Supporting Information

Laccase-mediated Michael Addition of ^{15}N-Sulfapyridine to a Model Humic Constituent

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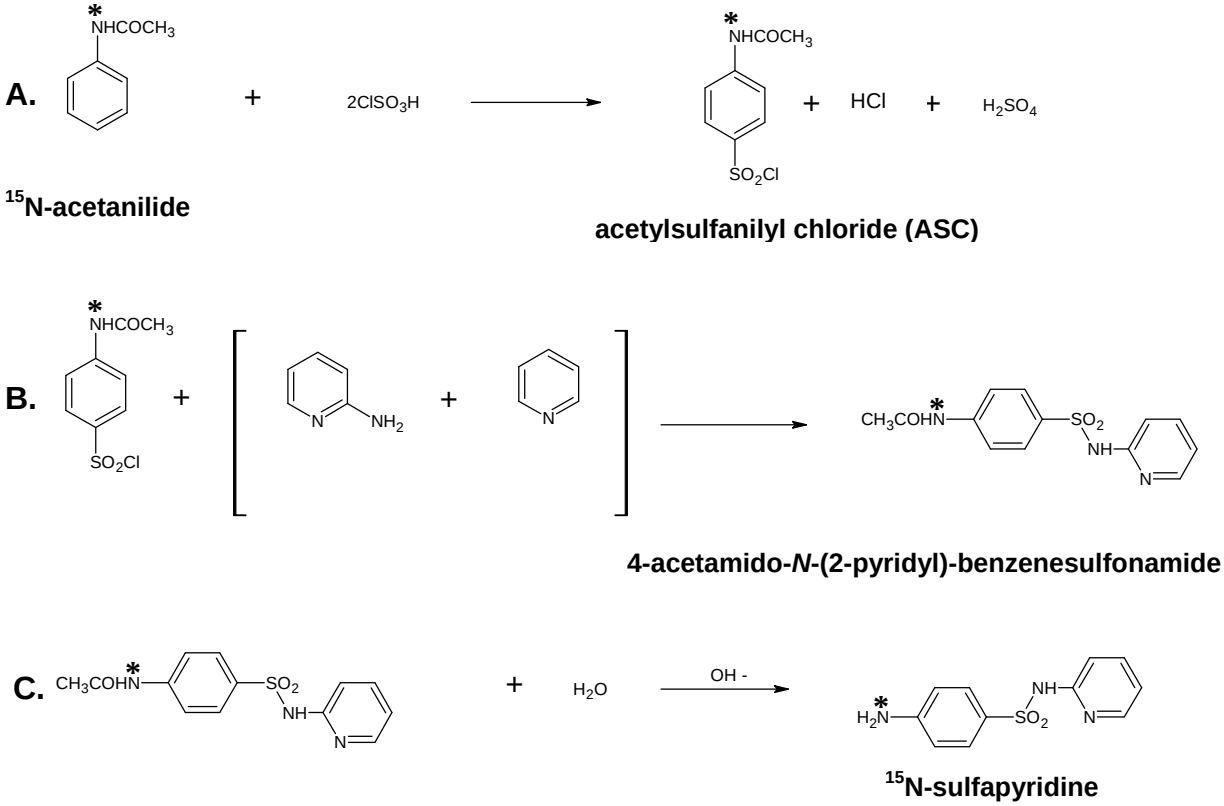


SCHEME S-1. Synthesis of ^{15}N-sulfapyridine: (A) preparation of acetylsulfanilyl chloride (ASC) by chlorosulfonation of ^{15}N-acetanilide; (B) reaction of ASC with 2-aminopyridine in the presence of pyridine to form 4-acetamido-*N*-(2-pyridyl)-benzenesulfonamide; (C) ^{15}N-sulfapyridine generated by deacetylation of 4-acetamido-*N*-(2-pyridyl)-benzenesulfonamide. The asterisk signifies the ^{15}N-enriched nitrogen. Scheme adapted from Lehman (*1*). Significant alterations to the original method were required for reproducible ^{15}N-sulfapyridine synthesis including (1) removal of residual acid from the ASC filtrate, (2) overnight freeze-drying of the ASC sample, (3) prevention of water contamination by transfer of dry solvents via a cannula, (4) the use of excess 2-aminopyridine and pyridine, and (5) extension of the reaction time of ASC with 2-aminopyridine to ~12 h. ASC is easily hydrolyzed to release sulfonic acid. The presence of 1-2% of sulfonic acid in the reaction mixture inhibits the reaction of ASC with 2-aminopyridine (Scheme S-1, Reaction B). Therefore, ensuring the ASC remains thoroughly dry is crucial to the success of the synthesis.

