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Band-Selective 2D HSQMBC: A Universal Technique for Detection and Measurement of ^{35,37}Cl Isotope Effects for ^{13}C Nuclei

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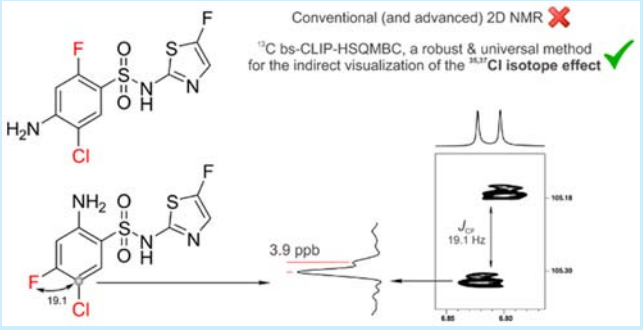
Supporting Information

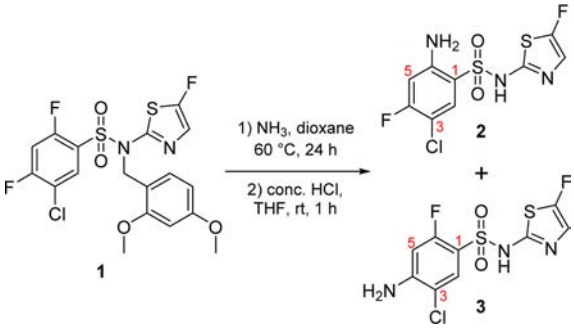
ABSTRACT: A novel technique that allows efficient measurement of the ^{35,37}Cl isotope pattern for any ^{13}C resonance has been developed. The band-selective CLIP-HSQMBC experiment is reliable and universally applicable for the indirect measurement of the ^{35,37}Cl isotope shift of ^{13}C resonances. The experiment provides advantages over conventional 1D ^{13}C NMR and the recently developed bs-HSQC experiments. The utility and performance of the bs-CLIP-HSQMBC experiment is demonstrated for polyhalogenated synthons in a synthetic route and for a polyhalogenated marine natural product.



Isotope effects have been utilized extensively in reaction mechanism studies and to a much lesser extent in NMR spectroscopy. The earliest report of ^{35,37}Cl isotope effects on ^{13}C chemical shifts was the 1975 communication by Buchner and Scheutzow[1] in a study of the assignments of polychlorinated hydrocarbons. That initial study was followed by further studies of chlorinated hydrocarbons.[2−5] These early reports demonstrated a range of isotope effects for δ ^{13}C{^{35,37}Cl} from −3.4 to −14.0 ppb. ^{35,37}Cl isotope effects have been successfully utilized to unequivocally assign ^{13}C shifts of chlorine-bearing carbons when traditional NMR techniques could not facilitate the assignment.[6−9] Most recently, Molinski and co-workers[10] reported the utilization of a band-selective HSQC (bs-HSQC) 2D NMR experiment to observe ^{35,37}Cl isotope effects that allowed them to differentiate protonated chlorine-bearing from bromine-bearing aliphatic carbons in a series of polyhalogenated marine natural products isolated from red algae and sponges. While very useful for protonated chlorine-bearing carbons, the bs-HSQC technique is not applicable to quaternary carbons. Also of note is the fact that ^{35,37}Cl isotope effects are significantly smaller for quaternary sp^2 carbons than for protonated carbons,[1,2] thus making them more difficult to observe. While more challenging, unambiguous identification of chlorinated quaternary carbons can be especially critical for structure elucidation of proton-deficient molecules as the vast majority of NMR structure elucidation experiments are proton-based.[11] Until now, the only way to measure ^{35,37}Cl isotope effects for quaternary carbons was by using 1D ^{13}C NMR. This approach requires larger samples and time-consuming data acquisition. Here, we describe the utilization of a modified, ^{13}C band-selective HSQMBC experiment to observe ^{35,37}Cl isotope effects for quaternary carbons, an experiment that evolved from the need for unequivocal assignments of polyhalogenated synthons for a planned synthetic scheme. We have expanded the application to NMR assignments of a polyhalogenated marine natural product that is independent of *ab initio* calculations (DFT) of ^{13}C chemical shift or estimations based on empirical substituent chemical shift increments.

