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Effective synthesis of sulfate metabolites of chlorinated phenols

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HIGHLIGHTS

- Chlorophenol sulfates were prepared via trichloroethyl-protected sulfate diesters.
- Ammonium salts of chlorophenol sulfates are stable for several months at −20 °C.
- Crystal structure analysis confirmed molecular structure of selected sulfate esters.

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ABSTRACT

Chlorophenols are an important class of persistent environmental contaminants and have been implicated in a range of adverse health effects, including cancer. They are readily conjugated and excreted as the corresponding glucuronides and sulfates in the urine of humans and other species. Here we report the synthesis and characterization of a series of ten chlorophenol sulfates by sulfation of the corresponding chlorophenols with 2,2,2-trichloroethyl (TCE) chlorosulfate using *N*,*N*-dimethylaminopyridine (DMAP) as base. Deprotection of the chlorophenol diesters with zinc powder/ammonium formate yielded the respective chlorophenol sulfate ammonium salts in good yield. The molecular structure of three TCE-protected chlorophenol sulfate diesters and one chlorophenol sulfate monoester were confirmed by X-ray crystal structure analysis. The chlorophenol sulfates were stable for several months if stored at −20 °C and, thus, are useful for future toxicological, environmental and human biomonitoring studies.

1. Introduction

Chlorophenols are a group of ubiquitous, persistent environmental contaminants and are used in industrial applications ranging from pesticides to synthetic intermediates (Jensen, 1996; ATSDR, 1999; Olaniran and Igbinosa, 2011). For example, pentachlorophenol (PCP) has been used worldwide as pesticide, antiseptic, and wood preservative (Czaplicka, 2004; Simpson and Sefton, 2007). Although PCP's use was restricted in the United States after 1992 (Nistor and Emneus, 2003), it is still manufactured in some countries, e.g. Mexico and China (Trevino-Quintanilla et al., 2011). Chlorophenols are also inadvertently formed during anthropogenic processes, such as the combustion of organic matter, chlorination of drinking water and paper production (Czaplicka, 2004; Olaniran and Igbinosa, 2011). Furthermore, mammals can metabolize dichlorobenzenes (Hawkins et al., 1980; Hissink et al., 1997, 1996; Klos and Dekant, 1994) or other chlorinated compounds, such as lindane (Chadwick et al., 1981), to chlorophenols. Chlorophenols have been classified as group 2B carcinogens "possibly carcinogenic to humans" by the International Agency for Research on Cancer (IARC) and can cause liver and kidney toxicities. Several chlorophenols, including PCP, are listed on the Priority Pollutant List of the United States Environmental Protection Agency (US EPA Website).

There is considerable evidence that sulfates and glucuronides are important biotransformation products of chlorophenols and other chlorinated environmental pollutants in many species, including fish (Layiwola et al., 1983; Oikari and Anas, 1985; Stehly and Hayton, 1989; Cravedi et al., 1999), rats (Chadwick et al., 1981; Hawkins et al., 1980; Hissink et al., 1997, 1996; Klos and Dekant, 1994; Pascal-Lorber et al., 2012) and humans (Renner and Mucke, 1986; Pekari et al., 1991; Ye et al., 2005). Particularly noteworthy is an investigation of saw-mill workers exposed to chlorophenols, which suggests that sulfation is a dominant urinary excretion pathway for low concentrations of chlorophenols (Pekari et al., 1991). In agreement with these earlier studies, Gulcan et al. recently demonstrated that PCP and other chlorophenols are substrates for human hydroxysteroid sulfotransferase hSULT2A1 (Gulcan et al., 2008). There is also evidence that structurally

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related phenols, such as brominated phenols (Ho et al., 2012) and certain hydroxylated polychlorinated biphenyls (Liu et al., 2006), are substrates for sulfotransferases.

Although sulfate monoesters are important metabolites of chlorophenols and other phenolic pollutants, their toxicity and occurrence in environmental and human samples are poorly investigated, partly because pure sulfate monoesters are not available from commercial sources. To overcome this gap in our knowledge, we herein report the synthesis and characterization of a series of ten chlorophenol sulfate monoesters. These well characterized and highly pure compounds are now available for toxicological, environmental and human biomonitoring studies.

2. Materials and methods

2.1. General

All chlorophenol sulfate monoesters were characterized by ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR and mass spectrometry. The NMR spectra were recorded on a Bruker DRX 400 Digital NMR spectrometer. All ^{1}H and ^{13}C chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as internal standard. Gas chromatography–mass spectrometry (GC–MS) was carried out using an Agilent 6890N GC with an Agilent 5975inert detector. Accurate mass determinations of all chlorophenol sulfates were obtained from the High Resolution Mass Spectrometry Facility of the University of California, Riverside (CA, USA). Elemental analyses were performed by Atlantic Microlab (Atlanta, GA, USA). Ultraviolet–visible (UV/Vis) spectra were measured using a Perkin Elmer Lambda 650 UV/Vis spectrometer at 23 °C. Melting points (mp) were determined using a MelTemp apparatus and are uncorrected. The chlorophenols **1a-i** were obtained from Fisher Scientific (Fairlawn, NJ, USA) or Sigma–Aldrich (St. Louis, MO, USA) and used without further purification. 2,2,2-Trichloroethyl chlorosulfate was synthesized according to a published procedure (Hedayatu et al., 1971). Sulfuric acid, 4-bromo-2-chloro-phenyl ester 2,2,2-trichloroethyl ester (**2j**) was obtained as described previously (Li et al., 2010d).

