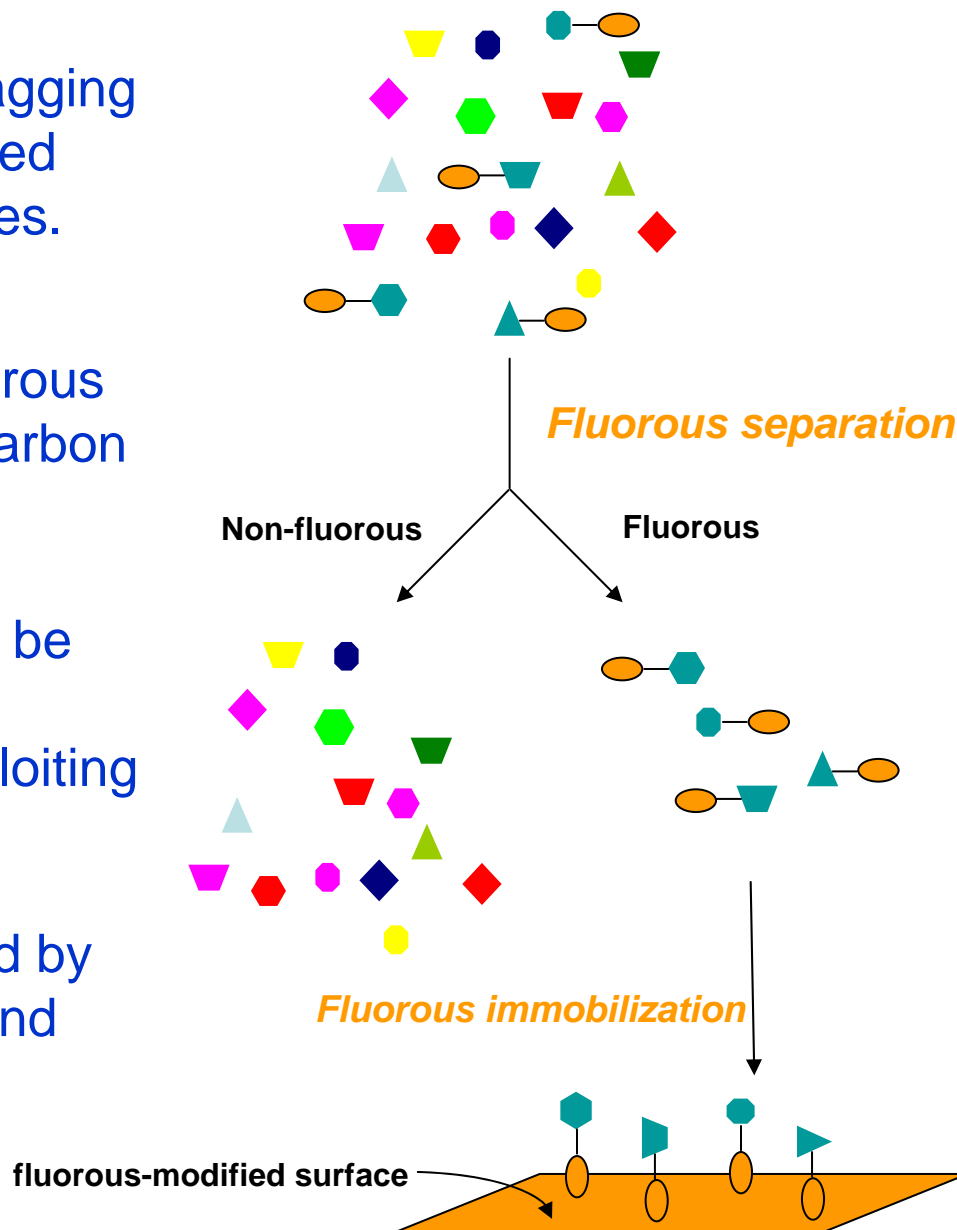


Fluorous Chemistry Based Synthesis and Analysis

- **Introduction**
- **Fluorous Separation Techniques**
- **Small Molecule Synthesis and Purification**
 - **Scavenging reactions**
 - **Acylation reactions**
 - **Mitsunobu reactions**
 - **Fluorous tagged libraries**
 - **Other purification techniques**
- **Fluorous Oligonucleotide, Peptide and Carbohydrate Chemistry**
- **Fluorous Proteomics and Metabolomics**
 - **Reverse fluorous SPE**
 - **Heavy fluorous liquid-liquid extraction**
- **Fluorous Products Available through Sigma-Aldrich**

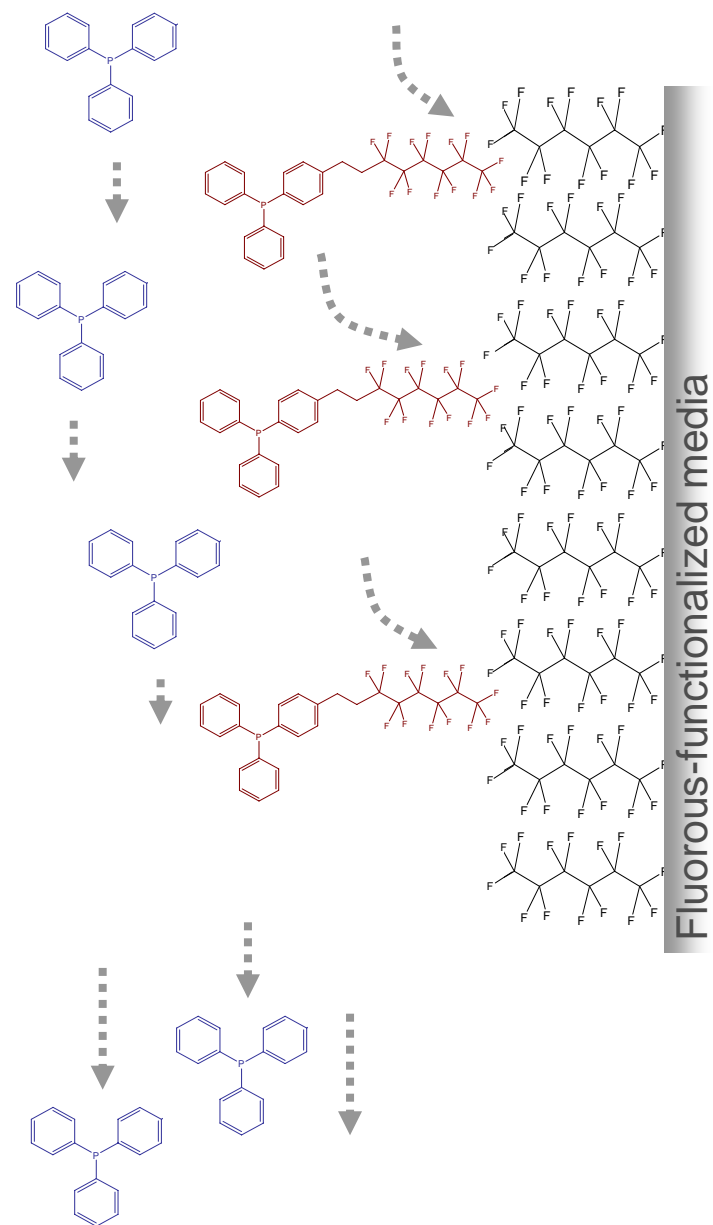
What is Fluorous Technology?

- Fluorous chemistry is a novel tagging technology that separates desired molecules from complex mixtures.
- Molecules can be rendered fluorous by the attachment of perfluorocarbon domains.
- Fluorous tagged molecules can be separated from non-fluorous molecules and immobilized exploiting fluorophilicity.
- Fluorous techniques are marked by high selectivity, low reactivity, and exceptional breadth



Fluorous Separation and Immobilization

- Fluorous tagged molecules partition preferentially into fluorous environments
- Non-fluorous molecules do not partition into fluorous environments.
- Novel mechanism orthogonal to other separation/ immobilization technologies
- Allows diverse chemistry and versatile separation options



Examples of Fluorous Molecules

Compounds with permanent fluorinated domains (e.g. reagents):



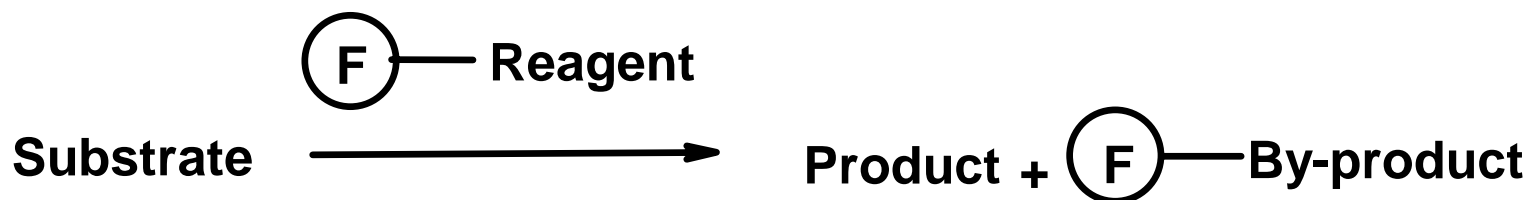
Compounds with temporary fluorous tags (e.g. substrates):



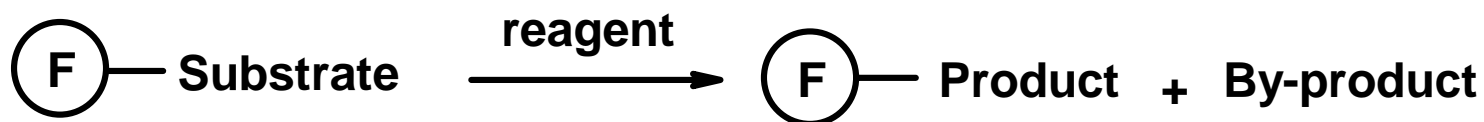
Fluorous tagged molecules are designed to react similarly to their non-fluorous analog

Two Fundamental Approaches

- Fluorous Reagents/Scavengers

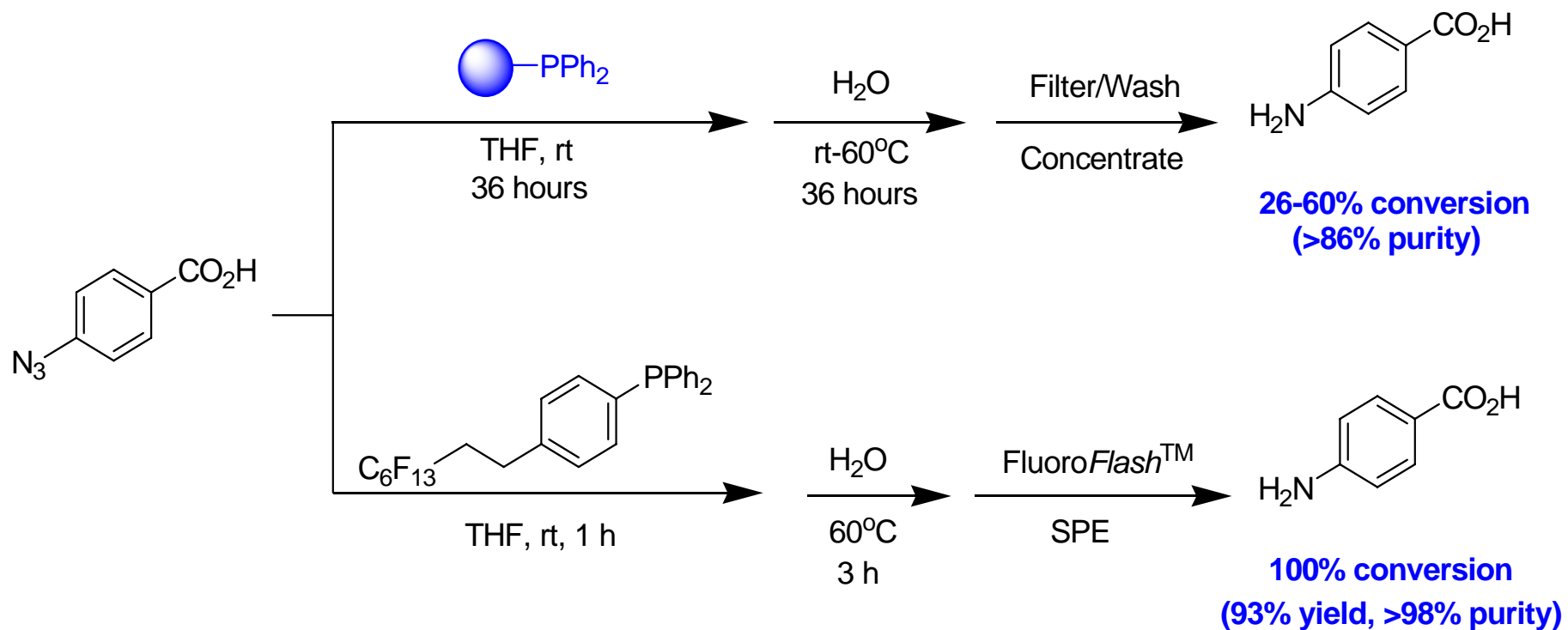


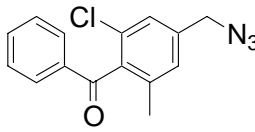
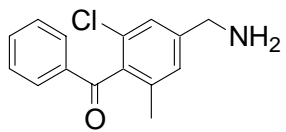
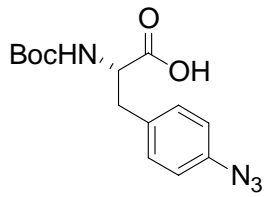
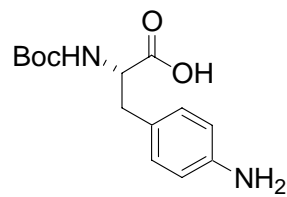
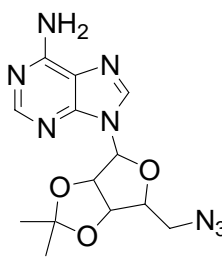
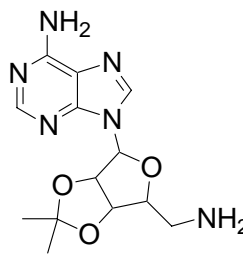
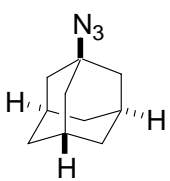
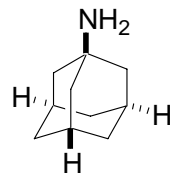
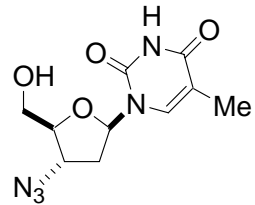
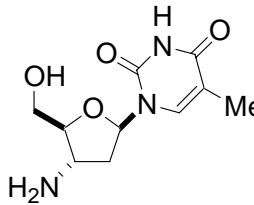
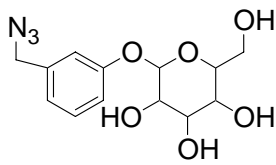
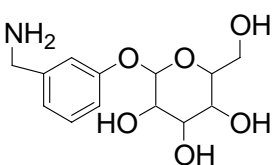
- Tagged Substrates



Fluorous tagged molecules can be analyzed by TLC, IR, MS, NMR and readily separated by F-SPE or F-HPLC

The Solution Phase Advantage



entry	RN ₃	RNH ₂	yield (%)	purity (%)
1			86	98/95
2			91	98/95
3			88	98/95
4			92	98/95
5			82	95/92
6			80	97/93

Broad reaction compatibility

Metal catalysis

- Suzuki
- Heck
- Buchwald
- Stille
- Co, Rh

Lewis acidic

- Friedel-Crafts
- BBr_3

Redox

- LAH
- hydrogenation
- H_2O_2
- Swern

Fluorous



Ionic

- Enolate
- Grignard,
- lithiate
- cationic

Free radical

- cyclization
- dehalogenation
- deoxygenation

All of the above reaction conditions have been used in the presence of fluorous tags.

Fluorous Separation Techniques

■ Liquid-Liquid Extraction

- “Heavy” fluorous technique
- Generally requires large F content, ~60%



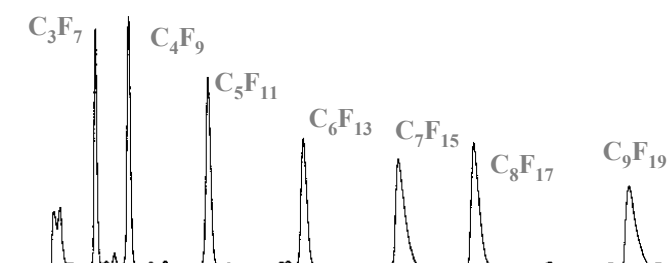
■ Fluorous Solid Phase Extraction (F-SPE)

- “Light” fluorous technique
- Separates fluorous from non-fluorous
- No fluorous solvents used



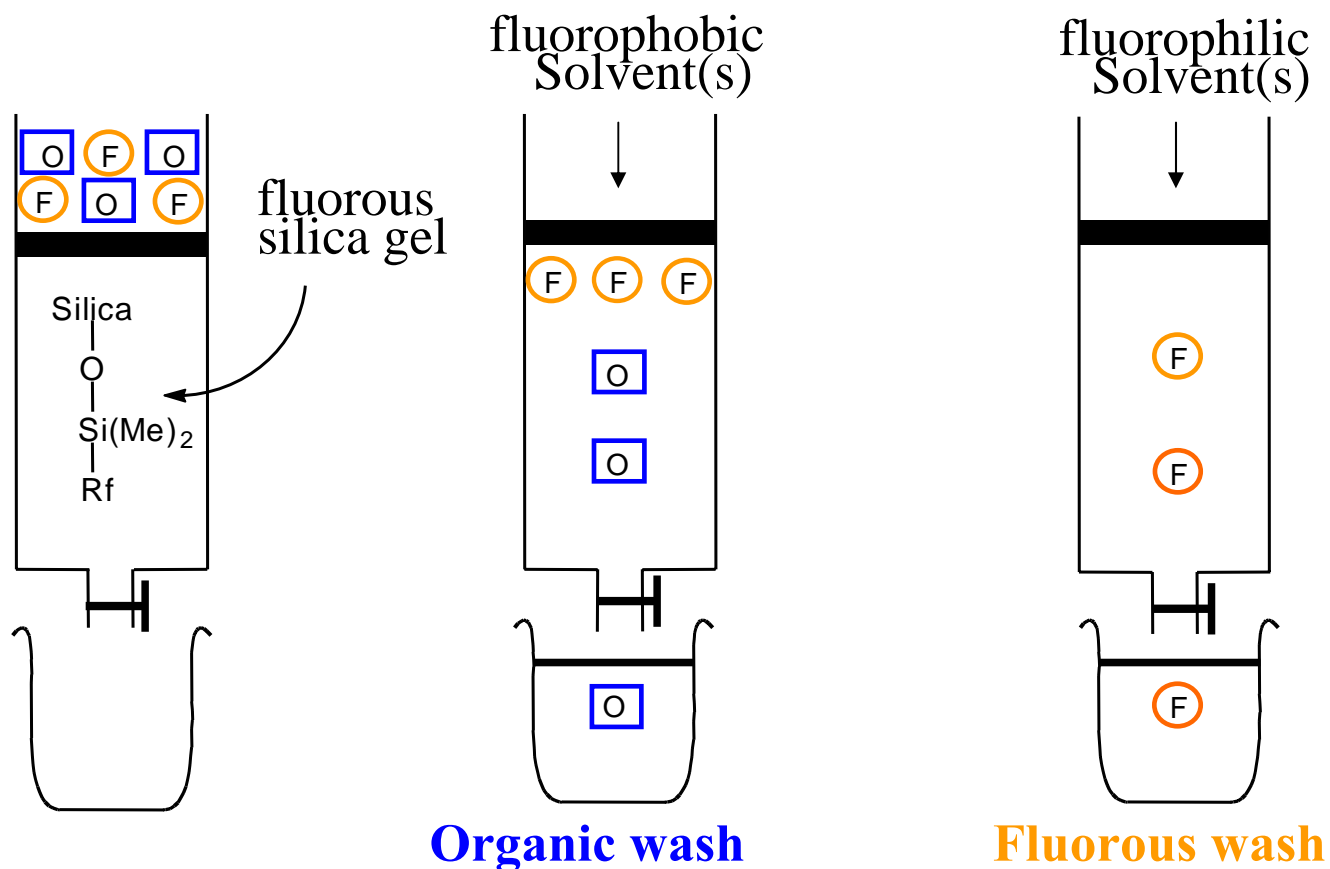
■ Fluorous Chromatography (F-HPLC)

