Part 1: Statistical Comparison of Computed and Experimental NMR Coupling Constants: A Model Study

Part 2: Reaction Titration of Hydride Solutions by No-D NMR Spectroscopy

A Thesis

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by

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This is to certify that I have examined this copy of a Master's Thesis by

Andrew William Aspaas

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1. Statistical Comparison of Computed and Experimental NMR Coupling Constants: A Model Study

1.1. Background

Often when a new natural product is isolated, some or all of its stereocenters are undefined when the molecule is first presented in the literature. In the past, the only way to correctly identify the stereocenters was to synthesize several or all of the stereoisomers of the molecule and compare the ^{1}H NMR spectra of the synthesized products to the natural product. For a natural product with several undefined stereocenters, this can be a tedious and inefficient process. The need has therefore arisen to investigate methods that will remove the element of trial-and-error from natural product total synthesis. If the stereochemical configuration of a natural product could be predicted, only that one likely configuration would need to be synthesized, instead of all the possible stereoisomers.

Qualitative methods have been developed to analyze the relative stereochemical configuration of complicated molecules through the use of NMR spectroscopy. By using advanced two-dimensional NMR techniques, $^3J_{C,H}$ coupling can be correlated with dihedral angles in difficult cases such as large, conformationally flexible molecules where simple qualitative analysis of $^3J_{H,H}$ coupling constants gives far less useful data.[1] Murata and coworkers have used this method to successfully assign the relative configurations of unknown stereocenters in large, acyclic natural products.[1] This method can be used along with the quantitative methods described below to make a convincing argument for the configuration of unknown stereocenters.

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[1] (a) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999,** *64,* 866-876. (b) Murata, M.; Matsuoka, S.; Matsumori, N.; Paul, G. K.; Tachibana, K. *J. Am. Chem. Soc.* **1999,** *121,* 870-871.

The infiltration of computational methods into synthetic organic chemistry has provided a way to quantify the relationship between empirical NMR data and the configuration of unknown stereocenters in complex natural products. In 1984, E. J. Corey presented the use of molecular modeling programs to calculate key dihedral angles in the possible configurations of hygrolidin, bafflomycin C_1, and Merck L-681.[2] The modified Karplus equation was used to calculate $^3J_{H,H}$ coupling constants from the dihedral angles for protons attached to the unknown stereocenters. The calculated *J* values were compared to NMR data in the literature for these compounds. The calculated molecular configuration that most closely matched the NMR data was presumed to contain the correct stereochemical configurations of that natural product. Their predictions were later confirmed to be correct through synthesis of these natural products.

Several projects in the Hoye group at the University of Minnesota have involved molecular modeling computations to determine the *J* values of the different possible stereoisomers of natural products.[3] These calculated coupling constants are compared to the experimental values from the natural product's original ^{1}H NMR spectrum, but due to the complexity of these molecules, the data are analyzed statistically as a means to quantitatively predict the stereochemical assignment of the natural product.

One such project involved the assignment of the configuration of two distinct stereoisomers of latrunculin B which were isolated from the sponge *Latruncula magnifica*.[3b] MacroModel 6.0 was used to run MM2* molecular modeling calculations to predict the structures of the possible stereoisomers of these conformationally flexible

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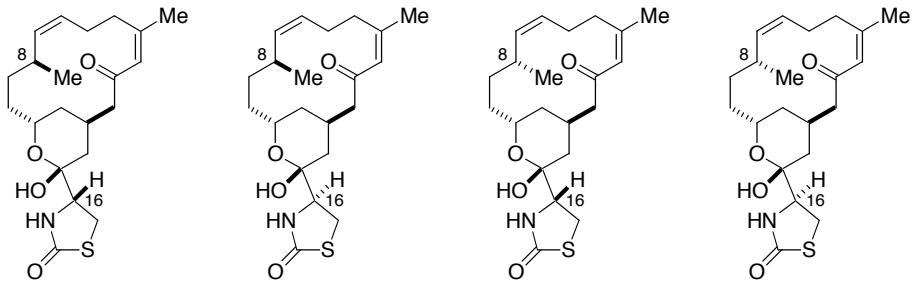
[2] Corey, E. J.; Ponder, J. W. *Tetrahedron Lett.* **1984,** *25,* 4325-4328.

[3] (a) Ayyad, S. N.; Judd, A. S.; Shier, W. T.; Hoye, T. R. *J. Org. Chem.* **1998**, *63,* 8102-8106. (b) Hoye, T. R., Ayyad, S. N.; Eklov, B. M.; Hashish, N. E.; Shier, W. T.; El Sayed, K. A.; Hamann, M. T. *J. Amer. Chem. Soc.* **2002**, *124*, 7405-7510. (c) Hoye, R. C.; Wang, J. Unpublished work. 2002.

molecules, and the same program was able to calculate Boltzmann-averaged $^{3}J_{H,H}$ coupling constants from the calculated conformations. The $\chi^{2'}$ method, which will be described in detail later in this thesis, was used to statistically compare the calculated coupling constants from the molecular modeling computations of the different possible diastereomers of latrunculin B, labeled ***A***, ***B***, ***C***, and ***D*** in Table 1.

Table 1. $\chi^{2'}$ comparison of experimental *J* values for latrunculin B (***A***) and 16-*epi*-latrunculin B (***B***) vs computed *J* values for latrunculin B (***A***), 16-*epi*-latrunculin B (***B***), 8-*epi*-latrunculin B (**C**), and 8,16-*bisepi*-latrunculin B (***D***).[3b]



A latrunculin B | ***B*** 16-*epi*-latrunculin B | ***C*** 8-*epi*-latrunculin B | ***D*** 8,16-*bisepi*-latrunculin B

		$\chi^{2'}$	
entry	data sets	no solvation	$CHCl_3$ solvation
1	$\boldsymbol{A}_{exp}$ vs $\boldsymbol{A}_{calc}$	**19**	**23**
2	$\boldsymbol{A}_{exp}$ vs $\boldsymbol{B}_{calc}$	27	35
3	$\boldsymbol{A}_{exp}$ vs $\boldsymbol{C}_{calc}$	158	207
4	$\boldsymbol{A}_{exp}$ vs $\boldsymbol{D}_{calc}$	148	182
5	$\boldsymbol{B}_{exp}$ vs $\boldsymbol{A}_{calc}$	29	26
6	$\boldsymbol{B}_{exp}$ vs $\boldsymbol{B}_{calc}$	**21**	**25**
7	$\boldsymbol{B}_{exp}$ vs $\boldsymbol{C}_{calc}$	166	212
8	$\boldsymbol{B}_{exp}$ vs $\boldsymbol{D}_{calc}$	153	189

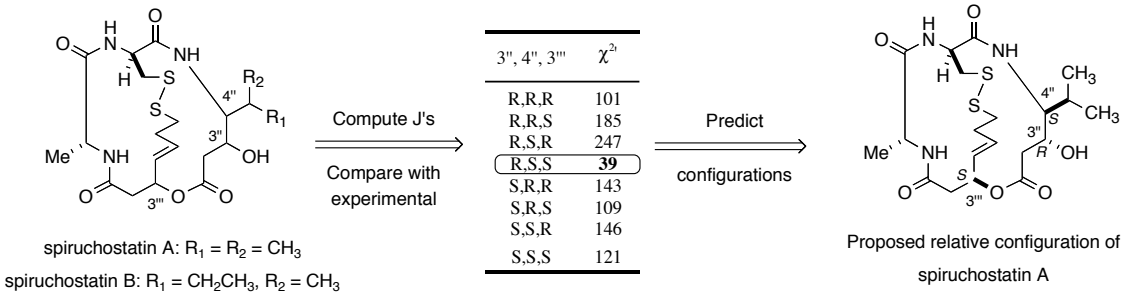
Smaller $\chi^{2'}$ values indicate a closer correlation, so as Table 1 shows, the experimental coupling constants for compound ***A*** closely matched the calculated coupling

constants for latrunculin B, and the experimental coupling constants for compound ***B*** closely matched the calculated coupling constants for 16-*epi*-latrunculin B. These results are in agreement with the subsequently verified structures of latrunculin B and 16-*epi*-latrunculin B.[3b]

However, before this success with the latrunculins, the Hoye group applied this same methodology to the ottelione family of natural products, but with a less successful outcome.[3a] The statistical analysis method was unable to correctly predict the correct stereochemical configuration of these molecules, even though their structure is more conformationally rigid than the latrunculins.

Weaknesses in this approach were also revealed when it was applied to the isolated natural product Spiruchostatin A.[4] As reported, the configurations of the 3'', 4'', and 3''' stereocenters were undefined. A series of NMR experiments yielded the full complement of *J* values for the isolated compound. The 8 compounds representing all possibilities were entered into MacroModel and all computed *J* values were recorded. When the values were compared with the $\chi^{2'}$ method, the *R, S, S* compound had the closest correlation between experimental and computed *J* values, as shown in Scheme 1.

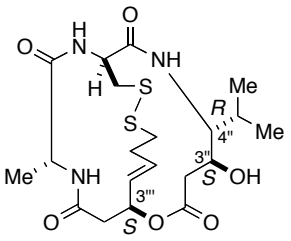
Scheme 1. Prediction of the configuration of unknown stereocenters in spiruchostatin A.



[4] (a) Masuoka, Y.; Nagai, A.; Shin-ya, K.; Furihata, K.; Nagai, K.; Suzuki, K.; Hayakawa, Y.; Seto, H. *Tetrahedron Lett*. **2001**, *42*, 41-44. (b) Hoye, T. R.; Hoye, R. C., unpublished work. (c) Hoye, T. R.; Wang, J., unpublished work.

Spiruchostatin A was successfully synthesized in 2004,[5] and the synthetic NMR spectrum matched the isolated product's spectrum, but the configuration of the 3 previously unknown stereocenters were not in agreement with the Hoye group's predictions (Figure 1). The correct structure was the *S, R, S* diastereomer, where the *R, S, S* diastereomer had been predicted.

Figure 1. Correct structure of spiruchostatin A.



spiruchostatin A

synthesized structure

3'', 4'', 3'''	χ^{2}	
R,R,R	101	
R,R,S	185	
R,S,R	247	
R,S,S	**39**	⟸ Predicted structure
S,R,R	143	
S,R,S	109	⟸ Correct structure
S,S,R	146	
S,S,S	121	

The need has therefore arisen to closely investigate both the computational method as well as the actual method of statistical comparison in order to gauge this method's effectiveness in correctly correlating experimental and calculated coupling constants.

1.2. Studies

A model study was devised whereby all stereoisomers of a simple molecule would be synthesized and all ^{1}H NMR coupling constants would be measured. The coupling constants for all isomers would also be computed using the Monte Carlo MM2* routines in MacroModel® 6.0, and the computed and experimental values would be

[5] Yurek-George, A.; Habens, F.; Brimmell, M.; Packham, G.; Ganesan, A.; *J. Am. Chem. Soc.* **2004**, *126*, 1030-1031.

compared statistically. Menthol and its other three diastereomers (Figure 2) were chosen for this study, due to their three stereocenters and their semi-rigid substituted cyclohexane structure. If the computed coupling constants closely match the experimental coupling constants, the usefulness of the technique will be reinforced. The statistical method of comparison will also be investigated, such that the correct pair of computed and experimental coupling constant sets will be most easily differentiated from the incorrect pairs. To our knowledge, no researchers have obtained complete sets of coupling constants for all four menthol diastereomers, so the NMR data will also be a new contribution to the scientific community.

Figure 2. Menthol and its diastereomers.

menthol ***1*** isomenthol ***2*** neomenthol ***3*** neoisomenthol ***4***

Menthol (**1**) and isomenthol (**2**) are commercially available. To prepare the remaining two diastereomers, the Mitsunobu reactions of menthol and isomenthol were explored to invert their carbinol centers to form neomenthol (**3**) and neoisomenthol (**4**), respectively. Stereoselective reductions of the parent ketones menthone and isomenthone were also investigated to form **3** and **4**. High-resolution ^{1}H NMR spectroscopy (500 and 800 MHz) was employed to extract coupling constants from each of the four isomers. NMR techniques such as resolution enhancement, decoupling, and 2-dimensional *J*-resolved spectroscopy were used to electronically analyze overlapping peaks, while

mixed-solvent systems and selective deuteration helped to simplify the spectra. The experimental coupling constants were compared to calculated Boltzmann-averaged *J* values from MM2* Monte Carlo multi-conformational searches using MacroModel®.

The process of comparing computed coupling constants with experimental values has promise of becoming a standard technique chemists can use with relative ease to predict the relative configurations of natural products. This project seeks to verify the reliability of the technique by using a simpler set of molecules than those natural products to which this method has been applied before.[1] The quality of the correlation between the experimental and computed values in this model study will be examined, and the statistical method used to compare the values will be explored to give the best fit possible, so that larger molecules with less clearly defined conformations can be more accurately compared with the computational data.