2.2. General procedure for the synthesis of trichloroethyl esters ***2a-i***

A solution of chlorosulfuric acid 2,2,2-trichloroethyl ester (1.0 equiv) in dry tetrahydrofuran (THF) (10 mL) was added drop wise over a period of 15 min to a solution of phenol **1a-i** (500 mg), triethylamine (1.2 equiv) and DMAP (1.0 equiv) in dry THF (20 mL) (Liu et al., 2004; Li et al., 2010d). The solution was stirred for 2 h at room temperature, ethyl acetate (100 mL) was added and the solution washed with H_2O (40 mL), 1.0 N HCl (2 × 40 mL), H_2O (40 mL) and saturated brine (40 mL). The organic layer was dried with Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using hexane–ethyl acetate (10:1) as eluent to yield the trichloroethyl esters **2a-i** in 43–83% yield.

2.2.1. Sulfuric acid 2-chlorophenyl 2,2,2-trichloroethyl ester (2a)

Yield: 42%; white solid; mp: 32–33 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 4.93 (s, 2H, CH_2), 7.27–7.36 (m, 2H), 7.48–7.54 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm 80.8, 92.4, 123.1, 126.8, 128.5, 129.0, 131.4, 146.1. EI–MS *m/z* (relative intensity, %): 338 (9, $C_8H_6Cl_4O_4S^{\cdot+}$), 141 (12), 128 (100), 99 (76), 73 (25), 63 (19). Anal. Calcd. for $C_8H_6Cl_4O_4S$: C 28.26, H 1.78, S 9.43. Found: C 28.19, H 1.65, S 9.18.

2.2.2. Sulfuric acid 3-chlorophenyl 2,2,2-trichloroethyl ester (2b)

Yield: 87%; white solid; mp: 31–32 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 4.82 (s, 2H, CH_2), 7.25–7.27 (m, 1H), 7.32–7.39 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm 80.7, 92.4, 119.6, 122.1, 128.5, 131.1, 135.7, 150.3. EI–MS *m/z* (relative intensity, %): 338 (21, $C_8H_6Cl_4O_4S^{\cdot+}$), 267 (15), 221 (10), 208 (21), 141 (16), 128 (100), 99 (53), 73 (11), 63 (22). Anal. Calcd. for $C_8H_6Cl_4O_4S$: C 28.26, H 1.78, S 9.43. Found: C 28.00, H 1.72, S 9.29.

2.2.3. Sulfuric acid 4-chlorophenyl 2,2,2-trichloroethyl ester (2c) (Liu et al., 2004)

Yield: 67%; white solid; mp: 28–29 °C (lit. mp: 27–28 °C (Liu et al., 2004)); ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 4.81 (s, 2H, CH_2), 7.28 (dd, 2H, AA′XX′ system), 7.39 (dd, 2H, AA′XX′ system). ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm 80.7, 92.4, 122.8, 130.5, 133.9, 148.6. EI–MS *m/z* (relative intensity, %): 338 (6, $C_8H_6Cl_4O_4S^{\cdot+}$), 208 (13), 128 (100), 99 (57), 73 (18), 63 (16). Anal. Calcd. for $C_8H_6Cl_4O_4S$: C 28.26, H 1.78, S 9.43. Found: C 27.96, H 1.66, S 9.14.

2.2.4. Sulfuric acid 2,4-dichlorophenyl 2,2,2-trichloroethyl ester (2d)

Yield: 73%; white solid; mp: 55–57 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 4.92 (s, 2H, CH_2), 7.29 (dd, 1H, J = 2. Hz, J = 8.8 Hz), 7.47 (d, 1H, J = 2.4 Hz), 7.59 (d, 1H, J = 8.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm 81.0, 92.3, 124.0, 127.9, 128.7, 131.1, 132.0, 144.7. EI–MS *m/z* (relative intensity, %): 372 (10, $C_8H_5Cl_5O_4S^{\cdot+}$), 162 (100), 133 (67), 63 (22). Anal. Calcd. for $C_8H_5Cl_5O_4S$: C 25.66, H 1.35, S 8.56. Found: C 25.93, H 1.32, S 8.38.

2.2.5. Sulfuric acid 2,5-dichlorophenyl 2,2,2-trichloroethyl ester (2e)

Yield: 79%; white solid; mp: 36–38 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 4.93 (s, 2H, CH_2), 7.27 (dd, 1H, J = 2.0 Hz, J = 8.8 Hz), 7.42 (d, 1H, J = 8.8 Hz), 7.55 (d, 1H, J = 2.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm 81.0, 92.3, 123.7, 125.5, 129.3, 131.8, 133.8, 146.1. EI–MS *m/z* (relative intensity, %): 372 (10, $C_8H_5Cl_5O_4S^{\cdot+}$), 162 (100), 133 (67), 95 (12), 73 (14), 63 (22). Anal. Calcd. for $C_8H_5Cl_5O_4S$: C 25.66, H 1.35, S 8.56. Found: C 25.81, H 1.23, S 8.57.

