JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

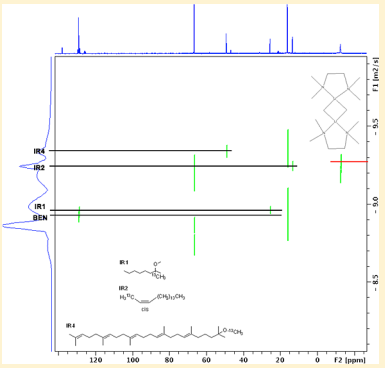
Isotopically Enriched ^{13}C Diffusion-Ordered NMR Spectroscopy: Analysis of Methyllithium

Chicheung Su, Russell Hopson, and Paul G. Williard*

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

Supporting Information

ABSTRACT: We report the development of isotopic-labeled ^{13}C diffusion-ordered NMR spectroscopy (DOSY) NMR with diffusion coefficient-formula weight (*D*-FW) analysis and its application in characterizing the aggregation state of methyllithium aggregates and complexes with several widely used diamines. Commercially available ^{13}C-labeled benzene and several easily synthesized ^{13}C-labeled compounds using ^{13}C-labeled iodomethane as the isotopic source are developed as internal references for diffusion-formula weight analysis (*D*-FW). The technique greatly expands the applicability of DOSY *D*-FW analysis to a much wider variety of compounds because of isotopic labeling. These results reveal that methyllithium exists as a tetrasolvated tetramer in diethyl ether and exclusively as bis-solvated dimers with chelating diamines.



INTRODUCTION

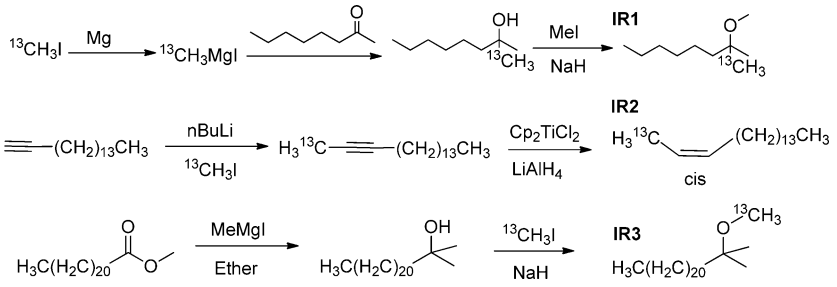
Diffusion-ordered NMR spectroscopy (DOSY) with the application of diffusion coefficient-formula weight (*D*-FW) correlation analysis is an efficient method for determination of formula weights of complexes in solution.[1] We have been developing DOSY NMR with internal references (IRs) for the determination of formula weights of reactive complexes by *D*-FW correlation analysis.[2] A linear regression plot of the logarithms of NMR determined relative diffusion coefficients against the known formula weights of added reference compounds allows us to deduce the formula weight of unknown complexes. We have successfully utilized this technique to probe the aggregation state and solvation state of several organometallic complexes. A major focus of our group is to expand this *D*-FW DOSY methodology for the structural analysis of alkali metal complexes in solution. Until now, our group has successfully utilized internal references for ^{13}C, ^{31}P DOSY and both hydrophilic and hydrophobic references for ^{1}H DOSY. Moreover, we have also demonstrated the use of physically separated reference systems to isolate the reference and the analyte solutions.[3] The internally referenced ^{1}H DOSY technique is used because of its simplicity and fast acquisition time. However, the technique often suffers from the drawback of overlapping resonances in the chemical shift dimension owing to the relatively narrow frequency range of ^{1}H NMR. Although ^{31}P DOSY has a very wide chemical shift range and fast acquisition time due to its 100% natural abundance, it is constrained to complexes containing phosphorus atoms. ^{13}C INEPT DOSY with *D*-FW analysis was reported by our group five years ago and proved useful in characterizing the aggregation states of an LDA−THF complex and a dimeric chiral lithium amide derived from (*S*)-*N*-isopropyl-*O*-triisopropylsilyl valinol.[2a,h,4] Although the dispersion of ^{13}C spectra provides ample separation for the resonances with similar structures, the very low natural abundance (1.1%) and long relaxation times of ^{13}C nuclei render ^{13}C DOSY with *D*-FW analysis a very long experiment. For example, a sample with reasonably high concentration (1 M) requires at least six hours for the signal acquisition. Such lengthy experiments preclude the analysis of short-lived intermediates. To address this problem, we prepared isotopically enriched ^{13}C-labeled internal references for *D*-FW analysis. The labeled internal references are either commercially available or can be synthesized readily using relatively inexpensive ^{13}C-labeled iodomethane. Use of ^{13}C-enriched DOSY greatly reduces the experiment time, affording the analysis of less concentrated and short-lived intermediates, which are easily identified in the pool of carbon resonances. Moreover, the cost and labor required to prepare isotopically labeled materials is offset because standard, protonated solvents can be used in place of expensive deuterated solvents such as diethyl ether and *n*-pentane. This is especially beneficial in experiments described herein in which the NMR solvents are also used as the solvent in the reactions to prepare the analytes.

We utilize ^{13}C-labeled DOSY NMR to establish the aggregation state of methyllithium solvated by various diamines by *D*-FW analysis. This is significant because organolithium reagents are the most widely used reagents in organic synthesis.[5] Alkyl-lithium reagents are typically utilized to generate a wide variety of carbanions such as lithium amides, acetylides, and alkoxides. Moreover, the low boiling point alkane formed as a byproduct is inert and easily eliminable. It has become evident that the reactivity and reaction pathways of

Received: August 8, 2013
Published: October 18, 2013

   dx.doi.org/10.1021/jo401740g | *J. Org. Chem.* 2013, 78, 11733−11746

Scheme 1. Synthesis of ^{13}C-Labeled Internal References



these alkyllithium reagents are related to their aggregation state[6] and also to the formation of mixed aggregates.[7] Hence, knowledge of the aggregation and solvation state helps optimize the reactivity of these reagents and provides a starting point for development of reaction mechanisms.

Methyllithium is not normally used in hydrocarbon solvents without a coordinating ligand due to its low solubility. In diethyl ether and tetrahydrofuran, it exists as a solvated tetramer in both solution and solid states.[8] Single crystal X-ray diffraction studies revealed that methyllithium is a tetrameric polymer in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA)[9] or 1,3,5-trimethyl-1,3,5-triazacyclo-hexane (TMTAC)[10] in solid state, while it is a dimer in the presence of (−)-sparteine or *N*,*N*,*N*′,*N*′-tetramethyl-1,2-diaminocyclohexane (TMCDA) in solid state.[11] However, there is no evidence that the solution state structure of methyllithium solvated by common diamine ligands is the same as that in solid state. Numerous calculations of possible structures and bonding in methyl lithium aggregates were reported.[12] Previous NMR studies and spectroscopic studies of methyl lithium are also reported.[13] Herein, we demonstrate that methyllithium forms dimeric complexes in diethyl ether solution in the presence of common diamine ligands including TMEDA, TMCDA, (−)-sparteine, 3,7-dimethyl-3,7-diazabicyclo[3.3.1]nonane (*N*,*N*′-dimethylbispidine) and *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (PMDTA). The formation of dimeric structures is corroborated by ^{13}C−^{6}Li coupling and ^{13}C-labeled DOSY results.

First we discuss the design, synthesis, and *D*-FW analysis protocol of the ^{13}C-labeled internal reference system for DOSY NMR. Next we demonstrate their utility in characterizing the tetrasolvated tetramer of methyl lithium in diethyl ether and in determining bis-solvated, dimeric structures of methyllithium in diethyl ether in the presence of diamines.

RESULTS AND DISCUSSION

Design, Synthesis and *D*-FW Analysis Protocol of the ^{13}C-Labeled Internal Reference System for DOSY NMR.

The criteria for internal molecular weight references include lack of reactivity and coordinating ability toward the analyte, easily recognizable resonances, solubility in NMR solvents, and desirable formula weight range.[2] In ^{1}H and natural abundance ^{13}C DOSY, many commercially available aromatic and alkene compounds satisfy these criteria. However, several reference compounds that we have previously utilized (benzene, cyclooctene, 1-tetradecene, and squalene) are not readily available with ^{13}C isotopic enrichment with the exception of benzene. Syntheses of these isotopically enriched ^{13}C-labeled compounds is cumbersome. Therefore, we report new internal, ^{13}C-labeled references that are easily prepared with isotopic enrichment.

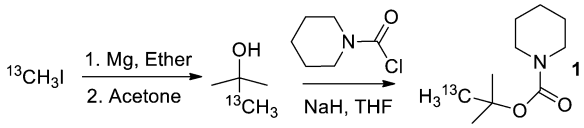
Design and Synthesis of ^{13}C-Labeled Internal References (IRs). We chose benzene-$^{13}C_6$ (BEN) as our first internal reference because it is the cheapest ^{13}C-labeled aromatic compound that is inert to our analyte. Additionally, a very small quantity is required for each experiment since it has six labeled carbon atoms. Cyclooctene, 1-tetradecene and squalene are not useful because their ^{13}C-labeled analogues are not available commercially and ^{13}C-labeled, synthetic precursors are expensive. Therefore, we focused on developing three new internal references using ^{13}C-labeled iodomethane as the starting material because this material represents an inexpensive ^{13}C atom source. These materials are depicted as **IR1**−**3** in Scheme 1.

Although two different ethers are used in our internal reference system, that is, **IR1** and **IR3**, we determined that they are not competitive in coordinating to methyl lithium aggregates since most of the experiments we performed were conducted in ethereal solvents. The concentration of these ethereal, ^{13}C-labeled internal references is many orders of magnitude less than the solvent. Additionally, these ethers are sterically hindered. Alternatively a significant amount of a strong chelating ligand such as TMEDA and PMDTA was also present in several experiments. Furthermore, it has been shown that the coordinating power of diethyl ether, THF or common diamine ligands to alkyl lithium reagents is much stronger than methyl *tert*-butyl ether (MTBE).[2g,14] Since the ethers we used as internal references are even more hindered than MTBE, it is reasonable to assume that they do not coordinate with our complexes significantly in diethyl ether or THF to an observable extent.

***D*-FW Analysis of the ^{13}C-Labeled Internal Reference System.** DOSY NMR separates resonances by their diffusion coefficients as is typically depicted along the vertical axis is a two-dimensional plot. Chemical shifts are typically displayed along the horizontal axis. These spectra are presented as typical two-dimensional NMR plots; however, in this experiment, only a single Fourier transform in the chemical shift dimension is utilized.[15] We first applied DOSY NMR with internal references to study *n*-BuLi aggregates without the *D*-FW protocol.[16] Subsequently, we have incorporated *D*-FW analysis into the experiment.[2c] A linear regression plot of the logarithm of relative diffusion coefficients determined by DOSY NMR against known formula weights of reference standards is used to extrapolate the formula weight of observable complexes. We used ^{13}C-labeled *N*-Boc-piperidine **1** synthesized from piperidine-1-carbonyl chloride and ^{13}C-labeled *tert*-butanol (Scheme 2) to establish the effectiveness of this ^{13}C-labeled, internal reference system.[17] Thus, ^{13}C-labeled *N*-Boc-piperidine and the ^{13}C-labeled internal references depicted in Scheme 1 were

dx.doi.org/10.1021/jo401740g | J. Org. Chem. 2013, 78, 11733−11746

Scheme 2. Synthesis of ^{13}C-Labeled *N*-Boc-piperidine 1



dissolved in a mixture of diethyl ether and cyclohexane and all ^{13}C-labeled atoms are easily discernible and assigned in the ^{13}C DOSY (Figure 1). A subsequent the *D*-FW analysis as shown in

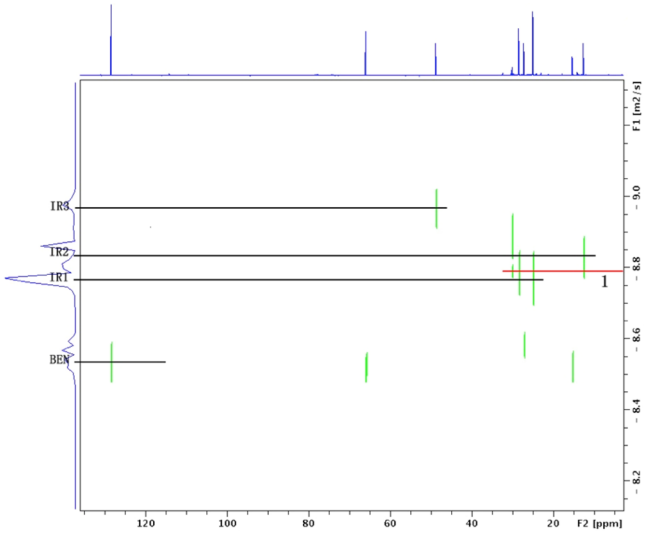


Figure 1. ^{13}C DOSY of **BEN**, **IR1**, **IR2**, **IR3** and **1** at 20 °C.

