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Electron-capture detection: difluorobenzyl and related electrophores

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Abstract

Difluorobenzyl derivatives (several isomers were tested) of 4-hydroxyacetophenone were synthesized and found to have similar properties (retention and response) by both reversed-phase HPLC and GC–ECD relative to each other, and also relative to that of a corresponding conventional pentafluorobenzyl derivative. The same was true for a representative difluorobenzyl derivative of thymine and 1-naphthoic acid. Overall, the responses by GC–ECD for the same core structure were only about two- to four-fold lower for a difluorobenzyl compared to a corresponding pentafluorobenzyl derivative. This makes a difluorobenzyl derivative attractive as an HPLC–UV retention marker, and sometimes as a substitute for a pentafluorobenzyl derivative (to help overcome an interference) in a method based on detection by electron capture. We also observed, somewhat as an aside, that the GC–ECD response of the benzyl derivative of 4-hydroxyacetophenone was only seven-fold lower than that of the corresponding pentafluorobenzyl derivative, and that this former benzyl derivative gave a $2 \cdot 10^4$ higher response than acetophenone. Thus, replacing the ring hydrogen atoms of a benzyl group with fluorine atoms had a relatively small impact on both the hydrophobicity and electron capture properties of the compounds tested here.

Keywords: 4-Hydroxyacetophenone; Difluorobenzyl derivatives

1. Introduction

Pentafluorobenzyl bromide (PFBzBr) has been widely employed as an electrophoric derivatizing agent in chemical analysis. It has been applied to a diversity of analytes, and the final products are detected by gas chromatography–electron-capture detection (GC–ECD), or gas chromatography–electron-capture mass spectrometry (GC–ECMS). Early examples of its application were reviewed in 1981 [1]. More recent examples include nonylphenols [2], chloride [3], nitrite and nitrate [4], hydroxy-substituted polynuclear aromatic hydrocarbons [5], alkylphosphates [6], fatty acids [7], abscisic acid [8], prostaglandins and their metabolites [9–11], tryptophan and kynurenine [12], amino acids [13], amino acids and dipeptides [14], histamine [15], DNA adducts [16] and electrophoric release tags [17]. Detection limits have reached the attomole level for standards (e.g. [18]) and generally the picomole/femtomole level for analytes derived from real samples.

Several analogs of PFBzBr have been studied as substitutes for this reagent: 3,5-bis(trifluoromethyl)benzyl bromide (BTFMBzBr), 4-(trifluoromethyl)tetrafluorobenzyl bromide, 4-(trifluoromethyl)benzyl bromide, pentafluorophenyldiazo methane, 1-(pentafluorophenyl)diazoethane and pentafluorobenzyl-*p*-toluenesulphonate. They were selected either arbitrarily, or promoted as PFBzBr analogs. An analog can be of interest since it may avoid certain impurities or side products that arise

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with PFBzBr, potentially eliminating an interfering peak. Murray et al. [19] applied BTFMBzBr to the measurement of heterocyclic amines (e.g. 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline) by GC–ECMS in foods. This group also tested both BTFMBzBr and 4-(trifluoromethyl)benzyl bromide as derivatization reagents for the analyte N-(dicyclopropylmethyl)amino-2-oxazoline, although the two derivatization products were only compared qualitatively, and a 3,5-bis(trifluoromethyl)benzoyl derivative was selected instead [20]. In the GC–ECMS method for uracil, Blount and Ames selected BTFMBzBr after first trying PFBzBr [21], and we arbitrarily selected this reagent to derivatize glycolate [22]. We found that 4-(trifluoromethyl)tetrafluorobenzyl and PFBz derivatives of N7-(2′-hydroxyethyl)guanine gave similar responses by GC–ECMS [23]. The two pentafluorophenyldiazoalkanes cited above were used to derivatize standards of carboxylic acids [24], and pentafluorobenzyl *p*-toluene sulphonate was applied to inorganic anions such as bromide, iodide, thiocyanate and nitrite [25].

