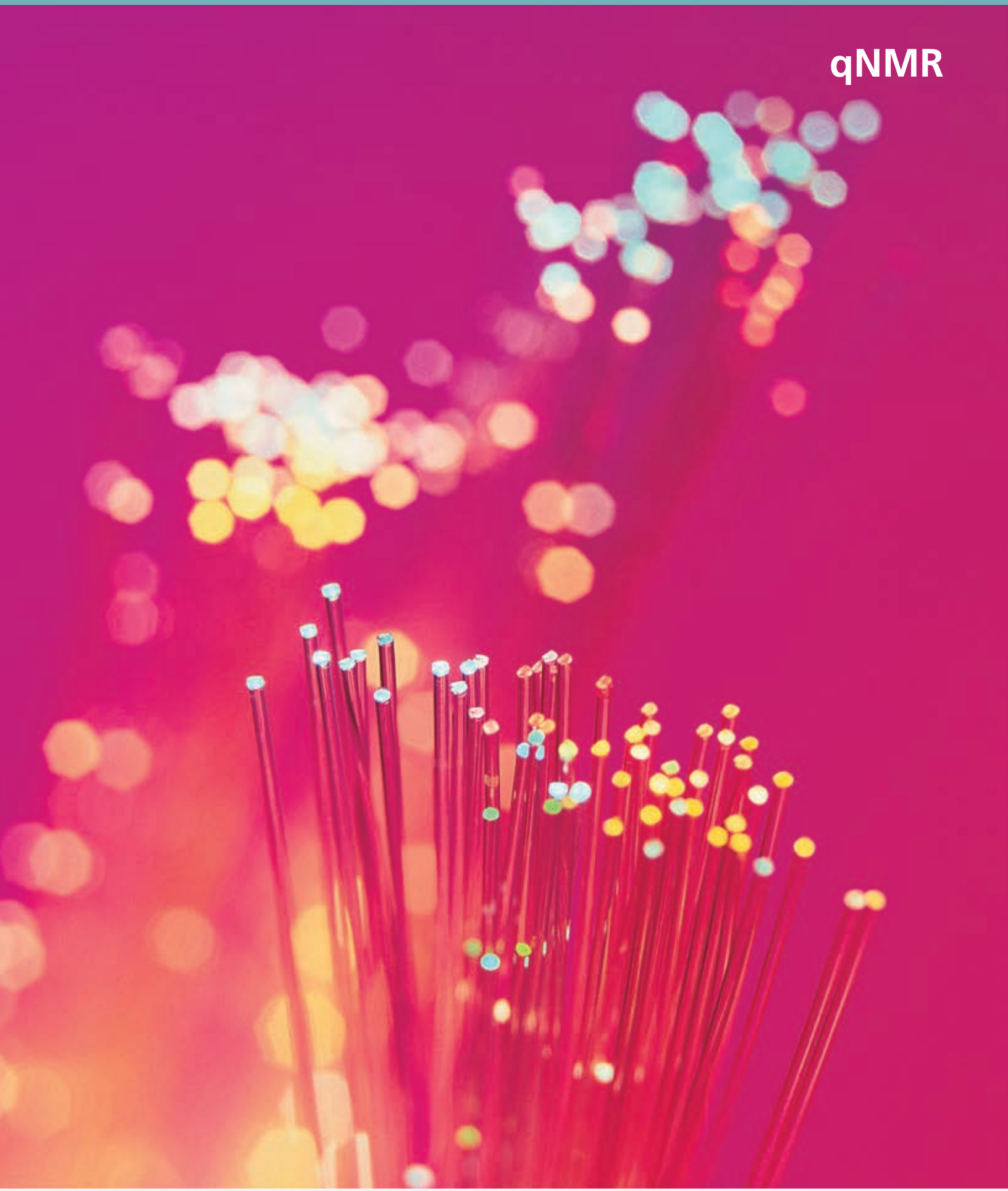
qNMR



Standards for qNMR

Quantitative ^{1}H-NMR (qNMR) continues to be utilized with much success in the pharmaceutical, chemical and food industries and in many facets of academic research. Regardless of the application, all qNMR methods require a calibration signal whose integrated signal intensity originates or is traceable to a known number of protons. Calibration for qNMR is made using either internal or external referencing methods. External methods rely on the use of a standard solution packaged in a defined NMR tube or capillary to obtain an integral that can be used for sample quantification, whereas internal methods rely on the use of a known amount of standard that is co-dissolved in the sample itself.