Text S1. Characterization of ^{15}N-sulfapyridine

The initially recrystallized ^{15}N-sulfapyridine (^{15}N-SPD) product was examined by TLC to qualitatively separate and visualize any impurities. Based on the R_f values of the reactants, no of impurities from starting materials (viz., ASC, aminopyridine, and pyridine) were apparent. ^{15}N-acetanilide was not monitored in the TLC study.

The purity of ^{15}N-SPD was further assessed by ^{1}H-^{15}N Heteronuclear Multiple Bond Correlation (HMBC) experiments (Figure S1). Spectra exhibited three- and four-bond couplings associated with ^{15}N-sulfapyridine, as well as crosspeaks resulting from the ^{15}N-acetanilide reactant (see Scheme S1, Reaction A). A second recrystallization step was required to reduce the acetanilide below levels detectable by NMR (Figure 1).

Proton resonances were assigned to the subsequently purified ^{15}N-sulfapyridine by one-dimensional ^{1}H NMR (Figure S2) and ^{1}H-^{1}H COSY (Figure 2). COSY experiments detect protons belonging to the same coupled spin system (*2)*. Diagonal peaks in a COSY spectrum correspond to the normal ^{1}H one-dimensional spectrum while off-diagonal peaks indicate correlations of protons within the same coupled spin system (*2*). The COSY spectrum of ^{15}N-SPD (Figure 2) revealed that the crosspeaks appearing at δ_H = 6.5 ppm and 7.5 ppm in the HMBC spectrum (Figure 1) resulted from protons at positions **B** and **C** (Figure 2). The two protons *ortho* to the anilinic nitrogen are chemically equivalent as are those *meta* to this moiety resulting in identical chemical shifts for the protons at each position. The proton at position **B** (δ_H = 6.5 ppm) is correlated to the anilinic nitrogen through three bonds while that at position **C** (δ_H = 7.5 ppm) is correlated to this nitrogen through four bonds (Figure 1). These protons correlate with each other (off-diagonal peaks) and no others because no other neighboring carbon atoms have attached hydrogens.

A comparison of proton NMR spectra of ^{15}N-enriched SPD with commercially available unlabeled SPD (Sigma), demonstrates that both compounds display identical chemical resonances for all protons (Figure S2). (Proton A of the enriched sulfonamide is split due to the two spin states of the ^{15}N nucleus). The one-dimensional spectrum (Figure S2) also reflects the interactions of protons (observed in the COSY spectrum; see Figure 2) as indicated by apparent peak splittings. For example, the hydrogen at δ_H ~6.9 ppm (**D**) is coupled to two other hydrogens in the heterocyclic ring, **F** and **G**, resulting in a triplet peak. The proton at δ_H ~7.1 ppm (**E**) is coupled to the hydrogen at δ_H ~7.66 ppm (**F**) resulting in the observed doublet. This is expected due to the presence of only one neighboring carbon attached to a hydrogen. The proton (**F**) is coupled to two other hydrogens (**E** and **D**) (forming a triplet) while the split singlet (appearing as a doublet), **G**, is coupled to its single hydrogen neighbor assigned to position **D**.

Proton **H** appears at the same δ_H (~10.8 ppm) in both the SPD standard (Figure S3) and the ^{15}N-enriched SPD (data not shown). The significant broadening of this peak may have been due to hydrogen-bonding or tautomerization of the pyridine nitrogen. The protons associated with the anilinic and amide nitrogens, for example, can hydrogen-bond with the sulfoxide moieties of other sulfonamide compounds present in solution (*3*). Tautomerization of the amide to the imide (*3*) over the timescale of the NMR experiment may have also contributed to the broadening of this resonance. Although sulfonamides are known to hydrogen-bond via protons attached to the anilinic and amide nitrogens, broadening of peaks associated with anilinic hydrogens were not observed suggesting that the observed broadening was due to tautomerization. Integration of the protons in spectrum B (Figure S2) resulted in ratios consistent with the COSY assignments (Figure 2).

