^{13}C INEPT Diffusion-Ordered NMR Spectroscopy (DOSY) with Internal References
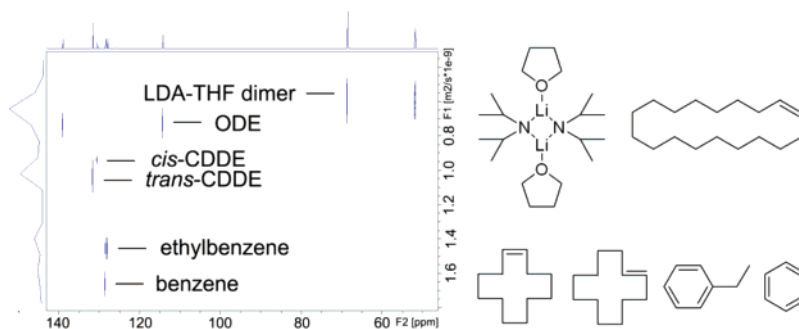
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ABSTRACT

^{13}**C INEPT Diffusion-ordered NMR spectroscopy (DOSY) with an internal reference system was developed to study the aggregation state of THF-solvated LDA dimeric complex. Six components are clearly identified in the diffusion dimension, and their DOSY-generated ^{13}C INEPT spectrum slices agree extremely well with their respective INEPT spectra. The correlation between log *D* and log FW of the linear least-squares fit to reference points of all components is exceptionally high: (*r* = 0.9985).**

Pulsed gradient spin–echo (PGSE) diffusion NMR spectroscopy was designed to measure diffusion coefficients and deduce the hydrodynamic radii of molecules in solution.[1] By incorporation of this technique in a two-dimensional experiment—now referred to as DOSY[2]—one can, in principle, determine formula weights of different components in solution.[3] Reports of ^{1}H DOSY experiments continue to increase, however very few ^{13}C INEPT DOSY spectra have been reported[4] although they provide better resolution, a wider chemical shift range than proton spectra and absence of homonuclear coupling.

DOSY spectra often include artifacts generated by temperature fluctuation, convection, and viscosity change.[5] Hence, we sought to develop an internal reference method[6] to avoid these effects while taking advantage of the benefits of ^{13}C INEPT DOSY spectra. We report that by utilizing known molecules as internal references, we can correlate relative diffusion coefficients with formula weights of aggregates quantitatively. Consequently, we determine aggregation numbers and solvation states accordingly. This

(1) (a) Cohen, Y.; Avram, L.; Frish, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 520–554. (b) Pregosin, P. S.; Kumar, P. G. A.; Fernandez, I. *Chem. Rev.* **2005**, *105*, 2977–2998. (c) Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, *42*, 288–292. (d) Tanner, J. E. *Rev. Sci. Instrum.* **1965**, *36*, 1086–1087. (e) Stejskal, E. O. *J. Chem. Phys.* **1965**, *43*, 3597–3603.

(2) (a) Nilsson, M.; Morris, G. A. *J. Chem. Soc., Chem. Commun.* **2007**, 933–935, (b) Johnson, C. S. *Prog. Nucl. Magn. Reson. Spectrosc.* **1999**, *34*, 203–256. (c) Morris, K. F.; Johnson, C. S. *J. Am. Chem. Soc.* **1992**, *114*, 3139–3141.

(3) (a) Jacobson, M. A.; Keresztes, I.; Williard, P. G. *J. Am. Chem. Soc.* **2005**, *127*, 4965–4975. (b) Viel, S.; Capitani, D.; Mannina, L.; Serge, A. *Biomacromolecules* **2003**, *4*, 1843–1847. (c) Keresztes, I. Ph.D. Dissertation, Brown University, 2002. (d) Chen, A.; Johnson, C. S. *J. Am. Chem. Soc.* **1995**, *117*, 7965–7970.

(4) (a) Schlorer, N. E.; Cabrita, E. J.; Berger, S. *Angew. Chem., Int. Ed.* **2002**, *41*, 107–109. (b) Kapur, G. S.; Findeisen, M.; Berger, S. *Fuel* **2000**, *79*, 1347–1351. (c) Wu, D. H.; Chen, A. D.; Johnson, C. S. *J. Magn. Reson., Ser. A* **1996**, *123*, 215–218.