1.2.1. MacroModel computations

The complete set of MacroModel calculations for menthol (**1**), isomenthol (**2**), neomenthol (**3**), and neoisomenthol (**4**) were completed using MacroModel 6[6] with the computing resources of the Minnesota Supercomputing Institute. A Monte Carlo multi-conformational search was set up using MacroModel's "normal" sequence and "automatic setup" routine to identify torsion and closure bonds. 1,000 Monte Carlo generated conformations were minimized using the MM2* force field with $CHCl_3$ solvent simulation. The Boltzmann-averaged coupling constants were measured using the "CoplF" routine. All coupling constants obtained showed agreement with previous

[6] (a) Karplus, M. *J. Chem. Phys.* **1959,** *30,* 11-15. (b) Haasnoot, C. A. G.; DeLeeuw, F. A. A. M.; Altona, C. *Tetrahedron.* **1981,** *36,* 2783-2792. (c) Garbisch, E. W., Jr. *J. Am. Chem. Soc.* **1964,** *86,* 5561-5564.

calculations performed by B. M. Eklov from the Hoye group[7] to within 0.1 Hz. These computed coupling constants appear in Table 2.

1.2.2. Synthesis of menthol diastereomers

1.2.2.1. Menthol

Using 500 MHz ^{1}H NMR, all $^3J_{H\text{-}H}$ coupling constants of menthol (**1**) were easily assigned, and were in good agreement with previous studies by Eklov.[5] These values are shown in Table 3. In order to statistically compare the computed coupling constants with the experimental values, $\chi^{2\prime}$ was chosen as a statistical analysis. This $\chi^{2\prime}$ method [$\chi^{2\prime} = \Sigma(A_{observed} - A_{expected})^2$] was chosen instead of the traditional χ^2 value [$\chi^2 = \Sigma(A_{observed} - A_{expected})^2 / A_{expected}$] that is used in most least-squares analyses so that the importance of small J values would be the same as large J values (the extra division by $A_{expected}$ amplifies the statistical importance of smaller values).[1b] In this case, $\chi^{2\prime} = \Sigma(J_{exp} - J_{calc})^2$. Results of the $\chi^{2\prime}$ analysis are shown in the right four columns of Table 2. For menthol, the $\chi^{2\prime}$ value for the correct pair of calculated and experimental coupling constant sets is 2.6, much smaller than the other values of 174.4, 174.8, and 283.8. This shows a very good correlation between experimental and calculated values; the correct match has nearly 1/100 the $\chi^{2\prime}$ value of the other diasteromers. More complex statistical manipulations will be explored in section 1.2.3.

[7] Eklov, B. M. Unpublished work. 2002.

Table 2. Comparison of experimental and calculated coupling constants of (a) menthol (**1**), (b) isomenthol (**2**), and (c) neomenthol (**3**). Computational data for neoisomenthol (**4**) is also included in each table.

a.

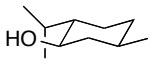
Coupling	Experimental *J*, Hz Menthol (1)	Calculated *J*, Hz 1	2	3	4
1-2	10.5	10.4	7.7	1.9	2.6
$1\text{-}6_{eq}$	4.4	4.5	3.8	3.7	5.4
$1\text{-}6_{ax}$	10.4	11.3	8.6	2.4	3.2
2-7	2.9	2.5	5.7	8.8	9.6
$2\text{-}3_{eq}$	3.2	2.9	3.4	3.1	3.4
$2\text{-}3_{ax}$	12.4	12.3	9.0	12.3	10.0
$3_{eq}\text{-}4_{eq}$	3.4	3.1	6.6	3.1	5.6
$3_{eq}\text{-}4_{ax}$	3.4	3.3	3.5	3.3	3.5
$3_{ax}\text{-}4_{eq}$	3.4	3.5	3.3	3.5	3.4
$3_{ax}\text{-}4_{ax}$	12.5	13.2	9.9	13.2	10.8
$4_{eq}\text{-}5$	3.4	3.3	5.7	3.3	4.7
$4_{ax}\text{-}5$	11.8	12.3	4.2	12.3	4.4
$5\text{-}6_{eq}$	3.4	3.3	5.7	3.3	4.6
$5\text{-}6_{ax}$	11.8	12.3	4.2	12.3	4.6
5-10	6.7	6.4	6.4	6.4	6.4
7-8/9	7.0	6.4	6.5	6.5	6.5
$\Sigma\lvert\Delta\rvert$	0	**5.20**	38.2	26.6	47.3
$\Sigma\Delta^2$	0	**2.66**	174	175	284
$\Sigma\lvert\Delta\rvert^5$	0	**1.69**	1042	1355	1980
coeff (β_1)	1	**0.932**	1.06	0.626	0.388
RMSE	0	**0.321**	3.35	0.321	3.94
t-stat	∞	**2.91**	0.317	1.95	0.0985
F-stat	∞	**2242**	6.66	10.8	0.940
std. err.	0	**0.0197**	0.412	0.190	0.400
coeff/std.err.	∞	**47.4**	2.58	3.29	0.969
intercept (β_0)	0	**0.444**	0.660	3.02	4.76
R^2	1	**0.994**	0.322	0.437	0.0629

b.

Coupling	Experimental *J*, Hz Isomenthol (2)	Calculated *J*, Hz 1	2	3	4
1-2	7.5	10.4	7.7	1.9	2.6
$1\text{-}6_{eq}$	3.8	4.5	3.8	3.7	5.4
$1\text{-}6_{ax}$	8.3	11.3	8.6	2.4	3.2
2-7	5.5	2.5	5.7	8.8	9.6
$2\text{-}3_{eq}$	3.5	2.9	3.4	3.1	3.4
$2\text{-}3_{ax}$	8.7	12.3	9.0	12.3	10.0
$3_{eq}\text{-}4_{eq}$	7.1	3.1	6.6	3.1	5.6
$3_{eq}\text{-}4_{ax}$	3.5	3.3	3.5	3.3	3.5
$3_{ax}\text{-}4_{eq}$	3.5	3.5	3.3	3.5	3.4
$3_{ax}\text{-}4_{ax}$	9.5	13.2	9.9	13.2	10.8
$4_{eq}\text{-}5$	5.8	3.3	5.7	3.3	4.7
$4_{ax}\text{-}5$	4.1	12.3	4.2	12.3	4.4
$5\text{-}6_{eq}$	6.2	3.3	5.7	3.3	4.6
$5\text{-}6_{ax}$	4.6	12.3	4.2	12.3	4.6
5-10	6.3	6.4	6.4	6.4	6.4
7-8/9	6.7	6.4	6.5	6.5	6.5
$\Sigma\lvert\Delta\rvert$	0	43.3	**3.70**	48.4	23.3
$\Sigma\Delta^2$	0	211	**1.20**	261	79.0
$\Sigma\lvert\Delta\rvert^5$	0	1288	**0.500**	1626	337
coeff (β_1)	1	1.11	**1.06**	0.489	0.673
RMSE	0	3.72	**0.269**	4.18	2.24
t-stat	∞	0.298	**3.94**	0.117	0.300
F-stat	∞	5.17	**904**	0.797	5.24
std. err.	0	0.487	**0.0352**	0.548	0.294
coeff/std.err.	∞	2.27	**30.1**	0.893	2.29
intercept (β_0)	0	0.385	**-0.374**	3.32	1.56
R^2	1	0.270	**0.985**	0.0538	0.272

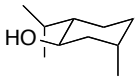
c.

Coupling	Experimental *J*, Hz Neomenthol (3)	Calculated *J*, Hz 1	2	3	4
1-2	2.5	10.4	7.7	1.9	2.6
$1\text{-}6_{eq}$	3.6	4.5	3.8	3.7	5.4
$1\text{-}6_{ax}$	2.5	11.3	8.6	2.4	3.2
2-7	9.2	2.5	5.7	8.8	9.6
$2\text{-}3_{eq}$	4.1	2.9	3.4	3.1	3.4
$2\text{-}3_{ax}$	13.1	12.3	9.0	12.3	10.0
$3_{eq}\text{-}4_{eq}$	3.8	3.1	6.6	3.1	5.6
$3_{eq}\text{-}4_{ax}$	3.7	3.3	3.5	3.3	3.5
$3_{ax}\text{-}4_{eq}$	3.8	3.5	3.3	3.5	3.4
$3_{ax}\text{-}4_{ax}$	13.1	13.2	9.9	13.2	10.8
$4_{eq}\text{-}5$	3.8	3.3	5.7	3.3	4.7
$4_{ax}\text{-}5$	12.3	12.3	4.2	12.3	4.4
$5\text{-}6_{eq}$	3.6	3.3	5.7	3.3	4.6
$5\text{-}6_{ax}$	12.0	12.3	4.2	12.3	4.6
5-10	6.5	6.4	6.4	6.4	6.4
7-8/9	6.5	6.4	6.5	6.5	6.5
$\Sigma\lvert\Delta\rvert$	0	29.1	46.5	**5.67**	28.8
$\Sigma\Delta^2$	0	188	246	**3.35**	142
$\Sigma\lvert\Delta\rvert^3$	0	1475	1556	**2.40**	954
coeff (β_1)	1	0.607	0.515	**0.965**	1.10
RMSE	0	3.21	3.00	**0.345**	3.00
t-stat	∞	0.189	0.172	**2.79**	0.365
F-stat	∞	9.48	1.10	**2018**	12.9
std. err.	0	0.197	0.492	**0.0215**	0.305
coeff/std.err.	∞	3.08	1.05	**44.9**	3.59
intercept (β_0)	0	2.29	3.47	**0.508**	0.431
R^2	1	0.404	0.0727	**0.993**	0.480



menthol

1

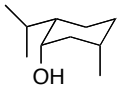


isomenthol

2

neomenthol

3



neoisomenthol

4

1.2.2.2. Isomenthol

The 500 MHz ^{1}H, COSY, HMQC and 2D-*J* NMR spectra of isomenthol (**2**) were then obtained in $CDCl_3$. Most of the peaks were overlapping in the ^{1}H spectrum, however. There was significant enough non-first order character in nearly all of the multiplets that their coupling constants could not be determined in these conditions. Non-first order character made the 2D-*J* spectrum inaccurate, as erroneous resonances appeared in several places in the spectrum. Titration of benzene-d_6 into the chloroform-*d* allowed for the deconvolution of some of the protons in the spectrum. Sine-bell and Gaussian apodization functions applied to the FID (known casually as "resolution enhancement") as well as careful adjustment of the amount of benzene added allowed for some of the peaks to be resolved, but many remained obscured.

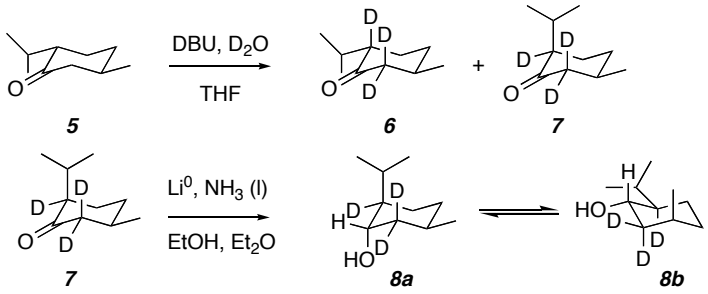
To aid in NMR assignments, isomenthol-d_3 (**8**)[8] was prepared (Scheme 2), which yielded a greatly simplified spectrum. Menthone (**5**) was trideuterated α to the carbonyl using DBU and D_2O in THF to provide an equilibrium mixture of 81% menthone-d_3 (**6**) and 19% isomenthone-d_3 (**7**). The mixture was separated using medium-pressure liquid chromatography, and isomenthone-d_3 was subjected to a dissolving-metal reduction to give exclusively isomenthol-d_3 (**8)** in 79% yield. Its ^{1}H NMR spectrum contained 3 fewer resonances and, as such, allowed for more of the peaks to be cleanly visible, and also simplified the remaining resonances.

In order to further resolve the peaks in the ^{1}H spectrum, the University of Minnesota Department of Biochemistry 800 MHz NMR spectrometer was used to obtain ^{1}H NMR spectra of isomenthol in $CDCl_3$ and C_6D_6. This enhanced the peak separation enough that at least one coupling constant was visible for each coupling pair, and in many

[8] Solodar, J. *J. Org. Chem.* **1976,** *41,* 3461-3464.

cases, the *J* values were found in both multiplets for each *J* coupling interaction. An overview of the spectra in $CDCl_3$ and C_6D_6 and at both 500 MHz and 800 MHz is shown in Figure 3. In all cases, the coupling constants were equal plus or minus 0.3 Hz for the multiplets in $CDCl_3$ compared to those in C_6D_6. Such manipulations allowed for a complete set of coupling constants to be determined.[9] Its experimental coupling constants also closely matched the computed values, with a $\chi^{2'}$ value of 1.2 (Table 2).

