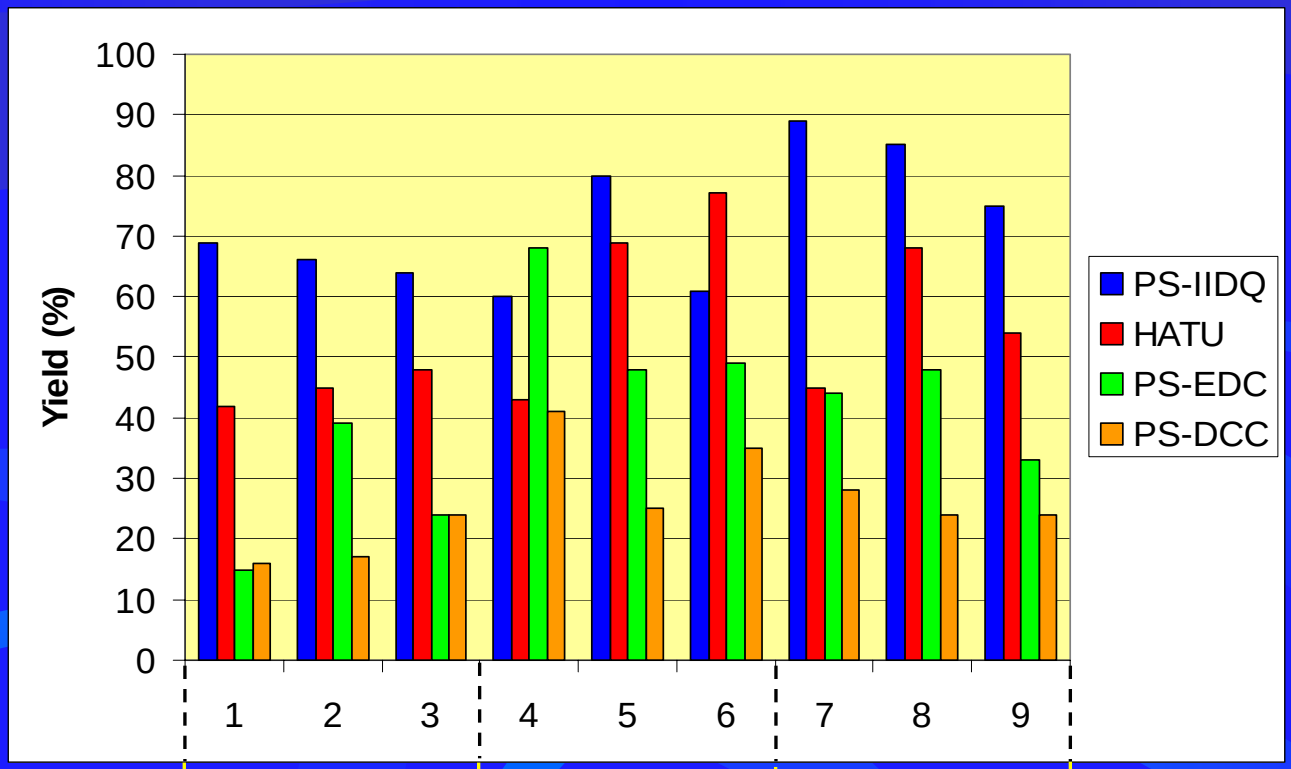
(b). Polymer-Supported IIDQ: Comparisons

Coupling reagents tested : **PS-IIDQ, HATU, PS-EDC, PS-**



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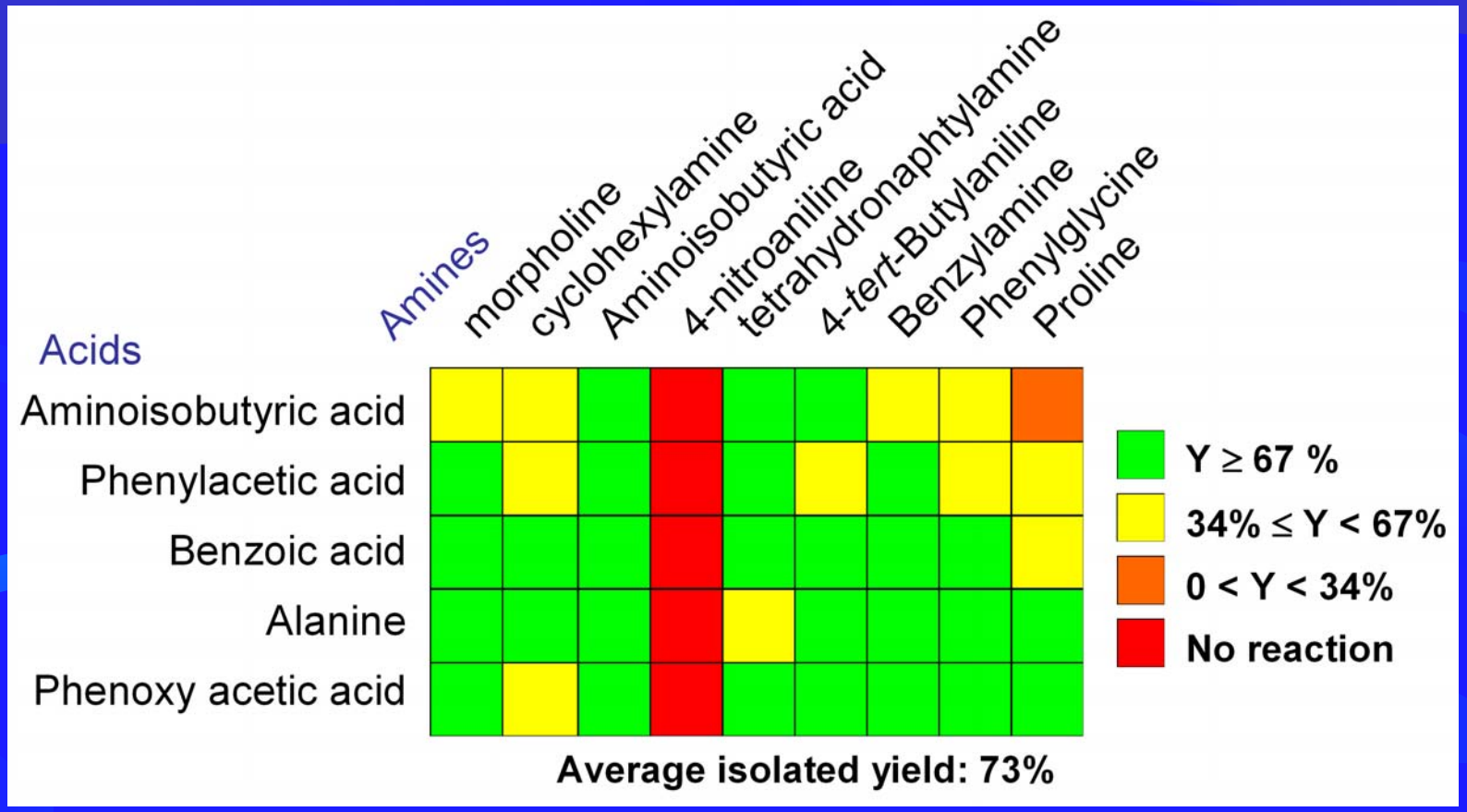
(b). Polymer-Supported IIDQ



