An automated framework for NMR chemical shift calculations of small organic molecules

Yasemin Yesiltepe1,2, Jamie R. Nuñez2, Sean M. Colby2, Dennis G. Thomas2, Mark I. Borkum2, Patrick N. Reardon3, Nancy M. Washton2, Thomas O. Metz2, Justin G. Teeguarden2, Niranjan Govind2 and Ryan S. Renslow1,2*

Abstract

When using nuclear magnetic resonance (NMR) to assist in chemical identification in complex samples, researchers commonly rely on databases for chemical shift spectra. However, authentic standards are typically depended upon to build libraries experimentally. Considering complex biological samples, such as blood and soil, the entirety of NMR spectra required for all possible compounds would be infeasible to ascertain due to limitations of available standards and experimental processing time. As an alternative, we introduce the in silico Chemical Library Engine (ISiCLE) NMR chemical shift module to accurately and automatically calculate NMR chemical shifts of small organic molecules through use of quantum chemical calculations. ISiCLE performs density functional theory (DFT)-based calculations for predicting chemical properties—specifically NMR chemical shifts in this manuscript—via the open source, high-performance computational chemistry software, NWChem. ISiCLE calculates the NMR chemical shifts of sets of molecules using any available combination of DFT method, solvent, and NMR-active nuclei, using both user-selected reference compounds and/or linear regression methods. Calculated NMR chemical shifts are provided to the user for each molecule, along with comparisons with respect to a number of metrics commonly used in the literature. Here, we demonstrate ISiCLE using a set of 312 molecules, ranging in size up to 90 carbon atoms. For each, calculation of NMR chemical shifts have been performed with 8 different levels of DFT theory, and with solvation effects using the implicit solvent Conductor-like Screening Model. The DFT method dependence of the calculated chemical shifts have been systematically investigated through benchmarking and subsequently compared to experimental data available in the literature. Furthermore, ISiCLE has been applied to a set of 80 methylcyclohexane conformers, combined via Boltzmann weighting and compared to experimental values. We demonstrate that our protocol shows promise in the automation of chemical shift calculations and, ultimately, the expansion of chemical shift libraries.

Keywords: Chemical shift, Density functional theory, DFT, Metabolomics, NMR, NWchem, Python, Quantum chemistry

Background

Metabolomics is being increasingly applied in biomedical and environmental studies, despite the technical challenges facing comprehensive and unambiguous identification of detected metabolites [1–3]. The capability to routinely measure and identify even a modicum
of biologically important molecules within all of chemical space—greater than $10^{60}$ compounds [4]—remains a grand challenge in biology. The prevention and treatment of metabolic diseases, determining the interactions between plant and soil microbial communities, and uncovering the building blocks that led to abiogenesis will all strongly depend on confidently identifying small molecules, and thus understanding the mechanisms involved in the complex processes of metabolic networks [5–7]. The current gold standard for chemical identification requires matching chemical features to those measured from an authentic chemical standard. However, this is not the case with the vast majority of molecules. For example, only 17% of compounds found in the Human Metabolome Database (HMDB) and less than 1% of compounds found in exposure chemical databases like the U.S. Environmental Protection Agency (EPA) Distributed Structure-Searchable Toxicity (DSSTox) Database [8] can be purchased in pure form [9, 10]. Although analytical techniques like nuclear magnetic resonance (NMR) spectroscopy [11–13] and mass spectrometry (MS) [14–16] have been applied for the identification of metabolites and to build libraries [17–21], determining the complete composition of entire metabolomes is still non-trivial for both technical and economic reasons. In this regard, libraries constructed of experimentally obtained data are too limited, expensive, and slow to build, even for libraries with thousands of metabolites [22–25].

The most practical approach to expand reference libraries for comprehensive identification of compounds detected in metabolomics studies is through in silico calculation of molecular attributes. Molecular properties that can be both accurately predicted computationally and consistently measured experimentally may be used in “standards free” metabolomics identification approaches. The metabolomics community has made many advances in calculations of measurable chemical attributes, such as chromatographic retention time [26, 27], tandem mass spectra [28–30], ion mobility collision cross section [31, 32], and NMR chemical shifts [33]. Recently, high throughput computation of chemical properties has been demonstrated using machine learning approaches [34–37]. These tools are a good resource for the metabolomics community, however, machine learning methods are limited by the size and scope of the initial training set, and thus ultimately limited by the number of authentic chemical standards available for purchase. In contrast, structure-based approaches, utilizing first principles of quantum chemical calculations, leverage our understanding of the underlying chemistry and physics to directly predict chemical properties of any chemically valid molecule. Thus, quantum chemical calculations enable us to overcome the reliance on authentic chemical standards in metabolomics. In this study, we focus on expanding the utility of density functional theory (DFT), a widely used electronic structure approach, which has been applied to predict NMR chemical shifts [38–41]. DFT enables examination of molecular conformers [42–45] and allows custom solvent conditions [46–48]. Ultimately, computational modeling can be used in the rapid identification and study of thousands of metabolites, culminating in in silico metabolome libraries of multiple chemical properties. Furthermore, the same tools that can be used to aid identification of small molecules in complex samples can also be used for structure confirmation and correction. For example, we recently used the tool described in this manuscript to help correct the misidentification of the isoflavonoid wrightiadione to the actual structure as an isobaric isostere, the alkaloid tryptantrin [49].

Metabolomics researchers unfamiliar with DFT or similar calculations may find the application of quantum chemical calculations complicated or challenging to apply quickly, and thus avoid these techniques. To this end, and to help bring DFT calculations to large sets of small organic molecules relevant to the mainstream metabolomics community, we have developed a Python-based workflow and analysis package, the ISiCLE (in silico Chemical Library Engine) NMR chemical shift module employs DFT methods through use of NWChem [50], a high-performance quantum chemistry software package developed at Pacific Northwest National Laboratory (PNNL). The module automates calculations of NMR chemical shifts, including solvent effects, via the COnductor-like Screening Model (COSMO) [51] of user-specified NMR-active nuclei for a given set of molecules for multiple DFT methods. ISiCLE also calculates the corresponding errors if experimental values are available. In this paper, we describe ISiCLE’s NMR module, provide a working tutorial example, demonstrate its use through the calculation of chemical shifts for a large set of small molecules, and, finally, show how ISiCLE can be applied to rapidly calculate chemical shifts of arrays of Boltzmann-weighted conformers to yield high accuracy chemical shift calculations.

**Methods**

**In silico Chemical Library Engine (ISiCLE)—NMR module**

ISiCLE is a Python module that provides straightforward automation of DFT using NWChem, an open source, high-performance computational quantum chemistry package, developed at Pacific Northwest National Laboratory (PNNL), for geometry optimization and chemical shift and solvent effect calculations. Figure 1 shows a schematic representation of ISiCLE. For typical use, ISiCLE requires only a list of molecules and a list of desired levels of DFT theory from
To start, users must prepare File A, containing a list of molecules, and File B, containing a list of DFT combinations, which both are required to be in Excel format (.xls or .xlsx). File A must contain all input molecules either as (i) International Union of Pure and Applied Chemistry (IUPAC) International Chemical Identifier (InChI) strings [52, 53] or (ii) XYZ files, a free-format text file having XYZ coordinates of atoms. In subsequent versions, alternative file formats will be supported, such as TSV for inputs and outputs.

Once prepared, the user runs ISiCLE. First, ISiCLE opens File A for the input molecules. OpenBabel, an open-source chemical informatics toolbox available with Python wrappers [54, 55], is called to generate geometry files. For InChI inputs, OpenBabel generates .xyz files for each molecule, unless .xyz files are provided, and converts InChI to InChIKey for naming files (otherwise, the base names of XYZ files are used for naming subsequent files). Next, OpenBabel applies the Merck molecular force field (MMFF94) [56] to generate a rough three-dimensional (3D) structure for each molecule, resulting in associated .mol files. ISiCLE then prepares NWChem input files based on the specified DFT methods, solvents, shielding parameters and regarding task directives given by the user-prepared File B. Finally, ISiCLE submits the appropriate files to, if relevant, a remote NWChem installation (typically on a non-local, networked, high-performance computer), and then retrieves the output files once the calculations are complete. Additional information and further details about ISiCLE is provided in Additional file 1 (S1). Note that future versions of ISiCLE will automatically generate conformers of a given molecule, as part of the seamless pipeline.