In a discovery chemistry research effort, the S_NAr reaction of the difluorobenzenesulfonamide **1** with NH_3 (Scheme 1), followed by deprotection of the DMB group, often leads to two regioisomers, e.g., putative structures **2** and **3**, that result from alternate displacements of fluoride leaving groups. The ability

Scheme 1. Polyhalogenated Synthons



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to unambiguously assign the structure of the product from the two possible constitutional isomers is critical in planning subsequent reactions and commonly occurs in broader drug discovery efforts within this chemical space. The structure elucidation of compounds **2** and **3** by NMR is purely an issue of regiochemistry of the nucleophilic substitution reaction; however, this deceptively simple problem could not be successfully addressed by conventional 1D and 2D NMR techniques. ^{1}H NMR shows only J_{HF} coupling constants ($^{3}J_{H5-F}$ = 11.7 Hz, $^{4}J_{H2-F}$ = 8.2 Hz), which do not differentiate **2** from **3**. In principle, with sufficient material to acquire a ^{13}C spectrum, the resonances of the halogenated phenyl ring could be assigned on the basis of $^{n}J_{CF}$ coupling constants and chemical shift considerations. However, in the present case, this was not possible because predicted ^{13}C chemical shifts for the chlorine- and sulfonamido-bearing carbons were nearly identical (see Figure S1). Likewise, HMBC spectra (see Figure S2) could not be used to unequivocally assign the structures because of the indeterminate nature of the $^{n}J_{CH}$ couplings, where n = 2−4. 1,1-ADEQUATE[12] data, which establish carbon−carbon connectivities separated by one bond, can identify the carbons directly adjacent to the two protonated carbons but cannot differentiate the structures without resorting to chemical shift considerations. The 1,*n*-ADEQUATE[13] experiment is hampered by the same ambiguities of the HMBC experiment, while the INADEQUATE[14] experiment, in addition to burdensome sample requirements, is hampered by the same ambiguities of the 1,1-ADEQUATE experiment.

A method that can unambiguously resolve constitutional isomers relies on the visualization of the ^{35,37}Cl isotope effect on the chlorine-bearing quaternary carbon, which would immediately establish the position of the fluorine-bearing carbon, *para* vs *ortho* to the chlorine, based on the presence (or absence) of the typical $^{2}J_{CF}$, which is at least 1 order of magnitude larger than a $^{4}J_{CF}$. Thus far, only 1D ^{13}C NMR experiments have been reported in the literature to show the ^{35,37}Cl isotope pattern of nonprotonated carbons.[1−9] This approach, however, is time-consuming, depends on sample availability, and requires the acquisition of extremely high-resolution ^{13}C spectra to differentiate the two isotopomers with expected chemical shift differences $\Delta\delta$ of 3−5 ppb. Observation of ^{35,37}Cl isotope patterns in 1D ^{13}C NMR may also be hampered by resonance overlap.

Interrogation of the chlorine-bearing carbon from a 2D long-range heteronuclear correlation experiment provides an elegant alternative to direct ^{13}C observation. Because of the resolution requirement of $\Delta\delta$ 3−5 ppb, optimal lineshapes of the observed cross-peak multiplet structures, in addition to sufficient F_1 resolution, are both critical. To avoid any distortion in the line shape due to J_{HH} evolution and to generate pure in-phase multiplets with respect to both J_{HH} and $^{n}J_{CH}$, we used selective ^{1}H inversion pulses during the INEPT transfers of a modified refocused HSQMBC pulse sequence. Unlike the recently reported bs-HSQC,[10,15] the use of selective inversion proton pulses to avoid J_{HH} modulation is necessary in long-range heteronuclear correlation experiments since both J_{HH} and $^{n}J_{CH}$ evolve during the INEPT transfers because they are of the same magnitude. A selective refocusing carbon pulse is also used to ensure the required ultrahigh F_1 digital resolution. The pulse sequence used for this purpose, namely band-selective CLIP-HSQMBC (bs-CLIP-HSQMBC), is a slight modification of the CLIP-HSQMBC[16] experiment, which incorporates the band-selective carbon-refocusing pulse mentioned above (see Figure S7). This pulse sequence was originally proposed for the accurate measurement of long-range heteronuclear coupling constants.[16−18]

