Investigation and Development of NMR Tools for Chiral Identification

SAND2014-16136D

Vitaliy Dernov*[1], Todd M. Alam[2], Paul Clem[2]
[1]Temple University, Philadelphia, PA 19122; Biochemistry, May 2016
[2]Department of Electronic, Optical, and Nanostructured Materials-1816
Sandia National Laboratories, Albuquerque, NM 87185
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 organo-fluorophosphate (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. Cyclodextrins (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 shifts 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 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). Enantioseparation in ^{19}F NMR spectra of $SNL_{OP\text{-}I}$ and α-CD could only be measured at the 1:1 ratio with no enantioseparation evident at higher concentrations of α-CD.

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].

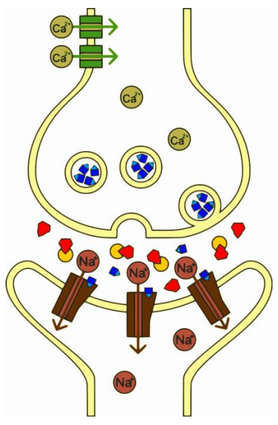

Figure 1. *Diagram of sarin (red) inhibition of acetylcholinestarase (yellow) and the build up of acetylcholine (blue) in the synaptic junction.*

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

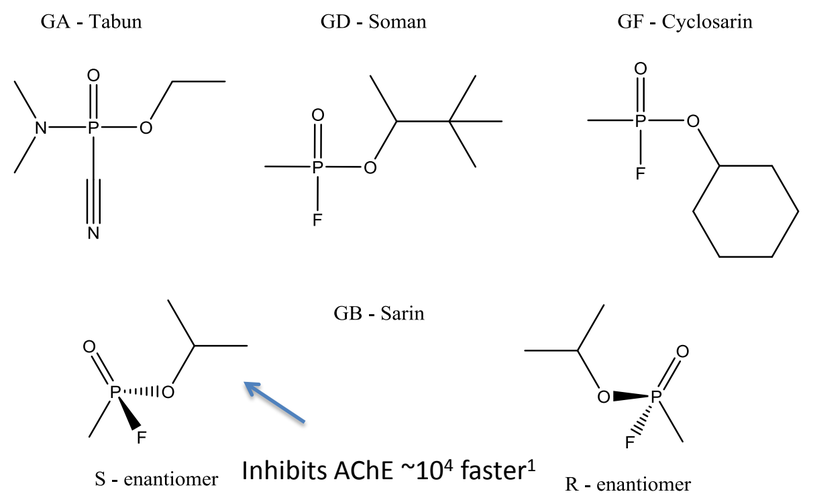

Figure 2. *Structures of G-series CWAs. Notice the organo-fluorophosphate (OFP) backbone. (Organophosphate for Tabun).*

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.).

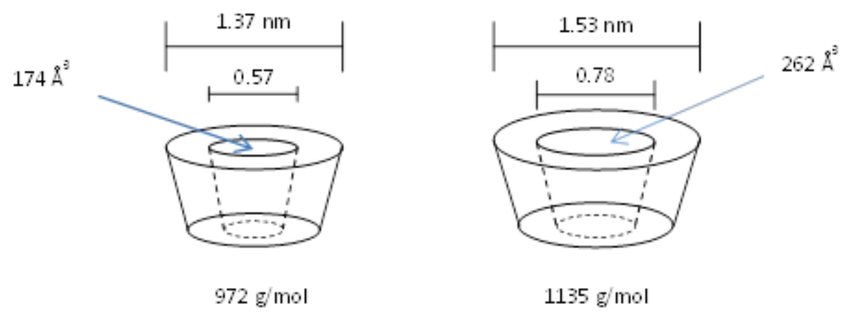

Figure 3. *Structure of α-CD and β-CD with focus on the cavity (Adopted from Szejtli 1998)*

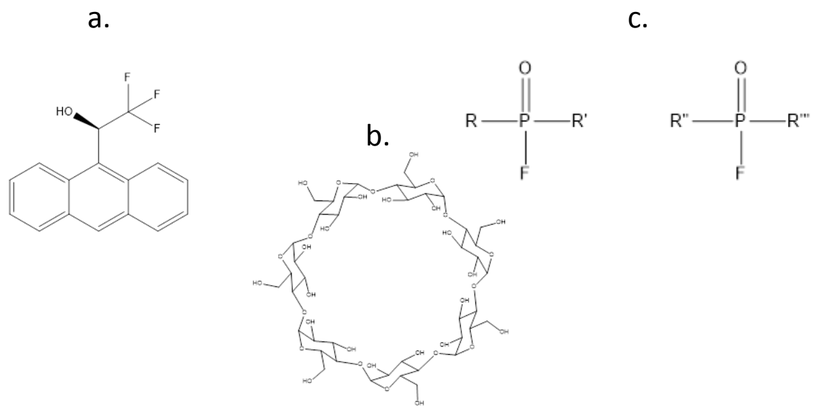

Figure 4a. *Structure of TFAE;* **Figure 4b.** *Structure of β-CD;* **Figure 4c.** *General structure of $SNL_{OP\text{-}I}$ and $SNL_{OP\text{-}II}$*

Methods

All spectra were obtained at 298K on a Bruker 500 with a 5mm BBO 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 R groups, including a branched alkane structure. 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 spectra were obtained to determine the default peak positions.