For each molecule, ISiCLE generates MDL Molfiles (.mol) [57] that contain isotropic shieldings and NMR chemical shifts. ISiCLE exports isotropic shieldings for each molecule and appends them to a MDL Molfile in the same atomic order of the original XYZ files. Then, ISiCLE converts isotropic shieldings to NMR chemical shifts by subtracting the isotropic shielding constants for the specified nuclei of the molecule of interest from those of a reference compound computed at the same level of theory (Eq. 1). For this manuscript, tetramethylsilane (TMS) is used as a reference compound. The experimental chemical shifts of TMS are assigned a value of zero, thus the calculation of NMR chemical shifts needs only isotropic shieldings of TMS [58–61]. Any molecule can be used as reference in ISiCLE as long as it has the specified nuclei and its experimental (or calculated) chemical shifts are supplied. It is explained in detail in a supplemental tutorial how a user inputs experimental data. The equation for calculating chemical shifts from isotropic shieldings is:

\[
\text{Chemical Shift} = \text{Isotropic Shielding}_{\text{Reference Compound}} - \text{Isotropic Shielding}_{\text{Molecule of Interest}}
\]
\[ \delta_i = \sigma_{\text{ref}} - \sigma_i + \delta_{\text{ref}} \]  

where \( \delta_i \) and \( \delta_{\text{ref}} \) are the chemical shifts of atom \( i \) (of the molecule of interest) and the reference molecule, respectively. \( \sigma_i \) and \( \sigma_{\text{ref}} \) are the isotropic shielding constants of atom \( i \) and the reference molecule, respectively.

ISiCLE also calculates errors in NMR chemical shifts if experimental data is provided in the MDL Molfiles in a required way as explained in the tutorial. The errors are quantified in terms of mean absolute error (MAE) (Eq. 2), corrected mean absolute error (CMAE) (Eq. 3), root mean square error (RMSE) (Eq. 4), and maximum absolute error (Eq. 5).

\[
\text{MAE} = \frac{\sum_{i=1}^{N} |\delta_{\text{exp}} - \delta_{\text{calc}}|}{N}
\]  

\[
\text{CMAE} = \frac{\sum_{i=1}^{N} |\delta_{\text{exp}} - (\delta_{\text{calc}} - \text{intercept})/\text{slope}|}{N}
\]  

\[
\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{N} (\delta_{\text{exp}} - \delta_{\text{calc}})^2}{N}}
\]  

\[
\max_{i=1, 2, ..., N} |\delta_{\text{exp}} - \delta_{\text{calc}}|
\]  

where \( N \) is the total number of chemical shifts, and \( \delta_{\text{calc}} \) and \( \delta_{\text{exp}} \) are the lists of calculated and experimental chemical shifts, respectively.

Empirical scaling of isotropic shieldings or NMR chemical shifts is the most common approach to remove systematic errors. If experimental data is provided, ISiCLE uses two optional approaches for its linear regression method, where slope and intercept values are derived from (i) regression of computed NMR chemical shifts versus experimental NMR chemical shifts using (Eq. 6), and/or (ii) regression of computed isotropic shieldings versus experimental NMR chemical shifts using (Eq. 7).

\[
\delta_{\text{exp}} = \frac{\text{intercept} - \delta_{\text{calc}}}{-\text{slope}}
\]  

\[
\delta_{\text{exp}} = \frac{\text{intercept} - \sigma_{\text{calc}}}{-\text{slope}}
\]  

where \( \sigma_{\text{calc}} \) is the list of isotopic shielding constants of molecules.

Alternatively, if the user does not provide experimental NMR chemical shifts, ISiCLE can scale NMR chemical shifts using provided intercept and slope values. The scaled NMR chemical shifts are appended to MDL Molfiles.

A detailed description of InChIs and InChIKeys, and why they were chosen, can be found in the Additional file 1 (S2). Similarly, justification for the use of MDL Molfiles is explained in Additional file 1 (S2). In the next version, ISiCLE will be compatible with other file formats, such as the NMReDATA [62] format that has been recently designed for NMR data use. To help ease the use of our data, we provide NMReDATA files for the demonstration set in the Additional file 2.

Furthermore, installation details for OpenBabel and other required Python packages are provided in the tutorial (see Additional file 2). The Windows-based tutorial provides step-by-step instructions for running ISiCLE for the first time, including information for installation of packages, properly preparing input files, running a calculation, and obtaining output files. The tutorial includes example molecules with anticipated output files for use as a practice set and for benchmarking purposes. It is designed to guide users of ISiCLE and NWChem in the use of the input files and scripts, demonstrated using three small molecules: methanol, methyl-isothiocyanate, and nitromethane. Calculation time may vary (depending on network speed, local computational power, etc.), but it is expected to take less than 10 min.

**Demonstration set**

For an initial demonstration of ISiCLE, we have compiled a molecule set of 312 compounds from previous studies: Alver [63], Asiri et al. [64], Bally and Rablen [65], Bagno et al. [66], Borkowski et al. [67], Coruh et al. [68], Fulmer et al. [69], Hill et al. [70], Içgi et al. [71], Karabacak et al. [72], Krishnakumar et al. [73–75], Kwan and Liu [45], Li et al. [76], Lomas [77], Osmialowski et al. [78], Parlak et al. [79], Perez et al. [80], Rablen et al. [81], Sarotti and Pellegrinet [82, 83], Sebastian et al. [84], Seca et al. [52], Senyel et al. [85, 86], Sridevi et al. [87], Tormena and da Silva [88], Vijaya and Sankaran [89], Watts et al. [53], Wiitala et al. [90, 91], Willoughby et al. [92], and Yang et al. [93]. We aimed to cover a broad chemical space and distribution of sizes. Our criteria also included the existence of all \(^1\)H and/or \(^13\)C NMR experimental data in chloroform solvent, referenced to TMS at room temperature, for comparisons. Note that the NMR spectra of each molecule set were not recorded at the same magnetic field strengths. A summary of the demonstration set compounds are given in Table 1. Detailed information about the individual sets is given in Additional file 1 (S3).
Computational details
As a first demonstration of ISiCLE, a benchmark study was performed with 8 different DFT methods to predict 
$^1$H and $^{13}$C NMR chemical factors for the calculations of chemical shifts in chloroform. Each compound was optimized with the Becke three-parameter Lee–Yang–Parr
Chloroform solvation effects were simulated using the Gauge Including Atomic Orbitals (GIAO) formalism. Chemical shifts were computed relative to TMS using subsequent Boltzmann weighting, two sets of axial and chemical shifts, along with frequency calculations (and chemical shifts. Relative free energies of the conformations and Boltzmann weighted NMR chemical shifts were compared to those found in the literature [92, 107–109].

All results shown in this manuscript were generated using the Cascade high-performance computer (1440 compute nodes, 23,040 Intel Xeon E5-2670 processor cores, 195,840 Intel Xeon Phi 5110P coprocessor cores, and 128 GB memory per compute node [110]), in EMSL (a U.S. national scientific user facility) located at PNNL. Cascade is available for external users through a free, competitive proposal process. ISiCLE can utilize local clusters or high-performance computing resources available to the user. NWChem is freely available and can be downloaded from the website [111, 112].

Results and discussion

NMR chemical shift calculations have been used successfully to identify new molecules, determine metabolite identifications, and eliminate structural misassignments [59, 113]. In the last two decades, many research groups have performed benchmark DFT studies on the accuracy of optimized molecular geometry [92, 114–116], functionals [117, 118], basis sets [88, 119], and solvation models [90, 120, 121] for NMR chemical shifts [60, 122–124]. Each group uses a molecule set focusing on a unique chemical class [78, 125–129] and several groups have recommended different exchange–correlation (XC) energy functionals with a different basis set for a particular condition or suitable to specific chemical functionalities and properties [70, 77, 130–133]. The prevailing opinion is that reliable isotropic NMR chemical shifts strongly depend on accurate calculations of molecular geometries and inclusion of HF exchange in selected DFT methods, to an extent [134, 135]. On the other hand, the size of the basis set does not increase the accuracy after a point [136, 137].

