An introduction to *SPOS*: Supports, linkers, and analytical methods for ***S***olid ***P***hase ***O***rganic ***S***ynthesis

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Key sources of information

- WWW
  - Diversity information pages [http://www.5z.com/divinfo/].
  - Unofficial combinatorial chemistry website [http://www.combinatorial.com/].
- Books
  - *The combinatorial index*, B.A.Bunin, AP, SanDiego, **1998**. (this is updated regularly on the www at: [http://www.combinatorial.com/]).
- Reviews
  - 'Preparation, structure and morphology of polymer supports', D.C.Sherrington, *Chem. Commun.* **1998**, 2275.
  - 'Linkers for solid phase organic synthesis', I.W.James, *Tetrahedron*, **1999**, *55*, 4855.
  - 'Solid phase organic reactions III-a review of the literature Nov96-Dec97', P.H.H.Hermkens *et al. Tetrahedron*, **1998**, *54*, 15385, & previous reviews in series.
  - 'Solid phase synthesis', A.R.Brown *et al. Synlett*, **1998**, 817.

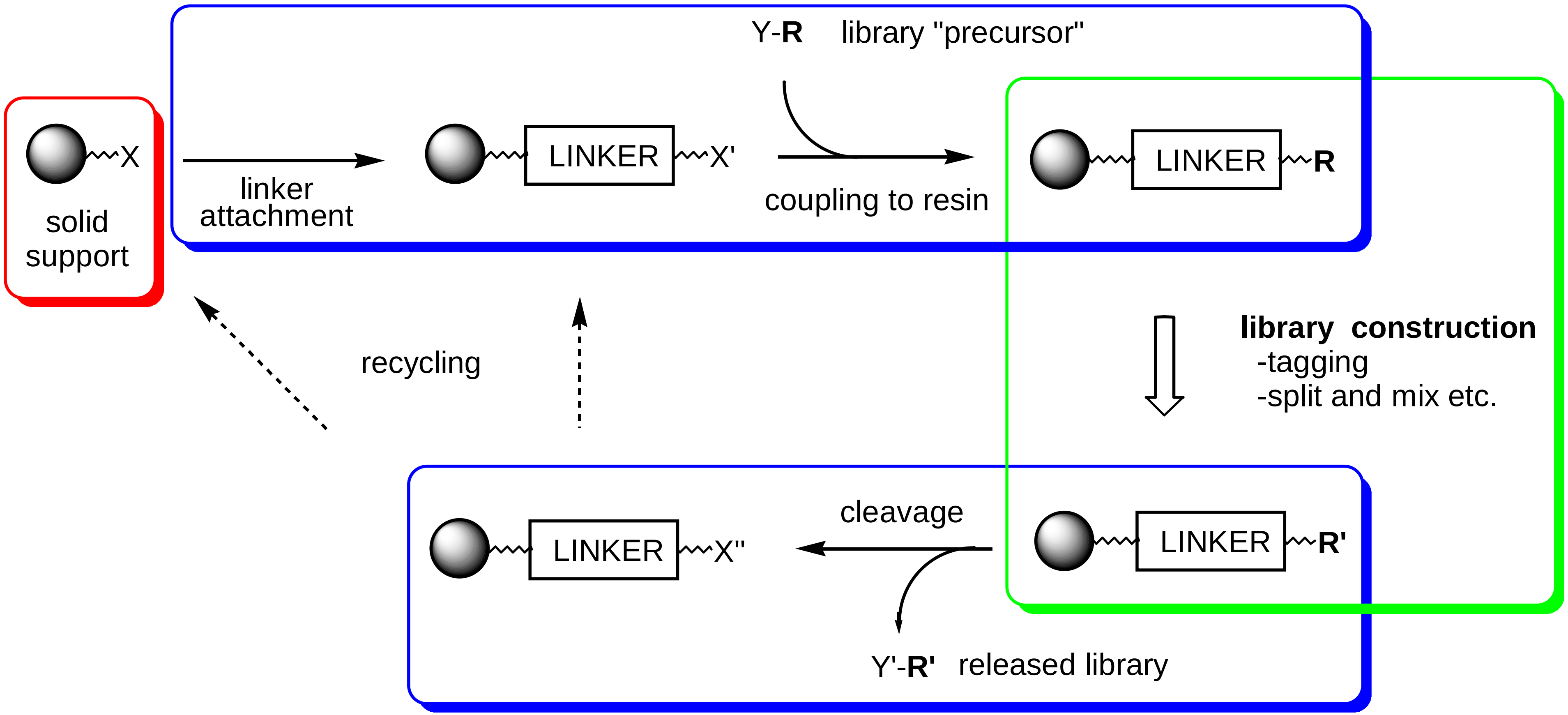
Format and scope of lecture

- What is *SPOS*?
- Why use *SPOS*?
- Types of solid support for *SPOS*: resins.
- Practicalities: working with resins.
- Getting molecules on and off resins: linkers.
- Monitoring reactions on resins.

What is ***S***olid ***P***hase ***O***rganic ***S***ynthesis (*SPOS*)?

- *SPOS* is the practice of organic synthesis on molecules which are covalently attached to an insoluble polymer which swells in the presence of the reaction solvent.
- It was pioneered for peptide synthesis by Bruce Merrifield in 1963 but has only recently been extended to ‘small molecule’ synthesis and particularly drug discovery.

Solid ***P***hase ***O***rganic ***S***ynthesis: overview



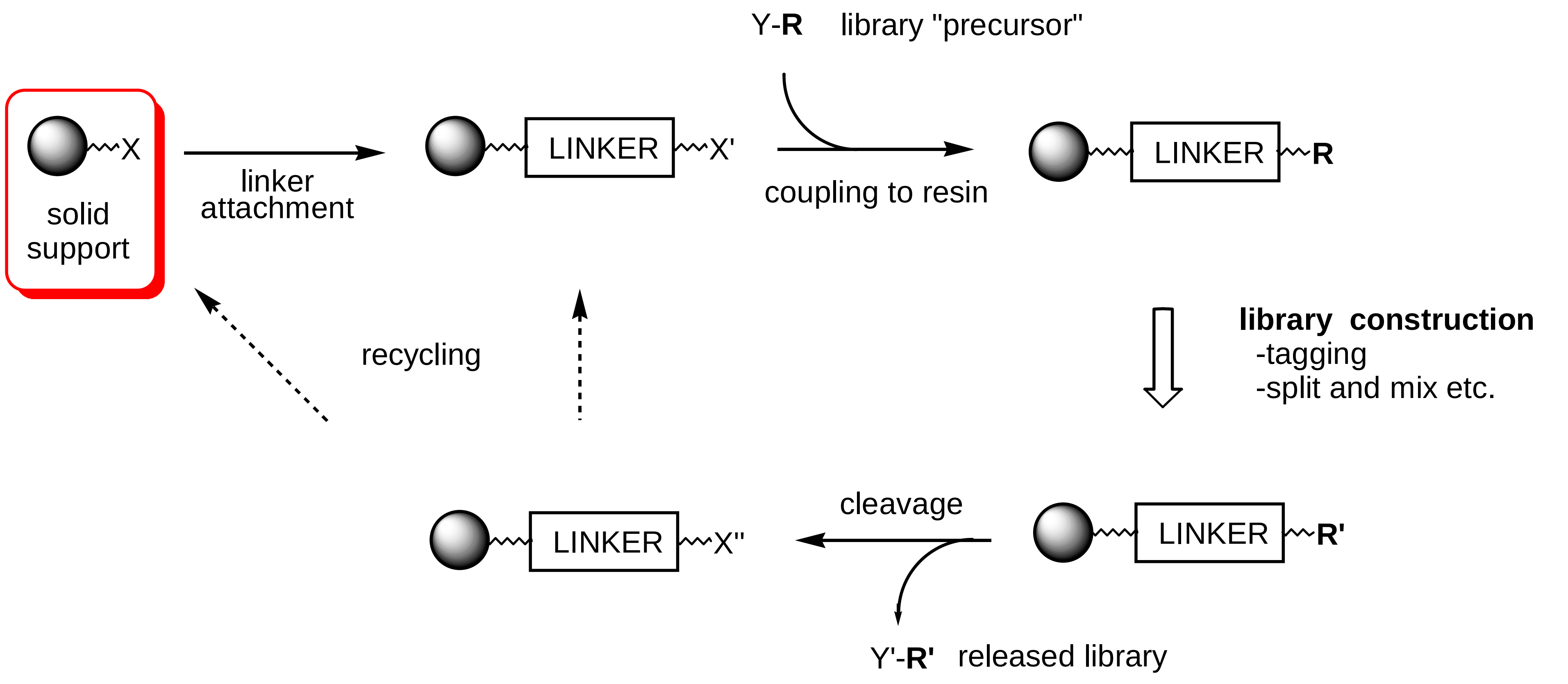
Advantages of *SPOS* relative to solution synthesis

- Reagents can be used in excess without subsequent separation problems and so reactions can be driven to completion.
- Ease of purification of support bound product by washing.
- Ease of automation of reaction sequences.
- Site isolation/pseudo-dilution effects.

Disadvantages of *SPOS* relative to solution synthesis

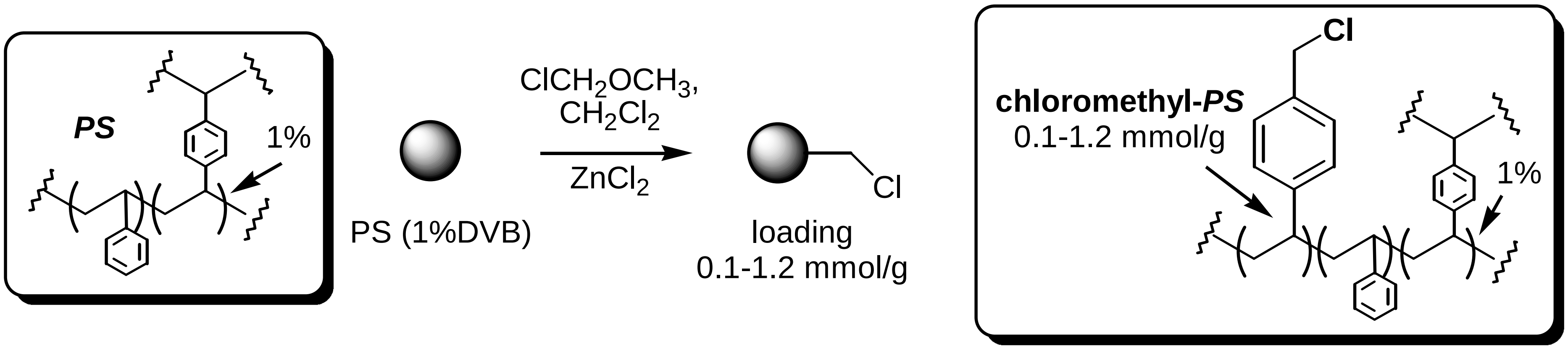
- Additional reaction steps for linkage to and cleavage from support required.
- Support and/or linker may limit the range of possible chemistry (heterogeneity, solvation problems).
- A relatively new technique, therefore expensive in terms of development time.
- Scale-up to produce large quantities of product impractical & expensive.
- Methods for analytical monitoring of reactions not well developed (esp. ‘real time’).