Two titrations were performed with the addition of ~2.5mM of the $SNL_{OP\text{-}I}$ organophosphate compound 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 spectra were obtained for analysis, including the determination of chemical shifts (Δδ) and enantiomeric discrimination (ΔΔδ). Table 1 below contains the data indicating the general chemical shifts and, if any 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 spectra were obtained for analysis and determination of chemical shifts (Δδ) and enantiomeric discrimination (ΔΔδ).

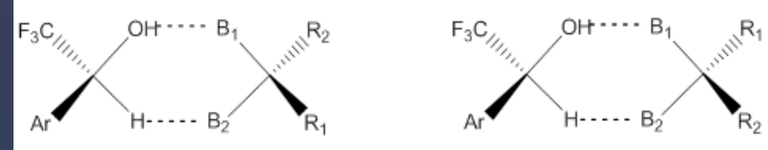

Figure 5. *A model of the primary intermolecular interactions between a chiral compound and TFAE (Adopted from Pirkle and Hoover 1982).*

Acknowledgements. The NMR characterization of these materials is supported by the Laboratory Directed Research Development (LDRD) program at Sandia. Partial funding (V.D.) provided through the Department of Homeland Security HS-STEM Summer Internship Program. DHS Education Programs are administered by the Oak Ridge Institute for Science and Education (ORISE) and Oak Ridge Associated Universities (ORAU). Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.
SAND No. XXXX-XXXXP

Results

β-CD

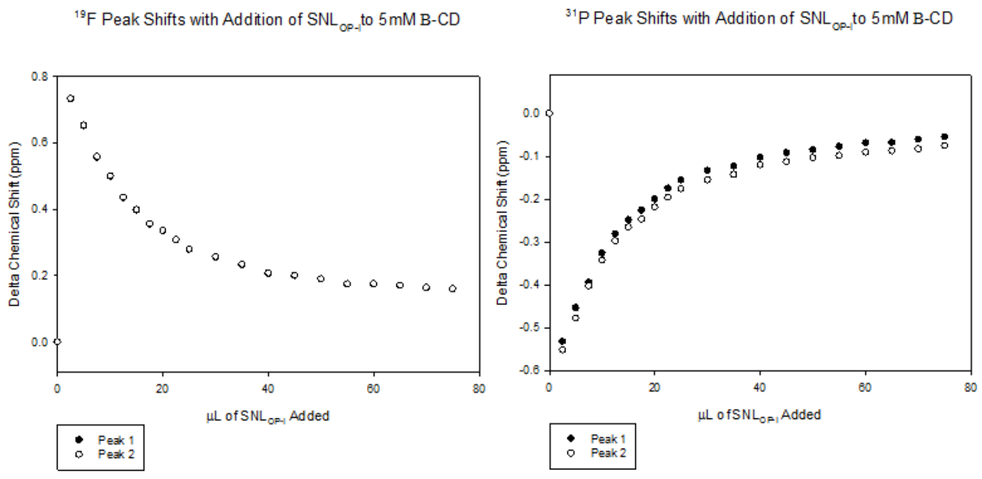

Figure 6. *Titration curves based on ^{19}F and ^{31}P spectra from SNL_{OP-I} titration with 5 mM β-CD at 298K*