The ISiCLE software can be installed locally. As seen in Fig. 1, it requires only two input files, prepared in Excel: a sequence of InChI or XYZ molecule geometry files, and a sequence of DFT methods of the user’s choice. Preparation of NWChem “run files,” 3D molecule geometry files, and/or Linux/Unix shell script “drivers” are not required. As output, ISiCLE prints isotropic shielding, calculated to a reference molecule and/or application of a user-specified linear regression technique. ISiCLE is a promising tool contributing to standards-free metabolomics, which depends on the ability to calculate properties for thousands of molecules and their associated conformers.

Application 1: chemical shift calculations for a demonstration set of molecules

To test ISiCLE, we generated a set of 312 molecules. This set is large relative to other metabolomic molecule sets found in the literature, which in our literature survey averaged 34 molecules (Table 1). Our molecule set ranges from small- to large-sized molecules (number of carbon atoms ranging from 1 to 90), and experimental $^{13}$C and $^{1}$H NMR data in chloroform were available for each of
them. Our set also spans a wide array of chemical classes, including acetylides, alkaloids, benzenoids, hydrocarbons, lipids, organohalogens, and organic nitrogen and oxygen compounds. ISiCLE was used to successfully perform DFT calculations for this set under chloroform solvation using eight different levels of DFT theory (4 different functionals and 2 basis sets for $^{13}$C and $^1$H).

A total of 2494 carbon nuclei and of 3127 hydrogen nuclei were calculated for all 312 molecules of the demonstration set and compared with experimental data. Deviation bars indicating MAE and MAXAE are plotted for each method in Fig. 3. For both $^{13}$C and $^1$H NMR chemical shifts, the MAE of each method with cc-pVTZ is higher than those with cc-pVDZ. For $^{13}$C, the MAE of each method with cc-pVTZ (7–10 ppm) is higher than those with cc-pVDZ (5–6 ppm). MAE of methods with a larger basis set deviate more compared to those with a smaller basis set. The smallest deviations are observed for B3LYP and B35LYP, both in MAE and MAXAE results. The same situation is observed for $^1$H NMR chemical shifts as well: MAE of each method with cc-pVTZ (~0.35 ppm) is higher than those with cc-pVDZ (~0.30 ppm). In contrast to $^{13}$C NMR chemical shifts, $^1$H NMR chemical shifts are better predicted with methods using larger basis sets (cc-pVTZ). Although the error differences among each method may be too low to confidently identify the outperforming method, B3LYP/cc-pVDZ is the most successful combination in the calculation of $^{13}$C and $^1$H NMR chemical shifts for our application shown here.

Figure 4 shows computational costs of DFT combinations for the demonstration set. We found that the smaller basis set (cc-pVDZ) in the calculation of both

![Computational costs of DFT methods performed for the demonstration set. Each bar is for two DFT methods with basis sets of cc-pVDZ and cc-pVTZ. The grey bars represent CPU times for the methods with cc-pVDZ and the black bars represent those with cc-pVTZ.](image)

![Mean absolute errors (MAE) and maximum absolute errors (MAXAE) of chemical shifts for the demonstration set. The grey bars represent MAE, the black bars represent MAXAE. For all methods, geometries are optimized at B3LYP/6-31G(d) in chloroform](image)


$^{13}$C and $^1$H NMR chemical shifts was an acceptable compromise between accuracy and computational performance, compared with the larger cc-pVTZ basis. This finding is similar to a recent benchmark study [138] that showed B3LYP/cc-pVTZ is a reliable combination, balancing accuracy with computational cost in $^{13}$C chemical shifts calculation. The larger basis set (cc-pVTZ) took 2–3 times longer to complete than cc-pVDZ (in terms of total CPU time). The computational times of the isotropic shielding and chemical shift calculations for this demonstration set are given in the file of Demonstration-Set_CPUtimes.xlsx in the Additional file 2.

**Effect of scaling by linear regression**

We performed the most general approach to error reduction, empirical scaling. Our molecule set has 1554 and 1830 experimental $^{13}$C and $^1$H NMR chemical shifts, respectively. It provides confidence for applying linear regression effectively as it reduces the possibility of overfitting. Empirical scaling was applied to the data obtained with the best combination, B3LYP/cc-pVDZ, using two different relationships: computed shifts versus experimental chemical shifts (Eq. 6), and computed isotropic shieldings versus experimental shifts (Eq. 7). Once the empirical scaling was applied, the accuracy for $^{13}$C chemical shifts and $^1$H chemical shifts improved by 0.7 and 0.11 ppm, respectively. Our computed NMR chemical shifts and shieldings deviate from unity (desired slope = 1) by 0.02 for both $^{13}$C and $^1$H NMR chemical shifts. Linear fits with correlation coefficients of 0.99 (Fig. 5a, b) and 0.93 (Fig. 5c, d) for $^{13}$C and $^1$H NMR chemical shifts, respectively, were observed, which also shows that B3LYP/cc-pVDZ is able to produce data free from random error. Results of linear regression to the $^{13}$C and $^1$H NMR chemical shifts obtained by other DFT methods are given in the Additional file 1 (S5).

**Detailed look at $^{13}$C NMR chemical shifts**

The carbon ($^{13}$C) magnetic shieldings and chemical shifts derived from the various DFT methods are highly correlated, as shown by a correlation coefficient of 0.99 (Fig. 5). The inclusion of a scaling factor enhances the performance of theoretical calculations with B3LYP/cc-pVDZ//B3LYP/6-31G(d) and decreases the MAE in $^{13}$C NMR chemical shifts for this set by approximately 13%.

There has been a trend toward using multiple references, such that each molecule should be referenced to a molecule with similar properties to improve accuracy of NMR chemical shifts [123, 134, 139]. Sarotti et al. examined the influence of the reference compound used in the $^{13}$C [83] and $^1$H [82] NMR chemical shift calculations over a set of organic compounds, all of which were included in our calculations. They recommended the use of benzene and methanol as a reference standard in the calculations of chemical shifts of sp-sp, sp and sp$^3$ hybridized carbon atoms, respectively, instead of TMS for all type of carbon atoms [140]. Propelled by the discussion in the study of Grimblat et al. [141] about the distribution of the errors observed in sp$^2$- and sp$^3$- carbons, we determined the distribution of the data of chemical shifts of sp$^2$ (933 carbons) and sp$^3$ (745 carbons) hybridized carbons (Fig. 6, the sp$^2$- and sp$^3$- derived series of carbons show two separate chemical shift distributions and two separate error distributions over a much larger variety of compounds than Grimblat et al. For our demonstration set, both errors between calculated and experimental sp$^2$- and sp$^3$- chemical shifts more closely resemble a Student’s t-distribution [58, 142], rather than a normal distribution [138, 143]. The correlation coefficients of the errors of sp$^2$- and sp$^3$- carbons are 0.93 and 0.78, and 0.98 and 0.95 for Student’s t-distribution and normal distribution, respectively.

Furthermore, we looked for the bonded neighbors of each carbon and hydrogen extensively in Fig. 7. For carbon shifts, error was measured for carbon (n = 1709), chlorine (n = 149), fluorine (n = 8), hydrogen (n = 1161), nitrogen (n = 199), oxygen (n = 251) and sulfur (n = 20) attachments. The largest deviations occur in carbon–chlorine and carbon–sulfur attachments with MAEs of 11.2 and 5.8 ppm and MAXAE 39.7 and 16.5 ppm, respectively. The study by Li et al. [76], which used a set of chlorinated carbons, reports the same conclusion: calculation accuracy decreases as the size of the basis set used increases, but improvement was obtained after linear regression corrections for B3LYP/6-31+G(d,p) with slope of 0.98. Other than chlorine and sulfur, carbon–hydrogen attachments also make the $^{13}$C NMR chemical shift DFT calculations deviate significantly from experimental values, with MAE of 4.7 and 3.9 ppm and with MAXAE of 51.6 and 34.9 ppm. Carbon was found in rings in 70% of the cases, and these carbons show a MAE of 4.6 ppm. Also, the MAE of $^{13}$C NMR chemical shift is 3.9 ppm for carbons bonded to a hydrogen atom but reaches 9.7 ppm in all other cases.

