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# 1 Identification, Quantitation, and Sensory Evaluation of Thiols in 2 Bordeaux Red Wine with Characteristic Aging Bouquet

3 Liang Chen,\* Emilio De Longhi, Alexandre Pons, Thierry Buffeteau, Nicolas Daugey, Pascaline Redon,  
4 Svitlana Shinkaruk, and Philippe Darriet

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5 **ABSTRACT:** Great Bordeaux red wines are known for their distinctive aging bouquet. However, the nature of volatile chemicals  
6 underpinning this sensory expression is not fully understood. This work investigated the empyreumatic aging bouquet of a collection  
7 of premium Bordeaux red wines using silver-ion ( $\text{Ag}^+$ ) solid-phase extraction, cryogenic heart-cutting multidimensional gas  
8 chromatography mass spectrometry/olfactometry, and comprehensive two-dimensional gas chromatography time-of-flight mass  
9 spectrometry. In doing so, a substantial number of “meaty” odors were revealed. Three detected “meaty” notes were tentatively or  
10 unequivocally attributed to furan thiols. Among them, 2-methyltetrahydrofuran-3-thiol (**1**) with a pleasant “meaty” aroma was  
11 reported in wine for the first time. Its *trans* isomer (*trans*-**1a**) was resolved from its racemate by chemical modification, which  
12 confirmed its presence in wine. The odor detection threshold of *trans*-**1a** in the model wine was determined at 55 ng/L. Moreover,  
13 an additive effect between **1** and literature-known 2-methyl-3-furanthiol was observed. By a new ultra high-performance liquid  
14 chromatography quadrupole Orbitrap high-resolution MS method, the concentration of *trans*-**1a**, in addition to those of 2-methyl-3-  
15 furanthiol and 2-furfuryl thiol, was measured in the wines at ng/L levels.

16 **KEYWORDS:** *meaty, thiols, aging bouquet, aroma, wine*

## 1. INTRODUCTION

17 Premium red wines produced in Bordeaux are renowned for  
18 their symbolic “Bordeaux style” aging bouquet associated with  
19 a great diversity of aromatic attributes, previously described as  
20 “dominant fruity/floral notes and some roasted and licorice  
21 odors, together with hints of empyreumatic nuances.”<sup>1</sup>  
22 Analytical and sensorial characterization of aging bouquet  
23 has been described for premium aged red wines produced in  
24 Bordeaux<sup>2,3</sup> and other regions.<sup>4</sup> The aroma nuances of a wine  
25 are driven by the volatile molecules in the headspace acting as  
26 stimuli for the human olfactory system.<sup>5</sup> The volatile  
27 compounds identified in a wine are diverse in chemical  
28 families, concentration ranges, and sensory contributions.<sup>6</sup>  
29 Those with exceedingly low odor detection thresholds  
30 (ODTs) are often aroma-critical compounds because of their  
31 greater sensory impacts.<sup>7</sup> Volatile thiols are such aroma-active  
32 odorants that have been actively attracting research interest<sup>8</sup>  
33 since the identification of 4-methyl-4-sulfanyl-pentan-2-one  
34 (4MSP, boxtree odor, ODT 0.8 ng/L in the model wine), the  
35 first thiol reported in wines.<sup>9</sup> Other subsequently identified  
36 volatile thiols such as 3-sulfanylhexas-1-ol (3SH, grapefruit  
37 odor), 3-sulfanylhexasyl acetate (3SHA, passion fruit odor), 2-  
38 furfurylthiol (FFT, coffee-like odor), and 2-methyl-3-furanthiol  
39 (2M3FT, cooked meat odor) have become widely recognized  
40 among the most potent volatile aroma compounds in wine.<sup>10</sup>  
41 Certain thiols were particularly linked to the empyreumatic  
42 notes in aged wines.<sup>11–13</sup> The identification of thiols ignited  
43 research focusing on thiol biosynthesis and fermentative  
44 biotransformation, demonstrating the significance of discover-

ing new volatile compounds, particularly those having  
45 meaningful sensory contributions, for aroma research.<sup>5</sup> 46

47 From the perspective of analytical chemistry, qualitative and  
48 quantitative analyses of thiols in wine is intrinsically  
49 challenging<sup>5</sup> because these highly reactive molecules are  
50 mostly present at ultratrace quantities (~ng/L) in a complex  
51 wine matrix.<sup>10</sup> Historically, many of the first identifications of  
52 thiols in wines were achieved by odorant screening with  
53 conventional gas chromatography mass spectrometry/olfac-  
54 tometry (GC–MS/O) after selective extraction of thiols from  
55 wine. In short, thiols were selectively extracted by reversible  
56 affinity chromatography.<sup>10</sup> Afterward, the prepared organic  
57 extracts were analyzed by GC–MS/O for odorous zones  
58 (OZs) of interest. If the retention index, odor descriptors, and  
59 mass spectra of the selected OZs are obtained and match those  
60 of pure reference standards, then full identification can be  
61 concluded. The identification of 4MSP, 3SH, 3SHA, FFT, and  
62 2M3FT in wine, along with many other more,<sup>14</sup> was achieved  
63 using such an analytical approach due to the extraordinarily  
64 sensitive human olfactory system employed in GC–MS/O  
65 screening.<sup>10</sup> However, severely toxic organomercuric chemicals  
66 are universally involved in the selective thiol extraction step,  
67 posing health and environmental concerns. Meanwhile, more

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68 robust chromatography separation and detection systems  
 69 facilitated more thiol identifications in wine.<sup>14,15</sup> A recently  
 70 proposed protocol described the identification of new thiols in  
 71 red wine through oak-wood accelerated reductive treatment.<sup>16</sup>  
 72 Another alternative approach to identify thiols in foods and  
 73 beverages<sup>10</sup> was to conduct thiol-specific chemical derivatiza-  
 74 tion and to screen thiol derivatives by high-performance liquid  
 75 chromatography (HPLC) combined with high-resolution MS  
 76 (HRMS) or tandem MS (MS/MS). Thiol-specific derivatiza-  
 77 tion was needed to facilitate HPLC separation and to provide  
 78 diagnostic ions for MS screening.

79 Recently, a solid-phase extraction (SPE) method using  
 80 silver-ion (Ag<sup>+</sup>) cartridges for selective thiol extraction was  
 81 developed in our laboratory, with a particular focus on  
 82 qualitative thiol screening.<sup>17</sup> In reported demonstrative  
 83 applications, Ag<sup>+</sup> SPE was carried out to isolate thiol fractions  
 84 from two Bordeaux red wines. Preserved free thiols were  
 85 analyzed by Deans switch facilitated heart-cutting (H/C)  
 86 multidimensional (MD) GC–MS/O.<sup>17</sup> This method offered a  
 87 new analytical workflow to explore unknown trace volatile  
 88 thiols in wine.

89 In this study, we investigated a pool of premium Bordeaux  
 90 red wines with marked empyreumatic<sup>11,12</sup> aging bouquet in the  
 91 hope to decipher unknown volatiles involved in their typical  
 92 aging aroma expression. Preliminary benchtop sensory  
 93 evaluation after the addition of traces of copper (Cu<sup>2+</sup>)  
 94 instantly depleted the empyreumatic aging bouquet of selected  
 95 wines, indicating the involvement of thiols. Therefore, thiol  
 96 fractions were isolated by selective Ag<sup>+</sup> SPE protocol.<sup>17</sup> The  
 97 obtained thiol fractions were screened for the OZs of interest  
 98 by cryogenic H/C MDGC–MS/O. A large number of OZs  
 99 reminiscent of pleasant “meaty,” “boxtree,” and “thiols” was  
 100 detected, demonstrating the organoleptic importance of thiols  
 101 to the aging bouquet of the selected wines. A “meaty” smelling  
 102 thiol, 2-methyltetrahydrofuran-3-thiol (**1**), was identified in  
 103 wine for the first time. Chemical modification and density  
 104 functional theory (DFT) calculations confirmed the structural  
 105 assignment of *trans*-**1a** in wine. Tentatively, structures of two  
 106 additional “meaty” OZs are proposed based on their mass  
 107 spectra. In addition to H/C MDGC–MS/O screening, Ag<sup>+</sup>  
 108 SPE thiol fractions were further explored by comprehensive  
 109 two-dimensional gas chromatography time-of-flight mass  
 110 spectrometry (GC × GC–TOF/MS), revealing more thiol  
 111 candidates. The knowledge demonstrated in this study has  
 112 expanded our understanding of the contribution of thiols to  
 113 the aging bouquet of wine.

## 2. MATERIALS AND METHODS

114 **2.1. Chemicals.** The following chemicals were purchased from  
 115 commercial suppliers: D-(–)-ribose (≥99%, Sigma-Aldrich, Saint-  
 116 Quentin-Fallavier, France), L-cysteine (≥99.5%, Sigma-Aldrich),  
 117 ethylenediaminetetraacetic acid (EDTA, ≥ 99.995%, Sigma-Aldrich),  
 118 *cis/trans*-2-methyltetrahydrofuran-3-thiol (**1**, food grade, ≥ 97%,  
 119 Sigma-Aldrich), 2M3FT (≥95%, TCI Europe, Zwijndrecht, Belgium),  
 120 FFT (≥98%, Sigma-Aldrich), and 2-sulfanylpropanoate (≥95%,  
 121 Enamine, Kyiv, Ukraine). Solvents (VWR, Paris, France) used for  
 122 sample preparation were of HPLC grade or higher. Dichloromethane  
 123 (CH<sub>2</sub>Cl<sub>2</sub>) was distilled before use. Water was purified by a Milli-Q  
 124 ultrapure water purification system (Merck Millipore, Guyancourt,  
 125 France). Solvents used for HRMS analyses were of Optima LC/MS  
 126 grade (Thermo Fisher Scientific, Illkirch-Graffenstaden, France). The  
 127 stock solutions of thiol standards were prepared by weighing each  
 128 pure compound on a balance and making the desired volumes with  
 129 HPLC-grade ethanol. Working solutions were obtained by volumetri-

cally diluting the respective stock solutions. EDTA (~1 mg/mL) was  
 added to thiol solutions to minimize oxidations. Solutions of **1** and  
 FFT were prepared every 2 months to ensure their integrity. 2M3FT  
 was always prepared fresh. Thiol solutions were stored in an inert  
 atmosphere, protected from light, and kept at –20 °C.

