
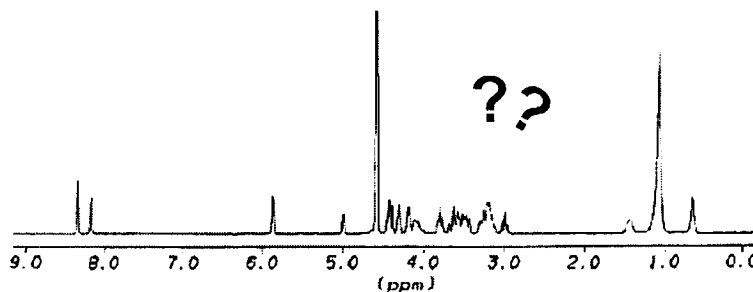


DOSY - NMR Chromatography-

\$ Introduction

One ^1H NMR chart is shown. 
It seems to be a spectrum of mixture....
What compounds are the components?

In such a situation, separation and isolation of each components makes it easy to identify the compounds.



So, to get clear NMR spectra, silica gel column chromatography or recrystallization etc. are usually required. However, unstable compounds, intermediates or metal complex can not be applied such purifying techniques.....



DOSY NMR ! is one of the choice to solve such problems.

DOSY : Diffusion-Orded NMR Spectroscopy

(Diffusion : spreading over a larger area)

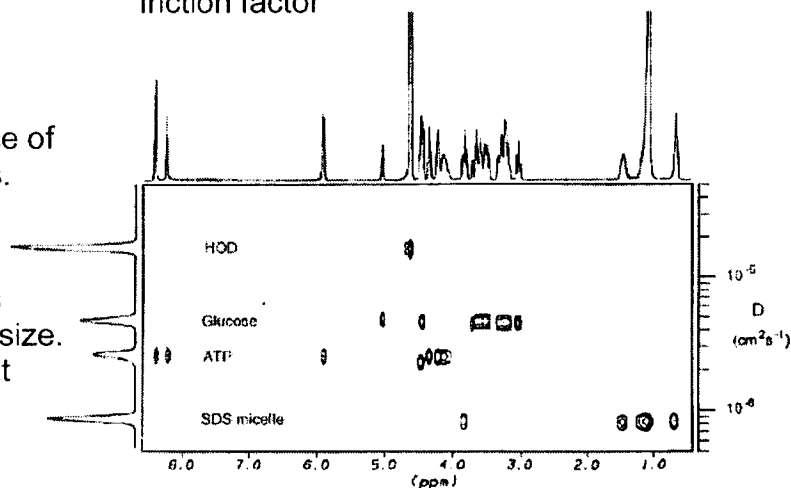
$$\text{Stokes-Einstein equation, } D = \frac{kT}{6\pi\eta R_H}$$

(k: Boltzmann coefficient
T: absolute temp.
 η : medium viscosity
 R_H : hydrodynamic radius)

friction factor

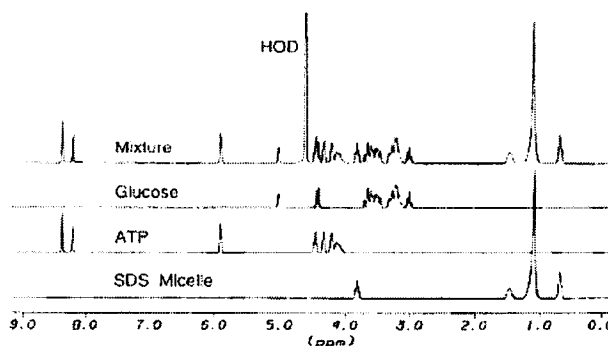
"Diffusion-Ordered" means...
to separate NMR spectra by the difference of diffusion coefficient of each compounds.
(See the figure on right side)

DOSY allow us to distinguish compounds according to differences in their effective size.
(Diffusion of chemical species in a solvent depends on its molecular size and its hydrodynamic volume.)



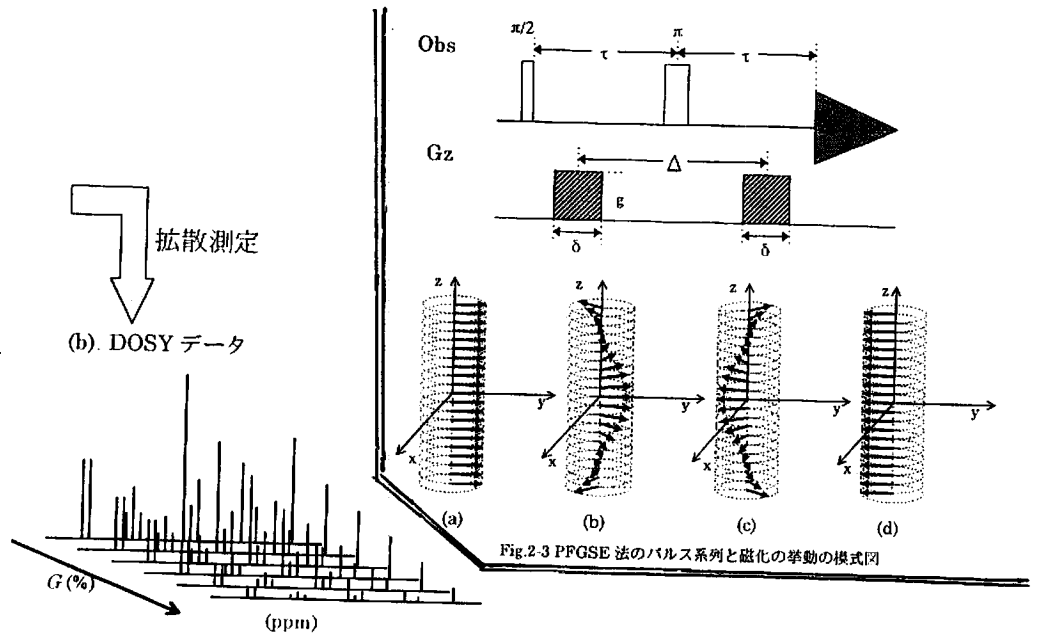
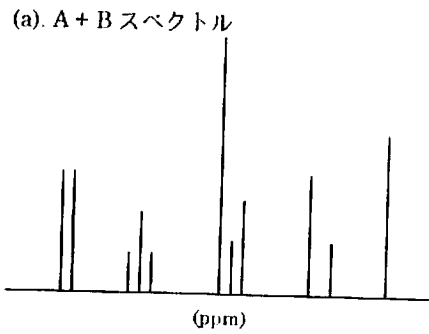
\$ Contents

1. Brief Explanation of Fundamental Principles
2. Examples for Organic Compounds
3. Examples for Metal Complex
4. Instruction for Measurement