2.2.6. Sulfuric acid 2,6-dichlorophenyl 2,2,2-trichloroethyl ester (2f)

Yield: 83%; colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 5.03 (s, 2H, CH_2), 7.26 (pseudo t, 1H, $J \sim$ 8.4 Hz), 7.40 (d, 2H, J = 8.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm 81.0, 92.5, 129.1, 129.8, 143.7. EI–MS *m/z* (relative intensity, %): 372 (12, $C_8H_5Cl_5O_4S^{\cdot+}$), 162 (100), 133 (96), 97 (14). 73 (19), 63 (28). Anal. Calcd. for $C_8H_5Cl_5O_4S$: C 25.66, H 1.35, S 8.56. Found: C 25.66, H 1.23, S 8.31.

2.2.7. Sulfuric acid 2,4,6-trichlorophenyl 2,2,2-trichloroethyl ester (2g)

Yield: 56%; white solid; mp: 81–82 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 5.01 (s, 2H, CH_2), 7.42 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm 81.2, 92.4, 129.7, 130.5, 134.3, 142.6. EI–MS *m/z* (relative intensity, %): 406 (12, $C_8H_4Cl_6O_4S^{\cdot+}$), 196 (100), 167 (54), 133 (22), 97 (53), 62 (19). Anal. Calcd. for $C_8H_4Cl_6O_4S$: C 23.50, H 0.99, S 7.84. Found: C 23.70, H 0.84, S 7.90.

2.2.8. Sulfuric acid 2,4,5-trichlorophenyl 2,2,2-trichloroethyl ester (2h)

Yield: 72%; colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 4.93 (s, 2H, CH_2), 7.61 (s, 1H), 7.65 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm 81.1, 92.2, 124.8, 126.1, 132.0, 132.4, 133.0, 144.4. EI–MS *m/z* (relative intensity, %): 406 (10, $C_8H_4Cl_6O_4S^{\cdot+}$), 198 (100), 167 (58), 131 (27), 97 (35), 61 (18). Anal. Calcd. for $C_8H_4Cl_6O_4S$: C 23.50, H 0.99, S 7.84. Found: C 23.26, H 0.83, S 7.89.

2.2.9. Sulfuric acid 2,3,4,5,6-pentachlorophenyl 2,2,2-trichloroethyl ester (***2i***)

Yield: 43%; white solid; mp: 138–139 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 5.03 (s, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$):

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δ/ppm 81.3, 92.2, 128.6, 133.0, 133.9, 143.5. EI–MS *m*/*z* (relative intensity, %): 475 (19, $C_8H_2Cl_8O_4S^+$), 266 (75), 237 (100), 165 (37), 141 (22), 132 (35), 117 (20), 97 (20). Anal. Calcd. for $C_8H_2Cl_8O_4S$: C 20.11, H 0.42, S 6.71. Found: C 20.17, H 0.36, S 6.67.

2.3. General procedure for the synthesis of chlorophenol sulfates **3a-j**

Ammonium formate (0.77 g, 12 mmol) was added to a solution of the TCE-protected chlorophenol sulfate diesters **2a-j** (2 mmol) in methanol (5 mL) (Liu et al., 2004; Li et al., 2010d). Zinc dust (0.26 g, 4 mmol) was added after the ammonium formate had dissolved completely and the reaction mixture was stirred until the TCE ester **2a-j** was completely consumed, as determined by thin layer chromatography. The solution was filtered through Celite and concentrated under reduced pressure at temperatures below 35 °C to minimize the decomposition of **3a-j**. The product was purified by column chromatography on silica gel using a mixture of dichloromethane, methanol and ammonium hydroxide (15:3:0.5, *v*/*v*) as eluent. The solvent was removed under reduced pressure at temperatures below 35 °C to yield **3a-j**. The R_f values of all chlorophenol sulfate monoesters **3a-j** were approximately $R_f = 0.3$ (CH_2Cl_2:CH_3OH:NH_4OH = 15:3:0.3, *v*/*v*/*v*).

2.3.1. 2-Chlorophenylsulfate, ammonium salt (3a)

Yield: 78%; white solid; mp: 122 °C (dec.); ^{1}H NMR (400 MHz, CD_3OD): δ/ppm 7.11–7.15 (m, 1H), 7.25–7.29 (m, 1H), 7.40–7.42 (m, 1H), 7.60–7.62 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ/ppm 124.0, 126.8, 127.8, 128.7, 131.3, 150.1. UV/Vis: $\lambda_{\mathbf{3a},max}$(MeOH) = 270 nm, $\varepsilon_{\mathbf{3a}} = 0.94 \times 10^4$ L mol^{-1} cm^{-1} ($\lambda_{\mathbf{2a},max}$(MeOH) = 280 nm, $\varepsilon_{\mathbf{2e}} = 0.21 \times 10^4$ L mol^{-1} cm^{-1}). HRMS (ESI, negative): $[M-NH_4]^-$ found *m*/*z* 206.9528, calculated for $C_6H_4ClO_4S$ 206.9524.