Scheme 2. Synthesis of deuterated isomenthol (**8a** and **8b**).



[9] For a convenient method of determining coupling constant values, see Hoye, T. R.; Zhao, H. *J. Org. Chem.* **2002**, *67*, 4014-4016.

Figure 3. ^{1}H NMR spectra of isomenthol (**2**). (a) 500 MHz, $CDCl_3$; (b) 800 MHz, $CDCl_3$; (c) 500 MHz, C_6D_6; (d) 800 MHz, C_6D_6.

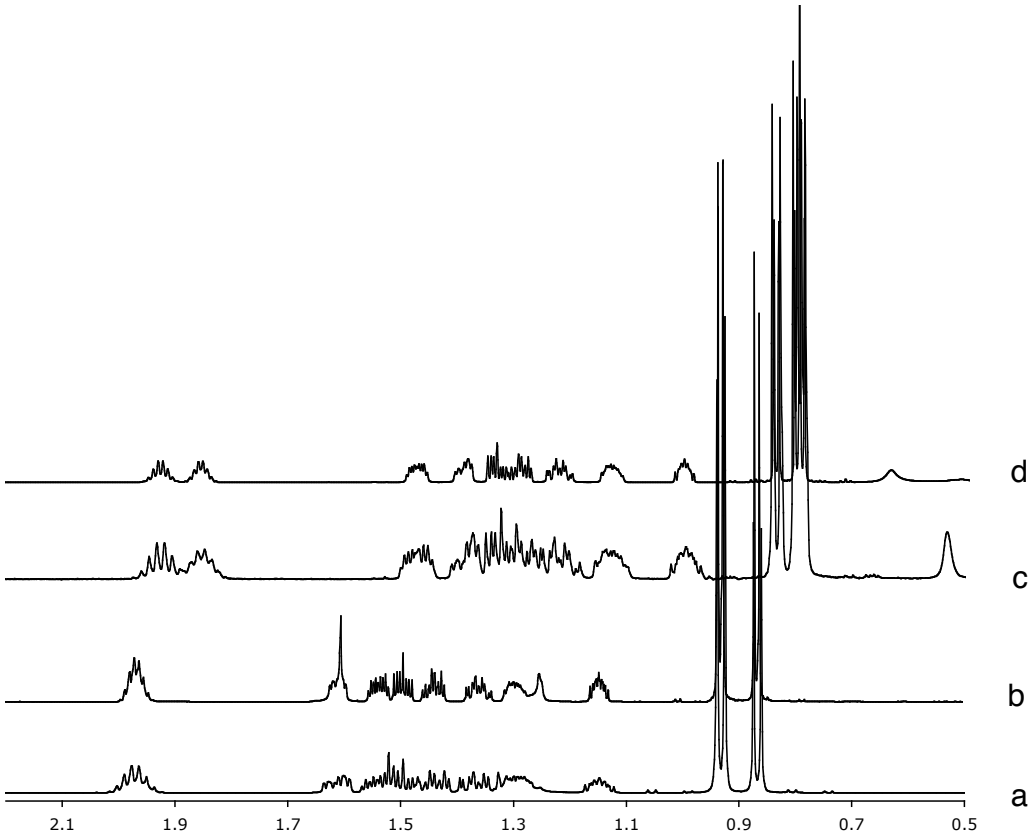
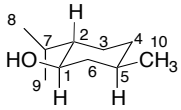
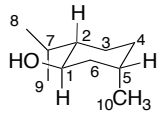
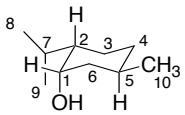


Table 3. Proton chemical shifts in $CDCl_3$ and C_6D_6 and coupling constants for menthol (**1**), isomenthol (**2**), and neomenthol (**3**).

Menthol (1)	**Proton**	**δ (ppm) $CDCl_3$**	**Mult.**	***J* (Hz)**
 menthol ***1***	1	3.41	ddt	2.5, 3.4, 10.5, 10.5
	2	1.11	tdd	3.0, 3.0, 10.0, 12.0
	3ax	0.97	dddd	3.3, 11.8, 12.0, 12.9
	3eq	1.61	qd	3.3, 3.3, 3.3, 12.9
	4ax	0.84	dddd	3.3, 11.8, 12.0, 12.3
	4eq	1.66	dqd	2.0, 3.5, 3.5, 3.5, 12.5
	5	1.43	tqt	3.5, 3.5, 6.5, 6.5, 6.5, 12.0, 12.0
	6ax	0.92	dt	10.5, 12.0, 12.0
	6eq	1.96	dddd	2.0, 3.4, 4.1, 12.0
	7	2.17	d sept	2.5, 7

Isomenthol (2)	**Proton**	**δ (ppm) $CDCl_3$**	**C_6D_6**	**Mult.**	***J* (Hz)**
 isomenthol ***2***	1	3.80	3.58	ddd	3.8, 7.5, 8.3
	2	1.15	1.01	dddd	3.5, 5.5, 7.5, 8.7
	3ax	1.36	1.24	dddd	3.5, 8.7, 9.5, 13.5
	3eq	1.54	1.49	tdd	3.5, 3.5, 7.1, 13.5
	4ax	1.44	1.31	dddd	3.5, 4.1, 9.5, 13.0
	4eq	1.30	1.14	ddddd	1.3, 3.5, 5.8, 7.1, 13.0
	5	1.97	1.86	octet	7.3
	6ax	1.50	1.37	ddd	4.6, 8.3, 13.0
	6eq	1.61	1.40	dddd	1.3, 3.8, 6.2, 13.0
	7	1.97	1.92	octet	6.7

Neomenthol (3)	**Proton**	**δ (ppm) $CDCl_3$**	**C_6D_6**	**Mult.**	***J* (Hz)**
 neomenthol ***3***	1	4.11	3.86	td	2.5, 2.5, 3.6
	2	0.87	0.67	dddd	2.5, 4.1, 9.2, 13.1
	3ax	1.27	1.32	dq	3.8, 13.1, 13.1, 13.1
	3eq	1.84	1.86	dddd	3.7, 3.8, 4.1, 13.1
	4ax	0.89	0.76	dddd	3.7, 12.3, 13.1, 13.4
	4eq	1.71	1.63	dqd	2.5, 3.8, 3.8, 3.8, 13.4
	5	1.67	1.64	ddqdd	3.6, 3.8, 6.5, 6.5, 6.5, 12.0, 12.3
	6ax	1.09	1.54	ddd	2.5, 12.0, 13.9
	6eq	1.73	1.57	dtd	2.5, 3.6, 3.6, 13.9
	7	1.52	1.69	sept d	6.5, 9.2

1.2.2.3. Neomenthol

Neomenthol (**3**) was synthesized by first performing the Mitsunobu inversion of menthol (**1**)[10] using *p*-nitrobenzoic acid, triphenylphosphine, and diethyl azodicarboxylate (Scheme 3) to produce **2** in 81% yield. The resulting *p*-nitrobenzoate ester was saponified with $LiOH{\cdot}H_2O$ to yield neomenthol (**3**) in 54% yield. Its 500 MHz NMR spectrum contained several overlapping peaks, however. There was slightly better peak separation when the spectrum was taken in C_6D_6. The 800 MHz ^1H NMR spectrum was obtained and showed dramatically fewer overlapping peaks, but the set of coupling constants was still incomplete due to two resonances that were obscured by the three large methyl doublets. Through experimentation with several different solvent compositions, the remaining resonances were revealed by using a 50:50 $CDCl_3$:C_6D_6 solvent mixture. Finally, by use of resolution enhancement, the full complement of coupling constants was determined. These spectra are compared in Figure 4. The complete set of coupling constants was compared with the calculated values and are in good agreement with the computed values; $\chi^{2'} = 3.3$ (Table 2). To confirm the assignments, deuterated neomenthol could be prepared by reducing deuterated menthone (**6**) with $LiAl(OCH_3)_3H$.[11] This bulkier hydride source is necessary to prevent formation of menthol, which is inseparable from neomenthol even by HPLC.[9]

[10] Dodge, J. A.; Trujillo, J. I.; Presnell, M. *J. Org. Chem.* **1994**, *59*, 234-236.
[11] Haut, S. A.; *J. Agric. Food Chem.* **1985,** *33*, 278-280.

Scheme 3. Mitsunobu reactions of menthol (1) and isomenthol (2) and reduction of isomenthone.

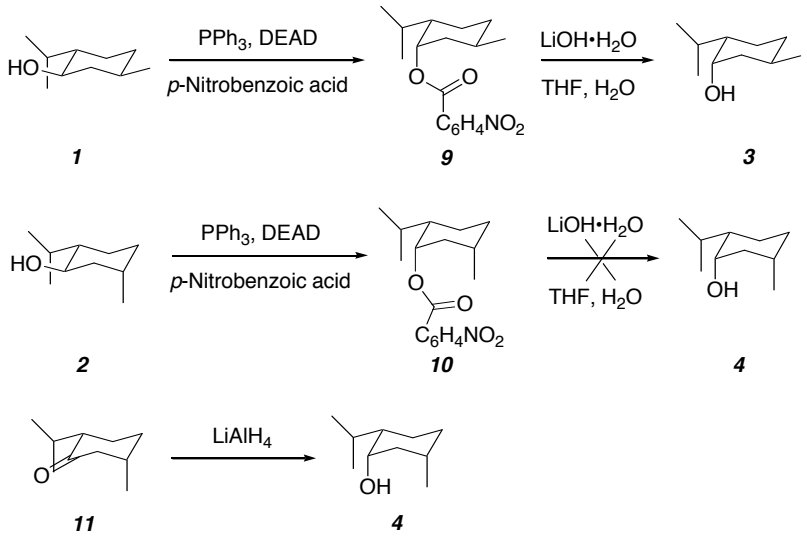
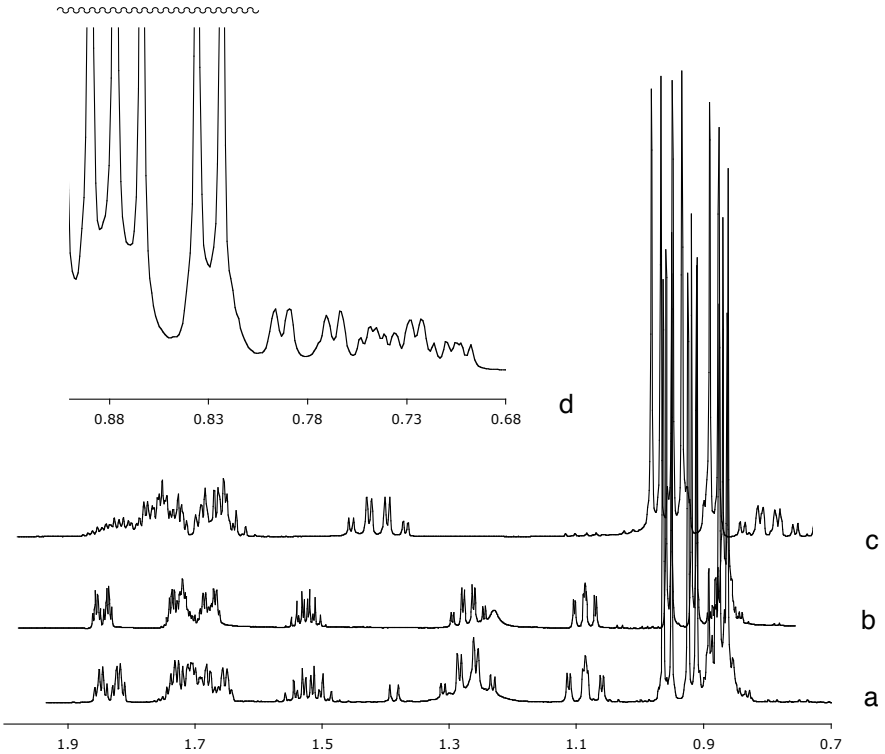


Figure 4. ^{1}H NMR spectra of neomenthol (**3**). (a) 500 MHz, $CDCl_3$; (b) 800 MHz, $CDCl_3$; (c) 500 MHz, C_6D_6; (d) 500 MHz, 50:50 $CDCl_3$:C_6D_6.



