Determination of Absolute Configuration using Vibrational Circular Dichroism (VCD)

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Boston, Massachusetts
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Outline

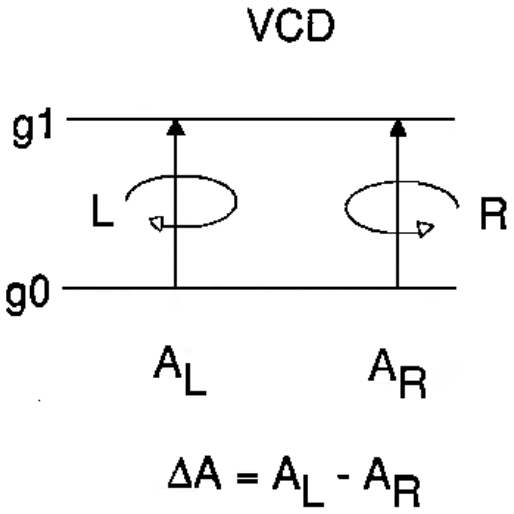
- Definitions of VOA
- Measurement and Calculation of VOA
- Use of VCD for Absolute Configuration and Conformation Determination
  - Following Chirality in Along Synthetic Pathway
  - Determination of Drug-Protein Binding Conformations
- Reaction Monitoring of Chiral Molecules
- Solid-Phase VCD Measurements
  - Use of VCD for Chiral Raw Material ID
  - Quality Control of Chirality in Formulated Drug Solids
- Conclusions

VIBRATIONAL OPTICAL ACTIVITY

Differential Interaction of a Chiral Molecule with Left and Right Circularly Polarized Radiation During Vibrational Excitation

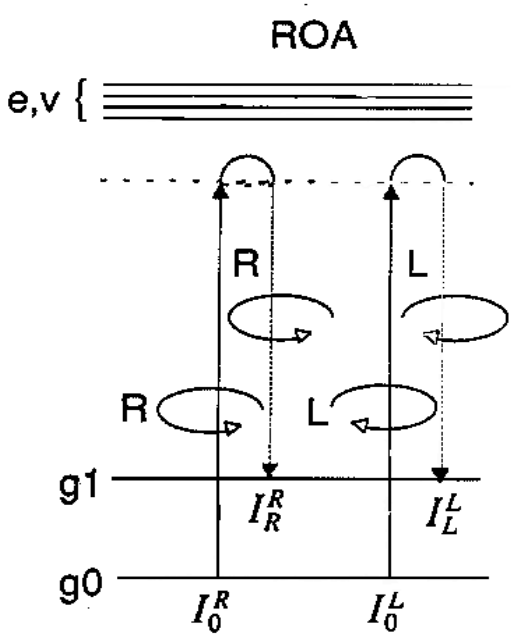
VIBRATIONAL CIRCULAR DICHROISM

Differential Absorption of Left and Right Circularly Polarized Infrared Radiation



RAMAN OPTICAL ACTIVITY

Differential Raman Scattering of Left and Right Incident and/or Scattered Radiation



DCP$_I$-ROA: $\Delta I_I = I_R^R - I_L^L$

Forms of Vibrational Optical Activity

VCD $$\Delta A(\bar{\nu}) = A_L(\bar{\nu}) - A_R(\bar{\nu})$$

ICP-ROA $$\Delta I_\alpha(\bar{\nu}) = I_\alpha^R(\bar{\nu}) - I_\alpha^L(\bar{\nu})$$

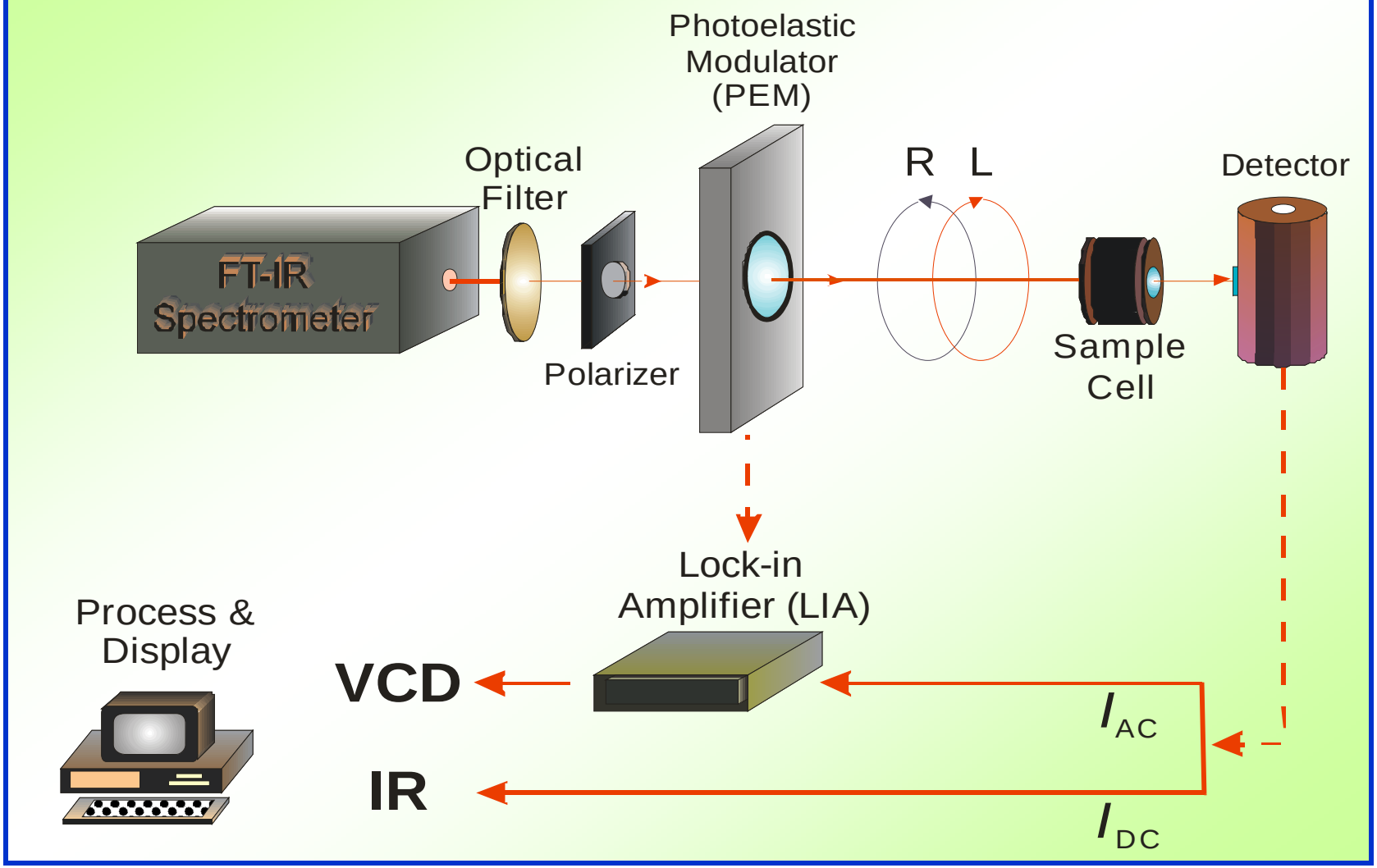
SCP-ROA $$\Delta I^\alpha(\bar{\nu}) = I_R^\alpha(\bar{\nu}) - I_L^\alpha(\bar{\nu})$$

DCP_I-ROA $$\Delta I_I(\bar{\nu}) = I_R^R(\bar{\nu}) - I_L^L(\bar{\nu})$$

DCP_{II}-ROA $$\Delta I_{II}(\bar{\nu}) = I_L^R(\bar{\nu}) - I_R^L(\bar{\nu})$$

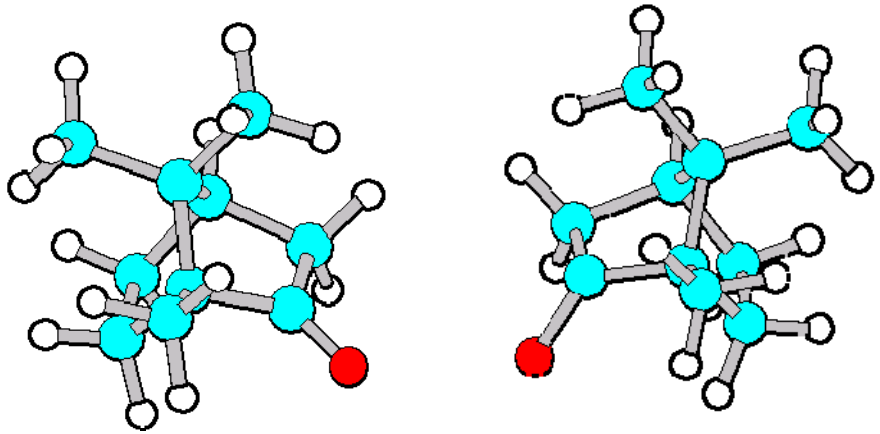
FT-VCD Measurements

FT-VCD Instrumental Layout

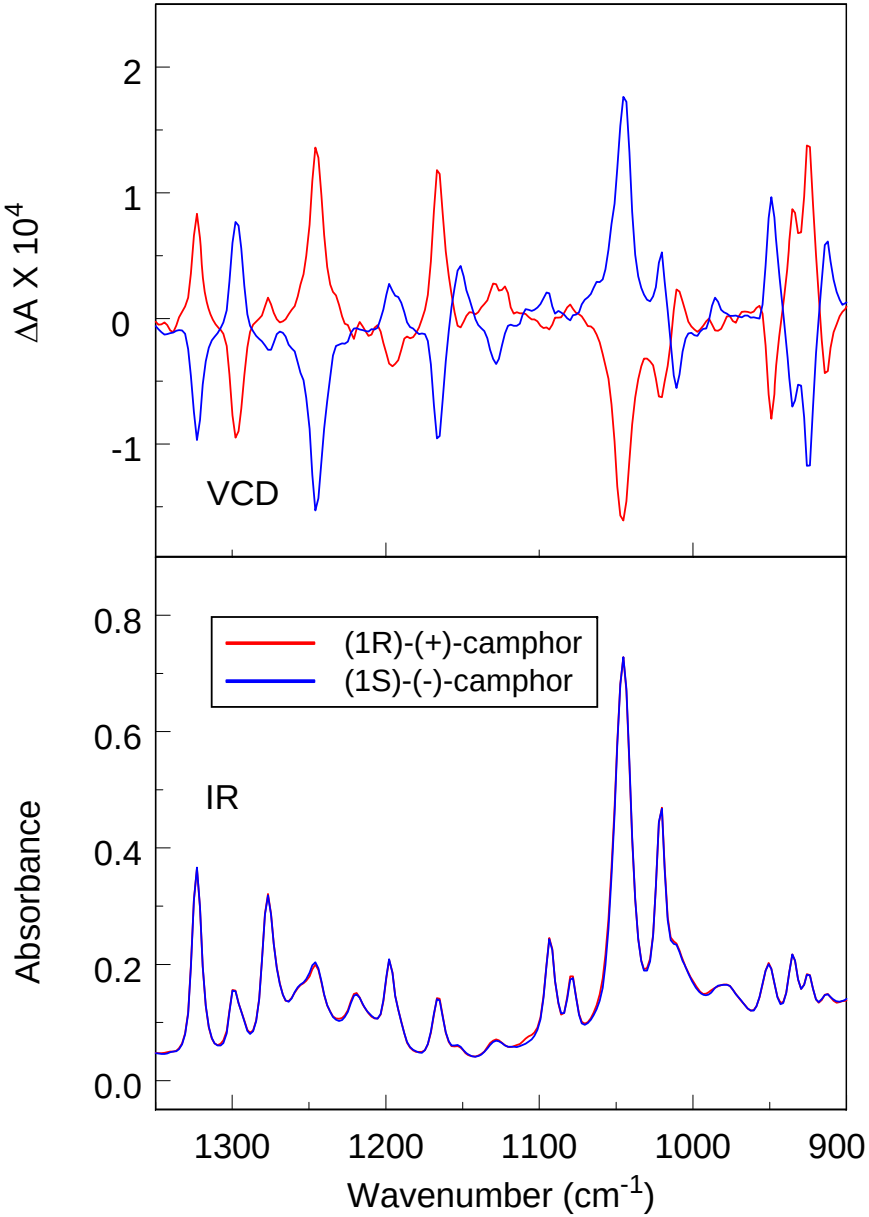



Chiral
BioTools BOMEM

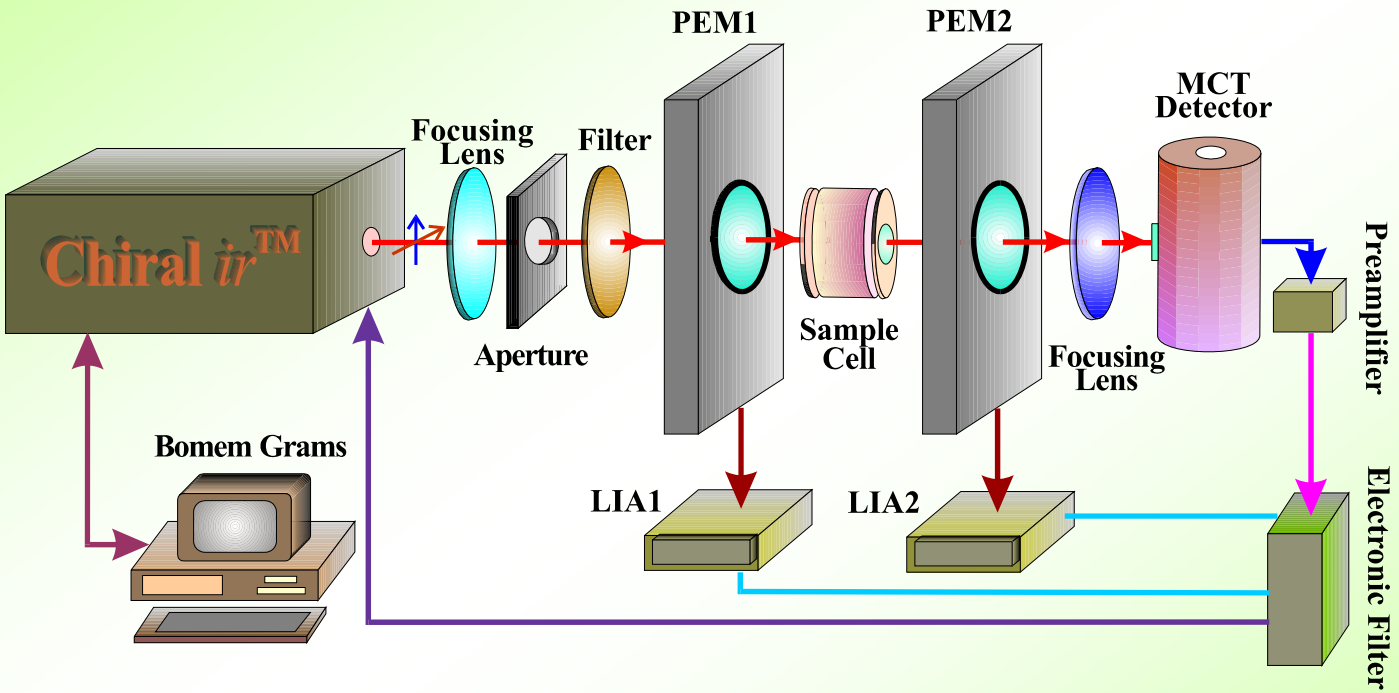
The IR spectra of enantiomers are identical, but their VCD spectra are opposite in sign



