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4-(4'-Methyltetrafluorophenyl)-2,3,5,6-tetrafluorobenzyl bromide: A New Electrophoric Derivatizing Reagent

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Abstract

Commercially-available 4,4'-dimethyloctafluorobiphenyl was converted in a single step to 4-(4'-methyltetrafluorophenyl)-2,3,5,6-tetrafluorobenzyl bromide (MTFB-TFBBBr) for the purpose of providing a new electrophoric derivatizing reagent. When reacted with this reagent, 2-fluoro-O⁶-(2'-hydroxyethyl)hypoxanthine, a model analyte, gave a mixture of isomeric products (apparently substituted at N7 and N9, analogous to its known reaction with pentafluorobenzyl bromide), and 53 femtograms of the mixture was detected at S/N = 10 by gas chromatography electron capture mass spectrometry (GC-EC-MS). As intended, the volatility of the MTFB-TFBBBr derivative was much less (2-fold) than that of the corresponding pentafluorobenzyl derivative. It is anticipated that MTFB-TFBBBr sometimes will be useful in providing an electrophoric derivative that encounters less background noise in analysis by electrophore derivatization/GC-EC-MS.

1. Introduction

Gas chromatography electron capture mass spectrometry (GC-EC-MS) is applied both to analytes which are inherently electrophoric and electrophore-derivatized compounds. Most notable is the high sensitivity it can provide, reaching the low amol level for standard compounds having high gas phase electron affinity and thermal stability [1-3]. Recent applications have been reviewed [4].

Having a diversity of electrophoric derivatizing reagents is important for GC-EC-MS as a means to tune up sensitivity either by increasing signal or decreasing noise. The strategy for decreasing noise is to test different electrophoric derivatives until one is found that elutes in a relatively clean region of the GC-EC-MS mass chromatogram. This topic has been discussed in more detail in a review of GC-EC-MS concerned with the detection of DNA adducts [5].

Towards a goal of increasing the menu of electrophoric derivatizing reagents in terms of volatility characteristics, we report here the synthesis of a relatively nonvolatile electrophoric derivatizing agent. In part, the preparation and testing of this compound was motivated by the

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continuing emergence of GC columns with high temperature stability. Fewer interferences tend to be encountered in the higher retention region of GC.

2. Experimental

2.1 Reagents

4,4'-Dimethyloctafluorobiphenyl, pentafluorobenzyl bromide, N-bromosuccinimide, benzoylperoxide, potassium carbonate, sodium nitrite, and sodium hydroxide were purchased from Aldrich (Milwaukee, WI, USA). 4,4'-Dimethyloctafluorobiphenyl currently is available from Fluorochem USA, West Columbia, SC, USA. O⁶-methylguanine was purchased from Chemsyn Science Laboratories (Lenexa, KS, USA). Tetrafluoroboric acid was purchased from Johnson Matthey Company (Ward Hill, MA USA). HPLC grade organic solvents were purchased from Doe and Ingalls (Medford, MA USA). Gases for GC-EC-MS were from Med-Tech (Medford, MA USA). 2-Fluoro-O⁶-methylhypoxanthine was prepared as described [5].

2.2 Equipment

A Model 5988A mass spectrometer from Hewlett-Packard (Palo Alto, CA, USA) was used for electron capture monitoring. The gas chromatograph, Hewlett-Packard 5890 Series II, was connected to the mass spectrometer with the capillary interface kept at 300°C. A Hewlett-Packard 59970 MS Chemstation data system was used to record the data. Methane (2 Torr) and He (20 p.s.i.) were used as reagent and carrier gases, respectively. Injections were made in an on-column mode onto an HP Ultra 2 (5% phenylmethyl silicone gum phase) (Hewlett-Packard) 25 m × 0.2 mm I.D., 0.33 µm film thickness capillary column. The oven was programmed from 120 to 290°C at 70°C/min, followed by 12 min hold for the analysis of the MTFP-TFBBr derivatives. A liquid chromatograph with a diode array detector (Hewlett-Packard model 1090), was used with a Microsorb C₁₈ bonded silica reversed-phase column, 25 cm length × 10 mm I.D., 5 µm particle size (Rainin Instrument Company, Inc. Woburn, MA, USA) for preparative HPLC. Thin layer chromatographic separations to verify completeness of reactions were performed with GHLF silic agel Uniplates with a UV lamp detector. Evaporation under reduced pressure was done using a SpeedVac[®] concentrator (Savant Instruments, Inc. Farmingdale, NY, USA).