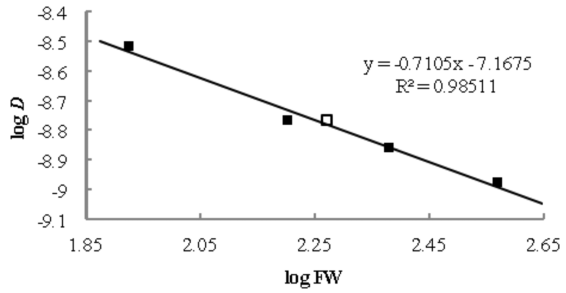


Figure 2. *D*-FW analysis of ^{13}C DOSY data. Internal references (■) and *N*-Boc-piperidine **1** (□).

Figure 2 displays a high correlation (r^2 = 0.985) and based upon this, the predicted formula weight of labeled *N*-Boc-piperidine is 179 g mol^{-1}, which is indeed very close to the actual formula weight of 186 g mol^{-1} (3.8% difference).

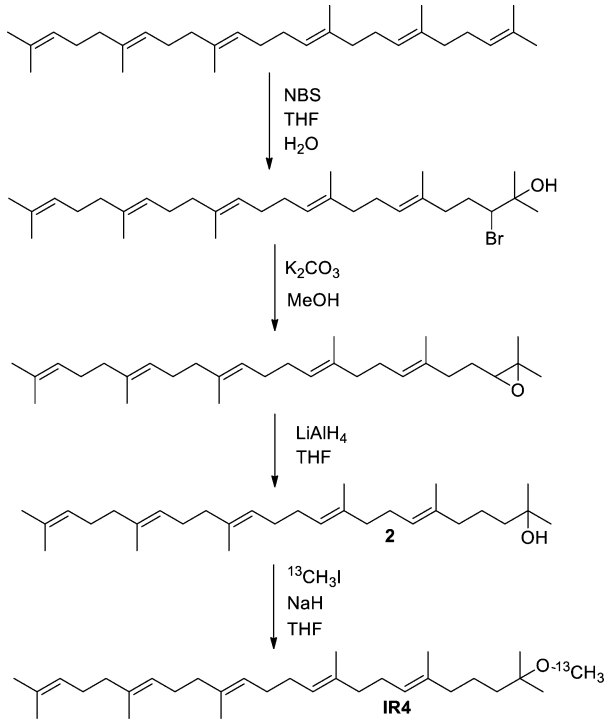
Modification of the ^{13}C-Labeled Internal Reference System. The high correlation (r^2 = 0.985) of the internal reference system given in Table 1 coupled with the small error in the prediction of formula weight of the ^{13}C-labeled internal reference system established for us its validity to predict formula weights using ^{13}C-labeled compounds. We note however that internal reference compound **IR3**, which is a solid at room temperature, precipitates easily from diethyl ether when the temperature drops below 0 °C. Since the investigation of organolithium complexes is usually performed significantly below 0 °C, **IR3** was replaced by another internal reference that remains soluble at low temperature. For simple

Table 1. *D*-FW Analysis of ^{13}C DOSY of BEN, IR1, IR2, IR3 and 1 at 20° C

entry	compd	FW (gmol^{-1})	10^{-9} *D* (m^2/s)	predicted FW (gmol^{-1})	% error
1	**BEN**	84.07	3.050	78	6.1
2	**IR1**	159.3	1.716	177	−11.4
3	**IR2**	239.4	1.383	240	−0.4
4	**IR3**	369.7	1.055	351	4.8
5	**1**	186.3	1.704	179	3.8

^{1}H *D*-FW analysis, we typically utilize squalene as our highest formula weight reference. Hence, in lieu of synthesizing ^{13}C-labeled squalene itself, we can readily modify squalene into the ^{13}C-labeled internal reference compound **IR4** depicted in Scheme 3. Thus tertiary alcohol **2** is readily prepared from

Scheme 3. Synthesis of Internal Reference Derived from Squalene



squalene in a few simple steps and yields **IR4** easily upon reaction with ^{13}C-labeled iodomethane (Scheme 3). Compound **IR4** has a formula weight of 443.8 g mol^{-1} and is a liquid at room temperature. There is no precipitation observed for the diethyl ether solution of **IR4** at −60 °C.

***D*-FW Analysis of the New ^{13}C-Labeled Internal Reference System.** The analysis and verification experiment described above was repeated utilizing **IR4** as the highest molecular weight standard. The ^{13}C DOSY spectrum is depicted in Figure 3. The *D*-FW plot of the internal references also produces an excellent correlation and the *D*-FW analysis yields a predicted formula weight of ^{13}C−*N*-Boc-piperidine **1** as 198 g mol^{-1} (Figure 4, Table 2).

Replacement of **IR3** by **IR4** greatly expands the temperature range for the application of this ^{13}C-labeled internal reference DOSY protocol. Therefore, we turned our attention to the use of these ^{13}C-labeled internal reference compounds for the DOSY analyses of the methyllithium diamine complexes at −20

dx.doi.org/10.1021/jo401740g | *J. Org. Chem.* 2013, 78, 11733−11746

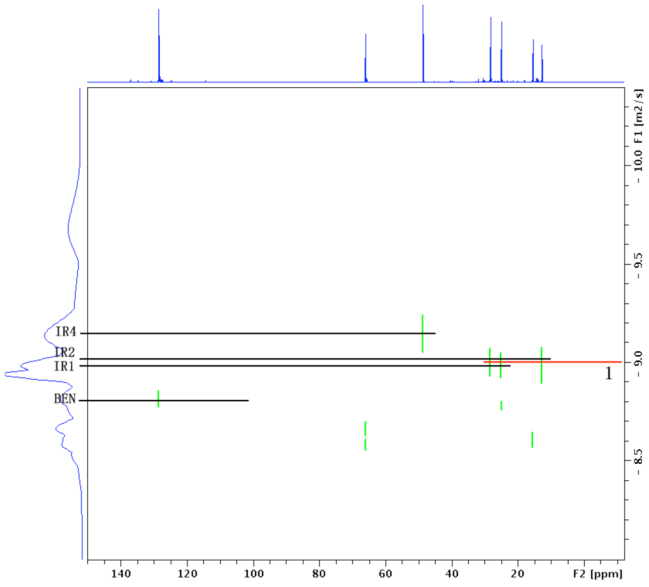


Figure 3. ^{13}C DOSY of **BEN**, **IR1**, **IR2**, **IR4** and **1** at 20 °C.

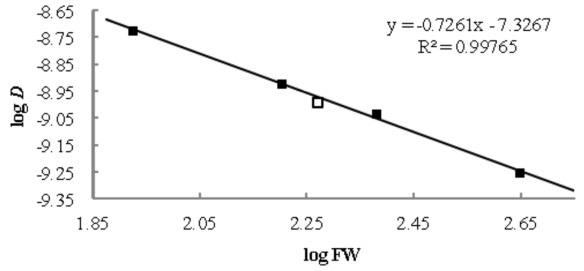


Figure 4. *D*-FW analysis of ^{13}C DOSY data. Internal references (■) and *N*-Boc-piperidine **1** (□).

Table 2. *D*-FW Analysis of ^{13}C DOSY of BEN, IR1, IR2, IR4 and 1 at 20° C

entry	compd	FW ($gmol^{-1}$)	10^{-10} *D* (m^2/s)	predicted FW ($gmol^{-1}$)	% error
1	**BEN**	84.07	18.69	85	−1.4
2	**IR1**	159.3	11.80	160	−0.8
3	**IR2**	239.4	9.150	227	4.8
4	**IR4**	443.8	5.526	456	−2.8
5	**1**	186.3	10.12	198	−6.5

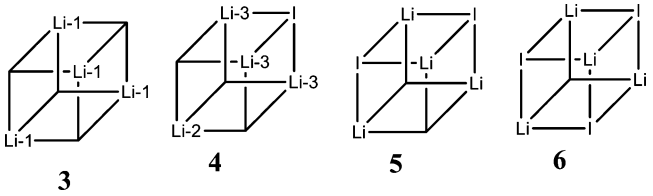
to −40 °C. As a caveat we note that since the internal references **IR1**, **IR3** and **IR4** are ethers, this fact cannot be overlooked when they are used for the study of reactive organolithium complexes in pure hydrocarbon solvents in the absence of any additional coordinating ligands or solvents. However, as noted previously, these references are indeed applicable in the investigation of many organolithium compounds and reactions since these reagents/reactions are usually performed in ethereal solvents with the addition of a stoichiometric amount of a strongly coordinating ligand such as a chelating diamine.

Analysis of Methyl Lithium and Methyllithium-Tertiary Diamine Complexes in Diethyl Ether. To demonstrate the utility of these ^{13}C-labeled internal reference materials, we chose to determine the aggregate state of methyllithium (MeLi) dissolved in diethyl ether (DEE) with and without the presence of several tertiary diamine ligands. Hence we describe comprehensive results of DOSY-*D*-FW analysis of the ^{13}C-labeled methyllithium in diethyl ether solution.

Characterization of $^{13}CH_3{}^6Li$–DEE Tetramer. Double-labeled (^{13}C, ^{6}Li) methyllithium was prepared using Maddaluno's method except that DEE was used instead of tetrahydrofuran-d_8.[1a] We typically observe approximately 5–10% lithium iodide present in the MeLi solution utilizing this procedure.

In 1972, Brown and co-workers demonstrated that MeLi exists as a tetramer $(MeLi)_4$ **3** and as a $(MeLi)_3(LiI)_1$ mixed tetramer **4** in diethyl ether in the presence of lithium iodide (Scheme 4).[8c] Later, Gunther and co-workers reported mixed

Scheme 4. MeLi Tetramer 3 and MeLi–LiI Mixed Tetramer 4



tetramers **5** and **6** when the mole ratio of LiI to MeLi approaches 1:1.[8a] Our ^{6}Li NMR spectrum of the $^{13}CH_3{}^6Li$–DEE solution at −30 °C showed two distinct peaks depicted in Figure 5. Since the mole ratio of LiI to MeLi is far less than 1:1,

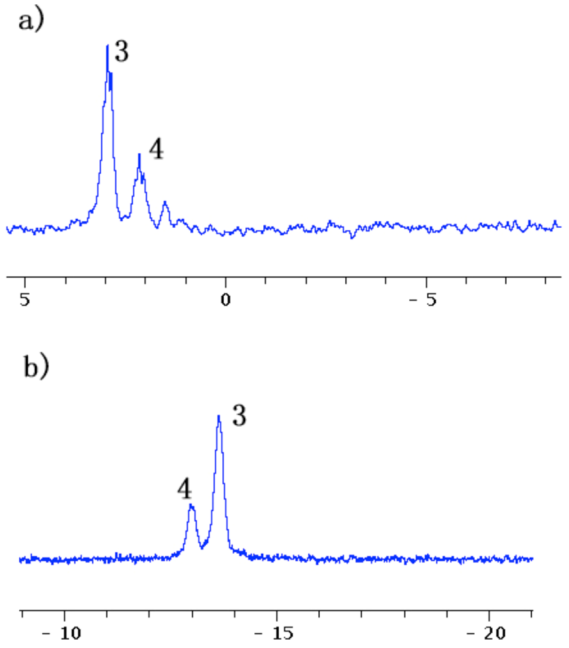


Figure 5. NMR spectra of 0.06 M $^{13}CH_3{}^6Li$–DEE solution at −40 °C. (a) ^{6}Li spectrum and (b) ^{13}C spectrum.

the presence of tetramer **5** or **6** is not significant. Therefore, we assign the large downfield peak in the ^{6}Li spectrum to Li-1 in the tetramer **3** and the small upfield peak to Li-3 in the $(MeLi)_3(LiI)_1$ mixed tetramer **4**. According to Brown and Gunther, Li-2 of tetramer **3** appears as a downfield shoulder of the Li-1 peak at −70 °C. However, we cannot resolve the shoulder peak at −40 °C possibly because of fast intramolecular exchange. The ^{13}C spectrum of $^{13}CH_3{}^6Li$–DEE solution

dx.doi.org/10.1021/jo401740g | J. Org. Chem. 2013, 78, 11733–11746

displays two distinct peaks in the upfield region. The peaks at −12.9 ppm and −13.7 ppm are assigned to the resonances of the methyl group of tetramer **3** and tetramer **4**, respectively.