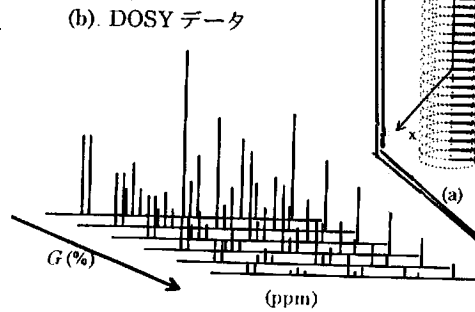


1. Brief Explanation of Fundamental Principles

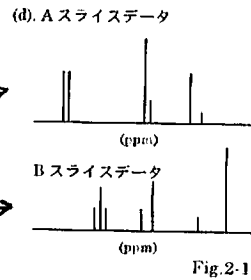
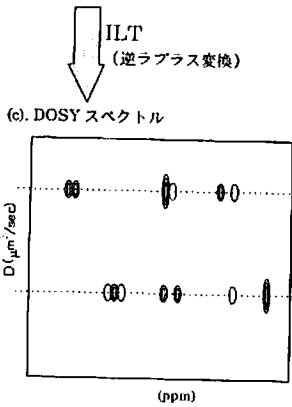
ref) JEOL user's meeting textbook 2000 and 2002
 Prog. NMR Spectrosc. 1999, 34, 203.



To detect signal decay by changing gradient in array.



Results are displayed as 2D spectra with NMR chemical shift on the horizontal axis and the derived diffusion coefficients on the vertical axis.

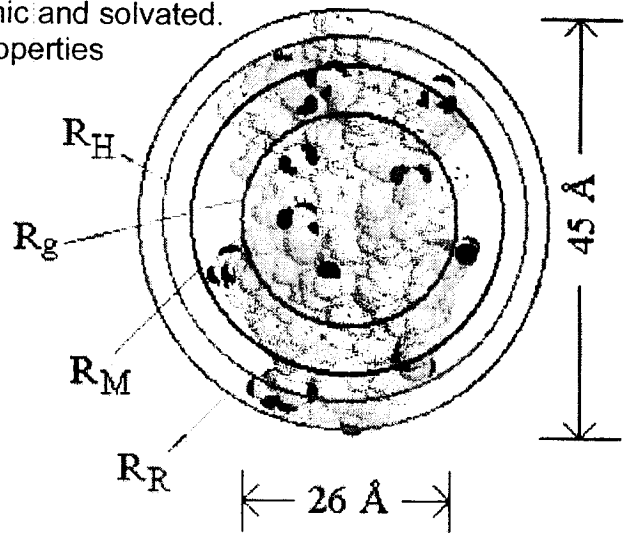


What is the hydrodynamic radius (R_H)?

Macromolecules in solution are non-spherical, dynamic and solvated. As such, the radius calculated from the diffusional properties of the particle is indicative of the apparent size of the dynamic hydrated/solvated particle, which includes both solvent (hydro) and shape (dynamic) effects.

$$R_g \text{ (gyration): } R_g^2 = \frac{\sum m_i r_i^2}{\sum m_i}$$

R_M : Equivalent radius of a sphere with the same mass and particle specific volume.
 R_R : Radius established by rotating the compound about the geometric center



2. Examples for Organic Compounds

Diffusion-Ordered Two-Dimensional Nuclear Magnetic Resonance Spectroscopy

← In this paper, "DOSY" was named.

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Department of Chemistry, University of North Carolina
Chapel Hill, North Carolina 27599-3290

Received December 9, 1991

J. Am. Chem. Soc. 1992, 114, 3139.

J. Am. Chem. Soc. 1993, 115, 4291.

(b)
DOSY spectrum for a sample containing
10.0 mM tetramethylammonium chloride (TMA)
with mixed micelles.
(SDS and octaethylene glycol dodecyl ether)

(a) Diffusion Ordered Spectroscopy

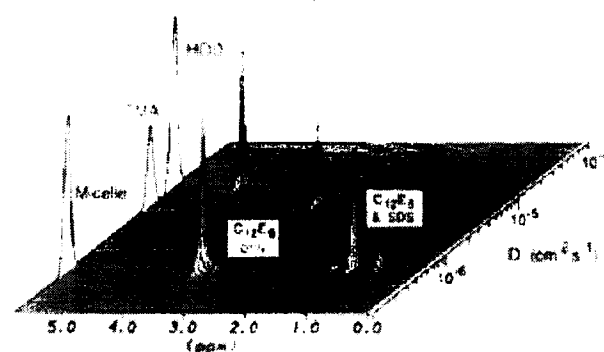


Figure 2. Sucrose and phospholipid vesicle in D₂O

Vesicle in the form of a spherical shell
enclose an aqueous region.
The diffusion peaks for free sucrose and
sucrose entrapped in the vesicle were easily
and clearly resolved with diffusion coefficients.

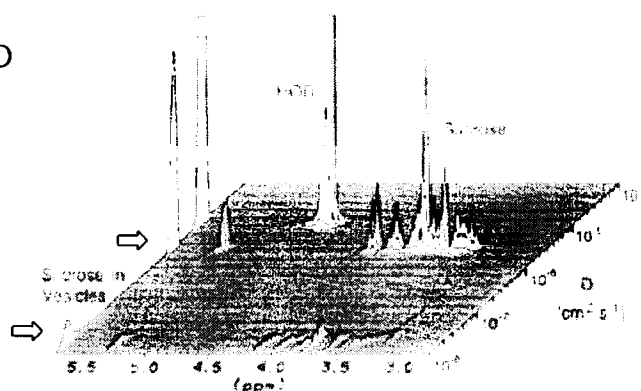


Figure 2. The diffusion-ordered 2D-NMR spectrum of a sample containing sucrose and phospholipid vesicles in D₂O.

This result matched the fact that phospholipid bilayer are highly permeable to water but not sucrose.

The qualitative probing of hydrogen bond strength by
diffusion-ordered NMR spectroscopy

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DOSY as a useful probe to qualitatively
understand relative strength of
hydrogen bonds.

Tetrahedron Lett. 2000, 41, 7181.

Magn. Reson. Chem. 2001, 39, S142.

For a mixture containing
phenol **1** and cyclohexanol
2 studying their interaction
with H-bond acceptors.

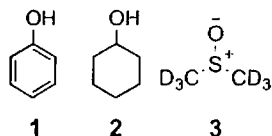


Figure 1. (b)

Phenol and DMSO are
spending sufficient time
together as an associated
molecular system.