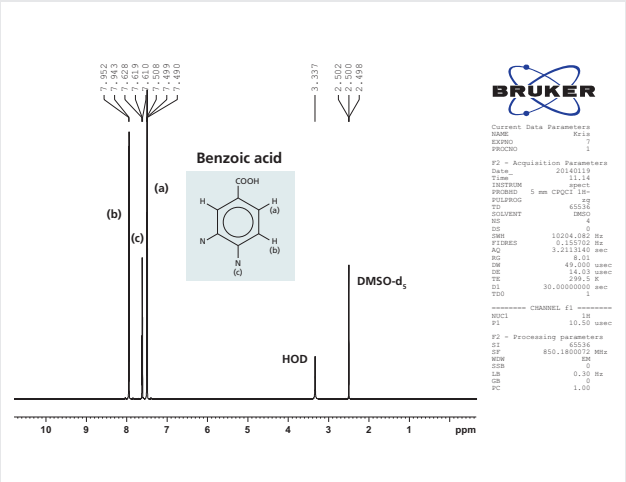


Figure 1. 850 MHz ^{1}H-NMR spectrum of benzoic acid in DMSO-d_6. Resonances from the aromatic protons of benzoic acid, HOD and DMSO-d_5 are assigned. The acid proton resonance from benzoic acid (~12-13 ppm) is not shown. (Courtesy Joe Ray, Baxter Healthcare Corporation, Round Lake, IL)

External Calibration Standards

CIL is pleased to offer external calibration standards for qNMR. The standards are formulated using CIL's high-quality DMSO-d_6 and benzoic acid from NIST (SRM 350(b)), a standard reference material for acidometry. Both 5 mM and 15 mM benzoic acid concentrations are available. The concentration and associated expanded uncertainty of the benzoic acid has been accurately determined using metrological techniques and verified using qNMR. The ^{1}H-NMR spectrum of benzoic acid in DMSO-d_6 is presented in Figure 1.

CIL is currently offering these standards in presealed NMR tubes. Please see the information below for details regarding NMR tubes and fill volumes. Other NMR tubes and concentrations may be available upon request.

qNMR Standard for External Referencing

Catalog No.	Description*	NMR Tube**	Part No.	Fill Volume
DLM-9491A	5 mM Benzoic acid in DMSO-d_6	1.7 mm O.D.	Bruker Part No. Z106462	50 µL
DLM-9491B	5 mM Benzoic acid in DMSO-d_6	3 mm O.D.	Wilmad Part No. 335-PP-9	160 µL
DLM-9491C	5 mM Benzoic acid in DMSO-d_6	5 mm O.D.	Wilmad Part No. 528-PP-8	750 µL
DLM-7061A	15 mM Benzoic acid in DMSO-d_6	1.7 mm O.D.	Bruker Part No. Z106462	50 µL
DLM-7061B	15 mM Benzoic acid in DMSO-d_6	3 mm O.D.	Wilmad Part No. 335-PP-9	160 µL
DLM-7061C	15 mM Benzoic acid in DMSO-d_6	5 mm O.D.	Wilmad Part No. 528-PP-8	750 µL

* The benzoic acid concentration and associated uncertainty are reported.

** All tubes are flame-sealed to ensure longevity.

Other NMR fill volumes and tubes are available. Please inquire.

Standards for qNMR *(continued)*

Internal Calibration Standards

The internal reference method commonly gives errors of <1% and is considered to be the most accurate and reproducible method available to obtain quantitative ^{1}H-NMR spectra. Unfortunately, the reference standard is typically weighed into each sample solution, an action that requires time and effort, and has been reported to the largest source of error with this method.

