

Synthesis and Analysis of Cyclohexyl trideuteromethylphosphonic acid

AUTHORS

Peech S. Reddy PhD, Huahua Jian PhD, Isil Dilek PhD, Ryan Carrell, Ning Chang PhD, Uma Sreenivasan PhD

Abstract

- Phosphorus compounds containing the P-C bond are not particularly abundant in nature but they have diverse biological activity and have attracted considerable synthetic and pharmacological interest. Aside from beneficial applications, they are used in the production of chemical warfare agents (CWAs) such as soman (GD), tabun (GA), sarin (GB), and cyclosarin (GF).
- Many of these agents are prone to hydrolysis to produce the corresponding O-alkyl hydrogen alkylphosphonates. These hydrolyzed products are persistent in the environment and provide good evidence of the specific agent used or produced. Stable isotope labeled derivatives of O-alkyl hydrogen alkylphosphonates are useful in detection and quantitation of CWAs.
- We will present the synthesis of cyclohexyl trideuteromethylphosphonic acid, a breakdown product of cyclosarin, and analytical data related to deuterium/hydrogen exchange. Synthesis by the traditional route used for the native analog afforded labeled cyclohexyl trideuteromethylphosphonic acid that showed significant deuterium exchange. An alternate route was developed to obtain isotopically pure cyclohexyl trideuteromethylphosphonic acid. LCMS and GCMS were used to track exchange and isotopic purity.

INTRODUCTION

- Organophosphorus compounds have diverse biological activity ranging from insecticides and herbicides to nerve agents. They are also widely used in industry as solvents, plasticizers and additives. Organophosphorus nerve agents such as **soman**, **tabun**, **sarin** and **cyclosarin** (Figure 1) are acetylcholinesterase inhibitors and represent a class of Chemical Warfare Agents (CWAs) that have been widely used around the world.
- CWAs breakdown to their corresponding O-alkyl hydrogen alkylphosphonates. These hydrolyzed products are persistent in the environment and provide good evidence of the specific agent used or produced. Stable isotope labeled derivatives of O-alkyl hydrogen alkylphosphonates are useful as internal standards in the detection and quantitation of CWAs. Several are available with isotopic substitution on the O-alkyl group.
- We were interested in the preparation of a stable isotope labeled derivative of the breakdown product of CYCLOSARIN, cyclohexyl methylphosphonic acid. Considerations for selection of the label included the fragmentation pattern of cyclosarin in

