Explaining Separation Phenomenon Through The Use Of Molecular Modeling and NMR

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Merck & Co., Inc

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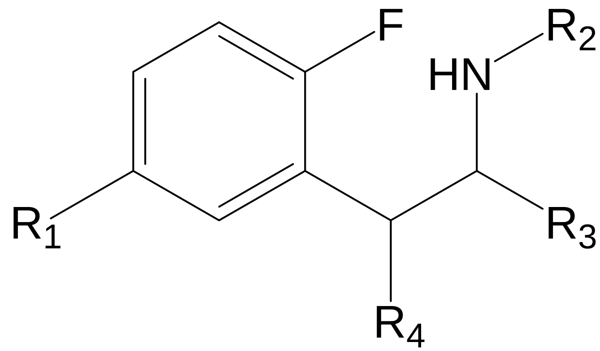
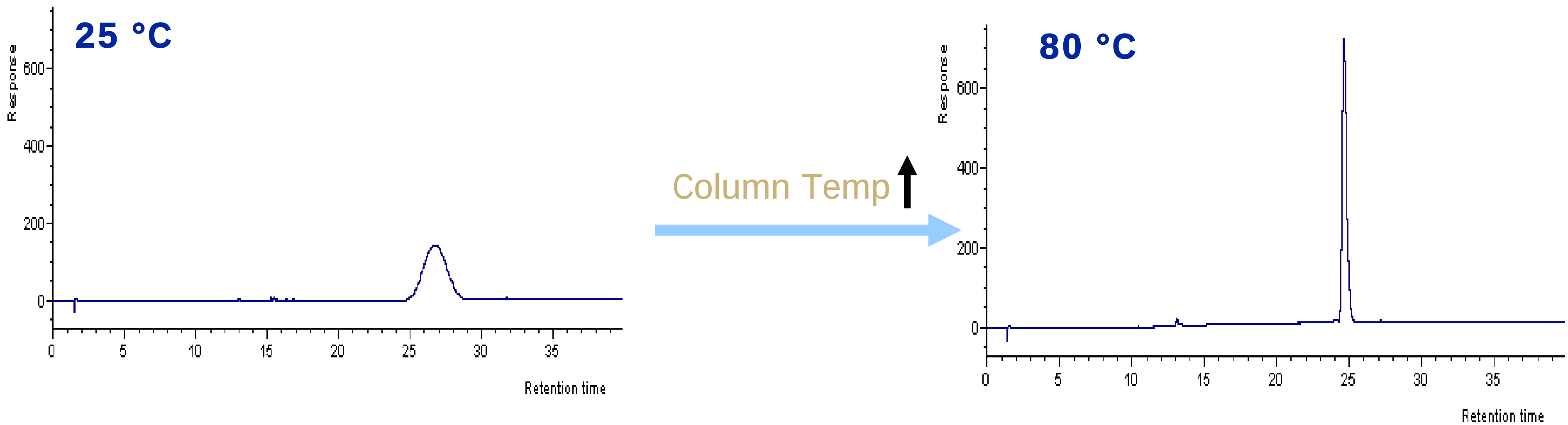
Issue 1: Development of an Impurity Profile Assay



RPLC Method: Thermo Hypercarb 100 mm x 4.6 mm x 3 mm; A=0.1%Perchloric acid, B=Acetonitrile; 10% B to 90% B in 40 min; Flow Rate 1.0 mL/min; 220 nm UV detection



Peak Broadening was observed in achiral reversed phase liquid chromatography (RPLC).

Temperature had a significant effect on peak width.

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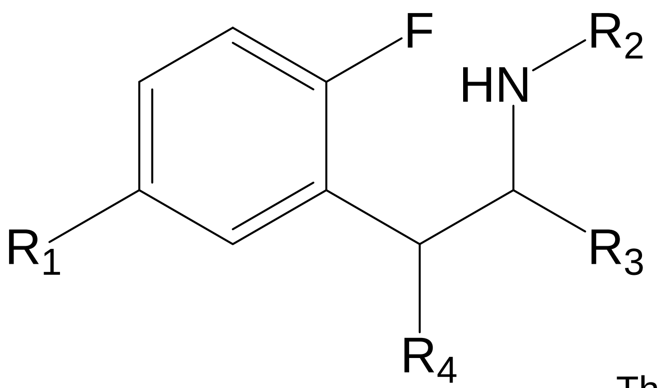
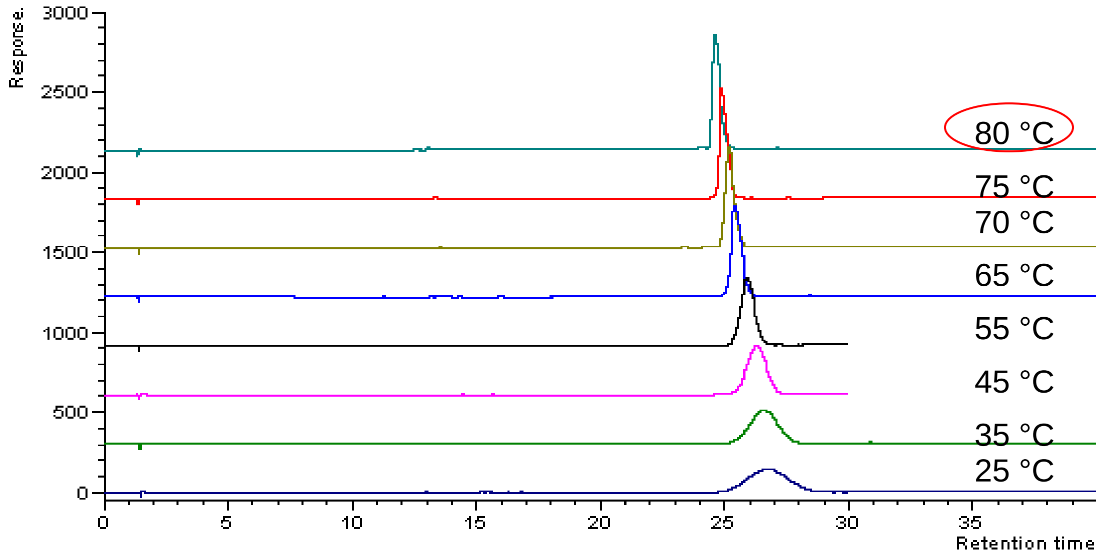
Effect of Temp on the Impurity Profile Assay is Dramatic

