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C. A. Valdez, R. N. Leif

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Carlos A. Valdez[1,2,*] *and Roald N. Leif*[1,2]

[1]*Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA, USA 94550*

[2]*Forensic Science Center, Lawrence Livermore National Laboratory, Livermore, CA, USA 94550*

*Corresponding author:
Dr. Carlos A. Valdez
Physical and Life Sciences Directorate
Lawrence Livermore National Laboratory
7000 East Avenue, L-091,
Livermore, CA 94550, USA
Email: valdez11@llnl.gov
Phone: + 1 925 423 1804
Fax: + 1 925 423 9014

Abstract

A derivatization method that employs diethyl (bromodifluoromethyl) phosphonate (DBDFP) to efficiently tag the endocrine disruptor pentachlorophenol (PCP) and other chlorinated phenols (CPs) along with their reliable detection and analysis by NMR is described. The method accomplishes the efficient alkylation of the hydroxyl group in CPs with the difluoromethyl (CF_2H) moiety in extremely rapid fashion (2 min.), at room temperature and in an environmentally benign manner. The approach proved successful in difluoromethylating a panel of 18 chlorinated phenols, yielding derivatives that displayed unique ^{1}H and ^{19}F NMR spectra allowing for the discrimination between isomerically related CPs. Furthermore, the method shows that PCP along with other CPs can be selectively derivatized in the presence of other various aliphatic alcohols, underscoring the superiority of the approach over other general derivatization methods that indiscriminately modify all analytes in a given sample. The present work represents the first application of NMR on the analysis of these highly toxic and environmentally persistent species.

Keywords: Pentachlorophenol, endocrine disruptor, difluoromethylation, NMR.

Introduction

Chlorinated phenols (CPs) in general, have posed a serious environmental concern due to their high levels of production and toxicity to organisms at extremely low concentrations, earning them a notorious profile and their induction in the list of endocrine disruptor compounds (EDC) [1]. Due to the wide usage of these compounds in industry as antiseptics, insecticides, wood preservatives and as important intermediates in the production of pharmaceutical products [2-4], their derivatives as well as their degradation products can be found worldwide in surface and ground waters, bottom sediments, and atmospheric air and solids. In order to mitigate the impact of CPs in the environment and human health, their use has been subject to severe restriction policies and even banning in several countries [4]. Nevertheless, although their usage has experienced a steady decline, these chemicals still remain in the environment due to their chemical stability aided by additional physical properties in the water, soil or sediment that harbors them such as pH and temperature [5].

Methods for the extraction, detection and monitoring of CPs using numerous analytical techniques exist. The majority of these rely heavily on their intrinsic UV-absorption (*e.g.* LC-MS) [6] while others depend on their semi-volatility brought upon by their proclivity for derivatization (GC-MS) [7]. Although the power of these aforementioned techniques is well established in the analytical chemistry realm, they still represent one-dimensional approaches for the study of these analytes. It is only when we stir away from them and start to dwell into analyses procured by two-dimensional methods and/or hyphenated GC and LC methods that we truly see their power in studying these species. An approach centered on the well-established and powerful NMR spectroscopy technique offers a multidimensional series of analysis that can all be carried out in one sample and in a non-destructive manner, a quality that is completely absent on the previously mentioned methods. However, a NMR approach to the analysis of even CPs could be demandingly challenging, particularly when a mixture of other similar species such as alcohols and a given matrix is present. Due to the ubiquitous presence of the proton (^{1}H) in most organic molecules (including those in a given matrix), analysis of even a mixture of CPs can be tedious and complex. Therefore, a method that selectively tags CPs amid a mixture of other analytes would be an invaluable tool that can be used in the data deconvolution in a given analysis. Furthermore, if this selective tag offers a means to analyze the CPs in a separate NMR channel (*e.g.* ^{19}F, ^{13}C), it would tremendously aid in the unequivocal identification of each CP component in the mixture. After surveying several tagging functionalities for alcohols, particularly acidic