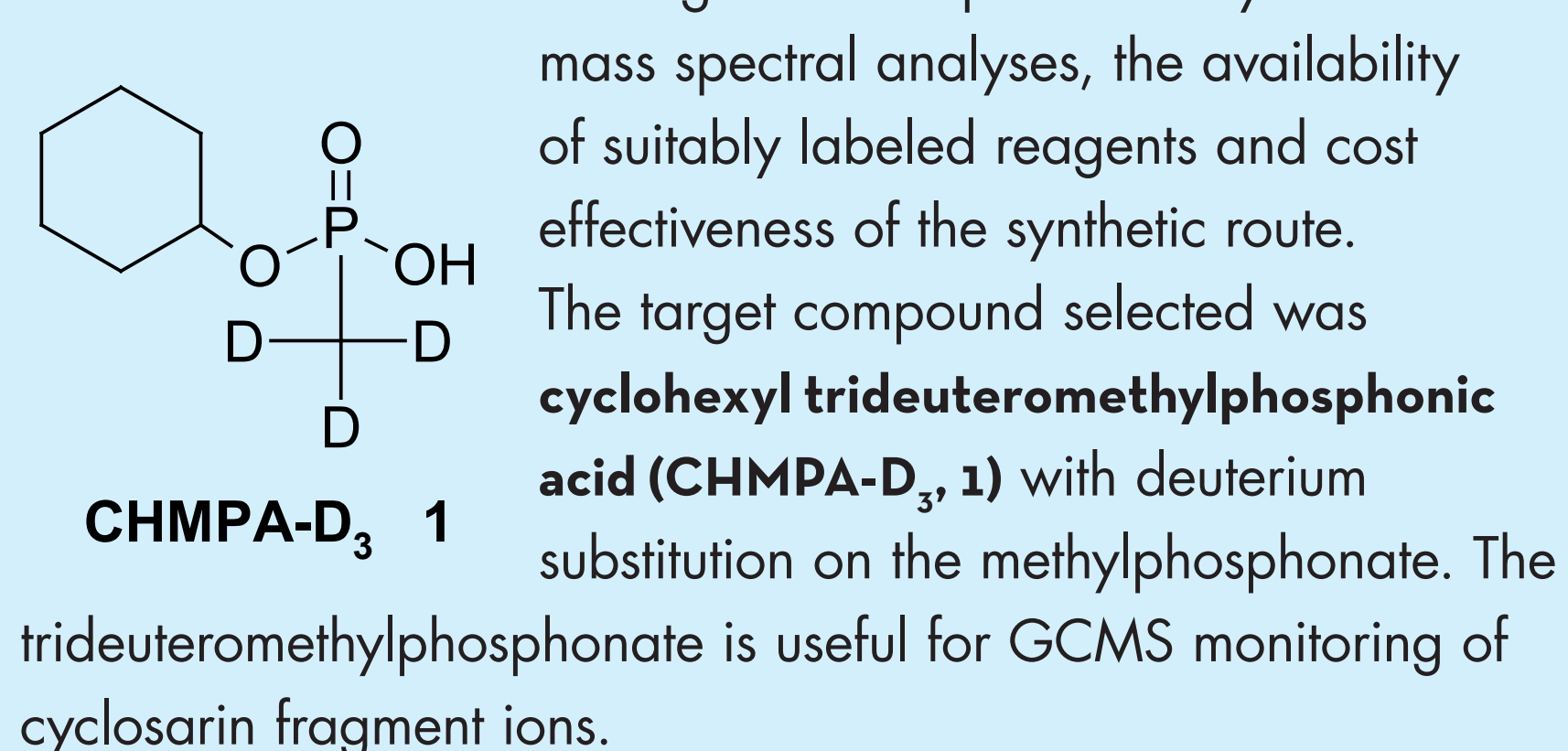
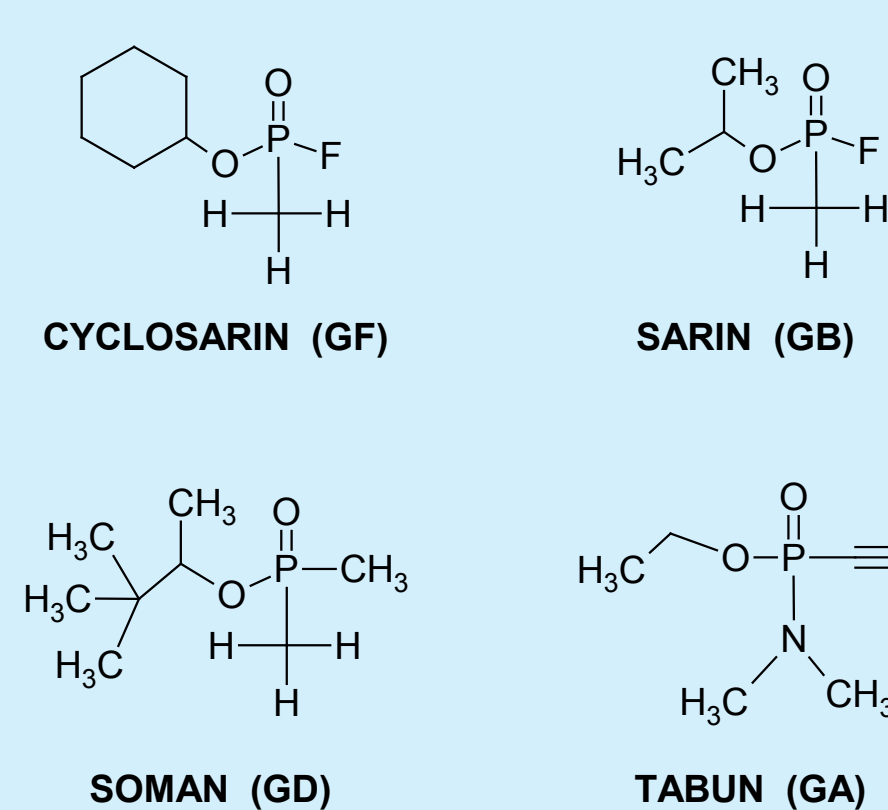
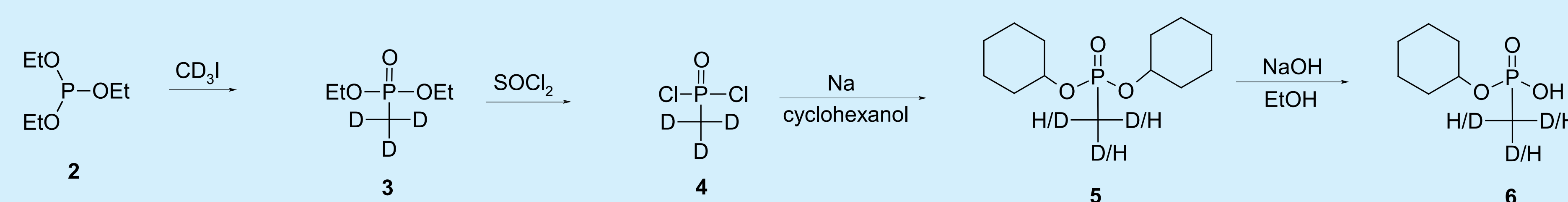