The feasibility of observing the chlorine isotope effect was investigated using the bs-CLIP-HSQMBC experiment by interrogating the carbons in question: C1 and C3 in compounds **2** and **3**. The starting point was always proton H5 since it correlates strongly to both carbons in both compounds through a $^{3}J_{CH}$ heteronuclear coupling. The bs-CLIP-HSQMBC on compound **2** revealed a ^{35,37}Cl isotope shift pattern for the carbon resonating at δ 105.2 ppm. A J_{CF} coupling constant of 19.5 Hz establishes this chlorine-bearing carbon as *ortho* to the fluorine-bearing carbon, thus unequivocally assigning the regiochemistry. In contrast, when the carbon resonating at δ 120.3 ppm was selectively refocused, no chlorine isotope shift was observed. In this case, the J_{CF} coupling constant was measured as 2.1 Hz, which is consistent with a four-bond coupling. As a consequence, the sulfonamido-bearing carbon must be in the *para*-position with respect to the fluorine atom, which further corroborates that compound **2** is the reaction product with the chlorine and the fluorine *ortho* to each other.

As illustrated in Figure 1, optimal parameter choices in data processing can significantly improve the ease of observation and

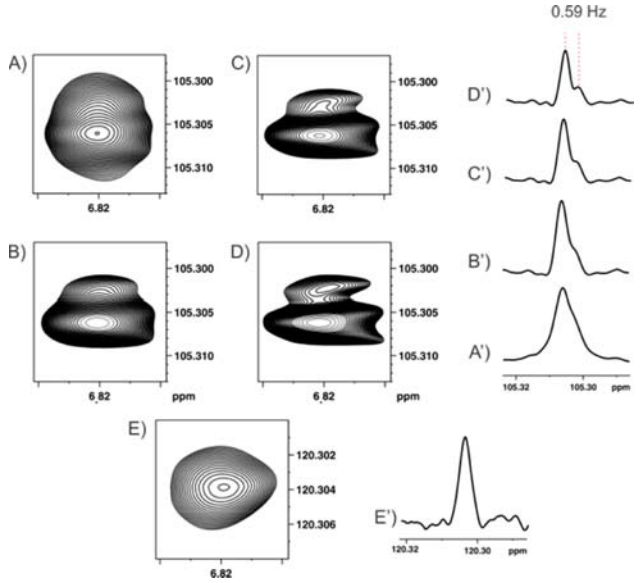

Figure 1. bs-CLIP-HSQMBC spectra in compound **2** after selective inversion of proton H5 resonating at 6.82 ppm and selective refocusing of (A−D) the carbon at 105.2 ppm and (E) the carbon at 120.3 ppm. 1D vertical slices are shown in panels A′−E′.

measurement of the isotope effect. When a conventional cosine-squared apodization function is used (Figure 1A,A′) the chlorine isotope shift is almost imperceptible. A traditional approach to extract isotope shift information from such data would be to use signal deconvolution. The deconvolution of bs-CLIP-HSQCMBC data processed using conventional cosine-squared apodization afforded a ^{35,37}Cl isotope shift value of 3.4 ± 0.4 ppb (see Figure S8 for details). However, resolution enhancement provides a simpler and easier way to visualize the ^{35,37}Cl isotope pattern. When a 90°-shifted Gaussian apodization function is applied in order to achieve resolution enhancement, the chlorine isotope shift becomes visible albeit

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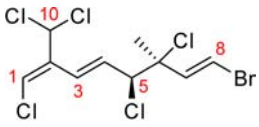
at the expense of the signal-to-noise ratio. After applying Lorentzian broadening (lb) of −1.0 Hz and a Gaussian broadening (gb) factor of 0.30 (Figure 1B,B′) and 0.35 (Figure 1C,C′), the chlorine isotope shift in compound **2** began to emerge. When a gb of 0.40 was applied (Figure 1D,D′), the isotope shift, 3.9 ppb (0.59 Hz), was resolved and in good agreement with deconvolution results. Generally, as the magnitude of the isotope shift diminishes in size, the optimal gb parameter will be progressively larger. In practice, the choice of resolution enhancement parameters should be based on the expected range of the isotope values.[1,2] Further details for processing parameters are found in Figure S9. No isotope effect was observed for the non-chlorine-bearing carbon resonating at δ 120.3 ppm even with resolution-enhancement processing (Figure 1E,E′).