Solid ***P***hase ***O***rganic ***S***ynthesis: types of solid support

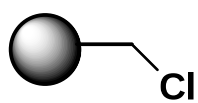


Polystyrene (*PS*)

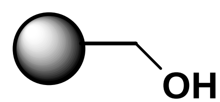
- **1-5% divinylbenzene (*DVB*) cross-linked *PS*.**
- Chloromethylated derivative introduced by Merrifield in early 1960's for peptide synthesis:



- Relatively high loading (0.1-1.2 mmol/g).
- Acceptable swelling only for limited number of solvents e.g. DMF and CH_2Cl_2.
- Not suitable for continuous flow automation (pores 'shrink' at moderate pressures).
- Hydroxymethyl-*PS*, chloromethyl-*PS*, and linker pre-functionalised-*PS* are all relatively cheap:



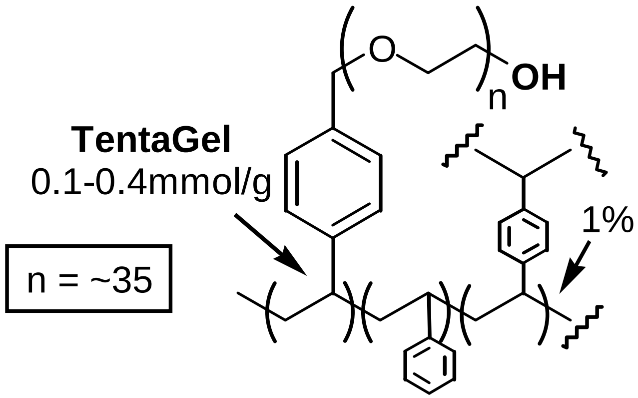
chloromethyl-*PS*

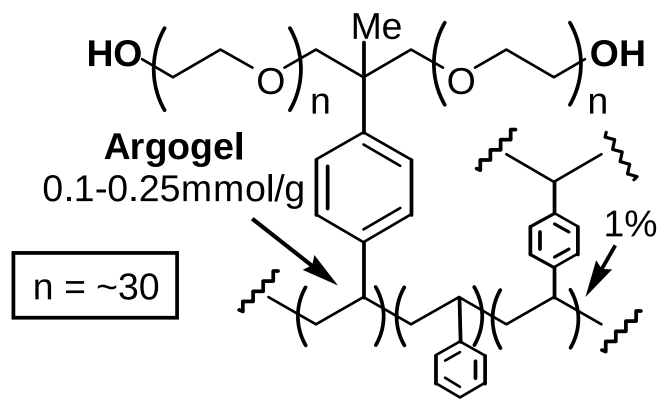


hydroxymethyl-*PS*

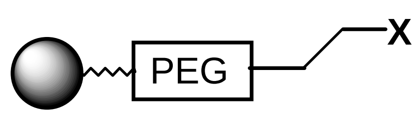
Polyethyleneglycol grafted *PS* (*PEG-PS*)

- ***PS* (1% *DVB*) grafted to *PEG*.**
- Common trade names include: TentaGel & Argogel (both contain ~70% *PEG* by weight):





- Relatively low loading (0.1-0.5 mmol/g).
- Good swelling over wide range of solvents including THF, CH_3CN, water, MeOH.
- Suitable for continuous flow automation.
- Hydroxyethyl-*PEG-PS*, bromoethyl-*PEG-PS*, aminoethyl-*PEG-PS*, and linker pre-functionalised-*PEG-PS* are all commercially available at a price!

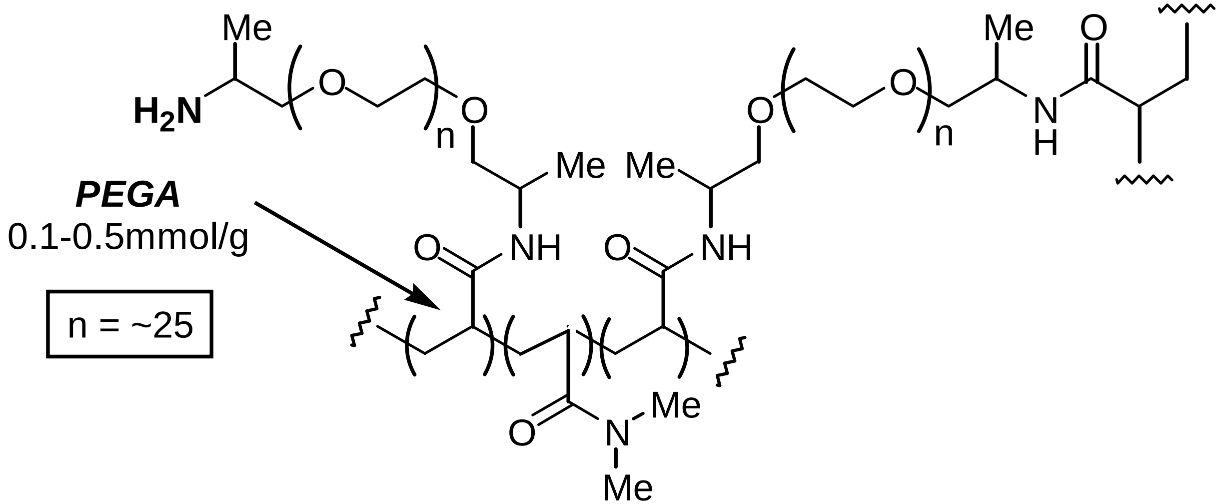


PS (1%DVB)

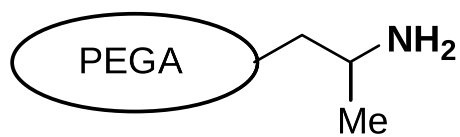
X = OH hydroxyethyl-PEG-PS
X = Br bromoethyl-PEG-PS
X = NH_2 aminoethyl-PEG-PS

Acrylamidopropyl-*PEG* (*PEGA*)

– **Acrylamidopropyl-*PEG-N,N*-dimethylacrylamide:**



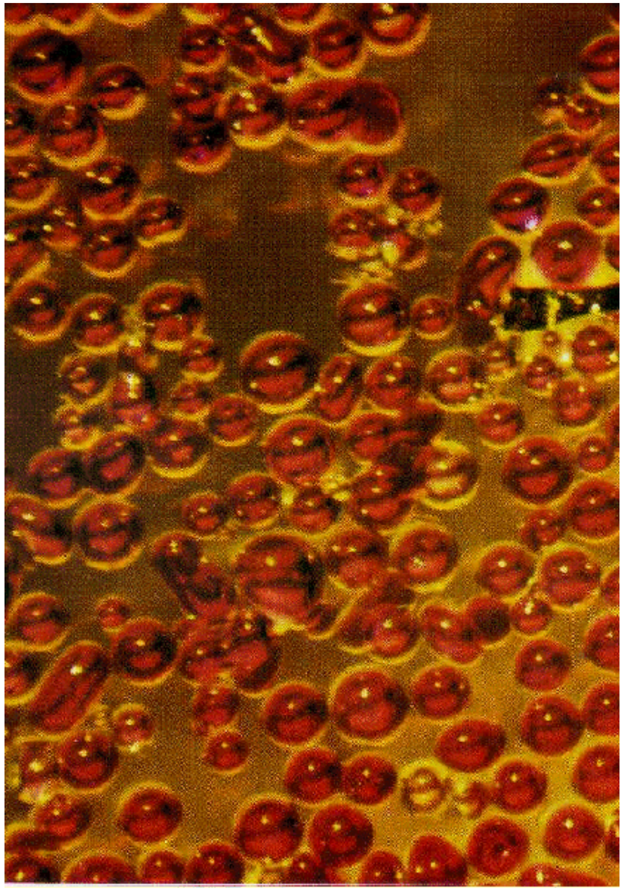
– Good swelling properties with polar solvents.
– No internal chromophores therefore excellent for on-bead colormetric assaying.
– Suitable for continuous flow peptide synthesis.
– Relatively low loading (0.1-0.5 mmol/g).
– Commercially available amino-functionalised:



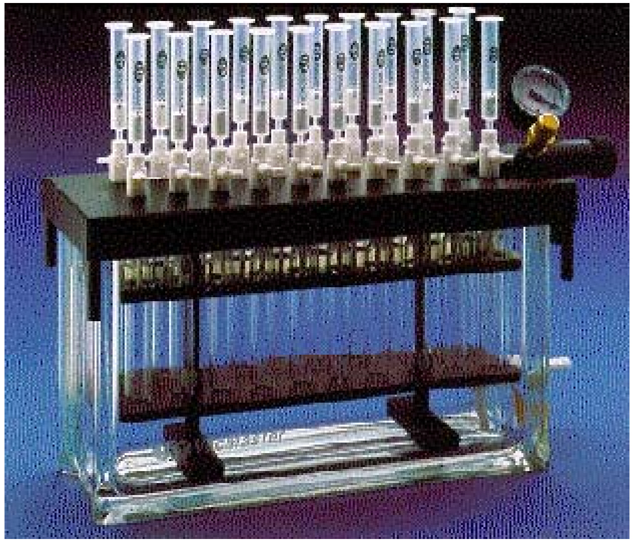
Other supports for *SPOS*...

- Kieselguhr-polyamide (Pepsyn)
  - Inorganic matrix with fixed pore size grafted to polyamide.
  - Low loading and poor mechanical stability.
  - Suitable for continuous flow peptide synthesis.
- Controlled pore glass (*CPG*)
  - Inorganic matrix with fixed pore size
  - low loading, poor mechanical stability.
  - Suitable for continuous flow oligonucleotide synthesis.
- Micropins and etched silicon surfaces
  - Polymers grafted to plastic pegs forming an array suitable for dipping into e.g. 96-well microtitre plates.
  - Various proprietry hydroxy-functionalised, spatially addressable surfaces.

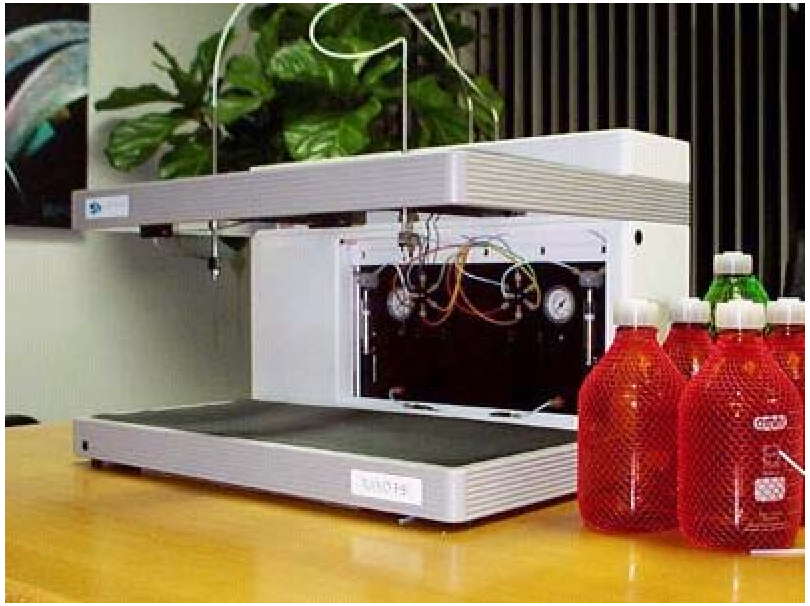
Handling solid support resins...

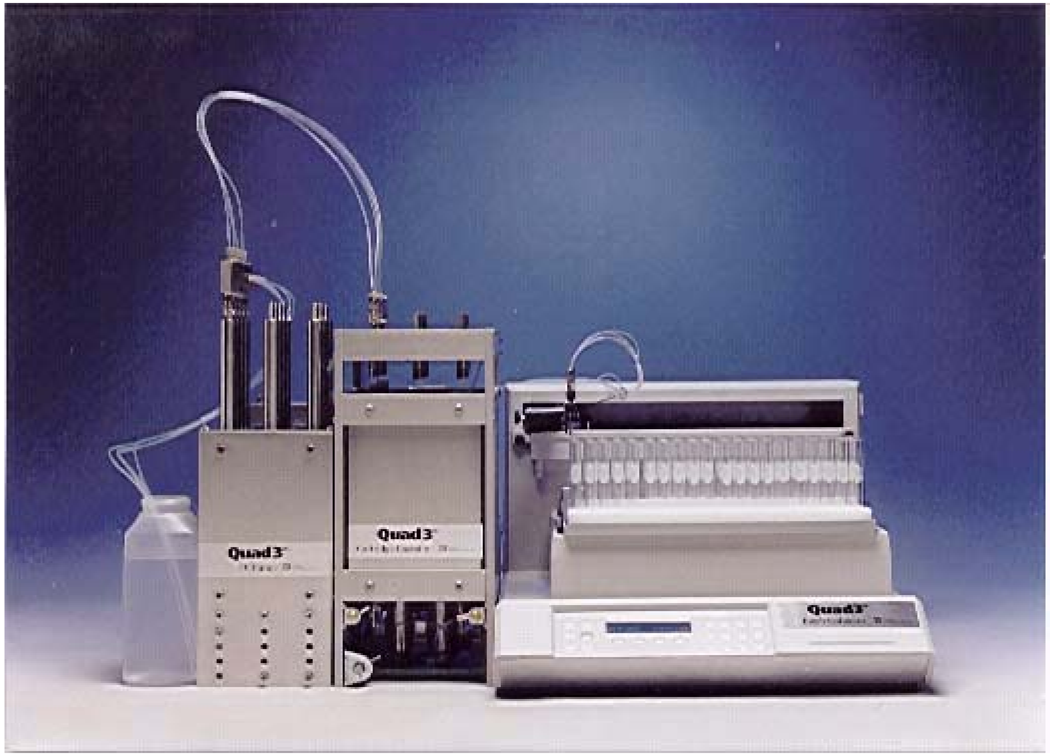


Handling solid support resins...