1.2.2.4. Neoisomenthol

Neoisomenthol (**4**) was planned to be produced in an analogous manner to neomenthol as above, with a Mitsunobu inversion of isomenthol (**2**) instead of menthol (**1**). Mitsunobu inversion of isomenthol (**2**) provided *p*-nitrobenzoate ester **10** successfully (Scheme 2). However, after unsuccessfully attempting a variety of hydrolysis conditions (using LiOH and KOH in methanol, water, and THF at temperatures approaching 120 °C in a sealed tube, as well as in ethylene glycol at temperatures approaching 170 °C), it was decided to synthesize neoisomenthol (**4**) by $LiAlH_4$ reduction of isomenthone (**11**, Scheme 3). [8]

An $LiAlH_4$ solution was prepared by dissolving a solid $LiAlH_4$ pellet in dry diethyl ether. The concentration of the solution was determined by No-D NMR spectroscopy, which will be described in detail in Part 2.

With an accurate concentration of the $LiAlH_4$ solution in hand, reduction of isomenthone (**11**) proceeded uneventfully, and **4** was obtained in 67% yield, and purified by MPLC (Scheme 3). Its NMR spectrum was not as clearly resolved as the other compounds, however. There was significant overlapping and non-first-order character in most of the spectrum (nearly all the methylene protons lie within a 0.2 ppm area). In order to simplify the spectrum, neoisomenthol-d_3 was synthesized by the $LiAlH_4$ reduction of isomenthone-d_3. Unfortunately, even with the three alpha-protons removed from the spectrum, the remaining resonances still were too overlapped and distorted to yield any meaningful experimental coupling constant data in this study. Nevertheless, the calculated coupling constants for neoisomenthol are included in the statistical analysis to provide one more set of data against which the three other isomers could be compared,

understanding that the calculated neoisomenthol data should match *none* of the experimentally-determined sets.

Table 4. $\chi^{2'}$ values determined for comparison of experimental and calculated coupling constants of isomenthol, menthol, and neomenthol. Small $\chi^{2'}$ values indicate a strong correlation.

Experimental configuration	Menthol (calculated)	Isomenthol (calculated)	Neomenthol (calculated)	Neoisomenthol (calculated)
Menthol (1R, 2S, 5R)	**2.6**	174.4	174.8	283.8
Isomenthol (1R, 2S, 5S)	211.2	**1.2**	261.2	79.0
Neomenthol (1S, 2S, 5R)	188.4	246.5	**3.3**	141.7

1.2.3. Statistical operations

Table 4 shows the $\chi^{2'}$ values used to compare experimental and calculated coupling constants for the three stereoisomers whose coupling constants were successfully measured experimentally in this study (menthol, isomenthol, and neomenthol). The $\chi^{2'}$ values corresponding to the correct matching of data sets (*e.g.* experimental menthol values compared to calculated menthol values) are remarkably low compared to the incorrect pairs of data. As such, if one were to isolate one of these stereoisomers with unknown stereocenter configurations, a simple comparison to the computed data for each of the possible isomers would yield a definitive identification.

Unfortunately in the case of more complicated natural product molecules, the $\chi^{2'}$ tests have failed to produce as clear-cut matches as these data. Therefore, the need has arisen to investigate the statistical comparison model in order to determine if another

analysis might produce a match that is even more numerically distinct from the other apparently "incorrect" matches.

A statistical correlation test was run on each of the data sets, using the correlation function of the Microsoft Excel Data Analysis Toolpack. This produces a matrix of Pearson correlation coefficients that show the extent to which each of the data sets is similar to each of the others. We are simply interested in the first column of the provided correlation matrix, which indicates how the single experimental data set compares to the four computed data sets. These data are shown in Table 5.

Table 5. Correlation coefficient (R^2) values determined to date for comparison of experimental and calculated coupling constants of isomenthol, menthol, and neomenthol. R^2 values closest to 1 indicate the strongest correlation.

Experimental configuration	Menthol (calculated)	Isomenthol (calculated)	Neomenthol (calculated)	Neoisomenthol (calculated)
Menthol (1R, 2S, 5R)	**0.9969**	0.5679	0.6607	0.2508
Isomenthol (1R, 2S, 5S)	0.5193	**0.9923**	0.2320	0.5217
Neomenthol (1S, 2S, 5R)	0.6354	0.2695	**0.9965**	0.6927

A correlation coefficient of 1 indicates that two data sets are identical, and a value of 0 indicates that the data sets have no correlation. The values shown in Table 5 correctly predict the correct matched pairs: each have correlation coefficient values greater than 0.99. However, this method is less desirable than the $\chi^{2\prime}$ data due to the finite nature and small size of the scale (possible values can only fall between 0 and 1) and relatively small amount of separation between the correct match and the incorrect matches. Also to note, for the experimental isomenthol data, the correlation coefficients

for the computed neomenthol and menthol values are very similar, 0.5217 and 0.5193, respectively, whereas the $\chi^{2\prime}$ differentiate these incorrect matches much more clearly, with values of 79.0 and 211.2, respectively.

Also, the rationale behind adopting $\chi^{2\prime}$ as opposed to the standard χ^2 least-squares test was assessed. Recall that $\chi^2 = \Sigma(A_{observed} - A_{expected})^2 / A_{expected}$ whereas $\chi^{2\prime} = \Sigma(A_{observed} - A_{expected})^2$. χ^2 values for these pairs of data are shown in Table 6.

Table 6. χ^2 values determined to date for comparison of experimental and calculated coupling constants of isomenthol, menthol, and neomenthol. Small χ^2 values indicate a strong correlation.

Experimental configuration	Menthol (calculated)	Isomenthol (calculated)	Neomenthol (calculated)	Neoisomenthol (calculated)
Menthol (1R, 2S, 5R)	**0.4**	35.8	69.9	71.2
Isomenthol (1R, 2S, 5S)	27.4	**0.2**	54.3	21.2
Neomenthol (1S, 2S, 5R)	31.9	45.8	**0.9**	29.5

Since these values are calculated in a similar manner to the least-squares R^2 values from Table 5, they show some of the same inconsistencies. The χ^2 method fails to adequately differentiate between the second-best matches in the isomenthol tests (when the experimental isomenthol data are compared with the calculated menthol and neoisomenthol data, χ^2 is 27.4 and 21.2, respectively), although it does still have a large differentiation between correct and incorrect sets.

More sophisticated statistical tests may be the answer to this problem. Due to the power and convenience of Microsoft Excel, tests that would have previously required an expensive statistics package such as SPSS can be easily run using the Regression Function Data Analysis Toolpack in Excel. Student's t-test and the F-test are two that can

be easily applied in this case. These tests analyze the slopes, intercepts, and errors of all the theoretical regression lines that could be generated if each of these pairs would be graphed against each other, and as such, they are a very powerful method of assessing correlation between data groups. A regression was performed on each of the pairs of data, and the t-stat and F-stat values were recorded. In both cases, a larger number indicates a stronger correlation between the data sets. If these tests were performed on two identical data sets, both the t-stat and the F-stat would be infinitely large. Table 7 shows the results of these tests. The differentiation between correct and incorrect pairs is tremendous, especially in the F-stat, where there are differences of hundreds or thousands between the correct pair and the incorrect pairs. It may prove useful to redo the statistical analysis with the t-test and F-test on the past failed attempts to predict successful configurations.

Table 7. (a) t-Test and (b) F-test values determined to date for comparison of experimental and calculated coupling constants of isomenthol, menthol, and neomenthol.

a. **t-Stat values**

Experimental configuration	Menthol (calculated)	Isomenthol (calculated)	Neomenthol (calculated)	Neoisomenthol (calculated)
Menthol (1R, 2S, 5R)	**2.91**	0.317	1.95	0.0985
Isomenthol (1R, 2S, 5S)	0.298	**3.94**	0.117	0.300
Neomenthol (1S, 2S, 5R)	0.189	0.172	**2.79**	0.365

b. **F-stat values**

Experimental configuration	Menthol (calculated)	Isomenthol (calculated)	Neomenthol (calculated)	Neoisomenthol (calculated)
Menthol (1R, 2S, 5R)	**2242**	6.66	10.8	0.940
Isomenthol (1R, 2S, 5S)	5.17	**904**	0.797	5.24
Neomenthol (1S, 2S, 5R)	9.48	1.10	**2018**	12.9

1.2.4. Conclusion

In this study complete ^{1}H NMR data were obtained for menthol (**1**), isomenthol (**2**), and neomenthol (**3**). The coupling constant data for these compounds were statistaclly compared to coupling constants computed with MacroModel, as a basis to test the effectiveness of this methodology for use in the synthesis of natural products. Neoisomenthol (**4**) was also synthesized but its resonances were too overlapped to provide useful information for this study.

The $\chi^{2\prime}$ method seems to be a very effective way of quantifying the similarity between sets of data like these. In the case of the menthol diastereomers where MacroModel's force field is parameterized very well for the calculations, the $\chi^{2\prime}$ are very small: 1.2, 2.6, and 3.3, especially when compared to the much larger numbers obtained in larger molecules such as the latrunculins, otteliones, and spiruchostatin A; values are often well into the 100's.

Other statistical comparison methods were evaluated, and the T-test and F-test show promise in more powerfully analyzing the similarities between data sets. These methods should be investigated further as new natural products are discovered with unknown stereochemical configurations.

Regardless of the statistical method used, problems with the quality of $\chi^{2\prime}$ matches in the past using more complicated molecules suggests that the computations themselves may be at fault. In the time since the inconsistent computations were run, methods in the Hoye group have been developed to ensure that the entire potential energy surface of the molecules are sufficiently covered by the Monte Carlo search, and there are methods to re-minimize the conformations found as the result of a primary Monte Carlo

search. These methods have shown promise to be beneficial in more correctly predicting the energetics and structure of the more complicated molecules. Likewise, work has been performed by members of the Hoye group to subject the conformations produced by the Monte Carlo search to more rigorous semi-empirical and *ab-initio* calculations in order to obtain more accurate thermodynamic data.

1.3. Experimental

All reactions requiring anhydrous conditions were carried out in oven-dried glassware under a nitrogen atmosphere. Anhydrous dichloromethane, tetrahydrofuran, diethyl ether, and toluene were obtained by passage through a column of activated alumina. Pyridine was distilled from potassium hydroxide and stored over 4 Å molecular sieves. Other solvents were distilled from calcium hydride or benzophenone ketyl.

All chemicals unless otherwise noted were purchased from Acros Chemical Company, Aldrich Chemical Company, Avocado Chemical Compnay, Flinn Scientific Inc., Fluka Chemical Company, or VWR Scientific, Inc.

^{1}H and ^{13}C NMR spectra were run on a 500 MHz or 300 MHz Varian Inova spectrometer. Chemical shifts are referenced to TMS (0.0 ppm) for spectra collected in $CDCl_3$ and to benzene (7.26 ppm) for spectra collected in C_6D_6.

1.3.1. Computational experiments

All computational experiments were performed in MacroModel 6[12] with the computing resources of the Minnesota Supercomputing Institute. To determine the coupling constants of a compound, a Monte Carlo multi-conformational search was set up using MacroModel's "normal" sequence and "automatic setup" routine to identify torsion and closure bonds. 1,000 Monte Carlo-generated conformations were minimized using the MM2* force field with $CHCl_3$ solvent simulation. The resultant set of conformations were then reminimized with the MM2* force field and any duplicates were automatically discarded. The Boltzmann-averaged coupling constants were then measured using the "CoplF" routine.

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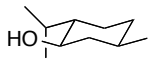
[12] (a) Karplus, M. *J. Chem. Phys.* **1959,** *30,* 11-15. (b) Haasnoot, C. A. G.; DeLeeuw, F. A. A. M.; Altona, C. *Tetrahedron.* **1981,** *36,* 2783-2792. (c) Garbisch, E. W., Jr. *J. Am. Chem. Soc.* **1964,** *86,* 5561-5564.

1.3.2. Menthol (1)



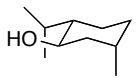
1

A sample of *l*-menthol was obtained from Aldrich Chemical Company and its NMR spectra were recorded without further purification.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.41 (dddd, *J* = 2.5, 3.4, 10.5, 10.5 Hz, 1H, C<u>H</u>OH), 2.17 [d sept, *J* = 2.5, 7.0 Hz, 1H, C<u>H</u>$(CH_3)_2$], 1.96 (dddd, *J* = 2.0, 3.4, 4.1, 12.0 Hz, 1H, C<u>H</u>$_2$ eq), 1.66 (ddddd, *J* = 2.0, 3.5, 3.5, 3.5, 12.5 Hz, 1H, C<u>H</u>$_2$ eq), 1.61 (dddd, *J* = 3.3, 3.3, 3.3, 12.9 Hz, 1H, CH_2 eq), 1.43 (tqt, *J* = 3.5, 6.5, 12.0 Hz, 1H, C<u>H</u>CH_3), 1.11 (dddd, *J* = 3.0, 3.0, 10.0, 12.0 Hz, 1H C<u>H</u>*i*-Pr), 0.97 (dddd, *J* = 3.3, 11.8, 12.0, 12.9 Hz, 1H, C<u>H</u>$_2$ ax), 0.93 (d, *J* = 7.0 Hz, 3H, CH_3), 0.92 (ddd, *J* = 10.5, 12.0, 12.0 Hz, 1H, C<u>H</u>$_2$ ax), 0.91 (d, *J* = 7.0 Hz, 3H, CH_3), 0.84 (dddd, *J* = 3.3, 11.8, 12.0, 12.3 Hz, 1H C<u>H</u>$_2$ ax), 0.81 (d, *J* = 7.0 Hz, 3H, CH_3).