Oxygen and nitrogen attachments to carbon led to $^{13}$C NMR chemical shifts with MAE up to 3.1 and 4.2 ppm and MAXAE of 32.5 and 23.6 ppm, respectively. Interestingly, half of the C–O attachments found in ring-form had chemical shifts with a MAE of 2.8 ppm, compared to the chemical shifts of C–O attachments not found in a ring, which had a relatively higher MAE of 3.2 ppm, leading to a percent difference of 14.1%. NMR chemical shifts of C–N attachments, present in a ring or not, show close MAE of 4.19 and 4.26 ppm, respectively, a percent difference of 1.6%. This is to be expected, since C–O attachments are expected to show some deviation in chemical
shift due to the polarization of the electron distribution caused by the high electronegativity of oxygen, while nitrogen atoms have a lower electronegativity, leading to a lower deviation in C–N chemical shifts.

The correlation plot (Fig. 5a, b) shows a linear pattern with only minor deviations of the predicted $^{13}$C shieldings or $^{13}$C chemical shifts from the fitted line. It is verified by the correlation coefficient of 0.99, as observed in previous studies [130, 144], that the deviation of the slope from unity within the range of 0.95 and 1.05 is an indicator of a reliable method. However, when placed in subgroups of different attachment types, distant outliers are observed, with some more than 15 ppm away (Fig. 7a). Most outliers are observed in the C–C and C–H attachments, respectively. C–Cl and C–N chemical shifts have a high occurrence of outliers, which may be due to the chemical properties of chlorine and nitrogen such as being remarkably close to first ionization energies. We

\[
y = -0.98x + 188.83 \\
R^2 = 0.98
\]

\[
y = 0.98x + 5.21 \\
R^2 = 0.98
\]

\[
y = -0.98x + 31.59 \\
R^2 = 0.93
\]

\[
y = 0.98x + 0.01 \\
R^2 = 0.93
\]

Fig. 5 Linear correlation plots of a $^{13}$C and c $^1$H isotropic shielding values, and b $^{13}$C and d $^1$H NMR chemical shifts versus experimental NMR chemical shifts. Chemical shifts are calculated using the GIAO/B3LYP/cc-pVDZ/B3LYP/6-31G(d) level of theory for the demonstration set in CDCl₃ (312 molecules (1554 carbons and 1830 hydrogens)). $R^2$ indicates the correlation coefficient.
suggested that some cases of high calculation errors could be due to the consideration of only a single conformation. For the highest accuracy, proper conformational sampling must be considered, as demonstrated below in “Application 2: Boltzmann-weighted NMR chemical shifts of methylcyclohexane” section.

**Detailed look at $^1$H NMR chemical shifts**

Proton ($^1$H) chemical shifts are significantly affected by intermolecular interactions, particularly in aqueous states, especially compared to $^{13}$C chemical shifts. Agreement with experimental values improves as empirical linear scaling is performed for $^1$H chemical shifts. GIAO/B3LYP/cc-pVDZ/B3LYP/6-31G(d) yields scaled $^1$H chemical shifts in chloroform solution having a MAE of 0.30 ppm in comparison with solution experimental values. The $^1$H chemical shifts in the range of 10–17 ppm show the largest deviation, occurring higher than 5 ppm.

In Fig. 7b, error bars are shown for $^1$H chemical shifts when the hydrogen attaches to carbon ($n=1793$),
nitrogen \((n=17)\), and oxygen \((n=41)\). Oxygen-bound hydrogen nuclei have the largest errors (up to 10 ppm), which is to be expected due to the electronegative property of oxygen atoms, as discussed in the previous section. It is followed by less electronegative nitrogen-bound hydrogen atoms, with an MAE of 0.71 ppm and a MAXAE of 2.25 ppm. About 95% of the \(^1\)H NMR chemical shifts calculated for this set are from H–C attachments. These chemical shifts had a MAE of 0.27 ppm and a MAXAE of 4.41 ppm. The high occurrence of outliers could be evidence of how \(^1\)H NMR chemical shifts are sensitive to intermolecular interactions.

H–O attachments are highly sensitive (to concentration, solvent, temperature, etc.), and it is non-trivial to determine the NMR chemical shift value of arbitrary protons experimentally as well as predict them by using a single, “catch all” DFT method, which explains the relatively low correlation coefficient of 0.93 (Fig. 5c–d). For future studies, we may need to consider the use of different DFT methods, including the use of explicit solvation, particularly in the calculation of \(^1\)H NMR chemical shifts in the presence of H–O attachments.

Application of empirical scaling to functional groups are given in detail in the Additional file 1 (see S7 and S8).

Cross-validation
As a final assessment for the data collected with the demonstration set, we assessed the stability and accuracy of the linear regression approach using cross-validation [145]. Cross-validation is a technique mostly used in prediction problems to evaluate how much a given model generalizes to an independent set of data. Specifically, we performed Monte Carlo cross-validation [146, 147]. The procedure of application of Monte Carlo cross-validation method is explained in Additional file 1 (S9).

We observed that the estimated linear model parameters (i.e. slope and intercept) from the training set do not differ from that of the entire set. Therefore, the predictive linear model is stable to be accurately estimated and the subsets of \(^{13}\)C and \(^1\)H NMR chemical shifts generalize well to the groups that are not represented in the training fold.

**Application 2: Boltzmann-weighted NMR chemical shifts of methylcyclohexane**

Metabolites were experimentally interrogated using solution-state NMR, where the observed signal arises from the combined signals of present conformers. It is routine that NMR chemical shifts calculations are carried out on a single dominant conformer. However, it is well known that metabolites do not comprise a single conformer in solution and are instead found in a collection of various conformers [148], and the accuracy of NMR chemical shifts heavily depends on molecular geometries and conformation consideration [46]. It has been shown that for the highest accuracy NMR chemical shift calculations, consideration of conformers is critical, even for relatively small molecules [149]. As a second demonstration of ISiCLE, a conformational analysis based on DFT was performed on a set of 80 conformers of methylcyclohexane using a Boltzmann distribution technique. Boltzmann weighting determines the fractional population of each conformer based on its energy level [92]. High-throughput and straightforward DFT-based NMR chemical shift calculations of all 80 methylcyclohexane conformers was performed by ISiCLE then compared to experimental values.

It has been shown by Willoughby et al. [92] that the effects of molecular flexibility on NMR chemical shifts can be captured by Boltzmann weighting analysis, as demonstrated with methylcyclohexane (Fig. 8). Methylcyclohexane is a well-studied small molecule [150–154]. It is flexible, composed of a single methyl group attached to a six-membered ring, and known to exist as an assembly of two chair conformers. There are two distinct conformations of which chair–chair interconversion is
rapid and dominated by equatorial to axial conformation. We weighted 40 axial and 40 equatorial conformers in chloroform and obtained a relative free energy of 1.99 kcal/mol with NWChem, similar to calculations using Gaussian [155] by Willoughby et al. [92], and similar to experimental findings (1.73 kcal/mol [156], 1.93 kcal/mol [108]) and computed (2.15–2.31 kcal/mol [107] and 1.68–2.48 kcal/mol [109]) values. We compared the Boltzmann-weighted $^1$H and $^{13}$C chemical shifts (a ratio of 3% axial to 97% equatorial) to experimental values reported by Willoughby et al. [92]. The Boltzmann-weighted, scaled MAE was 0.017 ppm for $^1$H chemical shifts ($\delta_{\text{exp}} = 1.00 \times \delta_{\text{comp}} + \sim 0.00$), similar to the experimental value of 0.018 ppm in the study of Willoughby et al. Also, the MAE for $^{13}$C chemical shifts was 4.4 ppm and decreases to 0.8 ppm when the chemical shifts were scaled ($\delta_{\text{exp}} = 0.99 \times \delta_{\text{comp}} + 0.13$) (Fig. 9). Further details can be found in the Additional file 1 (S10).