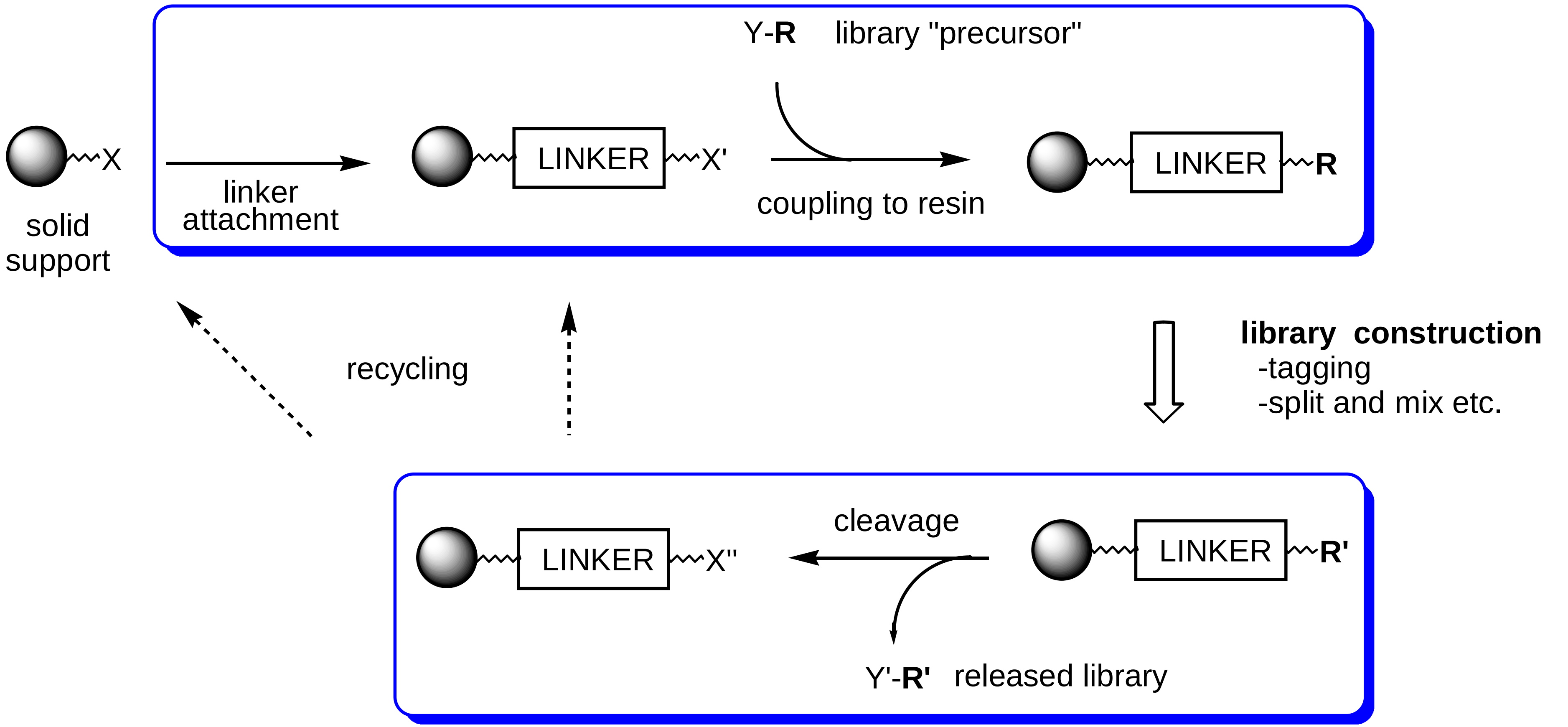
Handling solid support resins...





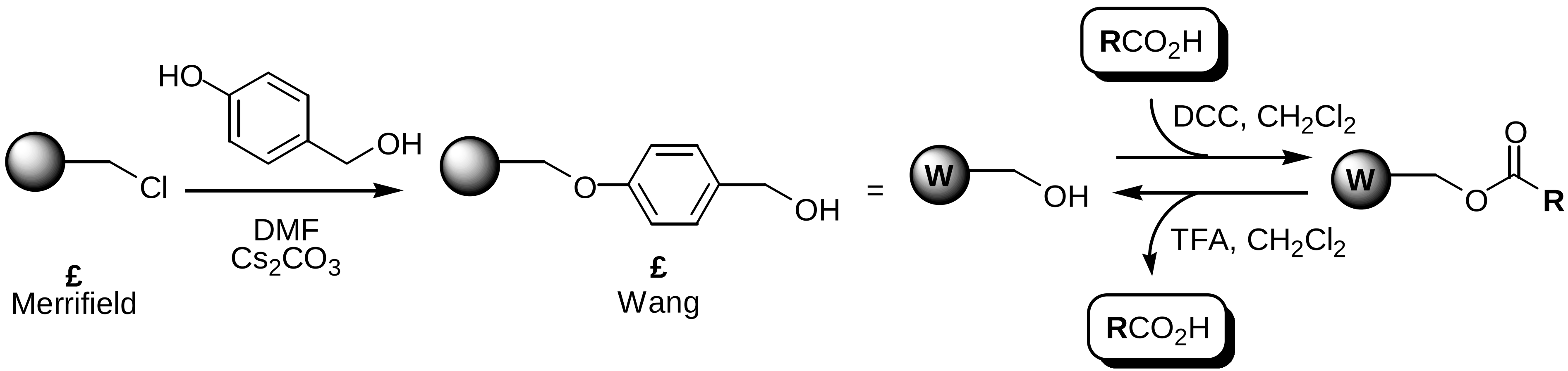
Handling solid support resins...

Solid ***P***hase ***O***rganic ***S***ynthesis: the role of linkers

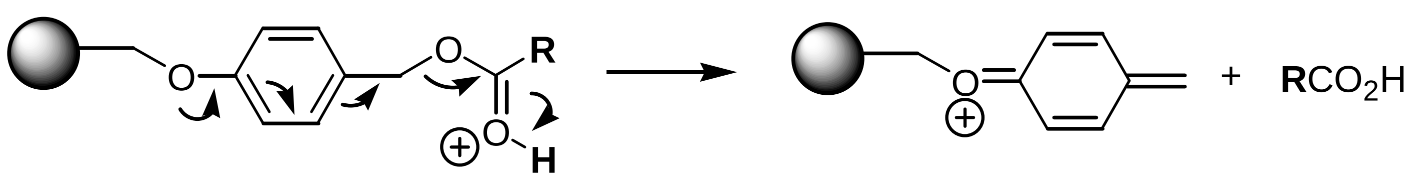


Acid labile carboxylic acid linker

- **Wang ester linker.**
- Wang *J. Am. Chem. Soc.* **1973**, *95*, 1328.
- Cleaved using 50% TFA in CH_2Cl_2 (30 min).

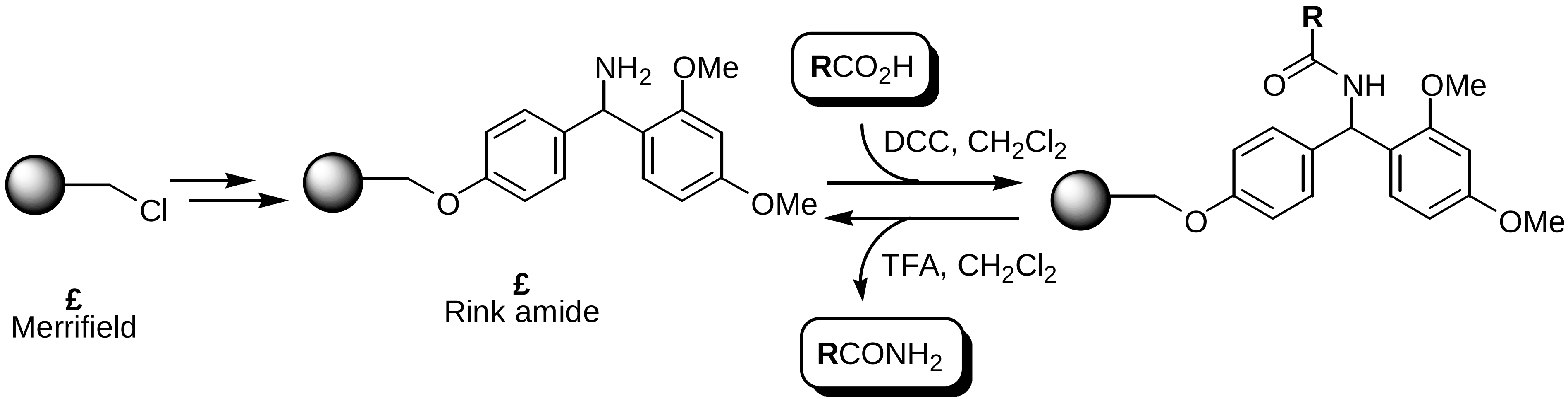


- Mechanism of cleavage:

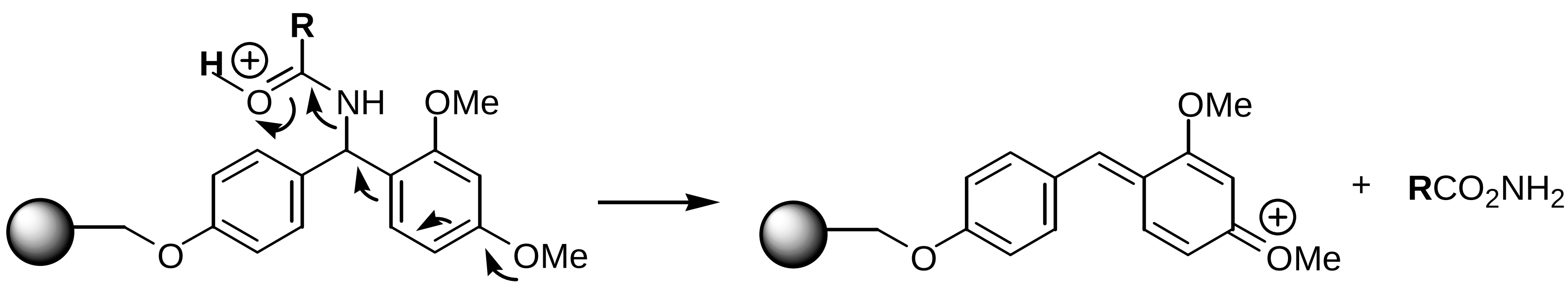


Acid labile amide linker

– **Rink amide linker.**
– Rink *Tet. Lett.* **1987**, *28*, 3787.
– Cleaved using 50% TFA in CH_2Cl_2 (15 min).