As reported in several previous studies,[1−9] the chlorine isotope shift can, in principle, also be observed from the 1D ^{13}C NMR spectrum if acquired with sufficiently high resolution. For comparison purposes, we acquired a ^{13}C NMR spectrum with a spectral width of 12 ppm centered at the chlorine-bearing quaternary carbon frequency. Due to poor signal-to-noise, the data were unusable for detection of the ^{35,37}Cl isotope shifts when acquired with the same total acquisition time as the bs-CLIP-HSQMBC (75 min). Even when ^{13}C NMR acquisition was extended to 14 h, the chlorine isotope shift on the chlorine-bearing quaternary carbon was only barely observed, and both sensitivity and resolution were insufficient for accurate measurement (see Figure S10). Hence, the bs-CLIP-HSQMBC experiment provides an elegant and efficient means for reliable detection and accurate measurement of ^{35,37}Cl isotope shifts for ^{13}C resonances, thus leading to answers to challenging regiochemical questions.

To further evaluate the potential of the bs-CLIP-HSQMBC experiment, we also acquired data on a limited sample (0.50 mg) of a polyhalogenated triene marine natural product **4** (Scheme 2). Historically, the assignments and placement of Br and Cl on carbons in polyhalogenated terpenoids have been notoriously equivocal due to deviations of expected chemical shift from predictions of δ based on simple additivity rules alone.[19] The issue is underscored by the fact that several structures of such natural products were incorrectly assigned and had to be revised.[9,20−22] The known bromopentachloroterpene **4** was purified from MeOH extracts of red alga *Plocamium cartilagineum* using a modification of the procedure described by Mynderse and Faulkner.[23]

Scheme 2. Bromopentachloroterpene



4

We initially acquired two bs-CLIP-HSQMBC data sets for **4** in order to measure the ^{35,37}Cl isotope shift of the chlorine-bearing quaternary carbon C6. Selective inversion of the H9 methyl resonance and selective inversion of the H5 methine proton were employed as both correlate via $^{2}J_{CH}$ to chlorine-bearing C6. As in the previous example, observation and measurement of the isotope shift is facilitated by the optimal choice of the processing parameters to provide the necessary resolution enhancement (see Figure 2). Resolution of the

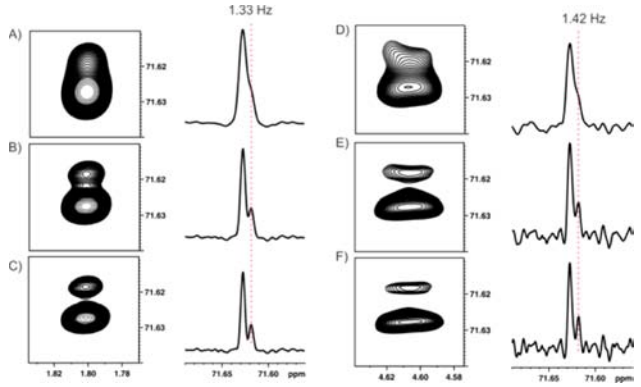


Figure 2. bs-CLIP-HSQMBC spectra in compound **4** after selective inversion of the methyl protons resonating at 1.80 ppm (panels A−C) and the methine proton resonating at 4.61 ppm (panels D−F). Refocusing carbon selective pulse was applied at the carbon chemical shift of 71.6 ppm in all cases.

^{35,37}Cl isotope shift (Figure 2A,D) begins to appear after a gb factor of 0.075 is applied, while a fully resolved isotope shift is clearly visible when gb factors of 0.15 (Figure 2B,E) and 0.2 (Figure 2C,F) are applied. Both experiments show comparable results: 8.9 ppb (1.33 Hz) and 9.5 ppb (1.42 Hz). Using a 1D ^{13}C experiment, the ^{35,37}Cl isotope shift of carbon C6 was measured to be 8.9 ppb (1.33 Hz). Consistent with observations made previously for **2** and **3**, the bs-CLIP-HSQMBC experiment yielded higher quality data than 1D ^{13}C data even when its total acquisition time was one-quarter of the directly observed ^{13}C experiment (see Figure S11).