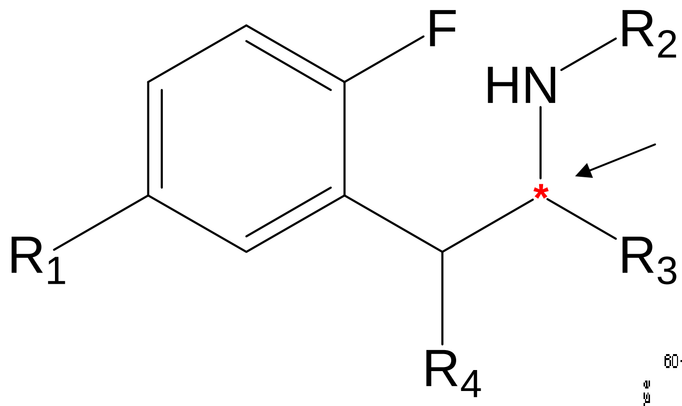


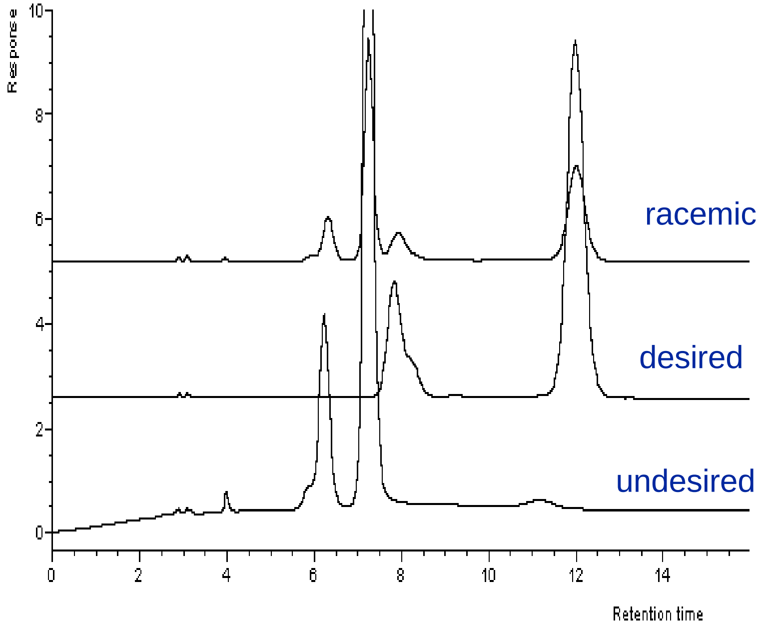
Thermo Hypercarb 100 mm x 4.6 mm x 3 mm



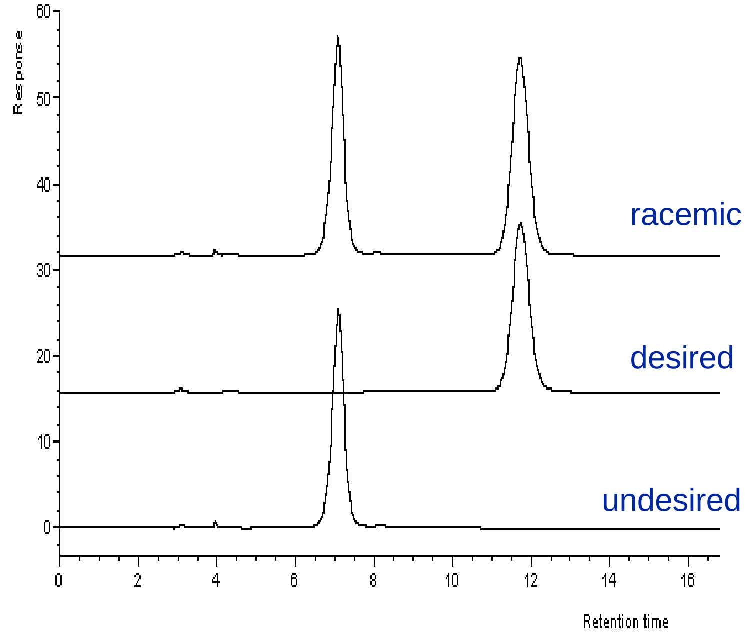
RPLC Method: A=0.1%Perchloric acid, B=Acetonitrile; 10% B to 90% B in 40 min; Flow Rate 1.0 mL/min; 220 nm UV detection

Issue 2: The Development of an Chiral Purity Assay





Change diluent



Diluent: Methanol

Diluent: Acetonitrile

NPLC Method: Chiralpak AD-H, 250 X 4.6 mm X 5 µm; A=IPA, B=heptane; 85%B isocratic for 17 min; Temp 40 °C; Flow Rate 1.0 mL/min; 220 nm UV detection