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(b). Polymer-Supported IIDQ

Characteristics :

PS-IIDQ obtained easily in three steps from Merrifield resin

High loading resin (1.4-1.6 mmol/g)

Stable under normal laboratory storage conditions

Efficiency:

Works well in most cases of general amide bond formation

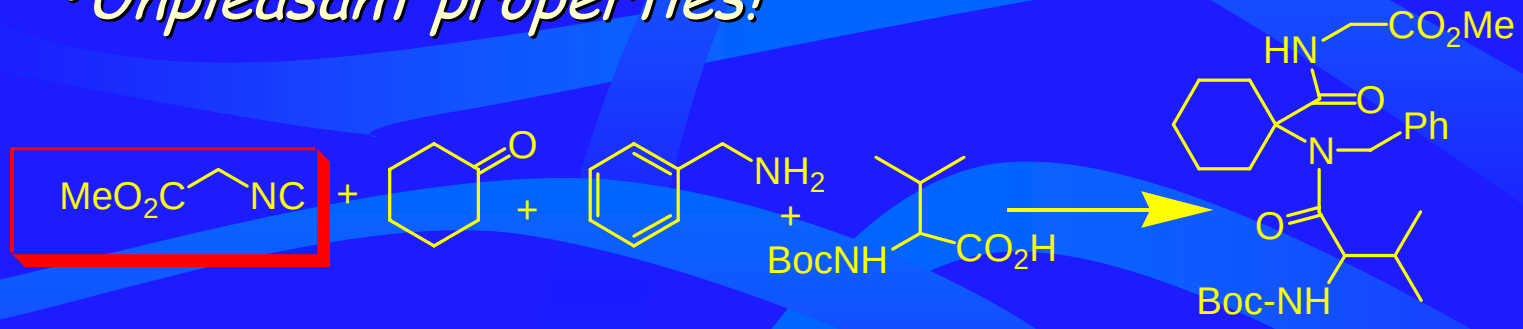
Tests at Evotec OAI on substituted coumarins confirmed the best results over other supported coupling reagents

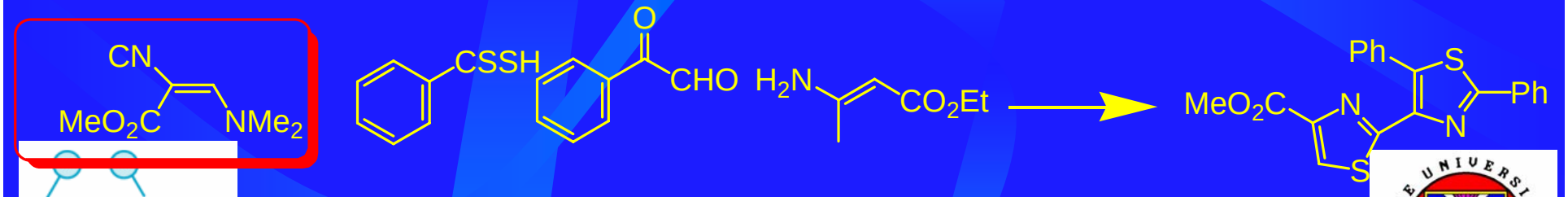
Limitation :

Hindered amines (Proline type) give carbamate species when coupling to carboxylic acids containing Methyl groups in the a position

(b). Multi-Component Reactions

- Isonitriles are important constituents of many MCR's due to their unusual "split reaction personality"
- Limited number commercially available
- Unpleasant properties!