FIGURE 1. EXAMPLES OF ORGANOPHOSPHORUS CHEMICAL WARFARE AGENTS



INITIAL APPROACH TO THE SYNTHESIS OF CHMPA-D₃

SCHEME 1



- The initial attempt to synthesize trideuterocyclohexyl methylphosphonic acid (CHMPA-D₃, 1) followed the traditional route used for the native analog as shown in **Scheme 1**.¹
- Diethyl methylphosphonate-D₃ (3) was prepared from triethylphosphite and methyl iodide-D₃ in accordance with the Arbuzov reaction in 95% yield.²
- Phosphonate (3) was reacted with thionyl chloride at reflux to obtain methylphosphonic dichloride-D₃ (4) in 47% yield.
- Compound (4) was reacted with cyclohexanol sodium salt to form dicyclohexyl methylphosphonate-D₃ (5) in 38% yield.
- Reaction of (5) with sodium hydroxide in ethanol produced compound (6) in 90% yield and 99.3% chromatographic purity, but isotopic purity analysis showed >10% D₀.

Although the synthetic pathway produced material of high chromatographic purity in excellent yield, LCMS and GCMS of (6) showed significant deuterium exchange with hydrogen.

ISOTOPIC DISTRIBUTION OF COMPOUND (6) BY LCMS ANALYSIS

| ION | %D _n | D _n /D ₃ |
|----------------|-----------------|--------------------------------|
| D ₀ | 10.4% | 33.3% |
| D ₁ | 17.0% | 54.6% |
| D ₂ | 22.8% | 73.2% |
| D ₃ | 31.2% | - |

- This level of isotopic purity is not suitable for internal standard applications. The hydrogen-deuterium (H/D) exchange likely arises from the acidity (pKa) of the methyl protons of the methylphosphonate in (5) and (6) that allows for H/D exchange in the presence of cyclohexanol sodium salt in step 3 and NaOH in step 4.

