# ANALYTICAL TECHNIQUES FOR COMBINATORIAL CHEMISTRY

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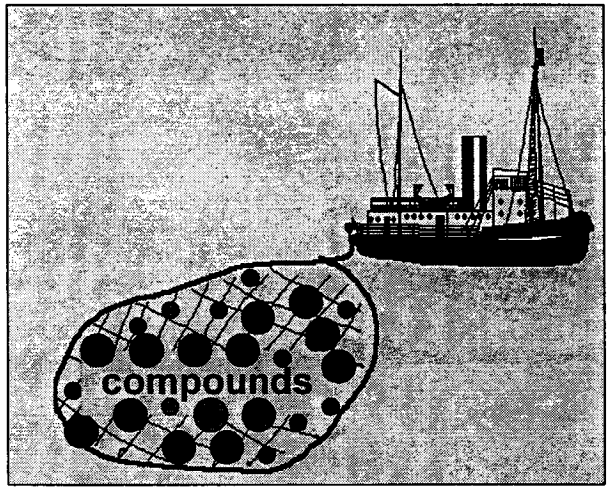
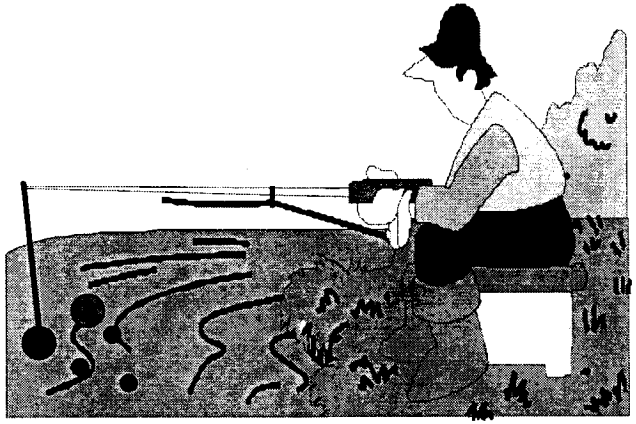
# Combinatorial Chemistry

- **From peptide synthesis**
- **To large arrays of small organic molecules (Libraries)**
- **Widely used for**
  - ♦ **biomedical research**
  - ♦ **agricultural research**
  - ♦ **fine chemical industry**

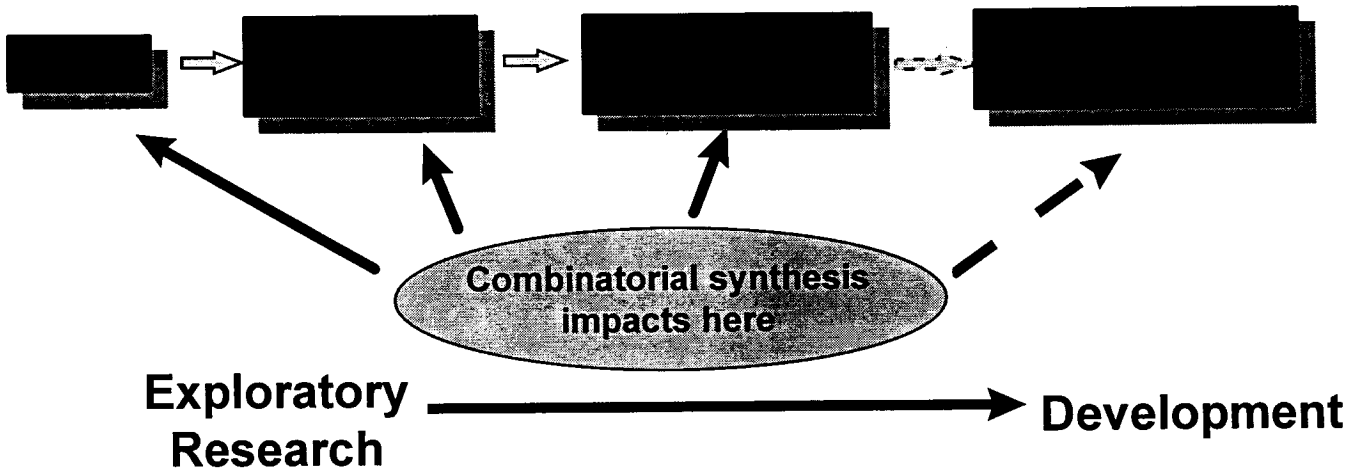
# From one compound.....



...to several compounds...

# Accelerating drug discovery

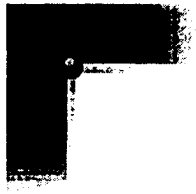


# Combinatorial Chemistry

⇒ **Solution Phase**

⇒ **“Solid phase”**

⇒ **Mixed Solution/Solid phase**



# Library construction

- **Identification of the core structure of a library**
- **Selection of the format and the type**
  - pools *Vs* discrete
  - solid *Vs* solution phase
- **Chemical Assessment**
- **Monomer Selection**
- **Library Rehearsal**
- **Library Production**

# Common Reaction Monitoring Tools

SOLUTION

* TLC
* HPLC/GC
* MS
* FT- IR
* NMR

SOLID

# Analytical Techniques for Solid Phase synthesis

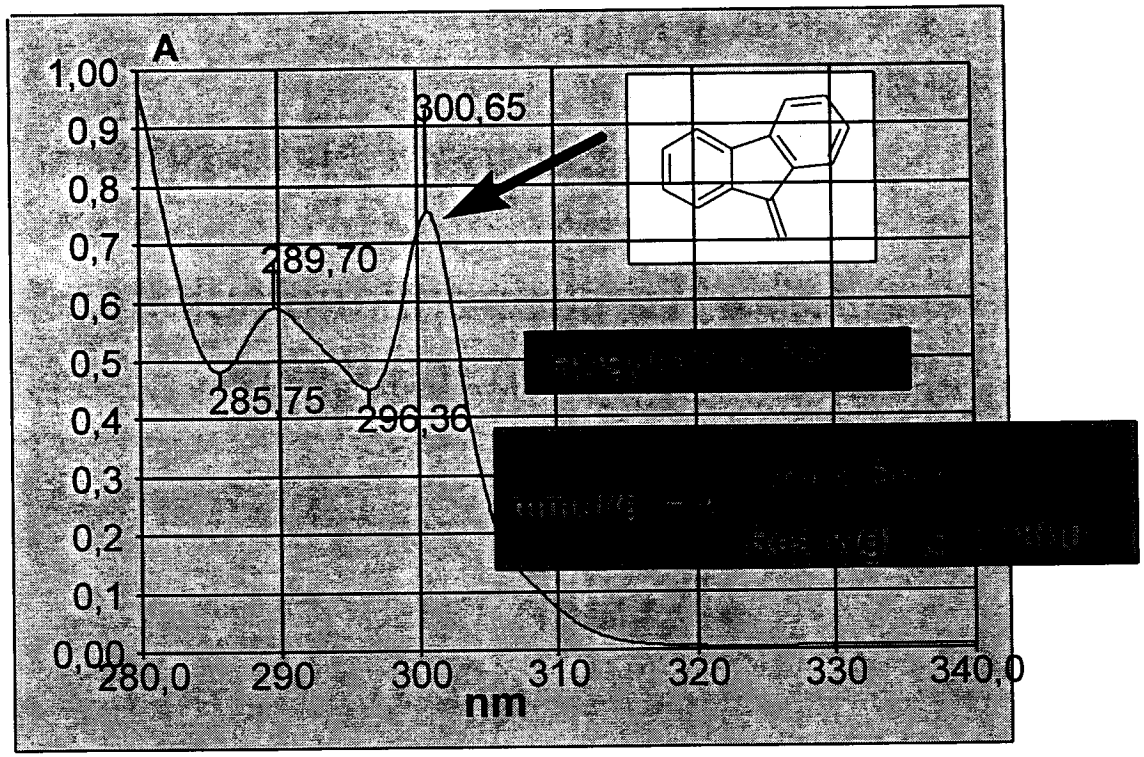
- **Destructive**
  - ♦ **Color & Colorimetric tests**
  - ♦ **HPLC, HPLC-MS, LC-NMR, LC-IR**
  - ♦ **MS**
- **Non-Destructive**
  - ♦ **NMR**
  - ♦ **IR on beads**