(1R)-(+)-camphor (1S)-(-)-camphor



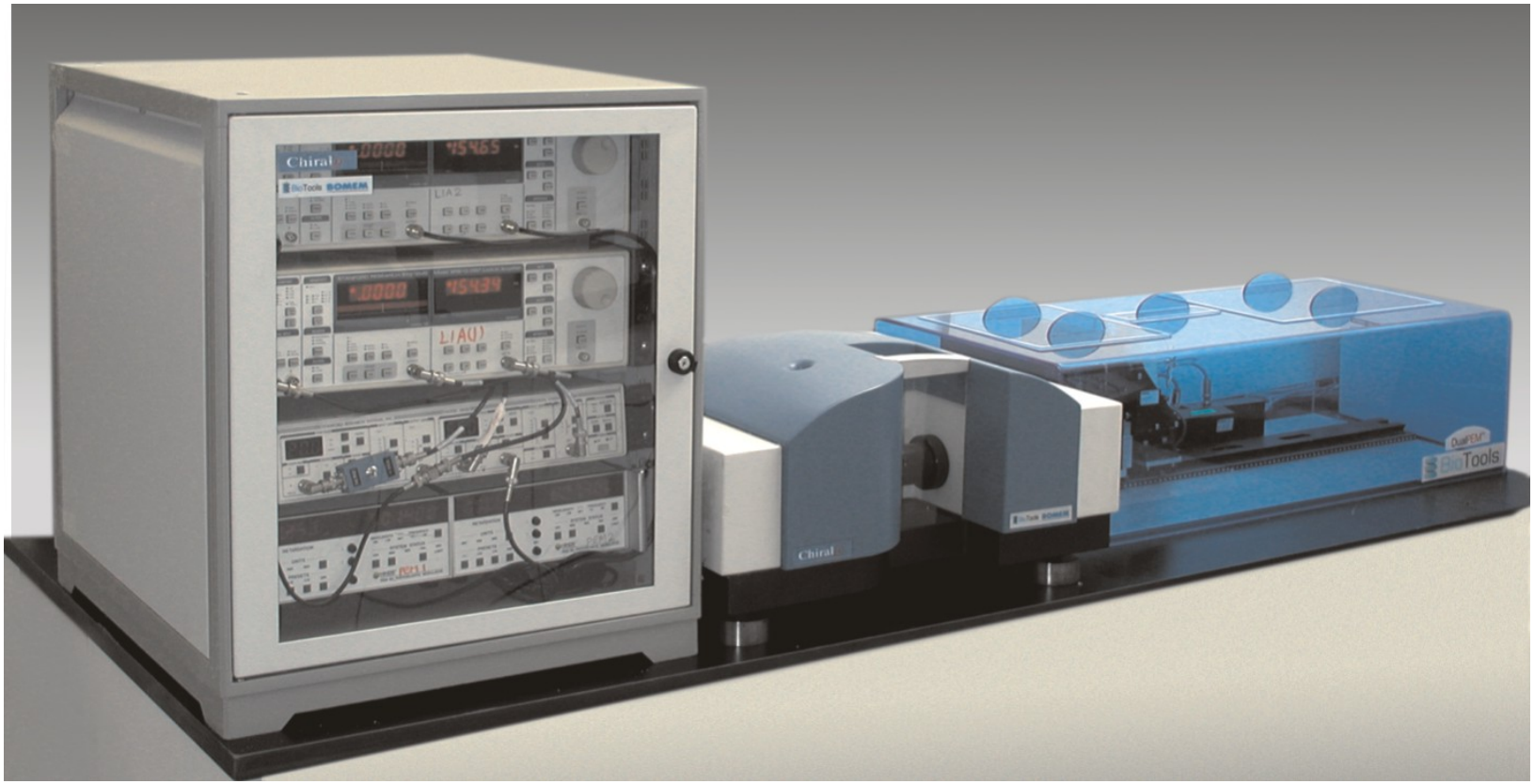
Setup of Dual Polarization Modulation



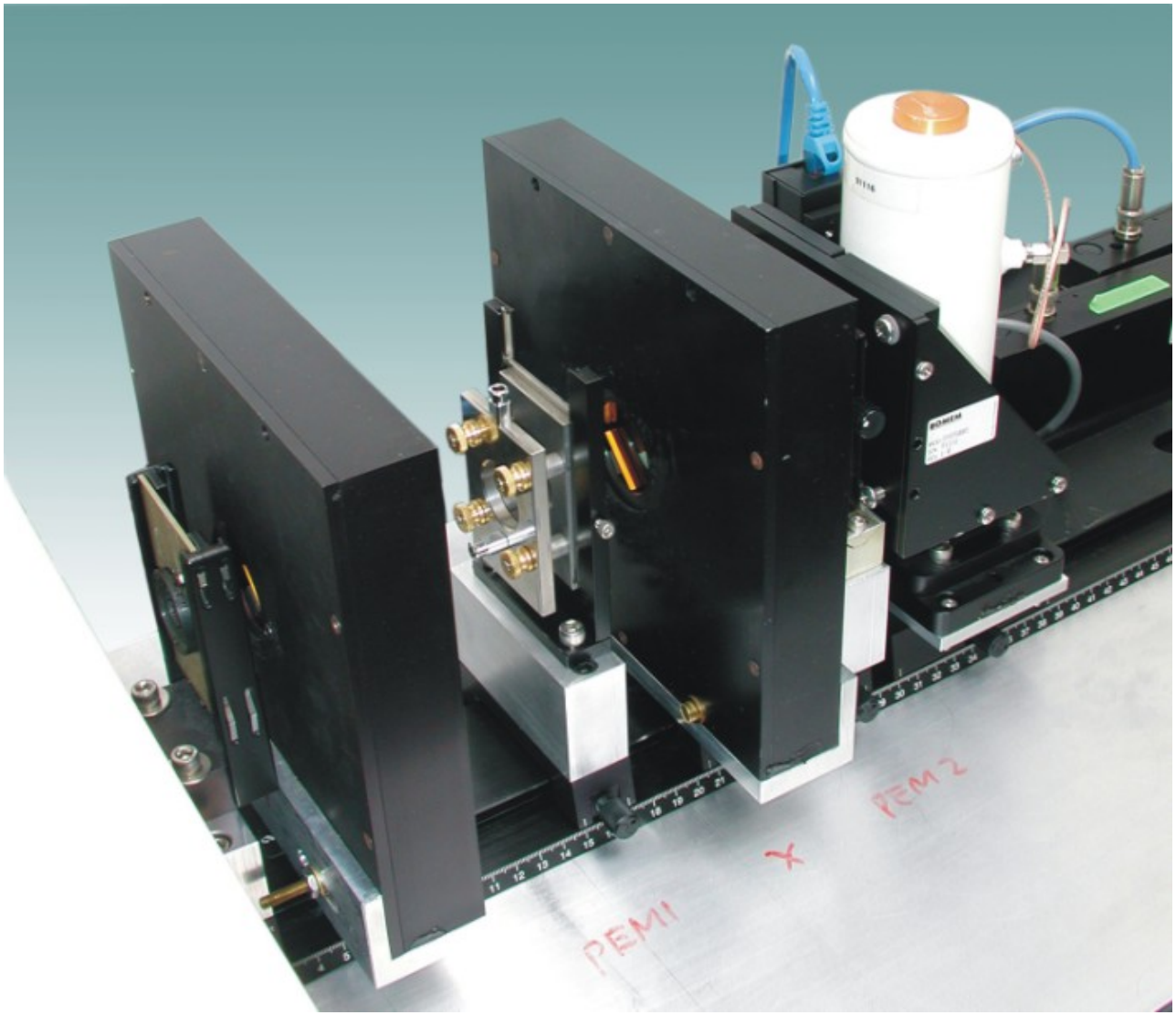
LIA=Lock-in Amplifier; PEM=Photoelastic Modulator with Axes at 45 degrees from Vertical

L.A.Nafie, *Applied Spectroscopy*, 2000, *54*, 1643

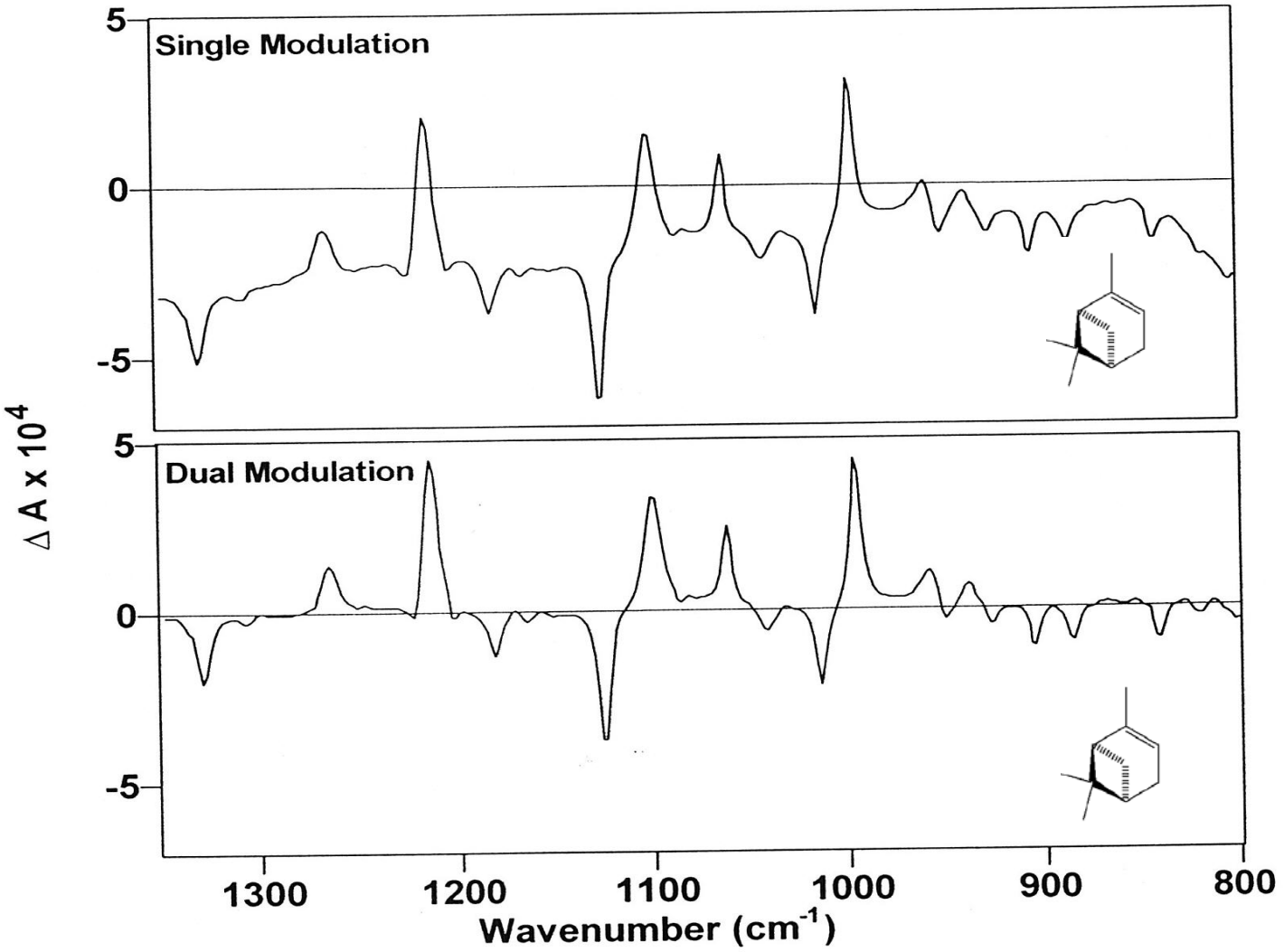
Chiral*IR* with Dual*PEM* accessory



View of Sample Area of Dual*PEM* Setup

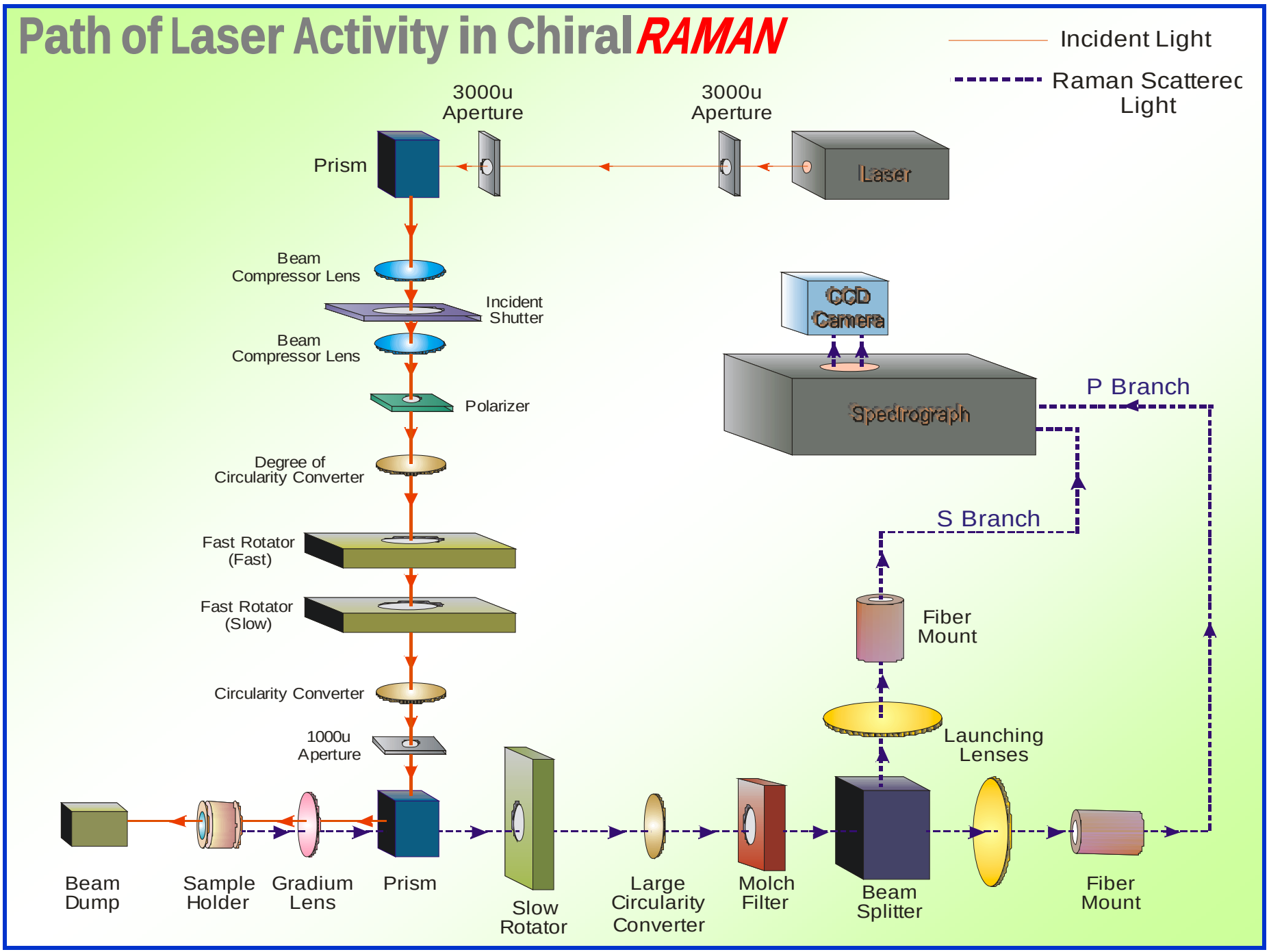


Effect of Dual Modulation
Automatic Baseline Correction

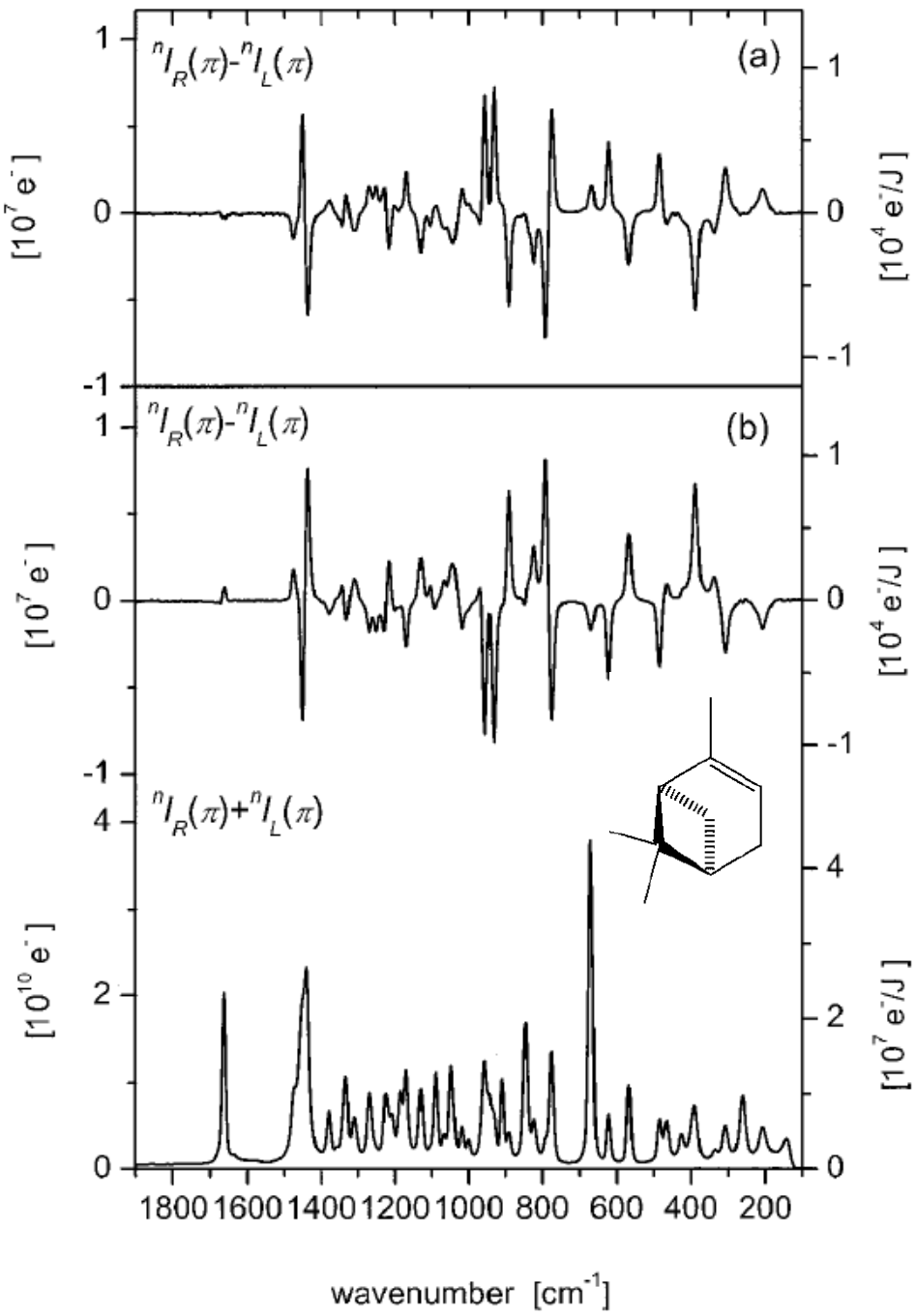


CCD-ROA Measurements

Path of Laser Activity in Chiral *RAMAN*



Chiral*RAMAM* SCP-ROA Spectrometer



Measured SCP-ROA Spectra

(a) ROA of
S-(-)-α-pinene

(b) ROA of
R-(+)-α-pinene

(c) Raman spectrum of
α-pinene

Determination of Absolute Configuration

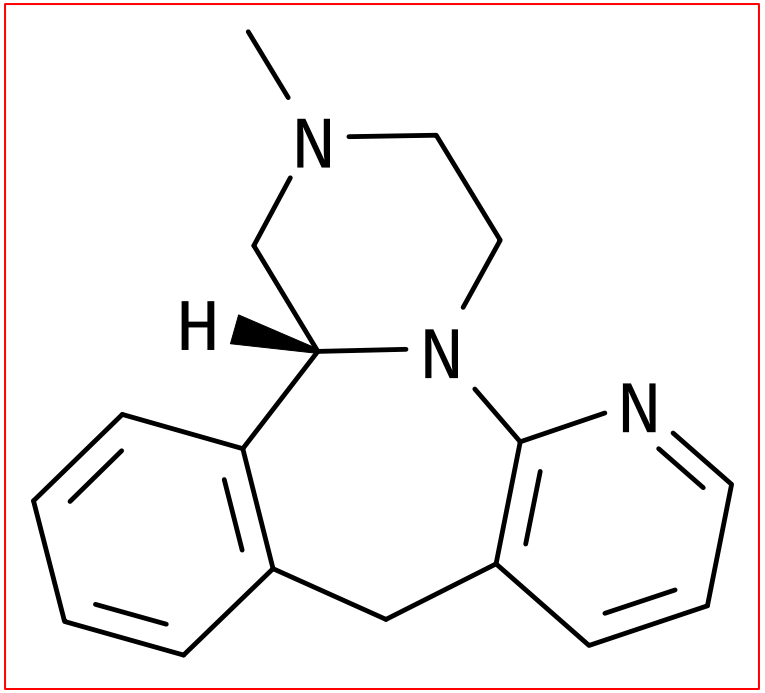
Absolute Configuration

- VCD provides unambiguous determination of Absolute Configuration in solution
- VCD is 'accepted' by regulatory agencies as proof of Absolute Configuration
- VCD is used by major pharmaceutical companies: Abbott, Amgen, Astra-Zeneca (2), Boehringer-Ingelheim, Bristol-Myers-Squibb, Cell Therapeutics, Eli Lilly, Glaxo-Smith-Kline, Johnson & Johnson, Merck, Novartis, Organon, Pfizer, Roche, Sanofi-Aventis, Wyeth, and U.S. FDA