phenols like CPs; the difluoromethyl moiety (CF_2H) was selected. The choice of the difluoromethyl tag, for labeling CPs was supported by several factors that can be directly attributed to the versatility of the fluorine atom in NMR based experiments. For example, using the difluoromethyl tag immediately introduces two readily detectable nuclei by NMR, that as a result of their natural abundance enjoy great sensitivity in this form of spectroscopic analysis (Figure 1). In the realm of NMR, ^{19}F is the most abundant isotope of the element and, as mentioned briefly above, it introduces NMR as a sensitive method for the detection of fluorine-containing species in a rapid manner (lowest possible number of scans for a given sample). In addition, the large chemical shift range exhibited by the ^{19}F channel allows for the clear resolution of structurally similar CPs. ^{1}H NMR also finds beneficial application for these analyses as the chemical shift of the $CF_2\underline{H}$ proton occurs between 6.7-6.9 ppm, well outside the resonances of aliphatic and olefinic substrates. Additionally, use of the aromatic proton signals from the CP except for pentachlorophenol (PCP) further aids in their structure determination. Consequently the protocol described in this work efficiently achieves the chemical tagging of CPs resulting in species that can be studied by ^{1}H, ^{19}F and ^{13}C NMR spectroscopy (Fig. 1).

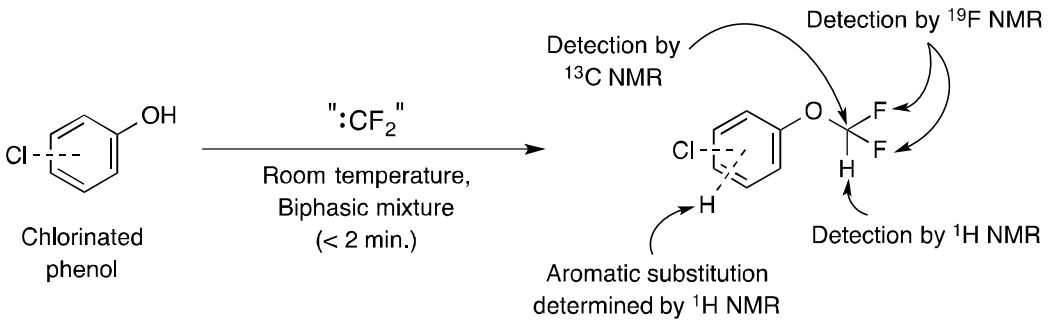


Figure 1. Features of the tagging strategy described in this work and its application for the analysis of CPs by NMR spectroscopy.

Materials and methods

All chemicals were purchased from commercial suppliers and used as received. Acetonitrile, methylene chloride and the used chlorophenols were purchased from Sigma-Aldrich chemicals (St. Louis, MO.) and diethyl (bromodifluoromethyl) phosphonate was purchased from Matrix Scientific Inc. (Columbia, SC.). Deuterated acetonitrile (CD_3CN) was purchased from Alfa Aesar (Ward Hill, MA).

Derivatization protocol The chlorinated phenol (0.08 mmol) was placed in a glass autosampler vial equipped with a small stir bar. The phenol was treated sequentially via pipette with deuterated acetonitrile [9] (CD_3CN, 600 μL) and diethyl (bromodifluoromethyl) phosphonate (21.4 μL, 0.12 mmol, 1.5 equiv. to phenol). To the above solution, aqueous saturated potassium hydroxide (300 μL) was added via pipette. The vial was capped and stirred at ambient temperature for 2 minutes. After the stirring was done, the mixture was allowed to stand to reveal a biphasic mixture and 500 μL of the top layer (CD_3CN) was aliquoted into another glass autosampler vial containing anhydrous sodium sulfate (Na_2SO_4, 50 mg). The dried, organic fraction was filtered into a 5 mm NMR tube through a cotton plug and treated with 100 μL of a 0.17 M solution of hexafluorobenzene in CD_3CN (^{19}F NMR internal standard).