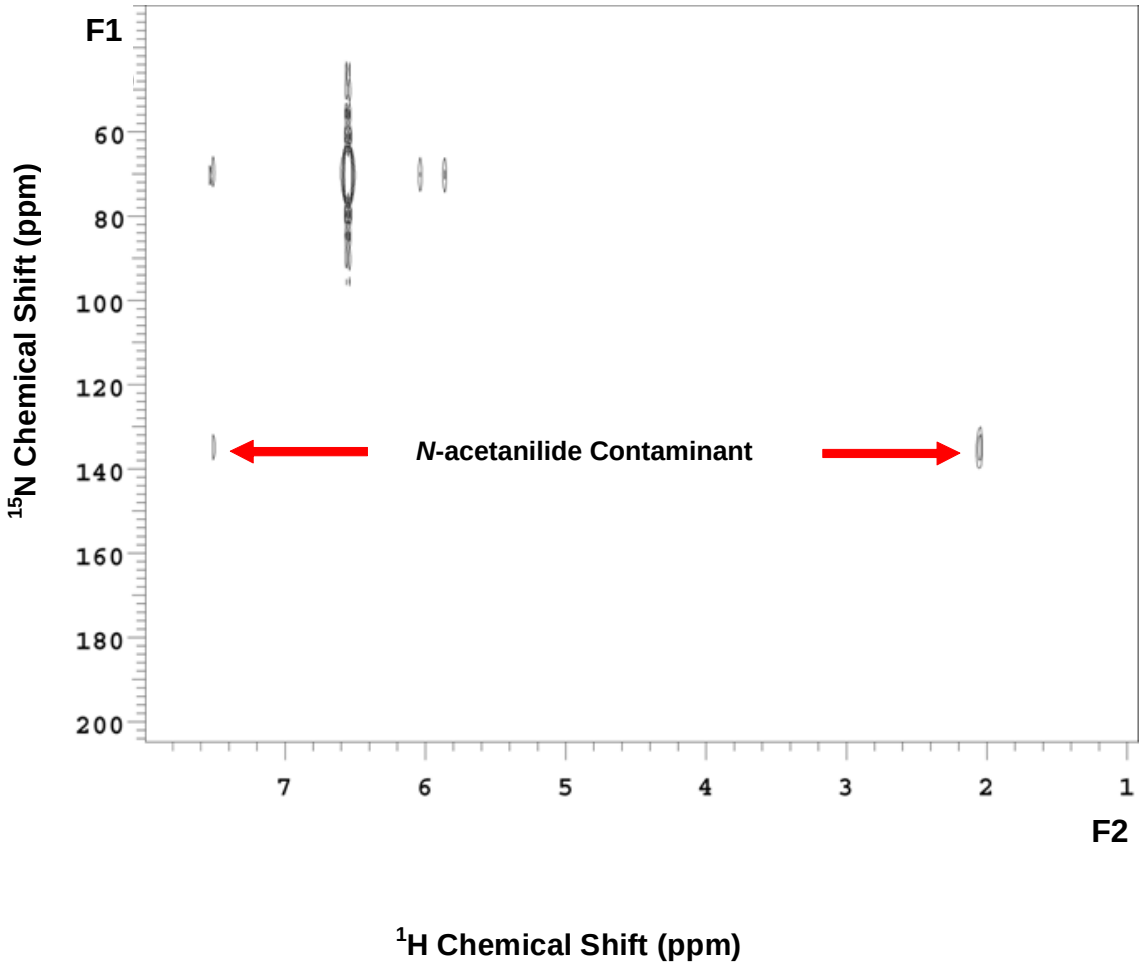
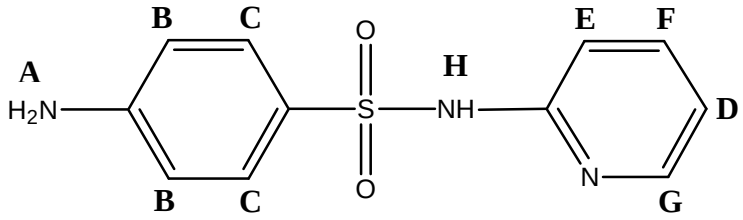
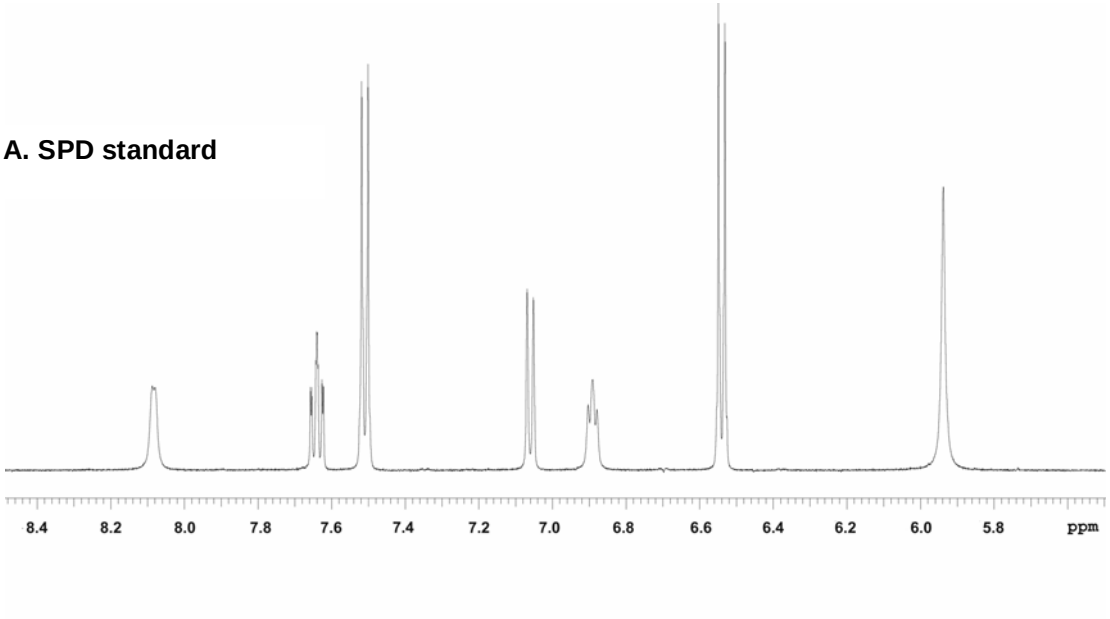