# Colorimetric tests

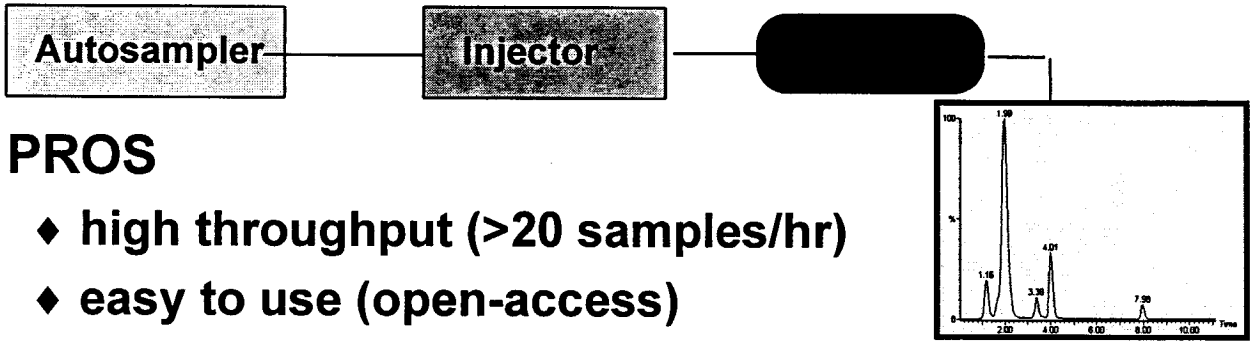
- Primary Amines
  - Kaiser test ( Ninhydrin)
  - Isatin
  - Trinitrobenzene sulphonic acid
  - Bromophenol Blue
- Thiols
  - Ellman

# QUANTITATIVE ANALYSIS ON BEAD

## UV Absorbing group (Fmoc loading)

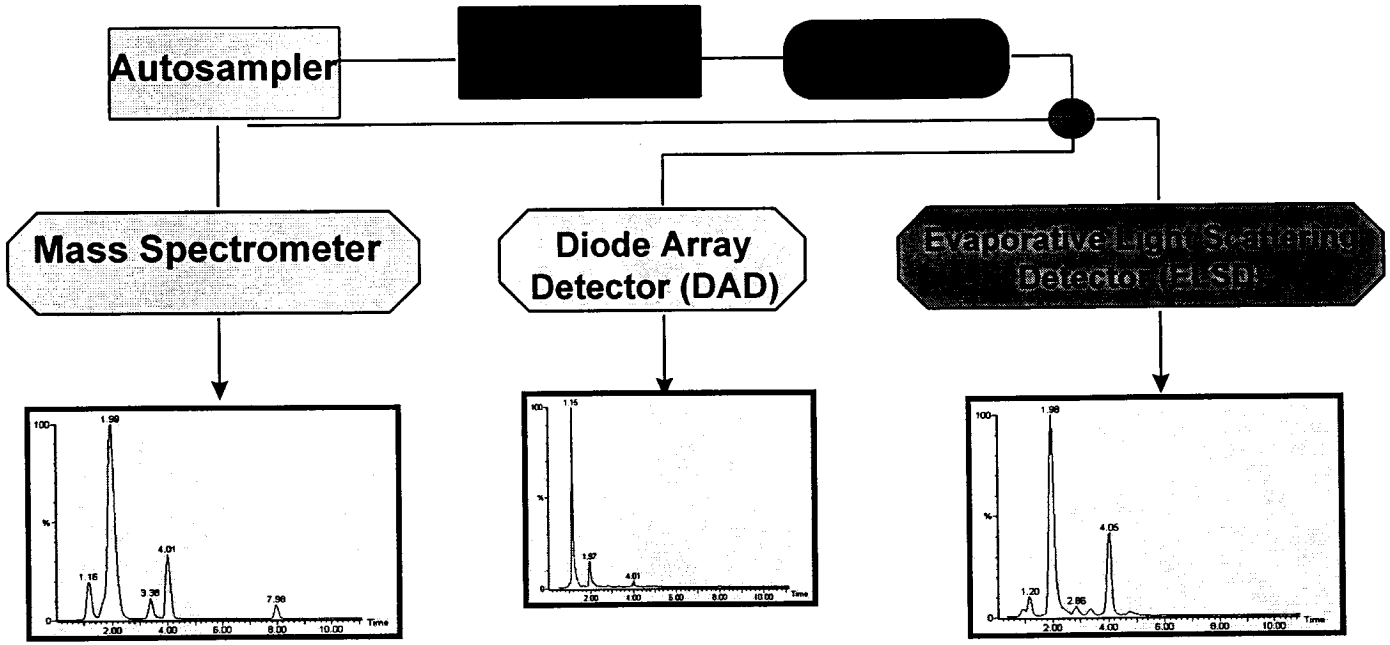


# Rapid Injection MS




- **PROS**
  - ♦ **high throughput (>20 samples/hr)**
  - ♦ **easy to use (open-access)**
  - ♦ **software support for data handling**
- **CONS**
  - ♦ **mixtures**
  - ♦ **poor structural information**