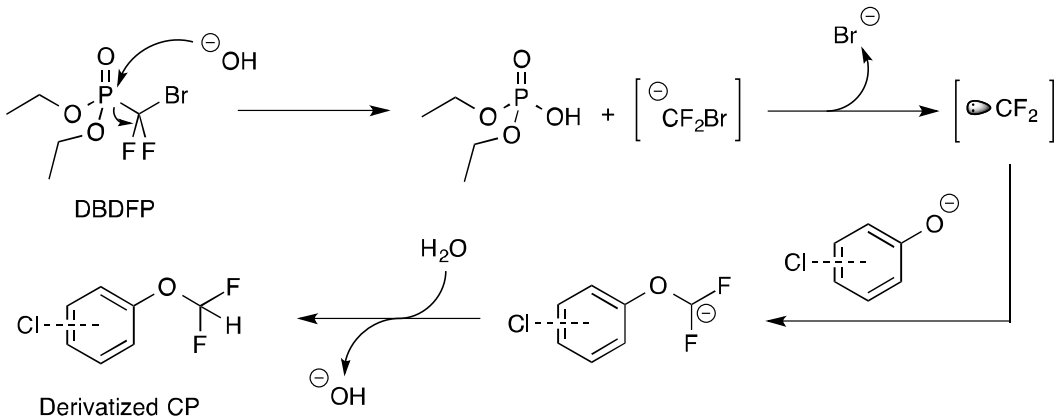
NMR Analysis Spectra were obtained using a Bruker Avance III 600 MHz instrument equipped with a Bruker TCI 5 mm cryoprobe (Bruker Biospin, Billerica, MA) at 30.0 ± 0.1 ^{o}C. ^{1}H NMR (600 MHz), ^{19}F NMR (565 MHz) and ^{13}C NMR (150 MHz) were recorded in CD_3CN. ^{1}H NMR chemical shifts are calibrated with respect to residual partially deuterated acetonitrile centered at 1.94 ppm. ^{19}F NMR chemical shifts are calibrated with respect to the singlet given by hexafluorobenzene at -164.9 ppm. Lastly, for ^{13}C NMR the septet centered at 1.79 ppm from CD_3CN was used for the spectral calibration.

GC-MS Analysis A 6890 Agilent GC with 5975 MS detector equipped with a split/splitless injector was used for the analysis. The GC column used for the analysis was an Agilent DB-5MS capillary column (30 m x 0.25 mm id x 0.25 μm i.f.). Ultra high purity helium was used as the carrier gas at 0.8 mL/min. The injector temperature was 250°C, and the injection volume was 1 μL. The oven temperature program was as follows: 40°C, held for 3 min, increased at 8°C/min to 300°C, held for 3 min. The MS ion source and quadrupole temperatures were 230°C and 150°C, respectively. Electron ionization was used with ionization energy of 70 eV. The MS was operated to scan from *m/z* 29 to *m/z* 600 in 0.4 sec.

Results and discussion

The difluoromethyl (CF_2H) moiety has been a key player in the pharmaceutical industry where its role as an isosteric group for the methyl moiety has, and continues to be exploited for its superior chemical attributes. Even though its size is comparable to that of the methyl group, its electronic properties are significantly different due to the presence of the fluorine atoms in its makeup. Due

to the importance of this group for the aforementioned reason, it is no surprise that many methods have been developed for its introduction in molecular targets [10]. Although some of the methods thus far developed require the use of heating and long reaction times, a method that makes use of diethyl (bromodifluoromethyl) phosphonate (DBDFP) developed by the Zafrani group provides a rapid and environmentally benign option [11]. Due to its high reactivity under basic conditions (pH ~ 12), reactions using DBDFP are often carried out at low temperatures (-78 ^{o}C) followed by warming up the mixture to room temperature once the reagent has been used to modify a phenolic moiety. The proposed mechanism for the overall transformation is outlined in Scheme 1. Thus, in the highly basic conditions that the reaction is carried out, DBDFP rapidly reacts with the base to produce a bromodifluoromethyl carbanion that spontaneously decomposes yielding the bromide anion and difluorocarbene. Reaction of the fleeting difluorocarbene with the nucleophilic phenoxide ion produces an anionic intermediate that rapidly protonates to furnish the difluoromethylated phenol [12]. The ease of formation of the phenoxide ion, especially in the case of the chlorinated phenols [13], is greatly favored under these conditions due to their low pK_a values (Table 1). Talk more about the Table 1, perhaps about its features of 1H, 19F and 13C NMR. The fact that DBDFP can accomplish the efficient derivatization of PCP in a very short amount of time, compounded to its liquid state at room temperature, make it an attractive alternative for the derivatization and study of chlorinated phenols not only by NMR spectroscopy but by other analytical techniques such as LC- and GC-MS.