- Separates fluorous from fluorous
- More fluorous = Greater retention



Fluorous Solid Phase Extraction

A Light Fluorous Technique

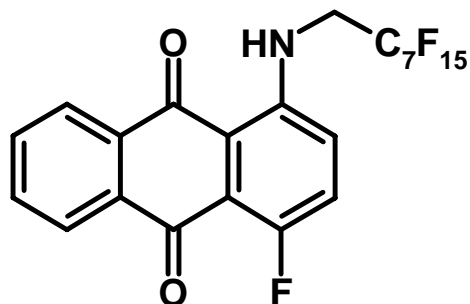


Fluorophobic

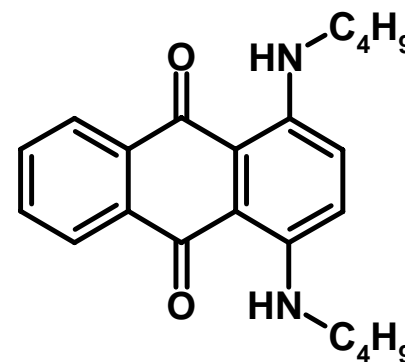
Typical Solvents for F-SPE

Fluorophilic





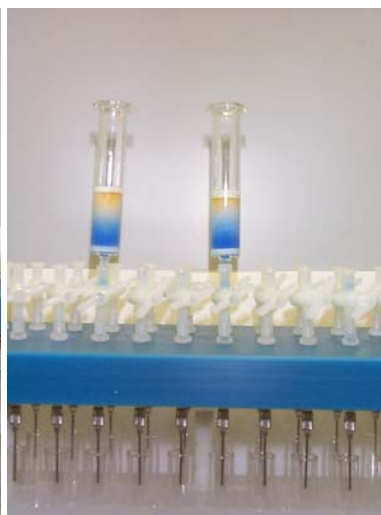
Fluorous Dye
(orange)



Non-fluorous Dye
(blue)



1. Load sample

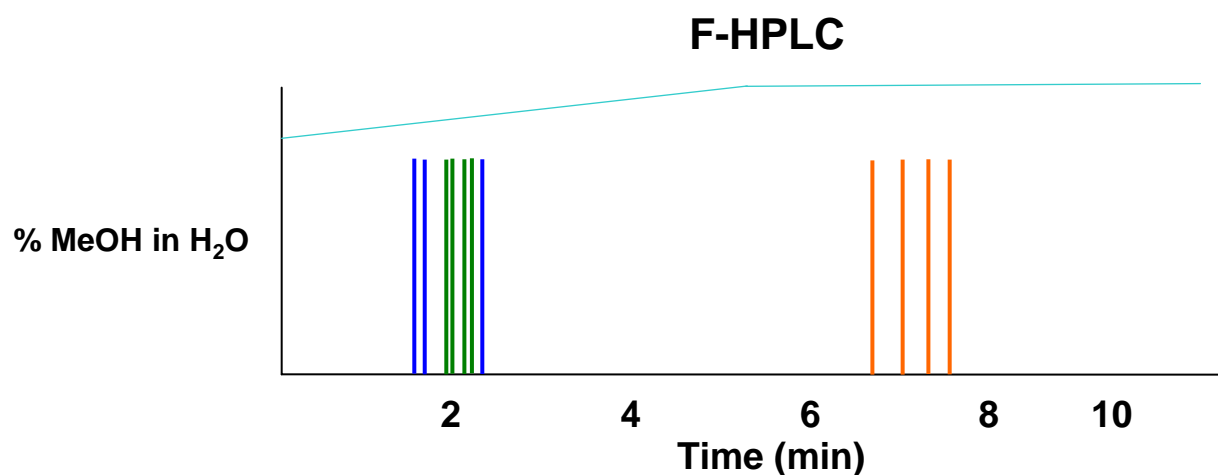
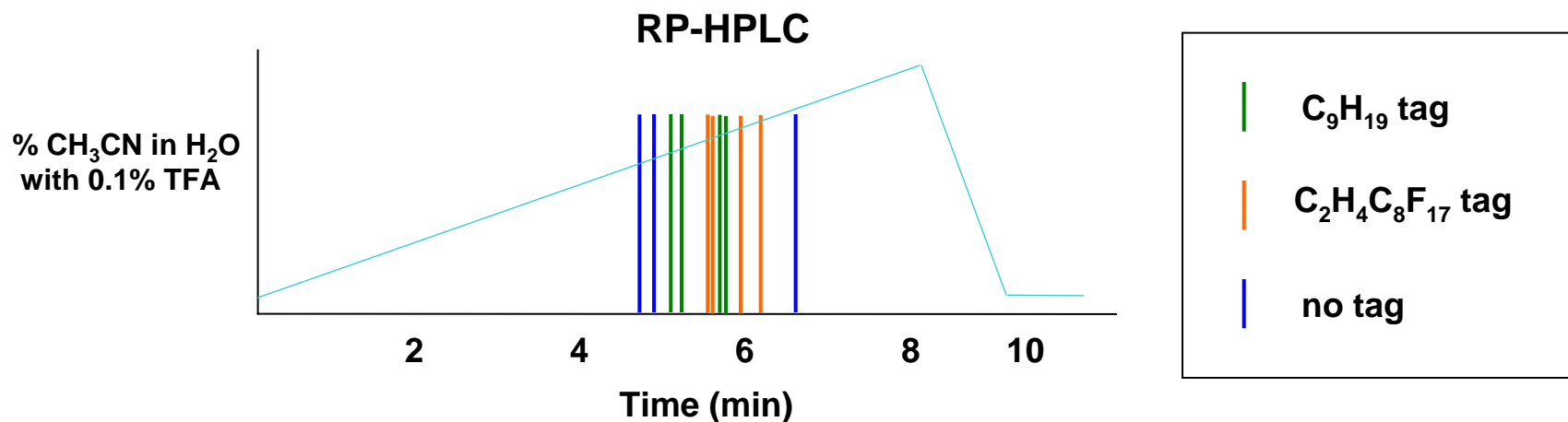


2. Wash non-fluorous dye
with MeOH-H₂O (85:15)



3. Wash fluorous dye
with MeOH

Fluorous Tags vs. Hydrophobic Tags



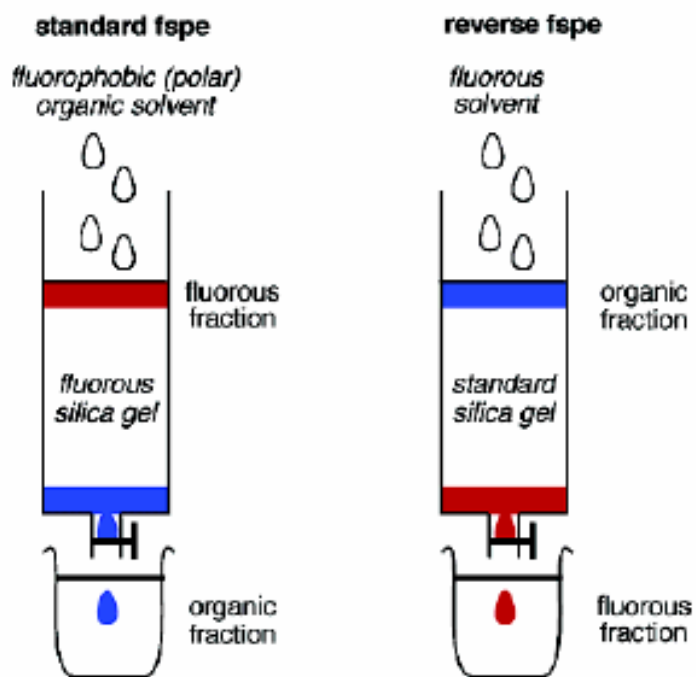
Tagged amino acids are Ser, Glu, Phe, and Trp.

The untagged controls were galactosyl pentaacetate, (Boc-Cys-OH)₂, and PPh₃.

Fluorous compounds are hydrophobic *and* lipophobic.

Reverse Fluorous Solid Phase Extraction

A Light Fluorous Technique



Standard FSPE

fluorous stationary phase
fluorophobic mobile phase
non-fluorous compounds washed

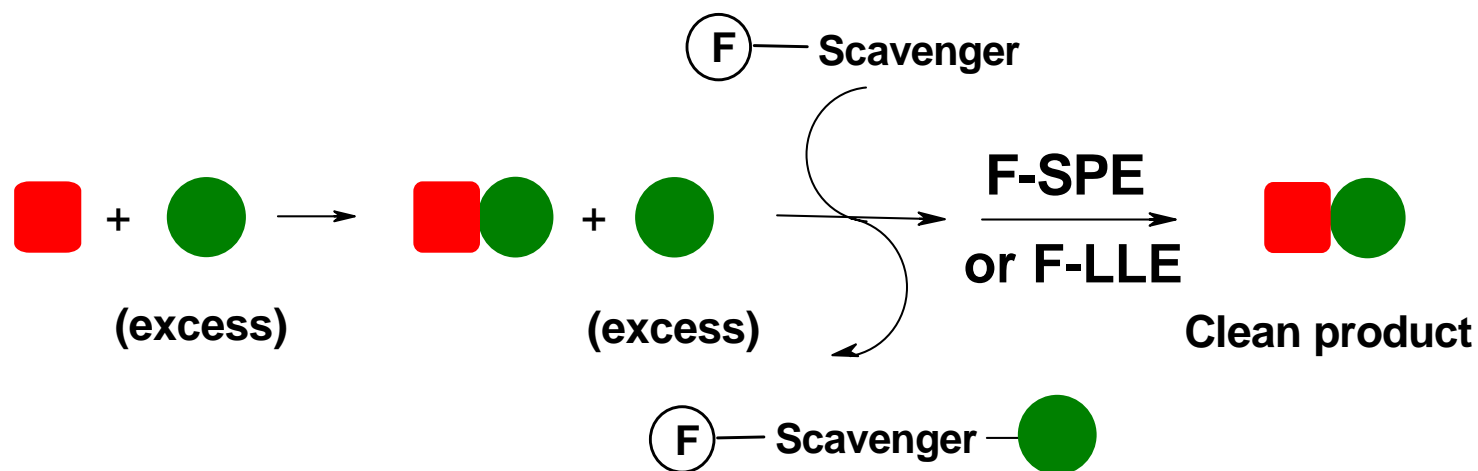
Reverse FSPE

standard stationary phase
fluorous mobile phase
fluorous compounds washed

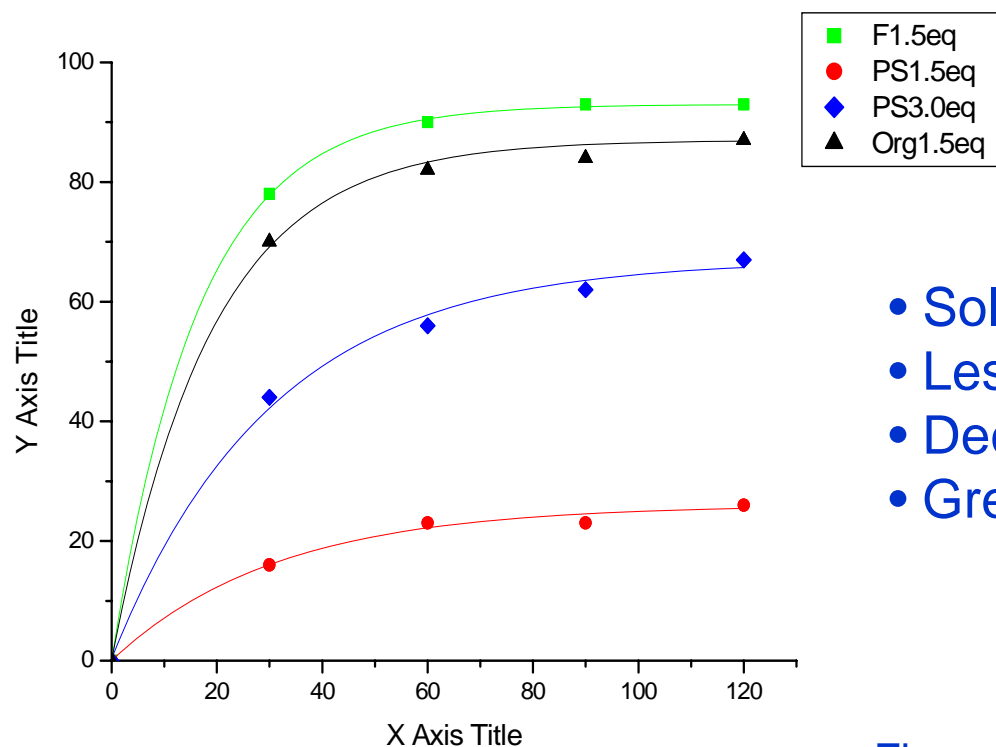
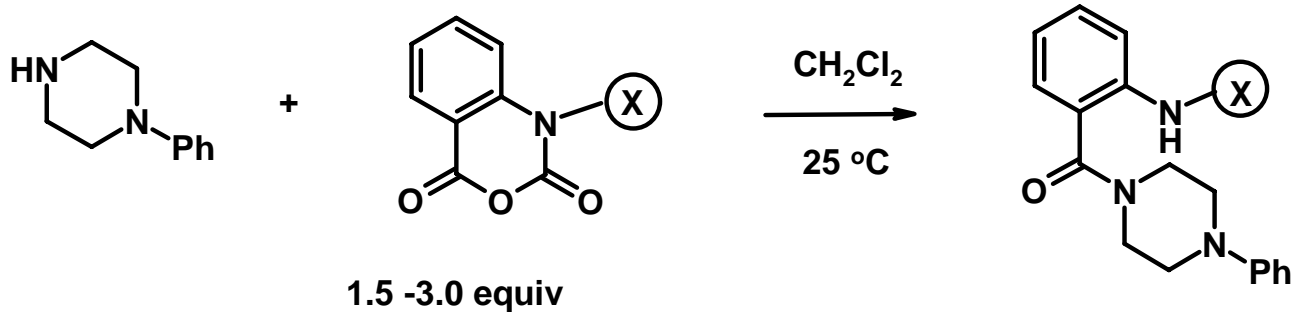
Small Molecule Synthesis and Purification Examples

- **Scavenging reactions**
- **Acylation reactions**
- **Mitsunobu reactions**
- **Fluorous tagged libraries**
- **Other purification techniques**

A Strategic Alternative to Resin bound Scavengers

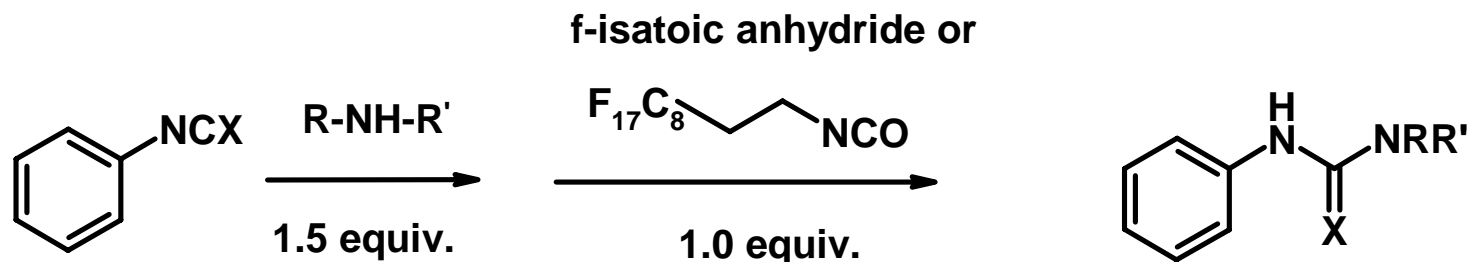


- Both reaction and scavenging carried out in homogenous solution phase
 - Favorable solution phase kinetics
 - Complete reaction monitoring, i.e. TLC, GC, LC, NMR
 - Adaptable to SPE, HPLC or liquid extraction workup
- Complete control of reagent stoichiometry



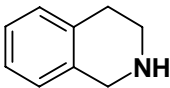
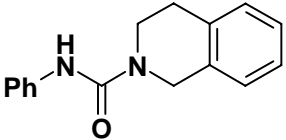
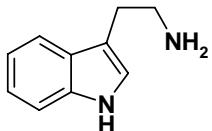
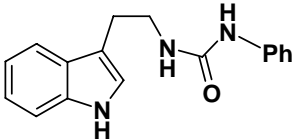
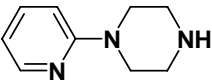
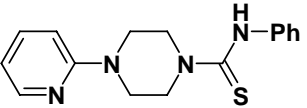
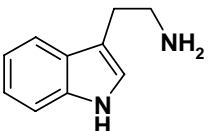
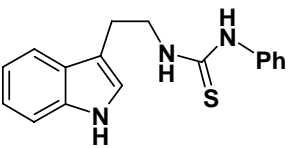
- Solution phase kinetics
- Less equivalents used
- Decreased loss of desired product
- Greater generality

Electrophilic Fluorous Scavengers

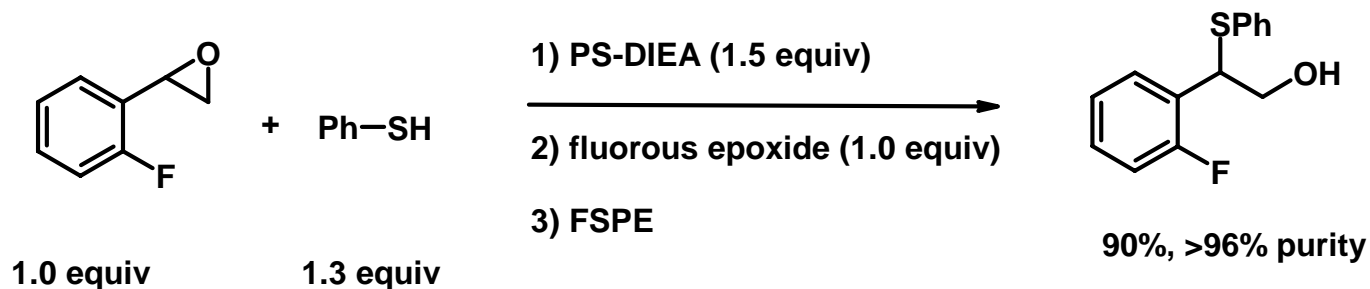
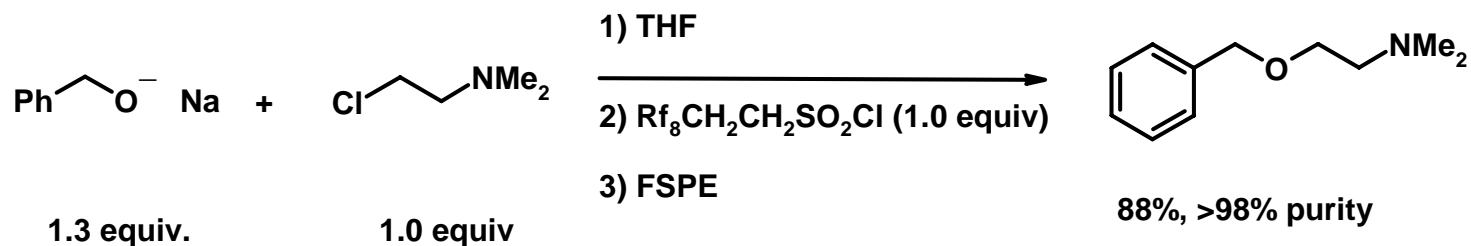
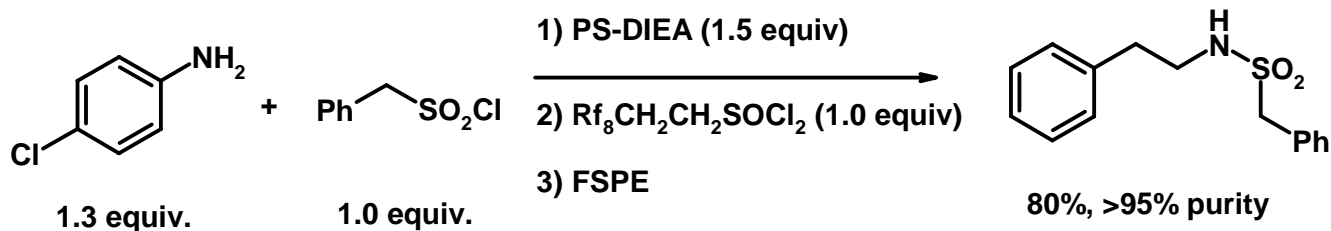


X = O or S

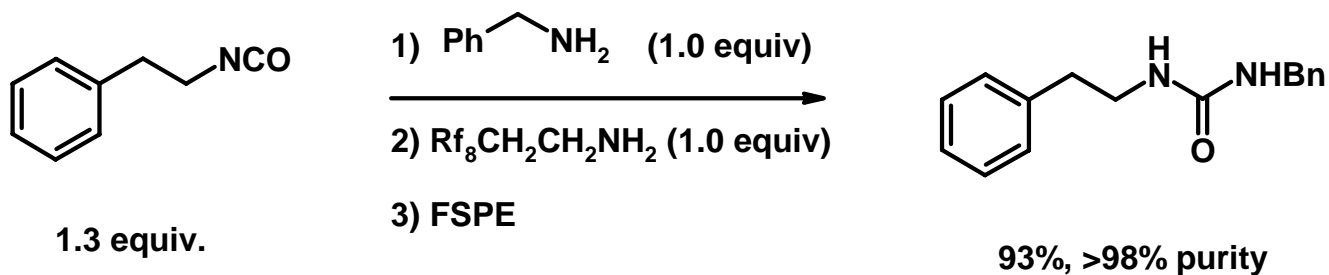
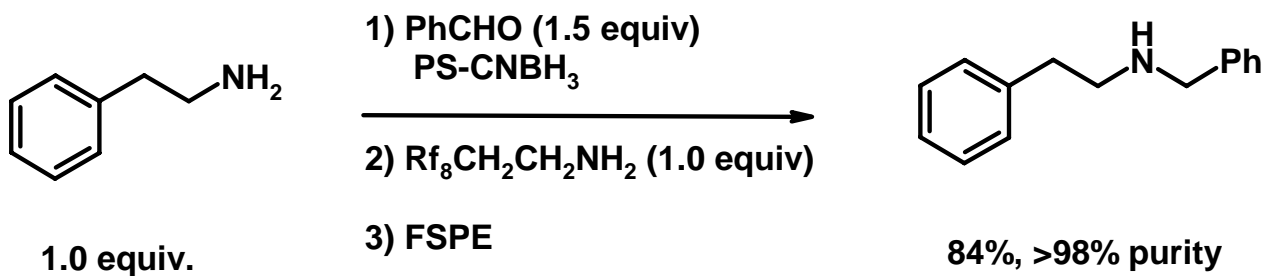
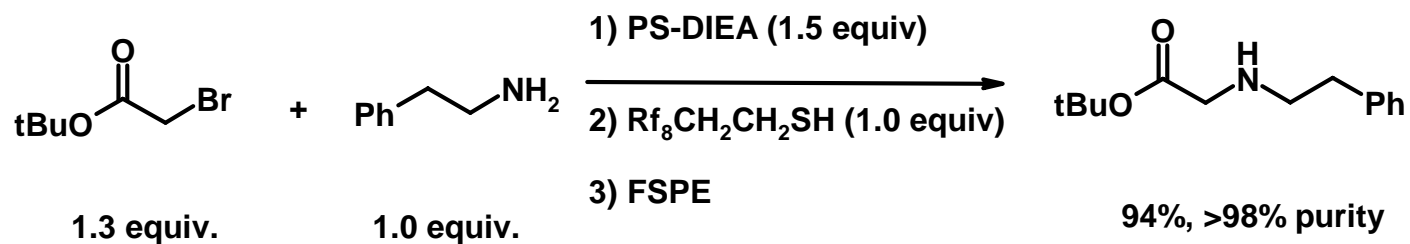
X = O or S