A. Domling - Current Opinion in Chem Biol

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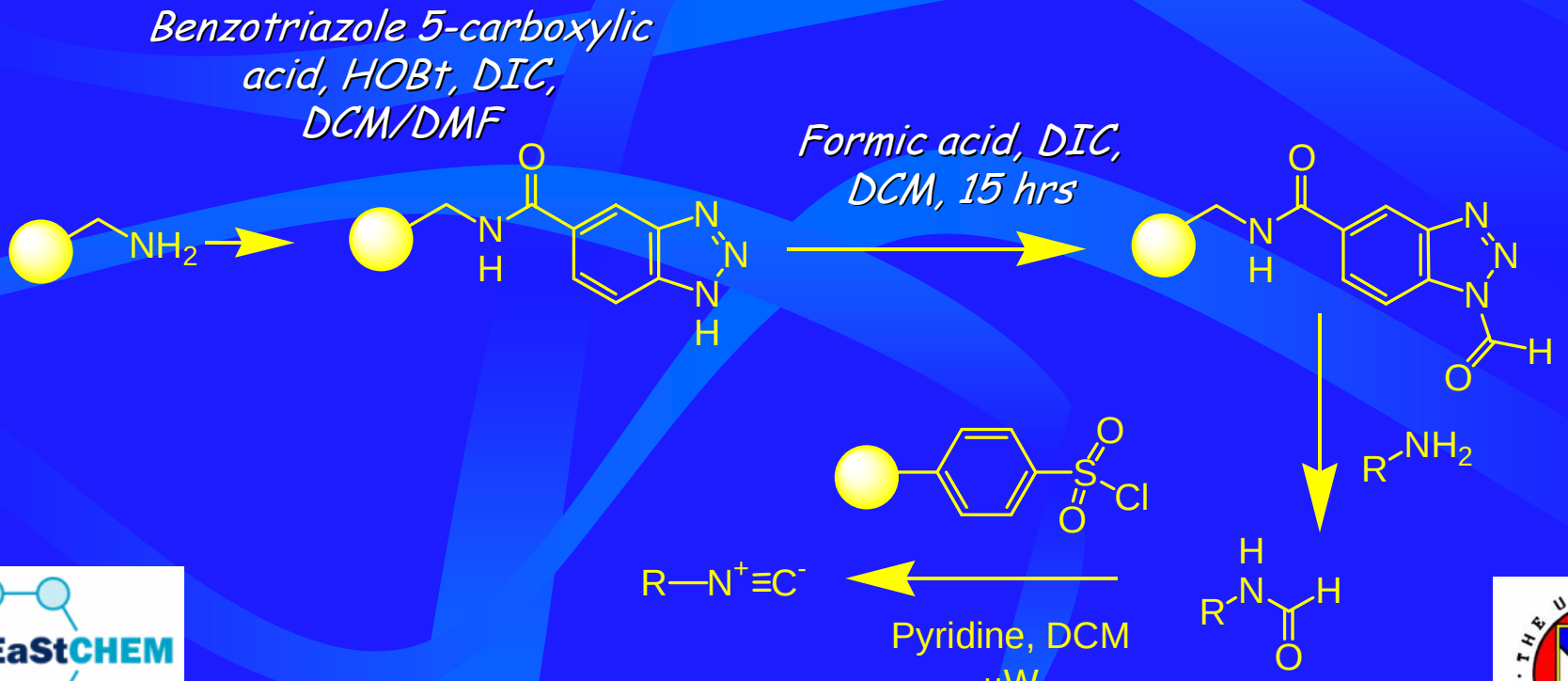
(b). Supported Reagents - Isonitrile Synthesis

- Isonitriles are important constituents of many MCR's due to their unusual "split reaction personality"
- Development of new methods for the synthesis of isocyanides using polymer-supported reagents



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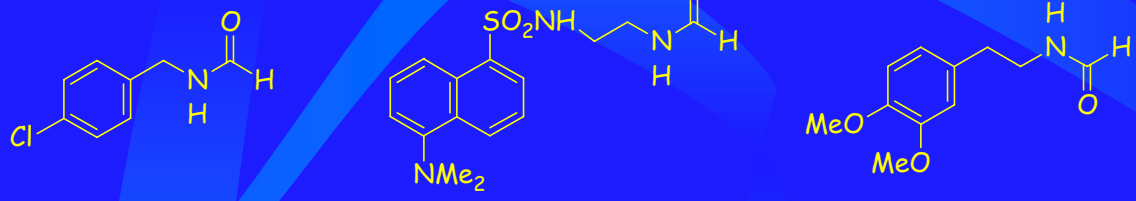
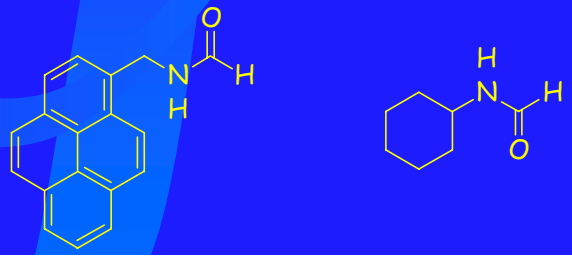
Delphine Launay TL

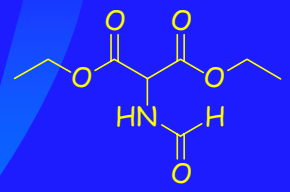
(b). Isonitrile Synthesis

Entry	Formamide	Sulfonyl chloride resin	Pyridine in DCM	Isocyanide (% purity)
A	1	3 eq.	50 eq.	100 %
B	1	1.5 eq.	25 eq.	76 %
C	1	2 eq.	50 eq.	90 %
D	2	3 eq.	50 eq.	81 %
E	3	3 eq.	50 eq.	96 %
F	4	3 eq.	50 eq.	100 %
G	5	3 eq.	50 eq.	95 %
H	6	3 eq.	50 eq.	18 %

Microwave: 100°C for 10 minutes.







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(c). Heterogeneous Pd Catalysts

- Recover and reuse without loss of activity
- Increase stability under air and/or moisture
- Compatible with most of solvents especially water
- Increase of activity for aryl halide

Cost & availability: I < Br < Cl
Reactivity: I > Br > Cl

- Easy to separate and handle

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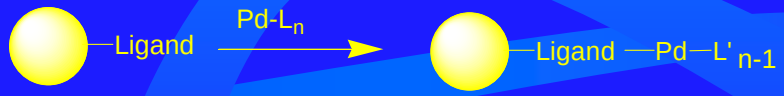
(c). Heterogeneous Pd Catalysts

(1) Conventional method

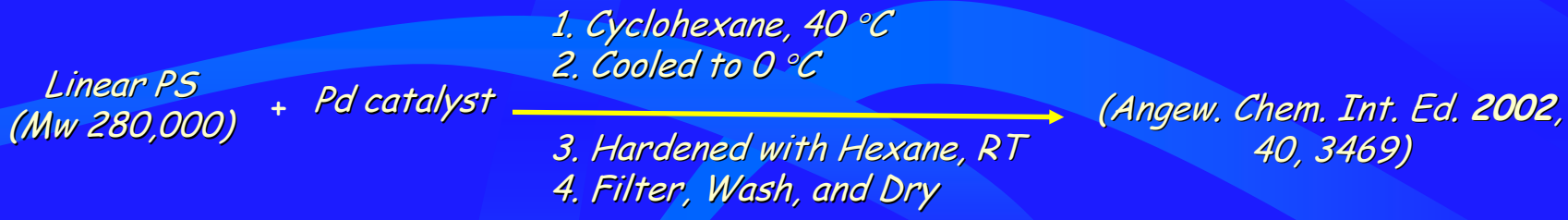
(a) Pd on activated carbon (Pd/C)

