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Communication

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"NMR chemosensing" using monolayer-protected nanoparticles as receptors

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Supporting Information Placeholder

ABSTRACT: A new sensing protocol, based on NMR magnetization transfer sequences and on the molecular recognition abilities of nanoparticles, allows the detection and identification of organic molecules in complex mixtures.

According to its definition, a chemosensor is "a molecule of abiotic origin that signals the presence of matter or energy".[1] This concept has been commonly translated into a supramolecular receptor that, upon selective binding (recognition) of its target (analyte), undergoes a measurable change of its properties.[2] Such a change, properly monitored, reveals the presence of the analyte in the sample under investigation. Typical molecular properties used for signal generation include luminescence,[3] absorbance,[4] redox potential,[5] relaxivity[6] and many others. Albeit several advantages justify the widespread interest in chemosensors, one intrinsic limitation of such an approach is that the reliability of a chemosensor response depends crucially on its selectivity. Indeed, the signal produced arises from a property of the chemosensor itself and, as such, it does not provide any direct information on the identity of the analyte detected. The user must presume he is measuring the presence of the desired target, and not of a known or unknown interferent, because he trusts the recognition ability of the chemosensor. Indeed, the design of new sensing schemes that allow for a direct individuation of the target in complex environments represent a major challenge. Our interest in understanding the structure and properties of monolayer protected gold nanoparticles, particularly using new tools based on NMR spectroscopy,[7] led us to the development of a new sensing protocol that allows also analyte identification and quantification in complex mixtures. Moreover, the results here reported also address the more classical, but still actual, problem of realizing systems capable to detect organic molecules in water, where most receptors fail in efficiently recognizing their targets.

As a test mixture, we selected a group of three water soluble aromatic compounds of similar size and features, namely sodium salicylate (**4**, Chart 1), sodium *p*-toluensulphonate (**7**) and disodium benzene-1,3-disulfonate (**8**). The identification of the single components from a ^{1}H-NMR spectrum (Figure 1a) of this fairly simple mixture is not trivial at all, and this is a well-known drawback that heavily limits its usefulness in

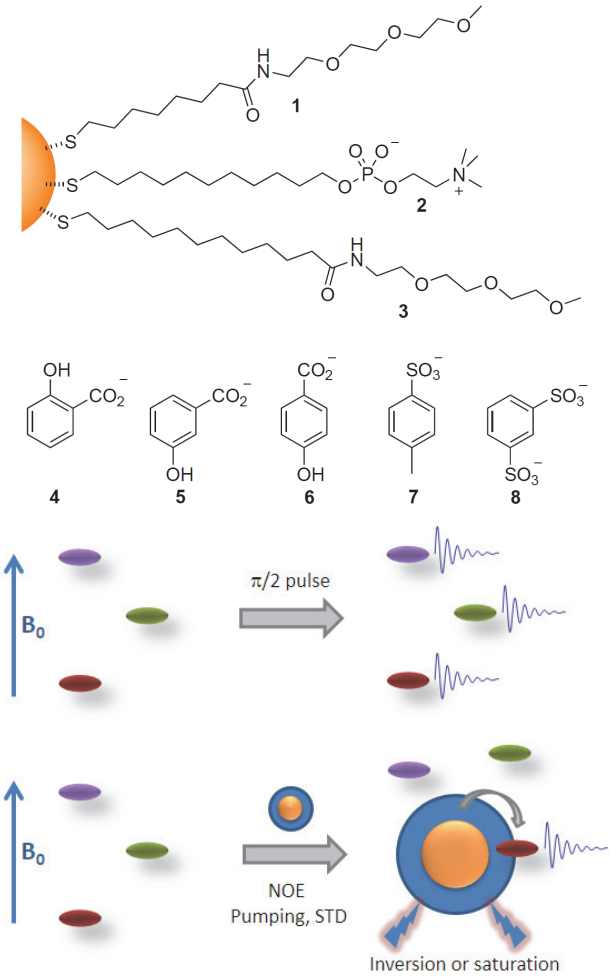

Chart 1. *Upper*) Nanoparticles coating thiols and analytes used in this article. *Lower*) Nanoparticle-based NMR sensing with NOE pumping experiments: working scheme.

the case of complex mixtures. However, when we mixed the same sample with gold nanoparticles (2 nm gold core diameter) protected with thiol **1** (Chart 1), we were able to extract just those signals arising from salicylate (Figure 1b) by means of diffusion-assisted nuclear Overhauser effect experiments (NOE pumping).[8]