(5) Sorland, G. H.; Aksnes, D. *Magn. Reson. Chem.* **2002**, *40*, S139–S146. (b) Jerschow, A.; Mueller, N. *J. Magn. Res.* **1997**, *125*, 372.

(6) (a) Groves, P.; Palczewska, M.; Molero, M. D.; Batta, G.; Canada, F. J.; Jiminez-Barbero, J. *Anal. Biochem.* **2004**, *331*, 395–397. (b) Cabrita, E. J.; Berger, S. *Magm. Reson. Chem.* **2001**, *39*, S142–S148.

methodology extends and simplifies the current NMR techniques such as analysis of quadrapole coupling,[7] multiplicity and bimolecular exchange,[8] HMPA titration[9] and Job plots[10] that have been employed for determining aggregation states of reactive intermediates in solution.

To establish the effectiveness of the internal reference ^{13}C INEPT DOSY technique, we report initial studies utilizing commercially available THF-solvated lithium diisopropylamide (LDA) dimer because LDA is the most prominent non-nucleophilic base used in organic synthesis and also an ideal candidate for investigating organolithium aggregation states.[11] It exists as a single form, THF disolvated dimer, in THF solution as depicted in Figure 1.[12] The solid structure of this aggregate has also been determined by X-ray crystallography so its size and shape are well established.[13] In this study we choose 1-octadecene (ODE), cyclododecene (CDDE),[14] and benzene as the internal references due to their chemical and NMR properties, such as solubility in different solvents, low

Figure 1. ^{13}C INEPT DOSY of THF-solvated LDA dimeric aggregate in toluene-d_8 with internal references at 25 °C. The *X*-axis is the regular ^{13}C INEPT dimension and the *Y*-axis is the diffusion dimension.

reactivity or coordination with other species, chemical shift dispersion, and desirable molecular weight distribution.

The ^{13}C INEPT DOSY spectrum of THF-solvated LDA dimer with the three internal references indicated above in toluene-d_8 solution separates into six components in the diffusion dimension. These are clearly identifiable in the DOSY spectrum reproduced in Figure 1. In increasing order of diffusion coefficient (decreasing radii) these are the LDA−THF dimer ($C_{20}H_{44}Li_2N_2O_2$, MW 351.5), ODE ($C_{18}H_{36}$, MW 252.3), *cis*-CDDE ($C_{12}H_{22}$, MW 166.3), *trans*-CDDE ($C_{12}H_{22}$, MW 166.3), ethylbenzene (C_8H_{10}, MW 106.2),[15] and benzene (C_6H_6, MW 78.1). It is noteworthy that diffusion dimension separation (Table S1) of these components was achieved, especially for *cis*- and *trans*-CDDE as they have exactly the same formula weight and are observed to exhibit a 3.17% difference in relative diffusion coefficient. The ^{13}C INEPT signals of the oxygen-attached carbons in THF ($\delta = 68.5$ ppm) and the LDA methane carbons ($\delta = 51.8$ ppm) have identical diffusion coefficients. This evidence corroborates that THF and LDA moieties are in the same aggregate and remain attached under the experimental conditions. The ^{1}H NMR integration also shows that THF and LDA have a 1:1 ratio in the aggregate (Figure S16).

An added bonus of 2D-INEPT ^{13}C DOSY spectra is to extract a one-dimensional (1D) ^{13}C INEPT spectrum slice at the diffusion coefficient of a particular species. Hence, one author has previously referred to the DOSY technique as "chromatography by NMR".[16] To emphasize this point, we have depicted peaks of LDA ($\delta = 68.5$ ppm) and THF ($\delta = 51.8$ ppm) in the slice of LDA−THF aggregate (Figure 2b) to be compared with the spectrum of the pure, authentic sample (Figure 2b′). Slices taken at other diffusion coefficients of the components agree extremely well with their