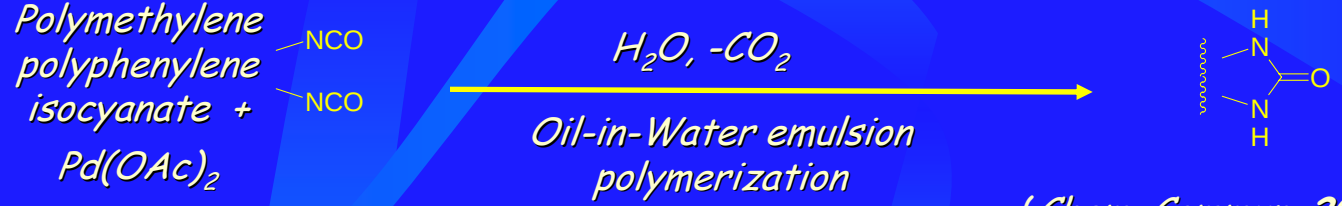
(b) Pd adsorption on inorganic supports (silica, alumina, etc)

(c) Coordination of Pd catalyst via resin-bound ligands



(2) Microencapsulation





(Chem. Commun. 2002, 1132)

Interaction between vacant orbital of Pd and functionality of polymer backbone e.g. π-orbital of phenyl ring or carbonyl group

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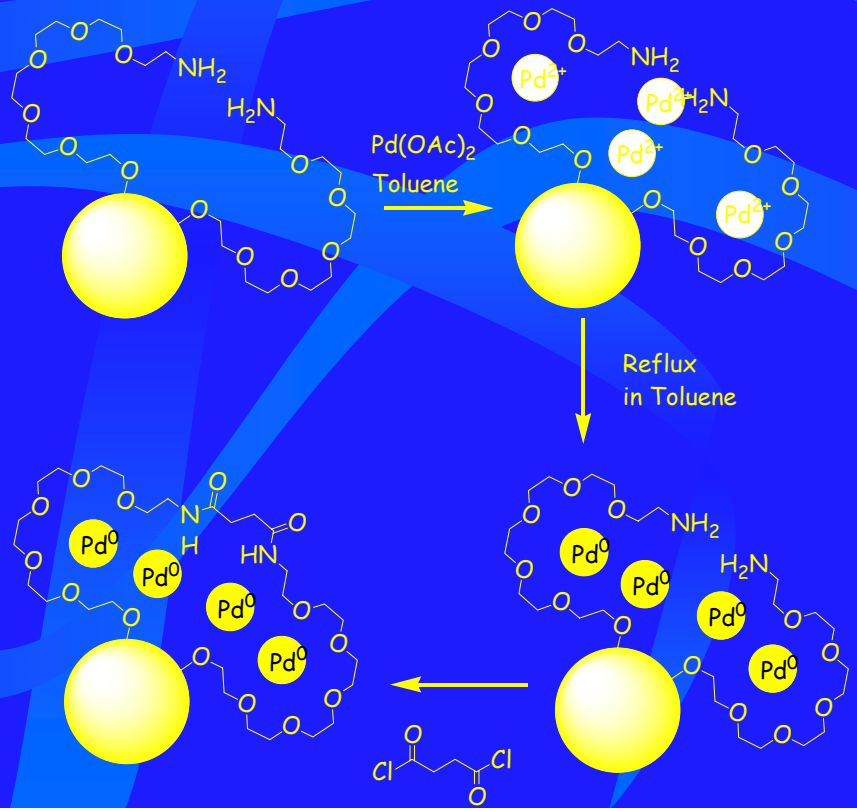
(c). Resin-Captured (RC) Pd

Pd catalyst could be entangled in the form of nano particles on the PS-PEG resin and fixed by cross linking

Interaction between π-electrons of the PS and/or unshared electrons of the PEG and vacant orbitals of Pd



0.36 mmol of Pd/g based on Pd analysis by ICP-AES

JACS, 2006

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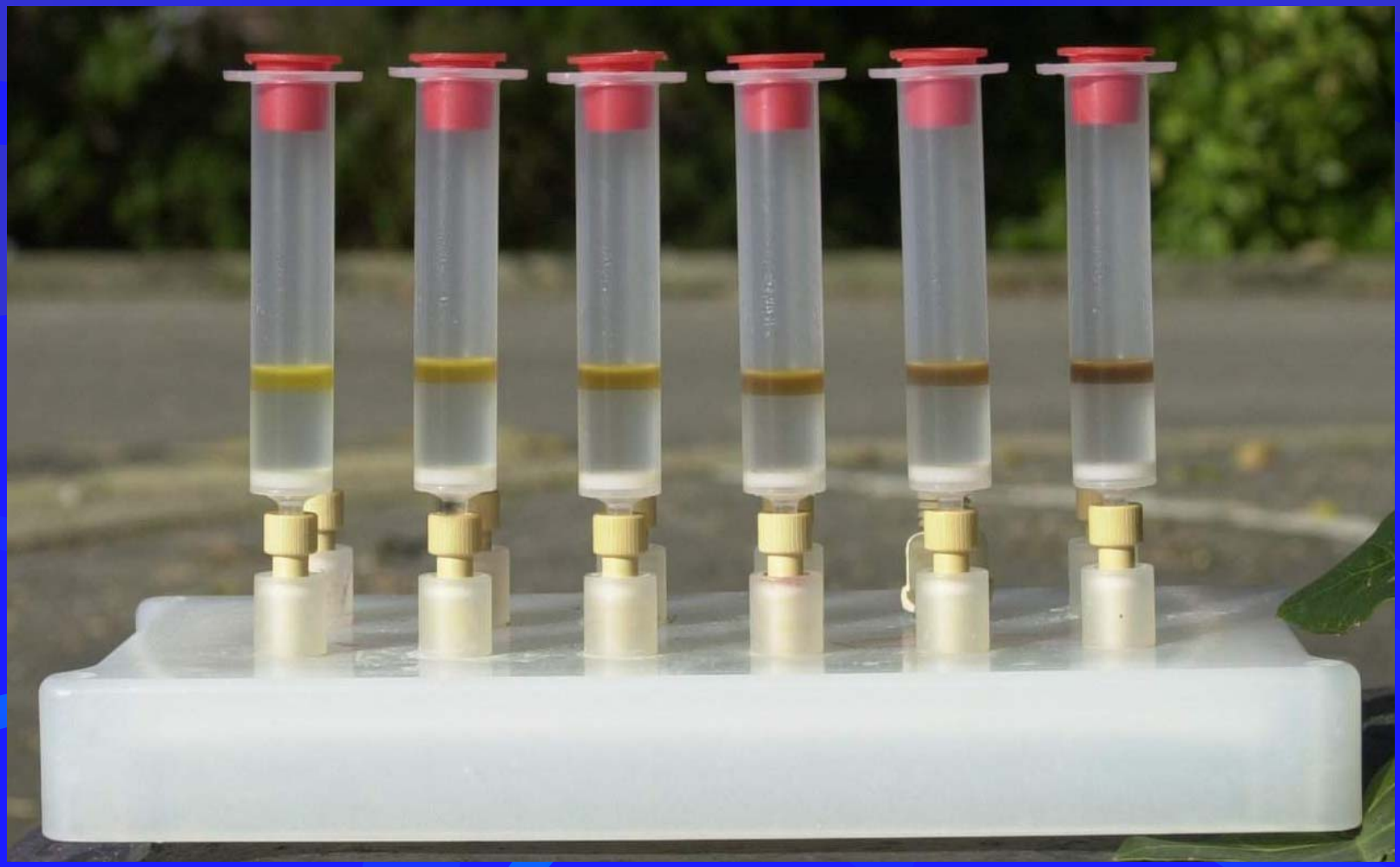

