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Short Communication

4-(Trifluoromethyl)-2,3,5,6-tetrafluorobenzyl bromide as a new electrophoric derivatizing reagent

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ABSTRACT

4-(Trifluoromethyl)-2,3,5,6-tetrafluorobenzyl bromide (TTBB) was synthesized in a single step from α,α,α,2,3,5,6-heptafluoro-*p*-xylene. The purpose of TTBB is to function as an analogue of pentafluorobenzyl bromide (PFBB) in electrophoric derivatization reactions prior to detection by gas chromatography–electron-capture negative ion mass spectrometry (GC–ECNI-MS). In more detail, it was anticipated that TTBB could be used along with, or as a substitute for, PFBB to help control some interferences and confirm results. This is because a TTBB-product (of an analyte) would have different retention and sometimes *m/z* characteristics than a corresponding PFBB product in GC–ECNI-MS, while the two products should be similar in their ease of formation and yields. Results demonstrating these expectations were achieved by derivatizing and detecting two analytes with these reagents: N7-(2-hydroxyethyl)xanthine, and 2,3-pyrenedicarboxylic acid.

INTRODUCTION

Pentafluorobenzyl bromide (PFBB) is used as a derivatizing reagent to enhance the detectability of susceptible compounds by gas chromatography–electron-capture negative ion mass spectrometry (GC–ECNI-MS) and GC with electron-capture detection (ECD). Examples of such compounds are some of the normal and modified DNA nucleobases [1–3], arachidonic acid metabolites [4–5], drugs [6–9], phenols [10], plant metabolites [11], indole-amine metabolites [12], fatty acids [13], herbicides [14], inorganic anions [15] and histamine [16]. A low detection limit can be achieved especially when a derivative is formed which gives essentially a single ion in GC–ECNI-MS. However, this compromises the specificity by making it difficult to know whether an interference is present. When GC–ECNI-MS is used for environmental analysis, it is useful to monitor more than one ion to help confirm results [17]. Increased demands are therefore placed on prior sample cleanup in chemical analysis when pentafluorobenzyl derivatives are formed that give single ions in GC–ECNI-MS. Use of a second GC column can help to confirm a result but is not always practical.

Along these lines, it can be useful to derivatize a given analyte with 2,3,5,6-tetrafluorobenzyl bromide. By producing a different but analogous product (having a different GC retention time,

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m/z or both), the use of this reagent can overcome a persistent interference or help to confirm peak identity [18]. For the latter purpose, it may be attractive to use the two reagents separately (on two aliquots of the sample), or as a mixture on a single sample.

We considered that it would be useful to have a third reagent of this type for additional flexibility in coping with interferences and confirming results. Partly this is because interferences tend to become increasingly random as analytical methods are progressively applied to smaller amounts of trace analytes. Thus, as reported here, we have prepared and tested a second analog of pentafluorobenzyl bromide, in which a trifluoromethyl group rather than a hydrogen atom replaces the *para* fluorine atom in pentafluorobenzyl bromide. The chemical and physical properties of the trifluoromethyl group have been reviewed [19].

EXPERIMENTAL

Reagents

Pentafluorobenzyl bromide, potassium hydroxide and tetrabutyl ammonium hydrogen sulphate (Bu_4NHSO_4) were purchased from Aldrich (Milwaukee, WI, USA). HPLC-grade organic solvents were purchased from Doe and Ingalls (Medford, MA, USA). α,α,α,2,3,5,6-Heptafluoro-*p*-xylene and benzoylperoxide were from Aldrich). Gases for GC–ECNI-MS were from Med-Tech (Medford, MA, USA). 2,3-Pyrenedicarboxylic acid was synthesized as described [20], as was N7-(2-hydroxyethyl)xanthine [3].

Equipment

A Model 5988A mass spectrometer from Hewlett-Packard (Palo Alto, CA, USA) was used. The gas chromatograph, a Hewlett-Packard 5890 Series II, was connected to the mass spectrometer with the capillary interface kept at 290°C and the ion source at 250°C. A Hewlett-Packard 59970 MS Chemstation data system was used to record the data. Methane (2 Torr; 1 Torr = 133.322 Pa) and He (20 p.s.i.; 1 p.s.i. = 6894.76 Pa) were used as reagent and carrier gases respectively. Injections were made in an on-column mode onto an HP Ultra 1 (dimethylpolysiloxane), 25 m × 0.2 mm I.D., 0.11 μm film thickness capillary column, and the oven was programmed from 110 to 250°C at 70°C/min (then 10 min hold) for compounds **1** to **3**, and from 140 to 300°C at 70°C/min for **4**, with a hold of 13 min.

Methods

4-(Trifluoromethyl)-2,3,5,6-tetrafluorobenzyl bromide (TTBB). A mixture of α,α,α,2,3,5,6-heptafluoro-*p*-xylene (5 g, 21.5 mmol), N-bromosucciminide (3.45 g, 19.4 mmol) and benzoylperoxide (500 mg, 2.0 mmol) in 50 ml of CCl_4 was refluxed under nitrogen for 10 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 chromatography (bed volume: 15 cm × 2 cm) with isooctane, yielding a colorless oil (2.7 g, 40%). ^{1}H NMR (300 MHz, C^2HCl_3) δ 4.55 (2H, s). This reagent is now available from Aldrich.

