

RESEARCH ARTICLE

# Synthesis and antioxidant properties of diphenylmethane derivative bromophenols including a natural product

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## Abstract

Bromination of bis(3,4-dimethoxyphenyl)methanone (**5**) gave four products (**6–9**) with mono, di, tri, and tetra Br under different conditions. Reduction and demethylation reactions of product **9** with tetra Br were performed, consecutively and a natural product, 5,5'-methylene bis(3,4-dibromobenzene-1,2-diol) (**1**), was obtained with a 53% yield. Five derivatives, (**13–17**) (bromophenols), of **1** were also synthesised. The antioxidant and radical scavenging activities of bromophenols **1** and **13–17** were determined by employing various *in vitro* assays such as 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH<sup>•</sup>), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS<sup>•+</sup>), N,N-dimethyl-p-phenylenediamine dihydrochloride radical cation (DMPD<sup>•+</sup>), and superoxide anion radical (O<sub>2</sub><sup>•-</sup>) scavenging, reducing ability determination by the Fe<sup>3+</sup>-Fe<sup>2+</sup> and Cu<sup>2+</sup>-Cu<sup>+</sup> cupric reducing antioxidant capacity (CUPRAC) transformation methods, hydrogen peroxide scavenging, and ferrous ion (Fe<sup>2+</sup>) chelating activities. Moreover, these activities were compared to those of synthetic standard antioxidant compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT),  $\alpha$ -tocopherol, and trolox. The results showed that the synthesised bromophenols had effective antioxidant power.

**Keywords:** Antioxidant activity; bromination; bromophenols; natural product; radical scavenging

## Introduction

Naturally occurring organohalogen compounds are abundantly found in marine life [1]. Bromophenols, frequently isolated from red algae of the family Rhodomelaceae, have some important biological activities [1, 2].

2,2',3,3'-Tetrabromo-4,4',5,5'-tetrahydroxydiphenylmethane (**1**) and 3-bromo-4-(2,3-dibromo-4,5-dihydroxybenzyl)-5-methoxymethylpyrocatechol (**2**) were first isolated in 1977 [3]. Since then they have been obtained from different sources many times [4–10]. Compounds **1** and **2** exhibit important biological activities such as enzyme inhibition, cytotoxicity, feeding deterrent, and anti-microbial activities [4–9]. It was reported that bromophenol **1** extracted from red algae is an inhibitor of protein tyrosine phosphatase, and it can be used to treat and prevent diabetes mellitus and obesity [11–13] (Figure 1).

Other diphenylmethane derivatives, 2,2',3,5,6-Pentabromo-3',4,4',5'-tetrahydroxydiphenylmethane (**3**) and bis(2,3,6-tribromo-4,5-dihydroxyphenyl)methane (**4**), exhibit significant aldose reductase inhibitory activity [14] (Figure 1). The bromophenols, **4** and 2-(2,3,6-tribromo-4,5-dihydroxybenzyl)cyclohexanone, exhibit antioxidant activity

[15,16]. To our knowledge, the first synthesis of the bromophenol **1** was carried out in low yield (0.3%) by Lundgren et al. [17]. Two synthesis of bromophenol **1** have been published recently [18,19].

Recently, interest in finding antioxidants for foods, cosmetics or medicines has increased considerably. Among the various antioxidants, phenolic compounds are reported to act by quenching oxygen-derived free radicals through donating a hydrogen atom or an electron to the free radical. Many phenolic compounds have been reported to possess potent antioxidant activity and also to have anticarcinogenic, antimutagenic, antibacterial, antiviral, or anti-inflammatory activities. Their physiological and pharmacological functions may originate from their antioxidant properties. The antioxidant activities are related to the structures of phenolic compounds [20,21]. Compound **2** [22] has also been studied. It is thought that the bromophenol **1** will have important biological activities and some of its new derivatives will show useful antioxidant activities so methods of synthesis of bromophenol **1** will be very important. Therefore, the synthesis and antioxidant activities of the bromophenol **1** and some of its derivatives were investigated in the present study.

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## Materials and methods

All chemicals and solvents are commercially available and were used after distillation or treatment with drying agents. Column chromatography (CC): silica gel ( $\text{SiO}_2$ ; 60 mesh, Merck-Darmstadt, Germany). Preparative thick-layer chromatography (PLC): 1 mm of  $\text{SiO}_2$  60 PF (Merck) on glass plates. Mp: cap. melting-point apparatus (BUCHI 530 - Flawil, Switzerland); uncorrected. IR Spectra: solns. in 0.1 mm cells with a Mattson 1000 FT-IR spectrophotometer (Cambridge, England).  $^1\text{H}$ - and  $^{13}\text{C}$ - NMR spectra: 200 (50) and 400 (100)-MHz Varian spectrometer (Danbury, USA);  $\delta$  in ppm;  $\text{Me}_4\text{Si}$  as the internal standard. Elemental analyses: Leco CHNS-932 apparatus (Michigan, USA). Antioxidant activities of samples was determined in a spectrophotometer (Shimadzu, UV-1208, Japan).

### Synthesis

Bis(3,4-dimethoxyphenyl)methanone (**5**) was obtained from the reaction of 3,4-dimethoxybenzoic acid and veratrole in polyphosphoric acid (PPA) [23]. Reactions of compound **5** under different conditions were investigated for the synthesis of the bromophenol **1** and its new derivatives. Aromatic compounds give selective bromination with ceric ammonium nitrate (CAN)/LiBr [24]. By applying the same procedure, bromination of **5** with 1.1 and 2.2 equivalents of LiBr/CAN gave mono- and dibromides respectively. An AB-system in the aromatic region was present in the  $^1\text{H}$ -NMR spectrum of monobromide while it was absent in that

of dibromide. Two protons (H-5 and H-6) of one aromatic ring in monobrominated **6** should give AB-system because of their being vicinal protons. Mono- and dibromides exhibit seventeen and nine lines respectively, in the  $^{13}\text{C}$ -NMR spectra. According to their NMR spectra, the monobromide is unsymmetric while the dibromide is symmetric. Isomeric structures are possible for mono- and dibromides. Their melting points are consistent with that of known bromides [25,26]. Therefore, their structures should be monobromide **6** and dibromide **7** (Scheme 1, Table I).

For the synthesis of the tribromide **8** and tetrabromide **9**, the reactions of **5** with 3.3 and excess equivalents of LiBr/CAN at room temperature were performed. In these reactions, only the dibromide **7** was obtained. A complex mixture of products was found to be obtained from the reaction of **5** with excess equivalents (such as 7 equivalent) of LiBr/CAN at reflux temperature (in MeCN). Reactions of **5** with molecular bromine in  $\text{CHCl}_3$  at room and reflux temperatures gave **7**, **8**, and **9** (Scheme 1, Table I). The aromatic hydrogen atoms of **8** resonate at 7.15, 7.06, and 6.96 ppm as singlet(s) with relative intensities of 1:1:1, while the aromatic hydrogen atoms of **9** resonate at 7.04 ppm as s. The NMR data of **8** and **9** are consistent with the proposed structures. To make sure of the positions of the aromatic hydrogen atoms of **9**, the exact structure of compound **9** was determined by X-ray diffraction analysis (Figure 2A).

The compound **9** crystallises in the centrosymmetric space group  $P-1$  (no:2) with  $Z=2$ . The C-Br bonds range

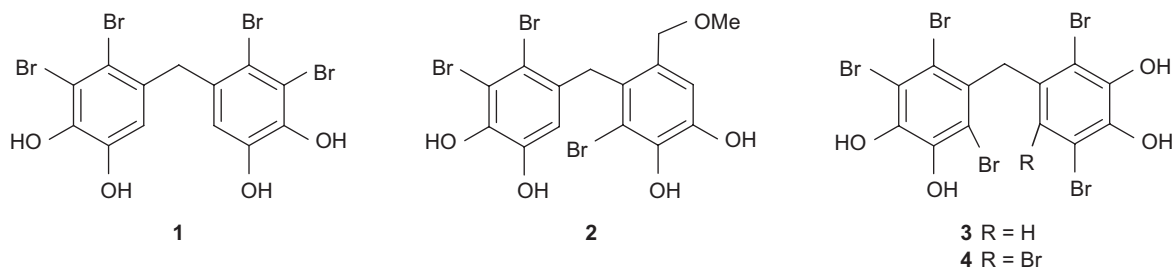
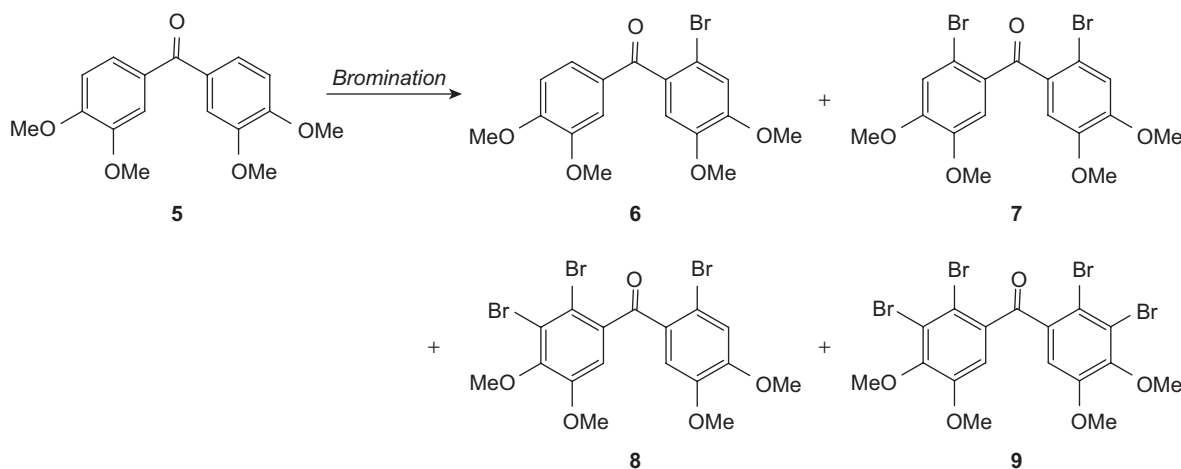


Figure 1. Some naturally occurring bromophenols.



