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Final Report for the DHS HS-STEM Program (2014)

"Development and Investigation of NMR Tools for Chiral Compound Identification"

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Summary:

The goal behind the assigned summer project was to investigate the ability of nuclear magnetic resonance spectroscopy (NMR) to identify enantiomers of select chiral organo-fluorophosphates (OFPs) compounds which are analogs of chemical warfare agents (CWAs, e.g. Sarin). This involved investigations utilizing chiral solvating agents (CSAs) and characterizing the binding phenomena with cyclodextrins. The resolution of OFPs enantiomers using NMR would be useful for research into toxicodynamics and toxicokinetics in biological systems due to the widely differing properties of the CWA enantiomers [1]. The optimization of decontamination abilities in the case of a CWA events, with this method's potential rapidity and

robustness, as well as the development of models correlating chiral compounds with CSAs for optimal resolution are all rational benefits of this research.

Background:

The most researched method for the chiral recognition of OFPs is through gas chromatography (GC). Past attempts at chiral analysis of nerve agent stereoisomers used tools such as the capillary Chirasil Val column for GC. The clever use of both the Chirasil Val and Carbowax columns in series was only able to provide complete enantiomeric resolution for the chemical G agent soman (GD). Attempts to observe the separate enantiomers of sarin (GB) and other OFPs were not successful. A later study (Benschop and De Jong 1988) did see success in observing ^{1}H NMR enantioseparation of OFPs with the use of Lanthanide shift reagents. There are numerous issues with this method, one of which is the formation of water complexes which results in hydrolysis[2]. The researchers in that case used GC and NMR spectroscopy in a way that complemented each other [1]. The goal of the current research effort is to obtain rapid and robust enantiomer identification and quantification using only NMR spectroscopy [3].

As an example, Sarin is classified as a nerve agent. It is also categorized as a G-series CWA with the abbreviation “GB”. The other G-series agents are tabun “GA”, soman “GD” and cyclosarin “GF”, as illustrated in Figure 1. One of the key structural features of such agents, also often similar to pesticides in structure (but not potency), is the OFP structure. The deadliness of sarin is attributed to its ability to inhibit acetylcholinesterase; an enzyme that typically breaks down acetylcholine. Acetylcholine is responsible for locomotion by having an excitatory role at neuromuscular junctions of the central nervous system (CNS) and the peripheral nervous system

(PNS). Sarin was used in two terrorist attacks that took place in Japan in 1994 and 1995. More recently, sarin has been in the news due to its use in Syria [4].

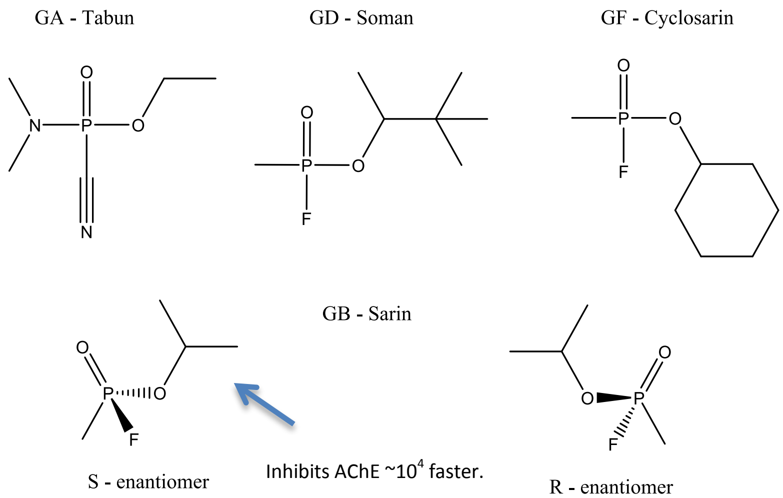

Figure 1 - *G-series chemical warfare agents (CWAs)*

Sarin was developed in 1938 by German researchers at IG Farben who were looking for effective pesticides. The name “sarin” is an acronym derived from **S**chrader, **A**mbros, **R**itter and L**in**de - the four scientists credited for its synthesis. The chemical composition of sarin is such that it exists as a colorless, odorless and tasteless liquid when in pure form (S enantiomer). It is one of the deadliest chemical warfare agents (CWAs), with toxicity that has been estimated to be approximately 26 times more deadly than cyanide. The common synthetic pathway for creating sarin is not stereospecific, so both the R and the S enantiomers are produced with a chiral center at the phosphorous atom (see Figure 1). However, the rate constant for inhibition of acetylcholinesterase by the S enantiomer has been measured to be approximately 10^4 times faster than inhibition by the R enantiomer [1], [4], [5]**.** The principle of being able to differentiate between enantiomers with NMR using CSAs is a matter of enantio-selective interactions between the chiral selector and enantiomers of the agent [3]. This can be explained by electrostatic interactions, van der Waals forces and H-bonding. As an example, in β-cyclodextrin (as well as α-CD and other cyclodextrins) a host/guest complex is formed, where a molecule enters the "donut hole" cavity that exists in such supramolecules. For each enantiomer, these interactions will vary due to steric effects caused by different geometries and should theoretically be reflected with a difference in the chemical shift between the enantiomers (ΔΔδ) on an NMR spectrum of the different nuclei present in the agent (^{1}H, ^{13}C, ^{19}F, ^{31}P, etc.).

The first reference to a cyclodextrin was by Villiers in 1891. He reported that a crystalline substance was the product of starch metabolism by *Bacillus amylobacter*. About a decade later a paper published by Schardinger, a bacteriologist, clarified Villiers' publication by identifying the bacterial strain as *Bacillus macerans*. He also found that it was possible to visually differentiate between the two cyclodextrins (likely α and β) by adding I_2 solution. A cyclodextrin/iodine complex would form where the α-CD appears blue/green and the β-CD appears red/brown. From 1911 to 1935, the key contributor to cyclodextrin research was Pringsheim. His group's main contribution was the discovery that cyclodextrins have a tendency to form complexes with other compounds [6].

The next several decades led scientists to numerous research adventures involving cyclodextrins. The CD inclusion phenomena were particularly interesting, so a great portion of the research dealt with the energetics and kinetics of inclusion with a myriad of different hosts, using NMR, FT-IR, etc. The pharmaceutical application of CDs was also particularly interesting due to the fact that many potential drug molecules have poor solubility and are sensitive to oxidation and light. Often, the polarity, size and structure of novel drug candidates make them great choices for host/guest interactions with cyclodextrins. As a result of this research, several drugs are currently marketed in the form of a complex with cyclodextrin [6]. The sublingual version of Nicorette smoking cessation aid is an example of such a drug complex.

Published research (Desire *et al.* 1986) indicates that β-CD is also able to act as a catalyst for the hydrolysis of OFPs – namely soman. The paper reported that the hydrolysis occurs rapidly at 25°C and a pH of 7.4 [7]. The degradation of our OFPs is a concern, namely because of the potential impact this may have during a titration and its effect on data that is gathered. Due to this, the status of the OFP/β-CD complex in the present research will be monitored for any degradation that may occur over time at our storage conditions (-20°C).

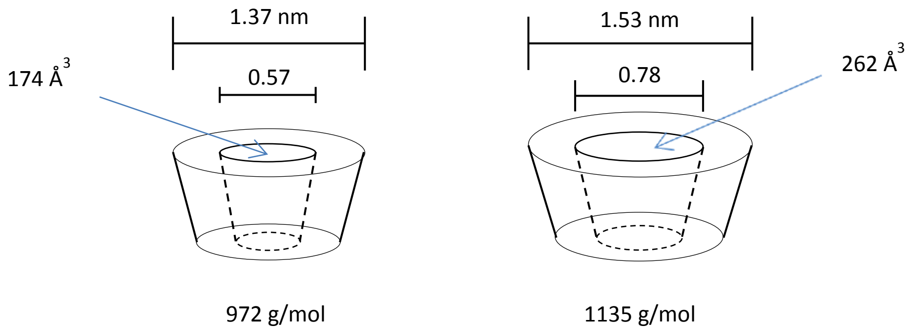

Figure 2a - *Structure of α-CD and β-CD with focus on the internal cavity (Adapted from Szejtli 1998)*

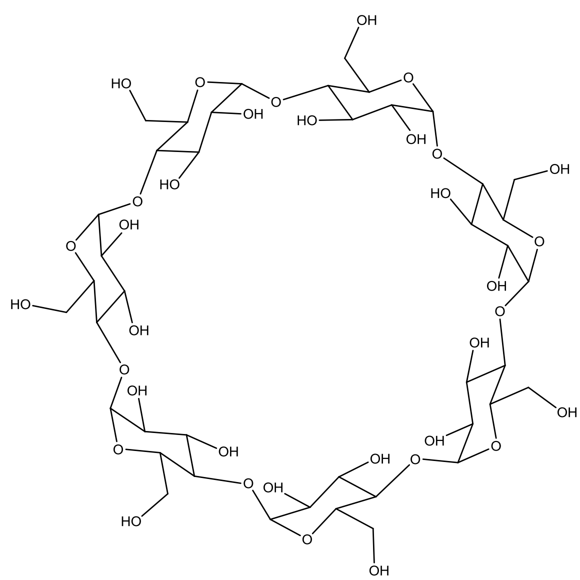

Figure 2b - *Structure of β-CD*

The other CSA of interest in this research project was R-(-)-2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE), also known as Pirkle's alcohol, as illustrated by Figure 3. The use of TFAE as a CSA in NMR studies was first reported by Pirkle in the 1960s. This molecule is in a different class of CSAs due to the high diamagnetic anisotropy of the anthracene, and its lack of a cyclodextrin-type cavity for a host/guest relationship. This source of anisotropy is what allows for differentiation of enantiomers with NMR due to the varying perturbations of their magnetic environment. Evidence exists which indicates that lower temperatures result in a greater nonequivalence with this molecule. With regards to modeling the interactions of such fluoro-alcohols, it is a matter of predicting the primary and secondary interactions, as well as the anisotropic environment of the nucleus being studied [8].

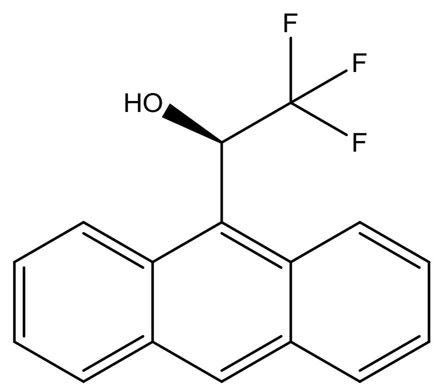

Figure 3 - *Structure of TFAE (also known as Pirkle's Alcohol)*

Figure 4a - *A model of the primary intermolecular interactions between a chiral compound and TFAE (Adapted from Pirkle and Hoover 1982)*

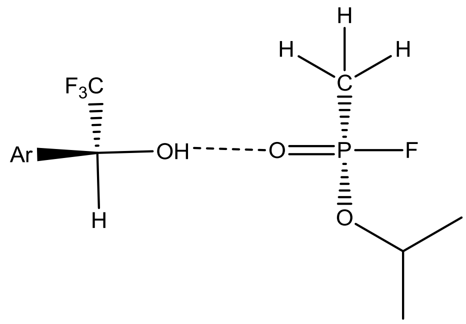

Figure 4b - *A proposed model for TFAE and S-sarin interactions.*

Results and Discussion:

α- and β-Cyclodextrins

To emulate the chemical structure and properties of sarin, two organo-fluorophosphate (OFPs) compounds (Figure 4) with a stereocenter at the phosphorous atom, bonded to an oxygen atom, a fluorine atom and organic R groups were used. The titration was performed only on SNL_{OP-I} due to the compound's greater stability in aqueous solutions. Initial ^{1}H, ^{31}P and ^{19}F NMR spectra were obtained to determine the default peak positions.

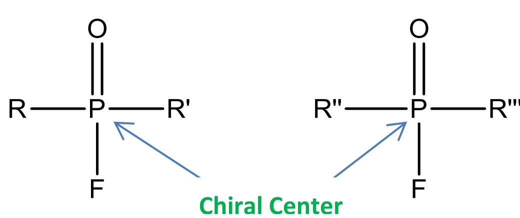

Figure 5 - *General structure of SNL_{OP-I} and SNL_{OP-II}*

Two titrations were performed with the addition of ~2.5mM of the SNL_{OP-I} OFP compound up to 15 mM and then ~5.0mM to a 5mM of α-CD or 5mM β-CD until a plateau with regards to chemical shifts was reached. With each addition, the ^{1}H, ^{31}P and ^{19}F NMR spectra were obtained for analysis, including the determination of chemical shift variations ($\Delta\delta = \delta_{free} - \delta_{complex}$) and enantiomeric discrimination ($\Delta\Delta\delta = |R\text{-}S|$). Table 1 below contains the data indicating the general chemical shifts and, if any enantioseparation was observed, and the enantiomer separation distance. The enantiomer separation distance is based on an arbitrary assignment of R and S enantiomers, since the actual enantiomeric identities of the peaks are unknown.

Table 1 – *Chemical shifts and enantioseparation of $SNL_{OP\text{-}I}$ with α-CD and β-CD in D_2O at 298K (Ratio: CD/SNL_{OP})*

				|R-S| separation		
Compound	CSA	Δδ ^{19}F (ppm)	Δδ ^{31}P (ppm)	ΔΔδ ^{19}F (ppm)	ΔΔδ ^{31}P (ppm)	ΔΔδ ^{1}H (ppm)
$SNL_{OP\text{-}I}$	α-CD					
1:1		-0.0413	0.0809	0.0221	-	-
1:3		-0.0192	0.0575	0.0164	-	-
1:6		-0.0034	0.0515	0.0113	-	-
1:9		-0.0009	0.0390	0.0084	-	-
1:12		0.0004	0.0361	0.0060	-	-
1:15		-0.0017	0.0332	0.0042	-	-
$SNL_{OP\text{-}I}$	β-CD					
1:1		0.6496	-0.4537	0.2170	-	-
1:3		0.3970	-0.2485	0.1170	-	-
1:6		0.2552	-0.1334	0.0670	-	-
1:9		0.1993	-0.0917	0.0438	-	-
1:12		0.1736	-0.0687	0.0359	-	-
1:15		0.1587	-0.0544	0.0272	-	-

Maximum enantiomeric shift

The enantiomer separation distance (ΔΔδ) could only be calculated from the ^{19}F NMR spectrum, since neither the ^{31}P nor ^{1}H NMR spectra showed any peak splitting due to different enantiomers at any of the concentrations. The results gathered indicate that the cavity sizes of α-CD (174 Å^3) and β-CD (262 Å^3) are sufficient for a host/guest relationship with $SNL_{OP\text{-}I}$. We are currently in the process of deriving a k value from the graphed chemical shifts obtained from the ^{19}F and ^{31}P NMR spectra (Figures 6c, 6d), with the exception of ^{31}P NMR α-CD titration results due to insufficient changes in chemical shift.

However, it is obvious from the results shown in Table 1 and Figures 6a and 6b that intermolecular interactions between the enantiomers of SNL_{OP-I} and the two cyclodextrins are not identical. The enantiomeric separation with β-CD is approximately 10x than with α-CD. Molecular modeling and simulations are needed to make definitive conclusions about why this is the case.