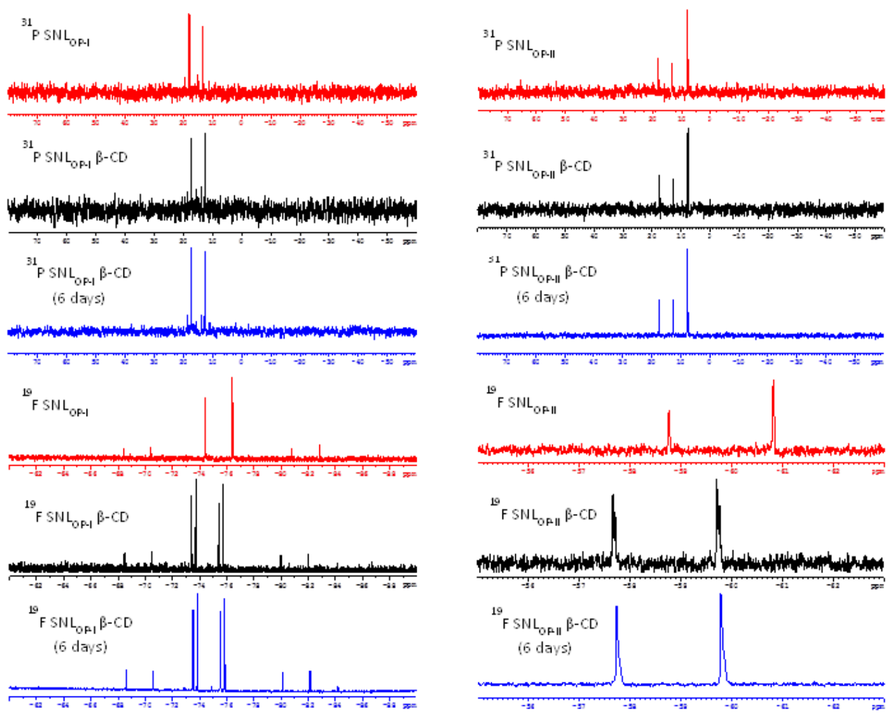

Figure 7. *SNL_{OP-I} and SNL_{OP-II} with 1:2 β-CD in D_2O at 298K*

TFAE

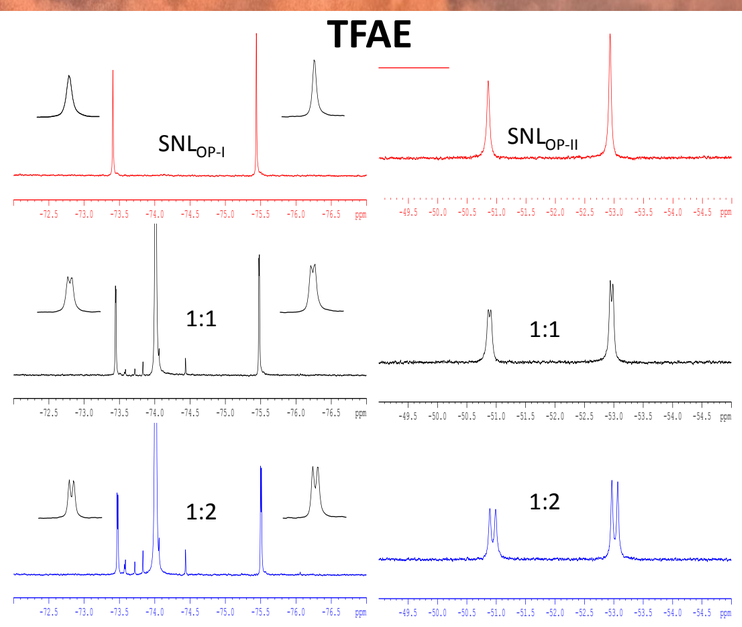

Figure 8. *^{19}F spectra of SNL_{OP-I} and SNL_{OP-II} with 1:1 and 1:2 TFAE in $CDCl_3$ at 298K*

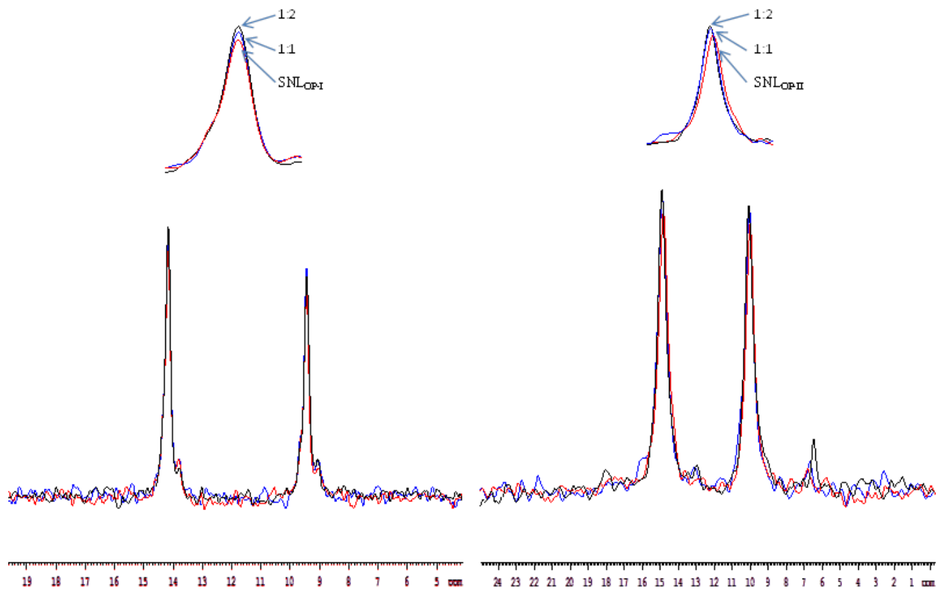

Figure 9. *^{31}P spectra of SNL_{OP-I} and SNL_{OP-II} with 1:1 and 1:2 TFAE in $CDCl_3$ at 298K*