Scheme 1. Bromination of compound 5.

**Table I.** Bromination reactions of the compound **5** (1.0 eq.) at different conditions and % yield of the product(s).

Reagent(s) and equivalent(s)	Solvent	Temperature	Time (day)	% yield of the product(s)			
				6	7	8	9
LiBr (1.1 Eq.) / CAN (1.1)	CH <sub>3</sub> CN	RT	0.5	95	0	0	0
LiBr (2.2 Eq.) / CAN (2.2)	CH <sub>3</sub> CN	RT	0.5	0	93	0	0
LiBr (Excess) / CAN (Excess)	CH <sub>3</sub> CN	RT	3	0	93	0	0
LiBr (Excess) / CAN (Excess)	CH <sub>3</sub> CN	Reflux	1	Mixture of complex products			0
Bromine (2.2 Eq.)	CHCl <sub>3</sub>	RT	15	11	78	0	0
Bromine (10 Eq.)	CHCl <sub>3</sub>	RT	15	0	0	4	70
Bromine (5.2 Eq.)	CHCl <sub>3</sub>	Reflux	1	0	19	37	23

CAN, Ceric ammonium nitrate; RT, Room temperature.

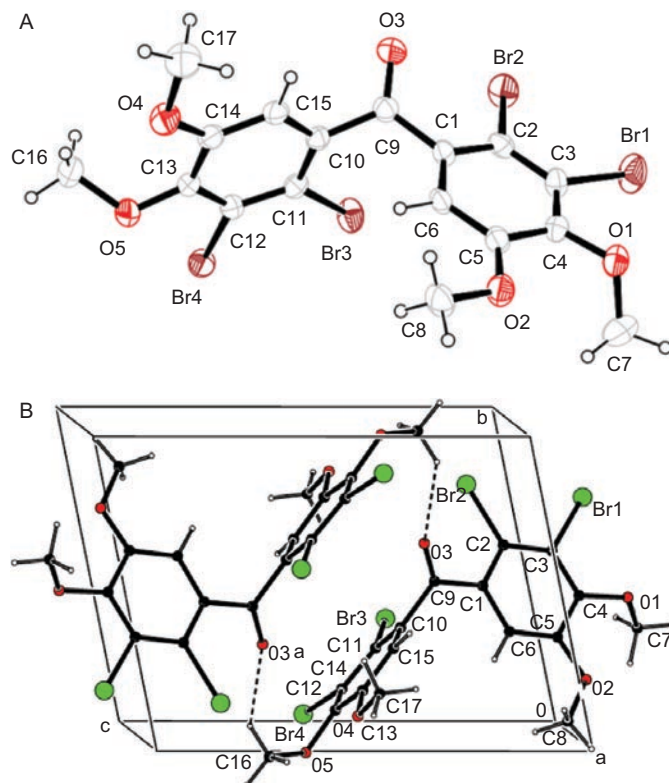
between 1.880(4)-1.884(4) Å. Due to the strong steric hindrance between Br(3), Br(2) and O(3) of the ketone [C(9)-O(3)=1.207(5) Å] atoms, the aromatic rings are extremely twisted along the C(1)-C(9)-C(10) molecular axis. Moreover, methoxy and ketone moieties are joined by two C(16)···O(3) (C(16)-H···O(3)<sup>a</sup> = 3.286(3) Å, C(16)-H···O(8)<sup>a</sup> = 131°, symmetry code (a); 2-x, 1-y, 1-z) H bonds, which lead to the formation of a centrosymmetric dimer of the molecule in the crystal unit cell (Figure 2B).

The compound **9** is a precursor of the natural product **1**. The reduction of the **9** with NaBH<sub>4</sub> in tetrahydrofuran (THF)-MeOH gave the alcohol **10** which was converted to the pentabromide **11** by treatment with PBr<sub>3</sub>-NEt<sub>3</sub>. In the synthesis of the natural product **1**, selective reduction of benzylic bromide in **11** was the crucial step. Both benzylic and aromatic bromide may be reduced with different reducing agents [27,28]. Therefore, we assumed that (*n*-Bu)<sub>3</sub>SnH could be used in the selective reduction of **11** [29]. As expected, the reduction of **11** (1.28 equiv.) with (*n*-Bu)<sub>3</sub>SnH (1.00 equiv.) selectively gave compound **12** in high yield and ether cleavage of **12** with BBr<sub>3</sub> lead to the natural product **1** (Scheme 2). This work corresponded to published papers of Li et al. and Oh et al. [18, 19].

As known, phenol and bromophenols are biologically active compounds [1-13,20,21]. Besides their biological importance, bromophenols are also useful for chemical purposes for instance they are used as precursors in synthetic organic chemistry [30]. Bromophenols **13-17** were synthesised from the corresponding compounds **5-9** by ether cleavage (Scheme 3). Natural products **1** and **13** contain OH-Br and OH-CO, respectively, while the compounds **14-17** contain HO, Br and CO as functional groups. The comparison of the antioxidant properties of compounds **1** and **13-17** with that of phenol without Br and CO will also be important. Consequently, the Wolff-Kishner reduction and demethylation of ketone **5** were performed, consecutively, and known [8,9,31] compound **19** was synthesised via **18** by an alternative method (Scheme 3).

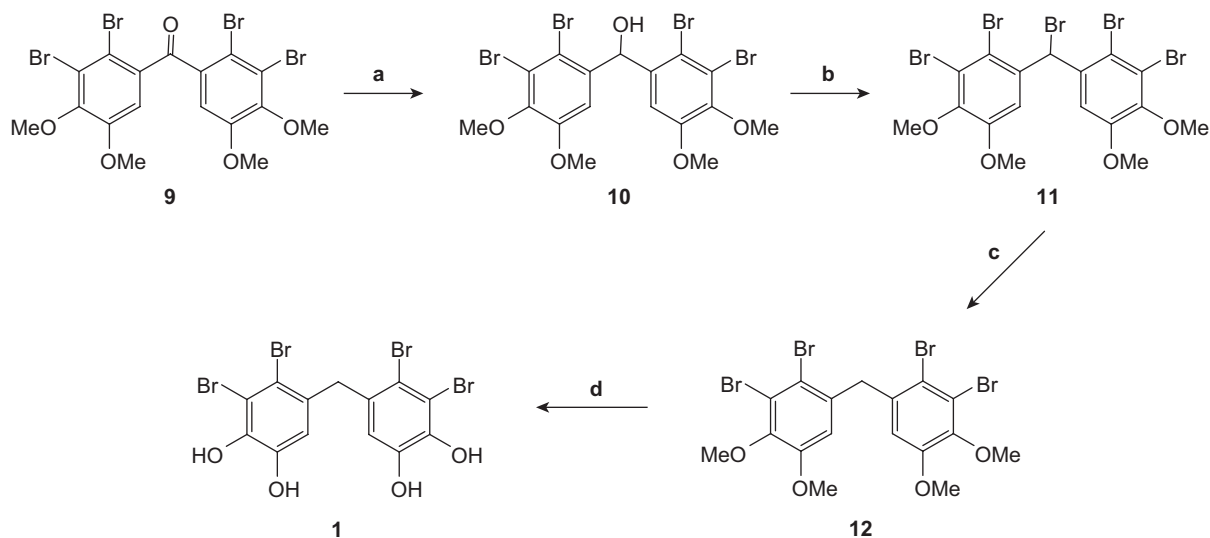
### Antioxidant properties

The antioxidant properties of bromophenols **1**, **13-17**, and **19** were investigated using different antioxidant assays. The Fe<sup>3+</sup>-Fe<sup>2+</sup> reducing power of bromophenols **1**, **13-17**, and **19** was measured by the direct reduction of Fe<sup>3+</sup>(CN)<sub>6</sub><sup>-</sup> to Fe<sup>2+</sup>(CN)<sub>6</sub><sup>-</sup>, and was determined by measuring absorbance