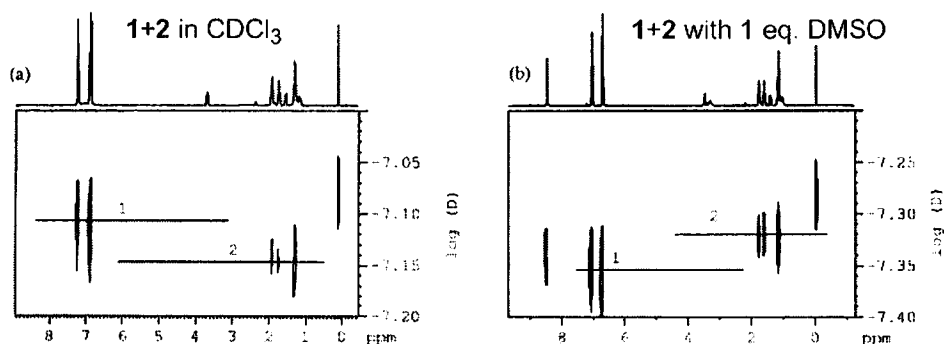


Figure 1. (a) The ¹H-DOSY spectrum of a mixture containing phenol (**1**) and cyclohexanol (**2**) in CDCl₃ containing TMS as a reference; and **(b)** The ¹H-DOSY spectrum of the same mixture containing DMSO-*d*₆ (**3**) as an additional component. The spectra were recorded on a Bruker DRX-400 instrument using the stimulated echo sequence incorporating bipolar gradients with an longitudinal eddy delay (BPPL-ED). The gradient strengths of 1 μs duration were incremented in 32 steps, with diffusion times of 50 ms

Figure 2. ^{31}P COSY
To accept a hydrogen bond should be highest in 7.

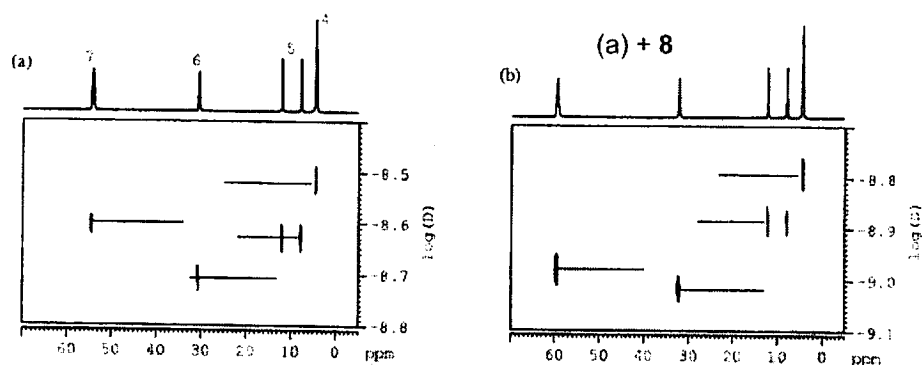
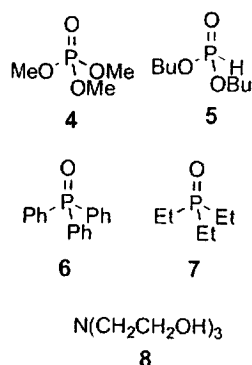


Figure 2. (a) The ^{31}P -DOSY spectrum of a mixture containing 4 phosphorous components viz. trimethyl phosphate (4), dibutyl phosphite (5), triphenylphosphine oxide (6), and triethylphosphine oxide (7); and (b) The ^{31}P -DOSY spectrum of the same mixture containing triethanol amine (8) as an additional component. The BPPLIED sequence was used with gradient strengths of 2 ms duration incremented in 32 steps, with diffusion times of 200 ms for spectrum (a), and 500 ms for spectrum (b)

HR-DOSY as a new tool for the study of chemical exchange phenomena

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By measuring a series of DOSY spectra of a two-site exchange system, where exchange occurs between sites with different diffusion coefficients, it is possible to extract information about the relative exchange rates of protons with the bulk solvent and therefore infer their solvent accessibility.

If exchange is slow compared with the diffusion period, then two diffusion coefficients will result for the same chemical shift. On the other hand, if exchange is fast, then a weighted average diffusion coefficient results.

DOSY of saccharose (Figure 1) was taken in the presence of water.
(DMSO-d₆ : water = 4 : 1)

Are there any differences between hydroxy groups ?

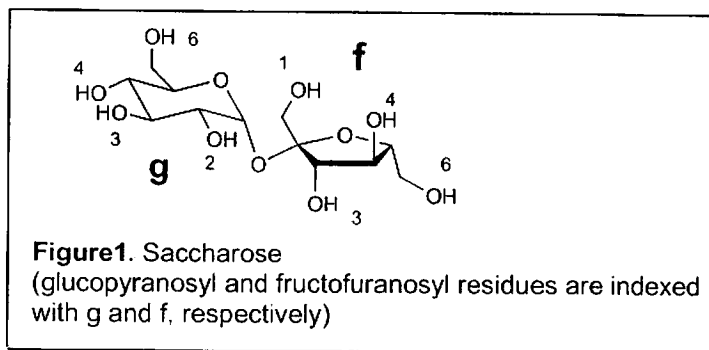


Figure2 (a) : Hydroxy groups are "sugar like".
 (b) : "g3" seems to have different tendency....
 (c) : Hydroxy groups are "water like".