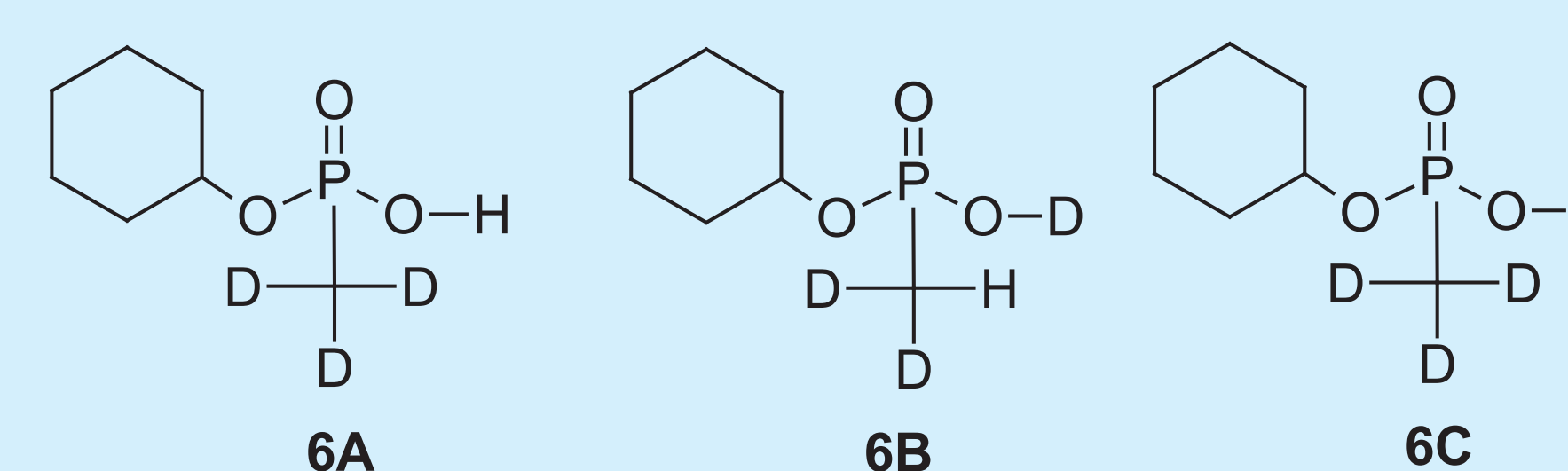
- All steps in scheme 1 were investigated to determine the source of exchange. The labeled starting material iodomethane-D₃ was procured with 99% deuterium enrichment. GCMS analysis indicated a distribution of 82.3% D₃, 1.3% D₂, 13.4% D₁, and 0.1% D₀, quite different from that of compound (6). The product of step 1, (3), has 62.95% D₂, 32.68% D₃, 0.35% D₁, and 0.03% D₀, suggesting that step 1 proceeded with little to no exchange. Intermediate (4) is unstable and was not tested. The GCMS spectrum of compound (5) produced a very low abundance of molecular ion which appeared to be predominantly D₂ ion while the fragment ion showed an inconsistent isotopic distribution pattern.
- The analysis of the precursors to (6) suggested that isotope exchange occurred at both steps 3 and 4 of the synthesis, i.e. under basic conditions.

USE OF DEUTERIUM SOLVENTS IN EXCHANGE REACTIONS

Exchange reactions on compound (6) failed to improve the isotopic purity. Conversion of (5) to (6) provided inconclusive results.

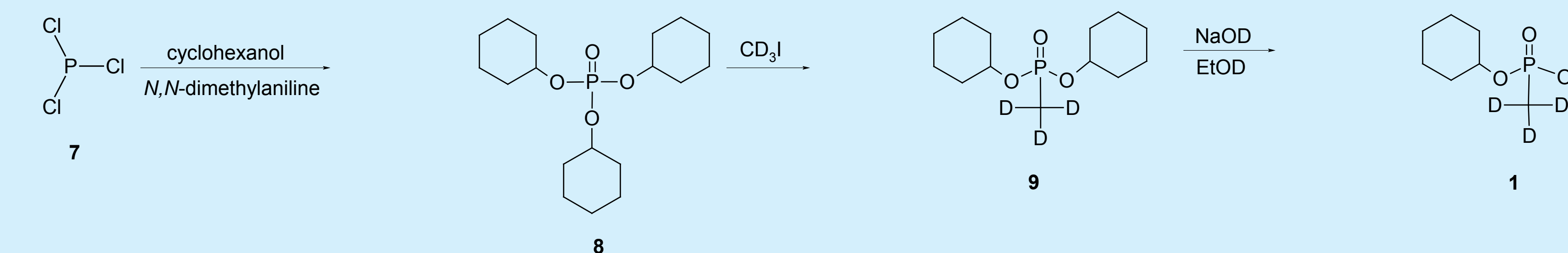
| STARTING MATERIAL | REAGENTS | RESULT |
|-------------------|-----------------|--|
| 6 | NaOD, EtOD, DCl | Product with significant H/D exchange %D ₀ = 10.40% and D ₀ /D ₃ = 45.50% |
| 6 | EtOD, DCl | Product with significant H/D exchange %D ₀ = 10.43% and D ₀ /D ₃ = 45.70% |
| 5 | NaOD, EtOD, DCl | Product with significant H/D exchange* %D ₀ = 0.19%, %D ₁ = 2.67%, %D ₂ = 31.04%, %D ₃ = 32.99%, %D ₄ = 30.73%, and D ₀ /D ₃ = 0.57% |