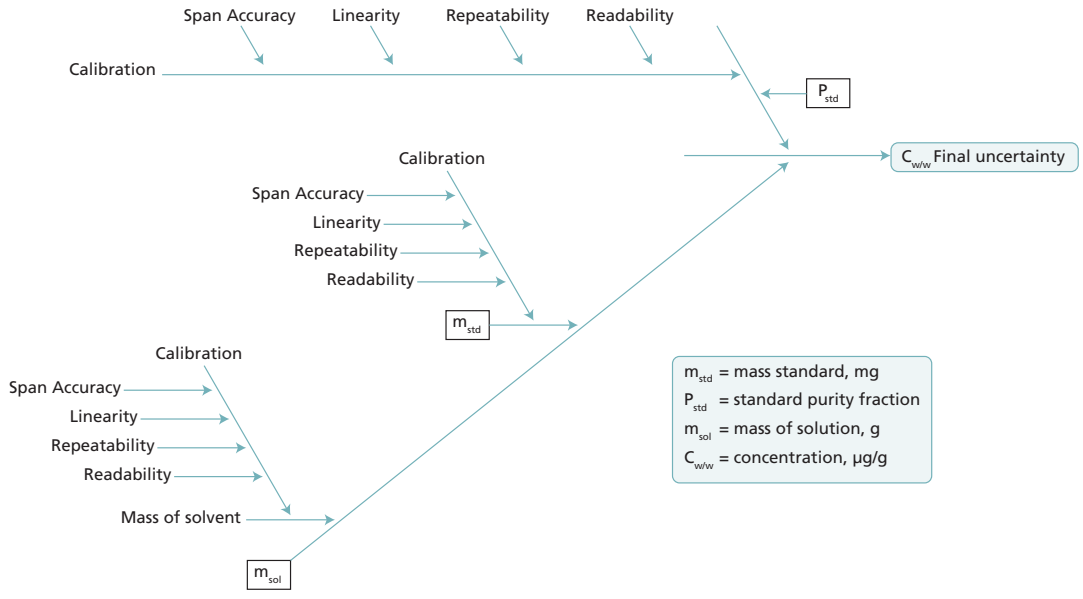
CIL is pleased to offer a ready-to-use DMSO-d_6 solution containing a known amount of benzoic acid for internal referencing. Because this solution is preformulated, the user does not need to weigh a standard material. The elimination of this step will reduce effort and time in sample preparation and also may bring about more accurate results than if the user performs this formulation. To use this product, the sample must be soluble in DMSO-d_6, physically and chemically inert toward benzoic acid and stable in acidic pH. Ideally, there will be no resonances from the sample in the region of benzoic acid aromatic protons (7.4-8.1 ppm), HOD (~3 ppm but is variable) and DMSO-d_5 (2.5 ppm). The benzoic acid concentration with associated uncertainty is presented on the certificate of analysis.

qNMR Standard for Internal Referencing

Catalog No.	Description	Ampoule	Comments
DLM-9491D	5 mM Benzoic acid in DMSO-d_6	1 g	The benzoic acid concentration and associated uncertainty is reported.
DLM-7061D	15 mM Benzoic acid in DMSO-d6	1 g	The benzoic acid concentration and associated uncertainty is reported.

CIL Formulation Procedure

The procedure that CIL uses to formulate qNMR external calibration reference standard bulk solutions allows for the expanded uncertainty of the concentration of the calibration standard (e.g., benzoic acid) to be determined. Traceability to SI is maintained through the use of weight sets with calibration traceable to NIST and laboratory balances with NIST-traceable calibration certificates, maintaining an unbroken chain of calibration to the kilogram. The factors contributing to the uncertainty of the benzoic acid concentration[1] is shown in below.



Cause-and-effect diagram of factors contributing to the uncertainty of the benzoic acid concentration in the qNMR standard formulation.

Reference

1. EURACHEM CITAC Guide CG 4, "Quantifying Uncertainty in Analytical Measurement," Third Edition, QUAM:2012

Synthesis

Stable Isotope-Labeled Synthetic Intermediates

CIL offers over 15,000 stable isotope-labeled products for your synthetic applications. We offer many labeling patterns for common starting materials. For more than 30 years CIL has offered:

- Expertise and quality service from your initial quote request through delivery of your order
- Flexibility of scale for custom and catalog products from milligram to multi-kilogram quantities
- A large selection of labeled compounds, including deuterated reagents and solvents
- cGMP suite for manufacturing clinical trial grade materials (CTM)
- Quantity discounts