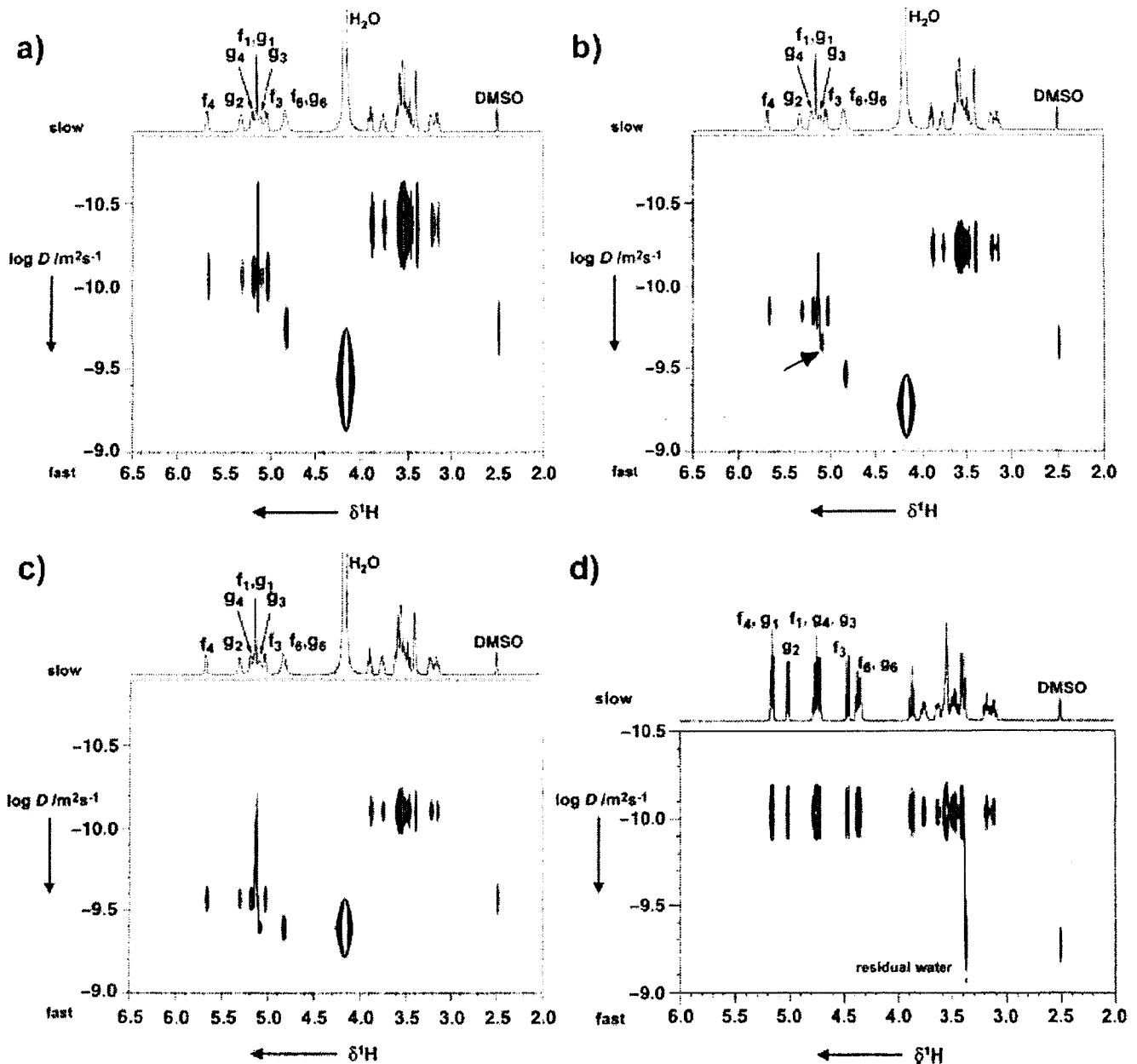
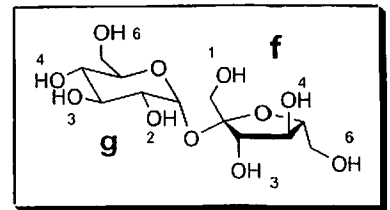


Figure 2. DOSY spectra of sample A acquired for different diffusion times (Δ) of (a) 25, (b) 50 and (c) 200 ms. (d) DOSY spectrum of saccharose in DMSO (sample B before the addition of water) acquired for a diffusion time of 50 ms (the spectra acquired for the diffusion times of 20 and 200 ms are identical). In the projection the hydroxyl protons are assigned using the indexing of Fig. 1; g1 is the anomeric proton.

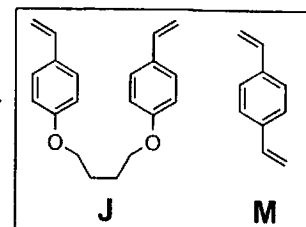
High-Resolution Diffusion-Ordered Spectroscopy To Probe the Microenvironment of JandaJel and Merrifield Resins

Céline Ganbs, Tobin J. Dickerson, Suresh Mahajan, Laura B. Pasternack, and Kim D. Janda*

J. Org. Chem. 2003, 68, 3673.
Tetrahedron Lett. 1999, 40, 6329.

Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

JandaJel: polystyrene resin containing tetrahydrofuran derived cross-linker
Merrifield Resin: polystyrene resin containing divinylbenzene cross-linker



To provide a more detailed picture of the matrix, research has turned to NMR for the determination of the diffusion coefficients of solvents and small molecules in swollen beads to provide a means to compare the microenvironment of swollen beads.

TABLE 1. Measured Diffusion Coefficients ($D_r \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$) for Solvents

	self-diffusion	J-NH ₂	J-OH	M-NH ₂	M-OH
THF	30.5 ± 3.3	24.8 ± 0.3	17.8 ± 0.02	17.8 ± 0.2	14.0 ± 0.1
DMF	17.8 ± 0.4	10.6 ± 0.2	9.1 ± 0.1	8.1 ± 0.04	6.8 ± 0.001
Toluene	30.1 ± 1.2	14.7 ± 0.1	11.9 ± 0.2	12.1 ± 0.2	7.3 ± 0.1

Figure 1

- 1) D_R are 20-30% higher for JandaJel.
- 2) D_R decrease in the order of THF > DMF > toluene.
- 3) D_R are higher for resin-NH₂.

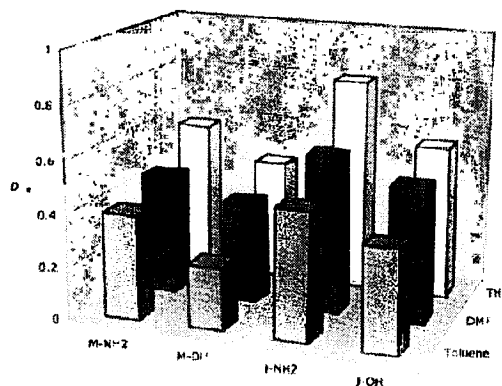


FIGURE 1. Reduced self-diffusion coefficients (D_r) of toluene, DMF, and THF.

Figure 3

Boc-glycine: An ion pair forms between the free carboxylic acid of Boc-glycine and the primary amino group of the resin.

TABLE 2. Measured Diffusion Coefficients for Small Molecules, ($D_r \times 10^{-10} \text{ m}^2 \text{ sec}^{-1}$)

molecule examined	solvent	solution	J-NH ₂	J-OH	M-NH ₂	M-OH
Boc-glycine	DMF- <i>d</i> ₇	8.0 ± 0.3	1.6 ± 0.01	3.2 ± 0.1	1.0 ± 0.03	2.0 ± 0.04
Boc-glycine methyl ester	DMF- <i>d</i> ₇	9.8 ± 0.1	4.2 ± 0.001	3.6 ± 0.1	3.9 ± 0.1	2.5 ± 0.1
<i>p</i> -xylene	DMF- <i>d</i> ₇	14.8 ± 0.2	6.9 ± 0.4	5.8 ± 0.3	5.7 ± 0.4	3.8 ± 0.2
<i>p</i> -xylene	toluene- <i>d</i> ₈	31.7 ± 0.04	9.2 ± 0.4	8.0 ± 0.2	7.9 ± 0.3	5.4 ± 0.2

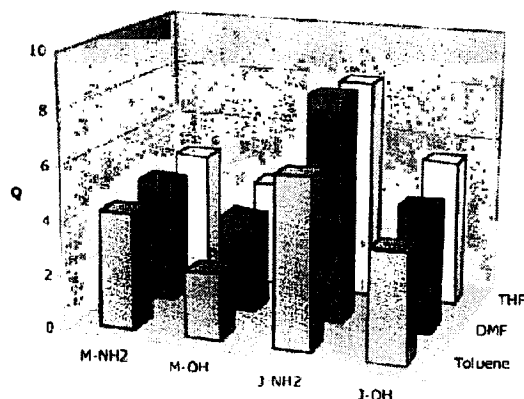


FIGURE 2. Swelling ratio (Q) of resins in DMF, THF, and toluene.