<u>Entry</u>	<u>X</u>	<u>Amine</u>	<u>Scavenger</u>	<u>Product</u>	<u>Yield (purity)</u>
1	O		f-IA		100% (>95%)
2	O		f-isocyanate		100% (95%)
3	S		f-IA		100% (95%)
4	S		f-isocyanate		34% (93%)

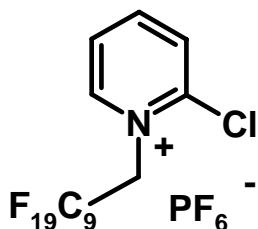
Electrophilic Fluorous Scavengers



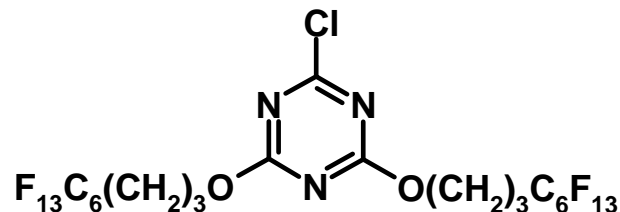
Nucleophilic Fluorous Scavengers



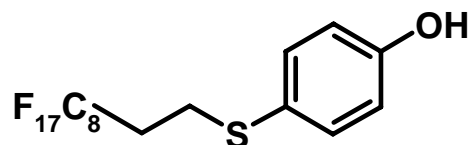
Fluorous Acylation Reagents



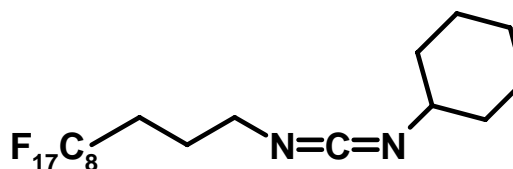
fluorous Mukaiyama's salt



fluorous CDMT



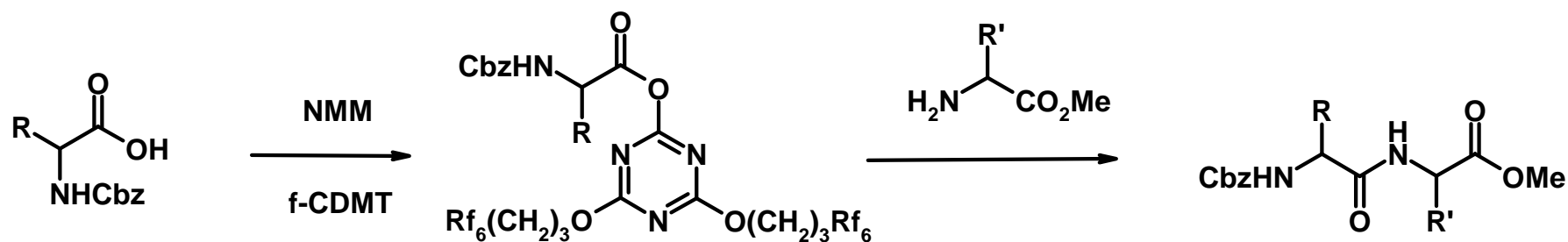
FluoMar



fluorous DCC

- Facilitate acylation reactions
- Facile purification by F-SPE
- Design Flexibility
 - Solution Phase or Hybrid Solid/Solution Phase

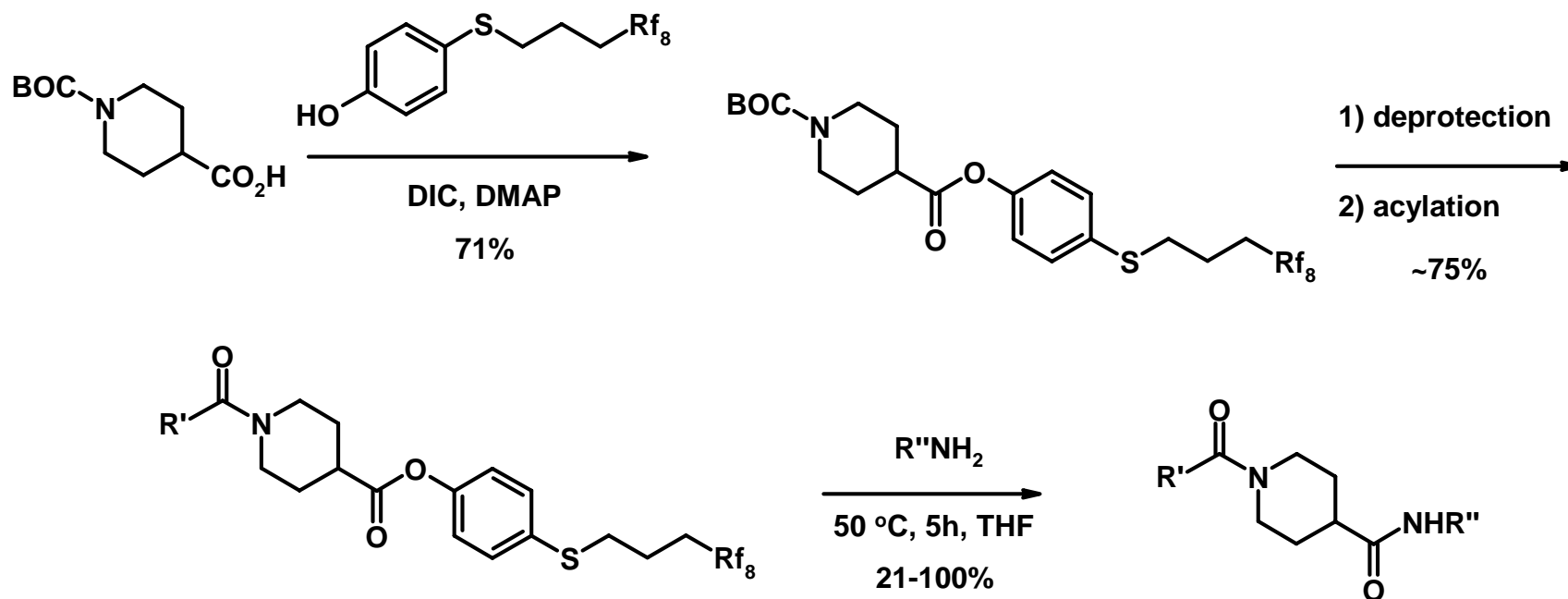
Fluorous Acylation Reactions



<u>Entry</u>	<u>Amide/Peptide</u>	<u>Yield(%)</u>	<u>Lit. yield(%)</u>
1	Cbz-Ala-Ala-OMe	98	94
2	Cbz-Pro-Ser-OMe	96	89
3	Cbz-Phe-Met-OMe	91	73
4	Cbz-Ala-Ala-Ala-OMe	93	75

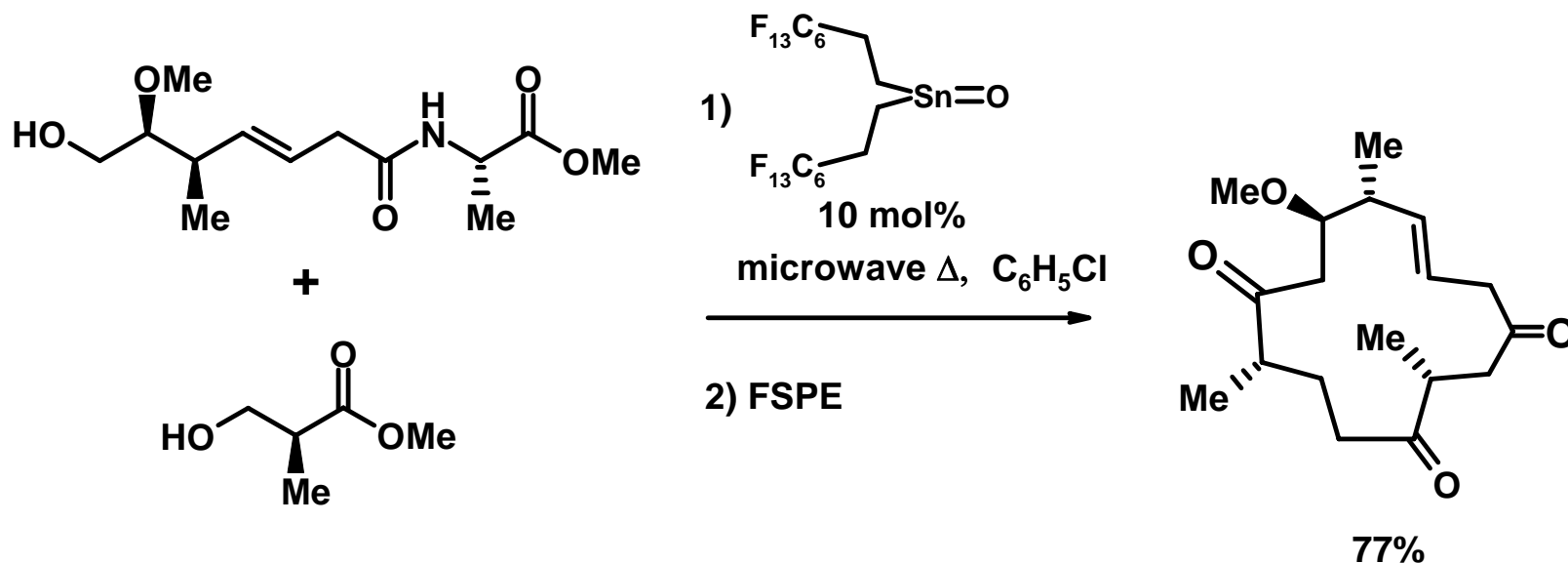
- No racemization observed
- f-CDMT suitable for use with α,α -disubstituted acids
- FSPE purification possible

Fluorous Acylation Reactions



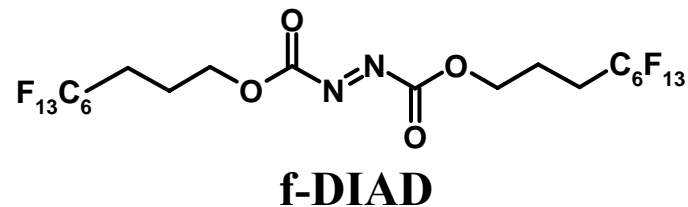
- All intermediates and final products purified by FSPE
- FluoMar used as a tag as well as an activating group
- FluoMar can be recovered and reused


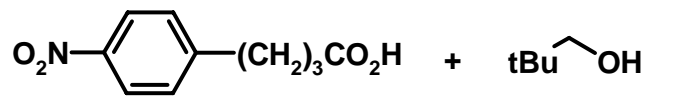
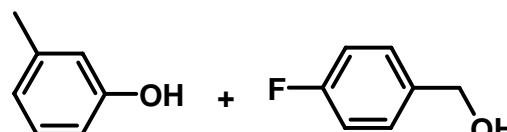
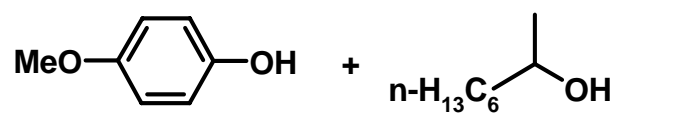
Fluorous Acylation Reactions



- Fluorous tin oxide catalyzed microwave assisted macrolactonization in higher yield than using dibutyl tin oxide.
- Macrolide library consisting of 127 isolated products with 83% of compounds in >90% purity (ELSD).
- FSPE reduced residual tin content to ~30 ppm. Further reduction to <10 ppm was easily accomplished by treatment with TAA resin.

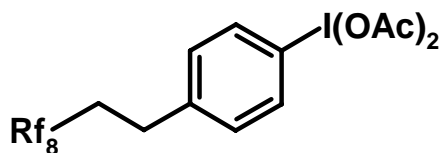
Fluorous Mitsunobu Reactions



Entry	Reactants	Conditions	% Yield (% purity)
1		TPP, DIAD	95
		f-TPP, f-DIAD	92(99)
2		TPP, DIAD	94
		f-TPP, f-DIAD	94(96)
3		TPP, DIAD	75
		f-TPP, f-DIAD	60(97)
4		TPP, DIAD	74
		f-TPP, f-DIAD	55(97)

FSPE purification effectively removes all fluorous by-products

Other Fluorous Reagents



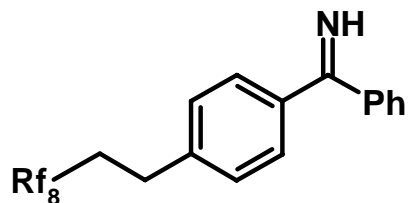
Co(f-salen)

hypervalent iodine oxidations

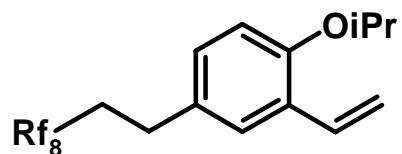
epoxidations



Radical mediated reductions
and cyclizations

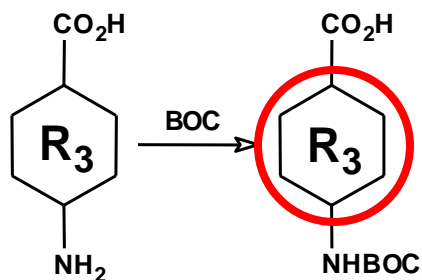
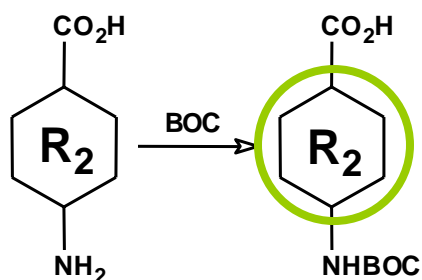
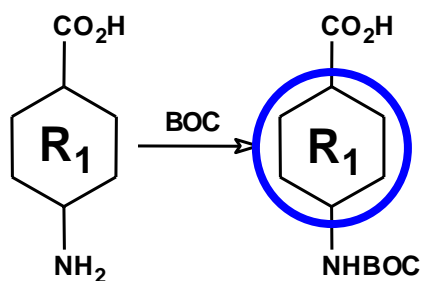


Buchwald-type aminations

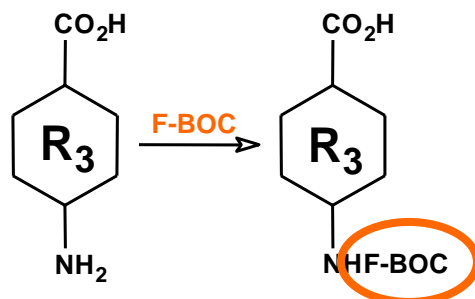
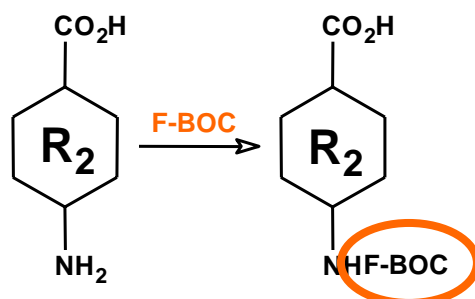
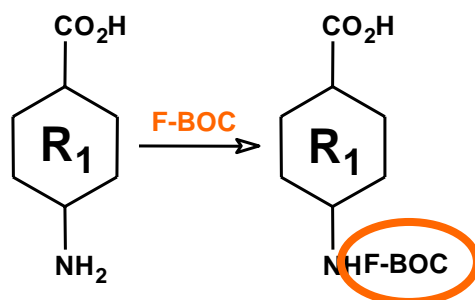


Olefin metathesis ligand

Non-fluorous



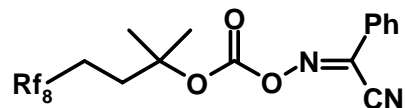
Fluorous



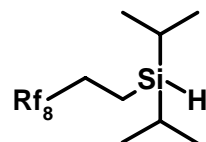
Non-fluorous: multiple chromatographic species, since separation controlled by variable domain.

Fluorous: single chromatographic species using single method on fluorous sorbent, since separation controlled by non-variable fluorous domain