Cross-linking %

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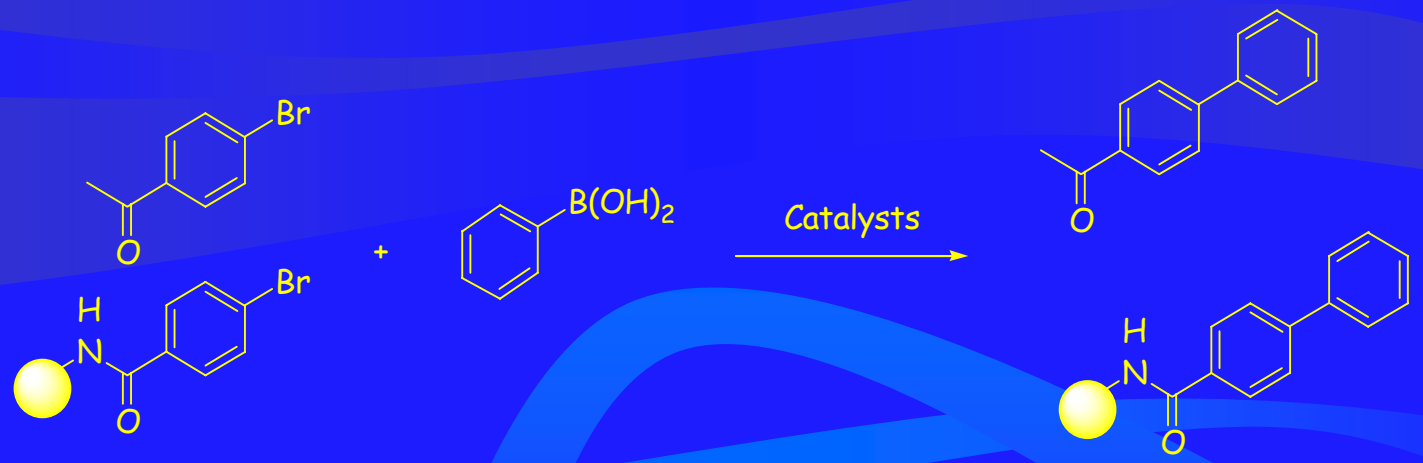
(c). 3 Phase Reaction Test



Pd Catalyst	Yield (solution)	Yield (solid)
Pd/C	98%	95%
Pd/Alumina	98%	95%
Pd coordinated by resin-bound phosphine ligand	>99%	85%
Pd captured by resin	99%	**17%**
Pd captured by resin followed by cross-linking (40%)	98%	**4%**

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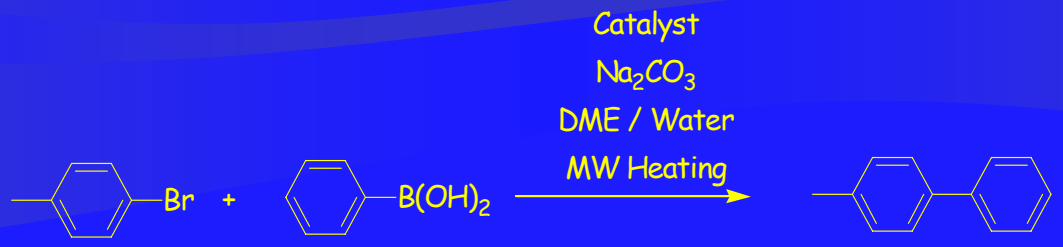
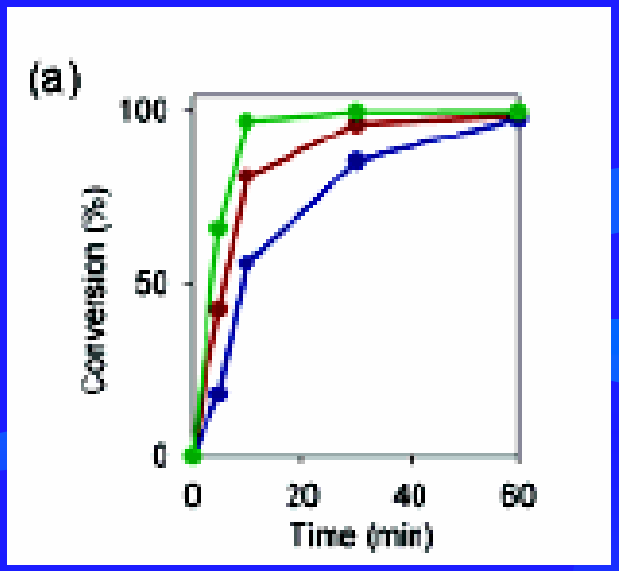
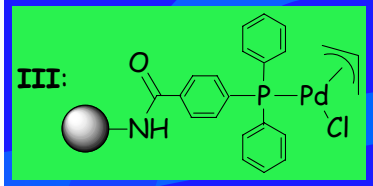
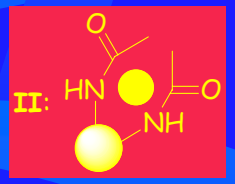
J. Am. Chem. Soc. ***2001****, 123, 10139-10140.*