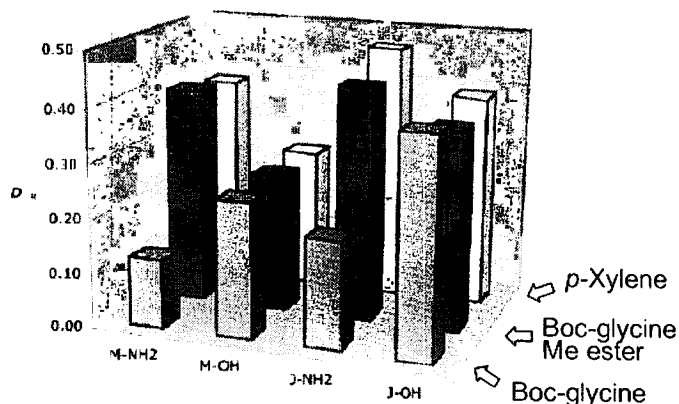


FIGURE 3. Reduced diffusion coefficients (D_r) of Boc-glycine (blue), Boc-glycine methyl ester (maroon), and *p*-xylene (yellow) solutions in DMF-*d*₇.

3. Examples for Metal Complex

Diffusion-Ordered NMR Spectroscopy (DOSY) of THF Solvated *n*-Butyllithium Aggregates

Ivan Keresztes and Paul G. Williard*

Department of Chemistry, Brown University
Providence, Rhode Island, 02912

J. Am. Chem. Soc. **2000**, *122*, 10228.

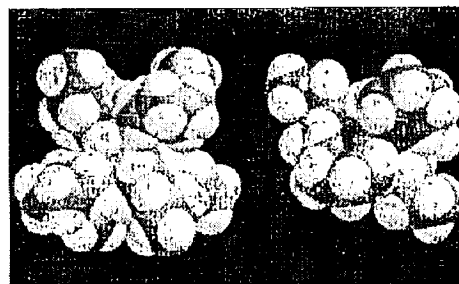


Figure 1. PM3 optimized structures of $[n\text{-BuLi}]_4 \cdot \text{THF}_4$ and $[n\text{-BuLi}]_2 \cdot \text{THF}_4$.

n-BuLi is known to exist in equilibrium between tetrasolvated dimeric and tetrameric aggregates in THF solution. (See Figure 1)

Table 1. Calculated and Measured Diffusion Coefficients for THF Solvated *n*-BuLi Aggregates at -84°C ($\times 10^{11} \text{ m}^2 \text{ s}^{-1}$)

	calculated		experimental ^a	
	X-ray	PM3	1H	7Li
tetramer	8.89	8.54	8.83 ± 0.45	8.63 ± 0.06
dimer	9.94 ^b	9.53	10.11 ± 0.28	n.d. ^c
diff (%)	10.6	10.4	13.2 ± 2.2^d	

^a Average of four experiments. Errors reported are three standard deviations. ^b TMEDA solvated aggregate. ^c Not determined. ^d Determined directly from experimental data.

Figure 4

The first successful example of DOSY to distinguish different organolithium aggregates in solution.

The CH₃ peaks that are not resolved in the NMR spectrum are not resolved in the diffusion dimension either.

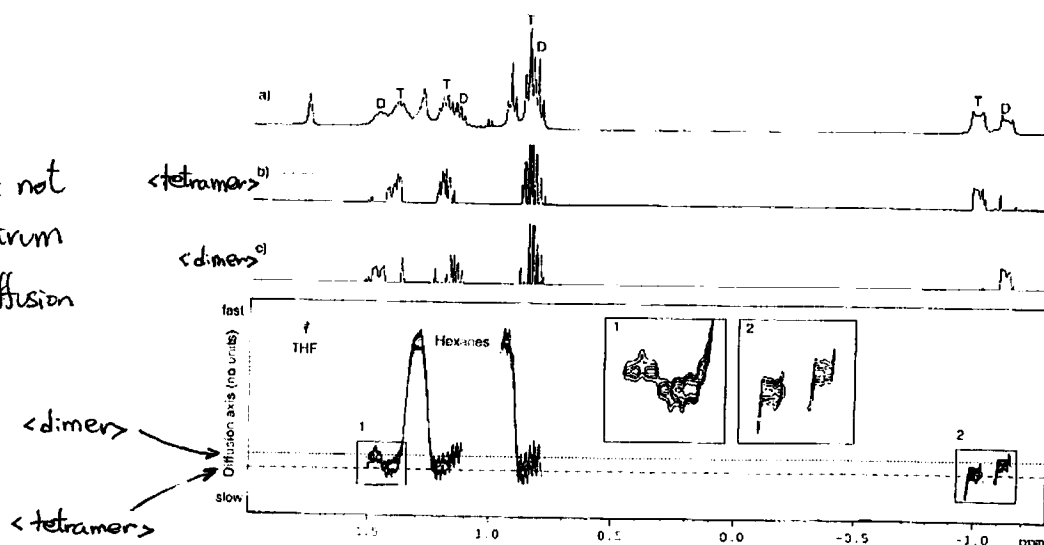
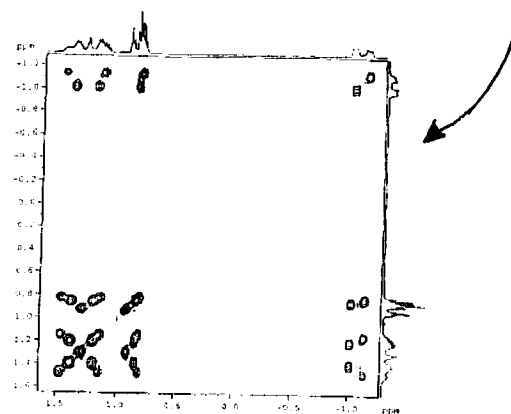
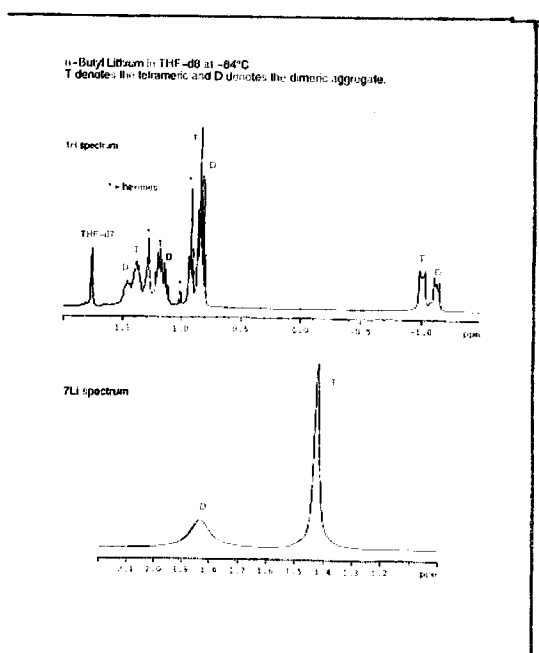