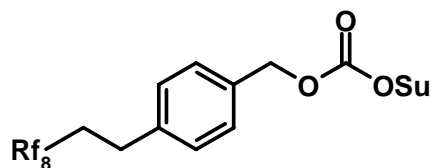
f-BOC-ON



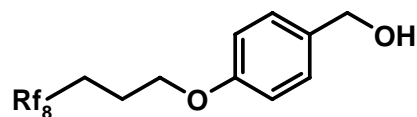
f-silane



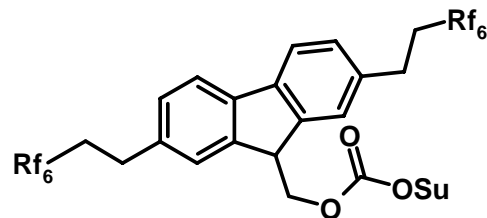
f-Cbz-OSu



f-PMB

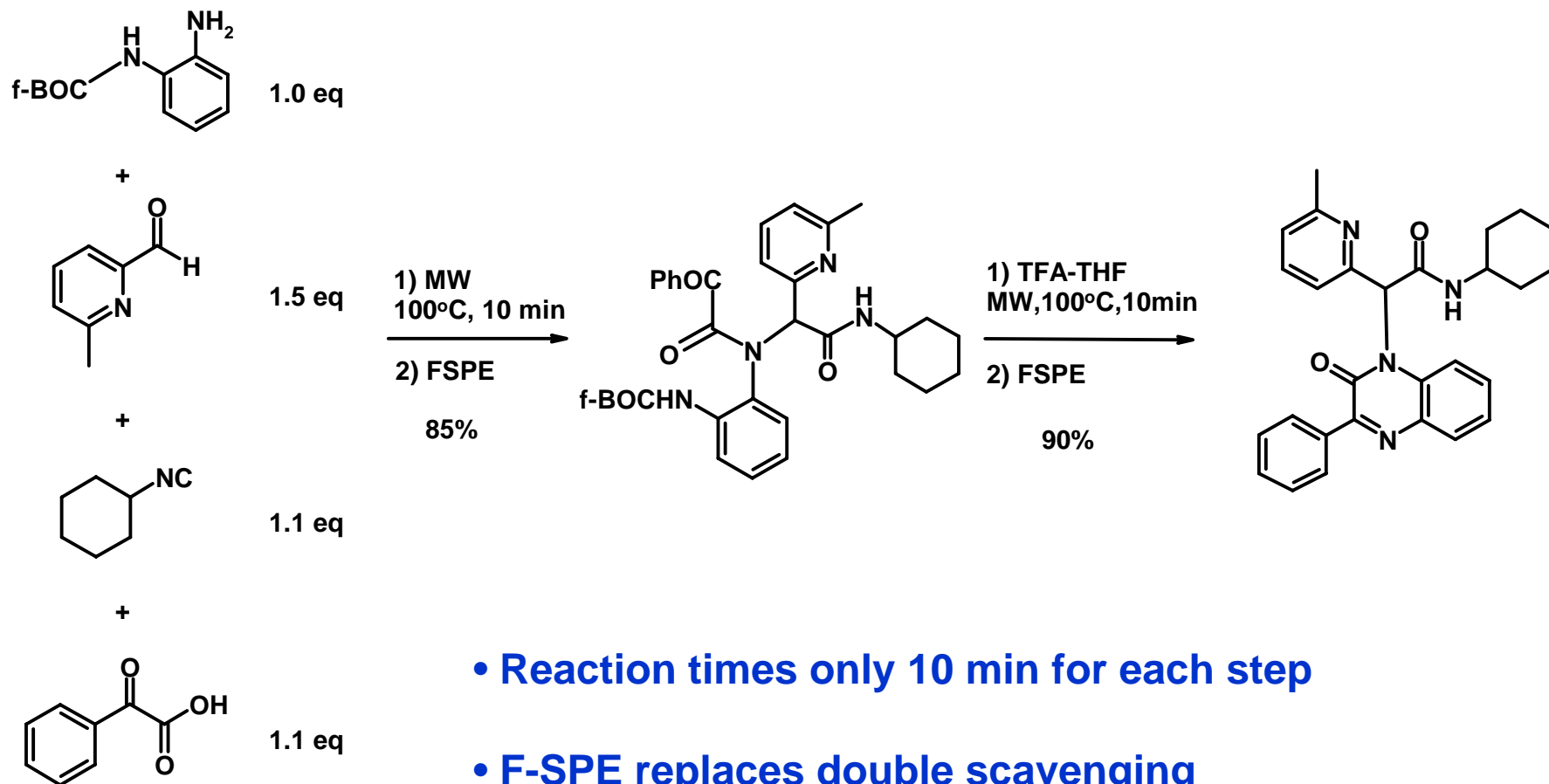


f-Fmoc

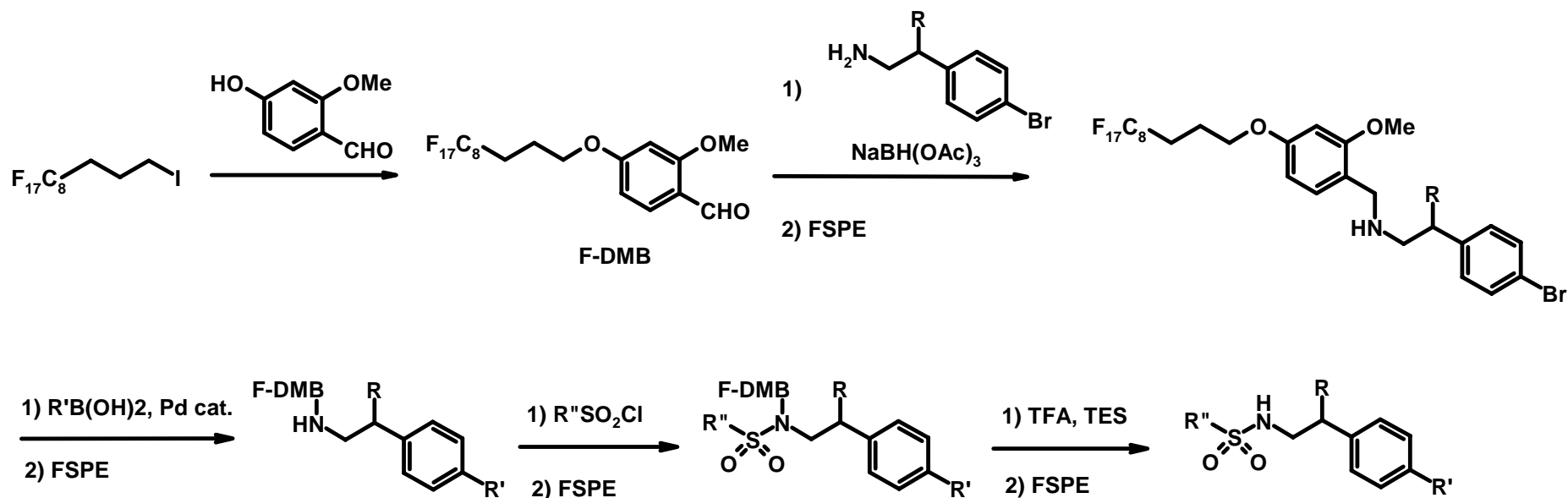


Fluorous tags behave similar to traditional protecting groups, but provide a handle for facile purification.

Fluorous Ugi Reaction

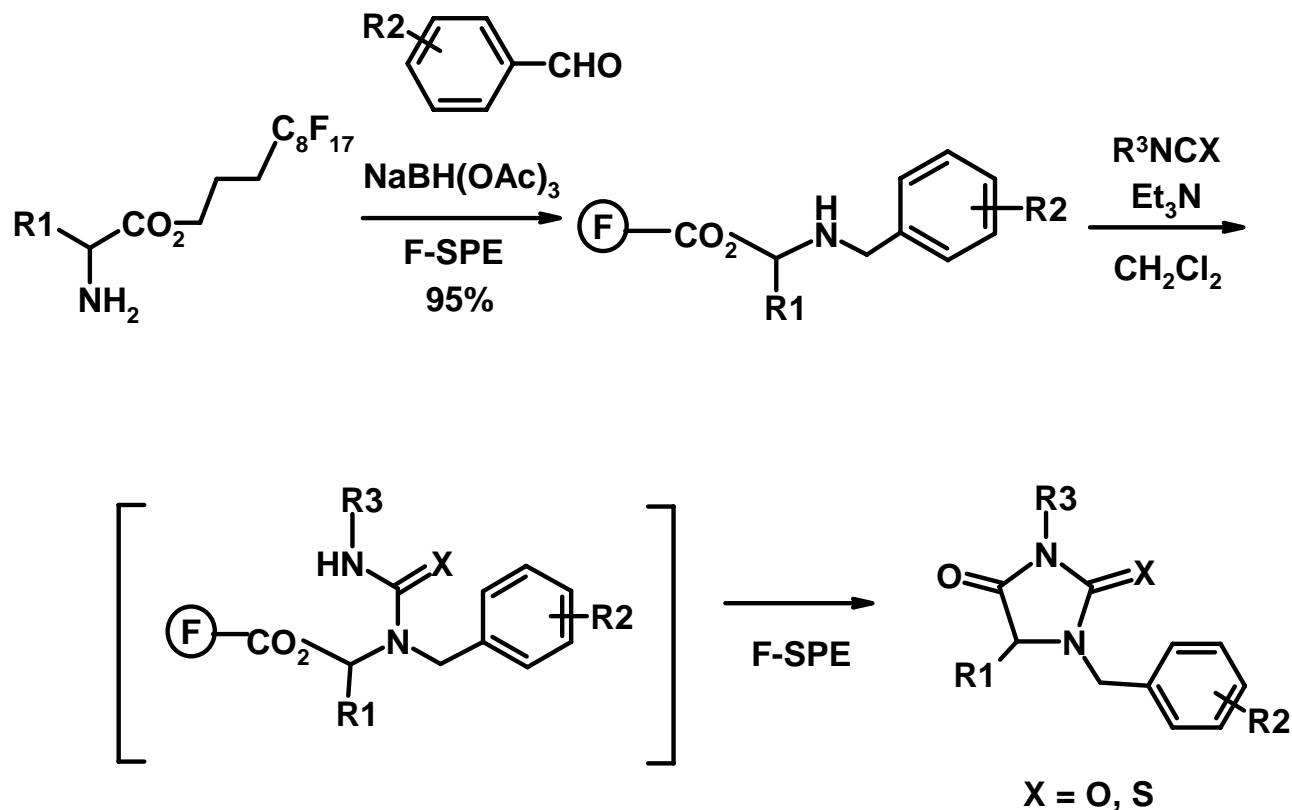


Fluorous Parallel Synthesis



- 27 member sulfonamide array with 3 diversity points produced
- All intermediates and products purified by FSPE
- All members of library >95% purity with no HPLC
- 18 member amide array also described using similar strategy

Synthesis of Hydantoin Library

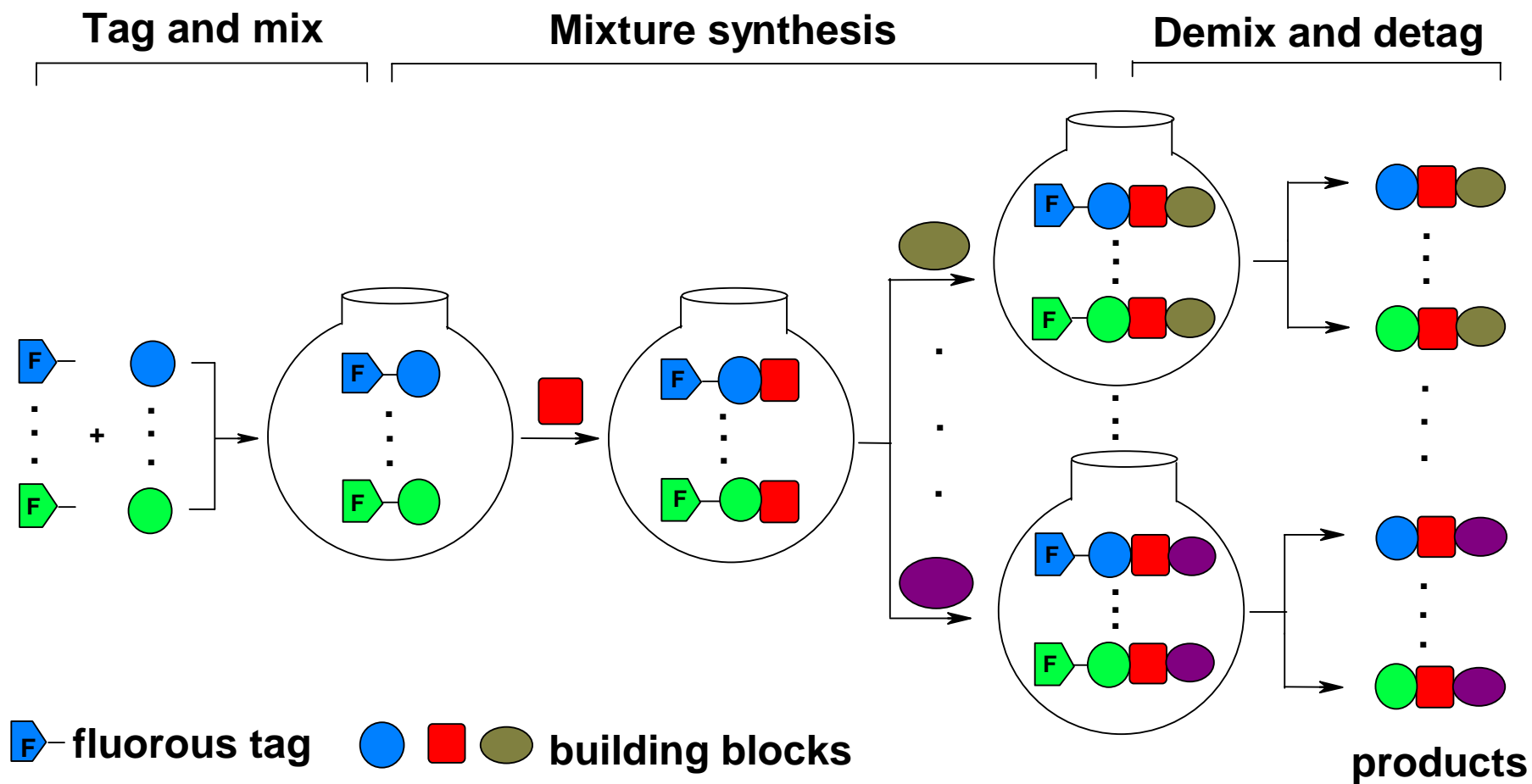


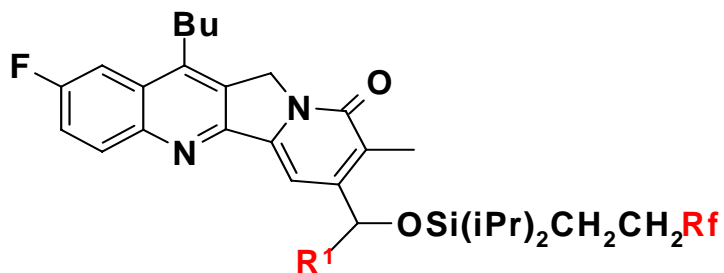
- 120 compound library produced. No HPLC purification
- Avg. yield = 30 mg (90% of compounds in >50% overall yield)
- 88% of compounds had >90% LC purity (MS detection)

- R_f derivatives are recommended for parallel synthesis.
- Most organic solvents can be used without issue. If solvation is a problem, the addition of BTF can help.
- Always try and design reactions to contain either one organic or one fluororous species.
- Fluororous compounds are generally hydrophobic.
- Highly polar functionalities (e.g. ammonium salts) can shorten fluororous retention times
- Fluororous TLC and HPLC can be valuable analytical tools for FSPE evaluation.

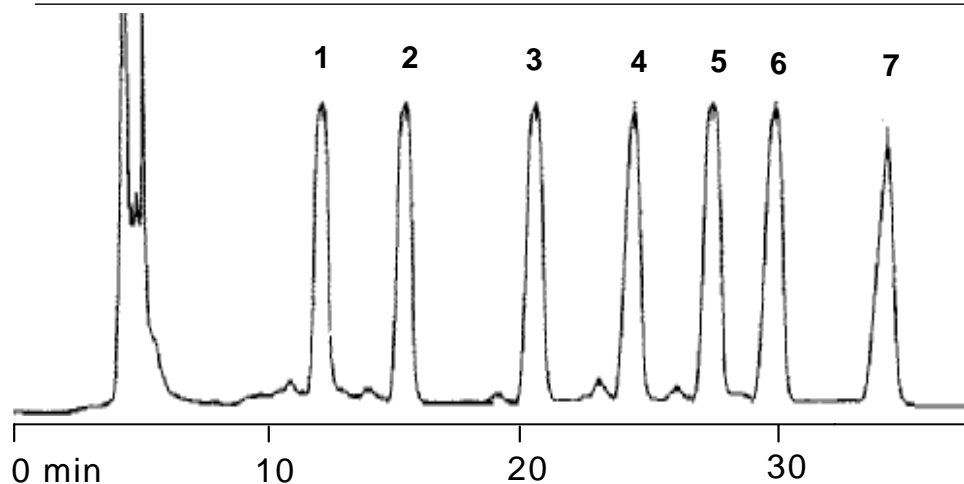
- Generally run using a SPE vacuum manifold available from numerous vendors
- Cartridges are available in 2, 5, 10, and 20 gram sizes.
- Maximum loading capacity of 20%, although 10-15% is recommended.
- Cartridge should be pre-treated with 80:20 MeOH:H₂O and sample loaded using a minimum of solvent. For recommended loading solvents and volumes, please consult the FSPE application note.
- First wash 80:20 MeOH:H₂O and second wash 100% MeOH.
- Cartridge can be reused multiple times after washing with MeOH or THF.

Concept of Fluorous Mixture Synthesis (FMS)





	1	2	3	4	5	6	7
R_f	C ₃ F ₇	C ₄ F ₉	C ₆ F ₁₀	C ₇ F ₁₅	C ₈ F ₁₇	C ₉ F ₁₉	C ₁₀ F ₂₁
R₁	Me	Pr	Et	s-Bu	i-Pr	c-C ₆ H ₁₁	CH ₂ CH ₂ -c-C ₆ H ₁₁



F-HPLC column (20 x 250 mm, 5 mm), gradient 88:12 MeOH-H₂O to 100% MeOH in 28 min, then to 100% THF in 7 min, 12 mL/min

- Components of a fluorous mixture are separated based on fluorine content
- FMS was used to produce library of analogs to mappacine.
- 560 member library produced in half of the number of reactions vs. parallel synthesis.



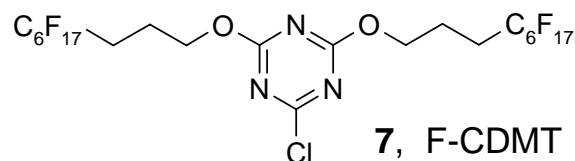
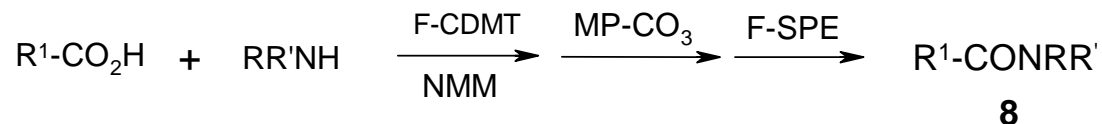
VacMaster-96™

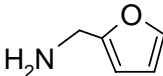
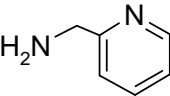
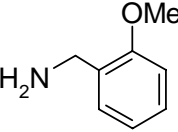
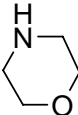
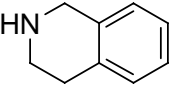
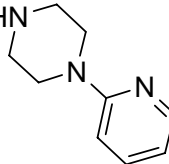
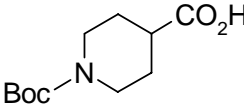
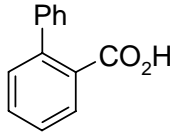
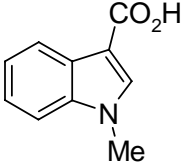
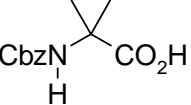


Whatman receiving plate

- 6 mL cartridges with 3 g of FluoroFlash silica
- Receiving well volume of 10 mL
- Receiving plate can be directly placed in a Genevac vacuum centrifuge
- Scavenging and amide coupling reactions reported
- Procedures using 48 and 96 well plate format being developed

Plate-to-Plate Fluorous SPE Amide Coupling



						
	81(100)*	50(83)	57(91)	80(90)	100(60)	67(100)
	68(99)	45(88)	53(95)	70(100)	84(95)	62(93)
	36(99)	37(92)	34(100)	67(97)	76(92)	47(95)
	58(81)	58(81)	58(92)	45(90)	63(77)	37(99)

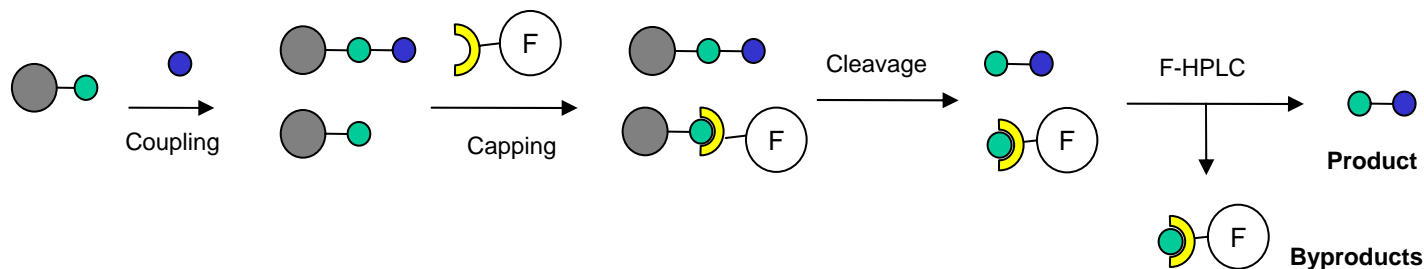
Fluorous Oligonucleotide, Peptide, and Carbohydrate Chemistry

Fluorous oligomer synthesis strategies

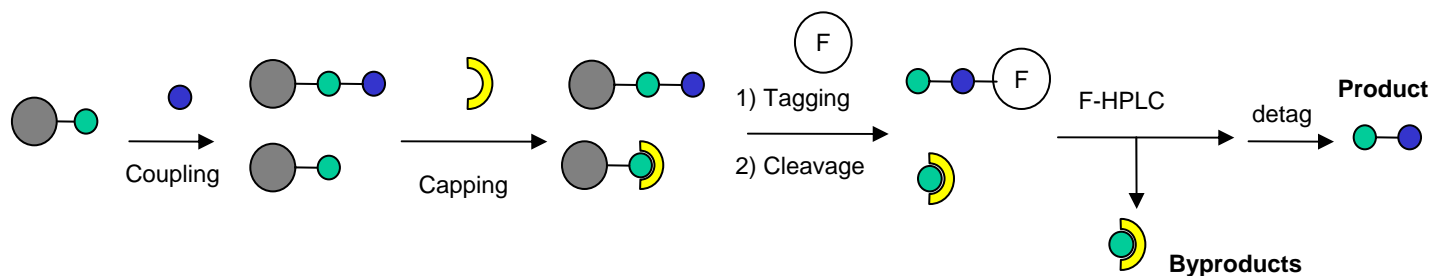
- **Solid-supported synthesis with fluorous *tagging***
 - Conventional solid phase synthesis with terminal fluorous tagged monomer.
 - General method to purify oligonucleotides and peptides.
 - FSPE or FHPLC used for simple pre-purification prior to final HPLC, increasing throughput.
- **Solid-supported synthesis with fluorous *capping***
 - Conventional solid phase synthesis with fluorous capping of deletion sequences
 - Purification by precipitation or FSPE
- **Solution phase synthesis with fluorous supports / tags**
 - Solution phase chemistry
 - Suitable for shorter sequences
 - Potential strategies for condensations and ligations