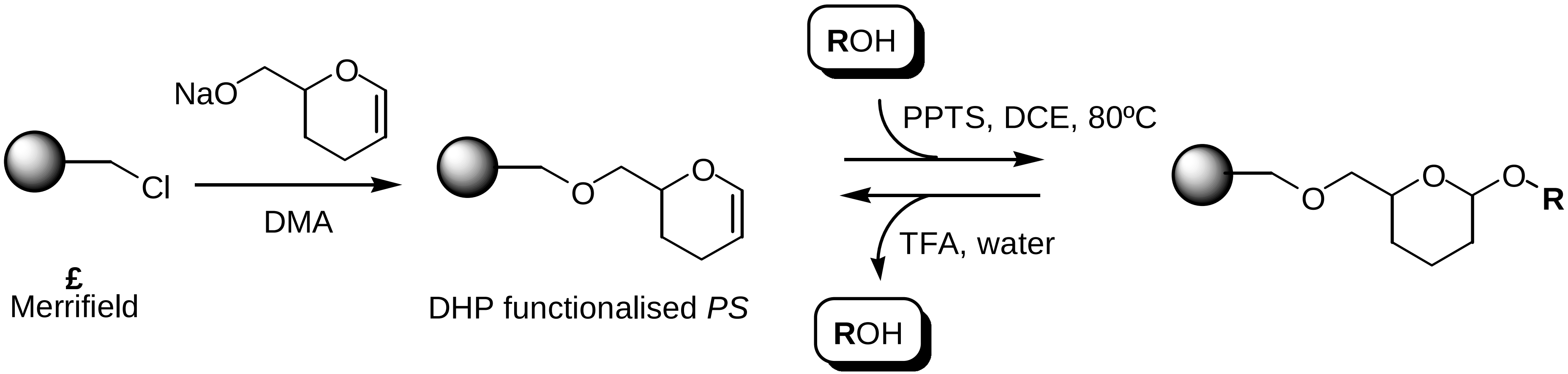


– Mechanism of cleavage:

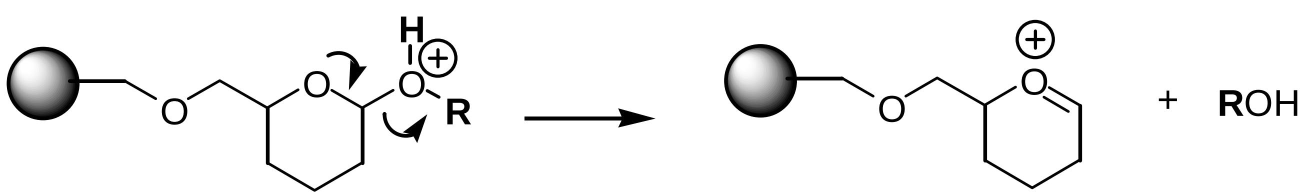


Acid labile alcohol linker

- **Tetrahydropyranyl (*THP*) acetal linker.**
- Ellman *Tet. Lett.* **1994**, *35*, 9333.
- Cleaved using 95% TFA in water.

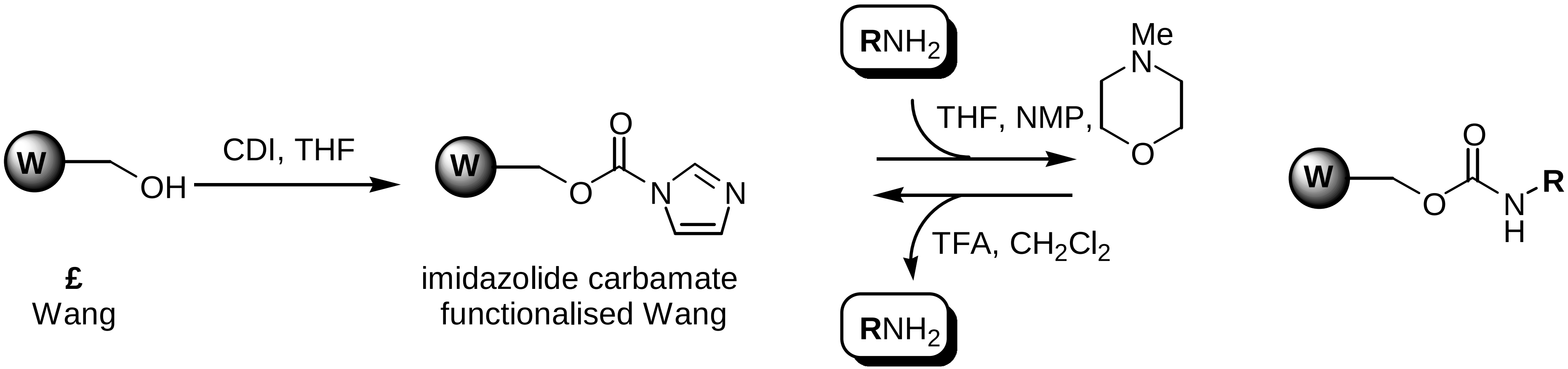


- Mechanism of cleavage:

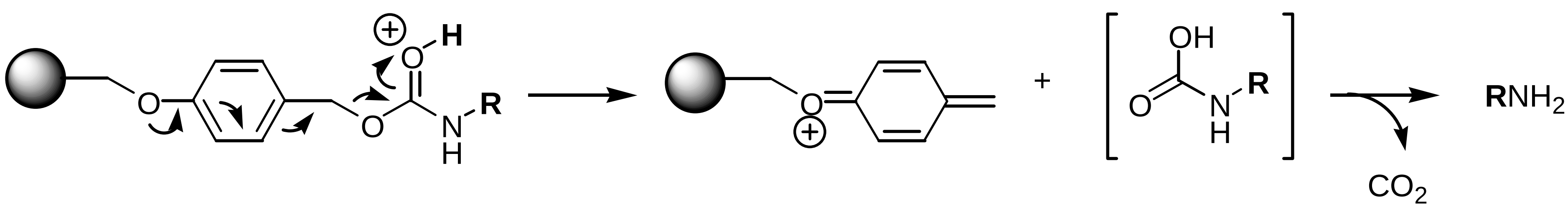


Acid labile amine linker

- **Carbamate linker (solid phase 'Cbz').**
- Rotella *J. Am. Chem. Soc.* **1996**, *118*, 12246.
- Cleaved using 50% TFA in CH_2Cl_2.

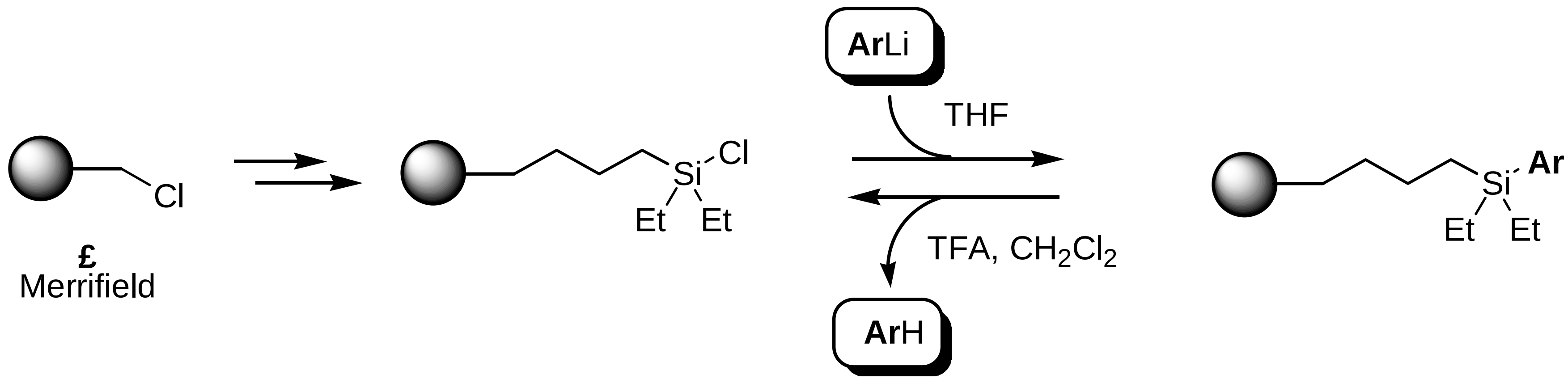


- Can also be cleaved by hydrogenolysis: $Pd(OAc)_2$, H_2 (45psi), DMF.
- Mechanism of cleavage:

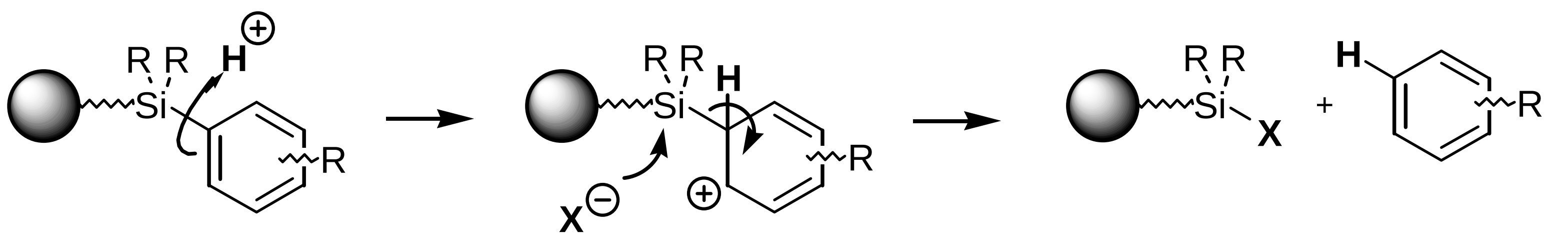


Traceless linker for aromatics

- **Hu's arylsilane linker.**
- Hu *J. Org. Chem.* **1998**, *63*, 4518.
- Cleaved using 50% TFA in CH_2Cl_2 or Xs. TBAF in CH_2Cl_2-THF.



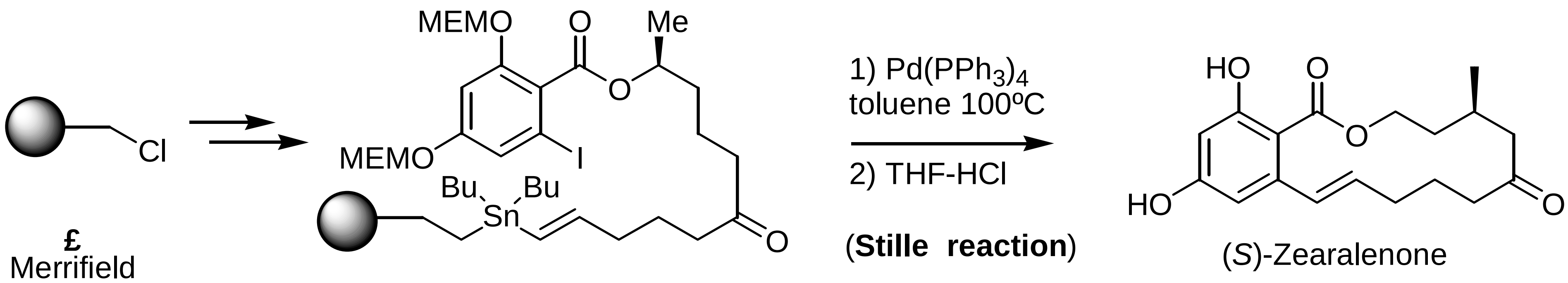
- Mechanism of cleavage:



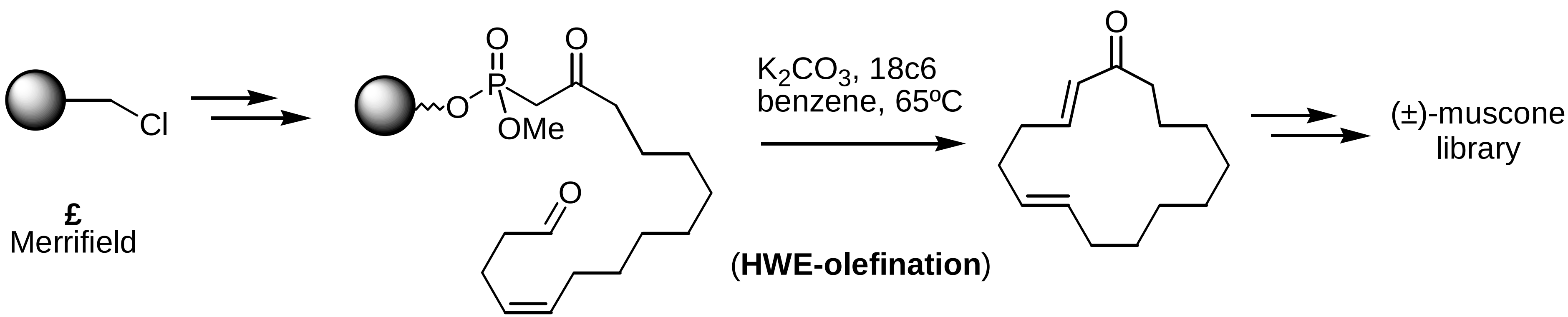
Wheland intermediate
stabilised by β-effect of silicon

Traceless cyclorelease linkers

- **Nicolaou's zearalenone synthesis (vinylstannane linker).**
- Nicolaou *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2534.