^{13}C NMR ($CDCl_3$, 125 MHz) δ 71.5, 50.2, 45.1, 34.6, 31.7, 25.9, 23.3, 22.2, 21.0, 16.2.

1.3.3. Isomenthol (2)



2

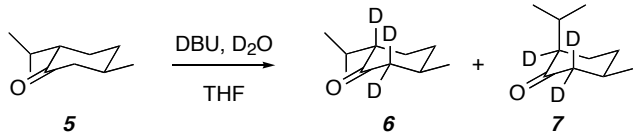
A sample of *l*-isomenthol was obtained from Aldrich Chemical Company and its NMR spectra were recorded without further purification.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.80 (ddd, *J* = 3.8, 7.5, 8.3 Hz, 1H, C<u>H</u>OH), 1.97 (octet, *J* = 7.3 Hz, 1H, C<u>H</u>CH_3), 1.97 [octet, *J* = 6.7 Hz, 1H, C<u>H</u>$(CH_3)_2$], 1.61 (dddd, *J* = 1.3, 3.8, 6.2, 13.0 Hz, 1H, C<u>H</u>$_2$ eq), 1.54 (dddd, *J* = 3.5, 3.5, 7.1, 13.5 Hz, 1H, C<u>H</u>$_2$ eq), 1.50 (ddd, *J* = 4.6, 8.3, 13.0 Hz, 1H, C<u>H</u>$_2$ ax), 1.44 (dddd, *J* = 3.5, 4.1, 9.5, 13.0 Hz, 1H, C<u>H</u>$_2$ ax), 1.36 (dddd, *J* = 3.5, 8.7, 9.5, 13.5 Hz, 1H, C<u>H</u>$_2$ ax), 1.30 (ddddd, *J* = 1.3, 3.5, 5.8, 7.1, 13.0 Hz, 1H, C<u>H</u>$_2$ eq), 1.15 (dddd, *J* = 3.5, 5.5, 7.5, 8.7 Hz, 1H, C<u>H</u>*i*-Pr), 0.93 (d, *J* = 6.7 Hz, 3H, CH_3), 0.93 (d, *J* = 6.7 Hz, 3H, CH_3), 0.87 (d, *J* = 6.7 Hz, 3H, CH_3).

^{1}H NMR (C_6D_6, 500 MHz) δ 3.58 (ddd, *J* = 3.8, 7.5, 8.3 Hz, 1H, C<u>H</u>OH), 1.92 [octet, *J* = 6.7 Hz, 1H, C<u>H</u>$(CH_3)_2$], 1.86 (octet, *J* = 7.3 Hz, 1H, C<u>H</u>CH_3), 1.50 (dddd, *J* = 1.3, 3.8, 6.2, 13.0 Hz, 1H, C<u>H</u>$_2$ eq), 1.49 (dddd, *J* = 3.5, 3.5, 7.1, 13.5 Hz, 1H, C<u>H</u>$_2$ eq), 1.37 (ddd, *J* = 4.6, 8.3, 13.0 Hz, 1H, C<u>H</u>$_2$ ax), 1.31 (dddd, *J* = 3.5, 4.1, 9.5, 13.0 Hz, 1H, C<u>H</u>$_2$ ax), 1.24 (dddd, *J* = 3.5, 8.7, 9.5, 13.5 Hz, 1H, C<u>H</u>$_2$ ax), 1.14 (ddddd, *J* = 1.3, 3.5, 5.8, 7.1, 13.0 Hz, 1H, C<u>H</u>$_2$ eq), 1.01 (dddd, *J* = 3.5, 5.5, 7.5, 8.7 Hz, 1H, C<u>H</u>*i*-Pr), 0.86 (d, *J* = 7.0 Hz, 3H, CH_3), 0.83 (d, *J* = 7.0 Hz, 3H, CH_3), 0.82 (d, *J* = 7.0 Hz, 3H, CH_3).

^{13}C NMR (C_6D_6, 125 MHz) δ 67.14, 49.50, 40.11, 30.55, 27.52, 25.97, 20.89, 19.85, 19.65, 19.10 ppm.

1.3.4. Menthone-d_3 (6), isomenthone-d_3 (7)



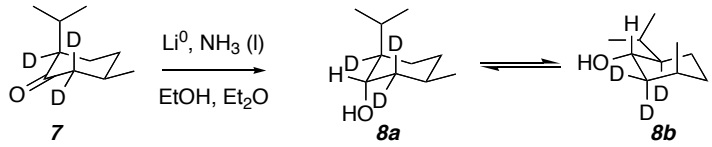
5 *6* *7*

A commercial mixture of 85:15 *l*-menthone:isomenthone (1.00 g, 6.48 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (9.67 mL, 64.8 mmol), and deuterium oxide (3 mL, 150 mmol) were added to THF (5 mL) in a 20 mL screw-cap culture tube. The mixture was allowed to stir at room temperature overnight, and the reaction mixture was extracted with methylene chloride (3 x 30 mL), washed with water and brine, and dried over magnesium sulfate. The solution was concentrated *in vacuo* to give a yellow oil which was purified by MPLC (SiO_2, 20:1 Hex:EtOAc) in two batches to give menthone-d_3 (636 mg, 62.5%) and isomenthone-d_3 (149.2 mg, 14.7%) as colorless oils.

Menthone-d_3: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.13 [sept, J = 6.8 Hz, C<u>H</u>$(CH_3)_2$], 2.06 (td, J = 3.3, 12.6 Hz, 1H, CH_2 eq), 1.90 (td, J = 3.6, 12.6 Hz, 1H, CH_2 eq), 1.84 (m, 1H, CH ax), 1.35 (m, 1H, CH_2 ax), 1.33 (m, 1H, CH_2 ax), 1.01 (d, J = 6.3, 3H, CH_3), 0.92 (d, J = 6.7, 3H, CH_3), 0.91(d, J = 6.7, 3H, CH_3).

Isomenthone-d_3: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.01 [sept, J = 6.8 Hz, 1H, C<u>H</u>$(CH_3)_2$], 1.99 (m, 1H, CH_2 eq), 1.91 (m, 1H, CH_2 eq), 1.70 (m, 1H, CH_2 eq), 1.66 (m, 1H, CH_2 ax), 1.47 (m, 1H, CH_2 ax), 0.98 (d, J = 6.3, 3H, CH_3), 0.93 (d, J = 6.7, 3H, CH_3), 0.91 (d, J = 6.7, 3H, CH_3).

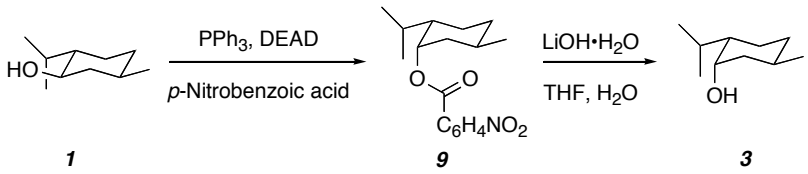
1.3.5. Isomenthol-d_3 (**8a** and **8b**)



A 50 mL 3-necked round-bottom flask was fitted with a magnetic stir bar, rubber septa, and a coldfinger condenser filled with dry ice and acetone, and was flushed with nitrogen. The flask was chilled to -78 °C and to this flask was added isomenthone-d_3 (**7**) (156 mg, 1.0 mmol), diethyl ether (10 mL), and ethanol (1.0 mL). NH_3 gas was introduced via a needle through a septum on the top of the condenser and approximately 20 mL of liquid NH_3 was allowed to condense into the reaction flask. Lithium wire (200 mg, 10 mmol) was added in portions over 30 minutes and the mixture was allowed to stir for 2 h. The flask was warmed to room temperature and the NH_3 was allowed to boil off. The reaction mixture was dissolved in water (17 mL) and was acidified to pH 2 with 10% HCl. The solution was extracted with methylene chloride (3 x 20 mL), washed with brine, and dried over magnesium sulfate. The solution was concentrated *in vacuo* to give a colorless oil, which was purified by MPLC (SiO_2, 4:1 Hex:EtOAc) to give exclusively isomenthol-d_3 (130 mg, 83%) as a white solid.

^{1}H NMR (C_6D_6, 500 MHz) δ 3.57 (s, 1H, C<u>H</u>OH), 1.93 [sept, J = 6.7 Hz, 1H, C<u>H</u>$(CH_3)_2$], 1.86 (sextet, J = 6.7 Hz, 1H, C<u>H</u>CH_3), 1.48 (ddd, J = 3.5, 7.1, 13.5 Hz, 1H, CH_2 eq), 1.31 (dddd, J = 3.5, 4.1, 9.5, 13.0, 1H, CH_2 ax), 1.24 (ddd, J = 3.5, 9.5, 13.5 Hz, 1H, CH_2 ax), 1.14 (ddddd, J = 1.3, 3.5, 5.8, 7.1, 13.0 Hz, 1H, CH_2 eq), 0.87 (d, J = 6.7 Hz, 3H, CH_3), 0.84 (d, J = 6.7 Hz, 3H, CH_3), 0.83 (d, J = 6.7 Hz, 3H, CH_3).

1.3.6. Neomenthol (3)



1 *9* *3*

A 50 mL round-bottom flask was fitted with a stirbar and septum and flushed with nitrogen. To this flask was added (*l*)-menthol (156 mg, 1.0 mmol), triphenylphosphine (426 mg, 1.2 mmol), *p*-nitrobenzoic acid (384 mg, 1.2 mmol), and THF (10 mL). Diethyl azodicarboxylate (0.623 mL, 1.2 mmol) was added dropwise to this solution over the course of 30 minutes, and the solution was allowed to stir overnight. The solution was concentrated *in vacuo* and then dissolved in diethyl ether (10 mL) to facilitate precipitation of triphenylphosphine oxide. After standing 30 minutes, the mixture was filtered through a plug of silica gel and concentrated *in vacuo*. The resulting yellow oil was purified by MPLC (SiO_2, 9:1 Hex:EtOAc) to give neomenthol *p*-nitrobenzoate ester (247 mg, 81%) as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.30 (td, *J* = 2.1, 9.0 Hz, 2H, ArH), 8.21 (td, *J* = 2.1, 9.0 Hz, 2H ArH), 5.50 (q, *J* = 3.3 Hz, 1 H, C<u>H</u>OAr), 2.09 (qd, *J* = 3.5, 14.4 Hz, 1H, CH_2), 1.86 (m, 1H, CH_2), 1.68 (m, 1H, CH_2), 1.53 (m, 1H, CH_2), 1,49 (m, 1H, CH_2), 1.19 (ddd, *J* = 2.6, 12.3, 14.3 Hz, 1H, CH_2), 1.02 (m, 1H, CH_2), 0.96 (m, 1H, CH_2), 0.93 (d, *J* = 6.4 Hz, 3H, CH_3), 0.90 (d, *J* = 6.4 Hz, 3H, CH_3), 0.88 (d, *J* = 6.4 Hz, 3H, CH_3).