**2.2. Wine Samples.** Eight Bordeaux red wines (Table 1) were  
 obtained from local producers for their expression of aging bouquet

**Table 1. Selected Premium Bordeaux Red Wines with Distinctive Emphyreumatic Aging Bouquet for GC–MS/O and H/C MDGC–MS/O Screening**

wine code	vintage	variety <sup>a</sup>	alcohol (v/v, %)	volume (mL/bottle)	Appellation d'Origine Contrôlée
W1	2009	Merlot 65%, Cabernet Franc 30%, Cabernet Sauvignon 5%	13.5	1500	Saint-Emilion <sup>b</sup>
W2	2007	Cabernet Sauvignon 55%, Merlot 35%, Cabernet Franc 5%	13.0	1500	Saint-Julien <sup>b</sup>
W3	2007	Merlot, Cabernet Sauvignon*	13.5	750	Saint-Julien
W4	2001	Merlot, Cabernet Sauvignon*	13.5	750	Saint-Julien
W5	2015	Merlot, Cabernet Sauvignon*	13.5	750	Saint-Julien
W6	2009	Merlot, Cabernet Sauvignon*	13.0	3000	Listrac-Médoc
W7	2009	Merlot, Cabernet Sauvignon*	13.0	750	Listrac-Médoc
W8	2006	Cabernet Sauvignon 65%, Merlot 35%	12.5	375	Pessac-Léognan <sup>b</sup>

<sup>a</sup>Blending percentage not specified. <sup>b</sup>Classified growth.

with emphyreumatic nuances and were subjected to thiol screening by  
 GC–MS/O and H/C MDGC–MS/O. Two wines (W9, W10, 1500  
 mL/bottle) from the same producer as W1, vintage 2011 and 2012,  
 respectively, were used for assessing the GC × GC–TOF/MS  
 performance.

**2.3. Ag<sup>+</sup> SPE.** The extraction of thiols from wine was conducted  
 according to a recently described protocol.<sup>17</sup> A large-volume SPE  
 barrel was fitted onto a Ag<sup>+</sup> SPE cartridge (MetaSep IC-Ag cartridge,  
 GL Sciences, Tokyo, Japan). An aliquot of wine (one or several 750 or  
 1500 mL bottles) was extracted by CH<sub>2</sub>Cl<sub>2</sub> (5% v/v solvent to sample  
 ratio) three times. The resulting organic phases were pooled. Ag<sup>+</sup> SPE  
 cartridge was preconditioned by 10 mL of CH<sub>2</sub>Cl<sub>2</sub>. Afterward, the  
 collected organic extracts were loaded onto a Ag<sup>+</sup> SPE cartridge,  
 followed by three washes, using CH<sub>2</sub>Cl<sub>2</sub> (10 mL), acetonitrile (20  
 mL), and CH<sub>2</sub>Cl<sub>2</sub> (10 mL) in sequence. Final elution was conducted  
 by 5 mL of H<sub>2</sub>O, 20 mL of freshly prepared L-cysteine solution (10 g/  
 L in H<sub>2</sub>O, sparged with N<sub>2</sub> before use), and 10 mL of CH<sub>2</sub>Cl<sub>2</sub>. The  
 collected elutes (5 mL of H<sub>2</sub>O, 20 mL of L-cysteine solution, and 10  
 mL of CH<sub>2</sub>Cl<sub>2</sub>) were combined together and stirred for 15 min, and  
 the organic phases were pooled, dried on anhydrous Na<sub>2</sub>SO<sub>4</sub>, and  
 concentrated under a gentle stream of N<sub>2</sub>. For method blanks<sup>18</sup>  
 (negative control samples), the entire extraction protocol was carried  
 out as described above, whereas the wine sample (0 mL) was omitted,  
 and the same batch solvents, reagents, laboratory ware, and  
 apparatuses were used to gauge any possible artifact inputs originated  
 from the laboratory environment and sample preparation.

**2.4. Maillard Reaction Generating “Meaty” Flavor Compounds.**  
 A thermal reaction was conducted to generate “meaty”  
 flavor compounds. A mixture was prepared by dissolving L-cysteine  
 (100 mg) and D-(–)-ribose (90 mg) in 20 mL of phosphate buffer  
 (0.2 M, pH 6.0).<sup>19</sup> Five aliquots (5 × 4 mL) of mixture were  
 transferred to five 10 mL thick-walled glass tubes (Pyrex) and sealed  
 with Teflon-coated screw caps. The tubes were protected from light  
 and autoclaved at 120 °C for 1 h. The autoclaved mixture was cooled  
 to room temperature, combined, and extracted by the Ag<sup>+</sup> SPE

172 protocol described above. The final extract (~50  $\mu$ L) was analyzed by  
173 H/C MDGC–MS/O.

174 **2.5. GC–MS/O and H/C MDGC–MS/O.** GC–MS/O and H/C  
175 MDGC–MS/O were conducted according to a previously described  
176 method<sup>17</sup> using a 7890B gas chromatograph (Agilent Technologies,  
177 Palo Alto, CA) coupled to a 5977A MS detector (Agilent  
178 Technologies). The selectable MDGC–MS/O system was fitted  
179 with a G4513A autosampler (Agilent Technologies), an olfactometry  
180 detection port (ODP3, Gerstel, Mülheim an der Ruhr, Germany)  
181 connected to a flow of humidified N<sub>2</sub> (Air Liquide, Floirac, France), a  
182 low thermal mass series II (LTM-II) module, a Deans switch plate, a  
183 three-way splitter, and a cryogenic trapping system (CTS2, Gerstel)  
184 using liquid nitrogen as the coolant. Helium (Air Liquide) was used as  
185 the carrier gas. The GC conditions were as follows–injector: liquid  
186 injection in the splitless mode at 240 °C; host GC oven kept at  
187 constant temperature of 240 °C; LTM <sup>1</sup>D column (30 m × 0.25 mm  
188 i.d., 0.25  $\mu$ m, DB-5MS or DB-35MS, Agilent J&W) with a flow rate of  
189 1.5 mL/min; LTM <sup>2</sup>D column (30 m × 0.25 mm i.d., 0.25  $\mu$ m, DB-  
190 WAX or DB-35MS, Agilent J&W) with a flow rate of 2.5 mL/min.  
191 For the conventional GC–MS/O analysis, the Deans switch was  
192 deactivated to bypass the <sup>2</sup>D column. In the H/C mode, Deans switch  
193 was activated, and cryogenic trapping was simultaneously applied.  
194 CTS2 was kept at 240 °C prior to the H/C, decreased at a rate of  
195 –720 °C/min to –150 °C (the duration of H/C), and increased to  
196 240 °C at a rate of 720 °C/min (after H/C). H/C window was  
197 selected based on GC–O data to target particular OZs of interest. MS  
198 transfer line temperature, MS source, and MS quadrupole were at 260  
199 °C, 230 °C, and 100 °C, respectively. Mass spectra were recorded in  
200 the full scan mode (35–350 *m/z*) using electron ionization at 70 eV.  
201 ODP was kept at 230 °C. A maximum of 2  $\mu$ L of extracts was  
202 injected. Two trained expert panelists were instructed to use a free  
203 vocabulary to describe the odors that were perceived at the sniffing  
204 port and to rank the intensity of perceived odors on a numerical scale  
205 from 1 to 5, with 1 being “weakest” and 5 as “strongest.” The panel  
206 was encouraged to focus on pleasant thiol-like odors. Linear retention  
207 indices (LRIs) were calculated by C<sub>8</sub>–C<sub>20</sub> *n*-alkanes (Sigma-Aldrich)  
208 for conventional GC–MS/O and for the <sup>1</sup>D of MDGC–MS/O runs.  
209 LRIs for the <sup>2</sup>D of the MDGC–MS/O were calculated according to a  
210 previously described procedure.<sup>20</sup> The collected sensory descriptors  
211 and their LRIs were compiled and compared to our in-house database  
212 and to NIST 2014. Method blank and instrument blank were analyzed  
213 to validate possible new identification of unknown thiols.

214 **2.6. Comprehensive GC × GC–TOF/MS.** GC × GC–TOF/MS  
215 system consisted of an Agilent 7890A gas chromatograph (Agilent  
216 Technologies), a Pegasus BT4D time-of-flight mass spectrometer  
217 (Leco Corporation, Saint Joseph, MI), and a L-PAL3 GC autosampler  
218 (Leco Corporation). A 0.5  $\mu$ L organic extract was injected at 230 °C  
219 in the splitless mode (1 min). Helium (Air Liquide) was used as the  
220 carrier gas at a flow rate of 1.2 mL/min. Capillary columns, DB-5MS  
221 50 m × 0.25 mm i.d., 0.25  $\mu$ m film thickness (Agilent J&W) and  
222 RTX-i-17Sil MS 1.3 m × 0.25 mm i.d., 0.25  $\mu$ m film thickness (Restek,  
223 Bellefonte, PA), were used for the <sup>1</sup>D and <sup>2</sup>D separation, respectively.  
224 The oven program for the first column was 55 °C (1 min) and a 4  
225 °C/min ramp to 280 °C (1 min). The secondary oven was kept at 5  
226 °C higher relative to the primary oven temperature. The modulator  
227 was held at 15 °C higher relative to the secondary oven temperature.  
228 The modulation period was set for 5 s and was performed by using a  
229 QuadJet dual-stage thermal modulator with liquid nitrogen. The  
230 transfer line temperature was set at 260 °C. MS ion source  
231 temperature was 250 °C. MS was operated in the electron ionization  
232 mode at 70 eV, and the mass scan range was 40–400 *m/z* with an  
233 acquisition rate of 200 spectra/s. Data acquisition and processing  
234 were performed by using LECO ChromaTOF software (version  
235 5.51). *n*-Alkane standards (C<sub>8</sub>–C<sub>20</sub>, Sigma-Aldrich) were analyzed for  
236 LRIs.

237 **2.6.1. Data Curation.** For each sample, automated baseline  
238 correction, deconvolution, and integration were processed using  
239 LECO ChromaTOF software for peaks with signal-to-noise (S/N) >  
240 25. Furthermore, peak identification was obtained by comparing to

commercial libraries (NIST 2017, FFNSC 3) for  $\Delta$ LRI  $\leq$  30 and/or a  
241 minimum mass spectrum similarity score (MSSS) of 800 out of 1000.

242 **2.7. Preparation of 1a and 1b.** The *trans*- and *cis*- stereoisomers  
243 of **1** (*trans*-**1a** and *cis*-**1b**, respectively) were obtained from 244  
commercially available racemic **1** through the following procedures 245  
(Figure 3a). The initial stereoisomeric ratio of *trans*-**1a** and *cis*-**1b** in 246  
racemic **1** was determined by GC–EI–MS. 247

**Step 1:** S-(2-methyloxolan-3-yl) benzenecarbothioate (**2**): To a 248  
suspension of potassium carbonate (10.6 g, 76.3 mmol) in 60 mL of 249  
acetone were added racemic **1** (2.6 mL, 3.0 g, 25.4 mmol) and 250  
benzoyl chloride (4.4 mL, 5.4 g, 38.2 mmol). The reaction mixture 251  
was stirred at room temperature. Thin-layer chromatography (TLC) 252  
was conducted on silica gel 60 F<sub>254</sub> TLC aluminum sheets (Merck) 253  
to monitor reaction. Spots were revealed with UV at 254 nm and 255  
potassium permanganate stain. The total conversion of **1** was achieved 256  
in 3.5 h. The mixture was extracted with EtOAc (3 × 40 mL). The 257  
organic phases were pooled and washed by H<sub>2</sub>O (3 × 40 mL), 258  
neutralized with saturated NaHCO<sub>3</sub> solution (120 mL), and washed 259  
by H<sub>2</sub>O (2 × 120 mL) until pH to 7. The organic phase was 260  
separated, dried over anhydrous magnesium sulfate, and filtered. The 261  
filtrate was collected, and the solvent was removed under a vacuum to 262  
obtain a crude colorless liquid **2** (5.95 g).