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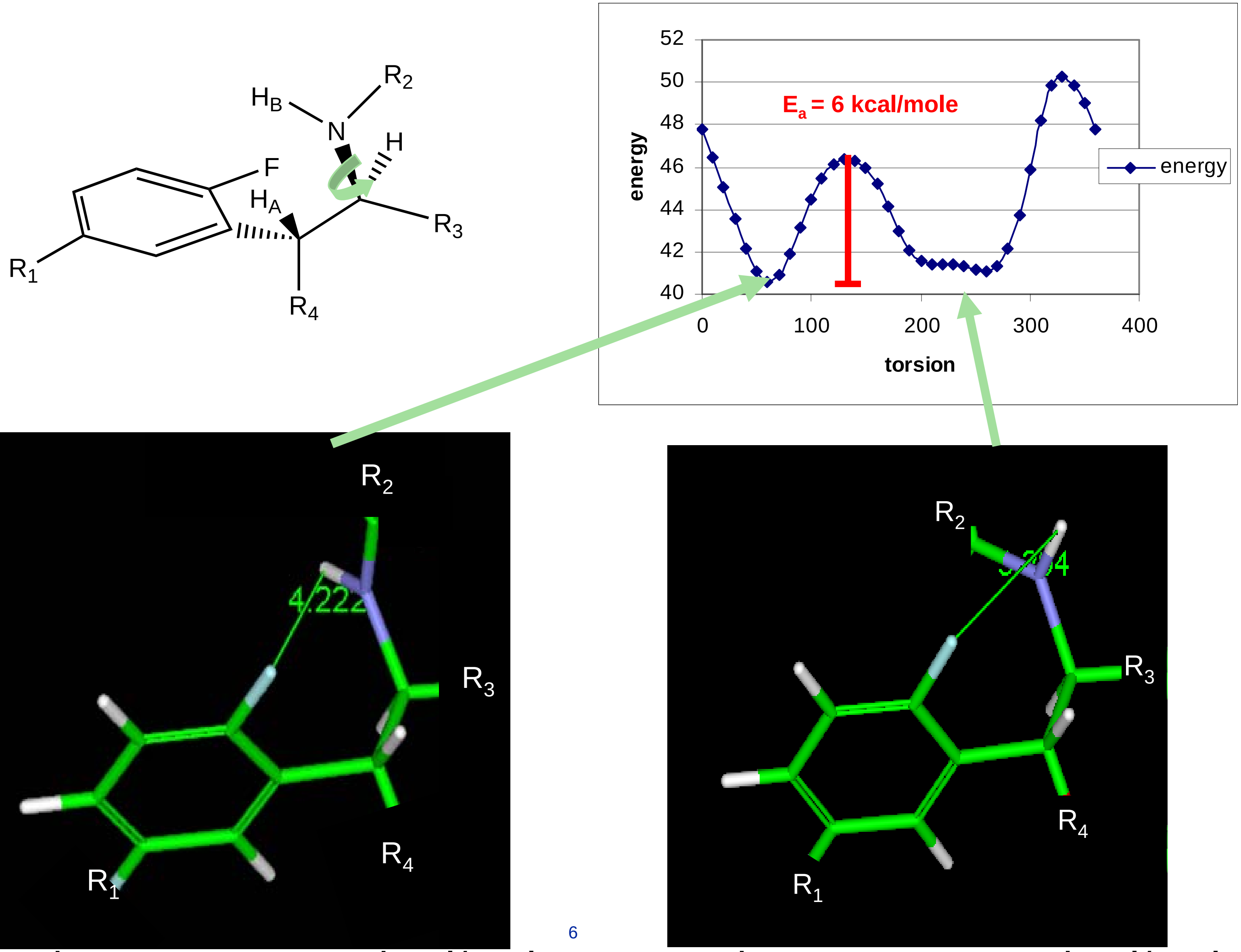
Can Molecular Modeling Offer any Insight ?