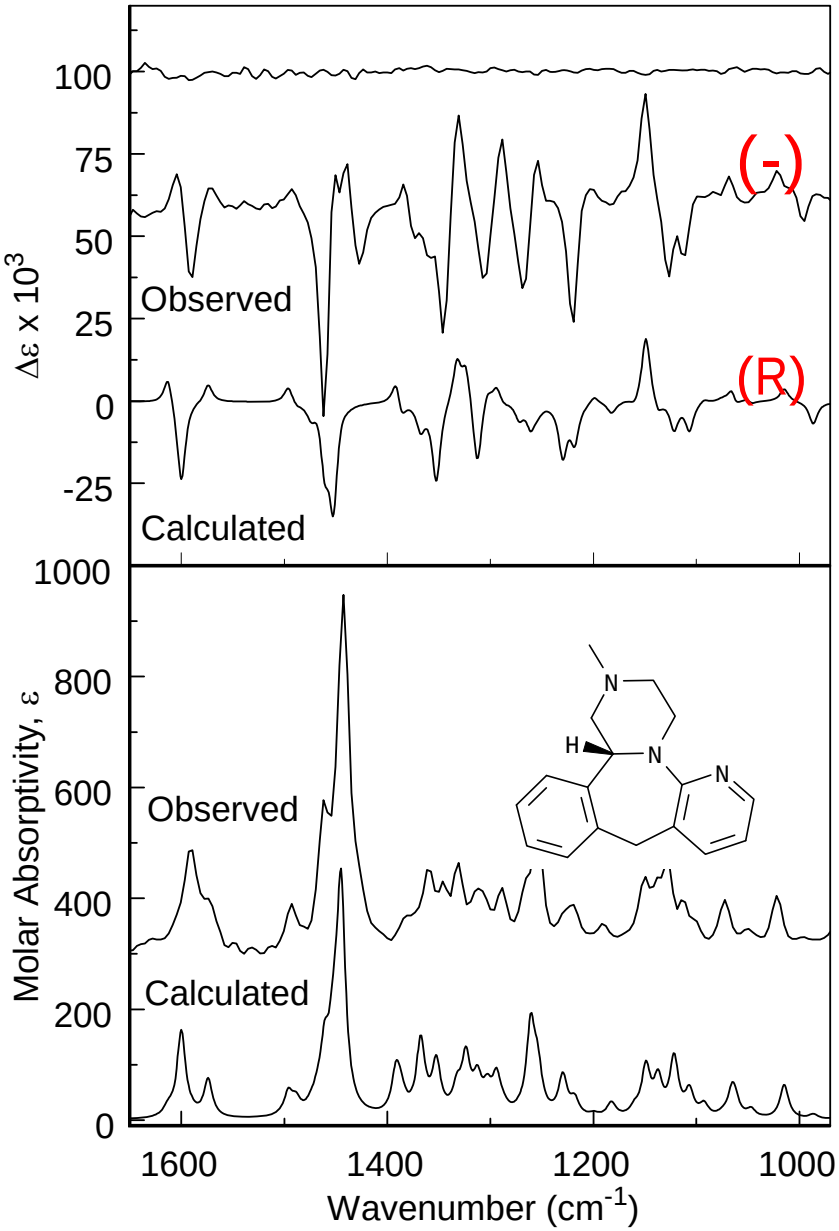
To our estimate over 1000 AC determinations have been performed!

Absolute configuration of a Mirtazapine enantiomer

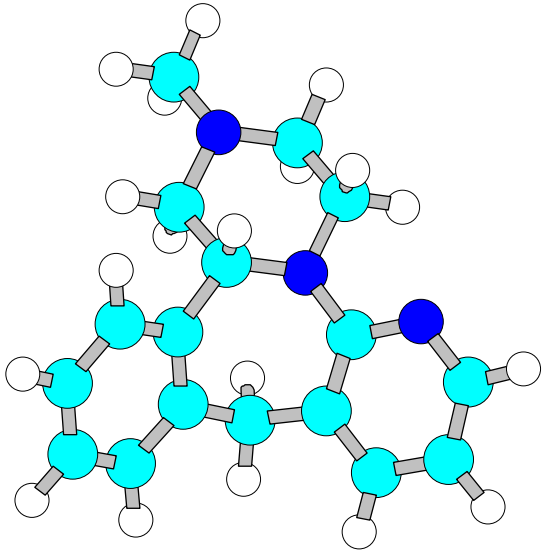
- Mirtazapine the active ingredient of an antidepressant drug
- 20 heavy atoms
- 1 chiral center



Drs. Edwin Kellenbach, Organon Laboratories, Riom, France & Petr van Hoof, Organon NV

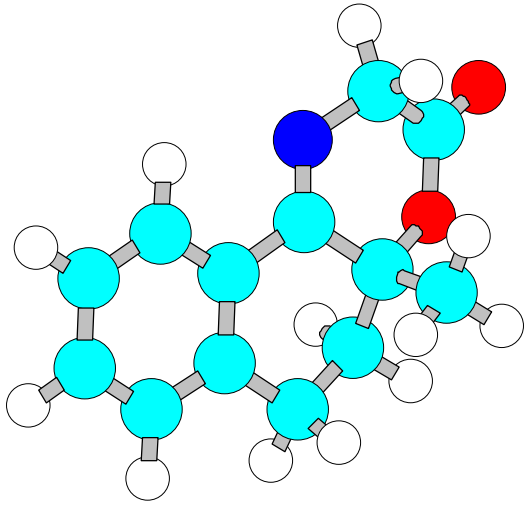


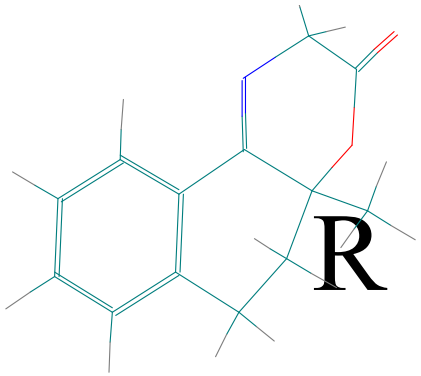
Helv. Chim. Acta, 85, 1160 (2002)

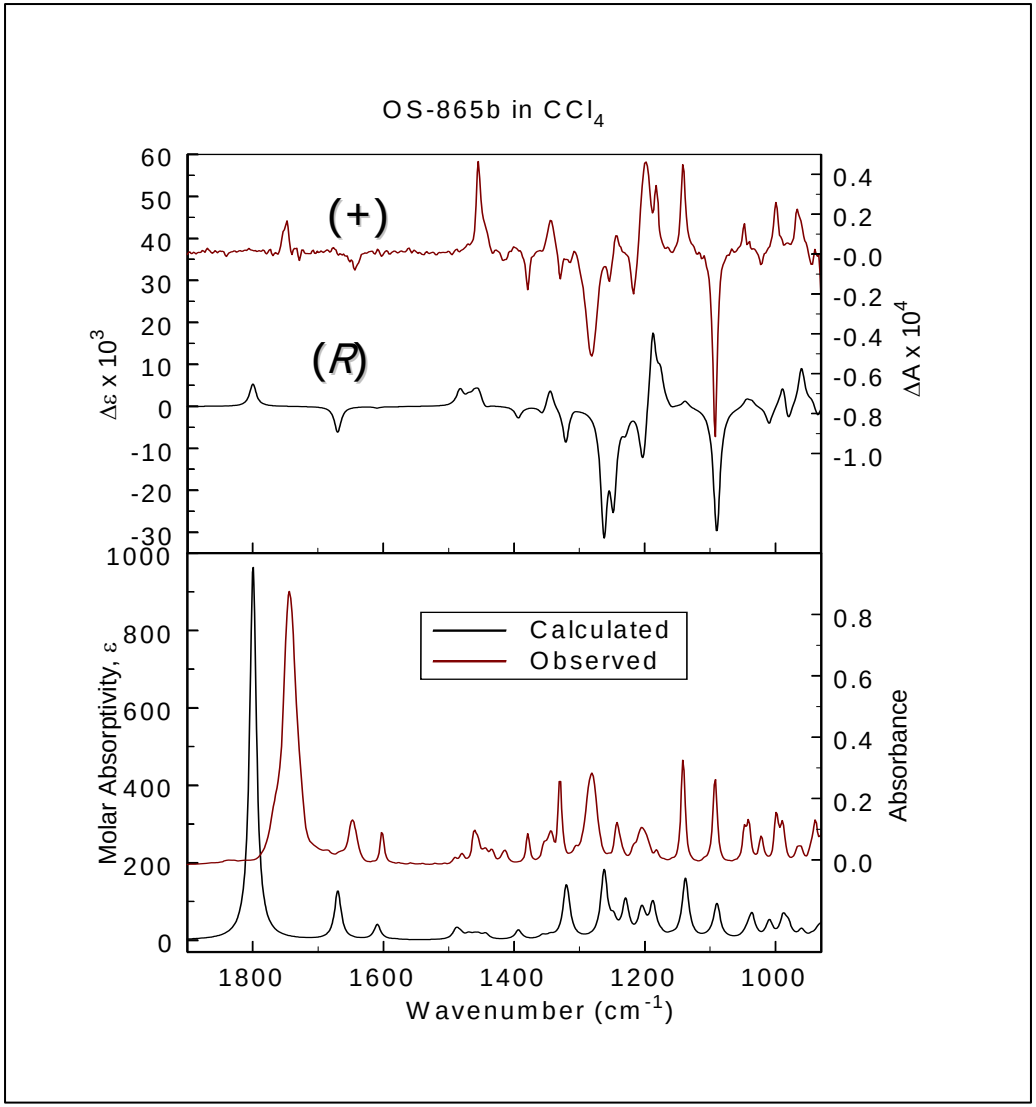


Vibrational circular dichroism (VCD) is used to identify ***unambiguously*** the absolute configuration of Mirtazapine as ***(-)-R*** and solution conformation as shown above

New Iminolactone: 16 heavy atoms, 1 chiral center







Professor Arlette Solladie-Cavallo, University of Strasbourg

Tetrahedron Asymmetry, 12, 2703, 2001

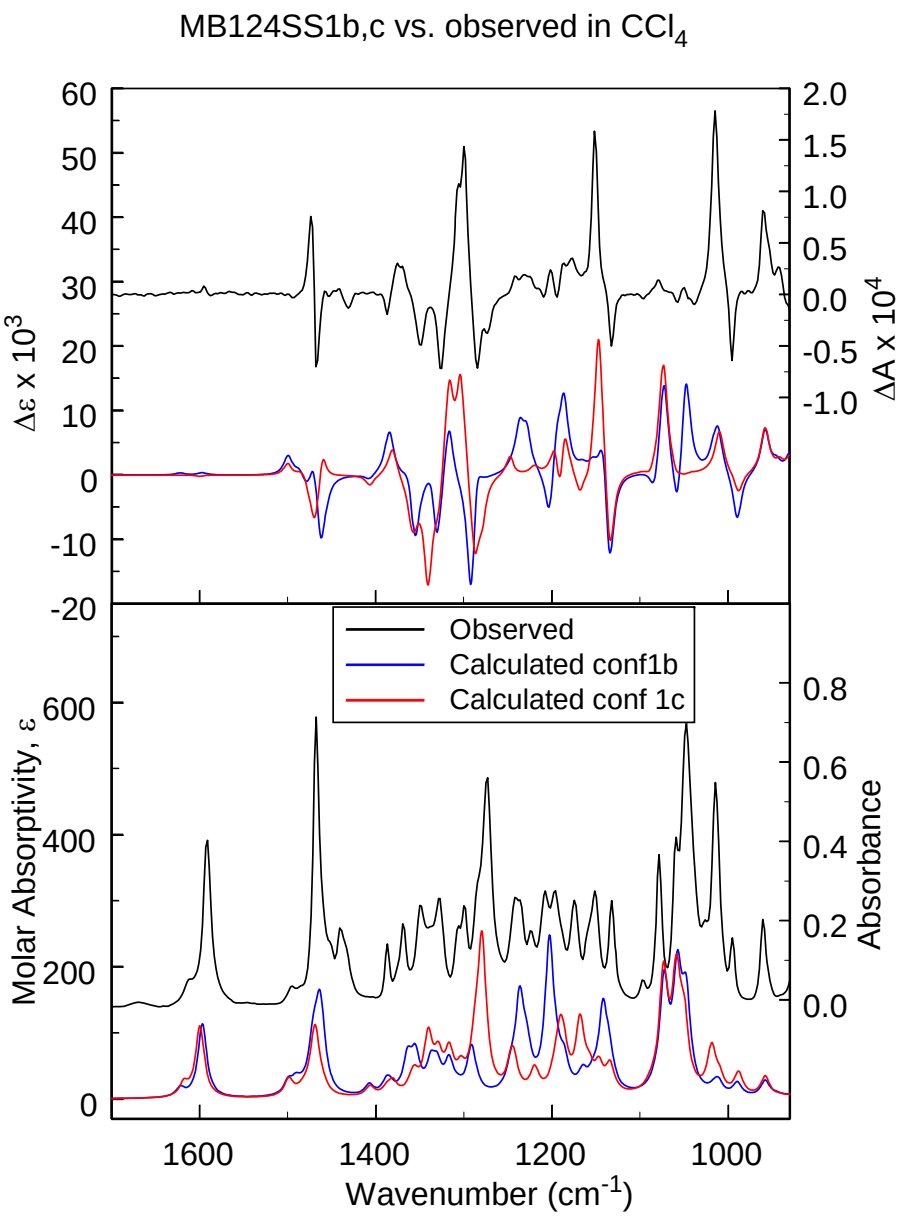
Absolute Configuration with VCD

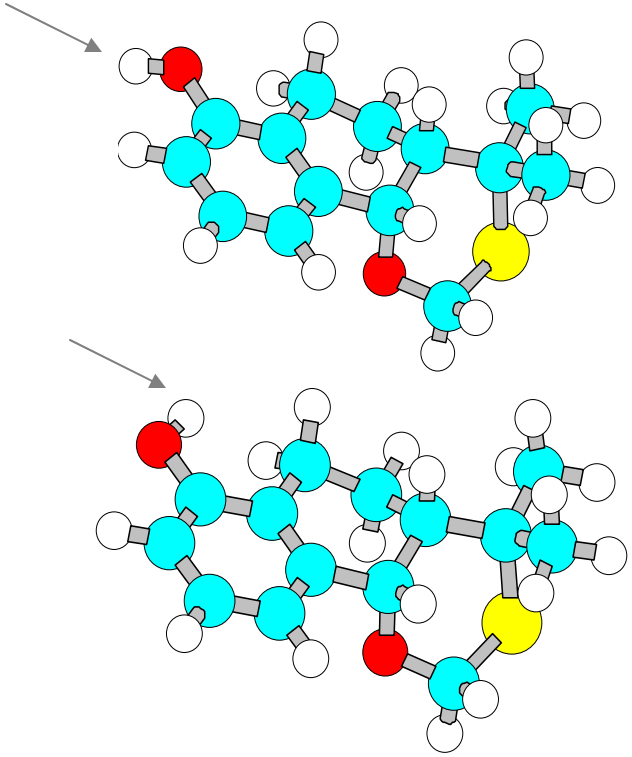
- No need for crystallization
- Solution phase sampling only
- *Ab-initio* calculations require only ground electronic state calculation
- Solution conformation obtained from analysis
- Software for VCD calculation is now commercially available (Gaussian98/03 – *gaussian.com* and BioTools, *btools.com*)

Solution-State Conformational Analysis

Key Points of VCD Conformational Analysis

- Optical spectra are linear superpositions of conformational populations with sub-picosecond temporal resolution
- X-ray conformational structures are limited to single-crystal packing environments
- NMR conformational analysis is limited to populations exchanging slower than the NMR timescale: microseconds
- Matching experimental VCD spectra to ab initio VCD spectra determines the solution state conformation of the molecule