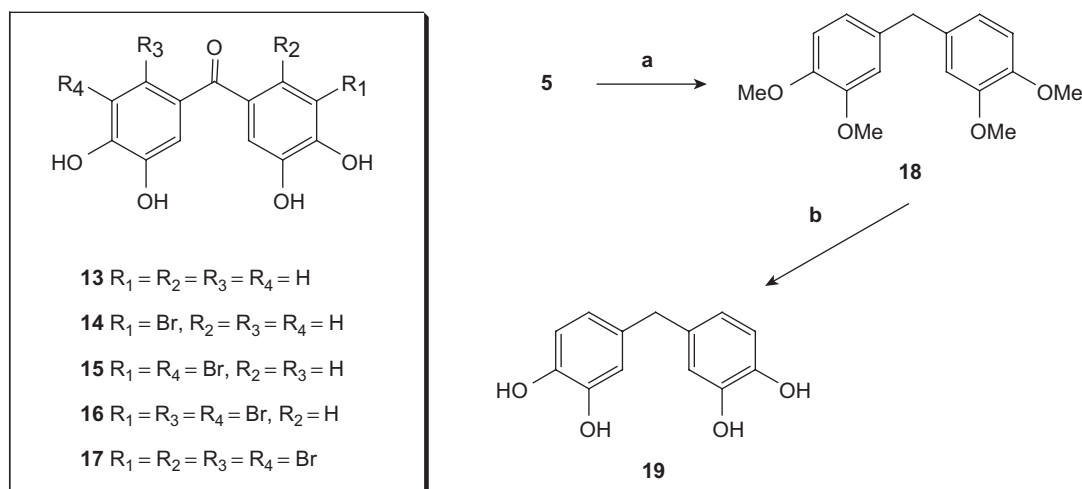


**Figure 2.** (A) The molecular structure of tetrabromide **9** showing the atom numbering scheme. Thermal ellipsoids are drawn at the 40% probability level. (B) packing diagram and H bonding geometry along the *a*-axis. (symmetry code a: 2-x, 1-y, 1-z).

that resulted from the formation of the Perl's Prussian blue complex following the addition of excess ferric ions (Fe<sup>3+</sup>). This method is based on the reduction of (Fe<sup>3+</sup>) ferricyanide in stoichiometric excess relative to the antioxidants [32]. On the other hand, in order to determine the cupric ion (Cu<sup>2+</sup>) reducing ability of bromophenols **1**, **13-17**, and **19**, the method proposed by Apak et al. was used with slight modification [33]. Ferrous ion (Fe<sup>2+</sup>) chelating activity of bromophenols **1**, **13-17**, and **19** was measured by inhibiting the formation of Fe<sup>2+</sup>-ferrozine complex after treatment of the test material with Fe<sup>2+</sup>. The Fe<sup>2+</sup>-chelating ability of bromophenols **1**, **13-17**, and **19** was monitored using the absorbance of ferrous iron-ferrozine complex at 562 nm [34].



**Scheme 2.** Reagents and conditions: (a) NaBH<sub>4</sub>/THF-MeOH, 0°C -RT, 12h; then dilute HCl, 93%; (b) PBr<sub>3</sub>/NEt<sub>3</sub>, benzene, 0°C -RT, 12h, 93%; (c) n-Bu<sub>3</sub>SnH, AIBN/THF, reflux, 77%; (d) BBr<sub>3</sub>/CH<sub>2</sub>Cl<sub>2</sub>, 0°C -RT, 24h; then MeOH, 97%.



**Scheme 3.** Reagents and conditions: (a) KOH-NH<sub>2</sub>NH<sub>2</sub>/(OHCH<sub>2</sub>)<sub>2</sub>, 110-190°C, 6 h, 86%; (b) BBr<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 96%.

The total radical scavenging capacity of bromophenols **1**, **13-17**, and **19** was determined and compared to that of BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene),  $\alpha$ -tocopherol, and trolox using the DPPH· (1,1-diphenyl-2-picryl-hydrazyl free radical), ABTS<sup>•+</sup> [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical cation], DMPD<sup>•+</sup> (N,N-dimethyl-p-phenylenediamine dihydrochloride radical cation), and O<sub>2</sub><sup>•-</sup> (superoxide anion radical) radical scavenging methods. The DPPH· solution, a deep violet colour, and the radical scavenging activity of antioxidant compounds can be measured spectrophotometrically at 517 nm by the loss of the absorbance as the pale yellow non-radical form (DPPH-H) produced. The method previously described by Gülçin [35] was used to assess the DPPH· free radical scavenging capacity of bromophenols **1**, **13-17**, and **19**. The DPPH radical shows absorbance at 517 nm, but its absorption decreases upon reduction by an antioxidant or a radical. When a hydrogen atom or electron was transferred to the odd electron in DPPH·, the absorbance at 517 nm

decreased proportional to the increases in the non-radical forms of DPPH [35].

The second test is based on the ability of antiradical molecules to quench the ABTS<sup>•+</sup>, a blue-green chromophore with characteristic absorption at 734 nm; the addition of antioxidants to the preformed radical cation reduces it to ABTS, determining decolourisation [21]. In this method, an antioxidant is added to a pre-formed ABTS radical solution, and after a period of time the remaining ABTS<sup>•+</sup> is quantified spectrophotometrically at 734 nm [21]. The ABTS<sup>•+</sup> was produced by reacting 2 mM ABTS in distilled water with 2.45 mM potassium persulphate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) [36].

The superoxide anion radical activity of bromophenols **1**, **13-17**, and **19** was determined as described by Zhishen and co-workers [37] with slight modification [38]. Superoxide radicals were generated in riboflavin, methionine illuminated, and assayed by the reduction of nitroblue tetrazolium (NBT) to form blue formazan. All solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The



photo-induced reactions were performed using fluorescent lamps (20 W) [37].

Finally, antiradical capacity was analysed by DMPD<sup>•+</sup> assay. The DMPD radical scavenging ability of bromophenols **1**, **13–17**, and **19** was determined according to Fogliano et al. [39] with some modification [33]. In the presence of Fe<sup>3+</sup>, a coloured DMPD radical cation is generated; antioxidant compounds able to transfer a hydrogen atom to DMPD<sup>•+</sup> cause a decolouration of the solution measured by the decrease in absorbance at 505 nm [33].

The percentage scavenging effects of bromophenols **1**, **13–17**, and **19** and standard compounds were calculated using the following equation (1):

$$\text{Scavenging effect (\%)} = \left(1 - \frac{A_s}{A_c}\right) \times 100 \quad (1)$$

where  $A_c$  is the absorbance of the control and  $A_s$  is the absorbance in the presence of synthesised bromophenol compounds. The control contains only FeCl<sub>2</sub> and ferrozine [34].

#### Standard procedure for selective bromination of bromophenol derivatives with LiBr/CAN

*(2-Bromo-4,5-dimethoxyphenyl)(3,4-dimethoxyphenyl) methanone (6)*: To a solution of **5** (0.45 g, 1.5 mmol) and LiBr (0.14 g, 1.65 mmol) in CH<sub>3</sub>CN (6.5 mL) a solution of CAN (0.91 g, 1.65 mmol) in CH<sub>3</sub>CN (6.5 mL) was added dropwise at room temperature (RT) and under nitrogen (N<sub>2</sub>) over 15 min. After the solution was stirred at RT and under N<sub>2</sub> for 12 hours (h), water (10 mL) was added and the mixture was extracted with ethyl acetate (3 × 30 mL). The organic phase was washed with solutions (5%, 2 × 15 mL) of NaHCO<sub>3</sub>, water (2 × 15 mL), and brine (15 mL), then dried over Na<sub>2</sub>SO<sub>4</sub> and the solvents evaporated. Monobromide **6** (0.55 g, 95%), the sole product, was crystallised from ethyl acetate/CHCl<sub>3</sub> as colourless crystals. Mp 132–134°C (lit. Mp 134°C) [25]. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 200 MHz): δ 7.52 (d, <sup>4</sup>J = 1.9 Hz, 1H); 7.23 (dd, A part of AB-system, <sup>4</sup>J = 1.9, <sup>3</sup>J = 8.4 Hz, 1H); 7.04 (s, 1H); 6.84 (s, 1H); 6.82 (d, B part of AB-system, <sup>3</sup>J = 8.4 Hz, 1H); 3.89 (s, OCH<sub>3</sub>, 9H), 3.8 (s, OCH<sub>3</sub>, 3H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 50 MHz): δ 196.2 (CO); 155.9 (C); 152.6 (C); 151.3 (C); 150.2 (C); 134.8 (C); 131.5 (C); 128.2 (CH); 117.8 (CH); 114.1 (CH); 113.4 (CH); 112.6 (C); 112.0 (CH); 58.3 (OCH<sub>3</sub>); 58.2 (OCH<sub>3</sub>); 58.2 (OCH<sub>3</sub>); 58.1 (OCH<sub>3</sub>). IR (CH<sub>2</sub>Cl<sub>2</sub>, cm<sup>-1</sup>): 3084, 3011, 2945, 2910, 2845, 1663, 1595, 1562, 1510, 1470, 1445, 1377, 1331, 1265, 1206, 1173, 1140, 1061, 1020, 936, 862. Anal. calcd for C<sub>17</sub>H<sub>17</sub>BrO<sub>5</sub>: C 53.54, H 4.46; found: C 53.25, H 4.46%.