Conclusions
We introduce the first release of ISiCLE, which predicts NMR chemical shifts of any given set of molecules relevant to metabolomics for a given set of DFT techniques. ISiCLE calculates the unscaled or scaled NMR chemical shifts (depending on the user’s choice of DFT method) and writes the data to appended MDL Molfiles. It also quantifies the error in calculated NMR chemical shifts if the user provides experimental values.

The functionality of ISiCLE is demonstrated on a molecule set consisting of 312 molecules, with experimental chemical shifts reported in chloroform solvent. 1H and 13C NMR chemical shifts were calculated using 8 different levels of DFT (BLYP, B3LYP, B35LYP, BHLYP and, cc-pVDZ, and cc-pVTZ), referenced to TMS in chloroform by carrying initial geometry optimizations out at B3LYP/6-31G(d) for all molecules. The optimal combination for this set was found to be B3LYP/cc-pVDZ//B3LYP/6-31G(d) with mean absolute error of 0.33 and 3.93 ppm for proton and carbon chemical shifts, respectively. We show that DFT calculations followed by linear scaling do in fact provide an analytically useful degree of accuracy and reliability. Finally, we used ISiCLE for the calculation of NMR chemical shifts of 80 Boltzmann-weighted conformers of methylcyclohexane and compared our results with earlier studies in the literature.

ISiCLE is a promising automated framework for accurate NMR chemical shift calculations of small organic molecules. Through this tool, we hope to expand chemical shift libraries, without the need for chemical standards run in the laboratory, which could lead to significantly more identifiable metabolites. Future work includes wrapping individual steps of the ISiCLE NMR module into a formal workflow management system such as Snakemake, to include better fault tolerance, modularization, and improved data provenance. Furthermore, additional chemical properties will be included, such as ion mobility collision cross section and infrared spectra. Finally, ISiCLE will be adapted to run seamlessly on cloud computer resources such as Amazon AWS, Microsoft Azure, and Google Cloud. ISiCLE is a promising tool contributing to standards-free metabolomics, which depends on the ability to calculate properties for thousands of molecules and their associated conformers.

Additional files

Additional file 1. Supporting information document.
Additional file 2. Supporting information files. Including tutorial, code, and all other files.

Authors’ contributions
YY designed the tool, gathered the dataset, performed the calculations and provided the analysis. NG provided technical support. RR conceived and supervised the project. All authors participated in the manuscript preparation. All authors read and approved the final manuscript.

Author details
1 The Gene and Linda Voiland School of Chemical Engineering and Biome- eneering, Washington State University, Pullman, WA, USA. 2 Earth and Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA, USA. 3 Nuclear Magnetic Resonance Facility, Oregon State University, Corvallis, OR 97331, USA.
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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
Project name: ISiCLE. Project home page: github.com/pnnl/isicle. Program availability: Source code, and tutorial are provided in the additional files.

