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^{1}H Indirect Detected ^{13}C Low-Abundance Single-Transition Correlation Spectroscopy (HICLASS)—^{13}C Homonuclear Correlation at Natural Abundance

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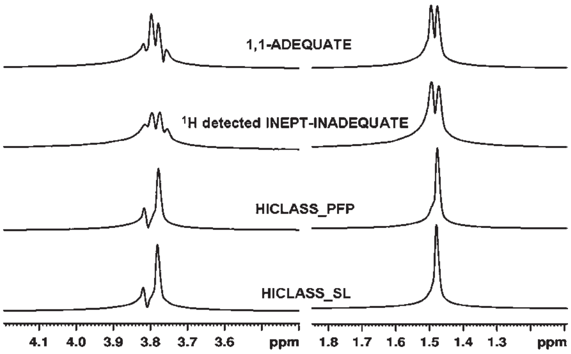
ABSTRACT



A novel proton-detected ^{13}C homonuclear correlation experiment is reported at natural abundance, viz., ^{1}H Indirect detected ^{13}C Low-Abundance Single-transition correlation Spectroscopy (HICLASS). HICLASS is based on the evolution of ^{13}C single-quantum single transitions, followed by their mixing, and ^{1}H detection subsequent to heteronuclear transfer. Reduced relaxation losses during the evolution time and partial selectivity in the ^{1}H multiplet structure result in enhanced sensitivity of HICLASS. The superior performance of HICLASS is demonstrated for ^{1}H-detected ^{13}C correlation work.

INADEQUATE[1] is arguably the nearest that NMR comes to directly visualizing bonding, and it is employed to trace the bonding topology of organic molecules at natural ^{13}C abundance. 2D INADEQUATE[2−11] maps ^{13}C pair correlations by suppressing signals from isolated ^{13}C spins (i.e., those that have no homonuclear coupling). However, the sensitivity of this experiment is low by definition, and there have been ongoing efforts to improve it in this regard. Strategies for sensitivity enhancement belong broadly to one of two categories, viz., direct and indirect detection methods, depending on the observed nucleus. In direct detection mode, it appears optimal to employ transition-selective experiments.

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2D INADEQUATE CR[12–16] and CLASSY[17]/LASSY[18] are two different experiments of this genre, both employing optimal double-quantum (DQ) reconversion, leading to ^{13}C single transitions (STs) instead of doublets. However, CLASSY/LASSY is optimal with respect to relaxation losses in addition, since it involves evolution of STs, which relax slower than DQ coherences (DQCs).

A fundamentally different approach for sensitivity enhancement in ^{13}C correlation spectroscopy involves the reverse transfer of the ^{13}C correlation information to the abundant spin (usually, ^{1}H) that is bound to one member of the ^{13}C pair, enabling "indirect" (^{1}H) detection. In principle, ^{1}H detection could improve sensitivity by a factor of up to 32 in comparison to the corresponding direct detection ^{13}C DQ experiments. In practice, however, this enhancement factor is reduced considerably due to the generation of "unused" coherence components in the indirect detection mode, as well as the presence of homonuclear ^{1}H couplings which distribute the available signal over a number of ^{1}H multiplet components. Further, the enhancement due to the INEPT front-end module in the indirect detection mode is partially offset by the NOE in the direct detection mode. Nevertheless, indirect detection remains advantageous in terms of enhanced sensitivity.

Various indirect detection experiments for rare spin correlation work, such as INSIPID (INadequate Sensitivity Improvement by Proton Indirect Detection),[19] ^{1}H-detected INEPT-INADEQUATE,[20] ADEQUATE,[21–24] and ^{1}H-detected DEPT-INADEQUATE,[25] are all based on the evolution of ^{13}C DQC and correlate ^{13}C DQ frequencies with the coupled ^{1}H single-quantum (SQ) frequency. In principle, two experiments are required to identify the two partners in the ^{13}C DQC unequivocally; covariance processing of ADEQUATE-HSQC data has been proposed recently as one possible approach.[26,27] Modified versions of ^{1}H-detected INEPT-INADEQUATE and ADEQUATE, including J-resolved and J-modulated (JM-ADEQUATE) methods, are also available for ^{13}C$-^{13}$C coupling measurements[28–34] in this milieu.