Fluorous Strategies in Solid Phase Synthesis

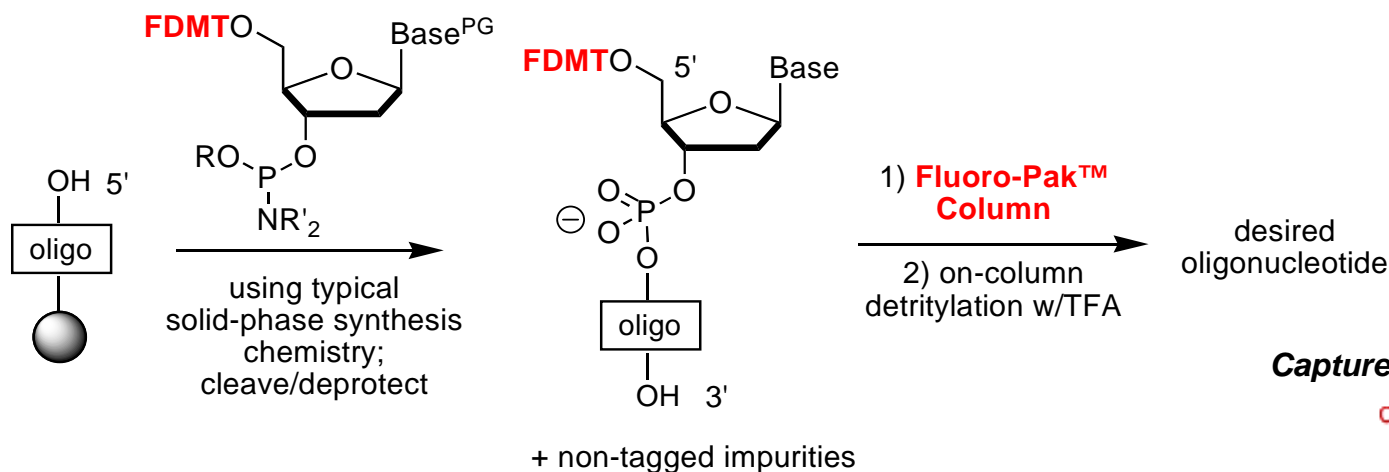
Cap undesired sequences



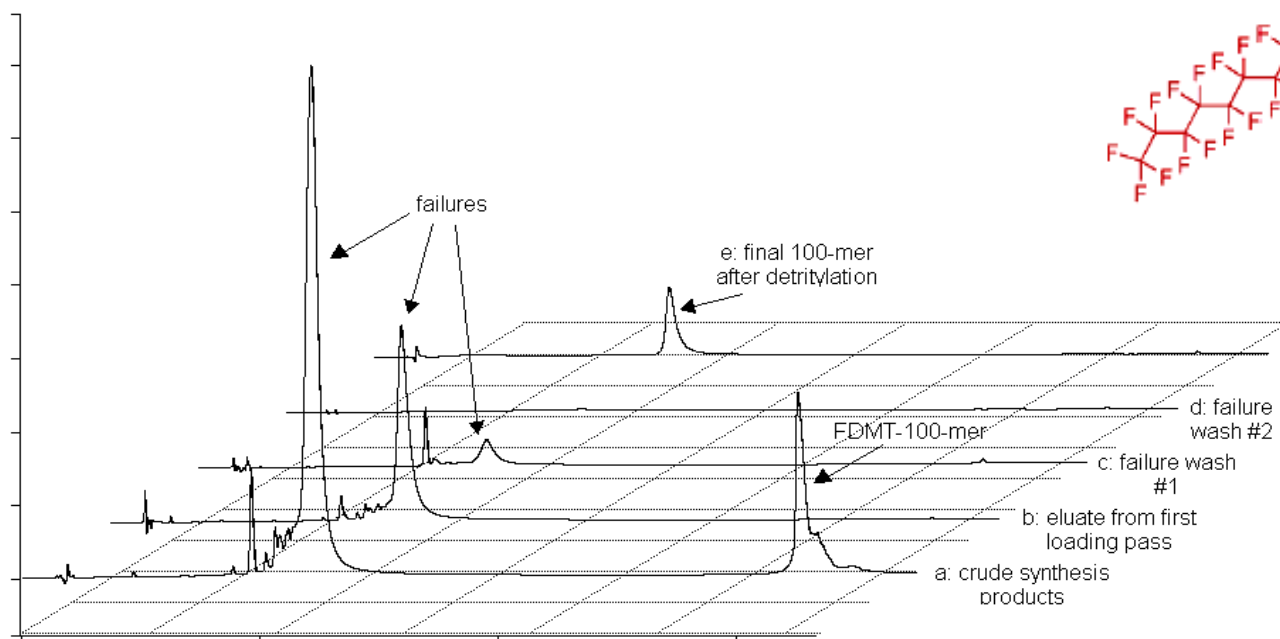
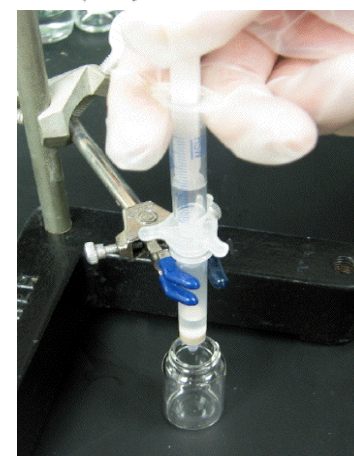
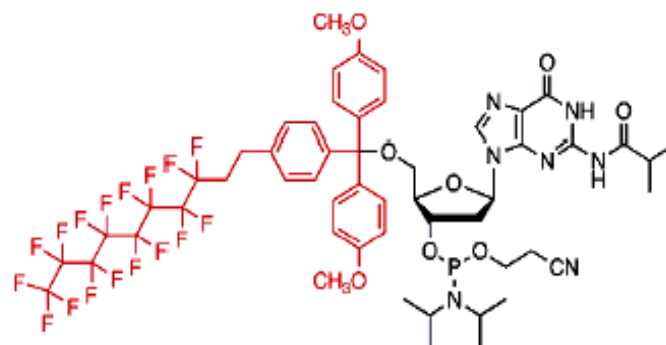
Tag desired sequence

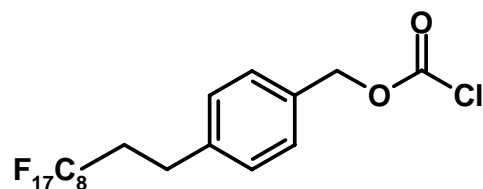


The Tag Approach

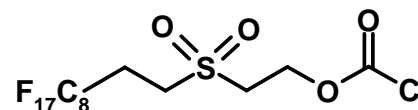


Capture and on-column detag





f-CbzCl

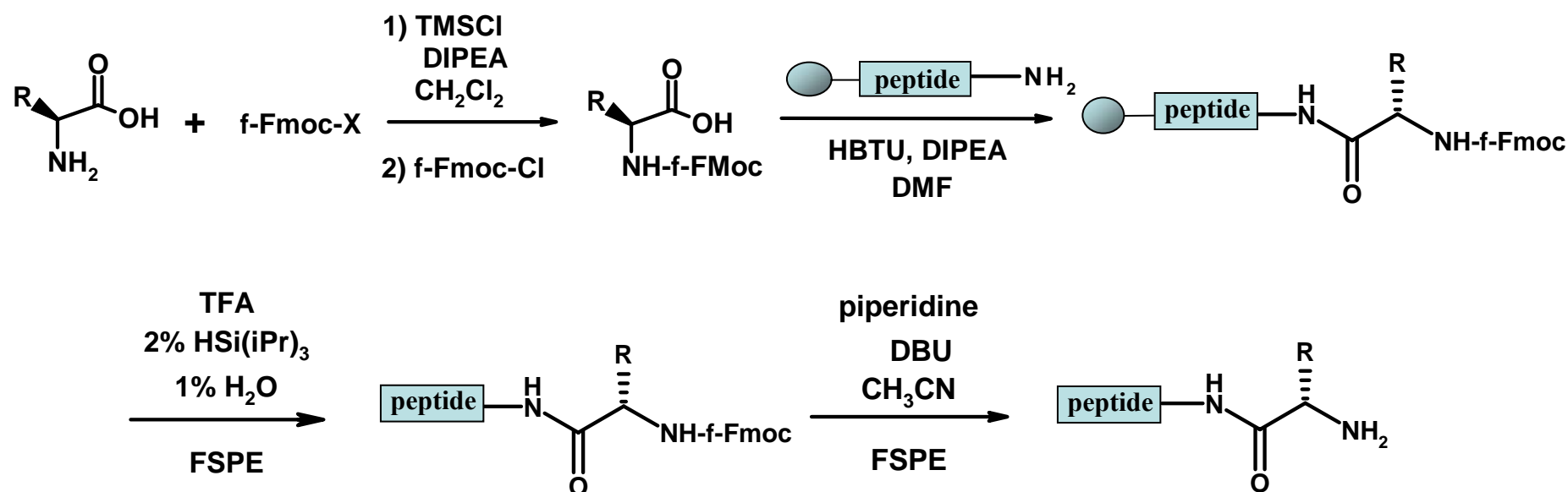
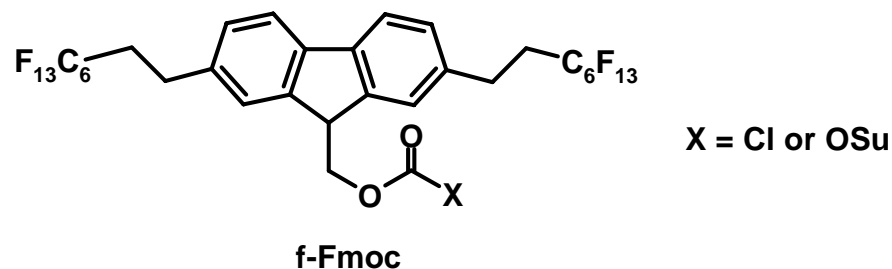


f-MscCl

Peptide	N-tag	Purification method	Yield(%)	Purity(%)
GVWPLFLLLLALPPKAYAG	f-Cbz	column	35	
GCCSLPPCALNNPDYC	F-Msc	FHPLC	37	98
	F-Msc	FSPE	59	91
RQIKIWFQNRRMKWKK	F-Msc	FHPLC	10	94
	F-Msc	FSPE	7	72
SELDDRADALQAGFSPFES SAAKLKRKYWWKNLK	F-Msc	FHPLC	21	99

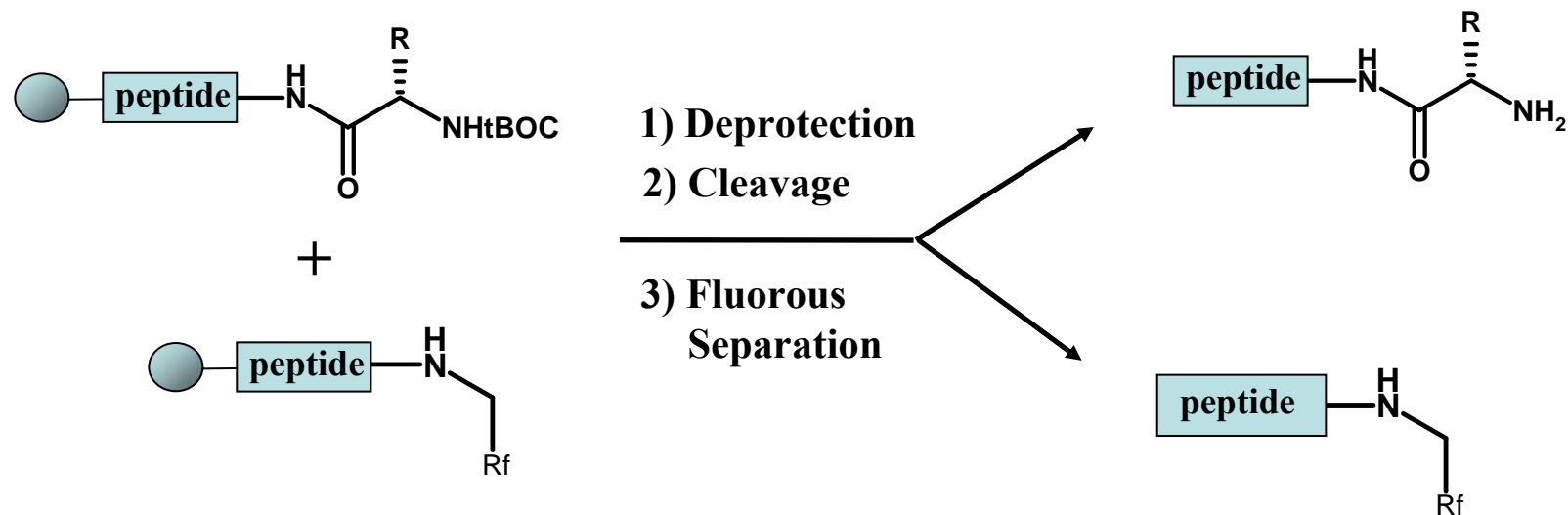
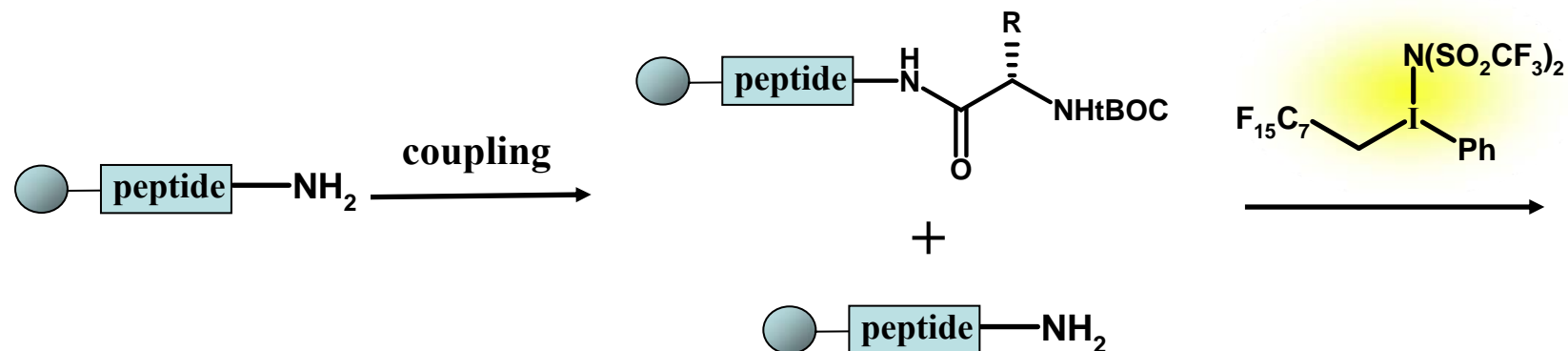
Fluorous Fmoc has been found to outperform f-Msc-Cl

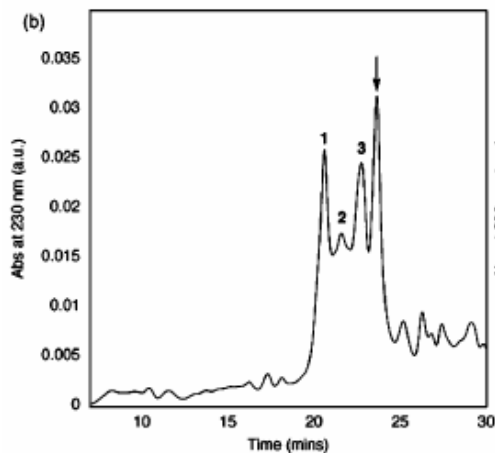
Fluorous Fmoc Peptide Synthesis



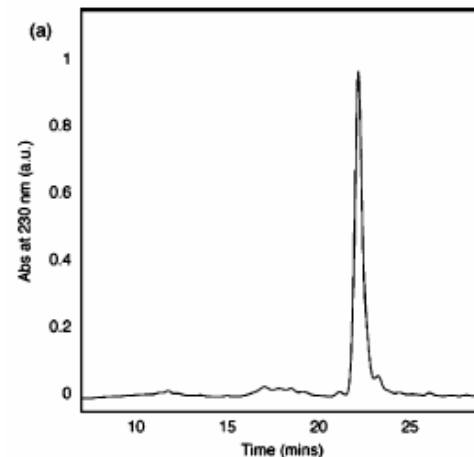
- “Standard” conditions using Novagel resin employed throughout
- FSPE conducted using 1:1 CH₃CN: water (fluorophobic wash) followed by CH₃CN (fluorophilic wash)

SPPS with fluorous capping...

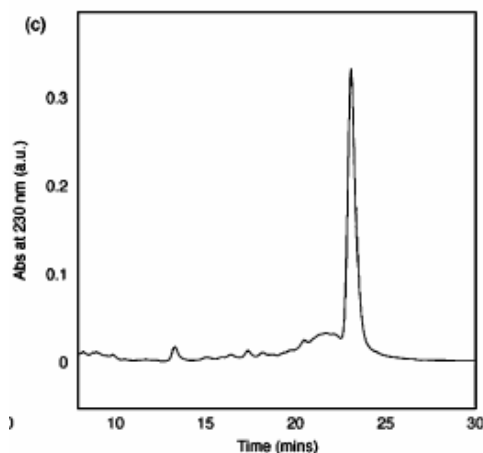




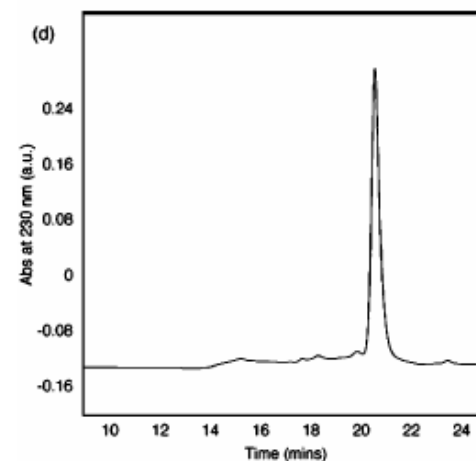
Ac-NH-RAV*KVY*ADAA*EDESAEFAEF-CONH₂
(no capping)



Ac-NH-VEA*AID*YI*DA-CONH₂

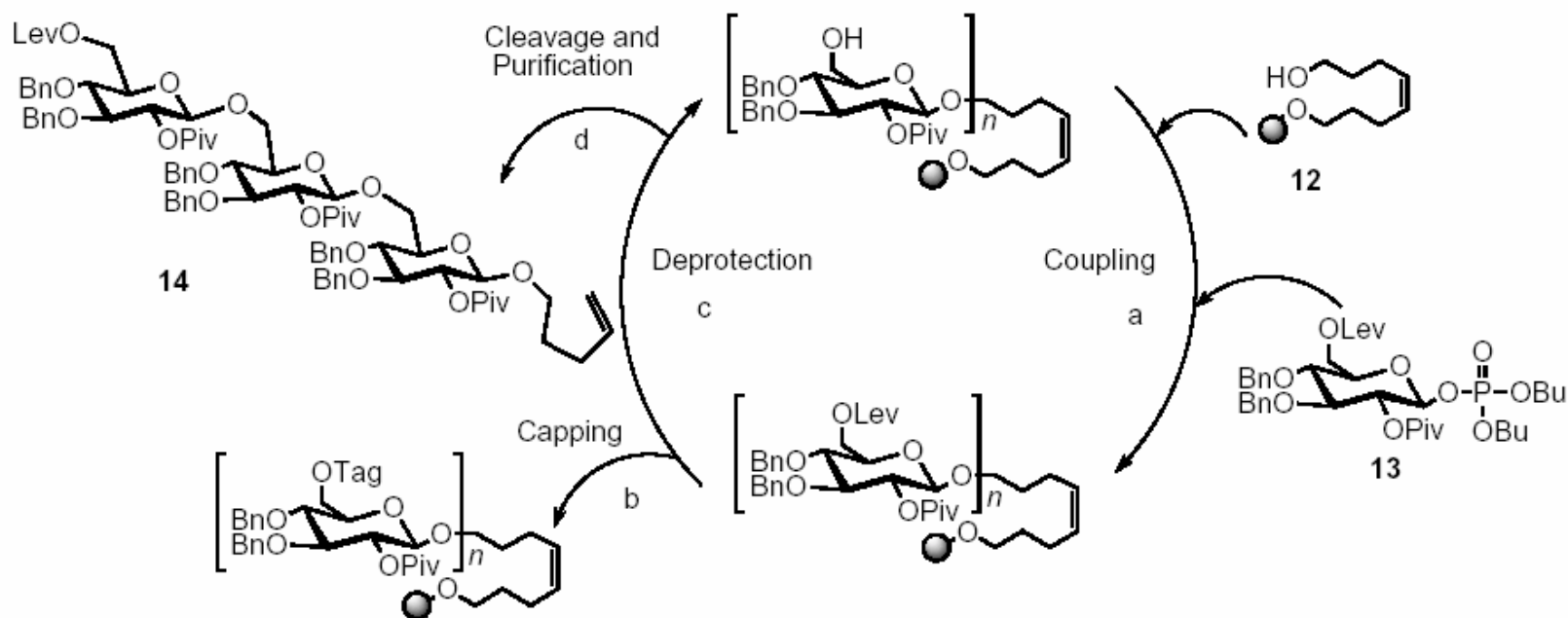


Ac-NH-RAV*KVY*ADAA*EDESAEFAEF-CONH₂

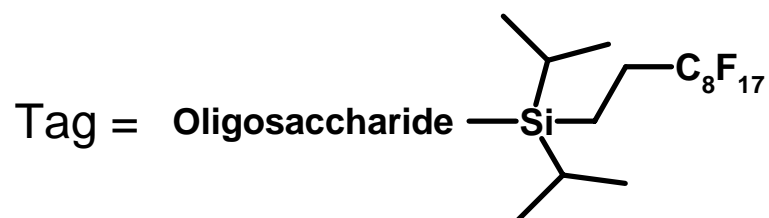


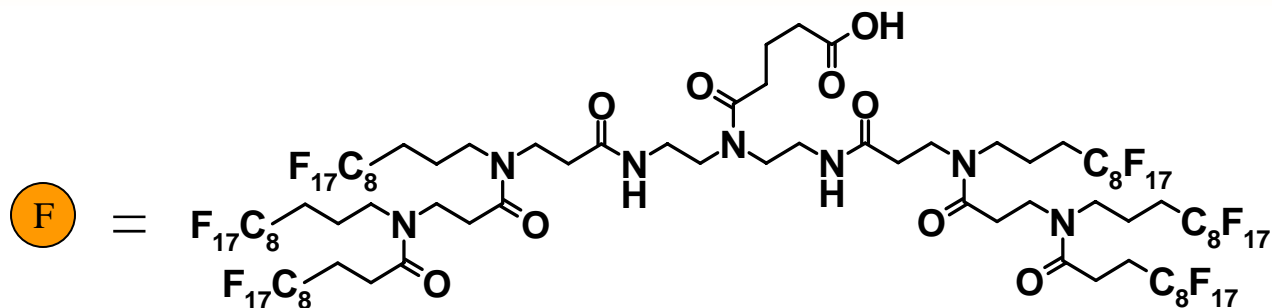
Ac-NH-PT*GYGS*SSRRAPET-CONH₂

Oligosaccharide synthesis with fluororous capping



- Tagging conducted after each coupling
- Tagged deletion sequences removed by FSPE (*quick intermediate purification in solution phase synthesis*)