Figure 4. 2D diffusion ordered ^1H spectrum (DOSY) of *n*-BuLi in THF- d_8 at -84°C . (a) ^1H spectrum at 187 K, the labeled butyl resonances of dimeric (D) and tetrameric (T) *n*-BuLi were assigned by 2D-TOCSY. (b) Slice of the 2D DOSY spectrum at the diffusion coefficient of the tetramer (---). (c) Slice of the 2D DOSY spectrum at the diffusion coefficient of the dimer (—)



Applications of Pulsed Field Gradient Spin-Echo Measurements to the Determination of Molecular Diffusion (and Thus Size) in Organometallic Chemistry

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Laboratory of Inorganic Chemistry, ETH Zentrum, CH-8092 Zurich, Switzerland

Organometallics 2000, 19, 2551.

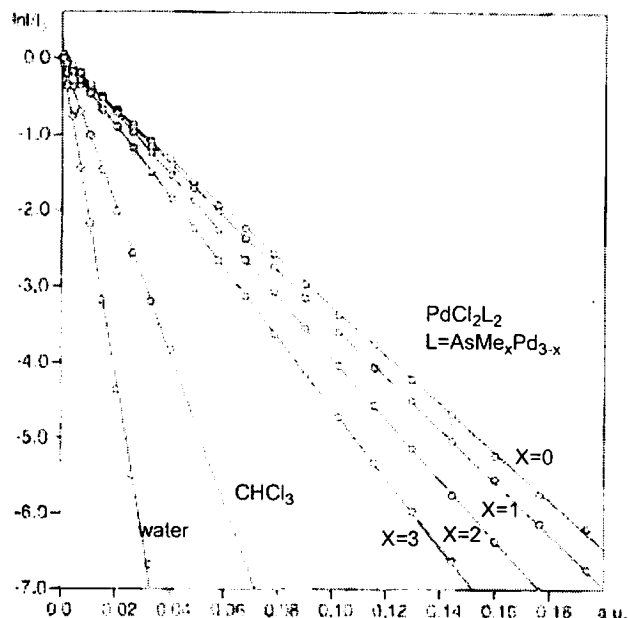


Figure 1. Plot of $\ln(I/I_0)$ vs arbitrary units proportional to the square of the gradient amplitude. The slopes of the lines are related to the diffusion coefficients, D . The six lines stem from water, CHCl_3 , and the four Pd-arsine complexes PdCl_2L_2 ($\text{L} = \text{AsMe}_x\text{Ph}_{3-x}$ ($x = 3-0$)) (increasing molecular weight from left to right). The absolute value of the slope decreases with increasing molecular weight.

$$\ln\left(\frac{I}{I_0}\right) = -\gamma\delta^2 G^2 \left(\Delta - \frac{\delta}{3}\right) D$$

The smaller AsMe_3 complex moves faster than the analogous AsMe_2Ph , which is then faster than the heavier analogues.

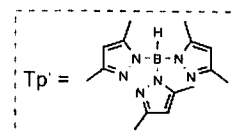
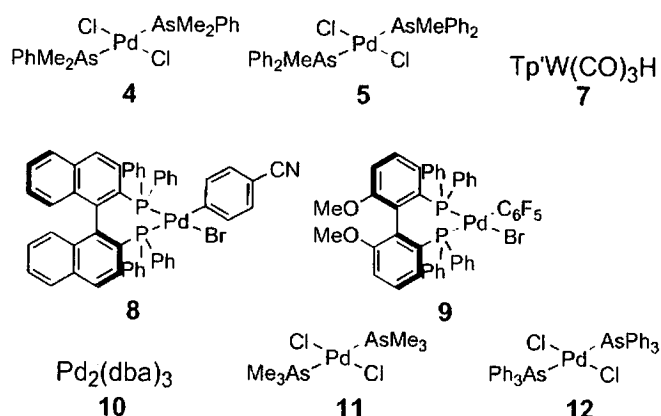


Table 2. Estimated Hydrodynamic Radii, r_H

compd ^a	hydrodynamic radius	radius from X-ray
4	4.8	4.8 ^{b,16}
5	5.4	5.4 ^{b,17}
7	4.6	5.0 ¹⁸
8	7.1	6.8 ¹⁹
9	6.0	6.2 ²⁰
10	6.2	6.3 ²¹
11	4.2	4.1 ^{d,22}
12	5.8	5.8 ^{c,23}

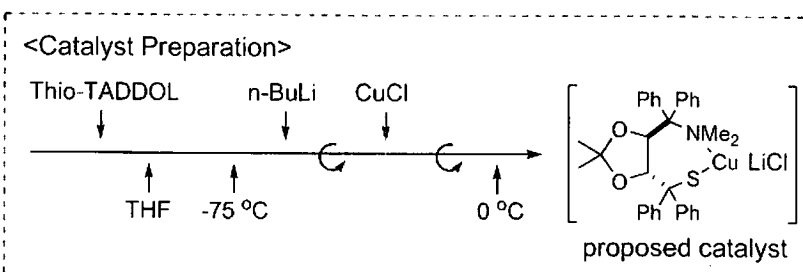
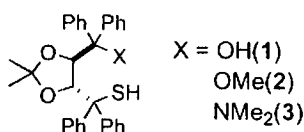
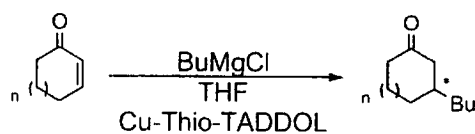
^a For compounds 4, 5, 11, and 12 the radii in the solid state were estimated using reported structures for closely related phosphine, instead of arsine complexes. ^b *trans*- $\text{PdCl}_2(\text{PMe}_2\text{Ph})_2$. ^c *cis*- $\text{PdCl}_2(\text{PMePh})_2$. ^d *cis*- $\text{PdCl}_2(\text{PMe}_3)_2$. ^e *trans*- $\text{PdCl}_2(\text{PPh}_3)_2$.