The ^{13}C DOSY spectrum of this $^{13}CH_3{}^6Li$–DEE solution with added internal references is reproduced in Figure 6. The

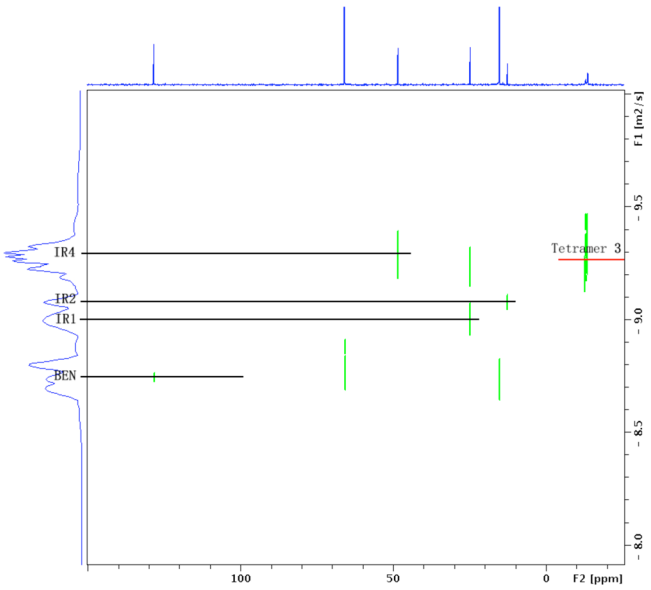


Figure 6. ^{13}C DOSY of $^{13}CH_3{}^6Li$–DEE solution at −40 °C.

correlation between log FW and log *D* of the linear regression is very high ($r^2 > 0.99$) and the predicted formula weight for the resonance at −13.7 ppm is 407 g mol^{-1}, which is very close to the formula weight of the tetrameric $^{13}CH_3{}^6Li$ with each lithium atom coordinated to a single DEE (384.7 g mol^{-1}, −6.0% difference). However, the predicted formula weight for the resonance at −12.9 ppm is 408 g mol^{-1}, which represents a 17.5% difference from the calculated formula weight (495.5 g mol^{-1}) of tetra-solvated tetramer **4** (Figure 7, Table 3). We suggest that the high inaccuracy in the formula weight prediction of tetra-solvated tetramer **4** is very likely due to the presence of the highly dense iodine atom in the complex.

We note that an alternative explanation for the formula weight of the tetramer **4** deduced from the *D*-FW analysis is consistent with a tris-solvated tetramer rather than a tetra solvated tetramer. The formula weight of an unknown complex is deduced from its experimental diffusion coefficient through the linear regression plot of the logarithms of NMR determined diffusion coefficients against the known formula weights of

Table 3. *D*-FW Analysis of ^{13}C DOSY of BEN, IR1, IR2, IR4, 3 and 4 at −40° C

entry	compd	FW (gmol^{-1})	10^{-10} *D* (m^2/s)	predicted FW (gmol^{-1})	% error
1	**BEN**	84.07	17.72	83	1.0
2	**IR1**	159.3	10.50	164	−3.2
3	**IR2**	239.4	8.013	233	2.4
4	**IR4**	443.8	4.882	445	−0.3
5	**3**[a]	384.7[a]	5.223	407	−6.0
6	**4**[b]	495.5[b]	5.213	408	17.5

[a]Formula weight of $(^{13}CH_3{}^6Li)_4(DEE)_4$ complex. [b]Formula weight of $(^{13}CH_3{}^6Li)_3I(DEE)_4$ complex.

reference compounds according to the empirical equation log *D* = *A* log FW + *C*. The validity of the empirical equation depends on the similarity of the density of internal references and complexes.[2] From a comparison of the densities of many crystal structures of organolithium complexes, it is apparent that the density of tetramer **4** is very different from the density of tetramer **3** and also from the density of the internal references owing to the incorporation of the iodine atom. Therefore, the predicted formula weight that is deduced from the linear regression plot deviates significantly from the calculated formula weight of tetra-solvated tetramer **4** as we have previously observed in analyzing compounds containing heavy atoms and whose density differs significantly from the reference standards. Our results for the tetramer **3** comport with those of both Brown and Gunther as expected with the enhancement that they permit a determination of the solvation state directly. Furthermore, these results serve to demonstrate a limitation of the *D*-FW analysis when applied to compounds that differ significantly in density.

Characterization of $^{13}CH_3{}^6Li$ Complexes with TMEDA, (rac)-TMCDA, *N*,*N*′-Dimethylbispidine, PMDTA or (−)-Sparteine. For these analyses, samples were prepared by adding the tertiary amines separately to $^{13}CH_3{}^6Li$–DEE solutions at −40 °C as described. Individual results and interpretation of each separate experiment are provided.

Upon addition of 3 equiv of TMEDA, the two peaks of complexes **3** and **4** disappeared and were replaced by a new peak at −12.7 ppm. The new peak resolved into a quintet with *J* = 8.2 Hz, when the temperature was lowered to −70 °C (Figure 8). As depicted in Figure 9, the ^6Li spectrum of $^{13}CH_3{}^6Li$–DEE solution with 3 equiv TMEDA at −70 °C is a triplet with *J* = 8.0 Hz. Clearly these results suggest the formation of a oligomeric, methyllithium-TMEDA complex. Within this complex, the single carbon of methyllithium

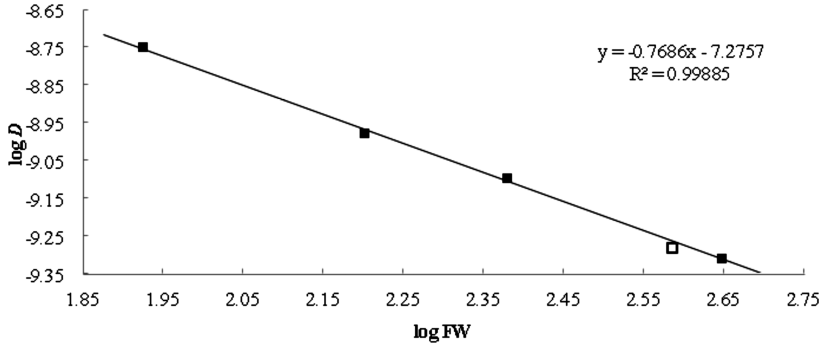


Figure 7. *D*-FW analysis of ^{13}C DOSY data. Internal references (■) and the tetra-solvated tetramer **3** (□).

dx.doi.org/10.1021/jo401740g | J. Org. Chem. 2013, 78, 11733−11746

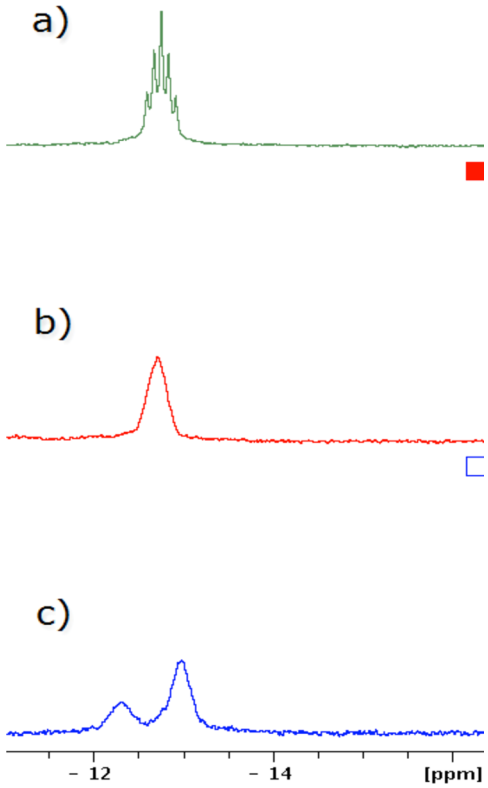


Figure 8. ^{13}C spectra of $^{13}CH_3{}^6Li$. (a) ^{13}C spectrum of 0.12 M $^{13}CH_3{}^6Li$ in DEE at −70 °C. (b) ^{13}C spectrum of 0.12 M $^{13}CH_3{}^6Li$ in DEE with 3 equiv TMEDA at −40 °C. (c) ^{13}C spectrum of 0.12 M $^{13}CH_3{}^6Li$ in DEE with 3 equiv TMEDA at −70 °C.

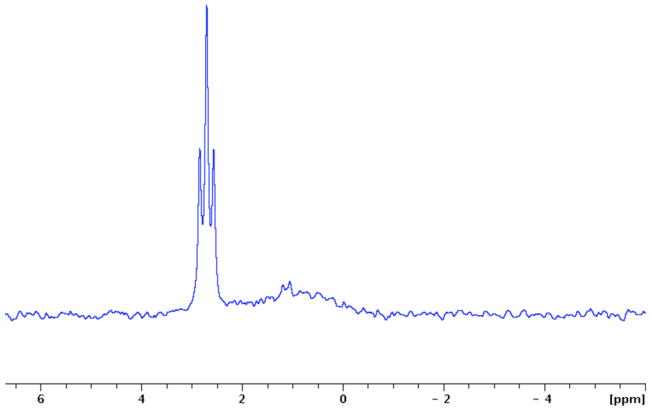
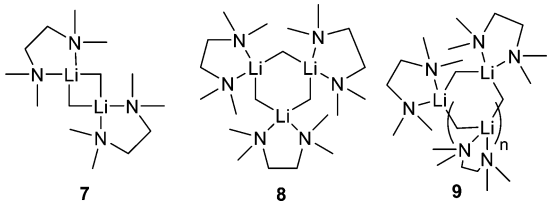


Figure 9. ^6Li NMR spectrum of 0.12 M $^{13}CH_3{}^6Li$–DEE solution with 3 equiv TMEDA at −70 °C.

interacts with two lithium atoms and each lithium atom interacts with two carbon atoms. Although the structure of dimer **7** as depicted in Scheme 5 is consistent with the NMR results, it is not the only possible structure because the cyclic trimer **8** and higher order cyclic oligomers are also consistent with these NMR spectra. It is noteworthy that these NMR results are inconsistent with the structure of the methyllithium-TMEDA complex determined by X-ray diffraction analysis by Weiss.[9] The tetrameric methyllithium unit observed for the crystal structure would appear as a septet for the ^{13}C and a quintet for the ^6Li spectrum.

To establish the dimeric nature of the MeLi–TMEDA complex, we performed a ^{13}C DOSY with ^{13}C-labeled internal

Scheme 5. MeLi–TMEDA Dimer 7, Cyclic Trimer 8, and Cyclic Oligomer 9



references **IR1**, **IR2** and **IR4**. Isotopic labeling with ^{13}C was necessary in this experiment because the natural abundance ^{13}C spectrum with nonlabeled internal references required a relatively high concentration (>1.0 M and much precipitation was observed when the DEE solution of 1.0 M MeLi with 3 equiv TMEDA was kept at −40 °C for the time required to complete this experiment, approximately 6–8 h. Moreover, ^1H DOSY and ^{13}C DOSY with regular internal references required the use of expensive DEE-d_{10} as solvent and this became prohibitive on a routine basis.

The ^{13}C DOSY spectrum with the isotopically labeled sample is depicted in Figure 10. The *D*-FW plot exhibits a reliably high

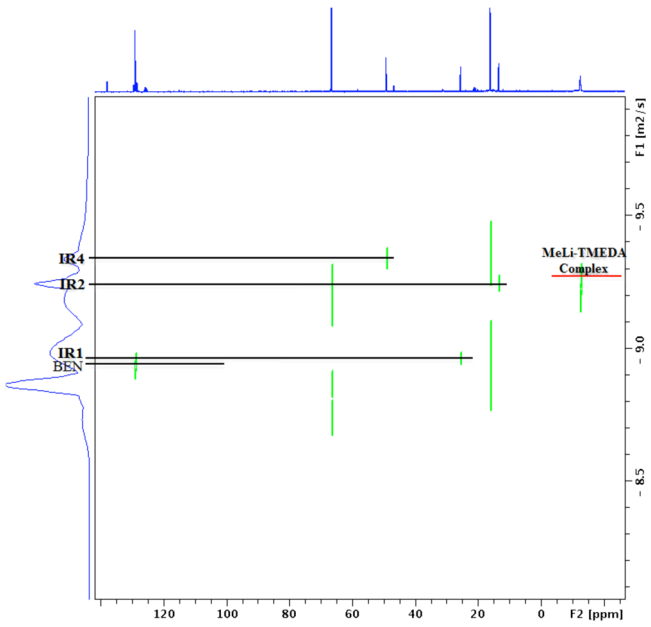


Figure 10. ^{13}C DOSY of $^{13}CH_3{}^6Li$–DEE solution with 3 equiv TMEDA at −50 °C.