- **Fluorous Supported Peptide Synthesis**

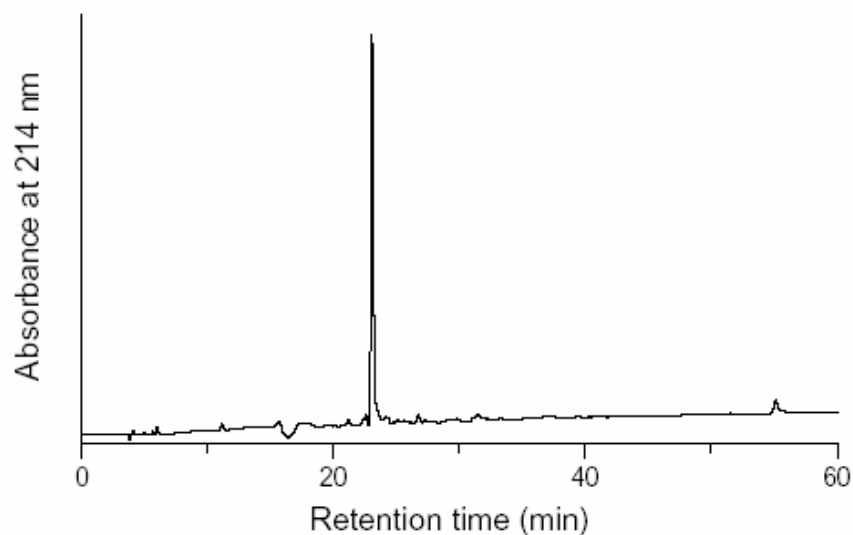
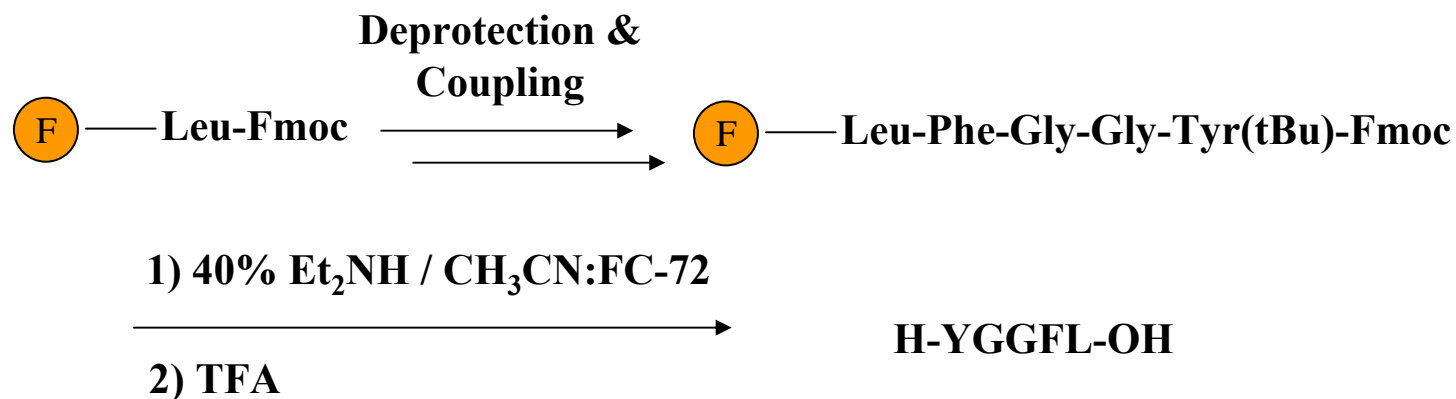
(Inazu, T. *et al*, Chem. Comm. 2003, 972.)

Tripeptide produced in 67% yield in excellent purity using liquid-liquid extraction and final HPLC purification.

- **Fluorous Supported Oligosaccharide Synthesis**

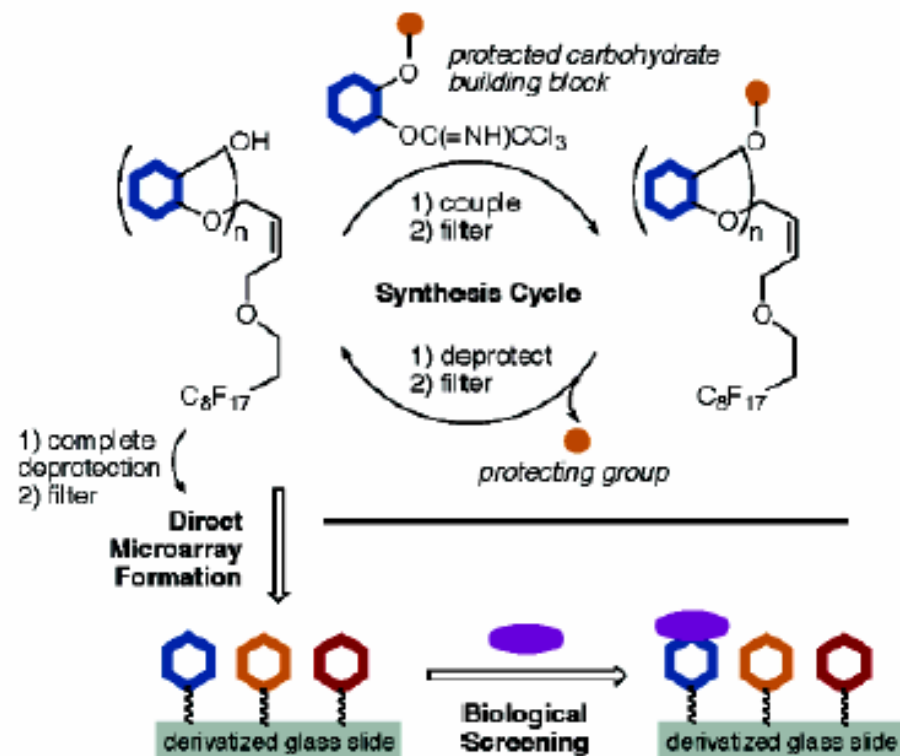
(Inazu, T. *et al*, Angew. Chem. Int. Ed. 2003, 42, 2047.)

Trisaccharide produced in 42% yield. Final purification by column chromatography after detachment from fluorous support.



- **Overall 70% yield over nine steps**
- **Final purification by RP-HPLC**
- **All reactions easily monitored**

Fluorous Based Synthesis and Immobilization



Man (1)

GlcNAc (2)

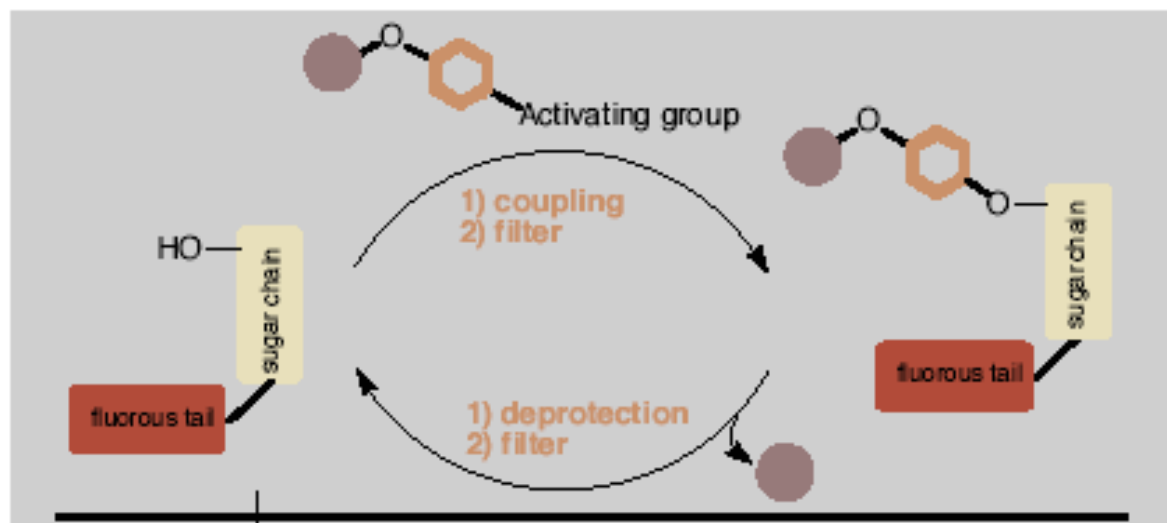
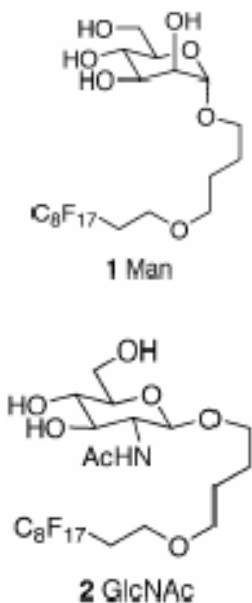
Gal (3)

Fuc (4)

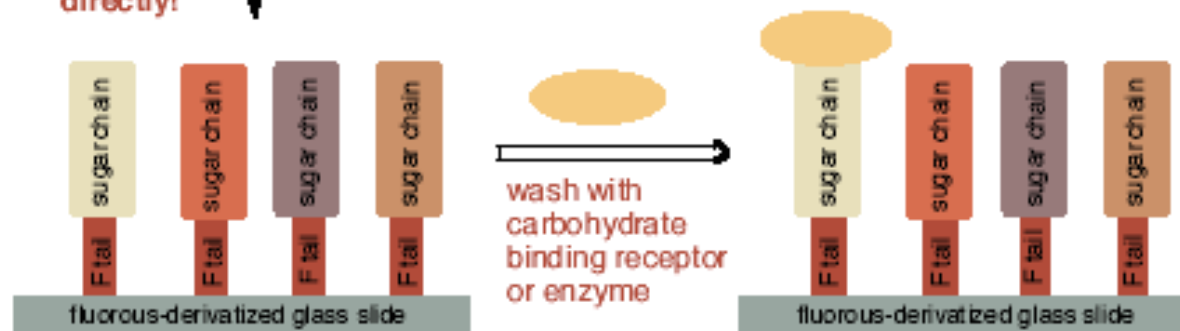


Fluorous supported oligosaccharide synthesis with direct microarraying onto fluorous derivatized glass slide

Fluorescence images of arrayed carbohydrates probed with FITC labeled lectins



microarray directly!

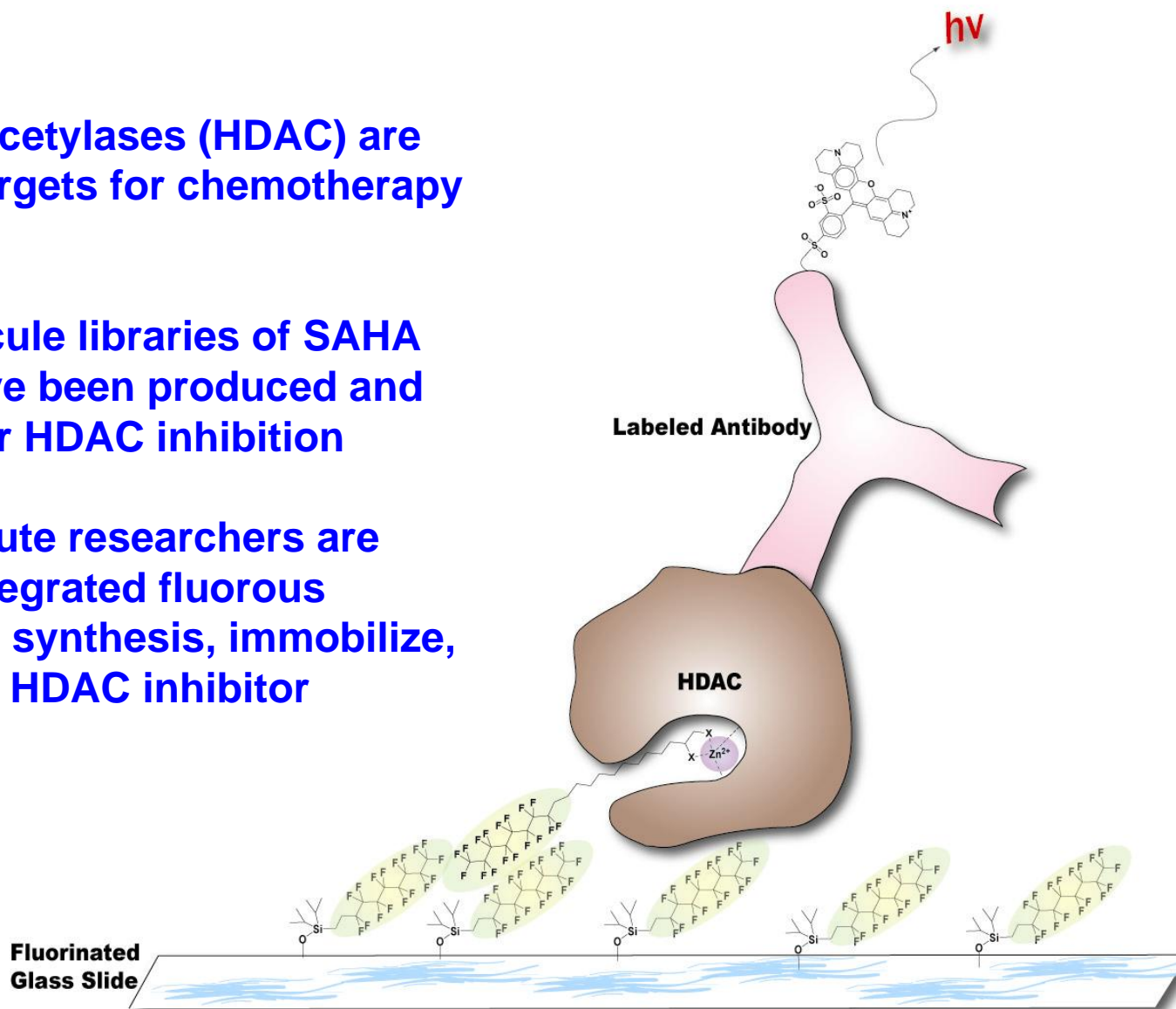


scan slide



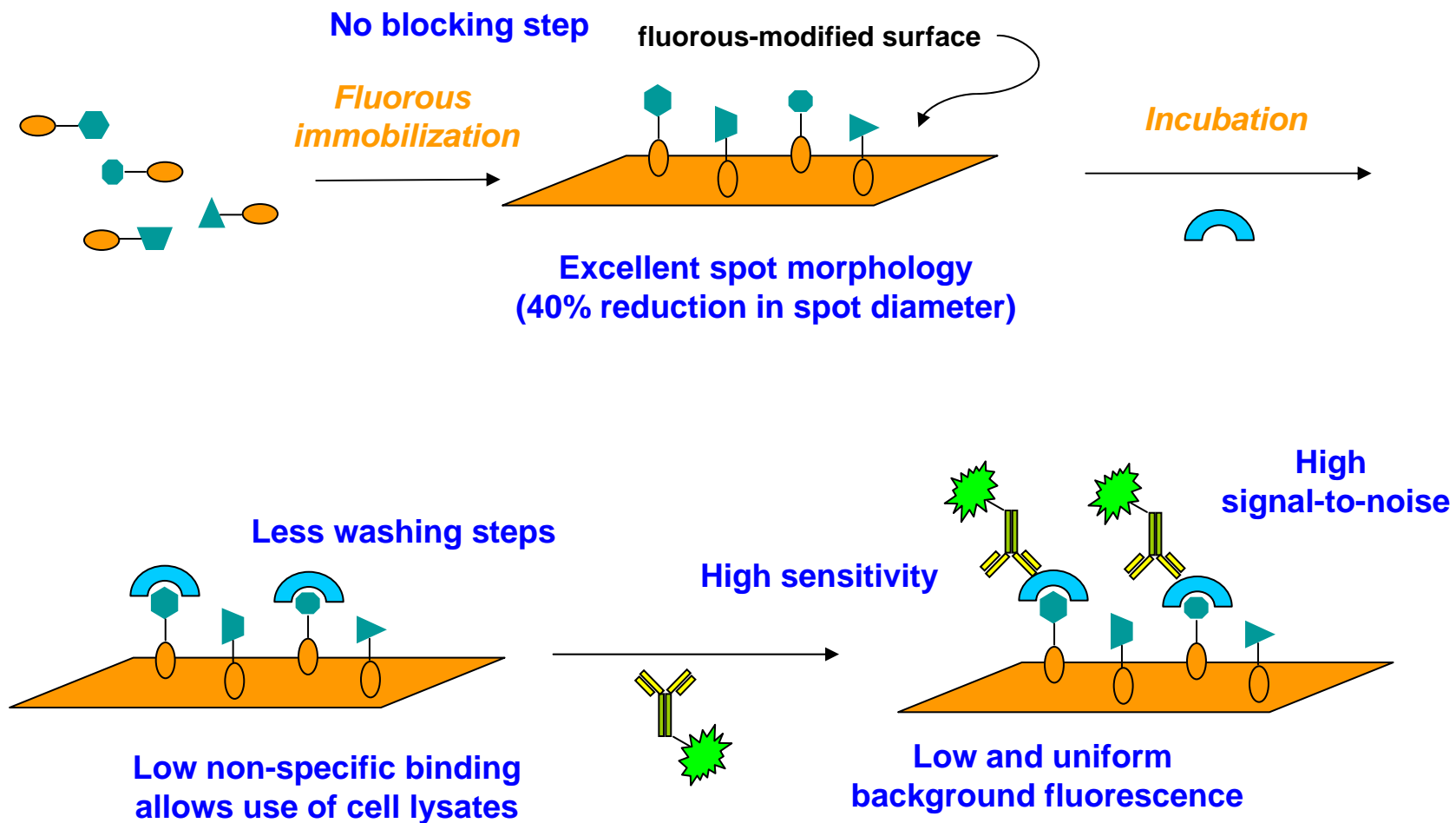
Application of Fluorous Microarrays

- Histone deacetylases (HDAC) are attractive targets for chemotherapy agents
- Small molecule libraries of SAHA analogs have been produced and screened for HDAC inhibition
- Broad Institute researchers are using an integrated fluorous approach to synthesis, immobilize, and analyze HDAC inhibitor libraries.



Vegas, A.J.; Bradner, J.E.; Tang, W.; McPherson, O.M.; Greenberg, E.F.; Koehler, A.N.; Schreiber, S.L. Unpublished results.