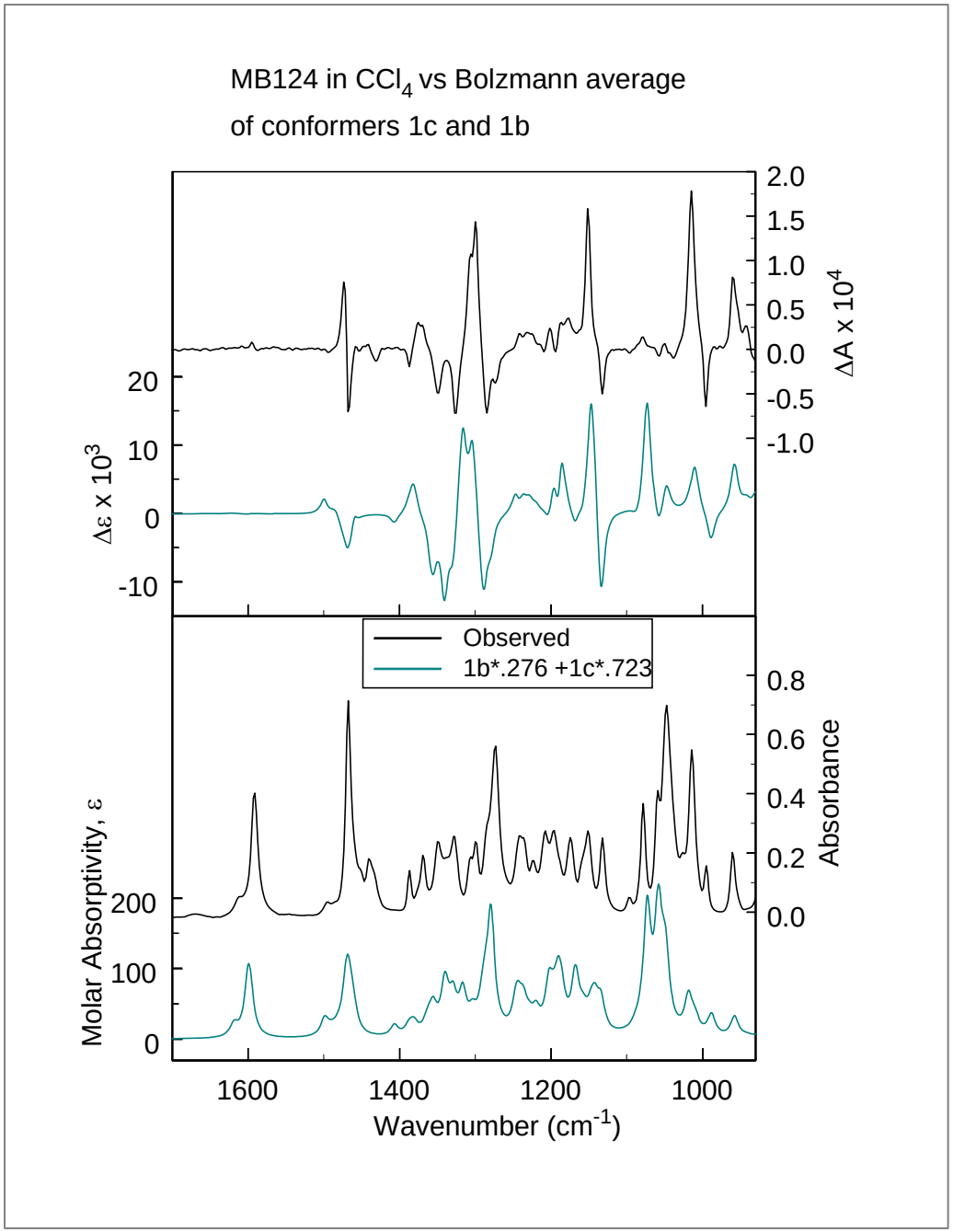




New Oxathiane:
17 heavy atoms
2 chiral centers

Professor Arlette Solladie-Cavallo, University of Strasbourg

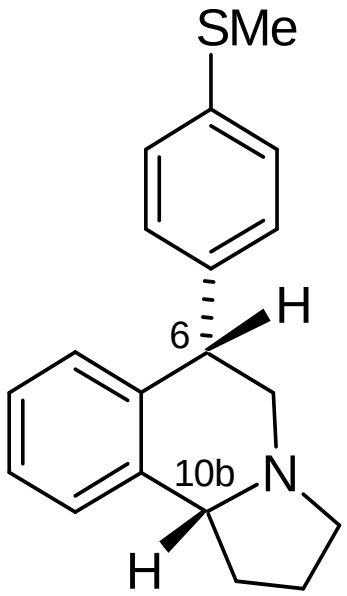
Tetrahedron Asymmetry, 12, 2605, 2001



New Oxathiane:
17 heavy atoms
2 chiral centers

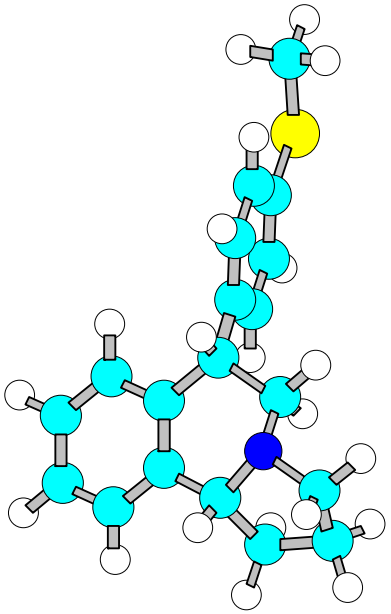
Boltzmann Average:
27% Conformer 1b
and
73% Conformer 1c

McN 5652-X Inhibitor of Seratonin Reuptake

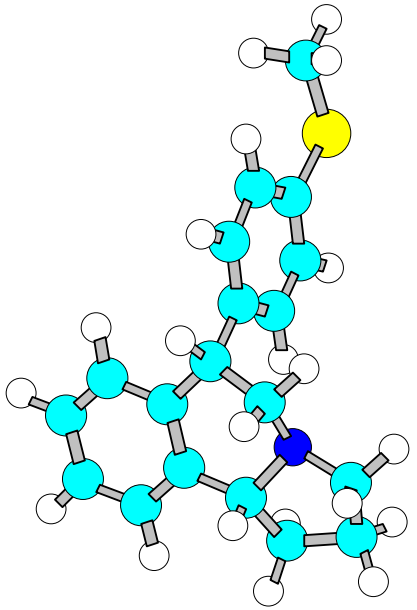


(+)-**1** (McN-5652-X)

trans-(6*S*,10b*R*)

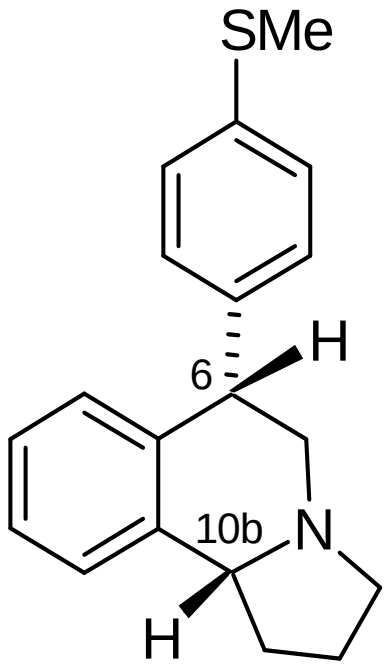


Conformer SRa
+1.63 kcal/mol



Conformer SRb
0.0 kcal/mol

Bruce E. Maryanoff, David F. McComsey, Rina K. Dukor, Laurence A. Nafie, Teresa B. Freedman, Xiaolin Cao, and Victor W. Day, *Biorg. Med. Chem.*, **11**, 2463-2470, (2003).



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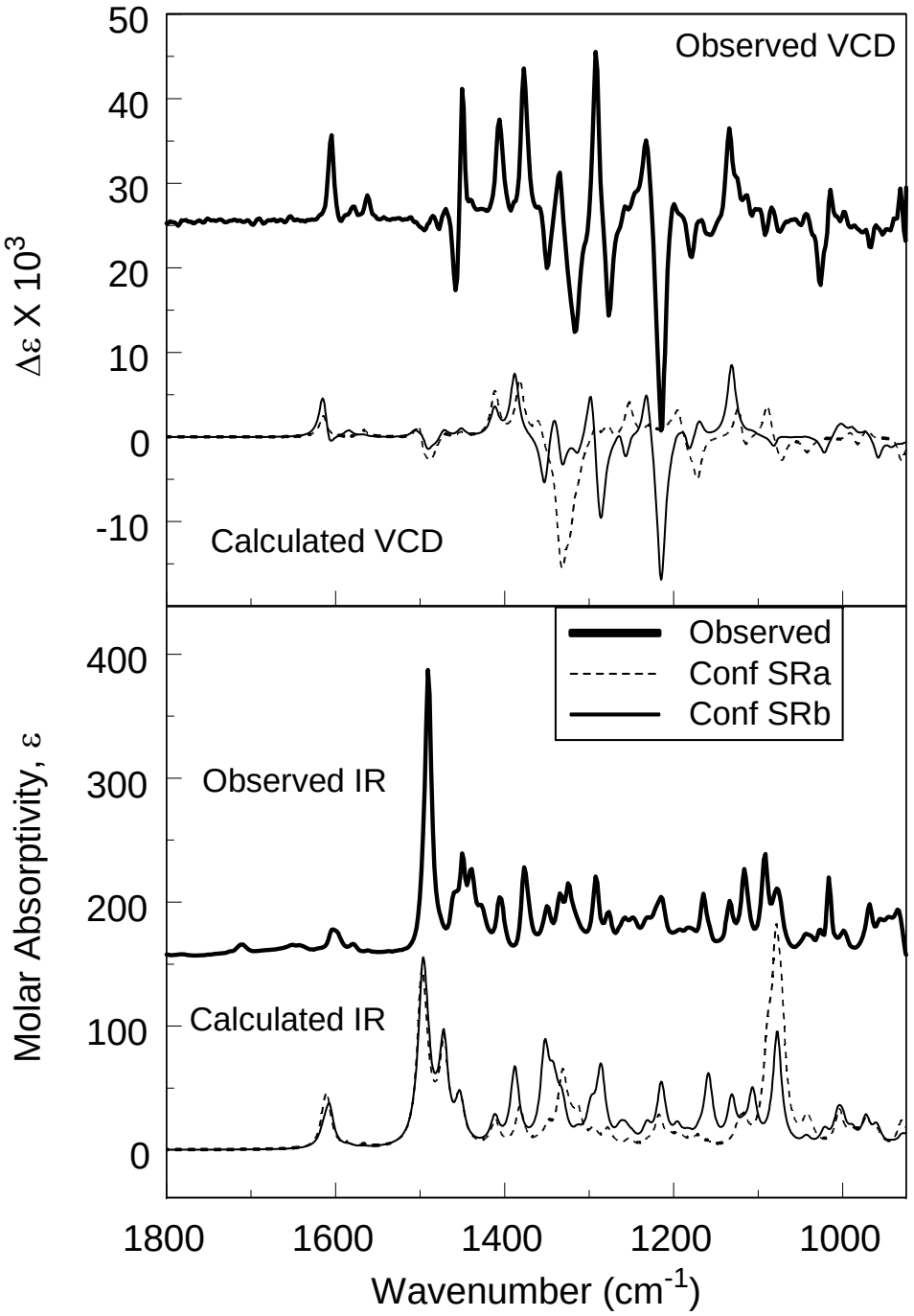
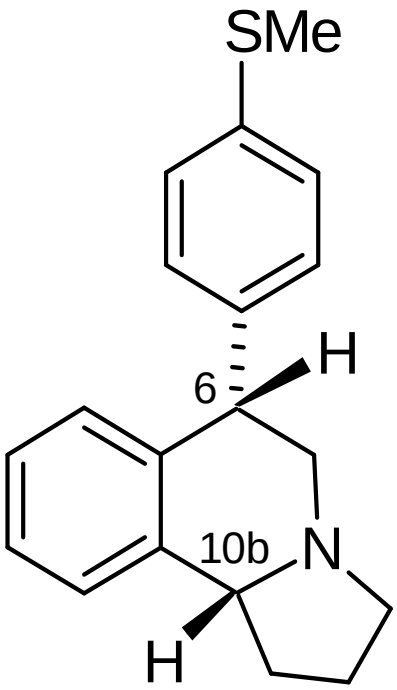
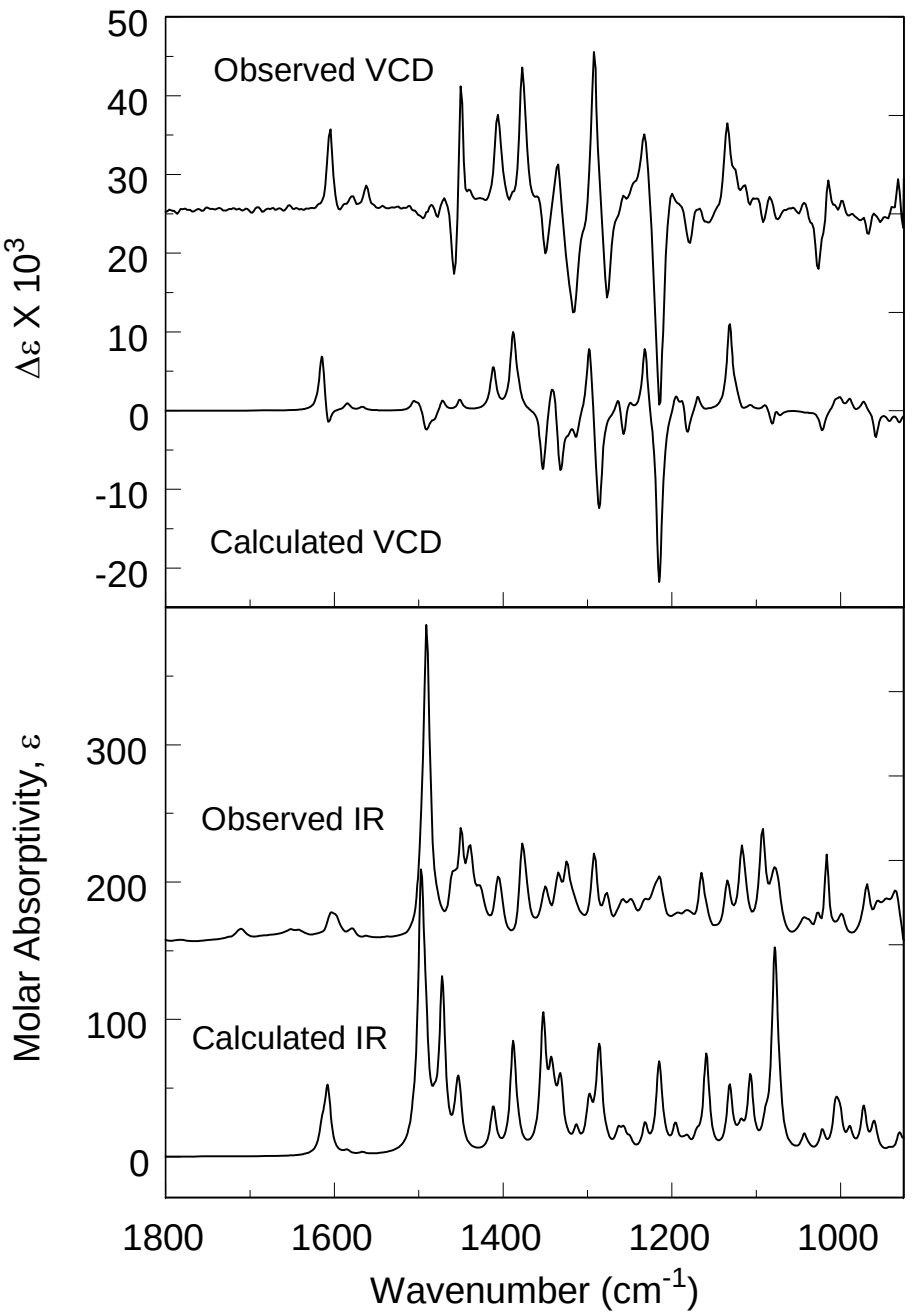




(+)-**1** (McN-5652-X)

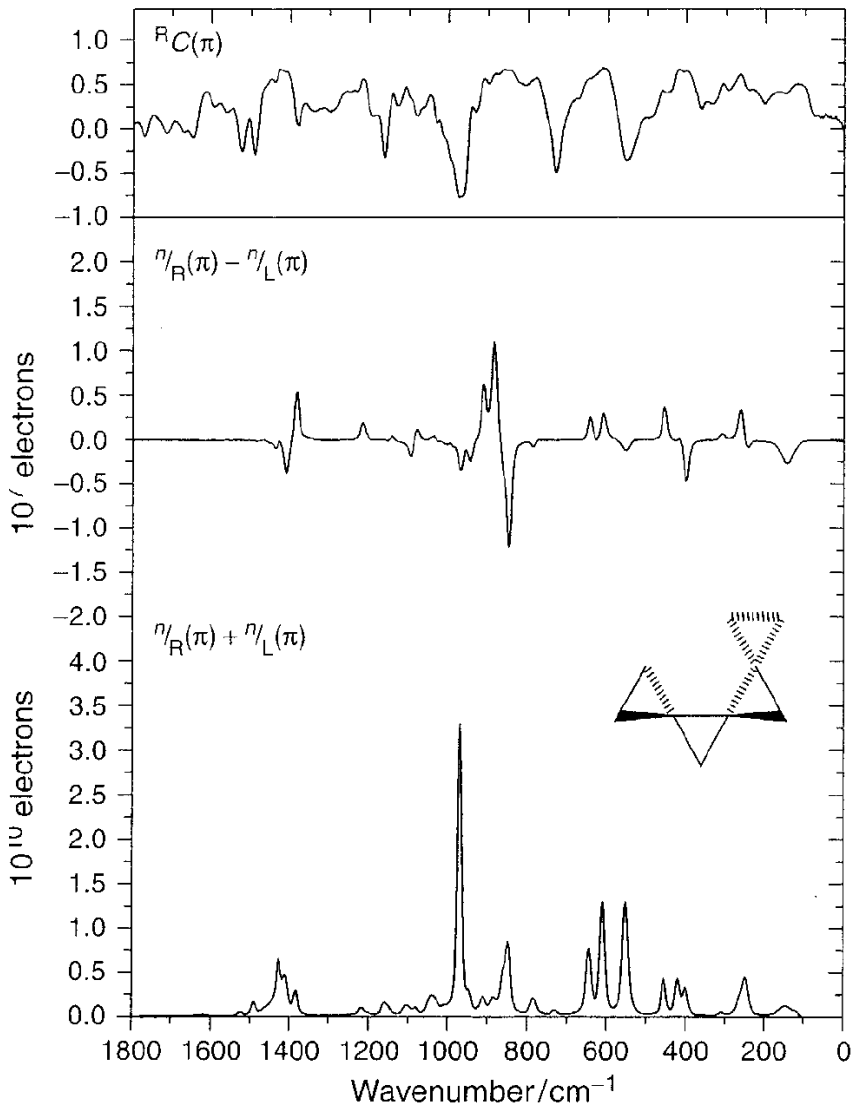
trans-(6*S*,10b*R*)