2.3.2. 3-Chlorophenylsulfate, ammonium salt (3b)

Yield: 73 %; white solid; mp: 178 °C (dec.); ^{1}H NMR (400 MHz, CD_3OD): δ/ppm 7.15–7.23 (m, 2H). 7.29–7.36 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ/ppm 121.0, 122.8, 125.9, 131.3, 135.4, 155.0. UV/Vis: $\lambda_{\mathbf{3b},max}$(MeOH) = 270 nm, $\varepsilon_{\mathbf{3b}} = 1.32 \times 10^4$ L mol^{-1} cm^{-1} ($\lambda_{\mathbf{2b},max}$(MeOH) = 280 nm, $\varepsilon_{\mathbf{2b}} = 0.20 \times 10^4$ L mol^{-1} cm^{-1}). HRMS (ESI, negative): $[M-NH_4]^-$ found *m*/*z* 206.9529, calculated for $C_6H_4ClO_4S$ 206.9524.

2.3.3. 4-Chlorophenylsulfate, ammonium salt (3c) (Liu et al., 2004)

Yield: 94%; white solid; mp: 95 °C (dec.); ^{1}H NMR (400 MHz, CD_3OD): δ/ppm 7.26–7.34 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD): δ/ppm 124.2, 130.3, 131.2, 152.7. UV/Vis: $\lambda_{\mathbf{3c},max}$(MeOH) = 270 nm, $\varepsilon_{\mathbf{3c}} = 1.16 \times 10^4$ L mol^{-1} cm^{-1} ($\lambda_{\mathbf{2c},max}$(MeOH) = 280 nm, $\varepsilon_{\mathbf{2c}} = 0.17 \times 10^4$ L mol^{-1} cm^{-1}). HRMS (ESI, negative): $[M-NH_4]^-$ found *m*/*z* 206.9531, calculated for $C_6H_4ClO_4S$ 206.9524.

2.3.4. 2,4-Dichlorophenylsulfate, ammonium salt (3d)

Yield: 97%; white solid; mp: 175 °C (dec.); ^{1}H NMR (400 MHz, CD_3OD): δ/ppm 7.29 (dd, 1H, *J* = 8.8 Hz, *J* = 2.5 Hz), 7.47 (d, 1H, *J* = 2.5 Hz), 7.58 (d, 1H, *J* = 8.8 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ/ppm 125.0, 128.79, 128.84, 130.9, 131.2, 149.2. UV/Vis: $\lambda_{\mathbf{3d},max}$(MeOH) = 270 nm, $\varepsilon_{\mathbf{3d}} = 1.01 \times 10^4$ L mol^{-1} cm^{-1} ($\lambda_{\mathbf{2d},max}$(MeOH) = 290 nm, $\varepsilon_{\mathbf{2d}} = 0.24 \times 10^4$ L mol^{-1} cm^{-1}). HRMS (ESI, negative): $[M-NH_4]^-$ found *m*/*z* 240.9128, calculated for $C_6H_3Cl_2O_4S$ 240.9135.

2.3.5. 2,5-Dichlorophenylsulfate, ammonium salt (3e)

Yield: 69%; white solid; mp: 110 °C (dec.); ^{1}H NMR (400 MHz, CD_3OD): δ/ppm 7.13 (dd, 1H, *J* = 8.6 Hz, *J* = 2.5 Hz), 7.38 (d, 1H, *J* = 8.6 Hz), 7.66 (d, *J* = 2.5 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ/ppm 123.8, 126.3, 126.4, 132.1, 133.7, 150.8. UV/Vis: $\lambda_{\mathbf{3e},max}$(MeOH) = 270 nm, $\varepsilon_{\mathbf{3e}} = 0.99 \times 10^4$ L mol^{-1} cm^{-1} ($\lambda_{\mathbf{2e},max}$(MeOH) = 280 nm, $\varepsilon_{\mathbf{2e}} = 0.39 \times 10^4$ L mol^{-1} cm^{-1}). HRMS (ESI, negative): $[M-NH_4]^-$ found *m*/*z* 240.9135, calculated for $C_6H_3Cl_2O_4S$ 240.9135.

2.3.6. 2,6-Dichlorophenylsulfate, ammonium salt (3f)

Yield: 81%; white solid; mp: 160 °C (dec.); ^{1}H NMR (400 MHz, CD_3OD): δ/ppm 6.80 (pseudo t, 1H, *J* ~ 8.0 Hz), 7.24–7.27 (m, 2H).

Table 1
X-ray crystallographic data for TCE-protected chlorophenol sulfate diesters **2g**, **2i** and **2j** and chlorophenol sulfate monoesters **3f**.