Additional work along these lines is reported here, where we focus mostly on difluorobenzyl bromides (DFBzBrs) as additional substitutes for PFBzBr. Also *m*-fluorobenzyl bromide and benzyl bromide are tested. The aim of this work was to compare the polarity and GC–ECD response of DFBz as opposed to PFBz derivatives for two reasons. First, we wanted to consider DFBz derivatives as potential retention markers in HPLC (based on UV absorbance detection) to monitor purification of corresponding trace PFBz derivatives at levels below UV absorbance detection. Second, in regard to our work with electrophore release tags [17], we wanted to learn whether a DFBz group could provide a comparable response by electron capture to a PFBz group, while maintaining moderate polarity.

2. Experimental

2.1. Materials

All chemicals were obtained from Aldrich (Milwaukee, WI, USA) unless otherwise stated. A Zorbax R_x C_{18} (150×4.6 mm I.D.) reversed-phase column (Mac-Mod Analytical, Chadds Ford, PA, USA) was employed for HPLC. The gas chromatograph (HP-5890 Series II) column (25 m×0.32 mm, 0.52 μm film thickness, Ultra 2), and electron-capture detector were from Hewlett-Packard (Palo Alto, CA, USA). For NMR (Varian XL-300, Suger Land, TX, USA), the reference signals for ^1H, ^{13}C and ^{19}F were the proton signal of tetramethylsilane (0 ppm), the center carbon peak of $CDCl_3$ (77.0 ppm) and the F-19 signal of $CFCl_3$ (0 ppm), respectively. All ^{13}C NMRs were conducted with the decoupler (^1H) on. Preparative TLC plates were purchased from Analtech (Newark, DE, USA).

2.2. Synthesis

2.2.1. 4-(2′,3′,4′,5′,6′-Pentafluorobenzyloxy)-acetophenone, 1

Sodium hydroxide (0.12 g, 2.7 mmol) was dissolved in 1 ml of water in a 50-ml round-bottomed flask. 4′-Hydroxyacetophenone (0.36 g, 2.7 mmol) in 5 ml methanol was added to the flask, followed by vigorous stirring for 5 min. α-Bromo-2,3,4,5,6-pentafluorotoluene (0.76 g, 2.4 mmol) in 20 ml methanol was added dropwise, followed by refluxing at 60–70°C for 4–5 h, giving a white precipitate. Water (25 ml) was added and the mixture was cooled to room temperature. Paper filtration (suction) followed by potassium carbonate (5%) and water rinse (20 ml each) gave a white solid that was recrystallized from methanol–water (usual technique: add water to cloud point into a hot methanol solution), m.p. 85–86°C. Yield: 86%. ^1H NMR (300 MHz, $CDCl_3$) δ 8.0 (d, J=9 Hz, 2H), 7.0 (d, J=9 Hz, 2H), 5.2 (s, 2H), 2.6 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.0, 162.0, 136.4–147.9 (m), 131.5, 131.0, 114.7, 109.7 (t, J=5 Hz) 57.7, 26.7. ^{19}F NMR (282 MHz, $CDCl_3$) δ −142.0 (t, J=11 Hz, 2F), 152.0 (t, J=20 Hz, 1F), 161.3 (m, 2F).

2.2.2. 4-(3′,5′-Difluorobenzyloxy)acetophenone, Ia

The same procedure for compound **1** but with α-bromo-3,5-difluorotoluene gave 80% of white flakes, m.p. 78–79°C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.9 (d, J=9 Hz, 2H), 7.0 (m, 4H), 6.7 (tt, J=9 Hz, 1 Hz, 1H), 5.1 (s, 2H), 2.5 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.6, 163.2 (dd, J=250, 13 Hz), 161.9,

140.3 (m), 130.7, 114.5, 109.7 (dd, J=17, 8 Hz), 103.5 (t, J=25 Hz), 68.7, 26.3. ^{19}F NMR (282 MHz, $CDCl_3$) δ −109.1 (s, 2F).