Results/Conclusions

α- and β-cyclodextrins

					|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)	ΔΔδ ^{1}H (ppm)
SNL_{OP-I}	α-CD						
1:1		-0.0845	0.0416	-0.0238	0.0116	-	-
1:3		-0.0534	0.0096	-0.0088	-	-	-
1:6		-	-	-	-	-	-
1:9		-	-	-	-	-	-
1:12		-	-	-	-	-	-
1:15		-	-	-	-	-	-
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	-	-

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

The enantiomer separation distance could only be calculated from the ^{19}F spectrum, since neither the ^{31}P nor ^{1}H spectra showed any peak splitting at any of the concentrations. Since the main purpose behind the titration was to determine whether there would be concentration-related peak splitting in the ^{19}F spectrum, the titration was stopped once it was clear that no peak splitting would be seen in the 1:3, 1:6, 1:9, 1:12 or 1:15 spectra.

The results gathered indicate that the cavity size of β-CD is sufficient for a host-guest relationship with SNL_{OP-I}. A k value of … was derived from the graphed chemical shifts obtained from the ^{19}F and ^{31}P spectra (Figure 6).

The inability to differentiate between the enantiomers in the ^{19}F spectrum at ≥ 1:3 ratio of SNL_{OP-I} and α-CD, and the plateau-like characteristic of the peaks, may simply be an averaged effect between the peak splitting seen at 1:1 and the non-splitting at higher concentrations. This may be an issue of low/insufficient α-CD concentration for complex formation between the newly added SNL_{OP-I} and the initial 5 mM α-CD. Perhaps the 262 $Å^3$ cavity of the β-CD allows for two molecules of $SNL_{OP-I,}$ while only one molecule may enter the 174 $Å^3$ cavity of α-CD.

Further investigations on the behavior of β-CD with our OFPs were prompted by a published study indicating the catalytic role of β-CD in the hydrolytic cleavage of soman [4]. Figure 7 shows the ^{31}P and ^{19}F spectra of SNL_{OP-I} and SNL_{OP-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 were visible in either the ^{31}P or ^{19}F spectra taken after 6 days.

Results/Conclusions

TFAE

					|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)	ΔΔδ ^{1}H (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	-	-
SNL_{OP-II}	TFAE						
1:1		0.0293	-0.0649	0.0270	0.0404	-	-
1:2		0.0834	-0.0838	0.0592	0.0994	-	-

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 could only be seen within the ^{19}F spectrum. The largest chemical shift for SNL_{OP-I} with one equivalent TFAE was observed in the ^{19}F spectrum. However, for SNL_{OP-II} with one equivalent of TFAE, the largest chemical shift was observed in the ^{31}P 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 spectra and 1.5x versus 2.2x in the ^{1}H 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 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

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 spectrum, and should involve novel OFPs as well as other CSA molecules (like γ-CD).

Future Work

- Molecular modeling simulations would elucidate the primary and secondary interactions between our and other OFPs with cyclodextrins.
- Continue monitoring the potential hydrolysis of OFPs catalyzed by β-CD.
- ^{19}F and ^{31}P INEPT optimizations could also be investigated for ability to aid in the differentiation and quantification of enantiomers.

Works Cited

1. Benschop, H. P. and L. P. A. De Jong (1988). "Nerve agent stereoisomers: analysis, isolation and toxicology." Accounts of Chemical Research **21**(10): 368-374.
2. Todd M. Alam, C. J. Pearce and Janelle Jenkins, "Developing a Molecular Understanding of Water-CWA-Surface Interactions"
3. Pirkle, W. H. and D. J. Hoover (1982). NMR Chiral Solvating Agents. Topics in Stereochemistry, John Wiley & Sons, Inc.: 263-331.
4. Desire, B. and S. Saint-Andre (1986). "Interaction of soman with B-cyclodextrin." Toxicological Sciences **7**(4): 646-657.
5. Szejtli, J. (1998). "Introduction and general overview of cyclodextrin chemistry." Chemical reviews **98**(5): 1743-1754.

Acknowledgements. The NMR characterization of these materials is supported by the Laboratory Directed Research Development (LDRD) program at Sandia. Partial funding (V.D.) provided through the Department of Homeland Security HS-STEM Summer Internship Program. DHS Education Programs are administered by the Oak Ridge Institute for Science and Education (ORISE) and Oak Ridge Associated Universities (ORAU). Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.
SAND No. XXXX-XXXXP

Sandia National Laboratories