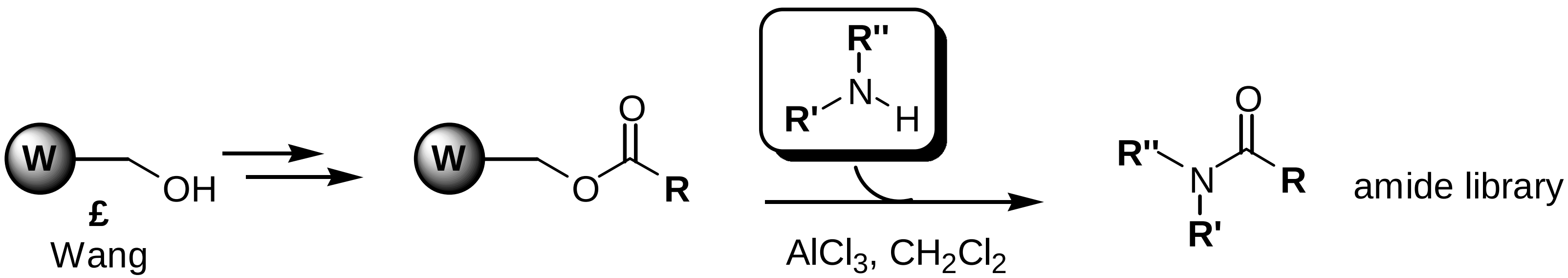


- **Nicolaou's muscone synthesis (ketophosphonate linker).**
- Nicolaou *J. Am. Chem. Soc.* **1998**, *120*, 5132.

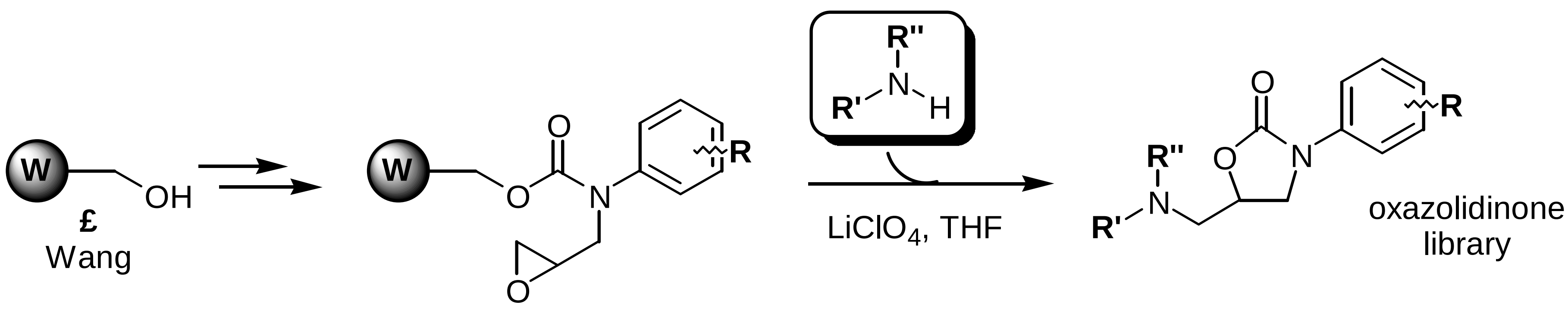


Combined cleavage/diversification

- **Lewis acid promoted cleavage of Wang with amines.**
- Rees *Tet. Lett.* **1996**, *37*, 3213.

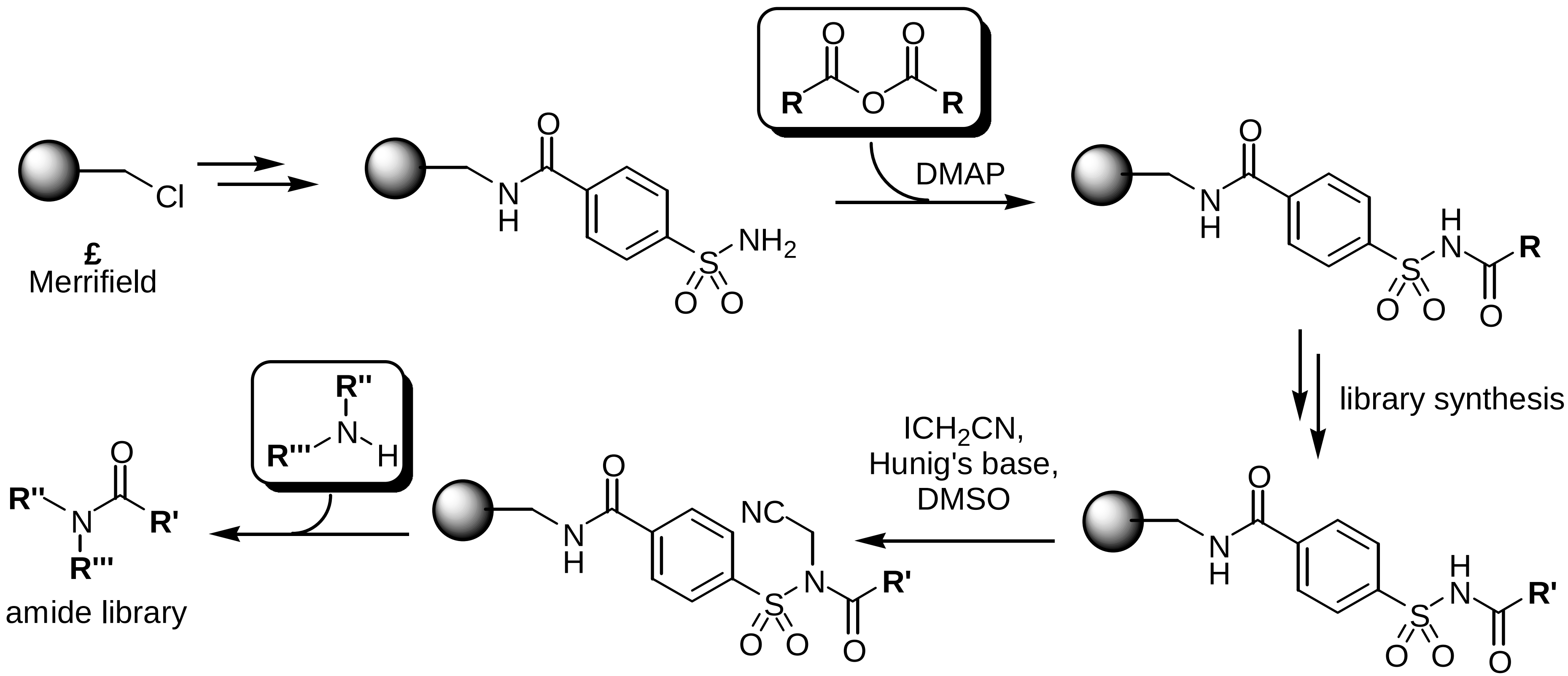


- **Merck's diversification/cyclorelease oxazolidinones.**
- Buchstaller *Tetrahedron* **1998**, *54*, 3465.



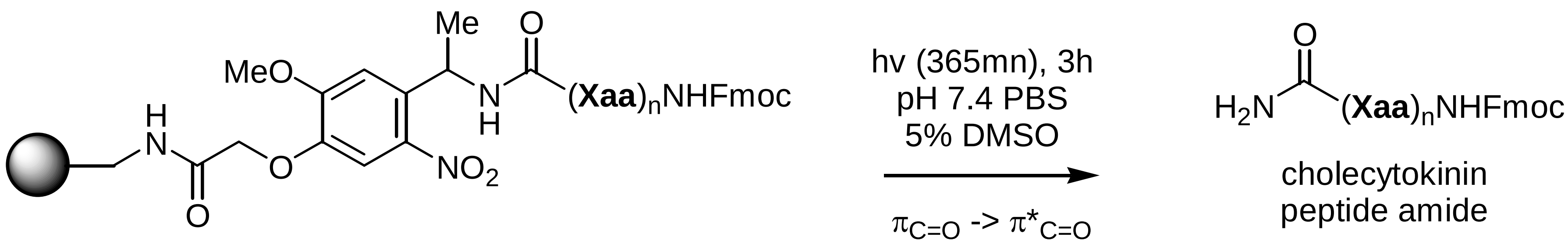
Safety-catch linkers

- **Ellman's acylsulfonamide safety-catch linker.**
- Ellman *J. Am. Chem. Soc.* **1996**, *118*, 3055.
- Completely stable to strongly acidic, basic and nucleophilic agents prior to 'activation' using iodoacetonitrile.