N1,N3-Bis-[4-(trifluoromethyl)-2,3,5,6-tetrafluorobenzyl]-N7-{2-[4-(trifluoromethyl)-2,3,5,6-tetrafluorobenzyloxy]ethyl}xanthine (3). To a stirred solution of N7-(2-hydroxyethyl)xanthine (N7-HEX; 2 mg, 0.01 mmol) in 100 μl of 1 N KOH were added CH_2Cl_2, (300 μl), Bu_4NHSO_4 (10 mg, 0.0027 mmol) and TTBB (10 mg, 3.2 μmol). After stirring for 48 h at room temperature the reaction mixture was partitioned between 1 ml each of water and CH_2Cl_2. The organic layer was separated and the aqueous layer was extracted 3× with 1 ml of CH_2Cl_2. The combined CH_2Cl_2 fractions were dried over Na_2SO_4, and the filtered solution was evaporated under vacuum. The crude product was purified on an analytical silica TLC plate with ethyl acetate–hexane (1:1), to give a white solid (2 mg, 22%). ^{1}H NMR (300 MHz, C^2HCl_3): δ 7.30 (s, 1H), 5.20 (s, 2H), 5.06 (s, 2H), 4.26 (s, 2H), 4.13 (t, 2H), 3.60 (t, 2H).

1,2-Bis[4-(trifluoromethyl)-2,3,5,6-tetrafluorobenzyl]pyrene dicarboxylate (4). To a stirred solution of 2,3-pyrene dicarboxylic acid (2.01 mg, 6.9 μmol) in 200 μl of CH_3CN was added K_2CO_3 (25 mg, 0.17 mmol) and TTBB (10 μl, 0.065 mmol). The reaction mixture was stirred for 20 h at room temperature and filtered

(paper). The filtrate was evaporated under vacuum, and the product was purified by silica TLC using an analytical plate (ethyl acetate–hexane, 5:95) for development; $R_F = 0.45$, followed by ethyl acetate for band extraction, giving 1.34 mg (26%). ^1H NMR (C^2HCl_3): δ 8.73 (s, 1H), 8.35–8.12 (m, 7H), 5.71 (s, 2H); 5.56 (s, 2H).

Trace reactions (each in duplicate)

Derivatization of N7-HEX with PFBB. Step 1: N7-HEX (95 pg, 0.49 pmol) in 10 μl of acetic acid–water (1:1), was evaporated under nitrogen. Potassium carbonate (5 mg, 36 μmol), acetonitrile (100 μl) and PFBB (1 μl, 0.38 μmol) were added. After stirring for 20 h at room temperature, the reaction mixture was evaporated under nitrogen.

Step 2: The residue obtained in step 1 was treated with a mixture of 50 μl of 1 *M* KOH containing 50 μg of Bu_4NHSO_4, 150 μl of CH_2Cl_2 and 10 μl (3.8 μmol) of PFBB. After stirring for 20 h at room temperature, the product, N1,N3-bis(pentafluorobenzyl)-N7-[2-(pentafluorobenzyloxy)ethyl]xanthine (**1**), was isolated and quantified by GC–ECNI-MS as described [21].

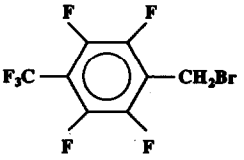
Derivatization of N7-HEX with PFBB and then TTBB. After step 1 as above, TTBB was used in step 2. The product N1,N3-bis-(pentafluorobenzyl)-N7-{2-[4-(trifluoromethyl)tetrafluorobenzyloxy]ethyl}xanthine (**2**) was isolated and quantified by GC–ECNI-MS.

Trace derivatization of N7-HEX with PFBB and then a 1:1 mixture of pentafluorobenzyl bromide and TTBB. After step 1 as above, a 1:1 mixture of pentafluorobenzyl bromide and TTBB was used in step 2. The product mixture of **1** and **2** was isolated and quantified by GC–ECNI-MS.

Trace derivatization of N7-HEX with TTBB. The same procedure was used as above in steps 1 and 2, except TTBB was substituted for pentafluorobenzyl bromide, and the product (**3**) was quantified by GC–ECNI-MS.

RESULTS AND DISCUSSION

In order to establish a new analogue of pentafluorobenzyl bromide (PFBB) for derivatization purposes, we converted α,α,α,2,3,5,6-heptafluoro-*p*-xylene to 4-(trifluoromethyl)-2,3,5,6-tetrafluorobenzyl bromide (TTBB).