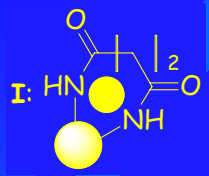
(c). Different Immobilised Pd Catalysts



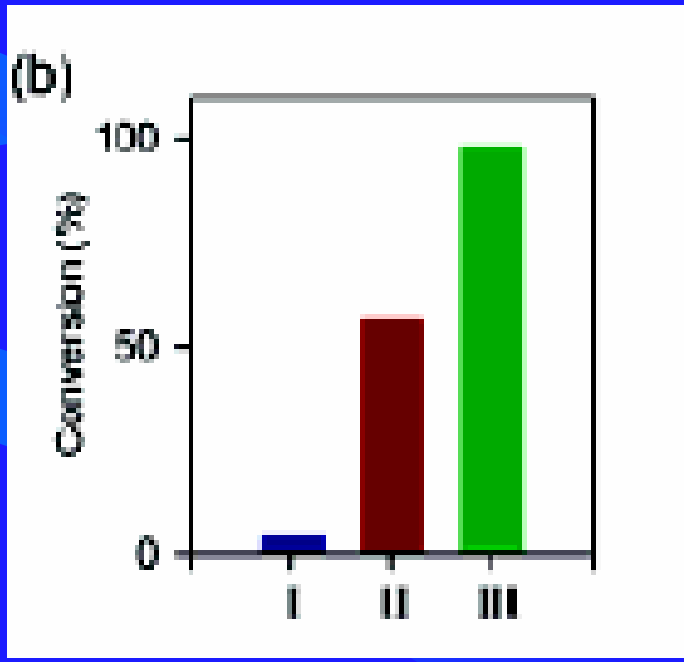








Activity of Filtrate



*The Pd content in the filtrate from **I** was 0.09 ppm based on elemental analysis using ICP-OES.*

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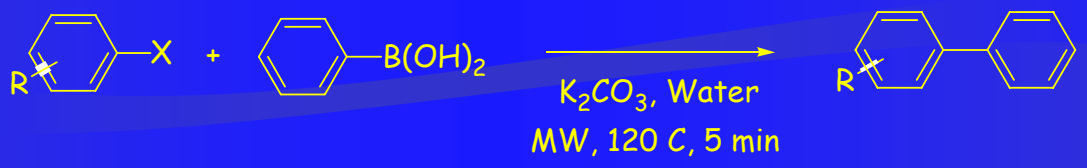
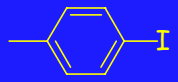
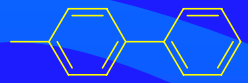
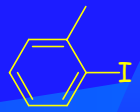
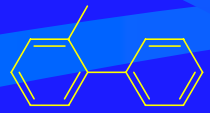
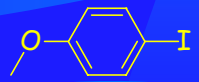
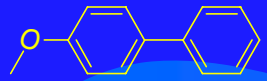
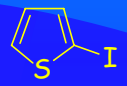
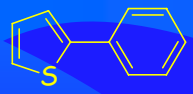
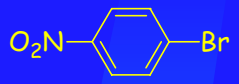
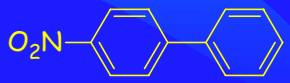
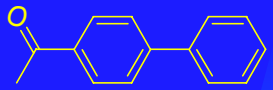
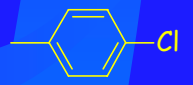
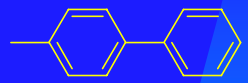


Reactant	Product	Yield (%)
		87
		85
		93
		91
		93
		99
		11

(c). Suzuki Reaction with RC Pd and Crosslinking

	R_1	R_2	yield (%)
Bromophenol blue	Br	H	41 (33)
Bromocresol purple	Me	H	57 (56)
Bromothylmol blue	*i-Bu*	Me	28 (25)

Isolated yield of mono-substituted products and compared with yields obtained using homogeneous catalyst ($Pd(OAc)_2$)

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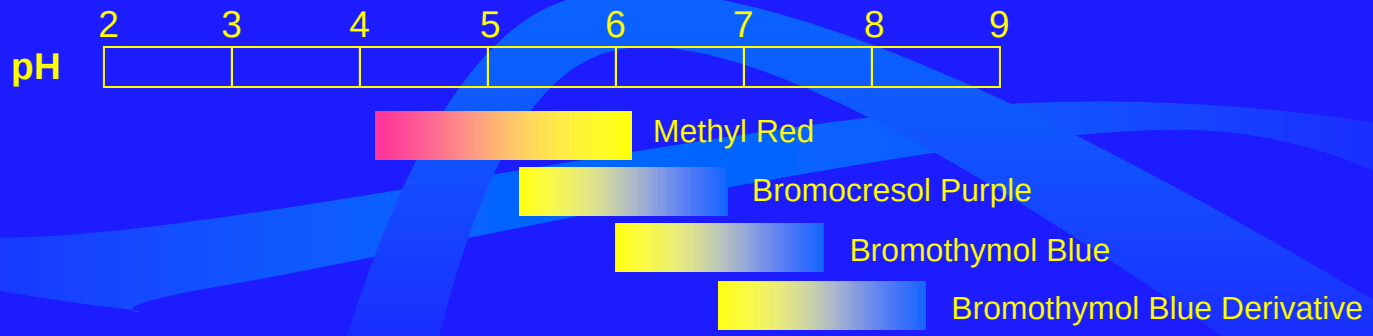
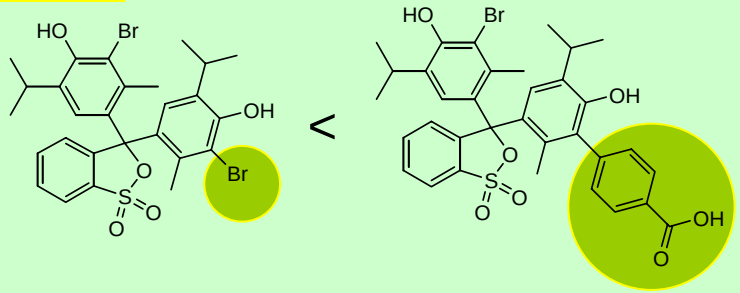
pH Indicator Transitions