Fluorous Microarray Benefits



Fluorous Proteomics and Metabolomics

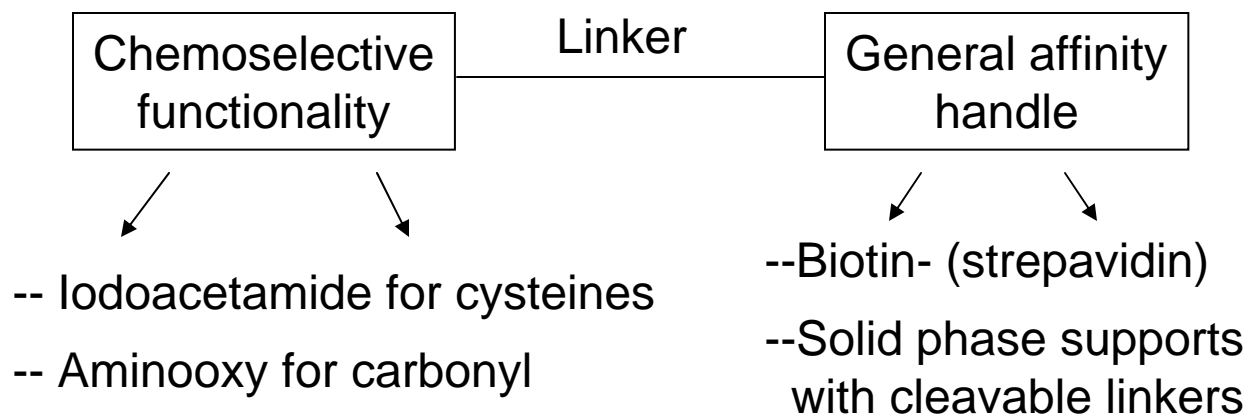
Two general enrichment strategies:

1) Moiety of interest directly interacts with affinity reagent:

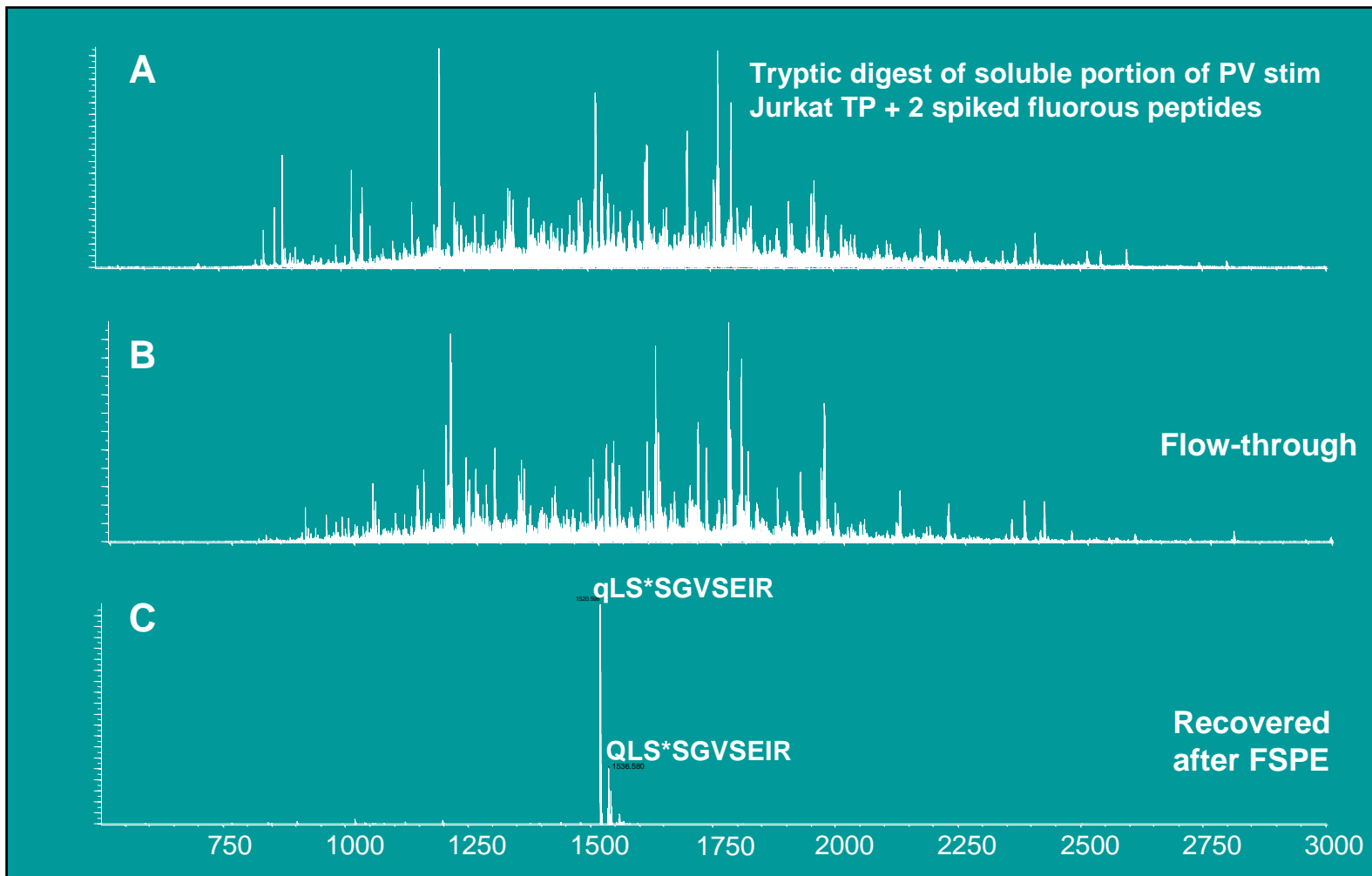
- Immunoprecipitation
- Phosphopeptides with IMAC
- Glycopeptides/ proteins with various lectins

{ Powerful, but highly specific

2) Multifunctional reagents containing:

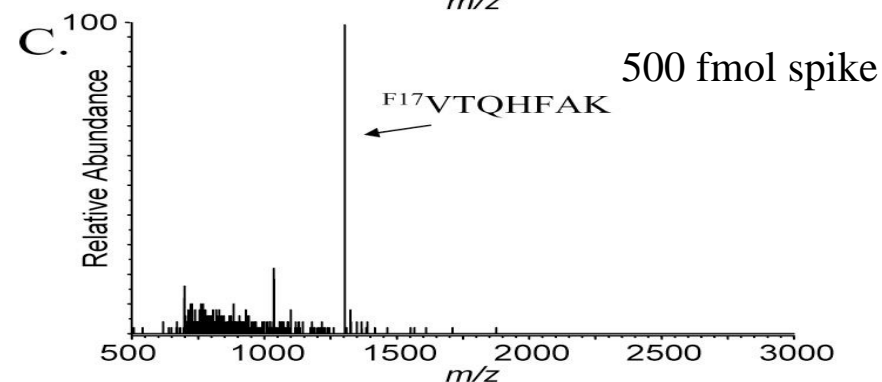
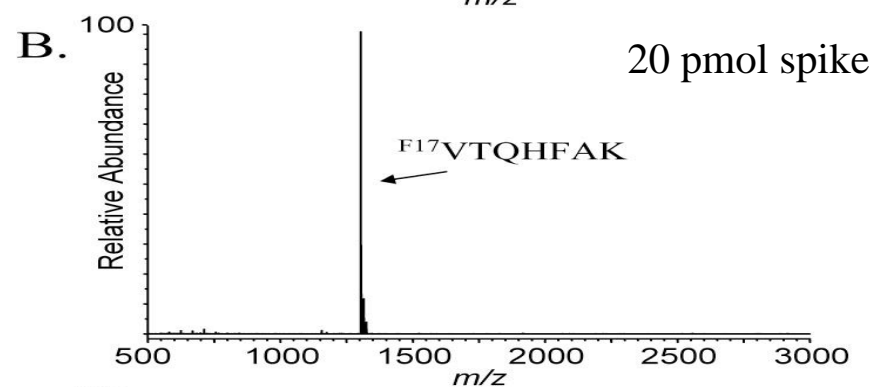
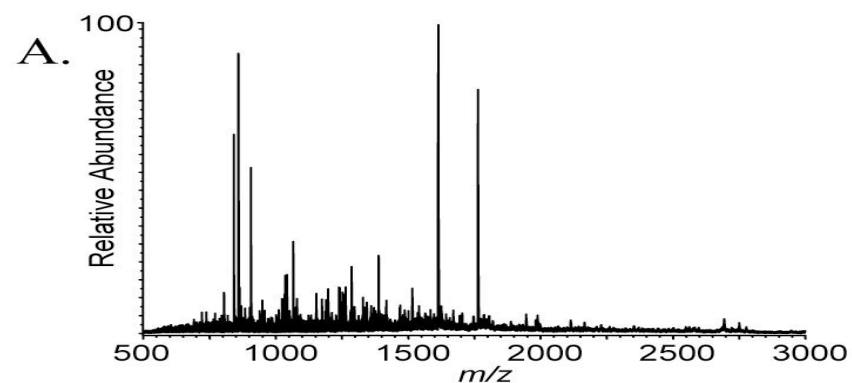


{ General format, but several operational issues + \$



Highly selective fluorous purification of complex peptide mixture

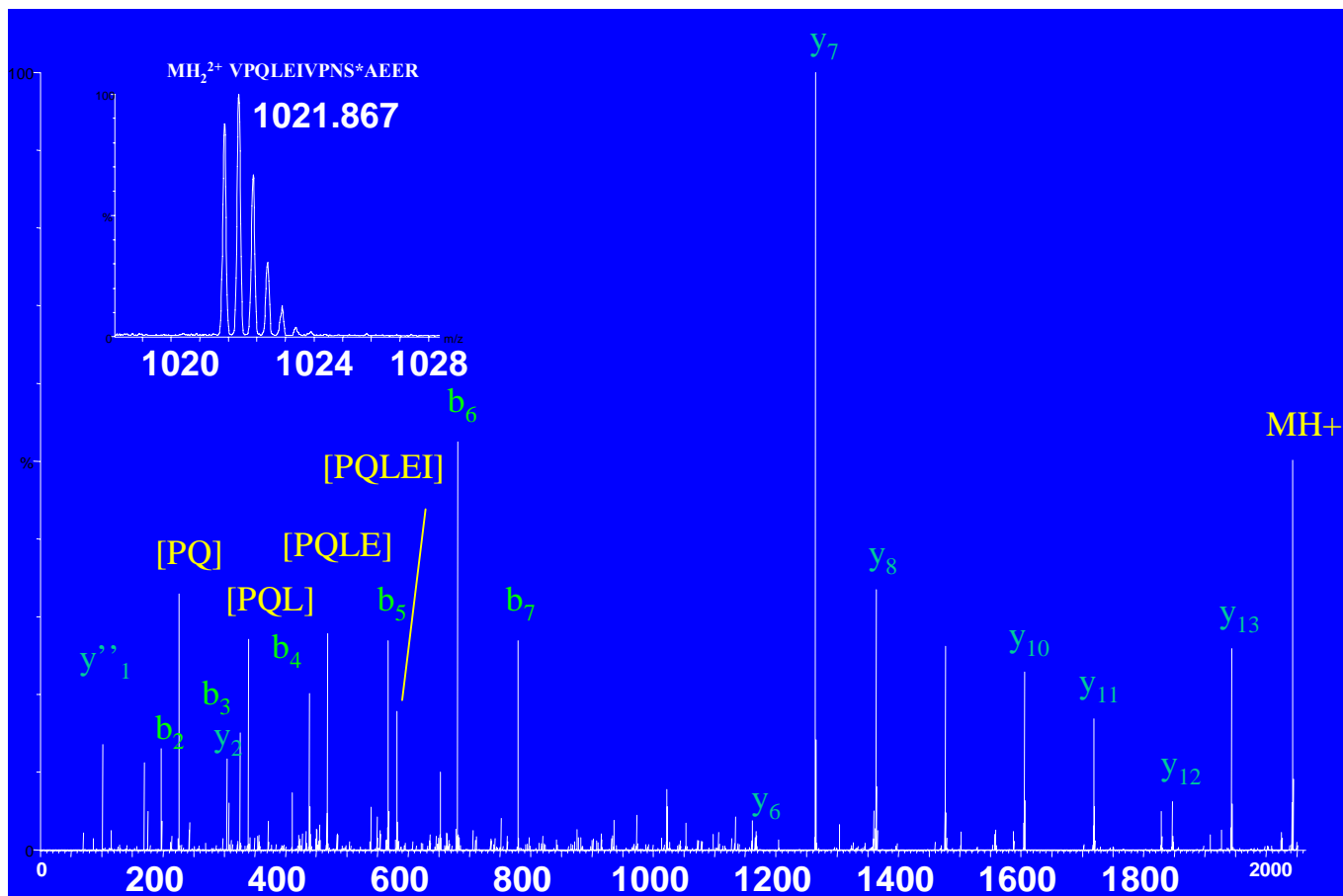
FSPE: Initial Evaluation of Recovery



Standard fluorous tagged peptide synthesized and also used for construction of calibration curve.

FIA analysis shows >50% recovery

Site of modification does not effect isolation

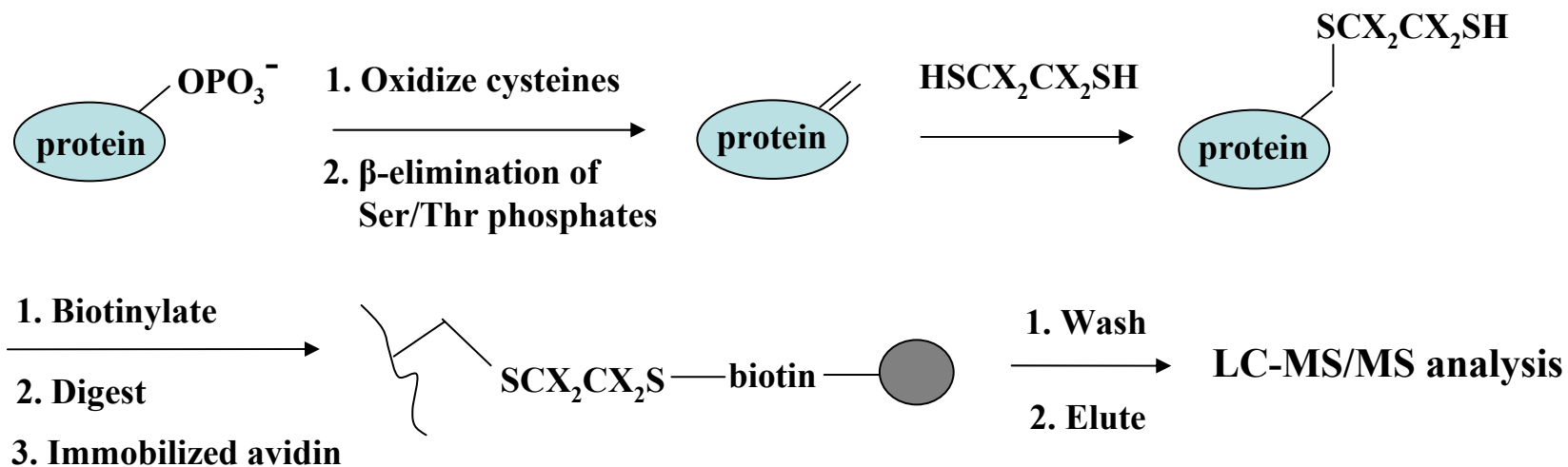


Fluorous tags exhibit excellent MS characteristics:

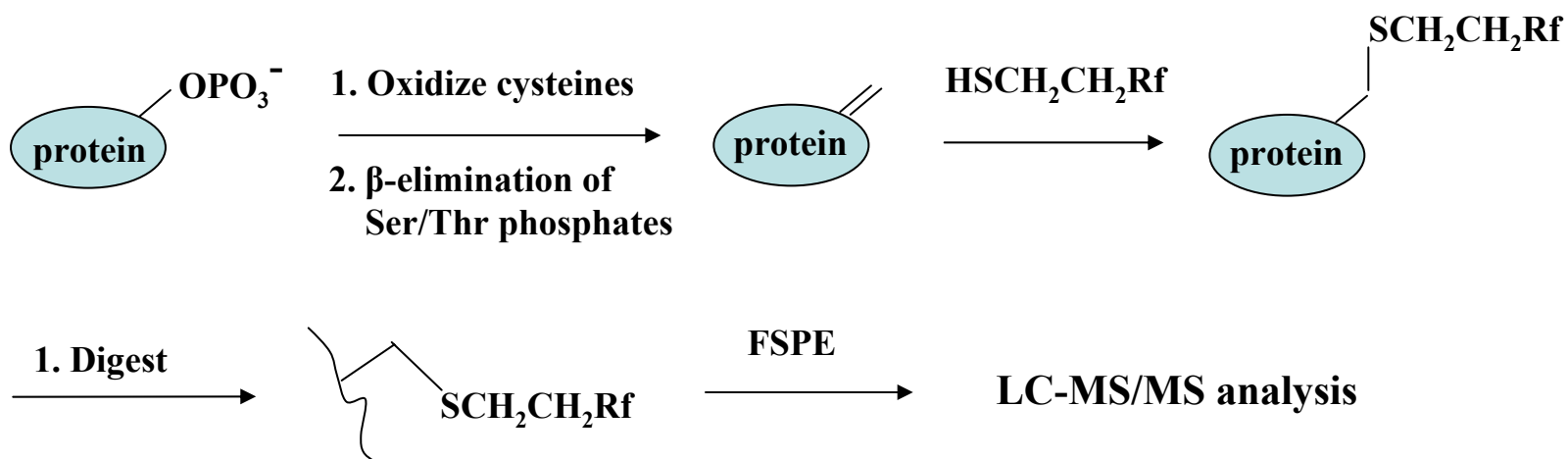
- Tag is non-fragmentary and non-suppressive
- Fluorine mass defect readily identifies tagged species
- Compatible with MASCOT and SEQUEST

Brittain, S.M.; Ficcaro, S.B.; Brock, A.; Peters, E.C. *Nature Biotech.* **2005**, 23, 463.

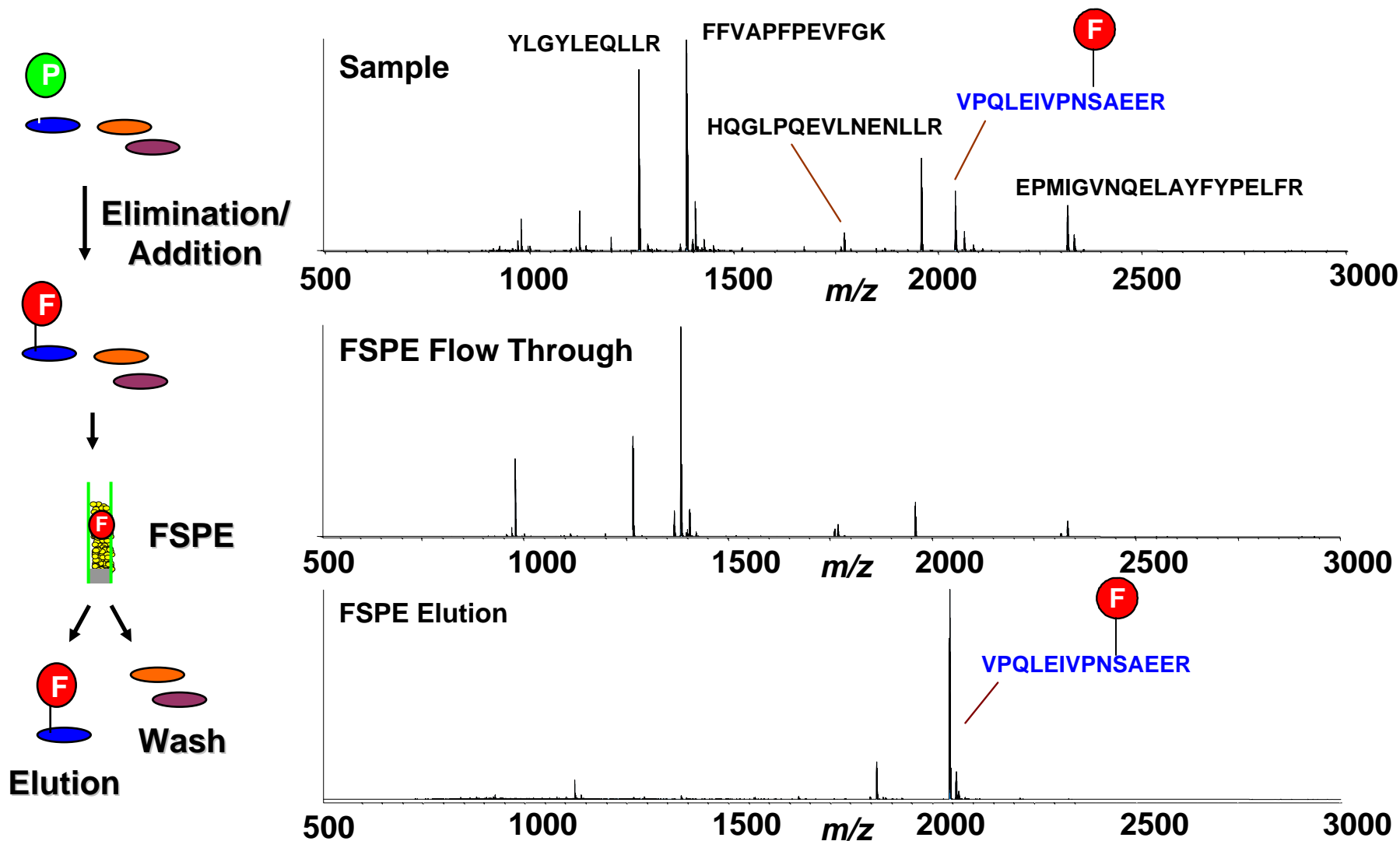
β -elimination (Oda, Y. et al, *Nat. Biotechnol.* 2001, **19**, 379)