(r^2 > 0.99) correlation. The predicted formula weight for the resonance of MeLi–TMEDA complex at −12.7 ppm is 288 g mol^{-1}, which represents a 4.4% difference from the calculated formula weight 276.5 g mol^{-1} for dimer **7** (Figure 11, Table 4). However, the calculated formula weight of cyclic trimer **8** is 414.8 g mol^{-1}, a 30.4% difference from the observed formula weight. Therefore, we rule out the possibility that the MeLi–TMEDA complex in DEE solution as a cyclic trimer or a higher oligomer.

Racemic, trans-TMCDA was added to the $^{13}CH_3{}^6Li$–DEE solution at −40 °C to the extent of 2 equiv of TMCDA per MeLi as depicted in Figure 12.[18] Upon addition of 0.25 equiv TMCDA, the resonance assigned to the $(MeLi)_3(LiI)_1$ mixed tetramer **4** disappeared and two new peaks emerge at −9.5 and −11.0 ppm. As more TMCDA was added to the sample, the

dx.doi.org/10.1021/jo401740g | J. Org. Chem. 2013, 78, 11733–11746

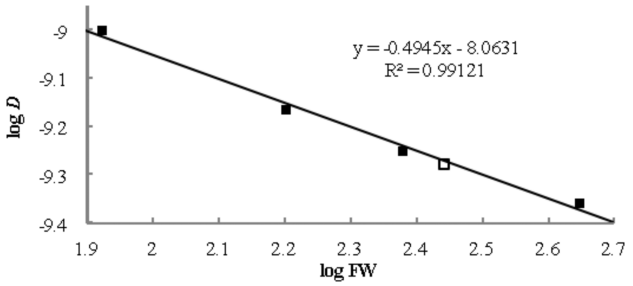


Figure 11. *D*-FW analysis of ^{13}C DOSY data. Internal references (■) and the MeLi–TMEDA dimer **7** (□).

Table 4. *D*-FW Analysis of ^{13}C DOSY of BEN, IR1, IR2, IR4 and 7 at −50° C

entry	compd	FW (gmol^{-1})	10^{-10} *D* (m^2/s)	predicted FW (gmol^{-1})	% error
1	**BEN**	84.07	9.944	79	5.6
2	**IR1**	159.3	6.838	169	−6.3
3	**IR2**	239.4	5.613	233	−5.4
4	**IR4**	443.8	4.367	445	5.5
5	**7**^{a}	276.5^a	5.253	288	−4.4
6	**8**^{b}	414.8^b	5.253	288	30.4

^{a}Formula weight of dimer $(^{13}CH_3{}^6Li)_2(TMEDA)_2$ **7**. ^{b}Formula weight of trimer $(^{13}CH_3{}^6Li)_3(TMEDA)_3$ **8**.

peak at −11.0 ppm increased at the expense of the resonance of tetramer **3**. Meanwhile, the intensity of peak at −9.5 ppm did not change significantly. These results are consistent with the formation of a MeLi–TMCDA complex with a resonance at −11.0 ppm and a $(MeLi)_m(LiI)_n$–TMCDA mixed complex with resonance at −9.5 ppm. The major peak at −11.0 ppm appears as a quintet with J = 7.7 Hz whereas we interpret the minor peak at −9.5 ppm as two quintets with J = 9.8 Hz overlapping with each other. The major resonance in the ^{6}Li spectrum of the sample is a triplet with J = 7.7 Hz, whereas the minor peak is a doublet with J = 9.7 Hz (Figure 13). Taken together, these one-dimensional NMR results support the formation of homodimer **10** as the resonance at −11.0 ppm and heterodimer **11** as the resonance at −9.5 ppm (Scheme 6). Because racemic TMCDA was used in the experiment, two diastereomeric heterodimers **11** can be formed. We suggest that formation of diastereomers of complex **11** accounts for the appearance of the resonance at −9.5 ppm as two overlapping quintets.

In analogy to the MeLi–TMEDA complex, these one-dimensional NMR results for the MeLi–TMCDA complex are also consistent with a cyclic trimer or higher cyclic oligomer. However, the NMR results of MeLi–LiI–TMCDA mixed complex are not consistent with any mixed cyclic trimer or cyclic oligomer depicted in Scheme 7. Thus, we performed ^{13}C DOSY with ^{13}C-labeled internal references to establish the dimeric structure of the MeLi–TMCDA complex in DEE. This ^{13}C DOSY spectrum is depicted in Figure 14. The extrapolated formula weight for the resonance at −11.0 ppm is 359 g mol^{-1}, a 6.6% difference from the calculated formula weight 384.7 g mol^{-1} for the dimer **10** (Figure 15, Table 5). This experiment establishes that the major species in the sample is the MeLi–TMCDA dimer **10** represents the same motif as the crystal structure obtained by Strohmann.[11] The low intensity of the minor peak prevented us from conducting a *D*-FW analysis

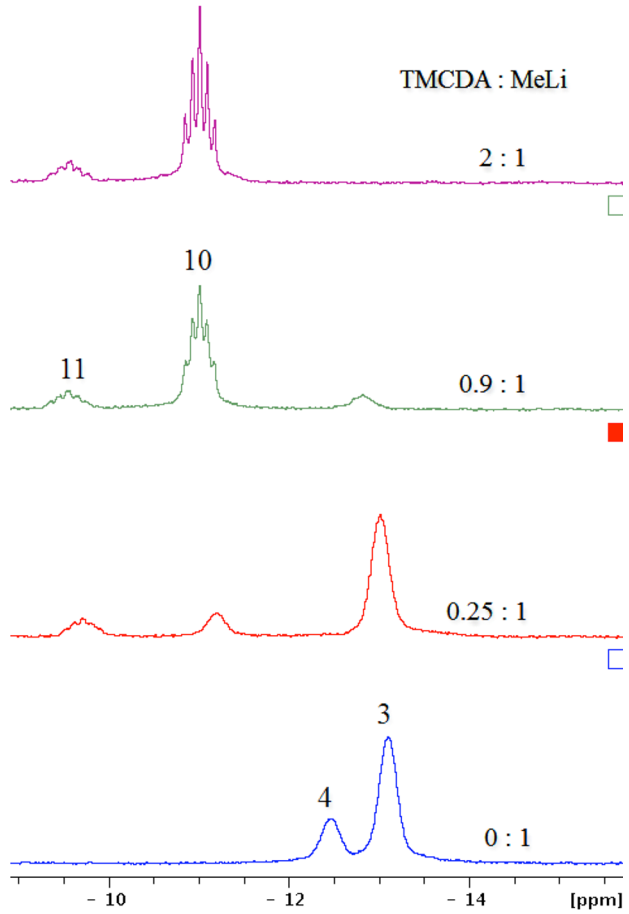


Figure 12. ^{13}C NMR spectra of TMCDA titration of 0.15 M $^{13}CH_3{}^6Li$–DEE solution at −40 °C. Peak 3 represents the resonance of tetramer **3**, 4 represents the resonance tetramer **4**, 10 represents the resonance of dimer **10**, and 11 represents the resonance of mixed dimer **11**.

because we were unable to obtain a reasonable attenuation in the DOSY experiment.

A sample was prepared by addition of 2 equiv *N*,*N*′-dimethylbispidine (DMB) to a $^{13}CH_3{}^6Li$–DEE solution at −40 °C. Two quintets appeared in the upfield region of ^{13}C spectrum after the addition of *N*, *N*′-dimethylbispidine (Figure 16a). The major peak at −14.0 ppm with J = 8.3 Hz is assigned as the methyllithium carbon of complex **12** whereas the minor peak at −12.3 ppm with J = 10.1 Hz is assigned as the methyllithium carbon of complex **13** (Scheme 8). The assignment of the carbon signals is supported by the ^{6}Li NMR which consist of a major triplet (J = 8.2 Hz) and a minor doublet (J = 10.1 Hz), see Figure 16b. Obviously, the ^{6}Li doublet is coupled to the ^{13}C quintet at −12.3 ppm while the ^{6}Li triplet is coupled to the ^{13}C quintet at −14.0 ppm. The splitting and coupling constants support our assignment as complexes **12** and **13** respectively. It is noteworthy that a significant peak emerged at −4.1 ppm after the addition of DMB. We believe that this new peak corresponds to the ^{13}C-labeled methane from the reaction between methyllithium and the water introduced with DMB. Moreover, there is also a sharp peak located downfield of the resonances of complex **12** and **13** in the ^{6}Li spectrum which is likely to be the corresponding resonance of lithium hydroxide.

dx.doi.org/10.1021/jo401740g | J. Org. Chem. 2013, 78, 11733–11746

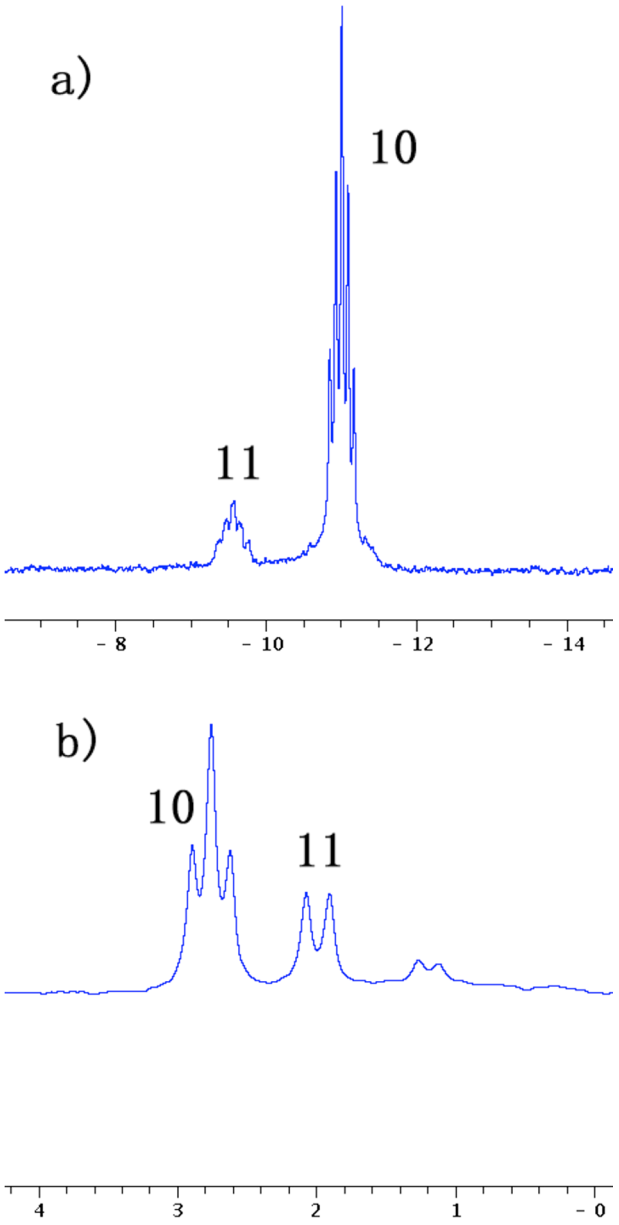
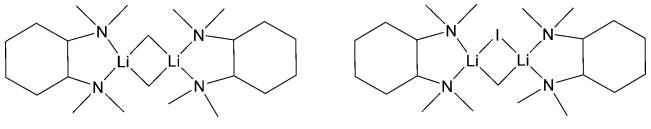


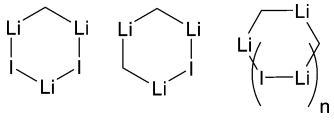
Figure 13. NMR spectra of 0.15 M $^{13}CH_3{}^6Li$–DEE solution with 2 equiv TMCDA at −40 °C. ^{13}C spectrum is depicted in (a) and ^6Li spectrum is depicted in (b).

Scheme 6. MeLi–TMCDA Homodimer 10 and MeLi–LiI–TMCDA Heterodimer 11



10 **11**

Scheme 7. MeLi–LiI Cyclic Trimers and Oligomers



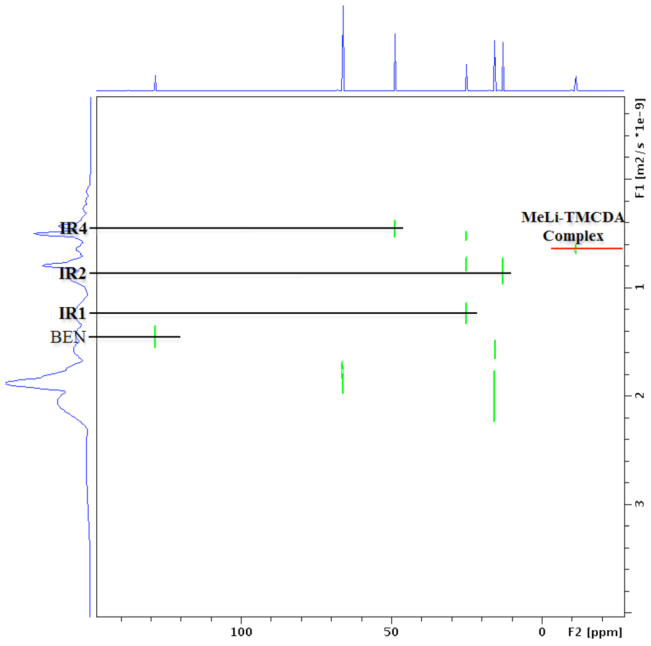


Figure 14. ^{13}C DOSY of $^{13}CH_3{}^6Li$–DEE solution with 2 equiv TMCDA at −40 °C.