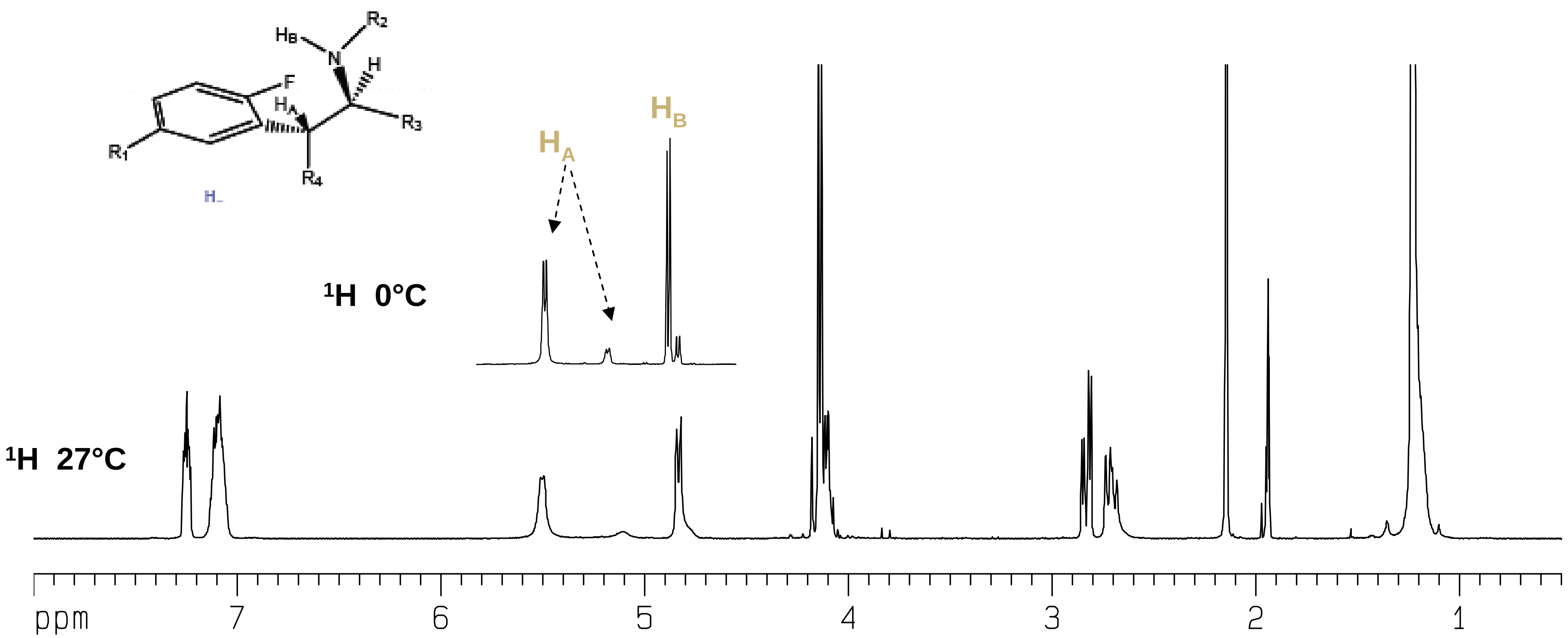


min1; E = 40.5975 kcal/mol

min2; E = 41.0636 kcal/mol

Rotamers are Detected by Solution NMR

Solution NMR at low temperature can often detect rotamers



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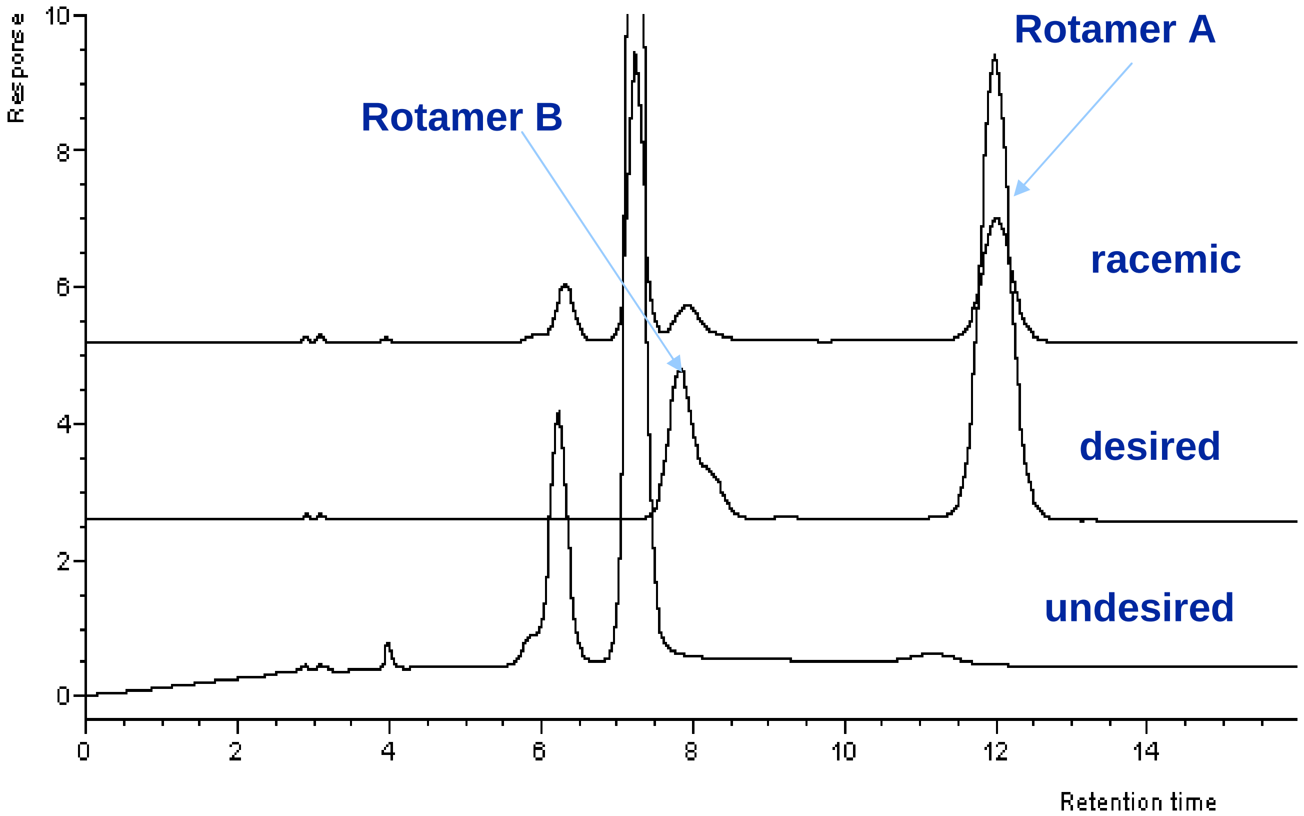
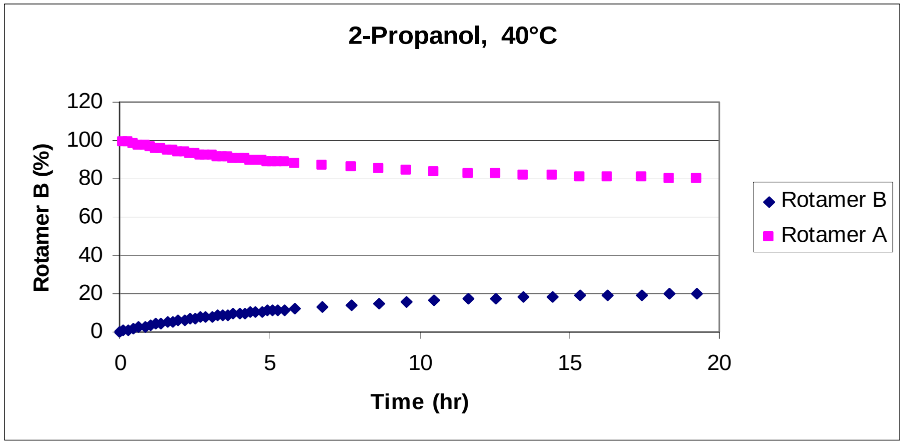
Kinetics of Rotamer

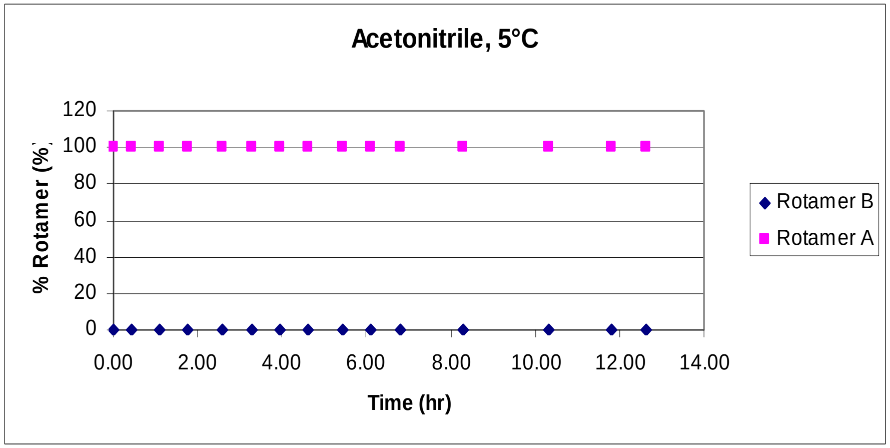


Rotamer A $\leftrightarrow$ Rotamer B

NPLC Method: Chiralpak AD-H, 250 X 4.6 mm X 5 μm; A=IPA, B=heptane; 85%B isocratic for 17 min; Temp 40 °C; Flow Rate 1.0 mL/min; 220 nm UV detection

Cont'd Kinetics of Rotamer





Rate = -Δ[Rotamer A]/ Δt = *k* [Rotamer A]

[Rotamer A] = relative area% of Rotamer A

$$\ln [\text{Rotamer A}]_t = -kt + \ln [\text{Rotamer A}]_0$$

$$y = mx + b$$

$$k = Ae^{-Ea/RT}$$

$$\ln k = -Ea/RT + \ln A$$

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Effect of Rotamer Conversion in Various Solvent Systems and Temperatures