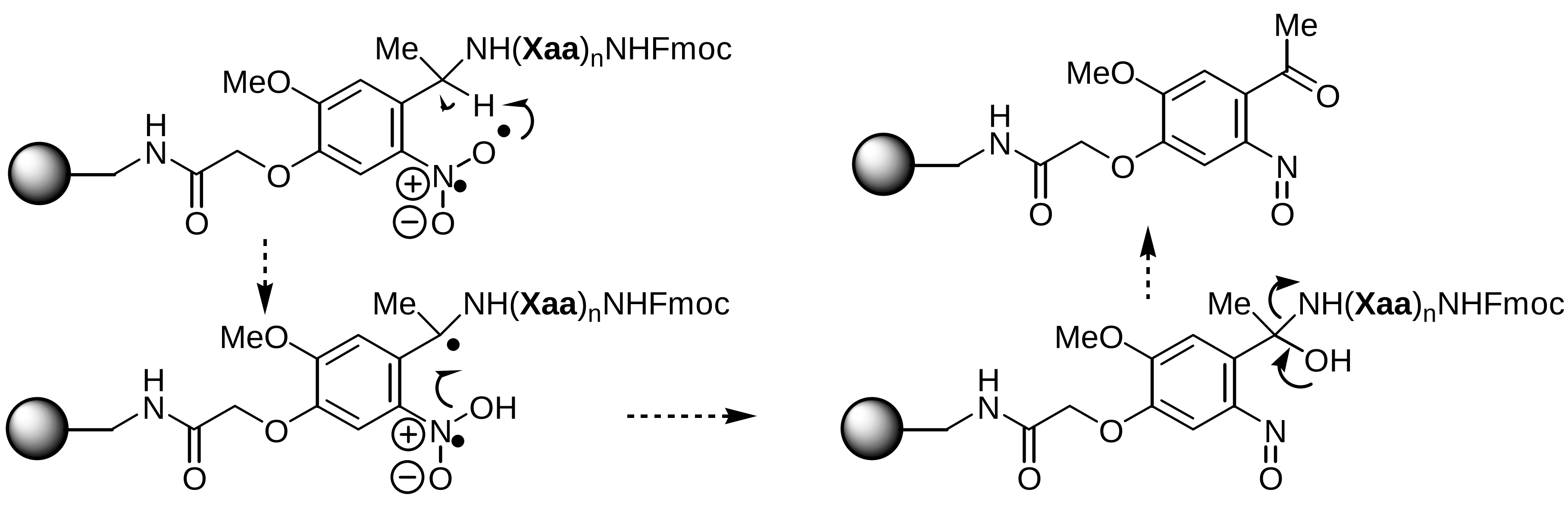


Photolabile linkers

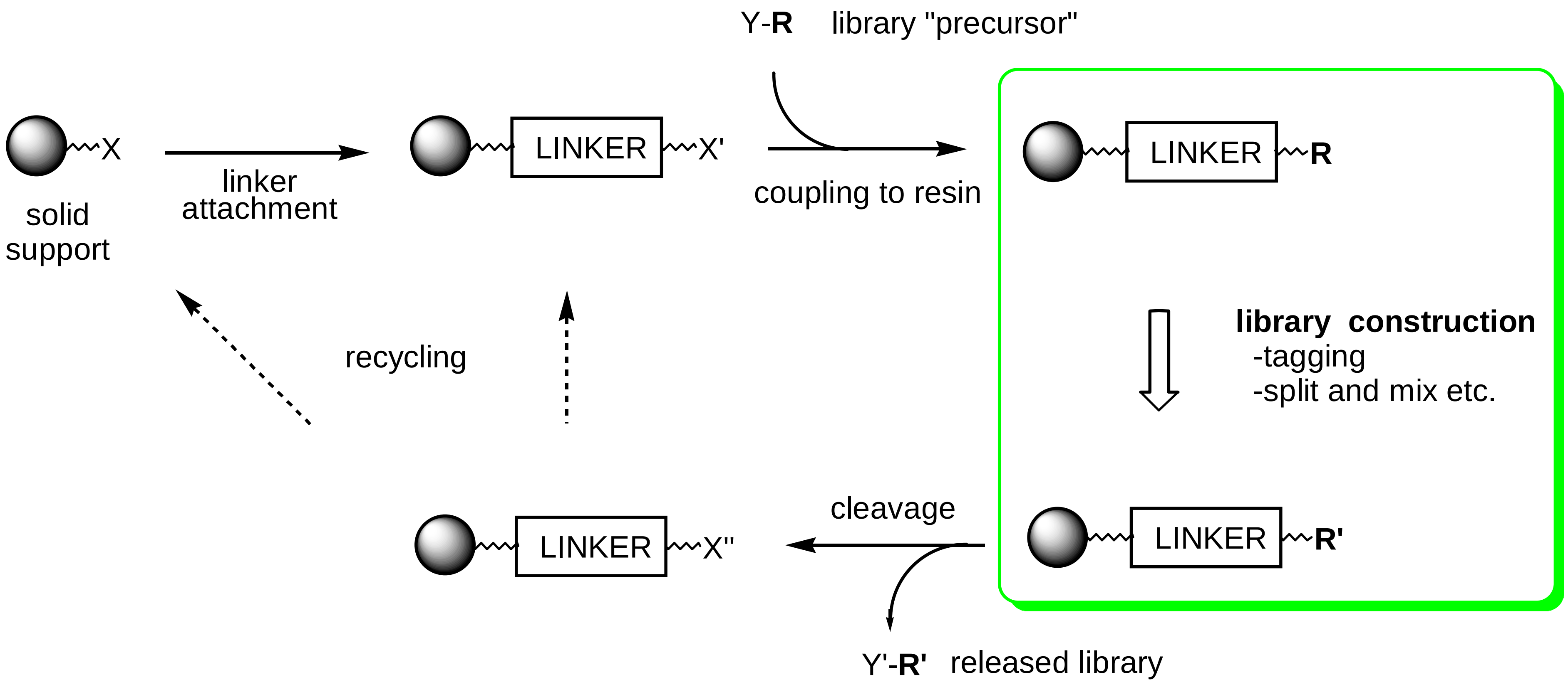
- **Holmes's *o*-nitrobenzyl linker.**
- Holmes *J. Org. Chem.* **1995**, *60*, 2318.
- Cleaved by irradiation with UV light (365nm) in pH 7.4 PBS buffer-5%DMSO.



- Mechanism:



Solid ***P***hase ***O***rganic ***S***ynthesis: monitoring reactions



The problem...

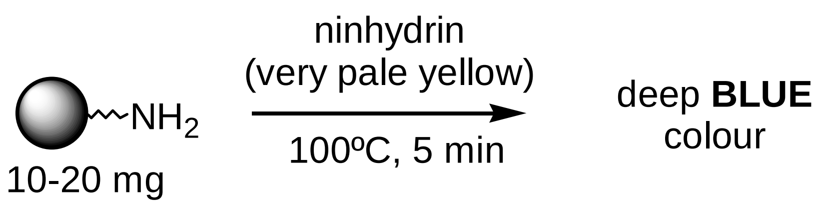
"Doing organic chemistry on solid support has been likened to working with a blindfold on because of the limited analytical techniques available relative to those available in solution."

Bunin, *The Combinatorial Index*, AP, SanDiego, **1998**.

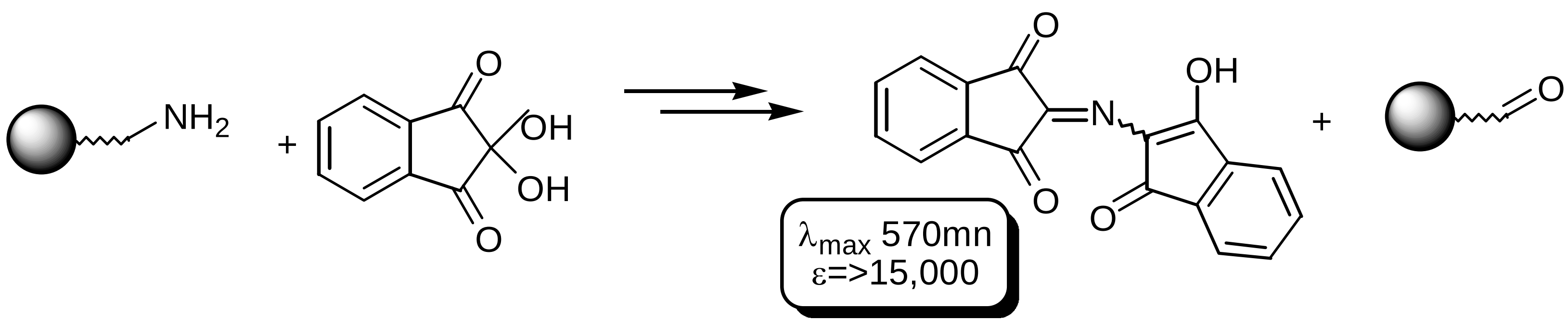
Simple ‘on bead’ chemical tests

- **Simple colour tests are very useful for qualitatively monitoring the course of certain reactions (*cf.* TLC for solution phase reactions):**

- **Ninhydrin** test for primary amines.

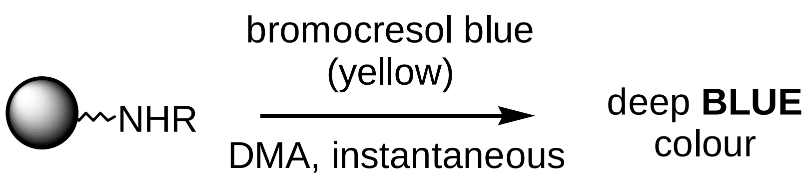


**(destructive
but even 5 μmol/g can be detected)**

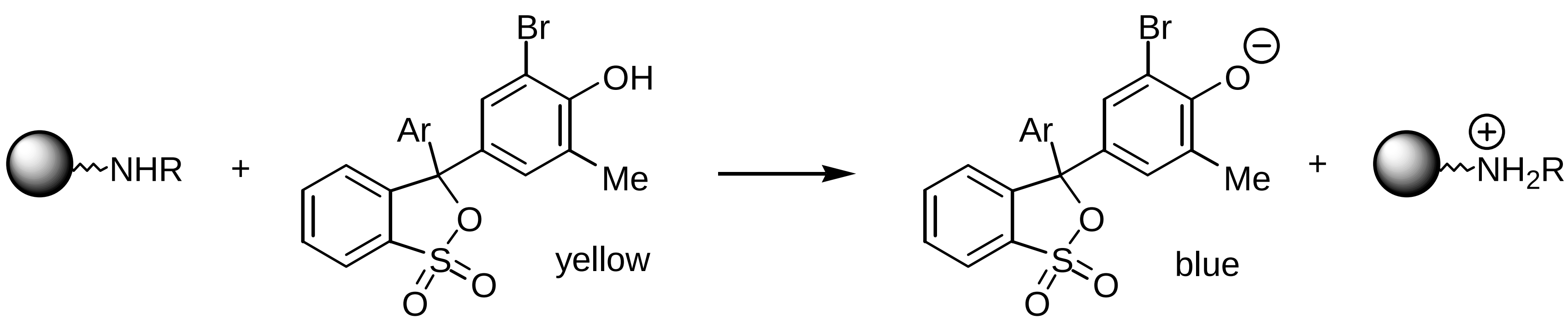


Simple ‘on bead’ chemical tests

– **Bromophenol blue** test for basic secondary and tertiary amines.

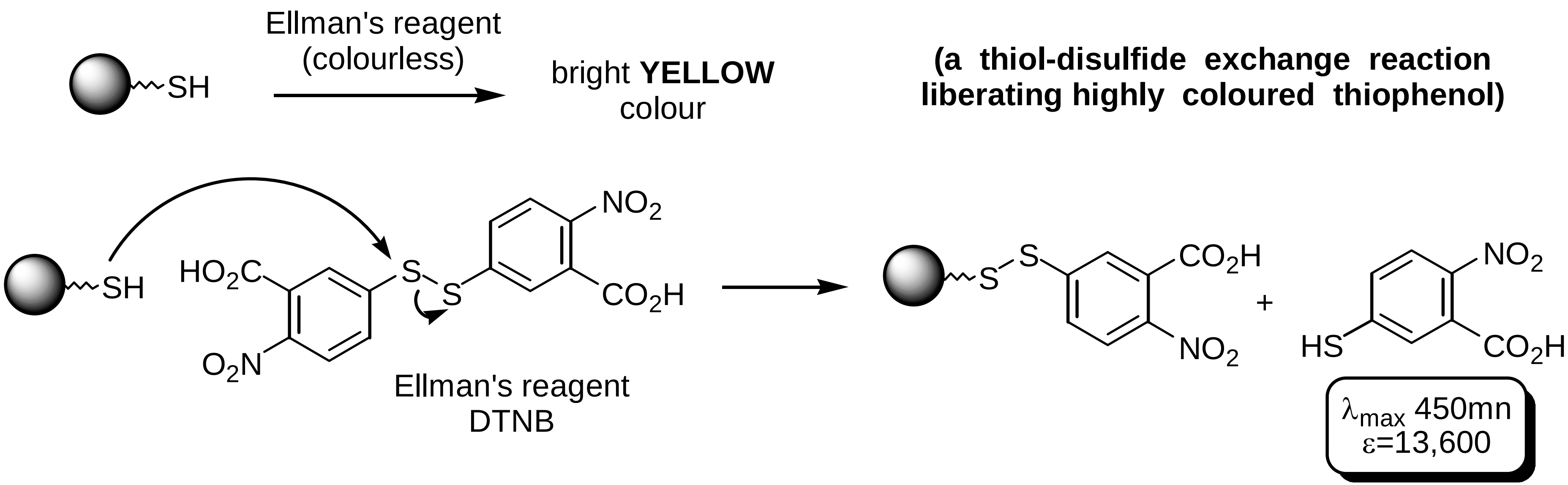


(non-destructive can be washed off and synthesis continued)



Simple ‘on bead’ chemical tests

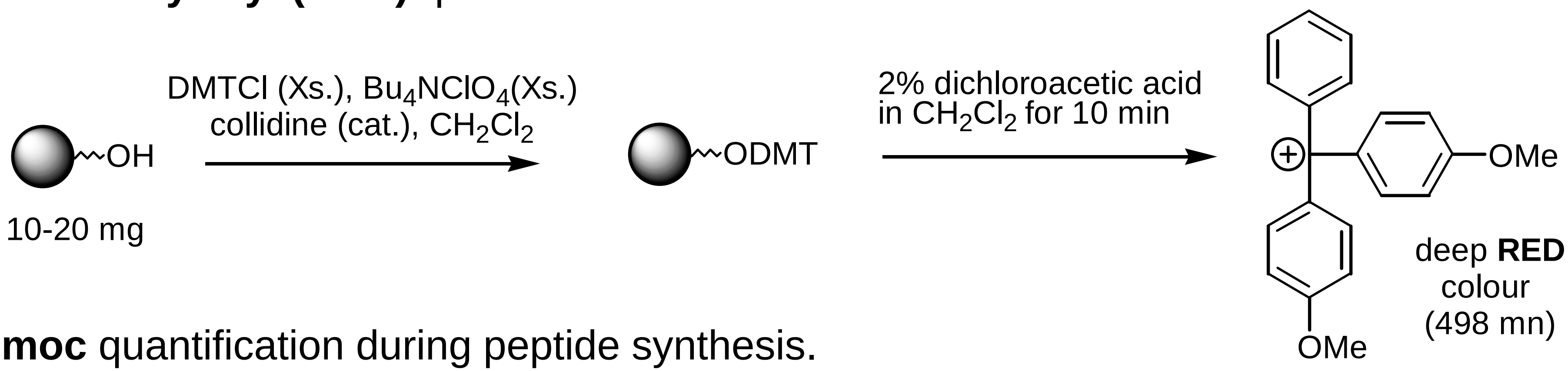
– **Ellman** test for thiols.