Catalog No.	Description	Amount
DLM-9	Acetone-d_6 (D, 99.9%)	Multiple sizes
DLM-112	Acetaldehyde (D_4, 99%)	5 g
DLM-247	Acetyl chloride (D_3, 98%)	10 g
DLM-1	Benzene-d_6 (D, 99.5%)	Multiple sizes
DLM-494	Biphenyl (D_{10}, 98%)	1 g
DLM-1945	*bis*(2-Chloroethoxy) methane (chloroethoxy-D_8, 98%)	0.1 g
DLM-1315	Borane (D_3, 98%) (1 molar in THF) (+0.005M $NABD_4$)	0.25 L
DLM-4747	Borane methylsulfide complex (D_3, 99%)	5 g
DLM-398	Bromobenzene (D_5, 99%)	25 g
DLM-1116	*tert*-Butylchloride (D_9, 98%)	Multiple sizes
DLM-263	Chlorobenzene-d_5 (D, 99%)	5 g
CNLM-7289	Cyanamide (^{13}C, 99%; $^{15}N_2$, 98%) (stabilized with < 0.1% acetic acid)	Please inquire
DLM-4	Deuterium oxide (D, 99.9%)	Multiple sizes
DLM-3903	Dimethyl carbonate (D_6, 99%)	1 g
DLM-196	Dimethyl sulfate (D_6, 98%)	Please inquire
DLM-805	Formaldehyde (D_2, 98%) (~20% w/w in D_2O)	20 mL of solution

Catalog No.	Description	Amount
DLM-1023	Iodoethane (1,1-D_2, 98%) + copper wire	5 g
DLM-1024	Iodoethane (2,2,2-D_3, 98%) + copper wire	5 g
DLM-272	Iodoethane (D_5, 99%) + copper wire	5 g
DLM-1981	Methanesulfonic acid (D_4, 97-98%)	5 g
DLM-24	Methanol-d_4 (D, 99.8%) methyl alcohol	Multiple sizes
DLM-651	Methyl formate (formyl-D, 99%)	5 g
DLM-362	Methyl iodide + copper wire (D_3, 99.5%)	Multiple sizes
DLM-289	Methylamine·HCl (methyl-D_3, 98%)	1 g
DLM-3484	Morpholine (2,2,3,3,5,5,6,6-D_8, 98%)	1 g
DLM-295	2-Nitrophenol (ring-D_4, 98%)	0.1 g
DLM-296	4-Nitrophenol (ring-D_4, 98%)	0.1 g
DLM-300	Paraformaldehyde (D_2, 99%)	5 g
DLM-370	Phenol (D_6, 98%)	5 g
DLM-788	Phthalic anhydride (D_4, 98%)	0.5 g
DLM-226	Sodium borodeuteride (D_4, 99%) CP 90-95%	Multiple sizes
DLM-1361	Sodium formate (D, 98%)	5 g

CP = chemical purity

Find a complete listing of synthetic intermediates here...

Getting Started with Using Deuterated Products? Or Looking for New Ideas?

A Sampling of Recent and Not-so-Recent Literature Articles, Reviews and Procedures

Comprehensive Tables of Chemical Shifts for the Synthetic Chemist

The following two articles are required reading for every synthetic chemist who uses NMR as an identification method. They provide the necessary information to identify NMR peaks that arise from contaminants in the desired synthetic compound, whether the sample is an in-process or final product.

The first in the list is the original paper and tabulates proton and ^{13}C chemical shifts of over 30 compounds that are routinely used in organic synthesis as reagents, solvents or lubrication materials and how the chemical shift varies depending on the deuterated solvent used in the NMR experiment. Additionally, the authors report the temperature dependence of the chemical shift of residual HOD.

The second article, written by two of the original authors with several others, is more comprehensive. It includes additional potential contaminants, as well as several additional deuterated solvents that are used in organometallics synthesis.

Gottlieb, H.E.; Kotlyar, V.; Nudelman, A. **1997**. NMR chemical shifts of common laboratory solvents as trace impurities. *J Org Chem, 62.21:* 7512-7515.

Fulmer, G.R., et al. **2010**. NMR chemical shifts of trace impurities: common laboratory solvents, organics, and gases in deuterated solvents relevant to the organometallic chemist. *Organometallics, 29.9:* 2176-2179.

H/D Exchange Mass Spectrometry Studies in Protein Structure and Dynamics

The following two articles (one review, one book chapter) describe the coupled use (HDX-MS) of rates of hydrogen/deuterium exchange of amide protons in protein with LC/MS to monitor structure function of protein therapeutics. The first article reviews the current state of the method, and the second article describes in detail a procedure one can follow to use this method in the laboratory.