Ester **9** (100 mg, 0.4 mmol) was added to a 5 mL screw-cap culture tube fitted with a magnetic stir bar, followed by lithium hydroxide monohydrate (42 mg, 1 mmol), water (500 μL, 28 mmol) and THF (1.5 mL). The cap was sealed and the solution was heated to 60 °C and stirred for 6 hours. The solution was cooled to room temperature and water (2 mL) was added. The solution was extracted with ethyl acetate (3 x 10 mL),

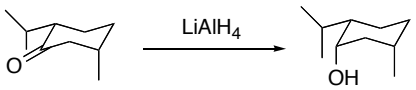
washed with saturated $NaHCO_3$ and brine, and dried over magnesium sulfate. The solution was concentrated *in vacuo* and the resulting yellow solid was purified first through a plug of silica gel (6:1 Hex:EtOAc) and then by MPLC (SiO_2, 20:1 Hex:EtOAc) to give neomenthol (**3**) as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.11 (ddd, *J* = 2.5, 2.5, 3.6 Hz, 1H, C<u>H</u>OH), 1.84 (dddd, *J* = 3.7, 3.8, 4.1, 13.1 Hz, 1H, CH_2 eq), 1.73 (dddd, *J* = 2.5, 3.6, 3.6, 13.9 Hz, 1H, CH_2 eq), 1.71 (ddddd, *J* = 2.5, 3.8, 3.8, 3.8, 13.4 Hz, 1H, CH_2 eq), 1.67 (ddqdd, *J* = 3.6, 3.8, 6.5, 12.0, 12.3 Hz, 1H, C<u>H</u>CH_3), 1.52 [sept d, *J* = 6.5, 9.2 Hz, 1H, C<u>H</u>$(CH_3)_2$], 1.27 (dddd, *J* = 3.8, 13.1, 13.1, 13.1 Hz, 1H, CH_2 ax), 1.09 (ddd, *J* = 2.5, 12.0, 13.9 Hz, 1H, CH_2 ax), 0.96 (d, *J* = 6.5 Hz, 3H, CH_3), 0.92 (d, *J* = 6.5 Hz, 3H, CH_3), 0.89 (dddd, *J* = 3.7, 12.3, 13.1, 13.4 Hz, 1H, CH_2 ax), 0.87 (dddd, *J* = 2.5, 4.1, 9.2, 13.1 Hz, 1H, C<u>H</u>*i*-Pr), 0.87 (d, *J* = 6.5 Hz, 3H, CH_3).

^{1}H NMR (C_6D_6, 500 MHz) δ 3.86 (ddd, *J* = 2.5, 2.5, 3.6 Hz, 1H, C<u>H</u>OH), 1.86 (dddd, *J* = 3.7, 3.8, 4.1, 13.1 Hz, 1H, CH_2 eq), 1.69 [sept d, *J* = 6.5, 9.2 Hz, 1H, C<u>H</u>$(CH_3)_2$], 1.64 (ddqdd, *J* = 3.6, 3.8, 6.5, 12.0, 12.3 Hz, 1H, C<u>H</u>CH_3), 1.63 (ddddd, *J* = 2.5, 3.8, 3.8, 3.8, 13.4 Hz, 1H, CH_2 eq), 1.57 (dddd, *J* = 2.5, 3.6, 3.6, 13.9 Hz, 1H, CH_2 eq), 1.54 (ddd, *J* = 2.5, 12.0, 13.9 Hz, 1H, CH_2 ax), 1.32 (dddd, *J* = 3.8, 13.1, 13.1, 13.1 Hz, 1H, CH_2 ax), 0.92 (d, *J* = 6.5 Hz, 3H, CH_3), 0.89 (d, J = 6.5 Hz, 3H, CH_3), 0.84 (d, *J* = 6.5 Hz, 3H, CH_3).0.76 (dddd, *J* = 3.7, 12.3, 13.1, 13.4 Hz, 1H, CH_2 ax), 0.67 (dddd, *J* = 2.5, 4.1, 9.2, 13.1 Hz, 1H, C<u>H</u>*i*-Pr),

^{13}C NMR ($CDCl_3$, 125 MHz) δ 67.8, 48.1, 42.7, 35.2, 29.2, 25.9, 24.2, 22.4, 21.2, 20.7.

1.3.7. Neoisomenthol (4)

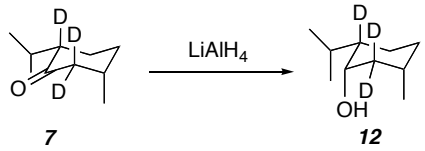


11 *4*

Isomenthone (**11**) (50.0 mg, 0.3 mmol) was added to a 5 mL culture tube followed by THF (1.0 mL). Lithium aluminum hydride solution in ether (206 μL, 1.45 M, 0.3 mmol) was added dropwise and the mixture was allowed to stir for 30 minutes. The reaction was quenched by sequential dropwise addition of water (0.5 mL), 15% NaOH (0.5 mL), and water (0.5 mL) to the mixture. The solution was extracted with diethyl ether (3 x 5 mL), washed with brine, and dried over magnesium sulfate. Concentration *in vacuo* gave a colorless oil, which was purified by MPLC (9:1 Hex:EtOAc) to yield neoisomenthol (**4**) as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.03 (ddd, J = 3.3, 3.3, 6.3 Hz, 1H, C<u>H</u>OH), 1.76 (m, 1H, CH_2), 1.67 (m, 1H, CH_2), 1.58 (m, 1H, CH_2), 1.47 (m, 1H, CH_2), 1.43 (m, 1H, CH_2), 1.40 (m, 1H, CH_2), 1.35 (m, 1H, CH_2), 1.24 (m, 1H, CH_2), 1.12 (m, 1H, CH_2), 1.07 (d, J = 7.3 Hz, 3H, CH_3), 1.00 (d, J = 6.7 Hz, 3H, CH_3), 0.93 (d, J = 6.7 Hz, 3H, CH_3).

1.3.8. Neoisomenthol-d_3 (12)



7 *12*

Isomenthone-d_3 (**7**) (50.0 mg, 0.3 mmol) was added to a 5 mL culture tube followed by 1.0 mL THF. Lithium aluminum hydride solution in ether (206 μL, 1.45 M, 0.3 mmol) was added dropwise and the mixture was allowed to stir for 30 minutes. The reaction was quenched by sequential dropwise addition of water (0.5 mL), 15% NaOH (0.5 mL), and water (0.5 mL) to the mixture. The solution was extracted with diethyl ether (3 x 5 mL), washed with brine, and dried over magnesium sulfate. Concentration *in vacuo* gave a colorless oil, which was purified by MPLC (9:1 Hex:EtOAc) to yield neoisomenthol-d_3 (**12**) as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.03 (s, 1H, C<u>H</u>OH), 1.76 (m, 1H, CH_2), 1.58 (m, 1H, CH_2), 1.47 (m, 1H, CH_2), 1.35 (m, 1H, CH_2), 1.24 (m, 1H, CH_2), 1.12 (m, 1H, CH_2), 1.07 (d, J = 7.3 Hz, 3H, CH_3), 1.00 (d, J = 6.7 Hz, 3H, CH_3), 0.93 (d, J = 6.7 Hz, 3H, CH_3).

2. Reaction titration of hydride solutions by No-D NMR spectroscopy

2.1. Background

Reactive anion species prepared and stored as solutions are often times a part of a practicing organic chemist's daily routine, and the integrity of these solutions is not often readily known. Many are air- or water-sensitive and their effective concentration decreases over time. Therefore, the concentration stated on the bottle is often inaccurate, and it becomes necessary to determine an accurate concentration of these reagents to ensure a successful and predictable reaction.

Previously it was necessary to perform a titration in order to determine the concentration. A colorimetric titration involves a measured amount of an acid, which is deprotonated by the anion solution. The endpoint is signaled by an indicator that changes color when deprotonated; bipyridyl is a common choice for titrating organolithium reagents. A gas-evolution titration may be performed with hydride solutions, whereby the hydride is treated with a proton source and the volume of evolved hydrogen gas is measured.

While these methods are reliable, many organic chemists would agree that performing a colorimetric or gas evolution titration is not an efficient way to spend valuable laboratory time. As a result, concentrations tend to be estimated and a titration might only performed as a last resort. A more convenient method to titer[13] an anionic

[13] In this document, the verb "to titer" is used to simply mean "to determine the concentration." The term "titration" is traditionally viewed in the narrow context of an acid-base reaction to determine a concentration by incremental addition of a titrant, but for convenience in this document, a titration refers to any method used to determine a concentration.

solution would allow a chemist to work more efficiently and reduce the number of failed reactions caused by a deteriorated reagent.

One method that can be conveniently used analyze these types of solutions is No-D NMR (no-deuterium proton NMR) spectroscopy. Recent studies by the Hoye group have shown No-D NMR spectroscopy to be an indispensable tool for gleaning diagnostic information on many types of organic solutions *in situ*.[14] Conventional ^{1}H NMR spectroscopy for organic compounds is normally performed in an NMR-silent, deuterated solvent. This restriction has limited its usefulness as a diagnostic tool for reactions; in order to monitor a reaction by traditional NMR spectroscopy, an aliquot of the reaction solution must be worked up and dissolved in deuterated solvent, or the entire reaction must be run in deuterated solvent. No-D NMR spectroscopy allows for a solution of *any* solvent to be analyzed directly without the hassle of extra workups, and without the added cost and wastefulness of running a reaction in deuterated solvent.

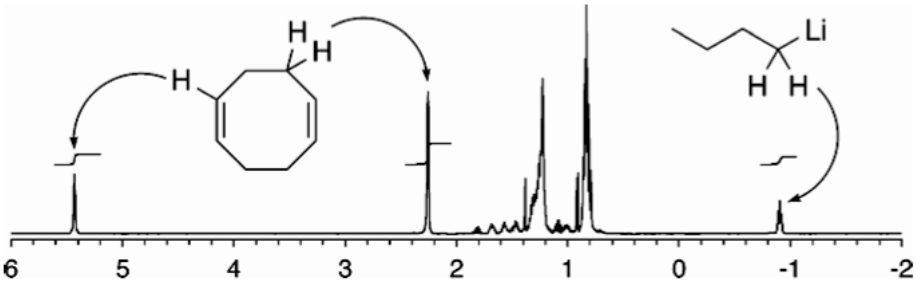
The first step of collecting a conventional NMR spectrum in deuterated solvent is to "lock" the signal on the deuterium resonance. This was designed to ensure that field drift would not broaden the peaks while the spectrum is being recorded. However, most modern NMR spectrometers have sufficiently low field drift that they can be run in unlocked mode—certainly so for relatively short collections. The deuterium resonance is also traditionally used to shim the spectrometer by maximizing its signal in responses to voltages changed in the shims. In No-D NMR spectroscopy, the spectrometer can be shimmed by iteratively adjusting the shims and simply monitoring the magnitude of the

[14] (a) Hoye, T. R.; Eklov, B. M.; Ryba, T. D.; Voloshin, M.; Yao, L. J. *Org. Lett.* **2004,** *6,* 953-956. (b) Hoye, T. R.; Eklov, B. M.; Voloshin, M. *Org. Lett.* **2004,** *6,* 2567-2570. (c) Hoye, T. R.; Kabrhel, J. E.; Hoye, R. C. *Org. Lett.* **2005,** *7,* 275-277. (d) Hoye, T. R.; Aspaas, A. W.; Eklov, B. M.; Ryba, T. D. *Org. Lett.* **2005,** *7,* 2205-2208.

real-time FID display. In fact, this method of hand-shimming often produces spectra with narrower peaks than samples shimmed with a deuterium lock. While the No-D method does produce large solvent resonances, the solute resonances are still present with an excellent signal-to-noise ratio at most practical reaction concentrations.

Because of its ability to directly analyze any solution without modification, No-D NMR spectroscopy is well-suited for determining the concentration of solutions. For solutions where the solute resonances are reliably visible by No-D NMR, such as organolithiums, Grignard reagents, or lithium diisopropylamide, the direct observation method is useful.[14b] In this method, a known amount of an integration standard is added to a precise volume of the solution, and the integrals are used to calculate the concentration. Cyclooctadiene is an optimal integration standard due to its low reactivity with anionic reagents and its pair of resonances, which appear downfield of most solvent resonances. In order to obtain reliable integral values, the acquisition time and relaxation delay were increased significantly to allow the protons to fully demagnetize between each scan. An NMR spectrum indicative of this type of titration is shown in Figure 5.

Figure 5. No-D NMR direct observation titration of *n*-butyllithium in hexanes using cyclooctadiene as the added integration standard. Reproduced from reference 13b.



The accuracy of the No-D NMR direct observation titration method was verified by performing standard colorimetric titrations on the solutions. In all cases, the margin of error between the two methods was minimal, and the data obtained from the No-D NMR method were actually more reproducible than by the colorimetric titration method.

The elegance of the direct observation method is indisputable. However, not all anionic solutions are directly observable by ^{1}H NMR. This problem is especially notable with hydride solutions such as lithium aluminum hydride ($LiAlH_4$) and diisobutyl aluminum hydride
(DIBAL-H).[15] Aluminum-27 has a nuclear spin of 5/2, which will significantly complicate and broaden the NMR resonances of any coupled protons. Also, hydride resonances themselves may not always be seen in ^{1}H NMR spectra. A different No-D NMR titration method was devised specifically for these solutions, whose anions are not directly observable by NMR: an indirect method of titration by analyzing the reaction of an NMR-visible compound with the hydride reagent.