FIGURE S1. ^{1}H-^{15}N HMBC spectrum of synthesized ^{15}N-SPD following initial recrystallization. The appearance of additional crosspeaks at δ_N ~133 ppm indicated the presence of ^{15}N-acetanilide contaminant (Scheme S-1). For anilides, δ_N ~135 ppm (*4*). The sample was referenced to DMSO-d_6. The acetanilide impurity was removed by a subsequent recrystallization (Figure 1).





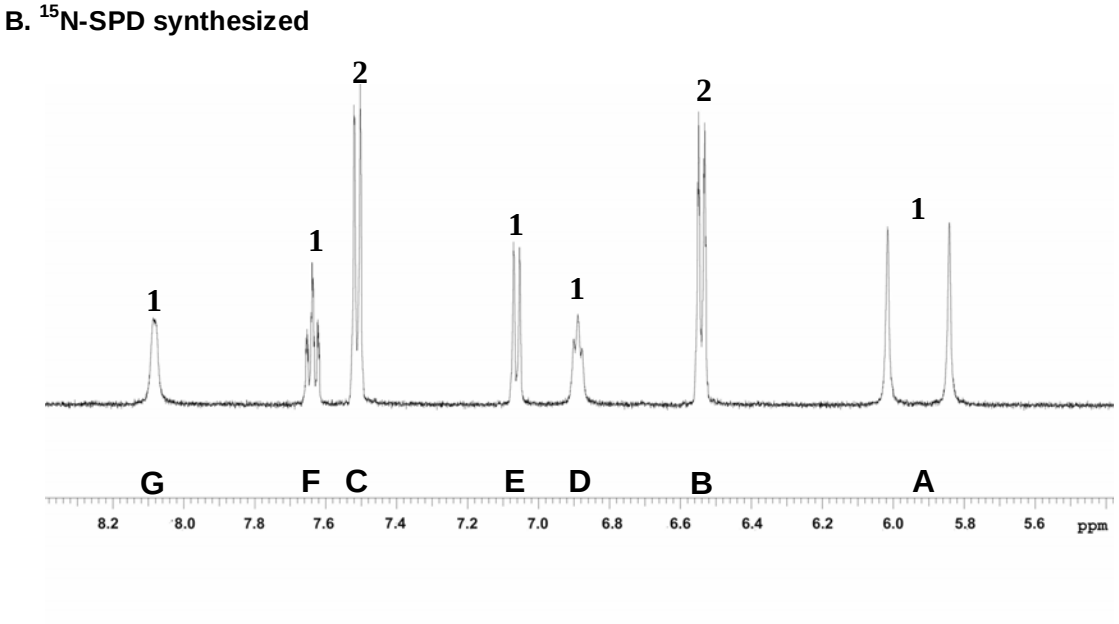


FIGURE S2. ^{1}H NMR spectra of (A) unlabeled SPD obtained from Sigma and (B) synthesized ^{15}N-SPD. The letters below the peaks signify the location of the protons in the sulfonamide structure. The numbers above the peaks indicate the ratios of the hydrogens (determined by spectral integration) and are consistent with the letter assignments. The sharp singlet at δ_H ~5.9 ppm in spectrum A was assigned to the protons of the anilinic nitrogen. In spectrum B, the singlet split into a doublet as a result of the two spin states of the enriched ^{15}N nucleus. This splitting was also observed in the ^{1}H-^{15}N HMBC (Figure 1) and the ^{1}H spectrum in Figure S1. The matched proton resonances between the two spectra demonstrate the purity of the synthesized ^{15}N-SPD. Both spectra were referenced to DMSO-d_6.