Chemical shift (Δδ) could only be calculated from the ^{19}F and ^{31}P NMR spectrum. The chemical shifts observed in the ^{1}H NMR spectrum were inconsistent and, due to this, the chemical shift was not reported for either the α-CD or β-CD titration. However, it is quite obvious from Table 1 and Figures 6a and 6b that the greatest chemical shift changes are observable in the ^{19}F NMR spectrum. The fact that the greatest success with chemical shift, as well as enantiomer separation distance, was observed in the ^{19}F NMR spectrum can likely be attributed the electronegativity of fluorine.

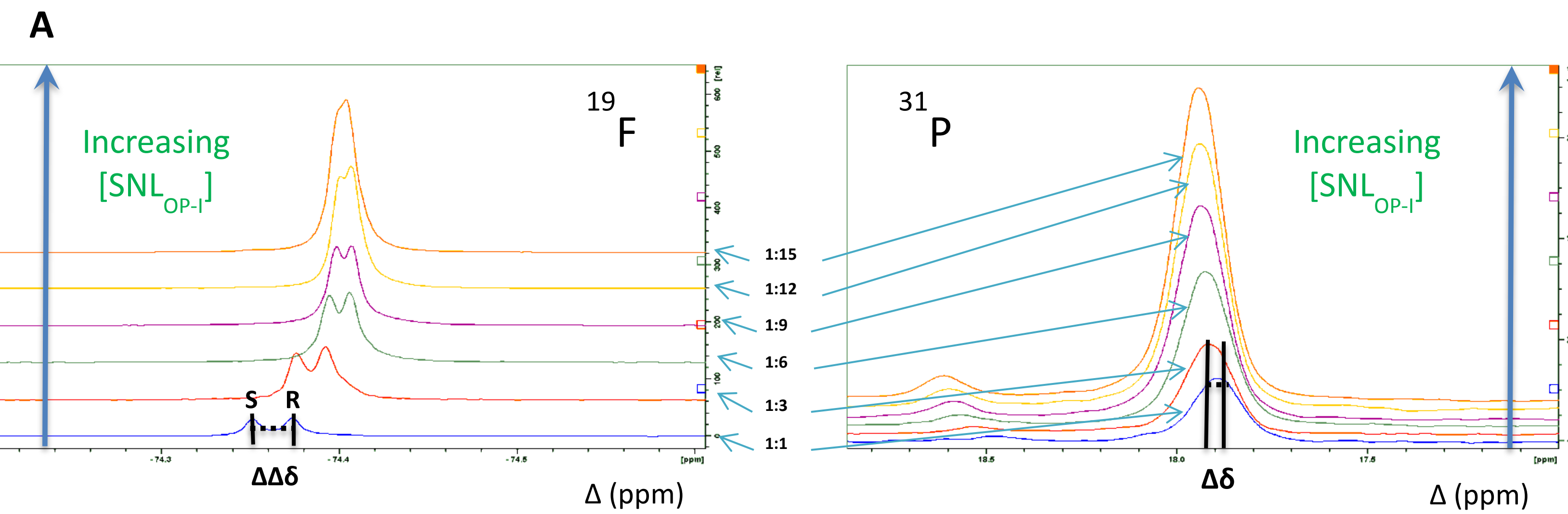

Figure 6a – *^{19}F and ^{31}P NMR spectra illustrating ΔΔδ and Δδ from SNL_{OP-I} titration with 5 mM α-CD at 298K (only most deshielded peak of the $^{19}F/^{31}P$ doublet is shown).*

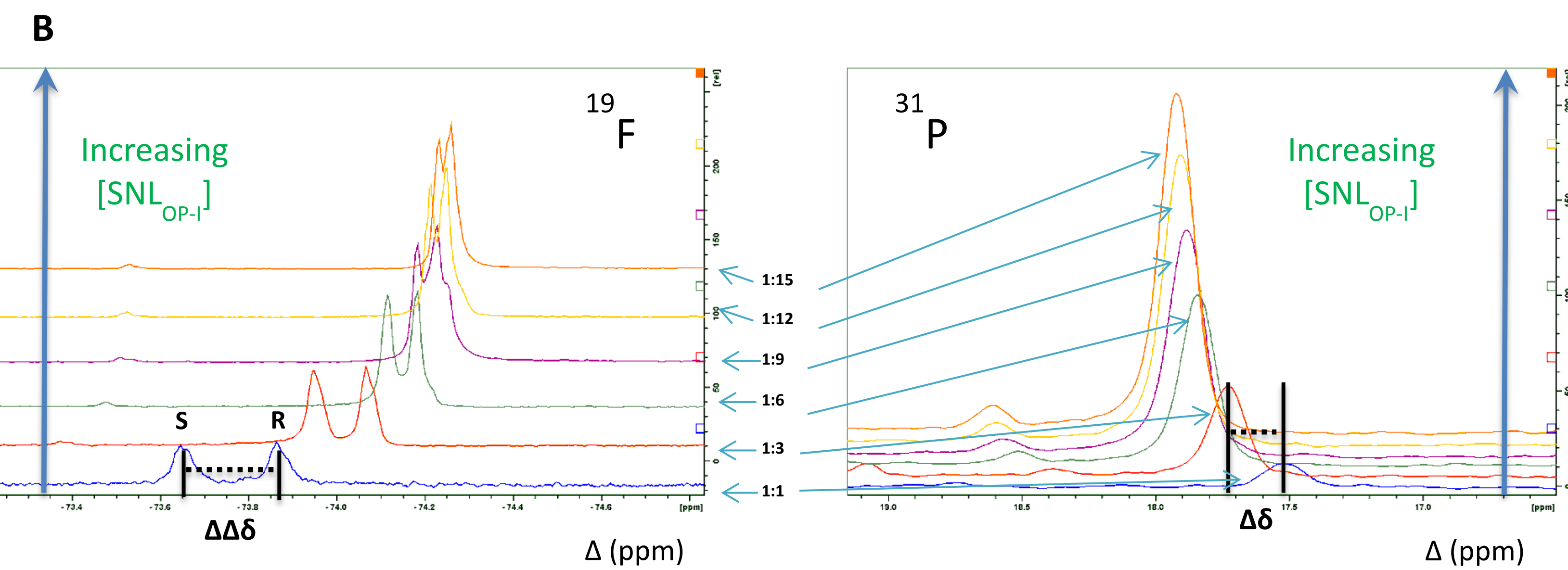

Figure 6b - *^{19}F and ^{31}P NMR spectra illustrating ΔΔδ and Δδ from SNL_{OP-I} titration with 5 mM β-CD at 298K (only most deshielded peak of the $^{19}F/^{31}P$ doublet is shown).*

C

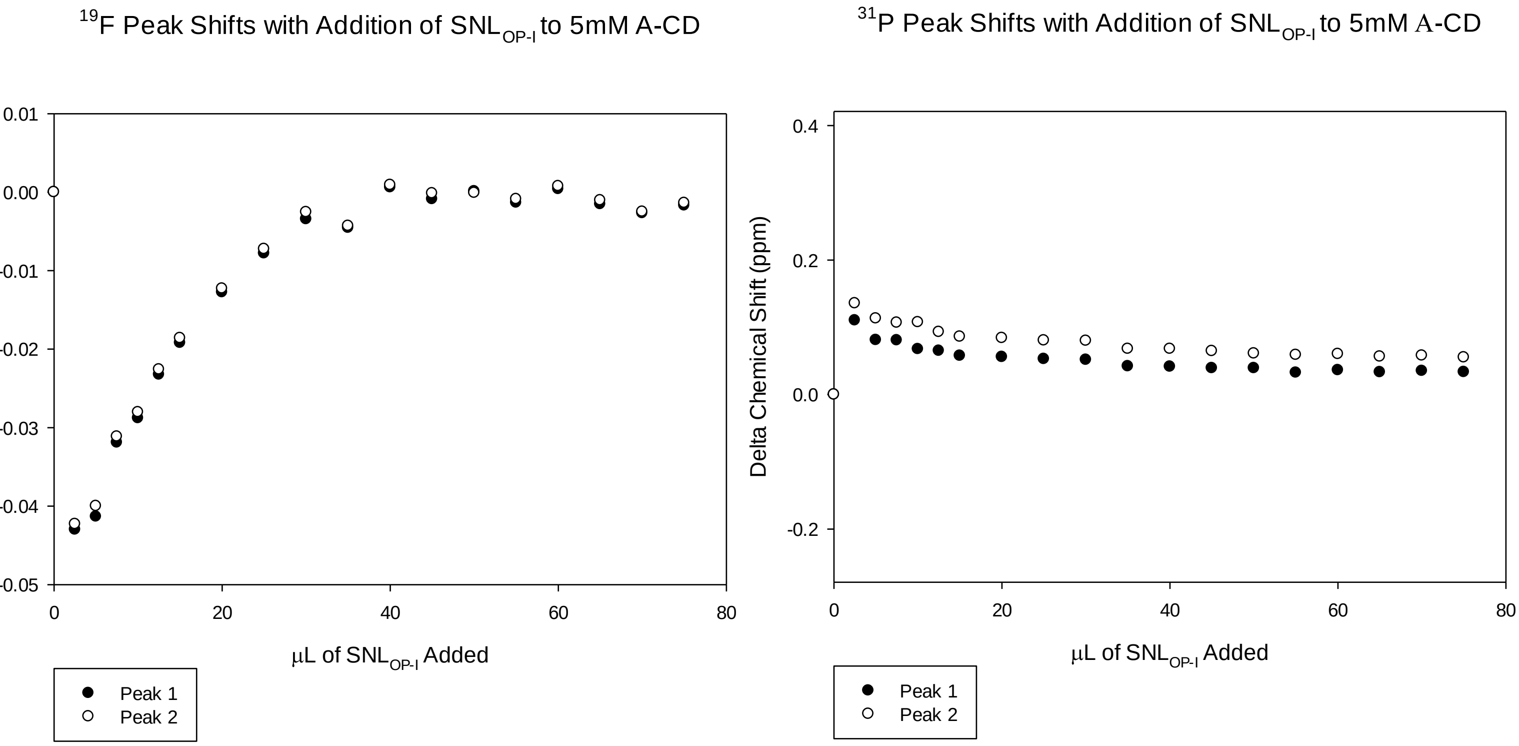

Figure 6c - *Titration curves based on ^{19}F and ^{31}P NMR spectra from $SNL_{OP\text{-}I}$ titration with 5 mM α-CD at 298K*

D

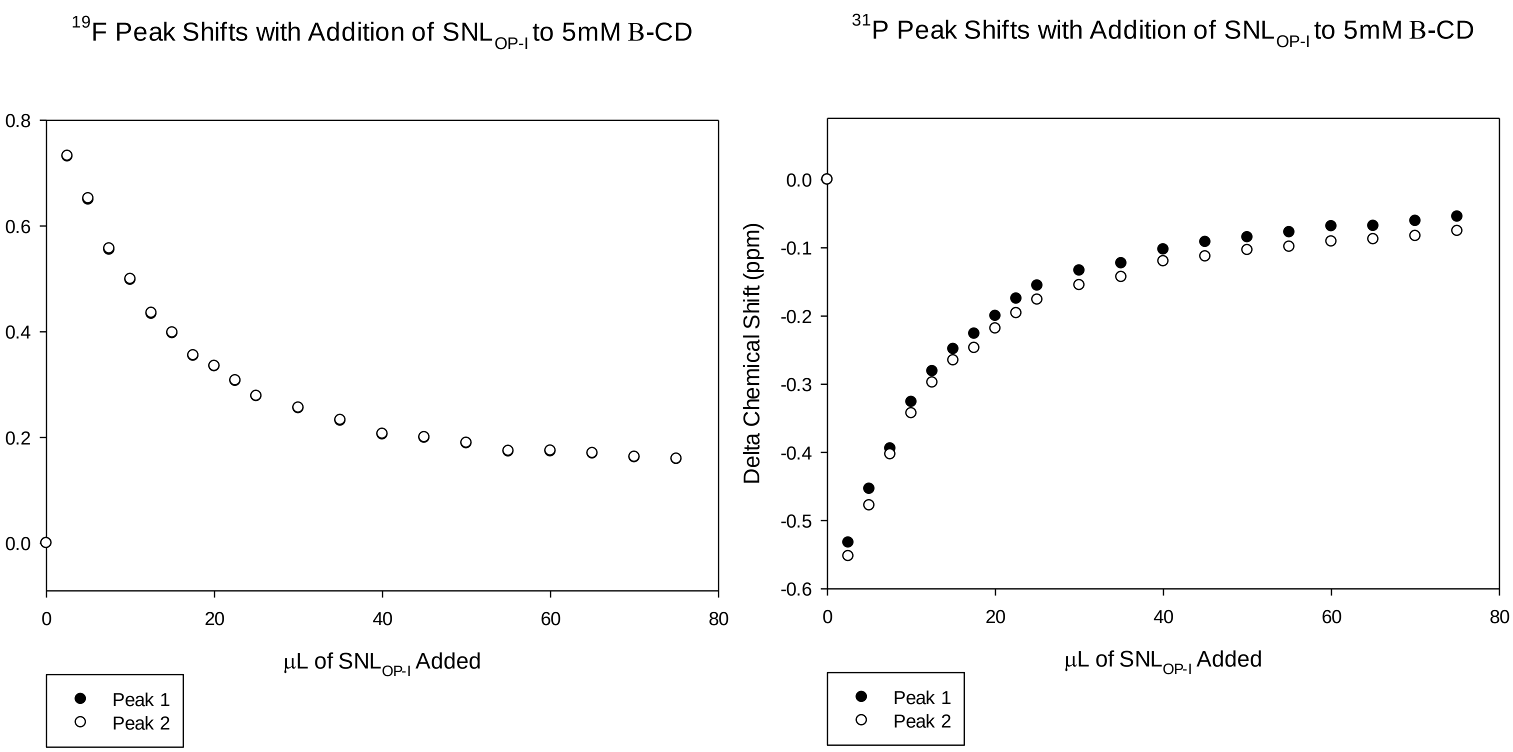

Figure 6d - *Titration curves based on ^{19}F and ^{31}P NMR spectra from $SNL_{OP\text{-}I}$ titration with 5 mM β-CD at 298K*

Figure 7 shows the ^{31}P and ^{19}F NMR spectra of $SNL_{OP\text{-}I}$ and $SNL_{OP\text{-}II}$ without β-CD, with β-CD at 1:2 and with β-CD at 1:2 after 6 days at -20°C. No obvious breakdown products are visible in either the ^{31}P or ^{19}F NMR spectra taken after 6 days. More recent spectra would need to be obtained to continue tracking the status of the compounds and to reach a definitive conclusion about the effect of β-CD on our OFPs. The paper on soman reported that the hydrolysis rapidly occurs at 25°C and a pH of 7.4, so perhaps our conditions are not be favorable to any hydrolysis and none will be observed in any future spectra. Even if decomposition is observed, it would be difficult to ascertain whether it occurred due to some effect of β-CD or due to other factors.