*LCMS results after conversion of (5) to (6) using NaOD/Ethanol-D followed by treatment with DCl showed approximately 30% each of D₂, D₃ and D₄ products. The D₃ and D₄ products could arise from a mix of compounds (6A), (6B) and (6C). The 4th deuterium in (6C) is exchangeable and would be expected to revert to a D₃ product in solution. In a similar manner, (6B) would exchange to a D₂ product in solution. We decided to try a more robust synthesis of CHMPA-D₃.



SUCCESSFUL SYNTHESIS OF CHMPA-D₃

SCHEME 2



- Phosphorus trichloride (7) was reacted with cyclohexanol to obtain tricyclohexyl phosphite (8) in 61% yield.³
- The trideuteromethyl substituent was introduced by treating compound (8) with iodomethane-D₃ under **neutral conditions** to obtain phosphonate (9). Crude phosphonate (9) was treated directly with sodium deuterioxide to obtain CHMPA-D₃ (1) in 17% yield after purification. Purity by GC/FID was 99.1%. Isotopic purity results are presented below.

LCMS METHOD FOR CHMPA-D₃

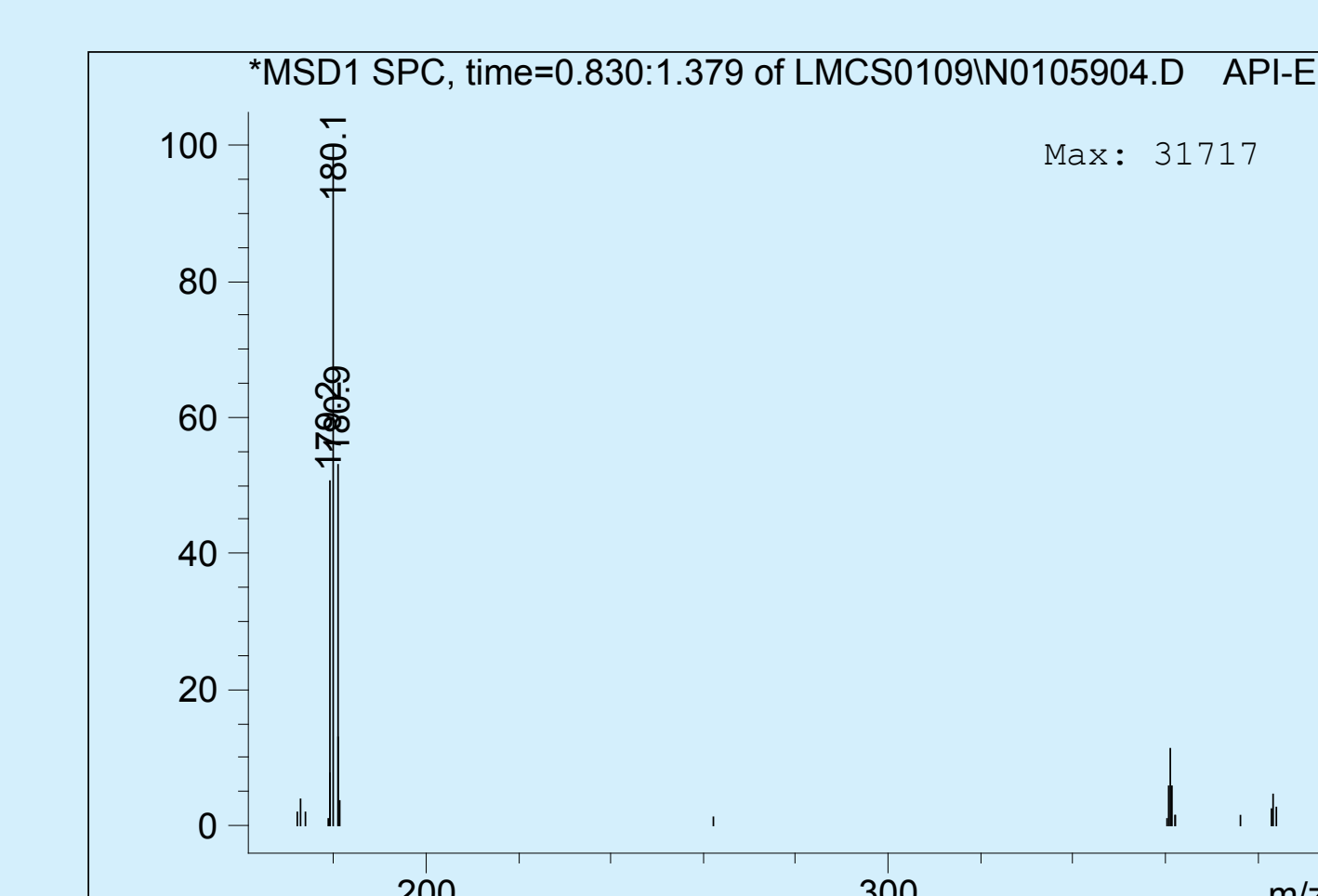
| | |
|------------------------|--|
| Instrument | Agilent 1100 LCMS |
| Detector | ESI |
| Polarity | Negative scan |
| Column | Luna 3μ C18(2), 100 x 2.0 mm |
| Mobile Phase | A: Acetonitrile B: 10 mM Ammonium Acetate |
| Gradient | Isocratic, A:B = 70:30 |
| Temperature | Ambient |
| Injection Volume | 1 μL |
| Flow | 0.2 mL/min |
| Drying Gas Temperature | 350 °C |
| Nebulizer Pressure | 35 psi |
| Capillary Voltage | 4000 V |