*Bis(2-bromo-4,5-dimethoxyphenyl)methanone (7)*: This reaction was performed according to standard procedure described for **6**. In this reaction, the moles of **5**, CAN and LiBr used were 1.5, 3.3 and 3.3, respectively. The dibromide **7** (0.63 g, 93%) was the sole product and was crystallised from ethyl acetate/hexane as colourless crystals. Mp 175–177°C (lit. Mp (172.5–173.2°C)[18] and (176–177°C) [26]). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz): δ 7.041 (s, 2H); 7.039 (s, 2H); 3.93 (s, OCH<sub>3</sub>, 6H); 3.86 (s, OCH<sub>3</sub>, 6H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz): δ 194.6 (CO); 152.1 (C); 148.5 (C); 131.9 (C); 116.4 (CH); 114.0 (CH); 113.4

(C); 56.6 (OCH<sub>3</sub>); 56.4 (OCH<sub>3</sub>). IR (CH<sub>2</sub>Cl<sub>2</sub>, cm<sup>-1</sup>): 3084, 3063, 3003, 2984, 2945, 2918, 2839, 2594, 2039, 1663, 1595, 1562, 1510, 1464, 1437, 1385, 1339, 1265, 1213, 1173, 1146, 1067, 1028, 941. Anal. calcd for C<sub>17</sub>H<sub>16</sub>Br<sub>2</sub>O<sub>5</sub>: C 44.35, H 3.48; found: C 44.43, H 3.42%. Reaction of **5** with excess equivalents (such as **7** equiv) of LiBr/CAN at room and under reflux gave only the dibromide **7** and a complex mixture of products, respectively.

#### Reaction of ketone 5 with bromine under different conditions

Bromination by 2.2 equivalent bromine at room temperature: To a stirred solution of ketone **5** (0.5 g, 1.66 mmol) in CHCl<sub>3</sub> (25 mL) was added a solution of bromine (0.583 g, 3.64 mmol, 2.2 eq.) in CHCl<sub>3</sub> (20 mL) dropwise at RT over 5 min. The mixture was stirred at the same temperature for three days, the solvent evaporated, and chromatography of the residue on silica gel (SiO<sub>2</sub>, 70 g) with ethyl acetate/hexane (1:9) gave the dibromide **7** (0.595 g, 78%) and monobromide **6** (0.07 g, 11%), respectively.

Bromination by 10 equivalent bromine at room temperature: To a stirred solution of ketone **5** (2 g, 6.6 mmol) in CHCl<sub>3</sub> (40 mL) was added a solution of bromine (10.59 g, 66.2 mmol, 10 eq.) in CHCl<sub>3</sub> (40 mL) dropwise at RT over 10 min. After the reaction mixture was stirred at RT for 15 days, water (150 mL) and NaHCO<sub>3</sub> solution (5%, 150 mL) were added consecutively. The organic phase was separated and the water phase extracted with CHCl<sub>3</sub> (2 × 50 mL). The combined organic phase was washed with Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution (saturated, 180 mL) and brine (180 mL). Then it was dried over Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> and the solvent was evaporated. The residue was subjected to column chromatography on silica gel (SiO<sub>2</sub>, 100g, Merck-Darmstadt, Germany) and eluted using a solvent gradient (5–20% EtOAc in hexane) to give the tribromide **8** (0.14 g, 4%) and tetrabromide **9** (2.85 g, 70%). Both bromides **8** and **9** were crystallised from ethyl acetate/hexane.

*(2-Bromo-4,5-dimethoxyphenyl)(2,3-dibromo-4,5-dimethoxyphenyl)methanone (8)*: Colourless crystals, Mp 158–159°C. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ 7.16 (s, 1H); 7.06 (s, 1H); 6.96 (s, 1H); 3.94 (s, OCH<sub>3</sub>, 3H); 3.91 (s, OCH<sub>3</sub>, 3H); 3.86 (s, OCH<sub>3</sub>, 6H). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>): δ 194.0 (CO); 152.89 (C); 152.85 (C); 149.7 (C); 148.5 (C); 138.1 (C); 130.1 (C); 123.3 (C); 116.9 (CH); 114.66 (CH); 114.57 (C); 114.54 (C); 113.1 (CH); 61.0 (OCH<sub>3</sub>); 56.63 (OCH<sub>3</sub>); 56.62 (OCH<sub>3</sub>); 56.5 (OCH<sub>3</sub>). IR (CH<sub>2</sub>Cl<sub>2</sub>, cm<sup>-1</sup>): 3090, 3011, 2970, 2945, 2845, 1668, 1595, 1510, 1464, 1424, 1377, 1339, 1312, 1265, 1206, 1167, 1080, 1047, 1007, 936. Anal. calcd for C<sub>17</sub>H<sub>15</sub>Br<sub>3</sub>O<sub>5</sub>: C 37.85, H 2.78; found: C 37.88, H 2.80%.

*Bis(2,3-dibromo-4,5-dimethoxyphenyl)methanone (9)*: Colourless crystals, Mp 110–111°C. (lit. Mp 120.7–121.4°C) [18] <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ 7.03 (s, 2H); 3.91 (s, OCH<sub>3</sub>, 6H); 3.86 (s, OCH<sub>3</sub>, 6H). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>): δ 194.0 (CO); 152.7 (C); 150.5 (C); 136.6 (C); 123.8 (C); 115.5 (C); 114.1 (CH); 61.0 (OCH<sub>3</sub>); 56.7 (OCH<sub>3</sub>). IR (CH<sub>2</sub>Cl<sub>2</sub>, cm<sup>-1</sup>): 3084, 3057, 3011, 2978, 2945, 2845, 2567, 1682, 1576, 1543, 1470, 1424, 1371, 1312, 1265, 1206, 1167, 1100, 1053, 1007, 936. Anal. calcd for C<sub>17</sub>H<sub>14</sub>Br<sub>4</sub>O<sub>5</sub>: C 33.01, H 2.27; found: C 33.04, H, 2.28%.

**Bromination by 5.2 equivalent bromine at reflux temperature.** To a stirring solution of ketone **5** (7.1 g, 23.5 mmol) in  $\text{CHCl}_3$  (50 mL) was added a solution of bromine (6.1 mL, 65.2 eq.) in  $\text{CHCl}_3$  (40 mL) dropwise at RT over 5 min. Then the reaction mixture was heated and refluxed for 30 h. After the mixture was cooled, the solvent was evaporated. Chromatography of the residue on silica gel ( $\text{SiO}_2$ , 100g, Merck-Darmstadt, Germany) with ethyl acetate/hexane (1:9) gave the dibromide **7** (2.05 g, 19%), tribromide **8** (4.69 g, 37%), and tetrabromide **9** (3.49 g, 24%), consecutively.

**Bis(2,3-dibromo-4,5-dimethoxyphenyl)methanol (10):** A solution (60 mL) of tetrabromide **9** (1.99 g, 3.22 mmol) in THF/methanol (1:1) was cooled to 0°C.  $\text{NaBH}_4$  (0.125 g) was carefully added over 5 min and the resulting mixture was stirred at the same temperature for 15 min. After the cold bath was removed, it was stirred at RT for 1 day. Water (30 mL) was added and then the mixture was acidified with HCl (5%) until its pH was 3. The solvent (methanol and THF) was removed by rotary evaporation and it was extracted with  $\text{CH}_2\text{Cl}_2$  (2 × 50 mL). The combined organic phase was dried over  $\text{Na}_2\text{SO}_4$  and the solvent was carefully evaporated. The product effervesced while the solvent was removed. Tetrabromo alcohol **10** (1.86 g, 93%) was obtained as a white amorphous solid and crystallised from ethyl acetate/ $\text{CHCl}_3$ . Mp 175–176°C.  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.88 (s, 2H); 6.33 (bs, H-CO, 1H); 3.85 (s,  $\text{OCH}_3$ , 6H); 3.77 (s,  $\text{OCH}_3$ , 6H); 2.99 (bs, OH, 1H).  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  152.8 (C); 147.4 (C); 138.6 (CH); 126.6 (C); 117.3 (C); 111.6 (C); 76.6 (OCH); 60.8 ( $\text{OCH}_3$ ); 56.5 ( $\text{OCH}_3$ ). IR ( $\text{CH}_2\text{Cl}_2$ ,  $\text{cm}^{-1}$ ): 3400, 2938, 2845, 1583, 1551, 1467, 1421, 1373, 1279, 1189, 1161, 1066, 1037, 1005, 853, 821, 736, 699. Anal. calcd for  $\text{C}_{17}\text{H}_{16}\text{Br}_4\text{O}_5$ : C 32.94, H 2.60; found: C 32.77; H 2.61%.