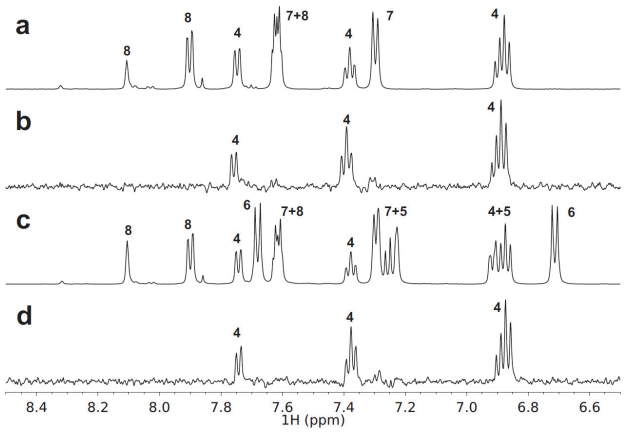

Figure 1. a) ^{1}H-NMR subspectrum of a mixture of molecules 4, 7 and **8** (7 mM in D_2O). b) NOE-pumping subspectrum of the same sample in the presence of **1**-coated gold nanoparticles (70 μM). c) ^{1}H-NMR spectrum of a mixture of molecules **4**-**8** (7 mM in carbonate buffer 100 mM, pD = 10)[9]. d) NOE-pumping subspectrum of the same sample in the presence of **1**-coated gold nanoparticles (70 μM).

In designing this experiment, we had in mind two starting points. First, it is well known that water-soluble monolayer-protected gold nanoparticles can incorporate hydrophobic molecules in the protecting monolayer.[10] Second, several NMR experiments have been devised to identify compounds with binding affinity to proteins and to other biomacromolecules, based on the transfer of net magnetization (or saturation) from large systems to small bound molecules.[8,11] Among these, the NOE-pumping experiment originally proposed by Shapiro[8] looked particularly suitable to our purposes. This experiment consists of two blocks: i) first, the signals of the small molecules in the sample are cancelled (dephased) with a diffusion filter, ii) second, a NOE experiment is started immediately after. In this way, only the signals of the small molecules interacting with the macromolecule in fast exchange regime can be detected, as they arise from the magnetization transferred from the macromolecule, which survives the diffusion filter. Hence, in our experiment the nanoparticles can be considered as "self-organized" supramolecular receptors that recognize their substrate and label it by magnetization transfer, which in turn generates the detected response (Chart 1). Note that the signal produced in this way does not arise from the chemosensor but from the analyte itself and, containing as much information as a NMR spectrum does, it allows not only the detection but also the unambiguous identification of the target.

The selectivity we found using such protocol is somehow surprising. NOE-pumping of a water solution containing **1**-coated nanoparticles and the three isomers salicylate (**4**), 3-hydroxybenzoate (**5**), and 4-hydroxybenzoate (**6**), which differ only for the relative positions of the two functional groups, revealed the presence of the sole salicylate signals (see Supporting Information). Such a selectivity remained unaffected even when all the compounds **4**-**8** so far studied were mixed together in a single tube with the nanoparticles. Again, only salicylate signals emerge from the NOE-pumping experiment (Figure 1c-d), with a substantial simplification of the spectrum of the mixture and clear detection of the presence of salicylate in the sample.

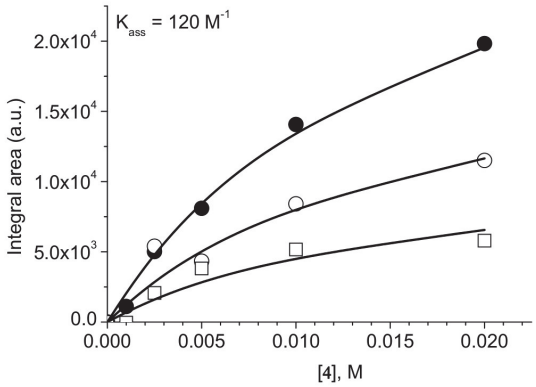

Figure 2. Relative area of the salicylate (**4**) signal in the NOE-pumping experiment as a function of its concentration. Solid line: best fit of the data (● = 6.8 ppm, ○ = 7.4 ppm, □ = 7.75 ppm). Conditions: [**1**-nanoparticles]= 70 μM, carbonate buffer 100 mM, pD = 10.

Besides selective detection, it is important to note that the method reported also allows the quantitative determination of the analyte. In a series of experiments performed with different concentrations of salicylate (Figure 2) we found that the integrals of the analyte signals increase following a saturation profile as expected for a binding process. Fitting of the relative intensity data with a 1:1 binding model provides an apparent association constant (K_{ass}) value of 120±5 M^{-1}. This value is comparable with those reported by Pasquato and Lucarini[10] for the association of organic radicals to **1**-coated nanoparticles of similar size and confirms that an analyte quantification, upon construction of a calibration curve, is possible with this method. In fact, when the concentration of salicylate in the independent experiment of figure 1d was recalculated using the parameters obtained from the fitting of data in figure 2 (See Supporting Information), the value of 6.8±0.4 mM was obtained, very close to the analytical value (7.0 mM). In the condition used (4 hours acquisition, 70 μM nanoparticles concentration), the experiment reported in figure 2 also allowed to set a detection limit of 2.5 mM for sodium salicylate.

The effective binding of salicylate to nanoparticles was confirmed by selective NOE experiments (see Supporting Information). Upon selective inversion of the nanoparticles resonances we observed that the signals arising from salicylate displayed clear NOEs, confirming the direct interaction of the two species. NOE intensities appear to be larger when the signals relative to the alkyl chains of thiol **1** are inverted. This observation suggests that salicylate is probably localized in the inner, hydrophobic, region of the monolayer.