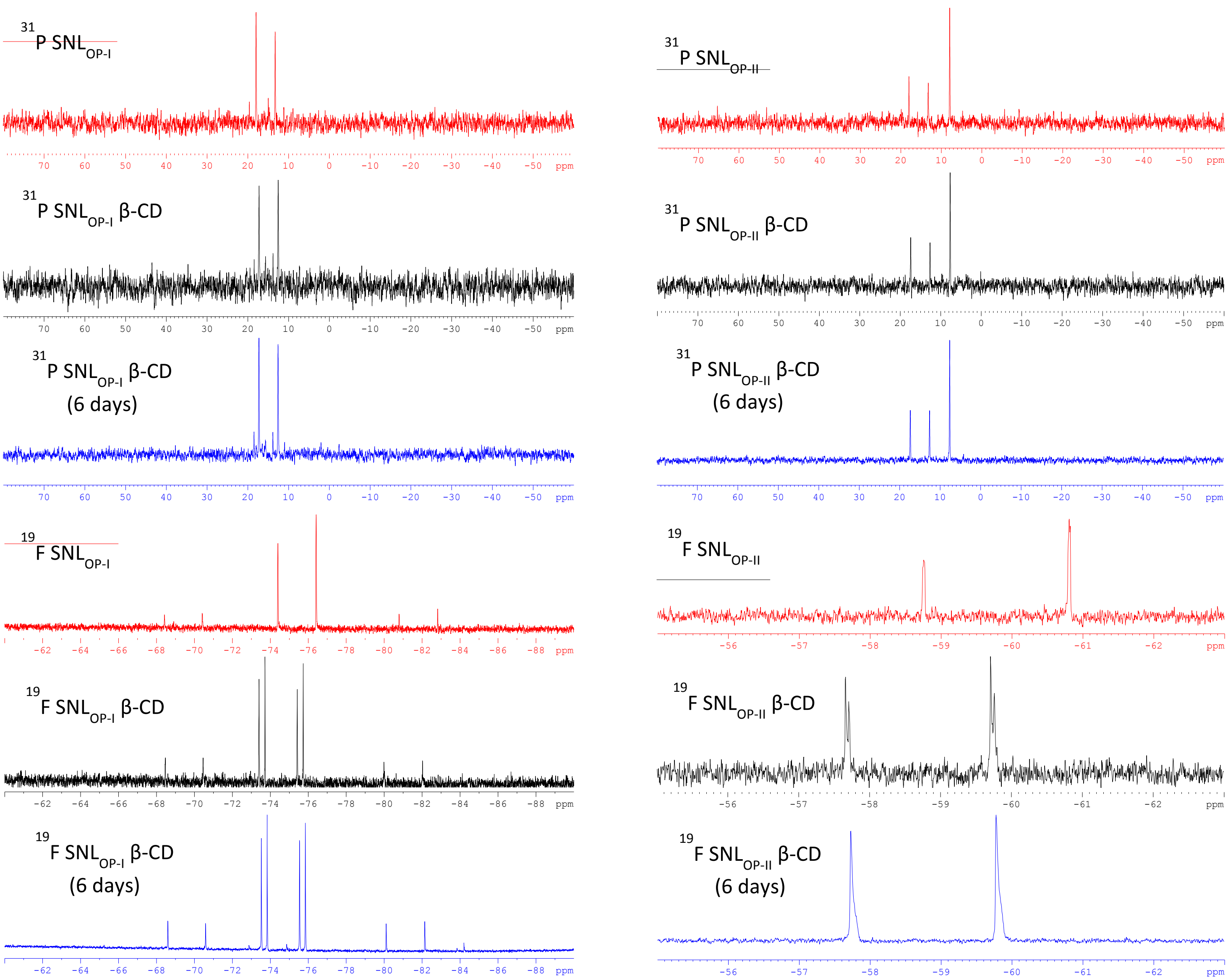

Figure 7 – *^{31}P and ^{19}F NMR spectra of SNL_{OP-I} and SNL_{OP-II} with 1:2 β-CD in D_2O at 298K*

TFAE

For further studies of the $SNL_{OP\text{-}I}$ and $SNL_{OP\text{-}II}$ OFPs and the ability to resolve their enantiomers, we used R-(-)-2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE), a compound characterized as a CSA. Table 2 below contains the results from 1:1, 1:2 and 1:4 (SNL_{OP}/TFAE) studies for both $SNL_{OP\text{-}I}$ and $SNL_{OP\text{-}II}$.

Table 2 – *Chemical shifts and enantioseparation of $SNL_{OP\text{-}I}$ and $SNL_{OP\text{-}II}$ in $CDCl_3$ with TFAE at 298K (Ratio: SNL_{OP}/TFAE)*

<table>
<tr><th colspan="5"></th><th colspan="3">|R-S| separation</th></tr>
<tr><th>Compound</th><th>CSA</th><th>$\Delta\delta\ ^{19}F$ (ppm)</th><th>$\Delta\delta\ ^{31}P$ (ppm)</th><th>$\Delta\delta\ ^{1}H$ (ppm)</th><th>$\Delta\Delta\delta\ ^{19}F$ (ppm)</th><th>$\Delta\Delta\delta\ ^{31}P$ (ppm)</th><th>$\Delta\Delta\delta\ ^{1}H$ (ppm)</th></tr>
<tr><td>$SNL_{OP\text{-}I}$</td><td>TFAE</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>1:1</td><td></td><td>0.0396</td><td>-0.0085</td><td>0.0171</td><td>0.0074</td><td>-</td><td>-</td></tr>
<tr><td>1:2</td><td></td><td>0.0629</td><td>-0.0030</td><td>0.0219</td><td>0.0131</td><td>-</td><td>-</td></tr>
<tr><td>1:4</td><td></td><td>0.1556</td><td>0.0033</td><td>0.0470</td><td>0.0282</td><td>-</td><td>-</td></tr>
<tr><td>$SNL_{OP\text{-}II}$</td><td>TFAE</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>1:1</td><td></td><td>0.0293</td><td>-0.0649</td><td>0.0270</td><td>0.0404</td><td>-</td><td>-</td></tr>
<tr><td>1:2</td><td></td><td>0.0834</td><td>-0.0838</td><td>0.0592</td><td>0.0994</td><td>-</td><td>-</td></tr>
<tr><td>1:4</td><td></td><td>0.1715</td><td>-0.1650</td><td>0.1098</td><td>0.1985</td><td>-</td><td>-</td></tr>
</table>

Maximum enantiomeric shift

As with the cyclodextrins, peak splitting could only be seen within the ^{19}F NMR spectrum. The largest chemical shift for $SNL_{OP\text{-}I}$ with one equivalent TFAE was observed in the ^{19}F NMR spectrum. However, for $SNL_{OP\text{-}II}$ with one equivalent of TFAE, the largest chemical shift was observed in the ^{31}P NMR spectrum. Overall, $SNL_{OP\text{-}II}$ with TFAE had more significant chemical shifts with the addition of a 2nd equivalent of TFAE – 1.7x versus 2.8x in the ^{19}F NMR spectra and 1.5x versus 2.2x in the ^{1}H NMR spectra of $SNL_{OP\text{-}I}$ and $SNL_{OP\text{-}II}$, respectively. This indicates that stronger intermolecular interactions between TFAE and $SNL_{OP\text{-}II}$ exist (versus TFAE and $SNL_{OP\text{-}I}$) which can be attributed to the differences in structure and steric effects of the R groups of the two OFPs (Fig. 5). Although further studies should focus on the ability of the CSA to induce peak splitting in the ^{19}F NMR spectrum, it is obvious that the chemical shifts in the ^{31}P NMR spectrum should not be discounted.

Figure 8 clearly shows the peak splitting in the ^{19}F NMR spectra, allowing for enantiomeric differentiation between the R and S enantiomers of SNL_{OP-I} and SNL_{OP-II}, with more obvious peak splitting at the 1:2 ratio of SNL_{OP} to TFAE. Figure 9, the corresponding ^{31}P NMR spectra, shows the observed variations in chemical shifts are less than a hundredth of a ppm for SNL_{OP-I} and just under a tenth of a ppm for SNL_{OP-II}.

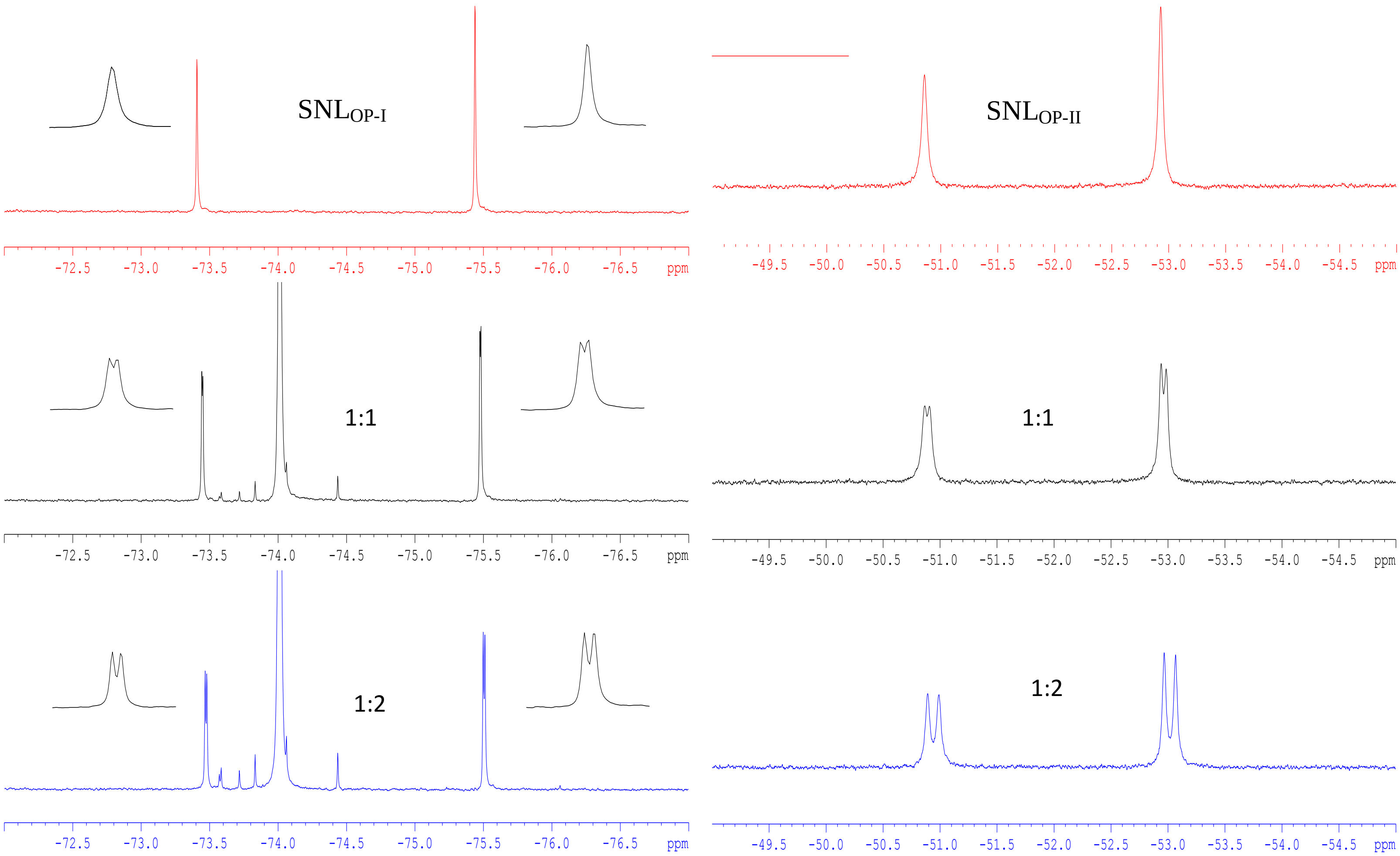

Figure 8 - *^{19}F NMR spectra of SNL$_{OP-I}$ and SNL$_{OP-II}$ with 1:1 and 1:2 TFAE in $CDCl_3$ at 298K*

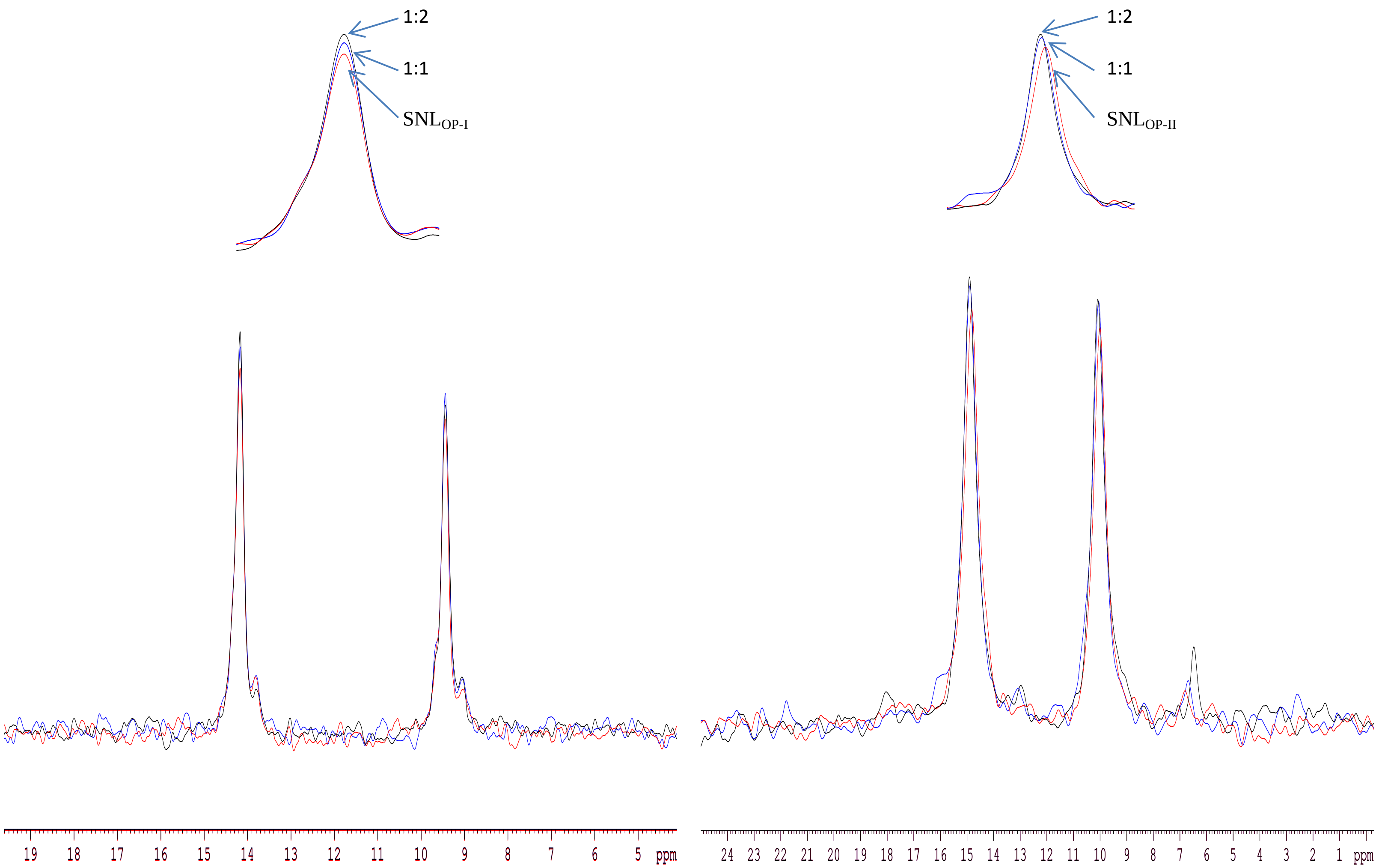

Figure 9 - *^{31}P NMR spectra of SNL_{OP-I} and SNL_{OP-II} with 1:1 and 1:2 TFAE in $CDCl_3$ at 298K*

Conclusions:

Overall, the best resolution of enantiomers of these OFPs was observed with β-CD, with a chemical shift of 0.2170 ppm at 1:1 with SNL_{OP-I}. For comparison, at 1:1, α-CD with SNL_{OP-I} resulted in a chemical shift of 0.0221 ppm, TFAE with SNL_{OP-I} resulted in a chemical shift of 0.0074 ppm, and TFAE with SNL_{OP-II} resulted in a chemical shift of 0.0404 ppm. These results make it even more tempting to expand and attempt this experiment with γ-CD, a cyclodextrin with 8 total sugars (1 more than β-CD). It is also tempting to assume that the larger chemical shifts observed with TFAE and SNL_{OP-II} would result in larger chemical shifts had SNL_{OP-II} been tested with α-CD and β-CD. Unfortunately, the relatively rapid decomposition rate of SNL_{OP-II} in solution would make it challenging to attempt a titration like the ones completed with the cyclodextrins and SNL_{OP-I} (many many hours of nearly non-stop work; several times on the weekend and once overnight). This summer's work has resulted in a very good basis for future experiments, but there are many more experiments that need to be completed to have a robust method for chiral recognition of OFPs.