# LC-MS SYSTEM

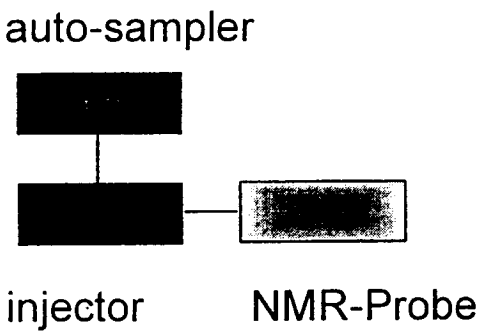

Autosampler
Mass Spectrometer
Diode Array Detector (DAD)
Evaporative Light Scattering Detector (ELSD)

# MS Techniques

- Matrix-assisted laser desorption ionization time-of-flight MS (MALDI-TOF-MS)
- Imaging time-of-flight secondary ion MS (TOF-SIMS)
- Electrospray MS (ES-MS)

- Problem
  - ♦ sample preparation (TFA vapor or light)

# "High-Throughput" NMR Configuration

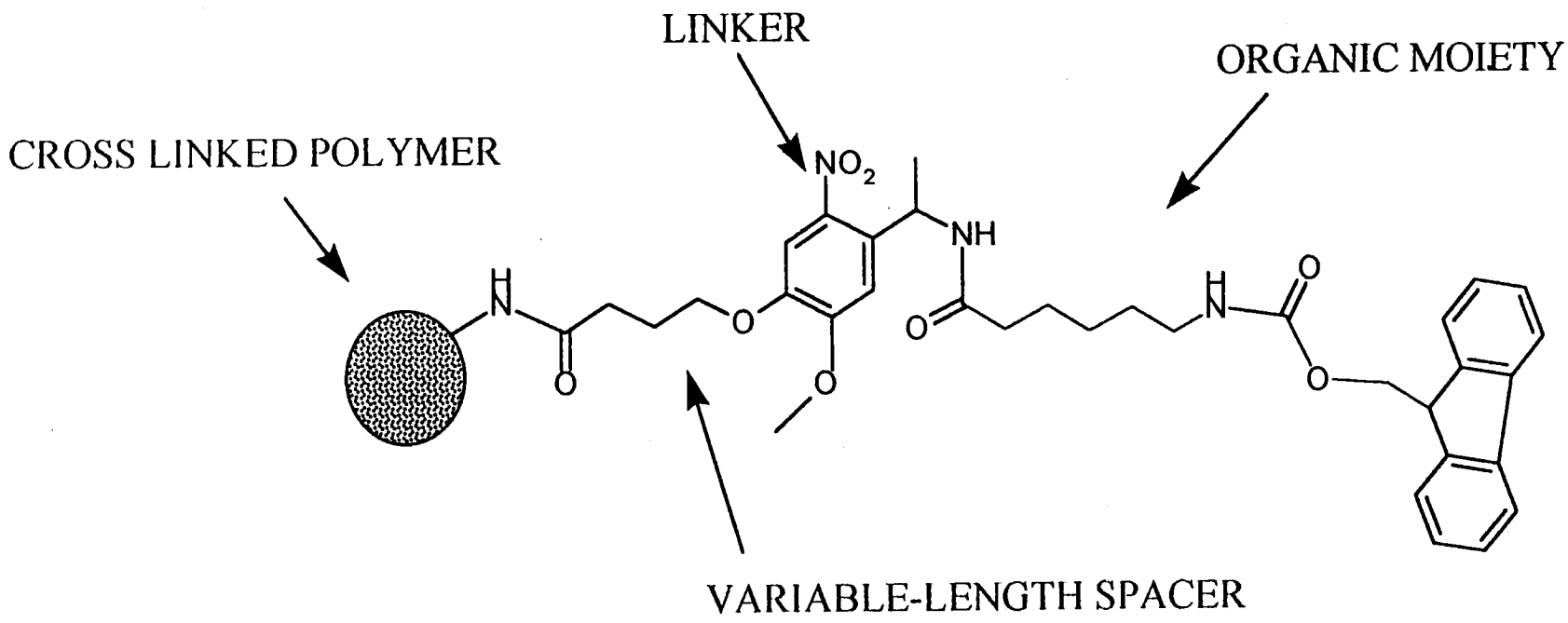



- Gilson 215 Liquid Handler
- Standard LC-Probe

# High Throughput NMR: LC-NMR

- Faster Throughput
  - ♦ 4 minute to inject/recover/rinse/$^{1}$H-acquire
- Higher Probe Sensitivity (Flow Probe)
- No NMR Tubes
- No need for protonated solvents
  - ♦ multiple-resonance solvent suppression techniques
- Use of Combinatorial 96-well microtiter-plate samples (1mg/mL) with autosampler for all steps

# Solid Phase Synthesis



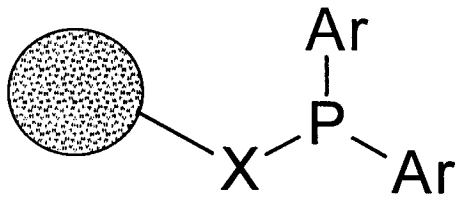
# NMR in Solid Phase Synthesis

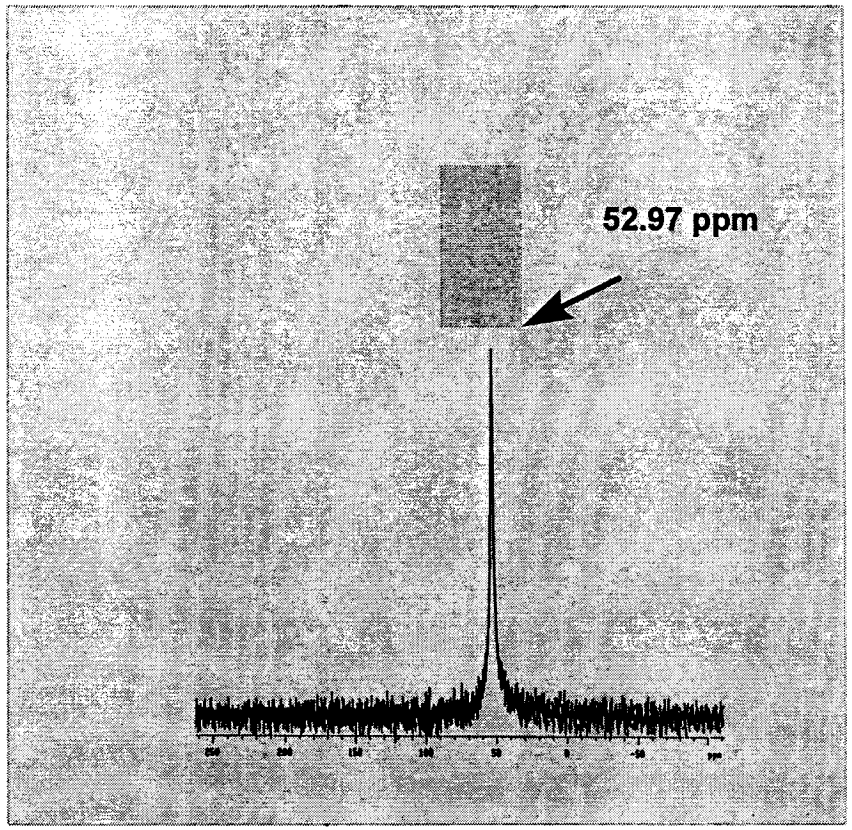
- **Gel-Phase NMR**
- **$^{13}C$ Magic Angle Spinning NMR**
- 
- **MAS Gel-phase NMR**
- 
- **Single-bead NMR**
- 