**Step 2:** The crude product **2** (3 g) was purified by silica gel 60 263  
(Merck, 70–230 mesh, 0.040–0.063 mm) flash chromatography (*n*- 264  
pentane/Et<sub>2</sub>O, 1/9, v/v) to give two colorless liquids: **2a** (330 mg, R<sub>f</sub> 265  
= 0.28) and **2b** 1.195 g (R<sub>f</sub> = 0.20). 266

For atom numbering used for NMR description, refer to Figure S1 267  
of the Supporting Information. 268

*trans*-(±)-3-S-Benzoyl-2,5-anhydro-1,4-dideoxy-3-thiopentitol (**2a**): 269  
<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  1.35 (d, *J* = 6.1 Hz, 3H, CH<sub>3</sub>), 1.98 270  
(dddd, *J* = 13.1 (4a–4b), 7.8 (4b–5a), 6.4 (3–4b), 5.3 (4b–5b) Hz, 271  
1H, H4b), 2.59 (dddd, *J* = 13.1 (4a–4b), 8.6 (3–4a), 7.6 (4a–5b), 272  
6.9 (4a–5a) Hz, 1H, H4a), 3.75 (app. td, *J* = 8.5, 6.8, 1H, H3), 3.98– 273  
3.81 (m, 2H, H2, H5a), 4.03 (ddd, *J* = 8.7 (5a–5b), 7.7 (4a–5b), 5.4 274  
(4b–5b) Hz, 1H, H5b), 7.46 (m, 2H, H<sub>ar</sub>3',5'), 7.58 (tt, *J* = 7.4, 1.3 275  
Hz, 1H, H<sub>ar</sub>4'), 7.95 (dd, *J* = 8.4, 1.3 Hz, 2H, H<sub>ar</sub>2',6'), 7.5 MHz, CDCl<sub>3</sub>):  $\delta$  19.54 (CH<sub>3</sub>), 33.63 (C4), 46.88 (C3), 66.98 (C5), 276  
80.32 (C2), 127.38 (C<sub>ar</sub>2',6'), 128.80 (C<sub>ar</sub>3',5'), 133.66 (C<sub>ar</sub>4'), 277  
136.98 (C1'), 191.59 (C=O). 279

*cis*-(±)-3-S-Benzoyl-2,5-anhydro-1,4-dideoxy-3-thiopentitol (**2b**): 280  
<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  1.27 (d, *J* = 6.0 Hz, 3H, CH<sub>3</sub>), 281  
2.07 (dddd, *J* = 13.3 (4a–4b), 8.0 (4b–5b), 6.1 (4b–5a), 4.3 (3–4b) 282  
Hz, 1H, H4b), 2.57 (dddd, *J* = 13.3 (4a–4b), 8.6 (4a–5a), 7.5 (3– 283  
4a), 6.1 (4a–5b) Hz, 1H, H4a), 3.82 (app. td, *J* = 8.4 (4a–5a and 284  
5a–5b), 6.5 (4b–5a) Hz, 1H, H5a), 4.01 (app. td, *J* = 8.0 (5a–5b and 285  
4b–5b), 6.1 (4a–5b) Hz, 1H, H5b), 4.21–4.31 (m, 2H, H2, H3), 286  
7.45 (m, 2H, H<sub>ar</sub>3',5'), 7.58 (tt, *J* = 7.5, 1.3 Hz, 1H, H<sub>ar</sub>4'), 7.95 (dd, *J* 287  
= 8.4, 1.4 Hz, 2H, H<sub>ar</sub>2',6'). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  17.13 288  
(CH<sub>3</sub>), 33.84 (C4), 46.53 (C3), 66.25 (C5), 76.92 (C2), 127.38 289  
(C<sub>ar</sub>2',6'), 128.77 (C<sub>ar</sub>3',5'), 133.60 (C<sub>ar</sub>4'), 137.08 (C-1'), 191.65 290  
(C=O). 291

**Step 3:** **2a** (330 mg, 1.5 mmol) was added to a degassed aqueous 292  
lithium hydroxide solution (LiOH, 0.1 mM, 45 mL), and the reaction 293  
mixture was stirred under an argon atmosphere for 5 h. The reaction 294  
mixture was extracted with *n*-pentane (2 × 20 mL). The aqueous 295  
phases were separated and acidified with 5 M HCl to bring pH to 3– 296  
4. The aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (20 mL), dried over 297  
anhydrous magnesium sulfate, and filtrated. The filtrate was 298  
concentrated by gentle rotary evaporation, and a white precipitate 299  
was formed. The white precipitate was washed with a small amount of 300  
ice-cold *n*-pentane. The organic phase was recovered and gently 301  
removed by rotary evaporation to afford colorless liquid *trans*-**1a** (96 302  
mg, 55% yield, purity 97% determined by GC–EI–MS). 303

*trans*-(±)-2-Methyltetrahydrofuran-3-thiol (*trans*-**1a**): <sup>1</sup>H NMR 304  
(300 MHz, CDCl<sub>3</sub>):  $\delta$  1.29 (d, *J* = 6.1 Hz, 3H, CH<sub>3</sub>), 1.58 (d, *J* = 305  
7.3 Hz, 1H, SH), 1.84 (app. dtd, *J* = 12.65 (4a–4b), 8.3 (3–4a and 306  
4a–5b), 6.6 (4a–5a) Hz, 1H, H4a), 2.44 (app. dtd, *J* = 12.65 (4a– 307  
4b), 8.0 (3–4b and 4b–5a), 5.8 (4b–5b) Hz, 1H, H4b), 2.77 (app. 308  
qt, *J* = 8.2 (8.0 for (2–3 and 3–4b) and 8.3 (3–4a), 7.3 (3-SH) Hz, 309  
1H, H3), 3.59 (dq, *J* = 8.0 (2–3), 6.1 (2–6) Hz, 1H, H2), 3.54– 310

311 9.94:3.86 (app.td,  $J = 8.6$  (8.3 (4a–5b) and 8.9 (5a–5b)), 5.8 (4b–  
312 5b) Hz, 1H, H5b), 3.91 (ddd,  $J = 8.9$  (5a–5b), 8.0 (4b–5a), 6.6 (4a–  
313 5a) Hz, 1H, H5a).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  18.20 ( $\text{CH}_3$ ),  
314 36.86 (C4), 42.66 (C3), 66.21 (C5), 83.70 (C2).

315 EI–HRMS ( $m/z$ ): Calculated for  $\text{C}_5\text{H}_{10}\text{OS}^+$ ,  $[\text{M}]^+$ , 118.0447;  
316 found 118.045. EI–MS, 70 eV,  $m/z$  (%): 74 (100), 41 (56), 84 (31),  
317 45 (15), 56 (14), 118 (13,  $\text{M}^+$ ), 43 (11), 73 (11), 55 (9), 59 (8).  
318  $\text{LRI}^{\text{DB-SMS}}$ , 908.  $\text{LRI}^{\text{DB-Wax}}$ , 1308.

319 The same procedure was applied for the deprotection of **2b** (1.18 g,  
320 5.5 mmol) to give *cis*-**1b** as a colorless liquid (399 mg, 64% yield,  
321 purity 96% determined by GC–EI–MS).

322 *cis*-(±)-2-Methyltetrahydrofuran-3-thiol (*cis*-**1b**):  $^1\text{H}$  NMR (300  
323 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.29 (d,  $J = 6.2$  Hz, 3H,  $\text{CH}_3$ ), 1.44 (d,  $J = 7.6$  Hz,  
324 1H, SH), 1.97 (dddd,  $J = 13.15$  (4a–4b), 7.9 (4a–5b), 5.4 (4a–5a),  
325 3.9 (3–4a) Hz, 1H, H4a), 2.46 (app. ddt,  $J = 13.15$  (4a–4b), 8.7  
326 (4b–5a), 6.9 (3–4b and 4b–5b) Hz, 1H, H4b), 3.40 (app. tt,  $J = 7.3$   
327 (7.6 (3-SH) and 6.9 (3–4b)), 4.3 (4.8 (2–3) and 3.9 (3–4a)) Hz,  
328 1H, H3), 3.77 (app. td,  $J = 8.6$  (5a–5b and 5a–4b), 5.4 (4a–5a) Hz,  
329 1H, H5a), 3.98–4.07:4.02 (ddd,  $J = 8.6$  (5a–5b), 7.9 (4a–5b), 6.9  
330 (4b–5b) Hz, 1H, H5b), 4.03 (qd,  $J = 6.2$  (2–6) 4.8 (2–3) Hz, 1H,  
331 H2).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.91 ( $\text{CH}_3$ ), 36.96 (C4), 42.04  
332 (C3), 65.61 (C5), 77.36 (C2).

333 EI–HRMS ( $m/z$ ): Calculated for  $\text{C}_5\text{H}_{10}\text{OS}^+$ ,  $[\text{M}]^+$ , 118.0447;  
334 found 118.0480. EI–MS, 70 eV,  $m/z$  (%): 74 (100), 41 (55), 84 (29),  
335 118 (16,  $\text{M}^+$ ), 45 (16), 43 (12), 56 (12), 73 (11), 55 (9), 59 (9).  
336  $\text{LRI}^{\text{DB-SMS}}$ , 950.  $\text{LRI}^{\text{DB-Wax}}$ , 1390.

337 **2.8. DFT Calculations.** All DFT calculations were carried out with  
338 Gaussian 16.<sup>21</sup> The calculation of the IR spectra began by  
339 conformational analysis of compounds (*R,R*)-**2** (*cis*-form) and  
340 (*S,R*)-**2** (*trans*-form). Preliminary conformer distribution search of  
341 (*R,R*)-**2** and (*S,R*)-**2** was performed at the molecular mechanics level  
342 of theory, employing MMFF94 force fields incorporated in Gauss-  
343 View 6.1 software package.<sup>22</sup> Ten conformers of (*R,R*)-**2** (11  
344 conformers for (*S,R*)-**2**) were found within roughly 4 kcal/mol of  
345 the lowest energy conformer. Their geometries were optimized at the  
346 DFT level using the B3LYP functional and 6-31G\*\* basis set, leading  
347 to four different conformers for (*R,R*)-**2** and (*S,R*)-**2**. Finally, only the  
348 three lowest energetic geometries for (*R,R*)-**2** and (*S,R*)-**2** were kept  
349 within 2.678 kJ/mol ((*R,R*)-**2**) and 2.232 kJ/mol ((*S,R*)-**2**).  
350 Vibrational frequencies and IR and VCD intensities were calculated  
351 at the same level of theory. The spectra were calculated for the  
352 isolated molecule in vacuo. For comparison to experiment, the  
353 calculated frequencies were scaled by 0.97, and the calculated  
354 intensities were converted to Lorentzian bands with a full width at  
355 half-maximum (fwhm) of 7  $\text{cm}^{-1}$ .