Scheme 1. Proposed mechanism for the difluoromethylation reaction.

Entry	CP	MW	CAS #	mp (^{o}C)	pK_a	^{1}H (δ)^{a}	^{19}F (δ)^{b,c}	^{13}C (δ)^{d}
1	2-CP	128.6	95-57-8	9	8.65	6.815	-83.326	118.14
2	3-CP	128.6	108-43-0	33	9.12	6.758	-83.887	117.86
3	4-CP	128.6	106-48-9	43	9.37	6.792	-83.692	117.95
4	2,3-DiCP	163.0	576-24-9	57-59	7.7	6.818	-83.924	118.01
5	2,4-DiCP	163.0	120-83-2	45	7.85	6.785	-83.895	117.98
6	2,5-DiCP	163.0	583-78-8	59	7.51	6.820	-84.187	117.61
7	2,6-DiCP	163.0	87-65-0	68-69	6.91	6.75	-82.492	118.82

8	3,4-DiCP	163.0	95-77-2	68	8.59	6.784	-84.260	117.75
9	3,5-DiCP	163.0	591-35-5	68	8.59	6.809	-84.538	117.62
10	2,3,5-TriCP	197.5	933-78-8	62	n.a.	6.839	-84.406	117.82
11	2,3,6-TriCP	197.5	933-75-5	58	5.9	6.759	-82.425	118.74
12	2,4,5-TriCP	197.5	95-95-4	68-70.5	7	6.812	-84.303	117.81
13	2,4,6-TriCP	197.5	88-06-2	65.9	5.99	6.735	-82.495	118.40
14	3,4,5-TriCP	197.5	609-19-8	101	7.83	6.806	-84.709	117.57
15	2,3,4,5-TetraCP	231.9	4901-51-3	116-117	5.64	6.842	-84.457	117.78
16	2,3,5,6-TetraCP	231.9	935-95-5	115	5.3	6.766	-82.369	118.69
17	2,3,4,6-TetraCP	231.9	58-90-2	70	5.22	6.752	-82.430	118.66
18	PCP	266.3	87-86-5	191	4.74	6.755	-82.370	118.39

Table 1. Physical properties and NMR data on difluoromethylated CPs described in this work. [a]All signals are triplets with $J_{H\text{-}F}$ = 72.5 Hz and their δ value is referenced relative to the CHD_2CN residual peak signal centered at δ = 1.94 ppm; [b]All signals are singlets (H-F decoupled experiment) thus providing greater signal enhancement; [c]All signals are referenced to an internal standard of hexafluorobenzene (δ = -164.89 ppm); [d]All signals are referenced to CD_3CN (δ = 1.79 ppm).

After having evaluated the efficiency of the approach in derivatizing CPs, we proceeded to test its selectivity for tagging these species in the presence of other structurally diverse alcohols. To this end, we prepared a mixture in CD_3CN consisting of PCP (0.08 mmol) with 12 other structurally diverse alcohols (each spiked at 0.08 mmol) and these included: primary (2-methyl-1-butanol, 2-methyl-1-pentanol), secondary (pinacolyl alcohol), tertiary (1-methylcyclopentanol, 3-ethyl-3-pentanol), N-substituted *β*-amino alcohols (*N*,*N*-diisopropylaminoethanol, *N*,*N*-dimethylaminoethanol, *N*-methyldiethanolamine), long-chained alcohols (2-decanol, 1-nonanol) and a thioether- and sulfone-containing alcohols (2,2'-thiodiethanol, 2,2'-sulfonyldiethanol) (Figure X, SI). Once the mixture was treated sequentially with DBDFP and aqueous KOH solution (pH = 12.2), it was stirred for 2 minutes and the organic layer analyzed by ^{1}H and ^{19}F NMR after drying over Na_2SO_4. Treatment of the equimolar alcohol mixture with 1, 1.5, 2 and 4 equivalents of DBDFP resulted in the efficient and selective derivatization of only PCP. The use of excess of DBDFP does not cause a surge of interfering signals in the vicinity of the OCF_2H-derived triplet at ~ δ = 6.76 ppm, but signals localized in the δ = 0.7-1.1 ppm range arising from the reagent's hydrolysis and by-products begin to increase in magnitude (Figure X, SI). This observation was further confirmed by the GC-MS analysis of the mixture where 1.5 equivalents of DBDFP were used for the derivatization. The GC chromatogram demonstrated that the only products arising from the procedure belong to PCP and its minor contaminant 2,3,4,6-tetrachlorophenol, while the 12 alcohols in the mixture remained underivatized (Figure Y, SI). Discuss more on how great this derivatization is as it is not only rapid, easy to perform, applicable to both aqueous and organic mixtures where acidic phenols are present, and specific for these substances. Due to the biphasic nature of the process, the procedure can be adapted for