**5,5'-(Bromomethylene)bis(3,4-dibromo-1,2-dimethoxybenzene) (11):** Tetrabromo alcohol **10** (0.99 g, 1.6 mmol) was dissolved in benzene (25 mL) and  $\text{NEt}_3$  (0.178 g, 1.76 mmol) was added. After the mixture was cooled to 0°C, a solution of  $\text{PBr}_3$  (0.48 g, 1.1 eq.) in benzene (10 mL) was added dropwise in the dark over 5 min and the mixture was stirred for 12 h. The mixture was left at RT during the stirring. After the solvent was evaporated, water (40 mL) and ether (50 mL) were added, consecutively. The organic phase was separated and the water phase was extracted with ether (2 × 50 mL). The combined organic phases were washed with HCl (10%, 5 mL),  $\text{NaHCO}_3$  (saturated, 10 mL), and water (50 mL), dried over  $\text{Na}_2\text{SO}_4$ , and the solvent was evaporated. Pentabromide **11** (1.02 g, 93%) was obtained and crystallised from  $\text{CH}_2\text{Cl}_2$  as white crystals. Mp 148–149°C.  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.14 (s, 2H); 6.87 (s, H-CBr, 1H); 3.87 (s,  $\text{OCH}_3$ , 6H); 3.82 (s,  $\text{OCH}_3$ , 6H).  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  152.7 (C); 148.0 (C); 136.2 (C); 122.9 (C); 117.6 (C); 114.2 (CH); 60.9 ( $\text{OCH}_3$ ); 56.7 (CHBr); 56.5 ( $\text{OCH}_3$ ). IR ( $\text{CH}_2\text{Cl}_2$ ,  $\text{cm}^{-1}$ ): 3003, 2967, 2936, 2844, 1583, 1546, 1467, 1421, 1373, 1321, 1295, 1275, 1261, 1198, 1160, 1056, 1005, 989, 826, 737. Anal. calcd for  $\text{C}_{17}\text{H}_{15}\text{Br}_5\text{O}_4$ : C 29.90, H 2.21; found: C 29.93, H 2.21%.

**Bis(2,3-dibromo-4,5-dimethoxyphenyl)methane (12):** To a solution in THF (80 mL) of the pentabromide **11** (1 g, 1.46 mmol) was added a solution of *n*- $\text{Bu}_3\text{SnH}$  (0.33 g, 1.14 mmol)

in THF (15 mL) at RT. After the solution was heated and refluxed for 15 min, azobisisobutyronitrile (AIBN) (0.05 g, Merck-Schuchardt) was added and refluxed for 3 days. The mixture was cooled to RT and the solvent was evaporated. The reaction mixture was subjected to column chromatography on silica gel ( $\text{SiO}_2$ , 100g, Merck-Darmstadt, Germany) and eluted with EtOAc/hexane (1:10). Tetrabromomethane **12** (0.68 g, 77%) and unreacted pentabromide **11** (0.16 g, 0.23 mmol) were isolated, consecutively. It was crystallised from  $\text{CH}_2\text{Cl}_2$  as white crystals. Mp 150–151°C (lit. Mp (159–160°C) [3] and (149.9–150.2°C) [18]).  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.58 (s, 2H); 4.24 (s,  $\text{CH}_2$ , 2H); 3.85 (s,  $\text{OCH}_3$ , 6H); 3.74 (s,  $\text{OCH}_3$ , 6H).  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  152.8 (C); 146.7 (C); 136.2 (C); 122.3 (C); 118.1 (C); 113.6 (CH); 60.8 ( $\text{OCH}_3$ ); 56.5 ( $\text{OCH}_3$ ); 45.5 ( $\text{CH}_2$ ). Anal. calcd for  $\text{C}_{17}\text{H}_{16}\text{Br}_4\text{O}_4$ : C 33.81, H 2.67; found: C 33.75, H 2.67%.

#### Standard procedure for demethylation of compounds with OMe by ether cleavage synthesis of 5,5'-methylene bis(3,4-dibromobenzene-1,2-diol) (1)

A solution of tetrabromomethane **12** (0.76 g, 1.25 mmol) in  $\text{CH}_2\text{Cl}_2$  (12 mL) was cooled to 0°C and then a solution of  $\text{BBr}_3$  (0.8 mL, 8.3 mmol) in  $\text{CH}_2\text{Cl}_2$  (8.5 mL) was added dropwise under  $\text{N}_2$  (g) over 5 min. After the cold bath was removed, the mixture was stirred at RT and under  $\text{N}_2$  for one day. Methanol (30 mL) was slowly added over 15 min and then the solvent was evaporated. After water (40 mL) and EtOAc (50 mL) were added, the mixture was shaken. The organic phase was separated and the water phase was extracted with EtOAc (2 × 30 mL). The combined organic phases were dried over  $\text{Na}_2\text{SO}_4$  and the solvent was evaporated. The natural product tetrabromotetrol **1** (0.7 g, 97%) was obtained and crystallised from Et<sub>2</sub>O as pale orange crystals. Mp 204–206°C (lit. Mp (196–199°C) [17]. and (199.0–199.8°C) [18]).  $^1\text{H-NMR}$  (200 MHz,  $\text{CD}_3\text{COCD}_3$ ):  $\delta$  8.95 (s, OH, 2H); 8.34 (s, OH, 2H); 6.57 (s, 2H); 4.05 (s,  $\text{CH}_2$ , 2H).  $^{13}\text{C-NMR}$  (50 MHz,  $\text{CD}_3\text{COCD}_3$ ):  $\delta$  147.6 (C); 145.9 (C); 134.4 (C); 118.7 (C and CH); 115.9 (C); 46.8 ( $\text{CH}_2$ ). Anal. calcd for  $\text{C}_{13}\text{H}_8\text{Br}_4\text{O}_4$ : C 28.5, H 1.47. found: C 28.5; H 1.46%.

**Bis(3,4-dimethoxyphenyl)methane (18):** To a solution of the methanone **5** (0.604 g, 2 mmol) in  $\text{OHCH}_2\text{CH}_2\text{OH}$  (4.5 mL) were added KOH (0.452 g) and hydrazine hydrate (1.1 mL), consecutively, at RT and under  $\text{N}_2$  (g). After the mixture was heated to 110°C and stirred at this temperature for 1 h, it was then stirred at 190°C for 6 h. The mixture was cooled to RT, acidified with HCl (10%), and extracted with EtOAc (2 × 30 mL). The combined organic phases were dried over  $\text{Na}_2\text{SO}_4$  and the solvent was evaporated. The residue was filtrated from small silica gel column ( $\text{SiO}_2$ , 100g, Merck-Darmstadt, Germany) and **18** (0.50 g, 86%) was obtained. Mp 63–65°C (lit. Mp (68–69°C) [23]).  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.8 (d,  $^3J = 8.07$  Hz, 2H); 6.71 (dd,  $^3J = 8.07$ ,  $^4J = 1.47$  Hz, 2H); 6.7 (bs, 2H); 3.88 (s,  $\text{CH}_2$ , 2H); 3.85 (s,  $\text{OCH}_3$ , 3H); 3.82 (s,  $\text{OCH}_3$ , 3H).  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  149.2 (C); 147.6 (C); 134.2 (C); 121 (CH); 112.4 (CH); 111.5 (CH); 56.1 ( $\text{OCH}_3$ ); 56 ( $\text{OCH}_3$ ); 41.2 ( $\text{OCH}_3$ ).

**Synthesis of phenols 13–17 and 19 from 5–9 and 18:** The standard procedure described above for the synthesis of **1**



with  $\text{BBr}_3$  was applied to **5-9** and **18**. From these reactions, phenols **13-17** and **19** were obtained.

**Bis (2,3-dihydroxyphenyl) methanone (13):** This was obtained and crystallised from ether as pale yellow crystals (98%); Mp 231-233°C (lit. for hydrated **13**: 230-231°C) [40].  $^1\text{H-NMR}$  (400 MHz,  $\text{CD}_3\text{COCD}_3$ ):  $\delta$  8.63 (s, 2 OH, 2H); 8.38 (s, 2 OH, 2H); 7.32 (d,  $J=1.83$  Hz, 2H); 7.2 (dd,  $^3J=8.06$ ,  $^4J=1.83$  Hz, 2H); 6.92 (d,  $^3J=8.06$  Hz, 2H);  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CD}_3\text{COCD}_3$ ):  $\delta$  193.4 (CO); 149.5 (C); 144.9 (C); 130.7 (C); 123.5 (CH); 117.1 (CH); 114.7 (CH).