X-Ray, Molecular Diffusion, and NOESY NMR Studies of Chiral, Tetranuclear Cu^I Catalysts Based on Monodentate Thiol Analogues of TADDOL**

Arkadiusz Pichota, Paul S. Pregosin,*
Massimiliano Valentini, Michael Würle, and
Dieter Seebach*

Angew. Chem. Int. Ed. 2000, 39, 153.
Helv. Chim. Acta 1997, 80, 2515.



{ (S)-product in up to 84% ee (with 1)
{ (R)-product in up to 84% ee (with 2&3)

Figure 2 X-ray crystal structure of 4
-Tetranuclear
-Monodentate thiolate complex

Second donor atom of the TADDOL derivative is not complexed !! (3.9-4.3 Å)

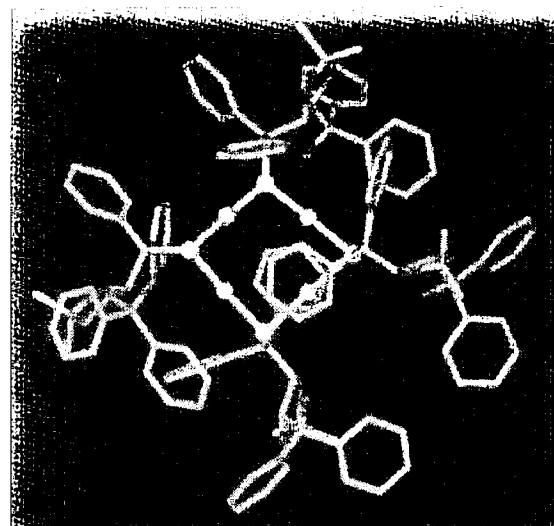
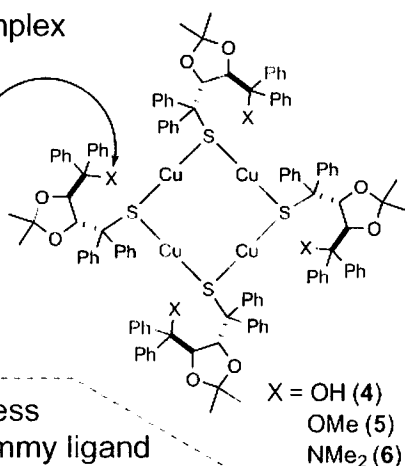


Figure 2. Structure of the tetranuclear Cu complex 4 based on X-ray diffraction data. The H atoms have been omitted for clarity; O atoms are indicated in red, S atoms in yellow, and Cu atoms in blue. The view has been generated with the program insight II (v. 98.0).

4-6 were treated with excess *t*-Butylisocyanide as a dummy ligand to afford the corresponding isocyanide complex 7-9 as shown below.

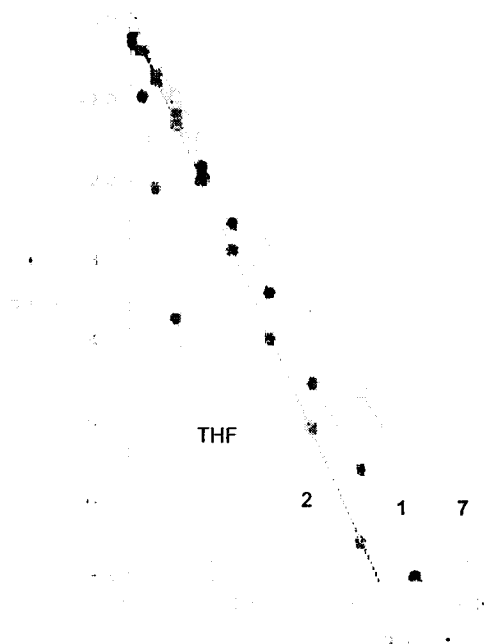


Figure 3. Results of the diffusion measurements using pulsed field gradients³¹ for the isocyanide complex 7 (yellow), the ligand 1 (red), ligand 2 (green), and solvent THF (blue). The slopes are proportional to the diffusion coefficient (*D*) and to the hydrodynamic radii of 7, 1, 2, and THF. The y axis represents a measure of the relative spin-echo signal intensity and the x axis represents the square of the gradient amplitude in arbitrary units (a.u.).

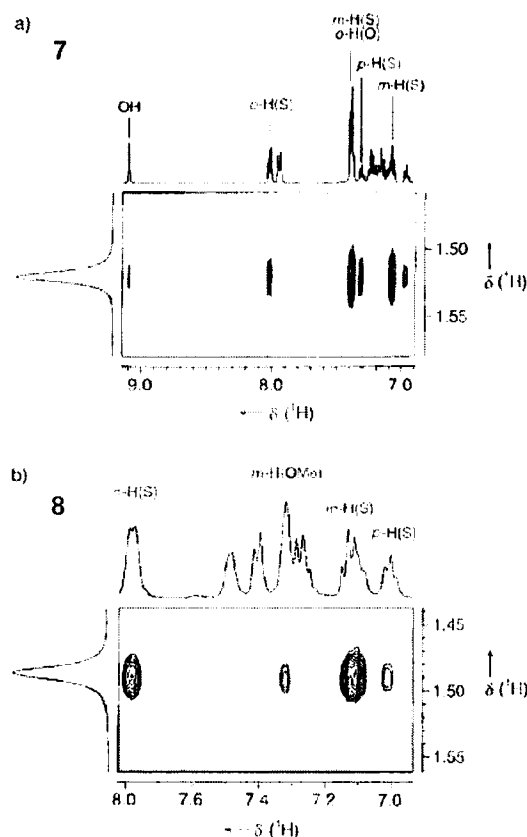
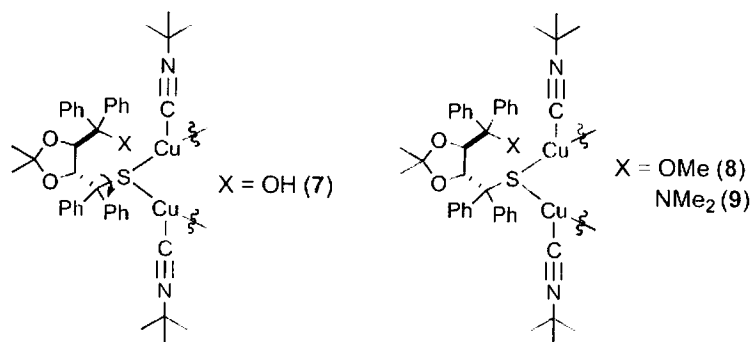


Figure 4. Sections of the NOESY NMR spectrum for the isocyanide complexes 7 (a) and 8 (b). In (a) there are NOEs from the OH proton and the *ortho* phenyl protons of one of the phenyl rings of the PhC(OH) moiety to the methyl protons of the isocyanide. In (b) the only strong NOEs arise from the *ortho* phenyl protons of one of the proximate PhC(S) rings to the methyl protons of the isocyanide. The dynamics for 7 are slightly different than for 8. The letters in parenthesis (O, S, and OMe) refer to aryl protons of the PhC(OH), PhC(S), and PhC(OMe) groups, respectively (400 MHz, [D]₂THF, ambient temperature, $\tau = 650 - 800$ ms).