As previously mentioned, a rapid and robust method for enantiomer identification of unknown/new OFPs would be very useful for national security. Although G-series agents have been banned and classified as weapons of mass destruction (WMD) by the United Nations, it is quite obvious that certain actors in the world are not willing to destroy their supplies of CWAs. It is obvious that there is still interest in using and perhaps developing new CWAs - Novichok agents for example. One of the objectives of those in charge of developing these new CWAs probably includes creating an OFP compound that cannot be detected with current NATO tools.

Continued threats in the Middle East (case in point: Syria), prove that we cannot be too careful and cannot assume that banning CWAs has had the full effect we've desired. Given

Russia's close relationships with certain countries in the Middle East, as well as China, and their willingness to sell them Russian weapons, etc., we should be suspicious and vigilant of such potential threats.

Et cetera:

Given the fact that my only previous NMR experience involved using a 60 MHz Anasazi NMR a handful of times, this summer experience has taught me the what, why and how of NMR. In organic chemistry class, NMR was described as a tool for identifying compounds by correlating the J-coupling and deshielding of the peaks with certain positions and functional groups. This is very far from what NMR can be used for and is being used for. This summer, I learned this first hand by observing and performing (at least once, often times more) HSQCs (Heteronuclear Single Quantum Coherence SpectroscopY), NOESYs (Nuclear Overhauser Effect SpectroscopY), COSYs (Correlation SpectroscopY) and DOSYs (Diffusion-Ordered SpectroscopY). All of these, and many many more (the limiting factor is the number of pulse sequences that can be created), are very useful analytical tools.

While at Sandia National Laboratories in Albuquerque, New Mexico, I've had numerous enrichment opportunities and have attended several of them. These include talks given by Sandia researchers on their current research, i.e. trapping ions as a method for improving the entropy behind encryption methods. Additionally, I attended a talk given by General Kehler (Retired, U.S. Air Force) on the enduring role of nuclear weapons in U.S. national security. We had weekly meetings where we presented and discussed the science behind our projects. Dr. Alam was also able to arrange visits to the Mind Institute at University of New Mexico and ABQMR. While at the Mind Institute, I was able to observe various MRI techniques first hand and get a better understanding the incredible usefulness of magnets. ABQMR introduced me to the innovations being made for NMR to be used beyond the lab bench. I've met with numerous scientists at Sandia who work closely with DHS on key issues. Overall, there was no shortage of activities (ran my first 5K) and events that I could attend. I can say with certainty that these experiences have greatly enriched and expanded my scientific and worldly knowledge.

Presentations/Posters/Publications:

1) Vitaliy Dernov* and Todd M. Alam, "Investigation and Development of NMR Tools for Chiral Compound Identification", *Sandia National Laboratories Student Intern Symposium*, Albuquerque, NM, July 2014 (Poster)

2) Vitaly Dernov*, "Development and Investigation of NMR Tools for Chiral Compound Identification Exploration/Optimization of Enantiomer Identification with Chiral Solvating Agents (CSAs) Using Organo-Fluorophosphate (OFP) Analogs of Chemical Warfare Agents (CWAs)", *Departmental Seminar*, Albuquerque, NM, July 2014 (Presentation).

3) Todd M. Alam and Vitaliy Dernov, "(U) Chiral NMR Separation of Select Organo-Fluorophosphates", SAND-XXXX (2014) *In preparation*[Classified Report].

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Appendix (PDF Copies attached)

a) Symposium Poster

b) Departmental Seminar

Investigation and Development of NMR Tools for Chiral Compound Identification

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7/31/2014

Abstract

The use of NMR spectroscopy with the assistance of chiral solvating agents (CSAs) for the identification and quantification of organo-fluorophoshates (OFPs) has not been thoroughly investigated. The optimization of existing methods for the enantiomeric discrimination and quantification of OFP analogs of chemical warfare agents (CWAs) like sarin would assist the development of decontamination techniques and modeling efforts for optimal resolution of chiral compounds. Additionally, it would assist the development of development of a rapid and a robust method for chiral recognition of unknown/new OFPs. Cyclodextrins (CDs, cyclic oligosaccharides) like α-CD and β-CD are supramolecules with an ability to form host-guest relationships with certain polar compounds. R-(-)-2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE/Pirkle's Alcohol) is a compound with a high diamagnetic anisotropy due to its anthracene ring, and has been reported to alter the magnetic environments of chiral compounds. Both of these CSA classes were used in our attempts to determine the chemical shift variations and to separate the enantiomers of two OFP compounds - $SNL_{OP\text{-}I}$ and $SNL_{OP\text{-}II}$. Enantioseparation was observed at all concentrations used in the ^{19}F NMR spectra of $SNL_{OP\text{-}I}$ with α- and β-CD (1:1 …1:15) and in the ^{19}F NMR spectra of $SNL_{OP\text{-}I}$ and $SNL_{OP\text{-}II}$ with TFAE (1:1 and 1:2).

Introduction

One of the major current methods for chiral recognition of OFPs is through gas chromatography (GC). Past attempts at chiral analysis of nerve agent stereoisomers used tools such as the capillary Chirasil Val column for GC. It was only partially able to resolve stereoisomers and a clever use of a Carbowax column in series was needed for complete stereoisomer resolution. The researchers in that case used GC and nuclear magnetic resonance (NMR) spectroscopy in a way that complemented each other [1]. The goal of the current effort is to obtain enantiomer identification and quantification using only NMR spectroscopy.

As an example, Sarin is classified as a nerve agent. It is also categorized as a G-series CWA with the abbreviation "GB". The other G-series agents referenced in Figure 2 are tabun "GA", soman "GD" and cyclosarin "GF". One of the key structural features of such agents, which are often similar to pesticides in structure (but not potency), is the organo-fluorophosphate structure[2]. The deadliness of sarin is attributed to its ability to inhibit acetylcholinesterase (as illustrated by Figure 1) – an enzyme that typically breaks down acetylcholine. Acetylcholine is responsible for locomotion by having an excitatory role at neuromuscular junctions of the central nervous system (CNS) and the peripheral nervous system (PNS) [3].

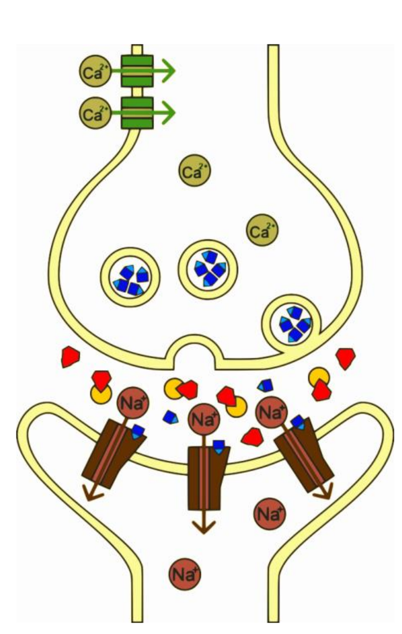

Figure 1. *Diagram of sarin (red) inhibition of acetylcholinestarase (yellow) and the build up of acetylcholine (blue) in the synaptic junction.*

http://en.wikipedia.org/wiki/Sarin#mediaviewer/File:Sarin_Biological_effects.svg

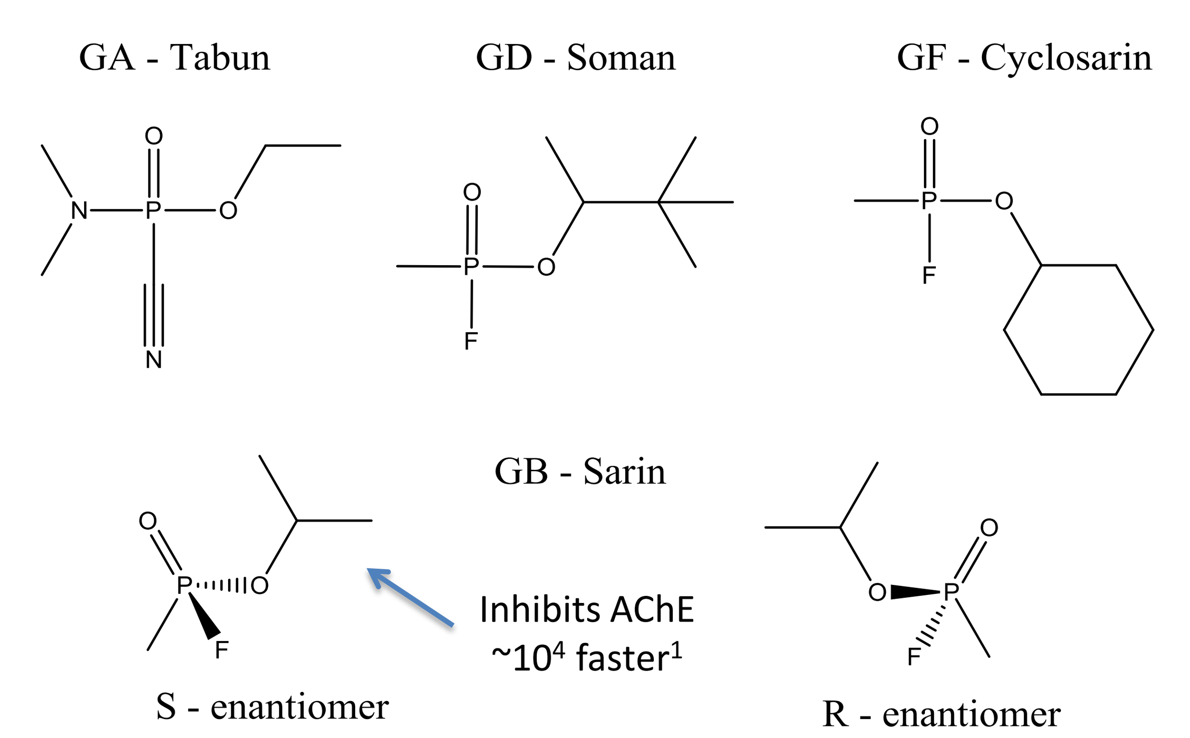

Figure 2. *Structures of G-series CWAs. Notice the organo-fluorophosphate (OFP) backbone. (Organophosphate for Tabun).*

The principle of being able to differentiate between enantiomers with NMR using CSAs is a matter of enantioselective interactions between the chiral selector and enantiomers. This can be explained by electrostatic interactions, van der Waals forces and H-bonding. As an example, in β-cyclodextrin (as well as α-CD and other cyclodextrins) a host/guest complex is formed, where a molecule enters the "donut hole" that exists in such supramolecules (Figure 3). For each enantiomer, these interactions will vary due to steric effects and should be reflected by a difference in the chemical shift between the enantiomers on an NMR spectrum (^{1}H, ^{13}C, ^{19}F, ^{31}P, etc.).

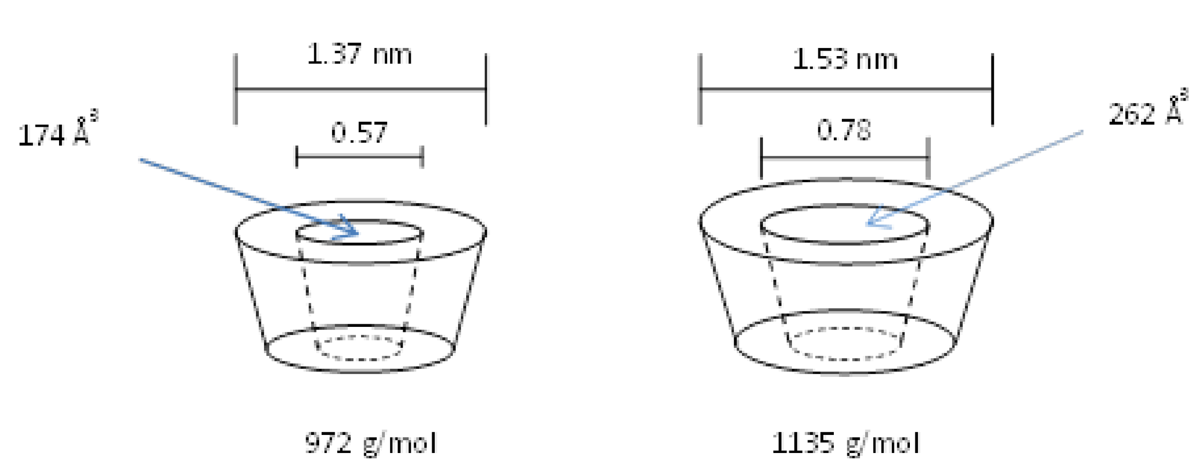

Figure 3. *Structure of α-CD and β-CD with focus on the cavity (Adapted from Szejtli 1998)*

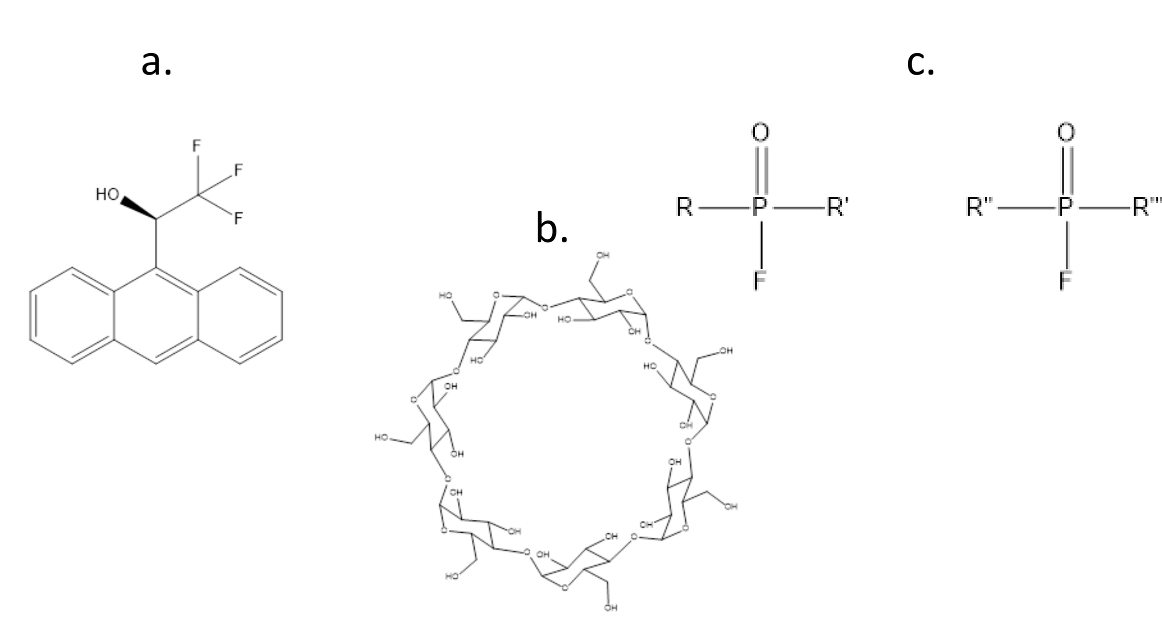

Figure 4 a) *Structure of TFAE,* **b)** *Structure of β-CD,* **c)** *General structure of $SNL_{OP\text{-}I}$ and $SNL_{OP\text{-}II}$*

Methods

All spectra were obtained at 298K on a Bruker 500 with a 5 mm broad band probe.