# Gel-Phase NMR

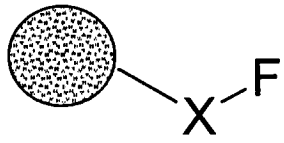
- **First application in 1971 to study crosslinked polymers**
- **Application in 1980 to peptide chemistry**
- **$^{19}F$, $^{31}P$, $^{13}C$ & $^{13}C$- enriched materials**

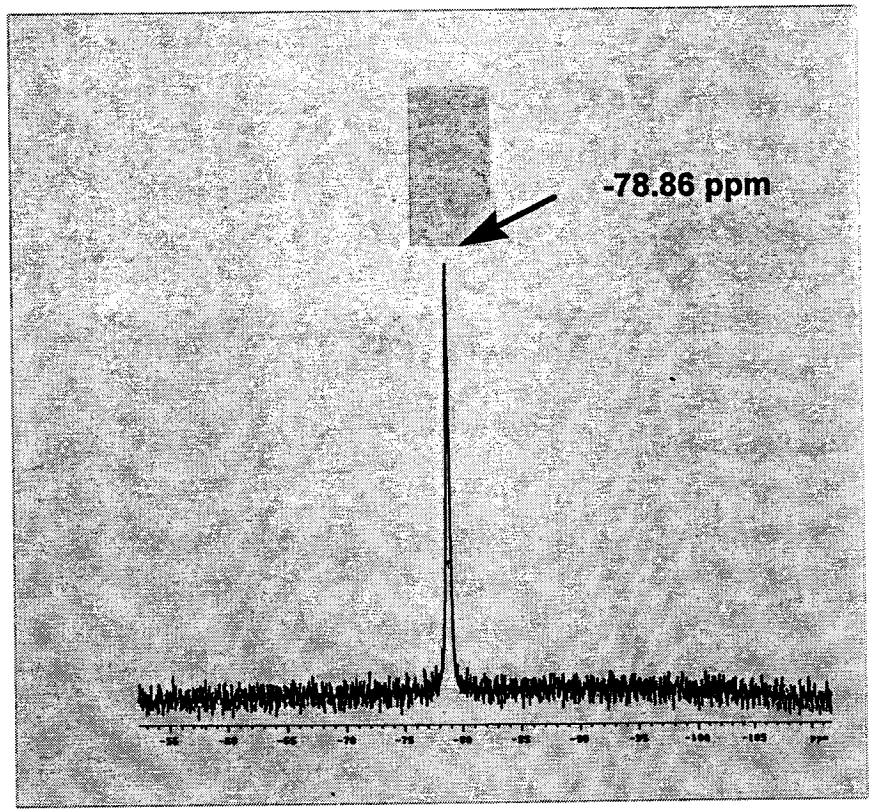
# $^{31}$P-NMR: Reaction Monitoring



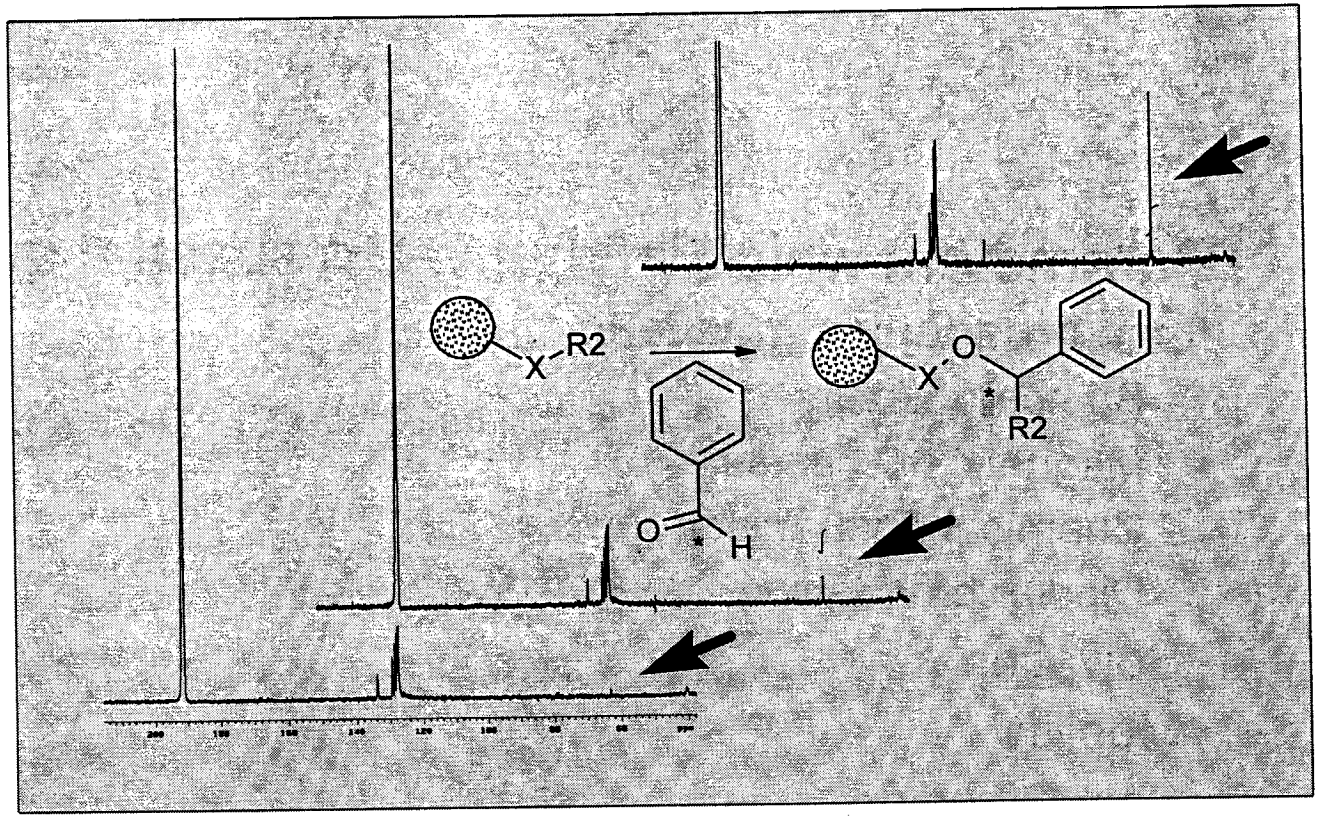



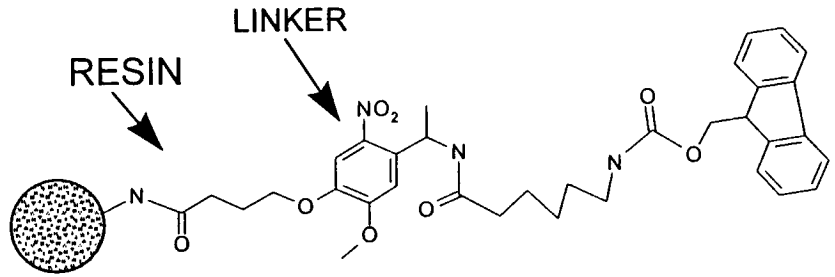
# $^{19}$F-NMR: Reaction Monitoring



# $^{13}$C-NMR: Reaction Monitoring

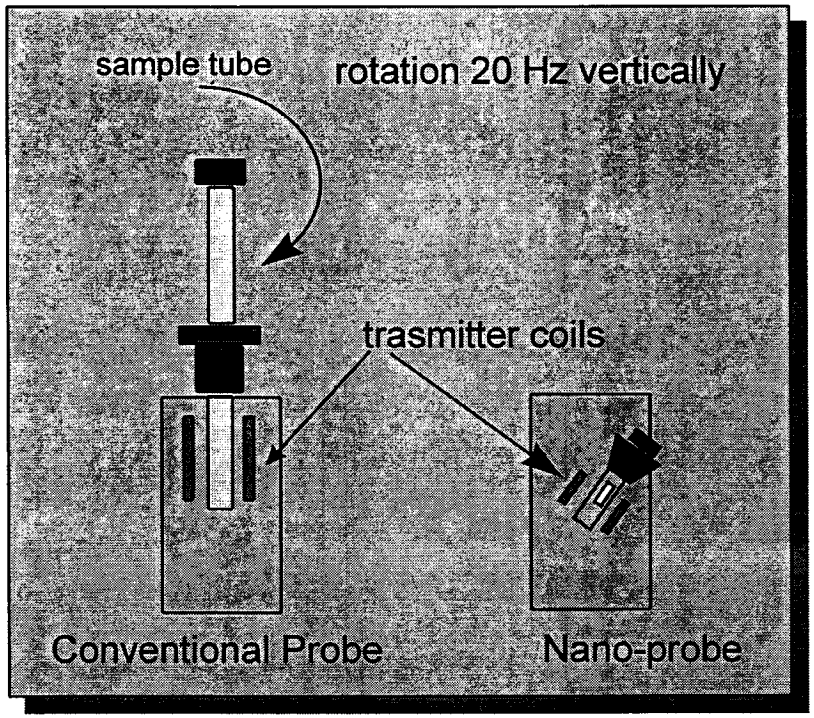





# Parameters which influence the spectral quality:

- Line broadening mechanism
  - Bo homogeneity
  - Magnetic susceptibility
  - Chemical Shift Anisotropy
  - Dipolar broadening
- Solvation
- Mobility
- Spin Diffusion