4. Instruction for Measurement ref) JEOL user's meeting textbook 2000 and 2002 ECX-500 user's manual

- No need for special equipments
(ECX-500 is our choice.)
- Make your sample as stable as possible.
- Density gradient is unfavorable.
- Sample height should be ~40mm.
- Thermal convection generates considerable error on diffusion value.
VT method is strongly recommended.
- Noise effects the resulting spectra. (S/N >500 is better.)
Adjustment of shims is quite important.
- For duplicated peaks, it is difficult to get reliable answer.
- $D = 1 \times 10^{-11} \text{ (m}^2/\text{s)}$ is the minimum limit to detect the signal decay,
which corresponds to $M = 2.19 \times 10^5$ polystyrene.
- Main parameters are Δ , δ and G. ($3[\text{mT/m}] < G < 0.25[\text{T/m}]$)
(Δ is ~100 [ms].)
(δ is ~5 [ms].)



To solve remaining problems, various pulse sequences have been developed.
New sequence and analysing algorithm are still created.
DOSY is developing methodology.

Major applications have been...

- { Analysis on food, perfume and so on.
- { Size of aggregates
- { Supramoleculer
- { Liquid crystalline polymer
- { Structure investigation

Figure 1

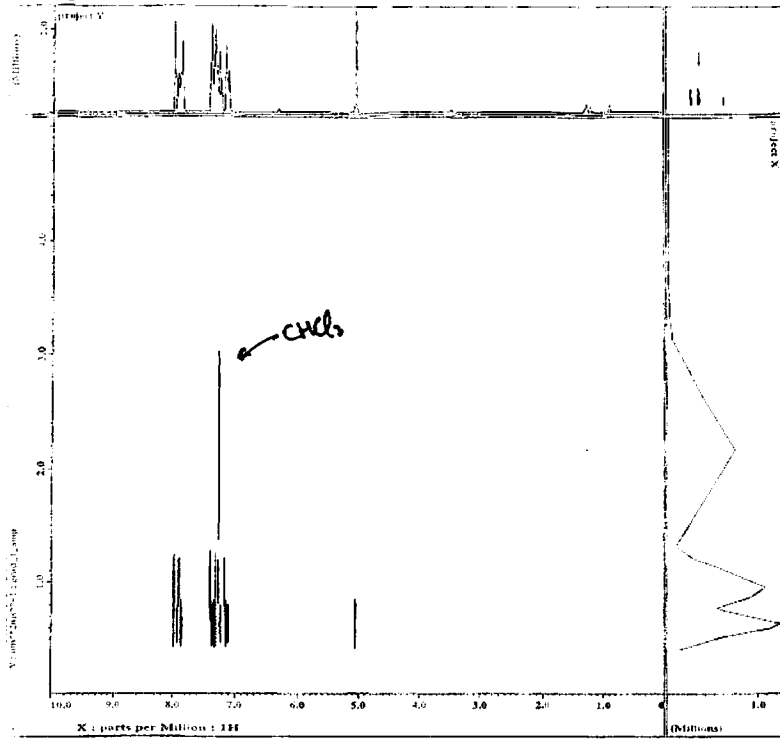
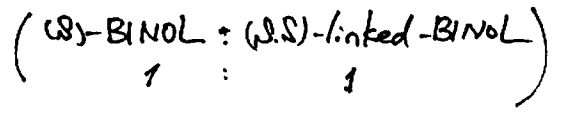


Figure 1.



in $CDCl_3$ at $25^\circ C$

← BINOL
← linked-BINOL

Figure 2.

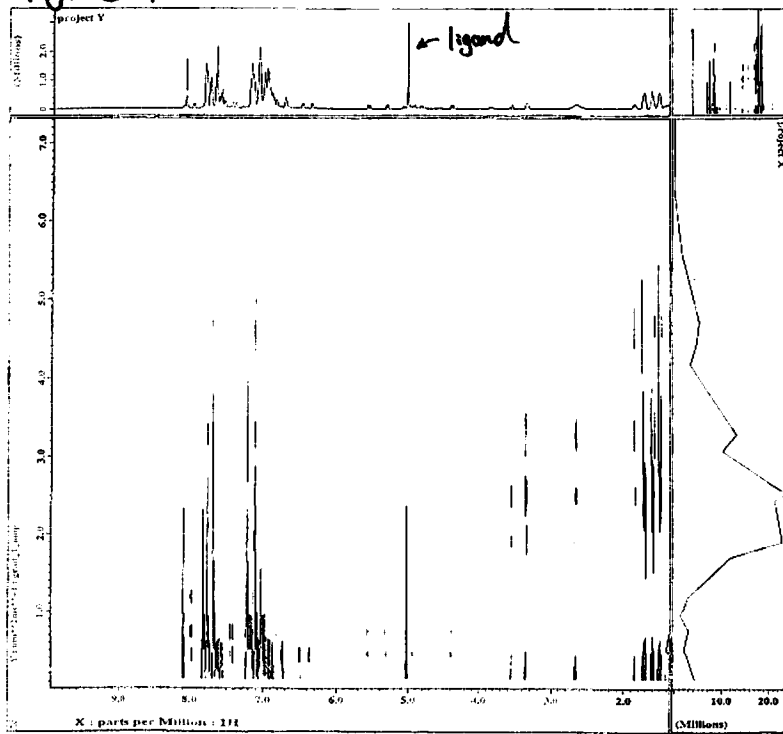
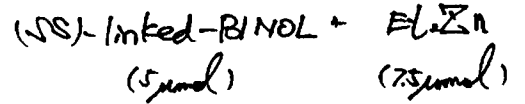


Figure 2.



in $THF-d_8$ at $25^\circ C$

← linked-BINOL ??

] 2:3 complex ??