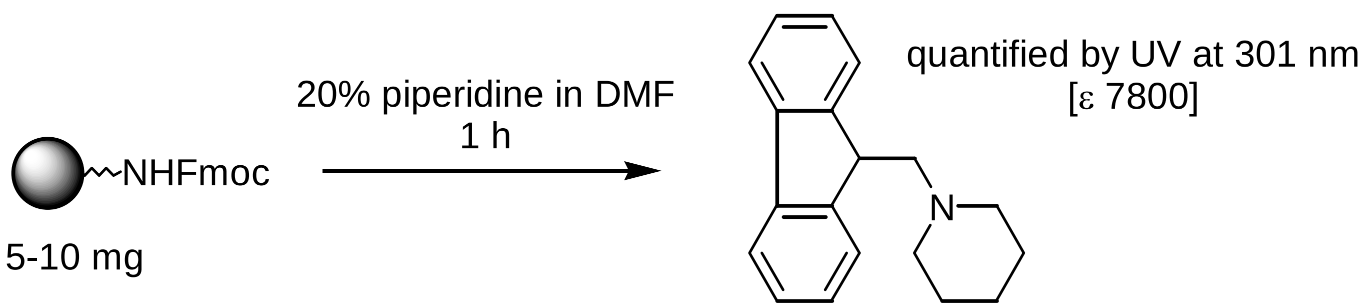
Colorimetric quantification of compounds released from resin

- **Quantitative monitoring of some reactions using UV spectroscopy is possible following cleavage of compounds from small aliquots of resin:**

- **Dimethoxytrityl (DMT)** quantification of free amines or alcohols.

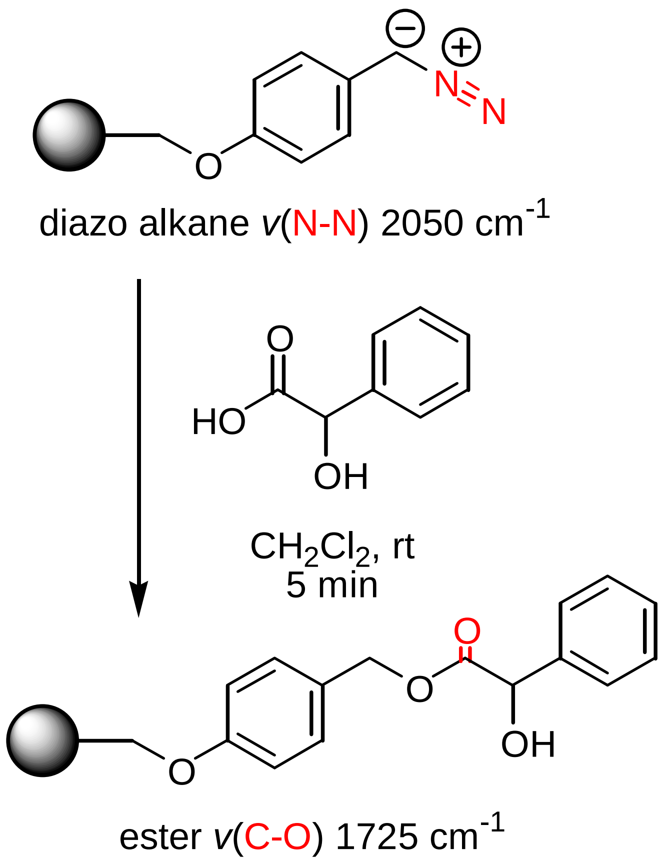


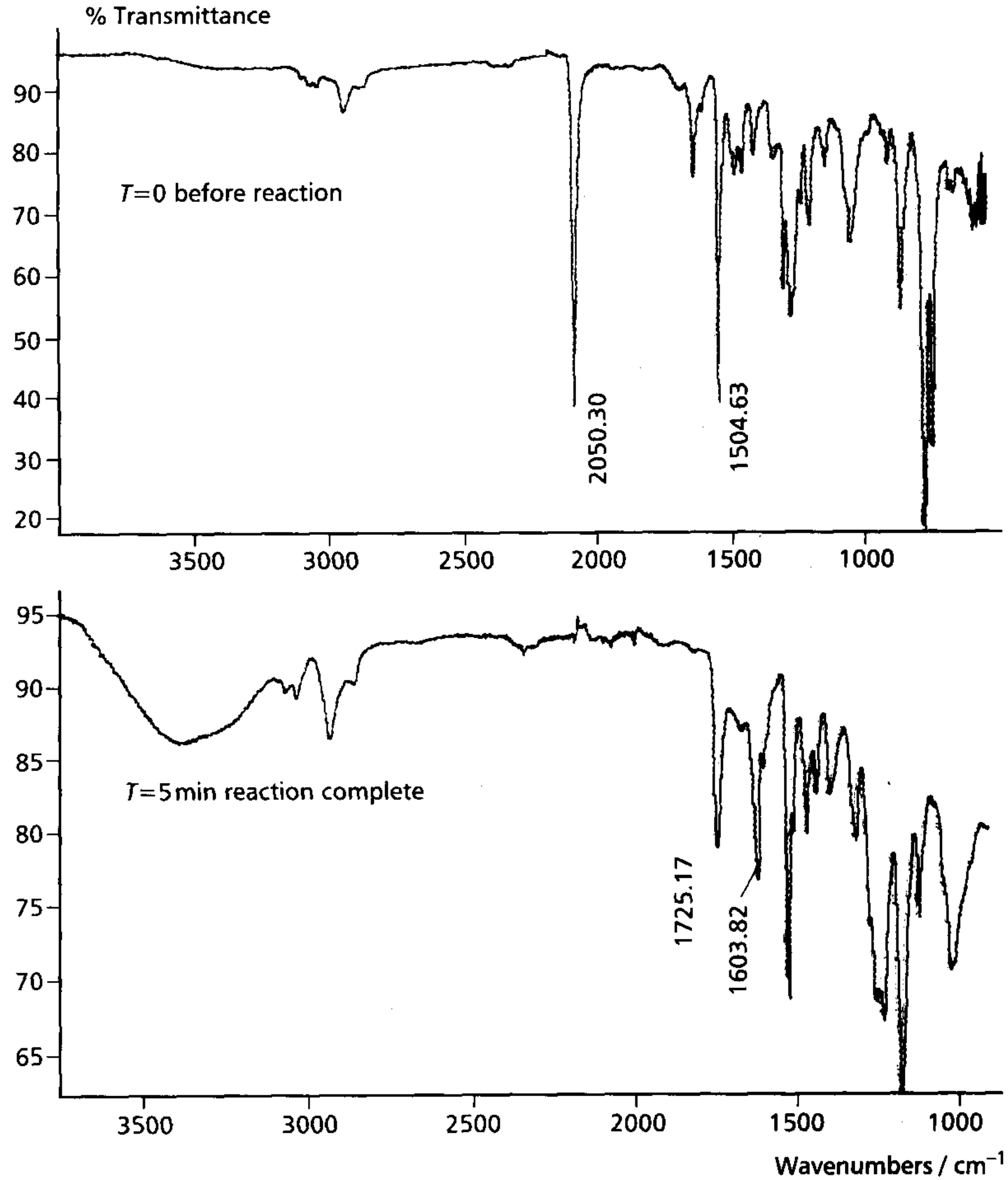
- **Fmoc** quantification during peptide synthesis.



‘On bead’ Fourier transform IR (FT-IR)

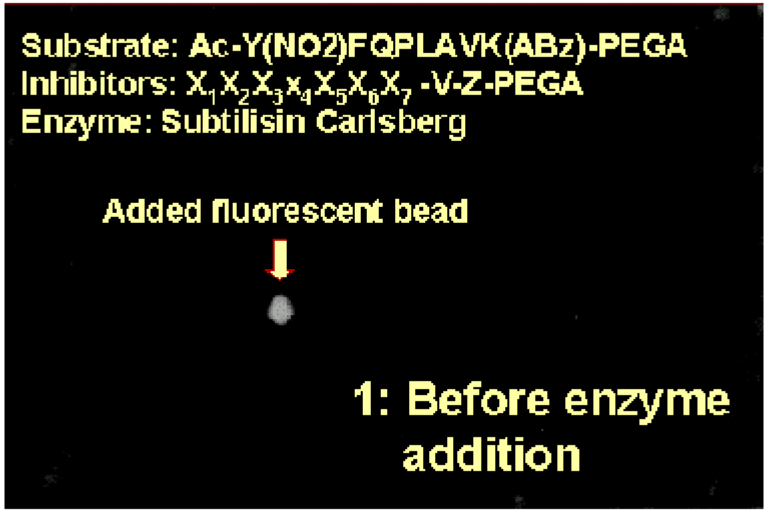
- **Monitoring the attachment of a carboxylic acid onto Norvartis’ diazo based linker.**





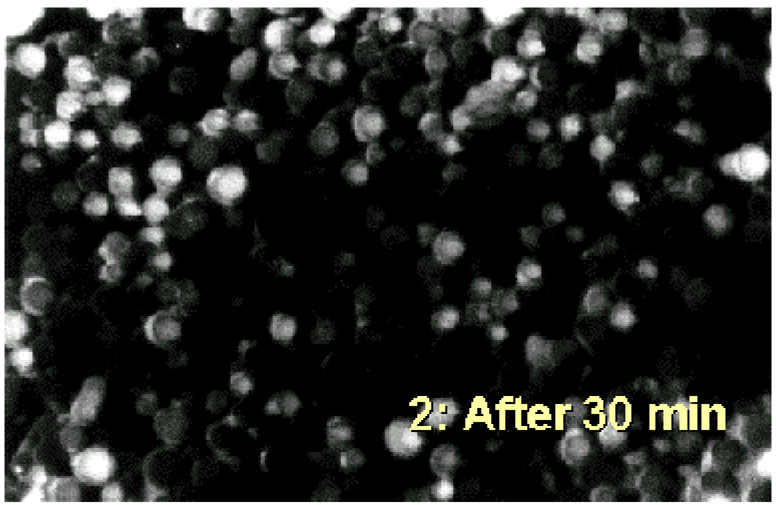
‘On bead’ fluorescence

1

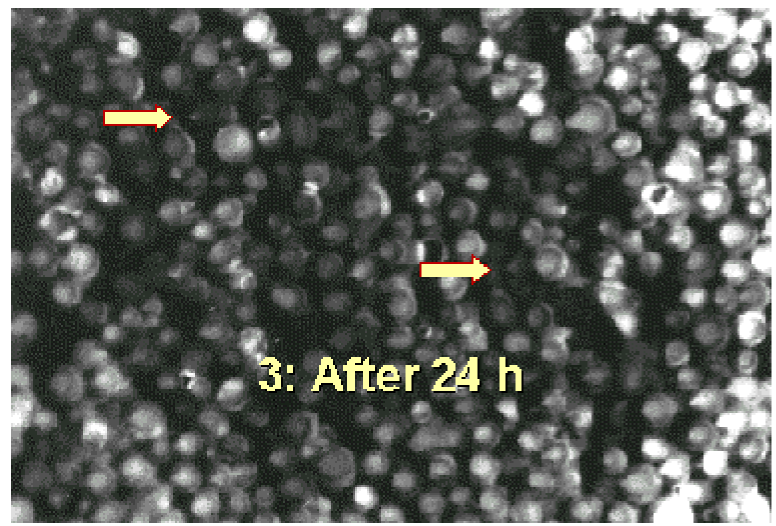


- **Assay to identify peptoid inhibitors of *Subtilisin Carlsberg*.**
- A ‘one-bead one-peptoid’ library is prepared.
- All beads are also partially loaded with a natural substrate peptide which has been modified to fluoresce on turnover.
- Incubation with the enzyme results in an ‘on bead’ competitive inhibition experiment.
- Dark beads indicate no turnover, therefore good inhibition (i.e. ‘hits’).