Wei, H., et al. **2014**. Hydrogen/deuterium exchange mass spectrometry for probing higher order structure of protein therapeutics: methodology and applications. *Drug Discovery Today, 19.1:* 95-102.

Singh, H.; Busenlehner, L.S. **2014**. Probing backbone dynamics with hydrogen/deuterium exchange mass spectrometry. *Protein Dynamics.* Humana Press. 81-99.

Deuteration Aids Mass Spectrometry Imaging in Neuropsychopharmacology Research

The article listed below describes the use of mass spec imaging to study distribution of large (proteins, lipids) and small (drugs, metabolites) molecules in brain tissue *in situ*. The samples are typically prepared with matrix crystals that can interfere with the interpretation of the data. The use of deuterated matrix crystals has improved the analysis by revealing previously unobserved compounds. In addition, deuterated reference standards (cocaine, imipramine) improve the accuracy of quantitation of signal.

Shariatgorji, M.; Svenningsson, P.; Andrén, P.E. **2014**. Mass spectrometry imaging, an emerging technology in neuropsychopharmacology. *Neuropsychopharmacology 39.1:* 34-49.

Effects of Deuteration of Proteins on Proton NMR Parameters

Deuteration of proteins has been successfully used to aid in the study of structure and dynamics. The effect of deuteration on proton and ^{13}C-NMR parameters has been reported in many publications. This review comprehensively describes the quantitative effects of deuteration of proteins specifically on chemical shifts, coupling constants and relaxation parameters. Theoretical treatment is presented along with experimental results that tabulate deuterium isotope shifts.

Tugarinov, V. **2014**. Indirect use of deuterium in solution NMR studies of protein structure and hydrogen bonding. *Prog Nucl Magn Reson Spectrosc, 77:* 49-68.

The Use of Deuterated Solvents and Deuterium Exchange Methods in Metabolite Identification

This book chapter describes a detailed procedure to streamline metabolite identification for drug-discovery studies. The use of fast LC-MS/MS with multiple-stage mass analysis provides a single analysis tool to identify lead candidates in drug development.

Lam, W.W., et al. **2014**. Metabolite Identification in Drug Discovery. *Optim Drug Discovery,* Humana Press. 445-459.

(continued)

Deuterium Isotope Effects in Pharmacology Studies on Living Cells, Organisms and Animals

This article is a comprehensive review on the history, use and effects of D_2O on living tissue. It includes a review of potential clinical effects of D_2O and the potential beneficial use of deuterated drugs, antimicrobials and insecticides. See the following two articles for recent applications.

Kushner, D. J.; Baker, A.; Dunsta ll T.G. **1999**. Pharmacological uses and perspectives of heavy water and deuterated compounds. *Can J Physiol Pharmacol, 77.2:* 79-88.

Honeybees Discriminate between Protonated and Deuterated Compounds

Related to the above Review article, this interesting study shows that honeybees can detect differences between isotopomers of the same compound. The authors present data that point to the theory that the honeybees discriminate between compounds based in intramolecular vibration difference, not on structural differences.

Gronenberg, W., et al. **2014**. Honeybees (*Apis mellifera*) learn to discriminate the smell of organic compounds from their respective deuterated isotopomers. *Proc R Soc B, 281.1778:* 20133089.

Deuterated C-Aryl Glycoside as a Potential Drug in the Treatment of Type 2 Diabetes

An emerging field in deuterated pharmaceuticals takes advantage of the kinetic deuterium isotope effect. The effect of deuterium in some compounds can block the formation of potentially harmful metabolites and also improve the effect of the drug on its target. This study describes one such example that may be useful in the treatment of Type 2 diabetes.

Xu, G., et al. **2014**. Design, Synthesis, and Biological Evaluation of Deuterated C-Aryl Glycoside as Potent and Long-Acting Renal Sodium-Dependent Glucose Cotransporter 2 (SGLT2) Inhibitor for the Treatment of Type 2 Diabetes. *J Med Chem, 57(4),* 1236-1251.

Deuterium Aids in Organic Mechanism and Kinetic Studies on Metal Surfaces

Ethanol decomposition on Pd(111) surface was studied using deuterated isotopomers of ethanol to determine the order of bond scission that the O-H bond cleaves prior to the C-H bond.