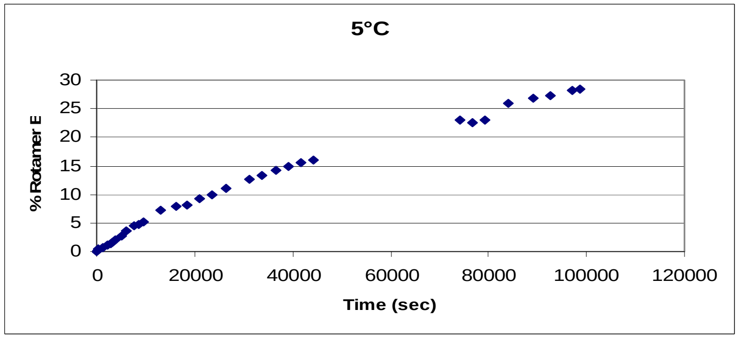
Diluents

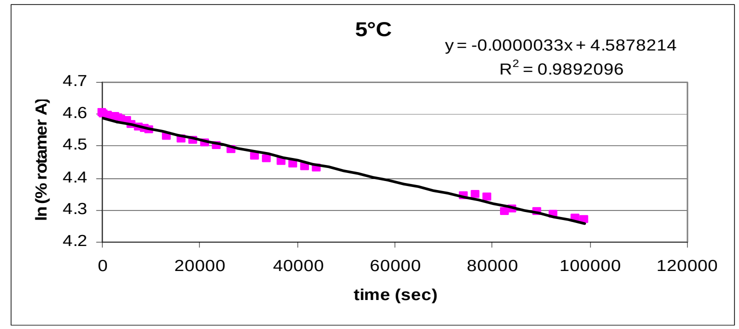
Non-alcohol	1º alcohol	2º alcohol	3º alcohol
Acetonitrile	Ethanol	2-propanol	tert-Butanol
	1-Propanol	2-Butanol	
	1-Butanol		

Temperatures

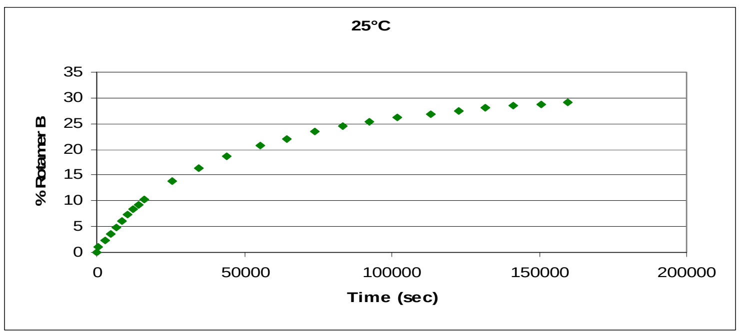
5°C, 25°C, and 40°C for all solvents except for tert-butanol where 25°C, 30°C, and 40°C were studied.

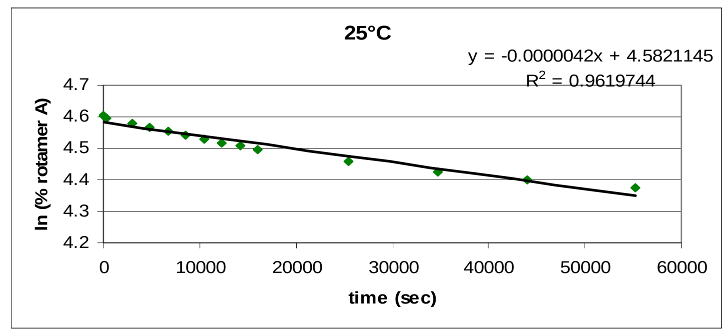
Activation Energy (Ea) Calculation by LC method

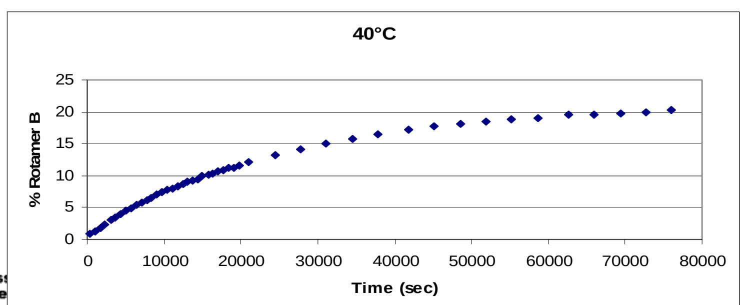


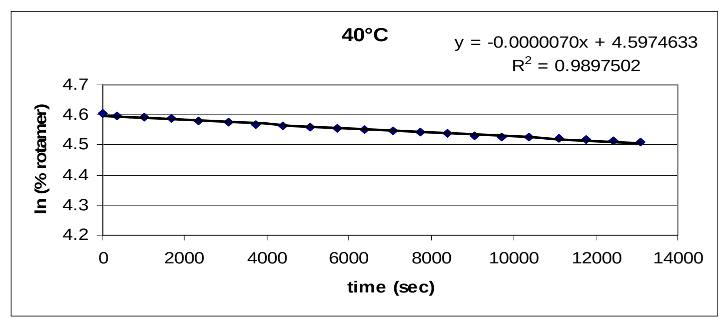


– Example: 2-Propanol



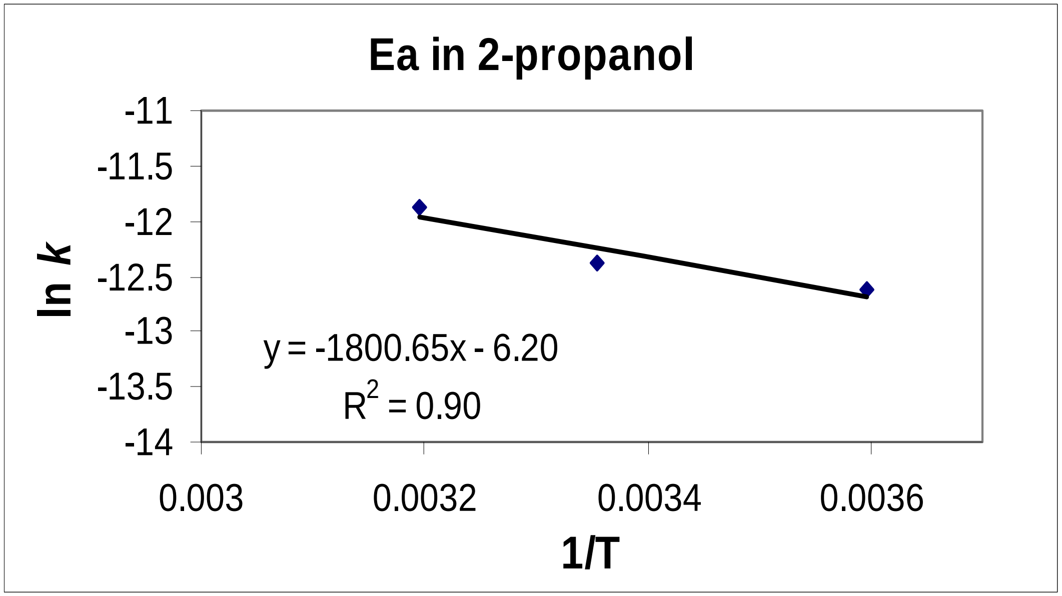






Cont'd Activation Energy (Ea) Calculation by LC method

Temp (K)	*k* (1/sec)	1/T	ln *k*
278	0.000003	0.0036	-12.62
298	0.000004	0.0034	-12.38
313	0.000007	0.0032	-11.87