(7) (a) Jackman, L. M.; Cizmeciyan, D.; Williard, P. G.; Nichols, M. A. *J. Am. Chem. Soc.* **1993**, *115*, 6262−6267. (b) Jackman, L. M.; Chen, X. *J. Am. Chem. Soc.* **1992**, *114*, 403−411. (c) Jackman, L. M.; Bortiatynski, J. *Adv. Carbanion Chem.* **1992**, *1*, 45−87. (d) Jackman, L. M.; Scarmoutzos, L. M.; Smith, B. D.; Williard, P. G. *J. Am. Chem. Soc.* **1988**, *110*, 6058−6063. (e) Jackman, L. M.; Scarmoutzos, L. M.; DeBrosse, C. W. *J. Am. Chem. Soc.* **1987**, *109*, 5355−5361. (f) Jackman, L. M.; Szeverenyi, N. M. *J. Am. Chem. Soc.* **1977**, *99*, 4954−4962.

(8) (a) Fraenkel, G.; Qiu, F. *J. Am. Chem. Soc.* **2000**, *122*, 12806−12812. (b) Kim, Y.-J.; Streitwieser, A.; Chow, A.; Fraenkel, G. *Org. Lett.* **1999**, *1*, 2069−2071. (c) Fraenkel, G.; Duncan, J. H.; Martin, K.; Wang, J. *J. Am. Chem. Soc.* **1999**, *121*, 10538−10544. (d) Fraenkel, G.; Subramanian, S.; Chow, A. *J. Am. Chem. Soc.* **1995**, *117*, 6300−6307. (e) Fraenkel, G.; Martin, K. *J. Am. Chem. Soc.* **1995**, *117*, 10336−10344. (f) Fraenkel, G.; Chow, A.; Winchester, W. R. *J. Am. Chem. Soc.* **1990**, *112*, 6190−6198. (g) Fraenkel, G.; Henrichs, M.; Hewitt, M.; Su, B. M. *J. Am. Chem. Soc.* **1984**, *106*, 255−256. (h) Fraenkel, G.; Pramanik, P. *J. Chem. Soc., Chem. Commun.* **1983**, 1527−1529. (i) Fraenkel, G.; Henrichs, M.; Hewitt, J. M.; Su, B. M.; Geckle, M. J. *J. Am. Chem. Soc.* **1980**, *102*, 3345−50. (j) Fraenkel, G.; Fraenkel, A. M.; Geckle, M. J.; Schloss, F. *J. Am. Chem. Soc.* **1979**, *101*, 4745−4747.