**2-Bromo-4,5-dihydroxyphenyl(3,4-dihydroxyphenyl) methanone (14):** This was obtained as amorphous (95%). Mp 67-69°C.  $^1\text{H-NMR}$  (200 MHz,  $\text{CD}_3\text{COCD}_3$ ):  $\delta$  8.95-8.2 (m, OH, 4H); 7.33 (d,  $^4J=2.1$  Hz, 1H); 7.17 (dd, A part of AB-system,  $^3J=8.3$ ,  $^3J=2.1$  Hz, 1H); 7.11 (s, 1H); 6.01 (d, B part of AB-system,  $^3J=8.3$  Hz, 1H); 6.86 (s, 1H).  $^{13}\text{C-NMR}$  (50 MHz,  $\text{CD}_3\text{COCD}_3$ ):  $\delta$  195.9 (CO); 153.4 (C); 150.1 (C); 147.7 (C); 147.2 (C); 135.5 (C); 132.2 (C); 126.9 (CH); 122.1 (CH); 119.3 (C); 118.8 (CH); 118 (CH); 111.1 (C). IR ( $\text{CH}_3\text{OH}$ ,  $\text{cm}^{-1}$ ): 3399, 1589, 1505, 1442, 1414, 1369, 1295, 1220, 1187, 1137, 1114, 1056, 1033, 1016, 953, 881, 852, 827, 862, 783, 636. Anal. calcd for  $\text{C}_{13}\text{H}_9\text{BrO}_5$ : C 48.03, H 2.79; found: C 48.04; H 2.80%.

**Bis-(2-bromo-4,5-dihydroxyphenyl) methanone (15):** This was obtained and crystallised from ether as brown crystals (91%). Mp 217-219°C.  $^1\text{H-NMR}$  (200 MHz,  $\text{CD}_3\text{COCD}_3$ ):  $\delta$  8.93-8.5 (m, OH, 4H); 7.12 (s, 2H); 6.97 (s, 2H).  $^{13}\text{C-NMR}$  (50 MHz,  $\text{CD}_3\text{COCD}_3$ ):  $\delta$  194.9 (CO); 151.8 (C); 147.1 (C); 134 (C); 123.1 (C); 121 (CH); 113.4 (C). IR ( $\text{CH}_3\text{OH}$ ,  $\text{cm}^{-1}$ ): 3230, 1589, 1503, 1416, 1358, 1212, 1179, 1038, 862, 795, 634. Anal. calcd for  $\text{C}_{13}\text{H}_8\text{Br}_2\text{O}_5$ : C 38.65, H 2. found: C 38.64; H 2.02%.

**2-Bromo-4,5-dihydroxyphenyl(2,3-dibromo-4,5-dihydroxyphenyl) methanone (16):** This was obtained as yellow amorphous solid (85%). Mp 126-128°C.  $^1\text{H-NMR}$  (200 MHz,  $\text{CD}_3\text{COCD}_3$ ):  $\delta$  9.23 (m, OH, 1H); 8.95 (m, OH, 1H); 8.85 (m, OH, 1H); 8.52 (m, OH, 1H); 7.14 (s, 1H); 7 (s, 1H); 6.96 (s, 1H).  $^{13}\text{C-NMR}$  (50 MHz,  $\text{CD}_3\text{COCD}_3$ ):  $\delta$  195 (CO); 152.2 (C); 149.3 (C); 147.2 (C); 147.1 (C); 136.4 (C); 132.8 (C); 123.6 (CH); 121.7 (CH); 118.6 (CH); 116.9 (C); 115.8 (C); 114.1 (C). IR ( $\text{CH}_3\text{OH}$ ,  $\text{cm}^{-1}$ ): 3270, 1648, 1589, 1504, 1467, 1394, 1330, 1283, 1216, 1184, 1055, 1012, 863, 774, 737. Anal. calcd for  $\text{C}_{13}\text{H}_7\text{Br}_3\text{O}_5$ : C 32.33, H 1.46; found: C 32.32; H 1.47%.

**Bis-(2,3-dibromo-4,5-dihydroxyphenyl) methanone (17):** This was obtained as amorphous solid (88%, brown). Mp 204-206°C.  $^1\text{H-NMR}$  (200 MHz,  $\text{CD}_3\text{COCD}_3$ ): 9.25 (m, 2 OH, 2H), 9.05 (m, 2 OH, 2H), 7 (s, 2H).  $^{13}\text{C-NMR}$  (50 MHz,  $\text{CD}_3\text{COCD}_3$ ):  $\delta$  195.0 (CO), 149.9 (C), 146.9 (C), 135.1 (C), 119.3 (CH), 117.4 (C), 116.5 (C). IR ( $\text{CH}_3\text{OH}$ ,  $\text{cm}^{-1}$ ): 3434, 2844, 2078, 1638, 1474, 1412, 1341, 1282, 1195, 1161, 1051, 1015, 857, 663. Anal. calcd for  $\text{C}_{13}\text{H}_6\text{Br}_4\text{O}_5$ : C 27.79, H 1.08; found: C 27.8; H 1.12%.

**4,4'-Methylenedibenzene-1,2-diol (19):** This was obtained as amorphous solid (86%). Mp 141-142°C.  $^1\text{H-NMR}$  (400 MHz,  $\text{CD}_3\text{COCD}_3$ ):  $\delta$  7.66 (s, 2 OH, 2H); 7.59 (s, 2 OH, 2H); 6.72 (d,  $^3J=8.04$  Hz, 2H); 6.64 (d,  $^4J=2.20$  Hz, 2H); 6.53 (dd,  $^3J=7.13$ ,  $^4J=1.85$  Hz, 2H).  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CD}_3\text{COCD}_3$ ):  $\delta$  145.1 (C); 143.4 (C); 134 (C); 120.2 (CH); 116.1 (CH); 115.2 (CH); 60.5 ( $\text{CH}_2$ ). Anal. calcd for  $\text{C}_{13}\text{H}_{12}\text{O}_4$ : C 67.23, H 5.21; found: C 67.45, H 5.2. (The NMR data are consistent with literature [41]).

### X-Ray structure determination for 9

For the crystal structure determination, the single-crystals of tetrabromide **9** were used for data collection on a four-circle Rigaku R-AXIS RAPID-S diffractometer (equipped with a two-dimensional area imaging plate (IP) detector) [42]. The graphite-monochromatised Mo  $\text{K}_\alpha$  radiation ( $\lambda=0.71073$  Å) and oscillation scans technique with  $\Delta\omega=5^\circ$  for one image were used for data collection. The lattice parameters were determined by the least-squares methods on the basis of all reflections with  $F^2>2\sigma(F^2)$ . Integration of the intensities, correction for Lorentz and polarisation effects and cell refinement was performed using CrystalClear (Rigaku/MSC) software [42]. The structures were solved by direct methods using SHELXS-97 [43] and refined by a full-matrix least-squares procedure using the program SHELXL-97 [43]. H atoms were positioned geometrically and refined using a riding model, fixing the aromatic C-H distances at 0.93 Å and methyl C-H distances at 0.96 Å [ $U_{\text{iso}}(\text{H})=1.2U_{\text{eq}}(\text{C})$  and  $U_{\text{iso}}(\text{H})=1.5U_{\text{eq}}(\text{methyl C})$ ]. The final difference Fourier maps showed no peaks of chemical significance.

**Crystal data for 9:**  $\text{C}_{17}\text{H}_{14}\text{Br}_4\text{O}_5$ , crystal system, space group: triclinic, P-1; (no:2); unit cell dimensions:  $a=9.834(4)$ ,  $b=9.964(4)$ ,  $c=11.822(5)$  Å,  $\alpha=72.95(5)$   $\beta=81.6(6)$ ,  $\gamma=63.33(5)^\circ$ ; volume: 989.5(3) Å<sup>3</sup>;  $Z=2$ ; calculated density: 2.07 mg/m<sup>3</sup>; absorption coefficient: 8.159 mm<sup>-1</sup>;  $F(000)$ : 592;  $\theta$  range for data collection 2.3-30.5°; refinement method: full-matrix least-square on  $F^2$ ; data/parameters: 6005/239; goodness-of-fit on  $F^2$ : 1.321; final  $R$  indices [ $I>2\sigma(I)$ ]:  $R_1=0.084$ ,  $wR_2=0.125$ ;  $R$  indices (all data):  $R_1=0.153$ ,  $wR_2=0.138$ ; largest diff. peak and hole: 0.513 and -0.478 e Å<sup>-3</sup>; CCDC-710557.

### Antioxidant assays

**Fe<sup>3+</sup> reducing power assay:** The  $\text{Fe}^{3+}(\text{CN}^-)_6$  to  $\text{Fe}^{2+}(\text{CN}^-)_6$  reducing power of bromophenol derivatives **1**, **13-17**, and **19** was measured as described by Oyaizu [44] with slight modification [20]. For this purpose, 10 µg/mL of bromophenol derivatives **1**, **13-17**, and **19** in 0.75 mL of distilled water were mixed with 1.25 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] (1%). The mixture was incubated at 50°C for 20 min. After 20 min of incubation, the reaction mixture was acidified with 1.25 mL of trichloroacetic acid (10%). Finally, 0.5 mL of  $\text{FeCl}_3$  (0.1%) was added to this solution, and the absorbance was measured at 700 nm in a spectrophotometer (UV<sub>mini</sub>-1240, Shimadadzo, Kyoto/Japan) [45].