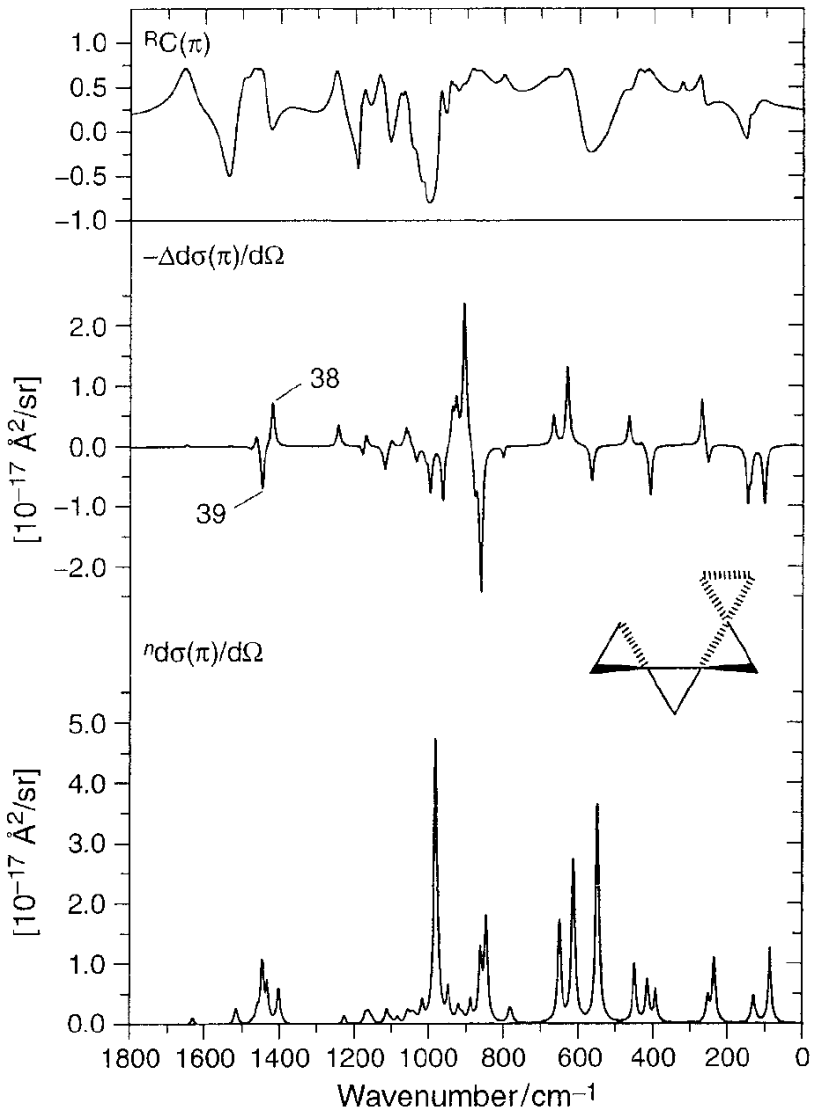
Composite VCD
85% SRb
15% SRa



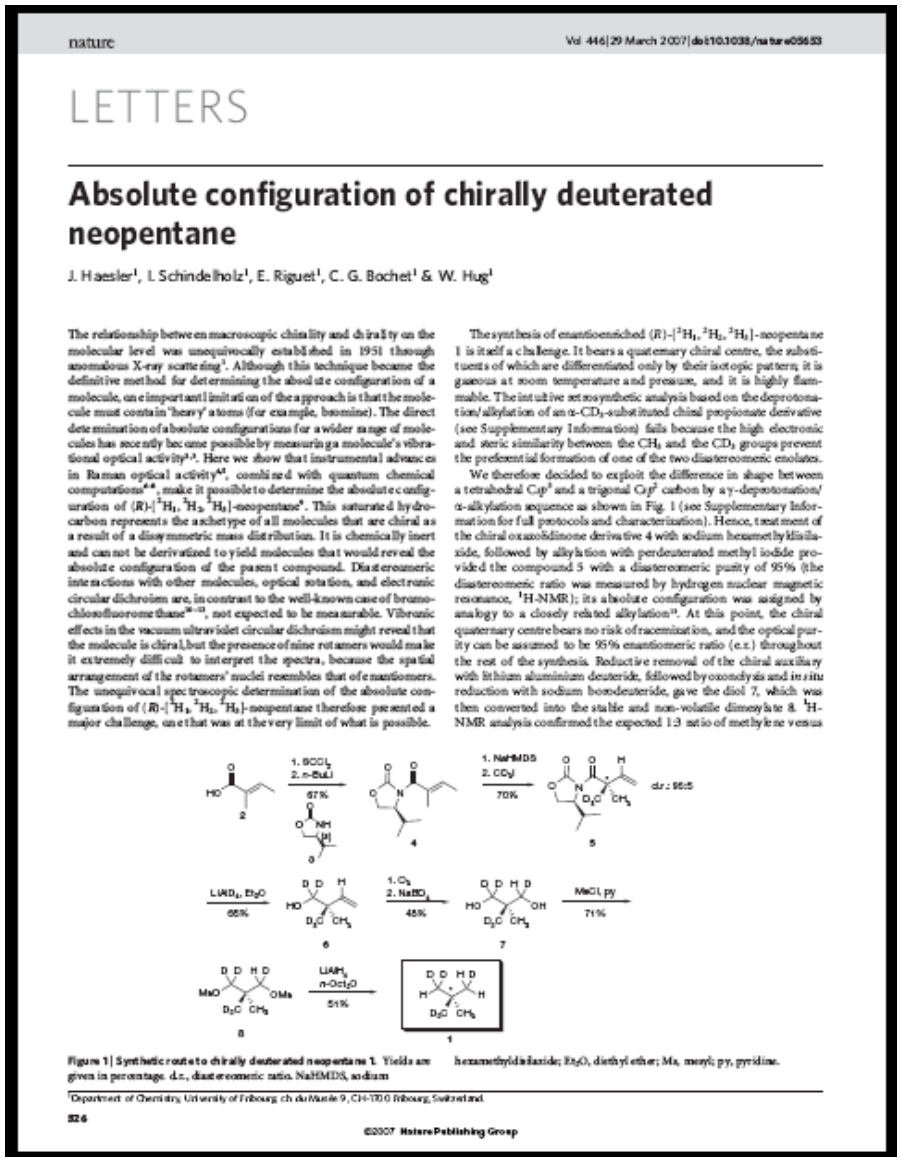
Absolute Configuration Determination using Raman Optical Activity

Comparison of Measured and Calculated SCP-ROA





Absolute Configuration using ROA



nature Vol 446|29 March 2007|doi:10.1038/nature05653

LETTERS

Absolute configuration of chirally deuterated neopentane

J. Haesler[1], I. Schindelholz[1], E. Riguet[1], C. G. Bochet[1] & W. Hug[1]

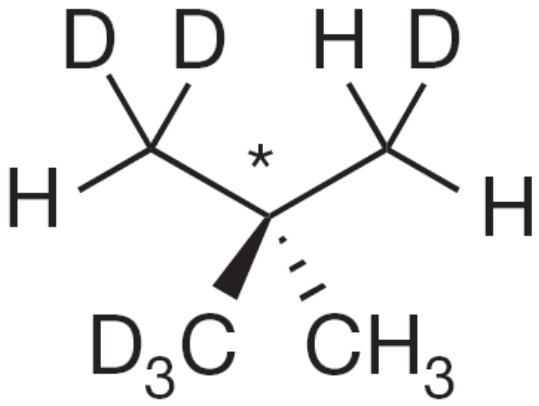
The relationship between macroscopic chirality and chirality on the molecular level was unequivocally established in 1951 through anomalous X-ray scattering[1]. Although this technique became the definitive method for determining the absolute configuration of a molecule, one important limitation of the approach is that the molecule must contain 'heavy' atoms (for example, bromine). The direct determination of absolute configurations for a wider range of molecules has recently become possible by measuring a molecule's vibrational optical activity[2,3]. Here we show that instrumental advances in Raman optical activity[4,5], combined with quantum chemical computations[6–8], make it possible to determine the absolute configuration of (*R*)-[^{1}H$_1$, ^{2}H$_2$, ^{3}H$_3$]-neopentane[9]. This saturated hydrocarbon represents the archetype of all molecules that are chiral as a result of a dissymmetric mass distribution. It is chemically inert and cannot be derivatized to yield molecules that would reveal the absolute configuration of the parent compound. Diastereomeric interactions with other molecules, optical rotation, and electronic circular dichroism are, in contrast to the well-known case of bromochlorofluoromethane[10–13], not expected to be measurable. Vibronic effects in the vacuum ultraviolet circular dichroism might reveal that the molecule is chiral, but the presence of nine rotamers would make it extremely difficult to interpret the spectra, because the spatial arrangement of the rotamers' nuclei resembles that of enantiomers. The unequivocal spectroscopic determination of the absolute configuration of (*R*)-[^{1}H$_1$, ^{2}H$_2$, ^{3}H$_3$]-neopentane therefore presented a major challenge, one that was at the very limit of what is possible.

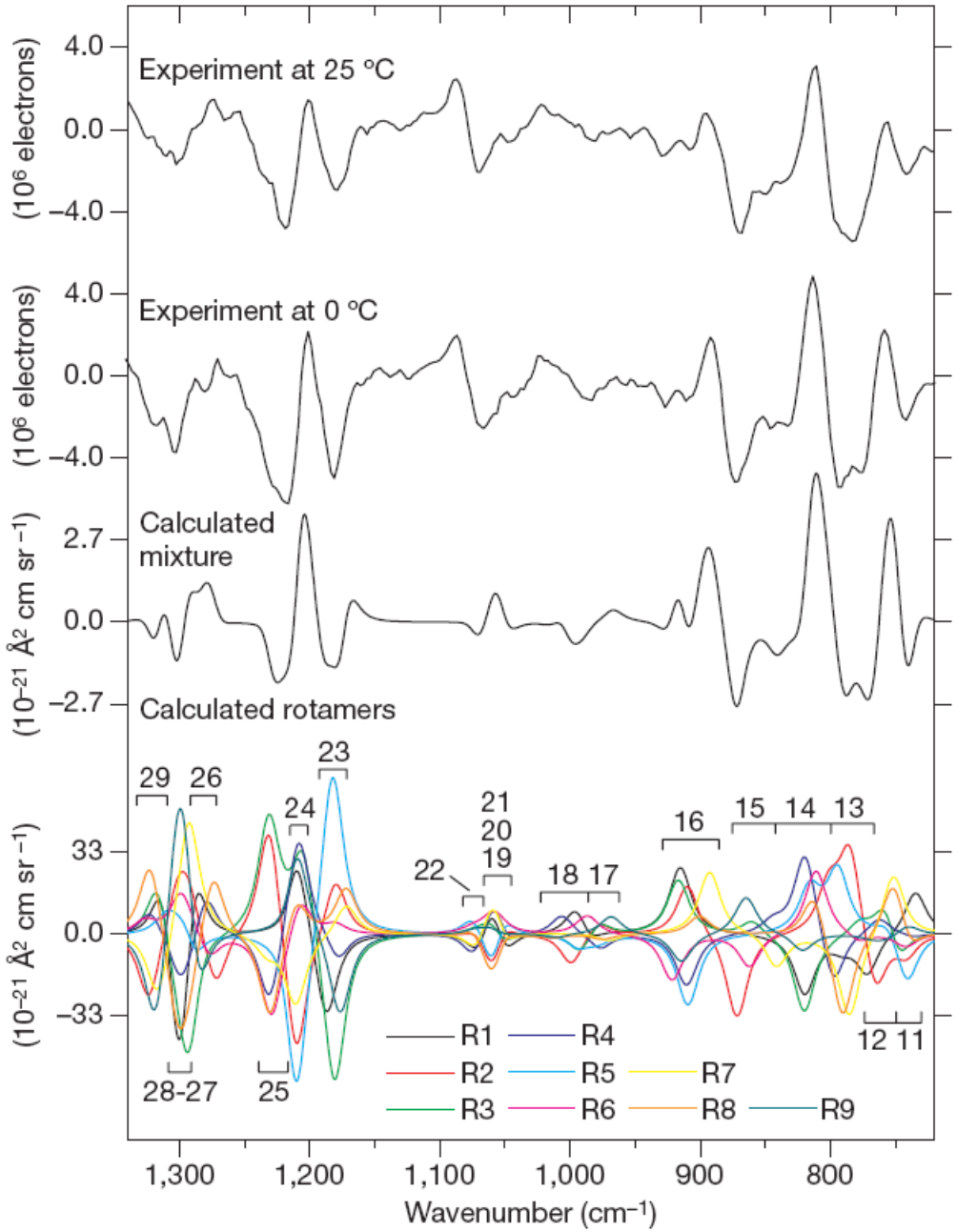
The synthesis of enantioenriched (*R*)-[^{2}H$_1$, ^{2}H$_2$, ^{2}H$_3$]-neopentane 1 is itself a challenge. It bears a quaternary chiral centre, the substituents of which are differentiated only by their isotopic pattern; it is gaseous at room temperature and pressure, and it is highly flammable. The intuitive retrosynthetic analysis based on the deprotonation/alkylation of an α-CD$_3$-substituted chiral propionate derivative (see Supplementary Information) fails because the high electronic and steric similarity between the CH$_3$ and the CD$_3$ groups prevent the preferential formation of one of the two diastereomeric enolates.

We therefore decided to exploit the difference in shape between a tetrahedral C(sp^3) and a trigonal C(sp^2) carbon by a γ-deprotonation/α-alkylation sequence as shown in Fig. 1 (see Supplementary Information for full protocols and characterization). Hence, treatment of the chiral oxazolidinone derivative 4 with sodium hexamethyldisilazide, followed by alkylation with perdeuterated methyl iodide provided the compound 5 with a diastereomeric purity of 95% (the diastereomeric ratio was measured by hydrogen nuclear magnetic resonance, ^{1}H-NMR); its absolute configuration was assigned by analogy to a closely related alkylation[14]. At this point, the chiral quaternary centre bears no risk of racemization, and the optical purity can be assumed to be 95% enantiomeric ratio (e.r.) throughout the rest of the synthesis. Reductive removal of the chiral auxiliary with lithium aluminium deuteride, followed by ozonolysis and *in situ* reduction with sodium borodeuteride, gave the diol 7, which was then converted into the stable and non-volatile dimesylate 8. ^{1}H-NMR analysis confirmed the expected 1:3 ratio of methylene versus

Figure 1 | Synthetic route to chirally deuterated neopentane 1. Yields are given in percentage. d.r., diastereomeric ratio. NaHMDS, sodium hexamethyldisilazide; Et_2O, diethyl ether; Ms, mesyl; py, pyridine.

[1]Department of Chemistry, University of Fribourg, ch. du Musée 9, CH-1700 Fribourg, Switzerland.





Comparison of Measured SCP-ROA at two temperatures and Calculated SCP-ROA as a composite of nine conformations of chirally deuterated neopentane

Further Applications of Absolute Configuration Determination

Following Chirality in Along Synthetic Pathway

VCD spectra of each isolated species is a check on absolute configuration and can be calculated if desired to check on absolute configuration

Further Applications of Absolute Configuration Determination

Determination of Drug-Protein Binding Conformations

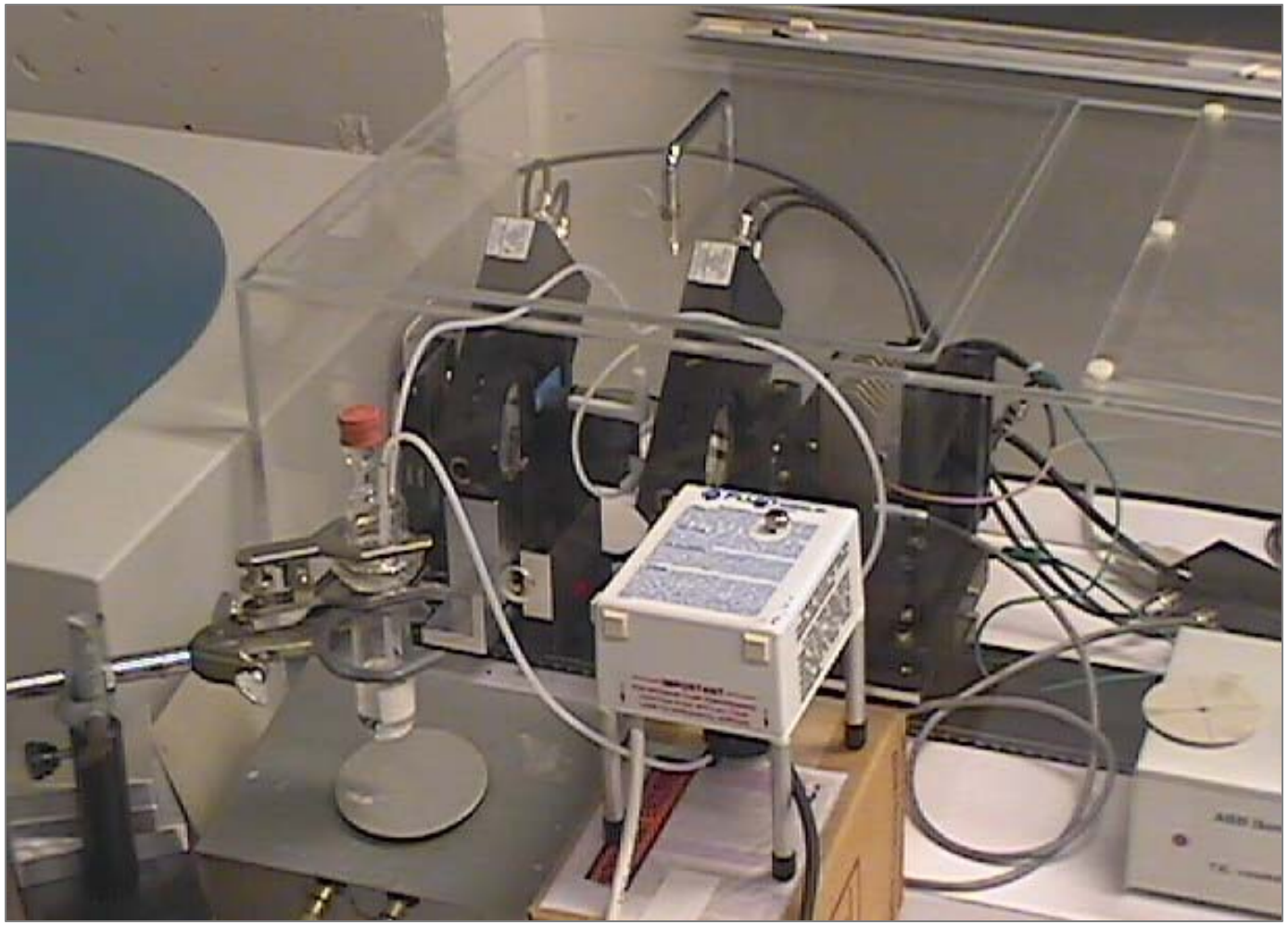
Active Conformation is typically one of the important conformations for VCD spectral matching. Small molecule protein interaction