The remarkable selectivity obtained with **1**-coated nanoparticles should hence arise from the different hydrophobicity of the five mixture components. As a matter of fact, computationally predicted *n*-octanol/water partition coefficients at pH 7.4 (log D) follow the order **4** (-1.14) > **6** (-1.35) ~ **5** (-1.47) > **7** (-2.57) >> **8** (-7.14),[12] with salicylate **4** being the most hydrophobic of the series. A similar correlation was obtained by submitting the mixture of the five compounds to HPLC separation using a C18 reversed-phase column (Supporting Information). Here, the order of elution times was: **8** << **7** < **6** <

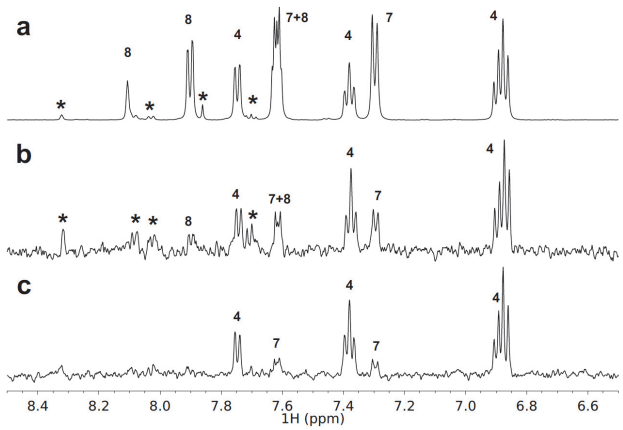

Figure 3. a) ^{1}H-NMR subspectrum of a mixture of molecules **4, 7** and **8** (7 mM in D_2O). b) NOE-pumping subspectrum of the same sample in the presence of **2**-coated gold nanoparticles (70 μM). c) NOE-pumping subspectrum of the same sample in the presence of **3**-coated gold nanoparticles (70 μM). The asterix identify the singals of an impurity in compound **8**.

5 << **4**. This observations indicate that the **1**-coated nanoparticles act as selective receptors for hydrophobic molecules, with a threshold than can be set at calculated log D (pH 7.4) values around -1.2 or HPLC retention times around 9-10 minutes in the adopted conditions.

However, other structural parameters influence the recognition process. One of the main advantages brought about by the use of nanoparticles as detection agents is the ease of their modification. Indeed, the use of nanoparticles coated with a different thiol, namely the phosphorylcholine derivative **2**, led to a different selectivity. When used to analyze the mixture of molecules **4** , **7** and **8**, these nanoparticles were able to reveal, by NOE-pumping, the presence not only of salicylate but also of *p*-toluensulphonate **7** (Figure 3b), benzenedisulphonate **8** and, with remarkable sensitivity, also of an impurity contained in the latter. Hence, a change of the features of the nanoparticles-coating monolayer led in this case to a broadening of the affinity toward different molecules. In order to identify the features of the coating thiol **2** that are responsible for such an affinity modification (as thiol **2** features both a longer, 11 carbon atoms alkyl chain, and a different, phosphoryl choline, headgroup), we synthesized thiol **3**, featuring an 11 carbon-long alkyl chain, same as **2**, and a tri(ethylene)glycol headgroup, same as **1**. Results of NOE-pumping experiments performed with **3**-coated nanoparticles (Figure 3c) revealed a selectivity in-between that of **1**- and **2**-coated nanoparticles, indicating that both the size of the hydrophobic region in the coating monolayer and specific interaction with the thiols headgroups may affect the binding affinity, a well-known phenomenon for HPLC stationary phases. Such observations also indicate that hydrophobicity is not the only source of recognition. In fact, hydroxybenzoates **5** and **6**, albeit more hydrophobic than *p*-toluensulphonate **7**, are not associated to the nanoparticles and hence are not revealed by the NOE-pumping experiment (see Supporting Information). Likely, an amphyphylic structure, with quite well defined hydrophilic and hydrophobic regions, is required for the inclusion of the analyte in the monolayer.

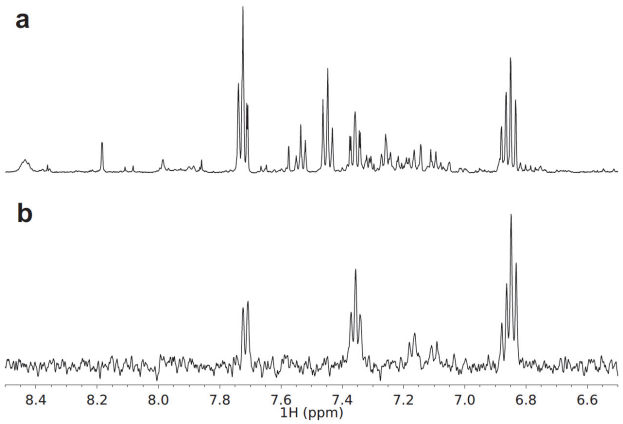

Figure 4. a) ^{1}H-NMR subspectrum of human urine containing 5 mM sodium salycilate. b) NOE-pumping subspectrum of the same sample in the presence of **1**-coated gold nanoparticles (70 μM).