**Cupric ions (Cu<sup>2+</sup>) reducing assay:** The cupric ion ( $\text{Cu}^{2+}$ ) reducing ability (CUPRAC assay) of bromophenol derivatives **1**, **13-17**, and **19** was determined according to the method proposed by Apak and co-workers [46]. Briefly, 0.25 mL of  $\text{CuCl}_2$  solution (0.01 M), 0.25 mL ethanolic neocuproine solution ( $7.5 \times 10^{-3}$  M) and 0.25 mL of  $\text{NH}_4\text{Ac}$  buffer solution (1 M) were added to a test tube, followed by mixing with 10 µg/mL concentration of bromophenol derivatives **1**, **13-17**, and **19**. Then, total volume was adjusted to 2 mL with distilled water, and mixed well. The tubes were stoppered and kept at room temperature. Absorbance was measured at 450 nm against a reagent blank 30 min later.

**Chelating activity on ferrous ions ( $Fe^{2+}$ ):** The ferrous ion ( $Fe^{2+}$ ) chelating activity was measured by inhibiting the formation of  $Fe^{2+}$ -ferrozine complex after treatment of the test material with  $Fe^{2+}$ , following the method described by Dinis and co-workers [47]. The ferrous ion ( $Fe^{2+}$ ) chelating ability of bromophenol derivatives **1**, **13–17**, and **19** was monitored using the absorbance of the ferrous iron-ferrozine complex at 562 nm. Briefly, 10  $\mu\text{g}/\text{mL}$  concentrations of bromophenol derivatives **1**, **13–17**, and **19** in 0.4 mL of methanol were added to a solution of  $FeCl_2$  (0.6 mM, 0.1 mL). The reaction was initiated by the addition of ferrozine (5 mM, 0.1 mL) dissolved in methanol. Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm [48].

**DPPH· scavenging activity:** The DPPH radical scavenging activity of bromophenol derivatives **1**, **13–17**, and **19** was determined as previously described by Gülçin [49]. Briefly, a solution (0.1 mM) of DPPH· was prepared in ethanol and 0.5 mL of this solution was added to 1.5 mL of bromophenol derivatives **1**, **13–17**, and **19** solutions in ethanol at different concentrations (10–30  $\mu\text{g}/\text{mL}$ ). These solutions were vortexed thoroughly, and incubated in the dark for 30 min. Another 30 min later, the absorbance was measured at 517 nm against blank samples lacking scavenger. A standard curve was prepared using different concentrations of DPPH·. The DPPH· scavenging capacity was expressed as mM in the reaction medium, and calculated from the calibration curve determined by linear regression ( $r^2$ : 0.9845):

$$\text{Absorbance}(\lambda_{517}) = 0.5869 \times [\text{DPPH}] + 0.0134 \quad (2)$$

**ABTS<sup>•+</sup> scavenging activity:** In the ABTS<sup>•+</sup> scavenging method, an antioxidant is added to a pre-formed ABTS radical solution, and after a fixed time period, the remaining ABTS<sup>•+</sup> is quantified spectrophotometrically at 734 nm [50,51]. The ABTS<sup>•+</sup> was produced by reacting ABTS (2 mM) in  $H_2O$  with potassium persulphate (2.45 mM,  $K_2S_2O_8$ ), stored in the dark at room temperature for 6 h. Then, 0.5 mL of ABTS<sup>•+</sup> solution was added to 1.5 mL of bromophenol derivatives **1**, **13–17**, and **19** solution in ethanol at different concentrations (10–30  $\mu\text{g}/\text{mL}$ ). The absorbance was recorded 30 min after mixing, and the percentage of radical scavenging was calculated for each concentration relative to a blank, containing no scavenger. The extent of decolourisation is calculated as the percentage reduction in absorbance [52,53]. For preparation of a standard curve, different concentrations of ABTS<sup>•+</sup> (0.033–0.33 mM) were used. The ABTS<sup>•+</sup> concentration (mM) in the reaction medium was calculated from the following calibration curve, determined by linear regression ( $r^2$ : 0.9899):

$$\text{Absorbance}(\lambda_{734}) = 2.5905 \times [\text{ABTS}^{\bullet+}] \quad (3)$$

**DMPD<sup>•+</sup> scavenging activity:** The DMPD<sup>•+</sup> scavenging ability of bromophenol derivatives **1**, **13–17**, and **19** was determined according to Fogliano et al. [39]. DMPD (100 mM) was prepared by dissolving 209 mg of DMPD in 10 mL of deionised water, and 1 mL of this solution was added to

100 mL of 0.1 M acetate buffer (pH 5.25), and the coloured radical cation (DMPD<sup>•+</sup>) was obtained by adding 0.2 mL of a solution of 0.05 M ferric chloride ( $FeCl_3$ ). Different concentrations of bromophenol derivatives **1**, **13–17**, and **19** (10–30  $\mu\text{g}/\text{mL}$ ) were added to test tubes, and the total volumes were adjusted to 0.5 mL with distilled water. The absorbance was measured at 505 nm ten minutes later. A one millilitre aliquot of the DMPD<sup>•+</sup> solution was directly added to the reaction mixture, and its absorbance was measured at 505 nm. The buffer solution was used as a blank sample. The DMPD<sup>•+</sup> concentration (mM) in the reaction medium was calculated from the following calibration curve, determined by linear regression ( $r^2$ : 0.9993):

$$\text{Absorbance}(\lambda_{505}) = 0.0088 \times [\text{DMPD}^{\bullet+}] \quad (4)$$

## Discussion

In the present study, we demonstrated the antioxidant and radical scavenging effects of bromophenols **1**, **13–17**, and **19** using different *in vitro* bioanalytical methodologies. Antioxidant capacity is widely used as a parameter for medicinal bioactive components. The antioxidant and radical scavenging activities of bromophenols **1**, **13–17**, and **19** were compared to those of BHA, BHT,  $\alpha$ -tocopherol, and its water-soluble analogue trolox. These comparisons were performed using a series of *in vitro* tests including DPPH·, ABTS<sup>•+</sup>, DMPD<sup>•+</sup> and  $O_2^{\bullet-}$  radicals scavenging activities, and reducing power by two methods ( $Fe^{3+}$ - $Fe^{2+}$  transformation and CUPRAC assays), hydrogen peroxide scavenging and metal chelating on ferrous ion ( $Fe^{3+}$ ) activities.

The reducing power, reflects the electron donating capacity of bioactive compounds and is associated with antioxidant activity. Antioxidants can be reductants and inactivators of oxidants. The reducing capacity of a compound can be measured by the direct reduction of  $Fe[(CN)_6]_3$  to  $Fe[(CN)_6]_2$ . Addition of free  $Fe^{3+}$  to the reduced product leads to the formation of the intense Perle's Prussian blue complex  $Fe_4[Fe(CN)_6]_3$ , which has strong absorbance at 700 nm. As can be seen from Table II, all of the bromophenols **1**, **13–17**, and **19** have powerful  $Fe^{3+}$  reducing ability. Similarly, the bromophenols showed marked cupric ion ( $Cu^{2+}$ ) reducing ability.

Ferrous ions are the most effective pro-oxidants in food systems; the chelating effect would be beneficial and removal of free iron ions from circulation could be a promising approach to prevent oxidative stress-induced diseases. When an iron ion is chelated, it may lose pro-oxidant properties. Iron, in nature, can be found as either ferrous ( $Fe^{2+}$ ) or ferric ions ( $Fe^{3+}$ ), with the latter form predominant in foods. Ferrous chelation may render important antioxidative effects by retarding metal-catalysed oxidation. Ferrous ion chelating activities of bromophenol derivatives **1**, **13–17**, and **19**, BHA, BHT,  $\alpha$ -tocopherol, and Trolox are shown in Table II. This shows that the most powerful metal chelating properties were found in **15**. In addition, the other bromophenols have marked  $Fe^{2+}$  chelating activity.



The free radical chain reaction is widely accepted as a common mechanism of lipid peroxidation. Radical scavengers may directly react with and quench peroxide radicals to terminate the peroxidation chain reactions, and improve the quality and stability of food products. Assays based upon the use of DPPH·, ABTS<sup>+</sup>, DMPD<sup>+</sup>, and O<sub>2</sub><sup>·-</sup> radicals are among the most popular spectrophotometric methods for determination of the antioxidant capacity of molecules. These radicals can directly react with antioxidants. Additionally, these radical scavenging assays have been used to evaluate the antioxidant activity of compounds due to the simple, rapid, sensitive, and reproducible procedures. These tests are standard assays in antioxidant

activity studies, and offer a rapid technique for screening the radical scavenging activity of specific compounds. As can be seen in Table III, DPPH·, ABTS<sup>+</sup>, DMPD<sup>+</sup>, and O<sub>2</sub><sup>·-</sup> radical scavenging activities of bromophenol derivatives were evaluated. In particular, the IC<sub>50</sub> values of the bromophenol derivatives used in the DPPH·, ABTS<sup>+</sup>, and O<sub>2</sub><sup>·-</sup> assays are lower than those of standard antioxidant compounds.