2.2.3. 4-(2′,6′-Difluorobenzyloxy)acetophenone, ***1b***

The procedure for **1** but with α-bromo-2,6-difluorotoluene gave 70% of white needles, m.p. 74–75°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.0 (d, J=9 Hz, 2H), 7.4 (m, 1H), 6.9–7.1 (m, 4H), 5.2 (s, 1H), 2.6 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.7, 162.3, 161.8 (dd, J=251, 7 Hz), 131.0 (t, J=10 Hz), 130.7, 130.6, 114.4, 111.3–112.0 (m), 58.0, 26.3. ^{19}F NMR (282 MHz, $CDCl_3$) δ −114.5 (s, 2F).

2.2.4. 4-(2′,4′-Difluorobenzyloxy)acetophenone, ***1c***

The procedure for compound **1** but with α-bromo-2,4-difluorotoluene gave 89% of a white needles, m.p. 69–70°C. ^{1}H NMR (300 Mhz, $CDCl_3$) δ 7.9 (d, J=9 Hz, 2H), 7.5 (dt, J=7, 8 Hz, 1H), 7.0 (d, J=9 Hz, 2H), 6.9 (m, 2H), 5.1 (s, 2H), 2.5 (s, 3H). ^{13}C NMR (75 Mhz, $CDCl_3$) δ 196.8, 163.5 (dd, J=248, 12 Hz), 162.4, 160.3 (dd, J=249, 12 Hz), 131.0–131.1 (m), 130.9, 130.8, 119.6 (d, J=11 Hz), 114.6, 111.6 (d, J=19 Hz), 104.2 (t, J=25 Hz), 63.6, 26.5. ^{19}F NMR (282 MHz, $CDCl_3$) δ −109.4 (br, 1F), −114.2 (br, 1F).

2.2.5. 4-(3′,4′-Difluorobenzyloxy)acetophenone, ***1d***

The procedure for compound **1** but with α-bromo-3,4-difluorotoluene gave 85% of white needles, m.p. 63–64°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.9 (d, J=9 Hz, 2H), 7.1–7.3 (m, 3H), 7.0 (d, J=9 Hz, 2H), 5.1 (s, 2H), 2.6 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.8, 162.3, 150.6 (dd, J=247, 13 Hz), 150.3 (dd, J=247, 12 Hz), 133.5 (m), 131.0, 130.8, 123.5 (dd, J=6, 4 Hz), 117.7 (d, J=17 Hz), 116.6 (d, J=18 Hz), 114.6, 68.9, 26.5. ^{19}F NMR (282 MHz, $CDCl_3$) δ −137.0 (br, 1F), −138.3 (br, 1F).

2.2.6. 4-(2′,5′-Difluorobenzyloxy)acetophenone, ***1e***

The procedure for compound **1** but with α-bromo-2,5-difluorotoluene gave 92% of white needles, m.p. 95–96°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.0 (d, J=9 Hz, 2H), 7.2 (m, 1H), 7.1 (m, 2H), 7.0 (d, J=9 Hz, 2H), 5.2 (s, 2H), 2.6 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.8, 162.2, 159.1 (dd, J=244, 3 Hz), 156.3 (dd, J=241, 2 Hz), 131.2, 130.9, 125.4 (dd, J=16, 8 Hz), 115.3–116.5 (m), 114.7, 63.5, 26.6. ^{19}F NMR (282 MHz, $CDCl_3$) δ −118.3 (br, 1F), 124.8 (br, 1F).