Since there are no limitations on the chemical structure both of the analytes and of the nanoparticles' coating thiols, the NOE-pumping experiment has the advantage of a very general applicability. However we found that, in some cases, the experimental times and detection limits of the protocol can be substantially improved by using Saturation Transfer Difference (STD) experiments.[11] While conceptually similar to a NOE experiment, STD provides stronger signals because saturation can be driven for longer periods as compared with typical NOE mixing times. The final result is that the intensity of the signals stemming from interacting molecules decrease and are revealed by subtraction from a reference spectrum. The use of STD is however limited by the requirement that no overlap must occur between the signals of the analyte and the macromolecule. Salicylate and **1**-coated nanoparticles meet such conditions and, using STD experiments, we were able to cut the acquisition times down to 30 min and increase the detection limit down to 250 μM (see Supporting Information).

Finally, we decided to test our nanoparticle-based NMR sensing protocol in a situation as challenging as the analysis of drug metabolites in urines. Indeed, NMR-based metabolomics is an area of utmost interest, which could substantially benefit from any protocols that expand the amount of information attainable.[13] In this framework, we added **1**-coated nanoparticles to a human urine sample containing 5 mM sodium salicylate, a concentration similar to that found in urines after medium-dose administration of acetylsalicylic acid.[14] Clean detection of salicylate in the ^{1}H NMR spectrum of urine samples (Figure 4a) is quite difficult, since its signals are mixed with many others of similar intensities and chemical shifts in the aromatic region (e.g. those of hippuric acid). On the other hand, a NOE-pumping experiment cancels out the whole bunch of matrix signals, leaving only those of salicylate (figure 4b) and allowing for a clear and unambiguous detection of the target molecule. A similar experiment (see Supporting Information) also allowed us to reveal and identify another metabolite of acetylsalicylic acid (salyciluric acid), excreted in urines in different physiological conditions.

To our knowledge, all the NMR-based chemosensors reported so far follow the "classic" approach were the recognition causes modifications of a chemosensor property, such as the ability to affect water relaxivity[6] or the chemical shift of a receptor's heteronucleus.[15] In this way, most of the meaningful information associated with NMR is lost. More similar to our approach is the "chromatographic NMR spectroscopy", where interactions with a stationary phase are used to perturb the diffusion coefficients of the sample components in such way that the NMR signals can be separated by means of a DOSY experiment.[16] However, besides the intrinsic difficulty of spreading the diffusion rates with a chromatographic medium, this approach suffers from limitations such as the need of high resolutions in both the frequency and the diffusion domains, along with non-trivial spectral inversion problems. The "NMR chemosensing" approach introduced here retains all the structural information provided by NMR spectroscopy, can be very easily implemented on standard instruments and can benefit from the features of monolayer protected nanoparticles, which can be easily tailored to meet the recognition requirements of different classes of analytes.

ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization of the organic compounds and nanoparticles; additional NMR experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interests.