α- and β-cyclodextrins

To emulate the chemical structure and properties of CWAs like sarin, we used two organo-fluorophosphate (OFP) compounds (Figure 4c) with a stereocenter at the phosphorous atom and with various organic R groups. Titrations were performed only on $SNL_{OP\text{-}I}$ due to the compound's greater stability in aqueous solutions. Initial ^{1}H, ^{31}P and ^{19}F NMR spectra were obtained to determine the default peak positions.

Two titrations were performed with the initial addition of ~2.5mM of the $SNL_{OP\text{-}I}$ OFP compound up to 15mM and then ~5.0mM to 5mM of α-CD or 5mM β-CD until a plateau with regards to chemical shifts was reached. With each addition, the ^{1}H, ^{31}P and ^{19}F NMR spectra were obtained for analysis, including the determination of chemical shifts ($\Delta\delta = \delta_{free} - \delta_{complex}$) and enantiomeric discrimination ($\Delta\Delta\delta = |R\text{-}S|$). Table 1 below contains the data indicating the general chemical shifts and, if any enantioseparation was observed, the enantiomer separation distance.

TFAE

For further studies of the $SNL_{OP\text{-}I}$ and $SNL_{OP\text{-}II}$ OFPs and the ability to resolve their enantiomers, we used R-(-)-2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE), a compound characterized as a CSA (Figure 4a). Table 2 below contains the results from 1:1 and 1:2 (SNL_{OP}/TFAE) studies for both $SNL_{OP\text{-}I}$ and $SNL_{OP\text{-}II}$.

As with the α-CD and β-CD, initial ^{1}H, ^{31}P and ^{19}F NMR spectra were obtained for analysis and determination of chemical shifts (Δδ) and enantiomeric discrimination (ΔΔδ).

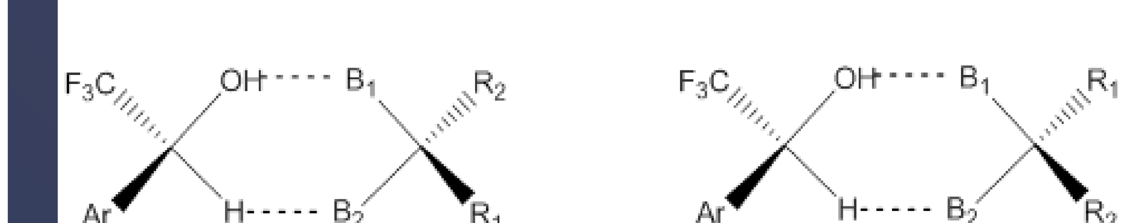

Figure 5. *A model of the primary intermolecular interactions between a chiral compound and TFAE (Adapted from Pirkle and Hoover 1982).*

α-CD Titration

Results

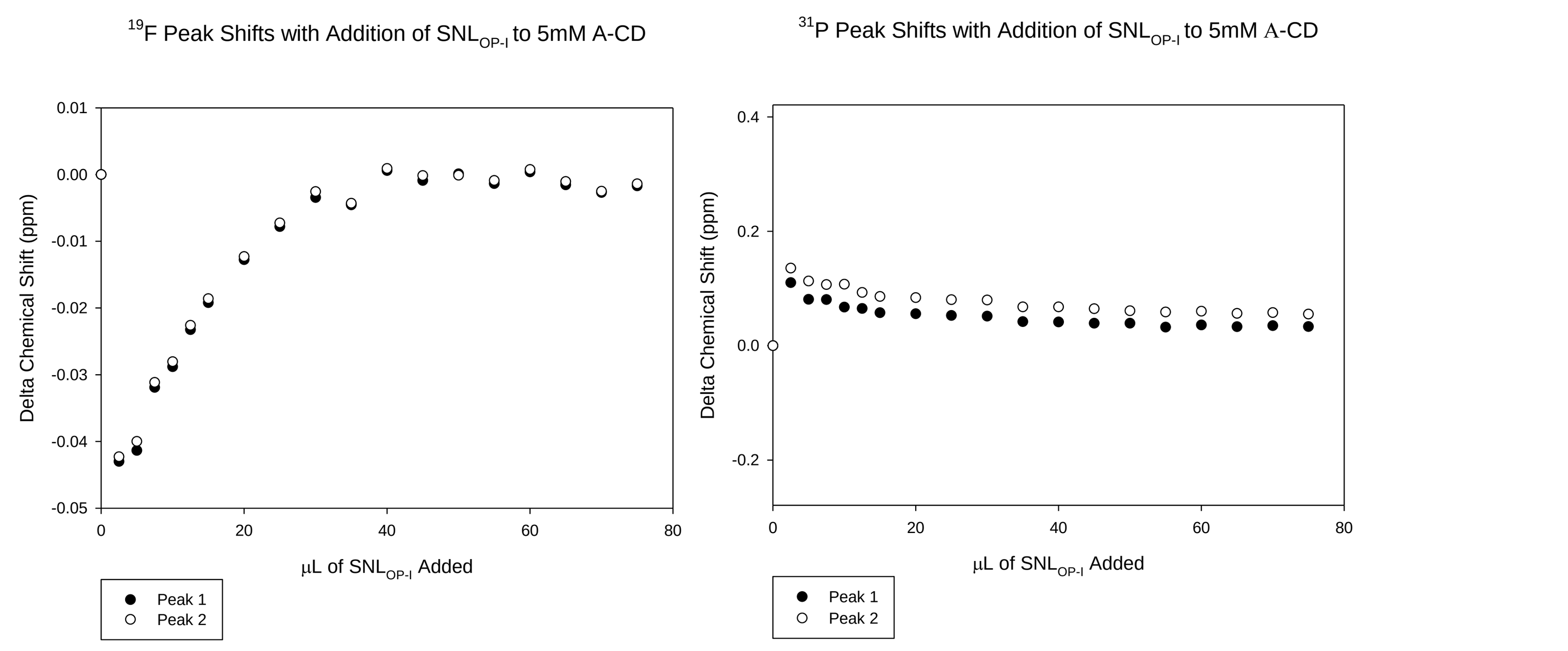

Figure 6. *Titration curves based on ^{19}F and ^{31}P NMR spectra from SNL$_{OP-I}$ titration with 5 mM α-CD at 298K*

TFAE

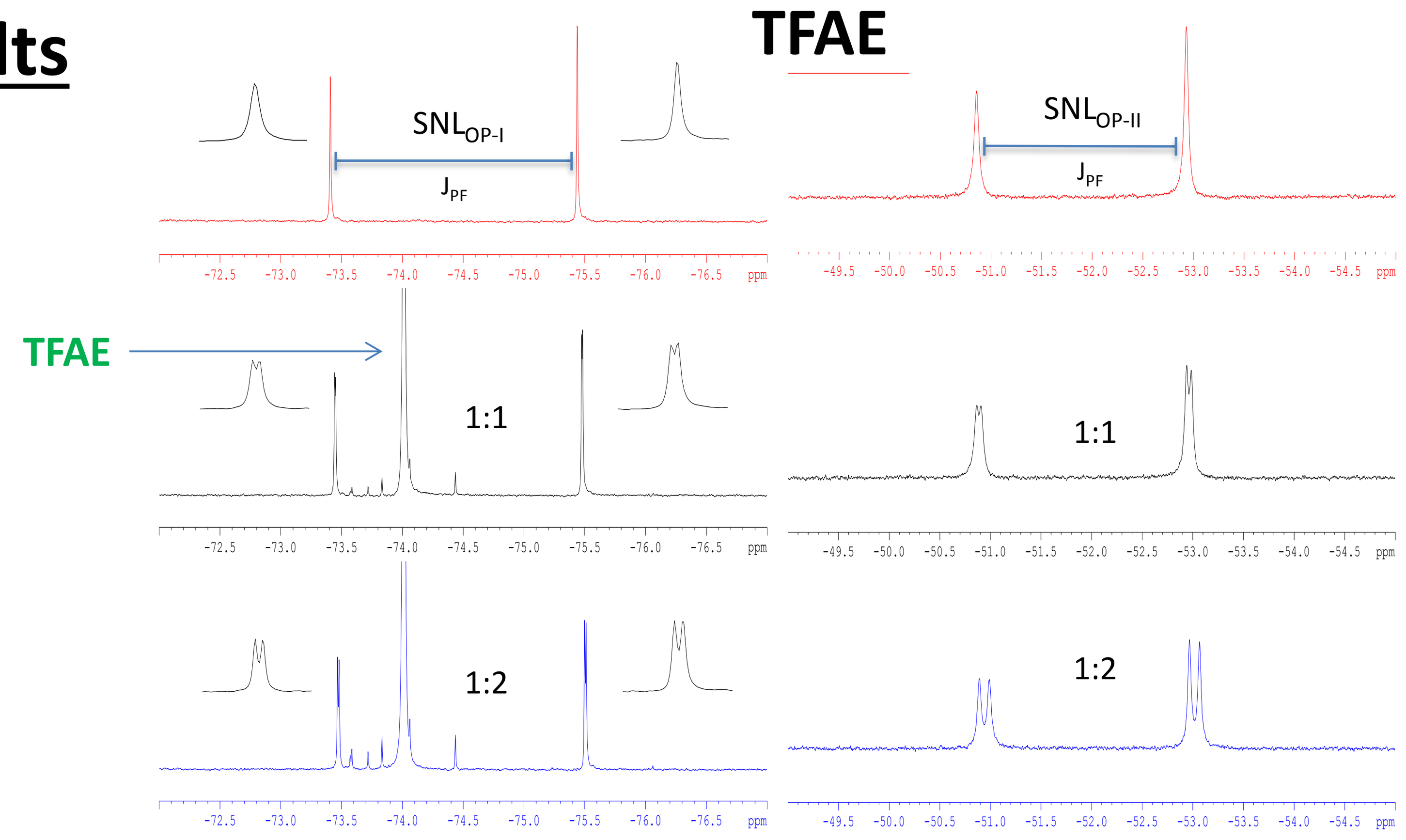

Figure 8. *^{19}F NMR spectra of SNL$_{OP-I}$ and SNL$_{OP-II}$ with 1:1 and 1:2 TFAE in CDCl$_3$ at 298K*

β-CD Titration

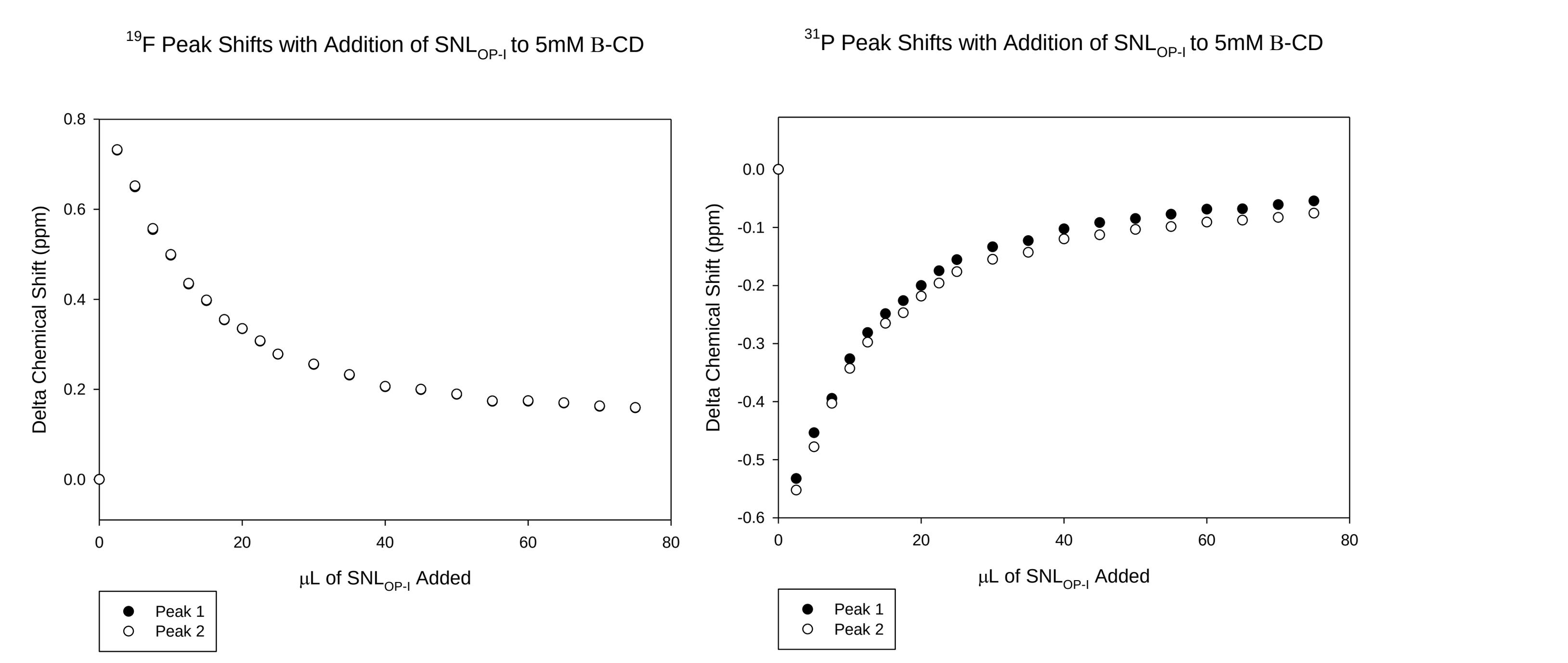

Figure 7. *Titration curves based on ^{19}F and ^{31}P NMR spectra from SNL$_{OP-I}$ titration with 5 mM β-CD at 298K*