Property	**2g**	**2i**	**2j**	**3f**
Formula	$C_8H_4Cl_6O_4S$	$C_8H_2Cl_8O_4S$	$C_8H_5BrCl_4O_4S$	$C_6H_7Cl_2NO_4S$
M	408.87	477.76	418.89	260.09
T/K	90.0(2)	90.0(2)	90.0(2)	90.0(2)
Wavelength (Å)	0.71073	0.71073	0.71073	0.71073
Space group	Monoclinic, $P2_1/c$	Monoclinic, $P2_1$	Orthorhombic, *P b c a*	Monoclinic, $P2_1/c$
a (Å)	8.2269 (4)	6.3617 (1)	12.7893 (2)	10.8336 (3)
b (Å)	11.1533 (6)	11.2063 (2)	9.3856 (3)	7.2384 (2)
c (Å)	15.7373 (9)	10.8794 (3)	22.5651 (5)	13.5811 (4)
α (°)	90.000	90.000	90.000	90.000
β (°)	99.777 (2)	94.9092 (10)	90.000	106.7814 (11)
γ (°)	90.000	90.000	90.000	90.000
V (Å^3)	1423.04 (13)	772.76 (3)	2708.61 (11)	1019.65 (5)
Z	4	2	8	4
Calculated density (mg m^{-3})	1.908	2.053	2.054	1.694
Absorption coefficient (mm^{-1})	1.356	1.599	3.978	0.828
F(000)	808	468	1632	528
Crystal size (mm)	0.40 × 0.22 × 0.02	0.11 × 0.06 × 0.03	0.25 × 0.15 × 0.08	0.25 × 0.20 × 0.13
θ range (°)	2.25–27.44	1.88–27.47	1.80–27.49	1.96–27.49
Limiting indices	$-10 \leqslant h \leqslant 10$ $-14 \leqslant k \leqslant 14$ $-20 \leqslant l \leqslant 20$	$-8 \leqslant h \leqslant 8$ $-14 \leqslant k \leqslant 14$ $-14 \leqslant l \leqslant 14$	$-16 \leqslant h \leqslant 16$ $-12 \leqslant k \leqslant 12$ $-29 \leqslant l \leqslant 29$	$-13 \leqslant h \leqslant 14$ $-9 \leqslant k \leqslant 9$ $-17 \leqslant l \leqslant 17$
Reflections collected/unique	18319/3240	18363/3539	29859/3111	6648/2336
R (int)	0.0830	0.0620	0.0700	0.0450
Completeness to θ = 27.44	99.9%	99.9%	99.9%	99.9%
Max. and min. transmission	0.973 and 0.613	0.954 and 0.844	0.741 and 0.436	0.900 and 0.820
Date/restraints/parameters	3240/0/172	3539/1/190	3111/0/163	2336/0/139
Goodness-of-fit on F^2	1.050	1.079	1.071	1.119
Final *R* indices *I*>2σ(*I*)	$R_1 = 0.0518$; $wR_2 = 0.1196$	$R_1 = 0.0420$; $wR_2 = 0.0915$	$R_1 = 0.0329$; $wR_2 = 0.0677$	$R_1 = 0.0350$; $wR_2 = 0.0836$
R indices (all data)	$R_1 = 0.0903$; $wR_2 = 0.1366$	$R_1 = 0.0581$; $wR_2 = 0.0996$	$R_1 = 0.0559$; $wR_2 = 0.0741$	$R_1 = 0.0449$; $wR_2 = 0.0892$
Largest diff. peak and hol (eÅ^{-3})	0.911 and −0.726	0.469 and −0.410	0.668 and −0.509	0.360 and −0.525

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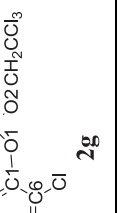
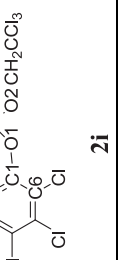
Table 2
Selected bond lengths and bond angles of TCE-protected chlorophenol sulfate diesters **2g**, **2i** and **2j** and chlorophenol sulfate monoester **3f**.

Property	 **2g**	 **2i**	 **2j**	 **3f**
Bond length (Å)				
C1–O1	1.406	1.388	1.420	1.387
S1–O1	1.604	1.606	1.588	1.639
S1–O2	1.564	1.564	1.569	1.453
S1–O3	1.418	1.415	1.419	1.441
S1–O4	1.420	1.414	1.411	1.441
Bond angles (°)				
O1–S1–O2	101.76	101.92	102.62	101.68
O1–S1–O3	110.02	110.03	110.64	106.01
O1–S1–O4	104.44	104.60	104.82	105.47
O2–S1–O3	105.75	105.50	104.50	115.16
O2–S1–O4	109.96	110.06	110.35	112.27
O3–S1–O4	122.98	122.85	122.30	114.63
Deviation of O1 from Ar plane (Å)	0.108(5)	0.023(6)	0.058(5)	0.065(3)

^{13}C NMR (100 MHz, CD_3OD): δ/ppm 126.0, 128.7, 130.3, 145.8. UV/Vis: $\lambda_{\mathbf{3f},max}$(MeOH) = 270 nm, $\varepsilon_{\mathbf{3f}} = 1.38 \times 10^4$ L mol^{-1} cm^{-1} ($\lambda_{\mathbf{2f},max}$(MeOH) = 280 nm, $\varepsilon_{\mathbf{2f}} = 0.15 \times 10^4$ L mol^{-1} cm^{-1}). HRMS (ESI, negative): $[M-NH_4]^-$ found *m*/*z* 240.9138, calculated for $C_6H_3Cl_2O_4S$ 240.9135.