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References
1. Fiehn O (2002) Metabolomics—the link between genotypes and phenotypes. Plant Mol Biol 48(1–2):155–171
2. Summer LW, Mendes P, Dixon RA (2003) Plant metabolomics: large-scale phytochemistry in the functional genomics era. Phytochemistry 62(6):817–836
3. Bino RJ et al (2004) Potential of metabolomics as a functional genomics tool. Trends Plant Sci 9(9):418–425
4. Dobson CM (2004) Chemical space and biology. Nature 432(7019):824–828
5. Nicholson JK et al (2002) Metabonomics: a platform for studying drug toxicity and gene function. Nat Rev Drug Discov 1(2):153–161
6. Berendsen RL, Preters CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8):478–486
7. Griffin JL, Bollard ME (2004) Metabolomics: its potential as a tool in toxicology for safety assessment and data integration. Curr Drug Metab 5(5):389–398
8. Richard AM, Gold LS, Nicklaus MC (2006) Chemical structure indexing of toxicity data on the internet: moving toward a flat world. Curr Opin Drug Discov Dev 9(3):314–325
9. Davis B (2005) Growing pains for metabolomics. Scientist 199(8):25–28
10. Nicholson JK, Wilson ID (2003) Understanding global systems biology: metabolomics and the continuum of metabolism. Nat Rev Drug Discov 2(8):668–676
11. Beckonert O et al (2007) Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. Nat Protoc 2(11):2692–2703
12. Nicholson JK, Lindon JC, Holmes E (1999) Metabonomics: underlining the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 29(11):1181–1189
13. Nicholson JK et al (1995) 750 MHz 1H and 1H-13C NMR spectroscopy of human blood plasma. Anal Chem 67(5):795–811
14. Soga T et al (2003) Quantitative metabolome analysis using capillary electrophoresis mass spectrometry. J Proteome Res 2(5):488–494
15. Smith CA et al (2006) XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. Anal Chem 78(3):779–787
16. Dettmer K, Amonov PA, Hammock BD (2007) Mass spectrometry-based metabolomics. Mass Spectrom Rev 26(1):51–78
17. Smith CA et al (2005) METLIN: a metabolite mass spectral database. Ther Drug Monit 27(6):747–751
18. Wishart DS et al (2013) HMDB 3.0—the human metabolome database in 2013. Nucleic Acids Res 41(Database issue):D801–7
19. Ulrich EL et al (2008) BioMagResBank: Nucleic Acids Res 36(Database issue)D402–8
20. Pence HE, Williams A (2010) ChemSpider: an online chemical information resource. J Chem Educ 87(11):1123–1124
21. Tautehnhrn R et al (2012) XCMS Online: a web-based platform to process untargeted metabolomic data. Anal Chem 84(11):5035–5039
22. Little JL et al (2012) Identification of “known unknowns” utilizing accurate mass data and ChemSpider. J Am Soc Mass Spectrom 23(1):179–185
23. Little JL, Cleven CD, Brown SD (2011) Identification of “known unknowns” utilizing accurate mass data and chemical abstracts service databases. J Am Soc Mass Spectrom 22(2):348–359
24. Patti GI et al (2013) A view from above: cloud plots to visualize global metabolomic data. Anal Chem 85(2):798–804
25. Wishart DS et al (2009) HMDB: a knowledgebase for the human metabolome. Nucleic Acids Res 37(Database issue)D603–10
26. Randazzo GM et al (2017) Enhanced metabolite annotation via dynamic retention time prediction: steroidsigeneal alterations as a case study. J Chomatogr B, Anal Technol Biomed Life Sci 1071:11–18
27. Vinaixa M et al (2016) Mass spectral databases for LC/MS- and GC/MS-based metabolomics: state of the field and future prospects. TrAC Trends Anal Chem 78:23–35
28. Bocker S (2017) Searching molecular structure databases using tandem MS data: are we there yet? Curr Opin Chem Biol 36:1–6
29. Allen F, Greiner R, Wishart D (2015) Competitive fragmentation modeling of ESI-MS/MS spectra for putative metabolite identification. Metabolomics 11(1):98–110
30. Wolf S et al (2010) In silico fragmentation for computer assisted identification of metabolite mass spectra. BMC Bioinform 11:148
31. Zheng XY et al (2017) Structural elucidation of cis/trans dicaffeoylquinic acid photosomerization using ion mobility spectrometry–mass spectrometry. J Phys Chem Lett 8(7):1381–1388
32. Metz TO et al (2017) Integrating ion mobility spectrometry into mass spectrometry-based exposome measurements: what can it add and how far can it go? Bioanalysis 9(1):81–98
33. Graham TR et al (2016) Precursor ion–ion aggregation in the Brust–Schiffrin synthesis of alkanethiol nanoparticles. J Phys Chem C 120(35):19837–19847
34. Zhou ZW et al (2016) Large-scale prediction of collision cross-section values for metabolites in ion mobility–mass spectrometry. Anal Chem 88(2):11084–11091
35. Zhou ZW et al (2017) LipidCCS: prediction of collision cross-section values for lipids with high precision to support ion mobility-mass spectrometry-based lipidomics. Anal Chem 89(17):9559–9566
36. Brockherde F et al (2017) Bypassing the Kohn–Sham equations with machine learning. Nat Commun 8:8872
37. Sarotti AM (2013) Successful combination of computationally inexpensive GIAO C-13 NMR calculations and artificial neural network pattern recognition: a new strategy for simple and rapid detection of structural misassignments. Org Biomol Chem 11(29):4847–4859
38. Forsyth DA, Sebag AB (1997) Computed C-13 NMR chemical shifts via empirically scaled GIAO shieldings and molecular mechanics geometries. Conformation and configuration from C-13 shifts. J Am Chem Soc 119(40):9483–9494
39. Casabianca LB, De Dios AC (2008) Ab initio calculations of NMR chemical shifts. J Chem Phys 128(5):052201
40. Auer AA, Gauss J, Stanton JF (2003) Quantitative prediction of gas-phase C-13 nuclear magnetic shielding constants. J Chem Phys 118(23):10407–10417
41. Mothana B, Ban FQ, Boyd RJ (2005) Validation of a computational scheme to study N-15 and C-13 nuclear shielding constants. Chem Phys Lett 401(1–3):7–12
42. Saito H (1986) Conformation-dependent C-13 chemical-shifts—a new means of conformational characterization as obtained by high-resolution solid-state C-13 NMR. Magn Reson Chem 24(10):835–852
43. Jaime C et al (1991) C-13 NMR chemical-shifts—a single rule to determine the conformation of calix[4]arenes. J Org Chem 56(10):3372–3376
44. Yannoni CS et al (1991) C-13 NMR-study of the C60 cluster in the solid-state — molecular-motion and carbon chemical-shift anisotropy. J Phys Chem 95(19):10
45. Kwan EE, Liu RY (2015) Enhancing NMR prediction for organic compounds using molecular dynamics. J Chem Theory Comput 11(1):5083–5089
46. Malkin VG et al (1996) Solvent effect on the NMR chemical shieldings in water calculated by a combination of molecular dynamics and density functional theory. Chem Eur J 2(4):452–457
47. Casanovas J et al (2001) Calculated and experimental NMR chemical shifts of p-methane-3,5-diols. A combination of molecular dynamics and quantum mechanics to determine the structure and the solvent effects. J Org Chem 66(11):3775–3782
48. Benzi C et al (2004) Reliable NMR chemical shifts for molecules in solution by methods rooted in density functional theory. Magn Reson Chem 42:557–567
49. Garcellano RC et al (2018) Isolation of tryptanthrin and reassessment of evidence for its isobaric isostere wrightiadione in plants of the Wrightia Genus. J Nat Prod. https://doi.org/10.1021/acs.jnatprod.8b00567
50. Valiev M et al (2010) NWChem: a comprehensive and scalable open-source solution for large scale molecular simulations. Comput Phys Commun 181(9):1477–1489
51. Klamt A, Schuurmann G (1993) Cosmo — a new approach to dielectric screening in solvents with explicit expressions for the screening and its gradient. J Chem Soc Perkin Trans 2(5):799–805
52. Seca AML et al (2000) Chemical composition of the light petroleum extract of Hibiscus cannabinus bark and core. Phytochem Anal 11(6):345–350
53. Watts HD, Mohamed MNA, Kubicki JD (2011) Comparison of multi-standard and TMS-standard calculated NMR shifts for coniferyl alcohol and application of the multistandard method to lignin dimers. J Phys Chem B 115(9):1958–1970
54. The Open Babel Package version 2.3.1. http://openbabel.org. Accessed 16 Oct 2018
55. O’Boyle NM et al (2011) Open Babel: an open chemical toolbox. J Cheminform 3:33
56. Baládi A et al (1992) Description of several chemical-structure file formats used by computer-programs developed at Molecular Design Limited. J Chem Inf Comput Sci 32(3):244–255
57. Smith SG, Goodman JM (2010) Assigning stereochemistry to single diastereoisomers by GIAO NMR calculation: the DP4 probability. J Am Chem Soc 132(37):12946–12959
58. Brown SG, Jansma MJ, Hoye TR (2012) Case study of empirical and computational chemical shift analyses: reassignment of the relative configuration of phomopsichalasin to that of diaiporthichalasin. J Nat Prod 75(7):1326–1331
59. Aliev AE, Courtier-Murias D, Zhou S (2009) Scaling factors for carbon NMR chemical shifts obtained from DFT B3LYP calculations. J Mol Struct Theorchem 893(1–3):1–5
60. Baldrige KK, Siegel JS (1999) Correlation of empirical delta(TMS) and absolute NMR chemical shifts predicted by ab initio computations. J Phys Chem A 103(20):4038–4042
61. Papier M et al (2018) NMRData, a standard to report the NMR assignment and parameters of organic compounds. Magn Reson Chem 56(8):703–715
62. Alver O (2011) DFT, FT-Raman, FT-IR, solution and solid state NMR studies of 2,4-dimethoxyphenylboronic acid. C R Chim 14(5):446–455
63. Asin AM et al (2011) Synthesis, molecular conformation, vibrational and electronic transition, isomeric chemical shift, polarizability and hyperpolarizability analysis of 3-(4-Methoxy-phenyl)-2-(2-nitrophenyl)- acrylonitrile: a combined experimental and theoretical analysis. Spectrochim Acta Part A Mol Biomol Spectrosc 82(1):444–453
64. Bally T, Rablen PR (2011) Quantum-chemical simulation of H-1 NMR spectra. 2. Comparison of DFT-based procedures for computing proton–proton coupling constants in organic molecules. J Org Chem 76(12):4818–4830
65. Bagno A, Rastrelli F, Saielli G (2008) Predicting the NMR spectra of nucleotides by DFT calculations: cyclic uridine monophosphate. Magn Reson Chem 46(6):518–524
66. Borkowski EJ, Suzie JD, Enriz RD (2015) Advances in correlation between experimental and DFT/GIAO computed C-13 NMR chemical shifts: a theoretical study on pentacyclic terpenoids (ferrenes). J Mol Struct Theorchem 953(1–3):83–90
67. Coruh A et al (2011) Synthesis, molecular conformation, vibrational, electronic transition, and chemical shift assignments of 4-(thio- phene-3-ylmethoxy)phthalonitrile: a combined experimental and theoretical analysis. Struct Chem 22(1):45–56
68. Füller GR et al (2010) NMR chemical shifts of trace impurities: common laboratory solvents, organics, and gases in deuterated solvents relevant to the organometallic chemist. OrganometaIcals 29(9):2176–2179
69. Hill DE, Vásquez N, Holland JP (2015) Evaluating the accuracy of density functional theory for calculating H-1 and C-13 NMR chemical shifts in drug molecules. Comput Theor Chem 1051:161–172
70. Izgi T et al (2007) FT-IR and NMR investigation of 2-(1-cyclohexenyl)ethylamine: a combined experimental and theoretical study. Spectrochim Acta Part A Mol Biomol Spectrosc 68(1):55–62