2.2.7. 1-Naphthoic acid, 2′,3′,4′,5′,6′-pentafluorobenzyl ester, ***2***

Potassium hydroxide (0.11 g, 1.9 mmol) was dissolved in 1 ml of water in a 50-ml round-bottomed flask, 1-naphthoic acid (0.33 g, 1.9 mmol) in 5 ml acetonitrile was added, and the mixture was stirred vigorously for 5 min. α-Bromo-2,3,4,5,6-pentafluorotoluene (0.5 g, 1.9 mmol) in 20 ml acetonitrile was added dropwise, and the mixture was refluxed at 60–70°C for 4–5 h, giving a white precipitate. Water (25 ml) was added and the mixture was cooled down to room temperature. Paper filtration followed by potassium carbonate (5%) and water rinse gave 85% of white needles, m.p. 119–120°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.9 (d, J=8 Hz, 1H), 8.2 (d, J=7 Hz, 1H), 8.0 (d, J=8 Hz, 1H), 7.4–7.7 (m, 3H), 5.5 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 136.1–147.8 (m), 134.1, 131.6, 130.1, 128.8, 128.3, 126.6, 126.1, 125.8, 124.6, 110.0 (m), 54.0. ^{19}F NMR (282 MHz, $CDCl_3$) d −142.1 (t, J=11 Hz, 2F), −152.0 (t, J=21 Hz, 1F), −161.2 (m, 2F).

2.2.8. 1-Naphthoic acid, 3′,5′-difluorobenzyl ester, ***2a***

The procedure for compound **2** but with α-bromo-3,5-difluorotoluene gave 80% of white needles, m.p. 93–94°C . ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.9 (d, J=8 Hz, 1H), 8.3 (d, J=7 Hz, 1H), 8.0 (d, J=8 Hz, 1H), 7.9 (d, J=8 Hz, 1H), 7.1–7.4 (m, 3H), 7.0 (d, J=6 Hz, 2H), 6.8 (t, J=9 Hz, 1H), 5.4 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.8, 163.3 (dd, J=248, 13 Hz), 140.2 (t, J=9 Hz), 134.0, 133.9, 131.6, 130.5, 128.7, 128.0, 126.4, 125.8, 124.5, 110.6 (dd, J=17, 8 Hz), 103.6 (t, J=24 Hz), 65.3. ^{19}F NMR (282 MHz, $CDCl_3$) δ −109.2 (s, 2F).

2.2.9. 1,3-Bis(2′,3′,4′,5′6′-pentafluorobenzyl)thymine, ***3***

Potassium hydroxide (0.18 g, 3.2 mmol) was dissolved in 1 ml of water in a 50-ml round-bottomed flask. Thymine (0.2 g, 1.6 mmol) in 5 ml acetonitrile was added, and the mixture was stirred

vigorously for 5 min. α-Bromo-2,3,4,5,6-pentafluorotoluene (0.83 g, 3.2 mmol) in 20 ml acetonitrile was added dropwise, and the mixture was refluxed at 60–70°C for 4–5 h, giving a white precipitate that was recrystallized from methanol–water. Water (25 ml) was added and the mixture was then cooled down to room temperature. Paper filtration followed by potassium carbonate (5%) and water rinse gave a needle-like white solid, that was recrystallized from methanol–water, m.p. 110–111°C. Yield: 67%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.1 (s, 1H), 5.2 (s, 2H), 5.0 (s, 2H), 1.9 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.0, 150.9, 136.0–147.5 (m), 138.3, 110.9, 110.2 (m), 109.1 (m), 41.3, 34.1, 13.2. ^{19}F NMR (282 MHz, $CDCl_3$) δ −141.1 (d, J=25 Hz, 2F), −142.8 (d, J=21 Hz, 2F), −152.0 (t, J=20 Hz, 1F), −155.4 (t, J=20 Hz, 1F), −160.6 (m), −162.6 (m).

2.2.10. 1,3-Bis(3′,5′-difluorobenzyl)thymine, ***3a***

The procedure for **3** was used but with α-bromo-3,5-difluorotoluene. After refluxing for 5 h, the mixture was cooled to room temperature. Potassium carbonate (20 ml, 5%) was added, along with another 150 ml of water. The mixture was then extracted with methylene chloride (30 ml×2). The organic extract was dried over sodium sulfate, and the methylene chloride was removed by rotary evaporation, yielding a sticky oil. Purification by preparative silica TLC (CH_2Cl_2) gave a colorless oil which later turned to a white solid, m.p. 103–104°C. Yield: 49%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.0 (m, 2H), 6.6–6.8 (m, 5H), 5.1 (s, 2H), 4.9 (s, 2H), 2.0 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.3 (dd, J=249, 12 Hz), 163.3, 162.9 (dd, J=247, 13 Hz), 151.6, 140.3 (m), 139.2 (m), 138.0, 137.9, 111.3 (m), 110.6 (m), 104.1 (t, J=25 Hz), 103.3 (t, J=25 Hz), 51.5, 44.1, 13.2. ^{19}F NMR (282 MHz $CDCl_3$) δ −108.1 (br, 2F), −109.9 (br, 2F).