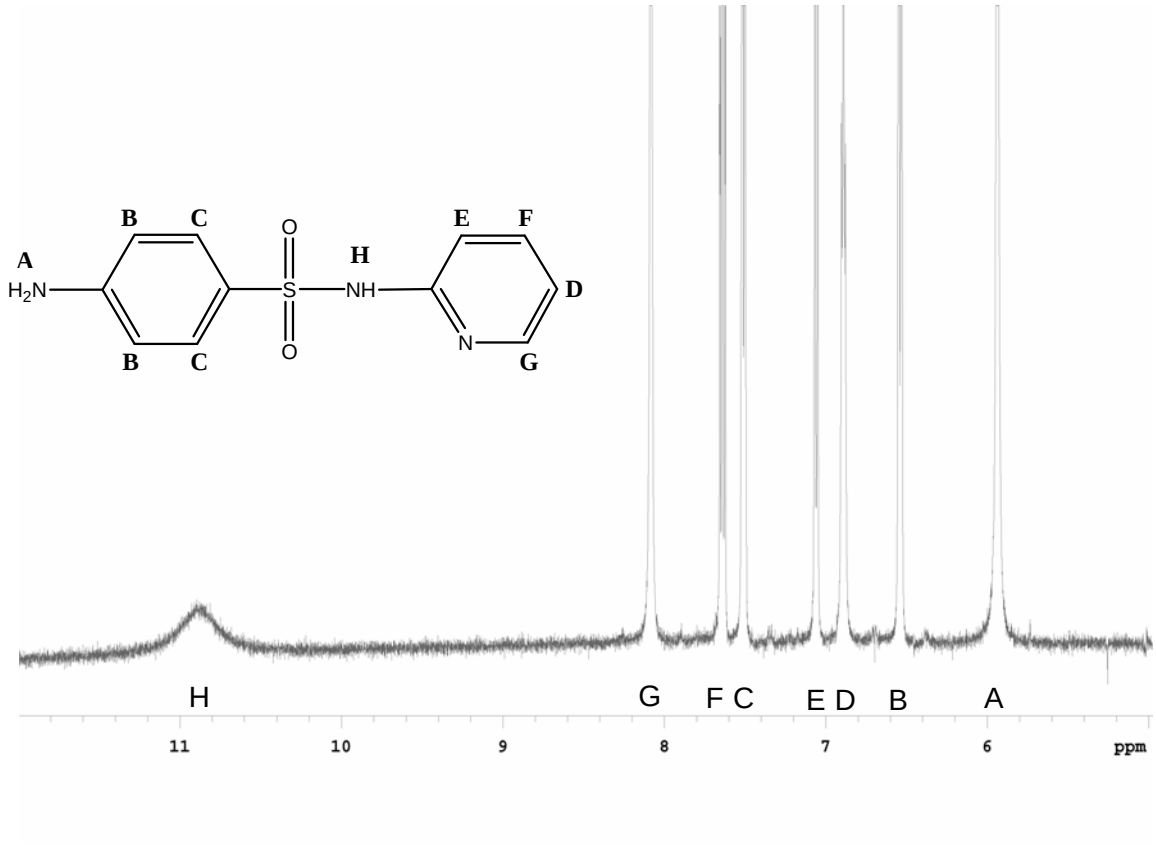


FIGURE S3. A focused ^{1}H spectrum of unlabeled SPD (Sigma). The proton (**H**) resonates at δ_H ~10.8 ppm. The significant broadening of this peak was likely due to tautomerization between the amide and imide forms over the timescale of the NMR experiment. This proton in ^{15}N-enriched SPD appears at the same chemical shift position (data not shown). The spectrum was referenced to DMSO-d_6.

Text S2. Literature Cited

(1) Lehman, J.W. *Operational Organic Chemistry*; Allyn and Bacon: Boston, MA, 1981.

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(3) Grant, D.; Adsmond, D.A. Hydrogen bonding in sulfonamides. *J. Pharm. Sci.* **2001**, *12*, 2058-2077.

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