Synthesis and Analysis of Cyclohexyl trideuteromethylphosphonic acid

AUTHORS

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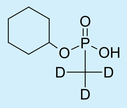
Abstract

- 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.

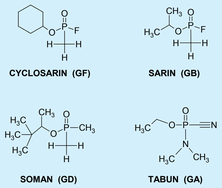
INTRODUCTION

- 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 acetylcholinestrase 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 mass spectral analyses, the availability of suitably labeled reagents and cost effectiveness of the synthetic route. The target compound selected was **cyclohexyl trideuteromethylphosphonic acid (CHMPA-D_3, 1)** with deuterium substitution on the methylphosphonate. The trideuteromethylphosphonate is useful for GCMS monitoring of cyclosarin fragment ions.



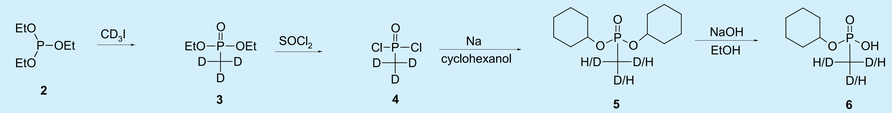
CHMPA-D_3 1

FIGURE 1. EXAMPLES OF ORGANOPHOSPHORUS CHEMICAL WARFARE AGENTS



INITIAL APPROACH TO THE SYNTHESIS OF CHMPA-D_3

SCHEME 1



- The initial attempt to synthesize trideuterocyclohexyl methylphosphonic acid (CHMPA-D_3, **1**) followed the traditional route used for the native analog as shown in **Scheme I**.[1]
- Diethyl methylphosphonate-D_3 **(3)** was prepared from triethylphosphite and methyliodide-D_3 in accordance with the Arbusov reaction in 95% yield.[2]
- Phosphonate **(3)** was reacted with thionyl chloride at reflux to obtain methylphosphonic dichloride-D_3 **(4)** in 47% yield.
- Compound **(4)** was reacted with cyclohexanol sodium salt to form dicyclohexyl methylphosphonate-D_3 **(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% D_0.

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%	-

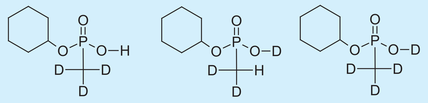
- This level of isotopic purity is not suitable for internal standard applications. The hydrogen-deuterium (H/D) exchange likely arises from the acidity (pKa) of the methyl protons of the methylphosphonate in **(5)** and **(6)** that allows for H/D exchange in the presence of cyclohexanol sodium salt 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-D_3 was procured with 99% deuterium enrichment. GCMS analysis indicated a distribution of 82.3% D_3, 1.3% D_2, 13.4% D_1 and 0.1% D_0, quite different from that of compound **(6)**. The product of step 1, **(3)**, has 62.95% D_3, 32.68% D_2, 0.35%D_1 and 0.03% D_0, 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 D_2 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.

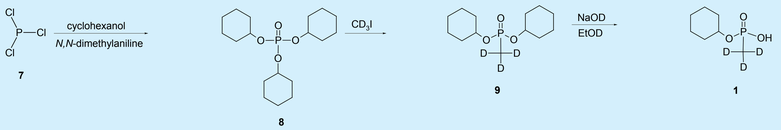
Starting Material	Reagents	Result
6	NaOD, EtOD, DCl	Product with significant H/D exchange %D_0 = 10.40% and D_0/D_3 = 45.50%
6	EtOD, DCl	Product with significant H/D exchange %D_0 = 10.43% and D_0/D_3 = 45.70%
5	NaOD, EtOD, DCl	Product with significant H/D exchange* %D_0= 0.19%, %D_1= 2.67%, %D_2= 31.04%, %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 D_2, D_3 and D_4 products. The D_3 and D_4 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 D_3 product in solution. In a similar manner, **(6B)** would exchange to a D_2 product in solution. We decided to try a more robust synthesis of CHPMA-D_3.



SUCCESSFUL SYNTHESIS OF CHMPA-D_3

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-D_3 under **neutral conditions** to obtain phosphonate **(9)**. Crude phosphonate **(9)** was treated directly with sodium deuteroxide to obtain CHMPA-D_3 **(1)** in 17% yield after purification. Purity by GC/FID was 99.1%. Isotopic purity results are presented below.

LCMS METHOD FOR CHMPA-D_3

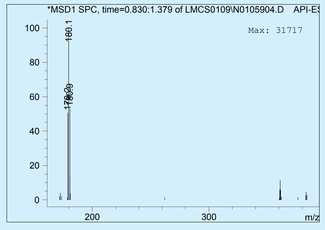
Instrument	Agilent 1100 LCMS
Detector	ESI
Polarity	Negative scan
Column	Luna 3µ C18(2), 100 x 2.0 mm
Mobile Phase	A: Acetonitrile B: 10 mM Ammonium Acetate
Gradient	Isocratic, A:B = 70:30
Temperature	Ambient
Injection Volume	1 µL
Flow	0.2 mL/min
Drying Gas Temperature	350 °C
Nebulizer Pressure	35 psi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

ISOTOPIC PURITY OF CHMPA-D_3 BY LCMS ANALYSIS

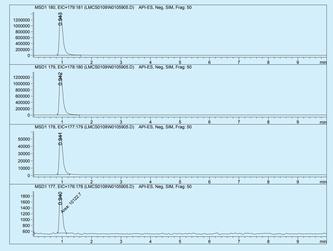
Ion	%D_n	D_n/D_3
D_0	0.04%	0.08%
D_1	1.98%	3.55%
D_2	47.3%	93.4%
D_3	50.7%	-

- This level of isotopic purity is suitable for internal standard applications.
- The relatively high level of D_2 ions is due to natural abundance of M-1 and some H/D chemical exchange that occurs despite the use of deuterated solvents.

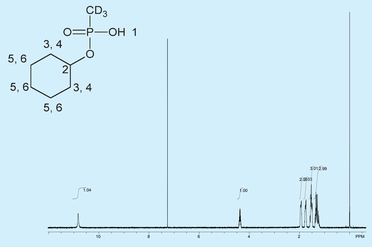
MASS SPECTRUM OF CHMPA-D_3



LCMS-SIM CHROMATOGRAM OF CHMPA-D_3



^{1}H-NMR SPECTRUM OF CHMPA-D_3 ($CDCl_3$)



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. Christol, H.; Levy, M.; Marty, C.; *J. Organomet.Chem.* **1968**, 12, 459
2. Arbusov BA.; *Pure. Appl. Chem.* **1964**, 9, 307 3. Saunders, B.C.; Stark, B.P.; *Tetrahedron* **1958**, 4, 169

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