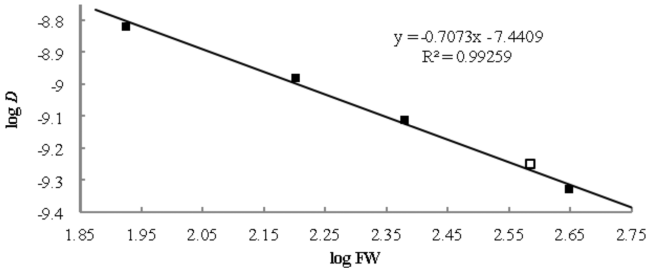


Figure 15. *D*-FW analysis of ^{13}C DOSY data. Internal references (■) and the MeLi–TMCDA dimer **10** (□).

Table 5. *D*-FW Analysis of ^{13}C DOSY of BEN, IR1, IR2, IR4 and 10 at −40° C

entry	compd	FW ($gmol^{-1}$)	10^{-10} *D* (m^2/s)	predicted FW ($gmol^{-1}$)	% error
1	**BEN**	84.07	15.18	88	−5.5
2	**IR1**	159.3	10.48	169	6.0
3	**IR2**	239.4	7.747	233	4.1
4	**IR4**	443.8	4.691	445	−5.1
5	**10**[a]	384.7[a]	5.643	359	6.6

[a]Formula weight of dimer $(^{13}CH_3{}^6Li)_2(TMCDA)_2$ **10**.

^{13}C DOSY *D*-FW analysis with the ^{13}C-labeled internal references verified the assignment as a dimer. The extrapolated formula weight for the resonance at −14.0 ppm is 361 g mol^{-1} differs from the calculated formula weight 352.6 g mol^{-1} of the homodimer **12** by 2.5%, see Figure 17 and Table 6. However, the extrapolated formula weight of the peak at −12.3 ppm is 309 g mol^{-1}, a 33.2% difference from the calculated formula weight 463.5 g mol^{-1} of heterodimer **13**. Similar to the tetramer **4**, heterodimer **13** contains an iodine atom that makes the density of the complex differ significantly from the internal references. Therefore, the extrapolated formula weight differs significantly from the calculated one. Notwithstanding the major discrepancy in the *D*-FW analysis of **13**, it is evident that

dx.doi.org/10.1021/jo401740g | J. Org. Chem. 2013, 78, 11733–11746

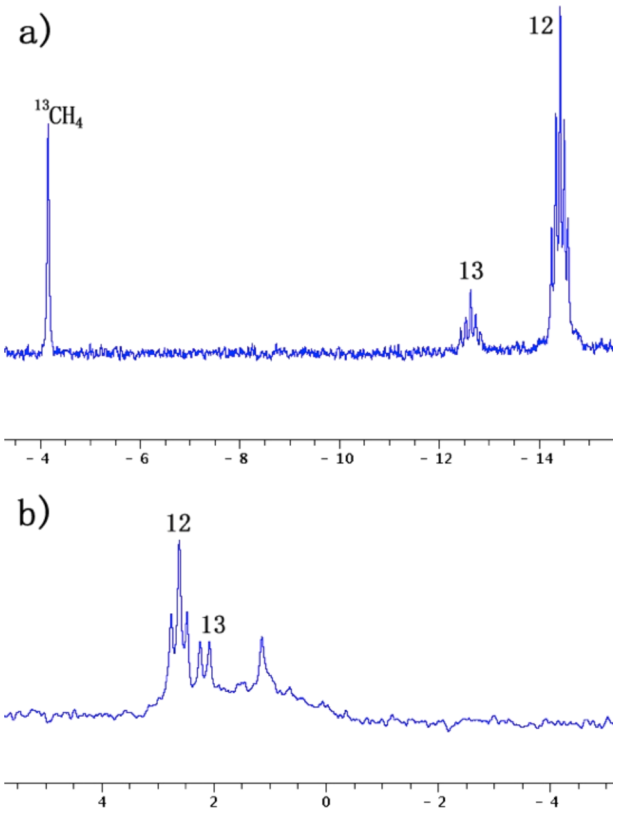
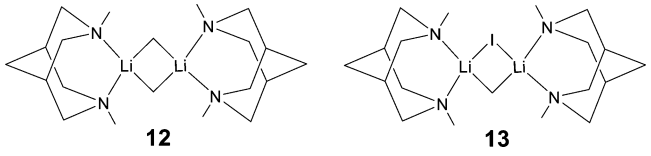


Figure 16. NMR spectra of 0.09 M $^{13}CH_3{}^6Li$–DEE solution with 2 equiv DMB at −40 °C. (a) ^{13}C spectrum and (b) ^6Li spectrum.

Scheme 8. MeLi–DMB Homodimer 12 and MeLi–LiI–DMB Heterodimer 13



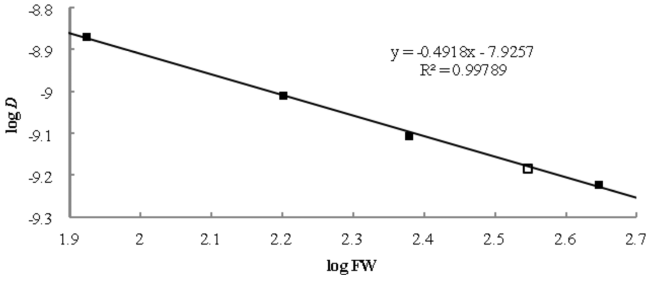


Figure 17. *D*-FW analysis of ^{13}C DOSY data. Internal references (■) and the MeLi–DMB dimer **12** (□).

homodimer **12** is formed and is the major species upon addition of DMB into the MeLi–DEE solution.

A sample was prepared by adding 2 equiv PMDTA into a $^{13}CH_3{}^6Li$–DEE solution at −40 °C followed by cooling to −70 °C which resolved the splitting of ^{13}C resonances coupled with ^6Li. As depicted in Figure 18, the carbon spectrum of this sample is a quintet at −11.6 ppm (J = 8.1 Hz) and a small broad peak at −8.8 ppm. The major peak in the ^6Li spectrum is a triplet (J = 7.9 Hz) and the minor is a small broad peak at −70 °C. These results are consistent with the formation of

Table 6. *D*-FW Analysis of ^{13}C DOSY of BEN, IR1, IR2, IR4, 12 and 13 at −40° C

entry	compd	FW (gmol^{-1})	10^{-10} D (m^2/s)	predicted FW (gmol^{-1})	% error
1	**BEN**	84.07	13.52	82	1.5
2	**IR1**	159.3	9.815	158	0.3
3	**IR2**	239.4	7.842	250	−4.7
4	**IR4**	443.8	6.002	431	2.7
5	**12**	352.6[a]	6.551	361	−2.5
6	**13**	463.5[b]	7.067	309	33.2

[a]Formula weight of homodimer $(^{13}CH_3{}^6Li)_2(DMB)_2$ **12**. [b]Formula weight of heterodimer $(^{13}CH_3{}^6Li)I(DMB)_2$ **13**.

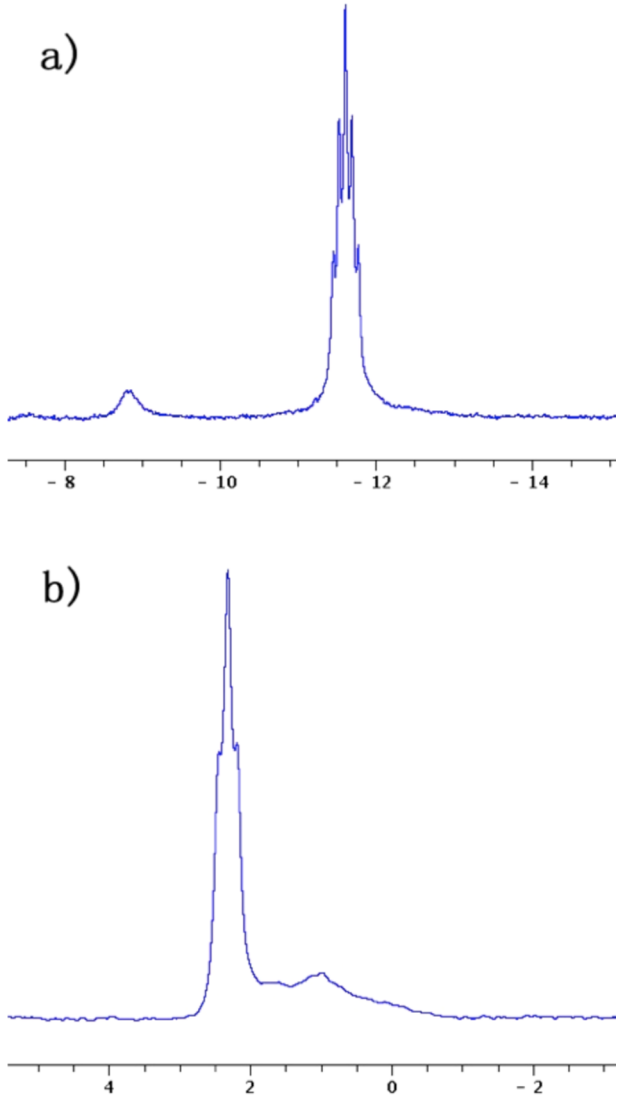
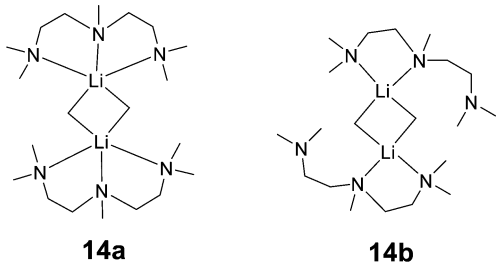


Figure 18. NMR spectra of 0.21 M $^{13}CH_3{}^6Li$–DEE solution with 2 equiv PMDTA at −70 °C. (a) ^{13}C spectrum and (b) ^6Li spectrum.

homodimer **14a** or **14b** depicted in Scheme 9. However, we are not able to differentiate between **14a** and **14b**. In light of the results of MeLi–LiI–diamine complexes previously described, it is not unreasonable to assign the minor resonance as the MeLi–LiI–PMDTA heterodimer; however, there is no definitive evidence to support this assignment. The ^{13}C DOSY *D*-FW formula weight derived from the resonance at −11.6 ppm is 377 g mol^{-1} and differs by 3.4% from the

dx.doi.org/10.1021/jo401740g | J. Org. Chem. 2013, 78, 11733–11746

Scheme 9. MeLi–PMDTA Homodimers 14a and 14b



calculated formula weight 390.7 g mol^{-1} of the homodimer **14**, see Figure 19 and Table 7.

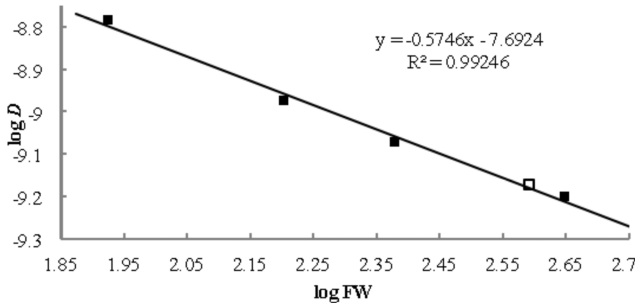


Figure 19. *D*-FW analysis of ^{13}C DOSY data. Internal references (■) and the MeLi–PMDTA dimer **14** (□).

Table 7. *D*-FW Analysis of ^{13}C DOSY of BEN, IR1, IR2, IR4, 14 at −40° C

entry	compd	FW (gmol^{-1})	10^{-10} *D* (m^2/s)	predicted FW (gmol^{-1})	% error
1	**BEN**	84.07	16.41	79	5.2
2	**IR1**	159.3	10.64	169	−6.3
3	**IR2**	239.4	8.503	250	−4.5
4	**IR4**	443.8	6.298	421	5.0
5	**14**	390.7[a]	6.712	377	3.4

[a]Formula weight of homodimer ($^{13}CH_3{}^6Li)_2(PMDTA)_2$ **14a** or **14b**.