Phenol derivatives **1**, **13–17**, and **19** showed high antioxidant activities. In most of the phenol derivatives, CO and Br are present as functional groups. Antioxidant activities of the phenols with CO are higher than those of the others. Among phenols with Br and without Br, these activities are almost the same, while they are similar in phenols that include different numbers of Br.

The reason for the higher antioxidant activities in the phenols with CO may be the CO group itself. Phenol derivatives have two OH groups on each ring as ortho or 3,4 and

**Table II.** Comparison of ferric ions (Fe<sup>3+</sup>) reducing ability by Fe<sup>3+</sup>-Fe<sup>2+</sup> transformation methods, Cu<sup>+</sup> reducing ability by CUPRAC method, ferrous ion (Fe<sup>2+</sup>) chelating activity of bromophenol derivatives (**1**, **13–17** and **19**) and standard antioxidant compounds such as BHA, BHT, α-tocopherol and trolox at the same concentration (10 μg/mL).

Tested compounds	Fe <sup>3+</sup> -Fe <sup>2+</sup> reducing ability*	Cu <sup>2+</sup> -Cu <sup>+</sup> reducing ability*	Fe <sup>2+</sup> chelating activity**
Control	1.633	2.251	100
BHA	1.038	2.099	50.2
BHT	0.673	0.808	36.7
Trolox	1.017	1.144	56.5
α-Tocopherol	1.757	2.843	26.6
Natural product			
<b>1</b>	1.633	2.251	19.9
<b>13</b>	1.737	2.107	62.0
<b>14</b>	1.609	1.82	40.1
<b>15</b>	1.229	1.336	67.1
<b>16</b>	1.605	1.965	30.4
<b>17</b>	1.542	1.56	17.7
<b>19</b>	1.681	2.542	56.1

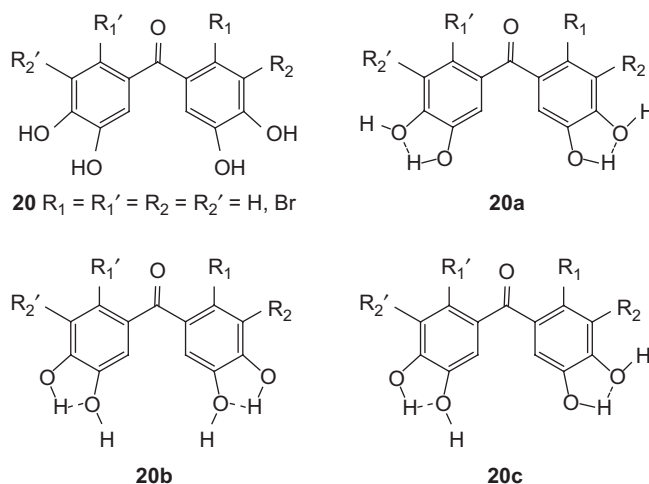
\*The values were expressed as absorbance. High absorbance indicates high reducing power ability. \*\*The values were expressed as percentage chelating of ferrous ions Fe<sup>2+</sup>. Lower chelating values showed stronger metal chelating effect.

BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene

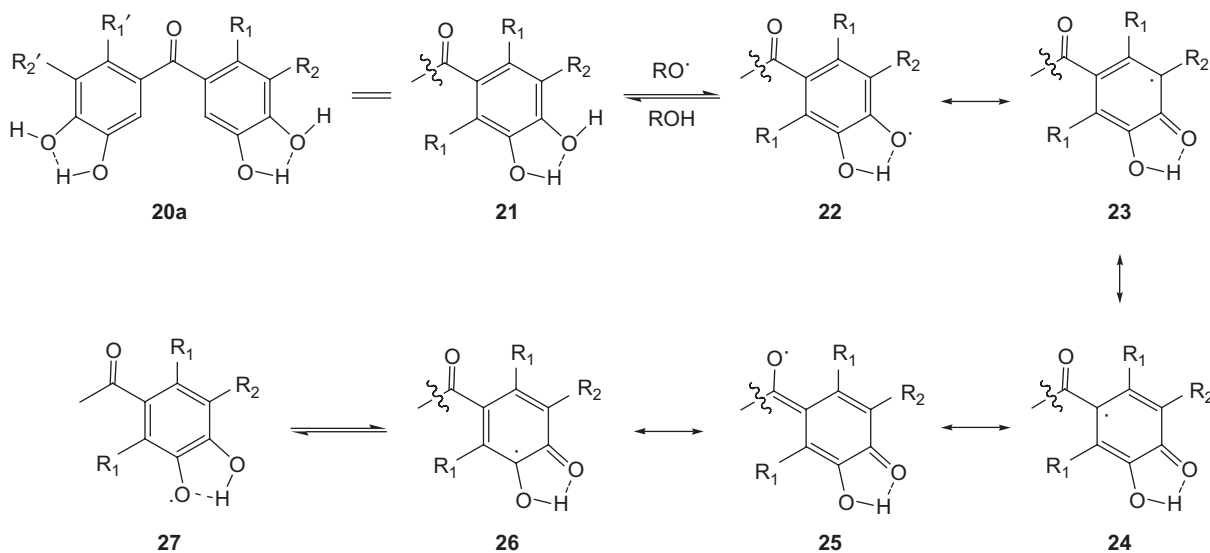
**Table III.** Concentration required for 50% scavenging (IC<sub>50</sub>) of DPPH·, ABTS<sup>+</sup>, DMPD<sup>+</sup> and O<sub>2</sub><sup>·-</sup> radical scavenging activities of bromophenol derivatives (**1**, **13–17** and **19**) and standard antioxidant compounds such as BHA, BHT, α-tocopherol and trolox (DPPH·: 1,1-diphenyl-2-picryl-hydrazyl free radical. ABTS<sup>+</sup>: 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid. DMPD<sup>+</sup>: N,N-dimethyl-p-phenylenediamine dihydrochloride radical. O<sub>2</sub><sup>·-</sup>: superoxide anion radicals).

Tested compounds	DPPH·-scavenging activity (IC <sub>50</sub> )*	ABTS <sup>+</sup> -scavenging activity (IC <sub>50</sub> )*	DMPD <sup>+</sup> -scavenging activity (IC <sub>50</sub> )*	O <sub>2</sub> <sup>·-</sup> scavenging activity (IC <sub>50</sub> )*
BHA	16.1	19.6	43.5	12.8
BHT	51.2	24.6	—	19.5
Trolox	13.1	28.4	21.4	25.4
α-Tocopherol	16.4	24.9	—	51.2
Natural product				
<b>1</b>	8.9	10.1	24.9	11.8
<b>13</b>	8.8	9.1	66.4	12.5
<b>14</b>	9.4	10.2	44.1	13.6
<b>15</b>	9.2	11.5	30.1	12.4
<b>16</b>	9.2	9.9	20.1	11.8
<b>17</b>	8.8	12.1	27.3	13.6
<b>19</b>	9.5	7.1	42.1	12.3

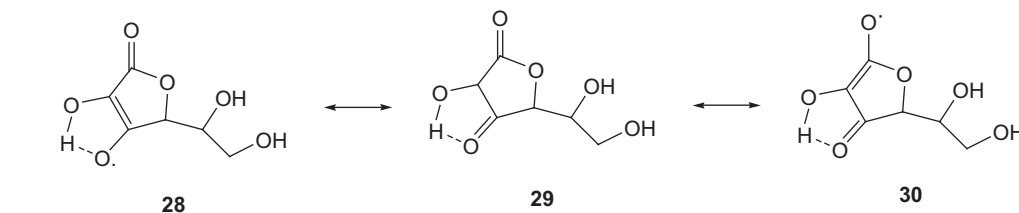
\*The values were expressed as μg/mL concentration. Lower IC<sub>50</sub> values indicate higher radical scavenging activity. BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene.



**Figure 3.** Structures of hydrogen bonds between OH groups in the one ring of phenol **20**.



Scheme 4. Stabilization of radicals by phenol 20.



Scheme 5. Stabilization of radicals by ascorbic acid (vitamin C).

3',4'. As shown in Figure 3, OH groups in one ring of the molecule may create intramolecular hydrogen bonds to each other.

An antioxidant compound is an inhibitor used to control auto-oxidation. For inhibitory effects of these phenol derivatives, the following mechanism may be proposed (Scheme 4). From the structures of phenol **20**, **20a** may be taken and abbreviated as **21**. The reaction of **21** with a radical ( $\text{RO}\cdot$ ) can give **22**, which converts to structures **23-26** by resonance and **27** by equilibrium. The structure **25** is an enol radical of a radical with CO. Ten resonance structures may be given for **20** in total because five resonance structures, **22-26**, are written for one ring of **20**. Therefore, phenols with CO will be better inhibitors than those without CO.

Ascorbic acid (vitamin C) is also a natural antioxidant and, like it forms a resonance-stabilised radical (Scheme 5) [54]. The structure **30** is an enol structure of ester CO. It is reported that CO groups [55] stabilise radicals and ketone [56,57] is formed in the reduction of aryl ketone.

## Declaration of interest

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