Reaction Monitoring of Chiral Molecules

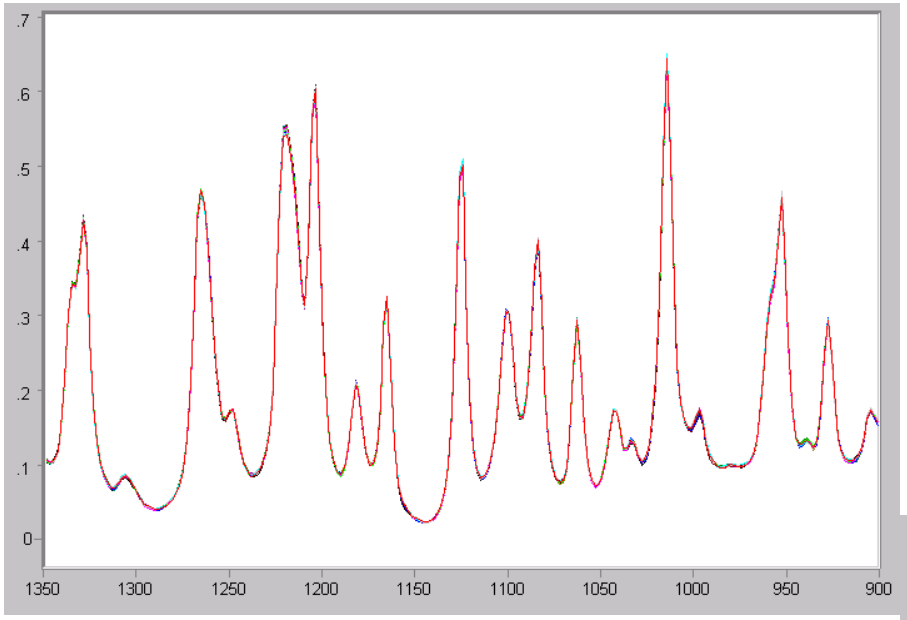
Key Aspects of %EE Determinations Using VCD

- VCD magnitude directly proportional to %EE
- Need one reference spectrum for each compound to determine absolute %EE
- Chromatographic separation not needed
- VCD is multiple spectral point measure of %EE
- IR and Molecular Structure information obtained simultaneously with %EE

Flow-Cell Setup for %EE VCD Measurements

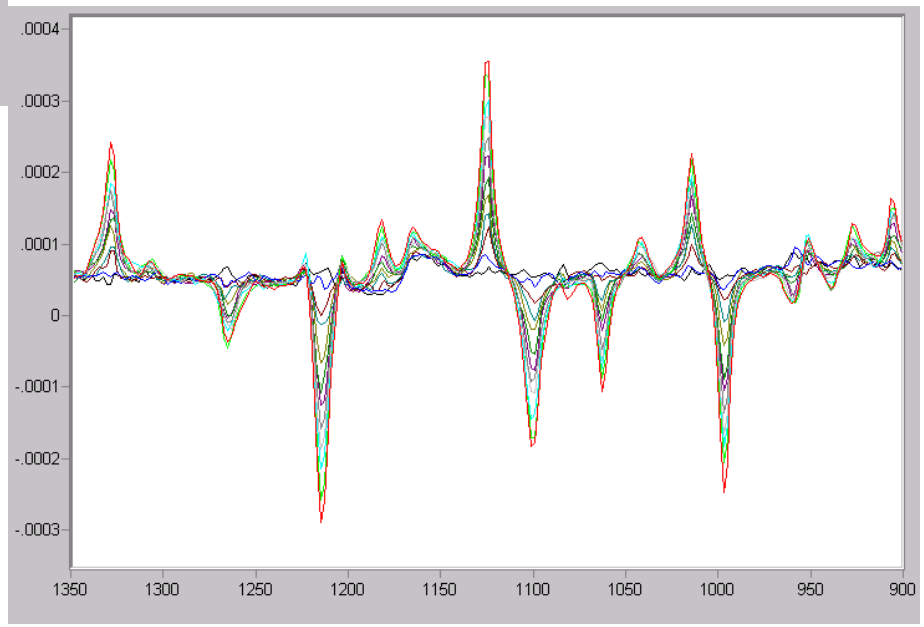


Flow cell experiment for 3.14M α-pinene enantiomers CCl_4 solution



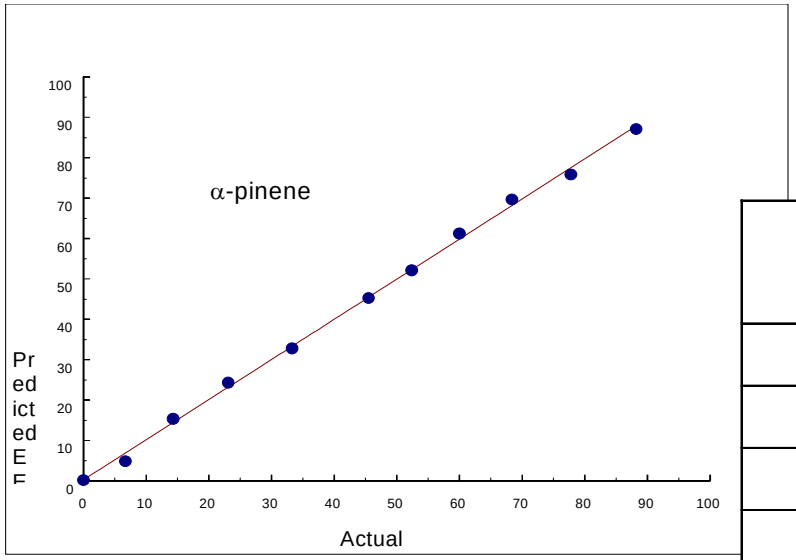
%EE VARIATION

(a) 11 IR spectra of α-pinene in a flow-cell



(b) VCD spectra. From top to bottom, the EE% (R-S) of α-pinene solution are 100, 88.2, 77.8, 68.4, 60.0, 52.4, 45.5, 33.3, 23.1, 14.3, 6.7.

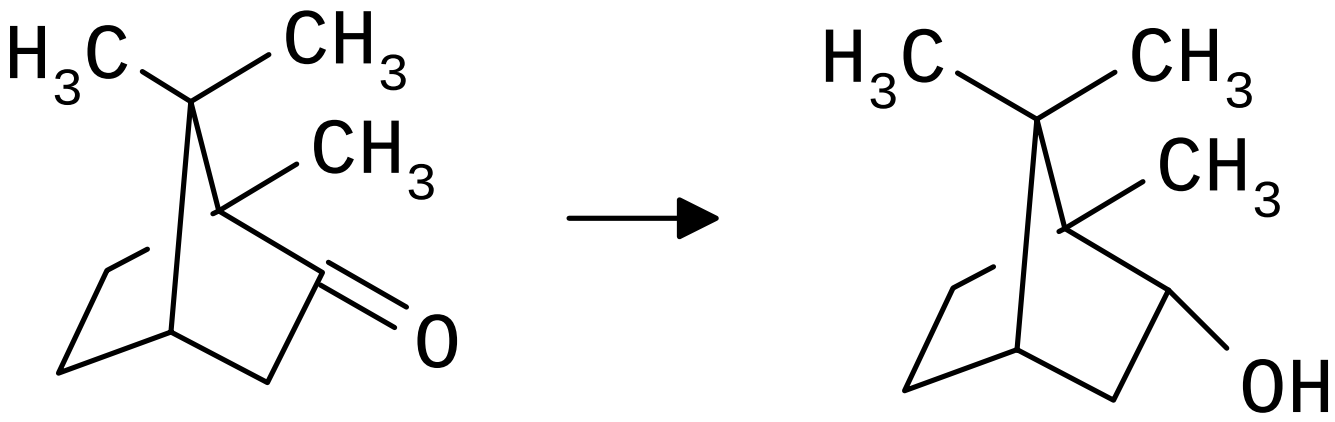
PLS analysis of VCD spectra for α-pinene: %EE variation



Actual EE%	Predicted EE%	Variance (EE%)
88.2	87.2	1.0
77.8	75.9	1.9
68.4	69.7	-1.3
60	61.3	-1.3
52.4	52.2	0.2
45.5	45.3	0.2
33.3	32.8	0.5
23.1	24.4	-1.3
14.3	15.4	-1.1
6.7	4.9	1.8
0	0.3	-0.3
RMSD	1.15	
STDEV	1.21	

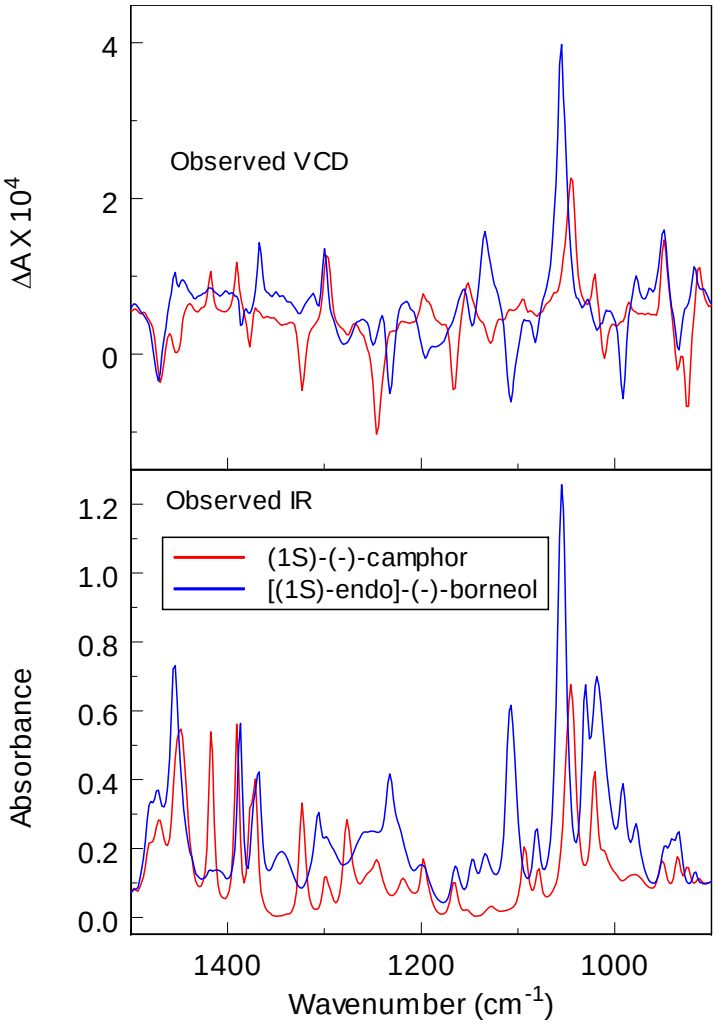
Reaction Simulation:

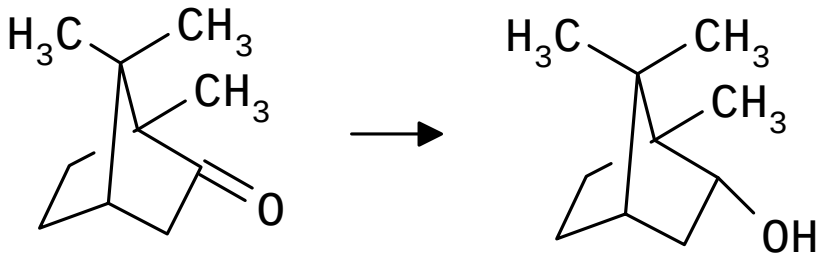
CAMPHOR → BORNEOL



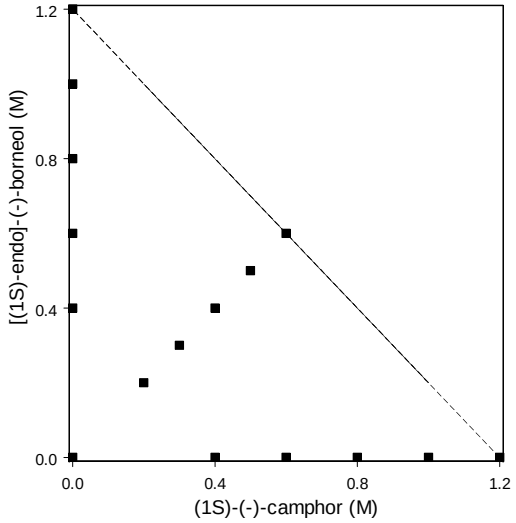
Two-component Mixture

Camphor & Borneol



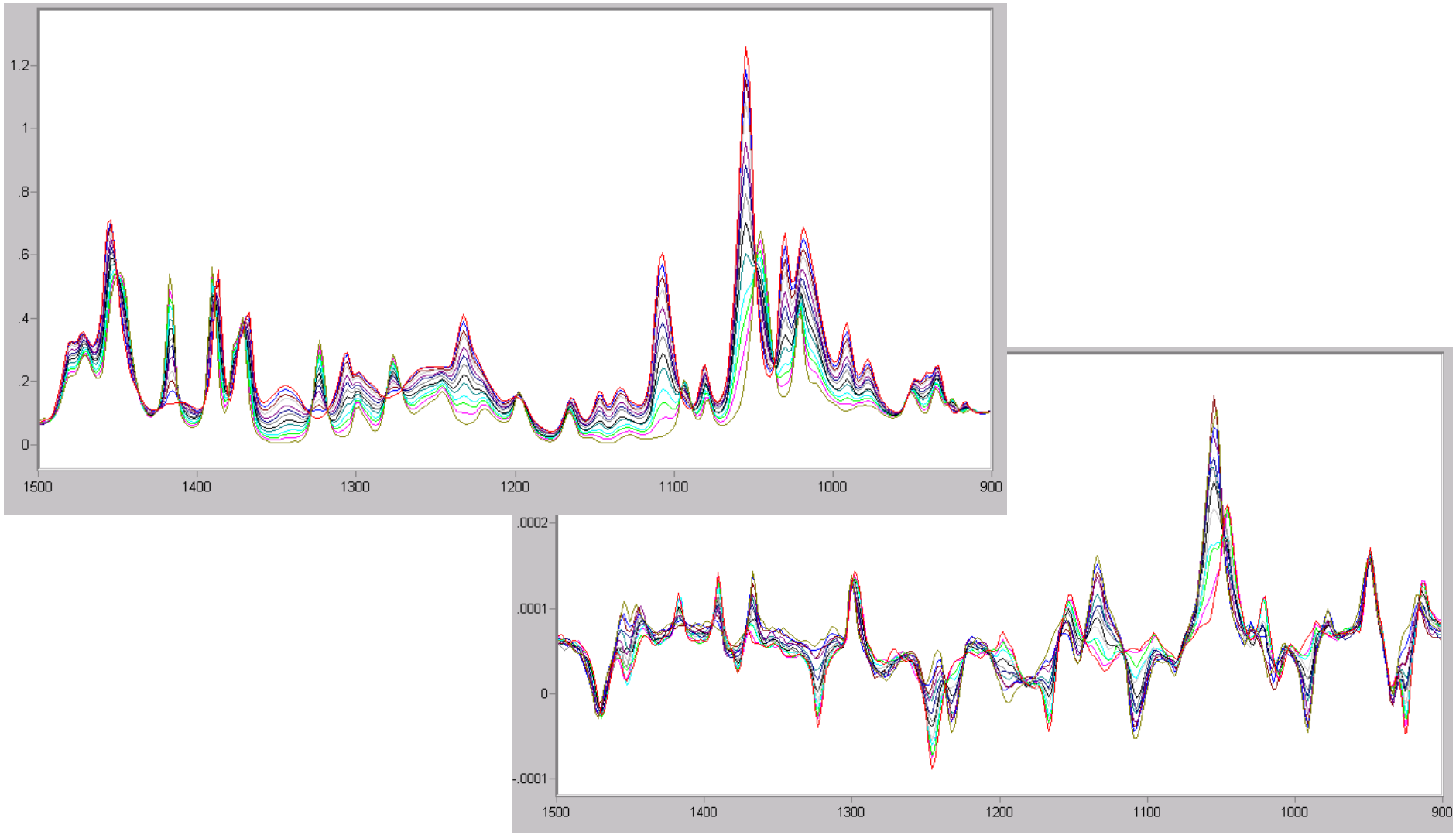


PLS training set

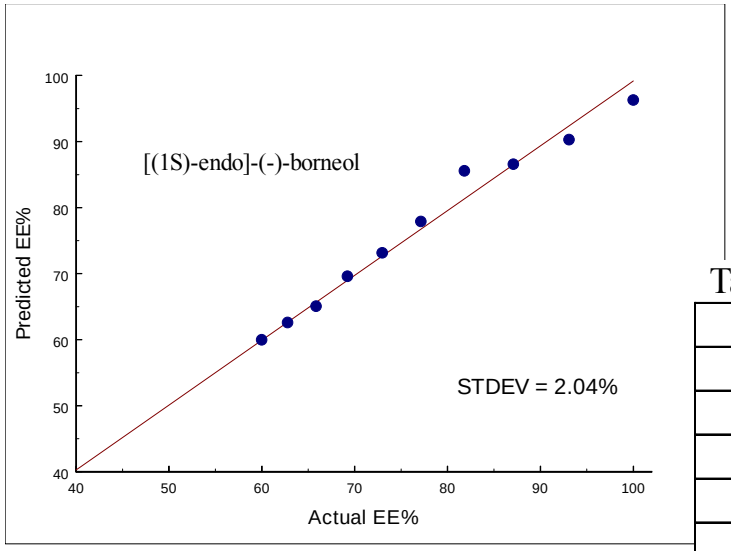


Number of factors = 4-5

Reaction Monitoring: Flow Cell Simulation



PLS Analysis of VCD spectra: %EE with *CHANGING COMPOSITION*



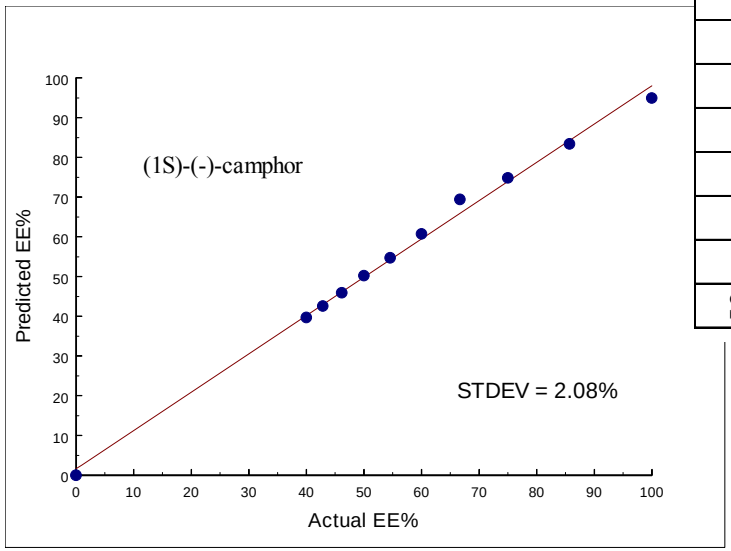


Table 4. Predicted EE% (calculated from IR and VCD prediction results)