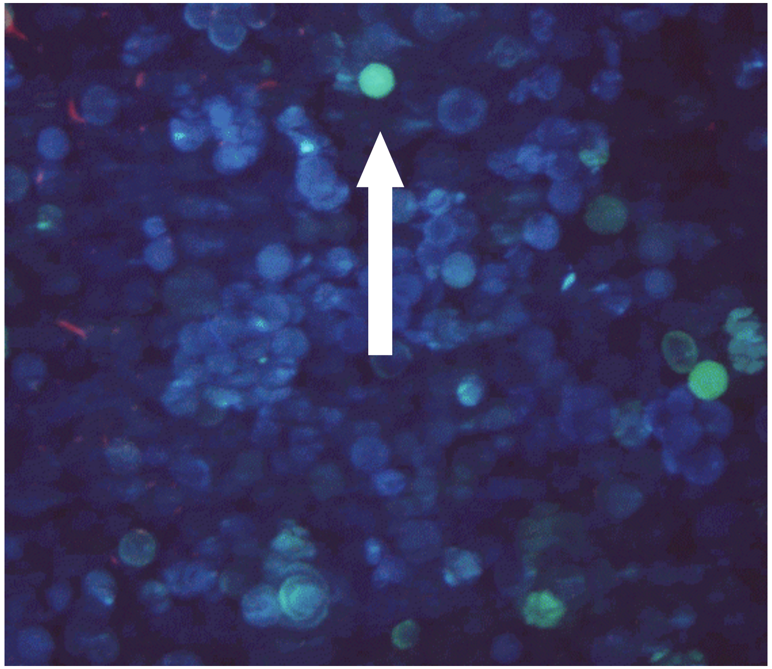
2



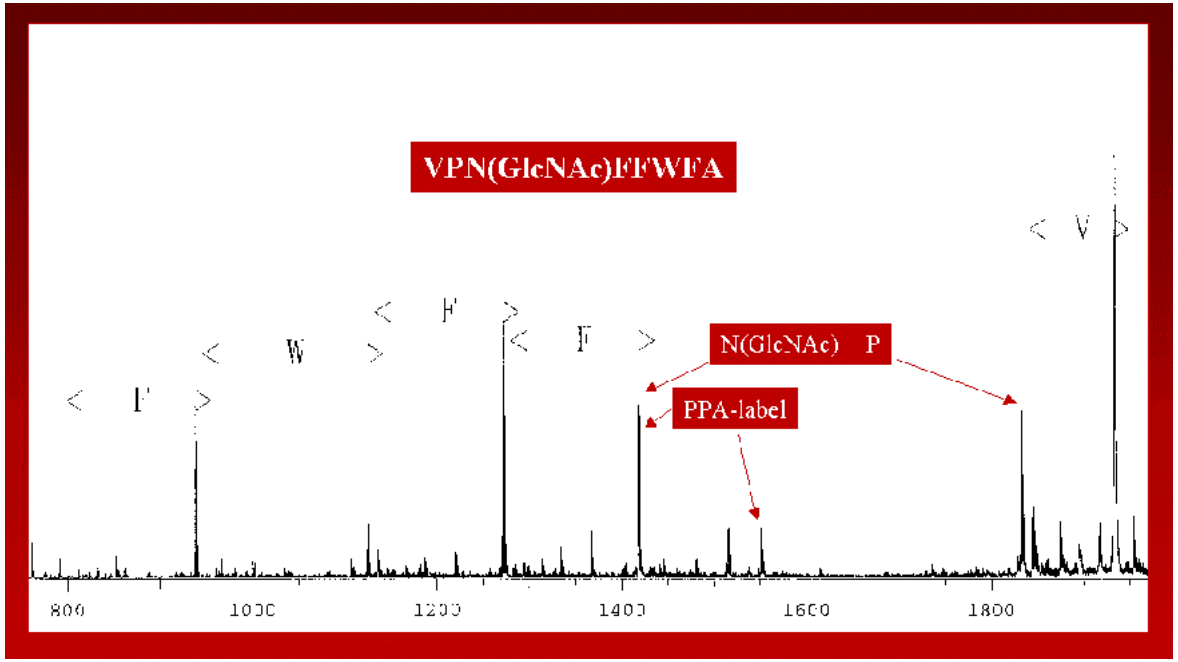
3



‘On-bead’ mass spectrometry (*MS*)

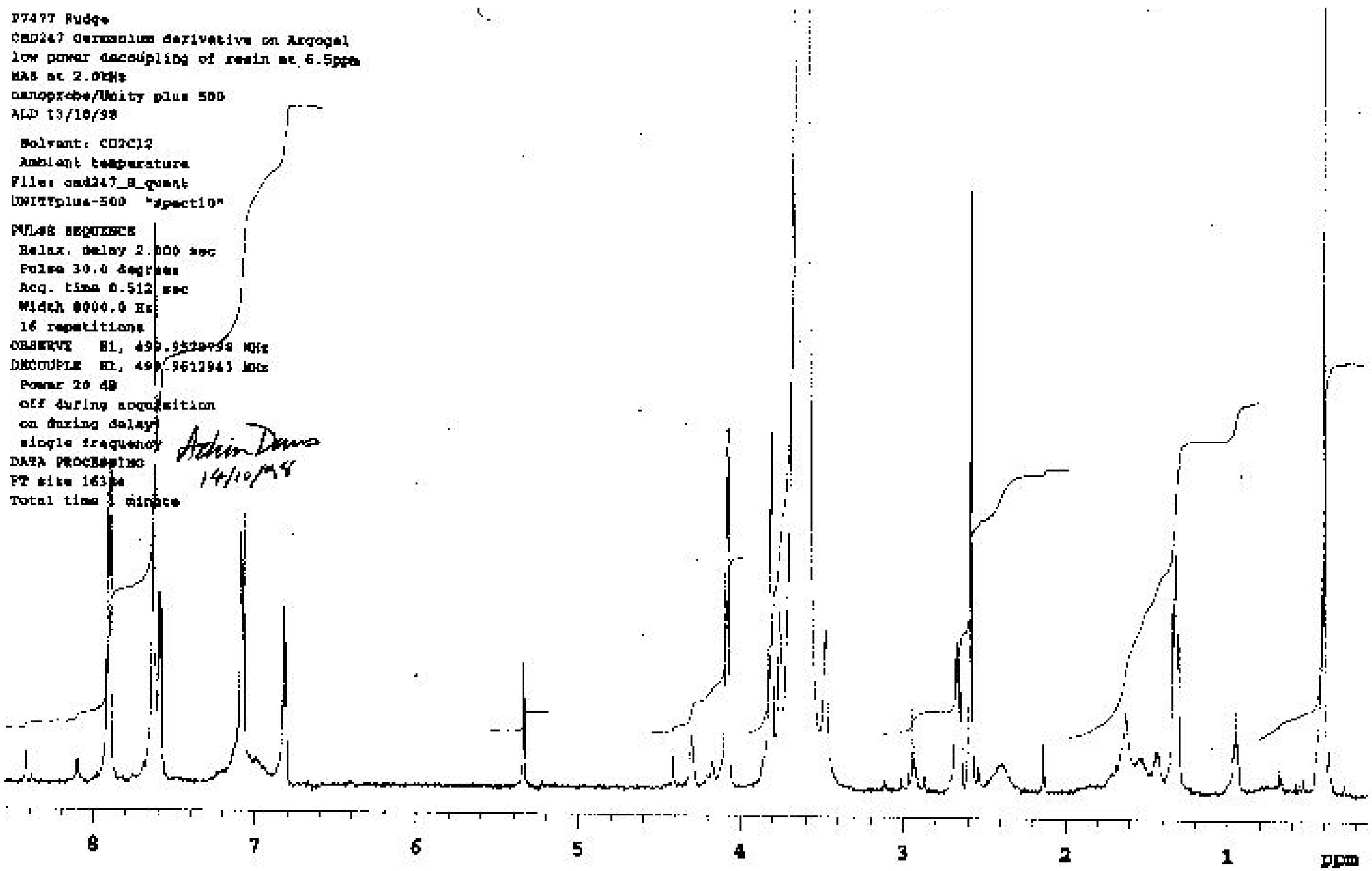


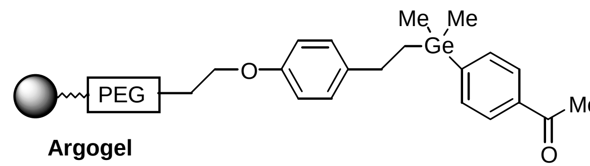
- **Assay to identify binding motifs for porcine glycopeptide binding protein.**
- A ‘one-bead one-glycopeptide’ library is prepared.
- This library is incubated with fluorescien labelled porcine glycopeptide binding protein.
- Following washing, only beads having glycopeptides which bind strongly to the labelled protein remain fluorescent (i.e. ‘hits’).



- All fluorescent beads are isolated.
- Single bead sequencing is performed by MALDI-TOF *MS* analysis (cleavage from bead by e.g. TFA vapour stream entering ionisation chamber).

'On bead' Magic Angle Spinning (*MAS*)-^1H *NMR*

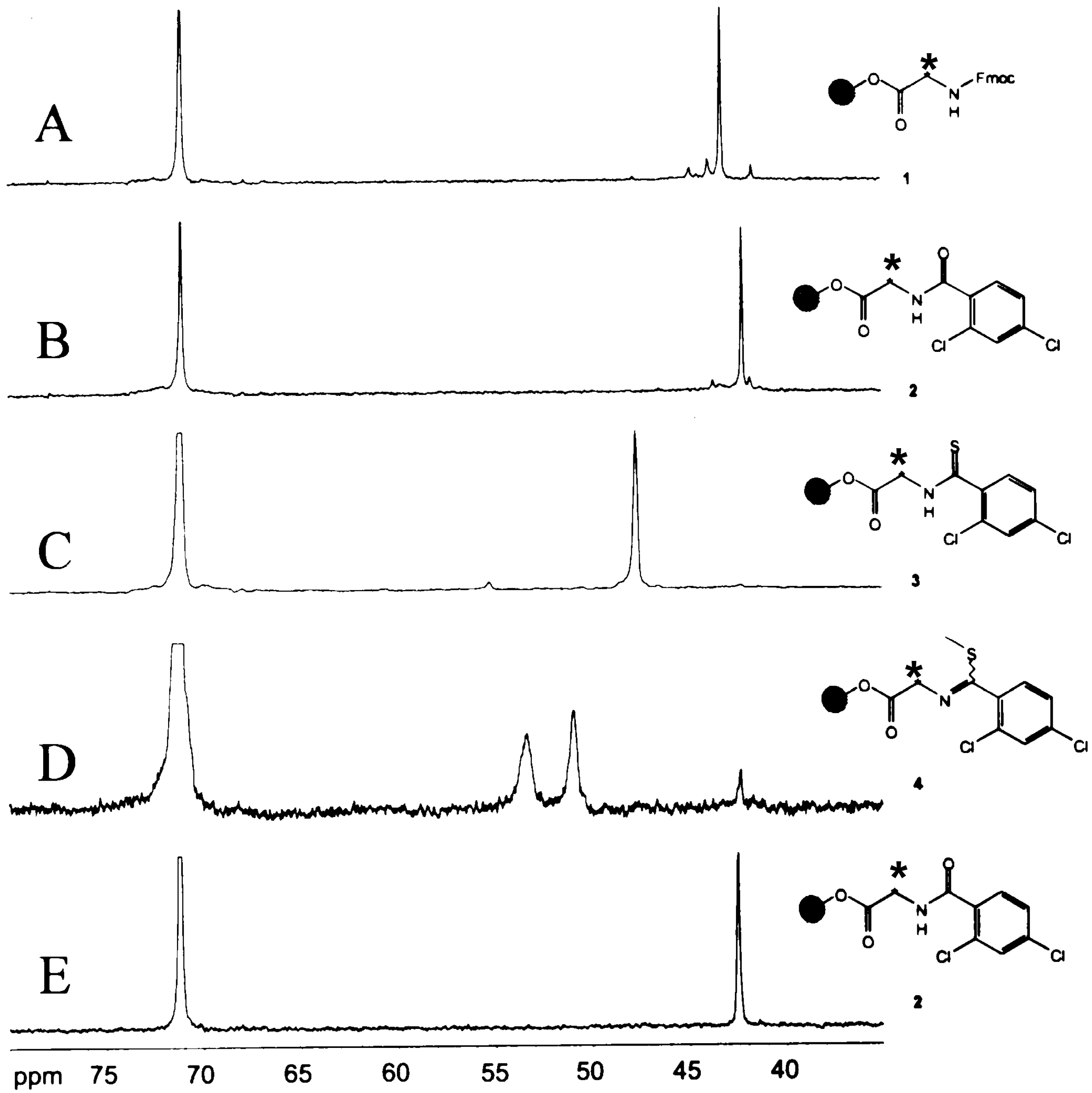




- *PEG-PS* type resins essential for high resolution *MAS*-^1H *NMR*
- Requires a special *NMR* spectrometer.
- Line-widths not dissimilar to those in solution

‘On bead’ gel ^{13}*C NMR*

- **Monitoring *N*-derivitisation of a resin bound glycine.**
- Glycine building block is ^{13}C-labelled (*****) at α-carbon.
- 20-30 mg of resin was dispersed in benzene.
- The resin was PS in this case. PEG-PS resin is also suitable.
- Spectra recorded on Bruker DRX600 in ~15 min.



Summary

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- Getting molecules on and off resins: linkers.
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