CP analysis on organic as well as aqueous samples as once the derivatization has taken place, the tagged CP will be soluble in the organic phase.

Conclusion

Our experiments have demonstrated the efficacy of diethyl (bromodifluoromethyl) phosphonate at difluoromethylating the endocrine disruptor pentachlorophenol and related CPs for their detection and analysis by multinuclear NMR. The reagent is convenient to use as it is a liquid at room temperature and once it has reacted its products and environmentally benign. Furthermore, the derivatization reaction is expedient in nature (2 min) and can be conveniently carried out at room temperature. The difluoromethylated derivatives exhibit unique chemical shifts in the ^{1}H NMR channel and the analyses of the derivatives is further enhanced as each possesses unique ^{19}F and ^{13}C NMR chemical shifts. In addition, the methodology demonstrates that CPs can be selectively derivatized in the presence of various kinds of aliphatic alcohols, underscoring the potential superiority of the approach over other general derivatization methods that indiscriminately modify all analytes in a given sample. Due to the ease and mild conditions involved in its execution this methodology should find wide applicability in the derivatization and analysis of not only CPs but other environmentally relevant phenolic compounds as well. Most importantly, and as briefly alluded to in the present work, the protocol lends itself as an additional, non-destructive technique for the analysis of acidic phenols in in conjunction with other techniques such as GC-MS and LC-MS.

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References

[1] F. Orton, I. Lutz, W. Kloas, E. J. Routledge. *Environ. Sci. Technol*. **2009**, *43*, 2144.

[2] M. Czaplicka. *Sci. Total Environ*. **2004**, *322,* 21.

[3] A. O. Olaniran, E. O. Igbinosa. *Chemosphere* **2011**, *83*, 1297.

[4] W. Zheng, X. Wang, H. Yu, X. Tao, Y. Zhou, W. Qu. *Environ. Sci. Technol*. **2011**, *45*, 4668.

[5] Y. Arcand, J. Hawari, S. R. Guiot. *Wat. Res*. **1995**, *29*, 131.

[6] M. R. Quintana, L. Ramos. *Trends Anal. Chem*. **2008**, *27*, 418.

[7] D. Li, J. Park, J-R. Oh. *Anal. Chem*. **2001**, *73,* 3089.

[8] The use of acetonitrile as a solvent is important, as once the derivatized sample has been analyzed by NMR, it can be directly employed in GC- and LC-MS analyses with no need for solvent exchange.

[9] Mild heating (45-50 ^{o}C) for 1-2 hours is needed when the difluorocarbene generating compound fluorosulfonyldifluoroacetic acid is used: Q. Y. Chen, S. W. Wu. *J. Fluorine Chem*. **1989**, *44*, 433; whereas for the agent sodium chlorodifluoroacetate, heating at 100 ^{o}C for 16 hours is required: T. Sifferlen, R. Koberstein, E. Cottreel, A. Boller, T. Weller, J. Gatfield, C. Brisbare-Roch, F. Jenck, C. Boss. *Bioorg. Med. Chem. Lett*. **2013**, *23*, 3857.

[10] Y. Zafrani, G. Sod-Moriah, Y. Segall. *Tetrahedron* **2009**, *65,* 5278.

[11] R. M. Flynn, D. J. Burton, D. M. Wiemers. *J. Fluorine Chem*. **2008**, *129*, 583.

[12] D. L. S. Brahms, W. P. Dailey. *Chem. Rev*. **1996**, *96*, 1585.

[13] Due to the close pK_a values amongst phenolic species, this method is expected to result in their difluoromethylation. As originally presented in Ref. 10, the propensity of the alkylation increases as the pK_a value of a phenol decreases.