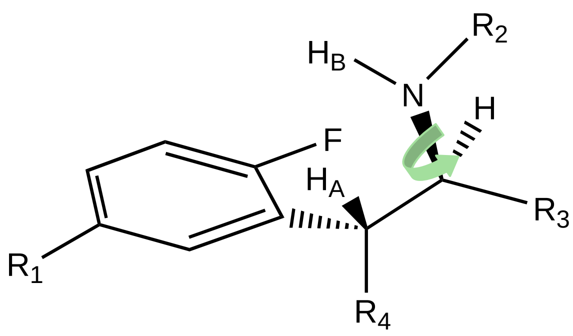
E_a in 2- Propanol = 15 kJ/mole = 4 kcal/mole

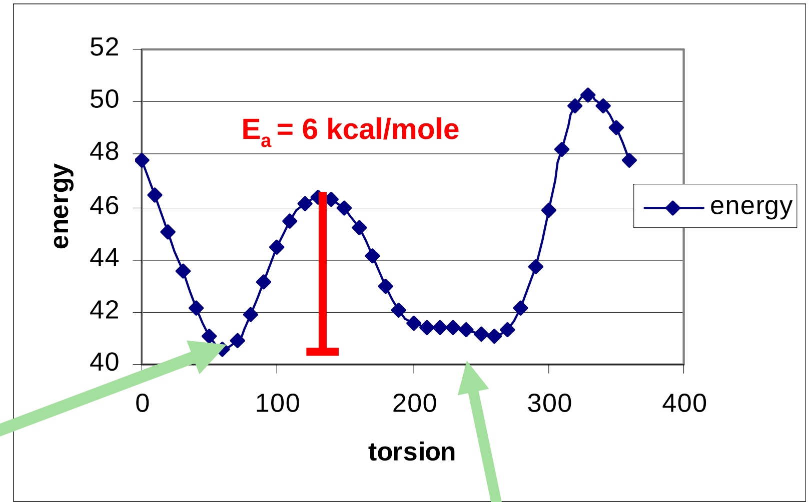
Activation Energy (Ea) in Various Solvent Systems by LC Method

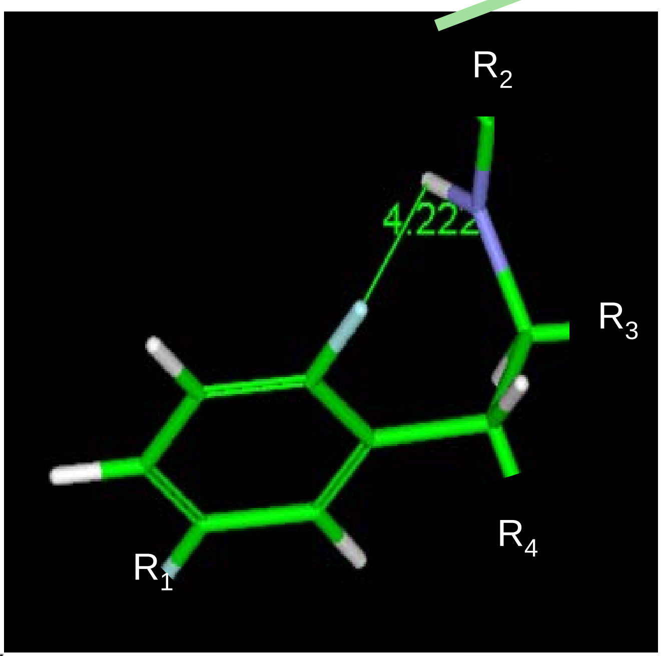
Non-alcohol		1º alcohol/ Ea kcal/mol		2º alcohol / Ea kcal/mol		3º alcohol / Ea kcal/mol	
Acetonitrile	N/A*	Ethanol	6	2-Propanol	4	tert-Butanol	4
		1-Propanol	6	2-Butanol	5		
		1-Butanol	6				

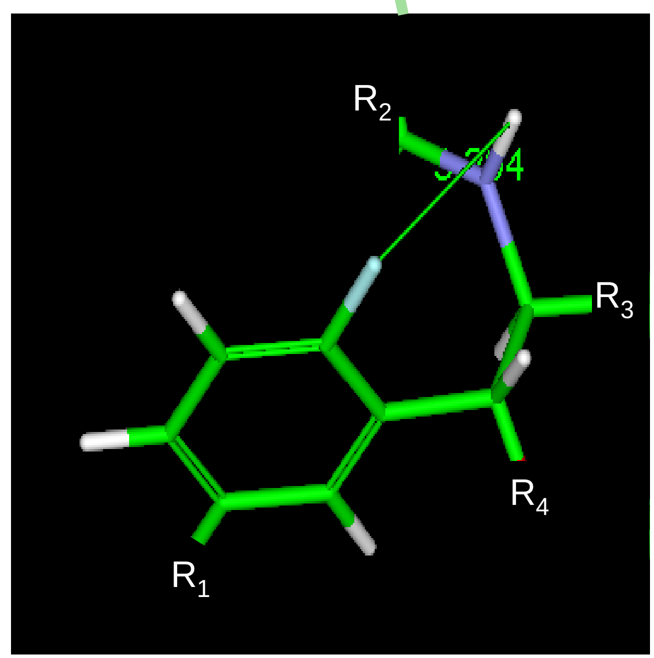
* % rotamer B change not observed in MeCN over 7 hrs at 40C and 24 hours at 5C