71. Karabacak M et al (2009) Experimental (UV, NMR, IR and Raman) and theoretical spectroscopic properties of 2-chloro-6-methylaniline. Mol Phys 107(3):253–264
72. Krishnakumar V et al (2012) Molecular structure, vibrational spectra, HOMO, LUMO and NMR studies of 2-chloro-4-nitrotoluene and 4-chloro-2-nitrotoluene. Spectrochim Acta Part A Mol Biomol Spectrosc 91:1–10
73. Krishnakumar V, Barathi D, Mathammal R (2012) Molecular structure, vibrational spectra, HOMO, LUMO and NMR studies of 1,2-dichloro-4-nitrobenzene and 2,3,5,6-tetrachloro-1-nitrobenzene based on density functional calculations. Spectrochim Acta Part A Mol Biomol Spectrosc 86:196–204
74. Krishnakumar V et al (2012) Molecular structure, spectroscopic studies (FTIR, FT-Raman and NMR) and HOMO–LUMO analysis of 6-chloro-o-cresol and 4-chloro-3-methyl phenol by density functional theoretical study. Spectrochim Acta Part A Mol Biomol Spectrosc 97:144–154
75. Li Y et al (2011) Screening and characterization of natural antioxidants in four Glycyrrhiza species by liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. J Chromatogr A 1218(45):8181–8191
76. Lomas JS (2016) H-1 NMR spectra of alcohols in hydrogen bonding solvents: DFT/GIAO calculations of chemical shifts. Magn Reson Chem 54(1):28–38
77. Osmialowski B, Kolehmainen E, Gavinecki R (2001) GIAO/DFT calculated chemical shifts of tautomeric species. 2-Phenacylpyridines and (2Z)-2-(2-hydroxy-2-phenylvinyl)pyridines. Magn Reson Chem 39(6):334–340
78. Park J et al (2008) Molecular structure, NMR analyses, density functional theory and ab initio Hartree–Fock calculations of 4,4′-diaminooc-tfluorobenzene. J Mol Struct 891(1–3):151–156
79. Perser J et al (2006) Accuracy versus time dilemma on the prediction of NMR chemical shifts: a case study (chloropyrimidines). J Org Chem 71(8):3103–3110
80. Rablen PR, Pearlman SA, Finkbiner J (1999) A comparison of density functional methods for the estimation of proton chemical shifts with chemical accuracy. J Phys Chem A 103(36):7357–7363
81. Sarotti AM, Pellegrinet SC (2012) Application of the multi-standard methodology for calculating H-1 NMR chemical shifts. J Org Chem 77(14):6059–6065
82. Sarotti AM, Pellegrinet SC (2009) A multi-standard approach for GIAO C-13 NMR calculations. J Org Chem 74(19):7254–7260
83. Sebastian S et al (2011) Quantum mechanical study of the structure and spectroscopic (FT-IR, FT-Raman, C-13, H-1 and UV), first order hyperpolarizabilities, NBO and TD-DFT analysis of the 4-methyl-2-cyanobenzophenone. Spectrochim Acta Part A Mol Biomol Spectrosc 78(2):590–600
84. Senyel M, Ulan A, Alver O (2009) Molecular structure, NMR analyses, density functional theory and ab initio Hartree–Fock calculations of 3-phenylpropylamine. C R Chim 12(6–7):808–815
86. Senyel M, Alver O, Parlak C (2008) H-1, C-13, N-15 NMR and (n)J(C, H) coupling constants investigation of 3-piperidino-propylamine: a combined experimental and theoretical study. Spectrochim Acta Part A Mol Biomol Spectrosc 71(3):830–834
87. Sridevi C, Shanthi G, Velraj G (2012) Structural, vibrational, electronic, NMR and reactivity analyses of 2-amino-4H-chromene-3-carbonitrile (ACC) by ab initio HF and DFT calculations. Spectrochim Acta Part A Mol Biomol Spectrosc 89:46–54
88. Tormena CF, da Silva GVI (2004) Chemical shifts calculations on aromatic systems: a comparison of models and basis sets. Chem Phys Lett 398(4–6):466–470
89. Vijaya P, Sankaran KR (2015) A combined experimental and DFT study of H-1 and C-13 chemical shifts in chloroform solution. J Chem Theory Comput 2(4):1085–1092
90. Wittala KW, Hoye TR, Cramer CJ (2006) Hybrid density functional methods empirically optimized for the computation of C-13 and H-1 chemical shifts in chloroform solution. J Chem Theory Comput 2(4):1085–1092
91. Wittala KW et al (2007) Evaluation of various DFT protocols for computing H-1 and C-13 chemical shifts to distinguish stereoisomers: diastereomeric 2-, 3-, and 4-methylcyclohexanols as a test set. J Phys Chem 111(3):345–354
92. Willoughby JH, Jansma MJ, Hoye TR (2014) A guide to small-molecule structure assignment through computation of (H-1 and C-13) NMR chemical shifts. Nat Protoc 9(3):643–660
93. Yang J, Huang SX, Zhao QS (2008) Structure revision of hassananes with H-1 and C-13 coupling constants investigation of 3-piperidino-propylamine: a combined experimental and theoretical study. Spectrochim Acta Part A Mol Biomol Spectrosc 71(3):830–834
94. Becke AD (1993) Density-functional exchange-energy approximation with correct asymptotic-behavior. Phys Rev A 38(6):3098–3100
95. Lee CT, Yang WT, Parr RG (1988) Development of the Colle–Salvetti correlation-energy formula into a functional of the electron-density. Phys Rev B 37(2):785–789
96. Becke AD (1993) Density-functional thermochemistry. 3. The role of exact exchange. J Chem Phys 98(7):5648–5652
97. Hehe WJ (1986) Ab initio molecular orbital theory. Wiley-Interscience, New York
98. Ruiz E, Nunzi F, Alvarez S (2006) Magnetic communication through functionalized nanotubes: a theoretical study. Nano Lett 6(3):380–384
99. Johnson JRT, Panas I (2001) Water adsorption and hydrolysis on molecular Al oxides and hydroxides—solution versus cluster formation. Phys Chem Chem Phys 3(24):5482–5488
100. Becke AD (1993) A new mixing of Hartree–Fock–Slatvei correlation-energy formula into a functional of the electron-density. Phys Rev B 37(2):785–789
101. Dunning TH (1989) Gaussian-basis sets for use in correlated molecular calculations. 1. The atoms boron through neon and hydrogen. J Chem Phys 90(2):1007–1023
102. Feller D (1996) The role of databases in support of computational chemistry calculations. J Comput Chem 17(13):1571–1586
103. Schuchardt KL et al (2007) Basis set exchange: a community database for computational sciences. J Chem Inf Model 47(3):1045–1052
104. Basis Set Exchange. https://bse.pnl.gov/bse/portal. Accessed 16 Oct 2018
105. Dupuis P (2001) New integral transforms for molecular properties and application to a massively parallel GIAO-SCF implementation. Comput Phys Commun 134(2):150–166
106. Case DA, Babin V, Berryman JT, Betz RM, Cai Q, Cerutti DS, Cheatham TE, III, Darden TA, Duke RE, Gohlke H, Goetz AW, Gussarov S, Homeyer N, Janowski P, Kaus J, Kolossvary I, Kovalenko A, Lee TS, LeGrand S, Luchko T, Luo R, Madjek B, Merz KM, Paesani F, Roe DR, Roitberg A, Sagu M, Salomon-Ferrer R, Seabra G, Simmerling CL, Smith W, Svatis J, Walker RC, Wang J, Wolf RM, Wu X, Kollman PA (2014) AMBER 14, University of California, San Francisco
107. Ribeiro DS, Rittner R (2003) The role of hyperconjugation in the conformational analysis of methylcyclohexane and methylcyclohexanols. J Org Chem 68(17):6780–6787
108. Abraham RJ, Ribeiro DS (2001) Conformational analysis. Part 36. A variable temperature C-13 NMR study of conformational equilibria in methyl substituted cycloalkanes. J Chem Soc Perkin Trans 2(3):302–307
109. Freeman F, Kasner ML, Hehe WJ (2001) An ab initio molecular orbital theory study of the conformational free energies of 2-methyl-, 3-methyl-, and 4-methyltetrahydro-2H-pyran. J Mol Struct Theochem 574:19–26
110. Cascade Supercomputer. https://www.emsl.pnl.gov/services/inst ruments/computing-cascade-atiga-1440-intel-xeon-phi-node-fdr-infin iband-linux-cluster. Accessed 16 Oct 2018
111. NWChem: Open source high-performance computational chemistry. www.nwchem-sw.org. Accessed 16 Oct 2018
112. NWChem: Open source high-performance computational chemistry—Github. https://github.com/nwchemgit. Accessed 16 Oct 2018
113. Tuan NO et al (2017) A grayanotox-9(11)-ene derivative from Rhodo dendron brachycarpum and its structural assignment via a protocol combining NMR and DP4 plus application. Phytochemistry 133:45–50
114. Barone G et al (2002) Determination of the relative stereochemistry of flexible organic compounds by ab initio methods: conformational analysis and Boltzmann-averaged GIAO C-13 NMR chemical shifts. Chem Eur J 8(14):3240–3245
115. Barone G et al (2002) Structure validation of natural products by quantum-mechanical GIAO calculations of C-13 NMR chemical shifts. Chem Eur J 8(14):3233–3239
116. Remya K, Suresh Ch (2013) Which density functional is close to CCSD accuracy to describe geometry and interaction energy of small noncovalent dimers? A benchmark study using gaussian09. J Comput Chem 34(15):1341–1353
117. Zhao Y, Truhlar DG (2008) Improved description of nuclear magnetic resonance chemical shielding constants using the M06-L meta-generalized-gradient-approximation density functional. J Phys Chem A 112(30):6794–6799
118. Magyarfalvi G, Pulay P (2003) Assessment of density functional methods for nuclear magnetic resonance shielding calculations. J Chem Phys 119(3):1350–1357
119. Cimino P et al (2004) Comparison of different theory models and basis sets in the calculation of C-13 NMR chemical shifts of natural products. Magn Reson Chem 42:526–533
120. Cramer CJ, Truhlar DG (1999) Implicit solvation models: equilibria, structure, spectra, and dynamics. Chem Rev 99(8):2161–2200
121. Reddy G, Yethiraj A (2006) Implicit and explicit solvent models for the simulation of dilute polymer solutions. Macromolecules 39(24):8536–8542
122. Bagno A, Rastelli F, Saelli G (2003) Predicting C-13 NMR spectra by DFT calculations. J Phys Chem A 107(46):9964–9973
123. Wang B et al (2001) Accurate prediction of proton chemical shifts. I. Substituted aromatic hydrocarbons. J Comput Chem 22(16):1887–1895
124. Wang B, Hinton JF, Pulay P (2002) Accurate prediction of proton chemical shifts. II. Peptide analogues. J Comput Chem 23(4):492–497
125. Smirnov SN et al (1996) Hydrogen deuteration isotope effects on the NMR chemical shifts and geometries of intermolecular low-barrier hydrogen-bonded complexes. J Am Chem Soc 118(17):4094–4101
126. Benedict H et al (1996) Hydrogen/deuteration isotope effects on the N-15 NMR chemical shifts and geometries of low-barrier hydrogen bonds in the solid state. J Mol Struct 378(1):11–16
127. Gidley MJ, Boecik SM (1988) C-13 Cp/MAS NMR-studies of amylose inclusion complexes, cyclodextrins, and the amorphous phase of starch—relationships between glycosidic linkage conformation and solid-state C-13 chemical-shifts. J Am Chem Soc 110(12):3820–3829
128. Buckingham AD (1960) Chemical shifts in the nuclear magnetic resonance spectra of molecules containing polar groups. Can J Chem Rev Can Chim 38(2):300–307
129. Gauss J (1995) Effects of electron correlation in the calculation of nuclear-magnetic-resonance chemical-shifts. J Chem Phys 93(5):3629–3643
130. Lodewyck MW, Siebert MR, Tantillo DJ (2012) Computational prediction of 1H and 13C chemical shifts: a useful tool for natural product, mecha- nistic, and synthetic organic chemistry. Chem Rev 112(3):1839–1862
131. Flagg D et al (2014) Benchmarking hydrogen and carbon NMR chemical shifts at HF, DFT, and MP2 levels. J Chem Theory Comput 10(2):572–578
132. Gregor T, Mauri F, Car R (1999) A comparison of methods for the calculation of NMR chemical shifts. J Chem Phys 111(5):1815–1822
133. Teale AM et al (2013) Benchmarking density-functional theory calculations of NMR shielding constants and spin–rotation constants using accurate coupled-cluster calculations. J Chem Phys 138(2):024111