(9) (a) Reich, H. J.; Goldenberg, W. S.; Sanders, A. W.; Jantzi, K. L.; Tzschucke, C. C. *J. Am. Chem. Soc.* **2003**, *125*, 3509−3521. (b) Reich, H. J.; Goldenberg, W. S.; Gudmundsson, B. O.; Sanders, A. W.; Kulicke, K. J.; Simon, K.; Guzei, I. A. *J. Am. Chem. Soc.* **2001**, *123*, 8067−8079. (c) 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. (d) Reich, H. J.; Gudmundsson, B. O. *J. Am. Chem. Soc.* **1996**, *118*, 6074−6075. (e) Reich, H. J.; Borst, J. P.; Dykstra, R. R.; Green, P. D. *J. Am. Chem. Soc.* **1993**, *115*, 8728−8741. (f) Reich, H. J.; Borst, J. P. *J. Am. Chem. Soc.* **1991**, *113*, 1835−1837. (g) Reich, H. J.; Green, D. P. *J. Am. Chem. Soc.* **1989**, *111*, 8729−8731.
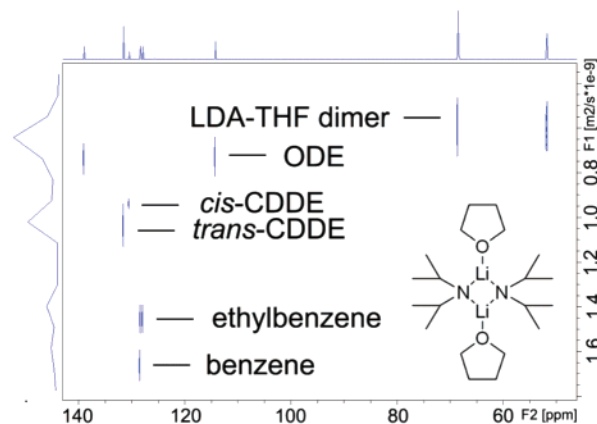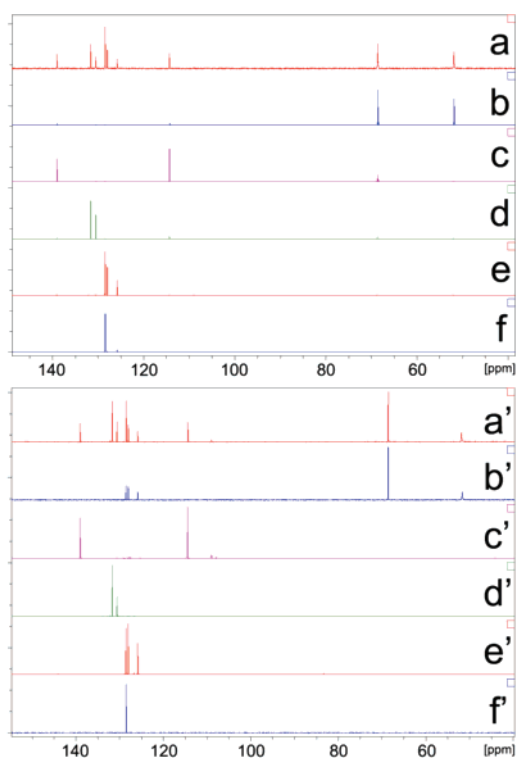
(10) (a) McNeil, A. J.; Collum, D. B. *J. Am. Chem. Soc.* **2005**, *127*, 5655−5661. (b) McNeil, A. J.; Toombes, G. E. S.; Gruner, S. M.; Lobkovsky, E.; Collum, D. B.; Chandramouli, S. V.; Vanasse, B. J.; Ayers, T. A. *J. Am. Chem. Soc.* **2004**, *126*, 16559−16568. (c) McNeil, A. J.; Toombes, G. E. S.; Chandramouli, S. V.; Vanasse, B. J.; Ayers, T. A.; O'Brien, M. K.; Lobkovsky, E.; Gruner, S. M.; Marohn, J. A.; Collum, D. B. *J. Am. Chem. Soc.* **2004**, *126*, 5938−5939.

(11) (a) Ma, Y.; Ramirez, A.; Singh, K. J.; Keresztes, I.; Collum, D. B. *J. Am. Chem. Soc.* **2006**, *128*, 15399−15404. (b) Singh, K. J.; Collum, D. B. *J. Am. Chem. Soc.* **2006**, *128*, 13753−13760. (c) Ramirez, A.; Sun, X. F.; Collum, D. B. *J. Am. Chem. Soc.* **2006**, *128*, 10326−10336.

(12) Collum, D. B.; McNeil, A. J.; Ramirez, A. *Angew. Chem., Int. Ed.* **2007**, *46*, 3002−3017.

(13) Williard, P. G.; Salvino, J. M. *J. Org. Chem.* **1993**, *58*, 1−3.

(14) Cyclododecene (CDDE) was purchased from Aldrich Chemical Company as a trans:cis (60:40) mixture. Olefin stereochemistry was confirmed by HSQC and ^{1}H NMR homodecoupling experiments utilizing ^{1}H CW off-resonance spectra. See: (a) Radeglia, R. *J. Prakt. Chem./Chem.-Ztg.* **1993**, *335*, 291−293. (b) Radeglia, R.; Poleschner, H.; Haufe, G. *Magn. Reson. Chem.* **1993**, *31*, 1054−1056. (c) Radeglia, R.; Poleschner, H.; Haufe, G. *Magn. Reson. Chem.* **1993**, *31*, 639−641. (d) Radeglia, R.; Poleschner, H.; Theil, F. *J. Prakt. Chem./Chem.-Ztg.* **1993**, *335*, 673−679. (e) Supporting Information Figures S19 and S20.