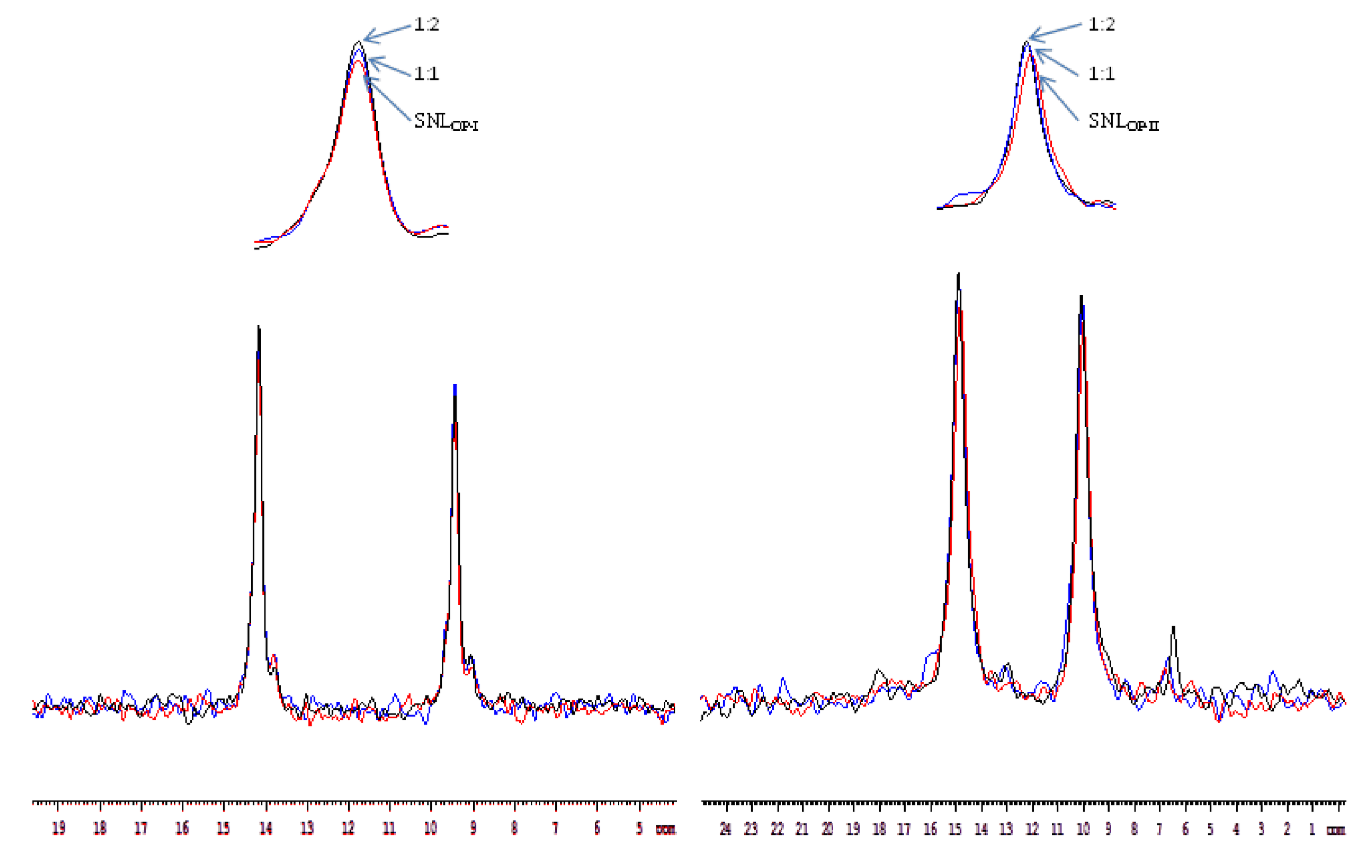

Figure 9. *^{31}P NMR spectra of SNL$_{OP-I}$ and SNL$_{OP-II}$ with 1:1 and 1:2 TFAE in CDCl$_3$ at 298K*

Results/Conclusions

α- and β-cyclodextrins

Compound	CSA	Δδ ^{19}F (ppm)	Δδ ^{31}P (ppm)	Δδ ^{1}H (ppm)	|R-S| separation ΔΔδ ^{19}F (ppm)	ΔΔδ ^{31}P (ppm)
SNL$_{OP-I}$	α-CD					
1:1		-0.0413	0.0809		0.0221	-
1:3		-0.0192	0.0575		0.0164	-
1:6		-0.0034	0.0515		0.0113	-
1:9		-0.0009	0.0390		0.0084	-
1:12		0.0004	0.0361		0.0060	-
1:15		-0.0017	0.0332		0.0042	-
SNL$_{OP-I}$	β-CD					
1:1		0.6496	-0.4537		0.2170	-
1:3		0.3970	-0.2485		0.1170	-
1:6		0.2552	-0.1334		0.0670	-
1:9		0.1993	-0.0917		0.0438	-
1:12		0.1736	-0.0687		0.0359	-
1:15		0.1587	-0.0544		0.0272	-

Maximum enantiomeric shift

Table 1. *Chemical shifts and enantioseparation of SNL$_{OP-I}$ with α-CD and β-CD in D$_2$O at 298K (Ratio: CD / SNL$_{OP}$)*

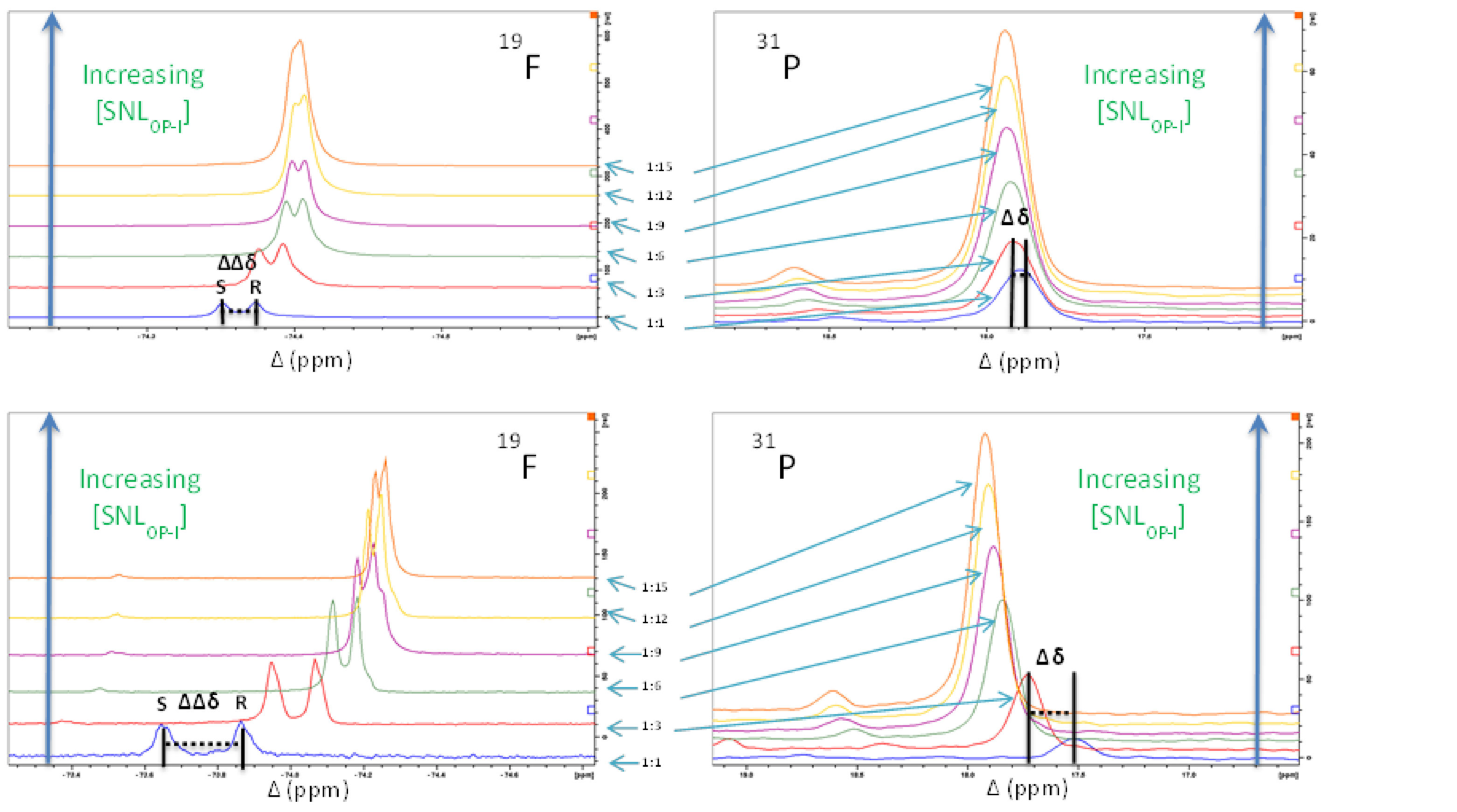

Figure 10. *^{19}F and ^{31}P NMR spectrum illustrating the chemical shifts and enantioseparation of SNL$_{OP-I}$ with α-CD and β-CD in D$_2$O at 298K (Ratio: CD / SNL$_{OP}$)*

The enantiomer separation distance could only be calculated from the ^{19}F NMR spectrum, since neither the ^{31}P nor ^{1}H NMR spectra showed any peak splitting at any of the concentrations. The results gathered indicate that the cavity sizes of α-CD (174 Å^3) and β-CD (262 Å^3) are sufficient for a host-guest relationship with SNL$_{OP-I}$. We are in the process of deriving a k value from the graphed chemical shifts obtained from the ^{19}F and ^{31}P NMR spectra (Figure 6), with the exception of ^{31}P NMR α-CD titration results due to insufficient changes in chemical shift.

However, it is obvious from the results shown in Table 1 and Figure 10 that the intermolecular interactions between the enantiomers of SNL$_{OP-I}$ and the two cyclodextrins are not identical. The enantiomeric separation with β-CD is approximately 10x than with α-CD. Molecular modeling and simulations are needed to make definitive conclusions about why this is the case.

Results/Conclusions

TFAE

Compound	CSA	Δδ ^{19}F (ppm)	Δδ ^{31}P (ppm)	Δδ ^{1}H (ppm)	|R-S| separation ΔΔδ ^{19}F (ppm)	ΔΔδ ^{31}P (ppm)
SNL$_{OP-I}$	TFAE					
1:1		0.0396	-0.0085	0.0171	0.0074	-
1:2		0.0665	-0.0030	0.0252	0.0123	-
1:4		0.1556	0.0033	0.0470	0.0282	
SNL$_{OP-II}$	TFAE					
1:1		0.0293	-0.0649	0.0270	0.0404	-
1:2		0.0834	-0.0838	0.0592	0.0994	-
1:4		0.1715	-0.1650	0.1098	0.1985	

Maximum enantiomeric shift

Table 2. *Chemical shifts and enantioseparation of SNL$_{OP-I}$ and SNL$_{OP-II}$ with TFAE in CDCl$_3$ at 298K (Ratio: SNL$_{OP}$ / TFAE)*

As with the cyclodextrins, peak splitting was only observed within the ^{19}F NMR spectrum. The largest chemical shift for SNL$_{OP-I}$ with one equivalent TFAE was observed in the ^{19}F NMR spectrum. However, for SNL$_{OP-II}$ with one equivalent of TFAE, the largest chemical shift was observed in the ^{31}P NMR spectrum. Overall, SNL$_{OP-II}$ with TFAE had more significant chemical shifts with the addition of a 2nd equivalent of TFAE – 1.7x versus 2.8x in the ^{19}F NMR spectra and 1.5x versus 2.2x in the ^{1}H NMR spectra of SNL$_{OP-I}$ and SNL$_{OP-II}$, respectively. This indicates that stronger intermolecular interactions between TFAE and SNL$_{OP-II}$ exist (versus TFAE and SNL$_{OP-I}$) which can be attributed to the differences in structure and steric effects of the R groups of the two OFPs (Figure 4c).

Fig. 8 clearly shows the peak splitting, allowing for enantiomeric differentiation between the R and S enantiomers of SNL$_{OP-I}$ and SNL$_{OP-II}$, with more obvious peak splitting at the 1:2 ratio of SNL$_{OP}$ to TFAE. Fig. 9, the corresponding ^{31}P NMR spectra, shows the observed chemical shifts – less than a hundredth of a ppm for SNL$_{OP-I}$ and just under a tenth of a ppm for SNL$_{OP-II}$.

Discussion/Future Work

Our results show promising leads that will help to optimize NMR chiral recognition of OFPs. Further studies should focus on the ability of the CSA to induce peak splitting in the ^{19}F NMR spectrum, and should involve novel OFPs as well as other CSA molecules (like γ-CD and Mosher's Acid). Molecular modeling simulations would elucidate the primary and secondary interactions between our and other OFPs with cyclodextrins. Monitoring for β-CD – catalyzed hydrolysis of OFPs would be helpful.