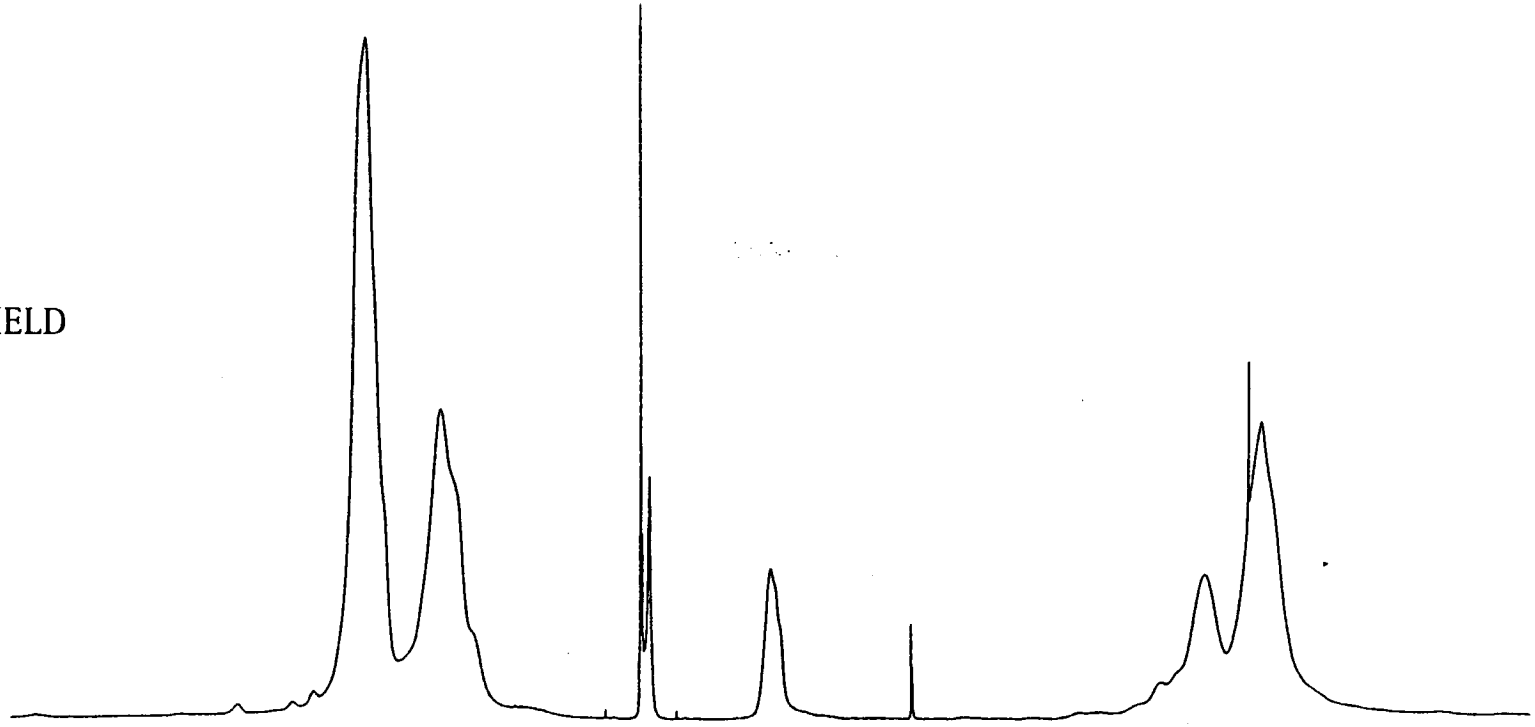
# On-Bead NMR



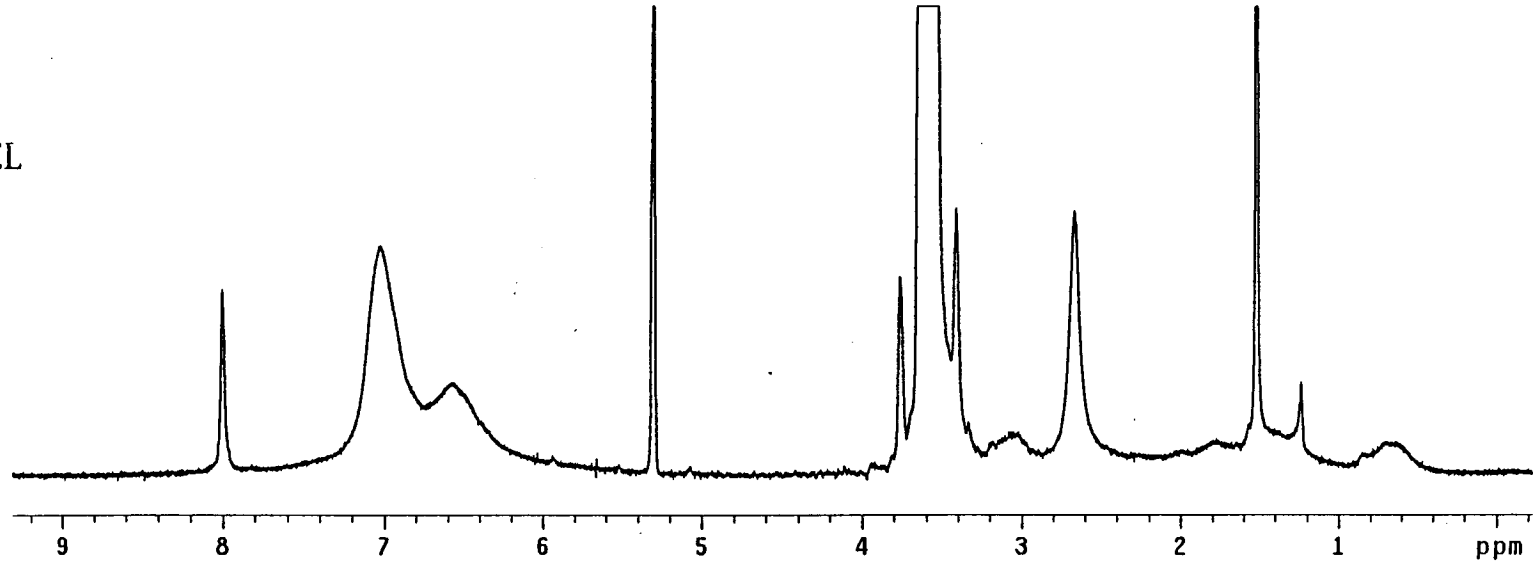

**Magic Angle 54.736 degrees**

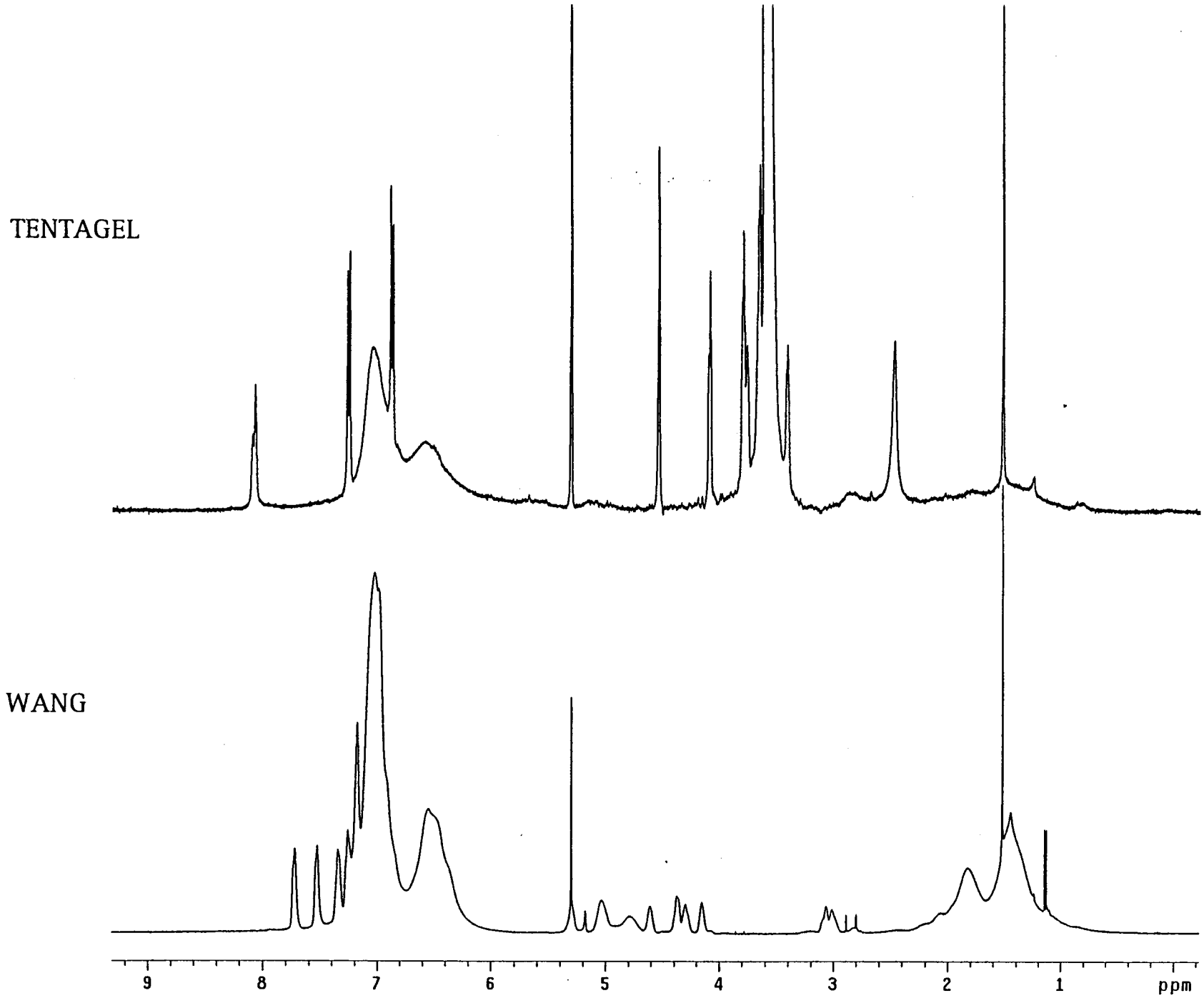
**rotating at 2-3 KHz**

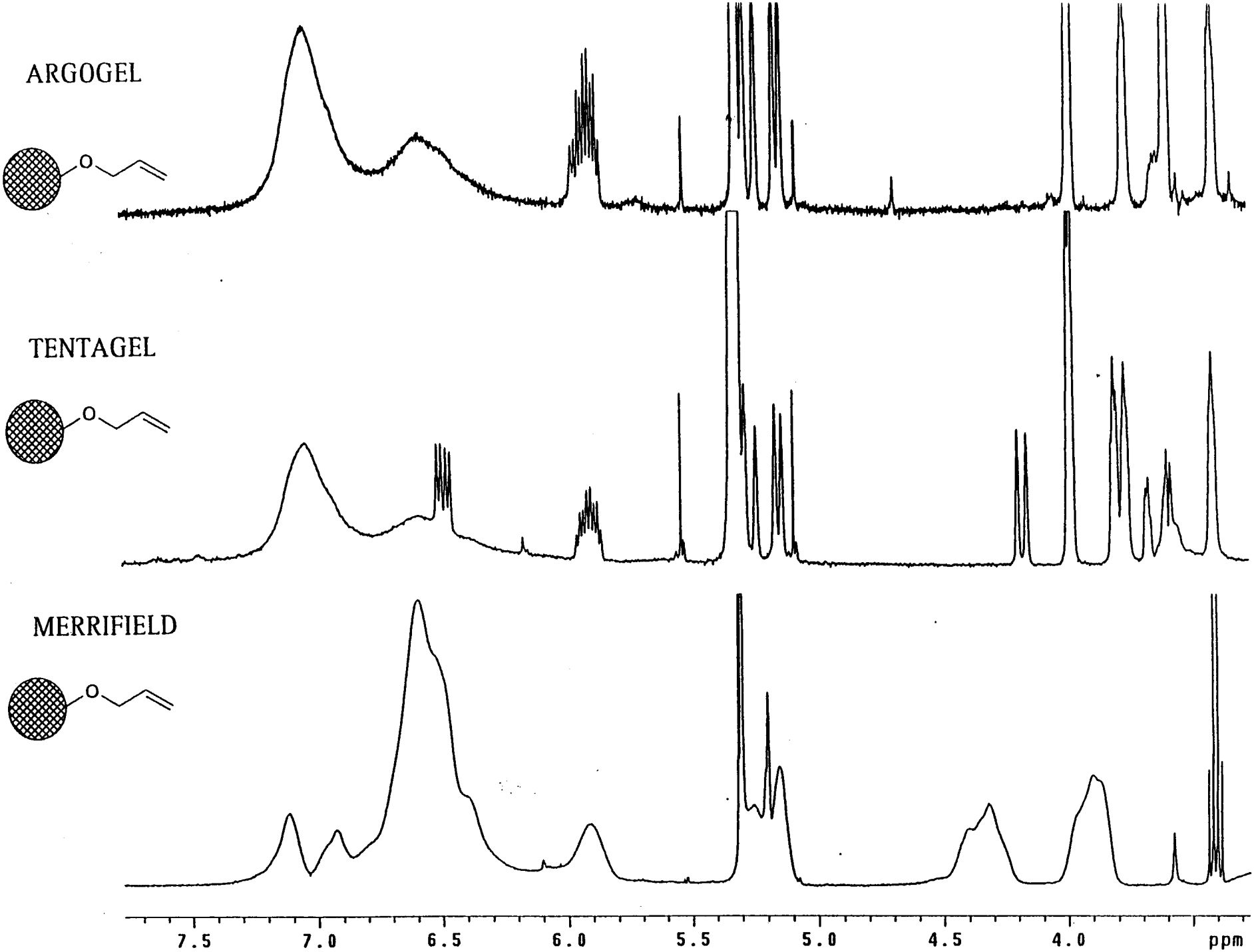
MERRIFIELD

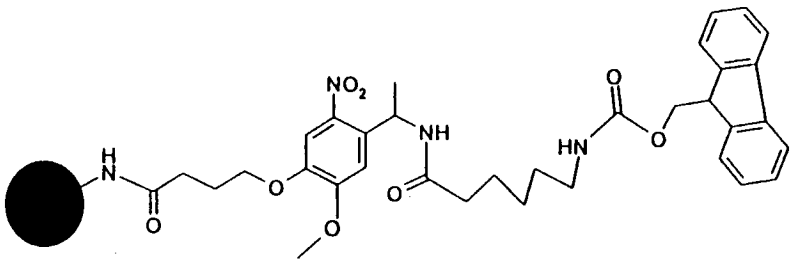


ARGOGEL

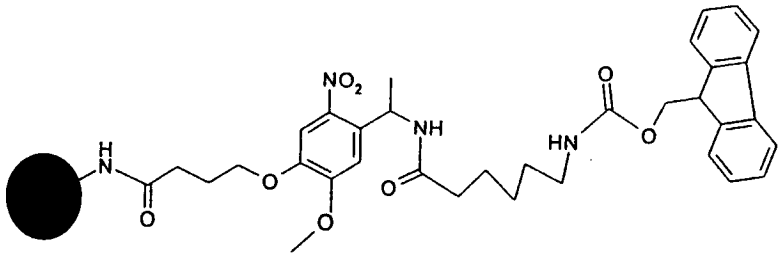


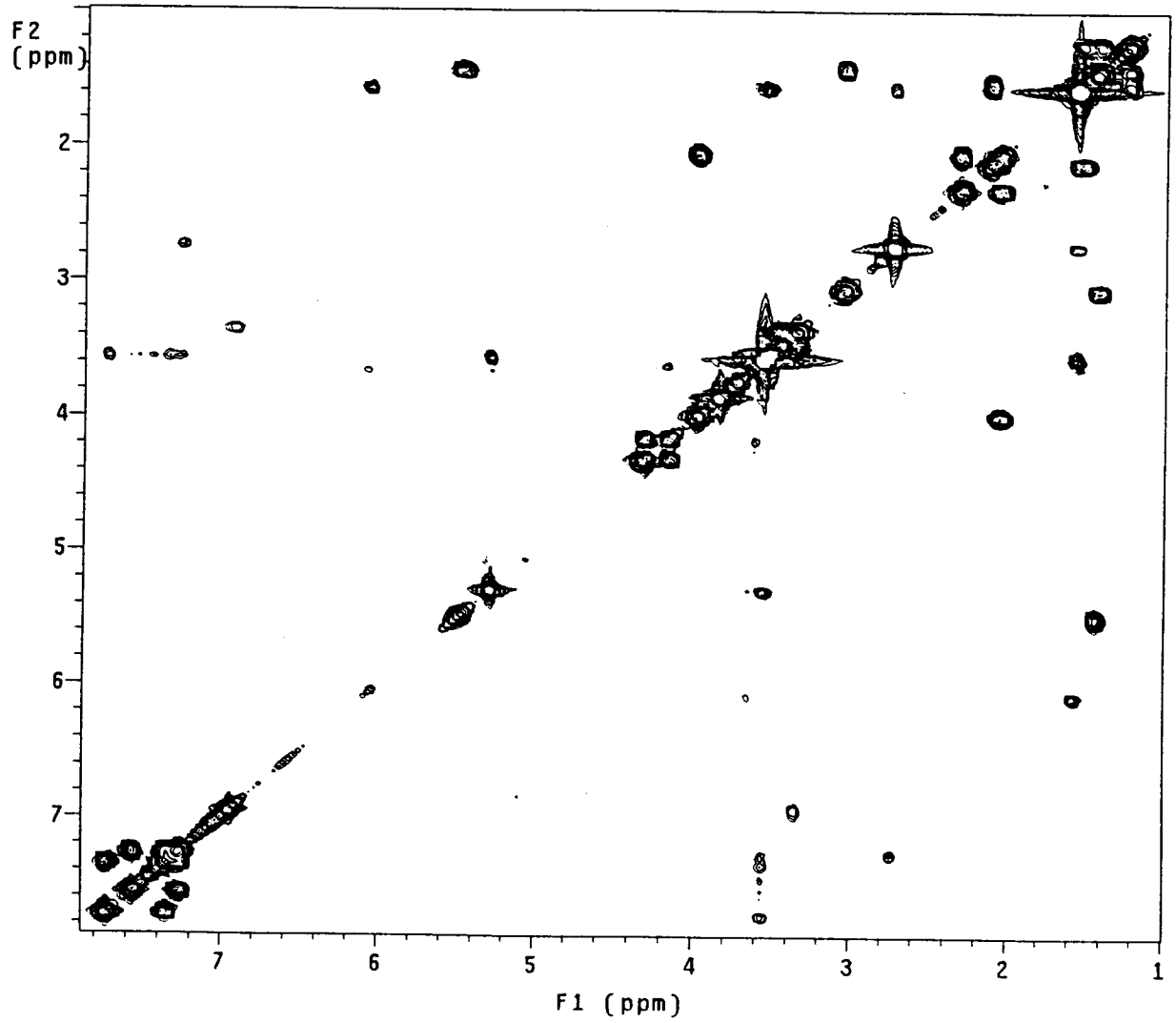

TENTAGEL
WANG
9
8
7
6
5
4
3
2
1
ppm


ARGOGEL
O
TENTAGEL
O
MERRIFIELD
O