ISOTOPIC PURITY OF CHMPA-D₃ BY LCMS ANALYSIS

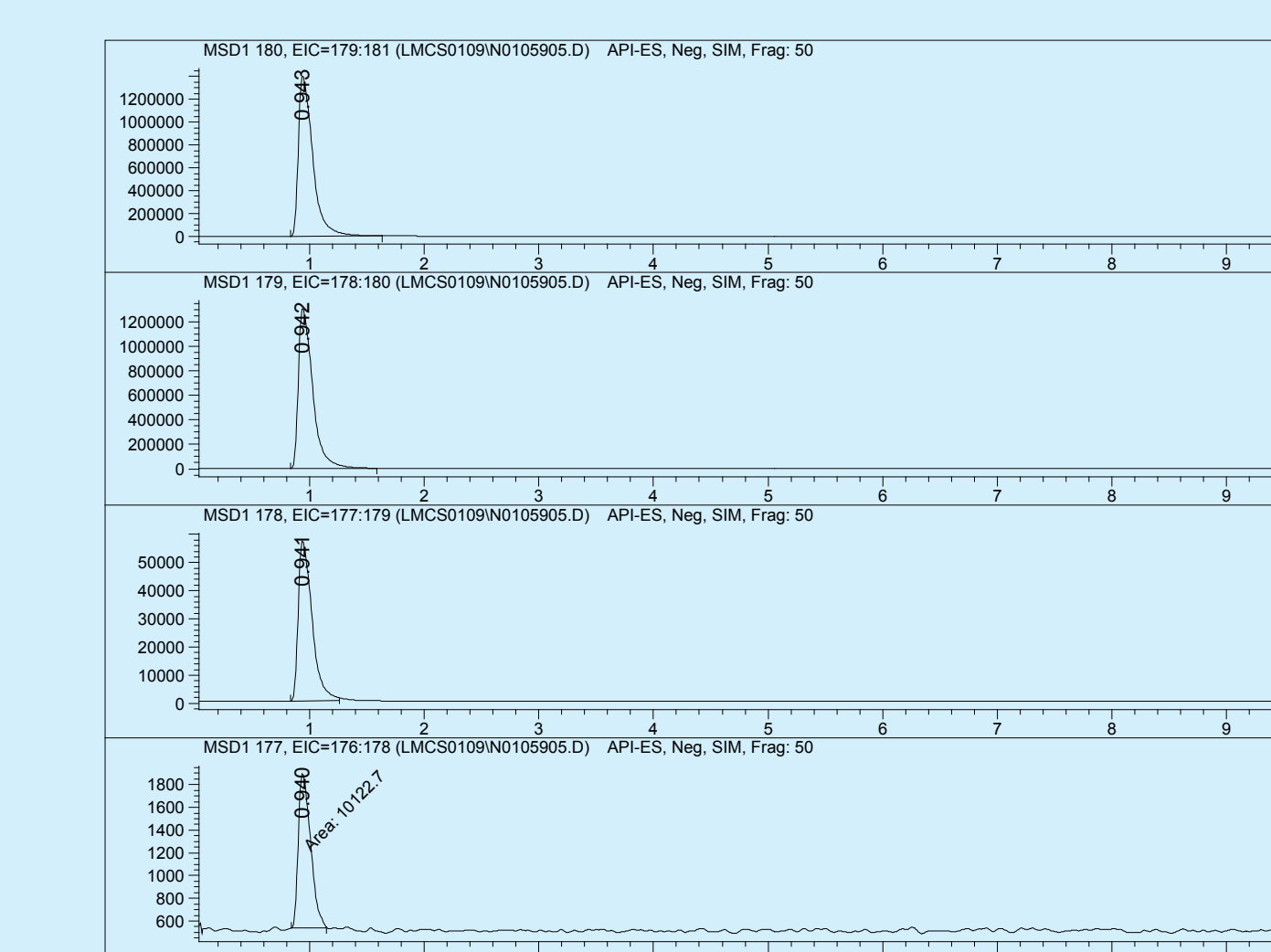
| ION | %D _n | D _n /D ₃ |
|----------------|-----------------|--------------------------------|
| D ₀ | 0.04% | 0.08% |
| D ₁ | 1.98% | 3.55% |
| D ₂ | 47.3% | 93.4% |
| D ₃ | 50.7% | - |

- This level of isotopic purity is suitable for internal standard applications.
- The relatively high level of D₂ ions is due to natural abundance of M-1 and some H/D chemical exchange that occurs despite the use of deuterated solvents.