356 **2.9. IR Spectroscopy.** IR spectra of **2a** and **2b** were recorded with  
357 a Thermo Nicolet Nexus 670 FTIR spectrometer at a resolution of 4  
358  $\text{cm}^{-1}$  by coadding 50 scans. Samples were held in a 250  $\mu\text{m}$  path  
359 length cell with  $\text{BaF}_2$  windows. IR spectra of **2a** and **2b** were  
360 measured in  $\text{CDCl}_3$  at a concentration of 60 mM. The solvent  
361 absorption was subtracted out in the presented IR spectra.

362 **2.10. GC–TOF/MS.** Spectra and accurate masses of *trans*-**1** and  
363 *cis*-**1b** were obtained on an Agilent 7980A gas chromatograph coupled  
364 to a JMS-T100GCGC TOF mass spectrometer (JEOL Ltd., Akishima,  
365 Tokyo, Japan). 1  $\mu\text{L}$  of sample was injected in a split/spiltless injector  
366 that was kept at 230  $^\circ\text{C}$ . A DB-5 capillary column (50 m  $\times$  0.22 mm  
367 i.d., 0.25  $\mu\text{m}$ , Agilent J&W) was used for separation following the  
368 oven gradient: 45  $^\circ\text{C}$  (1 min) to 240  $^\circ\text{C}$  at 4  $^\circ\text{C}/\text{min}$  held for 5 min.  
369 Helium (6.0, Messer) was used as the carrier gas with a flow rate of  
370 1.0 mL/min. The transfer line was kept at 250  $^\circ\text{C}$ . Ion source was set  
371 at 250  $^\circ\text{C}$ . Mass spectra were recorded in the full scan mode (45–300  
372  $m/z$ ) with mass resolution at 5,000 using electron ionization at 70 eV.  
373 Data acquisition was performed using JEOL Mass Center software.  
374 Solutions of *trans*-**1a** and *cis*-**1b** were prepared in  $\text{CH}_2\text{Cl}_2$  at a  
375 concentration of  $\sim 1$  mg/L.

376 **2.11. Nuclear Magnetic Resonance Spectroscopy.** The  $^1\text{H}$   
377 and  $^{13}\text{C}$  nuclear magnetic resonance (NMR) experiments were  
378 performed using a Bruker Avance 300 spectrometer at 300 and 75  
379 MHz, respectively, at 297 K with deuterated chloroform ( $\text{CDCl}_3$ ) as  
380 the solvent. The spectra were referenced using the lock frequency of

deuterated solvent. Chemical shifts ( $\delta$ ) and coupling constants ( $J$ ) are  
381 expressed in ppm and Hz, respectively. NMR spectra data of  
382 synthesized *trans*-**1a**, *cis*-**1b**, **2a**, and **2b** are available in Figures S2–S7  
383 of the Supporting Information.

384  
385 **2.12. Thiol Quantitation.** Thiols *trans*-**1a**, 2M3FT, and FFT  
386 were quantified by a new UPLC quadrupole Orbitrap HRMS method.  
387 Sample preparation consisted of chemical derivatization described  
388 previously.<sup>23</sup> Liquid chromatographic separation was performed using  
389 a Vanquish system (Thermo Fisher Scientific) consisting of a binary  
390 pump, an autosampler, and a temperature-controlled column  
391 chamber. HRMS analyses were performed on a Thermo Fisher  
392 Scientific Exactive Plus Orbitrap mass spectrometer fitted with a  
393 heated electrospray ionization source (HESI-II) probe operated in the  
394 positive ion mode. Data acquisition and instrument control were  
395 managed with Xcalibur 4.3 and Tune 2.9 software (Thermo Fisher  
396 Scientific). The details of the method development and validation will  
397 be presented in a subsequent publication from our research  
398 laboratory.

399 **2.13. ODT.** ODT determination experiments were carried out in  
400 the sensory facility at ISVV. The ODT of **1** (commercial racemate)  
401 was measured in the model wine following the ISO 13301:2018  
402 guideline.<sup>24</sup> The model wine solution was freshly prepared by  
403 dissolving food-grade L-(+)-tartaric acid (5 g/L) in a 12% (v/v)  
404 ethanol–water solution, and the pH was adjusted to 3.2 using NaOH  
405 pellets. To limit the change of the concentration of **1**, fresh standard  
406 solutions of **1** were prepared, stored in an inert atmosphere at  $-20$   
407  $^\circ\text{C}$ , and used for sensory analysis within 24 h. The addition of **1** to the  
408 model wine solution was performed 1 h before the sensory sessions. A  
409 panel of 25 participants (M 12, F 13, average age: 31, students and  
410 researchers at ISVV) were presented with six sets of triangular tests  
411 arranged in an ascending order in terms of the concentration of **1** (5,  
412 15, 45, 135, 405, and 1215 ng/L). Each set consisted of three samples,  
413 in which one was different from the other two. Standard clear wine  
414 glasses were coded with a three-digit number, filled with 25 mL of the  
415 sample, and covered with lids. The participants were instructed to  
416 smell each sample within each set and to choose the sample exhibiting  
417 different aroma. The panelist was asked to provide descriptors for the  
418 aroma perceived. The collected sensory data were processed  
419 according to the group best estimated threshold procedure outlined  
420 in ISO 13301:2018.<sup>24</sup> ODT of *trans*-**1a** was determined in the same  
421 manner in a separate session by the same panel within the same day.

422 **2.14. Sensory Interaction between **1** and 2M3FT.** The  
423 sensory experiment was conducted in the ISVV sensory laboratory  
424 with a panel of 26 participants (M 8, F 18) recruited from ISVV. The  
425 general protocol and model wine solution preparation were identical  
426 to the ODT determination described above. Three sessions were  
427 performed. Session 1 aimed to assess the panel's ODT of 2M3FT in  
428 model wine using seven concentration levels of 0.5, 1, 2, 4, 8, 16, and  
429 32 ng/L. Session 2 targeted the ODT of **1** in model wine with  
430 ascending concentrations of 5, 10, 20, 40, 80, 160, and 320 ng/L. For  
431 session 3, the base model wine was first supplemented with 2M3FT to  
432 reach a final concentration of 0.5 ng/L. Increasing concentration of **1**  
433 was added to the modified model wine to reach concentrations of 5,  
434 10, 20, 40, 80, and 320 ng/L. Three sessions were conducted within  
435 the same day by the same panel. Group best estimated thresholds  
436 were obtained following the ISO 13301:2018 guideline.<sup>24</sup>

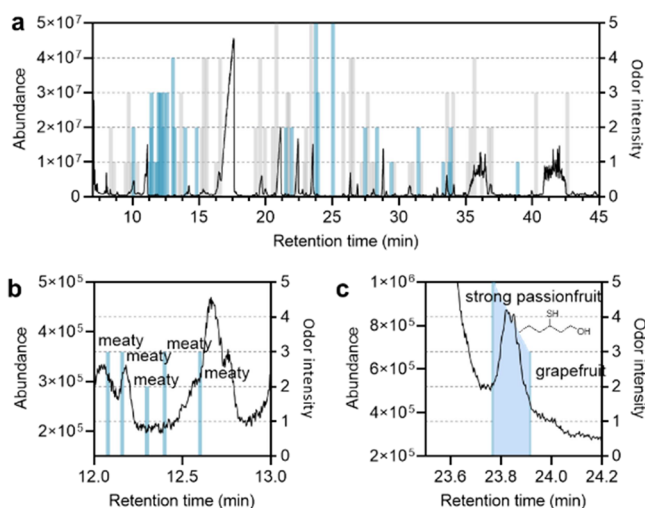
437 **2.15. Statistical Analyses.** Data reduction and treatment were  
438 performed using Microsoft Excel.

### 3. RESULTS AND DISCUSSION

439 **3.1. GC–MS/O Profile of  $\text{Ag}^+$  SPE Extracts of Selected**  
440 **Wines.** A total of eight red wines (vintage 2001 to 2015, aged  
441 8 to 22 years) from the Bordeaux region (Table 1) were  
442 selected for their distinctive empyreumatic aging bouquet. To  
443 search potentially new volatile thiols, the  $\text{Ag}^+$  SPE protocol  
444 recently developed in our laboratory<sup>17</sup> was used to isolate thiol  
445 fractions from red wines. Because of the trace quantity of thiols  
446 in red wines and the effluent splitting between MS and O, extra

447 measures were taken during sample preparation to ensure  
 448 maximum MS/O detection, including using a relatively large  
 449 volume of wine (one to several 750 or 1500 mL bottles) and  
 450 concurrently concentrating the final  $\text{Ag}^+$  SPE extracts to a  
 451 small volume ( $\leq 50 \mu\text{L}$ ). This corresponded to a minimal  
 452 concentration factor of 15,000. The obtained volatile thiol  
 453 extracts were first analyzed by GC-MS/O and then by H/C  
 454 MDGC-MS/O to screen OZs of interest by two experienced  
 455 sniffers throughout this project.

456 A representative total ion chromatogram (TIC) of the GC-  
 457 MS/O analysis of the  $\text{Ag}^+$  SPE extract on a DB-5MS column is  
 458 shown in Figure 1a. The chemical and sensorial complexity of



**Figure 1.** (a) Representative TIC overlaid with the aromagram of a Bordeaux red wine (W1) analyzed by  $\text{Ag}^+$  SPE and GC-MS/O fitted with a DB-5MS column (30 m  $\times$  0.25 mm i.d., 0.25  $\mu\text{m}$ ). (b, c) Expanded sections of (a). Vertical lines represent perceived OZs; in blue: OZs of interest, in gray: other OZs. Shaded area in (c) indicates that the odor was continuously perceived.

