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SelEXSIDE: Fast and Easy Measurement of Multiple-Bond $^{1}H,^{13}C$ Coupling Constants for Stereochemical Analysis

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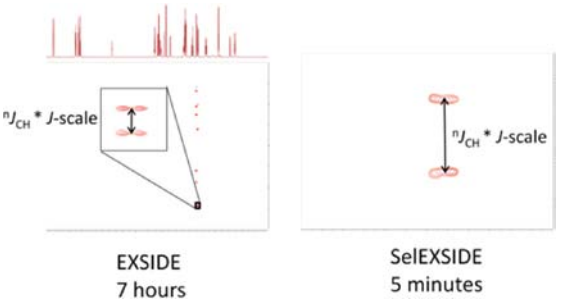
ABSTRACT



Long-range $^{1}H,^{13}C$ coupling constants ($^{n}J_{CH}$) are underutilized parameters for stereochemical structure determination, primarily because they are not easy to measure. This report describes a rapid and easily interpreted method for the measurement of one or more $^{n}J_{CH}$ values based on a ^{13}C band-selective EXSIDE (SelEXSIDE), which reduces experiment times from many hours down to a few minutes while allowing a simple and straightforward readout of $^{n}J_{CH}$ values from the resulting in-phase doublet signal.

Organic chemists are generally familiar with the use of NMR spectroscopy as a tool for studying molecular conformation and stereochemistry. It is commonly recognized that $^{3}J_{HH}$ scalar coupling constants and NOE measurements are the primary NMR parameters for this, often combined with computational methods to maximize the speed and certainty of structural elucidations, exemplified by approaches such as '*J*-based configurational analysis' to acyclic systems.[1] What is less well-recognized is that J_{CH} couplings, analogous to J_{HH} couplings, are equally facile for structure determination, albeit more challenging to measure. This is perhaps surprising as stereochemical analyses of complex molecules are often underdetermined; i.e., the number and accuracy of measured parameters may not be sufficient to account for all of the possible configurations and conformations of the system. Thus increasing both the quantity and the accuracy of NMR experimental data can be substantially beneficial to stereochemical and conformational structure determinations. This principal has been applied recently by demonstrating remarkable accuracy in the analysis of NOEs[2] which allowed the measurement of fine details of a three-dimensional structure in solution, for example identifying very small populations (~2%) of molecular conformers,[3] accurately assessing the rotational populations of a flexible alkyl chain,[4] and allowing stereochemical elucidation of contiguous quaternary centers[5] as well as semiflexible molecules.[6]

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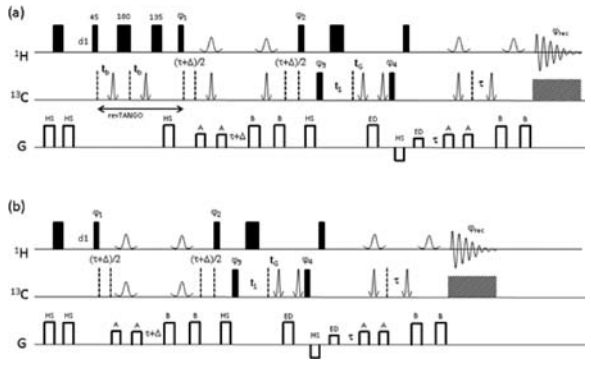

Figure 1. (a) Standard EXSIDE pulse sequence and (b) modified SelEXSIDE sequence. Narrow blocks = 90° pulse, wide blocks = 180° pulse, otherwise flip angles are noted. Narrow curves = broad-band adiabatic 180° wurst2i pulses, wide curves on ^{1}H = band-selective 180° q3 pulses, wide curves on ^{13}C = band-selective 180° wurst2i pulses. "HS" = homospoil gradients, "A" and "B" = DPFGSE echo gradients, "ED" = encode/decode selection gradients (second ED is γ_C/γ_H times the first ED gradient and is alternatingly inverted for echo-antiecho phase selection in F1). All gradients are 1−2 ms. Delays: τ = 1/(4* $^{n}J_{CH}$), Δ = t_1*J-scaling factor, t_G = encoding gradient time, t_b 1/(4 * $^{1}J_{CH}$). Phases: φ_1 = (x,x,$-x$,$-x$), φ_2 = y, φ_3 = (x,$-x$), φ_4 = (4*x, 4* − x), φ_{rec} = (x,$-x$,$-x$,x, $-x$,x,x,$-x$), all other phases are x.