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm


NO2
NH
HN
O
O
O
O
O
H
N


12
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
1.32
0.62
0.93
8.90
1.81
2.27
71.57
1.41
4.74
6.21
0.22


NO2
NH
HN
O
H
N
O
O
O
O
O


F2
(ppm)
2
3
4
5
6
7
7
6
5
4
3
2
1
F1 (ppm)

# HR-MAS NMR and Nano*nmr Probes

- The structure of the resin affects the NMR resolution. Tentagel resins provide the highest resolution NMR spectra.

- The choice of solvent used to swell the resin also has a large effect. $CD_2Cl_2$ (or DMSO-$d_6$) provide the narrowest lines for most resins.

# HR-MAS NMR and Nano*nmr Probes

- High resolution NMR spectra can be obtained nondestructively.

- The sensitivity is sufficient to allow NMR to be used to detect the amount of material bound to a single bead of resin.

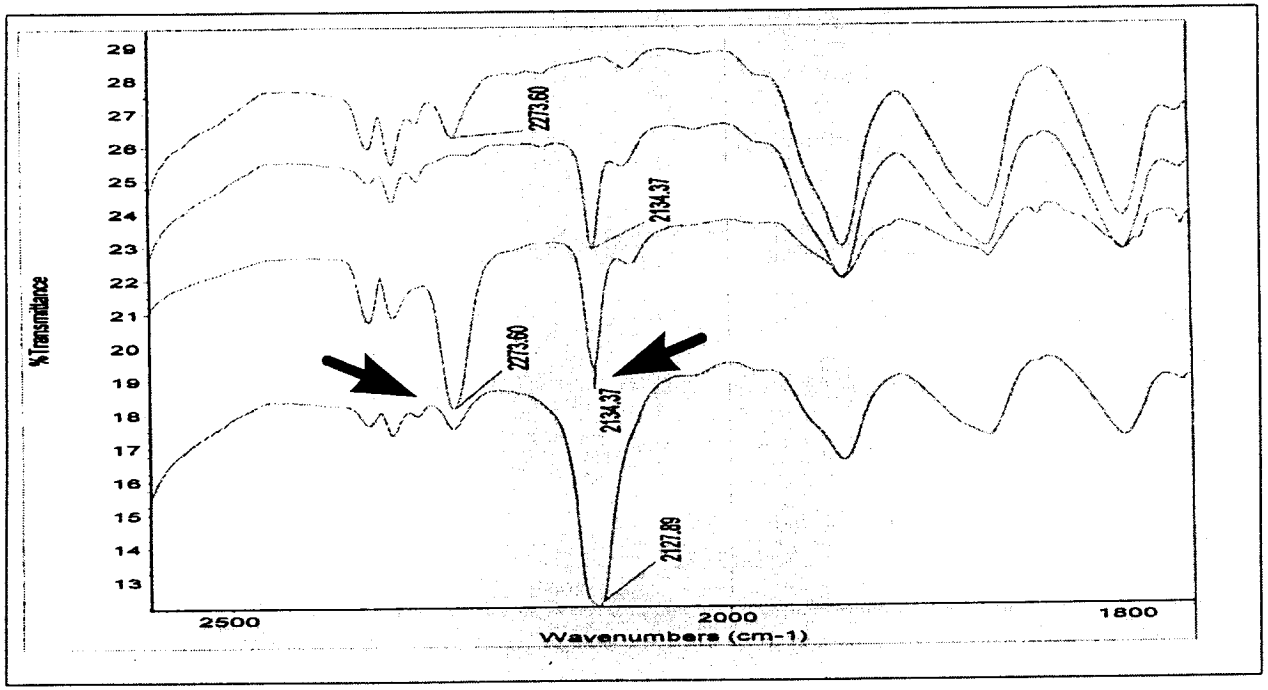
# IR in Solid Phase Synthesis

- **From 1971 KBr disks of ground beads**
- **On-bead FT-IR**
  - ➔ microscope (transmission or reflectance mode)
  - ➔ microscope (attenuated total reflection ATR)
  - ➔ ATR
  - ➔ photoacustic (PAS)
  - ➔ diffuse reflectance DRIFT

# IR in Solid Phase Synthesis

- **Reaction Monitoring**
- **Quantitative Analysis**
  - ➔ deuterium-labeled reporter groups (C-D str)
  - –
- **Future Development**
  - ➔ 2D FT-IR
  - ➔ High Throughput IR

# IR: Reaction Monitoring

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