The (−)-sparteine complex was prepared by adding 3 equiv (−)-sparteine into a $^{13}CH_3{}^6Li$–DEE solution at −40 °C. After the addition of sparteine, the resonances of tetramers **3** and **4** disappeared and a new quintet (J = 8.1 Hz) appeared at −8.1 ppm as depicted in Figure 20. A small broad peak next to the quintet is present in this ^{13}C spectrum. The ^{6}Li spectrum consists of a major triplet (J = 8.4 Hz) and some minor peaks that were not characterized. Strohmann and co-workers characterized the crystal structure of a homodimer of MeLi and (−)-sparteine.[10] Our NMR spectra are consistent with the same homodimer **15**, see Scheme 10. We suggest that the small board peak at −7.2 ppm is the resonance of the methyllithium in the MeLi–LiI heterodimer **16**, although we have no clear evidence for this. The formula weight for the resonance at −8.1 ppm determined by ^{13}C DOSY *D*-FW analysis is 462 g mol^{-1}, a 9.7% difference from the calculated formula weight 512.9 g mol^{-1} of the homodimer **15**, see Figure 21 and Table 8. Therefore, we conclude that the structure of the MeLi–sparteine in solution is the same as the solid-state structure.

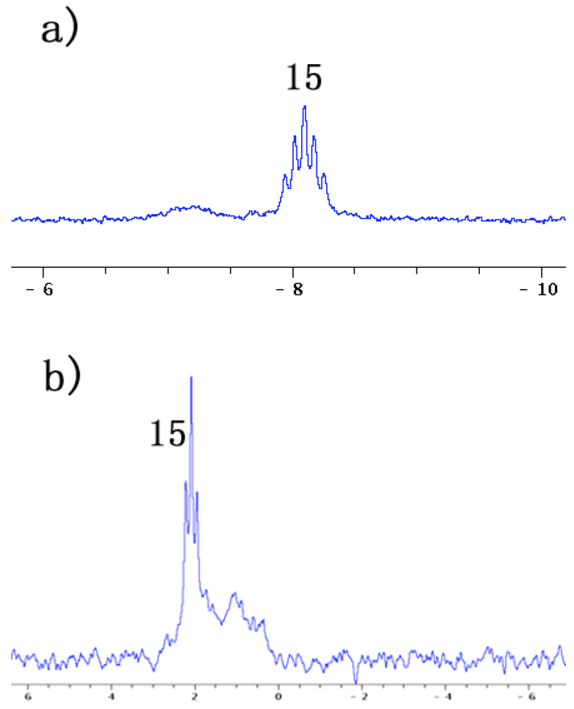
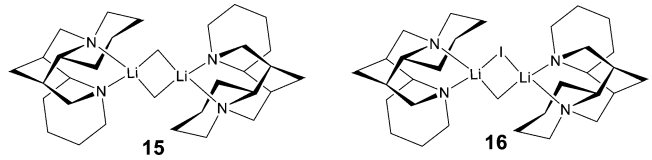


Figure 20. NMR spectra of 0.08 M $^{13}CH_3{}^6Li$–DEE solution with 3 equiv (−)-sparteine at −40 °C. (a) ^{13}C spectrum and (b) ^{6}Li spectrum.

Scheme 10. MeLi–SP Homodimers 15 and MeLi–LiI–SP Heterodimer 16



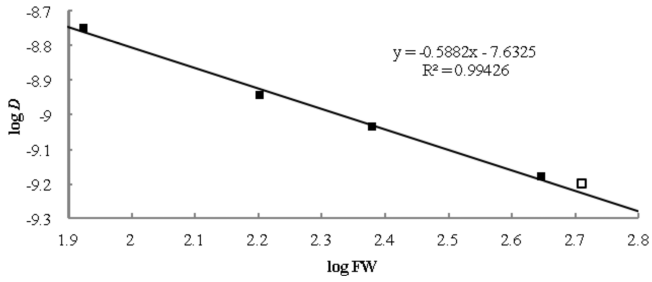


Figure 21. *D*-FW analysis of ^{13}C DOSY data. Internal references (■) and the MeLi–SP dimer **15** (□).

Table 8. *D*-FW Analysis of ^{13}C DOSY of BEN, IR1, IR2, IR4, 15 at −40° C

entry	compd	FW (gmol^{-1})	10^{-10} *D* (m^2/s)	predicted FW (gmol^{-1})	% error
1	**BEN**	84.07	17.69	80	4.7
2	**IR1**	159.3	11.35	170	−7.0
3	**IR2**	239.4	9.202	243	−1.7
4	**IR4**	443.8	6.605	427	3.6
5	**15**	512.9[a]	6.306	462	9.7

[a]Formula weight of homodimer ($^{13}CH_3{}^6Li)_2(SP)_2$ **15**.

dx.doi.org/10.1021/jo401740g | J. Org. Chem. 2013, 78, 11733–11746

CONCLUSION

The successful development of ^{13}C-labeled internal reference correlated DOSY with *D*-FW analysis is a significant step forward for the DOSY *D*-FW analysis, because it extends the applicability of the technique for the study of reactive intermediates or sensitive complexes. It is especially important when ^{1}H DOSY cannot be used owing to the overlap of resonances in the ^{1}H spectra. Isotopically enriched ^{13}C internal referenced DOSY is clearly preferable to natural abundance ^{13}C internal referenced DOSY because samples with relatively low concentration (<1 M) and compounds that decompose in the time required for natural abundance ^{13}C spectra can be analyzed. Moreover, analysis of the ^{13}C spectrum is considerably simplified because the isotopically enriched ^{13}C-labeled compounds are readily identified and assigned effortlessly. Furthermore, the use of ^{13}C-labeled internal reference DOSY is strongly preferable because the solvents used in the preparation of the sample are normal protonated solvents instead of expensive deuterated solvents such as diethyl ether-d_{10} and methylcyclopentane-d_{12}.

The solution structures of methyllithium complexes with various common tertiary diamines were characterized by utilizing $^{13}C-^{6}Li$ double-labeled methyllithium, as well as the ^{13}C-labeled internal reference DOSY with *D*-FW analysis. These NMR results prove that the methyllithium - diamine complexes are homodimeric in solution. Furthermore, evidence was also obtained for the presence of minor amounts of mixed dimeric complexes consisting of one equivalent of MeLi and one equivalent of LiI with the *N*,*N*,*N*′,*N*′-tetramethyl-1,2-diaminocyclohexane and *N*,*N*′-dimethylbispidine samples. These results not only demonstrate the validity of the newly formulated ^{13}C-labeled internal reference DOSY, but also further our knowledge of the methyllithium−diamine complexes in solution state. These results are important to the interpretation of the mechanism and reactivity of methyllithium.

EXPERIMENTAL SECTION

Procedure for NMR Experiments. NMR samples of methyllithium complexes were prepared in tubes sealed with rubber septa and parafilm. NMR tubes were evacuated, flame-dried and filled with argon before use. ^{1}H chemical shifts were referenced to toluene-d_8 at 7.09 ppm, and ^{13}C chemical shifts were referenced to toluene-d_8 at 137.86 ppm. For the NMR spectra of pure internal references and ^{13}C-labeled *N*-Boc-piperidine, ^{1}H chemical shifts were referenced to the chloroform peak at 7.27 ppm in the ^{1}H spectrum and the peak at 77.0 ppm in the ^{13}C spectrum or alternatively to the benzene peak at 7.16 ppm, and the benzene-d_6 peak at 128.36 ppm. All NMR experiments were acquired on a 400 MHz spectrometer equipped with a *z*-axis gradient amplifier and an ATMA BBO probe with a *z*-axis gradient coil. Maximum gradient strength was 0.214 T/m. ^{1}H-DOSY was performed using the standard dstebpgp3s program, employing a double stimulated echo sequence, bipolar gradient pulses for diffusion, and 3 spoil gradients. Diffusion time was 200 ms, and the rectangular gradient pulse duration was 600−800 μs. Gradient recovery delays were 200 μs. Individual rows of the quasi-2-D diffusion databases were phased and baseline corrected. Actual diffusion coefficients used for *D*-FW analysis were obtained with commercial software using the T1/T2 analysis module.

The ^{13}C-labeled internal references were first mixed in a ratio of 1:6:6:6 for BEN, **IR1**, **IR2**, and **IR4** respectively (a ratio of 1:6:6:6 for BEN, **IR1**, **IR2**, and **IR3** respectively). The mixed internal references were titrated into the NMR tube and monitored by ^{13}C NMR. The titration was stopped when the peak intensity of benzene was about a half of the major methyllithium carbon peak.

Preparation of *n*-Bu^{6}Li Solution. The *n*-Bu^{6}Li solution was prepared in heptane according to the method that we have previously employed.[2j]

Preparation of Double-labeled (^{13}C, ^{6}Li) Methyllithium DEE Solution. The double-labeled (^{13}C, ^{6}Li) methyllithium was prepared according to Maddaluno.[1a] A solution of 1 equiv *n*-Bu^{6}Li was added dropwise to 60 μL $^{13}CH_3I$ dissolved in 1.5 mL of pentane at −78 °C in a centrifugation tube. The solution was then stirred at −30 °C until white precipitates formed. After that, the mixture was stirred at 0 °C for 20 min before centrifuging for 15 min, followed by removal of the solvent with a syringe. The solid residue was placed under vacuum for 30 min, followed by the addition of 2.5 mL of diethyl ether yielding a 0.1−0.2 M methyllithium solution. The mixture was transferred to an NMR tube via a syringe with the intervention of a PTFE filtration owing to the presence of a small amount of white solid precipitate in the solution. An additional 0.05 mL of toluene-d_8 was added to the NMR samples to facilitate deuterium locking.

Preparation of Tertiary Diamines. TMEDA, PMDTA and (−)-sparteine are commercially available. Racemic TMCDA was prepared according to Collum's method.[14a] *N*,*N*′-Dimethylbispidine was prepared according to Zones[19] by reaction of *N*-methyl-4-piperidone with a mixture of acetic acid, paraformaldehyde and methylamine hydrochloride. The resulting oil was then reduced to *N*,*N*′-dimethylbispidine by Wolff-Kischner reduction.

Synthesis of 2-Methoxy-2-methyloctane-1-^{13}C IR1. Step 1: To a flame-dried two-neck round-bottom flask fitted with a condenser under argon atmosphere was placed 0.16 g of magnesium (6.6 mmol, 1.4 equiv) and then 8 mL of anhydrous diethyl ether was added with stirring. A solution of 0.836 g of $^{13}CH_3I$ (5.85 mmol, 1.25 equiv) dissolved in 5 mL of diethyl ether was added dropwise to the magnesium DEE mixture using a syringe pump at room temperature. After the addition, the gray mixture was allowed to stir for 30 min at room temperature before transferring to a flame-dried 50 mL round-bottom flask flushed with argon and cooled to 0 °C. A solution of 0.60 g of 2-octanone (4.68 mmol, 1 equiv) in 5 mL of anhydrous DEE was slowly added to the gray methylmagnesium iodide solution using a syringe pump at 0 °C. After that, the reaction mixture was allowed to stir at room temperature overnight before quenching with 5 mL of 0.5 M HCl. The mixture was extracted with 20 mL of Et_2O three times and the combined organic phase was washed by 10 mL of brine and dried over anhydrous Na_2SO_4. The solvent was then removed by rotary evaporation. The resulting 2-methyloctan-2-ol-1-^{13}C was used in the next step without further purification.

Step 2: Into a flame-dried flask was placed 0.75 g of 60% sodium hydride (18.7 mmol, 4 equiv) in mineral oils under an argon atmosphere. The solid was washed by 5 mL of dry pentane three times before adding 12 mL of anhydrous THF and 2-methyloctan-2-ol-1-^{13}C obtained in the previous step. The resulting solution was then refluxed for 1 h before adding 1.00 g of iodomethane (7.05 mmol, 1.5 equiv). This reaction mixture was then refluxed overnight and quenched with 5 mL of saturated ammonium chloride at 0 °C. The mixture was extracted with 20 mL of pentane three times and the combined organic phase was washed by 10 mL of brine and dried over anhydrous Na_2SO_4. The solvent was then removed by rotary evaporation. The residue was purified chromatigraphically on a flash column with 150 mL of pentane. After the removal of solvent, 0.47 g of **IR1** (2.95 mmol, 63%) was obtained as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.18 (s, 3H), 1.49−1.39 (m, 2H), 1.36−1.19 (m, 9.5H), 0.98 (s, 1.5H), 0.89 (t, 3H, *J* = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 74.8, 74.4, 49.0, 39.8, 31.9, 29.9, 25.0, 23.8, 22.7, 14.1; MS *m*/*z* 158 $[M-H]^-$, 144, 86, 74, 69, 56, 45.