2.3.7. 2,4,6-Trichlorophenylsulfate, ammonium salt (3g)

Yield: 67%; white solid; mp: 170 °C (dec); ^{1}H NMR (400 MHz, CD_3OD): δ/ppm 7.46 (s, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ/ppm 129.9, 131.7, 132.6, 146.6. UV/Vis: $\lambda_{\mathbf{3g},max}$(MeOH) = 270 nm, $\varepsilon_{\mathbf{3g}} = 1.06 \times 10^4$ L mol^{-1} cm^{-1} ($\lambda_{\mathbf{2g},max}$(MeOH) = 290 nm, $\varepsilon_{\mathbf{2g}} = 0.26 \times 10^4$ L mol^{-1} cm^{-1}). HRMS (ESI, negative): $[M-NH_4]^-$ found *m*/*z* 274.8742, calculated for $C_6H_2Cl_3O_4S$ 240.8745.

2.3.8. 2,4,5-Trichlorophenylsulfate, ammonium salt (3h)

Yield: 88%; white solid; mp: 155 °C (dec); ^{1}H NMR (400 MHz, CD_3OD): δ/ppm 7.63 (s, 1H), 7.79 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ/ppm 125.0, 127.2, 129.2, 131.95, 131.99, 149.6. UV/Vis: $\lambda_{\mathbf{3h},max}$(MeOH) = 270 nm, $\varepsilon_{\mathbf{3h}} = 1.47 \times 10^4$ L mol^{-1} cm^{-1} ($\lambda_{\mathbf{2h},max}$(MeOH) = 290 nm, $\varepsilon_{\mathbf{2h}} = 0.11 \times 10^4$ L mol^{-1} cm^{-1}). HRMS (ESI, negative): $[M-NH_4]^-$ found *m*/*z* 274.8747, calculated for $C_6H_2Cl_3O_4S$ 274.8745.

2.3.9. 2,3,4,5,6-Pentachlorophenylsulfate, ammonium salt (3i)

Yield: 60%; white solid; mp: 220 °C (dec); ^{13}C NMR (100 MHz, CD_3OD): δ/ppm 130.9, 131.3, 132.8, 148.4. UV/Vis: $\lambda_{\mathbf{3i},max}$(MeOH) = 310 nm, $\varepsilon_{\mathbf{3i}} = 0.32 \times 10^4$ L mol^{-1} cm^{-1}($\lambda_{\mathbf{2i},max}$(MeOH) = 270 nm, $\varepsilon_{\mathbf{2i}} = 1.29 \times 10^4$ L mol^{-1} cm^{-1}). HRMS (ESI, negative): $[M-NH_4]^-$ found *m*/*z* 342.7972, calculated for $C_6Cl_5O_4S$ 342.7965.

2.3.10. 4-Bromo-2-chloro-phenolsulfate, ammonium salt (3j)

Yield: 94%; white solid; mp: 200 °C (dec); ^{1}H NMR (400 MHz, CD_3OD): δ/ppm 7.41 (dd, 1H, *J* = 8.8 Hz, *J* = 2.4 Hz), 7.52 (d, 1H, *J* = 8.8 Hz), 7.59 (d, *J* = 2.4 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ/ppm 118.2, 125.3, 129.1, 131.8, 133.7, 149.8. UV/Vis: $\lambda_{\mathbf{3j},max}$(MeOH) = 270 nm, $\varepsilon_{\mathbf{3j}} = 1.30 \times 10^4$ L mol^{-1} cm^{-1} ($\lambda_{\mathbf{2j},max}$(MeOH) = 270 nm, $\varepsilon_{\mathbf{2j}} = 1.22 \times 10^4$ L mol^{-1} cm^{-1}). HRMS (ESI, negative): $[M-NH_4]^-$ found *m*/*z* 284.8629, calculated for $C_6H_3BrClO_4S$ 284.8629.

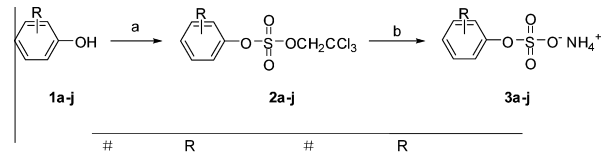
2.4. Single crystal structure determinations

Crystals suitable for crystal structure analysis were obtained by slow crystallization of TCE sulfate esters **2g**, **2i** and **2j** as well as sulfate monoester **3f** from methanol. X-ray diffraction data were collected at 90.0(2) K on a Nonius KappaCCD diffractometer as described previously (Li et al., 2010d). The crystal data and the related parameters are summarized in Tables 1 and 2. Additional crystallographic data have been deposited with the Cambridge Crystallographic Data Center (CCDC) as Supplementary Publications CCDC 946880-946883. Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

3. Results and discussion

3.1. Synthesis

Numerous sulfation reagents and conditions have been utilized for the synthesis of sulfates, including SO_3-pyridine (Lee et al., 2004), SO_3-trimethylamine (Tully et al., 2004), SO_3-triethylamine$_3$ (Dusza et al., 1985), SO_3-DMF (Young and Kiessling, 2002), sulfuric acid/*N*,*N*′-dicyclohexylcarbodiimide (Mumma, 1966) and chlorosulfuric acid (Ho et al., 2012). Recent efforts



#	R	#	R
2a, 3a	2-Cl	**2f, 3f**	2,6-diCl
2b, 3b	3-Cl	**2g, 3g**	2,4,6-triCl
2c, 3c	4-Cl	**2h, 3h**	2,4,5-triCl
2d, 3d	2,4-diCl	**2i, 3i**	2,3,4,5,6-pentaCl
2e, 3e	2,5-diCl	**2j, 3j**	2-Cl-4-Br

Scheme 1. Synthesis of the ammonium salts of chlorophenol sulfate monoesters **3** via the corresponding TCE-protected chlorophenol sulfate diesters **2**. (a) 2,2,2-Trichloroethyl chlorosulfate, DMAP, dry CH_2Cl_2, 2 h; (b) Zn powder, HCO_2NH_4, MeOH, 1 h.