Ea by Molecular Modeling

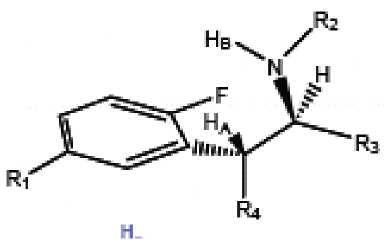








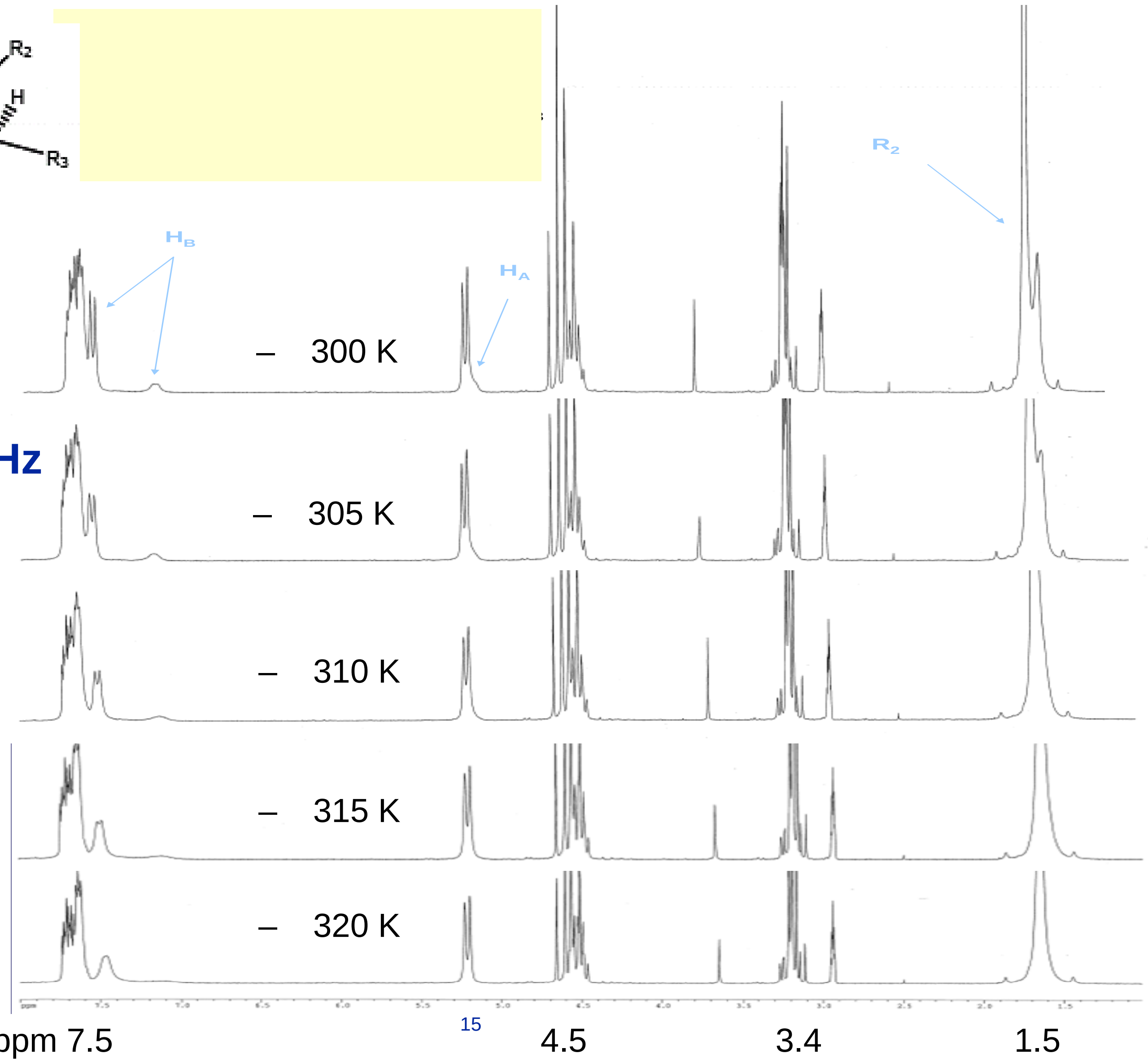
Ea by Solution NMR



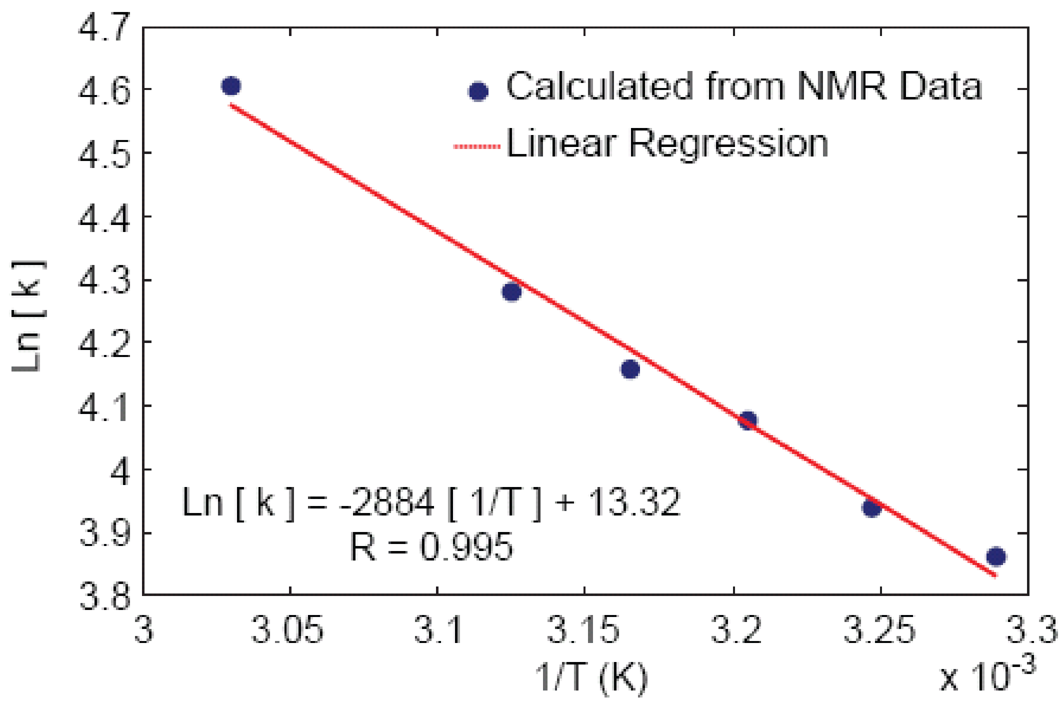
d3-DMSO

Magnet = 300 MHz

Spin = 20 kHz



Cont'd Ea by Solution NMR



– Activation Energy = 6 kcal/mole

Comparison of Ea by Various Techniques

Technique	Ea (kcal/mol)
Proton NMR	6
HPLC- NP	4-6
Molecular Modeling	6

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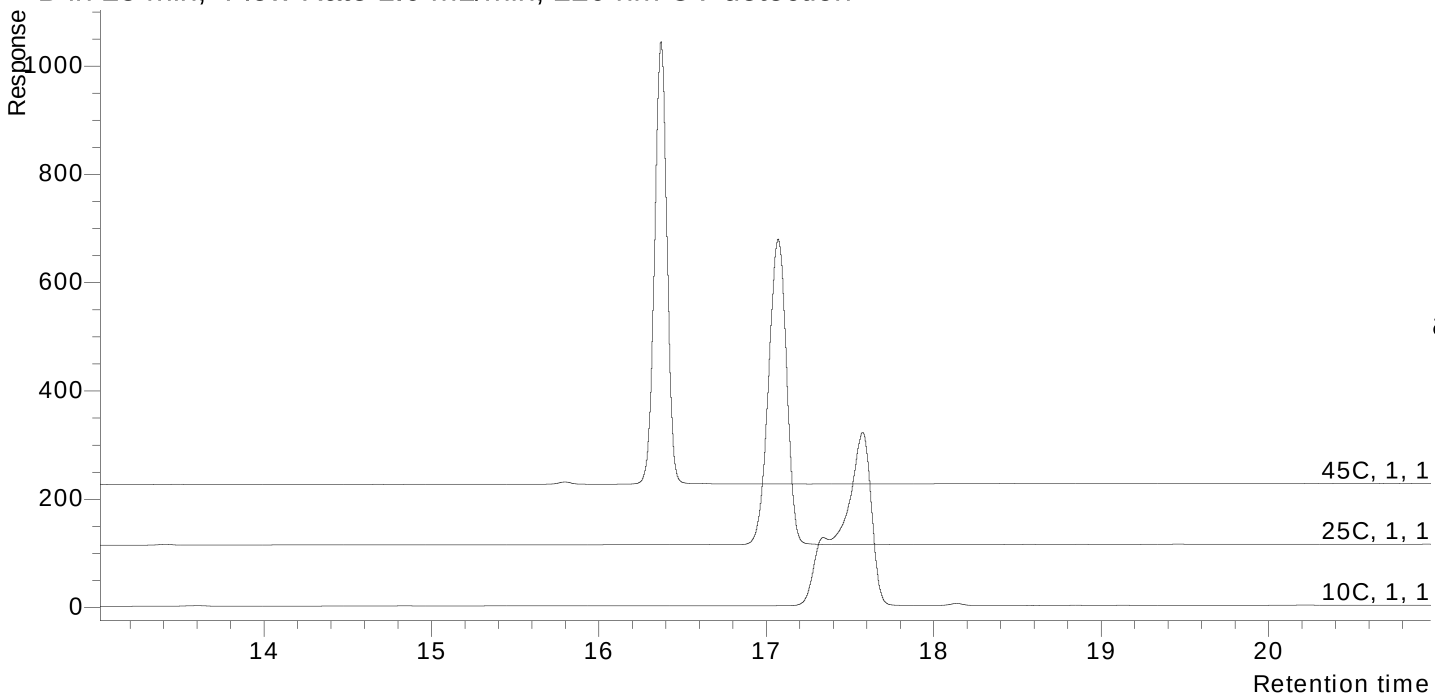
So How is This Study Practical ?

RPLC Method: Waters Symmetry Shield C18 100 mm x 4.6 mm x 3 mm; A=0.1%Phosporic Acd, B=Acetonitrile; 10% B to 90% B in 25 min; Flow Rate 1.0 mL/min; 220 nm UV detection



– LC-MS and DAD confirmed that the two species have an identical UV trace and MS/MS fragmentation pattern

Ultimately, this API was found to contained rotamers. (Molecular Modeling and NMR also confirm)

When the processing team tried to crystallize the API at room temp, a poor crystal structure was obtained (if any at all). However, heating the system to 80 °C converted the rotamers to one conformational state so that when the antisolvent was added the API could easily be crystallized.

Conclusion

- **The energy of activation (Ea) of an intermediate of active pharmaceutical ingredient (API) determined by the normal phase chiral liquid chromatography in different diluents ranges from 4-6 kcal/mole. The Ea determined by H1-NMR in is 6 kcal/mole. Both calculated Ea corresponds well to the predicted value of 6 kcal/mole from modeling.**