Williams, R.M.; Pang, S.H.; Medlin, J.W. **2014**. OH versus CH bond scission sequence in ethanol decomposition on Pd (111). *Surf Sci, 619:* 114-118.

Chiral transfer in Pd catalyzed amination of allyl alcohols is reported through the use of kinetic studies using deuterated isotopomers of allyl acohol.

Sawadjoon, S., et al. **2014**. Mechanistic Insights into the Pd-Catalyzed Direct Amination of Allyl Alcohols: Evidence for an Outer-sphere Mechanism Involving a Palladium Hydride Intermediate. *Chem – Eur J, 20(6),* 1461-1764.

A mechanistic study of gold catalyzed heterocyclization reactions is accomplished through the use of both deuterated solvents and deuterated urea reveals dual σ,π-gold activation in addition to the well-established π-activation.

Gimeno, A., et al. **2014**. Competitive Gold-Activation Modes in Terminal Alkynes: An Experimental and Mechanistic Study. *Chem – Eur J, 20.3:* 683-688.

The mechanism of platinum mediated carbon-carbon bond formation in organoboron compounds using dioxygen is studied using partially deuterated reagents coupled with reaction in methanol-d_4 and reveals C=C coupling at the boron center.

Pal, S.; Zavalij, P.Y.; Vedernikov, A.N. **2014**. Oxidative C (sp^3)-H bond cleavage, C-C and C=C coupling at a boron center with O_2 as the oxidant mediated by platinum (ii). *Chem Commun, 50(40),* 5376-5378.

New Methods for Quantitation of Environmental Pollutants

A Sampling of Recent and Not-so-Recent Literature Articles, Reviews and Procedures

Isotope Dilution Mass Spectrometry LC-MS/MS Analysis of Water Pollutants

The authors validated a new IDMS multi-analyte method to quantitate four artificial sweeteners in samples of lake and river waters exposed to wastewater effluent and agricultural run-off. Deuterated analogs were necessary to reduce uncertainty while improving accuracy.

Perkola, N.; Sainio, P. **2014**. Quantification of four artificial sweeteners in Finnish surface waters with isotope dilution mass spectrometry. *Environ Pollut,184,* 391-396.

GC/MS and NMR Analysis of Atmospheric Pollutants

The authors describe methods for identification of toluene photooxidation products in aerosols. Deuterated toluene aided in the analysis. The MS results include previously unidentified compounds in aerosols. NMR was used to identify several functional groups arising from oligomeric material that cannot be identified using traditional MS methods.

White, S.J.; Jamie, I.M.; Angove, D.E. **2014**. Chemical characterisation of semi-volatile and aerosol compounds from the photooxidation of toluene and NO_x. *Atmos Environ, 83,* 237-244.

Deuterated Conductive Polymers Exhibit Improved Characteristics

This paper describes a comprehensive experimental and theoretical study of the optoelectronic behavior of deuterated conducting polymers. The neutron scattering and Raman results reveal differences in morphology vibrational modes. Distinct changes in device performance and optical properties are also observed.

Shao, M., et al. **2014**. The isotopic effects of deuteration on optoelectronic properties of conducting polymers. *Nat Commun, 5*.

Use of Neutron Imaging to Understand Durability of High Temperature Fuel Cells

The degradation of high T polymer electrolyte fuel cells is accelerated by evaporation of H_3PO_4 along with other processes. Neutron imaging, combined with *in situ* H/D exchange in phosphoric acid, allowed a properly referenced study to determine the distribution of phosphoric acid in fuel cells and its effect on the durability of these cells.

Boillat, P., et al. **2014**. Evaluation of Neutron Imaging for Measuring Phosphoric Acid Distribution in High Temperature PEFCs. *J Electrochem Soc, 161,3:* F192-F198.

Enhancement of Room Temperature Phosphorescence Yield in Deuterated Compounds

Aromatic compounds are useful building blocks for creating new materials with photosensitive applications. One goal is to increase the phosphorescence lifetime and yield at room temperature. This study shows the effect of deuteration position in purely aromatic compounds to phosphorescence yield at room temperature.

Hirata, S., et al. **2014**. Relationship between room temperature phosphorescence and deuteration position in a purely aromatic compound. *Chem Phys Lett, 591,* 119-125.

Reference our complete Environmental product offerings here...