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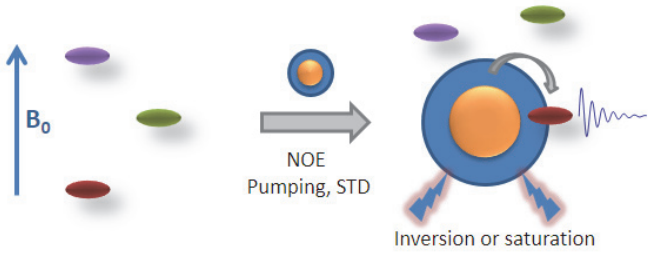
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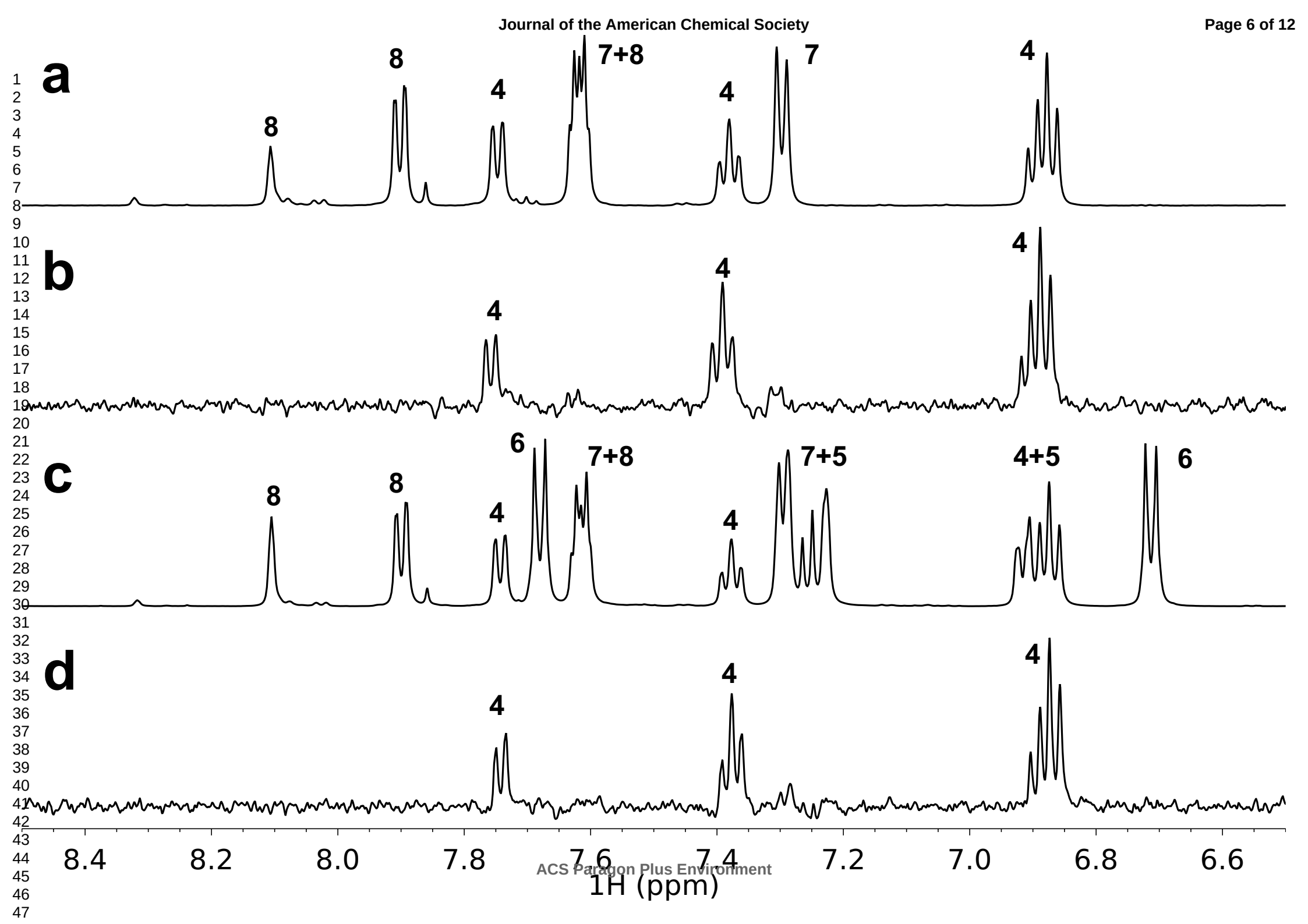