The present work reports the development of the indirect detected version of ^{13}C ST correlation spectroscopy, seeking to combine the advantages of the indirect detection mode with those of the transition-selective mode—the result is a new indirect detection experiment with enhanced sensitivity for ^{13}C correlations. We term this sequence HICLASS (^{1}H Indirect detected Carbon Low-Abundance Single-transition correlation Spectroscopy). In HICLASS, ^{1}H magnetization is first transferred to a coupled ^{13}C through a standard INEPT module, followed by preparation of ^{13}C DQC. This is immediately converted to ^{13}C SQ STs employing a composite refocusing (CR) module. Following their time evolution, homonuclear coherence transfer of STs is effected by coherence order-selective (COS) mixing,[35] which has two alternative implementations. Finally, ^{13}C STs are transferred to coupled protons by a heteronuclear COS[36] module. Homonuclear mixing and heteronuclear transfer may be optimized jointly by setting the duration of the former to $(1-x)$ times its nominal value ($J_{CC}{}^{-1}$), with $x = J_{CC}/J_{CH}$.

Calculation of the HICLASS experiment for an $H_1-C_1-C_2-H_2$ fragment shows that this sequence results in a "remote"correlation, e.g., (H_1-C_2) between a proton (H_1) and the carbon (C_2) that is coupled to the one bearing H_1, while HICLASS with "no mixing" (NM) gives the direct (H_1-C_1) correlation; *both spectra result from molecules with* C_1-C_2 *pairs*. From these two experiments we may infer the ^{13}C$-^{13}$C correlations, e.g., C_1-C_2 in this case. More simply, the HICLASS experiment with mixing could be compared with the standard HSQC experiment to trace ^{13}C correlations.

Remarkably, POMA[37] calculations and experiments show that the following ^{1}H multiplet patterns result in F_2 from various molecular fragments, under the influence of intrafragment ^{1}H$-^{1}$H couplings:

HC−CH:both doublets are reduced to STs

H$_2$C−CH:the CH$_2$ doublet reduces to an ST; the CH triplet remains as such

H$_3$C−CH:the CH$_3$ doublet is reduced to an ST; the CH quartet reduces to two transitions separated by $2J_{HH}$

H$_2$C−CH$_2$:two triplets result

H$_3$C−CH$_2$:the CH$_3$ triplet remains as such; the CH$_2$ quartet reduces to two transitions separated by $2J_{HH}$

It may be noted that *all reductions in multiplicity are accompanied by sensitivity enhancement*.

Thus, HICLASS goes some way in optimizng the sensitivity enhancement of indirect detection experiments by reducing the multiplet structure arising from intrafragment homonuclear ^{1}H couplings, and it also benefits from reduced relaxation losses in t_1; these gains typically overcome relaxation losses during the

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extended DQ reconversion and mixing modules by a substantial margin.

The efficiency of HICLASS is to be compared with that of ADEQUATE and ^{1}H-detected INEPT-INADEQUATE. Since the ST evolution increment in HICLASS is double that in experiments that involve DQ evolution, HICLASS may be performed with half the number of evolution increments ("1 td") and double the number of scans for comparisons of efficiency. This choice ensures matched evolution windows as well as equal measurement time. Figure 1 shows the comparison of HICLASS with other known methods for 0.05 M 2,3-^{13}C-α-alanine in D_2O.

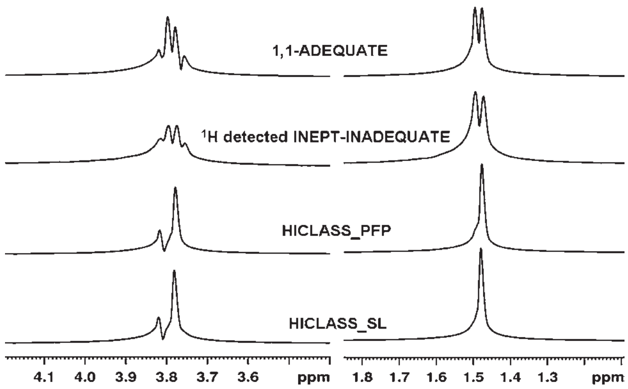

Figure 1. F_2 partial sum of the "remote" correlation for the high-field peak (right) and the low-field peak (left) of 0.05 M 2,3-^{13}C-alanine in D_2O. Two variants of HICLASS, that implement the homonuclear COS module under "spin lock" (SL) or under "pulsed free precession" (PFP), are benchmarked. The former requires only half the mixing time of the latter but employs relatively high radio frequency fields for the duration $(2J_{CC})^{-1}$.

The predicted partial transition selectivity of HICLASS is clearly observed in Figure 1. Partial transition selectivity may, of course, be switched to other multiplet components by changing the component selection in the CR module.