2.2.11. 4-(3′-Fluorobenzyloxy)acetophenone, ***4***

The procedure for compound **1** but with α-bromo-3-fluorotoluene in acetonitrile–water media gave 78% of white needles, m.p. 83–84°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.9 (d, J=9 Hz, 2H), 6.9–7.4 (m, 6H), 5.1 (s, 2H), 2.5 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.8, 163.2 (d, J=245 Hz), 162.5, 139.0 (d, J=7 Hz), 130.9, 130.5 (d, J=8 Hz), 122.9 (d, J=3 Hz), 115.3 (d, J=21 Hz), 114.8, 114.4 (d, J=22 Hz), 114.6, 69.4, 26.6. ^{19}F NMR (282 MHz, $CDCl_3$) δ −112.8 (s, 1F).

2.2.12. 4-Benzyloxyacetophenone, ***5***

The procedure for compound **1** but with α-bromotoluene in acetonitrile–water gave 84% of white needles, m.p. 90–91°C. ^{1}H NMR (300 MHz, $CDCl_3$) 87.9 (d, J=9 Hz, 2H), 7.2–7.5 (m, 5H), 7.0 (d, J=9 Hz, 2H), 5.1 (s, 2H), 2.5 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.0, 162.9, 136.5, 130.9, 130.8, 129.0, 128.5, 127.8, 114.8, 70.3, 26.6.

2.2.13. 4-[3′,5′-Bis(trifluoromethyl)benzyloxy] acetophenone, ***6***

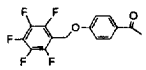
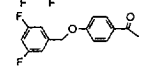
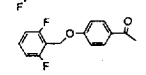
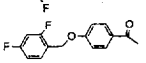
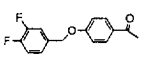
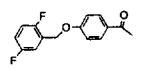
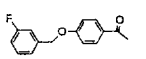
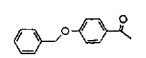
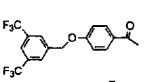
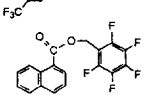
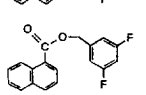
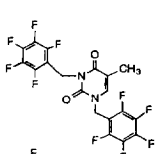
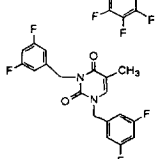
The procedure for **1** but with 3,5-bis(trifluoromethyl)benzyl bromide gave 80% of white needles, m.p. 113–114°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.8–8.1 (m, 5H), 7.0 (d, J=9 Hz, 2H), 5.2 (s, 2H), 2.6 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.6, 161.7, 138.9, 131.6 (q, J=33 Hz), 131.0, 130.7, 127.6, 123.1 (q, J=271 Hz), 122.1, 114.4, 68.4, 26.3. ^{19}F NMR (282 MHz, $CDCl_3$) δ −63.3 (s, 6F).

3. Results and discussion

Of the variety of analytes that have been converted into PFBz derivatives for detection by an electron capture technique, most of them have been phenols, carboxylic acids or heterocyclics such as nucleobases. In this overall technique, one or more of the reactive NH or OH hydrogens is replaced with a PFBz moiety, and the resulting derivative then ordinarily undergoes dissociative electron capture with loss of the PFBz moiety as a neutral radical. In an EC–MS, the complementary anionic fragment is detected.