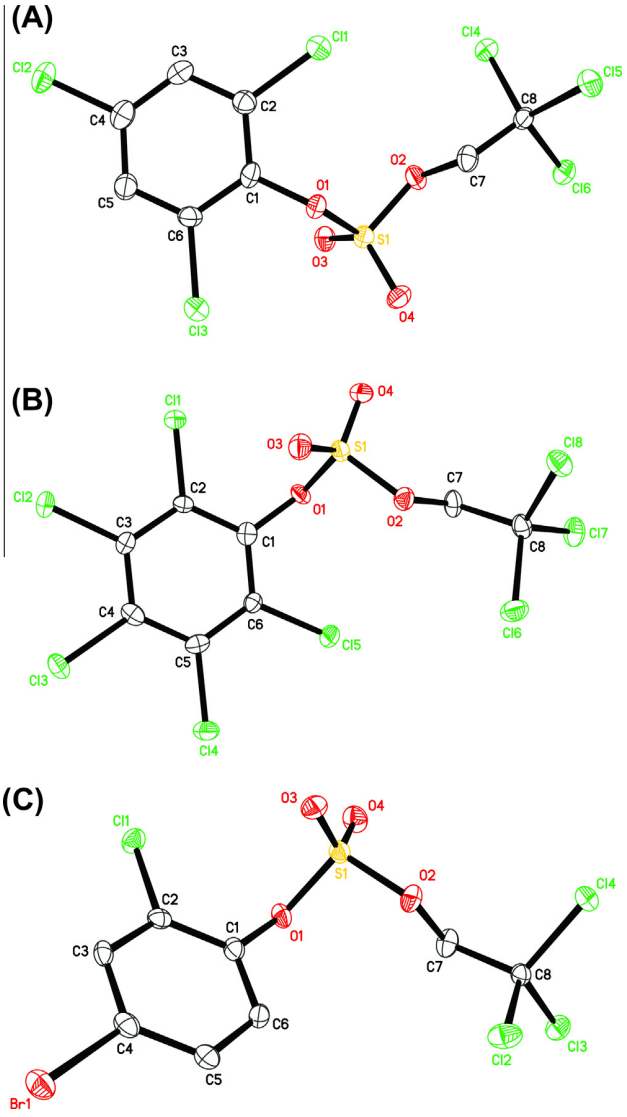


Fig. 1. Molecular structure of (A) sulfuric acid, 2,4,6-trichlorophenyl ester 2,2,2-trichloroethyl ester (**2g**), (B) sulfuric acid, 2,3,4,5,6-pentachlorophenyl ester 2,2,2-trichloroethyl ester (**2i**), and (C) sulfuric acid, 4-bromo-2-chloro-phenyl ester 2,2,2-trichloroethyl ester (**2j**) showing the atom numbering scheme. Displacement ellipsoids are drawn at the 50% probability level.

have focused on the introduction of the sulfate group via protected sulfate diesters (Al-Horani and Desai, 2010). In particular the TCE group has emerged as an effective protecting group in

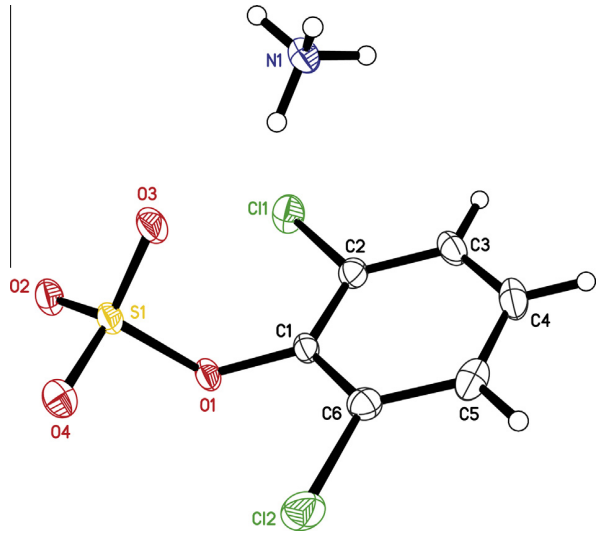


Fig. 2. Molecular structure of 2,6-dichlorophenylsulfate ammonium salt (**3f**) showing the atom numbering scheme. Displacement ellipsoids of **3f** are drawn at the 50% probability level.