TTBB

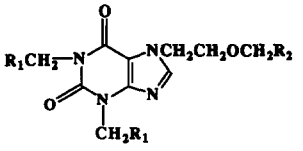
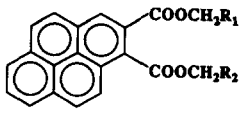
PFBB is a useful reagent both in terms of its reactivity as an alkylating agent, and the GC–ECD or GC–ECNI-MS properties of the derivatives it forms with a diversity of target compounds [1–16]. Thus, in order to test the usefulness of TTBB, we compared it in these two respects with representative compounds. For convenience, we selected two analytes from our current work on the detection of DNA adducts by pentafluorobenzylation/GC–ECNI-MS: N7-(2-hydroxyethyl)xanthine [21] and 2,3-pyrenedicarboxylic acid [22]. Together these two compounds provide three types of functional groups for derivatization: ring NH, hydroxyethyl OH, and carboxyl.

The derivatives that we formed are shown in Table I, along with reaction yields (preparative starting from 2 mg of N7-HEX, and analytical from 95 pg) and relative molar responses by GC–ECNI-MS. No effort was made to optimize the reaction yields with TTBB (arbitrarily the conditions currently in use with PFBB were adopted). Yet the analytical yields for products **1–3** are all reasonable (as absolute yields at the picogram level), as are the preparative yields of **3** and **4**. These latter reactions were conducted with only 2 mg of starting material in each case. A co-injection of **1**, **2** and **3** gave GC retention times of 7.67, 7.40 and 7.27 min, respectively.

Molar responses in GC–ECNI-MS depend on both the recoveries and electron capture characteristics of the compounds tested in the system. Active sites in the injector, column, and ion source can all contribute to the former, and many parameters can influence the yield of ions from the source [23]. Thus one should be careful not to over-interrupt relative molar response

TABLE I

YIELD AND RESPONSE OF ELECTROPHORIC PRODUCTS

Structure	Yield		Response by GC–ECNI-MS[a]
	Preparative	Analytical	
			
1: $R_1 = R_2 = C_6F_5$	–	27, 45[b]	1.0
2: $R_1 = C_6F_5$; $R_2 = (CF_3)C_6F_4$	–	66, 84[c]	–[d]
3: $R_1 = R_2 = (CF_3)C_6F_4$	27	40, 66	0.2
			
4: $R_1 = R_2 = (CF_3)C_6F_4$	26		1.5

[a] Molar response relative to **1** based on peak area.
[b] This yield is from prior work [18], and the structure of this compound was established previously [2].
[c] Analytical yield assuming that **1** and **2** have the same relative molar response.
[d] The molar response is not available since this product was not prepared preparatively.

values. We conclude, from the molar response values presented in Table I, that TTBB is similar to pentafluorobenzyl bromide in its ability to provide sensitive derivatives of the compounds tested for GC–ECNI-MS.

The chromatograms A and B in Fig. 1 were obtained by derivatizing 95 pg of N7-(2-hydroxyethyl)xanthine on the N1 and N3 positions with PFBB, followed by derivatization of the hydroxyethyl OH with a 1:1 molar mixture of PFBB and TTBB. Chromatograms of corresponding blank reactions (no analyte) are shown in Fig. 1A′ and B′. As seen, a peak with nearly same retention time as **2**, but with 1/4 of the abundance, is present in A′ but not in B′. For the individual sample leading to chromatogram B, more background peaks are seen, but the corresponding blank chromatogram B′ is the cleanest of all. This kind of variation in background peaks as a function of m/z, or even one vial *vs.* another, is encountered sometimes at this level of sensitivity by GC–ECNI-MS in our ex-

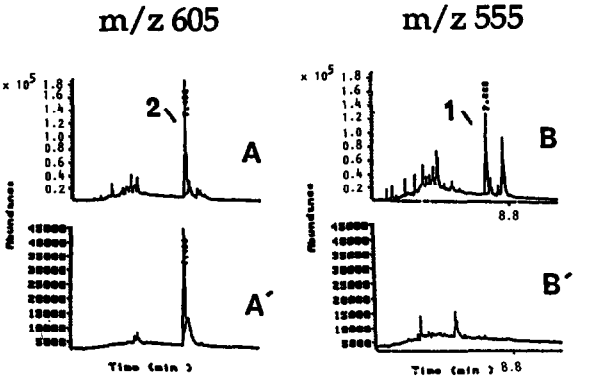


Fig. 1. GC–ECNI-MS chromatograms from the derivatization of N7-(2-hydroxethyl)xanthine (95 pg) in two steps (see Experimental): step 1 with PFBB, step 2 with PFBB–TTBB, 1:1. Corresponding chromatograms from blank reactions (no analyte) are shown (A′, B′). Retention times (min): **1** = 7.67 min; **2** = 7.40 min; highest peak in A′, 7.43 min. Note that the abundance scale is expanded by 4× in A′, B′ relative to A, B.

perience, and supports the usefulness of forming more than derivative.

In conclusion, the availability now of two analogues of pentafluorobenzyl bromide (H or CF_3 at the *para* position instead of F) provides additional flexibility in this area of derivatization to cope with both consistent and random interferences, helping to confirm results. Conveniently, the same column and conditions can be used to quantify these products. Their similar chemical and physical characteristics also allow them to be formed together and co-purified.

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