In this report, we address the ease of measurement of long-range ^{13}C−^{1}H scalar coupling constants ($^{n}J_{CH}$) from NMR spectroscopy. The contribution of $^{n}J_{CH}$ values to conformational analysis is clear, as there are generally more $^{n}J_{CH}$ than $^{n}J_{HH}$ interactions in organic molecules. In particular $^{3}J_{CH}$ values have Karplus-like dependencies on dihedral angles which can be analyzed relatively easily by empirical approaches in an analogous fashion to $^{3}J_{HH}$.[7] Further, $^{2}J_{CH}$ values can give conformational detail on an electronegative substituent, typically O and N. Unfortunately, in practical terms $^{n}J_{CH}$ values are challenging to extract from NMR spectra, partially because of the low natural abundance and insensitivity of the ^{13}C nucleus, but also due to the coevolution of homonuclear (J_{HH}) as well as the desired heteronuclear (J_{CH}) couplings which complicate the resulting spectra. Current methods for measuring $^{n}J_{CH}$ generally suffer from at least one of a number of problems: complex line shapes, e.g. HR-HMBC,[8,9] which require line-shape fitting in order to extract J_{CH}; TOCSY-based methods[10,11] are unable to measure J_{CH} values for quaternary carbons. Complex pulse trains and multiple magnetization transfers may lead to substantial signal loss.[10,12] Finally, the dominance of ^{12}C isotopomers complicates operation and interpretation of 1D methods,[11] and substantial data collection times are typically required for 2D-methods which encode J_{CH} in the F1 dimension due to the requirement for large numbers of F1 (^{13}C) data points, e.g. EXSIDE[12] or HR-HMBC.[8,9] Clearly more straightforward and rapid methods for establishing one or more $^{n}J_{CH}$ values will be of substantial benefit to organic chemists.[13]

In our experience utilizing NMR methods for measuring J_{CH}, EXSIDE[12] is among the easiest to use as it gives a simple in-phase doublet in the F1 (^{13}C) dimension corresponding to each $^{n}J_{CH}$ value. This doublet gives $^{n}J_{CH}$ very easily by simply dividing the splitting by the user-selected J-scaling factor (which makes couplings of a few Hz measurable with "only" a few hundred t_1 increments). The downside of EXSIDE, shared with most 2D-methods of this sort, is the substantial experiment time, typically hours required to measure those hundreds of t_1 increments.

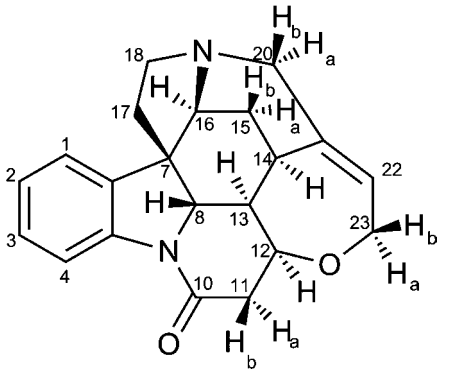

Figure 2. Labeled structure of strychnine.

Herein, we demonstrate that the time constraint of EXSIDE can be essentially eliminated through its conversion to be doubly selective, i.e. in *both* the ^{13}C and ^{1}H domains, hereafter referred to as the SelEXSIDE experiment. The SelEXSIDE sequence reduces a multihour experiment to a matter of minutes for each coupling constant measured.

A standard pulse sequence for the EXSIDE experiment is shown in Figure 1a which includes optional gradient-90-gradient purge pulses and reverse-TANGO[14] one-bond suppression elements. The critical elements of this sequence are the DPFGSE steps incorporating excitation-sculpted ^{1}H 180° pulses which refocus the ^{1}H−^{1}H homonuclear coupling in the F1 dimension for the selected proton(s) while retaining ^{1}H−^{13}C coupling evolution.