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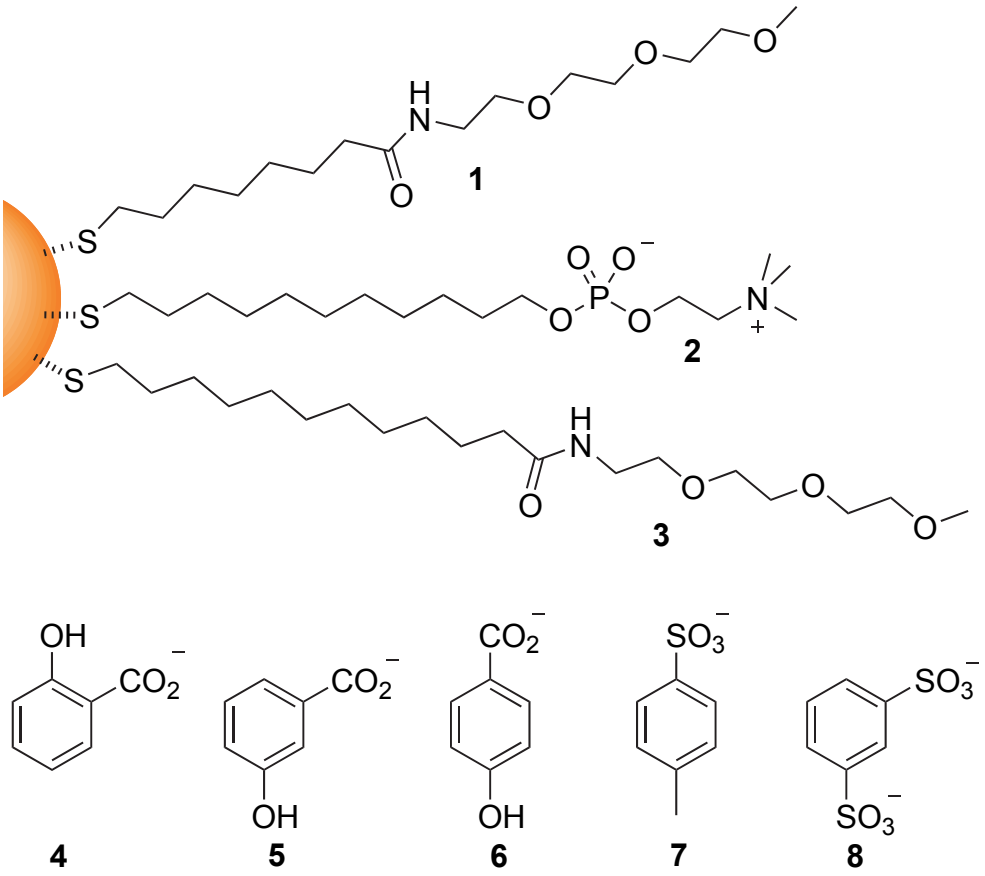
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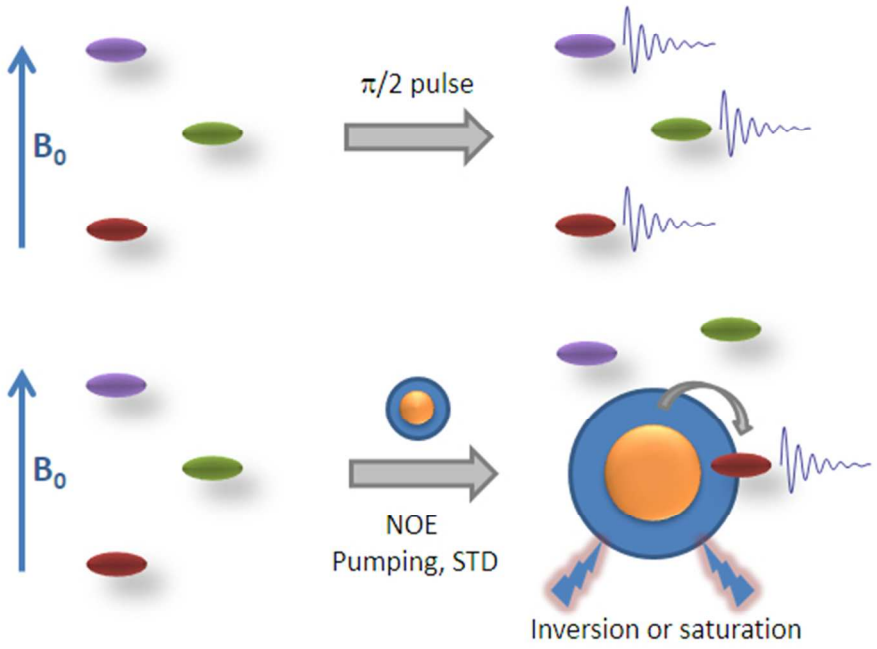