2.2. Studies

2.2.1. No-D NMR reaction titrations

Conventionally, a hydride solution is titered either by gas titration[16] (treatment of the solution with excess water or methanol, and quantitative measurement of the evolved H_2 gas) or by colorimetric titration through the use of various indicators.[17] A more

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[15] (a) Horne, D. *J. Am. Chem. Soc.* **1980**, *102*, 6011-6014. (b) Andrianarson, M. M.; Avent, A. G.; Ellerby, M. C.; Gorrell, I. B.; Hitchcock, P. B.; Smith, J. D.; Stanley, D. R. *J. Chem. Soc., Dalton Trans.* **1998**, 249-253.

[16] Brown, H. C.; Kramer, G. W.; Levy, A. B.; Midland, M. M. *Organic Synthesis* V*ia Boranes*; John Wiley and Sons: New York, 1973; pp 241-244)

[17] (a) Brown, E.; Lézé, A.; Touet, J. *Tetrahedron Lett.* **1991**, *32*, 4309-4310. (b) Love, B. E.; Jones, E. G. *J. Org. Chem.* **1999**, *64*, 3755-3756.

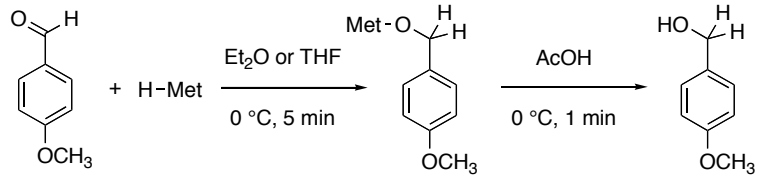
convenient method of titration was explored, making use of No-D NMR spectroscopy. If an approximately two-fold excess of a reducible compound such as an aldehyde was treated with a hydride solution, the concentration of the hydride solution could be calculated based upon exactly how much of the aldehyde had been reduced and how much remained as unreacted starting material. This method is well-suited to the same No-D NMR spectroscopy techniques performed in other studies in the Hoye group. The reaction mixture can be observed directly without the hassle and potential error introduced by quenching, extracting, and drying an aliquot (the product alcohol may be water-soluble, for instance). With the spectrometer settings modified sufficiently to provide reliable integration values, the accurate and reproducible titer of many types of hydride solutions can be obtained in less than 20 minutes of an experimentalist's time.

The reactive compound to be reduced by the hydride solution was chosen carefully. The compound should be easy to handle and measure, it should be relatively shelf-stable, and its diagnostic ^{1}H NMR resonances should appear in a convenient region of the spectrum and be sufficiently modified by the transformation from aldehyde to alcohol. We settled on the use of *p*-methoxybenzaldehyde (referred to in this document by its common name, *p*-anisaldehyde, **13**). Its nominal ^{1}H NMR spectrum has a number of easily differentiable resonances: the aldehyde proton at 9.9 ppm, two aromatic protons between 6.5 and 8 ppm, and the methoxy protons near 3.8 ppm. All of these resonances are significantly modified when *p*-anisaldehyde is reduced to *p*-methoxybenzyl alcohol (**14**, Scheme 4), but the most diagnostic are the two aromatic resonances, which are shifted upfield upon conversion to the more shielded alcohol product. See Figure 6 for a representative spectrum illustrating this.

2.2.1.1. $LiAlH_4$ and DIBAL-H solutions

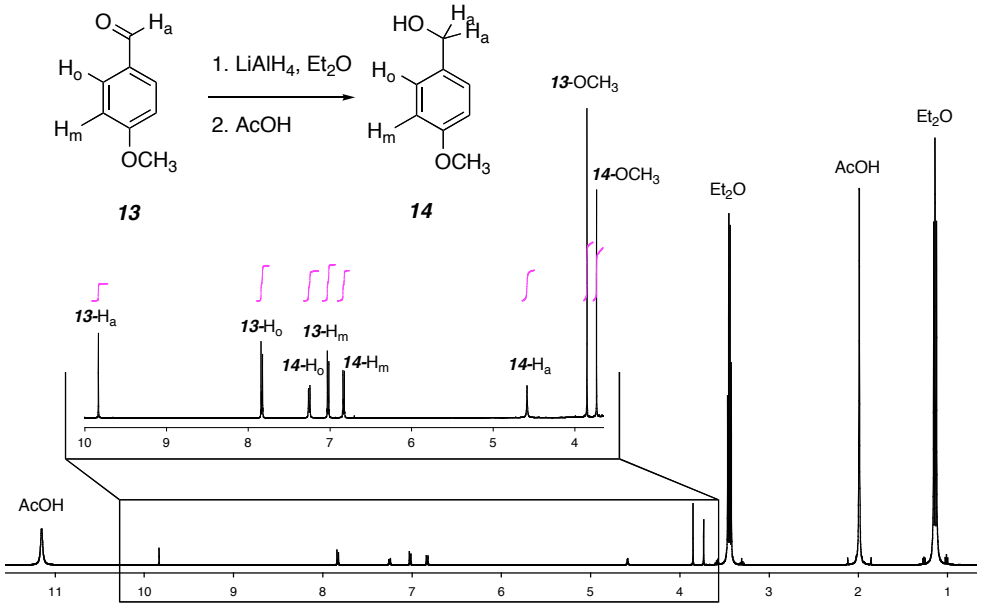
When an ethereal solution of *p*-anisaldehyde (**13**) is treated with a hydride solution such as $LiAlH_4$ or DIBAL-H, and the solution is analyzed by No-D NMR spectroscopy, often the aluminum alkoxide (**15**) is observed (Scheme 4). Most metal hydroxides like this are insoluble in organic solvents, and indeed the solution is usually thick and cloudy. While No-D NMR spectroscopy is versatile enough to get meaningful information out of this solution, we found the peaks are sharper and the data are more reliable once the alkoxide (**15**) was protonated *in situ* using glacial acetic acid to produce *p*-methoxybenzyl alcohol (**14**). This homogenizes and clarifies the solution, and spectral quality is improved immensely. The added acetic acid does not interfere with any of the diagnostic resonances of the aldehyde or alcohol.

Scheme 4. Conversion of *p*-anisaldehyde (**13**) to its metal alkoxide (**15**) and *p*-methoxybenzyl alcohol (**14**).



13 ***15*** ***14***

Figure 6. No-D NMR reaction titration of $LiAlH_4$ solution in Et_2O using *p*-anisaldehyde (**13**). A concentration of 0.81 *M* was found for this solution.



After the solution was homogenized with acetic acid, the No-D ^{1}H NMR spectrum was recorded. Acquisition time and relaxation delay were adjusted to improve integral accuracy (exact spectrometer parameters are given in the experimental section). Care was taken that the integral lines were leveled and tilted correctly, and the integrals were cut in consistent areas above a flat baseline but inside the ^{13}C satellite peaks for each resonance.[18]

With accurate integral values, the concentration of the hydride reagent could be calculated. First, percent conversion of the aldehyde **13** was calculated as follows:

$$\%\ \text{conversion} = \frac{\text{integral}(\mathbf{14})}{\text{integral}(\mathbf{14}) + \text{integral}(\mathbf{13})} \qquad (1)$$

[18] Pauli, G. F.; Jaki, B. U.; Lankin, D. C. *J. Nat. Prod.* **2005,** *68,* 133-149.

then the molar concentration of the hydride species, [Met-H], was calculated as follows:

$$[\text{Met-H}] = \frac{(\text{mmol } \mathbf{13})(\%\text{ conversion})}{(\text{mL Met-H soln})} \quad (2)$$

This process was typically performed three times for each spectrum, giving separate concentration values for the pair of ortho proton resonances, the pair of meta proton resonances, and the pair of methoxy proton resonances. This ensures internal consistency in the spectrum, and may serve as a diagnostic in case some integrals were incorrectly cut. The three values were generally in good agreement, however, and were averaged to give a final molar concentration for the hydride solution.

The process was performed by the author without incident for solutions of $LiAlH_4$ in Et_2O and in THF[19] as well as solutions of DIBAL-H in hexanes. The values have tended to be quite precise, with errors on multiple trials usually less than ± 0.01 *M*. Other members of the Hoye group have performed the procedure on solutions of L-Selectride (lithium-tri-*sec*-butylborohydride) in THF with similarly predictable results.[13d] However, since L-Selectride is not an aluminum hydride, it can be directly observed by No-D NMR spectroscopy. The reaction titration and direct observation titration were performed on the same solution and the concentration values obtained were quite close (1.00 *M* ± 0.01 and 1.04 *M* ± 0.01 for the two methods respectively).

[19] We prefer to prepare solutions of $LiAlH_4$ on an as-needed basis, by dissolving a solid $LiAlH_4$ pellet in Et_2O. The grey suspension that forms settles quickly allowing for the clear supernatant to be withdrawn. While THF is a good solvent for $LiAlH_4$, we find that the grey particles suspended in the solution do not settle as easily as with Et_2O, even with centrifugation.

2.2.1.2. Red-Al™ solution

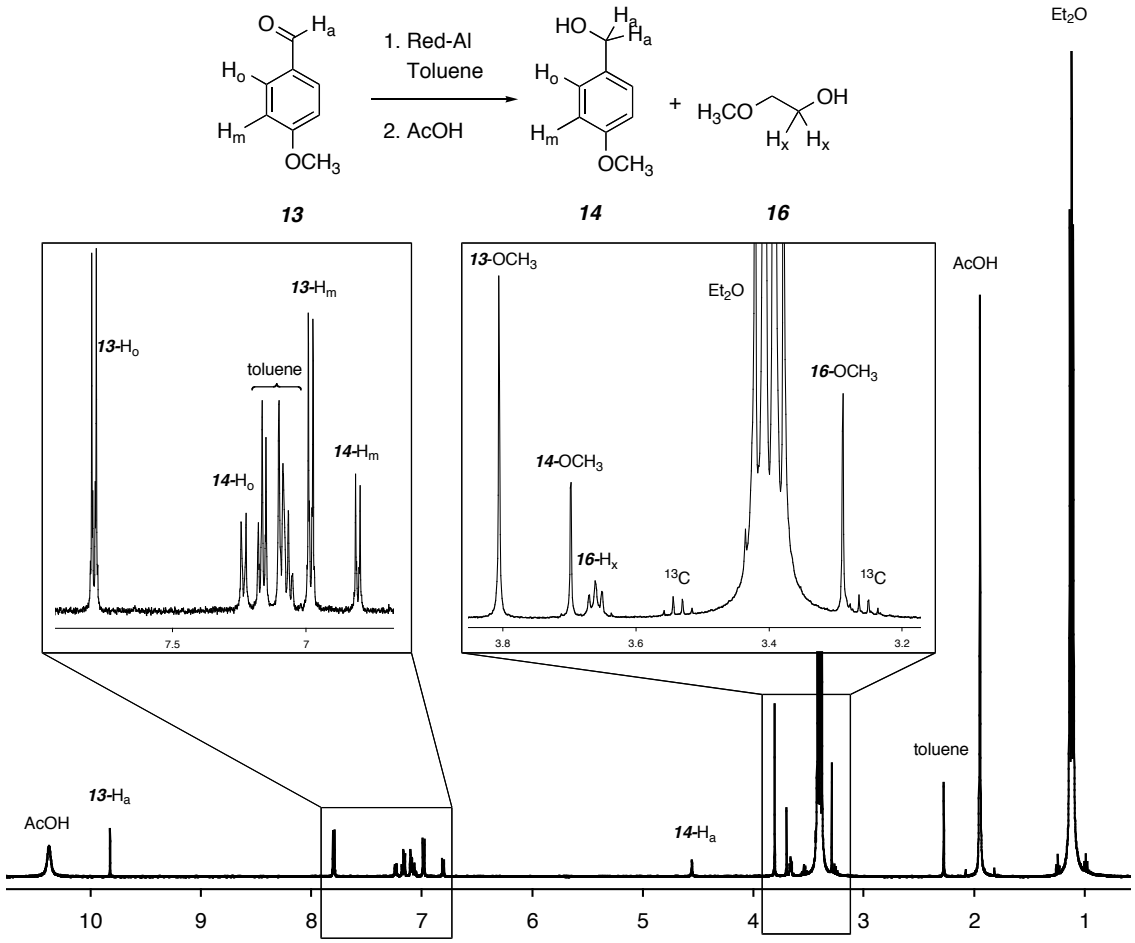
Red-Al™ [sodium bis(2-methoxyethoxy)aluminum hydride, Vitrol™] is a useful hydride reducing agent, and we found that it can be effectively titered using the reaction titration method with *p*-anisaldehyde (**13**). A commercial 65 wt% solution of Red-Al™ in toluene was subjected to an identical procedure as with $LiAlH_4$ and DIBAL-H. In the No-D NMR spectrum (Figure 7), the aromatic toluene resonances appear distinctly between H_o of **14** and H_m of **13**. Also, 2-methoxyehtanol (**16**), the protonolysis biproduct of Red-Al™ can be readily identified at 3.6 ppm and 3.3 ppm. Integration provides a concentration of 3.18 *M*. Nominally, the commercial product is stated 65 wt%, or 3.2 *M*.