2.3 Methods

4-(4'-methyltetrafluorophenyl)-2,3,5,6-tetrafluorobenzyl bromide (MTFP-TFBBr)—A mixture of 4,4'-dimethyloctafluorobiphenyl (1 g, 2.94 mmol), N-bromosuccinimide (0.4 g, 2.25 mmol) and benzoylperoxide (60 mg, 0.25 mmol) in 10 ml of CCl₄ was refluxed under nitrogen for 110 h. The hot reaction mixture was filtered through a Buchner funnel (Celite). The solvent was evaporated and the crude product was purified by silica flash column chromatography (bed volume: 15 cm × 2 cm) with isooctane, yielding a white solid (470 mg, 40%). This reagent was further purified by semi-preparative reversed-phase HPLC with a mobile phase of methanol/water (95:5).

2-Fluoro-O⁶-methyl-N7 and N9-(4-[4"-methyltetrafluorophenyl]-2',3',-5',-6'-tetrafluorobenzyl)—Dry potassium carbonate (16 mg, 0.12 mmol) was added to a stirred solution of 2-fluoro-O⁶-methylhypoxanthine (1.3 mg, 8 µmol) in 5 ml acetone/acetonitrile (1:1). After 5 min of stirring, MTFP-TFBBr (5 mg dissolved in 500 µl acetonitrile, 0.012 mmol) was added and stirring was continued for 24 h. The reaction product was purified by silica flash column chromatography, washed with hexane and eluted with ethyl acetate. The ethyl acetate extract of the product was evaporated under reduced pressure to give a white solid. This product was further purified by analytical reversed-phase HPLC with a mobile phase of acetonitrile/water (85:15) at a flow rate of 1 ml/min. No attempt was made to separate the co-eluting N7 and N9 isomers.

3. Results and Discussion

The structure of our new electrophoric derivatizing reagent, 4-(4'-methyltetrafluorophenyl)-2,3,5,6-tetrafluorobenzyl bromide (MTFP-TFBBr) is shown in Figure 1. It was readily synthesized from the corresponding, commercial available dimethyl compound.

MTFP-TFBBr

We chose to evaluate MTFP-TFBBr as an electrophoric derivatization agent by reacting it with 2-fluoro-O⁶-(2'-hydroxyethyl)hypoxanthine. The latter compound is known from prior work in our laboratory as an intermediate in the conversion of O⁶-methylguanine, a DNA adduct, to 2-fluoro-O⁶-(2'-hydroxyethyl)-N-(7 and 9)-(pentafluorobenzyl)hypoxanthines [6]. In this prior work, where pentafluorobenzyl bromide was employed as the electrophoric derivatizing reagent, two isomers were formed (N7 and N9) in nearly equal yields. However, the response of the N9 isomer was about 4 times higher than that of the N7 isomer. The N9 isomer also eluted earlier (3.3 min) than the N7 isomer (3.7 min) by GC-EC-MS. The difference in the retention and responses of these compounds was hypothesized to arise from the N7 compound having a polar footprint, making it not only less volatile by GC, but more susceptible to adsorption losses or decomposition in the GC-EC-MS system [5,6].

The measurement of MTFP-TFBBr-derivatized 2-fluoro-O⁶-(2'-hydroxyethyl)hypoxanthine by GC-EC-MS is shown in Figure 1. Considering the similar prior results with the corresponding pentafluorobenzyl derivative, we assume that the early eluting compound is the N9 isomer. As seen, the response is excellent since 54 femtograms of the combined compounds (apparently N9 and N7 derivatives) are detected at a signal-to-noise of about 10. Under the same GC-EC-MS conditions, the corresponding pentafluorobenzyl derivatives have retention times about 2-fold smaller (data not shown), and give the same product anions (*m/z* 167) arising from loss of the electrophoric moiety as a neutral from the parent anion radical.

4. Conclusions

We have reached our goal of introducing a new electrophoric derivatizing reagent which has the following, demonstrated advantages: takes advantage of the derivatization conditions already well established for pentafluorobenzyl bromide; is easy to synthesize; forms a strong electrophoric derivative with an appropriate compound; and provides a much less volatile derivative than pentafluorobenzyl bromide when needed. This new reagent will increase the usefulness of GC-EC-MS.

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Figure 1. GC-EC-MS chromatogram of isomeric MTFP-TFBBr derivatives of 2-fluoro-O⁶-methylhypoxanthine. One μ l of acetonitrile containing 53 fg of the combined isomers (apparently N9 at 7.9 min, N7 at 10.1 min) was injected, and m/z 167 was monitored.

GC-EC-MS chromatogram of isomeric MTFP-TFBBr derivatives of 2-fluoro-O⁶-(2'-hydroxyethyl)hypoxanthine. One μ l of acetonitrile containing 53 fg of the combined isomers (apparently N9 at 7.9 min, N7 at 10.1 min) was injected, and m/z 167 was monitored.