We prepared and tested both PFBz and DFBz derivatives of model analytes representing these three classes of analytes as summarized in Table 1. While several isomers of DFBzBr are commercially available, we applied all of these reagents to only one of the analytes, 4-hydroxyacetophenone. For the other analytes, 1-naphthoic acid and thymine, we arbitrarily selected 3,5-DFBzBr.

Table 1
HPLC and GC–ECD characterization of electrophores

Structure		Retention time (min)		Relative molar ECD response
		HPLC	GC-ECD	
	**1**	11.3[a]	6.2	1
	**1a**	11.0[a]	6.5	0.40[c], 0.48[d] (**1a/1**)
	**1b**	10.6[a]	6.5	0.40[d] (**1b/1**)
	**1c**	10.8[a]	6.5	0.35[d] (**1c/1**)
	**1d**	10.8[a]	6.5	0.49[d] (**1d/1**)
	**1e**	10.8[a]	6.5	0.57[d] (**1e/1**)
	**4**	10.7[a]	6.5	0.45[d] (**4/1**)
	**5**	10.9[a]	6.5	0.15[d] (**5/1**)
	**6**	12.4[a]	6.0	0.72[d] (**6/1**)
	**2**	11.3[b]	7.2	0.64[c], 1.20[d], 1.00[e] (**2/1**)
	**2a**	11.0[b]	7.5	0.22[c], 0.24[d], 0.25[e] (**2a/1**)
				0.34[c], 0.20[d], 0.25[e] (**2a/2**)
	**3**	10.3[b]	7.9	1.64[c], 1.68[d] (**3/1**)
	**3a**	10.0[b]	8.6	0.78[c], 0.65[d] (**3a/1**)
				0.48[c], 0.39[d] (**3a/3**)

[a] HPLC conditions I: acetonitrile–water (10:90, v/v) for the first 5 min, then change to acetonitrile–water (60:40) within 1 min and keep at that concentration.

[b] HPLC conditions II: acetonitrile–water (10:90) for the first 5 min, then change to acetonitrile–water (80:20) within 1 min and keep at that concentration.

[c] Data from coinjection (one injection) of equimolar amounts of compounds **1**–**1a**, **2**–**2a** and **3**–**3a** on the Ultra 2 column.

[d] Data from individual injections (three times for each compound) on Ultra 2 column.

[e] Data from coinjections (five injections) of equimolar amounts of compounds **1**, **2** and **2a** on the Ultra 2 column on a different day from that of c and d. HPLC peak widths at the base (defined by tangents from the sides of the peaks) were ≤0.35 min. Standard deviations for the retention times in GC were ≤0.08.

The first column of data in Table 1 gives the retention times for the derivatives on a reversed-phase HPLC column. We were motivated to obtain this data because of our interest in the hydrophobicity of fluoro compounds (tied to our study of electrophoric release tags [17]), as well as a need for UV retention markers for PFBz derivatives. When such derivatives are purified at the trace level by HPLC (for subsequent off-line detection by MS), UV absorbance detection may not be possible. In order to monitor the separation and collect the right fraction, it is helpful to include one or more related compounds at a higher concentration as markers. The similar retention times of corresponding PFBz and DFBz derivatives in Table 1 suggest that the latter should make good HPLC markers for corresponding PFBz derivatives. Two important advantages of DFBz derivatives for this purpose are: (1) they can be prepared in the same way as the PFBz derivatives; and (2) they should track the PFBz derivatives closely under different chromatographic conditions because of their structural similarities. More generic UV retention markers for HPLC, such as acylphenones [26] and alkylbenzenes [27], cannot always provide close tracking.

Of course, a suitable marker for retention time in HPLC must not interfere with the subsequent detection of the analyte by GC. As seen in Table 1, each mono-DFBz derivative elutes 0.3 min later by GC than the corresponding mono-PFBz derivative. For thymine, which forms a di-DFBz derivative, the retention time is 0.7 min later than that of the corresponding di-PFBz derivative. However, one will need to be cautious since the DFBz derivative as an HPLC marker needs to be present in a much higher concentration than the PFBz derivative, risking peak overlap in the gas chromatograph. Nevertheless, in general, one would probably prefer to inject the DFBz derivative separately anyway as a marker to minimize general contamination of the sample, which, along with the difference in subsequent GC retention times, should avoid any problems. This difficulty is absent for an analyte that contains two or more active hydrogens (like thymine), along with detection by mass spectrometry, where the attachment of two or more PFBz moieties means that a different anion will form anyway for the PFBz and DFBz derivatives.