- **Molecular modeling was successfully used to predict and explain chromatographic behaviors .**

Acknowledgements

Bing Mao

Peter Skrdla

Ahmed Abrahim

Zhihong Ge

Literature References

- http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TGW-41YG7VJ-19&_user=5891&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000001418&_version=1&_urlVersion=0&_userid=5891&md5=4dff50ba8e2f7fbe02d8f299e948d239
  - Activation energy for this complicated cluster is 5 kJ/mol

- For typrophan the free energy of activation ranges from 4-10 kcal/mol
  - http://www.biophysj.org/cgi/content/full/91/3/816/TBL2

- For these platinum complexes the activation energy between rotamers is 14 kcal/mol
  - http://www3.interscience.wiley.com/cgi-bin/fulltext/104544669/PDFSTART\

- 2-(2'-aminophenyl) benzimidazole
- Has activation energy between 0.5 kcal/mol and 9 kcal/mol depending on ground state vs excited singlet state for rotamer conversion
- http://www.ingentaconnect.com/content/els/03010104/1998/00000226/00000003/art00311;jsessionid=191ohjt4d063n.alice?format=print

- 1,2-diacetyl benzene, 1,2-dipropanoyl benzene - rotamer activation energy 4.9 kcal/mol
  - http://pubs.acs.org/cgi-bin/abstract.cgi/joceah/1997/62/i22/abs/jo970669x.html

- http://article.pubs.nrc-cnrc.gc.ca/ppv/RPViewDoc?issn=1480-3291&volume=43&issue=8&startPage=2135
  - DMFEa of 6.5 kcal/mole, rotamers observed by NMR