Synthesis of (*Z*)-Heptadec-2-ene-1-^{13}C IR2. Step 1: A solution of 1.2 equiv *n*-BuLi was added slowly to a solution of 0.55 g of 1-hexadecyne (2.5 mmol, 1 equiv) dissolved in 20 mL of anhydrous THF in a flame-dried bottom flask under argon atmosphere and cooled to −78 °C. After 5 min, the reaction mixture was warmed up to room temperature and stirred for 30 min. The resulting solution was placed in an ice bath and 0.53 g of $^{13}CH_3I$ (3.7 mmol, 1.5 equiv) was added all at once. The resulting solution was stirred at room temperature for 2 h before quenching with 5 mL of saturated

dx.doi.org/10.1021/jo401740g | J. Org. Chem. 2013, 78, 11733−11746

ammonium chloride. The mixture was extracted with 20 mL of hexanes three times and the combined organic phase was washed by 10 mL of brine and dried over anhydrous Na_2SO_4. The solvent was then removed by rotary evaporation. The resulting heptadec-2-yne-1-^{13}C was used in the next step without further purification.

Step 2: The heptadec-2-yne-1-^{13}C was reduced to (*Z*)-heptadec-2-ene-1-^{13}C by Ashby's method.[20] To a solution of 0.060 g of titanocene dichloride (0.24 mmol, 0.1 equiv) dissolved in 12 mL of THF in a flame-dried flask under argon atmosphere was added the resulting heptadec-2-yne-1-^{13}C at room temperature. The resulting solution was allowed to stir for 20 min before cooling down to 0 °C. After that, 0.19 g of lithium aluminum hydride (5.0 mmol, 2 equiv) mixed with 2 mL of anhydrous THF was added slowly to the reaction mixture. The resulting mixture was allowed to stir at room temperature overnight before cooling back to 0 °C. The reaction was then quenched by adding 1 mL of water and then 4 mL of 0.5 M HCl. The mixture was extracted with 20 mL of hexanes three times and the combined organic phase was washed by 10 mL of brine and dried over anhydrous Na_2SO_4. The solvent was then removed by rotary evaporation. The residue was purified by a flash column with 150 mL of hexanes. After removal of solvent, 0.49 g of **IR2** (2.1 mmol, 83%) was obtained as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.50−5.32 (m, 2H), 2.09−2.00 (m, 2H), 1.63 (dd, 3H, *J* = 125, 6.2 Hz), 1.36−1.17 (m, 24H), 0.91 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 130.9, 123.7, 123.4, 31.9, 29.7, 29.7, 29.6, 29.4, 29.3, 22.7, 21.1, 17.9, 14.4, 14.1, 13.8, 13.6, 12.7; HRMS-EI *m*/*z*: $[M]^+$ Calcd for $C_{16}{}^{13}CH_{34}$: 239.2689, found: 239.2682.

Synthesis of 2-(Methoxy-^{13}C)-2-methyltricosane IR3. Step 1: To a flame-dried two-neck round-bottom flask fitted with a condenser under argon atmosphere was placed 0.41 g of magnesium (17 mmol) and then 25 mL of anhydrous diethyl ether was added and stirring initiated. A solution of 2.00 g of iodomethane (14 mmol) dissolving in 10 mL of diethyl ether was added dropwise to the magnesium DEE mixture at room temperature using a syringe pump. After the addition, the gray mixture was allowed to stir for 30 min at room temperature before transferring to a flame-dried round-bottom flask flushed with argon at 0 °C. A solution of 1.75 g of methyl behenate (4.93 mmol) dissolved in 10 mL of DEE was slowly added to the gray methylmagnesium iodide solution using a syringe pump at 0 °C. After that, the reaction mixture was allowed to stir at room temperature overnight before quenching with 15 mL of 0.5 M HCl. The mixture was extracted with 40 mL of Et_2O three times and the combined organic phase was washed by 30 mL of brine and dried over anhydrous Na_2SO_4. The solvent was then removed by rotary evaporation. The resulting solid was recrystallized in ethyl acetate to yield 2-methyltricosan-2-ol.

Step 2: Into a flame-dried flask was placed 0.23 g of 60% sodium hydride (5.7 mmol, 4 equiv) in mineral oils under argon atmosphere. The solid was washed by 2.5 mL of dry pentane three times before adding 10 mL of anhydrous THF and 0.50 g of solid 2-methyltricosan-2-ol (1.4 mmol, 1 equiv). The resulting solution was refluxed for 4 h before adding 0.30 g of $^{13}CH_3I$ (2.1 mmol, 1.5 equiv). The resulting solution was then refluxed overnight before quenching with 5 mL of saturated ammonium chloride at 0 °C. The mixture was extracted with 30 mL of hexanes three times and the combined organic phase was washed by 20 mL of brine and dried over anhydrous Na_2SO_4. The solvent was removed by rotary evaporation. The residue was purified chromatographically on a flash column with 150 mL of hexanes. After the removal of solvent, 0.45 g of **IR3** (1.2 mmol, 86%) was obtained as white solid. ^{1}H NMR (Benzene-d_6, 400 MHz) δ 3.06 (d, 3H, *J* = 139 Hz), 1.46−1.39 (m, 4H), 1.39−1.23 (m, 36H), 1.09 (s, 6H), 0.92 (t, 3H, *J* = 6.5 Hz); ^{13}C NMR (Benzene-d_6, 100 MHz) δ 74.5, 74.5, 49.2, 40.9, 40.9, 32.7, 31.1, 30.6, 30.5, 30.2, 25.5, 25.5, 24.6, 23.5, 14.7.

Synthesis of (6E,10E,14E,18E)-23-(Methoxy-^{13}C)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18-pentaene IR4. **IV4** was synthesized from 2,2-dimethyl-3-((3E,7E,11E,15E)-3,7,12,16,20-pentamethylhenicosa-3,7,11,15,19-pentaen-1-yl)oxirane, which was made by Tsangarakis' method from commercially available squalene.[21]

Step 1: To a solution of 1.40 g of 2,2-dimethyl-3-((3E,7E,11E,15E)-3,7,12,16,20-pentamethylhenicosa-3,7,11,15,19-pentaen-1-yl)oxirane (3.28 mmol) dissolved in 20 mL of anhydrous THF in a flame-dried round-bottom under argon atmosphere was added slowly 0.25 g of lithium aluminum hydride (6.6 mmol) mixed with 4 mL of anhydrous THF at 0 °C. The reaction mixture was allowed to stir at room temperature overnight before quenching with 5 mL of 0.5 M HCl at 0 °C. The mixture was extracted with 30 mL of Et_2O three times and the combined organic phase was washed by 10 mL of brine and dried over anhydrous Na_2SO_4. The solvent was then removed by rotary evaporation. Purification by flash column chromatography (7:1 hexanes to EtOAc) yielded 1.24 g of (6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-6,10,14,18,22-pentaen-2-ol (2.89 mmol) as a light-yellow oil.

Step 2: Into a flame-dried flask was placed 0.19 g of 60% sodium hydride (4.8 mmol, 4 equiv) in mineral oil under argon atmosphere. The solid was washed by 2 mL of dry pentane three times before adding 10 mL of anhydrous THF and 0.50 g of (6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-6,10,14,18,22-pentaen-2-ol (1.2 mmol, 1 equiv). The resulting solution was refluxed for 1 h before adding 0.25 g of $^{13}CH_3I$ (1.7 mmol, 1.5 equiv). The resulting solution was then refluxed overnight before quenching with 5 mL of saturated ammonium chloride at 0 °C. The mixture was extracted with 30 mL of hexanes three times and the combined organic phase was washed by 20 mL of brine and dried over anhydrous Na_2SO_4. The solvent was then removed by rotary evaporation. The residue was purified chromatographically on a flash column with 250 mL of hexanes. After the removal of solvent, 0.45 g of **IR4** (1.0 mmol, 83%) was obtained as a light-yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.21−5.06 (m, 5H), 3.19 (d, 3H, *J* = 140 Hz), 2.14−2.06 (m, 5H), 2.06−1.89 (m, 12H), 1.71 (s, 3H), 1.65−1.58 (m, 15H), 1.46−1.40 (m, 5H), 1.16 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 135.1, 134.9, 134.9, 131.2, 124.4, 124.4, 124.3, 124.3, 74.6, 58.5, 57.6, 55.7, 49.0, 40.1, 39.8, 39.8, 39.7, 39.3, 39.3, 28.3, 26.8, 26.7, 25.7, 25.0, 22.2, 17.7, 16.0, 16.0, 15.8; MS *m*/*z* 443 [M]+, 413, 341, 273, 231, 203, 175, 143, 137, 107, 95, 81, 69, 55.

Synthesis of 2-Methylpropan-2-yl-1-^{13}C Piperidine-1-carboxylate 1. The synthesis of 2-methylpropan-2-yl-1-^{13}C piperidine-1-carboxylate **1** was divided into 3 steps.

Step 1: A solution of 0.250 g of 1-allylpiperidine (2.00 mmol, 1 equiv) was prepared by dissolution in 5 mL of dry DCM under an atmosphere of argon at 0 °C. To this solution was added dropwise 0.207 g of triphosgene (0.698 mmol, 0.35 equiv) dissolved in 2 mL of DCM. The solution was allowed to warm to room temperature and stirred for 4 h. Water was then added to the reaction mixture and the resulting solution was then extracted with 20 mL of DCM three times. The combined organic phase was washed with saturated sodium bicarbonate solution and brine and dried over $NaSO_4$. The solvent was removed by rotary evaporation and then by oil pump to yield the piperidine-1-carbonyl chloride. The resulting piperidine-1-carbonyl chloride was used in step 3 without further purification.

Step 2: To a flame-dried two-neck round-bottom flask fitted with a condenser under argon atmosphere was placed 0.12 g of magnesium (4.9 mmol, 2.5 equiv) and then 10 mL of anhydrous diethyl ether was added and stirring initiated. A solution of 0.642 g of $^{13}CH_3I$ (4.49 mmol, 2.25 equiv) dissolving in 5 mL of diethyl ether was added dropwise to the magnesium DEE mixture using a syringe pump at room temperature. After the addition, the gray mixture was allowed to stir for 30 min at room temperature before transferring to a flame-dried 50 mL round-bottom flask flushed with argon at 0 °C. A solution of 0.170 g of acetone (2.99 mmol, 1.5 equiv) in 5 mL of anhydrous DEE was slowly added to the gray methylmagnesium iodide solution using a syringe pump at 0 °C. After that, the reaction mixture was allowed to stir at room temperature for 4 h before quenching with 5 mL of 0.5 M HCl. The mixture was extracted with 20 mL of Et_2O three times and the combined organic phase was washed by 5 mL of brine and dried over anhydrous Na_2SO_4. The solvent was then removed by simple distillation at 45 °C until no more ether distilled out. The resulting 2-methylpropan-2-ol-1-^{13}C was used in next step without further purification.

dx.doi.org/10.1021/jo401740g | J. Org. Chem. 2013, 78, 11733−11746

Step 3: To a flame-dried flask was placed 0.32 g of 60% sodium hydride (8.0 mmol, 4 equiv) in mineral oil under an argon atmosphere. The solid was washed by 3 mL of dry pentane three times before adding 20 mL of anhydrous THF and the 2-methylpropan-2-ol-1-^{13}C obtained in step 2. The resulting mixture was refluxed for 1 h before adding the piperidine-1-carbonyl chloride all at once. The mixture was then refluxed overnight before cooling down to room temperature and quenched by 1 M ammonium chloride solution. The mixture was then extracted with 30 mL of ethyl acetate three times and the organic phase was washed with brine and dried over $NaSO_4$. The solvent was removed by rotary evaporation and then by oil pump yielding a light-yellow liquid of 2-methylpropan-2-yl-1-^{13}C piperidine-1-carboxylate **1** (0.32 g, 1.7 mmol, 86%), which was then characterized by NMR spectroscopy and GC-MS. Further purification by flash column with 200 mL of 10:1 hexanes to EtOAc yielded 0.27 g of colorless 2-methylpropan-2-yl-1-^{13}C piperidine-1-carboxylate **1**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.35 (t, 4H, J = 5.4 Hz), 1.45 (d, 2H, J = 126 Hz), 1.62−1.40 (m, ^{12}H; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.9, 79.3, 78.8, 44.6, 28.5, 25.7, 24.5; HRMS-EI m/z: [M]+ Calcd for $C_9{}^{13}CH_{19}NO_2$: 186.1444, found: 186.1442.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplemental NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Email: pgw@brown.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported through NSF grant 1058051.