Works Cited

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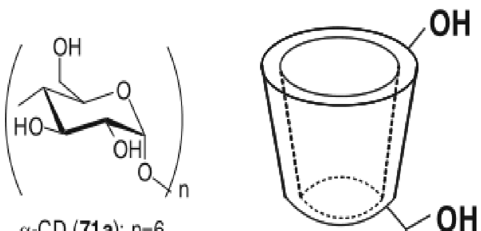

α-CD (71a): n=6
β-CD (71b): n=7
γ-CD (71c): n=8

Development and Investigation of NMR Tools for Chiral Compound Identification

Exploration/Optimization of Enantiomer Identification with Chiral Solvating Agents (CSAs) Using Organo-Fluorophosphate (OFP) Analogs of Chemical Warfare Agents (CWAs)

Vitaliy Dernov
Temple University – B.S. in Biochemistry 2016

Partial funding provided by the DHS HS-STEM Summer Internship Program

Student Internship Program Presentation at SNL
7/31/14

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Exceptional service in the national interest

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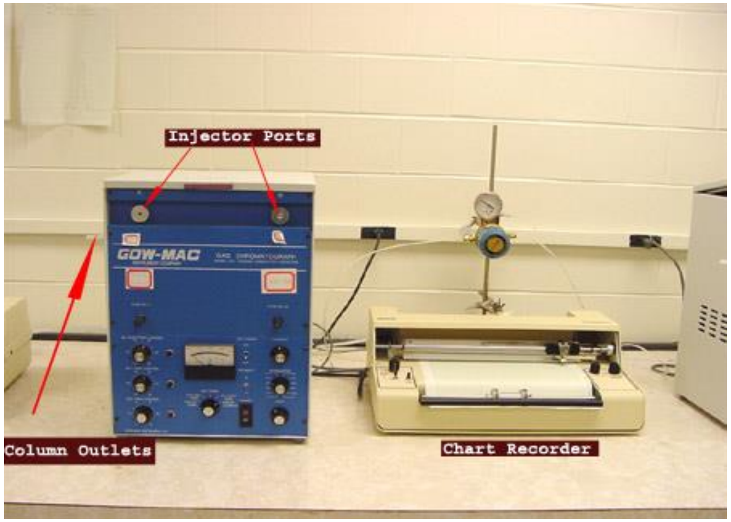
Goals

- Investigate NMR for identification of organo-fluorophosphate (OFP) enantiomers
- Test several types of chiral solvating agents (CSAs)
  - Cyclodextrins – H/G
  - TFAE - High diamagnetic anisotropy
- Electrostatic interactions, Van der Waals forces and H-bonding
- Characterize their binding by calculating Δδ ($\delta_{free} - \delta_{complex}$) and ΔΔδ (|R-S|) – both in ppm.

$$Chiral\ Compound + CSA \ \leftrightarrow \ R/CSA\ Complex + S/CSA\ Complex$$

Motivation

- Most researched method for chiral recognition of OFPs/OPs is gas chromatography (GC)
- Chirasil Val Column and Carbowax Column
- Incomplete resolution
  - Satisfactory results only for deuterated soman in 1984 Benschop *et al.* study
- Benschop and De Jong 1988 study had success in chiral NMR analysis with Lanthanide shift reagents
  - Several downsides --- i.e. water complexes with Lanthanide shift reagents and causes hydrolysis



- DECON optimizations in case of CWA event
- Development of models correlating chiral compounds and CSA
- **Robust** method for identification of unknown/new OFPs – request from Edgewood Chemical Biological Center

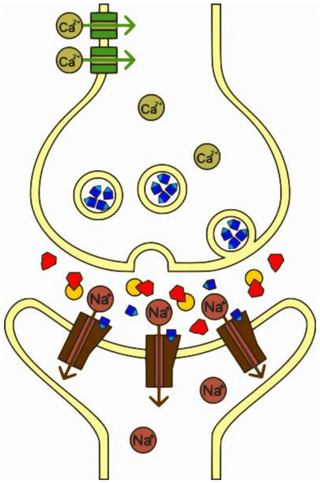
CWAs Background (Sarin)

Sarin:

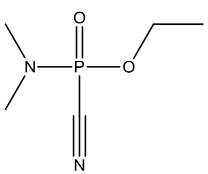
- Developed in Germany at IG Farben – looking for pesticides.
- **S**chrader, **A**mbros, **R**itter and L**in**de
- Sarin is colorless and odorless in pure form.
- 26 times more deadly than cyanide
- Easy to synthesize – but racemic mixture.

Modus Operandi of G-Series CWAs:

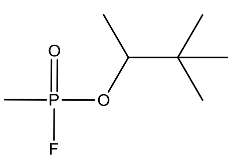
- Acetylcholinesterase (AChE)
- Acetylcholine plays an excitatory role at neuromuscular junctions in CNS and PNS.



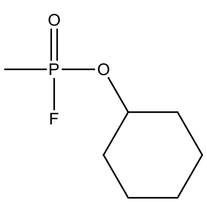
GA - Tabun



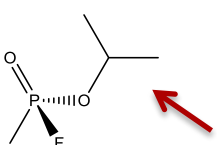
GD - Soman



GF - Cyclosarin

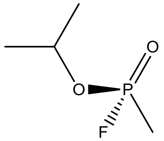


GB - Sarin

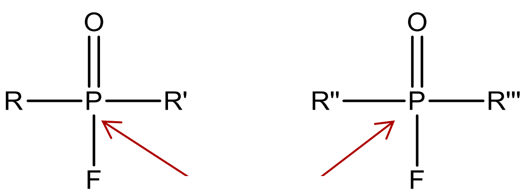


S - enantiomer

Inhibits AChE ~10^4 faster.



R - enantiomer



Chiral Center

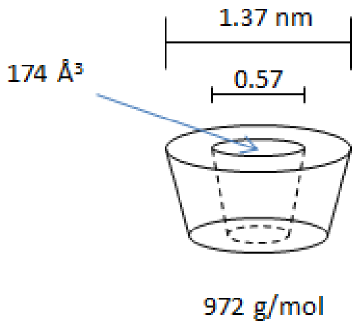
SNL_{OP-I} SNL_{OP-II}

General structure of the 2 SNL compounds

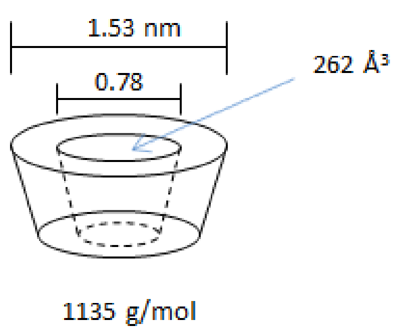
CSAs: Cyclodextrins

- Cyclic oligosaccharides (sugar molecules)
- Discovered in 1891 by Villiers.
- Schardinger clarified bacterial strain as *Bacillus macerans* and knew there were two cyclodextrins (CDs).
- From 1911 to 1935, Pringsheim's main contribution was that CDs forms complexes.
- CD inclusion phenomena, pharmaceutical, *etc.*

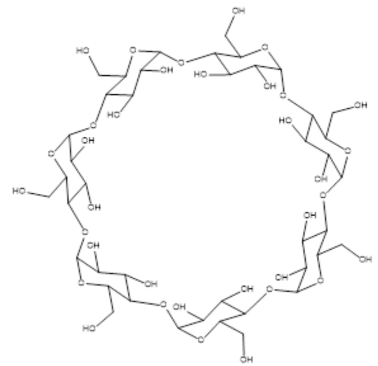
- Host/guest complex – guest enters a "donut hole".

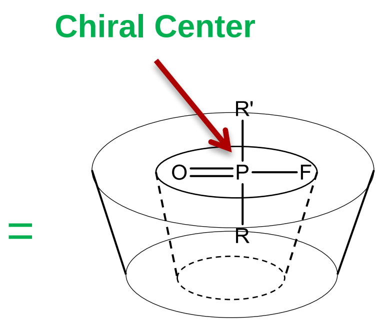


α-CD



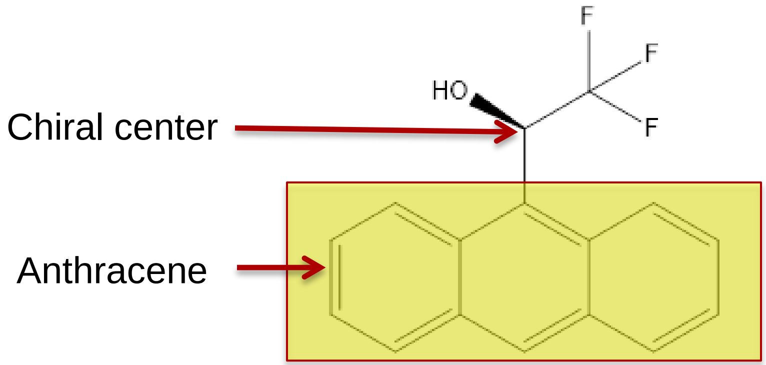
β-CD

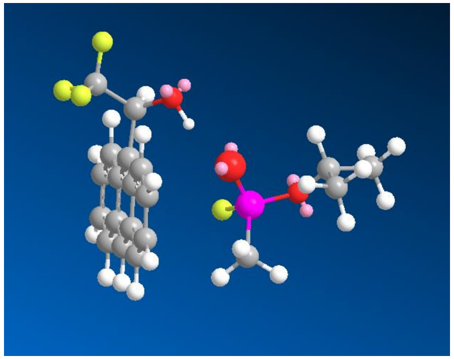


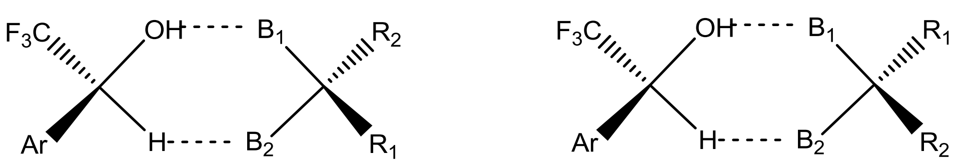


CSA: TFAE

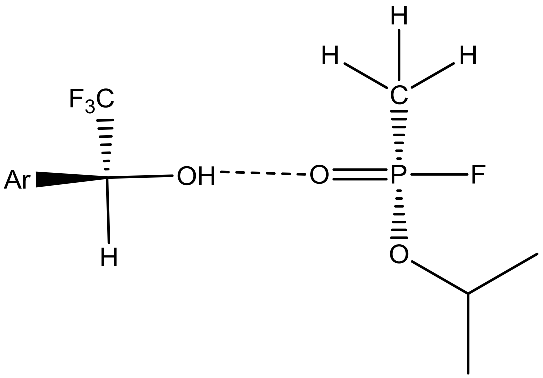
- R-(-)-2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE), also known as Pirkle's Alcohol
- Use of TFAE as a CSA in NMR studies reported by Pirkle in 1960s.
- Different from CDs due to high diamagnetic anisotropy of anthracene and lack of CD-type cavity.
- Anisotropy allows for NMR differentiation of enantiomers by causing perturbations of their magnetic environment.





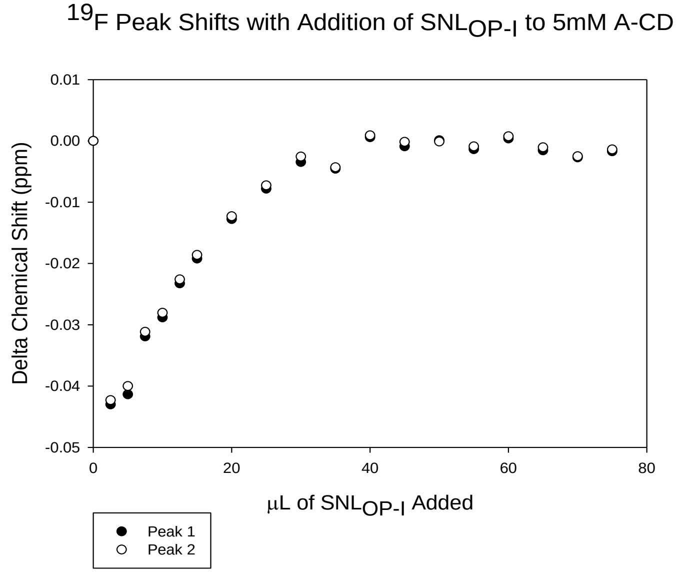


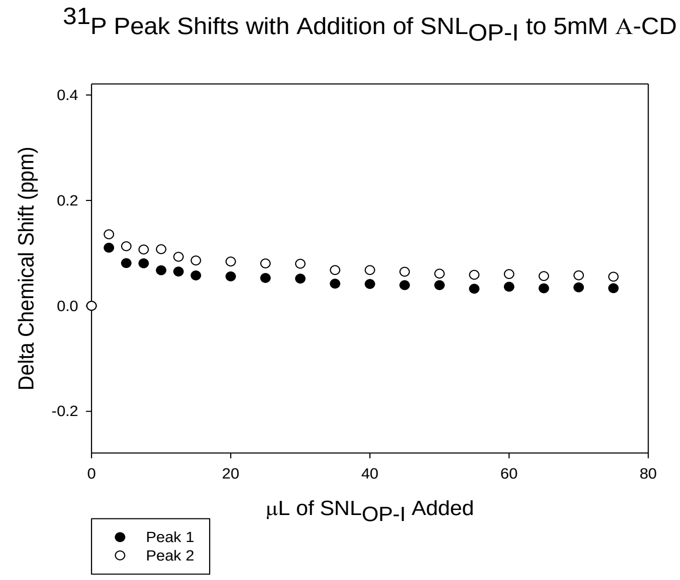
A model of the primary intermolecular interactions between a chiral compound and TFAE
(Adapted from Pirkle and Hoover 1982.)