The universality of bs-CLIP-HSQMBC was demonstrated by observing the ^{35,37}Cl isotope effect for a protonated carbon, C5. Since C5 is protonated, the same isotope effect could be measured using either bs-CLIP-HSQMBC or bs-HSQC, thus allowing a direct comparison between the two methods. The measured values of the ^{35,37}Cl isotope shift were nearly identical: 8.1 ppb (1.21 Hz) for the former and 8.3 ppb (1.24 Hz) for the latter (Figure 3).

Interestingly, for the same total acquisition time, the bs-CLIP-HSQMBC experiment yielded data with slightly higher resolution and sensitivity than bs-HSQC (Figure 3). We attribute this result to two factors: heteronuclear decoupling during HSQC acquisition causes slight line shape distortions, while signal intensity is largely controlled by a very long carbon evolution time necessary to achieve sufficiently high resolution in F_1 dimension. Under these conditions, the difference in INEPT delays between HSQC and HSQMBC (3.5 and 60 ms, respectively) becomes negligible compared to a total t_1 evolution time of 1.7s. In addition, J_{HH} modulation is completely avoided in the bs-CLIP-HSQMBC due to the utilization of proton-selective inversion pulses. We also investigated the ^{35,37}Cl isotope effect on carbon C10. Using bs-CLIP-HSQMBC with selective inversion of the H1 resonance, we have been able to reproduce the same isotope pattern as reported previously using bs-HSQC[15] (see Figure S12).

The ^{13}C band-selective CLIP-HSQMBC experiment is reliable and universally applicable for the indirect detection and measurement of the ^{35,37}Cl isotope shift of ^{13}C resonances. The experiment is not limited by the protonation state of the carbons and allows measurement of the ^{35,37}Cl isotope effect of quaternary carbons much more efficiently than when 1D ^{13}C

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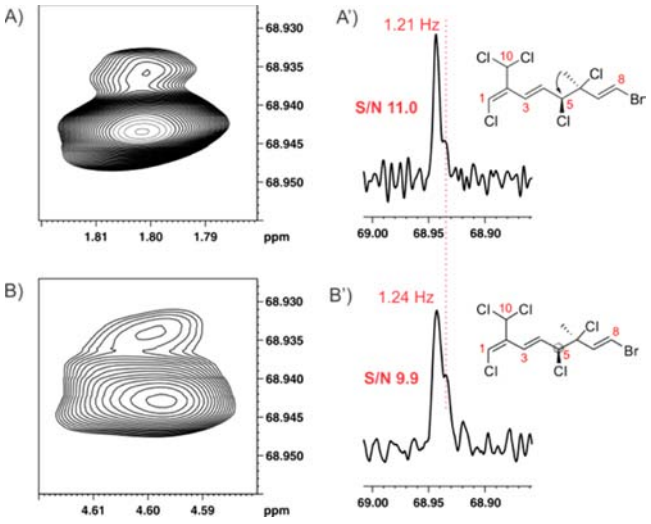

Figure 3. Comparison of ^{35,37}Cl isotope pattern of C5 of **4** observed using bs-CLIP-HSQMBC (A) and bs-HSQC (B). Selective inversion of the methyl protons H9 resonating at 1.80 ppm was used in (A). Selective carbon-refocusing pulse was applied at a carbon chemical shift of 68.9 ppm in both cases.

NMR is used. bs-CLIP-HSQMBC is an efficient method for measuring the ^{35,37}Cl isotope shift of protonated carbons, outperforming bs-HSQC[10,15] in some aspects. The bs-CLIP-HSQMBC experiment allows the rapid resolution of complex regiochemical problems that are not easily solved otherwise. Finally, the experiment constitutes a universal and reliable tool that allows a researcher to quickly ascertain whether a carbon atom is chlorine-bearing or not.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02158.

NMR data, pulse sequence, chemical shift analysis, and deconvolution analysis (PDF)

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Notes

The authors declare no competing financial interest.

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