pKa



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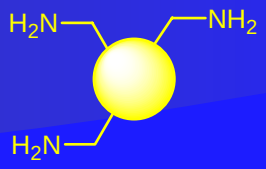
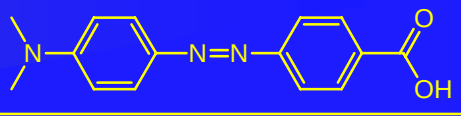
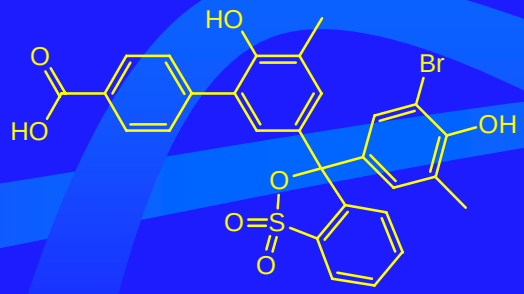
pH-Indicating Resin





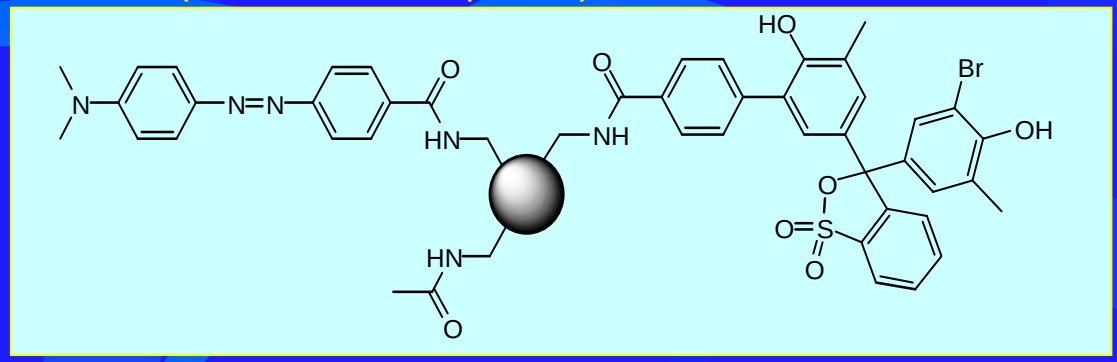
(Methyl Red)

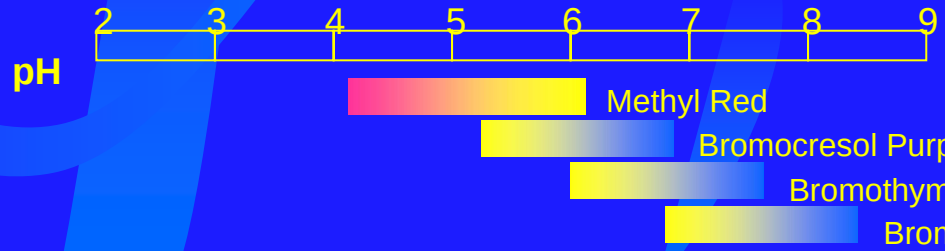


(Bromocresol Purple der)

TFFH/TEA used as coupling agents

Capping by acetylation





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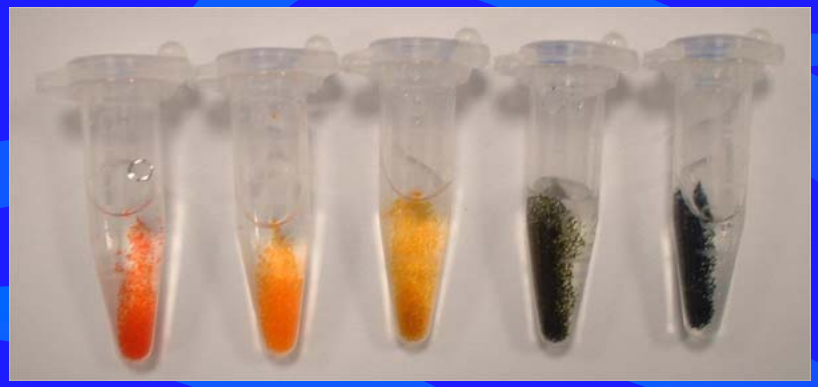
Colour Change of Indicator Resin According to pH



pH

1	3	5	7	9

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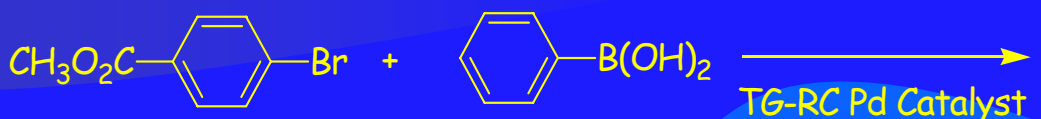
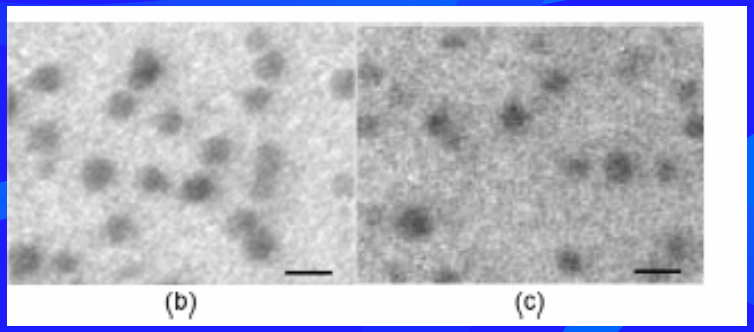
(c). Recycling of RC Pd for Suzuki Reactions



98%
94% (2nd)
92% (3rd)
95% (4th)
90% (5th)
96% (6th)

(isolated yields)



Pd Nanoparticles (Left) Before and (Right) After 6 Reuses

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(b). Heterogeneous Pd Catalysts