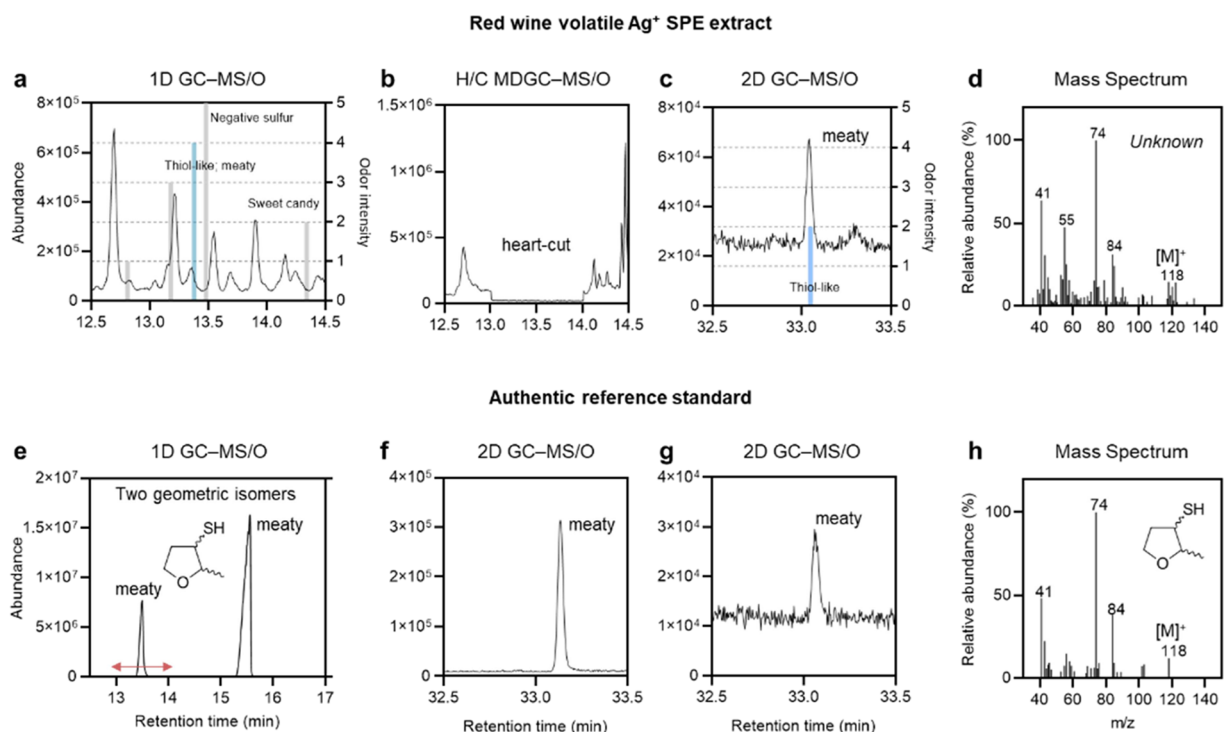
459 the  $\text{Ag}^+$  SPE fraction was apparent. Twenty-nine OZs of  
 460 interest exhibiting “thiol-like,” “boxtree,” “meaty,” “roasted,”  
 461 “cooked food,” and “grilled” notes that warranted further  
 462 investigations were perceived in addition to forty-five OZs  
 463 displaying disagreeable “sulfur,” “rubber,” “garlic,” “pungent,”  
 464 or other aroma nuances. The detection of negative sulfurous or  
 465 nonthiol OZs was related to certain sulfur-containing [e.g., 3-  
 466 (methylthio)-1-propanol, 3-(methylthio)propanal], nitrogen-  
 467 containing (e.g., pyrazines), and aromatic (e.g., phenylethyl  
 468 alcohol) compounds which were simultaneously isolated by  
 469 the  $\text{Ag}^+$  SPE sorbent used. Our observation on the affinity of  
 470  $\text{Ag}^+$  sorbent toward multiclass of molecules was mirrored by a  
 471 recent report.<sup>25</sup> Nevertheless, the long-lasting odors of  
 472 abundant wine volatile compounds such as acetates, alcohols,  
 473 and acids were absent on GC-MS/O, showing sufficient  
 474 selectivity of this sample preparation method targeting thiols.

475 The olfactometric profile of the thiol fractions by GC-MS/  
 476 O was challenging to interpret. For instance, a total of five  
 477 “meaty” OZs were subsequently noticed in an approximate 30  
 478 s elution time window (Figure 1b). The OZs of interest from  
 479 GC-MS/O analysis of sample W8 on a DB-5MS column are  
 480 listed in Table S1 of the Supporting Information. Each OZ was  
 481 examined for their corresponding chromatographic peak and  
 482 mass spectrum. Initial identification of OZs was difficult,  
 483 mostly hindered by severe coelutions. Only for very limited

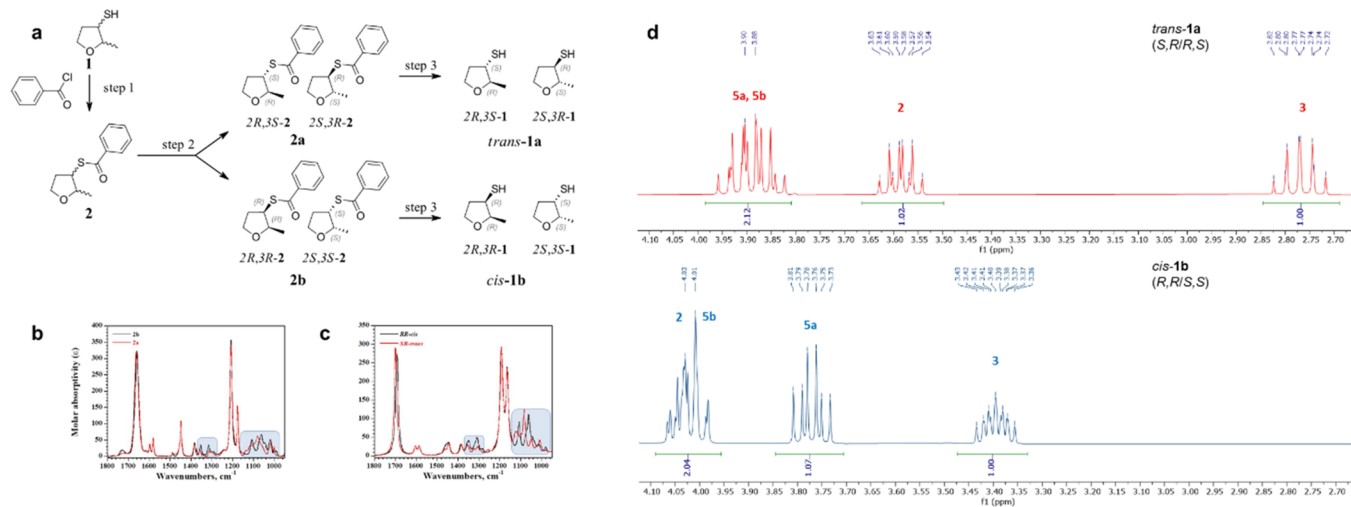
examples can identification of known thiols based on a clearly  
 484 resolved peak on GC-MS/O be made, such as for 3SH  
 485 (Figure 1c). To resolve coelutions and obtain meaningful MS/  
 486 O information, H/C MDGC-MS/O was necessary.

**3.2. H/C MDGC-MS/O Profile of  $\text{Ag}^+$  SPE Extracts of Selected Wines.** Table S1 of the Supporting Information also  
 489 presents respective OZs of interest detected after nine  
 490 scheduled heart-cuts of sample W8. The first evident  
 491 observation was that H/C MDGC successfully resolved  
 492 persistent coelutions that were otherwise observed by conven-  
 493 tional GC-MS/O using a single column (<sup>1</sup>D-olfactometry).  
 494 However, the odors detected after the second column by  
 495 MDGC-MS/O (<sup>2</sup>D-olfactometry) were not always consis-  
 496 tent with those perceived on the <sup>1</sup>D. This discrepancy in  
 497 perceived odors between the <sup>1</sup>D separations and <sup>2</sup>D was likely  
 498 to be the results of perceptual interaction of coeluting odorants  
 499 on the <sup>1</sup>D which were resolved by the <sup>2</sup>D. The next obvious  
 500 point was that a number of OZs reminiscent of “meaty” and  
 501 “cooked food” were detected. Although “meaty” aroma has  
 502 been mentioned to contribute to the aging bouquet of fine  
 503 Bordeaux red wines,<sup>2,3,26,27</sup> the associated molecules were  
 504 limited to half a dozen known volatile thiols of which only  
 505 2M3FT exhibits a “meaty” or “roasted” odor. In another GC-  
 506 MS/O study on aged red wine, FFT and 2M3FT were  
 507 identified as contributing aroma compounds to the “savory” or  
 508 “umami-type” aroma.<sup>28</sup> Our H/C MDGC-MS/O results  
 509 clearly illustrated a deep involvement of “meaty” “cooked  
 510 food” odors in the overall aroma space of premium Bordeaux  
 511 red wines. Another interesting observation was that “meaty”/  
 512 “cooked food” OZs (LRI<sup>DB-WAX</sup>  $\sim 964$ ) occurred in all nine H/  
 513 C runs despite being derived from different heart-cuts.  
 514 Regardless, most resolved chromatographic peaks on the <sup>2</sup>D  
 515 were still extremely small, which is not surprising for trace thiol  
 516 analytes. In many instances, meaningful mass spectra for  
 517 intriguing odors were still not obtainable. With  
 518 effortful manual peak picking and mass spectrum interpreta-  
 519 tion, the following previously known thiols 3-sulfanyl-2-  
 520 methylpropan-1-ol, ethyl 2-sulfanylacetate, 3SH, 4MSP,  
 521 2M3FT, and FFT were found in the selected Bordeaux red  
 522 wines. Certain detected thiols have been particularly associated  
 523 with the positive aroma expression (3SH, 2M3FT, and FFT)  
 524 of aged red wines<sup>2,28</sup> and empyreumatic notes (FFT) in  
 525 various wines.<sup>12,13,28</sup>

**3.3.  $\text{Ag}^+$  SPE H/C MDGC-MS/O False Positive Identification.** One OZ (GC-MS/O LRI<sup>DB-SMS</sup>  $\sim 848$ )  
 528 exhibiting a strong “boxtree” and “thiol” odor was noted, but  
 529 no identification could be concluded. Hence, LRI range  
 530 (LRI<sup>DB-SMS</sup>  $848 \pm 5 = 843-857$ ) was heart-cut, and the same  
 531 odor was detected on the <sup>2</sup>D, accompanied by a resolved peak.  
 532 NIST 2014 library matched it as methyl 2-sulfanylpropanoate  
 533 (CAS 53907-46-3, matching score 785). The identity of this  
 534 peak was confirmed by an authentic standard (MS, LRI, and  
 535 odor quality) analyzed under the same analytical condition. Its  
 536 tentative identification in wine by LC-HRMS was previously  
 537 suggested.<sup>29</sup> It seemed that methyl 2-sulfanylpropanoate  
 538 naturally occurred in wine, but a method blank indisputably  
 539 proved that this identification was false in our wine samples.  
 540 Further investigation (data not shown) showed that methyl 2-  
 541 sulfanylpropanoate was an artifact that originated from the L-  
 542 cysteine that was used for  $\text{Ag}^+$  SPE elution. This false positive  
 543 identification highlighted the importance of running method  
 544 blanks as negative control.



**Figure 2.** Compiled H/C MDGC–MS/O identification results of one isomer of 2-methyltetrahydrofuran-3-thiol **1** in a 2007 Bordeaux red wine (W3). (a) A segment (12.5–14.5 min) of GC–MS/O TIC of a wine Ag<sup>+</sup> SPE extract overlaid with detected OZs showing a distinct “thiol-like” “meaty” odor with an intensity level at 4. (b) A segment (12.5–14.5 min) of H/C MDGC–MS/O TIC of the same extract showing the heart-cut retention time = 13–14 min. (c) A segment (32.5–33.5 min) of H/C MDGC–MS/O TIC of the same extract showing a clearly resolved peak on the 2D. (d) Mass spectra of the peak showed in (c). (e) A segment (12.5–13.5 min) of GC–MS/O TIC of an authentic reference standard of 2-methyltetrahydrofuran-3-thiol analyzed under the identical instrument condition as that for (a). (f) A segment (32.5–33.5 min) of H/C MDGC–MS/O TIC of a high concentration of the same reference standard analyzed under the identical instrument condition as that for (c). (g) A segment (32.5–33.5 min) of H/C MDGC–MS/O TIC of a diluted authentic reference standard analyzed under the identical instrument condition as that for (c). (h) Mass spectra of the peak showed in (g).