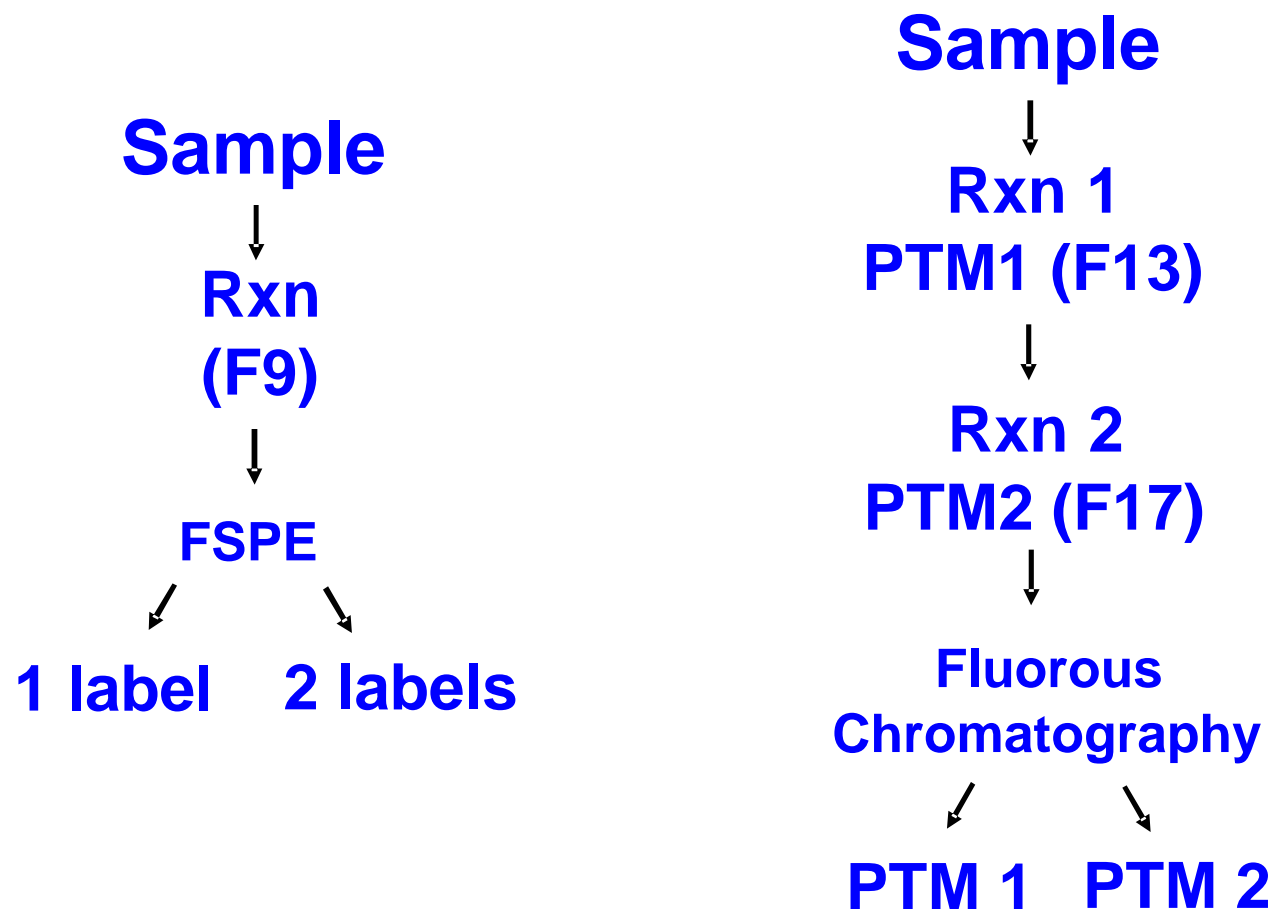
Fluorous β -elimination



Fluorous Phosphopeptide Enrichment

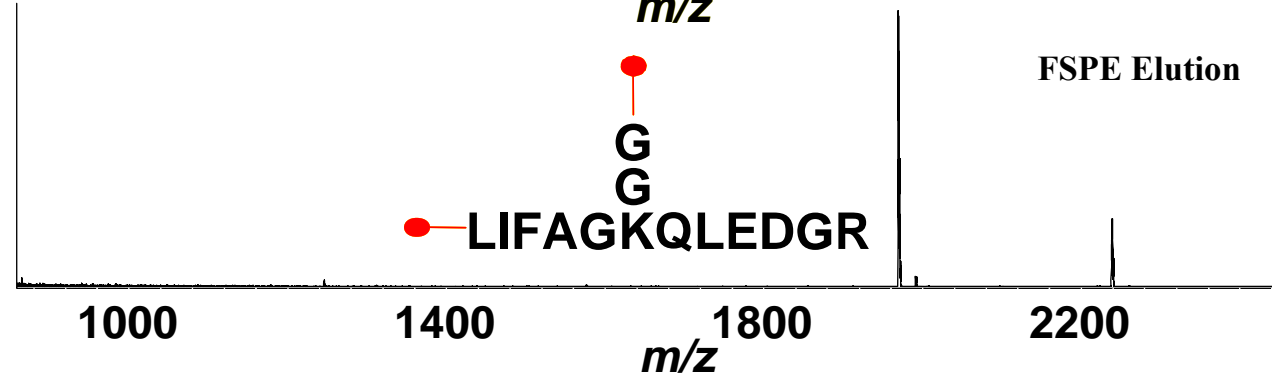
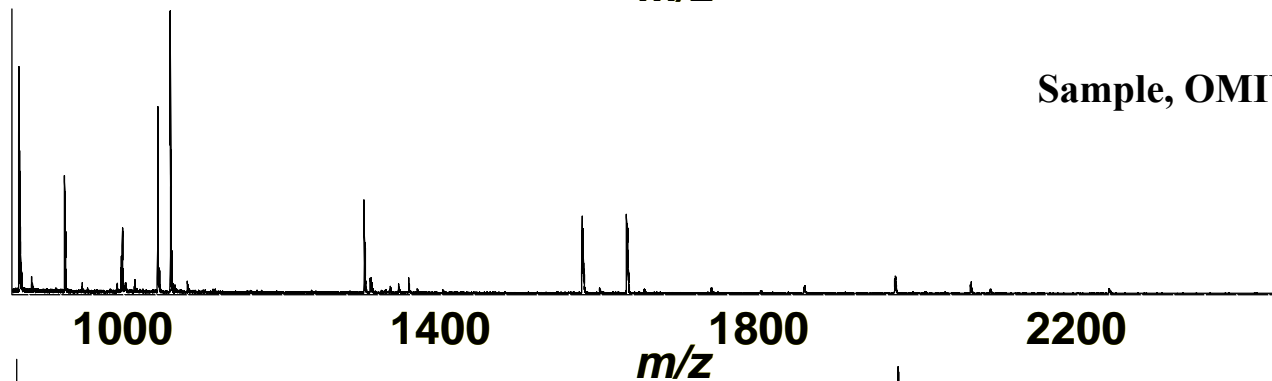
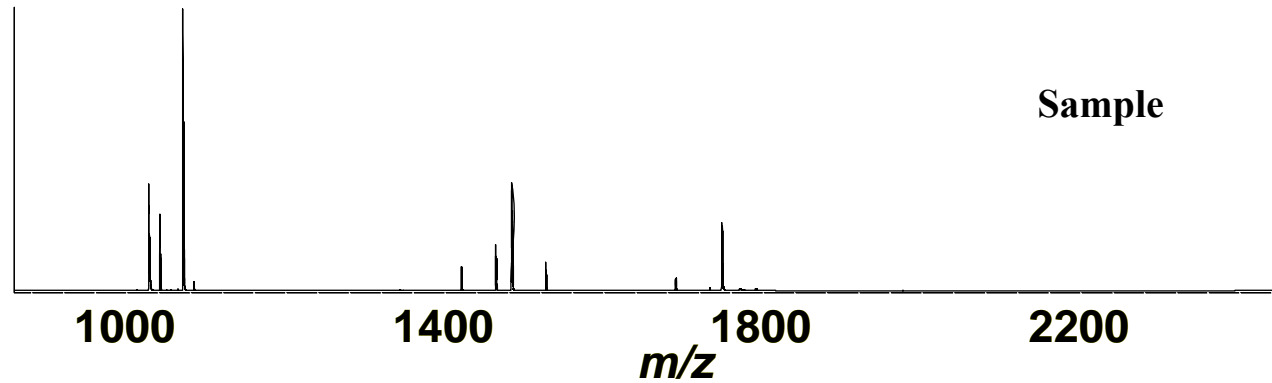
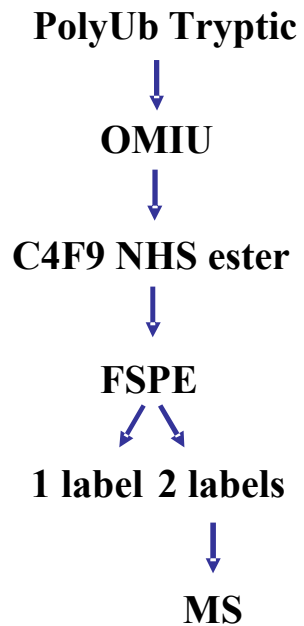


Separations based on fluororous content

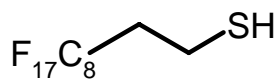


Fluorous tagged strategies can distinguish between singly and multiply labeled species

Fluorous isolation of ubiquitin peptides

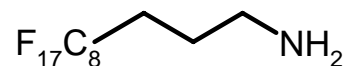


Fluorous Products Available through Sigma-Aldrich



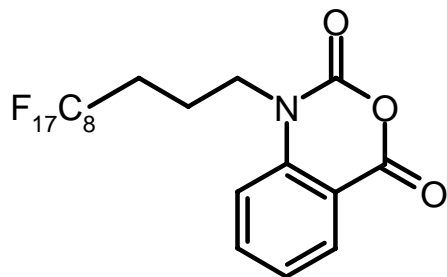
f-thiol

nucleophilic
scavenger



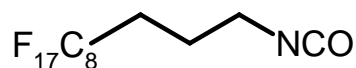
f-amine

nucleophilic
scavenger



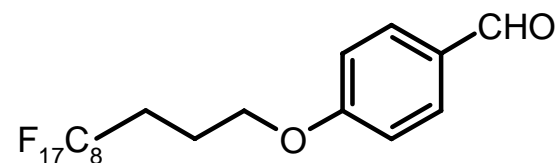
f-isatoic anhydride

electrophilic
scavenger



f-isocyanate

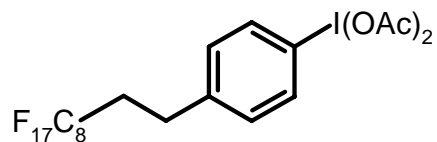
electrophilic
scavenger



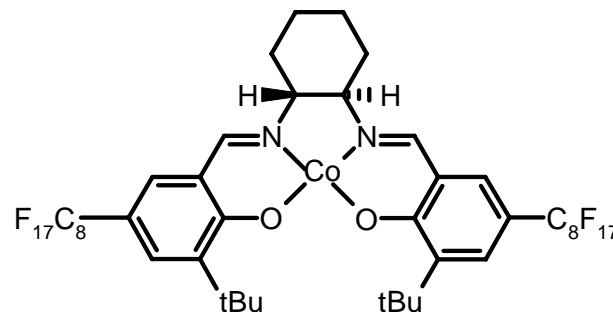
f-benzaldehyde

amine
scavenger

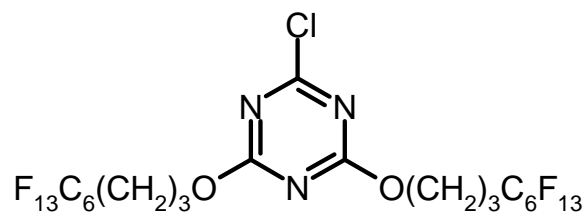
Fluorous Synthesis Reagents



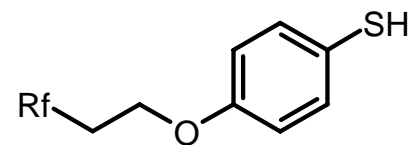
f-DAIB
(oxidations)



Co(f-salen)
(oxidations)

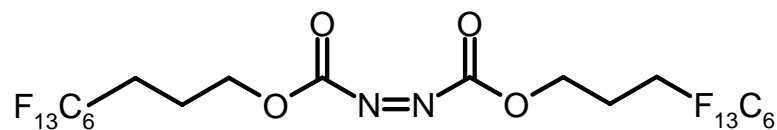


f-CDMT
(amide coupling)



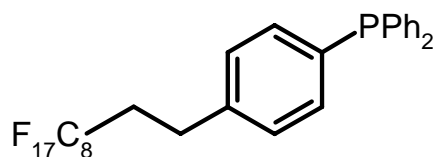
Rf = C₆F₁₃ or C₈F₁₇

FluoMar
(amide coupling)



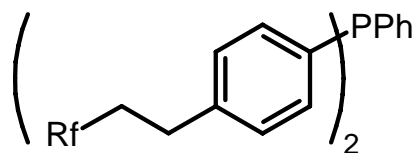
f-DIAD
(Mitsunobu chemistry)

Fluorous Phosphine Reagents



single chain f-TPP

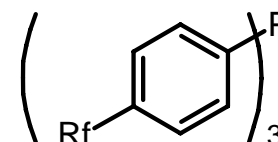
(Mitsunobu and other applications,
purification by FSPE)



Rf = C₆F₁₃ or C₈F₁₇

double chain f-TPP

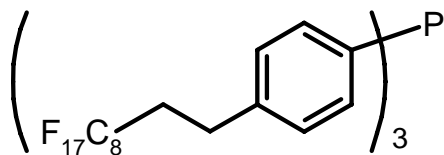
(purification by FSPE or
liquid-liquid extraction)



Rf = C₆F₁₃ or C₈F₁₇

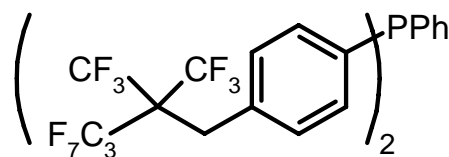
triple chain f-TPP

(purification by liquid-liquid extraction)



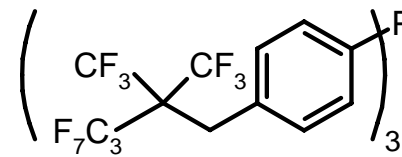
triple chain f-TPP

(purification by liquid-liquid extraction)



double branched chain f-TPP

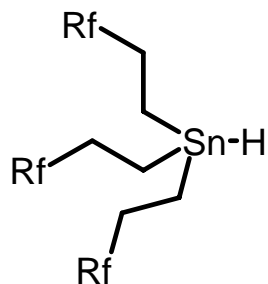
(Wittig chemistry, purification by
FSPE or liquid-liquid extraction)



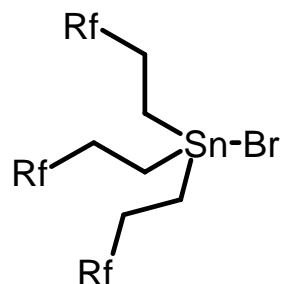
triple branched chain f-TPP

(Wittig chemistry, purification by
liquid-liquid extraction)

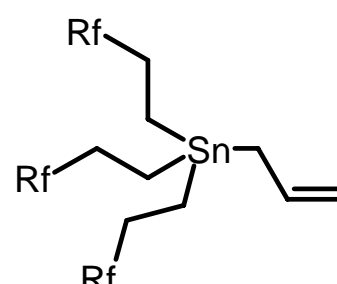
Fluorous Tin Reagents



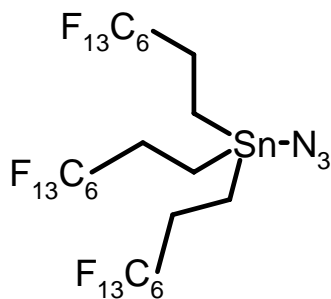
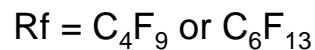
f-tin hydride
(reductions &
free radical reactions)



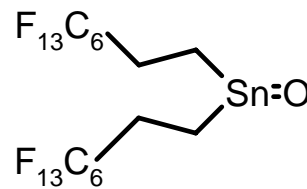
f-tin hydride
(stannylating reagent)



f-tin hydride
(allylating reagent)

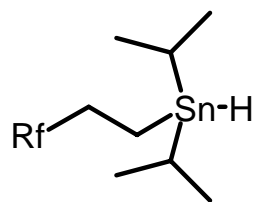


f-tin azide
(azide reagent)

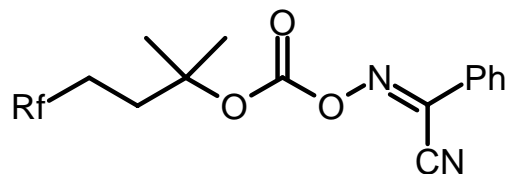


f-tin oxide
(acylation and sulfonylation
catalyst)

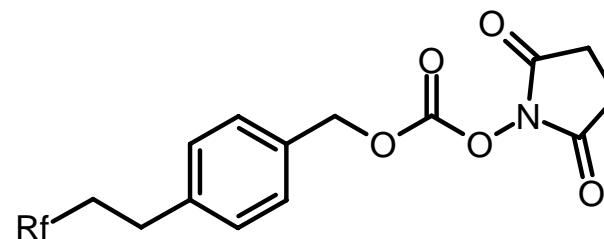
Fluorous Tags for Parallel Synthesis and FMS



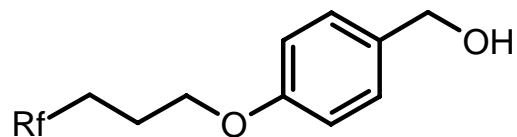
f-silane



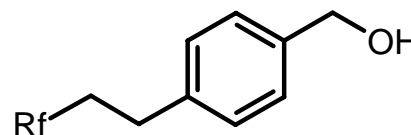
f-Boc-ON



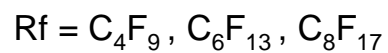
f-Cbz-OSu



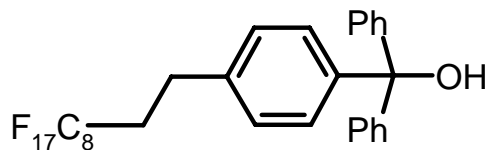
f-PMB-OH



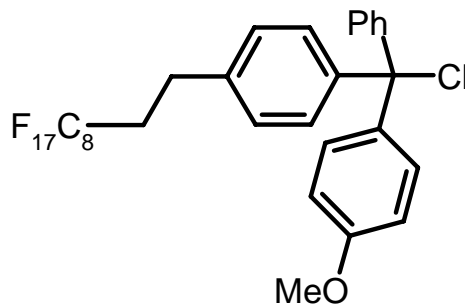
f-Benzyl-OH



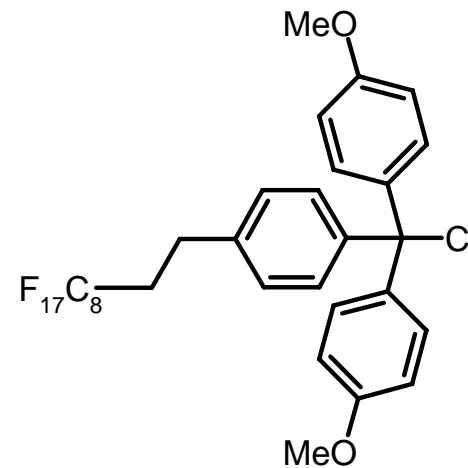
Fluorous Tags for Oligomer Synthesis



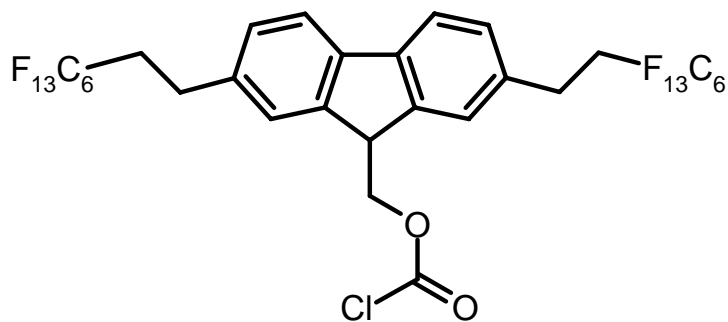
f-trityl-OH



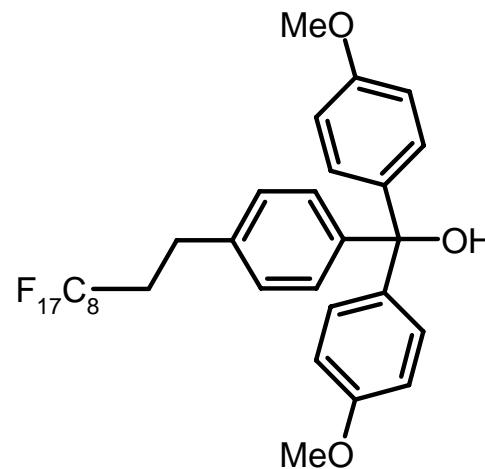
f-MMT-Cl



f-DMT-Cl

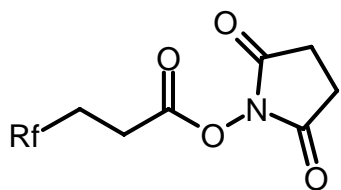


f-Fmoc-Cl

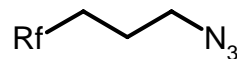


f-DMT-OH

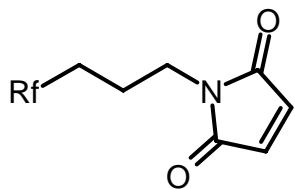
Fluorous Proteomics Reagents



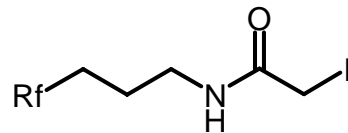
amine reactive tag



click chemistry tag

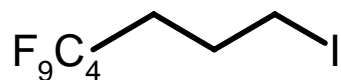


Cys reactive tag

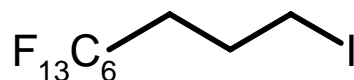


Cys reactive tag

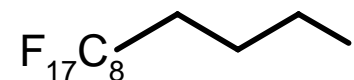
Rf = C₆F₁₃ or C₈F₁₇



Rf₄ propyl iodide



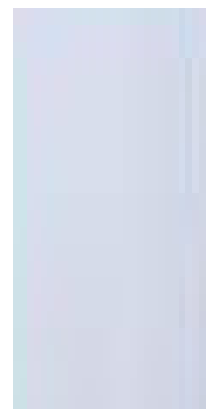
Rf₆ propyl iodide



Rf₈ propyl iodide



FSPE cartridges



F-TLC plates



Bulk fluorinated silica gel