It is interesting that the DFBz derivatives all have retention times by reversed-phase HPLC that are similar to those of the corresponding PFBz derivatives. This reveals the similar hydrophobicity of the PFBz and DFBz groups under the aqueous conditions examined. It is well known that fluorination of an organic compound can increase or decrease the lipophilicity depending on the structural details [28]. Differences in the size, electronegativity, and resonance properties of a fluorine vs. a hydrogen atom can all play a role. For example, a pentafluorophenyl group withdraws electrons inductively more strongly than a phenyl group, but much less than a trifluoromethyl group [29]. Consistent with this, trifluorodiazaethane fails to derivatize carboxylic acids, but such derivatization is successful with pentafluorophenyldiazomethane [24].

Also interesting are the small differences in the retention times by reversed-phase HPLC for some of the DFBz derivatives **1a–1e**. Perhaps subtle differences (magnitude, orientation, location, exposure) of the dipole moment associated with the different DFBz groups in these compounds account for these variations. For example, the two fluorine atoms might act cooperatively with the CH_2O substituent in creating a large dipole moment in **1b**, but not in **1a** where their individual contributions are more opposed. This could make **1b** and **1a** the most and least polar compounds, respectively, in the series **1a–1e**, consistent with their HPLC retention times.

Whatever differences are involved in the polarity details of **1a–1e** under aqueous conditions, these differences play no role in the GC–ECD, where all of these DFBz compounds have the same retention time. In the GC–ECD, all of the DFBz derivatives elute slightly later than the corresponding PFBz derivatives (by 0.3 min for one such substituent, and by 0.7 min when there two, as pointed out above).

The last column in Table 1 gives the relative molar responses by GC–ECD for the derivatives. Compound **1**, 4-(2′,3′,4′,5′,6′-pentafluorobenzyloxy) acetophenone, is a reference compound throughout. Also, for 1-naphthoic acid and thymine, the PFBz derivative is a second reference compound.

The main point in regard to the ECD response data is that the DFBz derivatives provide respectable responses, that is, their responses are only about two- to four-fold lower than that of the corresponding

PFBz derivatives for the same substrate. Thus, a DFBz derivative indeed can be considered as a replacement for a PFBz derivative when the latter encounters an interfering peak, and there is sensitivity to spare. Small differences in the response values in Table 1 should not be overinterpreted, since ECD response depends on several parameters in the instrument [30,31]. This includes the unknown recovery of the compound from the injection port and GC column into the electron capture region. For example, the pattern of ECD responses for a series of related analytes was found in one study to be a function of which GC–ECD was used for the separation [31]. Fig. 1 shows a GC–ECD chromatogram from a sample containing some of the derivatives.

The similar properties of PFBz and DFBz derivatives in terms of HPLC retention time, GC retention time and GC–ECD molar response motivated us to similarly test a monofluorobenzyl and also benzyl derivative, as well as a BTFMBz derivative. Thus we prepared such derivatives of 4-hydroxyacetophenone, arbitrarily selecting to test just one isomer (*meta*) of monofluorobenzyl bromide. This led to compounds **4**–**6** as shown in Table 1. As seen, the properties of these latter compounds continue, basically, to be relatively similar to those of the PFBz derivative, aside from the six-fold lower response of the benzyl relative to the PFBz derivative by GC–ECD. Conjugated carbonyls are known to be electrophoric [32]. Does this part of **5** alone account for its moderately strong response, or is the response promoted as well by the *p*-benzyloxy group? Since the minimal detectable concentration (gas phase, 100°C) of *p*-chloroacetophenone is 100× lower than that of acetophenone [33], we assumed that the latter was true. We confirmed this by observing that the response by GC–ECD of **5** is 2×10^4 higher than that of acetophenone (data not shown in Table 1), but never anticipated that the latter compound would give such a relatively low response.