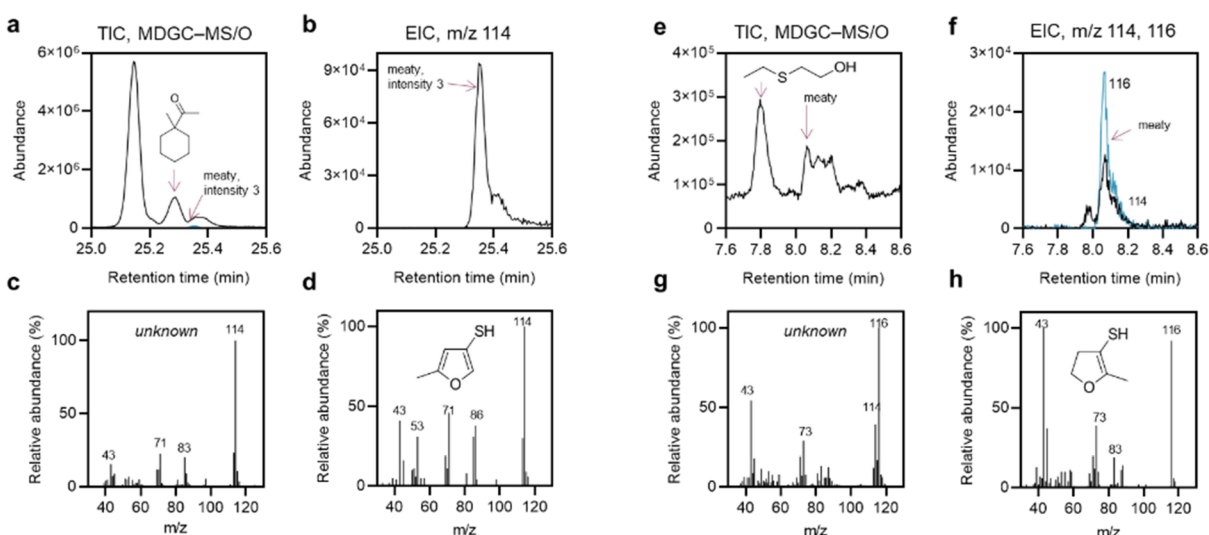


**Figure 3.** (a) Chemical synthesis routes to pure *trans* (*trans-1a*) and *cis* (*cis-1b*) isomers of 2-methyltetrahydrofuran-3-thiol from commercial **1**. Step 1: benzoyl chloride, K<sub>2</sub>CO<sub>3</sub>, acetone, rt, 3.5h. Step 2: flash chromatography (*n*-pentane/Et<sub>2</sub>O, 1/9, v/v). Step 3: LiOH, H<sub>2</sub>O, rt, 5h. (b) Experimental IR spectra of **2a** (in red) and **2b** (in black); shaded areas indicate spectra difference. (c) Calculated DFT spectra of *RS-2* (in red) and *RR-2* (in black); shaded areas indicate spectra difference. (d) Attribution of <sup>1</sup>H NMR spectra of H2, H3, and H5 of *trans-1a* and *cis-1b*. For atom numbering, refer to Figure S1 of Supporting Information.

546 **3.4. Identification of 2-Methyl-tetrahydrofuran-3-**  
 547 **thiol (1) in Wine by Ag<sup>+</sup> SPE H/C MDGC–MS/O.** A  
 548 pleasant “meaty”/“roasted meat” odor was noticed at  
 549 ~LRI<sup>DB-SMS</sup> 900 during GC–MS/O screening (Figure 2a) of  
 550 a 2007 Bordeaux red wine (W3). However, no positive

identification can be reached due to coeluting peaks. 551  
 Therefore, a one min heart-cut (centered at LRI<sup>DB-SMS</sup> 900, 552  
 Figure 2b) was performed. A total of six OZs were perceived 553  
 on the <sup>2</sup>D of which one had the same “cooked/roasted meat” 554  
 odor that was noted on GC–MS/O. A fully resolved 555





**Figure 4.** Chromatography and mass spectra of two unknown “meaty” smelling odors detected in the  $\text{Ag}^+$  SPE extracts of selected Bordeaux red wines (W5 and W3) and two reference mass spectra. (a, e) TICs of the  $^2\text{D}$  showing the respective retention regions; (b, f) EICs of the  $^2\text{D}$  revealing the detection of ions  $m/z$  114 and/or 116 during the detection of “meaty” odors; (c, g) experimental mass spectra of two unknown peaks; archived reference mass spectra (internal database from Firmenich) of (d) 5M3FT and (h) DH2M3FT.

556 chromatographic peak was recorded at the same retention time  
 557 when this odor was detected on  $^2\text{D}$  (Figure 2c). Mass  
 558 spectrum search in the NIST 2014 database indicated the  
 559 compound to be *cis/trans*-2-methyl-3-tetrahydrofuranthiol **1**.  
 560 Its identity was fully confirmed by a commercial reference  
 561 standard (CAS 57124-87-5, racemic mixture, Figure 2e–h). At  
 562 this stage, identification was only confirmed for one isomer of  
 563 **1**, not yet for the *cis*- or *trans*- form. Method blanks were  
 564 conducted for both GC–MS/O and H/C MDGC–MS/O,  
 565 and **1** was not detected in either run, validating its genuine  
 566 occurrence in wines.

567 In the context of foods and beverages, **1** was mentioned in  
 568 meat flavorings<sup>30</sup> and coffee products<sup>31,32</sup> in a few cases. In a  
 569 previously published review,<sup>33</sup> it was stated that **1** had been  
 570 found and quantified in wines, but this claim was questionable  
 571 and cannot be backed up by the two originally cited source  
 572 references.<sup>34,35</sup> Therefore, we believe that this is the first time  
 573 that thiol **1** was positively identified in wine. When the  
 574 authentic reference standard was used to confirm the presence  
 575 of **1** in wine, we noticed that the identified single isomer of **1**  
 576 had an  $\text{LRI}^{\text{DB-SMS}}$  value very similar to that of FFT, a previously  
 577 known thiofuran with extremely potent coffee-like odor (ODT  
 578 0.4 ng/L in model wine solution and 0.0084 ng/L in air<sup>36</sup>). In  
 579 retrospective, their coelution on a classic nonpolar column  
 580 during GC–O screening might partially explain why **1** was not  
 581 identified in wine until now.

582 **3.5. Preparation of *cis*-1b and *trans*-1a.** **1** has two chiral  
 583 centers at the C2 and C3 positions (Figure 3a), affording a pair  
 584 of *cis/trans* stereoisomers and two pairs of enantiomers. The  
 585 reference standard of **1** from a commercial supplier was a  
 586 mixture of *cis*- and *trans*- isomers with an approximate ratio 1:5  
 587 or 5:1 (determined by GC–EI–MS with a DB-5MS column,  
 588 Figure 2e) or 1:4.2–4.4 or 4.2–4.4:1 (later determined by the  
 589 integration of SH peak on the  $^1\text{H}$  NMR spectrum of  
 590 commercial **1**). During our GC–MS/O and H/C GC–MS/  
 591 O screening experiments, the “meaty” odor noticed only  
 592 corresponded to one isomer on nonchiral GC columns DB-  
 593 SMS (Figure 2a)/DB-35MS/DB-WAX. Based on available  
 594 literature,<sup>37</sup> four enantiomers of **1** possessed distinguishable

odor properties and different ODTs ( $\sim\text{pg}$  levels, in the 595  
 596 unspecified matrix). Therefore, the ratio of *cis/trans* of **1** in the  
 597 commercial racemate was determined to confirm the peak  
 598 identity. NMR analysis of the commercial *cis/trans* mixture of  
 599 **1** was carried out, but the spectrum was too difficult to  
 600 interpret and did not allow confidently distinguishing between  
 601 the two pairs of enantiomers (data not shown). Therefore,  
 602 chemical synthesis (Figure 3a) was conducted to separate *cis*-  
 603 **1b** and *trans*-**1a** from the commercial mixture. The chemical  
 604 modification of the sulfhydryl group in **1** was achieved by the  
 605 reaction with benzoyl chloride to form thioester **2**. The steric  
 606 hindrance caused by the thioester formation facilitated the  
 607 chromatographic separation of the *cis/trans* mixture by classical  
 608 flash chromatography on silica gel to give two fractions: **2a** and  
 609 **2b**. The fractions **2a** and **2b** were studied by IR spectroscopy  
 610 to discriminate the two pairs of *cis* (*RR/SS*) and *trans* (*SR/RS*)  
 611 diastereomers. IR spectra recorded in  $\text{CDCl}_3$  solution at a  
 612 concentration of 60 mM are reported in Figure 3b in the  
 613 1800–950  $\text{cm}^{-1}$  spectral range. The IR spectra of **2a** and **2b**  
 614 are similar, except around 1300  $\text{cm}^{-1}$  and in the 1150–1000  
 615  $\text{cm}^{-1}$  region. The band around 1300  $\text{cm}^{-1}$  is related to the  
 616 bending  $\delta\text{OCH}$  and wagging  $\omega\text{CH}_2$  of the furan group,  
 617 whereas the bands in the 1150–1000  $\text{cm}^{-1}$  region are related  
 618 to the asymmetric  $\nu_a\text{COC}$  and symmetric  $\nu_s\text{COC}$  stretching  
 619 modes of the furan group coupled with rocking  $\text{CH}_2$  and  $\text{CH}_3$ .  
 620 The bands associated with the thio-benzoyl group are not  
 621 sensitive to the stereochemistry of the molecule ( $\nu\text{C}=\text{O}$  at  
 622 1660  $\text{cm}^{-1}$ ,  $\nu_{8a}\text{C}=\text{C}$  and  $\nu_{8b}\text{C}=\text{C}$  at 1598 and 1582  $\text{cm}^{-1}$ ,  
 623  $\nu_{19a}\text{C}=\text{C}$  and  $\nu_{19b}\text{C}=\text{C}$  at 1489 and 1449  $\text{cm}^{-1}$ ,  $\delta_{\text{ip}}\text{CH}$  of  
 624 phenyl at 1208 and 1177  $\text{cm}^{-1}$ ).

The predicted spectra calculated at the B3LYP/6-31G\*\* 625  
 626 level for the *RR*-**2** and *SR*-**2** configurations are reported in  
 627 Figure 3c in the 1800–950  $\text{cm}^{-1}$  spectral range. As observed in  
 628 the experimental IR spectra, significant changes are present in  
 629 the predicted DFT spectra for modes involving furan group  
 630 around 1300  $\text{cm}^{-1}$  and in the 1150–1000  $\text{cm}^{-1}$  spectral range.  
 631 Comparison of experimental and predicted spectra clearly  
 632 establishes the *trans*-**2a** and *cis*-**2b** configurations.