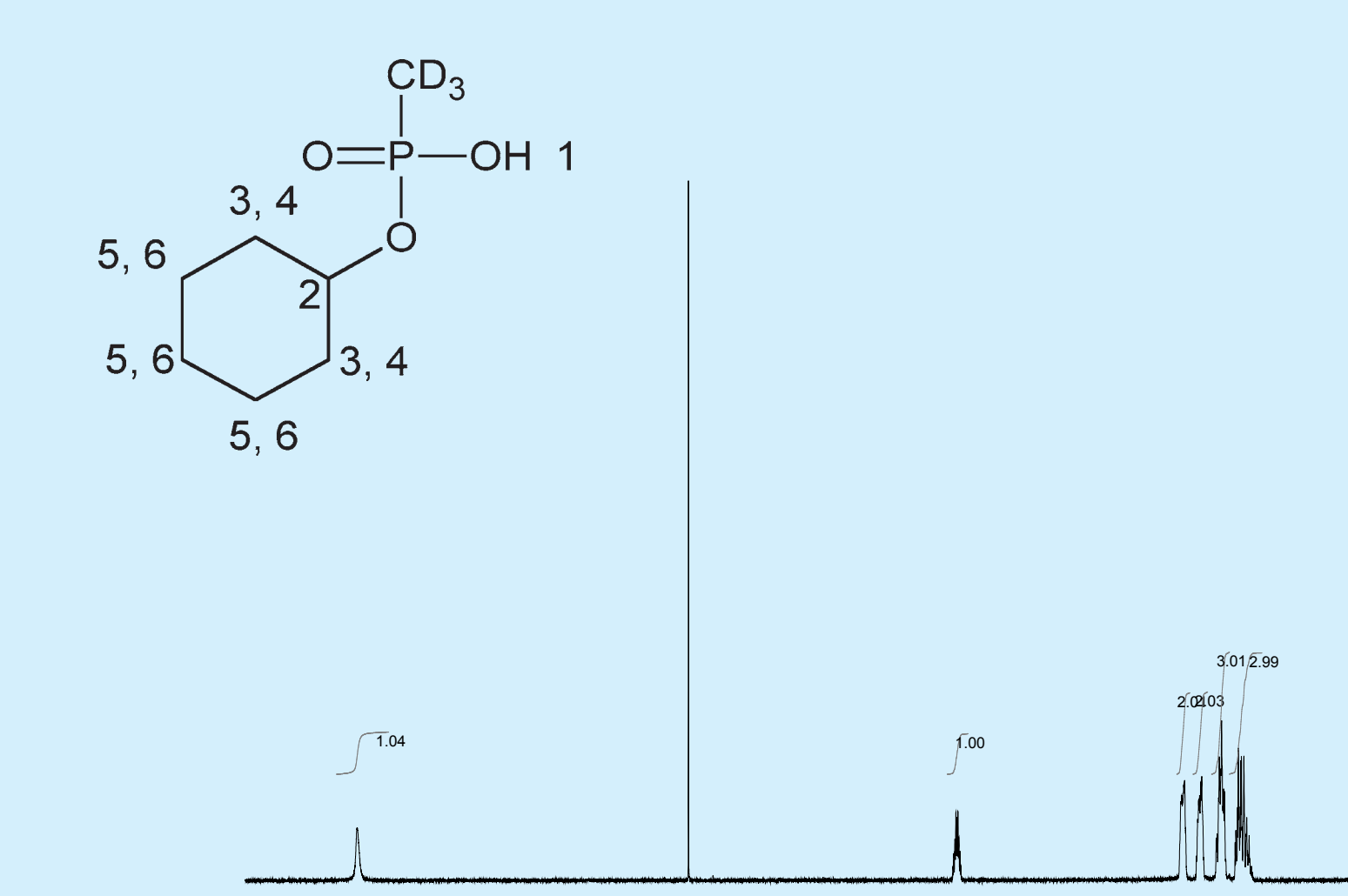
MASS SPECTRUM OF CHMPA-D₃



LCMS-SIM CHROMATOGRAM OF CHMPA-D₃



¹H-NMR SPECTRUM OF CHMPA-D₃ (CDCl₃)



CONCLUSIONS

Stable labeled derivatives of CWAs are important as reference standards for the detection and quantitation of CWAs in the environment. In the development of an internal standard one must consider the appropriate synthetic pathway to produce material that meets not only yield and chromatographic purity requirements, but also isotopic purity specifications that are appropriate for its intended use. Often the most obvious synthetic pathway is not the best option for the production of labeled internal standards. The initial synthetic method, based on the synthesis of the native analog illustrates how H/D exchange can influence synthetic design in the development of stable labeled reference materials. The development of the alternate route represents a successful synthesis of CHMPA-D₃ with D₀/D₃ 0.083%. Cyclohexyl trideuteromethylphosphonic acid (1) of high isotopic purity was synthesized for use as an internal standard in the detection and quantitation of cyclosarin and its breakdown products.

REFERENCES 1. Christ, H.; Levy, M.; Morry, C. J. *Organomet Chem.* **1968**, *12*, 459
2. Arbuzov, B. A. *Proc. Appl. Chem.* **1944**, *9*, 307 3. Saunders, B.C.; Stark, B.P. *Tetrahedron* **1968**, *4*, 169

ACKNOWLEDGEMENTS Cerilliant Colleagues: Greg Kirkovits PhD, Karan Yasar