On comparing the F_2 partial sums of the methyl signal, HICLASS under "spin lock" (SL) is seen to have 32% higher signal intensity in comparison to 1,1-ADEQUATE and 26% more in comparison to ^{1}H-detected INEPT-INADEQUATE, while HICLASS under "pulsed free precession" (PFP) has 27% and 21% higher intensity, respectively. For the low-field peak, HICLASS SL results in 23% and 81% higher intensity in comparison to ADEQUATE and ^{1}H-detected INEPT-INADEQUATE, respectively, while HICLASS PFP gives 13% and 67% higher intensity, respectively. The extended mixing time of the PFP version reduces the sensitivity enhancement in HICLASS PFP in comparison to HICLASS SL.

Figure 2 shows the HICLASS spectra of 0.5 M sucrose in D_2O at natural abundance. As a typical exercise in identifying coupled ^{13}C partners, we may note the following: on comparing HICLASS NM (Figure 2a) with HICLASS SL (Figure 2b) or with HICLASS PFP (Figure 2c), the G1, 1 correlation in the former spectrum is seen to have moved to a new frequency in the ^{13}C dimension in the latter two cases, resulting in peaks designated G2,1, all at the same frequency in the ^{1}H dimension. This signifies that C_1 is coupled to C_2; i.e., C_1 is adjacent to C_2 in the molecule. [The first number in the peak designation indicates the carbon index, while the second number indicates the proton index.] By similar analysis of all the observed correlations, we may trace the carbon skeleton of the molecule.

The enhanced sensitivity of HICLASS SL compared to ^{1}H-detected INEPT-INADEQUATE and 1,1-ADEQUATE is clearly demonstrated from the F_2 partial sums shown in Figure 3. HICLASS reduces the active (intrafragment) proton splitting to a ST (e.g., proton coupling between $F(H_3)$ and $F(H_4)$ reduces to proton STs), whereas the passive spin (extrafragment) proton splitting, if any, remains intact (e.g., for the $F(C_2{-}H_3)$ correlation, the passive coupling to $F(H_4)$ remains).

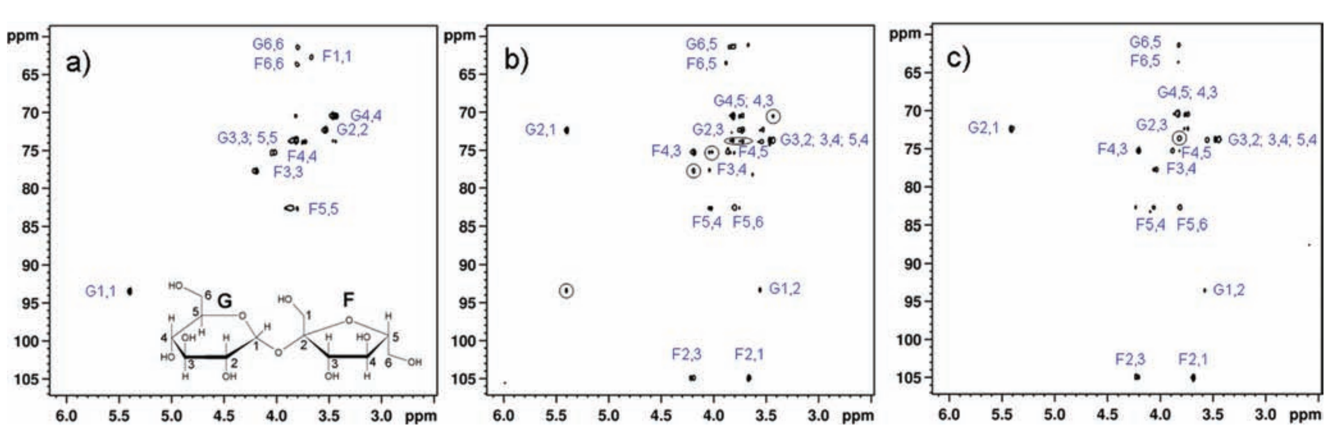

Figure 2. (a) HICLASS NM, (b) HICLASS SL, and (c) HICLASS PFP. Circled peaks correspond to the unsuppressed direct correlations.