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(13) During preparation of this paper, an elegant solution to the global measurement of $^{n}J_{CH}$ values was reported by Parella et al. through the HSQMBC-TOCSY experiment, by which the sign and magnitude of the majority of couplings in a molecule can be measured in a single 2D experiment in 1−2 h. Sauri, J.; Espinosa, J. F.; Parella, T. *Angew. Chem., Int. Ed.* **2012**, *51*, 3919–3922.

(14) For a discussion of these elements, see: Claridge, T. D. W. *High-Resolution NMR Techniques in Organic Chemistry*; Elsevier Science & Technology, Elsevier Science Ltd.: Oxford, U.K., 2008.

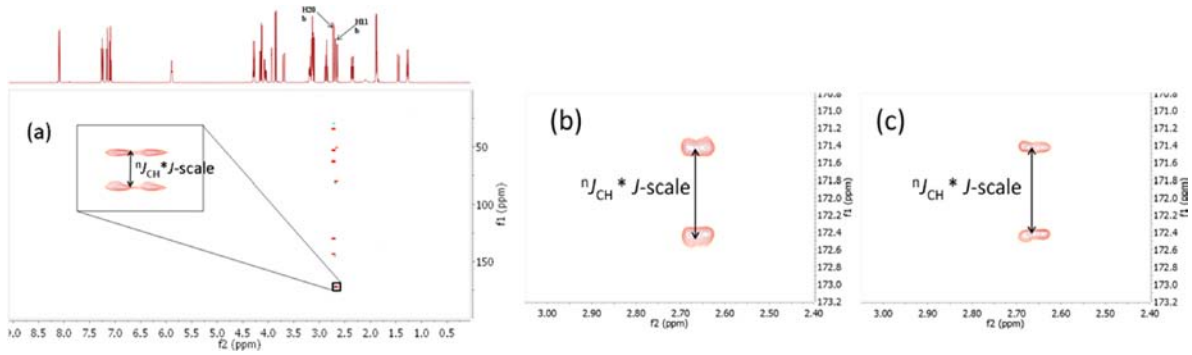

Figure 3. (a) EXSIDE spectrum (> 7h) for H11b and H20b of strychnine with H11b-C10 highlighted (inset) with 200 ppm F1 width, 1675 t_1 increments, 4 scans/increment. (b) SelEXSIDE spectrum (22 min) of H11b-C10 with 10 ppm F1 width, 83 t_1 increments, 4 scans/increment. (c) SelEXSIDE spectrum (4 min) of H11b-C10 with 4 ppm F1 width, 32 t_1 increments, 2 scans/increment.

The $^1H-^{13}C$ coupling is also *J*-scaled (by a factor of ~15 typically) in order to reduce the F1-resolution demands of the experiment. An EXSIDE spectrum for H20b and H11b of strychnine (Figure 2) is shown in Figure 3a (full details in Supporting Information) giving seven correlations corresponding to H20b-C14/C16/C18/C22/C21 and H11b-C12/C10. All correlations are simple in-phase doublets, with splittings of 77, 101, 41, 64, 30, 102, and 119 Hz, respectively (see Table S1) corresponding to J_{CH} values of 5.1, 6.7, 2.7, 4.2, 2.0, 6.8, and 7.9 Hz (*J*-scaling factor of 15). However, to obtain sufficient F1 resolution to measure *J*-scaled splittings as small as 15 Hz (i.e., resolve J_{CH} of ~1 Hz), this experiment required ~7.5 h to run. This is clearly a substantial amount of NMR instrument time to expend in establishing only a few J_{CH} values, with only some being of interest.

