## **Supporting Information**

## Ultra high-resolution HSQC: Application to the efficient and accurate measurement of heteronuclear coupling constants

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## **Experimental Section**

NMR experiments were acquired on a Bruker AVANCE spectrometer (Bruker BioSpin, Rheinstetten, Germany) operating at 400.13 MHz proton frequency, equipped with a 5 mm BBOF probe and a z-axis pulsed field gradient accessory (maximum strength of 53.5 G/cm). All spectra were collected on 20 mg of sample (2-fluoropyridine, 2,3difluoropyridine, albacanazole and allyltriphenylphosphonium bromide) dissolved in 0.6 ml of CDCl<sub>3</sub> at a temperature T = 298 K, and processed with the software TOPSPIN 3.1. The sample for measuring J(CD) and J(HD) was prepared mixing 120µl of commercial acetonitrile-d<sub>3</sub> (99.80%), acetone-d<sub>6</sub> (99.80%), DMSO-d<sub>6</sub> (99.80%), methanol-d<sub>4</sub> (99.80%) and CD<sub>2</sub>Cl<sub>2</sub> (99.90%).

The non-selective 180° <sup>1</sup>H pulses were of 12.5 $\mu$ s duration. The 2D <sup>1</sup>H-<sup>13</sup>C HSQC spectra were acquired using the pulse sequence hsqcetgpsi (Bruker's library) and optimized to 140-160 Hz ( $\Delta$ =1/(2\*J<sub>CH</sub>)) depending of the sample (see A+C below). Two scans were collected for each of the 128 t<sub>1</sub> values with 2048 complex points in the corresponding FID. Data were transformed with a shifted sine window function along both the F1 and F2 dimensions and with a zero-filling to 1K in F1. The total experimental time was about 7 minutes for each 2D spectrum. The analog pure-shift HSQC spectra were collected under the same experimental conditions as described in ref. 14 (see A+C below). Broadband homodecoupling during acquisition was achieved applying 130 loops (n) with  $\tau$ =8-10 ms.





Figure S1: Spectral-aliased HSQC spectrum of 2-fluoropyridine, acquired A) with and B) without broadband <sup>13</sup>C heteronuclear decoupling during acquisition. From this latter dataset, a complete set of magnitudes and signs of <sup>1</sup>J(CH), J(FH) and J(FC) coupling values can be simultaneously measured from each individual high-resolved cross-peak. A SW(<sup>13</sup>C) of 2 ppm was recorded using 128 t<sub>1</sub> increments, as described in Fig. 2 of the main manuscript.



Figure S2: Experimental effects on the 2D signal resolution and sensitivity after reducing the <sup>13</sup>C spectral width from 60 to 2 ppm. In the lasts 1D slices (5 and 2 ppm), sensitivity is decreased by a factor of 2 because the corresponding cross-peak are resolved along the F1 dimension in two differentiated components.



Figure S3: 2D  $^{1}$ H- $^{13}$ C HSQC spectrum of 2,3-difluoropyridine acquired with SW( $^{13}$ C)= 1 ppm and 128 t<sub>1</sub> increments.



Figure S4: Expansion extracted from spectral aliased spectra of Fig. 4 showing the enhanced signal simplification and dispersion achieved by applying broadband homonuclear decoupling. The cross-peak corresponds to the H2 proton of allyltriphenylphosphonium bromide.





Figure S5: Spectral aliased  ${}^{1}\text{H}{-}{}^{13}\text{C}$  HSQC-TOCSY of of allyltriphenylphosphonium bromide acquired with a SW( ${}^{13}\text{C}$ ) of 2 ppm. Mixing time= 60 ms and number of scans of 16 per t<sub>1</sub> increment. Whereas the analysis of the positive/negative slope of a single cross-peak only provides information about if the involved couplings have the same or opposite sign, the analysis of the positive/negative slope for a set of cross-peaks into the same column (they have the same J(HP) coupling) or the same row (they have the same J(CP)) help in the absolute determination of the positive/negative sign of the involved couplings.



Figure S6: 2D <sup>1</sup>H-<sup>13</sup>C SIS-J-HSQC spectra after selective excitation of the H22 proton (20 ms Gaussian) on a degradated sample of strychnine in CDCl3. A) Standard experiment recorded with SW(<sup>13</sup>C)=160ppm, TD1=128w and J-scaling=20; B) SW(<sup>13</sup>C) reduced to 5 ppm and J-scaling=3; C) SW(<sup>13</sup>C) reduced to 1 ppm and without J-scaling. The E.COSY pattern provides the sign and the magnitude of J(H<sub>x</sub>-H22) and <sup>n</sup>J(C<sub>x</sub>-H22) between the excited proton (passive spin-H22) and the observed CH cross-peak.