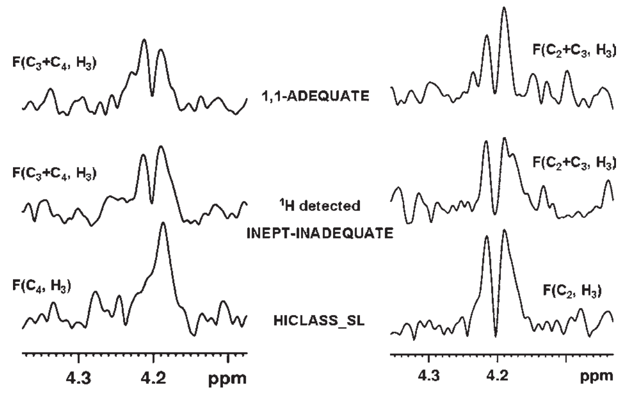

Figure 3. F_2 partial sum of the remote correlation from two representative sites for 0.5 M sucrose in D_2O.

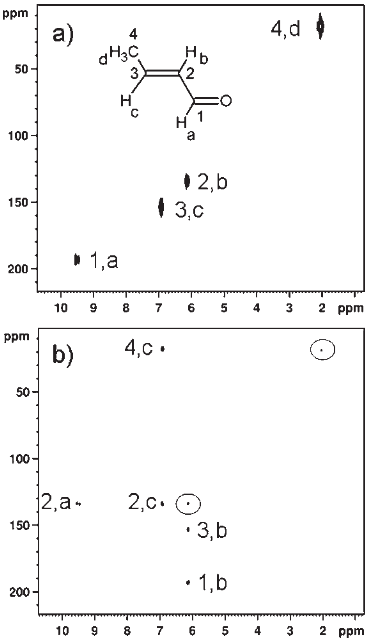

Figure 4. (a) HSQC and (b) HICLASS DBR spectra of crotonaldehyde in $CDCl_3$. Circled peaks are the unsuppressed direct correlations.

In order to compensate strong offset effects that arise with the wide spectral widths typical in ^{13}C NMR, we have employed composite pulses[38] in the HICLASS experiments, replacing all pulses in the ^{13}C channel by the corresponding composite pulses in the PFP version of HICLASS. Figure 4b shows the HICLASS spectrum of crotonaldehyde in $CDCl_3$ obtained using the composite pulse version of HICLASS PFP. On comparing the HICLASS and HSQC spectra, it is clear that the (3,c) correlation in the HSQC spectrum has shifted to two new frequencies in F_1 for HICLASS, viz., (2,c) and (4,c), both at the same F_2 frequency. This signifies that C_3 is coupled to both C_2 and C_4; i.e., C_3 is adjacent to two other carbons in the molecule, viz., C_2 and C_4. By similar analysis of all the observed correlations, we may trace the molecular carbon skeleton.

For intensity comparison, ADEQUATE and ^{1}H-detected INEPT-INADEQUATE were also performed with composite pulses. Traces of F_2 partial sums from two representative sites are shown in Figure 5. On average, HICLASS PFP gives 26% extra intensity in comparison to ADEQUATE and 33% extra in comparison to ^{1}H-detected INEPT-INADEQUATE; it also exhibits considerably reduced spectral crowding. The SL version of HICLASS may not be expedient for wider spectral widths, as the radio frequency amplitude required for SL tends to become larger than commercial solution-state probeheads can handle safely.

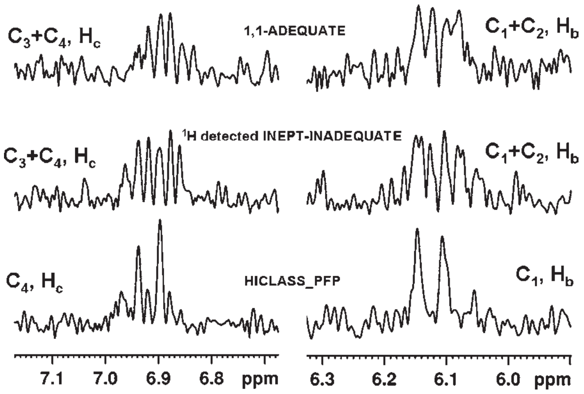

Figure 5. F_2 partial sum of the remote correlation from two representative sites for crotonaldehyde in $CDCl_3$.

We have also employed successfully the composite pulse version of HICLASS PFP to trace the ^{13}C skeleton of a larger molecule, strychnine. The details and the spectrum are given in the Supporting Information.

In conclusion, we have demonstrated a novel indirect detection experiment with enhanced sensitivity for rare spin correlation spectroscopy, and we have shown its superior performance over methods reported in the literature.

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Supporting Information Available. Pulse sequence diagrams, experimental details, and density matrix calculation. This material is available free of charge via the Internet at http://pubs.acs.org.

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