The SelEXSIDE pulse sequence (Figure 1b) was generated[15] by replacement of the third and fourth ^{13}C inversion pulses in the EXSIDE sequence (Figure 1a) with band-selective (wurst2i) excitation sculpted pulses, in an analogous fashion to that reported for band-selective HMBC.[16] This allows a substantial reduction (up to ~50-fold) in the ^{13}C-spectrum width and, thus, the number of required t_1 data points, without the danger of any aliased signals folding on top of the signal(s) of interest. Essentially this retains high F1 resolution (~15 Hz with a *J*-scaling factor of 15) but with up to an ~50-fold saving in experiment time.

Figure 3b and 3c show SelEXSIDE spectra for the H11b-C10 correlation (*cf.* full EXSIDE correlation, inset of Figure 3a) which were obtained with 10 ppm (b) and 4 ppm (c) ^{13}C windows in 22 and 4 min respectively. The SelEXSIDE spectra were measured with identical F1 resolution to the full EXSIDE spectrum shown in Figure 3a and gave comparable $J_{H11b,C10}$ values; however the latter experiment required ~7.5 h to collect. The 4 ppm ^{13}C window spectrum in Figure 3c was collected with only 2 scans/increment (rather than 4 scans/increment for the other two data sets) in order to demonstrate a reasonable minimum experiment time for SelEXSIDE spectra. It should be noted that further reductions in the ^{13}C spectrum window can be made in theory (down to around 200 Hz/1.5 ppm with a *J*-scaling of 15); however this exacerbates the well-established loss of signal in *J*-scaled 2D experiments (such as EXSIDE) from relaxation during long *J*-scaled t_1 delays. A more detailed discussion of this can be found in the Supporting Information.

To verify the reliability of the SelEXSIDE sequence, all of the measurable 2- and 3-bond $^1H-^{13}C$ coupling constants for strychnine were determined in a series of data collections (Table S1, Supporting Information). These gave $^nJ_{CH}$ values which are comparable to experimental[17] and calculated[18] values reported previously. Only one $^nJ_{CH}$ value ($^3J_{H12,C10}$) measured by SelEXSIDE differed by > 1.5 Hz from the average of current literature data; $^3J_{H12,C10}$ was assessed by SelEXSIDE to be 1.7 Hz, while Edden et al. had previously reported an experimental value of 5.8 Hz. The SelEXSIDE data for $^3J_{H12,C10}$ are, however, in line with the value calculated by Bagno et al. (1.3 Hz). Further, inspection of the crystallographic[19] and computed[18] geometries of strychnine found that the H12−C12−C11−C10 dihedral angle is ~109° which corresponds to a coupling constant of ~1−2 Hz; hence the SelEXSIDE result appears to be robust.

It should be noted that J_{CH} values for the strongly coupled AB systems of H17a/b and H23a/b could not be measured using SelEXSIDE as the correlations appear as multiplets in the F1 dimension in these cases. This multiplicity arises from a breakthrough of the $^1H-^1H$ scalar coupling within the AB systems. This is a known limitation of the EXSIDE approach, for which the ^1H-selective pulse

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should only be applied to multiple spins if they are not coupled to each other.[12] One further potential limitation is sensitivity, where several milligrams of material are needed in order to measure the spectrum in only 2 or 4 scans. This limited sensitivity is not out of line with other standard proton-detected multiple bond correlation experiments, e.g. HMBC, but will be exacerbated where fast T2-relaxation can further reduce signal intensities; again this is also true of all *J*-scaled 2-D experiments such as EXSIDE.

In summary, we present the SelEXSIDE experiment for measuring $^nJ_{CH}$ values as a rapid alternative to the readily interpreted, but very slow, EXSIDE experiment. SelEXSIDE allows $^nJ_{CH}$ values to be assessed in minutes rather than hours, with no significant data processing or interpretation required other than measuring the separation between peaks of the resulting in-phase doublets. While only one or two couplings might be measured in any given SelEXSIDE experiment, the ~50-fold improvement in experiment time makes multiple selective experiments both practical and possible.

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Supporting Information Available. Larger-scale image of pulse sequence, general experimental details, full table of $^nJ_{CH}$ couplings for strychnine, notes regarding sensitivity, and details of implementation in Agilent VNMRJ software. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.