■ REFERENCES

(1) (a) Lecachey, B.; Oulyadi, H.; Lameiras, P.; Harrison-Marchand, A.; Gerard, H.; Maddaluno, J. *J. Org. Chem.* **2010**, *75*, 5976−5983. (b) Consiglio, G. B.; Queval, P.; Harrison-Marchand, A.; Mordini, A.; Lohier, J.; Delacroix, O.; Gaumont, A.; Gerard, H.; Maddaluno, J.; Oulyadi, H. *J. Am. Chem. Soc.* **2011**, *133*, 6472−6480. (c) Armstrong, D. R.; Garcia-Alvarez, P.; Kennedy, A. R.; Mulvey, R. E.; Robertson, S. D. *Chem.—Eur. J.* **2011**, *17*, 6725−6730. (d) Li, W.; Chung, H.; Daeffler, C.; Johnson, J. A.; Grubbs, R. H. *Macromolecules* **2012**, *45*, 9595−9603.

(2) (a) Li, D.; Hopson, R.; Li, W.; Liu, J.; Williard, P. G. *Org. Lett.* **2008**, *10*, 909−911. (b) Li, D.; Kagan, G.; Hopson, R.; Williard, P. G. *J. Am. Chem. Soc.* **2009**, *131*, 5627−5634. (c) Li, D.; Keresztes, I.; Hopson, R.; Williard, P. G. *Acc. Chem. Res.* **2009**, *42*, 270−280. (d) Kagan, G.; Li, W.; Hopson, R.; Williard, P. G. *Org. Lett.* **2009**, *11*, 4818−4821. (e) Kagan, G.; Li, W.; Hopson, R.; Williard, P. G. *Org. Lett.* **2010**, *12*, 520−523. (f) Socha, A. M.; Kagan, G.; Li, W.; Hopson, R.; Sello, J. K.; Williard, P. G. *Energy Fuels* **2010**, *24*, 4518−4521. (g) Kagan, G.; Li, W.; Sun, C.; Hopson, R.; Williard, P. G. *J. Org. Chem.* **2011**, *76*, 65−70. (h) Kagan, G.; Li, W.; Li, D.; Hopson, R.; Williard, P. G. *J. Am. Chem. Soc.* **2011**, *133*, 6596−6602. (i) Su, C.; Hopson, R.; Williard, P. G. *J. Org. Chem.* **2013**, *78*, 7288−7292. (j) Su, C.; Hopson, R.; Williard, P. G. *Eur. J. Inorg. Chem.* **2013**, *2013*, 4136−4141. (k) Su, C.; Hopson, R.; Williard, P. G. *J. Am. Chem. Soc.* **2013**, *135*, 12400−12406. (l) Su, C.; Hopson, R.; Williard, P. G. *J. Am. Chem. Soc.* **2013**, *135*, 14367−14379.

(3) (a) Li, W.; Kagan, G.; Yang, H.; Cai, C.; Hopson, R.; Sweigart, D. A.; Williard, P. G. *Org. Lett.* **2010**, *12*, 2698−2701. (b) Li, W.; Kagan, G.; Yang, H.; Cai, C.; Hopson, R.; Dai, W.; Sweigart, D. A.; Williard, P. G. *Organometallics* **2010**, *29*, 1309−1311.

(4) Li, D.; Sun, C.; Liu, J.; Hopson, R.; Li, W.; Williard, P. G. *J. Org. Chem.* **2008**, *73*, 2373−2381.

(5) (a) Collum, D. B.; McNeil, A. J.; Ramirez, A. *Angew. Chem., Int. Ed.* **2007**, *46*, 3002−3017. (b) Wu, G.; Huang, M. *Chem. Rev.* **2006**, *106*, 2596−2616. (c) Rappoport, Z., Marek, I., Eds. *The Chemistry of Organolithium Compounds*; John Wiley & Sons, Ltd.: West Sussex, 2004. (d) Hodgson, D. *Organolithiums in Enantionselective Synthesis*; Springer: New York, 2003. (e) Clayden, J. *Organolithiums: Selectivity for Synthesis*; Pergamon: Oxford, 2002. (f) Lucht, B.; Collum, D. *Acc. Chem. Res.* **1999**, *32*, 1035−1042. (g) Trost, B. M., Fleming, I., Eds. *Comprehensive Organic Synthesis*; Pergamon: Oxford, 1991. (h) Snieckus, V. *Chem. Rev.* **1990**, *90*, 879−933.

(6) (a) Ince, H. H.; Dedeoglu, B.; Gul, S.; Aviyente, V.; Coldham, I. *Mol. Phys.* **2012**, *110* (6), 353−359. (b) Khartabil, H. K.; Gros, P. C.; Fort, Y.; Ruiz-Lopez, M. F. *J. Am. Chem. Soc.* **2010**, *132* (7), 2410−2416. (c) Riggs, J. C.; Singh, K. J.; Yun, M.; Collum, D. B. *J. Am. Chem. Soc.* **2008**, *130* (41), 13709−13717. (d) Jones, A. C.; Sanders, A. W.; Bevan, M. J.; Reich, H. J. *J. Am. Chem. Soc.* **2007**, *129*, 3492−3493. (e) Yakimansky, A.; Wang, G.; Janssens, K.; Van Beylen, M. *Polymer* **2003**, *44* (21), 6457−6463. (f) Rutherford, J. L.; Hoffmann, D.; Collum, D. B. *J. Am. Chem. Soc.* **2002**, *124*, 264−271. (g) Reich, H. J.; Green, D. P.; Medina, M. A.; Goldenberg, W. S.; Gudmundsson, B. O.; Dykstra, R. R.; Phillips, N. H. *J. Am. Chem. Soc.* **1998**, *120*, 7201−7210. (h) Lucht, B. L.; Bernstein, M. P.; Remenar, J. F.; Collum, D. B. *J. Am. Chem. Soc.* **1996**, *118*, 10707−10718. (i) Ogle, C. A.; Johnson, H. C., IV; Wang, X. L.; Strickler, F. H.; Bucca, D.; Gordon, B., III *Macromolecules* **1995**, *28*, 5184−5191. (j) Bates, T. F.; Clarke, M. T.; Thomas, R. D. *J. Am. Chem. Soc.* **1988**, *110*, 5109−5112. (k) McGarrity, J. F.; Ogle, C. A.; Brich, Z.; Loosli, H. *J. Am. Chem. Soc.* **1985**, *107*, 1810−1815. (l) West, P.; Waack, R.; Purmort, J. I. *J. Am. Chem. Soc.* **1970**, *92*, 840−845.

(7) (a) Khartabil, H. K.; Gros, P. C.; Fort, Y.; Ruiz-Lopez, M. F. *J. Am. Chem. Soc.* **2010**, *132* (7), 2410−2416. (b) Khartabil, H. K.; Gros, P. C.; Fort, Y.; Ruiz-Lopez, M. F. *J. Org. Chem.* **2008**, *73* (23), 9393−9402. (c) Walfort, B.; Lameyer, L.; Weiss, W.; Herbst-Irmer, R.; Bertermann, R.; Rocha, J.; Stalke, D. *Chem.−Eur. J.* **2001**, *7* (7), 1417−1423.

(8) (a) Fox, T.; Hausmann, H.; Gunther, H. *Magn. Reson. Chem.* **2004**, *42*, 788−794. (b) Ogle, C. A.; Huckabee, B. K.; Johnson, H. C., IV; Sims, P. F.; Winslow, S. D. *Organometallics* **1993**, *12*, 1960−1963. (c) Novak, D. P.; Brown, T. L. *J. Am. Chem. Soc.* **1972**, *94*, 3793−3798.

(9) Koster, H.; Thoennes, D.; Weiss, E. *J. Organomet. Chem.* **1978**, *160*, 1−5.

(10) Kamps, I.; Neumann, B.; Stammler, H.-G.; Mitzel, N. W. *Organometallics* **2010**, *29*, 4746−4748.

(11) (a) Strohmann, C.; Strohfeldt, K.; Schildbach, D.; McGrath, M. J.; O'Brien, P. *Organometallics* **2004**, *23*, 5389−5391. (b) Strohmann, C.; Gessner, V. H. *J. Am. Chem. Soc.* **2007**, *129*, 8952−8953.

(12) (a) Khartabil, H. K.; Gros, P. C.; Fort, Y.; Ruiz-Lopez, M. F. *J. Org. Chem.* **2008**, *73* (23), 9393−9402. (b) Ohta, Y.; Demura, A.; Okamoto, T.; Hitomi, H.; Nagaoka, M. *J. Phys. Chem. B* **2006**, *110* (25), 12640−12644. (c) Gerard, H.; De la Lande, A.; Maddaluno, J.; Parisel, O.; Tuckerman, M. E. *J. Phys. Chem. A* **2006**, *110* (14), 4787−4794. (d) Fressigne, C.; Lautrette, A.; Maddaluno, J. *J. Org. Chem.* **2005**, *70* (20), 7816−7828. (e) Jemmis, E. D.; Gopakumar, G. *Chem. Organolithium Compd.* **2004**, *1*, 1−45. (f) Verstraete, P.; Deffieux, A.; Fritsch, A.; Rayez, J. C.; Rayez, M. T. *J. Mol. Struct. THEOCHEM* **2003**, *631*, 53−66. (g) Ponec, R.; Roithova, J.; Girones, X.; Lain, L.; Torre, A.; Bochicchio, R. *J. Phys. Chem. A* **2002**, *106* (6), 1019−1025. (h) Kwon, O.; Sevin, F.; McKee, M. L. *J. Phys. Chem. A* **2001**, *105* (5), 913−922. (i) Frischknecht, A. L.; Milner, S. T. *J. Chem. Phys.* **2001**, *114* (2), 1032−1050. (j) Grotjahn, D. B.; Pesch, T. C.; Brewster, M. A.; Ziurys, L. M. *J. Am. Chem. Soc.* **2000**, *122* (19), 4735−4741. (k) Kirschenbaum, L. J.; Howell, J. M. *Struct. Chem.* **1998**, *9* (5), 327−337. (l) Kirschenbaum, L. J.; Howell, J. M.; Singman, E. L. *Struct. Chem.* **1996**, *7* (1), 79−83.

(13) (a) Pate, F.; Oulyadi, H.; Harrison-Marchand, A.; Maddaluno, J. *Organometallics* **2008**, *27* (14), 3564−3569. (b) Knorr, R.; Menke, T.; Ferchland, K.; Mehlstaeubl, J.; Stephenson, D. S. *J. Am. Chem. Soc.* **2008**, *130* (43), 14179−14188. (c) Gohaud, N.; Begue, D.; Pouchan,

C. *Chem. Phys.* **2005**, *310* (1−3), 85−96. (d) McKeever, L. D.; Waack, R.; Doran, M. A.; Baker, E. B. *J. Am. Chem. Soc.* **1968**, *90*, 3244.
(14) (a) Remenar, J. F.; Lucht, B. L.; Collum, D. B. *J. Am. Chem. Soc.* **1997**, *119*, 5567−5572. (b) Lucht, B. L.; Collum, D. B. *J. Am. Chem. Soc.* **1996**, *118*, 2217−2225.
(15) (a) Morris, K. F.; Johnson, C. S., Jr. *J. Am. Chem. Soc.* **1992**, *114* (2), 776−777. (b) Morris, K. F.; Johnson, C. S., Jr. *J. Am. Chem. Soc.* **1992**, *114* (8), 3139−3141.
(16) Keresztes, I.; Williard, P. G. *J. Am. Chem. Soc.* **2000**, *122*, 10228−10229.
(17) This particular urethane was selected for analysis because it is a precursor and analog of synthetically useful carbanions; *c.f.*:. Beng, T. K.; Tyree, W. S.; Parker, T.; Su, C.; Williard, P. G.; Gawley, R. E. *J. Am. Chem. Soc.* **2012**, *134* (40), 16845−16855.
(18) It is curious that the use of optically pure TMCDA in this experiment leads to extensive precipitation precluding the possibility of performing the NMR analyses.
(19) Wagner, P.; Nakagawa, Y.; Lee, G. S.; Davis, M. E.; Elomari, S.; Medrud, R. C.; Zones, S. I. *J. Am. Chem. Soc.* **2000**, *122*, 263−273.
(20) Ashby, E. C.; Noding, S. A. *J. Org. Chem.* **1980**, *45*, 1035−1041.
(21) Tsangarakis, C.; Raptis, C.; Arkoudis, E.; Stratakis, M. *Adv. Synth. Catal.* **2008**, *350*, 1587−1600.