134. Wu A et al (2007) Systematic studies on the computation of nuclear magnetic resonance shielding constants and chemical shifts: the density functional models. J Comput Chem 28(15):2431–2442

135. Giesen DJ, Zumbulyadis N (2002) A hybrid quantum mechanical and empirical model for the prediction of isotropic C-13 shielding constants of organic molecules. Phys Chem Chem Phys 4(22):5498–5507

136. Jain R, Bally T, Rablen PR (2009) Calculating accurate proton chemical shifts of organic molecules with density functional methods and modest basis sets. J Org Chem 74(11):4017–4023

137. Gao HW et al (2010) Comparison of different theory models and basis sets in the calculations of structures and C-13 NMR spectra of [Pt(en)(CBDCA-O, O′)], an analogue of the antitumor drug carboplatin. J Phys Chem B 114(11):4056–4062

138. Xin D et al (2017) Development of a (1)C NMR chemical shift prediction procedure using B3LYP/cc-pVDZ and empirically derived systematic error correction terms: a computational small molecule structure elucidation method. J Org Chem 82(10):5135–5145

139. Zhang Y et al (2006) OPBE: a promising density functional for the calculation of nuclear shielding constants. Chem Phys Lett 421(4–6):383–388

140. d’Antuono P et al (2008) A joint theoretical–experimental investigation on the 1H and 13C NMR signatures of defects in poly(vinyl chloride). J Phys Chem B 112(47):14804–14818

141. Grimbilat N, Zanardi MW, Sarotti AM (2015) Beyond DP4: an improved probability for the stereochemical assignment of isomeric compounds using quantum chemical calculations of NMR shifts. J Org Chem 80(24):12526–12534

142. Navarro-Vazquez A (2017) State of the art and perspectives in the application of quantum chemical prediction of H-1 and C-13 chemical shifts and scalar couplings for structural elucidation of organic compounds. Magn Reson Chem 55(1):29–32

143. Ermanis K et al (2017) Doubling the power of DP4 for computational structure elucidation. Org Biomol Chem 15(42):8998–9007

144. Pierens GK (2014) H-1 and C-13 NMR scaling factors for the calculation of chemical shifts in commonly used solvents using density functional theory. J Comput Chem 35(18):1388–1394

145. Kohavi R (1995) A study of cross-validation and bootstrap for accuracy estimation and model selection. In: International joint conference on artificial intelligence

146. Dubitzky W, Granzow M, Berrar DP (2007) Fundamentals of data mining in genomics and proteomics. Springer, Berlin

147. Xu Q-S, Liang Y-Z (2001) Monte Carlo cross validation. Chemom Intell Lab Syst 56(1):1–11

148. Li DW, Bruschweiler R (2010) Certification of molecular dynamics trajectories with NMR chemical shifts. J Phys Chem Lett 1(1):246–248

149. Di Micco S et al (2013) Plakilactones G and H from a marine sponge. Stereochemical determination of highly flexible systems by quantitative NMR-derived interproton distances combined with quantum mechanical calculations of C-13 chemical shifts. Beilstein J Org Chem 9:2940–2949

150. Hirsch JA (1967) Table of conformational energies. Top Stereochem 1:199

151. Beckett CW, Pitzer KS, Spitzer R (1947) The thermodynamic properties and molecular structure of cyclohexane, methylcyclohexane, ethylcyclohexane and the seven dimethylcyclohexanes. J Am Chem Soc 69(10):2488–2495

152. Streitwieser A, Heathcock CH, Kosower E (1992) Introduction to organic chemistry. Macmillan, New York

153. Smith MB, March J (1992) March’s advanced organic chemistry. Wiley, New York

154. Solomons TWG (2000) Organic chemistry. Wiley, New York

155. Frisch MJ et al (2016) Gaussian 16 Rev. B.01. Gaussian, Inc, Wallingford

156. Booth H, Everett. JR (1980) Experimental-determination of the conformational free-energy—enthalpy, and entropy differences for alkyl-groups in alkylcyclohexanes by low-temperature C-13 magnetic-resonance spectroscopy. J Chem Soc Perkin Trans 2(2):255–259