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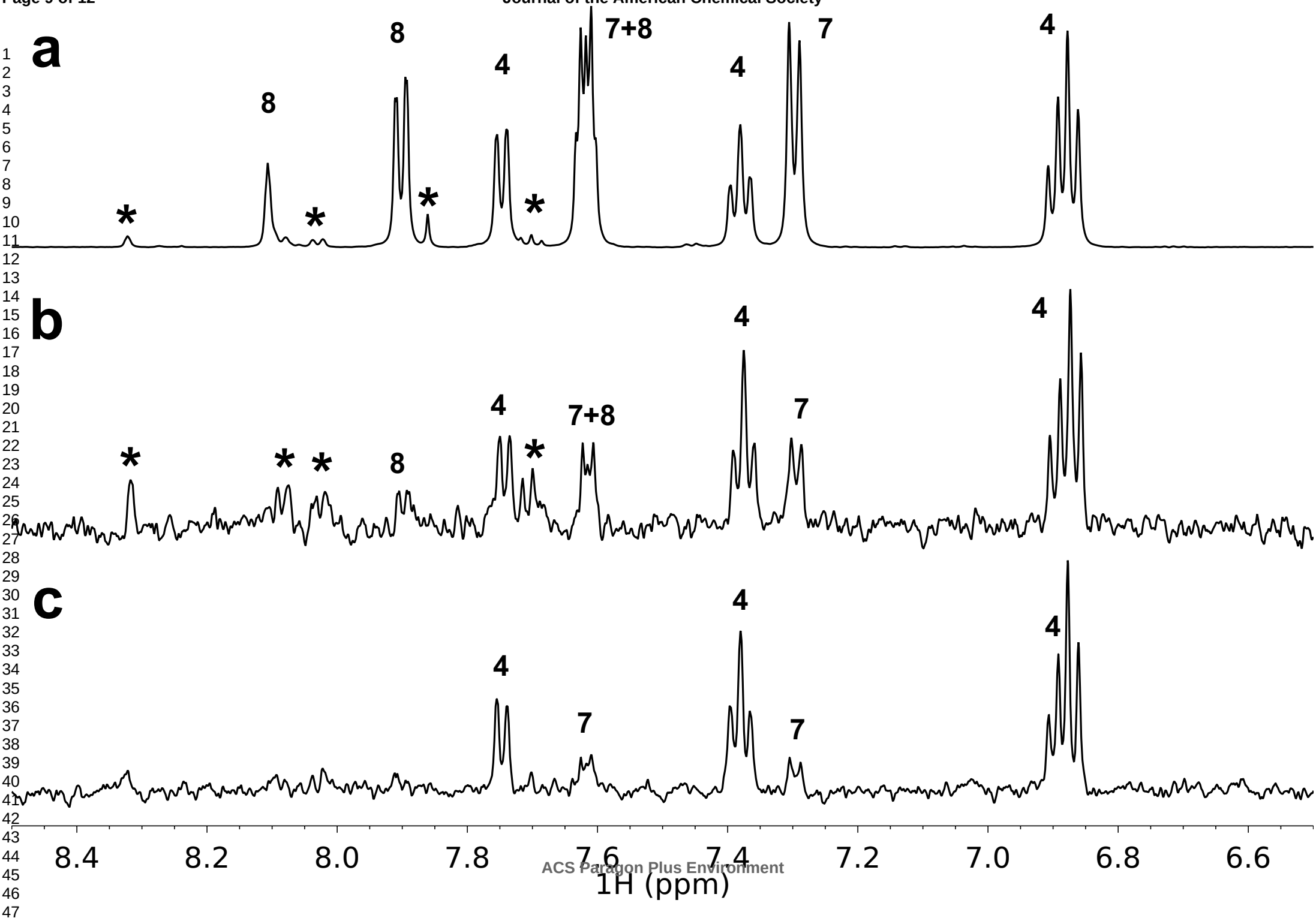
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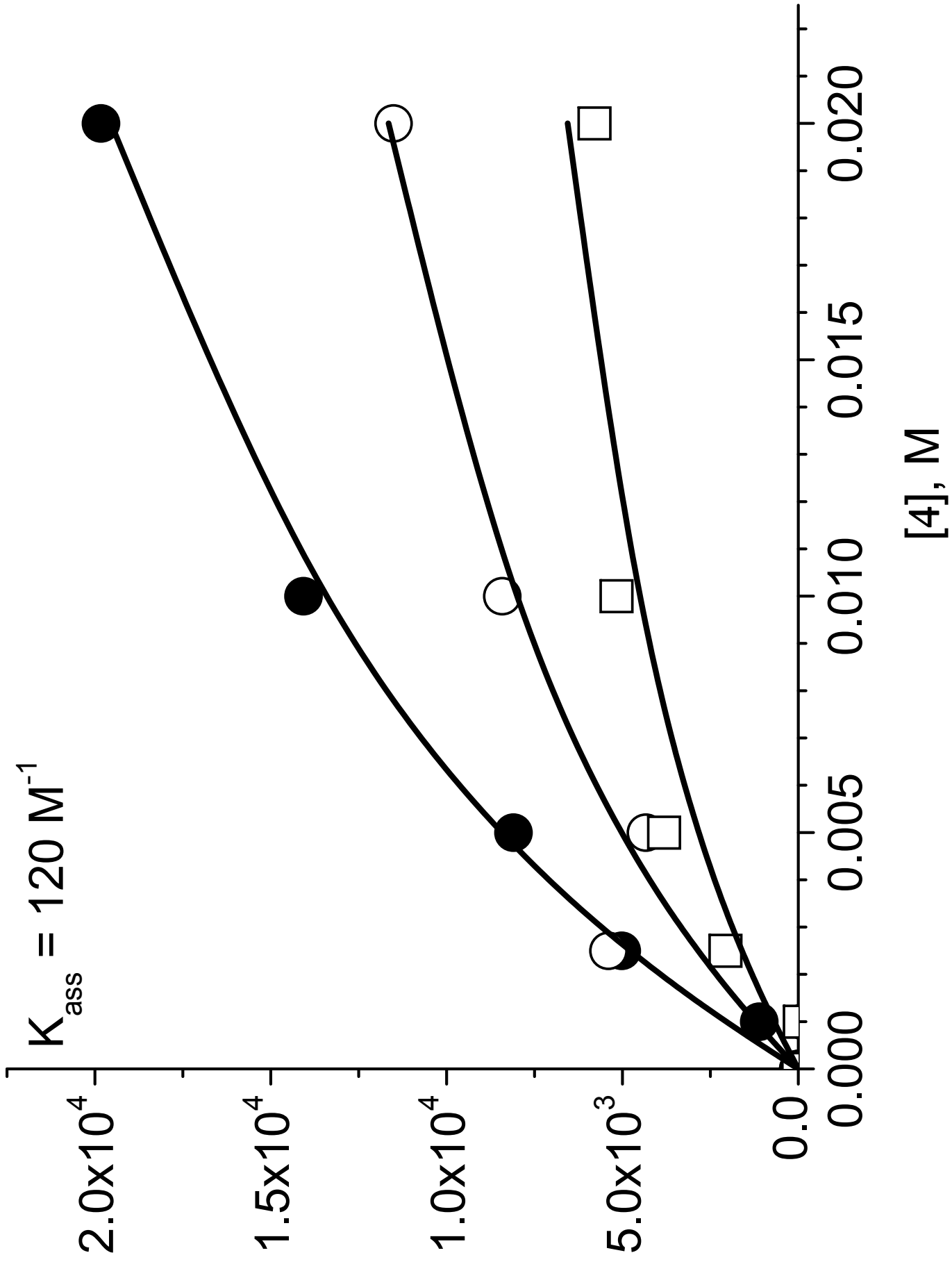