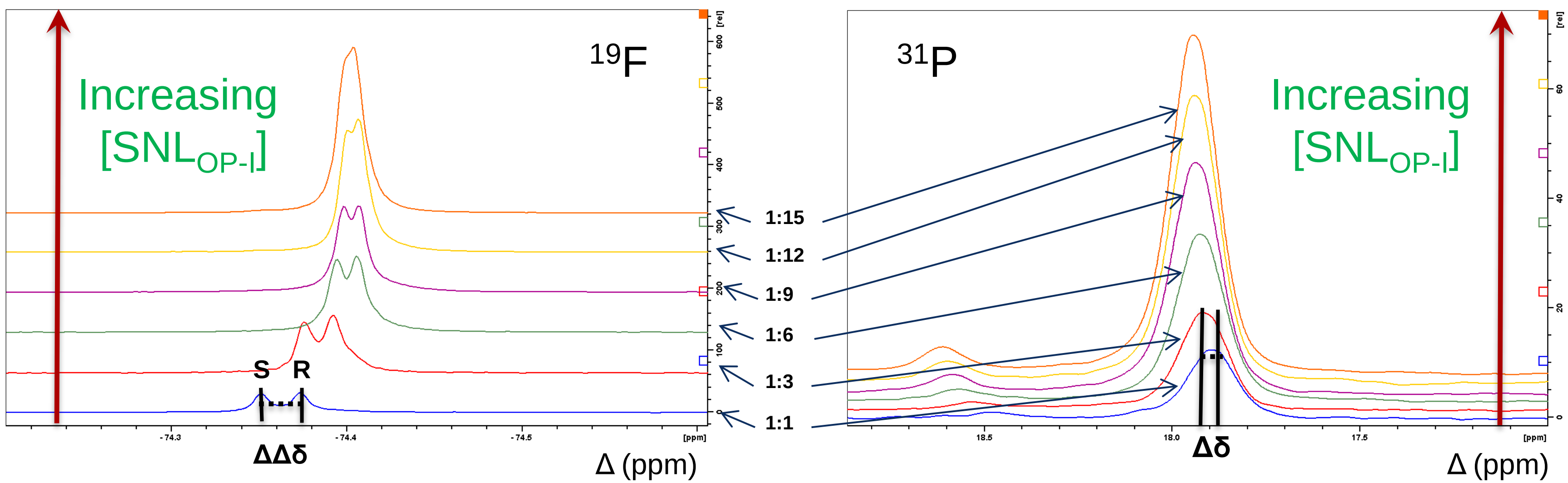


Proposed TFAE/S-Sarin interactions

α-CD Titration Results

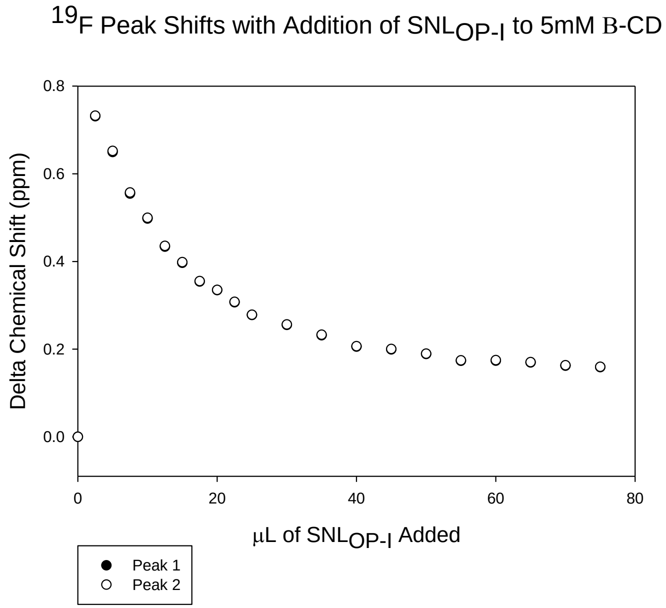


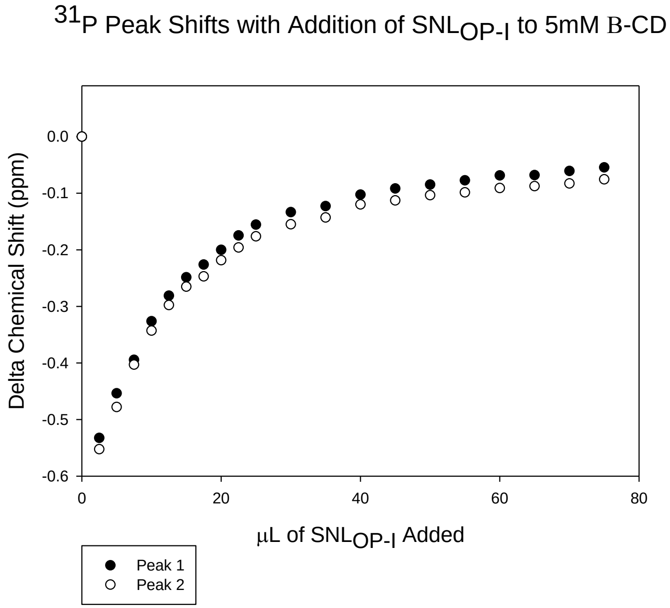


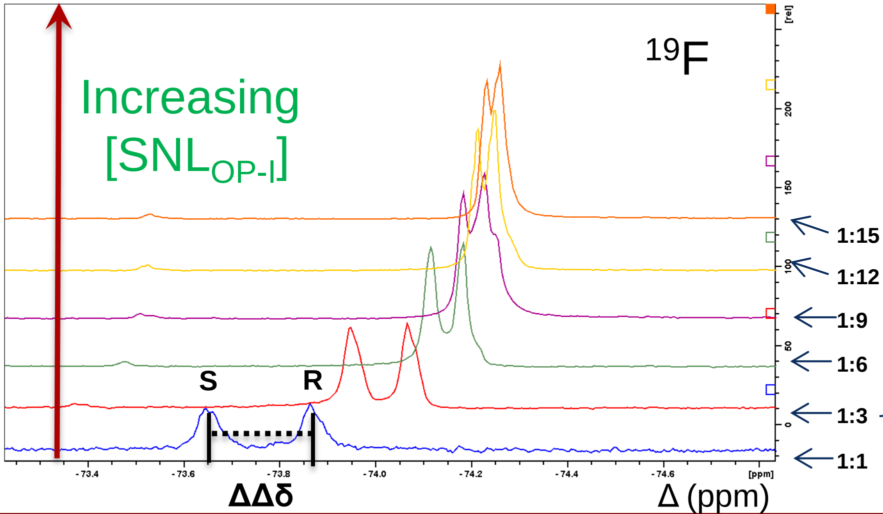


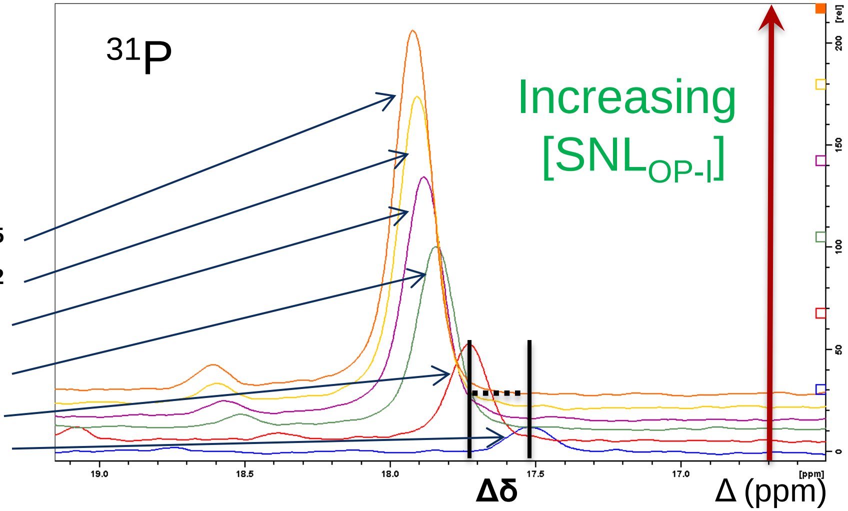
Arbitrary assignment of S and R enantiomers

β-CD Titration Results









Arbitrary assignment of S and R enantiomers

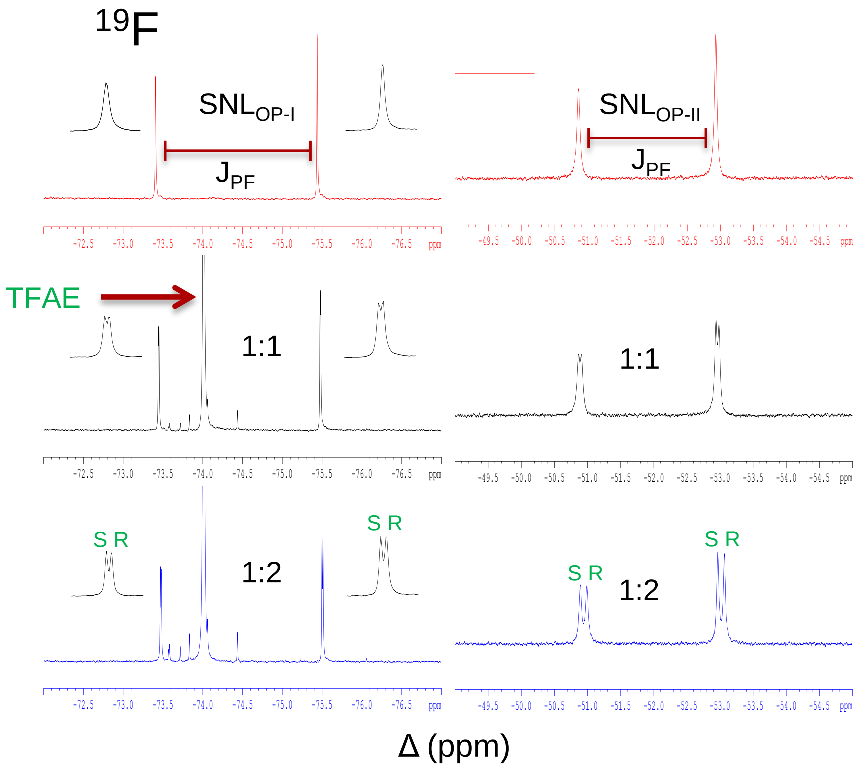
α/β-CD Titration Results

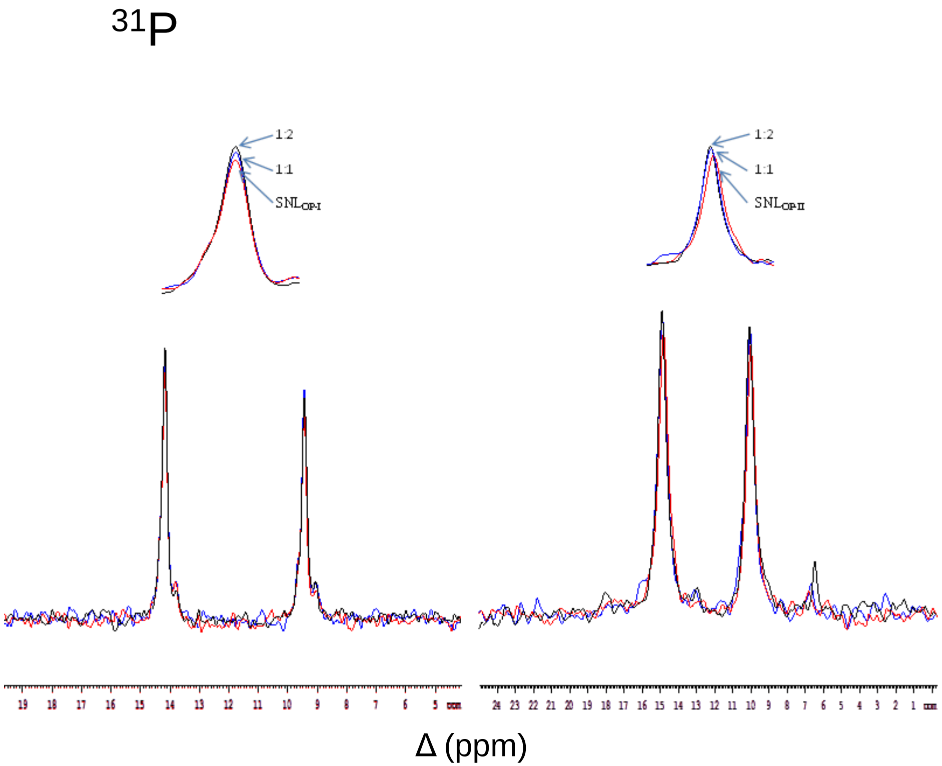
- $\Delta\Delta\delta$ could only be calculated from the ^{19}F NMR spectrum.
- Cavity sizes of α-CD (174 $Å^3$) and β-CD (262 $Å^3$) sufficient for a G/H interactions with SNL_{OP-I}.

				|R-S| separation	
Compound	CSA	$\Delta\delta$ ^{19}F (ppm)	$\Delta\delta$ ^{31}P (ppm)	$\Delta\Delta\delta$ ^{19}F (ppm)	$\Delta\Delta\delta$ ^{31}P (ppm)
α-CD/SNL_{OP-I}	α-CD				
1:1		-0.0413	0.0809	0.0221	-
1:3		-0.0192	0.0575	0.0164	-
1:6		-0.0034	0.0515	0.0113	-
1:9		-0.0009	0.0390	0.0084	-
1:12		0.0004	0.0361	0.0060	-
1:15		-0.0017	0.0332	0.0042	-
β-CD/SNL_{OP-I}	β-CD				
1:1		0.6496	-0.4537	0.2170	-
1:3		0.3970	-0.2485	0.1170	-
1:6		0.2552	-0.1334	0.0670	-
1:9		0.1993	-0.0917	0.0438	-
1:12		0.1736	-0.0687	0.0359	-
1:15		0.1587	-0.0544	0.0272	-

Maximum enantiomeric shift

TFAE Results





- As with the cyclodextrins, peak splitting could only be seen within the ^{19}F NMR spectrum.
- The largest chemical shift for SNL$_{OP-I}$ with one equivalent TFAE was observed in the ^{19}F NMR spectrum.
- However, for SNL$_{OP-II}$ with one equivalent of TFAE, the largest chemical shift was observed in the ^{31}P NMR spectrum.
- ^{31}P NMR spectrum indicates Δδ less than a hundredth of a ppm for SNL$_{OP-I}$ and Δδ just under a tenth of a ppm for SNL$_{OP-II}$.

TFAE Results

- Overall, SNL_{OP-II} with TFAE had more significant Δδ with the addition of a 2nd equivalent of TFAE – 1.7x versus 2.8x in the ^{19}F NMR spectra and 1.5x versus 2.2x in the ^{1}H NMR spectra of SNL_{OP-I} and SNL_{OP-II}, respectively.
- Indication of stronger intermolecular interactions between TFAE and SNL_{OP-II}.
  - Differences in R groups; steric effects.

					|R-S| separation	
Compound	CSA	Δδ ^{19}F (ppm)	Δδ ^{31}P (ppm)	Δδ ^{1}H (ppm)	ΔΔδ ^{19}F (ppm)	ΔΔδ ^{31}P (ppm)
SNL_{OP-I}/TFAE	TFAE					
1:1		0.0396	-0.0085	0.0171	0.0074	-
1:2		0.0665	-0.0030	0.0252	0.0123	-
1:4		0.1556	0.0033	0.0470	0.0282	
SNL_{OP-II}/TFAE	TFAE					
1:1		0.0293	-0.0649	0.0270	0.0404	-
1:2		0.0834	-0.0838	0.0592	0.0994	-
1:4		0.1715	-0.1650	0.1098	0.1985	

Maximum enantiomeric shift

Discussion/Future Endeavors

Discussion:

- Results show promising leads for future research on optimizing NMR chiral recognition of OFPs.
- Indications that peak splitting by CSAs should be monitored in the ^{19}F NMR spectrum for enantiomeric discrimination.
- Chemical shifts serve as indicators of primary and secondary interactions between CSA and chiral molecule.

Future Endeavors:

- Molecular modeling for elucidation of primary and secondary interactions between our OFPs and CSAs.
- Monitoring β-CD – catalyzed hydrolysis of OFPs.
- Using novel OFPs and other CSA molecules (γ-CD and Mosher's Acid).

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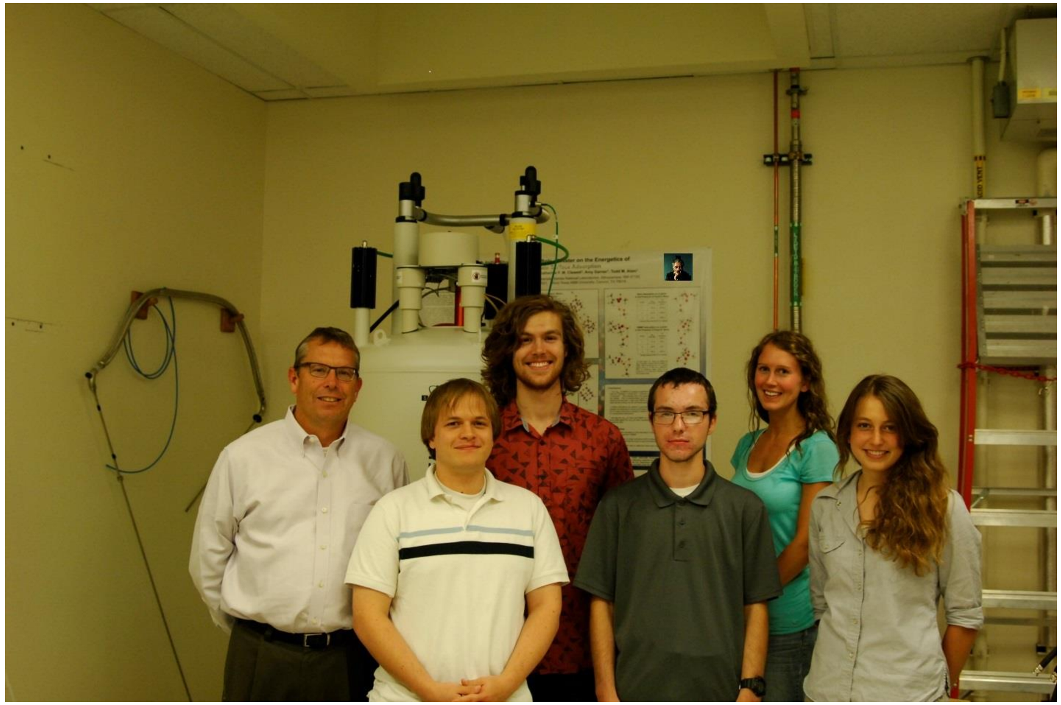
Acknowledgements



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