The relative response of these derivatives as a function of the temperature of the ECD is not studied here; we arbitrarily used 350°C. Such a high temperature tends to favor dissociative relative to associative electron capture, and may have enhanced the response of **5** relative to that of acetophenone. This was not tested.

Which isomer of DFBzBr is the best one? It is difficult to recommend one over the others since

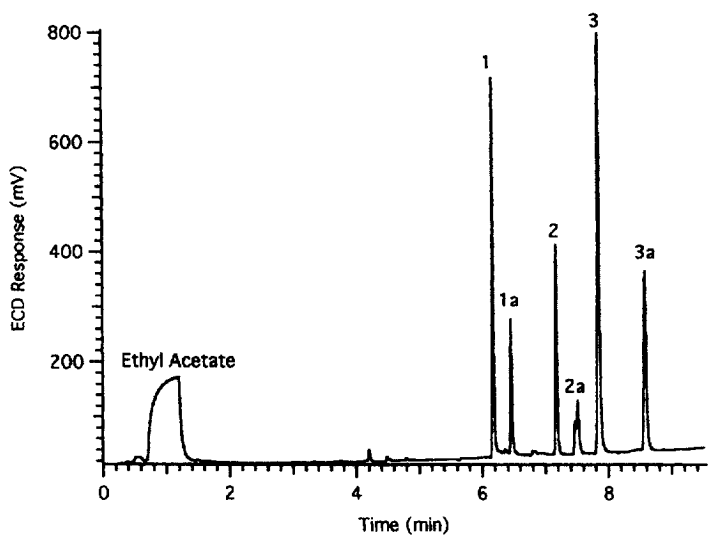


Fig. 1. GC–ECD chromatogram for some of the compounds shown in Table 1. One μl of ethyl acetate containing 0.63 pmol each of the compounds shown was injected. The injector temperature was increased from 60°C to 300°C within the first 2 min and maintained at 300°C. The temperature of the GC oven was kept at 50°C for the first 2 min, was increased to 220°C at 70°C/min, and then was raised to 280°C at 10°C/min where it was held. Detector temperature: 350°C. The unidentified peak that partly overlaps the front of peak **2a** is a system peak in the GC–ECD, apparently coming from the injector.

their performance here is so similar. Perhaps the 2,5-DFBzBr is a good first choice. Although we pointed out that the response of the compounds basically should be regarded as similar, at least the response for the 2,5-DFBz derivative of 4-hydroxyacetophenone was the highest in this study.

4. Conclusions

This work shows that difluoro, monofluoro and even nonfluoro analogs of PFBzBr can be considered as substitute or complementary reagents for this latter compound, both in regard to HPLC (UV retention markers) and GC–ECD (alternative derivatives to overcome an interference). While the PFBz derivative always gave a maximum response by GC–ECD, the responses of the others are lower by only a factor of about two- to seven-fold (about two- to four-fold for difluoro) for the model analytes tested. For each model analyte that was derivatized, the retention time by reversed-phase HPLC for the alternative derivative was similar to that of the PFBz derivative, suggesting that any of them could be employed as a UV marker compound to guide the HPLC collection of a trace, PFBz-derivatized analyte during sample preparation. In most cases the marker, since it would be used at a much higher concentration than the analyte, would be injected separately from the samples to minimize contamination of the analyte by this additive and its impurities. The technique fits in nicely with sample cleanup by satellite HPLC [34], since it provides an additional way to avoid contamination of the HPLC column with analyte, beyond the use of a parent HPLC to define retention times. For the second subject of electrophoric release tags [17], the conclusion of this work is that a difluorobenzyl group indeed can be used in place of a pentafluorobenzyl group in such tags, without significantly compromising either electron capture response or polarity.

Acknowledgments

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