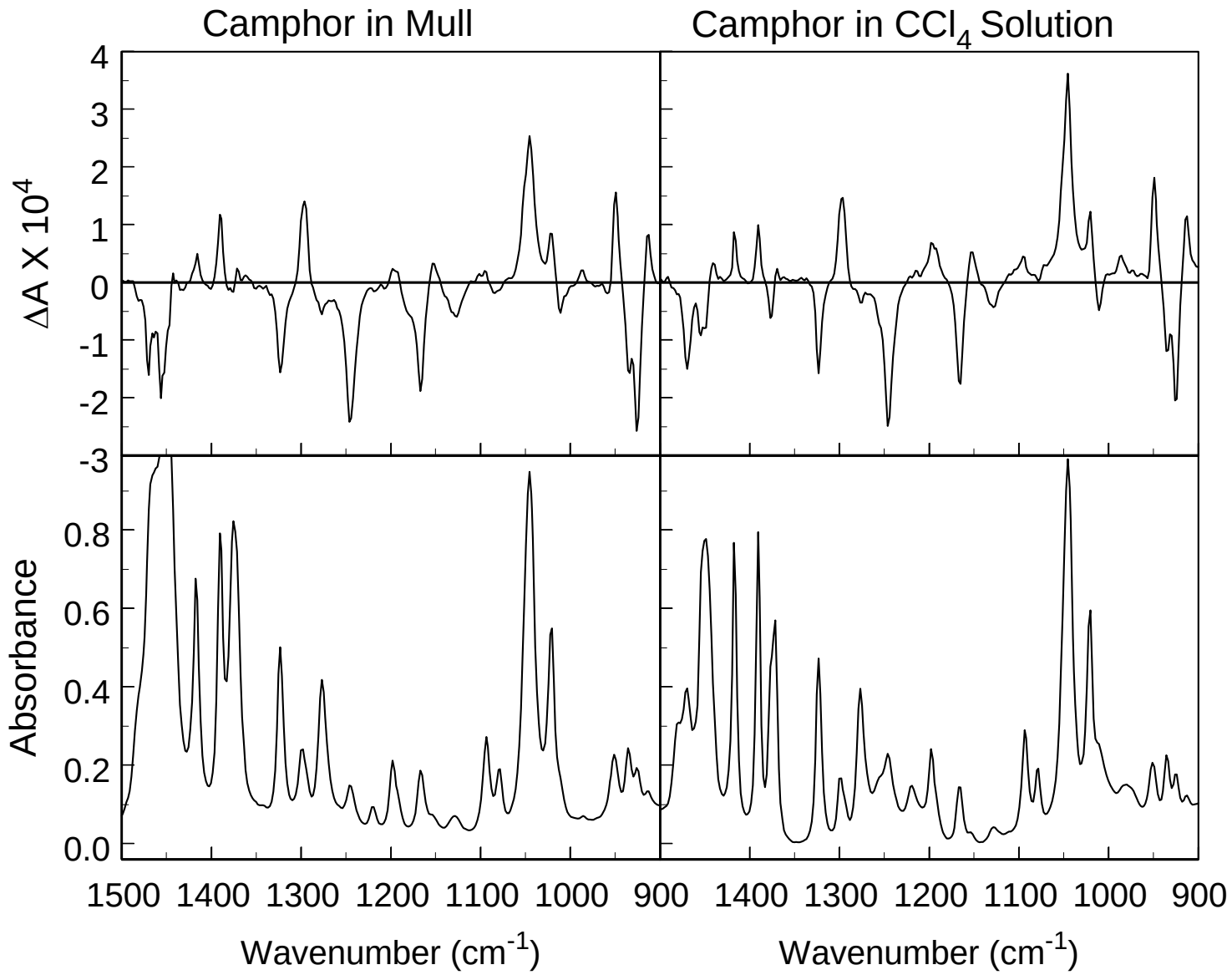
	EE% of [(1S)-endo]-(-)-borneol			EE% of (1S)-(-)-Camphor		
	Actual	Predicted	Variance	Actual	Predicted	Variance
1	100.00	96.30	-3.70	100.00	94.99	-5.01
2	93.10	90.30	-2.80	85.71	83.42	-2.29
3	87.10	86.59	-0.50	75.00	74.87	-0.13
4	81.82	85.58	3.76	66.67	69.45	2.79
5	77.14	77.91	0.76	60.00	60.77	0.77
6	72.97	73.16	0.19	54.55	54.75	0.21
7	69.23	69.61	0.38	50.00	50.27	0.27
8	65.85	65.09	-0.76	46.15	45.96	-0.19
9	62.79	62.62	-0.17	42.86	42.58	-0.28
10	60.00	60.00	0.00	40.00	39.74	-0.26
RMSD	1.93			1.98		
STDEV	2.04			2.08		

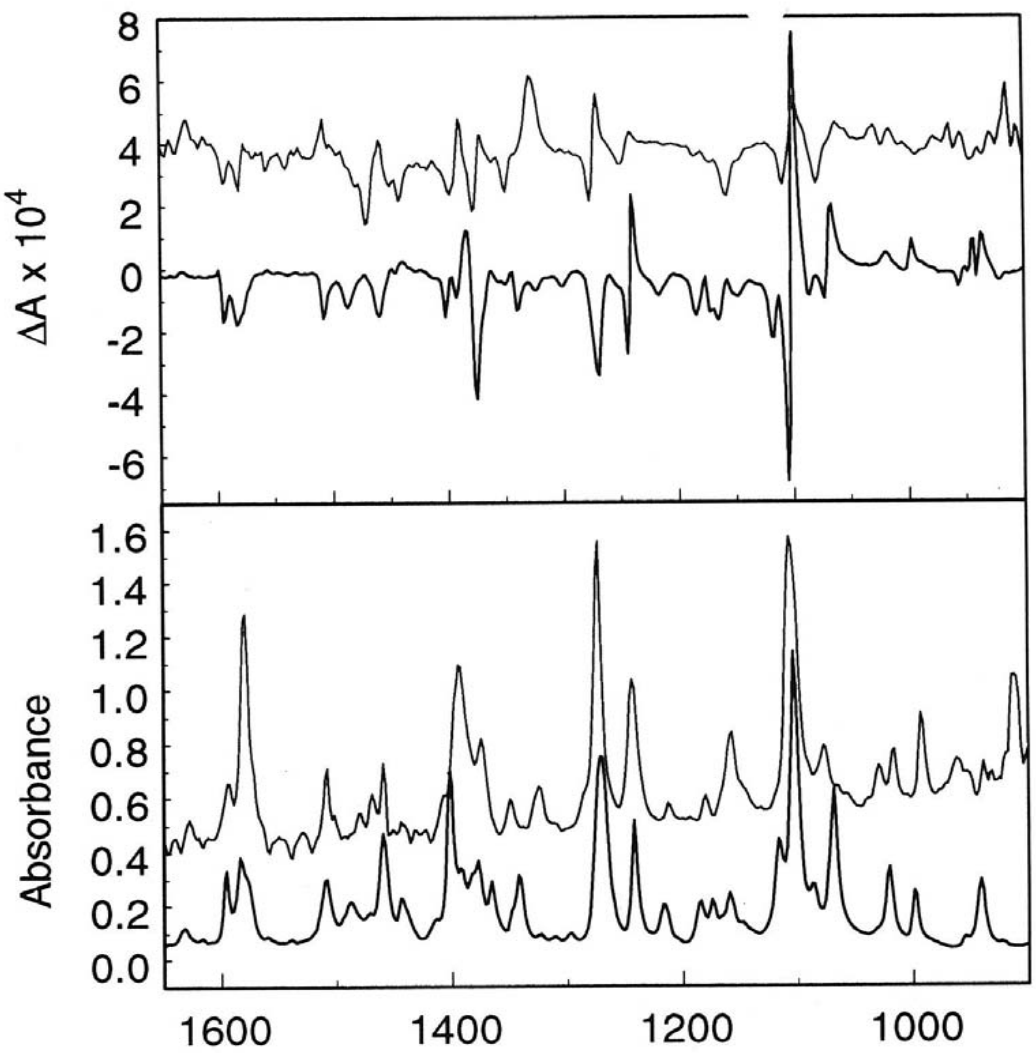
Solid-Phase VCD Measurements

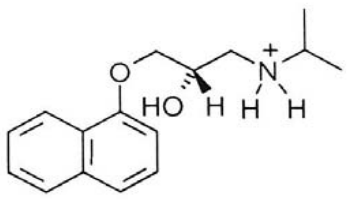
Key Elements of Solid Phase VCD

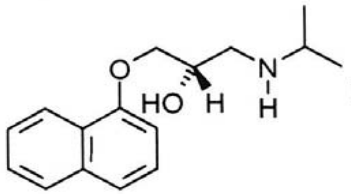
- Mulls, Particle-Size Scattering
  - Grind to Particle Size smaller than wavelength of the light
- Films, Birefringence Effects
- Spray-Dried Samples
- Excipients and Formulated Products
- Quality control applications

Comparison of Solid and Solution VCD



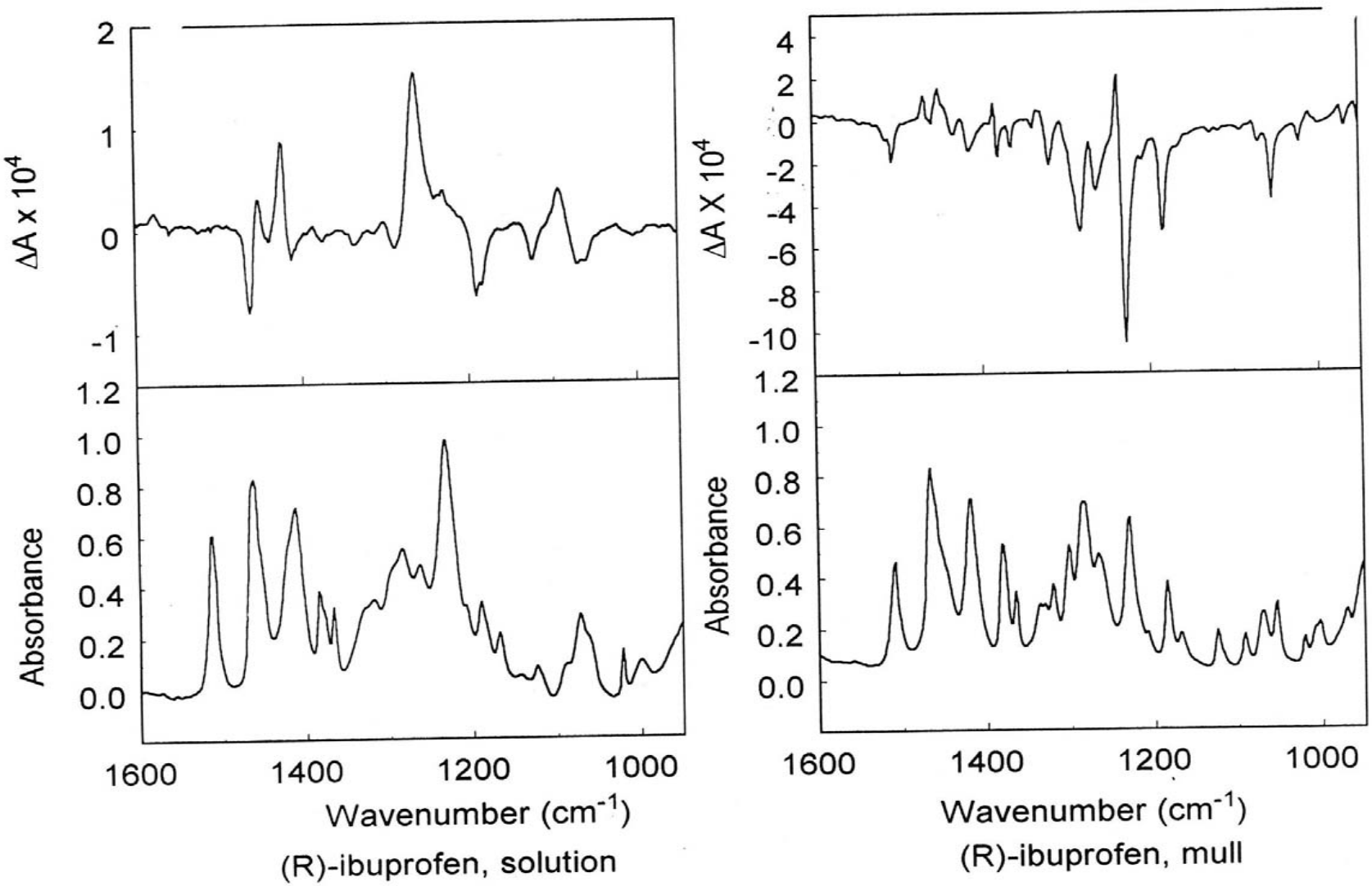






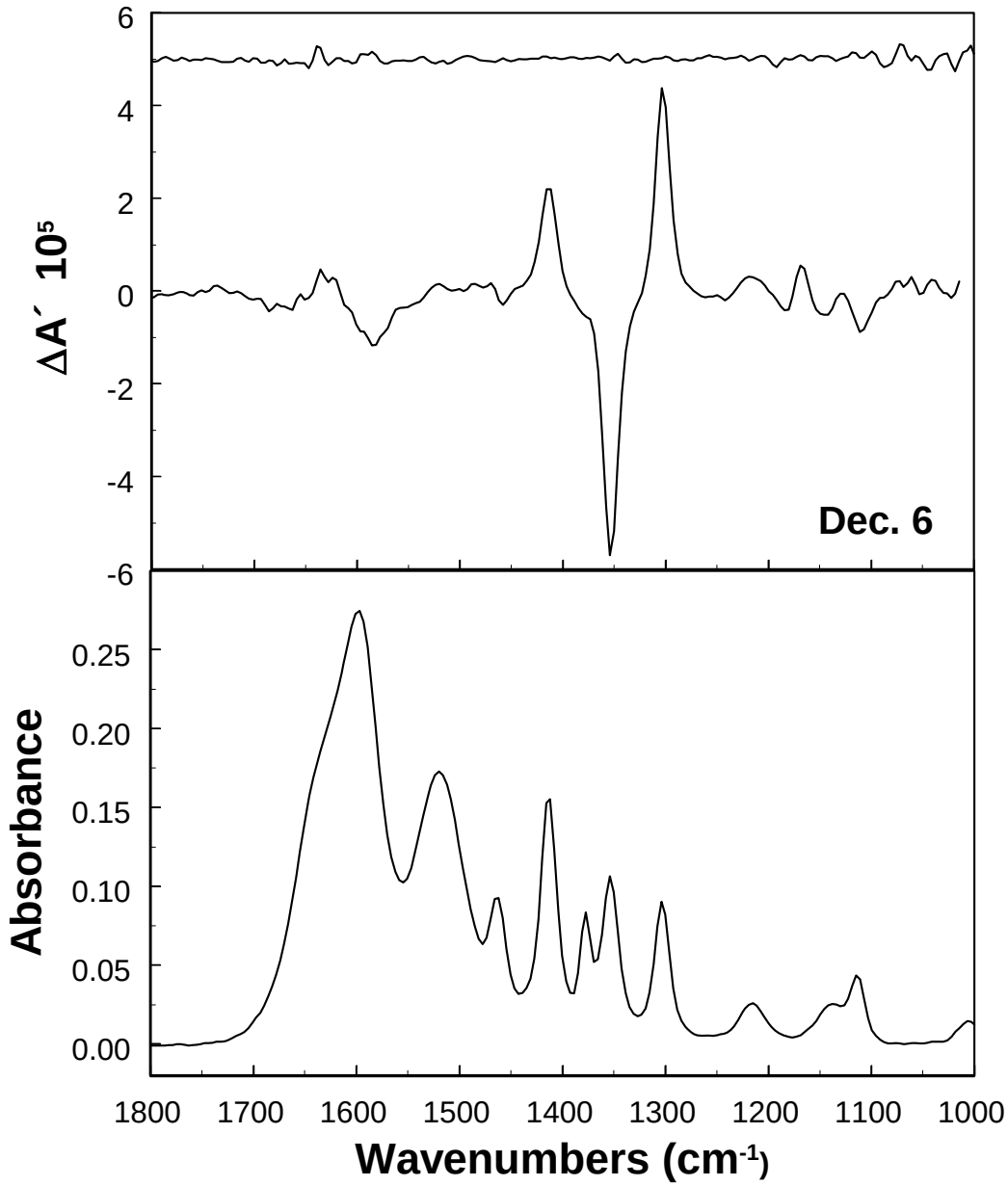
(S)-propranolol, free base, mull (black)