(15) Ethylbenzene is present in the LDA−THF solution obtained from Aldrich Chemical Company. Supporting Information Figures S2 and S3.

(16) Gounarides, J. S.; Chen, A.; Shapiro, M. J. *J. Chromatogr., B* **1999**, *725*, 79−90.

Figure 2. Comparison between slices of ^{13}C INEPT DOSY spectra (a−f) with ^{13}C INEPT NMR spectra of authentic samples (a′−f′). (a) and (a′)−LDA dimer with internal references, (b) and (b′)−LDA dimer without internal references, (c) and (c′)−ODE, (d) and (d′)−*cis*- and *trans*-CDDE mixture, (e) and (e′)−ethylbenzene, (f) and (f′)−benzene.

respective ^{13}C INEPT spectra. Hence, the extracted INEPT spectra determined from the single 2D INEPT DOSY experiment resolved chemical shift information of every component in the mixture. We note that the 1D ^{13}C INEPT spectrum of commercially available LDA−THF solution (Figure 2b′) includes signals of ethylbenzene (δ = 125.8, 127.9, and 128.4 ppm); however, these signals do not appear in the DOSY slice (Figure 2b). The DOSY slices also illustrate the complete chemical shift resolution of nearly identical signals of benzene (δ = 128.38 ppm) and ethylbenzene (δ = 128.41 ppm) (Figure S21). We also call attention to the fact that, although the difference of relative diffusion coefficients between *cis*- and *trans*-CDDE is observed to be only 3.17%, spectra of the pure *trans*- and nearly pure *cis*-olefin isomers are resolvable (Figure S22).

Previously we have proposed a relationship between diffusion coefficient and formula weight utilizing ^{1}H DOSY experiments and we have utilized this correlation to study vinylic lithiation of allylamine derivatives.[3a,c] Analogously, the ^{13}C INEPT DOSY results strongly suggest that the

diffusion coefficients and formula weights (Table S1) of LDA−THF dimer, ethylbenzene, and the three internal references, ODE, CDDE, and benzene can also be utilized to define a linear correlation between the relative log D (diffusion coefficient) and log FW (formula weight). The correlation between log FW and log D of the linear least-squares fit to reference points of all components in this mixture is extremely high, r = 0.9985 (Figure 3). This
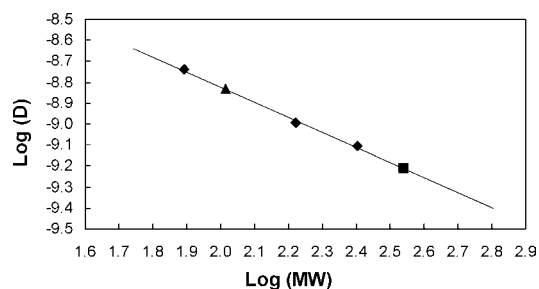
Figure 3. ^{13}C INEPT DOSY of the five major species in toluene-d_8. Diamonds represent reference compounds. Solid square represents THF-solvated LDA dimer. Triangle represents ethylbenzene. Solid line is a linear least-squares fit to the reference points.

remarkable result highlights the ability to use suitable internal references in ^{13}C INEPT DOSY experiments to interpolate relative diffusion coefficients and formula weights and, by inference, solvation and aggregation states of the bis-THF-solvated LDA dimer.

The following issues, noted by a perceptive reviewer, are worthy of consideration: applicability of the Stokes−Einstein equation to nonspherical molecules, secondary solvent shell solvation, ligand exchange, and temperature/viscosity dependence. We have also been concerened about these issues, and we note that these factors do not interefere with the interpretation of results reported in this manuscript. We are applying this methodology to study the solvation and aggregation of additional organometallic intermediates, and we are also probing the scope and limitations of this internal reference DOSY methodology vis-à-vis the issues noted above.

Supporting Information Available: The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, and ^{13}C INEPT DOSY spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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