633 The  $^1\text{H}$  NMR analysis of **2a** and **2b** fractions was difficult  
634 because of the signal overlapping of protons at the 2, 3, and 5  
635 positions (Figure S2 of the Supporting Information) but  
636 seemed to confirm the configuration determined by IR  
637 measurements and DFT calculations. Indeed, protons H2  
638 and H3 are more strongly shielded for compound **2a** which is  
639 in agreement with theoretical calculations. Moreover, the  
640 proton H3 of **2a** is split to the apparent doublet of triplets  
641 (Figure S3 of the Supporting Information). Deeper analysis of  
642 this signal by the deconvolution process presented it as a ddd  
643 with  $^3J = 8.9, 8.2,$  and  $6.5$  Hz which can be attributed to (2–3),  
644 (3–4a), and (3–4b) H–H coupling, respectively. According  
645 to the Karplus curve, $^3J = 8.9$  Hz corresponds to the *trans*  
646 coupling of the protons at 2 and 3 positions.

647 The deprotection of the sulfhydryl groups of **2a** and **2b** gave  
648 *trans-1a* and *cis-1b*, respectively. The  $^1\text{H}$  NMR analysis  
649 confirmed the configuration assignment as *trans-1a* and *cis-*  
650 **1b** (Figure 3d). The experimental vicinal coupling constants  
651 were  $^3J_{2-3} = 8.0$  Hz for *trans-1a* and  $^3J_{2-3} = 4.8$  Hz for *cis-1b*.  
652 These values are in accordance with the Karplus dependence  
653 of the H–H coupling constants on the dihedral angle, which is  
654 larger for the *trans*-isomer. In the same way as for protected  
655 forms, protons H2 and H3 were more shielded for compound  
656 *trans-1a*.

657 Therefore, the compound detected in the wine samples was  
658 confirmed as *trans-1a*.

### 659 3.6. Two “Meaty” OZs by $\text{Ag}^+$ SPE H/C MDGC–MS/O.

660 Two intense and pleasant “roasted meat”/“meaty” OZs were  
661 detected during the GC–MS/O screening of  $\text{Ag}^+$  SPE wine  
662 extracts. The odor qualities of these two OZs were almost  
663 indistinguishable from those of 2M3FT and *trans-1a*, but again,  
664 positive identification was not possible at this stage due to  
665 coelutions. Neither the OZ corresponded to known thiofurans  
666 (2M3FT, FFT, 2,5-dimethylfuran-3-thiol, and *trans-1a*).

667 The first intense ( $I = 5$ ) “meaty” OZ was detected on the  $^1\text{D}$   
668 at  $\sim \text{LRI}^{\text{DB-SMS}} 937$  (rt = 15.20 min) of five bottles of W5. A 30  
669 s heart-cut from 15 to 15.5 min was performed and resolved on  
670 the  $^2\text{D}^{\text{DB-3SMS}}$ . On the  $^2\text{D}$ , two “meaty” and two “boxtree”  
671 odors were perceived. However, severe chromatographic  
672 coelutions appeared. One “meaty” odor ( $I = 4$ ) was recorded  
673 in the valley region of two adjacent GC peaks both in decent  
674 abundances (Figure 4a). NIST 2014 library search suggested  
675 the first peak to be 1-(1-methylcyclohexyl)-ethanone, unlikely  
676 to correspond to any “meaty” odor. With a close inspection of  
677 the retention time region close to the beginning of the second  
678 peak, detection of  $m/z$  114 was spotted. Extracted ion  
679 chromatogram (EIC,  $m/z$  114) is presented in Figure 4b,  
680 which perfectly aligned with the detection of the intense  
681 “meaty” odor. The subtracted mass spectrum of this peak is  
682 given in Figure 4c, a mass spectrum very similar to that of  
683 2M3FT. However, the identity of this peak cannot be 2M3FT  
684 as 2M3FT eluted outside this heart-cut event. Further  
685 comparison of our experimental mass spectrum to an external  
686 MS database (Firmenich) indicated a possible match to 5-  
687 methyl-3-furanthiol (5M3FT). The historically archived mass  
688 spectrum of the pure standard of 5M3FT is given in Figure 4d.  
689 5M3FT is a constitutional isomer to 2M3FT, differing in the  
690 position of the methyl group. It was once mentioned in  
691 Maillard reaction products, but its identification  
692 ( $\text{LRI}^{\text{CPWAX57CB}}=1304$ ) then was only based on mass spectrum  
693 comparison to that of 2M3FT.<sup>19</sup> The same authors also  
694 suggested<sup>19</sup> that the detected isomer to 2M3FT could also be  
695 4-methyl-3-furanthiol (4M3FT). Elsewhere, a similar mass

spectrum of a compound originated from the model Maillard  
696 reaction was proposed to be 2-methyl-4-furanthiol (2M4FT,  
697 no LRI information available),<sup>38</sup> another isomer to 2M3FT.  
698 Without a pure reference standard, only tentative identification  
699 can be reached. None of the suspected isomers to 2M3FT  
700 were commercially available, and several attempts to synthesize  
701 5M3FT were made, but none was successful (data not shown).  
702

Another strong-smelling ( $I = 5$ ) “meaty” odor was detected  
703 in the  $\text{Ag}^+$  SPE extract (20  $\mu\text{L}$ ) prepared from W3 at  
704  $\sim \text{LRI}^{\text{DB-SMS}} 941$ . To enhance detectability, repeated injections  
705 ( $n = 5$ ) were performed, and five heart-cuts were cryogenically  
706 focused on the head of the  $^2\text{D}$  column and released at once for  
707 MDGC–MS/O analyses. TIC shows persistent coelutions  
708 (Figure 4e). A small but partially resolved peak was noticed for  
709 the “meaty” aroma ( $I = 4$ ) on the  $^2\text{D}$ . Ions  $m/z$  114 and 116  
710 were observed at the corresponding retention time (Figure 4f).  
711 A clean mass spectrum was obtained for this partially resolved  
712 peak (Figure 4g). Again, no probable hits could be retrieved  
713 from the NIST 2014 library. After comparison to the same  
714 external database (Firmenich), the peak identity was indicated  
715 as 4,5-dihydro-2-methyl-3-furanthiol (DH2M3FT). The  
716 archived reference mass spectrum of DH2M3FT is provided in  
717 Figure 4h. This thiofuran was also reported as the volatile  
718 component from Maillard reaction that had “roasted meat”  
719 aromas.<sup>38–40</sup> DH2M3FT was previously patented as a flavoring  
720 substance<sup>41</sup> and its formation pathways were proposed.<sup>42</sup>  
721 DH2M3FT had LRI values reported as 927<sup>40</sup> on a DB-1  
722 column and 939<sup>39</sup> on a HP-5MS column, which was close to  
723 our data (941 on a DB-SMS column). Substantial efforts for its  
724 synthesis were devoted but eventually proved fruitless in  
725 producing this targeted thiol even at a trace quantity (data not  
726 shown). Alternatively, we investigated the possibility of  
727 generating these two thiofurans (DH2M3FT and 5M3FT)  
728 through the Maillard reaction, which was known to produce  
729 “meaty” aromas. Following a literature protocol,<sup>19</sup> a Maillard  
730 reaction was performed. The resulting Maillard reaction  
731 mixture exhibited extremely intense “meaty” aromas. Although  
732 the mass spectra very similar to those of suspected thiofurans  
733 (Figure 4d,h) were noticed in the Maillard reaction mixture by  
734 GC–MS/O, due to the restraint on time and resources, the  
735 isolation of targeted thiol fractions from the Maillard reaction  
736 mixture and subsequent identification were not pursued.  
737 Therefore, the structural identify of these two “meaty” smelling  
738 compounds remains to be elucidated upon obtaining pure  
739 reference standards. The access to the unavailable standards  
740 may be achieved by novel chemical synthesis by fractionating  
741 Maillard reaction mixture using  $\text{Ag}^+$  SPE and preparative scale  
742 MDGC with a cryogenic fraction trap, or by other alternative  
743 means.<sup>43</sup> Although  $\text{Ag}^+$  SPE with preparative MDGC for  
744 isolating trace thiofurans from Maillard reaction extracts is  
745 without precedent, the concept of the technique is well-known  
746 for small molecule discovery.<sup>44</sup> One analytical system adapting  
747 single- dimension preparative GC with the fraction collector  
748 has already allowed the separation, collection, and identi-  
749 fication of volatile compounds in wine.<sup>45</sup> 750

**3.7.  $\text{Ag}^+$  SPE and GC  $\times$  GC–TOF/MS for Non-Targeted**  
**Thiol Screening in Wine.** As demonstrated earlier,  $\text{Ag}^+$  SPE  
752 and H/C MDGC–MS/O are very effective in thiol screening  
753 due to the highly sensitive human olfactory system, but its  
754 associated data acquisition (particularly for olfactometry) and  
755 interpretation require rigorous attention from panelists/  
756 analysts. Additionally, the conventional single quadrupole  
757 mass spectrometer operated in the full scan mode in 758

759 MDGC–MS/O struggles to provide sufficient detectability  
760 needed for trace thiol analytes in complex samples. By  
761 comparison, GC × GC–TOF/MS offers higher resolution  
762 power, lower detection limits, as well as automatic data  
763 deconvolution features than H/C MDGC–MS/O. This  
764 technique has been reported for nontargeted screening of  
765 (thiols) unknowns in wine<sup>14,15</sup> and coffee,<sup>15,32</sup> in which various  
766 sample cleanup protocols were applied. Here, Ag<sup>+</sup> SPE  
767 coupling with GC × GC–TOF/MS as an enhanced solution  
768 for thiol screening in red wines was demonstrated. First, the  
769 sample complexity significantly impacted GC × GC–TOF/  
770 MS performance. Crude organic extract of two wines (W9,  
771 W10) and two Ag<sup>+</sup> SPE extracts (first CH<sub>2</sub>Cl<sub>2</sub> washing and the  
772 final elute) prepared from the same wines were collected.  
773 According to previous data,<sup>17</sup> these fractions had various levels  
774 of complexity. Each fraction was analyzed by GC × GC–  
775 TOF/MS, and the data summarizing the number of  
776 identifications in relation to data filtering criteria are presented  
777 in Table S2 of the Supporting Information. The total number  
778 of sulfur-containing compounds (119–152 matches) was quite  
779 similar across different fractions. Approximately, half of the  
780 matches were retained after mass spectra similarity score  
781 greater than 800 was applied. ΔLRI ≤ 30 filtered out an  
782 average of 87% the initial matches. The final Ag<sup>+</sup> SPE extract  
783 showed more than double the number of sulfur compound  
784 identifications compared to those in other organic extracts.  
785 This trend, in a much more pronounced manner, was observed  
786 for thiols. For W9, a total of 18 thiols fitting either the mass  
787 spectra similarity score or ΔLRI criteria were seen in the final  
788 Ag<sup>+</sup> SPE extracts compared to just two in other fractions  
789 (Table S2 of the Supporting Information). Compared to  
790 previously reported GC × GC–TOF/MS data for similar red  
791 wine varietal,<sup>15</sup> thiol-rich Sauvignon blanc wine,<sup>46</sup> and other  
792 wines,<sup>14</sup> a considerably higher number of thiols reported in this  
793 study clearly suggests the superiority of applying Ag<sup>+</sup> SPE as  
794 sample cleanup prior to nontargeted thiol screening by GC ×  
795 GC–TOF/MS.