2.2.1.3. Sodium and potassium hydride

Sodium and potassium hydride are useful bases but their high reactivity with water makes it difficult to know their exact amount of active hydride. An acid-base reaction titration and No-D NMR spectroscopy could be used in this case to determine the exact amount of hydride present in the reagent. The author briefly investigated the use of diethyl malonate as a titrant since one of its alpha-protons is sufficiently acidic to be deprotonated by sodium or potassium hydride, and the resulting anion is visible by No-D NMR spectroscopy, but its use was abandoned due to unfortunate overlapping of some key resonances.

A more effective titrant, ethyl diethylphosphonoacetate in THF, was investigated by other members of the Hoye group, and was shown to perform very reliably in reaction titration conditions with No-D NMR spectroscopy.[13d]

Figure 7. No-D NMR reaction titration of Red-Al™ solution in toluene using *p*-anisaldehyde (**13**), with formation of 2-methoxyethanol (**16**) visible. A concentration of 3.18 *M* was found for this solution.



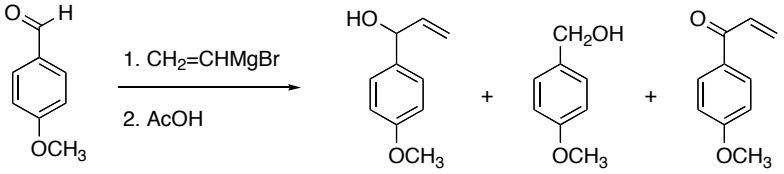
2.2.1.4. Organolithium and Grignard solutions

The possibility was explored that solutions of organolithium and Grignard reagents could be titered by the reaction titration method with *p*-anisaldehyde (**13**) as well. However, this method was abandoned once products from Cannizarro-like redox events were observed in the reaction mixtures (Scheme 5). The formation of *p*-methoxybenzyl alcohol (**14**) and enone **15** along with the expected alcohol **17** from the

reaction of *p*-anisaldehyde (**13**) with vinyl magnesium bromide is strong evidence that this redox event is occurring.

This side-reaction complicates the No-D NMR spectrum for the reaction titration of any organolithium or Grignard reagent with *p*-anisaldehyde (**13**). Although a different titrant may be found which would be less susceptible to these complications, the matter was not studied further, due mostly to the ease with which these compounds can be titrated by the direct observation method.

Scheme 5. Cannizarro-like redox events produce undesirable side products (**14** and **16**) when organolithium and Grignard reagents are reacted with *p*-anisaldehyde (**13**).



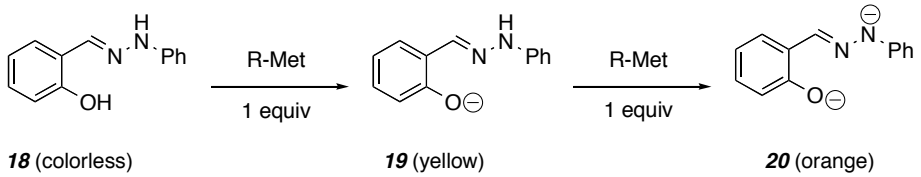
13 ***17*** ***14*** ***16***

2.2.2. Colorimetric and H_2 gas evolution titrations

2.2.2.1. Salicylaldehyde phenylhydrazone as an indicator in colorimetric titration of hydride solutions

Some conventional methods for titrating hydride agents were explored to assess the accuracy of the reaction titration method. Colorimetric titration with the use of salicylaldehyde phenylhydrazone (**18**) has been shown to be an effective method for titrating numerous hydride reagents.[17b] Since **18** is a diprotic acid, the hydroxyl proton is first removed. The resultant monoanion **19** is light yellow colored. Once the hydroxide has been completely deprotonated, the phenylhydrazone N–H proton begins to be removed, and the resultant dianion **20** is bright orange in color (Scheme 6).

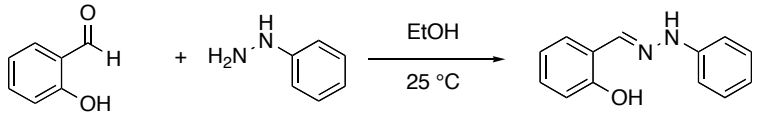
Scheme 6. Salicylaldehyde phenylhydrazone as an acid-base indicator.



18 (colorless) ***19*** (yellow) ***20*** (orange)

Phenylhydrazone **18** is easily synthesized from salicylaldehyde (**21**) and phenylhydrazine (**22**, Scheme 7). When **21** and **22** are combined in ethanol, **18** begins to crystallize almost immediately. Love and coworkers state the phenylhydrazone can be used without further purification,[17b] but in order for the solution to be light enough to see the endpoint, **18** had to be quickly purified through a pad of silica gel, since phenylhydrazine used in this study had a dark-colored impurity. Nonetheless, pure **18** was produced efficiently in 83.8% yield.

Scheme 7. Synthesis of salicylaldehyde phenylhydrazone (**18**).



21 ***22*** ***18***

Colorimetric titration was performed on a $LiAlH_4$ solution in Et_2O twice and values of 0.83 *M* and 0.85 *M* were obtained. Reaction titration on that same sample yielded 0.81 *M* and 0.82 *M*. The values from reaction titration are slightly smaller, the values are close enough to ensure that our reaction titration method is indeed accurate. This method was not investigated further.

2.2.2.2. H_2 gas evolution titration

Another conventional method for titering a hydride solution is a gas evolution titration, where the hydride is quenched by a proton source and the volume of hydrogen gas produced is measured. This method, although straightforward in principle, is relatively cumbersome to perform experimentally, especially while keeping the reaction flask under pressure. To perform this procedure, an excess of a protic solvent such as methanol is placed in a round-bottom flask which is connected to Teflon tubing by an air-tight junction to a large inverted buret filled with water. A carefully-measured volume of the hydride reagent is added by syringe to the reaction flask, and as hydrogen gas is produced, it passes through the tubing and is collected underwater in the inverted buret. Septa are, by nature, not completely airtight once they have been punctured by a needle, so this method ended up being inexact at best. However, after significant trial and error, the author was able to reproduce a titer of approximately 0.50 *M* on a solution of DIBAL-H which was determined to be 0.54 *M* by reaction titration. Slight air leaks can account for the slightly lower concentration found by the gas titration method. Nonetheless, this was enough for us to determine that the reaction titration method is accurate, and is certainly far more convenient than the gas titration method.

2.3. Conclusion

We have shown that reaction titration is a convenient and reliable method for titering hydride solutions. An NMR-based solution such as this will most likely be more appealing to a practicing organic chemist than tedious colorimetric or gas evolution titration methods, and as a result, if this method is adopted, the barrier for regularly titering reactive hydride solutions will hopefully be lowered significantly.

The method is of course not limited to the hydride solutions presented here. We see no reason why the same method would not work with other common reactive reagents such as boranes, hydridoborates, Super-Hydride, etc. The ease with which this method has been adapted towards several different solutions is evidence of its utility and is another practical application of No-D NMR spectroscopy.

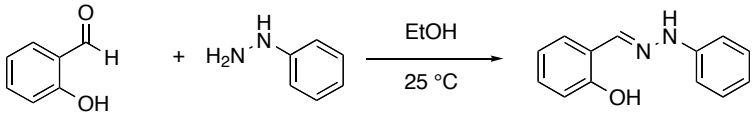
2.4. Experimental

2.4.1. Reactive titration procedures

p-Anisaldehyde (300 μL, 2.47 mmol) was added to a tared, N_2 flushed, oven dried, septum capped flask under a nitrogen atmosphere, and the mass of the aldehyde was noted. Diethyl ether (3 mL) was added to the aldehyde, and the solution was cooled in an ice bath. Enough hydride solution was added to reduce approximately one half of the aldehyde (ca. 1.23 mmol). The solution was stirred for 5 minutes, and excess acetic acid (2 mL) was added. A ^{1}H NMR spectrum of this solution was acquired with the following acquisition time, delay, transmitter power, and number of transients: at=20, d1=20, tpwr=46, nt=16. Shimming was accomplished by acquiring a series of one-pulse spectra and iteratively maximizing the signal of the free induction decay (FID), and if necessary (from within the Varian acquisition window) minimizing the linewidth of known solvent peaks. Care was taken to achieve a flat spectrum and integral baseline by phasing the spectrum and adjusting the level/tilt of the integral. Defining integral stops and applying automatic baseline correction ("bc") is helpful in obtaining reliable and consistent integral values. After collection of the spectra, the concentration was calculated by comparing the integration of resonances from the remaining anisaldehyde to those of the reduced para-methoxybenzyl alcohol.

^{1}H NMR (500 MHz, Et_2O) δ 10.67 (br s, 1H, AcOH), 9.90 (s, 1H, CHO), 7.87 (d, J = 8.3 Hz, 1H, aldehyde ArH), 7.30 (d, J = 8.3 Hz, 1H, alcohol ArH), 7.07 (d, J = 8.3 Hz, 1H, aldehyde ArH), 6.87 (d, J = 8.3 Hz, 1H, alcohol ArH), 4.61 (s, 2H, C$\underline{H_2}$OH), 3.90 (s, 3H, aldehyde OCH_3), 3.78 (s, 3H, alcohol OCH_3), 3.47 (q, J = 7.2 Hz, 4H, Et_2O), 2.01 (s, 3H, C$\underline{H_3}CO_2H$), 1.19 (t, J = 7.2 Hz, 6H, Et_2O).

2.4.2. Salicylaldehyde phenylhydrazone synthesis



21 *22* *18*

To a 250 mL round-bottom flask was added 95% EtOH (84 mL), phenylhydrazine (9.09 mL, 92.47 mmol), and salicylaldehyde (9.85 mL, 92.47 mmol) while cooling to -10 °C. The solution was slowly agitated and left to stand for 30 minutes without stirring while the solid product crystallized. The yellow solid was collected by vacuum filtration and the colored polar impurity was removed by silica gel chromatography (9:1 Hex:EtOAc) to provide salicylaldehyde phenylhydrazone (**18**) as a white solid (16.42 g, 83.8%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.87 (s, 1H, C<u>H</u>=N), 7.86 (br s, NH), 7.31 (t, *J* = 7.5 Hz, 2H, ArH), 7.24 (t, *J* = 7.5 Hz, 1H, ArH), 7.15 (dd, *J* = 2.1, 7.5 Hz, 1H, ArH), 6.99 (t, *J* = 7.5 Hz, 3H, ArH), 6.93 (t, *J* = 7.5 Hz, 1H, ArH), 6.90 (t, *J* = 7.5 Hz, 1H, ArH), 4.81 (br s, 1H, OH).

2.4.3. Colorimetric titration procedure

Salicylaldehyde phenylhydrazone (**18**) (typically between 80 - 100 mg) was added to a tared, N_2 flushed, oven dried, septum capped flask under a nitrogen atmosphere. Anhydrous THF (10 mL) was added and the solid was allowed to dissolve. The hydride reagent was added dropwise by syringe through the septum. An initial yellow color gives way to a bright orange endpoint once the titration is completed.

2.4.4. H_2 gas evolution titration procedure

An excess of methanol (10 mL) is added to a round-bottomed flask fitted with a brand new septum. A length of thin Teflon spaghetti tubing was fed through a 16-gauge needle and inserted into the septum. A large 1-L buret was graduated in 50 mL increments, and inverted over a water reservoir. Vacuum was applied to the tip of the buret and the stopcock was opened to allow water to fill the buret to the top. The line of spaghetti tubing was then placed underwater below the opening of the buret. A carefully measured volume of the hydride reagent (usually 1.0 mL) was added to the reaction flask very slowly, allowing gas evolution to completely cease before continuing addition. The volume of hydrogen gas produced was measured by the graduations on the buret. Additional trials could be run in the same reaction flask provided sufficient excess protic solvent was present at the beginning.

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[13] In this document, the verb "to titer" is used to simply mean "to determine the concentration." The term "titration" is traditionally viewed in the narrow context of an acid-base reaction to determine a concentration by incremental addition of a titrant, but for convenience in this document, a titration refers to any method used to determine a concentration.

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[19] We prefer to prepare solutions of $LiAlH_4$ on an as-needed basis, by dissolving a solid $LiAlH_4$ pellet in Et_2O. The grey suspension that forms settles quickly allowing for the clear supernatant to be withdrawn. While THF is a good solvent for $LiAlH_4$, we find that the grey particles suspended in the solution do not settle as easily as with Et_2O, even with centrifugation.