- Recover and reuse without loss of activity
- Increased stability under air and/or moisture
- Compatible with most of solvents including water
- Easy to separate and handle

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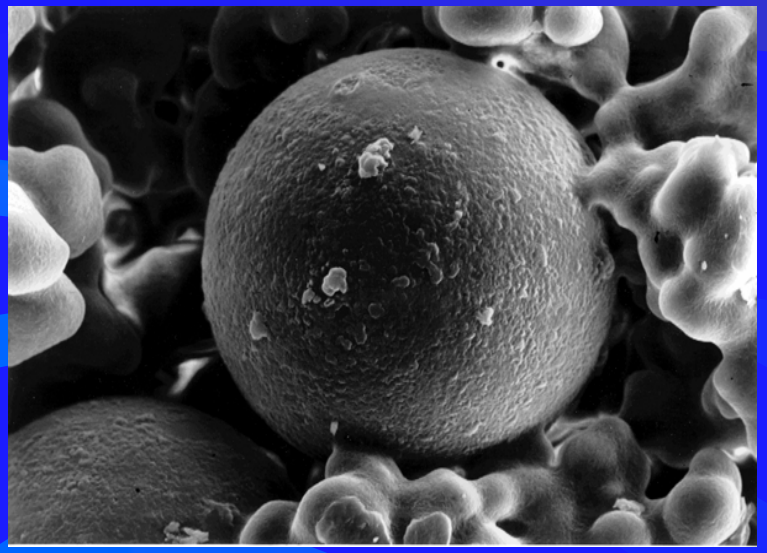
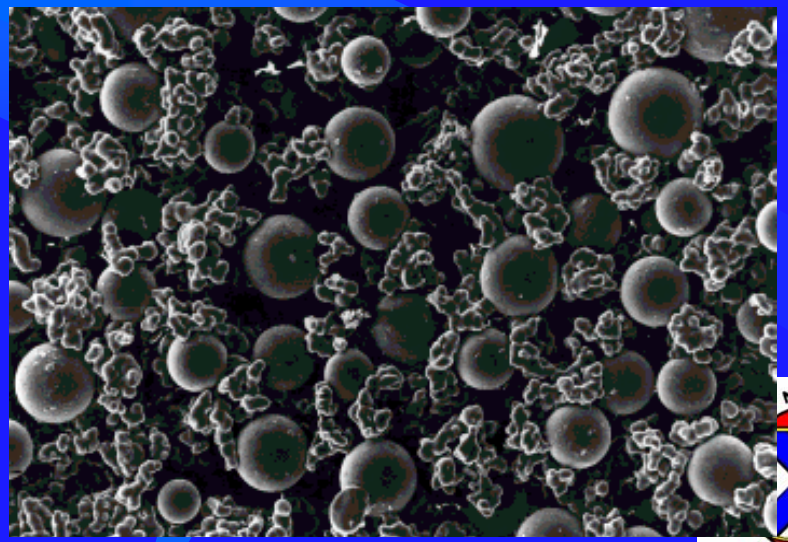
Resin Plugs





Resin composites
Polystyrene resin and
inert porous filler

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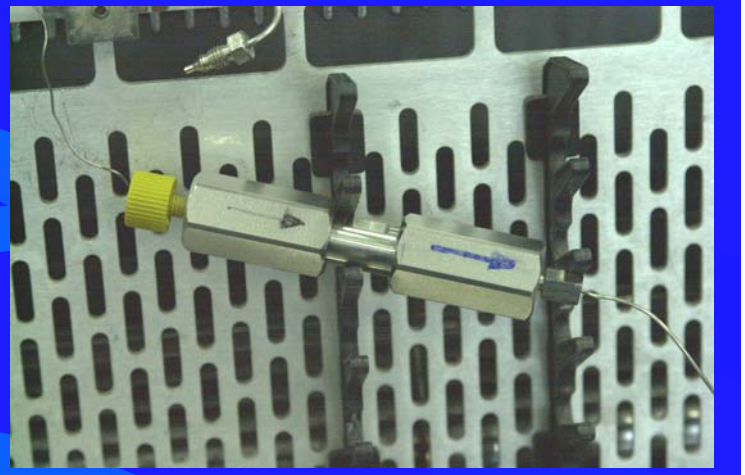
Flow-Through Synthesis



5-10 cartridges in a single Column

Catalysts, Reagents, Scavengers etc…

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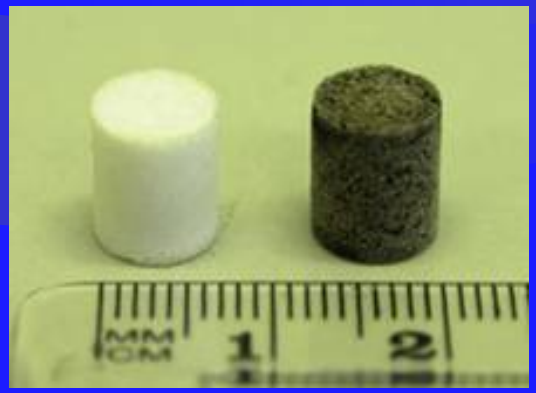
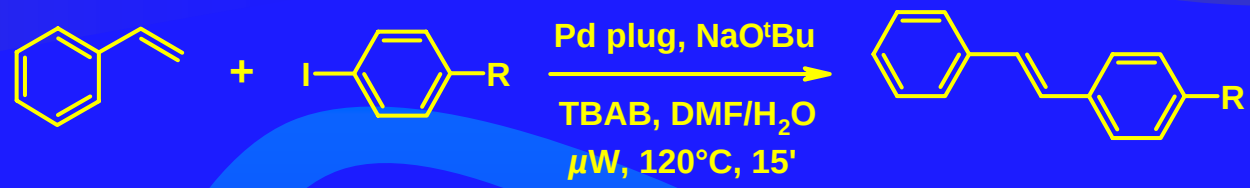
(c). Plugs – Modular Resin Captured Pd





R	Catalyst quantity (mol %)	Isolated yield (%)
OMe	6.7	93
CF_3	0.7	94
NO_2	6.7	79
Me	2.7	96
H	6.7	94
COMe	2.7	82

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