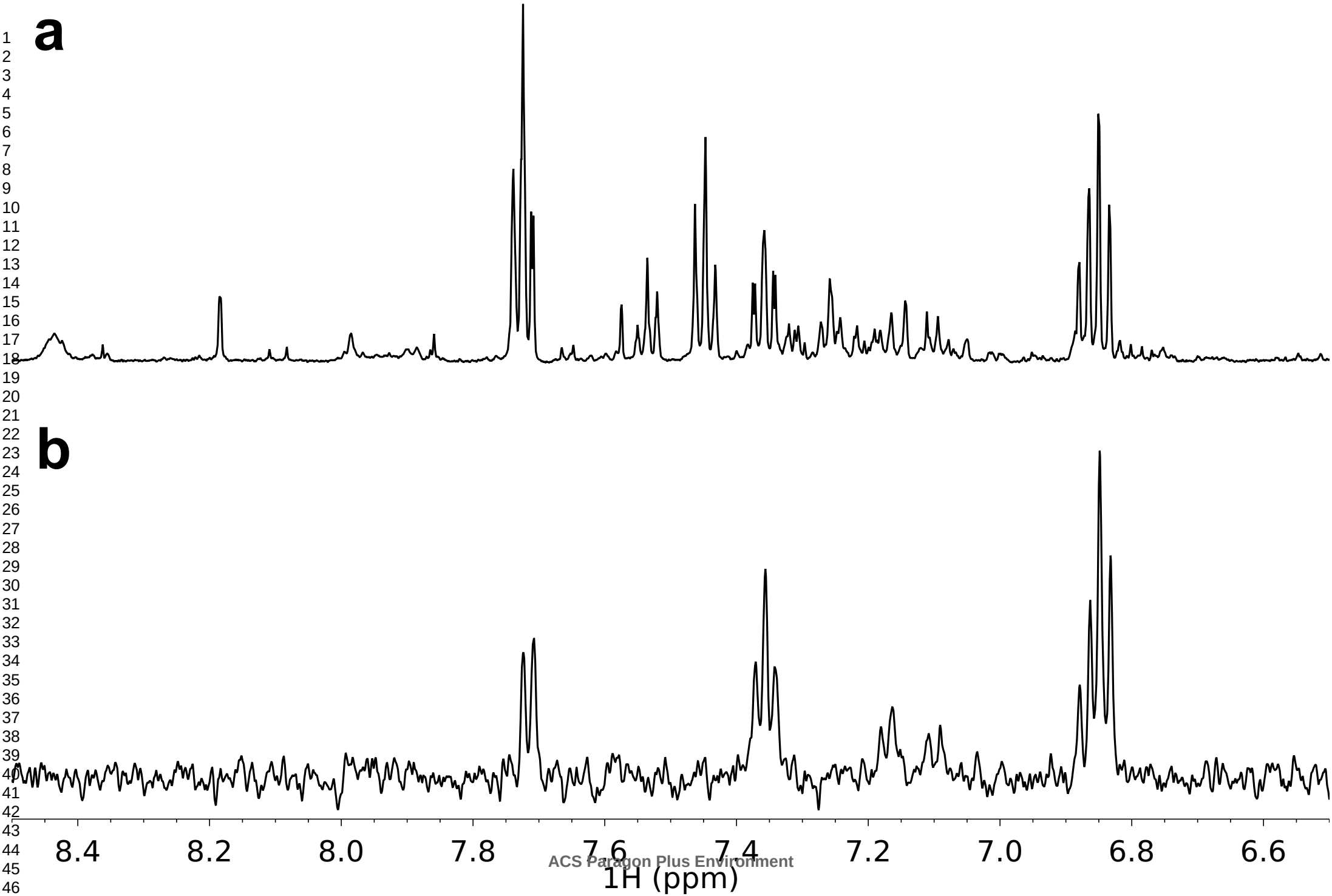

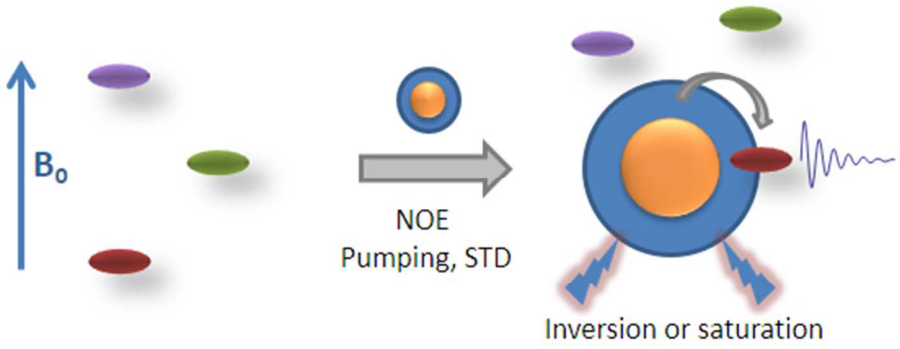

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