(S)-propranolol, HCl salt, mull (red)

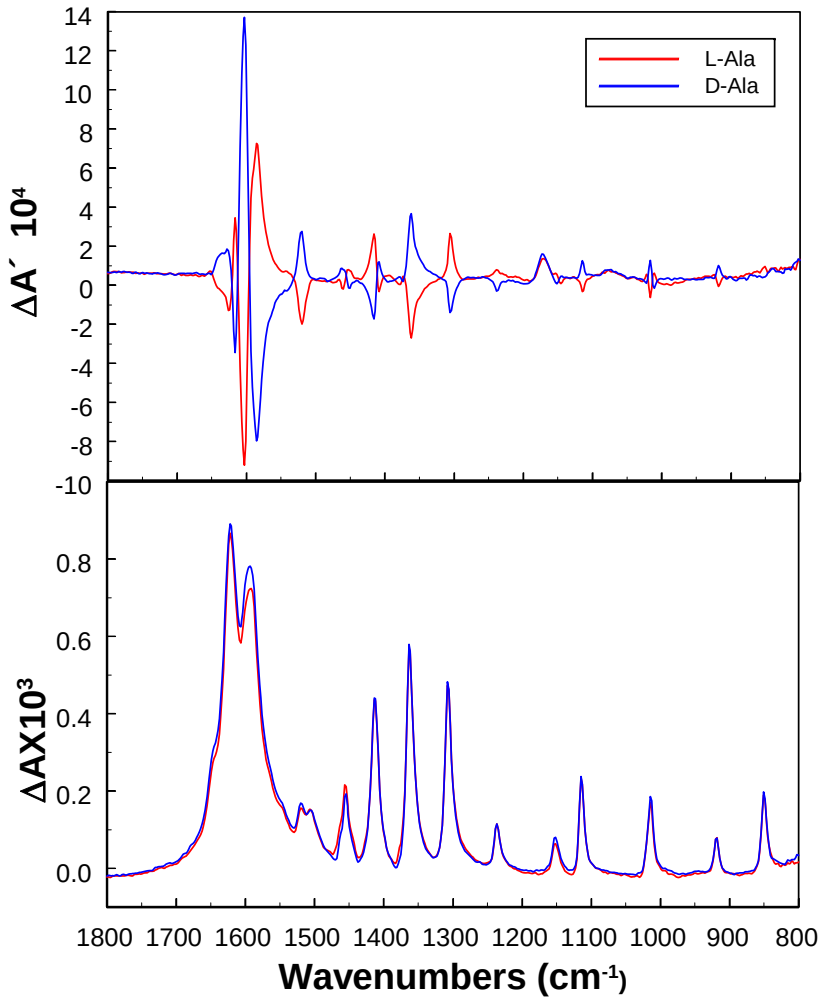

ΔA x 10^4
2
1
0
-1
Absorbance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1600
1400
1200
1000
Wavenumber (cm^-1)
(R)-ibuprofen, solution
ΔA X 10^4
4
2
0
-2
-4
-6
-8
-10
Absorbance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1600
1400
1200
1000
Wavenumber (cm^-1)
(R)-ibuprofen, mull

Alanine VCD and IR in H_2O using 6 micron pathlength cell

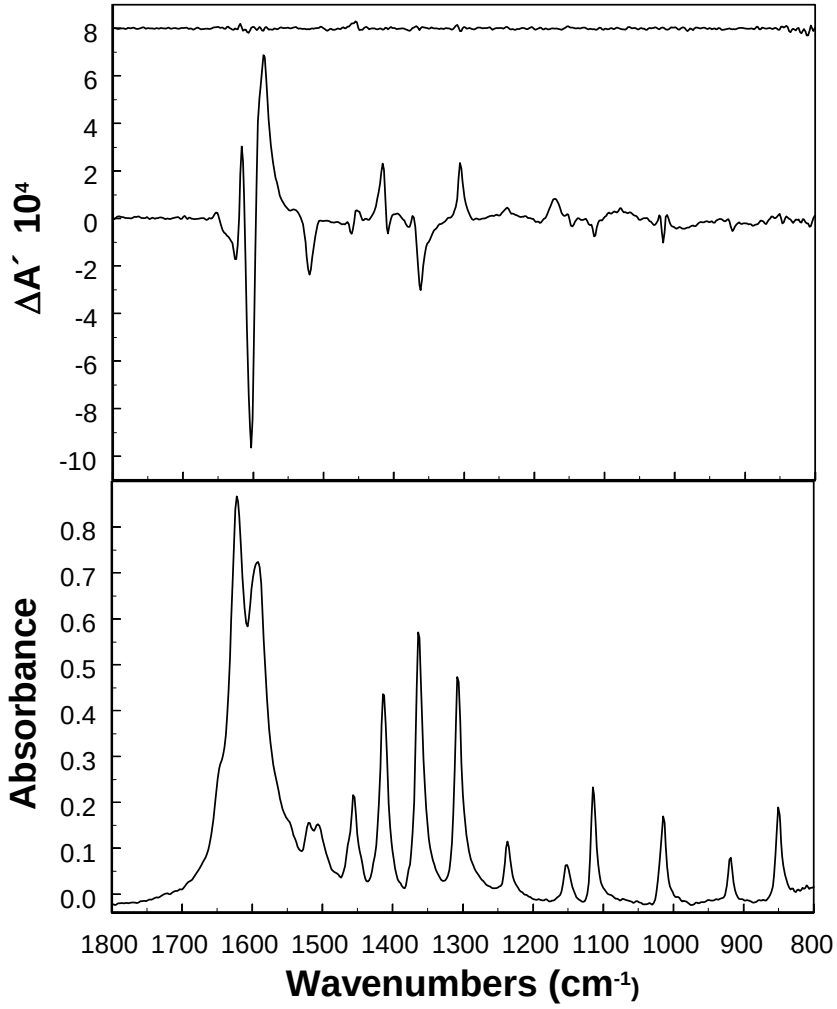
VCD max is ~0.04% of IR intensity



Mull VCD/IR of Alanine VCD max is ~0.2% of IR intensity



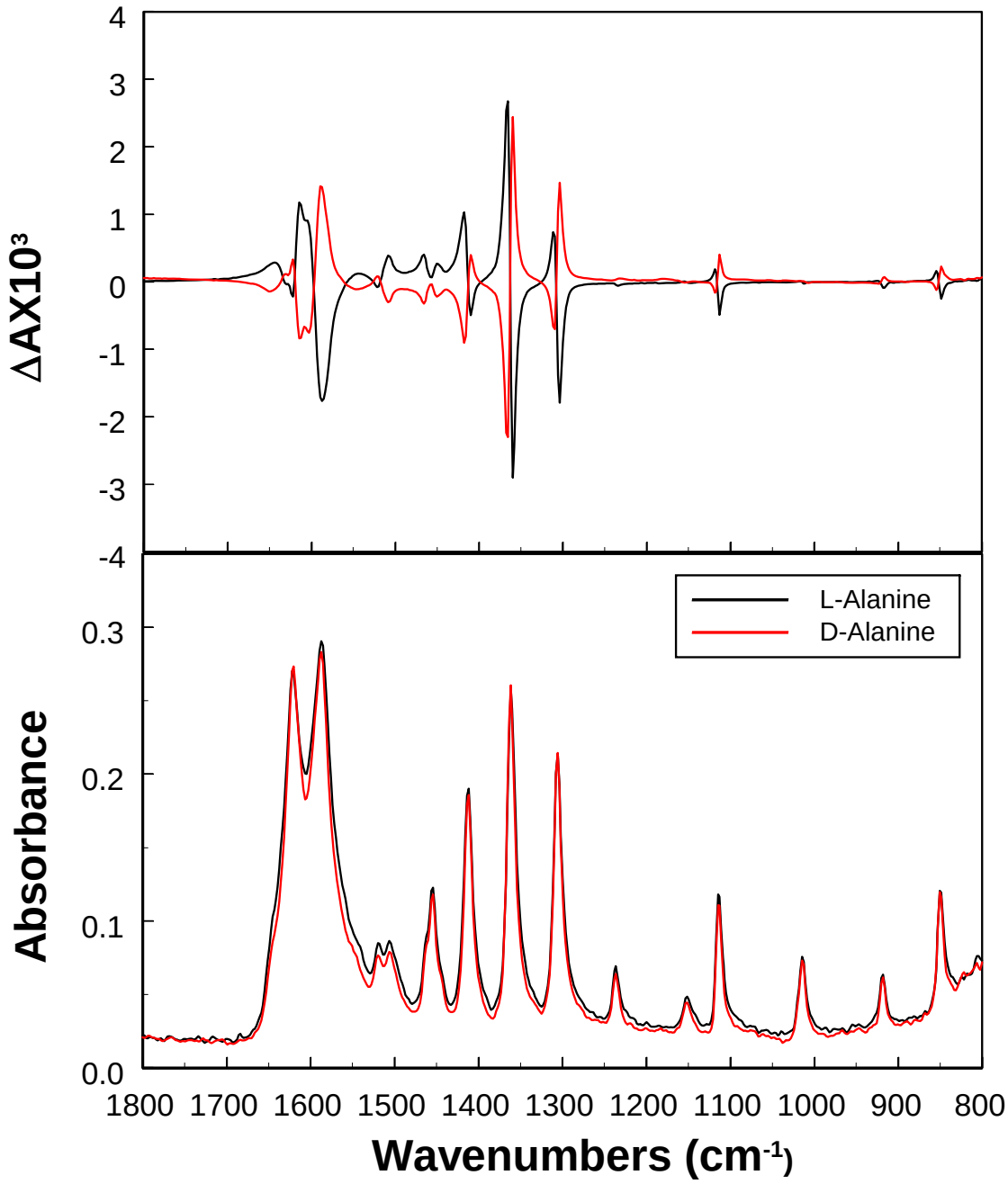
L- & D-Alanine in nujol, 4cm^{-1}, 20 min each. 1400cm^{-1}



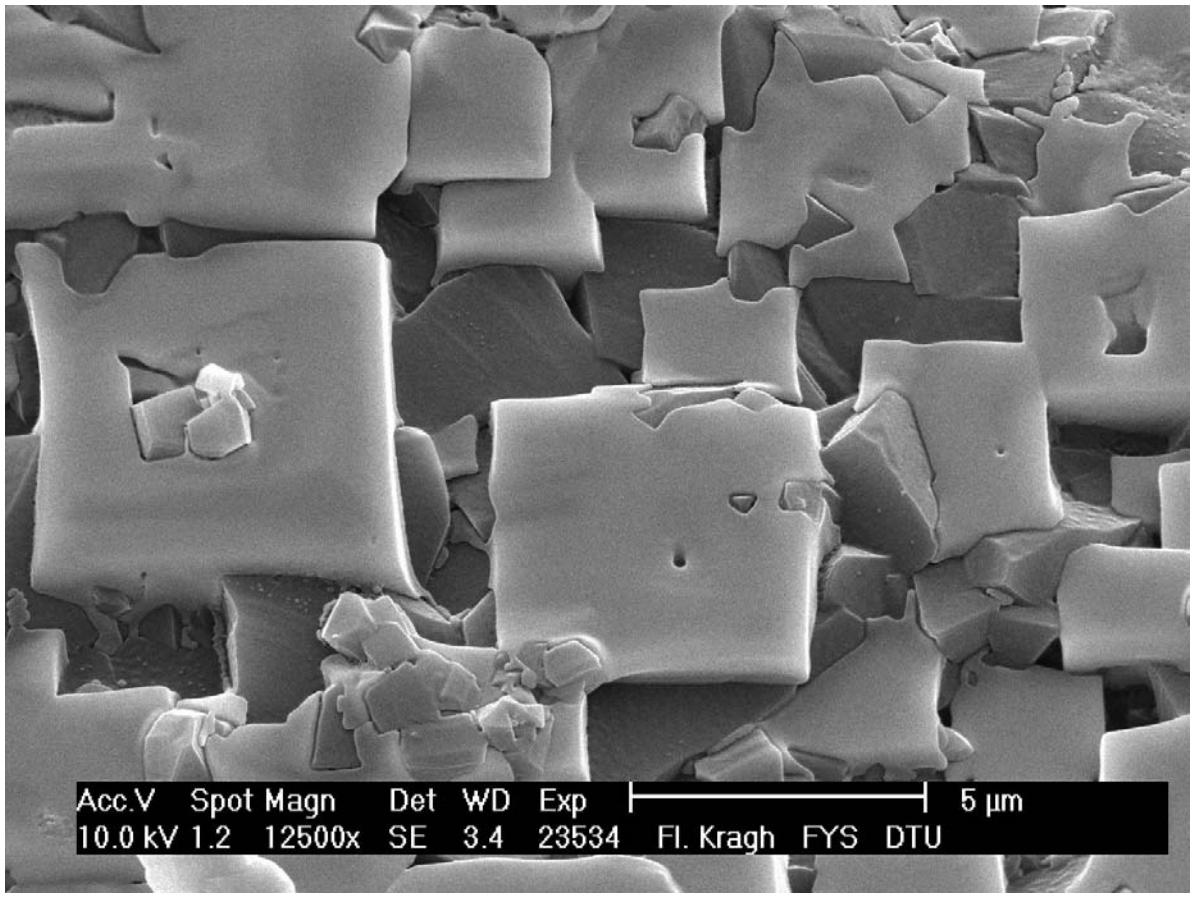
L-Alanine in nujol (IR corrected), 4cm^{-1}, 20min, PEM=1400cm^{-1}

L- and D-Alanine neat H_2O solution sprayed on IR window

VCD max is ~2% of IR intensity

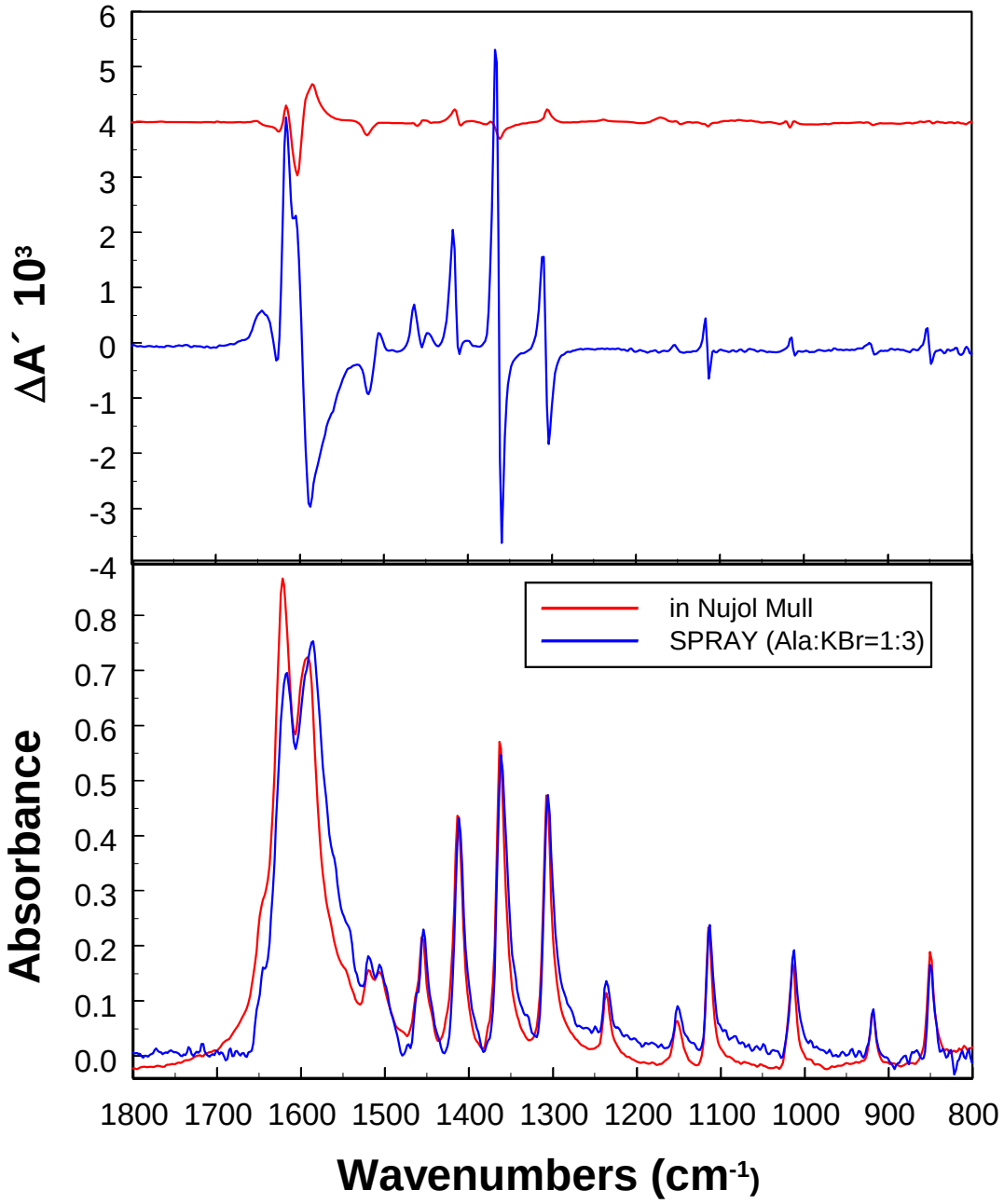


SEM Image of Alanine Spray-Dried Film



Comparison of L-Ala mull and spray at Ala:KBr 1:3 in aqueous solution sprayed on IR window.

VCD max spray is 2% of IR intensity



VCD of Solids can now be routinely run opening up pharmaceutical applications in the areas of:

1. Raw material ID of incoming chiral starting materials
2. Chiral excipients and excipient drug mixtures
3. Final formulated solid pharmaceutical products

Conclusions

- VCD and ROA are a sensitive spectroscopic probes of absolute molecular stereochemistry of all classifications of chiral molecules
- VOA offers an alternative to X-ray crystallography for a priori determination of absolute configuration
- VOA is a new probe of solution-state conformational population determinations
- VOA can be used *in situ* to measure %EE of multiple chiral components in a reaction mixture
- VCD can be measured in solids, films, powders, sprays and other solid phase sampling environments

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