the synthesis of aryl sulfates because it can be easily removed in the final step of the synthesis (Liu et al., 2004; Li et al., 2010d). In the present study we utilized this approach for the synthesis of chlorophenol sulfate monoesters **3a-j**. In short, TCE-protected chlorophenol sulfate diesters **2a-j** were obtained in 42–87% yield by adding a solution of TCE chlorosulfate in dry THF to a solution of the respective chlorophenol, triethylamine and DMAP in dry THF at room temperature, followed by stirring for 2 h (Scheme 1). The TCE-protected chlorophenol sulfate diesters **2a-j** were treated with zinc powder/ammonium formate in methanol to yield the ammonium salts of the corresponding chlorophenol sulfate monoesters in good yields (75–94%). The chlorophenol sulfate monoesters **3a-j** were stable at −20 °C for several months without any detectable decomposition, but degraded within days to the corresponding phenol **1** in methanolic solution. It is noteworthy that we were able to isolate chlorophenol sulfate monoesters **3f**, **3g** and **3i** with two chlorine substituents *ortho* to the sulfate group. In contrast, polychlorinated biphenyl (PCB) sulfate monoesters with a similar substitution pattern appeared to be unstable and readily degraded to the corresponding hydroxylated PCB (Li et al., 2010d).

3.2. Solid state molecular structure of TCE-protected chlorophenol sulfate diesters *2g, 2i, 2j* and chlorophenol sulfate monoester *3f*

The X-ray crystal structure of several TCE-protected chlorophenol sulfate diesters (**2g**, **2i** and **2j**) and chlorophenol sulfate ammonium salt (**3f**) (Figs. 1 and 2; Tables 1 and 2) were determined to confirm their molecular structure. These solid state structures will aid future quantitative structure activity relationship studies of the interaction of chlorophenol sulfates with cellular target molecules, such as sulfotransferases (Stehly and Hayton, 1988; Czaplicka, 2004; Gulcan et al., 2008) or plasma binding proteins (Grimm et al., 2013). The C1–O1 and O1–S1 bond lengths of the sulfate ester group are of particular interest in this context because they are thought to correlate with the stability of the sulfate monoesters (Brandao et al., 2005). The C1–O1 bond of the TCE-protected chlorophenol sulfate diesters **2i**, **2g** and **2j** lies essentially in the plane of the phenyl ring system (Table 2). Its lengths followed the order **2i** < **2g** < **2j**, which is due to the decreasing number of

electronegative substituents in the aromatic ring system. The O1–S1 bonds of the *ortho*-disubstituted chlorophenol sulfate diesters **2g** and **2i** were slightly longer compared to the *ortho* monosubstituted O1–S1 bond of diester **2j**. These findings suggest that an increase in the number of electronegative substituents slightly weakens the O1–S1 ester bond (Brandao et al., 2005). However, further studies are needed to determine if the O1–S1 ester bond lengths of TCE-protected sulfate diesters indeed predicts environmentally and physiologically relevant differences in the stability of chlorophenol sulfates. The O–S–O bond angles of the TCE-protected chlorophenol sulfate diesters **2i**, **2g** and **2j** are comparable. Overall, similar trends in bond length and bond angles have been observed with structurally related TCE-protected sulfate diesters of polychlorinated biphenyls (Lehmler et al., 2013; Li et al., 2010a,b,c, 2008).

The C1–O1 bond length of the chlorophenyl sulfate monoester **3f** (1.387 Å) was shorter than that of TCE-protected PCB and chlorophenol sulfate diesters (1.393–1.449 Å) (Lehmler et al., 2013; Li et al., 2010a,b,c; Li et al., 2008), which is ultimately a result of the negative charge of the sulfate group. C–O bond lengths for other aromatic sulfate monosters were comparable and ranged from 1.374 to 1.409 Å (Brandao et al., 2005). The length of the S1–O1 ester bond of monoester **3f** (1.639 Å) was much longer compared to diesters **2g**, **2i** and **2j** (Table 2) due to the increased electron density of atom S1 in the negatively charged sulfate group. Comparable S–O bond lengths have been reported for structurally related aromatic sulfate monoesters, with S–O bond lengths ranging from 1.611 to 1.653 Å (Brandao et al., 2005). The other three S–O bond lengths of **3f** were comparable (1.441–1.453 Å). The corresponding S–O bond lengths in other aromatic sulfate monoesters ranged from 1.422 to 1.451 Å (Brandao et al., 2005). In contrast, the S1–O3 and S1–O4 bond lengths of diesters **2g**, **2i** and **2j** were shorter due to their double bond character.

4. Conclusions

Chlorophenols are an important group of persistent environmental pollutants. Although it is well established that chlorophenols are readily sulfated and subsequently excreted as sulfates with the urine in wildlife, laboratory animals, and humans, limited information is available about their toxicity and occurrence in the environment and humans. In the present study, a series of ten chlorophenol sulfates **3** was synthesized by sulfation of the corresponding chlorophenol **1** with 2,2,2-trichloroethyl chlorosulfate, followed by deprotection of the TCE-protected chlorophenol sulfate diester **2** with zinc powder/ammonium formate. The neat chlorophenol sulfates **3** can be stored at −20 °C for several months without detectable degradation and can be used for toxicological, environmental and human biomonitoring studies. A similar synthetic strategy can also be employed for the synthesis of sulfate monoesters of other phenolic environmental pollutants.

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Appendix A. Supplementary material

Supplementary data with NMR and mass spectra of compounds **2a-i** and **3a-j** can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2013.06.087.

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