796 Table S3 summarizes volatile sulfur compounds and thiols  
797 tentatively identified in the selected Premium Bordeaux red  
798 wines. It can be first concluded that GC × GC–TOF/MS  
799 achieved equal, if not better, identification performance in  
800 comparison to H/C MDGC–MS/O as nearly all thiols  
801 discovered using H/C MDGC–MS/O were captured by GC  
802 × GC–TOF/MS, including **1**. Many of these sulfur and thiol  
803 compounds exhibited “meaty” or “roasted” aromas, reflecting  
804 the aroma profile of the initial wine samples. Apart from  
805 literature-known thiols, a number of novel thiols were also  
806 tentatively uncovered based on comparison of mass spectra  
807 similarity score and LRI to commercial available databases,  
808 such as 4-propan-2-ylbenzenethiol, thiophene-2-thiol, thio-  
809 guaiacol, octane-1-thiol, and 5-methyl-2-furfurylthiol. This  
810 result shed new light on thiol composition in wine. For  
811 example, thioguaiacol was recently reported in smoke-exposed  
812 wines and thought to be unique to smoke tainted wines.<sup>47</sup> The  
813 detection and quantitation of thiophenols in our wines (data  
814 not shown) indicate a rather ubiquitous occurrence, which  
815 seemed to be unrelated to smoke exposure but to other  
816 sources. Further details on thiophenols in wines will be  
817 presented in upcoming manuscripts from our research group.<sup>16</sup>

818 **3.8. ODT.** The ODT of racemic **1** and *trans*-**1a** measured in  
819 model wine solution by an untrained panel was at 71 ng/L and  
820 55 ng/L, respectively, much higher than previously reported  
821 values (2–13 pg in unspecified matrix).<sup>37</sup> As detailed sensory

protocol and matrix information for the previous measure- 822  
ments were not provided,<sup>37</sup> it was hard to draw meaningful 823  
comparisons here. Threshold of racemic **1** was slightly higher 824  
than that of *trans*-**1a**. The threshold of 2M3FT reported in 825  
model wine was at 4 ng/L,<sup>26</sup> significantly lower than that of **1** 826  
and *trans*-**1a**. 2M3FT and *trans*-**1a** (and **1**) differ in the 827  
saturation degree of the furan ring, and this might result in a 828  
difference in their perception threshold. *Trans*-**1a** had a much 829  
lower detection threshold than that (300 ng/L) of another 830  
“meat-like” “roasty” sulfur-containing furan compound 2- 831  
methyl-3-(methylthio)furan previously identified in red 832  
wines.<sup>48</sup> The threshold of *cis*-**1b** was not measured. 833

A quick assessment for the aroma of the two isomers (*trans*- 834  
**1a** and *cis*-**1b**) was conducted with a small panel (*n* = 5) after 835  
transferring ~μL of each stock solution to a cellulose smelling 836  
strip. *Trans*-**1a** possessed very similar aroma properties to 837  
2M3FT, showing pleasant “meaty,” “cooked meat,” and “BBQ” 838  
aroma. Comparing the aroma qualities, *trans*-**1a** and **1** had a 839  
more rounded and pleasant meaty aroma profile than *cis*-**1b** 840  
which exhibit a hint of rubbery/pungent notes, and this 841  
observation was mirrored by previous statements.<sup>37</sup> Influence 842  
of structural modification and chirality on the odor quality of 843  
thiols was well documented.<sup>36</sup> 844

**3.9. Sensory Interaction between **1** and 2M3FT.** 845  
Intrigued by the sensorial and structural similarities between 846  
**1** and the well-known 2M3FT, we investigated the sensory 847  
interaction between these two thiofurans was investigated. A 848  
sensory panel (*n* = 26) first accessed the ODT of two 849  
thiofurans in model wine and found that the ODT was at 0.9 850  
and 60 ng/L for 2M3FT and **1**, respectively. The ODT at 60 851  
ng/L for **1** measured using a new panel was consistent with the 852  
previously determined value (71 ng/L) considering the panel 853  
variations. However, the ODT of 2M3FT at 0.9 ng/L was 854  
much lower than previously reported 4 ng/L.<sup>26</sup> This 855  
discrepancy could be attributed to a highly sensitive panel to 856  
2M3FT and/or the formation of trace 2M3FT disulfide 857  
(confirmed by GC–EI–MS, data not shown) that was 858  
extremely potent with an odor threshold reported at 2 parts 859  
in 10<sup>14</sup> parts of water.<sup>49</sup> The high reactivity of 2M3FT has 860  
been long known,<sup>26</sup> and it can be easily oxidized to its disulfide 861  
form.<sup>17</sup> Regardless, 0.5 ng/L 2M3FT (half of the group 862  
detection threshold) was supplemented to a model wine 863  
solution, to which increasing amounts of **1** were added. In the 864  
presence of 2M3FT, the ODT of **1** was measured to be at 23 865  
ng/L, a significantly decrease from 60 ng/L, indicating a strong 866  
additive effect between two investigated thiols. 2M3FT was 867  
previously reported to have a synergistic interaction with thiol 868  
3-methyl-3-sulfanylbutanal during the aroma extraction 869  
dilution analysis of Sauternes wines.<sup>50</sup> In other words, even 870  
at the subthreshold level, **1** may still have meaningful impacts 871  
on “meaty” “roasted meat” aroma in wine in the presence of 872  
other thiols. 873

**3.10. Quantitation of *trans*-**1a**, 2M3FT, and FFT.** The 874  
concentrations of three thiols, *trans*-**1a**, 2M3FT, and FFT, 875  
were assayed in selected wines by a newly developed UPLC 876  
quadruple Orbitrap HMRS method and are presented in Table 877 12  
**2**. In the surveyed wines, *trans*-**1a** was detected in all samples, 878 12  
indicating a ubiquitous occurrence. With concentrations 879  
ranging from 1.4 to 10.3 ng/L, under its threshold (55 ng/ 880  
L), *trans*-**1a** appeared to be unable to make a direct 881  
contribution to wine aroma quality. However, given the 882  
observed additive effect between **1** and 2M3FT, subthreshold 883  
concentrations of *trans*-**1a** might still impart real sensory 884

**Table 2. Concentration (ng/L)<sup>a</sup> of *trans*-1a, 2M3FT, and FFT in Selected Bordeaux Red Wines**

wine	<i>trans</i> -1	FFT	2M3FT
1	3.3 (1.7)	2.5 (2.2)	79.8 (3.8)
2	10.3 (0.4)	4.8 (1.1)	37.1 (1.2)
3	1.4 (2.5)	30.4 (5.0)	35.7 (1.8)
4	5.9 (0.5)	10.9 (7.2)	30.3 (6.6)
5	3.4 (2.5)	4.6 (2.1)	26.4 (1.0)
6	4.8 (0.6)	1.8 (7.0)	47.6 (2.3)
7	7.2 (1.5)	3.2 (5.5)	112.1 (8.5)
8	5.0 (3.4)	2.0 (3.1)	95.8 (1.8)
9	6.3 (0.8)	8.6 (3.0)	39.4 (3.3)
10	6.3 (1.5)	2.8 (2.2)	67.5 (3.7)

<sup>a</sup>Data derived from replicates ( $n = 3$  for wine 1–7,  $n = 2$  for wine 8–10), expressed as average values with relative standard deviation (% RSD) given in parentheses.

tentative identification based on LRIs and MSSS from GC × GC–TOF/MS analysis of Ag<sup>+</sup> SPE extract of 930 wine samples (PDF) 931

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885 impact. Moreover, in another ongoing project in our  
886 laboratory, *trans*-1a was noticed in quantities in wine well  
887 above its threshold (unpublished results). In a previous study,  
888 **1** (racemate) was quantitated in coffee brew samples at ~2 ng/  
889 L.<sup>31</sup> Regardless of the detected isomer(s),<sup>31</sup> *trans*-1a had a  
890 more pronounced concentration in our wines than in coffee  
891 brews.

892 FFT ranged from 1.8 to 30.4 ng/L, lower than that of  
893 2M3FT between 26.5 and 112.1 ng/L. Both furan thiols  
894 presented at concentrations similar to the historical data  
895 measured in Bordeaux red wines.<sup>2,26</sup> Out of 10 wines, 6 wine  
896 samples contained *trans*-1a presented at a higher concentration  
897 than that of FFT. No correlation was noticed among the three  
898 thiofurans. Considering the very limited samples that were  
899 analyzed here, a broader survey covering more wines is  
900 certainly required.

901 In summary, this study has reported identification of novel  
902 thiols in Bordeaux red wines with marked aging bouquet. 2-  
903 Methyltetrahydrofuran-3-thiol **1**, reminiscent of “meaty” odor,  
904 was identified. Tentative identifications of two furan thiols  
905 were proposed. This study was the first demonstration of  
906 applying Ag<sup>+</sup> SPE with H/C MDGC–MS/O and GC × GC–  
907 TOF/MS as effective approaches for thiol discovery.  
908 Quantitation and sensory evaluation of *trans*-1a were achieved.  
909 Moreover, **1** and 2M3FT exhibited an additive sensory  
910 interaction effect. Looking into the future, the following  
911 avenues may be explored. First and foremost, *trans*-1a needs to  
912 be surveyed in a larger set of wines of various varieties,  
913 vintages, and regions to provide more quantitative data. The  
914 enantiomeric chirality of *trans*-1a can be further investigated.  
915 Questions surrounding the origin, evolution, and reactivity of  
916 *trans*-1a in wine remain to be answered. Additional efforts  
917 could also be directed to interrogate other detected unknown  
918 “meaty” odorants for a better understanding of the aroma  
919 space of the distinctive aging bouquet of Bordeaux red wine.

## ASSOCIATED CONTENT 920

### Supporting Information 921

922 The Supporting Information is available free of charge at  
923 <https://pubs.acs.org/doi/10.1021/acs.jafc.3c05854>.

924 Chemical structures of *trans*-1a, *cis*-1b, **2a**, and **2b**; <sup>1</sup>H  
925 and <sup>13</sup>C NMR spectra of *trans*-1a, *cis*-1b, **2a**, and **2b**;  
926 GC–MS/O and H/C MDGC–MS/O odor screening  
927 results; nontargeted GC × GC–TOF/MS screening  
928 results of extracts with different complexities; and

987 **Notes**

988 The authors declare no competing financial interest.

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